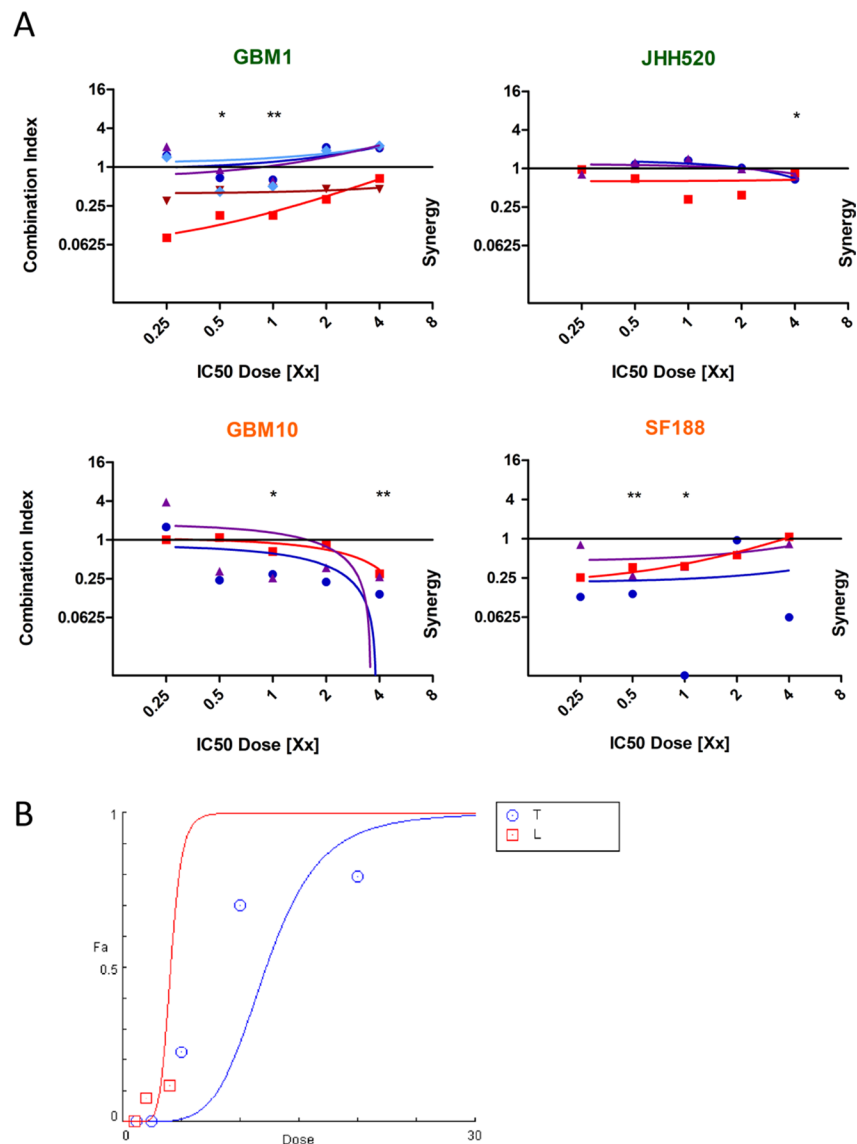
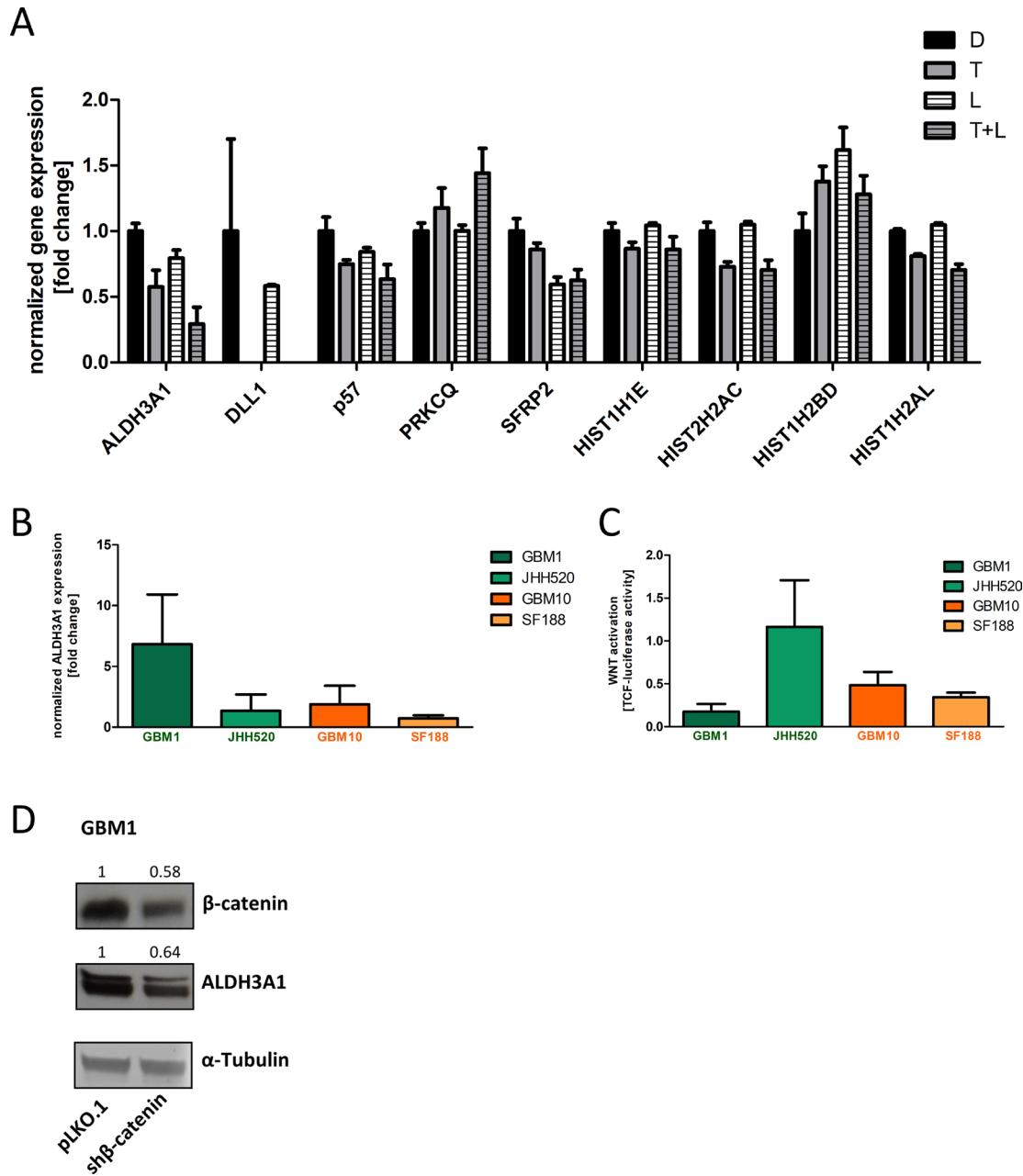


Inhibition of Wnt/beta-catenin signaling downregulates expression of aldehyde dehydrogenase isoform 3A1 (ALDH3A1) to reduce resistance against temozolomide in glioblastoma *in vitro*

SUPPLEMENTARY MATERIALS



Supplementary Figure 1: (A) LGK974 acts synergistically in combination with γ -irradiation. The x-axis represents different multiples of the detected IC_{50} dose for each treatment and cell line. The axis of ordinates demonstrates the calculated combination index. The combination index is calculated based on the median-effect equation, taking each value from γ -irradiation, LGK974 single treatment and combination of both treatments for one specific dose into account. In one experiment, the combination index is calculated for five different doses and represented by one colored line. If the combination index is less than 1, both treatments act synergistically. If it is equal 1, both treatments act additively. If the combination index is more than 1, the effects are antagonistic. Each color represents one independent experiment (GBM1 $n = 5$ independent experiments; JHH520, GBM10 and SF188 $n = 3$ independent experiments). MGMT methylated cell lines presented in green, MGMT unmethylated cell lines presented in orange. (B) Dose-effect curve of TMZ and LGK974 in GBM1. Effect (Fa) is represented on the axis of ordinates; dose is represented on the x-axis on linear scales. D: DMSO; T: TMZ; I: γ -irradiation; L: LGK974.



Supplementary Figure 2: (A) mRNA expression of 9 genes showing most pronounced differential expression in LGK974 and TMZ treated GBM1 cells as detected by microarray analysis. (B) Basal ALDH3A1 mRNA expression of all four cell lines. (C) Basal Wnt activation of all four cell lines assessed by T cell factor (TCF) luciferase reporters. (D) ALDH3A1 protein expression is reduced in GBM1 β -catenin-knock-down cells. pLKO.1 serves as the vector control. Data is presented as mean \pm standard deviation (SD). D: DMSO; T: TMZ; L: LGK974.

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Supplementary Figure 3: Genomic sequence on chromosome 17 (nucleotides 19753433-19748433) with binding sides for TCF in der *ALDH3A1* promoter marked in blue. The sequence was retrieved from NCBI GenBank.

Supplementary Table 1: Primer sequences used for qPCR analyses (A) and oligonucleotides used as RNA-targeting sequences for CRISPR/Cas9-mediated knock-down of ALDH3A1

A

Primer	fwd	rev
β2-microglobulin	GTTGCTCCACAGGTAGCTCTAG	ACAAGCTTTGAGTGCAAGAGATTG
ALDH3A1	TGTTCTCCAGCAACGACAAG	CTGACCTTCAGGCCTTCATC
P57	GCTGAACGCCGAGGACCAGAACCG	CGGGCACCGAGTCGCTGTCCACTT
DLL1	TGGCGCAGGCATCGA	GGCGGCTGATGAGTCTTTCT
PRKCQ	CTCCCTTATATGGCCCCTGG	CTTCTGCGATGCCACTGTAC
SFRP2	TTCCCCAAGCACACTCCTAG	TACAAGATTCGGGTGGGCTT
HIST1H1E	CCAAGAAGAGCGCCAAGAAG	CGCCTTGGGTTTAACTGCTT
HIST2H2AC	GTGGCAAACAAGGAGGCAAG	GTCTTCTTGTTGTCCTCCGAGC
HIST1H2BD	ACCGGCACCTTGATCTTGTA	GGCTGGGGAGTAAAGAGTGT
HIST1H2AL	GACAACAAGAAGACCCGCAT	CTCGGTCTTCTTGGGCAGTA
Sox2	TGGACAGTTACGCGCACAT	CGAGTAGGACATGCTGTAGGT
Nestin	GGCGCACCTCAAGATGTCC	CTTGGGGTCCTGAAAGCTG

B

Oligo	fwd	rev
Control	CACCGGGTGAACCGCATCGAGCTGA	AAACTCAGCTCGATGCGGTTACCC
crALDH3A1	CACCGTTCGACCATATCCTGTACAC	AAACGTGTACAGGATATGGTCGAAC
crALDH3A1 2nd	CACCGGGACACCCCATGATTACT	AAACAGTAATCAATGGGGGTGTCCC