Supplemental Information

Materials and Methods

Cells

Daudi lymphoma cells were cultured as described by the American Type Culture Collection. Human peripheral blood mononuclear cells (PBMCs) were isolated from a de-identified fresh spleen by ficoll density gradient separation. The spleen was obtained from the Cooperative Human Tissue Network, following Institutional Review Board approval as non-human subject research (#150139).

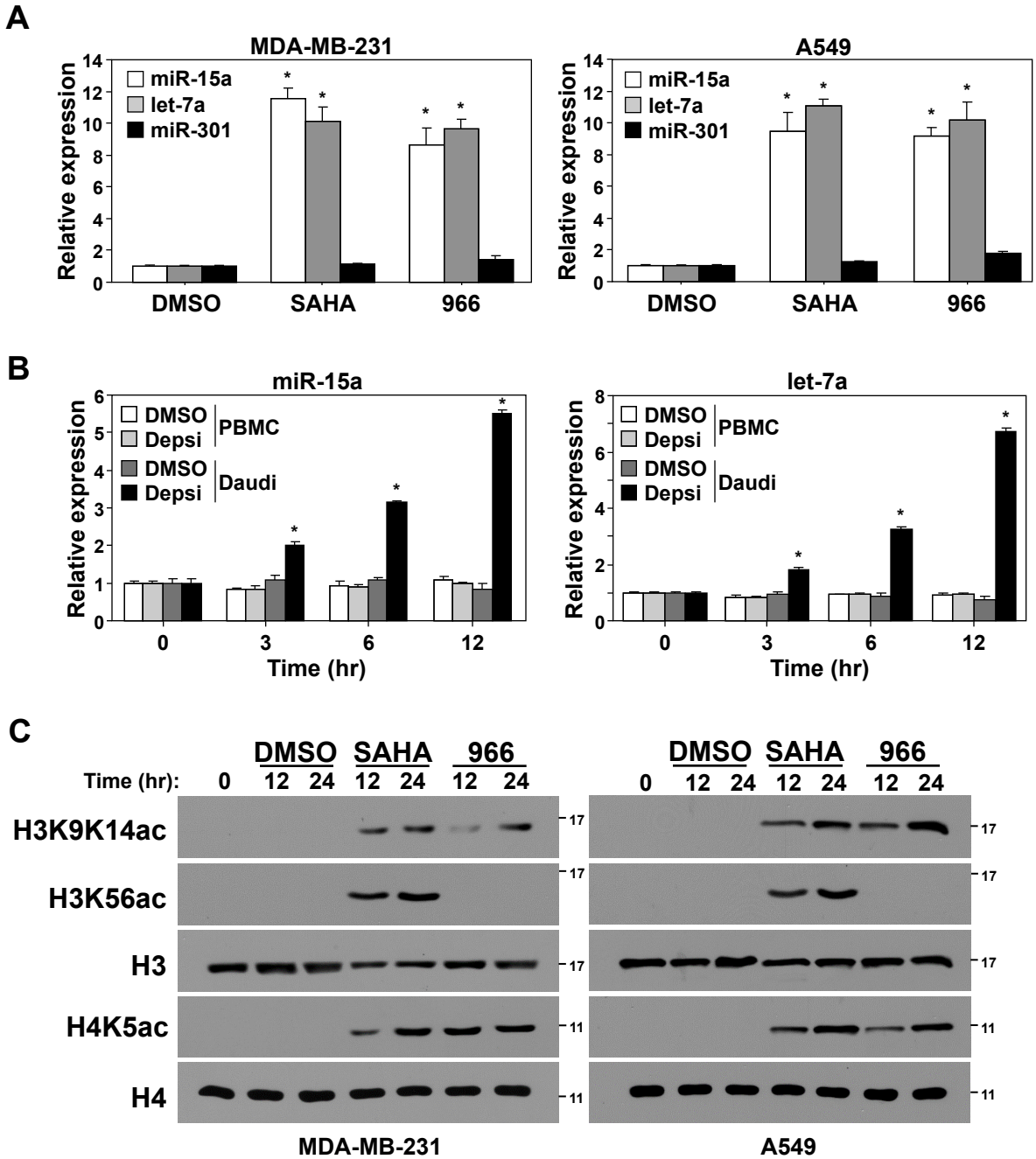
Supplemental Figure Legends

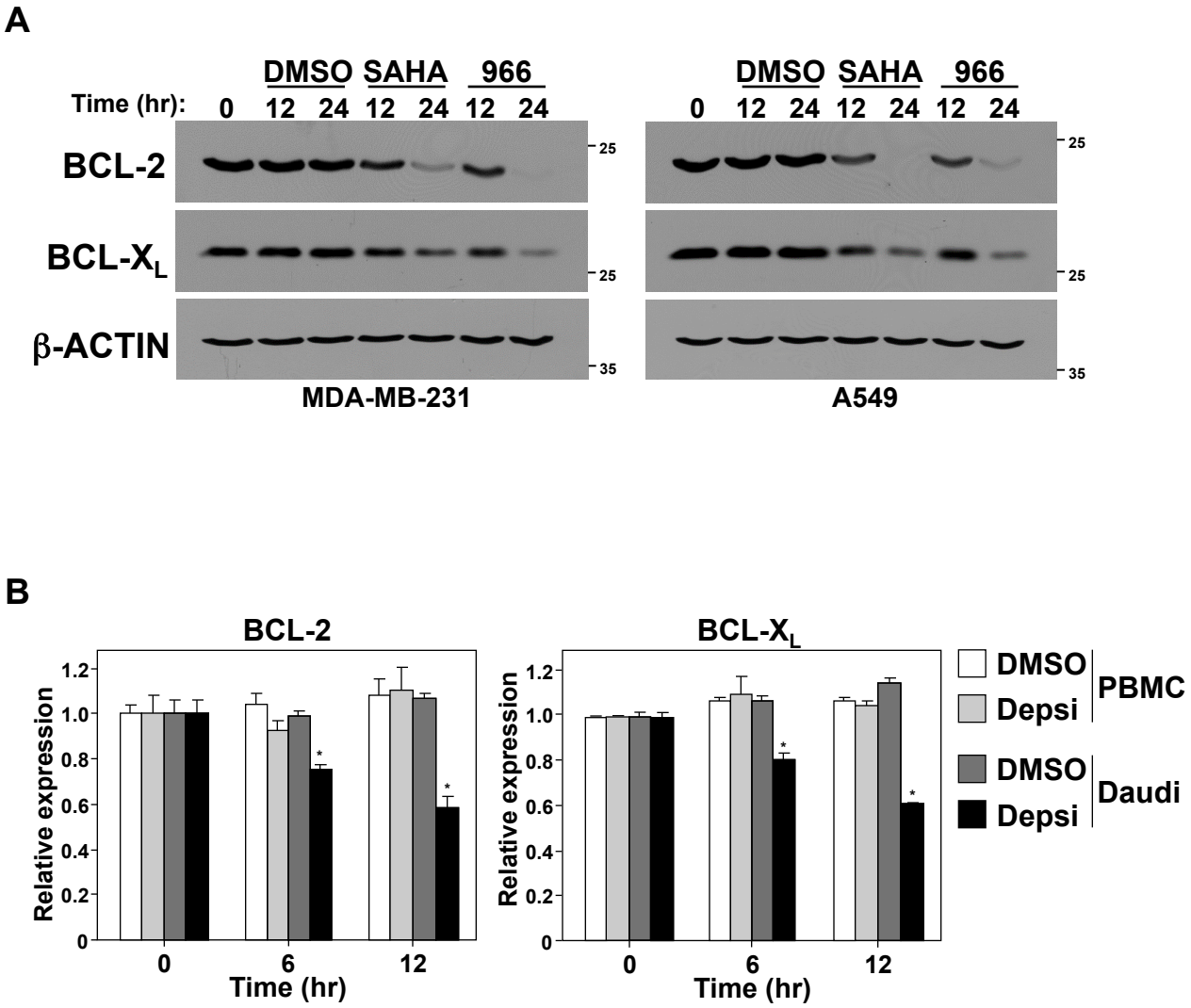
Figure 1. Upregulation of the miR-15 and let-7 families in transformed cells following HDACi. (A) Human breast (MDA-MB-231) and lung (A549) carcinoma cell lines were treated for 12 hours with vehicle control (DMSO), SAHA, or RGFP966 (966). Levels of the indicated miRNA were determined by qRT-PCR (triplicates) and normalized to small RNA *RNU6b* levels. (B) Human peripheral blood mononuclear cells (PBMCs) and a human B cell lymphoma cell line (Daudi) received vehicle control (DMSO) or Depsipeptide (Depsi). Following treatment for the indicated intervals, levels of miR-15a and let-7a were determined by qRT-PCR (triplicates) and normalized to small RNA *RNU6b* levels. Error bars are SEM; *$p<0.009$ for A and *$p<0.02$ for B were determined by comparison to DMSO. (C) Western blot analysis of the indicated proteins at intervals following treatment with SAHA, 966, or DMSO vehicle control. Molecular weight (kilodalton) indicated.

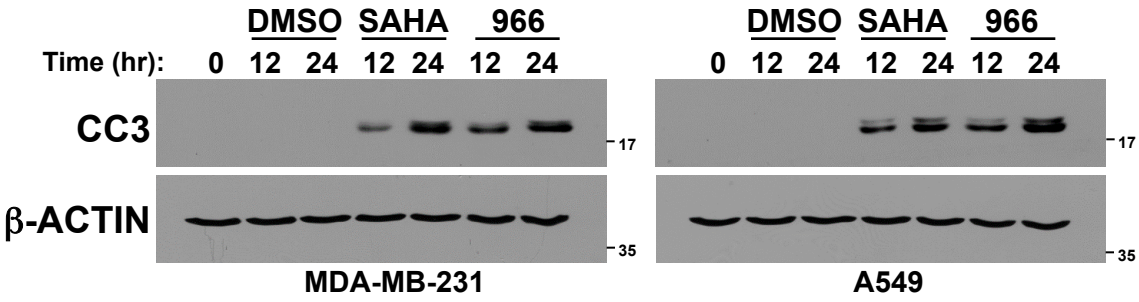
Figure 2. BCL-2 and BCL-X_L expression decreases after treatment with different HDAC inhibitors. (A) Western blots for the indicated proteins were performed with whole cell protein lysates of human breast (MDA-MB-231) and lung (A549) carcinoma cell lines following addition of SAHA, 966, or vehicle control (DMSO) for the indicated time. Molecular weight (kilodalton) indicated. (B) Human peripheral blood mononuclear cells (PBMCs) and a human B cell lymphoma cell line (Daudi) were left untreated or were treated with vehicle control (DMSO) or Depsipeptide (Depsi) for the indicated time. *BCL-2* and *BCL-X_L* mRNA levels were evaluated by qRT-PCR (triplicates) and are relative to *β-ACTIN* levels. Error bars are SEM; *$p<0.006$ was determined by comparison to DMSO.

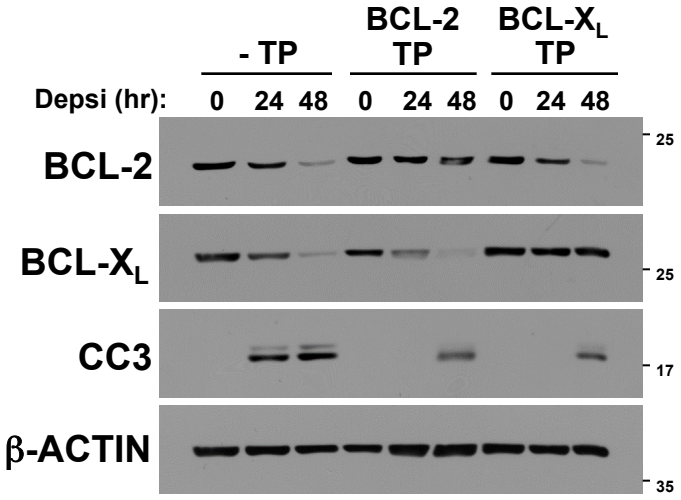
Figure 3. Different HDAC inhibitors induce apoptosis of human breast and lung carcinoma cells. Western blots for cleaved Caspase 3 (CC3) were performed with whole cell protein lysates of human breast (MDA-MB-231) and lung (A549) carcinoma cell lines following addition of SAHA, 966, or vehicle control (DMSO) for the indicated time. Molecular weight (kilodalton) indicated.

Figure 4. Protection from *BCL-2* and *BCL-X_L* down regulation inhibits apoptosis following HDACi. MDA-MB-231 breast cancer cells were transiently transfected with *BCL-2* or *BCL-X_L* Target Protectors (TP) that block the miR-15 family and let-7 family binding sites in the *BCL-2* and *BCL-X_L* 3'-untranslated region (UTR), respectively. Following addition of Depsipeptide (Depsi) for the indicated intervals, total cell protein lysates from MDA-MB-231 with or without (-TP) Target Protectors were Western blotted for the indicated proteins; cleaved Caspase 3, CC3. Molecular weight (kilodalton) indicated.


A
MDA-MB-231
A549
Relative expression
miR-15a
let-7a
miR-301
DMSO
SAHA
966
B
miR-15a
let-7a
DMSO
Depsi
PBMC
Daudi
Time (hr)
C
DMSO
SAHA
966
Time (hr):
0
12
24
H3K9K14ac
H3K56ac
H3
H4K5ac
H4
17
11
MDA-MB-231
A549


A
DMSO SAHA 966
Time (hr): 0 12 24 12 24 12 24
BCL-2
BCL-X$_L$
β-ACTIN
25
25
35
MDA-MB-231
A549
B
BCL-2
BCL-X$_L$
Relative expression
Time (hr)
DMSO
Depsi
PBMC
Daudi


DMSO
SAHA
966
Time (hr):
0
12
24
12
24
12
24
CC3
β-ACTIN
17
35
MDA-MB-231
A549


- TP
BCL-2 TP
BCL-XL TP
Depsi (hr):
0 24 48 0 24 48 0 24 48
BCL-2
BCL-XL
CC3
β-ACTIN
25
25
17
35