Supplementary Data

Figure legends

Figure S1. Knockdown of *ACTN4* disrupts E2 driven ERE-mediated reporter activity. *A*, The ACTN4 protein levels after ACTN4 knockdown using siACTN4-2 in MCF-7 cells. ACTN4 expression levels were normalized to α-tubulin expression with siCtrl treated with vehicle being set to 1. ***B*,** Knockdown of ACTN4 decreased ER-mediated reporter activity. The experiments were performed as described in Figures 2D-E except that siACTN4-2 was used to knockdown ACTN4.

Figure S2. ACTN4 knockdown does not affect subcellular localization of ERα in MCF-7 cells. MCF-7 cells were transfected with siCtrl or siACTN4 followed by treatment with either 10 nM E2 or vehicle for 24 h. Immunostaining was performed using α-ACTN4 and α-ERα antibodies followed by immunoflourescence microscopy. DNA was visualized by 4', 6-diamidino-2-phenylindole (DAPI) staining.

Figure S3. Subcellular fractionation of wild type and mutant (LXXAA) ACTN4 in MCF-7 cells. MCF-7 cells were transfected with expression plasmids for HA-ACTN4 (WT) and HA-ACTN4 (LXXAA) mutant. ***A*,** Immunostaining was performed using α-HA antibodies followed by immunoflourescence microscopy. DNA was visualized by 4', 6-diamidino-2-phenylindole (DAPI) staining. ***B*,** Cell fractionation was done as described in "Experimental Procedures" followed by Western blotting with the indicated antibodies.

Figure S4. Subcelluar localization of wild type and mutant (Δ831-869) ACTN4 in MCF-7 cells. Wild-type or mutant ACTN4 expression plasmids were transiently transfected into MCF-7 cells followed by immunostaining using α-HA antibodies. DNA was visualized by 4', 6-diamidino-2-phenylindole (DAPI) staining.

Figure S5. Knockdown of *ACTN4* reduces expression of endogenous ERα regulated genes. *A-B*, mRNA expression level of estrogen responsive genes in MCF-7 cells following ACTN4 knockdown by siACTN4-2. RNA was isolated as described in "Experimental Procedures" and

relative expression levels of *pS2* and *PR* were determined by qRT-PCR. The mRNA levels of *pS2 and PR* were normalized to mRNA level of GAPDH with siCtrl treated with vehicle being set to 1.

Figure S6. Knockdown of *HDAC7* increases expression of endogenous ERα regulated genes. *A-B*, mRNA expression level of estrogen responsive genes in MCF-7 cells following HDAC7 knockdown by siHDAC7-2. RNA was isolated as described in "Experimental Procedures". Relative expression levels of *pS2* and *PR* were analyzed by qRT-PCR. The mRNA levels of *ps2 and PR* were normalized to mRNA level of GAPDH with siCtrl treated with vehicle being set to 1.

Figure S7. The effects of ACTN4 or HDAC7 knockdown on MCF-7 cell proliferation. ***A,*** The experiment was carried out as described in Figure 8A. The effect of a second ACTN4 siRNA (siACTN4-2) on MCF-7 cell proliferation is shown. Fewer number of MCF-7 cells were used as compared to that in Figure 8A. ***B,*** *Left*, Western blotting of HDAC7 protein levels. A control siRNA (siCtrl) or siRNA against HDAC7 (siHDAC7-2) was transiently transfected into MCF-7 cells. An aliquot of cells were used for whole cell extract preparation and an aliquot of cells were used for proliferation assays. *Right*, The effect of a second HDAC7 siRNA on MCF-7 cell proliferation is shown. Proliferation assays were carried out as described in Figure 8B.

Figure S8. The effect of E2 on subcellular localization of ACTN4 in MCF-7 cells. ***A*,** MCF-7 cells were treated with or without 10 nM of E2 for 24 h followed by immunostaining and immunoflourescence microscopy for ACTN4 and ERα using α-ACTN4 and α-ERα antibodies. DNA was visualized by 4', 6-diamidino-2-phenylindole (DAPI) staining. ***B*,** The subcellular distribution of ACTN4 in cytoplasm and nuclear fractions after E2 treatment. MCF-7 cells were treated with E2 and harvested at the indicated periods of time followed by fractionation using the protocol described in "Experimental Procedures". Fractionated cytoplasmic and nuclear fractions were resolved on the SDS PAGE and immunoblotted with α-ACTN4, α-HDAC1 and α-GAPDH antibodies.

Figure S9. GRIP-1 does not interact with ACTN4 in pulldown assays. HEK293 cells were transfected with either VDR or with HA-GRIP expression plasmids. Forty-eight hours post-transfection, whole cell lysates were prepared and incubated with bacterially expressed GST-ACTN4 (WT). GST-pulldown assays were carried out in the presence or absence of vitamin D3 as indicated in the figure. Pulldown fractions were subjected to Western blotting with α-VDR and α-HA -antibodies. The arrow indicates the full-length GST-ACTN4 fusion protein.

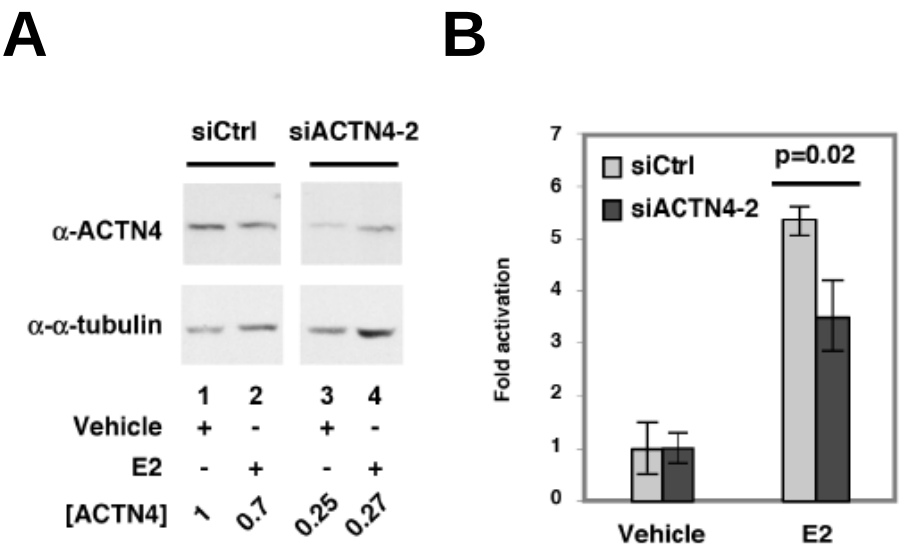

A
siCtrl
siACTN4-2
α-ACTN4
α-α-tubulin
1 2 3 4
Vehicle + - + -
E2 - + - +
[ACTN4] 1 0.7 0.25 0.27
B
siCtrl
siACTN4-2
p=0.02
Fold activation
0
1
2
3
4
5
6
7
Vehicle
E2

Fig. S1

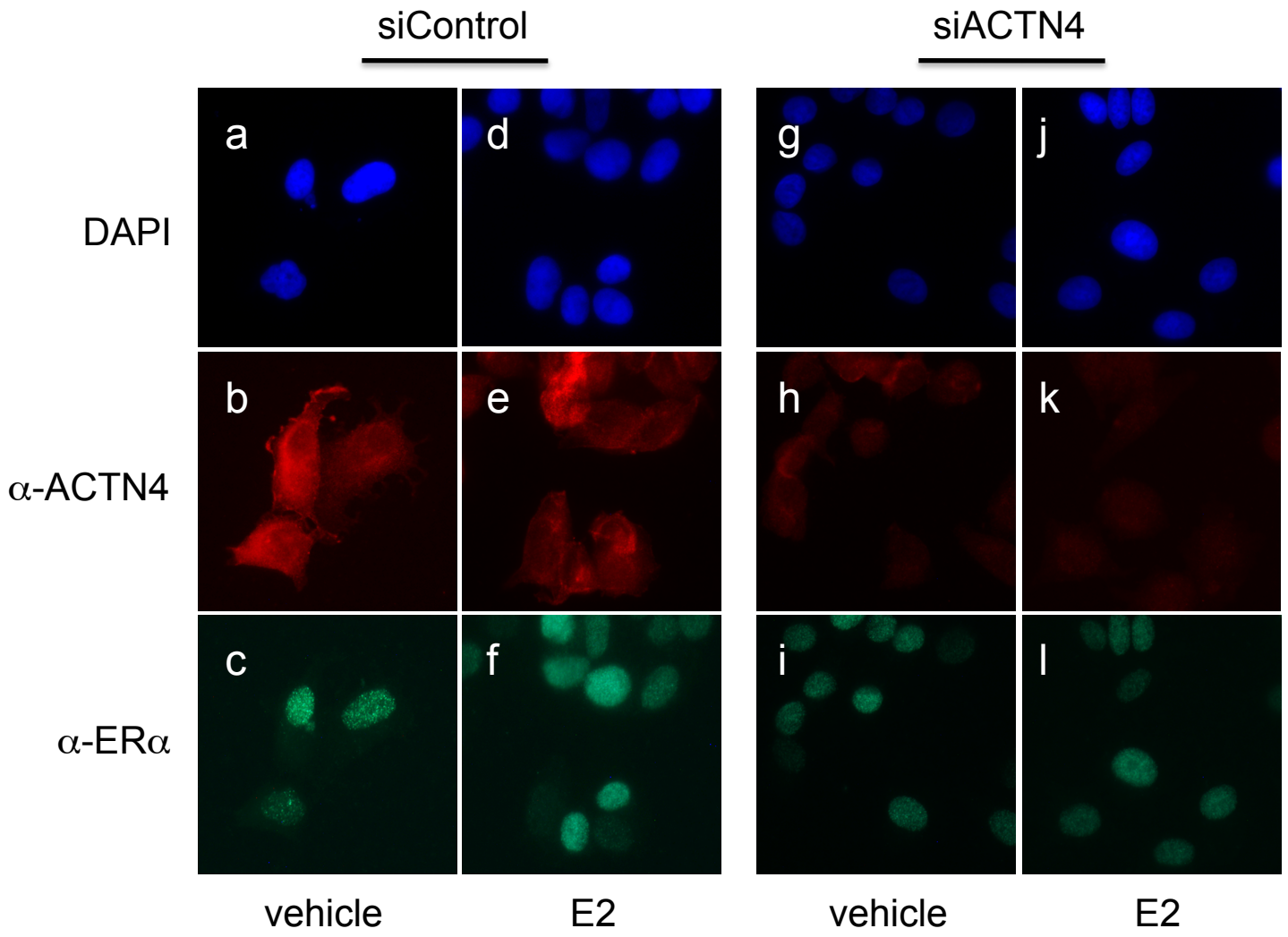

siControl
siACTN4
DAPI
α-ACTN4
α-ERα
a
b
c
d
e
f
g
h
i
j
k
l
vehicle
E2
vehicle
E2

Fig. S2

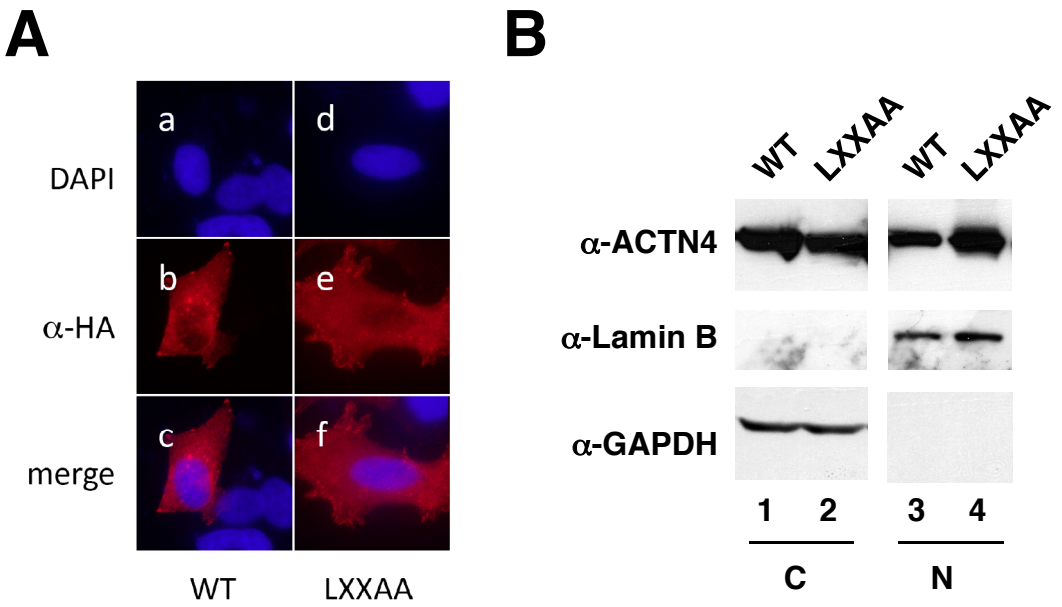

A
B
DAPI
α-HA
merge
a
d
b
e
c
f
WT
LXXAA
WT
LXXAA
WT
LXXAA
α-ACTN4
α-Lamin B
α-GAPDH
1
2
3
4
C
N

Fig. S3

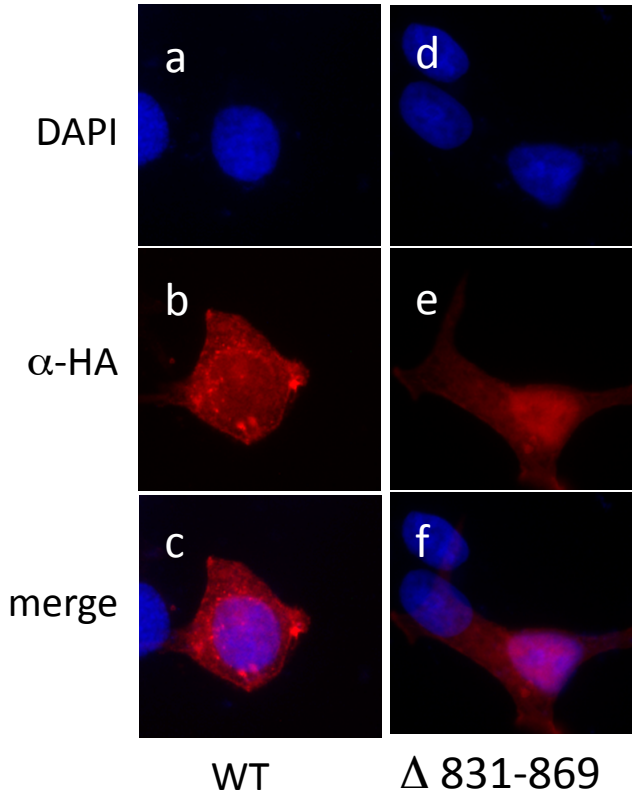


Fig. S4

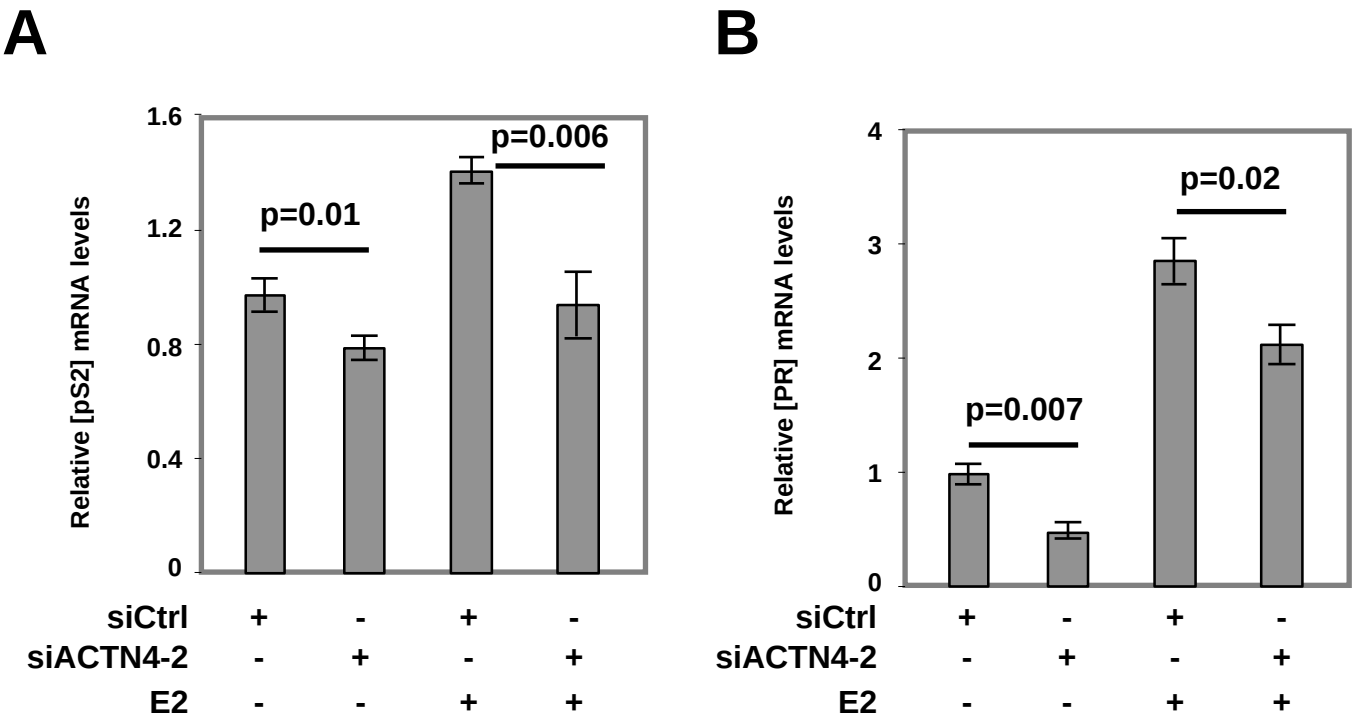

A
B
Relative [pS2] mRNA levels
1.6
1.2
0.8
0.4
0
p=0.01
p=0.006
siCtrl + - + -
siACTN4-2 - + - +
E2 - - + +
Relative [PR] mRNA levels
4
3
2
1
0
p=0.007
p=0.02
siCtrl + - + -
siACTN4-2 - + - +
E2 - - + +

Fig. S5

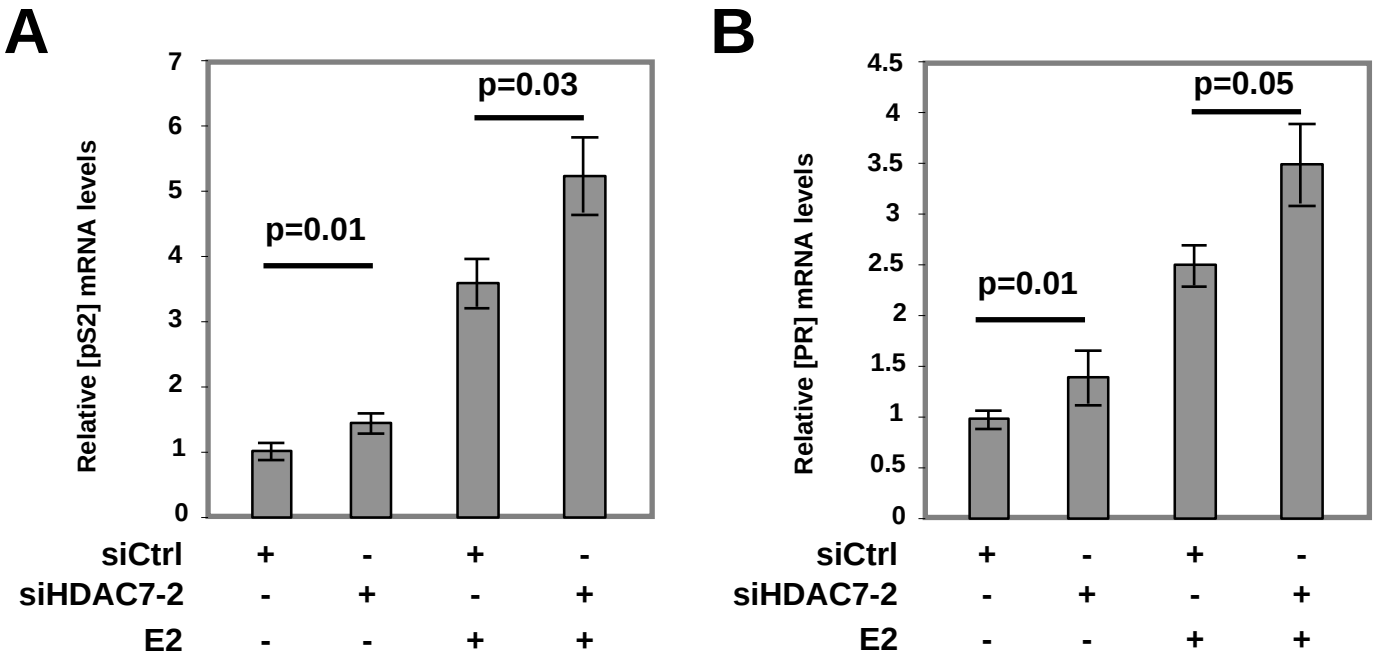

A
Relative [pS2] mRNA levels
7
6
5
4
3
2
1
0
p=0.01
p=0.03
siCtrl + - + -
siHDAC7-2 - + - +
E2 - - + +
B
Relative [PR] mRNA levels
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
p=0.01
p=0.05
siCtrl + - + -
siHDAC7-2 - + - +
E2 - - + +

Fig. S6

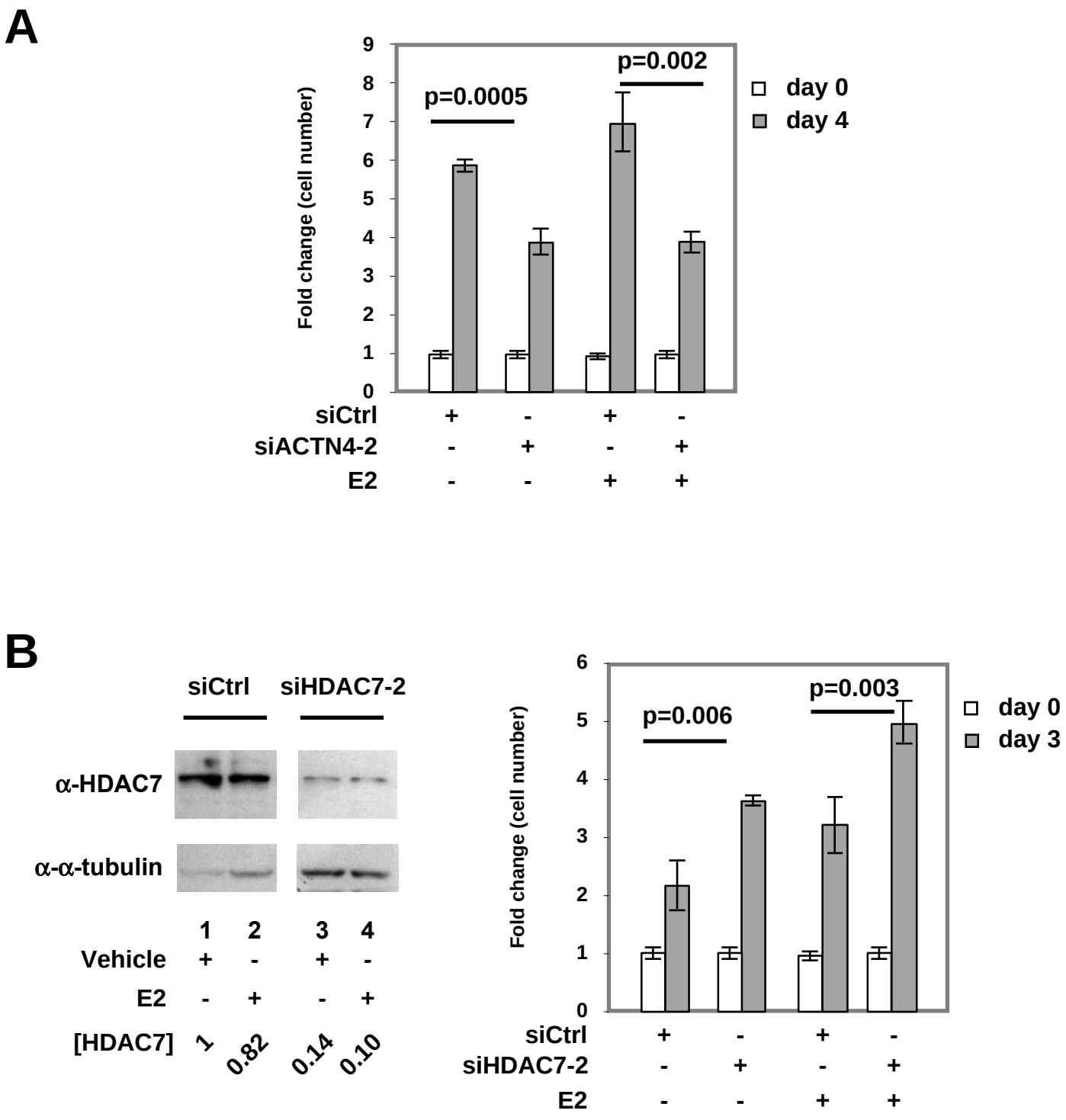
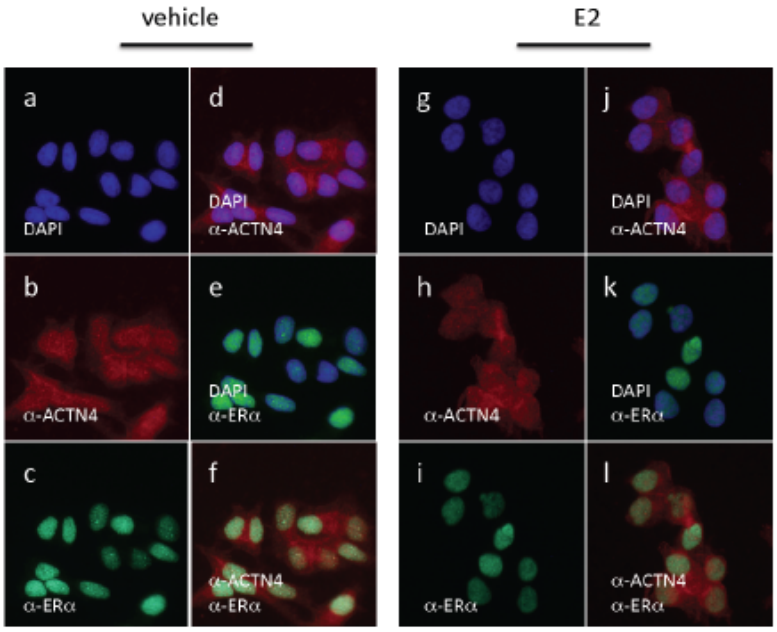

A
p=0.0005
p=0.002
day 0
day 4
Fold change (cell number)
siCtrl
siACTN4-2
E2
B
siCtrl
siHDAC7-2
α-HDAC7
α-α-tubulin
1 2 3 4
Vehicle
E2
[HDAC7]
1
0.82
0.14
0.10
p=0.006
p=0.003
day 0
day 3
Fold change (cell number)
siCtrl
siHDAC7-2
E2

Fig. S7

A


vehicle
E2
a
DAPI
d
DAPI
α-ACTN4
g
DAPI
j
DAPI
α-ACTN4
b
α-ACTN4
e
DAPI
α-ERα
h
α-ACTN4
k
DAPI
α-ERα
c
α-ERα
f
α-ACTN4
α-ERα
i
α-ERα
l
α-ACTN4
α-ERα

B

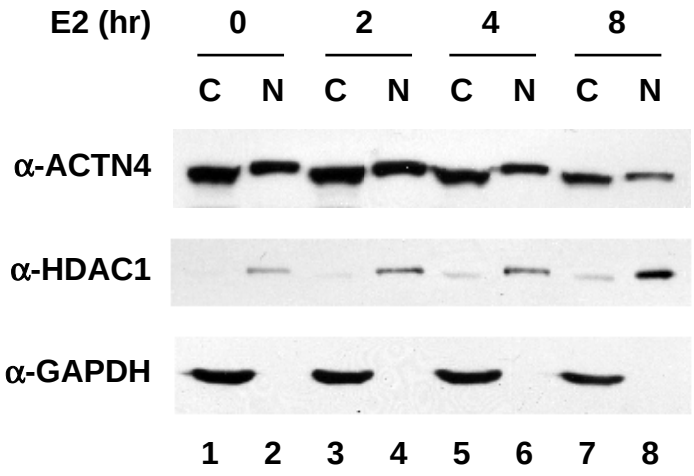

E2 (hr)
0
2
4
8
C N C N C N C N
α-ACTN4
α-HDAC1
α-GAPDH
1 2 3 4 5 6 7 8

Fig. S8

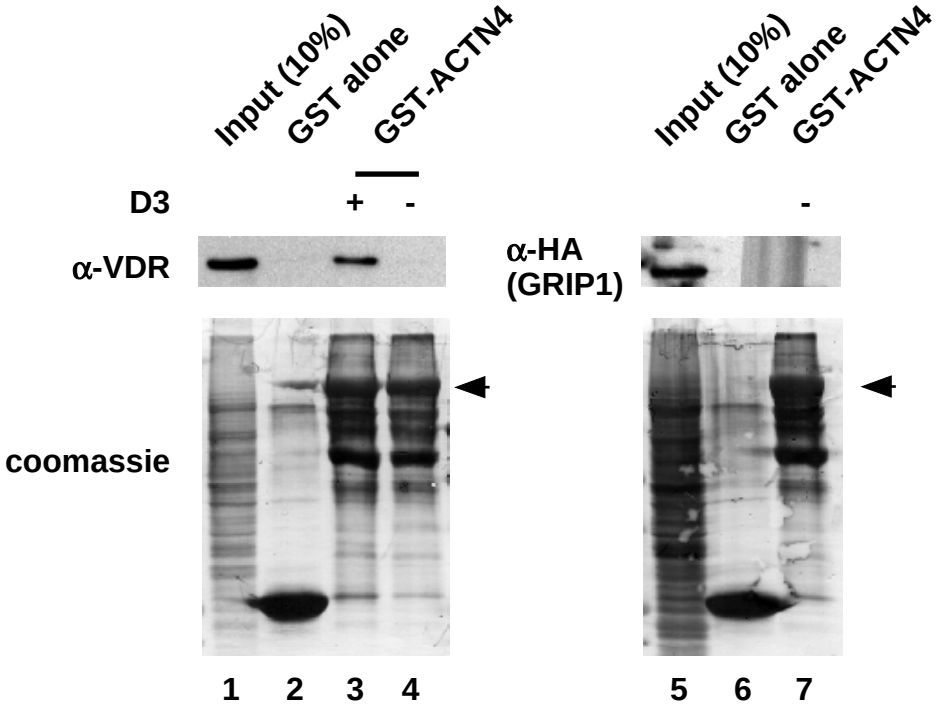

Input (10%)
GST alone
GST-ACTN4
Input (10%)
GST alone
GST-ACTN4
D3
+
-
-
α-VDR
α-HA
(GRIP1)
coomassie
1
2
3
4
5
6
7

Fig. S9