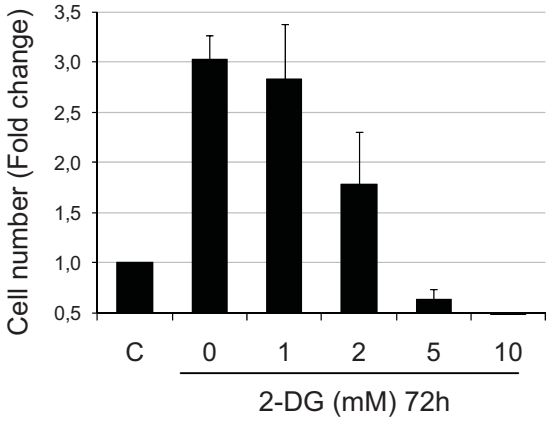
A

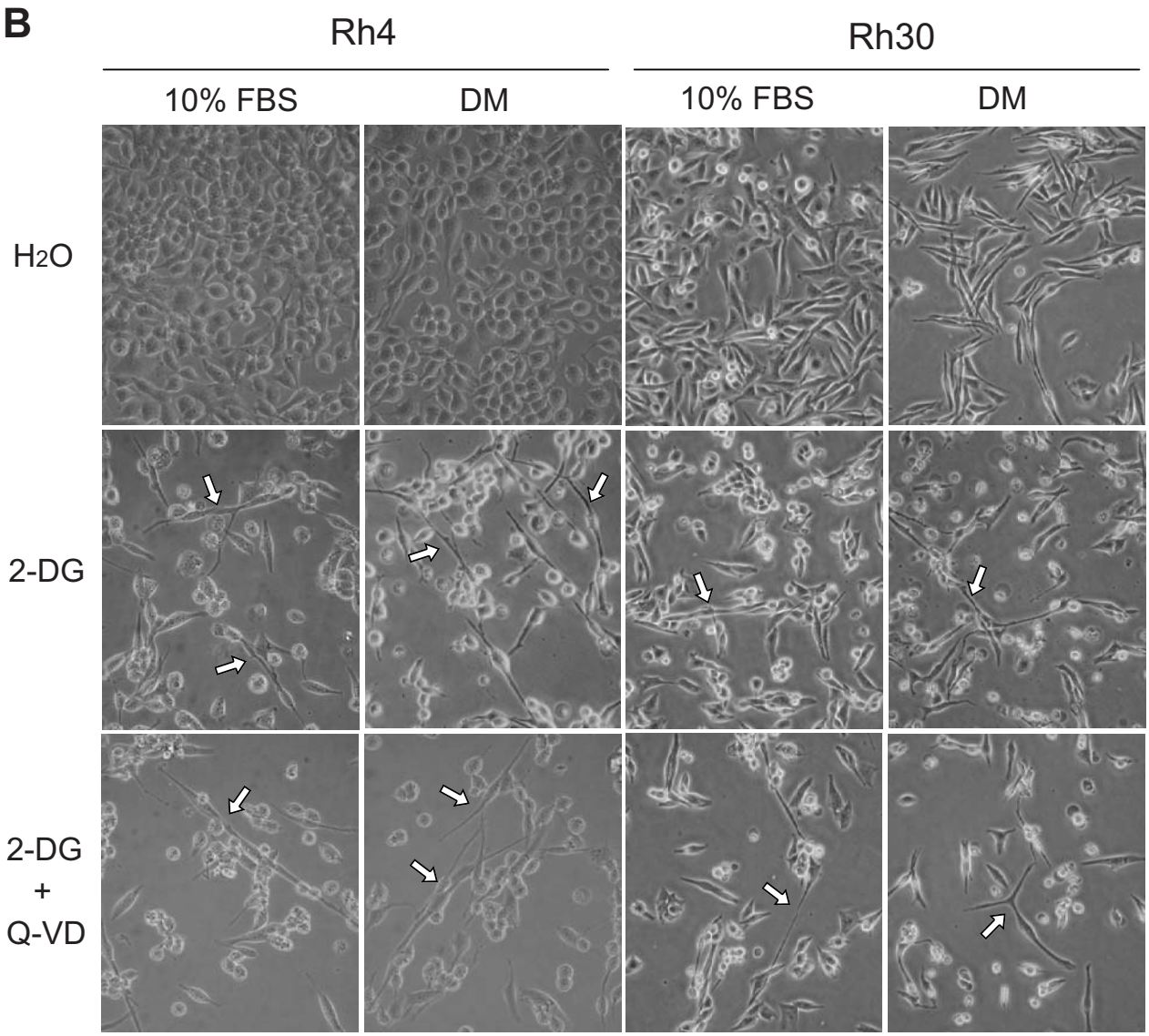


Supplementary Figure S1

2-deoxyglucose promotes growth arrest, cell death and differentiation of alveolar rhabdomyosarcoma.

A. Rh4 cells were plated at 20% confluence. 24h after plating, one well was stained with crystal violet (c, untreated control). The rest were stained after incubation for 72h in the absence or presence of 2-DG at indicated doses. Values are normalized to the value of the untreated control.

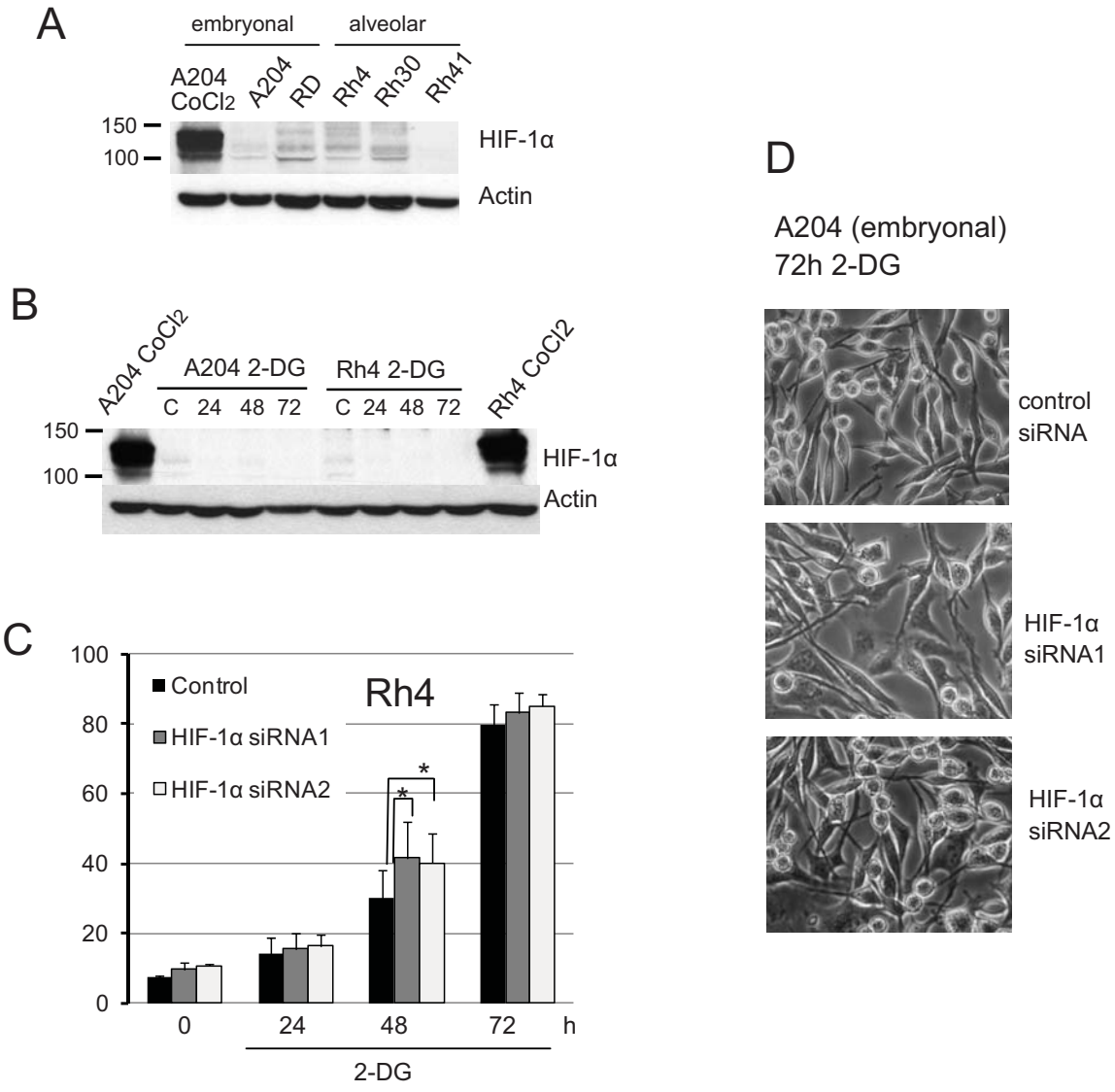
B



B. RH4 or RH30 cells were treated with 10mM 2-DG in the presence of 20μM Q-VD in complete media (RPMI + 10%FBS) or differentiation media (DM, serum-free RPMI) for 48h. Phase-contrast microscope pictures are shown. Arrows indicate fused cells with myotube morphology.

Supplementary Figure S2

HIF-1α expression does not explain differential sensitivity of alveolar and embryonal rhabdomyosarcoma cells to 2-deoxyglucose.



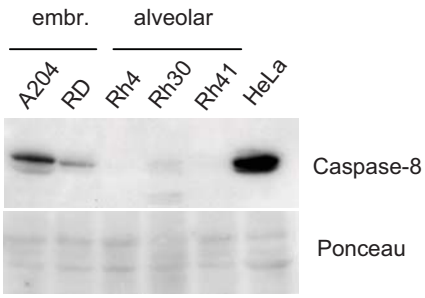
A. Indicated cell lines were analyzed for expression of HIF-1α. Positive control were A204 cells treated with CoCl2 200μM for 4hours

B. A204 and Rh4 were treated with CoCl2 200μM for 4hours or with 2-DG 10mM for indicated times and collected for analysis of HIF-1α expression by western blot.

C. Rh4 cells were transfected with control or HIF-1α siRNA . After 72h they were treated with 2-DG 10mM for indicated times and collected for subG1 analysis. Paired t-student test indicates that siRNA against HIF-1α produces a small although significant sensitization to 2-DG at 48h (p=0.049, n=4 experiments).

D. A204 cells are not sensitized to 2DG by pretreatment with siRNAs against HIF-1α. Same results were obtained by analysis of subG1 DNA content of cells pretreated with siRNAs for 48h or 72h (not shown).

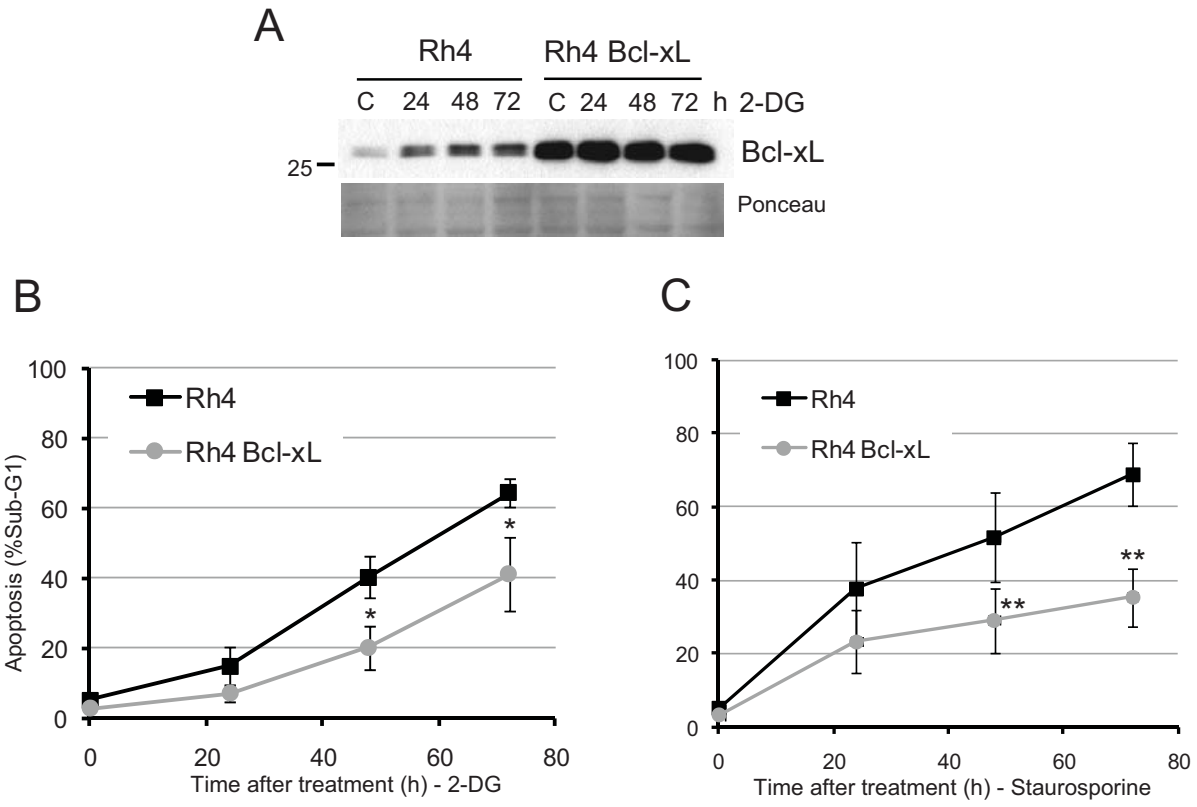
Suppl. Fig. S3



Rhabdomyosarcoma cell lines express low levels of caspase-8. Total cell extracts of embryonal (embr.) or alveolar rhabdomyosarcoma cell lines were analyzed for caspase-8 expression by western blot. HeLa cell lysate was used as a positive control for caspase-8 expression.

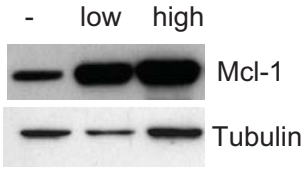
Suppl. Figure S4

Cells overexpressing Bcl-xL are protected from 2-deoxyglucose.

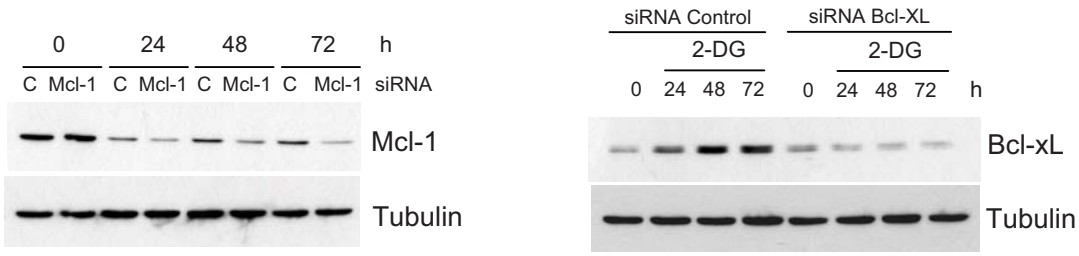


Cells from the parent cell line and Rh4 cells stably over-expressing Bcl-xL were subjected to western blot analysis (A) or cultured for the indicated times with 2-DG 10mM (B) or staurosporine 300nM (C) and collected for analysis of sub-G1 DNA content. Degree of protection from 2-DG is similar to protection from staurosporine. Results show average and SEM of 5 experiments.

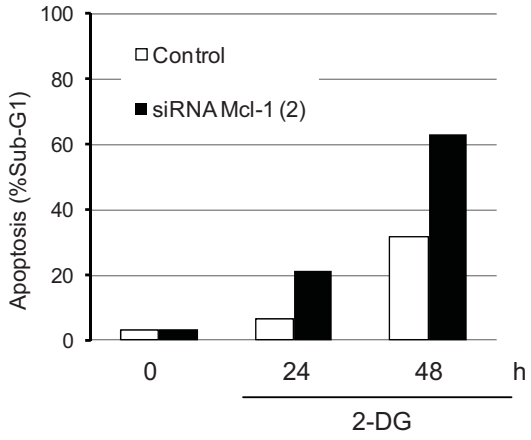
Suppl. Figure S5



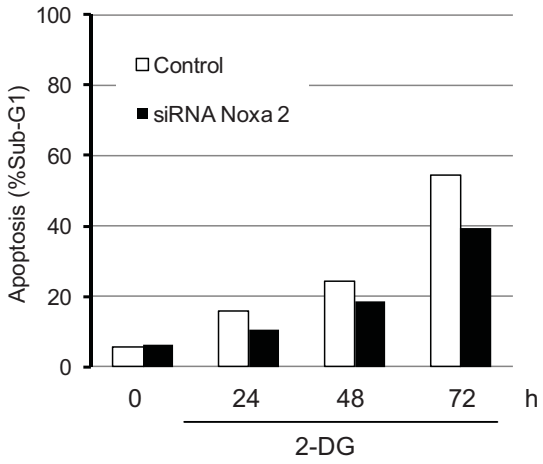
Western blot showing overexpression of Mcl-1 24h after transfection (see fig. 4A)



Western blot from samples analyzed in Figure 4C and 4D showing downregulation of Mcl-1 or Bcl-xL. "c", control siRNA.



Cells were treated like in Figure 4C but a different sequence to target Mcl-1 was used (5'-UCAAAAGAAACGCGGUAAU).



Cells were treated like in Figure 5C but a different sequence to target Noxa was used (5'-GCTACTCAACTCAGGAGATTT)

Suppl. Figure S6

Interactions between Bcl-2 family members.