

Fig. S1

Differentiation Day 0

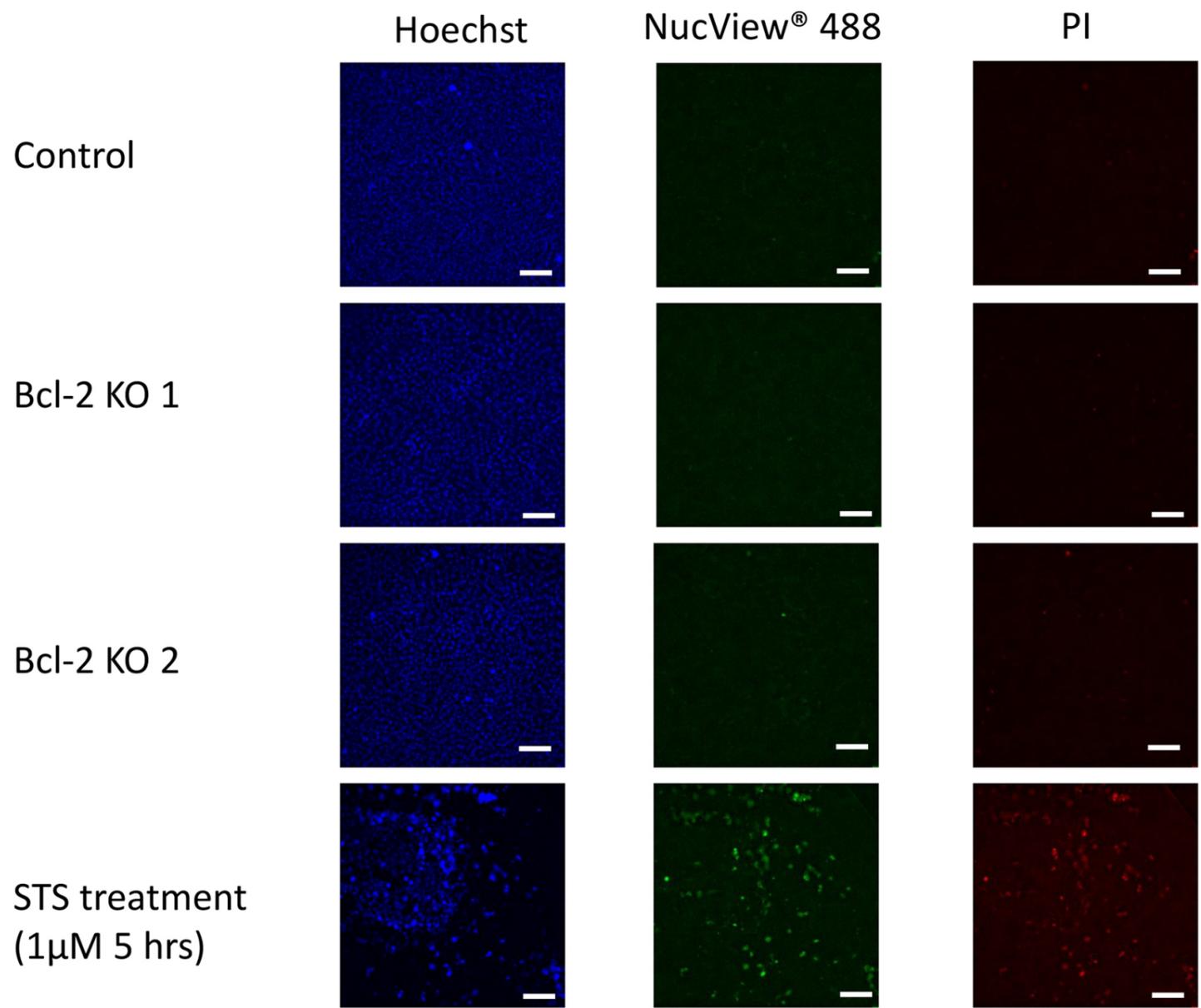


Fig. S2
Differentiation Day 7

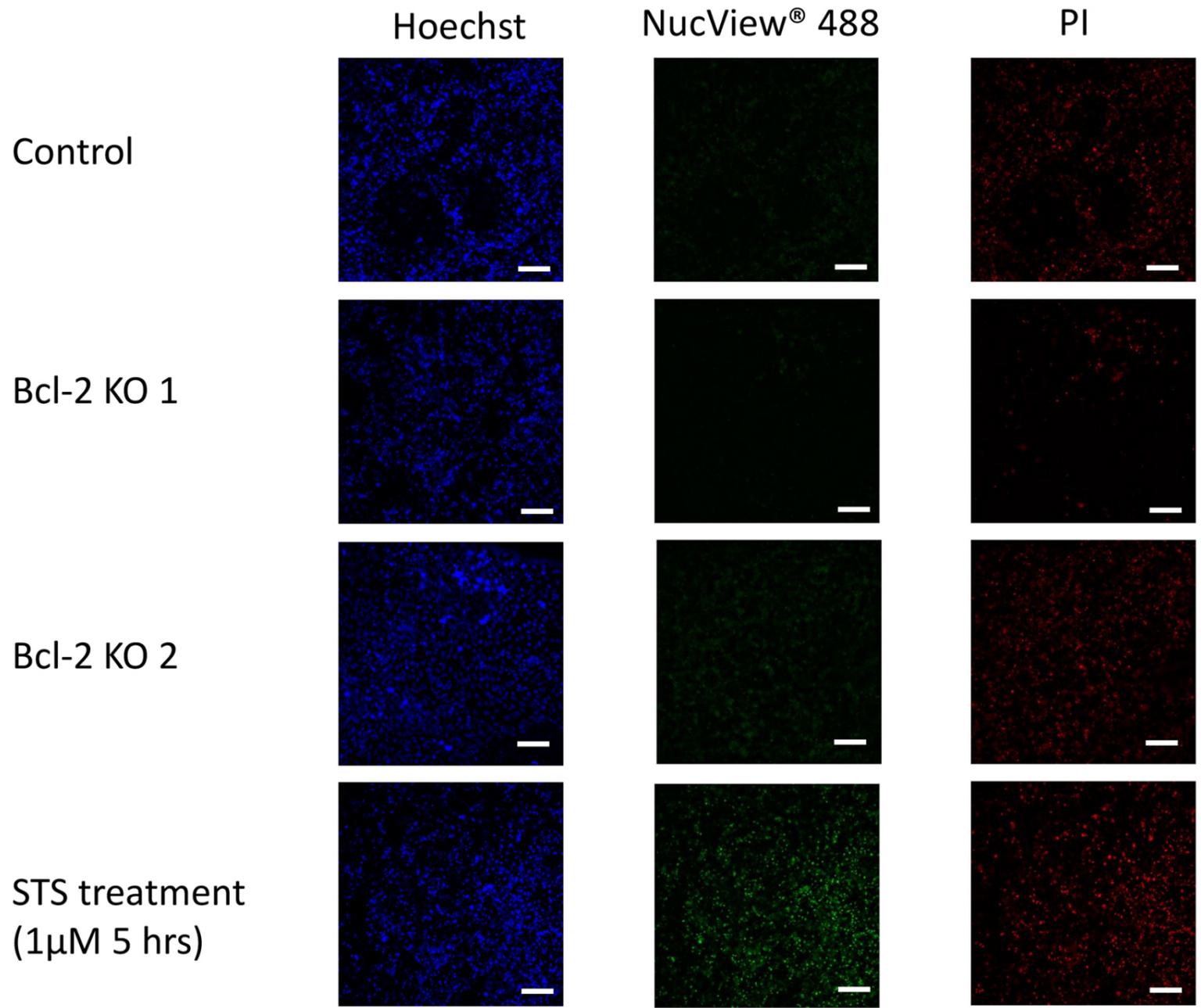


Fig. S3

Differentiation Day 14

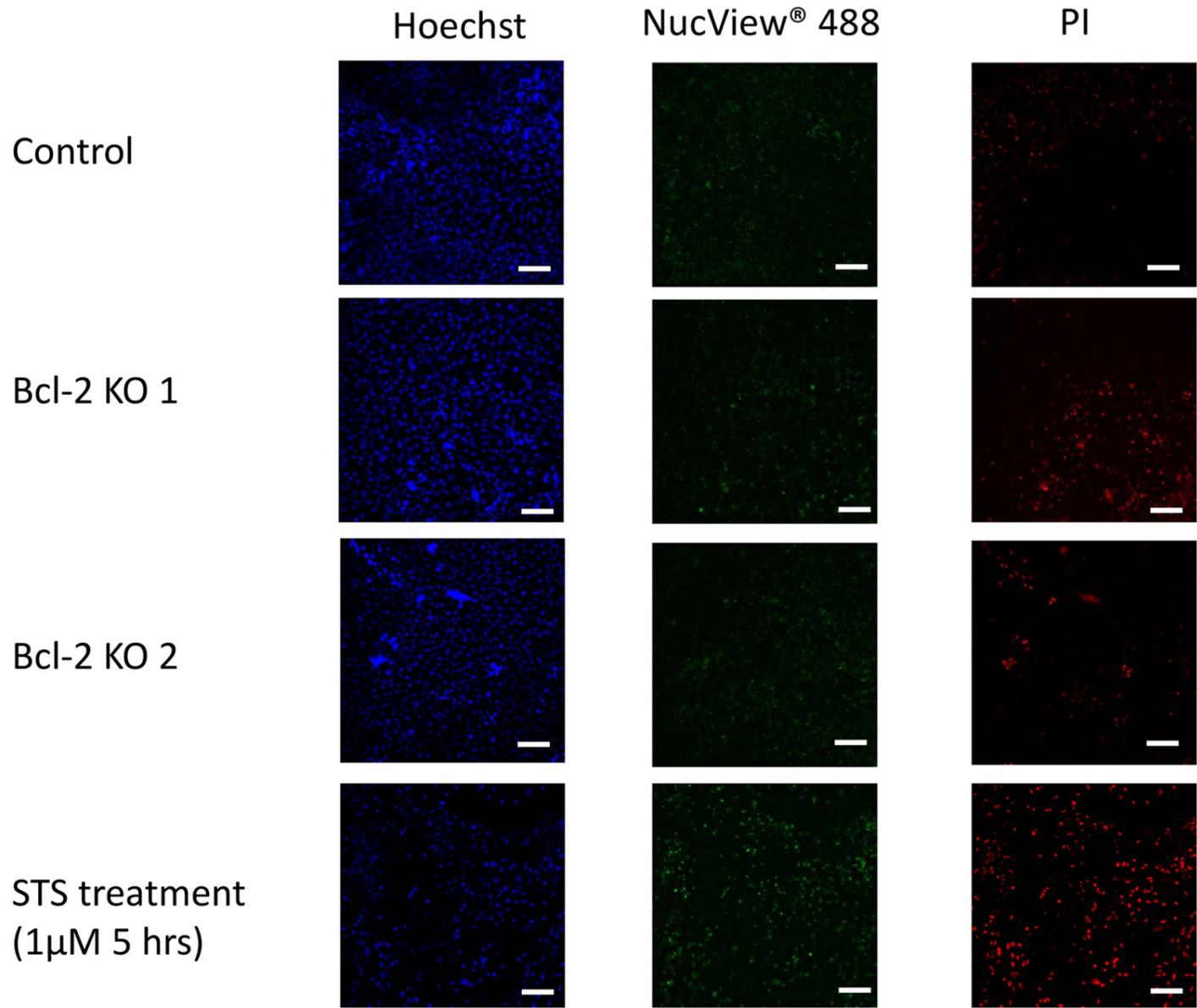
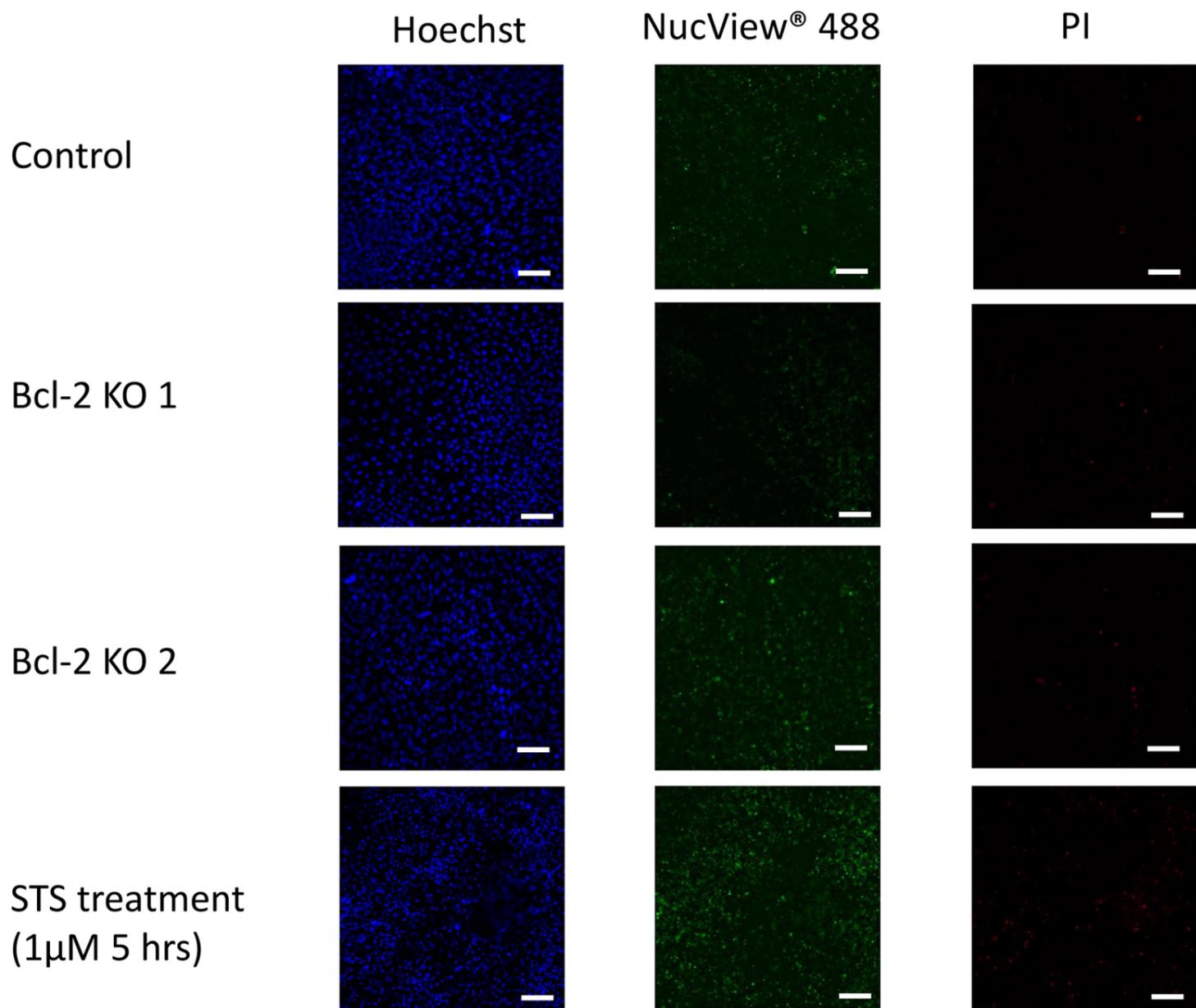


Fig. S4

Differentiation Day 21



Figs. S1-S4. Cell death induction is not altered upon KO of Bcl-2. Typical images taken on differentiation day 0, 7, 14 and 21 (supplemental figure 1, 2, 3, 4 respectively) of control and Bcl-2 KO conditions. Cells were stained 15 min prior to image acquisition with a cell permeable Hoechst stain, NucView®488 and propidium iodide (PI) to monitor total nucleus count, caspase 3 activity and cell integrity respectively. Each experimental day a staurosporine treatment (1 μ M for 5 hours) is taken with as positive control for the different dyes. The white scale bar corresponds to 100 μ M. Quantification of these experiment can be found in figure 2B in the main manuscript file.

Table S1. Antibodies used

Name	Source	Catalog number	Dilution	Validation/reference
anti-Vinculin	Merck	#V-9131	1/5000	PMID: 20098684
anti-c-Myc	Merck	#M4439	1/2000	M4439 product page
anti-Tubulin	BD Pharmingen	#556321	1/5000	PMID: 8743943
anti-Bcl-2 HRP	Santa Cruz	#sc7382HRP	1/500	PMID: 26436418
anti-phospho Ser70-Bcl-2	Santa Cruz	#sc-293128	1/1000	PMID: 35176677
anti- phospho-p70 S6 kinase	Cell Signalling	#9234S	1/1000	PMID: 35551922
anti-p70 S6 kinase	Cell Signalling	#9202	1/1000	PMID: 36270994
anti- phospho- p38MAPK	Cell Signalling	#9215S	1/1000	PMID: 36329021
anti- p38MAPK	Cell Signalling	#9212	1/1000	PMID: 36329021
anti-Bcl-X _L	Cell Signalling	#2764	1/1000	PMID: 36289220
anti-PARP	Cell Signalling	#9532S	1/3000	PMID: 36257929
anti-LC3	Nanotools	#0231-100/LC3- 5F10	1/500	PMID: 29358170
Anti-RyR (C3:33)	ThermoFisher Scientific	#MA3-916	1/1000	PMID: 23895152
anti-NCX (C2:C12)	ThermoFisher Scientific	#MA3-926	1/1000	PMID: 23511010
anti-cTnT	Abcam	#ab209813	1/2000	PMID: 33054489
anti-IP ₃ R2	Abiocode	#R2872-2	1/1000	R2872-2 product page
anti- SERCA2a	Dr. Wuytack (KU Leuven)		1/2000	PMID: 2244871

Table S2. List of primers used for genomic DNA screening

Gene	Primer direction	Primer sequence (5' > 3')
<i>BCL2</i>	forward	GTGCTGAAGATTGATGGGATCG
	reverse	CTCAAAGAAGGCCACAATCCTCC
<i>HAL insertion</i>	forward	GCGCGTCCTGCCTTCATTTATCC
	reverse	GACACTTACCGCATTGACAAGCACG
<i>HAR insertion</i>	forward	AGGCGGGCCATTTACCGTAAG
	reverse	GAGGAGAAGATGCCCGGTGC
<i>RFBOX</i>	forward	AGTCCCAGCCCTCTAATCACAAAG
	reverse	GGTGACTTTGGACAGGTGGCTCAG
<i>SLC2A9</i>	forward	ATTCTCACAAATCCCTGCCAGTGC
	reverse	GAAGGTGCAACACAATGACTCTGG
<i>ANKRD28</i>	forward	CAGACAGTGATCCTTAGGCTTC
	reverse	CACAAGGCGGAAATAGTCTGGCACC
<i>NEFL</i>	forward	GTTCCAGGATCTACGGCAATGTG
	reverse	TGCAATGTCCAACCAGTCAAGC
<i>SLC23A</i>	forward	CCTAACTAATACAGCCCTCACTGG
	reverse	CCAGGCTCGGGTGAGGGAGTTAC
<i>PTPRT</i>	forward	AGCATTGCCAACCCTAGCAGAAG
	reverse	TGATGCGGATGAAGAGGCTGAGTC
<i>NCKAP5</i>	forward	GAAGGCAGGGATAGGGCAGGACAAG
	reverse	GCAAAGTGAGGAGACGAGATAACC
<i>FRAS1</i>	forward	AAGCACAGGACCAGACTTGCCAG
	reverse	GGCTGGCAGTTTGGAACAGGTGTG
<i>DLG2</i>	forward	GAGGAAATGGATGGAGAGTGAGG
	reverse	AAGCCTTCAGGGAGTGACCATC
<i>PCDH19</i>	forward	CAGCAATCGACTCCAAAGAACC
	reverse	TTCACTGAGCCTAACCACCAAG

Table S3. List of primers utilized in this study for Quantitative Real-Time PCR.

Gene	Primer direction	Primer sequence (5' > 3')
<i>OCT4</i>	forward	CGAGCAATTTGCCAAGCTCCTGAA
	reverse	GCCGCAGCTTACACATGTTCTTGA
<i>NANOG</i>	forward	TGGCCGAAGAATAGCAATGGTGTG
	reverse	TTCCAGGTCTGGTTGCTCCACATT
<i>BRACH</i>	forward	ACCCAGTTCATAGCGGTGAC
	reverse	AAGCTTTTGCAAATGGATTG
<i>GATA4</i>	forward	CGACACCCCAATCTCGATATG
	reverse	GTTGCACAGATAGTGACCCGT
<i>NKX2.5</i>	forward	ACCTCAACAGCTCCCTGACTCT
	reverse	ATAATCGCCGCCACAACTCTCC
<i>MYH6</i>	forward	GCCCTTGACATTGCACTG
	reverse	CGGGACAAAATCTGGCTTTGA
<i>TNNI3</i>	forward	GATGCGGCTAGGGAACCTC
	reverse	GCATAAGCGCGGTAGTTGGA
<i>TNNT2</i>	forward	ACAGAGCGGAAAAGTGGGAAG
	reverse	TCGTTGATCCTGTTTCGGAGA
<i>GAPDH</i>	forward	TCAAGAAGGTGGTGAAGCAGG
	reverse	ACCAGGAAATGAGCTTGACAAA
<i>RPL13a</i>	forward	CCTGGAGGAGAAGAGGAAAGAGA
	reverse	TTGAGGACCTCTGTGATTTGTCAA

Table S4. *BCL2* homologues sequences used for CRISP/Cas9 approach

<p><i>BCL2</i> homologues sequence (HAL)</p> <p>TGTAATTTGCCGAGAAGGGGAAAACATCACAGGACTTCTGCGAATACCGGACTGAAAATTGTAATTC ATCTGCCGCCGCGCTGCCTTTTTTTTTTCTCGAGCTCTTGAGATCTCCGGTTGGGATTCTGCGGA TTGACATTTCTGTGAAGCAGAAGTCTGGGAATCGATCTGGAAATCCTCCTAATTTTACTCCCTCTC CCCGCGACTCCTGATTCATTGGGAAGTTCAAATCAGCTATAACTGGAGAGTGCTGAAGATTGATGG GATCGTTGCCTTATGCATTTGTTTTGGTTTTACAAAAGGAACTTGACAGAGGATCATGCTGTACT TAAAAAATAACAAGTAAGTTCTCTGCACAGGAAATGGTTTTAATGTAACCTTCAATGGAAACCTTTGA GATTTTTTACTTAAAGTGCATTCGAGTAAATTTAATTTCCAGGCAGC</p>
<p><i>BCL2</i> homologues sequence (HAR)</p> <p>TACATTCCTTTTAGCCGTGTTACTTGTAGTGTGTATGCCCTGCTTTCACTCAGTGTGTACAGGGAAA CGCACCTGATTTTTTACTTATTAGTTTGTTTTTCTTTAACCTTTTCAGCATCACAGAGGAAGTAGAC TGATATTAACAATACTTACTAATAATAACGTGCCTCATGAAATAAAGATCCGAAAGGAATTGGAATA AAAATTTCTGCATCTCATGCCAAGGGGAAAACACCAGAATCAAGTGTCCGCGTGATTGAAGACAC CCCCTCGTCCAAGAATGCAAAGCACATCCAATAAAATAGCTGGATTATAACTCCTCTTCTTCTCTG GGGCCGTGGGGTGGGAGCTGGGGCGAGAGGTGCCGTTGGCCCCGTTGCTTTTCTCTGGGAAGGA TGCGTAAGCTGGGAGAACAGGGTACGATAACCGTGAGATAGTGTA</p>

Fig. S5. Supplementary full length immunoblots for figures 1, 3, 5 and 6

All immunoblots are shown merged with the colorimetric image of the actual membrane in order to visualize exactly where the membranes were cut.

Full length blots Figure 1

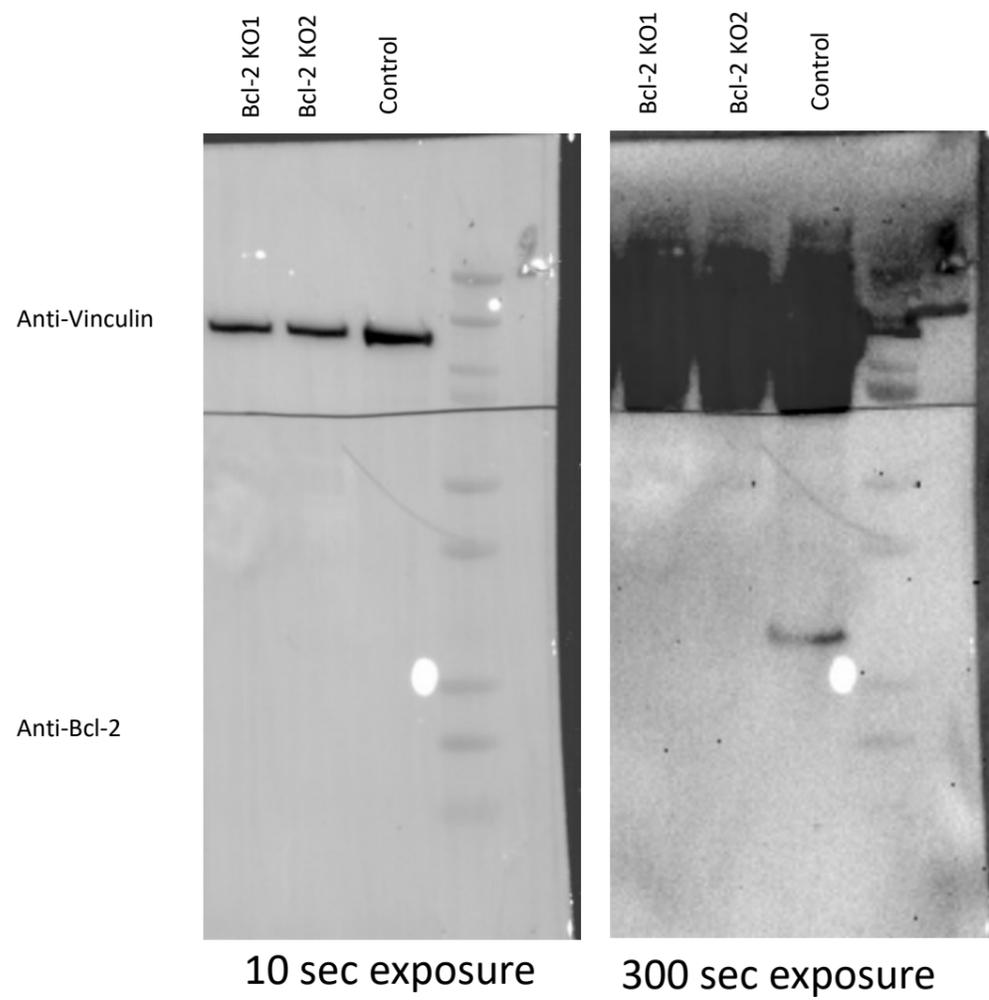
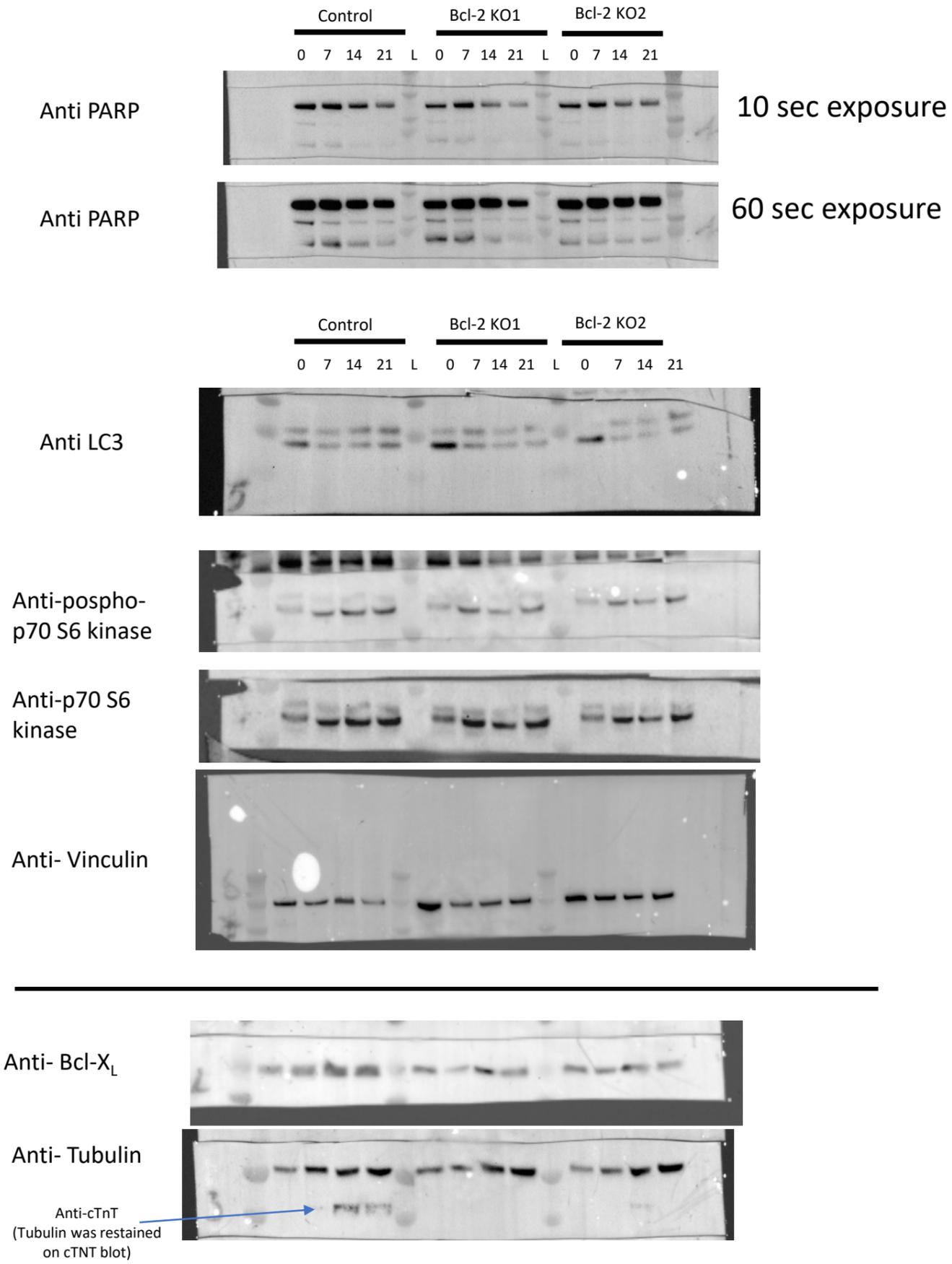
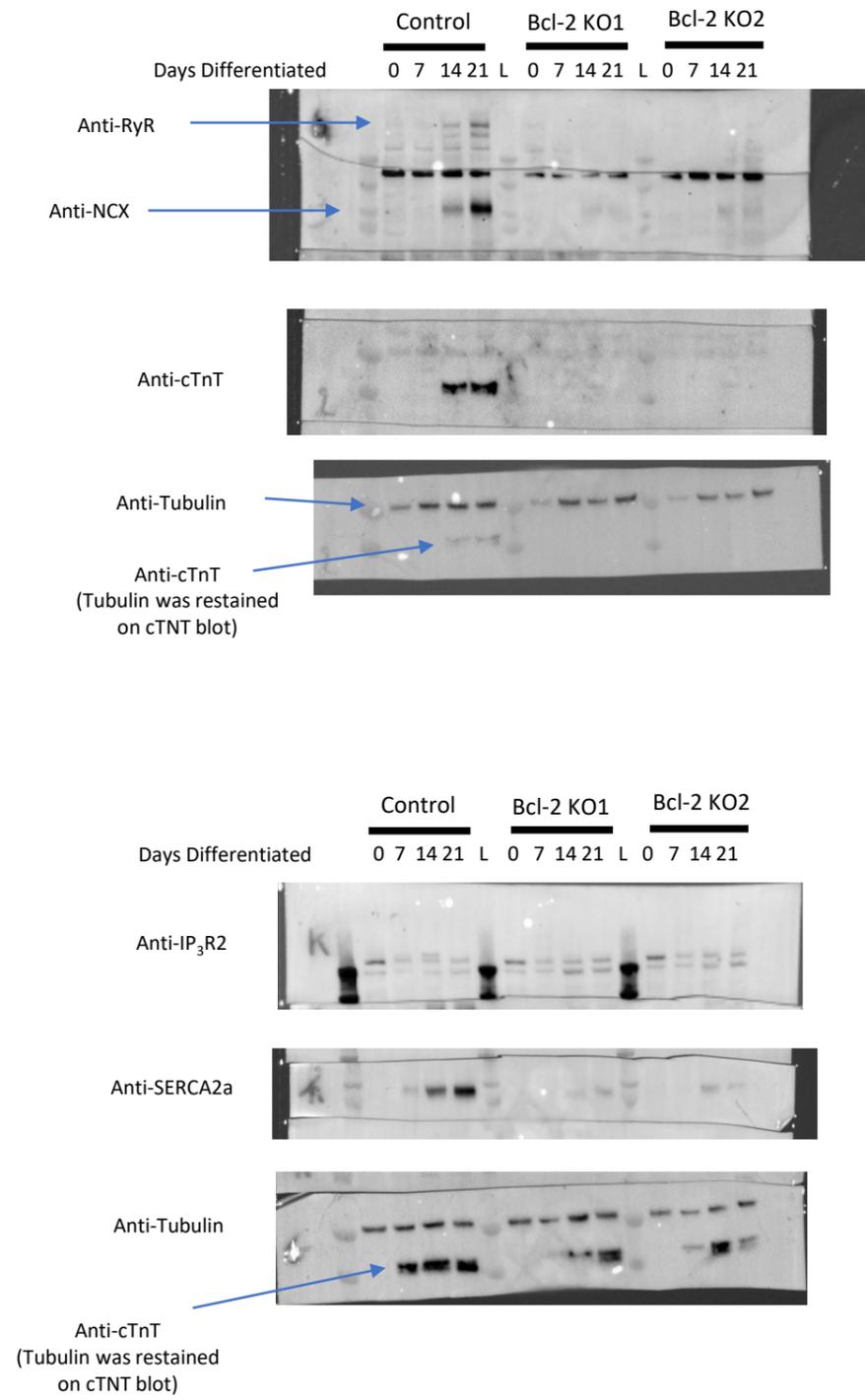


Figure 1

Full length blots Figure 3



Full length blots Figure 5



Full length blots Figure 6

