Supplemental figure legends

Supplemental Fig. 1: PEA-15 knockdown efficiency assessed by immunohistochemistry and qPCR

A, LβT2 cells were transfected with either scrambled or PEA-15 siRNA. Cells were then fixed and stained with an anti-PEA-15 antibody (red) and DAPI (blue), as indicated in *Materials and Methods*. **B,** Single-cell quantification of PEA-15 fluorescence using the 3D-CatFISH image analysis suite. Student-t test n= 117; *** $P<0.005$. **C,** Effect of PEA-15 siRNA knockdown on PEA-15 mRNA expression. PEA-15 transcripts were measured by qPCR.

Supplemental Fig. 2: PEA-15 knockdown efficiency assessed by immunohistochemistry and Western blot

A, LβT2 cells were transfected with either GFP or PEA-15 siRNA. Cells were then fixed and stained with an anti-PEA-15 antibody (red) and DAPI (blue), as indicated in *Materials and Methods*. Control cells transfected with scrambled siRNA are illustrated in Supplemental Fig 1A. **B,** LβT2 cells were transfected with PEA-15 siRNA. Whole-cell lysates were subjected to a Western blot analysis using an anti-PEA-15 antibody (left panel). PKCα was used as loading control. Quantification of Western blot densitometry was plotted as mean ± SEM (right panel). Student-t test n=3, * $P<0.05$.

Supplemental Fig. 3: Specificity of PEA-15 knockdown assessed by immunohistochemistry

A, LβT2 cells stably expressing GFP were transfected with either PEA-15 or GFP siRNA. GFP fluorescence was detected by fluorescence microscopy. Scale = 20μm. **B,** Single-cell quantification of GFP fluorescence using the 3D-CatFISH image analysis suite. Student-t test n= 165; *** $P<0.005$. Single-cell GFP fluorescence distribution is also presented in Supplemental Fig 4A.

Supplemental Fig. 4: Single-cell distribution of GFP *vs.* PEA-15 fluorescence in PEA-15 siRNA-transfected cells

LβT2 cells stably expressing GFP were transfected with either GFP or PEA-15 siRNA. GFP fluorescence was detected by fluorescence microscopy, while PEA-15 fluorescence was identified with an anti-PEA-15 antibody. Single-cell quantification was performed using the 3D-CatFISH image analysis suite. **A,** Histograms of single-cell GFP fluorescence distribution in GFP siRNA-transfected cells. **B,** Histograms of single-cell PEA-15 fluorescence distribution in PEA-15 siRNA-transfected cells. Indicated are the percentages of cells with a fluorescence level as specified by the length of the horizontal bar.

Supplemental Fig. 5: Single-cell distribution of nuclear phosphoERK fluorescence in PEA-15 siRNA-transfected GnRH-stimulated cells

LβT2 cells were serum-starved overnight, transfected with either GFP or PEA-15 siRNA, and stimulated or not with 100 nM GnRH for 5 min. Nuclear phospho-ERK fluorescence was detected using an anti-phospho-ERK antibody and DAPI (nuclear staining), and single-cell quantification was performed using the 3D-CatFISH image analysis suite. **A,** Histograms of single-cell phospho-ERK fluorescence distribution in GFP siRNA-transfected cells, in the presence or absence of GnRH. **B,** Histograms of single-cell phospho-ERK fluorescence distribution in PEA-15 siRNA-transfected cells, in the presence or absence of GnRH. Indicated are the percentages of cells with a fluorescence level as specified by the length of the horizontal bar.

Supplemental Fig. 6: Single-cell distribution of nuclear phosphoERK fluorescence in PEA-15 siRNA-transfected GnRH-stimulated cells (continued)

LβT2 cells were serum-starved overnight, transfected with either scrambled or PEA-15 siRNA, and stimulated or not with 100 nM GnRH for 5 min. Nuclear phospho-ERK fluorescence was detected using an anti-phospho-ERK antibody and DAPI (nuclear staining), and single-cell quantification was performed

using the 3D-CatFISH image analysis suite. **A,** Histograms of single-cell phospho-ERK fluorescence distribution in scrambled siRNA-transfected cells, in the presence or absence of GnRH. **B,** Histograms of single-cell phospho-ERK fluorescence distribution in PEA-15 siRNA-transfected cells, in the presence or absence of GnRH. Indicated are the percentages of cells with a fluorescence level as specified by the length of the horizontal bar.

Supplemental Fig. 7: Single-cell distribution of cytoplasmic *vs.* nuclear ERK2 fluorescence in PEA-15 siRNA-transfected cells

LβT2 cells were transfected with either scrambled or PEA-15 siRNA. Cytoplasmic *vs*. nuclear ERK2 fluorescence was detected using an anti-ERK2 antibody and DAPI (nuclear staining), and single-cell quantification was performed using the 3D-CatFISH image analysis suite. **A,** Histograms of single-cell ERK2 fluorescence distribution in scrambled siRNA-transfected cells. **B,** Histograms of single-cell ERK2 fluorescence distribution in PEA-15 siRNA-transfected cells. Indicated are the percentages of cells with a fluorescence level as specified by the length of the horizontal bar.

Supplemental Fig. 8: Nuclear *vs*. cytoplasmic distribution of phospho-ERK following GnRH stimulation

After being serum-starved overnight, LβT2 cells were stimulated or not with 100 nM GnRH for 5 min. Cells were fractionated into nuclear and cytoplasmic extracts, and aliquots of the nuclear and cytoplasmic fractions were subjected to a Western blot analysis using phospho-ERK-, PKCα-, and LSD1-specific antibodies. PKCα was used as a cytosolic marker/loading control for the cytoplasmic fraction, while LSD1 was used as a nuclear marker/loading control for the nuclear fraction.

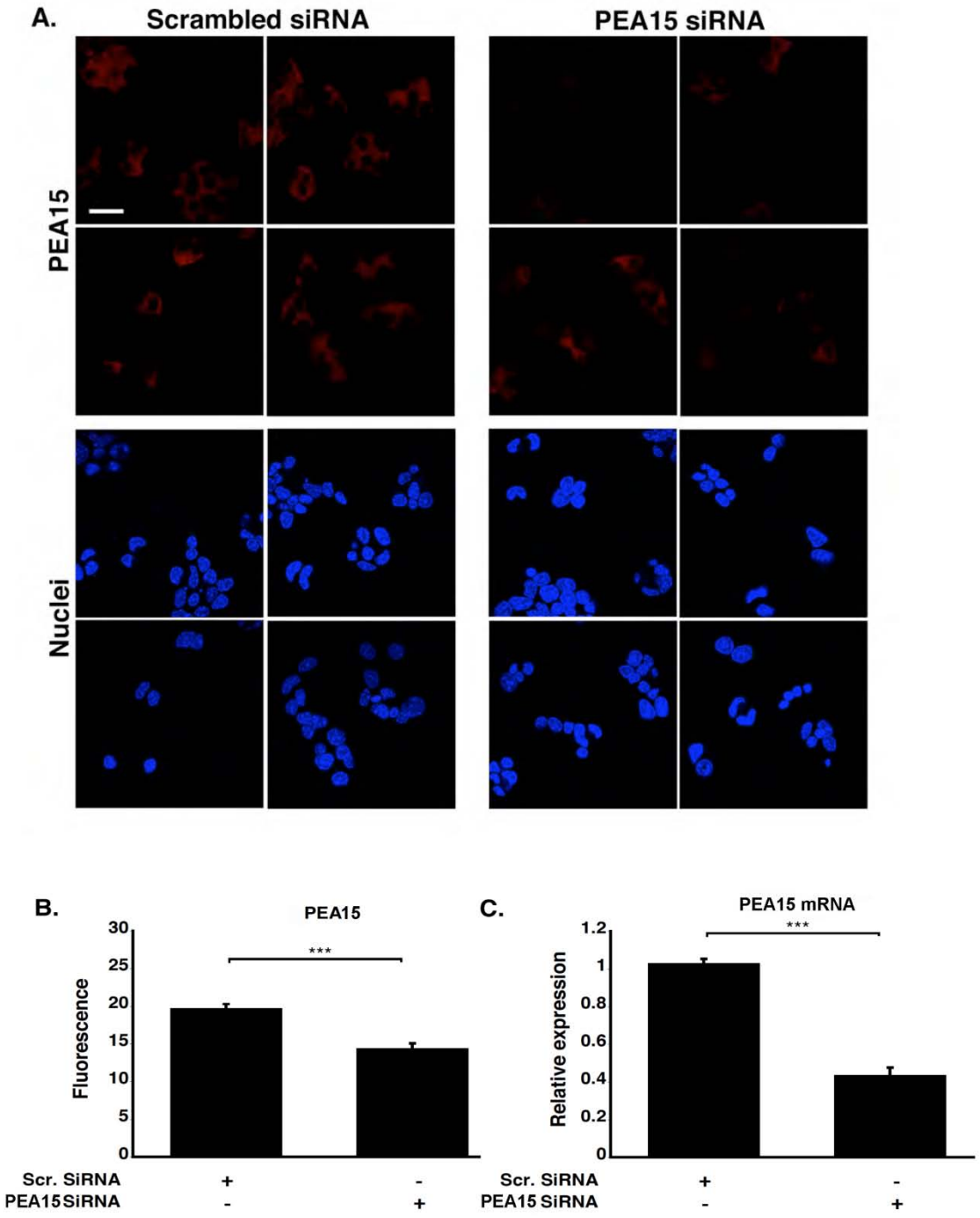
Supplemental Fig. 9: Effect of ERK inhibition on GnRH-induced RSK activation

LβT2 cells were serum-starved overnight, pretreated with 10 μM U0126 (or DMSO as a vehicle) for 30 min, and stimulated with 100 nM GnRH for 5 min. Whole-cell lysates were subjected to a Western blot

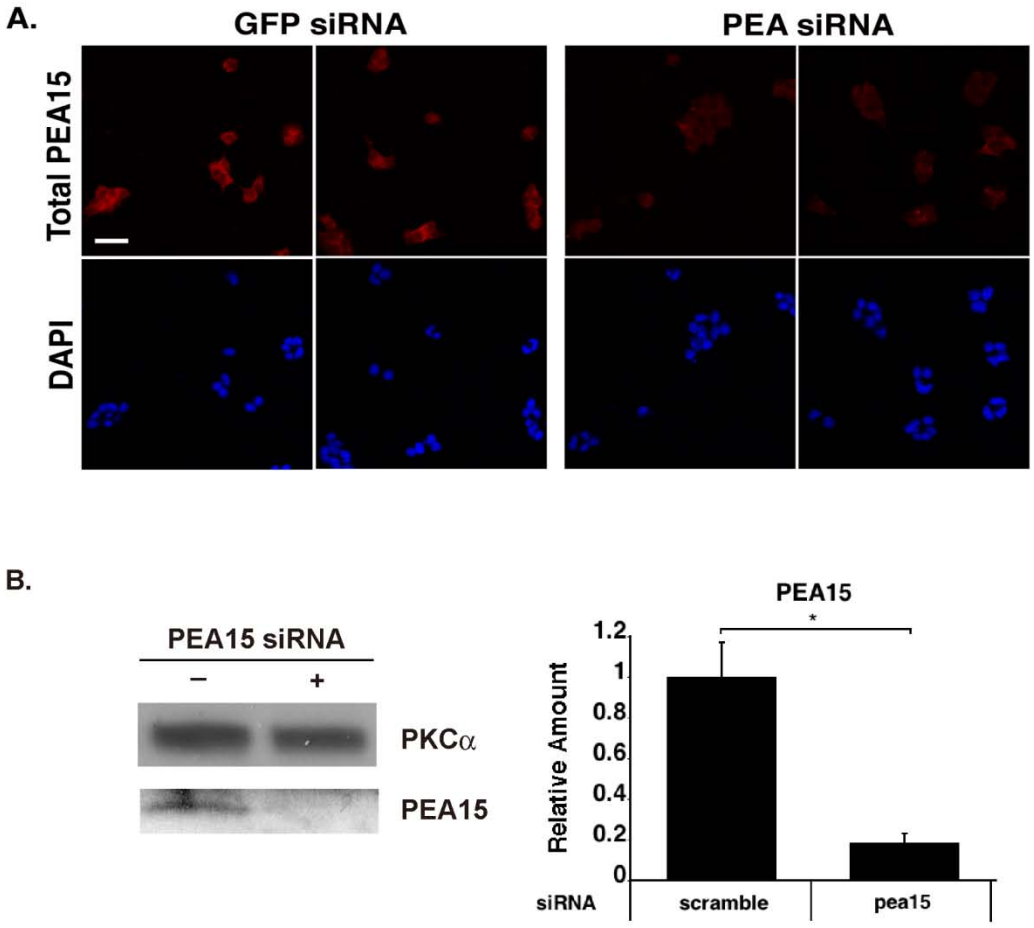
analysis using phospho-ERK- and phospho-RSK-specific antibodies. Total ERK was used as a loading control.

Supplemental Fig. 10: Coimmunoprecipitation of HA-tagged wild-type or mutant PEA-15 with anti-HA antibodies from transfected HEK293 cells

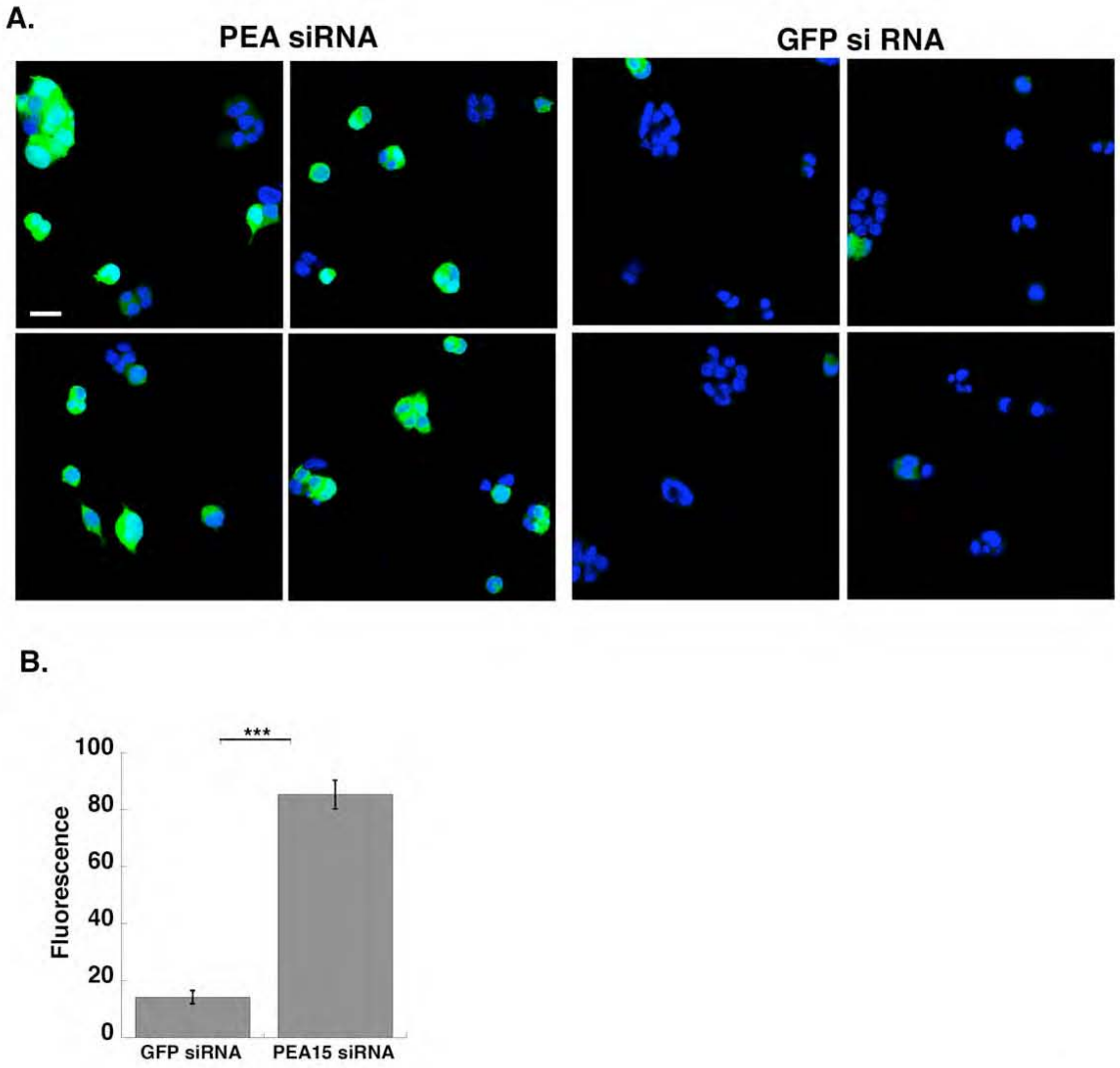
After being serum-starved overnight, HEK293 cells were transfected with either HA-tagged wild-type PEA-15 or mutant PEA-15/A, and stimulated with PMA for 5 min. Mock cells were transfected with an empty vector (pcDNA3). Cell lysates were immunoprecipitated with an HA antibody, and subjected to a Western blot analysis using RSK1-, HA-PEA-15-, and total ERK-specific antibodies (right panel). In parallel, whole-cell lysates were subjected to a Western blot analysis using RSK1-, HA-PEA-15-, and phospho-PEA-15-specific antibodies. Total ERK was used as a loading control (left panel). Three independent experiments were conducted, and one representative image is presented in each panel.


A.
Scrambled siRNA
PEA15 siRNA
PEA15
Nuclei
B.
PEA15
Fluorescence
30
25
20
15
10
5
0
***
Scr. SiRNA + -
PEA15 SiRNA - +
C.
PEA15 mRNA
Relative expression
1.2
1
0.8
0.6
0.4
0.2
0
***
Scr. SiRNA + -
PEA15 SiRNA - +

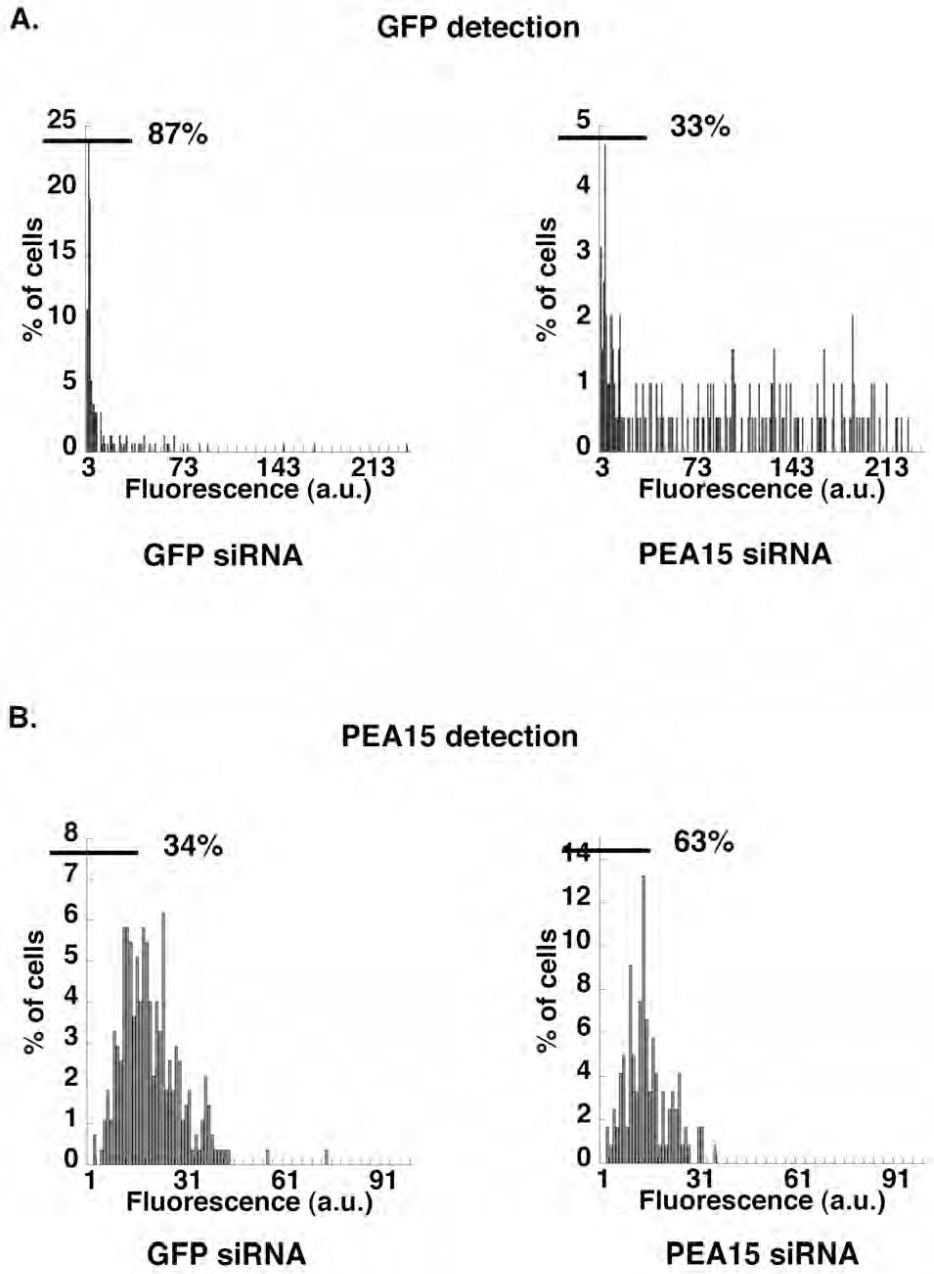
Suppl. Fig. 1


A.
GFP siRNA
PEA siRNA
Total PEA15
DAPI
B.
PEA15 siRNA
–
+
PKCα
PEA15
PEA15
*
Relative Amount
1.2
1
0.8
0.6
0.4
0.2
0
siRNA
scramble
pea15

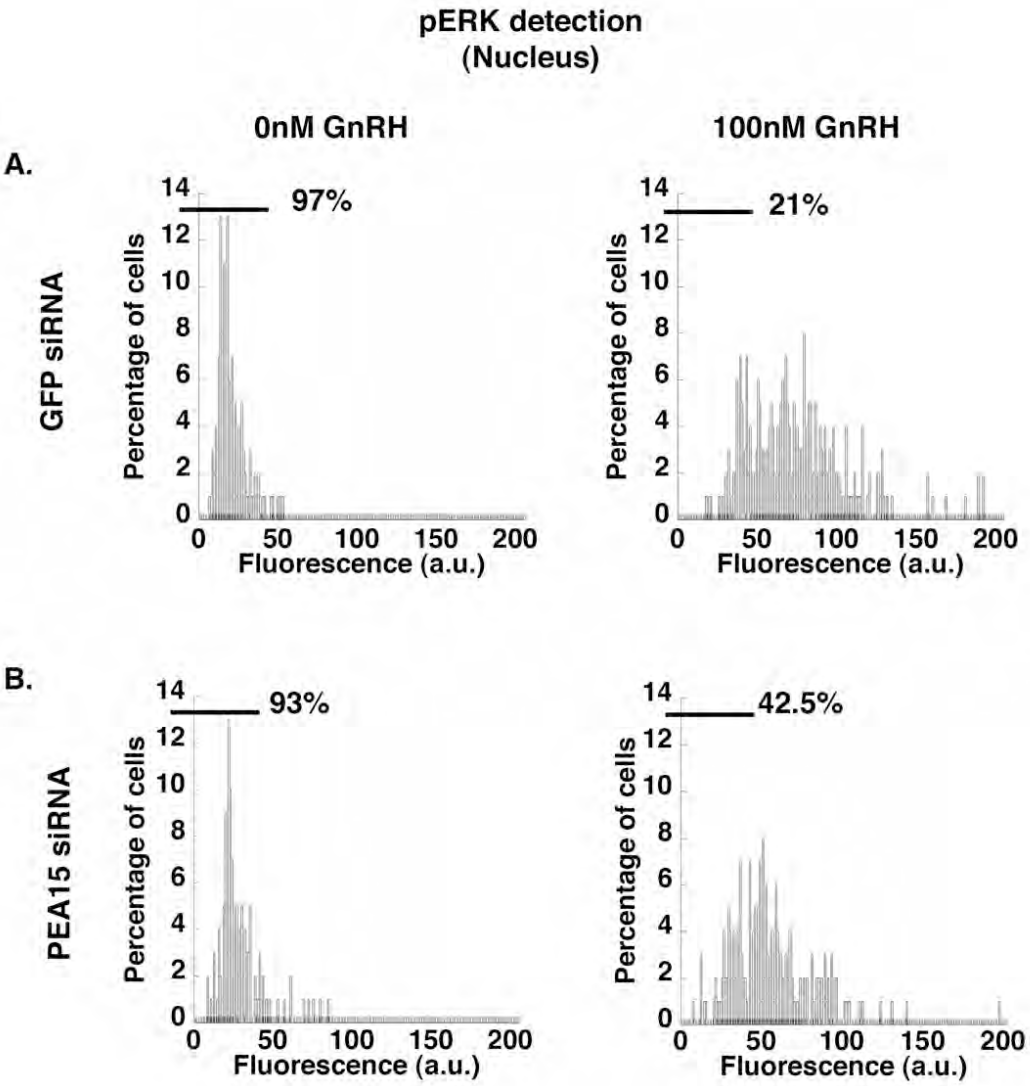
Suppl. Fig. 2


A.
PEA siRNA
GFP si RNA
B.
***
100
80
60
40
20
0
Fluorescence
GFP siRNA
PEA15 siRNA

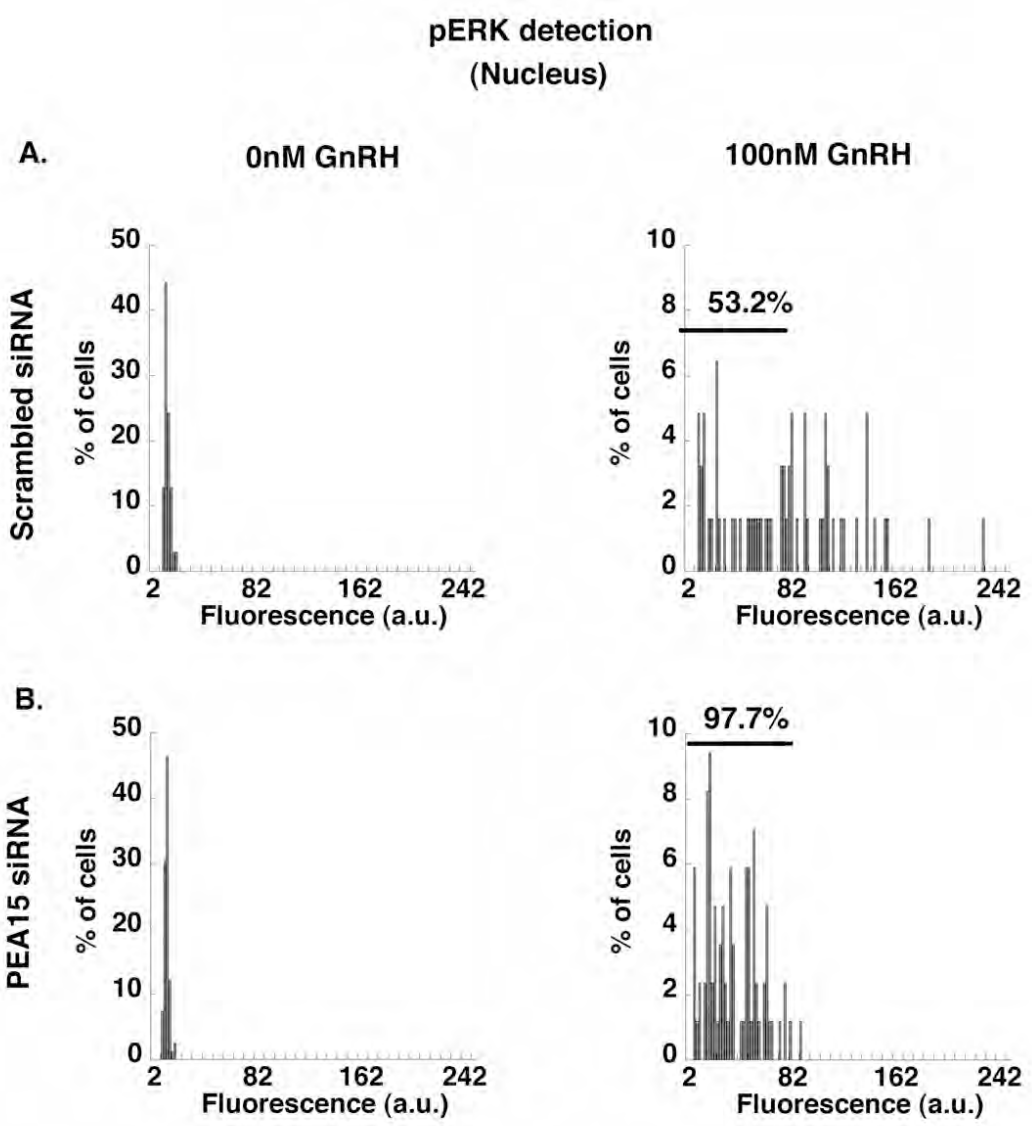
Suppl. Fig. 3


A.
GFP detection
87%
% of cells
Fluorescence (a.u.)
GFP siRNA
33%
% of cells
Fluorescence (a.u.)
PEA15 siRNA
B.
PEA15 detection
34%
% of cells
Fluorescence (a.u.)
GFP siRNA
63%
% of cells
Fluorescence (a.u.)
PEA15 siRNA

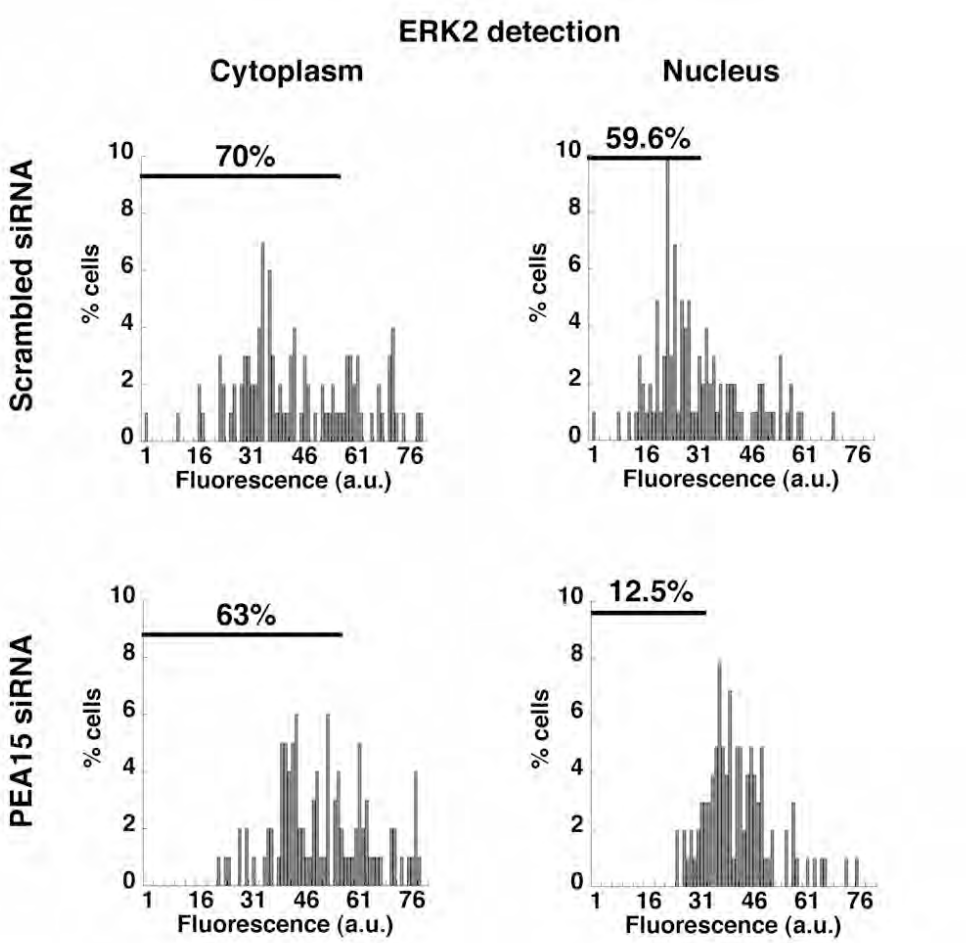
Suppl. Fig. 4


pERK detection
(Nucleus)
0nM GnRH
100nM GnRH
A.
GFP siRNA
97%
21%
B.
PEA15 siRNA
93%
42.5%
Percentage of cells
Fluorescence (a.u.)
0
2
4
6
8
10
12
14
0
50
100
150
200

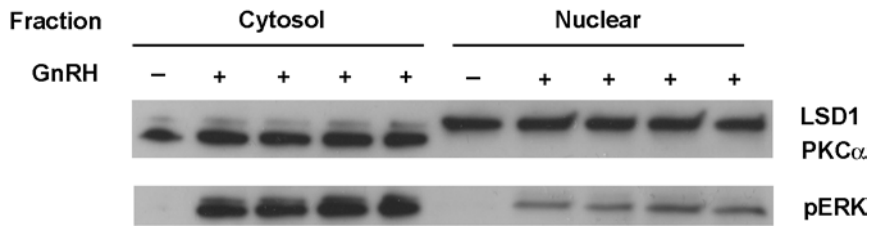
Suppl. Fig. 5


pERK detection
(Nucleus)
A.
0nM GnRH
100nM GnRH
Scrambled siRNA
% of cells
Fluorescence (a.u.)
53.2%
B.
PEA15 siRNA
97.7%

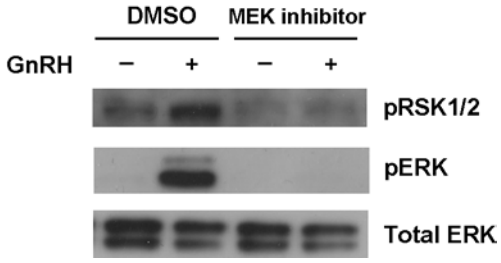
Suppl. Fig. 6


ERK2 detection
Cytoplasm
Nucleus
Scrambled siRNA
PEA15 siRNA
70%
59.6%
63%
12.5%
% cells
Fluorescence (a.u.)
0
2
4
6
8
10
1
16
31
46
61
76

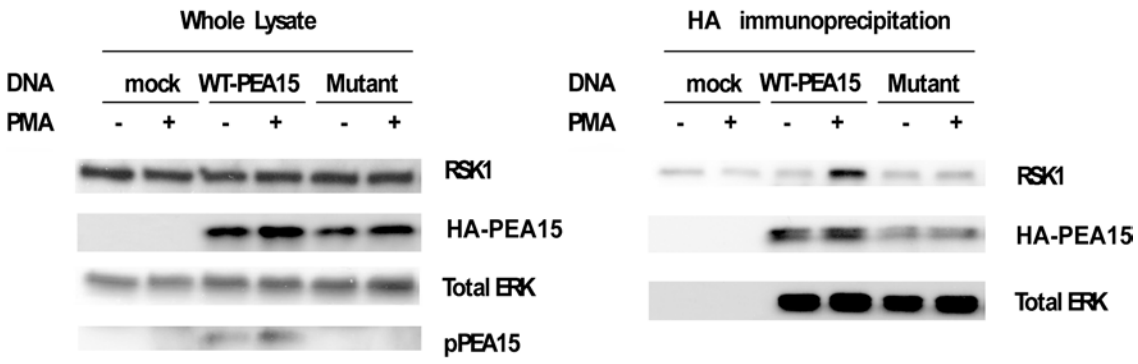
Suppl. Fig. 7



Suppl. Fig. 8



Suppl. Fig. 9


Whole Lysate
DNA
mock
WT-PEA15
Mutant
PMA
- + - + - +
RSK1
HA-PEA15
Total ERK
pPEA15
HA immunoprecipitation
DNA
mock
WT-PEA15
Mutant
PMA
- + - + - +
RSK1
HA-PEA15
Total ERK

Suppl. Fig. 10