

Supplementary Information

A phosphorylation switch in the Mediator MED15 controls cellular senescence and cognitive decline

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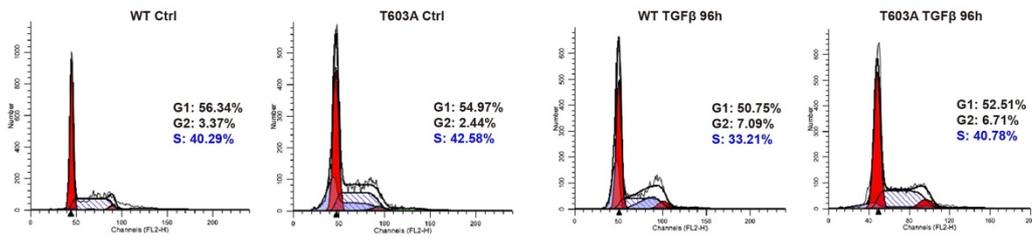


Figure S1. The MED15 T603A mutant confers resistance to TGFβ-induced cell cycle arrest. Flow cytometry analysis of the cell cycle distribution of WT and MED15 T603A mutant HaCaT cells treated without or with TGFβ.

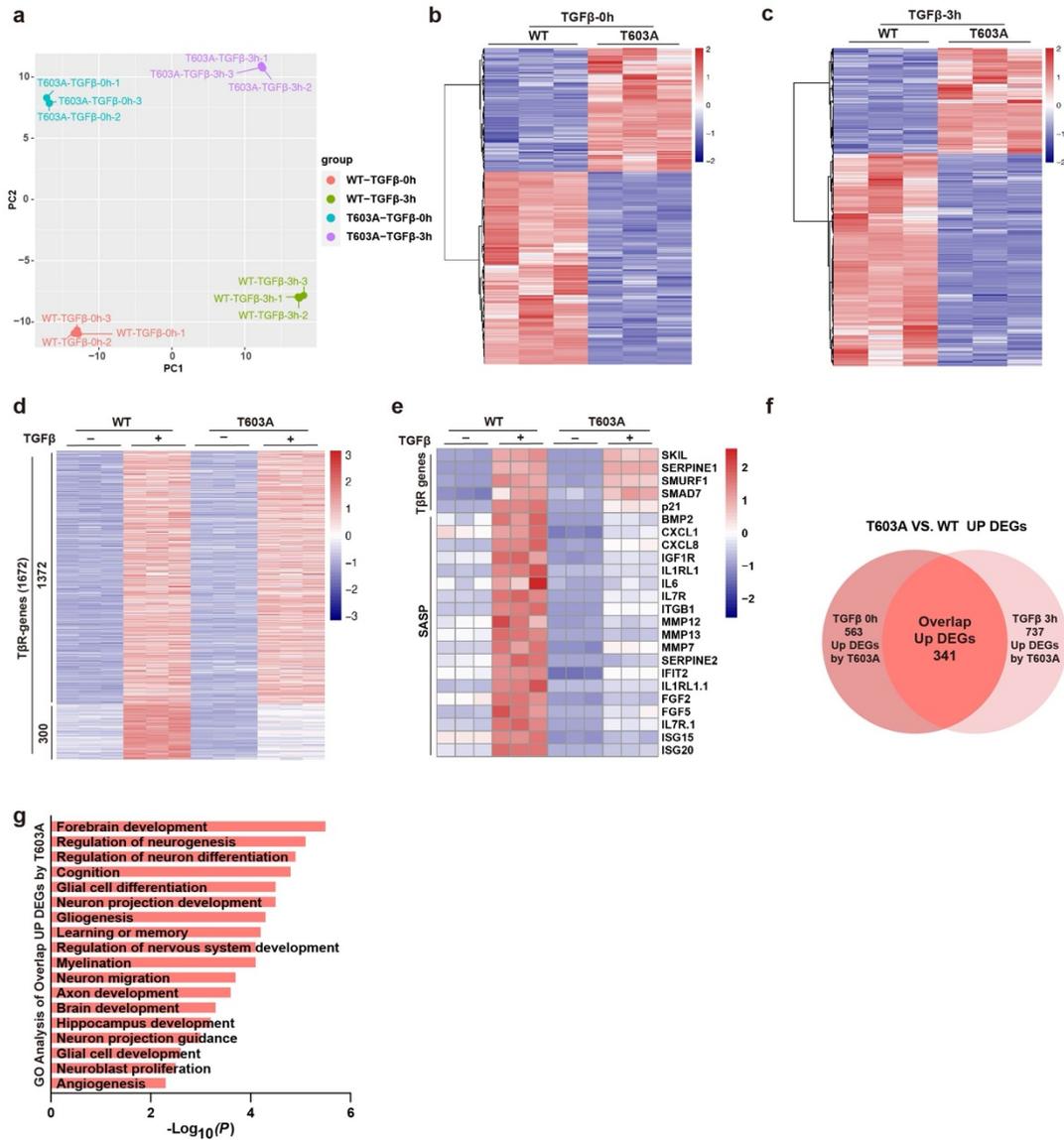


Figure S2. The MED15 T603A mutation relieves SASP

- (a) Principal component analysis (PCA) of the transcriptomes of WT and MED15 T603A mutant HaCaT cells treated without or with TGFβ.
- (b) Heatmap of differentially expressed genes (DEGs) identified by RNA-seq in WT and MED15 T603A mutant HaCaT cells in the steady state.
- (c) Heatmap of DEGs identified by RNA-seq in WT and MED15 T603A mutant HaCaT cells after 3 h of TGFβ treatment.
- (d) Heatmap of the TGFβ-responsive genes (TβR genes) identified by RNA-seq in WT and MED15 T603A mutant HaCaT cells treated without or with TGFβ.

- (e) Heatmap of the SASP genes and T β R genes identified by RNA-seq in WT and MED15 T603A mutant HaCaT cells treated without or with TGF β .
- (f) Venn diagram showing the 341 overlapping genes among the upregulated DEGs identified by RNA-seq in the MED15 T603A mutant HaCaT cells.
- (g) GO analysis of the 341 overlapping DEGs associated with the MED15 T603A mutation in HaCaT cells.

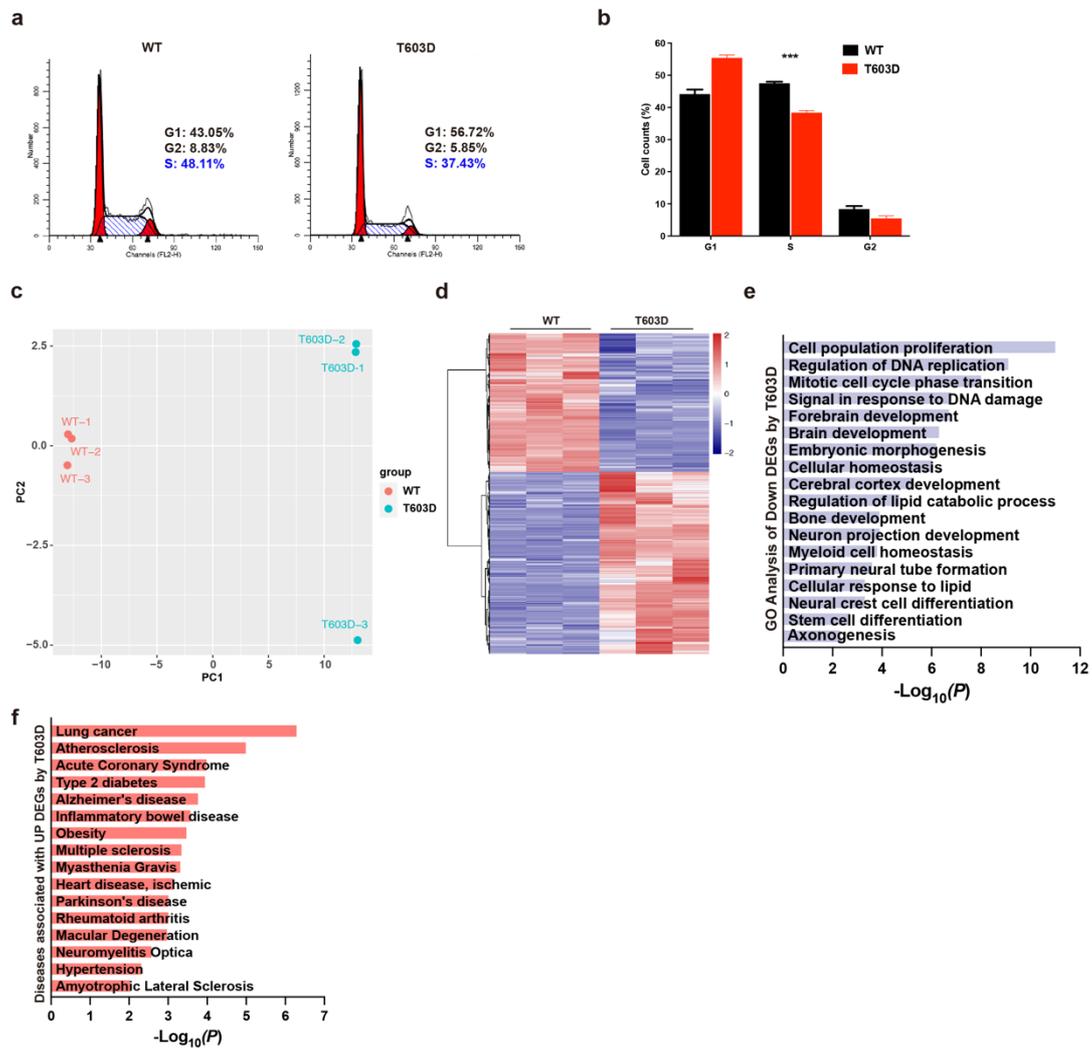


Figure S3. The MED15 T603D mutation exacerbates the SASP.

(a) Flow cytometry assays and statistical analysis (b) were used to analyze the cell cycle distribution of WT and MED15 T603D mutant MCF7 cells. The values are presented as the means \pm SDs. *** $P < 0.001$.

(c) PCA of the transcriptomes of WT and MED15 T603D mutant MCF7 cells.

(d) Heatmap of DEGs identified by RNA-seq in WT and MED15 T603D mutant MCF7 cells.

(e) GO analysis of DEGs downregulated by the MED15 T603D mutation.

(f) Diseases predicted to be associated with upregulated genes by the MED15 T603D mutation.

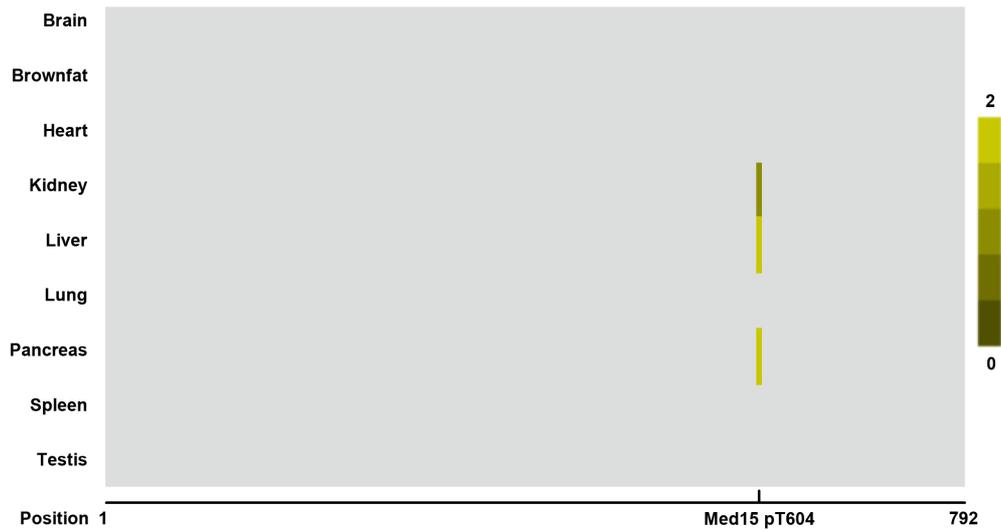


Figure S4. pT604 is the only known phosphosite of Med15 in mice.
Phosphosite analysis of Med15 in mice via the PhosphoMouse database.

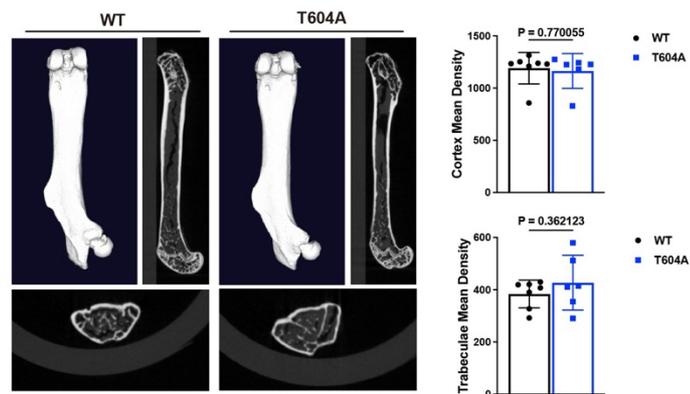


Figure S5. Bone density in aging WT and Med15 T604A mutant mice.
Representative μ CT images of tibias from old male WT mice (n=7) and Med15 T604A (n=6) mutant mice.

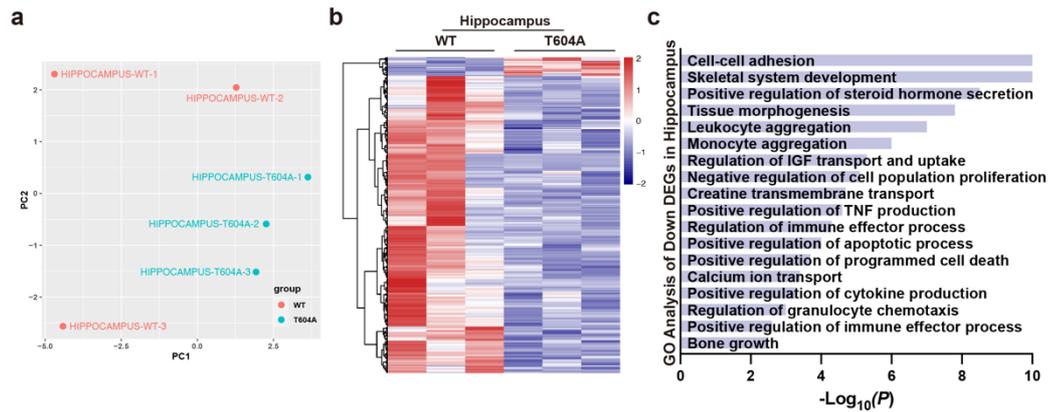


Figure S6. The Med15 T604A mutant alleviates the SASP in the hippocampus of aging mice.

- (a) PCA of the transcriptomes of the hippocampus of the old male WT and Med15 T604A mutant mice (n=3).
- (b) Heatmap of DEGs identified by RNA-seq in the hippocampus of old male WT and Med15 T604A mutant mice (n=3).
- (c) GO analysis of DEGs downregulated by the Med15 T604A mutation in the hippocampus of aging mice.

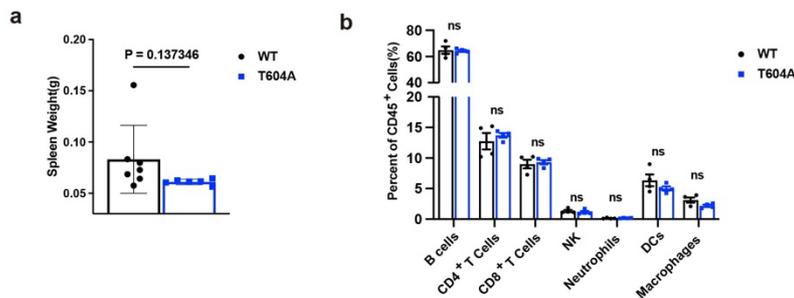


Figure S7. Proportion of splenic immune cells in aging WT and Med15 T604A mutant mice.

- (a) Spleen weights of old male WT (n=7) and Med15 T604A mutant (n=6) mice.
- (b) Proportions of splenic immune cells in old male WT and Med15 T604A mutant mice (n=3).

Table S1. Mendelian ratios of the outcome of Med15 T604A/+ mice breeding.

| Genotype | Number of mice(%) |
|-------------------|-------------------|
| Med15 +/+ | 75(27.7%) |
| Med15 T604A/+ | 128(47.2%) |
| Med15 T604A/T604A | 68(25.1%) |
| Total | 271(100%) |

Table S2. qRT-PCR primer sequences.

| Gene | Forward | Reverse |
|--------------|-------------------------|-------------------------|
| ACTIN | GCACGGCATCGTCACCAACT | CATCTTCTCGCGTTGGCCT |
| CDK1 | GGGTCAGCTCGTTACTCAAC | TGACATGGGATGCTAGGCTT |
| CTGF | CTGCAGGCTAGAGAAGCAGAG | GATGCACTTTTTGCCCTTCT |
| CXCL1 | CTGCTCCTGCTCCTGGTAG | TGGCTATGACTTCGGTTTGG |
| CXCL3 | GCAGGGAATTCACCTCAAGA | GGTGCTCCCCTTGTTCAAGTA |
| CXCL8 | CAGCCTTCTGATTTCTGC | ACTTCTCCACAACCCTCTGC |
| CXCL10 | CCATTCTGATTTGCTGCCTTA | CCTTTCCTTGCTAACTGCTTTC |
| IL1 α | TGGTAGTAGCAACCAACGGGA | ACTTTGATTGAGGGCGTCATTC |
| IL1 β | ACTTTGATTGAGGGCGTCATTC | CGTTATCCCATGTGTGCGAAGAA |
| IL6 | CCTGAACCTTCCAAAGATGGC | TTCACCAGGCAAGTCTCCTCA |
| MMP1 | GATGTGGAGTGCCTGATGTG | TGATGTCTGCTTGACCCTCA |
| MMP2 | AGTGGATGATGCCTTTGCTC | GAGTCCGTCCTTACCGTCAA |
| MMP3 | CGGTTCCGCCTGTCTCAAG | CGCCAAAAGTGCCTGTCTT |
| MMP12 | TTGATGGCAAAGGTGGAATC | AGCAGAGAGGCGAAATGTGT |
| MMP13 | CTTTGGCTTAGAGGTGACTGG | AGGCACTCCACA TCTTGGTTT |
| p21 | TTAGCAGCGGAACAAGGAGT | GCCGAGAGAAAACAGTCCAG |
| PAI1 | ATTCAAGCAGCTATGGGATTCAA | CTGGACGAAGATCGCGTCTG |
| SKIL | GAGGCTGAATATGCAGGACAG | CTATCGGCCTCAGCATGG |
| TNF α | AACCTCCTCTCTGCCATCAA | ATGTTCGTCCTCCTCACAGG |
| TNFSF10 | TGGCAACTCCGTCAGCTCGTTA | AGCTGCTACTCTCTGAGGACCT |

Table S3. siRNA sequences.

| Gene | Sense | Antisense |
|------------|-----------------------|-----------------------|
| si-Ctrl | UUCUCCGAACGUGUCACGUTT | ACGUGACACGUUCGGAGAATT |
| si-CDK1 #1 | GGCACUGAAUCAUCCAUAUTT | AUAUGGAUGAUUCAGUGCCTT |
| si-CDK1 #2 | GCACUCCCAAUAAUGAAGUTT | ACUUCAUUUUUGGGAGUGCTT |
| si-CDK1 #3 | GUCUUCAGGAUGUGCUUAUTT | AUAAGCACAUCCUGAAGACTT |

Table S4. GSEA (AGING gene set).

| | | | | | | | | |
|-------------|------------|------------|-----------------|----------|---------|-------------|--------------|---------|
| AREG | CXCR6 | IL31 | SERPINB1 | BCL10 | IFI27L1 | PLCH2 | TERF1 | SLC6A2 |
| AXL | FGF16 | IL31RA | SERPINB10 | BCL11A | IFI27L2 | PML | TERF2 | SLC6A9 |
| BMP1 | FGF17 | IL32 | SERPINB11 | BCL11B | IFI30 | POLG | TFAP2A | SLC7A8 |
| BMP10 | FGF18 | IL33 | SERPINB12 | BCL2 | IFI35 | PPARGC1A | TGFB1 | SLC9A3 |
| BMP15 | FGF19 | IL34 | SERPINB13 | BCL2L14 | IFI44 | PPARGC1B | TGFB11 | SLC9A9 |
| BMP2 | FGF21 | IL36A | SERPINB2 | BCL2L2 | IFI44L | PRKAA2 | TGFB2 | SLCO3A1 |
| BMP3 | FGF7 | IL36B | SERPINB3 | BCL3 | IFI6 | PRKAB1 | TGFB2-AS1 | |
| BMP4 | FGF8 | IL36G | SERPINB4 | BMI1 | IFIH1 | PRKAB2 | TGFB2-OT1 | |
| BMP5 | GDF1 | IL36RN | SERPINB5 | BSCL2 | IFIT1 | PRKACA | TGFB3 | |
| BMP6 | GDF10 | IL37 | SERPINB6 | CDKN1A | IFIT1B | PRKACB | TGFBR2 | |
| BMP7 | GDF11 | IL3RA | SERPINB7 | CDKN2A | IFIT2 | PRKAG1 | TIMM23 | |
| BMP7-AS1 | GDF15 | IL4 | SERPINB8 | CDKN2B | IFIT3 | PSEN1 | TIMM23B | |
| BMPER | GDF2 | IL4I1 | SERPINB9 | CEBPA | IFIT5 | PSEN2 | TIMM9 | |
| CCL1 | GDF3 | IL4R | SERPINB9P1 | CEBPB | IFITM1 | PTEN | TIMMDC1 | |
| CCL11 | GDF5 | IL5 | SERPINC1 | CEBPD | IFITM10 | PTGS1 | TIMP1 | |
| CCL13 | GDF6 | IL5RA | SERPIND1 | CETP | IFITM2 | PTGS2 | AREG | |
| CCL14 | GDF7 | IL6 | SERPINE1 | CHCHD1 | IFITM3 | PYCR1 | AXL | |
| CCL15 | GDF9 | IL6R | SERPINE2 | CHCHD10 | IFITM4P | PYCR2 | TOP1 | |
| CCL15-CCL14 | HGF | IL6ST | SERPINE3 | CHEK2 | IFITM5 | RECQL | TP53 | |
| CCL16 | IGF1 | IL7R | SERPINF1 | CREB1 | IGF1 | RECQL4 | TP53I11 | |
| CCL17 | IGF1R | IL9 | SERPINF2 | CREB3 | IGF1R | RECQL5 | TP53I3 | |
| CCL18 | IGF2 | ITGA1 | SERPING1 | CREBBP | IGF2 | REL | TP53INP1 | |
| CCL19 | IGF2-AS | ITGA10 | SERPINH1 | CSF2RB | IGF2BP1 | RELA | TP53 | |
| CCL2 | IGF2BP1 | ITGA11 | SERPINI1 | CSNK1E | IGF2BP2 | RELB | TP53I11 | |
| CCL20 | IGF2BP2 | ITGA3 | SERPINI2 | CSNK1G1 | IGF2R | RELL1 | TP53I3 | |
| CCL21 | IGF2R | ITGA4 | TNFRSF10A | CTF1 | IRS1 | RELL2 | TP53INP1 | |
| CCL22 | IGFALS | ITGA5 | TNFRSF10B | CTNNB1 | ITGA1 | RIPK1 | TP63 | |
| CCL23 | IGFBP1 | ITGA6 | TNFRSF10C | CTNND1 | ITGA10 | RIPK2 | TRAF3 | |
| CCL24 | IGFBP2 | ITGA8 | TNFRSF10D | DBN1 | ITGA11 | RIPK3 | TRAF3IP2 | |
| CCL25 | IGFBP3 | ITGA9 | TNFRSF11A | DCTN1 | ITGA2 | RPS6KA1 | TRAF6 | |
| CCL26 | IGFBP4 | ITGA9-AS1 | TNFRSF11B | DDIT3 | ITGA2B | RPS6KA2 | TRAP1 | |
| CCL27 | IGFBP5 | ITGAD | TNFRSF12A | DDIT4 | ITGA3 | RPS6KA2-AS1 | TRIM14 | |
| CCL28 | IGFBP6 | ITGAE | TNFRSF13B | DDX58 | ITGA4 | RPS6KA2-IT1 | TRIM16 | |
| CCL3 | IGFBP7 | ITGAL | TNFRSF13C | DDX60L | JUN | RPS6KA5 | TRIM16L | |
| CCL3L1 | IL10 | ITGAM | TNFRSF14 | EDARADD | JUND | S100A1 | TRIM21 | |
| CCL3L3 | IL10RA | ITGAV | TNFRSF17 | EGF | JUP | S100A10 | TRIM22 | |
| CCL4 | IL10RB | ITGAX | TNFRSF18 | EGR1 | KLF3 | S100A11 | TRIM25 | |
| CCL4L1 | IL10RB-AS1 | ITGB1 | TNFRSF19 | EGR2 | KLF4 | S100A12 | TRIM36 | |
| CCL4L2 | IL11 | ITGB1BP1 | TNFRSF1A | EGR3 | KLF5 | S100A13 | TRIM37 | |
| CCL5 | IL11RA | ITGB1BP2 | TNFRSF1B | EGR4 | KLF6 | S100A14 | TRIM38 | |
| CCL7 | IL12A | ITGB2 | TNFRSF21 | EIF4EBP1 | KLF7 | S100A16 | TRIM4 | |
| CCL8 | IL12A-AS1 | ITGB2-AS1 | TNFRSF4 | EPOR | KRAS | S100A2 | TRIM46 | |
| CD47 | IL12B | ITGB6 | TNFRSF6B | ERCC1 | LMNB1 | S100A3 | TRIM47 | |
| CD55 | IL12RB1 | ITGB8 | TNFRSF8 | ESR1 | LRP1 | S100A7 | TRIM5 | |
| CD58 | IL12RB2 | MMP1 | TNFRSF9 | FADS1 | LRP10 | S100A7A | TRIM6 | |
| CD59 | IL13 | MMP10 | TNFSF10 | FADS3 | LRP11 | S100P | TRPV1 | |
| CD6 | IL13RA1 | MMP11 | TNFSF11 | FAS | MAP3K4 | SDHC | SLC10A7 | |
| CD63 | IL13RA2 | MMP12 | TNFSF12 | FAS-AS1 | MAP3K5 | SESN1 | SLC11A2 | |
| CD68 | IL15 | MMP13 | TNFSF12-TNFSF13 | FGF1 | MAP3K8 | SESN2 | SLC12A2 | |
| CD69 | IL16 | MMP14 | TNFSF13 | FGF11 | MAP3K9 | SESN3 | SLC12A4 | |
| CD74 | IL17A | MMP19 | TNFSF13B | FGF13 | MAX | SHC1 | SLC12A6 | |
| CD81 | IL17B | MMP2 | TNFSF14 | FGF2 | MDM2 | SIN3A | SLC12A8 | |
| CD83 | IL17C | MMP20 | TNFSF15 | FGF5 | MSRA | SIRT1 | SLC15A3 | |
| CD9 | IL17RA | MMP21 | TNFSF18 | FGFBP1 | MSRB1 | SIRT2 | SLC16A1 | |
| CD93 | IL1A | MMP23A | TNFSF4 | FGFR2 | MSRB2 | SIRT3 | SLC16A13 | |
| CD96 | IL1B | MMP23B | TNFSF8 | FOS | MXD1 | SIRT4 | SLC16A8 | |
| CD99 | IL1F10 | MMP26 | TNFSF9 | FOSB | MXD3 | SIRT5 | SLC16A9 | |
| CSF1 | IL1R1 | MMP27 | ADH5 | FOSL1 | MXD4 | SIRT6 | SLC1A4 | |
| CSF1R | IL1R2 | MMP28 | ADH7 | FOSL2 | MXRA5 | SIRT7 | SLC20A2 | |
| CSF2 | IL1RAP | MMP3 | AGPAT4 | FOXL1 | MXRA7 | SNCG | SLC22A13 | |
| CSF2RA | IL1RAPL1 | MMP7 | APOC3 | FOXL2 | MYC | SOCS1 | SLC22A20 | |
| CSF2RB | IL1RAPL2 | MMP8 | APOE | FOXL2NB | NBN | SOCS2 | SLC24A1 | |
| CSF3 | IL1RL1 | OAS1 | APOF | GCK | NFE2 | SOCS2-AS1 | SLC25A12 | |
| CSF3R | IL1RL2 | OAS2 | APOH | GPX1 | NFE2L1 | SOCS3 | SLC25A21 | |
| CXCL1 | IL1RN | OAS3 | APOL1 | GPX2 | NFKB1 | SOCS4 | SLC25A21-AS1 | |
| CXCL10 | IL2 | OASL | APOL2 | GPX3 | NFKBIA | SOCS5 | SLC25A25-AS1 | |
| CXCL11 | IL21 | PTGES | APOL3 | GPX8 | NGFR | SOCS6 | SLC25A28 | |
| CXCL12 | IL21-AS1 | RPS6KA2 | APOL4 | GRN | NGFRAP1 | SOCS7 | SLC25A29 | |
| CXCL13 | IL21R | RPS6KA3 | APOL5 | GSK3B | NLRP2 | SOD1 | SLC26A1 | |
| CXCL14 | IL21R-AS1 | RPS6KC1 | APOL6 | GSS | NR3C1 | SOD2 | SLC26A11 | |
| CXCL16 | IL22 | SERP1 | AREG | GSTA4 | NR4A1 | SOD3 | SLC28A3 | |
| CXCL17 | IL22RA1 | SERP2 | ARNTL | HESX | NR4A3 | OAS1 | SLC2A6 | |
| CXCL2 | IL22RA2 | SERPINA1 | ARNTL2 | HELLS | NRG1 | OAS2 | SLC2A9 | |
| CXCL3 | IL23A | SERPINA10 | ARNTL2-AS1 | HGF | PAPPA | OAS3 | SLC30A9 | |
| CXCL5 | IL23R | SERPINA11 | ATF5 | HIF1A | PIAS1 | OASL | SLC36A1 | |
| CXCL6 | IL24 | SERPINA12 | ATG10 | HSP90AA1 | PIAS2 | STAT1 | SLC36A4 | |
| CXCL8 | IL25 | SERPINA13P | ATG101 | HSP90B2P | PIAS3 | STAT2 | SLC38A6 | |
| CXCL9 | IL26 | SERPINA2 | ATG14 | HSPA12A | PIAS4 | STAT3 | SLC39A11 | |
| CXCR1 | IL27 | SERPINA3 | ATG2A | HSPA14 | PIK3C2G | STAT4 | SLC39A8 | |
| CXCR2 | IL27RA | SERPINA4 | ATG2B | HSPA1A | PIK3CA | STAT5A | SLC39A9 | |
| CXCR2P1 | IL2RA | SERPINA5 | ATG4A | HSPA4L | PIK3CD | STAT5B | SLC5A5 | |
| CXCR3 | IL2RB | SERPINA6 | ATM | HTT | PLAU | STAT6 | SLC6A14 | |
| CXCR4 | IL2RG | SERPINA7 | BAK1 | IFI16 | PLAUR | STUB1 | SLC6A15 | |
| CXCR5 | IL3 | SERPINA9 | BAX | IFI27 | PLCG2 | TAB2 | SLC6A16 | |

Table S5. GSEA (SASP gene set).

| | | | | | | | |
|-------------|--------|---------|------------|-----------|-----------|------------|-----------------|
| AREG | CCL7 | CXCR2P1 | IL10RB | IL25 | ITGAE | SERPINA1 | TNFRSF10D |
| AXL | CCL8 | CXCR3 | IL10RB-AS1 | IL26 | ITGAL | SERPINA10 | TNFRSF11A |
| BMP1 | CD47 | CXCR4 | IL11 | IL27 | ITGAM | SERPINA11 | TNFRSF11B |
| BMP10 | CD55 | CXCR5 | IL11RA | IL27RA | ITGAV | SERPINA12 | TNFRSF12A |
| BMP15 | CD58 | CXCR6 | IL12A | IL2RA | ITGAX | SERPINA13P | TNFRSF13B |
| BMP2 | CD59 | FGF16 | IL12A-AS1 | IL2RB | ITGB1 | SERPINA2 | TNFRSF13C |
| BMP3 | CD6 | FGF17 | IL12B | IL2RG | ITGB1BP1 | SERPINA3 | TNFRSF14 |
| BMP4 | CD63 | FGF18 | IL12RB1 | IL3 | ITGB1BP2 | SERPINA4 | TNFRSF17 |
| BMP5 | CD68 | FGF19 | IL12RB2 | IL31 | ITGB2 | SERPINA5 | TNFRSF18 |
| BMP6 | CD69 | FGF21 | IL13 | IL31RA | ITGB2-AS1 | SERPINA6 | TNFRSF19 |
| BMP7 | CD74 | FGF7 | IL13RA1 | IL32 | ITGB6 | SERPINA7 | TNFRSF1A |
| BMP7-AS1 | CD81 | FGF8 | IL13RA2 | IL33 | ITGB8 | SERPINA9 | TNFRSF1B |
| BMPER | CD83 | GDF1 | IL15 | IL34 | MMP1 | SERPINB1 | TNFRSF21 |
| CCL1 | CD9 | GDF10 | IL16 | IL36A | MMP10 | SERPINB10 | TNFRSF4 |
| CCL11 | CD93 | GDF11 | IL17A | IL36B | MMP11 | SERPINB11 | TNFRSF6B |
| CCL13 | CD96 | GDF15 | IL17B | IL36G | MMP12 | SERPINB12 | TNFRSF8 |
| CCL14 | CD99 | GDF2 | IL17C | IL36RN | MMP13 | SERPINB13 | TNFRSF9 |
| CCL15 | CSF1 | GDF3 | IL17RA | IL37 | MMP14 | SERPINB2 | TNFSF10 |
| CCL15-CCL14 | CSF1R | GDF5 | IL1A | IL3RA | MMP19 | SERPINB3 | TNFSF11 |
| CCL16 | CSF2 | GDF6 | IL1B | IL4 | MMP2 | SERPINB4 | TNFSF12 |
| CCL17 | CSF2RA | GDF7 | IL1F10 | IL4I1 | MMP20 | SERPINB5 | TNFSF12-TNFSF13 |
| CCL18 | CSF2RB | GDF9 | IL1R1 | IL4R | MMP21 | SERPINB6 | TNFSF13 |
| CCL19 | CSF3 | HGF | IL1R2 | IL5 | MMP23A | SERPINB7 | TNFSF13B |
| CCL2 | CSF3R | IGF1 | IL1RAP | IL5RA | MMP23B | SERPINB8 | TNFSF14 |
| CCL20 | CXCL1 | IGF1R | IL1RAPL1 | IL6 | MMP26 | SERPINB9 | TNFSF15 |
| CCL21 | CXCL10 | IGF2 | IL1RAPL2 | IL6R | MMP27 | SERPINB9P1 | TNFSF18 |
| CCL22 | CXCL11 | IGF2-AS | IL1RL1 | IL6ST | MMP28 | SERPINC1 | TNFSF4 |
| CCL23 | CXCL12 | IGF2BP1 | IL1RL2 | IL7R | MMP3 | SERPIND1 | TNFSF8 |
| CCL24 | CXCL13 | IGF2BP2 | IL1RN | IL9 | MMP7 | SERPINE1 | TNFSF9 |
| CCL25 | CXCL14 | IGF2R | IL2 | ITGA1 | MMP8 | SERPINE2 | TGFB1 |
| CCL26 | CXCL16 | IGFALS | IL21 | ITGA10 | OAS1 | SERPINE3 | TGFB2 |
| CCL27 | CXCL17 | IGFBP1 | IL21-AS1 | ITGA11 | OAS2 | SERPINF1 | TGFB3 |
| CCL28 | CXCL2 | IGFBP2 | IL21R | ITGA3 | OAS3 | SERPINF2 | |
| CCL3 | CXCL3 | IGFBP3 | IL21R-AS1 | ITGA4 | OASL | SERPINF1 | |
| CCL3L1 | CXCL5 | IGFBP4 | IL22 | ITGA5 | PTGES | SERPINH1 | |
| CCL3L3 | CXCL6 | IGFBP5 | IL22RA1 | ITGA6 | RPS6KA2 | SERPINI1 | |
| CCL4 | CXCL8 | IGFBP6 | IL22RA2 | ITGA8 | RPS6KA3 | SERPINI2 | |
| CCL4L1 | CXCL9 | IGFBP7 | IL23A | ITGA9 | RPS6KC1 | TNFRSF10A | |
| CCL4L2 | CXCR1 | IL10 | IL23R | ITGA9-AS1 | SERP1 | TNFRSF10B | |
| CCL5 | CXCR2 | IL10RA | IL24 | ITGAD | SERP2 | TNFRSF10C | |