Supplementary Data

Supplementary Figure 1

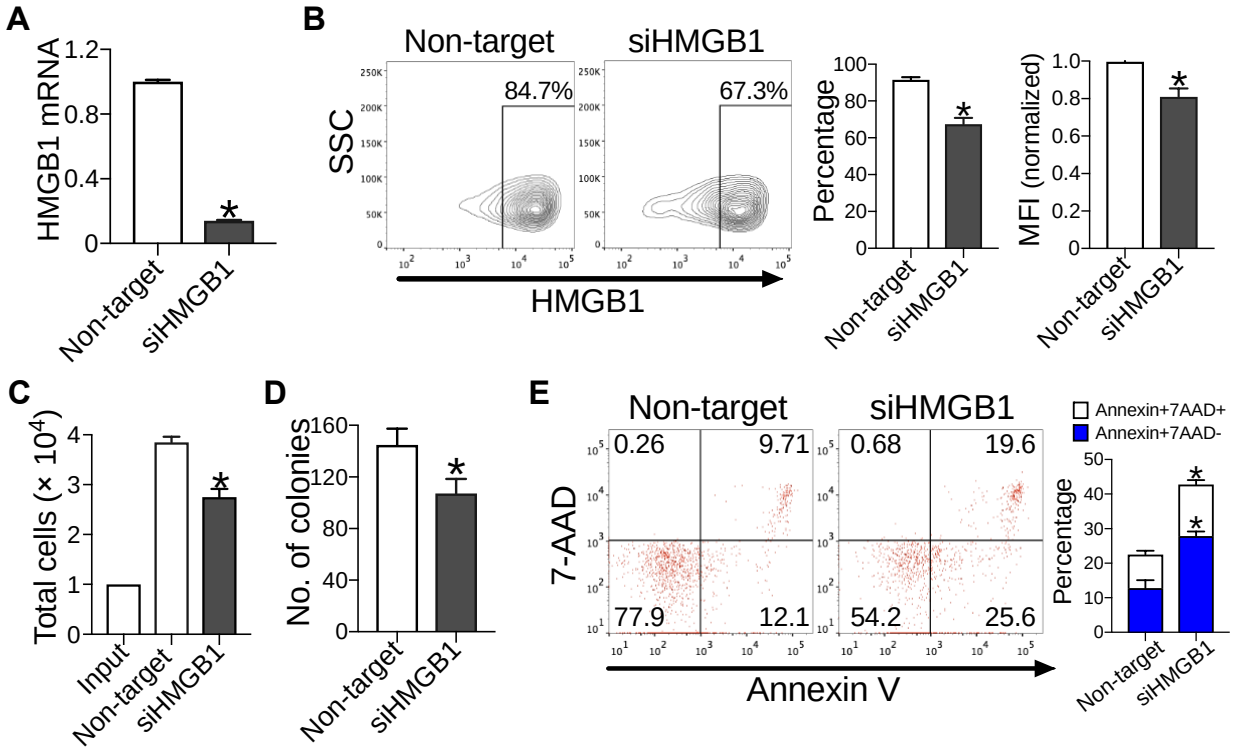

Figure S1. Inhibition HMGB1 impairs total cell expansion and colony-forming capacity of MDS-L. (**A**) RTPCR analysis of HMGB1 mRNA expression at 72 h in MDS-L cells with either non-targeting siRNA control (non-target) or HMGB1-specific siRNA (siHMGB1). **P* <0.0001 for siHMGB1 compared to non-target. *n*= 3/group. (**B**) Left, representative flow cytometric analysis of HMGB1 expression of HMGB1 at 72 h for specified treatments. SSC, side-scattered light. Right, bar graphs indicate percentage of HMGB1+ population and average expression of HMGB1 in mean fluorescence intensity (MFI) value. **P* <0.0001. *n*= 5-6/group. (**C**) Total cell expansion and (**D**) Number of colony-forming cells (CFCs) per 1000 cells per dish containing the indicated siRNA. **P* <0.05. *n*= 4-6/group. (**E**) Apoptotic (Annexin V+ 7AAD-) and necrotic (Annexin V+ 7AAD+) populations in MDS-L containing either non-target siRNA or HMGB1-

specific siRNA. *P= 0.001 and 0.02 for Annexin V+ 7AAD- and Annexin V+ 7AAD+, respectively. n= 4/group. Student's 2-tailed unpaired t tests were used in these analyses.

Supplementary Figure 2

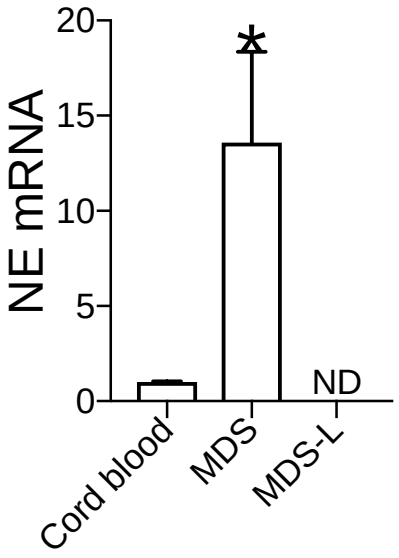

Figure S2. Neutrophil Elastase (NE) is differentially expressed in MDS cells compared to CD34+ Cord blood. mRNA expression of NE in primary MDS cells compared to CD34+ cord blood. NE was not detected (ND) in MDS-L cells. *P= 0.02 for MDS compared to cord blood by Mann-Whitney analysis. n= 3-6/group. Student's 2-tailed unpaired t test was used in this analysis.

Supplementary Figure 3

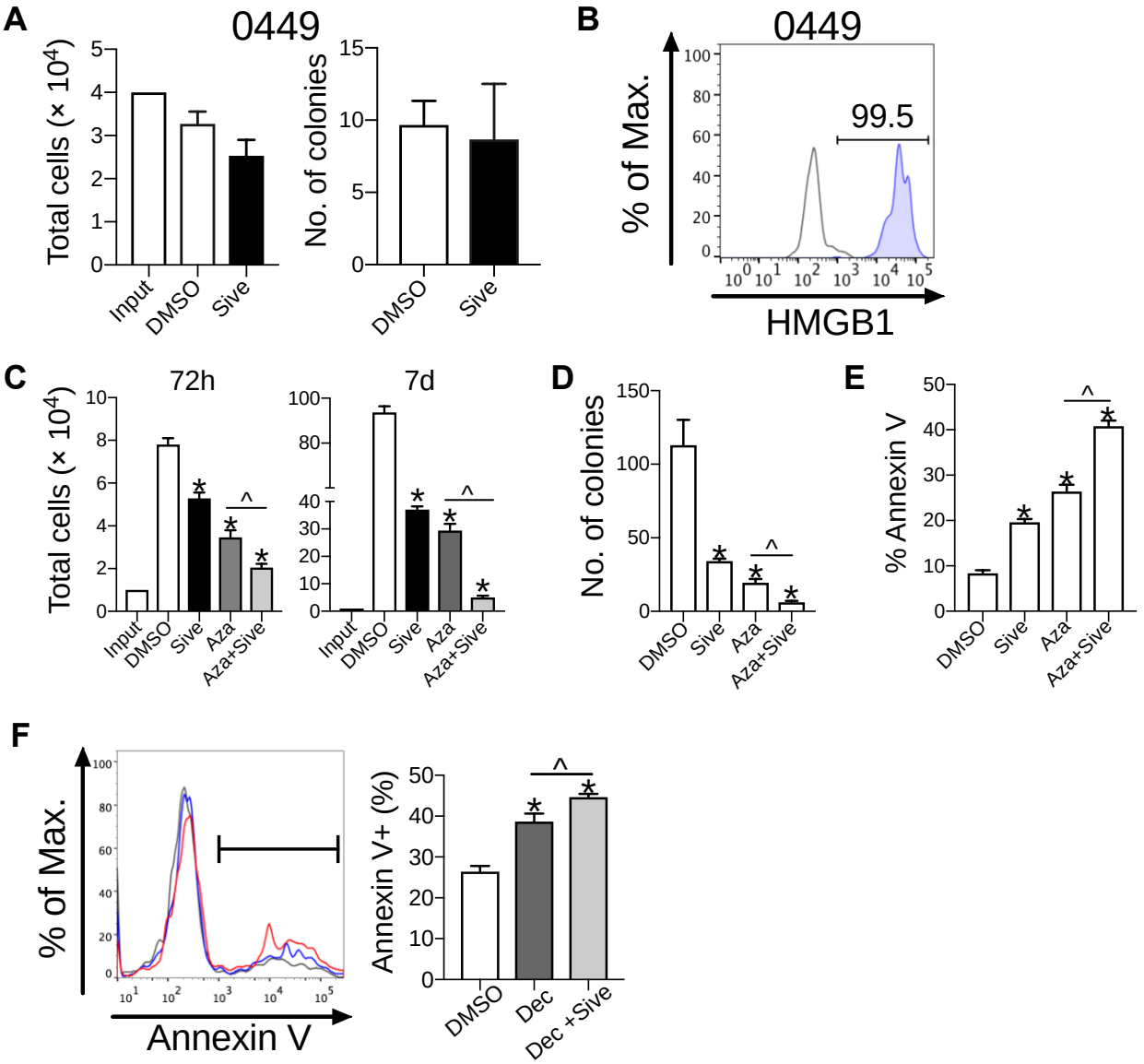

Figure S3. Dual therapy with sivelestat and chemotherapy is additive to impair MDS function compared to chemotherapy alone. (**A**) Left, total cell expansion of primary marrow cells from MDS patient 0449 after 72 h incubation with 300 μg/ml sivelestat (Sive) or DMSO. *n*= 4/treatment. Right, number of CFCs following the treatment with sivelestat or DMSO. 5,500 cells for 0449 were plated per dish. *n*= 3/group. (**B**) Flow cytometric analysis of HMGB1 expression in $CD34^+$ cells from MDS patient 0449. (**C**) Total cell expansion of MDS-L in cultures at 72 h and 7d with DMSO, 300 μg/ml sivelestat, 1 μM azacitidine, or both. **P* ≤0.001 for sivelestat, Aza and

Aza+Sive compared to DMSO. ^*P*= 0.005 and <0.0001 for Aza compared to Aza + Sive in 72h and 7d, respectively. *n*= 5-6/group. (**D**) CFCs from 72 h cultures with treatments indicated in (**C**). Number of cells from culture per dish: 1,000 cells. * *P* ≤0.001 for sivelestat, Aza and Aza+Sive compared to DMSO. ^*P*< 0.001 for Aza compared to Aza + Sive. *n*= 6/group. (**E**) Percentage annexin V+ cells by flow cytometric analysis for 72 h cultures at conditions indicated in (**C**). **P* <0.0001 for sivelestat, Aza and Aza+Sive compared to DMSO. ^*P* <0.0001 for Aza compared to Aza + Sive. *n*= 5/group. (**F**) Percentage Annexin V+ cells of primary CD34+ MDS cells in culture with DMSO, 75 nM decitabine (Dec) or 75 nM decitabine and 300 μg/ml sivelestat (Dec+Sive) for 7 days. DMSO is shown in gray, Dec in blue and Dec+Sive in red. **P*= 0.002 and <0.0001 for Dec and Dec+Sive compared to DMSO. ^*P*= 0.03 for Dec+Sive compared to Dec, respectively. *n*= 4/group. Student's 2-tailed unpaired *t* tests were used in these analyses.

Supplementary Figure 4

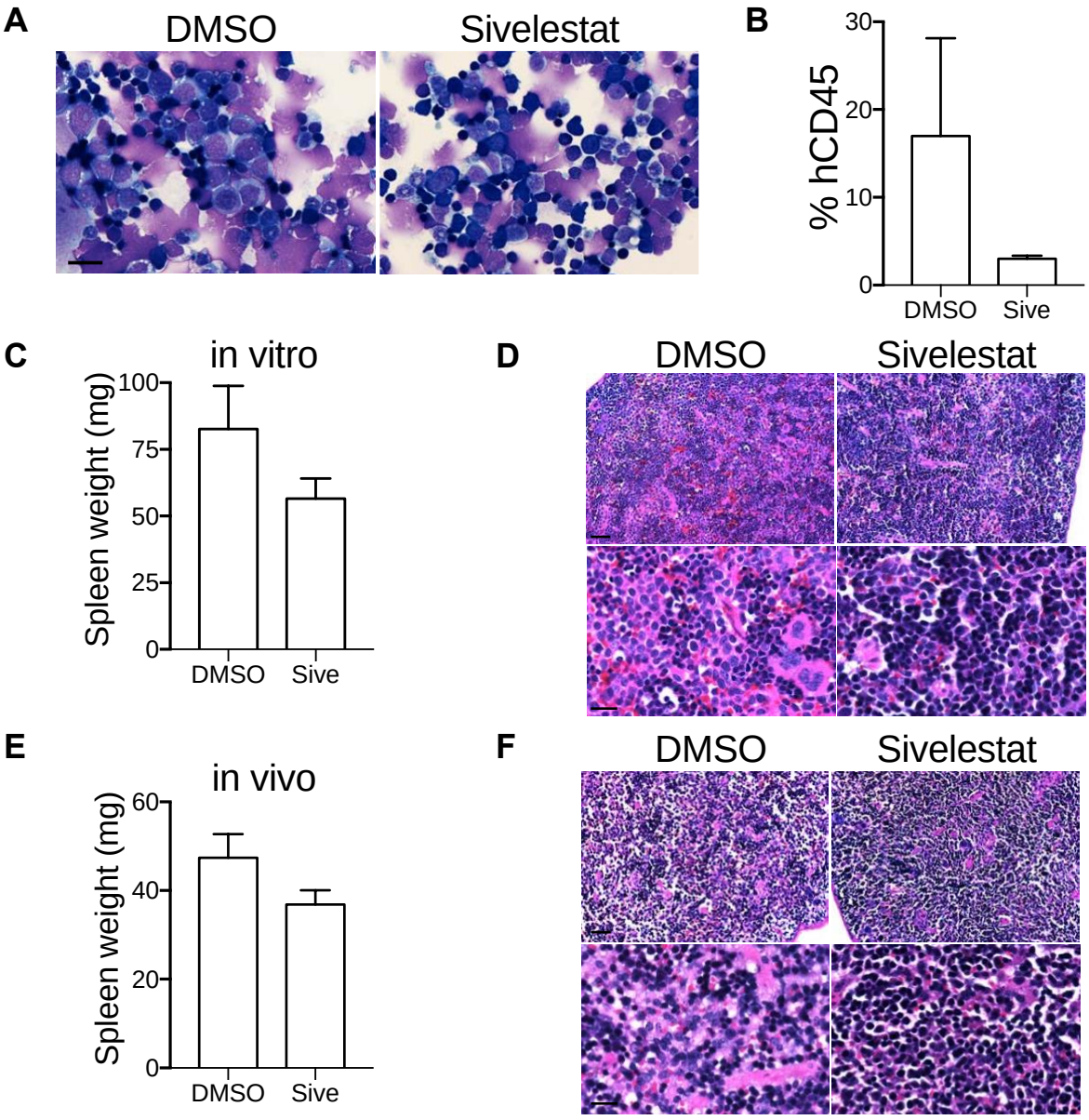

Figure S4. Sivelestat preserved splenic architecture in mice transplanted with MDS-L cells. The efficacy of sivelestat in an MDS xenograft model was tested in vitro and in vivo. NSG mice were transplanted with MDSL cells that were pretreated with 300 μg/ml sivelestat for 72h in vitro (a-d). Spleens were harvested for analysis at 17 weeks post-transplantation. (**A**) Wright-Giemsa staining of spleen aspirates showing a lower level of MDS-L engraftment in sivelestat-treated animals. (**B**) Percentages of human CD45 cell engraftment in the spleen aspirates by

flow cytometric analysis. (**C**) Spleen weights are shown. *n*= 4-5/group. (**D**) Hematoxylin and eosin stain of section of spleen. Scale bar is 20 μM. For testing drug in vivo, NSG mice were given sivelestat via IP injection (5 mg/kg for 7 days) 24 h after MDSL transplantation (E-F). (**E**) Spleen weights are shown. *n*= 5-7/group. (**F**) Hematoxylin and eosin stain of section of spleen. Scale bar 20 μM. Student's 2-tailed unpaired *t* tests were used in these analyses.

Supplementary Figure 5

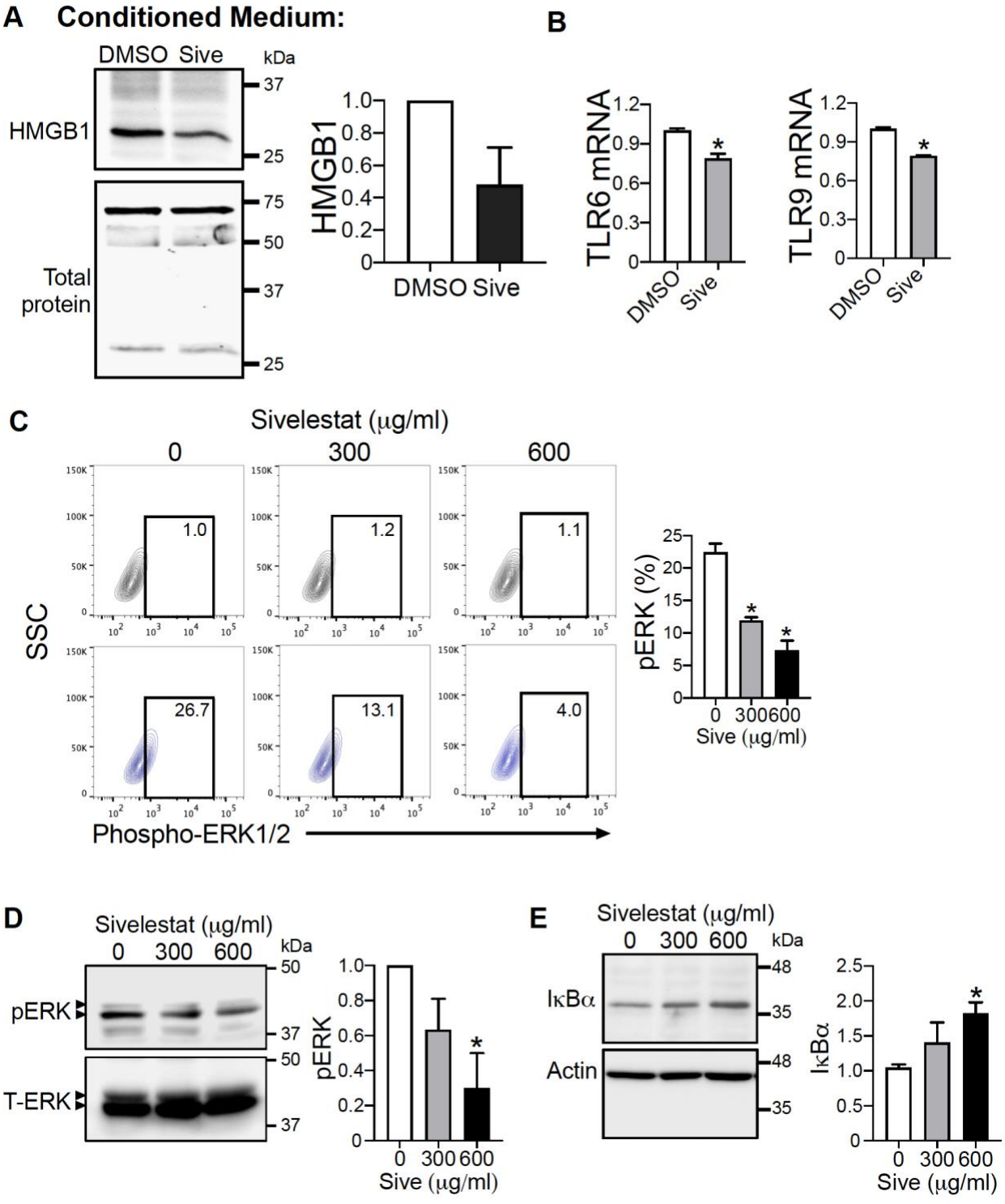

Figure S5. Sivelestat inhibits the release of HMGB1 in primary MDS, and modulates TLR-related signaling pathways. (**A**) HMGB1 protein expression by western analysis of conditioned

media from CD34+ MDS cells treated with 300 μg/ml sivelestat or DMSO for 12h. Right, quantification of HMGB1 level in conditioned media was normalized to REVERT Total Protein Stain. *n*= 1 for biologic sample; 2 technical replicates/group. (**B**) Quantitative RTPCR measurement of expression of TLR6 and TLR9 in MDS-L cells following culture with DMSO or 300 μg/ml sivelestat for 4-8 h. **P* = 0.006 and <0.0001for TLR6 and TLR9, respectively. *n*= 3/group. (**C**) Left, representative flow cytometry plots of isotype (grey) and anti-phospho-ERK1/2 (blue) in MDS-L cells after incubation with sivelestat for 12 h. Positive gating was defined by isotype control in the individual treatment. Right, quantification of phospho-ERK1/2. **P* = 0.0004 and <0.0001 for 300 μg/ml and 600 μg/ml sivelestat vs. DMSO, respectively. *n*= 4-8/group. (**D**) Levels of phospho-ERK1/2 and total ERK1/2 in MDS-L cells following 12h culture with sivelestat or DMSO. Right, quantification of phospho-ERK1/2 was normalized to total ERK for each sample. **P* =0.02 for sivelestat compared to DMSO, *n*=2-3/group. (**E**) Protein expression of IKBα in MDS-L cells treated with sivelestat or DMSO for 12h. **P* = 0.01 for sivelestat vs DMSO, *n*=4-5/group. Student's 2-tailed unpaired *t* tests were used in these analyses.

Supplementary Fig. 6

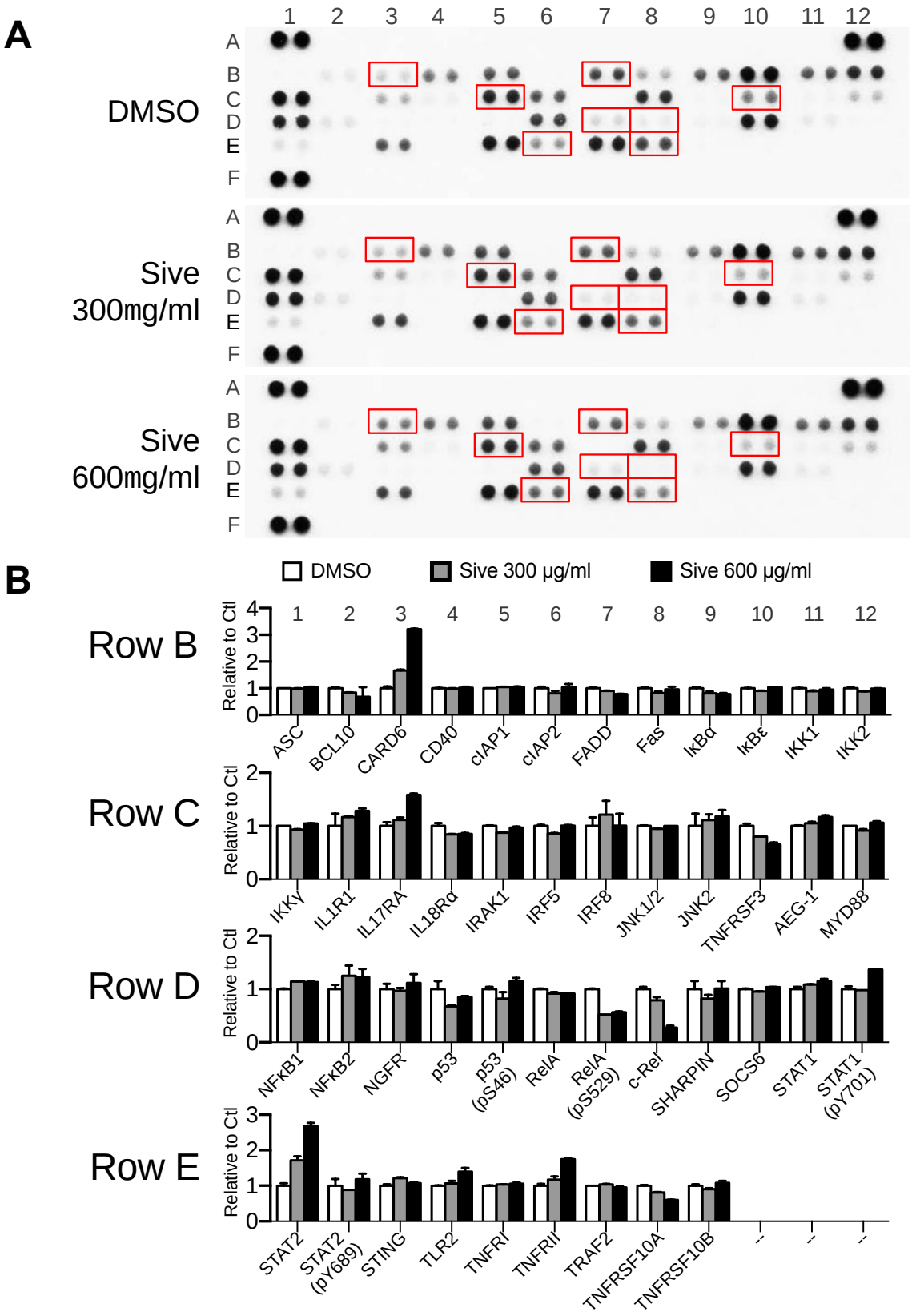

Figure S6. Sivelestat modulates the innate immune response in MDS-L cells via the NFκB pathway. Proteome Profiler Arrays for the NFκB pathway of protein lysates from MDS-L cells cultured with DMSO or sivelestat (300 or 600 μg/ml) for 24 h are shown. (**A**) Images of membranes from Proteome Profiler Arrays are shown following culture sivelestat compared to control DMSO. Four pairs of reference spots are shown at the following locations: A1, A12, F1 are positive references and F12 is negative control. Proteins were assayed in duplicates. Protein targets selected in Fig. 6 are highlighted with red boxes as follows: CARD6 (B3), FADD (B7), IRAK1 (C5), TNFRSF3 (C10), RelA pS529 (D7), c-Rel (D8), TNFRII (E6), and TNFRSF10A (E8). (**B**) Levels of proteins are quantified with ImageJ software following sivelestat treatment compared to control DMSO cultures.

Supplementary Figure 7

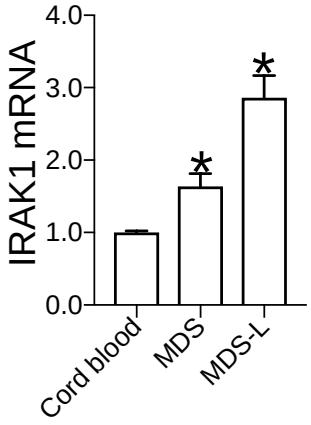

Figure S7. Interleukin Receptor Associated Kinase-1 (IRAK1) is differentially expressed in MDS cells compared to CD34+ Cord blood. Real-time PCR analysis of IRAK1 expression in primary MDS cells and MDS-L cells compared to CD34+ Cord blood. Data are normalized to GAPDH internal control and shown relative to CD34+ cord blood expression. **P*= 0.005 and 0.0001 for MDS and MDS-L compared to cord blood, respectively. *n*= 6/group. Student's 2-tailed unpaired *t* tests were used in these analyses.