Supplementary materials

Prostate-specific Oncogene OTUD6A Promotes Prostatic Tumorigenesis via Deubiquitinating and Stabilizing c-Myc

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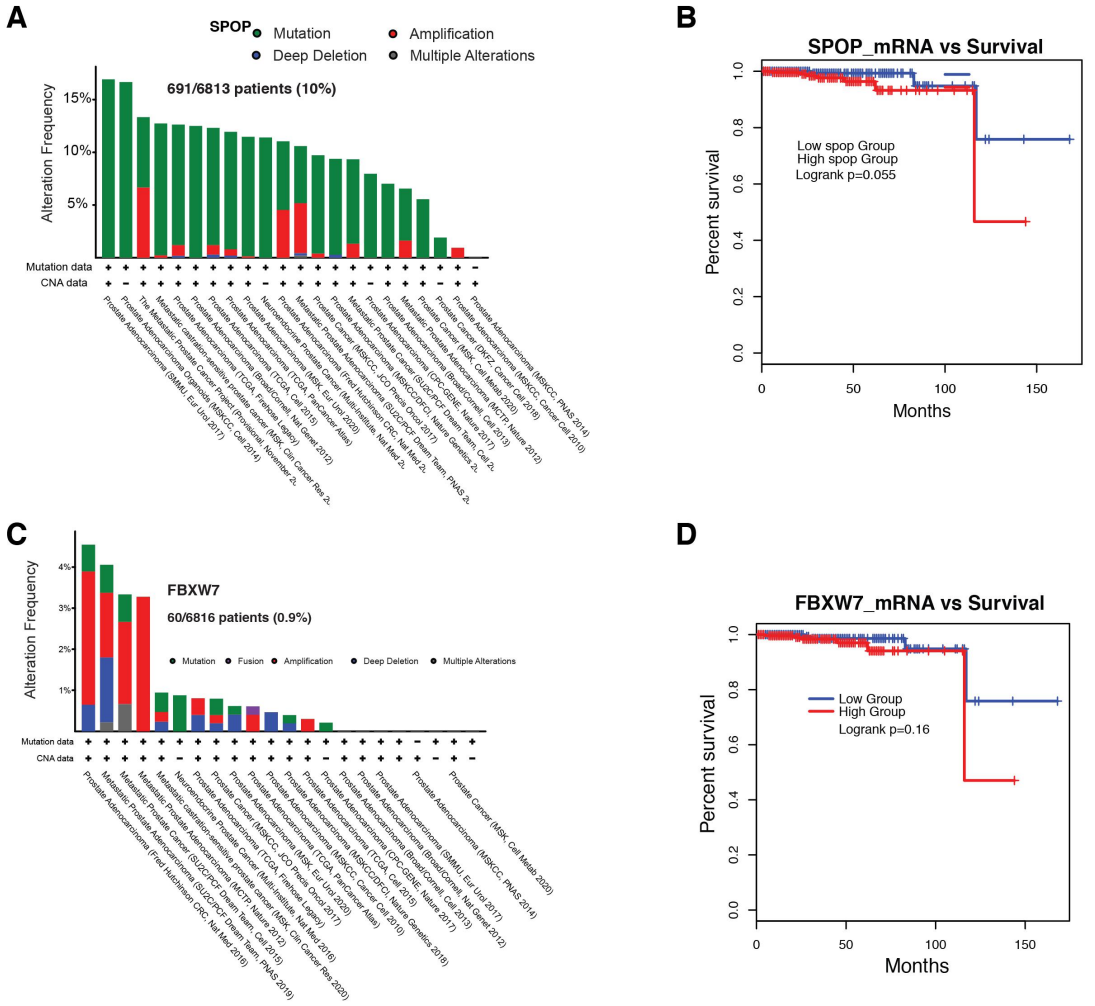
Supplementary Figures. 1-9



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21 **Supplementary figure 1. c-Myc protein overexpression in prostate cancer (PrCa)**

22 **patients might be due to both the transcriptional and post-translational regulation.**

23 **(A)** SPOP, a ubiquitin E3 ligase of c-Myc in PrCa setting is highly mutated in PrCa

24 patients. **(B)** SPOP expression is not a prognostic marker for PrCa patients. **(C)** The

25 tumor suppressive FBXW7, another ubiquitin E3 ligase of c-Myc is not frequently

26 mutated in PrCa patients. **(D)** FBXW7 is not a prognostic marker for PrCa patients.

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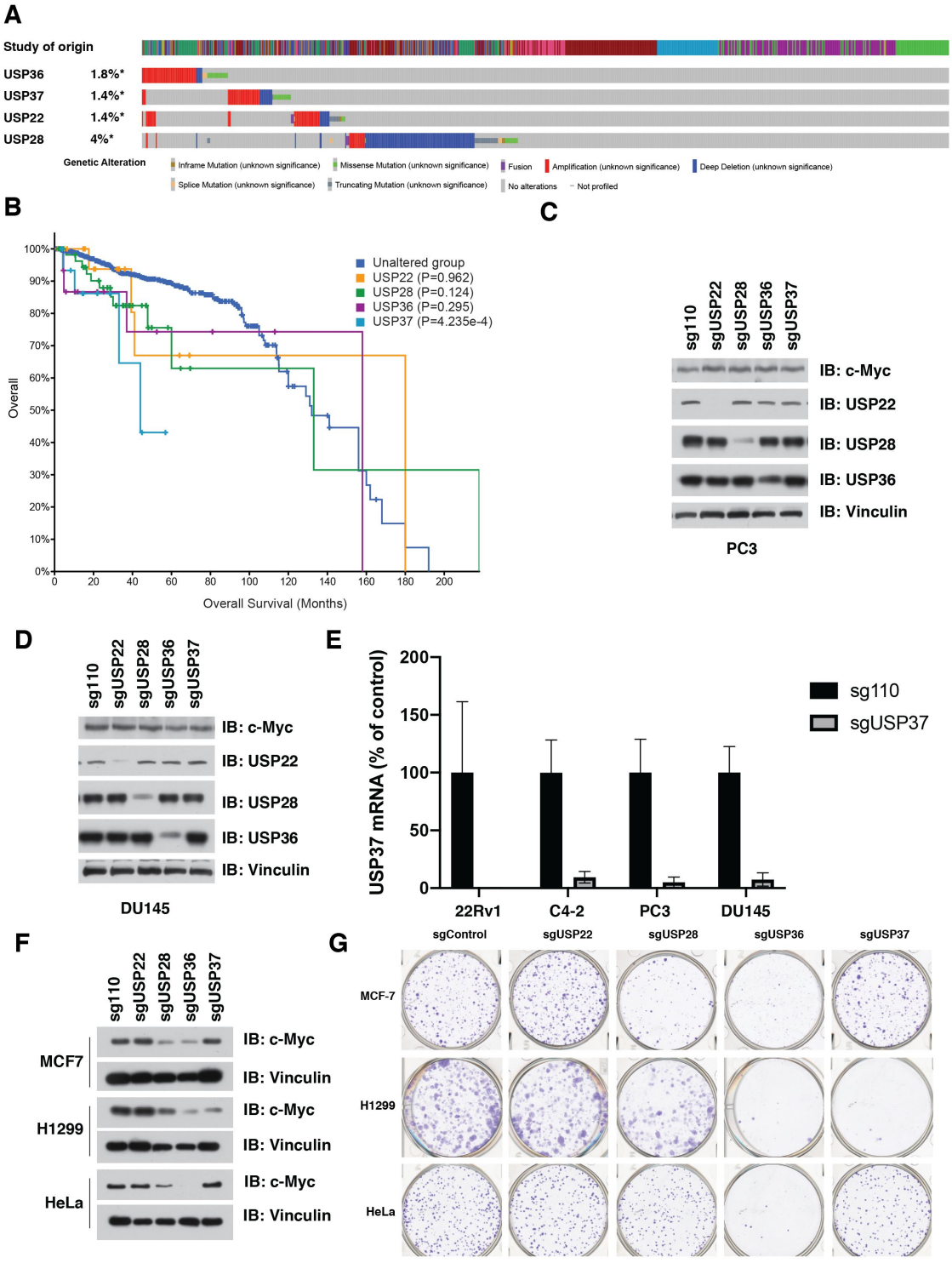
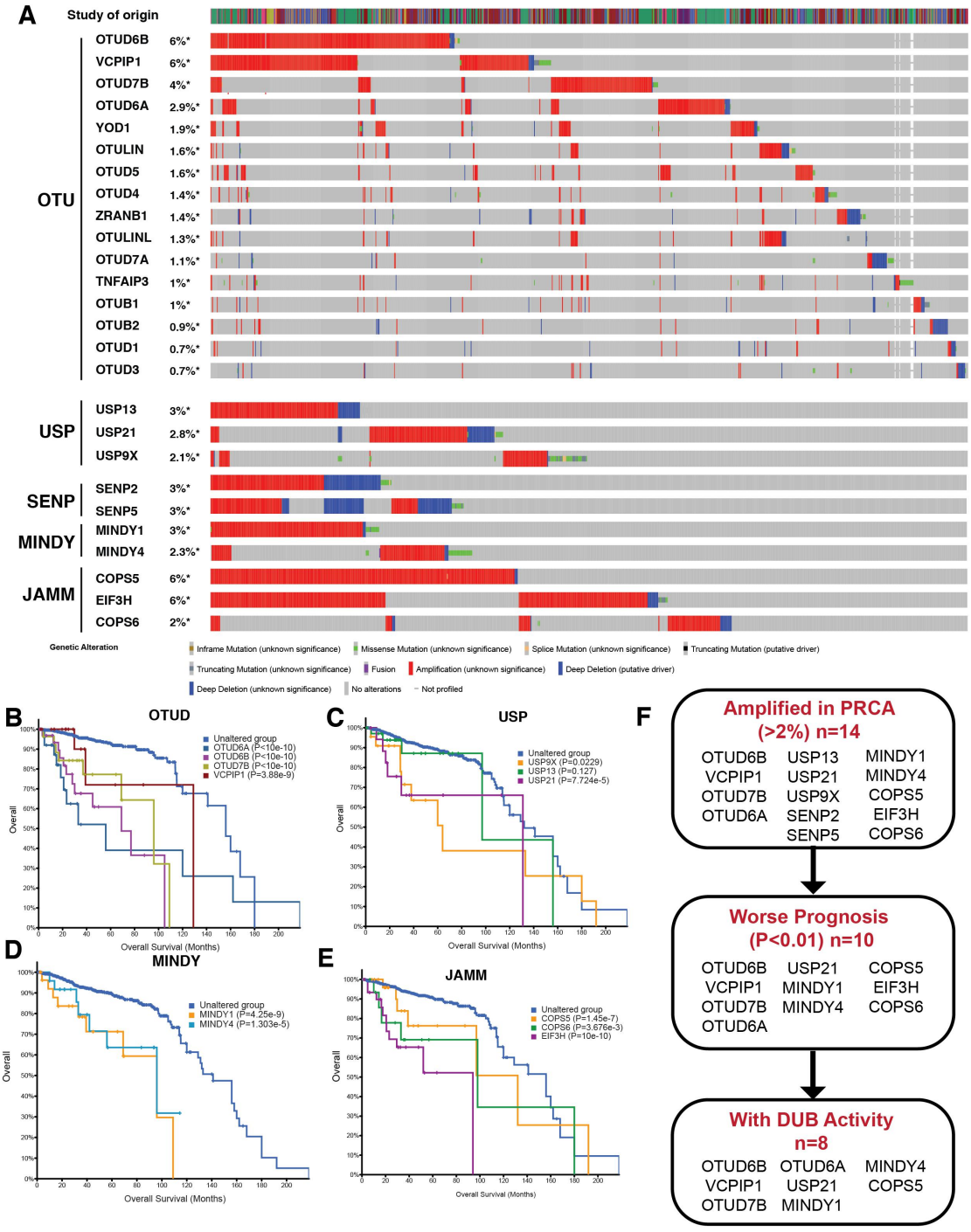


Supplementary figure 2. The USPs are not physiological DUBs for c-Myc oncoprotein in PrCa cells. (A) Genetic variations, including copy number alternation (CNA) or point mutations, of of USP22, USP28, USP36 and USP37 in human PrCa

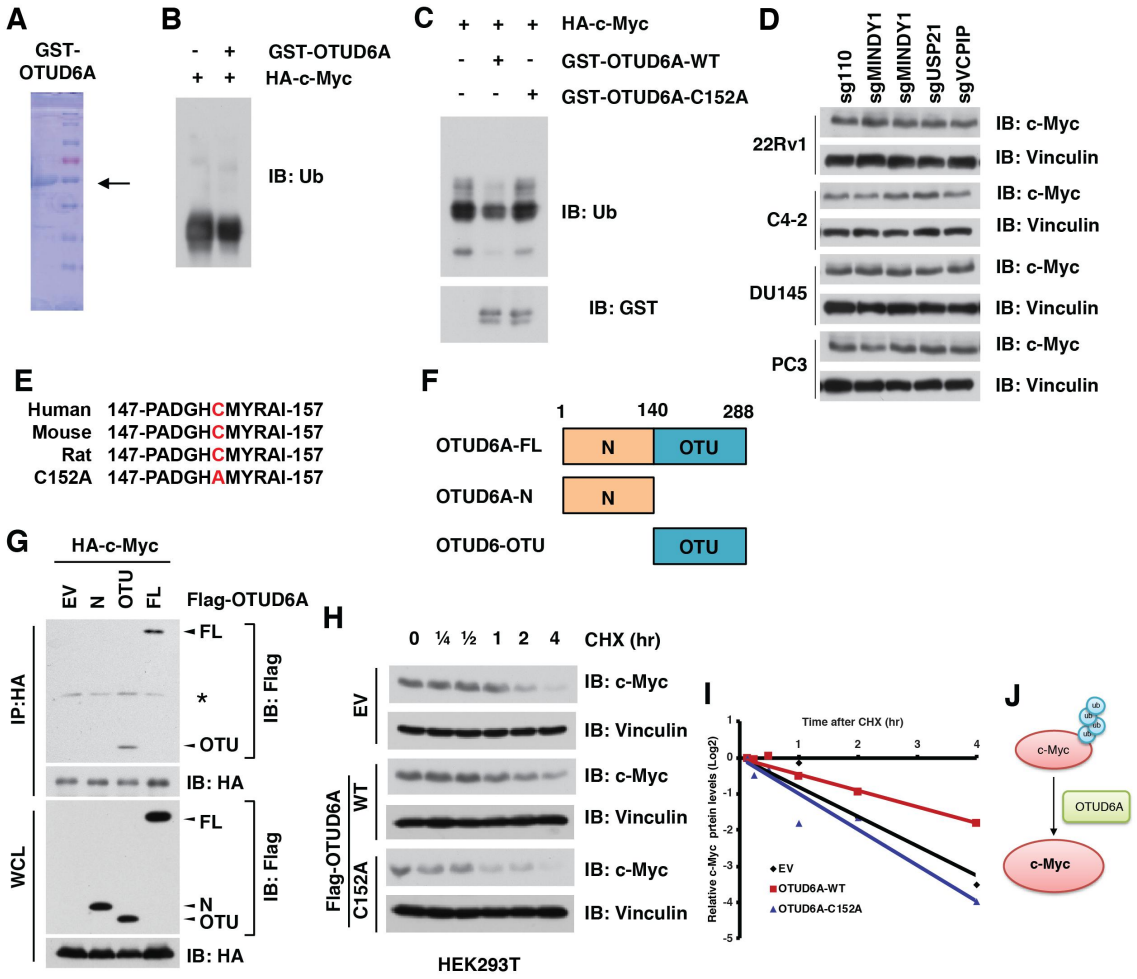
patients in TCGA database. **(B)** Genetic variations of USP22, USP28 or USP37 did not predict worse prognosis of PrCa patients. **(C. D)** Depletion of USP22, USP28, USP36 or USP37 did not change the protein level of c-Myc in PC3 (**C**) and DU145 (**D**) PrCa cells. (**E**) The USP37 mRNA in PrCa cells after depletion of *USP37* using CRISPR/Cas9. **(F)** Depletion of USP DUBs led to reduce in c-Myc in MCF7 breast cancer cells, NCI-H1299 lung cancer cells and HeLa cervical cancer cells. **(G)** Depletion of USP DUBs compromised the tumorigenesis ability of breast and lung cancer cell lines.

The relevant raw data are provided in Supplemental Materials.



Supplementary figure 3. Screening of physiological DUBs for c-Myc oncoprotein in PrCa setting. (A) Genetic variations, including copy number alternation (CNA) or point mutations, of DUBs in human PrCa. **(B-E)** The correlation between the genetic variations

of OTU (**B**), USP (**C**), MINDY (**D**) and JAMM (**E**) DUBs and prognosis of PrCa patients. **(F)** Screen pitfall of physiological DUBs for c-Myc oncoprotein in PrCa setting.



Supplementary figure 4. OTUD6A regulates the protein stability of c-Myc in PrCa setting. (A) GST-OTUD6A protein was purified from BL21 *E. Coli*. (**B**) OTUD6A deubiquitinated c-Myc *in vitro*. HA-c-Myc protein derived from HEK293T cells with poly-ubiquitin chain was incubated with bacterial purified GST-OTUD6A protein, and then was analyzed by SDS-PAGE. (**C**) OTUD6A deubiquitinated c-Myc in a catalytic activity dependent manner *in vitro*. **(D)** Depletion of MINDY1/4, USP21 or VCPIP did not affect the protein abundance of c-Myc oncoprotein. **(E)** Alignment of the conserved cysteine residual in OTUD6A among species. **(F)** A schematic diagram to show the full

length (FL) or N-terminal (N) and OTU-domain (OTU) of OTUD6A. **(G)** c-Myc binds with the OTU domain of OTUD6A. **(H, I)** Ectopic WT, but not the C152A mutant of OTUDA6 extends the protein half-life of c-Myc in 22Rv1 cells. Immunoblot (**H**) and quantification (**I**) of cell lysis derived from 22Rv1 cells transient transfected with either EV, OTUD6A-WT or OTUD6A-C152A, and treated with 200 μg/ml of CHX for indicated time. (**J**) A schematic diagram shows the deubiquitination of c-Myc by OTUD6A.

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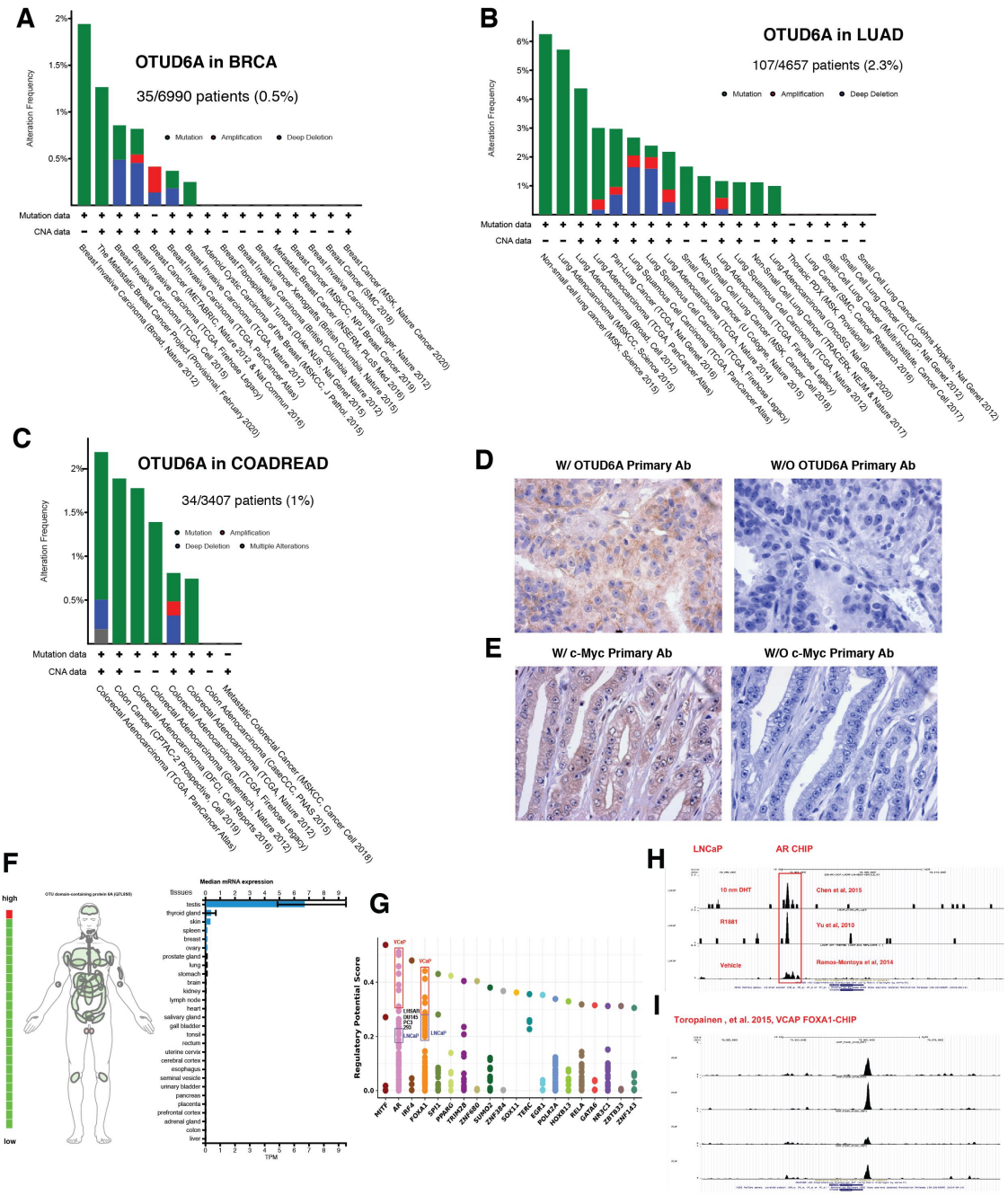
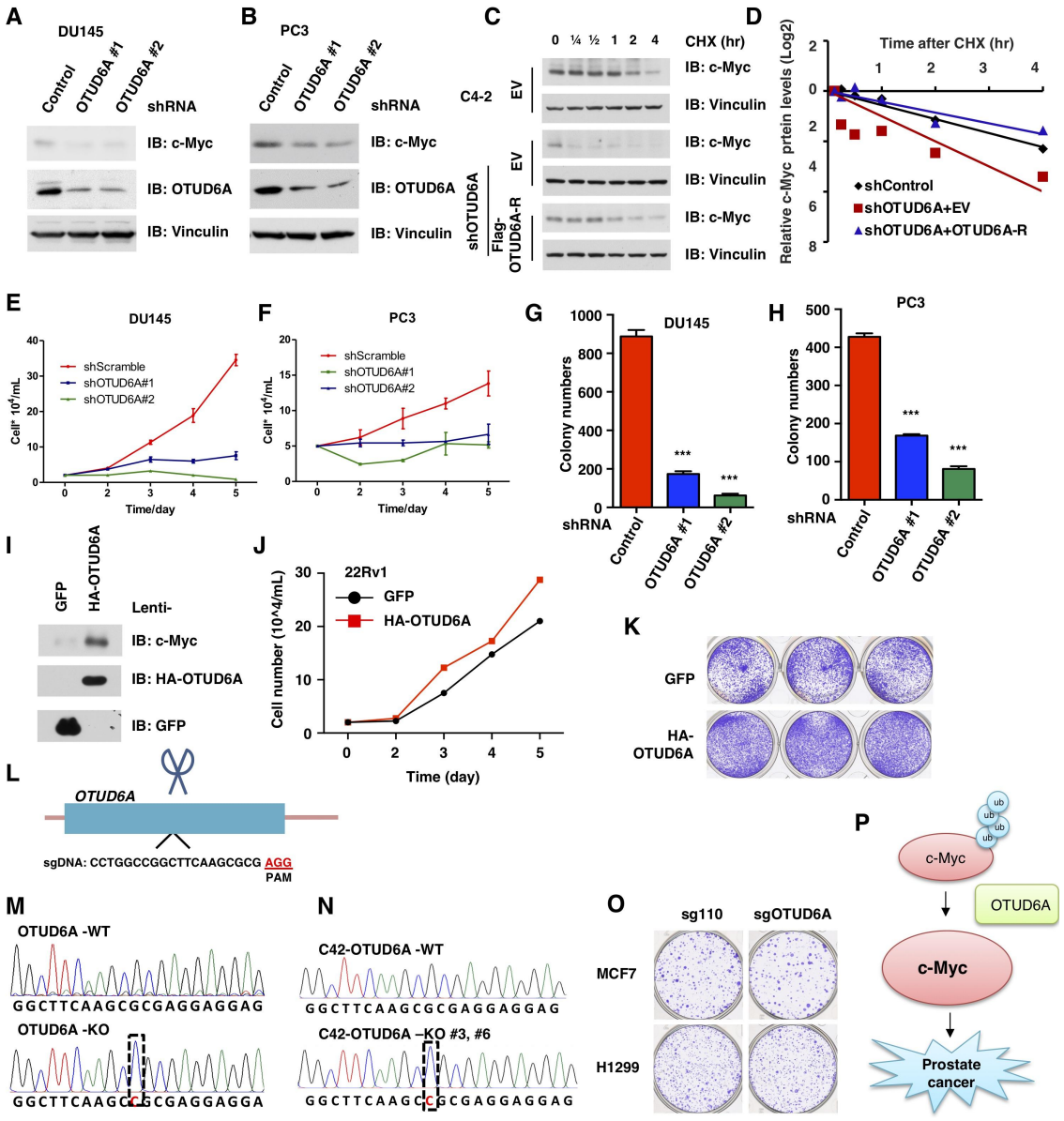


Supplementary figure 5. *OTUD6A* is a prostatic specific oncogene in PrCa. (A-C) *OTUD6A* is not amplified but mutated at very low frequency in breast cancer (BRCA), lung cancer (LUAD) and colorectal cancer (COADREAD). **(D, E)** Validation of IHC staining for OTUD6A (**D**) and c-Myc antibody (**E**), in which staining without primary

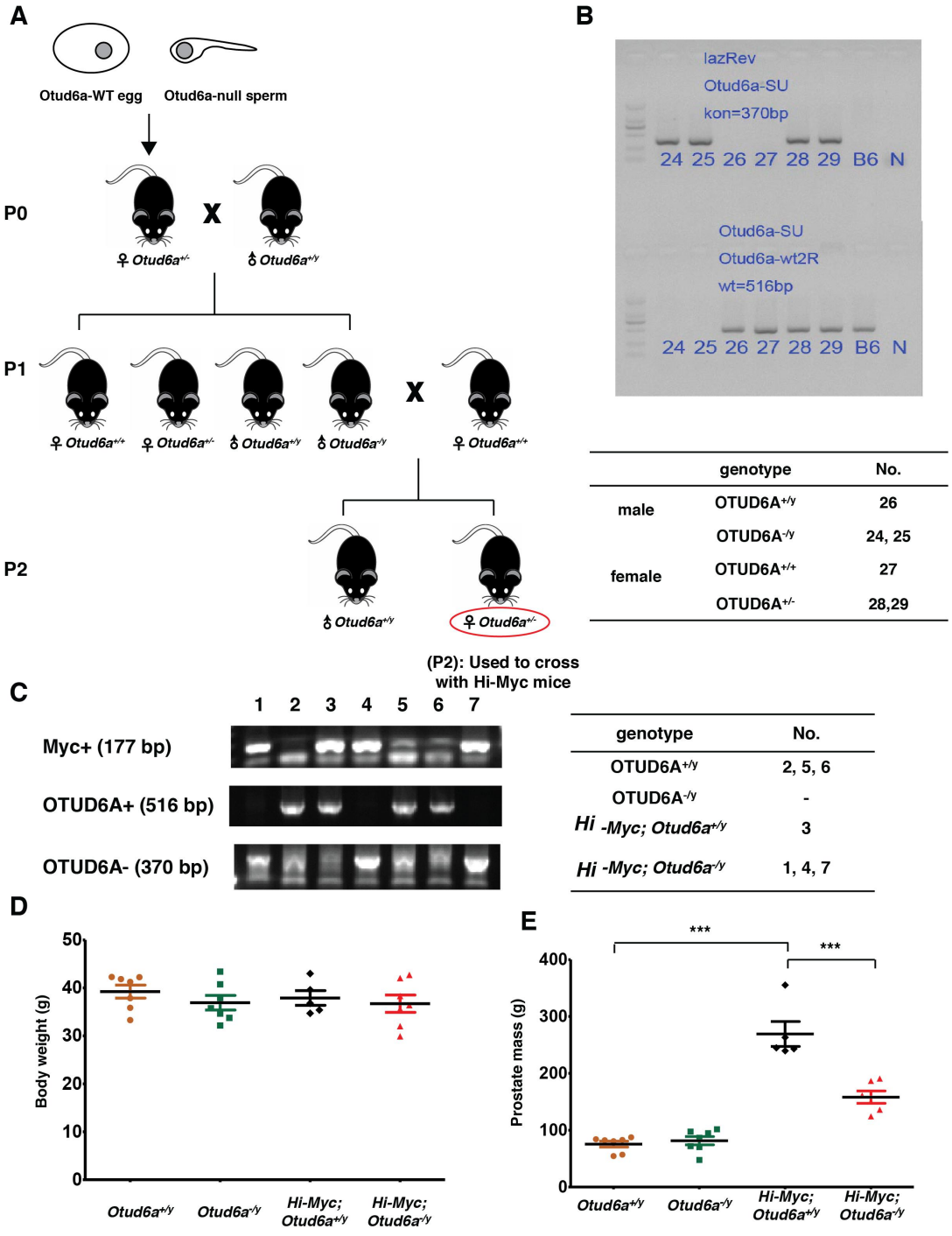
antibody as negative control. (**F**) The expression patten of OTUD6A in different tissue/organ in human. (**G**) AR and FOXA1 are predicted transcription factor of *OTUD6A* based on the prediction of Cistrome. (**H**) AR binds with the promoter region of OTUD6A in LNCaP PrCa cells. (**I**) FOXA1 binds with the promoter/enhancer region of OTUD6A in VCaP PrCa cells.



Supplementary figure 6. Depletion of endogenous *OTUD6A* represses the proliferation and tumorigenesis of PrCa cells. (A, B) Depletion of *OTUD6A* leads to reduced protein levels of c-Myc in DU145 and PC3 PrCa cells. DU145 (**A**) and PC3 (**B**) cells were infected with shControl or shOTUD6A lenti-virus and selected with puromycin for 3 days, followed by IB assay for indicated proteins. **(C, D)** Depletion of

OTUD6A reduces the half-life of c-Myc protein in C4-2 cells. C4-2 Cells were infected with shControl or shOTUD6A virus and selected with puromycin for 3 days, followed by transient transfection with EV or shRNA resistant Flag-OTUD6A constructs. The cells were treated with CHX (200 μg/ml) for indicated time before harvest, followed by IB assay for indicated proteins (**C**) and quantification (**D**). **(E, F)** Depletion of *OTUD6A* suppresses cell growth of DU145 (**E**) and PC3 cells (**F**). The cells as in **a** and **b** were subjected to growth curve analysis. **(G, H)** Depletion of *OTUD6A* suppresses colony growth of DU145 and PC3 PrCa cells. DU145 (**G**) and PC3 cells (**H)** as in **A** and **B** were subjected to colony formation assay. ***: $P<0.001$. (**I**) Overexpression of OTUD6A leads to increased protein level of protein levels of c-Myc in 22Rv1 cells. (**J**) Overexpression of OTUD6A leads to increased cell proliferation of 22Rv1 cells. (**K**) Overexpression of OTUD6A leads to an increase in the tumorigenesis of 22Rv1 cells in the colony formation assay. **(L)** The sequence of sgDNA for CRISPR knockout of OTUD6A. **(M, N)** Sequencing validation of *OTUD6A* knockout in 22Rv1 (**M**) and C4-2 cell (**N**). **(O)** Depletion of *OTUD6A* did not compromise the tumorigenesis of MCF7 BRCA cells and H1299 LUAD cells. **(P)** A schematic diagram to show the effect of OTUD6A in promoting PrCa through deubiquitinating c-Myc oncoprotein.

The relevant raw data are provided in Supplemental Materials.

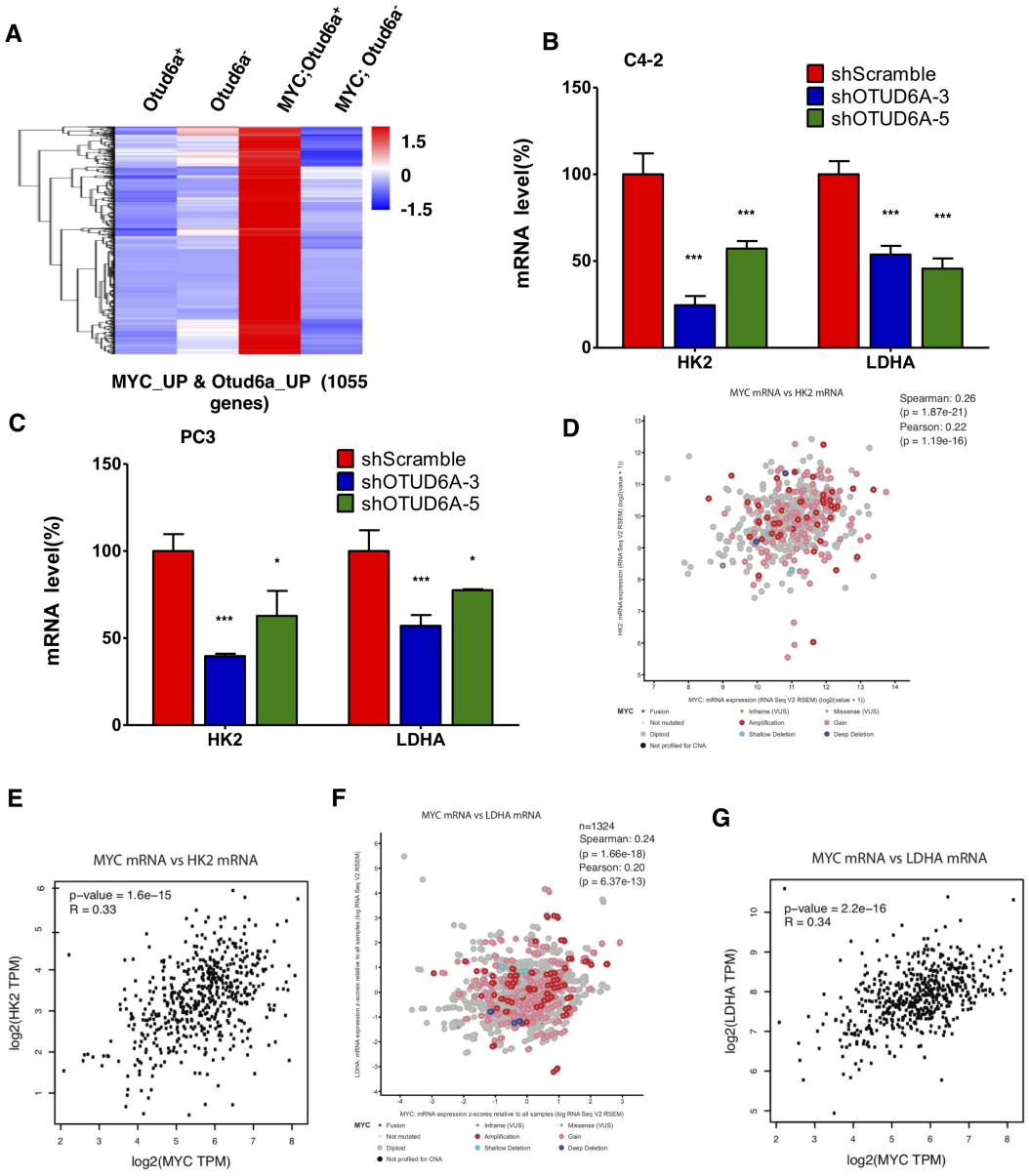


	genotype	No.
male	OTUD6A$^{+/y}$	26
	OTUD6A$^{-/y}$	24, 25
female	OTUD6A$^{+/+}$	27
	OTUD6A$^{+/-}$	28,29

genotype	No.
OTUD6A$^{+/y}$	2, 5, 6
OTUD6A$^{-/y}$	-
Hi-Myc; Otud6a$^{+/y}$	3
Hi-Myc; Otud6a$^{-/y}$	1, 4, 7

Supplementary figure 7. Breeding of Hi-Myc mice with/without depletion of endogenous *Otud6a*. **(A)** A schematic diagram shows that the breeding strategy for OTUD6A null mice. **(B)** Representative Genotyping results of OTUD6A WT and null mice. **(C)** Representative Genotyping results of Hi-Myc mice with/without depletion of

endougenous *Otud6a.* **(D, E)** Body weight (**D**) and the weight of prostate tissue (**E**) of mice as in **Fig. 6B**. N=7, 7, 5 and 6 for *Otud6a*$^{+/y}$, *Otud6a*$^{-/y}$, *Hi-Myc;Otud6a*$^{+/y}$ and *Hi-Myc;Otud6a*$^{-/y}$ group, individually. * P<0.05, *** P<0.001. One-way ANOVA.



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113 **Supplementary figure 8. The OTUD6A/c-Myc axis regulates the prostate cancer cell**

114 **metabolism. (A)** Heatmap shows the genes (DEG) in prostate tissue/tumors that

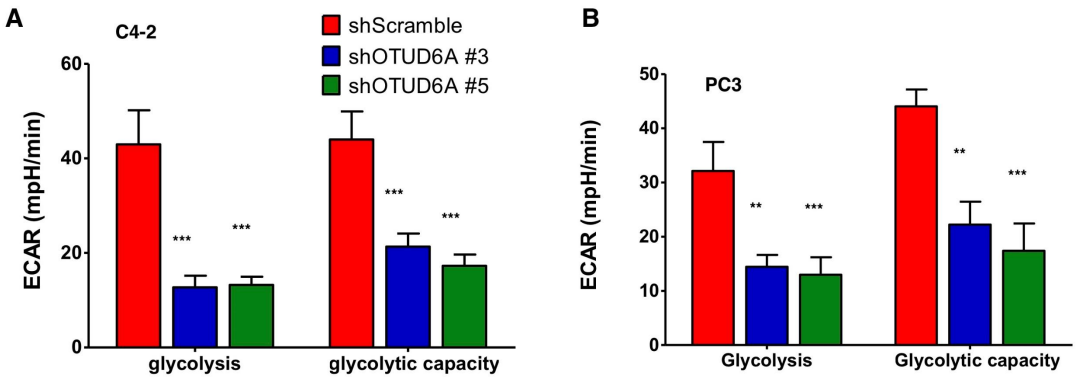
115 increased in Myc-driven mice and further decreased in *Otud6a* knockout mice. **(B, C)**

116 Depletion of *OTUD6A* led to reduced mRNA expressions of *HK2* and *LDHA* in C4-2 (**B**)

117 and PC3 PrCa cells (**C**). These PrCa cells were depleted of OTUD6A by shRNA, and the

118 mRNA levels were measured by qPCR. **(D-G)** c-Myc expression level is positively

correlated with the mRNA levels of LDHA (**D, E**) and HK2 (**F, G**) in TCGA database.



Supplementary figure 9. Depletion of OTUD6A leads to the reduce in glycolytic metabolism in PrCa cells. (A, B) Depletion of *OTUD6A* reduced the glycolysis level in C4-2 (**A**) and PC3 cells (**B**). These PrCa cells were depleted of *OTUD6A* by shRNA, and the cancer cell metabolism were measured by XF24 Seahorse extracellular flux analyzer.