

**Supplementary Figure 1.** (**A**) iBAQ plot showing MS-enrichment of FBW7 and interaction partners over a control IgG. (**B**) Western blots for indicated proteins in cells transfected with indicated shRNAs. (**C**) Western blots for indicated proteins in cells co-transfected with Flag-FBW7 $\beta$  and indicated shRNAs.



## Supplementary Figure 2. USP9X cleaves K48-linked polyubiquitin chains on FBW7.

(A) Recombinant USP9X does not affect polyubiquitination of c-JUN in an in vitro deubiquitylation reaction. (B) Schematic for experiment in C. (C) Ubiquitin chain restriction (UbiCRest) experiment with indicated deubiquitinases: USP2 (promiscuous), OTUBAIN1 (K48-linked), and AMSH (K63-linked).



**Supplementary Figure 3.** Accumulation of SCF(FBW7) substrates in cells transfected with *USP9X*-shRNA compared to a non-targeting control.



С

Usp9x<sup>+/+</sup>

Usp9x<sup>∆G</sup>

Β



Supplementary Figure 4. (A) IHC sections stained for proliferating cells (MCM6) from the intestine of indicated mice. Scale bar = 50 µm. (B) BrdU staining for proliferating cells in colonic crypts from indicated mice, quantification shown in right panel. Scale bar = 100  $\mu$ m, n = 3-4 mice/group. (C) In situ hybridization for Olfm4 (stem cells) on the sections from the intestine of indicated mice. Scale bar = 100 μm. (D and E) IHC for enterocytes (Alkaline phosphatase) and enteroendocrine cells (Chromogranin) in gut from indicated mice. Scale bars = 100  $\mu$ m. (F) qRT-PCR analysis showing mRNA levels of indicated genes normalised to actin and represented as fold change over control, in isolated crypts from  $Usp9x^{+/+}$  (wildtype) and  $Usp9x^{\Delta G}$ aut, n = 5-8 mice/group.



**Supplementary Figure 5.** (**A**) Average goblet cell number per villus from indicated mice. (**B**) Average BrdU+ cell number per crypt from indicated mice.



**Supplementary Figure 6.** (**A** and **B**) Weight curves presented as percent of starting weight in indicated mice from colitis-driven tumorigenesis experiment, n = 5-9 animals/genotype. *P* values were calculated by Log-rank (Mantel-Cox) test.

**Supplementary Table.** Shortlisted candidates from IP-mass spectrometry experiment using endogenous FBW7 as bait.

Gene Symbol	Description	Function
FBXW7	F-box/WD repeat-containing protein 7	E3 Ligase
SKP1	S-phase kinase-associated protein 1	SCF Component
UBAP2L	Ubiquitin-associated protein 2-like	Ubiquitylation
MYCBP2	MYC binding protein 2	Probable E3 ubiquitin-protein ligase
HERC2	E3 ubiquitin-protein ligase HERC2	E3 Ligase
HUWE1	E3 ubiquitin-protein ligase HUWE1	E3 Ligase
HECTD1	E3 ubiquitin-protein ligase HECTD1	E3 Ligase
USP9X	Probable ubiquitin carboxyl-terminal hydrolase FAF-X	Deubiquitinase
USP20	Ubiquitin carboxyl-terminal hydrolase 20	Deubiquitinase
FKBP8	Peptidyl-prolyl cis-trans isomerase FKBP8	Chaperon
FKBP4	Peptidyl-prolyl cis-trans isomerase FKBP4	Chaperon
PSMA2	Proteasome subunit alpha type-2	Proteasome subunit
PSMA3	Proteasome subunit alpha type-3	Proteasome subunit
PSMA6	Proteasome subunit alpha type	Proteasome subunit
PSMD3	26S proteasome non-ATPase regulatory subunit 3	Proteasome subunit
FOXP2	Forkhead box protein P2	Transcription factor
c-JUN	Transcription factor AP-1	Transcription factor
STAT1	Signal transducer and activator of transcription 1-alpha/beta	Transcription factor
MSH6	DNA mismatch repair protein Msh6	DNA replication/Genome integrity
MCM3	DNA replication licensing factor MCM3	DNA replication/Genome integrity
MCM7	DNA replication licensing factor MCM7	DNA replication/Genome integrity
TOP1	DNA topoisomerase 1	DNA replication/Genome integrity
RAD21	Double-strand-break repair protein rad21 homolog	DNA replication/Genome integrity
NAP1L1	Nucleosome assembly protein 1-like 1	DNA replication/Genome integrity
HDAC2	Histone deacetylase 2;Histone deacetylase	DNA replication/Genome integrity
CHD4	Chromodomain-helicase-DNA-binding protein 4	DNA replication/Genome integrity
MRE11A	Double-strand break repair protein MRE11A	DNA replication/Genome integrity
DNAJB6	DnaJ homolog subfamily B member 6	DNA replication/Genome integrity
MTOR	Serine/threonine-protein kinase mTOR	Kinase
STK3	Serine/threonine-protein kinase 3	Kinase
ΡΑΝΚ2	Pantothenate kinase 2, mitochondrial	Kinase
GALK1	Galactokinase	Kinase
DEK	Protein DEK	Kinase
CSNK1A1	Casein kinase I isoform alpha	Kinase
РІ4КВ	Phosphatidylinositol 4-kinase beta	Kinase
WEE1	Wee1-like protein kinase	Kinase
XPO1	Exportin-1	Nuclear export
CSE1L	Exportin-2	Nuclear export
KPNB1	Importin subunit beta-1	Nuclear import
RANBP1	Ran-specific GTPase-activating protein	Transport
LUC7L	Putative RNA-binding protein Luc7-like 1	RNA binding/processing

DDX39	ATP-dependent RNA helicase DDX39A	
MARS	Methionine-tRNA ligase, cytoplasmic	
RAE1	mRNA export factor	
BCAS2	Pre-mRNA-splicing factor SPF27	
LARS	LeucinetRNA ligase, cytoplasmic	
RBM39	RNA-binding protein 39	
RBM28	RNA-binding protein 28	
DDX24	ATP-dependent RNA helicase DDX24	
TARS	ThreoninetRNA ligase, cytoplasmic	
FMR1	Fragile X mental retardation protein 1	
IARS	IsoleucinetRNA ligase, cytoplasmic	
YBX2	DNA-binding protein A;Y-box-binding protein 2	
MLL	Histone-lysine N-methyltransferase	
RPN2	DPD glycosyltransferase subunit 2	
DPM1	Dolichol-phosphate mannosyltransferase	
PRMT1	Protein arginine N-methyltransferase 1	
ACLY	ATP-citrate synthase	
EIF4G1	Eukaryotic translation initiation factor 4 gamma 1	
EIF3F	Eukaryotic translation initiation factor 3 subunit F	
EIF2B4	Translation initiation factor eIF-2B subunit delta	
EIF5B	Eukaryotic translation initiation factor 5B	
MFF	Mitochondrial fission factor	
SSBP1	Single-stranded DNA-binding protein, mitochondrial	
ATP5J2	ATP synthase subunit f, mitochondrial	
MTCH2	Mitochondrial carrier homolog 2	
MAGED2	Melanoma-associated antigen D2	
ACTR2	Actin-related protein 2	
TUBA1C	Tubulin alpha-1C chain	
TUBB2A	Tubulin beta-2A chain	

RNA binding/processing Enzyme Enzyme Enzyme Enzyme Enzyme Translation Translation Translation Translation Mitochondrial Mitochondrial Mitochondrial Mitochondrial Cell adhesion Cytoskeleton Cytoskeleton Cytoskeleton