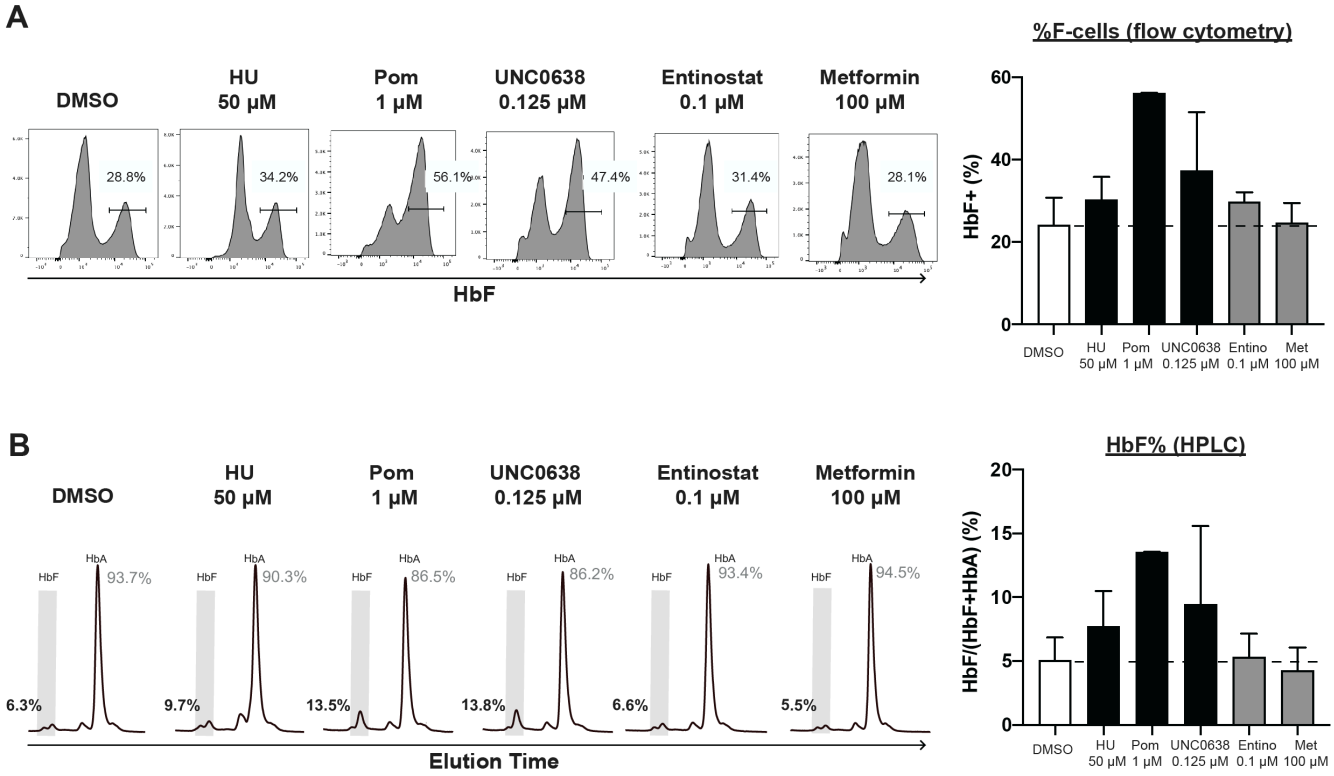
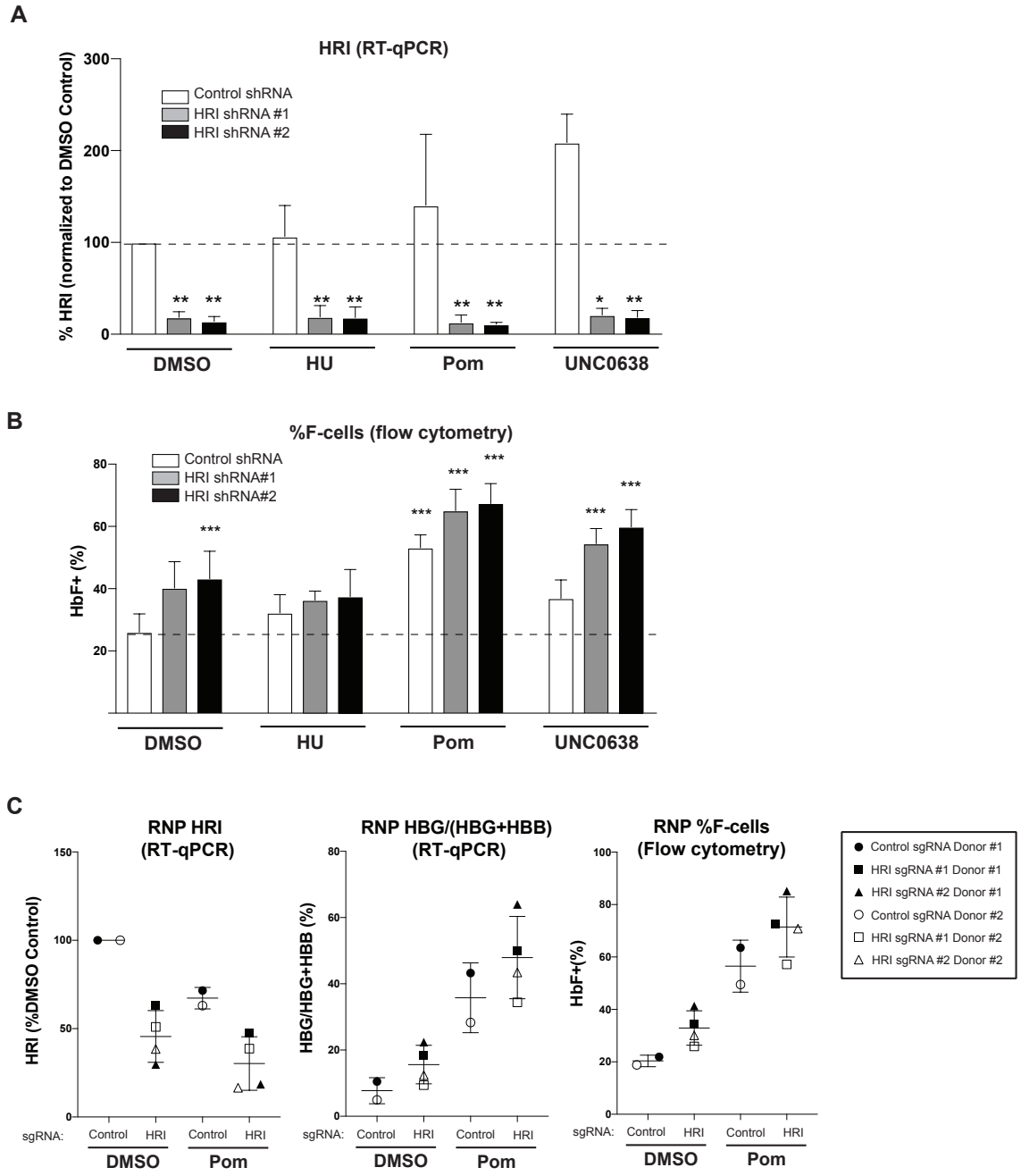
**Supplemental materials for Peslak et al. HRI depletion cooperates with pharmacologic inducers to strongly elevate fetal hemoglobin and reduce sickle cell formation**

## SUPPLEMENTAL FIGURE 1



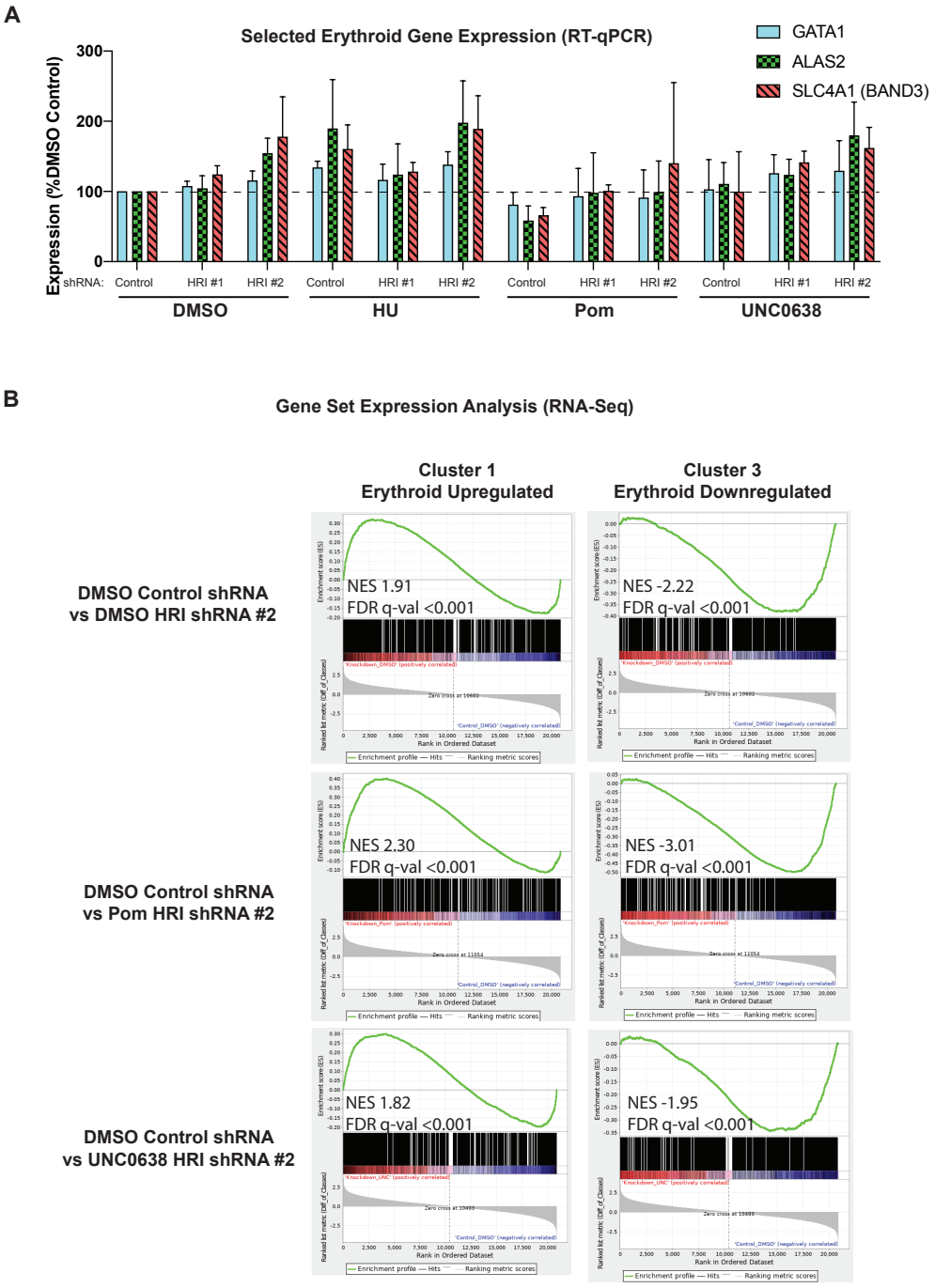

**Supplemental Figure 1.** ***Select pharmacologic HbF inducers raise HbF levels in vitro.*** **A.** F-cell quantitation by flow cytometry in $CD34^+$ healthy donors following treatment with DMSO control, hydroxyurea (HU), pomalidomide (Pom), UNC0638, entinostat, or metformin. % F-cells are quantified for each sample. **B.** HbF levels (quantified as percent of total HbF+HbA peaks) by HPLC. DMSO serves as pharmacologic vehicle control. Error bars represent standard deviation. N=2 independent biological replicates.

## SUPPLEMENTAL FIGURE 2



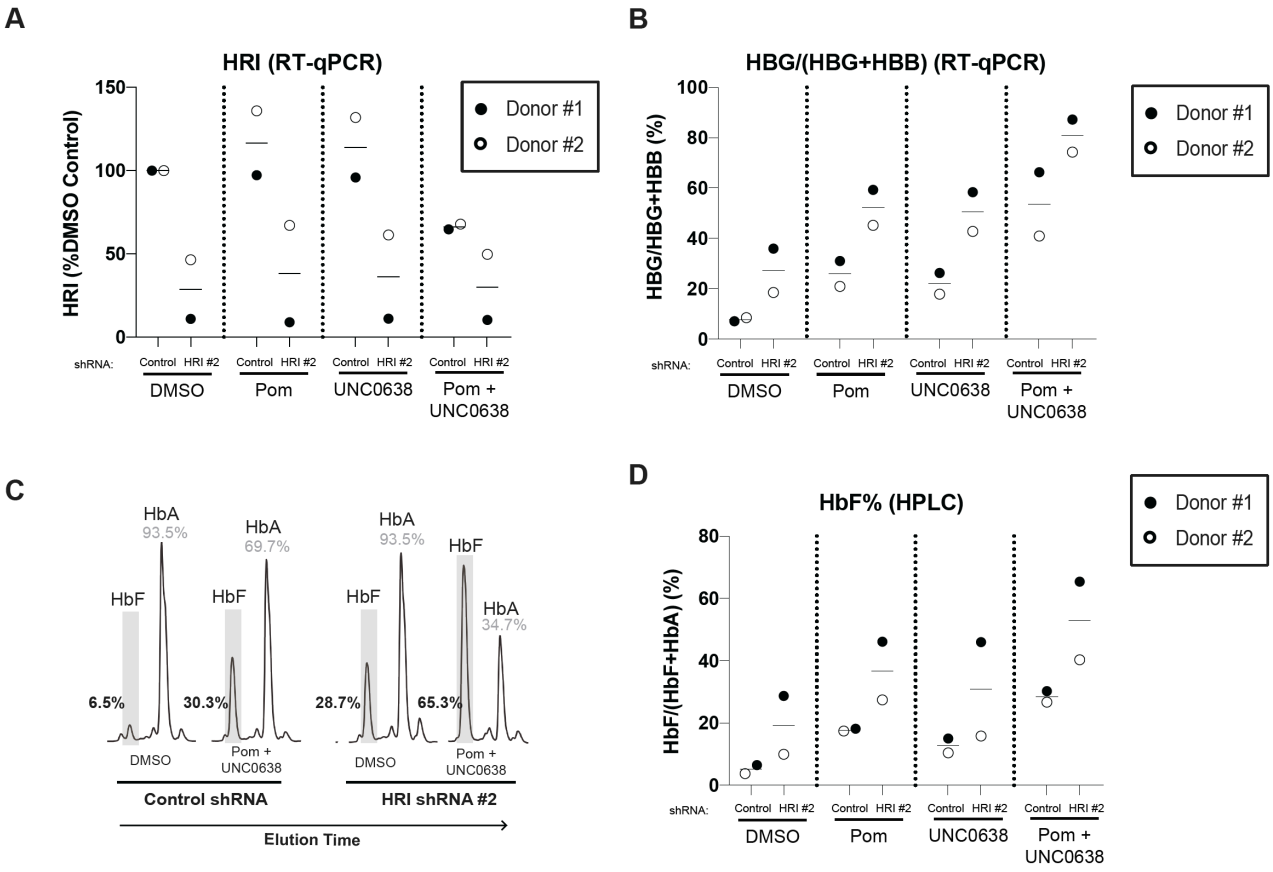

**Supplemental Figure 2.** ***HRI knockdown cooperates with pomalidomide or UNC0638 during HbF induction.*** **A.** HRI transcript levels (normalized to DMSO control) of healthy CD34$^{+}$ donors as measured by RT-qPCR following shRNA scrambled control or HRI depletion with two independent shRNA combined with either vehicle control or HbF pharmacologic induction (50 μM hydroxyurea (HU), 1 μM pomalidomide (Pom), or 0.125 μM UNC0638). **B.** F-cell quantitation by flow cytometry following shRNA scrambled control or HRI shRNA depletion combined with HbF pharmacologic induction. N=3 independent biological replicates for shRNA-based experiments. Statistical analyses done using two-way ANOVA analysis. * represents significance as compared to DMSO control. * $p<0.05$; ** $p<0.01$; *** $p<0.001$. **C.** Quantitation of HRI transcript (left panel, normalized to DMSO control); γ-globin transcript levels (center panel, expressed as γ-globin/γ-globin + β-globin); and % F-cells (right panel, obtained by flow cytometry) following RNP-based depletion of HRI combined with vehicle control or 1 μM pomalidomide treatment. N=2 independent biological replicates for RNP-based experiments. DMSO serves as pharmacologic vehicle control for all samples. Error bars represent standard deviation.

## SUPPLEMENTAL FIGURE 3



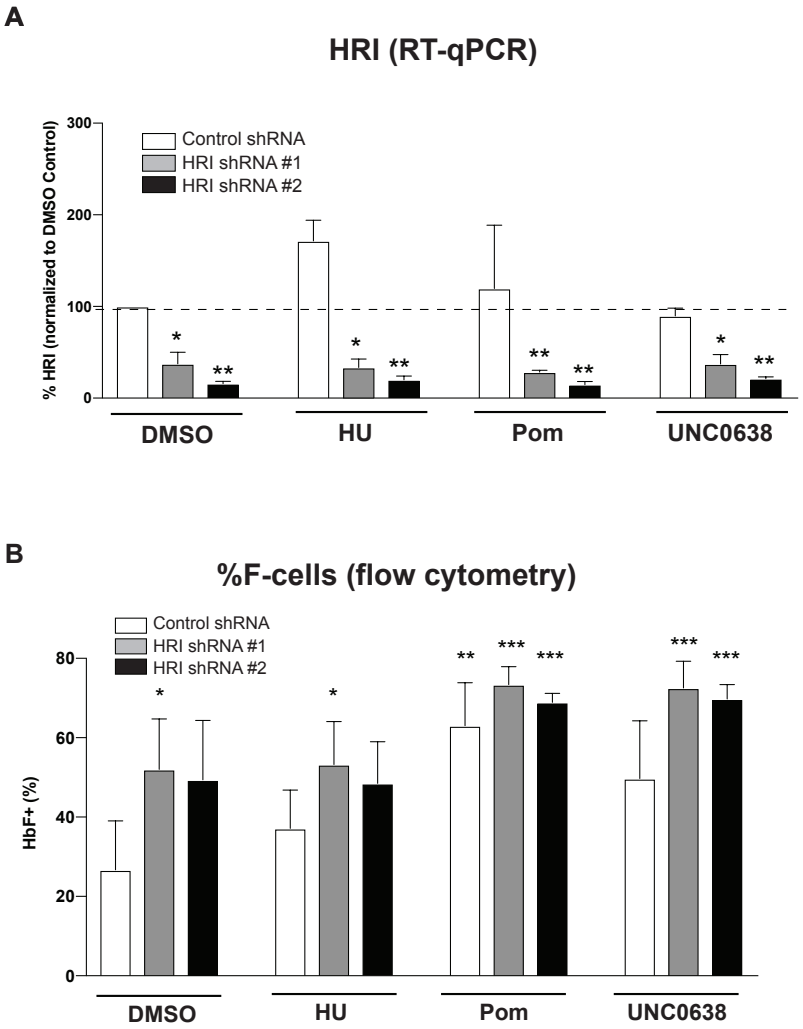

**Supplemental Figure 3**. ***Gene set enrichment analysis of HRI-depleted CD34$^+$ derived cells treated with either pomalidomide or UNC0638 using erythroid differentiation transcriptional signatures.*** **A.** Expression levels of select erythroid differentiation genes (normalized to DMSO control) as measured by RT-qPCR. N=3 independent biological replicates. Error bars represent standard deviation. **B.** Gene set enrichment analysis of RNA-Seq data from HRI-depleted CD34$^+$ derived cells treated with either DMSO control, 1 μM pomalidomide, or 0.125 μM UNC0638 using erythroid differentiation transcriptional signatures. Cluster 1 contains genes that are upregulated during erythroid differentiation and cluster 3 contains genes that are downregulated during erythroid differentiation. NES, normalized enrichment score; FDR, false discovery rate.

## SUPPLEMENTAL FIGURE 4



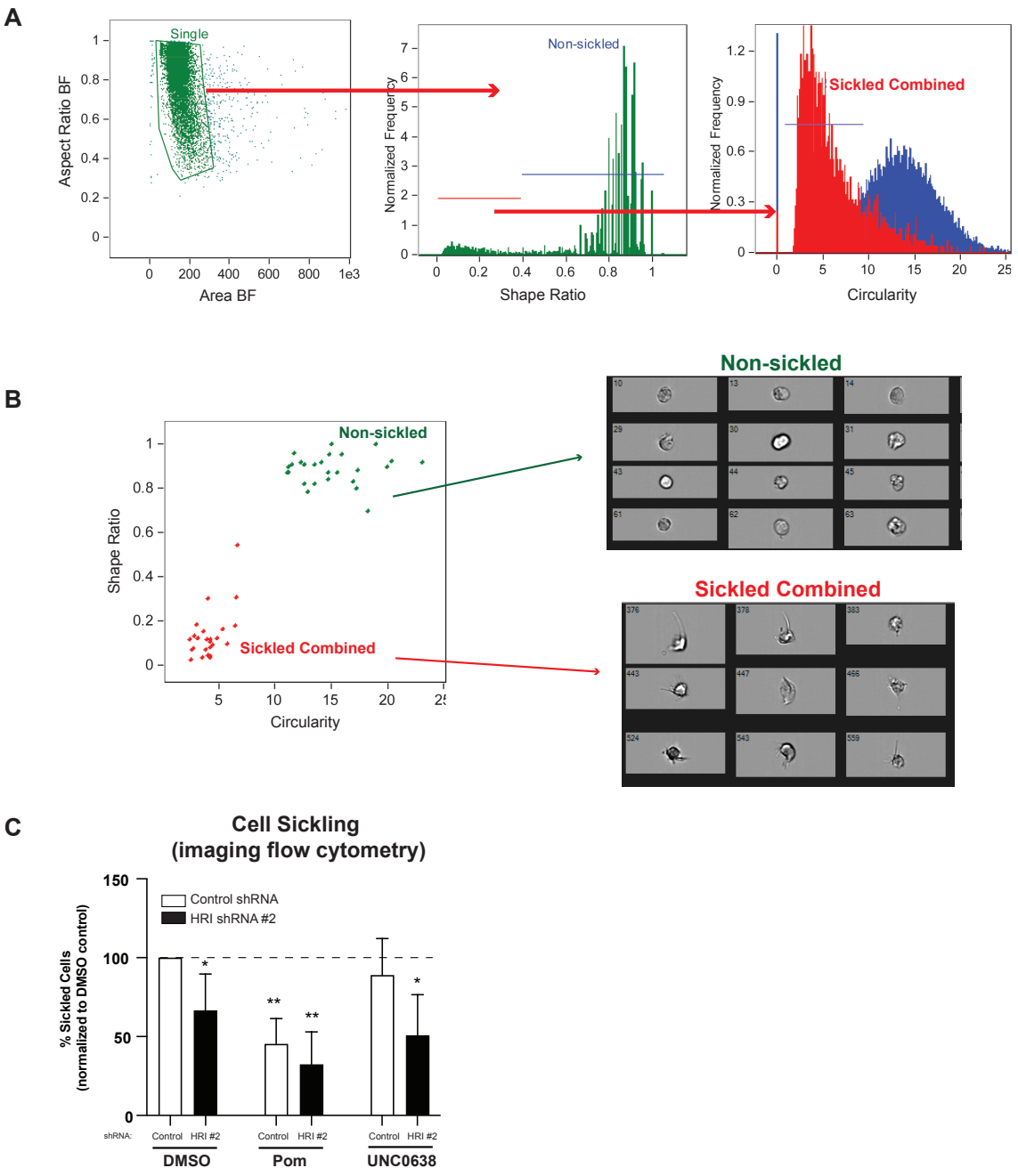

**Supplemental Figure 4**. ***HRI depletion combined with both pomalidomide and UNC0638 treatment leads to very high levels of HbF induction.*** **A.** HRI transcript levels following shRNA-mediated depletion of HRI combined with either vehicle control, 1 μM pomalidomide, 0.125 μM UNC0638, or 1 μM pomalidomide+0.125 μM UNC0638 treatment. **B.** γ-globin transcript levels (right panel, expressed as γ-globin/γ-globin + β-globin). **C.** Representative HPLC tracings of HbF and HbA. **D.** HbF levels (quantified as percent of total HbF+HbA peaks) by HPLC. N=2 independent biological replicates. DMSO serves as pharmacologic vehicle control. Error bars represent standard deviation.

## SUPPLEMENTAL FIGURE 5




**Supplemental Figure 5**. ***HRI knockdown cooperates with pharmacologic inducers during HbF induction in SCD patient-derived cells.*** **A.** HRI transcript levels (normalized to DMSO control) as measured by RT-qPCR in SCD patient-derived cells following shRNA scrambled control or HRI depletion with two independent shRNA combined with HbF pharmacologic induction (50 μM hydroxyurea (HU), 1 μM pomalidomide (Pom), or 0.125 μM UNC0638). **B.** F-cell quantitation by flow cytometry of SCD patient-derived cells following shRNA scrambled control or HRI shRNA depletion combined with HbF pharmacologic induction. Statistical analyses done using two-way ANOVA analysis. * represents significance as compared to DMSO control. N≥3 independent biological replicates. Error bars represent standard deviation. * $p<0.05$; ** $p<0.01$; *** $p<0.001$.

## SUPPLEMENTAL FIGURE 6




**Supplemental Figure 6.** ***Imaging flow cytometric analysis of cellular sickling.*** **A.** Gating strategy for identifying sickled vs non-sickled cells by imaging flow cytometry as modified from previously published methods.[27] Single focused cells were classified as sickled vs non-sickled utilizing a combination of shape ratio (ratio of short axis to long axis) and circularity. **B.** Representative flow cytometric images (60x) of sickled vs non-sickled cells. **C.** Quantitation by imaging flow cytometry of percent sickled cells (normalized to DMSO control) following low-$O_2$ exposure. Statistical analyses done using two-way ANOVA analysis. * represents significance as compared to DMSO control. N=3 independent biological replicates. Error bars represent standard deviation. * $p<0.05$; ** $p<0.01$; *** $p<0.001$.

## SUPPLEMENTAL TABLES

### SUPPLEMENTAL TABLE 1. shRNA oligonucleotides.

| Name | Sequence |
|---|---|
| Control shRNA F | CACCGGCAACAAGATGAAGAGCACCAACTCGAGTTGGTGCTCTTCATCTTGTTGTTTTTG |
| Control shRNA R | AATTCAAAAACAACAAGATGAAGAGCACCAACTCGAGTTGGTGCTCTTCATCTTGTTGCC |
| HRI shRNA #1 F | CACCGGCAGAGAGCAATGTGGTGTTAACTCGAGTTAACACCACATTGCTCTCTGTTTTTG |
| HRI shRNA #1 R | AATTCAAAAACAGAGAGCAATGTGGTGTTAACTCGAGTTAACACCACATTGCTCTCTGCC |
| HRI shRNA #2 F | CACCGGGCAGAAGTTCTAACAGGTTTACTCGAGTAAACCTGTTAGAACTTCTGCTTTTTG |
| HRI shRNA #2 R | AATTCAAAAAGCAGAAGTTCTAACAGGTTTACTCGAGTAAACCTGTTAGAACTTCTGCCC |

### SUPPLEMENTAL TABLE 2. sgRNA sequences.

| Name | Sequence |
|---|---|
| Control sgRNA | GCACTACCAGAGCTAACTCA |
| HRI sgRNA #1 | TTGTTGGCTATCACACCGCG |
| HRI sgRNA #2 | ATAGTCGAGAGAAACAAGCG |

### SUPPLEMENTAL TABLE 3. qRT-PCR primers.

| Name | Sequence |
|---|---|
| β-globin F | TGGGCAACCCTAAGGTGAAG |
| β-globin R | GTGAGCCAGGCCATCACTAAA |
| γ-globin F | TGGCAAGAAGGTGCTGACTTC |
| γ-globin R | GCAAAGGTGCCCTTGAGATC |
| HRI F | CTGGACCAACAGAAACGGGA |
| HRI R | TTCGGGTGAAGCGTACAGAC |
| BCL11A F | ACAAACGGAAACAATGCAATGG |
| BCL11A R | TTTCATCTCGATTGGTGAAGGG |
| LRF/ZBTB7A F | GCTTGGGCCGGTTGAATGTA |
| LRF/ZBTB7A R | GGCTGTGAAGTTACCGTCGG |
| GATA1 F | CTGTCCCCAATAGTGCTTATGG |
| GATA1 R | GAATAGGCTGCTGAATTGAGGG |
| ALAS2 F | CAGCGCAATGTCAAGCAC |
| ALAS2 R | TAGATGCCATGCTTGGAGAG |
| SLC4A1 F | ACCTCTCTCACCTCACCTTCTG |
| SLC4A1 R | AACCTGTCTAGCAGTTGGTTGG |
| RPS18 F | GTAACCCGTTGAACCCCATT |
| RPS18 R | CCATCCAATCGGTAGTAGCG |
| AHSP F | TGAAGGAGTTCAGCGTTCTG |
| AHSP R | CACCTGCTGCCTGTAATAGTTG |
| GAPDH F | ACCACAGTCCATGCCATCACT |
| GAPDH R | CCATCACGCCACAGTTTCC |

### SUPPLEMENTAL TABLE 4. Western blot primary and secondary antibodies.

| Name | Primary antibody | Secondary antibody |
|---|---|---|
| HRI | EIF2AK1 (1:1000 dilution)<br>MyBioSource, Cat. #MBS-9208228 | IRDye 800 donkey anti-rabbit IgG (1:15000 dilution)<br>Licor Cat. #926-32213 |
| BCL11A | CTIP1/BCL11A (1:1000 dilution)<br>Abcam, Cat. #19487 | IRDye 800 donkey anti-mouse IgG (1:15000 dilution)<br>Licor Cat. #926-32212 |
| LRF | Pokemon/LRF (1:1000 dilution) eBioscience<br>Cat. #14-3309-82 | Armenian Hamster IgG 680 (1:15000 dilution)<br>Rockland Cat. #620-144-440 |
| γ-globin | γ-globin (1:1000 dilution)<br>Novus Biologicals, Cat. #NB-110-41084 | IRDye 800 donkey anti-goat IgG (1:15000 dilution)<br>Licor Cat. #925-32214 |
| β-actin | β-actin (1:500 dilution)<br>Santa Cruz, Cat. #sc-47778) | IRDye 680 donkey anti-mouse IgG (1:15000 dilution)<br>Licor Cat. #926-68072 |

**SUPPLEMENTAL TABLE 5. Comparison of DMSO control shRNA versus DMSO HRI-depleted erythroid transcripts.** RNA-seq expression of selected erythroid maturation up- and down-regulated genes comparing DMSO control shRNA samples versus DMSO HRI shRNA #2 samples. Gene numbers correspond to labels in Figure 2D. Data are shown as read counts for DMSO control and DMSO HRI-depleted samples, $\log_2$ fold change, and DESeq adjusted p-values.

**DMSO Control shRNA vs DMSO HRI shRNA #2:**

| Category | Gene Number | Gene ID | DMSO control reads | DMSO HRI knockdown reads | Log2 fold change | Padj Value |
|---|---|---|---|---|---|---|
| Erythroid Upregulated | 1 | GATA1 | 5976.9 | 6199.0 | 0.0526 | 0.84 |
| | 2 | KEL | 7191.2 | 10356.6 | 0.5236 | 8.1E-09 |
| | 3 | ANK1 | 24876.2 | 26851.7 | 0.0738 | 0.95 |
| | 4 | KLF1 | 35505.9 | 34891.0 | -0.0259 | 0.92 |
| | 5 | FOXO3 | 36816.2 | 38826.9 | 0.0697 | 0.66 |
| | 6 | AHSP | 44995.0 | 83174.8 | 0.8843 | 4.6E-29 |
| | 7 | EPB42 | 48508.9 | 81487.9 | 0.7484 | 6.5E-24 |
| | 8 | GYPA | 80201.2 | 89299.5 | 0.1604 | 0.11 |
| | 9 | ALAS2 | 147138.6 | 226877.3 | 0.6293 | 1.2E-14 |
| | 10 | SLC4A1 (BAND3) | 187828.5 | 252594.0 | 0.401 | 0.46 |
| | 11 | SLC25A37 | 268505.8 | 282252.1 | 0.062 | 0.76 |
| Erythroid Downregulated | 12 | GATA2 | 90.4 | 106.3 | 0.1748 | 0.89 |
| | 13 | JUN | 549.8 | 555.4 | -0.0476 | 0.96 |
| | 14 | MYC | 868.7 | 455.6 | -0.8984 | 0.00001 |
| | 15 | CD44 | 1312.9 | 681.3 | -1.1007 | 0.11 |
| | 16 | MYB | 1750.7 | 1426.5 | -0.3408 | 0.061 |
| | 17 | KIT | 2634.3 | 1633.3 | -0.6856 | 2.3E-08 |
| | 18 | CASP3 | 3599.8 | 6636.8 | 0.9112 | 4.3E-12 |
| | 19 | PCNA | 24437.2 | 16850.0 | -0.5337 | 7.5E-11 |

**SUPPLEMENTAL TABLE 6. Comparison of DMSO control shRNA versus pomalidomide HRI-depleted erythroid transcripts.** RNA-seq expression of selected erythroid maturation up- and down-regulated genes comparing DMSO control shRNA samples versus 1 μM pomalidomide HRI shRNA #2 samples. Gene numbers correspond to labels in Figure 2E. Data are shown as read counts for DMSO control and pomalidomide HRI-depleted samples, $\log_2$ fold change, and DESeq adjusted p-values.

**DMSO Control shRNA vs Pom HRI shRNA #2:**

| Category | Gene Number | Gene ID | DMSO control reads | Pom HRI knockdown reads | Log2 fold change | Padj Value |
|---|---|---|---|---|---|---|
| Erythroid Upregulated | 1 | GATA1 | 5444.2 | 6598.0 | 0.2646 | 0.12 |
| | 2 | KEL | 6551.9 | 15543.9 | 1.249 | 9.7E-38 |
| | 3 | ANK1 | 22655.9 | 24078.9 | 0.0553 | 0.93 |
| | 4 | KLF1 | 32317.7 | 29729.6 | -0.1152 | 0.3 |
| | 5 | FOXO3 | 33486.3 | 40983.9 | 0.2823 | 0.00037 |
| | 6 | AHSP | 40978.9 | 76306.1 | 0.8952 | 1.4E-30 |
| | 7 | EPB42 | 44167.5 | 82705.4 | 0.903 | 2.8E-36 |
| | 8 | GYPA | 72995.1 | 64800.6 | -0.164 | 0.21 |
| | 9 | ALAS2 | 133919.9 | 246836.2 | 0.883 | 1.1E-29 |
| | 10 | SLC4A1 (BAND3) | 171052.5 | 220158.1 | 0.3307 | 0.57 |
| | 11 | SLC25A37 | 244373.2 | 256606.8 | 0.0664 | 0.65 |
| Erythroid Downregulated | 12 | GATA2 | 82.4 | 218.0 | 1.4024 | 0.000037 |
| | 13 | JUN | 500.8 | 2415.9 | 2.2701 | 6.5E-62 |
| | 14 | MYC | 792.4 | 322.5 | -1.2533 | 0.0000019 |
| | 15 | CD44 | 1194.9 | 741.0 | -0.9388 | 0.34 |
| | 16 | MYB | 1596.2 | 678.2 | -1.3099 | 1.6E-10 |
| | 17 | KIT | 2400.9 | 1384.1 | -0.7887 | 3E-09 |
| | 18 | CASP3 | 3274.5 | 6679.2 | 1.0553 | 9.8E-13 |
| | 19 | PCNA | 22268.5 | 9738.9 | -1.1983 | 1.2E-51 |

**SUPPLEMENTAL TABLE 7. Comparison of DMSO control shRNA versus UNC0638 HRI-depleted erythroid transcripts.** RNA-seq expression of selected erythroid maturation up- and down-regulated genes comparing DMSO control shRNA samples versus 0.125 μM UNC0638 HRI-depleted transcripts. Gene numbers correspond to labels in Figure 2F. Data are shown as read counts for DMSO control and UNC0638 HRI-depleted samples, $log_2$ fold change, and DESeq adjusted p-values**.**

**DMSO Control shRNA vs UNC0638 HRI shRNA #2:**

| Category | Gene Number | Gene ID | DMSO control reads | UNC0638 HRI knockdown reads | Log2 fold change | Padj Value |
|---|---|---|---|---|---|---|
| Erythroid Upregulated | 1 | GATA1 | 5856.5 | 7537.5 | 0.3588 | 0.0042 |
| | 2 | KEL | 7046.5 | 10822.1 | 0.6181 | 1.5E-11 |
| | 3 | ANK1 | 24374.7 | 24395.2 | -0.0021 | 1 |
| | 4 | KLF1 | 34787.8 | 32246.0 | -0.1024 | 0.53 |
| | 5 | FOXO3 | 36069.0 | 38316.5 | 0.0957 | 0.4 |
| | 6 | AHSP | 44087.7 | 75722.4 | 0.7742 | 0.0006 |
| | 7 | EPB42 | 47529.6 | 80603.6 | 0.7626 | 1.7E-23 |
| | 8 | GYPA | 78578.8 | 71719.9 | -0.124 | 0.24 |
| | 9 | ALAS2 | 144162.3 | 234142.3 | 0.7066 | 1.5E-22 |
| | 10 | SLC4A1 (BAND3) | 184040.2 | 234813.9 | 0.349 | 0.0000019 |
| | 11 | SLC25A37 | 263073.4 | 292281.0 | 0.1508 | 0.082 |
| Erythroid Downregulated | 12 | GATA2 | 88.6 | 81.3 | -0.1247 | 0.94 |
| | 13 | JUN | 538.7 | 480.9 | -0.1703 | 0.71 |
| | 14 | MYC | 851.3 | 498.5 | -0.7347 | 0.0071 |
| | 15 | CD44 | 1286.4 | 901.1 | -0.7066 | 0.51 |
| | 16 | MYB | 1715.6 | 1372.2 | -0.3461 | 0.052 |
| | 17 | KIT | 2581.4 | 1879.7 | -0.4638 | 0.027 |
| | 18 | CASP3 | 3526.8 | 5717.7 | 0.7248 | 0.00000017 |
| | 19 | PCNA | 23945.8 | 15138.6 | -0.6583 | 4.1E-16 |