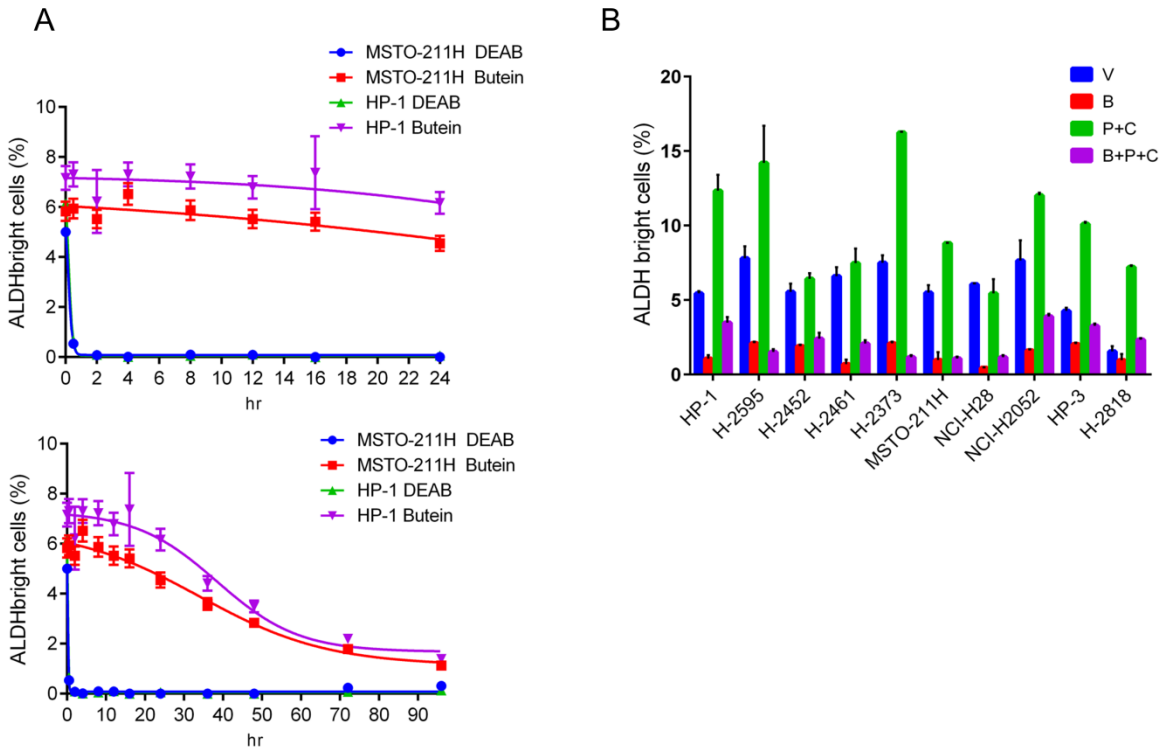
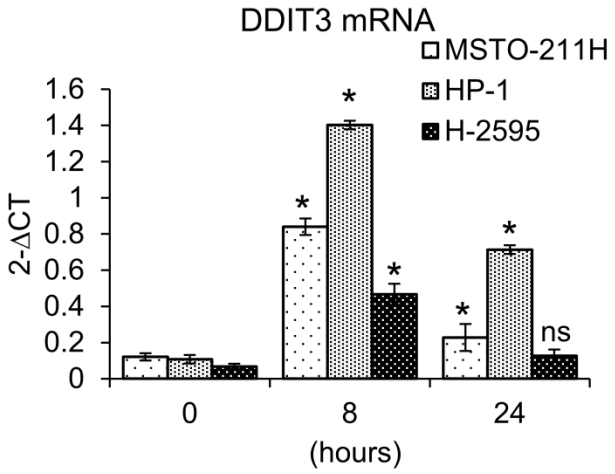
A STAT3-NFkB/DDIT3/CEBPβ axis modulates ALDH1A3 expression in chemoresistant cell subpopulations

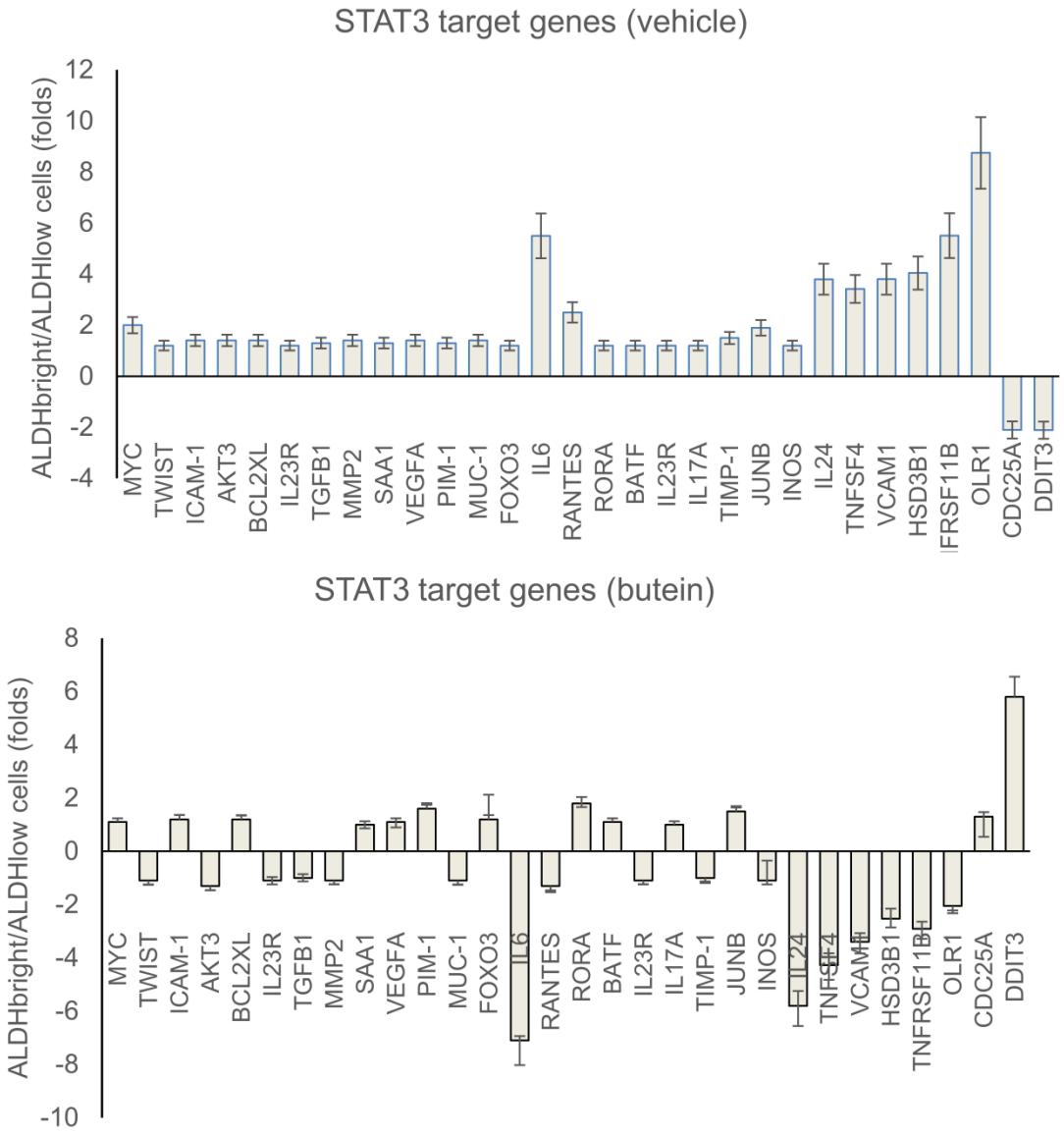
Supplementary Material



Suppl. Fig. 1: **Butein affects the ALDHbright cell number in MPM cell cultures**. **A.Butein does not inhibit the ALDH enzyme.** Graph showing the number of ALDH^{bright} cells in time (0-90h) after treatment with butein or DEAB (diethylaminobenzaldehyde, 5μM), a specific ALDH inhibitor. Average and s.e.m of triplicate experiments. **B.** Histograms show the percentage of ALDH^{bright} cells in the indicated MPM cell cultures treated for 24hrs with vehicle (V: DMSO 0.05%) and Butein (B: 18μM), alone or in combination with pemetrexed + cisplatin (P+C: 10 μM + 5 μM, respectively) and stained for ALDH activity at 96hrs. The percentage of ALDH^{bright} cells was determined over the same cells treated with a specific ALDH inhibitor (DEAB) immediately after adding the ALDH substrate (BAA). Average + s.e.m of triplicate experiments.



Suppl. Fig. 2: Butein modulates the DDIT3 mRNA levels. Quantitative PCR**.** DDIT3 mRNA levels of three representative MPM cell lines treated with butein (18μM) for the indicated times. Histogram bars represent the mean ± s.e.m of ≥ three experiments. Statistics: $p<0.05$. Statistics: * $p<0.05$; ns=not significant: ($p>0.05$). Student's t-test (comparing each sample to its control at 0hrs)



Suppl. Fig. 3: **Butein affects the levels of STAT3 target genes in ALDHbright and ALDHlow cells**. mRNA levels of multiple STAT3 target genes in MSTO-211H ALDH^{bright} vs ALDH^{low} cells, upon treatment with vehicle (upper) or butein (18μM) (lower) for 24hrs. Histogram bars show the average + s.e.m of duplicate experiments.