1   **Supplemental Figures- De La Garza et al.**

Sibling   36 hpf   *sf3b1* mutant   36 hpf
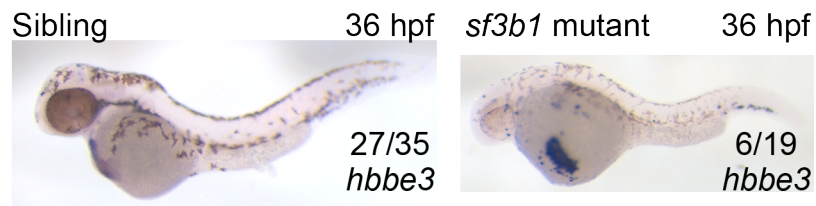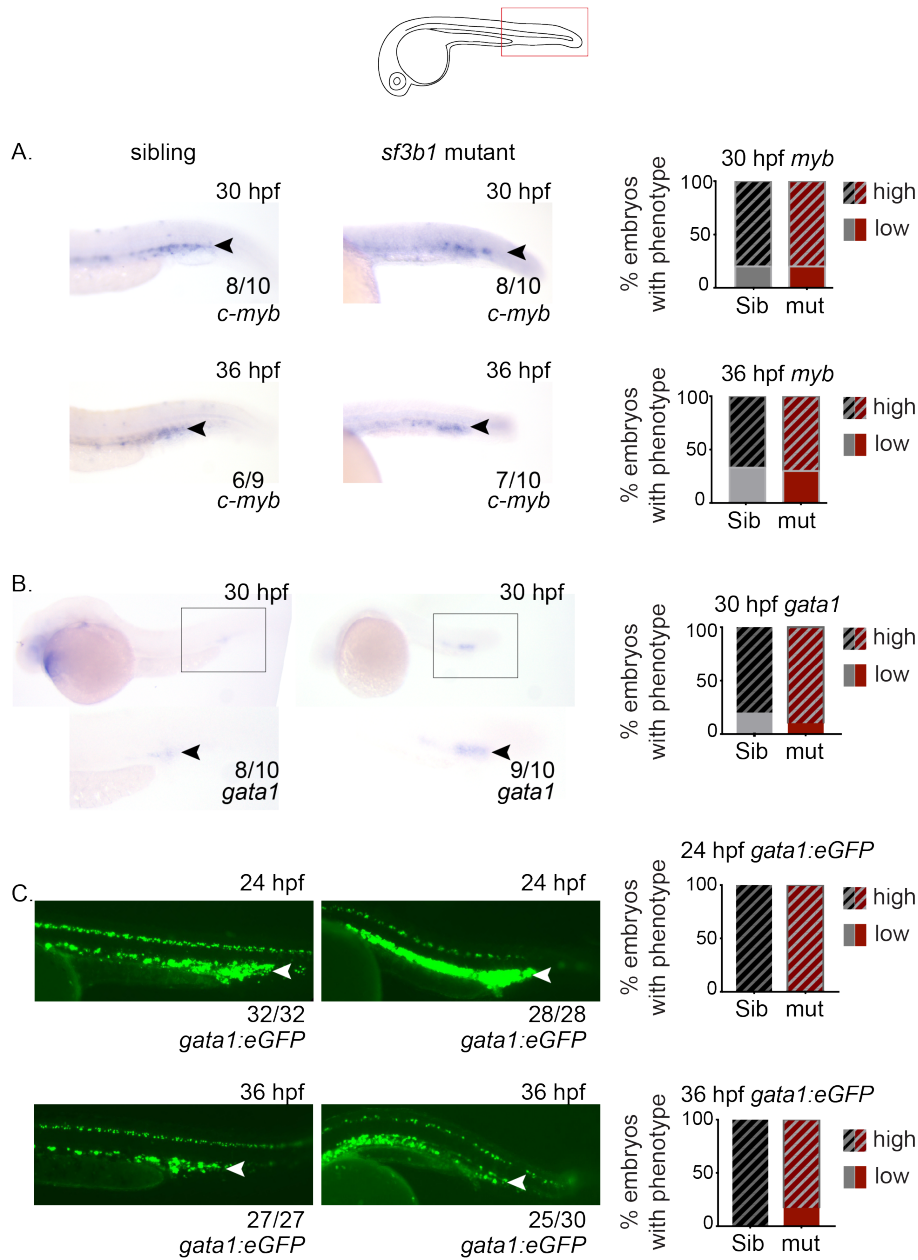

27/35   6/19
hbbe3   *hbbe3*
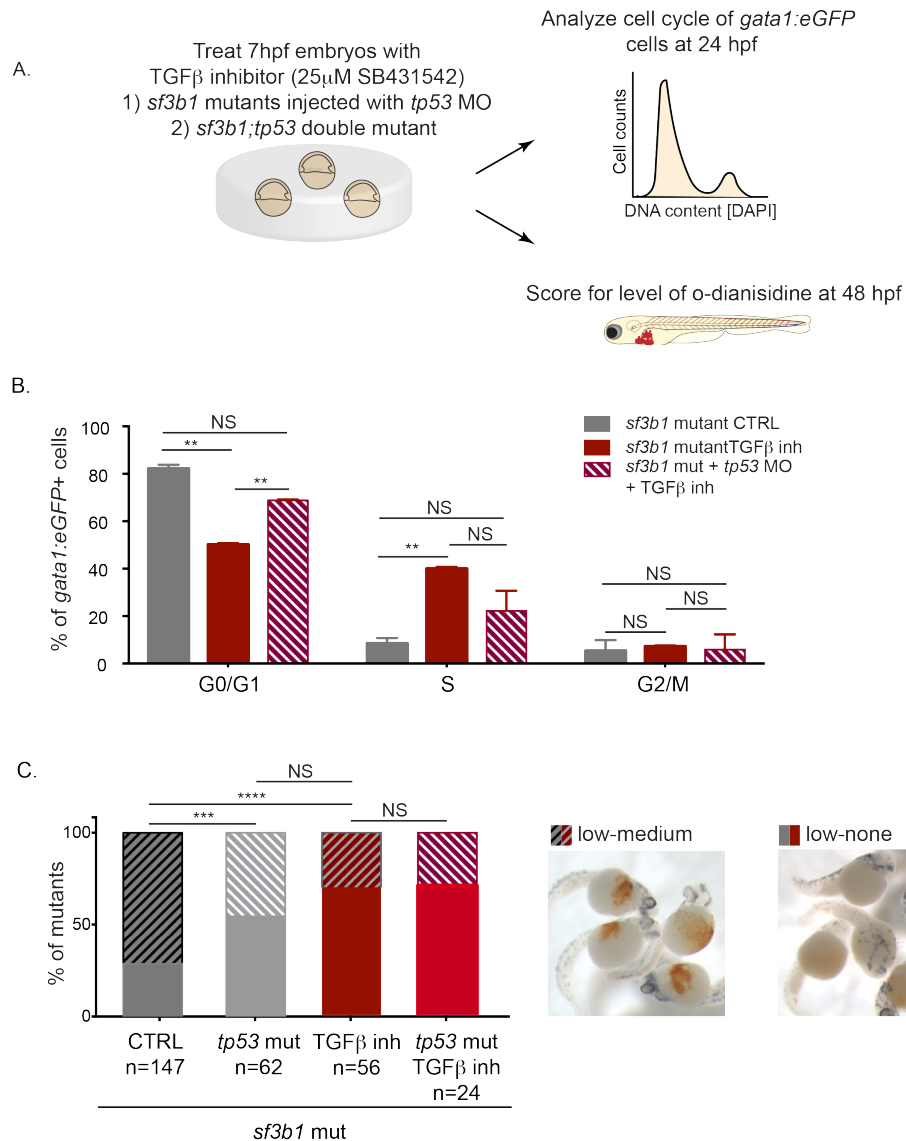
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3   **Figure S1. Embryonic globin expression aberrantly persists in *sf3b1* mutants.** *In situ*

4   hybridization of early globin *hbbe3* in siblings and *sf3b1* mutants at 36 hpf. Numbers in lower

5   right denote number of embryos that displayed a similar phenotype to the image.

Figure S2. EMP progenitors formation is intact in *sf3b1* mutants. A. *In situ* hybridization of *c-myb* in siblings and *sf3b1* mutants at 30 and 36 hpf. **B.** *In situ* hybridization of *gata1* in siblings and *sf3b1* mutants at 30 hpf. Inset below shows a higher magnification view of area boxed in the image above. **C.** Images of *gata1:eGFP* signal in the EMP region in siblings and *sf3b1* mutants at 24 and 36 hpf. Arrowheads denote the PBI region. For A-C, numbers in lower right denote number of embryos that displayed a similar phenotype to the image. Graphs to the right denote the percentage of embryos with the designated phenotype.

A. Treat 7hpf embryos with
TGFβ inhibitor (25μM SB431542)
1) *sf3b1* mutants injected with *tp53* MO
2) *sf3b1;tp53* double mutant

Analyze cell cycle of *gata1:eGFP* cells at 24 hpf

Score for level of o-dianisidine at 48 hpf

B.

C.

low-medium

low-none

Figure S3. Diminished Tp53 activity has minimal effect on TGFβ-mediated cell cycle arrest and *sf3b1* mutant anemia. A. Schematic of experiment to determine the effect of diminished *p53* function + TGFβ inhibition on erythroid cell cycle arrest or anemia in *sf3b1* mutants. **B.** Graph quantifying the percentage of *gata1:eGFP*-positive erythrocytes in G0/G1, S, or G2/M phases of the cell cycle at 24 hpf in *sf3b1* mutants treated with DMSO, 25μM TGFβ inhibitor SB431542, or *tp53* morpholino + SB431542. ANOVA with Bonferroni FDRmulti-testing correction **C.** Graph showing frequency of *sf3b1* mutants and *sf3b1;tp53* double mutants with designated levels of o-dianisidine-positive oxygenated erythrocytes at 48 hpf treated with DMSO or 25μM TGFβ inhibitor SB431542. Total number of mutants analyzed per treatment group is

24   listed below the graph. Images of *sf3b1* mutant embryos with low-medium or low-none o-
25   dianisidine-positive cells are shown to the right. Significance of comparison between groups
26   determined by Fisher's Exact Test. All experiments were done in biological triplicates. **<0.01,
27   ***<0.001, ****<0.0001.