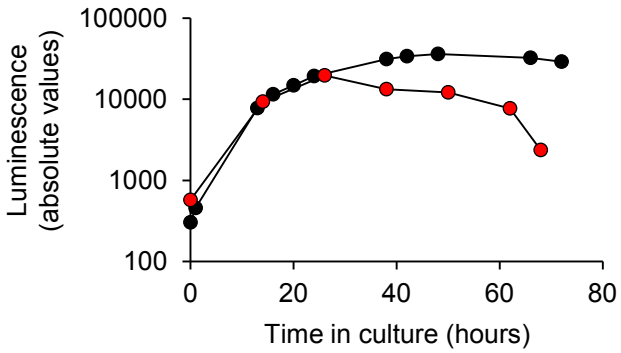

Figure S1. Proliferation of primary AML blast cells in cytokine supplemented media. Proliferation of primary AML cells was confirmed using the RealTime-Glo MT assay (Promega). Luminescence was measured at multiple time points for two primary AML samples (shown in plot).

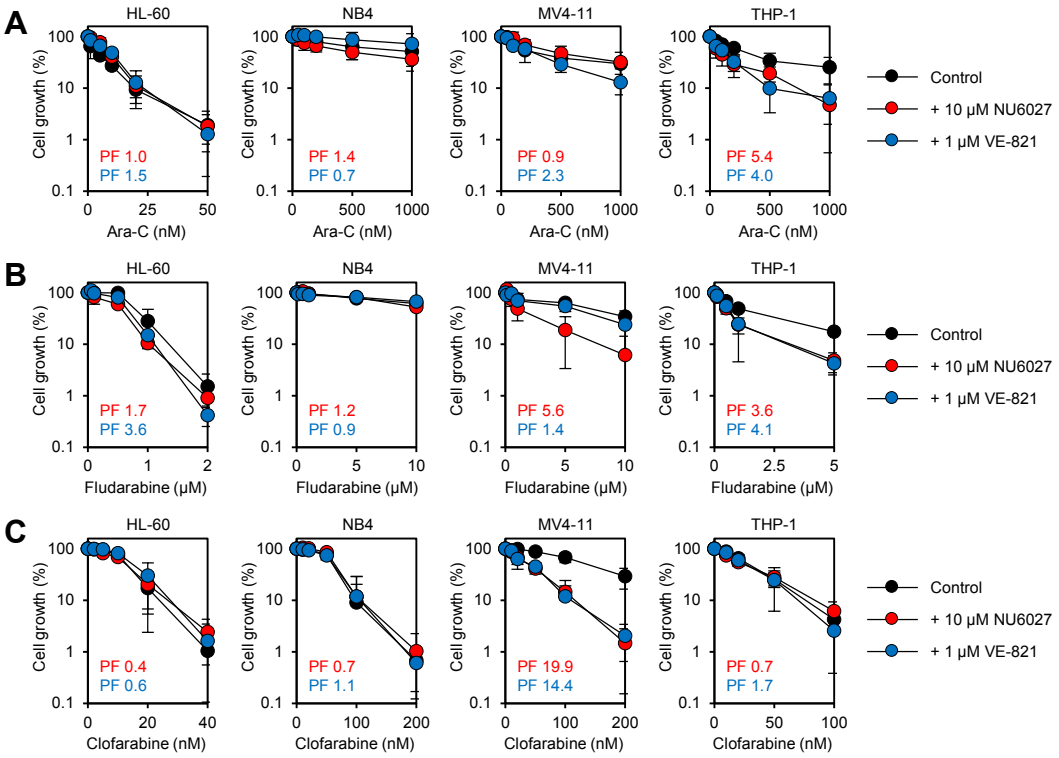

Figure S2. Small molecule ATR inhibition does not consistently potentiate the cytotoxic effects of chemotherapeutic nucleoside analogues in AML cell lines. Related to Figure 1. AML cell lines were treated with Ara-C (A), fludarabine (B) or clofarabine (C), either alone (black circles), in combination with 10 μM NU6027 (red circles) or in combination with 1 μM VE-821 (blue circles), and cell growth (relative to respective vehicle controls) was determined after 96 hr. PFs were calculated at the highest drug doses. Data represents the mean ± SD of 3 independent experiments.

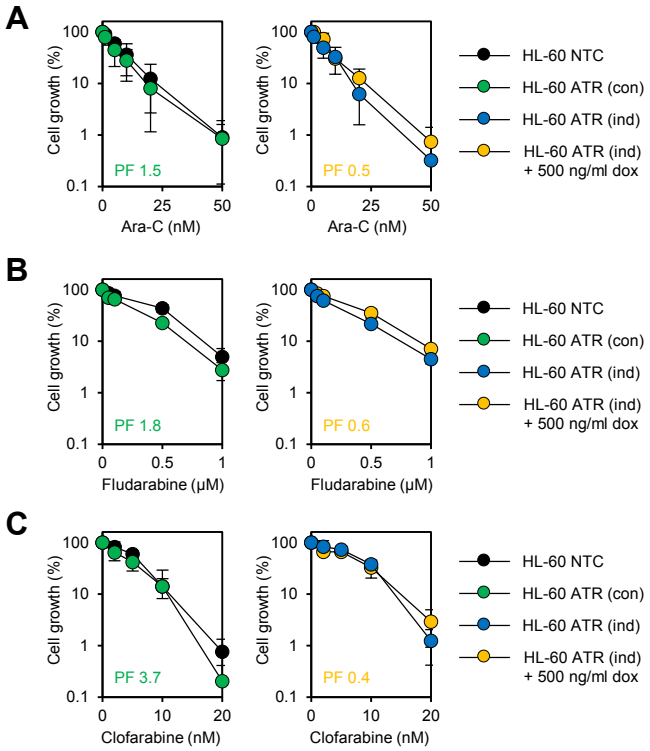

Figure S3. ATR depletion by shRNA-mediated gene knockdown does not potentiate the cytotoxic effects of chemotherapeutic nucleoside analogues in HL-60 AML cells. Related to Figure 1.

HL-60 ATR (con) cells with constitutive ATR knockdown (green circles; left hand charts) and HL-60 ATR (ind) cells with doxycycline-induced ATR knockdown (yellow circles; right hand charts) and their respective controls were treated with Ara-C (a), fludarabine (b) or clofarabine (c) and cell growth (relative to respective vehicle controls) was determined after 96 hr. PFs were calculated at the highest drug doses. Data represents the mean ± SD of 3 independent experiments.

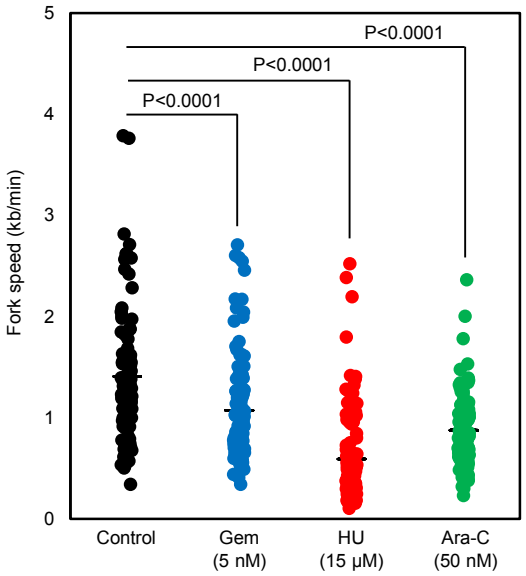

Figure S4. Treatment with gemcitabine, HU or Ara-C slows replication fork progression in MV4-11 AML cells. Related to Figure 3.

MV4-11 cells were pre-incubated with 5 nM gemcitabine, 15 mM hydroxyurea, 50 nM Ara-C or vehicle control for 1 hr then pulse labelled with 25 mM IdU for 40 min, followed by 250 mM CldU for 40 min (with maintenance of exposure to drug throughout). Data points represent fork speed progression of IdU tracts under each specified condition from 3 independent experiments. Solid lines indicate mean IdU fork speed. P values were calculated using the Mann Whitney U test.

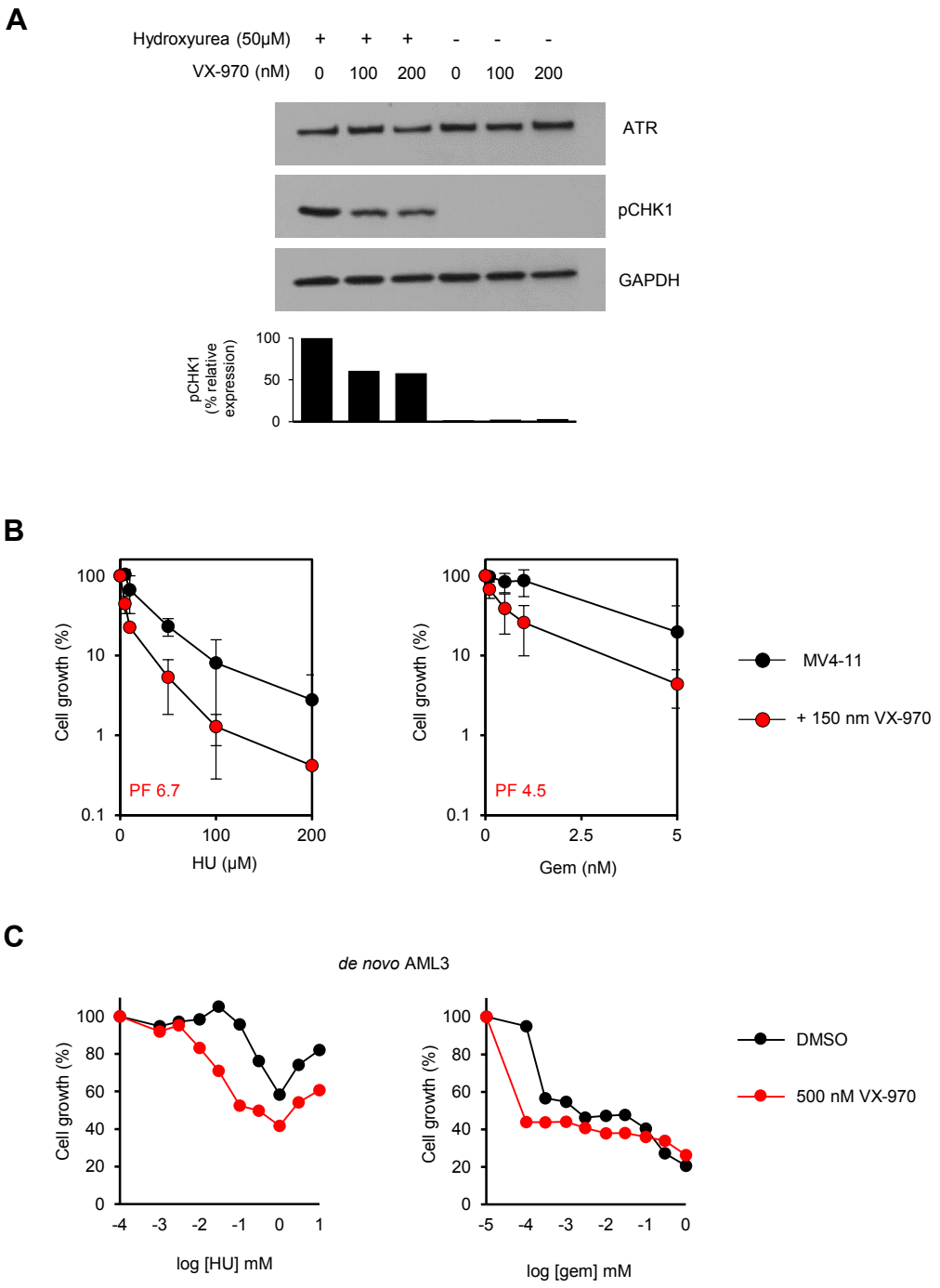

Figure S5. VX-970 (VE-822) inhibits pCHK1 and potentiates the cytotoxic effects of HU and gemcitabine in MV4-11 cells and primary AML cells. Related to Figure 1 and Figure 5.
A. To confirm target engagement, MV4-11 AML cells were exposed to 50 μM HU (or vehicle control) for 1hr in the presence of VX-970 (or DMSO control) and western blotting was performed for ATR and pCHK1 (Ser345). Expression of pCHK1 is expressed relative to the HU only lane. B. MV4-11 AML cells were treated with HU (left) or gemcitabine (right) alone (black circles) or in combination with 150 nM VX-970 (red circles) and cell growth (relative to respective vehicle controls) was determined after 96 hr. PFs were calculated at the highest dose of HU or gemcitabine. Data represents the mean and SD ± 3 independent experiments. C. Bone marrow mononuclear cells from a *de novo* AML patient were treated with HU (left) or gemcitabine (right) in combination with 500 nM VX-970 (red circles) or DMSO control (black circles). Cell growth (relative to respective vehicle controls) was determined after 72 hr by addition of resazurin sodium salt.

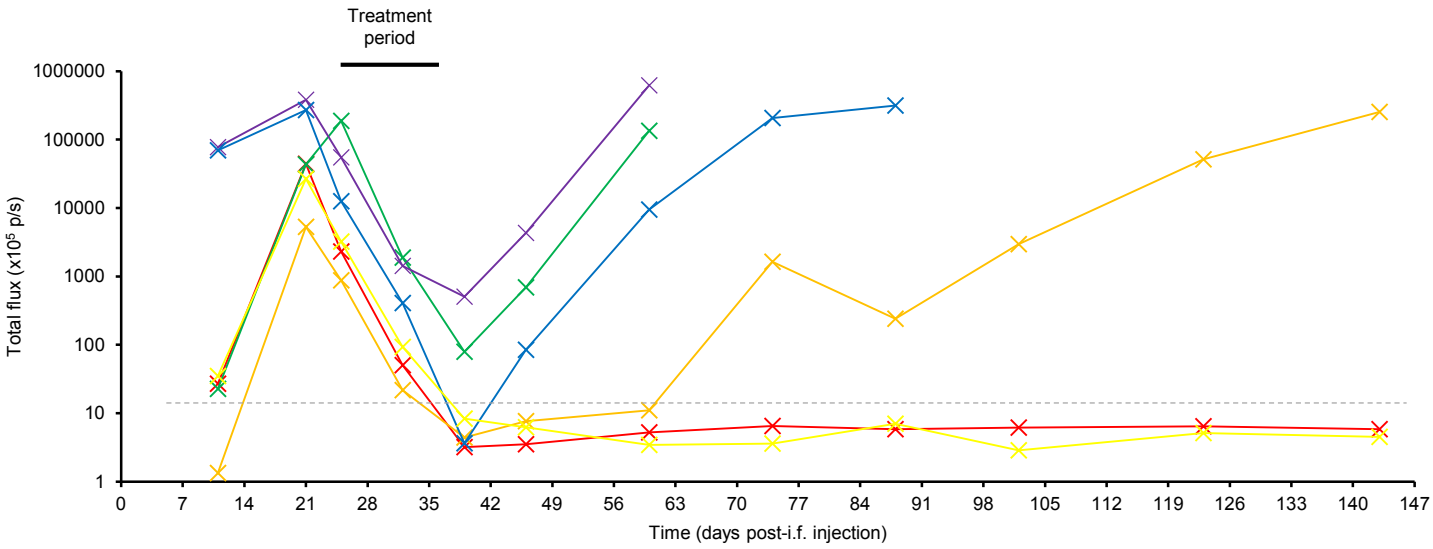

Figure S6. Delayed onset of treatment with gemcitabine and VX-970 reduces disease burden in mice with disseminated AML. Related to Figure 6 and Figure 7.
Six female *Rag2*$^{-/-}$ *gc*$^{-/-}$ mice were injected intrafemorally with 5x10^5 MV4-11 luc+ GFP+ cells and treatment with 60 mg/kg VX-970 and 100 mg/kg gemcitabine was administered on days 21-32 post-injection, as detailed in main text. Bioluminescent imaging was performed at regular intervals to monitor disease burden and total flux was measured from bioluminescent images. Chart shows total flux measurements from the 6 individual mice on indicated days post-i.f. injection. Dashed line indicates threshold for mean background flux.