cancers

MDPI

*Article*

# Oncogenic Kinase Cascades Induce Molecular Mechanisms that Protect Leukemic Cell Models from Lethal Effects of de novo dNTP Synthesis Inhibition

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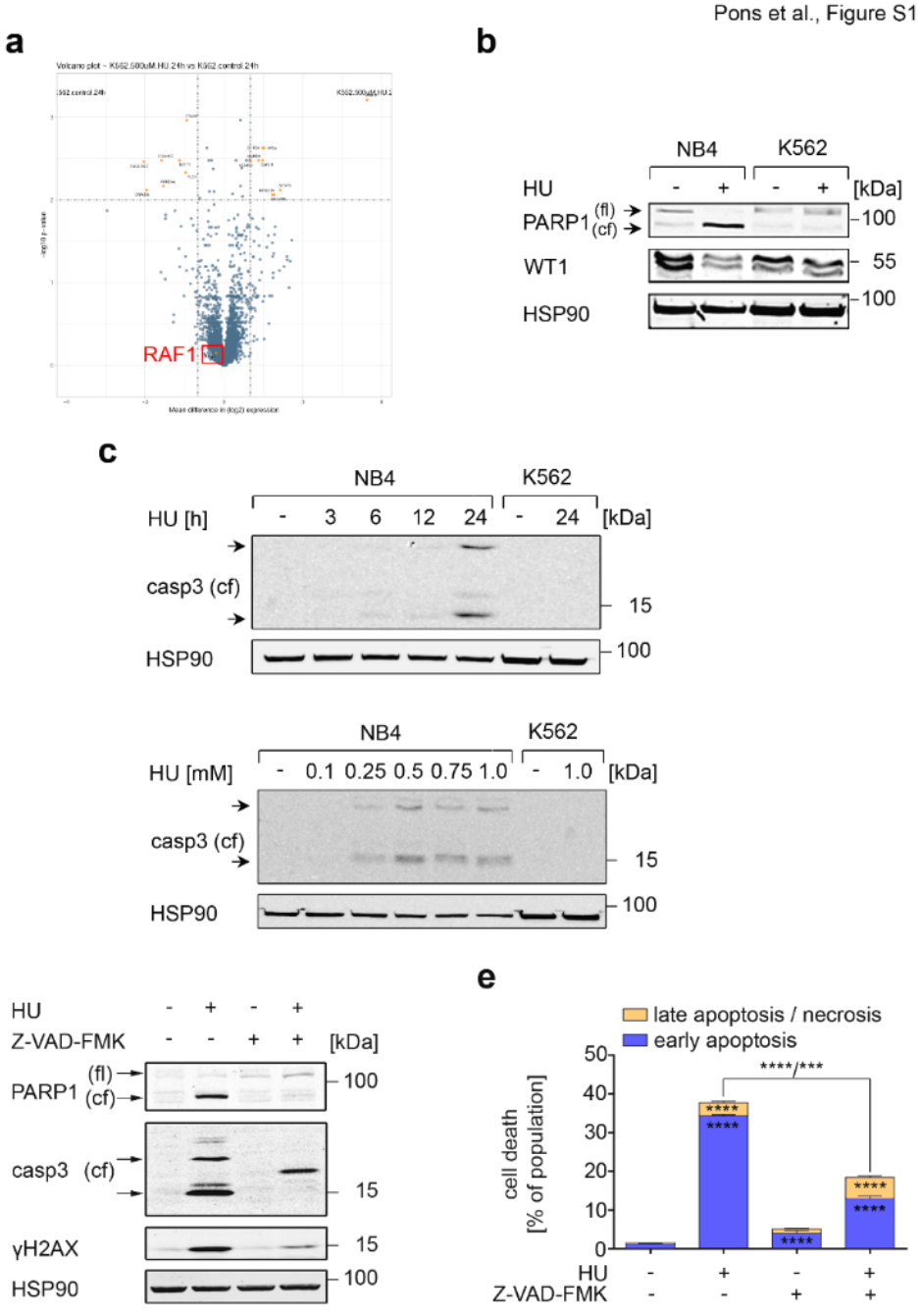
Supplementary Figures and Legends

Pons et al., Figure S1



**f**

**NB4 cells**

| Gene names | LFQ intensity - NB4 - con | LFQ intensity - NB4 - HU | Trend |
|---|---|---|---|
| Kinases | | | |
| **RAF1** | 1 | **0,00000001** | ↓↓↓↓ |
| TP53RK | 1 | 1,15 | ↑ |
| RBM4B;MST4 | 1 | 0,71 | ↓ |
| PAK2 | 1 | 0,37 | ↓ |
| EIF2AK2 | 1 | 0,75 | ↓ |
| CDK2 | 1 | 0,59 | ↓ |
| CMPK1 | 1 | 1,03 | ↔ |
| PIK3R1 | 1 | 2,27 | ↑ |
| CSK | 1 | 1,17 | ↑ |
| PXK | 1 | 0,85 | ↓ |
| MAPK1 | 1 | 1,78 | ↑ |
| SKP1 | 1 | 1,30 | ↑ |
| CDK1 | 1 | 0,49 | ↓ |
| ADK | 1 | 0,84 | ↓ |
| HK2 | 1 | 0,95 | ↓ |

red, higher/over 50% increase;
green, lower/over 50% decrease;
yellow, decrease or increase over 25% but below 50%;
↔ change below 5%; rise over 5%; ↓ decrease over 5%

**Figure S1.** Effects of hydroxyurea on apoptotic markers in NB4 and K562 cells. **a**) K562 cells were treated 5 mM HU for 24 h. Global scale proteomics was performed. Volcano plot shows no significant changes in RAF1 expression. **b**) Immunoblot of lysates from NB4 and K562 cells that were treated with 0.5 mM hydroxyurea (HU) for 24 h shows a loss of the apoptotic markers WT1 and PARP1 in NB4 cells; (fl) - full length, (cf) - cleaved form; HSP90 serves as loading control. **c**) Upper: NB4 and K562 cells were treated with 0.5 mM HU for 3-24 h. Cleaved caspase-3 (casp3 (cf), caspase 3 - cleaved form) was detected by immunoblot; HSP90 serves as loading control. Arrows indicate the active caspase-3 cleavage products. Lower: NB4 and K562 cells were treated with 0.1-1.0 mM HU for 24 h. Active caspase-3 was analyzed by immunoblot; HSP90 serves as loading control; arrows show active caspase-3 cleavage products. **d**) NB4 cells were treated with 50 μM Z-VAD-FMK for 1h before adding 0.5 mM HU for additional 24 h. Immunoblot shows expression of PARP1, caspase 3 and γH2AX; (fl) - full length, (cf) - cleaved form; HSP90 serves as loading control. **e**) Cells treated as in (e) were stained with annexin-V and PI and measured via flow cytometry for the induction of cell death. Calculation of cell death for K562 treated and stained as stated before; $n = 3 \pm$ SD; two-way ANOVA; Bonferroni's multiple comparisons test: ****$p < 0.0001$. **f**) NB4 cells were treated with 0.5 mM HU for 24 h. Global scale proteomics was performed. Heatmap shows how kinase expression changed following the treatment; $n = 3$.

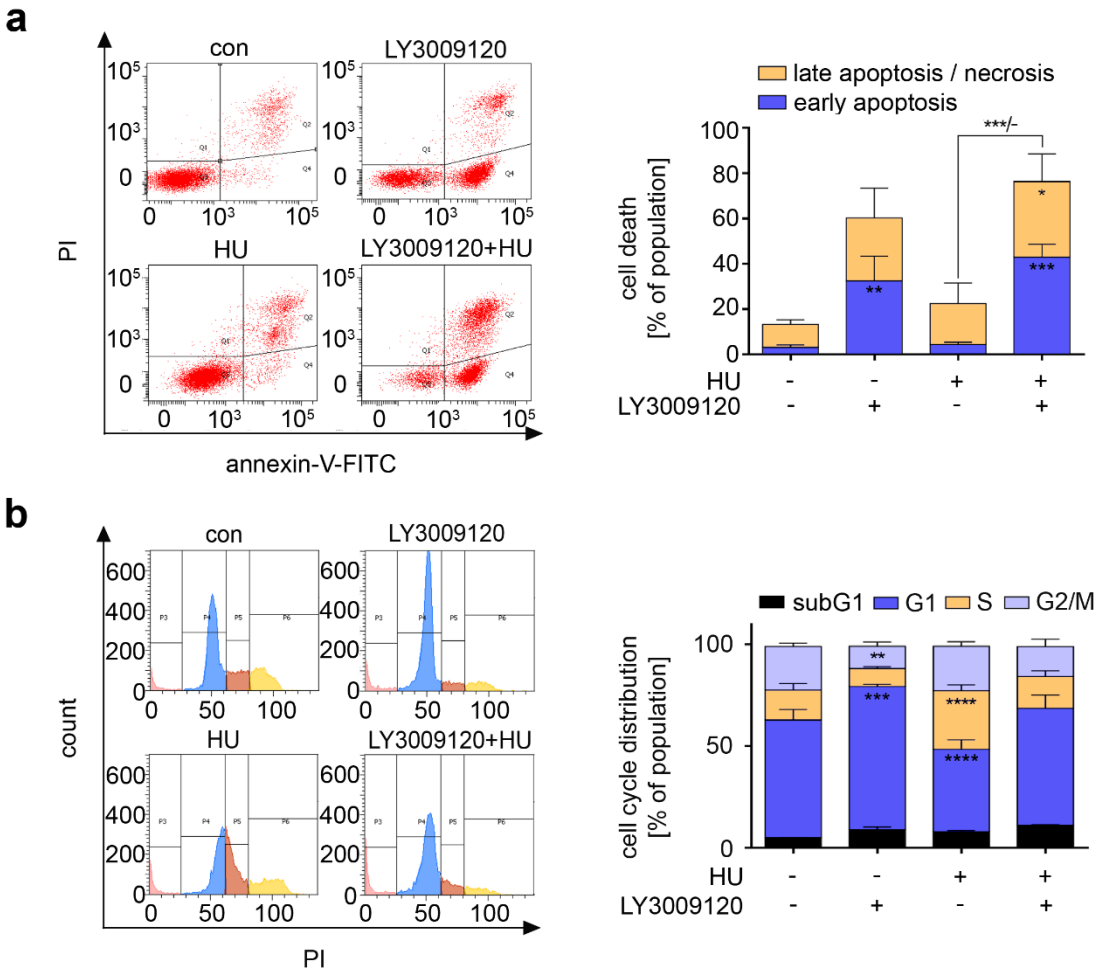



**Figure S2.** RAF Inhibition and hydroxyurea combine favorably against KYO-01 cells**.** a) Left: Exemplary dot plots of KYO-01 cells exposed to 1 µM LY3009120 and 1 mM hydroxyurea (HU) for 24 h. Cells were stained with annexin-V and PI and measured via flow cytometry for the induction of cell death. Right: Calculation of cell death for K562 treated and stained as stated before; $n = 3 \pm$ SD; two-way ANOVA; Bonferroni's multiple comparisons test: *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$. b) Left: Representative histograms of the cell cycle of fixed and PI stained KYO-01 cells that were treated with 1 µM LY3009120 and 1 mM HU for 24 h. Right: Cell cycle distributions of such cells; $n = 3 \pm$ SD; two-way ANOVA; Bonferroni's multiple comparisons test: **$p < 0.01$; ***$p < 0.001$.

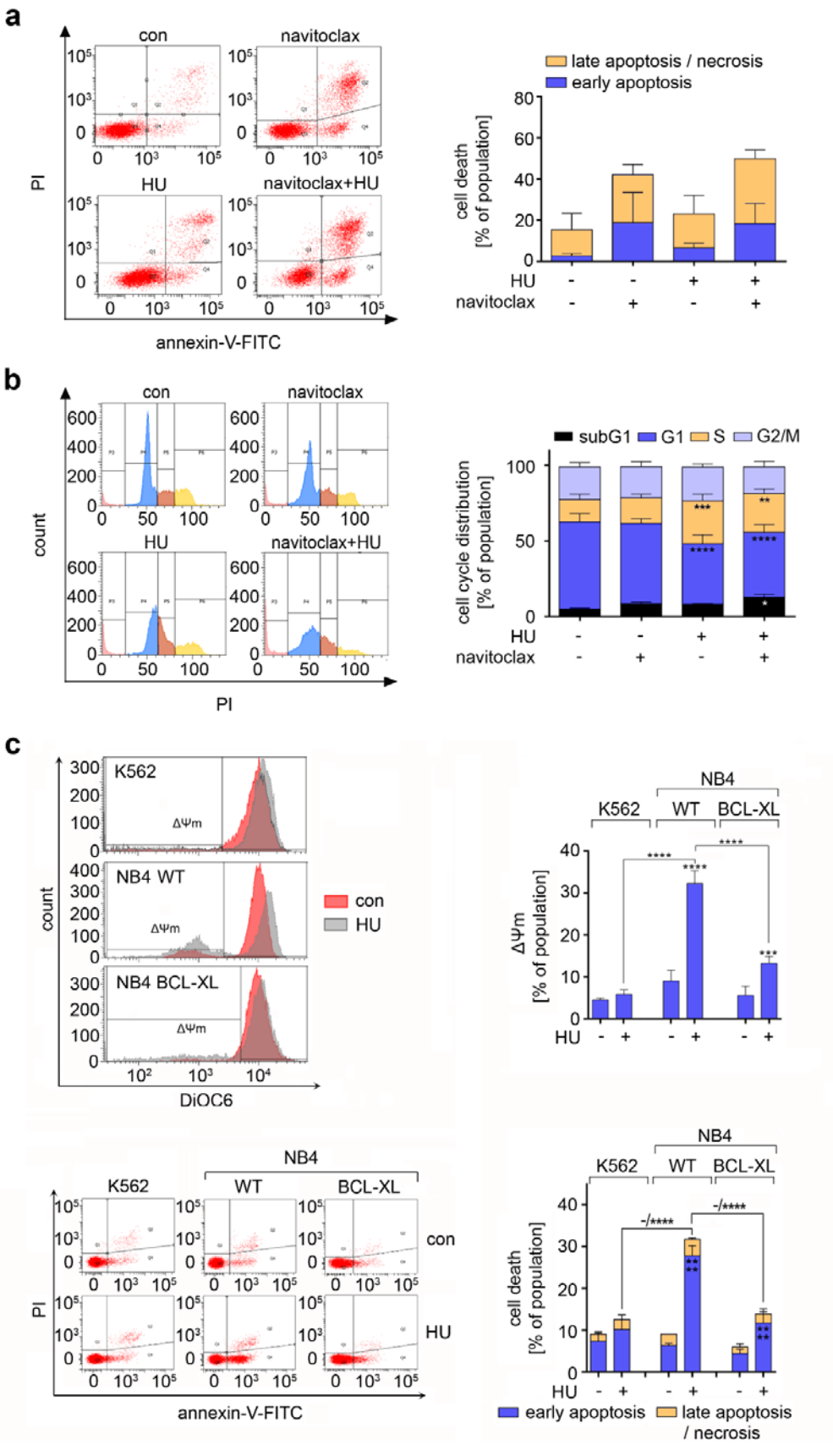



**Figure S3.** Navitoclax and hydroxyurea efficiently kill KYO-01 cells. **a**) Left: Exemplary dot plots of KYO-01 cells treated with 1 μM navitoclax and 1 mM hydroxyurea (HU) for 24 h. Cells were stained with annexin-V-FITC and PI and analyzed by flow cytometry. Right: Cell death calculations of annexin and PI stained KYO-01 cells treated as mentioned before; $n$ = 3 ± SD; two-way ANOVA; Bonferroni's multiple comparisons test; not significant. (**b**) Such cells were also analyzed for

cell cycle distributions by flow cytometry. Left: Shown are representative histograms. Right: Cell cycle distributions; two-way ANOVA; Bonferroni's multiple comparisons test: $^{**}p < 0.01$; $^{**}$ $p < 0.05$; $^{***}p < 0.001$. **c**) Upper left: Representative overlay histogram of K562 and NB4 wild-type (WT) cells or NB4 cells with BCL-XL overexpression were treated with 0.5 mM HU for 16 h. Cells were stained with DiOC6 to measure ΔΨm. Lower right: DiOC6 stained cells treated as indicted were analyzed for ΔΨm; $n$ = 3- ± SD; one-way ANOVA; Bonferroni's multiple comparisons test: $^{***}p < 0.001$; $^{***}p < 0.0$ = 01. Lower left: Exemplary dot plots of K562 an NB4 cells treated with 0.5 mM HU for 16 h. Cells were stained with annexin-V-FITC and PI and analyzed by flow cytometry. Upper right: Cell death calculations of annexin-V/PI-stained cells treated as mentioned before; $n = 3 \pm$ SD; two-way ANOVA; Bonferroni's multiple comparisons test: $^{****}p < 0.0001$.

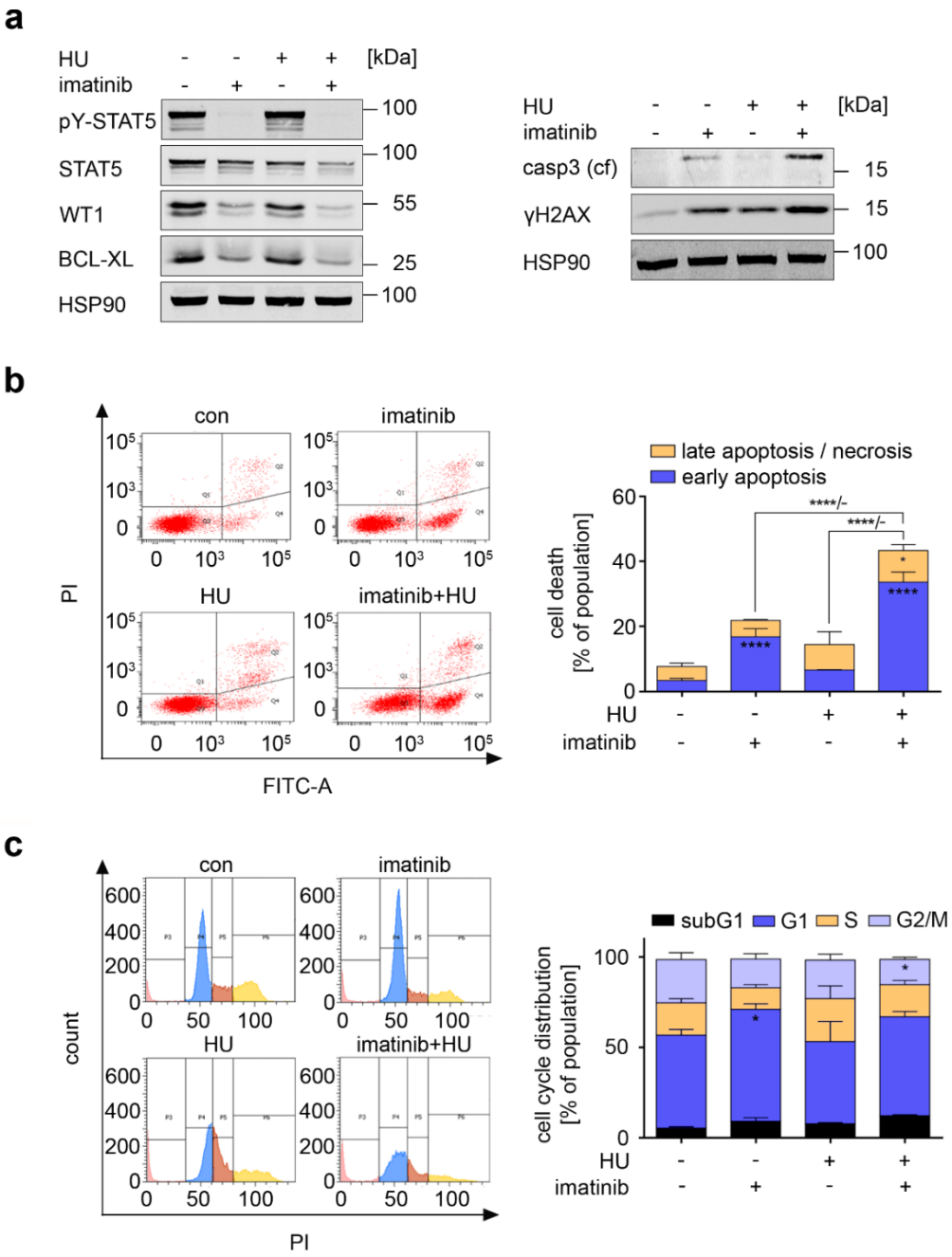



**Figure S4.** Imatinib and hydroxyurea combine favorably against KYO-01 cells **a)** KYO-01 cells were treated with 1 μM imatinib and 1 mM hydroxyurea (HU). Immunoblot analysis shows pY-STAT5, STAT5, WT1, BCL-XL, cleaved caspase-3 (casp3 (cf), caspase 3 - cleaved form), and γH2AX; HSP90 serves as loading control. **b**) Left: Representative dot plots of KYO-01 cells treated with 1 μM imatinib and 1 mM HU for 24 h. Cells were stained with annexin and PI to determine the induction of cell death. Right: Cell death was measured for cells treated as mentioned before; $n = 3 \pm$ SD; two-way ANOVA; Bonferroni's multiple comparisons test: $^{*}p < 0.05$; $^{****}p < 0.0001$. **c**) Cells treated as in B) were fixed and stained with PI to analyze cell cycle distributions. Left: Shown are exemplary histograms. Right: Cell cycle distributions of these cells; $n = 3$ ± SD; two-way ANOVA; Bonferroni's multiple comparisons test: $^{*}p < 0.05$.

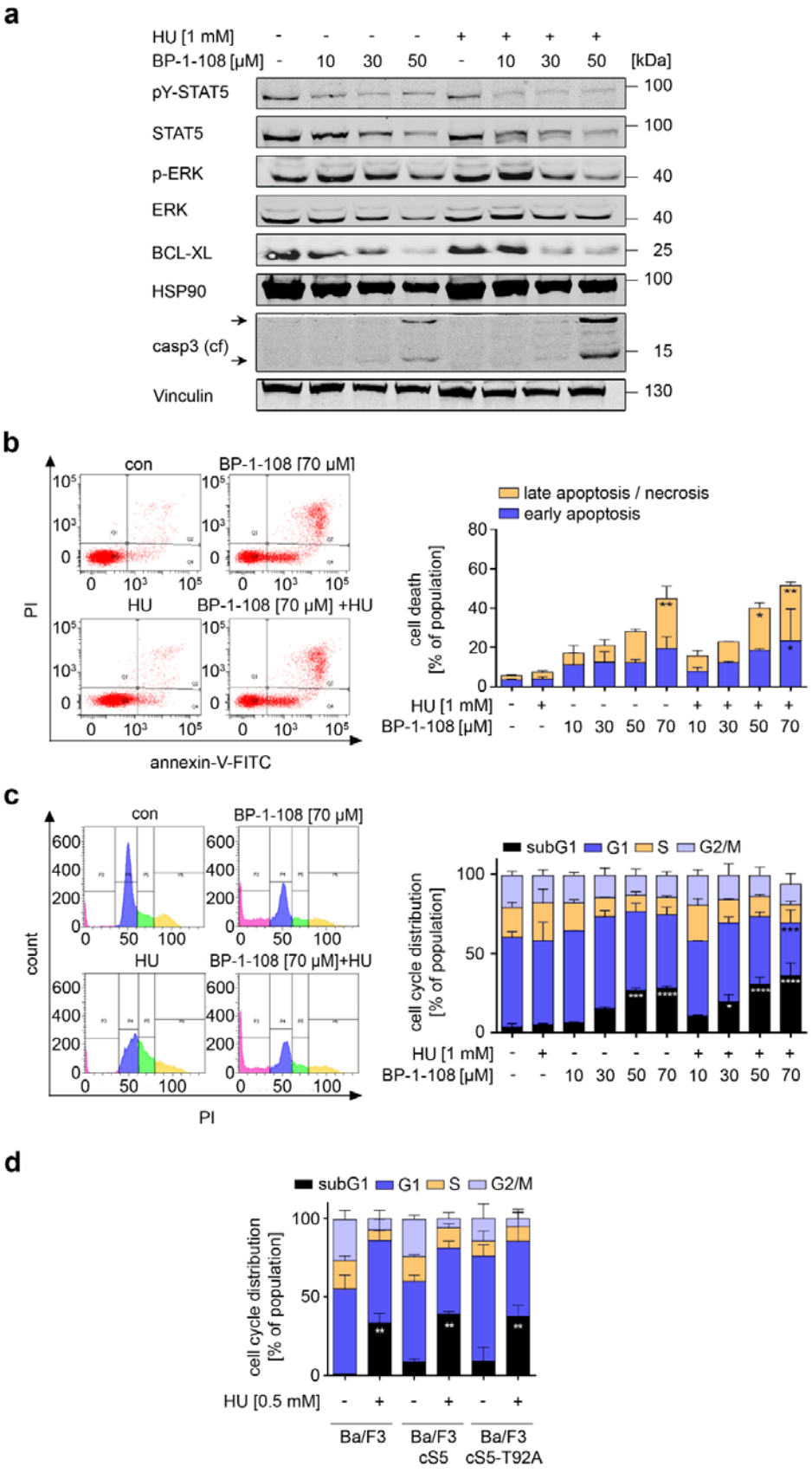



**Figure S5.** Inhibition of STAT5 is cytotoxic for K562 cells. **a**) Immunoblot of K562 cells treated with 1 mM hydroxyurea (HU) and increasing doses (10-50 μM) of the STAT5 inhibitor BP-1-108 for 24 h. Shown are pY-STAT5, STAT5, *p*-ERK, ERK, BCL-XL, and cleaved caspase-3 (casp3 (cf), caspase 3 - cleaved form); HSP90 and vinculin serve as loading controls. **b**) Left: Exemplary dot plots of K562 cells exposed to 70 μM BP-1-108 and 1 mM HU for 24 h. Cells were stained with annexin-V and PI and measured via flow cytometry for the induction of cell death. Right: Cell death calculations of annexin

and PI stained K562 cells treated with 1 mM HU and 10-70 μM of BP-1-108 for 24 h; $n = 3 \pm SD$; two-way ANOVA; Bonferroni's multiple comparisons test: $^{*}p < .05$; $^{**}p < 0.01$. **c**) K562 cells treated as in B) were fixed and stained with PI to analyze cell cycle distributions. Left: Shown are exemplary histograms. Right: Cell cycle distributions of K562 cells treated with 1 mM HU and 10-70 μM of BP-1-108 for 24 h; $n = 3 \pm SD$; two-way ANOVA; Bonferroni's multiple comparisons test: $^{*}p < 0.05$; $^{***}p < 0.001$; $^{****}p < 0.0001$. **d**) Parental and cS5 (active) or cS5-T92A (inactive) transfected Ba/F3 cells were treated with 0.5 mM HU for 24 h and analyzed for cell cycle distributions; $n = 2 \pm SD$; two-way ANOVA; Bonferroni's multiple comparisons test: $^{**}p < 0.01$.