Supplementary Figure legends

Figure S1. E1A enhances SAHA-induced apoptosis more potently than for other chemotherapeutic drugs. MDA-MB-435 cells were transiently transfected with either empty or E1A expression plasmid and treated with 1 mM 5-fluorouracil (for 16 h), 2.5 μM SAHA (12 h), 20 μM cisplatin (16 h), 25 μM etoposide (16 h), or 20 μM paclitaxel (12 h). The cells were then subjected to the caspase assay.

Figure S2. E1A enhances HDACi-induced cell death in MDA-MB-231 and MDA-MB-468 breast cancer cells and 2774-c10 ovarian cancer cells. (a) MDA-MB-231 control or E1A cells were treated with 250 nM of TSA or 5 μM of SAHA for the indicated periods, and caspase-3 activity was determined by the caspase assay using fluorescence substrate. (b) MDA-MB-231 control or E1A cells were treated with 5 μM of SAHA for 12 h. Cellular sensitivity to SAHA was determined by the clonogenic survival assay. (c and d) MDA-MB-468 (c) or 2774-c10 cells (d) were transiently transfected with either empty or E1A expression plasmid and then treated with 250 nM of TSA or 5 μM of SAHA for 14 h. Caspase-3 activity was then determined by the caspase-3 assay using fluorescence substrate (top). E1A and actin expression in the cells used here was verified by immunoblot analysis (bottom).

Figure S3. Comparison of the effects of E1A plus SAHA and E1A plus paclitaxel in MDA-MB-231. MDA-MB-231 cells were treated with the indicated concentrations of SAHA or paclitaxel for 36 h, and viability was determined by the trypan blue dye exclusion assay.

Figure S4. The combination of E1A gene therapy and SAHA exhibits less toxicity than the combination of E1A gene therapy and paclitaxel. CD1 mice were used to determine the toxicity of clinical doses of the drug combinations. The mice were injected with a single dose of E1A/liposome (50 μg/mouse) with paclitaxel (32 mg/mouse) or SAHA (130 mg/mouse). (a) Survival curve of the mice. (b) Serum ALT concentration for mice treated with E1A/liposome plus SAHA were measured at the biochemistry laboratory in the Department of Veterinary Medicine and Surgery. The corresponding value for a normal mice is 54 < AST < 298 (U/L).

Figure S5. The combination of E1A and SAHA activates the mitochondrial pathway of apoptosis in various cancer cells. (a,b) MDA-MB-231 control or E1A cells were treated with 250 nM of TSA or 5 μM of SAHA. (a) Ten hours after treatment, the cells were subjected to subcellular fractionation to separate the cytosol and membrane fractions. Each fraction was analyzed by immunoblot analysis with the indicated antibodies. (b) Ten hours after treatment, the Bax conformational change was determined by immunoprecipitation with anti-Bax 6A7 antibody, which recognizes only the active form of Bax. (c) SKOV3-ip1 control or E1A cells were treated with 250 nM of TSA or 5 μM of SAHA. Six hours after treatment, Bcl-XL mRNA levels was determined by RT-PCR. (d) MDA-MB-231 control or E1A cells were treated with 250 nM of TSA or 5 μM of SAHA. After the cells were treated for the indicated periods, the expressions of Bcl-XL, Bim, E1A, and actin were determined by immunoblot analysis. (e, f) 2774-c10, MDA-MB-435 (e), and MCF10A cells (f) were transiently transfected with either empty or E1A expression plasmid and then treated with 250 nM of TSA or 5 μM of SAHA for 16 h and subjected to immunoblot analysis with the indicated antibodies.

Figure S6. (a) SKOV3-ip1 E1A cells were transiently transfected with the reduced amounts of siRNA oligo against Bim (1/10 or 1/5 of Figure 4e) or control non-specific siRNA. 24 hours after transfection, the cells were treated with 5 μM of SAHA for 16 h and subjected to caspase assay. Bim and tubulin expressions are shown in the right panel. (b) SKOV3-ip1 cells were co-transfected with Bim promoter-luciferase plasmid together with renilla luciferase expression plasmid (internal control), E1A expression plasmid/empty vector, and dominant negative Egr-1expression plasmid/empty vector. Twenty-four hours after transfection, the cells were treated with 5 μM of SAHA for an additional 6 h and subjected to the dual luciferase assay. The luciferase activity is shown relative to that of untreated control transfected with empty vector.

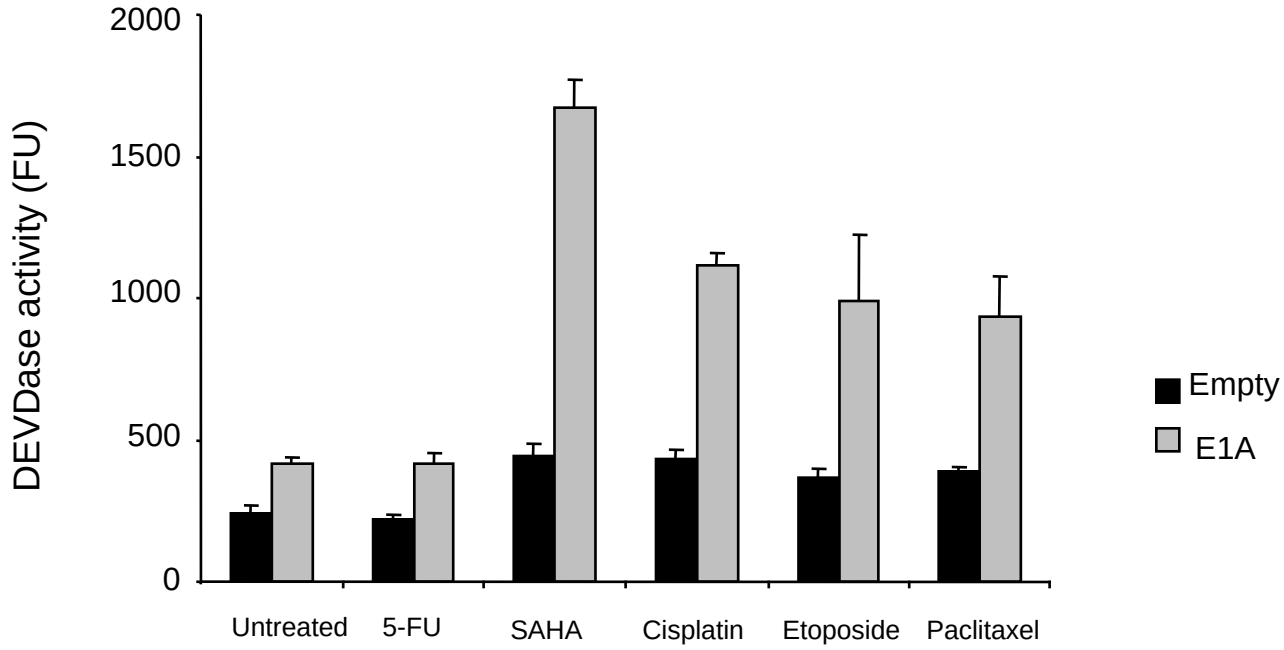

DEVDase activity (FU)
2000
1500
1000
500
0
Untreated
5-FU
SAHA
Cisplatin
Etoposide
Paclitaxel
Empty
E1A

Figure S1

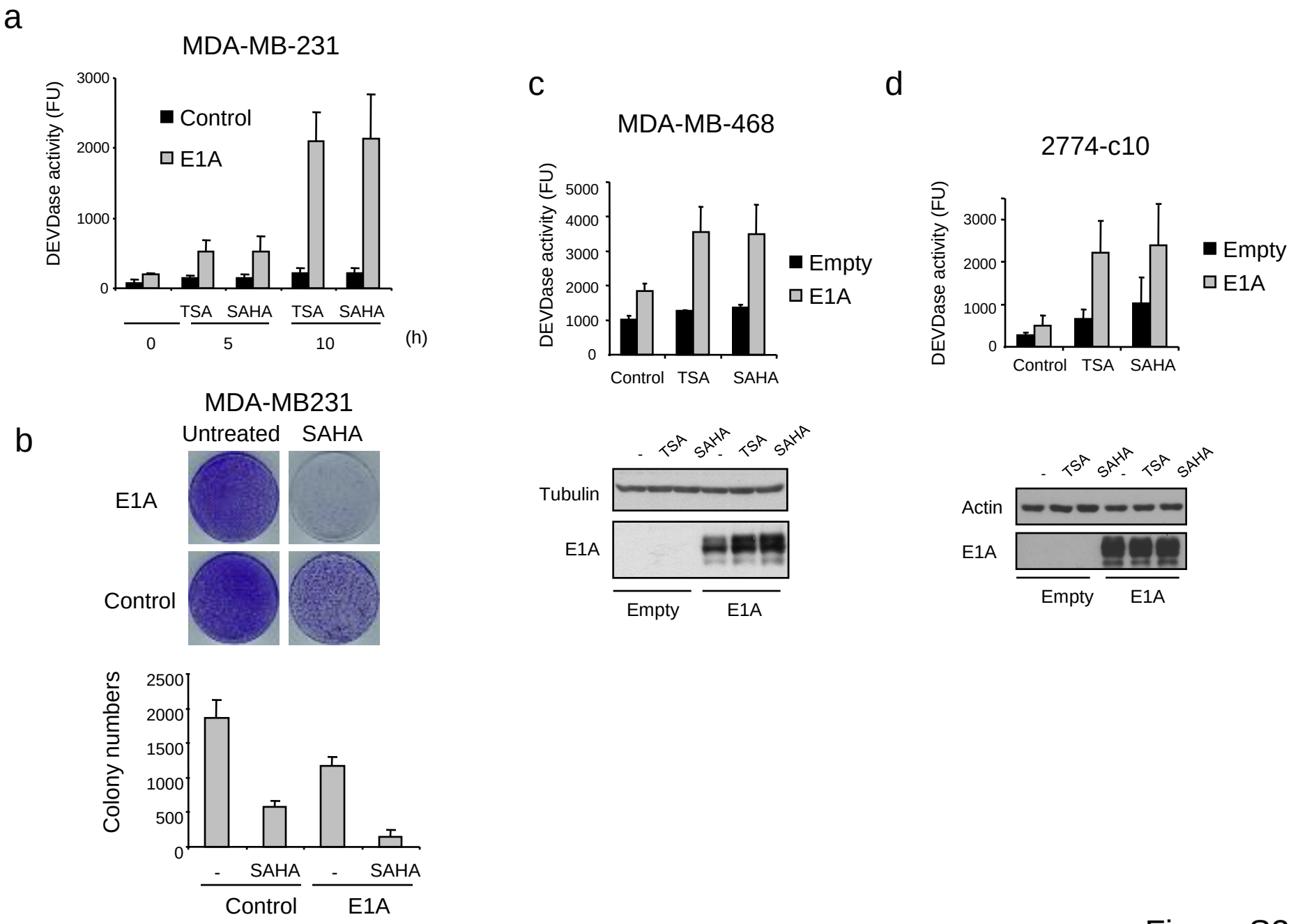
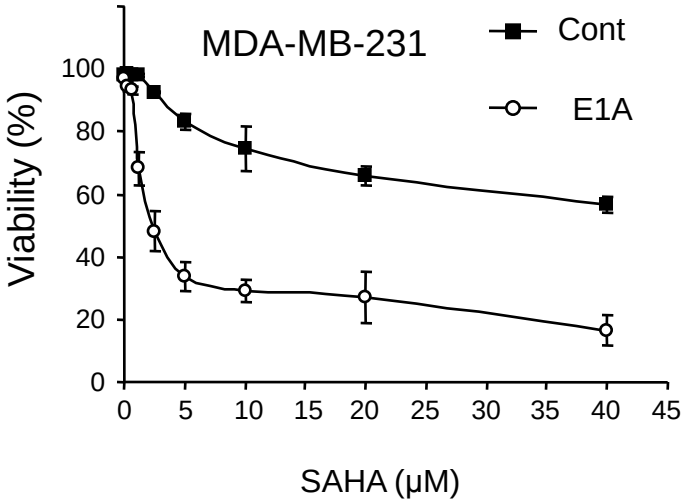
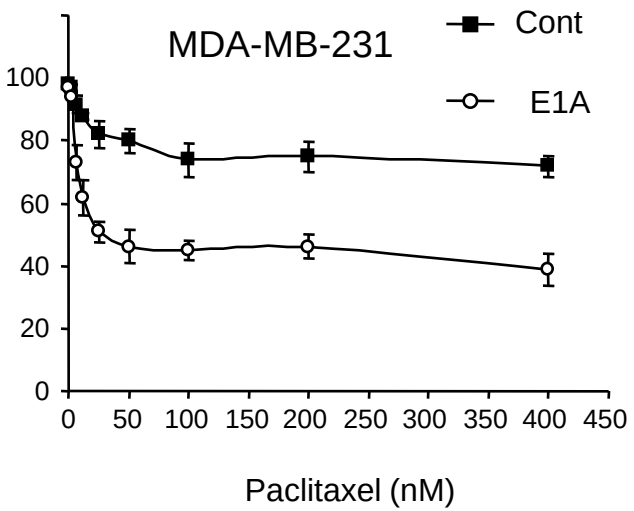

a
MDA-MB-231
DEVDase activity (FU)
3000
2000
1000
0
Control
E1A
TSA
SAHA
TSA
SAHA
0
5
10
(h)
b
MDA-MB231
Untreated
SAHA
E1A
Control
Colony numbers
2500
2000
1500
1000
500
0
-
SAHA
-
SAHA
Control
E1A
c
MDA-MB-468
DEVDase activity (FU)
5000
4000
3000
2000
1000
0
Empty
E1A
Control
TSA
SAHA
-
TSA
SAHA
TSA
SAHA
Tubulin
E1A
Empty
E1A
d
2774-c10
DEVDase activity (FU)
3000
2000
1000
0
Empty
E1A
Control
TSA
SAHA
-
TSA
SAHA
TSA
SAHA
Actin
E1A
Empty
E1A

Figure S2



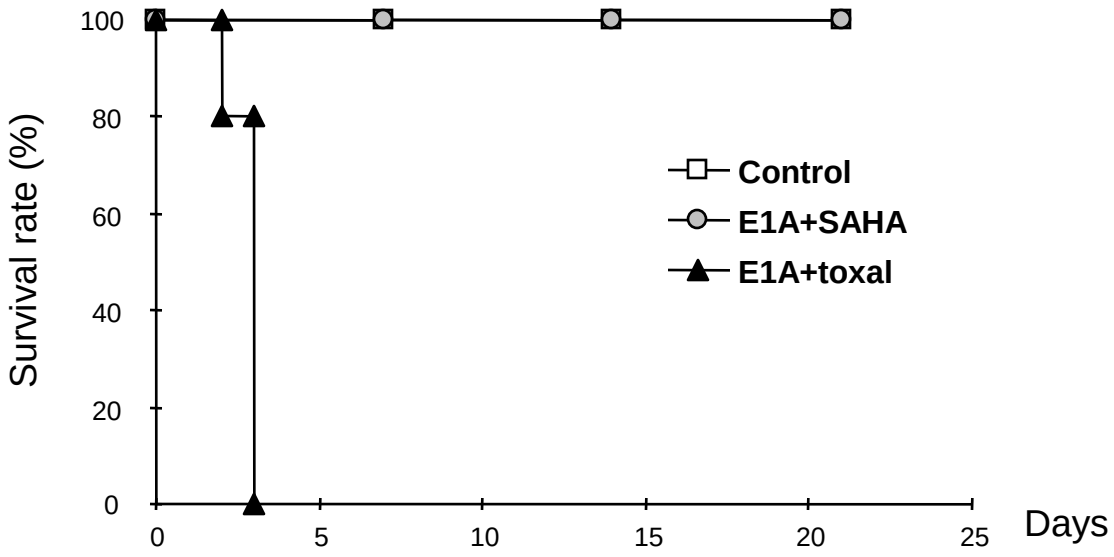
Cell death (6.6 μM): 20 % (control) vs. 68 % (E1A)



Cell death (50 nM): 20 % (control) vs. 54 % (E1A)

Figure S3

a


Survival rate (%)
100
80
60
40
20
0
0
5
10
15
20
25
Days
Control
E1A+SAHA
E1A+toxal

b

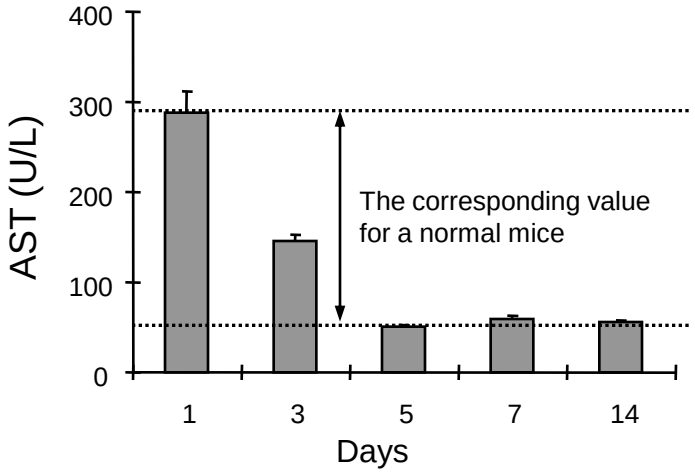

AST (U/L)
400
300
200
100
0
The corresponding value
for a normal mice
1
3
5
7
14
Days

Figure S4

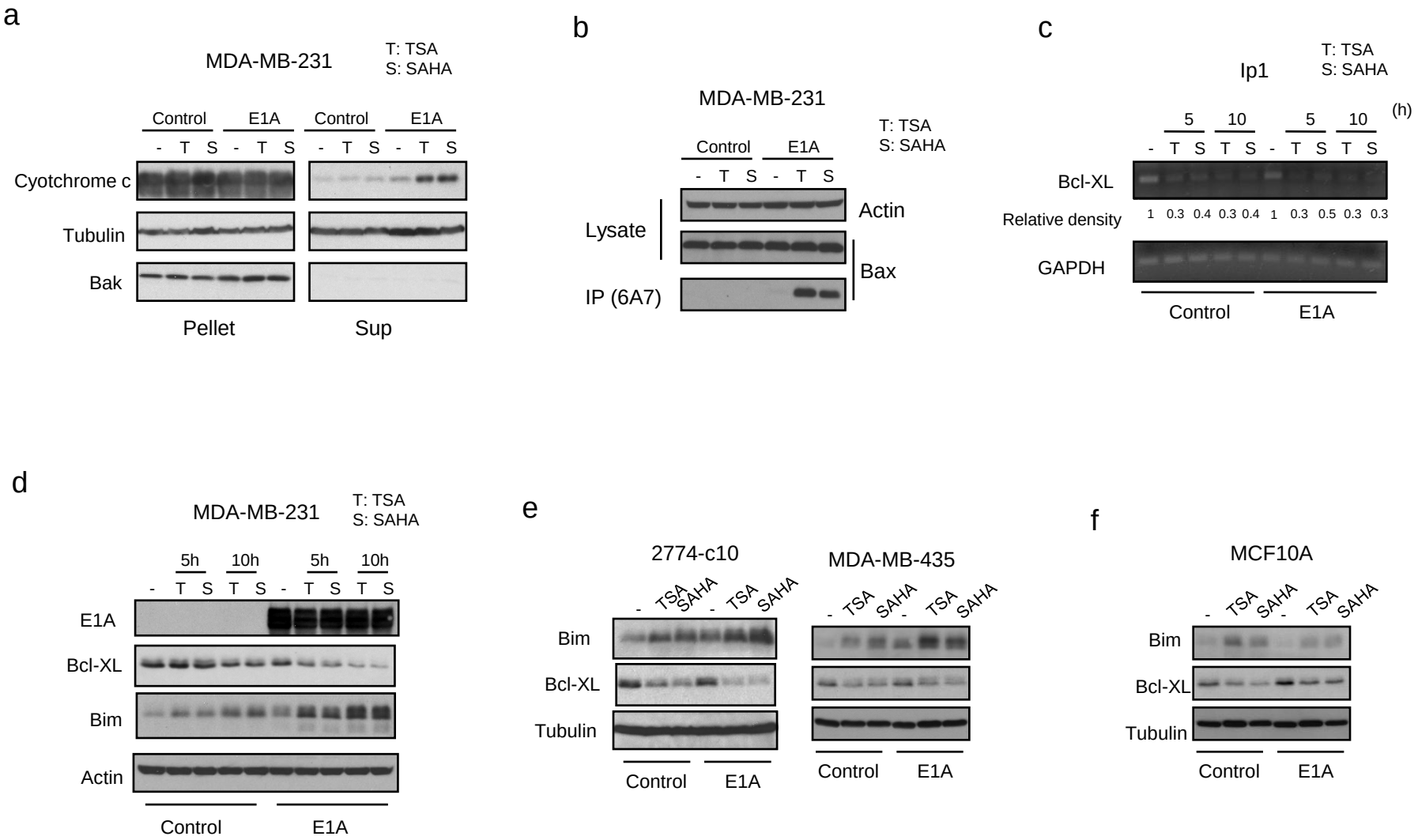
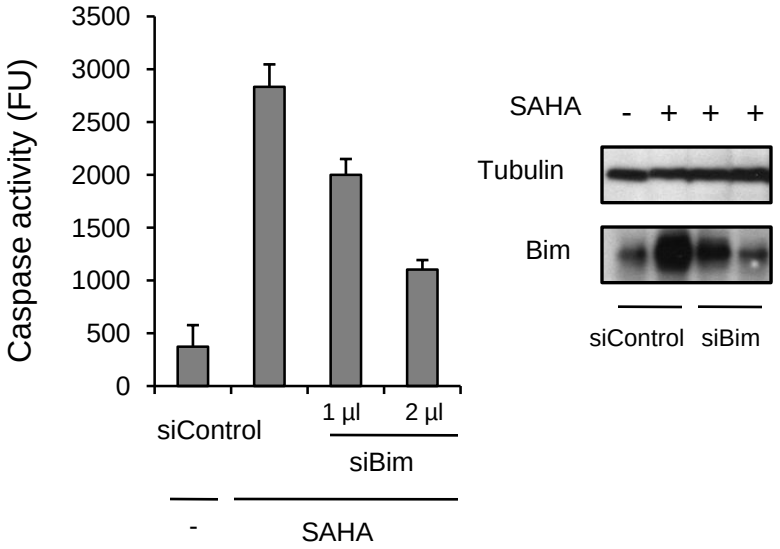

a
MDA-MB-231
T: TSA
S: SAHA
Control
E1A
Control
E1A
- T S - T S - T S - T S
Cyotchrome c
Tubulin
Bak
Pellet
Sup
b
MDA-MB-231
T: TSA
S: SAHA
Control
E1A
- T S - T S
Lysate
Actin
Bax
IP (6A7)
c
Ip1
T: TSA
S: SAHA
5
10
5
10
(h)
- T S T S - T S T S
Bcl-XL
Relative density
1 0.3 0.4 0.3 0.4 1 0.3 0.5 0.3 0.3
GAPDH
Control
E1A
d
MDA-MB-231
T: TSA
S: SAHA
5h
10h
5h
10h
- T S T S - T S T S
E1A
Bcl-XL
Bim
Actin
Control
E1A
e
2774-c10
MDA-MB-435
- TSA SAHA - TSA SAHA
- TSA SAHA - TSA SAHA
Bim
Bcl-XL
Tubulin
Control
E1A
Control
E1A
f
MCF10A
- TSA SAHA - TSA SAHA
Bim
Bcl-XL
Tubulin
Control
E1A

Figure S5

a


Caspase activity (FU)
3500
3000
2500
2000
1500
1000
500
0
siControl
1 µl
2 µl
siBim
-
SAHA
SAHA - + + +
Tubulin
Bim
siControl siBim

b

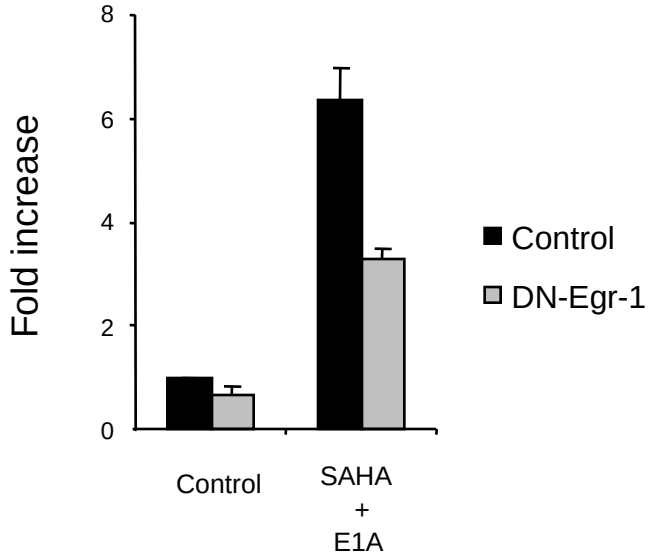

Fold increase
8
6
4
2
0
Control
SAHA + E1A
Control
DN-Egr-1

Figure S6