Supplemental information

# SEL1L-HRD1 ER-associated degradation suppresses hepatocyte hyperproliferation and liver cancer

Asmita Bhattacharya, Juncheng Wei, Wenxin Song, Beixue Gao, Chunyan Tian, Shuangcheng Alivia Wu, Jian Wang, Ligong Chen, Deyu Fang, and Ling Qi

# Supplementary materials

Supplementary Figures S1-S6 and Table S2

SUPPLEMENTARY FIGURES AND LEGENDS

Figure S1. ***Sel1L^{Alb}*** **liver tumors show increased proliferation, Related to Figure 1**. (A, B) Quantitation of CYCLIN D1 (A) and KI67 staining (B) at the end of the DEN-HFD experiment, as shown in Figure 1i, j respectively (n=5 per group). Values, mean ± SEM; **, p<0.01 by Student's *t* test.
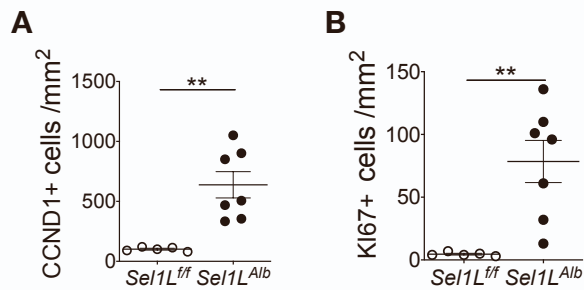
Figure S2. *Sel1L^Alb* **livers show elevated proliferation following injury, Related to Figure 3**.
(A-C) Two-thirds partial hepatectomy surgery of 10-week-old mice: (A) Quantitation of nuclear size as shown in Figure 3C; (B-C) Quantitation of CYCLIN D1 staining as shown in Figure 3D (n=4 per group); (D) Quantitation of KI67 staining as shown in Figure 3I (n=4-5 per group). (E-G) 2-month-old male mice were injected i.p. with 0.2 ml/kg body weight carbon tetrachloride (CCL4) dissolved in oil thrice a week for 3 months: (E-F) Serum levels of alanine aminotransferase (ALT) (E) and aspartate aminotransferase (AST) (F); (G) Representative images of hematoxylin-eosin staining;. Values mean ± SEM; **, p<0.01; ***, p<0.001; by Student's *t* test.
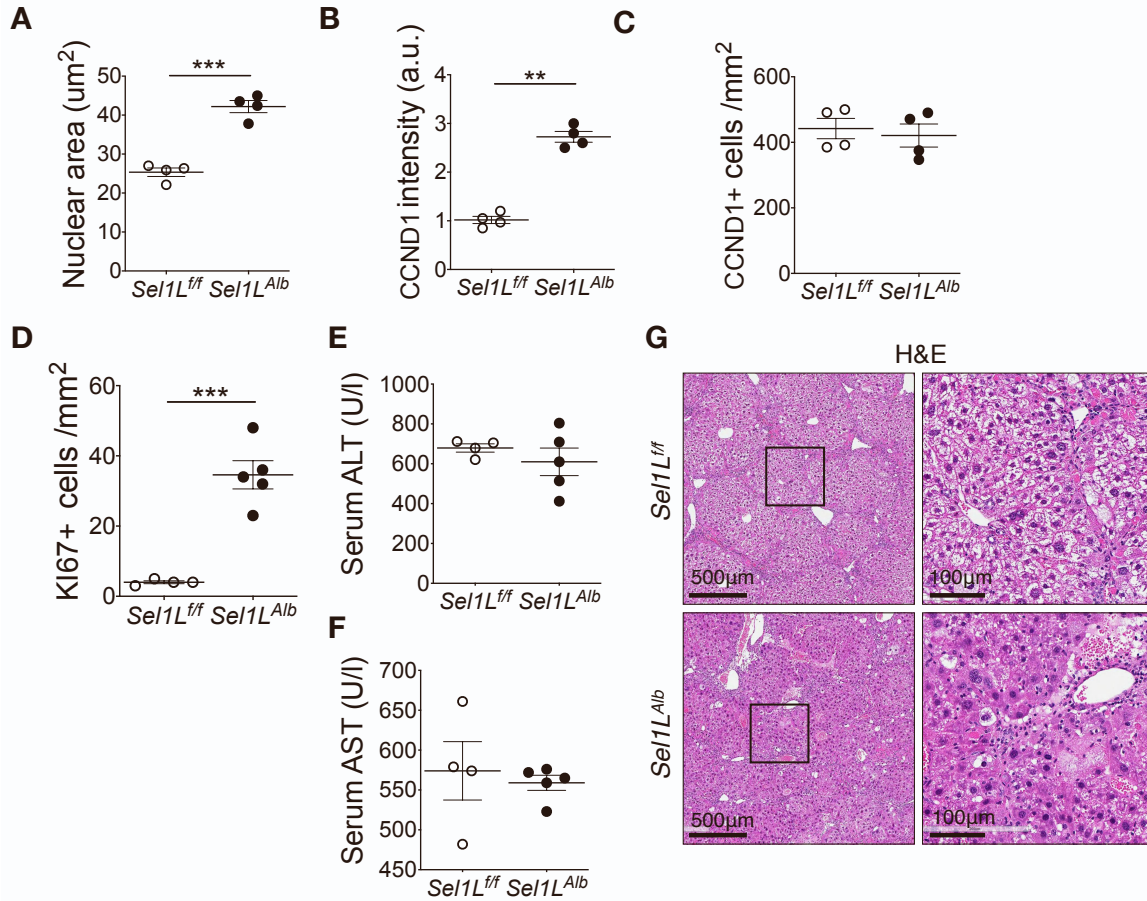
Figure S3. **Proliferation and WNT pathway gene expressions are altered in *Sel1L^Alb* livers, Related to Figure 4**. (A, B) qPCR mRNA analysis of *Wnt5A/B* (A) and cell cycle (B) genes in 2-month-old mouse livers (n=5 per group). (C) Hepatic cDNA microarray analyses of *Sel1L^Alb* and *Hrd1^Alb* livers as compared to *Sel1L^{f/f}* and *Hrd1^{f/f}* livers respectively: Heat map of genes associated with mature hepatocyte function as a logarithm of fold change (n=3 per group). *L32*, loading control. Values, mean ± SEM; *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$ by Student's *t* test.
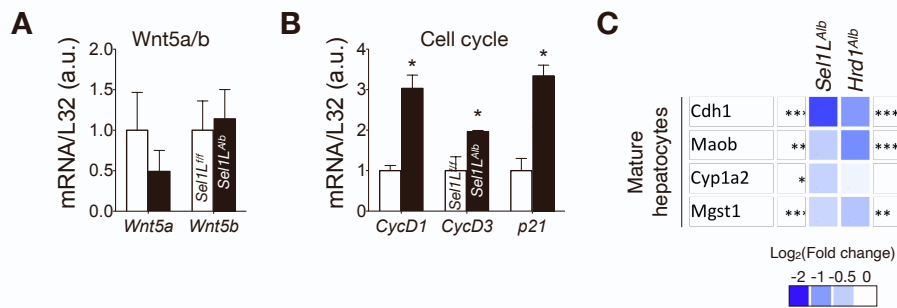
Figure S4. **WNT5B and WNT1 distribution are not altered in *Sel1L^Alb* livers, Related to Figure 5**. (A-B) Reducing and non-reducing SDS-PAGE and Western blot analyses of sucrose gradient fractions of liver lysates for WNT1 (A) and WNT5B (B) (n=3 per group). Loading controls are the same as those for Figure 5E.
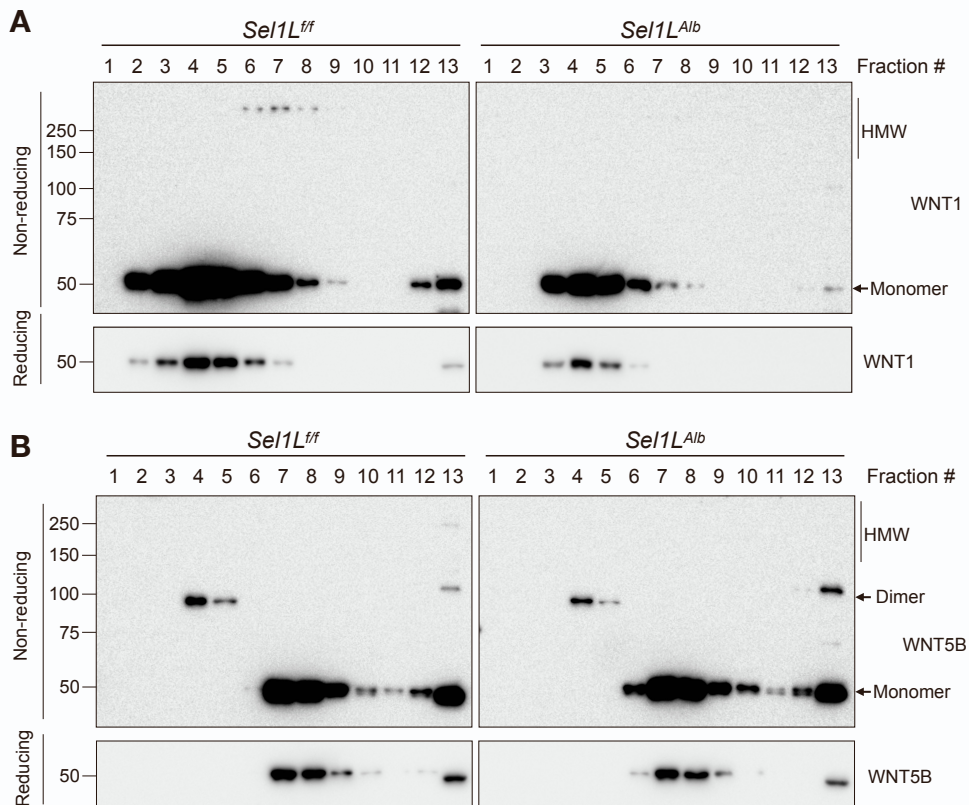
Figure S5. **WNT5A is a substrate of SEL1L-HRD1 ERAD, Related to Figure 6**. Western blot analysis of V5-WNT5A co-immunoprecipitated with SEL1L (A) and HRD1 (B) in HEK293T cells transfected with indicated plasmids (2 independent repeats). Hsp90, loading control.
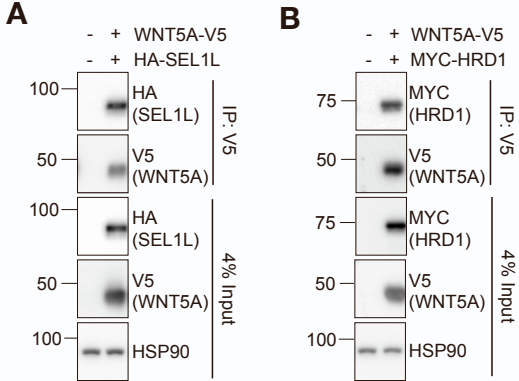
Figure S6, Related to Figure 7. **SEL1L-HRD1 have polymorphisms associated with human liver cancer**. (A) cBioPortal oncoprint of alterations in *SEL1L* and *HRD1* levels as analyzed from expression data obtained from 353 Liver Hepatocellular Carcinoma TCGA Pan Cancer Atlas samples. (B-C) Details of SNPs identified from the Liver Hepatocellular Carcinoma TCGA Pan Cancer Atlas dataset for *SEL1L* (B) and *HRD1* (C).

A
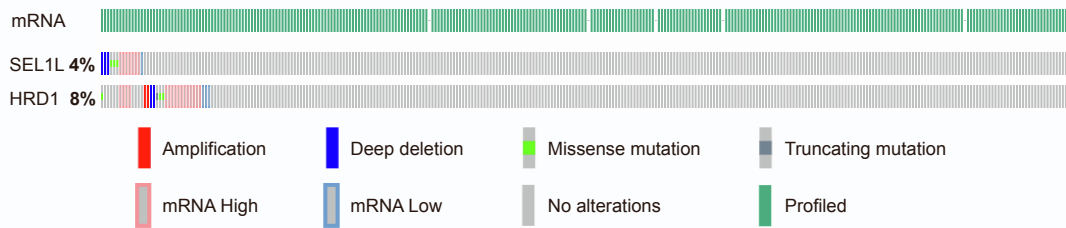
B

SEL1L			
AA change	Mutation type	Frequency of allele in tumor	# of mutations in sample
I5M	Missense	0.33	70
M455I	Missense	0.14	335
M735V	Missense	0.3	98

C

HRD1			
AA change	Mutation type	Frequency of allele in tumor	# of mutations in sample
F116V	Missense	0.17	48
F243L	Missense	0.32	87
A44=	Splice	0.36	124
A10V	Missense	0.1	93

Table S2, Related to STAR Methods. Primers and siRNA sequences used in this study.

Wnt5A_F	CAAATAGGCAGCCGAGAGAC
Wnt5A_R	CTCTAGCGTCCACGAACTCC
Wnt5B_F	CTGCTTGCGTAATGAGACCA
Wnt5B_R	AAAGCAACACCAGTGGAACC
CycD1_F	CACAACGCACTTTCTTTCCA
CycD1_R	ACCAGCCTCTTCCTCCACTT
CycD3_F	TAGGCGCCTGCTCTATGTCT
CycD3_R	ATCTGTGGGAGTGCTGGTCT
p21_F	ACAAGAGGCCCAGTACTTCC
p21_R	GGGCACTTCAGGGTTTTCTC
L32_F	GAGCAACAAGAAAACCAAGCA
L32_R	TGCACACAAGCCATCTACTCA
siRNA_Wnt5A_1	CCACTTGTATCAGGACCACAT
siRNA_Wnt5A_2	GAGTTCGTGGACGCTAGAGAA
siRNA_luciferase	GTTGCGCGGAGGAGTTGTG