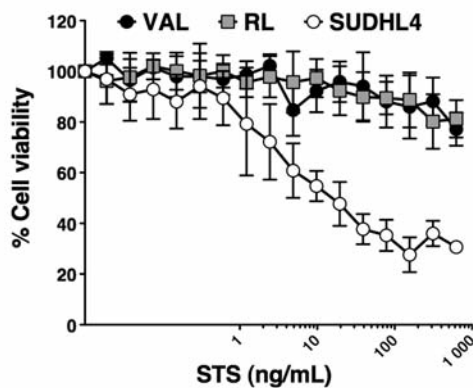


# Quercetin-mediated Mcl-1 and survivin downregulation restores TRAIL-induced apoptosis in non-Hodgkin's lymphoma B cells

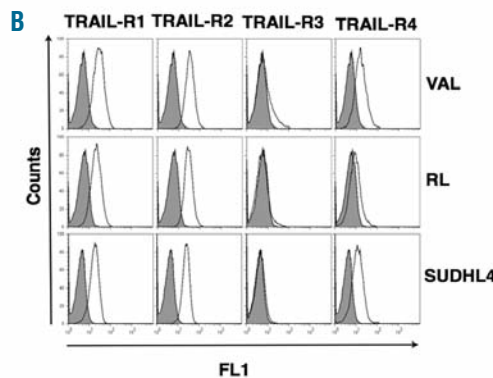
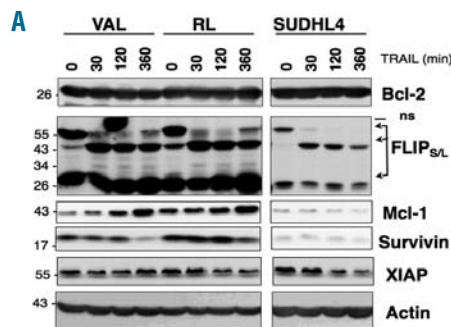
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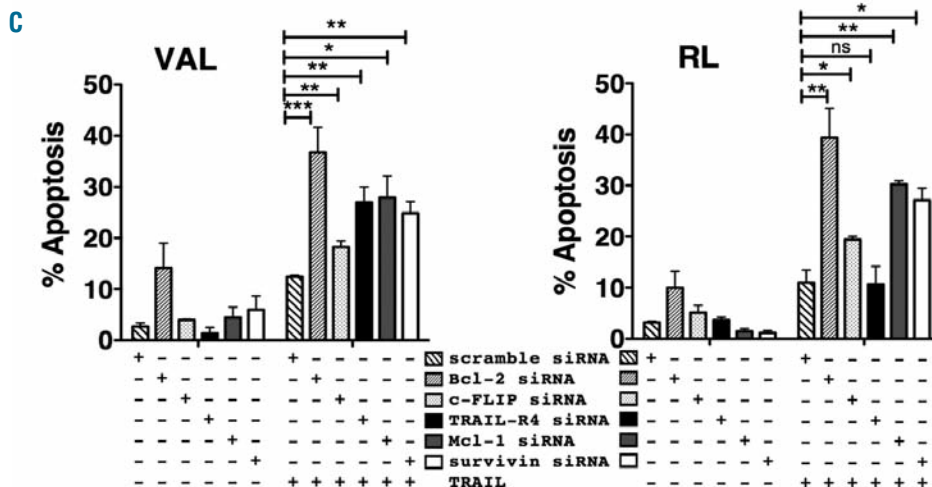
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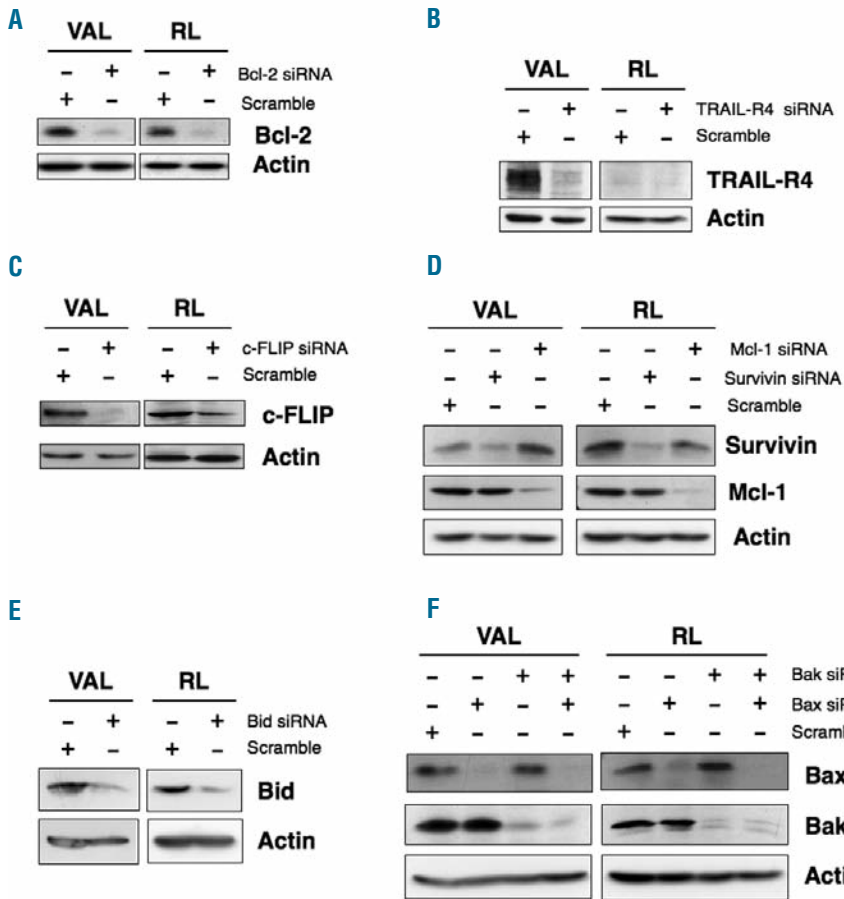


**Online Supplementary Figure S1.** VAL and RL, but not SUDHL4, are resistant to staurosporine-induced cell death. Sensitivity to staurosporine-induced cell death of the non-Hodgkin's B-lymphoma cell lines VAL, RL and SUDHL4. Cells were treated with different concentrations of staurosporine (STS) for 24 h. Cell viability was measured by the AlamarBlue method. Error bars indicate SD of triplicate experiments.

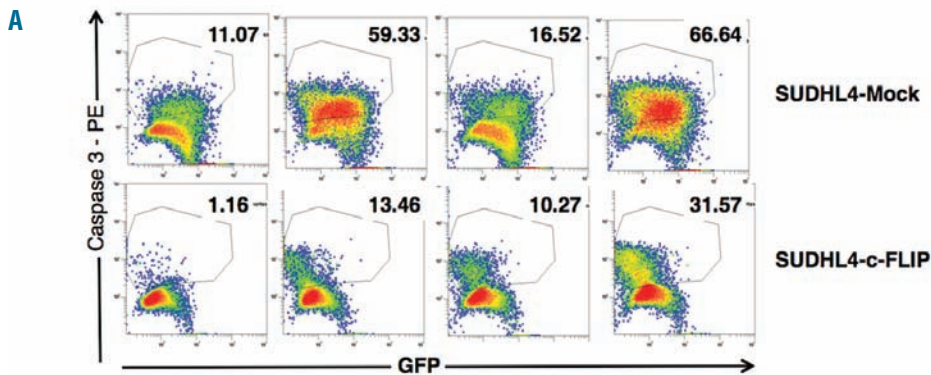


**Online Supplementary Figure S2.** Bcl-2, Mcl-1 and survivin account for the resistance to TRAIL of VAL and RL lymphoma B-cell lines. (A) Western blot analysis of antiapoptotic proteins upon stimulation with His-TRAIL (500 ng/mL) for the indicated times. (B) Expression of TRAIL receptors on VAL, RL and SUDHL4 cells, at the membrane level, was measured by flow cytometry (unfilled peaks). Shaded peaks correspond to the isotype control antibody staining. (C) Effect of siRNA-mediated knockdown of Mcl-1, survivin, Bcl-2, c-FLIP or TRAIL-R4 on the sensitivity to TRAIL-induced apoptosis. Forty-eight hours after electroporation with a specific siRNA or a control siRNA (scramble), VAL and RL cells were treated with TRAIL at 500 ng/mL for 3 h. Apoptosis was measured by flow cytometry after annexin V staining. Data presented are means plus or minus SD n=3; \*P<0.05; \*\*P<0.01 or \*\*\*P<0.001 respective to scramble siRNA.

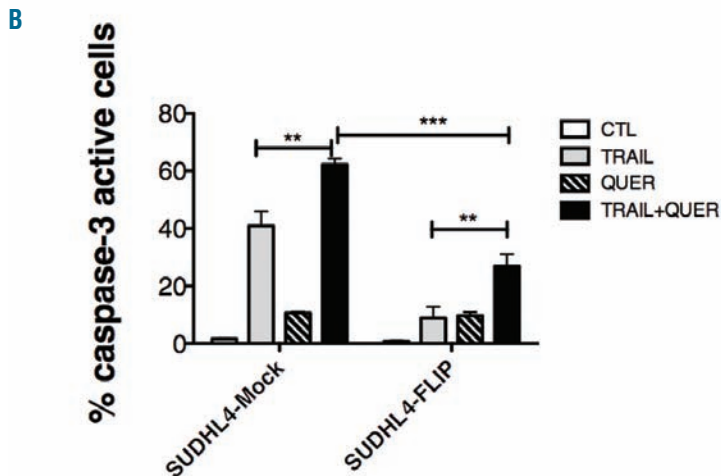


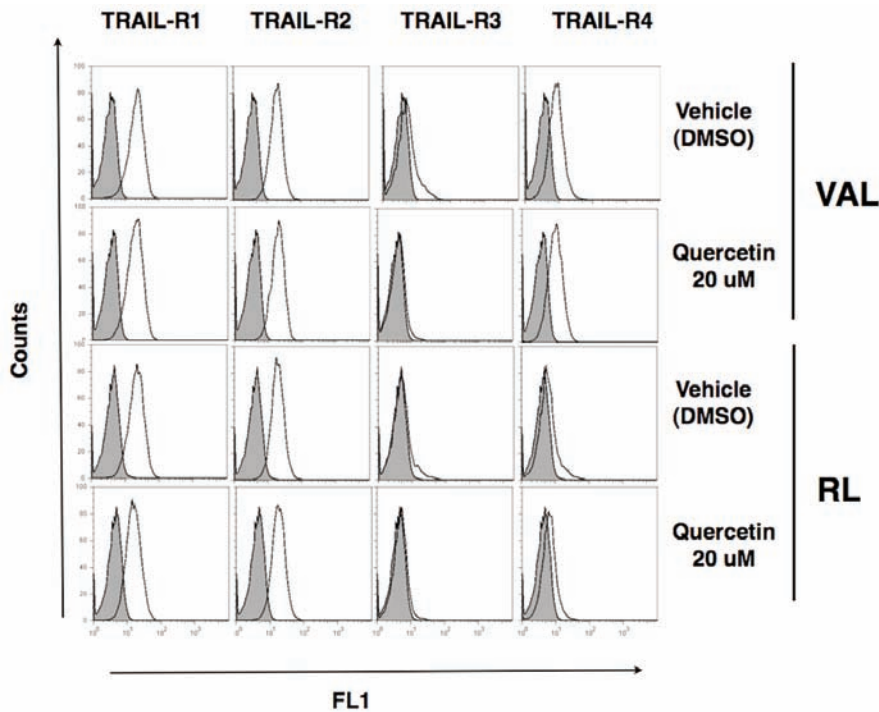


Online Supplementary Figure S3. siRNA-mediated downregulation of Mcl-1, survivin, TRAIL-R4, Bid, c-FLIP, Bcl-2, Bax and Bak. (A-F) Forty-eight hours after electroporation with a specific siRNA or with a control siRNA (scramble), VAL and RL cells were lysed in a NP40-containing buffer, as described in the *Design and Methods* section and the efficiency of each siRNA was assessed by Western blot.

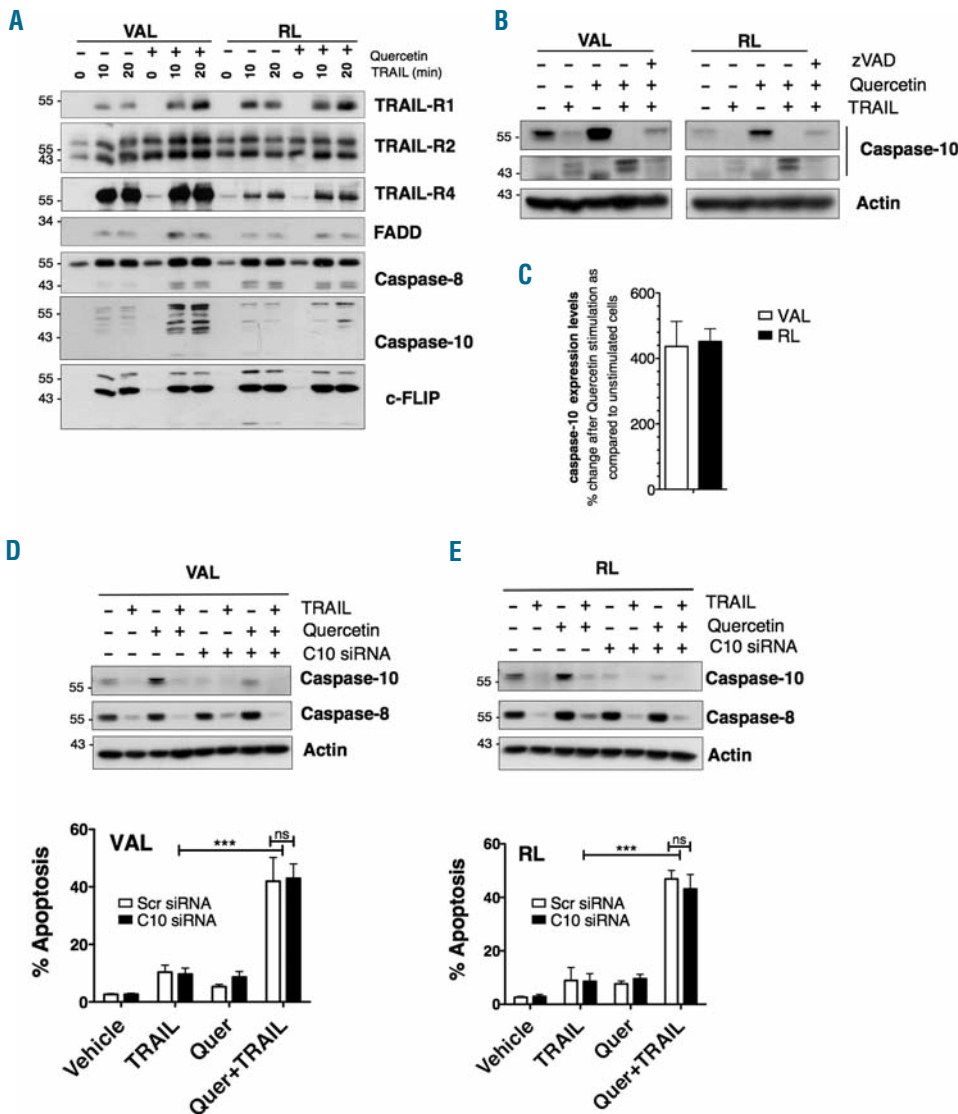


Online Supplementary Figure S4. Overexpression of c-FLIP inhibits TRAIL-induced apoptosis in combination with quercetin. Twenty-four hours after Quercetin treatment (20  $\mu$ M), SUDHL4-mock and SUDHL4-cFLIP cell death were induced with a stimulation of killerTRAIL (100 ng/mL) for 6 h. Apoptosis was evaluated by flow cytometry with anti-active-caspase-3 staining. (A) Representative dot plots from one experiment. (B) Error bars indicate SD of triplicate experiments.



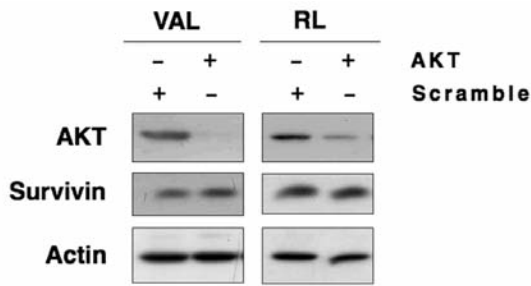


**Online Supplementary Figure S5.** Analysis of TRAIL receptor expression after quercetin stimulation. Levels of TRAIL receptor expression at the surface of VAL and RL cells after treatment with quercetin (24 h at 20  $\mu$ M) or DMSO (vehicle). Cells were analyzed by flow cytometry after staining with specific (unshaded areas) or control (shaded areas) antibodies.



**Online Supplementary Figure S6.** Quercetin induces caspase-10 upregulation and recruitment to the DISC, but sensitization to TRAIL occurs independently of caspase-10. (A) Analysis of TRAIL-induced DISC formation. VAL and RL cells were treated with quercetin (20  $\mu$ M, 24 h) and stimulated with TRAIL (500 ng/mL, 6 h) and/or zVAD-fmk (20  $\mu$ M, 30 min before TRAIL). (B) Western blot analysis of caspase-10 expression after treatment with quercetin (20  $\mu$ M, 24 h) and/or TRAIL (500 ng/mL, 6 h) and/or zVAD-fmk (20  $\mu$ M, 30 min before TRAIL). (C) Relative expression of caspase-10 mRNA by qPCR after treatment with quercetin (20  $\mu$ M, 24 h). Results correspond to % fold change mRNA expression compared with cells treated with DMSO, and were normalized to L32 levels. (D-E) Effect of siRNA-mediated caspase-10 knockdown on the efficiency of the combined treatment with quercetin and TRAIL. 24 h after electroporation with a specific siRNA or a control siRNA (scramble), VAL and RL cells were treated with quercetin (20  $\mu$ M) for 24 h, followed by TRAIL (500 ng/mL) for 3 h. Apoptosis was measured by flow cytometry after annexin V staining. Efficiency of the caspase-10 siRNA was evaluated by Western blotting. (D-E) Data are means plus or minus SD n=3; \*\*\* $P$ <0.001 respective to TRAIL alone or to quercetin+TRAIL in the presence or the absence of Caspase-10 siRNA. ns: not statistically relevant.

**Online Supplementary Figure S7.** Effect of siRNA-mediated knock-down of Akt on survivin expression. Twenty-four 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 was evaluated by Western blot.



**Online Supplementary Figure S8.** 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  $\mu$ M) for 12 h and cells were co-treated or not with killerTRAIL (1  $\mu$ g/mL), during 6 h. B-cell apoptosis was evaluated with an anti-active caspase 3 staining by flow cytometry on selectively gated CD19<sup>+</sup> CD20<sup>+</sup> active caspase-3 positives cells. Results are expressed in arbitrary units of caspase-3 activation relative to unstimulated cells.

