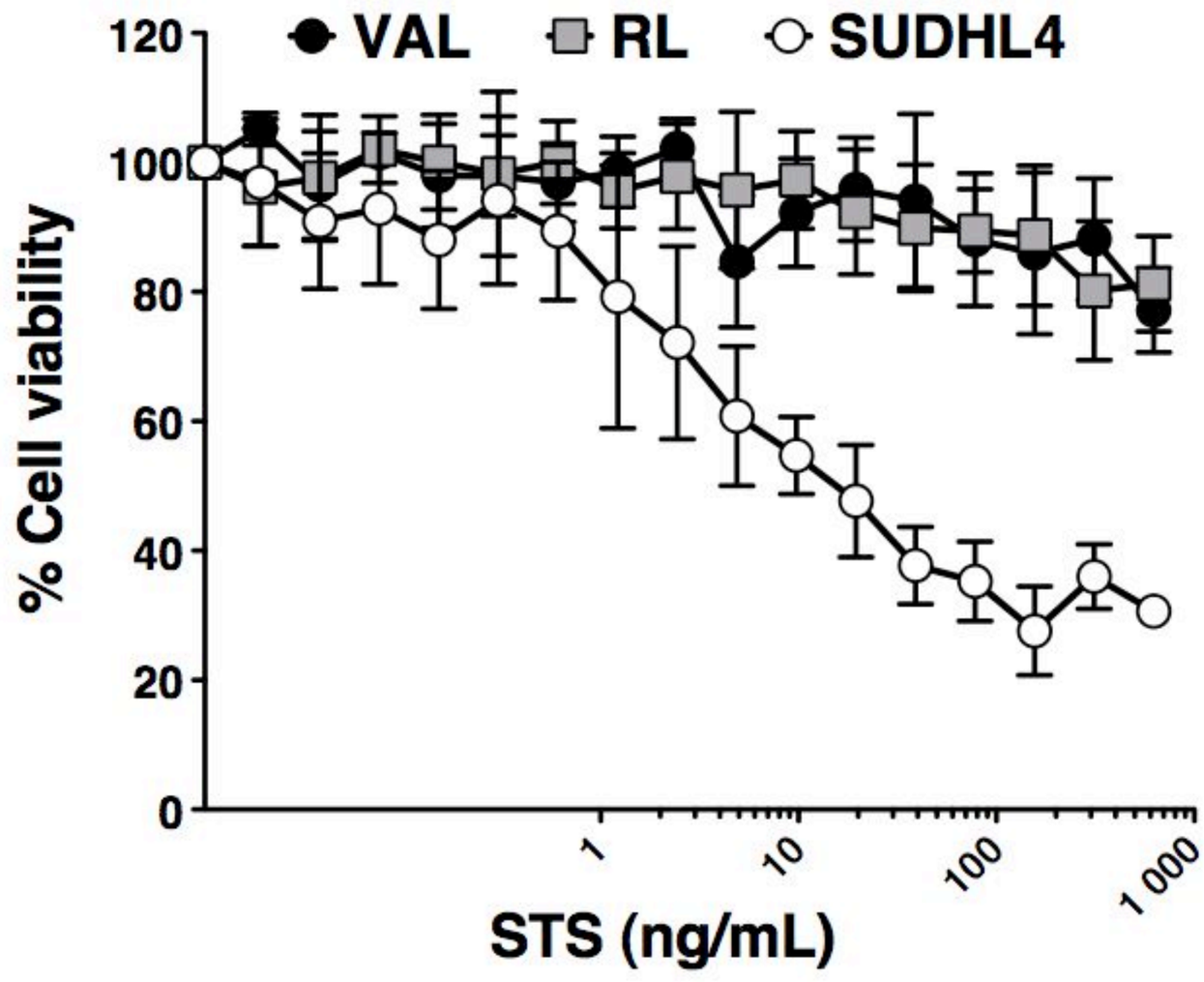
Supplemental #1



Supplemental #2

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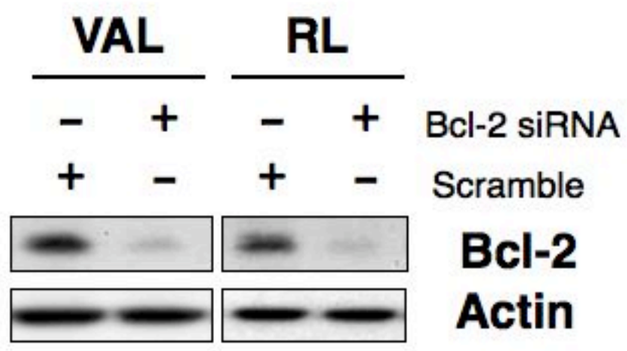

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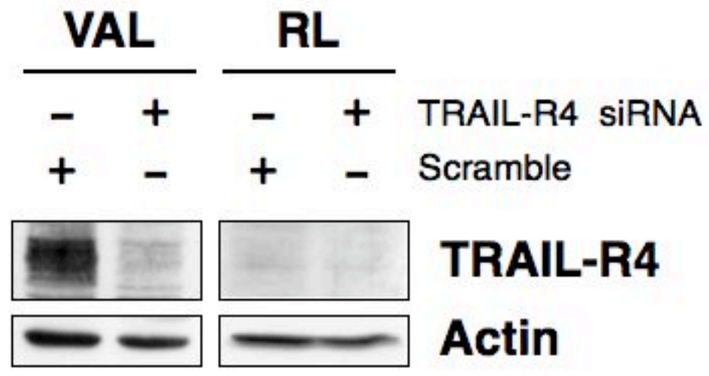

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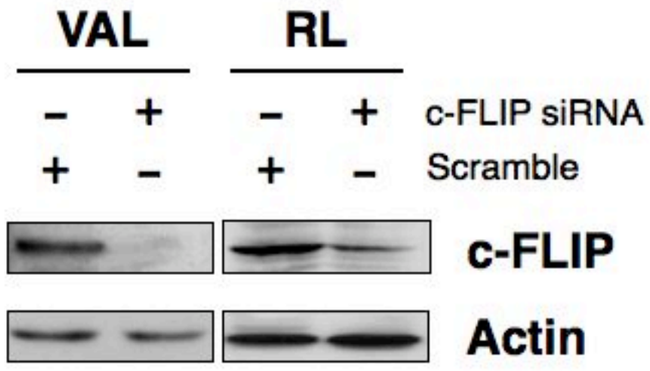

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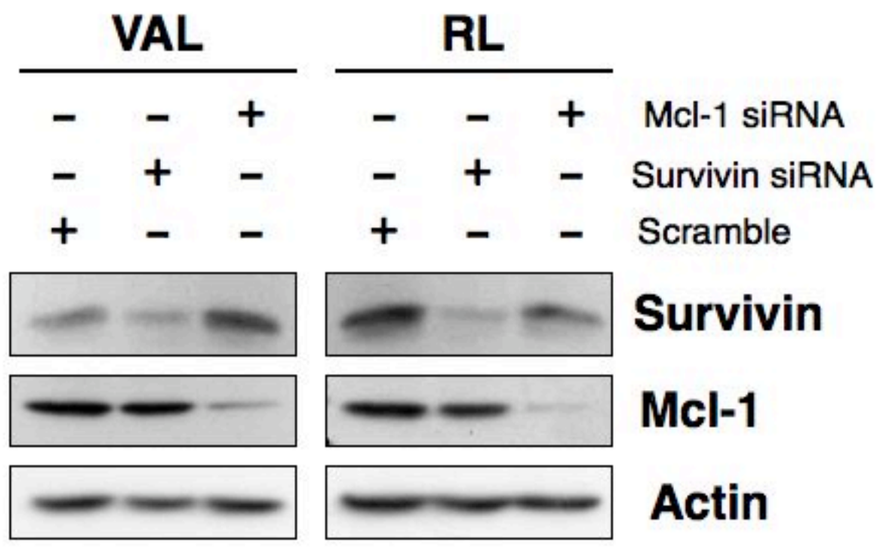

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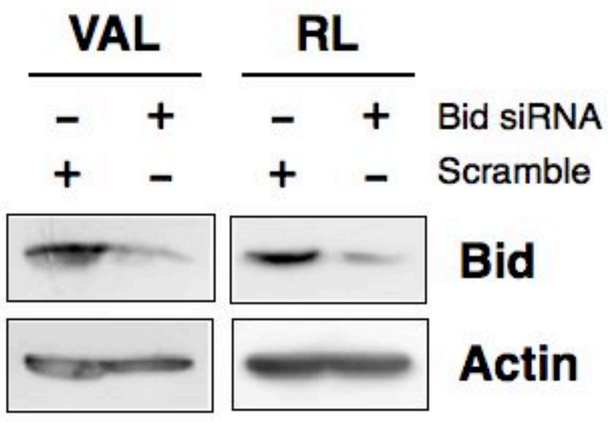

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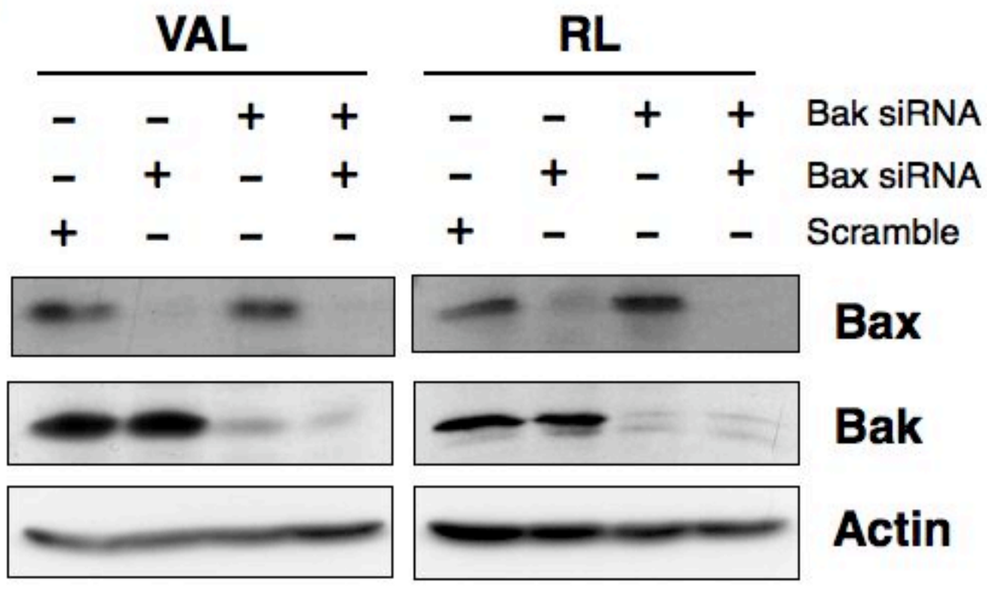

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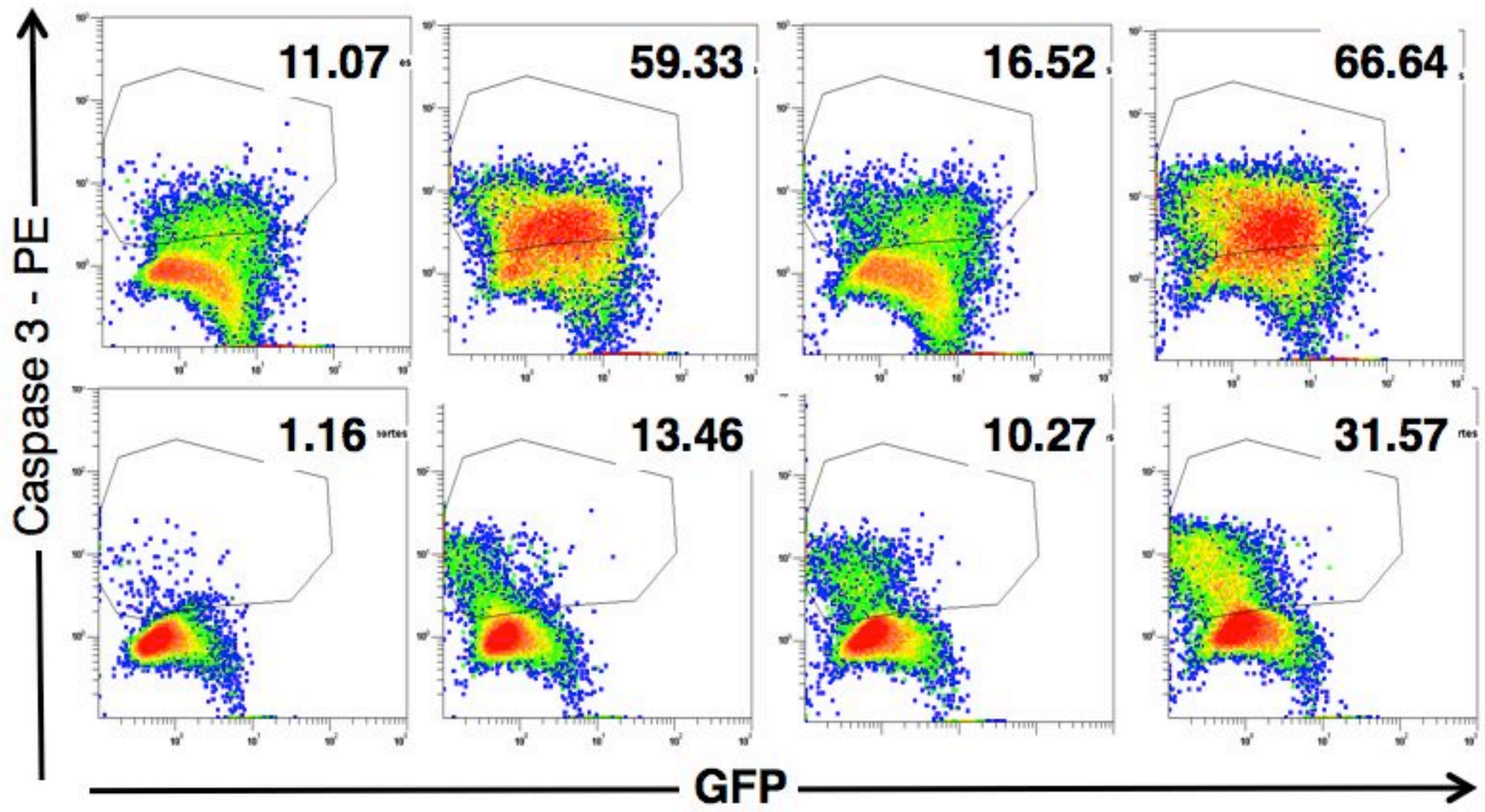
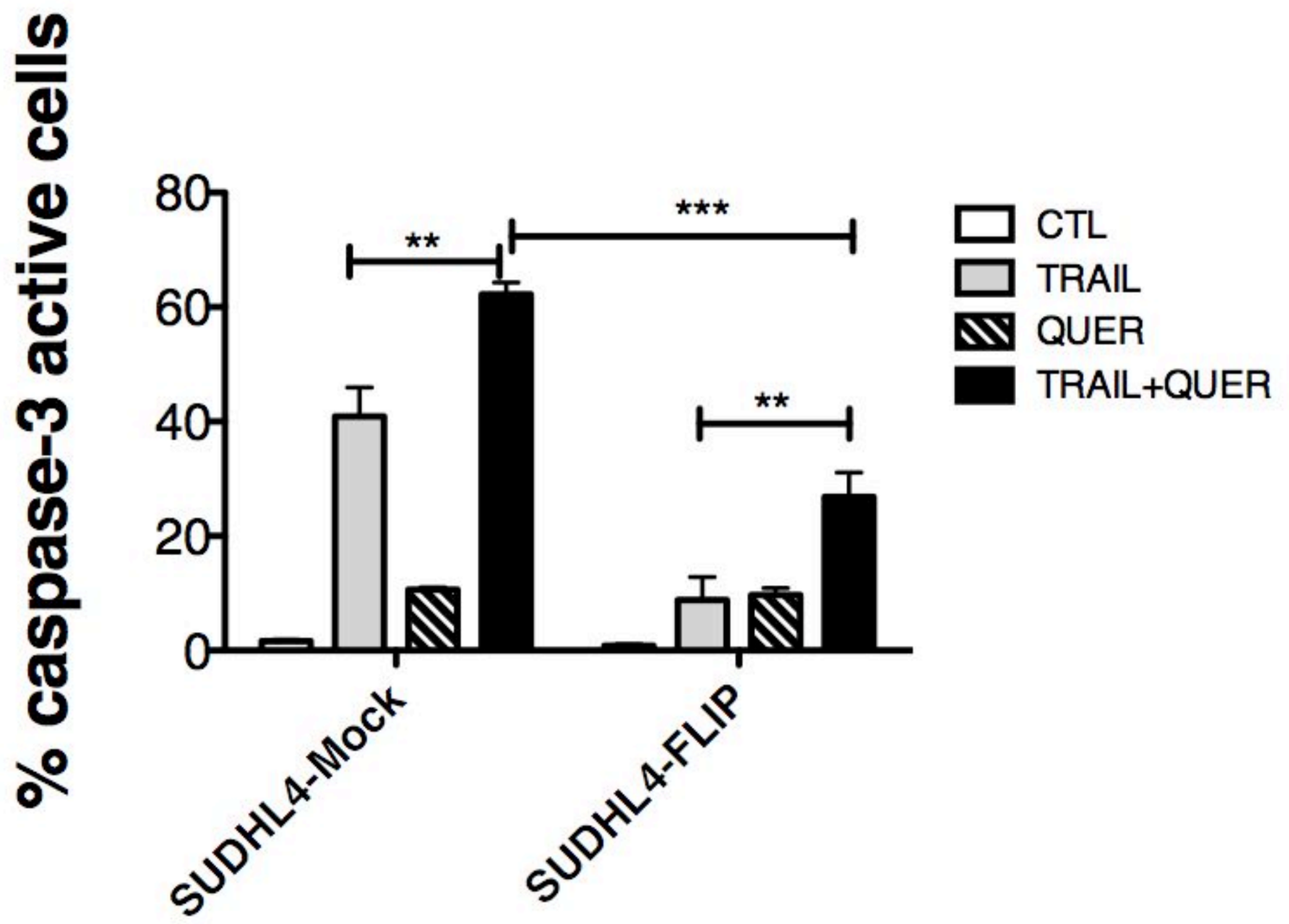
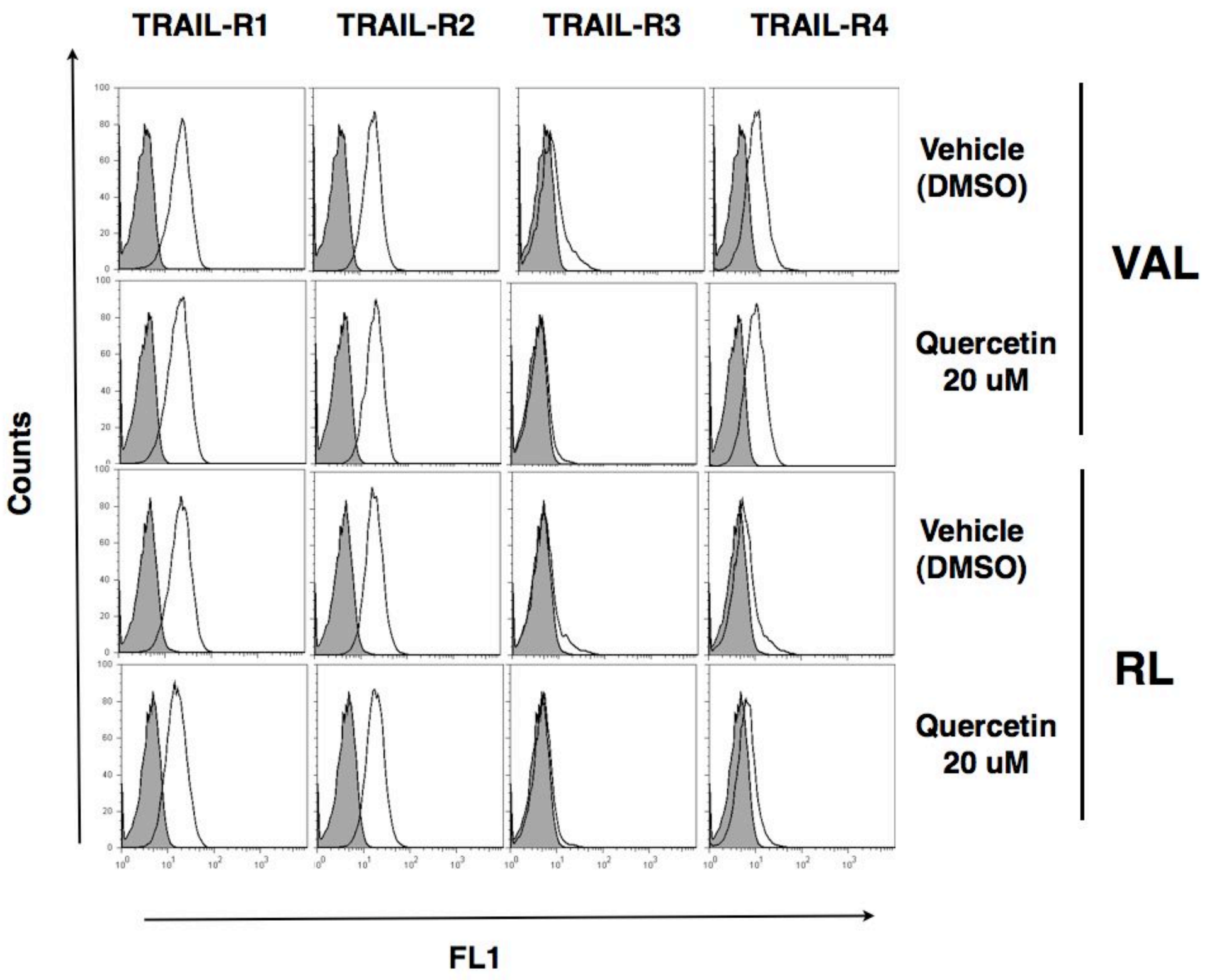

11.07
59.33
16.52
66.64
1.16
13.46
10.27
31.57
Caspase 3 - PE
GFP
SUDHL4-Mock
SUDHL4-c-FLIP

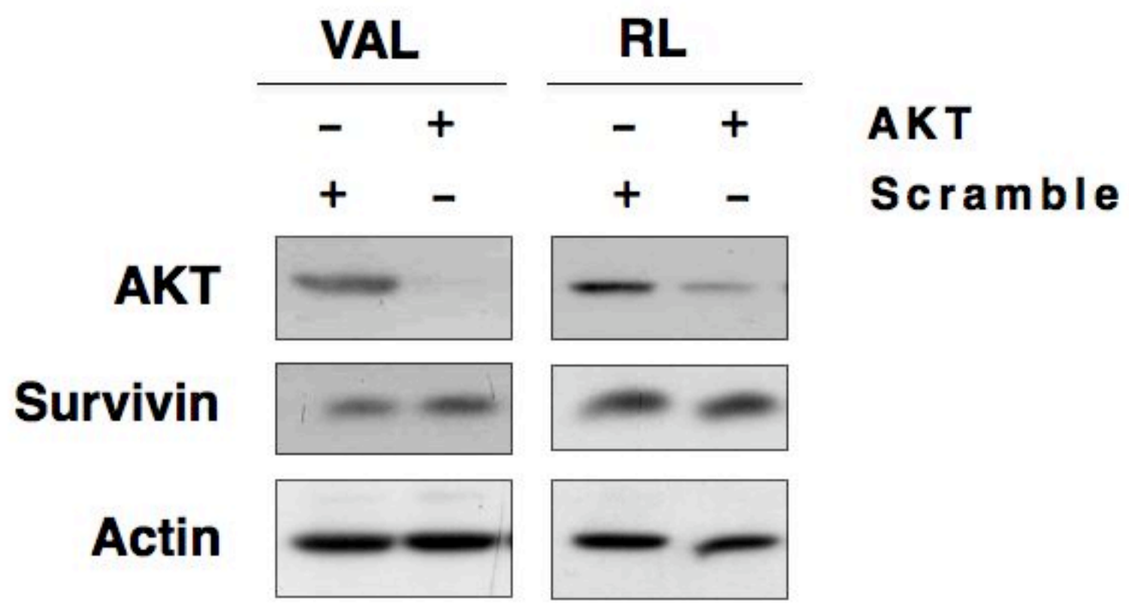
B


% caspase-3 active cells
80
60
40
20
0
**
***
**
CTL
TRAIL
QUER
TRAIL+QUER
SUDHL4-Mock
SUDHL4-FLIP

Supplemental #4



Supplemental #5



Supplemental #6

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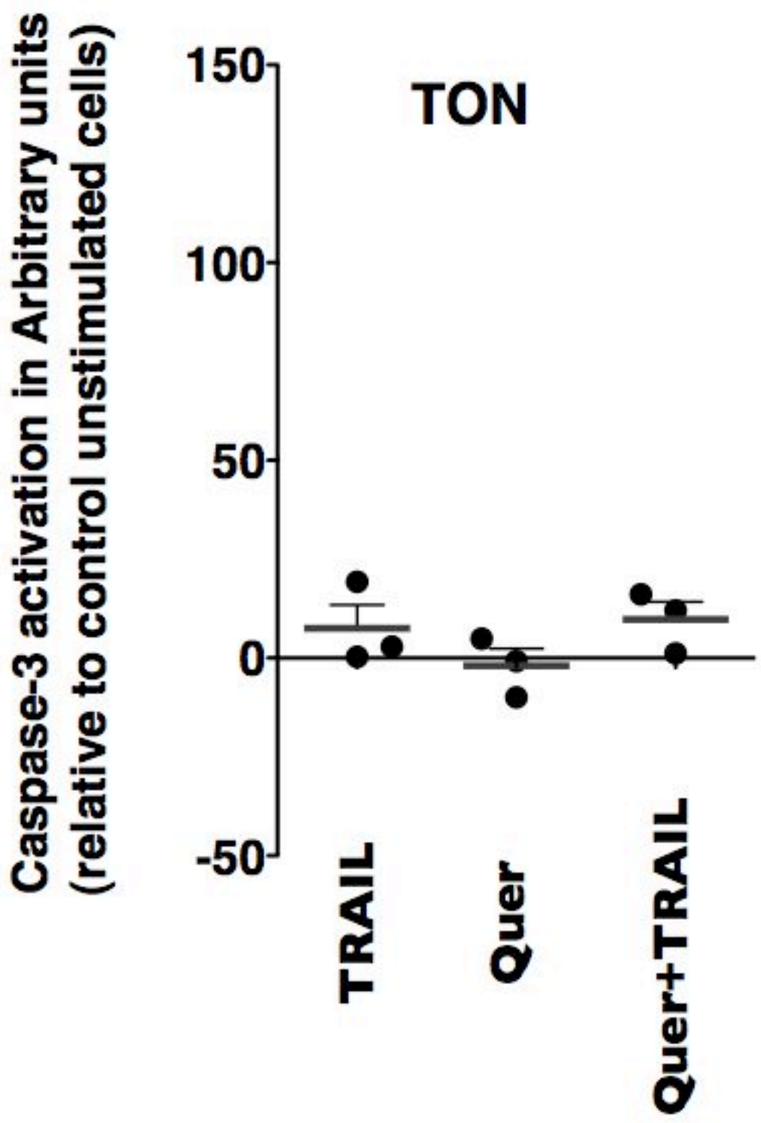

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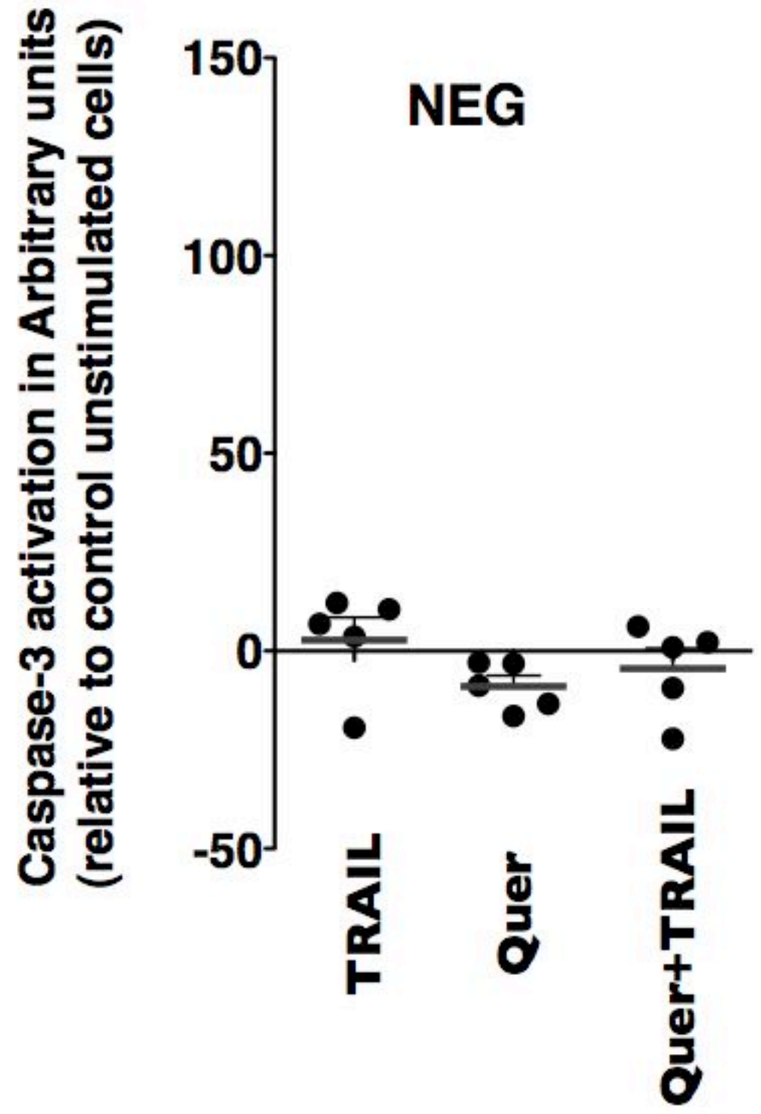

SUPPLEMENTAL MATERIAL & METHODS

Cell culture and treatments :

Samples were collected in the University Hospital of Rennes from 5 patients with reactive non-malignant lymph nodes (NEG) and from 3 children undergoing routine tonsillectomy (TON) considered as normal. Patient recruitment followed institutional review board approval and written informed consent process according to the Declaration of Helsinki. Lymph nodes and tonsils were cut into pieces and flushed using syringe and needle. Cell suspensions were cultured at $7.5x10^5$ cells/ml in RPMI 1640 (Invitrogen), supplemented with 10% FCS (Invitrogen) and antibiotics (penicillin/streptomycin, Invitrogen). SUDHL4-mock and SUDHL4-cFLIP, which express the GFP and which overexpress or not c-FLIP long isoform, have previously been described {Travert, 2008 #1300}. These cells were cultured in RPMI 1640 supplemented with 10% fetal bovine serum and regularly checked for GFP positivity by flow cytometry. To assess cell sensitivity to TRAIL and/or quercetin stimulation, 8.10^5 cells were treated with Quercetin 20 μM for 24 hours and stimulated or not with 100 ng/ml killerTRAIL from Alexis (Coger, Paris, France) for 6 hours. Caspase-3 activation was evaluated by flow cytometry using a PE-conjugated anti-active caspase-3 from BD Biosciences (BD biosciences, Le Pont de Claix, France).

Western blot analysis:

The rabbit polyclonal anti-Akt antibody was from Cell Signalling (Millipore, Molsheim, France), remaining antibodies are described in the Material and Methods.

SUPPLEMENTAL FIGURE LEGENDS

Supplemental figure #1. VAL and RL, but not SUDHL4 are resistant to staurosporine-induced cell death. Sensitivity to staurosporine-induced cell death of the non-Hodgkin B lymphoma cell lines VAL, RL and SUDHL4. Cells were treated with different concentrations of staurosporine (STS) for 24 hours. Cell viability was measured by the AlamarBlue method. Error bars indicate SD of triplicate experiments.

Supplemental figure #2. siRNA-mediated downregulation of Mcl-1, survivin, TRAIL-R4, Bid, c-FLIP, Bcl-2, Bax and Bak. (A-F) 48 hours after electroporation with a specific siRNA or with a control siRNA (scramble), VAL and RL cells were lysed in a NP40-containing buffer, as described in "Materials and Methods" and the efficiency of each siRNA was assessed by western blot.

Supplemental figure #3. Overexpression of c-FLIP inhibits TRAIL-induced apoptosis in combination with Quercetin. 24 hours after Quercetin treatment (20 μM), SUDHL4-mock and SUDHL4-cFLIP cell death were induced with a stimulation of killerTRAIL (100 ng/ml) for 6 hours. Apoptosis was evaluated by flow cytometry with anti-active-caspase-3 staining. (A) Representative dot plots from one experiment. (B) Errors bars indicate SD of triplicate experiments.

Supplemental figure #4. Analysis of TRAIL receptor expression after quercetin stimulation. Levels of TRAIL receptors expression at the surface of VAL and RL cells after treatment with quercetin (24 hours at 20 μM) or DMSO (Vehicle). Cells were analysed by flow cytometry after staining with specific (unshaded areas) or control (shaded areas) antibodies.

Supplemental figure #5. Effect of siRNA-mediated knockdown of Akt on survivin expression. 24 hours after electroporation using a selective AKT siRNA or a control siRNA (scramble, VAL and RL cells were lysed and the expression of survivin, Akt or actin were evaluated by western blot.

Supplemental figure #6. Quercetin and TRAIL alone or in combination fail to trigger caspase-3 activation in normal cells. Normal B cells from Lymph nodes and tonsils were cultured alone or with Quercetin (20 μM) for 12 hours and cells were co-treated or not with killerTRAIL (1 μg/ml), during 6 hours. B cell apoptosis was evaluated with an anti-active caspase 3 staining by flow cytometry on selectively gated CD19+ CD20+ active caspase-3 positives cells. Results are expressed in arbitrary units of caspase-3 activation relative to unstimulated cells.