**Supplemental Figure 1. Androgen down-regulates TM4SF3 mRNA**

(**A**) LNCaP cells were treated with either ethanol (-) or 1 nM R1881 (+) and measured for expression of (**A**) TM4SF3 mRNA by Q-RT-PCR using four different sets of primers or (**B**) expression of either TM4SF3 or PSA mRNA by Q-RT-PCR. Diagram in **A** below shows the regions of the TM4SF3 mRNA that the primers cover; primers are described in Materials and Methods. In **B**, the CDS primer was used for TM4SF3. Data points represent averages of three independent experiments plus standard deviations. The Student T–test was performed to show statistical significance ($p < 0.05$) as indicated by asterisks.

**Supplemental Figure 2. TM4SF3 induces invasion and migration of prostate cancer cells.**

Western blotting was used to measure TM4SF3 and AR expression in (**A**) LNCaP, VCaP, or PrEC (primary prostate epithelial cells) cells or (**B**) in LNCaP cells grown for 0-24 hrs. Cells were treated with either ethanol (-) or 1 nM R1881 (+). (**C-E**) C81cells were treated with either ethanol (-) or 1 nM R1881 (+) and measured for expression of TM4SF3 and AR by (**C, D**) immunocytochemistry or (**E**) Western blotting of cell fractions [cytosol (C), membrane (M), and nuclear (N)]. Note that β-tubulin was used as a cytosolic marker, Na+/K+ ATPase as a membrane marker, Lamin A as a nuclear marker, and β-actin to control for protein loading. (**F**) C-81 cells treated with ethanol (-) or 1 nM R1881 (+) and whole-cell extracts were subjected to IP using antibodies against AR or TM4SF3, or IgG. For **A, B,** and **E**, the numbers above the gel represent quantification of the TM4SF3 and AR Western blot

signals, standardized to β-actin, and relative to the first lane or third lane, which was set to 1.

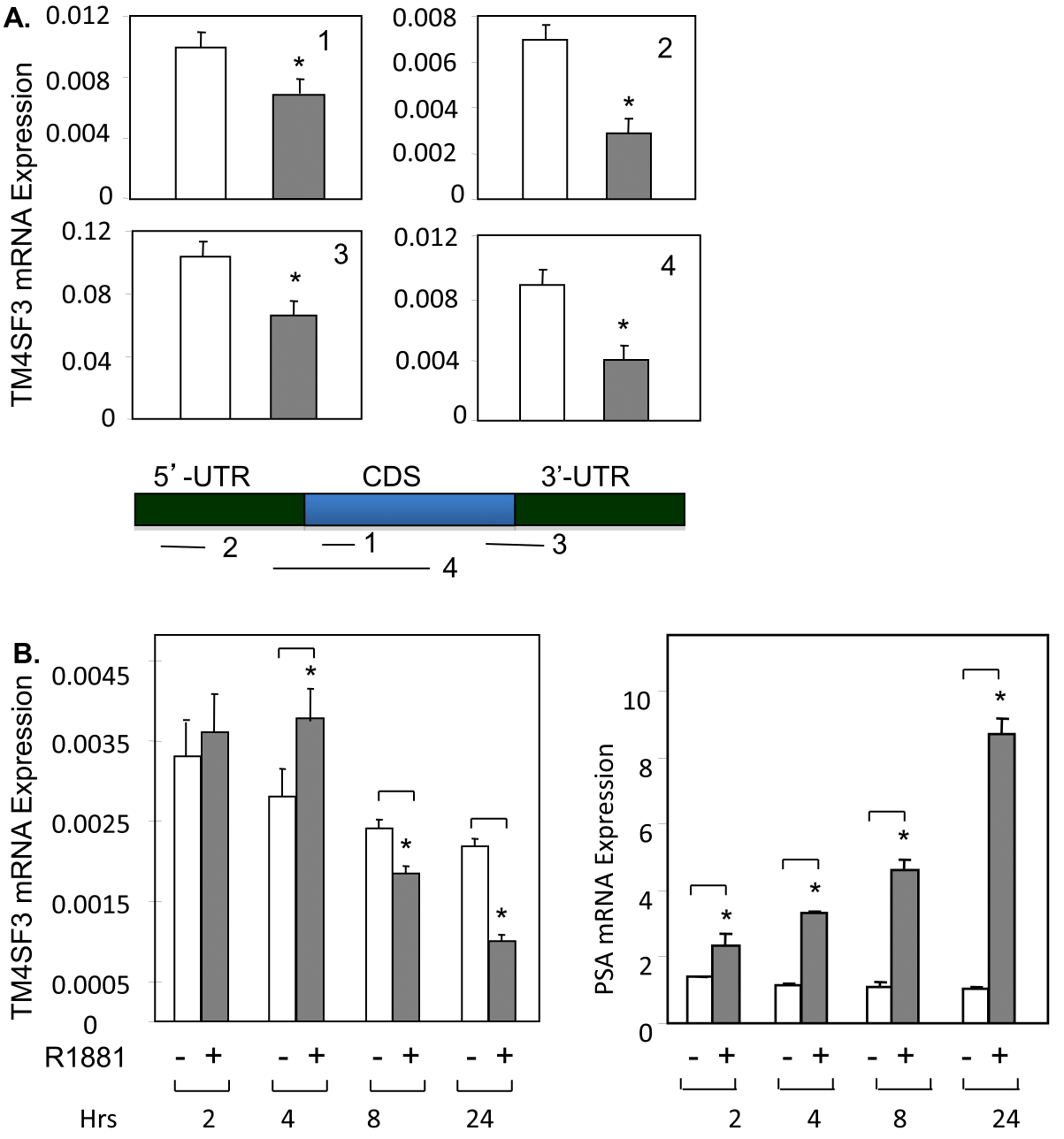
**Supplemental Figure 3. Androgen up-regulates and induces nuclear localization of exogenous TM4SF3 in prostate cancer.**

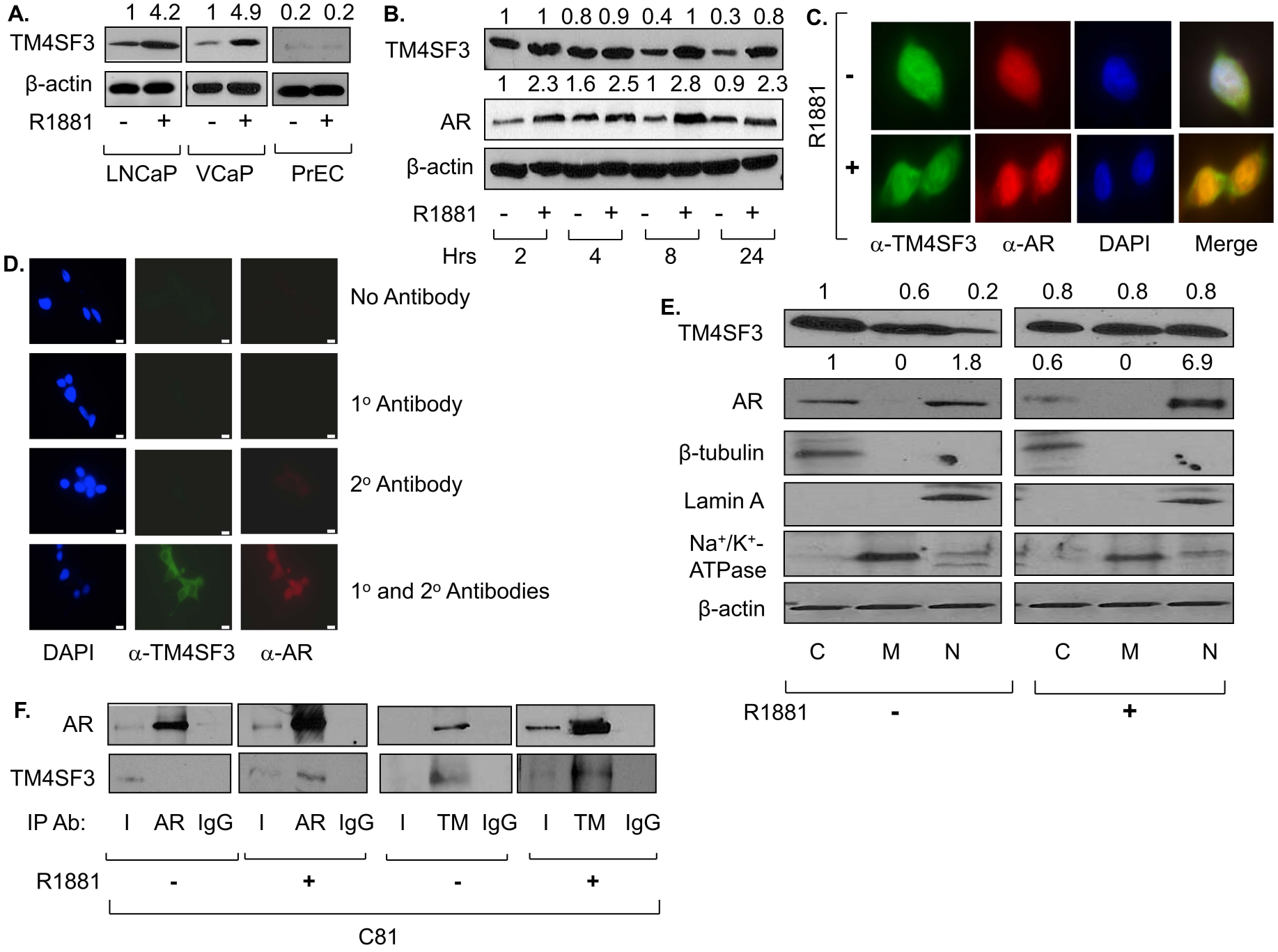
LNCaP cells, treated with either ethanol (-) or 1 nM R1881 (+), were transfected with (**A, C**) Empty vector or TM4SF3-Flag or (**B**) tGFP (from Origene) or TM4SF3-tGFP and expression was measured by Western blotting or tGFP fluorescence. Note that in **A** TM4F3 expression was measured using antibodies against TM4SF3 or Flag and in **B** TM4SF3-Flag was measured in different cell fractions [Cytosol (C), membrane (M), and nuclear (N)] Cell fractions markers are GAPDH (cytosol) marker, Na+/K+ ATPase (membrane), and Lamin A (nuclear marker). Note that β-actin was used as a loading control in **A** and DAPI was used to stain the nuclei in **B**. For **A**, the numbers above the gel represent quantification of the TM4SF3 and AR Western blot signals, standardized to β-actin, and relative to the first lane or third lane, which was set to 1.

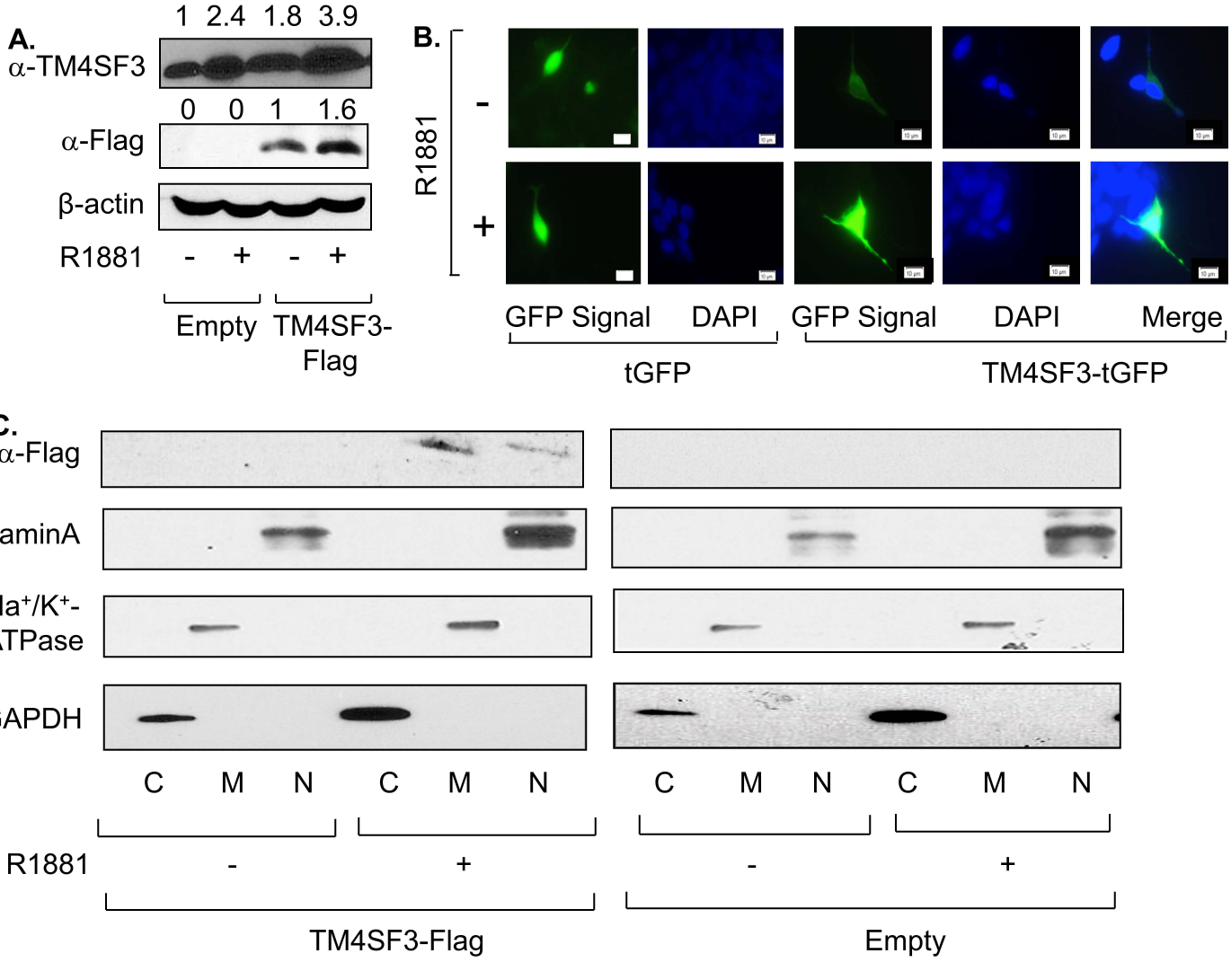
**Supplemental Figure 4. TM4SF3 up-regulates the AR protein and androgen signaling.**

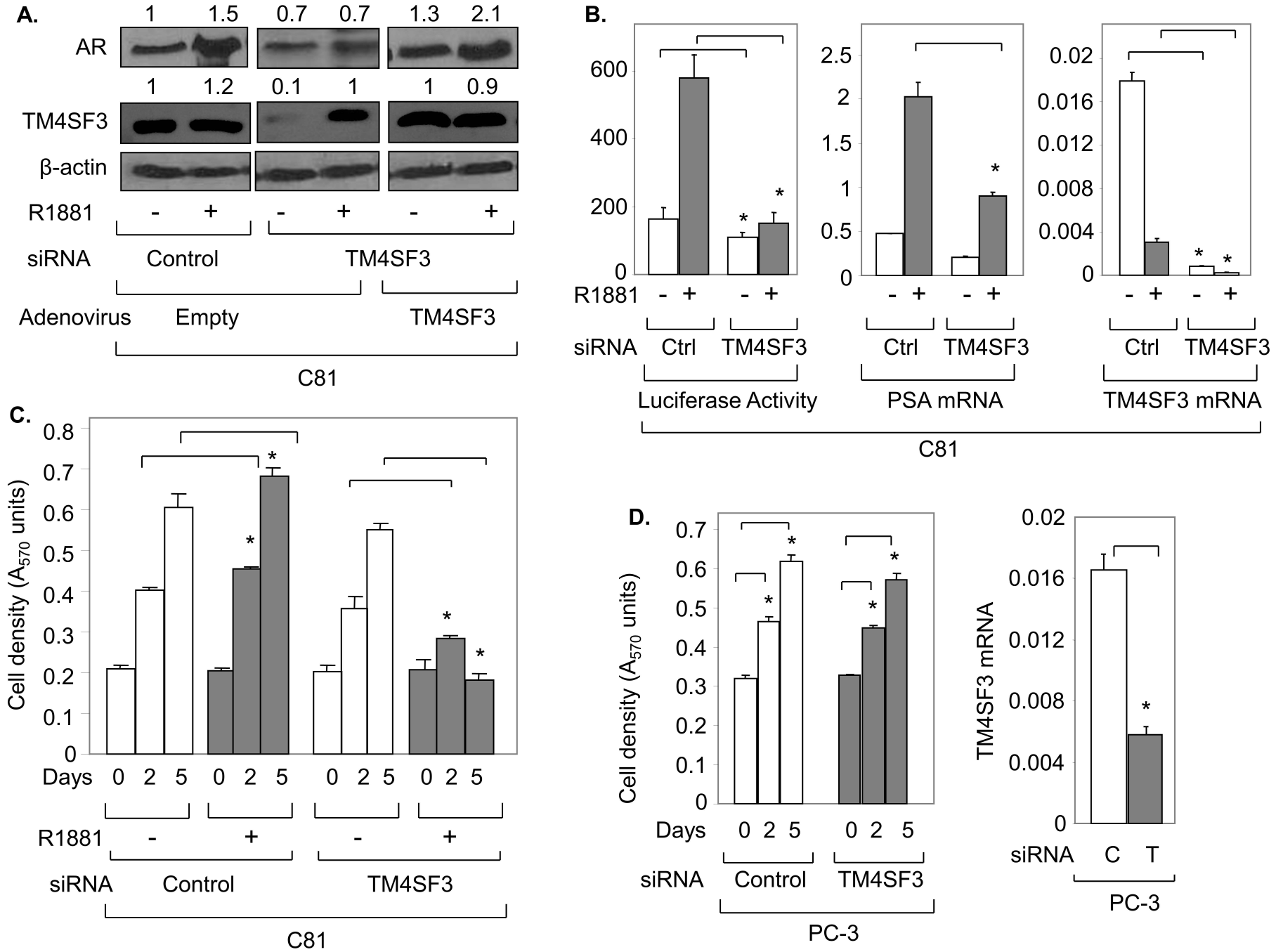
(**A**) C81 cells were transfected with control or TM4SF3 siRNA and infected with empty or TM4SF3 adenovirus in the presence of ethanol (-) or 1 nM R1881 (+) and measured for expression of TM4SF3 and AR by Western blotting. (**B, C**) C81 cells were transfected with Control or TM4SF3 siRNA and treated with ethanol (-) or 1 nM R1881 (+) and then measured for (**B**) reporter activity of transfected Luciferase or expression of endogenous PSA or TM4SF3 by Q-RT-PCR or (**C**) cell proliferation. (**D**) PC-3 cells were transfected with control or TM4SF3 siRNA and measured for cell

proliferation or TM4SF3 mRNA expression by Q-RT-PCR. In **B**, **C**, and **D**, bar graphs represents averages of three independent experiments plus standard deviations. The Student T-test was performed to show statistical significance when comparing cells transfected with TM4SF3 siRNA to control siRNA ($p<0.03$), as indicated by asterisks.


A.
TM4SF3 mRNA Expression
0.012
0.008
0.004
0
1
*
0.008
0.006
0.004
0.002
0
2
*
0.12
0.08
0.04
0
3
*
0.012
0.008
0.004
0
4
*
5' -UTR
CDS
3'-UTR
2
1
3
4
B.
TM4SF3 mRNA Expression
0.0045
0.0035
0.0025
0.0015
0.0005
0
R1881
- + - + - + - +
Hrs
2
4
8
24
PSA mRNA Expression
10
8
6
4
2
0
- + - + - + - +
2
4
8
24


A.
1 4.2 1 4.9 0.2 0.2
TM4SF3
β-actin
R1881 - + - + - +
LNCaP VCaP PrEC
B.
1 1 0.8 0.9 0.4 1 0.3 0.8
TM4SF3
1 2.3 1.6 2.5 1 2.8 0.9 2.3
AR
β-actin
R1881 - + - + - + - +
Hrs 2 4 8 24
C.
R1881
-
+
α-TM4SF3 α-AR DAPI Merge
D.
No Antibody
1° Antibody
2° Antibody
1° and 2° Antibodies
DAPI α-TM4SF3 α-AR
E.
1 0.6 0.2 0.8 0.8 0.8
TM4SF3
1 0 1.8 0.6 0 6.9
AR
β-tubulin
Lamin A
Na+/K+-ATPase
β-actin
C M N C M N
R1881 - +
F.
AR
TM4SF3
IP Ab: I AR IgG I AR IgG I TM IgG I TM IgG
R1881 - + - +
C81


A.
1 2.4 1.8 3.9
α-TM4SF3
0 0 1 1.6
α-Flag
β-actin
R1881 - + - +
Empty
TM4SF3-Flag
B.
R1881
-
+
GFP Signal
DAPI
GFP Signal
DAPI
Merge
tGFP
TM4SF3-tGFP
C.
α-Flag
LaminA
Na+/K+-ATPase
GAPDH
C M N C M N
C M N C M N
R1881
- +
- +
TM4SF3-Flag
Empty


A.
1 1.5 0.7 0.7 1.3 2.1
AR
1 1.2 0.1 1 1 0.9
TM4SF3
β-actin
R1881 - + - + - +
siRNA Control TM4SF3
Adenovirus Empty TM4SF3
C81
B.
600
400
200
0
2.5
2
1.5
1
0.5
0
0.02
0.016
0.012
0.008
0.004
0
R1881 - + - + - + - + - + - +
siRNA Ctrl TM4SF3 Ctrl TM4SF3 Ctrl TM4SF3
Luciferase Activity
PSA mRNA
TM4SF3 mRNA
C81
C.
Cell density (A570 units)
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Days 0 2 5 0 2 5 0 2 5 0 2 5
R1881 - + - +
siRNA Control TM4SF3
C81
D.
Cell density (A570 units)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Days 0 2 5 0 2 5
siRNA Control TM4SF3
PC-3
TM4SF3 mRNA
0.02
0.016
0.012
0.008
0.004
0
siRNA C T
PC-3