Supplemental tables

Table S1. The detail information of the lentivirus library

Gene name	shRNA-derived lentivirus number
Ctrl	Negative control（Non-targeting shRNA）
PC	Oncogene X specific-targeting shRNA
OTUB2	PSC40498mix
USP6	PSC49026mix
USP34	PSC49023mix
USP7	PSC49020mix
USP9X	PSC49014mix
PAN2 （USP52）	PSC49011mix
UCHL5	PSC49005mix
USP29	PSC48993mix
Cyld	PSC48990mix
OTUD7B （Cezanne）	PSC48984mix
USP5	PSC49032mix
USP32	PSC48987mix
USP3	PSC49035mix
USP24	PSC48999mix
USP1	PSC49038mix
USP33	PSC49029mix
Mpnd	PSC49017mix
USP21	PSC49002mix
USP15	PSC49008mix
OTUD4	PSC48996mix

Table S2. The primers for PCR amplification of the regulatory regions of USP5

Regulatory sequence Region	Primer Direction	Sequences (5'-3')
-2000/+32	Forward	**GGGGTACC**TGCCACCGTGCTTTTGTGTGTGT
	Reverse	**CCCAAGCTT**GACACCGGCAGCAGCTTCTCC
-1500/+32	Forward	**GGGGTACC**TCCTCCCCATATTCCAGGAAG
	Reverse	**CCCAAGCTT**GACACCGGCAGCAGCTTCTCC
-1000/+32	Forward	**GGGGTACC**GCAGCTGACAAAATGGCTCGTTC
	Reverse	**CCCAAGCTT**GACACCGGCAGCAGCTTCTCC
-500/+32	Forward	**GGGGTACC**GATAGTAGCTTTATTGGTTGAC
	Reverse	**CCCAAGCTT**GACACCGGCAGCAGCTTCTCC
-300/+32	Forward	**GGGGTACC**TGGACTCTAAGGGTCCCGGAGC
	Reverse	**CCCAAGCTT**GACACCGGCAGCAGCTTCTCC
-230/+32	Forward	**GGGGTACC**GCTGCTCTACGTGCGCTCCCG
	Reverse	**CCCAAGCTT**GACACCGGCAGCAGCTTCTCC
-160/+32	Forward	**GGGGTACC**GCTTCTCATTGGCGTCAGTCA
	Reverse	**CCCAAGCTT**GACACCGGCAGCAGCTTCTCC
-100/+32	Forward	**GGGGTACC**CCAACTGCCATTCTCGCGCGTCGT
	Reverse	**CCCAAGCTT**GACACCGGCAGCAGCTTCTCC
-74/+32	Forward	**GGGGTACC**CCGCGGCGCATGCCCCTA
	Reverse	**CCCAAGCTT**GACACCGGCAGCAGCTTCTCC
-230/-75	Forward	**GGGGTACC**GCTGCTCTACGTGCGCTCCCG
	Reverse	**CCCAAGCTT**AGACGACGCGCGAGAATGGCA

Table S3. Multivariable OS analyses for USP5 expression in CRC cells in CRC patients

	OS	
	HR (95%CI)	***p***
USP5 Positive	2.148(1.032-4.624)	**0.041***
Age	1.035(1.006-1.065)	**0.017***
Gender	0.815(0.395-1.682)	0.581
M stage	3.589(1.244-10.358)	**0.018***

NOTE: Multivariable analysis adjusted for age, gender, M stages. *p<0.05.

Supplemental figures and figure legends

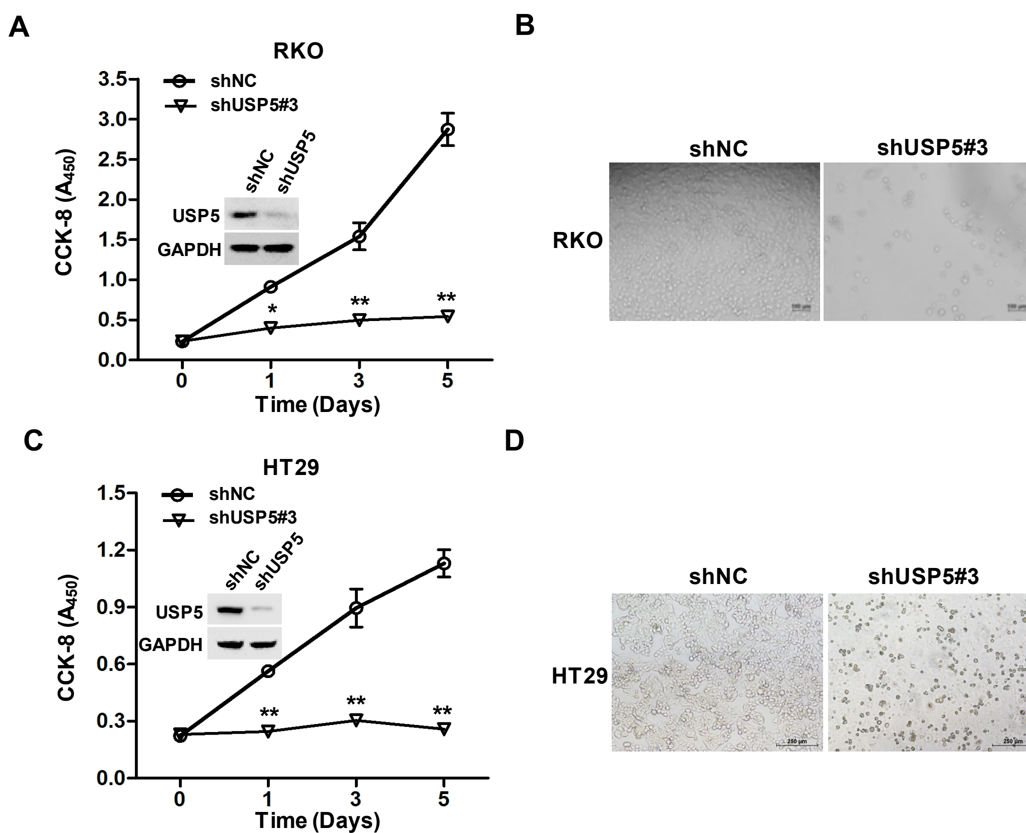

Figure S1. Knockdown of USP5 inhibits cell growth in RKO and HT29 cells. A & B. RKO cells were stably infected with lentiviral shUSP5#3 or control, followed by CCK-8 staining at day 0, 2, 4 and 6 (A). Immunoblotting assay was also performed against USP5 and GAPDH at day 5 (A). At the end of the experiment, the photos were taken (B). **C & D.** HT29 cells were stably infected with lentiviral shUSP5#3 or control, followed by CCK-8 staining at day 0, 2, 4 and 6 (C). Immunoblotting assay was also performed against USP5 and GAPDH at day 5 (C). At the end of the experiment, the photos were taken (D). $^{*}p$<0.01; $^{**}p$<0.01.

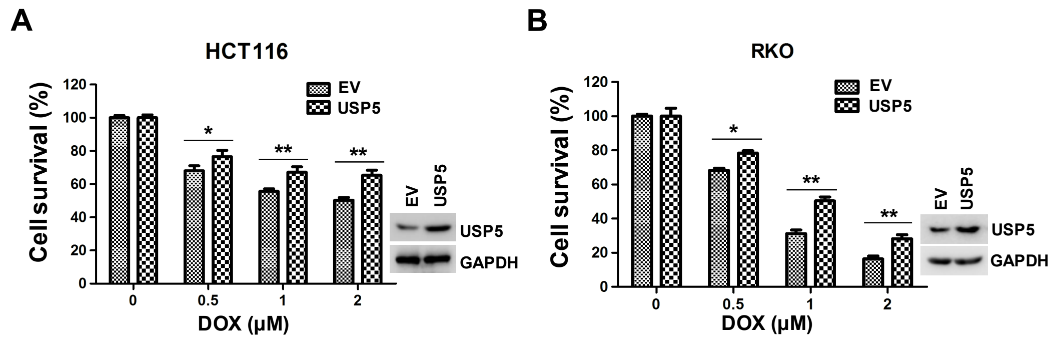

Figure S2. Overexpression of USP5 decreases the sensitivity of doxorubicin to colorectal cancer cells. A & B. Empty vector (EV) or USP5 plasmids were transfected into HCT116 (A) or RKO cells (B). Twenty-four hours later, the cells were treated with indicated concentrations of doxorubicin (DOX) overnight and then evaluated by CCK-8 assay or prepared for immunoblotting analysis.

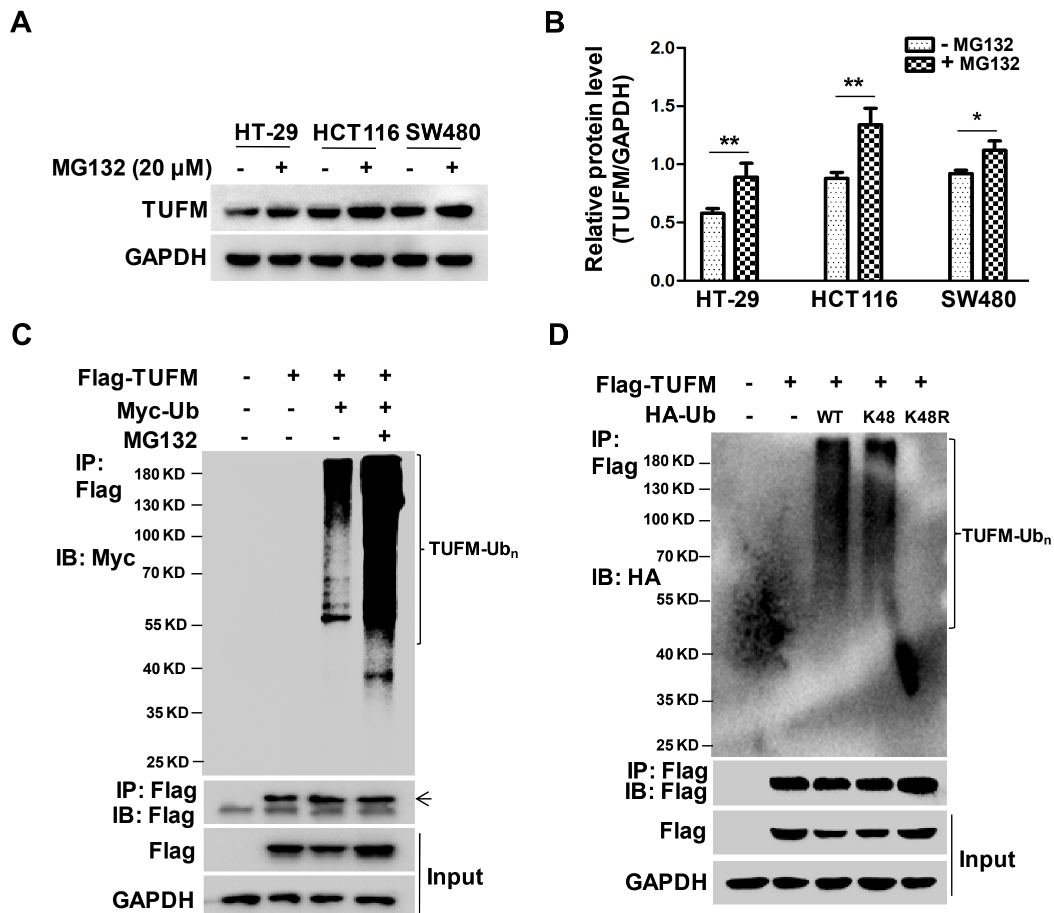

Figure S3. MG132 upregulates endogenous TUFM expression in colorectal

cancer cells, and TUFM ubiquitination was mediated by Ub-K48. A. HT-29, HCT116 and SW480 cells were treated by 20 μM MG132 for 6 hours, and then cells were prepared for immunoblotting against TUFM. GAPDH was used as a loading control. **B.** Statistically analysis of Figure A (mean +/- SD). **C.** HEK293T cells were transfected with Flag-TUFM or Myc-Ub. Twenty-four hours later, cells were treated with 20 μM MG132 for 6 hours, and cells were prepared for co-immunoprecipitation to detect the protein ubiquitination of TUFM. Whole cell lysates were used for immunoblotting against Flag and GAPDH. **D.** HEK293T cells were transfected with Flag-TUFM, HA-Ub-WT, HA-Ub-K48 or HA-Ub-K48R. Thirty-six hours later, cells were prepared for co-immunoprecipitation to detect the protein ubiquitination of TUFM. Whole cell lysates were used for immunoblotting against Flag and GAPDH.

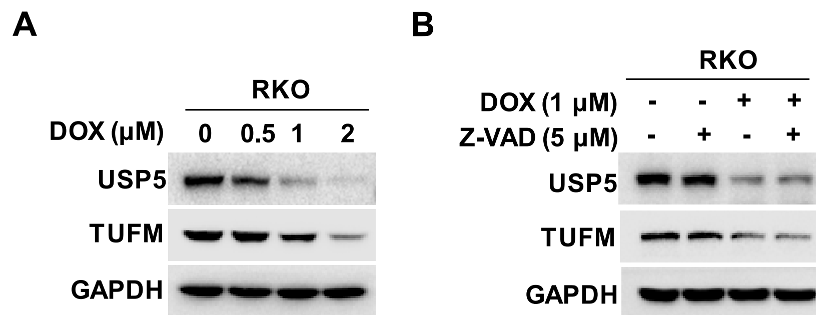

Figure S4. Doxorubicin inhibits USP5-TUFM axis. A. RKO cells were treated with doxorubicin (DOX) for 24 hours, and then cells were prepared for immunoblotting against USP5, TUFM and GAPDH. **B.** RKO cells were treated with DOX or Caspase inhibitor Z-VAD for 24 hours, followed by immunoblotting against USP5 and TUFM. GAPDH was used as a loading control.

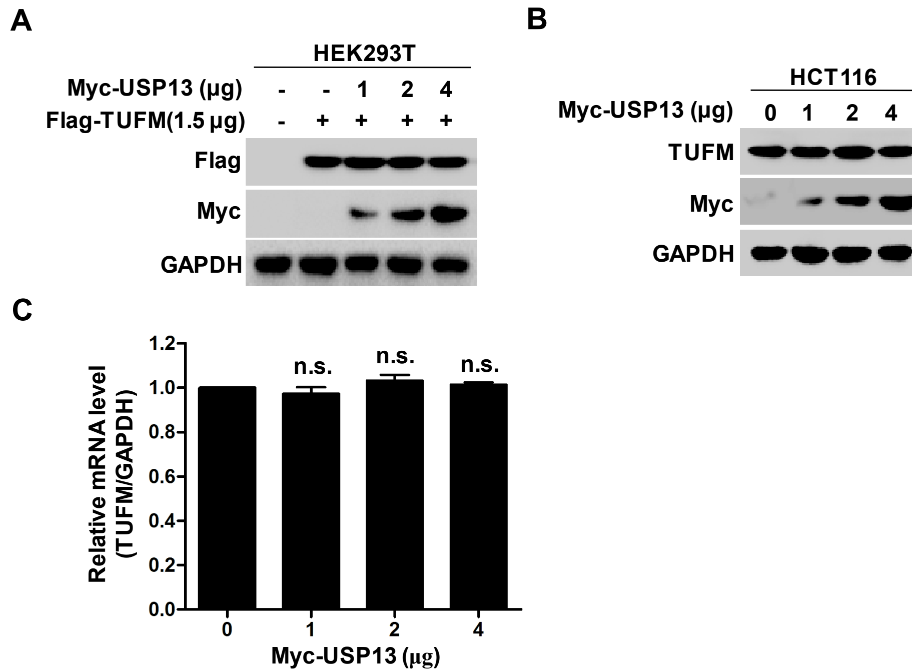

Figure S5. USP13 does not play roles in TUFM stabilization. A. Myc-USP13 and Flag-TUFM were co-transfected into HEK293T cells. Twenty-four hours later, cells were prepared for immunoblotting analysis against Flag, Myc and GAPDH. **B & C.** HCT116 cells were transfected with increased Myc-USP13 plasmids, followed by immunoblotting against TUFM, Myc and GAPDH (B), or qRT-PCR against TUFM and GAPDH (C).