SUPPLEMENTARY FIGURE LEGENDS

Figure S1. CSN4 does not affect mRNA expression of sGCα1, p53, or CSN5. (**A**) LNCaP and (**B**) CWR-22Rv1 cells were transfected with Ctrl siRNA or CSN4 siRNA and Q-RT-PCR was used to measure mRNA expression of CSN4, sGCα1, p53, and CSN5.

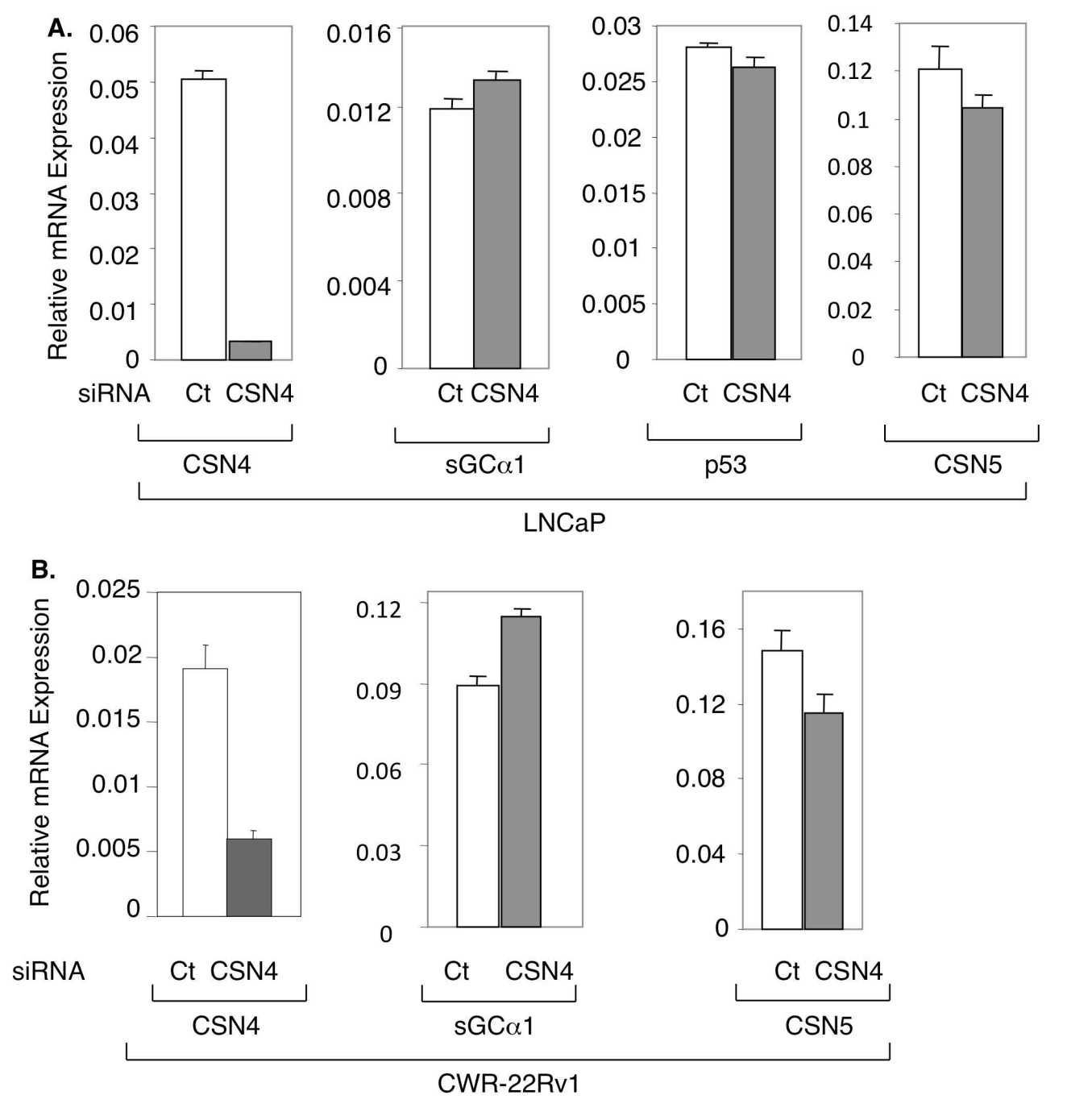
Figure S2. CSN4 affects the growth of AR-positive prostate cancer cells, but not AR-negative cells. (**A**) LNCaP, C81, and PC-3 cells were transfected with Ctrl siRNA or CSN4 siRNA, with or without CSN4 expression plasmid, and cell density was measured by MTT assay. Data points represent averages of three independent experiments plus standard deviations. Asterisks indicate statistical significance ($p < 0.03$), using the Student's T-test. (**B**) LNCaP and CWR-22Rv1 cells were transfected with Ctrl siRNA (-) or CSN4 siRNA (+), with or without sGCα1 expression plasmid, and subjected to Western blotting to measure p53, sGCα1, and CSN4. β-actin was used as loading control and the molecular weights of the proteins are 53 kDa (p53), 77 kDa (sGCα1), 49 kDa (CSN4), and 42 kDa (β-actin).

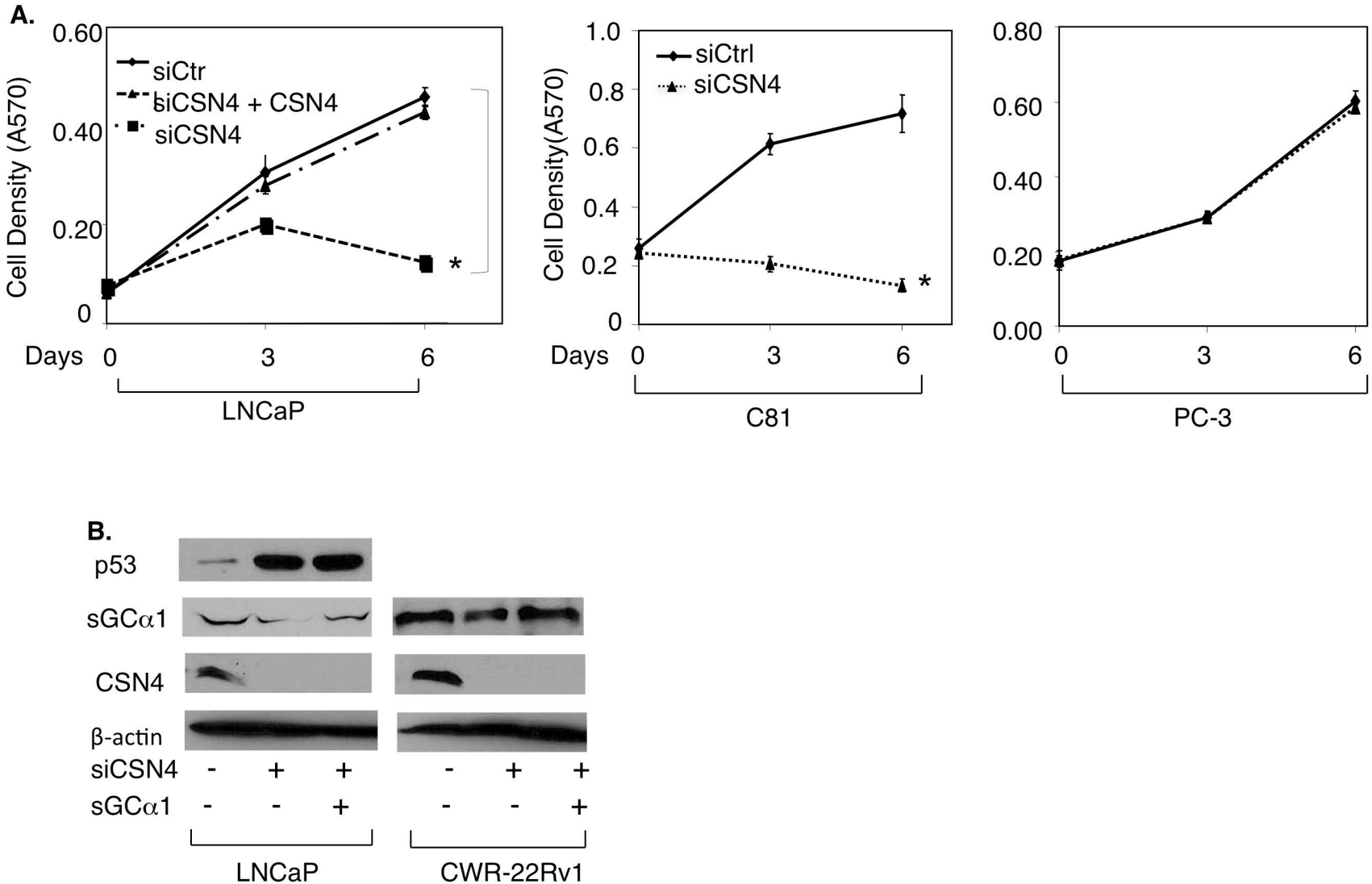
Figure S3. Cyclase-deficient sGCα1 rescues the growth of prostate cancer cells depleted for CSN4. LNCaP cells were transfected with Ctrl siRNA or CSN4 siRNA, with or without sGCα1(D531A) expression plasmid, and (**A**) MTT assay was used to measure cell density or (**B**) Western blotting to measure expression of CSN4, sGCα1, and sGCα1(D531A). Asterisks indicate statistical significance ($p < 0.05$), using the Student T-test. (**C**) LNCaP and CWR-22Rv1 cells were transfected with Ctrl siRNA or CSN4

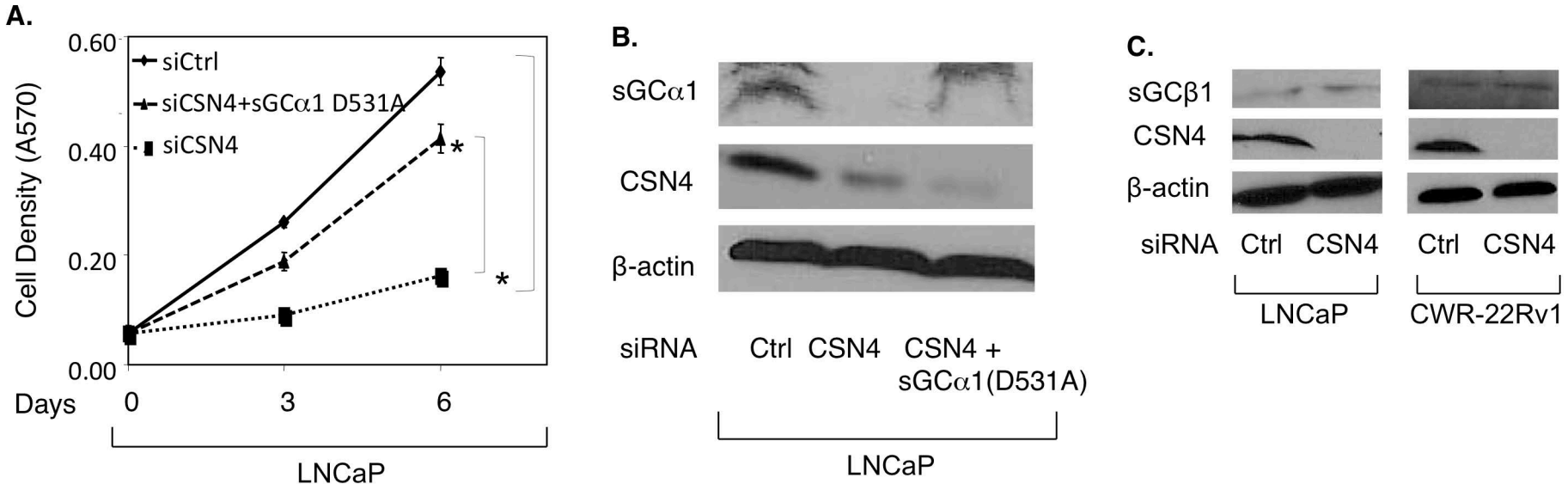
siRNA and subjected to Western blotting to measure sGCβ1, and CSN4. In B and C, β-actin was used as loading control and the molecular weights of the proteins are 53 kDa (p53), 77 kDa (sGCα1), 77 kDa (sGCα1-D531A), 49 kDa (CSN4), 72 kDa (sGCβ1), and 42 kDa (β-actin).

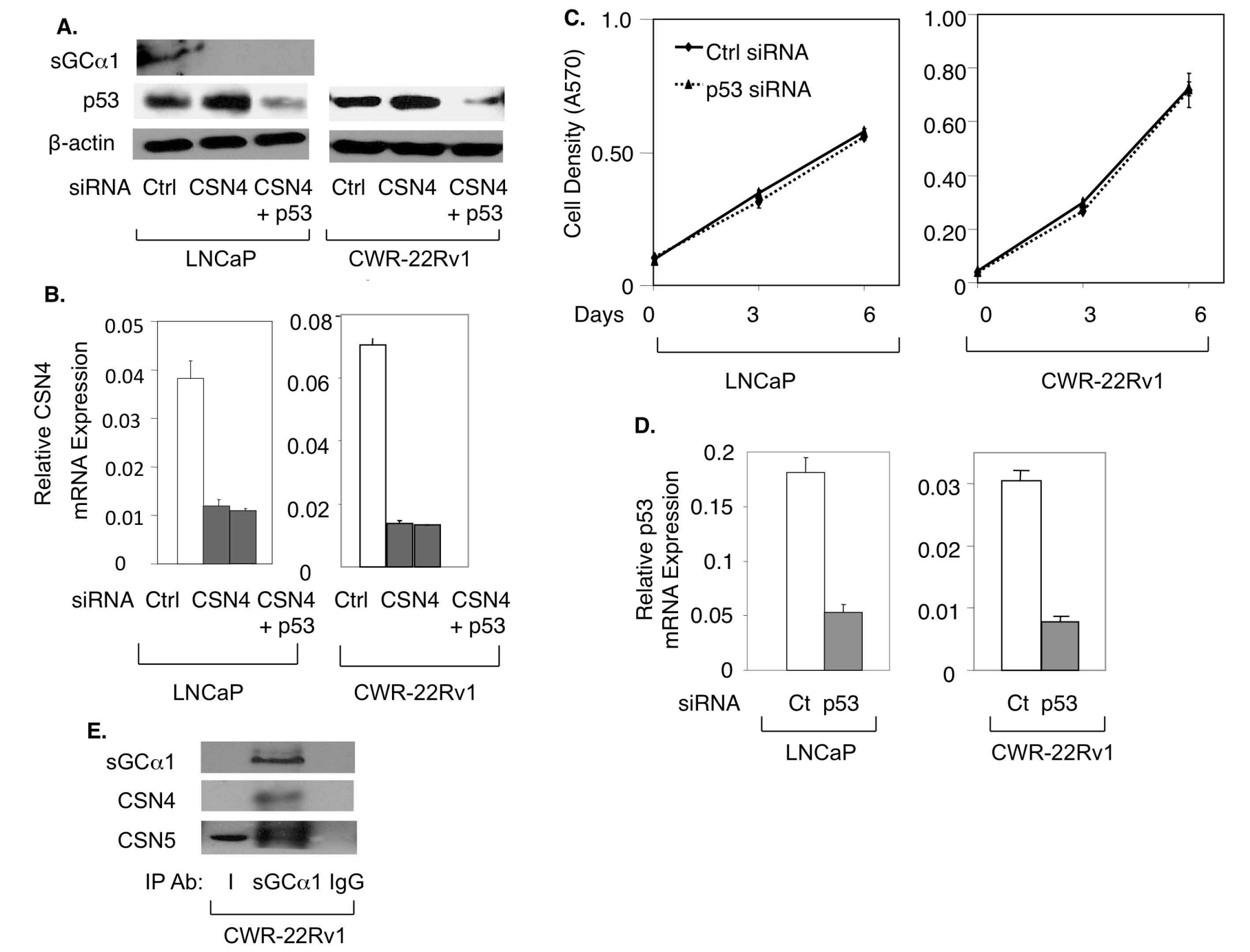
Figure S4. p53 does not affect sGCα1 protein levels nor the growth of prostate cancer cells. (A) LNCaP and CWR-22Rv1 cells were transfected with Ctrl siRNA or CSN4 siRNA, with or without p53 siRNA, and subjected to Western blotting to measure expression of sGCα1 and p53 or (**B**) Q-RT-PCR to measure expression of CSN4. LNCaP and CWR-22Rv1 cells were transfected with Ctrl siRNA or p53 siRNA, and subjected to (**C**) MTT to measure cell density or (**D**) Q-RT-PCR to measure expression of p53. (**E**) CWR-22Rv1 cell extracts were subjected to IP using antibody against sGCα1. IgG was used as negative control IP. Western blotting was used to measure sGCα1 (77 kDa), CSN4 (49 kDa), and CSN5 (38 kDa).

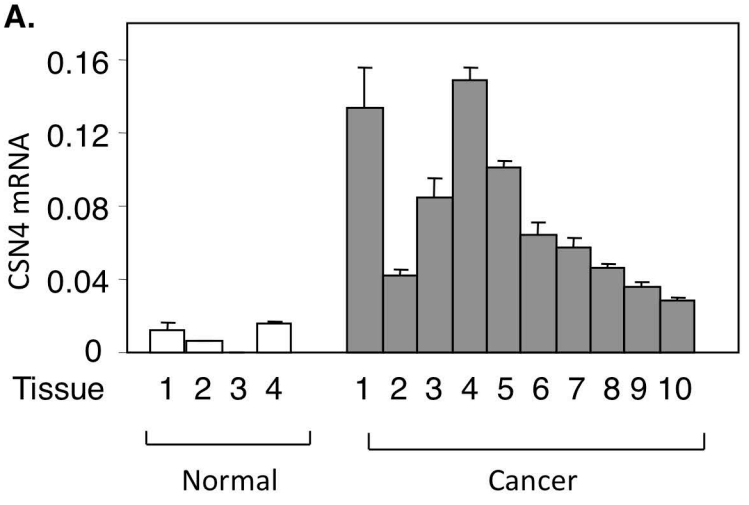
Figure S5. CSN4 mRNA is over-expressed in prostate tumors. (**A**) Q-RT-PCR was used to measure expression of CSN4 in normal prostate tissues or prostate tumors. (**B**) LNCaP and CWR-22Rv1 cells were treated with 25μM or 50 μM CID and Western blotting was used to measure sGCα1 and p53. β-actin was used as loading control.

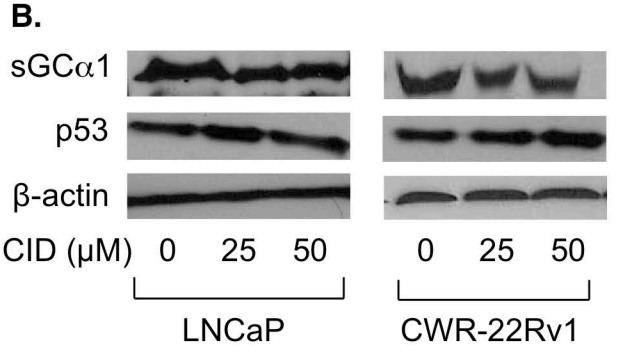

A.
Relative mRNA Expression
0.06
0.05
0.04
0.03
0.02
0.01
0
siRNA
Ct
CSN4
CSN4
0.016
0.012
0.008
0.004
0
Ct CSN4
sGCα1
0.03
0.025
0.02
0.015
0.01
0.005
0
Ct CSN4
p53
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
Ct CSN4
CSN5
LNCaP
B.
Relative mRNA Expression
0.025
0.02
0.015
0.01
0.005
0
siRNA
Ct CSN4
CSN4
0.12
0.09
0.06
0.03
0
Ct CSN4
sGCα1
0.16
0.12
0.08
0.04
0
Ct CSN4
CSN5
CWR-22Rv1


A.
Cell Density (A570)
0.60
0.40
0.20
0
siCtr
siCSN4 + CSN4
siCSN4
*
Days
0
3
6
LNCaP
Cell Density(A570)
1.0
0.8
0.6
0.4
0.2
0
siCtrl
siCSN4
*
Days
0
3
6
C81
0.80
0.60
0.40
0.20
0.00
0
3
6
PC-3
B.
p53
sGCα1
CSN4
β-actin
siCSN4 - + + - + +
sGCα1 - - + - - +
LNCaP
CWR-22Rv1


A.
0.60
0.40
0.20
0.00
Cell Density (A570)
siCtrl
siCSN4+sGCα1 D531A
siCSN4
*
*
Days
0
3
6
LNCaP
B.
sGCα1
CSN4
β-actin
siRNA
Ctrl
CSN4
CSN4 +
sGCα1(D531A)
LNCaP
C.
sGCβ1
CSN4
β-actin
siRNA
Ctrl
CSN4
Ctrl
CSN4
LNCaP
CWR-22Rv1


A.
sGCα1
p53
β-actin
siRNA Ctrl CSN4 CSN4 + p53 Ctrl CSN4 CSN4 + p53
LNCaP
CWR-22Rv1
B.
Relative CSN4 mRNA Expression
0.05
0.04
0.03
0.02
0.01
0
0.08
0.06
0.04
0.02
0
siRNA Ctrl CSN4 CSN4 + p53 Ctrl CSN4 CSN4 + p53
LNCaP
CWR-22Rv1
C.
Cell Density (A570)
1.0
0.50
0
Ctrl siRNA
p53 siRNA
Days 0 3 6
LNCaP
1.00
0.80
0.60
0.40
0.20
0
0 3 6
CWR-22Rv1
D.
Relative p53 mRNA Expression
0.2
0.15
0.1
0.05
0
siRNA Ct p53
LNCaP
0.03
0.02
0.01
0
Ct p53
CWR-22Rv1
E.
sGCα1
CSN4
CSN5
IP Ab: I sGCα1 IgG
CWR-22Rv1


A.
0.16
0.12
0.08
0.04
0
CSN4 mRNA
Tissue
1 2 3 4
1 2 3 4 5 6 7 8 9 10
Normal
Cancer


B.
sGCα1
p53
β-actin
CID (μM) 0 25 50
0 25 50
LNCaP
CWR-22Rv1