

cells at different stages of differentiation stained are shown in (C). Cell images were obtained with 40X objectives as describe in the legends of Fig. 3B. (D) Percentages of cells at the basophilic erythroblast (Baso), polychromatic erythroblast (Poly) and orthochromatic erythroblast (Ortho) stages as well as benzidine-positive cells were determined by scoring the Giemsa-benzidine stained cells.

Fig. 6 Modulation of Hri signaling by salubrinal in β -thalassemic erythroid precursors

(A) eIF2 α P levels in *Hri*^{+/-}*Hbb*^{-/-} reticulocytes. Cells were treated for 6 hours with concentrations of salubrinal indicated. (B) Globin protein synthesis in *Hri*^{+/-}*Hbb*^{-/-} reticulocytes. After treatment with 100 μ M salubrinal for 2 hours, globin protein synthesis at times indicated was measured in the supernatant and pellet fractions. Vertical lines have been inserted to indicate a repositioned gel lane. The middle lane between Control and Sal in the bottom rows (pellet), which was the salubrinal treated sample at time zero prior to ³⁵S-Met/Cys labeling, was removed. (C) eIF2 α P and Chop in *Hri*^{+/-}*Hbb*^{-/-} FL erythroid precursors. Cells were treated for 12 hours with salubrinal at concentrations indicated. Numbers in (A) and (C) denote the ratio of eIF2 α P/ eIF2 α or Chop/eIF2 α . Numbers in B denote the ratio of ³⁵S-globin/total globin. (D) eIF2 α P levels and protein synthesis in Ter119⁺ cells from *Hri*^{+/-}*Hbb*^{-/-} spleen. Cells were treated for 3.5 hours with salubrinal as indicated, and labeled with ³⁵S-Met/Cys for the last 3 hours. Total protein syntheses in the cell lysates are shown in the middle panel. Newly synthesized ³⁵S-Atf4 immunoprecipitated with antibody (Abnova) is shown in bottom panel. Numbers indicate ratio of eIF2 α P/eIF2 α or globin and Atf4 syntheses relative to 0 μ M controls.

Fig. 7 A schematic illustration of Hri-eIF2 α P-Atf4 signaling pathway in mitigating stress and during erythropoiesis. (A) Inhibition of general protein synthesis and enhancement of Atf4 translation by eIF2 α P. Upon various stress conditions, eIF2 α kinases are activated and phosphorylate eIF2 α . The first order of action by eIF2 α P is to inhibit protein synthesis to prevent proteotoxicity resulted from accumulation of excessive unfolded proteins. In the erythroid precursors, Hri is necessary to inhibit globin synthesis in heme deficiency to prevent accumulation of denatured heme-free globins. The second action by eIF2 α P is to selectively enhance the translation of some mRNAs with open reading