Supplementary Tables

Table 1. Primers for PCR amplification for CRISPR/Cas9 mouse models

Primer	**Primer sequence (5'-3')**	**Purpose**
T7-sgRNA F(S186A)1	**ttaatacgactcactatagg**AAGCAGCTGTCGGAAGACCTgttttagagctagaaatagc	In vitro transcription sgRNA
T7-sgRNA F(S186A)2	**ttaatacgactcactatagg**TTCCGACAGCTGCTTTCGCTgttttagagctagaaatagc	
T7-sgRNA R	AAAAGCACCGACTCGGTGCC	In vitro transcription sgRNA
T7-Cas9 F (for px330)	**ttaatacgactcactatag**GGAGAATGGACTATAAGGACCACGAC	In vitro transcription of Cas9
T7-Cas9 R (for px330)	GCGAGCTCTAGGAATTCTTAC	

Table 2. ssODN oligos

	Forward
ssODN-1 (S186A)	AGTCAAAATATGGTTCCAGAACAGACGCTATAAGACCAAGCGAAAGCAGCTGGCGGAAGACCTGGGAGTCTTGGAGAAGAACTCACCATTGTCTTTGCCAGCCCTGAAAGATGACAGCCTG

Table 3. Primers for genotyping of mouse mutation

	Forward	**Reverse**
S186A	TGGAGAGGAAGTTCAGCC	CTTCCGACTCCTTGACATC
Nkx3.1+/-	TTCCACATACACTTCATTCTCAGT	GCCAACCTGCCTCAATCACTAAGG

Table 4. Antibodies used for immunofluorescence staining

Antigen	**Supplier**	**Ig type**	**Dilution**
Nkx3.1	Athena #0315	Rabbit IgG	1:200
β-catenin	BD transduction lab #610153	Mouse IgG	1:500
Ki-67	eBioscience #14-5698-82	Rat IgG	1:100
CK5	BioLegend #905901	Chicken IgG	1:500
CK8	Abcam #	Rabbit IgG	1:400
γH2AX	Millipore #05-636	Mouse IgG	1:1000
pATM (pS1981)	Rockland #200-301-400	Mouse IgG	1:1000
53BP1	Cell Signaling #4937	Rabbit IgG	1:500
Nitrotyrosine	Millipore #06-284	Rabbit IgG	1:50
8-oxoG	Millipore #MAB3560	Mouse IgG	1:250
pS6	Cell Signaling #2211S	Rabbit IgG	1:1000
OGG1	Santa Cruz	SC-33181	1:50
Cleaved caspase 3	Cell Signaling #D3E9	Rabbit IgG	1:200

Supplementary Figure 1

A. Amino acid sequences of human (top) and murine (bottom) NKX3.1 showing consensus (central) sequence and location of serine 185 and 186 indicated by the box in the figure.

B. Effect of S186A mutation on half-life of murine Nkx3.1.

A

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1    MLRVPEPRPGEAKAEG----AAPPTPSKPLTSFLIQDILRDGAQRQGGRTSS-QRQRDPE  55
     MLRV EPR    +A G    AAPPT SK LTSFLIQDILRD A+R GG + + Q   DP
1    MLRVAEPREPRVEAGGRSPWAAPPTQSKRLTSFLIQDILRDRAERHGGHSGNPQHSPDPR  60

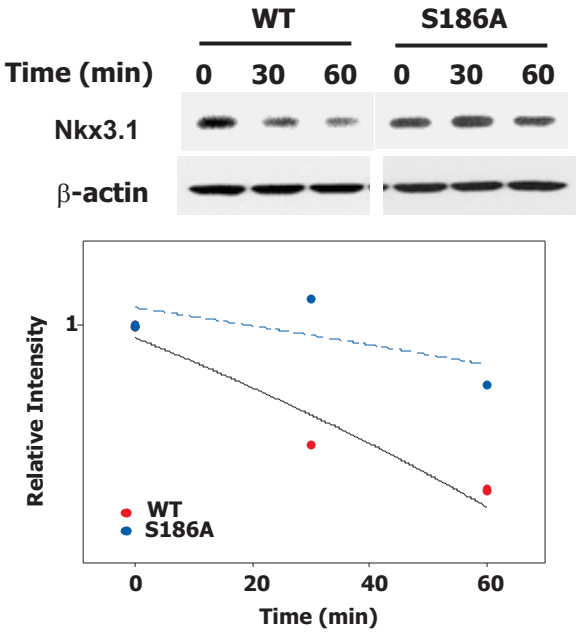
56   PEPEPEPEGGRSRAGAQNDQLSTGPRAAPDEAETLAETEPERHLGSYLLDSENTSGALPR  115
      +  PEP+    R  A  D  S   R +P  AET  E E + H  +YLLD E+  G L
61   RDSAPEPDKAGGRGVAPEDPPSI--RHSP--AETPTEPESDAHFETYLLDCEHNPGDLAS  116

116  LPQTPKQPQKRSRAAFSHTQVIELERKFSHQKYLSAPERAHLAKNLKLTETQVKIWFQNR  175
      PQ  KQPQKRSRAAFSHTQVIELERKFSHQKYLSAPERAHLAKNLKLTETQVKIWFQNR
117  APQVTKQPQKRSRAAFSHTQVIELERKFSHQKYLSAPERAHLAKNLKLTETQVKIWFQNR  176

176  RYKTKRKQLSSELGDLEKHS--SLPALKEEAFSRASLVSVYNSYPYYPYLHCVGSWSPAF  233
     RYKTKRKQLS +LG LEK+S  SLPALK+++    SLVSVY SYPYYPYL+C+GSW P+F
177  RYKTKRKQLSEDLGVLEKNSPLSLPALKDDSLPSTSLVSVYTSYPYYPYLYCLGSWHPSF  236

234  W  234
     W
237  W  237
```

B

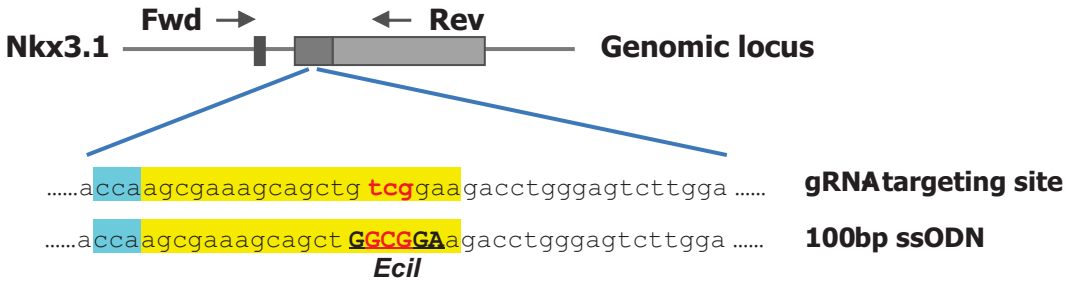


Supplementary Figure 2

A. Schematic of targeted sites for mutation at *Nkx3.1* locus.

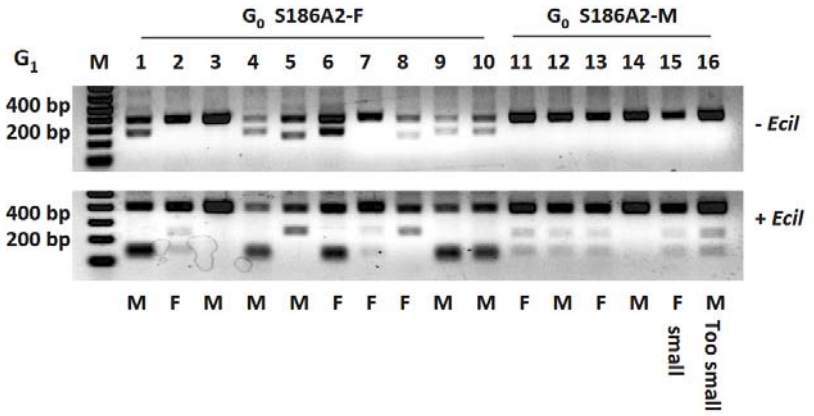
B. Germline transmission of the Nkx3.1(S186A) mutation generated using CRISPR/Cas9 genome engineering. PCR was performed with genotyping primer pairs (Supplementary Table 3). PCR products were incubated with 0.4 unit of EciI at 37deg C for 1 hour and visualized on a 2% agarose gel.

A. S186A2 mutation (sgRNA2)



sgRNA sequence
PAM motif
NNN mutation sites
UPPERCASE: restriction enzyme cutting site for genotyping

B.



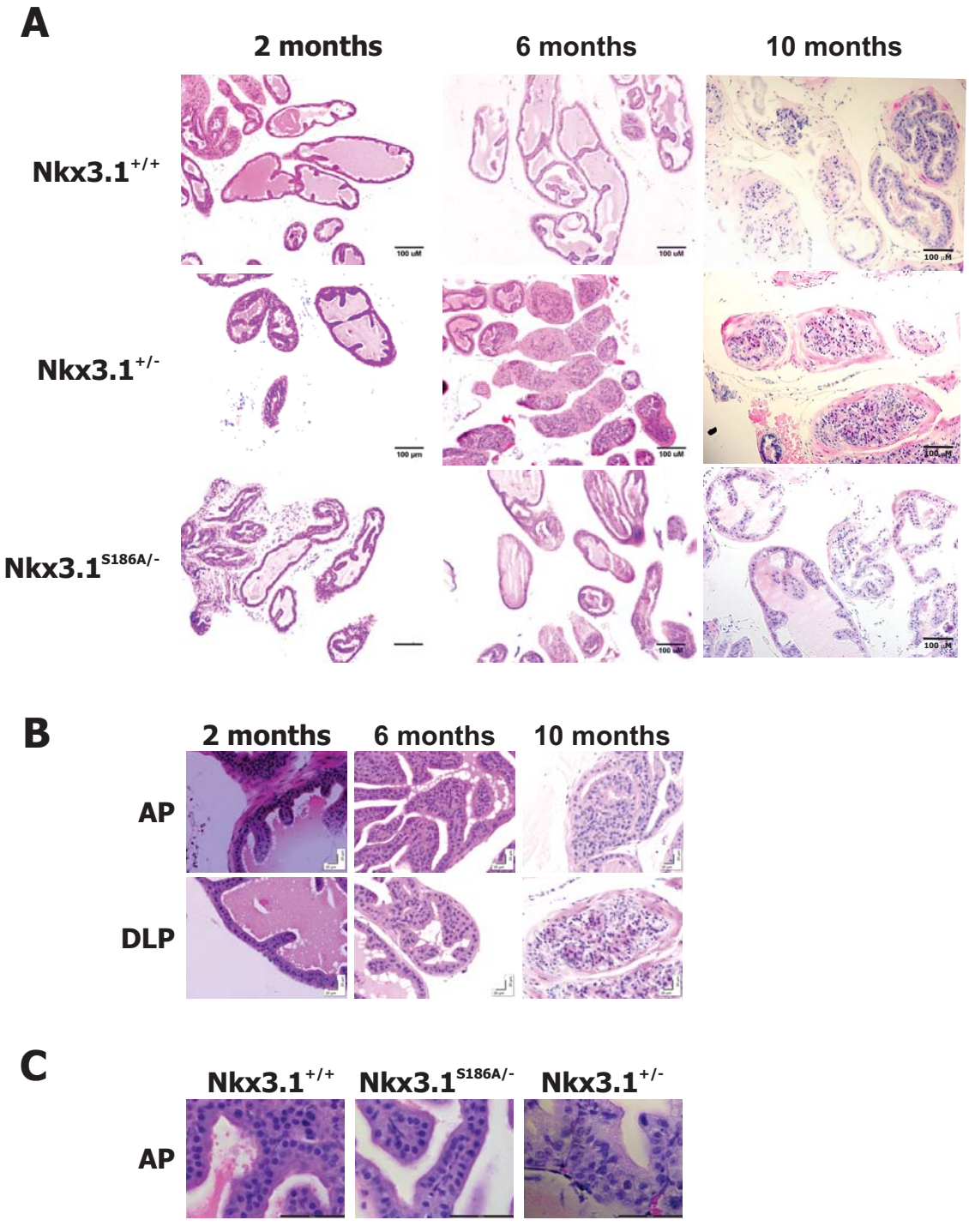
WT	5′ AGACGCTATAAGACCAAGCGAAAGCAGCTGTCGGAAGACCTGGGAGTCTTGGAGAAGA 3′	
Number of mice	gS186A2-G1 sequence	Deletion
2 11 12 13	5′ AGACGCTATAAGACCAAGCGAAAGCAGCTGGCGGAAGACCTGGGAGTCTTGGAGAAGA 3′	
4 6 9 10	5′ AGACGCTATAAGACCAAGCGAAAGCAGCTGGCGGAAGAC---------- 3	-111 bp
5	5′ AGACGCTATAAGACCAAGCGAAAGCAGCTG---------- 3′	-158 bp
7	5′AGACGCTATAAGACCAAGCGAAAGCAGCTGGCGGAAGACCTGGGAGTCTTGGAGAAGAA CTCAcCATTGTCTTTGCCAGCCCTGAAAGATGACAGCCTGCCCAGTACCTCCTTGGTCTCCG TGTATACTAGCTATCCCTACTACCCCTAC-TGTACTGTCTGTACTGTACTG 3′	

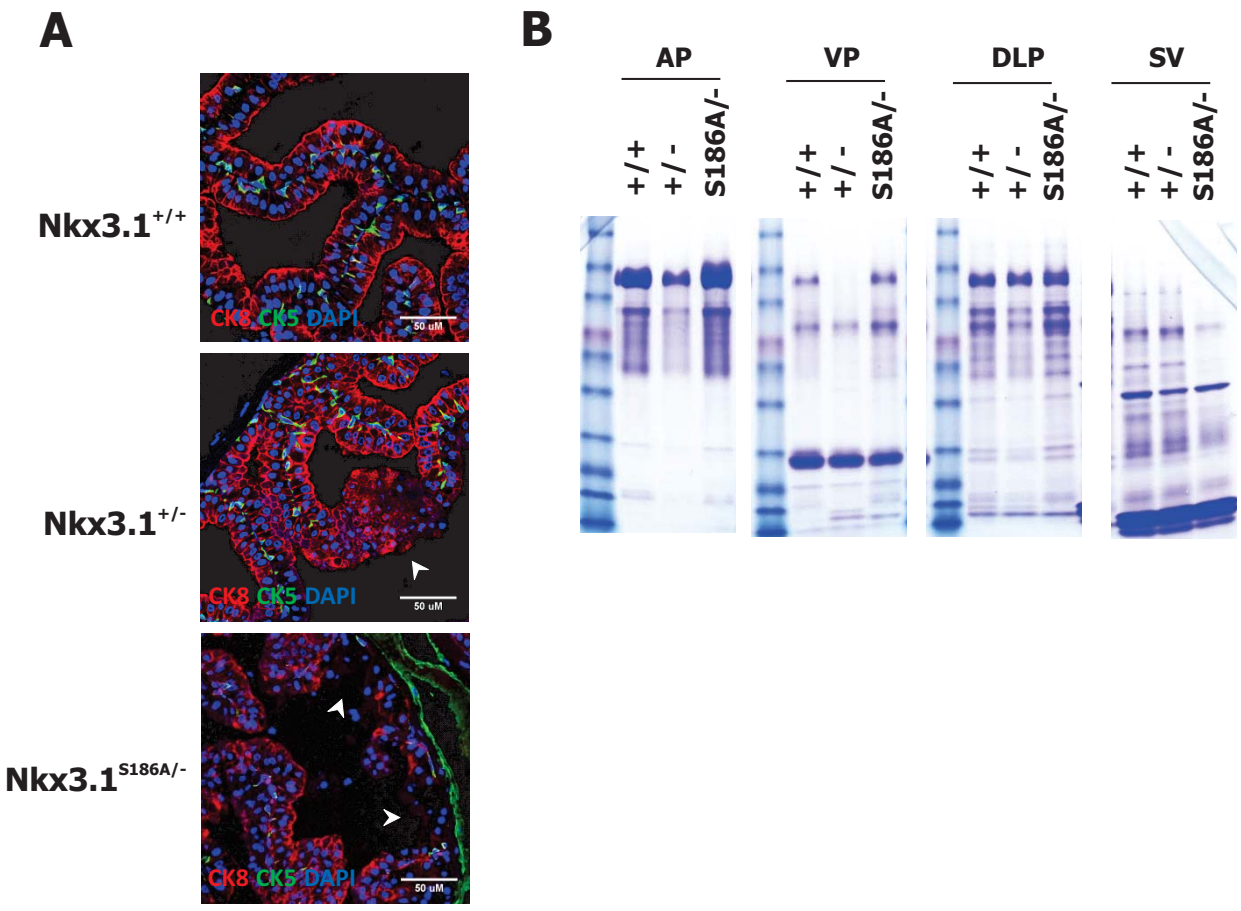
Supplementary Fig 3

A. H&E staining of dorsolateral prostatic lobes at different time points. Results similar to those seen with anterior prostates are shown in the figure.

B. H&E staining of anterior (AP) and dorsolateral (DLP) prostatic lobes at 400x demonstrating the progression of cytologic abnormalities over time in Nkx3.1$^{+/-}$ mice.

C. High-power image of H&E staining of anterior prostatic lobe at 10 months of age from mice of the indicated genotype. Note the architecural and cytologic disruption in NKX3.1$^{+/-}$ mice.





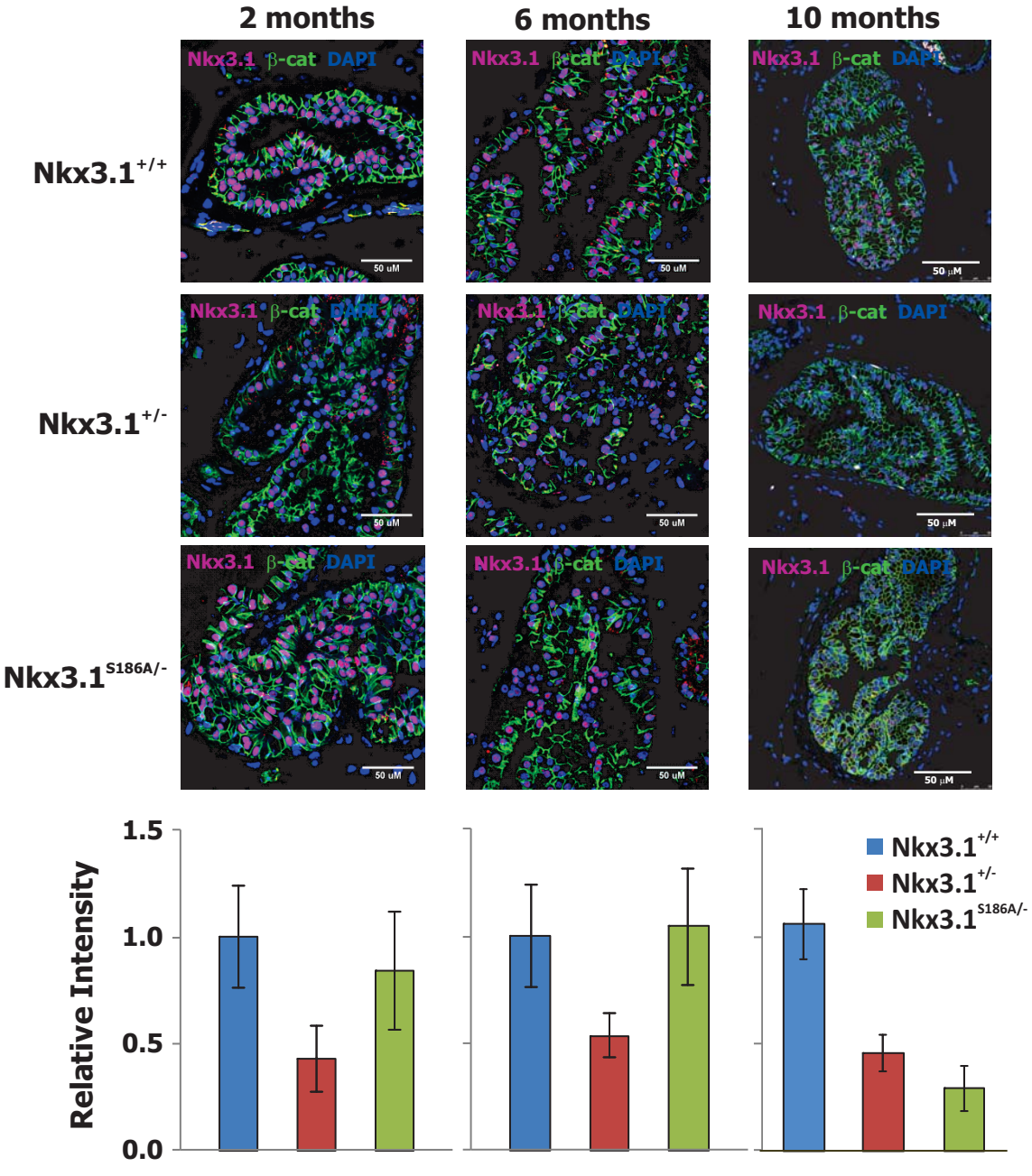
Supplementary Fig 4

A. Immunohistochemistry for cytokeratins 5 and 8 in 2-month old anterior prostate lobes. The arrow in the section from Nkx3.1$^{+/-}$ show an area of early hyperplasia. The arrows in the section from Nkx3.1$^{S186A/-}$ show areas of epithelial detachment.

B. Polyacrylamide gel separation of Coomassie blue-stained proteins from prostate lobes and seminal vesicle from mice of three genotypes as indicated.

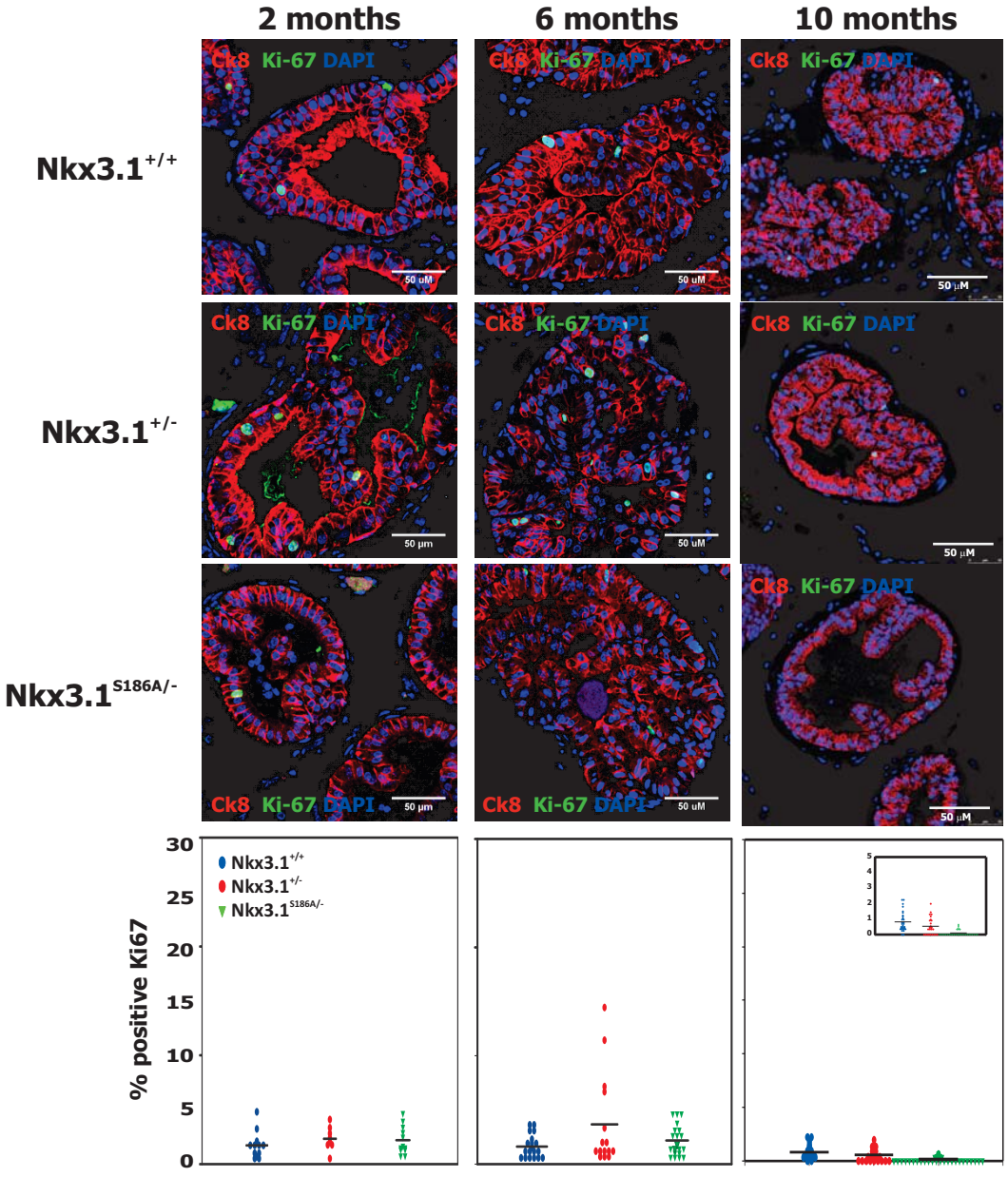
Supplementary Figure 5

Nkx3.1 expression. Nkx3.1 was detected in paraffin sections from dorsolateral prostate lobes of Nkx3.1$^{+/+}$, Nkx3.1$^{+/-}$, and Nkx3.1$^{S186A/-}$ mice at 2, 6, and 10 months of age. Counter stain was done with antibody to β-catenin and DAPI. Confocal images were quantitated with image J and Nkx3.1 expression was plotted relative to adjacent β-catenin expression. Relative intensity of Nkx3.1 expression in Nkx3.1$^{S186A/-}$ mice differed from both Nkx3.1$^{+/+}$ and Nkx3.1$^{+/-}$ mice by t-test with $p<0.01$.



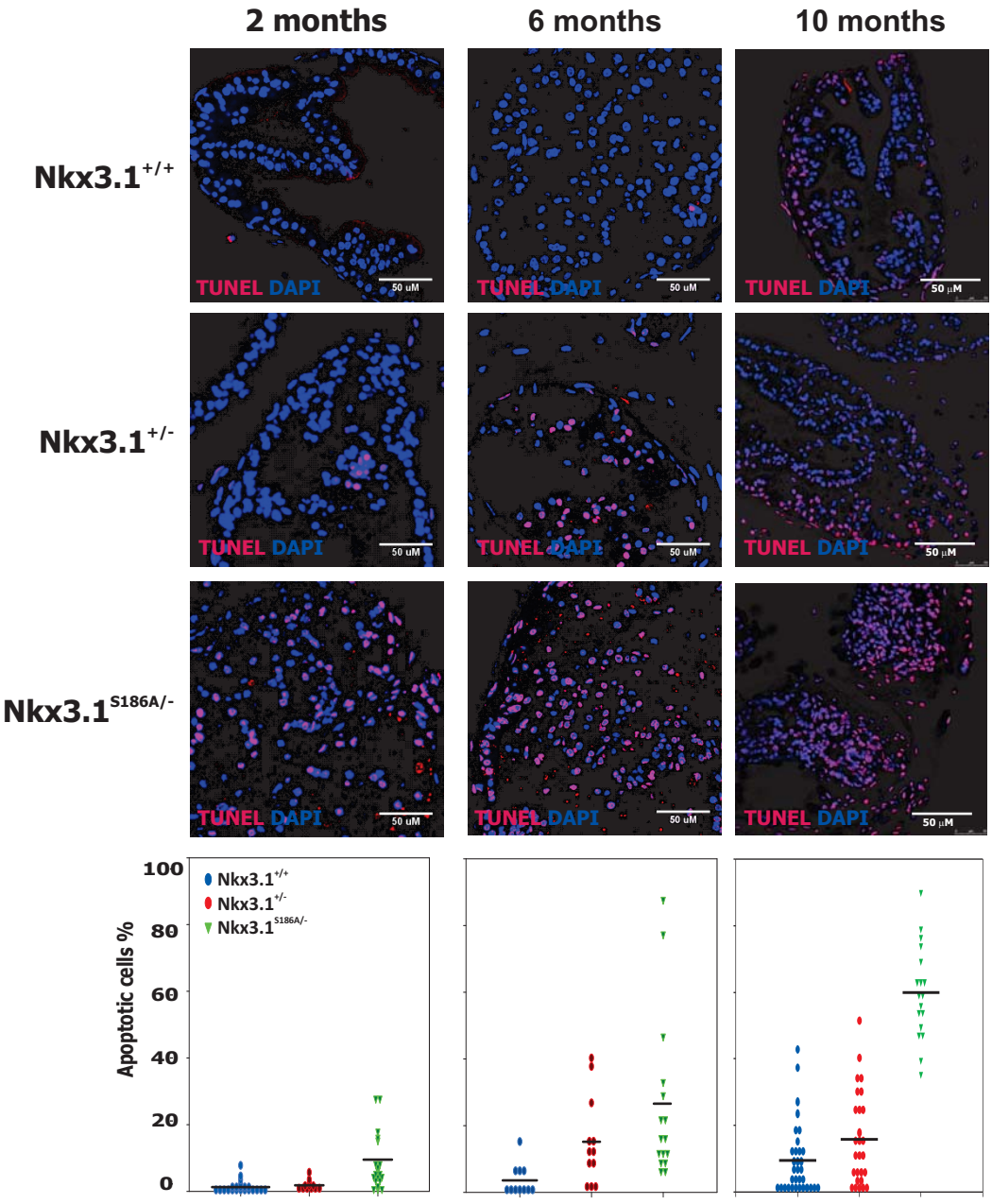
Supplementary Fig 6

Cell proliferation. Immunostaining for Ki-67 was done with cytokeratin 8 and DAPI as counterstains in sections from DLP. Confocal images were quantitated with image J and the number of Ki-67-positive cells per field as a percentage of all nuclei in the field were recorded. The fraction of Ki-67 staining cells in Nkx3.1$^{+/-}$ prostates differed from both Nkx3.1$^{+/+}$ and Nkx3.1$^{S186A/-}$ prostates at 6 months by t-test with $p<0.01$.



Supplementary Figure 7

TUNEL staining was done on paraffin sections from dorsolateral prostate lobes of Nkx3.1$^{+/+}$, Nkx3.1$^{+/-}$, and Nkx3.1$^{S186A/-}$ mice at 2, 6, and 10 months of age. Counterstain was done with DAPI. Confocal images were quantitated with image J and the number of TUNEL-positive cells per field were recorded compared to the total number of nuclei seen in the field. Nkx3.1$^{S186A/-}$ mice differed from both Nkx3.1$^{+/+}$ and Nkx3.1$^{+/-}$ mice by t-test with $p<0.01$.



Supplementary Figure 8

A. Cleaved caspase 3 staining was done on paraffin sections from both anterior and dorsolateral prostate lobes of Nkx3.1$^{+/+}$, Nkx3.1$^{+/-}$, and Nkx3.1$^{S186A/-}$ mice at 2, 6, and 10 months of age. Counterstain was done with DAPI.

B. Cleaved caspase 3 staining of prostates from Nkx3.1$^{+/+}$ mice unirradiated and 2 hours after 15Gy irradiation.