

Supplemental material

Muto et al., <https://doi.org/10.1084/jem.20180900>

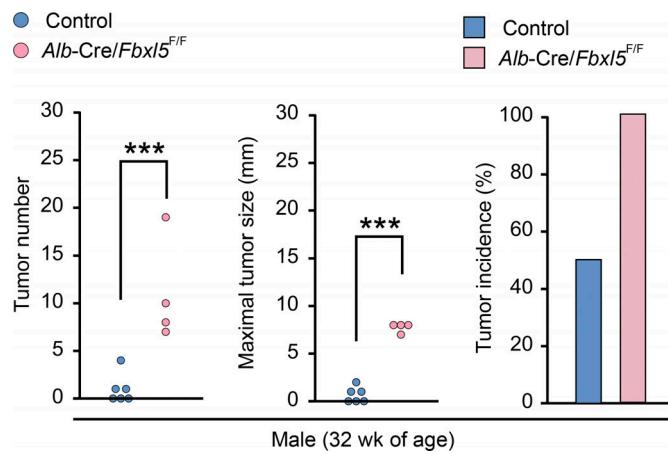


Figure S1. FBXLS deficiency increases DEN-induced hepatic tumor incidence. Tumor multiplicity, maximal tumor diameter, and tumor incidence were determined for the liver of DEN-injected *Fbxl5*^{F/F} ($n = 6$) or *Alb-Cre/Fbxl5*^{F/F} ($n = 4$) male mice at 32 wk of age. Horizontal bars are means. ***, $P < 0.005$ (Mann-Whitney *U* test).

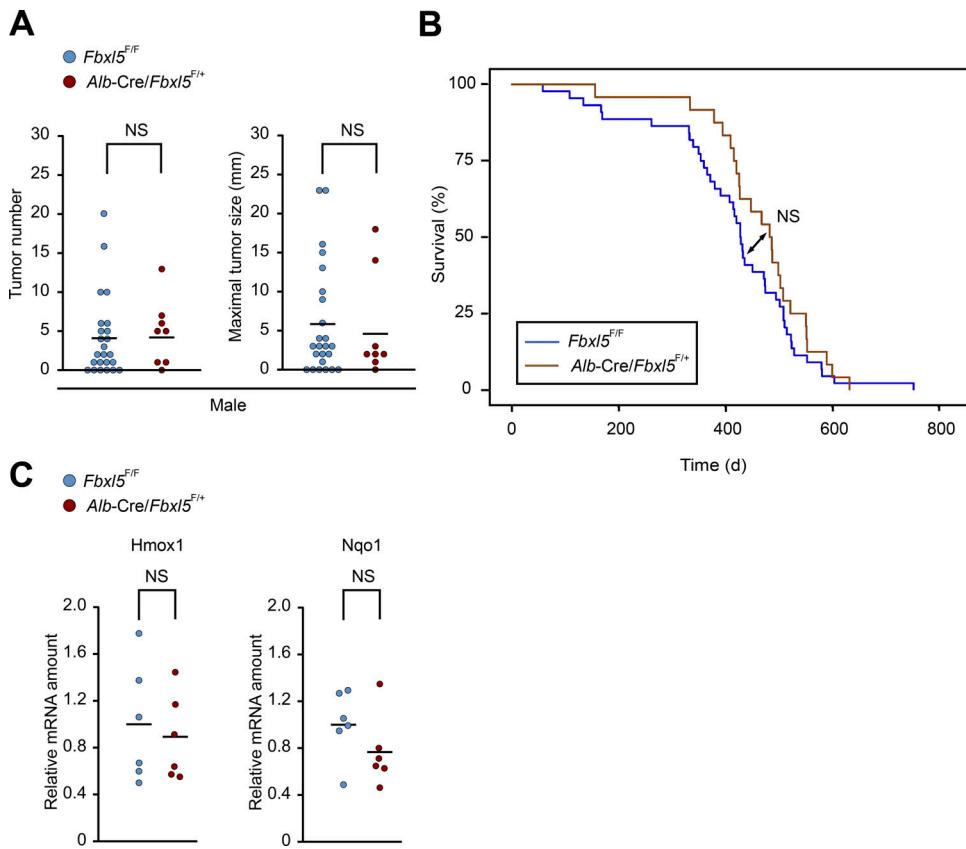


Figure S2. *Alb-Cre* activity is not associated with DEN-induced liver carcinogenesis. **(A)** Tumor multiplicity and maximal tumor diameter for the liver of DEN-injected *Fbxl5*^{F/F} ($n = 24$, also shown in Fig. 1 B) or *Alb-Cre/Fbxl5*^{F/+} ($n = 8$) male mice at 36 wk of age. Statistical analysis was performed with the Mann–Whitney *U* test. **(B)** Survival rate of DEN-injected *Fbxl5*^{F/F} ($n = 44$, also shown in Fig. 2 A) or *Alb-Cre/Fbxl5*^{F/+} ($n = 24$) male mice. Statistical analysis was performed with the log-rank test. **(C)** RT and real-time PCR analysis of mRNAs for proteins related to oxidative stress in nontumor liver tissue of DEN-injected *Fbxl5*^{F/F} ($n = 6$) or *Alb-Cre/Fbxl5*^{F/+} ($n = 6$) male mice at 36 wk of age. Horizontal bars are means. Statistical analysis was performed with the Student's *t* test.

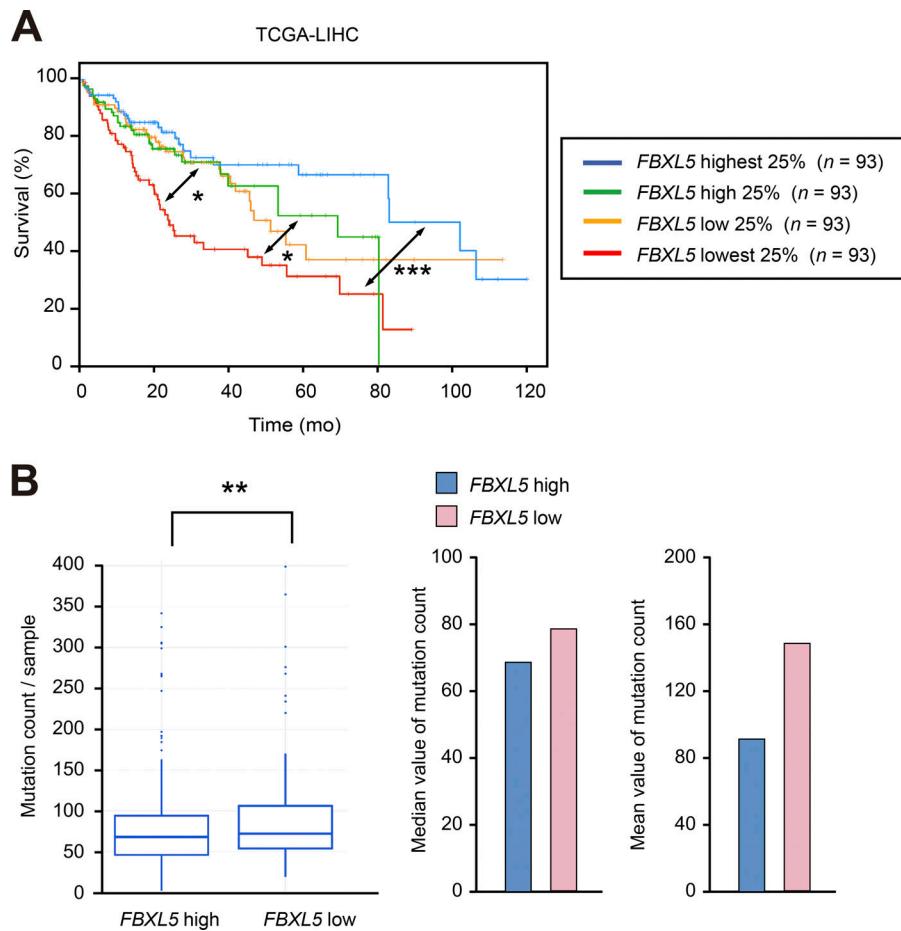


Figure S3. Low *FBXL5* expression is associated with increased mutation load in HCC. **(A)** Survival curves for HCC patients in the TCGA-LIHC dataset subdivided into quartile groups ($n = 93$) based on *FBXL5* expression level. *, $P < 0.05$; ***, $P < 0.005$ (log-rank test). **(B)** Box-and-whisker plots as well as median and mean values for mutation load in HCC with high or low levels of *FBXL5* expression for the TCGA-LIHC data shown in Fig. 7 A. Box-and-whisker plots show median, interquartile range, and most extreme data point less than 1.5 interquartile range from box. **, $P < 0.01$ (Mann-Whitney *U* test).

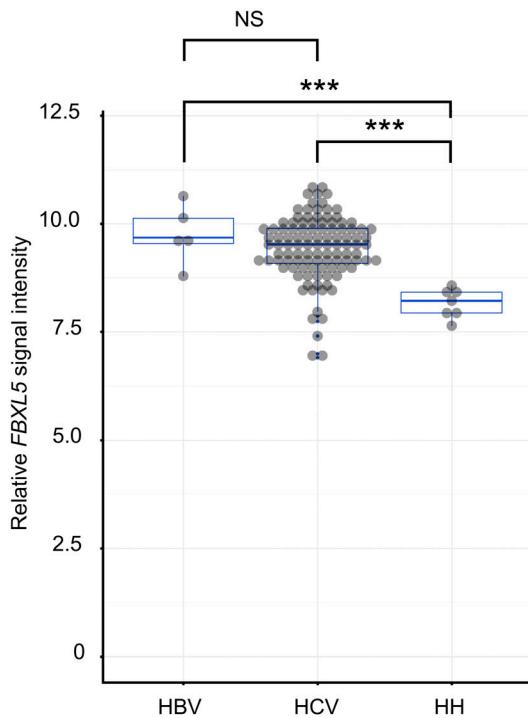


Figure S4. *FBXL5* expression is down-regulated in HCC with hemochromatosis. Microarray analysis of *FBXL5* expression in HCC of patients with HBV infection ($n = 5$), HCV infection ($n = 107$), or hemochromatosis (HH, $n = 7$) is shown for the E-MTAB950 dataset. Each point represents an individual patient. ***, $P < 0.005$; NS (Mann-Whitney U test). Box-and-whisker plots show median, interquartile range, and most extreme data point less than 1.5 interquartile range from box.

Table S1. Clinical information for the HCC patients and signal intensities of the immunoblot bands in Fig. 8

| Patient ID | Survival | Status | IRP2 signal | TFR1 signal |
|------------|----------|----------|-------------|-------------|
| 1 | 1,383 | Deceased | 6,313.669 | 2,721.062 |
| 2 | 2,516 | Deceased | 22,057.74 | 6,578.79 |
| 3 | 3,161 | Alive | 2,686.527 | 636.991 |
| 4 | 951 | Alive | 19,694.79 | 42,420.447 |
| 5 | 560 | Deceased | 23,775.205 | 11,140.426 |
| 6 | 3,994 | Alive | 6579.234 | 5,088.355 |
| 7 | 3,932 | Alive | 11,023.669 | 9,340.841 |
| 8 | 185 | Deceased | 10,163.841 | 18,567.79 |
| 9 | 381 | Alive | 9,278.012 | 1,344.941 |
| 10 | 78 | Deceased | 4,482.598 | 321.577 |
| 11 | 683 | Deceased | 9,651.134 | 534.82 |
| 12 | 2,909 | Alive | 5,382.598 | 1,008.527 |
| 13 | 1,075 | Deceased | 5,826.669 | 2,587.012 |
| 14 | 2,946 | Alive | 6,863.012 | 1,681.941 |
| 15 | 2,885 | Alive | 7,232.719 | 4,373.477 |
| 16 | 1,947 | Alive | 14,233.548 | 8,869.184 |
| 17 | 1,444 | Deceased | 23,442.548 | 7,677.598 |
| 18 | 2,779 | Alive | 41,271.861 | 3,678.82 |
| 19 | 641 | Deceased | 10,006.355 | 27,774.083 |
| 20 | 519 | Deceased | 7,475.82 | 26,817.012 |
| 21 | 152 | Alive | 40,459.569 | 41,790.447 |
| 22 | 2,370 | Alive | 4,816.012 | 4,494.012 |
| 23 | 2,328 | Alive | 3,603.205 | 385.092 |