

Additional File 1

**A post-transcriptional program of chemoresistance by AU-rich elements and TTP in quiescent
leukemic cells**

List of Additional Files

1. Additional File 1:

Supplemental Figures S1-S6 and legends

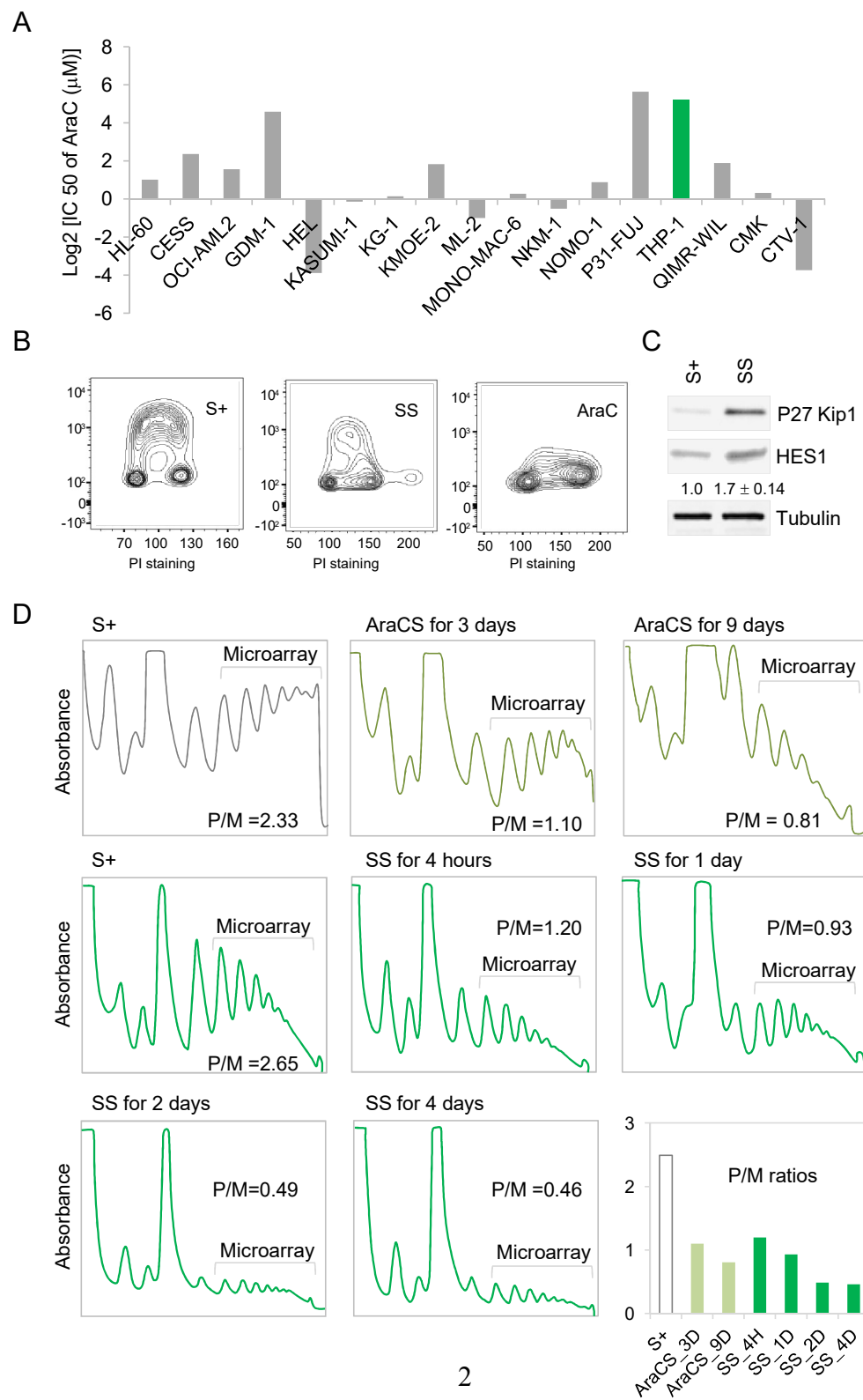
Supplemental Tables legends (for Additional File 2: Table S1 and Additional File 3: Table S2)

References

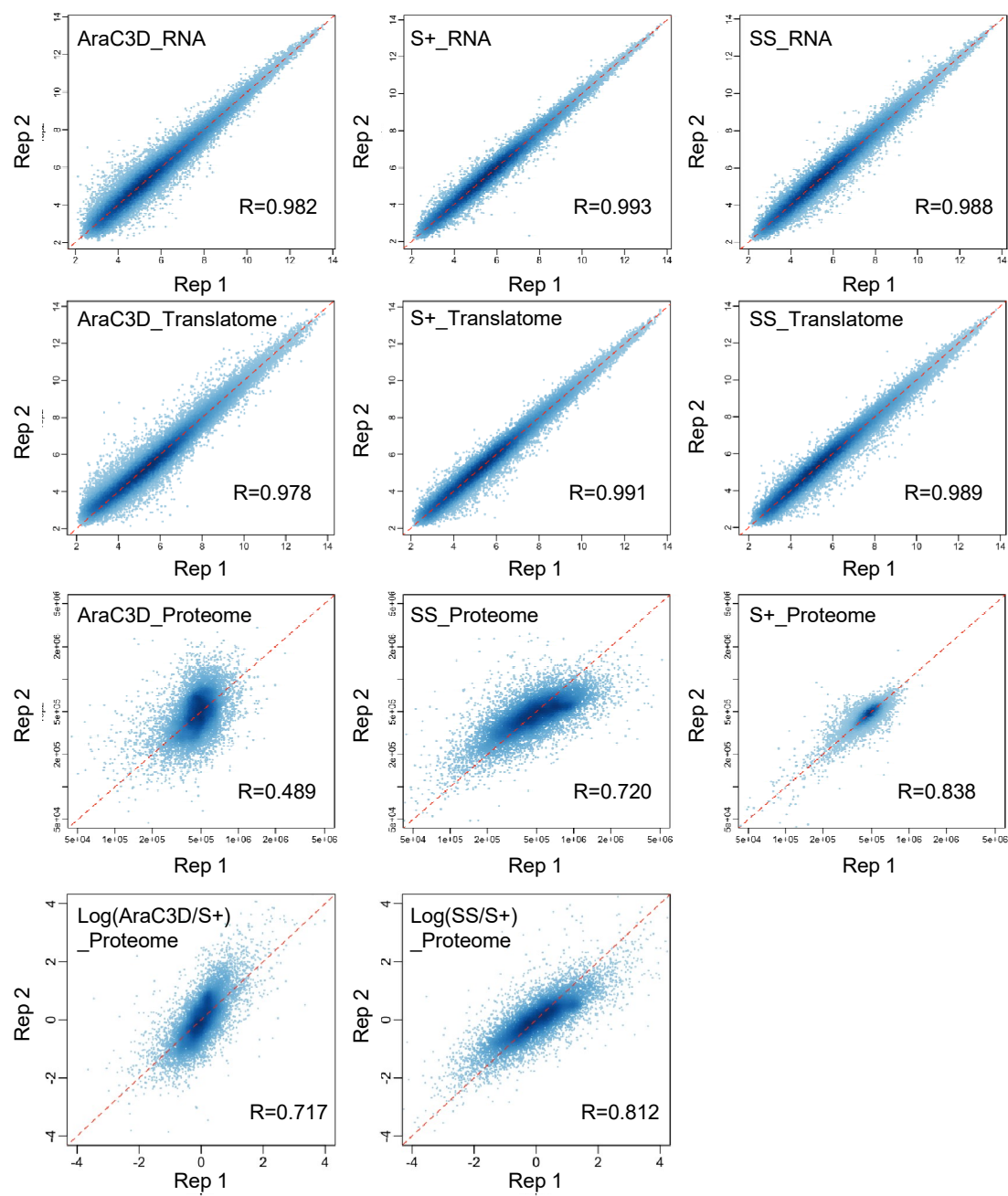
2. Additional File 2: Table S1

3. Additional File 3: Table S2

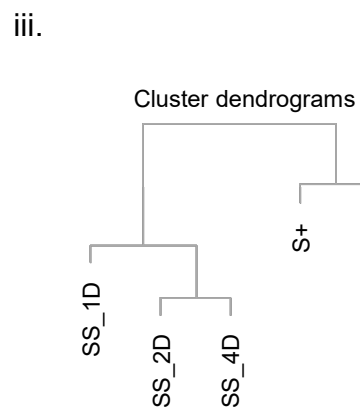
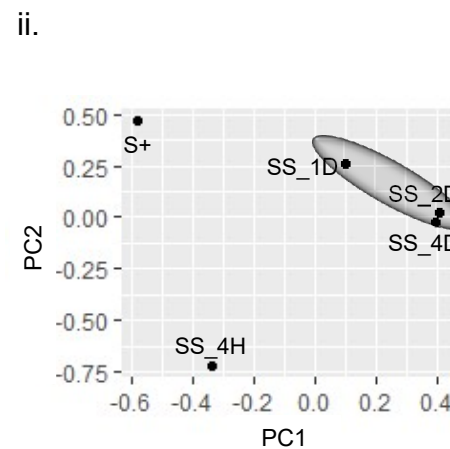
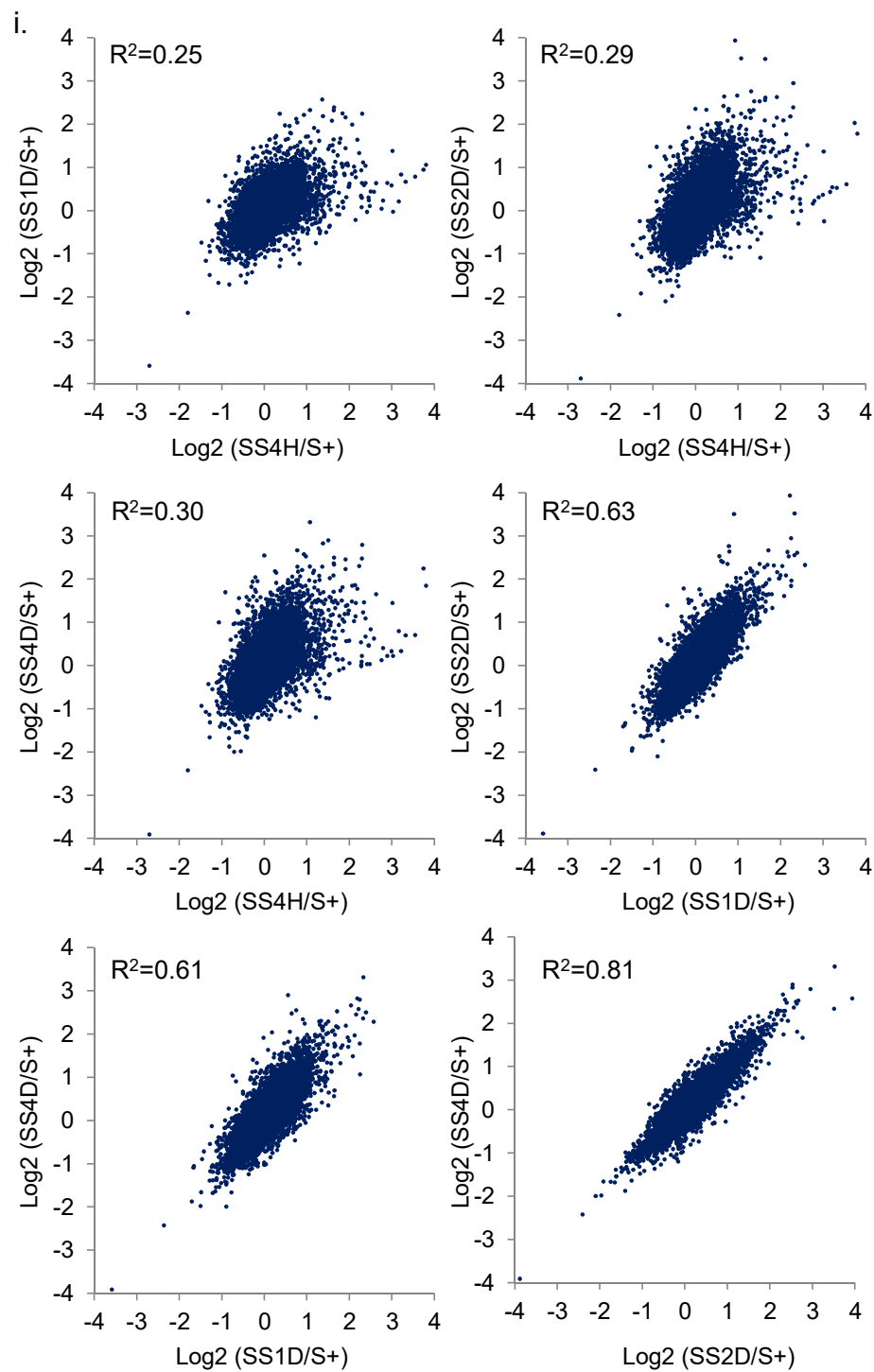
4. Additional File 4: uncropped Western blots for Main and Supplemental Figures



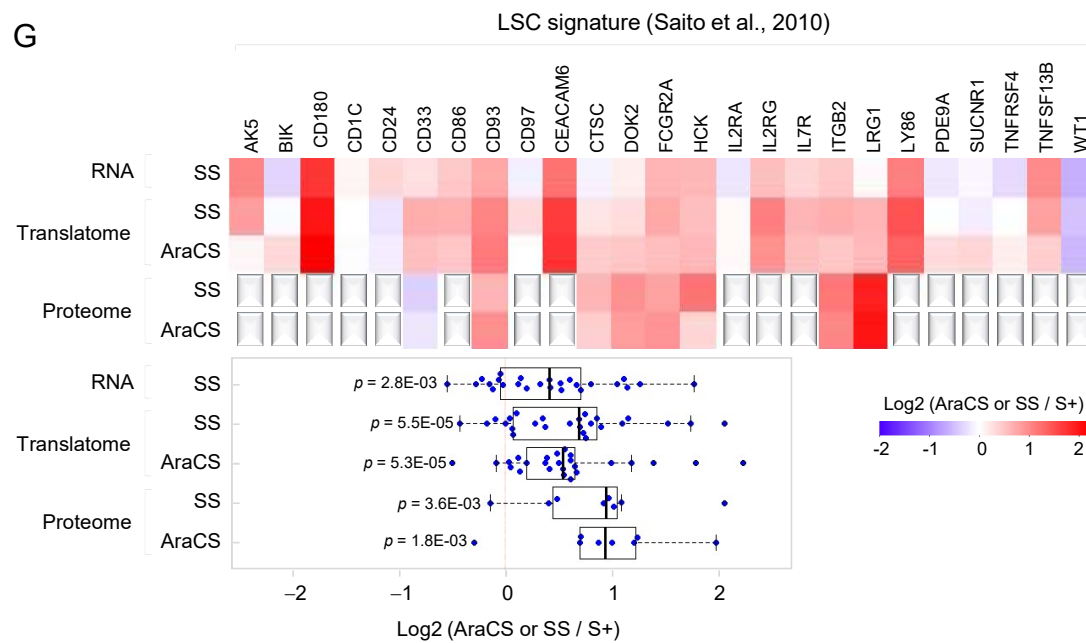
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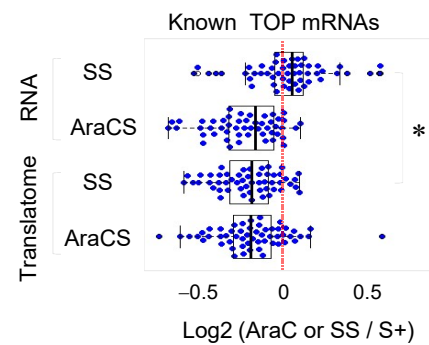
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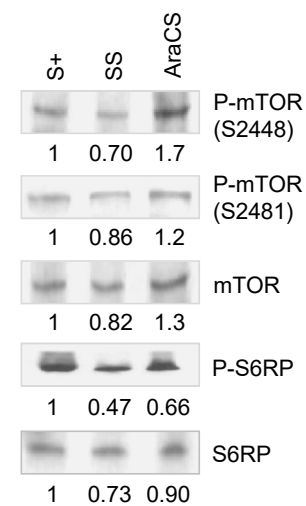
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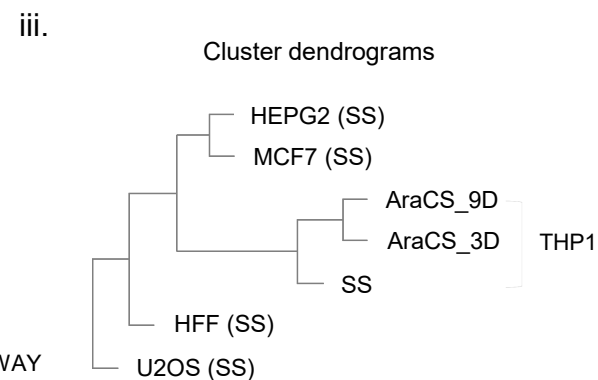
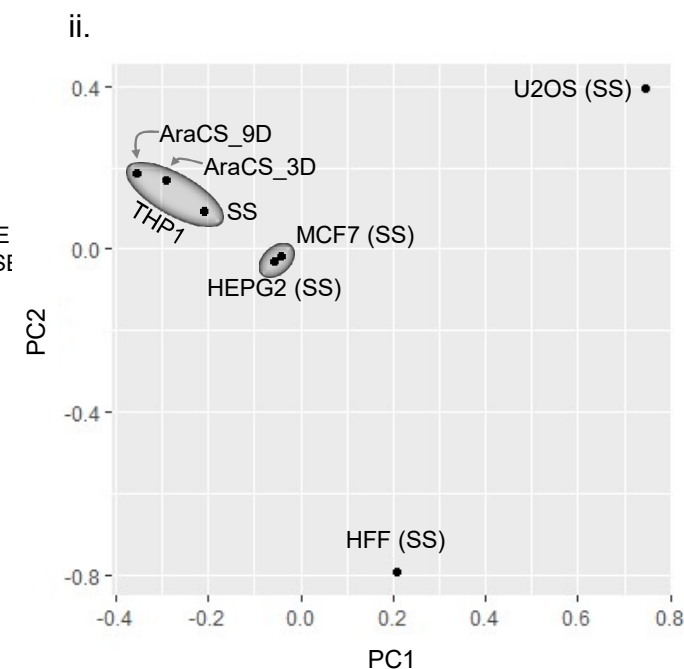
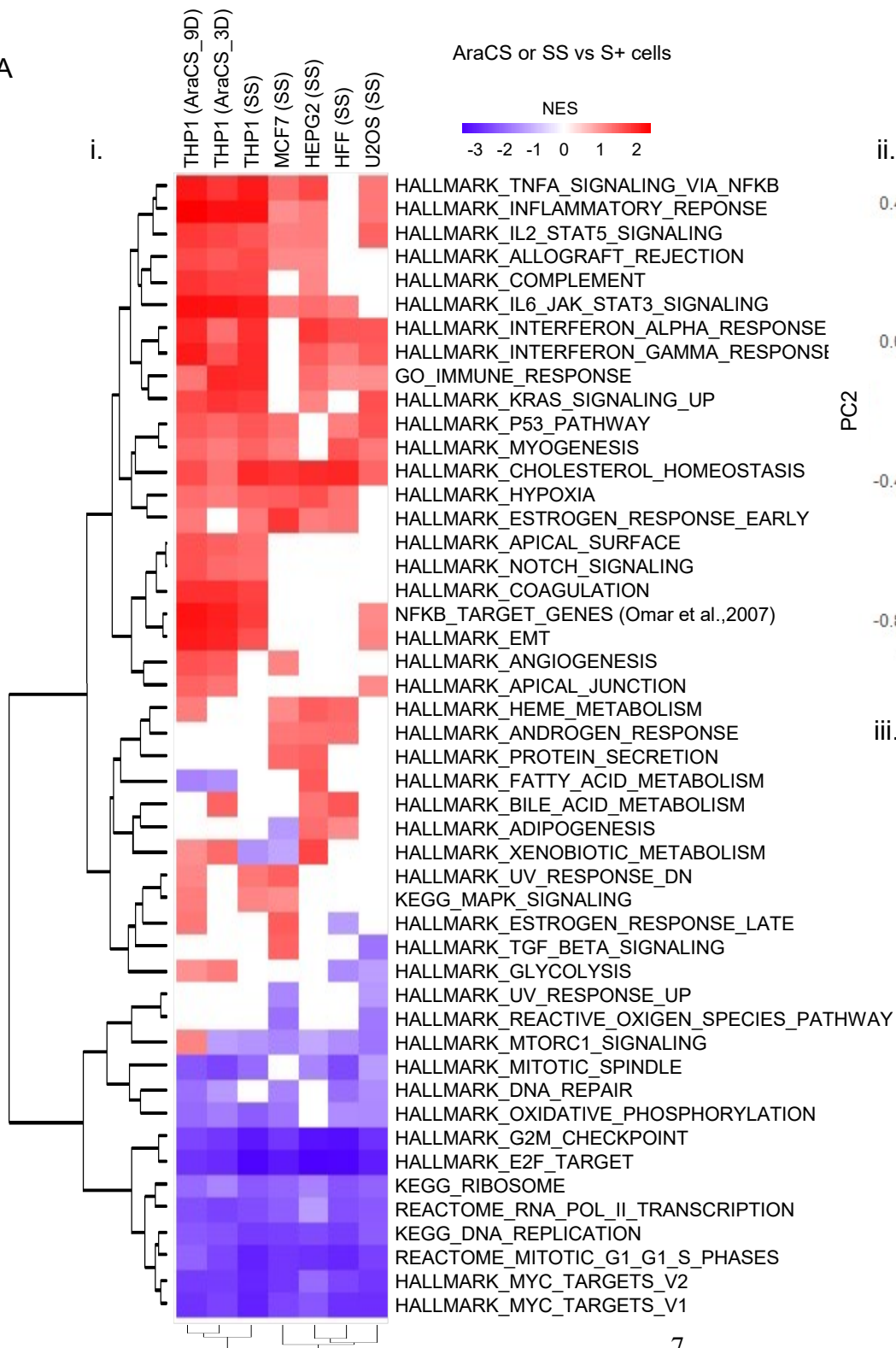


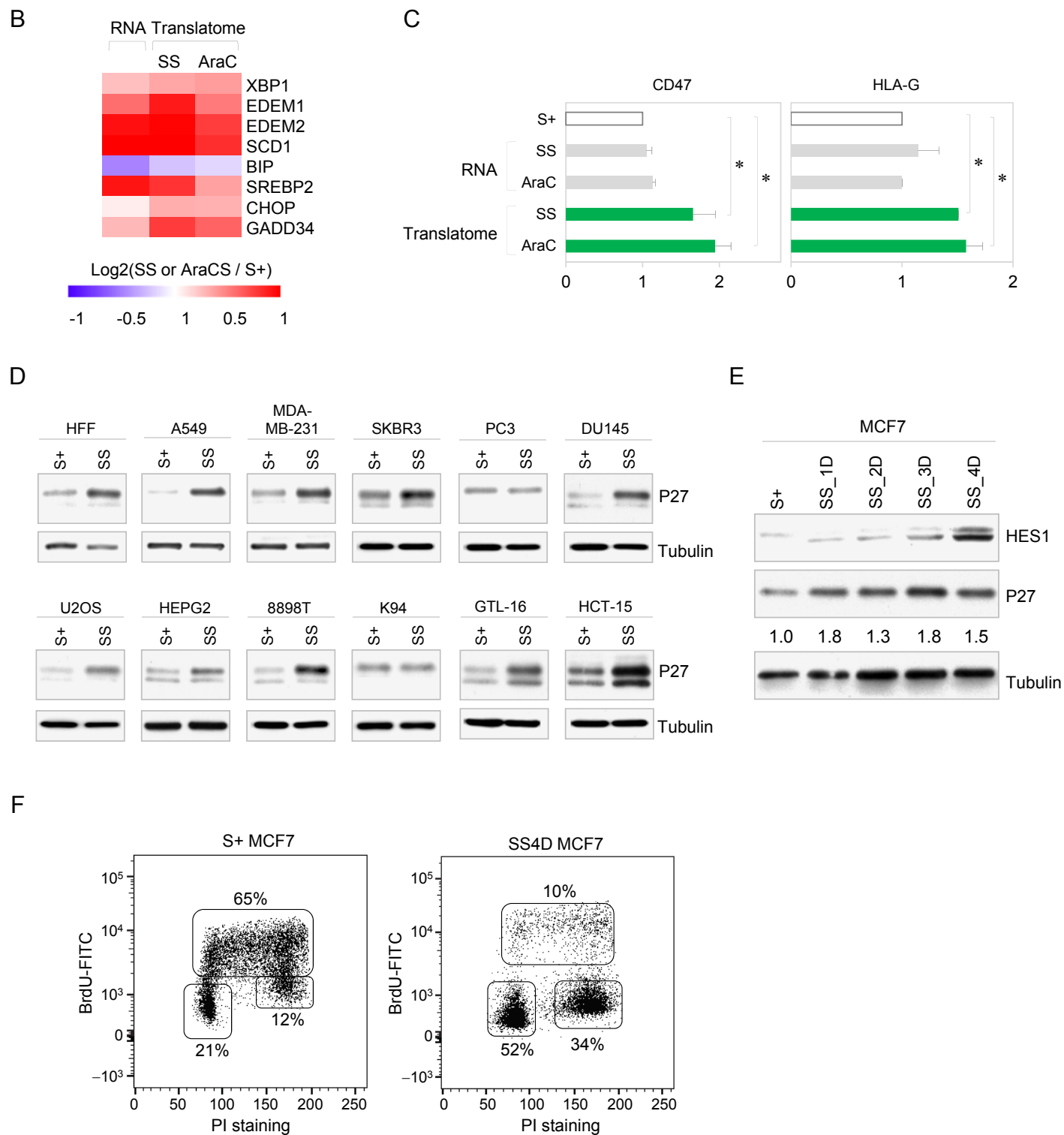
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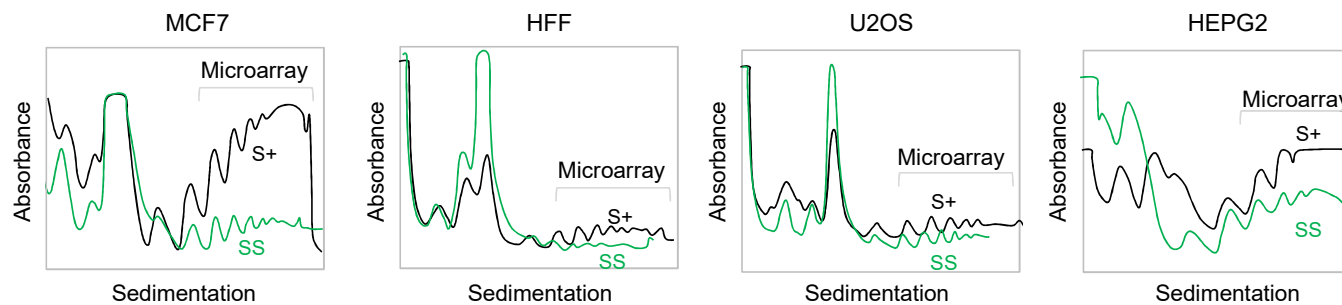
Additional File 1: Fig. S1. Related to main figure 1. A. IC50 values of standard anti-leukemic chemotherapy, AraC, in AML cell lines (1). THP1 cell line was selected for this study as it shows strong resistance to AraC. **B.** Flow cytometric profiles of S+, SS (serum starvation for 4 days) and AraCS (5 μ M AraC treatment for 3 days), using BrdU and PI staining. **C.** G0 arrest of serum-starved THP1 is assessed by Western analysis of p27 and Hes1 levels. **D.** Polysome profiles of S+, SS (serum starvation for 4 hours, 1 day, 2 days and 4 days) and AraCS (5 μ M AraC treatment for 3 days and 9 days) THP1 cells. Heavy polysomes (≥ 3 ribosomes)-associated mRNAs were analyzed by microarray. 'P/M' indicates polysome to monosome ratios. **E.** Scatter plots and correlation coefficients between biological replicates for S+, SS and AraCS cells in the transcriptome, translome, and proteome levels. Additionally, two plots at the bottom show correlation between two replicates from AraCS and SS normalized by the corresponding S+ replicates of the proteome profiles. **F.** Scatter plots and correlation coefficients (i), principal component analysis (PCA) analysis (ii) and unbiased hierarchical clustering (iii) of the translomes of cells that were serum-starved for indicated times. Heatmap and boxplot of the expression of LSC gene signature (2) in AraCS and SS cells. **H.** Boxplot of the transcriptome and translome changes of known TOP mRNAs in response to SS or AraC treatment. **I.** Western blot of mTOR phosphorylation (Serine 2481 and 2448 sites), compared to total mTOR, and of phospho-S6 and total S6, in SS and AraCS cells (3-13).

A



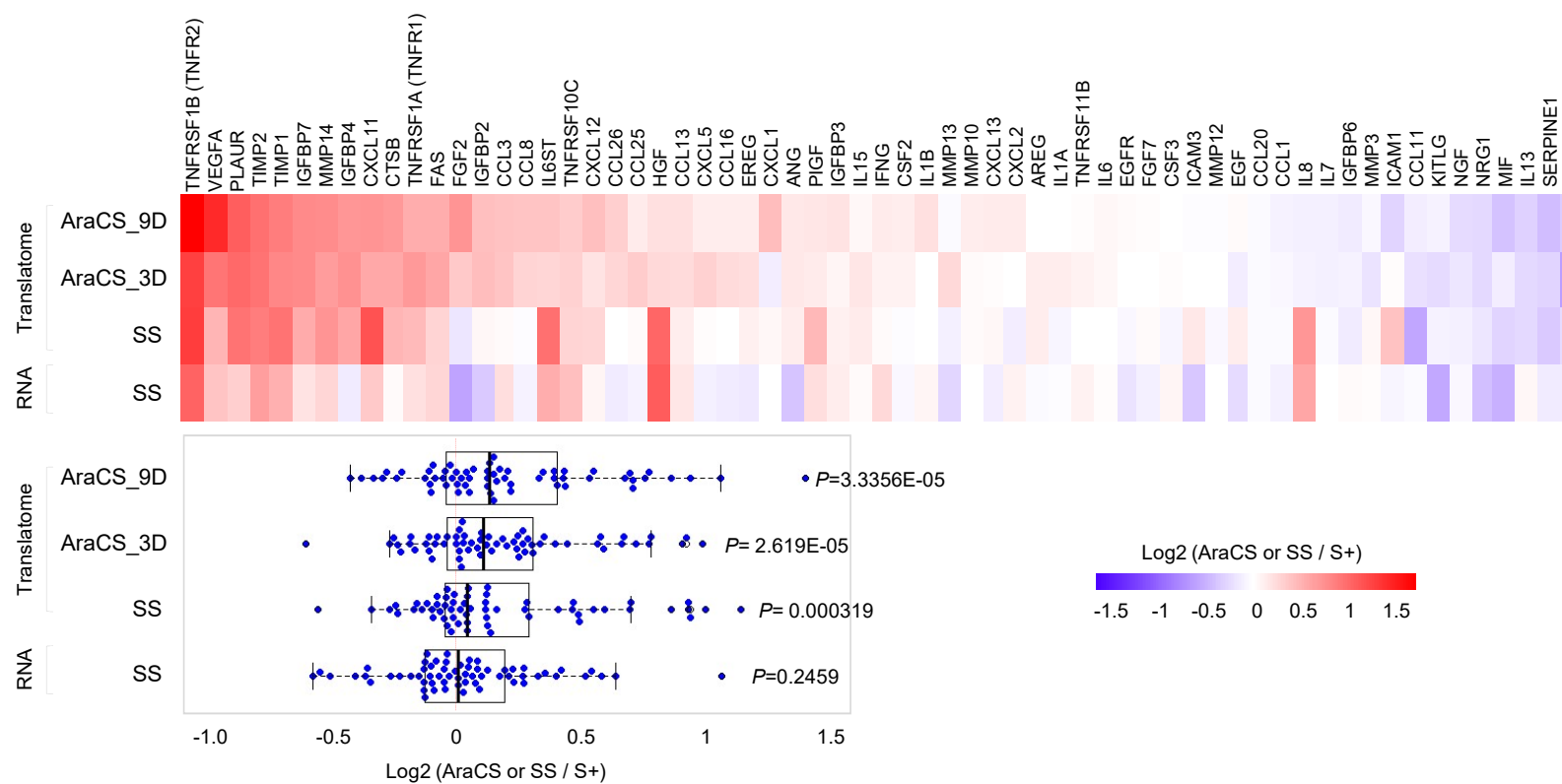


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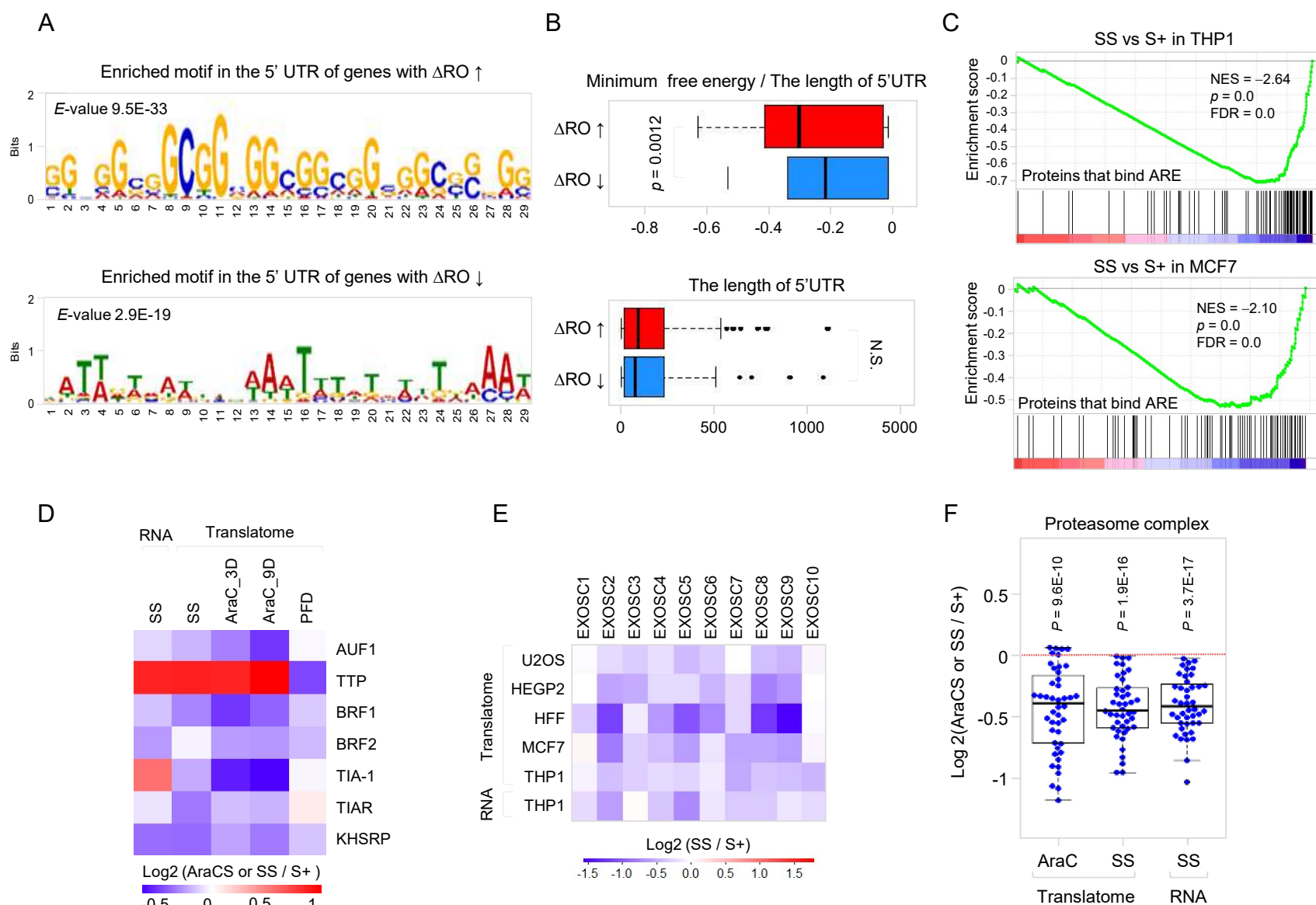


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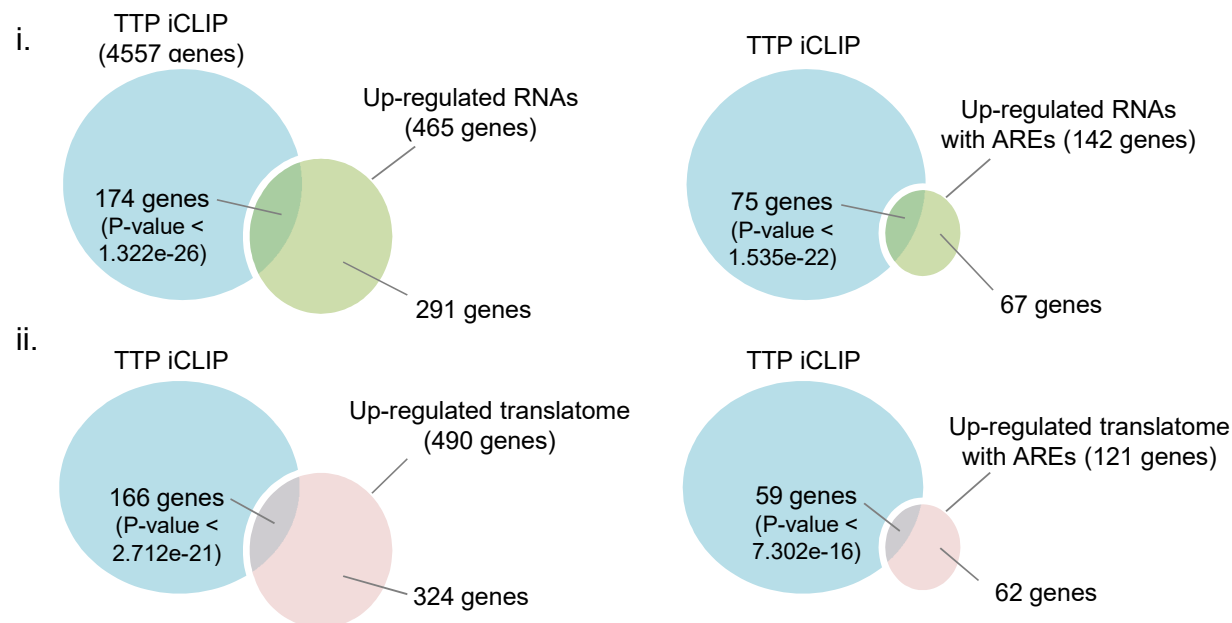
The Senescence-Associated Secretory Phenotype (SASP)



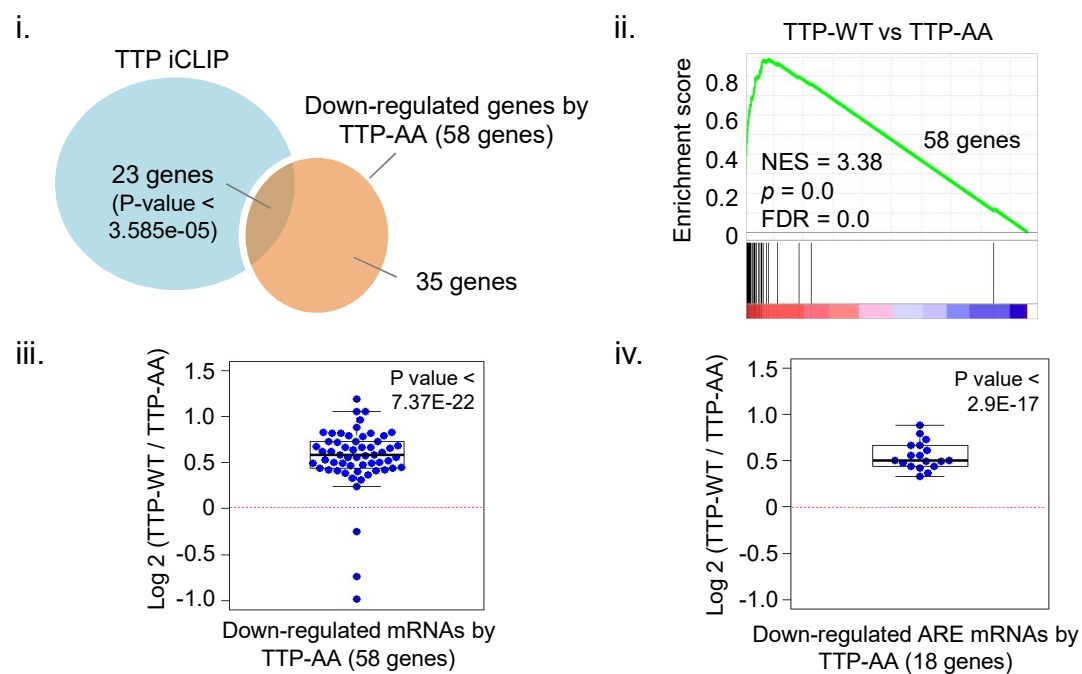
Additional File 1: Fig. S2. Related to main figure 2. **A.** Comparison of translomes of G0 cells from five different cell lines. GSEA was performed using all 50 gene sets of the Hallmark, KEGG, reactome, and GO pathways from the Molecular Signatures Database (MSigDB). Heatmap of normalized enrichment score (NES) for (i) each gene set , (ii) PCA analysis , and (iii) unbiased hierarchical clustering of translomes are shown. **B.** Heatmap of the expression of ER stress related genes in SS and AraCS cells. **C.** Bar graphs showing the expression of CD47 and HLA-G in S+, SS and AraCS cells. **D.** Western analysis of p27KIP1 (p27) in S+ and SS cells as a marker for G0/G1 arrest, shows that G0 cells are induced by serum-starvation in a number of cancer cell lines. **E.** G0 arrest of serum-starved MCF7 is assessed by Western analysis of p27 and Hes1 levels. **F.** Flow cytometric analysis of S+ and SS cells from MCF7 using BrdU and PI staining. **G.** Polysome profiles of S+ and SS cells from MCF7, U2OS, HEPG2 and non-cancerous HFF fibroblasts cell lines. Heavy polysomes (≥ 3 ribosomes) were analyzed by microarray. **H.** Heatmap and boxplot of the expression of SASP signature genes in SS and AraCS THP1 cells. * $p \leq 0.05$ Data are represented as average $\pm$ SEM.



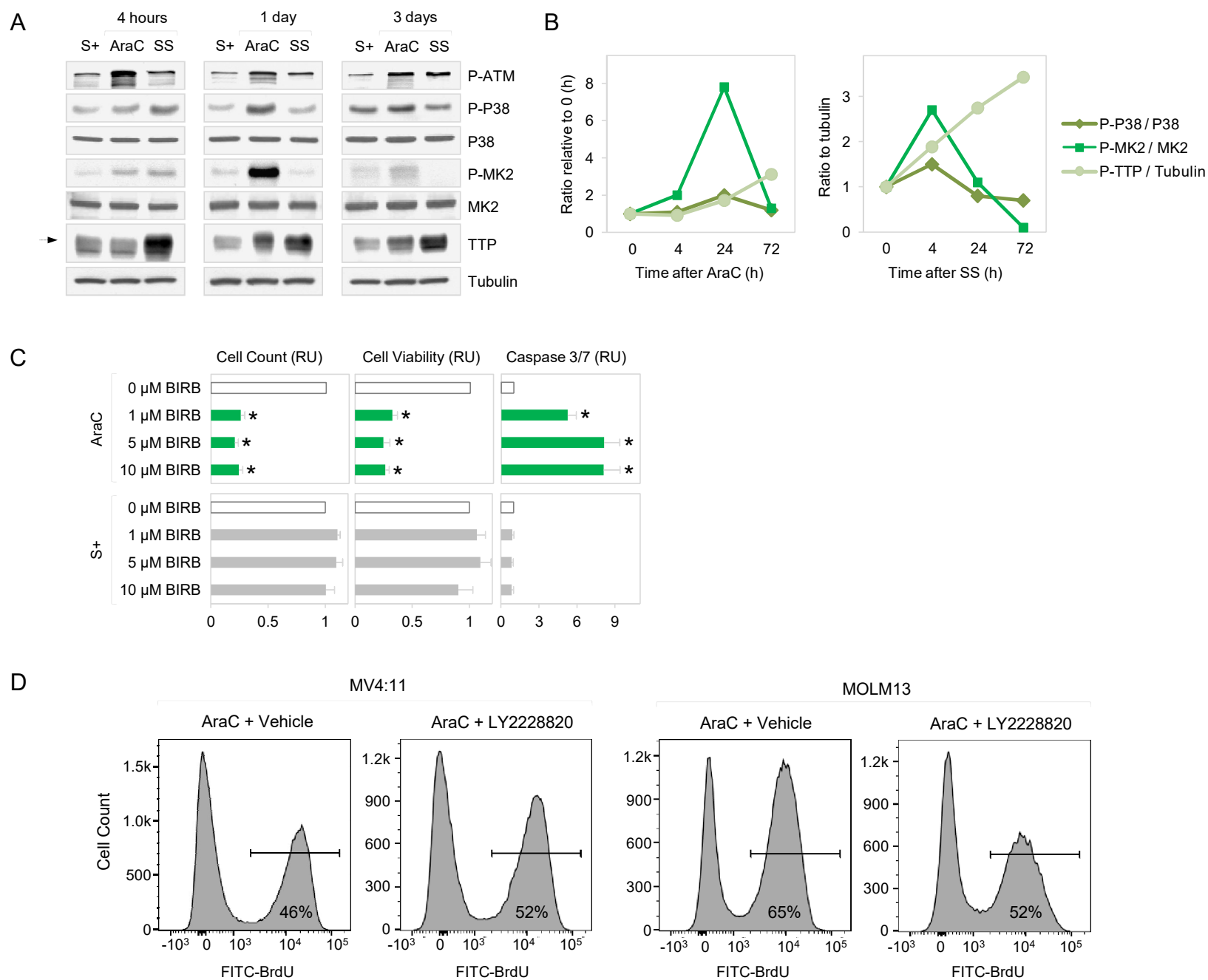
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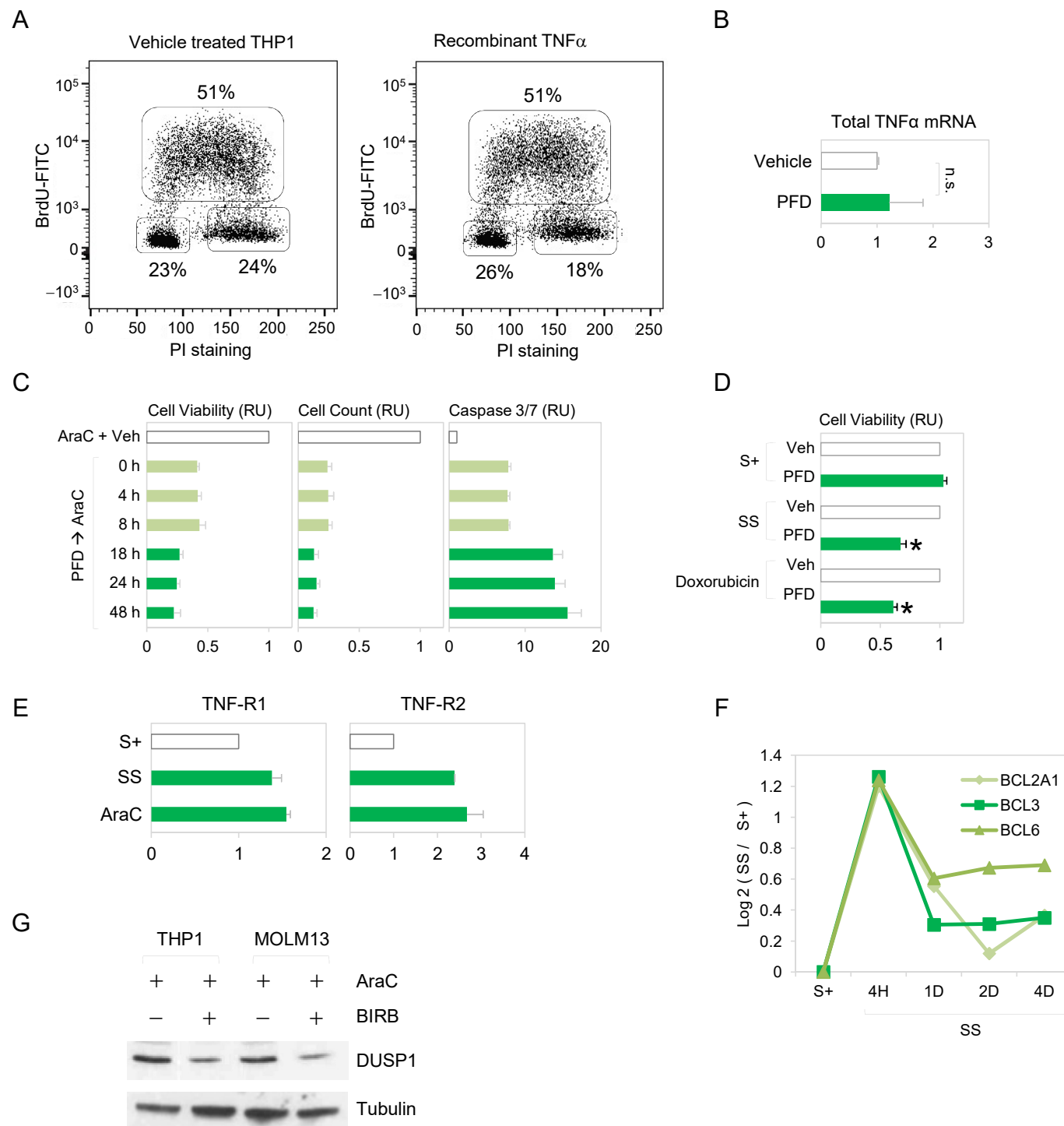
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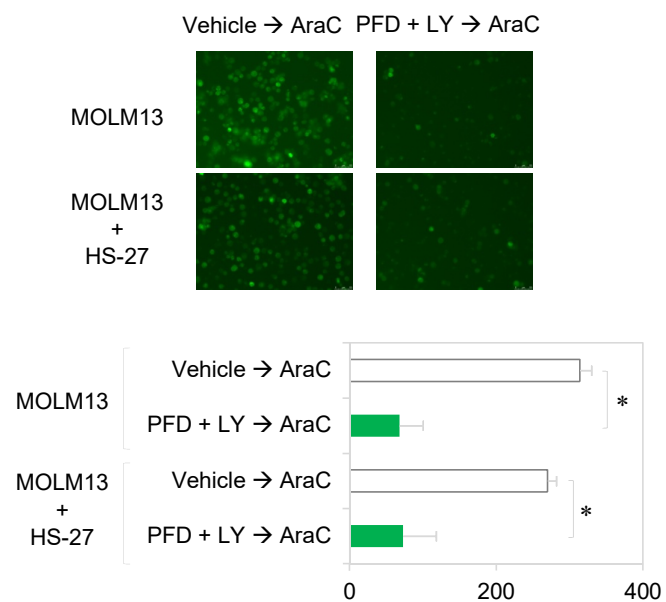
Additional File 1: Fig. S3. Related to main Figure 3. **A.** Distinct motifs enriched in 5' UTRs of genes where ribosome occupancy is significantly increased ($\Delta RO \uparrow$, top panel) or decreased ($\Delta RO \downarrow$, bottom panel) in G0 chemoresistant cells. **B.** Minimum free energy of RNA secondary structure and length of 5'UTRs of genes. **C.** Expression of genes involved in the decay of ARE mRNAs in SS THP1 (top) or SS MCF7 (bottom) compared to S⁺ cells is shown by GSEA. **D.** Expression of ARE-binding proteins in G0 leukemic cells are shown as a heatmap. These proteins are known to cause ARE mRNA decay or translation repression. **E.** Heatmap of the expression of exosome complex genes (3'-5' exonuclease RNA decay and processing complex) in G0 cancer cells. **F.** Boxplot showing reduced expression of proteasome complex genes in G0 leukemic cells. **G.** (i) Venn diagram comparison of TTP targets (TTP-iCLIP) (14) with the translome or ARE-bearing translome up-regulated in G0 cells and (ii) of TTP targets with the mRNAs or ARE mRNAs up-regulated in G0 cells. **H.** Transcriptome profiles using microarray, of AraC-treated cells knocked out for endogenous TTP and expressing either TTP-AA or TTP-WT. (i) Venn diagram of TTP targets and the transcriptome downregulated by TTP-AA compared to TTP-deficient cells in AraCS cells. (ii) GSEA of 58 genes down-regulated by TTP-AA in cells expressing TTP-WT compared to TTP-AA. (iii) Relative RNA levels of 58 genes targeted by TTP-AA in cells expressing TTP-WT compared to TTP-AA. (iv) Relative RNA levels of 18 ARE-bearing genes targeted by TTP-AA in cells expressing TTP-WT compared to TTP-AA. Data are represented as average $\pm$ SEM.



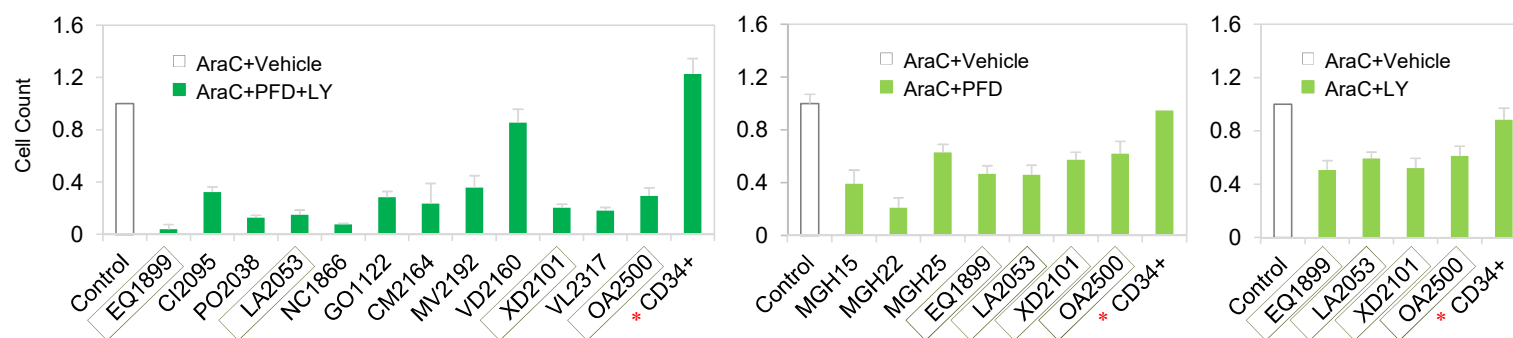
Additional File 1: Fig. S4. Related to main Figures 4-6. **A.** Western analysis of indicated proteins in THP1 cells at time points after serum starvation or 5 μ M AraC treatment. **B.** Relative ratio of phospho-p38 MAPK to total p38 MAPK, phospho-MK2 to total MK2, or TTP to tubulin (loading control), at indicated time points upon AraC or SS treatment. **C.** Effect of p38 MAPK inhibition on survival of AraC-resistant cells. MOLM13 leukemic cells were pre-treated with various concentrations of BIRB796 (BIRB or BB), followed by AraC or vehicle treatment. Cell viability and death were assessed by cell counting, MTS and caspase 3/7 assays. RU=relative units. **D.** Flow cytometric profiles of MV4:11 or MOLM13 cells treated with AraC or AraC plus LY2228820 for one day. Percentages of BrdU-positive cells are shown. * $p \leq 0.05$ Data are represented as average $\pm$ SEM.



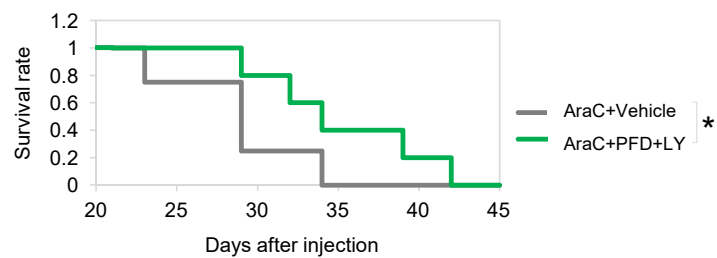
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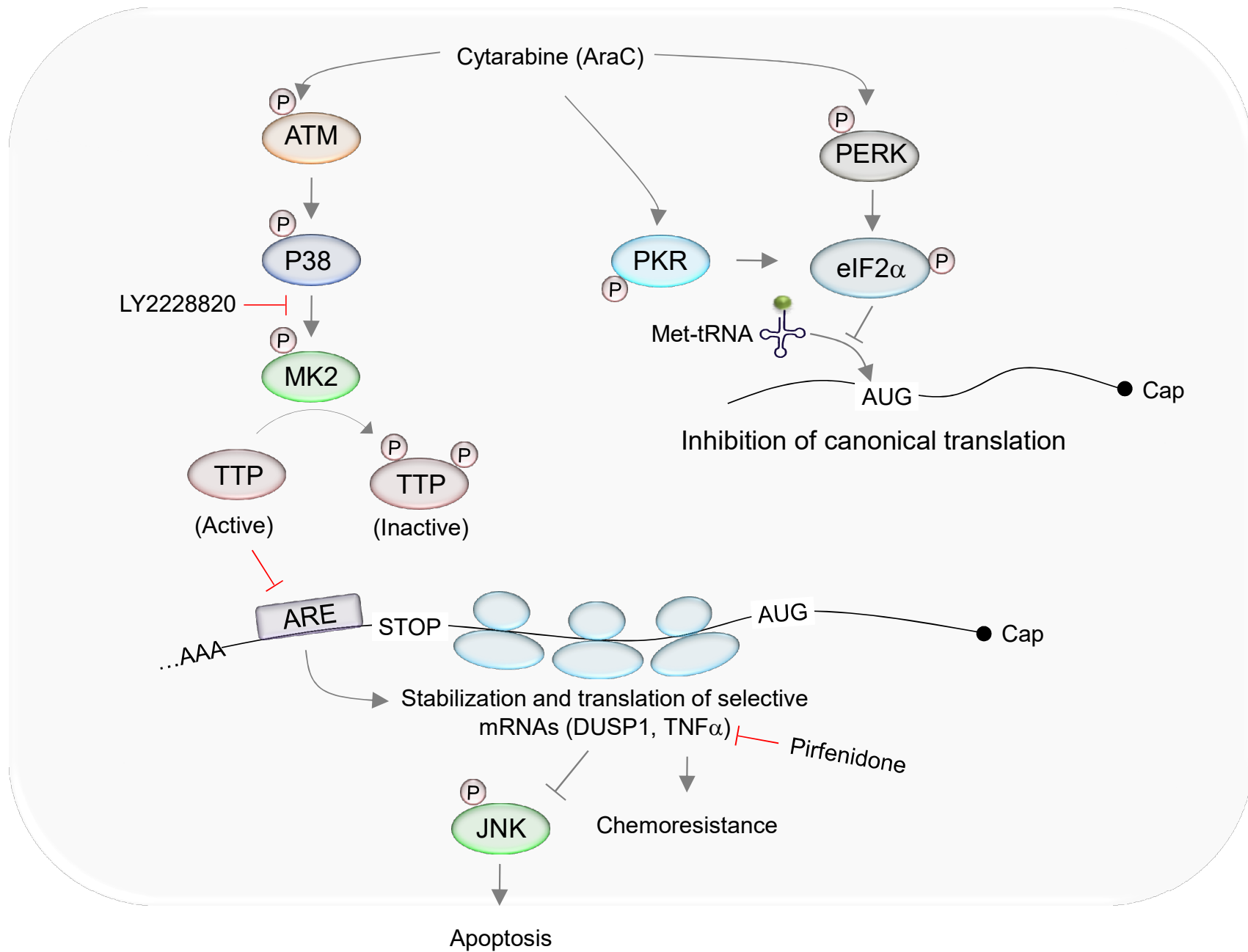
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Additional File 1: Fig. S5. Related to main Figures 5-7. **A.** Flow cytometric profiles of THP1 cells treated with vehicle or recombinant TNF α . **B.** TNF α RNA levels in SS cells treated with vehicle or PFD. **C.** Effect of PFD on survival of AraC-resistant cells. THP1 cells pre-treated with 300 μ g/ml PFD for indicated time followed by 5 μ M AraC. Cell viability and death were measured. **D.** MCF7 cells were pre-treated with 300 μ g/ml PFD or vehicle 1 day before treatment with serum starvation or 150 nM doxorubicin. Cell viability of PFD-treated compared to vehicle-treated cells is shown. **E.** TNF-R1 and TNF-R2 expression at the transcriptome level. **F.** Transcriptome expression of BCL2A1, BCL3 and BCL6 at indicated time points after serum starvation. **G.** Western blot of DUSP1 in AraC THP1 and MOLM13 cells treated with BIRB or vehicle. Tubulin serves as loading control. **H.** MOLM13-GFP-luciferase cells were cultured with or without HS-27 cells and treated with PLA therapy or AraC. Representative microscopic images (top panel) and quantification of luciferase activity (bottom panel) are shown. **I.** Viability of primary cells from AML patients and normal CD34 $^{+}$ cells (marked by a red asterisk) from healthy donors after indicated treatments, normalized to DMSO+AraC treatment for each patient sample (represented as a white bar and set to 1). Patient samples that were compared with individual treatments and PLA and shown in Fig. 8A are marked by a box. **J.** Kaplan-Meier survival curves of C57BL/6 mice engrafted with primary HOXA9/Meis1 cells and treated with PLA therapy or AraC in figure 8F. * $p \leq 0.05$ Data are represented as average $\pm$ SEM.



Additional File 1: Fig. S6. Related to main Figures 1-8. A model for chemoresistance in AML. Post-transcriptional and translational regulation of gene expression leads to chemoresistance and G0 cell survival, and is regulated by DNA damage and stress signaling, triggered in subpopulations of cancer by chemotherapy and serum-starvation. AraC or SS treatment induces and enriches for quiescent, chemoresistant leukemic cells. Even though canonical translation is inhibited, ARE-bearing mRNAs are increased and highly translated in G0 cells. Mechanistically, the p38 MAPK-MK2 pathway stabilizes ARE mRNAs such as TNF α and DUSP1 via phosphorylation of TTP and inactivation of its RNA decay activity. DUSP1 inhibits JNK-mediated apoptosis and TNF α increases anti-apoptotic genes and cell survival, leading to chemoresistance. Inhibition of ARE mRNA expression by p38 MAPK and TNF α inhibitors or TTP-AA mutant sensitizes resistant leukemic cells to AraC treatment.

Legend for Additional File 2: Table S1. Related to main Figures 1-8. RNAs up-regulated at the translome level and their RO changes in G0 cells induced by SS or AraC.

Legend for Additional File 3: Table S2. Related to main Figures 1-8. RNAs bearing AU-rich elements (AREs) and up-regulated at the translome level in G0 cells induced by SS or AraC.

References

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