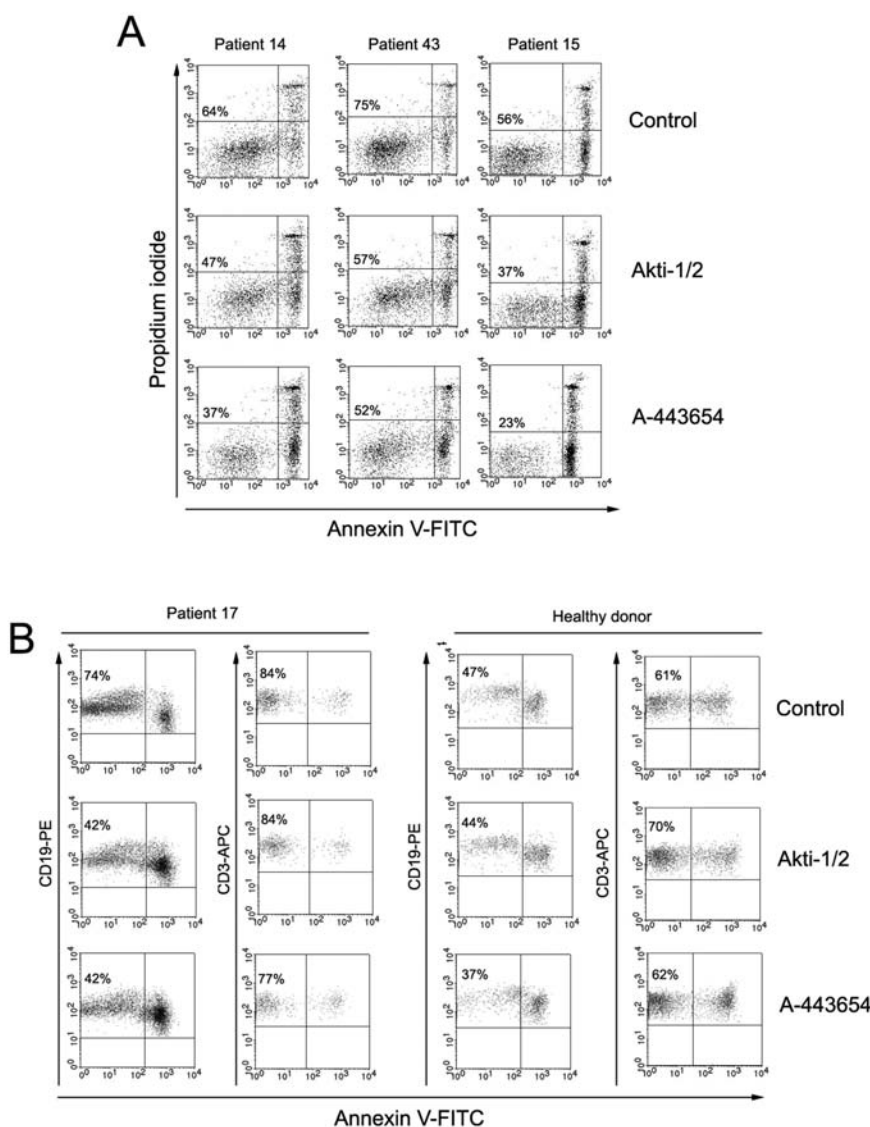


Akt inhibitors induce apoptosis in chronic lymphocytic leukemia cells

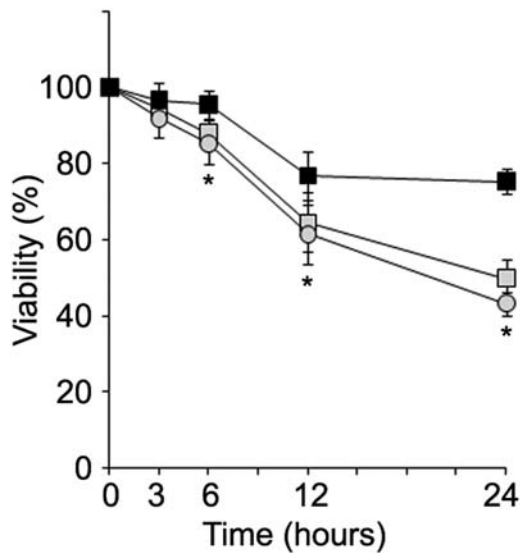
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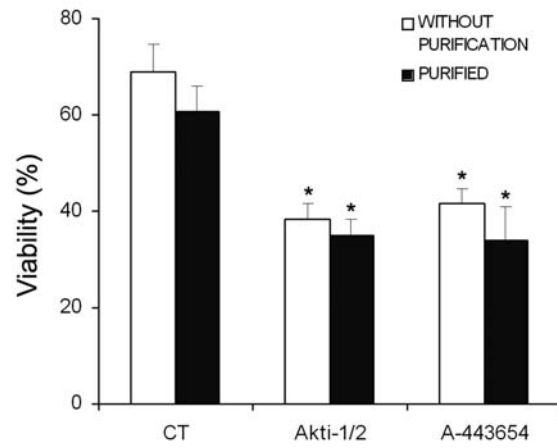
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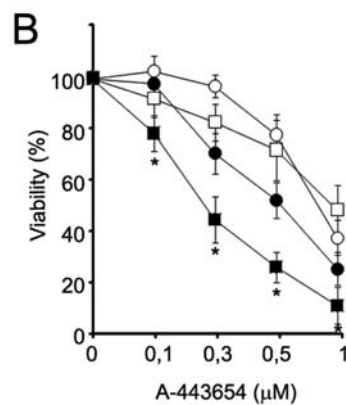
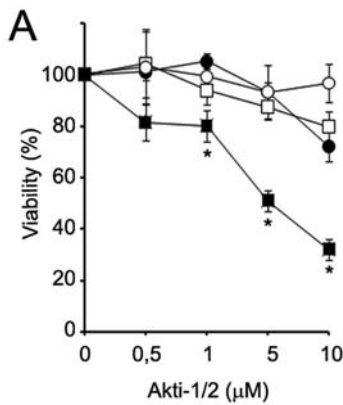
Online Supplementary Figure S1. Flow cytometry analysis of the cytotoxic effect of Akt inhibitors. (A) CLL cells were treated for 24 h with or without 5 μ M Akti-1/2 or 0.5 μ M A-443654. The cytotoxic effect was analyzed by performing an annexin V-fluorescein isothiocyanate (FITC)/propidium iodide double staining. The top rows show cells without treatment, the middle rows present cells treated with 5 μ M Akti-1/2 and the bottom rows show results of cells treated with 0.5 μ M A-443654. The percentage of non-apoptotic cells (lower-left quadrant) is determined and expressed inside each row. Results from three representative patients are shown. (B) CLL cells and peripheral blood lymphocytes from healthy donors were treated for 24 h with or without 5 μ M Akti-1/2 or 0.5 μ M A-443654. The differential cytotoxic effect on B and T cells was analyzed by performing a triple staining with CD3-allophycocyanin, CD19-phycoerythrin (PE) and annexin V-FITC. The top rows show cells without treatment, the middle rows show cells treated with 5 μ M Akti-1/2 and the bottom rows show results from cells treated with 0.5 μ M A-443654. The left rows represent cells stained with CD19/annexin V and the right rows represent cells stained with CD3/annexin V. The percentages of non-apoptotic cells (left-upper quadrant) are expressed inside each row. Results from one CLL sample and one healthy donor are shown.



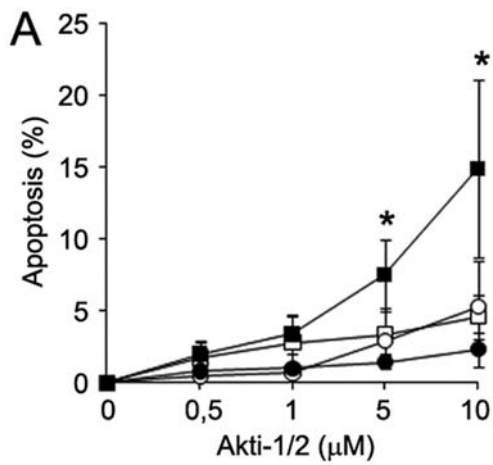
Online Supplementary Figure S2. Time-course of effect of Akt inhibitors on CLL cells. CLL cells were untreated (■) or treated with 5 μM Akti-1/2 (□) and 0.5 μM A-443654 (●) during 3, 6, 12 and 24 h, and cell viability was measured by analysis of phosphatidylserine exposure and propidium iodide uptake as described in the Design and Methods section. Viability is expressed as the percentage of the viability of untreated cells at 0 h (100%). Data are shown as the mean value ± SEM (n=6). * $p < 0.005$ treated versus untreated.



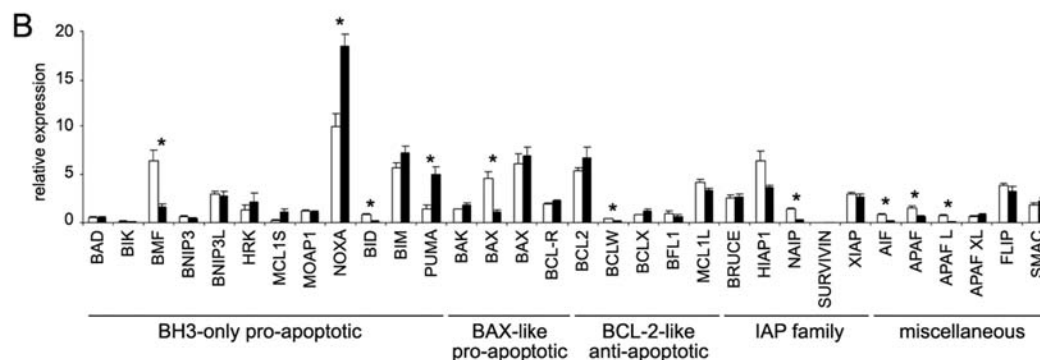
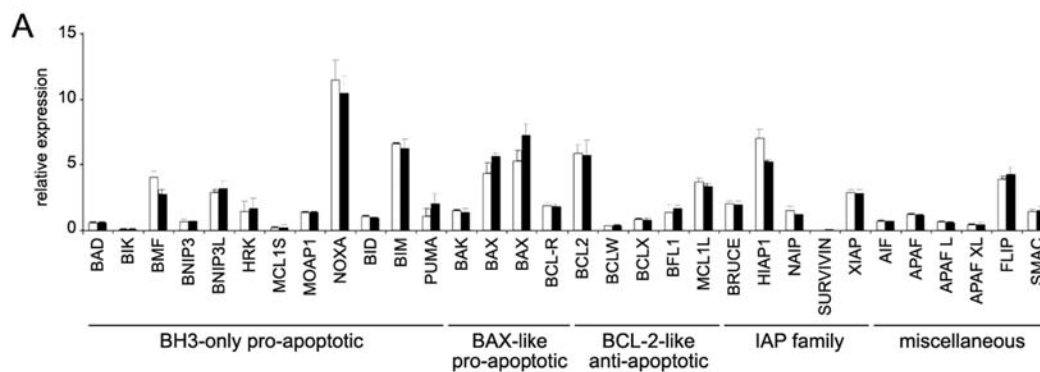
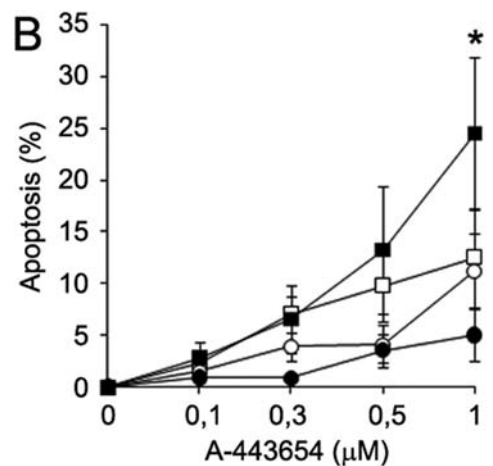
Online Supplementary Figure S3. Cytotoxic effect of Akt inhibitors on CLL cells before and after purification. Cells from the same CLL samples before (white filled bars) or after (black filled bars) the CD19⁺ isolation procedure were incubated for 24 h without (CT) or with 5 μM Akti-1/2 or 0.5 μM A-443654 (n = 3). Viability was measured by analysis of phosphatidylserine exposure and propidium iodide uptake as described in the Design and Methods section. Data are shown as the mean value ± SEM. * $p < 0.05$ treated versus untreated.



Online Supplementary Figure S4. Cytotoxic effect of Akt inhibitors on CLL cells and on peripheral blood lymphocytes (PBL) from healthy donors. Cells from CLL patients and healthy donors were incubated for 48 h with or without various doses of Akti-1/2 (A, n=9 and n=3, respectively) and A-443654 (B, n=11 and n=5, respectively). Viability was measured as non-apoptotic CD3⁺/CD19⁻ T cells from PBL (○) and CLL (●) or CD3⁻/CD19⁺ B cells from PBL (□) and CLL (■) as described in the Design and Methods section. Viability is expressed as the percentage of the viability of untreated cells. Data are shown as the mean value ± SEM. * $p < 0.005$, B cells versus T cells from patients with CLL.



Online Supplementary Figure S5. Apoptotic effect of Akt inhibitors on CLL cells and on peripheral blood lymphocytes (PBL) from healthy donors. Cells from CLL patients ($n=6$) and healthy donors ($n=4$) were incubated for 24 h with or without various doses of Akti-1/2 (A) and A-443654 (B). Apoptosis was measured as the quantification of the sub- G_0 peak of the $\text{CD}3^+$ T cells from PBL (\circ) and CLL (\bullet) or $\text{CD}19^+$ B cells from PBL (\square) and CLL (\blacksquare) as described in the Design and Methods section. Apoptosis is expressed as the increase in the sub- G_0 peak relative to untreated cells. Data are shown as the mean value \pm SEM. $*p<0.05$, B cells versus T cells from CLL.



Online Supplementary Figure S6. Apoptosis-related gene expression profile induced by Akti-1/2 and A-443654. Cells from CLL patients were untreated (open bars) or treated (black filled bars) with 5 μM Akti-1/2 (A) or 0.5 μM A-443654 (B) for 24 h. Cells were lysed, and the expression profile of apoptosis-related genes was analyzed by RT-MLPA as described in the Design and Methods section. The sum of all peak data was set at 100% to normalize for fluctuations in total signal between samples, and individual peaks were calculated relative to the 100% value. The results are shown as the mean value \pm SEM of four and eight different experiments for Akti-1/2 and A-443654, respectively. $*p<0.005$ treated versus untreated cells.

