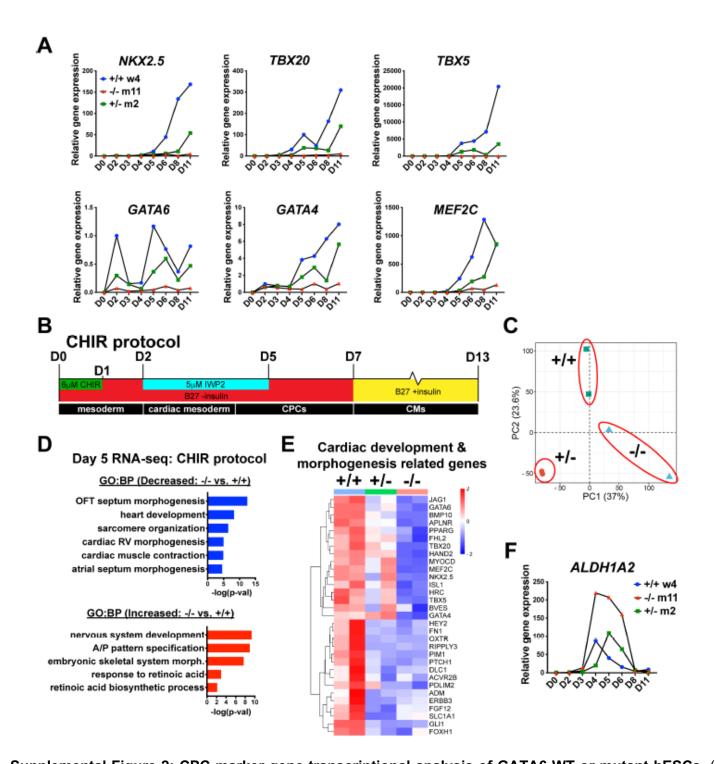


lines. (A) GATA6 CRISPR targeting scheme using H1 iCas9 hESCs was described in Shi et al. (Ref. 20). Two CRISPR gRNAs were used targeting the C-terminal zinc finger domain (GATA6-Cr1 and Cr2 gRNA target sequence in green, red indicates the PAM). (B) Table describing H1-GATA6-hESC clonal lines, genotype designation, and gRNA used in gene editing. Predicted protein describes mutant alleles according to the Human Genome Variation Society (HGVS) guidelines, fs indicates frameshift mutation. (C) Western blots from $GATA6^{+/+}$, $GATA6^{+/-}$, and $GATA6^{-/-}$ protein lysates at day 2 or 5 of cardiac differentiation probed for GATA6 with β-actin used as a loading control. (D) Immunofluorescence for the sarcomere marker α-Actinin and co-stained with DAPI on day 23 lactate-purified CMs. (E) Schematic for CRISPR-based correction of the GATA6 c.1071delG iPSC mutant allele to WT sequence ($GATA6^{corr/+}$). A pair of gRNAs flanking the G deletion site were used for targeting with Nickase-Cas9 and a WT repair template to allow for homology directed repair (HDR) of the mutant allele. A single base substitution of G to A (indicated by brown) was used in the WT repair template to induce a silent mutation in the gRNA 2 recognition sequence to prevent additional Cas9 activity after HDR. (F) Table describing the GATA6 iPSC clonal lines. The GATA6 c.1071delG mutant allele is indicated as the protein V358Cfs

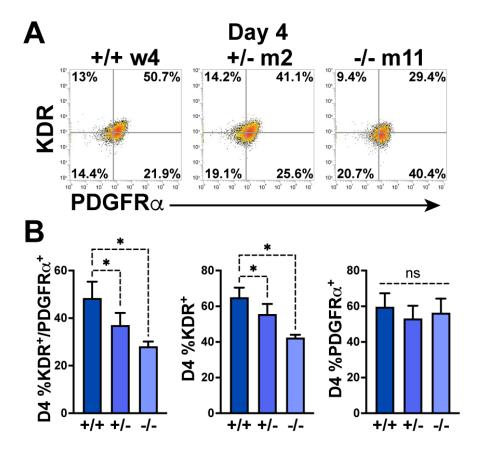
(according to HGVS guidelines). (G) Immunofluorescence for the pluripotency markers NANOG or SOX2 on

Supplemental Figure 1: CRISPR gene editing and characterization of mutant GATA6 hESC and iPSC

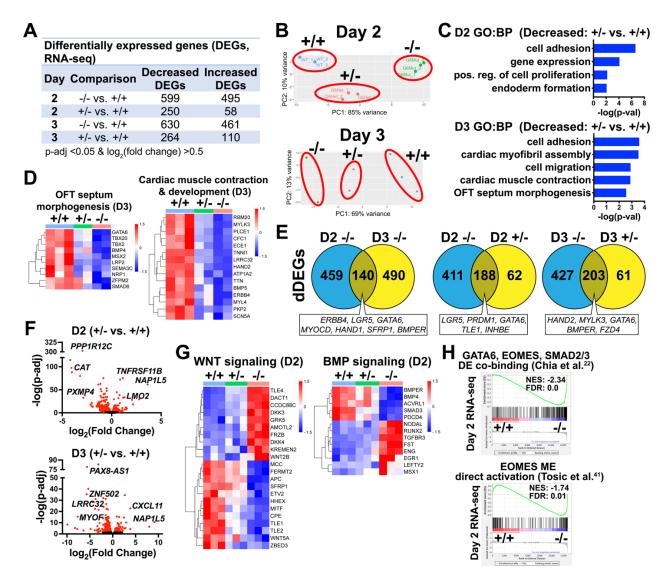
 $GATA6^{\text{corr}/+}$ or $GATA6^{1071\text{delG}/+}$ iPSC colonies. (**H**) Flow cytometry quantification for %cTnT⁺ CMs following cardiac directed differentiation for days 13 to 16 of $GATA6^{\text{corr}/+}$ or $GATA6^{1071\text{delG}/+}$ iPSCs. Data represents the mean ± SEM, dots represent independent biological replicates. Significance defined as **p<0.01 using the two-tailed Student's T-test. (**I**) Immunofluorescence for cTnT and co-stained with DAPI on day 23 lactate-purified iPSC-CMs. (**J**) Representative karyogram for $GATA6^{\text{corr}/+}$ iPSCs. (**K**) Western blots from $GATA6^{\text{corr}/+}$ or $GATA6^{\text{1071delG}/+}$ protein lysates at day 5 of cardiac differentiation probed for GATA6 with β-actin used as a loading control.



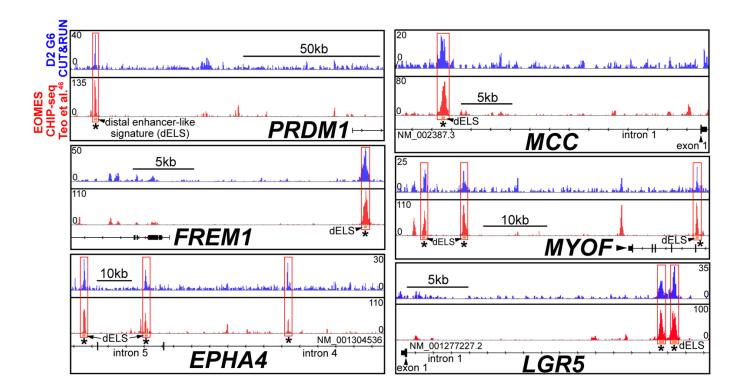
Supplemental Figure 2: CPC marker gene transcriptional analysis of GATA6 WT or mutant hESCs. (A) RT-qPCR time course for CPC markers (and *GATA6*) in differentiating *GATA6*^{+/-}, *GATA6*^{+/-}, and *GATA6*^{-/-} hESCs (normalized to day 2 WT gene expression levels). (B) Schematic for *in vitro* CM directed differentiation CHIR protocol. (C) Principal component analysis (PCA) for comparisons of day 5 bulk RNA-seq from *GATA6*^{+/-}, *GATA6*^{+/-}, and *GATA6*^{-/-} hESCs using the CHIR protocol (n=2). (D) Gene ontology (GO) analysis for biological process (BP) using differentially expressed genes (DEGs) from day 5 RNA-seq comparing *GATA6*^{-/-} to WT. (E) Heatmap for cardiac development related genes from day 5 RNA-seq. Color gradient indicates relative gene expression level. (F) RT-qPCR time course for *ALDH1A2* transcript levels in differentiating *GATA6*^{+/-}, *GATA6*^{+/-}, and *GATA6*^{-/-} hESCs using the cytokine-based protocol.



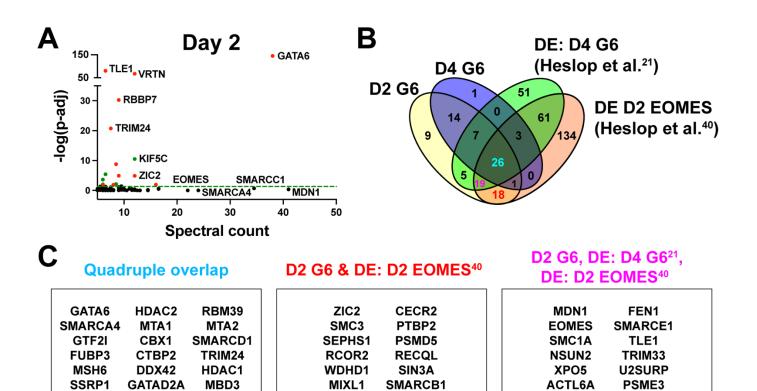
Supplemental Figure 3: KDR and PDGFR α cardiac mesoderm analysis at day 4. (A) Representative flow cytometry plots at day 4 of cardiac differentiation analyzing the % KDR and PDGFR α double-positive cells (%K⁺P⁺) from $GATA6^{+/+}$, $GATA6^{+/-}$, or $GATA6^{-/-}$ hESCs. (B) Quantification of day 4 flow cytometry data for %K⁺P⁺ double-positive, %KDR⁺ single positive, or %PDGFR α ⁺ single positive cells (n=6). Data represent the mean \pm SEM, with statistical significance indicated as *p<0.05 and ns indicating not significant by one-way repeated measures ANOVA with Holm-Šídák's multiple comparison test.



Supplemental Figure 4: RNA-seq analysis following cardiac differentiation at day 2 or day 3. (A) Number of DEGs identified when comparing $GATA6^{+/-}$ and $GATA6^{+/-}$ samples to $GATA6^{+/-}$ controls from day 2 and 3 RNA-seq data. B) PCA analysis for $GATA6^{+/-}$, $GATA6^{+/-}$, and $GATA6^{+/-}$ profiles from day 2 or day 3 RNA-seq. Data is representative of at least 2 independent biological replicates per sample. (C) Day 2 and 3 GO (BP) analysis using dDEGs identified comparing $GATA6^{+/-}$ to WT samples. (D) Heatmaps for OFT septum morphogenesis and cardiac development related genes from day 3 RNA-seq. Color gradient indicates relative gene expression levels. (E) Venn diagram comparisons of dDEGs (relative to WT) identified by day 2 or day 3 RNA-seq of $GATA6^{+/-}$ and $GATA6^{+/-}$ samples. (F) Volcano plots for day 2 or 3 $GATA6^{+/-}$ sample RNA-seq gene expression data relative to WT. Dots represent genes, red indicates p-adj<0.05 and black indicates p-adj>0.05. (G) Heatmaps for WNT and BMP related genes from day 2 RNA-seq. (H) Gene set enrichment analysis (GSEA) analysis of day 2 RNA-seq gene expression data ($GATA6^{+/-}$ relative to WT) using the GATA6, EOMES, and SMAD2/3 co-binding during hESC-DE differentiation dataset from Chia et al. (Ref. 22) and EOMES ME direct activation dataset from Tosic et al. (Ref. 41). NES indicates normalized enrichment score; FDR indicates false discovery rate.



Supplemental Figure 5: GATA6 CUT&RUN peaks overlap with previously published EOMES CHIP-seq peaks. Human genome browser representations of GATA6 CUT&RUN data at day 2 of cardiac differentiation (blue tracks) and EOMES CHIP-seq identified genes at day 2 of hESC-DE differentiation from Teo et al. (Ref. 46) (red tracks). Orange rectangles represent approximate location for distal enhancer-like signatures (dELS) via the ENCODE Project (indicated by black arrows). Asterisks indicate significant GATA6 binding peaks (p<0.003 relative to IgG controls) and significant EOMES binding peaks as defined by Teo et al. (Ref. 46).



OGT

SMARCC2

SART3

VRTN

KDM1A

SALL4

MTA3

RBBP7

CTBP1

MSH₂

RBM4

Supplemental Figure 6: Extended GATA6-RIME analysis. (**A**) Graph depicts GATA6-RIME enriched proteins (spectral count) identified at day 2 of cardiac differentiation and their corresponding day 2 RNA-seq gene expression level significance (p-adj, *GATA6*^{-/-} relative to WT). Dots indicate genes, black indicates p-adj>0.05, red indicates decreased expression level (p-adj<0.05), green indicates increased expression level (p-adj<0.5). (**B**) Venn diagram comparisons for GATA6-RIME enriched proteins from day 2 (D2 G6) and day 4 (D4 G6) of cardiac differentiation from the present study with day 4 GATA6-RIME (DE: D4 G6) or day 2 EOMES-RIME (DE: D2 EOMES) enriched proteins reported during hESC-DE differentiation from Heslop et al. (Ref. 21 and Ref. 39). (**C**) Proteins identified in the Venn diagram comparisons of RIME data described in (B).

ARID1B

DNAJC8

RNF40

EP300

SMARCD2

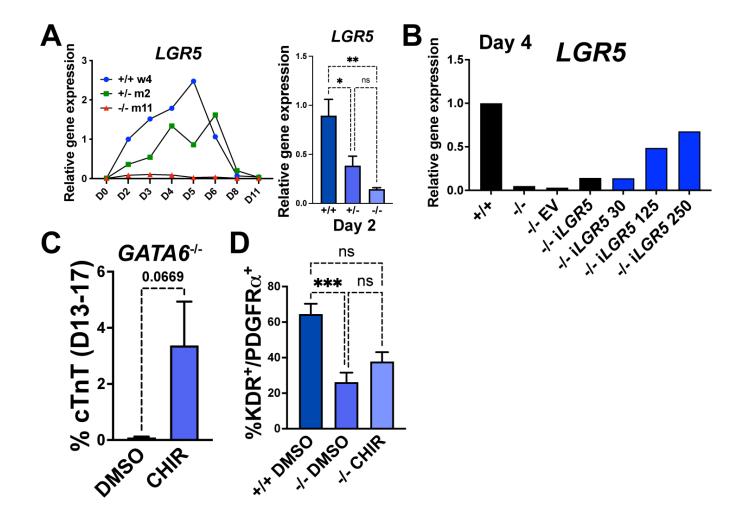
PPM1G

BUB3

SRRT

HAT1

PRPF40A



Supplemental Figure 7: Extended data for i*LGR5* and early CHIR treatment. (A) RT-qPCR time course for relative *LGR5* expression in differentiating $GATA6^{+/+}$, $GATA6^{+/-}$, and $GATA6^{+/-}$ hESCs normalized to day 2 WT gene expression level (on left). Day 2 RT-qPCR quantification for relative *LGR5* expression level normalized to WT (on right, n=6). (B) Day 4 RT-qPCR for relative *LGR5* expression level (normalized to WT) in i*LGR5* or EV transduced $GATA6^{-/-}$ hESCs treated with or without varying concentrations of DOX (30, 125, and 250 indicate ng/mL concentrations of DOX). (C) %cTnT+ CMs from days 13 to 17 of cardiac differentiation quantified by flow cytometry in $GATA6^{-/-}$ cells treated with CHIR (3 μ M) or vehicle (n≥8). (D) Flow cytometry quantification for %K+P+ double-positive cells at day 5 of cardiac differentiation from $GATA6^{-/-}$ hESCs treated with CHIR and vehicle treated $GATA6^{-/-}$ or $GATA6^{-/-}$ hESCs controls (n≥3). Data represents the mean ± SEM, significance indicated by *p<0.05, **p<0.01, ***p<0.001, by two-tailed Student's t-test (C) or one-way ANOVA with Tukey's multiple comparisons test (A, D).

Supplemental Table 1: GATA6-RIME Enriched Proteins

| Gene Name | 99 common elements in Day_2_G6_R1 and Day_2_G6_R2 Protein Name | Spectral Count (Avg) |
|---------------------|---|-------------------------|
| MDN1 | Midasin | 4 |
| GATA6 SMARCC1 | Transcription factor GATA-6 SWI/SNF complex subunit SMARCC1 | 34. |
| SMARCA4 | Transcription activator BRG1 | 24.0 |
| EOMES | Eomesodermin homolog | 22 |
| KLC3 MRPL15 | Kinesin light chain 3 39S ribosomal protein L15 mitochondrial | 16.5 16.5 |
| DZIP3 | E3 ubiquitin-protein ligase DZIP3 | 16 |
| DNM2 GTF2I | Dynamin-2 General transcription factor II-I | 14.5 14.5 |
| FUBP3 | Far upstream element-binding protein 3 | 13 |
| KIF5B MSH6 | Kinesin-1 heavy chain DNA mismatch repair protein Msh6 | 13 12.5 |
| SSRP1 | FACT complex subunit SSRP1 | 12.5 |
| ARID1A | AT-rich interactive domain-containing protein 1A | 12 |
| GADD45GIP1 KIF5C | Growth arrest and DNA damage-inducible proteins-interacting protein 1 Kinesin heavy chain isoform 5C | 12 |
| VRTN | Vertnin | 12 |
| ZIC2 KDM1A | Zinc finger protein ZIC 2 Lysine-specific histone demethylase 1A | 12 11.5 |
| SALL4 | Sal-like protein 4 | 11.5 |
| TOP2B HDAC2 | DNA topoisomerase 2 (Fragment) Histone deacetylase 2 | 11.5 |
| MRPL44 | 39S ribosomal protein L44 mitochondrial | 10.5 |
| ELAVL1 | ELAV-like protein 1 | 10 |
| MTA1 SMC1A | Metastasis-associated protein MTA1 Structural maintenance of chromosomes protein | 10 |
| CBX1 | Chromobox protein homolog 1 | 9.5 |
| PYCR2 SMARCA5 | Pyrroline-5-carboxylate reductase 2 SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily A member 5 | 9.5 9.5 |
| SMARCA5 SMC3 | Structural maintenance of chromosomes protein 3 | 9.5 |
| CTBP2 | C-terminal-binding protein 2 | 9 |
| DDX42 GATAD2A | ATP-dependent RNA helicase DDX42 Transcriptional repressor p66-alpha | 9 |
| MTA3 | Metastasis-associated protein MTA3 | g |
| NSUN2 RBBP7 | tRNA (cytosine(34)-C(5))-methyltransferase | 9 |
| SF1 | Histone-binding protein RBBP7 Splicing factor 1 | 9 |
| SMU1 | WD40 repeat-containing protein SMU1 | g |
| XPO5 ACTL6A | Exportin-5 Actin-like protein 6A | 8.5 |
| SEPHS1 | Selenide water dikinase 1 | 8.5 |
| CTBP1 EP300 | C-terminal-binding protein 1 | 8 |
| RBM39 | Histone acetyltransferase p300 RNA-binding protein 39 | 8 |
| RCOR2 | REST corepressor 2 | 8 |
| SMARCD2 DAZAP1 | SMARCD2 protein DAZ-associated protein 1 | 7.5 |
| HNRNPUL1 | Heterogeneous nuclear ribonucleoprotein U-like protein 1 | 7.5 |
| MRPL4 MTA2 | 39S ribosomal protein L4 mitochondrial (Fragment) Metastasis-associated protein MTA2 | 7.5 7.5 |
| SMARCD1 | SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily D member 1 | 7.5 |
| TRIM24 | Transcription intermediary factor 1-alpha | 7.5 |
| WDHD1 HDAC1 | WD repeat and HMG-box DNA-binding protein 1 Histone deacetylase 1 | 7.5 |
| MIXL1 | Homeobox protein MIXL1 | 7 |
| OGT PPM1G | UDP-N-acetylglucosaminepeptide N-acetylglucosaminyltransferase 110 kDa subunit | 7 |
| SMARCC2 | Protein phosphatase 1G SWI/SNF complex subunit SMARCC2 | 7 |
| XRN2 | 5'-3' exoribonuclease 2 | 7 |
| BUB3 DUT | Mitotic checkpoint protein BUB3 (Fragment) DUTP pyrophosphatase isoform CRA_c | 6.5 6.5 |
| FEN1 | Flap endonuclease 1 | 6.5 |
| MBD3 MSH2 | Methyl-CpG-binding domain protein 3 DNA mismatch repair protein Msh2 | 6.5 |
| RBM4 | RNA-binding protein 4 | 6.5 |
| SART3 | Squamous cell carcinoma antigen recognized by T-cells 3 | 6.5 |
| SMARCE1 SNX9 | SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily E member 1 (Fragment) Sorting nexin-9 | 6.5 6.5 |
| THRAP3 | Thyroid hormone receptor-associated protein 3 | 6.5 |
| TLE1 TRIM33 | Transducin-like enhancer protein 1 E3 ubiquitin-protein ligase TRIM33 (Fragment) | 6.5 6.5 |
| U2SURP | U2 snRNP-associated SURP motif-containing protein | 6.5 |
| CECR2 | Cat eye syndrome critical region protein 2 | 6 |
| MRPL38 MRPL47 | 39S ribosomal protein L38 mitochondrial 39S ribosomal protein L47 mitochondrial | 6 |
| PSME3 | Proteasome activator complex subunit 3 | 6 |
| PTBP2 | Polypyrimidine tract-binding protein 2 | 6 |
| SRRT USP9X | Serrate RNA effector molecule homolog Probable ubiquitin carboxyl-terminal hydrolase FAF-X | 6 |
| DDX46 | Probable ATP-dependent RNA helicase DDX46 | 5.5 |
| HAT1 HINT1 | Histone acetyltransferase type B catalytic subunit Histidine triad nucleotide-binding protein 1 | 5.5 5.5 |
| MRPL13 | 39S ribosomal protein L13 mitochondrial | 5.5 |
| MRPL20 MRPL50 | 39S ribosomal protein L20 mitochondrial | 5.5 5.5 |
| MSI1 | 39S ribosomal protein L50 mitochondrial RNA-binding protein Musashi homolog 1 | 5.5 |
| PSMD5 | 26S proteasome non-ATPase regulatory subunit 5 | 5.5 |
| RECQL SIN3A | ATP-dependent DNA helicase Q1 Paired amphipathic helix protein Sin3a | 5.5 5.5 |
| SMARCB1 | SWI/SNF related matrix associated actin dependent regulator of chromatin subfamily b member 1 isoform CRA_c | 5.5 |
| SNRNP70 | U1 small nuclear ribonucleoprotein 70 kDa | 5.5 |
| ARID1B DNAJC8 | AT-rich interactive domain-containing protein 1B DnaJ homolog subfamily C member 8 | 5 |
| MRPL43 | 39S ribosomal protein L43 mitochondrial (Fragment) | Ę |
| MRPL49 MSI2 | 39S ribosomal protein L49 mitochondrial | |
| MSI2 PRPF40A | RNA-binding protein Musashi homolog 2 Pre-mRNA-processing factor 40 homolog A | 5 |
| RNF40 | E3 ubiquitin-protein ligase BRE1B | |
| RNF40 | E3 ubiquitin-protein ligase BRE1B | 1 |

| 52 common elements in Day 4 G6 R1 and Day 4 G6 R2 | | |
|---|---|-------------|
| | | Spectral |
| Gene Name | Protein Name | Count (Avg) |
| DNM2 | Dynamin-2 | 24.5 |
| GATA6 | Transcription factor GATA-6 | 24 |
| SMARCC1 | SWI/SNF complex subunit SMARCC1 | 24 |
| PYCR2 | Pyrroline-5-carboxylate reductase 2 | 23.5 |
| DZIP3 | E3 ubiquitin-protein ligase DZIP3 | 21.5 |
| SMARCA4 | Transcription activator BRG1 | 18.5 |
| FUBP3 | Far upstream element-binding protein 3 | 15.5 |
| MRPL15 | 39S ribosomal protein L15 mitochondrial | 13 |
| MRPL44 | 39S ribosomal protein L44 mitochondrial | 13 |
| SNX9 | Sorting nexin-9 | 12 |
| GADD45GIP1 | Growth arrest and DNA damage-inducible proteins-interacting protein 1 | 11.5 |
| SSRP1 | FACT complex subunit SSRP1 | 11.5 |
| KIF5B | Kinesin-1 heavy chain | 10.5 |
| MSH6 | DNA mismatch repair protein Msh6 | 9.5 |
| SALL4 | Sal-like protein 4 | 9.5 |
| VRTN | Vertnin | 9.5 |
| GTF2I | General transcription factor II-I | 9 |
| TRIM24 | Transcription intermediary factor 1-alpha | 9 |
| ELAVL1 | ELAV-like protein 1 | 8.5 |
| KDM1A | Lysine-specific histone demethylase 1A | 8.5 |
| SMU1 | WD40 repeat-containing protein SMU1 | 8.5 |
| HDAC2 | Histone deacetylase 2 | 8 |
| MSI2 | RNA-binding protein Musashi homolog 2 | 7.5 |
| MTA1 | Metastasis-associated protein MTA1 | 7.5 |
| RBBP7 | Histone-binding protein RBBP7 | 7.5 |
| SMARCD1 | SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily D member 1 | 7.5 |
| CBX1 | Chromobox protein homolog 1 | 7 |
| CTBP1 | C-terminal-binding protein 1 | 7 |
| DDX42 | ATP-dependent RNA helicase DDX42 | 7 |
| KLC3 | Kinesin light chain 3 | 7 |
| MSH2 | DNA mismatch repair protein Msh2 | 7 |
| MSI1 | RNA-binding protein Musashi homolog 1 | 7 |
| MTA3 | Metastasis-associated protein MTA3 | 7 |
| SF1 | Splicing factor 1 | 7 |
| TAF15 | TATA-binding protein-associated factor 2N | 7 |
| CTBP2 | C-terminal-binding protein 2 | 6.5 |
| RBM39 | RNA-binding protein 39 | 6.5 |
| SNRPN | Small nuclear ribonucleoprotein-associated protein N (Fragment) | 6.5 |
| TIAL1 | Nucleolysin TIAR | 6.5 |
| GATAD2A | Transcriptional repressor p66-alpha | 6 |
| MRPL47 | 39S ribosomal protein L47 mitochondrial | 6 |
| MTA2 | Metastasis-associated protein MTA2 | 6 |
| RBM4 | RNA-binding protein 4 | 6 |
| TOP2B | DNA topoisomerase 2 (Fragment) | 6 |
| USP9X | Probable ubiquitin carboxyl-terminal hydrolase FAF-X | 6 |
| ARID1A | AT-rich interactive domain-containing protein 1A | 5.5 |
| HDAC1 | Histone deacetylase 1 | 5.5 |
| HNRNPUL1 | Heterogeneous nuclear ribonucleoprotein U-like protein 1 | 5.5 |
| MRPL4 | 39S ribosomal protein L4 mitochondrial (Fragment) | 5.5 |
| ADAR | Double-stranded RNA-specific adenosine deaminase | 5 |
| MBD3 | Methyl-CpG-binding domain protein 3 | 5 |
| MRPL49 | 39S ribosomal protein L49 mitochondrial | 5 |

$\label{lem:continuous} \textbf{Supplemental Table 2: Cell lines}, \textbf{primer sequences}, \textbf{and antibodies used}.$

Cell Lines

| Name | Source | Notes |
|---|--|-------|
| | Parent line: WiCell Research Institute, H1 (WA01), | |
| H1-GATA6-hESCs | NIHhESC-10-0043 | Male |
| GATA6 c.1071delG iPSCs | Yu et al., 2014 (Ref: 15) | Male |
| 293T | ATCC: human embryonic kidney cells, CRL-11268 | |
| CF1 Mouse Embryonic Fibroblasts, irradiated | MTI Global Stem: CF-1 MEF 2M IRR | |

gRNAs sequences

| Name | Sequence 5' to 3' | Cell line used | Notes |
|------------|---------------------------|------------------------|----------------------------------|
| G6-Cr1 | GGCGTTTCTGCGCCATAAGG | H1-GATA6-hESCs | |
| G6-Cr2 | TTATGGCGCAGAAACGCCG | H1-GATA6-hESCs | |
| gRNA 1 - F | caccgCACAGCCTGCAGAGCCGCGC | GATA6 c.1071delG iPSCs | gRNA target sequence capitalized |
| gRNA 1 - R | aaacGCGCGGCTCTGCAGGCTGTGc | GATA6 c.1071delG iPSCs | gRNA target sequence capitalized |
| gRNA 2 - F | caccgGTGCAGCACGGGGTCTCGAA | GATA6 c.1071delG iPSCs | gRNA target sequence capitalized |
| gRNA 2 - R | aaacTTCGAGACCCCGTGCTGCAc | GATA6 c.1071delG iPSCs | gRNA target sequence capitalized |

Primer sequences

| Name | Sequence 5' to 3' | Application |
|----------------|--------------------------------|--|
| G6-iPSC-F | GCCCCTACTCGCCCTACG | PCR genotyping |
| G6-iPSC-R | CACCCGGACGAGAAAGTCCT | PCR genotyping |
| | | Full Length LGR5 PCR (using day 2 cDNA). |
| LGR5-cloning-F | TTCGTACGTACTTCGGGCACCATGGA | Includes BsiWI site. |
| - | | Full Length LGR5 PCR (using day 2 cDNA). |
| LGR5-cloning-R | GGCAATTGTTAGAGACATGGGACAAATGCC | Includes Mfel site. |
| LGR5-seq1 | GAAAGAAATGCTTTGATGGG | LGR5 Sanger sequencing primer 1 |
| LGR5-seq2 | ATGCATTTTCCACTTTGCCA | LGR5 Sanger sequencing primer 2 |
| LGR5-seq3 | CTGGTGGGAGAATGGGGTTG | LGR5 Sanger sequencing primer 3 |
| CS-TRE-F | ATTTTCGGGTTTATTACAGG | Lentiviral vector Sanger sequencing primer |
| CS-TRE-R | GGAAAGGAGCTGACAGGT | Lentiviral vector Sanger sequencing primer |
| HPRT-F | ACCAGTCAACAGGGGACATAA | RT-qPCR |
| HPRT-R | CTTCGTGGGGTCCTTTTCACC | RT-qPCR |
| NKX2.5-F | AGCCGAAAAGAAGAGCTGTGCG | RT-qPCR |
| NKX2.5-R | GACCTGCGCCTGCGAGAAGAG | RT-qPCR |
| TBX20-F | GGCGACGGAGAACACAATCAA | RT-qPCR |
| TBX20-R | CTGGGCACAGGACGACTTC | RT-qPCR |
| TBX5-F | AGCAGTGACTTCCTACCAGAAC | RT-qPCR |
| TBX5-R | TGACATTCTGTGCAGCTCCAT | RT-qPCR |
| GATA6-F | CTGCGGGCTCTACAGCAAG | RT-qPCR |
| GATA6-R | GTTGGCACAGGACAATCCAAG | RT-qPCR |
| GATA4-F | AAAGAGGGGATCCAAACCAG | RT-qPCR |
| GATA4-R | TTGCTGGAGTTGCTGGAAG | RT-qPCR |
| MEF2C-F | CTGGTGTAACACATCGACCTC | RT-qPCR |
| MEF2C-R | GATTGCCATACCCGTTCCCT | RT-qPCR |
| ALDH1A2-F | GGAGTCCCTTTGACCCCACCACT | RT-qPCR |
| ALDH1A2-R | CCCTTTCGGCCCAGTCCTTTGC | RT-qPCR |
| LGR5-F | GTTTCCCGCAAGACGTAACT | RT-qPCR |
| LGR5-R | CAGCGTCTTCACCTCCTACC | RT-qPCR |
| T-F | ACCCAGTTCATAGCGGTGAC | RT-qPCR |
| T-R | CCATTGGGAGTACCCAGGTT | RT-qPCR |
| EOMES-F | CTGCCCACTACAATGTGTTCG | RT-qPCR |
| EOMES-R | GCGCCTTTGTTATTGGTGAGTTT | RT-aPCR |

Antibodies

| Antibody | Source | Application |
|--|---------------------------------|---|
| mouse monoclonal α-Actinin | Sigma-Aldrich A7811 | immunocytochemistry 1:200 |
| rabbit polyclonal NANOG | Cell Signaling Technology 3850 | immunocytochemistry 1:250 |
| rabbit polyclonal SOX2 | Invitrogen 48-1400 | immunocytochemistry 1:100 |
| mouse monoclonal cardiac troponin T | Invitrogen MA5-12960 | immunocytochemistry 1:200, flow cytometry 1:500 |
| rabbit monoclonal GATA6 | Cell Signaling Technology 5851 | western blotting 1:1000 |
| mouse monoclonal β-actin | Sigma-Aldrich A1978 | western blotting 1:50000 |
| mouse monoclonal anti-human KDR PE conjugated | R&D Systems FAB357P-100 | flow cytometry 10μL per million cells |
| mouse monoclonal anti-human PDGFR α PE conjugated | R&D Systems FAB1264A | flow cytometry 10μL per million cells |
| goat polyclonal anti-human/mouse Brachyury PE-conjugated | R&D Systems IC2085P | flow cytometry 1:40 |
| rabbit polyclonal SMAD2/3 | Cell Signaling Technology 3102 | western blotting 1:500 |
| rabbit monoclonal Phospho-SMAD2 (Ser465/467)/SMAD3 (Ser423/425) (D27F4) | Cell Signaling Technology 8828 | western blotting 1:500 |
| rabbit monoclonal Phospho-SMAD1 (Ser463/465)/ SMAD5 (Ser463/465)/ SMAD9 (Ser465/467) (D5B10) | Cell Signaling Technology 13820 | western blotting 1:500 |
| rabbit monoclonal non-phospho (Active) β- Catenin (Ser33/37/Thr41) (D13A1) | Cell Signaling Technology 8814 | western blotting 1:1000 |
| rabbit polyclonal β-Catenin | Cell Signaling Technology 9562 | western blotting 1:1000 |
| rabbit monoclonal SMARCC1/BAF155 (D7F8S) | Cell Signaling Technology 11956 | western blotting 1:1000 |
| rabbit polyclonal TBR2 / Eomes | Abcam ab23345 | western blotting 1:500 |
| goat anti-mouse IgG1 Alexa Fluor™ 488 | Invitrogen A-21121 | immunocytochemistry 1:500 |
| goat anti-rabbit Alexa Fluor™ 488 | Invitrogen A-11008 | immunocytochemistry 1:1000 |
| goat anti-mouse Alexa Fluor™ 488 | Invitrogen A-10667 | immunocytochemistry 1:500 |
| goat anti-mouse Alexa Fluor™ 647 | Invitrogen A-21236 | flow cytometry 1:400 |
| goat anti-rabbit HRP | Bio-Rad 1706515 | western blotting 1:2000 - 1:10000 |
| goat anti-mouse HRP | Bio-Rad 1706516 | western blotting 1:2000 - 1:10000 |
| mouse monoclonal IgG1 APC-conjugated | R&D Systems IC002A | flow cytometry 10µL per million cells |
| mouse monoclonal IgG1 PE-conjugated | R&D Systems IC002P | flow cytometry 10µL per million cells |
| goat polyclonal IgG PE-conjugated | R&D Systems IC108P | flow cytometry 1:40 |