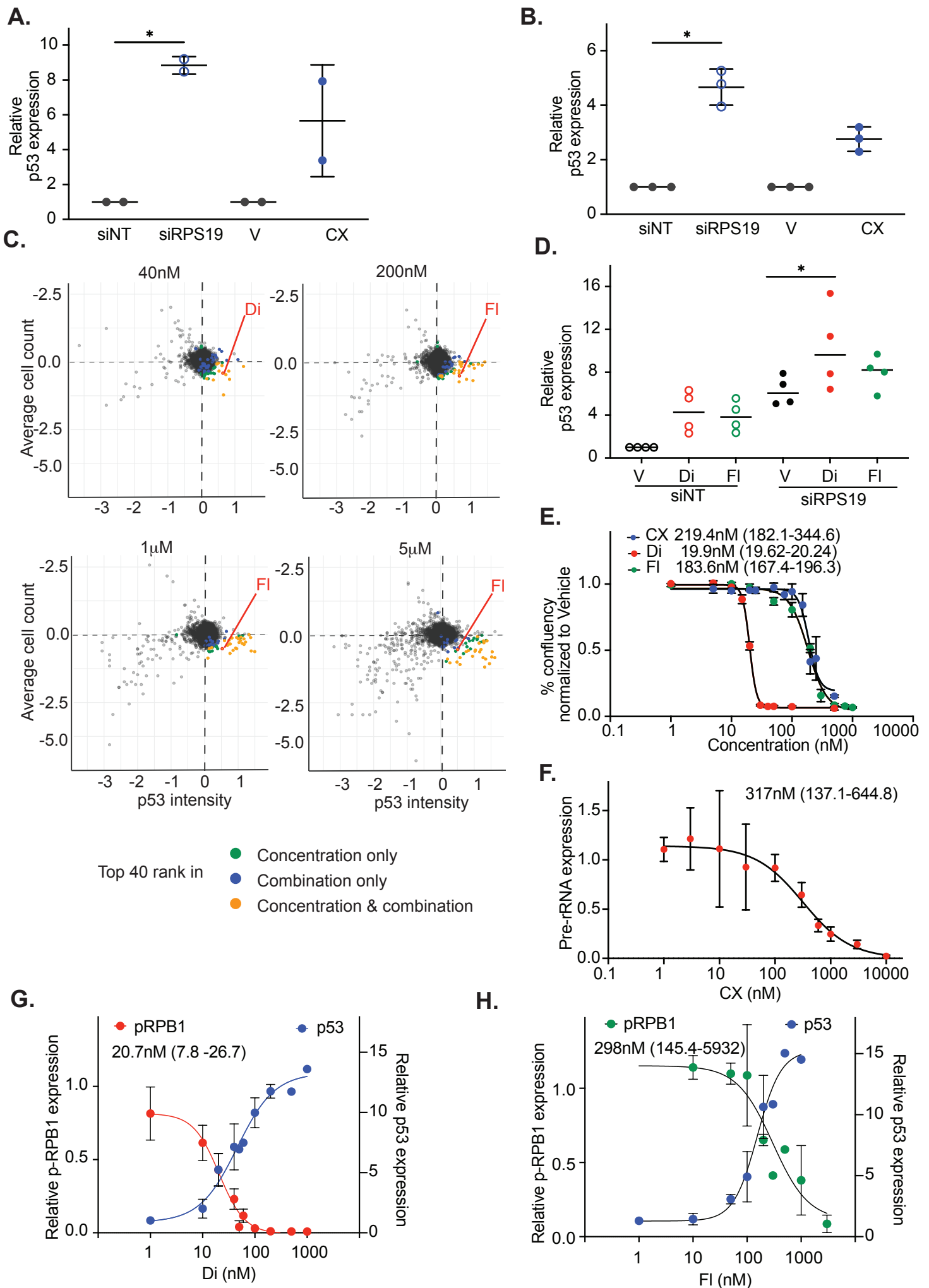
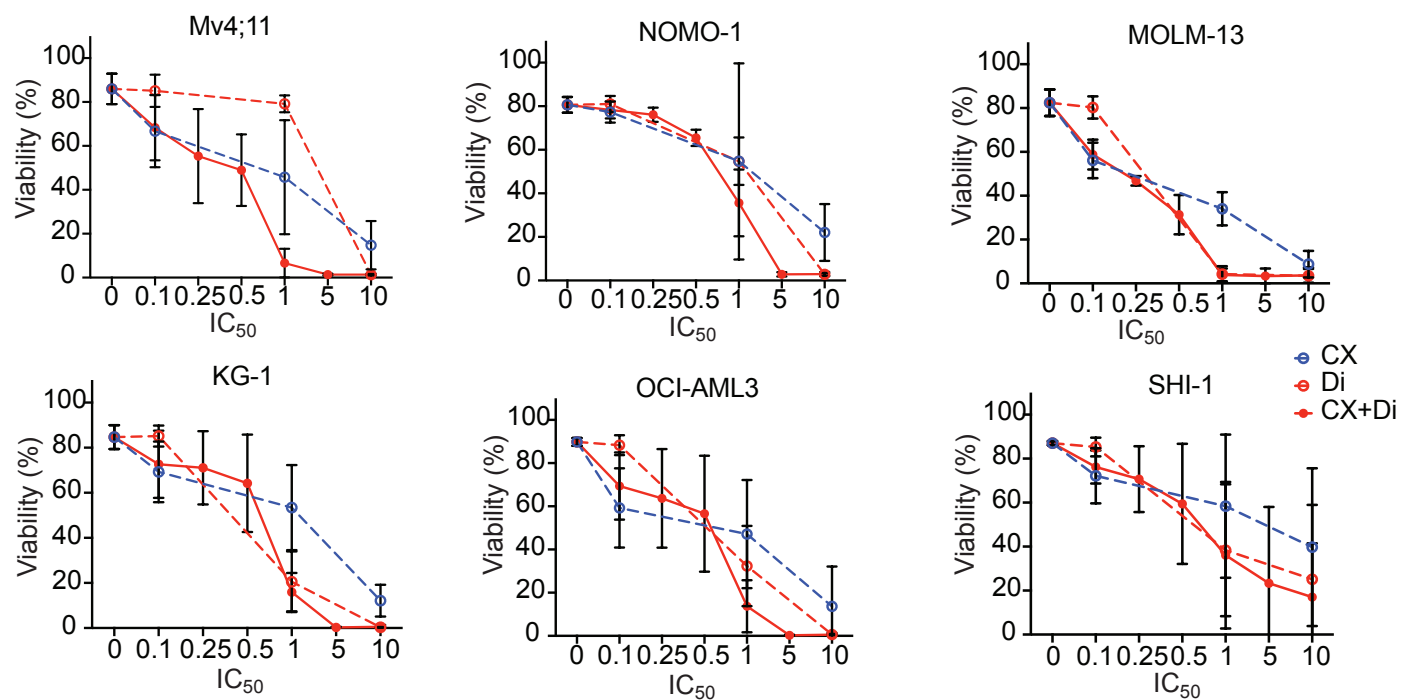




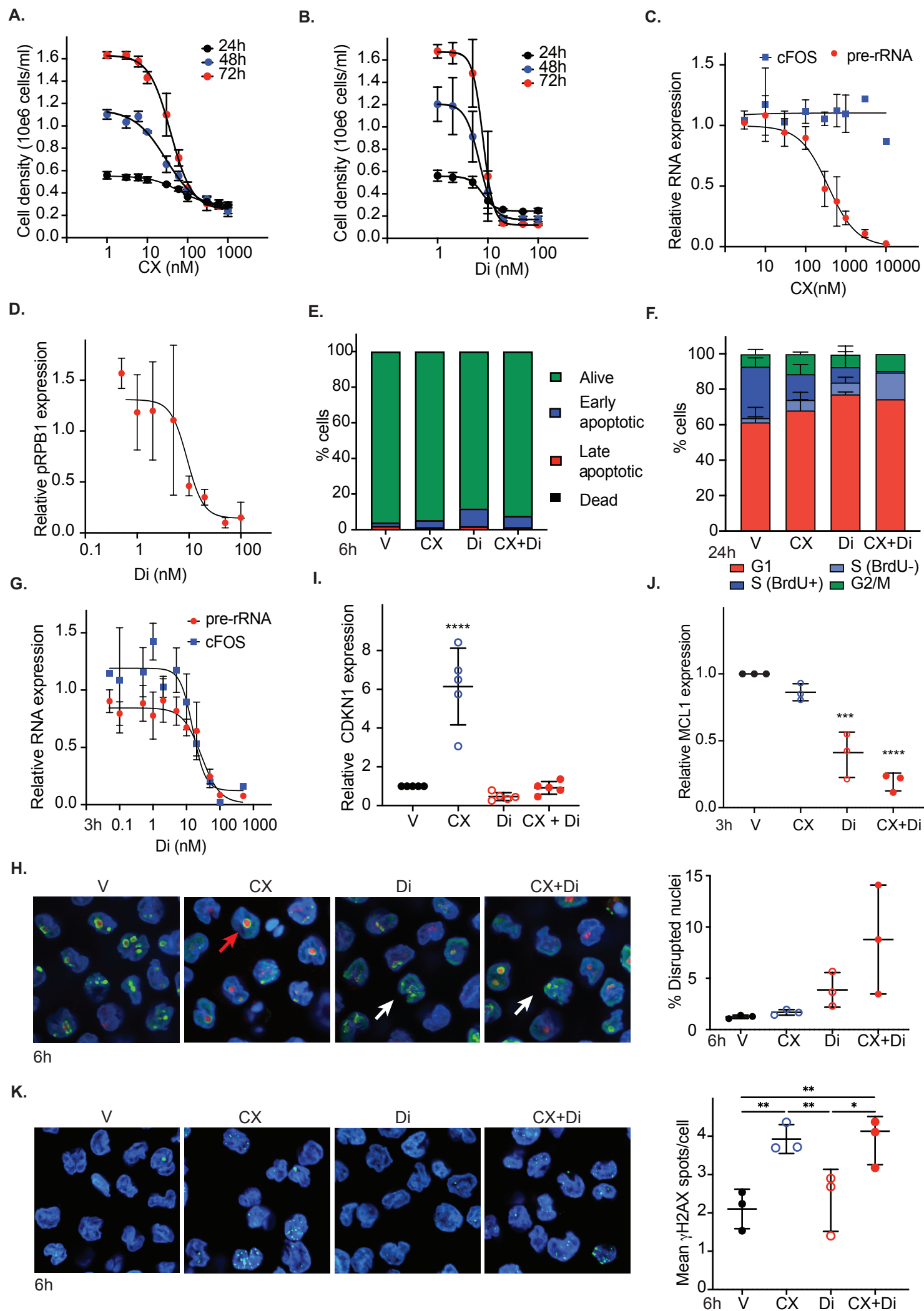
Supplementary Figure 1

Supplementary Figure 1. A-B. Quantification of immunoblotting (A) and immunofluorescence (B) shown in figures 1A and 1B, respectively. **C.** Distribution of Mahalanobis distance of each library compound across 40nM, 200nM, 1 μ M and 5 μ M drug concentrations. Top 40 ranked drugs identified only in a single concentration, only in the combined data from all concentrations tested or in both single and combine data depicted in green, blue and orange, respectively. Dinaciclib was identified at 40nM concentration as well as combined concentrations. Flavopiridol was identified at 200nM, 1 μ M and 5 μ M concentrations as well as in combined concentrations. **D.** Quantification of p53 protein abundance in A549 cells transfected for 48h with siNT or siRPS19 and treated for 3h with Dinaciclib or Flavopiridol. Densitometry values were normalized to Vehicle within each biological replicate. **E.** Dose response curves showing percentage confluency of A549 cells treated with increasing concentrations of CX-5461, Dinaciclib and Flavopiridol (0.3nM to 10 μ M), normalize to vehicle used for GIC50 calculation. **F.** Dose response curve showing inhibition of pre-rRNA, normalized to housekeeping gene *B2M* and Vehicle, in A549 cells treated for 3h with increasing concentrations of CX-5461 (1nM to 10 μ M) used for TIC₅₀ calculation. **G-H.** Quantification of pRBP1 and p53 protein abundance in A549 cells treated for 3h with increasing concentrations (1nM to 1 μ M) of Dinaciclib (**G**) or Flavopiridol (**H**). Densitometry values were normalized to Vehicle within each biological replicate and TIC₅₀ was calculated. Graphs represent mean $\pm$ SD of n=2 (A), n=3 (B, E-H) or n=4 (D) independent experiments. V: Vehicle; CX: CX-5461; Di: Dinaciclib; Fl: Flavopiridol. Significance was calculated using ordinary one-way ANOVA with Bonferroni's multiple comparison test. * p<0.05.



Supplementary Figure 2

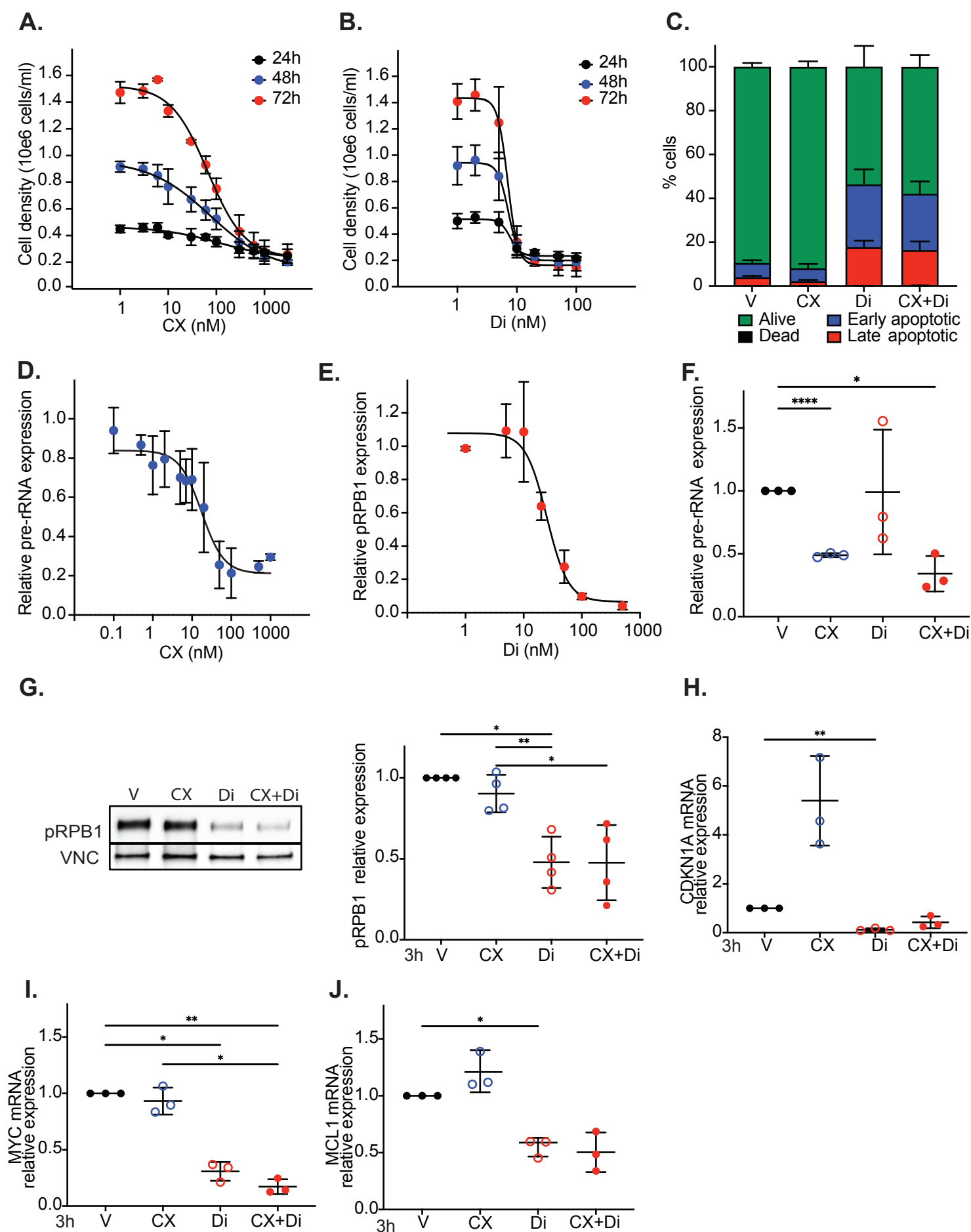
Supplementary Figure 2. Dose response curves showing percentage viability in human AML cell lines treated with variable CX-5461(CX) and Dinaciclib (Di) concentrations. Concentration used are factor of the pre-determine IC₅₀ values. Mv4,11 and MOLM-13: 300nM CX-5461, 20nM Dinaciclib; NOMO-1: : 65nM CX-5461, 20nM Dinaciclib; KG1, OCI-AML3, SHI-1 and OCI-M2: 930nM CX-5461, 20nM Dinaciclib. Graphs represent mean +/- SD of n= 3 independent.



Supplementary Figure 3

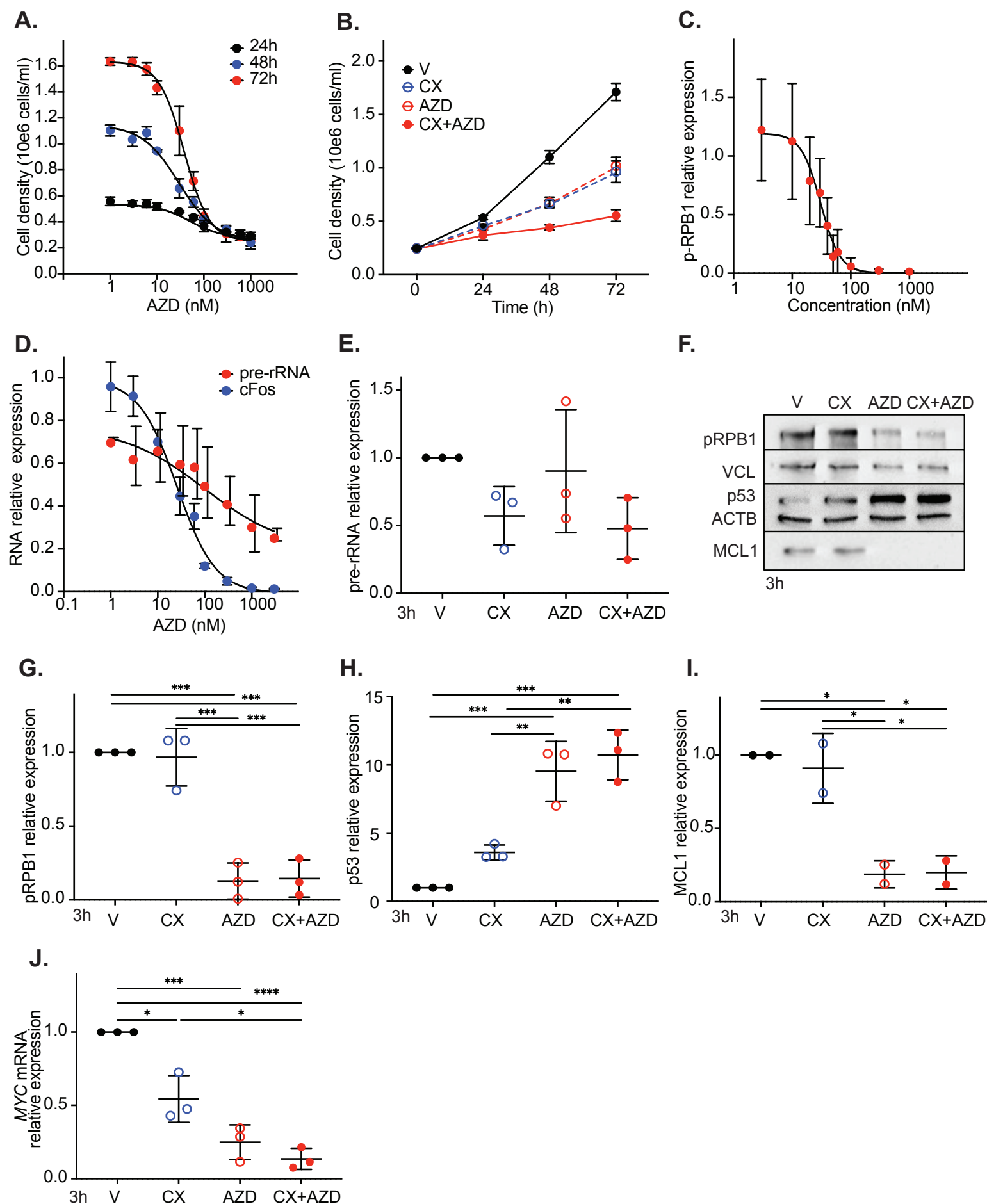
Supplementary Figure 3. A-B. Cell density of Mv4;11 cells treated with increasing concentrations of CX-5461 (A) or Dinaciclib (B) for 24, 48 and 72 hours. Dose response curves at 72 hours was used to calculate GIC₅₀ for CX-5461 (37.9nM; CI: 33.95-43.4;) and Dinaciclib (7.7nM; CI: 4.5-30.9). **C.** Relative pre-rRNA (Pol I transcribed gene) and cFOS (Pol II transcribed gene) expression of Mv4;11 cells treated with increasing concentrations (1nM to 10μM) of CX-5461 for 3 hours. Dose response curve was used to calculate TIC₅₀ for CX-5461 (295nM; CI:196.1-492.0). **D.** Quantification of pRPB1 protein abundance in Mv4;1 cells treated for 3h with increasing concentrations of Dinaciclib (0.3nM to 100nM). Densitometry values were normalized to vehicle within each biological replicate and target IC₅₀ was calculated for Dinaciclib (10nM). **E.** Apoptosis analysis of Mv4;11 treated for 6 hours with vehicle, 300nM CX-5461, 10nM Dinaciclib and combination showing low levels of apoptosis (n=1). **F.** Cell cycle analysis of Mv4;11 cells treated for 24 hours with vehicle, 300nM CX-5461, 10nM Dinaciclib and combination of both drugs. Sub-G1 (dead) cells were excluded from analysis. Graphs represent mean +/- SD of n= 3 with the exception of combo treatment where 2 biological replicates show very high levels of cell death preventing analysis. **G.** Dose response curves showing pre-rRNA and cFOS RNA expression in Mv4;11 cells treated with increasing concentrations of Dinaciclib (0.03nM to 1μM) normalize to vehicle showing a concentration-dependent reduction in both cFOS and pre-rRNA expression. **H.** Representative images of Fibrillarin (red), Nucleolin (green) and DAPI (cyan) immunofluorescence (left; 40X) and quantification of nuclear disruption (right) in MV4;11 cells treated for 3h with GIC₅₀ concentrations of CX-5461 (60nM) and Dinaciclib (10nM), or a combination of both drugs. **I.** Quantification of CDKN1 mRNA level 3h post-treatment with vehicle CX-5461 (300nM), Dinaciclib (10nM) or combination of both drugs. **J.** Quantification of MCL1 protein level, 6h post-treatment with vehicle CX-5461 (300nM), Dinaciclib (10nM) or combination of both drugs. **K.** Representative images of γH2AX (green) and DAPI (cyan) immunofluorescence on Mv4;11 cells treated for 6 hours with CX-5461 (300nM) and Dinaciclib (20nM) alone, or in

combination (left) and quantification of average number of γ H2AX foci per nucleus (right). All graphs display mean $\pm$ standard deviation of 3 biological replicates. Significance was calculated using ordinary one-way ANOVA with Bonferroni's multiple comparison test. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$. V: Vehicle; CX: CX-5461; Di: Dinacicib.



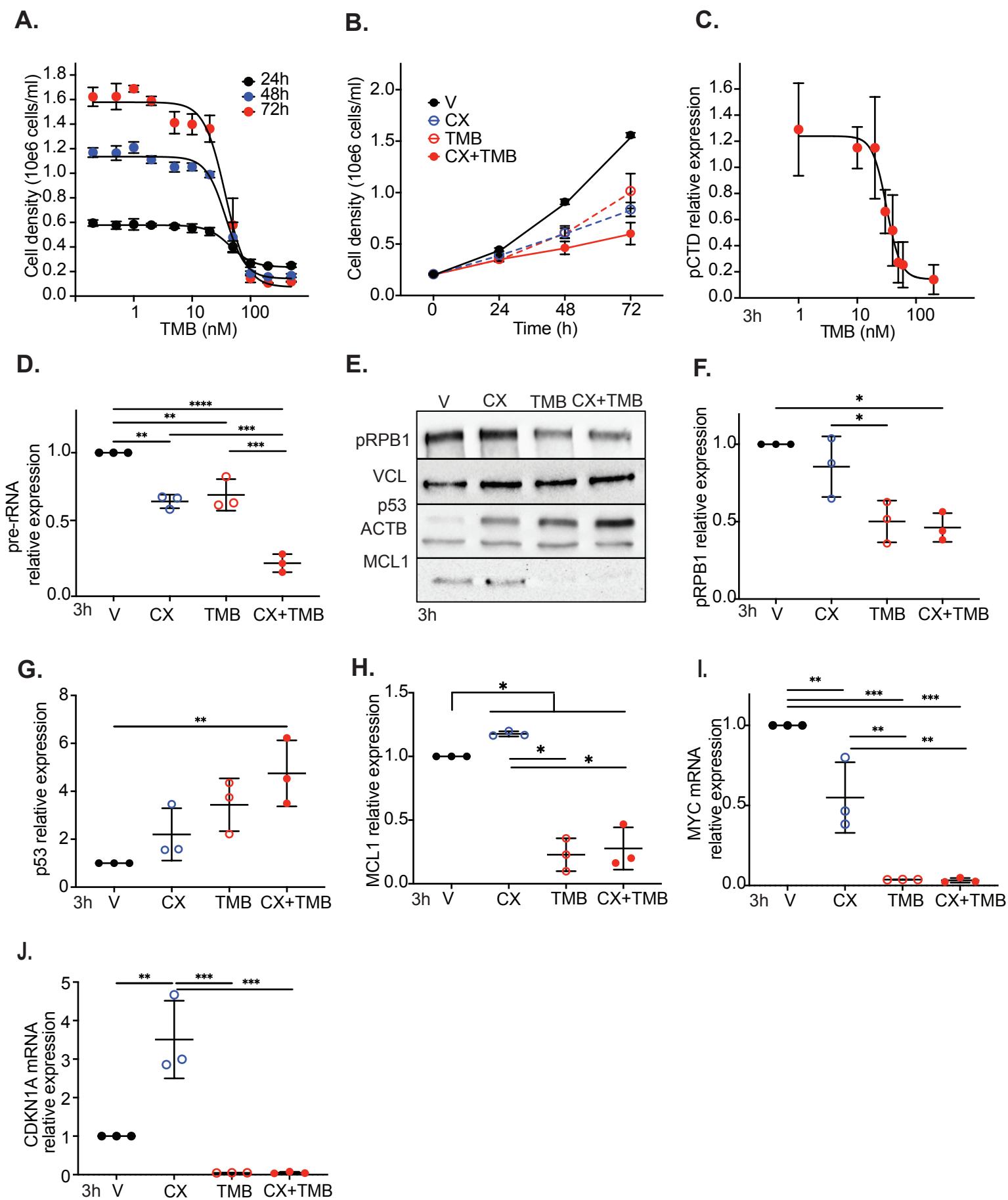
Supplementary Figure 4

Supplementary Figure 4. **A.** Cell density of KG1 cells treated with increasing concentrations of CX-5461 (A) or Dinaciclib (B) for 24, 48 and 72 hours. Dose response curves at 72 hours was used to calculate HIC_{50} of CX-5461 (66.6nM; CI: 55.70-90.49) and Dinaciclib (7.1nM; CI: 6.23-8.06). **C.** Apoptosis analysis of KG1 treated for vehicle, 70nM CX-5461, 10nM Dinaciclib and combination of both drugs for 48 hours showing showing increase levels of apoptosis in Dinaciclib- and combination-treated cells. **D.** Relative pre-rRNA (Pol I transcribed gene) expression in KG1 cells treated with increasing concentrations (1nM to 10 μ M) of CX-5461 for 3 hours. Dose response curve was used to calculate TIC_{50} for CX-5461 (396.9nM; CI: 160.1-2879). **E.** Quantification of pRBP1 protein abundance in KG-1 cells treated for 3h with increasing concentrations of Dinaciclib (1nM to 600nM). Densitometry values were normalized to vehicle within each biological replicate, and TIC_{50} was calculated for Dinaciclib (30.61nM; CI: 26.91-43.85). **F.** Pre-rRNA expression levels, normalized to housekeeping gene *B2M* and Vehicle, of KG-1 cells treated for 3h with vehicle, 400nM Cx-5461, 25nM Dinaciclib and combination. **G.** Representative immunoblot for Serine 2 phospho-RBP1 (pRBP1) and Vinculin (VNC) (left) and protein levels normalized to loading control VNC and vehicle (right) of KG1 cells treated for 3h with vehicle, 400nM CX-5461, 25nM Dinaciclib and combination. **H-J.** Quantification of *CDKN1* (H), *MYC* (I) and *MCL1* (J) mRNA levels, normalized to *B2M* and vehicle and vehicle, of KG1 cells treated for 3h with vehicle, 400nM Cx-5461, 25nM Dinaciclib and combination. All graphs display mean $\pm$ standard deviation of at least 3 biological replicates. Significance was calculated using ordinary one-way ANOVA with Bonferroni's multiple comparison test. * $p < 0.05$, ** $p < 0.01$, **** $p < 0.0001$. Vehicle; CX: CX-5461; Di: Dinaciclib.



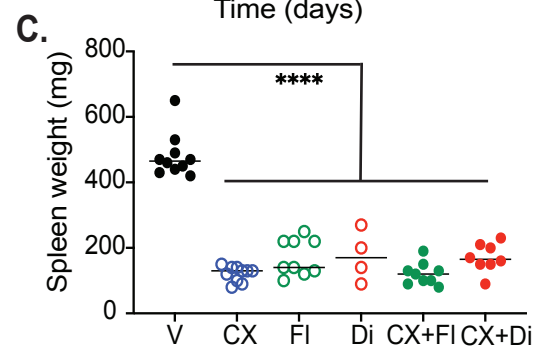
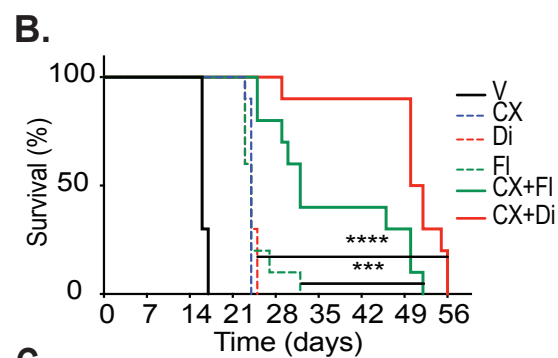
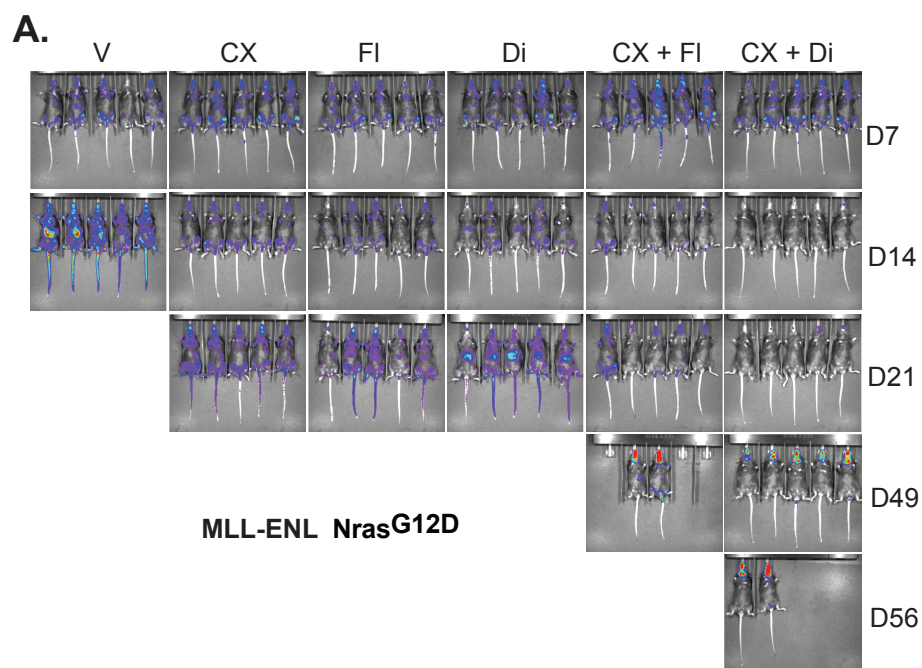
Supplementary Figure 5

Supplementary Figure 5. A. Cell density of Mv4;11 cells treated with increasing concentrations of AZD4573 for 24, 48 and 72 hours. Dose response curves at 72 hours was used to calculate growth IC_{50} (GIC_{50} =16.69nM; CI: 15.21-18.42; n=3). **B.** Growth curves of Mv4;11 cells treated with GIC_{50} concentrations of CX-5461 (30nM), AZD4573 (10nM) or combination of both for 72 hours. **C.** Quantification of pRPB1 protein abundance in Mv4;11 cells treated for 3h with increasing concentrations of AZD4573 (1nM to 1 μ M). Densitometry values were normalized to vehicle within each biological replicate and target IC_{50} was calculated for AZD4573 (TIC_{50} =25.1nM; CI: 18.47-37.72; n=3). **D.** Relative pre-rRNA (Pol I transcribed gene) and cFOS (Poll II transcribed gene) expression in Mv4;11 cells treated with increasing concentrations (1nM to 1 μ M) of AZD4573 for 3 hours. **F.** Representative immunoblots for Serine 2 phospho-RPB1(pRBP1), Vinculin (VNC), p53, Beta-Actin (ACTB) and in Mv4;11 cells treated for 3h with vehicle, 300nM CX-5461, 30nM AZD4573 and combination. **G-I.** Quantification of pRPB1(G), p53 (H), and MCL1 (I) protein levels, normalized to VCL (pRPB1) or ACTB (p53, MCL1) and vehicle Mv4;11 cells treated for 3h with vehicle, 300nM CX-5461, 30nM AZD4573 and combination. **J.** Quantification of *MYC* mRNA levels, normalized to *B2M* and vehicle Mv4;11 cells treated for 3h with vehicle, 300nM CX-5461, 30nM AZD4573 and combination. All graphs display mean $\pm$ standard deviation of at least 3 biological replicates. Significance was calculated using ordinary one-way ANOVA with Bonferroni's multiple comparison test. * $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$. V: Vehicle; CX: CX-5461; AZD: AZD4573.



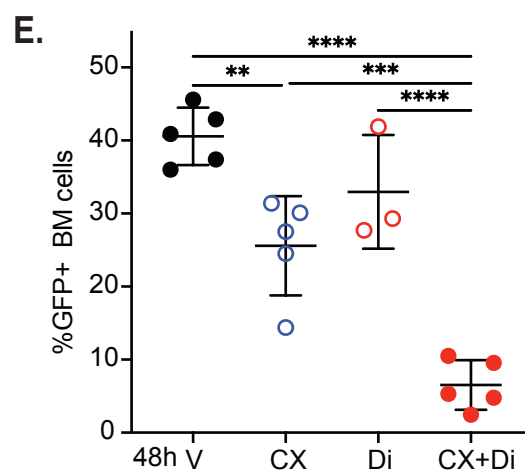
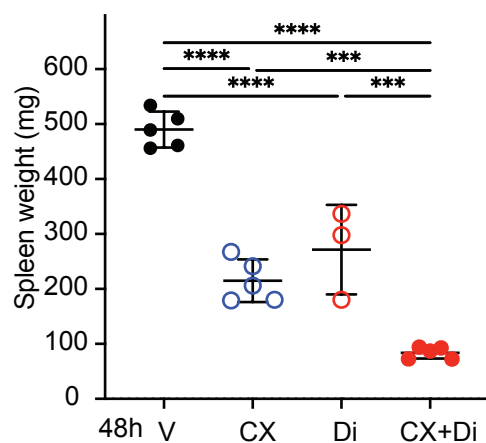
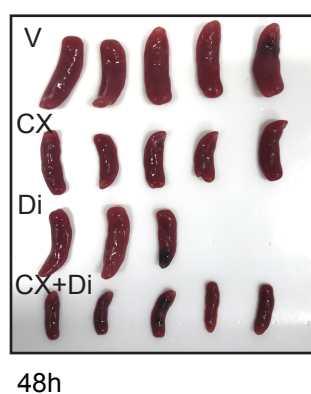
Supplementary Figure 6

Supplementary Figure 6. A. Cell density of Mv4;11 cells treated with increasing concentrations of Tambiciclib (TMB) for 24, 48 and 72 hours. Dose response curves at 72 hours was used to calculate growth IC_{50} ($GIC_{50}=37.2nM$; CI: 30.88-42.73; $n=3$). **B.** Growth curves of Mv4;11 cells treated with GIC_{50} concentrations CX-5461 (30nM), AZD4573 (30nM) or combination of both for 72 hours. **C.** Quantification of pRPB1 protein abundance in Mv4;11 cells treated for 3h with increasing concentrations of Tambiciclib (1nM to 1 μ M). Densitometry values were normalized to vehicle within each biological replicate, and target IC_{50} was calculated for Tambiciclib ($TIC_{50}=31.6$; CI: 23.92-45.73; $n=3$). **D.** Relative pre-rRNA (Pol I transcribed gene) expression in Mv4;11 cells treated for 3h with vehicle, 300nM CX-5461, 30nM Tambiciclib and combination. **F.** Representative immunoblots for Serine 2 phospho-RPB1(pRBP1), Vinculin (VNC), p53, Beta-Actin (ACTB) and MCL1 in Mv4;11 cells treated for 3h with vehicle, 300nM CX-5461, 30nM Tambiciclib and combination. **G-I.** Quantification of pRPB1(G), p53 (H) and MCL1 (I) protein levels, normalized to VCL (pRPB1) or ACTB (p53, MCL1) and vehicle Mv4;11 cells treated for 3h with vehicle, 300nM CX-5461, 30nM Tambiciclib and combination. **J.** Quantification of *MYC* mRNA levels, normalized to *B2M* and vehicle Mv4;11 cells treated for 3h with vehicle, 300nM CX-5461, 30nM Tambiciclib and combination. All graphs display mean $\pm$ standard deviation of at least 3 biological replicates. Significance was calculated using ordinary one-way ANOVA with Bonferroni's multiple comparison test. * $p<0.5$, ** $p<0.05$, *** $p<0.005$. V: Vehicle; CX: CX-5461, TMB: Tambiciclib.



D.

AML1-ETO9a NrasG12D



Supplementary Figure 7. A. Representative images of bioluminescence imaging used to monitor disease progression. **B.** Kaplan-Meier plot showing overall survival. Data for the survival study was analysed by log-rank (Mantel-Cox) test with Benjamini and Hochberg correction, n=10 mice/group. *** $p < 0.001$, **** $p < 0.0001$. **C.** Spleen weight at ethical endpoint of MLL-ENL Nras^{G12D} AML model treated with vehicle, CX-5461, Dinaciclib, Flavopiridol or combination therapies. **Note:** Data for Vehicle, CX-5461, Dinaciclib and CX-5461-Dinaciclib combination is the same as in Fig 5A. **D.** Representative images (left) and spleen weights (right) of AML1-ETO9a Nras^{G12D} mice treated for 48 hours with CX-5461 and Dinaciclib monotherapy, and combination therapy. **E.** Percentage of tumor (GFP+) cells in bone marrow of AML1-ETO9a mice treated for 48 hours with CX-5461 and Dinaciclib monotherapy, and combination therapy. n=5 mice/group at the start of treatment. Data was analyzed by ordinary one-way ANOVA with Tukey's multiple comparisons test. Significance * $p < 0.05$, ** $p < 0.01$, *** $p < 0.0001$, **** $p < 0.0001$. CX: CX-5461; Fl: Flavopiridol; Di: Dinaciclib.