

SUPPLEMENTAL MATERIAL

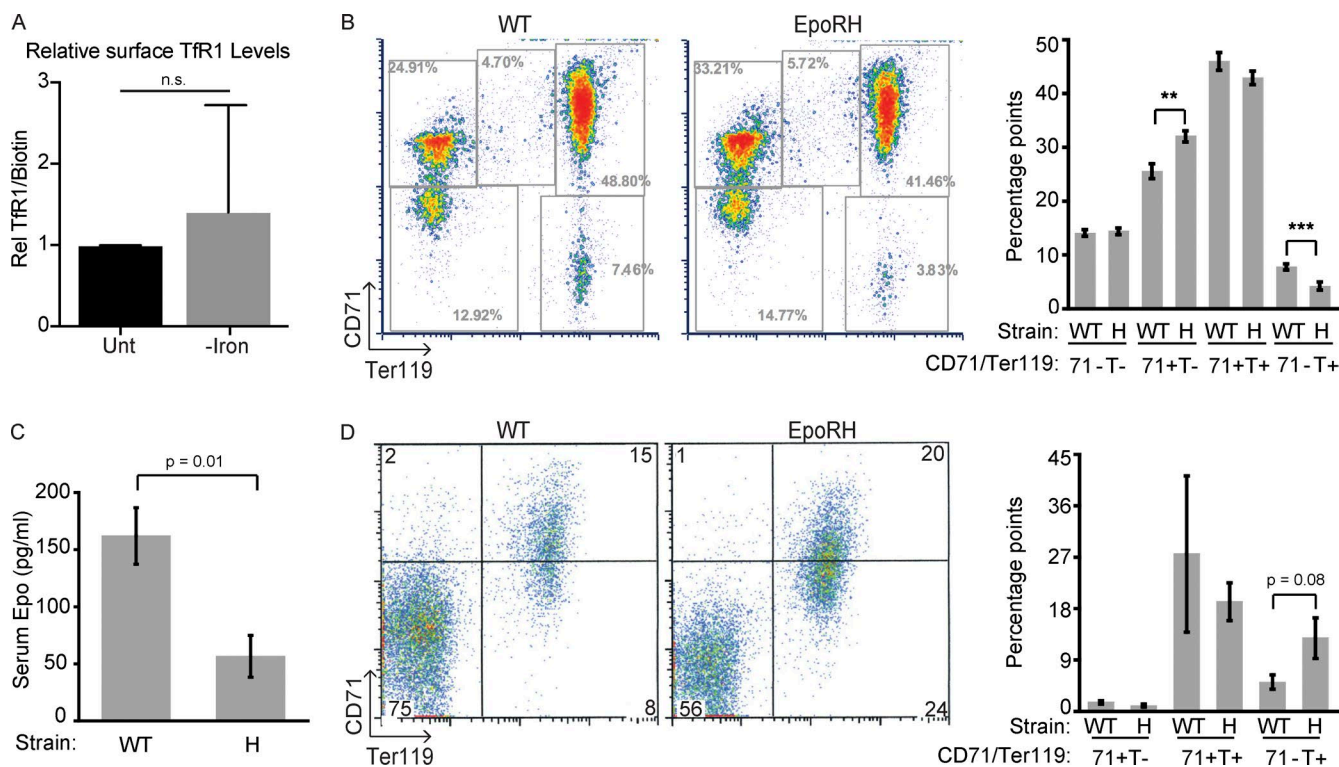
Khalil et al., <https://doi.org/10.1084/jem.20170396>

Figure S1. **Impact of erythroid iron deprivation on surface TfR1 and further characterization of *EpoR-H* animals.** (A) Human erythroid progenitors cultured \pm iron deprivation underwent surface biotinylation, streptavidin pull-down, immunoblot, and scanning densitometry exactly as in Fig. 1 (A and B). The graph depicts relative surface TfR1 levels normalized to total biotinylated protein ($n = 3$, Student's t test). (B) Flow cytometry plots for marrow erythroid maturation of iron-replete WT and *EpoR-H* mice. Spleens were similarly analyzed but showed no detectable erythropoiesis in either group. The graph summarizes maturation stages from the four corner quadrants of the plots ($n = 5$ per group, Student's t test). (C) Serum Epo levels in same animals as in B ($n = 5$ per group, Student's t test). (D) FACS plots of splenic erythroid maturation in iron-deficient (6 wk on low iron diet) WT and *EpoR-H* mice. The graph summarizes maturation stages from four quadrants ($n = 5$ for WT and 6 for *EpoR-H* [1 WT was eliminated as an outlier based on Grubb's test], Student's t test). Note that marrows from iron-deficient animals showed no differences between strains. **, $P < 0.01$; ***, $P < 0.005$; n.s., not significant. H, *EpoR-H* mice. Graphs depict mean \pm SEM from the indicated number of independent experiments.

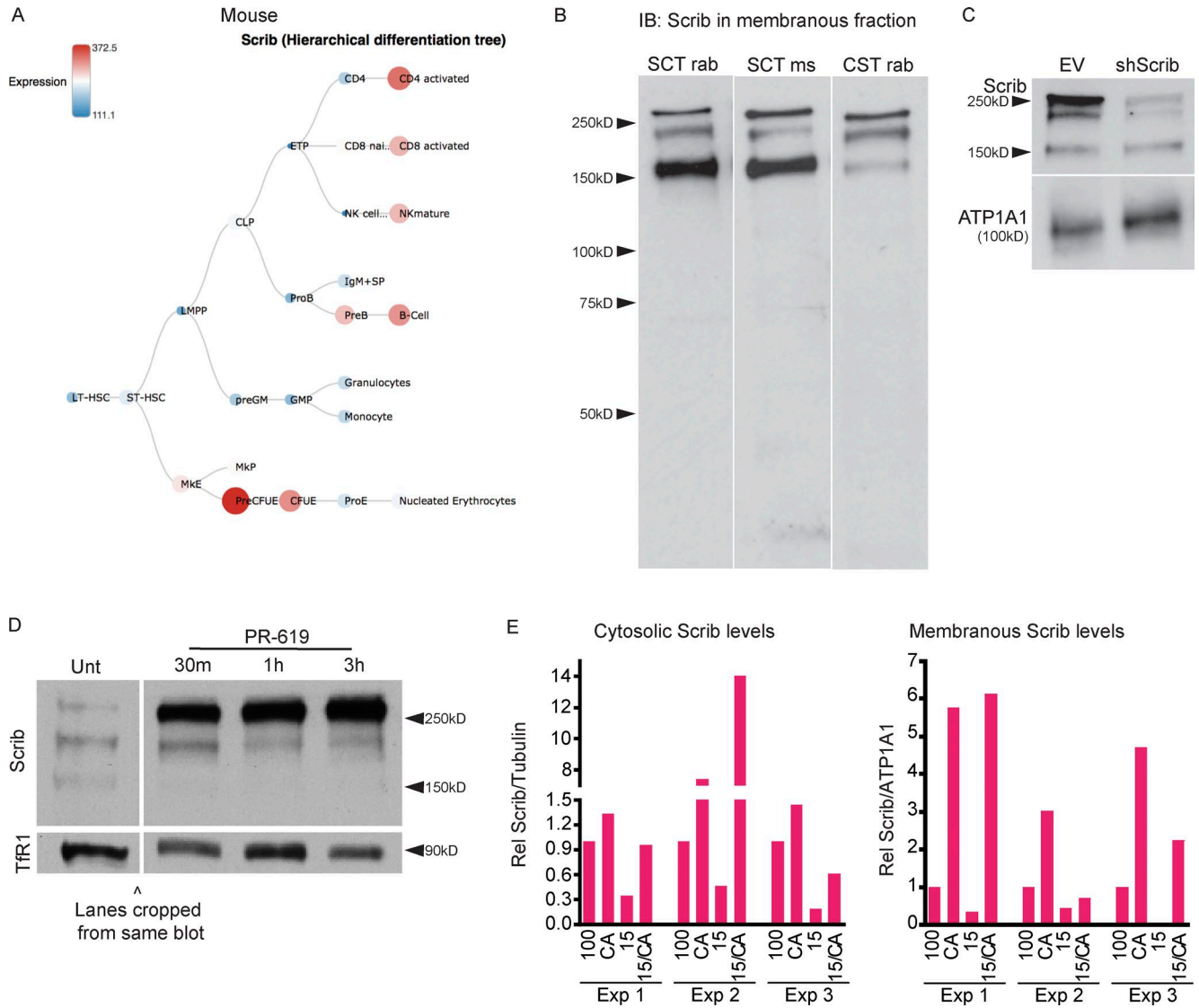


Figure S2. **Scrib** expression in mouse hematopoiesis, confirmation of three distinct protein species as Scribble, and densitometry for Scribble modulation by iron deprivation and cathepsin inhibition. **(A)** Heat map of *Scrib* expression levels in hematopoietic hierarchy from BloodSpot server using mouse normal hematopoietic system. **(B)** Immunoblot (IB) of membrane fractions from human progenitors cultured in erythroid medium. Identical samples run on three separate lanes were probed with three independent antibodies to Scribble: polyclonal rabbit from Santa Cruz Biotechnology (SCT rab: sc-28737), monoclonal mouse from Santa Cruz Biotechnology (SCT ms: sc-55543), and polyclonal rabbit from Cell Signaling Technology (CST rab: 4475). **(C)** Immunoblot of membrane fractions from progenitors transduced with control (EV) or Scribble-targeting (shScrib) lentiviral shRNA constructs and cultured in erythroid medium. **(D)** Immunoblot of membrane fractions from progenitors cultured in erythroid medium and treated with the deubiquitylase inhibitor PR-619 for 30 min, 1 h, and 3 h. **(E)** Densitometry from three independent experiments for Scribble levels in progenitors cultured in erythroid medium with indicated TSATs \pm cathepsin inhibitor CA074me (CA). Immunoblot signals from indicated subcellular fractions are normalized for loading, and values are expressed relative to Scrib levels with 100% TSAT and no cathepsin inhibitor.

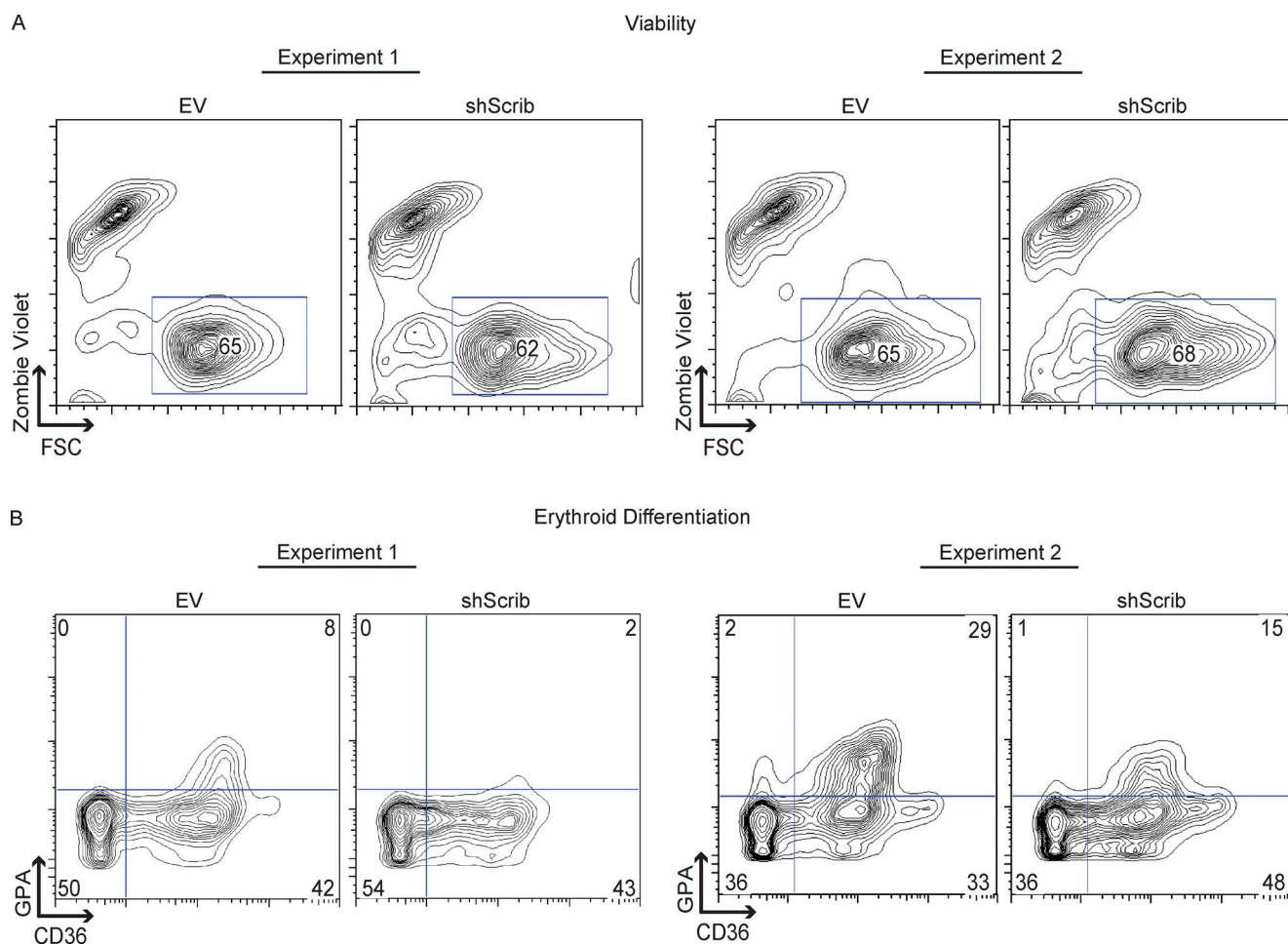


Figure S3. **Phenotypic consequences of Scribble knockdown on human erythropoiesis.** **(A)** Flow cytometry assessment of viability in primary progenitors transduced with control or Scribble-targeting lentiviral shRNA constructs and cultured for 3 d in erythroid medium, with gating on all cells. **(B)** Flow cytometry assessment of early and later erythroid markers, CD36 and GPA, in progenitors transduced with lentiviral shRNA constructs and cultured for 3 d in erythroid medium with gating on live cells. EV, lentiviral shRNA control; shScrib, Scribble-targeting lentiviral shRNA construct.

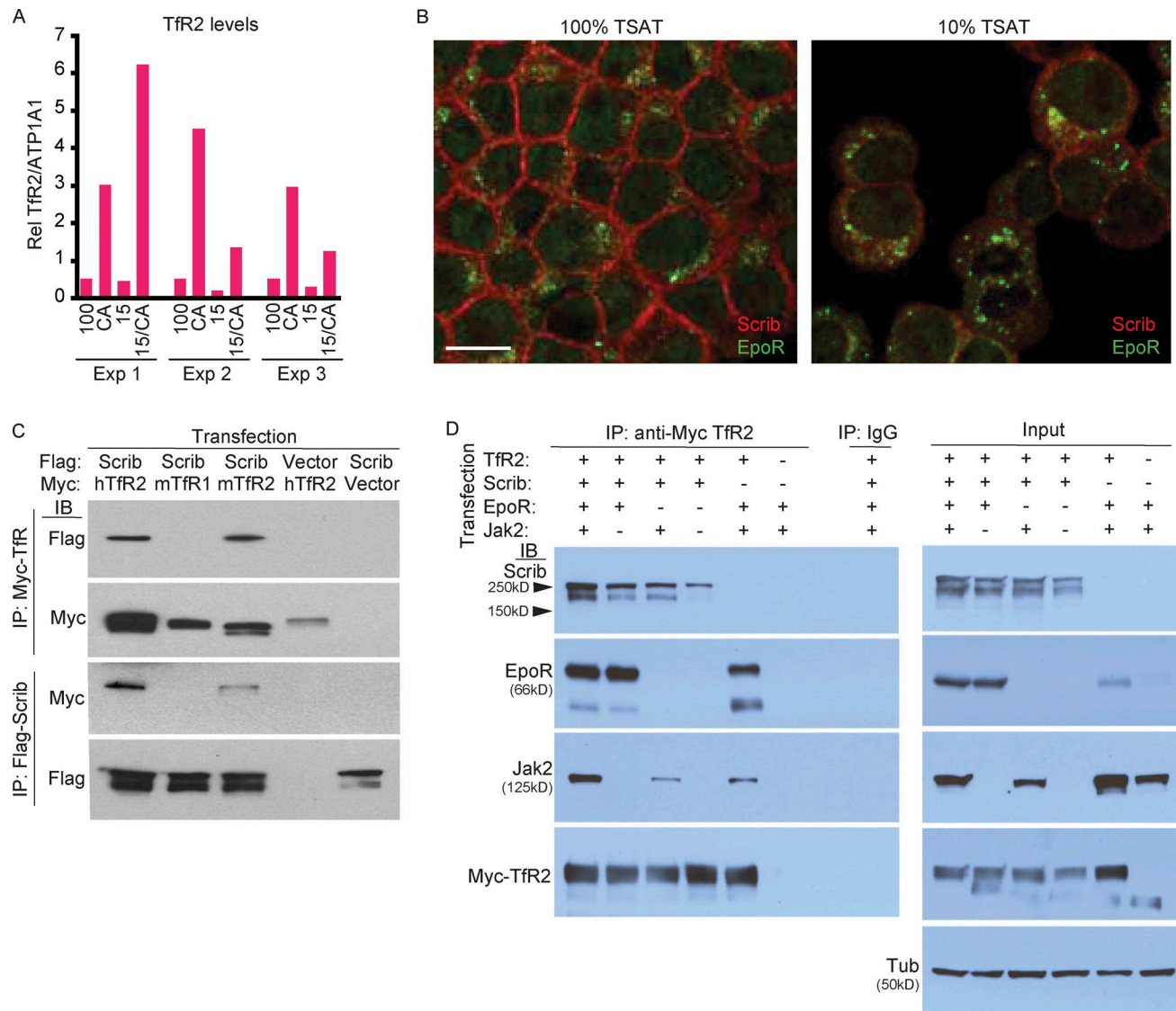


Figure S4. Densitometry for Tfr2 modulation by iron deprivation and cathepsin inhibition, immunofluorescence for Scribble and EpoR, and interaction of Tfr2 with Scribble, EpoR, and JAK2. (A) Densitometry for Tfr2 levels from immunoblots of membrane fractions from human erythroid progenitors cultured with indicated TSATs \pm cathepsin inhibitor (CA; $n = 3$). Representative immunoblot shown in Fig. 4 C. (B) Immunofluorescence costaining for Scribble and EpoR in human erythroid progenitors cultured with the indicated TSATs with visualization by confocal microscopy. Representative results from two independent experiments. Bar, 15 μ m. (C) IP of epitope-tagged proteins (Myc-human Tfr2, Myc-mouse Tfr1, Myc-mouse Tfr2, and Flag-human Scribble) from extracts of HEK293T cotransfectants followed by immunoblot for indicated epitope tags. Representative results from three independent experiments. (D) IP of Myc-tagged Tfr2 from extracts of HEK293T cotransfectants followed by immunoblot detection of coexpressed factors and input immunoblot. IB, immunoblot. Representative results from three independent experiments.