

Supporting Information

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SI Materials and Methods

Northern Blot Analysis. A murine tissue mRNA blot (Mouse MTN Blot) containing 2 μ g of mRNA from eight different tissues was purchased from Clontech Laboratories, Inc. Human hematopoietic cell line mRNA was extracted using TRIzol (Invitrogen), followed by Oligo-Tex (Takara Bio Inc.), and 4 μ g of mRNA from each line was transferred to a nylon membrane. Both filters were hybridized with mouse *hemp* cDNA or a β -actin cDNA probe.

Construction of a Targeting Vector and Generation of *hemp* Knockout Mice. A BAC clone containing the mouse *hematopoietic expressed mammalian polycomb (hemp)* gene was isolated from a mouse genomic BAC library (Genome Systems, Inc.) using a portion of the mouse *hemp* cDNA as a probe. A 12-kb genomic fragment from an ApaI site in intron 2 to an EcoRV site in exon 6, and a 4-kb genomic fragment from a ClaI site in exon 9 to an ApaI site in intron 11 were used as the 5' and 3' arms of the knockout vector, respectively. A fragment containing *IRE5/GFP* (derived from *pIRE52-EGFP*; Clontech Laboratories Inc.), an *SV40* polyadenylation signal, and a *floxed Neo* resistance gene were inserted between the 5' and 3' arms, and a *diphtheria toxin A (DTA)* gene was attached to the 5' end of the vector as a negative selector. ES cells (1×10^5) from E14 (kindly provided by Nobuaki Yoshida, Institute of Medical Science, University of Tokyo, Japan) were electroporated with 30- μ g linearized vector and selected with 400 μ g/mL of G418. Individual candidate clones were screened by genomic PCR using the following primers: 5' P1 (5'-CAGACTTCAGGTAACGGTACAGAAACCTTC-3') and P2 (5'-AAGACCCCTAGGAATGCTCGTCAAGAAGAC-3'), followed by Southern blottings using an external 3' probe. Correctly targeted cells were microinjected into blastocysts derived from C57BL/6 \times BDF1 mice. Chimeric male mice were crossed with C57BL/6 female mice to for transmit transmission of the mutant allele to the progeny. The *Neo* resistance gene was removed by microinjecting a *Cre* expression vector into fertilized eggs obtained from crossing *hemp* heterozygotes with C57BL/6 mice. Mice that had been backcrossed to the C57BL/6-Ly5.2 background at least seven times were used for these studies. All mice were maintained according to the guidelines of the Institute of Laboratory Animal Science, Hiroshima University.

Generation of an Anti-Hemp Antibody, Immunoprecipitation, and Western Blot Analysis. A 5' segment of mouse *hemp* cDNA, corresponding to amino acids 5 to 91 of the protein product, was amplified using the following primers: 5'-GACGGATCCGATAGCTGCAGT-3' and 5'-CCAGAATTCTTGCCTTCTTA-3', where the underlined nucleotides were substituted to create restriction-enzyme sites. The PCR product was subcloned into the bacterial expression vector, *pGEX1* (Amersham Pharmacia). Recombinant protein was purified using an anti-GST column and used to immunize rabbits to generate a polyclonal antibody against the N-terminal region of mouse Hemp (HempN). To detect Hemp protein, aliquots of \sim 1 mg of protein were incubated with the HempN antibody (1:400) coupled to Protein A. Then, the immunoprecipitated protein was separated by 7.5% SDS/PAGE, transferred to a polyvinylidene membrane, and blotted with the same antibody (1:3,000). Signals were detected using the ECL chemiluminescent system (Amersham).

Skeletal Analysis. Skeletal analysis was performed essentially as previously described (1). In brief, mouse embryos were fixed in 95% ethanol, defatted in acetone, and subjected to staining with

Alizarin red S for bone and Alcian blue for cartilage. The stained specimens were cleared with 1% KOH and stored in glycerol.

Quantitative Real-Time PCR. Quantitative real-time PCR was performed as previously described (2). PCR primers for murine *hemp* were purchased from Takara Bio Inc.

Immunohistochemical Analysis. Preparation of sagittal sections of the embryos and immunohistochemical staining were performed as previously described using the HempN antibody (1:50) (3).

Flow Cytometric Analysis. Flow cytometric analysis was performed essentially as previously described (4). Monoclonal antibodies (mAbs) used in this study are: anti-CD4-PE, anti-CD4-allophycocyanin (RM4-5), anti-CD8-PE (53-6.7), anti-CD3-PE (145-2C11), anti-CD25-allophycocyanin (PC61), anti-c-Kit-allophycocyanin (2B8), anti-B220-PE (RA3-6B2), anti-Mac1-PE (M1/70), anti-Gr1-allophycocyanin (RB6-8C5), anti-Ter-119-PE, anti-CD45.1-PE (A20), anti-CD45.2-FITC (104), anti-CD32/CD16 (2.4G2) (to block Fc receptors).

CFU-C, HPP-CFC, and Competitive Repopulation Assays. Sorted CD150+ LSK cells from FL were cultured in MethoCult GF3434 (StemCell Technologies Inc.) and colonies were counted on day 8 (CFU-C) or day 16 (HPP-CFC). For the competitive repopulation assay, sorted CD150+ LSK cells from E14.5 FL (Ly5.2) were transplanted into irradiated (9.5 Gy) Ly5.1 recipients together with Ly5.1 bone marrow mononuclear cells. Peripheral blood and bone marrow chimerisms were analyzed as previously described (5).

Cell Cycle and Apoptosis Assays. The short-term BrdU incorporation assay with sorted cells was performed as previously described (2). To detect apoptotic cells, sorted cells were attached to glass slides, fixed, stained with anticlaved caspase-3 antibody (Cell Signaling Technology Inc.), and visualized using a fluorophore-conjugated secondary antibody and DAPI counterstain. Apoptotic cleaved caspase-3-positive cells were analyzed and counted using a confocal microscope.

DNA Microarray Analysis, Microarray Scanning, and Data Processing. Total RNA was isolated using TRIzol reagent (Invitrogen) from lineage (lin)⁻/c-kit^{high} cells isolated from E12.5 *hemp*^{+/+} and *hemp*^{-/-} fetal livers using TRIzol reagent (Invitrogen). The amplification and preparation of amino allyl RNA (aRNA) and the labeling of aRNA with the fluorescence dyes, Cy3 and Cy5, were performed with the Amino Allyl MessageAmp II aRNA Amplification Kit (Ambion Inc.). The Cy3- or Cy5-labeled aRNAs were hybridized to the probes on the 3D-Genemouse oligo chip 24k (Toray Industries Inc.) according to the manufacturer's instructions.

Microarrays were scanned using a ScanArray Lite Scanner (Perkin-Elmer) and the photomultiplier tube levels were adjusted to achieve 0.1% to 0.5% pixel saturation. Each tagged-image file-format image was analyzed using GenePix Pro-6.0 (Molecular Devices). Data were filtered to remove low-confidence measurements, and the data were globally normalized globally per array such that the median log₂ (Cy3/Cy5 fluorescence ratio) was 0 after normalization. Results were further processed for Gene Ontology term enrichment using PathwayStudio6 software (Ariadne Genomics Inc.). Genes with global normalization scores greater than 50 in either *hemp*^{+/+} or *hemp*^{-/-} samples, and that differed by more than 1.5-fold between samples, were classified into "Biological process" and "Molecular function" categories. Those genes whose with expression levels that differed by more than twofold were characterized by MultiExperiment Viewer version 4.3.02 software (<http://www.tm4.org/mev.html>).

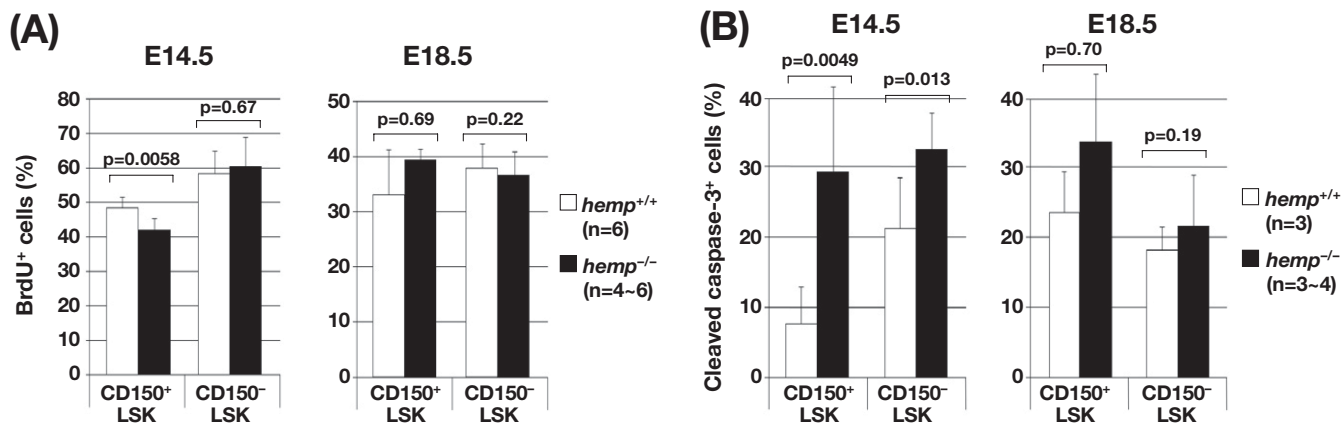


Fig. 53. Proliferative and apoptotic assays of *hemp*^{+/+} and *hemp*^{-/-} FL cells. (A) Quantification of short-term BrdU and incorporation in *hemp*^{+/+} (open bar) and *hemp*^{-/-} (closed bar) CD150⁺ and CD150⁻ LSK cells from E14.5 and E18.5 FLs. Cells were isolated after 90 min in vivo BrdU-labeling, stained with an anti-BrdU antibody, and analyzed by fluorescent microscopy. Frequencies of BrdU-positive cells were quantified (data ± SD). (B) Quantification of cleaved-caspase-3-positive cells in *hemp*^{+/+} (open bar) and *hemp*^{-/-} (closed bar) CD150⁺ and CD150⁻ LSK cells from E14.5 and E18.5 FLs. Cells were stained with an anti-cleaved-caspase-3 antibody, analyzed by fluorescent microscopy, and the frequencies of cleaved caspase-3-positive cells were quantified (data ± SD).

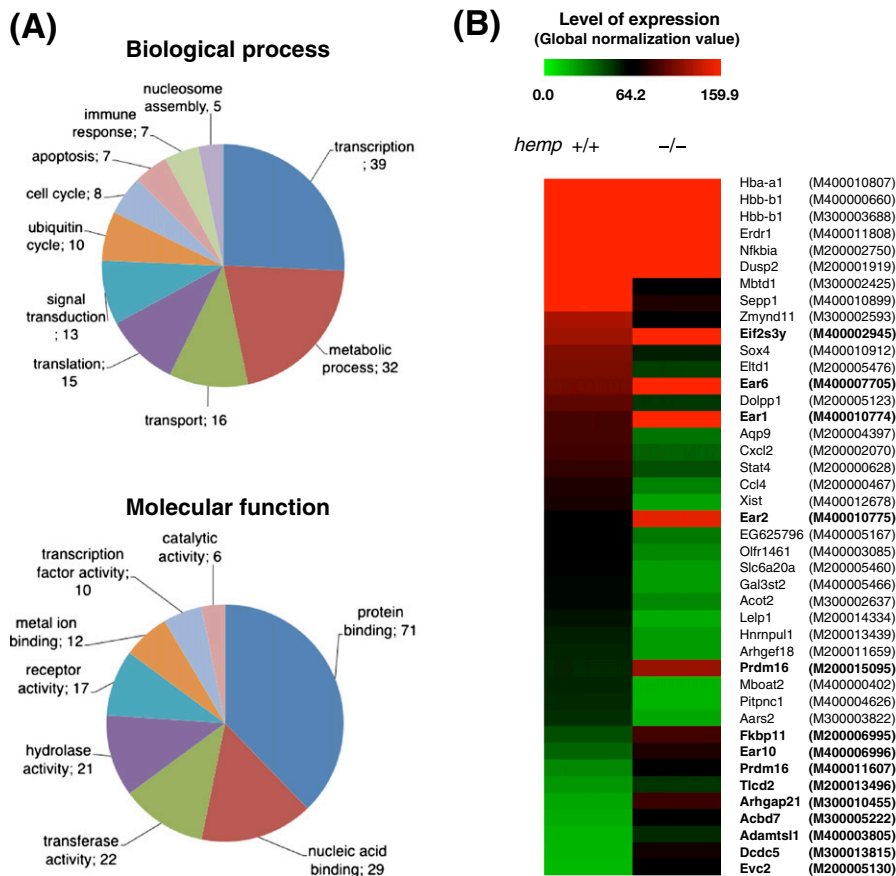


Fig. 54. Analysis of gene expression profiles in *lin*^{-/-}*kit*^{high} cells of E12.5 in *hemp*^{+/+} and *hemp*^{-/-} FLs. (A) The genes whose expression levels were more than 50 in *hemp*^{+/+} or *hemp*^{-/-} arrays and were altered more than 1.5-fold, and were classified into “Biological process” and “Molecular function” categories, according to the Gene Ontology terms. The classifications and gene numbers are shown. (B) Clustering of genes whose expression levels were more than 50 in *hemp*^{+/+} or *hemp*^{-/-} arrays and were altered more than 2.0-fold. Gene names are shown in the right with the probe IDs, and those with more than twofold increase in the expression in *hemp*^{-/-} FL are shown in boldface.

Table S2. Genes with expression levels showing greater than 1.5-fold up-regulation in *hemp*^{-/-} arrays compared with *hemp*^{+/+} arrays

Gene Symbol	Description	Global normalization ratio(Cy3/Cy5)	Global normalization Cy5_ Hemp WT	Global normalization Cy3_ Hemp KO	Probe ID	Entrez Gene ID
<i>Dcdc5</i>	Doublecortin domain containing 5	4.4	16.8	74	M300013815	329482
<i>Arhgap21</i>	ρ GTPase activating protein 21	4.1	21.4	87.5	M300010455	71435
<i>Evc2</i>	Ellis van Creveld syndrome 2 homolog (human)	3.9	16.3	63.7	M200005130	68525
<i>Acbd7</i>	Acyl-CoA binding domain containing 7	3.3	19.3	64.4	M300005222	78245
<i>Adamts1</i>	ADAMTS-like 1	2.9	17.6	51.6	M400003805	77739
<i>Eif2s3y</i>	Eukaryotic translation initiation factor 2, subunit 3, structural gene Y-linked	2.3	123.3	280.4	M400002945	26908
<i>Prdm16</i>	PR domain containing 16	2.3	29	65.4	M400011607	70673
<i>Prdm16</i>	PR domain containing 16	2.3	52.7	120.6	M200015095	70673
<i>Ear1</i>	Eosinophil-associated, ribonuclease A family, member 1	2.2	93.3	201.9	M400010774	13586
<i>Ear2</i>	Eosinophil-associated, ribonuclease A family, member 2	2.2	67.1	150.6	M400010775	13587
<i>Fkbp11</i>	FK506 binding protein 11	2.1	43.1	90.9	M200006995	66120
<i>Ear10</i>	Eosinophil-associated, ribonuclease A family, member 10	2.1	37.3	77.4	M400006996	93725
<i>Ear6</i>	Eosinophil-associated, ribonuclease A family, member 6	2	107.4	217.8	M400007705	93719
<i>Tlcd2</i>	TLC domain containing 2	2	24.8	50.1	M200013496	380712
<i>Top1mt</i>	DNA topoisomerase 1, mitochondrial	1.9	156.4	294.9	M200012085	72960
<i>Caskin2</i>	Cask-interacting protein 2	1.9	26.8	50.8	M200013352	140721
<i>4932438A13Rik</i>	RIKEN cDNA 4932438A13 gene	1.9	39.7	76.6	M300010838	229227
<i>D10627</i>	cDNA sequence D10627	1.9	36.9	70.6	M400007003	234358
<i>Hist1h2bg</i>	Histone cluster 1, H2bg	1.9	518.7	962.8	M300001635	319181
<i>EG626903</i>	Predicted gene, <i>EG626903</i>	1.9	520.5	1001.5	M400006972	626903
<i>Cd59a</i>	CD59a antigen	1.8	47.8	84.4	M200002712	12509
<i>Pcbd1</i>	Pterin 4 α carbinolamine dehydratase/dimerization cofactor of hepatocyte nuclear factor 1 α (TCF1) 1	1.8	64.1	113.7	M200008194	13180
<i>Eif3e</i>	Eukaryotic translation initiation factor 3, subunit E	1.8	1973.8	3457.1	M300003218	16341
<i>Mgmt</i>	O-6-methylguanine-DNA methyltransferase	1.8	145.9	267.1	M400010845	17314
<i>Olf47</i>	Olfactory receptor 47	1.8	36.1	64.7	M400007344	18346
<i>Map4k1</i>	Mitogen-activated protein kinase kinase kinase 1	1.8	408.9	737.9	M300010878	26411
<i>Gcat</i>	Glycine C-acetyltransferase (2-amino-3-ketobutyrate-CoA ligase)	1.8	136.7	241.6	M200003649	26912
<i>Snhg8</i>	Small nucleolar RNA host gene (nonprotein coding) 8	1.8	545.7	1006.8	M300017518	66487
<i>1190017012Rik</i>	RIKEN cDNA 1190017012 gene	1.8	375.9	687.9	M400003958	68936
<i>Zfp566</i>	Zinc finger protein 566	1.8	51.7	90.6	M400012176	72556
<i>Ng23</i>	Ng23 protein	1.8	81.3	150.4	M200013291	78376
<i>Hsd3b7</i>	Hydroxy- δ -5-steroid dehydrogenase, 3 β - and steroid δ -isomerase 7	1.8	65.5	120.8	M200009913	101502
<i>Rdh9</i>	Retinol dehydrogenase 9	1.8	38.8	68.2	M400003618	103142
<i>Gipr</i>	Gastric inhibitory polypeptide receptor	1.8	42	76.5	M400001263	381853
<i>LOC546711</i>	Similar to 40S ribosomal protein S25	1.8	869	1532.3	M400010160	546711
<i>Hesx1</i>	Homeo box gene expressed in ES cells	1.7	251.9	427.9	M200001963	15209
<i>Khk</i>	Ketohexokinase	1.7	889	1512.6	M300006522	16548
<i>Prg2</i>	Proteoglycan 2, bone marrow	1.7	385	655.8	M200009412	19074
<i>Tcte3</i>	t-Complex-associated testis expressed 3	1.7	92.2	152.3	M400001917	21647
<i>B9d1</i>	B9 protein domain 1	1.7	31.9	54.7	M200007973	27078
<i>2810405K02Rik</i>	RIKEN cDNA 2810405K02 gene	1.7	37.7	62.4	M300006458	66469
<i>Esf1</i>	ESF1, nucleolar prerRNA processing protein, homolog (<i>Saccharomyces cerevisiae</i>)	1.7	268.9	457.4	M300011875	66580
<i>Cgrrf1</i>	Cell growth regulator with ring finger domain 1	1.7	73.5	122.8	M400005060	68755
<i>1700029101Rik</i>	RIKEN cDNA 1700029101 gene	1.7	498.2	824.3	M400009318	70005
<i>Bbs7</i>	Bardet-Biedl syndrome 7	1.7	32.6	56.5	M300010870	71492
<i>Mterfd3</i>	MTERF domain containing 3	1.7	65.7	113.2	M300019805	74238
<i>Scpep1</i>	Serine carboxypeptidase 1	1.7	71.3	119.9	M300000050	74617

Table S2. Cont.

Gene Symbol	Description	Global normalization ratio(Cy3/Cy5)	Global normalization Cy5_ Hemp WT	Global normalization Cy3_ Hemp KO	Probe ID	Entrez Gene ID
<i>Rfp14</i>	Ret finger protein-like 4	1.7	122.8	204.6	M200006154	192658
<i>Socs2</i>	Suppressor of cytokine signaling 2	1.7	41.7	71	M200001605	216233
<i>Zfp503</i>	Zinc finger protein 503	1.7	29.2	50	M200004050	218820
<i>Shprh</i>	SNF2 histone linker PHD RING helicase	1.7	37.5	61.9	M200004083	268281
<i>EG435392</i>	Predicted gene, <i>EG435392</i>	1.7	71.8	124.4	M400001592	435392
<i>Scye1</i>	Small inducible cytokine subfamily E, member 1	1.6	1311.1	2098.3	M300005908	13722
<i>Igtp</i>	IFN- γ induced GTPase	1.6	38.7	63.4	M400011289	16145
<i>Phyh</i>	Phytanoyl-CoA hydroxylase	1.6	37	60.8	M200005407	16922
<i>Phb</i>	Prohibitin	1.6	1573.6	2532.7	M300011808	18673
<i>Pik3ca</i>	Phosphatidylinositol 3-kinase, catalytic, α polypeptide	1.6	32	52.6	M200013803	18706
<i>Tfrc</i>	Transferrin receptor	1.6	971.2	1556.3	M200006124	22042
<i>Ctr9</i>	Ctr9, Paf1/RNA polymerase II complex component, homolog (<i>S. cerevisiae</i>)	1.6	45.6	75.1	M200000180	22083
<i>Chek2</i>	CHK2 checkpoint homolog (<i>Schizosaccharomyces pombe</i>)	1.6	169.3	277.2	M200013896	50883
<i>Zfp644</i>	Zinc finger protein 644	1.6	68.3	111.9	M200004912	52397
<i>Nola2</i>	Nucleolar protein family A, member 2	1.6	190.5	298.8	M200005882	52530
<i>Ireb2</i>	Iron responsive element binding protein 2	1.6	70.6	110.9	M300008167	64602
<i>Lsm7</i>	LSM7 homolog, U6 small nuclear RNA associated (<i>S. cerevisiae</i>)	1.6	688.2	1121	M400011474	66094
<i>Ccdc72</i>	Coiled-coil domain containing 72	1.6	1629.5	2565.8	M400006621	66167
<i>1110036O03Rik</i>	RIKEN cDNA 1110036O03 gene	1.6	32	50.2	M300000865	66180
<i>1810014B01Rik</i>	RIKEN cDNA 1810014B01 gene	1.6	827.4	1291.9	M400008225	66263
<i>Chchd7</i>	Coiled-coil-helix-coiled-coil-helix domain containing 7	1.6	1155.3	1882.4	M300013556	66433
<i>Nuf2</i>	NUF2, NDC80 kinetochore complex component, homolog (<i>S. cerevisiae</i>)	1.6	442.9	730.2	M300005239	66977
<i>Nkap</i>	NF κ B activating protein	1.6	35.6	57.8	M200013737	67050
<i>Rpl14</i>	Ribosomal protein L14	1.6	793.9	1262.3	M200014008	67115
<i>Hist1h2bc</i>	Histone cluster 1, H2bc	1.6	434.4	679.2	M400011439	68024
<i>Rpl22l1</i>	Ribosomal protein L22 like 1	1.6	3538.1	5523.3	M300012040	68028
<i>6720467C03Rik</i>	RIKEN cDNA 6720467C03 gene	1.6	238.6	376.1	M200005112	68099
<i>Rpl39l</i>	Ribosomal protein L39-like	1.6	56.5	92	M200004670	68172
<i>Apoo</i>	Apolipoprotein O	1.6	344	563.3	M300019988	68316
<i>Mitd1</i>	MIT, microtubule interacting and transport, domain containing 1	1.6	192.9	308.2	M300004929	69028
<i>Mllt3</i>	Myeloid/lymphoid or mixed-lineage leukemia (trithorax homolog, <i>Drosophila</i>)	1.6	35.3	56.2	M300006125	70122
<i>5730469M10Rik</i>	RIKEN cDNA 5730469M10 gene	1.6	200.7	325	M200005469	70564
<i>Acn9</i>	ACN9 homolog (<i>S. cerevisiae</i>)	1.6	134.6	212.6	M300013732	71238
<i>Hspb11</i>	Heat-shock protein family B (small), member 11	1.6	84.5	133.7	M200004218	72938
<i>2410076I21Rik</i>	RIKEN cDNA 2410076I21 gene	1.6	38.3	61.6	M200015162	73673
<i>Josd3</i>	Josephin domain containing 3	1.6	715.5	1129.8	M300007986	75316
<i>Fdps</i>	Farnesyl diphosphate synthetase	1.6	588.2	931.7	M400011865	110196
<i>Zfp295</i>	Zinc finger protein 295	1.6	48.5	75.8	M400013580	114565
<i>AI987944</i>	Expressed sequence AI987944	1.6	30.4	50.1	M400005490	233168
<i>Sept7</i>	Septin 7	1.6	228	360.6	M300000235	235072
<i>EG236844</i>	Predicted gene, <i>EG236844</i>	1.6	3123.1	4841.1	M400003082	236844
<i>EG270499</i>	Predicted gene, <i>EG270499</i>	1.6	116.4	190	M400008464	270499
<i>Rpl17</i>	Ribosomal protein L17	1.6	481.1	771.9	M300004829	319195
<i>Hcfc1r1</i>	Host cell factor C1 regulator 1 (XPO1-dependent)	1.6	184.5	293.1	M300003807	353502
<i>EG383613</i>	Predicted gene, <i>EG383613</i>	1.6	1106.7	1791.1	M400006417	383613
<i>EG383815</i>	Predicted gene, <i>EG383815</i>	1.6	57.9	91.7	M400005919	383815
<i>EG432950</i>	Predicted gene, <i>EG432950</i>	1.6	2330.7	3623.8	M400006352	432950

Table S2. Cont.

Gene Symbol	Description	Global normalization ratio(Cy3/Cy5)	Global normalization Cy5_ Hemp WT	Global normalization Cy3_ Hemp KO	Probe ID	Entrez Gene ID
<i>EG623924</i>	Predicted gene, <i>EG623924</i>	1.6	2439.2	3863.3	M400006401	623924
<i>Hmgb11</i>	High mobility group box 1-like	1.6	192.8	307.6	M400007851	625781
<i>LOC676847</i>	Similar to ribosomal protein S15a	1.6	1601	2565.1	M400005666	676847
<i>Cenpc1</i>	Centromere protein C1	1.5	84.8	130.6	M400001150	12617
<i>Cstb</i>	Cystatin B	1.5	398.7	598.8	M200002288	13014
<i>Sdpr</i>	Serum deprivation response	1.5	39.2	59.2	M300016875	20324
<i>Trip6</i>	Thyroid hormone receptor interactor 6	1.5	182.4	277.5	M400000672	22051
<i>Dkk11</i>	Dickkopf-like 1	1.5	41.4	63.6	M200005492	50722
<i>Nckap1</i>	NCK-associated protein 1	1.5	57.5	87.3	M300005431	50884
<i>Ccnc</i>	Cyclin C	1.5	69.8	104.9	M200001678	51813
<i>C1d</i>	Nuclear DNA binding protein	1.5	90.4	138.9	M200000938	57316
<i>Dnajc15</i>	DnaJ (Hsp40) homolog, subfamily C, member 15	1.5	567.2	873.9	M200005658	66148
<i>Esf1</i>	ESF1, nucleolar prerRNA processing protein, homolog (<i>S. cerevisiae</i>)	1.5	161.9	249.2	M400015785	66580
<i>Asb17</i>	Ankyrin repeat and SOCS box-containing 17	1.5	104.3	160.3	M200008852	66772
<i>Ptgr1</i>	Prostaglandin reductase 1	1.5	690.9	1051	M200007590	67103
<i>Ccdc77</i>	Coiled-coil domain containing 77	1.5	74.4	111.9	M200014317	67200
<i>Ndufb4</i>	NADH dehydrogenase (ubiquinone) 1 β subcomplex 4	1.5	2138.7	3214.6	M400008997	68194
<i>Mrpl13</i>	Mitochondrial ribosomal protein L13	1.5	957	1459.8	M200002853	68537
<i>2310001H12Rik</i>	RIKEN cDNA 2310001H12 gene	1.5	467.5	703.2	M400009683	69504
<i>Bdh1</i>	3-hydroxybutyrate dehydrogenase, type 1	1.5	109.9	169.3	M200006708	71911
<i>Mettl10</i>	Methyltransferase like 10	1.5	453.8	699.6	M200006100	72096
<i>Kif2c</i>	Kinesin family member 2C	1.5	176.1	270.4	M300006226	73804
<i>2700078E11Rik</i>	RIKEN cDNA 2700078E11 gene	1.5	52.3	78.5	M200007079	78832
<i>Pecr</i>	Peroxisomal trans-2-enoyl-CoA reductase	1.5	246.6	376.7	M300004977	111175
<i>Ccdc52</i>	Coiled-coil domain containing 52	1.5	43.7	67.3	M300014152	212514
<i>BC002059</i>	cDNA sequence BC002059	1.5	695.7	1056.2	M400007106	213811
<i>Pstk</i>	Phosphoseryl-tRNA kinase	1.5	116.1	174.3	M300016933	214580
<i>Rfesd</i>	Rieske (Fe-S) domain containing	1.5	1087.8	1643.2	M300014263	218341
<i>Nup214</i>	Nucleoporin 214	1.5	163.7	251.2	M300000236	227720
<i>Tmem159</i>	Transmembrane protein 159	1.5	42.2	64.7	M300007492	233806
<i>Zfp512</i>	Zinc finger protein 512	1.5	43.7	65.6	M200007002	269639
<i>2610524H06Rik</i>	RIKEN cDNA 2610524H06 gene	1.5	199.4	304.7	M300018590	330173
<i>Wdr51b</i>	WD repeat domain 51B	1.5	32.9	51	M200014833	382406
<i>EG383538</i>	Predicted gene, <i>EG383538</i>	1.5	182.5	277.1	M400006855	383538

Table S3. Genes with expression levels showing greater than 1.5-fold down-regulation in *hemp*^{-/-} arrays compared with *hemp*^{+/+} arrays

Gene symbol	Description	Global normalization ratio(Cy3/Cy5)	Global normalization Cy5_ Hemp WT	Global normalization Cy3_ Hemp KO	Probe ID	Entrez Gene ID
<i>Mbtd1</i>	Mbt domain containing 1	0.3	268.8	67.7	M300002425	103537
<i>Mboat2</i>	Membrane bound O-acyltransferase domain containing 2	0.3	52.6	18	M400000402	67216
<i>Lelp1</i>	Ate cornified envelope-like proline-rich 1	0.3	57.3	19.2	M200014334	69332
<i>Pitpnc1</i>	Phosphatidylinositol transfer protein, cytoplasmic 1	0.3	52.3	17.5	M400004626	71795
<i>Xist</i>	Inactive X specific transcripts	0.3	76.8	22	M400012678	213742
<i>Dusp2</i>	Dual specificity phosphatase 2	0.4	485.5	214.6	M200001919	13537
<i>Hba-a1</i>	Hemoglobin α , adult chain 1	0.4	40556	14779.3	M400010807	15122
<i>Ccl4</i>	Chemokine (C-C motif) ligand 4	0.4	78.8	30.7	M200000467	20303
<i>Cxcl2</i>	Chemokine (C-X-C motif) ligand 2	0.4	88.5	38.4	M200002070	20310
<i>Aqp9</i>	Aquaporin 9	0.4	92.7	34.4	M200004397	64008
<i>Arhgef18</i>	Rho/rac guanine nucleotide exchange factor (GEF) 18	0.4	53.8	23.9	M200011659	102098
<i>Slc6a20a</i>	Solute carrier family 6 (neurotransmitter transporter), member 20A	0.4	61.3	23.5	M200005460	102680
<i>Eltf1</i>	EGF, latrophilin seven transmembrane domain containing 1	0.4	110	47.3	M200005476	170757
<i>Erdr1</i>	Erythroid differentiation regulator 1	0.4	5458.1	2350	M400011808	170942
<i>Aars2</i>	Alanyl-tRNA synthetase 2, mitochondrial (putative)	0.4	50.4	20.6	M300003822	224805
<i>Hnrnpul1</i>	Heterogeneous nuclear ribonucleoprotein U-like 1	0.4	55.1	23.3	M200013439	232989
<i>Olf1r1461</i>	Olfactory receptor 1461	0.4	64	28.7	M400003085	258299
<i>Gal3st2</i>	Galactose-3-O-sulfotransferase 2	0.4	59.2	23.3	M400005466	381334
<i>Cd69</i>	CD69 antigen	0.5	122.3	67.2	M200015388	12515
<i>Hbb-b1</i>	Hemoglobin, β adult major chain	0.5	17427	7892.9	M400000660	15129
<i>Hbb-b1</i>	Hemoglobin, β adult major chain	0.5	13575.8	7111	M300003688	15129
<i>Nr4a1</i>	Nuclear receptor subfamily 4, group A, member 1	0.5	196.1	101	M200000038	15370
<i>Junb</i>	Jun-B oncogene	0.5	121	63.1	M400004256	16477
<i>Lmnb2</i>	Lamin B2	0.5	54.3	27.4	M400007668	16907
<i>Nfkbia</i>	Nuclear factor of κ light polypeptide gene enhancer in B-cells inhibitor, α	0.5	642.4	309.5	M200002750	18035
<i>Ripk1</i>	Receptor (TNFRSF)-interacting serine-threonine kinase 1	0.5	50.2	25.9	M200001212	19766
<i>Sepp1</i>	Selenoprotein P, plasma, 1	0.5	159.9	79.7	M400010899	20363
<i>Siah2</i>	Seven in absentia 2	0.5	72.6	36.9	M400001030	20439
<i>Sox4</i>	SRY-box containing gene 4	0.5	112.6	53.9	M400010912	20677
<i>Stat4</i>	Signal transducer and activator of transcription 4	0.5	86	42.6	M200000628	20849
<i>Surf4</i>	Surfeit gene 4	0.5	72.8	38.3	M200001059	20932
<i>Acot1</i>	Acyl-CoA thioesterase 1	0.5	90.2	49.4	M300013759	26897
<i>Rgs1</i>	Regulator of G protein signaling 1	0.5	290.7	152.9	M200008591	50778
<i>Dolpp1</i>	Dolichyl pyrophosphate phosphatase 1	0.5	100.7	48.6	M200005123	57170
<i>Sertad2</i>	SERTA domain containing 2	0.5	56.9	31.1	M200008525	58172
<i>Zmynd11</i>	Zinc finger, MYND domain containing 11	0.5	128.5	62.8	M300002593	66505
<i>Ppp1r14a</i>	Protein phosphatase 1, regulatory (inhibitor) subunit 14A	0.5	53	27.6	M400011578	68458
<i>4931431F19Rik</i>	RIKEN cDNA 4931431F19 gene	0.5	79	40.7	M300022239	70980
<i>Prr3</i>	Proline-rich polypeptide 3	0.5	51	27.6	M300011587	75210

Table S3. Cont.

Gene symbol	Description	Global normalization ratio(Cy3/Cy5)	Global normalization Cy5_ Hemp WT	Global normalization Cy3_ Hemp KO	Probe ID	Entrez Gene ID
<i>Acot2</i>	Acyl-CoA thioesterase 2	0.5	58.5	28.7	M300002637	171210
<i>Senp1</i>	SUMO1/sentrin specific peptidase 1	0.5	50.9	26.5	M200009018	223870
<i>Muc20</i>	Mucin 20	0.5	50.1	26.2	M300010024	224116
<i>D330017J20Rik</i>	RIKEN cDNA D330017J20 gene	0.5	67.7	36.9	M400015990	320609
<i>OTTMUSG00000016703</i>	Predicted gene, <i>OTTMUSG00000016703</i>	0.5	50.9	26.9	M400004839	329436
<i>EG625796</i>	Predicted gene, <i>EG625796</i>	0.5	65.4	32.8	M400005167	625796
<i>Angpt1</i>	Angiopoietin 1	0.6	82.7	48.4	M300003197	11600
<i>Arx</i>	Aristaless related homeobox gene (<i>Drosophila</i>)	0.6	52.2	32.2	M200014722	11878
<i>Btg2</i>	B-cell translocation gene 2, antiproliferative	0.6	62.3	34.8	M200000321	12227
<i>Cd48</i>	CD48 antigen	0.6	368.5	236.9	M400000228	12506
<i>CtsG</i>	Cathepsin G	0.6	117.4	71.7	M200001999	13035
<i>Phc1</i>	Polyhomeotic-like 1 (<i>Drosophila</i>)	0.6	86.6	50	M200002435	13619
<i>Eef1a1</i>	Eukaryotic translation elongation factor 1 α 1	0.6	57659	34308.2	M400002052	13627
<i>Egr1</i>	Early growth response 1	0.6	152.1	91.8	M200012044	13653
<i>Eln</i>	Elastin	0.6	63.9	41	M400001192	13717
<i>Ext1</i>	Exostosin (multiple) 1	0.6	76.7	44.7	M300011671	14042
<i>Fjx1</i>	Four jointed box 1 (<i>Drosophila</i>)	0.6	61.7	39.7	M400011012	14221
<i>Srgap2</i>	SLIT-ROBO Rho GTPase activating protein 2	0.6	54.1	31.8	M300005098	14270
<i>Ggpi1</i>	Geranylgeranyl diphosphate synthase 1	0.6	93.5	59.7	M300002685	14593
<i>Gypa</i>	Glycophorin A	0.6	608.5	364.9	M300007861	14934
<i>Hbb-b2</i>	Hemoglobin, β adult minor chain	0.6	2157.4	1247.7	M400011276	15130
<i>Hbb-y</i>	Hemoglobin β , β -like embryonic chain	0.6	96	57.7	M300003689	15135
<i>Hdac6</i>	Histone deacetylase 6	0.6	62	37.6	M200006690	15185
<i>Ier3</i>	Immediate early response 3	0.6	511.4	307.9	M200005156	15937
<i>Itpr3</i>	Inositol 1, 4, 5-triphosphate receptor 3	0.6	54.2	30.9	M200005377	16440
<i>PsmB8</i>	Proteasome (prosome, macropain) subunit, β type 8 (large multifunctional peptidase 7)	0.6	426.5	238.6	M200003254	16913
<i>Lyn</i>	Yamaguchi sarcoma viral (v-yes-1) oncogene homolog	0.6	56.8	34.8	M400002593	17096
<i>Lyz2</i>	Lysozyme 2	0.6	191.9	118.5	M400005459	17105
<i>Lyz1</i>	Lysozyme 1	0.6	148	86.9	M400000367	17110
<i>Marcks1</i>	MARCKS-like 1	0.6	467.8	269.9	M300018771	17357
<i>Myd116</i>	Myeloid differentiation primary response gene 116	0.6	76.8	43.6	M200001567	17872
<i>Pnlipr2</i>	Pancreatic lipase-related protein 2	0.6	71.4	45.2	M400000796	18947
<i>Prodh</i>	Proline dehydrogenase	0.6	67.9	42.8	M400011110	19125
<i>Raly</i>	hnRNP-associated with lethal yellow	0.6	408.2	252.8	M400011426	19383
<i>Rbp1</i>	Retinol binding protein 1, cellular	0.6	194.5	125.9	M300017309	19659
<i>Sub1</i>	SUB1 homolog (<i>S. cerevisiae</i>)	0.6	56.1	34.3	M300003141	20024
<i>Sema4a</i>	Sema domain, Ig domain (Ig), transmembrane domain (TM) and short cytoplasmic domain, (semaphorin) 4A	0.6	81.9	50.7	M200004279	20351
<i>Serf1</i>	Small EDRK-rich factor 1	0.6	216.7	124.8	M200000687	20365
<i>Spin1</i>	Spindlin 1	0.6	57.7	35.4	M200013917	20729
<i>Spnb1</i>	Spectrin β 1	0.6	94.3	58.7	M300002533	20741
<i>Stat3</i>	Signal transducer and activator of transcription 3	0.6	100	62.5	M200012506	20848
<i>Cmpk2</i>	Cytidine monophosphate (UMP-CMP) kinase 2, mitochondrial	0.6	60.2	37	M300002295	22169
<i>Zyx</i>	Zyxin	0.6	193	108.8	M200002019	22793

Table S3. Cont.

Gene symbol	Description	Global normalization ratio(Cy3/Cy5)	Global normalization Cy5_ Hemp WT	Global normalization Cy3_ Hemp KO	Probe ID	Entrez Gene ID
<i>Klf6</i>	Kruppel-like factor 6	0.6	176.2	105.7	M200012730	23849
<i>Iqgap1</i>	IQ motif containing GTPase activating protein 1	0.6	113.8	72.4	M200014838	29875
<i>Fbxl6</i>	F-box and leucine-rich repeat protein 6	0.6	232.3	136.3	M200005638	30840
<i>Park2</i>	Parkin	0.6	73.9	43.4	M200013858	50873
<i>Mcrs1</i>	Microspherule protein 1	0.6	210.8	134.7	M400003319	51812
<i>Zfp622</i>	Zinc finger protein 622	0.6	94.5	57.1	M400004051	52521
<i>D12Ert647e</i>	DNA segment, Chr 12, ERATO Doi 647, expressed	0.6	55.9	36.1	M400008599	52668
<i>Arhgef5</i>	ρ Guanine nucleotide exchange factor (GEF) 5	0.6	66.8	42.4	M400002303	54324
<i>Tmem115</i>	Transmembrane protein 115	0.6	151	97.1	M200005500	56395
<i>Nudt3</i>	Nudix (nucleotide diphosphate linked moiety X)-type motif 3	0.6	95.2	61.3	M200016350	56409
<i>Dnajb12</i>	DnaJ (Hsp40) homolog, subfamily B, member 12	0.6	51.7	33.6	M200008554	56709
<i>Pdpx</i>	Pyridoxal (pyridoxine, vitamin B6) phosphatase	0.6	112.6	64.3	M400011359	57028
<i>Terf2ip</i>	Telomeric repeat binding factor 2, interacting protein	0.6	115.4	73.2	M200013251	57321
<i>Lrp10</i>	Low-density lipoprotein receptor-related protein 10	0.6	50.2	32.2	M200006018	65107
<i>Twsg1</i>	Twisted gastrulation homolog 1 (<i>Drosophila</i>)	0.6	79.8	51.3	M200002883	65960
<i>Ogfod2</i>	2-oxoglutarate and iron-dependent oxygenase domain containing 2	0.6	54.6	34.8	M400011506	66627
<i>5730470L24Rik</i>	RIKEN cDNA 5730470L24 gene	0.6	59.1	36.5	M200008245	66641
<i>Agtppb1</i>	ATP/GTP binding protein 1	0.6	68.3	42.2	M200014357	67269
<i>Cep70</i>	Centrosomal protein 70	0.6	50.5	29.8	M200003700	68121
<i>1110018G07Rik</i>	RIKEN cDNA 1110018G07 gene	0.6	58.9	35.5	M200004898	68497
<i>Ctdspl</i>	CTD (carboxyl-terminal domain, RNA polymerase II, polypeptide A) small phosphatase-like	0.6	73.5	47.5	M200006149	69274
<i>Rnf113a1</i>	Ring finger protein 113A1	0.6	78.2	46.2	M300010421	69942
<i>Tspan15</i>	Tetraspanin 15	0.6	51.5	31.7	M400012571	70423
<i>Slc25a33</i>	Solute carrier family 25, member 33	0.6	145	86.1	M300006413	70556
<i>Acy3</i>	Aspartoacylase (aminoacylase) 3	0.6	65.7	38.6	M200006624	71670
<i>Ppp2r2a</i>	Protein phosphatase 2 (formerly 2A), regulatory subunit B (PR 52), α isoform	0.6	173.2	106.2	M400000535	71978
<i>Ssbp3</i>	Single-stranded DNA binding protein 3	0.6	73.8	47.2	M300006197	72475
<i>MacroD2</i>	MACRO domain containing 2	0.6	77.6	47.9	M400001005	72899
<i>Psd</i>	Pleckstrin and Sec7 domain containing	0.6	55.5	31.2	M300013961	73728
<i>1200011M11Rik</i>	RIKEN cDNA 1200011M11 gene	0.6	69.9	44.4	M200004598	74133
<i>2310066E14Rik</i>	RIKEN cDNA 2310066E14 gene	0.6	76.9	46.6	M200013530	75687
<i>4930569F06Rik</i>	RIKEN cDNA 4930569F06 gene	0.6	50.1	32.3	M200011419	78055
<i>5730596K20Rik</i>	RIKEN cDNA 5730596K20 gene	0.6	57.9	35.6	M200007535	109168
<i>Phka2</i>	Phosphorylase kinase α 2	0.6	112	68.3	M300007653	110094
<i>Ms4a3</i>	Membrane-spanning 4-domains, subfamily A, member 3	0.6	70	45.3	M200008917	170813
<i>Fchsd2</i>	FCH and double SH3 domains 2	0.6	70.3	41.9	M300007360	207278
<i>Mgst2</i>	Microsomal GST 2	0.6	69.8	40.2	M300019764	211666
<i>Frat2</i>	Frequently rearranged in advanced T-cell lymphomas 2	0.6	88.1	55.6	M400003320	212398

Table S3. Cont.

Gene symbol	Description	Global normalization ratio(Cy3/Cy5)	Global normalization Cy5_ Hemp WT	Global normalization Cy3_ Hemp KO	Probe ID	Entrez Gene ID
<i>Tnrc6b</i>	Trinucleotide repeat containing 6b	0.6	56.4	35.3	M200004519	213988
<i>Sidt2</i>	SID1 transmembrane family, member 2	0.6	77.3	48.2	M200005570	214597
<i>Wipf1</i>	WAS/WASL interacting protein family, member 1	0.6	55.3	30.5	M300013345	215280
<i>BC005764</i>	cDNA sequence BC005764	0.6	68.7	42.3	M300010107	216152
<i>Ckap4</i>	Cytoskeleton-associated protein 4	0.6	240.3	133.6	M300017714	216197
<i>Mlh3</i>	mutL homolog 3 (<i>Escherichia coli</i>)	0.6	214	135.8	M200008501	217716
<i>Ankrd54</i>	Ankyrin repeat domain 54	0.6	62.7	39.6	M200004849	223690
<i>Pim3</i>	Proviral integration site 3	0.6	80.7	46.2	M200006785	223775
<i>Zdhhc14</i>	Zinc finger, DHHC domain containing 14	0.6	83.5	46	M200004358	224454
<i>Ppig</i>	Peptidyl-prolyl isomerase G (cyclophilin G)	0.6	110	64.8	M400002584	228005
<i>Prkcbp1</i>	Protein kinase C binding protein 1	0.6	51.1	32.7	M200011615	228880
<i>Tmem41b</i>	Transmembrane protein 41B	0.6	94.4	58.4	M200013985	233724
<i>Zbtb44</i>	Zinc finger and BTB domain containing 44	0.6	90.5	58.1	M400001969	235132
<i>Usp3</i>	Ubiquitin specific peptidase 3	0.6	80.5	47.9	M200008148	235441
<i>Tsp50</i>	Testes-specific protease 50	0.6	201.2	126.8	M300019526	235631
<i>Chsy1</i>	Chondroitin sulfate synthase 1	0.6	51	33	M300008360	269941
<i>Taf1</i>	TAF1 RNA polymerase II, TATA box binding protein (TBP)-associated factor	0.6	56.5	36.7	M300007669	270627
<i>D11Wsu47e</i>	DNA segment, Chr 11, Wayne State University 47, expressed	0.6	59.5	35.1	M200007483	276852
<i>Hist1h2ao</i>	Histone cluster 1, H2ao	0.6	52676	33715.3	M400007165	319171
<i>Hist1h2ab</i>	Histone cluster 1, H2ab	0.6	42329	25035.8	M400012322	319172
<i>Hist2h2ac</i>	Histone cluster 2, H2ac	0.6	41327	23798.6	M400012323	319176
<i>Jph4</i>	Junctophilin 4	0.6	57.4	37.3	M300003142	319984
<i>Heatr2</i>	HEAT repeat containing 2	0.6	73.7	46.8	M300004809	433956
<i>EG627648</i>	Predicted gene, EG627648	0.6	532.8	318.5	M300000928	627648
<i>Ccng2</i>	Cyclin G2	0.7	57.7	37.5	M300006655	12452
<i>Spsb2</i>	SplA/ryanodine receptor domain and SOCS box containing 2	0.7	79.9	53.2	M200000999	14794
<i>H2-T3</i>	Histocompatibility 2, T region locus 3	0.7	69.7	45.6	M400004713	15043
<i>Htt</i>	Huntingtin	0.7	59.2	39.4	M300006492	15194
<i>Ifi203</i>	IFN-activated gene 203	0.7	62.6	41.5	M300005163	15950
<i>Kcnk5</i>	Potassium channel, subfamily K, member 5	0.7	69.6	45.7	M200015255	16529
<i>Cog1</i>	component of oligomeric golgi complex 1	0.7	79.2	52.4	M200012092	16834
<i>Ltb</i>	Lymphotoxin B	0.7	63.2	42.1	M200000678	16994
<i>Cd93</i>	CD93 antigen	0.7	93.3	61	M200000240	17064
<i>Rai1</i>	Retinoic acid induced 1	0.7	52.4	34.7	M200001924	19377
<i>Ren2</i>	Renin 2 tandem duplication of Ren1	0.7	63.1	42	M400011721	19702
<i>Sla</i>	Src-like adaptor	0.7	82	53.9	M200002576	20491
<i>Spint2</i>	Serine protease inhibitor, Kunitz type 2	0.7	235.6	157	M300007301	20733
<i>Cd27</i>	CD27 antigen	0.7	59.1	38.5	M400001252	21940
<i>Arih1</i>	Ariadne ubiquitin-conjugating enzyme E2 binding protein homolog 1 (<i>Drosophila</i>)	0.7	73.2	48.2	M200012926	23806

Table S3. Cont.

Gene symbol	Description	Global normalization ratio(Cy3/Cy5)	Global normalization Cy5_ Hemp WT	Global normalization Cy3_ Hemp KO	Probe ID	Entrez Gene ID
<i>Sgta</i>	Small glutamine-rich tetratricopeptide repeat (TPR)-containing, α	0.7	97.8	64.1	M200006817	52551
<i>Ptges3</i>	Prostaglandin E synthase 3 (cytosolic)	0.7	353.7	235.7	M300012510	56351
<i>Ttyh1</i>	Tweety homolog 1 (<i>Drosophila</i>)	0.7	54.2	35.3	M400010682	57776
<i>Tmem176a</i>	Transmembrane protein 176A	0.7	76.1	50	M200005402	66058
<i>Gmpr</i>	Guanosine monophosphate reductase	0.7	708.2	467.2	M300000048	66355
<i>Crip2</i>	Cysteine rich protein 2	0.7	72.9	48.3	M400000130	68337
<i>Lysmd2</i>	LysM, putative peptidoglycan-binding, domain containing 2	0.7	60.3	39.5	M400001435	70082
<i>Zdhhc2</i>	Zinc finger, DHHC domain containing 2	0.7	50.4	33	M300012190	70546
<i>Synpr</i>	Synaptopodin	0.7	66.7	43.9	M400005462	72003
<i>Jam3</i>	Junction adhesion molecule 3	0.7	54.7	36.1	M400001423	83964
<i>Oosp1</i>	Oocyte secreted protein 1	0.7	64.3	42.3	M200004987	170834
<i>V1rc17</i>	Vomer nasal 1 receptor, C17	0.7	67	44.2	M300019477	171190
<i>Nrbp1</i>	Nuclear receptor binding protein 1	0.7	84	55.7	M200004273	192292
<i>Trib2</i>	Tribbles homolog 2 (<i>Drosophila</i>)	0.7	93.9	62.3	M300002269	217410
<i>Slc39a10</i>	Solute carrier family 39 (zinc transporter), member 10	0.7	72.8	48.2	M300004873	227059
<i>Rtn4rl2</i>	Reticulon 4 receptor-like 2	0.7	173	114.6	M300021558	269295
<i>Tpm4</i>	Tropomyosin 4	0.7	130	85.3	M300007904	326618
<i>Tubgcp6</i>	Tubulin, γ complex associated protein 6	0.7	62.3	41.5	M300010075	328580
<i>Pram1</i>	PML-RAR α -regulated adaptor molecule 1	0.7	56.5	37.5	M300008414	378460
<i>EG544809</i>	Predicted gene, <i>EG544809</i>	0.7	82.1	54.7	M300016054	544809
<i>ENSMUSG00000053270</i>	Predicted gene, <i>ENSMUSG00000053270</i>	0.7	160.9	105.6	M400004407	791285