1 **The human glucocorticoid receptor variant rs6190 promotes blood cholesterol and atherosclerosis**<br>2<br>**Authors:**<br>Hima Bindu Durumutla<sup>1,2</sup>, April Haller<sup>3</sup>, Greta Noble<sup>1</sup>, Ashok Daniel Prabakaran<sup>1</sup>, Kevin McFarland<sup>1</sup>, H 2<br>3<br>4<br>5<br>6 3 **Authors:**<br>4 Hima Bind<br>5 Latimer<sup>1</sup>, (<br>6 **Affiliation**<br>7 <sup>1</sup>Molecula Hima Bindu Durumutla<sup>1,2</sup>, April Haller<sup>3</sup>, Greta Noble<sup>1</sup>, Ashok Daniel Prabakaran<sup>1</sup>, Kevin McFarland<sup>1</sup> Hima Bindu Durumutla<sup>1,2</sup>, April Haller<sup>3</sup>, Greta Noble<sup>1</sup>, Ashok Daniel Prabakaran<sup>1</sup>, Kevin McFarland<sup>1</sup>, Hannah<br>Latimer<sup>1</sup>, Olukunle Akinborewa<sup>1,4</sup>, Bahram Namjou-Khales<sup>2,4</sup>, David Y. Hui<sup>2</sup>, Mattia Quattrocelli<sup>1\*</sup><br> Latimer<sup>1</sup>, Olukunle Akinborewa<sup>1,4</sup>, Bahram Namjou-Khales<sup>2,4</sup>, David Y. Hui<sup>2</sup>, Mattia Quattrocelli<sup>1\*</sup>

- <sup>1</sup>Molecular Cardiovascular Biology, Heart Institute, Cincinnati Children's Hospital Medical Center, Cincinnati,
- 
- 5<br>5<br>5<br>7<br>8<br>9<br>5 6 **Affiliations:**<br>
7 <sup>1</sup>Molecular C<br>
8 OH, USA.<br>
9 <sup>2</sup>Dept. Pediat<br>
0 Cincinnati Co<br>
1 <sup>4</sup>Center for A  $^{2}$ Dept. Pediatrics;  $^{3}$ Department of Pathology;  $^{4}$
- <sup>7</sup> Molecular Cardiovascular Biology, Heart Institute, Cincinnati Children's Hospital Medical Center, Cincinnati,<br>
9 <sup>2</sup> Dept. Pediatrics; <sup>3</sup> Department of Pathology; <sup>4</sup> Dept Pharmacology, Physiology and Neurobiology; Un 8 OH, USA.<br>9 <sup>2</sup>Dept. Pec<br>0 Cincinnati<br>1 <sup>4</sup>Center for<br>2 USA. <sup>2</sup> Dept. Pediatrics; <sup>3</sup> Department of Pathology; <sup>4</sup> Dept Pharmacology, Physiology and Neurobiology; University of<br>
2 Cincinnati College of Medicine, Cincinnati, OH, USA.<br>
<sup>4</sup> Center for Autoimmune Genomics and Etiology, Cincinnati College of Medicine, Cincinnati, OH, USA.<br>11 <sup>4</sup>Center for Autoimmune Genomics and Etiology, Cinc<br>12 USA.<br><sup>2</sup> **Corresponding Author:**<br>15 Mattia Quattrocelli, PhD, Molecular Cardiovascular Bi <sup>4</sup>Center for Autoimmune Genomics and Etiology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH,
- 

- <sup>4</sup>Center for Autoimmune Genomics and Etiology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH,<br>12 USA.<br><sup>2</sup> Corresponding Author:<br><sup>2</sup> Corresponding Author:<br>15 Mattia Quattrocelli, PhD, Molecular Cardiovascul 12 USA.<br>
13 **Corre**<br>
14 **Corre**<br>
15 Mattia<br>
16 ical Corre<br>
17 513-5 14<br>15<br>16<br>17<br>18 14 **\*Corresponding Author:**<br>15 Mattia Quattrocelli, PhD, I<br>16 ical Center, 240 Albert Sa<br>17 513-517-1221<br>18 **Keywords** – Rs6190, E Mattia Quattrocelli, PhD, Molecular Cardiovascular Biology, Heart Institute, Cincinnati Children's Hospital Medical Center, 240 Albert Sabin Way T4.676, Cincinnati, OH 45229. Email: <u>mattia.quattrocelli@cchmc.org</u>, tel: +1
- 
- 16 ical Center, 240 Albert Sabin Way T4.676, Cincinnati, OH 45229. Email: <u>mattia.quattrocelli@cchmc.org,</u> tel: +1-<br>17 513-517-1221<br>18 **Keywords** Rs6190, ER22/23K, glucocorticoid receptor, SNP, cholesterol, atheroscleros 17 513-517-1221<br>
18<br>
19 **Keywords –** 1<br>
20 hepatocytes, L<br>
21<br>
22 **Word count** (in 19<br>
20<br>
21<br>
22<br>
23 19 **Keywords – Rs6190**, ER22/23K, glucocorticoid receptor, SNP, cholesterol, atherosclerosis, liver, hiPSCs,<br>
10 hepatocytes, LDL receptor, PCSK9, transactivation.<br>
12 **Word count** (including title page, full text, referen
- 
- 
- 

Interpretations, LDL receptor, PCSK9, transactivation.<br>
21 **Word count** (including title page, full text, reference:<br>
23 **Data availability – RNA-seq and ChIP-seq dataset**<br>
25 **GSE280572.**  $\frac{22}{23}$ <br> $\frac{23}{24}$ <br> $\frac{25}{26}$ **Word count** (including title page, full text, references, figure legends) – 10,266 words.<br>
23 **Data availability –** RNA-seq and ChIP-seq datasets reported here are available on G<br>
25 **GSE280572.**<br>
26 **Author contributions**  $\frac{24}{25}$ <br> $\frac{25}{26}$ <br> $\frac{27}{28}$ 

**Data availability –** RNA-seq and ChIP-seq datasets reported here are available on GEO as GSE280494 and<br>
25 GSE280572.<br> **Author contributions** –HBD, AH, GN, ADP, KMF, HL, OA, BNK: Data curation, Formal analysis, Investigat 25 GSE280572.<br>26<br>27 **Author contr**<br>28 AJ, DYH: Res<br>29<br>30 **Conflicts of I** 

- 
- 27<br>
28<br>
29<br>
30<br>
31 **Author contributions** –HBD, AH, GN, ADP, KMF, HL, OA, BNK: Data curation, Formal analysis, Investigation;<br>
29<br> **29 Conflicts of Interest** – MQ is listed as co-inventor on a patent application related to intermittent glu 28 AJ, DYH: Resources; MQ: Conceptualization, Formal analysis, Funding acquisition, Supervision.<br>
29<br> **29 Conflicts of Interest** – MQ is listed as co-inventor on a patent application related to intermittent<br>
31 use filed  $30$ <br> $31$ <br> $32$ <br> $33$ <br> $34$ 30 **Conflicts of Interest –** MQ is listed as co-inventor on a patent application related to intermittent glucocorticoid<br>31 use filed by Northwestern University (PCT/US2019/068,618), unrelated to any aspects of this study. 31 use filed by Northwestern University (PCT/US2019/068,618), unrelated to any aspects of this study. All other<br>32 authors declare they have no competing interests.<br>33<br>34<br>35 32 authors declare they have no competing interests.<br>33<br>34<br>35<br>36
- 
- 34<br>35<br>36
- 
- $\frac{35}{36}$ 35

### <sup>37</sup>**Graphical Abstract**



- 38<br>39
- 
- 39

## <sup>41</sup>**Abstract**

13 Elevated cholesterol poses a significant cardiovascular risk, particularly in older women. The glucocorticoid re-14 ceptor (GR), a crucial nuclear transcription factor that regulates the metabolism of virtually all major nutrients, 15 harbors a still undefined role in cholesterol regulation. Here, we report that a coding single nucleotide polymor-16 phism (SNP) in the gene encoding the GR, *rs6190*, associated with increased cholesterol levels in women ac-17 cording to UK Biobank and All Of Us datasets. In SNP-genocopying transgenic mice, we found that the rs6190 18 SNP enhanced hepatic GR activity to transactivate *Pcsk9* and *Bhlhe40*, negative regulators of low-density lip-19 oprotein (LDL) and high-density lipoprotein (HDL) receptors in liver respectively. Accordingly, in mice the 50 rs6190 SNP was sufficient to elevate circulating cholesterol levels across all lipoprotein fractions and the risk 51 and severity of atherosclerotic lesions on the pro-atherogenic *hAPOE\*2/\*2* background. The SNP effect on 52 atherosclerosis was blocked by in vivo knockdown of Pcsk9 and Bhlhe40 in liver. Remarkably, we found that 53 this mechanism was conserved in human hepatocyte-like cells using CRISPR-engineered, SNP-genocopying 54 human induced pluripotent stem cells (hiPSCs). Taken together, our study leverages a non-rare human variant 55 to uncover a novel GR-dependent mechanism contributing to atherogenic risk, particularly in women.

 $57$ 

 $58$ <br> $59$ <br> $50$ <br> $51$ 58 **Introduction**<br>59 Hypercholest<br>50 disease, part<br>51 have demons<br>52 going to incre 59 Hypercholesterolemia, i.e. elevated plasma cholesterol, is a major risk factor for atherosclerotic cardiovascular<br>50 disease, particularly in older women (1, 2). Although advancements in drug therapies and lifestyle in disease, particularly in older women (1, 2). Although advancements in drug therapies and lifestyle interventions<br>have demonstrated efficacy, the identification of genetic and epigenetic factors regulating cholesterol is st

Figure 1.6 have demonstrated efficacy, the identification of genetic and epigenetic factors regulating cholesterol is still on-<br>going to increase our mechanistic understanding and better predict and manage hypercholesterol going to increase our mechanistic understanding and better predict and manage hypercholesterolemia.<br>
13 Despite its involvement in virtually every nutrient metabolism, the glucocorticoid receptor (GR) remains<br>
14 Is define bespite its involvement in virtually every nutrient metabolism, the glucocorticoid receptor (GR) remains a poor-<br>by defined nuclear factor in cholesterol homeostasis. The GR is a ligand-activated nuclear transcription fact isty defined nuclear factor in cholesterol homeostasis. The GR is a ligand-activated nuclear transcription factor<br>
that exerts multifaceted effects on nutrient metabolism (3, 4) by transactivating or transrepressing large that exerts multifaceted effects on nutrient metabolism (3, 4) by transactivating or transrepressing large gene<br>
programs in a tissue-specific manner (5). While traditionally recognized for its role in immune regulation, G programs in a tissue-specific manner (5). While traditionally recognized for its role in immune regulation, GR<br>profoundly influences metabolic processes, including glucose and lipid metabolism (6). Prior studies employing<br> profoundly influences metabolic processes, including glucose and lipid metabolism (6). Prior studies employing<br>
GR knockdown in liver and adipose tissue have shown promising outcomes in mitigating hypercholesterolemia<br>
and 6R knockdown in liver and adipose tissue have shown promising outcomes in mitigating hypercholesterolemia<br>
and associated metabolic abnormalities in obese diabetic mice (7). Retrospective studies involving<br>
pathomorphologi and associated metabolic abnormalities in obese diabetic mice (7). Retrospective studies involving<br>pathomorphological data obtained from human autopsies have provided insights into potential relationships<br>between glucocort The pathomorphological data obtained from human autopsies have provided insights into potential relationships<br>
11 between glucocorticoid treatments and atherogenesis (8-11). However, the direct link between the hepatic GR<br> 71 between glucocorticoid treatments and atherogenesis (8-11). However, the direct link between the hepatic GR<br>72 and regulation of cholesterol levels remains elusive. Indeed, although the glucocorticoid-GR axis has been i and regulation of cholesterol levels remains elusive. Indeed, although the glucocorticoid-GR axis has been im-<br>plicated in apolipoprotein expression (12) and cholesterol efflux in macrophages (13, 14), the epigenetic and<br>t plicated in apolipoprotein expression (12) and cholesterol efflux in macrophages (13, 14), the epigenetic and<br>transcriptional mechanisms enabled by the GR in hepatocyte-autonomous cholesterol uptake remain still poor-<br>ly

The transcriptional mechanisms enabled by the GR in hepatocyte-autonomous cholesterol uptake remain still poor-<br>
17 defined.<br>
17 Previously, several genetic variants in the GR gene (NR3C1; OMIM #138040) have been described 75 ly defined.<br>
76 Previously,<br>
77 man popula<br>
78 get genes,<br>
79 of an asso Previously, several genetic variants in the GR gene (*NR3C1;* OMIM #138040) have been described in the human population. These genetic variants can affect the transcriptional activity of the GR and its downstream target ge man population. These genetic variants can affect the transcriptional activity of the GR and its downstream tar-<br>get genes, potentially influencing nutrient regulation (15-18). Epidemiological studies have provided eviden 78 get genes, potentially influencing nutrient regulation (15-18). Epidemiological studies have provided evidence<br>
79 of an association between specific GR polymorphisms and variation in lipid profiles (15, 19, 20). Notabl of an association between specific GR polymorphisms and variation in lipid profiles (15, 19, 20). Notably, the<br> *rs6190* (c.68G>A; p.R23K) coding single nucleotide polymorphisms (SNP) - also known as "E22R/E23K" due<br>
its c *rs6190* (c.68G>A; p.R23K) coding single nucleotide polymorphisms (SNP) - also known as "E22R/E23K" due<br>its complete linkage to the silent p.E22E *rs6189* SNP - is a missense mutation at codon 23 in the N-terminus of<br>the G 81 its complete linkage to the silent p.E22E *rs6189* SNP - is a missense mutation at codon 23 in the N-terminus of<br>82 the GR protein, resulting in an amino acid change from arginine (R) to lysine (K) (21). This mutation h 82 the GR protein, resulting in an amino acid change from arginine (R) to lysine (K) (21). This mutation has been<br>83 linked to alterations in several parameters of metabolic homeostasis in humans, including cholesterol (17 Inked to alterations in several parameters of metabolic homeostasis in humans, including cholesterol (17).<br>
184 However, the precise molecular mechanisms through which this polymorphism skews GR activity to perturb<br>
185 ch

84 However, the precise molecular mechanisms through which this polymorphism skews GR activity to perturb<br>85 cholesterol remain poorly characterized.<br>86 In this study, we harnessed the human rs6190 SNP to identify a direct 85 cholesterol remain poorly characterized.<br>86 In this study, we harnessed the human rs<br>87 ic cholesterol regulation and its associati<br>88 correlated with increased levels of choles<br>89 cholesterol and atherosclerosis in tr In this study, we harnessed the human *rs6190* SNP to identify a direct GR-mediated program governing hepat-<br>ic cholesterol regulation and its association with atherogenic risk. We found that this low-frequency coding SNP<br> 87 ic cholesterol regulation and its association with atherogenic risk. We found that this low-frequency coding SNP<br>88 correlated with increased levels of cholesterol in women from UK Biobank and All of Us cohorts, and pr correlated with increased levels of cholesterol in women from UK Biobank and All of Us cohorts, and promoted<br>cholesterol and atherosclerosis in transgenic mice according to the number of SNP alleles (ho-<br>mo>hetero>referenc 89 cholesterol and atherosclerosis in transgenic mice according to the number of SNP alleles (ho-<br>
89 mo>hetero>reference). Our transcriptomic and epigenetic datasets revealed that the mutant GR perturbed<br>
89 cholesterol l 90 mo>hetero>reference). Our transcriptomic and epigenetic datasets revealed that the mutant GR perturbed<br>
91 cholesterol levels through transactivation of *Pcsk9* and *Bhlhe40*, negative regulators of LDL and HDL receptor 91 cholesterol levels through transactivation of *Pcsk9* and *Bhlhe40*, negative regulators of LDL and HDL receptors<br>92 in the liver and previously unknown targets of GR. Our study identifies rs6190 as a potential risk fac 92 in the liver and previously unknown targets of GR. Our study identifies rs6190 as a potential risk factor for ath-<br>in the liver and previously unknown targets of GR. Our study identifies rs6190 as a potential risk facto

- 93 erosclerosis, particularly in women, and reports unanticipated mechanisms through which the hepatic GR im-<br>94 pacts cholesterol levels in the circulation.
- 94 pacts cholesterol levels in the circulation.

95 **Results**<br>96 *rs6190 \*<br>97 To inves<br>98 the Unit<br>99 rs6190 \ rs6190 SNP increases plasma cholesterol levels in women according to allele zygosity.<br>
To investigate the influence of rs6190 variant on cholesterol regulation, we probed the<br>
the United Kingdom (UK) Biobank, comprising of 97 To investigate the influence of rs6190 variant on cholesterol regulation, we probed the large adult cohort from<br>98 the United Kingdom (UK) Biobank, comprising of 485,895 at the age of 40-70 years. In this cohort, the GR 98 the United Kingdom (UK) Biobank, comprising of 485,895 at the age of 40-70 years. In this cohort, the GR<br>99 rs6190 variant (*NR3C1* gene, transcript ENST00000231509.3 (-strand); c.68G>A; p.R23K) exhibited a minor<br>90 all 99 rs6190 variant (*NR3C1* gene, transcript ENST00000231509.3 (-strand); c.68G>A; p.R23K) exhibited a minor<br>10 allele frequency of 2.75% (25,944 heterozygous, 413 homozygous individuals), categorizing it as a low-<br>11 frequ of allele frequency of 2.75% (25,944 heterozygous, 413 homozygous individuals), categorizing it as a low-<br>frequency variant (21). We screened the quantitated parameters from the NMR metabolomics dataset within<br>the UK Bioba 11 frequency variant (21). We screened the quantitated parameters from the NMR metabolomics dataset within<br>
12 the UK Biobank dataset (120,356 individuals comprising of 65156 women and 55380 men; same age range as<br>
13 gene the UK Biobank dataset (120,356 individuals comprising of 65156 women and 55380 men; same age range as<br>
general dataset, 40-70 years) for rs6190 associations disaggregated by sex. All analyses were adjusted for<br>
age, body 93 general dataset, 40-70 years) for rs6190 associations disaggregated by sex. All analyses were adjusted for<br>
94 age, body mass index (BMI), top 10 principal components, and genotype information for 12 commonly-<br>
95 refe age, body mass index (BMI), top 10 principal components, and genotype information for 12 commonly-<br>referenced, hypercholesterolemia-associated SNPs within *PCSK9, CELSR2, APOB, ABCG8, SLC22A1, HFE,*<br>*MYLIP, ST3GAL4, NYNRIN* of the set all the percholesterolemia-associated SNPs within *PCSK9, CELSR2, APOB, ABCG8, SLC22A1, HFE, MYLIP, ST3GAL4, NYNRIN, LDLR,* and *APOE* genes (22). Importantly, none of these 12 classical variants were in the n *MYLIP, ST3GAL4, NYNRIN, LDLR,* and *APOE* genes (22). Importantly, none of these 12 classical variants<br>were in the neighborhood of rs6190 and did not show significant pairwise LD (linkage disequilibrium) effect (r<sup>2</sup><br>< 0. were in the neighborhood of rs6190 and did not show significant pairwise LD (linkage disequilibrium) effect (r<sup>2</sup> 07 68  $\times$  0.001) at the genomic level. While no associations were significant after multiple testing in men, rs6190 SNP<br>
99 significantly associated with many cholesterol parameters in women, accounting for 23 out of 33 tot

99 significantly associated with many cholesterol parameters in women, accounting for 23 out of 33 total plasma<br>
10 parameters with a significant rs6190 effect (adjusted p<0.005) (**Figure 1A**).<br>
11 We then stratified tota parameters with a significant rs6190 effect (adjusted p<0.005) (**Figure 1A**).<br>
11 We then stratified total, LDL-, and HDL-cholesterol values from women acc<br>
12 fining here homozygous carriers of the reference allele (contr 11 We then stratified total, LDL-, and HDL-cholesterol values from women according to SNP zygosity. We are de-<br>
12 fining here homozygous carriers of the reference allele (control population) as  $\text{GR}^{\text{ref/ref}}$ , heterozyg fining here homozygous carriers of the reference allele (control population) as GR<sup>ref/ref</sup>, heterozygous SNP carfining here homozygous carriers of the reference allele (control population) as  $GR^{ref/net}$ , heterozygous SNP carriers as  $GR^{ref/ALT}$ , and homozygous SNP carriers as  $GR^{ALT/ALT}$ . We performed linear regressions with a mixed model riers as GR<sup>ref/ALT</sup>, and homozygous SNP carriers as GR<sup>ALT/ALT</sup>, We performed linear regressions with a mixed The risk as GR<sup>ref/ALI</sup>, and homozygous SNP carriers as  $GR^{AL1/AL1}$ . We performed linear regressions with a mixed<br>
14 model correcting for age, BMI, diabetic status and triacylglycerols. In parallel, we also compared media model correcting for age, BMI, diabetic status and triacylglycerols. In parallel, we also compared median confi-<br>
15 dence intervals across rs6190 genotypes. Remarkably, total, LDL-, and HDL-cholesterol showed a modest bu 15 dence intervals across rs6190 genotypes. Remarkably, total, LDL-, and HDL-cholesterol showed a modest but<br>16 significant elevation of median levels according to the number of SNP alleles in women (**Figure 1B**). The<br>17 16 significant elevation of median levels according to the number of SNP alleles in women (**Figure 1B**). The zygosity-dependent trends were not significant in men (Suppl. Fig. 1A). Considering the effects on cholesterol, 27 zygosity-dependent trends were not significant in men **(Suppl. Fig. 1A)**. Considering the effects on cholesterol, we probed the total UK Biobank dataset for hypercholesterolemia and cardiovascular disease mortality odd 18 we probed the total UK Biobank dataset for hypercholesterolemia and cardiovascular disease mortality odds<br>
19 ratios. In alignment with the trends in cholesterol,  $GR^{ALT/ALT}$  women displayed an increased odds ratio of 1 ratios. In alignment with the trends in cholesterol, GR<sup>ALT/ALT</sup> 19 ratios. In alignment with the trends in cholesterol,  $GR^{ALI/ALI}$  women displayed an increased odds ratio of 1.34 (95% CI: 1.02 – 1.76; P=0.0092) for hypercholesterolemia (total cholesterol >240 mg/dl) and 2.37 (95% CI: 1.05 – 5.9; P=0.01) for death due to cardiovascular diseases, compared to GR<sup>ref/ref</sup> women (**Figure 1C-D**).

20 (95% CI: 1.02 – 1.76; P=0.0092) for hypercholesterolemia (total cholesterol >240 mg/dl) and 2.37 (95% CI:<br>
21 1.05 – 5.9; P=0.01) for death due to cardiovascular diseases, compared to GR<sup>ref/ref</sup> women (**Figure 1C-D**). 1.05 – 5.9; P=0.01) for death due to cardiovascular diseases, compared to GR<sup>reme</sup> women (**Figure 1C-D**).<br>
22 To probe these rs6190 correlations in a more genetically diverse human dataset, we queried the All Of Us<br>
23 tas <sup>22</sup> To probe these rs6190 correlations in a more genetically diverse human dataset, we queried the All Of Us da-<br><sup>23</sup> taset, where we found the SNP at a variable minor allele frequency ranging from low-frequency to rare a 23 taset, where we found the SNP at a variable minor allele frequency ranging from low-frequency to rare across<br>
24 ancestries: African/African-American, 0.49%; American Admixed/Latino, 0.84%; East Asian, 0.061%; Europe-<br> 24 ancestries: African/African-American, 0.49%; American Admixed/Latino, 0.84%; East Asian, 0.061%; Europe-<br>25 an, 2.67%; Middle Eastern, 1.43%; South Asian, 1.49%. In the All Of Us subset of 245,385 individuals with<br>26 19 25 an, 2.67%; Middle Eastern, 1.43%; South Asian, 1.49%. In the All Of Us subset of 245,385 individuals with<br>26 rs6190 genotype annotation encompassing all ancestries and ages, we repeated the linear regressions cor-<br>27 re 26 rs6190 genotype annotation encompassing all ancestries and ages, we repeated the linear regressions cor-<br>27 rected for age, BMI, diabetes, triacylglycerols, as well as the median comparisons. The analyses in the All of<br> 27 rected for age, BMI, diabetes, triacylglycerols, as well as the median comparisons. The analyses in the All of<br>28 Us dataset confirmed a significant correlation between rs6190 zygosity and total, LDL and HDL cholesterol <sup>28</sup> Us dataset confirmed a significant correlation between rs6190 zygosity and total, LDL and HDL cholesterol<br>29 levels in women (Figure 1E), while correlations were not significant once again in men (Suppl. Fig. 1B). 29 levels in women **(Figure 1E)**, while correlations were not significant once again in men **(Suppl. Fig. 1B)**.

- 
- 

30 Taken together, our findings highlight the association of the rs6190 SNP with modest but significant and potentially consequential elevations of cholesterol in women from the UK Biobank and the All Of Us cohorts.<br>32 The 31 tially consequential elevations of cholesterol in women from the UK Biobank and the All Of Us cohorts.<br>32<br>33 The rs6190 SNP is sufficient to increase plasma cholesterol and promotes GR transactivation of Po<br>34 Bhlhe40 i

 $33$ <br> $34$ <br> $35$ <br> $36$ The rs6190 SNP is sufficient to increase plasma cholesterol and promotes GR transactivation of Pcsk9 and<br>
34 Bhlhe40 in mice.<br>
35 To elucidate the extent to which the mutant GR promotes cholesterol elevation, we introduced 34 *Bhlhe40 in mice.*<br>35 To elucidate the<br>36 the rs6190 SNF<br>37 ortholog of the h<br>38 ing CRISPR-med 35 To elucidate the extent to which the mutant GR promotes cholesterol elevation, we introduced a genocopy of<br>36 the rs6190 SNP into the endogenous *Nr3c1* (GR gene) locus on the C57BL/6J background. The murine<br>37 ortholog 36 the rs6190 SNP into the endogenous *Nr3c1* (GR gene) locus on the C57BL/6J background. The murine ortholog of the human GR-R23K mutation is GR-R24K due to an additional amino acid in position 10. Employing CRISPR-mediat 37 ortholog of the human GR-R23K mutation is GR-R24K due to an additional amino acid in position 10. Employing CRISPR-mediated knock-in recombination, the murine GR gene was targeted at the orthologous codon 24 resulting 38 ing CRISPR-mediated knock-in recombination, the murine GR gene was targeted at the orthologous codon 24 resulting in C>T mutation in the forward strand (c.71G>A mutation in the codon, reverse strand) leading to a p.R24 39 resulting in C>T mutation in the forward strand (c.71G>A mutation in the codon, reverse strand) leading to a<br>
29 p.R24K amino acid substitution (**Suppl. Figure 2A**). In concordance with human carriers, we define here h p.R24K amino acid substitution (**Suppl. Figure 2A**). In concordance with human carriers, we define here ho-<br>mozygous mice for wild-type allele as "GR<sup>re/ref</sup>" (control), heterozygous SNP mice as "GR<sup>ref/ALT</sup>", and homozy-<br> mozygous mice for wild-type allele as " $GR^{refen}$ " (control), heterozygous SNP mice as " $GR^{refl}(R^{1.1})$ , and homozy-<br>gous SNP mice as " $GR^{ALT/ALT}$ . In female littermate mice under normal chow conditions, total plasma cholestergous SNP mice as "GR<sup>ALT/ALT</sup> qous SNP mice as " $\text{GR}^{\text{ALL}/\text{ALL}}$ ". In female littermate mice under normal chow conditions, total plasma cholester-<br>ol increased according to SNP zygosity in both fasted and fed states (**Figure 2A**). Using the standard ol increased according to SNP zygosity in both fasted and fed states (**Figure 2A**). Using the standard fast-<br>performance liquid chromatography (FPLC) method, we found that the GR SNP elicited an increase in choles-<br>terol l 14 performance liquid chromatography (FPLC) method, we found that the GR SNP elicited an increase in cholesterol 15 terol levels across all lipoprotein fractions – VLDL-, LDL- and HDL-cholesterol - according to SNP allele terol levels across all lipoprotein fractions – VLDL-, LDL- and HDL-cholesterol - according to SNP allele num-<br>ber, in conditions of either regular chow or 16-week long Western diet in female (**Figure 2B**), but not male m 16 ber, in conditions of either regular chow or 16-week long Western diet in female (**Figure 2B**), but not male mice<br>
17 (Suppl. Fig. 2B). This sex-specific effect in mice paralleled the correlations within human datasets 47 **(Suppl. Fig. 2B)**. This sex-specific effect in mice paralleled the correlations within human datasets and<br>48 prompted us to focus on female mice for the bulk of our histological, physiological and mechanistic analyses prompted us to focus on female mice for the bulk of our histological, physiological and mechanistic analyses.<br>
After Western diet exposure, in 3 out of 5 analyzed  $GR^{ALT/ALT}$  female mice, we found histological evidence of<br>
i After Western diet exposure, in 3 out of 5 analyzed GR<sup>ALT/ALT</sup> 49 After Western diet exposure, in 3 out of 5 analyzed  $GR^{ALIALI}$  female mice, we found histological evidence of<br>49 immature plaque formation in the aortic root (**Suppl. Fig. 2C**), a remarkable finding in the absence of pro 50 immature plaque formation in the aortic root (**Suppl. Fig. 2C**), a remarkable finding in the absence of pro-<br>51 atherogenic genetic backgrounds. Moreover, considering that the GR naturally responds to diurnal oscillatio 51 atherogenic genetic backgrounds. Moreover, considering that the GR naturally responds to diurnal oscillations<br>52 in endogenous glucocorticoids (corticosterone in mice), we followed the circadian oscillations in choleste in endogenous glucocorticoids (corticosterone in mice), we followed the circadian oscillations in cholesterol<br>across genotypes. The SNP effect on cholesterol elevation was significant through the circadian cycle and par-<br>t 53 across genotypes. The SNP effect on cholesterol elevation was significant through the circadian cycle and par-<br>54 ticularly acute during the dark phase (corticosterone peak in mice), without significant changes in<br>55 co ist ticularly acute during the dark phase (corticosterone peak in mice), without significant changes in corticosterone levels per se (**Suppl. Fig. 2D**). Our findings provide evidence that, in homogeneous genetic settings, 55 corticosterone levels per se (**Suppl. Fig. 2D**). Our findings provide evidence that, in homogeneous genetic set-<br>56 tings, the SNP is sufficient to modestly but significantly elevate total, LDL-, and HDL-cholesterol in

tings, the SNP is sufficient to modestly but significantly elevate total, LDL-, and HDL-cholesterol in females according to an incomplete dominance model, i.e. commensurate to SNP zygosity.<br>We then focused our mechanistic 57 cording to an incomplete dominance model, i.e. commensurate to SNP zygosity.<br>
58 We then focused our mechanistic analyses on  $\text{GR}^{\text{ref/ref}}$  vs  $\text{GR}^{\text{ALT/ALT}}$  liver compar<br>
59 role of this organ in cholesterol regul We then focused our mechanistic analyses on GR $^{\text{ref/ref}}$  vs GR $^{\text{ALT/ALT}}$ 58 We then focused our mechanistic analyses on  $\text{GR}^{\text{ReII/RLI}}$  vs  $\text{GR}^{\text{ALI/ALI}}$  liver comparisons, considering the primary<br>59 role of this organ in cholesterol regulation (23). In primary hepatocytes, the mutant GR Follow for this organ in cholesterol regulation (23). In primary hepatocytes, the mutant GR showed an increased<br>
Fig. 26). We therefore conducted RNA-sequencing and GR ChIP-sequencing in liver to identify poten-<br>
fial diff 50 epigenetic activity both at baseline and after glucocorticoid stimulation, assayed through a luciferase reporter<br>
51 (Suppl. Fig. 2E). We therefore conducted RNA-sequencing and GR ChIP-sequencing in liver to identify po 51 (**Suppl. Fig. 2E**). We therefore conducted RNA-sequencing and GR ChIP-sequencing in liver to identify potential differential targets of GR transactivation based on GR SNP genotype. The liver GR ChIP-seq was validated by ial differential targets of GR transactivation based on GR SNP genotype. The liver GR ChIP-seq was validated<br>by enrichment for the canonical GRE motif in unbiased motif analysis (**Suppl. Figure 2F**). Compared to the<br>contro by enrichment for the canonical GRE motif in unbiased motif analysis (**Suppl. Figure 2F**). Compared to the control GR, the increased epigenomic activity of the mutant GR was evidenced by increased GR signal on GRE sites ge 64 control GR, the increased epigenomic activity of the mutant GR was evidenced by increased GR signal on<br>65 GRE sites genome-wide and on the *Fkbp5* promoter, a canonical marker for GR activity (24, 25) (**Suppl. Fig.**<br>67 65 GRE sites genome-wide and on the *Fkbp5* promoter, a canonical marker for GR activity (24, 25) (**Suppl. Fig.** 

**2G-H**). No statistical differences were noted in overall peak number or genomic peak distribution, which clustered preferentially in proximal promoter regions for both genotypes (**Suppl. Fig. 2I-J**). Liver RNA-seq reveale for tered preferentially in proximal promoter regions for both genotypes (**Suppl. Fig. 2I-J**). Liver RNA-seq revealed<br>
368 genes with differential expression by the mutant GR (**Suppl. Fig. 2K**). The overlay of both dataset 368 genes with differential expression by the mutant GR (**Suppl. Fig. 2K**). The overlay of both datasets unveiled 236 genes exhibiting both differential expression and a gain of mutant GR signal on their promoters (**Figure** Figure 20. Gene ontology (GO) analysis revealed a significant enrichment for cholesterol metabolism. Nota-<br>
10 (Figure 2C). Gene ontology (GO) analysis revealed a significant enrichment for cholesterol metabolism. Nota-<br>
1 70 (Figure 2C). Gene ontology (GO) analysis revealed a significant enrichment for cholesterol metabolism. Nota-<br><sup>71</sup> bly, within this pathway, proprotein convertase subtilisin/kexin type 9 (*Pcsk9*) was the highest hit. Th 71 bly, within this pathway, proprotein convertase subtilisin/kexin type 9 (*Pcsk9*) was the highest hit. The in-<br>72 creased transactivation of *Pcsk9* in liver by the mutant GR was validated at mRNA and protein levels ( The 73 creased transactivation of *Pcsk9* in liver by the mutant GR was validated at mRNA and protein levels (**Figure**<br> **2D-E**). Besides indirect and direct inhibition of VLDL-cholesterol clearance (26, 27), PCSK9 plays a **2D-E**). Besides indirect and direct inhibition of VLDL-cholesterol clearance (26, 27), PCSK9 plays a pivotal<br>role in increasing circulating LDL cholesterol by promoting the degradation of the main LDL-cholesterol recep-<br> role in increasing circulating LDL cholesterol by promoting the degradation of the main LDL-cholesterol receptor, LDLR, at the protein level (28, 29). Accordingly, the gain in PCSK9 levels correlated with a reduction in p 75 tor, LDLR, at the protein level (28, 29). Accordingly, the gain in PCSK9 levels correlated with a reduction in protein but not mRNA levels of LDLR in  $GR^{ALT/ALT}$  compared to  $GR^{ref/ref}$  liver tissues (**Figure 2D-E**). Addit tein but not mRNA levels of LDLR in GR<sup>ALT/ALT</sup> compared to GR<sup>ref/ref</sup> The tein but not mRNA levels of LDLR in GR<sup>ALI/ALI</sup> compared to GR<sup>remer</sup> liver tissues (**Figure 2D-E**). Additionally,<br>within the "rhythmic process" pathway from the ChIP-seq/RNA-seq overlay, the top hit for mutant GR tran within the "rhythmic process" pathway from the ChIP-seq/RNA-seq overlay, the top hit for mutant GR transactivation was *Bhlhe40* (**Figure 2C**), a transcriptional repressor involved in many processes including circadian clo vation was *Bhlhe40* (**Figure 2C**), a transcriptional repressor involved in many processes including circadian<br>clock homeostasis (30, 31). Using an ENCODE-mining platform for transcription factor target prediction (32),<br>we For the screened for putative *Bhlhe40* targets in the promoters of down-regulated genes in mutant versus WT livers. This analysis revealed Scavenger Receptor Class B Type I (SR-B1), encoded by *Scarb1*, as a unique hypot we screened for putative *Bhlhe40* targets in the promoters of down-regulated genes in mutant versus WT livers. This analysis revealed Scavenger Receptor Class B Type I (SR-B1), encoded by *Scarb1*, as a unique hypothetica 81 ers. This analysis revealed Scavenger Receptor Class B Type I (SR-B1), encoded by *Scarb1*, as a unique hy-<br>
82 pothetical target of BHLHE40 from our RNA-seq datasets. SR-B1 is the main receptor for reverse HDL-<br>
84 cho bothetical target of BHLHE40 from our RNA-seq datasets. SR-B1 is the main receptor for reverse HDL-<br>cholesterol transport in the liver (33). Consistent with our prediction, *Bhlhe40* upregulation correlated with SR-<br>B1 do cholesterol transport in the liver (33). Consistent with our prediction, *Bhlhe40* upregulation correlated with SR-<br>B1 downregulation at both mRNA and protein levels in GR<sup>ALT/ALT</sup> compared to GR<sup>ref/ref</sup> liver tissues (**F** B1 downregulation at both mRNA and protein levels in GR $^{\text{ALT/ALT}}$  compared to GR $^{\text{ref/ref}}$ 84 B1 downregulation at both mRNA and protein levels in GR<sup>ALI/ALI</sup> compared to GR<sup>retrier</sup> liver tissues (**Figure 2D-**<br> **E**). Additionally, to confirm the in-silico prediction of SR-B1 transcriptional repression by BHLHE4 85 **E**). Additionally, to confirm the in-silico prediction of SR-B1 transcriptional repression by BHLHE40, we compared Scarb1 expression and SR-B1 protein levels in liver tissues from *Bhlhe40<sup>-M/mull</sup>* (34) *(Bhlhe40-KO)* pared *Scarb1* expression and SR-B1 protein levels in liver tissues from *Bhlhe40<sup>null/null*</sup> pared *Scarb1* expression and SR-B1 protein levels in liver tissues from *Bhlhe40<sup>-humm</sup>* (34) (*Bhlhe40-KO*) vs<br>their wild-type littermate controls (*Bhlhe40-WT*). As hypothesized, SR-B1 was upregulated in the *Bhlhe40-KO* their wild-type littermate controls (*Bhlhe40-WT*). As hypothesized, SR-B1 was upregulated in the *Bhlhe40-KO*<br>livers compared to WT controls (**Suppl. Fig. 2L**). We then asked the extent to which the mutant GR effect on<br>L Iivers compared to WT controls (**Suppl. Fig. 2L**). We then asked the extent to which the mutant GR effect on<br>
LDLR and SR-B1 downregulation was biologically significant on hepatocyte biology. We probed fluorescently-<br>
labe 89 LDLR and SR-B1 downregulation was biologically significant on hepatocyte biology. We probed fluorescently-<br>
90 labeled LDL and HDL uptake assays in primary hepatocytes to assess this propensity in the absence of body-<br> 90 labeled LDL and HDL uptake assays in primary hepatocytes to assess this propensity in the absence of body-<br>91 wide confounders. In line with the LDLR and SR-B1 changes, the  $\text{GR}^{\text{ALT/ALT}}$  hepatocytes showed decreased wide confounders. In line with the LDLR and SR-B1 changes, the GR<sup>ALT/ALT</sup> wide confounders. In line with the LDLR and SR-B1 changes, the GR<sup>ALI/ALI</sup> hepatocytes showed decreased<br>LDL and HDL uptake *in vitro* compared to GR<sup>ref/ref</sup> control hepatocytes (**Figure 2H**). Collectively, our findings<br>s LDL and HDL uptake *in vitro* compared to GRref/ref 92 LDL and HDL uptake *in vitro* compared to GR<sup>remer</sup> control hepatocytes (**Figure 2H**). Collectively, our findings support a mechanism for the rs6190 SNP effect on cholesterol through which the SNP skews the hepatic GR e 93 support a mechanism for the rs6190 SNP effect on cholesterol through which the SNP skews the hepatic GR<br>
94 epigenetic activity and promotes transactivation of *Pcsk9* and *Bhlhe40*, which in turn decreases LDL and HDL<br> 94 epigenetic activity and promotes transactivation of *Pcsk9* and *Bhlhe40*, which in turn decreases LDL and HDL<br>
95 cholesterol uptake in liver by repressing LDLR and SR-B1 levels respectively.<br>
97 CRISPR-engineered hiPS

cholesterol uptake in liver by repressing LDLR and SR-B1 levels respectively.<br>96<br>CRISPR-engineered hiPSC-derived hepatocytes confirm the mouse-to-huma<br>98 nism.  $37$ <br> $38$ <br> $39$ <br> $30$ 

CRISPR-engineered hiPSC-derived hepatocytes confirm the mouse-to-human relevance for the SNP mecha-<br>
1918 In tandem with our murine mouse studies, we questioned whether the molecular SNP mechanism identified<br>
1929 In tande 98 *nism.*  In tandem with our murine mouse studies, we questioned whether the molecular SNP mechanism identified<br>30 was translatable to human hepatocytes. We, therefore, generated SNP heterozygous and homozygous lines<br>31 from the sam was translatable to human hepatocytes. We, therefore, generated SNP heterozygous and homozygous lines<br>from the same parental GR<sup>ref/ref</sup> hiPSC line through a CRISPR-knockin system. Individual founding clones of<br>from the sa from the same parental GR<sup>ref/ref</sup> 01 from the same parental GR<sup>ret/ret</sup> hiPSC line through a CRISPR-knockin system. Individual founding clones of<br>
11 from the same parental GR<sup>ret/ret</sup> hiPSC line through a CRISPR-knockin system. Individual founding clones

isogenic GR<sup>ref/ref</sup> (control), GR<sup>ref/ALT</sup> (het), and GR<sup>ALT/ALT</sup> (homo) hiPSCs were verified through Sanger sequencisogenic GR<sup>reme</sup> (control), GR<sup>reme</sup> (homo) and GR<sup>ALITALI</sup> (homo) hiPSCs were verified through Sanger sequenci-<br>ing and quality-controlled for pluripotency marker expression (**Figure 3A; Suppl. Fig. 3A**). Despite no dif ing and quality-controlled for pluripotency marker expression (**Figure 3A; Suppl. Fig. 3A**). Despite no differences in pluripotency markers, the SNP significantly skewed the GR to a higher rate of glucocorticoid-driven GR ences in pluripotency markers, the SNP significantly skewed the GR to a higher rate of glucocorticoid-driven<br>
05 GR translocation in hiPSCs, as shown by serial imaging after a dexamethasone pulse (**Suppl. Fig. 3B**) and<br>
06 OF GR translocation in hiPSCs, as shown by serial imaging after a dexamethasone pulse (**Suppl. Fig. 3B**) and<br>
26 consistent with our previous findings with the mutant GR in murine hepatocytes luciferase assay and liver<br>
27

consistent with our previous findings with the mutant GR in murine hepatocytes luciferase assay and liver<br>
ChIP-seq.<br>
To investigate whether the SNP-mediated molecular mechanism was conserved in human hepatocytes, we<br>
subj 07 ChIP-seq.<br>
07 To investig<br>
09 subjected<br>
10 cells (HLC<br>
11 ration (36-3 To investigate whether the SNP-mediated molecular mechanism was conserved in human hepatocytes, we subjected the isogenic lines of hiPSCs to a 23-day differentiation protocol to generate mature hepatocyte-like cells (HLCs) subjected the isogenic lines of hiPSCs to a 23-day differentiation protocol to generate mature hepatocyte-like<br>cells (HLCs) (35). Given the well-established role of GR as a regulator of hepatocyte differentiation and matu 10 cells (HLCs) (35). Given the well-established role of GR as a regulator of hepatocyte differentiation and matu-<br>11 ration (36-38), we sought to investigate whether the presence of the GR SNP influenced the differentiati 11 ration (36-38), we sought to investigate whether the presence of the GR SNP influenced the differentiation process. To address this, we examined the expression profiles of differentiation markers at multiple time points the definitive endoderm stage (40); *AFP* and *OCT4* at the pluripotent stage (39); *SOX17* and *FOXA2* at the definitive endoderm stage (40); *AFP* and *HNF1A* at the immature hepatocyte stage (41); *ALB* and *CY18*, morp during the differentiation process: *NANOG* and *OCT4* at the pluripotent stage (39); *SOX17* and *FOXA2* at the definitive endoderm stage (40); *AFP* and *HNF1A* at the immature hepatocyte stage (41); *ALB* and *CY18*, mo 14 definitive endoderm stage (40); *AFP* and *HNF1A* at the immature hepatocyte stage (41); *ALB* and *CY18*, mor-<br>15 phology, and albumin secretion at the mature hepatocyte stage (42). We did not detect any SNP-driven sig 15 phology, and albumin secretion at the mature hepatocyte stage (42). We did not detect any SNP-driven significant alterations in the *in vitro* maturation process of hiPSC-derived hepatocytes (**Suppl. Fig. 3C-D**). Howev 16 cant alterations in the *in vitro* maturation process of hiPSC-derived hepatocytes (**Suppl. Fig. 3C-D**). However,<br>17 the hiPSC-derived hepatocytes reproduced the zygosity-dependent increase in GR nuclear translocation ( 17 the hiPSC-derived hepatocytes reproduced the zygosity-dependent increase in GR nuclear translocation (**Fig-**<br>
18 **ure 3B**) and the SNP-mediated effects on *PCSK9* and *BHLHE40* transactivation, as well as post-translati 18 **ure 3B**) and the SNP-mediated effects on *PCSK9* and *BHLHE40* transactivation, as well as post-translational<br>19 repression of LDLR and SR-B1 (Figure 3C). Furthermore, the GR<sup>ALT/ALT</sup> hiPSC-derived hepatocytes displaye repression of LDLR and SR-B1 (**Figure 3C**). Furthermore, the GRALT/ALT 19 repression of LDLR and SR-B1 (Figure 3C). Furthermore, the GR<sup>ALI/ALI</sup> hiPSC-derived hepatocytes displayed<br>
20 decreased uptake of HDL and LDL-cholesterol compared to GR<sup>ref/ref</sup> control cells (Figure 3D-E). Taken toge decreased uptake of HDL and LDL-cholesterol compared to GR<sup>ref/ref</sup> decreased uptake of HDL and LDL-cholesterol compared to GR<sup>remer</sup> control cells (**Figure 3D-E**). Taken together, our hiPSC-derived hepatocyte data confirm that the molecular SNP mechanism is conserved in human cells and ap 21 er, our hiPSC-derived hepatocyte data confirm that the molecular SNP mechanism is conserved in human cells<br>22 and appears autonomous to hepatocytes in the absence of *in vivo* body-wide physiology.<br>23 and 30 and 28 and

22 and appears autonomous to hepatocytes in the absence of *in vivo* body-wide physiology.<br>23<br>24 rs6190 GR SNP promotes atherosclerosis *in vivo*.<br>25 Despite our results so far linking the mutant GR to cholesterol regulati  $\frac{24}{5}$ <br> $\frac{25}{17}$ rs6190 GR SNP promotes atherosclerosis in vivo.<br><sup>25</sup> Despite our results so far linking the mutant GR t<br><sup>26</sup> enabled program significantly impacts atheroscl<br><sup>27</sup> which the rs6190 SNP contributes to atherogenic<br><sup>28</sup> our mut <sup>25</sup> Despite our results so far linking the mutant GR to cholesterol regulation, the extent to which the overall SNP-<br><sup>26</sup> enabled program significantly impacts atherosclerosis *in vivo* remains unknown. To evaluate the e enabled program significantly impacts atherosclerosis *in vivo* remains unknown. To evaluate the extent to<br>which the rs6190 SNP contributes to atherogenic risk *in vivo* in conditions of genetic homogeneity, we crossed<br>our which the rs6190 SNP contributes to atherogenic risk *in vivo* in conditions of genetic homogeneity, we crossed<br>
28 our mutant SNP mice with the atherogenic background characterized by homozygous expression of the human<br>
2 28 our mutant SNP mice with the atherogenic background characterized by homozygous expression of the human  $APOE*2$  variant (43, 44). The h $APOE*2/*2$  mice are well-established transgenic mice known for their susceptibility to *APOE\*2* variant (43, 44). The h*APOE\*2/\*2* mice are well-established transgenic mice known for their susceptibility to atherosclerosis while maintaining cholesterol distribution across all three major lipoprotein compart 30 bility to atherosclerosis while maintaining cholesterol distribution across all three major lipoprotein compart-<br>31 ments (44, 45), unlike other atherogenic backgrounds like  $ApoE-KO$ . We also excluded the *LdIr-KO* back

ments (44, 45), unlike other atherogenic backgrounds like *ApoE-KO*. We also excluded the *LdIr-KO* back-<br>32 ground as a direct genetic confounder of our LDLR-involving hypothesis.<br>33 For these analyses, we focused on GR<sup>A</sup> 32 ground as a direct genetic confounder of our LDLR-involving hypothesis.<br>33 For these analyses, we focused on  $\text{GR}^{\text{ALT/ALT}}$  vs  $\text{GR}^{\text{ref/ref}}$  female mice.<br>34 regular chow diet,  $\text{GR}^{\text{ALT/ALT}}$  mice exhibited elev For these analyses, we focused on GR<sup>ALT/ALT</sup> vs GR<sup>ref/ref</sup> 33 For these analyses, we focused on  $GR^{ALI/ALI}$  vs  $GR^{IBI/IF}$  female mice. On the h*APOE\*2/\*2* background and regular chow diet,  $GR^{ALI/ALI}$  mice exhibited elevated levels of VLDL-, LDL- and HDL-cholesterol in the FPLC curv regular chow diet, GR<sup>ALT/ALT</sup> 34 regular chow diet, GR<sup>ALI/ALI</sup> mice exhibited elevated levels of VLDL-, LDL- and HDL-cholesterol in the FPLC<br>35 curves compared to control littermates, and this was reinforced even more after a 16-week-long Western diet 35 curves compared to control littermates, and this was reinforced even more after a 16-week-long Western diet<br>36 exposure (Figure 4A). We focused on mice exposed to Western diet for atherosclerotic plaque analyses. 36 exposure (**Figure 4A**). We focused on mice exposed to Western diet for atherosclerotic plaque analyses.

Compared to GR<sup>ref/ref</sup>, GR<sup>ALT/ALT</sup> mice exhibited a significant increase in atherosclerotic plaque incidence as 37 Compared to GR<sup>ret/ret</sup>, GR<sup>ALT/ALT</sup> mice exhibited a significant increase in atherosclerotic plaque incidence as<br>38 quantitated through overall plaque/total aorta area ratio in en face whole aorta staining and imaging 38 quantitated through overall plaque/total aorta area ratio in en face whole aorta staining and imaging (**Figure 4B, left**). Furthermore, histological analysis of the aortic root cross-sections and Oil Red O staining reve **4B, left**). Furthermore, histological analysis of the aortic root cross-sections and Oil Red O staining revealed a significant increase in atherosclerotic lesion size (plaque/lumen ratio) and lipid accumulation in  $GR^{ALT/$ significant increase in atherosclerotic lesion size (plaque/lumen ratio) and lipid accumulation in GR<sup>ALT/ALT</sup> significant increase in atherosclerotic lesion size (plaque/lumen ratio) and lipid accumulation in GR<sup>ALI/ALI</sup> ver-<br>sus GR<sup>ref/ref</sup> mice (**Figure 4B, right**). Finally, considering our hypothesis of *Pcsk9* and *Bhlhe40* a sus GR<sup>ref/ref</sup> sus GR<sup>rettrer</sup> mice (**Figure 4B, right**). Finally, considering our hypothesis of *Pcsk9* and *Bhlhe40* as mechanistic<br>mediators of the SNP effect, we tested the effect of *in vivo* knock-down of these genes on the SNP-med mediators of the SNP effect, we tested the effect of *in vivo* knock-down of these genes on the SNP-mediated<br>effect on cholesterol and atherosclerosis through AAV8 vectors. For *Pcsk9* knockdown we used a previously<br>report 43 effect on cholesterol and atherosclerosis through AAV8 vectors. For *Pcsk9* knockdown we used a previously<br>
44 reported AAV vector (46) and confirmed its max knockdown effect in liver in vivo in Apo<sup>\*</sup>2/<sup>\*</sup>2 mice on We Fig. 4B). At 2 months of age, GR<sup>ALT/ALT</sup> vs GR<sup>Fef/ref</sup> female mice on the Apo<sup>\*2/\*2</sup> mice on Western<br>15 Diet with a 10^13vg/mouse dose (Suppl. Fig. 4A). For *Bhlhe40*, we combined two AAVs with different shRNAs<br>16 under 15 Diet with a 10^13vg/mouse dose (Suppl. Fig. 4A). For *Bhlhe40*, we combined two AAVs with different shRNAs<br>
16 under the U6 promoter, as they showed synergistic effect on *Bhlhe40* knockdown in Apo\*2/\*2 livers (Suppl.<br> under the U6 promoter, as they showed synergistic effect on *Bhlhe40* knockdown in Apo\*2/\*2 livers (Suppl.<br> **Fig. 4B)**. At 2 months of age, GR<sup>ALT/ALT</sup> vs GR<sup>ref/ref</sup> female mice on the ApoE\*2/\*2 background were injected **Fig. 4B)**. At 2 months of age,  $\textsf{GR}^\textsf{ALT/ALT}$  vs  $\textsf{GR}^\textsf{ref/ref}$ Fig. 4B). At 2 months of age,  $GR^{ALI/ALI}$  vs  $GR^{BU/REI}$  female mice on the ApoE\*2/\*2 background were injected ret-<br>ro-orbitally (r.o.) with  $3x10^{13}$  vg/mouse AAV-scramble or  $10^{12}$  vg/mouse/vector AAV-antiPcsk9 (1 vecto ro-orbitally (r.o.) with 3x10<sup>13</sup> vg/mouse AAV-scramble or 10<sup>12</sup> 18 ro-orbitally (r.o.) with  $3x10^{13}$  vg/mouse AAV-scramble or  $10^{12}$  vg/mouse/vector AAV-antiPcsk9 (1 vector) + AAV-<br>antiBhlhe40 (2 vectors) immediately before starting the 16-week-long Western Diet exposure. At endpo 49 antiBhlhe40 (2 vectors) immediately before starting the 16-week-long Western Diet exposure. At endpoint, we validated target gene knockdown (**Fig. 4C)** and we focused on FPLC cholesterol curves and atherosclerotic plaq validated target gene knockdown **(Fig. 4C)** and we focused on FPLC cholesterol curves and atherosclerotic<br>plaques as read-outs. Compared to scramble, the knockdown vectors reduced the cholesterol levels across<br>ippoprotein plaques as read-outs. Compared to scramble, the knockdown vectors reduced the cholesterol levels across<br>ipoprotein fractions in  $GR^{ALT/ALT}$  mice to  $GR^{ref/ref}$ -like levels (Fig. 4D), and blunted the SNP-mediated effect on<br>plaq lipoprotein fractions in GR $^{\sf ALT/ALT}$  mice to GR $^{\sf ref/ref}$ is a lipoprotein fractions in  $GR^{ACL/ALL}$  mice to  $GR^{fert/IEL}$  like levels (**Fig. 4D**), and blunted the SNP-mediated effect on<br>plaque incidence (**Fig. 4E**) and severity (**Fig. 4F**). We also noted that the knockdown vectors red plaque incidence (**Fig. 4E**) and severity (**Fig. 4F**). We also noted that the knockdown vectors reduced VLDL-<br>cholesterol and plaque incidence but not histological plaque severity in GR<sup>ref/ref</sup> mice compared to scramble.<br> cholesterol and plaque incidence but not histological plaque severity in GR<sup>ref/ref</sup> cholesterol and plaque incidence but not histological plaque severity in GR<sup>remer</sup> mice compared to scramble.<br>
Taken together, our findings demonstrate that the rs6190 SNP promotes hypercholesterolemia and atheroscle-<br>
ros 55 Taken together, our findings demonstrate that the rs6190 SNP promotes hypercholesterolemia and atheroscle-<br>56 rosis *in vivo* through upregulation of *Pcsk9* and *Bhlhe40* in liver.<br>57 56 rosis *in vivo* through upregulation of *Pcsk9* and *Bhlhe40* in liver.

 $58$ 

59 **Discussion**<br>50 The glucocor<br>51 modulating<br>52 governs cho<br>53 cocorticoid The glucocorticoid receptor (GR) is well-known for its involvement in orchestrating large gene programs and<br>modulating hepatic lipid and glucose metabolism. However, the precise mechanisms by which hepatic GR<br>governs chole modulating hepatic lipid and glucose metabolism. However, the precise mechanisms by which hepatic GR<br>governs cholesterol regulation remains elusive. Despite the well-established association between chronic glu-<br>cocorticoid 52 governs cholesterol regulation remains elusive. Despite the well-established association between chronic glu-<br>
62 cocorticoid exposure and hypercholesterolemia with concomitant metabolic stress (47), a direct link betwe 63 cocorticoid exposure and hypercholesterolemia with concomitant metabolic stress (47), a direct link between<br>64 GR and atherosclerosis remains unclear. In this study, we leveraged a naturally occurring human mutation, th 664 GR and atherosclerosis remains unclear. In this study, we leveraged a naturally occurring human mutation, the<br>55 rs6190 SNP, to unveil a direct GR-mediated program governing hepatic cholesterol regulation and its conse rs6190 SNP, to unveil a direct GR-mediated program governing hepatic cholesterol regulation and its conse-<br>quential implication for atherogenic risk. We focused here on the hepatic transactivation targets of the mutant<br>GR quential implication for atherogenic risk. We focused here on the hepatic transactivation targets of the mutant<br>
GR based on ChIP-seq-RNA-seq overlay, and consequently validated *Pcsk9* and *Bhlhe40* as mediators of the<br>
S 67 GR based on ChIP-seq-RNA-seq overlay, and consequently validated *Pcsk9* and *Bhlhe40* as mediators of the<br>67 SNP effect on LDLR and SR-B1 levels in liver, as well as on overall cholesterol levels and atherosclerosis in 58 SNP effect on LDLR and SR-B1 levels in liver, as well as on overall cholesterol levels and atherosclerosis in<br>59 the  $hAPOE*2/*2$  background. We recognize that our study did not address potential mutant GR effects on<br>70 a for the *hAPOE\*2/\*2* background. We recognize that our study did not address potential mutant GR effects on apolipoproteins (e.g. ApoE itself) or macrophages, both critical determinants of atherosclerosis and in turn regul apolipoproteins (e.g. ApoE itself) or macrophages, both critical determinants of atherosclerosis and in turn reg-<br>
11 ulated by glucocorticoids and/or GR (12, 13). While beyond the focus of the present study, these are com It ulated by glucocorticoids and/or GR (12, 13). While beyond the focus of the present study, these are compel-<br>
Ing questions to address to expand significance of our findings for overall hypercholesterolemia and athero-<br>

Improvides to expand significance of our findings for overall hypercholesterolemia and atherosclerosis risk in SNP carriers.<br>
24 Our mixed-model regressions in the UK Biobank and the All Of Us datasets have unveiled an une sclerosis risk in SNP carriers.<br>74 Our mixed-model regressions<br>75 sociation between the rs6190<br>76 portantly, the impact of the rsf<br>77 according to the number of "r 74 Our mixed-model regressions in the UK Biobank and the All Of Us datasets have unveiled an unexpected as-<br>5 sociation between the rs6190 SNP and elevated levels of total, LDL-, and HDL-cholesterol in women. Im-<br>76 portan ociation between the rs6190 SNP and elevated levels of total, LDL-, and HDL-cholesterol in women. Im-<br>portantly, the impact of the rs6190 genetic variant demonstrated an additive effect based on SNP zygosity, i.e.<br>accordin portantly, the impact of the rs6190 genetic variant demonstrated an additive effect based on SNP zygosity, i.e.<br>according to the number of "risk" alleles. Additionally in the UK Biobank, the rs6190 SNP correlated with in-<br> according to the number of "risk" alleles. Additionally in the UK Biobank, the rs6190 SNP correlated with in-<br>creased odds ratio for hypercholesterolemia and cardiovascular-related mortality. It was compelling to find<br>anal reased odds ratio for hypercholesterolemia and cardiovascular-related mortality. It was compelling to find<br>analogous correlations in two cohorts that are quite different with regards to genetic ancestry composition. In<br>the 29 analogous correlations in two cohorts that are quite different with regards to genetic ancestry composition. In<br>
20 the All Of Us cohort, the highest minor allele frequency for the SNP was in individuals with European a 80 the All Of Us cohort, the highest minor allele frequency for the SNP was in individuals with European ancestry<br>81 and closely matched the minor allele frequency of the UK Biobank, where the "white British ancestry" inde 81 and closely matched the minor allele frequency of the UK Biobank, where the "white British ancestry" indeed<br>82 accounts for almost 90% of the cohort (48). Beyond SNP correlations in human datasets, we sought to gain the accounts for almost 90% of the cohort (48). Beyond SNP correlations in human datasets, we sought to gain the<br>mechanistic insight in mice and hiPSCs of the extent to which the rs6190 SNP is sufficient to regulate choles-<br>te mechanistic insight in mice and hiPSCs of the extent to which the rs6190 SNP is sufficient to regulate choles-<br>terol. Our findings in murine liver and hiPSC-derived hepatocytes show the SNP is indeed sufficient to elevate<br> 84 terol. Our findings in murine liver and hiPSC-derived hepatocytes show the SNP is indeed sufficient to elevate<br>85 cholesterol and promote atherosclerosis through a specific change in the GR activity. In principle, this 85 cholesterol and promote atherosclerosis through a specific change in the GR activity. In principle, this is a nov-<br>86 el mechanism of SNP action that is independent from the genomic context. Future studies will be neede el mechanism of SNP action that is independent from the genomic context. Future studies will be needed to<br>articulate the genetic modifiers that potentiate or contrast this mechanism across ancestries in the human pop-<br>dati

articulate the genetic modifiers that potentiate or contrast this mechanism across ancestries in the human pop-<br>alation.<br>Given the well-established role of GR as a potent transcription factor, we examined the potential alt 88 ulation.<br>
89 Given the epig<br>
91 the mutate pression<br>
92 pression Siven the well-established role of GR as a potent transcription factor, we examined the potential alterations in<br>the epigenetic activity of GR induced by the rs6190 mutation. At the molecular level, our findings revealed t 90 the epigenetic activity of GR induced by the rs6190 mutation. At the molecular level, our findings revealed that<br>
91 the mutant GR exhibited increased epigenetic activity and nuclear translocation, leading to the differ 91 the mutant GR exhibited increased epigenetic activity and nuclear translocation, leading to the differential ex-<br>pression of 236 genes, including key regulators of cholesterol metabolism. Notably, the mutant GR upregula 92 pression of 236 genes, including key regulators of cholesterol metabolism. Notably, the mutant GR upregulated<br>93 Pcsk9, a key regulator of VLDLR and LDLR degradation, and Bhlhe40, a circadian transcriptional repressor<br>9 93 *Pcsk9*, a key regulator of VLDLR and LDLR degradation, and *Bhlhe40*, a circadian transcriptional repressor<br>
94 that is implicated in SR-B1 control. At present, additional experiments are required to ascertain the exte 94 that is implicated in SR-B1 control. At present, additional experiments are required to ascertain the extent to

which the increase in cholesterol is independent of general changes in lipidemia. However, we emphasize that<br>
96 our regression analyses in women from the UK Biobank dataset took into account triacylglycerols as co-<br>
97 va

96 our regression analyses in women from the UK Biobank dataset took into account triacylglycerols as co-<br>97 variate, and still found a significant zygosity-dependent effect on total and LDL-cholesterol.<br>98 To confirm the variate, and still found a significant zygosity-dependent effect on total and LDL-cholesterol.<br>
98 To confirm the conservation of the SNP-mediated mechanism, we utilized isogenic hiPSC-<br>
99 carrying the rs6190 SNP. These h 98 To confirm the conservation of the SNP-mediated mechanism, we utilized isogenic hiPSC-derived hepatocytes<br>
99 carrying the rs6190 SNP. These hiPSC-derived hepatocytes exhibited increased expression of *PCSK9* and<br> *BHLH* 99 carrying the rs6190 SNP. These hiPSC-derived hepatocytes exhibited increased expression of *PCSK9* and<br>
90 *BHLHE40*, consistent with murine model findings. Moreover, these hepatocytes demonstrated reduced uptake<br>
91 of *BHLHE40*, consistent with murine model findings. Moreover, these hepatocytes demonstrated reduced uptake<br>of HDL and LDL cholesterol, providing direct evidence that the SNP influences cholesterol regulation and this<br>mechan of HDL and LDL cholesterol, providing direct evidence that the SNP influences cholesterol regulation and this<br>mechanism is conserved in human cells. Although the rs6190 SNP is described in ClinVar as associated with<br>"gluco mechanism is conserved in human cells. Although the rs6190 SNP is described in ClinVar as associated with<br>
"glucocorticoid resistance," our analyses in hiPSCs and hiPSC-derived hepatocytes revealed that the mutant<br>
GR is m <sup>33</sup> "glucocorticoid resistance," our analyses in hiPSCs and hiPSC-derived hepatocytes revealed that the mutant GR is more susceptible to glucocorticoid-induced activation than the reference GR isoform. This observation s GR is more susceptible to glucocorticoid-induced activation than the reference GR isoform. This observation<br>
suggests that the SNP may confer increased "glucocorticoid sensitivity" in addition to its effects on cholesterol of suggests that the SNP may confer increased "glucocorticoid sensitivity" in addition to its effects on cholesterol<br>
16 regulation. The evidence in support of "glucocorticoid resistance" is mostly limited to one study, w 16 regulation. The evidence in support of "glucocorticoid resistance" is mostly limited to one study, where targeted<br>
16 limited analyses found that rs6190 decreased dexamethasone-driven activation of *GILZa* in immune ce limited analyses found that rs6190 decreased dexamethasone-driven activation of *GILZa* in immune cells (49).<br>
107 However, several subsequent studies have failed to find correlation between rs6190 and reduced sensitivity Mowever, several subsequent studies have failed to find correlation between rs6190 and reduced sensitivity to<br>
19 glucocorticoids, including the seminal study that first discovered the rs6190 polymorphism (21, 24, 47, 50-<br> glucocorticoids, including the seminal study that first discovered the rs6190 polymorphism (21, 24, 47, 50-<br>
10 53). Further *in vitro* experiments are warranted to investigate the extent to which the mutant GR activates n 10 53). Further *in vitro* experiments are warranted to investigate the extent to which the mutant GR activates new-<br>11 ly identified glucocorticoid response elements (GREs) dependently or independently from other key nucl

12 ly identified glucocorticoid response elements (GREs) dependently or independently from other key nuclear<br>12 factors for cholesterol regulation.<br>13 **Limitations of this study** – Besides specific limitations and consider 12 factors for cholesterol regulation.<br>
13 **Limitations of this study** – Besi<br>
14 in this study we have not formally<br>
15 ous study in a limited cohort fou<br>
16 levels in men but not in women (1 **Limitations of this study –** Besides specific limitations and considerations reported above for specific results,<br>in this study we have not formally assessed the impact of sexual dimorphism on the SNP effect. While a prev 14 in this study we have not formally assessed the impact of sexual dimorphism on the SNP effect. While a previ-<br>15 ous study in a limited cohort found a significant association between the rs6190 SNP and lower cholesterol 15 ous study in a limited cohort found a significant association between the rs6190 SNP and lower cholesterol<br>16 levels in men but not in women (17), our investigations in the large UK Biobank and All Of Us cohorts reveale levels in men but not in women (17), our investigations in the large UK Biobank and All Of Us cohorts revealed<br>a significant association between the SNP and increased cholesterol levels in women but not in men. Our stud-<br>i 17 a significant association between the SNP and increased cholesterol levels in women but not in men. Our stud-<br>18 is in SNP mice further confirmed a significant or larger magnitude of SNP effect in female rather than mal 18 ies in SNP mice further confirmed a significant or larger magnitude of SNP effect in female rather than male<br>
19 mice. These sex-specific observations will require well-powered studies to disentangle the interplay betwe mice. These sex-specific observations will require well-powered studies to disentangle the interplay between<br>the mutant GR and sex-specific nuclear receptor cascades from the sexual dimorphism in downstream choles-<br>terol r

20 the mutant GR and sex-specific nuclear receptor cascades from the sexual dimorphism in downstream choles-<br>21 terol regulations, complex experiments that go beyond the mechanistic discovery focus of this initial study.<br>2 21 terol regulations, complex experiments that go beyond the mechanistic discovery focus of this initial study.<br>
22 **Conclusions and overall impact** – In conclusion, our study leverages the rs6190 SNP as genetic linch<br>
23 22 **Conclusions and overall impact** – In conclusion, our study leverages the rs6190 SNP as genetic linchpin to advance our understanding of the GR-driven regulation of cholesterol through genetic and epigenetic mechanisms. 23 advance our understanding of the GR-driven regulation of cholesterol through genetic and epigenetic mecha-<br>24 nisms. Our data support early and proactive monitoring for cholesterol in carriers of this non-rare variant, 24 nisms. Our data support early and proactive monitoring for cholesterol in carriers of this non-rare variant, par-<br>25 ticularly in women.<br>26 25 ticularly in women.<br>
26<br>
27<br>
28

- 
- $\frac{27}{28}$
- $\frac{28}{ }$

### UK Biobank plasma NMR metabolomics (2, N=65156; age 40-70yo)



 $33343678$ Figure 1 – rs6190 correlates with cholesterol increase in women from the UK Biobank and All of US datasets. (A) Unbiased ranking of UK Biobank plasma NMR parameters for significant rs6190 effect in women.<br>
Cholesterol-rela tasets. (A) Unbiased ranking of UK Biobank plasma NMR parameters for significant rs6190 effect in women.<br>
33 Cholesterol-related parameters are highlighted in bold text and red bars. P values were adjusted for age, BMI<br>
34 33 Cholesterol-related parameters are highlighted in bold text and red bars. P values were adjusted for age, BMI<br>34 and canonical hypercholesterolemia-associated SNPs. (B) Linear regressions (blue lines; shaded area repre 34 and canonical hypercholesterolemia-associated SNPs. **(B)** Linear regressions (blue lines; shaded area represents 95% C.I.; corrected for age, diabetes, triacylglycerols) and median confidence intervals (Kruskal-Wallis t 35 sents 95% C.I.; corrected for age, diabetes, triacylglycerols) and median confidence intervals (Kruskal-Wallis test) show zygosity-dependent trends in elevation of total, LDL- and HDL-cholesterol in women. **(C-D)** Comp 36 test) show zygosity-dependent trends in elevation of total, LDL- and HDL-cholesterol in women. **(C-D)** Compared to non-carriers, homozygous SNP carriers showed increased odds ratio for hypercholesterolemia and cardiovas 37 pared to non-carriers, homozygous SNP carriers showed increased odds ratio for hypercholesterolemia and cardiovascular disease deaths according to ICD10 codes; Chi-square test. (E) Linear regressions and median compari 38 cardiovascular disease deaths according to ICD10 codes; Chi-square test. **(E)** Linear regressions and median comparisons correlated rs6190 genotype with cholesterol elevation in women from the All of Us dataset, includi 39 comparisons correlated rs6190 genotype with cholesterol elevation in women from the All of Us dataset, includ-<br>30 ing all ancestries and ages. \*, P<0.05; \*\*, P<0.01; \*\*\*, P<0.001; \*\*\*\*, P<0.0001.<br>31 40 ing all ancestries and ages. \*, P<0.05; \*\*, P<0.01; \*\*\*, P<0.001; \*\*\*\*, P<0.0001.

30

# 42 **Figure 2**



 $13$ 

 $14$  15 16 17 18 19 10 11 Figure 2. The rs6190 SNP is sufficient to increase cholesterol and skew the liver GR to a gene program repressing liver cholesterol uptake in mice. (A) Zygosity-dependent increases in cholesterol in both fed and fasted st **the system is and the system of cholesterol across lipoprotein fractions (arrows highlight increases in LDL- and HDL-chole** 46 fasted states in littermates control vs SNP-carrier mice. **(B)** Analogous trends with regular and Western diets,<br>47 as assayed through FPLC distribution of cholesterol across lipoprotein fractions (arrows highlight inc 47 as assayed through FPLC distribution of cholesterol across lipoprotein fractions (arrows highlight increases in LDL- and HDL-cholesterol). (C) RNA-seq and ChIP-seq overlay in liver tissue identifies *Pcks9* and *Bhlhe4* 18 LDL- and HDL-cholesterol). **(C)** RNA-seq and ChIP-seq overlay in liver tissue identifies *Pcks9* and *Bhlhe40* as<br>19 putative transactivation targets of the mutant GR. **(D-E)** ChIP-seq and RNA-seq, as well as validation putative transactivation targets of the mutant GR. **(D-E)** ChIP-seq and RNA-seq, as well as validation WB val-<br>io ues for PCSK9, BHLHE40 and their putative targets LDLR and SR-B1. **(F)** Uptake of LDL and HDL particles<br>(tr 50 ues for PCSK9, BHLHE40 and their putative targets LDLR and SR-B1. **(F)** Uptake of LDL and HDL particles<br>51 (traced by red fluorescence) is lower in GR<sup>ALT/ALT</sup> than GR<sup>ref/ref</sup> primary hepatocytes. N=3-10<sup>0</sup>/group, 3-6 51 (traced by red fluorescence) is lower in GR<sup>ALT/ALT</sup> than GR<sup>ref/ref</sup> primary hepatocytes. N=3-10♀/group, 3-6mo; A:<br>52 1w ANOVA + Sidak; D-F: Welch's t-test; \*, P<0.05; \*\*, P<0.01; \*\*\*, P<0.001; \*\*\*\*, P<0.0001. 52 1w ANOVA + Sidak; D-F: Welch's t-test; \*, P<0.05; \*\*, P<0.01; \*\*\*, P<0.001; \*\*\*\*, P<0.0001.



 $,4$  $55$  $56$ 

54557859505157 55 57 58 59 50 51 52 52  $-7890123$ Figure 3. The SNP molecular effects are replicated in hiPSC-derived hepatocytes. (A) Sanger sequencing<br>of SNP genotype and brightfield representative images for isogenic hiPSCs and derived hepatocytes with no,<br>one or two r 58 of SNP genotype and brightfield representative images for isogenic hiPSCs and derived hepatocytes with no,<br>59 one or two rs6190 SNP alleles. (B) Rate of GR nuclear signal enrichment in hiPSC-hepatocytes increased be-<br>5 59 one or two rs6190 SNP alleles. **(B)** Rate of GR nuclear signal enrichment in hiPSC-hepatocytes increased be-<br>tween 20-60min after dexamethasone addition according to SNP zygosity. **(C)** Zygosity-dependent effects on<br>FCS 60 tween 20-60min after dexamethasone addition according to SNP zygosity. **(C)** Zygosity-dependent effects on  $PCSK9$  and  $BHLHE40$  upregulation at the hepatocyte level, as well as on protein level downregulation for LDLR and Frank *PCSK9* and *BHLHE40* upregulation at the hepatocyte level, as well as on protein level downregulation for<br>
LDLR and SR-B1. **(D-E)** SNP zygosity replicated the effects on HDL and LDL fluorescent particle uptake in<br>
h 62 LDLR and SR-B1. **(D-E)** SNP zygosity replicated the effects on HDL and LDL fluorescent particle uptake in hiPSC-hepatocytes. Each dot represents an independent differentiation replicate; N=3-6/group. B: 2w ANOVA + Sidak impted in the state of the presents an independent differentiation replicate; N=3-6/group. B: 2w ANOVA + Sidak; C-E: 1w ANOVA + Sidak. \*, P<0.05; \*\*, P<0.01; \*\*\*, P<0.001; \*\*\*\*, P<0.0001.<br>55 64 + Sidak; C-E: 1w ANOVA + Sidak. \*, P<0.05; \*\*, P<0.01; \*\*\*, P<0.001; \*\*\*\*, P<0.0001.

 $\frac{1}{2}$ 



 $58$ 

59 70 71 72 73 74 75 Figure 4. The SNP promotes atherosclerosis in vivo. (A) FPLC curves show the additive effect of SNP<br>
70 genotype on the hAPOE\*2/\*2-driven hypercholesterolemia across lipoprotein fractions in both normal and<br>
71 Western di 70 genotype on the hAPOE\*2/\*2-driven hypercholesterolemia across lipoprotein fractions in both normal and<br>
71 Western diets (arrows). **(B)** Compared to GR<sup>ref/ref</sup> mice, GR<sup>ALT/ALT</sup> mice on the *hAPOE\*2/\*2* background sho Western diets (arrows). **(B)** Compared to GRref/ref mice, GRALT/ALT Western diets (arrows). **(B)** Compared to GR<sup>remer</sup> mice, GR<sup>ALI/ALI</sup> mice on the *hAPOE\*2/\*2* background showed<br>higher incidence (as quantitated from en face analyses) and severity (as quantitated through Oil Red O stain higher incidence (as quantitated from en face analyses) and severity (as quantitated through Oil Red O stain-<br>
ing in aortic root sections) of atherosclerotic plaques. (C) qPCR validation of target knockdown in liver. (D-73 ing in aortic root sections) of atherosclerotic plaques. **(C)** qPCR validation of target knockdown in liver. **(D-F)**<br>
74 AAV-mediated knockdown of *Pcsk9* and *Bhlhe40* in adult mice blunted the SNP effect on VLDL-, LDL 74 AAV-mediated knockdown of *Pcsk9* and *Bhlhe40* in adult mice blunted the SNP effect on VLDL-, LDL- and<br>
75 HDL-cholesterol (FPLC), plaque incidence in en face aorta assays, and histological severity of aortic root<br>
76 75 HDL-cholesterol (FPLC), plaque incidence in en face aorta assays, and histological severity of aortic root<br>
76 plaques. N=4-7 $\frac{2}{\sqrt{3}}$  (group, 6mo; B: Welch's t-test; E-F: 2w ANOVA + Sidak; \*, P<0.05; \*\*, P<0.01; \*\* 76 plaques. N=4-7♀/group, 6mo; B: Welch's t-test; E-F: 2w ANOVA + Sidak; \*, P<0.05; \*\*, P<0.01; \*\*\*, P<0.001;<br>\*\*\*\*, P<0.0001.<br>\*\*\*\*, P<0.0001. 77 \*\*\*\*, P<0.0001.



### 79

# 30<br>31<br>32<br>33<br>34

- Supplementary Figure 1. Related to Figure 1. Additional data from UK Biobank and All Of Us datasets.<br>
2ygosity-dependent correlations of rs6190 with total, LDL and HDL cholesterol were not significant in men from<br>
either U 81 Zygosity-dependent correlations of rs6190 with total, LDL and HDL cholesterol were not significant in men from<br>82 either UK Biobank (A) or All of Us (B) datasets. Linear regressions were corrected for age, diabetes,<br>83
- 82 either UK Biobank (A) or All of Us (B) datasets. Linear regressions were corrected for age, diabetes,<br>83 triacylglycerols; median intervals were compared through Kruskal-Wallis test.<br>84<br>85 83 triacylglycerols; median intervals were compared through Kruskal-Wallis test.<br>84<br>85<br>86
- 
- 
- 
- 
- $35$ <br> $36$ <br> $37$ <br> $38$ 36<br>37<br>38<br>39 37<br>38<br>39<br>39
- 38
- 
- 39
- $30<sup>°</sup>$
- 38<br>39<br>30<br>30<br>31 39<br>39<br>30<br>31  $\overline{)}$ <br> $\overline{)}$ <br> $\overline{)}$ <br><br><br><br><br><br><br><br><br><br><br><br><br><br><br>  $\overline{)1}$  $31$



**Supplementary Figure 2. Related to Figure 2. Additional analyses related to mutant GR effects in mu-<br>
ine liver. (A) Diagram highlighting the human-mouse GR sequence orthology and the SNP genocopy intro-<br>
duced through CR** Fine liver. (A) Diagram highlighting the human-mouse GR sequence orthology and the SNP genocopy intro-<br>
95 duced through CRISPR-Cas9. (B) Differently than in female mice, male mice blunted the SNP effect on choles-<br>
97 ter 96 duced through CRISPR-Cas9. **(B)** Differently than in female mice, male mice blunted the SNP effect on cholesterol elvetion according to SNP zygosity, according to FPLC cholesterol levels across lipoprotein fractions. **(** 97 terol elevation according to SNP zygosity, according to FPLC cholesterol levels across lipoprotein fractions. **(C)**<br>98 In 3 out of 5 female mice analyzed from the parental WT genetic background after Western diet exposu 98 In 3 out of 5 female mice analyzed from the parental WT genetic background after Western diet exposure,<br>99 emerging immature plaques were noted in the aortic roots (arrows; insets: high magnification). **(D)** The SNP<br>90 99 emerging immature plaques were noted in the aortic roots (arrows; insets: high magnification). **(D)** The SNP<br>effect on cholesterol in female mice followed circadian fluctuations and peaked in the active phase (dark<br>phas of the mumber of peaks than the control GR, although the relative genomic distribution did not change. (K) Volcano of peaks than the control GR, although the relative genomic distribution (K) Juninum and peaked in the acti base; arrow), in phase with the endogenous corticosterone elevation in mice. However, no SNP-dependent<br>
effects were noted in either peak or trough corticosterone values (right histogram). (E) The SNP increased ba-<br>
sal an effects were noted in either peak or trough corticosterone values (right histogram). **(E)** The SNP increased ba-<br>sal and steroid-driven GR activity on a GRE luciferase reporter transfected in primary hepatocytes. **(G)** Unb Sal and steroid-driven GR activity on a GRE luciferase reporter transfected in primary hepatocytes. **(G)** Unbi-<br>
36 showed increased GR occupancy genome-wide on GRE motifs (arrow). **(I)** Representative peak traces for a<br> ased motif analysis validates ChIP-seq datasets through enrichment for GRE motif (arrows). **(H)** Mutant GR<br>showed increased GR occupancy genome-wide on GRE motifs (arrow). **(I)** Representative peak traces for a<br>canonical m bowed increased GR occupancy genome-wide on GRE motifs (arrow). (**I)** Representative peak traces for a canonical marker of GR epigenetic activity, the *Fkbp5* distal promoter (arrow). (**J)** Mutant GR had a higher number o of canonical marker of GR epigenetic activity, the *Fkbp5* distal promoter (arrow). (**J)** Mutant GR had a higher number of peaks than the control GR, although the relative genomic distribution did not change. (**K)** Volca or number of peaks than the control GR, although the relative genomic distribution did not change. **(K)** Volcano<br>
18 plot of SNP-dependent differentially expressed genes in liver per RNA-seq datasets. **(L)** Compared to *B* 98 plot of SNP-dependent differentially expressed genes in liver per RNA-seq datasets. **(L)** Compared to *Bhlhe40-WT* littermates, *Bhlhe40-KO* livers showed upregulation of SR-B1 levels at mRNA (*Scarb1*, gene name for S 09 *WT* littermates, *Bhlhe40-KO* livers showed upregulation of SR-B1 levels at mRNA (*Scarb1*, gene name for SR-<br>
09 B1), supporting the notion of Bhlhe40 as transcriptional repressor of SR-B1 in liver. N=3-5/group, ∂ in 10 B1), supporting the notion of Bhlhe40 as transcriptional repressor of SR-B1 in liver. N=3-5/group,  $\circ$  in B,  $\circ$  in 16. L, 6mo; D, E, J: 2w ANOVA + Sidak; I, L: Welch's t-test; \*, P<0.05; \*\*, P<0.01; \*\*\*, P<0.001; \*\* 11 C-L, 6mo; D, E, J: 2w ANOVA + Sidak; I, L: Welch's t-test; \*, P<0.05; \*\*, P<0.01; \*\*\*, P<0.001; \*\*\*\*, P<0.0001.<br>12<br>13<br>14

- 
- 
- 13<br>14<br>15<br>16 l4<br>l5<br>l6<br>l7
- 
- 
- 
- $17$
- 
- 15<br>15<br>16<br>17<br>18 16<br>17<br>18<br>19 17<br>18<br>19<br>20 18<br>19<br>20 18
- 19<br>20<br>20 L9
- $\frac{1}{2}$  $20$



23<br>24<br>25<br>26<br>27<br>28<br>29<br>20 **Supplementary Figure 3. Related to Figure 3. Additional analyses related to SNP-mutant hiPSCs. (A)**<br>
Pluripotency marker validation of isogenic hiPSC lines with CRISPR-knock-in engineering of a SNP genocopy<br>
25 in the end Pluripotency marker validation of isogenic hiPSC lines with CRISPR-knock-in engineering of a SNP genocopy<br>
25 in the endogenous *NR3C1* gene locus. **(B)** The SNP promoted GR translocation at the undifferentiated hiPSC<br>
26 25 in the endogenous *NR3C1* gene locus. **(B)** The SNP promoted GR translocation at the undifferentiated hiPSC<br>
26 stage. **(C)** The SNP genotype did not impact the overall progression of differentiating hiPSCs across the s 27 of hepatocyte differentiation: hiPSC, pluripotent; DE, definitive endoderm; IMH, immature hepatocytes; MH,<br>
28 mature hepatocytes. (D) Albumin production (staining) and secretion (ELISA) confirmed hepatocyte maturation<br> 27 of hepatocyte differentiation: hiPSC, pluripotent; DE, definitive endoderm; IMH, immature hepatocytes; MH,<br>
28 mature hepatocytes. (D) Albumin production (staining) and secretion (ELISA) confirmed hepatocyte maturation<br> 28 mature hepatocytes. (D) Albumin production (staining) and secretion (ELISA) confirmed hepatocyte maturation<br>
29 comparable across SNP genotypes. Each dot represents an independent differentiation replicate; N=3/group.<br> 29 comparable across SNP genotypes. Each dot represents an independent differentiation replicate; N=3/group.<br>
20 2w ANOVA + Sidak; \*, P<0.05; \*\*, P<0.01; \*\*\*, P<0.001; \*\*\*\*, P<0.0001.<br>
31<br>
32<br>
33 30 2w ANOVA + Sidak; \*, P<0.05; \*\*, P<0.01; \*\*\*, P<0.001; \*\*\*\*, P<0.0001.<br>31<br>32<br>33

- 
- $\frac{32}{33}$ 32

vector combination for Bhlhe40 knockdown В



36  $37$ 

- $\frac{37}{38}$  39 10 11 12 13 38<br>39<br>40<br>41<br>42<br>43 **Supplementary Figure 4 – Preliminary validations of dosage and combinations for AAV8-mediated knockdowns in vivo. (A)**  $10^{13}$ y/mouse maximized *Pcsk9* knockdown in liver with AAV8-antiPcsk9 compared to scramble. (B) Co **knockdowns in vivo. (A)** 10^13vg/mouse maximized *Pcsk9* knockdown in liver with AAV8-antiPcsk9 compared to scramble. **(B)** Combination of both AAV8-antiBhlhe40 shRNA vectors was synergistic in maximizing *Bhlhe40* knock 10 pared to scramble. **(B)** Combination of both AAV8-antiBhlhe40 shRNA vectors was synergistic in maximizing<br>
11 *Bhlhe40* knockdown in liver compared to scramble. N=5<sup>0</sup>/group, 6mo; 1w ANOVA + Sidak; \*, P<0.05; \*\*,<br>
12 P< 41 *Bhlhe40* knockdown in liver compared to scramble. N=5♀/group, 6mo; 1w ANOVA + Sidak; \*, P<0.05; \*\*, P<0.01; \*\*\*, P<0.001; \*\*\*\*, P<0.0001.<br>43<br>59.0001; \*\*\*, P<0.001; \*\*\*\*, P<0.0001.
- 42 P<0.01; \*\*\*, P<0.001; \*\*\*\*, P<0.0001.
- 

14 **METHODS**<br>15 **Animals ar**<br>16 Mice used i<br>17 Medical As<br>18 mittee (IAC 45 **Animals and diet**<br>46 Mice used in this s<br>47 Medical Associatio<br>48 mittee (IACUC) at<br>49 carried out in acco Mice used in this study were maintained in a pathogen-free facility in accordance with the American Veterinary<br>
Medical Association (AVMA) and under protocol fully approved by the Institutional Animal Care and Use Com-<br>
mi Medical Association (AVMA) and under protocol fully approved by the Institutional Animal Care and Use Com-<br>mittee (IACUC) at Cincinnati Children's Hospital Medical Center (#2023-0002). Euthanasia of the mice was<br>carried ou mittee (IACUC) at Cincinnati Children's Hospital Medical Center (#2023-0002). Euthanasia of the mice was<br>carried out in accordance with ethical guidelines. Carbon dioxide inhalation was utilized as the initial method<br>for e

49 carried out in accordance with ethical guidelines. Carbon dioxide inhalation was utilized as the initial method<br>49 for euthanasia, followed by cervical dislocation and removal of the liver tissue.<br>41 animals were maint 50 for euthanasia, followed by cervical dislocation and removal of the liver tissue.<br>
51 All animals were maintained in a temperature-controlled environment with a<br>
52 fasting group, mice were subjected to an 18-hour starv 51 All animals were maintained in a temperature-controlled environment with a 12h/12h light/dark cycle. For the<br>52 fasting group, mice were subjected to an 18-hour starvation period. Mutant GR mice were generated using<br>53 52 fasting group, mice were subjected to an 18-hour starvation period. Mutant GR mice were generated using CRISPR/Cas9 genome editing by genocopying the rs6190 SNP in the endogenous *Nr3c1* locus on the C57BL/6J backgroun 53 CRISPR/Cas9 genome editing by genocopying the rs6190 SNP in the endogenous *Nr3c1* locus on the C57BL/6J background. This genetic modification was performed by the Transgenic Animal and Genome Editing Core Facility at 54 C57BL/6J background. This genetic modification was performed by the Transgenic Animal and Genome Editing Core Facility at CCHMC. To ensure genetic background homogeneity and control for potential confounding variables, is ing Core Facility at CCHMC. To ensure genetic background homogeneity and control for potential confounding<br>
variables, the colonies were maintained through heterozygous matings. This approach allowed us to compare<br>
thr 36 variables, the colonies were maintained through heterozygous matings. This approach allowed us to compare<br>37 three distinct groups of mice as littermates:  $GR^{\text{ref/ref}}$  (control WT),  $GR^{\text{ref/ALT}}$  (heterozygous SNP carrie Three distinct groups of mice as littermates:  $GR^{left}$  (control WT),  $GR^{left}$  (heterozygous SNP carriers), and  $GR^{ALT/ALT}$  (homozygous SNP carriers). All animals used in this study were approximately 3-4 months of age at the t  ${\sf GR}^{\sf ALT/ALT}$ 58 GR<sup>ALI/ALI</sup> (homozygous SNP carriers). All animals used in this study were approximately 3-4 months of age at<br>59 the time of experimentation. As the primary atherogenic model,  $hAPOE$ <sup>\*2</sup>/<sup>\*2</sup> homozygous mice were origin 59 the time of experimentation. As the primary atherogenic model,  $hAPOE*2/*2$  homozygous mice were originally<br>50 obtained from the Maeda Laboratory at the University of North Carolina (44) and maintained as breeding colo-<br>5 60 obtained from the Maeda Laboratory at the University of North Carolina (44) and maintained as breeding colo-<br>hy from Dr. David Hui's lab at the University of Cincinnati. These mice were crossed with the R24K mutant<br>mice 61 ny from Dr. David Hui's lab at the University of Cincinnati. These mice were crossed with the R24K mutant<br>
62 mice. To induce hypercholesterolemia and atherosclerosis, R24K mice crossed on  $hAPOE^*2/*2$  background<br>
63 wer

mice. To induce hypercholesterolemia and atherosclerosis, R24K mice crossed on *hAPOE\*2/\*2* background<br>were subjected to cholate-free western diet, which contained 21% fat and 0.2% cholesterol for 16 weeks.<br>For systemic AA were subjected to cholate-free western diet, which contained 21% fat and 0.2% cholesterol for 16 weeks.<br>
For systemic AAV experiments, wild-type and homozygous SNP-mutant littermate mice on  $hAPOE^*/2$ <br>
ground were injected For systemic AAV experiments, wild-type and homozygous SNP-mutant littermate mice on *hAPOE\*2/\*2* back-<br>ground were injected retro-orbitally with either  $3x10^{13}$  vg/mouse of AAV8-scramble shRNA or  $1x10^{13}$  vg/mouse<br>for ground were injected retro-orbitally with either 3x10<sup>13</sup> vg/mouse of AAV8-scramble shRNA or 1x10<sup>13</sup> ground were injected retro-orbitally with either  $3x10^{13}$  vg/mouse of AAV8-scramble shRNA or  $1x10^{13}$  vg/mouse<br>for each of the knockdown combination vectors, i.e. one AAV8-anti*Pcsk9* (46) and two AAV8-*Bhlhe40shRNA*<br>v for each of the knockdown combination vectors, i.e. one AAV8-anti*Pcsk9* (46) and two AAV8-*Bhlhe40*shRNA<br>vectors (Vector Builder vectors # VB010000-0023jze, VB230421-1310pka, VB230421-1312ydp; Addgene<br>#163025; scramble sh vectors (Vector Builder vectors # VB010000-0023jze, VB230421-1310pka, VB230421-1312ydp; Addgene<br>
#163025; scramble shRNA sequence: CCTAAGGTTAAGTCGCCCTCG; anti-*Bhlhe40* shRNA sequences:<br>
GCGAGGTTACAGTGTTTATAT, GTAGTGGTTTGG 4163025; scramble shRNA sequence: CCTAAGGTTAAGTCGCCCTCG; anti-*Bhlhe40* shRNA sequences:<br>GCGAGGTTACAGTGTTTATAT, GTAGTGGTTTGGGCAAATTTC) while under inhaled isoflurane anesthesia.<br>All AAV8 injections were diluted in sterile 6CGAGGTTACAGTGTTTATAT, GTAGTGGTTTGGGCAAATTTC) while under inhaled isoflurane anesthesia.<br>
All AAV8 injections were diluted in sterile PBS. To prepare and isolate AAV virions, we followed the procedures<br>
We previously repor

All AAV8 injections were diluted in sterile PBS. To prepare and isolate AAV virions, we followed the procedures<br>
11 we previously reported (54, 55).<br>
12 **RNA extraction and RT-qPCR**<br>
13 Total RNA was extracted from cryo-p 71 we previously reported (54, 55).<br>
72 **RNA extraction and RT-qPCR**<br>
73 Total RNA was extracted from cr<br>
74 (Cat #15596026, Thermo Fishe<br>
75 VILO<sup>TM</sup> Master Mix (Cat #11756 **RNA extraction and RT-qPCR**<br>
73 Total RNA was extracted from cr<br>
74 (Cat #15596026, Thermo Fishe<br>
75 VILO™ Master Mix (Cat #11756<br>
76 using 1X SYBR Green Fast qP( Total RNA was extracted from cryo-pulverized liver tissues and hiPSC-derived hepatocyte-like cells using Trizol<br>
(Cat #15596026, Thermo Fisher Scientific) and 1 ug RNA was reverse-transcribed using SuperScript<sup>™</sup> IV<br>
75 (Cat #15596026, Thermo Fisher Scientific) and 1 ug RNA was reverse-transcribed using SuperScript™ 74 (Cat #15596026, Thermo Fisher Scientific) and 1 ug RNA was reverse-transcribed using SuperScript<sup>1M</sup> IV<br>
75 VILO<sup>™</sup> Master Mix (Cat #11756050, Thermo Fisher Scientific). RT-qPCRs were conducted in three replicates<br>
7  $VILO<sup>TM</sup>$  Master Mix (Cat #11756050, Thermo Fisher Scientific), RT-qPCRs were conducted in three replicates  $VILO<sup>IM</sup> Master Mix (Cat #11756050, Thermo Fisher Scientific). RT-qPCRs were conducted in three replicates using 1X SYBR Green Fast qPCR machine (Bio-Rad, Hercules, CA; thermal profile: 95C, 15 sec; 60C, 30sec; 40x; melting curve). The 2- $\triangle\triangle CT$  method was used to calculate relative gene expression. GAPDH was used as the internal control. Primers were selected among validated primer sets from MGH PrimerBank:$ The using 1X SYBR Green Fast qPCR machine (Bio-Rad, Hercules, CA; thermal profile: 95C, 15 sec; 60C, 30sec;<br>
40x; melting curve). The 2-ΔΔCT method was used to calculate relative gene expression. GAPDH was used as<br>
18 the 77 40x; melting curve). The 2-∆∆CT method was used to calculate relative gene expression. GAPDH was used as 78 the internal control. Primers were selected among validated primer sets from MGH PrimerBank:



79

30<br>31<br>32<br>33 80 **Western blotting**<br>
81 Protein analyses in<br>
82 RIPA buffer (Cat #<br>
83 #78440, Thermo F 81 Protein analyses in liver were performed on ∼ 25 ug total lysates. Cyro-pulverized liver tissue was incubated in<br>82 RIPA buffer (Cat #89900Thermo Fisher Scientific) supplemented with 1x protease/phosphatase inhibitor (

#78440, Thermo Fisher Scientific) for 30 mins and sonicated for 10 secs twice. The samples were then centri-83 #78440, Thermo Fisher Scientific) for 30 mins and sonicated for 10 secs twice. The samples were then centri-<br>#78440, Thermo Fisher Scientific) for 30 mins and sonicated for 10 secs twice. The samples were then centri-

fuged at 12,000 rpm for 10 mins at  $4^{\circ}$ C. Supernatant containing the protein is transferred into a new tube and tuged at 12,000 rpm for 10 mins at  $4^{\circ}$ C. Supernatant containing the protein is transferred into a new tube and<br>used as a total lysate. For total cell lysates from culture cells, cells were harvested and resuspended in used as a total lysate. For total cell lysates from culture cells, cells were harvested and resuspended in RIPA<br>buffer containing 1x protease and phosphatase inhibitors. Lysates were incubated for 30 mins and centrifuged<br>a 86 buffer containing 1x protease and phosphatase inhibitors. Lysates were incubated for 30 mins and centrifuged<br>87 at 12,000 rpm for 10 mins at 4°C. The supernatant was used as a total cell lysate. The protein concentrati at 12,000 rpm for 10 mins at  $4^{\circ}$ at 12,000 rpm for 10 mins at  $4^{\circ}$ C. The supernatant was used as a total cell lysate. The protein concentrations<br>of the supernatants were determined using the Pierce BCA Protein Assay kit (Cat #23225, Thermo Fisher Sciof the supernatants were determined using the Pierce BCA Protein Assay kit (Cat #23225, Thermo Fisher Scientific). Equal amounts of protein were separated using SDS-PAGE and transferred to a PVDF membrane (Cat #1620177, Bi entific). Equal amounts of protein were separated using SDS-PAGE and transferred to a PVDF membrane (Cat #1620177, BioRad). Membranes were blocked in 5% milk in TBST for 1 hour at room temperature and then incubated overni 41620177, BioRad). Membranes were blocked in 5% milk in TBST for 1 hour at room temperature and then<br>incubated overnight at 4°C with primary antibodies: PCSK9 (Cat #A7860, 1:1000, ABclonal), BHLHE40 (Cat<br>#A6534, 1:1000, AB incubated overnight at  $4^{\circ}$ C with primary antibodies: PCSK9 (Cat #A7860, 1:1000, ABclonal), BHLHE40 (Cat 91 incubated overnight at 4°C with primary antibodies: PCSK9 (Cat #A7860, 1:1000, ABclonal), BHLHE40 (Cat #A6534, 1:1000, ABclonal), SR-B1 (Cat #A0827, 1:1000, ABclonal), LDLR (Cat #A14996, 1:1000, ABclonal), followed by i 4/46534, 1:1000, ABclonal), SR-B1 (Cat #A0827, 1:1000, ABclonal), LDLR (Cat #A14996, 1:1000, ABclonal),<br>followed by incubation with anti-rabbit IgG, HRP-conjugated secondary antibody (Cat #7074, 1:5000, Cell Sig-<br>naling) f followed by incubation with anti-rabbit IgG, HRP-conjugated secondary antibody (Cat #7074, 1:5000, Cell Sig-<br>
94 naling) for 1 hour at room temperature. Immunoreactive bands were visualized by chemiluminescence using<br>
95 P

94 naling) for 1 hour at room temperature. Immunoreactive bands were visualized by chemiluminescence using<br>95 Pierce Enhanced Chemiluminescent western blotting substrate (Cat #32106, Thermo Fisher Scientific)<br>96 RNA sequen Pierce Enhanced Chemiluminescent western blotting substrate (Cat #32106, Thermo Fisher Scientific)<br>
96 **RNA sequencing sample preparation and analysis**<br>
97 RNA-seq was conducted on RNA extracted from the liver tissue of wi RNA sequencing sample preparation and analysis<br>
PRNA-seq was conducted on RNA extracted from the l<br>
Bach liver was immediately snap frozen in 1 ml TRIsu<br>
each heart were extracted individually and re-purified<br>
90 seq was p RNA-seq was conducted on RNA extracted from the liver tissue of wild-type versus R24K homozygous mice.<br>
Bach liver was immediately snap frozen in 1 ml TRIsure (Bioline, BIO-38033) using liquid Nitrogen. RNAs from<br>
each hea Each liver was immediately snap frozen in 1 ml TRIsure (Bioline, BIO-38033) using liquid Nitrogen. RNAs from<br>each heart were extracted individually and re-purified using the RNeasy Mini Kit (Cat #74104, Qiagen). RNA-<br>seq w each heart were extracted individually and re-purified using the RNeasy Mini Kit (Cat #74104, Qiagen). RNA-<br>
99 was performed at the DNA core (CCHMC). 150 ng – 300 ng of total RNA determined by Qubit (Invitrogen)<br>
91 highor seq was performed at the DNA core (CCHMC). 150 ng – 300 ng of total RNA determined by Qubit (Invitrogen)<br>high-sensitivity spectrofluorometric measurement was poly-A selected and reverse transcribed using Illumina's<br>TruS high-sensitivity spectrofluorometric measurement was poly-A selected and reverse transcribed using Illumina's<br>
12 TruSeq stranded mRNA library preparation kit (Cat #20020595, Illumina, San Diego, CA). Each sample was<br>
13 f TruSeq stranded mRNA library preparation kit (Cat #20020595, Illumina, San Diego, CA). Each sample was<br>fitted with one of 96 adapters containing a different 8 base molecular barcode for high level multiplexing. After<br>15 cy 93 fitted with one of 96 adapters containing a different 8 base molecular barcode for high level multiplexing. After<br>15 cycles of PCR amplification, completed libraries were sequenced on an Illumina NovaSeqTM 6000, gener-<br> 15 cycles of PCR amplification, completed libraries were sequenced on an Illumina NovaSeqTM 6000, generating 20 million or more high quality 100 base long paired end reads per sample. A quality control check on the fastq f of the fasta files was performed using FastQC. Upon passing basic quality metrics, the reads were trimmed to re-<br>fasta files was performed using FastQC. Upon passing basic quality metrics, the reads were trimmed to re-<br>mov 1666 fastq files was performed using FastQC. Upon passing basic quality metrics, the reads were trimmed to re-<br>1666 move adapters and low-quality reads using default parameters in Trimmomatic [Version 0.33]. The trimmed<br>18 07 move adapters and low-quality reads using default parameters in Trimmomatic [Version 0.33]. The trimmed<br>
18 reads were then mapped to mm10 reference genome using default parameters with strandness (R for single-<br>
19 end reads were then mapped to mm10 reference genome using default parameters with strandness (R for single-<br>end and RF for paired-end) option in Hisat2 [Version 2.0.5]. Next, the transcript/gene abundance was deter-<br>mined usin of the transcript/gene abundance was deter-<br>mined using kallisto [Version 0.43.1]. We first created a transcriptome index in kallisto using Ensembl cDNA<br>sequences for the reference genome. This index was then used to quant mined using kallisto [Version 0.43.1]. We first created a transcriptome index in kallisto using Ensembl cDNA<br>sequences for the reference genome. This index was then used to quantify transcript abundance in raw counts<br>and c 11 sequences for the reference genome. This index was then used to quantify transcript abundance in raw counts<br>
12 and counts per million (CPM). Differential expression (DE genes, FDR<0.05) was quantitated through DESeq2.<br> 12 and counts per million (CPM). Differential expression (DE genes, FDR<0.05) was quantitated through DESeq2.<br>13 PCA was conducted using ClustVis. Gene ontology pathway enrichment was conducted using the Gene Ontol-<br>15 **Ch** 

PCA was conducted using ClustVis. Gene ontology pathway enrichment was conducted using the Gene Ontol-<br>14 ogy analysis tool.<br>15 **Chromatin immunoprecipitation sequencing**<br>16 Whole livers were cryopowdered using a liquid ni 14 ogy analysis tool.<br>15 **Chromatin immu**<br>16 Whole livers were<br>17 was then fixed in<br>18 Fixation was quer **Chromatin immunoprecipitation sequencing**<br>16 Whole livers were cryopowdered using a liquid<br>17 was then fixed in 10 ml of 1% paraformaldehyd<br>18 Fixation was quenched 1ml of 1.375 M glycine<br>19 for 5 min at room temperature. 16 Whole livers were cryopowdered using a liquid nitrogen-cooled RETSCH CryoMill. The cryopowdered tissue<br>17 was then fixed in 10 ml of 1% paraformaldehyde (PFA) for 30 mins at room temperature with gently nutation.<br>18 Fi 17 was then fixed in 10 ml of 1% paraformaldehyde (PFA) for 30 mins at room temperature with gently nutation.<br>
18 Fixation was quenched 1ml of 1.375 M glycine (Cat # BP381-5, Thermo Fisher Scientific) with gentle nutation<br> 18 Fixation was quenched 1ml of 1.375 M glycine (Cat # BP381-5, Thermo Fisher Scientific) with gentle nutation<br>19 for 5 min at room temperature. After centrifugation at 3000g for 5 mins at 4°C, the pellet was resuspended i for 5 min at room temperature. After centrifugation at 3000g for 5 mins at  $4^{\circ}$ C, the pellet was resuspended in 19 for 5 min at room temperature. After centrifugation at 3000g for 5 mins at  $4^{\circ}$ C, the pellet was resuspended in  $\frac{1}{2}$ 

20 cell lysis buffer as per reported conditions, supplementing the cell lysis buffer with cytochalasin B (3 ug/ml) and<br>
21 rotating for 10 min at 4°C. Nuclei were pelleted at 300g for 10 min at 4°C and subsequently proces rotating for 10 min at 4°C. Nuclei were pelleted at 300g for 10 min at 4° 21 rotating for 10 min at  $4^{\circ}$ C. Nuclei were pelleted at 300g for 10 min at  $4^{\circ}$ C and subsequently processed follow-<br>
22 ing the reported protocol with the adjustment of adding cytochalasin B (3ug/ml) into all solut 22 ing the reported protocol with the adjustment of adding cytochalasin B (3ug/ml) into all solutions for chromatin<br>23 preparation and sonication, antibody incubation, and wash steps. Chromatin was then sonicated for 15 c 23 preparation and sonication, antibody incubation, and wash steps. Chromatin was then sonicated for 15 cycles<br>
24 (30s, high power, 30s pause, and 500 µl volume) in a water bath sonicator set at 4°C (Bioruptor 300.<br>
25 D (30s, high power, 30s pause, and 500 µl volume) in a water bath sonicator set at  $4^{\circ}$ C (Bioruptor 300, 24 (30s, high power, 30s pause, and 500 µl volume) in a water bath sonicator set at  $4^{\circ}$ C (Bioruptor 300.<br>
25 Diagenode, Denville, NJ). After centrifuging at 10,000g for 10 min at  $4^{\circ}$ C, sheared chromatin was checke Diagenode, Denville, NJ). After centrifuging at 10,000g for 10 min at  $4^{\circ}$ C, sheared chromatin was checked on 25 Diagenode, Denville, NJ). After centrifuging at 10,000g for 10 min at  $4^{\circ}$ C, sheared chromatin was checked on<br>26 agarose gel for a shear band comprised between 150 and 600 bp. Two micrograms of chromatin were kept f 26 agarose gel for a shear band comprised between 150 and 600 bp. Two micrograms of chromatin were kept for<br>
27 pooled input controls, whereas 50 ug of chromatin was used for each pull-down reaction in a final volume of<br> 27 pooled input controls, whereas 50 ug of chromatin was used for each pull-down reaction in a final volume of<br>
28 2ml rotating at 4°C overnight. Rabbit polyclonal anti-GR (Cat #A2164, 1:100, ABclonal) was used as a prima 2ml rotating at  $4^{\circ}$ C overnight. Rabbit polyclonal anti-GR (Cat # A2164, 1:100, ABclonal) was used as a primary 28 2ml rotating at 4°C overnight. Rabbit polyclonal anti-GR (Cat # A2164, 1:100, ABclonal) was used as a primary<br>29 antibody. Chromatin complexes were precipitated with 100  $\mu$ l of Sheep Dynabead M-280 (Cat #11204, Therm 29 antibody. Chromatin complexes were precipitated with 100  $\mu$ l of Sheep Dynabead M-280 (Cat #11204, Thermo<br>
20 Fisher). After washing and elution, samples were treated with proteinase K (Cat #19131, Qiagen) at 55°C,<br>
2 Fisher). After washing and elution, samples were treated with proteinase K (Cat #19131, Qiagen) at  $55^{\circ}$ C, Fisher). After washing and elution, samples were treated with proteinase K (Cat #19131, Qiagen) at 55°C,<br>cross-linking was reversed through overnight incubation at 65°C. DNA was purified using a MinElute purifica-<br>ion kit cross-linking was reversed through overnight incubation at  $65^{\circ}$ C. DNA was purified using a MinElute purificacross-linking was reversed through overnight incubation at 65°C. DNA was purified using a MinElute purification kit (Cat #28004, Qiagen) and quantified using Qubit reader and reagents. Library preparation and sequencing we 32 tion kit (Cat #28004, Qiagen) and quantified using Qubit reader and reagents. Library preparation and se-<br>33 quencing were conducted at the NU Genomics Core, using TrueSeq ChIP-seq library prep (with size exclusion)<br>34 33 quencing were conducted at the NU Genomics Core, using TrueSeq ChIP-seq library prep (with size exclusion)<br>34 on 10 ng of chromatin per ChIP sample or pooled inputs and HiSeq 50-bp single-read sequencing (60 million<br>35 34 on 10 ng of chromatin per ChIP sample or pooled inputs and HiSeq 50-bp single-read sequencing (60 million read coverage per sample). Peak analysis was conducted using HOMER software (v4.10) after aligning fastq files to 35 read coverage per sample). Peak analysis was conducted using HOMER software (v4.10) after aligning fastq<br>36 files to the mm10 mouse genome using bowtie2. PCA was conducted using ClustVis. Heatmaps of peak densi-<br>37 ty w 36 files to the mm10 mouse genome using bowtie2. PCA was conducted using ClustVis. Heatmaps of peak densi-<br>37 ty were imaged with TreeView3. Peak tracks were imaged through WashU epigenome browser. Gene ontology<br>38 pathway

17 ty were imaged with TreeView3. Peak tracks were imaged through WashU epigenome browser. Gene ontology<br>
18 pathway enrichment was conducted using the gen ontology analysis tool.<br>
19 **Plasma measurements of total choleste** pathway enrichment was conducted using the gen ontology analysis tool.<br> **Plasma measurements of total cholesterol and total triglycerides**<br>
Blood samples were procured from ~3-month-old mice and collected i<br>
puncture metho **Plasma measurements of total cholesterol and total triglycerides**<br> **Blood samples were procured from ~3-month-old mice and collecte**<br> **puncture method following an overnight fasting. The blood samples v**<br> **centrifugation** 40 Blood samples were procured from ~3-month-old mice and collected in EDTA-treated tubes using cardiac<br>
41 puncture method following an overnight fasting. The blood samples were maintained on ice and subjected to<br>
42 ce quare method following an overnight fasting. The blood samples were maintained on ice and subjected to centrifugation at 2500 x g for 10 mins to isolate plasma. Following the centrifugation step, the obtained plasma was i 12 centrifugation at 2500 x g for 10 mins to isolate plasma. Following the centrifugation step, the obtained plasma<br>13 was immediately transferred into a clean microcentrifuge tube for plasma lipid measurements. The plasm was immediately transferred into a clean microcentrifuge tube for plasma lipid measurements. The plasma lev-<br>els of total cholesterol (TC) and total triglycerides (TG) were measured using Infinity<sup>TM</sup> Cholesterol kit (Cat els of total cholesterol (TC) and total triglycerides (TG) were measured using Infinity<sup>TM</sup> #TR13421, Thermo Fisher Scientific) and Infinity™ Triglyceride kit (Cat # TR22421, Thermo Fisher Scientific).

els of total cholesterol (TC) and total triglycerides (TG) were measured using Infinity<sup>1M</sup> Cholesterol kit (Cat<br>
#TR13421, Thermo Fisher Scientific) and Infinity<sup>TM</sup> Triglyceride kit (Cat #TR22421, Thermo Fisher Scientif #TR13421, Thermo Fisher Scientific) and Infinity<sup>™</sup> Triglyceride kit (Cat # TR22421, Thermo Fisher Scientific).<br> **Lipoprotein analysis**<br>
For lipoprotein separation through FPLC, fresh plasma samples were pooled, totaling 16 **Lipoprotein analysis**<br>
17 For lipoprotein separat<br>
18 least 5 mice per grou<br>
19 rangement of 2 Superc<br>
10 0.5 ml increments, mai For lipoprotein separation through FPLC, fresh plasma samples were pooled, totaling 250 µl, obtained from at<br>least 5 mice per group. Each group's pooled plasma underwent FPLC gel filtration, utilizing a tandem ar-<br>rangemen 18 least 5 mice per group. Each group's pooled plasma underwent FPLC gel filtration, utilizing a tandem arrangement of 2 Superose 6 columns (GE Healthcare). The elution process entailed the collection of fractions in 0.5 m 19 rangement of 2 Superose 6 columns (GE Healthcare). The elution process entailed the collection of fractions in<br>
10.5 ml increments, maintaining a steady flow rate of 0.5 ml/min. This procedure yields a total of fifty-on 50 0.5 ml increments, maintaining a steady flow rate of 0.5 ml/min. This procedure yields a total of fifty-one distinct<br>51 fractions, each of which is subjected to quantification of total triglyceride and cholesterol level 51 fractions, each of which is subjected to quantification of total triglyceride and cholesterol levels using the Infinity<br>52 Triglyceride and Cholesterol kits.<br>53 **Atherosclerotic lesion analysis** 52 Triglyceride and Cholesterol kits.<br>53 Atherosclerotic lesion analysis<br>53

# 53 **Atherosclerotic lesion analysis**

Mice under anesthesia were subjected to a perfusion procedure using a 10% formalin solution in buffered sa-<br>
ine for 5 mins. Following this perfusion, the hearts were carefully dissected to harvest aortic roots. These haris line for 5 mins. Following this perfusion, the hearts were carefully dissected to harvest aortic roots. These har-<br>vested tissues were subsequently preserved in 10% buffered formalin solution. To assess the distribution vested tissues were subsequently preserved in 10% buffered formalin solution. To assess the distribution of atherosclerosis, en face whole aorta lesion staining was performed with Oil Red O for 30 mins, followed by two 1x 57 atherosclerosis, en face whole aorta lesion staining was performed with Oil Red O for 30 mins, followed by two<br>58 1x PBS washes. Furthermore, the aortic root of the heart was embedded in OCT compound for the preparation 1x PBS washes. Furthermore, the aortic root of the heart was embedded in OCT compound for the preparation<br>
19 of frozen sections. Cross cryosections of the aortic roots, measuring 7µm in thickness and encompassing the<br>
19 59 of frozen sections. Cross cryosections of the aortic roots, measuring 7µm in thickness and encompassing the<br>50 aortic valve region, were stained with H&E, Oil Red O and Trichrome staining according to our established pr 60 aortic valve region, were stained with H&E, Oil Red O and Trichrome staining according to our established pro-<br>61 tocols. Images were obtained using a ZEISS Axio Imager.A2 microscope and histological analyses performed<br>

61 tocols. Images were obtained using a ZEISS Axio Imager.A2 microscope and histological analyses performed<br>61 using the ImageJ software (NIH).<br>61 Human iPSC cell line and maintenance<br>61 Human iPSC line 72\_3 with CRISPR kn 92 using the ImageJ software (NIH).<br>
53 Human iPSC cell line and maint<br>
54 Human iPSC line 72\_3 with CRIS<br>
55 homozygous for GR SNP were of<br>
56 maintained in feeder-free condition **Human iPSC cell line and maintenance**<br> **Human iPSC line 72\_3 with CRISPR knoc**<br> **homozygous for GR SNP were obtained f**<br> **maintained in feeder-free conditions using<br>
fied incubator at 37°C, 5% CO<sub>2</sub>. Human iF** 54 Human iPSC line 72\_3 with CRISPR knock-in for R23K in the *Nr3c1* gene locus to generate heterozygous and<br>55 homozygous for GR SNP were obtained from CCHM Pluripotent Stem Cell Facility (PSCF). The hiPSCs were<br>56 mainta 56 homozygous for GR SNP were obtained from CCHM Pluripotent Stem Cell Facility (PSCF). The hiPSCs were maintained in feeder-free conditions using mTeSR1 medium (Cat #85850, Stem Cell Technologies) in a humidified incubato 56 maintained in feeder-free conditions using mTeSR1 medium (Cat #85850, StemCell Technologies) in a humidi-<br>57 fied incubator at 37°C, 5% CO<sub>2</sub>. Human iPSCs were plated on six-well plates pre-coated with Cultrex obtained fied incubator at  $37^{\circ}$ C. 5% CO<sub>2</sub>. Human iPSCs were plated on six-well plates pre-coated with Cultrex obtained Fied incubator at 37°C, 5% CO<sub>2</sub>. Human iPSCs were plated on six-well plates pre-coated with Cultrex obtained<br>from the CCHMC PSCF. The isogenic cell lines were tested and confirmed mycoplasma-free during mainte-<br>nance and 58 from the CCHMC PSCF. The isogenic cell lines were tested and confirmed mycoplasma-free during mainte-<br>nance and before differentiation process. For maintenance of hiPSC, the cells at 70% confluency were pas-<br>saged usin saged using Gentle Cell Dissociation Reagent (GCDR) (Cat #100-0485, StemCell Technologies) into medium<br>clumps. The colonies were resuspended in mTeSR<sup>TM</sup>1 medium with 10  $\mu$ M Y-27632 (PSCF, CCHMC) and pas-<br>saged at split The colonies were resuspended in mTeSR<sup>™M</sup>1 medium with 10 µM Y-27632 (PSCF, CCHMC) and passaged at split ratios ranging from 1:6 to 1:9 as appropriate.<br> **Human iPSC-derived hepatocyte-like cells (HLCs) differentiation i** clumps. The colonies were resuspended in mTeSR $^{\text{\tiny{\textsf{TM}}}}$ 

clumps. The colonies were resuspended in mTeSR<sup>1M</sup>1 medium with 10 µM Y-27632 (PSCF, CCHMC) and passaged at split ratios ranging from 1:6 to 1:9 as appropriate.<br> **Human iPSC-derived hepatocyte-like cells (HLCs) different** saged at split ratios ranging from 1:6 to 1:9 as appropriate.<br> **Human iPSC-derived hepatocyte-like cells (HLCs) differ**<br>
When human iPSCs reached a confluency of approximate<br>
Dissociation Reagent (Cat #07920, StemCell Tech **Human iPSC-derived hepatocyte-like cells (HLCs) differentiation in vitro**<br>
74 When human iPSCs reached a confluency of approximately 95% they were<br>
Dissociation Reagent (Cat #07920, StemCell Technologies) and resuspended When human iPSCs reached a confluency of approximately 95% they were passaged with Accutase $^{\text{\tiny{\textsf{TM}}}}$ 74 When human iPSCs reached a confluency of approximately 95% they were passaged with Accutase<sup>1M</sup> Cell<br>
75 Dissociation Reagent (Cat #07920, StemCell Technologies) and resuspended as single cells in mTesR<sup>TM</sup>1 me-<br>
77 di Dissociation Reagent (Cat #07920, StemCell Technologies) and resuspended as single cells in mTesR<sup>™</sup> Dissociation Reagent (Cat #07920, StemCell Technologies) and resuspended as single cells in mTesR<sup>1M</sup>1 me-<br>
dium with 10 µM Y-27632 (Tocris Bioscience). The cells were seeded in six well plates pre-coated with Cultrex<br>
di dium with 10 µM Y-27632 (Tocris Bioscience). The cells were seeded in six well plates pre-coated with Cultrex<br>diluted in ice-cold DMEM/F12 (Thermo Fisher Scientific). After 24 hours, wash the cells with room temperature<br>DM diluted in ice-cold DMEM/F12 (Thermo Fisher Scientific). After 24 hours, wash the cells with room temperature<br>
DMEM/F12 and switch to RPMI 1640 (Cat #11875093, Thermo Fisher Scientific) with B27 supplement Minus<br>
Insulin ( 78 DMEM/F12 and switch to RPMI 1640 (Cat #11875093, Thermo Fisher Scientific) with B27 supplement Minus<br>
179 Insulin (Cat #A1895601, Thermo Fisher Scientific), along with 100 ng/ml Activin A (Cat #120-14P, Peprotech)<br>
181 Insulin (Cat #A1895601, Thermo Fisher Scientific), along with 100 ng/ml Activin A (Cat #120-14P, Peprotech)<br>
and 3 µM CHIR99021 (Cat #4423, Tocris Bioscience). Following 24-h treatment, CHIR99021 was withdrawn,<br>
and the ce and 3 µM CHIR99021 (Cat #4423, Tocris Bioscience). Following 24-h treatment, CHIR99021 was withdrawn,<br>and the cells were treated with RPMI 1690/B27 Minus Insulin basal medium with 100 ng/ml Activin A for anoth-<br>er 48 hours 81 and the cells were treated with RPMI 1690/B27 Minus Insulin basal medium with 100 ng/ml Activin A for anoth-<br>82 er 48 hours and renewed every day to generate definitive endoderm cells (DE). The differentiated endoderm<br>8 er 48 hours and renewed every day to generate definitive endoderm cells (DE). The differentiated endoderm<br>cells were further treated with RPMI 1640/B27 Minus Insulin along with 10 ng/ml basic fibroblast growth factor<br>(FGF) cells were further treated with RPMI 1640/B27 Minus Insulin along with 10 ng/ml basic fibroblast growth factor (FGF) (Cat 3100-18B, Peprotech) and 20 ng/ml Bone morphogenic factor 4 (BMP4) (Cat #120-05ET, Peprotech). The m 84 (FGF) (Cat 3100-18B, Peprotech) and 20 ng/ml Bone morphogenic factor 4 (BMP4) (Cat #120-05ET,<br>
85 Peprotech). The media was replaced every day for the next 5 days to generate hepatic progenitor (HP) cells.<br>
86 Next, th Beprotech). The media was replaced every day for the next 5 days to generate hepatic progenitor (HP) cells.<br>Next, the hepatic progenitors were further differentiated into immature hepatocytes (IMH) by replacing the me-<br>di Next, the hepatic progenitors were further differentiated into immature hepatocytes (IMH) by replacing the media with RPMI/B27 Minus Insulin, 20 ng/ml hepatocyte growth factor (HGF) (Cat #100-39, Peprotech), and 0.5% DMSO 37 dia with RPMI/B27 Minus Insulin, 20 ng/ml hepatocyte growth factor (HGF) (Cat #100-39, Peprotech), and<br>88 0.5% DMSO. The media was replaced every day for the next 5 days. To promote maturation of immature<br>89 hepatocyt 0.5% DMSO. The media was replaced every day for the next 5 days. To promote maturation of immature<br>By hepatocytes, the media was replaced with HCM<sup>™</sup> Hepatocyte Culture Medium Bulletkit™ (Cat # CC-3198,<br>Repatocytes, the hepatocytes, the media was replaced with HCM<sup>TM</sup> Hepatocyte Culture Medium Bulletkit<sup>TM</sup>  $39$  hepatocytes, the media was replaced with HCM<sup>IM</sup> Hepatocyte Culture Medium Bulletkit<sup>IM</sup> (Cat # CC-3198,

Lonza) except HEGF, 10 ng/ml HGF, 20 ng/ml Oncostatin M (Cat #300-10T, Peprotech), 100 nM Dexame-<br>
thasone (Cat # D2915, Sigma), and 0.5% DMSO for another 5 days with everyday media change.<br>
For the GR translocation assay 91 thasone (Cat # D2915, Sigma), and 0.5% DMSO for another 5 days with everyday media change.<br>
92 For the GR translocation assay and analysis, hiPSCs were exposed to either a vehicle control or<br>
93 methasone for various t For the GR translocation assay and analysis, hiPSCs were exposed to either a vehicle control or 1 μM Dexa-<br>methasone for various time intervals (20 mins, 40 mins, 60 mins, and 120 mins). Subsequently, an immunoflu-<br>oresc methasone for various time intervals (20 mins, 40 mins, 60 mins, and 120 mins). Subsequently, an immunoflu-<br>orescence assay was performed. To evaluate GR translocation in hiPSC-derived mature HLCs, the maturation<br>medium c 94 orescence assay was performed. To evaluate GR translocation in hiPSC-derived mature HLCs, the maturation<br>95 medium containing 100 nM Dexamethasone was removed, and the cells were cultured in hepatocyte mainte-<br>96 matur medium containing 100 nM Dexamethasone was removed, and the cells were cultured in hepatocyte mainte-<br>
hance (HCM) medium without dexamethasone for 24 hours. The following day, mature HLCs were treated with<br>
either a vehi 96 nance (HCM) medium without dexamethasone for 24 hours. The following day, mature HLCs were treated with<br>
97 either a vehicle control or 1 μM Dexamethasone for the aforementioned time intervals. Immunofluorescent<br>
98 st either a vehicle control or 1 μM Dexamethasone for the aforementioned time intervals. Immunofluorescent<br>staining was performed using GR (Cat #sc-393232, 1:200, Santa Cruz) and Alexa Fluor® 488 AffiniPure Don-<br>key Anti-Mou 98 staining was performed using GR (Cat #sc-393232, 1:200, Santa Cruz) and Alexa Fluor® 488 AffiniPure Don-<br>99 key Anti-Mouse IgG (H+L) (Cat #102650-156, 1:300, VWR). The analysis of GR translocation was carried out<br>90 usi

99 key Anti-Mouse IgG (H+L) (Cat #102650-156, 1:300, VWR). The analysis of GR translocation was carried out<br>90 using ImageJ software on 5-6 images per sample acquired from a Nikon Eclipse Ti-U microscope.<br>91 **Human Albumin** using ImageJ software on 5-6 images per sample acquired from a Nikon Eclipse Ti-U microscope.<br>
10 Human Albumin ELISA<br>
12 Cell supernatant containing the cell culture media from mature hiPSC-hepatocytes was collected<br>
13 f 11 **Human Albumin ELISA**<br>
12 **Cell supernatant containi**<br>
13 **fuged at 2000 x g for 10**<br>
14 **provided in the kit (Cat #**<br>
15 **lsolation of Primary mo** Olell supernatant containing the cell culture media from mature hiPSC-hepatocytes was collected and centri-<br>
13 fuged at 2000 x g for 10 mins to remove debris. Centrifuged samples were diluted 1:5 in Sample Diluent NS<br>
14

fuged at 2000 x g for 10 mins to remove debris. Centrifuged samples were diluted 1:5 in Sample Diluent NS<br>provided in the kit (Cat # ab179887, Abcam) and assayed according to the manufacturer's instructions.<br>**Isolation of** provided in the kit (Cat # ab179887, Abcam) and assayed according to the manufacturer's instructions.<br> **Isolation of Primary mouse hepatocytes**<br>
Primary hepatocytes were isolated from GR<sup>ref/ref</sup> (control), GR<sup>ref/ALT</sup> (h **Isolation of Primary mouse hepatocytes**<br>
06 Primary hepatocytes were isolated from GP<br>
17 genase perfusion method. The mice were<br>
18 a 24-gauge needle. HBSS – (Cat #1417;<br>
19 AM9260G, Thermo Fisher Scientific) was p Primary hepatocytes were isolated from GR<sup>ref/ref</sup> (control), GR<sup>ref/ALT</sup> (het), and GR<sup>ALT/ALT</sup> Firmary hepatocytes were isolated from  $GR^{ref/ReLU}$  (control),  $GR^{ref/ALL}$  (het), and  $GR^{ALL/AL}$  (homo) mice with colla-<br>genase perfusion method. The mice were anesthetized, and the inferior vena cava (IVC) was cannulated with<br>a 2 97 genase perfusion method. The mice were anesthetized, and the inferior vena cava (IVC) was cannulated with<br>
27 a 24-gauge needle. HBSS – (Cat #14175095, Thermo Fisher Scientific) containing 0.5 mM EDTA (Cat #<br>
29 AM9260 a 24-gauge needle. HBSS – (Cat #14175095, Thermo Fisher Scientific) containing 0.5 mM EDTA (Cat #<br>
AM9260G, Thermo Fisher Scientific) was perfused to chelate calcium. Next, HBSS + (Cat #14025092, Thermo<br>
Fisher Scientific) AM9260G, Thermo Fisher Scientific) was perfused to chelate calcium. Next, HBSS + (Cat #14025092, Thermo<br>
Fisher Scientific) containing 0.3 mg/ml collagenase X (Cat #035-17861, FUJUFILM Wako Chemicals) was per-<br>
tused to di 10 Fisher Scientific) containing 0.3 mg/ml collagenase X (Cat #035-17861, FUJUFILM Wako Chemicals) was per-<br>11 fused to dissociate extracellular matrix of the liver. After the liver dissection, cells were filtered with 100 11 fused to dissociate extracellular matrix of the liver. After the liver dissection, cells were filtered with 100 µm<br>12 mesh cell strainer (Cat #08-771-19, Fisher Scientific), and the hepatocytes were purified by 40% Perc 12 mesh cell strainer (Cat #08-771-19, Fisher Scientific), and the hepatocytes were purified by 40% Percoll (Cat #<br>
13 P1644, Sigma) gradient centrifugation method. Hepatocytes were suspended in William's E medium (Cat<br>
14 13 P1644, Sigma) gradient centrifugation method. Hepatocytes were suspended in William's E medium (Cat #12551032, Thermo Fisher Scientific) supplemented with 10% FBS (Cat # S11150, R&D systems) and 1x Anti-<br>15 Anti (Cat #1 14 #12551032, Thermo Fisher Scientific) supplemented with 10% FBS (Cat # S11150, R&D systems) and 1x Anti-<br>15 Anti (Cat #15240062, Thermo Fisher Scientific) for overnight and then replaced the next day with fresh medi-<br>17

15 Anti (Cat #15240062, Thermo Fisher Scientific) for overnight and then replaced the next day with fresh medi-<br>16 um.<br>**IT Immunostaining and image analysis**<br>18 Cells plated on cultrex-coated dishes containing sterile cove 16 um.<br>17 **Imm**<br>18 Cells<br>19 fixed<br>20 wasl I7 **Immunostaining and image analysis**<br>18 Cells plated on cultrex-coated dishes c<br>19 fixed with Fixation solution (2% formal<br>20 washed 3 times with 1x DPBS and trea<br>21 for 30 mins and then at room temperat 18 Cells plated on cultrex-coated dishes containing sterile cover glasses were washed gently with 1x DPBS and fixed with Fixation solution (2% formaldehyde in 1x PBS) for 15 mins at room temperature. The cells were washed 19 fixed with Fixation solution (2% formaldehyde in 1x PBS) for 15 mins at room temperature. The cells were<br>
20 washed 3 times with 1x DPBS and treated with permeabilization reagent (1% triton X-100 in 1x DPBS) at  $37^{\circ}$ washed 3 times with 1x DPBS and treated with permeabilization reagent (1% triton X-100 in 1x DPBS) at  $37^{\circ}$ C washed 3 times with 1x DPBS and treated with permeabilization reagent (1% triton X-100 in 1x DPBS) at  $37^{\circ}$ C<br>
21 for 30 mins and then at room temperature for 10 mins. Next, the cells were blocked with blocking buffer ( 21 for 30 mins and then at room temperature for 10 mins. Next, the cells were blocked with blocking buffer (10%<br>22 normal donkey serum in 1x DPBS) for 1 hour at room temperature and stained with primary antibodies: Nanog<br>2 22 normal donkey serum in 1x DPBS) for 1 hour at room temperature and stained with primary antibodies: Nanog<br>23 (Cat #D73G4, 1:200, Cell Signaling), OCT4 (Cat #A7920, 1:200, ABclonal), and Albumin (Cat #A1363, 1:200,<br>24 AB 23 (Cat #D73G4, 1:200, Cell Signaling), OCT4 (Cat #A7920, 1:200, ABclonal), and Albumin (Cat #A1363, 1:200, <br>24 ABclonal) diluted in 10% Donkey serum in 1x DPBS overnight. Next day, the cells were washed twice with 1x<br>4. A 24 ABclonal) diluted in 10% Donkey serum in 1x DPBS overnight. Next day, the cells were washed twice with 1x

25 DPBS and stained with secondary antibodies: Alexa Fluor® 594 AffiniPure Donkey Anti-Rabbit IgG (H+L) (Cat<br>
26 #102649-732, 1:300, VWR), and Alexa Fluor® 488 AffiniPure Donkey Anti-Rabbit IgG (H+L) (Cat #102649-726,<br>
27 26 #102649-732, 1:300, VWR), and Alexa Fluor® 488 AffiniPure Donkey Anti-Rabbit IgG (H+L) (Cat #102649-726,<br>27 1:300, VWR) diluted in 10% Donkey serum in 1x DPBS for 1 hour at room temperature. Cells were washed<br>28 three t 1:300, VWR) diluted in 10% Donkey serum in 1x DPBS for 1 hour at room temperature. Cells were washed<br>
three times in 1x DPBS. The coverslips were mounted on slides and imaged with Nikon Eclipse Ti – U micro-<br>
scope.<br> **Flu** 

28 three times in 1x DPBS. The coverslips were mounted on slides and imaged with Nikon Eclipse Ti – U micro-<br>29 scope.<br>**29 Fluorometric HDL and LDL uptake assay and quantitation**<br>21 Plate 3-4x10<sup>4</sup> cells/ well in a 96-wel 29 scope.<br>30 **Fluoro**<br>31 Plate 3<br>32  $37^{\circ}$ C in<br>33 (Cat #a Fluorometric HDL and LDL uptake assay and quantitation<br>
<sup>31</sup> Plate 3-4x10<sup>4</sup> cells/ well in a 96-well white clear-bottom cell<br>
<sup>37°</sup>C incubator. Next day, wash the cells with Assay buffer prov<br>
(Cat #ab204717, Abcam) and L Plate  $3-4\times10^4$ 31 Plate 3-4x10<sup>4</sup> cells/ well in a 96-well white clear-bottom cell culture plates and culture in media overnight at<br>32 37°C incubator. Next day, wash the cells with Assay buffer provided in this appropriate kit. For fluor 37°C incubator. Next day, wash the cells with Assay buffer provided in this appropriate kit. For fluorometric HDL 32 37°C incubator. Next day, wash the cells with Assay buffer provided in this appropriate kit. For fluorometric HDL<br>
33 (Cat #ab204717, Abcam) and LDL (Cat #770230-9, Kalen Biomedical) staining and quantitation, follow th 33 (Cat #ab204717, Abcam) and LDL (Cat #770230-9, Kalen Biomedical) staining and quantitation, follow the in-<br>34 structions according to the manufacturer. Protect from light and measure the fluorescence in a microplate<br>35

34 structions according to the manufacturer. Protect from light and measure the fluorescence in a microplate<br>35 reader.<br>36 **UK Biobank and All of Us analyses**<br>37 Our analyses were conducted under the UKB application number 35 reader.<br>36 **UK Bio**<br>37 Our ana<br>38 rw-0fb5.<br>39 sent. Al 36 **UK Biobank and All of Us analyses**<br>37 Our analyses were conducted under the<br>38 rw-0fb52975. We constructed a rs619<br>39 sent. All available values for the teste<br>40 and related parameters: Age: 21001 37 Our analyses were conducted under the UKB application number 65846 and All of Us workspace number aou-<br>
38 W-0fb52975. We constructed a rs6190 genotype-stratified cohort, excluding participants if they withdrew con-<br>
39 38 rw-0fb52975. We constructed a rs6190 genotype-stratified cohort, excluding participants if they withdrew con-<br>39 sent. All available values for the tested parameters were collected per genotype group. For UK Biobank, UD 39 sent. All available values for the tested parameters were collected per genotype group. For UK Biobank, UDI<br>30870-0.0; Total Cholesterol: 23400; ICD10 causes of death, primary 40001, secondary 40002. For initial dis-<br>30 and related parameters: Age: 21001-0.0; BMI: 21001-0.0; Glycemia (mM): 30740-0.0; Triglycerides (mM): 30870-0.0; Total Cholesterol: 23400; ICD10 causes of death, primary 40001, secondary 40002. For initial dis-<br>covery usin 30870-0.0; Total Cholesterol: 23400; ICD10 causes of death, primary 40001, secondary 40002. For initial dis-<br>
covery using the NMR metabolomics datasets, quantitative linear regression and conditional analyses were<br>
perfor 42 covery using the NMR metabolomics datasets, quantitative linear regression and conditional analyses were<br>performed using an additive genetic model adjusting for 10 PCs, sex; and age. In conditional analyses, the 12<br>esta performed using an additive genetic model adjusting for 10 PCs, sex; and age. In conditional analyses, the 12<br>established SNP dosage effects were also included as additional covariates. Regression analyses were per-<br>formed 44 established SNP dosage effects were also included as additional covariates. Regression analyses were per-<br>45 formed using second generation of PLINK (56). Before analyses, a series of standard QC measures were ap-<br>46 p 45 formed using second generation of PLINK (56). Before analyses, a series of standard QC measures were applied including sample call rates, sample relatedness, and sex inconsistency as well as marker quality (i.e., marke plied including sample call rates, sample relatedness, and sex inconsistency as well as marker quality (i.e., marker call rate, minor allele frequency (MAF), and Hardy-Weinberg equilibrium (HWE). Analyses were limited to 17 marker call rate, minor allele frequency (MAF), and Hardy-Weinberg equilibrium (HWE). Analyses were limited<br>18 to participants with call rates > $\square$ 98%, SNPs with call rates > $\square$ 99%, and SNPs with MAF > $\square$ 1% and HWE<br> to participants with call rates > $\square$ 98%, SNPs with call rates > $\square$ 99%, and SNPs with MAF > $\square$ 1% and HWE<br>
p $\square$ > $\square$ 0.0001. For independent association confirmation studies, multiple linear regression analysis was carri 9 p□>□0.0001. For independent association confirmation studies, multiple linear regression analysis was carried<br>30 out using R 4.3.2 (R Core Team, 2023) to explore the association of total cholesterol, clinical LDL, and H

out using R 4.3.2 (R Core Team, 2023) to explore the association of total cholesterol, clinical LDL, and HDL<br>
cholesterol versus separate sex (males/females) and correcting for BMI, glycemia, and triglycerides.<br> **Statistic** cholesterol versus separate sex (males/females) and correcting for BMI, glycemia, and triglycerides.<br> **Statistics**<br>
Unless differently noted, statistical analyses were performed using Prism software v8.4.1 (GraphPac<br>
CA). 52 **Statistics**<br>53 **Unless diff**<br>54 **CA). The F**<br>55 the two group<br>56 paring thre Unless differently noted, statistical analyses were performed using Prism software v8.4.1 (GraphPad, La Jolla, CA). The Pearson-D'Agostino normality test was used to assess data distribution normality. When comparing the t 54 CA). The Pearson-D'Agostino normality test was used to assess data distribution normality. When comparing<br>55 the two groups, a two-tailed Student's t-test with Welch's correction (unequal variances) was used. When com-<br> 55 the two groups, a two-tailed Student's t-test with Welch's correction (unequal variances) was used. When com-<br>56 paring three groups of data from one variable, one-way ANOVA with Sidak multi-comparison was used. When<br>57 56 paring three groups of data from one variable, one-way ANOVA with Sidak multi-comparison was used. When<br>57 comparing data groups for more than one related variable, two-way ANOVA was used. For ANOVA and t-test<br>58 analys 57 comparing data groups for more than one related variable, two-way ANOVA was used. For ANOVA and t-test<br>
58 analyses, a P value less than 0.05 was considered significant. When the number of data points was less than<br>
59 58 analyses, a P value less than 0.05 was considered significant. When the number of data points was less than<br>59 10, data were presented as single values (dot plots, histograms). Tukey distribution bars were used to empha 59 10, data were presented as single values (dot plots, histograms). Tukey distribution bars were used to empha-

- 
- 
- Size data range distribution in analyses pooling larger data points sets per group (typically > 10 data points).<br>
Analyses pooling data points over time were presented as line plots connecting medians of box plots showing<br> Analyses pooling data points over time were presented as line plots connecting medians of box plots showing<br>distribution of all data per time points. Randomization and blinding practices are followed for all experiments.<br>A
- 

distribution of all data per time points. Randomization and blinding practices are followed for all experiments.<br>
All the data from all animal cohorts and cell clone replicates is reported, whether outlier or not.<br> **Study** Fig. 23 All the data from all animal cohorts and cell clone replicates is reported, whether outlier or not.<br>
54 **Study approval**<br>
55 Mice were housed in a pathogen-free facility in accordance with the American Veterinary M 54 **Study approval**<br>55 Mice were house<br>56 (AVMA) and und<br>57 Cincinnati Childr<br>58 conducted under Mice were housed in a pathogen-free facility in accordance with the American Veterinary Medical Association<br>
(AVMA) and under protocols fully approved by the Institutional Animal Care and Use Committee (IACUC) at<br>
Cincinna

- 
- 66 (AVMA) and under protocols fully approved by the Institutional Animal Care and Use Committee (IACUC) at<br>67 Cincinnati Children's Hospital Medical Center (#2022-0020, #2023-0002). UKB and All of Us analyses were<br>65846 an 67 Cincinnati Children's Hospital Medical Center (#2022-0020, #2023-0002). UKB and All of Us analyses were<br>678 conducted under the UKB application number 65846 and All of Us workspace number aou-rw-0fb52975.<br>**Data availabi**
- 

- 68 conducted under the UKB application number 65846 and All of Us workspace number aou-rw-0fb52975.<br> **Data availability**<br>
RNA-seq and ChIP-seq datasets reported here are available on GEO as GSE280494 and GSE280572<br>
vidual 70 RNA-seq and ChIP-seq datasets reported here are available on GEO as GSE280494 and GSE280572. Indi-<br>71 vidual data for all charts presented here is available in the Supporting Data Values file.<br>71 vidual data for all cha
- 59 **Data availability**<br>
70 RNA-seq and Ch<br>
71 vidual data for all 71 vidual data for all charts presented here is available in the Supporting Data Values file.

- 
- 
- 
- Author contributions –HBD, AH, GN, ADP, KMF, HL, OA, BNK: Data curation, Formal analysis, Investigation;<br>AJ, DYH: Resources; MQ: Conceptualization, Formal analysis, Funding acquisition, Supervision.<br>**Acknowledgements -** Ne AJ, DYH: Resources; MQ: Conceptualization, Formal analysis, Funding acquisition, Supervision.<br>
74<br> **Acknowledgements -** Next-gen sequencing was performed thanks to the Cincinnati Children's I<br>
76 ing and Genotyping Facilit 75<br>76<br>77<br>78<br>79 75 **Acknowledgements -** Next-gen sequencing was performed thanks to the Cincinnati Children's DNA Sequencing and Genotyping Facility (RRID: SCR\_022630), with critical assistance by David Fletcher, Keely Icardi, Julia Flynn ing and Genotyping Facility (RRID: SCR\_022630), with critical assistance by David Fletcher, Keely Icardi, Julia<br>
Flynn, and Taliesin Lenhart. hiPSC generation, engineering and initial quality control/selection were perform Flynn, and Taliesin Lenhart. hiPSC generation, engineering and initial quality control/selection were performed<br>
thanks to the Cincinnati Children's Pluripotent Stem Cell Facility (RRID: SCR\_022634), with critical assistan The Cincinnati Children's Pluripotent Stem Cell Facility (RRID: SCR\_022634), with critical assistance<br>
29 by Chris Mayhew and Yueh-Chiang Hu. pAAV Alb-AAT KRAB-SadCas9 U6-mPcsk9 was a gift from Tonia Rex<br>
29 (Addgene plasm
- 19 by Chris Mayhew and Yueh-Chiang Hu. pAAV Alb-AAT KRAB-SadCas9 U6-mPcsk9 was a gift from Tonia Rex<br>
19 (Addgene plasmid # 163025 ; http://n2t.net/addgene:163025 ; RRID:Addgene\_163025).<br>
19 Grant support This work was s 80 (Addgene plasmid # 163025 ; http://n2t.net/addgene:163025 ; RRID:Addgene\_163025).<br>81 **Grant support** – This work was supported by R56HL158531-01, R01HL166356-01,<br>83 R01AG078174-01 (NIH) and RIP, GAP, CCRF Endowed Schola 32<br>33<br>34<br>35 82 **Grant support** – This work was supported by R56HL158531-01, R01HL166356-01, R03DK130908-01A1,<br>R01AG078174-01 (NIH) and RIP, GAP, CCRF Endowed Scholarship, HI Translational Funds (CCHMC) grants<br>to MQ; NIH grant RO1HL156 83 R01AG078174-01 (NIH) and RIP, GAP, CCRF Endowed Scholarship, HI Translational Funds (CCHMC) grants<br>84 to MQ; NIH grant RO1HL156954 to DYH.<br>85 84 to MQ; NIH grant RO1HL156954 to DYH.<br>85<br>86
	-

86<br>1<br>3<br>6<br>1<br>3<br>3<br>3<br>3<br>1<br>1<br>1<br>1<br>1<br>1<br>1<br>1<br>1<br>12.32<br/>33.33<br/>34.33<br/>35.33<br/>36.35<br/>37.35<br/>38.35<br/>39.35<br/>39.35<br/>30.35<br/>30.35<br/>30.35<br/>30.35<br/>30.35<br/>30.35<br/>30.35<br/>30.35<br/>30.35<br/>30.35<br/>30.35<br/>30.35<br/>30.35<br/>30.35<br/>30.35<br/>30.35<br/>30.35<br/>30

- 87 **References**<br>
88 1. Zamc<br>
89 hyper<br>
90 2023<br>
91 2. Iyen I<br>
92 with<br>
93 natio
- 88 1. Experimentated and poorly controlled compared to men. Sci Rep.<br>
88 1. 2023;13(1):1492.<br>
88 1. 2023;13(1):1492.<br>
98 1. 2023;13(1):1492.<br>
98 1. 2023;13(1):1492.<br>
98 1. 2021;13(1):1492.<br>
98 1. 2021;13(1):1492.<br>
98 1. 20 89 hypercholesterolemia phenotype are andertreated and poorly controlled compared to men. Scritep.<br>
89 appears 2023;13(1):1492.<br>
89 by the B, Qureshi N, Weng S, Roderick P, Kai J, Capps N, et al. Sex differences in cardiov 91 2. Iyen B, Qureshi N,<br>
92 with familial hype<br>
93 national hospital r<br>
94 3. de Guia RM, Rose<br>
95 *Investig*. 2014;19(<br>
96 4. Schaaf MJ, and Cit<br>
97 *Mol Biol.* 2002;83 92 2. It is millial hypercholesterolaemia: A retrospective cohort study of the UK Simon Broome register linked to<br>
93 3. Interval B, Rose AJ, and Herzig S. Glucocorticoid hormones and energy homeostasis. Horm Mol Biol Clin
- 
- 93 mational hospital records. Atherosclerosis. 2020;315:131-7.<br>
92 mational hospital records. Atherosclerosis. 2020;315:131-7.<br>
92 municipal metalsterolaemia: A retrospective condomnations and energy homeostasis. Horm Mol
- 93 national hospital records. Atherosclerosis. 2020;315:131-7. 94 9. de Guia RM, Rose AJ, and Herzig S. Glucocorticoid hormones and energy homeostasis. Horm Mol Biol Clin<br>
95 Investig. 2014;19(2):117-28.<br>
97 Mol Biol. 2002;83(1-5):37-48.<br>
98 5. Lim HW, Uhlenhaut NH, Rauch A, Weiner J, 95 Myssing. 2014;19(2):117-28.<br>
96 4. Schaaf MJ, and Cidlowski JA.<br>
97 Mol Biol. 2002;83(1-5):37-48<br>
99 and dimers mediates transcr<br>
90 44.<br>
91 6. Oakley RH, and Cidlowski JA.<br>
22 disease. *J Allergy Clin Immun* 96 4. Schola Mol, and Cidlowski JA. Molecular mechanisms of glucocorticoid action and resistance. J Steroid Biochem<br>
96 5. Lim HW, Uhlenhaut NH, Rauch A, Weiner J, Hubner S, Hubner N, et al. Genomic redistribution of GR mo Morbiol. 2002;83(1-5):37-48.<br>
97 Morbiol. 2002;83(1-5):37-48.<br>
99 and dimers mediates transcrip<br>
90 44.<br>
92 disease. *J Allergy Clin Immuno*<br>
93 7. Watts LM, Manchem VP, Leed<br>
94 glucocorticoid receptor expres
- 
- 99 and dimers mediates transcriptional response to exogenous glucocorticoid in vivo. *Genome Res.* 2015;25(6):836<br>
98 and dimers mediates transcriptional response to exogenous glucocorticoid in vivo. *Genome Res.* 2015;25( 99 and dimers mediates transcriptional response to exogenous glucocorticoid in vivo. Genome Res. 2015,25(6):836-<br>
99 44.<br>
99 disease. *J Allergy Clin Immunol.* 2013;132(5):1033-44.<br>
99 disease. *J Allergy Clin Immunol.* 20 00 44. disease. *J Allergy Clin Immunol.* 2013;132(5):1033-44.<br>
2013;132(5):1033-44.<br>
2014 Blucocorticoid receptor expression with antisense oligonucleotides improves hyperglycemia and hyperlipidemia<br>
2015 in diabetic rodents wit 32 disease. J Allergy Clin Immunol. 2013,132(3):1033-44.<br>
2013,132(3):1033-44.<br>
2013,132(3):1033-44.<br>
2013,132(3):1033-44.<br>
2013,132(3):1033-44.<br>
2013,132(3):1033-44.<br>
2013,132(3):1033-44.<br>
2013,132(3):1033-44.<br>
2013,132(3 93. Manchem Varian States Atterioscher States (igonucleotides improves hyperglycemia and hyperlipidemia<br>
193. Petrichenko IE, Daret D, Kolpakova GV, Shakhov YA, and Larrue J. Glucocorticoids stimulate cholesteryl ester<br>
19
- 8. Petrichenko IE, Daret D, Kolpakova GV, Shakhov YA, and Larrue J. Glucocorticoids stimulate cholesteryl e<br>17 formation in human smooth muscle cells. *Arterioscler Thromb Vasc Biol.* 1997;17(6):1143-51.<br>18 9. Nashel DJ. I
- 
- 04 glucocorticoid antagonism. *Diabetes.* 2005;54(6):1846-53.<br>
04 Glucocorticoids stimulate cholesteryl ester<br>
17 formation in human smooth muscle cells. Arterioscler Thromb Vasc Biol. 1997;17(6):1143-51.<br>
18 9. Nashel DJ.
- 06 9. Petrichenko Innes in human smooth muscle cells. Arterioscler Thromb Vasc Biol. 1997;17(6):1143-51.<br>
06 9. Mashel DJ. Is atherosclerosis a complication of long-term corticosteroid treatment? Am J Med. 1986;80(5):92<br>
1 07 Formation in human smooth muscle cells. Arterioscler Thromb Vasc Biol. 1997;17(0):1143-51.<br>
10. MacLeod C, Hadoke PWF, and Nixon M. Glucocorticoids: Fuelling the Fire of Atherosclerosis or<br>
10. MacLeod C, Hadoke PWF, an 08 9. Nasher DJ. Is atherosclerosis a complication of long-term corticosteroid treatment? Am J Med. 1986;80(5):925-9.<br>
10. MacLeod C, Hadoke PWF, and Nixon M. Glucocorticoids: Fuelling the Fire of Atherosclerosis or Therap Extinguishers? *Int J Mol Sci.* 2021;22(14).<br>
11. Pujades-Rodriguez M, Morgan AW, Cubbon RM, and Wu J. Dose-dependent oral glucocorticoid cardiovascu<br>
12. Trusca VG, Fuior EV, Fenyo IM, Kardassis D, Simionescu M, and Gafen Extinguishers? *Int J Mor Sci. 2021*, 22(14).<br>
11 11. Pujades-Rodriguez M, Morgan AW, Cubbc<br>
13 2020;17(12):e1003432.<br>
14 12. Trusca VG, Fuior EV, Fenyo IM, Kardassis L<br>
15 on apolipoprotein E gene expression in m<br>
16 13.
- 
- 11. Putation 11. Putation 11. Published inflammatory diseases: A population-based cohort study. PLoS Med.<br>
11. Pusca VG, Fujor EV, Fenyo IM, Kardassis D, Simionescu M, and Gafencu AV. Differential action of glucocorticoid<br> 13 a consider the minimum of the minimum of the states. A population-based cohort study. P Los Med.<br>
12 a Trusca VG, Fuior EV, Fenyo IM, Kardassis D, Simionescu M, and Gafencu AV. Differential action of glucocortice<br>
13 a 14 12. Trusca VG, Fuior EV, Fen<br>
15 on apolipoprotein E gen<br>
16 13. Ayaori M, Sawada S, Yon<br>
17 binding cassette transpo<br>
18 *Arterioscler Thromb Vas*<br>
19 14. Cavenee WK, Johnston I<br>
20 lipoproteins: dexametha 14 11 12. Trusca VG, Fuita Capacity is an applipable and hepatocytes. PloS one. 2017;12(3):e0174078.<br>
14 13. Ayaori M, Sawada S, Yonemura A, Iwamoto N, Ogura M, Tanaka N, et al. Glucocorticoid receptor regulates ATP-<br>
17 1 15 on apolipoprotein Eigene expression in macrophages and nepatocytes. Phosione. 2017;12(3):e0174078.<br>
16 13. Ayaori M, Sawada S, Yonemura A, Iwamoto N, Ogura M, Tanaka N, et al. Glucocorticoid receptor regulate<br>
17 bindin
- 18 Arterioscler Thromb Vasc Biol. 2006;26(1):163-8.<br>
19 14. Cavenee WK, Johnston D, and Melnykovych G. Regulation of cholesterol biosynthesis in HeLa S3G cells by serum<br>
19 14. Cavenee WK, Johnston D, and Melnykovych G. Re Friendscher Thromb Vasc Biol. 2000,20(1):163-6.<br>
19 14. Cavenee WK, Johnston D, and Melnykovych G. Re<br>
20 lipoproteins: dexamethasone-mediated interfere<br>
21 reductase. *Proc Natl Acad Sci U S A*. 1978;75(5):2<br>
22 15. van R 19 14. Cavanthasone-mediated interference with suppression of 3-hydroxy-3-methylglutaryl coenzyme A<br>
19 14. Cape Reductase. *Proc Natl Acad Sci U S A.* 1978;75(5):2103-7.<br>
19 15. The Regulation D, Recent Prog Horm Res. 200
- 16 13. Ayanori M, Sawada S, Yonemura A, Iwamoto N, Sawada S, 2004;59:333-57. 21 reductase. *Proc Natl Acad Sci U S A*. 1978;75(5):2103-7.<br>
22 15. van Rossum EF, and Lamberts SW. Polymorphisms in the glucocorticoid receptor gene and their associations with<br>
23 metabolic parameters and body compositi
- 
- 22 15. van Rossum EF, and Lamberts SW. Polymorphisms in the<br>
22 15. van Rossum EF, and Lamberts SW. Polymorphisms in the<br>
23 metabolic parameters and body composition. *Recent Pre*<br>
24 16. Yudt MR, and Cidlowski JA. The gl 22 15. van Rossum EF, Koper JW, Huizenga NA, Uitterlinden AG, Janssen JA, Brinkmann AO, et al. A polymorphism in the glucocorticoid receptor gene. Mol Endocrinol. 2002;16(8):1719-26.<br>25 17. van Rossum EF, Koper JW, Huizeng 24 16. Yudt MR, and Cidlowski JA. The glucocorticoid receptor: coding a diversity of proteins a<br>
25 single gene. *Mol Endocrinol.* 2002;16(8):1719-26.<br>
26 17. van Rossum EF, Koper JW, Huizenga NA, Uitterlinden AG, Janssen 25 single gene. *Mol Endocrinol.* 2002;16(8):1719-26.<br>
26 17. van Rossum EF, Koper JW, Huizenga NA, Uitterlinden AG, Janssen JA, Brinkmann AO, et al. A polymorphism in th<br>
27 glucocorticoid receptor gene, which decreases s 25 single gene. Mor Endocrmol. 2002;10(0):1719-20.<br>
26 17. van Rossum EF, Koper JW, Huizenga NA, Uitterlind<br>
27 glucocorticoid receptor gene, which decreases ser<br>
28 and cholesterol levels. Diabetes. 2002;51(10):312:<br>
29 1
- 
- 27 glucocorticoid receptor gene, which decreases sensitivity to glucocorticoids in vivo, is associated with low insulin<br>
28 and cholesterol levels. *Diabetes*. 2002;51(10):3128-34.<br>
29 18. Kino T. Single Nucleotide Variati 28 and cholesterol levels. Diabetes. 2002;51(10):3128-34.<br>
29 18. Kino T. Single Nucleotide Variations of the Human GR Gene Manifested as Pathologic Mutations or<br>
29 Polymorphisms. *Endocrinology.* 2018;159(7):2506-19.<br>
21 29 18. Kino T. Single Nucleotide Variations of the Human GR G<br>
29 18. Kino T. Single Nucleotide Variations of the Human GR G<br>
20 Polymorphisms. *Endocrinology*. 2018;159(7):2506-19.<br>
22 ER22/23EK polymorphism in the glucoc 21 19. Kino Many Schoology, 2018;159(7):2506-19.<br>
29 Polymorphisms. *Endocrinology*. 2018;159(7):2506-19.<br>
29 ER22/23EK polymorphism in the glucocorticoid receptor gene with survival and C-reactive protein leaderly men. *A* 30 Polymorphisms. Endocrinology. 2018;159(7):2506-19.
- 
- ER22/23EK polymorphism in the glucocorticoid receptor gene with survival and C-reactive protein levels in<br>
19. value of the survival AM, Vang V, Zhu K, Guan D, et al. Individual-specific functional epigenomics reveals gene ER22/23EK polymorphism in the glucocorticoid receptor gene with survival and C-reactive protein levels in<br>
133 elderly men. Am J Med. 2004;117(3):158-62.<br>
20. Hu W, Jiang C, Kim M, Yang W, Zhu K, Guan D, et al. Individual-33 elderly men. Am J Med. 2004;117(3):158-62.<br>34 20. Hu W, Jiang C, Kim M, Yang W, Zhu K, Guan D,<br>35 determinants of adverse metabolic effects of<br>36 21. Koper JW, Stolk RP, de Lange P, Huizenga NA, I<br>37 polymorphisms in th 35 determinants of adverse metabolic effects of glucocorticoids. *Cell Metab.* 2021;33(8):1592-609 e7.<br>36 21. Koper JW, Stolk RP, de Lange P, Huizenga NA, Molijn GJ, Pols HA, et al. Lack of association between five<br>37 poly 35 determinants of adverse metabolic effects of glucocorticoids. Cell Metab. 2021,33(8):1592-609 e7.<br>36 21. Koper JW, Stolk RP, de Lange P, Huizenga NA, Molijn GJ, Pols HA, et al. Lack of association between f<br>37 polymorph 37 polymorphisms in the human glucocorticoid receptor gene and glucocorticoid resistance. Hum Genet.<br>38 1997;99(5):663-8. 38 polymorphisms in the human glucocorticoid receptor gene and glucocorticoid resistance. Hum Genet.<br>38 1997;99(5):663-8. 38 1997;99<br>38 1997;99<br>(593-8.97):

- 
- 
- 39 13 23. Trapani L, Segatto M, and Pallottini V. Regulation and deregulation of cholesterol homeostasis: The liver<br>
39 13 24. Russo P, Tomino C, Santoro A, Prinzi G, Proietti S, Kisialiou A, et al. FKBP5 rs4713916: A Pote Added Strapen in a material in adults. NPJ Genom Med. 2021,0(1):50.<br>
11 23. Trapani L, Segatto M, and Pallottini V. Regulation and deregulation<br>
13 24. Russo P, Tomino C, Santoro A, Prinzi G, Proietti S, Kisialiou A, et a<br> metabolic "power station". World J Hepatol. 2012;4(6):184-90.<br>
24. Russo P, Tomino C, Santoro A, Prinzi G, Proietti S, Kisialiou A, et al. FKBP5 rs4713916: A Potential Genetic<br>
24. Predictor of Interindividual Different Re 13 24. Russo P, Tomino C, Santoro A, Prinzi G, Proietti S, Kisialiou A, et<br>14 Predictor of Interindividual Different Response to Inhaled Cortic<br>15 Pulmonary Disease in a Real-Life Setting. *Int J Mol Sci.* 2019;20(<br>16 25.
- Frank Predictor of Interindividual Different Response to Inhaled Corticosteroids in Patients with Chronic Obstrune Pulmonary Disease in a Real-Life Setting. *Int J Mol Sci.* 2019;20(8).<br>
25. Zannas AS, Wiechmann T, Gassen 24 Pulmonary Disease in a Real-Life Setting. *Int J Mol Sci.* 2019;20(8).<br>
25. Zannas AS, Wiechmann T, Gassen NC, and Binder EB. Gene-Stress-Epigenetic Regulation of FKBP5: Clinical and<br>
26. Hollstein T, Vogt A, Grenkowitz
- 
- Fulmonary Disease in a Real-Life Setting. *Int J Worl Sci. 2013*,20(6).<br>
46 25. Zannas AS, Wiechmann T, Gassen NC, and Binder EB. Gene-Stress-<br>
17 Translational Implications. *Neuropsychopharmacology*. 2016;41(1)<br>
48 26. H Translational Implications. *Neuropsychopharmacology*. 2016;41(1):261-74.<br>
18 26. Hollstein T, Vogt A, Grenkowitz T, Stojakovic T, März W, Laufs U, et al. Treatment with PCSK9 inhibitors reduces<br>
19 anners in a real-world 47 Translational Implications. Neuropsychopharmacology. 2016;41(1):201-74.<br>
48 26. Hollstein T, Vogt A, Grenkowitz T, Stojakovic T, März W, Laufs U, et al. Treatn<br>
49 atherogenic VLDL remnants in a real-world study. Vascul 44. Holdstein Text A, and Selection: Text A, and Selection C. 2019;116:8-15.<br>
48 27. Canuel M, Sun X, Asselin MC, Paramithiotis E, Prat A, and Seidah NG. Proprotein convertase subtilisin/kexin typ<br>
48 9 (PCSK9) can mediate 49 atherogenic VLDL remnants in a real-world study. Vascul Pharmacol. 2019,116:8-15.<br>
30 27. Canuel M, Sun X, Asselin MC, Paramithiotis E, Prat A, and Seidah NG. Proprotein continuous<br>
31 9 (PCSK9) can mediate degradation 51 9 (PCSK9) can mediate degradation of the low density lipoprotein receptor-related protein 1 (LRP-1). PloS one.<br>
52 2013;8(5):e64145.<br>
73 28. Maxwell KN, Fisher EA, and Breslow JL. Overexpression of PCSK9 accelerates the
- 51 9 (PCSK9) can mediate degradation of the low density lipoprotein receptor-related protein 1 (LRP-1). Plos one.<br>
51 2013;8(5):e64145.<br>
53 28. Maxwell KN, Fisher EA, and Breslow JL. Overexpression of PCSK9 accelerates the 33 28. Maxwell KN, Fisher<br>
54 post-endoplasmic r<br>
55 29. Lagace TA. PCSK9 a<br>
56 2014;25(5):387-93.<br>
57 30. Azmi S, Sun H, Ozo<sub>1</sub><br>
58 transcriptional repr<br>
59 31. Honma S, Kawamo
- 
- 
- 53 29. Lagace TA. PCSK9 and LDLR degradation: regulatory mechanisms in circulation and in cells. Curr Opin Lipidol.<br>
55 29. Lagace TA. PCSK9 and LDLR degradation: regulatory mechanisms in circulation and in cells. Curr Opi 55 29. Lagace TA. PCSK9 and LDLR degradation: regulatory mechanisms in circulation and in cells.<br>
56 2014;25(5):387-93.<br>
30. Azmi S, Sun H, Ozog A, and Taneja R. mSharp-1/DEC2, a basic helix-loop-helix protein functions<br>
5 Example TA. POSS and LDLR degradation. regulatory mechanisms in circulation and in cens. Curr Opin Lipidol.<br>
2014;25(5):387-93.<br>
Azmi S, Sun H, Ozog A, and Taneja R. mSharp-1/DEC2, a basic helix-loop-helix protein function
- 57 30. Azmi S, Sun H, Ozog<br>
58 transcriptional repre<br>
59 31. Honma S, Kawamot<br>
50 mammalian molecu<br>
51 32. Rouillard AD, Gunde<br>
52 collection of proces<br>
53 (Oxford). 2016;2016 58. It is transcriptional repressor of E box activity and Stra13 expression. *J Biol Chem.* 2003;278(22):20098-1<br>
59. 31. Honma S, Kawamoto T, Takagi Y, Fujimoto K, Sato F, Noshiro M, et al. Dec1 and Dec2 are regulators of 58 31. Honma S, Kawamoto T, Takagi Y, Fujimoto K, Sato F, Noshiro M, et al. Dec1 and Dec2 are regulators of th<br>50 mammalian molecular clock. *Nature*. 2002;419(6909):841-4.<br>51 32. Rouillard AD, Gundersen GW, Fernandez NF, 59 51. Hondian molecular clock. *Nature.* 2002;419(6909):841-4.<br>
51. Souillard AD, Gundersen GW, Fernandez NF, Wang Z, Monteiro CD, McDermott MG, et al. The harmonizor<br>
52 collection of processed datasets gathered to serve 60 mammalal molecular clock. Nature. 2002,419(6909):641-4.<br>
61 32 Rouillard AD, Gundersen GW, Fernandez NF, Wang Z, Monteir<br>
collection of processed datasets gathered to serve and mine I<br>
(Oxford). 2016;2016.<br>
54 33. Shen
- 
- 61 52. Collection of processed datasets gathered to serve and mine knowledge about genes and proteins. *Database*<br>
61 33. Shen WJ, Azhar S, and Kraemer FB. SR-B1: A Unique Multifunctional Receptor for Cholesterol Influx an 62 collection of processed datasets gathered to serve and mine knowledge about genes and proteins. Dutabase<br>
62 (Oxford), 2016;2016.<br>
63 Shen WJ, Azhar S, and Kraemer FB. SR-B1: A Unique Multifunctional Receptor for Choles 63 (Oxford): 2010,2010.<br>
54 33. Shen WJ, Azhar S, and<br>
55 Annu Rev Physiol. 201<br>
56 34. Hamilton KA, Wang Y,<br>
63 The Physiol. 201<br>
63 One. 2018;13(5):e019<br>
59 35. Peters DT, Henderson<br>
70 cell-surface marker fo Annu Rev Physiol. 2018;80:95-116.<br>
56 34. Hamilton KA, Wang Y, Raefsky SM, Berkowitz S, Spangler R, Suire CN, et al. Mice lacking the transcriptional<br>
57 regulator Bhlhe40 have enhanced neuronal excitability and impaired s Find Rev Physiol. 2018;80:95-110.<br>
56 34. Hamilton KA, Wang Y, Raefsky SM, I<br>
regulator Bhlhe40 have enhanced r<br> *one.* 2018;13(5):e0196223.<br>
59 35. Peters DT, Henderson CA, Warren C<br>
70 cell-surface marker for isolating h
- Figure 1.1 Hamilton Bhlhe40 have enhanced neuronal excitability and impaired synaptic plasticity in the hippocampus<br>
For all States DT, Henderson CA, Warren CR, Friesen M, Xia F, Becker CE, et al. Asialoglycoprotein recept Figure of Bhlhe40 have emailed neuronal excitability and impaired synaptic plasticity in the inppocampus. Thos<br>
one. 2018;13(5):e0196223.<br>
Peters DT, Henderson CA, Warren CR, Friesen M, Xia F, Becker CE, et al. Asialoglyco
- 68 one. 2016,13(5):e0150223.<br>
59 35. Peters DT, Henderson CA, W<br>
68 cell-surface marker for isola<br>
71 2016;143(9):1475-81.<br>
72 36. Lu H, Lei X, Winkler R, John<br>
73 receptor in the regulation of<br>
74 2022;21(1):46.<br>
75 37. O 69 35. Peters DT, Henderson CA, Warren CR, Friesen M, Xia F, Becker CE, et al. Asialoglycoprotein receptor 1 is a specific For the surface marker for isolating hepatocytes derived from human pluripotent stem cells. Development.<br>
2016;143(9):1475-81.<br>
2016;143(9):1475-81.<br>
2016;143(9):1475-81.<br>
2022;21(1):46.<br>
2022;21(1):46.<br>
2022;21(1):46.<br>
20 72 36. Lu H, Lei X, Winkler R,<br>
73 receptor in the regulat<br>
74 2022;21(1):46.<br>
75 37. Oyadomari S, Matsunc<br>
76 (HNF)-4alpha is activat<br>
77 2000;478(1-2):141-6.<br>
78 38. Engblom D, Kornfeld J
- 12 36. Lu H, Lei X, Winkler R, John S, Kumar D, Li W, et al. Crosstalk of hepatocyte nuclear factor 4a and glucocorticoid<br>
12 12 12 12 12 12 12 14 16 12 12 12 12 12 12 13 13 13 14 16 12 12 13 13 14 16 12 12 12 12 13 14 16
- 74 2022;21(1):46.<br>
75 7 2022;21(1):46.<br>
75 7 2020;271(1):46.<br>
77 2000;478(1-2):141-6.<br>
77 2000;478(1-2):141-6.<br>
78 38. Engblom D, Kornfeld JW, Schwake L, Tronche F, Reimann A, Beug H, et al. Direct glucocorticoid recepto<br> 75 37. Oyadomari S, N<br>
76 (HNF)-4alpha is<br>
77 2000;478(1-2):<br>
78 38. Engblom D, Kor<br>
79 interaction in h<br>
30 2007;21(10):11<br>
31 39. Bharathan SP, N 75 (HNF)-4alpha is activated by glucocorticoids and glucagon, and repressed by insulin in rat liver. FEBS Lett.<br>
77 2000;478(1-2):141-6.<br>
78 38. Engblom D, Kornfeld JW, Schwake L, Tronche F, Reimann A, Beug H, et al. Direc 2000;478(1-2):141-6.<br>
77 (2000;478(1-2):141-6.<br>
78 (2000;478(1-2):141-6.<br>
78 (2007;478(1-2):141-6.<br>
79 interaction in hepatocytes controls body size and maturation-related gene expression. *Genes Dev.*<br>
2007;21(10):1157-62 78 38. Engblom D, Kornfeld J<br>
79 interaction in hepatoc<br>
2007;21(10):1157-62.<br>
31 39. Bharathan SP, Manian<br>
32 markers used for the i<br>
33 40. Wang P, McKnight KD,<br>
34 definitive endoderm g
- 
- 18 18 18. Enteraction in hepatocytes controls body size and maturation-related gene expression. *Genes Dev.*<br>
2007;21(10):1157-62.<br>
2007;21(10):1157-62.<br>
2007;21(10):1157-62.<br>
2007;21(10):1157-62.<br>
2007;21(10):1157-62.<br>
20 2007;21(10):1157-62.<br>
2007;21(10):1157-62.<br>
39. Bharathan SP, Manian KV, Aalam SM, Palani D, Deshpande PA, Pratheesh MD, et al. Systematic eva<br>
markers used for the identification of human induced pluripotent stem cells. 81 39. Bharathan SP, Manian<br>
82 markers used for the is<br>
83 40. Wang P, McKnight KD,<br>
84 definitive endoderm g<br>
85 cells. *Stem Cells Dev.* 2<br>
86 41. Ghosheh N, Olsson B, l<br>
87 Expression of Lineage-
- 82. Bharaces used for the identification of human induced pluripotent stem cells. *Biol Open.* 2017;6(1):100-8.<br>
81 40. Wang P, McKnight KD, Wong DJ, Rodriguez RT, Sugiyama T, Gu X, et al. A molecular signature for purifie 82 markers used for the Nentmeation of human induced pluripotent stem cells. *Biol Open.* 2017;6(1):100-8.<br>
82 definitive endoderm guides differentiation and isolation of endoderm from mouse and human embryonic<br>
82 definit 84 definitive endoderm guides differentiation and isolation of endoderm from mouse and human embryoni<br>
85 cells. Stem Cells Dev. 2012;21(12):2273-87.<br>
86 41. Ghosheh N, Olsson B, Edsbagge J, Kuppers-Munther B, Van Giezen M 85 cells. Stem Cells Dev. 2012;21(12):2273-87.<br>
86 41. Ghosheh N, Olsson B, Edsbagge J, Kuppers-Munther B, Van Giezen M, Asplund A, et al. Highly Synchronized<br>
87 Expression of Lineage-Specific Genes during In Vitro Hepati 86 41. Ghosheh N, Olsson B, Edsbagge J, Kuppers-1<br>
87 Expression of Lineage-Specific Genes during<br>
88 Lines. *Stem Cells Int*. 2016;2016:8648356.<br>
99 42. Siller R, Greenhough S, Naumovska E, and S<br>
human pluripotent stem c 87 Expression of Lineage-Specific Genes during In Vitro Hepatic Differentiation of Human Pluripotent Stem Cell<br>
88 Lines. *Stem Cells Int*. 2016;2016:8648356.<br>
89 42. Siller R, Greenhough S, Naumovska E, and Sullivan GJ. S
- 88 Lines. *Stem Cells Int.* 2016;2016:8648356.<br>
87 Siller R, Greenhough S, Naumovska E, and Sullivan GJ. Small-molecule-driven hepatocyte differentiation of human pluripotent stem cells. *Stem Cell Reports.* 2015;4(5):939-88 Lines. Stem Cells Int. 2010,2016.0040356.<br>89 42. Siller R, Greenhough S, Naumovska E, and<br>80 human pluripotent stem cells. Stem Cell Re 89 30 human pluripotent stem cells. Stem Cell Reports. 2015;4(5):939-52. human pluripotent stem cells. Stem cell Reports. 2015;4(5):539-52.<br>human pluripotent stem cells.

- 
- 92 (Arg145Cys) mutation causes autosomal dominant type III hyperlipoproteinemia with incomplete penetral<br>
91 44. Sullivan PM, Mezdour H, Quarfordt SH, and Maeda N. Type III hyperlipoproteinemia and spontaneous<br>
95 atherosc 93 Arterioscler Thromb Vasc Biol. 1997;17(5):865-72.<br>
92 Arterioscler Thromb Vasc Biol. 1997;17(5):865-72.<br>
95 anterosclerosis in mice resulting from gene replacement of mouse Apoe with human Apoe\*2. J Clin Invest.<br>
92 199 44. Sullivan PM, Mezdour H, Quarfordt SH, and Maeda<br>
35 atherosclerosis in mice resulting from gene replace<br>
36 1998;102(1):130-5.<br>
37 45. Huang Y, Schwendner SW, Rall SC, Jr., and Mahley F<br>
39 46. Backstrom JR, Sheng J, W
- 
- atherosclerosis in mice resulting from gene replacement of mouse Apoe with human Apoe\*2. J Clin In<br>
1998;102(1):130-5.<br>
Huang Y, Schwendner SW, Rall SC, Jr., and Mahley RW. Hypolipidemic and hyperlipidemic phenotypes i<br>
tr 95 atherosclerosis in mice resulting from gene replacement of mouse Apoe with Human Apoe\*2. J Clin Invest.<br>
95 atherosclerosis in mice resulting from gene replacement of mouse Apoe with Human Apoe\*2. J Clin Invest.<br>
95 45. 97 45. Huang Y, Schwendne<br>
98 transgenic mice exp<br>
99 46. Backstrom JR, Sheng<br>
10 Elements for a Singl<br>
11 2020;19:139-48.<br>
12 Brovkina AF, Sychev<br>
13 effectiveness of glue 97 1971 1974.<br>
97 1974 1975. Huang Prince expressing human apolipoprotein E2. *J Biol Chem.* 1996;271(46):29146-51.<br>
97 19. Backstrom JR, Sheng J, Wang MC, Bernardo-Colón A, and Rex TS. Optimization of S. aureus dCas9 and 99 46. Backstrom JR, Sheng J, Wang MC, Bernardo-Colón A, and Rex TS. Optimization of S. aureus of<br>
199 Elements for a Single Adeno-Associated Virus that Targets an Endogenous Gene. *Mol Ther N*<br>
2020;19:139-48.<br>
47. Brovki
- Elements for a Single Adeno-Associated Virus that Targets an Endogenous Gene. *Mol Ther Methods Clin Dev.*<br>
99 2020;19:139-48.<br>
89 2020;19:139-48.<br>
89 2020;19:139-48.<br>
89 2010 11 2020;136 and Rex Toptimization of S. [Influ Elements for a Single Adeno-Associated Virus that Targets an Endogenous Gene. Mor Ther Methods Chin Dev.<br>
2020;19:139-48.<br>
Brovkina AF, Sychev DA, and Toropova OS. [Influence of CYP3A4, CYP3A5, and NR3C1 genes polymorphism 2020 47. Brovkina AF, Sych<br>
2020 47. Brovkina AF, Sych<br>
effectiveness of g<br>
2020 1225-32.<br>
2030 48. Constantinescu A<br>
2050 continental ances<br>
2050 8. Russcher H, Smit<br>
2080 glucocorticoid results
- 
- 12. Brow effectiveness of glucocorticoid therapy in patients with endocrine ophthalmopathy]. Vestn Oftalmol. 2020;136(6.<br>
12. Broven DA, 2020;125-32.<br>
13. Broom Continental ancestry groups of the UK Biobank. Hum Genomics. 03 effectiveness of glucocorticoid therapy in patients with endocrine opinium opathy]. Vestn Oftalmol. 2020;136(6.<br>
03 Vyp. 2):125-32.<br>
2005;90(10):5804-10.<br>
2005;90(10):5804-10.<br>
2005;90(10):5804-10.<br>
2006. EI-Fayoumi R, 95 48. Constantinescu<br>
06 continental ance<br>
07 49. Russcher H, Smi<br>
99 2005;90(10):58<br>
10 50. El-Fayoumi R, H:<br>
11 Polymorphisms
- 06 11 68. Continental ancestry groups of the UK Biobank. Hum Genomics. 2022;16(1):3.<br>
2023.16(1):3.<br>
2028. Constant H, Smit P, van den Akker EL, van Rossum EF, Brinkmann AO, de Jong FH, et al. Two polymorphisms<br>
2005;90(10 06 continental ancestry groups of the OK Biobank. Hum Genomics. 2022;16(1):3.<br>
17 49. Russcher H, Smit P, van den Akker EL, van Rossum EF, Brinkmann AO, de Jong F<br>
2005;90(10):5804-10.<br>
10 50. El-Fayoumi R, Hagras M, Aboze den B, Parama S, and Shinawi T. Association Between NR3C1 Gene<br>
2005;90(10):5804-10.<br>
10 50. El-Fayoumi R, Hagras M, Abozenadaha A, Bawazir W, and Shinawi T. Association Between NR3C1 Gene<br>
2005;90(10):5804-10.<br>
11 Polymor 98 glucocorticoid receptor gene directly direct glucocorticoid-regulated gene expression. J Clin Endocrinol Metab.<br>
10 50. El-Fayoumi R, Hagras M, Abozenadaha A, Bawazir W, and Shinawi T. Association Between NR3C1 Gene<br>
11 10 50. El-Fayoumi R, Hagras N<br>
11 Polymorphisms and To<br>
12 Leukemia. Asian Pac J<br>
13 51. Roerink SH, Wagenma<br>
14 receptor polymorphisr<br>
15 syndrome. *Endocrine*.<br>
16 52. Quax RA, Koper JW, Hu
- 11 Folymorphisms and Toxicity Induced by Glucocorticoids Therapy in Saudi Children with Acute Lymphob<br>
12 Leukemia. Asian Pac J Cancer Prev. 2018;19(5):1415-23.<br>
13 51. Roerink SH, Wagenmakers MA, Smit JW, van Rossum EF, N 12 Leukemia. Asian Pac J Cancer Prev. 2018;19(5):1415-23.<br>
13 S1. Roerink SH, Wagenmakers MA, Smit JW, van Rossum EF, Netea-Maier RT, Plantinga TS, et al. Glucocorticoid<br>
14 receptor polymorphisms modulate cardiometabolic Educemia. Asian at 9 currel Trev. 2016;19(3):1415-23.<br>
13 51. Roerink SH, Wagenmakers MA, Smit JW, van Rossum EF,<br>
14 receptor polymorphisms modulate cardiometabolic risk<br>
15 syndrome. *Endocrine*. 2016;53(1):63-70.<br>
16 52
- 14 receptor polymorphisms modulate cardiometabolic risk factors in patients in long-term remission of Cushin<br>15 syndrome. *Endocrine*. 2016;53(1):63-70.<br>16 52. Quax RA, Koper JW, Huisman AM, Weel A, Hazes JM, Lamberts SW,
- 14 receptor polymorphisms modulate cardiometabolic risk factors in patients in long-term remission of Cushing's<br>
15 syndrome. *Endocrine*. 2016;53(1):63-70.<br>
16 52. Quax RA, Koper JW, Huisman AM, Weel A, Hazes JM, Lamberts Syndrome. Endocrine. 2010,33(1):63-70.<br>
16 52. Quax RA, Koper JW, Huisman AM, Weel A<br>
17 receptor gene and in the glucocorticoid-<br>
18 response to glucocorticoid bridging thera<br>
19 53. Bouma EM, Riese H, Nolte IM, Oosterom<br> 17 receptor gene and in the glucocorticoid-induced transcript 1 gene are associated with disease activity and<br>
18 response to glucocorticoid bridging therapy in rheumatoid arthritis. *Rheumatol Int*. 2015;35(8):1325-33.<br>
1 18 response to glucocorticoid bridging therapy in rheumatoid arthritis. *Rheumatol Int.* 2015;35(8):1325-33.<br>
19 53. Bouma EM, Riese H, Nolte IM, Oosterom E, Verhulst FC, Ormel J, et al. No associations between single nucl 18 response to glucocorticoid bridging therapy in rheumatoid arthritis. Rheumatol Int. 2015;35(8):1325-33.
- 19 53. Bouma EM, Riese H, Nolte IM, Oosterom E, Verhulst FC, Ormel J, et al. No associations between single nucleotide 21 test in adolescents: the TRAILS study. *Behav Genet.* 2011;41(2):253-61.<br>
22 54. Durumutla HB, Prabakaran A, El Abdellaoui Soussi F, Akinborewa O, Latimer H, McFarland K, et al. Glucocorticoid<br>
23 chrono-pharmacology pr
- 22 54. Durumutla HB, Prabakaran A, El Abdellaoui Soussi F, Akinborewa O, Lati<br>
23 throno-pharmacology promotes glucose metabolism in heart through a<br>
24 transactivation program. JCl Insight. 2024.<br>
25 55. Prabakaran AD, Mc 22 12 22 5. Duramacology promotes glucose metabolism in heart through a cardiomyocyte-autonomous<br>
24 25. Drabakaran AD, McFarland K, Miz K, Durumutla HB, Piczer K, El Abdellaoui Soussi F, et al. Intermittent<br>
26 27 27 27 2 24 transactivation program. *JCl Insight*. 2024.<br>
25 chrono-pharmacology program. *JCl Insight*. 2024.<br>
25 chrono-pharmacology program. *JCl Insight*. 2024.<br>
26 glucocorticoid treatment improves muscle metabolism via the P 25 55. Prabakaran AD, McFarland K, Miz K, Durum<br>26 glucocorticoid treatment improves muscle<br>27 sarcopenia model. *J Clin Invest*. 2024;134(1<br>28 56. Chang CC, Chow CC, Tellier LC, Vattikuti S, F<br>29 challenge of larger and r 26 glucocorticoid treatment improves muscle metabolism via the PGC1alpha/Lipin1 axis in an aging-relat<br>
27 sarcopenia model. *J Clin Invest.* 2024;134(11).<br>
28 56. Chang CC, Chow CC, Tellier LC, Vattikuti S, Purcell SM, an
- 27 sarcopenia model. *J Clin Invest.* 2024;134(11).<br>
28 56. Chang CC, Chow CC, Tellier LC, Vattikuti S, Purcell SM, and Lee JJ. Second-generation PLINK: rising to the challenge of larger and richer datasets. *Gigascience*. 28 56. Chang CC, Chow CC, Tellier LC, Vattikuti S, Purc<br>
29 challenge of larger and richer datasets. *Gigasc*<br>
30 28 challenge of larger and richer datasets. *Gigascience*. 2015;4:7.<br>30 29 challenge of larger and richer datasets. Gigascience. 2015;4:7.<br>30
-