Supplemental Methods

Histologic analysis. Embryos and placentas were fixed in 4% paraformaldehyde at 4°C overnight, the tissues were washed in PBS, cryoprotected in 30% sucrose/PBS with gentle agitation at 4°C overnight, embedded in 7.5% w/v gelatin (Fluka, cat. No. 48723) containing 1.5% w/v sucrose and were frozen in 2-methylbutane at - 30°C. Specimens were cut into 7 μm sections in cryostat, collected on poly-L-lysine-coated slides and stained with hematoxylin/eosin (Sigma, cat. no HHS16) or subjected to in situ hybridization.

In situ-hybridization. The in situ hybridization with 4311, Tef5 and Pl1 probes for spongiotrophoblasts, syncytiotrophoblasts and giant cells respectively, was performed as previously described (31). Briefly, samples were fixed with 4 % PFA, incubated with proteinase K 20 μg/ml for 5 min at RT, washed with 0.2 % glycine in 1x PBS, fixed in 4 % PFA and washed in 1 x PBS for 5 min at RT. Then they were incubated with an acetylation mix containing triethenolamine 0.1 M (Merck, cat. no. 8379), NaOH 10N, and acetic anydrate 0.25% (Merck, cat. no. A6404) for 10 min, RT, washed with 1x PBS and hybridised with RNA probe in a concentration 1 ng/μl in hybridization buffer (50 % formamide 5 x Denhardts, 5x SSC, yeast tRNA 200 μg/ml, herring sperm DNA 0,5 mg/ml) at 55^{o}C for 16-18 hrs. After hybridization, samples were washed in 2× SSC for 30 min at 55°C, incubated in 20 μg/ml RNase for 30 min at 37°C, washed in 2× SSC / 50% formamide for 20 min and then 2× SSC (twice) at 55°C. Samples were blocked for non - specific

binding with 10% FCS for 1 hr at RT and then they were incubated with alkaline phosphatase (AP)-conjugated anti-digoxigenin (Roche cat.no. 11093274910) at 37°C, overnight. The hybridization was examined with NBT/BCIP solution (Roche, cat. no. 11681451001). Samples were photographed with 63x lens on a Zeis Axio Scope microscope fitted with ProgRess Jenoptik camera.

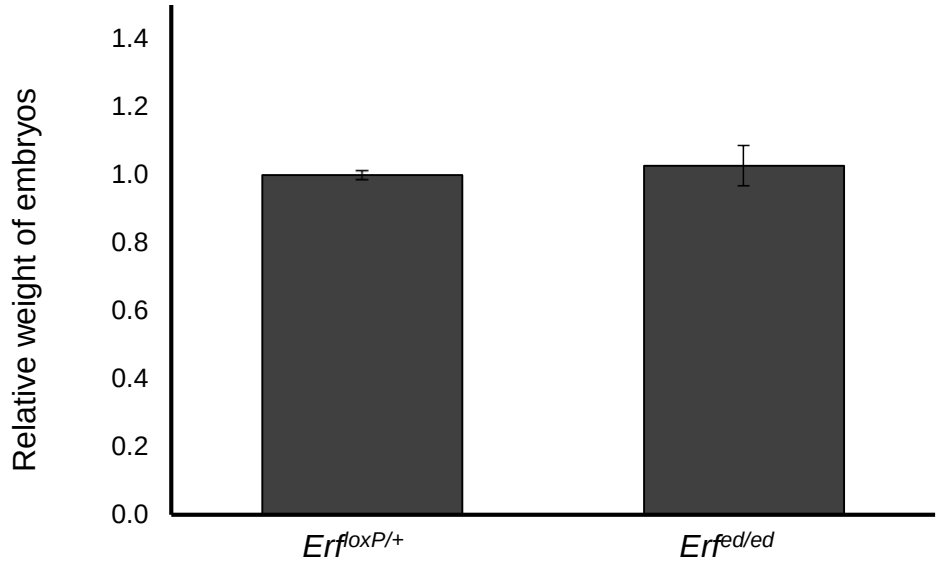
Real-time quantitative PCR for Erf. Total RNA was extracted from whole embryos, fetal livers and yolk sacs at E12.5, using Trizol reagent (Invitrogen, cat. no. 15596018) according to manufacturer's instructions. Reverse transcription of mRNA was employed by the SuperScript first strand synthesis kit (Invitrogen, cat. no. 11904-018). The reactions were performed with 100 ng of total cDNA utilizing the 2 x BrilliantIII SYBRGREEN QPCR mastermix (Stratagene, cat. no. 600882-51) in an Applied Biosystems StepOne plus Real-Time PCR machine. Expression levels of Erf were detected with the Erf primers (Fw: 5´ - TGTGGCACTTTATCCTGGAG - 3; Rv: 5´ - CTTGTAGGTGAACCGTTTCC – 3´). All expression levels were normalized to Gapdh levels (Fw: 5´ - CCAGTATGACTCCACTCACG.- 3´, Rv: 5´ - GACTCCACGACATACTCAGC - 3´) in the same cDNA. Annealing was performed at 56^{o}C for 20 sec for both Erf and Gapdh.

Fluorescence staining and confocal microscopy. Cryosections from E11.5 or E12.5 embryos were blocked in 5 % fetal bovine serum , 2% BSA, 0.5 % Triton solution in PBS for 1 hr and stained with the following antibodies: rat anti-Ter119 (Biolegend, cat. no.116203) or rat anti-CD71 antibody (Biolegend, cat. no.113805) for detection of all proerythroblasts to maturing enucleated

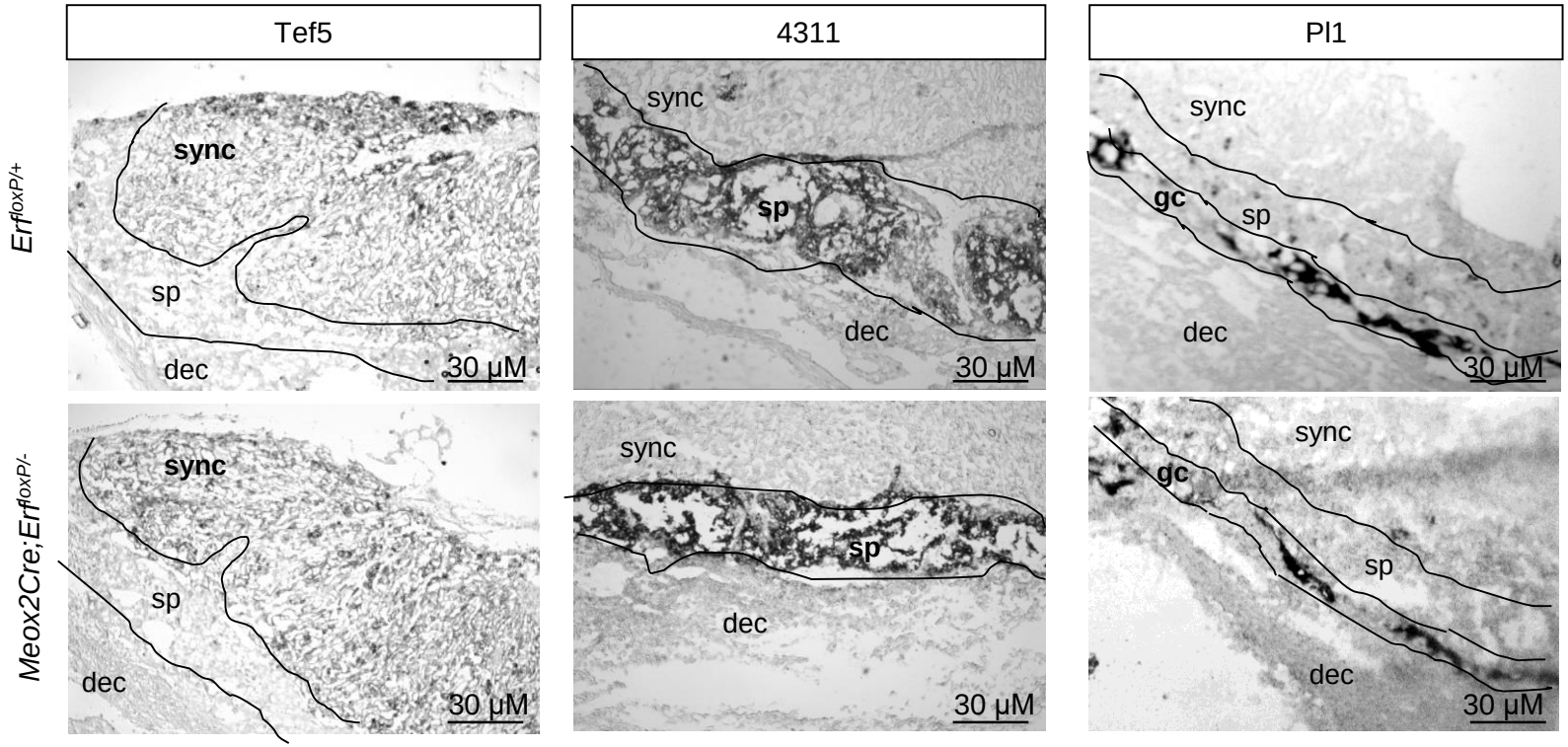
erythroblasts, rabbit anti-HNF4a for hepatocytes, rabbit anti - pH3 (Merck Millipore, cat. no, 07-424) for proliferating cells and rat anti - F4/80 (Biolegend, cat. no,123109) for the macrophages. The antibodies were diluted in 1 % fetal bovine serum, 0.4 % BSA in PBS and incubated overnight at 4°C, washed 3 times in PBS and stained with anti-rat CF555 (Sigma, cat. no. SAB4600060), anti-rabbit FITC (Sigma, cat. no. F0382) and anti-rabbit CF555 (Sigma, cat. no. SAB4600068), in 1:1000, 1:50 and 1:1000 dilution, respectively. Nuclei were stained with TO-PRO-3 iodide (Invitrogen cat. no. T3605) for 5 min at RT and the slides were mounted with mowiol (Sigma, cat. no. 81381). Analysis of apoptotic cells was performed with the In Situ cell dead kit TMR Red (Roche, cat. no. 12156792910) according to manual instructions.

BrdU staining in fetal livers was performed injecting pregnant mice with 50 μg Brdu (Sigma, cat. no B5002) per gr 2 hrs before sacrifice. Embryos were dissected, fixated and froze for cryosections. Sections were fixed in 4% paraformaldehyde in 1x PBS for 10 min, RT and then washed with 1xPBS for 3 times. Samples were incubated in a solution containing 2N HCl and 0.5% Triton-X100 in 1xPBS for 30 min, at 37^{o}C in a glass bottle and washed 3 times for 5 minutes with 0.1M sodium tetraborate neutralization buffer pH 8.5, blocked and incubated with rat anti-Brdu (Bio-rad, cat. no. OBT0030G) in dilution 1:800, overnight, at 4^{o}C. Next day, the samples were washed and incubated with anti-rat CF555 (Sigma, cat. no. SAB4600060) for 1 hr, RT. The staining of nuclei was performed as before with TOPRO-3. All samples were analyzed by confocal microscope and processed with Leica 2.6.0 confocal imaging software.

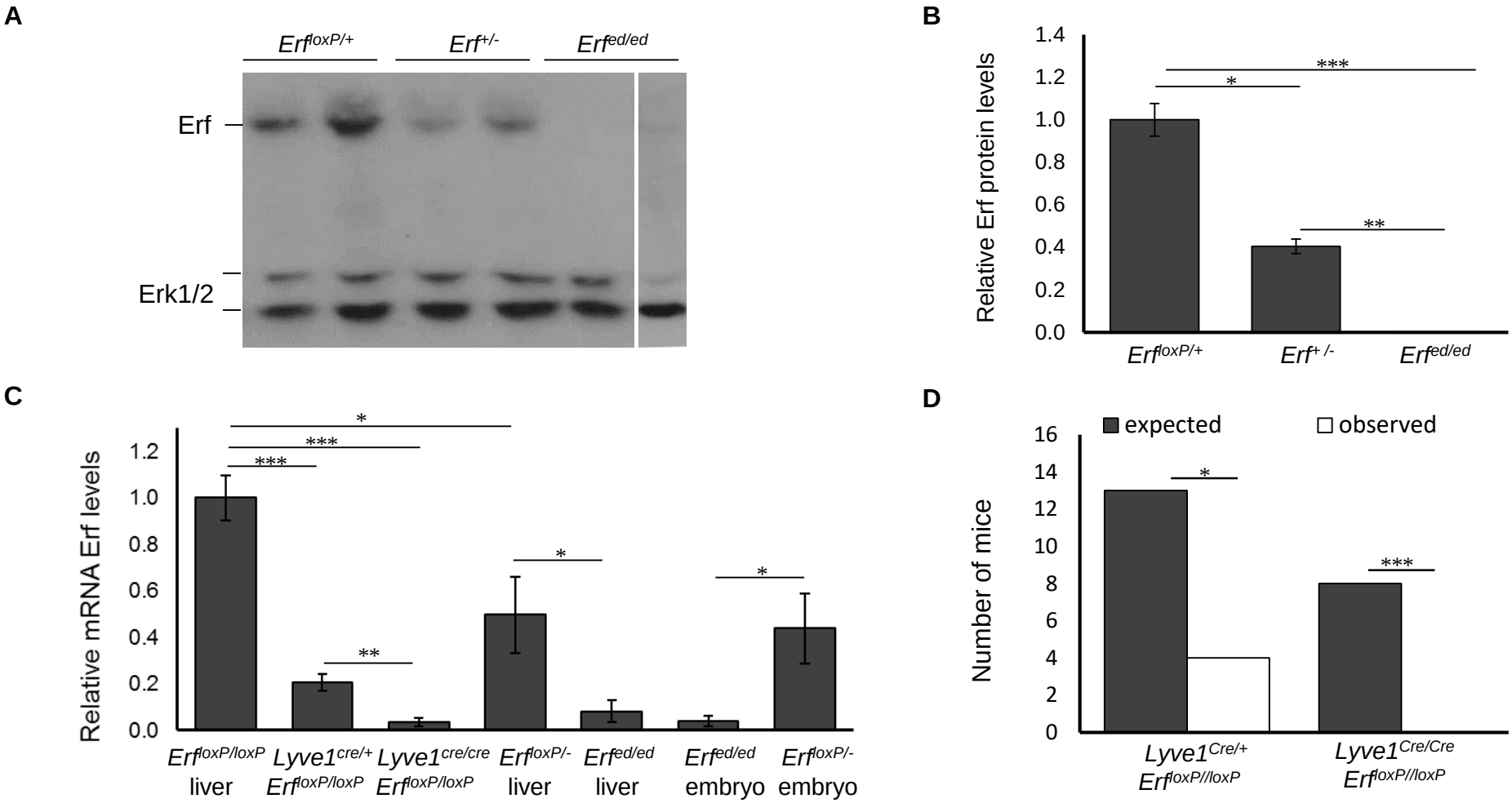
Cell cycle analysis. Fetal livers were isolated from E13.5 embryos and dissociated by gentle pipetting in IMDM with 2% FBS. Cells were counted in hematocytometer and cells were incubated in 0.25% Triton, 1% BSA in 1x PBS for 15 min at 4°C. Then, 5 x 10^5 cells were centrifuged at 300 g and stained with 300 µl of a PBS solution containing propidium iodide 20 µg/ml and RNAse A (Qiagen, cat. no. 19101) 250 µg/ml for 20 minutes, at 4°C. Samples were analyzed by BD FACSCalibur Flow cytometer.



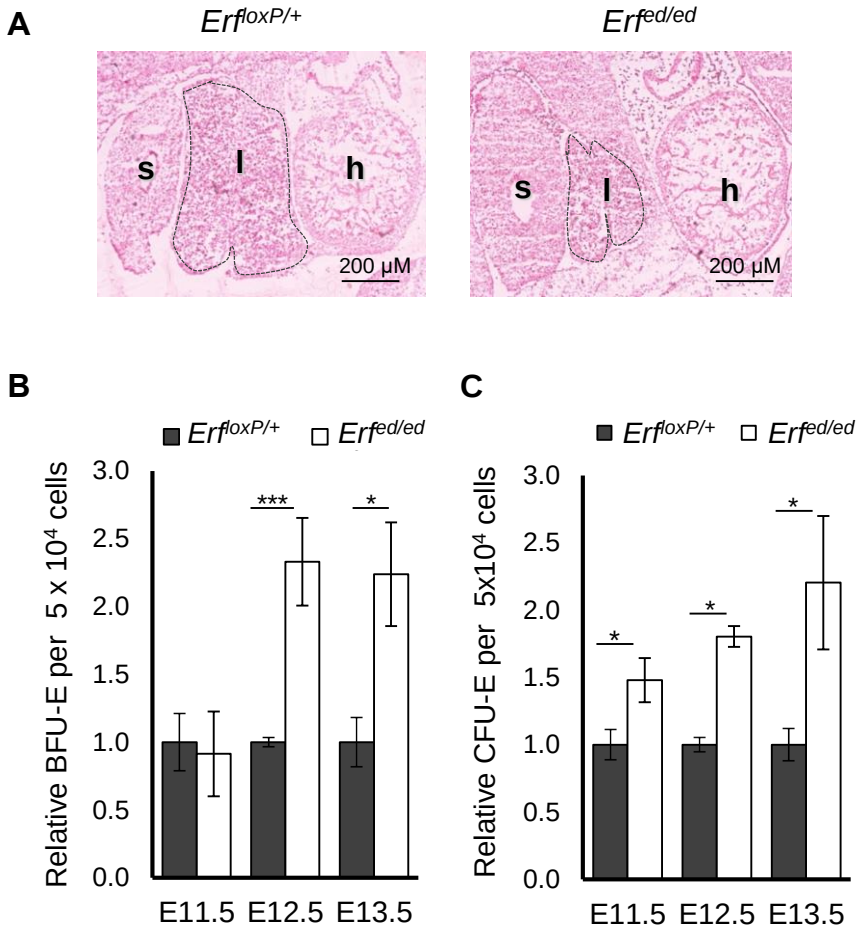
Supplemental Figure 1. Weight of *Erf*$^{ed/ed}$ embryos is comparable to their *Erf*$^{loxP/+}$ littermates. Dissected embryos were weighed and their weight was compared to the average weight of the *Erf*$^{loxp/+}$ embryos of each litter. The graph indicates the relative embryo weight of the respective genotype at embryonic day 13.5. All values are means ± SE of 10 biological samples from 8 litters. No statistical significance was observed using unpaired t-test with two-tailed distribution.



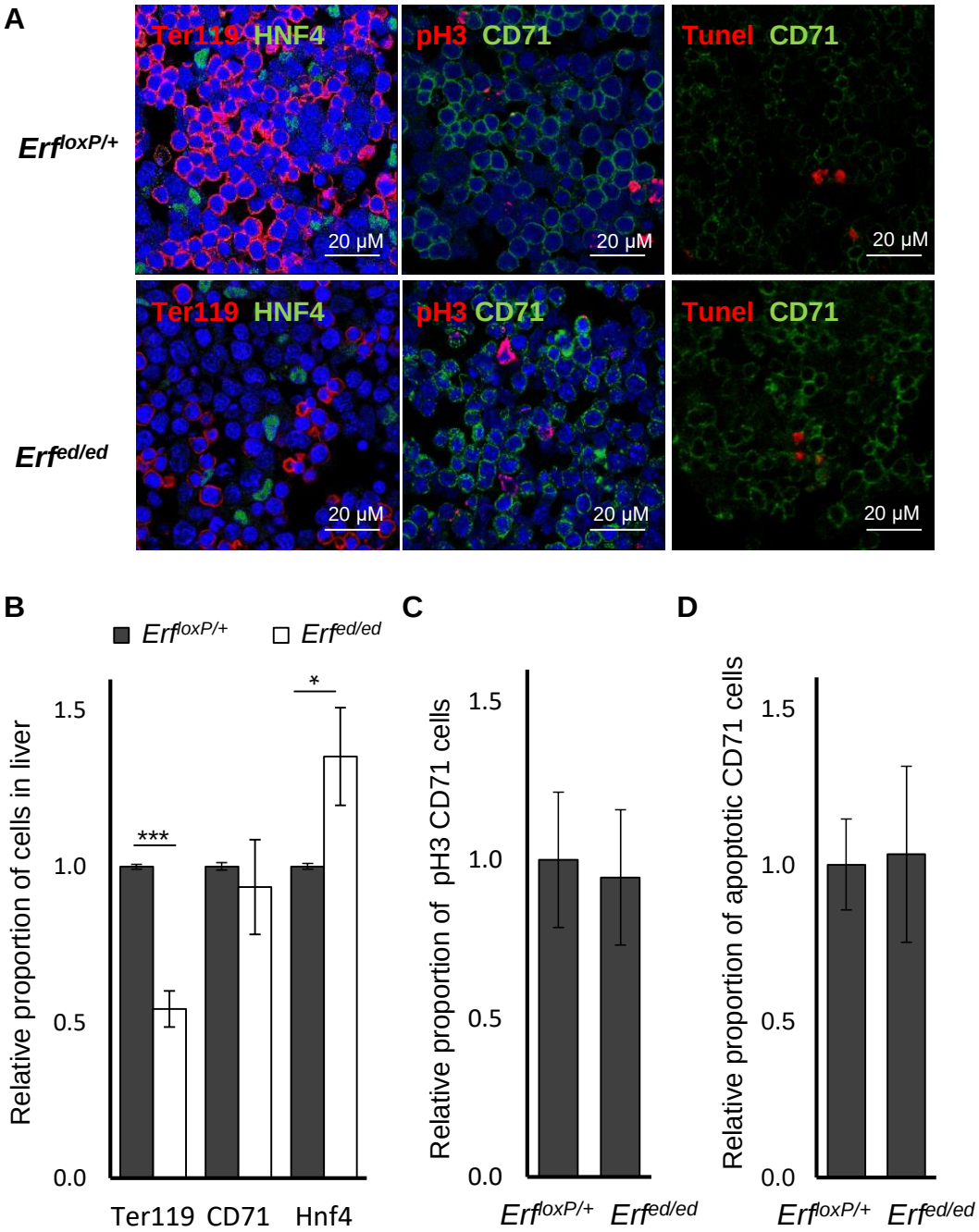
Supplemental Figure 2. *Erf*$^{ed/ed}$ embryos have no placenta defects. RNA in Situ hybridization on cryosections from E13.5 placentas with the cell type specific markers Tef5, 4311 that label the syncytiotrophoblast and the spongiotrophoblast respectively and from E12.5 placenta with the marker Pl1 that labels giant cells layer. Placentas of *Meox2cre;Erf*$^{floxP/-}$ concepti that nurture *Erf*$^{ed/ed}$ embryos appear comparable to their *Erf*$^{floxP/+}$ counterparts. sync: synciotrophoblast, sp: spongiotrophoblast, dec: decidua, gc: giant cells.



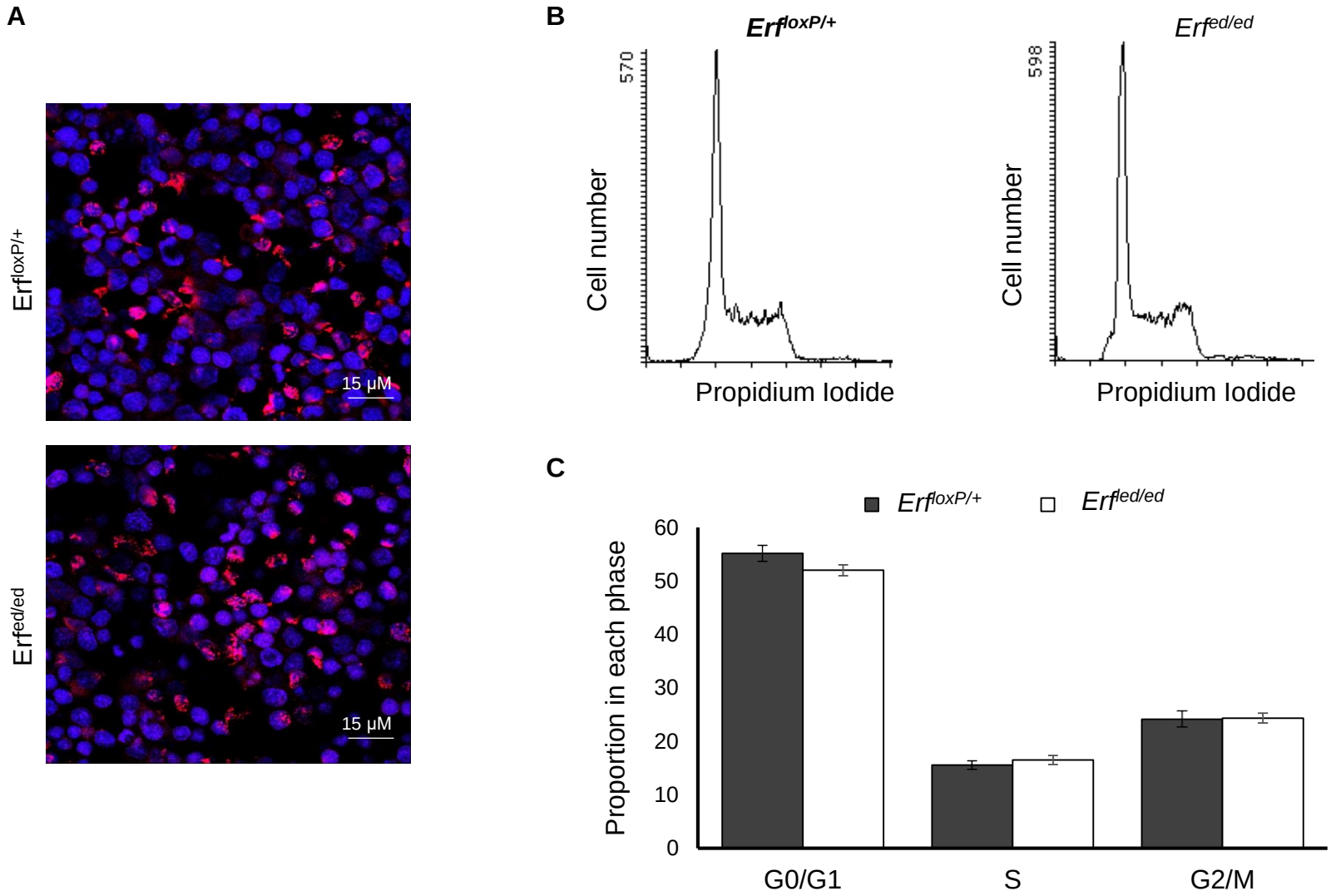
Supplemental Figure 3. Quantity differences in Erf expression. (A) Protein levels of Erf were determined by western blot from *Erf*$^{loxP/+}$, *Erf*$^{+/-}$ and *Erf*$^{ed/ed}$ E12.5 embryos. Erk1/2 antibody was used for normalization. All samples except for the last one were loaded in the same blot. **(B)** Quantification of Erf protein levels from *Erf*$^{loxP/+}$, *Erf*$^{+/-}$ and *Erf*$^{ed/ed}$ E12.5 embryos. Samples are represented as ratio to *Erf*$^{loxp/+}$ littermates after normalization with Erk1/2 quantities. **(C)** mRNA levels of Erf were determined in fetal livers of *Erf*$^{loxP/loxP}$, *Lyve1*$^{Cre/+}$ *Erf*$^{loxP/loxP}$ and *Lyve1*$^{Cre/Cre}$ *Erf*$^{loxP/loxP}$ E12.5 embryos as well in both livers and whole embryos of *Erf*$^{loxP/-}$ and *Erf*$^{ed/ed}$ at E12.5, employing the qPCR method. Samples were normalized to Gapdh mRNA levels and were quantified corresponding to the 2 loxP alleles. **(D)** Number of animals born with the *Lyve1*$^{Cre/+}$ *Erf*$^{loxP/loxP}$ or the *Lyve1*$^{Cre/+}$ *Erf*$^{loxP/loxP}$ genotype. Chi square test showed statistical important differences of the actual over expected numbers of total 100 mice from 16 different litters. All values are means ± SE of at least 5 samples from 5 litters**.** Statistical analysis was performed using the unpaired t-test with two-tailed distribution. *, $P < 0.05$, $P < 0.05$, ***, $P < 0.0005$.



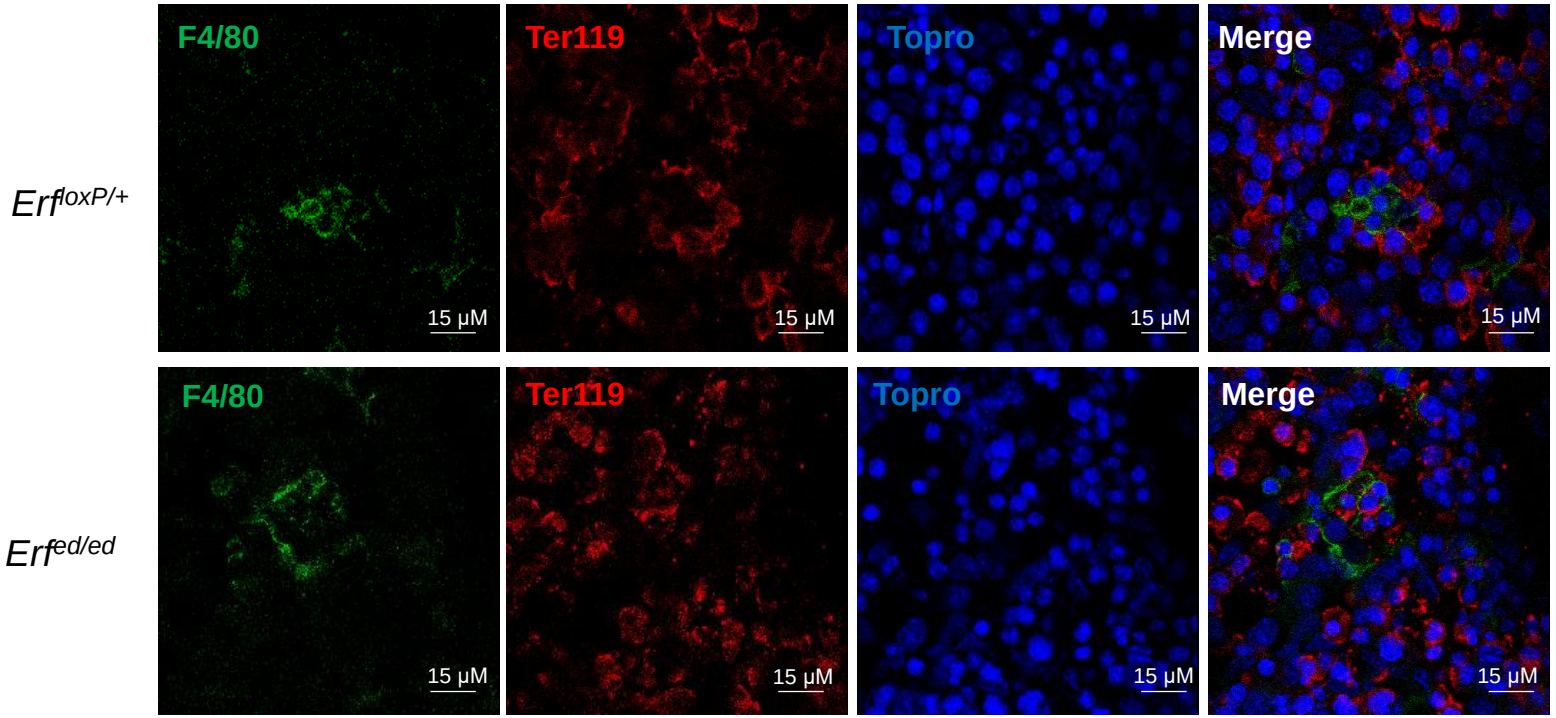
Supplemental Figure 4. *Erf*$^{ed/ed}$ livers have decreased numbers but increased proportion of BFU-Es and CFU-Es. **(A)** Sagittal sections from E11.5 *Erf*$^{ed/ed}$ and *Erf*$^{loxP/+}$ embryos stained with Hematoxylin and Eosin. s: stomach, l: liver, h: heart. **(B)** Proportion of BFU-E and **(C)** CFU-E at 11.5 – 13.5 d.p.c. Samples are represented as ratio to *Erf*$^{loxp/+}$ littermates. All values are means ± SE of samples from at least 6 litters per gestation day (Supplemental Table 4). Statistical analysis was performed using the unpaired t-test with two-tailed distribution. *, $P < 0.05$, ***, $P < 0.0005$.



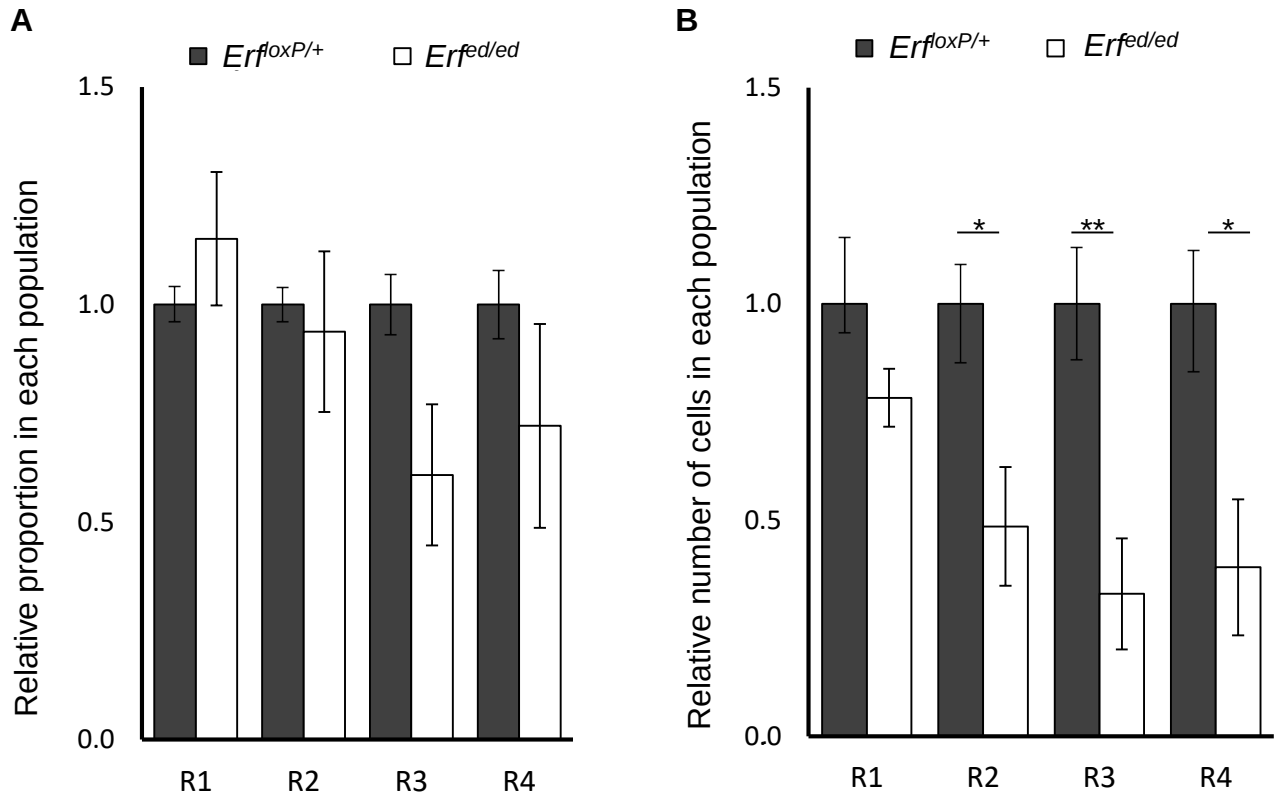
Supplemental Figure 5. *Erf* does not affect hepatic cells, growth rate or apoptosis. (A) Confocal microscopy images of sagittal sections of livers from E12.5 $Erf^{ed/ed}$ and $Erf^{loxP/+}$ embryos stained with the anti-Ter119 antibody for detection of the mature erythroblasts R3 – R5, anti-HNF4 antibody for hepatocytes (left panels), anti-CD71 antibody for the erythroblasts R1 – R4 and anti-pH3 antibody for proliferating cells (middle panels) or with In Situ cell dead kit TMR (Tunel) for detection of apoptotic CD71 cells (right panels). Nuclei were stained with TOPRO-3 (blue). **(B)** Proportion of Ter119, HNF4 and CD71 cells in E12.5 livers. The graph shows the values for $Erf^{ed/ed}$ and $Erf^{loxP/+}$ embryos, compared to the average value of the $Erf^{loxP/+}$ littermates. All values are means ± SE of at least 10 biological samples of each genotype from 8 litters. Statistical analysis was performed using the unpaired t-test with two-tailed distribution. ***, $P < 0.0005$. **(C)** Proportions of CD71 cells are positive for pH3 at E12.5 livers. The values for $Erf^{ed/ed}$ and $Erf^{loxP/+}$ embryos were compared to the average value of the $Erf^{loxP/+}$ littermates. All values are means ± SE of 4 biological samples of each genotype from 3 litters. *, $P < 0.05$. **(D)** Proportion of apoptotic cells (Tunel positive) at E12.5 livers. The values for $Erf^{ed/ed}$ and $Erf^{loxP/+}$ embryos were compared to the average value of the $Erf^{loxP/+}$ littermates. All values are means ± SE of at least 2 biological samples of each genotype from 2 litters. Statistical analysis was performed using the unpaired t-test with two-tailed distribution. *, $P < 0.05$.



Supplemental Figure 6. *Erf*$^{ed/ed}$ erythroblasts have normal cell cycle progression. (A) Confocal microscopy images of sagittal sections of livers from E12.5 *Erf*$^{ed/ed}$ and *Erf*$^{floxP/+}$ embryos stained with BrdU, a marker of proliferating cells (red). Nuclei were stained with TOPRO-3 (blue). **(B)** Representative flow cytometry profiles of cell cycle of E13.5 *Erf*$^{ed/ed}$ and *Erf*$^{floxP/+}$ fetal livers stained with propidium iodide. **(C)** Proportions of phases G0/G1, S and G2/M of cell cycle at E13.5 livers. All values are means ± SE of 6 biological samples of each genotype from 2 litters.



Supplemental Figure 7. *Erf*$^{ed/ed}$ have normal macrophages – erythroblasts contacts. Confocal microscopy images of sagittal sections of livers from E12.5 *Erf*$^{ed/ed}$ and *Erf*$^{loxP/+}$ embryos stained with anti-F4/80 antibody, a marker for macrophages (green) and Ter119, a marker for maturing erythroblasts (red). Nuclei were stained with TOPRO-3 (blue).



Supplemental Figure 8. *Erf*$^{ed/ed}$ have reduced progenitor erythroid cells at E11.5. (A) Proportions and **(B)** total cells of R1-4 populations from E11.5 embryonic liver cells. Liver cells were stained with anti-CD71 and anti-Ter119 antibodies and were analyzed by flow cytometry based on the dynamic expression of CD71 and Ter119 markers. The graphs show the values for *Erf*$^{ed/ed}$ and *Erf*$^{floxP/+}$ embryos compared to the average value of the *Erf*$^{floxP/+}$ littermates. All values are means ± SE of at least 6 biological samples of each genotype of at least 4 litters. Statistical analysis was performed using the unpaired t-test with two-tailed distribution. *, $P < 0.05$, **, $P < 0.005$. .

Supplemental Tables

Supplemental Table 1. Number of *Erf*$^{ed/ed}$ embryos in each gestation day, whose lethality was measured as shown in Figure 1A.

Embryonic day	No. of litters	No. of *Erf*$^{ed/ed}$ *embryos*	No. of total embryos
9.5	5	6	48
10.5	12	12	86
11.5	12	17	91
12.5	39	45	219
13.5	27	32	152
14.5	15	9	102
15.5	5	2	26
16.5	5	1	23
adult	38	0	248

Supplemental Table 2. Number of embryos, whose number of cells in bloodstream was counted in each gestation day (epiblast derived conditional mice) as shown in Figure 1C.

		No. of embryos for peripheral blood counts	
Embryonic day	litters	*Erf*$^{floxP/+}$	*Erf*$^{ed/ed}$
9.5	3	9	4
10.5	9	24	15
11.5	9	15	11
12.5	5	13	7
13.5	3	3	4
14.5	6	13	7

Supplemental Table 3. Number of liver samples in each gestation day and genotype, which was used for counting total cells in liver as shown in Figure 5A.

		No. of liver samples	
Embryonic day	litters	*Erf*$^{floxP/+}$	*Erf*$^{ed/ed}$
11.5	6	17	8
12.5	9	22	15
13.5	13	28	24

Supplemental Table 4. Number of samples used in each gestation day and genotype for counting proportion and numbers of BFU-E / CFU-E per liver as shown in Figure 5B, C and Supplemental figure 4B,C.

		No. of samples for BFU-E / CFU-E analysis	
Embryonic day	litters	*Erf*$^{floxP/+}$	*Erf*$^{ed/ed}$
11.5	3	6	5
12.5	5	10	9
13.5	4	9	8