

Supplemental Figure Legends

Supplemental Figure 1. Transcriptional changes are dependent upon Fpn and occur in peritoneal macrophages.

A. Mouse bone marrow macrophages were transfected with either WT Fpn-GFP or Fpn(D157G)-GFP. Eighteen h later cells were solubilized, RNA extracted and SOCS3, IL-7 and IL-17R mRNA levels were analyzed relative to actin mRNA by RT-PCR. **B.**

Thioglycolate-elicited peritoneal macrophages from C3H mice were isolated, cultured and incubated in presence or absence of FAC (10 μ M Fe) with or without hepcidin (1 μ g/ml). Six h later cells were solubilized, RNA extracted and Fpn, SOCS3, IL-7 and IL-17R mRNA levels were analyzed by RT-PCR.

Supplemental Figure 2. LPS does not induce hepcidin in iron-loaded macrophages.

A. Mouse bone marrow macrophages were incubated +/- FAC (10 μ M Fe) for 24 h and then incubated for two h +/-LPS (10 ng/ml). Cells were solubilized, RNA extracted and hepcidin and IL-6 mRNA were analyzed relative to actin mRNA by RT-PCR. **B.** Mouse bone marrow macrophages incubated as in A were fixed and CHIP performed with either no antibody or with anti-Stat3 antibody as described in the methods. The data was quantified by densitometry and normalized to actin.

Supplemental Figure 3. Fpn is down-regulated in the duodenum of mice injected with hepcidin.

Protein extraction from duodenum of mice injected with or without hepcidin was performed. Fpn levels were analyzed by Western blots using rabbit anti-Fpn followed by a peroxidase-conjugated goat anti-rabbit IgG. Actin was analyzed as a loading control using goat anti-actin followed by a peroxidase-conjugated donkey anti-goat IgG. Fpn levels were quantified by Bio-Rad QuantOne software.

Supplemental Figure 4. Effect of hepcidin injection on rectal temperature and activity of LPS treated mice.

A. C57BL/6 mice (five for each group) were treated with or without an intraperitoneal injection of hepcidin (100 μ g/mouse). Two h later, mice were injected with PBS or LPS (1 mg/kg) and four h later rectal temperature was measured. **B.** Mice as in (A) were placed on a Rota-rod that accelerates gradually. The time for the mice to fall from the rod was recorded automatically by digital display (maximum time 300 seconds). Each mouse was given three trials with 15-min intervals between trials.

Supplemental Figure 5. Effect of a high iron diet on LPS-mediated changes in rectal temperature and activity in wild type and HAMP^{-/-} mice.

A. C57BL/6 and HAMP^{-/-} mice were maintained on a normal (350 mg iron/kg mouse chow) or high iron (2 g iron/kg mouse chow) diet. After 48 h mice were sacrificed and sera collected. Hepcidin levels in sera were measured using a radioactive competition assay as described²⁰. **B.** C57BL/6 and HAMP^{-/-} mice maintained on a high iron diet (2 g/kg) for 48 h were treated with an intraperitoneal injection of PBS or LPS as in Supplemental figure 3. Rectal temperature was measured four h later. **C.** Mice as in (B) were placed on top of a Rota-rod that accelerates gradually. The time for the mice to fall from the rod was recorded automatically by digital display (maximum time 300 seconds). Each mouse was given three trials with a 15-min interval between trials.

Supplemental Figure 6. HFE^{-/-} mice show LPS-mediated cytokine responses and hepcidin-mediated transcriptional response.

A. Age and gender matched C57BL/6 and HFE^{-/-} mice (five for each group) were intraperitoneally injected with a sublethal (1 mg/kg) dose of LPS. After four h mice were sacrificed and mRNA was extracted from liver. mRNA levels for IL-6 and hepcidin were measured relative to actin mRNA using RT-PCR. **B.** Mouse bone marrow macrophages isolated from WT and HFE^{-/-} mice were incubated with FAC (10 μ M Fe) for 24 h and then incubated in presence of hepcidin (1 μ g/ml). After two h cells were solubilized, RNA extracted and SOCS3, IL-7 and IL-17R mRNA levels measured relative to actin mRNA using RT-PCR.

Supplemental Movie 1

Mice treated as described in Figure 3 were placed in a cage and videotaped for 90 sec. Mice pretreated with hepcidin were marked with an orange tag on the tail. Mice treated with LPS only were marked with a green tag on the tail.

Supplemental Table 1 Effect of hepcidin on LPS-induced cytokine secretion in macrophages.

	IL-6 (ng/ml media)	TNFα (ng/ml media)
-LPS -Hepcidin	1.4 \pm 0.98	1.9 \pm 1.1
+LPS -Hepcidin	91.2 \pm 2.69	78.3 \pm 3.14
-LPS +Hepcidin	1.3 \pm 1.87	2.36 \pm 0.87
+LPS +Hepcidin	21.0 \pm 2.54	18.51 \pm 4.02

Mouse bone marrow macrophages were incubated with FAC (10 μ M Fe) for 24 h and then incubated for two h in the absence or presence of hepcidin (1 μ g/ml). After two h +/- hepcidin LPS was added for an additional four h. Media were collected and levels of IL-6 and TNF α were determined by ELISA.

Supplemental Table 2 Effect of hepcidin on LPS-induced cytokine secretion in vivo.

	IL-6 (ng/ml serum)	TNFα (ng/ml serum)
-LPS -Hepcidin	81 \pm 1.36	74 \pm 2.25
+LPS -Hepcidin	964 \pm 2.60	515 \pm 3.69
-LPS +Hepcidin	79 \pm 0.48	64 \pm 1.24
+LPS +Hepcidin	369 \pm 2.78	247 \pm 4.17

Age and gender matched C57BL/6 mice (five for each group) were treated with an intraperitoneal injection of PBS or hepcidin (100 μ g/mouse). After two h a sublethal dose of LPS (1 mg/kg) was injected intraperitoneally. Sera from these mice were isolated four h post LPS injection and IL-6 (pg/ml serum) and TNF α (pg/ml serum) levels were measured.

Supplemental Table 3 Effect of a high iron diet on LPS-injected wild type mice.

	IL-6 (ng/ml serum)	TNFα (ng/ml serum)
-LPS -Hepcidin	88 \pm 1.36	68 \pm 2.58
+LPS -Hepcidin	869 \pm 2.60	501 \pm 4.12
-LPS +Hepcidin	78 \pm 1.90	54 \pm 3.69
+LPS +Hepcidin	401 \pm 3.98	214 \pm 1.54

Six C57BL/6 mice maintained on a high iron diet for two days were given an intraperitoneal injection of PBS or LPS (1 mg/kg). After four h mice were sacrificed, sera isolated and IL-6 (pg/ml serum) and TNF α (pg/ml serum) levels were measured.

Supplemental Table 4 Effect of a high iron diet on LPS-injected HAMP^{-/-} mice.

	IL-6 (ng/ml serum)	TNFα (ng/ml serum)
-LPS -Hepcidin	132 \pm 3.60	96 \pm 1.34
+LPS -Hepcidin	1782 \pm 3.60	812 \pm 2.46
-LPS +Hepcidin	132 \pm 2.1	91 \pm 6.70
+LPS +Hepcidin	1841 \pm 4.60	859 \pm 2.48

Six HAMP^{-/-} mice maintained on a high iron diet for two days were given an intraperitoneal injection of PBS or LPS (1 mg/kg). After four h mice were sacrificed, sera isolated and IL-6 (pg/ml serum) and TNF α (pg/ml serum) levels were measured.

Supplemental Table 5 Primer List

Target	Forward	Reverse
Hepcidin	agagctgcagcctttgcac	gaagatgcagatggggaagt
Jak2	tctgtgggagatctgcagtg	cacggatgacagctctgaaa
Stat3	gacccgccaacaaattaaga	tcgtggtaaactggacacca
IL-7	gtatttgaccgatgcctgct	ctgtttctgggcaaattggt
IL-17R	tgctgtggcactgaagtag	ttcatggctgcagtgaaaag
TNF α	agccccagctctgtatcctt	ctcccttgcagaactcagg
SOCS3	cctttgacaagcggactctc	gccagcataaaaacccttca
Actin	gacggccaagtcatcatcactattg	ccacaggattccataccaaga

Supplemental Table 6

Microarray (+Hepcidin 2 hr)

Cytokine-cytokine receptor interaction

Ratio	Direction	Gene Identifier	Gene Name
4.77	Up	BC012653	Mus musculus, Similar to chemokine (C-X3-C) receptor 1, clone MGC:13785 IMAGE:4017224, mRNA, complete cds.
3.26	Down	NM_009140	Mus musculus small inducible cytokine subfamily, member 2 (Scyb2), mRNA.
3.08	Up	D87747	Mus musculus mRNA for murine CXCR-4, complete cds.
3.04	Down	BG976607	ectodermal-neural cortex 1
2.94	Down	NM_009971	Mus musculus colony stimulating factor 3 (granulocyte) (Csf3), mRNA.
2.77	Up	AI573431	Mus musculus interleukin 7 receptor (Il7r), mRNA. interleukin 7 receptor /FL=gb:NM_008372.2 gb:M29697.1
2.71	Up	AI573431	gb:AF078906.1
2.7	Up	BC012653	Mus musculus chemokine (C-X3-C) receptor 1 (Cx3cr1), mRNA.
2.69	Down	NM_031168	Mus musculus interleukin 6 (Il6), mRNA.
2.44	Down	NM_008176	Mus musculus GRO1 oncogene (Gro1), mRNA.
2.26	Down	BB554288	ESTs
2.22	Up	NM_010228	Mus musculus FMS-like tyrosine kinase 1 (Flt1), mRNA.
2.16	Down	U50712	Mus musculus monocyte chemoattractant protein-5 precursor (MCP-5) mRNA, complete cds.
2.14	Up	AF252873	Mus musculus CXC chemokine MIP-2gamma precursor, mRNA, complete cds.
2.12	Down	BC011437	Mus musculus, Similar to interleukin 1 beta, clone MGC:18555 IMAGE:3989461, mRNA, complete cds.
1.99	Down	AV246615	expressed sequence AW061234
1.99	Down	X03019	Mouse mRNA for granulocyte-macrophage colony stimulating factor (GM-CSF).
1.94	Down	NM_008518	Mus musculus lymphotoxin B (Ltb), mRNA.
1.84	Down	BB554288	ESTs
1.8	Down	NM_013653	Mus musculus small inducible cytokine A5 (Scya5), mRNA. transforming growth factor, beta receptor I /FL=gb:D28526.1
1.74	Up	BM248342	gb:NM_009370.1 gb:D25540.1
1.73	Down	AV239587	bone morphogenetic protein 2 /FL=gb:NM_007553.1
1.62	Up	BG972377	tumor necrosis factor receptor superfamily, member 21 /FL=gb:AF322069.1 gb:AY043489.1 gb:NM_052975.1 gb:BC016420.1
1.62	Down	NM_013693	Mus musculus tumor necrosis factor (Tnf), mRNA.
1.62	Down	AF128218	Mus musculus strain B10.SJ small inducible cytokine A4 (ScyA4) mRNA, complete cds.
1.58	Up	BC023427	Mus musculus platelet derived growth factor, B polypeptide (Pdgfb), mRNA.
1.58	Up	AK010040	Mus musculus adult male tongue cDNA, RIKEN full-length enriched library, clone:2310065C03:interleukin 17 receptor, full insert sequence.
1.57	Up	BG972377	Mus musculus tumor necrosis factor receptor superfamily, member 21 (Tnfrsf21), mRNA.
1.56	Up	AK010040	Mus musculus interleukin 17 receptor (Il17r), mRNA.
1.55	Down	NM_007782	Mus musculus colony stimulating factor 3 receptor (granulocyte) (Csf3r), mRNA.
1.55	Up	AV231648	Mus musculus, chemokine (C-C) receptor 1, clone MGC:18743 IMAGE:3992755, mRNA, complete cds.
1.54	Up	BB815530	kit ligand /FL=gb:U44725.1 gb:NM_013598.1 gb:BC011322.1

			gb:M57647.1
1.51	Up	BC023427	Mus musculus, Similar to platelet derived growth factor, B polypeptide, clone IMAGE:5010396, mRNA, partial cds.

Cell adhesion molecules (CAMs)

Ratio	Direction	Gene Identifier	Gene Name
3.98	Down	M36005	Mus musculus selectin, lymphocyte (Sell), mRNA.
3.17	Down	M36005	Mouse lymph node homing receptor mRNA, complete cds.
2.88	Up	NM_009864	Mus musculus cadherin 1 (Cdh1), mRNA.
2.04	Up	BB350308	ESTs
1.97	Up	NM_133983	Mus musculus expressed sequence AU016588 (AU016588), mRNA.
1.89	Up	NM_013486	Mus musculus CD2 antigen (Cd2), mRNA.
1.78	Up	NM_018770	immunoglobulin superfamily, member 4 /FL=gb:NM_018770.1 gb:AF061260.1 gb:AB021965.1 gb:AB021964.1 gb:AB052293.1 gb:AF434663.1 immunoglobulin superfamily, member 4 /FL=gb:NM_018770.1 gb:AF061260.1 gb:AB021965.1 gb:AB021964.1 gb:AB052293.1 gb:AF434663.1
1.76	Up	NM_018770	
1.75	Up	BG144448	ESTs
1.73	Up	NM_018770	Mus musculus immunoglobulin superfamily, member 4 (Igsf4), mRNA.
1.73	Down	BQ175493	expressed sequence AI447669
1.69	Down	BB623587	Mus musculus integrin alpha8 mRNA, partial cds Mus musculus adult male hippocampus cDNA, RIKEN full-length enriched library, clone:2900073G06:immunoglobulin superfamily, member 4, full insert sequence.
1.67	Up	AK013775	
1.66	Down	NM_009259	Mus musculus sialophorin (Spn), mRNA.
1.62	Up	NM_010386	Mus musculus histocompatibility 2, class II, locus DMa (H2-DMa), mRNA.
1.62	Down	NM_008984	Mus musculus protein tyrosine phosphatase, receptor-type, M (Ptpm), mRNA.
1.61	Up	NM_010388	Mus musculus histocompatibility 2, class II, locus Mb2 (H2-DMb2), mRNA.
1.58	Down	NM_019388	Mus musculus, CD86 antigen, clone MGC:18471 IMAGE:4008635, mRNA, complete cds.
1.57	Down	NM_133721	RIKEN cDNA 2610002H11 gene /FL=gb:NM_133721.1
1.57	Up	AU021035	Mus musculus syndecan 2 (Sdc2), mRNA.
1.55	Up	AF349453	Mus musculus claudin-18A2.2 mRNA, complete cds, alternatively spliced.
1.53	Up	NM_007727	Mus musculus contactin 1 (Cntn1), mRNA.
1.51	Down	BM251152	chondroitin sulfate proteoglycan 2

MAPK signaling pathway

Ratio	Direction	Gene Identifier	Gene Name
3.04	Down	BG976607	ectodermal-neural cortex 1
2.18	Down	BE691393	expressed sequence AI452260
2.16	Up	AV295798	RIKEN cDNA 2310076D10 gene Mus musculus, Similar to interleukin 1 beta, clone MGC:18555 IMAGE:3989461, mRNA, complete cds.
2.12	Down	BC011437	
2.09	Up	NM_012044	Mus musculus phospholipase A2, group IIE (Pla2g2e), mRNA.

1.83	Up	BQ266161	ESTs
1.82	Down	X59274	M.musculus mRNA brain protein kinase C beta-I.
1.78	Up	NM_025846	Mus musculus related RAS viral (r-ras) oncogene homolog 2 (Ras2), mRNA.
1.78	Down	NM_013642	Mus musculus protein tyrosine phosphatase, non-receptor type 16 (Ptpn16), mRNA.
1.75	Down	BF660388	protein kinase C, beta /FL=gb:NM_008855.1
1.74	Up	BM248342	transforming growth factor, beta receptor I /FL=gb:D28526.1 gb:NM_009370.1 gb:D25540.1
1.69	Up	AK012530	Mus musculus 11 days embryo whole body cDNA, RIKEN full-length enriched library, clone:2700078F24:unclassifiable transcript, full insert sequence.
1.68	Down	NM_020013	Mus musculus fibroblast growth factor 21 (Fgf21), mRNA.
1.66	Down	BB121278	DNA segment, Chr 6, ERATO Doi 213, expressed
1.62	Down	NM_013693	Mus musculus tumor necrosis factor (Tnf), mRNA.
1.62	Down	NM_009008	Mus musculus RAS-related C3 botulinum substrate 2 (Rac2), mRNA.
1.58	Up	BC023427	Mus musculus platelet derived growth factor, B polypeptide (Pdgfb), mRNA.
1.56	Down	NM_011949	Mus musculus mitogen activated protein kinase 1 (Mapk1), mRNA.
1.52	Up	BB821318	expressed sequence AW124339
1.52	Down	AK018008	Mus musculus adult male thymus cDNA, RIKEN full-length enriched library, clone:5830453M24:unclassifiable transcript, full insert sequence.
1.52	Up	BB147462	expressed sequence AI848108
1.51	Up	BC023427	Mus musculus, Similar to platelet derived growth factor, B polypeptide, clone IMAGE:5010396, mRNA, partial cds.
1.5	Up	AV295798	RIKEN cDNA 2310076D10 gene

Focal adhesion

Ratio	Direction	Gene Identifier	Gene Name
4.3	Up	AB029929	Mus musculus mRNA for caveolin-1 alfa isoform, complete cds.
3.05	Up	NM_010580	Mus musculus integrin beta 5 (Itgb5), mRNA.
2.83	Up	NM_010580	Mus musculus integrin beta 5 (Itgb5), mRNA.
2.72	Up	BB543979	integrin beta 5
2.32	Down	AK007904	Mus musculus 10 day old male pancreas cDNA, RIKEN full-length enriched library, clone:1810059D21:cyclin D2, full insert sequence.
2.27	Down	NM_009829	cyclin D2 /FL=gb:M86182.1 gb:M83749.1 gb:NM_009829.1
2.22	Up	NM_010228	Mus musculus FMS-like tyrosine kinase 1 (Flt1), mRNA.
2.18	Down	BE691393	expressed sequence AI452260
2.11	Down	NM_009829	Mus musculus cyclin D2 (Cnd2), mRNA.
2.02	Down	NM_009829	cyclin D2 /FL=gb:M86182.1 gb:M83749.1 gb:NM_009829.1
1.94	Up	BI134907	catenin beta
1.94	Down	NM_009829	cyclin D2 /FL=gb:M86182.1 gb:M83749.1 gb:NM_009829.1
1.89	Down	BQ175880	EST AA087124
1.82	Down	X59274	M.musculus mRNA brain protein kinase C beta-I.
1.75	Down	BF660388	protein kinase C, beta /FL=gb:NM_008855.1
1.73	Down	BQ175493	expressed sequence AI447669
1.72	Up	BB667216	expressed sequence AI551257
1.7	Up	NM_007614	Mus musculus catenin beta (Catnb), mRNA.
1.69	Down	BB623587	Mus musculus integrin alpha8 mRNA, partial cds
1.62	Down	NM_009008	Mus musculus RAS-related C3 botulinum substrate 2 (Rac2), mRNA.

1.58	Up	BC023427	Mus musculus platelet derived growth factor, B polypeptide (Pdgfb), mRNA.
1.57	Down	NM_133721	RIKEN cDNA 2610002H11 gene /FL=gb:NM_133721.1
1.57	Up	BI134721	expressed sequence AI595373
1.56	Down	NM_011949	Mus musculus mitogen activated protein kinase 1 (Mapk1), mRNA.
1.55	Up	AI465173	expressed sequence AI465173
1.53	Down	AI385532	thrombospondin 1 /FL=gb:M87276.1 gb:NM_011580.1
1.51	Down	BF451748	Mus musculus telokin mRNA, complete cds /FL=gb:AF314149.1
1.51	Up	BC023427	Mus musculus, Similar to platelet derived growth factor, B polypeptide, clone IMAGE:5010396, mRNA, partial cds.

Regulation of actin cytoskeleton

Ratio	Direction	Gene Identifier	Gene Name
3.05	Up	NM_010580	Mus musculus integrin beta 5 (Itgb5), mRNA.
2.83	Up	NM_010580	Mus musculus integrin beta 5 (Itgb5), mRNA.
2.72	Up	BB543979	integrin beta 5
2.27	Up	AW492237	expressed sequence AI132431
2.23	Up	NM_019410	Mus musculus profilin 2 (Pfn2), mRNA.
2.18	Down	BE691393	expressed sequence AI452260
1.93	Down	BC003763	Mus musculus, Similar to phosphatidylinositol-4-phosphate 5-kinase, type 1 beta, clone MGC:5928 IMAGE:3591003, mRNA, complete cds.
1.78	Up	NM_025846	Mus musculus related RAS viral (r-ras) oncogene homolog 2 (Rras2), mRNA.
1.73	Down	BQ175493	expressed sequence AI447669
1.69	Down	BB623587	Mus musculus integrin alpha8 mRNA, partial cds
1.68	Down	NM_020013	Mus musculus fibroblast growth factor 21 (Fgf21), mRNA.
1.67	Down	BB822856	phosphatidylinositol-4-phosphate 5-kinase, type 1 beta
1.62	Down	NM_009008	Mus musculus RAS-related C3 botulinum substrate 2 (Rac2), mRNA.
1.59	Down	BQ176159	RIKEN cDNA 5730504C04 gene
1.59	Up	NM_019410	Mus musculus profilin 2 (Pfn2), mRNA.
1.58	Up	BC023427	Mus musculus platelet derived growth factor, B polypeptide (Pdgfb), mRNA.
1.57	Down	NM_133721	RIKEN cDNA 2610002H11 gene /FL=gb:NM_133721.1
1.56	Down	NM_011949	Mus musculus mitogen activated protein kinase 1 (Mapk1), mRNA.
1.52	Up	BB469215	ESTs
1.51	Down	BF451748	Mus musculus telokin mRNA, complete cds /FL=gb:AF314149.1
1.51	Up	BC023427	Mus musculus, Similar to platelet derived growth factor, B polypeptide, clone IMAGE:5010396, mRNA, partial cds.

Hematopoietic cell lineage

Ratio	Direction	Gene Identifier	Gene Name
3.56	Up	NM_009846	Mus musculus CD24a antigen (Cd24a), mRNA.
2.94	Down	NM_009971	Mus musculus colony stimulating factor 3 (granulocyte) (Csf3), mRNA.
2.85	Up	NM_009846	CD24a antigen /FL=gb:NM_009846.1 gb:M58661.1
2.77	Up	AI573431	Mus musculus interleukin 7 receptor (Il7r), mRNA.
2.71	Up	AI573431	interleukin 7 receptor /FL=gb:NM_008372.2 gb:M29697.1 gb:AF078906.1
2.69	Down	NM_031168	Mus musculus interleukin 6 (Il6), mRNA.

2.12	Down	BC011437	Mus musculus, Similar to interleukin 1 beta, clone MGC:18555 IMAGE:3989461, mRNA, complete cds.
2.11	Down	BE686894	ESTs
2.03	Down	NM_007646	Mus musculus CD38 antigen (Cd38), mRNA. Mouse mRNA for granulocyte-macrophage colony stimulating factor (GM-CSF).
1.99	Down	X03019	
1.89	Up	NM_013486	Mus musculus CD2 antigen (Cd2), mRNA.
1.7	Down	NM_010016	Mus musculus decay accelerating factor 1 (Daf1), mRNA.
1.62	Down	NM_013693	Mus musculus tumor necrosis factor (Tnf), mRNA. Mus musculus colony stimulating factor 3 receptor (granulocyte) (Csf3r), mRNA.
1.55	Down	NM_007782	
1.54	Up	BB815530	kit ligand /FL=gb:U44725.1 gb:NM_013598.1 gb:BC011322.1 gb:M57647.1
1.51	Down	NM_010016	decay accelerating factor 1 /FL=gb:NM_010016.1 gb:L41366.1 gb:BC011314.1 gb:D63679.1

Neuroactive ligand-receptor interaction

Ratio	Direction	Gene Identifier	Gene Name
7.36	Up	NM_008311	Mus musculus 5-hydroxytryptamine (serotonin) receptor 2B (Htr2b), mRNA.
1.95	Down	BF100813	expressed sequence AU022549
1.83	Down	D43775	Mouse mRNA for endothelin-1, complete cds.
1.79	Up	AW490413	ESTs
1.71	Down	AF209905	Mus musculus calcitonin receptor-like receptor mRNA, complete cds.
1.69	Down	NM_007577	Mus musculus complement component 5, receptor 1 (C5r1), mRNA.
1.68	Up	AW558570	ESTs
1.63	Down	NM_008039	Mus musculus formyl peptide receptor, related sequence 2 (Fpr-rs2), mRNA.
1.59	Down	BB552085	ESTs
1.54	Down	AV083350	ESTs, Highly similar to BETA-2 ADRENERGIC RECEPTOR (M.musculus)
1.52	Up	BB133079	endothelial differentiation sphingolipid G-protein-coupled receptor 1 /FL=gb:NM_007901.1
1.51	Down	AV344554	ESTs
1.5	Up	AK003144	Mus musculus 18 days embryo whole body cDNA, RIKEN full-length enriched library, clone:1100001A16:unclassifiable transcript, full insert sequence.

Calcium signaling pathway

Ratio	Direction	Gene Identifier	Gene Name
7.36	Up	NM_008311	Mus musculus 5-hydroxytryptamine (serotonin) receptor 2B (Htr2b), mRNA.
2.03	Down	NM_007646	Mus musculus CD38 antigen (Cd38), mRNA.
1.95	Down	BF100813	expressed sequence AU022549
1.94	Up	BB224034	Mus musculus strain ILS phospholipase C beta 4 (Plcb4) mRNA, complete cds /FL=gb:AF332071.1 gb:AF332072.1
1.82	Down	X59274	M.musculus mRNA brain protein kinase C beta-I.
1.79	Up	AW490413	ESTs
1.75	Down	BF660388	protein kinase C, beta /FL=gb:NM_008855.1

1.68	Up	AW558570	ESTs
1.57	Up	BB313689	expressed sequence AI852629
1.56	Down	NM_016745	Mus musculus, ATPase, Ca++ transporting, ubiquitous, clone MGC:28920 IMAGE:4924519, mRNA, complete cds.
1.54	Down	AV083350	ESTs, Highly similar to BETA-2 ADRENERGIC RECEPTOR (M.musculus)
1.51	Down	BF451748	Mus musculus telokin mRNA, complete cds /FL=gb:AF314149.1

Complement and coagulation cascades

Ratio	Direction	Gene Identifier	Gene Name
3.2	Down	NM_007976	coagulation factor V /FL=gb:NM_007976.1 gb:U52925.1
2.89	Up	NM_008871	Mus musculus serine (or cysteine) proteinase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), member 1 (Serpine1), mRNA.
2.43	Down	K02782	Mouse complement component C3 mRNA, alpha and beta subunits, complete cds.
2.14	Down	NM_007976	Mus musculus coagulation factor V (F5), mRNA.
2.11	Down	BE686894	ESTs
1.72	Up	BB667216	expressed sequence AI551257
1.7	Down	NM_010016	Mus musculus decay accelerating factor 1 (Daf1), mRNA.
1.69	Down	NM_007577	Mus musculus complement component 5, receptor 1 (C5r1), mRNA.
1.64	Up	NM_007572	Mus musculus complement component 1, q subcomponent, alpha polypeptide (C1qa), mRNA.
1.6	Up	NM_009378	Mus musculus thrombomodulin (Thbd), mRNA.
1.6	Up	AK015095	Mus musculus adult male testis cDNA, RIKEN full-length enriched library, clone:4930405E17:Zinc carboxypeptidase containing protein, full insert sequence.
1.59	Down	BB552085	ESTs
1.58	Up	NM_007574	Mus musculus complement component 1, q subcomponent, c polypeptide (C1qc), mRNA.
1.51	Down	NM_010016	decay accelerating factor 1 /FL=gb:NM_010016.1 gb:L41366.1 gb:BC011314.1 gb:D63679.1

Jak-STAT signaling pathway

Ratio	Direction	Gene Identifier	Gene Name
2.94	Down	NM_009971	Mus musculus colony stimulating factor 3 (granulocyte) (Csf3), mRNA.
2.77	Up	AI573431	Mus musculus interleukin 7 receptor (Il7r), mRNA.
2.71	Up	AI573431	interleukin 7 receptor /FL=gb:NM_008372.2 gb:M29697.1 gb:AF078906.1
2.69	Down	NM_031168	Mus musculus interleukin 6 (Il6), mRNA.
2.48	Down	NM_011487	Mus musculus signal transducer and activator of transcription 4 (Stat4), mRNA.
2.32	Down	AK007904	Mus musculus 10 day old male pancreas cDNA, RIKEN full-length enriched library, clone:1810059D21:cyclin D2, full insert sequence.
2.27	Down	NM_009829	cyclin D2 /FL=gb:M86182.1 gb:M83749.1 gb:NM_009829.1
2.11	Down	NM_009829	Mus musculus cyclin D2 (Ccmd2), mRNA.
2.02	Down	NM_009829	cyclin D2 /FL=gb:M86182.1 gb:M83749.1 gb:NM_009829.1
1.99	Down	AV246615	expressed sequence AW061234
1.99	Down	X03019	Mouse mRNA for granulocyte-macrophage colony stimulating factor (GM-CSF).

1.94	Down	NM_009829	cyclin D2 /FL=gb:M86182.1 gb:M83749.1 gb:NM_009829.1
1.89	Down	BQ175880	EST AA087124
1.8	Up	NM_007707	Mus musculus cytokine inducible SH2-containing protein 3 (Cish3), mRNA.
1.56	Up	NM_007706	Mus musculus cytokine inducible SH2-containing protein 2 (Cish2), mRNA.
1.55	Down	NM_007782	Mus musculus colony stimulating factor 3 receptor (granulocyte) (Csf3r), mRNA.

Melanogenesis

Ratio	Direction	Gene Identifier	Gene Name
2.47	Down	NM_009526	Mus musculus wingless-related MMTV integration site 6 (Wnt6), mRNA.
2.11	Down	AV308073	ESTs
1.95	Down	BF100813	expressed sequence AU022549
1.94	Up	BB224034	Mus musculus strain ILS phospholipase C beta 4 (Plcb4) mRNA, complete cds /FL=gb:AF332071.1 gb:AF332072.1
1.94	Up	BI134907	catenin beta
1.83	Down	D43775	Mouse mRNA for endothelin-1, complete cds.
1.82	Down	X59274	M.musculus mRNA brain protein kinase C beta-I.
1.75	Down	BF660388	protein kinase C, beta /FL=gb:NM_008855.1
1.7	Up	NM_007614	Mus musculus catenin beta (Catnb), mRNA.
1.61	Up	BB175494	transcription factor 4
1.56	Down	NM_011949	Mus musculus mitogen activated protein kinase 1 (Mapk1), mRNA. kit ligand /FL=gb:U44725.1 gb:NM_013598.1 gb:BC011322.1
1.54	Up	BB815530	gb:M57647.1
1.51	Up	BB175494	transcription factor 4

Adherens junction

Ratio	Direction	Gene Identifier	Gene Name
2.88	Up	NM_009864	Mus musculus cadherin 1 (Cdh1), mRNA.
2.18	Down	BE691393	expressed sequence AI452260
1.94	Up	BI134907	catenin beta
1.74	Up	BM248342	transforming growth factor, beta receptor I /FL=gb:D28526.1 gb:NM_009370.1 gb:D25540.1
1.7	Up	NM_007614	Mus musculus catenin beta (Catnb), mRNA.
1.62	Down	NM_009008	Mus musculus RAS-related C3 botulinum substrate 2 (Rac2), mRNA.
1.62	Down	NM_008984	Mus musculus protein tyrosine phosphatase, receptor-type, M (Ptpm), mRNA.
1.61	Up	BB175494	transcription factor 4
1.56	Down	NM_011949	Mus musculus mitogen activated protein kinase 1 (Mapk1), mRNA.
1.55	Up	AI465173	expressed sequence AI465173
1.51	Up	BB175494	transcription factor 4

Axon guidance

Ratio	Direction	Gene Identifier	Gene Name
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3.17	Down	AV348197	ESTs
3.08	Up	D87747	Mus musculus mRNA for murine CXCR-4, complete cds.
2.49	Down	Z93948	M.musculus mRNA for semaphorin Hv (3988 bp).
2.18	Down	BE691393	expressed sequence AI452260 ESTs, Weakly similar to TRANSFORMING PROTEIN RHOB (M.musculus)
2.17	Down	BE852181	Mus musculus sema domain, immunoglobulin domain (Ig), short basic domain, secreted, (semaphorin) 3E (Sema3e), mRNA.
2.04	Down	NM_011348	ESTs
1.68	Down	BB361326	ESTs
1.62	Down	NM_009008	Mus musculus RAS-related C3 botulinum substrate 2 (Rac2), mRNA.
1.56	Down	BQ032843	expressed sequence AI426163
1.56	Down	NM_011949	Mus musculus mitogen activated protein kinase 1 (Mapk1), mRNA.
1.54	Down	AI606937	ESTs

PPAR signaling pathway

Ratio	Direction	Gene Identifier	Gene Name
2.8	Up	NM_010174	Mus musculus fatty acid binding protein 3, muscle and heart (Fabp3), mRNA.
2.5	Up	NM_138648	Mus musculus oxidized low density lipoprotein (lectin-like) receptor 1 (Olr1), mRNA.
2.45	Down	AK017272	Mus musculus 6 days neonate head cDNA, RIKEN full-length enriched library, clone:5430405H20:lipoprotein lipase, full insert sequence.
2.14	Down	BC003305	Mus musculus lipoprotein lipase (Lpl), mRNA.
2.09	Up	NM_021272	Mus musculus fatty acid binding protein 7, brain (Fabp7), mRNA.
1.78	Down	BF683028	glycerol kinase /FL=gb:U48403.1 gb:Nm_008194.1 gb:BC003767.1
1.78	Down	BF683028	Mus musculus glycerol kinase (Gyk), mRNA.
1.62	Down	AI591480	protective protein for beta-galactosidase Mus musculus adult male testis cDNA, RIKEN full-length enriched library, clone:1700026H15:malic enzyme, supernatant, full insert sequence.
1.6	Up	AK006387	Mus musculus RIKEN cDNA 1300013A03 gene (1300013A03Rik), mRNA.
1.6	Down	NM_024264	mRNA.
1.52	Up	BC011081	Mus musculus malic enzyme, supernatant (Mod1), mRNA.
1.52	Down	BQ176629	ESTs

Purine metabolism

Ratio	Direction	Gene Identifier	Gene Name
1.81	Up	NM_007421	Mus musculus adenylosuccinate synthetase 1, muscle (Adss1), mRNA.
1.74	Down	AF055573	Mus musculus fragile histidine triad protein (Fhit) mRNA, complete cds.
1.72	Up	AF339910	Mus musculus ectonucleotide pyrophosphatasephosphodiesterase 1 allotype b (Enpp1) mRNA, Enpp1-b allele, complete cds.
1.69	Down	BM246564	phosphodiesterase 4B, cAMP specific /FL=gb:Nm_019840.1 gb:AF326556.1 gb:AF326555.1 gb:AF208023.2
1.59	Up	NM_021515	Mus musculus adenylate kinase 1 (Ak1), mRNA.
1.59	Down	BG793493	ESTs
1.58	Up	AW910504	RIKEN cDNA 4833416E15 gene
1.56	Up	NM_009647	Mus musculus adenylate kinase 4 (Ak4), mRNA.
1.53	Down	BB729239	RNA polymerase 1-4 (194 kDa subunit) /FL=gb:Nm_009088.1 gb:AF000938.1

1.51	Up	NM_009647	Mus musculus adenylate kinase 4 (Ak4), mRNA.
1.5	Down	NM_010266	Mus musculus guanine deaminase (Gda), mRNA.

TGF-beta signaling pathway

Ratio	Direction	Gene Identifier	Gene Name
1.98	Up	AF010133	Mus musculus Smad6 mRNA, complete cds. transforming growth factor, beta receptor I /FL=gb:D28526.1
1.74	Up	BM248342	gb:Nm_009370.1 gb:D25540.1
1.73	Down	AV239587	bone morphogenetic protein 2 /FL=gb:Nm_007553.1
1.64	Up	U43884	Mus musculus transcription factor Id1B (Id1B) mRNA, complete cds.
1.62	Down	NM_013693	Mus musculus tumor necrosis factor (Tnf), mRNA.
1.56	Down	NM_011949	Mus musculus mitogen activated protein kinase 1 (Mapk1), mRNA.
1.54	Up	NM_008321	Mus musculus inhibitor of DNA binding 3 (Idb3), mRNA.
1.53	Down	AI385532	thrombospondin 1 /FL=gb:M87276.1 gb:Nm_011580.1

Wnt signaling pathway

Ratio	Direction	Gene Identifier	Gene Name
2.47	Down	NM_009526	Mus musculus wingless-related MMTV integration site 6 (Wnt6), mRNA.
2.32	Down	AK007904	Mus musculus 10 day old male pancreas cDNA, RIKEN full-length enriched library, clone:1810059D21:cyclin D2, full insert sequence.
2.27	Down	NM_009829	cyclin D2 /FL=gb:M86182.1 gb:M83749.1 gb:Nm_009829.1
2.18	Down	BE691393	expressed sequence AI452260
2.11	Down	AV308073	ESTs
2.11	Down	NM_009829	Mus musculus cyclin D2 (Cnd2), mRNA.
2.02	Down	NM_009829	cyclin D2 /FL=gb:M86182.1 gb:M83749.1 gb:Nm_009829.1
1.94	Up	BB224034	Mus musculus strain ILS phospholipase C beta 4 (Plcb4) mRNA, complete cds /FL=gb:AF332071.1 gb:AF332072.1
1.94	Up	BI134907	catenin beta
1.94	Down	NM_009829	cyclin D2 /FL=gb:M86182.1 gb:M83749.1 gb:Nm_009829.1
1.89	Down	BQ175880	EST AA087124
1.82	Down	X59274	M.musculus mRNA brain protein kinase C beta-I.
1.75	Down	BF660388	protein kinase C, beta /FL=gb:Nm_008855.1
1.7	Up	NM_007614	Mus musculus catenin beta (Catnb), mRNA.
1.62	Down	NM_009008	Mus musculus RAS-related C3 botulinum substrate 2 (Rac2), mRNA.
1.61	Up	BB175494	transcription factor 4
1.54	Down	U34245	Mus musculus fos-related antigen-1 (Fra-1) mRNA, complete cds.
1.51	Up	BB175494	transcription factor 4

Arachidonic acid metabolism

Ratio	Direction	Gene Identifier	Gene Name
3.59	Down	M94967	Mouse glucocorticoid-regulated inflammatory prostaglandin GH synthase (griPGHS) mRNA, complete cds. Mus musculus RIKEN cDNA 1810054N16 gene (1810054N16Rik), mRNA.
2.48	Up	NM_024444	
2.09	Up	NM_012044	Mus musculus phospholipase A2, group IIE (Pla2g2e), mRNA.

1.71	Up	BC010758	Mus musculus, Similar to carbonyl reductase 2, clone MGC:18465 IMAGE:4219349, mRNA, complete cds.
1.71	Down	NM_022415	Mus musculus prostaglandin E synthase (Ptges), mRNA.
1.62	Down	BB730139	ESTs
1.57	Up	NM_011539	Mus musculus thromboxane A synthase 1, platelet (Tbxas1), mRNA.
1.55	Up	NM_009998	Mus musculus cytochrome P450, 2b10, phenobarbital inducible, type b (Cyp2b10), mRNA.
1.54	Down	NM_022415	Mus musculus, prostaglandin E synthase, clone MGC:35838 IMAGE:4950950, mRNA, complete cds.

ECM-receptor interaction

Ratio	Direction	Gene Identifier	Gene Name
3.05	Up	NM_010580	Mus musculus integrin beta 5 (Itgb5), mRNA.
2.83	Up	NM_010580	Mus musculus integrin beta 5 (Itgb5), mRNA.
2.72	Up	BB543979	integrin beta 5
1.73	Down	BQ175493	expressed sequence AI447669
1.72	Up	BB667216	expressed sequence AI551257
1.69	Down	BB623587	Mus musculus integrin alpha8 mRNA, partial cds
1.57	Down	NM_133721	RIKEN cDNA 2610002H11 gene /FL=gb:NM_133721.1
1.57	Up	AU021035	Mus musculus syndecan 2 (Sdc2), mRNA.
1.53	Down	AI385532	thrombospondin 1 /FL=gb:M87276.1 gb:NM_011580.1 Mus musculus 12 days embryo male wolffian duct includes surrounding region cDNA, RIKEN full-length enriched library, clone:6720466F14:hyaluronan mediated motility receptor (RHAMM), full insert sequence.
1.52	Up	AK020144	
1.51	Up	AF079222	Mus musculus hyaluronan receptor RHAMMV5 mRNA, complete cds.

Leukocyte transendothelial migration

Ratio	Direction	Gene Identifier	Gene Name
7.29	Down	NM_013599	Mouse mRNA for gelatinase, complete cds.
4.65	Down	NM_013599	Mus musculus matrix metalloproteinase 9 (Mmp9), mRNA.
3.08	Up	D87747	Mus musculus mRNA for murine CXCR-4, complete cds.
2.18	Down	BE691393	expressed sequence AI452260 Mus musculus CXC chemokine MIP-2gamma precursor, mRNA, complete cds.
2.14	Up	AF252873	
1.94	Up	BI134907	catenin beta
1.82	Down	X59274	M.musculus mRNA brain protein kinase C beta-I.
1.75	Down	BF660388	protein kinase C, beta /FL=gb:NM_008855.1
1.7	Up	NM_007614	Mus musculus catenin beta (Catnb), mRNA.
1.62	Down	NM_009008	Mus musculus RAS-related C3 botulinum substrate 2 (Rac2), mRNA.
1.55	Up	AF349453	Mus musculus claudin-18A2.2 mRNA, complete cds, alternatively spliced.

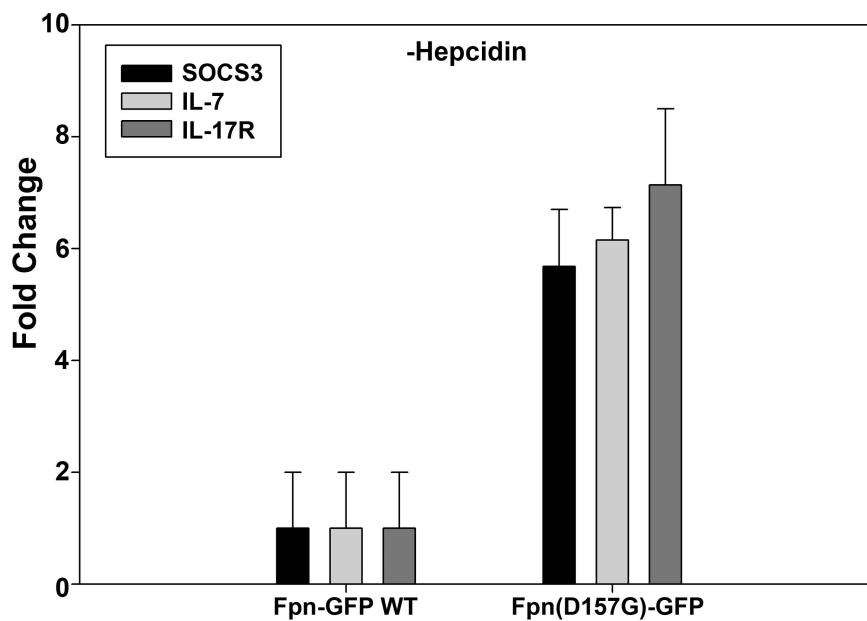
Toll-like receptor interaction

Ratio	Direction	Gene Identifier	Gene Name
2.69	Down	NM_031168	Mus musculus interleukin 6 (Il6), mRNA.

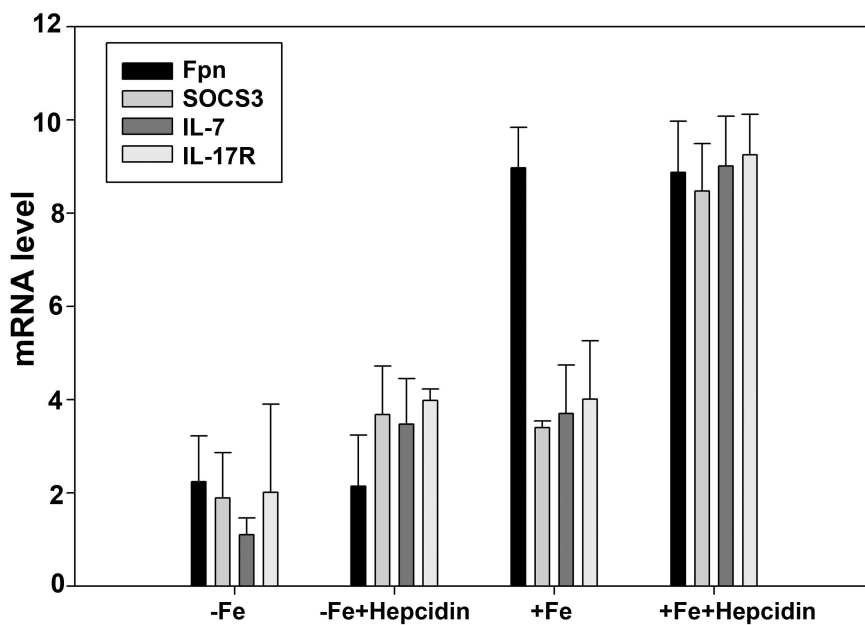
2.12	Down	BC011437	Mus musculus, Similar to interleukin 1 beta, clone MGC:18555 IMAGE:3989461, mRNA, complete cds.
1.8	Down	NM_013653	Mus musculus small inducible cytokine A5 (Scya5), mRNA.
1.62	Down	NM_013693	Mus musculus tumor necrosis factor (Tnf), mRNA.
1.62	Down	AF128218	Mus musculus strain B10.SJ small inducible cytokine A4 (ScyA4) mRNA, complete cds.
1.58	Down	NM_019388	Mus musculus, CD86 antigen, clone MGC:18471 IMAGE:4008635, mRNA, complete cds.
1.52	Up	BB821318	expressed sequence AW124339
1.52	Up	BB147462	expressed sequence AI848108

Supplemental Figure 1

A

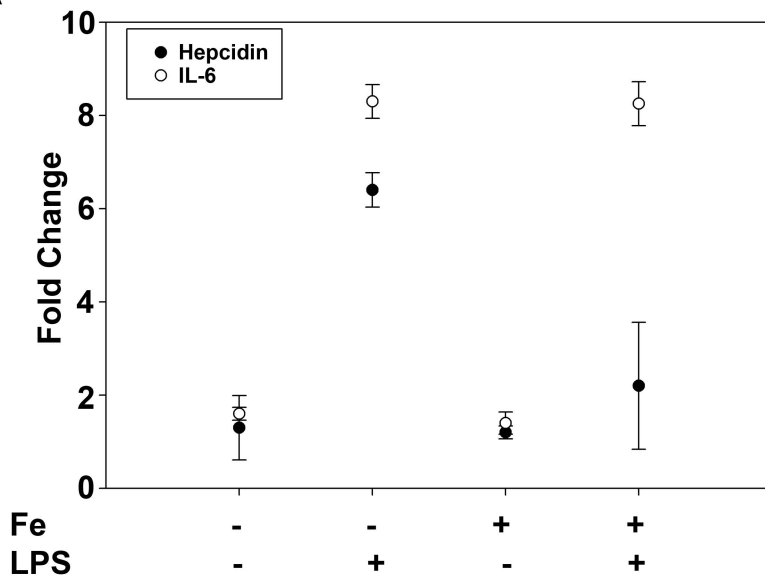


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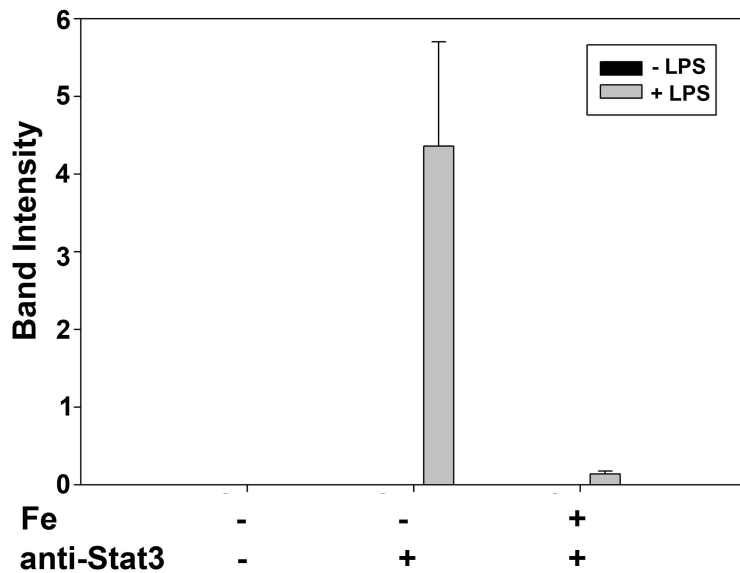


Supplemental Figure 2

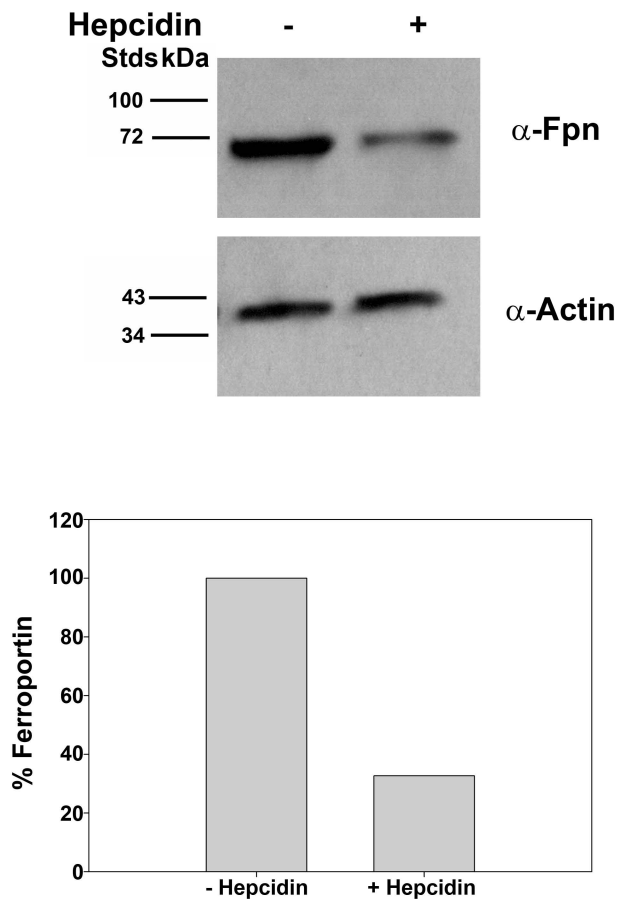
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B

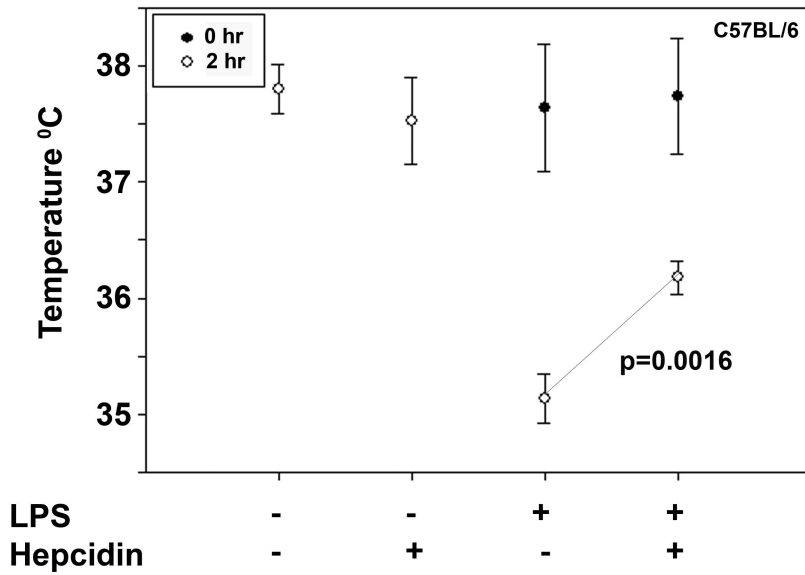


Supplemental Figure 3

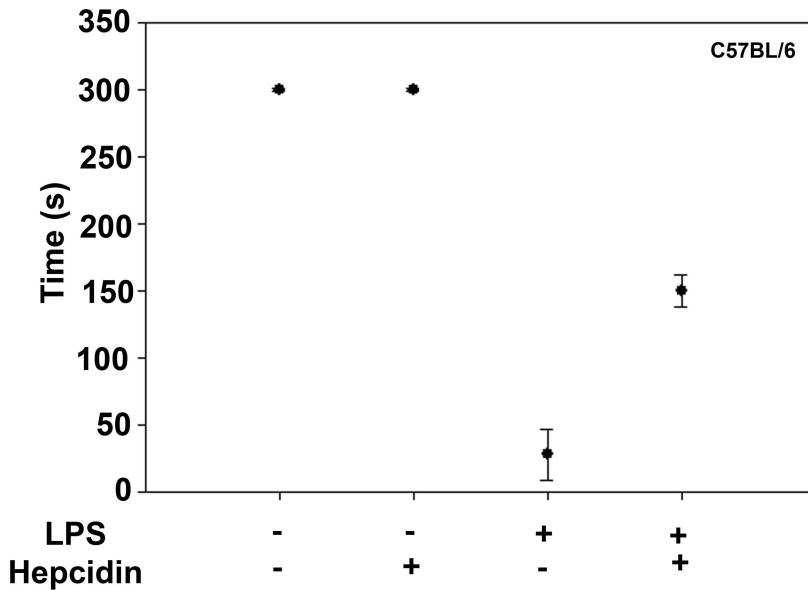


Supplemental Figure 4

A

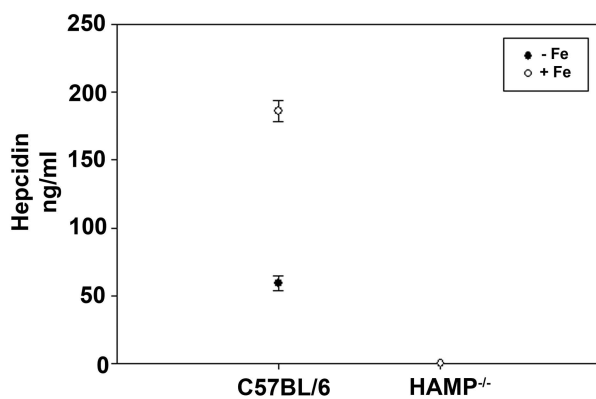


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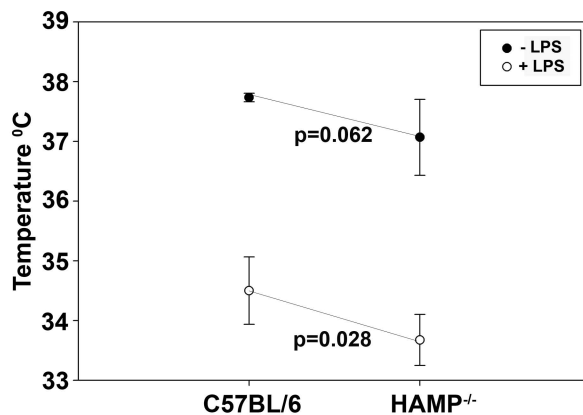


Supplemental Figure 5

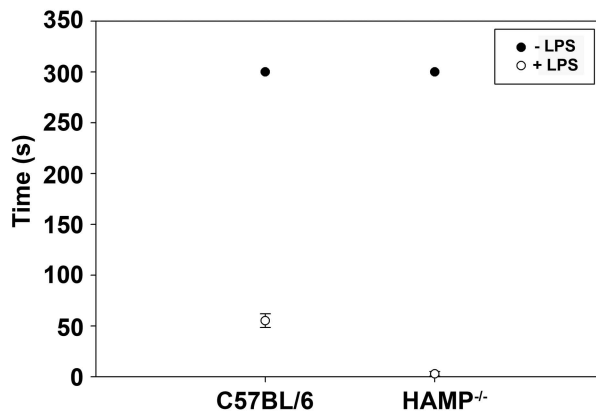
A



B

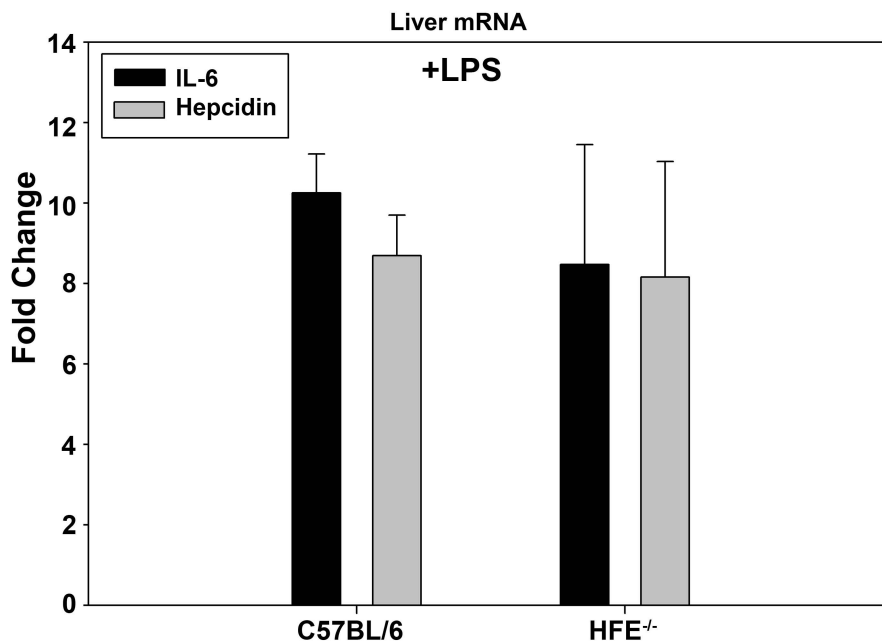


C



Supplemental Figure 6

A



B

