Supplemental Materials

Molecular Biology of the Cell

Di Donato et al.

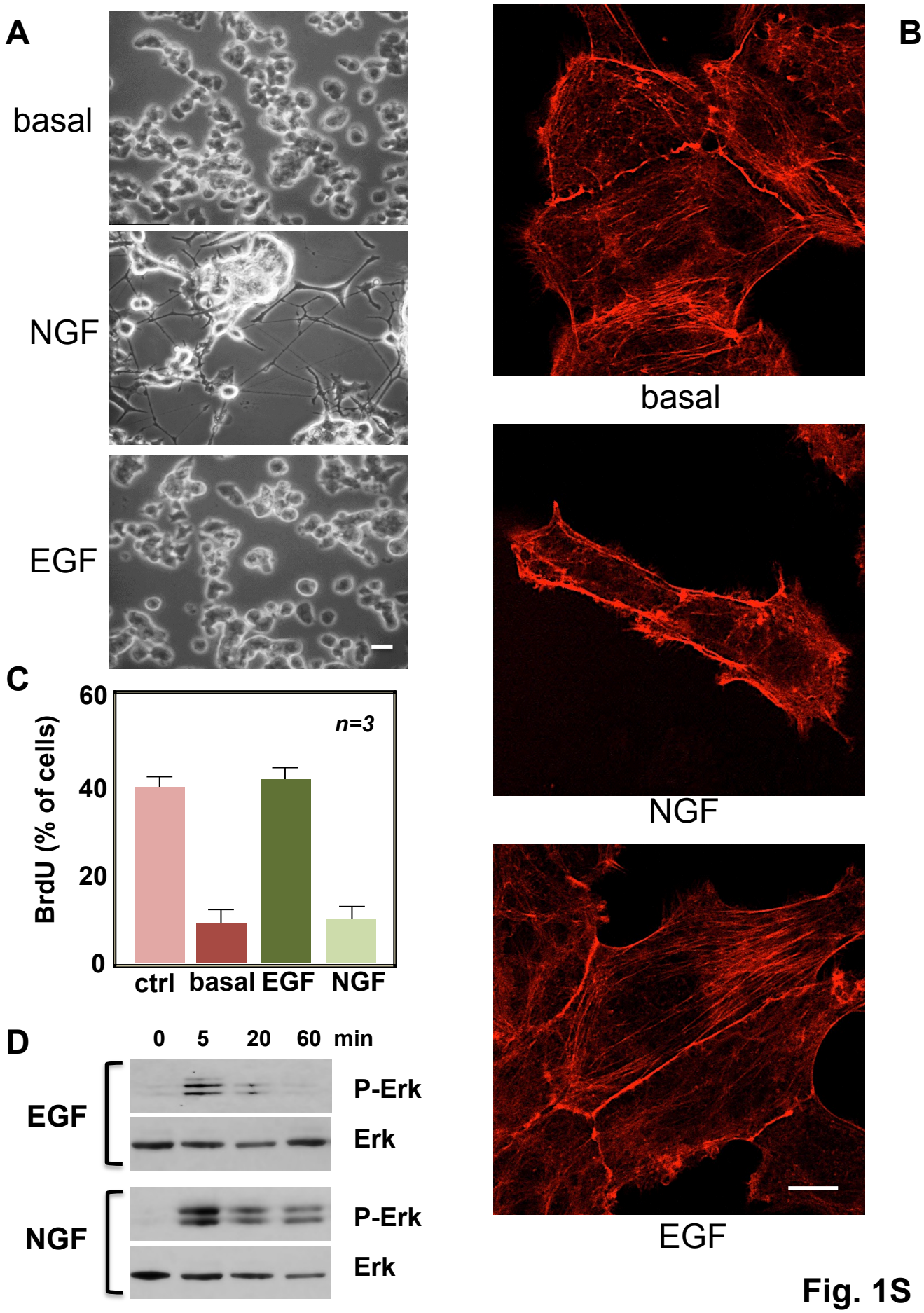

A
basal
NGF
EGF
B
basal
NGF
EGF
C
BrdU (% of cells)
60
40
20
0
n=3
ctrl
basal
EGF
NGF
D
0
5
20
60
min
EGF
P-Erk
Erk
NGF
P-Erk
Erk

Fig. 1S

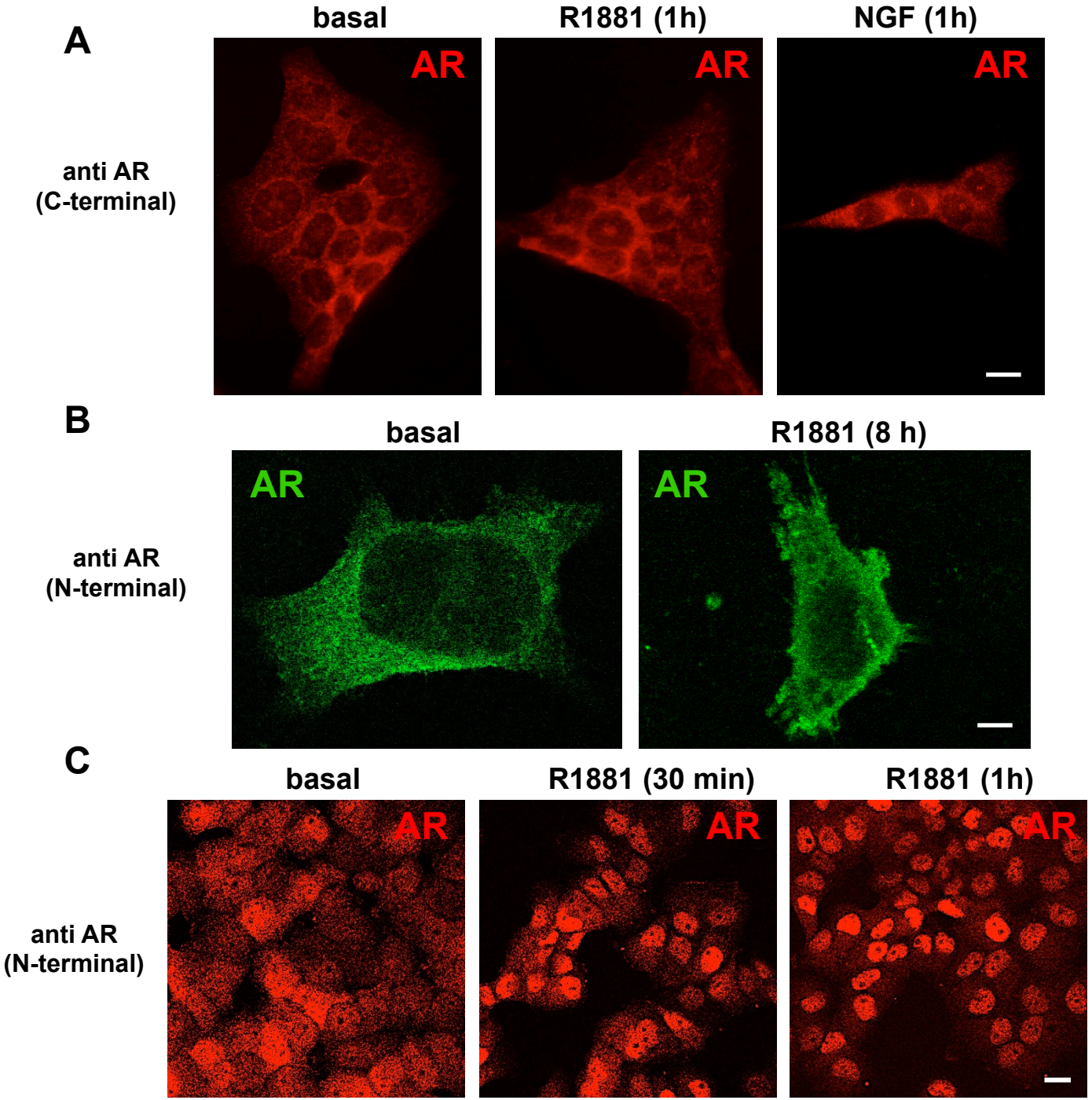
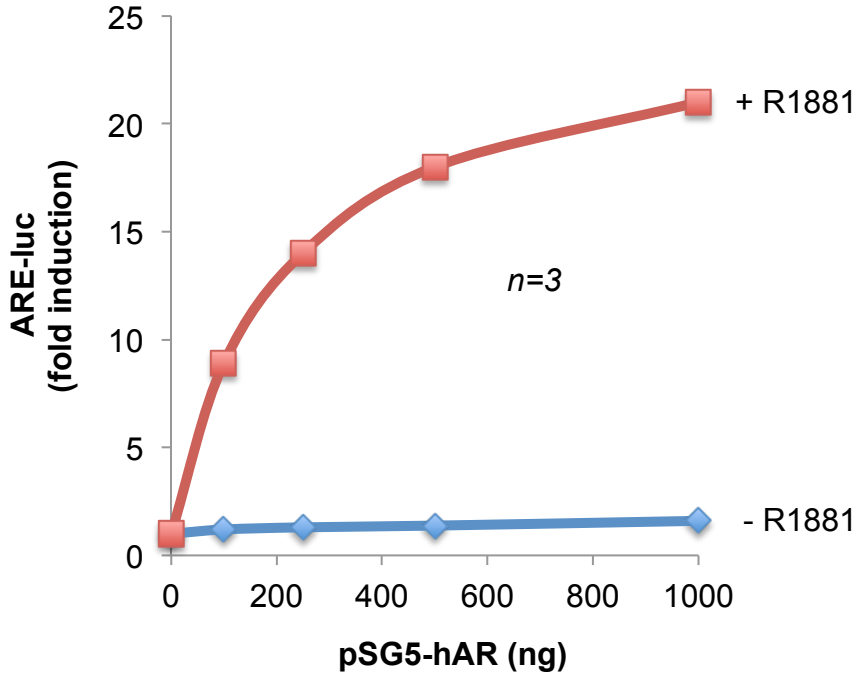

A
basal
R1881 (1h)
NGF (1h)
AR
AR
AR
anti AR
(C-terminal)
B
basal
R1881 (8 h)
AR
AR
anti AR
(N-terminal)
C
basal
R1881 (30 min)
R1881 (1h)
AR
AR
AR
anti AR
(N-terminal)

Fig. 2S

A

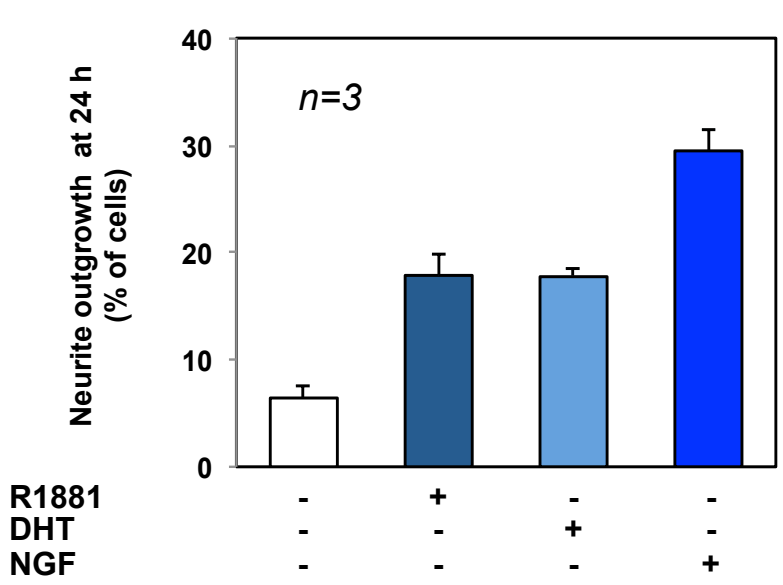

ARE-luc
(fold induction)
25
20
15
10
5
0
0
200
400
600
800
1000
pSG5-hAR (ng)
n=3
+ R1881
- R1881

B


ENSRNOP00000009129/1-2706
ENSP00000363822/1-2760

Fig. 3S

A


Neurite outgrowth at 24 h
(% of cells)
n=3
40
30
20
10
0
R1881 - + - -
DHT - - + -
NGF - - - +

B

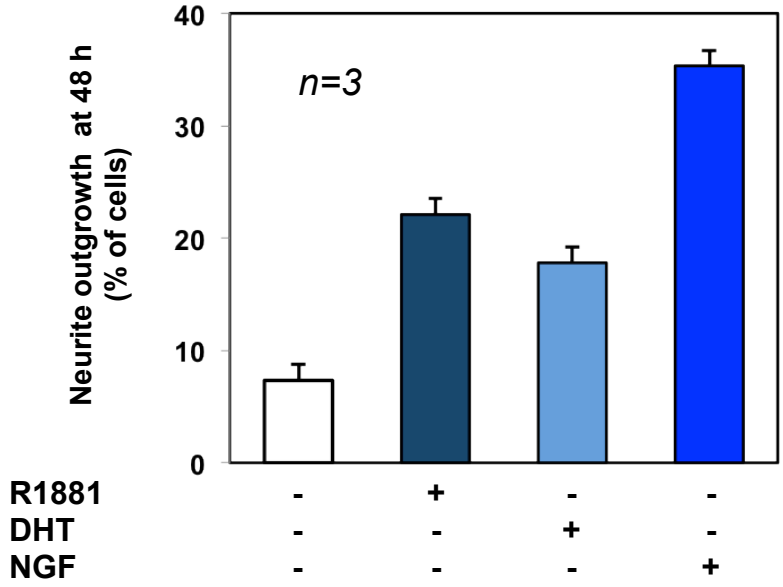
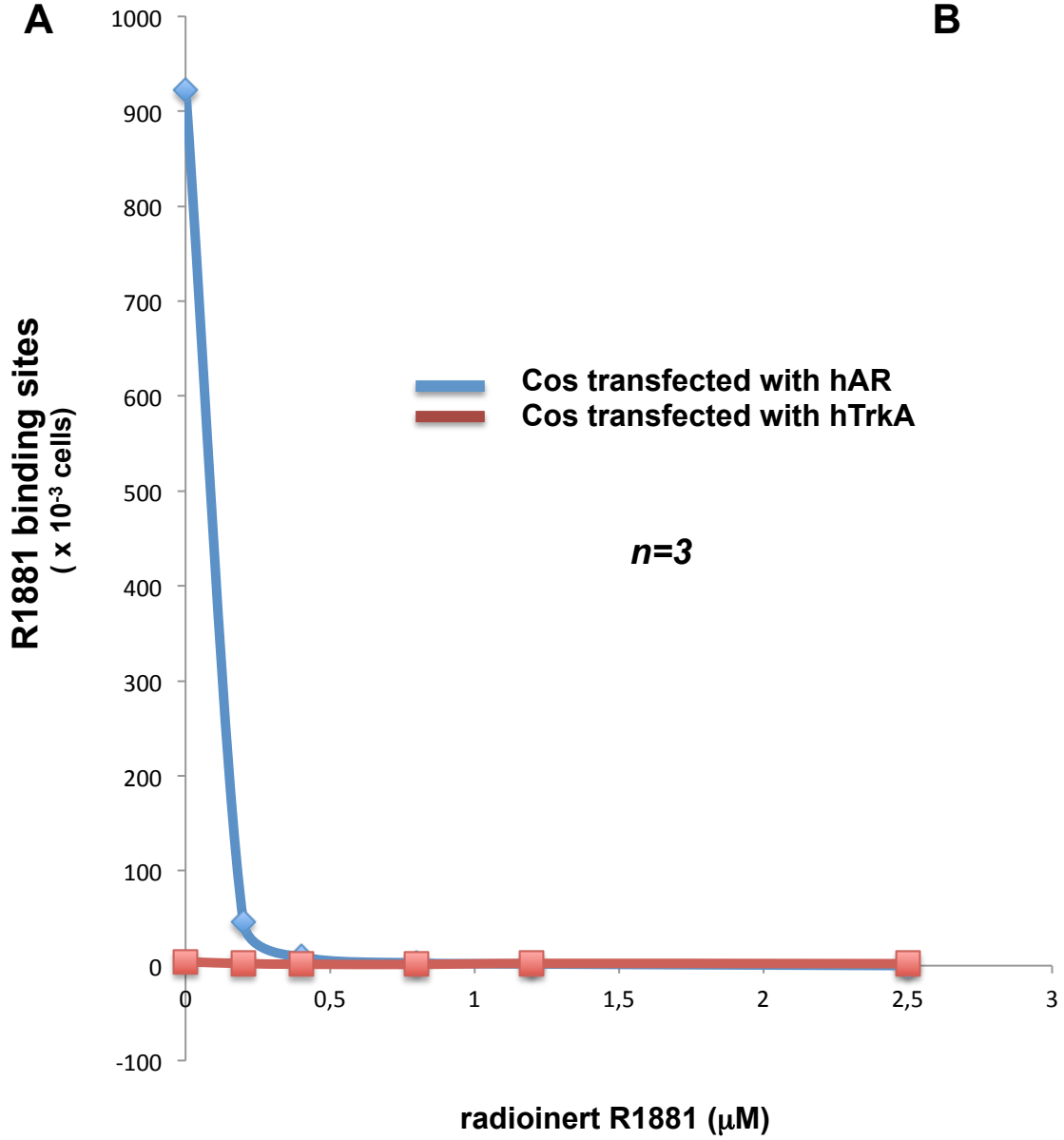

Neurite outgrowth at 48 h
(% of cells)
n=3
40
30
20
10
0
R1881 - + - -
DHT - - + -
NGF - - - +

Fig. 4 S

A



B

radioinert R1881 (μM)	Cos-hAR binding sites (x10^{-3} cells)	Cos-hTrkA binding sites (x10^{-3} cells)
0	922	4,1
0,2	46	2,2
0,4	9,4	1,5
0,8	2,8	1,3
1,2	1,7	2,4
2,5	0,03	2,1

C

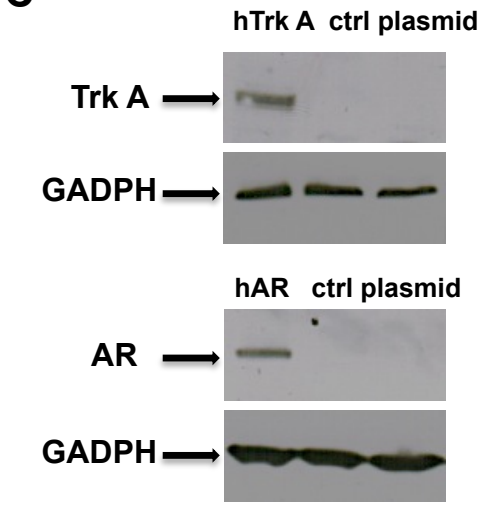
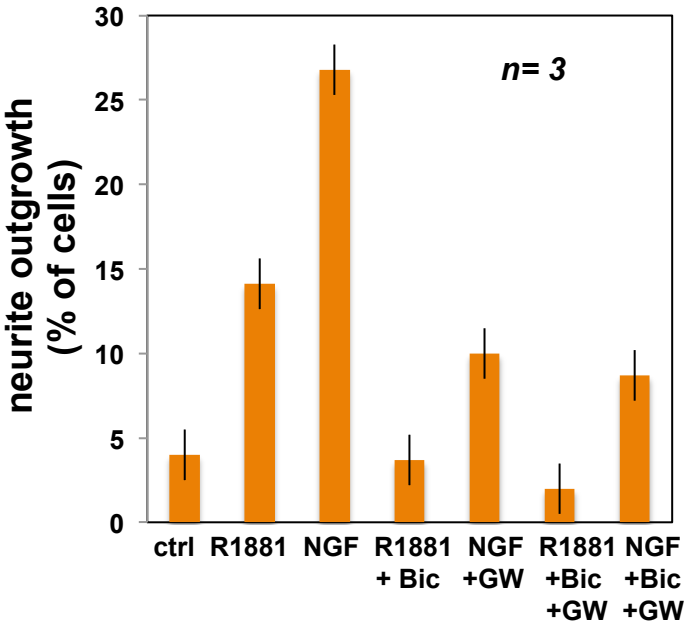


Fig. 5 S

A



B

Staple Peptide (**RH-2025u**): Ac-Stapled [A628S5, K632S5]

Human Androgen Receptor (628-646)-Amide

[Mol. wt. 2205.54 + (114.05 x 4)] = 2661.62

RH-2025u peptide

Fig. 6 S

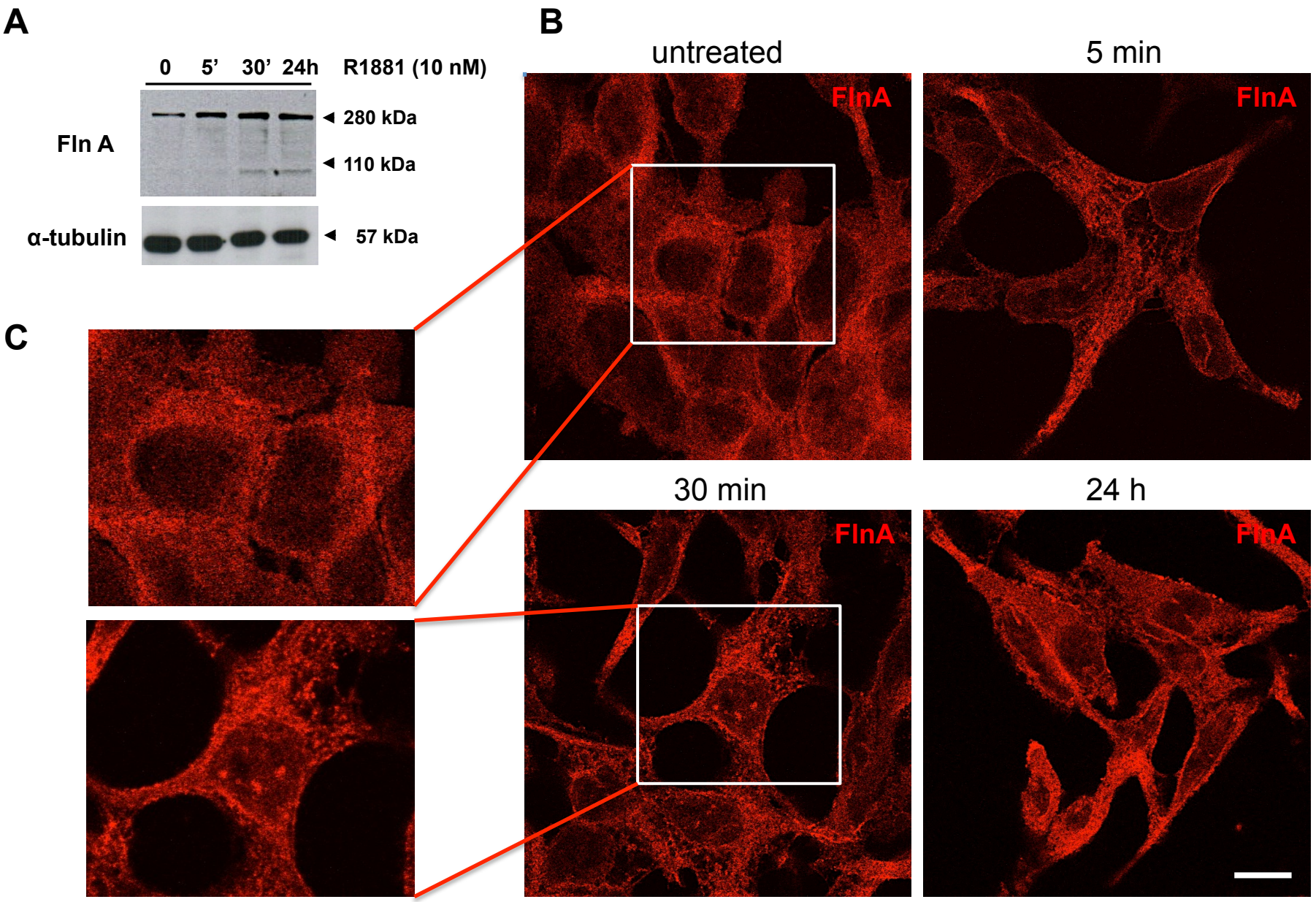

A
0
5'
30'
24h
R1881 (10 nM)
Fln A
280 kDa
110 kDa
α-tubulin
57 kDa
B
untreated
5 min
30 min
24 h
FlnA
C

Fig. 7 S

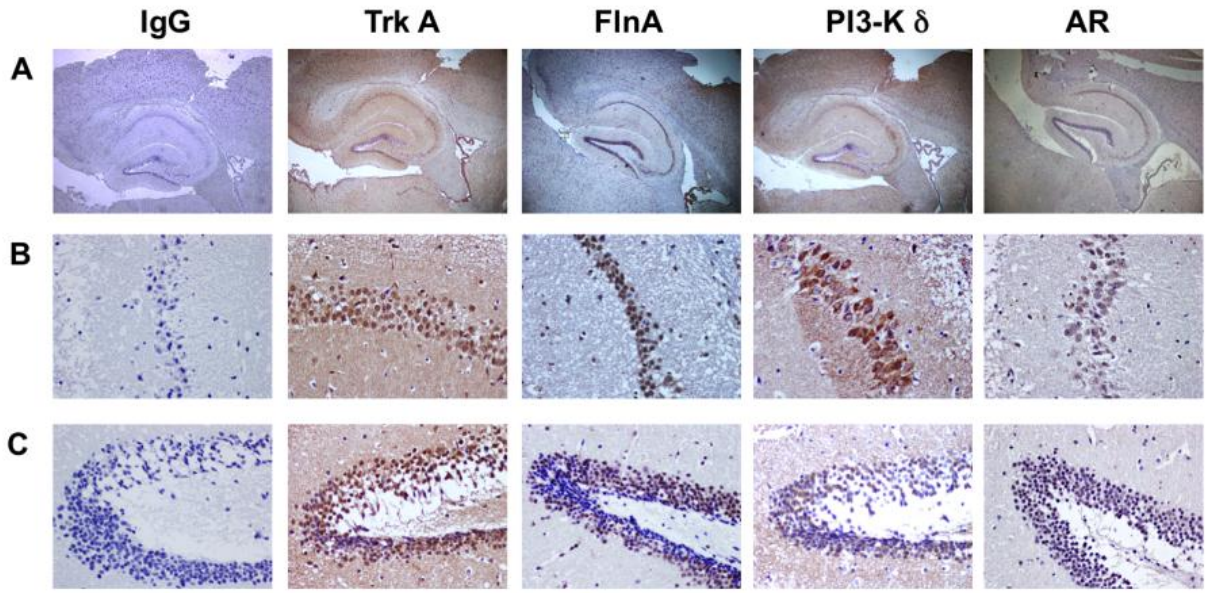


Fig. 8 S

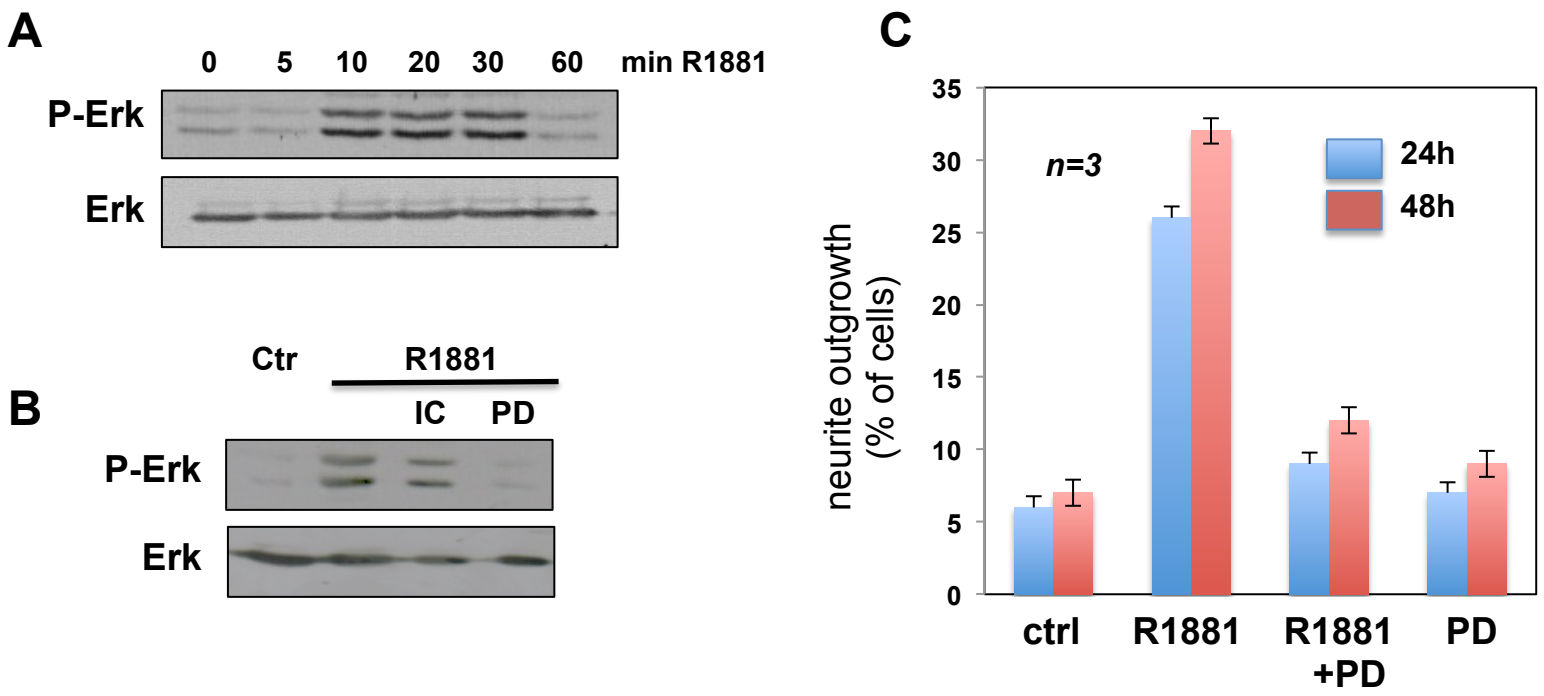

A
0
5
10
20
30
60
min R1881
P-Erk
Erk
B
Ctr
R1881
IC
PD
P-Erk
Erk
C
n=3
24h
48h
neurite outgrowth
(% of cells)
35
30
25
20
15
10
5
0
ctrl
R1881
R1881
+PD
PD

Fig. 9S

SUPPLEMENTAL DATA

Fig. 1S shows the effect of NGF or EGF stimulation of quiescent PC12 cells.

NGF or EGF were both used at 100 ng/ml. In **A** and **B**, cells on polylysine-coated coverslips were used. In **A**, quiescent cells left untreated (basal) or challenged for 48 h with NGF or EGF. Cells were analyzed by contrast phase microscopy or actin staining by confocal microscopy. Bar, 10 μM. In **C**, growing cells were left in complete medium (ctrl) or made quiescent and then left untreated (basal) or challenged for 24 h with NGF or EGF. After *in vivo* pulse with BrdU (100 μM), BrdU incorporation was analyzed by IF and expressed as % of total cells. Means and SEM are shown. In **D**, quiescent PC12 cells on plastic were left untreated or treated for the indicated times with EGF (upper) or NGF (lower). P-Erk was detected from lysate proteins using the appropriate antibody. Filters were re-probed with the anti-Erk antibody.

Fig.2S shows that R1881 and NGF do not significantly affect AR nuclear translocation in PC12 cells (A and B). R1881 induces nuclear translocation of AR in prostate cancer-derived LNCaP cells (C).

Quiescent PC12 cells on polylysine-coated coverslips were left untreated or treated for the indicated times with 10 nM R1881 or 100 ng/ml NGF. AR was visualized by IF (**A**), or confocal microscopy (**B**), using the anti AR antibodies indicated in Figure. Whatever the ligand stimulation or antibody used, AR is predominantly localized outside the nuclei in PC12 cells. Only a faint, background AR nuclear fluorescence is observed (*see also Ozanne et al. 2000; Ni et al., 2013 in Refs.*). Shown in the lower panels are the confocal microscopy images from quiescent LNCaP cells untreated (basal) or treated for 30 min or 1h with 10 nM R1881. (**A** and **B)**, bar, 10 μM. (**C)**, bar, 5 μM.

Fig. 3S shows the effect of increasing human AR amounts on androgen-triggered gene transcription in Cos-7 cells (A) and the nucleotide alignment of rat AR versus human AR coding sequence (B).

In **A,** AR-negative Cos-7 cells were transfected with 3424 ARE-Luc constructs with the indicated amounts of hAR-expressing plasmid. Cells were made quiescent, and left un-stimulated or stimulated for 18 h with 10 nM R1881. Luciferase activity was assayed, normalized using β-gal as an internal control, and expressed as fold induction. Data from several independent experiments were analyzed. Means and SEM are shown; ***n***, represents the number of experiments.

In **B,** the nucleotide alignment of rAR (line above) versus hAR (line below) coding sequence (CDS) is shown. Asterisks indicate identical nucleotides. The presence of CAG tri-nucleotide repeats is conserved in both species (blue boxes in hAR CDS; red boxes in rAR CDS). The set of oligos flanking the CAG repeats in rAR is highlighted in light blue (predicted size of the PCR product: 500 bp).

Fig. 4S shows the effect of R1881, DHT and NGF on neurite outgrowth in PC12 cells.

A and **B**, quiescent PC12 cells on polylysine-coated coverslips were left untreated or treated for 24 (**A**) or 48 (**B**) h with 10 nM R1881, 10 nM DHT or 100 ng/ml NGF. Neurite outgrowth was analyzed by contrast phase microscopy and expressed as % of total cells. Means and SEM are shown.

Fig. 5S shows the results of ligand binding displacement studies in Cos-7 cells.

Cos-7 cells were transfected with plasmid expressing hAR or hTrkA, as described in Methods. After transfection, the cells were made quiescent and then incubated with 10 nM [^{3}H] R1881 in the absence or presence of the indicated excess (from 0,2 μM to 2,5 μM) of radio inert compounds. Intracellular radioactivity was assayed as described in Methods. In **A** and **B**, data from three different experiments were collected. The mean of binding sites was calculated and expressed as R1881 binding sites x 10^{-3} cells. *n*= number of experiments. In **C**, lysate proteins from Cos-7 cells transfected with hTrkA or hAR plasmids were probed using the antibodies against the indicated proteins. Lysate proteins from cells transfected with the empty plasmids (ctrl plasmids) were probed in parallel. Filters were re-probed using the anti-GADPH antibody, as a loading control.

Fig. 6S A, shows the effect of bicalutamide (bic) and GW441,756 (GW) on neurite outgrowth induced by R1881 and NGF in PC12 cells.

Quiescent PC12 cells on polylysine-coated coverslips were left untreated or treated for 24 with the indicated compounds (10 nM R1881, 100 ng/ml NGF, 10 μM bic, 1 μM GW). Neurite outgrowth was analyzed by contrast phase microscopy and expressed as % of total cells. Means and SEM are shown.

Fig. 6S B shows the sequence of RH-2025u-stapled peptide.

Fig. 7S- Analysis of FlnA proteolysis and intracellular localization in LNCaP cells.

Panel **A,** shows the androgen-induced FlnA proteolysis in quiescent LNCaP cells challenged for the indicated times with 10 nM R1881**.** Panel **B,** shows the confocal microscopy images of FlnA from quiescent LNCaP cells challenged for the indicated times with 10 nM R1881. Panel C, the

upper microphotograph shows the magnification from the white box in **B** (untreated), while the lower microphotograph shows the magnification from the white box in **B** (30 min). Bar, 10 μM.

Fig. 8S shows the expression pattern of TrkA, FlnA, PI3-Kδ and AR in adult mouse hippocampus.

IHC analysis for TrkA, FlnA, PI3-K δ and AR in sagittal sections from C56/Bl6 mouse hippocampus was done. Sections were mounted and analyzed with a BX43 Olympus microscope (Olympus). Images were acquired using a DP21 Olympus camera (Olympus), equipped with 10x Plan CN (**A**) or 40x Plan CN (**B** and **C**) objectives. IHC control analysis (IgG) was performed in parallel. FlnA and TrkA are abundantly expressed in almost all the regions of hippocampus, whereas PI3-K δ and AR expression is restricted to Ammon's horn. In this area, all four proteins are expressed.

Fig. 9S- Role of MAPK activation in the androgen-triggered neurite outgrowth of PC12 cells.

Quiescent PC12 cells were used. In **A**, cells were left untreated or treated for the indicated times with 10 nM R1881. Lysate proteins were analyzed for Erk activation, using appropriate antibodies. The total amount of Erk was detected as a control. In **B**, cells were left untreated (Ctrl) or treated for 10 min with 10 nM R1881 in the absence or presence of the indicated inhbitors (PD98050 was used at 10 μM; the PI3-Kδ inhibitor, IC87114 was used at 5 μM). Lysate proteins were analyzed for P-Erk, using appropriate antibodies. The total amount of Erk was detected as a control. In C, quiescent cells on polylysine-coated coverslips were left untreated (ctrl) or treated for 24h (blue) or 48h (red) with 10 nM R1881, in the absence or presence of PD98050 (10 μM). Neurite outgrowth was analyzed by contrast phase microscopy and expressed as % of total cells. Means and SEM are shown.