Supporting Information

Gan et al. 10.1073/pnas.0810584105

SI Text

Administration of Tamoxifen. Littermates at 4-6 weeks of age were administered tamoxifen daily by i.p. injection (Sigma) in corn oil (12 μ g/uL in corn oil) at 132 μ g tamoxifen/per g of body weight per day for 5 consecutive days.

Administration of Rapamycin. For rapamycin treatment, mice at 30 DPI were administered rapamycin (LC Laboratories) by daily i.p. injection at 4 μ g per g of body weight per day, or mice were administered rapamycin daily along with tamoxifen treatment from the beginning. After tamoxifen treatment was finished, mice were treated with rapamycin for another 3 days. For rapamycin treatment in competitive transplantation setting, competitive transplantation was performed as described above. Six weeks after transplantation, when these mice showed stable chimerism, TSC1 was deleted by tamoxifen treatment for 5 days. At the time of tamoxifen treatment, rapamycin or vehicle was also administered daily to TSC1 KO and WT mice transplants for 5 days, followed by continuous treatment every 2 days for 16 weeks. Rapamycin was first reconstituted in absolute ethanol at 25 mg/mL and then diluted in 5% Tween-80 and 5% PEG-400 before injection to make 0.5 mg/mL solution for injection.

Autopsy and Histopathology. Animals were autopsied and all tissues were examined regardless of their pathological status. Tissue samples were fixed in 10% neutral-buffered formalin (Sigma) overnight, and washed once with $1 \times$ PBS and then transferred into 70% ethanol and stored at 4 °C. Tissues were processed by ethanol dehydration and embedded in paraffin (Histoserv) according to standard protocols. Sections (5 μ m) were prepared for antibody detection and H&E staining.

Competitive and Noncompetitive Repopulation Assays. In noncompetive repopulation assay, 1×10^6 bone marrow cells from both TSC1L/L, Rosa26-CreERT2 or littermate WT mice (both $CD45.2^+$) were injected into the lateral tail veins of lethally irradiated CD45.1⁺ recipient animals. In competitive repopulation assay, 0.5×10^6 bone marrow cells from both TSC1L/ L,Rosa26-CreERT2 or littermate WT mice (both CD45.2⁺) were mixed with 0.5×10^6 bone marrow cells from CD45.1⁺ WT mice, then injected into lateral tail veins of lethally irradiated CD45.1⁺ recipient animals (950 rad in 2 dosages, 2 h apart). Six weeks after transplantation, when these mice showed stable chimerism, TSC1 was deleted by tamoxifen treatment. Peripheral blood was collected at 1, 2, 4, 8, 12, and 16 weeks after tamoxifen treatment, and bone marrow from mice at 16 weeks was analyzed for contribution of CD45 congenic and lineage markers by flow cytometry. Tissues from the recipient mice at 16 weeks were collected for further analysis, including cell surface marker staining by flow cytometry, histology characterization, etc. Both noncompetitive and competitive transplants were carried out with 3-5 donors per genotype mice with 3 recipient mice per donor in each experiment. Generation of shRNA-expressing virus stock and virus infection of bone marrow cells were done as described (1). shRNA sequence information is available on request.

Flow Cytometric Analysis and Cell Sorting. Single-cell suspensions were prepared form spleen, thymus, and bone marrow (from femoral and tibial bones) by passing cells through a 70- μ m cell strainer. Cells were lysed on ice with red blood cell lysis solution (Sigma), washed in PBS + 2% FCS, then resuspended in PBS +2%FCS. Cells were incubated with fluorochrome-conjugated (or biotin-conjugated) antibodies for 30 min on ice, followed by washing once in PBS + 2% FCS. For lineage marker labeling, cells were stained with other fluorochrome-conjugated antibodies and biotin-conjugated lineage markers (CD3, B220, Gr-1, Mac-1, Ter119) (BD Bioscience) for 30 min on ice, followed by incubation with fluorochrome-conjugated Streptavidin (BD Bioscience) for 5 min. For the analysis of bone marrow lymphopoiesis, cells were stained with fluorochrome-conjugated anti-B220, CD19, CD43, IgM, and biotin-conjugated lineage markers (CD3, Gr-1, Mac-1, Ter119) (BD Bioscience) for 30 min on ice, followed by incubation with fluorochrome-conjugated Streptavidin for 5 min.

In Vitro Colony Assays. Myeloid and pre-B colony-plating assays were performed in methylcellulose-based medium (M3434 and M3630; Stem Cell Technologies). A total of 2×10^4 bone marrow and spleen cells were plated in duplicate and scored for colony formation at 10 and 14 days. For CFU-E assay, 100 000 bone marrow cells were plated per mL of serum-free methylcellulose (M3134; StemCell Technologies) supplemented with 10% FBS, 100 ng/ml of rmSCF (PeproTech), and 4 units/mL human erythropoietin (hEpo; Amgen). For BFU-E assay, 25,000 fresh bone marrow cells were plated in 1 mL of methylcellulose (M3134; StemCell Technologies) containing 10% FBS, 4 units/mL hEPO, 100 ng/mL rrSCF, 100 ng/mL G-CSF, and 20 ng/mL IL-3 (PeproTech). Colonies were scored on day 2 (CFU-Es) or day 10 (BFU-Es).

Quantitative Real-Time PCR analysis. RNA from various tissues was harvested by using TRIzol (Invitrogen) and the RNeasy kit (Qiagen). RNA was treated with RQ1 RNase-freeDNase (Promega), and cDNA was prepared by using SuperScript II Rnase H-Reverse transcriptase (Invitrogen). RNA from sorted cells was extracted with the PicoPure RNA Isolation Kit (Molecular Devices), and cDNA was prepared by using SuperScript II Rnase H-Reverse Transcriptase (Invitrogen). Quantitative real-time PCR was performed on cDNA samples with the Quantitative SYBR Green PCR kit (Qiagen) and was run on the Stratagene Mx3000P. Primer sequences are available on request.

Microarray Analysis. RNA from sorted LSK cells (10,000–20,000) was extracted by using the PicoPure RNA Isolation Kit (Molecular Devices), and cDNA was prepared by using SuperScript II Rnase H-Reverse transcriptase (Invitrogen). Gene expression profiling was performed with the Affymetrix 430 2.0 chips at Partners Healthcare Center for Genetics and Genomics at Harvard Medical School. dChip was used to normalize arrays and compute expression indices as described (2).

Thomas EK, et al. (2007) Rac guanosine triphosphatases represent integrating molecular therapeutic targets for BCR-ABL-induced myeloproliferative disease. Cancer Cell 12:467–478.

Paik JH, et al. (2007) FoxOs are lineage-restricted redundant tumor suppressors and regulate endothelial cell homeostasis. Cell 128:309–323.



Fig. S1. Somatic deletion of *TSC1* leads to fatal bone marrow failure. (*A*) *TSC1* deletion and subsequent mTORC1 activation in various hematopoietic organs. PCR genotyping, and Western blotting by phosphor-S6, S6 were performed on bone marrow, spleen, thymus, and peripheral blood samples from *Rosa26-CreERT2⁺*, *TSC1^{LL}* (*TSC1* KO), and *Rosa26-CreERT2⁻*, *TSC1^{LL}* (*TSC1* WT) at 3 DPI. (*B*) Quantitative TSC1 RT-PCR of bone marrow, spleen, thymus, and peripheral blood samples from *TSC1* KO and WT mice at 3 DPI. (*C*) Body weight of female mice with indicated genotypes at 40 DPI. *n* = 10 for each genotype. Male mice show similar results. (*D*) Scatter plot showing red blood cell (RBC), hemoglobin (Hgb), and hematocrit (HCT) counts of *TSC1* WT and *TSC1* KO mice at 40–50 DPI. *n* = 11 for each genotype. (*E*) Bar graph showing the percentage of Ter119-positive cells in bone marrow of *TSC1* WT and KO mice after 30 DPI. *n* = 8 for each genotype. (*F*) Bar graph showing increased percentages of apoptotic Ter119⁺ cells in *TSC1* KO mice. *n* = 3 for each genotyping. *P* < 0.01. (*G*) Bar graph showing increased percentages of apoptotic Ter119⁺ cells in *TSC1* KO mide. (*H*) Flow cytometry analysis of CMP, GMP, and MEP subpopulations at 4 DPI from representative *TSC1* KO and WT mice. The percentages of CMP, GMP and MEP; *P* > 0.1 for CMP. (*I*) Bar graph showing platelet counts in *TSC1* WT and KO mice after 30 DPI. *n* = 6 for each genotyping. *P* < 0.05.



Fig. 52. *TSC1* deletion leads to myeloproliferative disease. (*A*) Flow cytometric analysis of spleen (SP), peripheral blood (PB), and bone marrow (BM) from representative *TSC1* WT and KO mice confirmed an increased population of $Gr1^+/Mac1^+$ cells in *TSC1* KO animals. The averaged percentage of $Gr1^+/Mac1^+$ cells is also indicated in each case. (*B*) Chloroacetate esterase (CAE) staining showing increased positive staining cells in *TSC1* KO spleen and liver. (*C* and *D*) Scatter plot showing spleen weight to body weight ratio (Sw/Bw) (*C*) and the total cell numbers of spleen (*D*) of *TSC1* WT and KO mice at 30 DPI. (*E*) Bar graph showing increased colony formation units from *TSC1* KO splenic cells at 30 DPI. *n* = 3 for each genotype.



Fig. 53. *TSc1* deficiency causes impaired lymphoid lineage development. (*A* and *B*) Scatter plot showing decreased relative (*A*) and absolute (*B*) number of peripheral blood lymphocytes of *TSC1* WT and KO mice at 30 DPI. (*C*) Bar graph showing increased percentage of apoptotic and dead cells from B220⁺IgM⁻ bone marrow cells in *TSC1* KO mice. n = 3 for each genotype. (*D*) Bar graph showing decreased CFU pre-B colony formation in *TSC1* KO bone marrow cells at 10 DPI. n = 3 for each genotype. (*E*) Scatter plot showing thymus weight to body weight ratio (Tw/Bw) of *TSC1* WT and KO mice at 30 DPI. (*F*) Bar graph showing total numbers of thymocytes from *TSC1* WT and KO mice at 7, 21, and 35 DPI. n > 3 for each genotyping at each time point. *, P > 0.05; **, P < 0.01. (*G*) Flow cytometry analysis of thymocytes from representative *TSC1* WT and KO mice at 35 DPI by CD4, CD8. The averaged percentage of each population is also indicated. n > 3 for each genotype.



Fig. S4. Deletion of TSC1 leads to HSC short-term expansion, but long-term reduction of HSC reserves. (*A*) Quantitative RT-PCR of various sorted cells from *TSC1* KO and WT mice at 3 DPI. n = 3 for each genotyping. (*B*) Histogram of mean FSC-H comparing *TSC1* WT and KO LSKs at 3 DPI. (*C*) Bar graph showing increased relative mean FSC-H (corresponding to increased cell size) in *TSC1* KO LSKs. n = 3 for each genotyping. (*D*) Flow cytometric analysis of LSK, MPP, ST-HSC, and LT-HSC from bone marrow at 3 DPI from representative *TSC1* KO and WT mice. The averaged percentage of each population is also indicated. n > 3 for each genotyping. (*E*) Bar graph showing the absolute numbers of HSC, LT-HSC, and ST-HSC cells per femur and tibia at 3 DPI in *TSC1* KO and control mice. n = 3 for each genotype. *, P < 0.01.



Fig. S5. Deletion of *TSC1* results in defective HSC long-term repopulating ability in vivo. (A) In competitive transplantation assay, bone marrow cells from both *TSC1L/L*, *Rosa26-CreERT2* (experimental) or littermate WT mice (control) (both CD45.2⁺) were mixed with bone marrow cells from CD45.1⁺ WT mice (competitor) at 1:1 ratio and transplanted into lethally irradiated CD45.1⁺ recipient animals. Five to 6 weeks after transplantation, when these mice showed stable chimerism, *TSC1* was deleted by tamoxifen treatment. (*B*) Recipient mice from noncompetitive transplantation were analyzed by CD45 staining to examine the contribution of donor-derived cells in peripheral blood at various time points before or after tamoxifen treatment. Six recipients for each genotype were used in noncompetitive transplantation. ***, *P* > 0.1; ****, *P* < 0.01. (*C* and *D*) Recipient mice from competitive transplantation were analyzed for the contribution of various donor-derived hematopoietic lineages in bone marrow cells at 16 weeks after transplantation. Bar graph showing the relative fold change of percentage of various donor-derived hematopoietic lineages. *n* = 6 for each genotyping.



Fig. S6. TSC1 operates via both mTORC1 dependent and independent mechanisms to regulate HSC biology. (*A*) Rapamycin or vehicle was administered daily to *TSC1* KO and WT mice from 30 DPI. Mice were analyzed 7–15 days later. (*B* and *C*) Western blotting by phosphor-56, S6 performed on splenic cells (*B*) and sorted Mac-1/Gr-1 double positive (DP) cells from spleen (*C*). (*D*) Scatter plot showing red blood cell (RBC), hemoglobin (Hgb), and hematocrit (HCT) counts of the mice in *A*. n = 3 for each genotype. (*E*) Bar graph showing the percentage of Mac-1⁺/Gr-1⁺ cells in spleen from the mice in A. (*F*) Prophylactic rapamycin or vehicle was administered daily to *TSC1* KO and WT mice for 5 days. The continuously treated mice were analyzed at 4 DPI. (*G*) Bar graph showing the percentage of LSK cells from spleen in *TSC1* KO and WT mice from prophylactic rapamycin administration at 14 DPI as indicated. n = 3 for each genotype.

Table S1. Differentially expressed genes in TSC1 KO LSKs

| Rank | Gene symbol | Log fold change | Fold change |
|----------------------|-------------------------|-----------------|-------------|
| Down-regulated gapes | | | |
| 1 | Scin | -4 02924 | 0 061245832 |
| 2 | Cerg | -3 47705 | 0.089805486 |
| 3 | Ccr9 | -3 43194 | 0.09265826 |
| 4 | Hspa1b | -3.03007 | 0.122421274 |
| 5 | Klk1b22 /// Klk1b9 | -2.65294 | 0.158995475 |
| 6 | Dsp | -2.55318 | 0.170379465 |
| 7 | ld2 | -2.46354 | 0.181301699 |
| 8 | Hdc | -2.31921 | 0.200376716 |
| 9 | LOC100047138 /// Tesc | -2.15314 | 0.224823058 |
| 10 | Chdh | -2.07478 | 0.237371898 |
| 11 | F10 | -1.84654 | 0.278058135 |
| 12 | Ccnb1 | -1.81778 | 0.283657932 |
| 13 | Pttg1 | -1.81564 | 0.284077491 |
| 14 | Olfm1 | -1.73262 | 0.300904357 |
| 15 | Atp6v0a1 | -1.71691 | 0.304199853 |
| 16 | lfi205 /// Mnda | -1.69647 | 0.308540883 |
| 17 | Dntt | -1.6366 | 0.321613696 |
| 18 | Foxp1 | -1.59861 | 0.330195181 |
| 19 | Hspa1b | -1.56563 | 0.337829636 |
| 20 | Cnn3 /// LOC100047856 | -1.55333 | 0.340723833 |
| 21 | lfi202b | -1.50429 | 0.352504595 |
| 22 | Cdc42 | -1.5064 | 0.351988002 |
| 23 | Cab39l | -1.49587 | 0.354565841 |
| 24 | Lamp2 | -1.49432 | 0.354947695 |
| 25 | LOC100046998 /// Opa1 | -1.46439 | 0.362389957 |
| 26 | Evi2a | -1.39279 | 0.3808289 |
| 27 | Hmmr | -1.34422 | 0.393866374 |
| 28 | Stard5 | -1.31926 | 0.400740315 |
| 29 | Fcgr2b | -1.29914 | 0.406369179 |
| 30 | Sf3a3 | -1.28286 | 0.410979418 |
| 31 | Fen1 | -1.25092 | 0.420179185 |
| 32 | Aurkb | -1.21779 | 0.429939394 |
| 33 | | -1.2122 | 0.431610088 |
| 34 | Cnn3 /// LOC100047856 | - 1.20076 | 0.435047445 |
| 35 | Polr3k | - 1.19691 | 0.436207975 |
| 30 27 | | - 1.19022 | 0.438236629 |
| 37 20 | CIIIS /// LOC100047836 | - 1.10405 | 0.459009919 |
| 00 | | - 1.13974 | 0.455640066 |
| 39 | Mrps23 | -1.0064 | 0.437303890 |
| 40 | | -1 08085 | 0.407000075 |
| 41 | Old I Rangan1 | -1.08905 | 0.40980878 |
| 43 | LOC100044385 /// Ppp2cb | -1 08494 | 0.470000004 |
| 45 | Seh11 | -1 07249 | 0.47549773 |
| 45 | Ctr9 | -1 07204 | 0.475646531 |
| 46 | Ecgr2b | -1 06497 | 0 477981465 |
| 47 | Bzw1 | -1 0484 | 0 483504999 |
| 48 | Cycs /// LOC672195 | -1.0476 | 0.483771918 |
| 49 | Kif20a | -1.04337 | 0.485192566 |
| 50 | Aars | -1.0409 | 0.48602371 |
| 51 | Cdca3 | -1.00244 | 0.499156352 |
| 52 | Ppp4r1l | -1.00089 | 0.499691153 |
| 53 | Tacc3 | -0.99681 | 0.501106064 |
| 54 | Nuf2 | -0.96225 | 0.513255035 |
| 55 | Rbm28 | -0.96159 | 0.513490592 |
| 56 | Tmem192 | -0.95624 | 0.515398695 |
| 57 | P2rx4 | -0.93547 | 0.522873508 |
| 58 | Erp29 | -0.92604 | 0.526301952 |
| 59 | Tex9 | -0.91143 | 0.531658901 |
| 60 | Tex9 | -0.90463 | 0.533237431 |
| 61 | 5430435G22Rik | -0.90352 | 0.534579756 |
| 62 | Fuca2 | -0.90145 | 0.53534999 |
| 63 | Ms4a6d | -0.88584 | 0.541174149 |

| Rank | Gene symbol | Log fold change | Fold change |
|--------------------|--|-----------------|-------------|
| 64 | Snf8 | -0.88135 | 0.542860448 |
| 65 | 2010111I01Rik | -0.87236 | 0.546251318 |
| 66 | Cnn3 /// LOC100047856 | -0.86398 | 0.549434265 |
| 67 | Rrbp1 | -0.85415 | 0.553189263 |
| 68 | Tmem14c | -0.85379 | 0 553330618 |
| 69 | Psmd13 | -0.84749 | 0 555749823 |
| 70 | Δaas | -0.84725 | 0 55584404 |
| 70 | Dop7 | -0.83886 | 0.550083383 |
| 71 | LOC100044220 /// SrdEa2 | 0.03000 | 0.559005505 |
| 72 | Ctcz | 0.03337 | 0.500205750 |
| 75 | C132 | -0.83241 | 0.501569400 |
| 74 | ALDUID /// LOC030003 | -0.8290 | 0.502000555 |
| 75 | CDX1/// LOC10004/028 | -0.8172 | 0.50754127 |
| /0 77 | Smpa4 | -0.80884 | 0.570840864 |
| // | | -0.80573 | 0.572073182 |
| 78 | Slc25a5 | -0.80032 | 0.574220113 |
| 79 | Slc1a4 | -0.79552 | 0.576133551 |
| 80 | Dntt | -0.78893 | 0.578773663 |
| 81 | Ctsz | -0.78706 | 0.579522588 |
| 82 | Gpr137b /// Gpr137b-ps /// LOC100044979 | -0.77639 | 0.583827139 |
| 83 | Pgam1 | -0.77071 | 0.586130217 |
| 84 | Calr | -0.77041 | 0.58624958 |
| 85 | Nup43 | -0.76144 | 0.589907935 |
| 86 | Farsb | -0.75974 | 0.590602316 |
| 87 | Med1 | -0.7517 | 0.593902685 |
| 88 | Hdgf | -0.75005 | 0.594584834 |
| 89 | Bcl2l11 | -0.74722 | 0.595751684 |
| 90 | Eif4e2 | -0.74273 | 0.597607438 |
| 91 | Psap | -0.7395 | 0.598945239 |
| 92 | Calr | -0.73674 | 0.600092482 |
| 93 | Cdk2ap1 /// LOC100047490 | -0.72686 | 0.604215852 |
| 94 | Fgfr1op2 | -0.71701 | 0.608356187 |
| 95 | Psmd13 | -0.70789 | 0.612215888 |
| 96 | — | -0.70567 | 0.61315912 |
| 97 | LOC100042343 /// Tmed2 | -0.68781 | 0.6207949 |
| 98 | Anp32e | -0.68557 | 0.621760284 |
| 99 | Racgap1 | -0.68065 | 0.623882294 |
| 100 | Hmmr | -0.67475 | 0.626441359 |
| 101 | Psat1 | -0.67304 | 0.627185795 |
| 102 | Dlat | -0.67029 | 0.628380567 |
| 103 | Farsa | -0.67026 | 0.628392362 |
| 104 | Prpf40a | -0.66304 | 0.631547377 |
| 105 | Rrbp1 | -0.65726 | 0.634079909 |
| 106 | Pcmt1 | -0.65471 | 0.635203008 |
| 107 | Calr | -0.6472 | 0.63851628 |
| 108 | Ugt1a1 /// Ugt1a10 /// Ugt1a2 /// Ugt1a5 /// Ugt1a6a /// Ugt1a6b /// Ugt1a7c /// Ugt1a9 | -0.64635 | 0.638892567 |
| 109 | Ube2f | -0.64496 | 0.639509156 |
| 110 | 1600012H06Rik | -0.64285 | 0.640447217 |
| 111 | Gnptab | -0.61669 | 0.652163665 |
| 112 | Etf1 | -0.61514 | 0.652867264 |
| 113 | Tacc2 | -0.60603 | 0.657002284 |
| 114 | EG623818 /// Hmbs | -0.60132 | 0.659149924 |
| 115 | Trim37 | -0.59275 | 0.663076348 |
| 116 | Sdhc | -0.5925 | 0.663191302 |
| Up-regulated genes | | | |
| 95 | Dynlrb1 | 0.590738 | 1.506016431 |
| 94 | Ccnd2 | 0.605114 | 1.521099376 |
| 93 | Ccnl1 | 0.610854 | 1.527162508 |
| 92 | Ccdc102a | 0.63316 | 1.55095813 |
| 91 | Rab2b | 0.645669 | 1.564464584 |
| 90 | Myadm | 0.656723 | 1.576497683 |
| 89 | Gltscr2 | 0.659698 | 1.579752315 |
| 88 | Galm | 0.660373 | 1.580490803 |
| 87 | Tmem191c | 0.673207 | 1.594613513 |

| Rank | Gene symbol | Log fold change | Fold change |
|----------|--|-----------------|--------------|
| 86 | Bnip3l | 0.677381 | 1.599233712 |
| 85 | Irf2 | 0.692413 | 1.615984231 |
| 84 | Ldhb | 0.699237 | 1.623645776 |
| 83 | Serpinb6a | 0.701334 | 1.626007644 |
| 82 | Yipf2 | 0.748223 | 1.679722732 |
| 81 | LOC100039656 /// LOC100040416 /// | 0.767344 | 1.702133319 |
| | LOC100040605 /// LOC100044916 /// Rpl13 | | |
| 80 | Trfr2 | 0.779336 | 1.716340306 |
| 79 | Ptpn21 | 0.785822 | 1.724074934 |
| 78 | Spint2 | 0.791111 | 1.73040644 |
| 77 | 5730427N09Rik /// EG433230 /// LOC636306 | 0.796522 | 1.736908205 |
| 76 | Trfr2 | 0.82791 | 1.775111626 |
| 75 | Bsdc1 | 0.831437 | 1.7794569 |
| 74 | Mboat2 | 0.833819 | 1.782397618 |
| 73 | Sqrdl | 0.846176 | 1.797730113 |
| 72 | Ipo4 | 0.856541 | 1.810691719 |
| 71 | Pear1 | 0.868976 | 1.826365674 |
| 70 | Zfp161 | 0.88556 | 1.847480978 |
| 69 | Arl3 | 0.906315 | 1.87425192 |
| 68 | Foxo1 | 0.907301 | 1.875533523 |
| 67 | 1110031B06Rik | 0.913009 | 1.882969106 |
| 66 | Erbb2ip | 0.964307 | 1.951126585 |
| 65 | Slc2a1 | 0.970203 | 1.959116567 |
| 64 | Rbpms | 0.97803 | 1.9697733 |
| 63 | Cyp4v3 | 0.978748 | 1.970754474 |
| 62 | 9530028C05 | 1.003878 | 2.005383309 |
| 61 | EG434179 | 1.008297 | 2.01153486 |
| 60 | Grb10 | 1.010655 | 2.014825493 |
| 59 | Capg | 1.031799 | 2.044572881 |
| 58 | Ptgs1 | 1.034344 | 2.048182259 |
| 57 | Socs2 | 1.035782 | 2.050225106 |
| 56 | Zfhx3 | 1.061033 | 2.086425273 |
| 55 | Ogt | 1.069426 | 2.098598036 |
| 54 | Slc2a1 | 1.123709 | 2.179064121 |
| 53 | Cabc1 | 1.148888 | 2.217428952 |
| 52 | Ldhb | 1.159533 | 2.233851743 |
| 51 | VldIr | 1.203568 | 2.303085962 |
| 50 | Ltbp3 | 1.235172 | 2.354093855 |
| 49 | EG622782 /// EG625349 /// EG666200 /// EG666464 /// LOC100041709 /// LOC544983 /// LOC545175 /// LOC619711 /// LOC624831 | 1.248354 | 2.375702032 |
| 48 | I pm2 | 1.284564 | 2.436083965 |
| 4/ | H ITU | 1.285014 | 2.436844901 |
| 46 | | 1.303986 | 2.469101852 |
| 45 | Metti/a | 1.305839 | 2.4/22/4934 |
| 44 | Prmtz | 1.31/403 | 2.4922/5330 |
| 43 | SIC93512 Bitnem1 | 1.319794 | 2.49030411 |
| 42 | | 1.323374 | 2.300323002 |
| 41 | Puzk lipi | 1.455005 | 2.701209207 |
| 40 20 | Ciquez Obel1 | 1.454606 | 2.74001904 |
| 22 | Balura | 1.455557 | 2.749030053 |
| 20 27 | rgiyipz Arbaof12 | 1.403138 | 2.700950101 |
| 37 | Angenz | 1.467933 | 2.00400010 |
| 35 | Saco | 1.505925 | 2.047 500001 |
| 24 | 59Ce | 1.546261 | 2.920032000 |
| 33 | lten1 | 1.670406 | 2 1820/1250 |
| 32 | Chi3l3 /// Chi3l4 | 1 620126 | 2 22/12/200 |
| 31 | Tin1 | 1 601977 | 2 220756/11 |
| 30 | יקני Mllt3 | 1 600777 | 2 2/7221150 |
| 29 | C1adc2 | 1 713143 | 2 2727/1/172 |
| 23 | Hdac11 | 1 745586 | 2 25220065 |
| 20 | Tam2 | 1 824354 | 3 541484308 |
| 26 | Tam2 | 1.841642 | 3.584177584 |
| 25 | Sbf2 | 1.893012 | 3.714099229 |

| Rank | Gene symbol | Log fold change | Fold change |
|------|----------------------------------|-----------------|-------------|
| 24 | Tgm2 | 1.997634 | 3.993444739 |
| 23 | Coro2b | 2.02048 | 4.057186608 |
| 22 | Ndn | 2.026023 | 4.072806463 |
| 21 | Mapk12 | 2.09874 | 4.283352502 |
| 20 | ltsn1 | 2.151927 | 4.444209047 |
| 19 | Bgn | 2.179817 | 4.530960708 |
| 18 | Col1a2 | 2.183038 | 4.541087451 |
| 17 | Meg3 | 2.214383 | 4.640829773 |
| 16 | Vasn | 2.217946 | 4.652305221 |
| 15 | D10Ertd610e | 2.220844 | 4.661661794 |
| 14 | Ndn | 2.296636 | 4.913107786 |
| 13 | Nov | 2.407725 | 5.306370461 |
| 12 | Nope | 2.449155 | 5.460961894 |
| 11 | Elavl4 | 2.721321 | 6.594762802 |
| 10 | Lcn2 | 2.949104 | 7.722692566 |
| 9 | Nov | 3.062546 | 8.354454377 |
| 8 | Fscn1 | 3.136315 | 8.792754389 |
| 7 | Ear1 /// Ear12 /// Ear2 /// Ear3 | 3.233197 | 9.403494326 |
| 6 | Hba-a1 /// Hba-a2 | 3.705012 | 13.04126642 |
| 5 | Ceacam10 | 4.616695 | 24.53373958 |
| 4 | Slc4a1 | 4.880442 | 29.45502985 |
| 3 | Hbb-b1 /// Hbb-b2 | 5.707686 | 52.26182283 |
| 2 | Hba-a1 /// Hba-a2 | 7.521218 | 183.7013324 |
| 1 | Hba-a1 /// Hba-a2 | 7.582344 | 191.6518242 |

Table S2. Genes involved in cell movement and amino acid metabolism in TSC1 HSC transcriptome

| Gene symbol | |
|---|--|
| Genes involved in cell movement | |
| AURKB | |
| CCNB1 | |
| KIF20A | |
| LOC643751 | |
| PITPNM1 | |
| RacGAP1 | |
| CALR | |
| ID2 | |
| LCN2 | |
| MAPK12 | |
| TJP1 | |
| SLC2A1 | |
| FSCN1 | |
| NOV | |
| CCR9 | |
| CHI3L3 | |
| HMMR | |
| Genes involved in amino acid metabolism | |
| FARSA | |
| FARSB | |
| AARS | |
| SLC1A4 | |
| SLC4A1 | |
| PCMT1 | |
| PRMT2 | |
| HDC | |
| LDHB | |
| DLAT | |
| GALM | |
| PGAM1 | |