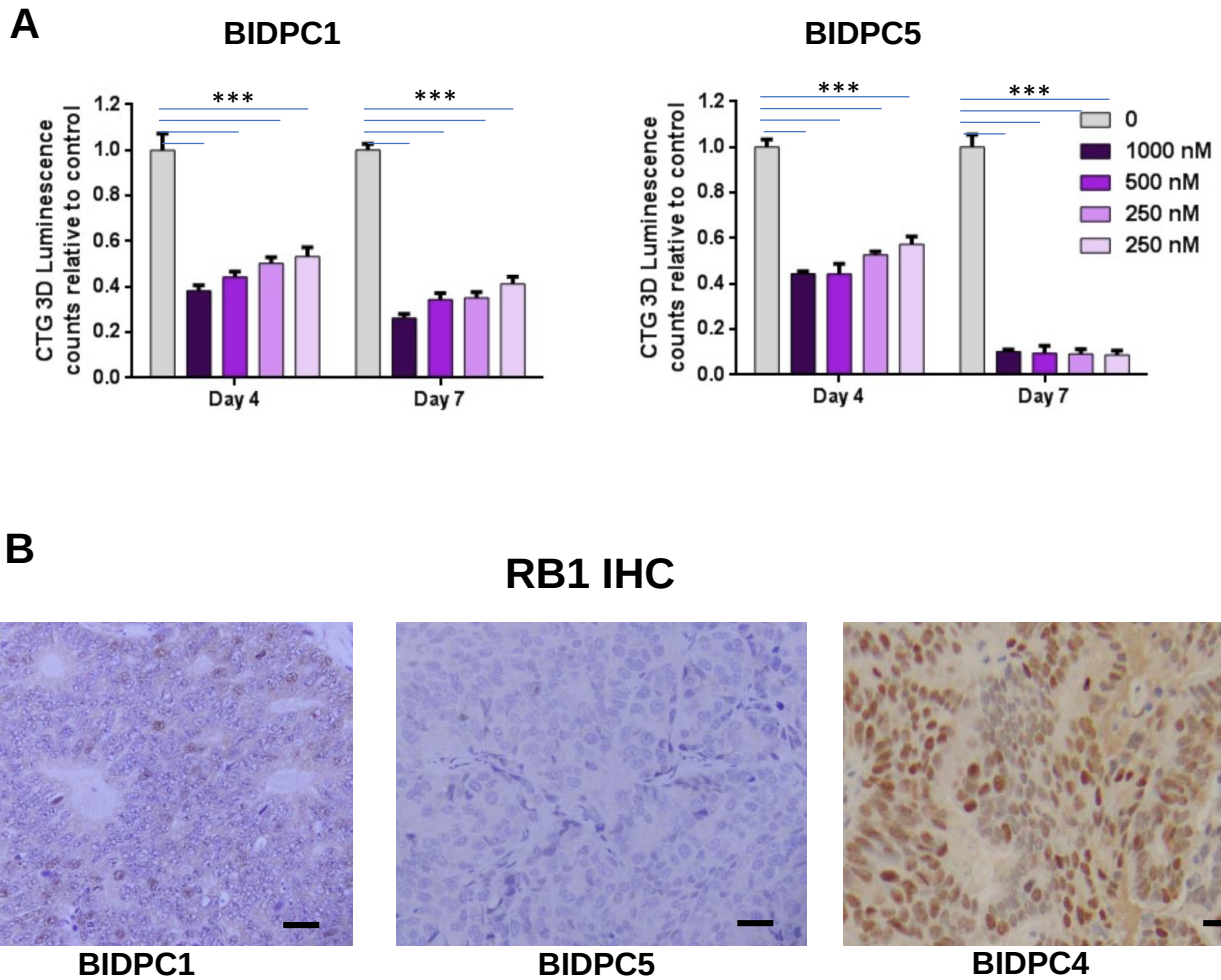
| Model | | Maximum Responses (% reduction from control) | | | IC50 Navitoclax (nM) | IC50 S63845 (nM) |
|---|---|---|---|---|---|---|
| | | Navitoclax | Venetoclax | S63845 | | |
| LuCaP Primary Cultures | 147 | 64 | 12 | 0 | 300 | - |
| | 176 | 78 | 3 | 4 | 300 | - |
| | 78 | 66 | 10 | 0 | 750 | - |
| | 23.1 | 50 | 17 | 10 | 1000 | - |
| | 136CR | 33 | 6 | 2 | - | - |
| | 189.4 | 26 | 24 | 14 | - | - |
| | 235.3 | 29 | 2 | 5 | - | - |
| 3D Organoids | BIDPC1 | 60 | 9 | 0 | 125 | - |
| | BIDPC4 | 7 | 10 | 13 | - | - |
| | BIDPC5 | 90 | 3 | 12 | 100 | - |
| | BIDPC6 | 70 | 23 | 53 | 250 | 500 |
| | BIDPC7 | 94 | 21 | 6 | <50 | - |
| | 15-442-12 | 74 | 16 | 38 | 250 | - |
| | LuCaP35CR | 10 | 5 | 80 | - | 40 |
| | LuCaP70CR | 20 | 25 | 90 | - | 50 |
| | LuCaP77 | 55 | 12 | 16 | 400 | - |
| Cell Lines | VCaP | 5 | 5 | 90 | - | 75 |
| | LNCaP | 5 | 0 | 6 | - | - |
| | C42 | 17 | 0 | 5 | - | - |
| | DU145 | 0 | 0 | 3 | - | - |
| | PC3 | 7 | 0 | 5 | - | - |

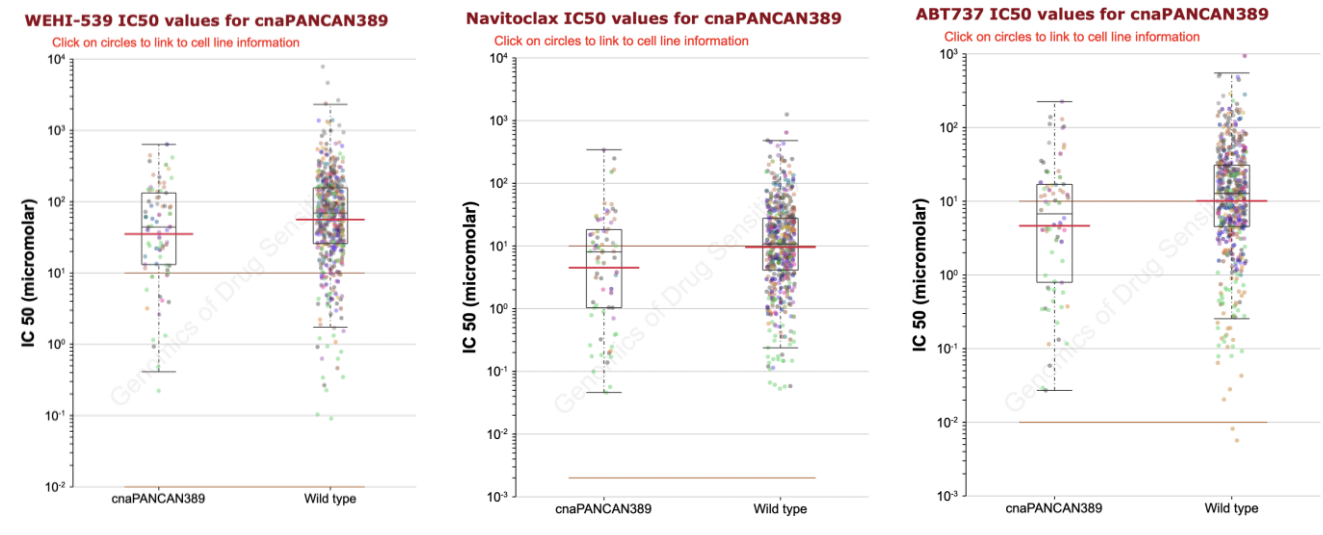
**Supplementary Table S1. BH3 mimetic screening in PCa organoids and cultures.** Maximum response and IC50 are shown.

| Drug | Target | Category | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | TKI | CDK | Spindle | PDK | MDM2 | PLK1 | Other |
| UK356618 | matrix metalloprotease-3 | | | | | | | other |
| Ascomycin | antibiotics | | | | | | | other |
| kbNB 14270 | selective protein kinase D (PKD) inhibitor | TKI | | | | | | |
| FK866 | nicotinamide phosphoribosyltransferase | | | | | | | other |
| SP2509 | histone demethylase LSD1 inhibitor | | | | | | | other |
| AS1517499 | STAT6 inhibitor | TKI | | | | | | |
| Defactinib | ocal adhesion kinase (FAK) inhibitor | TKI | | | | | | |
| MI-773 | MDM2 antagonist | | | | | MDM2 | | |
| YK4279 | EWS-FLI1 binding to RNA helicase A (RHA). | | | | | | | other |
| RO495 | Non-receptor tyrosine-protein kinase 2 (TYK2 | TKI | | | | | | |
| SB225002 | CXCR2 Antagonist | | | | | | | other |
| PD176252 | non-peptide gastrin-releasing peptide receptor (GRP-R, BB2) | | | | | | | other |
| MK886 | 5-lipoxygenase-activating protein (FLAP) | | | | | | | other |
| PHA 767491 | CDK9, CDK7 | | CDK | | | | | |
| PF514273 | CB1 receptor antagonist | | | | | | | other |
| GSK923295 | allosteric inhibitor of CENP-E kinesin motor ATPase | | | Kinesin | | | | |
| PF3758309 | TP-competitive, pyrrolopyrazole inhibitor of PAK4 | TKI | | | | | | |
| BX912 | ATP-Competitive PDK1 Inhibitor. | | | | PDK1 | | | |
| GSK2334470 | PDK1 Inhibitor | | | | PDK1 | | | |
| MG-132 | proteasome inhibitor | | | | | | | other |
| KPT-330 | Exportin-1 (XPO1) | | | | | | | other |
| Eltrombopag | thrombopoetin | | | | | | | 0ther |
| AZD8055 | mTOR kinase inhibitor | TKI | | | | | | |
| Volasertib | PLK1 inhibitor | | | | | | PLK1 | |
| BMN-673 | PARP1-2 inhibitor | | | | | | | other |
| CP-673451 | PDGFR inhibitor | TKI | | | | | | |
| OTSSP167 | MELK-selective inhibito | | | | | | | other |
| Dihydroartemisinin | artemisinin component/ free radicals | | | | | | | other |
| BAY61-3606 | Syk inhibitor | TKI | | | | | | |
| Ispinesib | kinesin spindle protein | | | kinesin | | | | |
| Obatoclax | BCL2, BCLXL, MCL1 | | | | | | | other |
| Nolatrexed | Thymidine Synthase inhibitor | | | | | | | other |
| Papaverine | phosphodiesterase | | | | | | | other |
| BI2536 | PLK1 PLK4 | | | | | | PLK1 | |
| L-779450 | B-Raf kinase inhibitor | TKI | | | | | | |
| LY2090314 | GSK-3 inhibitor | | | | | | | other |
| CCT241533 | CHK2 inhibitor | TKI | | | | | | |
| UNC2881 | Mer tyrosine kinase inhibitor | TKI | | | | | | |
| MBX-2982 | GPR119 agonist | | | | | | | other |
| Ro-3306 | CDK1 inhibitor | | CDK | | | | | |
| UNC0631 | Protein lysine methyltransferase G9a | | | | | | | other |
| BNC105 | tubulin polymerization inhibitor | | | tubulin | | | | |
| RG7388 | MDM2 inhibitor | | | | | MDM2 | | |
| MK2206 | allosteric Akt inhibitor | | | | | | | other |
| Nutlin | MDM2 inhibitor | | | | | MDM2 | | |
| ARRY520 | kinesin spindle protein (KSP) inhibitor | | | kinesin | | | | |
| Rupatadine | H1 histamine, PAFR antagonist | | | | | | | other |
| PF-431396 | FAK and PYK2 inhibitor | TKI | | | | | | |
| GSK126 | EZH2 methyltransferase inhibitor | | | | | | | other |
| AZD6482 | PI3Kβ inhibitor | TKI | | | | | | |
| SB525334 | PI3Kβ-selective inhibitor | TKI | | | | | | |
| CH5132799 | pan-PI3 kinase inhibitor | TKI | | | | | | |
| AZD5438 | CDK 1.2.9 | | CDK | | | | | |
| AZ20 | ATR inhibitor | TKI | | | | | | |
| Rimonabant | cannabinoid receptor CB1 | | | | | | | other |
| Abemaciclib | CDK4/6 | | CDK | | | | | |
| Spautin-1 | Beclin-1 degrater, autophaty | | | | | | | other |
| CCT128930 | Akt inhibitor | TKI | | | | | | |
| AXL1717 | IGF1R inhibitor | TKI | | | | | | |
| SB-743921 | kinesin spindle protein | | | kinesin | | | | |
| Epothilone | microtubule-stabilizing agents | | | microtubules | | | | |
| TAK960 | PLK1 | | | | | | PLK1 | |
| AZD1332 | neurotrophic tyrosine kinase receptors antagonist | TKI | | | | | | |
| Differine | naphthoic acid | | | | | | | other |
| PG01 | CFTR Cl- channel potentiator | | | | | | | other |
| BIP135 | glycogen synthase kinase-3 | | | | | | | other |
| Rotenone | Mitochondrial Complex I Inhibitor | | | | | | | other |
| BRD6929 | HDAC1 and HDAC2 inhibitor | | | | | | | other |
| JNJ10198409 | PDGFR inhibitor | TKI | | | | | | |
| Mozavaptan | vasopressing receptor antagonist | | | | | | | other |
| | | 20 | 4 | 6 | 2 | 3 | 3 | 32 |

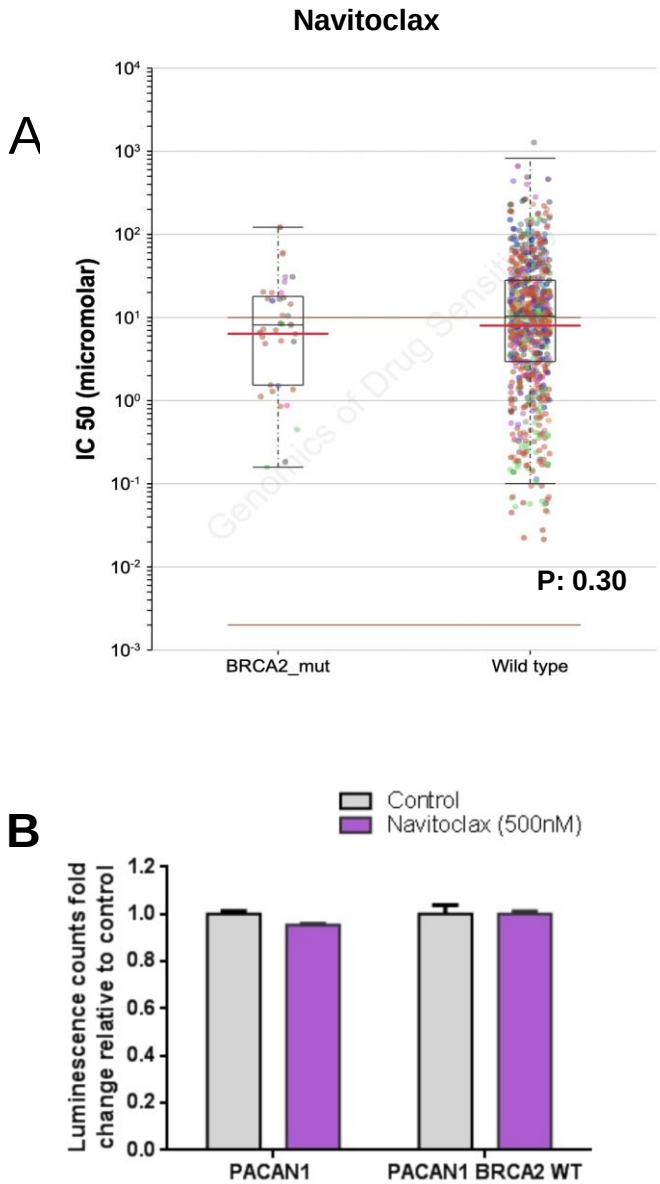
**Supplementary Table S2. Agents that enhancd responses to navitoclax in primary drug screen.**



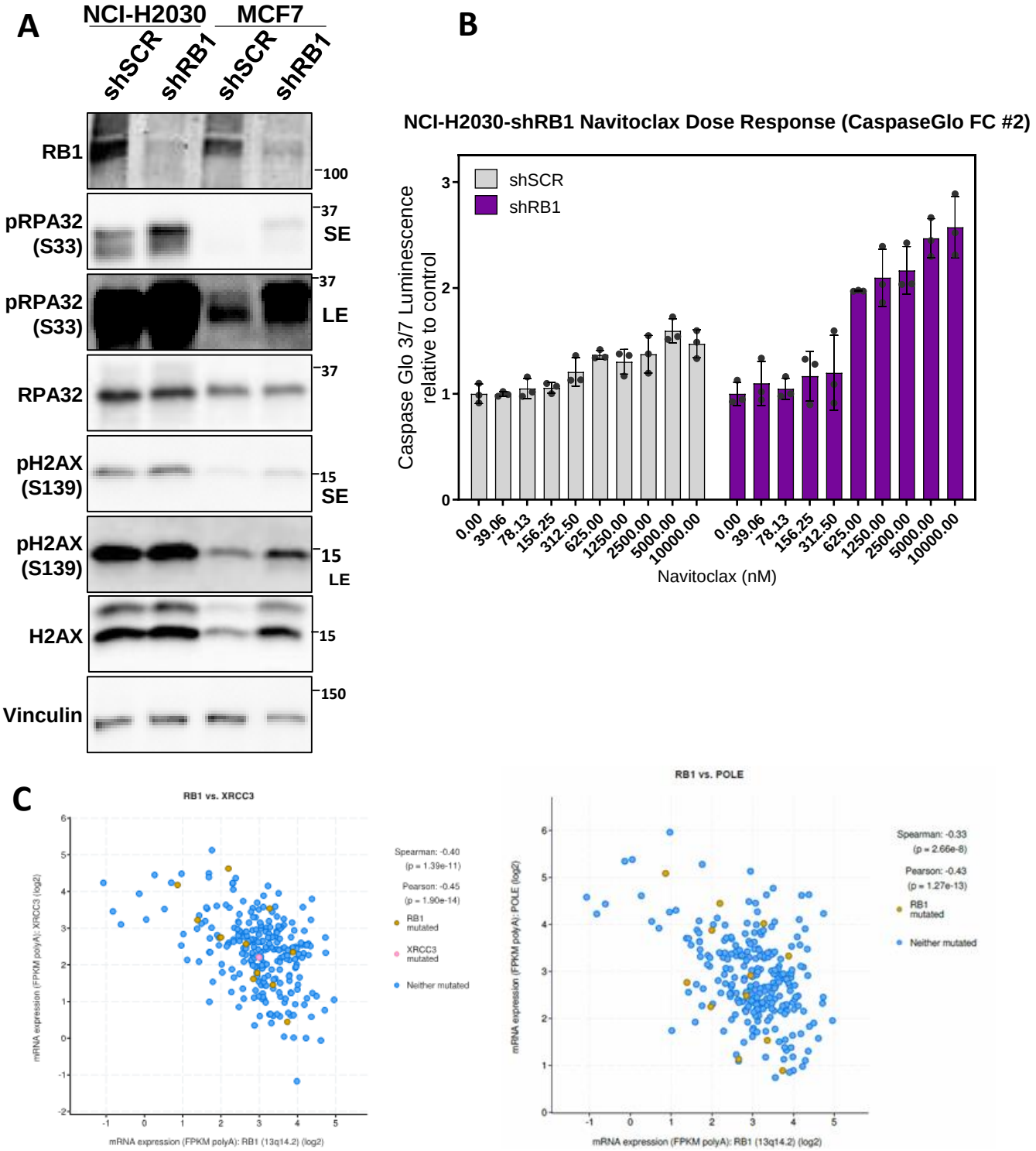

**Supplementary Figure S1. (A)** Sustained navitoclax responses in PCa organoids with longer culture duration. Primary organoid cultures from BIDPC1 or BIDPC5 PDXs were treated for 4 or 7 days with navitoclax at the indicated concentrations and assessed for caspase activation (Caspase Glo 3/7). Data show mean values and SEM from biological triplicates. One way ANOVA test was performed for comparison *** $p<0.001$. (**B)** FFPE blocks from untreated BIDPC1, 4, and 5 PDX were stained for RB1 (clone 4H1). Antibody dilution was 1:2000. Weak staining in subset of cells in BIDPC1 may be nonspecific or reflect small subset of positive cells. Scale bar is 20 μM.



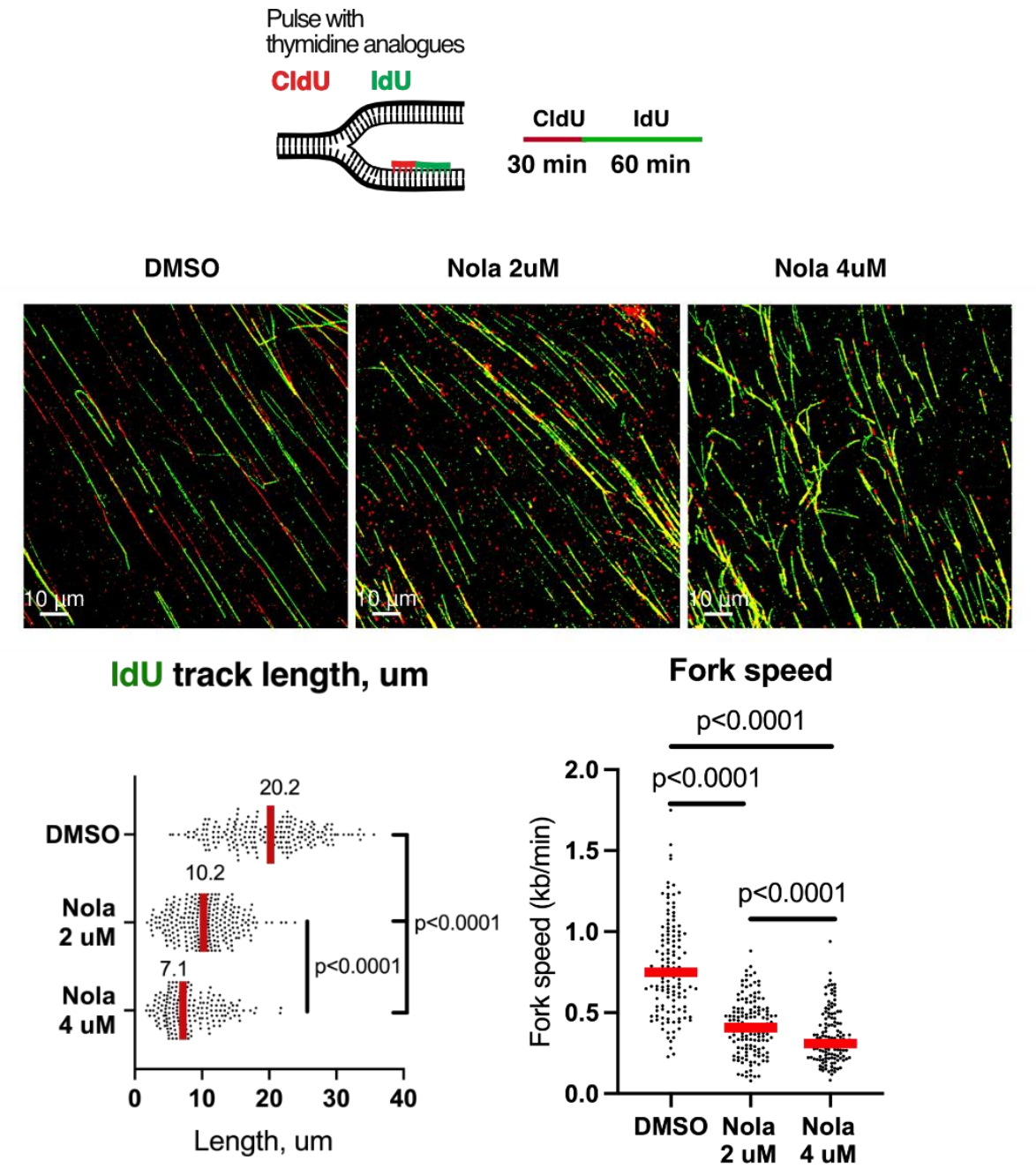

**Supplementary Figure S2. RB1 loss sensitizes to navitoclax.** Sanger data on IC50 for BH3 mimetics in solid tumor cells with *RB1* copy number loss (cnaPANCAN389).



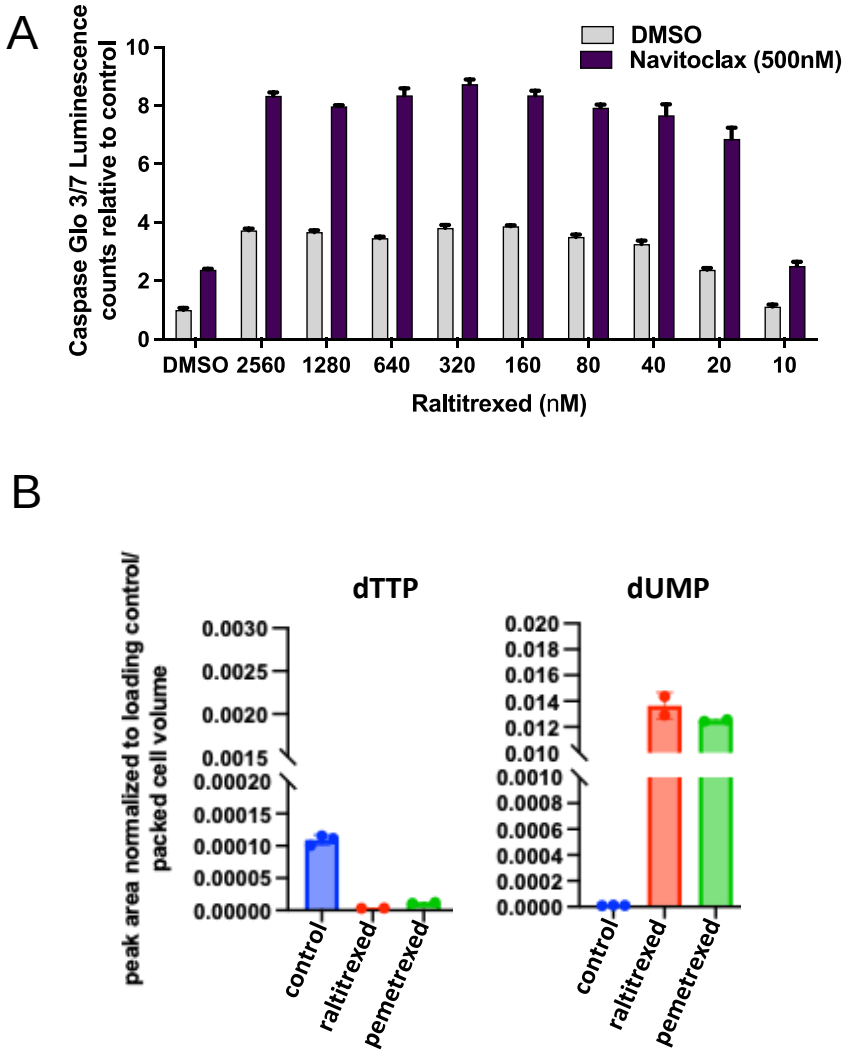

**Supplementary Figure S3. BRCA2 loss does not sensitize to navitoclax. (A)** Sanger data on IC50 for navitoclax in BRCA2 altered versus wild-type cells. Analysis by one way ANOVA indicated the difference was not significant (p=0.30). **(B)** Effect of navitoclax in BRCA2 deficient PANCAN1 cells and in an isogenic line with restored BRCA2. Cells were treated for 48 hrs with 500 nM navitoclax or vehicle control (DMSO). Data are mean and SEM from biological triplicates.



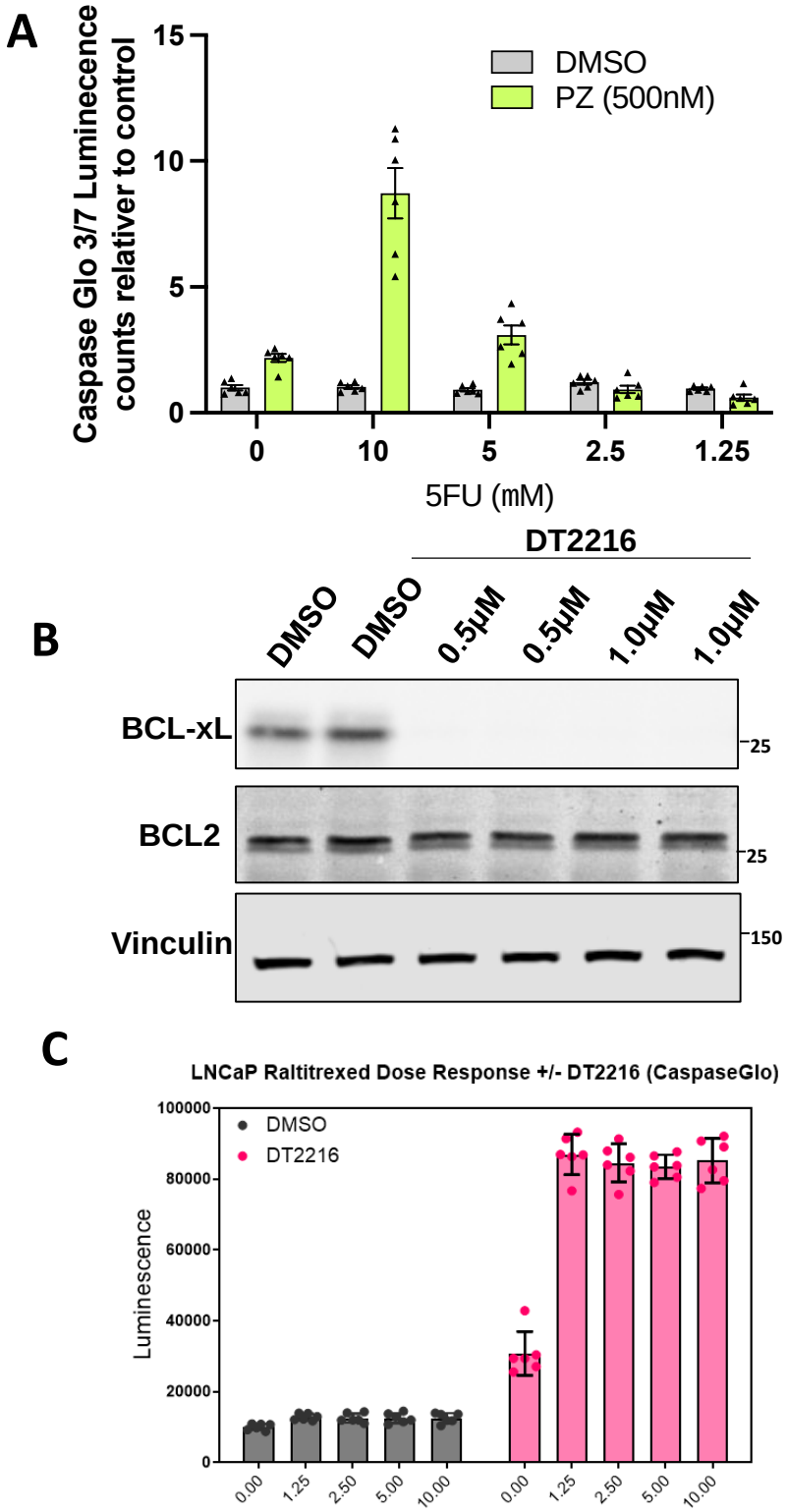

**Supplementary Figure S4. RB1 loss increases DNA damage. (A)** NCI-H2030 and MCF7 cells expressing doxycycline-regulated RB1 or scrambled control shRNA were treated with doxycycline (500 ng/ml) for 3 days to induce shRNA and then lysed and assessed by immunoblotting as indicated. Short (SE) and long (LE) exposures are shown for some proteins. **(B)** NCI-2030 cells expressing doxycyline-induced RB1 or control shRNA were treated with navitoclax for 24 hrs and assessed for caspase activation. Data are mean and SEM of biological triplicates, and are normalized to the respective no navitoclax controls. Analysis by two way ANOVA showed that the RB1 shRNA significantly increased levels of Caspase Glo activity in response to navitoclax ($p<0.001$). **(C)** DNA repair gene and RB1 expression are negatively correlated in primary PCa. XRCC3 and POLE expression were correlated with RB1 expression in TCGA primary PCa (data from cBioportal).



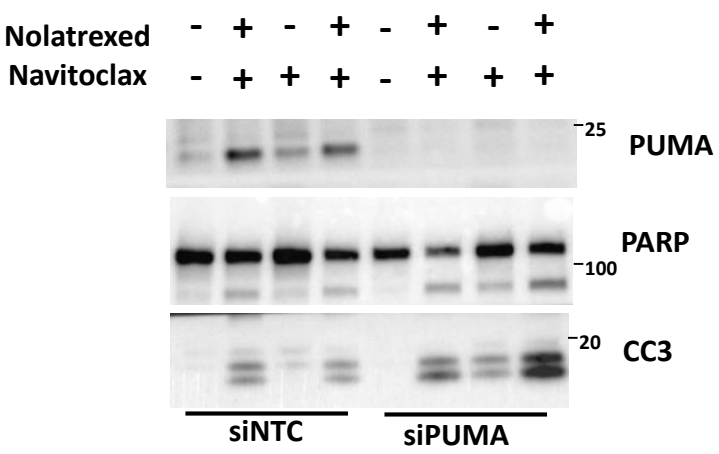

**Supplementary Figure S5.** Replication fork dynamics were analysed by DNA Fiber Assay. LNCaP cells were treated with either DMSO or nolatrexed (2 μM or 4 μM) for 16h. Cells were labelled with CldU followed by IdU as indicated. Representative images are shown, scale bar: 10 μm. Median IdU track lengths (μm) of at least 200 double-labelled fibers are shown in red. Mann–Whitney test was applied to test significance. Replication fork speed was calculated using conversion factor 1 μm=2.59 kb. Values were plotted and median fork speed is shown in red for each treatment condition. Mann–Whitney test was applied to test significance.



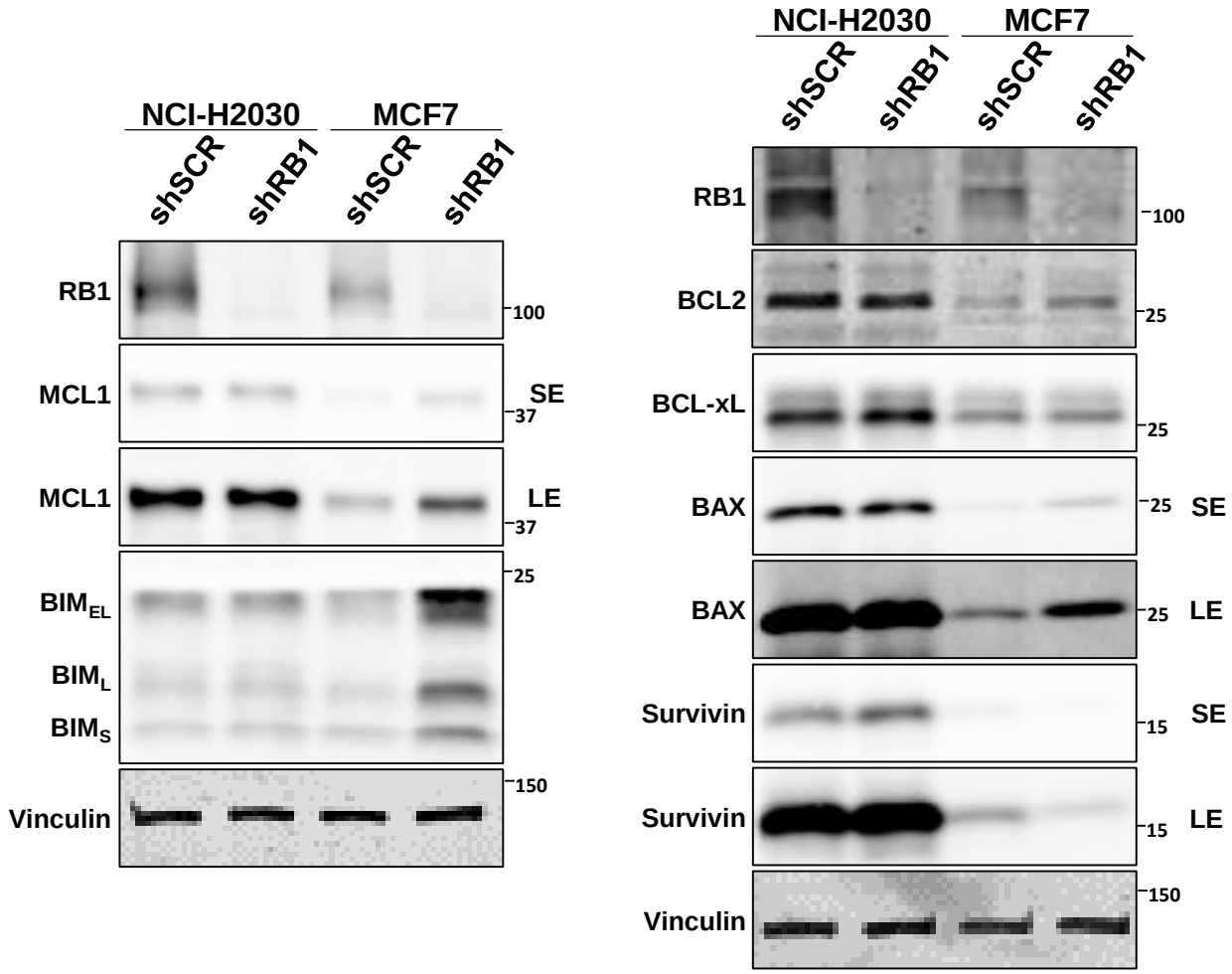

**Supplementary Figure S6. Nucleotide depletion due to thymidylate synthase inhibition correlates with sensitization to navitoclax. (A)** LNCaP cells were treated with raltitrexed for 48 hours followed by 6 hours treatment with navitoclax as indicated. Apoptosis was then assessed by Caspase Glo assay. Data shown are mean and SEM from biological triplicates and are normalized to cells that only got vehicle. Analysis by two way ANOVA showed that raltitrexed significantly enhanced the apoptotic response to navitoclax ($p<0.001$). **(B)** Levels of dTTP and dUMP were assessed by mass spectrometry in LNCaP cells treated for 24 hours with 100 nM raltitrexed or pemetrexed. Data are mean and SEM of biological triplicate samples.



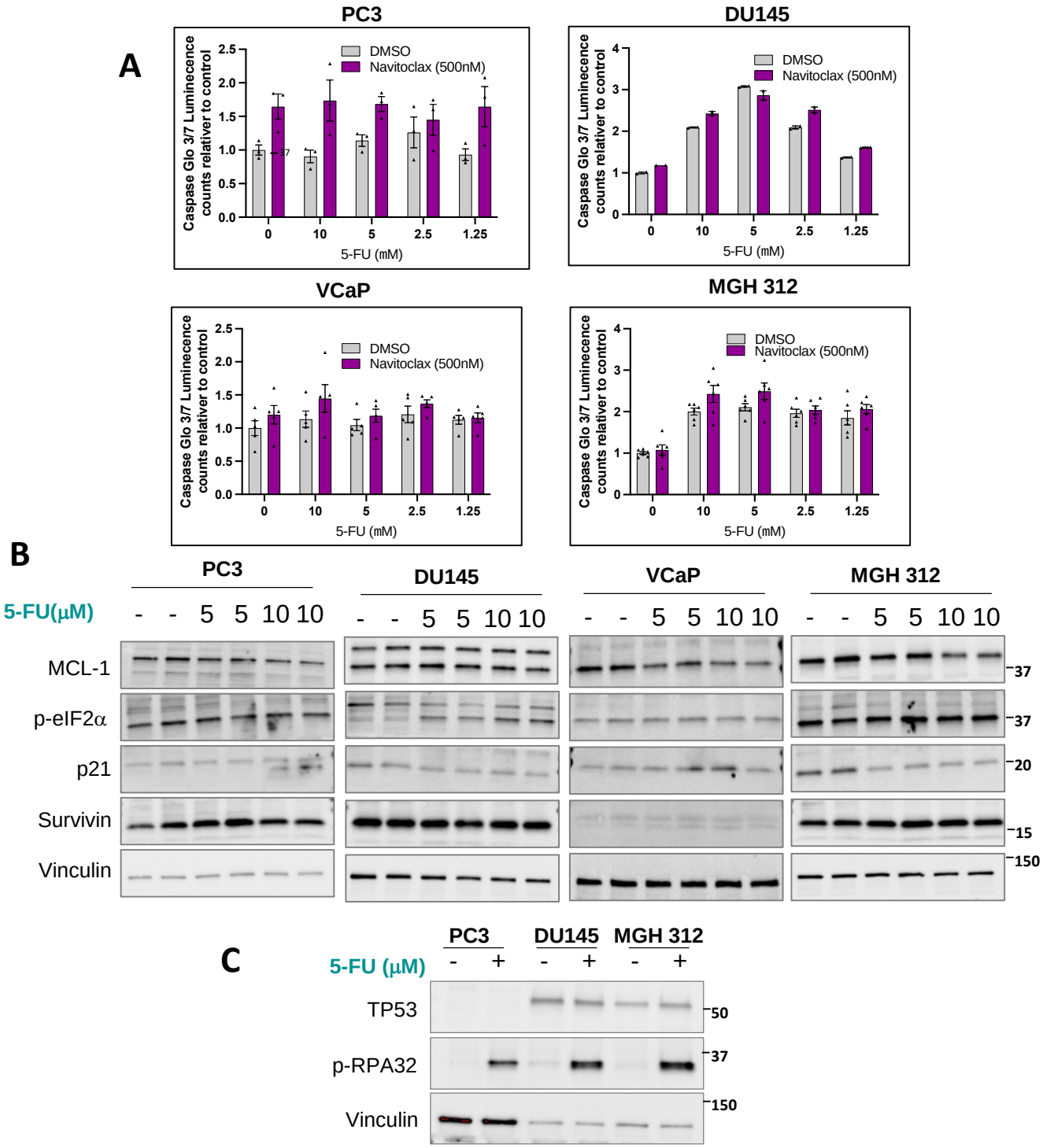

**Supplementary Figure S7. Cooperativity between thymidylate synthase inhibition and BCL-XL degraders. (A)** LNCaP cells were treated for 48 hours with 5-FU followed by PZ18755B (BCL2/BCL-XL degrader) for 16 hours, and apoptosis was assessed by Caspase Glo 3/7. Data are mean and SEM of biological replicates (n=6). Analysis by two way ANOVA showed that 5FU significantly enhanced the apoptotic response to navitoclax ($p<0.001$). **(B)** LNCaP cells were treated for 16 hours with DT2216 (BCL-XL selective degrader) and assessed by immunoblotting. (**C,**) LNCaP cells were treated for 48 hours with raltitrexed followed by 16 hours with DT2216 and Caspase Glo assay. Data are mean and SEM of biological replicates (n=6). Analysis by two way ANOVA showed that raltitrexed significantly enhanced the apoptotic response to navitoclax ($p<0.001$).



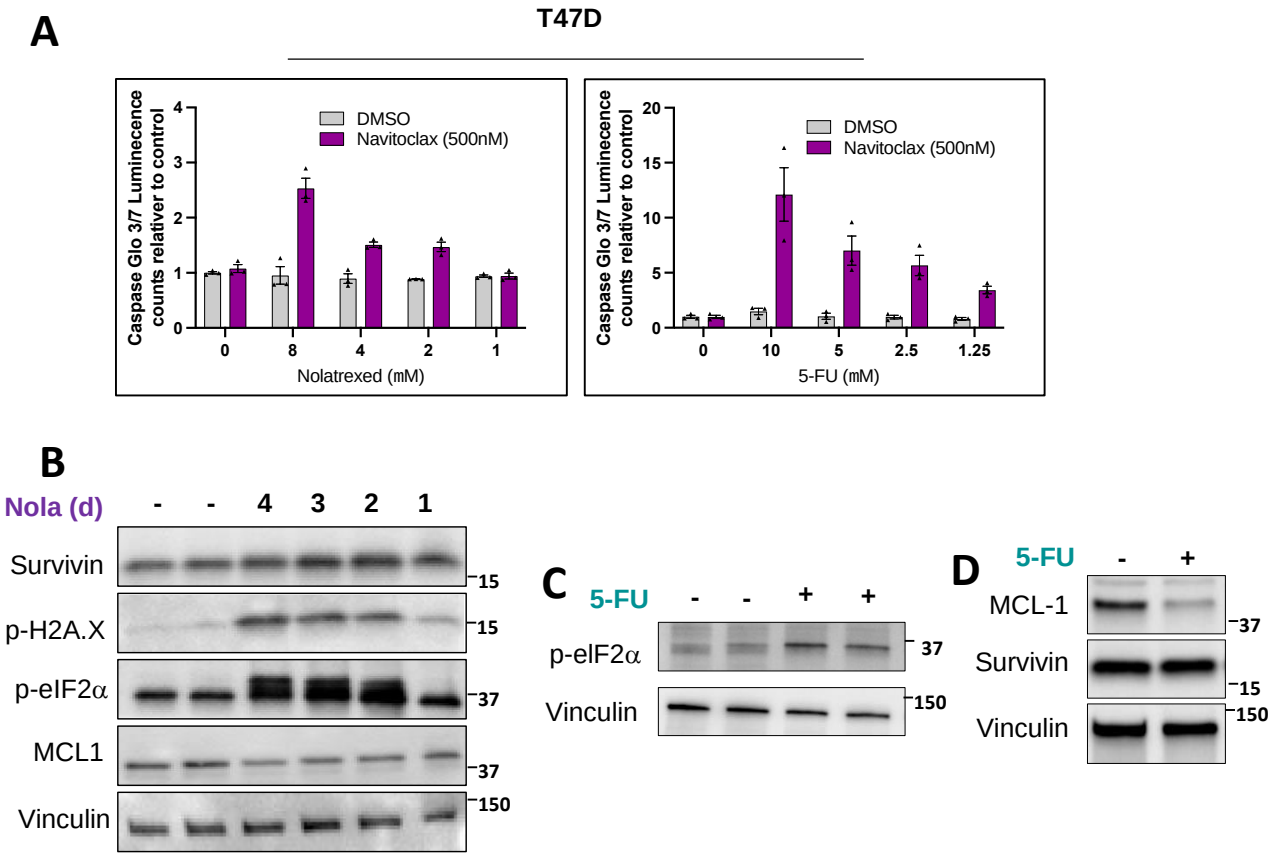

**Supplementary Figure S8. Nolatexed sensitization to navitoclax is not dependent on PUMA.** LNCaP cells were treated with PUMA or nontarget control siRNA for 3 days in total. During last 48 hours they were treated with nolatrexed (500 nM), and during last 6 hours with navitoclax (500 nM).



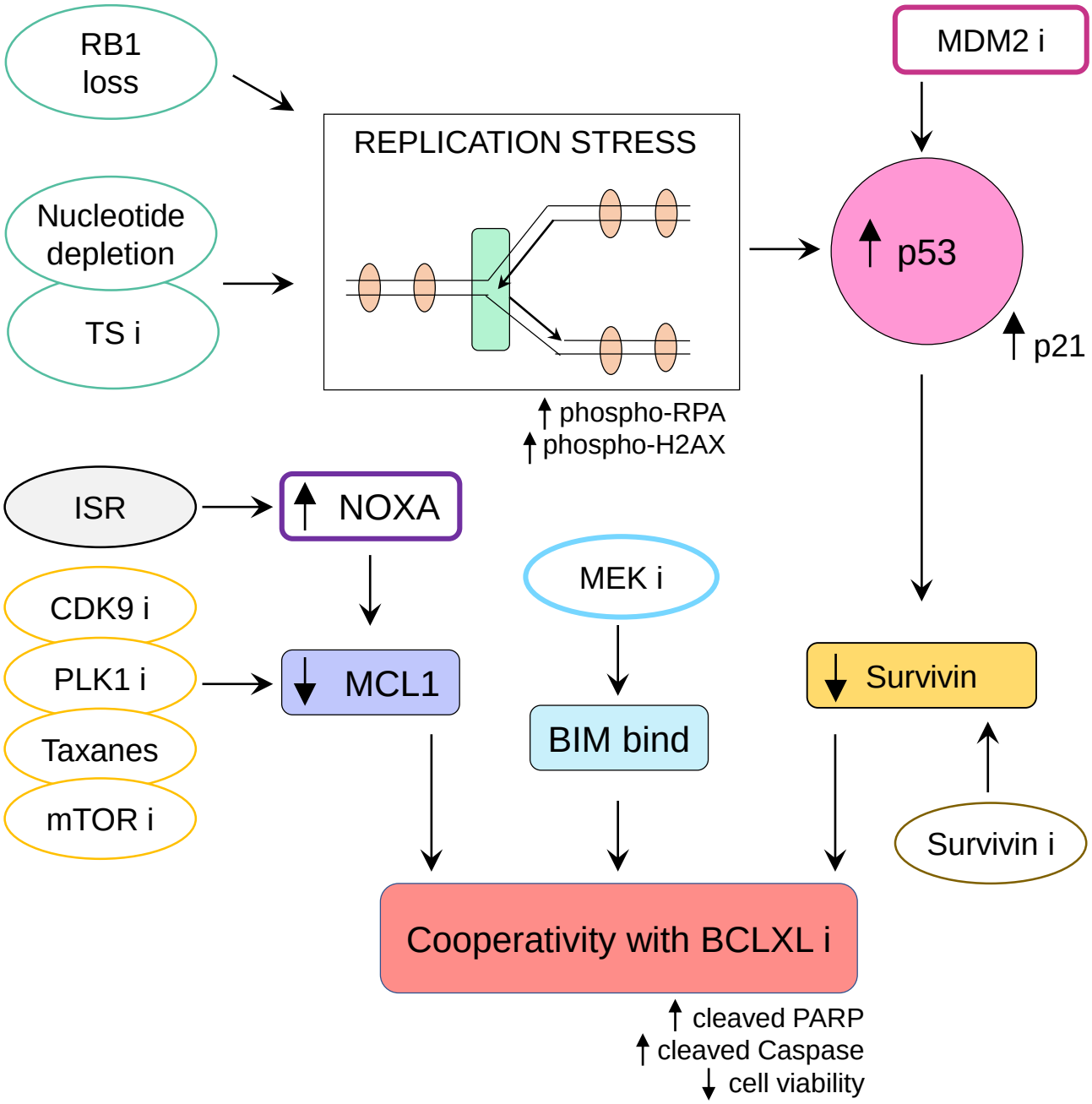

**Supplementary Figure S9. Effects of RB1 downregulation on apoptosis-related proteins.** NCI-H2030 and MCF7 cells expressing RB1 or scrambled control shRNA were lysed and assessed by immunoblotting as indicated. Short (SE) and long (LE) exposures are shown for some proteins.



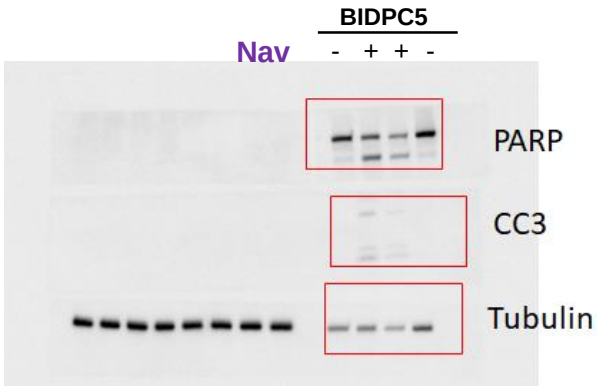

**Supplementary Figure S10. Thymidylate synthase sensitization to BCL-XL inhibition is p53 dependent. (A)** TP53 mutant solid tumor cell lines (PC3-prostate cancer, DU145-prostate cancer, VCaP-prostate cancer, MGH312-breast cancer) were treated with the combination of 5-FU (48 hours) and navitoclax (added for last 6 hours). Caspase 3/7 activity was assessed with immunofluorescence-based assay. Data are mean and SEM of biological replicates (n=5). (**B**) Immunoblotting analysis for p53, integrated stress response, and survivin in TP53 deficient cell lines treated with 5-FU for 48 hours. **(C)** Immunoblotting for replication stress marker (pRPA32) in TP53 deficient cell lines treated with 5-FU for 48 hours.



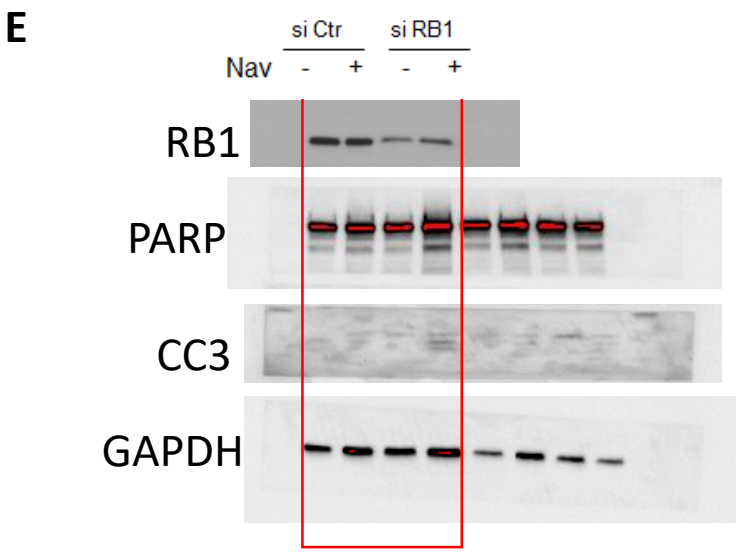

**Supplementary Figure S11. Thymidylate synthase inhibition sensitization to BCL-XL inhibition in T47D through activation of an integrated stress response**. (**A)** Caspase 3/7 activity in T47D (TP53 deficient breast cancer) cell line treated for 48 hours with the combination of nolatrexed plus navitoclax (left) and 5-FU plus navitoclax (right). Data are mean and SEM of biological triplicates. Analysis by two way ANOVA showed that both nolatrexed and 5-FU significantly enhanced the apoptotic response to navitoclax ($p<0.001$). (**B)** Immunoblotting analysis of integrated stress response marker (p-eIF2α) and apoptotic machinery proteins in T47D cells treated with nolatrexed (2 μM) for 1-4 days. (**C, D)** T47 cells were treated with 5-FU for 48 hours. Immunoblotting analysis was performed for integrated stress response marker p-eIF2α (C) or for MCL-1 and survivin (D).

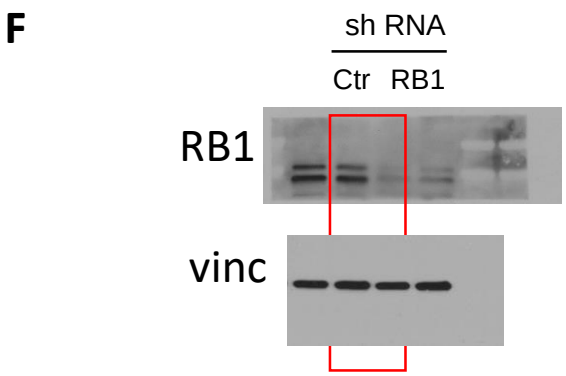



**Supplementary Figure S12. Overview of mechanisms that increase dependence on BCL-XL.** One group of mechanisms converges on MCL1 to reduce its expression or increase its degradation. A second group described here converges on survivin. Survivin expression is also cell cycle regulated and its activity may be modulated by phosphorylation (not shown).

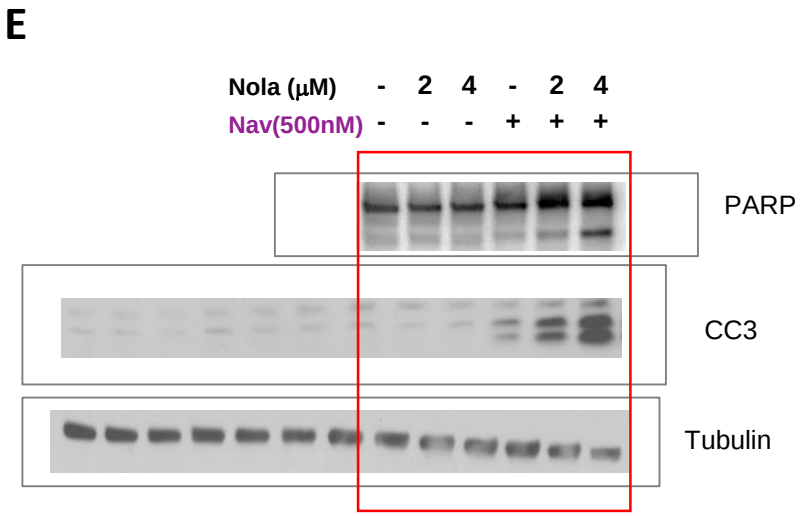



**Supplementary Figure S13.** Uncropped gels related to Figure 1

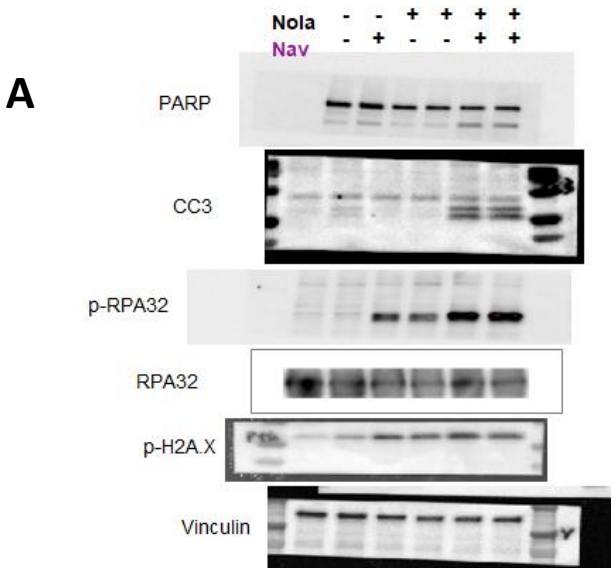


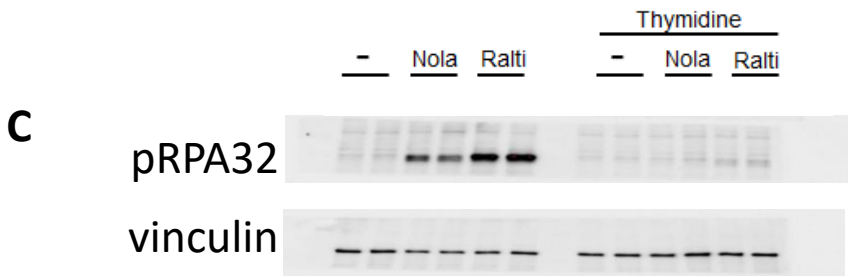



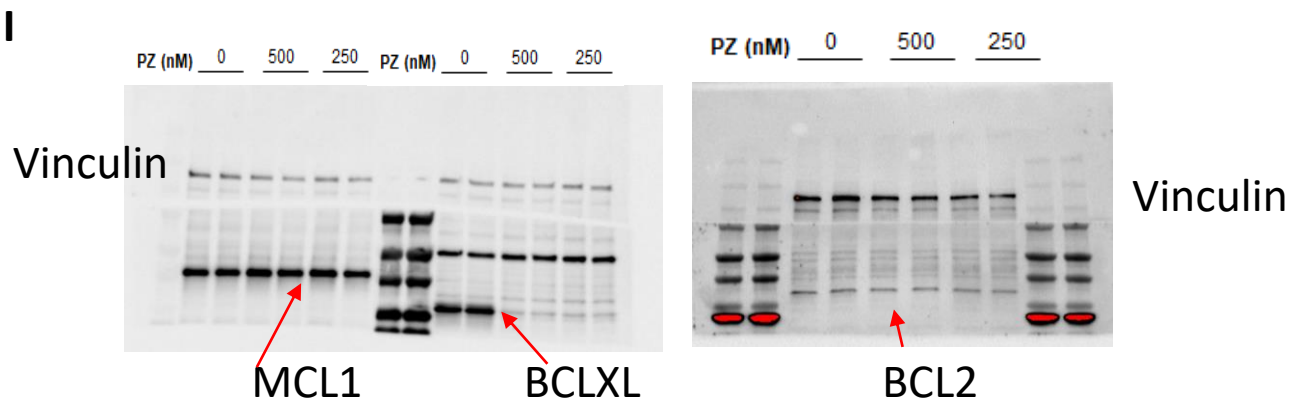

**Supplementary Figure S14.** Uncropped gels related to Figure 2.



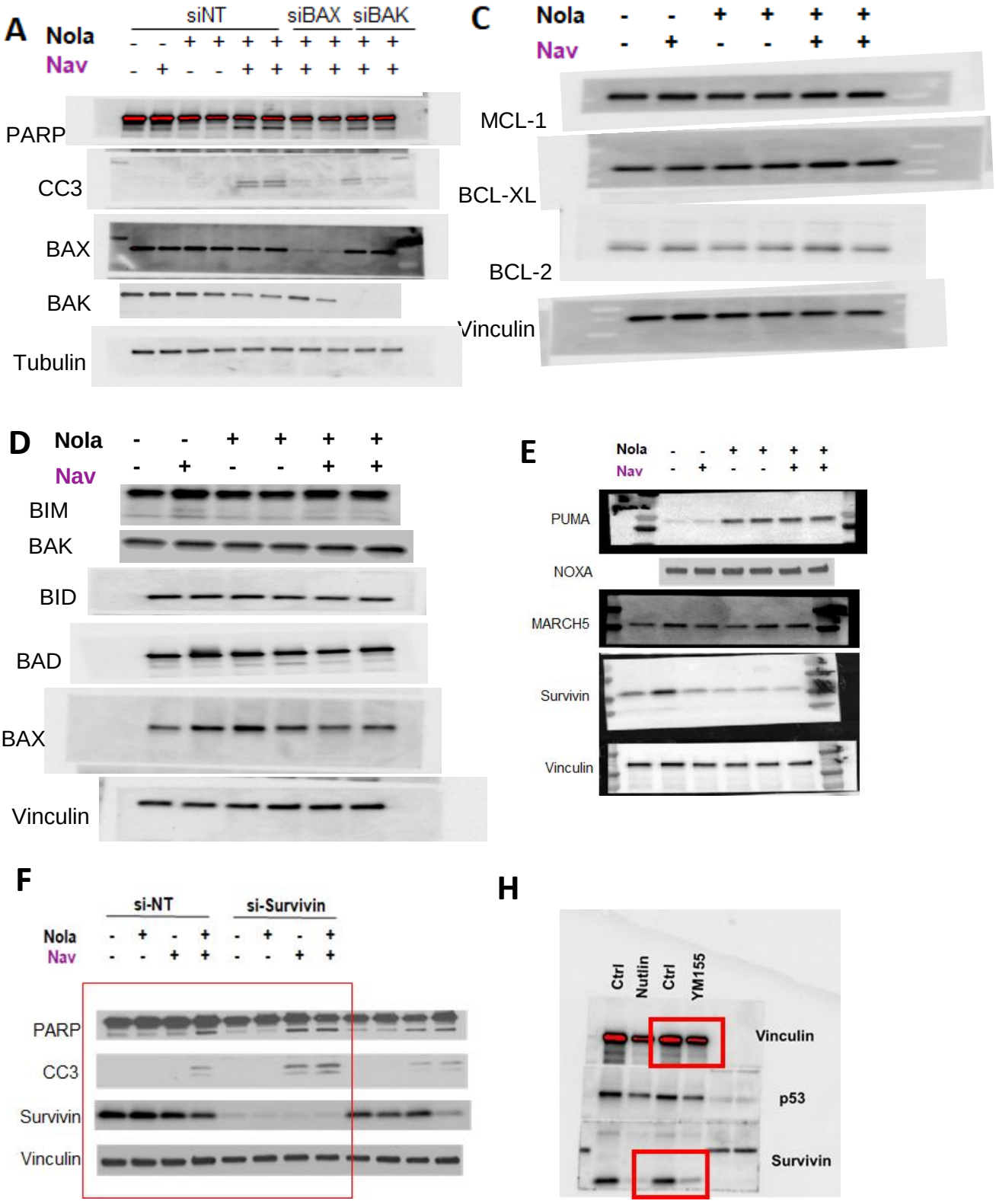

**Supplementary Figure S15.** Uncropped gels related to Figure 3.



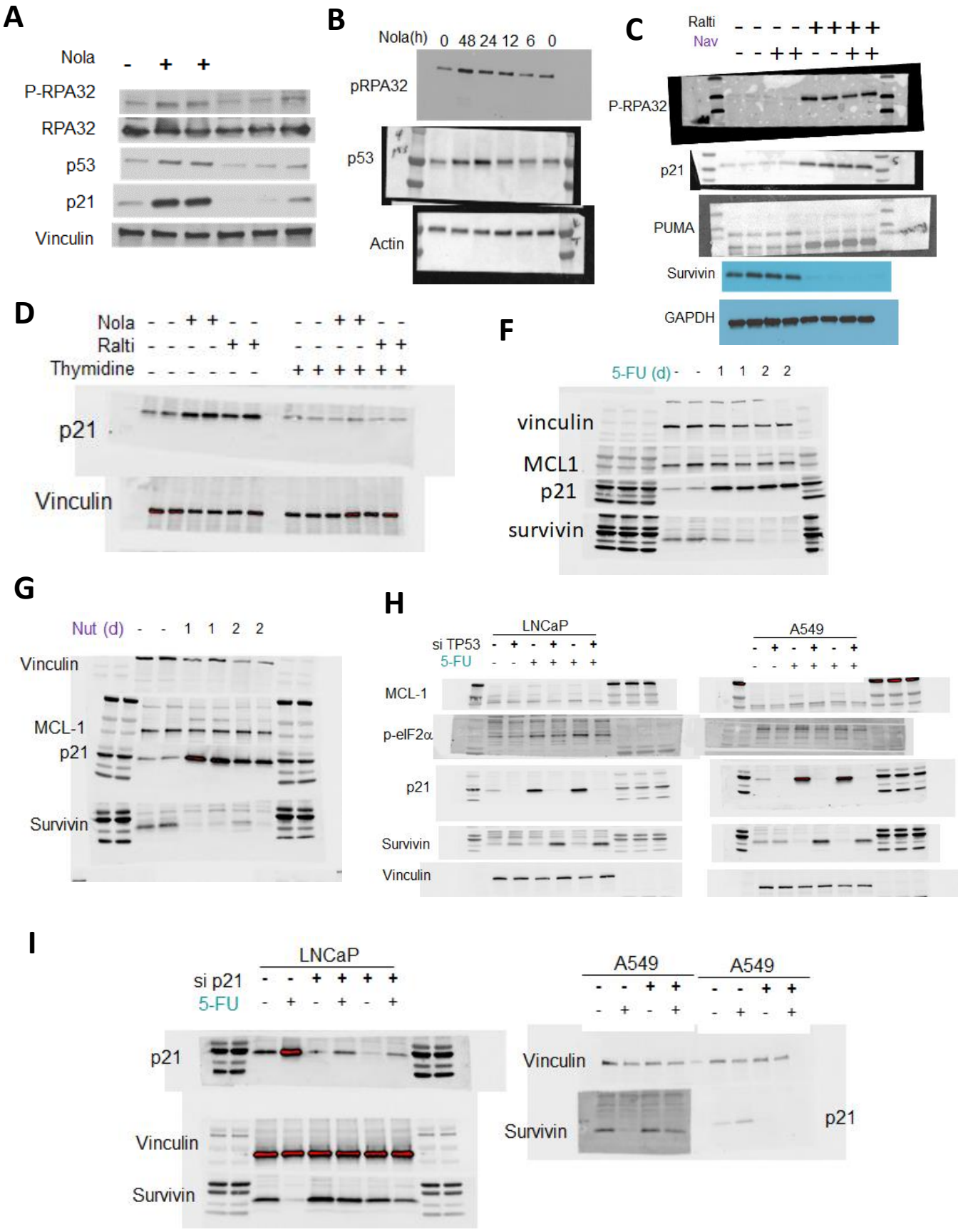



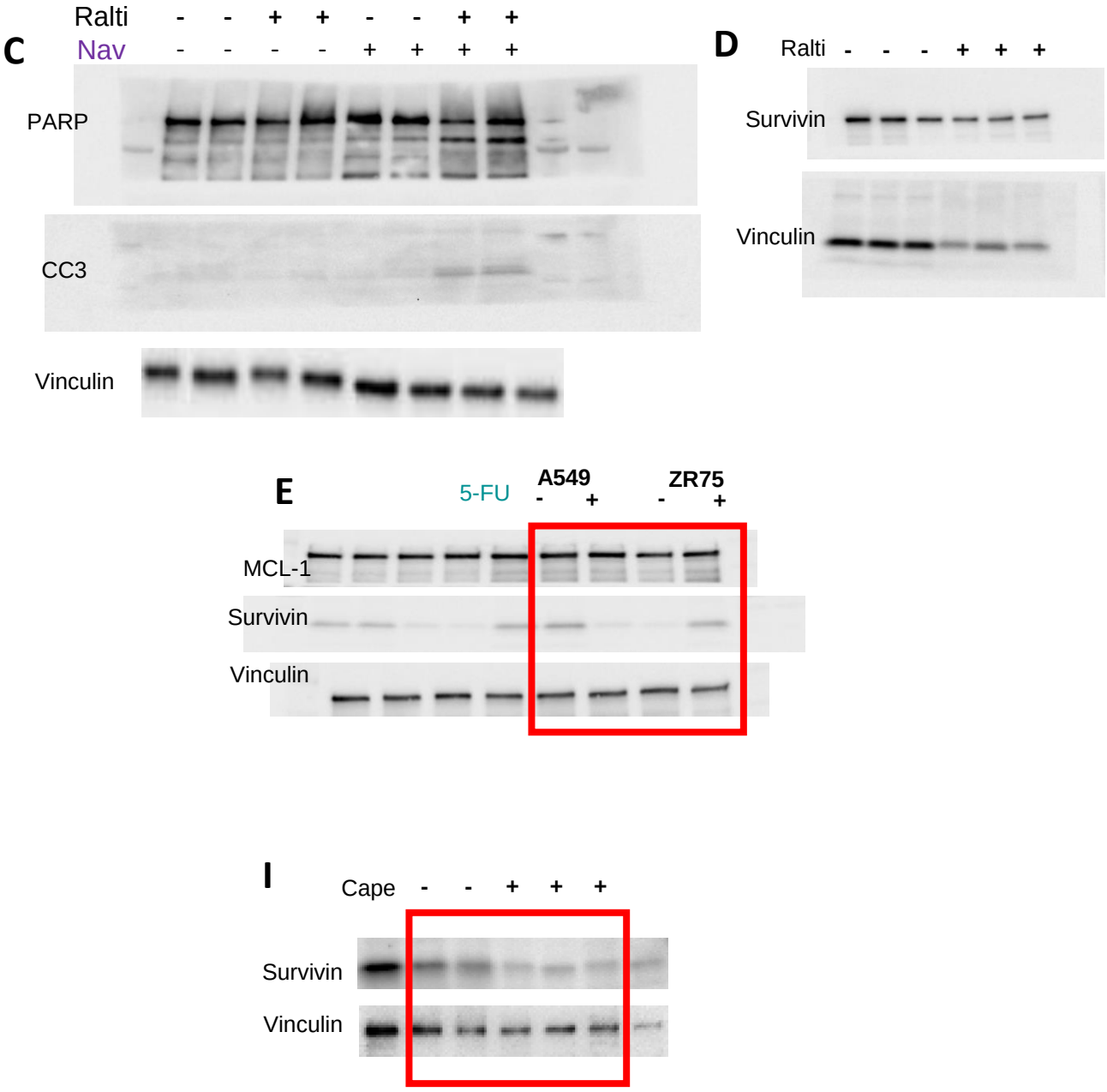

**Supplementary Figure S16.** Uncropped gels related to Figure 4.



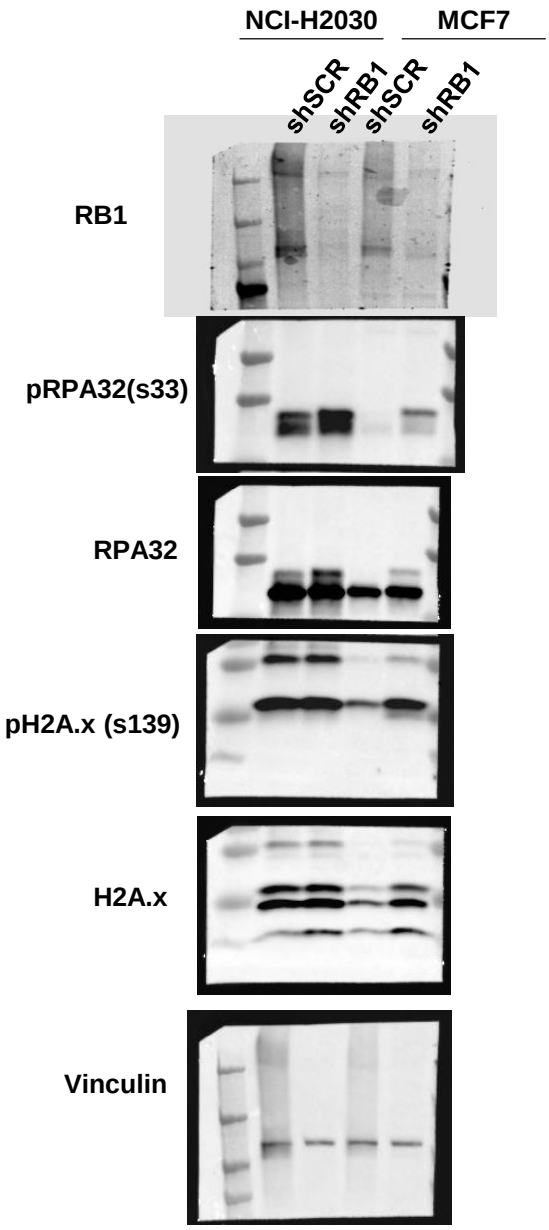

**Supplementary Figure S17.** Uncropped gels related to Figure 5.




**Supplementary Figure S18.** Uncropped gels related to Figure 6.

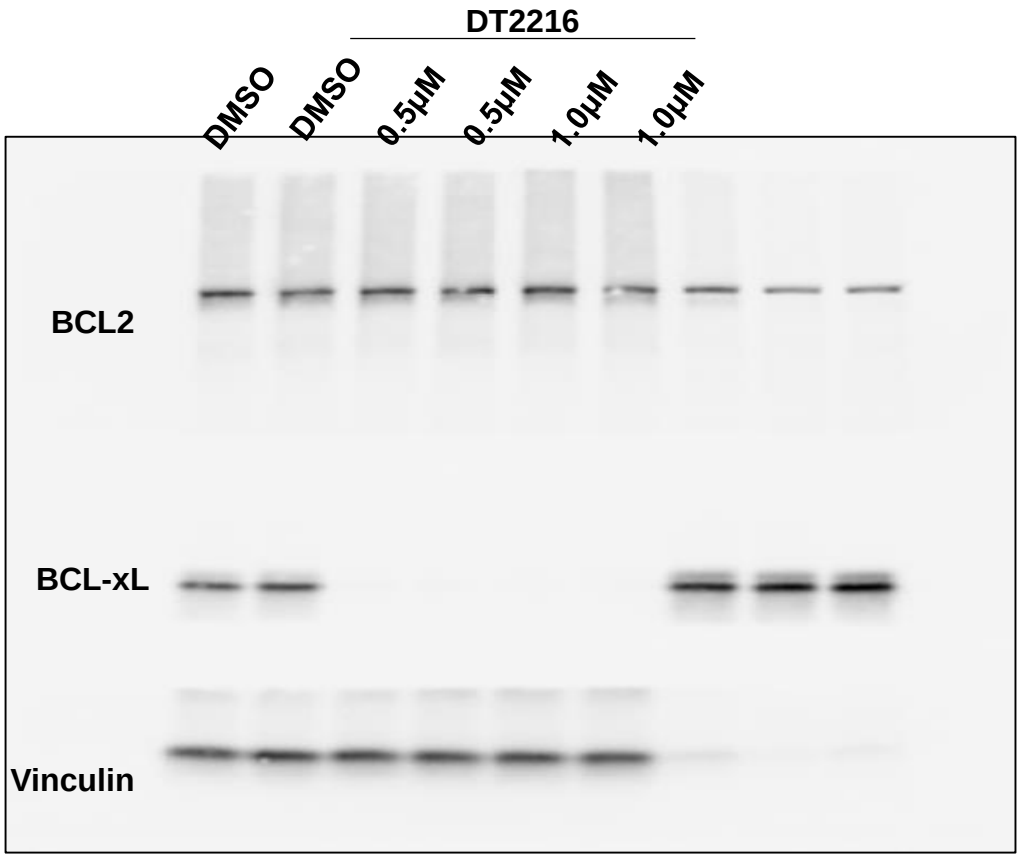



**Supplementary Figure S19.** Uncropped gels related to Figure 7.

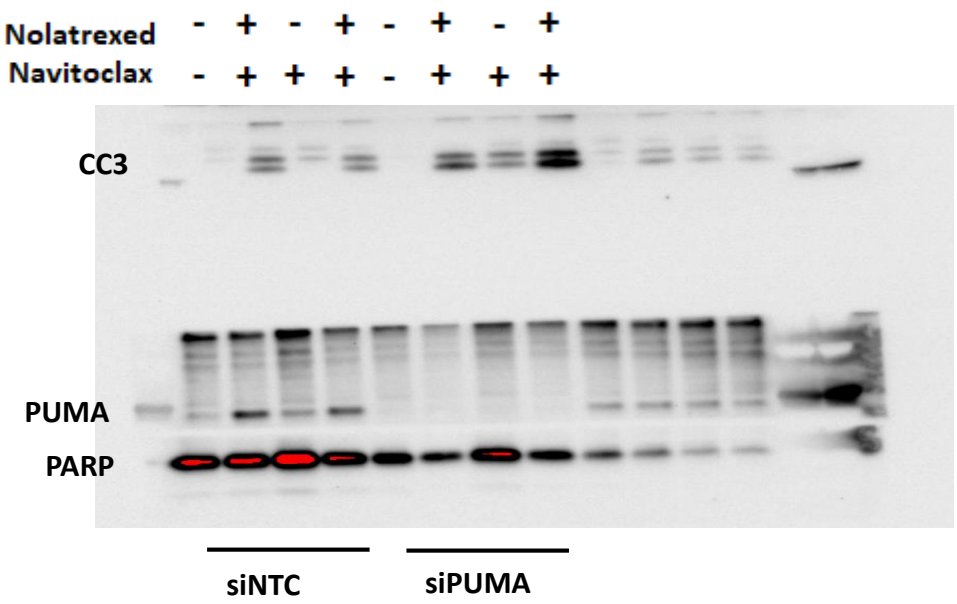



**Supplementary Figure S20.** Uncropped gels related to Figure S4.

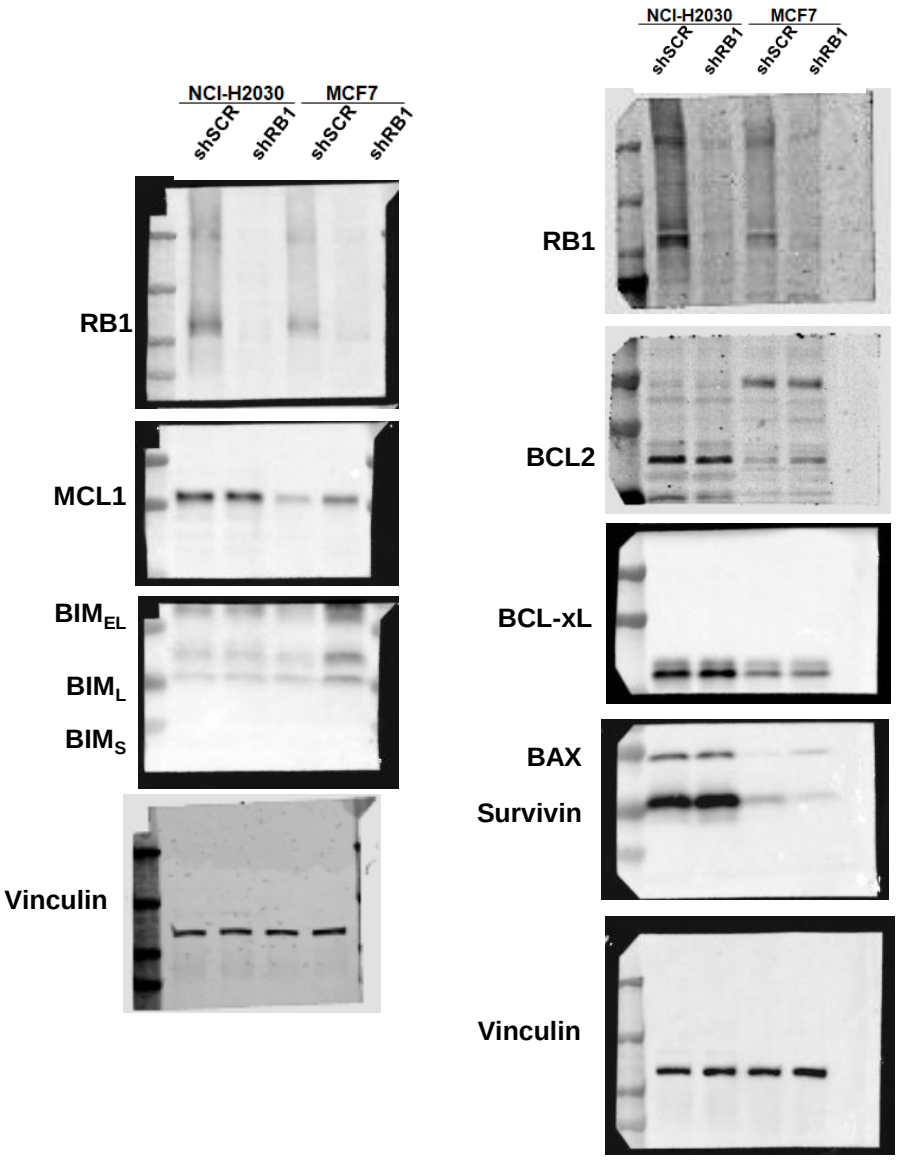
B



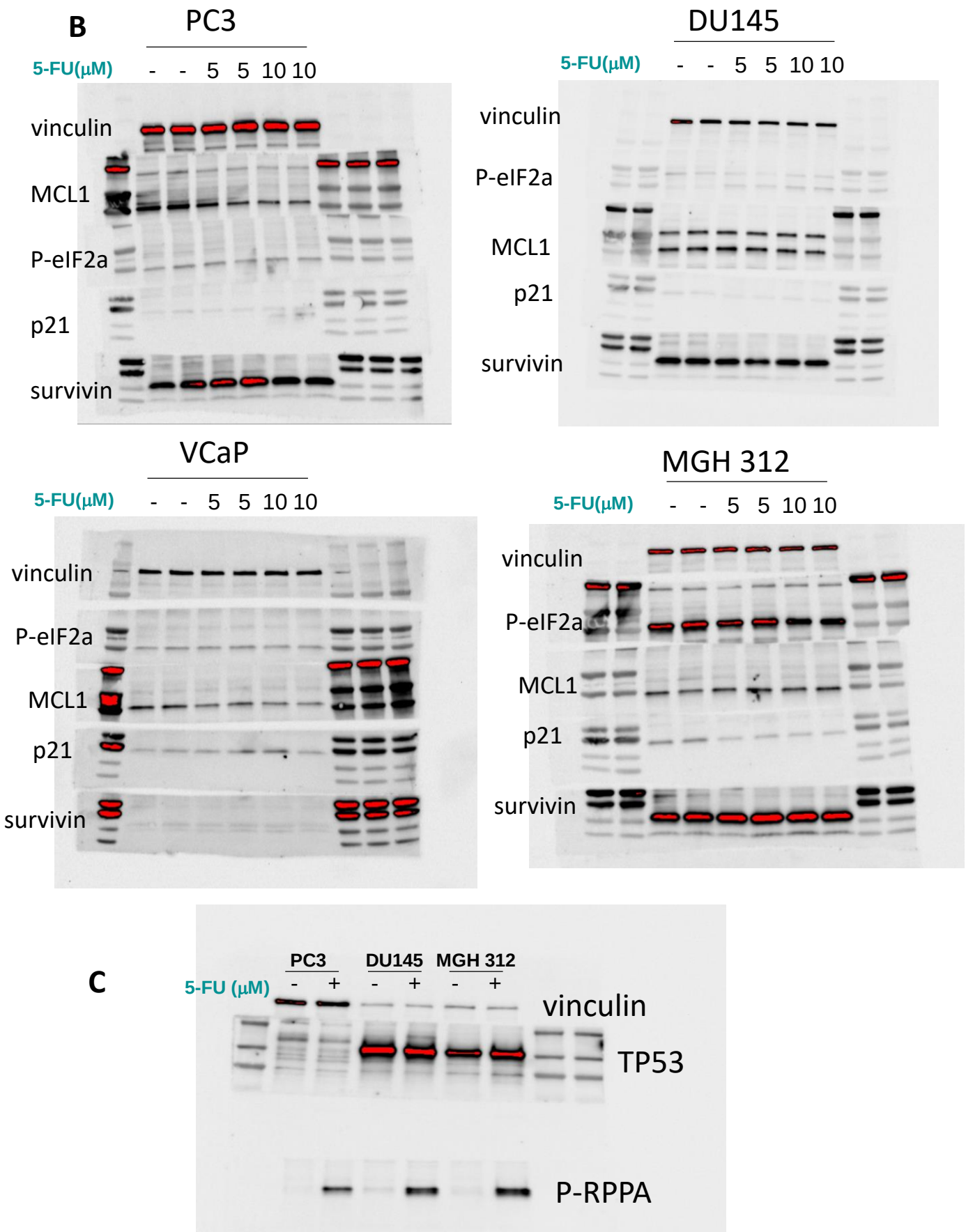

**Supplementary Figure S21.** Uncropped gels related to Figure S7.



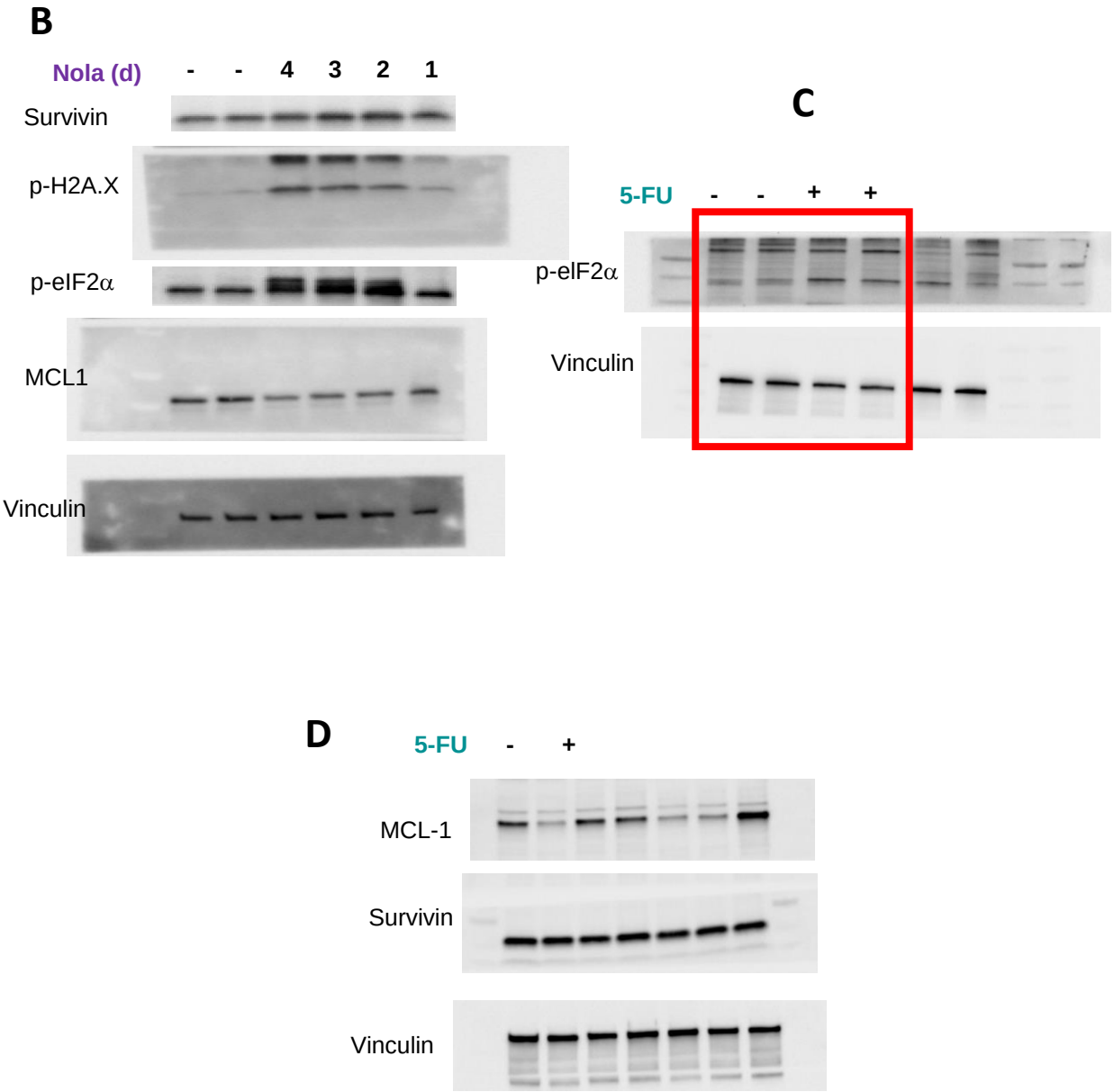

**Supplementary Figure S22.** Uncropped gels related to Figure S8.




**Supplementary Figure S23.** Uncropped gels related to Figure S9.




**Supplementary Figure S24.** Uncropped gels related to Figure S10.




**Supplementary Figure S25.** Uncropped gels related to Figure S11.