Supporting Information

Ghosh et al. 10.1073/pnas.1717993115

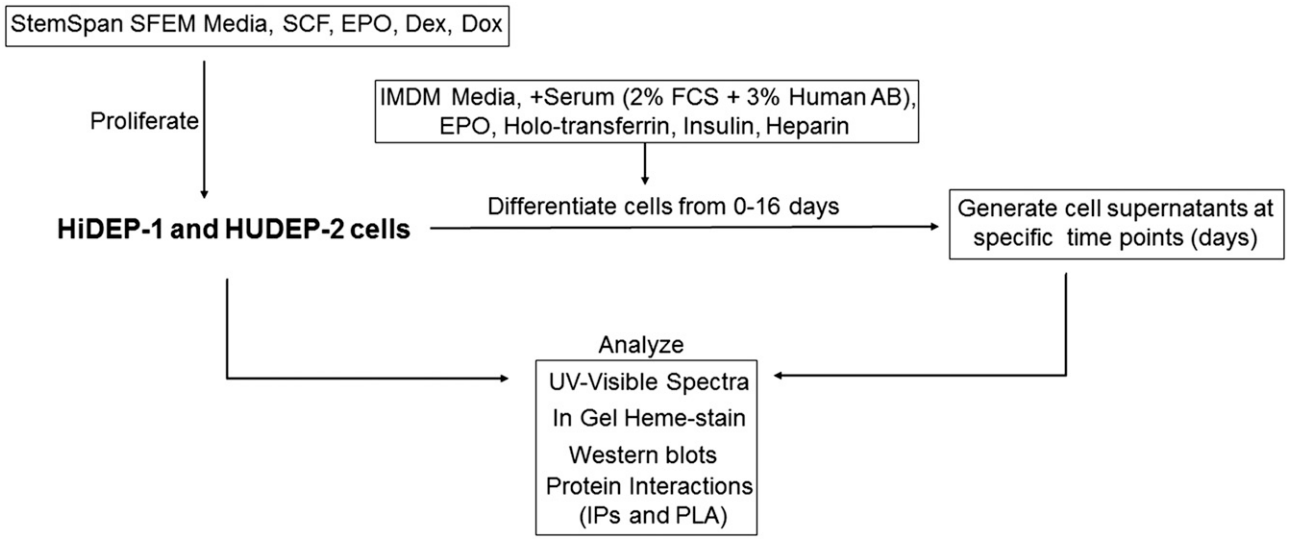

Fig. S1. General methods to monitor Hb maturation in HiDEP-1 and HUDEP-2 cells during proliferation and erythropoietic differentiation. See *Results* describing Figs. 3 and 4 and Fig. S1. Dex, dexamethasone; Dox, doxicyclin; EPO, erythropoietin; HiDEP-1, human iPS cell-derived erythroid progenitor-1; HUDEP-2, human umbilical cord blood-derived erythroid progenitor-2; IPs, immunoprecipitations; PLA, proximity ligation assay; SCF, stem cell factor.

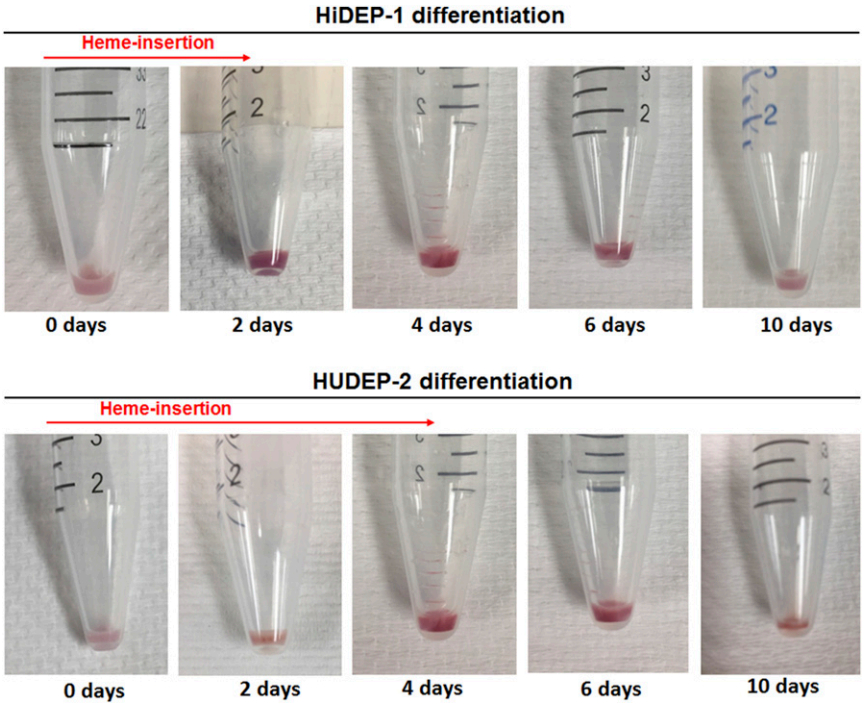

Fig. S2. Relative Hb maturation in erythroid progenitor cells. Representative cell pellets prepared at different days of differentiation depict relative content of mature heme-containing Hb in HiDEP-1 and HUDEP-2 cells. Red arrows depict time span from the onset up to the point of maximum heme insertion into Hb, as determined from spectral absorption measures on cell supernatants as depicted in Fig. 3 *A* and *G*. Experimental details are described in legends to Fig. 3 and Fig. S1.

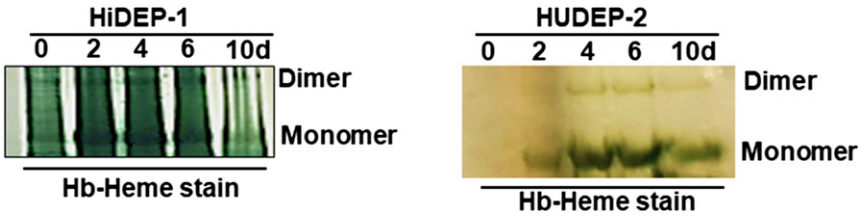

Fig. S3. Mature Hb present in differentiating HiDEP1 and HUDEP-2 cells as detected by heme-staining. Cell supernatants (equal protein) were run on SDS/PAGE, and the gel was subject to a heme-staining protocol to detect and compare levels of heme-replete Hb.

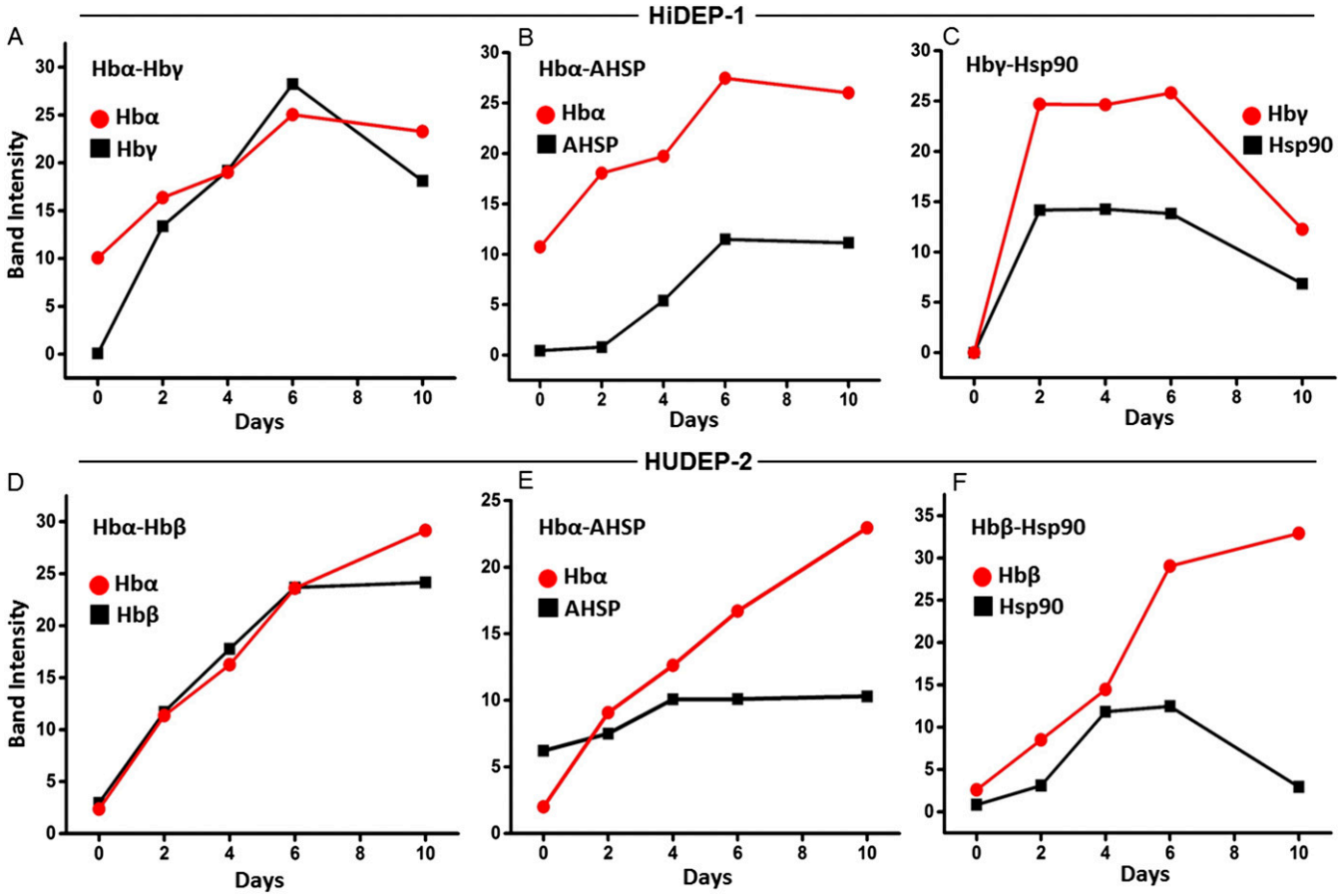

Fig. S4. Densitometric analysis of IP data indicating the Hbα–Hbγ/β, Hbα–AHSP, and Hbβ/γ–Hsp90 interactions in HiDEP-1 and HUDEP-2 cells during differentiation. *A*, *B*, *D*, and *E* depict mean densitometries ($n = 2$) of Hbα–Hbγ/β or Hbα–AHSP interactions similar to those shown in Fig. 3 *E* and *K*, respectively. *C* and *F* depict mean densitometries ($n = 2$) of Hbγ/β–Hsp90 interactions as depicted in Fig. 3 *F* and *L*, respectively.

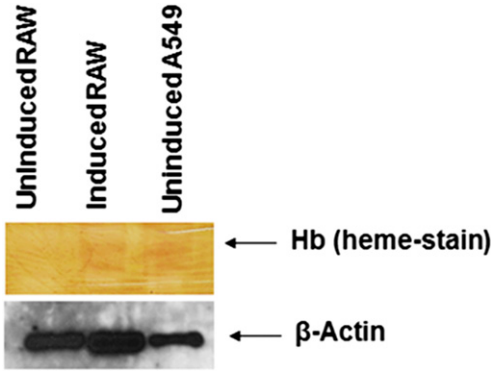

Fig. S5. Nonerythroid cells express heme-containing Hb. *Upper* panel shows Hb bands in heme-stained SDS/PAGE samples of RAW and A549 cells, and *Lower* shows β-actin blot run on separate gels as a loading control.

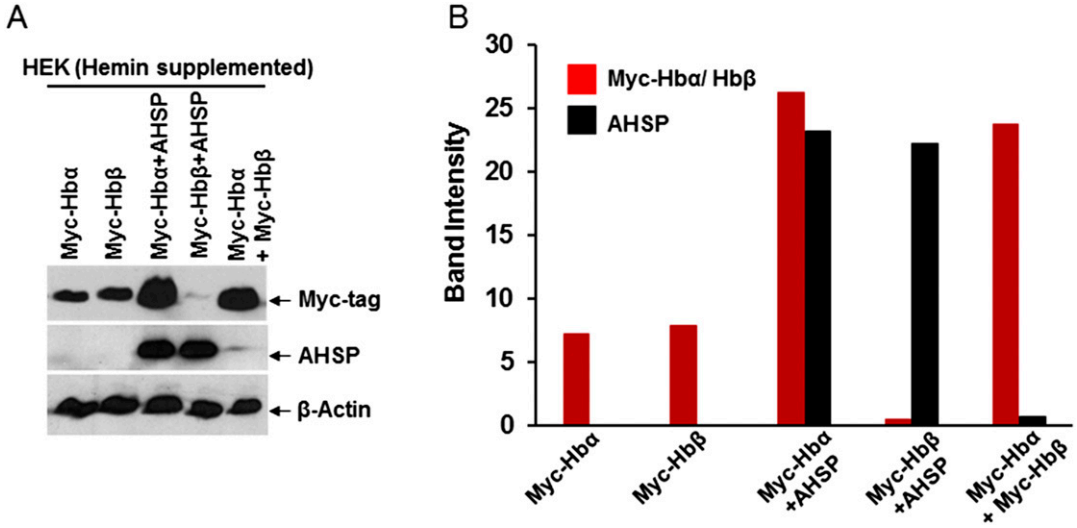

Fig. S6. AHSP inhibits Hb-β expression in nonerythroid cells even when hemin is added to the culture. HEK cells transiently expressing Myc-tagged globins either individually or in combination with AHSP were cultured with 5 μM hemin and then analyzed for protein expression. (*A*) Representative expression levels of Myc-tagged globins, AHSP, and loading control β-actin as indicated. (*B*) Corresponding densitometry of protein expression levels representing the mean of two independent experiments ($n = 2$).