PPAR-\(\alpha\) and glucocorticoid receptor synergize to promote erythroid progenitor self-renewal

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Many acute and chronic anaemias, including haemolysis, sepsis and genetic bone marrow failure diseases such as Diamond–Blackfan anaemia, are not treatable with erythropoietin (Epo), because the colony-forming unit erythroid progenitors (CFU-Es) that respond to Epo are either too few in number or are not sensitive enough to Epo to maintain sufficient red blood cell production1–5. Treatment of these anaemias requires a drug that acts at an earlier stage of red cell formation and enhances the formation of Epo-sensitive CFU-E progenitors. Recently, we showed that glucocorticoids specifically stimulate self-renewal of an early erythroid progenitor, burst-forming unit erythroid (BFU-E), and increase the production of terminally differentiated erythroid cells6–9. Here we show that activation of the peroxisome proliferator-activated receptor \(\alpha\) (PPAR-\(\alpha\)) by the PPAR-\(\alpha\) agonists GW7647 and fenofibrate synergizes with the glucocorticoid receptor (GR) to promote BFU-E self-renewal. Over time these agonists greatly increase production of mature red blood cells in cultures of both mouse fetal liver BFU-Es and mobilized human adult CD34+ peripheral blood progenitors, with a new and effective culture system being used for the human cells that generates normal enucleated reticulocytes. Although \(Ppara^{+/–}\) mice show no haematological difference from wild-type mice in both normal and phenylhydrazine (PHZ)-induced stress erythropoiesis, PPAR-\(\alpha\) agonists facilitate recovery of wild-type but not \(Ppara^{+/–}\) mice from PHZ-induced acute haemolytic anaemia. We also show that PPAR-\(\alpha\) alleviates anaemia in a mouse model of chronic anaemia. Finally, both in control and corticosteroid-treated BFU-E cells, PPAR-\(\alpha\) co-occupies many chromatin sites with GR; when activated by PPAR-\(\alpha\) agonists, additional PPAR-\(\alpha\) is recruited to GR-adjacent sites and presumably facilitates GR-dependent BFU-E self-renewal. Our discovery of the role of PPAR-\(\alpha\) agonists in stimulating self-renewal of early erythroid progenitor cells suggests that the clinically tested PPAR-\(\alpha\) agonists we used may improve the efficacy of corticosteroids in treating Epo-resistant anaemias.

The therapeutic effect of glucocorticoids in treating Epo-resistant anaemias such as Diamond–Blackfan anaemia (DBA) is well documented, although steroid therapy has severe side effects that limit its use10–13. Given the physiological importance and attractive drug targets of nuclear receptors (NRs)14–17, and in the hope of identifying other treatments of DEX in stimulating erythroid expansion of human CD34+ cells (Extended Data Fig. 2b). Addition of DEX and GW7647 does not interfere with DEX-promoted BFU-E self-renewal, indicating that DEX does not promote BFU-E self-renewal through activating the transcriptional activity of PPAR-\(\alpha\) (Extended Data Fig. 1g).

Adding GW7647 increases total cell number by an additional fourfold, yielding a 120,000-fold expansion in our newly developed synchronized human CD34+ erythroid culture system (Supplementary Discussion and Fig. 2a). In these cultures the numbers of BFU-Es and CFU-Es are highest during days 5–8 (Fig. 2b and Extended Data Fig. 2a). Addition of GW7647 with DEX increased total BFU-E numbers, which in turn subsequently increased total CFU-E numbers in the culture. Similar to mouse BFU-E cultures, GW7647 synergized with very low concentrations of DEX in stimulating erythroid expansion of human CD34+ cells (Extended Data Fig. 2b). Addition of DEX and GW7647 affects expression of many key erythroid genes (Supplementary Discussion).

As expected, knocking down PPAR-\(\alpha\) abrogated the ability of GW7647 to stimulate production of BFU-E cells or the total number of erythroid cells at the end of the culture (Fig. 2c and Extended Data Fig. 2b). The therapeutic effect of glucocorticoids in treating Epo-resistant anaemias such as Diamond–Blackfan anaemia (DBA) is well documented, although steroid therapy has severe side effects that limit its use10–13. Given the physiological importance and attractive drug targets of nuclear receptors (NRs)14–17, and in the hope of identifying other treatments...
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BFU-E cells were cultured in DEX the indicated treatment. Cell numbers were counted every 3 days. (right) BFU-E cells from embryonic day (E)14.5 mouse fetal livers were isolated with GR to promote BFU-E self-renewal during erythroid differentiation. Thus, in the human as well as in the mouse, only ligand-activated, not unactivated, PPAR-α agonists to stimulate red cell production in a mouse model of chronic anaemia, ‘neonatal anaemia’ (Nan) mice (Supplementary Discussion). While BFU-E numbers in spleens of Nan/+ mutant mice are similar to or slightly higher than that in wild-type mice, the average number of BFU-Es in the spleens of GW7647-injected Nan/+ mutant mice are higher than that in untreated mice (Extended Data Fig. 5b). Importantly, GW7647 injection increased haemoglobin level, haematocrit and red blood cell numbers in these anaemic mice (Fig. 3b), suggesting that the PPAR-α agonist alleviates anaemia in Nan/+ mutant mice by increasing BFU-E numbers in the spleen. GW7647 does not have any significant effects on either platelet or white blood cell numbers in peripheral blood from Nan/+ mice (Extended Data Fig. 5c).

To understand the molecular mechanism by which PPAR-α synergizes with GR to promote BFU-E self-renewal, we conducted chromatin immunoprecipitation followed by sequencing (ChIP-seq) recaptures RPS19 haploinsufficiency in DBA patients2,12 (Extended Data Fig. 3a). Addition of GW7647 substantially increased the fraction of CD71<sup>+</sup> cells at day 9 of culture after RPS19 knockdown as well as total cell numbers and the fraction of CD235α<sup>+</sup> cells at day 21 (Extended Data Figs 3b, c). Taken together, our data suggest that GW7647 facilitates BFU-E self-renewal and production of immature erythroid progenitors in both wild-type and RPS19-knockdown human cells.

We next tested the function of GW7647 in a PHZ-induced haemolytic anaemia mouse model in which the endogenous corticosteroid level becomes markedly increased<sup>11</sup>. Wild-type mice were treated with either dimethylsulfoxide (DMSO) or GW7647 for 3 days followed by treatment with PHZ; they were then injected with DMSO or GW7647 for another 7 days (Supplementary Discussion and Extended Data Fig. 4a). Treatment with GW7647 resulted in significantly higher levels of haemoglobin, red blood cell numbers, and haematocrit after PHZ injection compared to in control mice (Fig. 3a). In contrast, white blood cell counts were similar in the two groups (data not shown). Importantly, the function of GW7647 during stress erythropoiesis was dependent on PPAR-α, as Ppara<sup>−/−</sup> mice failed to respond to GW7647 treatment (Extended Data Fig. 4b).

We also tested the ability of PPAR-α agonists to stimulate red cell production in a mouse model of chronic anaemia, ‘neonatal anaemia’ (Nan) mice (Supplementary Discussion). While BFU-E numbers in spleens of Nan/+ mutant mice are similar to or slightly higher than that in wild-type mice, the average number of BFU-Es in the spleens of GW7647-injected Nan/+ mutant mice are higher than that in untreated mice (Extended Data Fig. 5b). Importantly, GW7647 injection increased haemoglobin level, haematocrits and red blood cell numbers in these anaemic mice (Fig. 3b), suggesting that the PPAR-α agonist alleviates anaemia in Nan/+ mutant mice by increasing BFU-E numbers in the spleen. GW7647 does not have any significant effects on either platelet or white blood cell numbers in peripheral blood from Nan/+ mice (Extended Data Fig. 5c).

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Figure 2 | Activation of PPAR-α signalling increased erythroid cell expansion in the ex vivo human CD34<sup>+</sup> erythroid culture system. a. Human CD34<sup>+</sup> cells were cultured as described in Methods. Total cell numbers were quantified by plating 1,000 cells at various time points during days 0–9 of the human CD34<sup>+</sup> erythroid culture on methylcellulose. b. BFU-E numbers (left) or total cell number (right) from control or PPAR-α-knockdown human CD34<sup>+</sup> cells cultured under the indicated condition were counted. shRNA, short hairpin RNA. c. Benzidine–Giemsa staining demonstrating cell morphology of the ex vivo human CD34<sup>+</sup> erythroid differentiation system. *P < 0.05, **P < 0.01, Student's t-test. Data are mean ± s.d. from three biological replicates.

Figure 1 | PPAR-α signalling synergizes with GR to promote self-renewal of mouse BFU-E erythroid progenitors. a. Wild-type (WT; left) and Ppara<sup>−/−</sup> (right) BFU-E cells from embryonic day (E)14.5 mouse fetal livers were isolated and cultured in serum-free erythroid liquid expansion (SFELE) medium with the indicated treatment. Cell numbers were counted every 3 days. b. Mouse BFU-E cells were cultured in DEX ± 10 µM GW7647 as indicated. Total erythroid cell numbers were counted at day 9. c. Colony-forming assays were conducted to determine BFU-E colony numbers from 100 mouse BFU-E cells cultured under the indicated conditions. Colony-forming assays were performed at 24-h intervals. Data are mean ± s.d. from three biological replicates; *P < 0.05, **P < 0.01, ***P < 0.001, Student’s t-test.

Data Fig. 2c). Consistent with the absence of a role for PPAR-α in normal haematopoiesis, knocking down PPAR-α in human CD34<sup>+</sup> cells did not affect the ability of DEX to stimulate BFU-E production or production of erythroid cells. Thus, in the human as well as in the mouse, only ligand-activated, not unactivated, PPAR-α synergizes with GR to promote BFU-E self-renewal during erythroid differentiation. We note that our cultures are highly synchronous on the basis of cell surface marker expression (Extended Data Fig. 2e) as well as morphology (Fig. 2d). Neither DEX nor GW7647 had any effects on terminal differentiation or enucleation (Extended Data Fig. 2h).

GW7647 significantly increased the number of both BFU-E and CFU-Es in our CD34<sup>+</sup> cell culture system after RPS19 knockdown, which
analysis to interrogate the genome-wide chromatin occupancy of GR and PPAR-α in mouse BFU-E cells. Compared to untreated cells, GR and PPAR-α occupancy were both induced upon DEX treatment alone; addition of GW7647 further enhanced PPAR-α but not GR occupancy (Fig. 4a). These results indicate that GW7647 enhances the recruitment of PPAR-α to GR-binding sites in BFU-Es. In BFU-Es co-treated with GW7647 and DEX, our ChIP-seq detected 1,058 GR and 1,623 PPAR-α peaks. GR and PPAR-α both predominately occupied distal intergenic (>63%) and intronic chromatin sites (>25%) (Extended Data Fig. 6a). Notably, in 719 peaks, GR and PPAR-α localize in close proximity (Extended Data Fig. 6b); 67.9% of total GR and 44.3% of PPAR-α peaks co-localize. The DNA sequences underlying these overlapping peaks are enriched for DNA-binding motifs for several transcription factors including PU.1, YY1, Smad3, Tal1, Klf4, Hif-2α and Myb (Extended Data Fig. 6c); most of these transcription factors are known to have crucial roles in stem-cell self-renewal. In addition, co-treatment with DEX and GW7647 also up-regulates many genes critical for stem-cell self-renewal (Supplementary Discussion and Extended Data Figs 7, 8).

DEX treatment of BFU-E cells leads to a slight increase in PPAR-α protein levels, and this is further increased to 1.6 times that of control cells by treatment with DEX and GW7647 (Fig. 4c). In contrast, the GR protein level is not altered in BFU-Es under these conditions. Consistent with these observations, addition of DEX to BFU-E cells induced the binding of both GR and PPAR-α to a chromatin site ~5 kb upstream of the transcription start site of Ppara, presumably part of a Ppara gene enhancer, and the binding of PPAR-α to this chromatin site was further enhanced by GW7647 addition (Fig. 4b). Our data suggest that there is a positive autoregulatory feedback loop of PPAR-α expression during BFU-E self-renewal promoted by DEX and GW7647.

Co-immunoprecipitation experiments demonstrated an interaction of GR and PPAR-α only in BFU-E cells treated with DEX with or without GW7647 (Fig. 4d). While addition of DEX to cultures of BFU-E cells stimulated PPAR-α binding to the GR, interactions between PPAR-α and GR were more pronounced after treatment with both DEX and GW7647. These results extend our ChIP-seq data, indicating that there is a physical interaction between GR and PPAR-α and probably other proteins that underlies DEX- and GW7647-enhanced BFU-E self-renewal (Supplementary Discussion and Extended Data Fig. 9).

Given that little is known concerning endogenous PPAR-α ligands, the precise role of PPAR-α in haematopoiesis, and erythropoiesis in particular, remains elusive. Nonetheless, our finding that agonists of PPAR-α lead to enhanced binding of PPAR-α to many chromatin sites and to an increase in corticosteroid-induced BFU-E self-renewal and erythroid expansion, point to a previously unappreciated role for this nuclear receptor in haematopoiesis (Extended Data Fig. 10). The function of PPAR-α has been mainly studied in nutrient metabolism and energy homeostasis, and fenofibrate is already an FDA-approved drug for dyslipidaemia treatment. Given that there is a very limited number of drugs that can be used to treat Epo-resistant anaemias, the development of new drugs or repurposing current drugs to treat these diseases is challenging. Our surprising discovery suggests a novel function of PPAR-α in self-renewal of early committed erythroid progenitors, which could potentially lead to new therapeutics to treat Epo-resistant anaemias such as DBA.
Online Content Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.

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**Author Contributions** H.-Y.L., X.G., L.L.P. and H.F.L. designed the experiments. H.-Y.L., X.G. and R.R.E. performed the experiments. M.I.B. and H.L. conducted bioinformatic analyses of ChIP-seq and RNA-seq. H.-Y.L., X.G. and H.F.L. wrote the manuscript with input from M.I.B. All authors discussed the results and commented on the manuscript.

**Author Information** RNA-seq and ChIP-seq data have been deposited in the Gene Expression Omnibus under accession numbers GSE63836 and GSE63837, respectively. Reprints and permissions information is available at www.nature.com/ reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to H.F.L. (lodish@wi.mit.edu).
METHODS

Reagents. Chemicals were obtained from Sigma. Human peripheral blood granulocytes—colonies stimulating factor (G-CSF)-mobilized haematopoietic stem/progenitor cells enriched for CD34+ were purchased from the Fred Hutchinson Cancer Research Center (FHCRC). StemSpan SFEM, CC100 cytokine cocktail, fetal bovine serum and BSA were purchased from STEMCELL Technologies. Holo human transferrin was from Regen. Recombinant human and murine stem cell factor (rhSCF, rmSCF) and interleukin-3 (rhIL-3, rmIL-3), recombinant murine interleukin-6 (rmIL-6) and recombinant murine insulin like growth factor-1 (rmIGF-1) were from Peprotech. Recombinant human erythropoietin (rhEpo) was from Amgen. Antibodies used were as follows. Santa Cruz: GR: H300 (sc-8992), M-20 (sc-11078), anti-human CD71 (sc-1004); PPAR-α (sc-9000). The number of reads mapped to each gene was calculated with the closestBed tool30. We selected peaks with fold enrichment ≥ 5 and a minimum length to keep 25 nucleotides (nt) of DNA. The closest gene using the closestBed tool30. We selected peaks with fold enrichment ≥ 5 and a minimum length to keep 25 nucleotides (nt) of DNA. The closest gene using the closestBed tool4. We selected peaks with fold enrichment ≥ 5 and a minimum length to keep 25 nucleotides (nt) of DNA. The closest gene using the closestBed tool.6. ChIP-PCR complexes were collected by incubation with protein A-Sepharose for 4 h at 4 °C. Loss-of-function assay in human CD34+ erythroid culture system. The lentiviral backbone vector plKO.1 and packaging plasmids were transfected into 293T cells. Supernatants containing viral particles were harvested at 48 and 72 h. Human primary CD34+ haematopoietic cells were transduced with lentivirus 1 day after thawing with the presence of 2 μg ml−1 polybrene (Sigma). Twenty four hours after viral transduction, cells were selected by growing in culture medium containing 1 μg ml−1 puromycin (Sigma) for 2 days. For the following experiments at 8 days, cells were infected with lentivirus encoding GFP and a shRNA targeting human PPAR-α (ref. 21) or a scrambled shRNA. Cells were then treated with DMSO or 0.01 μM, 0.1 μM or 1 μM GW7647. After 48 h, GFP-positive cells were isolated by flow cytometry and returned to culture. Colony-forming assays were conducted at day 6. CD71 expression was analysed at day 9 and CD235 expression was analysed at day 21 to determine the percentage of erythroid cells. Total cell numbers were also counted at the end of each differentiation stage. ChIP-seq and de novo motif discovery. Mouse ChIP-seq experiments in BFU-Es were conducted as described before24. 106 mouse BFU-E cells purified from E14.5 fetal liver with or without treatment were used for immunoprecipitation. Besides specific antibodies, we included species-matched IgG as control, and species-matched IgG yielded little to no signals. Purified DNA was sequenced according to a modified version of the Solexa genomic DNA protocol. ChIP-seq fragments as well as inputs were barcode and sequenced on an Illumina HiSeq sequencer. Approximately 30 million 40-nucleotide-long single reads per sample was obtained. Adapter sequences and reads shorter than 20 nucleotides were discarded. Reads were mapped with Bowtie1 (ref. 25). Peaks were called with MACS 1.4 using the corresponding input for each sample and “mfold” set to 5.0 (ref. 26). We selected peaks with fold enrichment ≥ 10. Plots showing density of reads around the peak summits were done with ngsplot. For de novo motif discovery, Homer was used with default settings to find motifs in GR and PPAR-α overlapping peaks27. RNA-seq. BFU-E cells from E14.5 mouse fetal livers were isolated as described29. Total RNA was purified from the mouse BFU-Es. Samples for paired-end mRNA-seq were prepared using the Solexa kit according to the manufacturer’s instructions. RNA samples from two replicates of cells untreated, or treated with DEX ± GW7647 for 12 h were sequenced on an Illumina HiSeq sequencer. Around 50 million 100-nucleotide paired-end reads were obtained from each sample. Reads were trimmed to remove low quality reads using FASTQ qualify trimmer with a quality threshold of 20. Reads that still had both pairs after the trimming step were mapped with TopHat2 using gene models from ENSEMBL Genes 67, Mus musculus genes NCBIM37 (Mus musculus.NCBIM37.67). The number of reads mapped to each gene was calculated with HTseq-count. Differential expression was assayed using DEseq230. For each of the 719 peaks bound by both GR and PPAR-α in the presence of GW7647 and DEX, we found the closest gene using the closestBed tool20. We filtered out any gene at a distance higher than 10 kb from a peak.

Immunoprecipitation. Mouse BFU-Es were isolated from E14.5 mouse fetal livers. Cells were cultured in SFEM medium alone or treated with 100 nM DEX or with or without 100 nM GW7647 for 12 h. Whole-cell lysates were prepared from 4 × 106 cells using RIPA lysis buffer (sc-24948, Santa Cruz Biotechnology) with protease inhibitor cocktail. The lysates were pre-cleared by incubating with protein A-Sepharose for 1 h at 4 °C and centrifugation. The supernatant was immunoprecipitated with 1 μg rabbit IgG or anti-GR antibody overnight at 4 °C. Immune complexes were collected by incubation with protein A-Sepharose for 4 h at 4 °C
and washed five times at 4 °C with lysis buffer. The immune complexes adsorbed to the beads were centrifuged and the supernatant was removed. Fifty microlitres of 1× loading buffer was added to the samples and boiled at 95 °C for 5 min. Proteins were resolved by SDS–PAGE and immunoblotted by GR and PPAR-α antibodies.

**Quantitative real-time RT-PCR.** Total RNA from mouse BFU-Es was purified with TRIzol (Invitrogen). cDNA was prepared from 1 μg RNA. Reaction mixtures (15 μl) contained 2.0 μl of cDNA, 7.5 μl of SYBR green master mix (Applied Biosystems) and appropriate primers. Product was monitored by SYBR green fluorescence. Control reactions lacking RT yielded little to no signal. Relative expression levels were determined from a ΔΔCt method and were normalized to 18S rRNA expression.

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Fold change (DEX vs. Untreated)

-3 -2 -1 0 1 2 3 4 5 6

Ppara
Rora
Rxrg
Mr
Vdr
Coup-TFI
Lhx-1
Rorb
Shp
Nurr1

**a**

**b**

**c**

**d**

**e**

**f**

**g**

**h**

Total BFU-E colony numbers from 100 BFU-E cells

**b**

**c**

**c**

**d**

**e**

**f**

**g**

**h**

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Extended Data Figure 1 | PPAR-α agonist GW7647 does not have adverse effects on erythroid differentiation and has no effects on CFU-E cells.

**a**, Gene expression changes of nuclear receptors in BFU-E cells from RNA-seq results published previously.**b**, Flow cytometry analyses of CD71 and Ter119 markers to demonstrate erythroid differentiation of mouse BFU-E cells after 9 days of culture with the indicated additions. **c**, Ppara gene expression in BFU-E, CFU-E and Ter119 erythroblasts. BFU-E, CFU-E and Ter119 erythroblasts were isolated from E14.5 mouse fetal livers as described. Total RNA was purified for quantitative PCR analysis. **d**, DNase I hypersensitivity (HS) analysis at Ppara promoter region in different mouse cells from Encode. **e**, Production of mouse erythroblasts from isolated CFU-E cells. Wild-type mouse CFU-E cells from E14.5 fetal livers were untreated (black line) or treated with DEX (blue line), GW7647 (red line) or fenofibrate (green line). Error bars represent mean ± s.d. from three independent experiments. **f**, Colony-forming assays were conducted at 48 h after compound treatment to determine BFU-E colony numbers from 100 mouse BFU-E cells cultured under the indicated conditions. *P < 0.05, Student’s t-test. Error bars represent mean ± s.d. from three independent experiments. **g**, At day 3, BFU-E colony numbers from 100 purified mouse BFU-E cells were quantified by colony forming assays. 100 purified mouse BFU-E cells were untreated or treated with DEX alone or DEX in combination with agonists or antagonists targeting PPAR receptors (α, γ or β). BFU-E colonies were quantified after 8 days in culture. *P < 0.05, **P < 0.01, Student’s t-test. Error bars represent mean ± s.d. from three independent experiments. **h**, Real-time PCR analysis of gene expression in DEX-treated and DEX + GW7647–treated wild-type or Ppara mice BFU-E cells. *P < 0.05, ***P < 0.001, Student’s t-test. Error bars represent mean ± s.d. from three independent experiments.
Extended Data Figure 2  | Human CD34⁺ erythroid differentiation system.

a, Total CFU-E colonies formed during days 0–9. CFU-E colony numbers were quantified by plating 1,000 cells from various time points during days 0–9 of the human CD34⁺ erythroid culture on methylcellulose. CFU-E colonies were quantified after 12–14 days. Total CFU-E colony numbers in culture under conditions without GW7647 (black line) or with GW7647 (red line) were calculated using the total cell numbers at corresponding time points in Fig. 2a.

b, Human CD34⁺ cells were treated at day 1 with 100 nM GW7647 with or without DEX at the concentration indicated in the figure. At day 6, total cell numbers were counted and cells were collected for BFU-E colony assays.

c, Protein expression of PPAR-α demonstrating shRNA knockdown efficiency via lentiviral transduction. LacZ shRNA is used as a control. shRNA-1 and -2 are both specific for PPAR-α. shRNA-2 has higher knockdown efficiency.

d, Cell pellets of 1 million cells demonstrating haemoglobin accumulation during the differentiation process. e, Flow cytometry analyses of erythroid markers during the 21-day human CD34⁺ erythroid culture. Top, c-KIT versus CD235a; middle, CD71 versus CD235a. Note the sequential induction of c-kit, CD71 and CD235a, as well as the sequential downregulation of c-kit and CD71. Bottom, enucleated reticulocytes are CD235a⁺ Hoechst⁻, nuclei are CD235a⁻ Hoechst⁺, and nucleated erythroblasts are CD235a⁺ Hoechst⁺. Enucleation rate is 32.6/(32.6 + 37.7) × 100% = 46.4%.

f, Summary of high-performance liquid chromatography (HPLC) results using haemolysates of cultured reticulocytes and normal human RBCs (control). Top, total protein composition of haemolysates. Bottom, haemoglobin composition of haemolysates. Cultured reticulocytes contain more than 90% of adult globins.

g, Size measurement of enucleated reticulocytes by both diameter and area. Scale bar, 10 μm.
h, Benzidine–Giemsa staining of human reticulocytes cultured with or without GW7647. Scale bar, 12 μm. *P < 0.05, Student’s t-test.

Error bars represent mean ± s.d. from three independent experiments.
Extended Data Figure 3 | GW7647 increases erythroid progenitors and CD235a⁺ cells in RPS19-knockdown human progenitor cells. a, Human CD34⁺ haematopoietic progenitors were transduced with lentivirus encoding GFP and either a scrambled shRNA or an shRNA targeting RPS19. Then transduced cells were treated with or without 100 nM GW7647. After 48 h, GFP⁺ cells were sorted by FACS and plated for BFU-E and CFU-E colony-forming assays. RPS19 knockdown efficiency is shown at the bottom. M.W., molecular weight. *P < 0.05, Student’s t-test. Error bars represent mean ± s.d. from three independent experiments. b, Sorted GFP⁺ cells were returned to culture with the indicated concentration of GW7647. Top, percentage of CD71⁺ cells at day 9 was determined by FACS. Bottom, percentage of CD235a⁺ cells at day 21 was determined by FACS. *P < 0.05, Student’s t-test. Error bars represent mean ± s.d. from three independent experiments. c, Total cell numbers generated from one GFP-positive cell at the indicated times of culture. *P < 0.05, Student’s t-test. Error bars represent mean ± s.d. from three independent experiments.
a
GW7647 or DMSO
Day
-3 -2 -1 0 1 2 3 4 5 6 7 8 9

b
HGB
Ppara^-/-
RBC
HCT

Day
Day
Day

Day

b

[Graphs showing changes in HGB, RBC, and HCT levels over time (Day 0 to 9).]

[Graphs showing changes in colony numbers (10^6 cells) in Spleen and Bone marrow under different conditions: WT: PHZ + DMSO, Ppara^-/-: PHZ + DMSO, WT: PHZ + GW7647, Ppara^-/-: PHZ + GW7647.

D

[Flow cytometry plots showing changes in CD71 and Ter119 expression under different conditions: WT: PHZ + DMSO, WT: PHZ + GW7647.]
Extended Data Figure 4 | GW7647 improves anaemia in two mouse models of anaemia. a, Experimental scheme for PHZ-induced haemolytic anaemia, used also in Fig. 3a. Wild-type or Ppara2/2 mice were pretreated with DMSO (control) or GW7647 (100 μg kg−1) for 3 days (days −3 to −1) before PHZ injection on day 0. Mice were subject to daily DMSO or GW7647 injections during days 0–6. Red arrows indicate days of blood sample collection. b, Ppara2/2 mice were treated with DMSO or GW7647 and then injected with PHZ. Haemoglobin (HGB), red blood cell numbers (RBC) and haematocrit (HCT) were measured on the days indicated. Error bars represent mean ± s.d. from six mice. c, BFU-E and CFU-E colony-forming assays of spleen or bone marrow cells. Wild-type or Ppara2/2 mice were treated with PHZ and DMSO (control) or GW7647 (100 μg kg−1) as described in earlier. *P < 0.05, Student’s t-test. Error bars represent mean ± s.d. from three independent experiments. d, Spleen and bone marrow cells were harvested. Representative flow cytometry analysis of spleen erythroblasts isolated from GW7647- or DMSO-treated wild-type mice at day 3 after PHZ injection. FSC-A, forward scatter area.
Extended Data Figure 5 | GW7647 increases BFU-E numbers in Nanl/− mutant mice. a. Corticosteroid levels in serum were measured in wild-type and Nanl/− mutant mice. Each dot represents one mouse. *P < 0.05, Student’s t-test. Error bars represent mean ± s.d. from all mice. b, Increase of BFU-E numbers in spleens from GW7647-treated wild-type and Nanl/− mutant mice at day 18. *P < 0.05, Student’s t-test. c, Total numbers of white blood cells (WBC) and platelets from peripheral blood samples were measured at day 0 and day 18. Each dot represents one mouse. Error bars represent mean ± s.d. from all mice.
Extended Data Figure 6 | Bioinformatic analyses of mouse BFU-E cells.

a, Genome-wide distribution of GR and PPAR-α chromatin occupancy sites in BFU-E cells. ChIP-seq analyses of GR and PPAR-α occupancy in mouse BFU-E cells isolated from DEX- and GW7647-treated wild-type E14.5 fetal livers. TSS, transcription start site; TTS, transcription termination site; UTR, untranslated region. Distal intergenic, regions greater than 3 kb from TSS. b, Venn diagram showing overlap between GR and PPAR-α chromatin occupancy sites. c, De novo motif searching of the overlapped chromatin sites occupied by GR and PPAR-α. The table depicts transcription-factor-binding motifs enriched at GR and PPAR-α overlapping sites relative to genomic background and associated P values. d, Real-time PCR analysis of Pu.1 gene expression in mouse BFU-E cells transduced with virus encoding either LacZ shRNA or Pu.1 shRNA. *P < 0.05, Student’s t-test. Error bars represent mean ± s.d. from three independent experiments. e, Colony-forming assays were conducted to determine BFU-E colony numbers from 100 mouse BFU-E cells infected with virus encoding either LacZ shRNA or Pu.1 shRNA. Cells were cultured in SFELE medium with or without DEX ± GW7647. Colony-forming assays were performed at 48 h. *P < 0.05, Student’s t-test. Error bars represent mean ± s.d. from three independent experiments.

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Extended Data Figure 7 | Kit is a target gene of GR and PPAR-α. a, Real-time RT–PCR analysis of Kit gene expression in wild-type and Ppara-/- mouse BFU-E cells untreated or treated with DEX with or without the addition of GW7647. *P < 0.05, Student's t-test. Error bars represent mean ± s.d. from three independent experiments. b, Human CD34+ cells were treated with or without GW7647 as described in Fig. 2. Top, at day 9 of culture, cell-surface KIT and CD71 expression were analysed by flow cytometry. Bottom, a representative histogram of KIT expression in cells treated or untreated with GW7647. c, ChIP-seq occupancy signal map of GR and PPAR-α across the Kit locus in BFU-E cells.
Extended Data Figure 8 | Pathways modulated by PPAR-α activation. Pathway analysis of RNA-seq data of genes that are up- or downregulated by more than 50%, comparing cultures treated with DEX alone or DEX+GW7647.
Extended Data Figure 9 | PPAR-α antagonist interferes with the function of PPAR-α but not GR. 

**a.** Quantitative ChIP analysis of GR and PPAR-α occupancy at Kit and Ppara loci in mouse BFU-E cells following the indicated treatments. Units are arbitrary; signals using rabbit IgG are represented by grey dot lines across the plots. *P < 0.05, **P < 0.01, Student’s t-test. Error bars represent mean ± s.d. from three independent experiments. 

**b.** Co-immunoprecipitation (IP) measuring interaction between GR and PPAR-α in mouse BFU-E cells isolated from E14.5 fetal livers in wild-type or Ppara−/− mice. BFU-E cells were untreated, or treated with DEX with or without GW7647 with or without GW6471. Whole-cell lysates were incubated with anti-GR antibody and immunoprecipitates were probed with specific antibodies as indicated. M.W., molecular weight. 

**c.** Colony-forming assays to determine BFU-E colony numbers from 100 mouse BFU-E cells cultured with the indicated treatments. *P < 0.05, Student’s t-test. Error bars represent mean ± s.d. from three independent experiments.
Extended Data Figure 10 | Model of synergism between PPAR-α and GR to promote BFU-E self-renewal. BFU-E cells normally undergo limited self-renewal to generate CFU-Es, which can differentiate into erythroblasts. GR is sequestered in the cytoplasm without glucocorticoids such as DEX. Upon glucocorticoid treatment, liganded GR will be translocated into the nucleus and bind to chromatin to regulate gene transcription important for BFU-E self-renewal. PPAR-α is often recruited to chromatin sites that are in close proximity to GR by glucocorticoid treatment alone without any function on BFU-E self-renewal. Upon glucocorticoid and PPAR-α agonist co-treatment, activated PPAR-α interacts with GR to modulate GR transcriptional activity. This leads to enhanced BFU-E self-renewal, and over time generates more CFU-Es and erythroblasts.