

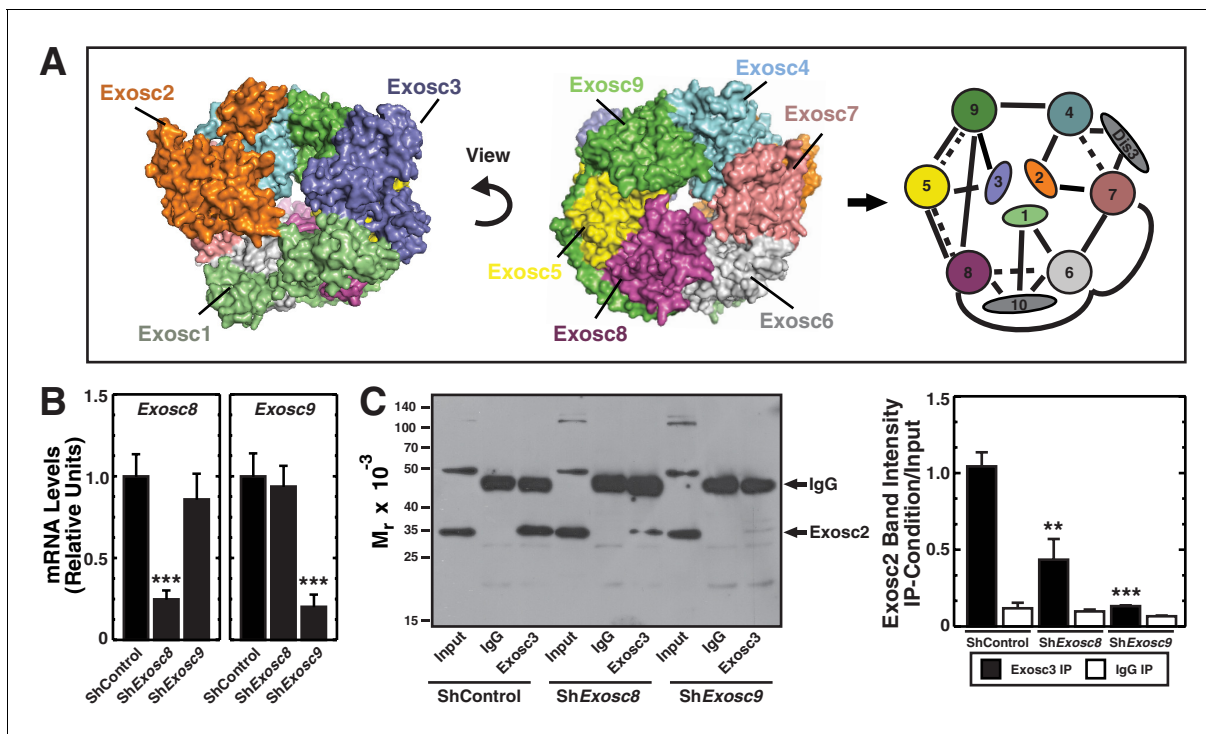


---

## Figures and figure supplements

Exosome complex orchestrates developmental signaling to balance proliferation and differentiation during erythropoiesis

**Skye C McIver et al**



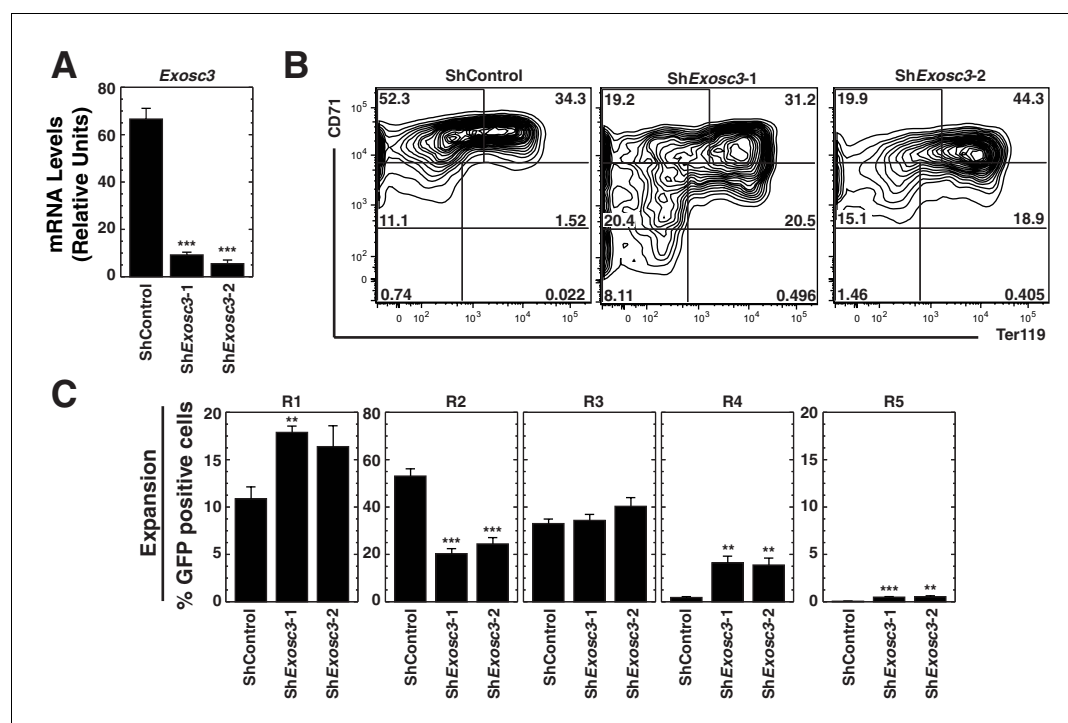
**Figure 1.** Exosc8 or Exosc9 downregulation disrupts protein-protein interactions within the exosome complex. (A) Crystal structure and model of the human exosome complex (Liu et al., 2006). Solid line, direct interactions; Dashed line, indirect interactions. (B) Real-time RT-PCR analysis of mRNA expression (mean  $\pm$  SE, 3 independent replicates) in G1E-ER-GATA-1 cells 48 hr post-infection with either *Exosc8* or *Exosc9* shRNA expressing retrovirus. Values normalized to 18S expression and relative to the control. (C) Left: representative image of a semi-quantitative Western blot of Exosc2 co-immunoprecipitated with anti-Exosc3 antibody in G1E-ER-GATA-1 whole cell lysates prepared 48 hr post-*Exosc8* or *Exosc9* knockdown. Right: densitometric analysis of band intensity relative to the input for each knockdown condition (mean  $\pm$  SE, 3 independent replicates). Statistical analysis of control and treatment conditions was conducted with the Student's T-test. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ . Source data is available in **Figure 1—source data 1**.

DOI: [10.7554/eLife.17877.003](https://doi.org/10.7554/eLife.17877.003)

The following source data is available for figure 1:

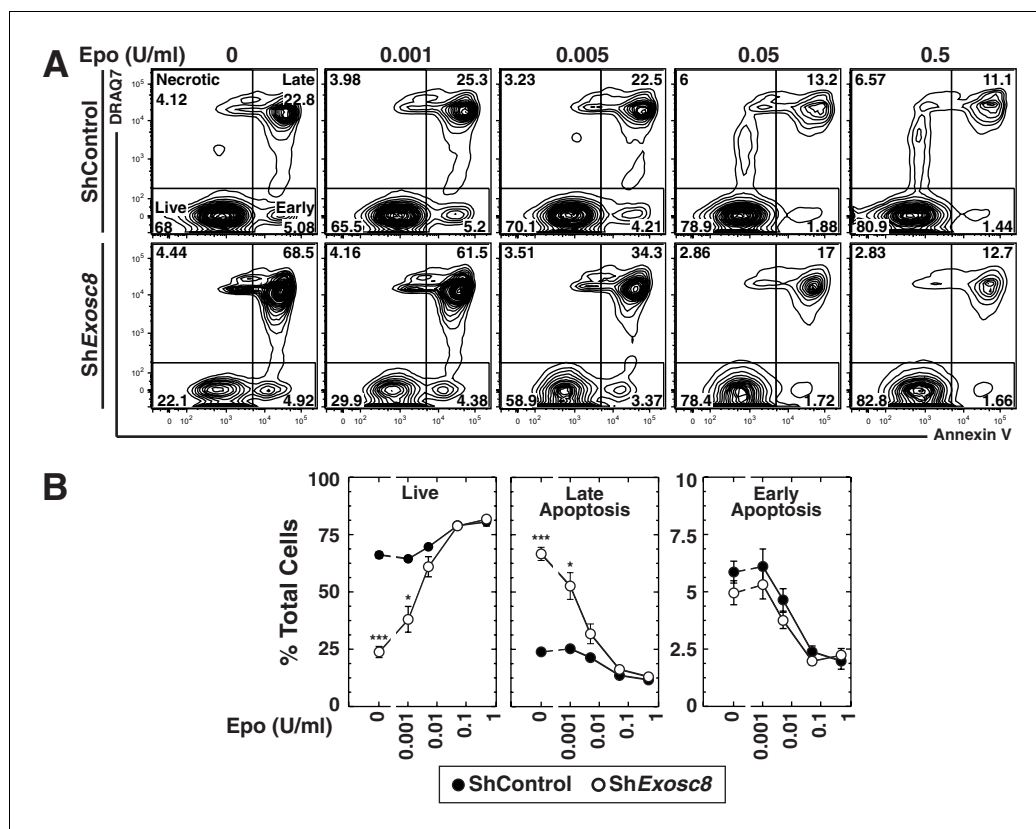
**Source data 1.** This Excel spreadsheet contains the values of each independent replicate for data presented as histograms (mean  $\pm$  SE) in **Figure 1**.

DOI: [10.7554/eLife.17877.004](https://doi.org/10.7554/eLife.17877.004)



**Figure 1—figure supplement 1.** The RNA binding exosome complex component *Exosc3* suppresses erythroid maturation. (A) qRT-PCR analysis of *Exosc3* mRNA in primary erythroid precursor cells 72 hr post-infection with shRNA-expressing retrovirus (mean  $\pm$  SE, 5 biological replicates). Values are normalized to 18S expression and relative to the control. (B) Erythroid maturation analyzed by flow cytometric quantitation CD71 and Ter119 staining 72 hr post-*Exosc3* knockdown in primary erythroid precursor cells. Representative flow cytometry plots, with the R1-R5 gates denoted (5 biological replicates). (C) Percentage of primary erythroid precursor cells in R1-R5 populations 72 hr after *Exosc3* knockdown (mean  $\pm$  SE, 5 biological replicates). Statistical analysis of control and treatment conditions was conducted with the Student's T-test. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ .

DOI: [10.7554/eLife.17877.005](https://doi.org/10.7554/eLife.17877.005)



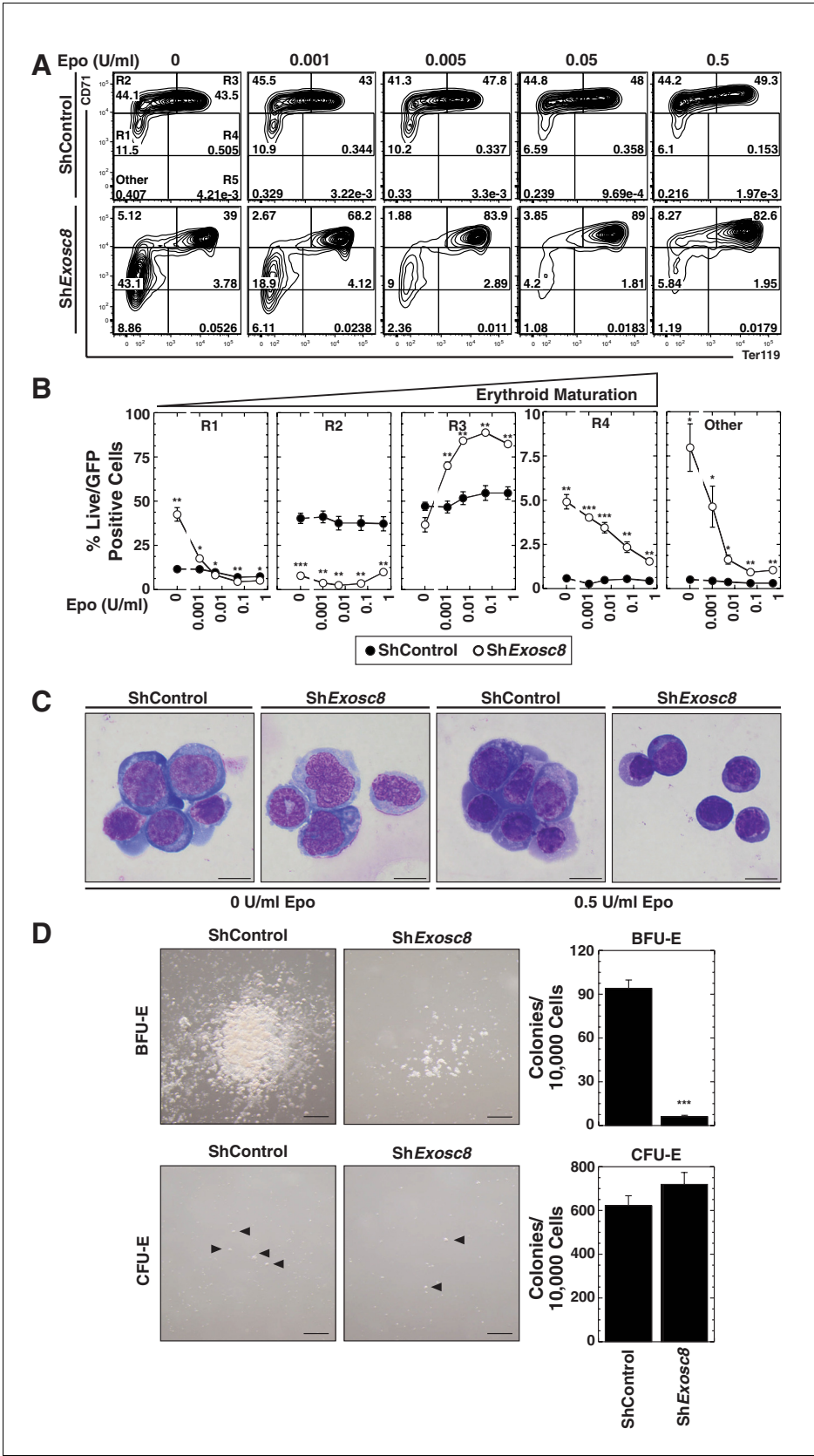
**Figure 2.** Exosome complex disruption renders primary erythroid cells hypersensitive to limiting erythropoietin concentrations. (A) Flow cytometric analysis with Annexin V and the membrane-impermeable dye DRAQ7 to quantitate apoptosis with control and *Exosc8*-knockdown primary erythroid cells expanded for 48 hr under Epo-limiting conditions. (B) Quantification of the percentage of primary erythroid cells in live, late and early apoptotic populations (mean  $\pm$  SE, 4 biological replicates). Statistical analysis of control and treatment conditions was conducted with the Student's T-test. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ . Source data is available in [Figure 2—source data 1](#).

DOI: [10.7554/eLife.17877.006](https://doi.org/10.7554/eLife.17877.006)

The following source data is available for figure 2:

**Source data 1.** This Excel spreadsheet contains the values of each biological replicate for data presented as line graphs (mean  $\pm$  SE) in [Figure 2](#).

DOI: [10.7554/eLife.17877.007](https://doi.org/10.7554/eLife.17877.007)



**Figure 3.** Erythropoietin is required for erythroid differentiation induced by disrupting the exosome complex. (A) Flow cytometric quantification of erythroid markers CD71 and Ter119 in live control and *Exosc8*-knockdown cells. (B) Erythroid maturation curves showing % Live/GFP positive cells vs Epo concentration. (C) Micrographs of erythroid colonies at 0 and 0.5 U/ml Epo. (D) BFU-E and CFU-E assay results showing colony formation per 10,000 cells.

Figure 3 continued on next page

*Figure 3 continued*

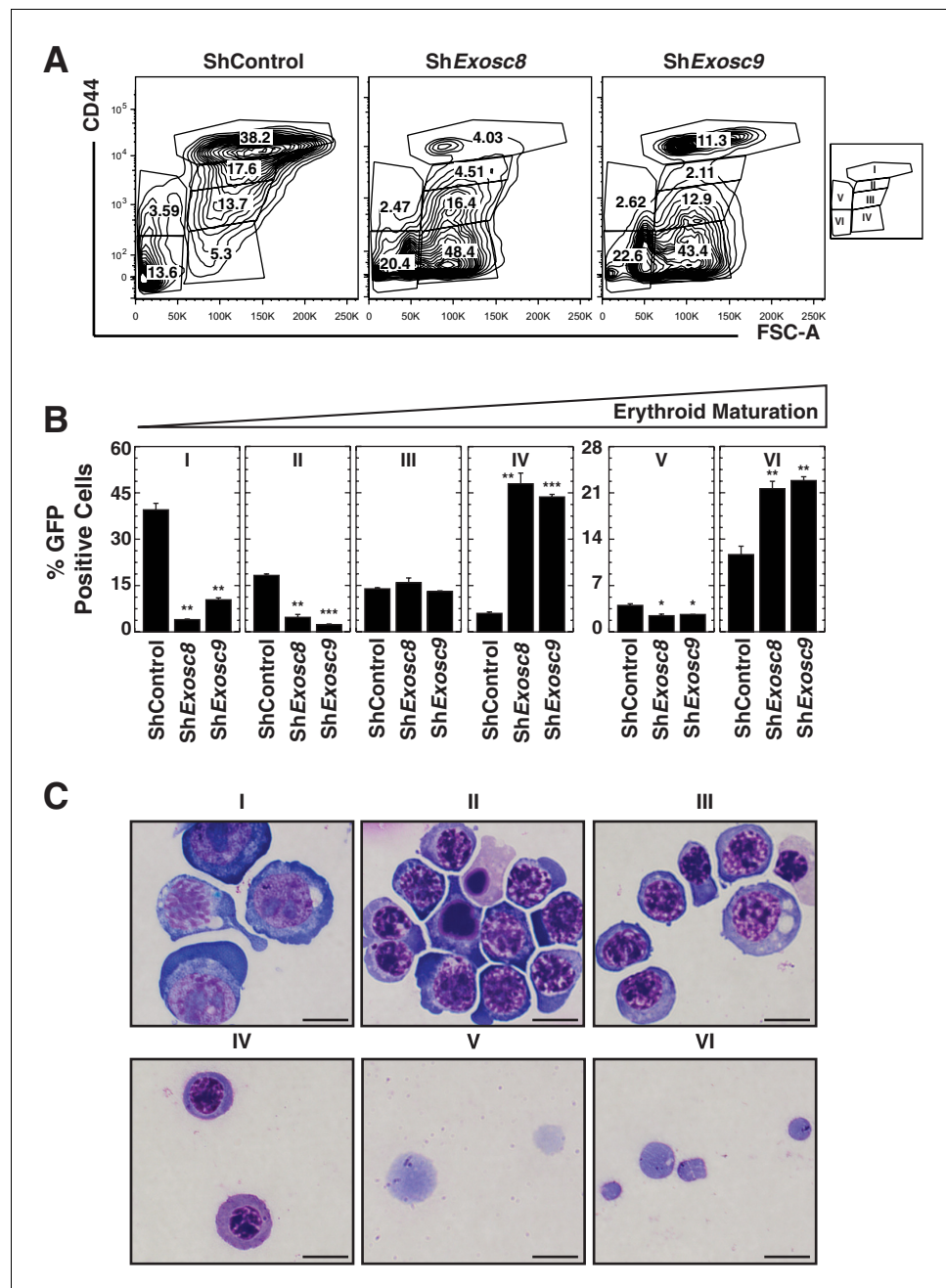
erythroid precursor cells cultured for 48 hr in Epo-limiting media. Representative plots with R1-R5 gates denoted. (B) Quantitation of the percentage of live cells in control and *Exosc8*-knockdown conditions from the R1-R4 and non erythroid gates (mean  $\pm$  SE, 4 biological replicates). (C) Representative images of Wright-Giemsa-stained, DRAQ7-negative erythroid precursor cells, infected with control or Sh*Exosc8* retrovirus. Cells were cultured with or without Epo for 48 hr (Scale bar, 10  $\mu$ m). (D) Representative images (left) and quantitation (right) of erythroid colony forming unit activity with FACS-sorted R1 cells 24 hr after *Exosc8* knockdown (mean  $\pm$  SE, 6 biological replicates) (Scale bar 200  $\mu$ m). Statistical analysis of control and treatment conditions was conducted with the Student's T-test. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ . Source data is available in **Figure 3—source data 1**.

[DOI: 10.7554/eLife.17877.008](https://doi.org/10.7554/eLife.17877.008)

The following source data is available for figure 3:

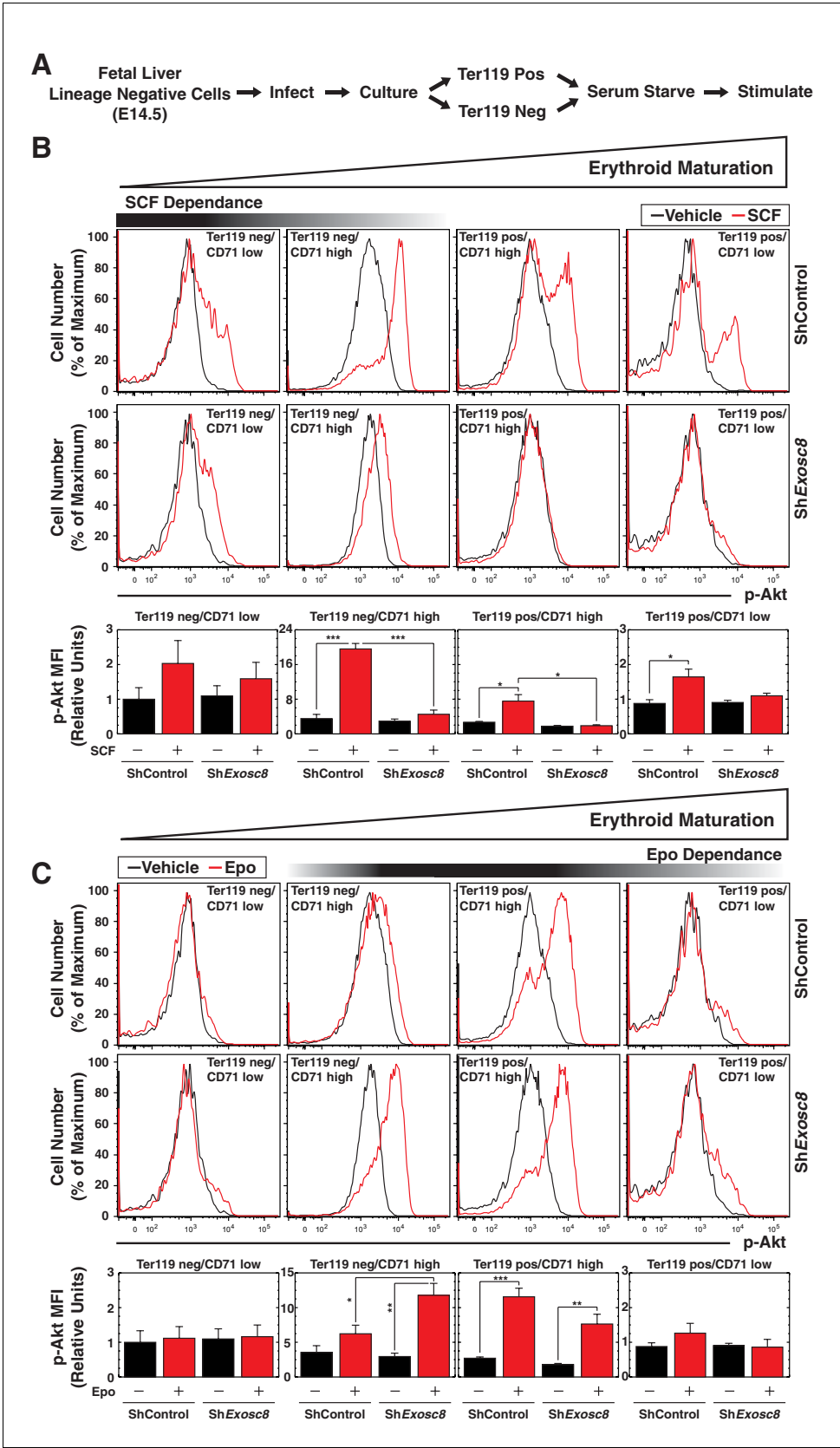
**Source data 1.** This Excel spreadsheet contains the values for each biological replicate for data presented as either line graphs or histograms (mean  $\pm$  SE) in **Figure 3**.

[DOI: 10.7554/eLife.17877.009](https://doi.org/10.7554/eLife.17877.009)



**Figure 3—figure supplement 1.** Analysis of the exosome complex-mediated erythroid maturation barricade using a distinct flow cytometric assay. (A) Erythroid maturation of primary erythroid precursor cells 72 hr post-infection with shExosc8- or shExosc9-expressing retroviruses analyzed by flow cytometric quantification of CD44 and side scatter (SSC). Representative flow cytometry plots with gates I to VI are depicted. (B) Percentage of erythroid cells detected in gates I through VI (3 biological replicates, mean  $\pm$  SE). (C) Representative images of Wright-Giemsa-stained erythroid cells from the sorted, gated (I-IV) populations under control conditions (Scale bar, 10  $\mu$ m). Statistical analysis of control and treatment conditions was conducted with the Student's T-test. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ .

DOI: [10.7554/eLife.17877.010](https://doi.org/10.7554/eLife.17877.010)



**Figure 4.** Exosome complex sustains proliferation signaling, while suppressing pro-differentiation signaling. (A) Experimental scheme: lineage-negative cells were isolated from E14.5 fetal livers, and infected with *luciferase* or *Exosome* complex. (B) SCF dependence of erythroid maturation. (C) Epo dependence of erythroid maturation. Figure 4 continued on next page



*Figure 4 continued*

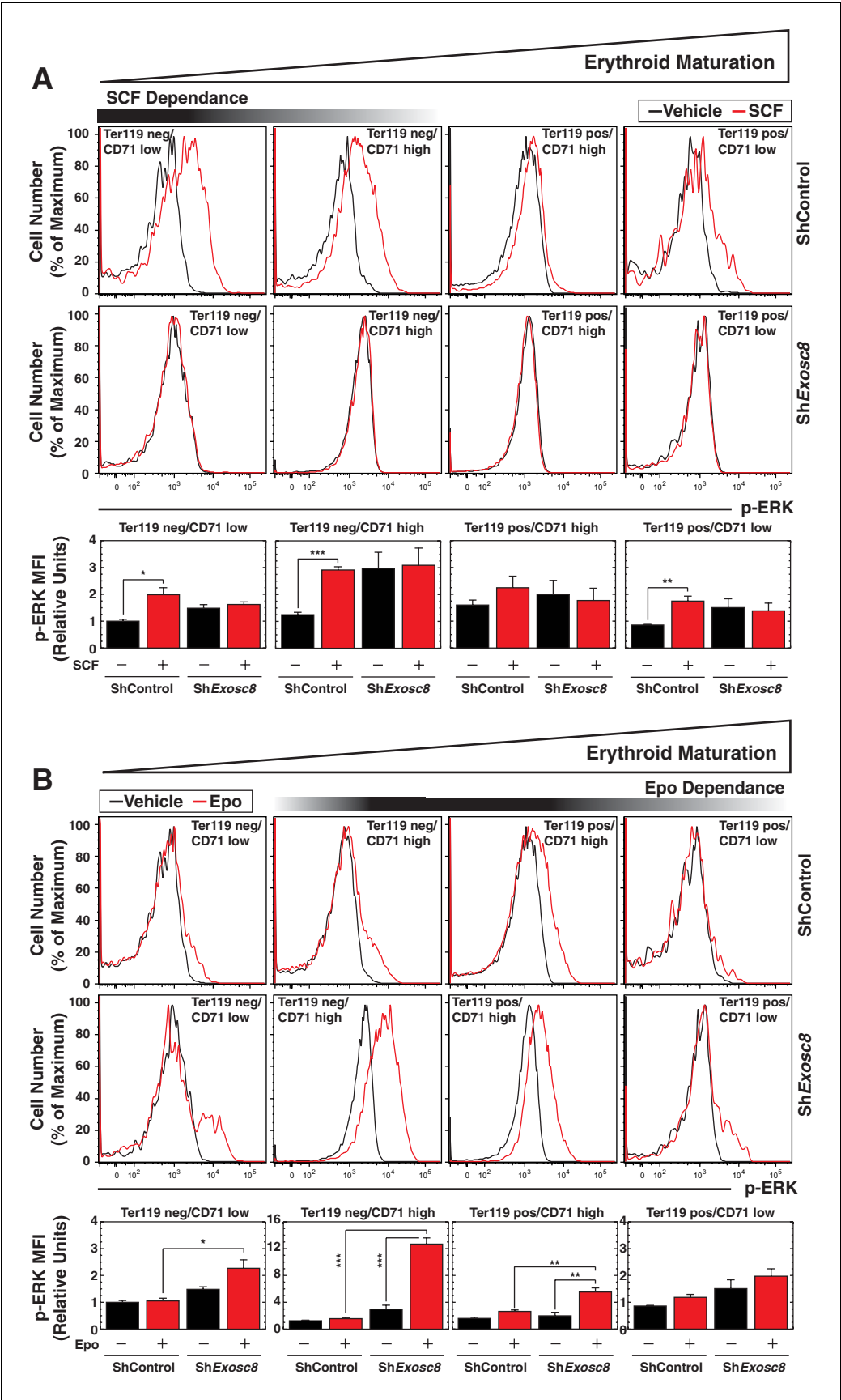
*Exosc8* shRNAs. Cells were cultured for 48 hr and sorted into Ter119<sup>+</sup> and Ter119<sup>-</sup> populations using beads. After 1 hr of serum-starvation, cells were stimulated for 10 min with 10 ng/ml SCF or 2 U/ml Epo and fixed/permeabilized before staining for CD71 and p-Akt. (B) Top: p-Akt staining after stimulation with 10 ng/ml SCF in control and *Exosc8*-knockdown cells (6 biological replicates). Bottom: Relative p-Akt MFI after stimulation with 10 ng/ml SCF in control and *Exosc8*-knockdown cells. MFI expressed relative to unstimulated Ter119<sup>-</sup>/CD71<sup>low</sup> control (mean ± SE, 6 biological replicates). (C) Top: p-Akt staining after stimulation with 2 U/ml EPO in control and *Exosc8*-knockdown cells (6 biological replicates). Bottom: Relative p-Akt MFI after stimulation with 2 U/ml Epo in control and *Exosc8*-knockdown cells. MFI expressed relative to unstimulated Ter119<sup>-</sup>/CD71<sup>low</sup> control (mean ± SE, 6 biological replicates). ANOVA identified any significant variation within the experiment, and a Tukey-Kramer test identified the statistical relationship between each pair of samples. \*p<0.05, \*\*p<0.01, \*\*\*p<0.001. Source data is available in **Figure 4—source data 1**.

DOI: [10.7554/eLife.17877.011](https://doi.org/10.7554/eLife.17877.011)

The following source data is available for figure 4:

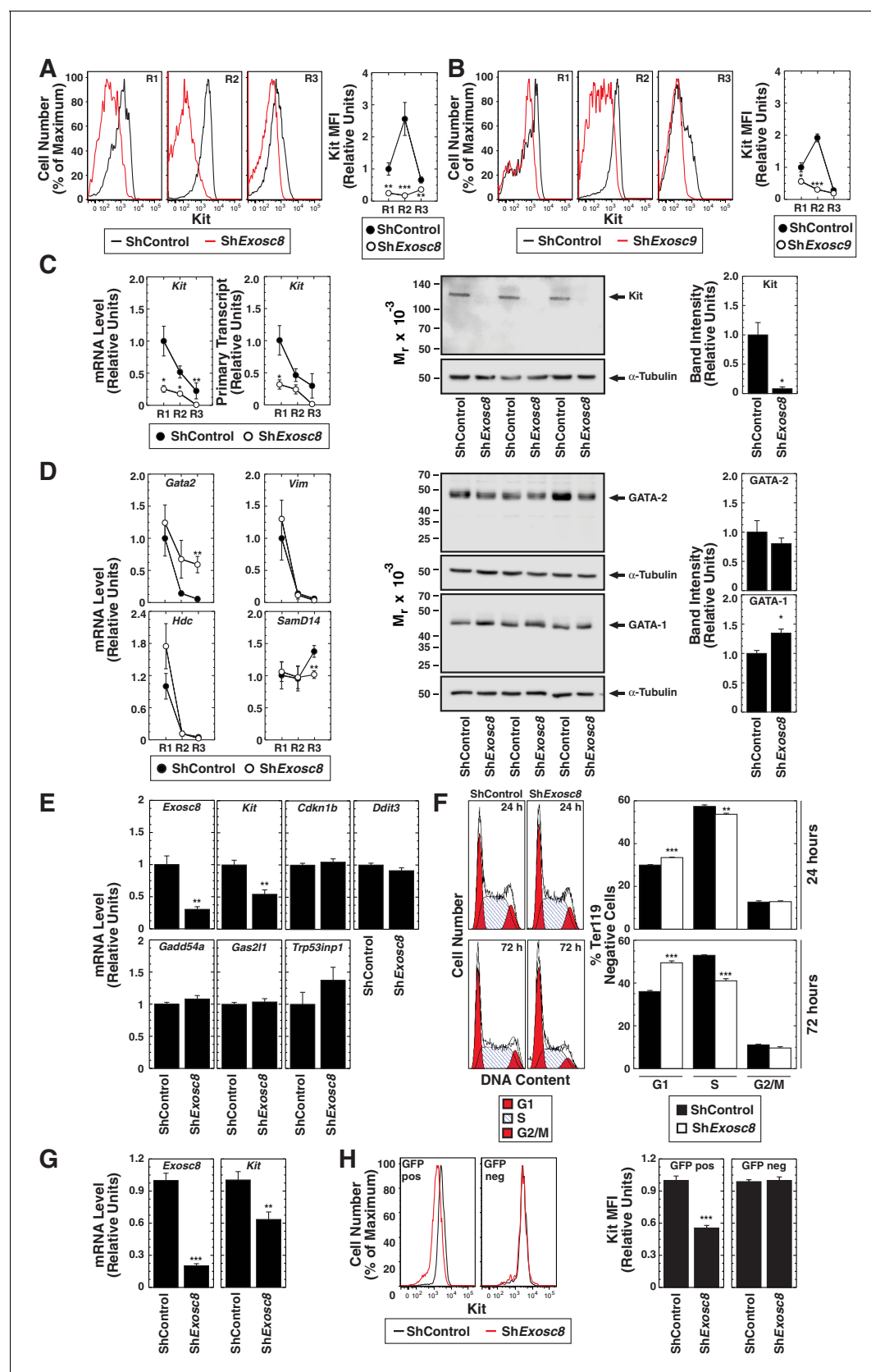
**Source data 1.** This Excel spreadsheet contains the values for each biological replicate for data presented as histograms (mean ± SE) in **Figure 4**.

DOI: [10.7554/eLife.17877.012](https://doi.org/10.7554/eLife.17877.012)



**Figure 4—figure supplement 1.** Flow cytometric analysis of ERK phosphorylation reveals Exosc8 requirement to confer Kit signaling and to suppress Epo signaling. **(A)** Top: p-ERK staining after 10 min stimulation with 10 ng/ml SCF in control and *Exosc8*-knockdown cells (5 biological replicates). Bottom: p-ERK MFI expressed relative to unstimulated Ter119<sup>+</sup>/CD71<sup>low</sup> control (mean  $\pm$  SE, 5 biological replicates). **(B)** Top: p-ERK staining after 10 min stimulation with 2 U/ml EPO in control and *Exosc8*-knockdown cells (5 biological replicates). Bottom: p-ERK MFI expressed relative to the Ter119<sup>+</sup>/CD71 low control (mean  $\pm$  SE, 5 biological replicates). Initially ANOVA identified any significant variation between experimental groups. A Tukey-Kramer test subsequently identified the statistical relationship between each pair of samples. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$

DOI: [10.7554/eLife.17877.013](https://doi.org/10.7554/eLife.17877.013)



**Figure 5.** Exosome complex requirement for Kit expression. (A) Left: surface Kit in R1-R3 populations 48 hr post-Exosc8 knockdown. Representative plots. Right: surface Kit MFI relative to control R1 (mean  $\pm$  SE, 5 biological replicates). (B) Left: surface Kit in R1-R3 cells 48 hr post-Exosc9 knockdown. Figure 5 continued on next page

## Figure 5 continued

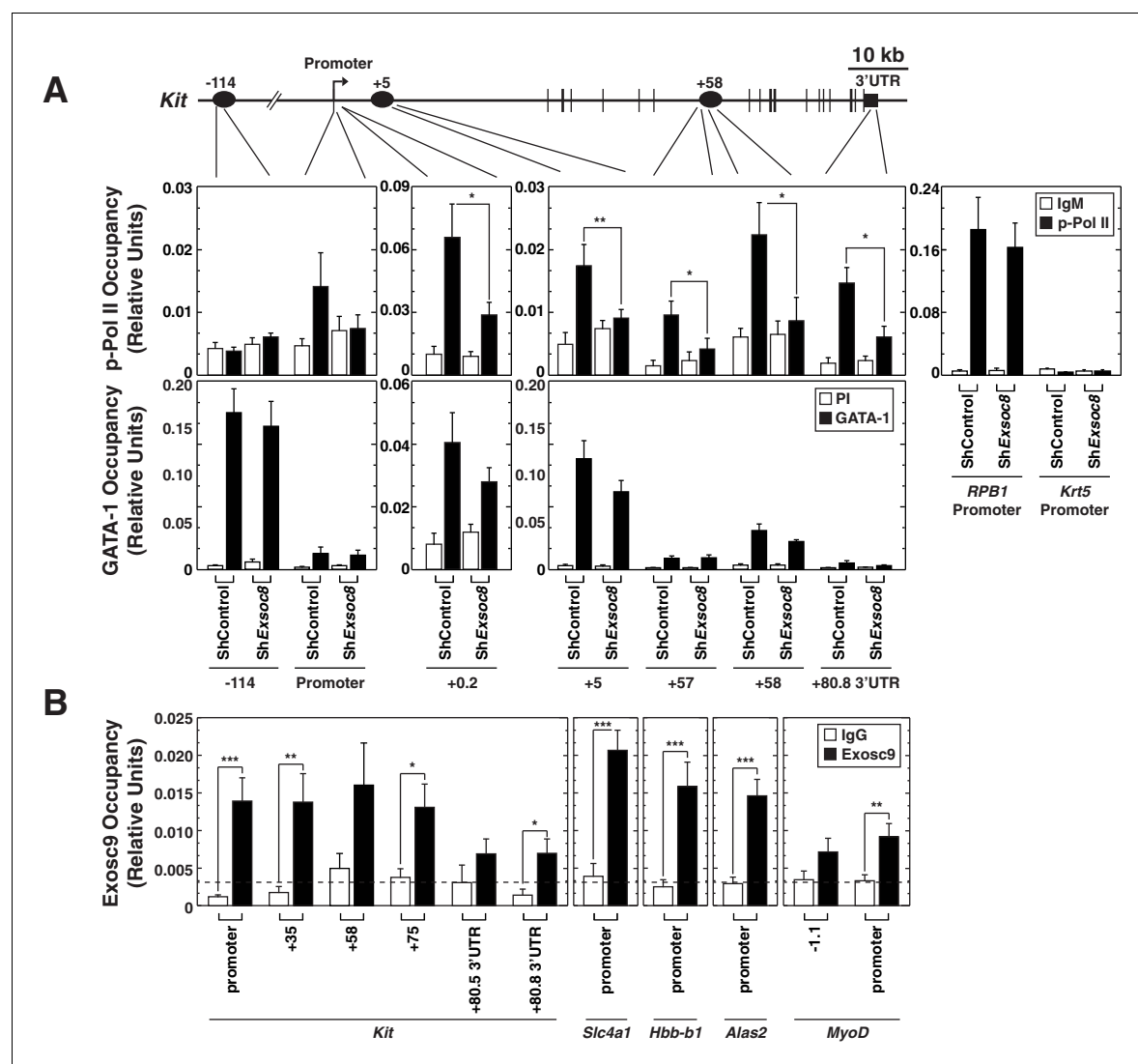
Representative plots. Right: surface Kit MFI relative to control R1 (mean  $\pm$  SE, 8 biological replicates). (C) Left: real-time RT-PCR of *Kit* mRNA and primary transcripts in sorted R1-R3 populations 72 hr post-infection with shControl or shExosc8 normalized to 18S and relative to control R1 (mean  $\pm$  SE, 6 biological replicates). Middle: Kit Western blot with Ter119<sup>+</sup> cells 24 hr post-infection (mean  $\pm$  SE, 3 biological replicates). (D) Left: real-time RT-PCR of erythroid mRNAs in sorted R1-R3 populations 72 hr post-infection with shControl or shExosc8 (mean  $\pm$  SE, 6 biological replicates). Middle: GATA-2 and GATA-1 Western blot with Ter119<sup>+</sup> cells 24 hr post-infection. Right: densitometric analysis normalized to tubulin and relative to shControl (mean  $\pm$  SE, 3 biological replicates). (E) qRT-PCR of *Exosc8* and *Kit* mRNA and GATA-1/*Exosc8*-regulated cell cycle arrest genes in primary erythroid precursor cells 24 hr post-infection. Normalized to 18S and relative to the control (mean  $\pm$  SE, 5 biological replicates). (F) Cell cycle analysis of control and *Exosc8*-knockdown Ter119<sup>+</sup> cells 24 (top) and 72 hr (bottom) post-infection (mean  $\pm$  SE, 6 biological replicates) (G) qRT-PCR analysis of *Exosc8* and *Kit* mRNA in G1E cells 48 hr post-infection with shControl or shExosc8 retrovirus, normalized to 18S and expressed relative to the control (mean  $\pm$  SE, 3 independent experiments) (H) Cell surface Kit expression in infected (GFP<sup>+</sup>) and uninfected (GFP<sup>-</sup>) populations of G1E cells 48 hr post-infection with shExosc8 (mean  $\pm$  SE, 3 independent experiments). Statistical analysis of control and treatment conditions was conducted with the Student's T-test \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ . Source data is available in **Figure 5—source data 1**.

DOI: [10.7554/eLife.17877.014](https://doi.org/10.7554/eLife.17877.014)

The following source data is available for figure 5:

**Source data 1.** This Excel spreadsheet contains the values for each biological replicate for data presented as either line graphs or histograms (mean  $\pm$  SE) in **Figure 5**.

DOI: [10.7554/eLife.17877.015](https://doi.org/10.7554/eLife.17877.015)



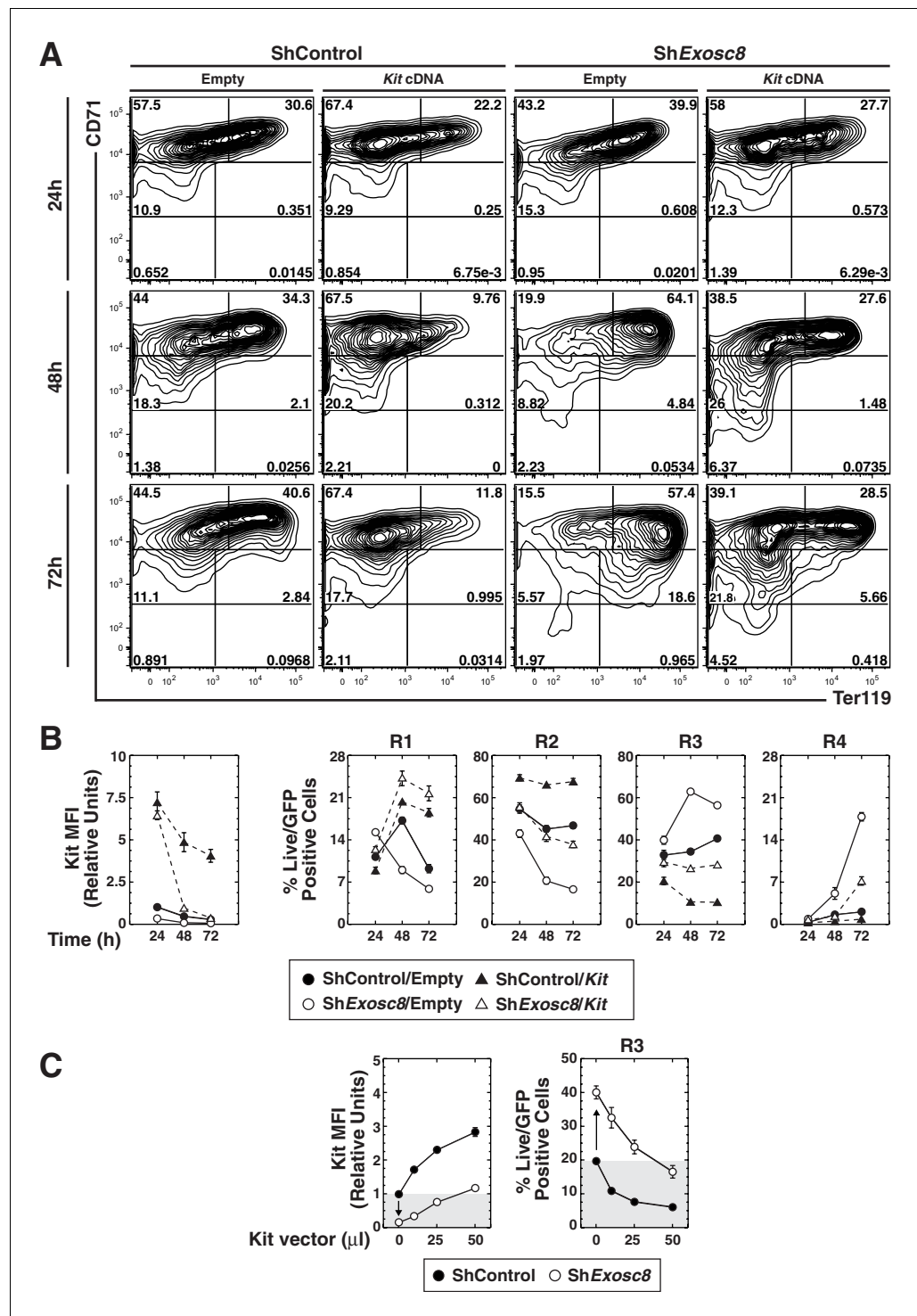
**Figure 6.** Exosome complex occupies the *Kit* locus and is required for active RNA Polymerase II occupancy at *Kit*. (A) qChIP of serine 5-phospho Pol II, and GATA-1 occupancy at *Kit* in control and Exosc8-knockdown Ter119<sup>+</sup> erythroid precursor cells 24 hr post-infection (mean  $\pm$  SE, 6 independent experiments). (B) qChIP of Exosc9 occupancy at *Kit* and promoters of other exosome complex-regulated erythroid genes (*Alas2*, *Hbb-b1* and *Slc4a1*) in erythroid precursor cells after culturing for 48 hr (mean  $\pm$  SE, 3 biological replicates). Statistical analysis of control and treatment conditions was conducted with the Student's T-test. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ . Source data is available in [Figure 6—source data 1](#).

DOI: [10.7554/eLife.17877.016](https://doi.org/10.7554/eLife.17877.016)

The following source data is available for figure 6:

**Source data 1.** This Excel spreadsheet contains the values for each biological replicate for data presented in histograms (mean  $\pm$  SE) in [Figure 6](#).

DOI: [10.7554/eLife.17877.017](https://doi.org/10.7554/eLife.17877.017)



**Figure 7.** Functional link between Kit downregulation and erythroid differentiation induced by disrupting the exosome complex. (A) Erythroid maturation analyzed by flow cytometric quantitation of CD71 and Ter119 post-*Exosc8* knockdown and/or Kit expression in primary erythroid precursor cells expanded for 72 hr. Representative flow cytometry plots, with the R1-R5 gates denoted. (B) Left: relative Kit MFI post-*Exosc8* knockdown and/or Kit overexpression (mean  $\pm$  SE, 4 biological replicates). Right: percentage of primary erythroid precursor cells in the R1-R4 gates (mean  $\pm$  SE, 4 biological replicates). (C) Left: relative Kit MFI 48 hr post-*Exosc8* knockdown in cells infected with increasing amounts of a Kit-expressing retrovirus. The arrow depicts Kit downregulation resulting from knocking-down *Exosc8*. Right: percentage of erythroid precursor cells in the R3 population 48 hr post-

Figure 7 continued on next page

*Figure 7 continued*

infection with shExosc8 in cells infected with increasing amounts of Kit-expressing retrovirus. The arrow depicts the increased R3 population post-Exosc8 knockdown. ANOVA identified any significant variation between experimental groups then a Tukey-Kramer test identified the statistical relationship between each pair of samples, \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ . Source data is available in **Figure 7—source data 1**

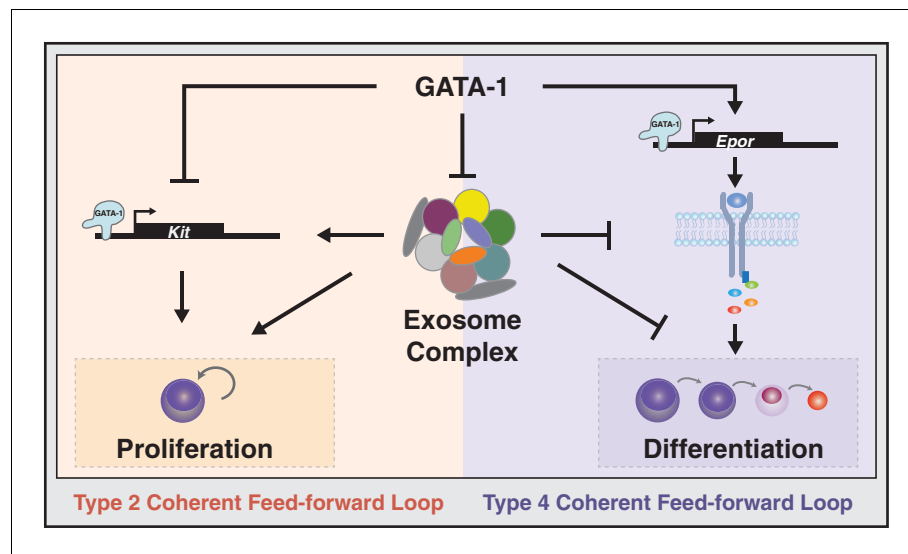
DOI: [10.7554/eLife.17877.018](https://doi.org/10.7554/eLife.17877.018)

The following source data is available for figure 7:

**Source data 1.** This Excel spreadsheet contains the values for each biological replicate for data presented in line graphs (mean  $\pm$  SE) in **Figure 7**.

DOI: [10.7554/eLife.17877.019](https://doi.org/10.7554/eLife.17877.019)





**Figure 8.** Exosome complex function to orchestrate developmental signaling pathways that control proliferation versus differentiation. The master regulator of erythropoiesis GATA-1 represses *Kit* transcription and upregulates *EpoR* transcription, thus establishing the developmental signaling circuitry for erythroid maturation. GATA-1 represses genes encoding exosome complex subunits, which promotes erythroid maturation. The exosome complex confers *Kit* expression and establishes competence for SCF-induced *Kit* signaling. Disruption of this mechanism abrogates *Kit* signaling and instigates *Epo* signaling, which favors erythroid precursor maturation versus self-renewal.

DOI: [10.7554/eLife.17877.020](https://doi.org/10.7554/eLife.17877.020)