Supplementary data to:

Alcohol directly stimulates epigenetic modifications in hepatic stellate cells

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Supplementary Materials and methods

Immunohistochemistry

Immunohistochemistry staining was carried out as previously described [1]. Briefly, sections of normal and diseased human liver were dewaxed in Clearene (Leica, UK) and serially dehydrated in alcohol. Endogenous peroxidase activity was blocked by hydrogen peroxide treatment for 15min. Antigen retrieval on sections was performed by microwaving in citric saline for 15min. Non-specific blocking was carried out using avidin/biotin blocking kit; slides were blocked with 20% pig serum and incubated with a 1:100 dilution of elastin (Abcam, catalogue number ab21610) or MLL1 (Bethyl, catalogue number A300-086A) antibody overnight at 4C. Slides were washed in PBS and incubated for 1h with a 1:200 dilution of swine anti-rabbit antibody (DAKO). After PBS washing, the slides were incubated with streptavidin biotin-peroxidase complex (Vector laboratories) and incubated at room temperature for 45min. Elastin or MLL1 positive cells by were visualised by 3,3′-diaminobenzidine tetrahydrochloride (DAB). Slides were counterstained with Mayers Haematoxylin for 30 sec, dehydrated, cleared in Clearene (Leica) and mounted in Pertex (HistoLab).

SDS-PAGE and immunoblotting

Whole-cell extracts were prepared and protein concentration of samples was determined using Bio-Rad DC Protein Assay (Bio-Rad, UK). Proteins were separated on 8% or 10% SDS polyacrylamide gel and transferred onto a nitrocellulose membrane in buffer containing 25 mM Tris, 192 mM glycine, and 20% methanol. Blots were blocked with TBS/Tween 20 (0.1% T-TBS) containing 5% milk protein before overnight incubation with primary antibodies- rabbit anti-MLL1 at 1:1000 (Bethyl, catalogue number A300-374A), rabbit anti-elastin at 1:500 (Abcam, catalogue number ab21610) or mouse anti-β actin (Sigma, catalogue number A5316) at 1:2000 dilution, respectively. Secondary HRP conjugated antibody (raised either against rabbit or mouse antibodies) was used at 1:2000 dilution. The antibody complexes were detected by chemiluminescence using Pierce ECL Western Blotting Substrate (Thermo Scientific).

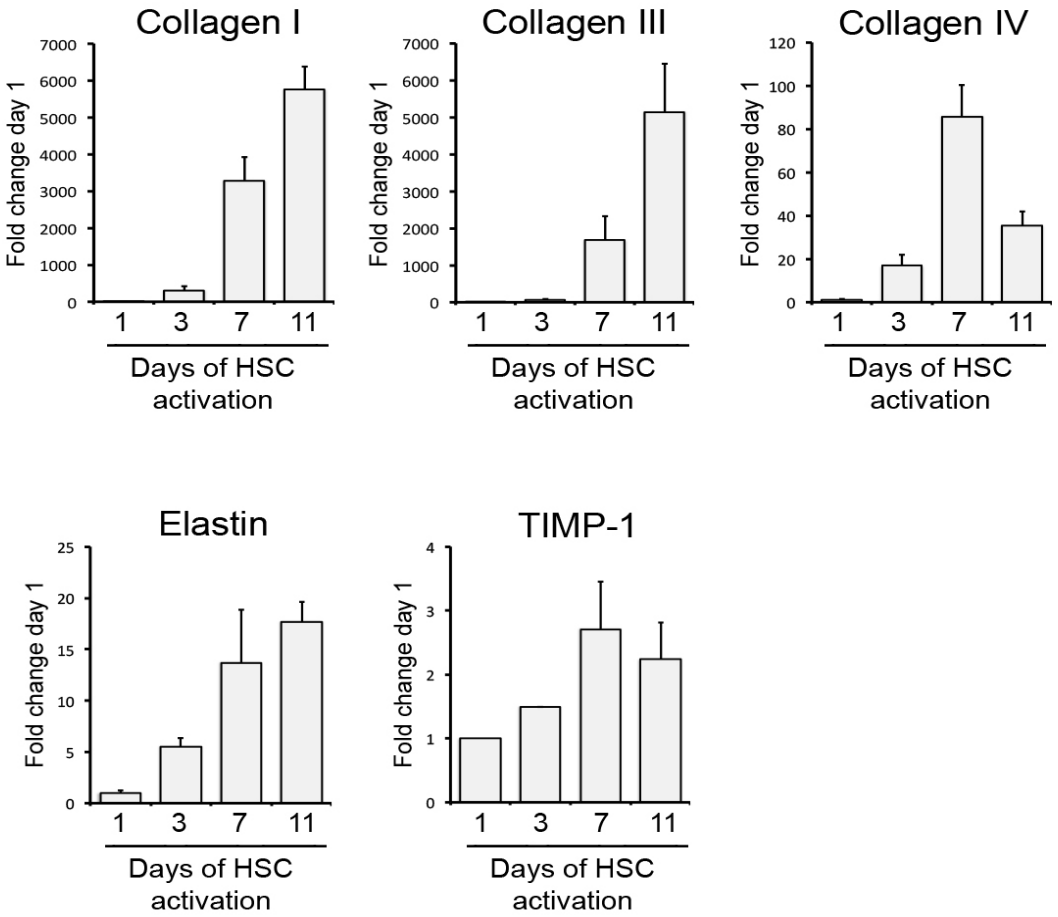
Quantitative PCR (RT-qPCR analysis)

Total RNA was purified from isolated cells using the Total RNA purification kit (Qiagen, UK) following manufacturer's instructions. One microgram of total RNA was DNAse treated (Promega) and used as template to generate cDNA using a random hexamer primer (p(dN)6) and MMLV reverse transcriptase (Promega). Primers for ECM components, histone lysine methyltransferases and histone lysine demethylases are included in Supplementary Table 1. SYBR Green quantitative RT-PCR reactions were performed in a total volume of 13 µl, containing 20ng of cDNA template, 6.5 µl of SYBR Green JumpStart Taq ReadyMix (Sigma), and 20pmols of forward and reverse primers (Supplementary Tables 1-3). The PCR reaction was carried out on a 7500 Fast Real-Time PCR System (Applied Biosystem, Warrington, Cheshire, UK) with the following parameters: 1 cycle at 95 °C for 10 sec followed by 40 cycles at 95 °C for 10 sec, 55 °C - 60 °C (primer pair specific annealing temperature, see Supplementary Table 1 and 2) for 30 sec, and finally 72°C for 30 sec. Melt curve analysis was employed to confirm presence of a single PCR product. All reactions were normalized to rat β-actin or human GAPDH internal control, and relative level of transcriptional difference calculated by using the following equation: (1/[2A]) ×100.

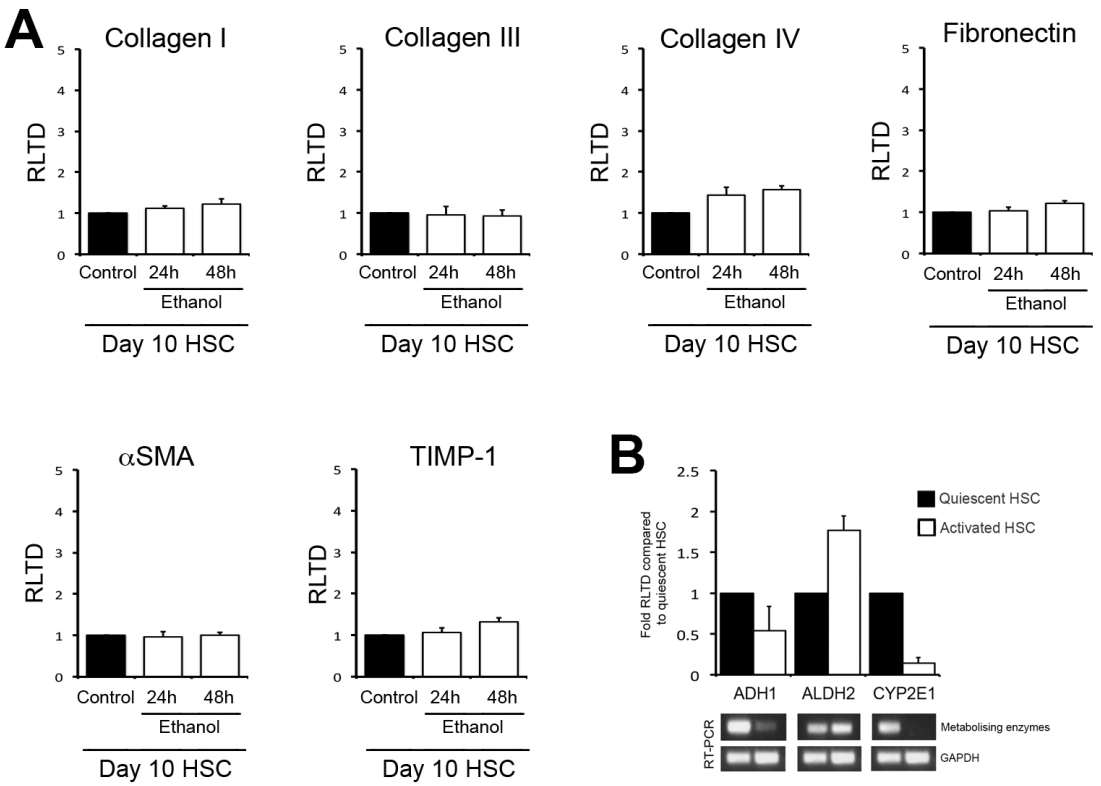
Micrococcal nuclease digestion

Native chromatin was prepared from day 2 rHSC treated with with 86 nM ethanol for 48 h, followed by nuclei isolation and suspension of nuclei to a final concentration of 0.5 mg/ml [2]. Samples were digested with 50 U of micrococcal nuclease (MNase) per one ml of 0.5 mg/ml DNA concentration within nuclei at 37°C, over a time course of 5 min. After each minute, 20-ul sample of digested DNA/nuclei was taken and the reaction stopped by adding 5mM EGTA. DNA was isolated from all samples using phenol/chloroform extraction. Chromatin fractions were analysed by electrophoresis of 10ul of each digested DNA sample loaded on a 1% agarose gel. In order to assess chromatin structure around the elastin promoter, quantitative PCRs were performed using primers: F1 (sense)- 5' GGAATCCCAGAGATCGATGA, and R1 (antisense) 5' CGATGCCTTGAATTCCTGAT.

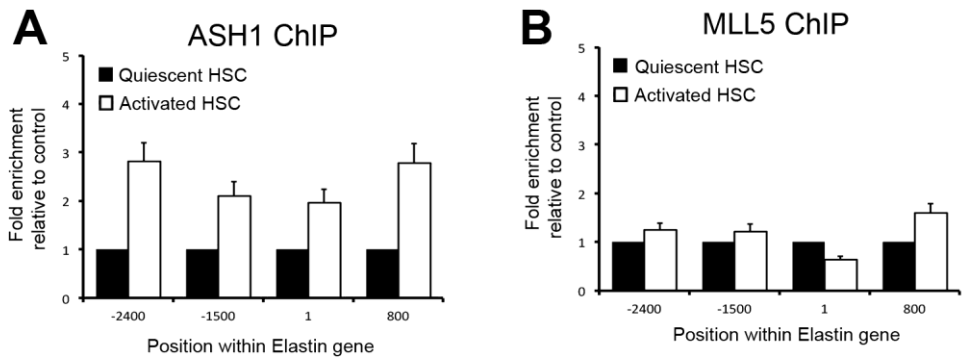
Supplementary Figures



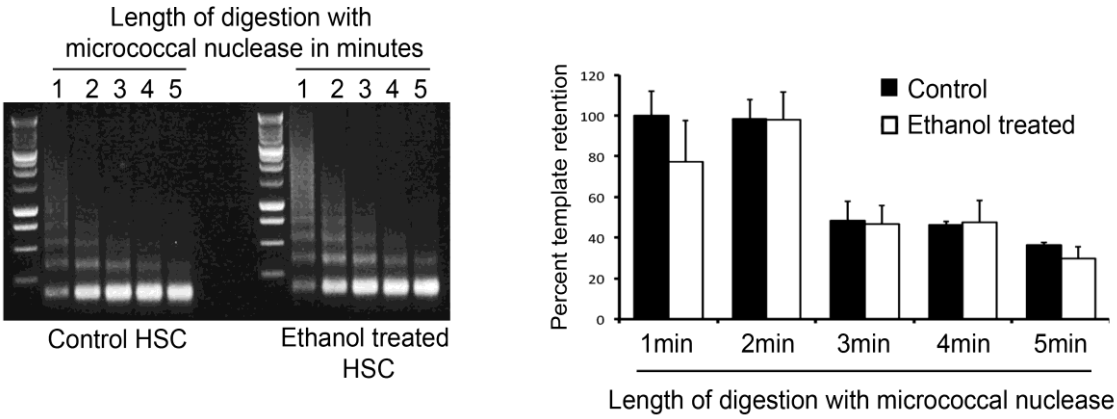
Supplementary Fig. 1. mRNA levels of collagen I, III, IV, elastin and TIMP-1 were quantified by qPCR in four separate preparations of primary rat HSCs at day 1, 3, 7 and 11 of cell culture.



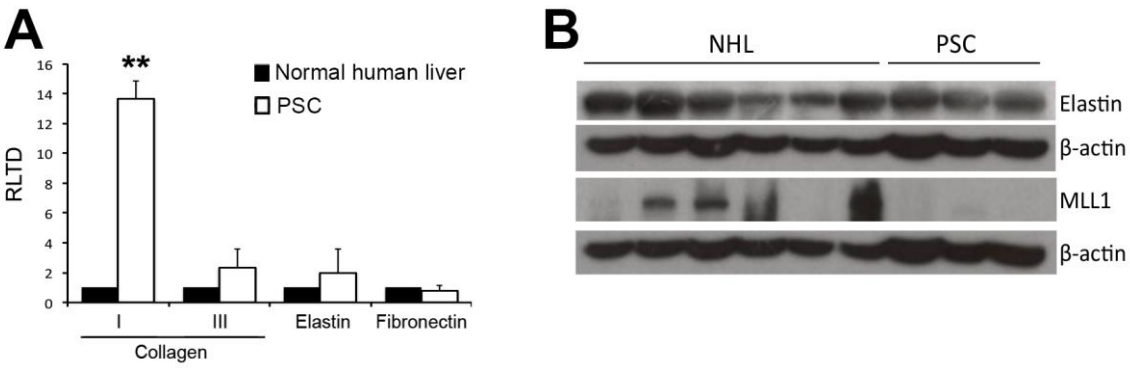
Supplementary Fig. 2. (A) mRNA levels of collagen I, III, IV, fibronectin, αSMA and TIMP-1 were quantified by qRT-PCR in four separate preparations of rat HSCs treated at day 10 of culture for 24 h or 48 h with 86 mM ethanol. (B) mRNA levels of *ADH1*, *ALDH2* and *CYP2E1* were quantified by RT-PCR and qRT-PCR in four separate preparations of quiescent (day 1) or activated (day 10) rat HSCs.



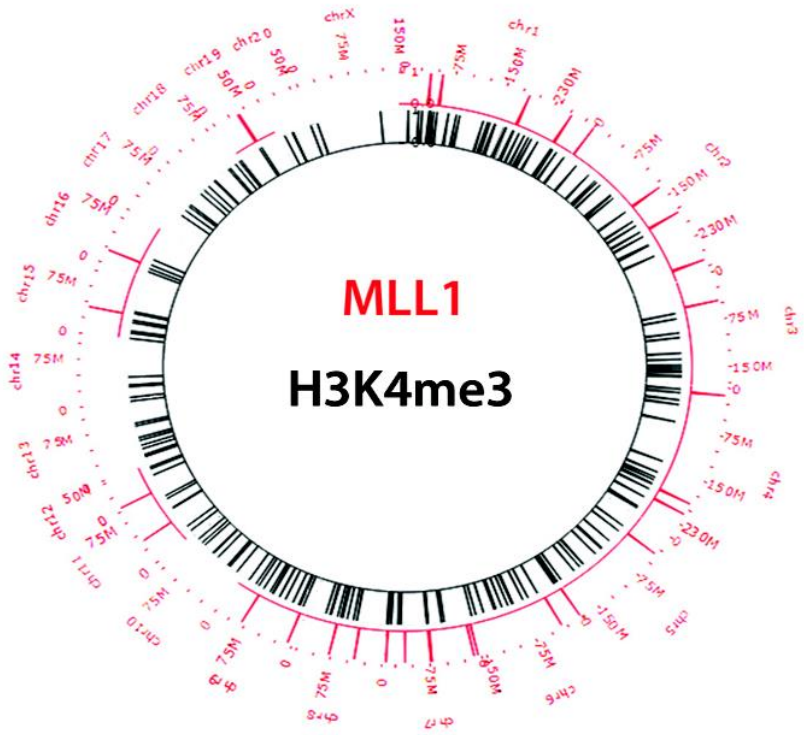
Supplementary Fig. 3. (A and B) 50 µg of cross-linked chromatin from quiescent rat HSCs or fully activated myofibroblasts was incubated with 5 µg of anti-ASH1 and MLL5 antibody and ChIP assay was carried out. Following ChIP, immunoprecipitated genomic DNA was used as template in qPCR reactions using primers specific for different regions of the elastin gene.



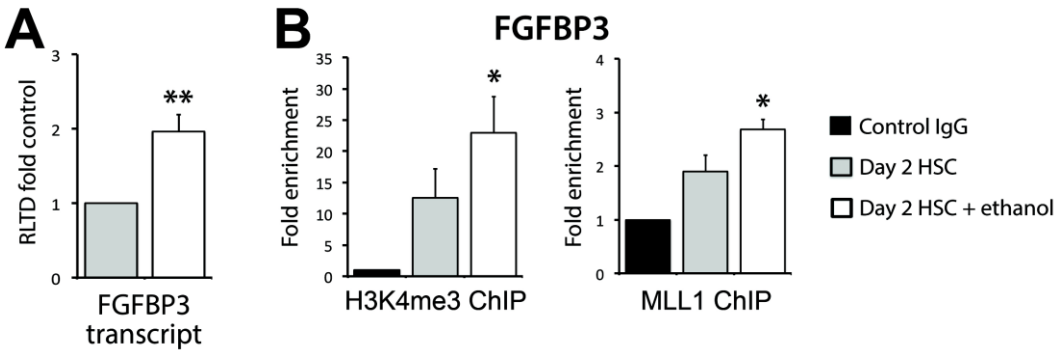
Supplementary Fig. 4. Native chromatin isolated from control and day 2 of rat HSCs, treated for 48 h with 86 mM ethanol was digested with micrococcal nuclease for 5 min with a sample taken every minute. Representative picture shows genomic DNA, isolated from timed digests, separated on 1% agarose gel (left panel). DNA isolated from timed digests was used as template in qPCRs, amplifying the elastin gene promoter (right panel).



Supplementary Fig. 5. (A) mRNA levels of extracellular components collagen I, III, elastin and fibronectin were quantified by qPCR in six separate preparations of normal human liver or PSC liver explants. Error bars represent mean values ± standard error of the mean (SEM). **p* <0.05. (B) 30 µg whole cell protein from six normal human liver (NHL) or three PSC explanted liver tissue samples were immunoblotted for MLL1, elastin and β-actin. Error bars represent mean values ± standard error of the mean (SEM). **p* <0.05.



Supplementary Fig. 6. Aligned reads from MLL1 and H3K4me3 ChIP-Seq (as in Fig. 4A and B) were analysed using MACS software to identify significantly enriched areas compared to the control for each library. Each spike represents gene enrichment after ethanol treatment mapped to its corresponding position on the chromosome. All peaks are based on differences where p <0.001.



Supplementary Fig. 7. (A) mRNA levels of *FGFBP3* were quantified by qPCR in four separate preparations of control or rat HSCs, treated at day 2 of culture for 48 h with 86 mM ethanol. (B). ChIP assay validation for MLL1 and H3K4me3 using cross-linked chromatin from control or day 2 HSCs, treated for 48 h with 86 mM of ethanol was carried out and binding to the *FGFBP3* promoter was tested. Error bars represent mean values ± standard error of the mean (SEM) **p* <0.05; ***p* <0.005.

Supplementary Tables

Supplementary Table 1. Quantitative PCR primers used for cDNA amplification.

Gene	Forward and reverse primer pair sequences	Annealing temperature (°C)
Rat COLLAGEN I	TTCACCTACAGCACGCTTGTG	55
	GATGACTGTCTTGCCCCAAGTT	
Rat COLLAGEN III	CTTCACCCCTCTCTTATTTT	55
	GGCTCATCATCACACATTAT	
Rat COLLAGEN IV	GCTCTGGCTGTGGAAAATGT	55
	CTCCAGCATCACCCTTTTGT	
Rat FIBRONECTIN	GAGCAAGAAGGACAACAGAG	55
	GGTCTGGGGTTGGTAAATAG	
Rat ELASTIN	CCCTGTCCCTGTTCCTTCTG	60
	CGCTCCCTATCCTCTTGTTG	
Human COLLAGEN I	CAAGAGGAAGGCCAAGTCGAGG	55
	CGTTGTCGCAGACGCAGAT	
Human COLLAGEN III	GCCAAATATGTGTCTGTGACTCA	55
	GGGCGAGTAGGAGCAGTTG	
Human FIBRONECTIN	ATGATGCCGACCAGAAGTTT	55
	CACGACCATTCCCAACACAC	
Human ELASTIN	GCAACCAAGGACCGTATCAT	60
	CTGTGCTTGTCCCTCCTCTA	
Rat LSD1 (KDM1A)	TCTCAGGAAGCAGCGTGTTT	55
	CTATGAACTCGGTGGACAAG	
Rat LSD2 (KDM1B)	CCAGCATCTTCTTCCTAAAC	55
	TCTGGGGTCCTCTTCCTACT	
Rat JARID1A (KDM5A)	CCTGGCAGTAGGAGCAAAAG	55
	ATCACACCCGTCACACAAAA	
Rat JARID1B (KDM5B)	CAAAACGGGAAAACGAGAAA	55
	TCATTGCCGCTGCCACATAA	
Rat JARID1C (KDM1C)	CCTGGCAGCAGCTGTACATA	55
	CATCTGGGCAGTCATAGCAA	
Rat JARID1D (KDM5D)	ACTGGTTCCATGGACAATGTG	55
	CCTCAGGCAGCCTTACAGAC	
Rat JHDM1B (KDM2B)	TATACCAGGGGGACTTCGTG	55
	GTGTTCACGTCCATCACGTC	
Rat JHDM2A (KDM3A)	CTCAGGTTGGAGCTGGAGAC	55
	AGTTCAGGAGCAGTGGCATT	
Rat JMJD2A (KDM4A)	TCCCAGGAAGTGCTCAAAGC	55
	CGACGGGTAGCAAAATTGGT	
Rat JMJD2C (KDM4C)	ACAGAGGGAGAAGAAAATGA	60
	CTTGCTACCTGCTGCTTTAC	

Rat JMJD3 (KDM6B)	AACAGCCACAGCAGTAGTCC	60
	AGGCAGGGCAAGAGTCAGTG	
Human/Rat MLL1 (KMT2A)	TCTGTGTTTTCCCCTCTA	58
	TTCAGGCTATCTTCTTTG	
Human/Rat MLL2 (KMT2D)	AGGAGGGCCCAGAACTAT	58
	GCAGTGTTTTCATGGATA	
Human/Rat MLL3 (KMT2C)	CAGCCGCAGACAAAAGAC	58
	ATTGTTCTTTGATTTCTG	
Human/Rat MLL5 (KMT2E)	CCATTGGGGGTTGATACA	58
	AGGAGGACGAGCACCATA	
Human/Rat ASH1 (ASH1L)	AATGATCTTTGCTGAGTGTT	58
	TCCCCAACCTTTTTCCTCAG	
Human/Rat GLP (EHMT1)	ACAAACAGCGTGGTCAAG	58
	TGGAAACGGTGAGAGATG	
Rat G9a (EHMT2)	GAGCCACCGAGAGAGTTC	58
	GGTGTCAGCCCCCTCATC	
Human/Rat Suv39H1	GGCGCCACCTACCTCTTTGA	58
	CGTTGTACACCTGCAGGTTG	
Human/Rat Suv39H2	CAAAAAGGCACACAGTATTC	58
	ACCTCTCCAACATATTCCAT	
Rat EZH2	CATTCATTTCATACGCTCTT	58
	TTTAGGTGGTGTCTTTATCC	
Rat MeCP2	AGGCAGGCAAAGCAGAGACATC	55
	GGCAAGGTGGGGTCATCATACA	
Rat TIMP-1	ACAGCTTTCTGCAACTCGGA	55
	AGTTTGCAAGGGATGGCTGA	
Rat α-SMA	CATGTCGTCCCAGTTGGTGAT	55
	CGAAGCGCAGAGCAAGAGA	
Rat ADH1	CATCAGCACCAGCACCTTCTC	58
	CGACAGACAGACCAACACCTC	
Rat ALDH2	GCCAGGTAGCCGAAGGGAACA	58
	TTGCCGTTGTCCAGGGTCTCC	
Rat CYP2E1	ATGGAAGGATGTGCGGAGGTT	58
	AATCAGAAATGTGGGGTCAAA	
Rat β-ACTIN	AGCCATGTACGTAGCCATCC	55-60
	CTCTCAGCTGTGGTGGTGAA	
Human GAPDH	GAAGGTGAAGGTCGGAGTC	55-60
	GAAGATGGTGATGGGATTTC	

Supplementary Table 2. ChIP primers for the elastin gene.

Region of elastin gene	Forward and reverse primer pair sequences	Annealing temperatures (°C)
Rat ELASTIN -2400	GGTGGTAGATCATGGGATGG	60
	GAAAGCTTGGGCACACATTT	
Rat ELASTIN -1500	ACGCCCTGTACTGGACAAGA	55
	ATGTGGGGTACTGGAGGACA	
Rat ELASTIN -800	ACATGGCTTTCACTCCCAAG	55
	GGCAAAATTGTCTGGGAGAA	
Rat ELASTIN +1	GCAATTACGCTTTGGGGATA	60
	ATCAGTCTTGCCACCTGGTC	
Rat ELASTIN +800	GTGAGCCCCAGCATGTAAGT	60
	CGATGCCTTGAATTCCTGAT	

Supplementary Table 3. Quantitative PCR and ChIP primers for ChIP-Seq validation.

Quantitative PCR primers

Gene	Forward and reverse primer pair sequences	Tm (°C)
Rat c-JUN	ACCCACCTCCACCTTCTTGC	56
	AGCCCTCATCCTCTTCACTG	
Rat FGFBP3	TAAATGAACTGTGCGTAAGA	60
	AGAAGGTGTGATGCCAAGTC	

ChIP assay primers

Gene	Forward and reverse primer pair sequences	Tm (°C)
Rat c-JUN	CATTCCTGGGACTGCACATC	60
	CCCCACCCTATGACATGGAA	
Rat FGFBP3	TAAATGAACTGTGCGTAAGA	60
	AGAAGGTGTGATGCCAAGTC	

Supplementary Table 4. Gene list generated from top hits of combined H3K4me3 and MLL1 ChIP-Seq.

MLL1 and H3K4me3 enriched genes following ethanol treatment		
Gene Symbol	**Description**	**Ensembl**
JUN	Jun proto-oncogene	ENSRNOG00000026293
ACTB	Actin, beta	ENSRNOG00000034254
EXOC8	Exocyst complex component 8	ENSRNOG00000019766
ZFP58	Protein Zfp580	ENSRNOG00000016274
ID3	Inhibitor of DNA binding 3	ENSRNOG00000026124
FGFBP3	Fibroblast growth factor binding protein 3	ENSRNOG00000022796
MAF	V-maf avian musculoaponeurotic fibrosarcoma oncogene homolog	ENSRNOG00000012428
FAU	Ubiquitin-like protein FUBI	ENSRNOG00000046393
CREBZF	CREB/ATF bZIP transcription factor	ENSRNOG00000018987
SF3B5	Splicing factor 3b, subunit 5	ENSRNOG00000014908
COX7C	Cytochrome c oxidase, subunit VIIc	ENSRNOG00000030237
SMIM4	Small Integral Membrane Protein 4	ENSRNOG00000028178
IMP3	U3 small nucleolar ribonucleoprotein, homolog (yeast)	ENSRNOG00000017460
MSX3	Msh homeobox 3	ENSRNOG00000046776
MGAT2	Mannosyl (alpha-1,6-)-glycoprotein beta-1,2-N-acetylglucosaminyltransferase (Mgat2)	ENSRNOG00000004234
DEXI	Dexamethasone-induced transcript	ENSRNOG00000002635

HIST2H3C2	Histone H3	ENSRNOG00000045644
ZFYVE9	Zinc finger, FYVE domain containing 9	ENSRNOG00000027183
CD28	Cd28 molecule	ENSRNOG00000010283
SRSF2	Serine/arginine-rich splicing factor 2	ENSRNOG00000000248
VOM2R37	Vomeronasal 2 receptor, 37	ENSRNOG00000043280
PIGP	Phosphatidylinositol N-acetylglucosaminyltransferase subunit P	ENSRNOG00000039850
TOR1AIP2	Torsin A interacting protein 2	ENSRNOG00000024849
IER3	Immediate early response 3	ENSRNOG00000000827
RUNDC1	Protein Rundc1	ENSRNOG00000023768
MUCF1	Microtubule-actin crosslinking factor 1	ENSRNOG00000016047
DST	Protein Dst	ENSRNOP00000017235
HERC2	HECT and RLD domain containing E3 ubiquitin protein ligase 2	ENSRNOG00000013718
RYR1	Ryanodine receptor 1, skeletal muscle	ENSRNOG00000020557
DNAH8	Dynein, axonemal, heavy chain 8	ENSRNOG00000000542
LRP1	Low density lipoprotein receptor-related protein 1	ENSRNOG00000025053
VPS13C	Vacuolar protein sorting 13C (yeast)	ENSRNOG00000030213
DNAH2	Protein Dnah2	ENSRNOG00000021356
DYNC1H1	Dynein, Cytoplasmic 1, Heavy Chain 1	ENSRNOG00000006178
DNAH1	Dynein, Axonemal, Heavy Chain 1	ENSRNOG00000026914
LAMA5	Laminin, Alpha 5	ENSRNOG00000006479
LYST	Lysosomal Trafficking Regulator	ENSRNOG00000012662
STAB2	Stabilin 2	ENSRNOG00000038948
USP34	Ubiquitin Specific Peptidase 34	ENSRNOG00000026034
ENSRNOP00000056216	Protein Neb	ENSRNOG00000006783
OBSCN	Obscurin, cytoskeletal calmodulin and titin-interacting RhoGEF	ENSRNOG00000022790

MLL1 enriched genes following ethanol treatment

Gene Symbol	Description	Ensembl
COL26A1	Collagen, Type XXVI, Alpha 1	ENSRNOG00000001422
INSR	Insulin receptor	ENSRNOG00000029986
PLOD3	Procollagen-Lysine, 2-Oxoglutarate 5-Dioxygenase 3	ENSRNOG00000001417
RCL1	RNA Terminal Phosphate Cyclase-Like 1	ENSRNOG00000015491
PLD	Phospholipase D1, Phosphatidylcholine-Specific	ENSRNOG00000028566
UNC5C	Unc-5 Homolog C (C. Elegans)	ENSRNOG00000029071
ATG7	Autophagy Related 7	ENSRNOG00000007486
MMP16	Matrix Metallopeptidase 16 (Membrane-Inserted)	ENSRNOG00000005708
CLIP4	CAP-GLY Domain Containing Linker Protein Family, Member 4	ENSRNOG00000008806
SDC2	Syndecan 2	ENSRNOG00000004936
OLR1	Oxidized Low Density Lipoprotein (Lectin-Like) Receptor 1	ENSRNOG00000008375
ARPC2	Actin Related Protein 2/3 Complex, Subunit 2	ENSRNOG00000014289
CTDSP1	CTD (Carboxy-Terminal Domain, RNA Polymerase II, Polypeptide A) Small Phosphatase 1	ENSRNOG00000015295
GRM	Glutamate Receptor, Metabotropic 1	ENSRNOG00000014290
UTRN	Utrophin	ENSRNOG00000011058
NELL1	NEL-like 1	ENSRNOG00000015675
PTGFRN	Prostaglandin F2 Receptor Inhibitor	ENSRNOG00000015655
CASC1	Cancer Susceptibility Candidate 1	ENSRNOG00000027630

LTBP1	Latent Transforming Growth Factor Beta Binding Protein 1	ENSRNOG00000033090
GALNTL6	UDP-N-Acetyl-Apha-D-Galactosamine:polypeptide N-Acetylgalactosaminyltransferase-Like 6	ENSRNOG00000047809
PHACTR1	Phosphatase And Actin Regulator 1	ENSRNOG00000014264
SSLP1	Spleen Protein 1 Precursor	ENSRNOG00000008563

H3K4me3 enriched genes following ethanol treatment

Gene Symbol	Description	Ensembl
STX7	Syntaxin 7	ENSRNOG00000015670
RGS17	Regulator Of G-Protein Signaling 17	ENSRNOG00000018690
IPCEF1	Interaction Protein For Cytohesin Exchange Factors 1	ENSRNOG00000018163
RSPH3	Radial Spoke 3 Homolog (Chlamydomonas)	ENSRNOG00000018762
LENG9	Leukocyte Receptor Cluster (LRC) Member 9	ENSRNOG00000018621
DNAJB13	DnaJ (Hsp40) Homolog, Subfamily B, Member 13	ENSRNOG00000017975
SPON1	Spondin 1, Extracellular Matrix Protein	ENSRNOG00000034303
EEF2K	Eukaryotic Elongation Factor-2 Kinase	ENSRNOG00000016448
RIC8A	RIC8 Guanine Nucleotide Exchange Factor A	ENSRNOG00000013256
PAPSS2	3'-Phosphoadenosine 5'-Phosphosulfate Synthase 2	ENSRNOG00000011068
ZFYVE27	Zinc Finger, FYVE Domain Containing 27	ENSRNOG00000014903
FAM160B1	Family With Sequence Similarity 160, Member B1	ENSRNOG00000017225
POLK	Polymerase (DNA Directed) Kappa	ENSRNOG00000025373
PTGER4	Prostaglandin E Receptor 4 (Subtype EP4)	ENSRNOG00000013240
FLAD1	Flavin Adenine Dinucleotide Synthetase 1	ENSRNOG00000020642
GSTM2	Glutathione S-Transferase Mu 2 (Muscle)	ENSRNOG00000019094
ATXN7L2	Ataxin 7-Like 2	ENSRNOG00000028749
SLC25A12	Solute Carrier Family 25 (Aspartate/Glutamate Carrier), Member 12	ENSRNOG00000022922
TTC7	Tetratricopeptide Repeat Domain 7A	ENSRNOG00000014879
API5	Apoptosis Inhibitor 5	ENSRNOG00000009689
TRIM44	Tripartite Motif Containing 44	ENSRNOG00000005191

TRIB3	Tribbles Pseudokinase 3	ENSRNOG00000007319
RBL1	Retinoblastoma-Like 1 (P107)	ENSRNOG00000006921
CTNNBL1	Catenin, Beta Like 1	ENSRNOG00000012021
NCOA5	Nuclear Receptor Coactivator 5	ENSRNOG00000017824
KCNB1	Potassium Voltage-Gated Channel, Shab-Related Subfamily, Member 1	ENSRNOG00000046949
MAD2L1	MAD2 Mitotic Arrest Deficient-Like 1 (Yeast)	ENSRNOG00000005376
HK2	Hexokinase 2	ENSRNOG00000006116
GATA2	GATA Binding Protein 2	ENSRNOG00000012347
UBE2R2	Ubiquitin-Conjugating Enzyme E2R 2	ENSRNOG00000010727
SVEP1	Sushi, Von Willebrand Factor Type A, EGF And Pentraxin Domain Containing 1	ENSRNOG00000033110
ALAD	Aminolevulinate Dehydratase	ENSRNOG00000015206
JAK1	Janus Kinase 1	ENSRNOG00000011157
YIPF1	Yip1 Domain Family, Member 1	ENSRNOG00000010512
ERMAP	Erythroblast Membrane-Associated Protein (Scianna Blood Group)	ENSRNOG00000000335
SRM	Spermidine Synthase	ENSRNOG00000011078
GNB1	Guanine Nucleotide Binding Protein (G Protein), Beta Polypeptide 1	ENSRNOG00000016638
CDC42EP3	CDC42 Effector Protein (Rho GTPase Binding) 3	ENSRNOG00000032136
AKAP6	A Kinase (PRKA) Anchor Protein 6	ENSRNOG00000004841
LRFN5	Leucine Rich Repeat And Fibronectin Type III Domain Containing 5	ENSRNOG00000005550
TIMM9	Translocase Of Inner Mitochondrial Membrane 9 Homolog (Yeast)	ENSRNOG00000008222
FCF1	FCF1 RRNA-Processing Protein	ENSRNOG00000004723
BTBD7	BTB (POZ) Domain Containing 7	ENSRNOG00000008598
SYT1	Synaptotagmin I	ENSRNOG00000006426
PAWR	PRKC, Apoptosis, WT1, Regulator	ENSRNOG00000005917
HRSP12	Heat-Responsive Protein 12	ENSRNOG00000005437

POP1	Processing Of Precursor 1, Ribonuclease P/MRP Subunit (S. Cerevisiae)	ENSRNOG00000005243
TXN2	Thioredoxin 2	ENSRNOG00000005614
ADSL	Adenylosuccinate Lyase	ENSRNOG00000018655
CYB5R3	Cytochrome B5 Reductase 3	ENSRNOG00000009592
IRAK4	Interleukin-1 Receptor-Associated Kinase 4	ENSRNOG00000005965
SLC48A1	Solute Carrier Family 48 (Heme Transporter), Member 1	ENSRNOG00000008192
SIK3	SIK Family Kinase 3	ENSRNOG00000045931
ZC3H12C	Zinc Finger CCCH-Type Containing 12C	ENSRNOG00000012470
TPM1	Tropomyosin 1 (Alpha)	ENSRNOG00000018184
BMP5	Bone Morphogenetic Protein 5	ENSRNOG00000010917
SNAP91	Synaptosomal-Associated Protein	ENSRNOG00000023861
SNX14	Sorting Nexin 14	ENSRNOG00000011348
ATR	Ataxia Telangiectasia And Rad3 Related	ENSRNOG00000010027
TJAP1	Tight Junction Associated Protein 1	ENSRNOG00000018980
PMS1	Postmeiotic Segregation Increased 1	ENSRNOG00000004076
STAT1	Signal Transducer And Activator Of Transcription 1	ENSRNOG00000014079
WDR12	WD Repeat Domain 12	ENSRNOG00000017340
NDUFV2	NADH Dehydrogenase (Ubiquinone) Flavoprotein 2	ENSRNOG00000042503
CLINT1	Clathrin Interactor 1	ENSRNOG00000005406
MGAT1	Mannosyl (Alpha-1,3-)-Glycoprotein Beta-1,2-N-Acetylglucosaminyltransferase	ENSRNOG00000031208
MYO1D	Myosin ID	ENSRNOG00000003276
VPS25	Vacuolar Protein Sorting 25 Homolog	ENSRNOG00000020441
COA3	Cytochrome C Oxidase Assembly Factor 3	ENSRNOG00000020487
ACOX1	Acyl-CoA Oxidase 1, Palmitoyl	ENSRNOG00000008755
LPP	LIM Domain Containing Preferred Translocation Partner In Lipoma	ENSRNOG00000031669

ALG3	Alpha-1,3- Mannosyltransferase	ENSRNOG00000001712
CCDC60	Coiled-Coil Domain Containing 60	ENSRNOG00000032684
NCKAP5	NCK-Associated Protein 5	ENSRNOG00000021553
FAIM3	Fas Apoptotic Inhibitory Molecule 3	ENSRNOG00000004441
RASSF5	Ras Association (RalGDS/AF-6) Domain Family Member 5	ENSRNOG00000005342
RGL1	Ral Guanine Nucleotide Dissociation Stimulator-Like 1	ENSRNOG00000002347
SLC10A6	Solute Carrier Family 10 (Sodium/Bile Acid Cotransporter), Member 6	ENSRNOG00000002057
TMEM156	Transmembrane Protein 156	ENSRNOG00000026518
PSME4	Proteasome (Prosome, Macropain) Activator Subunit 4	ENSRNOG00000006609
FAM149B1	Family With Sequence Similarity 149, Member B1	ENSRNOG00000006554
LGALS3	Lectin, Galactoside-Binding, Soluble, 3	ENSRNOG00000010645
SPATA13	Spermatogenesis Associated 13	ENSRNOG00000013707
HIST1H2A	Histone Cluster 1, H2a	ENSRNOG00000017175
PLXDC2	Plexin Domain Containing 2	ENSRNOG00000000142
USP14	Ubiquitin Specific Peptidase 14 (TRNA-Guanine Transglycosylase)	ENSRNOG00000014981
CDC25C	Cell Division Cycle 25C	ENSRNOG00000024008
HTR4	5-Hydroxytryptamine (Serotonin) Receptor 4, G Protein-Coupled	ENSRNOG00000019134
MALT1	Mucosa Associated Lymphoid Tissue Lymphoma Translocation Gene 1	ENSRNOG00000017181
SMAD2	SMAD Family Member 21	ENSRNOG00000018140
PHKB	Phosphorylase Kinase, Beta	ENSRNOG00000024101
RNASEH2A	Ribonuclease H2, Subunit A	ENSRNOG00000003504
HERC4	HECT And RLD Domain Containing E3 Ubiquitin Protein Ligase 4	ENSRNOG00000000381
RHOBTB1	Rho-Related BTB Domain Containing 1	ENSRNOG00000000633
GRIK2	Glutamate Receptor, Ionotropic, Kainate 2	ENSRNOG00000000368
FUNDC1	FUN14 Domain Containing 1	ENSRNOG00000003470
DDN	Dendrin	ENSRNOG00000014789

Supplementary Table 5. Gene list generated from top hits of combined H3K4me3 and MLL1 ChIP-Seq.

MLL1and H3K4me3 enriched genes in alcohol fed mice

Gene Symbol	Description	Ensembl
ADAM19	**ADAM** Metallopeptidase Domain 19	ENSMUST00000011400
DOCK4	Dedicator of Cytokinesis 4	ENSMUST00000037488
MAST4	Microtubule Associated Serine/Threonine Kinase Family Member 4	ENSMUST00000164111
MAML3	Mastermind-Like 3	ENSMUST00000121440
ASTN2	Astrotactin 2	ENSMUST00000084496
FGGY	**FGGY** Carbohydrate Kinase Domain Containing	ENSMUST00000130541
KAZN	Kazrin, Periplakin Interacting Protein	ENSMUST00000036476
PLXNA4	Plexin A4	ENSMUST00000115096
EXOC4	Exocyst Complex Component 4	ENSMUST00000052266
GRM 7	Glutamate Receptor, Metabotropic 7	ENSMUST00000172951
FGFR2	Fibroblast Growth Factor Receptor 2	ENSMUST00000124096
ARHGAP44	Rho GTPase Activating Protein 44	ENSMUST00000093001
RBFOX3	RNA Binding Protein, Fox-1 Homolog	ENSMUST00000120061
STXBP6	Syntaxin Binding Protein	ENSMUST00000143376
GPHN	Gephyrin	ENSMUST00000052472
PTPRN2	Protein Tyrosine Phosphatase, Receptor Type, N Polypeptide 2	ENSMUST00000070733
CLDN10	Claudin 10	ENSMUST00000071546
CDH18	Cadherin 18, Type 2	ENSMUST00000167623
CSMD3	CUB And Sushi Multiple Domains 3	ENSMUST00000162830

SAMD12	Sterile Alpha Motif Domain Containing 12	ENSMUST00000078673
GNB1L	Guanine Nucleotide Binding Protein (G Protein), Beta Polypeptide 1-Like	ENSMUST00000147739
ERG	V-Ets Avian Erythroblastosis Virus E26 Oncogene Homolog	ENSMUST00000171646
PTPRM	Protein Tyrosine Phosphatase, Receptor Type, M	ENSMUST00000037974
TRPM3	Transient Receptor Potential Cation Channel, Subfamily M, Member 3	ENSMUST00000087576
BTRC	Beta-Transducin Repeat Containing E3 Ubiquitin Protein Ligase	ENSMUST00000111936
PRPF18	Pre-MRNA Processing Factor 18	ENSMUST00000035721
LYPD6B	LY6/PLAUR Domain Containing 6B	ENSMUST00000028103
LRRC4C	Leucin Rich Repeat Containing 4C	ENSMUST00000162807
RBBP9	Retinoblastoma Binding Protein 9	ENSMUST00000028915
ZFP831	Zinc Finger Protein 831	ENSMUST00000059452
CDH4	Cadherin 4	ENSMUST00000108911
NFIA	Nuclear Factor I/A	ENSMUST00000107057
MAGI2	Membrane Associated Guanylate Kinase, WW And PDZ Domain Containing 2	ENSMUST00000115267
SDK1	Sidekick Cell Adhesion Molecule 1	ENSMUST00000074546
DENND2A	DENN/MADD Domain Containing 2A	ENSMUST00000036877
GRIN2B	Glutamate Receptor, Ionotropic, N-Methyl D-Aspartate 2B	ENSMUST00000053880
RASA3	RAS P21 Protein Activator 3	ENSMUST00000117551
GALNTL6	UDP-N-Acetyl-Alpha-D-Galactosamine:Polypeptide N-Acetylgalactosaminyltransferase-Like 6	ENSMUST00000077447
RNF50	Ring Finger protein 50	ENSMUST00000078525

MLL1 enriched genes in alcohol fed mice

Gene Symbol	Description	Ensembl
ITGB6	Integrin Beta 6	ENSMUST00000154764
FRAS1	Fraser Syndrome 1; Extracellular Matrix Protein	ENSMUST00000036019
ITGA9	Integrin Alpha 9	ENSMUST00000124360
ALDH8A1	Aldehyde Dehydrogenase 8 Family, Member A1	ENSMUST00000042699
LAMA2	Laminin	ENSMUST00000092639
JARID2	Jumonji, AT Rich Interactive Domain 2	ENSMUST00000174086
CYP2D34	Cytochrome P450, Family 2, Subfamily d, Polypeptide 34	ENSMUST00000109515
COL8A1	Collagen, Type VIII, Alpha 1	ENSMUST00000089332
PKHD1	Polycystic Kidney And Hepatic Disease 1	ENSMUST00000088448
JAK2	Janus Kinase 2	ENSMUST00000025705
FGF2	Fibroblast Growth Factor 2	ENSMUST00000091203
COL11A1	Collagen, Type XI, Alpha 1	ENSMUST00000123619
COL24A1	Collagen, Type XXIV, Alpha 1	ENSMUST00000029848
SPON1	Spondin 1; Extracellular Matrix Protein	ENSMUST00000084696
CREBBP	CREB Binding Protein	ENSMUST00000023165
MYO5B	Myosin	ENSMUST00000121875
CDC123	Cell Division Cycle 123	ENSMUST00000043864
IL18RAP	Interleukin 18 Receptor Accessory Protein	ENSMUST00000027237
FMN1	Formin 1	ENSMUST00000161731
IL7	Interleukin 7	ENSMUST00000168269
TNIK	TRAF2 And NCK Interacting Kinase	ENSMUST00000162777

CAMK2D	Calcium/Calmodulin-Dependent Protein Kinase II Delta	ENSMUST00000170149
NGR1	Neuronal Growth Regulator 1	ENSMUST00000106065
COL27A1	Collagen, Type XXVII, Alpha 1	ENSMUST00000036300
DMP1	Dentin Matrix Acidic Phosphoprotein 1[1]	ENSMUST00000066708
SPOCK3	Sparc/Osteonectin, Cwcv And Kazal-Like Domains Proteoglycan	ENSMUST00000093480
TGFBP2	Transforming Growth Factor, Beta Receptor II	ENSMUST00000035014
IL1RAPL1	Interleukin 1 Receptor Accessory Protein-Like 1	ENSMUST00000113964
TERF2	Telomeric Repeat Binding Factor 2	ENSMUST00000068421
CCDC79	Coiled-Coil Domain Containing 79	ENSMUST00000162014
LTBP1	Latent Transforming Growth Factor Beta Binding Protein 1	ENSMUST00000001927
GRIK2	Glutamate Receptor, Ionotropic, Kainate 2 (Beta 2)	ENSMUST00000105483
YIPF2	Yip1 Domain Family, Member 1	ENSMUST00000128284
BMP5	Bone Morphogenetic Protein 5	ENSMUST00000012281
SDR16C5	Short Chain Dehydrogenase/Reductase Family 16C, Member 5	ENSMUST00000040925
DPT	Dermatopontin	ENSMUST00000027861
GREM2	Gremlin 2 Homolog	ENSMUST00000055294
LIMS1	LIM And Senescent Cell Antigen-Like Domains 1	ENSMUST00000020078
CAR10	Carbonic Anhydrase 10	ENSMUST00000107863
FHIT	Fragile Histidine Triad Gene	ENSMUST00000160956
FERMT2	Fermitin Family Homolog 2	ENSMUST00000045905
PTK2B	PTK2 Protein Tyrosine Kinase 2 Beta	ENSMUST00000111121
PRKAA1	Protein Kinase, AMP-Activated, Alpha 1 Catalytic Subunit	ENSMUST00000051186
ADAMTS20	Disintegrin-Like And Metallopeptidase With Thrombospondin Type 1 Motif, 20	ENSMUST00000035342
ADAM33	Disintegrin And Metallopeptidase Domain 33	ENSMUST00000147333
ADAM12	Disintegrin And Metallopeptidase Domain 12	ENSMUST00000067680
ADAMTS17	Disintegrin-Like And Metallopeptidase With Thrombospondin Type 1 Motif, 20	ENSMUST00000107478

ADAMTSL3	Adamts Like 3	ENSMUST00000173828
FIBP	Fibroblast Growth Factor Intracellular Binding Protein	ENSMUST00000025847
LAMC3	Laminin Gamma 3	ENSMUST00000028187
KIF16B	Kinesin Family Member 16B	ENSMUST00000043589
FLT1	FMS Like Tyrosine Kinase 1	ENSMUST00000031653
CTSC	Cathepsin C	ENSMUST00000128791
SPOCK3	Sparc/ Osteonectin	ENSMUST00000093480
NKD1	Naked Cuticle 1 Homolog	ENSMUST00000169179
ST14	Supression of tumorigenicity 14	ENSMUST00000034478
NRG4	Neuregulin 4	ENSMUST00000164721
MAP2K1	Mitogen-Activated Protein Kinase 1	ENSMUST00000005066

H3K4me3 enriched genes in alcohol fed mice

Gene Symbol	Description	Ensembl
Col4A3	Collagen, Type IV, Alpha 3	ENSMUST00000113457
CASC3	Cancer Susceptibility Candidate 3	ENSMUST00000169695
MYL4	Myosin, Light Polypeptide 4	ENSMUST00000106956
RACGAP1	Rac GTPase-Activating Protein 1	ENSMUST00000023756
PEBP4	Phosphatidylethanolamine-Binding Protein 4	ENSMUST00000022678
DEXI	Dexamethasone-Induced Transcript	ENSMUST00000038281
IL1RAP	Interleukin 1 Receptor Accessory Protein	ENSMUST00000023156
CCDC80	Coiled-Coil Domain Containing 80	ENSMUST00000061050
ASH1I	Absent, Small, or Homeotic-Like	ENSMUST00000090933
FGF13	Fibroblast Growth Factor 13	ENSMUST00000124402
FLNA	Filamin A, Alpha	ENSMUST00000114299
RIPK1	Receptor (TNFRSF)-Interacting Serine-Threonine Kinase 1	ENSMUST00000171137
SPOCK1	Sparc/Osteonectin, Cwcv And Kazal-Like Domains Proteoglycan 1	ENSMUST00000172326
USP54	Ubiquitin Specific Peptidase 54	ENSMUST00000035340
CCDC50	Coiled-Coil Domain Containing 50	ENSMUST00000039443
EMILIN2	Elastin Microfibril Interfacer 2	ENSMUST00000024849
NRG2	Neuregulin 2	ENSMUST00000115705
IL33	Interleukin 33	ENSMUST00000144528
SETDB1	SET Domain, Bifurcated 1	ENSMUST00000107170
USP33	Ubiquitin Specific Peptidase 33	ENSMUST00000117492

RBBP4	Retinoblastoma Binding Protein 4	ENSMUST00000135585
MLL3	Lysine (K)-Specific Methyltransferase 2C	ENSMUST00000045291
ELFN1	Extracellular Leucine-Rich Repeat And Fibronectin Type III Domain Containing 1	ENSMUST00000050519
CFTR	Cystic Fibrosis Transmembrane Conductance Regulator	ENSMUST00000115406
WNT5B	Wingless-Related MMTV Integration Site 5B	ENSMUST00000119369
IL4I1	Interleukin 4 Induced 1	ENSMUST00000033015
NELL1	NELL-Like1	ENSMUST00000151721
VPS13C	Vacuolar Protein Sorting 13 Homolog C	ENSMUST00000077879
ADHFE1	Alcohol Dehydrogenase, Iron Containing, 1	ENSMUST00000144177
TERF1	Telomeric Repeat Binding Factor 1	ENSMUST00000027057
DST	Dystonin	ENSMUST00000115104
STAT4	Signal Transducer And Activator Of Transcription 4	ENSMUST00000027277
HTR2B	5-Hydroxytryptamine (Serotonin) Receptor 2B, G Protein-Coupled	ENSMUST00000027431
TRAF3IP1	TNF Receptor-Associated Factor 3 Interacting Protein 1	ENSMUST00000047242
TNFSF4	Tumor Necrosis Factor (Ligand) Superfamily, Member 4	ENSMUST00000028024
SMYD3	SET And MYND Domain Containing 3	ENSMUST00000128302
HHAT	Hedgehog Acyltransferase	ENSMUST00000154755
EGR2	Early Growth Response	ENSMUST00000127820
EFEMP1	EGF Containing Fibulin-Like Extracellular Matrix Protein 1	ENSMUST00000139713
MGAT1	Mannoside Acetylglucosaminyltransferase 1	ENSMUST00000109194
COL23A1	Collagen, Type XXIII, Alpha 1	ENSMUST00000102765
FRK	Fyn-Related Kinase	ENSMUST00000019913
EZH1	Enhancer of Zeste Homolog 1	ENSMUST00000100417
ITGB1BP1	Integrin Beta 1 Binding Protein 1	ENSMUST00000172834
HDAC9	Histone Deacetylase	ENSMUST00000085463
PTPRN2	Protein Tyrosine Phosphatase, Receptor Type, N Polypeptide 2	ENSMUST00000070733

DNAHC1	Dynein, Axonemal, Heavy Polypeptide 1	ENSMUST00000048603
TNFRSF19	Tumor Necrosis Factor Receptor Superfamily, Member 19	ENSMUST00000111236
SQSTM1	Sequestosome 1	ENSMUST00000143379
RCBTB2	Regulator Of Chromosome Condensation (RCC1) And BTB (POZ) Domain Containing Protein 2	ENSMUST00000110952
GPC6	Glypican 6	ENSMUST00000125435
SAMD12	Sterile Alpha Motif Domain Containing 12	ENSMUST00000078673
ITFG3	Integrin Alpha FG-GAP Repeat Containing 3	ENSMUST00000118487
MAPK4	Mitogen-Activated Protein Kinase 4	ENSMUST00000159162
JHDM1A	Lysine (K)-Specific Demethylase 2A	ENSMUST00000116571
RASGRP1	RAS Guanyl Releasing Protein 1	ENSMUST00000173252
CTDSPL2	CTD (Carboxy-Terminal Domain, RNA Polymerase II, Polypeptide A) Small	ENSMUST00000110574
RTEL1	Regulator Of Telomere Elongation Helicase 1	ENSMUST00000153112
TLR4	Toll-Like receptor 4	ENSMUST00000107365
USP24	Ubiquitin Specific Peptidase 24	ENSMUST00000094933
MAPK10	Mitogen-Activated Protein Kinase 10	ENSMUST00000112846
RTKN	Rhotekin	ENSMUST00000087938
USP5	Ubiquitin Specific Peptidase 5	ENSMUST00000122110
ITFG2	Integrin Alpha FG-GAP Repeat Containing 2	ENSMUST00000001559
FBL	Fibrillarin	ENSMUST00000042405
MYO9A	Myosin IXA	ENSMUST00000128341
USP3	Ubiquitin Specific Peptidase 3	ENSMUST00000174387
ALDH1A3	Aldehyde Dehydrogenase 1 Family, Member A3	ENSMUST00000015278
DST	Dystonin	ENSMUST00000115104
RGS17	Regulator of G-protein signaling 17	ENSMUST00000019909
API5	Apoptosis Inhibitor 5	ENSMUST00000028617

SYT1	Synaptotagmin I	ENSMUST00000156979
CXXC5	CXXC Finger 5	ENSMUST00000060722
ADHFE1	Alcohol Dehydrogenase, Iron Containing, 1	ENSMUST00000144177
INPP5D	Inositol Polyphosphate-5-Phosphatase D	ENSMUST00000167032
TNS3	Tensin3	ENSMUST00000020695
RPS6KA5	Ribosomal Protein S6 Kinase, Polypeptide 5	ENSMUST00000043599
HRH2	Histamine Receptor H2	ENSMUST00000038101
GPC6	Glypican 6	ENSMUST00000125435
GHR	Growth Hormane Receptor	ENSMUST00000110697
ADAMTS1	Disintegrin-Like And Metallopeptidase With Thrombospondin Type 1 Motif, 1	ENSMUST00000023610
FER	Fer Protein Kinase	ENSMUST00000038080
RPS6KA4	Ribosomal Protein S6 Kinase, Polypeptide 4	ENSMUST00000025903
ADAMTS3	Disintegrin-Like And Metallopeptidase With Thrombospondin Type 1 Motif, 3	ENSMUST00000061427
CDH11	Cadherin 11	ENSMUST00000075190
CXCR5	Chemokine Receptor 5	ENSMUST00000062215

Genes highlighted in red overlap with *in vitro* results

Supplementary References

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