

Supplementary Material

Western Blotting

Cells were grown to ~50-70% confluence in T75 flasks in standard growth medium (DMEM/F12) with 10% fetal bovine serum (FBS) and 1% antibiotic-antimycotic. Cells were then trypsinized, pelleted by centrifugation (2,000 rpm, 4°C, 5min) and lysed in NETN buffer (150 mM NaCl, 1 mM EDTA, 20 mM pH 8.0 Tris, 0.5% NP-40) supplemented with protease and phosphatase inhibitors. Whole cell lysates were collected, and protein concentrations were determined by BCA assay. Equal amounts of protein (30 µg per sample) were separated using SurePAGE precast polyacrylamide gels and transferred to PVDF membranes. Membranes were blocked for 1 hr at room temperature in 5% non-fat milk prepared in 1xTBST, then incubated overnight at 4°C with primary antibodies against the BCL2 protein family (1:1000) or vinculin (1:2000). After washing four times in 1xTBST, membranes were incubated with HRP conjugated secondary antibodies (1:2000) for 1 hr at room temperature. Protein bands were visualized using enhanced chemiluminescence and imaged with an Odyssey XF Imager.

Reagent/Anitbodies/Equipment	Source	Catalog Number
Antibiotic-antimycotic	Gibco	15240-062
Anti-mouse IgG HRP	Sigma	A4416
Anti-rabbit IgG HRP	Cell Signaling	7074V
MCL1	Cell Signaling	39224S
BCL2 (D5V5L)	Agilent	M088729-2
BCL-XL (54H6)	Cell Signaling	2764S
BCLW (31H4)	Cell Signaling	2724S
BAX (D3R2M)	Cell Signaling	14796S
BFL-1 (E3U2F)	Cell Signaling	4093S
DMEM/F12	JangoCell	104020
FBS	R&D Systems	S11550H
NaCl	ChemCruz	Sc-295833
Nonidet P-40 (same as IGEPAL CA-630)	Sigma	I8896
Phosphatase inhibitors	Roche	4906837001
Protease inhibitors	Roche	45-11836170001
SurePAGE gels	GenScript	M00661 or M00653
Vinculin (EPR8185)	Abcam	129002
Odyssey XF Imager	LICORbio	N/A

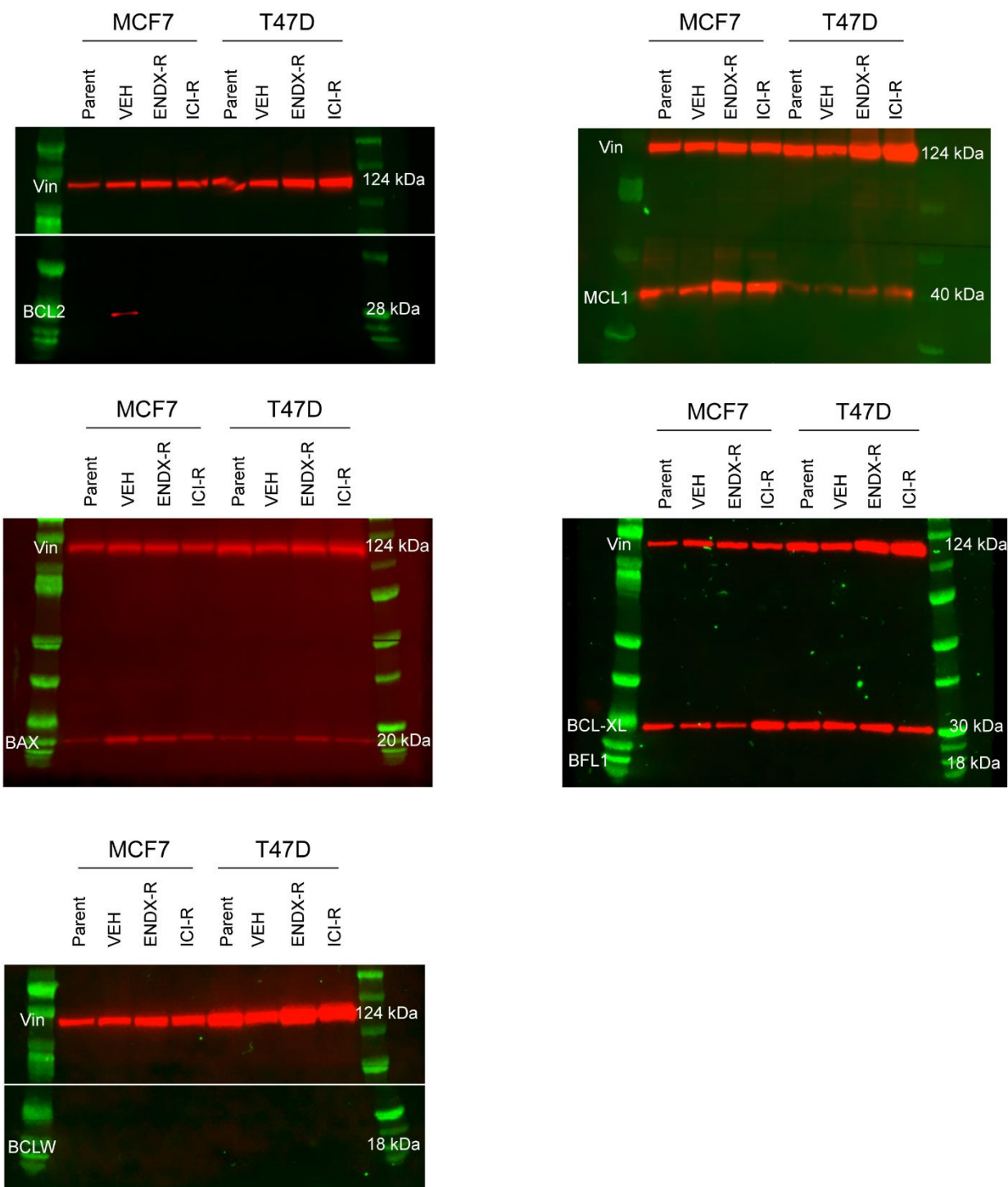


Fig. S1. Western blot analysis of BCL2 family members in endocrine therapy sensitive and resistant breast cancer cell lines. BCL2 family protein expression was assessed in MCF7 and T47D parental cells, vehicle (VEH) control cells, endoxifen-resistant cells (ENDX-R), and fulvestrant-resistant cells (ICI-R). Vinculin was used as a loading control.