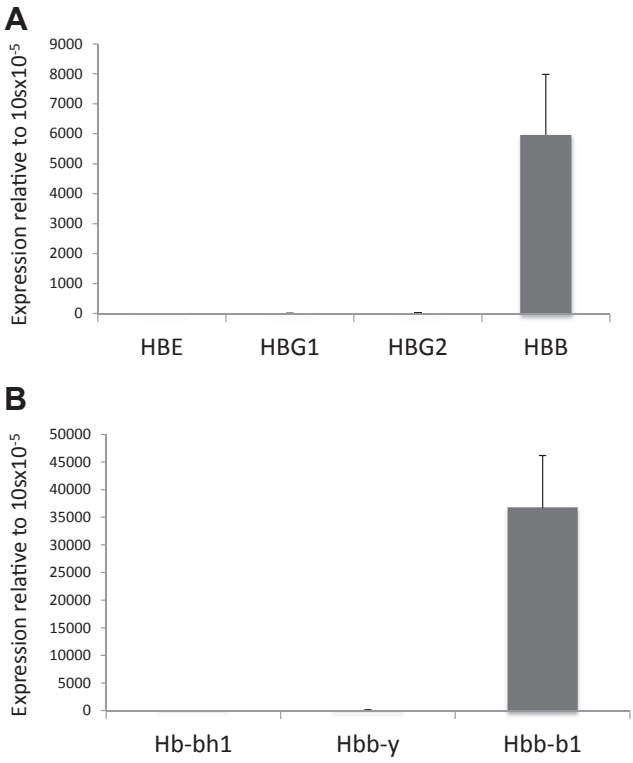
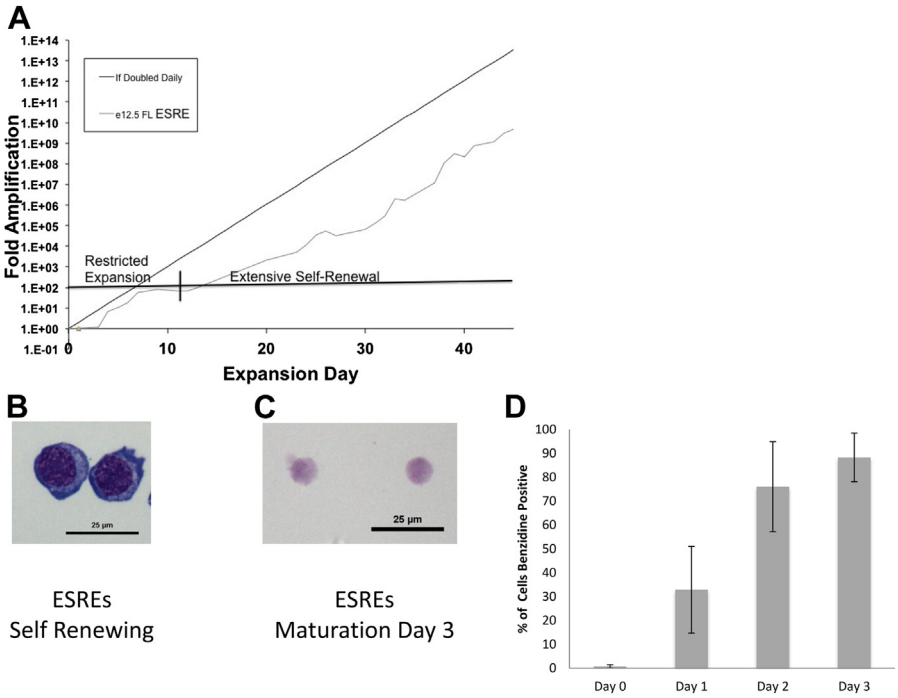
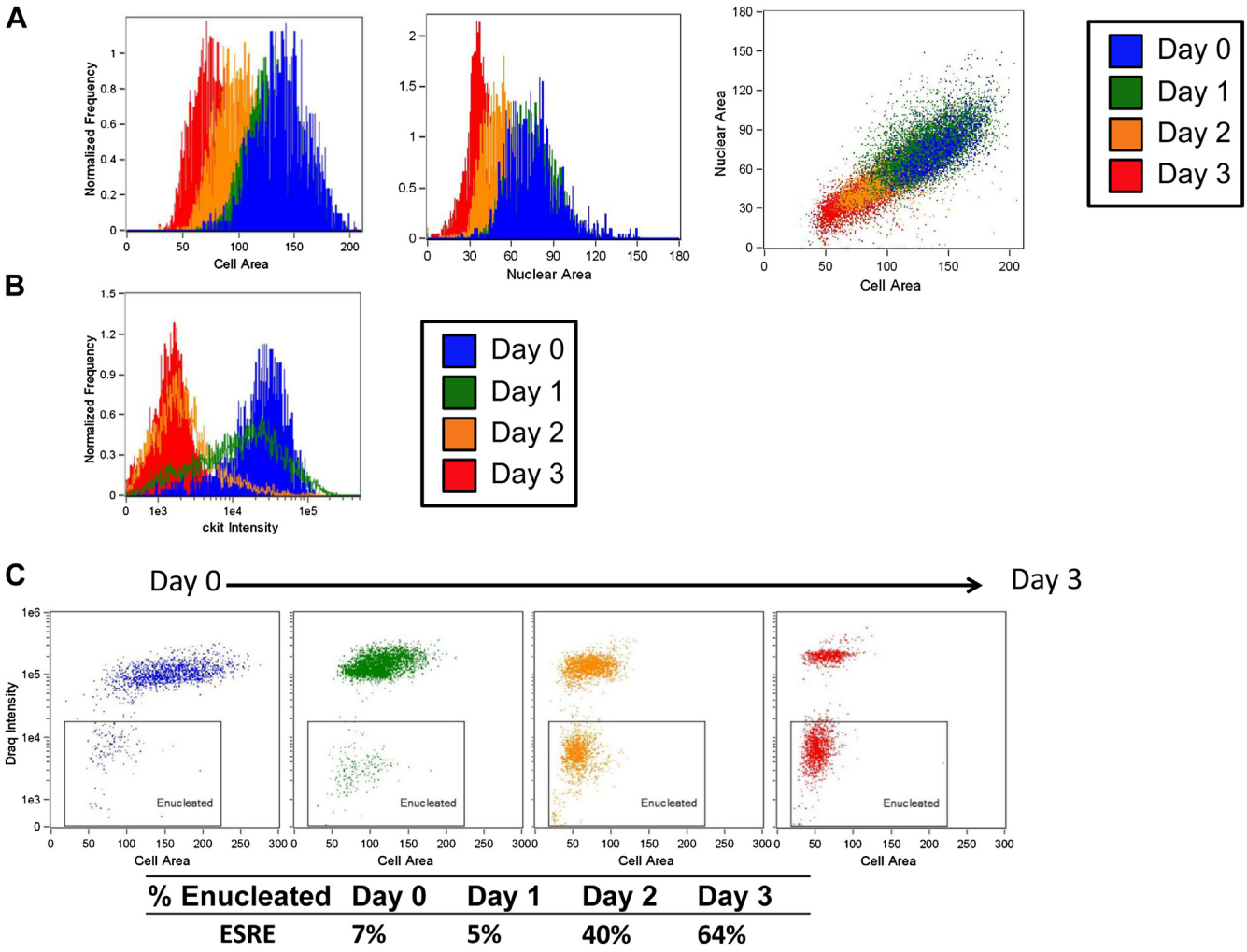


Supplementary Figure E1. Human **(A)** and murine **(B)** globin expression from β-yac ESREs derived from E9.5 yolk sac.

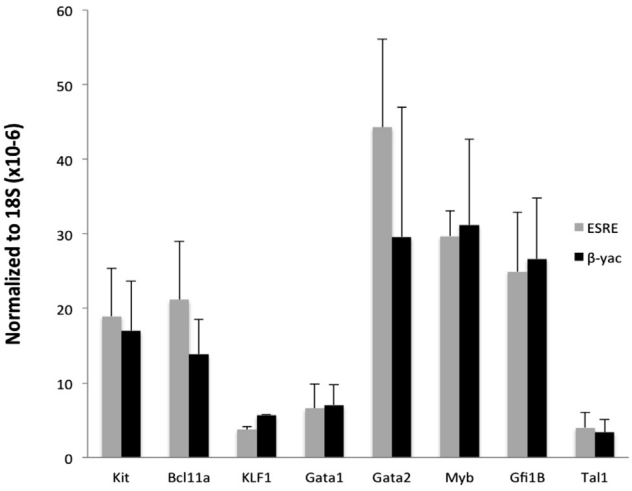


Supplementary Figure E2. Non-transgenic ESREs behave similarly to β-yac ESREs. **(A)** ESREs undergo an initial period of restricted proliferation prior to beginning extensive self-renewal. **(B)** Photomicrograph demonstrating that during extensive self-renewal, ESREs phenotypically resemble proerythroblasts. **(C)** Photomicrograph demonstrating that after three days in maturation media, the culture contains enucleated red cells. **(D)** After three days in maturation media, the majority of ESREs are benzidine positive. Data represent the mean and the SEM of three independent experiments.

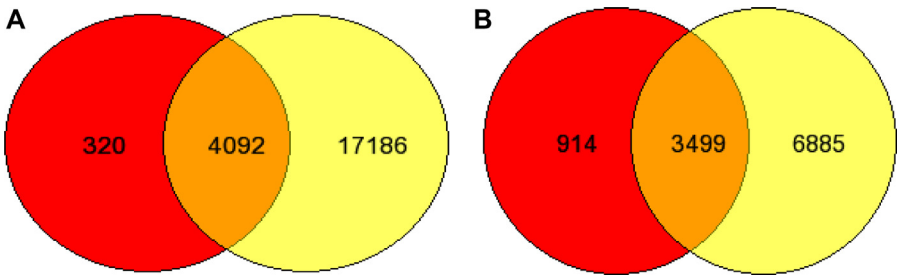


% Enucleated	Day 0	Day 1	Day 2	Day 3
ESRE	7%	5%	40%	64%

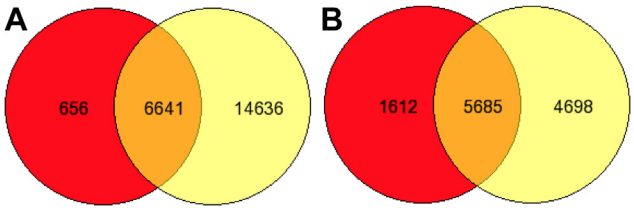
Supplementary Figure E3. Image stream analyses of ESRE maturation. One of three representative experiments is shown. **(A)** ESRE maturation is associated with a progressive decrease in cell and nuclear size. **(B)**. ESRE maturation is associated with a loss of Kit expression. **(C)** ESRE enucleation as determined by comparing draq intensity to cell area. After three days of maturation, over 64% of ESREs are enucleated.



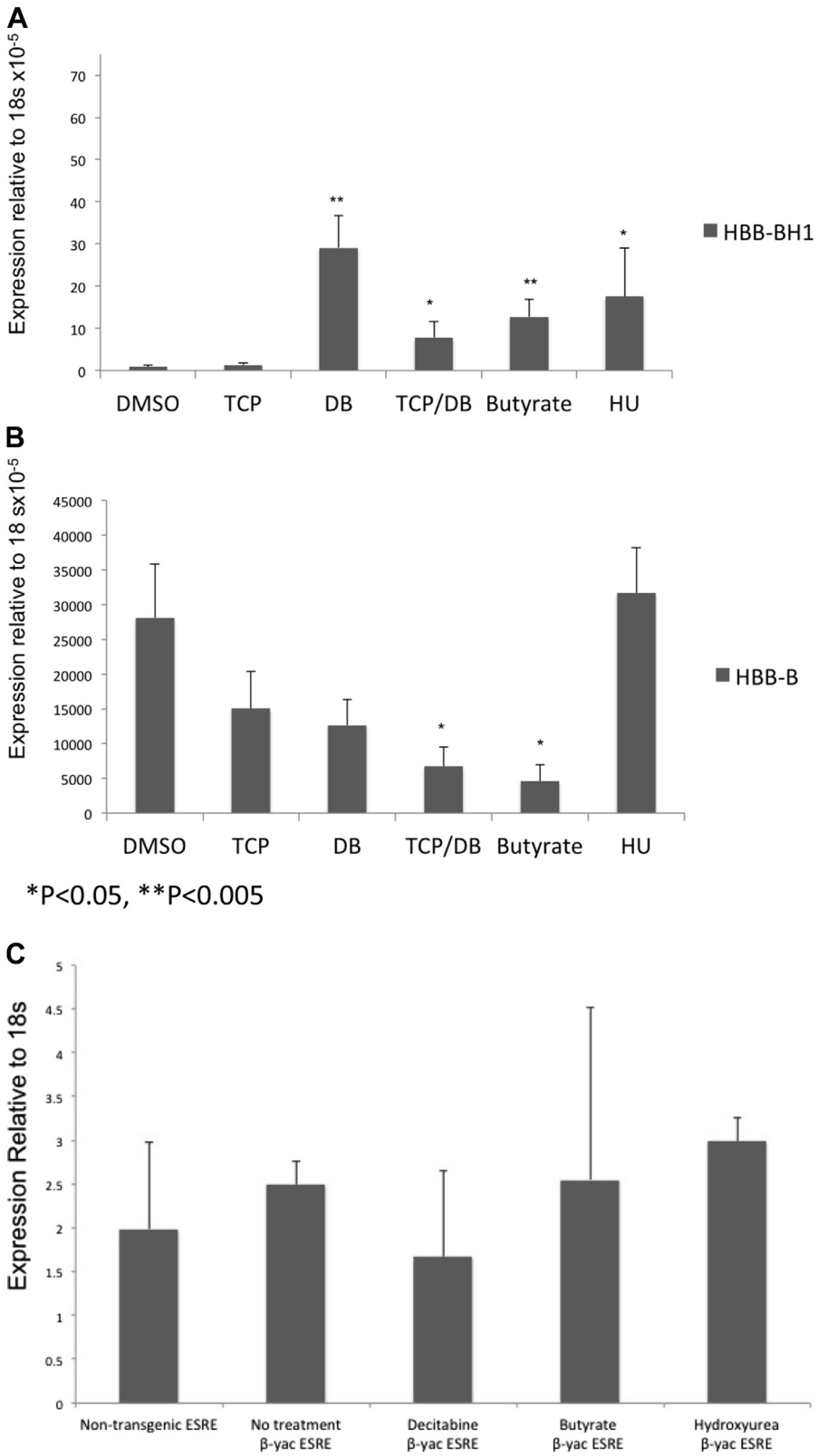
Supplementary Figure E4. Quantitative PCR demonstrates that the expression level of several key transcriptional regulators are similar in β-yac (black bars) and non-transgenic (gray bars) ESREs.



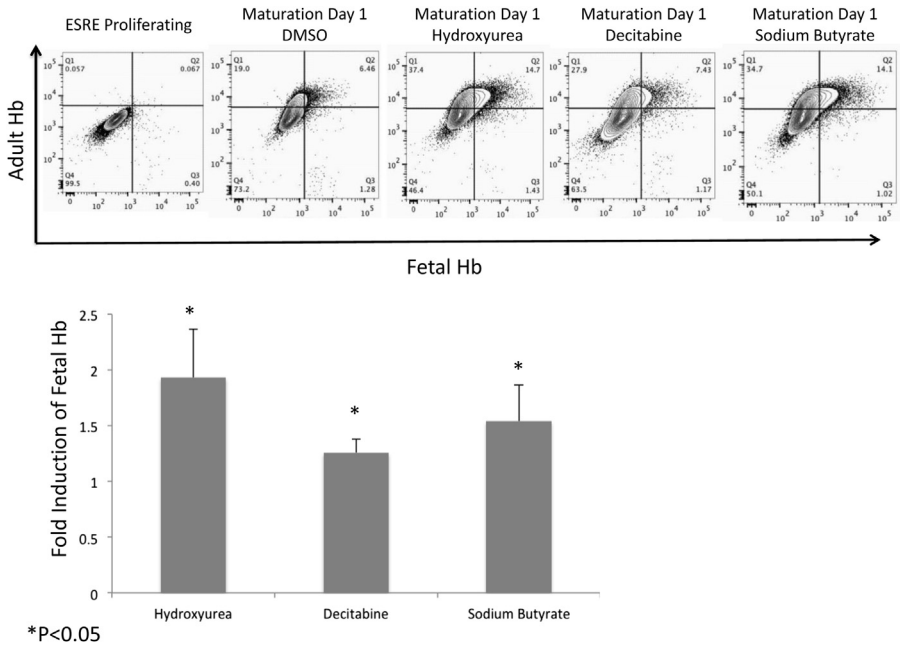
Supplementary Figure E5. There is significant RNA overlap between ESREs and uncultured fetal liver derived erythroid precursors. GennVenn (http://genevenn.sourceforge.net/) was used to determine overlap of mRNA expression. Red = Number of transcripts detected in uncultured fetal liver proerythroblasts and basophilic erythroblasts. Orange = Transcripts detected in both proliferating ESREs and uncultured fetal liver proerythroblasts and basophilic erythroblasts Yellow = Transcripts detected only in proliferating ESREs. **(A)** There is significant overlap between genes expressed in ESREs and genes expressed in primary fetal liver derived pro- and basophilic- erythroblasts. This data includes all transcripts detected by RNA-seq. **(B)** Even with a stringent expression cutoff of FPKM$>$ 0.5 applied to the RNA-seq data, there is significant transcriptome overlap between ESREs and fetal liver derived pro- and basophilic- erythroblasts.



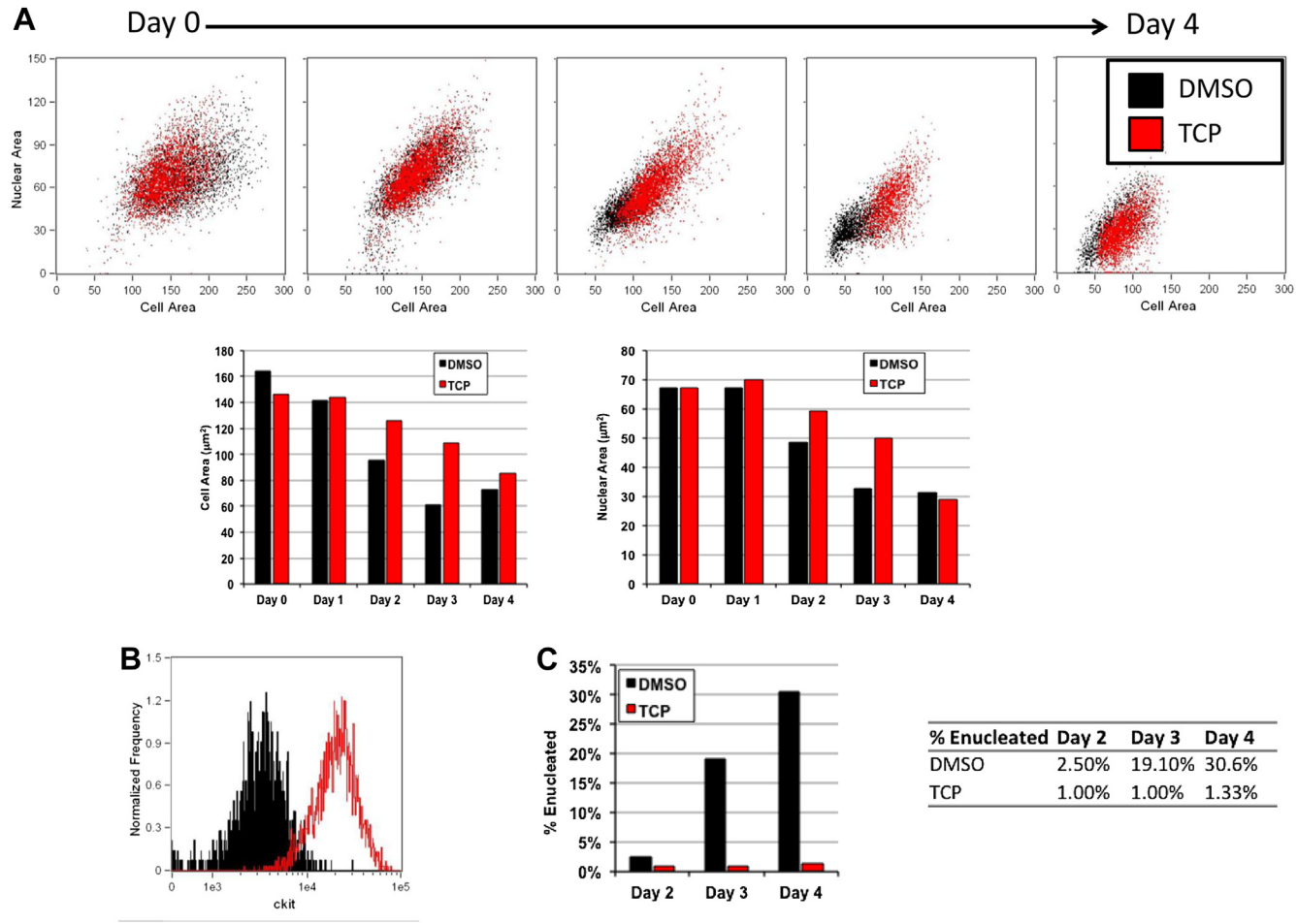
Supplementary Figure E6. There is significant RNA overlap between ESREs and uncultured adult bone marrow derived erythroid precursors. GennVenn (http://genevenn.sourceforge.net/) was used to determine overlap of mRNA expression. Red = Number of transcripts detected in uncultured adult bone marrow derived proerythroblasts and basophilic erythroblasts. Orange = Transcripts detected in both proliferating ESREs and uncultured adult bone marrow proerythroblasts and basophilic erythroblasts. Yellow = Transcripts detected only in proliferating ESREs. **(A)** There is significant overlap between genes expressed in ESREs and genes expressed in primary adult bone marrow derived pro- and basophilicerythroblasts. This data includes all transcripts detected by RNA-seq. **(B)** Even with a stringent expression cutoff of FPKM$>$ 0.5 applied to the RNA-seq data, there is significant transcriptome overlap between ESREs and adult bone marrow derived derived pro- and basophilic- erythroblasts.



Supplementary Figure E7. The effect of inhibitor treatment on endogenous murine globin expression. * denotes $p < 0.05$. Graphs represent mean and SEM of a minimum of two independent experiments. **(A)** The embryonic murine globin, βH1 is expressed at low levels in β-yac ESREs. Treatment of β-yac ESREs with decitabine, TCP and decitabine, sodium butyrate, or hydroxyurea leads to modest increases in βH1 expression. **(B)** Expression of murine β-globin in samples treated with TCP, decitabine, a combination of TCP and Decitabine, sodium butyrate, and hydroxyurea. **(C)** The level of alpha globin expression was similar between nontransgenic and of β-yac ESREs. Inhibitor treatment did not significantly effect α-globin expression.



Supplementary Figure E8. FACS analyses of fetal globin expression in β-yac ESREs. Top panels are representative FACS experiments demonstrating fetal and adult globin expression in proliferating β-yac ESREs and in β-yac ESREs matured in the presence of vehicle (DMSO), hydroxyurea, decitabine, and sodium butyrate. Bottom panel is the average fold induction of fetal hemoglobin compared to vehicle control. Data are the mean and SEM of three independent experiments. *indicates $p < 0.05$.



% Enucleated	Day 2	Day 3	Day 4
DMSO	2.50%	19.10%	30.6%
TCP	1.00%	1.00%	1.33%

Supplementary Figure E9. Image stream analyses of TCP and vehicle treated β-yac cells on maturation day 4. One representative experiment of three is shown. **(A)** The TCP treated cells have a slightly larger cell area. Nuclear area is similar between TCP and vehicle treated samples. **(B)** TCP treated cells continue to express kit. **(C)** Percent of enucleated cells, determined by comparing draq intensity to cell area. The enucleation rate of TCP treated samples remains low on maturation day 4.