

Supplemental data

Zhao et al

PRMT1 and JMJD6 dependent arginine methylation regulate HNF4 α expression and hepatocyte proliferation

Supplemental Figure Legends

Figure S1. **A.** Western blot analysis of ADMA modified proteins in cell extracts of Huh 7.5 cells overexpressing wild type PRMT1. **B.** Alcohol inhibits PRMT1 dependent arginine methylation of SAM68. Western blot analysis of SAM68 immunoprecipitated from livers of wild type mice fed alcohol or control liquid diet using anti-methyl-arginine antibody or IgG as a negative control.

Figure S2. Hepatocyte specific PRMT1 deletion results in an increase of serum ALT. **A.** Serum ALT levels in PRMT1 flox/flox mice and PRMT1 flox/flox Albumin-Cre littermates. N=3 per group. **B.** Serum ALT levels and relative Gpt mRNA levels in PRMT1 flox/flox mice received 5×10^{10} - 2×10^{11} gc of AAV-Cre or AAV-control.

Figure S3. PCR array analysis of genes differentially regulated in PRMT1 flox/flox mice and PRMT1 flox/flox Albumin-Cre mice. **A.** Top 20 upregulated and down regulated genes. **B.** Ingenuity pathway analysis of upstream transcriptional regulators.

Figure S4. Western blot analysis of protein levels in the livers of wild type mice fed control liquid diet (pair fed) or alcohol diet (alcohol) for 10 days (A) or 3 weeks (B).

Figure S5. **A.** Chromatin immunoprecipitation using H4R3me2a antibodies or IgG as a negative control from livers of mice that received either AAV-control or AAV-Cre. Data are presented as mean \pm SD. N=3 *p < 0.05. **B.** Relative mRNA levels in PRMT1 flox/flox mice received AAV-Cre or AAV-control for 12 weeks.

Figure S6. PRMT1 flox/flox mice after 3 weeks post injection of AAV8-TBG-Cre or AAV-control virus 10^{11} gc/mouse followed by second injection of AAV8-GFP-U6-m-JMJD6-shRNA or AAV8-GFP-U6-scramb-shRNA 2×10^{11} gc/mouse. Representative images of immunohistochemical staining of PRMT1 (A) and JMJD6 (B) in the livers of these mice. C. Serum ALT levels in these mice. Data are presented as mean \pm SD. N=4-6 per group.

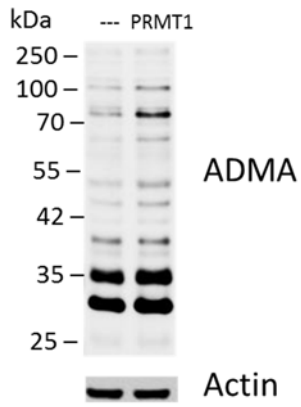
Figure S7. Wild type mice were injected 2×10^{11} gc/mouse AAV8-GFP-U6-m-JMJD6-shRNA or AAV8-GFP-U6-scramb-shRNA and fed control liquid diet (pair fed) or alcohol diet (alcohol) for 3 weeks. **A.** Western blot analysis of JMJD6 and HNF4 α protein expression levels in the livers of these mice **B.** Liver/body weight ratio in these mice. *p < 0.05, ***p < 0.001,

n=4-6 per group. C. Serum ALT levels in these mice. Data are presented as mean \pm SD. N=4-6 per group.

Figure S8 Relative PRMT1 protein levels in human HCC specimen. Data are presented as mean \pm SD. N=5 for donor samples N=7 for HCC (Tumor and Adjacent). *p < 0.05.

Figure S1

A



B



Figure S2

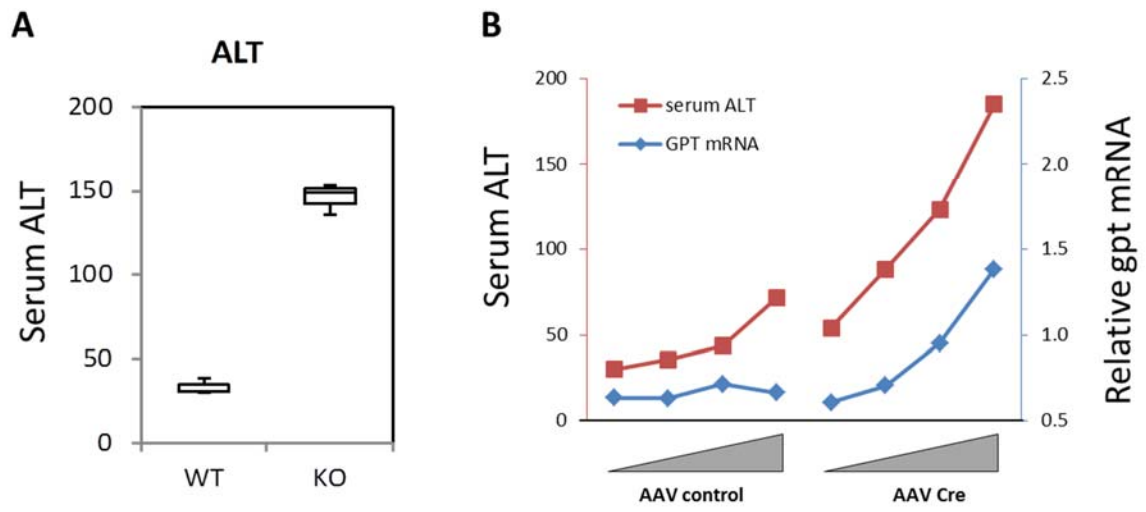


Figure S3

A

Top 20 upregulated In PRMT1 hepKO		Top 20 downregulated In PRMT1 hepKO	
Gene	log2	Gene	log2
Wnt1	4.15	Shh	-6.11
Ywhae	3.46	Cryab	-1.19
Melk	2.82	Rad50	-0.84
Tubb2a	2.75	Prkce	-0.79
Ttk	2.44	Nes	-0.76
Cdkn1a	2.42	Sirt1	-0.75
Ccnb1	2.08	Cdc45	-0.74
Btg2	1.86	Mre11a	-0.71
Src	1.78	Rictor	-0.69
Pbk	1.75	Tlk1	-0.69
Aurkb	1.73	Cdh13	-0.66
Rrm2	1.63	Cul4a	-0.64
Uhrf1	1.62	Ezh2	-0.62
Ccnb2	1.59	Rad17	-0.62
Ect2	1.56	Med12	-0.59
Cdk1	1.52	Kat2b	-0.58
Cdkn2a	1.49	Id4	-0.58
Ube2c	1.49	Brca2	-0.57
Foxm1	1.44	Prkaca	-0.56
Chek1	1.38	Smc1a	-0.55
Jun	1.37		

B

Upstream transcriptional regulators

	Z-score	p-value
FOXO1	3.461	8.01E-17
MITF	3.264	7.62E-11
CCND1	3.051	4.15E-20
CTNNB1	2.975	4.74E-07
E2F1	2.858	2.30E-29
FOXM1	2.646	4.43E-20
MYC	2.546	1.12E-19
RB1	-2.935	7.98E-22
CDKN2A	-2.445	3.46E-26
KDM5B	-2.309	4.29E-15
TP53	-2.265	4.64E-39
IRF4	-2.236	6.36E-05
HDAC2	-2.165	3.13E-05
NUPR1	-2.111	2.04E-07
E2F6	-2.000	9.16E-07
NRIP1	-2.000	9.16E-07
HDAC1	-1.951	2.56E-19
HNF4A	-1.941	1.10E-05

Figure S4

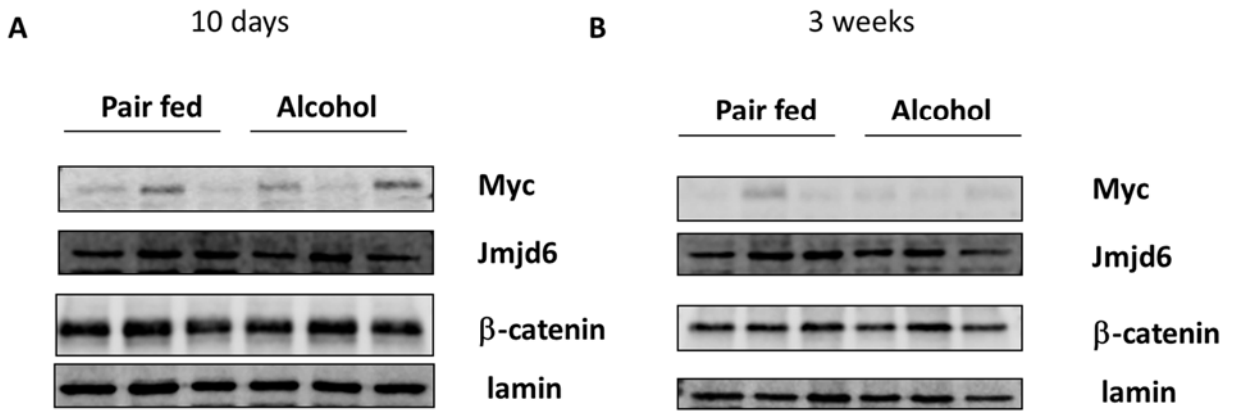


Figure S5

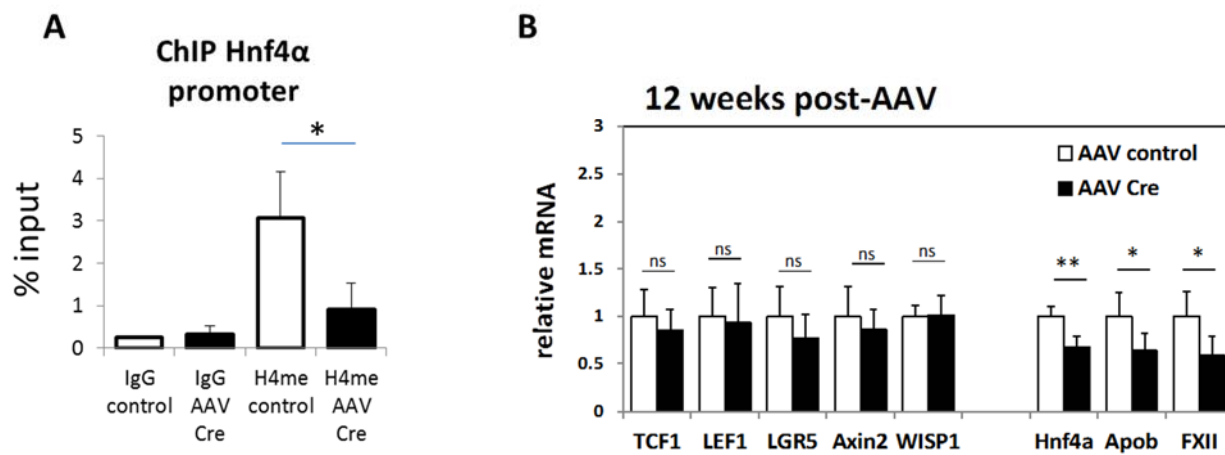
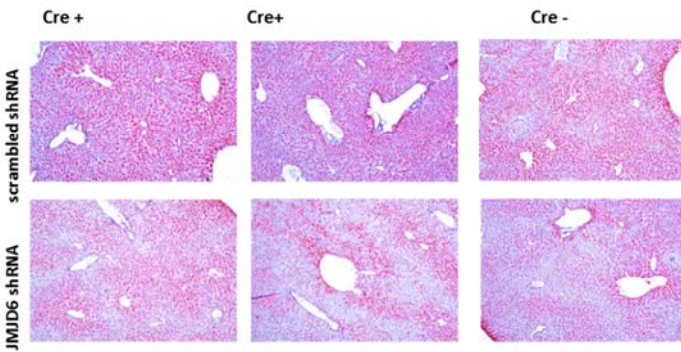


Figure S6

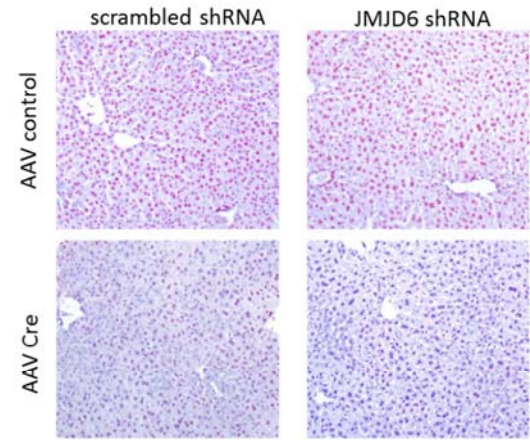
A

JMJD6 staining



B

PRMT1 staining



C

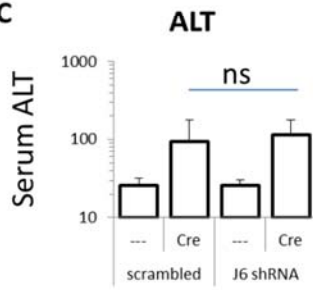
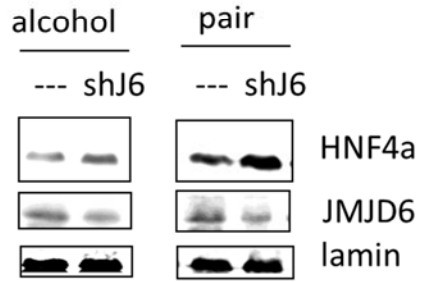
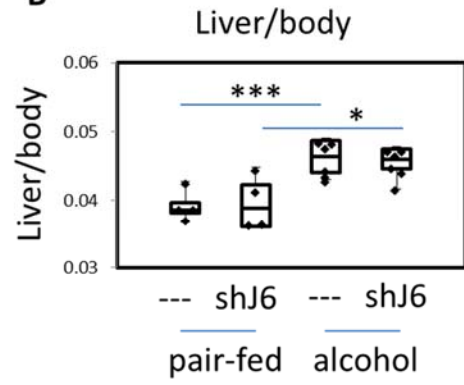


Figure S7

A



B



C

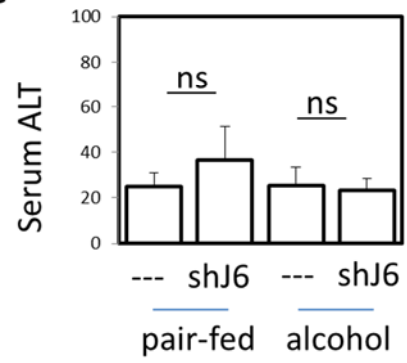


Figure S8

