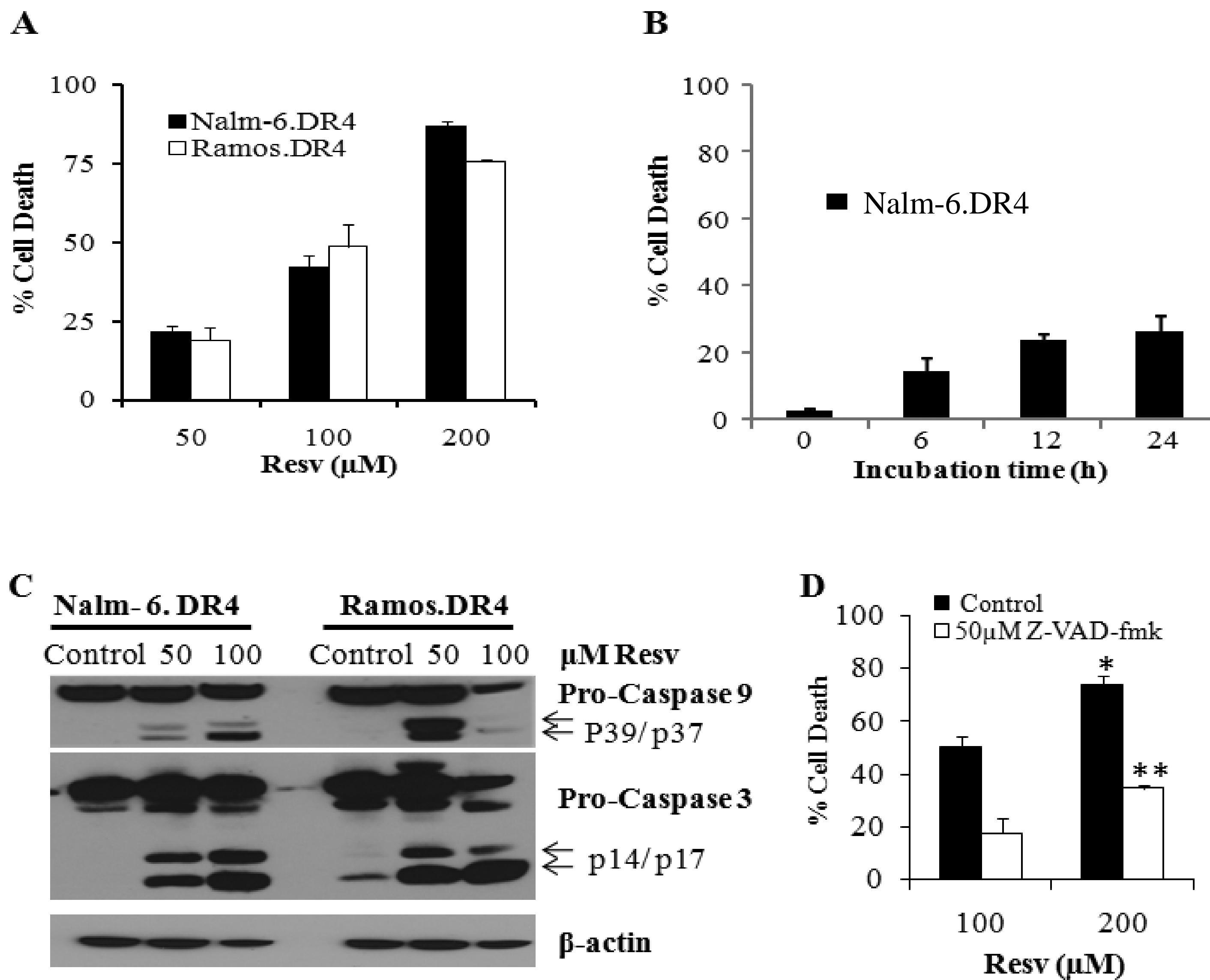
cleavage of Rab 4B proteins (arrow) following Resv treatment of Ramos.DR4 lymphoma cells. Cells were treated with vehicle alone or 50 µM of Resv for 24 h, and subjected to western blotting for Rab 4B molecules. Actin was used as a loading control. (B) Ramos.DR4 cells treated with vehicle alone or Resv were also washed in cold PBS, and biotinylated with sulfo-NHS-SS-biotin and divided into three groups. Cells in group I were kept on ice while cell in group II were warmed (37°C for 15 min) to allow internalization of these biotin-labeled surface molecules, followed by cleavage of residual biotin from cell surface proteins with a glutathione solution as described in the methods. The biotin-labeled endocytosed class II proteins were then detected in a capture ELISA, and the percent endocytosis was calculated as described. Data are representative of triplicate wells, and is expressed as percent class II endocytosis/recycling ± SEM.

Supplemental Figure 1. Resv induces apoptotic cell death in B-lymphoma cell lines. (A) Nalm-6.DR4 and Ramos.DR4 cells were seeded at 1×10^5 cells/well in 100 µl of appropriate culture medium in a flat-bottom 96-well plate, and treated with Resv (50, 100, 200 µM) for 24 h. Then 20 µl of MTS solution was added to each well and incubated for another 2 h according to the manufacturer's protocol. The resulting colored solution was measured at 490 nm. Cells treated with vehicle alone were used as controls. The percent cell death induced by Resv was calculated using the equation: [(Absorbance $_{control}$ – Absorbance $_{treated}$)/Absorbance $_{control}$] x100. The data shown are results of at least three separate experiments that were performed in triplicate wells. Error bars represent mean ± S.D. (B) Nalm-6.DR4 cells were also treated with 50 µM of Resv, and percent cell death was determined as described. (C) Nalm-6.DR4 and Ramos.DR4 were treated

with Resv (50 μM and 100 μM) or vehicle alone (control) for 24 h, and analyzed by western blotting for active caspase 9, and 3 protein expression as described in the methods. Arrows indicate cleaved (active) forms of caspases. β-actin was utilized as a loading control. Data are representative of three independent experiments. (D) Caspase inhibition assay was accomplished by the addition of 20 μl of caspase inhibitor Z-VAD-FMK to GA-A (100 μM and 200 μM) or vehicle treatment of Nalm-6.DR4 cells for 24 h, followed by the MTS cell viability assay as described. Data are representative of three independent experiments with similar patterns, and error bars represent average ± S.D.

Supplemental Figure 2. RT-PCR analysis of HLA-DRα, HLA-DRβ and CIITA mRNA expression in Resv-treated lymphoma cell lines. Nalm-6.DR4 and Ramos.DR4 cells were treated with vehicle alone or Resv (50 μM) for 24 h, and total RNA was reverse-transcribed, amplified, and separated by electrophoresis. Specific products were detected in these cells by RT-PCR using specific primers to HLA-DRα, HLA-DRβ and CIITA. The amplification of β-actin was included as a control.


A
100
75
50
25
0
% Cell Death
Nalm-6.DR4
Ramos.DR4
50
100
200
Resv (μM)
B
100
80
60
40
20
0
% Cell Death
Nalm-6.DR4
0
6
12
24
Incubation time (h)
C
Nalm- 6. DR4
Ramos.DR4
Control 50 100
Control 50 100
μM Resv
Pro-Caspase 9
P39/p37
Pro-Caspase 3
p14/p17
β-actin
D
Control
50μM Z-VAD-fmk
100
80
60
40
20
0
% Cell Death
*
**
100
200
Resv (μM)

Radwan et al. Supplemental Figure 2