# Supplemental Table S2

| **Ingenuity Canonical Pathways** | -log(p-value) |
|---|---|
| EIF2 Signaling | 28.70 |
| Protein Ubiquitination Pathway | 25.80 |
| Regulation of eIF4 and p70S6K Signaling | 17.20 |
| tRNA Charging | 13.20 |
| mTOR Signaling | 11.80 |
| p70S6K Signaling | 11.20 |
| Actin Cytoskeleton Signaling | 10.30 |
| Purine Nucleotides De Novo Biosynthesis II | 7.35 |
| PI3K/AKT Signaling | 7.27 |
| Systemic Lupus Erythematosus Signaling | 6.58 |
| RAN Signaling | 6.15 |
| 14-3-3-mediated Signaling | 6.01 |
| Cell Cycle: G2/M DNA Damage Checkpoint Regulation | 6.00 |
| Breast Cancer Regulation by Stathmin1 | 5.84 |
| VEGF Signaling | 5.82 |
| Role of NFAT in Regulation of the Immune Response | 5.77 |
| Signaling by Rho Family GTPases | 5.71 |
| ILK Signaling | 5.60 |
| Fcγ Receptor-mediated Phagocytosis in Macrophages and Monocytes | 5.40 |
| Glycolysis I | 5.20 |
| Clathrin-mediated Endocytosis Signaling | 5.10 |
| Tight Junction Signaling | 5.00 |
| Germ Cell-Sertoli Cell Junction Signaling | 4.90 |
| Gap Junction Signaling | 4.90 |
| Ephrin B Signaling | 4.80 |
| Huntington's Disease Signaling | 4.79 |
| B Cell Development | 4.67 |
| Integrin Signaling | 4.66 |
| CD28 Signaling in T Helper Cells | 4.65 |
| B Cell Receptor Signaling | 4.49 |
| DNA Double-Strand Break Repair by Non-Homologous End Joining | 4.44 |
| RhoA Signaling | 4.35 |
| Methionine Degradation I (to Homocysteine) | 4.24 |
| CTLA4 Signaling in Cytotoxic T Lymphocytes | 4.23 |
| PKCθ Signaling in T Lymphocytes | 4.10 |
| Protein Kinase A Signaling | 4.06 |
| FAK Signaling | 3.98 |
| fMLP Signaling in Neutrophils | 3.95 |
| PRPP Biosynthesis I | 3.88 |
| 5-aminoimidazole Ribonucleotide Biosynthesis I | 3.88 |
| G Beta Gamma Signaling | 3.87 |
| Virus Entry via Endocytic Pathways | 3.81 |
| RhoGDI Signaling | 3.74 |
| ERK/MAPK Signaling | 3.69 |
| PI3K Signaling in B Lymphocytes | 3.57 |
| Calcium Signaling | 3.54 |
| Cell Cycle Control of Chromosomal Replication | 3.50 |
| Phospholipase C Signaling | 3.50 |
| α-Adrenergic Signaling | 3.41 |
| Antigen Presentation Pathway | 3.34 |
| Telomerase Signaling | 3.27 |
| Crosstalk between Dendritic Cells and Natural Killer Cells | 3.26 |
| Telomere Extension by Telomerase | 3.18 |
| Sertoli Cell-Sertoli Cell Junction Signaling | 3.18 |
| Aldosterone Signaling in Epithelial Cells | 3.13 |
| Regulation of Actin-based Motility by Rho | 3.06 |
| Mismatch Repair in Eukaryotes | 3.03 |
| FcγRIIB Signaling in B Lymphocytes | 3.03 |

**Supplemental Table S2. PU-H71 proteomics reveals teHsp90 chaperoned pathways**. (**A**) The union of the proteins identified in OCI-Ly1 and OCI-Ly7 PU-H71 proteomics assay were subjected to Ingenuity Pathway Analysis. Significantly represented pathways are listed here. BCR pathway is highlighted in orange.

# Supplementary Table S3

**A**

| | Toxicity Day 13 | | | | Washout Day 27 | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Test** | **Control** | **Ibrutinib** | **PUH71** | **Combination** | **Control** | **Ibrutinib** | **PUH71** | **Combination** | **Reference** |
| WBC | 6.87±2.09 | 7.78±3.36 | 4.67±1.82 | 5.17±0.87 | 8.17±3.36 | 6.77±0.76 | 6.64±3.01 | 7.12±0.37 | 6.4±2.5 K/uL |
| RBC | 11.00±0.54 | 10.49±0.64 | 10±0.62 | 9.47±0.42 | 11.32±0.14 | 11.46±0.45 | 9.57±0.48 | 9.60±0.11 | 8.53±0.5 M/uL |
| Hemoglobin | 16.03±0.74 | 15.5±0.53 | 14.4±0.79 | 14.33±0.61 | 16.67±0.25 | 16.67±0.50 | 14.63±0.31 | 14.55±0.07 | 14.5±1.1 g/dL |
| Platelets | 878±17.35 | 491.67±138.60 | 562±229.1 | 681±230.12 | 713.67±299.95 | 612±369.78 | 1156±692.60 | 1385±31.11 | 799-1300 K/uL |
| Hematocrit | 57.8±3.75 | 54.17±4.40 | 48.6±3.72 | 44.83±3.18 | 56.83±1.98 | 57.60±2.17 | 48.83±2.80 | 48.80±0.42 | 32-54 % |
| Neutrophils | 1±0.89 | 0.98±0.97 | 0.77±0.23 | 1.54±0.92 | 1.51±1.81 | 1.64±1.05 | 1.04±0.22 | 1.31±0.28 | 0-1.8 K/uL |
| Lymphocytes | 5.28±2.04 | 6.33±2.65 | 3.71±1.87 | 3.32±157 | 6.29±4.47 | 4.51±1.08 | 5.08±2.40 | 5.33±0.64 | 2.5-10 K/uL |
| Monocytes | 0.35±0.19 | 0.34±0.15 | 0.11±0.04 | 0.22±0.13 | 0.28±0.33 | 0.44±0.35 | 0.31±0.31 | 0.25±0.00 | 0-0.2 K/uL |
| Eosinophils | 0.23±0.22 | 0.12±0.04 | 0.07±0.07 | 0.08±0.05 | 0.08±0.02 | 0.16±0.01 | 0.18±0.07 | 0.24±0.01 | 0-0.5 K/uL |
| Basophils | 0.01±0.00 | 0.007±0.006 | 0.01±0.00 | 0.02±0.01 | 0.01±0.00 | 0.01±0.01 | 0.02±0.02 | 0.01±0.01 | 0-0.4 K/uL |
| Albumin | 4.35±0.07 | 3.13±0.12 | 2.7±0.17 | 3.15±0.64 | 3.23±0.06 | 3.87±0.06 | 3.3±0.61 | 3.1±0.26 | 2.5-3.9 g/dL |
| Total Protein | 7.3±0.00 | 5.53±0.15 | 4.73±0.45 | 5.55±0.919 | 5.6±0.1 | 6.5±0.00 | 5.57±0.72 | 5.43±0.38 | 4.1-6.4 g/dL |
| Globulin | 3.0±0.07 | 2.4±0.1 | 2.03±0.47 | 2.4±0.28 | 2.37±0.06 | 2.63±0.06 | 2.27±0.12 | 2.33±0.12 | 1.3-2.8 g/dL |
| Total Bilirubin | 0.9±0.14 | 0.27±0.12 | 0.23±0.06 | 0.7±0.28 | 0.27±0.12 | 0.83±0.05 | 0.63±0.15 | 0.43±0.15 | 0-0.3 mg/dL |
| Creatinine | 0.30±0.07 | 0.26±0.06 | 0.16±0.02 | 0.2±0.07 | 0.27±0.06 | 0.26±0.02 | 0.24±0.02 | 0.23±0.03 | 0.1-0.6 mg/dL |
| Cholesterol | 246.5±3.54 | 111.33±16.01 | 155.5±23.33 | 159.5±58.69 | 95.67±8.74 | 154±13 | 117±27 | 97.67±15.63 | 70-100 mg/dL |

**Supplemental Table S3**. **Ibrutinib and PU-H71 combination is non-toxic in mice.** (**A**) Blood from mice tested for ibrutinib-PU-H71 toxicity as in Supplementary Figure 2 was collected after 12 days of treatment (Toxicity) and in a second cohort after a 2 week washout period (Washout). Blood was analyzed for differential blood cell count and chemistry panel.

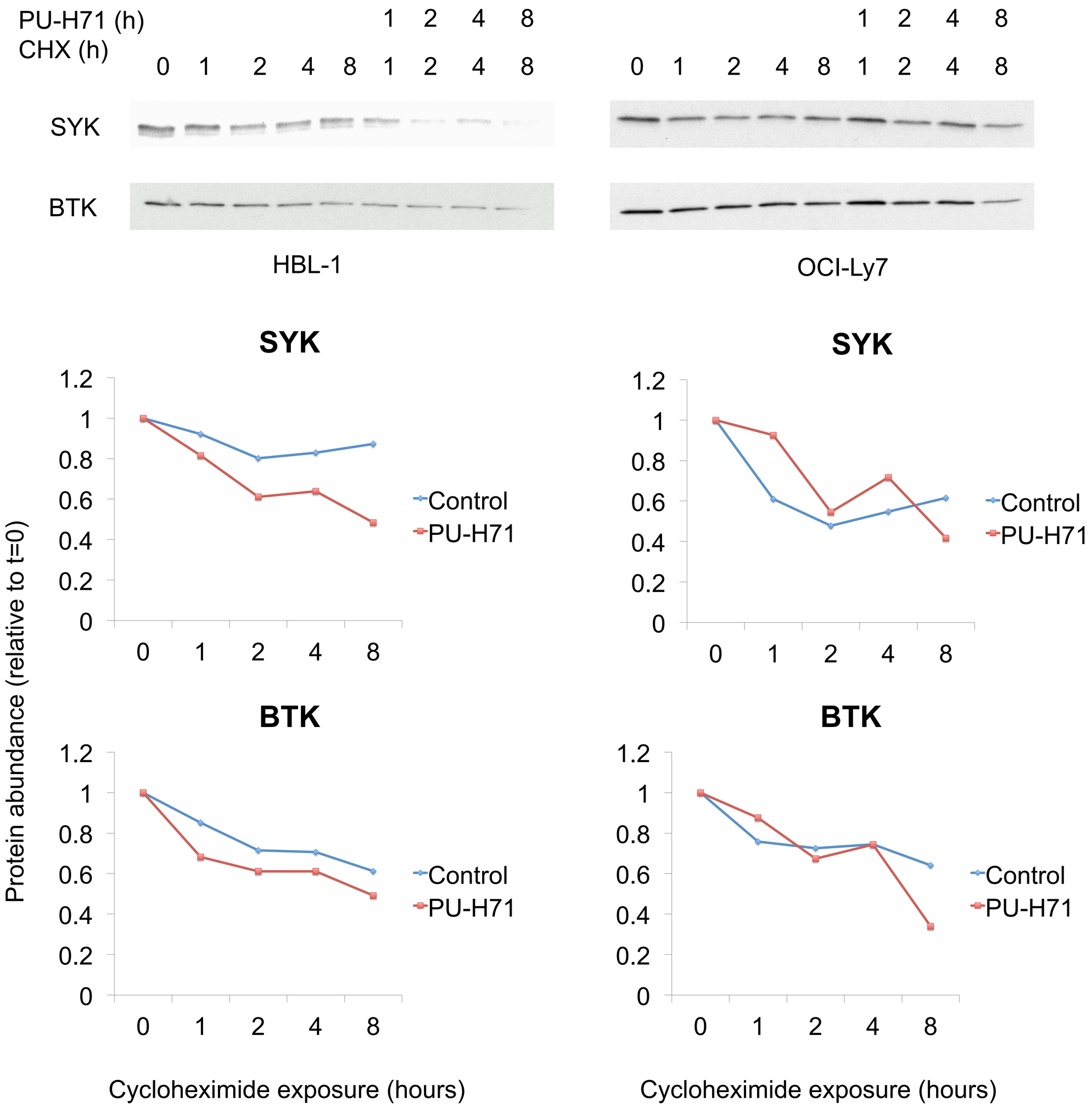
# Supplemental Table S4

**A**

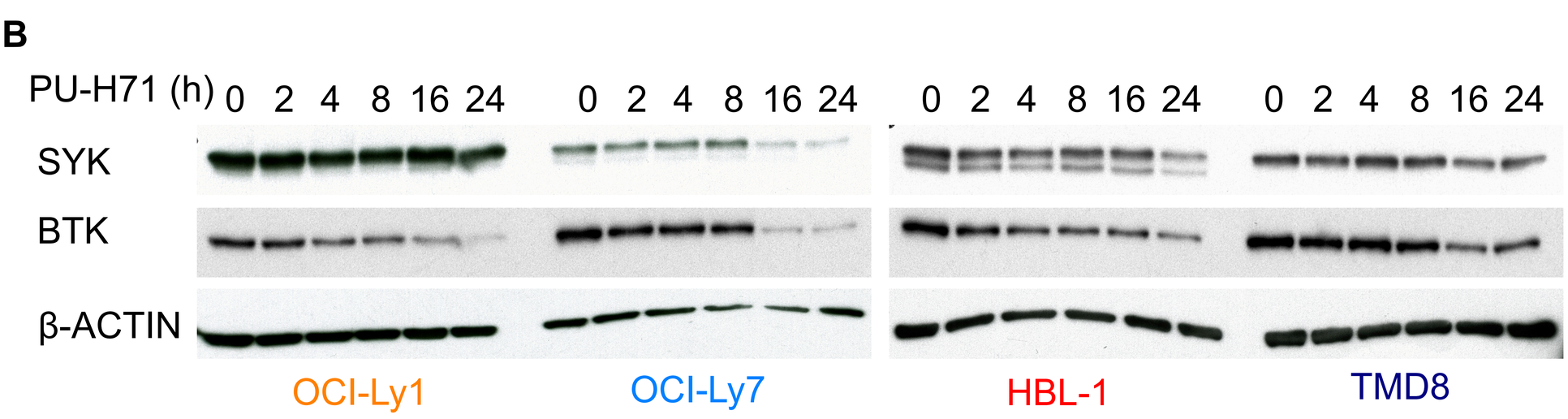
| Antigen | Clone | Catalog Number | Company |
|---|---|---|---|
| CD79A | HM47 | | Santa Cruz Biotechnology |
| CD79B | SN8 | | Santa Cruz Biotechnology |
| CD79B | FL-229 | | Santa Cruz Biotechnology |
| LYN | 44 | | Santa Cruz Biotechnology |
| SYK | 4D10 | | Santa Cruz Biotechnology |
| BTK | D3H5 | | Cell Signaling Technology |
| PLCγ2 | Q-20 | | Santa Cruz Biotechnology |
| BLNK | 2B11 | | Santa Cruz Biotechnology |
| HSP90 | | ab13495 | Abcam |
| HSP90 | H9010 | | StressMarq |
| β-Actin | AC-15 | | Sigma Aldrich |
| pSYK Y352 | | 2701 | Cell Signaling Technology |
| pBTK Y223 | | 5082 | Cell Signaling Technology |
| pPLCγ2 Y759 | | 3874 | Cell Signaling Technology |
| pLYN Y507 | | 2731 | Cell Signaling Technology |
| pLYN Y396 | | 1645-1 | Epitomics |
| AlexFluor 488 Mouse anti- SYK (pY348) | I120-722 | 560081 | BD Biosciences |
| AlexaFLuor 488 Mouse IgG1 K | | 2317666 | BD Biosciences |
| PE Mouse anti-BTK (pY223) | N35-86 | 562753 | BD Biosciences |
| PE Mouse IgG1 K | | 551436 | BD Biosciences |
| AlexaFluor 647 Mouse anti-PLCγ2 Y759 AlexaFluor647 | K86-689.37 | 558498 | BD Biosciences |
| AlexaFluor 647 MouseIgG1 K | | 557783 | BD Biosciences |

**Supplemental Table S4. Antibodies used for experiments**

# Supplemental Figure 1

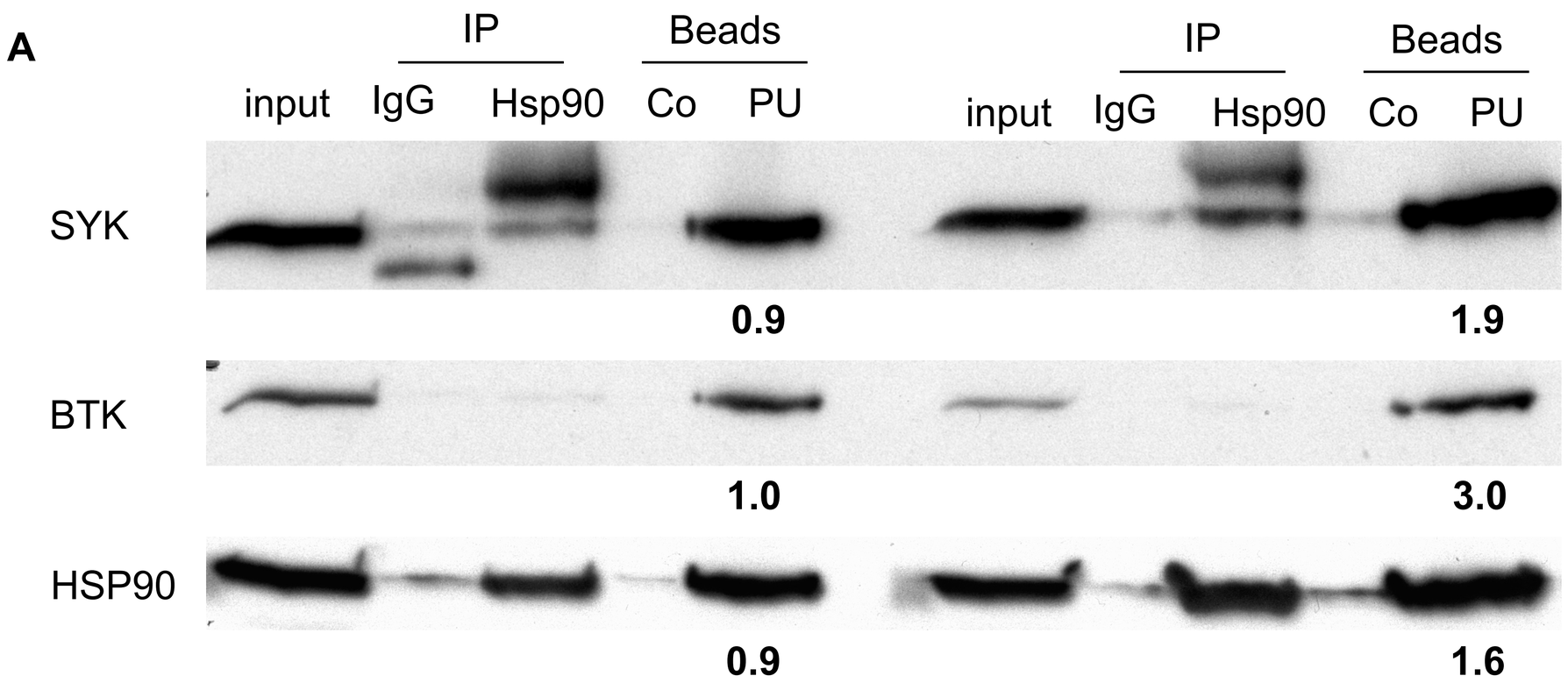



**B**



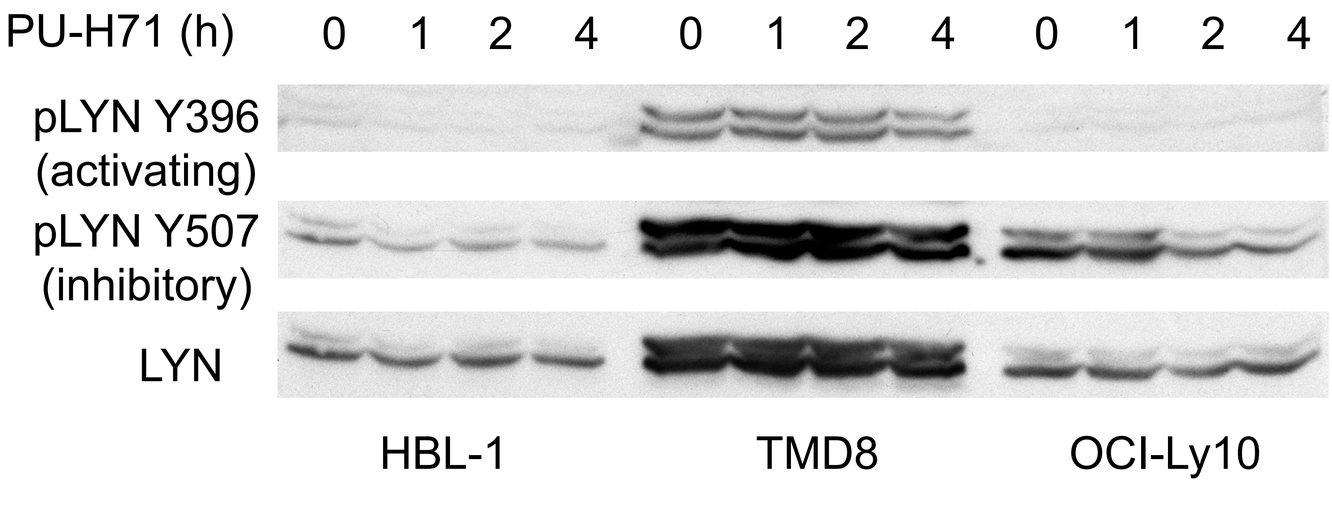

**Supplemental Figure 1. HSP90 inhibition induces degradation of SYK and BTK.** HBL-1 and OCI-Ly7 cells were exposed to cycloheximide (100 µg/mL) and/or PU-H71 (1 µM) for the times indicated. Lysates were subjected immunoblotting for the indicated proteins. The relative abundance of each protein relative to t = 0h was quantified by densitometry and is shown in the bar graph below. (**B**) Cells were exposed to PU-H71 (1 µM) for the times indicated. Lysates were subjected to immunoblotting with the indicated antibodies

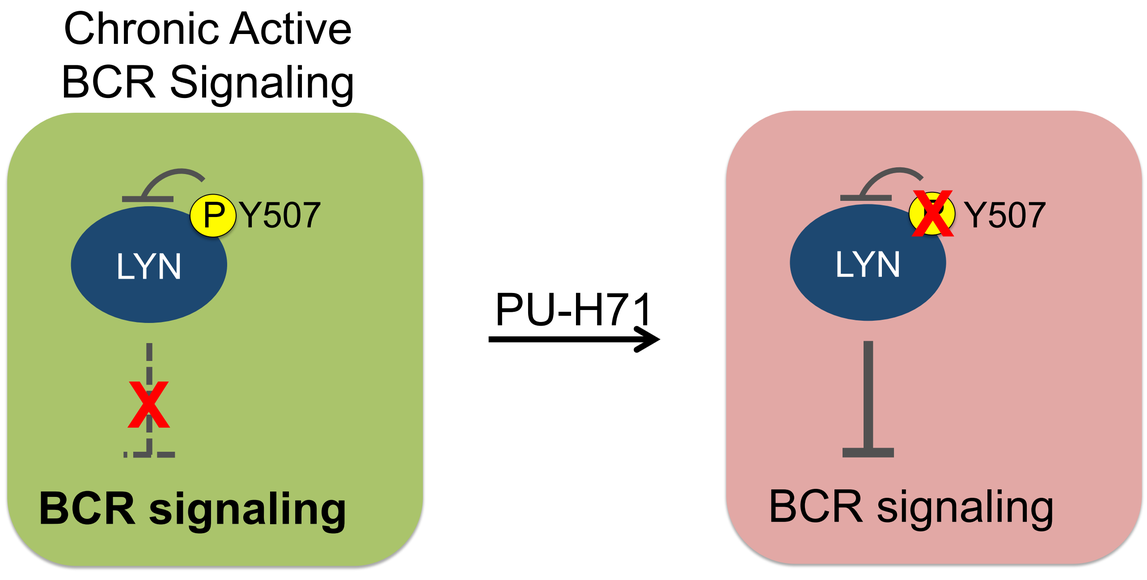
# Supplemental Figure 2




**Supplemental Figure 2. HSP90 complexes in DLBCL and normal cells.** (**A**) Lysates from $CD19^+$ cells from peripheral blood and OCI-Ly1 cells were subjected to immunoprecipitation with antibodies to IgG or HSP90 or chemical precipitation with PU-H71 (PU) or control chemical (CO) beads followed by immunoblotting for the indicated proteins. The relative abundance of protein precipitated by PU-H71 beads relative to input was quantified by densitometry and is shown numerically.

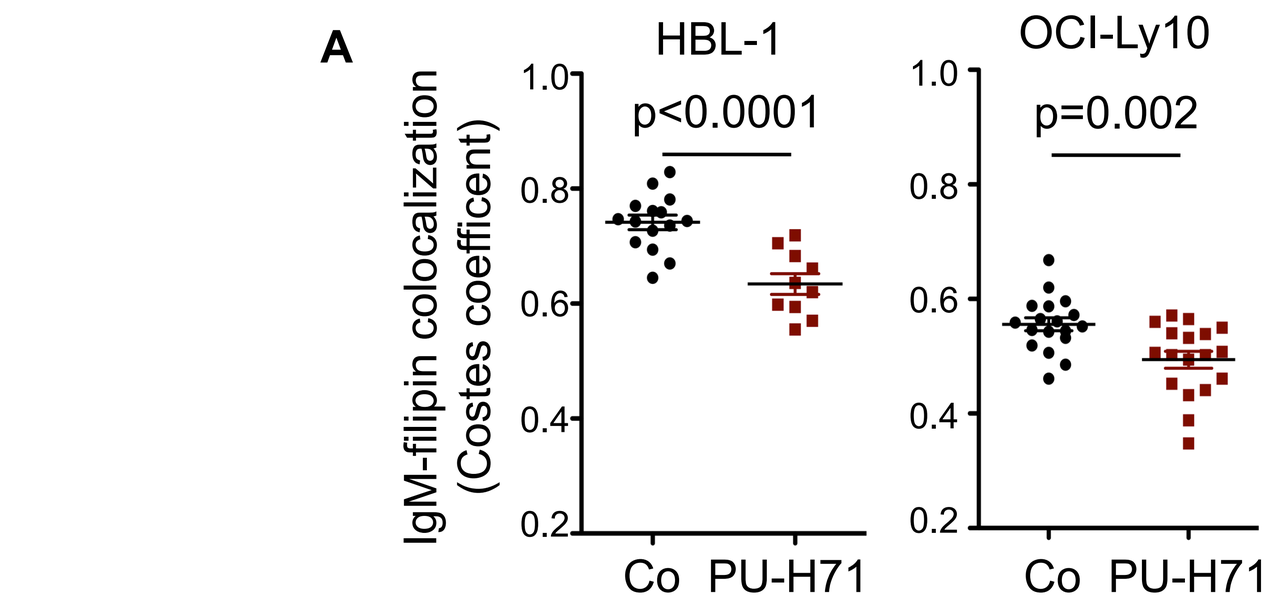
# Supplemental Figure 3

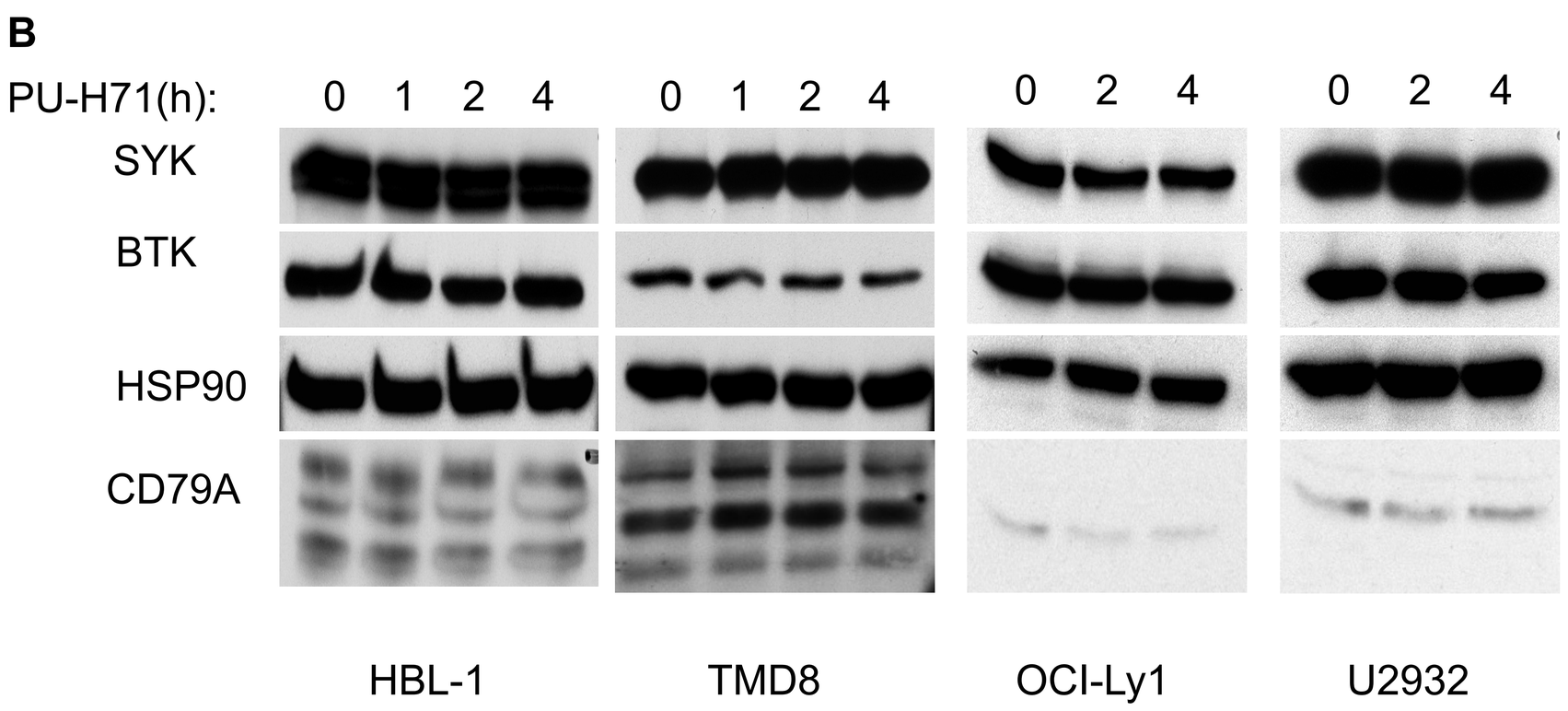


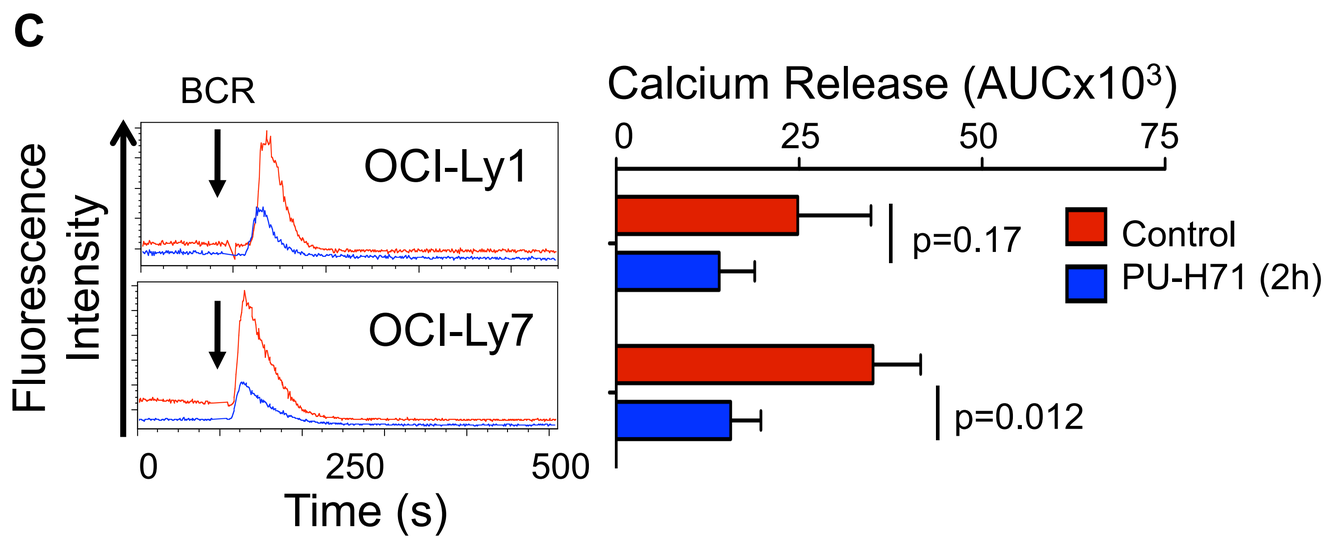



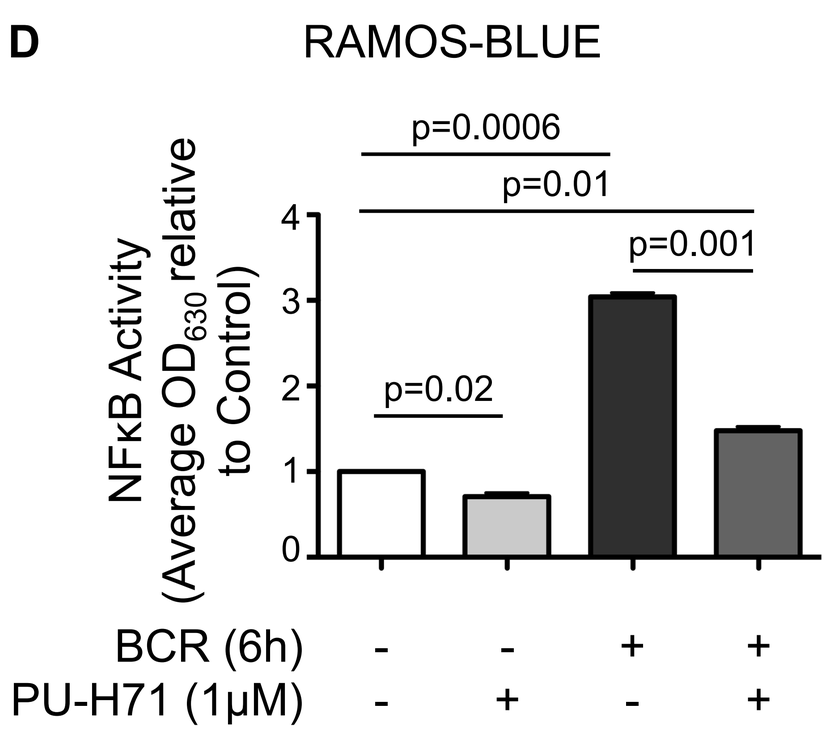

**Supplemental Figure 3. PU-H71 induces loss of LYN activity in DLBCL cells.** HBL-1, TMD8 and OCI-Ly10 cells were exposed PU-H71 (1 μM) for increasing time as indicated. Lysates were subjected to immunoblotting with the indicated antibodies.

# Supplemental Figure 4



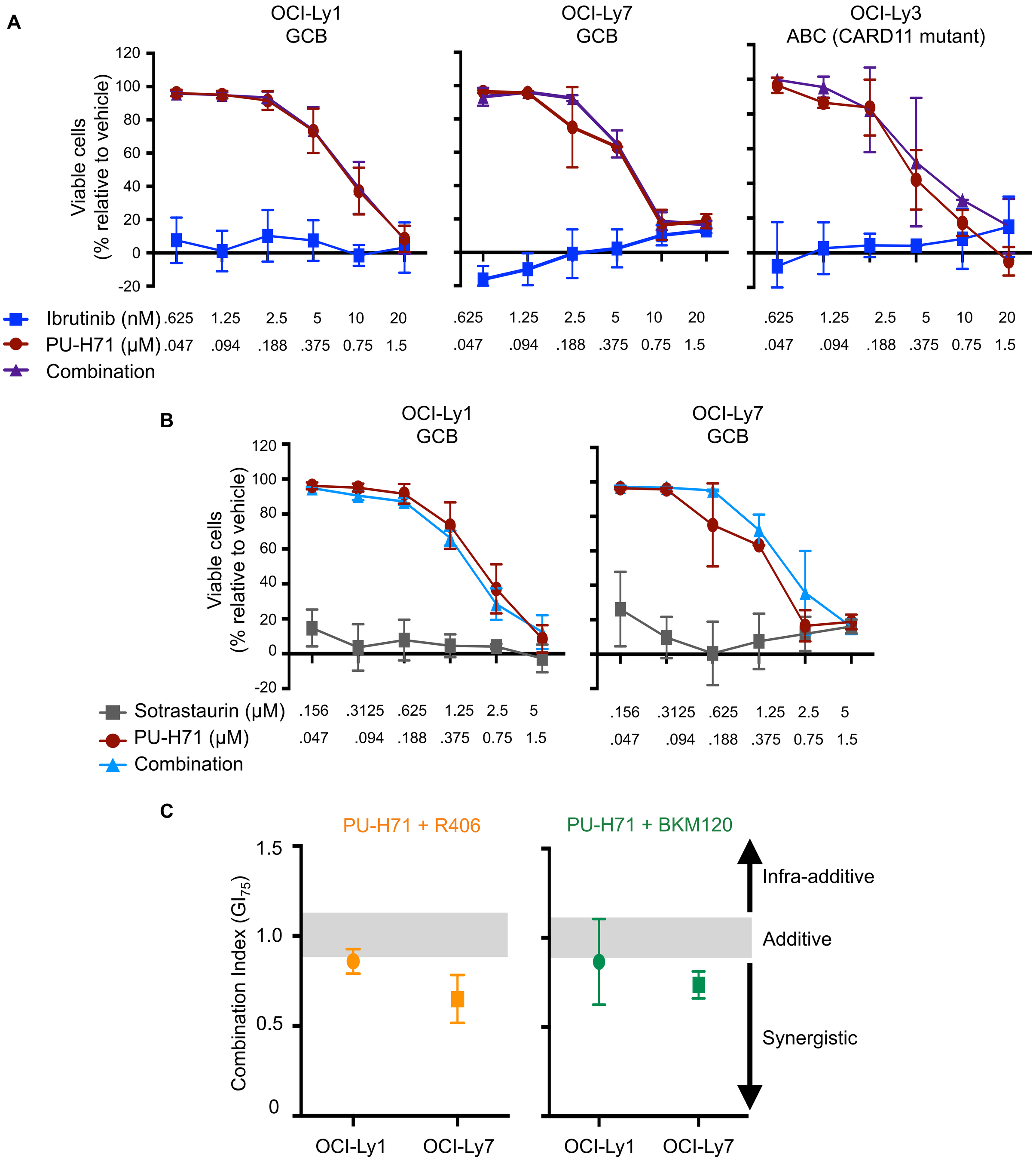



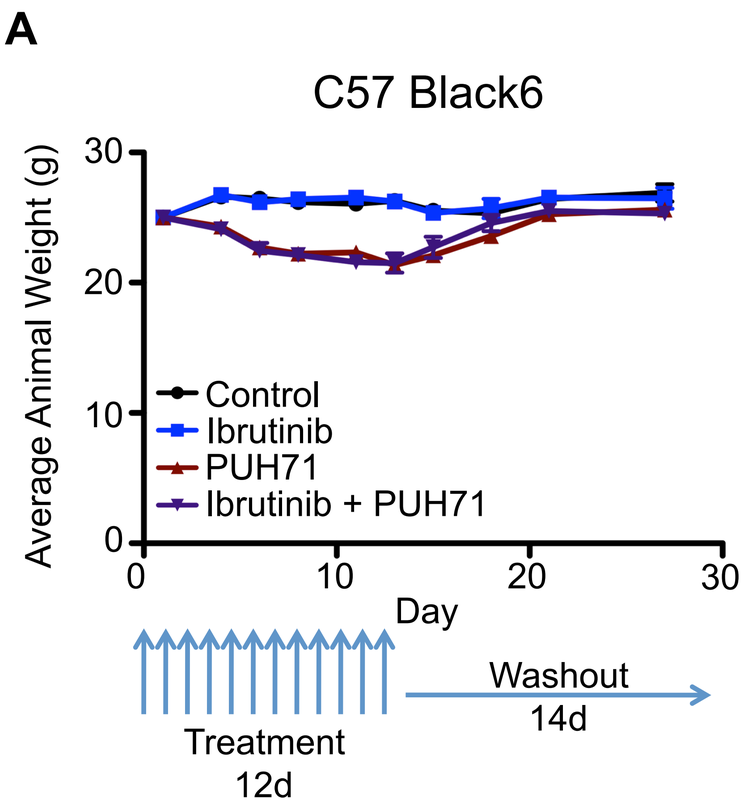

**Supplemental Figure 4. Inhibition of teHsp90 induces broad attenuation of BCR signaling at multiple nodes.** (**A**) IgM and filipin staining in HBL-1 and OCI-Ly10 cells exposed to vehicle or PU-H71 (1 μM, 4h) were visualized by confocal microscopy (60X). Colocalization was quantified (Costes method) for at least 10 high power fields. Two independent experiments. Unpaired t-test. (**B**) Lysates of DLBCL cell lines exposed to vehicle or PU-H71 (1 μM) were immunoblotted with the indicated antibodies. These immunoblots are the inputs for the immunoprecipitation experiments shown in Figure 4A. (**C**) OCI-Ly1 and OCI-Ly7 cells were treated with vehicle or PU-H71 (1 μM, 2h) then incubated with a fluorescent calcium indicator (Fluo 4AM, 2 μM, 30min). Calcium release was measured over time by flow cytometry before and after BCR stimulation (IgM+IgG 10 μg/mL at 120sec). Three biological replicates, unpaired t-test. (**D**) Ramos-Blue cells were treated with vehicle, PU-H71 (1 μM), IgM+IgG (10 μg/mL) or PU-H71 and IgM+IgG (6h). NFκB reporter activity was measured by colorimetric assay. Unpaired t-test.

# Supplemental Figure 5




**Supplemental Figure 5. PU-H71 and BCR pathway inhibitors combine in GCB DLBCL cells.** (**A**) OCI-Ly1, OCI-Ly7 and OCI-Ly3 cells were treated with a dose curve of ibrutinib, PU-H71 or the combination. Cell viability was measured using a luminescent ATP method and normalized to vehicle-treated controls. Dose-effect curves show mean ± SEM, 3 biological replicates. (**B**) OCI-Ly1 and OCI-Ly7 cells were treated with a dose curve of sotrastuarin, PU-H71 or the combination. Cell viability was measured using a luminescent ATP method and normalized to vehicle-treated controls. Dose-effect curves show mean ± SEM, 3 biological replicates. (**C**) OCI-Ly1 and OCI-Ly7 cells were treated with a dose curve of R406 or BKM120, PU-H71 or the combination indicated. Cell viability was measured using a luminescent ATP method and normalized to vehicle-treated controls. Synergy was assessed using Compusyn. Combination Index values <0.9 are synergistic. Mean ± SEM, 3 biological replicates.

# Supplemental Figure 6




**Supplemental Figure 6. Ibrutinib and PU-H71 combination is non-toxic in mice**. (**A**) C57 Black mice (n=6/group until day 13) were treated with vehicle, ibrutinib (12.5 mg/kg/d ad libitum), PU-H71 (75 mg/kg/d i.p.) or the combination for 12 days. Animals were weighed every other day. Half of each group (n=3) was sacrificed after treatment for microscopic analysis of organs and blood. The other half (n=3) continued untreated for two weeks to serve as a “washout” group and were weighed every other day until sacrifice for organ and blood analysis.