

Figure S1. A novel 2-step primitive erythroid culture assay recapitulates primitive erythroblast terminal maturation.

A. Schematic of the 2-step primitive erythroid culture assay. E8.5 mouse embryos are dissociated to single cell suspension with trypsin and cultured in gelatin-coated wells for 24 hours (d0-d1). Next, non-adherent cells are transferred to new, uncoated wells and cultured for a total of up to 4 days. B. Wright-Giemsa stained, progressively maturing primitive erythroblasts, from cultures supplemented with or deprived of EPO and sampled on successive days. Cells were photographed using a Nikon Optiphot microscope (40x objective, NA 0.60) and SPOT RT-slider digital camera (Diagnostic Instruments, Sterling Heights, MI).

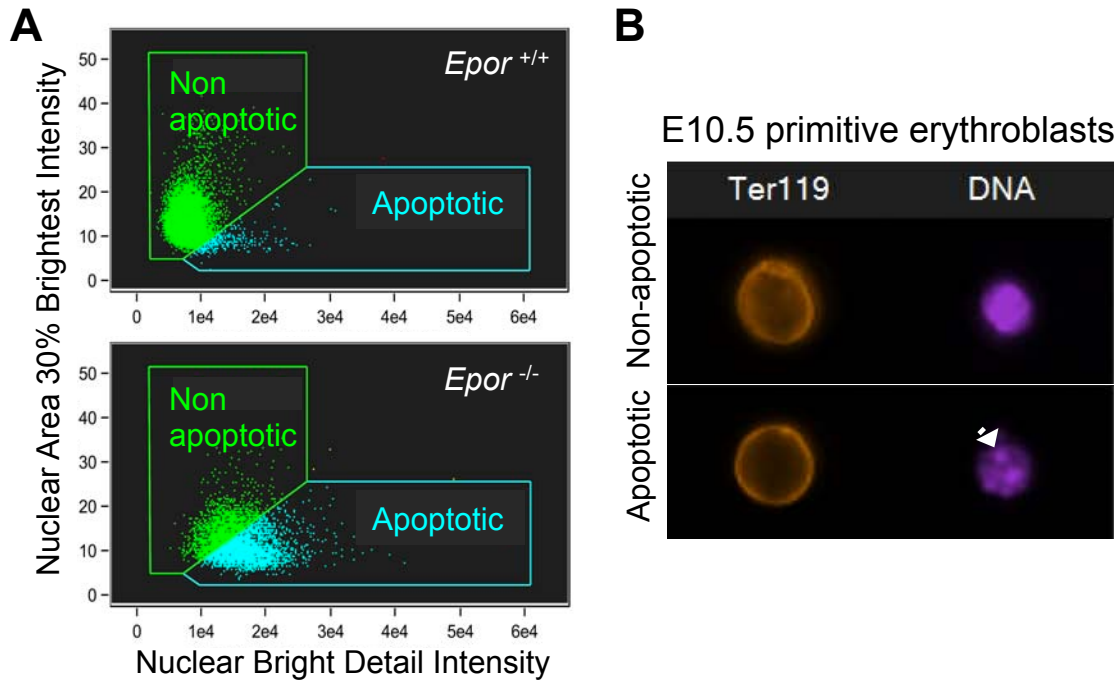


Figure S2. Identification of apoptotic primitive erythroblasts by imaging flow cytometry.

A. Gating strategy of nuclear features used to quantify apoptotic primitive erythroblasts from individual wild-type and *Epor*-null mouse embryos based on method described by Henery et al. B. Example of non-apoptotic and apoptotic E10.5 primitive erythroblasts as defined by the gates in (A). Arrow denotes areas of increased nuclear staining intensity indicative of apoptotic morphology.

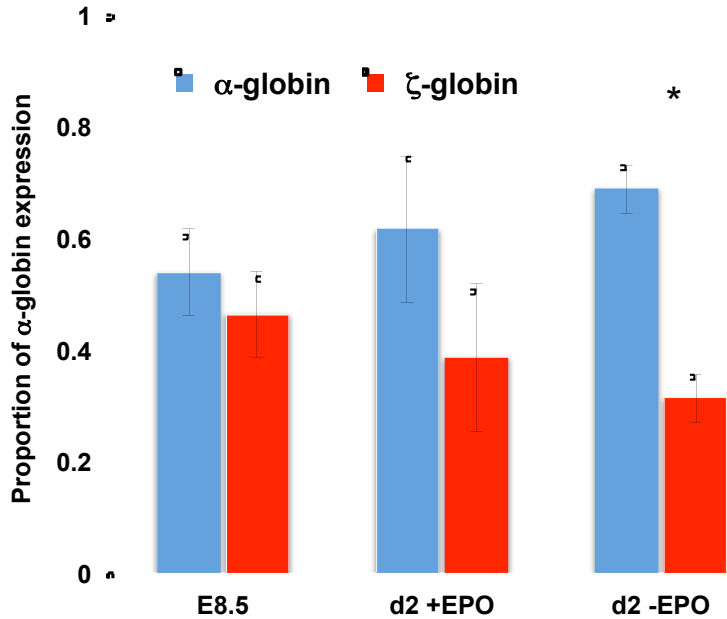


Figure S3. The primitive erythroblast maturational α -globin switch indicates that maturation is accelerated in the absence of EPO.

Measurement of ζ -globin and α -globin transcripts in cultures of wild-type primitive erythroblasts cultured for two days with (+EPO) and without (-EPO) EPO. ζ - and α -globin genes are expressed at similar levels at the start of the culture period (E8.5) and begin to “switch” with maturation (d2 +EPO). Consistent with the more rapid maturation of primitive erythroblasts lacking EpoR signaling, we find that the ζ - to α -globin switch has significantly progressed in the cultures lacking EPO (d2 -EPO); $p= 0.02$, $N=3$.