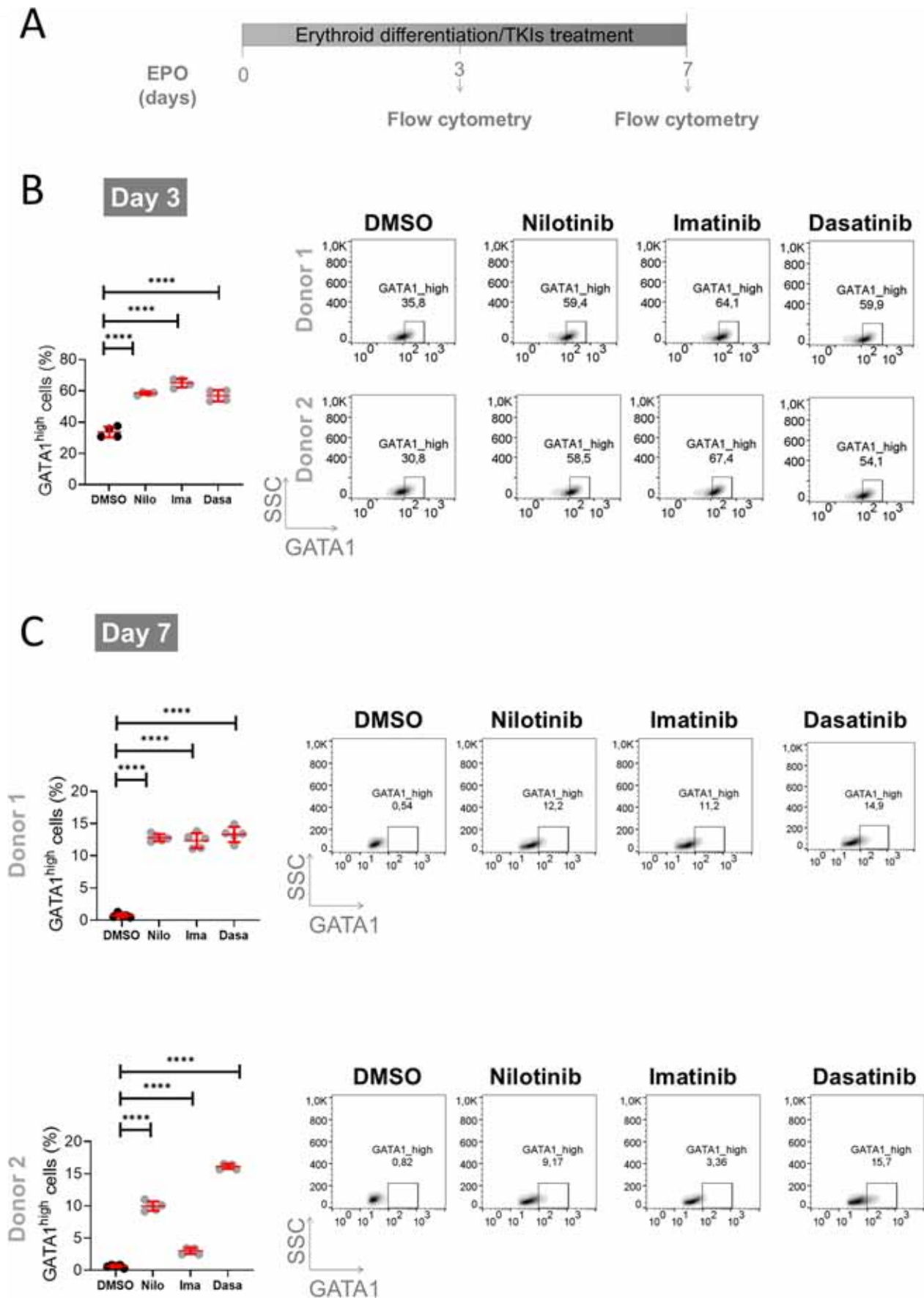


Expanded View Figures

Figure EV1. (related to Figs. 2 and 6). TKIs increased GATA1 protein amount during the erythroid differentiation of primary CD34⁺ HSPCs.

(A) Primary human CD34⁺ cells were purified from human cord blood and differentiated for 7 days with EPO in the presence of either DMSO, 100 nM nilotinib, 100 nM imatinib or 1 nM dasatinib. (B, C) Cells were stained with anti-GATA1-APC and GATA1^{high} cells analyzed by flow cytometry at 3 and 7 days post-differentiation. Representative dot plots at different differentiation times are also shown. Data are shown as the mean $\pm$ SEM. *P* values were calculated using one-way ANOVA and Tukey's multiple range test. All the significant comparisons have a $p < 0.0001^{****}$. Source data are available online for this figure.



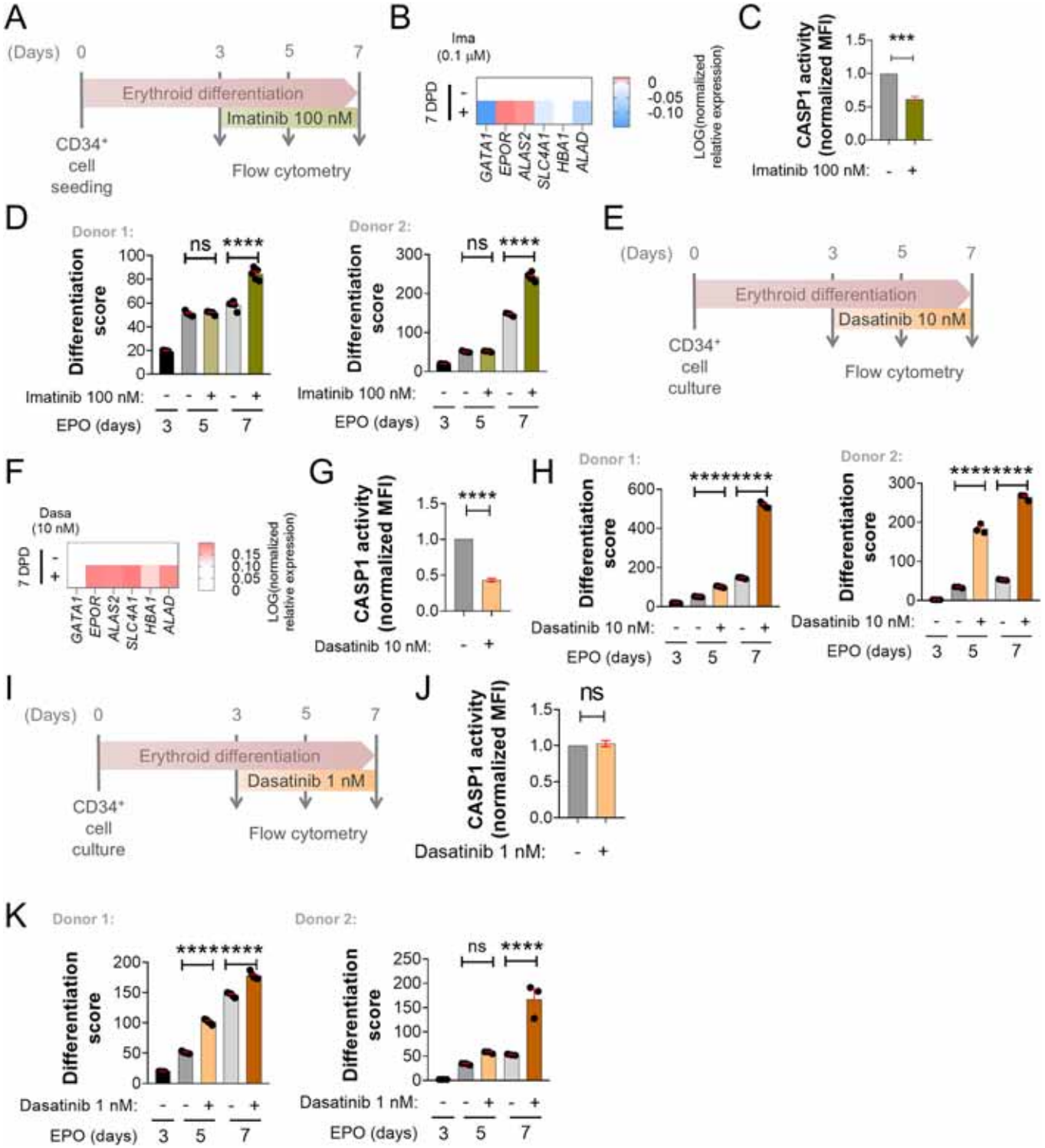




Figure EV2. (related to Figs. 2 and 6). Imatinib and dasatinib replicate the effects of nilotinib on human HSPCs from healthy donors.

Primary human CD34⁺ HSPCs from healthy donors (ZenBio or StemCell Technologies) were differentiated with EPO in the presence of 0.1 μ M imatinib (A–D), or 10 (E–H) and 1 nM (I–K) dasatinib from 3 to 7 days of culture. Cells were stained with anti-CD235A-APC (Glycophorin A) and anti-CD71-FITC (Transferrin Receptor), and erythroid differentiation was then analyzed by flow cytometry. The transcript levels of GATA1-dependent genes (B), caspase-1 activity determined with FAM FLICA (D, G) and the differentiation score calculated as the ratio between CD235A⁺/CD71⁺ (intermediate erythroid progenitors) and CD235A[−]/CD71⁺ (early erythroid progenitors) (D, H, K) at 7 dpd are shown. Data are shown as the mean $\pm$ SEM ($N = 3$). P values were calculated using one-way ANOVA and Tukey's multiple range test (D, H, K) or a Student's t -test (C, G). ns, non-significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.01$ and **** $p < 0.0001$. (C): $p = 0.0005^{***}$, (D): (Donor 1) DMSO_D7 wrt IMA_D7 $p < 0.0001$, (Donor 2) DMSO_D7 wrt IMA_D7 $p < 0.0001$, (G): $p < 0.0001^{****}$, (H): DMSO_D5 wrt DASA_D5 $p < 0.0001^{****}$, DMSO_D7 wrt DASA_D7 $p < 0.0001^{****}$, (K): DMSO_D5 wrt DASA_D5 $p < 0.0001^{****}$, DMSO_D7 wrt DASA_D7 $p < 0.0001^{****}$. Source data are available online for this figure.

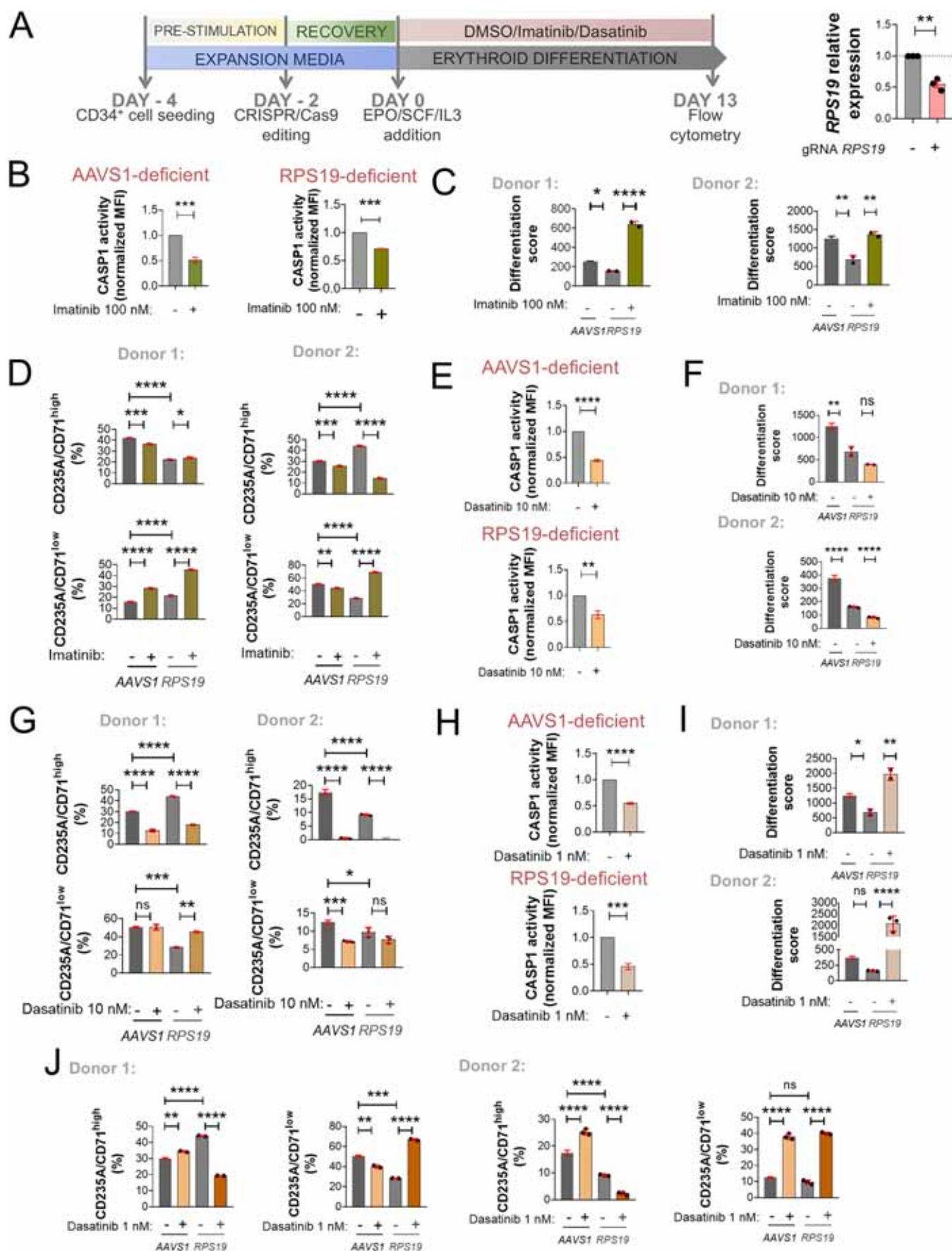


Figure EV3. (related to Figs. 2 and 6). Imatinib and dasatinib alleviate defective erythropoiesis of RPS19-deficient HSPCs.

(A) Primary human CD34⁺ from healthy donors (ZenBio or StemCell Technologies) were edited with CRISPR/Cas9 and differentiated for 13 days with EPO in the presence of 0.1 μ M imatinib (A–D) or 10 nM dasatinib (E–J) from 3 to 13 days of culture. Cells were stained with either FAM FLICA or anti-CD235A-APC (Glycophorin A) and anti-CD71-FITC (Transferrin Receptor), and CASP1 activity (B, H), erythroid differentiation (B–D, F, G, I, J) were then analyzed by flow cytometry. The differentiation score was calculated as the ratio between CD235A⁺/CD71⁺ (intermediate erythroid progenitors) and CD235A⁺/CD71⁺ (early erythroid progenitors) (C, F, I), and the percentage of CD235A⁺/CD71^{high} (erythroblasts) and CD235A⁺/CD71^{low} (reticulocytes) (D, G, J) at 13 days of culture (E). Data are shown as the mean $\pm$ SEM ($N = 3$). P values were calculated using one-way ANOVA and Tukey's multiple range test (C, D, F, G, I, J) or a Student's t -test (A, B, E, H). ns, non-significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.01$ and **** $p < 0.0001$. (A): $p = 0.0011^{**}$, (B): AAVS1-deficient $p = 0.0009^{***}$, RPS19-deficient $p = 0.0009^{***}$, (C): (donor 1) AAVS1_DMSO wrt RPS19_DMSO $p = 0.0271^{*}$, RPS19_DMSO wrt RPS19_IMA $p = 0.0002^{***}$, (donor 2) AAVS1_DMSO wrt RPS19_DMSO $p = 0.0043^{**}$, RPS19_DMSO wrt RPS19_IMA $p = 0.0034^{**}$, (D): (Donor 1) (Upper graph) AAVS1_DMSO wrt: AAVS1_IMA $p = 0.0005^{***}$ or RPS19_DMSO $p < 0.0001^{****}$, RPS19_DMSO wrt RPS19_IMA $p = 0.0446^{*}$, (lower graph) all $p < 0.0001^{****}$, (Donor 2) (Upper graph) AAVS1_DMSO wrt: AAVS1_IMA $p = 0.0006^{***}$ or RPS19_DMSO $p < 0.0001^{****}$, RPS19_DMSO wrt RPS19_IMA $p < 0.0001^{****}$, (lower graph) AAVS1_DMSO wrt: AAVS1_IMA $p = 0.0012^{**}$ or RPS19_DMSO $p < 0.0001^{****}$, RPS19_DMSO wrt RPS19_IMA $p < 0.0001^{****}$, (E): AAVS1-deficient $p < 0.0001^{****}$, RPS19-deficient $p = 0.0076^{**}$, (F): (Donor 1) AAVS1_DMSO wrt RPS19_DMSO $p = 0.0012^{**}$, (Donor 2) all significant comparison have $p < 0.0001^{****}$, (G): (Donor 1) (upper graph) all significant differences have $p < 0.0001^{****}$, (lower graph) AAVS1_DMSO wrt RPS19_DMSO $p = 0.0006^{***}$, RPS19_DMSO wrt RPS19_DMSO $p = 0.0018^{**}$, (Donor 2) (upper graph) all significant differences have $p < 0.0001^{****}$, (lower graph) AAVS1_DMSO wrt: AAVS1_DASA $p = 0.0002^{***}$ or RPS19_DMSO $p = 0.0142^{*}$, (H): AAVS1-deficient $p < 0.0001^{****}$, RPS19-deficient $p = 0.0006^{***}$, (I): (Donor 1) AAVS1_DMSO wrt RPS19_DMSO $p = 0.0311^{*}$, RPS19_DMSO wrt RPS19_DASA $p = 0.0015^{**}$, (Donor 2) RPS19_DMSO wrt RPS19_DASA $p < 0.0001^{****}$, (J): (Donor 1) (left) AAVS1_DMSO wrt: AAVS1_DASA $p = 0.0012^{**}$ or RPS19_DMSO $p < 0.0001^{****}$, RPS19_DMSO wrt RPS19_DASA $p < 0.0001^{****}$, (right) AAVS1_DMSO wrt AAVS1_DASA $p = 0.0015^{**}$ or RPS19_DMSO $p = 0.0001^{****}$, RPS19_DMSO wrt RPS19_DASA $p < 0.0001^{****}$, (Donor 2) all significant differences have $p < 0.0001^{****}$. Source data are available online for this figure.