

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

著者	Salah Mohammed, Nishimoto Yuuki, Kohno Susumu, Kondoh Atsushi, Kitajima Shunsuke, Muranaka Hayato, Nishiuchi Takumi, Ibrahim Ahmed, Yoshida Akiyo, Takahashi Chiaki
journal or publication title	Molecular Carcinogenesis
volume	55
number	12
page range	1974-1989
year	2016-12-01
URL	http://hdl.handle.net/2297/46761

doi: 10.1002/mc.22444

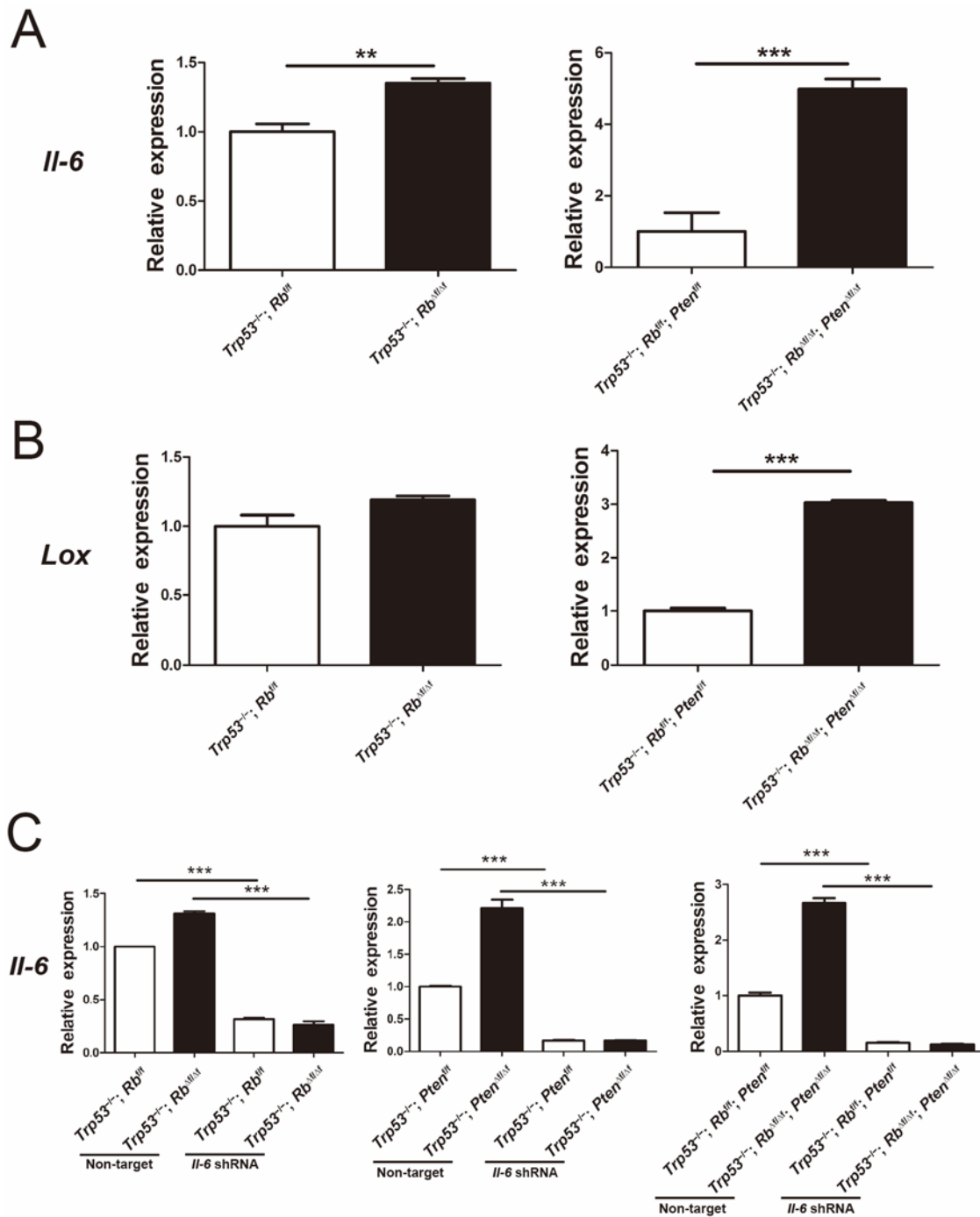
SUPPLEMENTAL MATERIALS

LEGENDS AND REFERENCES FOR SUPPLEMENTAL FIGURES

Molecular Carcinogenesis

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

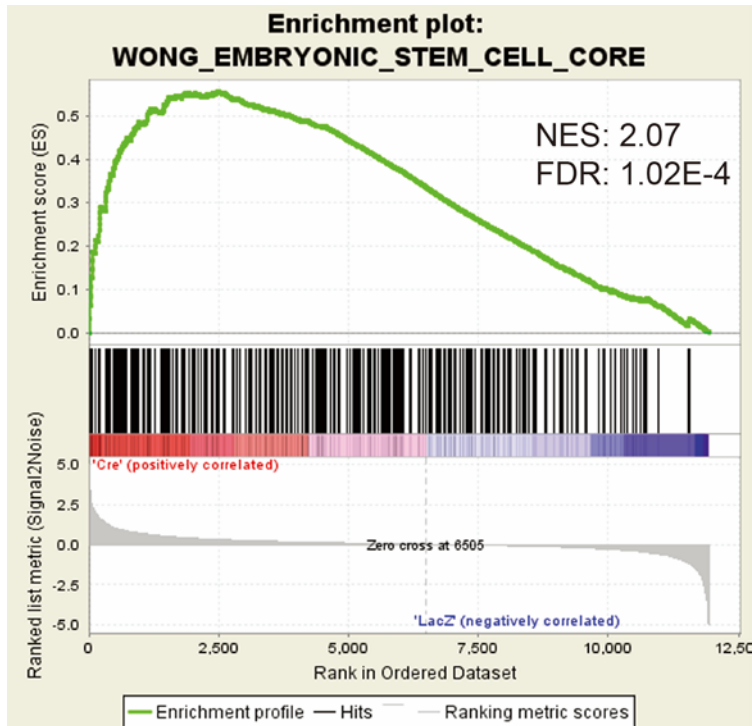
Mohammed Salah^{1,2}, Yuuki Nishimoto², Susumu Kohno², Atsushi Kondoh², Shunsuke Kitajima^{2,3}, Hayato Muranaka², Takumi Nishiuchi⁴, Ahmed Ibrahim^{1,5}, Akiyo Yoshida^{2,6} and Chiaki Takahashi^{2,*}



Supplemental Figure 1.

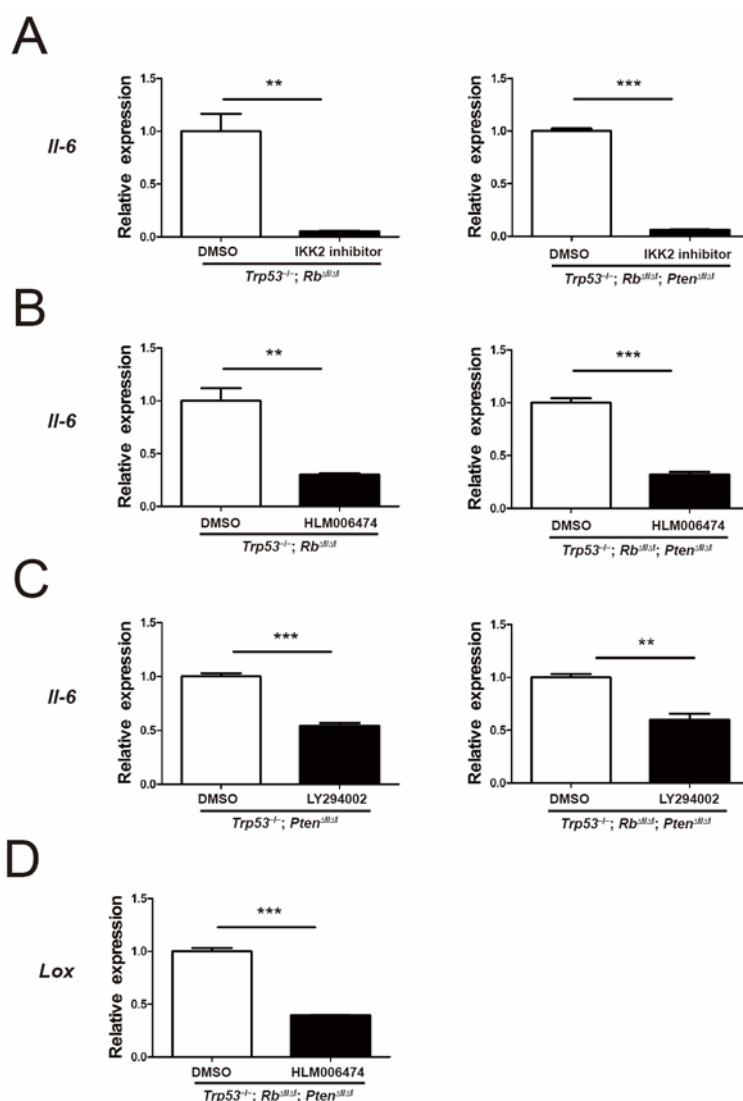
(A): *Il-6* expression detected by microarray analysis in the indicated genotype of prostate epithelial cells. Columns: relative frequency plus S.D. (N = 3 in the left panel and N = 4 in the right panel). ** $P < 0.01$ and *** $P < 0.001$ (Student's *t*-test). (B): *Lox* expression detected by microarray 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).



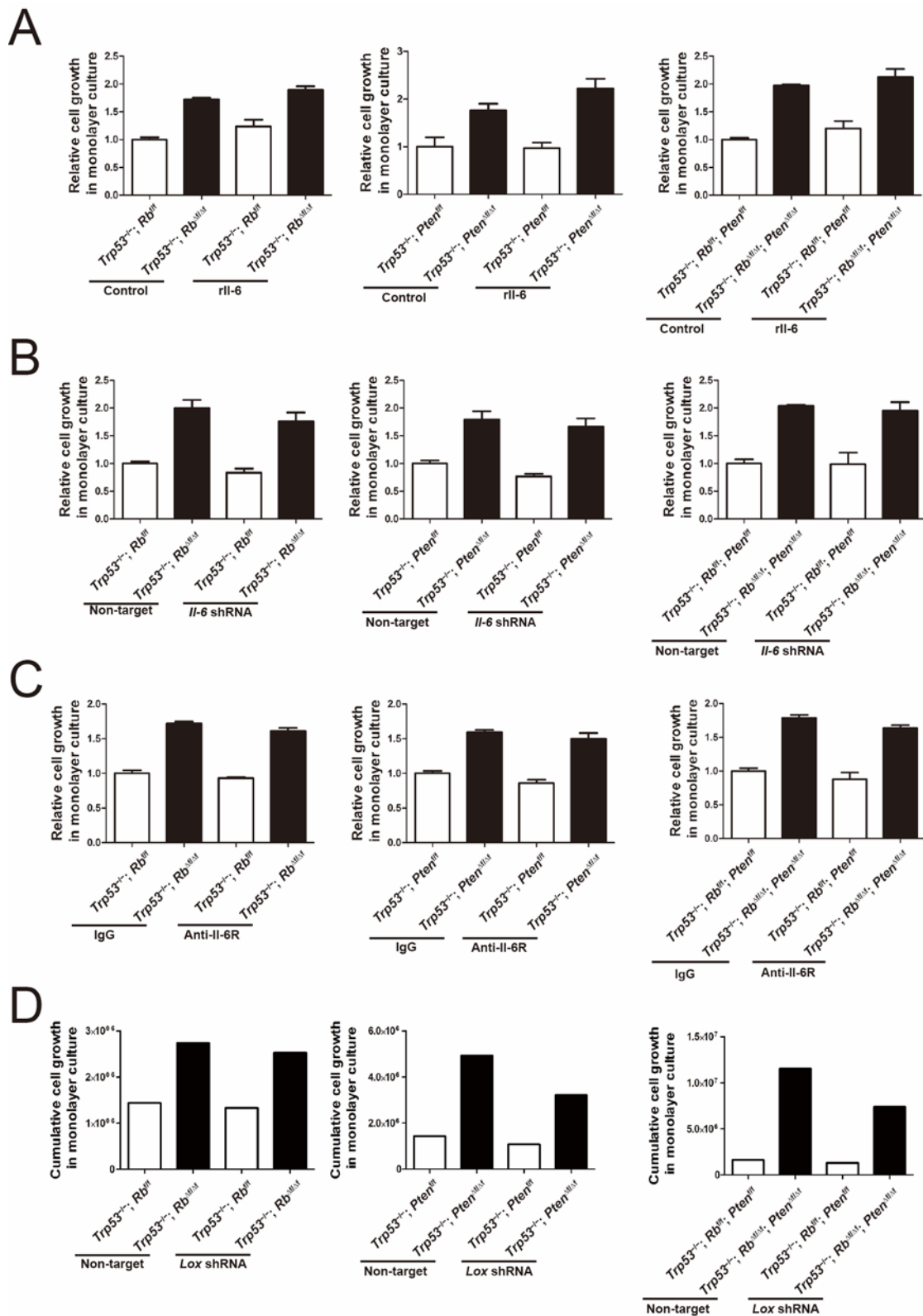
Supplemental Figure 2.

A GSEA result for genes upregulated in $p53^{-/-}; Rb^{ff}$ 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 20 μ M IKK-2 Inhibitor IV for 4 hr (A), 20 μ M HLM006474 for 24 hr (B) or 10 μ M 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 μ M 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 μ M 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, Bumber 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.