**Supplementary Materials**

# p27T187A knockin mutation identifies Skp2/Cks1 pocket inhibitors for advanced prostate cancer

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**Supplemental Tables**



**Supplemental Figures**



**Associated references**

Table S1 Skp2 protein expression in a prostate tissue microarray study (1)

| Study | Yang et al, 2002 | | |
|---|---|---|---|
| Prostate tissues[1] | Normal | HGPIN | Primary prostate cancer |
| Specimen #s | 4 | 74 | 622 |
| Skp2 labeling frequency % (Median) | 0.50 | 10.00 | 30.00 |
| *p* values compared to normal | | 0.025 | 0.0037 |
| *p* value compared to HGPIN | | | <0.0001 |

We adapted the data from a prostate tissue microarray study for Skp2 expression measured by IHC (1). [1] Prostate tissues for the tissue microarrays were obtained from prostatectomy. Pathological diagnosis of tissue areas used for tissue microarrays are indicated. *p* Values are by the Mann-Whitney test.

In the same study, Yang et al also reported that higher Skp2 labeling index correlated with lower p27 ($p = 0.0003$) and shorter biochemical recurrence-free survival ($p < 0.0363$, log-rank test).

Table S2. Expression of *Skp2* and its relationships to *RB1*, *TP53,* and *PTEN* inactivation

| | Primary prostate cancer | mCRPC |
|---|---|---|
| Studies | TCGA, 2015 | Robinson et al, 2015 |
| Specimen #s | 333 | 150 |
| *RB1* inactivation [1] | 0.9% | 8.6% |
| *TP53* inactivation [1] | 7.5% | 50% |
| *PTEN* inactivation [1] | 17% | 40% |
| *Skp2* activation [2] | 5.1% | 11% |
| Co-occurrence [3] | PS, PtS | **RS**, PS |
| Statistic significance [4] | | ***p* = 0.006** |

We retrieved the data sets from and analyzed them on cBioPortal. [1] Inactivation of *RB1*, *TP53*, and *PTEN* are queried for HOMDEL MUT. [2] Activation of Skp2 is queried for AMP EXP > 2. [3] Co-occurrence is by Log Odds Ratio; R, P, Pt, and S are short for *RB1*, *TP53*, *PTEN*, and *Skp2*, respectively, to indicate the pairs. [4] *p* Value is by Fisher Exact Test. $p < 0.05$ is considered statistically significant. Bold font marks the pair with the indicated statistically significant *p* value. Non-bold pairs show the tendencies with *p*-values between 0.092 (PS in mCRPC), 0.126 (PS in primary cancer), and 0.155 (PtS in primary cancer).

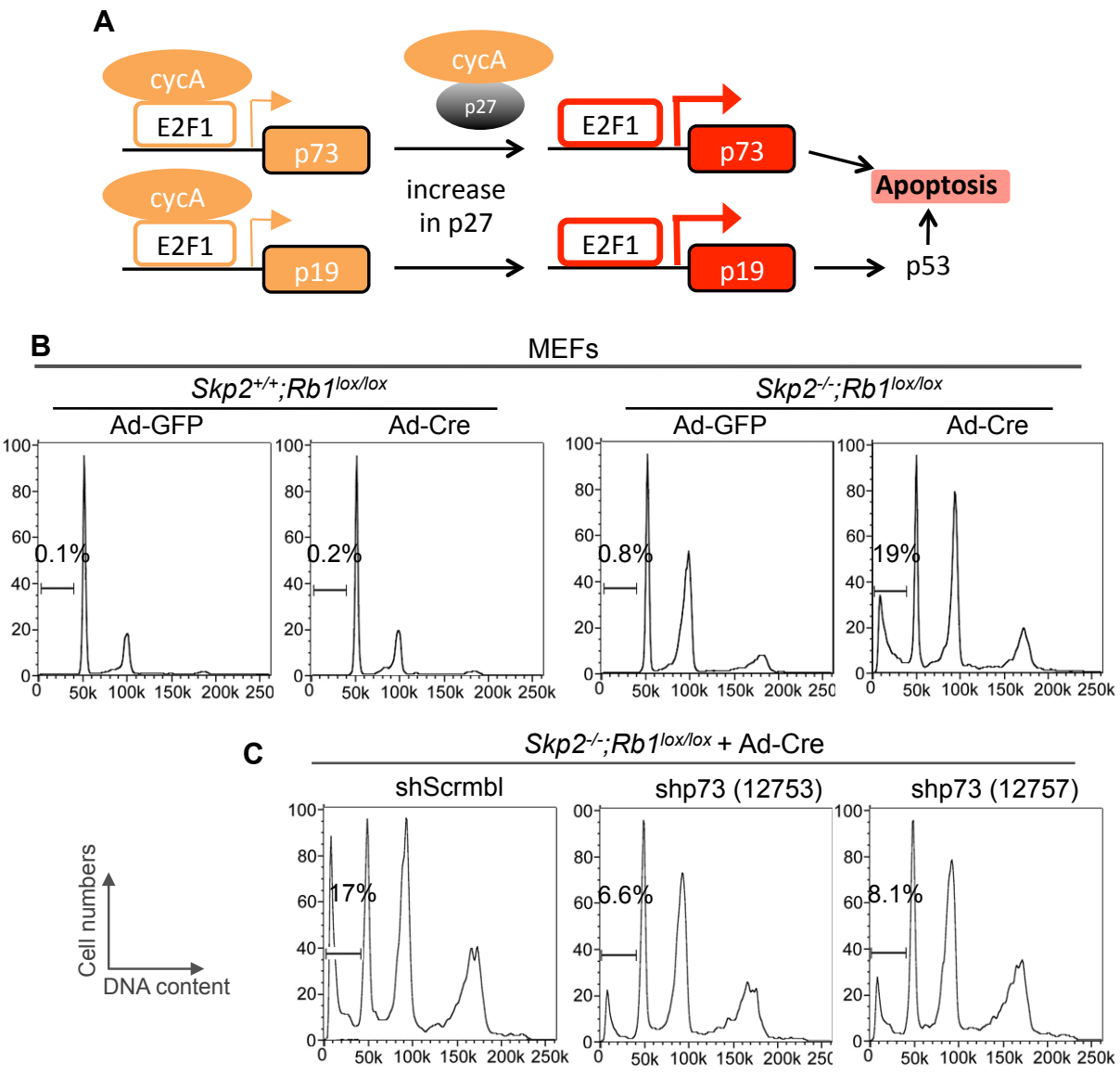


**Figure S1**. Contribution of p73 to the apoptosis induced by *Rb1* deletion in Skp2 KO cells. (**A**) Working model of how combined *Rb1* and Skp2 deletion led to further activation of E2F1, which can induce p53-dependent and -independent apoptosis. *Rb1* deletion activates E2F1 to increase expression of its target genes. The orange color indicates activated but restrained activity and expression when E2F1 is bound to cyclin A. Combined Skp2 deletion increased p27, which binds cyclin A competitively against E2F1 to relieve E2F1 from binding to and repression by cyclin A. The further elevated activity and expression is indicated by the red color. (**B**) Propidium iodide based DNA content FACS was used to detect and quantify apoptosis as sub-G1 cell populations shown above the brackets. Skp2 WT and Skp2 KO MEFs treated with Ad-GFP (as control) or Ad-Cre (to delete *Rb1*) were subjected to DNA content FACS to determine sub-G1 cell population sizes as indicated. (**C**) Same as in (A) except the indicated cells were additionally transduced with lentiviruses expressing shScrmbl (as control) or shp73 (to knockdown p73). Two separate shp73 constructs as indicated were used. Related to Figure 2.

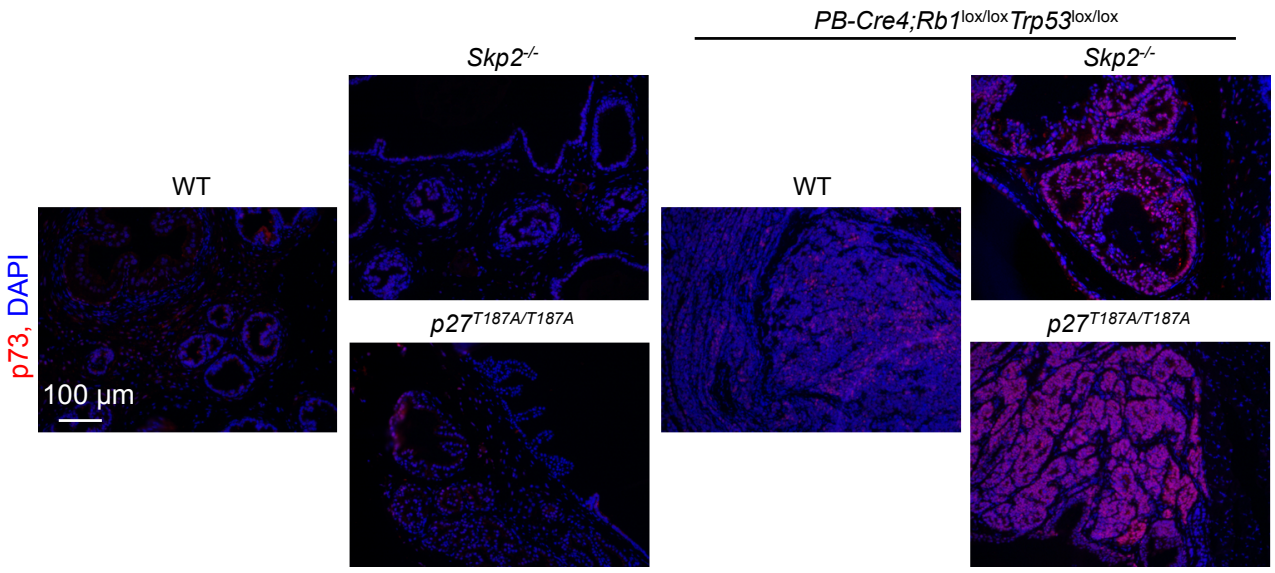


**Figure S2**. p73 protein increased in DKO prostate tumorigenesis in Skp2 KO, p27T187A KI, but not WT mice, nor in Skp2 KO or p27T187A KI mice. Prostate sections of the indicated genotypes were stained with anti-p73 with DNA counter stain by DAPI, as marked in red and blue, respectively. Related to Figure 2.

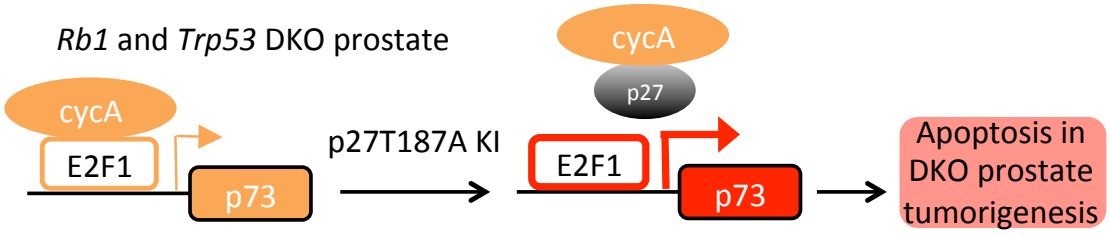


**Figure S3**. p27T187A KI activates a p27-E2F1-p73-apoptosis pathway in DKO prostate tumorigenesis. The orange color indicates activated but restrained activity and expression. p27T187A KI increased p27, which relieves E2F1 from binding to and repression by cyclin A. The further elevated activity and expression is indicated by the red color. Related to Figure 3.

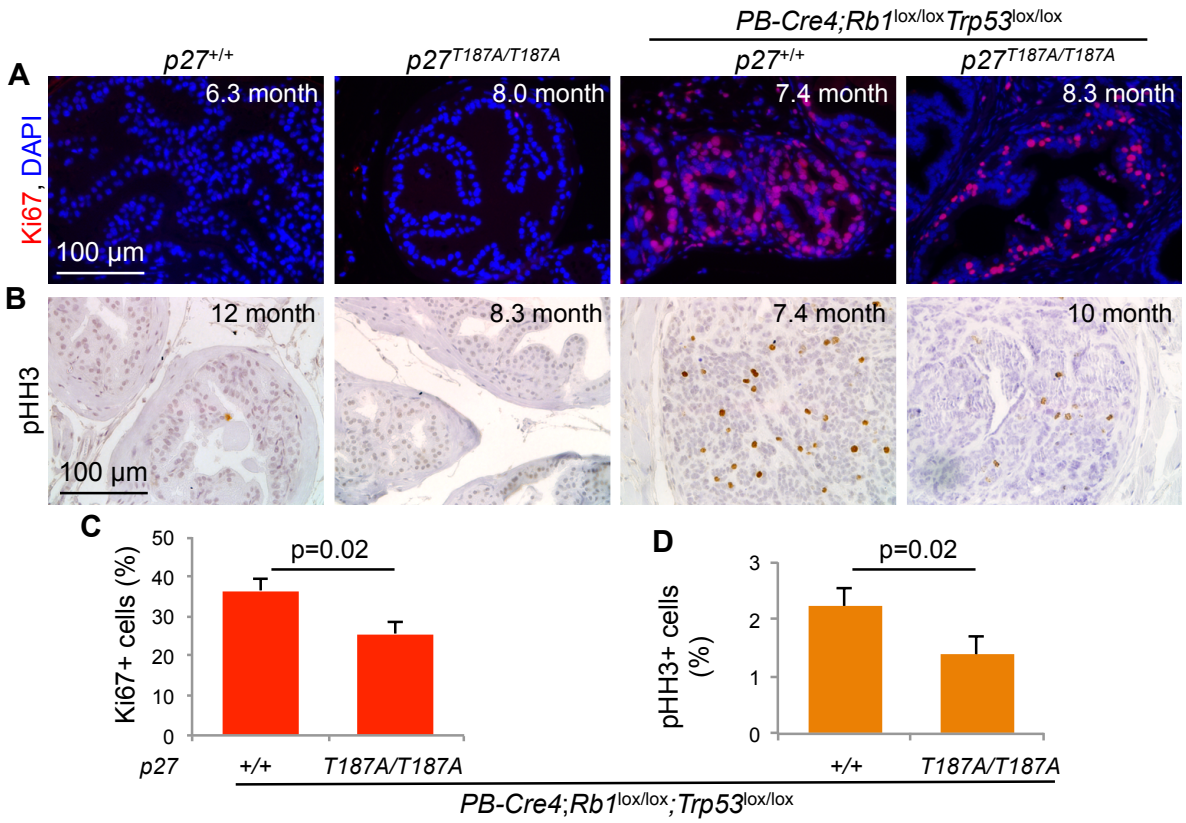


**Figure S4**. p27T187A KI inhibits cell proliferation in DKO prostate tumorigenesis. (**A**) Cell proliferation was measured by Ki67 IF with DNA counter stain by DAPI as indicated by red and blue, respectively. Ki67 positive cells in DKO prostate tumorigenesis in p27 WT and p27T187A KI mice were quantified in (**C**). (**B**) Cell proliferation was measured by pHH3 IHC, positive cells in DKO prostate tumorigenesis in p27 WT and p27T187A KI mice were quantified in (**D**)**.** Error bars represent SEM and p values are by two-sided *t* test. Related to Figure 4.

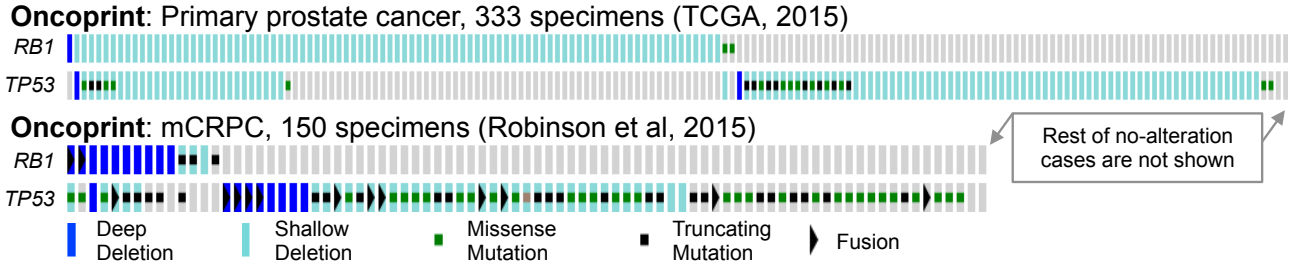


**Figure S5**. *RB1* and *TP53* gene mutations and deletions in one study of primary prostate cancer (2) and one study of mCRPC (3). Related to Table 1.

**Associated references**

1. Yang G, Ayala G, De Marzo A, Tian W, Frolov A, Wheeler TM, et al. Elevated Skp2 protein expression in human prostate cancer: association with loss of the cyclin-dependent kinase inhibitor p27 and PTEN and with reduced recurrence-free survival. Clin Cancer Res. 2002 Nov;8(11):3419-26. PubMed PMID: 12429629.

2. TCGA. The Molecular Taxonomy of Primary Prostate Cancer. Cell. 2015 Nov 5;163(4):1011-25. PubMed PMID: 26544944.

3. Robinson D, Van Allen EM, Wu YM, Schultz N, Lonigro RJ, Mosquera JM, et al. Integrative clinical genomics of advanced prostate cancer. Cell. 2015 May 21;161(5):1215-28. PubMed PMID: 26000489. Pubmed Central PMCID: 4484602.