Supplemental figure legends

Figure S1. PCR genotyping on CCSP-rtTA;Tet-on-Kras4b^{G12D} transgenic mice, and Kras(G12D) induced prostatic lesions responding to doxycycline withdrawal. (A) Genomic DNA was extracted from mice tails. The primers (listed in Supplemental Table 1) were used for detecting Kras and rtTA gene fragment by PCR amplification. For example, the mouse "8724" was detected as double CCSP-rtTA;Tet-on-Kras4b^{G12D} positive. This mouse was used for the experiment in Figure 1. **(B)** Phase images of Kras(G12D) grafts derived from 8 weeks of Dox induction through drinking water (+Dox) or 8 weeks Dox induction plus 4 weeks of Dox withdrawal (-Dox). (**C)** H&E staining of the regenerated tissues from (B). Scale bar, 50 μm.

Figure S2. Androgen levels in the host regulate the growth of Kras(G12D)+AR tumors. (A) Kras(G12D)+AR xenografts responded to the addition of external androgen in the host mice. Kras(G12D)+AR tumor xenografts were seeded subcutaneously in the flank sides of SCID mice. Additionally, an external androgen pellet was implanted subcutaneously on the back of host mice in one group, but not the other group (under physiological androgen levels). Xenografts were harvested for analysis after 8 weeks. The volume of the tumors was measured. The white arrow indicates the location of a tumor, and the blue arrow is a testosterone pellet. **(B)** H&E (a and b) and IHC staining of CK5 (red)/CK8 (green)/DAPI (blue) (c and d) in Kras(G12D)+AR tumor xenografts. Scale bar, 50 μm.

Figure S3. Physiological androgen levels were required for the regeneration of secondary tumors. A) Schematic representation of the experimental design for generating the secondary tumor under intact/castrated recipients. Dissociated single cells from a primary Kras(G12D)+AR

tumor were injected subcutaneously into intact or castrated SCID mice. Xenografts were monitored and harvested after 2 months. **B)** Secondary tumors were formed in the intact SCID mice, but not the castrated recipients. The white arrow indicates the location of a tumor. **C)** Red and green fluorescence images (scale bar, 2 mm), H&E and IHC staining of p63, CK5 (red)/CK8 (green)/DAPI (blue), and AR of secondary Kras(G12D)+AR tumors. Kras(G12D)+AR tumorigenic cells from our previous study (carrying both RFP and GFP markers) were implanted subcutaneously in intact or castrated recipient SCID mice. Intact recipients developed tumors with GFP/RFP markers. The expression of AR, p63 and CK5/CK8 in tumor tissues and the tumor pathology were similar to those previously described. Scale bar, 50 μm.

Figure S4. Localization of $p63^+$, $CK5^+$, $CK5^+/CK8^+$, and $CK8^+$ cells in Kras(G12D)+AR tumors after removal of the external androgen pellet. Related to Figure 2B, f and 2B, i. IHC staining of p63 **(A)** (a zoom-in of Figure 2B, f) and CK5 (red)/CK8 (green)/DAPI (blue) **(B)** (a zoom-in of Figure 2B, i) on Kras+AR tumor xenografts. Line (red in A and white in B) indicates tumor region and its surrounding stromal region. Multiple layers of $CK5^+$ cells (red), a lay of $CK5^+/CK8^+$ cells (yellow), and multiple layers $CK8^+$ cells (green) were distributed from basal layer toward luminal region. Red arrow indicates $p63^+$ cells. **C)** IHC staining of CK5 (red)/CK8 (green)/DAPI (blue) on normal prostate tissue, Kras(G12D)+AR tumors with the external androgen pellet or after withdrawal of the androgen pellet. Scale bar, 50 μm.

Figure 5 Gli1 and Gli2 expression levels were elevated in Kras+AR tumors. A) The expression levels of Gli1, Gli2, and 18S rRNA were analyzed by RT-PCR in regenerated prostate tissues derived from normal regenerated tissues or Kras+AR tumors from the second

recipient. **B)** The expression levels of Gli1 and Gli2 expression were analyzed by immunohistochemistry in the regenerated normal prostate tissues (control) or Kras+AR tumors from the second recipient. The red arrows indicate the staining of Gli1 or Gli2. ***:$p<0.005$.

Figure S6. Verification of AR(WT), AR mutants, and Gli3T expression. (A) Related to Figure 3. 293T cells were transduced with AR(WT) or its mutants including AR(ΔPro), AR(V518F), AR(ΔNLS), and AR(N705S) (with no GFP reporter) by lentiviral infection. The expression levels of AR(WT) and AR mutants were examined by western blot analysis. **(B) Related to Figure 4.** Schematic representation of Gli3 and C-terminally truncated form of Gli3 (Gli3T) that acts as a dominant repressor of Gli transcription. Gli3T was cloned into a lentiviral vector as described in the Supplemental Methods. Schematic of lentiviral constructs expressing Gli3T (labeled with RFP). **(C)** 293T cells were transduced with indicated volumes of Gli3T virus by lentiviral infection. The expression levels of Gli3T and endogenous Gli3 (endo. Gli3) were analyzed by Western analysis. **(D)** UGSM cells were transduced with Gli3T or control vector by lentiviral infection. The mRNA levels of Patched1 (*Ptc1*), *Bcl2*, and *GAPDH* were measured by RT-PCR. Ptc1 and Bcl2 were normalized to GAPDH and set as 1 in control vector. ***: $p<0.001$

Figure S7, related to Figure 5. Validation of Gli1/2 shRNA. (A) Schematic representation of the lentiviral vector for shRNA expression including RFP expression controlled by a separate CMV promoter. **B)** Three shRNAs targeting Gli1, and two shRNAs targeting Gli2 were created in the lentiviral vector. UGSM cells were transduced with the shRNAs by lentiviral infection. The expression level of Gli1 and Gli2 were analyzed by RT-PCR. The Gli1-shRNA-771 and Gli2-shRNA-3'UTR with the best knockdown efficiency were used for *in vivo* experiments in Figure 5 and 6. The mock was normalized to GAPDH and set as 1. ***: $p<0.001$

Figure S8. The effect of Gli signaling on normal prostate regeneration. Prostate epithelial cells derived from CCSP-rtTA;Tet-on-Kras4b^{G12D} transgenic mice were transduced with control vector, overexpression of Gli3T, shRNA-Gli1, or shRNA-Gli2. The transduced cells were mixed with UGSM and implanted under the renal capsule. Host SCID mice were fed with normal drinking water (no induction of Kras) for 8 weeks. The regenerated tissues became normal tissue due to no induction of Kras. **(A)** Phase and RFP images of normal regenerated tissues. Scale bar, 3 mm. **(B)** H&E, RFP fluorescence, and IHC staining of AR, p63, CK5 (red)/CK8 (green)/DAPI (blue) in regenerated tissues of Vector control, over-expression of Gli3T, shRNA-Gli1, and shRNA-Gli2. Scale bar, 50 μm.

Figure S9. The effect of Gli signaling on Kras mediated transformation. Prostate epithelial cells derived from CCSP-rtTA;Tet-on-Kras4b^{G12D} transgenic mice were transduced with control vector, overexpression of Gli3T, shRNA-Gli1, or shRNA-Gli2. The transduced cells were mixed with UGSM and implanted under the renal capsule. Host SCID mice were fed with Dox in the drinking water for 8 weeks for induction of Kras. **(A)** Phase and RFP images of the regenerated tissues with an induction of Kras. Scale bar, 5 mm. **(B)** H&E, RFP fluorescence, and IHC staining of AR, p63, CK5 (red)/CK8 (green)/DAPI (blue) in Kras-mediated tissues with vector control, over-expression of Gli3T, shRNA-Gli1, and shRNA-Gli2. Scale bar, 50 μm.

Figure S1

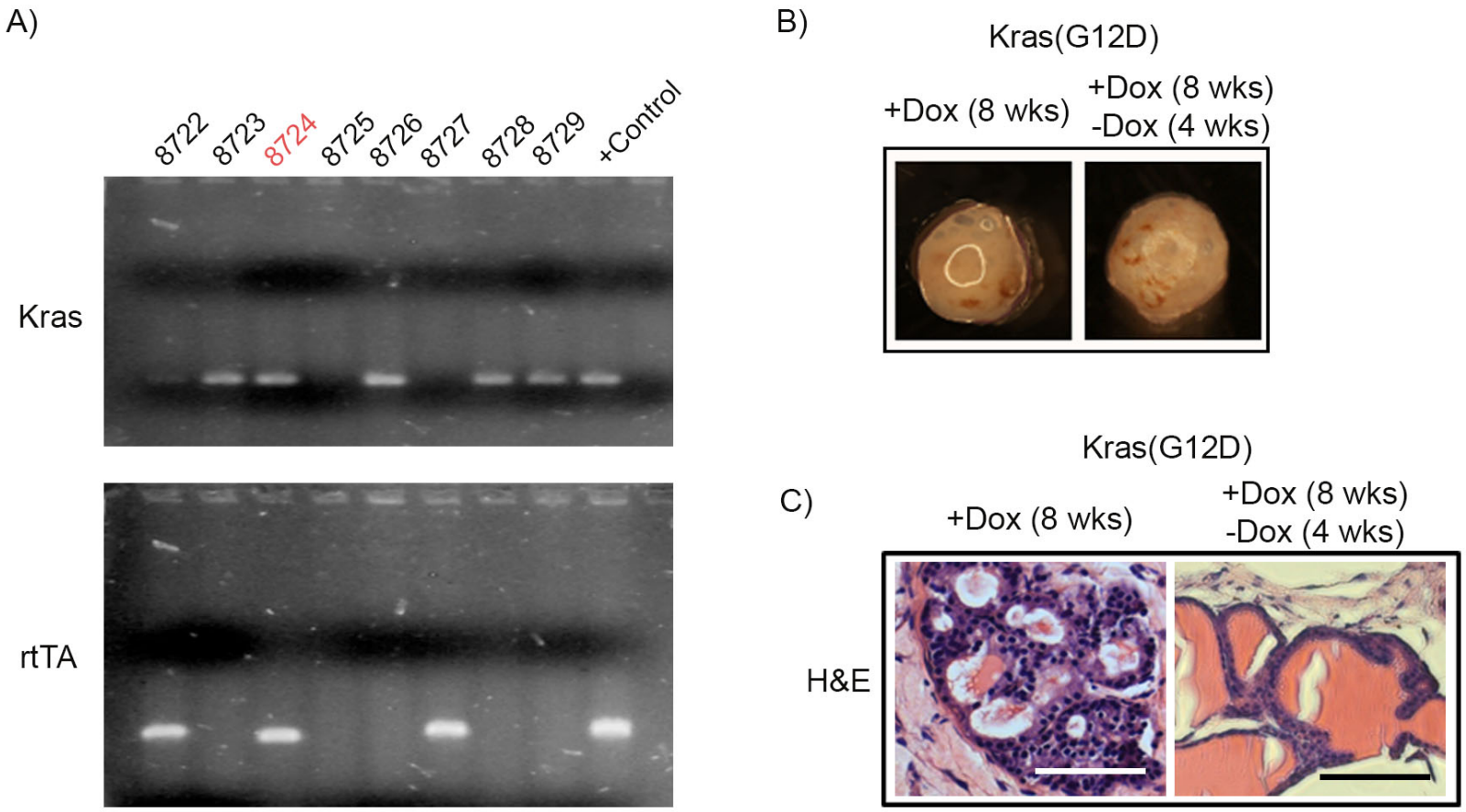

A)
8722
8723
8724
8725
8726
8727
8728
8729
+Control
Kras
rtTA
B)
Kras(G12D)
+Dox (8 wks)
+Dox (8 wks)
-Dox (4 wks)
C)
Kras(G12D)
+Dox (8 wks)
+Dox (8 wks)
-Dox (4 wks)
H&E

Figure S2

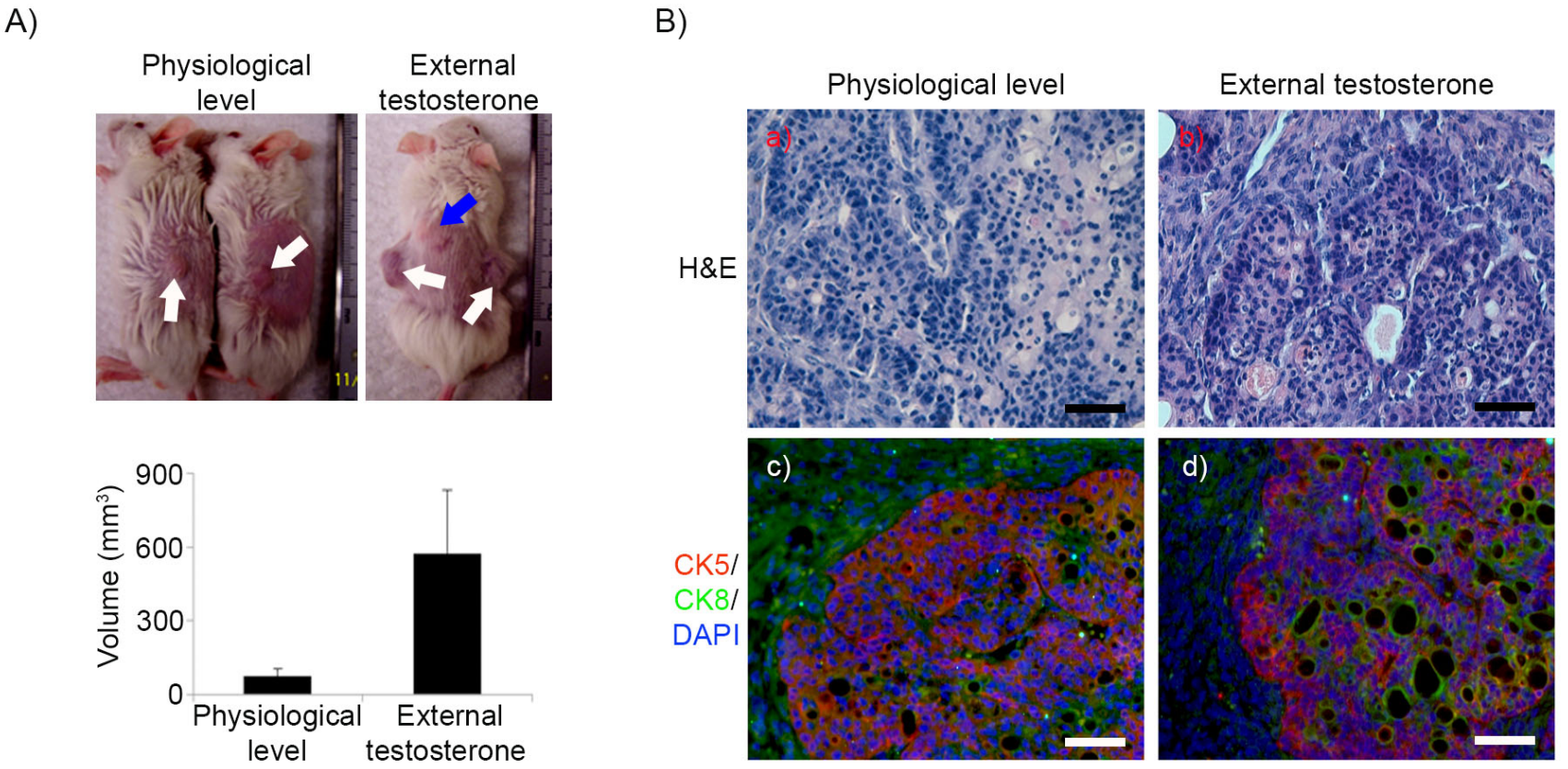

A)
Physiological level
External testosterone
900
600
300
0
Volume (mm3)
Physiological level
External testosterone
B)
Physiological level
External testosterone
H&E
a)
b)
CK5/
CK8/
DAPI
c)
d)

Figure S3

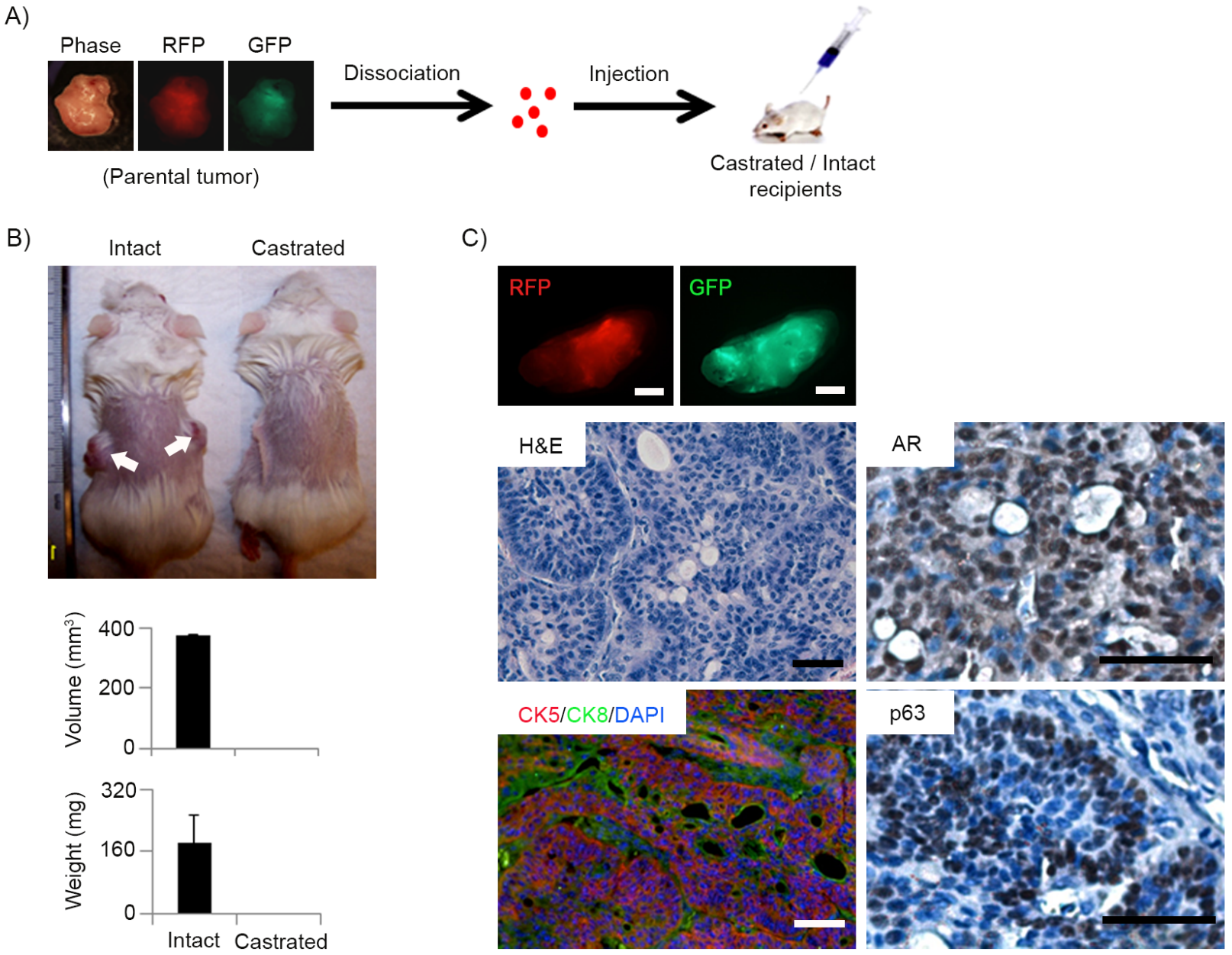

A)
Phase
RFP
GFP
(Parental tumor)
Dissociation
Injection
Castrated / Intact recipients
B)
Intact
Castrated
Volume (mm3)
400
200
0
Weight (mg)
320
160
0
Intact
Castrated
C)
RFP
GFP
H&E
AR
CK5/CK8/DAPI
p63

Figure S4

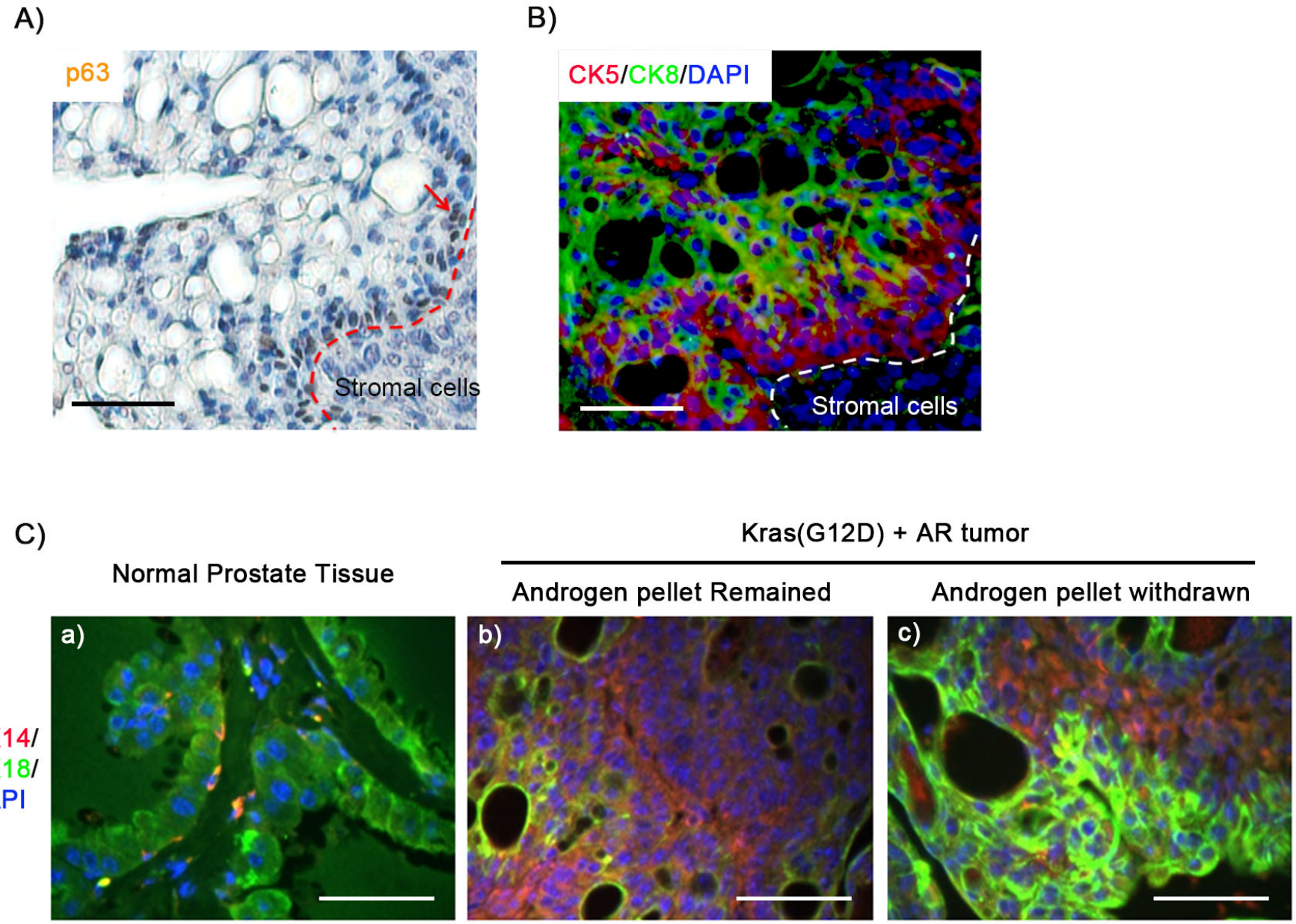

A)
p63
Stromal cells
B)
CK5/CK8/DAPI
Stromal cells
C)
Normal Prostate Tissue
Kras(G12D) + AR tumor
Androgen pellet Remained
Androgen pellet withdrawn
CK14/
CK18/
DAPI
a)
b)
c)

Figure S5

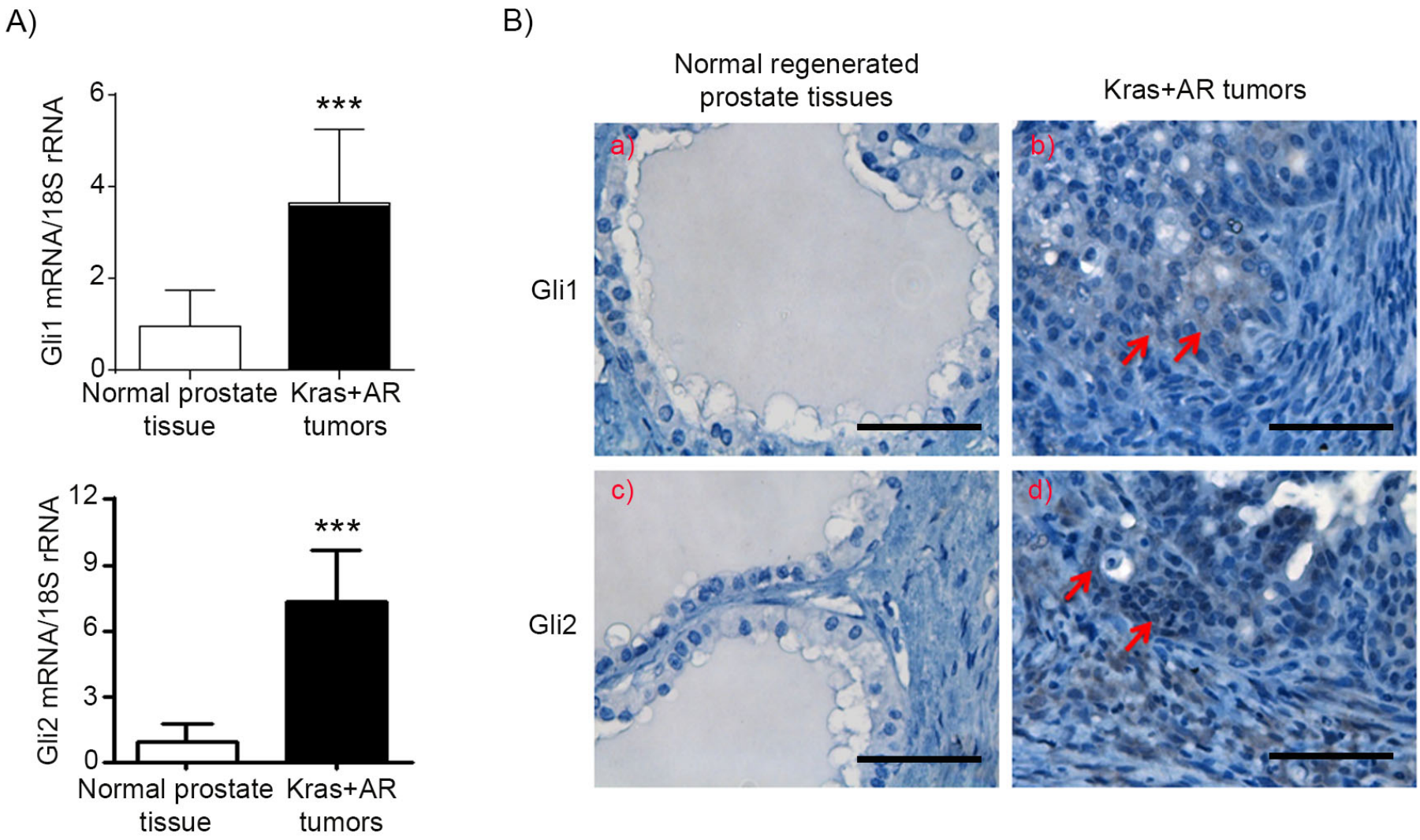

A)
Gli1 mRNA/18S rRNA
6
4
2
0
***
Normal prostate tissue
Kras+AR tumors
Gli2 mRNA/18S rRNA
12
9
6
3
0
***
Normal prostate tissue
Kras+AR tumors
B)
Normal regenerated prostate tissues
Kras+AR tumors
Gli1
Gli2
a)
b)
c)
d)

Figure S6

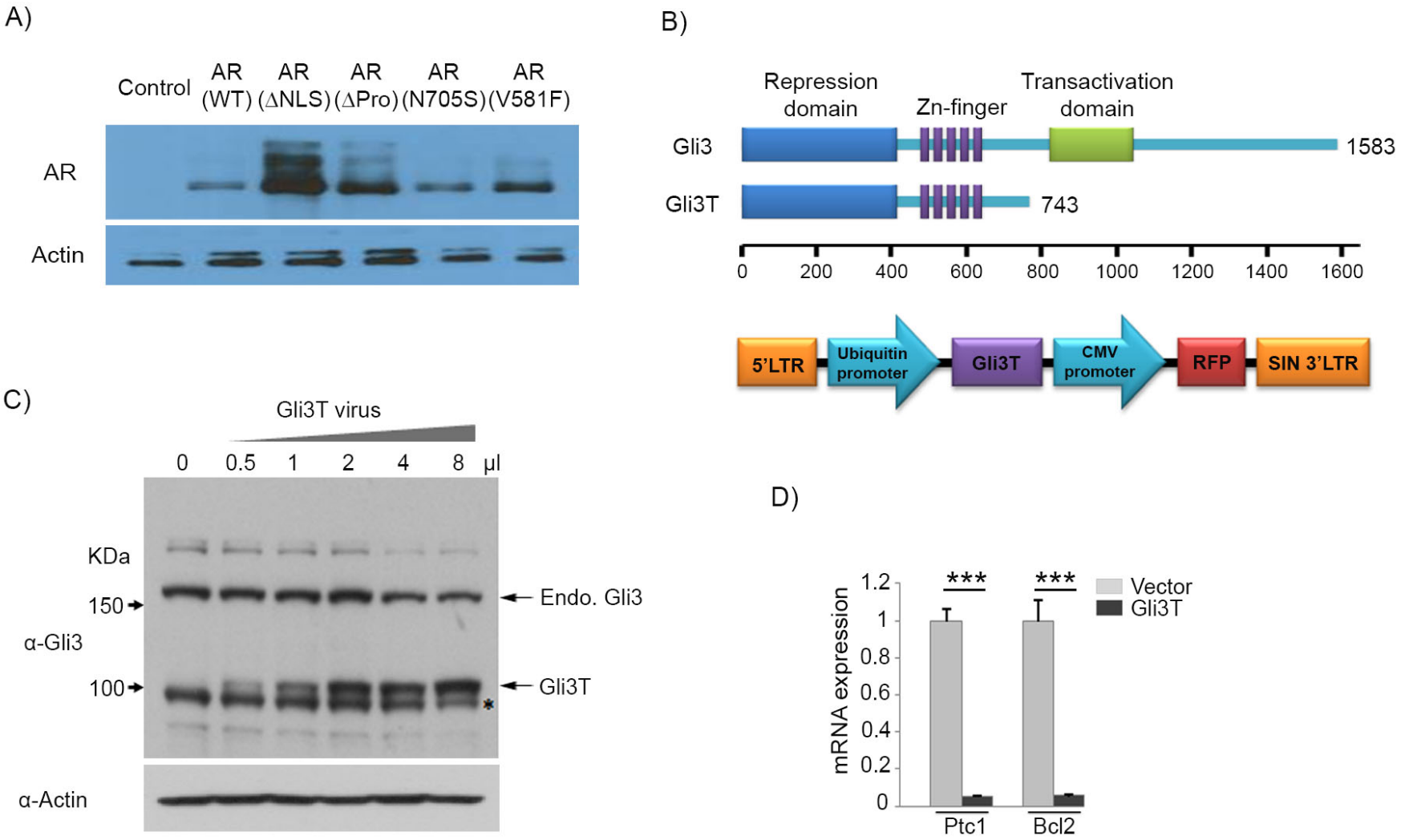
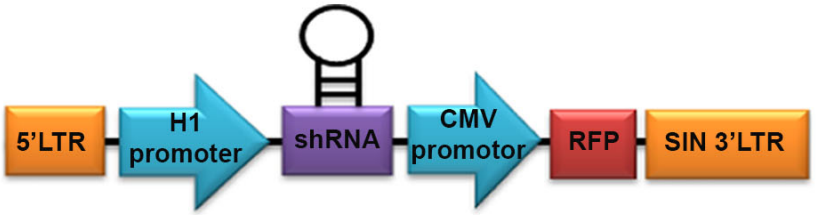

A)
Control
AR (WT)
AR (ΔNLS)
AR (ΔPro)
AR (N705S)
AR (V581F)
AR
Actin
B)
Repression domain
Zn-finger
Transactivation domain
Gli3
1583
Gli3T
743
0
200
400
600
800
1000
1200
1400
1600
5'LTR
Ubiquitin promoter
Gli3T
CMV promoter
RFP
SIN 3'LTR
C)
Gli3T virus
0
0.5
1
2
4
8
μl
KDa
150
α-Gli3
100
Endo. Gli3
Gli3T
α-Actin
D)
mRNA expression
1.2
1
0.8
0.6
0.4
0.2
0
***
***
Vector
Gli3T
Ptc1
Bcl2

Figure S7

A)


5'LTR
H1 promoter
shRNA
CMV promotor
RFP
SIN 3'LTR

B)

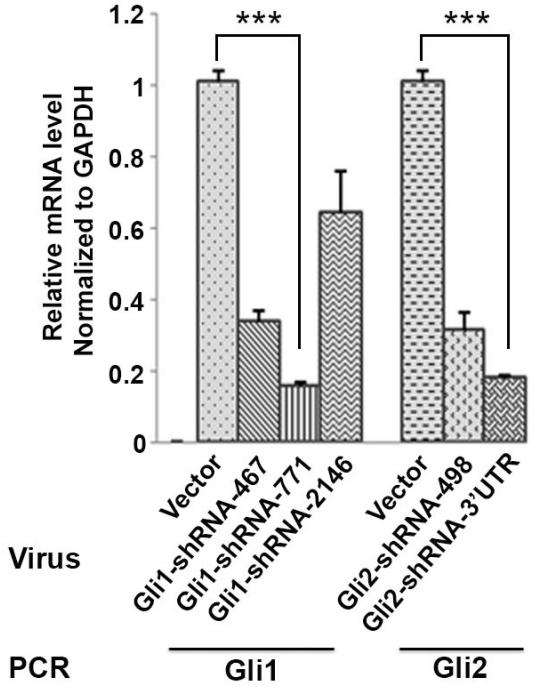

Relative mRNA level
Normalized to GAPDH
1.2
1
0.8
0.6
0.4
0.2
0
***
***
Vector
Gli1-shRNA-467
Gli1-shRNA-771
Gli1-shRNA-2146
Vector
Gli2-shRNA-498
Gli2-shRNA-3'UTR
Virus
PCR
Gli1
Gli2

Figure S8

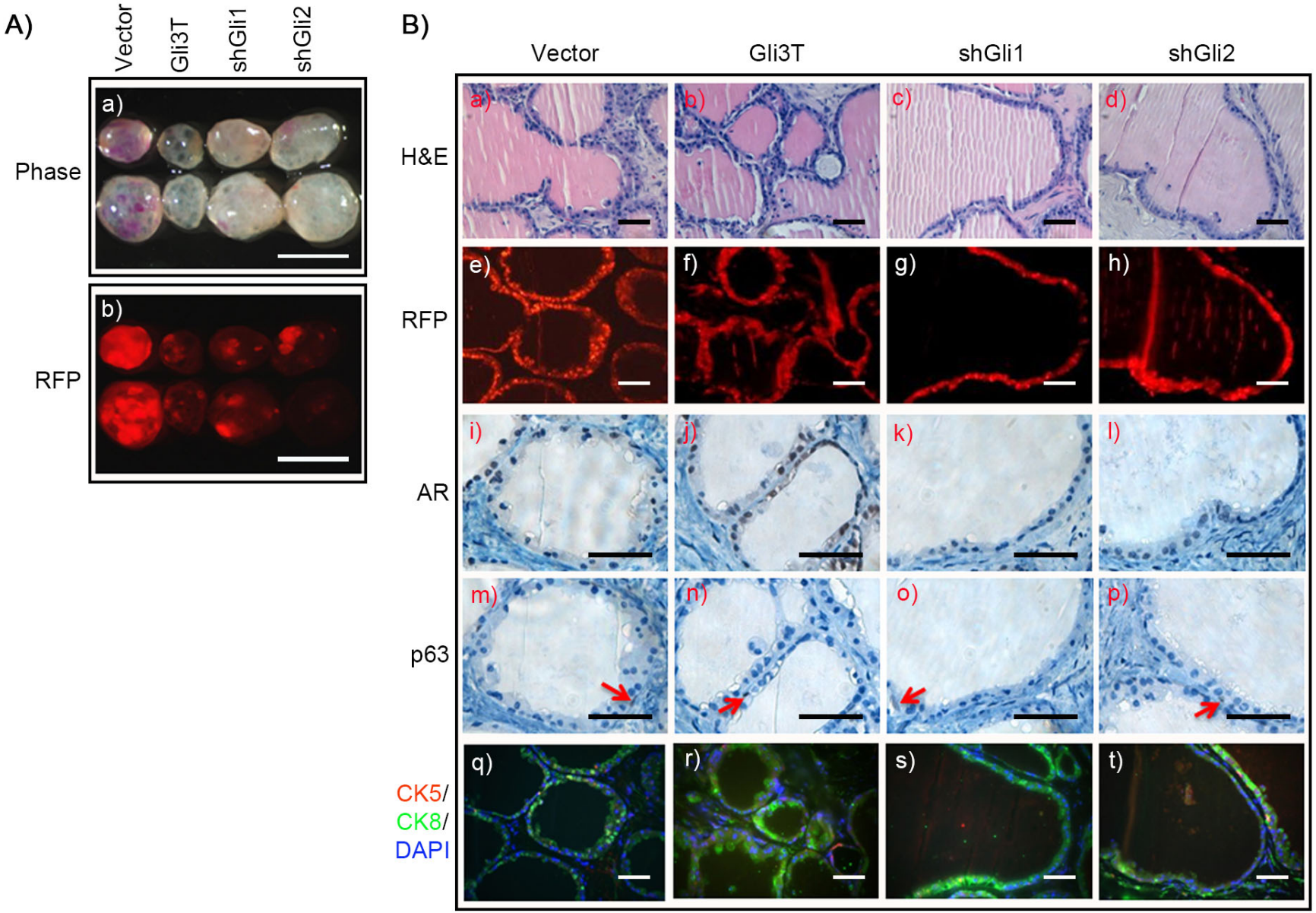

A)
Vector
Gli3T
shGli1
shGli2
Phase
RFP
a)
b)
B)
Vector
Gli3T
shGli1
shGli2
H&E
RFP
AR
p63
CK5/
CK8/
DAPI
a)
b)
c)
d)
e)
f)
g)
h)
i)
j)
k)
l)
m)
n)
o)
p)
q)
r)
s)
t)

Figure S9

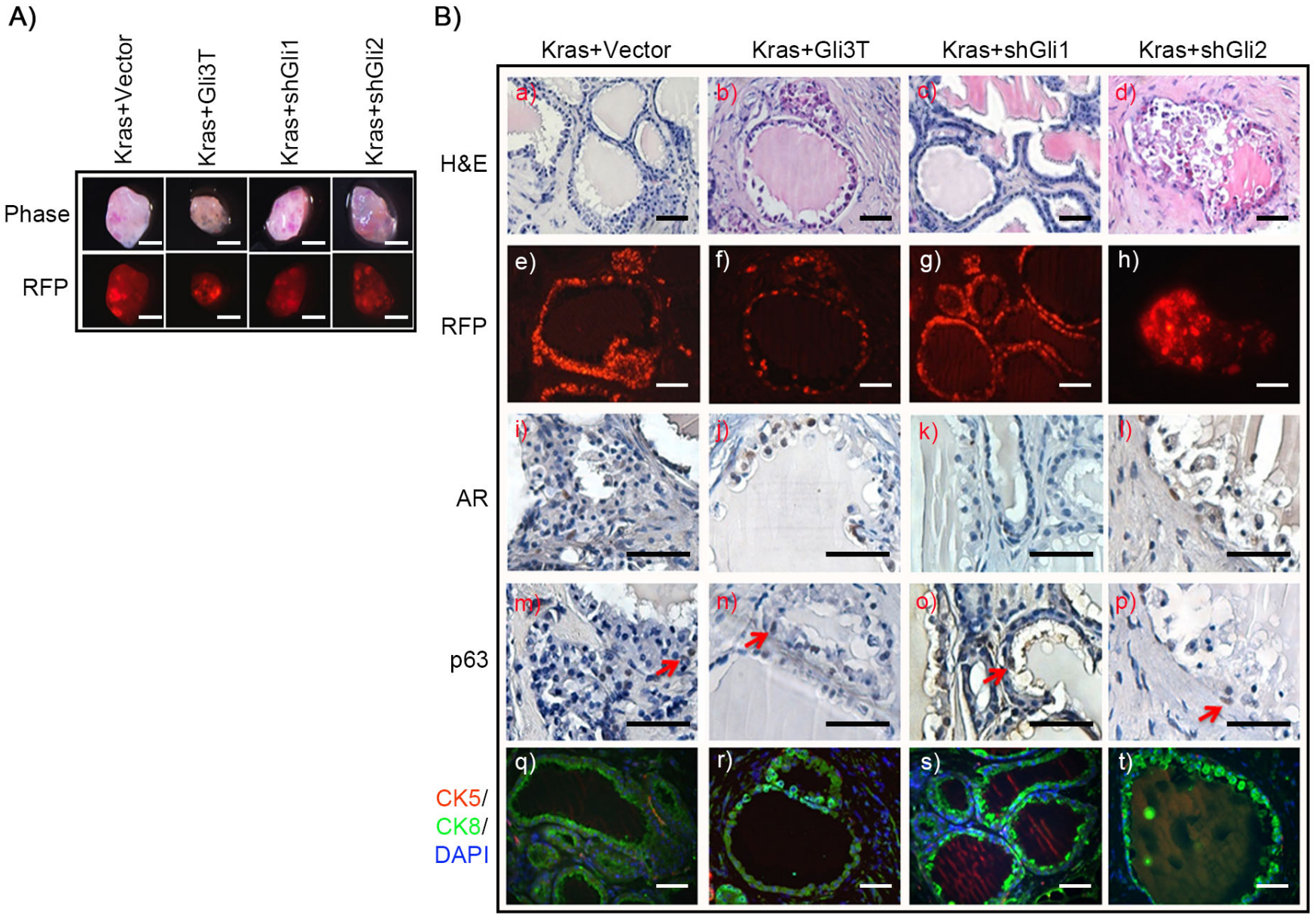

A)
Kras+Vector
Kras+Gli3T
Kras+shGli1
Kras+shGli2
Phase
RFP
B)
Kras+Vector
Kras+Gli3T
Kras+shGli1
Kras+shGli2
H&E
RFP
AR
p63
CK5/
CK8/
DAPI
a)
b)
c)
d)
e)
f)
g)
h)
i)
j)
k)
l)
m)
n)
o)
p)
q)
r)
s)
t)

Table S1. Oligonucleotide primers used for gene cloning and PCR analysis

Oligonucleotide	Sequence 5'-3'	Note
hGli3-F(XbaI)	AGTATCTAGAGCCGCCACCATGGAGGCCCAGTCC CACAGC	Cloning Gli3T (fragment of Gli3 from 1-743 amino acid)
hGli3-743R(XbaI)	CGCTTCTAGACTAGGCACTGAGGTCTCCTATACTA C	
shGli1-467-F	TCCC**CCTGTGTACCACATGACTCTA**TTCAAGAGA **TAGAGTCATGTGGTACACAGG**	Cloning shRNA-Gli1 in a lentiviral vector
shGli1-467-R	AAAA**CCTGTGTACCACATGACTCTA**TCTCTTGAA **TAGAGTCATGTGGTACACAGG**	
shGli1-771-F	TCCC**CCACATCAACAGTGAGCATAT**TTCAAGAGA **ATATGCTCACTGTTGATGTGG**	
shGli1-771-R	AAAA**CCACATCAACAGTGAGCATAT**TCTCTTGAA **ATATGCTCACTGTTGATGTGG**	
shGli1-2416-F	TCCC**CGACTTGAGCATTATGGACAA**TTCAAGAG A**TTGTCCATAATGCTCAAGTCG**	
shGli1-2416-R	AAAA**CGACTTGAGCATTATGGACAA**TCTCTTGA A**TTGTCCATAATGCTCAAGTCG**	
shGli2-498-F	TCCC**CACCAACCCTTCAGACTATTA**TTCAAGAGA **TAATAGTCTGAAGGGTTGGTG**	Cloning shRNA-Gli2 in a lentiviral vector
shGli2-498-R	AAAA**CACCAACCCTTCAGACTATTA**TCTCTTGAA **TAATAGTCTGAAGGGTTGGTG**	
shGli2-3UTR-F	TCCC**TATGTTTACCCGCTCCTATTT**TTCAAGAGA **AAATAGGAGCGGGTAAACATA**	
shGli2-3UTR-R	AAAA**TATGTTTACCCGCTCCTATTT**TCTCTTGAA **AAATAGGAGCGGGTAAACATA**	
mPtc1-F	CTCTGGAGCAGATTTCCAAGG	Real time-PCR primers. Bcl2-F and R could be used for detection of human Bcl2 gene as well
mPtc1-R	TGCCGCAGTTCTTTTGAATG	
mBCL2-F	GTGGATGACTGAGTACCTGAACC	
mBCL2-R	AGCCAGGAGAAATCAAACAGAG	
mGli2-F	ACTTTCTCCACACCCTGCTG	
mGli2-R	GGCTGCGAGGCTAAAGAGTC	
mGli1-F	CCGACGGAGGTCTCTTTGTC	
mGli1-R	GCGTCTCAGGGAAGGATGAG	
mGAPDH-F	AGGTCGGTGTGAACGGATTTG	

mGAPDH-R	TGTAGACCATGTAGTTGAGGTCA	
CCSP-F	ATCTGCCCAGGATTTCTTCA	
CCSP-R	TCTTGCTTACACAGAGGACTTGTT	
rtTA-F	AAGGTTTAACAACCCGTAAACTCG	
rtTA-R	ATCTCAATGGCTAAGGCG	
18S rRNA-F	GTCTGTGATGCCCTTAGATG	
18S rRNA-R	AGCTTATGACCCGCACTTAC	