An in vitro system to characterize prostate cancer progression identified signaling required for self-renewal

SUPPLEMENTAL MATERIALS

LEGENDS AND REFERENCES FOR SUPPLEMENTAL FIGURES

Molecular Carcinogenesis

An *in vitro* system to characterize prostate cancer progression identified signaling required for self-renewal

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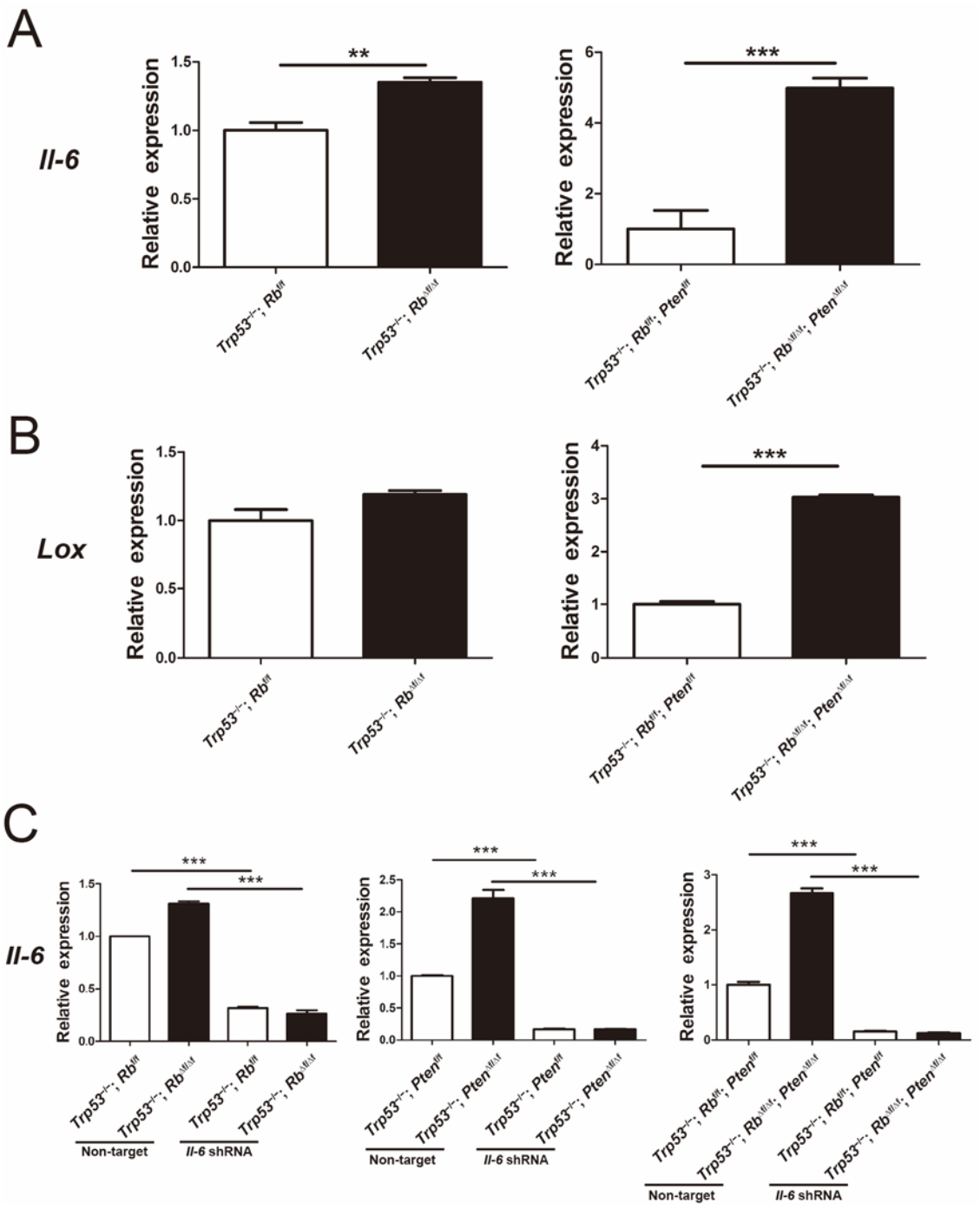
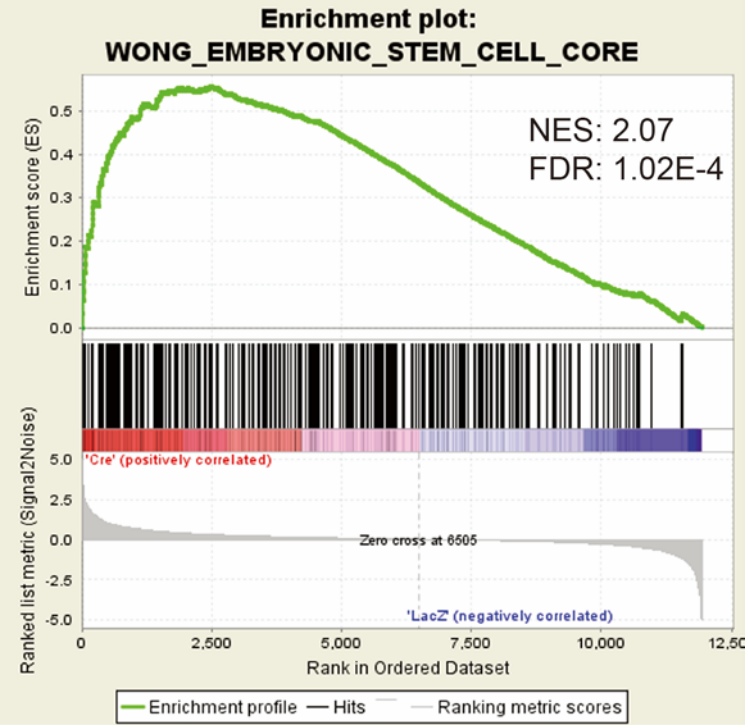


Supplemental Figure 1.

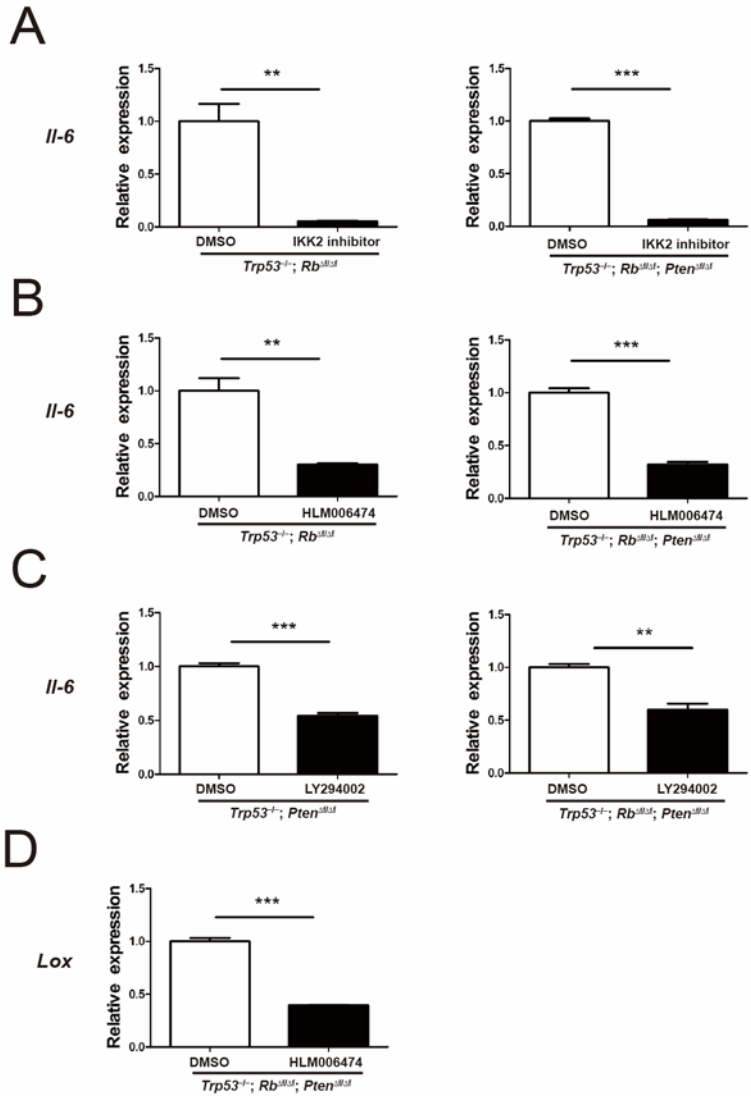
(A): *Il-6* expression detected by microarray analysis in the indicated genotype of prostate epithelial cells. Columns: relative frequency plus S.D. (N = 3 in the left panel and N = 4 in the right panel). $^{**}P < 0.01$ and $^{***}P < 0.001$ (Student′s *t*-test). (B): *Lox* expression detected by microarray analysis in the indicated genotype of prostate epithelial cells. Columns: relative frequency plus S.D. (N = 3 in the left panel and N = 4 in the right panel). $^{***}P < 0.001$ (Student′s *t*-test). (C): *Il-6* expression detected by RT-qPCR in the indicated genotype of prostate epithelial cells transduced with the

indicate shRNA. Columns: relative frequency plus S.D. (N = 3). ***$P < 0.001$ (Student′s *t*-test).



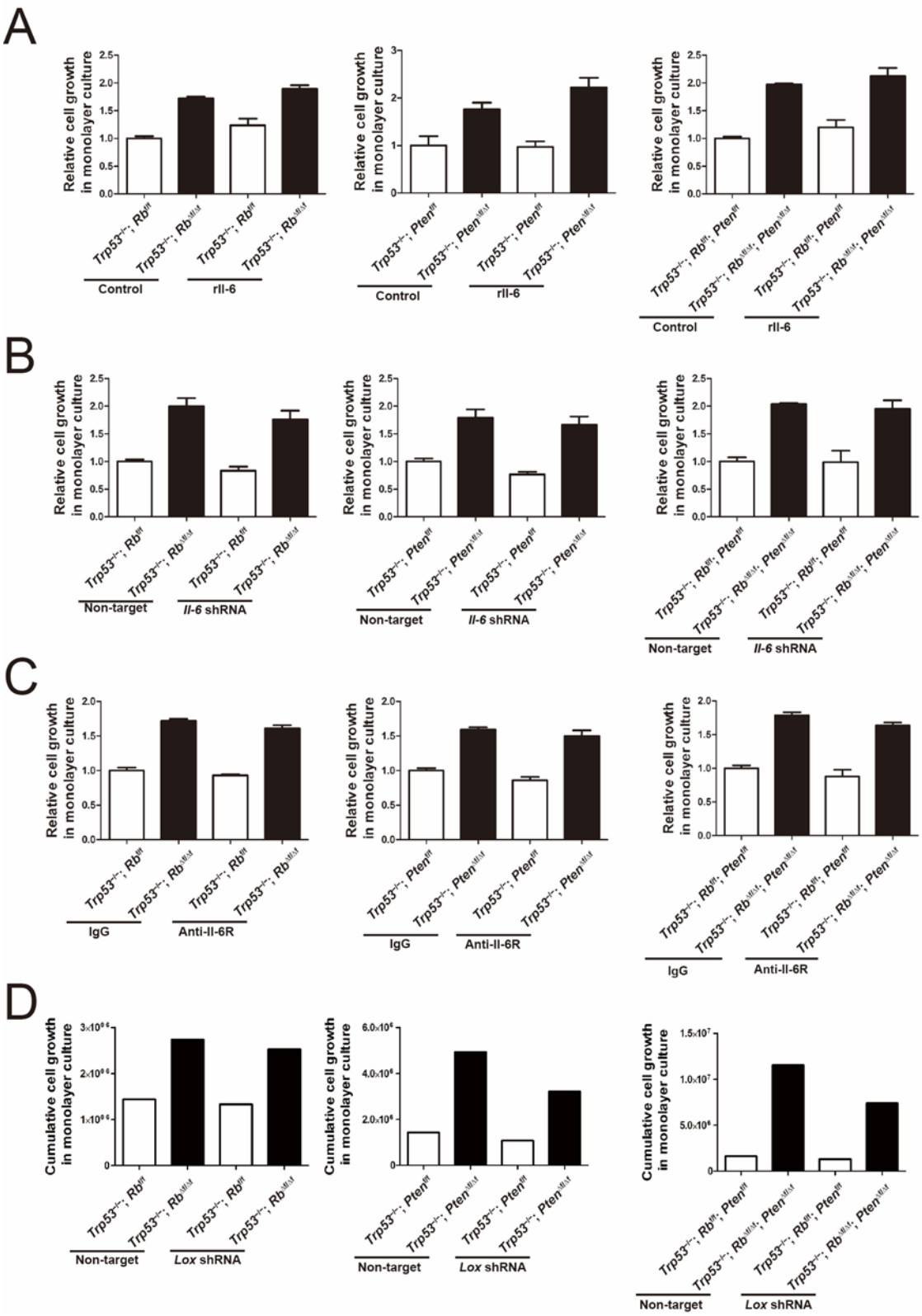
Supplemental Figure 2.

A GSEA result for genes upregulated in $p53^{-/-}$;$Rb^{f/f}$ cells after infection with Ad-Cre as compared to Ad-LacZ against one of gene sets that features genes expressed in embryonic stem (ES) cells (Wong et al., 2008).



Supplemental Figure 3.

RT-qPCR of *Il-6* in the indicated genotype of primary prostate cells treated with 20μM IKK-2 Inhibitor IV for 4 hr (A), 20 μM HLM006474 for 24 hr (B) or 10 μM LY294002 for 4 hr (C). Columns: relative frequency plus S.D. (N = 3). (D) RT-qPCR of *lox* in the indicated genotype of primary prostate cells treated with 20 μM HLM006474 for 24 hr. Dimethyl sulfoxide (DMSO): a vehicle. Columns: relative frequency plus S.D. (N = 3). **P* < 0.05 and ****P* < 0.001 (one-way ANOVA followed by post-hoc Tukey's test).



Supplemental Figure 4.

(A): Relative cell growth of the indicated genotype of prostate epithelial cells treated

with 20 ng/ml rIl-6 under monolayer culture condition for 48 hrs. Columns: relative frequency plus S.D. (N = 3). One-way ANOVA followed by post-hoc Tukey's test was used. The same analyses for cells transduced with *Il-6* shRNA (B), treated with 0.4 μM anti-Il-6R antibody for 48 hrs (C), and transduced with *Lox* shRNA and observed for 96 hrs (N = 1) (D).

SUPPLEMENTAL REFERENCES

1. Kondo Y, Shen L, Cheng AS, Ahmed S, Boumber Y, Charo C, Yamochi T, Urano T, Furukawa K, Kwabi-Addo B, Gold DL, Sekido Y, Huang TH, Issa JP. Gene silencing in cancer by histone H3 lysine 27 trimethylation independent of promoter DNA methylation. Nat Genet. 2008;40(6):741-750.

2. Wong DJ, Liu H, Ridky TW, Cassarino D, Segal E, Chang HY. Module map of stem cell genes guides creation of epithelial cancer stem cells. Cell Stem Cell. 2008;2(4):333-344.