Supporting Information

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SI Materials and Methods

Immunofluorescence. For immunofluorescence studies, liver tissue was fixed in 4% (vol/vol) paraformaldehyde for 2 h, transferred to a 30% sucrose solution (vol/vol) overnight at 4°C, and subsequently frozen in optimal cutting temperature (OCT) medium. To stain for GFP, frozen tissue sections (5 μm) were fixed in 4% paraformaldehyde (vol/vol), permeabilized, and then stained with anti-GFP antibody (1:1,000, Novus Biologicals). A tyramide signal amplification kit was used to amplify the GFP signal (Perkin-Elmer).

ALT and Necrotic Area Quantification. Serum alanine aminotransferase (ALT) levels were measured using an ALT-SL kit (Genzyme Diagnostics). To quantify necrotic cell area, liver sections were stained with H&E, and 10 random fields from each animal were analyzed, using ImageJ software.

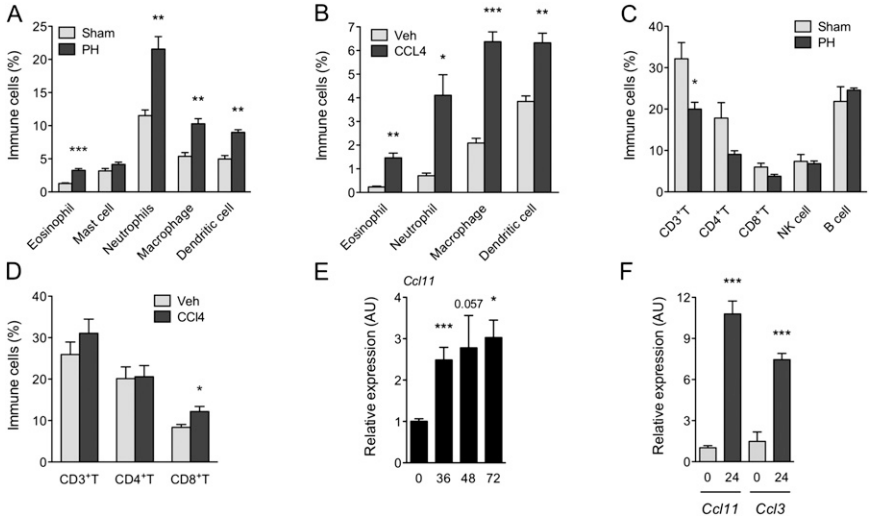

Fig. S1. Immune cell repertoire of regenerating livers. (*A* and *B*) Liver regeneration after partial hepatectomy (PH) or toxin-induced injury [carbon tetrachloride (CCl_4)] is associated with recruitment of innate immune cells. Infiltration by innate immune cells was analyzed 2 d after PH or CCl_4-mediated injury (n = 4 mice per group). (*C* and *D*) The percentages of adaptive immune cells were analyzed 2 d after PH and CCl_4-mediated injury in wild-type (WT) BALB/cJ mice (n = 4 mice per group). (*E* and *F*) Expression of eotaxin-1 (*Ccl11*) after PH and/or CCl_4-mediated injury (n = 3–8 mice per group and time). *$P < 0.05$, as determined by Student t test. All data are presented as mean ± SEM.

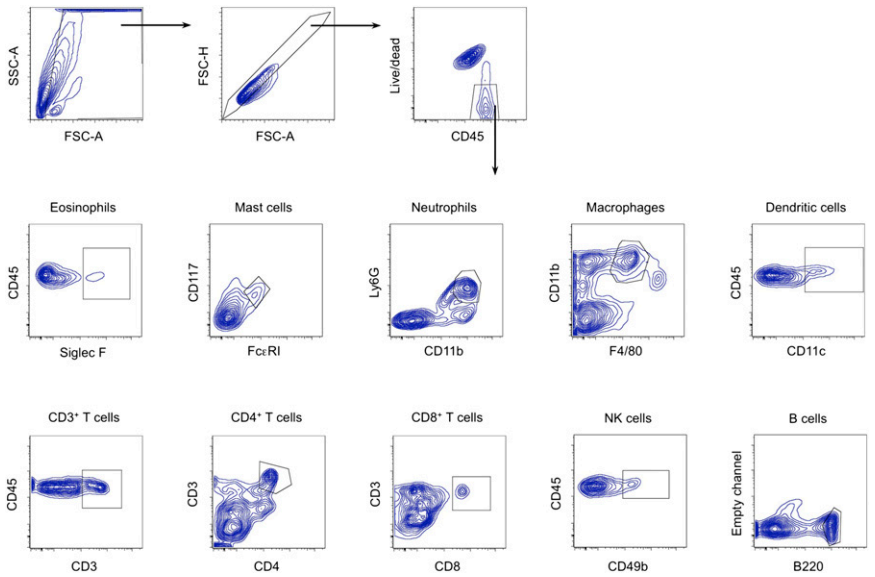

Fig. S2. Flow cytometric gating strategy for the identification of the immune cell repertoire of regenerating livers. Nonparenchymal cells were isolated from digested livers and gated for side- and forward-scatter (SSC/FSC), doublets, and live cells before the analysis of various cell surface markers of innate or adaptive immune cells. The following antibodies were used to identify eosinophils [sialic acid-binding Ig receptor (Siglec F)], mast cells (CD117 and FcεRI), neutrophils (Ly6G and CD11b), macrophages (F4/80 and CD11b), dendritic cells (CD11c), T cells (CD3, CD4 and CD8), NK cells (CD49b), and B cells (B220).

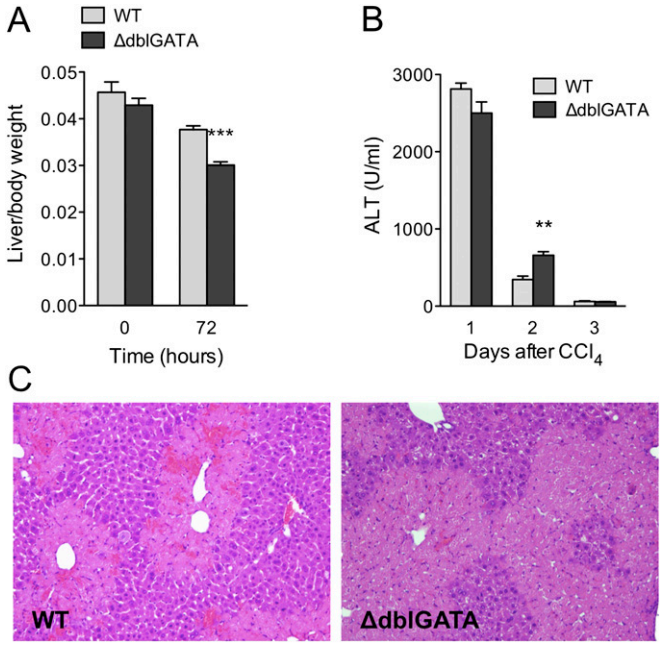

Fig. S3. Liver regeneration in WT and ΔdblGATA mice. (*A*) Liver/body weight ratio in WT and ΔdblGATA mice 72 h after partial hepatectomy. (*B*) Serum levels of ALT at the indicated times after administration of CCl_4. (*C*) Representative H&E staining of liver sections from WT and ΔdblGATA mice 2 d after administration of CCl_4 (n = 4–5 mice per genotype). $^{**}P < 0.01$ and $^{***}P < 0.001$, as determined by Student t test. All data are presented as mean ± SEM.

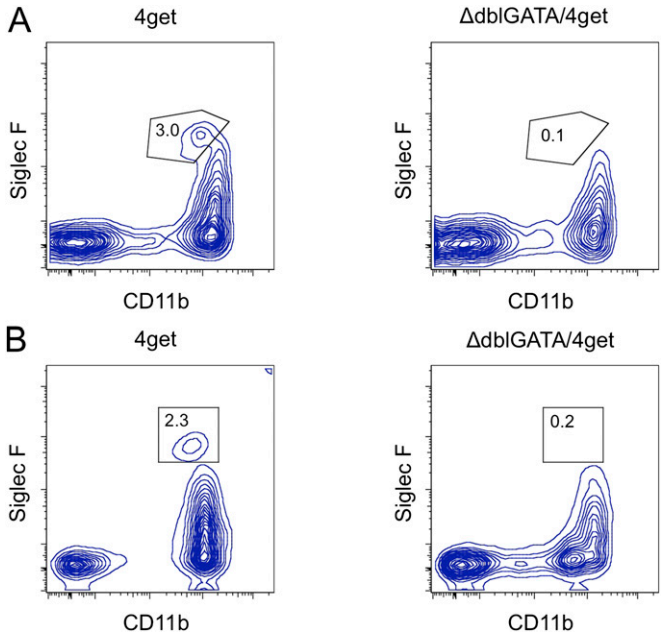

Fig. S4. Flow cytometric quantification of eosinophils. Eosinophils, which are identified as being Siglec-F^{+}CD11b^{+}, were enumerated in IL-4 reporter mice (4get mice) and ΔdblGATA/4get livers after partial hepatectomy (*A*) or CCl_4-induced injury (*B*).

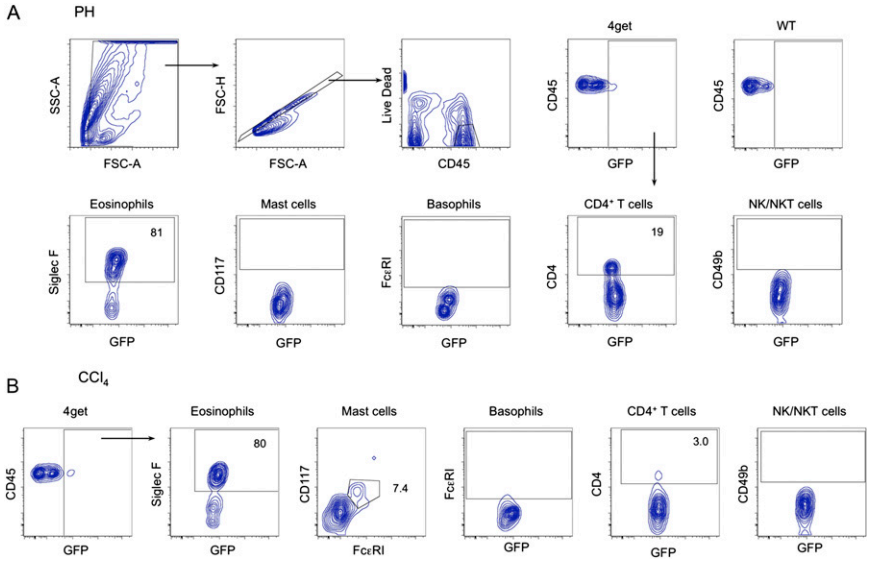

Fig. S5. Flow cytometric gating and quantification of IL-4-producing cells in regenerating livers. Immune cells competent for IL-4 secretion were identified in 4get mice 2 d after partial hepatectomy (*A*) or CCl_4 administration (*B*). Nonparenchymal cells were isolated and gated for SSC/FSC doublets, and live cells before the analysis of the CD45^{+} population. GFP+ cells, which are competent for IL-4 secretion, were subsequently analyzed for expression of markers of eosinophils, mast cells, basophils, CD4^{+} T, cells or NK cells.

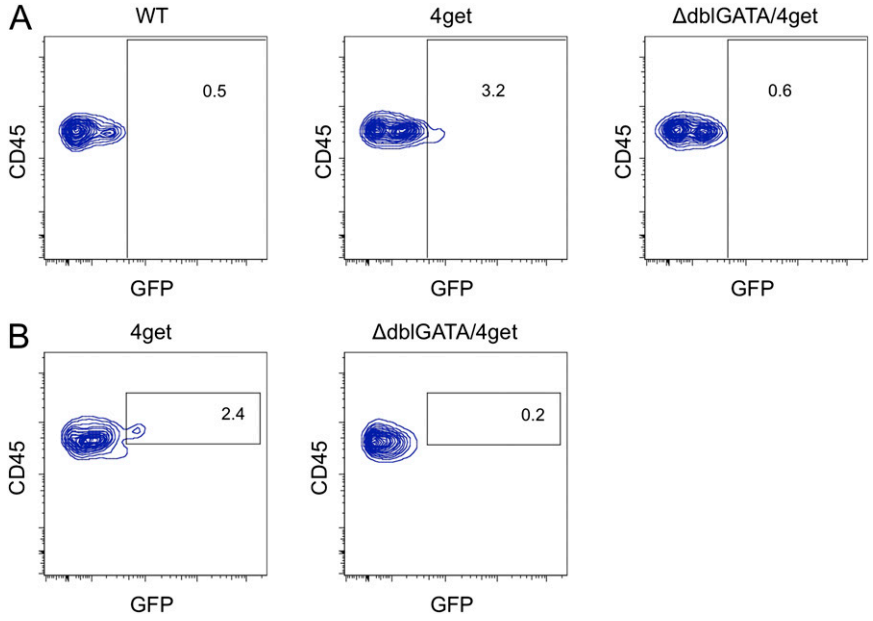

Fig. S6. Eosinophils are the predominant IL-4-producing cells in regenerating livers. Cells competent for IL-4 secretion were analyzed in WT, 4get, or ΔdblGATA/4get mice 2 d after partial hepatectomy (*A*) or CCl_4-induced liver injury (*B*).

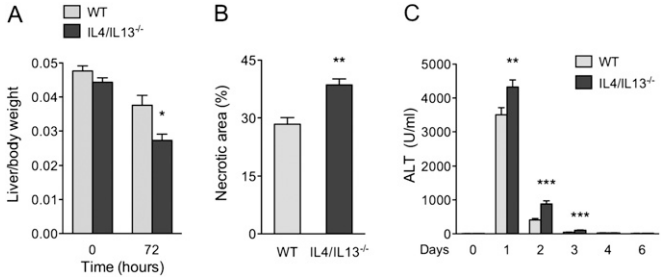

Fig. S7. Evaluation of CCl_4-induced liver injury in WT and IL-4/IL-13$^{-/-}$ mice. (*A*) Liver/body weight ratios in WT and IL-4/IL-13$^{-/-}$ mice 72 h after partial hepatectomy. (*B*) Necrotic area in WT and IL-4/IL-13$^{-/-}$ mice 2 d after CCl_4 administration. (*C*) Serum ALT levels of WT and IL-4/IL-13$^{-/-}$ mice at indicated times after injection of CCl_4. Data pooled from 3 independent experiments. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.005$, as determined by Student *t* test. All data are presented as mean ± SEM.

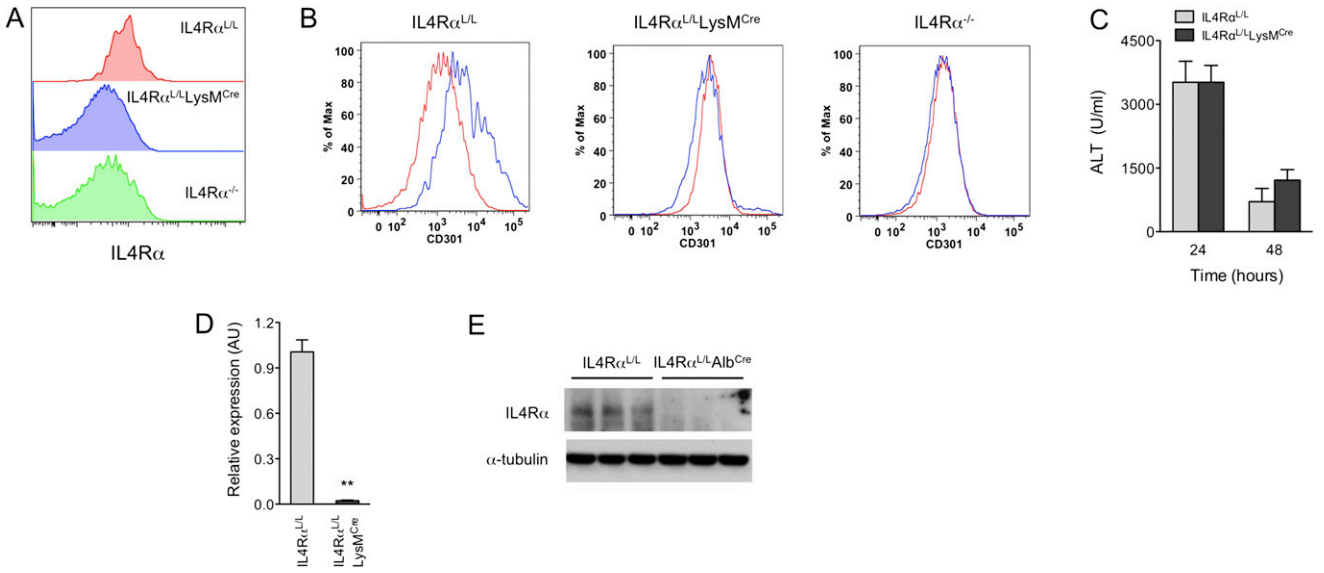

Fig. S8. Deletion of IL-4Rα in myeloid cells and hepatocytes. (*A*) Expression of IL-4Rα on peritoneal macrophages of *IL4Rα*$^{L/L}$, *IL4Rα*$^{L/L}$*LysM*^{Cre} (IL4Rα floxed allele crossed with Cre recombinase driven by Lysozyme 2 promoter), and IL4Rα$^{-/-}$ mice. (*B*) Expression of alternative activation marker CD301 in peritoneal macrophages. *IL4Rα*$^{L/L}$, *IL4Rα*$^{L/L}$*LysM*^{Cre}, and IL4Rα$^{-/-}$ mice that were given a single injection of IL-4 and CD301 expression were analyzed by flow cytometry on peritoneal macrophages 48 h later. (*C*) Serum levels of ALT in *IL4Rα*$^{L/L}$ and *IL4Rα*$^{L/L}$*LysM*^{Cre} mice at indicated times after injection of CCl_4 ($n = 4$–5 per genotype and time). (*D* and *E*) Expression of IL-4Rα in primary hepatocytes isolated from *IL4Rα*$^{L/L}$ and *IL4Rα*$^{L/L}$*Alb*^{Cre} (IL4Rα floxed allele crossed with Cre recombinase driven by Albumin promoter) mice; quantitative RT-PCR analysis of IL-4Rα mRNA (*D*) and immunoblot analysis of IL-4Rα protein (*E*). *$P < 0.05$, **$P < 0.01$.

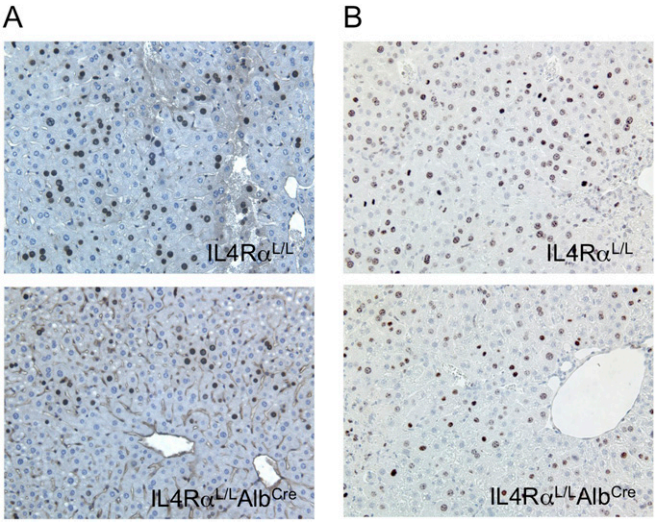

Fig. S9. IL-4Rα signaling in hepatocytes is required for liver regeneration after injury. (*A*) Representative liver sections from *IL4Rα*$^{L/L}$ and *IL4Rα*$^{L/L}$*Alb*^{Cre} mice were stained for BrdU 36 h after partial hepatectomy. (*B*) Representative liver sections from *IL4Rα*$^{L/L}$ and *IL4Rα*$^{L/L}$*Alb*^{Cre} mice were stained for K_i67 2 d after injury with CCl_4.

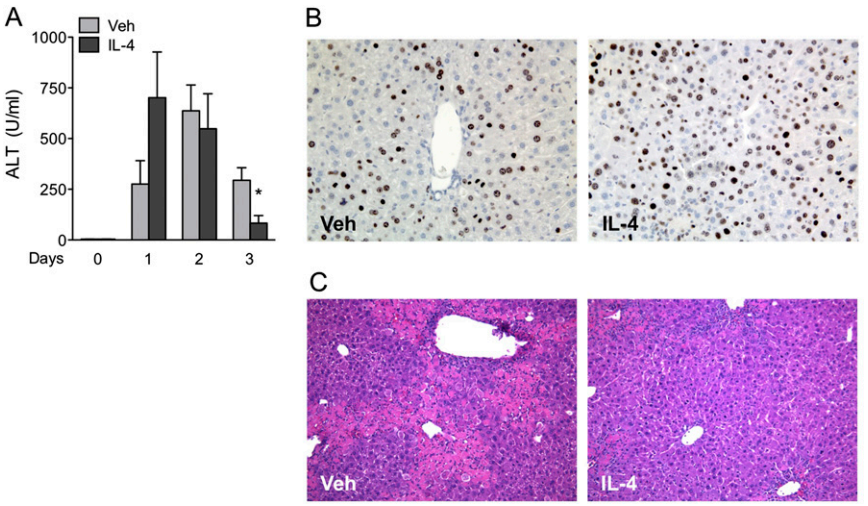

Fig. S10. Treatment with IL-4 protects mice from CCl_4-induced liver damage. (*A*) Serum levels of ALT in vehicle (Veh) and IL-4-treated mice after administration of CCl_4. (*B*) Representative liver sections from Veh and IL-4 mice were stained for K_i67 3 d after administration of CCl_4. (*C*) Representative H&E staining of liver sections from Veh and IL-4-treated mice 3 d after administration of CCl_4 ($n = 4$–5 mice per genotype). $^*P < 0.05$, as determined by Student *t* test. All data are presented as mean ± SEM.

Table S1. GO terms associated with genes that were differentially expressed in WT and IL-4/IL-13$^{-/-}$ mice

GO term	Gene no.	Genes	*P* value
GO:0022403~cell cycle phase	16	DBF4, TPX2, KIF18A, CDC23, NUSAP1, ANLN, CDC20, RAD51, C79407, NCAPD2, CCNB1, PLK1, SPAG5, F630043A04RIK, CDCA2, H2AFX	1.53E−07
GO:0000279~M phase	15	TPX2, KIF18A, CDC23, NUSAP1, ANLN, CDC20, RAD51, C79407, NCAPD2, CCNB1, PLK1, SPAG5, F630043A04RIK, CDCA2, H2AFX	1.57E−07
GO:0022402~cell cycle process	17	DBF4, TPX2, KIF18A, CDC23, NUSAP1, ANLN, CDC20, RAD51, C79407, NCAPD2, CCNB1, PLK1, SPAG5, GSK3B, F630043A04RIK, CDCA2, H2AFX	2.79E−07
GO:0000280~nuclear division	12	CCNB1, PLK1, SPAG5, F630043A04RIK, KIF18A, CDCA2, CDC23, NUSAP1, CDC20, ANLN, NCAPD2, C79407	7.79E−07
GO:0007067~mitosis	12	CCNB1, PLK1, SPAG5, F630043A04RIK, KIF18A, CDCA2, CDC23, NUSAP1, CDC20, ANLN, NCAPD2, C79407	7.79E−07
GO:0000087~M phase of mitotic cell cycle	12	CCNB1, PLK1, SPAG5, F630043A04RIK, KIF18A, CDCA2, CDC23, NUSAP1, CDC20, ANLN, NCAPD2, C79407	9.58E−07
GO:0048285~organelle fission	12	CCNB1, PLK1, SPAG5, F630043A04RIK, KIF18A, CDCA2, CDC23, NUSAP1, CDC20, ANLN, NCAPD2, C79407	1.11E−06
GO:0000278~mitotic cell cycle	13	DBF4, KIF18A, CDC23, NUSAP1, ANLN, CDC20, C79407, NCAPD2, CCNB1, SPAG5, PLK1, F630043A04RIK, CDCA2	1.36E−06
GO:0007049~cell cycle	19	PRC1, DBF4, KIF18A, TPX2, CDC23, NUSAP1, ANLN, CDC20, CHEK2, RAD51, C79407, NCAPD2, CCNB1, PLK1, SPAG5, GSK3B, F630043A04RIK, CDCA2, H2AFX	5.15E−06
GO:0051301~cell division	13	PRC1, CDC23, NUSAP1, ANLN, CDC20, C79407, NCAPD2, CCNB1, SPAG5, PLK1, F630043A04RIK, CDCA2, TOP2A	5.87E−06
GO:0001944~vasculature development	10	EDNRA, TCF21, HIF1A, HEY1, SOX18, COL1A1, FIGF, ENG, MMP2, COL5A1	3.27E−04
GO:0001568~blood vessel development	9	EDNRA, HIF1A, HEY1, SOX18, COL1A1, FIGF, ENG, MMP2, COL5A1	0.001291533
GO:0007018~microtubule-based movement	6	KIF23, KIF22, KIF2C, KIF4, KIF18A, TUBE1	0.00185112
GO:0007017~microtubule-based process	8	KIF23, KIF22, KIF2C, KIF4, TPX2, KIF18A, TUBE1, NUSAP1	0.002426587
GO:0007169~transmembrane receptor protein tyrosine kinase signaling pathway	7	GRB10, IRS2, TIAM1, PDGFRB, FGF20, FIGF, CSF1R	0.006565756
GO:0033554~cellular response to stress	10	KIF22, HIF1A, RAD23A, GSK3B, MAPK8IP2, H2AFX, KRT20, CHEK2, FEN1, RAD51	0.008592196
GO:0048754~branching morphogenesis of a tube	5	EDNRA, TCF21, GPC3, ENG, PXN	0.008718856
GO:0007167~enzyme-linked receptor protein signaling pathway	8	GRB10, IRS2, TIAM1, PDGFRB, FGF20, FIGF, ENG, CSF1R	0.009731471
GO:0030198~extracellular matrix organization	5	HSPG2, ADAMTS2, COL5A1, APLP1, EMILIN1	0.01156676
GO:0010171~body morphogenesis	3	GPC3, COL1A1, MMP2	0.012828367
GO:0030261~chromosome condensation	3	NUSAP1, TOP2A, NCAPD2	0.015421729
GO:0035239~tube morphogenesis	6	EDNRA, TCF21, GPC3, HIF1A, ENG, PXN	0.016664171
GO:0032963~collagen metabolic process	3	HIF1A, ADAMTS2, MMP2	0.01679561
GO:0060346~bone trabecula formation	2	COL1A1, MMP2	0.017293486
GO:0007059~chromosome segregation	4	F630043A04RIK, KIF18A, NUSAP1, TOP2A	0.018118481
GO:0044259~multicellular organismal macromolecule metabolic process	3	HIF1A, ADAMTS2, MMP2	0.018219658
GO:0030814~regulation of cAMP metabolic process	4	EDNRA, ADCY6, TIMP2, ADORA1	0.020447695
GO:0044236~multicellular organismal metabolic process	3	HIF1A, ADAMTS2, MMP2	0.021214429
GO:0000910~cytokinesis	3	PRC1, NUSAP1, ANLN	0.02278327

Table S1. Cont.

GO term	Gene no.	Genes	*P* value
GO:0010648~negative regulation of cell communication	6	DKK3, GRB10, GPC3, PAWR, RGS16, ADORA1	0.023038383
GO:0001763~morphogenesis of a branching structure	5	EDNRA, TCF21, GPC3, ENG, PXN	0.023458806
GO:0030799~regulation of cyclic nucleotide metabolic process	4	EDNRA, ADCY6, TIMP2, ADORA1	0.025577753
GO:0007242~intracellular signaling cascade	15	USP8, BAIAP2, ADCY6, IQGAP3, CHEK2, ADORA1, STK3, PXN, RLN1, TIAM1, GSK3B, MAPK8IP2, H2AFX, DEPDC1B, GADD45B	0.027155727
GO:0006140~regulation of nucleotide metabolic process	4	EDNRA, ADCY6, TIMP2, ADORA1	0.02742748
GO:0035295~tube development	7	EDNRA, TCF21, GPC3, HIF1A, ADAMTS2, ENG, PXN	0.027562353
GO:0043588~skin development	3	COL1A1, ADAMTS2, COL5A1	0.027764592
GO:0001525~angiogenesis	5	EDNRA, HIF1A, SOX18, FIGF, ENG	0.028620776
GO:0048514~blood vessel morphogenesis	6	EDNRA, HIF1A, HEY1, SOX18, FIGF, ENG	0.029144189
GO:0043062~extracellular structure organization	5	HSPG2, ADAMTS2, COL5A1, APLP1, EMILIN1	0.040830616
GO:0001957~intramembranous ossification	2	COL1A1, MMP2	0.042679218
GO:0007507~heart development	6	EDNRA, HIF1A, ALPK3, HSPG2, ENG, COL5A1	0.044917917
GO:0008285~negative regulation of cell proliferation	6	GPC3, PPARG, PAWR, TIMP2, ADORA1, H19	0.045637208

GO, gene ontology.