

Supporting Information

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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 $\mu\text{g}/\mu\text{L}$ in corn oil) at 132 μg 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 μg 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 $^{\circ}\text{C}$. Tissues were processed by ethanol dehydration and embedded in paraffin (Histoserv) according to standard protocols. Sections (5 μm) were prepared for antibody detection and H&E staining.

Competitive and Noncompetitive Repopulation Assays. In noncompetitive 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 from spleen, thymus, and bone marrow (from femoral and tibial bones) by passing cells through a 70- μm 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).

1. Thomas EK, et al. (2007) Rac guanosine triphosphatases represent integrating molecular therapeutic targets for BCR-ABL-induced myeloproliferative disease. *Cancer Cell* 12:467–478.
2. Paik JH, et al. (2007) FoxOs are lineage-restricted redundant tumor suppressors and regulate endothelial cell homeostasis. *Cell* 128:309–323.

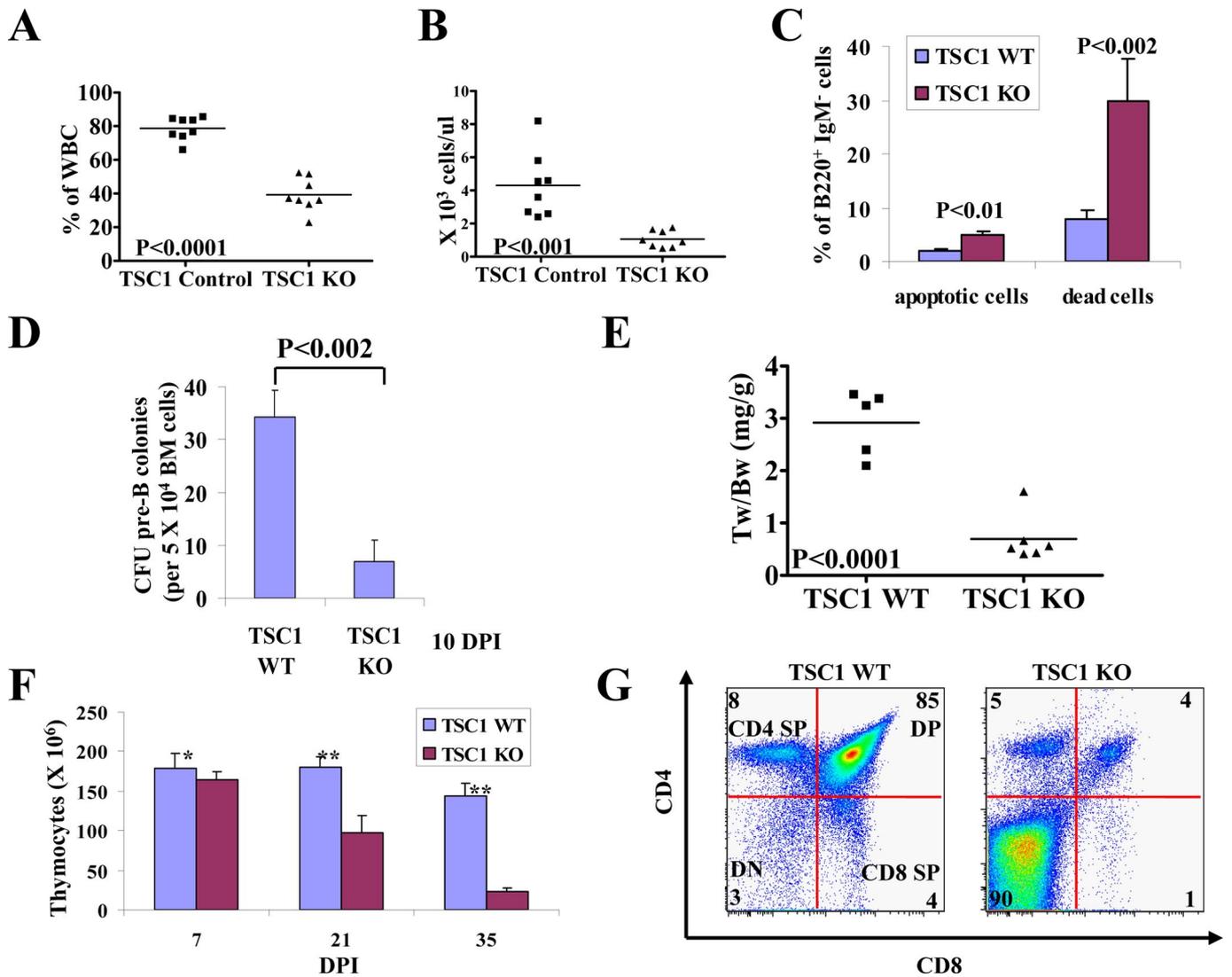
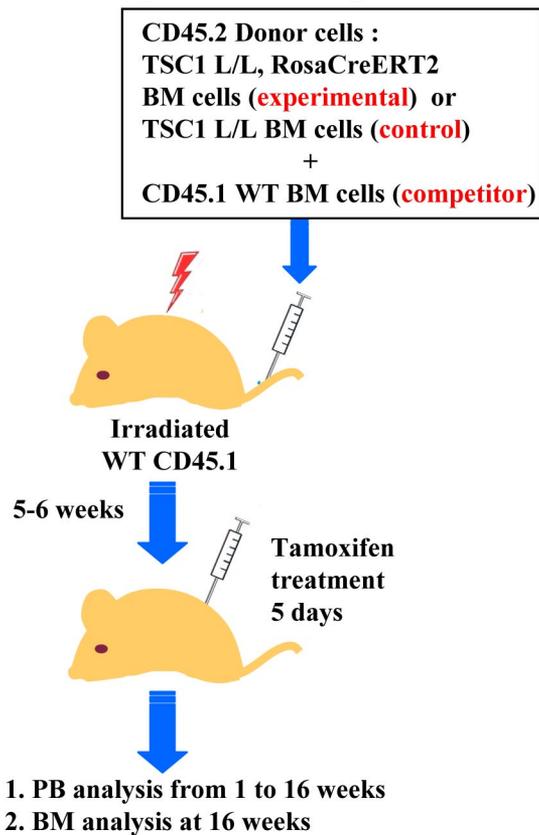


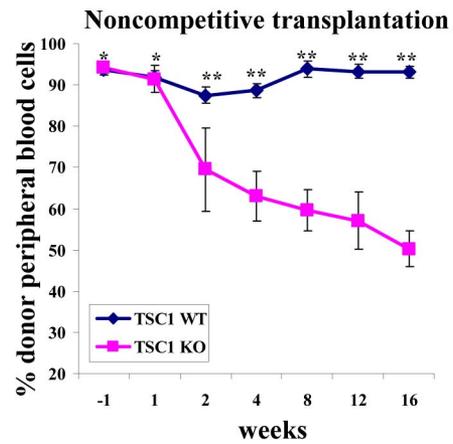
Fig. 53. *Tsc1* deficiency causes impaired lymphoid lineage development. (A and B) Scatter plot showing decreased relative (A) and absolute (B) number of peripheral blood lymphocytes of *Tsc1* WT and KO mice at 30 DPI. (C) Bar graph showing increased percentage of apoptotic and dead cells from B220⁺ 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.

A

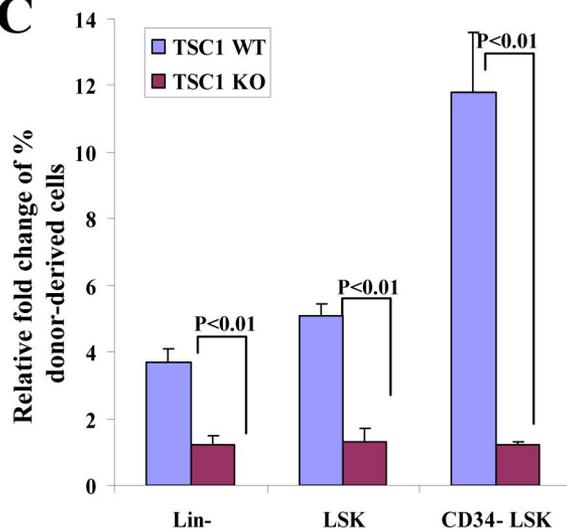
Competitive transplantation



B



C



D

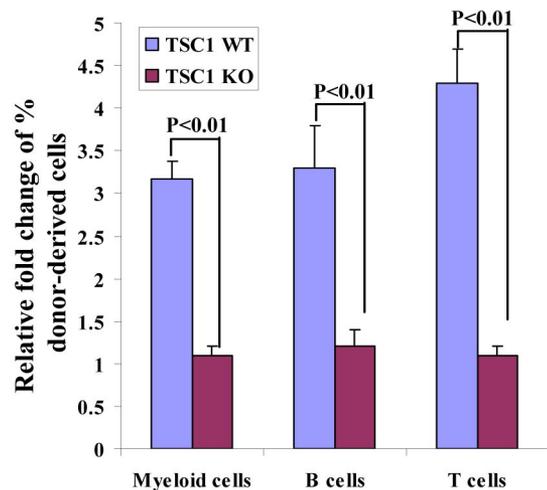


Fig. S5. Deletion of *TSC1* results in defective HSC long-term repopulating ability in vivo. (A) In competitive transplantation assay, bone marrow cells from both *TSC1*L/L, *Rosa26-CreERT2* (experimental) or littermate WT mice (control) (both CD45.2⁺) were mixed with bone marrow cells from CD45.1⁺ WT mice (competitor) at 1:1 ratio and transplanted into lethally irradiated CD45.1⁺ recipient animals. Five to six 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.

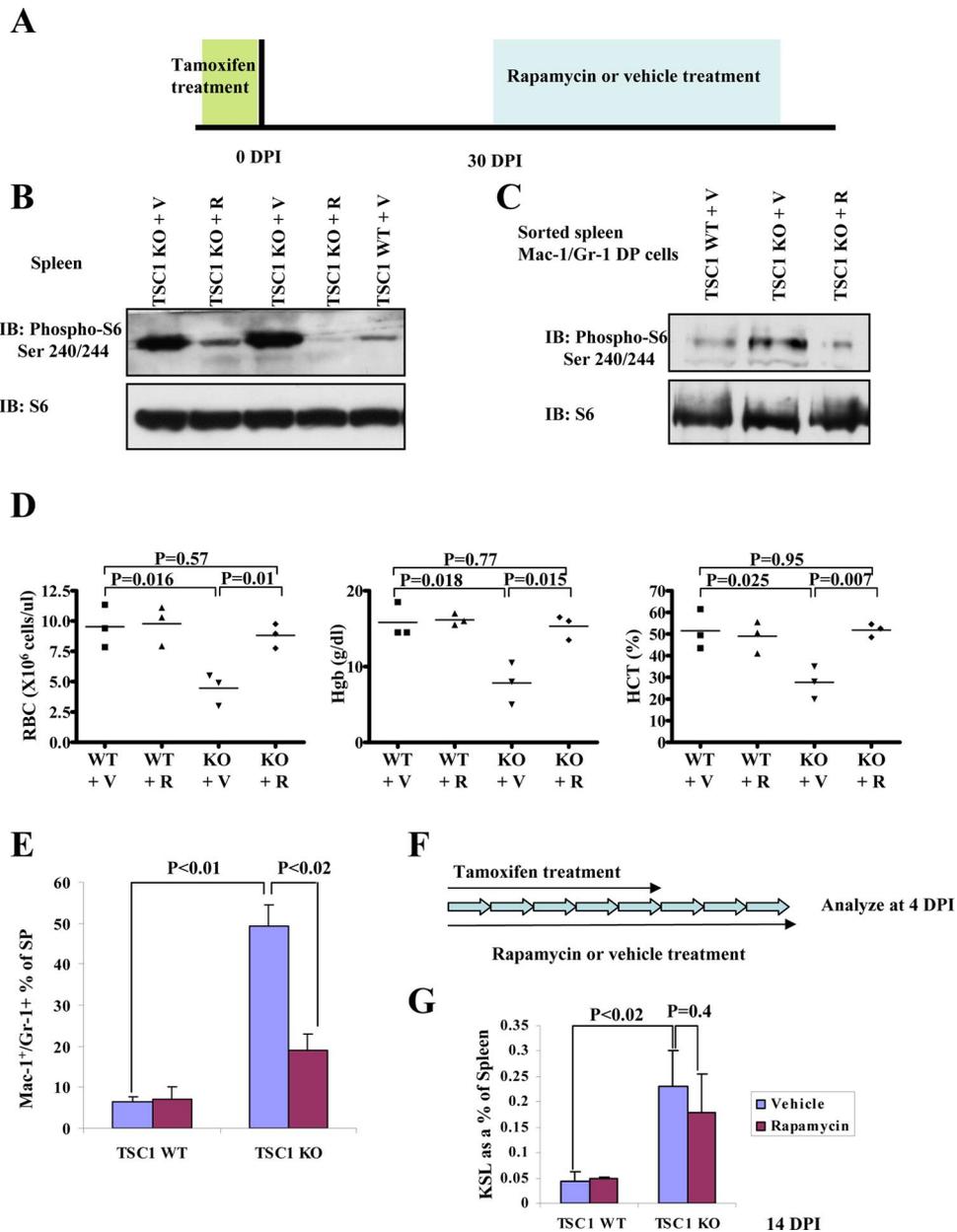


Fig. S6. TSC1 operates via both mTORC1 dependent and independent mechanisms to regulate HSC biology. (A) Rapamycin or vehicle was administered daily to *TSC1* KO and WT mice from 30 DPI. Mice were analyzed 7–15 days later. (B and C) Western blotting by phospho-S6, S6 performed on splenic cells (B) and 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 administration: at the time of tamoxifen treatment, 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 genes			
1	Scin	-4.02924	0.061245832
2	Ccr9	-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	Id2	-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	Ifi205 /// 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	Ifi202b	-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	Hsp90b1	-1.2122	0.431610088
34	Cnn3 /// LOC100047856	-1.20076	0.435047445
35	Polr3k	-1.19691	0.436207975
36	Fanca	-1.19022	0.438236629
37	Cnn3 /// LOC100047856	-1.18485	0.439869919
38	Mcm10	-1.13974	0.453840688
39	Aurkb	-1.12859	0.457363896
40	Mrps23	-1.0964	0.467680875
41	Ola1	-1.08985	0.46980878
42	Rangap1	-1.08905	0.47006994
43	LOC100044385 /// Ppp2cb	-1.08494	0.471410488
44	Seh1l	-1.07249	0.47549773
45	Ctr9	-1.07204	0.475646531
46	Fcgr2b	-1.06497	0.477981465
47	Bzw1	-1.0484	0.483504999
48	Cyca /// 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
86	Bnip3l	0.677381	1.599233712
85	Irf2	0.692413	1.615984231
84	Ldhb	0.699237	1.623645776
83	Serpib6a	0.701334	1.626007644
82	Yipf2	0.748223	1.679722732
81	LOC100039656 /// LOC100040416 /// LOC100040605 /// LOC100044916 /// Rpl13	0.767344	1.702133319
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	Bsdcl	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	Erbp2ip	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	Vldlr	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	Tpm2	1.284564	2.436083965
47	H1f0	1.285014	2.436844901
46	Nelf	1.303986	2.469101852
45	Mettl7a	1.305839	2.472274934
44	Prmt2	1.317463	2.492275336
43	Slc9a3r2	1.319794	2.49630411
42	Pitpnm1	1.325574	2.506325862
41	Pdzk1ip1	1.433605	2.701209207
40	C1qdc2	1.454608	2.74081984
39	Obsl1	1.459357	2.749856835
38	Pglyrp2	1.465158	2.760936161
37	Arhgef12	1.487933	2.80486816
36	Dos	1.509929	2.847960861
35	Sgce	1.546281	2.920632608
34	S100a8	1.612564	3.057948161
33	Itsn1	1.670406	3.183042368
32	Chi3l3 /// Chi3l4	1.689186	3.224747006
31	Tjp1	1.691872	3.230756411
30	Mllt3	1.699277	3.247381159
29	C1qdc2	1.713143	3.278744472
28	Hdac11	1.745586	3.35330965
27	Tgm2	1.824354	3.541484308
26	Tgm2	1.841642	3.584177584
25	Sbf2	1.893012	3.714099229

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	