

Early growth response 1 regulates hematopoietic support and proliferation in human primary bone marrow stromal cells

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1. Supplemental Materials and Methods

BM-MNC isolation

Bone marrow mononuclear cells (BM-MNC) from BM aspiration samples were isolated by density gradient centrifugation (LSM 1077 Lymphocyte, PAA, Pasching, Austria) either with or without prior incubation with RosetteSep Human Mesenchymal Stem Cell Enrichment Cocktail (STEMCELL Technologies, Vancouver, Canada) for lineage depletion (CD3, CD14, CD19, CD38, CD66b, glycophorin A).

BM-MNCs from fetal long bones and adult hip bones were isolated as reported previously¹ by gently crushing bones (femora, tibiae, fibulae, humeri, radii and ulna) in PBS+0.5% FCS subsequent passing of the cell suspension through a 40- μ m filter. Bone fragments were digested with 0.25% collagenase type I (STEMCELL Technologies) for 45 minutes at 37°C. Excess PBS was added to the solution and cells were filtered through a 40 μ m filter. Then, cell suspensions were washed once with PBS+0.5% FCS and erythrocytes were lysed with IOTest 3 lysing solution (Beckman Coulter, Brea, California, United States) according to manufacturer's instructions.

Generation of EGR1 knockdown and EGR1 overexpressing stroma cells

Sorted BMSCs (CD45⁻CD271⁺CD140a⁻) were cultured in standard MSC culture medium [StemMACS MSC Expansion Media, human (Miltenyi Biotec, Bergisch Gladbach, Germany) + 1% antibiotic-antimycotic solution (Sigma, St. Louis, USA)]. Medium was changed weekly and cells were passaged at 80% confluency after trypsinization (0.05% trypsin/EDTA, Invitrogen, Carlsbad, USA). EGR1 knockdown BMSCs, EGR1 overexpressing BMSCs and their respective controls were generated by infecting stromal cells (passage 2-4) with shEGR1-GFP, scramble control-GFP (both from OriGene, Rockville, United States), EGR1-

P2A-GFP and GFP-encoding lentivirus VSV-G (Virus Core Facility, Lund Stem Cell Center, Lund University, Lund, Sweden) at a MOI of 5. Lentiviral vectors carrying shEGR1 (TL313277) and scramble control (TR30021) were from OriGene. Three days after infection, GFP expressing cells were sorted by flow cytometry, followed by expansion in culture. For assessment of growth kinetics, population doubling times (PD) were calculated as $PD = [\text{culture duration}] / [\log_2(\text{final cell number}) - \log_2(\text{initial cell number})]$, with culture duration expressed in days.

Flow cytometry and fluorescence activated cell sorting (FACS)

Lineage-depleted BM-MNCs were incubated in blocking buffer [DPBS w/o Ca^{2+} , Mg^{2+} , 3.3 mg/ml human normal immunoglobulin (Gammanorm, Octapharm, Stockholm, Sweden), 1% FBS (Invitrogen)], followed by staining with monoclonal antibodies against CD271, CD140a, and CD45. Sorting gates were set according to the corresponding fluorescence-minus-one (FMO) controls and cells were sorted on a FACS Aria II or Aria III (BD Bioscience, Erembodegem, Belgium). Dead cells were excluded by 7-Amino-actinomycin (7-AAD, Sigma) staining and doublets were excluded by gating on FSC-H versus FSC-W and SSC-H versus SSC-W.

For sorting of BM cells for RNA-seq analysis, antibody incubations were performed in PBS/0.5% FCS for 20 minutes on ice in the dark. Cell populations of interest were sorted using a FACS Aria III Cell Sorter (BD). Dead cells were gated out using 7AAD (Stem-Kit Reagents) after MNC selection and doublets exclusion. Cells were directly sorted into 800 μ l Trizol (Ambion) for isolation of RNA for RNA-seq analysis. RNA for qPCR analysis of sorted cells was isolated as described below.

Cultured cells were harvested, washed, and unspecific binding was blocked with human normal immunoglobulin. Cells were stained (45 min, 4°C) with combinations of antibodies and samples were analyzed on a LSRII or LSRFortessa (BD).

ROS levels were measured by incubating cells with CellROX Deep Red Reagent (Thermo Fisher Scientific, Waltham, USA) at a final concentration of 5 µM at 37°C for 30 minutes followed by FACS analysis.

Cell cycle analysis was performed by flow cytometry (LSRII, BD) using Lysis buffer with 10 µl/ml DAPI and Stabilization buffer according to the manufacturer's instruction (ChemoMetec, Lillerod, Denmark).

Antibodies

For FACS analysis and cell sorting the following antibodies were used: CD45-FITC (fluorescein isothiocyanate) (clone 2D1), CD34-FITC (clone 581), HLA-DR-FITC (clone L243), CD14-PE (clone MφP9), CD19-PE (clone SJ25C1), CD73-PE (clone AD2), HLA-class I-PE (clone G46-2.6), CD90-APC (allophycocyanin) (clone 5E10), CD45-APC-Cy7 (clone 2D1), CD34-PE-Cy7 (clone 8G12) (all BD Bioscience), CD271-APC (clone ME20.4-1.H4, Miltenyi). Matching isotype controls were from BD Bioscience and R&D Systems. For unconjugated primary antibodies, goat anti-mouse IgG2a-FITC and goat anti-mouse IgM-FITC (Jackson ImmunoResearch Laboratories, Inc., Suffolk, UK) were used as secondary antibodies. Neutralizing antibody specific to CCL28 (clone MM0153-9G34), VCAM1 (clone B-K9) and IgG control (clone 15-6E10A7) were from Abcam (Cambridge, UK).

For RNA-seq cell sorting, the following antibody were used CD45 (clone HI30), CD271 (clone ME20.4), CD235a (clone HI264), CD31 (clone WM59), CD9 (clone HI9a) from Biolegend, and CD105 (clone SN6) from eBioscience.

CFU-F (colony-forming unit, fibroblast) assay

FACS-sorted cells were cultured at plating densities of 10-50 cells/cm² when assaying EGR1 knockdown and EGR1 overexpressing stromal cells and their respective controls. Colonies were counted after 14 days (1% Crystal Violet, Sigma). Generally, assays were set up in duplicates or triplicates.

***In vitro* differentiation assays**

Cultured BM mesenchymal stromal cells were differentiated towards the adipogenic, osteoblastic, and chondrogenic lineage as described previously². Briefly, cells were cultured for 14 days in AdipoDiff medium (Miltenyi) and cells were stained with Oil Red O (Sigma). For osteogenic differentiation, cells were cultured in osteogenesis induction medium for 21 days and calcium depositions were detected by Alizarin Red staining (Sigma). Osteogenesis induction medium: standard MSC medium supplemented with 0.05 mM L-ascorbic-acid-2-phosphate (Wako Chemicals, Neuss, Germany), 0.1 μM dexamethasone and 10 mM β-glycerophosphate (both from Sigma).

Chondrogenic differentiation was induced by culturing cell pellets (2.5×10^5 cells/pellet) for 28 days in chondrogenesis induction medium. Chondrogenesis induction medium: DMEM-high glucose supplemented with 0.1 μM dexamethasone, 1 mM sodium pyruvate, 0.35 mM L-proline (all from Sigma), 0.17 mM ascorbic acid, 1% ITS+ culture supplements (BD Biosciences) and 0.01 μg/ml TGF-β3 (R&D Systems).

Pellets were paraformaldehyde (PFA)-fixed, and frozen in O.C.T. Compound (Sakura, Zoeterwoude, Netherlands). Cryo sections were stained with antibodies against aggrecan, and nuclei were stained with 4',6-diamidino-2-phenylindole (DAPI, Invitrogen). Sections were

analyzed with an Olympus BX51 (Olympus, Solna Sweden) fluorescence microscope and a DP70 Olympus digital camera (DP manager software Version 1.1.1.71).

Cytokines

Stem cell factor (SCF), thrombopoietin (TPO), and FLT3-ligand (FLT3L), all at 25 ng/ml, [STF25] and 100 ng/ml of CCL28 were used in the CB co-culture expansion experiments. All growth factors were from Peprotech, Rocky Hill, USA.

Quantitative real-time PCR

RNA from sorted primary BM $\text{lin}^- \text{CD45}^- \text{CD271}^+ \text{CD140a}^-$ and $\text{lin}^- \text{CD45}^- \text{CD271}^+ \text{CD140a}^+$ and $\text{lin}^- \text{CD45}^- \text{CD271}^- \text{CD140a}^-$ populations was isolated from three individual donors. RNA was isolated using QIAshredder Homogenizers columns and the RNeasy Micro Kit (both from Qiagen, Hilden, Germany) according to the manufacturer's protocol. The concentration and purity of RNA was determined by Nanodrop (Thermo Fisher Scientific). cDNA was synthesized using SuperScript VILO cDNA synthesis kit (Life Technologies, Carlsbad, USA) on a C1000TM Thermal Cycler (Bio-Rad, Hercules, CA, USA). Quantitative real-time PCR analysis was carried out using Fast SYBR master mix (Applied Biosystems by Life Technologies) according to the manufacturer's instructions. The crossing point of each sample was measured and analyzed with StepOne Software v2.1 (Applied Biosystems). Each gene-specific mRNA was normalized to the housekeeping gene *Glyceraldehyde 3-phosphate dehydrogenase (GAPDH)* mRNA. The expression of each mRNA was determined using the $2^{-\Delta\Delta\text{CT}}$ threshold cycle method.

ROS inhibitor treatment

For the ROS inhibition assay experiments, L-ascorbic acid, citric acid monohydrate, 4-Aminobenzoic acid, LY2228820, apocynin and N-acetylcystein (NAC) were added to the cultures. ROS inhibitors used: L-ascorbic acid (Sigma, St. Louis, USA) at 0.378 mg/ml, citric acid monohydrate (Thermo Fisher, Pittsburgh, USA) at 0.158 mg/ml, myeloperoxidase (MPO) blocker 4-Aminobenzoic acid (Sigma) at 100 μ M, p38 mitogen-activated protein kinase (MAPK) inhibitor LY2228820 (Selleckchem, Houston, USA) at 500 nM, NADPH oxidase inhibitor apocynin at 100 μ M and N-acetylcystein (NAC) at 10 mM. Inhibitors were dissolved in dimethyl sulfoxide (DMSO) (Sigma, St. Louis, USA).

***In vivo* HSC repopulation assay**

Eight- to twelve-week old NOD.Cg-PrkdcscidIl2rgtmlWjl/SzJ (NOD/SCID-IL2R γ c null; NSG) mice (Jackson Laboratory, Bar Harbor, USA) were sublethally irradiated (250 cGy) 6 hours prior to transplantation. The culture equivalent of 50,000 input CD34⁺ cells of *ex vivo* co-cultured CB CD34⁺ cells was injected intravenously and human hematopoietic engraftment was assessed 8, 12 and 16 weeks after transplantation by flow cytometry (human-specific antibodies against CD45, CD15/CD33/CD66b and CD19). All animal procedures were approved by the local ethical committee on animal experiments.

CCL28 ELISA

The levels of secreted CCL28 protein were quantified using the CCL28 Human ELISA Kit (Abcam, Cambridge, UK) according to the manufacturer's instructions. Culture supernatants were collected, centrifuged at 1,000 \times g for 15 min at 4^o C, and assayed by ELISA. Microplate readings were analyzed with the SoftMax Pro 6.4 software (Molecular Devices, CA, USA).

Illumina microarray data analysis

Total RNA isolated from shEGR1-GFP, scramble-GFP, EGR1-OE-GFP and control-GFP stroma cells were subjected to a two-round amplification using the TargetAmp 2-round biotin-aRNA amplification kit (Epicentre Biotechnologies, Madison, USA). Microarray analysis was performed by the SCIBLU Swegene center at Lund University using Illumina Human HT-12 expression v4 BeadChips comprising of 48,107 probes according to the manufacturer's instructions. Microarray data were initially pre-processed and normalized using Quantile Normalization method ³. These analyses were performed using GenomeStudio software V2011.1. Non-annotated probe sets and probe sets with signal intensities below the median of the negative control intensities in 20% of the samples that did not belong to one condition were excluded. To identify significantly differentially expressed genes between shEGR1-GFP and scramble-GFP cells, between EGR1OE-GFP and control-GFP cells, we used significance analysis of microarrays (SAM) method with the q value of 0 for the comparison between EGR1 OE and control-GFP and q value of 10 for the comparison between shEGR1-GFP and scramble-GFP cells ⁴. SAM analyses were performed using TMEV v4.0 software ⁵. Up and down-regulated genes were tested for enriched GO terms using DAVID Bioinformatics Resources 6.8.

RNA-seq

RNA of sorted cells was extracted according to the manufacturer's instructions for RNA isolation with GenElute LPA (Sigma). cDNA was prepared using the SMARTer procedure (SMARTer Ultra Low RNA Kit, Clontech, Takara, St-Germain-en-Laye, France). Library preparation and RNA-seq was performed as previously described and validated for low input ⁶.

Proteome analysis

Non-transfected control, scrambled control and shEGR1 BM stromal cells were prepared for proteomic analysis as previously reported ⁷. For sample preparation, cells were lysed with 0.1% RapiGest (Waters) in 100 μ l 50 mM ammonium bicarbonate and extracted proteins were reduced/alkylated and digested with sequencing grade modified trypsin (Promega). Protein digests were differentially dimethyl labelled on columns as previously described ⁷. Briefly, samples were labelled by flushing the columns with labelling reagent (light, intermediate or heavy using $\text{CH}_2\text{O} + \text{NaBH}_3\text{CN}$, $\text{CD}_2\text{O} + \text{NaBH}_3\text{CN}$ or $^{13}\text{CD}_2\text{O} + \text{NaBD}_3\text{CN}$, respectively). Sample complexity was reduced by fractionation using OFFGEL isoelectric focusing (Agilent). The 12 fractions resolved were acidified and desalted with C18 Ultra-Micro SpinColumns (Harvard). Samples were reconstituted in 4% acetonitrile/0.1% formic acid prior to MS analysis.

MS analyses were carried out on an Orbitrap Fusion Tribrid MS system (Thermo Scientific) equipped with a Proxeon Easy-nLC 1000 (Thermo Fisher). On a 110 min gradient, one full scan spectrum from m/z 375 to 1500 at resolution 60,000 FWHM was followed by MS/MS scans (resolution 15,000 FWHM) of the most intense ions (up to 15) from the full scan MS.

MS raw data files were processed with MaxQuant (version 1.5.0.0) ⁸. The derived peak list was searched using the in-built Andromeda search engine in MaxQuant against the Uniprot human database (downloaded 2016.10.13) together with 265 frequently observed contaminants (Andromeda configured database) and reversed sequences of all entries. A 1% false discovery rate (FDR) was required at both the protein level and the peptide level. Criteria for top differentially expressed proteins was set as the product of \log_2 ratios of shEGR1 to the two controls. The protein identification was reported as an indistinguishable “protein group” if no unique peptide sequence to a single database entry was identified.

Deposition of gene expression and proteomics data

Normalized illumina array data have been deposited in the GEO database (GSE122831). RNA-seq data can be accessed at the European Genome-phenome Archive: EGAS00001002736. The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE ⁹ partner repository with the dataset identifier PXD011767.

Primer sequences for qRT-PCR analysis*

<i>GAPDH</i>	F	5'- CACTCCACCTTTGACGC -3'
	R	5'- GGTCCAGGGGTCTTACTCC -3'
<i>EGR1</i>	F	5'- ACCCCTCTGTCTACTATTAAGGC-3'
	R	5'- TGGGACTGGTAGCTGGTATTG-3'
<i>EGR2</i>	F	5'- TCAACATTGACATGACTGGAGAG-3'
	R	5'- AGTGAAGGTCTGGTTTCTAGGT-3'
<i>EGR3</i>	F	5'- GCGACCTCTACTCAGAGCC-3'
	R	5'- CTTGGCCGATTGGTAATCCTG-3'
<i>EGR4</i>	F	5'- GGAGGCTCGTTTTCCCGTAAT-3'
	R	5'- TGGGATAGAGTCTGTTGGCTG-3'

*All primers were obtained from Life Technologies.

2. Supplemental Figures

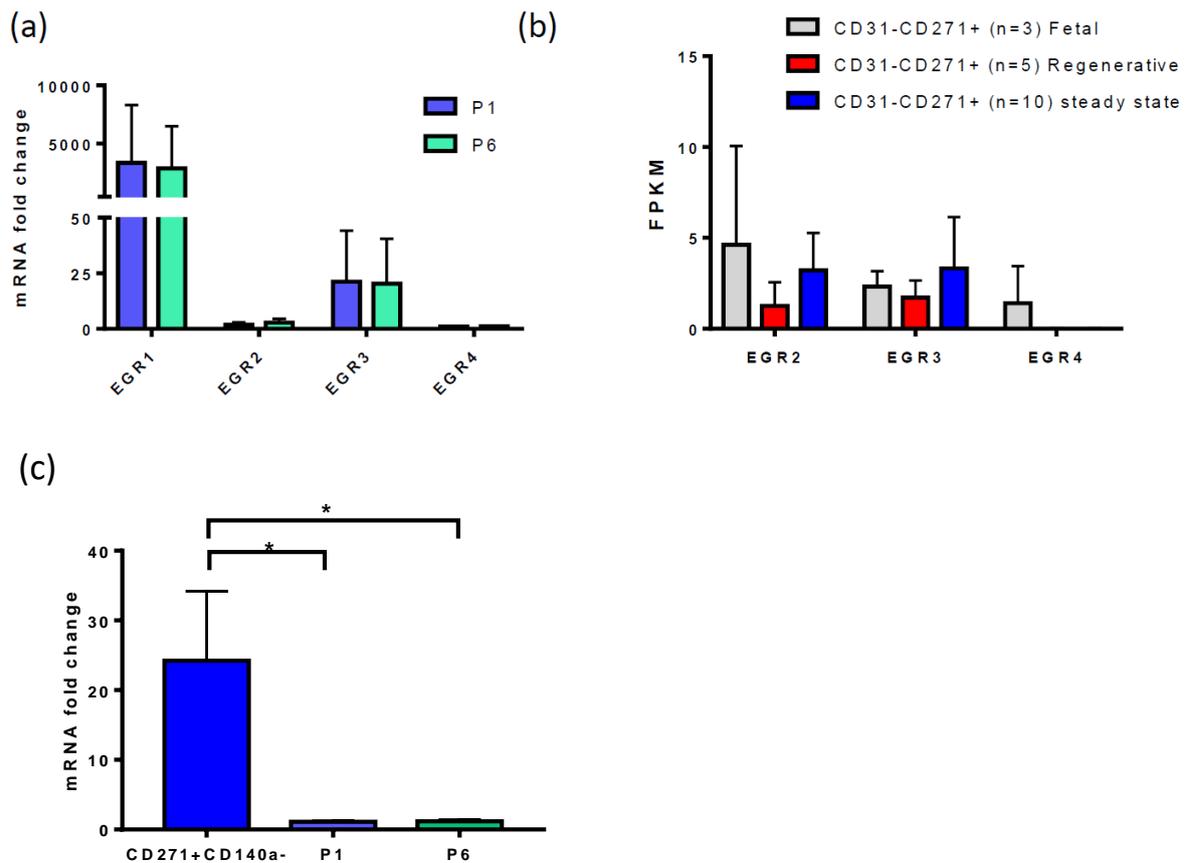
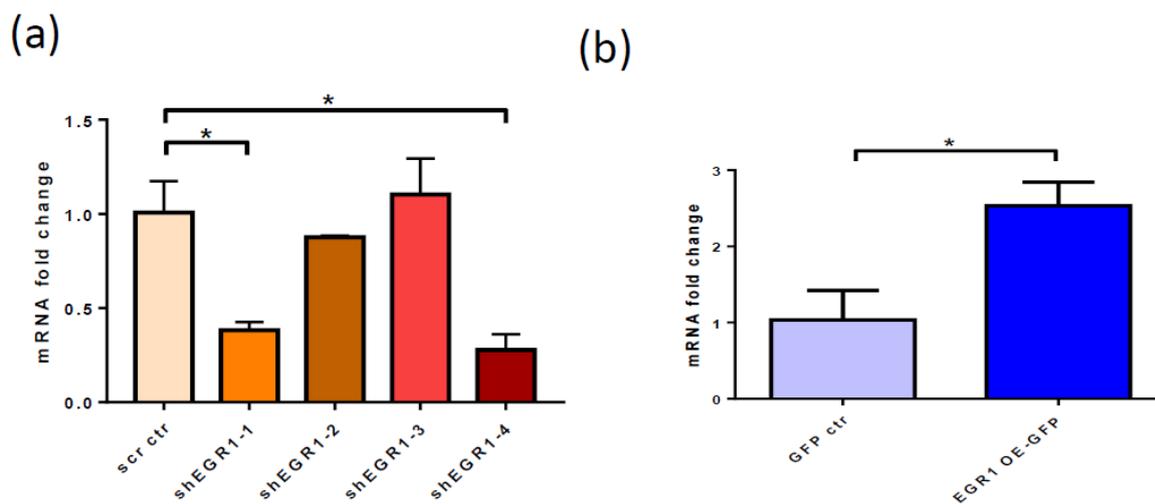
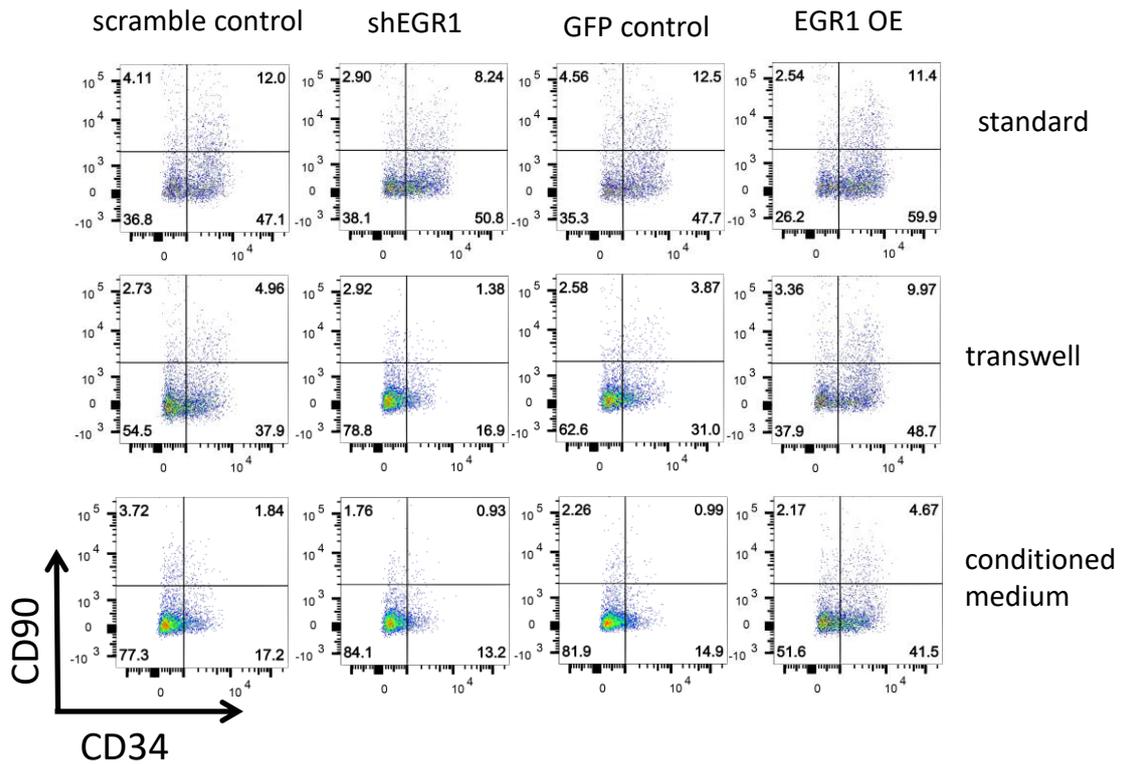


Figure S1

(a) Quantitative rtPCR of EGR1-4 expression in passage 1 (P1) and passage 6 (P6) BMSCs. Results are shown as fold mRNA change after standardizing with GAPDH levels. Data are shown as mean \pm SD (n=3). (b) Transcript analysis by massive parallel RNA sequencing of EGR2-4 expression in BMSCs (CD31⁻CD271⁺) isolated from human fetal, regenerative and steady-state BMSCs. Data are shown as mean \pm SEM for (a) and mean \pm SEM for (b), n=3-10. (c) EGR1 expression differences in freshly isolated Lin⁻/CD45⁻/CD271⁺/CD140a⁻ compared to cultured stromal cells in passage 1 (P1) and passage 6 (P6). Gene expression is calculated relative to P1 MSCs. Data are shown as mean \pm SD (n=3). *:p < 0.05

**Figure S2**

Quantitative rtPCR of EGR1 expression in BMSCs 72 h after infection with lentiviral particles encoding shEGR1 1-4 (a) or EGR1 overexpression plasmid (b). Data are shown as mean \pm SD (n=3). *: $p < 0.05$

**Figure S3**

Five thousand cord blood CD34⁺ cells were co-cultured for 4 days with 10,000 bone marrow derived feeder MSCs transfected with scramble control, shEGR1, GFP control and EGR1 overexpression plasmids, respectively, in SFEM supplemented with 25 ng/ml of SCF, TPO and Flt3L. Co-cultures were performed in either standard culture plates (standard) or transwell culture plates with the MSCs in the bottom well and CD34⁺ cells in the insert (transwell). For conditioned medium cultures, 10,000 bone marrow derived MSCs transfected with scramble control, shEGR1, GFP control and EGR1 overexpression plasmids, respectively, were cultured with 200 μ l SFEM supplemented with 25 ng/ml of SCF, TPO and Flt3L for four days. Conditioned media was collected and used to stimulate cultures with CB CD34⁺ cells (without feeder cells). Representative FACS profiles of co-culture generated cells are shown.

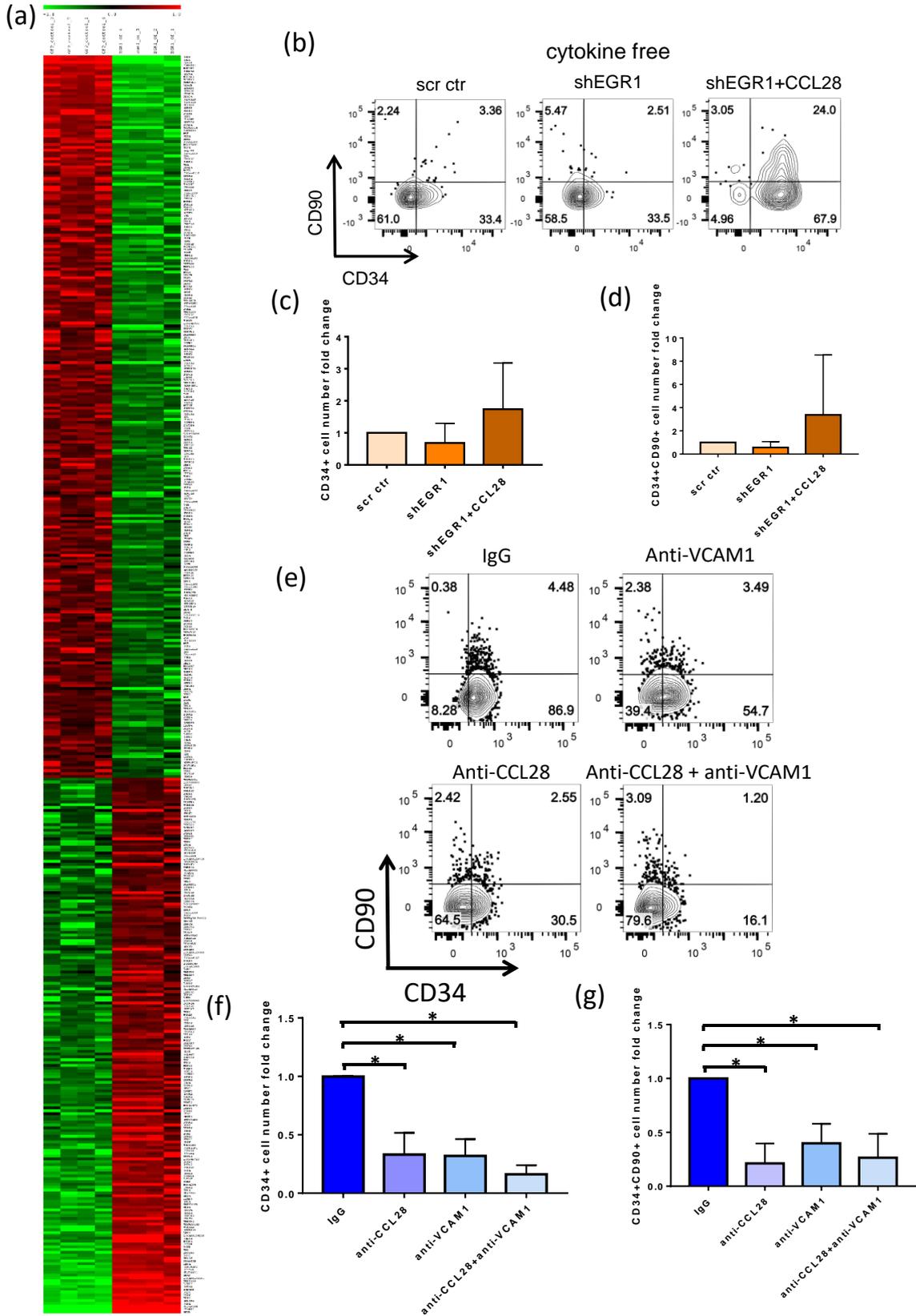
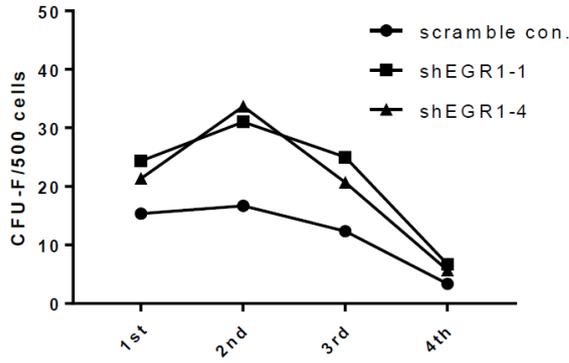


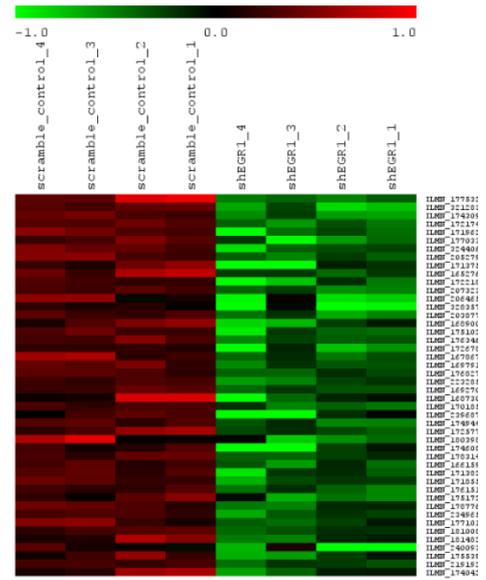
Figure S4

(a) Heatmap of significantly up- and down-regulated genes in EGR1 overexpressing cells versus controls. (b-d) 5,000 cord blood CD34⁺ cells were co-cultured for 4 days with 10,000 bone marrow derived feeder stromal cells transfected with scramble control and shEGR1 plasmids, respectively, in cytokine-free culture condition supplemented with or without 100 ng/ml CCL28. (b) Representative FACS profiles of co-culture generated cells in cytokine-free culture. The type of feeder cells is indicated on top of the FACS plots in. (c,d) Fold change of total numbers of CD34⁺ cells and CD34⁺CD90⁺ cells produced in cytokine-free cultures. (e-g) 5,000 cord blood CD34⁺ cells were co-cultured for 4 days with 10,000 EGR1 overexpressing cells as feeder cells in cytokine-free culture condition supplemented with neutralizing antibody against CCL28, VCAM1 and IgG control (all at 100 ng/ml) for 4 days. (e) Representative FACS profiles of co-culture generated cells. Total number of CD34⁺ cells (f) and CD34⁺CD90⁺ cells (g) produced in the co-cultures without/with neutralizing antibodies as indicated by the x-axis label. *: $p < 0.05$

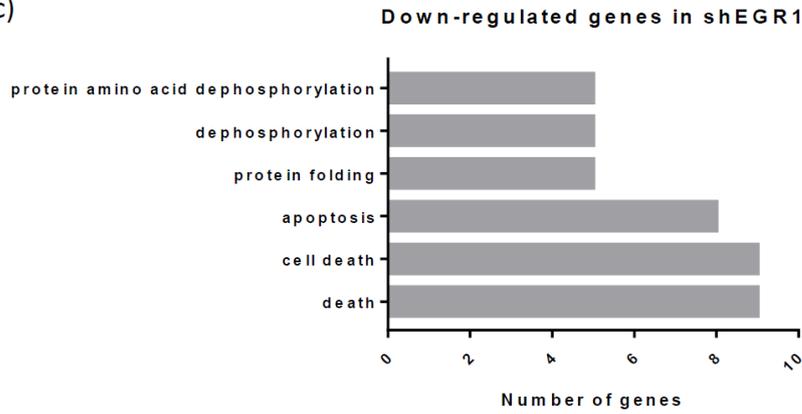
(a)



(b)



(c)



Down-regulated genes in EGR1OE

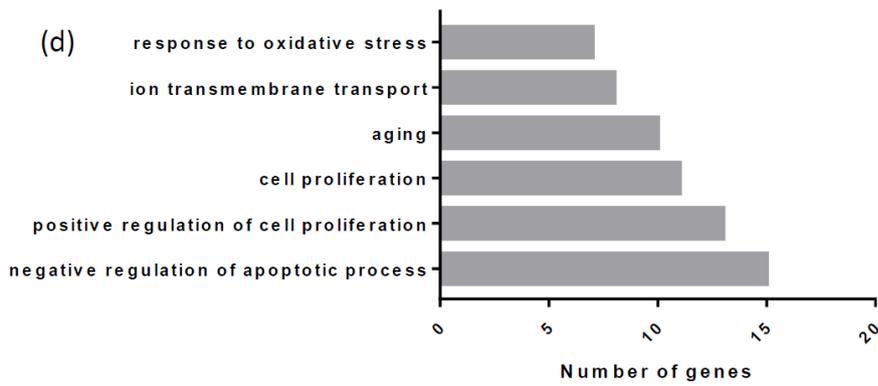


Figure S5

(a) EGR1 knockdown cells were harvested after 14 days and replated for CFU-F assay for four times. Representative CFU-F frequencies of three different experiments are presented as mean \pm SD. * $p < 0.05$. (b) Heatmap of significantly down-regulated genes in EGR1 knockdown cells versus controls. (c) Biological process annotations for down-regulated proteins in EGR1 knockdown cells and (d) EGR1 overexpressing cells were identified using the DAVID Bioinformatics Resources 6.8.

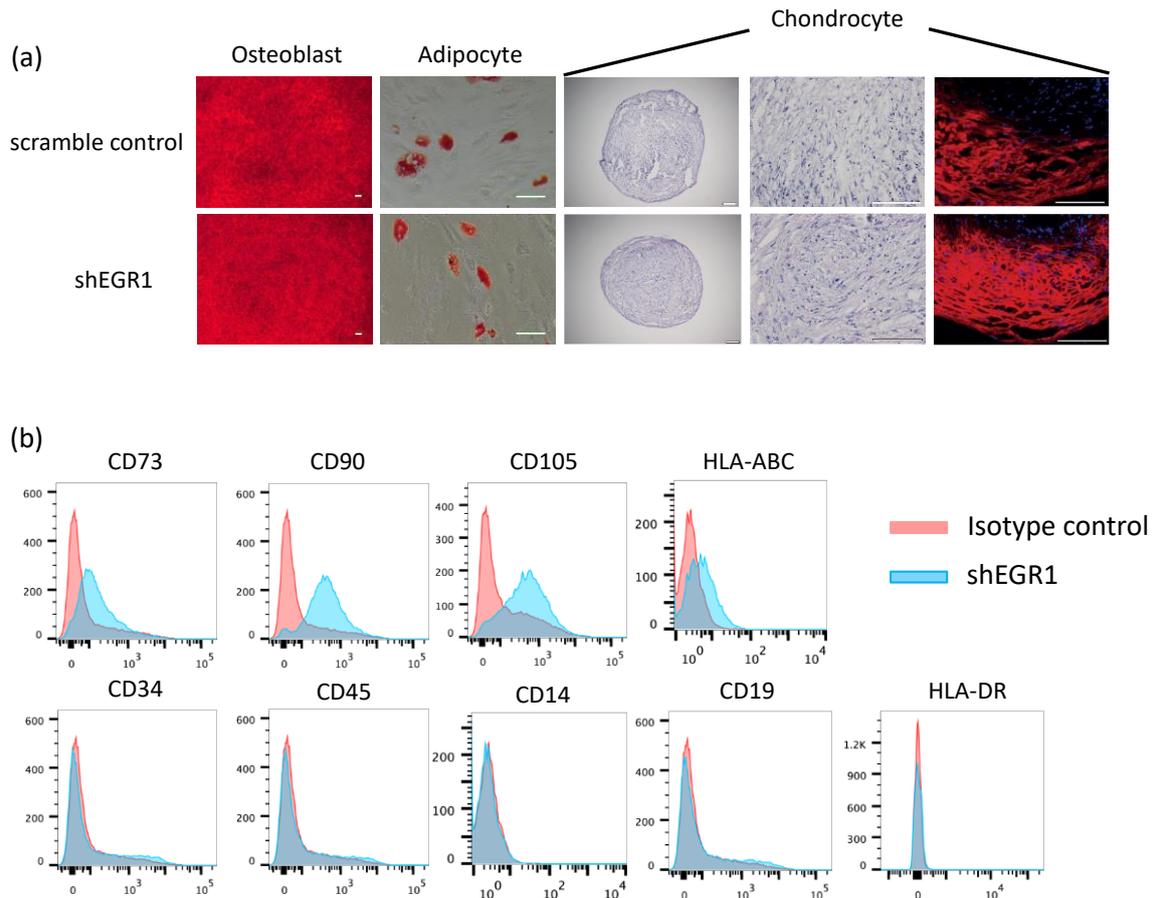


Figure S6

(a) Trilineage *in vitro* differentiation potential of EGR1 knockdown cells and controls. A representative set of pictures from a total of three replicates for each differentiation experiment is shown. Osteoblasts and adipocytes were stained with Alizarin red and oil-red-O, respectively. Chondrocyte pellets were stained with hematoxylin-eosin (HE) (left and middle panel) and antibodies against aggrecan (right panel). Scale bars represent 100 μm for all images. Differentiation capacity of EGR1 overexpressing cells could not be investigated due to insufficient cell numbers. (b) Typical BMSC surface marker profile of EGR1 knockdown cells; a representative set of data of a total of three experiments is shown. Blue: EGR1 knockdown cells; red: corresponding isotype control.

3. Supplemental Tables

Table S1. Up-regulated genes in EGR1 overexpressing cells

Gene name	Fold change*
<i>HEYL</i>	24.04682
<i>LOXL4</i>	5.469145
<i>LOC100132805</i>	5.333917
<i>CRABP2</i>	5.082554
<i>FLJ40504</i>	4.693288
<i>CALCA</i>	4.670778
<i>ABLIM1</i>	3.824112
<i>HBA2</i>	3.692209
<i>SYN1</i>	3.563409
<i>SYT7</i>	3.545673
<i>GSTA1</i>	3.504459
<i>LOC646723</i>	3.429366
<i>TMEM130</i>	3.394655
<i>PDPN</i>	3.379174
<i>TUBA4A</i>	3.363236
<i>DLX3</i>	3.342427
<i>ANKRD38</i>	3.334845
<i>PRAGMIN</i>	3.32348
<i>CCL28</i>	3.297285
<i>RPUSD2</i>	3.155637
<i>MFAP4</i>	3.100745
<i>CRLF1</i>	3.060728
<i>COL8A2</i>	3.015144
<i>LOC100134259</i>	3.014138
<i>HES4</i>	2.998961
<i>SEPT5</i>	2.925093
<i>RAB40B</i>	2.9152
<i>PCNX</i>	2.871909
<i>CPA4</i>	2.855123
<i>CDK5RAP2</i>	2.813803
<i>LITAF</i>	2.739239
<i>FLJ20021</i>	2.733041
<i>TNC</i>	2.722391
<i>CPSF4</i>	2.720022
<i>MARCH6</i>	2.71634
<i>NRCAM</i>	2.692568
<i>MGC4294</i>	2.668485
<i>EXTL1</i>	2.659776
<i>FBXO2</i>	2.639293
<i>LMO4</i>	2.637843

<i>DUSP6</i>	2.633063
<i>SLC29A1</i>	2.596552
<i>LMO4</i>	2.585314
<i>CCT6B</i>	2.559732
<i>TNFRSF10D</i>	2.525184
<i>COL8A2</i>	2.497526
<i>DIO2</i>	2.4887
<i>CCDC80</i>	2.481945
<i>RBM15</i>	2.478366
<i>SH3PXD2A</i>	2.456376
<i>ENPP2</i>	2.450576
<i>HLX1</i>	2.445158
<i>ADSS</i>	2.396962
<i>CENTD3</i>	2.341444
<i>P2RX6</i>	2.333991
<i>TMEM91</i>	2.333142
<i>TNFRSF21</i>	2.32309
<i>LEMD3</i>	2.32143
<i>RIMKLB</i>	2.314856
<i>TNC</i>	2.300494
<i>CCNB1IP1</i>	2.295839
<i>GOLGA7</i>	2.283916
<i>TMEM99</i>	2.268274
<i>SPG3A</i>	2.262277
<i>CXCL16</i>	2.255413
<i>TACSTD2</i>	2.254667
<i>SPG3A</i>	2.250582
<i>ITPK1</i>	2.24732
<i>GOT1</i>	2.231113
<i>ATL1</i>	2.224798
<i>AFAP1</i>	2.21616
<i>UPP1</i>	2.207567
<i>MRPS6</i>	2.196855
<i>KLHL29</i>	2.188431
<i>JAG1</i>	2.179362
<i>TMEM126B</i>	2.176408
<i>GLDN</i>	2.171981
<i>RYBP</i>	2.161275
<i>PPP1R8</i>	2.148596
<i>DUSP14</i>	2.130284
<i>FSTL3</i>	2.118973
<i>ENPP2</i>	2.115292
<i>HAPLN1</i>	2.108836
<i>CAVI</i>	2.105571

<i>NCBP2</i>	2.103025
<i>PRKCD</i>	2.095128
<i>LFNG</i>	2.091684
<i>DCBLD2</i>	2.082522
<i>ATP6V1E2</i>	2.071519
<i>FLJ10916</i>	2.062291
<i>WDR19</i>	2.05977
<i>EDNRA</i>	2.044538
<i>COL22A1</i>	2.024462
<i>ERRF1</i>	2.011895
<i>RAB22A</i>	2.011799
<i>MYH10</i>	2.007465
<i>LPHN2</i>	2.006611
<i>ROR2</i>	2.005633
<i>MGC15476</i>	1.995416
<i>CX3CL1</i>	1.994451
<i>MIZF</i>	1.986694
<i>LOC728006</i>	1.973569
<i>ABI2</i>	1.969274
<i>PTHR1</i>	1.96808
<i>AGPS</i>	1.957946
<i>ST3GAL5</i>	1.956943
<i>ALOX5AP</i>	1.954736
<i>HSPA12A</i>	1.95364
<i>C14ORF132</i>	1.952735
<i>SMYD3</i>	1.949609
<i>SC4MOL</i>	1.947637
<i>FLJ10986</i>	1.944654
<i>IGFBP2</i>	1.936061
<i>GLCE</i>	1.930212
<i>BHLHB3</i>	1.928226
<i>VASH1</i>	1.927925
<i>PRMT3</i>	1.92763
<i>BRPF1</i>	1.926086
<i>VDP</i>	1.924077
<i>TIMP3</i>	1.90994
<i>TNFRSF10B</i>	1.907431
<i>NPM3</i>	1.903561
<i>HSPB8</i>	1.898188
<i>LRRC32</i>	1.897707
<i>PRICKLE1</i>	1.895564
<i>RPL15</i>	1.891342
<i>TUBB2B</i>	1.891338
<i>LOC389662</i>	1.884076

<i>C16ORF80</i>	1.878068
<i>LRRC54</i>	1.865839
<i>SEC22A</i>	1.861806
<i>FBLN2</i>	1.853324
<i>LASS5</i>	1.853191
<i>KCNG1</i>	1.851116
<i>LOC402055</i>	1.84924
<i>LOC100129599</i>	1.84588
<i>NSMAF</i>	1.844945
<i>GCNT1</i>	1.837608
<i>PHLDA2</i>	1.833745
<i>TTC5</i>	1.832985
<i>ETS1</i>	1.831743
<i>TSPYL2</i>	1.823639
<i>ACVR1</i>	1.821737
<i>ATP6V0A2</i>	1.819122
<i>YRDC</i>	1.817355
<i>PHF13</i>	1.810271
<i>RWDD2A</i>	1.809382
<i>C6ORF64</i>	1.801124
<i>PTPLB</i>	1.795147
<i>ADCY3</i>	1.793846
<i>VCAM1</i>	1.792743
<i>LOC647150</i>	1.790474
<i>TEX2</i>	1.788576
<i>TMEM97</i>	1.7878
<i>INTS10</i>	1.786562
<i>FGGY</i>	1.785374
<i>C8ORF76</i>	1.775816
<i>CXXC5</i>	1.774037
<i>DAB2</i>	1.771974
<i>RRN3</i>	1.766272
<i>ZNF407</i>	1.763096
<i>THUMPD3</i>	1.762694
<i>EGFLAM</i>	1.762673
<i>PRPS2</i>	1.75994
<i>ATL1</i>	1.757244
<i>GNA12</i>	1.75662
<i>ZFAND5</i>	1.740144
<i>ZNF365</i>	1.738365
<i>FBLN2</i>	1.733752
<i>YWHAZ</i>	1.726912
<i>POLE3</i>	1.726341
<i>LOC100129759</i>	1.725305

<i>RDH13</i>	1.721423
<i>RPL9</i>	1.721108
<i>LOC730098</i>	1.718807
<i>UBL3</i>	1.716626
<i>DKFZP761P0423</i>	1.716354
<i>TRIP12</i>	1.709918
<i>PLAC9</i>	1.705565
<i>KIAA0649</i>	1.69279
<i>PRKAA1</i>	1.688524
<i>HNRPDL</i>	1.679661
<i>FAM131A</i>	1.675193
<i>BACE2</i>	1.672757
<i>NRCAM</i>	1.666867
<i>ZNF207</i>	1.666276
<i>PLEKHCl1</i>	1.663113
<i>PLXNB1</i>	1.630995
<i>FAM119B</i>	1.602944

Table S2. Down-regulated genes in EGR1 knockdown cells

Gene name	Fold change*
<i>MTG1</i>	0.4651335
<i>GLUD1</i>	0.49536812
<i>LOC728139</i>	0.5112009
<i>VCAN</i>	0.51561624
<i>CXorf40A</i>	0.5266048
<i>RABEP1</i>	0.5286921
<i>MCL1</i>	0.53835016
<i>RPAIN</i>	0.54282737
<i>IGFBP3</i>	0.5483111
<i>LOC647276</i>	0.5512289
<i>XRCC6</i>	0.5516438
<i>C2orf56</i>	0.5568179
<i>TNFRSF12A</i>	0.560885
<i>C9orf69</i>	0.56164235
<i>RNASEH1</i>	0.5644487
<i>LOC440589</i>	0.56846654
<i>ATPBD1B</i>	0.57813084
<i>SLC37A4</i>	0.5794197
<i>COL4A2</i>	0.5830043
<i>NONO</i>	0.5833543
<i>SERPINH1</i>	0.5835326
<i>NRP2</i>	0.59852964
<i>RPS9</i>	0.6036461

<i>TAGLN</i>	0.6049642
<i>DNAJC12</i>	0.6092027
<i>FTHL12</i>	0.6101501
<i>AMY1C</i>	0.6101859
<i>ALDH7A1</i>	0.6108177
<i>FTL</i>	0.6123927
<i>LOC649049</i>	0.6131814
<i>WHSC1L1</i>	0.613947
<i>LOC646294</i>	0.61468005
<i>LOC646723</i>	0.61522424
<i>M6PRBP1</i>	0.6191784
<i>PTGES</i>	0.619627
<i>AHSA1</i>	0.6199522
<i>ARL2BP</i>	0.6229743
<i>MIPEP</i>	0.62435114
<i>CAB39</i>	0.627086
<i>POLR2D</i>	0.6271075
<i>YWHAQ</i>	0.62757957
<i>C9orf50</i>	0.62804794
<i>PHF5A</i>	0.6306418
<i>CDS2</i>	0.63101745
<i>PARP12</i>	0.6341547
<i>TAF1C</i>	0.6348977
<i>PPP1CC</i>	0.63563496
<i>PTPRM</i>	0.6360393
<i>FAP</i>	0.63685125
<i>HEATR1</i>	0.63895655
<i>CXorf40B</i>	0.6391185
<i>MTMR4</i>	0.63995576
<i>DDIT4</i>	0.64001524
<i>TSPO</i>	0.64432764
<i>LSM12</i>	0.64659804
<i>ARPC1A</i>	0.6480679
<i>ILF2</i>	0.6497163
<i>EIF4G2</i>	0.6502523
<i>DCUN1D2</i>	0.6505436
<i>COBRA1</i>	0.65230817
<i>POP1</i>	0.6526774
<i>PTK2</i>	0.6546412
<i>RPS4Y1</i>	0.6546739
<i>HSPA8</i>	0.65502083
<i>WDR71</i>	0.6567855
<i>BFAR</i>	0.65868634
<i>PTP4A2</i>	0.66107935

<i>CSRP2BP</i>	0.66213995
<i>LOC91461</i>	0.6639797
<i>MOCOS</i>	0.6648766
<i>LOC441775</i>	0.6654071
<i>GOT2</i>	0.6665561
<i>ACLY</i>	0.66721296
<i>PEBP1</i>	0.6686973
<i>TIGD5</i>	0.6689023
<i>PTPRK</i>	0.670189
<i>SURF4</i>	0.67073953
<i>RGMB</i>	0.6738465
<i>MYC</i>	0.6739198
<i>ABCF3</i>	0.674448
<i>LOC653888</i>	0.6751951
<i>EIF3EIP</i>	0.67540044
<i>GLO1</i>	0.675774
<i>TBRG4</i>	0.6768825
<i>C9orf6</i>	0.67795897
<i>MAP3K4</i>	0.6812834
<i>LBH</i>	0.6816504
<i>LOC285176</i>	0.6857253
<i>GRN</i>	0.68770385
<i>LOC644029</i>	0.68839926
<i>EFCAB4A</i>	0.69079137
<i>MPDU1</i>	0.6982763
<i>PPIE</i>	0.7070499

Table S3. Down-regulated genes in EGR1 overexpressing cells

Gene name	Fold change*
<i>CDC20</i>	0.15815102
<i>GAS6</i>	0.24462053
<i>MICAL1</i>	0.2514341
<i>CD24</i>	0.27618173
<i>SEPT4</i>	0.28452358
<i>FBLN1</i>	0.28896004
<i>FHOD3</i>	0.33932027
<i>CABLES1</i>	0.34277028
<i>SLC1A3</i>	0.34325686
<i>FEZ1</i>	0.34732097
<i>VEGFB</i>	0.3489335
<i>PODXL</i>	0.36104074
<i>GPRC5C</i>	0.36204252
<i>ITGA10</i>	0.36207664

<i>C10ORF116</i>	0.366149
<i>EHP1L1</i>	0.3680063
<i>MAP4K2</i>	0.36807543
<i>TK1</i>	0.36876675
<i>TNFRSF14</i>	0.3702543
<i>RARRES3</i>	0.37226787
<i>ZNF34</i>	0.3805235
<i>NQO1</i>	0.38100612
<i>KIAA1199</i>	0.38758293
<i>SI00A10</i>	0.38770238
<i>IL18BP</i>	0.39480674
<i>DDIT4</i>	0.39773726
<i>CKAP2L</i>	0.3984352
<i>ANKRD9</i>	0.3992384
<i>STC2</i>	0.4000877
<i>CRIP1</i>	0.4026607
<i>PRC1</i>	0.40315002
<i>ZNF775</i>	0.40633786
<i>FAM64A</i>	0.4097832
<i>IDH2</i>	0.41060326
<i>NUSAP1</i>	0.4106741
<i>MVP</i>	0.41083208
<i>GRK5</i>	0.41301152
<i>TCIRG1</i>	0.41523704
<i>ANXA11</i>	0.41602263
<i>ENG</i>	0.41648003
<i>TAGLN2</i>	0.4168468
<i>DDEFL1</i>	0.41987488
<i>ANPEP</i>	0.42208642
<i>MGC87042</i>	0.42436945
<i>UBE2C</i>	0.4254324
<i>COL8A1</i>	0.42639893
<i>DNAL4</i>	0.43305585
<i>PTTG1</i>	0.43606496
<i>CRISPLD2</i>	0.4374978
<i>SIDT2</i>	0.4375223
<i>TMEM158</i>	0.4397441
<i>FAM39DP</i>	0.44066033
<i>LOC649366</i>	0.441047
<i>AKR1C3</i>	0.44206282
<i>FARP1</i>	0.44781372
<i>NPR2</i>	0.44874227
<i>BCAS4</i>	0.4491407
<i>ARSD</i>	0.44963104

<i>TGFB111</i>	0.45258313
<i>PLK4</i>	0.45277795
<i>TKT</i>	0.45310432
<i>IFI30</i>	0.45592034
<i>RAB3IL1</i>	0.46130627
<i>MT1A</i>	0.4619316
<i>ARHGDI1A</i>	0.46426922
<i>GPI</i>	0.46708283
<i>ATOH8</i>	0.46718782
<i>NUDT1</i>	0.46728435
<i>KCNMB1</i>	0.46744573
<i>MCM7</i>	0.46810037
<i>GPSM2</i>	0.46980724
<i>ACTN4</i>	0.47453827
<i>C17ORF68</i>	0.4781475
<i>EPDR1</i>	0.48006555
<i>CCNB2</i>	0.4811842
<i>CFD</i>	0.48141733
<i>RNF150</i>	0.48448664
<i>PLEKHH3</i>	0.48503172
<i>CHST14</i>	0.48529178
<i>EMP3</i>	0.48589405
<i>TRPV2</i>	0.48595846
<i>ADCY4</i>	0.48602852
<i>NT5C</i>	0.48680586
<i>PTGES</i>	0.48859593
<i>MOV10</i>	0.4891507
<i>SGSH</i>	0.48998314
<i>AURKA</i>	0.49018893
<i>SCNN1D</i>	0.49079683
<i>CLIC1</i>	0.4909556
<i>C11ORF80</i>	0.49207956
<i>MRPL23</i>	0.49284706
<i>P2RX4</i>	0.49391636
<i>TRPM4</i>	0.4951819
<i>CLTB</i>	0.49571034
<i>ATP6V0E2</i>	0.49876997
<i>C10ORF10</i>	0.5009529
<i>PRR6</i>	0.5039548
<i>PLAUR</i>	0.5072452
<i>ACSL5</i>	0.5081115
<i>MSRB2</i>	0.5081497
<i>MUTYH</i>	0.51013297
<i>WDR51A</i>	0.5104153

<i>GINS2</i>	0.5131264
<i>FADS1</i>	0.5139733
<i>DHRS3</i>	0.51461667
<i>C7ORF10</i>	0.51580465
<i>TBC1D2B</i>	0.5166551
<i>LARGE</i>	0.5176216
<i>ENO3</i>	0.51925373
<i>BCL2L1</i>	0.5207038
<i>STMN3</i>	0.52117985
<i>TFDP1</i>	0.5216644
<i>KIAA1644</i>	0.5226889
<i>SLC15A3</i>	0.5227549
<i>PEX16</i>	0.52379847
<i>CXCL12</i>	0.5237991
<i>BRI3</i>	0.5244612
<i>AXL</i>	0.52471435
<i>TBLIX</i>	0.5251542
<i>PLEKHQ1</i>	0.5264136
<i>SERPINF1</i>	0.5277494
<i>NME3</i>	0.52857774
<i>ALG9</i>	0.5301495
<i>SMPD1</i>	0.53085303
<i>PGD</i>	0.53159475
<i>IGFBP4</i>	0.5320994
<i>TALDO1</i>	0.53260577
<i>HCFC1R1</i>	0.5333419
<i>UNC93B1</i>	0.53511024
<i>ALDOA</i>	0.5357041
<i>FVT1</i>	0.53790134
<i>PUM1</i>	0.5390357
<i>IRAK1</i>	0.5391665
<i>TRIP13</i>	0.5391793
<i>PHF11</i>	0.53965515
<i>LOC644096</i>	0.54054904
<i>HNT</i>	0.54165065
<i>HLA-E</i>	0.54217863
<i>KRT81</i>	0.5422072
<i>EIF2B4</i>	0.54266125
<i>ICMT</i>	0.54313856
<i>LOC653210</i>	0.54332876
<i>FUCA1</i>	0.54445845
<i>C22ORF25</i>	0.5446214
<i>WDR71</i>	0.5462399
<i>TINF2</i>	0.5463321

<i>LTBR</i>	0.54634225
<i>TSPO</i>	0.54718184
<i>FHOD1</i>	0.5475933
<i>NINJI</i>	0.54775155
<i>NRP1</i>	0.5481697
<i>PGLS</i>	0.54995143
<i>RRAS</i>	0.5519728
<i>ATP2A2</i>	0.55233675
<i>ARHGAP23</i>	0.5541292
<i>C9ORF142</i>	0.5543595
<i>CSTB</i>	0.55454576
<i>OGFR</i>	0.55467445
<i>CD320</i>	0.5556943
<i>SFXN5</i>	0.5567052
<i>C10ORF33</i>	0.5568497
<i>IFITM2</i>	0.5569963
<i>TUBB3</i>	0.55735517
<i>SORT1</i>	0.5582776
<i>FAM89A</i>	0.5589613
<i>LENG4</i>	0.5607092
<i>MGAT1</i>	0.5610538
<i>ISG15</i>	0.56164014
<i>WDR22</i>	0.5651484
<i>DTX3</i>	0.56575805
<i>MT2A</i>	0.56733793
<i>SCARA3</i>	0.5676039
<i>LAMA4</i>	0.56816703
<i>LRFN4</i>	0.5692356
<i>SIN3A</i>	0.56927943
<i>SECISBP2</i>	0.5693249
<i>CPT1A</i>	0.5697325
<i>CENTA1</i>	0.5704499
<i>RALY</i>	0.5709072
<i>ZNF341</i>	0.5712033
<i>CCDC16</i>	0.5724743
<i>CD99</i>	0.5749912
<i>CCT5</i>	0.5762109
<i>SEPXI</i>	0.5763557
<i>FXD5</i>	0.5769112
<i>PLEC1</i>	0.57705307
<i>HMOX1</i>	0.57863784
<i>ABCC10</i>	0.57867277
<i>ATP2B4</i>	0.57875115
<i>CYB5R3</i>	0.5794876

<i>ECGF1</i>	0.5807397
<i>VIM</i>	0.5823382
<i>C20ORF55</i>	0.5834289
<i>FKSG30</i>	0.58455724
<i>ZFYVE19</i>	0.584842
<i>COMMD9</i>	0.5849753
<i>ACTB</i>	0.5851506
<i>IDE</i>	0.58596903
<i>PHF19</i>	0.5877516
<i>MRPL12</i>	0.588378
<i>CFL1</i>	0.5883804
<i>ADAM19</i>	0.58854985
<i>ANKS3</i>	0.58895904
<i>SLC7A6</i>	0.5892533
<i>ZSWIM4</i>	0.58998704
<i>RPS6KA2</i>	0.5903171
<i>CD44</i>	0.59168845
<i>NMB</i>	0.5917483
<i>PKM2</i>	0.5934822
<i>DCN</i>	0.59394175
<i>MOBKL2A</i>	0.59471256
<i>SLC35C1</i>	0.5953934
<i>FRAP1</i>	0.59569556
<i>ZBTB25</i>	0.5968993
<i>TUSC4</i>	0.5973328
<i>FAM129B</i>	0.5978567
<i>CIORF102</i>	0.59791
<i>DNAJC12</i>	0.5983575
<i>IDH3G</i>	0.59981424
<i>HABP4</i>	0.6004926
<i>PAOX</i>	0.6012136
<i>EGFR</i>	0.60265356
<i>RC3H2</i>	0.60266393
<i>APOBEC3F</i>	0.60370135
<i>C6ORF47</i>	0.60376835
<i>SERINC3</i>	0.604318
<i>VEZF1</i>	0.6046357
<i>LRRC20</i>	0.6047029
<i>NBL1</i>	0.60799634
<i>ARHGDIB</i>	0.6083563
<i>MRGPRF</i>	0.61021346
<i>MGC16824</i>	0.61232674
<i>NRM</i>	0.61590964
<i>CALR</i>	0.61610484

<i>MVD</i>	0.61764866
<i>CIB1</i>	0.6209554
<i>RIPK1</i>	0.62136376
<i>TACC3</i>	0.62401336
<i>CAPNS1</i>	0.62542814
<i>FBXW5</i>	0.62582606
<i>PSMD4</i>	0.6285216
<i>DDX54</i>	0.6328575
<i>MED16</i>	0.63317823

* Fold change represents the difference of the mean gene expression intensity in EGR1 overexpressing cells or EGR knock-down cells compared to control cells. Gene expression analysis was performed on sorted cells from four donors.

Table S4. Up-regulated proteins in EGR1 knockdown cells

Protein names	Gene names	Fold change (shEGR1/NT normalized)	Fold change (shEGR1/scrambled normalized)
Acetyl-CoA acetyltransferase, cytosolic	ACAT2	11.795	7.088
Leucine-rich repeat-containing protein 15	LRRC15	4.592	5.321
Transmembrane protein 205	TMEM205	14.703	2.405
Protein kinase C iota type	PRKCI	6.053	2.442
Serine palmitoyltransferase 1	SPTLC1	5.248	2.572
DNA replication licensing factor MCM3	HCC5;MCM3	1.946	9.482
Insulin receptor substrate 1	IRS1	2.659	4.460
Serine/arginine-rich splicing factor 3	SRSF3;SFRS3	3.535	3.101
Eukaryotic translation initiation factor 1A, X-chromosomal; Eukaryotic translation initiation factor 1A, Y-chromosomal	EIF1AX; EIF1AY	10.586	1.791
Chondroitin sulfate proteoglycan 4	CSPG4	3.194	3.241
DNA replication licensing factor MCM5	MCM5	2.639	3.951
Pre-mRNA-processing factor 19	PRPF19	2.905	3.459
Condensin complex subunit 2	NCAPH	2.224	4.762
Secretory carrier-associated membrane protein 1	SCAMP1	3.133	2.938
	TPCN1	3.571	2.616
Transcription elongation factor B polypeptide 1	TCEB1	2.811	3.066
Hematological and neurological expressed 1-like protein	HN1L	2.962	2.626
DCC-interacting protein 13-beta	APPL2	2.725	2.845
SEC23-interacting protein	SEC23IP	8.377	1.590
	TPM4	2.929	2.423
Exocyst complex component 5	EXOC5	2.719	2.561
Protein FAM50B;Protein FAM50A	FAM50A;FAM50B	2.909	2.386
High mobility group protein 20A	HMG20A	2.107	3.341
NADH dehydrogenase [ubiquinone] 1 alpha subcomplex assembly factor 5	NDUFAF5	2.380	2.768
Survival of motor neuron-related-splicing factor 30	SMNDC1	2.312	2.732
N-terminal kinase-like protein	SCYL1	6.760	1.551
Guanine nucleotide-binding protein G(q) subunit alpha	GNAQ	3.964	1.837
Peptidyl-prolyl cis-trans isomerase;Peptidyl-prolyl cis-trans isomerase G	PPIG	2.507	2.458
Nucleolysin TIA-1 isoform p40	TIA1	2.130	2.740

E3 ubiquitin-protein ligase TRIP12	TRIP12	4.268	1.689
E3 ubiquitin-protein ligase;E3 ubiquitin-protein ligase Itchy homolog	ITCH	12.343	1.350
Breast cancer anti-estrogen resistance protein 1	BCAR1	3.332	1.863
Transformer-2 protein homolog beta	DKFZp686F18120;TRA2B	2.088	2.735
DnaJ homolog subfamily C member 7	DNAJC7	2.928	1.967
Replication factor C subunit 5	RFC5	2.429	2.148
Liprin-beta-1	PPFIBP1	2.679	1.982
Protein pelota homolog	PELO	2.283	2.235
Casein kinase II subunit beta	CSNK2B;CSNK2B-LY6G5B-1181;CSNK2B-LY6G5B--991	1.739	3.237
Cadherin-2	CDH2	2.427	2.079
Overexpressed in colon carcinoma 1 protein	OCC1;C12orf75	2.195	2.277
Transmembrane protein 119	TMEM119	1.798	2.946
28S ribosomal protein S35, mitochondrial	MRPS35	5.750	1.432
Sickle tail protein homolog	KIAA1217	2.099	2.329
NHS-like protein 1	NHSL1	1.743	3.089
WD repeat-containing protein 26	WDR26	1.847	2.703
Zinc finger MYM-type protein 3	ZMYM3	2.136	2.200
DNA replication licensing factor MCM7	MCM7	1.466	4.606
Serine/arginine-rich splicing factor 2	SRSF2;SFRS2	1.891	2.495
28S ribosomal protein S18b, mitochondrial	MRPS18B	2.508	1.884
Nuclear autoantigenic sperm protein	NASP	3.557	1.569
Sepiapterin reductase	SPR	1.603	3.348
40S ribosomal protein S27-like;40S ribosomal protein S27	RPS27L;RPS27;LOC392748	2.873	1.705
Discoidin domain-containing receptor 2	DKFZp686D1354;DDR2	2.532	1.828
Putative E3 ubiquitin-protein ligase UBR7	UBR7	2.375	1.900
DNA-directed RNA polymerase II subunit RPB1	POLR2A	1.378	5.520
Stathmin	STMN1	2.589	1.777
Protein ELYS	AHCTF1	1.397	4.959
Putative protein phosphatase inhibitor 2-like protein 3;Protein phosphatase inhibitor 2	PPP1R2;PPP1R2P3	1.949	2.215
Nucleolar transcription factor 1	UBTF	3.444	1.536
Huntingtin-interacting protein 1	HIP1	1.918	2.248
Syncoilin	SYNC	2.114	1.998
HEAT repeat-containing protein 5B	HEATR5B	1.894	2.247

Ubiquitin-associated protein 2	UBAP2	2.210	1.910
Talin-2	TLN2	2.156	1.946
Nuclear migration protein nudC	NUDC	2.050	2.027
Na(+)/H(+) exchange regulatory cofactor NHE-RF2	SLC9A3R2	1.896	2.210
CD2 antigen cytoplasmic tail-binding protein 2	CD2BP2	2.088	1.987
CLIP-associating protein 1	CLASP1	1.928	2.152
Urokinase plasminogen activator surface receptor	PLAUR	2.460	1.748
Lysine-specific demethylase 3B	JMJD1B;KDM3B	2.928	1.586
Structural maintenance of chromosomes protein 1A	SMC1A;DKFZp686L19178	2.093	1.950
Ras GTPase-activating protein nGAP	RASAL2	1.775	2.360
Breast cancer anti-estrogen resistance protein 3	BCAR3	2.987	1.568
Protein SON	SON	2.319	1.793
Stathmin;Stathmin-2	STMN2	1.953	2.082

Table S5. Down-regulated proteins in EGR1 knockdown cells

Protein names	Gene names	Fold change (shEGR1/NT normalized)	Fold change (shEGR1/ scrambled normalized)
Ras-related protein Rab-32	RAB32	0.214	0.114
Glutaminase kidney isoform, mitochondrial	GLS	0.186	0.152
NHP2-like protein 1;NHP2-like protein 1, N-terminally processed	NHP2L1	0.245	0.143
Ganglioside-induced differentiation-associated protein 2	GDAP2	0.043	0.448
Protein Niban	FAM129A	0.346	0.120
Eukaryotic translation initiation factor 4E	EIF4E	0.250	0.211
HLA class I histocompatibility antigen, alpha chain E	HLA- E; HLA-B	0.198	0.306
Mammalian ependymin-related protein 1	EPDR1; UCC1	0.308	0.210
Neuropathy target esterase	PNPLA6	0.131	0.406
Thioredoxin domain-containing protein 17	TXNDC17	0.276	0.289
Protein-glutamine gamma-glutamyltransferase 2	TGM2	0.386	0.205
Coatomer subunit zeta-2	COPZ2	0.357	0.239
Vinexin	SORBS3	0.266	0.346

Non-specific lipid-transfer protein	SCP2	0.333	0.279
72 kDa type IV collagenase;PEX	MMP2	0.330	0.288
Cytochrome P450 1B1	CYP1B1	0.170	0.460
Endoplasmic reticulum mannosyl-oligosaccharide 1,2-alpha-mannosidase	DKFZp434I213; MAN1B1	0.409	0.235
Uveal autoantigen with coiled-coil domains and ankyrin repeats	UACA	0.340	0.301
Chloride intracellular channel protein 4	CLIC4	0.386	0.265
Protein disulfide-isomerase A5	PDIA5	0.291	0.376
Bis(5-nucleosyl)-tetraphosphatase [asymmetrical]	NUDT2	0.138	0.545
Sorting nexin-3	SNX3	0.366	0.332
Thioredoxin-dependent peroxide reductase, mitochondrial	PRDX3	0.417	0.286
Dynein light chain roadblock-type 1;Dynein light chain roadblock-type 2	DYNLRB1; DYNLRB2	0.554	0.162
WD repeat domain phosphoinositide-interacting protein 3	WDR45L; WDR45B	0.263	0.449
Glutathione S-transferase P	GSTP1	0.477	0.240
Transgelin	Q53GC9	0.309	0.408
Methylmalonyl-CoA mutase, mitochondrial	MUT	0.439	0.280
Fibronectin type-III domain-containing protein 3A	FNDC3A	0.299	0.423
Protein KIAA1199	KIAA1199	0.379	0.344
Sulfhydryl oxidase 1	QSOX1	0.583	0.148
Persulfide dioxygenase ETHE1, mitochondrial	ETHE1	0.303	0.429
Forkhead box protein P4	DKFZp762O213;FOXP4	0.498	0.238
Ragulator complex protein LAMTOR3	LAMTOR3	0.448	0.290
Prosaposin	PSAP	0.541	0.198
Preylcysteine oxidase 1	PCYOX1	0.388	0.352
Metalloproteinase inhibitor 1	TIMP1	0.493	0.248
Translocon-associated protein subunit alpha	SSR1	0.456	0.292
Glutathione peroxidase; Glutathione peroxidase 1	GPX1	0.512	0.253
Phosphoglucomutase-1	PGM1	0.470	0.300
Beta-galactosidase	GLB1	0.434	0.336
SRA stem-loop-interacting RNA-binding protein, mitochondrial	SLIRP	0.371	0.400
NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 8	NDUFA8	0.447	0.328

Lactoylglutathione lyase	GLO1	0.449	0.336
Stromal interaction molecule 1	STIM1	0.186	0.599
SH2 domain-containing protein 4A	SH2D4A	0.260	0.531
Syntenin-1	SDCBP	0.451	0.345
NADH dehydrogenase [ubiquinone] 1 beta subcomplex subunit 5, mitochondrial	NDUFB5	0.491	0.305
ATP synthase-coupling factor 6, mitochondrial	ATP5J	0.393	0.411
Aspartyl aminopeptidase	DNPEP	0.317	0.491
Ras-related GTP-binding protein A; Ras-related GTP-binding protein B	RRAGB; RRAGA	0.334	0.475
Neurogenic locus notch homolog protein 3; Notch 3 extracellular truncation; Notch 3 intracellular domain	NOTCH3	0.488	0.322
ADP-ribosylation factor-like protein 8A; ADP-ribosylation factor-like protein 8B	ARL8A; ARL8B	0.378	0.434
Integrin alpha-2	ITGA2	0.322	0.493
60S ribosomal protein L37a	RPL37A	0.554	0.267
ADP-ribose pyrophosphatase, mitochondrial	NUDT9	0.395	0.432
Copine-3	CPNE3	0.503	0.323
YTH domain family protein 1	YTHDF1	0.293	0.538
Phosphopantothenate--cysteine ligase	PPCS	0.349	0.497
ADP-ribosylation factor-like protein 3	ARL3	0.463	0.385
Bcl10-interacting CARD protein	C9orf89	0.502	0.348
NADH dehydrogenase [ubiquinone] 1 beta subcomplex subunit 1	NDUFB1	0.360	0.496
Leucyl-cystinyl aminopeptidase; Leucyl-cystinyl aminopeptidase, pregnancy serum form	LNPEP	0.366	0.493
Transaldolase	TALDO1	0.523	0.337
Acylphosphatase-2	ACYP2	0.329	0.533
Galectin-3	LGALS3	0.519	0.350
26S proteasome non-ATPase regulatory subunit 6	PSMD6	0.612	0.248
ATP synthase subunit O, mitochondrial	ATP5O	0.396	0.485
Cytochrome c	CYCS	0.524	0.354
Histidine triad nucleotide-binding protein 1	HINT1	0.490	0.391
2,4-dienoyl-CoA reductase, mitochondrial	DECR1	0.485	0.397
Eukaryotic initiation factor 4A-III;	EIF4A3	0.458	0.427

Eukaryotic initiation factor 4A-III, N-terminally processed			
Estradiol 17-beta-dehydrogenase 11	HSD17B11	0.550	0.337
Protein phosphatase 1 regulatory subunit 12C	PPP1R12C	0.455	0.443
40S ribosomal protein S5;40S ribosomal protein S5, N-terminally processed	RPS5	0.532	0.363
Protein S100-A10	S100A10	0.583	0.309
Tyrosine-protein kinase SgK223	SGK223	0.568	0.328
60S acidic ribosomal protein P1	RPLP1	0.654	0.227
RalBP1-associated Eps domain-containing protein 1	REPS1	0.340	0.560
Ubiquitin-conjugating enzyme E2 K	UBE2K;HIP2	0.539	0.366
Fatty acid desaturase 3	FADS3	0.510	0.406
Epidermal growth factor receptor kinase substrate 8-like protein 2	EPS8L2	0.376	0.544
Peptidyl-prolyl cis-trans isomerase C;Peptidyl-prolyl cis-trans isomerase	PPIC	0.427	0.497
Heterochromatin protein 1-binding protein 3	HP1BP3	0.449	0.476
Peptidyl-prolyl cis-trans isomerase B	PPIB	0.605	0.306
Probable E3 ubiquitin-protein ligase HERC4	HERC4	0.438	0.489
WAS protein family homolog 6;Putative WAS protein family homolog 3;WAS protein family homolog 2;WAS protein family homolog 1;Putative WAS protein family homolog 4	FLJ00075;DKFZp434K1323;WASH;WASH6P;DKFZp686C24272;WASH3P;WASH2P;WASH1;WASH4P	0.593	0.334
Phosphoserine aminotransferase	PSAT1	0.657	0.255
Arylsulfatase A;Arylsulfatase A component B;Arylsulfatase A component C	ARSA;DKFZp686G12235	0.505	0.433
Dolichol-phosphate mannosyltransferase	DPM1	0.589	0.342
Survival motor neuron protein	SMN1;SMN2	0.324	0.605
S-formylglutathione hydrolase	ESD	0.526	0.416
Alkaline phosphatase, tissue-nonspecific isozyme;Alkaline phosphatase	ALPL	0.655	0.266
Secernin-1	SCRN1	0.394	0.551
Arylsulfatase B	ARSB	0.485	0.469

Laminin subunit beta-2	LAMB2	0.355	0.591
Ubiquitin-like modifier-activating enzyme ATG7	ATG7	0.486	0.476
Hydroxyacylglutathione hydrolase, mitochondrial	HAGH	0.469	0.494
Proteasome subunit beta type;Proteasome subunit beta type-1	PSMB1	0.641	0.304
Pentraxin-related protein PTX3	PTX3	0.367	0.590
Cartilage-associated protein	CRTAP	0.486	0.482
Cytochrome b-c1 complex subunit 1, mitochondrial	UQCRC1	0.529	0.441
Trafficking protein particle complex subunit 8	TRAPPC8	0.450	0.521
Sulfurtransferase;3-mercaptopyruvate sulfurtransferase	MPST	0.670	0.276
EF-hand domain-containing protein D2	EFHD2	0.503	0.475
Nucleoporin Nup37	NUP37	0.405	0.570
Calpain-1 catalytic subunit	CAPN1	0.349	0.620
Copper transport protein ATOX1	ATOX1	0.671	0.288
A disintegrin and metalloproteinase with thrombospondin motifs 2	ADAMTS2	0.550	0.436
UPF0587 protein C1orf123	C1orf123	0.362	0.617
Vesicle-trafficking protein SEC22b	SEC22B	0.475	0.519
V-type proton ATPase 116 kDa subunit a isoform 1	ATP6V0A1; DKFZp686N0561	0.520	0.476
Up-regulated during skeletal muscle growth protein 5	USMG5	0.548	0.449

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