SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure 1: Analysis of human hematopoietic cell differentiation genomic mapping (dMap) data reveals a role for AhR in normal hematopoietic specification. (A) Computational analysis of comprehensive microarray data obtained through the Broad Institute's Differential Map Portal (dMAP). The genes were sorted based on hierarchical clustering with 1-Pearson correlation as the distance metric, and average linkage as the agglomeration rule [1]. (B) The normalized expression level of AhR within each cell population (sub-population) was computed and visualized by means of box-and-whiskers plots. For each population, the plot reports the median (thick mid line), the middle half (the box), and the Interquartile Range (IQR, the distance between the "whiskers") of the distribution of AhR values. The difference in the expression level of AhR among cell populations was tested by standard analysis-of-variance (anova) [2].

Supplementary Figure 2: Characterization of early-stage iPSC-derived hematopoietic progenitor cells. (A) Flow cytometry characterization of adherent and floating cell populations in D12 hematopoietic cultures. The cells were examined for a panel of markers representing early-stage hematopoietic (CD43, c-kit, CD34) and endothelial markers (Tie2, KDR). These results indicate that floating cells are enriched for CD43 positivity, a marker of committed definitive hematopoietic progenitors, while the adherent layer contains a high proportion of cells positive for early hematopoietic markers c-kit and CD34, in addition to endothelial markers Tie2 and KDR. (B) Colony forming cell assays from unsorted D15 non-adherent cell populations in which 28,000 cells per assay were grown in methylcellulose with hematopoietic growth factors for 12 days. Data are averages of three independent experiments and graphed as a percentage of total colonies formed. (no statistical difference was noted between colony types).

Supplementary Figure 3: iPSCs and hematopoietic progenitor cells are responsive to a spectrum of AhR agonists. (A) RT-PCR analysis of *CYP1B1* in iPSC treated with

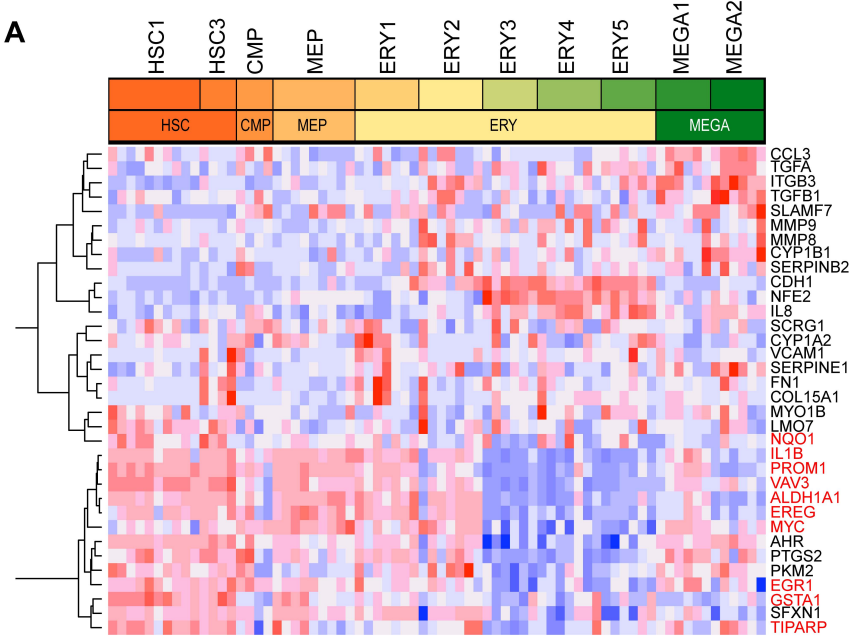
TCDD or β-NF for 4 days. Data are averages of duplicate wells ± SE and values are normalized to *GAPDH*. (B) RT-PCR analysis of *CYP1B1* in HP treated with β-NF or FICZ. Data are averages of two independent experiments ± SE and values normalized to *GAPDH*.

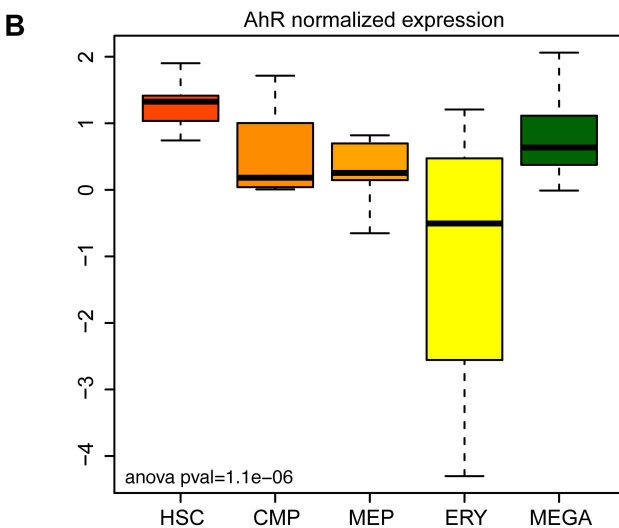
Supplementary Figure 4: iPSC-derived erythroid-lineage cells express a spectrum of embryonic and fetal globins. (A) Mass Spectrometry analysis of human peripheral blood in comparison to D30 iPSC-derived erythroid-lineage cells. In contrast to peripheral blood cells, which only express α-globin and β-globin (forming the most common form of hemoglobin in adult humans), iPSC-derived erythroid-lineage cells express α-globin, γ-globin (fetal), as well as two embryonic globins (ζ- and ε-globin). These results, as well as the lack of adult globin (β-globin) in this population suggest that iPSC-derived erythroid-lineage cells are at an embryonic/fetal developmental stage.

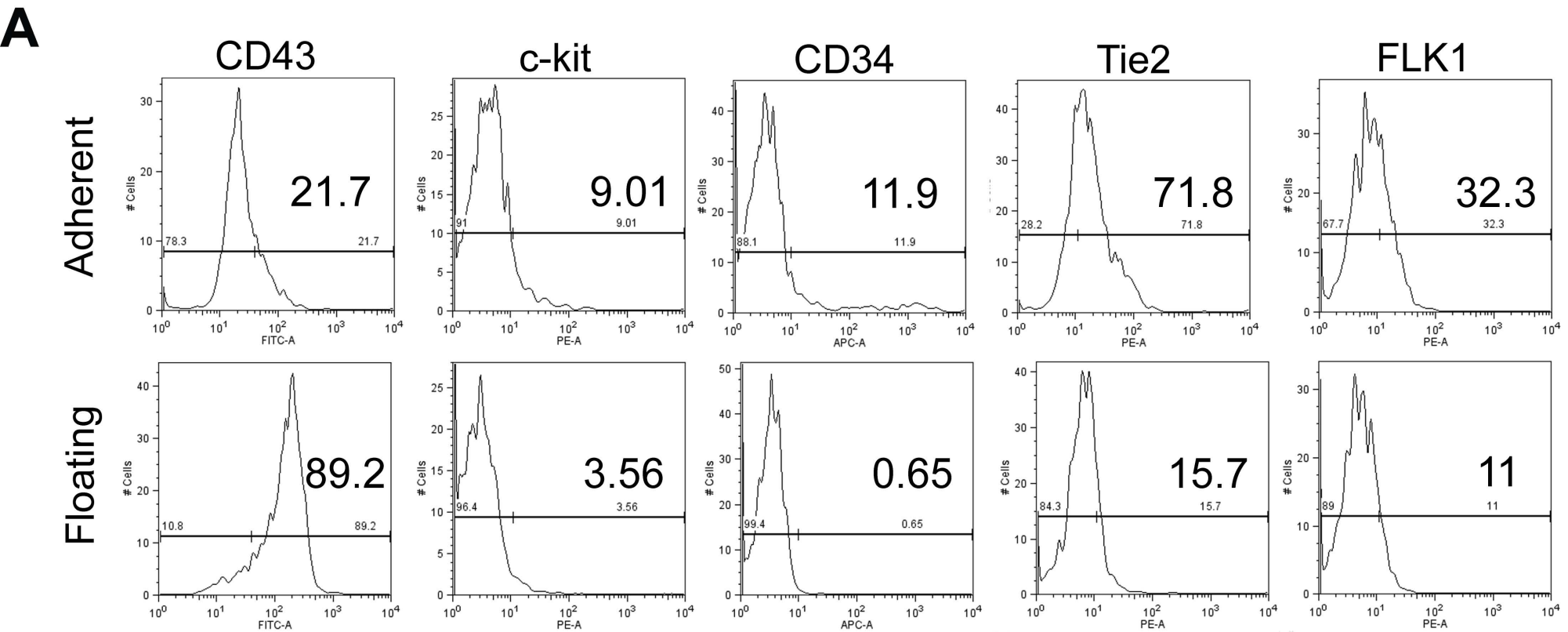
Supplementary Figure 5: Mechanistic diagram of AhR involvement in normal hematopoietic development. The AhR agonist FICZ allows for the production and exponential expansion of hematopoietic progenitor cells (HPs). Continued AhR agonism is permissive to erythroid-lineage cell maturation whereas AhR antagonism elicits a transcriptional switch, which preferentially directs HPs to become megakaryocytes.

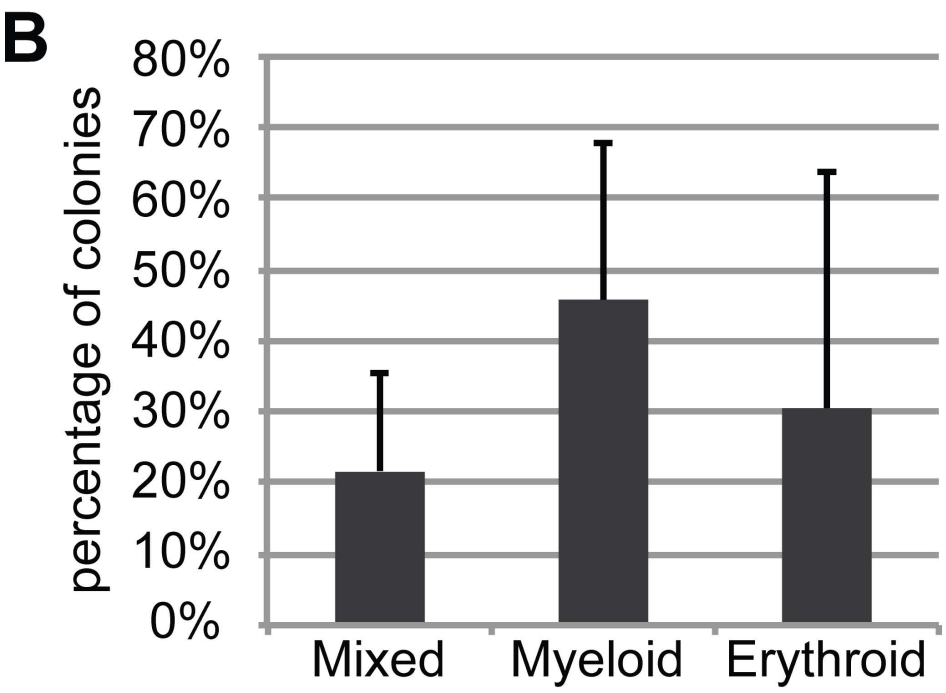
1. Eisen MB, Spellman PT, Brown PO, Botstein D. Cluster analysis and display of genome-wide expression patterns. *Proc Natl Acad Sci U S A*. 1998;95(25):14863-14868.

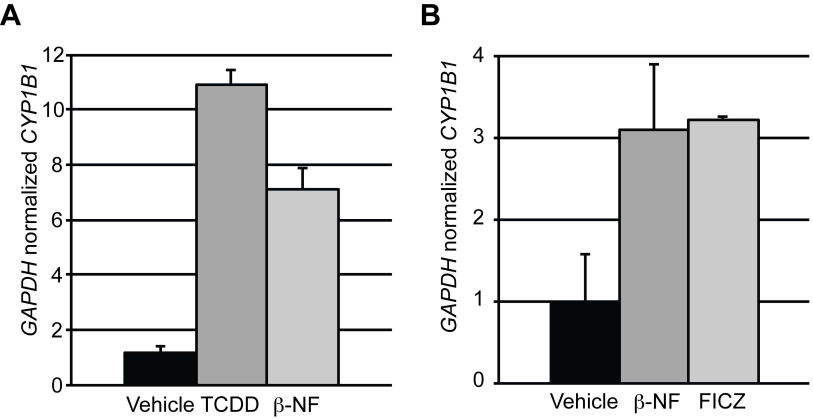
2. T.J. CJMH. Statistical Models in S: Chapman and Hall/CRC; 1991.

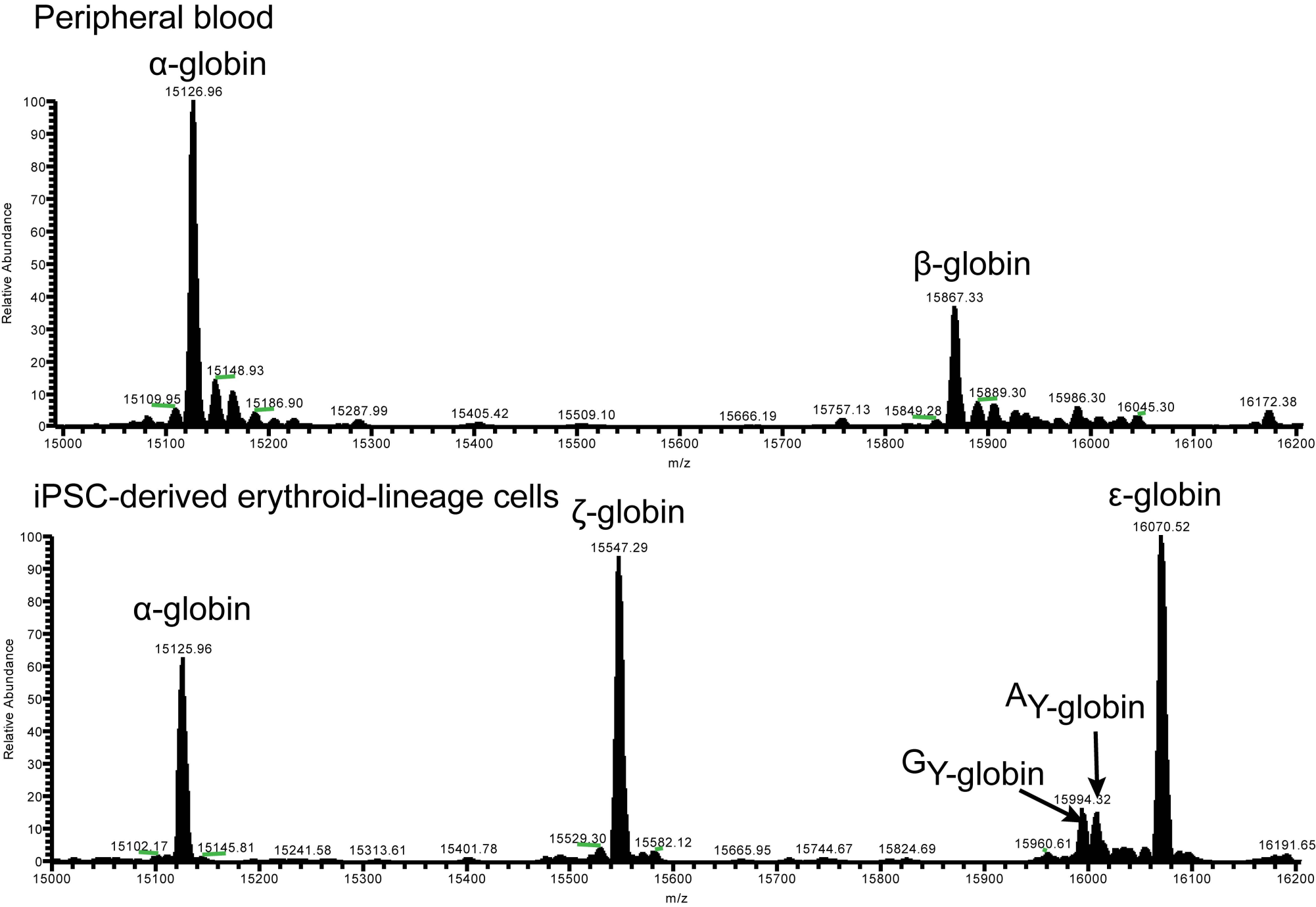

A
HSC1
HSC3
CMP
MEP
ERY1
ERY2
ERY3
ERY4
ERY5
MEGA1
MEGA2
HSC
CMP
MEP
ERY
MEGA
CCL3
TGFA
ITGB3
TGFB1
SLAMF7
MMP9
MMP8
CYP1B1
SERPINB2
CDH1
NFE2
IL8
SCRG1
CYP1A2
VCAM1
SERPINE1
FN1
COL15A1
MYO1B
LMO7
NQO1
IL1B
PROM1
VAV3
ALDH1A1
EREG
MYC
AHR
PTGS2
PKM2
EGR1
GSTA1
SFXN1
TIPARP

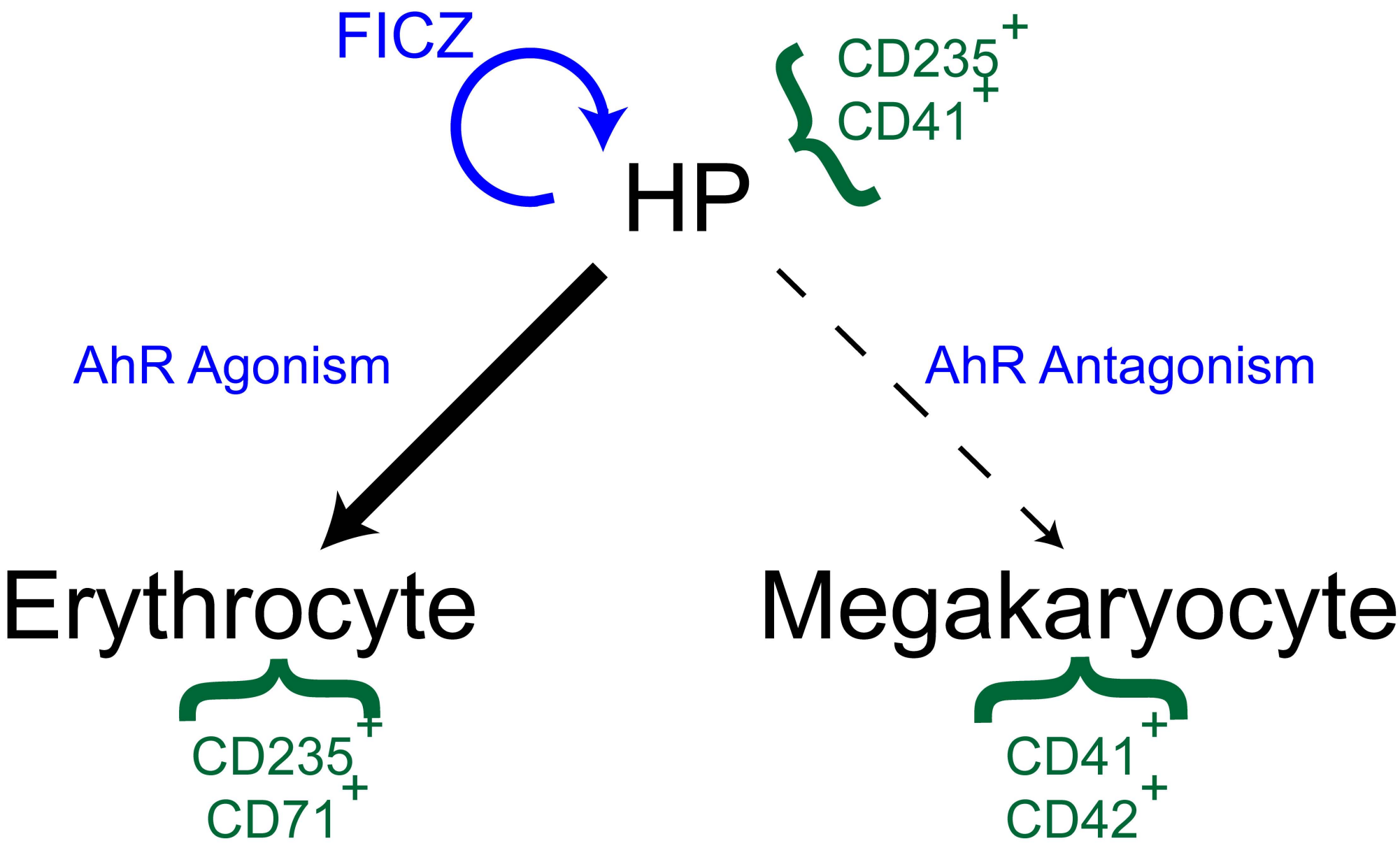

B
AhR normalized expression
2
1
0
−1
−2
−3
−4
anova pval=1.1e−06
HSC
CMP
MEP
ERY
MEGA


A
CD43
c-kit
CD34
Tie2
FLK1
Adherent
Floating
21.7
9.01
11.9
71.8
32.3
89.2
3.56
0.65
15.7
11
78.3
91
88.1
28.2
67.7
10.8
96.4
99.4
84.3
89
Cells
FITC-A
PE-A
APC-A


B
percentage of colonies
80%
70%
60%
50%
40%
30%
20%
10%
0%
Mixed
Myeloid
Erythroid


A
B
GAPDH normalized CYP1B1
0
2
4
6
8
10
12
Vehicle
TCDD
β-NF
GAPDH normalized CYP1B1
0
1
2
3
4
Vehicle
β-NF
FICZ


Peripheral blood
α-globin
15126.96
β-globin
15867.33
15148.93
15109.95
15186.90
15287.99
15405.42
15509.10
15666.19
15757.13
15849.28
15889.30
15986.30
16045.30
16172.38
Relative Abundance
m/z
iPSC-derived erythroid-lineage cells
ζ-globin
15547.29
ε-globin
16070.52
α-globin
15125.96
AY-globin
GY-globin
15994.32
15102.17
15145.81
15241.58
15313.61
15401.78
15529.30
15582.12
15665.95
15744.67
15824.69
15960.61
16191.65
Relative Abundance
m/z


FICZ
CD235+
CD41+
HP
AhR Agonism
AhR Antagonism
Erythrocyte
Megakaryocyte
CD235+
CD71+
CD41+
CD42+