Supplemental method

Generation and maintenance of Igf2bp2Δ/Δ mouse

A transient induction of CRE-recombinase, however, results in a mixture of normally oriented and invertedly oriented exon-3 sequences in the gene locus. We employed transgenic mice that express CRE recombinase in the germline to select for offspring that carry an inverted exon-3, resulting in a deletion of exon-3 (49 bp) in Igf2bp2 mRNA (Supplemental Figure 1A-B). The deletion of exon-3 leads to a frame shift and introduction of a premature stop codon resulting premature translation termination and complete abrogation of Igf2bp2 expression at protein level (Supplemental Figure 1C-E). Also a truncated protein could not be detected.

Mice with a heterozygous or homozygous inversion of exon-3 are referred to as Igf2bp2Δ/Δ or Igf2bp2Δ−/Δ. The mouse line was bred by Igf2bp2Δ/Δ x Igf2bp2Δ/Δ matings or Igf2bp2Δ/Δ (females) x Igf2bp2Δ−/Δ (males) matings. Igf2bp2Δ−/Δ females were infertile indicating that Igf2bp2 is a maternal factor as seen in another mouse model of Igf2bp2 deletion. All wild-type mice in our experiments were from the littermates of Igf2bp2Δ−/Δ crosses or from C57BL/6 Janvier (C57BL/6JRj). Animals used for experiments were on a C57BL/6JRj background (7 fold backcrossed) and maintained in a specific pathogen-free (SPF) animal facility at the Fritz Lipmann Institute (FLI), Jena, with 12:12 hours light/dark cycle and fed ad-libitum (Ssniff GmbH; V1524-786) with a standard mouse chow and water. All animal experiments were performed following the protocols approved by the state government of Thuringia (Application Nr. 03-053/16 ZRA, O.KLR.18-20, Nr. FLI-17-022, and Nr.03-051/16).

Flow cytometry sorting and analysis

Total bone marrow cells were harvest from bones including fore and hind limbs, pelvis and spine. cKit+ BM cells were enriched by magnetic-activated cell separation (Miltenyi Biotec; 130-090-855). cKit+ BM cells were incubated with lineage cocktail containing biotinylated antibodies against CD4 (Biolegend; 100508), CD8 (Biolegend; 100704), TER-119 (Biolegend; 116204), CD11b (Biolegend; 101204), Gr-1 (Biolegend; 108404), and B220 (Biolegend; 103204) on ice for 30 min. After washing, the cells were incubated with anti-cKit-APC (Biolegend; 105812), anti-Sca1-PE-Cy7
(Biolegend; 108114), anti-streptavidin-APC-Cy7 (Biolegend; 405208), anti-CD34-Alexafluor700 (eBioscience; 56-0341-82), and anti-CD150-BV605 (Biolegend; 115927) on ice for overnight. For isolation of the HSC-containing population of cells derived from cultured HSC, the cultured cells were stained with lineage cocktail on ice for 30 min. After washing, the cells were stained with anti-Sca1-PE-Cy7, anti-streptavidin-APC-Cy7, and anti-CD48-PerCP-Cy5.5 (Biolegend; 103422) on ice for 30 minutes and re-suspended in FACS staining medium with DAPI (1:1,000) after washing.

Peripheral blood was incubated with anti-CD4-APC (Biolegend; 100516), anti-CD8-APC (Biolegend; 100712), anti-Gr-1-APC-Cy7 (Biolegend; 108424), anti-CD11b-APC-Cy7 (Biolegend; 101226), anti-B220-APC (eBioscience; 17-0452-82), and anti-B220-APC-Cy7 (Biolegend; 103224) on ice for 30 minutes, followed by red blood cell lysis (BD Biosciences; 555899).

Freshly isolated BM cells (1x10^7) were incubated with lineage cocktail at 4 °C for 30 min, then stained with anti-cKit-APC, anti-Sca1-PE-Cy7, anti-streptavidin-APC-Cy7, anti-CD34-Alexafluor700, anti-CD150-BV605, anti-FcγR-FITC (Biolegend; 101306) or anti-CD48-FITC (Biolegend; 103404), anti-CD127-PerCP-Cy5.5 (Biolegend; 135022), anti-Flt3-PE (Biolegend; 135306) or anti-CD41-PE (Biolegend; 133906) on ice for overnight.

Cell purification and FACS analysis were performed by the LSR Fortessa cell analyzer and FACS Aria III cell sorter, respectively. Data were analyzed by FlowJo software.

**Homing assay**

Previous protocols^3 were used to examine the homing potential of myeloid-biased HSC. In brief, freshly purified myeloid biased HSC (CD150^{high}CD34-LSK; 3,000 cells) from donor mice (CD45.2) were transplanted into lethally γ-irradiated (12 Gy) recipients (CD45.1) by intravenous (i.v.) injection (5 recipients per experimental group). In this experiment, 5 mice irradiated but not transplanted were used as negative control to quantify leftover CFCs in the BM of the irradiated mice. 12 h after transplantation, total bone marrow cells were harvest from bones (including fore and hind limbs, pelvis and spine) of each irradiated mouse and were quantified. Total BM cells from recipients in experimental groups were seeded into methylcellulose medium
at 3\times 10^6 cells/6 replicates; and total BM cells from mice in negative control group were seeded into methylcellulose medium at 1\times 10^6/duplicate. The number of colonies was counted 10 days after culture. Prior to transplantation, the number of CFCs from 500 myeloid-biased HSC of donor mice were quantified 10 days after culture. The calculation used was as below.

\[
\% \text{ CFC homed} = \frac{\text{total colony number (recipient)} - \text{total colony number (negative)}}{\text{colony number per 3000 donor cells}}
\]

Total colony number = colony number per million BM cells × BM cell number/1000000

Colony number per 3000 donor cells = colony number per 500 donor cells × 6

**RNA purification, reverse transcription and quantitative real time PCR (RT-qPCR)**

Total RNA was extracted by using MagMax96 total RNA isolation kit (Ambion; AM1830) according to the manufacturer’s protocol. The GoScript Reverse transcription system (Promega; A5000) was used for cDNA synthesis. RT-qPCR was performed with the SYBR system by using the 384 CFX detection systems. The amount of target RNA was normalized to that of the endogenous control β-actin (Actb). The gene expression ratio was calculated following the Pfaffl formula with primer efficiency correction. The qPCR primer sequences are as follows: β-actin, Fw 5’-AAGGCCAACCGTGAAAAGAT-3’ and Re 5’-GTGGTACGACCAGAGGCATAC-3; Lin28b, Fw 5’-AGAATGCAGTCTACCTCCTAG-3’ and Re 5’-CCTCCACTTCTCTTGGTGC-3; Hmga2, Fw 5’-AACCTGTGAGCCCTCTAAG-3’ and Re 5’-GCCGTTTTTCTCAAATGGTC-3; Igf2bp2, Fw 5’-GAATCCAGATTCGGGAACATCC-3’ and Re 5’- GTTGACAACCGCAGTTCTG-3’.

**FACS analysis of phosphorylated-mTOR**

Freshly isolated bone marrow cells stained with antibodies against surface marker were fixed and permeabilized by using the Cytofix/Cytoperm kit (BD Biosciences; 554714). The level of p-mTOR in specific populations was analyzed by FACS using anti-p-mTOR-PE (pS2448; BD Biosciences; 563489).

**Cell cycle assay**
Cell cycle analysis for freshly isolated cells was performed via Ki67 staining (BD Biosciences; 556027) using Cytofix/Cytoperm kit (see above).

**Immunofluorescence staining**

5,000-10,000 HSPC were plated on 12-well slides that were pre-coated with 1x poly-L-lysine (Sigma-Aldrich; P8920) at room temperature (RT) for 30 min and kept in a humid chamber for 30 min allowing cells to settle down onto the slides. Cells were fixed with 100% methanol for 10 min at RT. After 3 washes with PBS, the fixed cells were permeabilized with 2% BSA containing 0.3% Triton X-100 for 30 min at RT. Following this, cells were blocked by 2% BSA at RT for 30 min. After blocking, cells were incubated with primary antibodies at RT for overnight: anti-phosphorylated AKT (Ser 473; 1:400 in 2% BSA). On the next day cells were washed 3 times with PBS and incubated with secondary antibodies against rabbit with a fluorescent dye (Cy3, 1:400 in 2% BSA) for 30 min at RT. After washing the stained cells 3 times with PBS, the stained cells were counterstained by mounting medium containing DAPI and covered by a glass coverslip. Pictures were taken at the ApoTome microscope. The median intensity of each cell was measured by ImageJ, and the median intensity per mouse was quantified from the median intensity from 100-200 cells of the mouse. In order to combine the independent experiments together, the fold change of median intensity was normalized to the average of median intensity of young HSC or MPP in each experiment.

**Respirometry analysis**

The cellular ATP production rate in living cells was detected by using Agilent Seahorse XF96 Technology according to the guideline of real-time ATP production rate assay. Briefly, freshly isolated HSPC (40,000 cells) were plated into poly-lysine-coated 96-well cell culture plate with 180 μl XF DMEM medium (Agilent technologies; 102353) with 1mM pyruvate, 2mM glutamine, 10 mM glucose, 50ng/ml mTPO, 50ng/ml mSFC, 100 U/ml penicillin and 100 μg/ml streptomycin. The plated cells were incubated at 37°C without CO₂ for 1h before measuring. The OCR and ECAR were measured every one hour for 6 h by XF96 Seahorse prior to the real-time ATP production assay following the manufacturer’s protocol. The concentration of drugs used in the experiment was 2μM oligomycin (Sigma-Aldrich; 75351) and 1 μM of rotenone (Sigma-Aldrich; R8875-1G)/Antimycin A (Sigma-Aldrich; A8674) (Rot/AA).
The OCR and ECAR measured by real-time ATP production assay was used to calculate glycoATP production rate and mitoATP production rate by the Agilent Seahorse XF Real Time ATP Rate Assay Report Generator. In brief, the glycoATP production rate is equivalent to glycolytic proton efflux rate (glycoPER). The glycoPER is equal to the subtraction of mitochondrial PER (CO₂-dependent proton) from total PER. The total PER is calculated from ECAR, and mitochondrial PER is related to mitochondrial OCR (subtraction of OCR after addition of Rot/AA from basal OCR). The ATP generated from mitochondria by the process of oxidative phosphorylation (OXPHOS), called mitoATP, is calculated from ATP-linked OCR that is subtracted the OCR after injection of oligomycin from basal OCR. For further details see manufactures instruction. ⁴

The mitochondrial function was assessed by Agilent Seahorse Cell Mito Stress assay according to the manufacturer’s protocol. Prior to the Mito Stress assay, purified HSPC (40,000 cells) were cultured by SFEM medium with 50ng/ml mTPO, 50ng/ml mSFC, 100 U/ml penicillin and 100 μg/ml streptomycin for 12 h. The Mito Stress assay was performed by XF96 Seahorse with injections of 2 μM oligomycin, 4 μM FCCP (Sigma-Aldrich, C2920), and 1 μM of Rot/AA. The measured OCR was used for quantification. In brief, the OCR for basal or maximal respiration was calculated by subtraction of non-mitochondrial OCR (after injection of Rot/AA) from the basal OCR or the OCR after FCCP injection, respectively. The OCR for ATP-linked respiration was calculated by subtraction of the OCR (after injection of oligomycin) from the basal OCR.

Lentivirus infection of HSC

The open reading frame (ORF) of Igf2bp2 cDNA was cloned into the SFLV-EGFP plasmid⁵, which up-regulates the Igf2bp2 expression at mRNA and protein level (Supplemental Figure 7A and B). The LentIX 293T producer cells were transfected according to established protocols.⁶

Cell culture and inhibitor treatment

Viral-infected HSC were plated in 400 μl of serum-free expansion medium (SFEM; Stem Cell; 09650) containing 50 ng/ml thrombopoietin (TPO; Peprotech; 315-14), and 50 ng/ml stem cell factor (SCF; Peprotech; 250-03) with PI3K inhibitor
(LY294002, 10 μM; Cell Signaling Technology; 9901) or mTOR inhibitor (rapamycin, 200 μM; LC Laboratories; R-5000).

**MitoRed measurement**

Two days after culture, cells were stained with antibodies against surface markers as described in supplemental methods. The stained cells were incubated with MitoRed (PromoCell; PK-CA707-70055) and analyzed by FACS.

**Bulk RNA sequencing and Gene ontology (GO) enrichment analysis**

Bulk RNA sequencing was conducted on myeloid-biased HSC from 3 month-old and 22-26 month-old \( \text{Igf2bp2}^{-/} \) versus \( \text{Igf2bp2}^{+/-} \) mice. Total RNA was extracted by MagMax96 total RNA isolation kit (Ambion; AM1830). Quantification and quality checks used an Agilent 4200 TapeStation. Sequencing was carried out by Illumina’s next-generation sequencing methodology. In brief, full-length cDNA was prepared using SMART-Seq v4 Ultra Low Input RNA Kit from 2 ng of input material and quantified using High Sensitivity D5000 ScreenTape. Starting with tagmentation, Nextera XT DNA Library Preparation Kit was used to further process the full-length cDNA to Illumina libraries. Subsequently, quantification and quality check of libraries was performed on D5000 ScreenTape. Illumina libraries were pooled and sequenced on NovaSeq 6000 System running (single-end; Read 1: 101 bp). Sequence information was converted to FASTQ format using bcl2fastq v2.20.0.422. The raw reads were pseudoaligned to the GRCm38 mouse transcriptome using Salmon (v1.4.0) with default parameters. The transcript per million values outputted by Salmon were imported to R (v4.0.2) and summarized into a gene-level matrix using the tximport R package (v1.16.1). Differential gene expression was carried out using the DESeq2 R package (v1.28.1). A gene was considered differentially expressed if the Benjamini-Hochberg adjusted p-value was less than 0.05. 1,421 differentially expressed genes (DEG) were identified in young myeloid-biased HSC from \( \text{Igf2bp2}^{-/} \) versus \( \text{Igf2bp2}^{+/-} \) mice; 26 DEG were identified in old myeloid-biased HSC from \( \text{Igf2bp2}^{-/} \) versus \( \text{Igf2bp2}^{+/-} \) mice.

An overlap analysis was performed on the DEG in young myeloid-biased HSC from \( \text{Igf2bp2}^{-/} \) vs. \( \text{Igf2bp2}^{+/-} \) mice with 83 identified target RNAs directly bound to IGF2BP2 in brown fat as reported previously.
Gene ontology (GO) enrichment analysis was performed on the differentially expressed genes that are down-regulated or up-regulated in young myeloid-biased HSC from \textit{lgl2bp2}\textsuperscript{−/−} vs. \textit{lgl2bp2}\textsuperscript{+/+} mice using the R package GOstats (v2.54.0). Significant GO terms were selected if the Benjamini-Hochberg adjusted p-value was less than 0.05. Taking the DEG down-regulated in young \textit{lgl2bp2}\textsuperscript{−/−} versus \textit{lgl2bp2}\textsuperscript{+/+} mice which are associated with the GO terms “Mitochondrial organization”, “Mitochondrial respiratory chain complex assembly”, “Peptide metabolic process”, “Proteasomal ubiquitin-independent protein catabolic process”, “Cellular amide metabolic process”, and “Translation”, we used the Quanto R package (v1.22.0) to evaluate the differences in the expression of these genes across groups of samples. The quanto function outputs an F statistic and the corresponding p-value for each pair of sample types: Young \textit{lgl2bp2}\textsuperscript{−/−} vs. Old \textit{lgl2bp2}\textsuperscript{+/+}, young \textit{lgl2bp2}\textsuperscript{−/−} vs. old \textit{lgl2bp2}\textsuperscript{−/−} and Young \textit{lgl2bp2}\textsuperscript{−/−} vs. Old \textit{lgl2bp2}\textsuperscript{+/+}.

**Single cell RNA sequencing (ScRNA-seq)**

Freshly isolated myeloid-biased HSC from male wildtype mice (6 weeks old) were used to conduct single cell RNA sequencing using 10x Genomics Chromium Controller and the Chromium Single Cell 3’ v3 chemistry following the standard manufacturer’s protocols. In brief, 20,000 freshly isolated myeloid biased HSC (CD150\textsuperscript{high}CD34 LSK) were loaded onto to the Chromium controller to recover 10,000 cells for library preparation and sequencing. After Post GEM-RT Cleanup, cDNA was amplified by 11 cycles. The total yield of cDNA was assessed on High Sensitivity DNA Assay (Agilent 2100 Bioanalyzer) resulting 540 ng. A total of 12 cycles was used for the Sample Index PCR reaction and final library was evaluated using D5000 ScreenTape. The library was sequenced on Illumina NextSeq 500 System (paired end; Read 1: 28 bp barcode & UMI; Read 2: 55 bp Insert). The initial analysis with Cell ranger (version: 3.1.0, parameter: -expect-cells=10000; bcl2fastq v2.20.0.422) estimated 7,906 cells with 46,552 reads/cell.

**Single cell RNA-seq (scRNA-seq) data normalization and quality control**

ScRNA-seq data were processed using 10X Genomics Cell Ranger (v5.0.0) mapped to the GRCm38 reference genome. Seurat R package (v3.2.3) was used for all further analysis. Data were read into R as a count matrix and log-transformed using the Seurat function SCTransform. For quality control, we began with 7,887 cells with
mean number of features 3,683.5, mean read count 14,037.9, and mean percentage of mitochondrial reads 8.2. We removed cells with fewer than 500 features, fewer than 3,000 reads, or over 50% mitochondrial reads, selecting 7,435 cells to use for further analysis.

**Visualization, clustering, and differential expression of scRNA-seq data using Seurat**

For Uniform Manifold Approximation and Projection (UMAP) and clustering, the first 30 principal components were used. UMAP plots of the expressions of *Lin28b, Hmga2, Igf2bp2, Igf2, Igf1, H19, Rian, and Cdkn1c* exclude the top 5%, 1%, 0%, 5%, 5%, 0%, 1%, and 1% of values, respectively (Figs. 3 and S4). Clustering was performed using the Seurat function FindClusters with resolution 0.3, resulting in 9 unique clusters. The Seurat function FindMarkers with a Bonferroni adjusted p-value cutoff of 0.01 was used to determine specific markers for each cluster. These markers were then compared with common hematopoietic lineage markers in order to identify which clusters were already primed towards a certain lineage. Clusters 1, 2, 6, and 7 were excluded based on their expression of: *Itga2b, Vwf, Pf4, Klf1, Mki67, Gata1, and Car1*, markers of megakaryocyte/thrombocyte and erythroid lineages (Supplemental Figure 5A-H).

Clusters 0, 3, 4, 5, and 8 were not clearly primed towards a certain lineage. KS tests were performed to compare *Igf2bp2* expression in Cluster 3 vs. Cluster 0, 4, 5, 8, respectively. We obtained the following p-values: 2.6x10^{-9}, 6.6x10^{-4}, 8.7x10^{-4}, 0.07. To perform differential expression on Cluster 3 relative to Cluster 0, 4, 5, and 8, we again used FindMarkers with an adjusted p-values cutoff of 0.01.

**Proteomics analysis of *Igf2bp2*-overexpressing stem cells**

Total CD150-positive (high and low) HSC were isolated from pools of old mice and transduced with virus particles containing *Igf2bp2*-cDNA or a vector control. 2.5 days after transduction, the infected HSC containing cell population (DAPI-GFP-CD48-LSK) was re-sorted for proteomic analysis, and protein amount was estimated based on cell number input. The sample preparation and data analysis were performed in the Core Facility Proteomics of the FLI.
In brief, cells were sorted in 10x lysis buffer (for a final concentration of 1% SDS, 100mM HEPES, 50mM DTT, pH8.5) and were sonicated by using a Bioruptor Plus Sonication Device with 10 cycles of 60 sec with interval of 30 sec resting at 20°C and heated to 95°C for 10 min. Following alkylation (15 mM iodoacetamide, 30 min, RT in the dark), proteins were precipitated by ice-cold acetone (8 x sample volume, overnight, -20°C). Protein pellets were obtained by centrifugation (20,000 g, 30min, 4°C), the pellets washed twice with 500 µL ice-cold 80% acetone/water. Pellets were vortexed and centrifuged (10mins after first wash, 2mins after second, at 20,000 g, 4°C), before re-suspension by sonication in the Bioruptor (as described before) in lysis buffer (100 mM HEPES, 3M Urea, pH 8.0). Digestion with Lys-C (1:100 enzyme/protein; Wako) was carried out for 4 h at 37°C, followed by 1:1 dilution with water and a secondary digestion with trypsin (1:100 enzyme/protein; Promega) performed overnight at 37°C. Digested peptides were acidified by the addition of 10% TFA to obtain pH 2 and then desalted using an Oasis® HLB µElution Plate (Waters Corporation). Digested peptides were spiked with the indexed retention time peptide (iRT) kit (Biognosys AG) and separated by the nanoAcquity M-Class Ultra-High Performance Liquid Chromatography system (Waters) fitted with a trapping (nanoAcquity Symmetry C18, 5µm, 180 µm x 20 mm) and an analytical column (nanoAcquity BEH C18, 1.7µm, 75µm x 250mm). The outlet of the analytical column was coupled directly to a Q exactive HF-X using the Proxeon nanospray source. Solvent A was water, 0.1 % FA and solvent B was acetonitrile, 0.1% FA. Samples were loaded at constant flow of solvent A at 5 µL/min onto the trap for 6 mins. Peptides were eluted via the analytical column at 0.3 µL/min and introduced via a Pico-Tip Emitter 360 µm OD x 20 µm ID; 10 µm tip (New Objective). A spray voltage of 2.2 kV was used. During the elution step, the percentage of solvent B increased in a non-linear fashion from 0 % to 40 % in 120 minutes. Total run time was 145 minutes. The capillary temperature was set at 300 °C. The RF lens was set to 40%. MS conditions were: Full scan MS spectra with mass range 350-1650 m/z were acquired in profile mode in the Orbitrap with resolution of 120000 FWHM. The filling time was set at maximum of 60 ms with limitation of 3 x 10^6 ions. DIA scans were acquired with 40 mass window segments of differing 20 widths across the MS1 mass range. The default charge state was set to 3+. HCD fragmentation (stepped normalized collision energy; 25.5, 27, 30%) was applied and MS/MS spectra were acquired with a resolution of 30000 FWHM with a fixed first mass of 200 m/z after accumulation of 3e6 ions or after
filling time of 35 ms (whichever occurred first). Data were acquired in profile mode. For data acquisition and processing of the raw data Xcalibur 4.0 (Thermo Scientific) and Tune version 2.9 were employed. For sample-specific spectral library generation, samples of Igf2bp2-cDNA infected and vector-infected digests were additionally analyzed by data-dependent acquisition (DDA), using the same gradients as the DIA analyses. Both DIA and DDA data were included in the library generation. The data were searched against the mouse Uniprot database (Swissprot entry only, release 2016_01, 16,747 entries) using the Pulsar search engine. The following modifications were included in the search: Carbamidomethyl (C) (Fixed) and Oxidation (M)/ Acetyl (Protein N-term) (Variable). A maximum of 2 missed cleavages for trypsin were allowed. The identifications were filtered to satisfy FDR of 1 % on peptide and protein level. The resulting library contained 79962 precursors corresponding to 5686 protein groups. Precursor matching, protein inference, and quantification were performed in Spectronaut using median peptide and precursors (no TopN). Relative quantification was performed in Spectronaut (version 12.0.20491.0.21234, Biognosys AG) using the paired samples from each condition across the replicates. Protein was considered differentially expressed which were >1.5-fold change and q-value<0.05. The data (candidate table) and data reports (protein quantities) were then exported and further data analyses and visualization were performed with R-studio (version 0.99.902) using in-house pipelines and scripts. Ingenuity pathway analysis (IPA) was performed on differentially expressed protein in Igf2bp2-overexpressing HSC compared to control vector-infected HSC and p-values were corrected by Benjamini-Hochberg.

**In vivo transplantation assay with Igf2bp2 overexpressing HSC**

10 h after transduction, viral-infected total CD150-positive (high and low) HSC (CD150^+^CD34^-^LSKs) from young and old WT mice (3 or 26 months old; CD45.2) were transplanted into recipients (7-month; CD45.2) by i.v. injection, along with 5x10^5^ competitor total BM cells (9-month; CD45.1). The initial transduction rate of HSC was determined on day 2.5 after culturing an aliquot of the same infected HSC that were used 10 h after infection for transplantation. The transduction efficiency did not show a significant difference in Igf2bp2 cDNA vs. vector-control infected HSC.

After transplantation, all the recipients were treated with antibiotic water (0.01%; Baytril) for one week and were monitored by weekly inspection until the end of the
experiments. Chimerism and lineage composition in PB from recipients was analyzed in 4 week intervals after transplantation by FACS.

**Genotyping of Igf2bp2**

Genotyping of the mice was performed by PCR amplification (Promega; M300) with genomic DNA from tail biopsies following the manufacturer’s protocol. The PCR primer sequences are as follows: Igf2bp2_GR, 5’-ACAGGCCTCAACCAATCAGA-3’ and INV_FP, 5’-AAAGCAACTGACCCTA-3’, the PCR product is 330 bp for the mutant allele; Lox_FP, 5’-AAGATTGTCCGTACGCTGCT-3’ and Lox_RP, 5’-AAATCTCCCACCTCCCAATC-3’, the PCR product is 150 bp for the wild type allele.

**Body weight and survival study**

For body weight study, mice were weighed in 2-week intervals after weaning. For survival study, mice were euthanized by CO₂ asphyxiation and recorded as “dead” when natural death occurred spontaneously or when human endpoints were reached including signs of morbidity, such as seizures, tumors, large non-healing skin erosions, large anal prolapses, sluggishness movements, or loss of >15% of body weight in two weeks.

**Preparation of mouse embryonic fibroblast (MEF)**

Embryos (E12.5) was aseptically minced with sterile scalpel in 2 ml of trypsin-EDTA, and incubated for 10 min at 37°C, 5% CO₂ after excision of head and liver. The head is used for genotyping as described before. The dissociated cells were plated onto new 10 cm dishes in 10 ml of DMEM medium with 10% FBS, 1% Non-essential amino acids (Gibco; 11140-035), 1% L-glutamine (Gibco; 25030-081), 1% Sodium pyruvate (Gibco; 11360-039) and 1% P/S. The cells were split at 1:2 ratio when freshly confluent, passaged two times to obtain protein lysate for western blotting.

**ROS measurement**

Freshly isolated bone marrow cells were stained with antibodies against surface makers as described above. The stained cells were incubated by CellRox detection (Thermo Scientific; C10444) and analyzed the intensity of ROS by FACS.

**Intracellular ATP measurement**
Intracellular ATP concentration in purified cells (1,500 cells) was determined by using ATP Determination Kit (Thermo Scientific; A22066) following the manufacturer’s protocol.

**Western blotting**

A piece of frozen liver, MEF cells or sorted cells with identical number were lysed with protein lysis buffer (RIPA lysis buffer with 1mM NaVO₃, 1mM DTT, Protease inhibitor and 1mM PMSF). The concentration of lysate was determined by the Bradford method using Bio-Rad Protein Assay Dye Reagent (Bio-Rad; 5000006). Equal amounts of protein were resolved through 12% SDS-PAGE. The proteins were wet-transferred using transfer buffer with 20% methanol onto nitrocellulose membranes. The total protein was detected for western blot normalization by Revert™ 700 Total Protein Stain Kit (LICOR Biosystems; 926-11010). The nitrocellulose membrane was blocked for 60 min with 5% milk in TBS buffer at RT and then incubated with the primary antibody against IGF2BP2 (D4R2F; 1:500; Cell Signaling Technology, 14672) in 5% milk in TBST (TBS with 0.01% Tween-20) for overnight. Next day the membrane was incubated with secondary antibody against Rabbit (1:10,000; IRDye 800CW Donkey anti-Rabbit; LI-COR) in 5% milk in TBST for 2 hrs, and image was acquired by the LI-COR Odyssey scanner. Then the membrane was blocked again and incubated with the primary antibody against β-actin (1:1,000; Sigma-Aldrich; A2066) at RT for 2 hrs. The last steps for secondary antibody incubation and scan were performed as described above.

**Bone marrow cell counting**

Bone marrow (BM) was isolated from fore and hind limbs, pelvis and spine. Freshly isolated BM cells were suspended by 15 ml staining medium, and measured the concentration of cells by CellCounter.

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Table S1: Top 200 GO terms conditioned on the significance of their child terms enriched for DEG down regulated in young *lgf2bp2*/− vs. *lgf2bp2*/+/+ mice

| Rank | GOBPID   | AdjPvalue                  | Count | Size | Term                                                          |
|------|----------|----------------------------|-------|------|---------------------------------------------------------------|
| 1    | GO:0007005 | 2.782656665316491E-10     | 29    | 300  | mitochondrion organization                                   |
| 2    | GO:0010499 | 2.00131450902744E-09      | 10    | 24   | proteasomal ubiquitin-independent protein catabolic process  |
| 3    | GO:0006518 | 8.0904883633362E-09       | 41    | 673  | peptide metabolic process                                    |
| 4    | GO:0034641 | 3.2592581004028E-07       | 157   | 5330 | cellular nitrogen compound metabolic process                 |
| 5    | GO:0043604 | 7.55111504610725E-07      | 39    | 732  | amide biosynthetic process                                   |
| 6    | GO:0032981 | 7.55111504610725E-07      | 10    | 45   | mitochondrial respiratory chain complex I assembly           |
| 7    | GO:0044238 | 1.25062983476723E-06      | 234   | 9156 | primary metabolic process                                    |
| 8    | GO:0017004 | 6.34073727455476E-06      | 8     | 32   | cytochrome complex assembly                                  |
| 9    | GO:1901566 | 1.26875727741665E-05      | 59    | 1528 | organonitrogen compound biosynthetic process                 |
| 10   | GO:0046034 | 1.32882855941542E-05      | 19    | 245  | ATP metabolic process                                        |
| 11   | GO:0001682 | 6.74803600808624E-05      | 5     | 12   | tRNA 5'-leader removal                                       |
| 12   | GO:0006412 | 0.000140744073659142     | 24    | 444  | translation                                                  |
| 13   | GO:0034471 | 0.000206364655962008     | 5     | 15   | ncRNA 5'-end processing                                     |
| 14   | GO:0044265 | 0.000485604046320963     | 40    | 1015 | cellular macromolecule catabolic process                     |
| 15   | GO:0033617 | 0.000649708076690648     | 5     | 19   | mitochondrial cytochrome c oxidase assembly                   |
|   | GO:          | p-value         | Count | Description                                                                 |
|---|-------------|-----------------|-------|-----------------------------------------------------------------------------|
| 16| GO:0034660  | 0.000956429574404362 | 22    | ncRNA metabolic process                                                     |
| 17| GO:0017144  | 0.000963094582954386  | 25    | drug metabolic process                                                      |
| 18| GO:0030163  | 0.0016027821937225   | 35    | protein catabolic process                                                   |
| 19| GO:0070584  | 0.00173142097569082   | 5     | mitochondrion morphogenesis                                                 |
| 20| GO:0032543  | 0.00176351096731206   | 8     | mitochondrial translation                                                   |
| 21| GO:0006364  | 0.00244506478217451   | 12    | rRNA processing                                                            |
| 22| GO:0022613  | 0.00259115789740638   | 20    | ribonucleoprotein complex biogenesis                                        |
| 23| GO:0051603  | 0.0027502525498714    | 9     | proteolysis involved in cellular protein catabolic process                   |
| 24| GO:0042773  | 0.00281867027253935   | 7     | ATP synthesis coupled electron transport                                     |
| 25| GO:1902600  | 0.00349540179824992   | 7     | proton transmembrane transport                                              |
| 26| GO:0009144  | 0.0046459528955488    | 8     | purine nucleoside triphosphate metabolic process                            |
| 27| GO:0045324  | 0.00496228750882052   | 4     | late endosome to vacuole transport                                           |
| 28| GO:0045333  | 0.00496228750882052   | 11    | cellular respiration                                                        |
| 29| GO:2000277  | 0.00496228750882052   | 2     | positive regulation of oxidative phosphorylation uncoupler activity         |
| 30| GO:0045039  | 0.00496228750882052   | 3     | protein insertion into mitochondrial inner membrane                          |
| 31| GO:0008033  | 0.00512279471211134   | 9     | tRNA processing                                                             |
| 32| GO:0070585  | 0.00629294861564953   | 8     | protein localization to mitochondrion                                       |
| 33| GO:0090201  | 0.00629294861564953   | 4     | negative regulation of release of cytochrome c from mitochondria            |
| GO:0044249 | 0.00767446512316412 | 122 | 4835 | cellular biosynthetic process |
| GO:0035456 | 0.00820606877977296 | 6 | 56 | response to interferon-beta |
| GO:0044267 | 0.00833265997704803 | 97 | 3948 | cellular protein metabolic process |
| GO:0006839 | 0.00884608779602929 | 11 | 181 | mitochondrial transport |
| GO:0015986 | 0.009264820920607 | 4 | 23 | ATP synthesis coupled proton transport |
| GO:009059 | 0.00934909188747073 | 104 | 4047 | macromolecule biosynthetic process |
| GO:0019068 | 0.0100088123880313 | 4 | 24 | virion assembly |
| GO:0035455 | 0.0100088123880313 | 4 | 24 | response to interferon-alpha |
| GO:0019068 | 0.0100088123880313 | 4 | 24 | response to interferon-alpha |
| GO:0006296 | 0.0100088123880313 | 2 | 3 | nucleotide-excision repair, DNA incision, 5'-to lesion |
| GO:0045764 | 0.0100088123880313 | 2 | 3 | positive regulation of cellular amino acid metabolic process |
| GO:0052547 | 0.0102461813760149 | 18 | 411 | regulation of peptidase activity |
| GO:0022900 | 0.0111700149718505 | 7 | 85 | electron transport chain |
| GO:0065003 | 0.0111700149718505 | 49 | 1635 | protein-containing complex assembly |
| GO:006396 | 0.0118836769876187 | 29 | 822 | RNA processing |
| GO:0032509 | 0.011836769876187 | 4 | 26 | endosome transport via multivesicular body sorting pathway |
| GO:0044085 | 0.0134772797543536 | 70 | 2633 | cellular component biogenesis |
| GO:1903900 | 0.0158092586362399 | 9 | 145 | regulation of viral life cycle |
| GO:0018364 | 0.0158092586362399 | 2 | 4 | peptidyl-glutamine methylation |
| GO:0043328 | 0.0158092586362399 | 2 | 4 | protein transport to vacuole involved in ubiquitin-dependent protein catabolic process via the multivesicular body sorting pathway |
| GO:0097250 | 0.0158092586362399 | 2 | 4 | mitochondrial respirasome assembly |
| GO:1905907 | 0.0158092586362399 | 2 | 4 | negative regulation of amyloid fibril formation |
| GO:1903624 | 0.0158508510693434 | 3 | 14 | regulation of DNA catabolic process |
| GO:0044237 | 0.0185384768007829 | 20 | 679 | cellular metabolic process |
| GO:0010917 | 0.0185384768007829 | 3 | 15 | negative regulation of mitochondrial membrane potential |
| GO:1902430 | 0.0185384768007829 | 3 | 15 | negative regulation of amyloid-beta formation |
| GO:0019884 | 0.0190131433713689 | 4 | 31 | antigen processing and presentation of exogenous antigen |
| GO:1900037 | 0.0207344162528091 | 3 | 16 | regulation of cellular response to hypoxia |
| GO:0007007 | 0.0207344162528091 | 2 | 5 | inner mitochondrial membrane organization |
| GO:1901575 | 0.0207344162528091 | 52 | 1846 | organic substance catabolic process |
| GO:0019348 | 0.0207344162528091 | 2 | 5 | dolichol metabolic process |
| GO:0033864 | 0.0207344162528091 | 2 | 5 | positive regulation of NAD(P)H oxidase activity |
| GO:0045541 | 0.0207344162528091 | 2 | 5 | negative regulation of cholesterol biosynthetic process |
|   | GO:ID       | Score       | M    | F    | Description                                           |
|---|------------|-------------|------|------|-------------------------------------------------------|
| 67| GO:0048550 | 0.0207344   | 2    | 5    | negative regulation of pinocytosis                   |
| 68| GO:1902512 | 0.0207344   | 2    | 5    | positive regulation of apoptotic DNA fragmentation    |
| 69| GO:1902952 | 0.0207344   | 2    | 5    | positive regulation of dendritic spine maintenance    |
| 70| GO:0090322 | 0.0229530   | 4    | 34   | regulation of superoxide metabolic process            |
| 71| GO:0010821 | 0.0245364   | 8    | 133  | regulation of mitochondrion organization              |
| 72| GO:0043161 | 0.0246286   | 16   | 394  | proteasome-mediated ubiquitin-dependent protein catabolic process |
| 73| GO:0045071 | 0.0257031   | 5    | 57   | negative regulation of viral genome replication       |
| 74| GO:2001233 | 0.0257031   | 16   | 399  | regulation of apoptotic signaling pathway             |
| 75| GO:0042127 | 0.0257031   | 47   | 1649 | regulation of cell population proliferation           |
| 76| GO:1903555 | 0.0264831   | 9    | 166  | regulation of tumor necrosis factor superfamily cytokine production |
| 77| GO:0010269 | 0.0264831   | 2    | 6    | response to selenium ion                              |
| 78| GO:0035795 | 0.0264831   | 2    | 6    | negative regulation of mitochondrial membrane permeability |
| 79| GO:0046070 | 0.0264831   | 2    | 6    | dGTP metabolic process                               |
| 80| GO:1905563 | 0.0264831   | 2    | 6    | negative regulation of vascular endothelial cell proliferation |
| 81| GO:0051205 | 0.0269966   | 4    | 37   | protein insertion into membrane                      |
|   | GO:0019941 | 0.0279923190091921 | 21 | 592 | modification-dependent protein catabolic process |
|---|------------|-------------------|----|-----|--------------------------------------------------|
| 83 | GO:0006725 | 0.0279923190091921 | 113| 4750| cellular aromatic compound metabolic process      |
| 84 | GO:0050790 | 0.0279923190091921 | 50 | 1798| regulation of catalytic activity                 |
| 85 | GO:1901564 | 0.280261358672338  | 95 | 4272| organonitrogen compound metabolic process         |
| 86 | GO:0009200 | 0.280261358672338  | 3  | 20  | deoxynucleoside triphosphate metabolic process    |
| 87 | GO:0033599 | 0.280261358672338  | 3  | 20  | regulation of mammary gland epithelial cell proliferation |
| 88 | GO:0042053 | 0.280261358672338  | 3  | 20  | regulation of dopamine metabolic process          |
| 89 | GO:1900409 | 0.280261358672338  | 3  | 20  | positive regulation of cellular response to oxidative stress |
| 90 | GO:0060337 | 0.280261358672338  | 4  | 39  | type I interferon signaling pathway               |
| 91 | GO:1903362 | 0.280261358672338  | 11 | 239 | regulation of cellular protein catabolic process  |
| 92 | GO:0055114 | 0.280261358672338  | 25 | 775 | oxidation-reduction process                       |
| 93 | GO:0009438 | 0.280261358672338  | 2  | 7   | methylglyoxal metabolic process                   |
| 94 | GO:0010760 | 0.280261358672338  | 2  | 7   | negative regulation of macrophage chemotaxis      |
| 95 | GO:0019673 | 0.280261358672338  | 2  | 7   | GDP-mannose metabolic process                     |
| 96 | GO:0060011 | 0.280261358672338  | 2  | 7   | Sertoli cell proliferation                         |
| 97 | GO:0070995 | 0.280261358672338  | 2  | 7   | NADPH oxidation                                   |
| GO ID       | GO Name                                                                 | P-value  | NES  | Description                                                                 |
|------------|--------------------------------------------------------------------------|----------|------|-----------------------------------------------------------------------------|
| GO:1902177 | positive regulation of oxidative stress-induced intrinsic apoptotic     | 0.0280261358672338 | 2    | signaling pathway                                                           |
| GO:0000413 | protein peptidyl-prolyl isomerization                                    | 0.0280261358672338 | 3    |                                                                             |
| GO:0006120 | mitochondrial electron transport, NADH to ubiquinone                    | 0.0280261358672338 | 3    |                                                                             |
| GO:0019430 | removal of superoxide radicals                                           | 0.0280261358672338 | 3    |                                                                             |
| GO:0032930 | positive regulation of superoxide anion generation                       | 0.0280261358672338 | 3    |                                                                             |
| GO:1901626 | regulation of postsynaptic membrane organization                         | 0.0280261358672338 | 3    |                                                                             |
| GO:0009206 | purine ribonucleoside triphosphate biosynthetic process                  | 0.0280261358672338 | 5    |                                                                             |
| GO:0045454 | cell redox homeostasis                                                   | 0.0280261358672338 | 5    |                                                                             |
| GO:0019748 | secondary metabolic process                                              | 0.0280261358672338 | 5    |                                                                             |
| GO:0032459 | regulation of protein oligomerization                                    | 0.0280261358672338 | 4    |                                                                             |
| GO:0061136 | regulation of proteasomal protein catabolic process                       | 0.0280261358672338 | 9    |                                                                             |
| GO:0046597 | negative regulation of viral entry into host cell                         | 0.0307334288045309 | 3    |                                                                             |
| GO:0051348 | negative regulation of transferase activity                              | 0.0312572952901065 | 11   |                                                                             |
| GO:1901360 | organic cyclic compound metabolic process                                | 0.0312572952901065 | 116  |                                                                             |
| GO:0046931 | pore complex assembly                                                    | 0.032304644334366 | 3    |                                                                             |
| GO:0071450 | cellular response to oxygen radical                                      | 0.032304644334366 | 3    |                                                                             |
| GO:0048678 | response to axon injury                                                  | 0.032304644334366 | 5    |                                                                             |
| GO        | Description                                                                 | EASE score | P value | q value | GO Tools | Description                                                                 |
|-----------|------------------------------------------------------------------------------|------------|---------|---------|----------|----------------------------------------------------------------------------|
| GO:2000179| positive regulation of neural precursor cell proliferation                  | 5          | 67      |         |          |                                                                             |
| GO:0090150| establishment of protein localization to membrane                             | 9          | 185     |         |          |                                                                             |
| GO:0046060| dATP metabolic process                                                       | 2          | 8       |         |          |                                                                             |
| GO:1990314| cellular response to insulin-like growth factor stimulus                      | 2          | 8       |         |          |                                                                             |
| GO:0060548| negative regulation of cell death                                            | 31         | 1031    |         |          |                                                                             |
| GO:0002181| cytoplasmic translation                                                      | 6          | 94      |         |          |                                                                             |
| GO:0043067| regulation of programmed cell death                                          | 42         | 1509    |         |          |                                                                             |
| GO:0034340| response to type I interferon                                                | 4          | 44      |         |          |                                                                             |
| GO:0010155| regulation of proton transport                                               | 3          | 24      |         |          |                                                                             |
| GO:0045862| positive regulation of proteolysis                                           | 13         | 325     |         |          |                                                                             |
| GO:0006164| purine nucleotide biosynthetic process                                       | 8          | 156     |         |          |                                                                             |
| GO:0051353| positive regulation of oxidoreductase activity                               | 4          | 46      |         |          |                                                                             |
| GO:0032760| positive regulation of tumor necrosis factor production                     | 6          | 98      |         |          |                                                                             |
| GO:1901657| glycosyl compound metabolic process                                         | 6          | 98      |         |          |                                                                             |
| GO:0032373| positive regulation of sterol transport                                      | 3          | 25      |         |          |                                                                             |
| GO:0051402| neuron apoptotic process                                                     | 12         | 293     |         |          |                                                                             |
| GO:0042743| hydrogen peroxide metabolic process                                         | 2          | 9       |         |          |                                                                             |
|   | GO:ID   | p-value   | q-value | Enrichment | Description                                                                 |
|---|---------|-----------|---------|------------|-----------------------------------------------------------------------------|
| 132 | GO:0009396 | 0.032304644334366 | 0.032304644334366 | 2 | 9 | folic acid-containing compound biosynthetic process |
| 133 | GO:0016559 | 0.032304644334366 | 0.032304644334366 | 2 | 9 | peroxisome fission |
| 134 | GO:0032471 | 0.032304644334366 | 0.032304644334366 | 2 | 9 | negative regulation of endoplasmic reticulum calcium ion concentration |
| 135 | GO:0034333 | 0.032304644334366 | 0.032304644334366 | 2 | 9 | adherens junction assembly |
| 136 | GO:0034551 | 0.032304644334366 | 0.032304644334366 | 2 | 9 | mitochondrial respiratory chain complex III assembly |
| 137 | GO:0042182 | 0.032304644334366 | 0.032304644334366 | 2 | 9 | ketone catabolic process |
| 138 | GO:0044597 | 0.032304644334366 | 0.032304644334366 | 2 | 9 | daunorubicin metabolic process |
| 139 | GO:0044598 | 0.032304644334366 | 0.032304644334366 | 2 | 9 | doxorubicin metabolic process |
| 140 | GO:0046666 | 0.032304644334366 | 0.032304644334366 | 2 | 9 | retinal cell programmed cell death |
| 141 | GO:1901033 | 0.032304644334366 | 0.032304644334366 | 2 | 9 | positive regulation of response to reactive oxygen species |
| 142 | GO:0044092 | 0.032304644334366 | 0.032304644334366 | 30 | 1006 | negative regulation of molecular function |
| 143 | GO:0072594 | 0.032304644334366 | 0.032304644334366 | 15 | 405 | establishment of protein localization to organelle |
| 144 | GO:0000154 | 0.032304644334366 | 0.032304644334366 | 3 | 26 | rRNA modification |
| 145 | GO:0000303 | 0.032304644334366 | 0.032304644334366 | 3 | 26 | response to superoxide |
| 146 | GO:0000737 | 0.032304644334366 | 0.032304644334366 | 3 | 26 | DNA catabolic process, endonucleolytic |
| 147 | GO:0006469 | 0.032304644334366 | 0.032304644334366 | 9 | 194 | negative regulation of protein kinase activity |
| 148 | GO:0051260 | 0.032304644334366 | 0.032304644334366 | 14 | 372 | protein homooligomerization |
| 149 | GO:0050435 | 0.032304644334366 | 0.032304644334366 | 4 | 48 | amyloid-beta metabolic process |
| GO        | p-value  | q-value | fold change | term                                                                 |
|-----------|----------|---------|-------------|----------------------------------------------------------------------|
| GO:0043066| 0.0323   | 0.323   | 26          | negative regulation of apoptotic process                             |
| GO:009394 | 0.0323   | 0.323   | 3           | 2'-deoxyribonucleotide metabolic process                             |
| GO:0008625| 0.0323   | 0.323   | 5           | extrinsic apoptotic signaling pathway via death domain receptors      |
| GO:006995 | 0.0323   | 0.323   | 2           | cellular response to nitrogen starvation                             |
| GO:009650 | 0.0323   | 0.323   | 2           | UV protection                                                        |
| GO:0072321| 0.0323   | 0.323   | 2           | chaperone-mediated protein transport                                 |
| GO:1904667| 0.0323   | 0.323   | 2           | negative regulation of ubiquitin protein ligase activity             |
| GO:0046483| 0.0323   | 0.323   | 109         | heterocycle metabolic process                                        |
| GO:0055086| 0.0323   | 0.323   | 17          | nucleobase-containing small molecule metabolic process               |
| GO:009142 | 0.0323   | 0.323   | 5           | nucleoside triphosphate biosynthetic process                         |
| GO:0010719| 0.0323   | 0.323   | 3           | negative regulation of epithelial to mesenchymal transition         |
| GO:009117 | 0.0323   | 0.323   | 15          | nucleotide metabolic process                                         |
| GO:2001056| 0.0323   | 0.323   | 7           | positive regulation of cysteine-type endopeptidase activity          |
| GO:009894 | 0.0323   | 0.323   | 25          | regulation of catabolic process                                      |
| GO:0050821| 0.0323   | 0.323   | 8           | protein stabilization                                                |
| GO:0030262| 0.0323   | 0.323   | 3           | apoptotic nuclear changes                                            |
| GO:2001243| 0.0323   | 0.323   | 5           | negative regulation of intrinsic apoptotic signaling pathway        |
|   | GO:0032464 | 0.0323046443334366 | 2 | 11 | positive regulation of protein homooligomerization |
|---|------------|---------------------|---|----|------------------------------------------------------------------|
| 168 | GO:0035999 | 0.0323046443334366 | 2 | 11 | tetrahydrofolate interconversion |
| 169 | GO:0043653 | 0.0323046443334366 | 2 | 11 | mitochondrial fragmentation involved in apoptotic process |
| 170 | GO:0002673 | 0.0323046443334366 | 4 | 53 | regulation of acute inflammatory response |
| 171 | GO:0001193 | 0.0323046443334366 | 1 | 1 | maintenance of transcriptional fidelity during DNA-templated transcription elongation from RNA polymerase II promoter |
| 172 | GO:0003106 | 0.0323046443334366 | 1 | 1 | negative regulation of glomerular filtration by angiotensin |
| 173 | GO:0006185 | 0.0323046443334366 | 1 | 1 | dGDP biosynthetic process |
| 174 | GO:0010797 | 0.0323046443334366 | 1 | 1 | regulation of multivesicular body size involved in endosome transport |
| 175 | GO:0014895 | 0.0323046443334366 | 1 | 1 | smooth muscle hypertrophy |
| 176 | GO:0015805 | 0.0323046443334366 | 1 | 1 | S-adenosyl-L-methionine transport |
| 177 | GO:0018323 | 0.0323046443334366 | 1 | 1 | enzyme active site formation via L-cysteine sulfinic acid |
| 178 | GO:0030961 | 0.0323046443334366 | 1 | 1 | peptidyl-arginine hydroxylation |
| 179 | GO:0032581 | 0.0323046443334366 | 1 | 1 | ER-dependent peroxisome organization |
| 180 | GO:0032775 | 0.0323046443334366 | 1 | 1 | DNA methylation on adenine |
| 181 | GO:0032976 | 0.0323046443334366 | 1 | 1 | release of matrix enzymes from mitochondria |
| 182 | GO:0034158 | 0.0323046443334366 | 1 | 1 | toll-like receptor 8 signaling pathway |
| 183 | GO:0036372 | 0.0323046443334366 | 1 | 1 | opsin transport |
| 184 | GO:0036471 | 0.0323046443334366 | 1 | 1 | cellular response to glyoxal |
| 185 | GO:0036526 | 0.0323046443334366 | 1 | 1 | peptidyl-cysteine deglycation |
| 186 | GO:0036527 | 0.0323046443334366 | 1 | 1 | peptidyl-arginine deglycation |
| 187 | GO:0036528 | 0.0323046443334366 | 1 | 1 | peptidyl-lysine deglycation |
| 188 | GO:0036529 | 0.0323046443334366 | 1 | 1 | protein deglycation, glyoxal removal |
| 189 | GO:0036531 | 0.0323046443334366 | 1 | 1 | glutathione deglycation |
| 190 | GO:0042543 | 0.0323046443334366 | 1 | 1 | protein N-linked glycosylation via arginine |
| 191 | GO:0043105 | 0.0323046443334366 | 1 | 1 | negative regulation of GTP cyclohydrolase I activity |
| 192 | GO:0044209 | 0.0323046443334366 | 1 | 1 | AMP salvage |
| 193 | GO:0046054 | 0.0323046443334366 | 1 | 1 | dGMP metabolic process |
| 194 | GO:0046491 | 0.0323046443334366 | 1 | 1 | L-methylmalonyl-CoA metabolic process |
| 195 | GO:0046711 | 0.0323046443334366 | 1 | 1 | GDP biosynthetic process |
| 196 | GO:0050668 | 0.0323046443334366 | 1 | 1 | positive regulation of homocysteine metabolic process |
| 197 | GO:0060311 | 0.0323046443334366 | 1 | 1 | negative regulation of elastin catabolic process |
| 198 | GO:0060785 | 0.0323046443334366 | 1 | 1 | regulation of apoptosis involved in tissue homeostasis |
|   | GO:0061078 | 0.0323046443334366 | 1  | 1  | positive regulation of prostaglandin secretion involved in immune response |
|---|------------|--------------------|----|----|--------------------------------------------------------------------------------|
| 200| GO:0070130 | 0.0323046443334366 | 1  | 1  | negative regulation of mitochondrial translation |
Table S2: Top 200 GO terms conditioned on the significance of their child terms enriched for DEG up regulated in young \textit{lgf2bp2}^{-/-} vs. \textit{lgf2bp2}^{+/+} mice

| Rank | GOBPID     | AdjPvalue             | Count | Size  | Term                                                      |
|------|------------|-----------------------|-------|-------|-----------------------------------------------------------|
| 1    | GO:0044238 | 9.23104288358855E-39  | 527   | 9156  | primary metabolic process                                  |
| 2    | GO:0036211 | 2.29999415326339E-29  | 259   | 3453  | protein modification process                              |
| 3    | GO:1901564 | 2.11298183675619E-21  | 277   | 4435  | organonitrogen compound metabolic process                 |
| 4    | GO:0046483 | 1.98544585315803E-17  | 284   | 4720  | heterocycle metabolic process                             |
| 5    | GO:0006725 | 7.01522075790976E-17  | 285   | 4791  | cellular aromatic compound metabolic process              |
| 6    | GO:0016070 | 2.12598353772816E-16  | 234   | 3687  | RNA metabolic process                                     |
| 7    | GO:1901360 | 3.73398022929563E-16  | 291   | 4997  | organic cyclic compound metabolic process                 |
| 8    | GO:0043170 | 1.17291265586009E-15  | 142   | 2421  | macromolecule metabolic process                           |
| 9    | GO:0044267 | 1.28192718824707E-15  | 156   | 2333  | cellular protein metabolic process                        |
| 10   | GO:0034641 | 2.20579221992963E-15  | 302   | 5330  | cellular nitrogen compound metabolic process              |
| 11   | GO:1901576 | 3.3198787845832E-15   | 187   | 2918  | organic substance biosynthetic process                    |
| 12   | GO:0043632 | 4.23509508570134E-15  | 69    | 602   | modification-dependent macromolecule catabolic process    |
| 13   | GO:0042886 | 2.40958301965545E-14  | 140   | 1878  | amide transport                                           |
| 14   | GO:0010605 | 1.55723244642524E-13  | 171   | 2581  | negative regulation of macromolecule metabolic process    |
|   | GO:ID          | p-value            | hits  | target | Description                                                                 |
|---|----------------|--------------------|-------|--------|-----------------------------------------------------------------------------|
| 15| GO:0009890     | 1.90684100550294E-13 | 118   | 1504   | negative regulation of biosynthetic process                               |
| 16| GO:0097659     | 3.7681774764402E-13 | 182   | 2814   | nucleic acid-templated transcription                                       |
| 17| GO:0048519     | 6.60669570362696E-13 | 168   | 2724   | negative regulation of biological process                                  |
| 18| GO:0070647     | 7.90095893875288E-12 | 48    | 394    | protein modification by small protein conjugation or removal               |
| 19| GO:0071702     | 8.19729951432942E-12 | 156   | 2391   | organic substance transport                                                 |
| 20| GO:0034654     | 8.6980937468926E-12 | 194   | 3179   | nucleobase-containing compound biosynthetic process                        |
| 21| GO:0044237     | 1.82927596067564E-11 | 79    | 1302   | cellular metabolic process                                                  |
| 22| GO:0045934     | 1.82927596067564E-11 | 98    | 1245   | negative regulation of nucleobase-containing compound metabolic process    |
| 23| GO:1902679     | 2.66218037821603E-11 | 95    | 1185   | negative regulation of RNA biosynthetic process                            |
| 24| GO:0065009     | 5.43845982573898E-11 | 138   | 2074   | regulation of molecular function                                            |
| 25| GO:0007030     | 8.60663021548485E-11 | 25    | 121    | Golgi organization                                                          |
| 26| GO:0051603     | 1.02038505506578E-10 | 58    | 575    | proteolysis involved in cellular protein catabolic process                 |
| 27| GO:0007049     | 2.26070449687253E-10 | 35    | 270    | cell cycle                                                                  |
| 28| GO:0043067     | 4.03623736835232E-10 | 110   | 1538   | regulation of programmed cell death                                        |
| 29| GO:1901575     | 6.83465687051356E-10 | 125   | 1855   | organic substance catabolic process                                         |
| 30| GO:0000122     | 8.02307087569772E-10 | 73    | 857    | negative regulation of transcription by RNA polymerase II                  |
|   | GO: Identifier | FDR | P-value | Count | Description                                    |
|---|---------------|-----|---------|-------|------------------------------------------------|
| 31| GO:0030163    | 9.4994028313801E-09 | 49   | 502  | protein catabolic process                       |
| 32| GO:0051301    | 2.16387806919067E-08 | 41   | 383  | cell division                                  |
| 33| GO:0022607    | 4.48134028968872E-08 | 136  | 2280 | cellular component assembly                     |
| 34| GO:0051130    | 5.73240839456416E-08 | 86   | 1198 | positive regulation of cellular component organization |
| 35| GO:0018193    | 1.09346230851413E-07 | 63   | 792  | peptidyl-amino acid modification                |
| 36| GO:0006650    | 1.3058760998349E-07  | 20   | 112  | glycerophospholipid metabolic process           |
| 37| GO:0045935    | 1.49211134057578E-07 | 114  | 1794 | positive regulation of nucleobase-containing compound metabolic process |
| 38| GO:0044770    | 1.83509170098268E-07 | 19   | 104  | cell cycle phase transition                     |
| 39| GO:0051338    | 3.97746034883717E-07 | 49   | 561  | regulation of transferase activity             |
| 40| GO:0000209    | 4.00541211274857E-07 | 20   | 120  | protein polyubiquitination                      |
| 41| GO:0000070    | 6.04016071093172E-07 | 12   | 42   | mitotic sister chromatid segregation            |
| 42| GO:0033674    | 6.2951577075664E-07  | 44   | 476  | positive regulation of kinase activity          |
| 43| GO:0016043    | 6.49375175879364E-07 | 71   | 1211 | cellular component organization                 |
| 44| GO:0006888    | 6.49375175879364E-07 | 20   | 123  | endoplasmic reticulum to Golgi vesicle-mediated transport |
| 45| GO:0065007    | 7.18047408058048E-07 | 274  | 6747 | biological regulation                           |
| 46| GO:0016567    | 8.33731333800214E-07 | 23   | 169  | protein ubiquitination                          |
| 47| GO:0001701    | 1.05978890015171E-06 | 47   | 538  | in utero embryonic development                  |
| 48| GO:0098813    | 1.07832968552138E-06 | 29   | 249  | nuclear chromosome segregation                  |
| # | Gene ID | P-Value  | MW  | K       | Process                                      |
|---|--------|----------|-----|---------|----------------------------------------------|
| 49 | GO:0032502 | 1.40863705277315E-06 | 200 | 4128 | developmental process |
| 50 | GO:0006793 | 1.60028129612288E-06 | 47  | 601   | phosphorus metabolic process |
| 51 | GO:0010942 | 1.97780721897141E-06 | 55  | 695   | positive regulation of cell death |
| 52 | GO:0007346 | 2.39886365486283E-06 | 31  | 293   | regulation of mitotic cell cycle |
| 53 | GO:0032270 | 3.03304533068727E-06 | 89  | 1396  | positive regulation of cellular protein metabolic process |
| 54 | GO:0043085 | 3.17899871582597E-06 | 45  | 542   | positive regulation of catalytic activity |
| 55 | GO:0015031 | 3.78588364422005E-06 | 32  | 333   | protein transport |
| 56 | GO:0016050 | 4.28089239712905E-06 | 28  | 252   | vesicle organization |
| 57 | GO:0006511 | 4.76070537564352E-06 | 21  | 159   | ubiquitin-dependent protein catabolic process |
| 58 | GO:0009891 | 5.07994813128319E-06 | 114 | 1932  | positive regulation of biosynthetic process |
| 59 | GO:0032989 | 5.30409554150901E-06 | 60  | 815   | cellular component morphogenesis |
| 60 | GO:0048194 | 5.30409554150901E-06 | 10  | 34    | Golgi vesicle budding |
| 61 | GO:0060548 | 5.30409554150901E-06 | 59  | 805   | negative regulation of cell death |
| 62 | GO:0000280 | 5.4968617469048E-06  | 25  | 214   | nuclear division |
| 63 | GO:0006325 | 5.96175799595545E-06 | 37  | 408   | chromatin organization |
| 64 | GO:0060322 | 6.38015584296174E-06 | 53  | 688   | head development |
| 65 | GO:0070507 | 6.49820962407204E-06 | 24  | 199   | regulation of microtubule cytoskeleton organization |
| 66 | GO:1902680 | 7.01438536007607E-06 | 95  | 1538  | positive regulation of RNA biosynthetic process |
|   | GO:       | p-value   | Count | Description                                                                 |
|---|-----------|-----------|-------|-----------------------------------------------------------------------------|
| 67| GO:0009792| 8.9257206772897E-06 | 60    | embryo development ending in birth or egg hatching                          |
| 68| GO:0006913| 9.5238977863853E-06 | 22    | nucleocytoplasmic transport                                                |
| 69| GO:0051174| 1.12458015290964E-05 | 74    | regulation of phosphorus metabolic process                                |
| 70| GO:1903047| 1.55505179560498E-05 | 28    | mitotic cell cycle process                                                 |
| 71| GO:0033365| 1.82002624722506E-05 | 43    | protein localization to organelle                                          |
| 72| GO:0048522| 1.82002624722506E-05 | 87    | positive regulation of cellular process                                    |
| 73| GO:0048858| 1.82407337627159E-05 | 52    | cell projection morphogenesis                                               |
| 74| GO:0051641| 2.02157005502507E-05 | 38    | cellular localization                                                      |
| 75| GO:0022603| 2.76135987050317E-05 | 70    | regulation of anatomical structure morphogenesis                            |
| 76| GO:0007264| 2.93336337219545E-05 | 40    | small GTPase mediated signal transduction                                  |
| 77| GO:0045937| 3.16604342040004E-05 | 72    | positive regulation of phosphate metabolic process                         |
| 78| GO:0034645| 3.49872810430653E-05 | 121   | cellular macromolecule biosynthetic process                                |
| 79| GO:0018105| 3.96890115007544E-05 | 30    | peptidyl-serine phosphorylation                                             |
| 80| GO:0042325| 4.77508286806648E-05 | 69    | regulation of phosphorylation                                               |
| 81| GO:0051674| 5.12093579894238E-05 | 94    | localization of cell                                                        |
| 82| GO:0010564| 5.53430583395541E-05 | 27    | regulation of cell cycle process                                            |
|   | GO: Identifier   | E-Value          | Up/Down   | Term Description                                                                 |
|---|-----------------|-----------------|-----------|----------------------------------------------------------------------------------|
| 83| GO:0051129      | 5.84237557787559E-05 | 41        | 523 negative regulation of cellular component organization                       |
| 84| GO:0051276      | 6.41735966350662E-05 | 17        | 136 chromosome organization                                                       |
| 85| GO:1902115      | 6.79262923444311E-05 | 22        | 200 regulation of organelle assembly                                               |
| 86| GO:0018205      | 6.79262923444311E-05 | 31        | 345 peptidyl-lysine modification                                                   |
| 87| GO:0045786      | 6.79262923444311E-05 | 28        | 298 negative regulation of cell cycle                                             |
| 88| GO:0040007      | 7.81414573321041E-05 | 54        | 789 growth                                                                         |
| 89| GO:1902850      | 8.21031973362559E-05 | 17        | 130 microtubule cytoskeleton organization involved in mitosis                     |
| 90| GO:0090110      | 8.21031973362559E-05 | 6         | 14 COPII-coated vesicle cargo loading                                              |
| 91| GO:0043065      | 9.51394318391474E-05 | 36        | 443 positive regulation of apoptotic process                                      |
| 92| GO:0043066      | 9.8766303564794E-05  | 41        | 541 negative regulation of apoptotic process                                      |
| 93| GO:0009987      | 0.000101384866956536| 52        | 1918 cellular process                                                              |
| 94| GO:0007051      | 0.000107246984383093| 18        | 148 spindle organization                                                           |
| 95| GO:0000902      | 0.000116644643942125| 33        | 404 cell morphogenesis                                                             |
| 96| GO:0120035      | 0.000128629744483153 | 53  | 772 regulation of plasma membrane bounded cell projection organization              |
| 97| GO:0042752      | 0.000136179932189572| 16        | 122 regulation of circadian rhythm                                                |
| 98| GO:0030098      | 0.000139642469104033 | 33        | 395 lymphocyte differentiation                                                     |
| 99| GO:1903320      | 0.000170514476435427 | 23        | 229 regulation of protein modification by small protein conjugation or removal     |
| GO:0070201 | 0.000170514476435427 | 52     | 761     | regulation of establishment of protein localization |
|-------------|------------------------|--------|---------|----------------------------------------------------|
| GO:0097549  | 0.000213924382378307   | 13     | 87      | chromatin organization involved in negative regulation of transcription |
| GO:0051302  | 0.000224055758628519   | 18     | 156     | regulation of cell division                        |
| GO:0033002  | 0.000226709441777809   | 18     | 157     | muscle cell proliferation                           |
| GO:0060429  | 0.000235865222091762   | 67     | 1090    | epithelium development                              |
| GO:0016236  | 0.000240825735659185   | 13     | 89      | macroautophagy                                     |
| GO:0051246  | 0.000240825735659185   | 44     | 661     | regulation of protein metabolic process            |
| GO:0007417  | 0.000240825735659185   | 58     | 895     | central nervous system development                 |
| GO:0006606  | 0.000240825735659185   | 14     | 102     | protein import into nucleus                        |
| GO:0060236  | 0.000240825735659185   | 6      | 17      | regulation of mitotic spindle organization         |
| GO:0090304  | 0.000240825735659185   | 33     | 475     | nucleic acid metabolic process                     |
| GO:0019637  | 0.000240825735659185   | 51     | 761     | organophosphate metabolic process                  |
| GO:0031123  | 0.000240825735659185   | 14     | 102     | RNA 3'-end processing                              |
| GO:0006891  | 0.000240825735659185   | 7      | 25      | intra-Golgi vesicle-mediated transport             |
| GO:0031326  | 0.000240825735659185   | 40     | 606     | regulation of cellular biosynthetic process        |
| GO:0045588  | 0.000240825735659185   | 4      | 6       | positive regulation of gamma-delta T cell differentiation |
| GO:0060304  | 0.000240825735659185   | 4      | 6       | regulation of phosphatidylinositol dephosphorylation |
| Gene ID | GO Term | p-value | Count | Total | Description |
|--------|---------|---------|-------|-------|-------------|
| 117    | GO:0022610 | 0.000313582137446708 | 77    | 1310  | biological adhesion |
| 118    | GO:0021987 | 0.000325053149595525 | 14    | 105   | cerebral cortex development |
| 119    | GO:0010556 | 0.000337414634638412 | 33    | 465   | regulation of macromolecule biosynthetic process |
| 120    | GO:0006260 | 0.0003740122984837072 | 16    | 135   | DNA replication |
| 121    | GO:0000082 | 0.000386974833037351 | 10    | 57    | G1/S transition of mitotic cell cycle |
| 122    | GO:0090630 | 0.000461097715945304 | 12    | 82    | activation of GTPase activity |
| 123    | GO:0048729 | 0.000500036363218249 | 46    | 677   | tissue morphogenesis |
| 124    | GO:0060341 | 0.000505855519641317 | 63    | 1029  | regulation of cellular localization |
| 125    | GO:0006886 | 0.000505855519641317 | 39    | 557   | intracellular protein transport |
| 126    | GO:1904594 | 0.000520678214941738 | 3     | 3     | regulation of termination of RNA polymerase II transcription |
| 127    | GO:0048661 | 0.000521694122965928 | 11    | 71    | positive regulation of smooth muscle cell proliferation |
| 128    | GO:0030258 | 0.000575257310020052 | 21    | 218   | lipid modification |
| 129    | GO:1902532 | 0.000582542272192119 | 37    | 506   | negative regulation of intracellular signal transduction |
| 130    | GO:0006267 | 0.000582542272192119 | 4     | 7     | pre-replicative complex assembly involved in nuclear cell cycle DNA replication |
| 131    | GO:0036388 | 0.000582542272192119 | 4     | 7     | pre-replicative complex assembly |
| 132    | GO:0046854 | 0.000582542272192119 | 8     | 38    | phosphatidylinositol phosphorylation |
| 133    | GO:2001251 | 0.000623053953913651 | 13    | 99    | negative regulation of chromosome organization |
|   | GO ID      | P-value       | Rank | Total | Description                                           |
|---|------------|---------------|------|-------|-------------------------------------------------------|
| 134 | GO:0080135 | 0.00065992436723702 | 34   | 457   | regulation of cellular response to stress           |
| 135 | GO:0043434 | 0.000660026010325452  | 27   | 324   | response to peptide hormone                         |
| 136 | GO:0033044 | 0.000717399882367531  | 7    | 30    | regulation of chromosome organization              |
| 137 | GO:0048511 | 0.000735993295718623  | 25   | 291   | rhythmic process                                    |
| 138 | GO:0016202 | 0.000824585663200282  | 18   | 176   | regulation of striated muscle tissue development     |
| 139 | GO:0032388 | 0.000824585663200282  | 19   | 192   | positive regulation of intracellular transport     |
| 140 | GO:0031399 | 0.000829142749957381  | 61   | 1047  | regulation of protein modification process          |
| 141 | GO:0010468 | 0.000842990028897118   | 43   | 724   | regulation of gene expression                       |
| 142 | GO:0007219 | 0.00086105111314719   | 18   | 177   | Notch signaling pathway                             |
| 143 | GO:0031323 | 0.00086105111314719   | 26   | 390   | regulation of cellular metabolic process            |
| 144 | GO:0003007 | 0.000866737546499747   | 15   | 132   | heart morphogenesis                                 |
| 145 | GO:0051783 | 0.000907880423323346  | 19   | 194   | regulation of nuclear division                      |
| 146 | GO:2000144 | 0.000959768436831571   | 7    | 31    | positive regulation of DNA-templated transcription, initiation |
| 147 | GO:0048193 | 0.000960681873875285   | 13   | 106   | Golgi vesicle transport                             |
| 148 | GO:0070828 | 0.00105920479476968    | 9    | 53    | heterochromatin organization                        |
| 149 | GO:0010563 | 0.00105920479476968    | 38   | 544   | negative regulation of phosphorus metabolic process |
| 150 | GO:0030866 | 0.00108008006781643    | 8    | 42    | cortical actin cytoskeleton organization             |
|   | GO:ID       | p-value       | Ranked | Total | Description                                           |
|---|-------------|---------------|--------|--------|-------------------------------------------------------|
| 151| GO:0001934 | 0.001080806781643 | 58     | 960    | positive regulation of protein phosphorylation        |
| 152| GO:0048534 | 0.0011353763308271 | 54     | 883    | hematopoietic or lymphoid organ development            |
| 153| GO:0033157 | 0.0011602358010702 | 20     | 215    | regulation of intracellular protein transport          |
| 154| GO:0016310 | 0.00121177868419467 | 20     | 235    | phosphorylation                                        |
| 155| GO:0051571 | 0.0012404274213238 | 5      | 15     | positive regulation of histone H3-K4 methylation       |
| 156| GO:0060560 | 0.0012404274213238 | 21     | 234    | developmental growth involved in morphogenesis         |
| 157| GO:0000045 | 0.0012484237920141 | 12     | 93     | autophagosome assembly                                  |
| 158| GO:0033047 | 0.00129850389179332 | 10     | 67     | regulation of mitotic sister chromatid segregation     |
| 159| GO:1901653 | 0.0013245758437273 | 24     | 287    | cellular response to peptide                           |
| 160| GO:0044089 | 0.00135371560172831 | 31     | 420    | positive regulation of cellular component biogenesis   |
| 161| GO:0032092 | 0.00146389020552491 | 13     | 109    | positive regulation of protein binding                 |
| 162| GO:0010647 | 0.00146389020552491 | 101    | 1936   | positive regulation of cell communication              |
| 163| GO:0045648 | 0.00147558882115683 | 6      | 24     | positive regulation of erythrocyte differentiation     |
| 164| GO:0048638 | 0.0015245840938114  | 30     | 401    | regulation of developmental growth                     |
| 165| GO:0007091 | 0.0015245840938114  | 3      | 4      | metaphase/anaphase transition of mitotic cell cycle    |
| GO  | Description                                                                                     | Adjusted p-value | p-value   | Fold Change | FDR   |
|-----|------------------------------------------------------------------------------------------------|------------------|-----------|-------------|-------|
| GO:0046726 | 0.00153763237707497 positive regulation by virus of viral protein levels in host cell | 5.8206167e-01    | 0.00153864700427697 | 3         | 4     | negative regulation of response to stimulus |
| GO:0048585 | 0.00153864700427697 cellular response to stress                                               | 1.6932099e-01    | 0.00153864700427697 | 26        | 352   | regulation of gamma-delta T cell activation |
| GO:0033554 | 0.00153864700427697 negative regulation of response to stimulus                               | 1.6459873e-01    | 0.00153864700427697 | 4         | 9     | regulation of gamma-delta T cell activation |
| GO:0046643 | 0.00153864700427697 maintenance of cell polarity                                               | 1.6760174e-01    | 0.00153864700427697 | 101       | 1942  | positive regulation of signaling            |
| GO:0030011 | 0.00158800081070809 positive regulation of signaling                                             | 7.9313452e-01    | 0.00158800081070809 | 5         | 16    | maintenance of cell polarity                |
| GO:0045893 | 0.00160919219081141 positive regulation of transcription, DNA-templated                         | 1.0358938e-01    | 0.00160919219081141 | 24        | 302   | negative regulation of cell communication   |
| GO:0010648 | 0.0016301830025447 erythrocyte homeostasis                                                       | 1.1891374e-01    | 0.0016301830025447 | 75        | 1352  | negative regulation of cell communication   |
| GO:0033554 | 0.00163593237707497 negative regulation of signaling                                             | 7.3025713e-01    | 0.00163593237707497 | 13         | 111   | DNA integrity checkpoint                    |
| GO:0034101 | 0.00173276218453138 erythrocyte homeostasis                                                     | 1.0358938e-01    | 0.00173276218453138 | 15        | 142   | erythrocyte homeostasis                     |
| GO:0023057 | 0.00173276218453138 negative regulation of signaling                                             | 1.0358938e-01    | 0.00173276218453138 | 75        | 1356  | negative regulation of signaling            |
| GO:0090087 | 0.00173319001381069 regulation of peptide transport                                               | 7.9313452e-01    | 0.00173319001381069 | 21        | 244   | regulation of peptide transport             |
| GO:0023057 | 0.0017435400208103 regulation of nucleocytoplasmic transport                                    | 1.0358938e-01    | 0.0017435400208103 | 13         | 112   | regulation of nucleocytoplasmic transport   |
| GO:0032275 | 0.00178452940165331 regulation of chromatin organization                                           | 1.0358938e-01    | 0.00178452940165331 | 17         | 175   | regulation of chromatin organization        |
| GO:0002011 | 0.00178452940165331 morphogenesis of an epithelial sheet                                           | 1.0358938e-01    | 0.00178452940165331 | 9          | 58    | morphogenesis of an epithelial sheet        |
| GO:2000145 | 0.00178452940165331 regulation of cell motility                                                   | 1.0358938e-01    | 0.00178452940165331 | 56         | 939   | regulation of cell motility                |
| ID  | GO ID             | FDR   | Count | Description                                                                 |
|-----|-------------------|-------|-------|-----------------------------------------------------------------------------|
| 182 | GO:0071559        | 0.0017971943954664 | 19    | 208 response to transforming growth factor beta                           |
| 183 | GO:0060996        | 0.0019466473370009 | 9     | 59 dendritic spine development                                             |
| 184 | GO:0048699        | 0.00198769940738496 | 80    | 1487 generation of neurons                                                 |
| 185 | GO:0046907        | 0.00198769940738496 | 21    | 263 intracellular transport                                                |
| 186 | GO:0045579        | 0.00200616594878531 | 5     | 17 positive regulation of B cell differentiation                           |
| 187 | GO:0006468        | 0.00201928835722702 | 35    | 538 protein phosphorylation                                                |
| 188 | GO:0051656        | 0.00204029794941234 | 22    | 264 establishment of organelle localization                                |
| 189 | GO:0043161        | 0.00210763073731539 | 18    | 198 proteasome-mediated ubiquitin-dependent protein catabolic process      |
| 190 | GO:0051348        | 0.00211355979004536 | 21    | 246 negative regulation of transferase activity                            |
| 191 | GO:0002315        | 0.00216927103778378 | 4     | 10 marginal zone B cell differentiation                                     |
| 192 | GO:0007220        | 0.00216927103778378 | 4     | 10 Notch receptor processing                                               |
| 193 | GO:0046543        | 0.00216927103778378 | 4     | 10 development of secondary female sexual characteristics                 |
| 194 | GO:0060982        | 0.00216927103778378 | 4     | 10 coronary artery morphogenesis                                            |
| 195 | GO:0009894        | 0.00218569269603798 | 31    | 442 regulation of catabolic process                                        |
| 196 | GO:0042176        | 0.00221883700453103 | 21    | 249 regulation of protein catabolic process                               |
| 197 | GO:0048812        | 0.00225200580313821 | 34    | 499 neuron projection morphogenesis                                        |
| 198 | GO:0016477        | 0.00232905705294305 | 29    | 410 cell migration                                                          |
| 199 | GO:0045859        | 0.00241119023116754 | 16    | 169 regulation of protein kinase activity                                  |
| 200 | GO:0002335 | 0.00241119023116754 | 6 | 27 | mature B cell differentiation |
Figure S1

A

WT *Igf2bp2* allele

Targeting vector

Mutant *Igf2bp2* allele

B

Mus musculus *Igf2bp2* mRNA

WT Liver *Igf2bp2* mRNA

WT Lung *Igf2bp2* mRNA

WT Intestine *Igf2bp2* mRNA

Mutant Liver *Igf2bp2* mRNA

Mutant Lung *Igf2bp2* mRNA

Mutant Intestine *Igf2bp2* mRNA

C

MEF

Igf2bp2-/-

Igf2bp2-/-

IGF2BP2

(66 kDa)

β-actin

(42 kDa)

D

Liver

Igf2bp2-/-

Igf2bp2-/-

Igf2bp2-/-

H2O

WT

(150 bp)

Mutant

(330 bp)
Figure S2
Figure S3

(A) Relative expression of \( \text{Igf2bp2} \) (normalized to \( \text{Actb} \)) in Young and Old mice.

- Balanced HSC: \( p = 0.0001 \)
- Myeloid-biased HSC: \( p = 0.0003 \)

- Male: \( p = 0.1008 \)
- Female: \( p < 0.0001 \)

(B) Myeloid-biased HSC

- 6 weeks old: \( p = 0.0001 \)
- 12 weeks old: \( p = 0.001 \)
- 36 weeks old: \( p < 0.0001 \)

(C) Myeloid-biased HSC

- Male: \( p = 0.001 \)
- Female: \( p < 0.0001 \)
Figure S4

A. Heatmap of top marker genes across different clusters.

B. UMAP plot showing expression levels of Hmga2 across different clusters.

C. Bar graph showing percentage of Hmga2 expressing cells in each cluster.

D. UMAP plot showing expression levels of Rian across different clusters.

E. Bar graph showing percentage of Rian expressing cells in each cluster.

F. UMAP plot showing expression levels of Cdkn1c across different clusters.

G. Bar graph showing percentage of Cdkn1c expressing cells in each cluster.

H. UMAP plot showing expression levels of H19 across different clusters.

I. Bar graph showing percentage of H19 expressing cells in each cluster.
Figure S5

(A) Myeloid-biased HSC

(B) Ilga2b

(C) Vwf

(D) Pf4

(E) Klf1

(F) Mki67

(G) Gata1

(H) Car1

(I) Igf2bp2 expression

Cluster number

0          3         4         5         8
Figure S6

Igf2bp2 deletion: effect size -14.31%, \( p=0.0014 \)

Igf2bp2 deletion: effect size -5.63%, \( p=0.401 \)
**Figure S7**

**A**

Relative expression of Igf2bp2 (normalized to Actb).

**B**

Intracellular ATP concentration (nM/1500 cells).

**C**

Comparison of OCR (pmol/min/4x10^4 cells) between young and old LSK Igf2bp2^-/- and Igf2bp2+/+.

**D**

Fold change of ROS MFI (normalized to young WT).

**E**

Rank GOBPID AdjPvalue Term

| Rank | GOBPID   | AdjPvalue | Term                                      |
|------|----------|-----------|-------------------------------------------|
| 19   | GO:0070584 | 0.00173   | mitochondrion morphogenesis               |
| 35   | GO:0035456 | 0.00821   | response to interferon-beta               |
| 59   | GO:1902430 | 0.01854   | negative regulation of amyloid-beta formation |
| 65   | GO:0033864 | 0.0207    | positive regulation of NAD(P)H oxidase activity |
| 70   | GO:0090322 | 0.023     | regulation of superoxide metabolic process |
| 71   | GO:0010821 | 0.0245    | regulation of mitochondrion organization  |
| 72   | GO:0043161 | 0.0246    | proteasome-mediated ubiquitin-dependent protein catabolic process |
| 73   | GO:0045071 | 0.0257    | negative regulation of viral genome replication |
| 89   | GO:1900409 | 0.028     | positive regulation of cellular response to oxidative stress |
| 90   | GO:0060337 | 0.028     | type I interferon signaling pathway        |
| 92   | GO:0055114 | 0.028     | oxidation-reduction process                |
| 97   | GO:0070995 | 0.028     | NADPH oxidation                           |
| 101  | GO:0019430 | 0.028     | removal of superoxide radicals             |
| 105  | GO:0045454 | 0.028     | cell redox homeostasis                     |
| 113  | GO:0071450 | 0.0323    | cellular response to oxygen radical        |
| 122  | GO:0034340 | 0.0323    | response to type I interferon              |
| 141  | GO:1901033 | 0.0323    | positive regulation of response to reactive oxygen species |
| 145  | GO:0000303 | 0.0323    | response to superoxide                     |
| 149  | GO:0050435 | 0.0323    | amyloid-beta metabolic process             |
| 169  | GO:0043653 | 0.0323    | mitochondrial fragmentation involved in apoptotic process |

**F**

Intracellular ATP concentration (nM/1500 cells).
Figure S8

(A) Fold change of GFP+ cells in CD45.2+ PB (normalized to initial GFP+ rate) over weeks after transplantation.

(B) Young vs. Old comparison of GFP+ cells in PB.

(C) Lineage composition of CD45.2+ GFP+ cells in PB.

- **Myeloid cells**
- **Lymphoid cells**

**Statistical Significance**:
- p = 0.1446 (ns)
- p = 0.019
- p = 0.0218
- p = 0.0255
Figure S11

A

Igf2bp2\textsuperscript{+/+}

Igf2bp2\textsuperscript{-/-}

PC1: 71% Variance

PC2: 19% Variance

B

LFC >1 or unadjusted \(p\)-value < 0.05

C

unadjusted \(p\)-value < 0.05

D

adjusted \(p\)-value < 0.05

E

\begin{tabular}{|c|c|c|c|}
\hline
Rank & GOBPID & AdjPvalue & Term \\
\hline
1 & GO:0051873 & 3.52E-07 & killing by host of symbiont cells \\
2 & GO:0031640 & 3.52E-07 & killing of cells of other organism \\
3 & GO:0051883 & 3.52E-07 & killing of cells in other organism involved in symbiotic interaction \\
4 & GO:0006952 & 4.91E-07 & defense response \\
5 & GO:0002376 & 4.91E-07 & immune system process \\
6 & GO:0070944 & 5.71E-07 & neutrophil-mediated killing of bacterium \\
7 & GO:0070943 & 1.3E-06 & neutrophil-mediated killing of symbiont cell \\
8 & GO:0070942 & 2.5E-06 & neutrophil mediated cytotoxicity \\
9 & GO:0001906 & 3.04E-06 & cell killing \\
\hline
\end{tabular}

F

\begin{tabular}{|c|c|c|c|}
\hline
Rank & GOBPID & AdjPvalue & Term \\
\hline
1 & GO:0006379 & 0.00506 & mRNA cleavage \\
2 & GO:0016601 & 0.0169 & Rac protein signal transduction \\
3 & GO:2000601 & 0.0169 & positive regulation of Arp2/3 complex-mediated actin nucleation \\
4 & GO:0009653 & 0.0194 & anatomical structure morphogenesis \\
5 & GO:0090501 & 0.0194 & RNA phosphodiester bond hydrolysis \\
6 & GO:0034315 & 0.0194 & regulation of Arp2/3 complex-mediated actin nucleation \\
\hline
\end{tabular}
Supplemental figure legend

Figure S1. Generation of Igf2bp2 knockout mice. (A) Targeting strategy for Igf2bp2 gene by the Cre-loxP recombination system. Exon-3 of Igf2bp2 is flanked with two loxP sites (orange triangles) that were in opposite orientation. Upon germline expression of CRE, offspring with inverted exon-3 were used to generate homozygous knockout mice, which are referred to as Igf2bp2−/− mice. Arrows indicate the position of primers used for genotyping. (B) Alignment of Igf2bp2 mRNA sequences from liver, lung and intestine of Igf2bp2−/− and Igf2bp2+/+ mice by Sanger sequencing. Alignment was conducted against the reference sequence of Mus musculus Igf2bp2 mRNA (NM_183029). (N=2 mice per genotype) (C) The western blot shows IGF2BP2 protein (66 kDa) expression in MEF (E12.5) of the indicated genotypes (2-3 mice per genotype). The actin control was developed from the same membrane. (D) The western blot shows IGF2BP2 protein (66 kDa) expression in adult liver of mice of the indicated genotype (n= 4 mice per group). The actin control was developed from the same membrane. (E) Genotyping PCR analysis of tail biopsies from 2-week-old mice (n=3 per group) reveals the expected bands indicative of Igf2bp2+/+, Igf2bp2+/− and Igf2bp2−/− genotypes.

Figure S2. Gating strategies of FACS analyses.
(A) Gating strategy of FACS analysis for hematopoietic stem cell and progenitor populations in BM cells.
LSK: Lineage−Sca1−cKit+; LK: Lineage−Sca1−cKit+; HSC: Lineage−Sca1−cKit−CD150−CD34−; MPP: Lineage−Sca1−cKit+CD34+; CLP: Lineage−cKitlowSca1lowFlt3+CD127+; CMP: Lineage−Sca1−cKit+CD34+FcγR−; GMP: Lineage−Sca1−cKit+CD34+FcγR++; MEP: Lineage−Sca1−cKit+CD34−FcγR−.
(B) Gating strategy of FACS analysis for mature cell populations in PB cells.
B-cell: CD45+B220+; T-cell: CD45+CD4+/CD8+; Myeloid cell: CD45+Gr1+/CD11b+.
(C) Gating strategy of FACS analysis for subpopulations of MPP in BM cells.
MPP1: Lineage−Sca1−cKit+CD150−CD48−CD34−Flt3−; MPP2: Lineage−Sca1−cKit+CD150−CD48−CD34−Flt3−; MPP3: Lineage−Sca1−cKit+CD150−CD48−CD34−Flt3−; MPP4: Lineage−Sca1−cKit+CD150−CD48−CD34−Flt3−.
(D) Gating strategy of FACS analysis of ex vivo culture HSC: DAPI−GFP+Lineage−Sca1+CD48−. FSC, forward scatter; SSC, side scatter.

**Figure S3.** Myeloid-biased HSC (CD150\textsuperscript{high}) from young, male mice have the highest expression of \textit{lgf2bp2} mRNA. The relative mRNA expression of \textit{lgf2bp2} (relative to \textit{Actb}) was analyzed (A) in balanced HSC (CD150\textsuperscript{low}CD34−LSKs) and myeloid-biased HSC (CD150\textsuperscript{high}CD34−LSKs) from young (3-4 months) and old (24-26 months) wildtype mice (n=6-7 mice per group), (B) in myeloid-biased HSC (CD150\textsuperscript{high}CD34−LSKs) from male wildtype mice of indicated age (n=3 mice per group), and (C) in myeloid-biased HSC from 9-month old wildtype mice of indicated genders, male and female (n=3 mice per group). (A-C) Statistical significance was assessed by two-way ANOVA on log2-transformed data followed by pairwise t-tests with Sidak's correction for multiple comparisons (A) or by one-way ANOVA on log2-transformed data followed by pairwise t-tests with Tukey's correction for multiple comparisons (B), or by Welch’s t-test on log2-transformed data (C). All data shown in this figure represent mean ± SD, and ns means “non-significant”.

**Figure S4.** \textit{lgf2bp2}-high cluster is enriched for the expression of imprinted genes that are expressed in long-term HSC. (A) Heat map showing top differentially expressed genes in each subcluster as depicted in Figure 3A. Color scale indicates the level of gene expression. (B-I) Feature plots and histograms on HSC-related, imprinted genes depicting the expression level and the percentage of positive cells in the HSC-subclusters (as shown in Fig. 3A) for (B,C) \textit{Hmga2}, (D,E) \textit{Rian}, (F,G) \textit{Cdkn1c} and (H,I) \textit{H19}. Gray dots indicate no expression, and red intensity indicates the expression level of each gene.

**Figure S5.** High expression of megakaryocyte/thrombocyte and erythroid marker genes indicates that HSC in clusters 1, 2, 6, and 7 are lineage primed. (A) UMAP plot with Seurat clustering. (B-H) Feature plots of common marker genes of megakaryocyte/thrombocyte and erythroid lineages: (B) \textit{Itga2b}, (C) \textit{Vwf}, (D) \textit{Pf4}, (E) \textit{Klf1}, (F) \textit{Mki67}, (G) \textit{Gata1}, (H) \textit{Car1}. (I) Violin plot of \textit{lgf2bp2} expression in Cluster-0, 3, 4, 5, 8 of unprimed myeloid-biased HSC. A KS test was performed to compare \textit{lgf2bp2} expression in Cluster 3 vs. each of Cluster-0 (\(p=2.6\times10^{-9}\)), Cluster-4 (\(p=6.6\times10^{-4}\)), Cluster-5 (\(p=8.7\times10^{-4}\)), Cluster-8 (\(p=0.07\)). The \textit{lgf2bp2} expression in
Cluster-3 is significantly up-regulated ($p=4.142\times10^{-9}$) compared to all of the unprimed clusters.

**Figure S6.** *Igf2bp2* deletion impairs colony forming capacity of balanced HSC of young mice. 500 freshly isolated, balanced HSC (CD150$^{low}$CD34$^{-}$LSK) from young (3-6 months) and aged (22-26 months) *Igf2bp2*+/− and *Igf2bp2*−/− mice were cultured in methylcellulose and serially replated for 3 rounds. The graph shows the number of colonies of the indicated groups per round of plating. Data points represent 8-12 mice per genotype. Statistical analysis by three-way ANOVA revealed significant effects on CFU capacity by genotype ($p=0.0047$) and round-of-plating ($p<0.0001$) but not by the age of the balanced HSC donors ($p=0.14$). Post-hoc testing with two-way ANOVA revealed that the genotype had stronger effects on suppression of CFU capacity on of balanced HSC of young mice (effect size -14.31 units, $p=0.0014$) compared to aged mice (effect size -5.63 units, $p=0.401$).

**Figure S7.** Lentivirus-mediated *Igf2bp2* overexpression and effects of *Igf2bp2* deletion on mitochondria respiration, ATP and ROS levels, and mitochondria stress related gene expression changes in hematopoietic stem and progenitor cells. (A-B) Freshly isolated LSK (Lineage−cKit$^{+}$Sca1$^{+}$) from wildtype mice were virally infected with *Igf2bp2* cDNA or an empty vector (control). Both constructs co-express GFP. On day 2.5 after transduction, DAPI$^{-}$GFP$^{+}$ cells were resorted for qPCR analysis and western blotting. (A) Relative mRNA expression of *Igf2bp2* (normalized to *Actb*) in infected LSK of the indicated groups from young (4 month) and old (24 month) mice. A total of 7 mice per group in two independent experiments. In one sample of vector-infected old LSK *Igf2bp2* was not detectable. Y-axis is in log-scale. Statistical test used two-way ANOVA on log2-transformed data followed by pairwise t-tests with Sidak’s correction for multiple comparisons. (B) Western blot showing IGF2BP2 protein level of LSK of 5 month old mice were infected with the indicated expression constructs (n=3 biological replicates, upper photograph). The total protein stain of the same membrane was determined as a loading control (lower photograph). (C) Representative kinetic profile of the oxygen consumption rate (OCR) of freshly isolated (upper profile) HSPC (Lineage−cKit$^{+}$Sca1$^{+}$ = LSK cells) from young (3-6 months), *Igf2bp2*−/− vs. *Igf2bp2*+/− mice and (lower profiles) from CD150$^{+}$ (high and low) HSC (CD150$^{+}$CD34$^{-}$LSK) and multipotent progenitors (CD34$^{+}$LSK) from aged (22-26 months).
months), *Igf2bp2*−/− vs. *Igf2bp2*+/+ mice. Arrows indicate the time for injections with oligomycin, FCCP, rotenone (Rot) and antimycin A (AA). (D) FACS analysis of ROS (by CellROX detection) in freshly isolated, living cells of *Igf2bp2*−/− vs. *Igf2bp2*+/+ mice at young (3-6 months) and old age (22-26 months). Fluorescence intensity values are plotted for CD150+ (high and low) HSC (CD150+CD34−LSK) and MPP (CD34+LSK). Data were normalized to HSC or MPP from young *Igf2bp2*+/+ mice set to 1. Data points represent 8-19 mice per group. No differences in ROS levels were observed in HSC or MPP from *Igf2bp2*−/− mice compared to *Igf2bp2*+/+ mice. (E) Mitochondrial stress related GO-terms that were significantly enriched and overall down-regulated in the bulk RNA sequencing of myeloid-biased HSC of young, *Igf2bp2*−/− vs. *Igf2bp2*+/+ mice (see Figure 2). (F) Intracellular ATP concentration was determined in 1,500 freshly isolated, CD150+ (high and low) HSC (CD150+CD34−LSK) and MPP (CD34+LSK) from young (4 months) and aged (22-26 months) *Igf2bp2*+/+ and *Igf2bp2*−/− mice. N=5-7 mice per group. (D and F) Statistical analysis by two-way ANOVA followed by pairwise t-tests with Sidak’s test correction for multiple comparisons. All data shown in this figure represent mean ± SD, and ns means “non-significant”.

**Figure S8.** *Igf2bp2* overexpression impairs maintenance and induces myeloid skewing of HSC, especially from aged donor mice. (A-C) CD150+ (high and low) HSC (CD150+CD34−LSKs) from young (3 month) and aged (26 month) WT mice were infected with virus containing *Igf2bp2*-cDNA or vector as control. 10 h after transduction, 5,000 viral-infected cells were transplanted along with 5x10^5 competitor total BM cells (CD45.1). 3-5 recipient per group. Chimerism in the peripheral blood (PB) from recipients was analyzed in 4-week intervals after transplantation by FACS. (A) Analysis of the relative change of GFP+ cells in CD45.2+ cells of PB from recipients of the indicated groups at the indicated time points after transplantation. Data were normalized to the initial transduction in HSC cells at 3 days after infection set to 1. (B) Representative FACS plots showing the percentage of GFP+ cells in CD45.2+ PB cells from recipients of the indicated groups, 12 weeks after transplantation. (C) The histogram shows the percentage of myeloid cells (Gr1+ and/or CD11b+) and lymphoid cells (B220+ or CD4+ or CD8+) in CD45.2+GFP+ PB cells of the indicated groups, 8 weeks after transplantation. (A and C) Statistical analysis by two-way ANOVA on original data (A), or on logit transformed data (C) followed by pairwise t-tests with
Sidak's test correction for multiple comparisons. All data shown in this figure represent mean ± SD, and ns means “non-significant”.

**Figure S9. Igf2bp2 deletion extends lifespan in female mice.** (A and B) Postnatal body weight curves of *Igf2bp2*+/+ and *Igf2bp2*−/− (A) male and (B) female mice. N=3-27 individual mice per group and per time point. The body weight was measured every two weeks after weaning. (C-E) Kaplan-Meier survival curve of *Igf2bp2*+/+ and *Igf2bp2*−/− mice, including (C) all animals (n=32-34 mice per group), (D) males (n=17-18 mice per group), and (E) females (n=15-16 mice per group). The statistical analysis was carried out by Log-rank test. Compared to the previous report, the reduction in body weight of knockouts vs. wildtype mice was less pronounced in our cohorts and the increase in lifespan was significant only in female mice but not in male mice. These differences may be related to differences in animal husbandry, in commensal bacteria/the microbiome, in animal chow, or in the genetic background.

**Figure S10. Influence of Igf2bp2 gene status on hematopoietic stem and progenitor cells.** (A-G) Freshly isolated bone marrow (BM) cells were analyzed to determine the number (per 1x10^6 BM cells) of the indicated cell populations and absolute number of total bone marrow cells in male and female, *Igf2bp2*+/+ and *Igf2bp2*−/− mice at young (3-6 months), middle (9-15 months), and old (18-27 months) age. The graphs show the combined analysis of multiple experiment, circles represent males, squares female. Cell number in the indicated groups of mice for (A) balanced HSC (CD150loswCD34−LSK), 6-24 mice per group; (B) CD41− HSC (CD41−CD150+CD34+LSK), 2-15 mice per group; (C) multipotent progenitor cells (MPP: CD34+LSK), 5-24 mice per group; (D) MPP subpopulations (MPP1: CD150+CD48−CD34+Flt3−LSK, MPP2: CD150+CD48+CD34+Flt3−LSK, MPP3: CD150−CD48+CD34+Flt3−LSK, and MPP4: CD150−CD48−CD34+Flt3−LSK), 2-10 mice per group; (E) common myeloid progenitors (CMP: CD34+FcyR−ScalcSca1^low^cKit^low^Lineage^−^), 2-12 mice per group; (F) absolute number of BM cells, 10-24 mice per group; (G) common lymphoid progenitor cells (CLP: Flt3+CD127+Sca1^low^cKit^low^Lineage^−^), 4-18 mice per group. (A,B,D) Y-axis is in log-scale. (A-G) Statistical analysis by two-way ANOVA on log-transformed data followed by pairwise t-tests with Sidak’s correction.
for multiple comparisons. (H) Representative FACS plots depicting myeloid cells (including Gr1⁺ cells and CD11b⁺) versus lymphoid cells (including B220⁺, CD4⁺, and CD8⁺ cells) in old donor-derived cells in peripheral blood (PB) 20 weeks after transplantation. All data shown in this figure represent mean ± SD, and ns means “non-significant”.

**Figure S11.** RNA sequencing data of long-term HSC (LT-HSC) from Yin et al.⁹ do not support the conclusion that *lgf2bp2* suppresses mitochondrial activity in HSC. (A) Principal component analysis plot of the four LT-HSC RNA-seq samples: two WT mice (red) and two *lgf2bp2*⁻/⁻ mice (black). (B-D) Volcano plots showing differentially expressed genes (DEG) highlighted in red, determined via different cutoff criteria. (B) DEG obtained using the criteria of Yin et al. of LFC > 1 or unadjusted p-value < 0.05 (8,332 genes). (C) DEG using the threshold of unadjusted p-value < 0.05 (1,394 genes). (D) DEG using the threshold of adjusted p-value < 0.05 (151 genes). Genes marked in Fig. 4A of Yin et al.⁹ are marked; only 2 (labeled blue) out of 18 are significant after correcting for multiple testing. (E) Top ten GO terms for the set of DEG that are up regulated in *lgf2bp2*⁻/⁻ mice as defined in (D). (F) Top ten GO terms for the set of DEG that are down regulated in *lgf2bp2*⁻/⁻ mice as defined in (D).