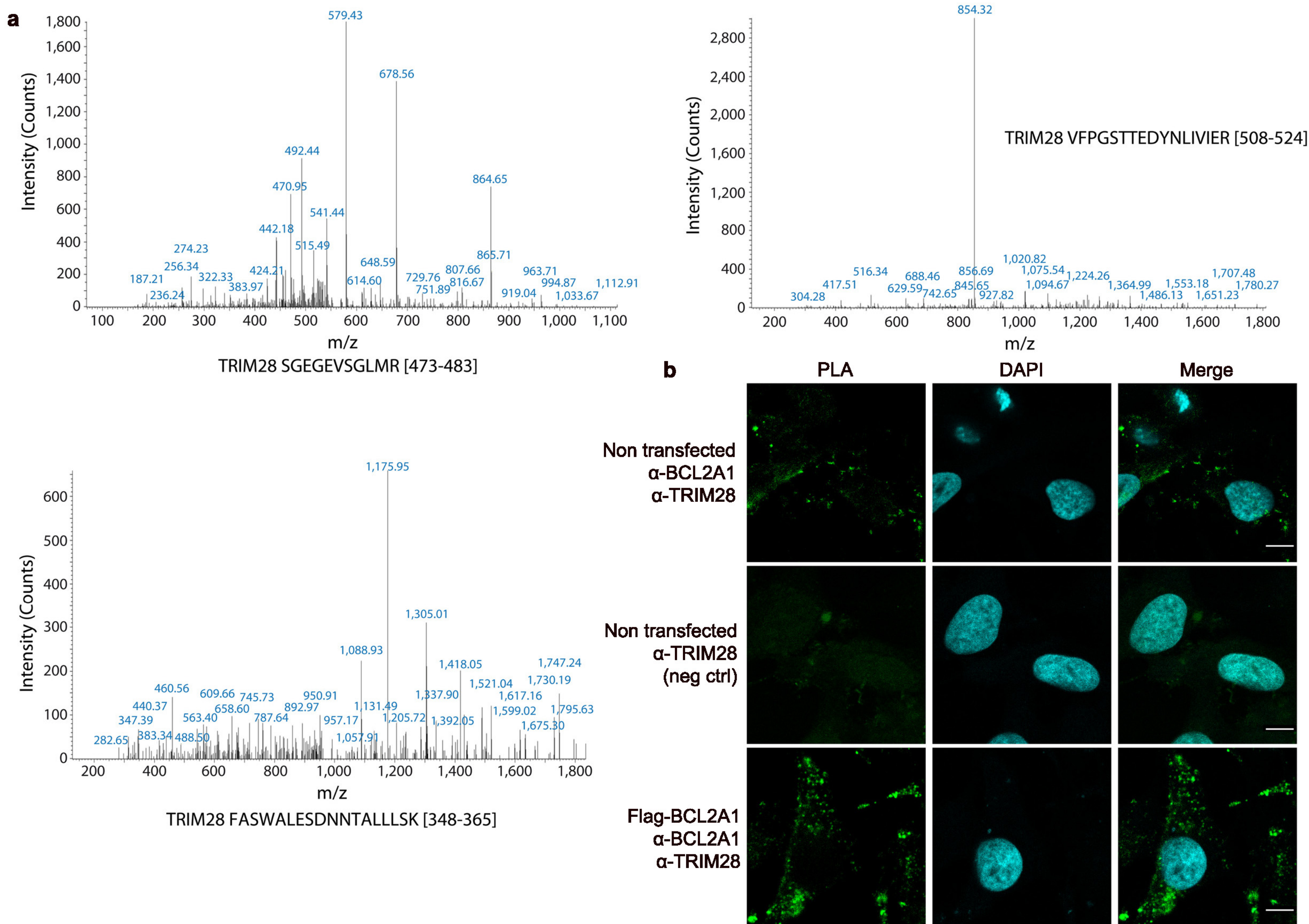


Figure S1: (a) Fragmentation spectrum of the three identified TRIM28 peptides. (b) In situ proximity ligation assay (PLA) was performed in SK-MEL-28 melanoma cells using anti-BCL2A1 and anti-TRIM28 antibodies. In one condition, Flag-BCL2A1 was ectopically expressed (third panel) which increased the PLA signal, validating the ability of the antibody to detect BCL2A1 and its interaction with TRIM28 in these conditions. Each green bright spot indicates the very close proximity of the two proteins. Negative control was obtained by omitting an antibody.

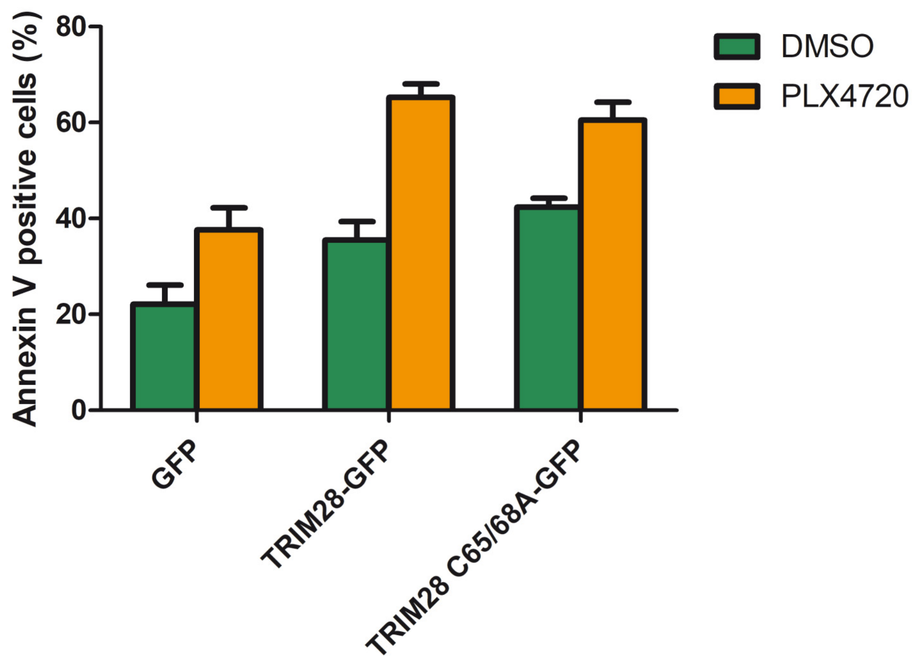


Figure S2: Alternative representation of the experiment depicted in Fig. 5e. SK-MEL-28 were transfected with GFP-tagged TRIM28 or TRIM28(C65A/C68A) for 24 h and subsequently treated with 20 µM PLX4720 for 24 h. Apoptosis was estimated in the GFP-positive cell population by flow cytometry using AnnexinV (APC) staining. Data are presented as % of annexin V positive cells and are the means ± SEM of four independent experiments.

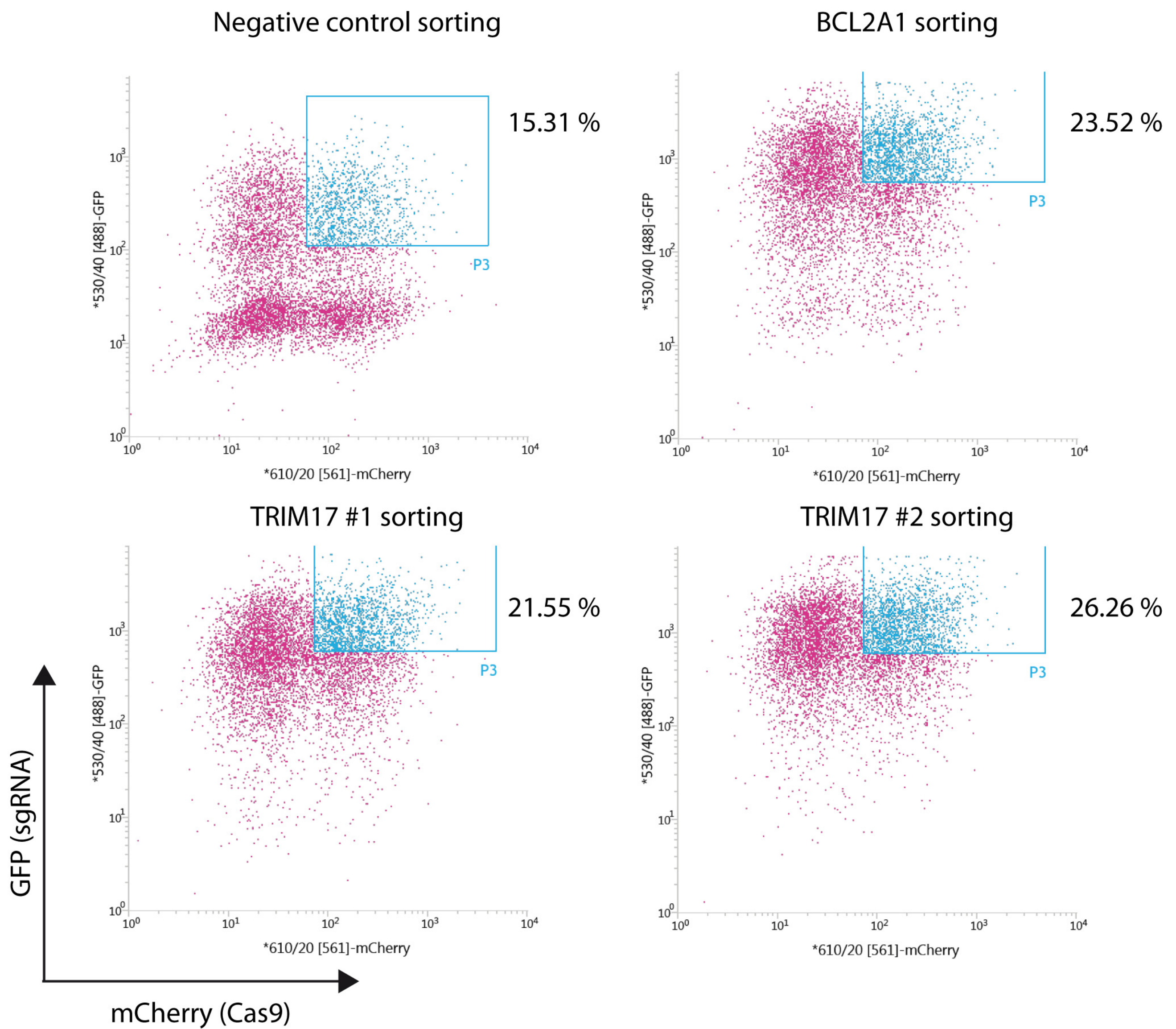


Figure S3: Representative examples of cell sorting of SK-MEL-28 cells expressing both Cas9 (reported by mCherry) and the indicated inducible sgRNA vectors (reported by constitutive eGFP expression) following double lentiviral transduction.

a

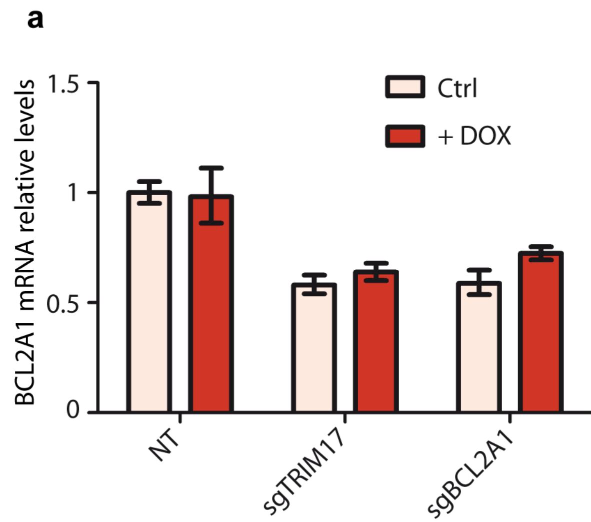
sgBCL2A1			# Reads
Ctrl sgRNA	No InDel	TCTGCAGTACGTCCTACAGATACCACA-ACCTGGATCAGGTCCAAGCAAAACGTCCAGAG	
sgBCL2A1	No InDel 6%	TCTGCAGTACGTCCTACAGATACCACA-ACCTGGATCAGGTCCAAGCAAAACGTCCAGAG	187
	InDel 94%	TCTGCAGTACGTCCTACAGATACCACAAACCTGGATCAGGTCCAAGCAAAACGTCCAGAG	996
		TCTGCAGTACGTCCTACAGATAC-------CTGGATCAGGTCCAAGCAAAACGTCCAGAG	876
		TCTGCAGTACGTCCTACAGATACCAC--ACCTGGATCAGGTCCAAGCAAAACGTCCAGAG	469
		TCTGCAGTACGTCCTACAGATACCAC----CTGGATCAGGTCCAAGCAAAACGTCCAGAG	340
		TCTGCAGTACGTCCTACAGATACCACATACCTGGATCAGGTCCAAGCAAAACGTCCAGAG	94
		TCTGCAGTACGTCCTACAG--------------------GTCCAAGCAAAACGTCCAGAG	91
		TCTGCAGTACGTCCTACAGATACCA---ACCTGGATCAGGTCCAAGCAAAACGTCCAGAG	86

sgTRIM17 #1			# Reads
Ctrl sgRNA	No InDel	GTCCCCGCAGAGGAACCTGCTGCCCAACC--GGCTGCTGACCAAGGTGGCCGAGATGGCGCA	
sgTRIM17 #1	No InDel 26%	GTCCCCGCAGAGGAACCTGCTGCCCAACC--GGCTGCTGACCAAGGTGGCCGAGATGGCGCA	1211
	InDel 74%	GTCCCCGCAGAGGAACCTGCTGA-----------------CCAAGGTGGCCGAGATGGCGCA	785
		GTCCCCGCAGAGGAACCTGCTGC-----------------CCAAGGTGGCCGAGATGGCGCA	554
		GTCCCCGCAGAGGAACCTGCTGC--------------TGACCAAGGTGGCCGAGATGGCGCA	528
		GTCCCCGCAGAGGAACCTGCTGCCCAACC-------------AAGGTGGCCGAGATGGCGCA	528
		GTCCCCGCAGAGGAACCTGCTGCCCAACCG-GGCTGCTGACCAAGGTGGCCGAGATGGCGCA	511
		GTCCCCGCAGAGGAACCTGCTGCCCAACCG---CTGCTGACCAAGGTGGCCGAGATGGCGCA	176
		GTCCCCGCAGAGGAACCTGCTGCCCAACC-----TGCTGACCAAGGTGGCCGAGATGGCGCA	165
		GTCCCCGCAGAGGAACCTGCTGCCCAACC--TGCTGCTGACCAAGGTGGCCGAGATGGCGCA	134
		GTCCCCGCAGAGGAACCTGCTGCCCAACCTTGGCTGCTGACCAAGGTGGCCGAGATGGCGCA	64

sgTRIM17 #2			# Reads
Ctrl sgRNA	No InDel	CATGGAGGCTGTGGAACTCGCCAGAAAAC-TGCAGGAGGAAGCTACGTGCTCCATCTGTCTG	
sgTRIM17 #2	No InDel 53%	CATGGAGGCTGTGGAACTCGCCAGAAAAC-TGCAGGAGGAAGCTACGTGCTCCATCTGTCTG	2159
	InDel 47%	CATGGAGGCTGTGGAACTCGCCAGGA-----------GGAAGCTACGTGCTCCATCTGTCTG	336
		CATGGAGGCTGTGGAACTCGCCAGAAAAC----AGGAGGAAGCTACGTGCTCCATCTGTCTG	328
		CATGGAGGCTGTGGAACTCGCCAGAAA--ATGCAGGAGGAAGCTACGTGCTCCATCTGTCTG	207
		CATGGAGGCTGTGGAACTCGCCAGAAAACCTGCAGGAGGAAGCTACGTGCTCCATCTGTCTG	159
		CATGGAGGCTGTGGAACTCGCCAGAAA-------GGAGGAAGCTACGTGCTCCATCTGTCTG	122
		CATGGAGGCTGTGGAACTC------------GCAGGAGGAAGCTACGTGCTCCATCTGTCTG	119
		CATGGAGGCTGTGGAACTCGCCAGAAA---TGCAGGAGGAAGCTACGTGCTCCATCTGTCTG	93
		CATGGAGGCTGTGGAACTC------------GCCAGAGGAAGCTACGTGCTCCATCTGTCTG	81
		CATGGAGGCTGTGGAACTCGCCAGAAA------AGGAGGAAGCTACGTGCTCCATCTGTCTG	77
		CATGGAGGCTGTGGAACTCGCCAGAAAACTTGCAGGAGGAAGCTACGTGCTCCATCTGTCTG	72
		CATGGAGGCTGTGGAACTCGCCAGAA-----------GGAAGCTACGTGCTCCATCTGTCTG	59
		CATGGAGGCTGTGGAACTCGCCAGAAAAC-------------CTACGTGCTCCATCTGTCTG	57
		CATGGAGGCTGTGGAACTCGCCAGAAA----------GGAAGCTACGTGCTCCATCTGTCTG	55
		CATGGAGGCTGTGGAACTCGCCAGAAAACATGCAGGAGGAAGCTACGTGCTCCATCTGTCTG	50
		CATGGAGGCTGTGGAACTCGCCAGAAA--CTGCAGGAGGAAGCTACGTGCTCCATCTGTCTG	46
		CATGGAGGCTGTGGAACTCGCCAGAA---------------GCTACGTGCTCCATCTGTCTG	44

b

Figure S4: Efficient InDel formation at the targeted loci by the inducible CRISPR/Cas9 system. (a) Genomic DNA from SK-MEL-28 cells expressing both Cas9 and the sgRNAs against TRIM17 or BCL2A1 was subjected to next-generation sequencing. The table shows sequencing reads with percentage of InDels detected in doxycycline treated cells. The negative control is a sgRNA targeting the mouse Bim gene. InDels are highlighted in yellow, PAM sequence in red. These data are representative of four independent experiments. (b) Percentage InDel reads from four independent experiments with and without doxycycline (DOX) treatment (72h). Data are mean ± SD.



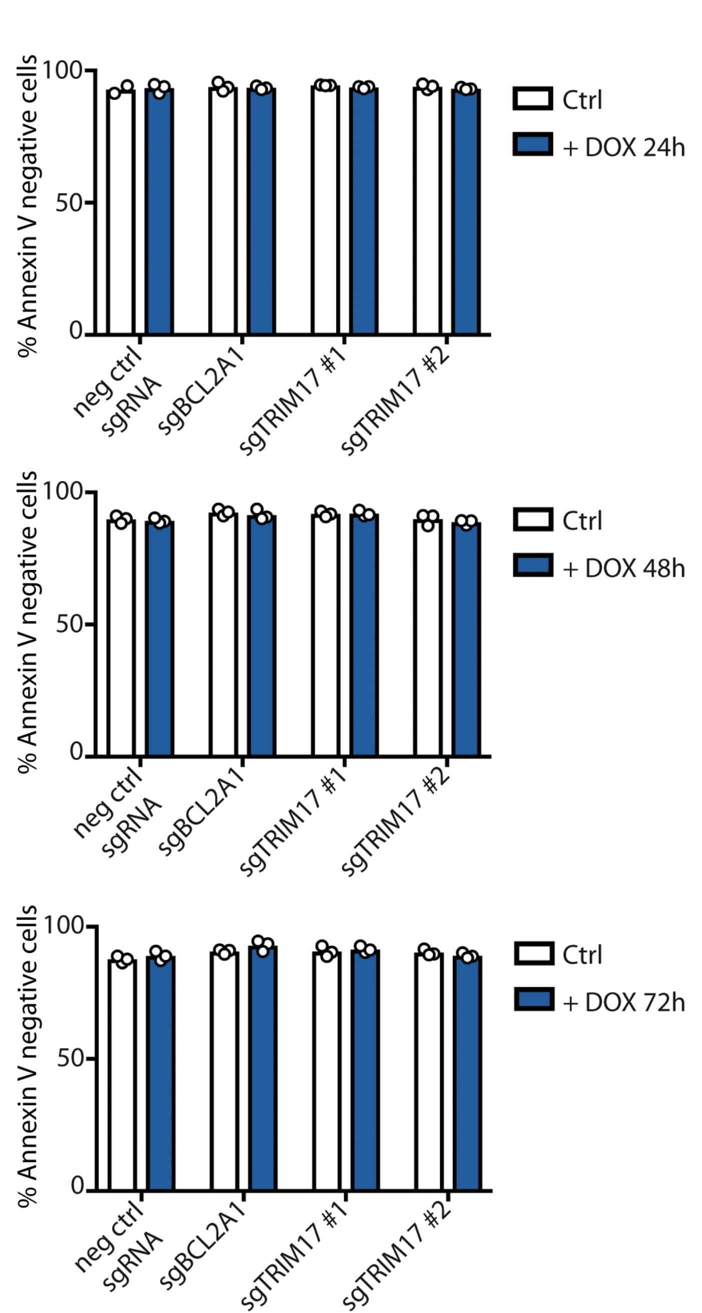


Figure S5: Doxycycline treatment per se has no impact neither on BCL2A1 expression nor on cell viability in SK-MEL-28 cells expressing dox-inducible sgRNAs. (a) Non transduced SK-MEL-28 cells (NT), or SK-MEL-28 cells expressing dox-inducible sgTRIM17#2 or sgBCL2A1 along with constitutive Cas9, were treated or not with 1 µg/ml doxycycline for 72 h. Total RNAs were extracted and the mRNA levels of BCL2A1 were estimated by quantitative RT-PCR in the different conditions. (b) SK-MEL-28 cells expressing dox-inducible negative control sgRNA, sgTRIM17#1, sgTRIM17#2 or sgBCL2A1 along with Cas9, were treated with 1 µg/ml doxycycline for 24 h, 48 h or 72 h as indicated. Apoptosis was quantified by flow cytometry using annexin V staining. The data of three independent experiments are presented as the percentage of annexin V negative cells.

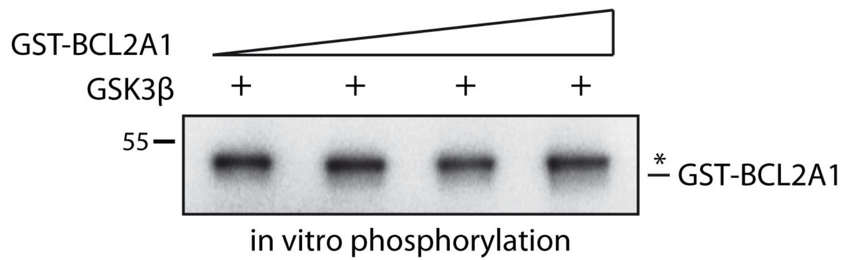


Figure S6: GSK3 does not phosphorylate full-length GST-BCL2A1 in vitro in the absence of BH3 peptide. Purified recombinant full-length GST-BCL2A1 protein was incubated with GSK3β recombinant protein kinase in the presence of [γ-32P]-ATP. The protein mix was resolved by SDS-PAGE and gel was then analyzed by autoradiography. As GSK3 autophosphorylation (indicated by *) and expected GST-BCL2A1 phosphorylation signals have similar molecular size, increasing amounts of GST-BCL2A1 were incubated to detect a potential increased BCL2A1 phosphorylation paralleling the amount of GST-BCL2A1 introduced in the reaction.