Supplementary Information

Phenotyping of cancer-associated somatic mutations in the BCL2 transmembrane domain

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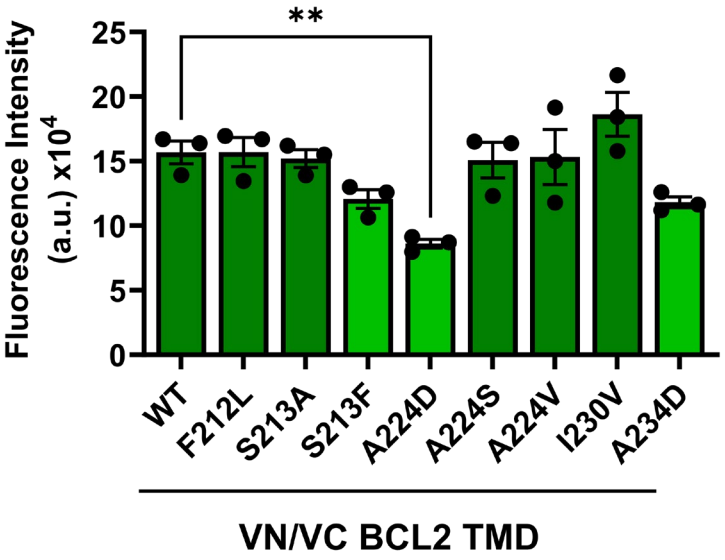
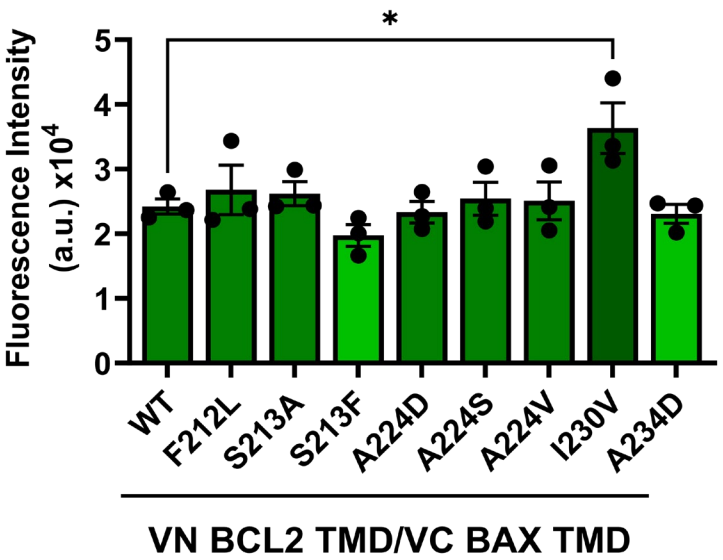
Supplementary Table I. Data of the patient samples where BCL-2 TMD mutations were found.

MUTANT	F212L	S213A	S213F	A224D	A224S	A224V	I230V	A234D
Attribute	Value	Value	Value		Value	Value	Value	Value
Number of Samples Per Patient	1	1	1				1	1
Overall Survival (Months)	16.1	3.1	10.1				21.7	
Overall Survival Status	0:LIVING	1:DECEASED	0:LIVING			0:LIVING	0:LIVING	1:DECEASED
Patient's Vital Status	ALIVE	DECEASED	ALIVE		10 years free survival		ALIVE	DECEASED
Sex	Male	Male	Male	Male	Male	Female	Female	Female
Smoking History	Prev/Curr Smoker	Prev/Curr Smoker	Prev/Curr Smoker		Heavy Smoker		Prev/Curr Smoker	Never
Attribute	P-0004865-T01-IM5	P-0006499-T01-IM5	P-0009189-T01-IM5		LUAD-S01405	SP82900	P-0002957-T01-IM3	P-0002136-T01-IM3
Mutation Count	146	12	67		301	1246	20	7
Fraction Genome Altered	0.0000	0.1660	0.0215				0.6923	0.5933
Cancer Type	Colorectal Cancer	Mature B-Cell Neoplasms	Melanoma	Liver neoplasm	Non-Small Cell Lung Cancer stage IIA	Melanoma	Non-Small Cell Lung Cancer	Ovarian Cancer
Cancer Type Detailed	Colon Adenocarcinoma	Diffuse Large B-Cell Lymphoma, NOS	Cutaneous Melanoma		Lung Adenocarcinoma	Melanoma malignant	Lung Adenocarcinoma	Mixed Ovarian Carcinoma
DNA Input	250	250	160.9				250	250
Matched Status	Matched	Unmatched	Matched		Matched		Matched	Matched
Oncotree Code	COAD	DLBCLNOS	SKCM		LUAD	MEL	LUAD	MXOV
Primary Tumor Site	Ascending Colon	Lymph node	Skin			SKIN	Lung	Ovary
Sample Class	Tumor	Tumor	Tumor			Tumor/Metastasis	Tumor	Tumor
Sample Collection Source	In-House	In-House	In-House	COSS2634306	CLCGP / MPI	SA432539	Outside (metastasis site lymph node)	Outside
Sample coverage	838	1037	254				349	402
Sample Type	Primary	Primary	Primary	tumor			Metastasis	Primary
Somatic Status	Matched	Unmatched	Matched		Matched		Matched	Matched
Specimen Preservation Type	DNA	FFPE	FFPE				FFPE	FFPE
Specimen Type	Resection	Biopsy	Biopsy				Biopsy	Resection
Tumor Purity	50	60	80				30	60

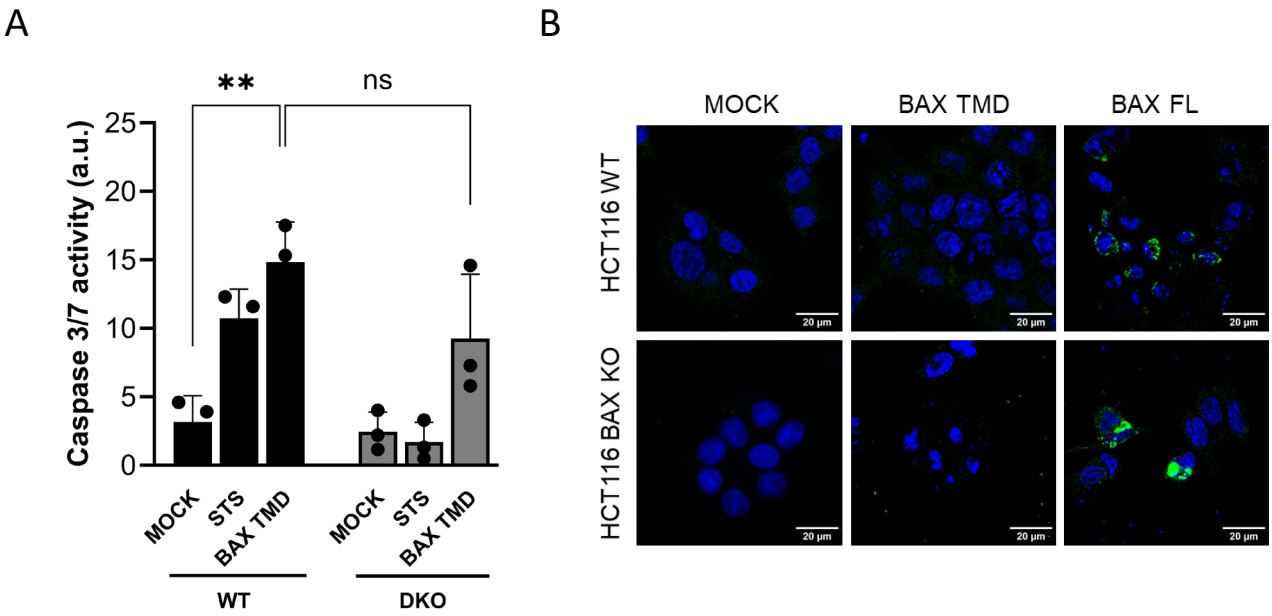
A



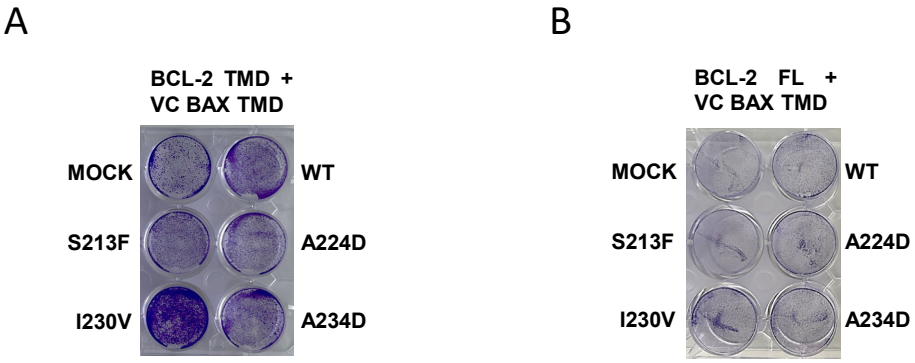
B



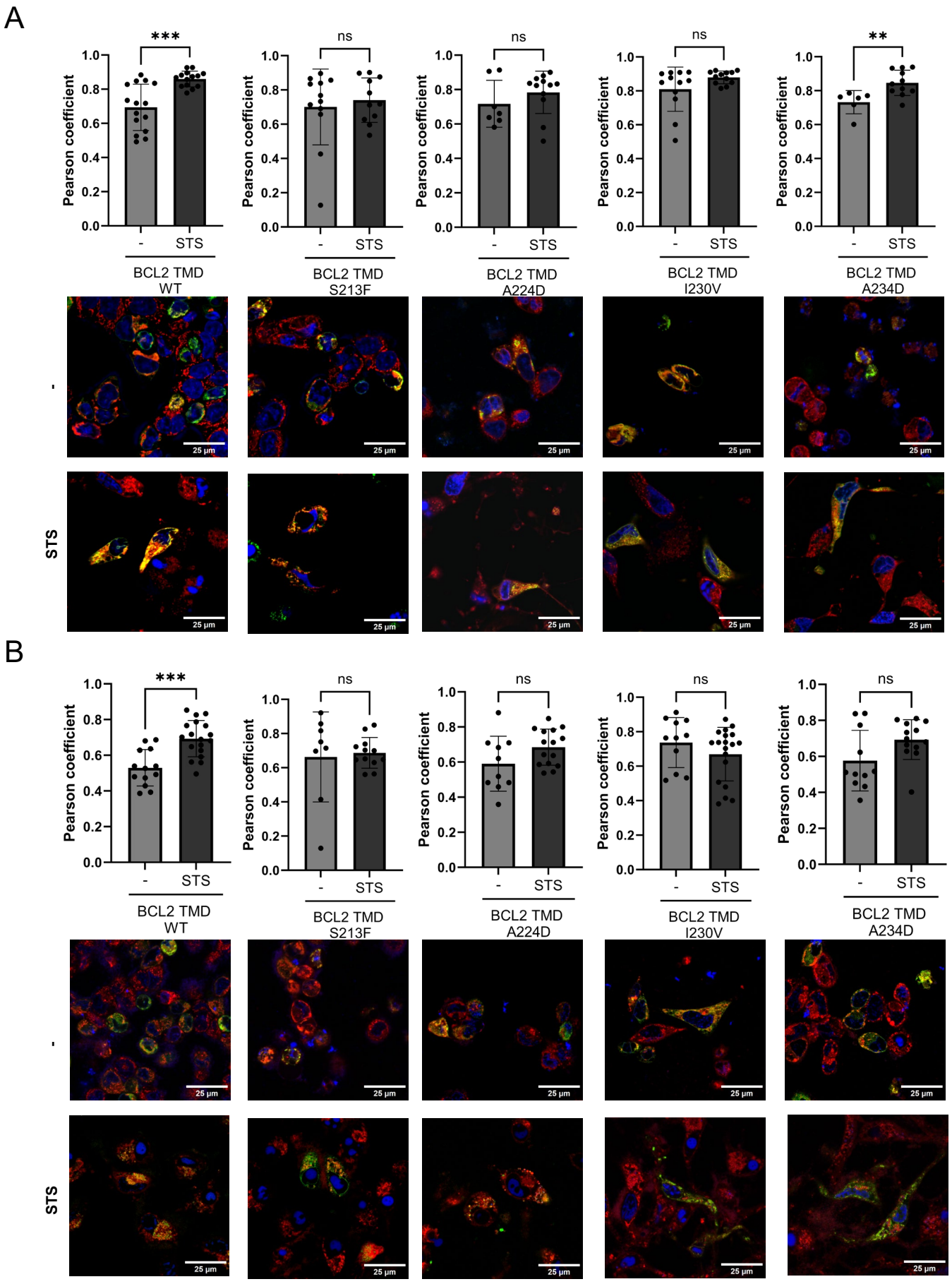
Supp Figure 1. Mutations affecting the BCL2 TMD interfere with oligomerization in HeLa cells. **A** Single amino acid mutations impacting the BCL2 TMD disrupt homo-oligomerization. BiFC signal measured in HeLa cells expressing wild-type (WT) or mutant BCL2 TMDs. **B** Single amino acid mutations disrupt BCL2/BAX TMD oligomerization. Interactions between BCL2 TMD mutants and BAX TMD were analyzed using the BiFC system. Data represented as mean ± SEM of at least n = 3. Significant differences compared to the WT BCL2 TMD analyzed using Dunnett's multiple comparison test (*p<0.05, **p<0.01).



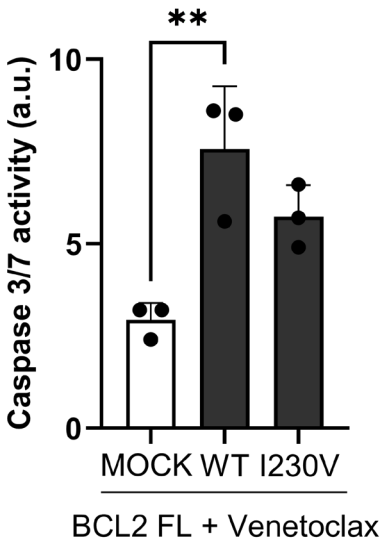
Supp Figure 2. BAX TMD induces apoptosis in WT and BAX/BAK DKO HCT116 cells. A Cell death measured by caspase 3/7 activity in HCT116 cells transfected with BAX TMD constructs in WT and BAX-/-BAK-/- cells (DKO). Cells treated with staurosporine (STS) 1 µM were included as positive control of apoptosis induction. MOCK refers to empty vector. Data represented as mean ± SEM of n = 3. Significant differences compared to the MOCK or BAX TMD analyzed using Šídák's multiple comparison tests (**p<0.01). **B** The transgenic BAX TMD do not displace the endogenous BAX protein to induce activation. Immunofluorescence of active BAX (clone 6A7; green signal) in HCT116 WT and BAX KO, overexpressing either BAX TMD or BAX FL, (MOCK = empty vector). Blue signal corresponds to nuclei, scale bar is 25 µm.



Supp Figure 3. Representative images of crystal violet staining in cell viability studies showing that some single amino acid mutations in the BCL2 TMD impair BCL2 apoptotic function. A HCT116 cells were transfected with BCL2 TMD mutants and BAX TMD. After 48 h, cells were fixed and stained with crystal violet. **B** HCT116 cells transfected with BCL2 FL mutants and BAX TMD and stained with crystal violet following the same procedure as A.



Supp Figure 4. Characterization of subcellular distribution of BCL2 TMDs in healthy and apoptotic cells. Representation of Pearson correlation coefficients between the BiFC BCL2 TMD constructs (green) and mitotracker (red) **(A)** or ER-tracker (red) **(B)** in healthy (-) and staurosporine (STS; 1µM) HCT116 treated cells. Representative confocal images are depicted below the graphs. Cell nuclei are stained with Hoescht (blue). Data represented as mean ± SEM of at least n = 3. Significant differences compared to WT BCL2 TMD analyzed using Dunnett's multiple comparison test (**p<0.01, ***p<0.001). Scale bar is 25 µm.



Supp Figure 5. Cell death induced by Venetoclax in cells overexpressing BCL2 FL I230V is lower than in WT. Caspase-3/7 activity measured in HCT116 cells transfected with BCL2 FL WT or I230V and treated with 25 µM Venetoclax for 24 h. Data represented as mean ± SEM (n = 3, **p<0.01). MOCK refers to cells transfected with pCDNA3.1 empty plasmid.