Cytosolic DNA sensing combined with differentiation therapy induces irreversible differentiation and cell growth arrest in myeloid leukemia cells

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Supplemental Figure Legends

Supplementary Figure 1. Apoptosis induction in HL-60 cells. Apoptosis analysis of HL-60 cells 24 h after treatment with 1 μM ATRA, 1 μM MRT, or ATRA+MRT. Representative results of FITC-conjugated annexin V and PI staining from three independent experiments are shown here. Percentages of PI^+ annexin V^-, PI^+ annexin V^+, and PI^- annexin V^+ cells among R1-gated cells were determined**.**

Supplementary Figure 2. Combined treatment with SBI and ATRA in HL-60 cells. (a) NBT staining of HL-60 cells 24 h after treatment with 1 μM ATRA, 1 μM MRT, 10 μM SBI, ATRA+MRT, or ATRA+SBI. Non-treated cells were used as a control. Representative results from three independent experiments are shown here**. (b)** Cell proliferation of HL-60 cells in drug-free medium after ATRA, SBI, or ATRA+SBI treatment for 24 h (n = 4). Cell proliferation was calculated by dividing the values of treated cells with those at 0 h.

Supplementary Figure 3. Induction of irreversible differentiation by combined treatment with ATRA and MRT in human myeloid leukemia cell lines. (a) Cell proliferation of THP-1, K562, and KG-1 cells in drug-free medium after treatment with 1 μM ATRA, 1 μM MRT, or ATRA+MRT for 48 h (n = 4). Untreated cells were used as a control. Cell proliferation was calculated by dividing the values of treated cells with those at 0 h. **(b)** Microscopic observation of each leukemia cell line 72 h after replating into drug-free fresh medium following 48 h exposure to the indicated

drugs. Representative results from four independent experiments are shown here.

Supplementary Figure 4. IL-1 β is dispensable for ATRA+MRT combined treatment-mediated myeloid differentiation. Expression of CD11b on HL-60 cells after combined treatment with 1 μM ATRA and 1 μM MRT in the presence of an anti-IL-1 β neutralizing mAb (clone; 8516, R and D Systems) or control mouse IgG1 (Agilent Dako) for 24 h. Representative results from three independent experiments are shown here.

Supplementary Figure 5. Alteration in the expression of leukocyte activation- and cell cycle-related genes by combined treatment with ATRA and MRT. Relative mRNA expression of the indicated genes in HL-60 cells 48 h after ATRA, MRT, or ATRA+MRT treatment. Untreated cells were used as a control. Data represent the mean ± SD from three independent experiments. $^{**}P < 0.01$ using Tukey-Kramer test.

Supplementary Figure 6. AIM2 KD modulates the ATRA+MRT combined treatment-mediated changes in the mRNA expression of leukocyte activation- and cell cycle-related genes. Relative mRNA expression of the indicated genes in shControl- and shAIM2-transduced HL-60 cells 24 h after ATRA+MRT or ATRA treatment. Data represent the mean ± SD from three independent experiments. $^{**}P < 0.01$ using two-sided Student's *t*-test.

Supplementary Figure 7. Inhibition of myeloid differentiation by AIM KD in THP-1 cells. Expression of CD11b **(a)** and p21 **(b)** in shControl- and shAIM2-transduced THP-1 cells after combined treatment with 1 μM ATRA and 1 μM MRT for 48 h. Fold increases in CD11b expression were calculated by dividing the values of treated cells with those of non-treated cells. Data represent the mean ± SD from

three independent experiments. **P < 0.01 using Tukey-Kramer test (a). Representative results and mean ± SD of MFI from three independent experiments are shown here. **P < 0.01 using Dunnett test (b).

Supplementary Figure 8. Validation of p21 KD in HL-60 cells. p21 expression in shControl- and shp21-transduced HL-60 cells, which were incubated in the presence or absence of 1 μM ATRA and 1 μM MRT for 48 h. The result of a single experiment is shown here.

Supplementary Figure 9. Morphological changes in MOLM-14 after combined treatment with quizartinib and MRT. Giemsa and NBT staining of MOLM-14 cells 24 and 48 h after treatment with 5 nM quizartinib or 5 nM quizartinib and 1 μM MRT in the presence of 10 ng/ml FGF2. Representative results from three independent experiments are shown here.

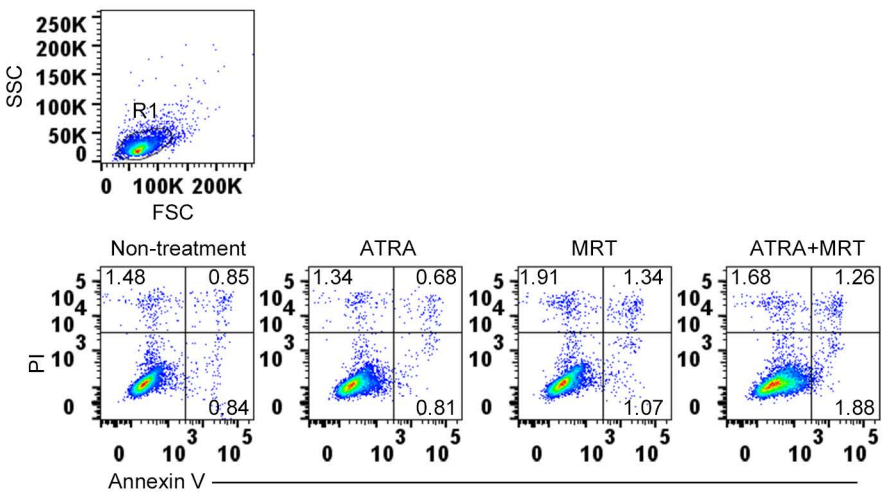
Supplementary Figure 10. AIM KD in MOLM-14 cells. (a) Effects of AIM2 KD on AIM2 expression in MOLM-14 cells. AIM2 expression levels were determined with the use of a flow cytometer and representative results from three independent experiments are shown here. Expression of p21 **(b)** and Giemsa staining **(c)** of shControl- and shAIM2-transduced MOLM-14 cells after combined treatment with 5 nM quizartinib and 1 μM MRT in the presence of 10 ng/ml FGF2. Representative results and mean ± SD of MFI from three independent experiments are shown here. *P < 0.05 using two-sided Student's t-test.

Supplementary Figure 11. ATRA-pretreatment potentiates the effects of combination treatment with ATRA and MRT. Cell proliferation of MOLM-13 cells in drug-free medium was determined 24 h **(a)** and 6 h **(b)** after combined incubation with 1 μM ATRA and 1 μM MRT following exposure to 1 μM ATRA for

48 h (n = 4).

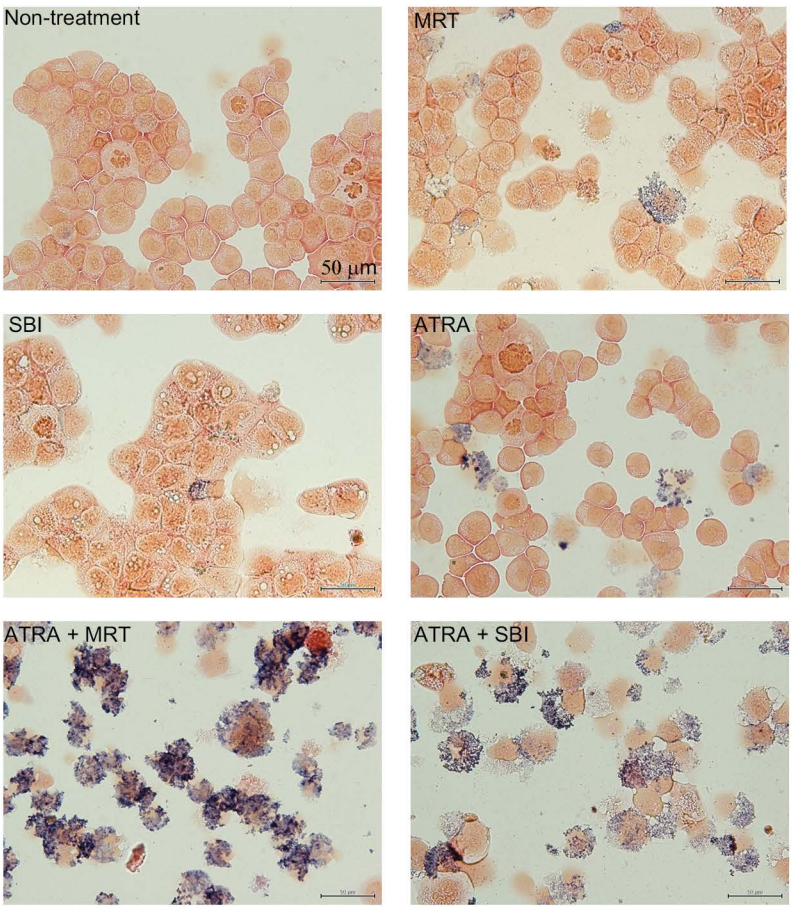
Supplementary Figure 12. Unabridged data of western blot analysis of p21 ubiquitination.

Supplementary Figure 13. Gating strategy of flow cytometric analysis. Representative data of light scattering of unfixed and fixed HL-60 cells are shown here.



Supplementary Figure 1

a



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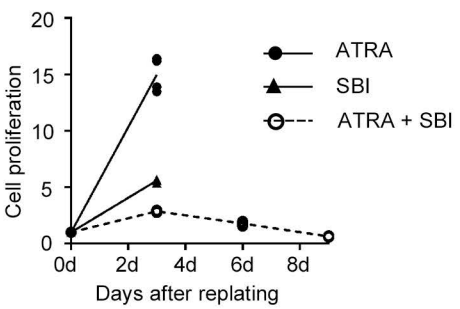
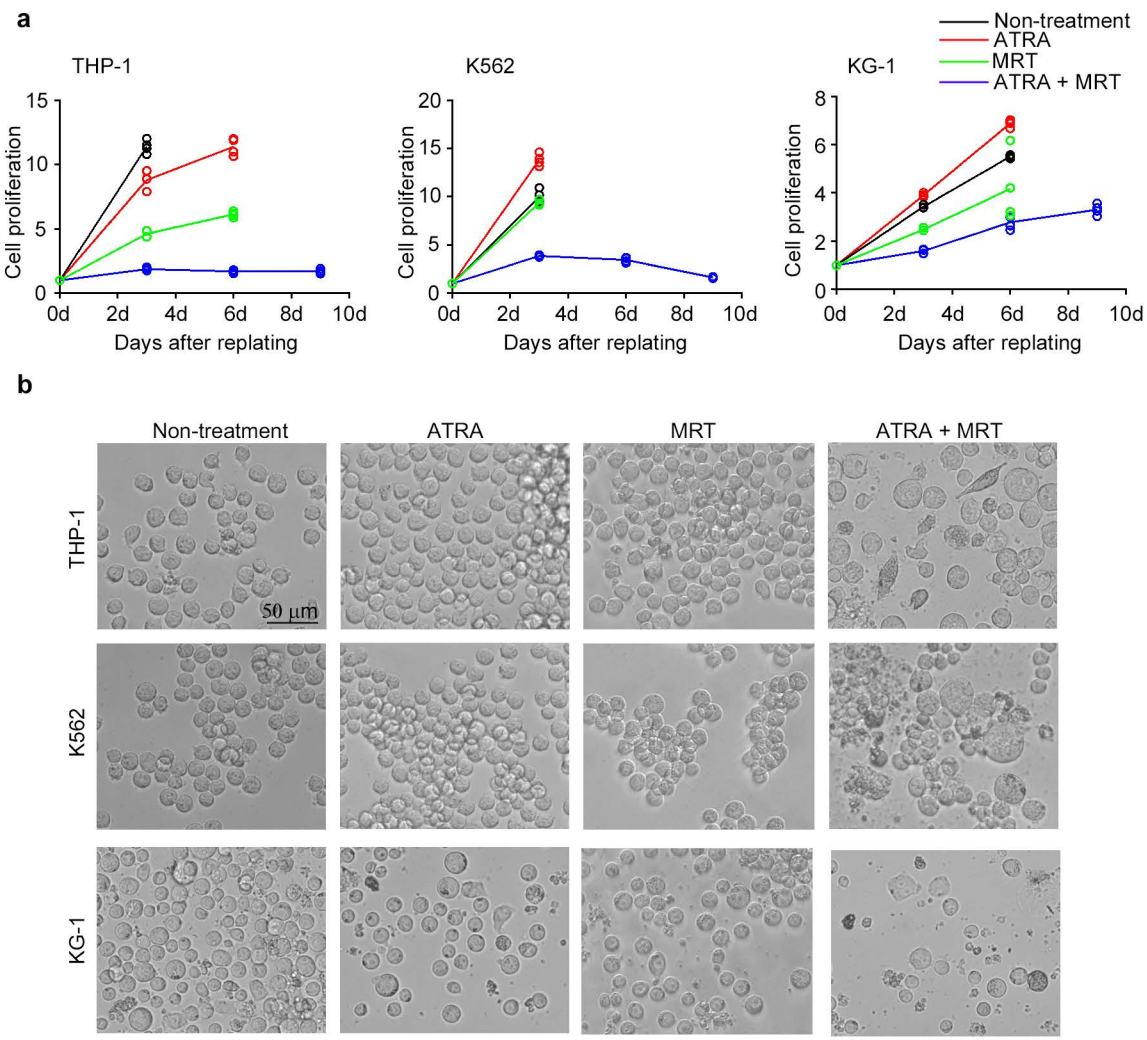
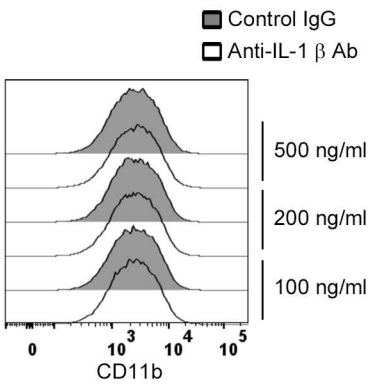


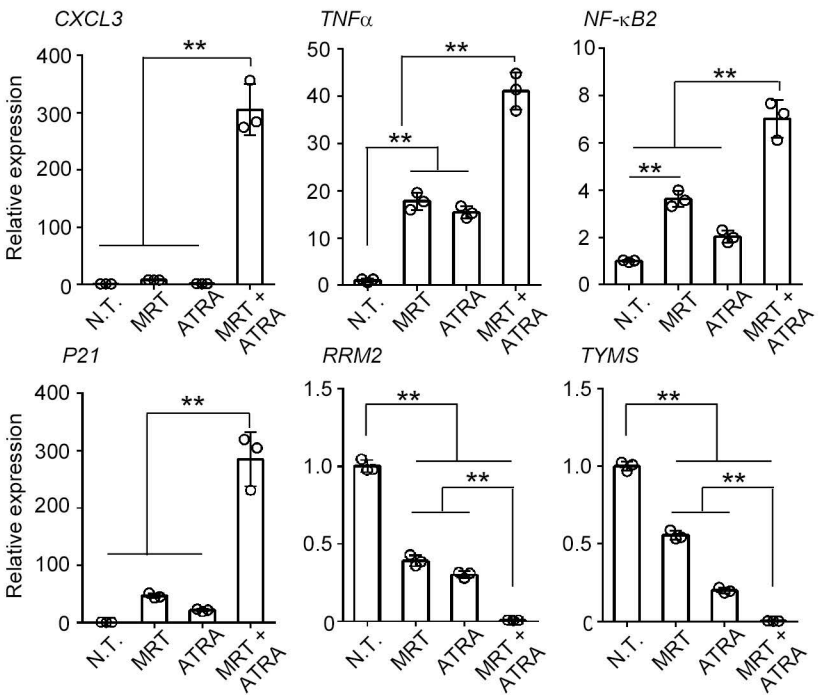
Supplementary Figure 2



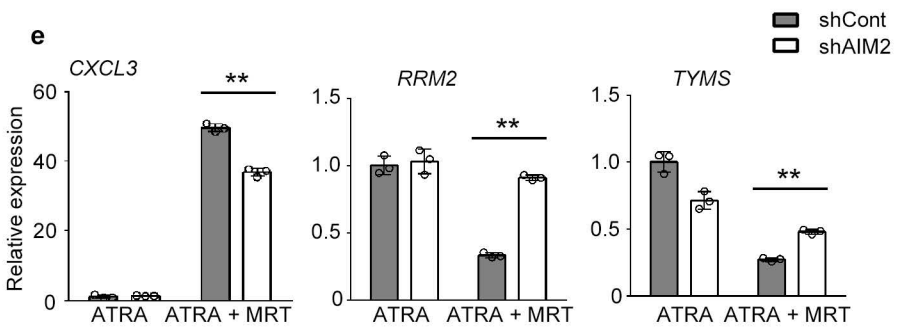
Supplementary Figure 3



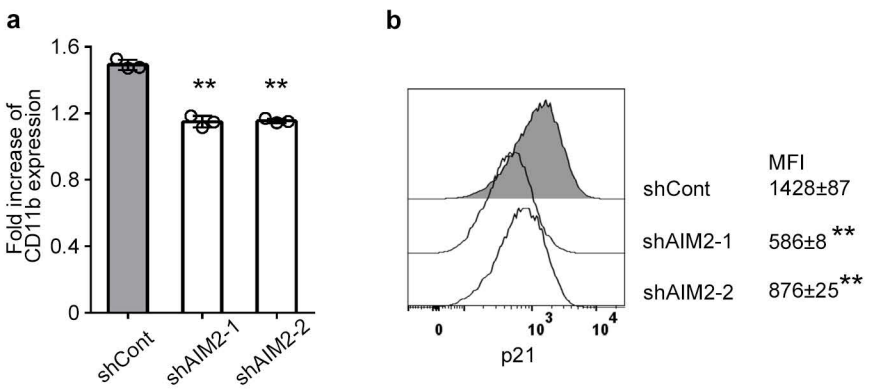
Supplementary Figure 4



Supplementary Figure 5



Supplementary Figure 6



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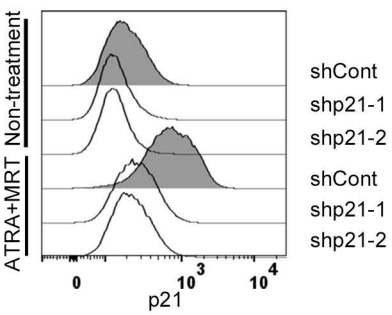

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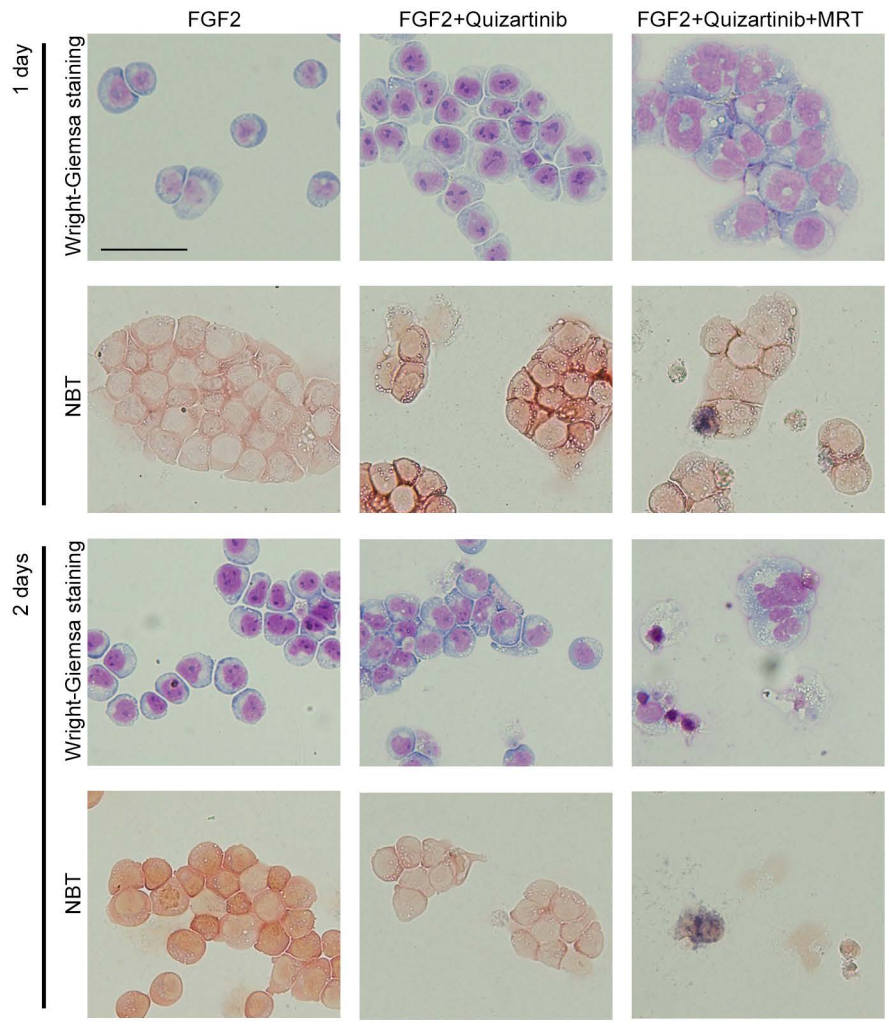
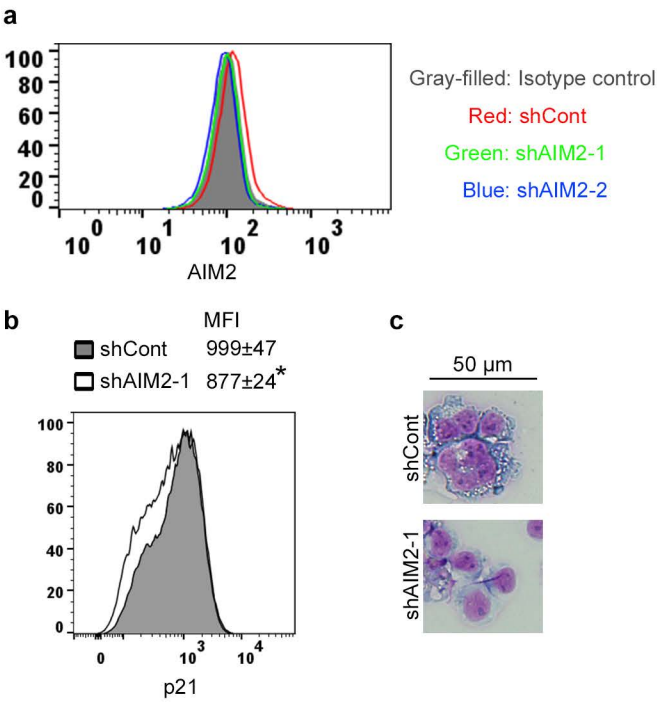
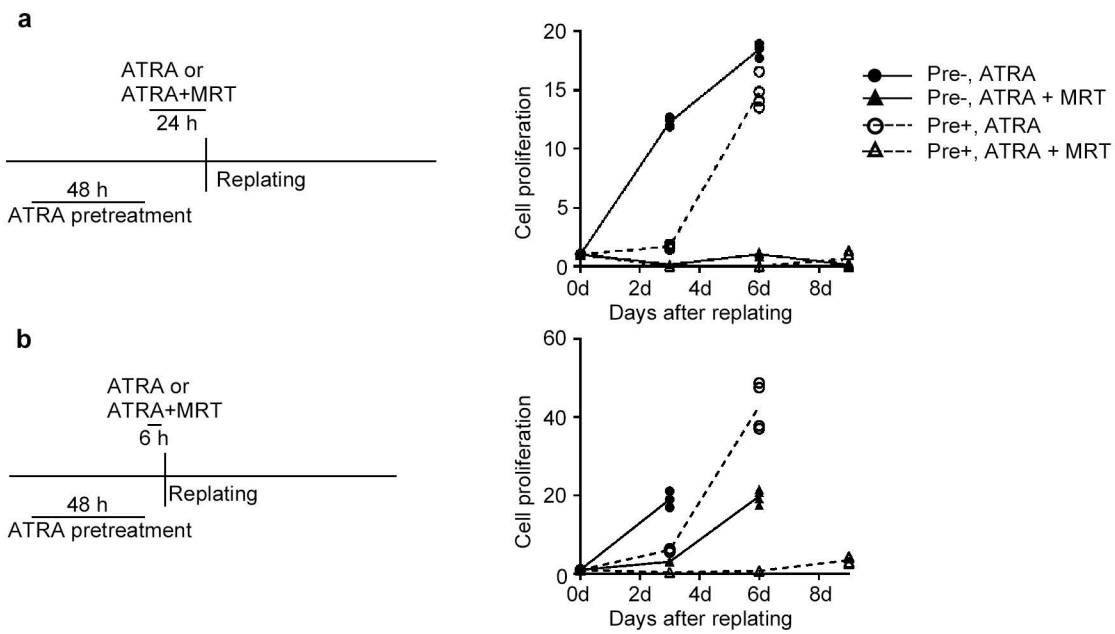


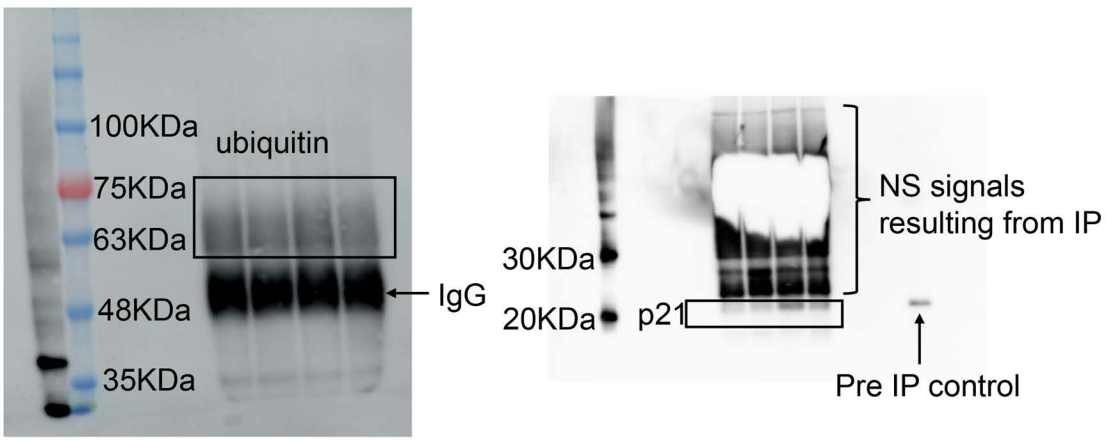
Supplementary Figure 9



Supplementary Figure 10

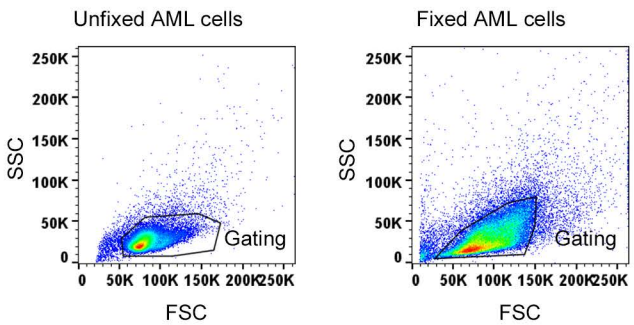


Supplementary Figure 11



Samples (From left to right):
DMSO-treated shControl cells, DMSO-treated shAIM2 cells,
ATRA+MRT-treated shControl cells, ATRA+MRT-treated shAIM2 cells

Supplementary Figure 12



Supplementary Figure 13