Supplementary Materials

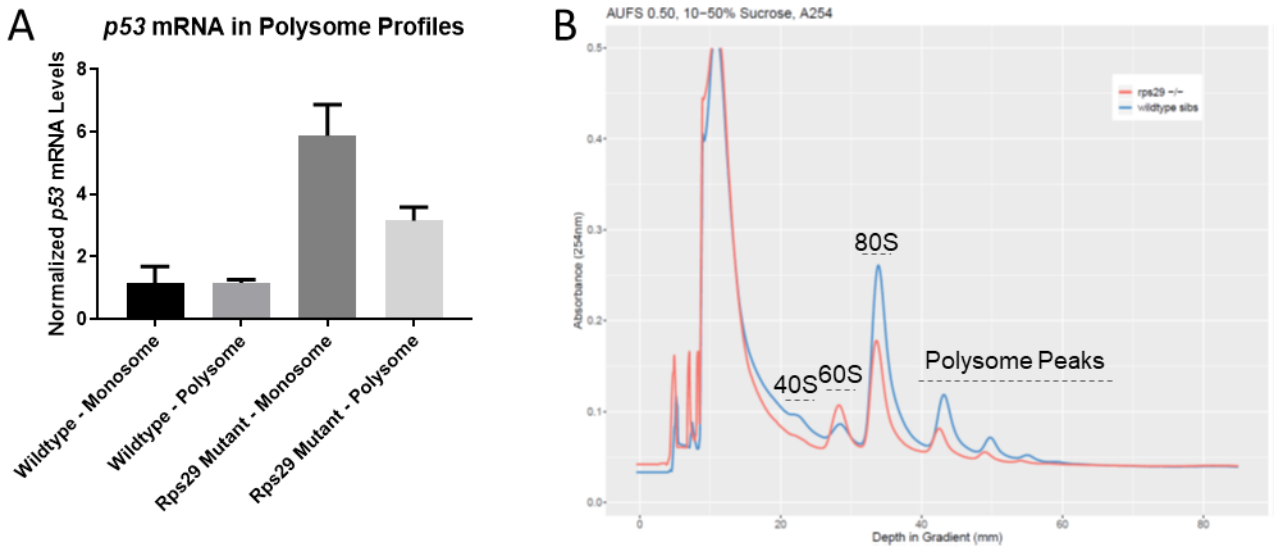

Fig. S1. *p53* is translated in higher amounts in a zebrafish model of Diamond-Blackfan anemia.

A. Polysome profiles were generated from 24 hpf wildtype (AB) zebrafish embryos and *rps29*$^{-/-}$ embryos. mRNA isolated from monosome and polysome fractions was analyzed by qPCR. Y-axis represents *p53* normalized to *tol2* mRNA spiked in the fractions.

B. Polysome profiles were generated from 24 hpf *rps29*$^{-/-}$ embryos and the sibling *rps29*$^{+/+}$ and *rps29*$^{+/-}$ embryos. Y-axis is absorbance, x-axis is sedimentation fraction.

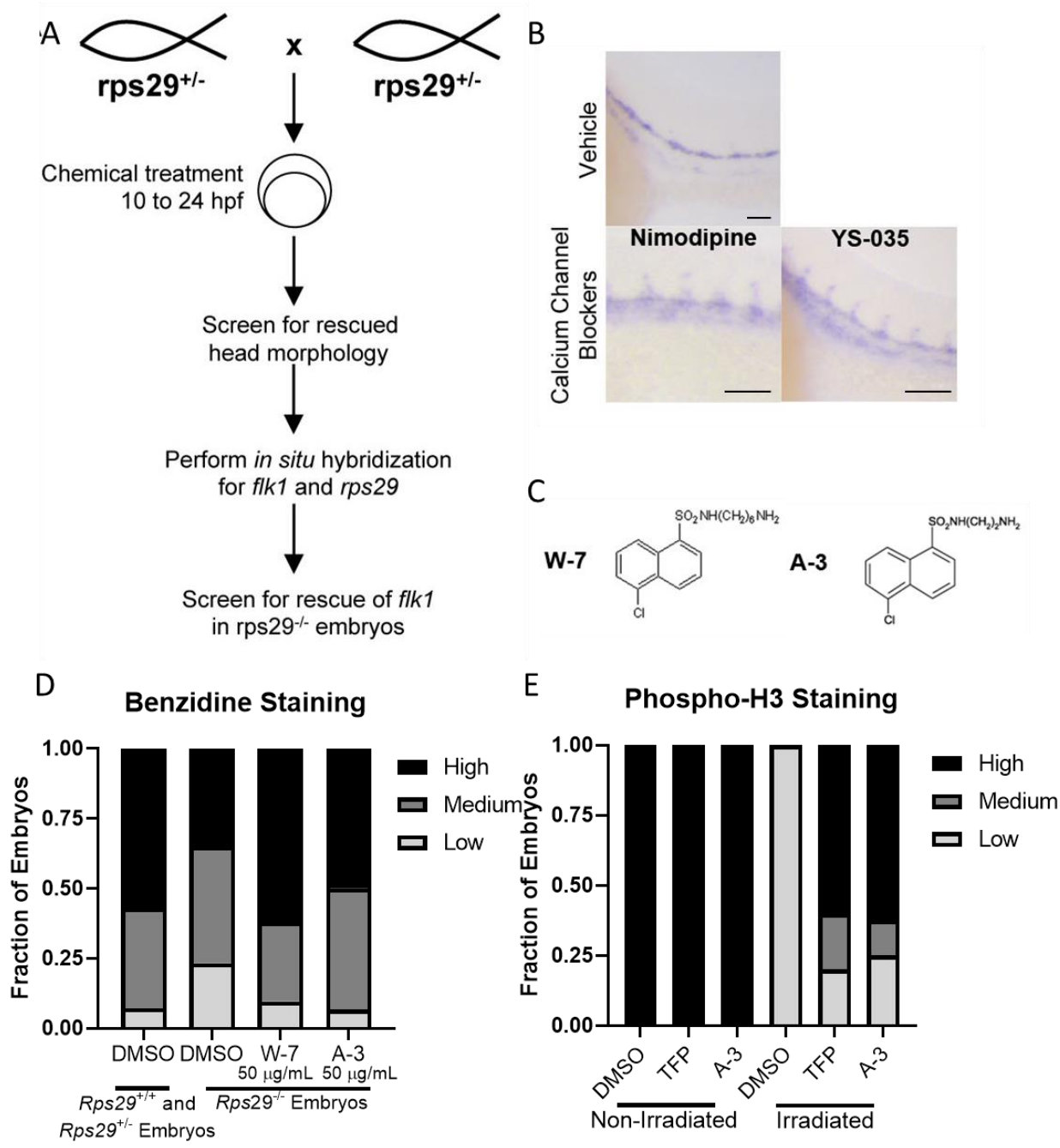

Fig. S2. Chemical screen identifies calmodulin inhibitors and calcium channel blockers.

A. Chemical screen design. *Rps29*$^{+/-}$ fish were incrossed and treated from bud (10 hpf) to 24 hpf with compounds of known bioactivity. At 24 hpf, mutant embryos were scored for

rescued head morphology. Embryos were then fixed for whole mount *in situ* hybridization (ISH) and stained for both *flk1* and *rps29*.

B. *Rps29*$^{-/-}$ embryos were treated with nimodipine or YS-035 at 10 hpf and collected at 24 hpf for *in situ* hybridization of *flk1*. Scale bar = 100 μm.

C. Chemical structures of naphthalenesulfonamides W-7 and A-3.

D. Embryos from an *rps29*$^{+/-}$ incross were treated with DMSO, A-3, or W-7 at 10 hpf and collected at 40 hpf for benzidine (*o*-dianisidine) staining of hemoglobinized cells. Samples were analyzed by binning of stain amount – high, medium, or low. Representative photographs in Figure 1D.

E. Wildtype embryos were treated with DMSO, A-3, or TFP at 50% epiboly (5.25 hpf), irradiated at 10 Gy at 24 hpf, and collected for phospho-H3 staining at 25.5 hpf. Samples were analyzed by binning of numbers of positively stained cells – high, medium, or low. Representative photographs in Figure 1E.

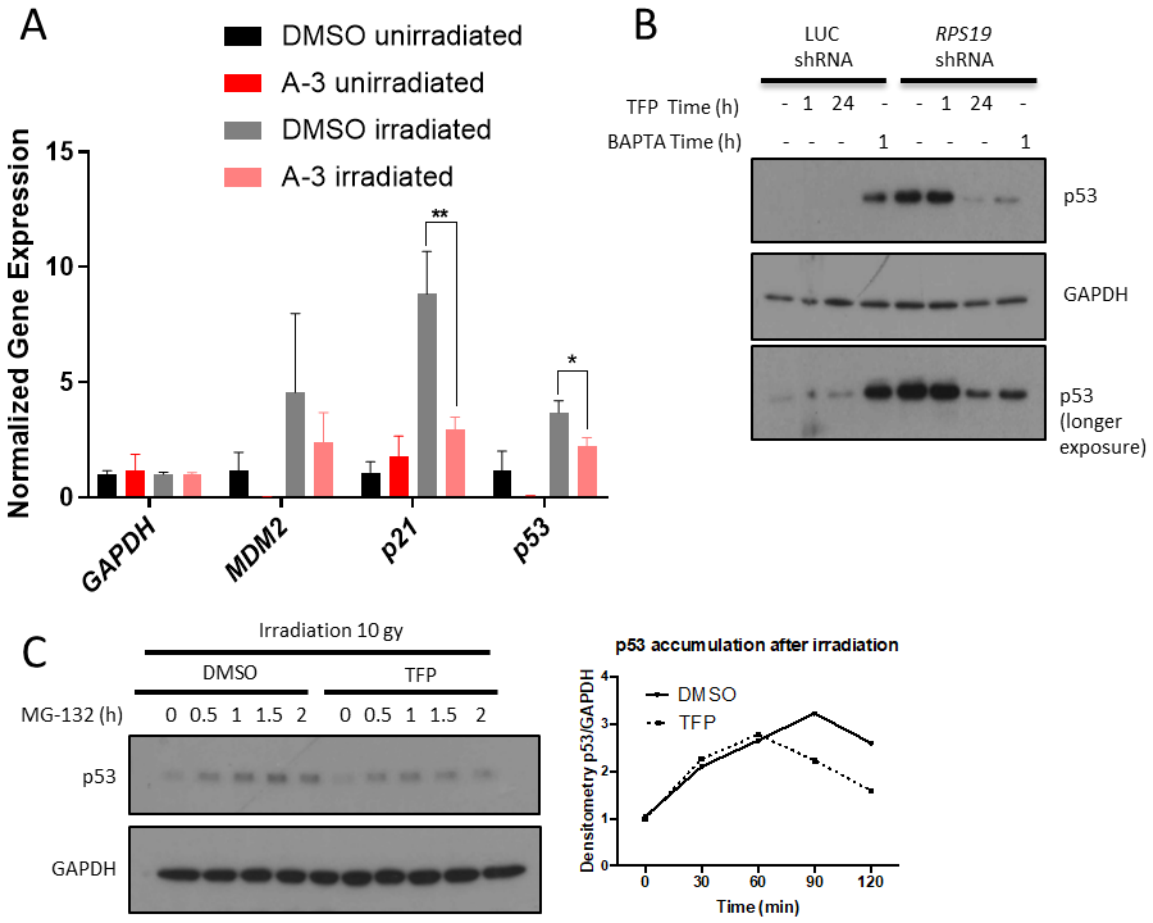

Fig. S3. TFP and BAPTA decreases p53 accumulation.

A. Wildtype embryos were treated with DMSO or A-3 at 50% epiboly (5.25 hpf), irradiated at 10 Gy at 24 hpf, and collected for RNA isolation and qPCR at 25.5 hpf.

B. $CD34^+$ cells were infected with shRNA against luciferase or *RPS19*, and GFP+ cells were selected for infection by FACS. Cells were treated with TFP or BAPTA, and Western blots for p53 and GAPDH were quantified by densitometry.

C. Irradiated $CD34^+$ cells were pre-treated with TFP for 2 hours before treatment with 20 µM MG-132 for increasing lengths of time, then lysates were collected for p53 and GAPDH protein quantification.

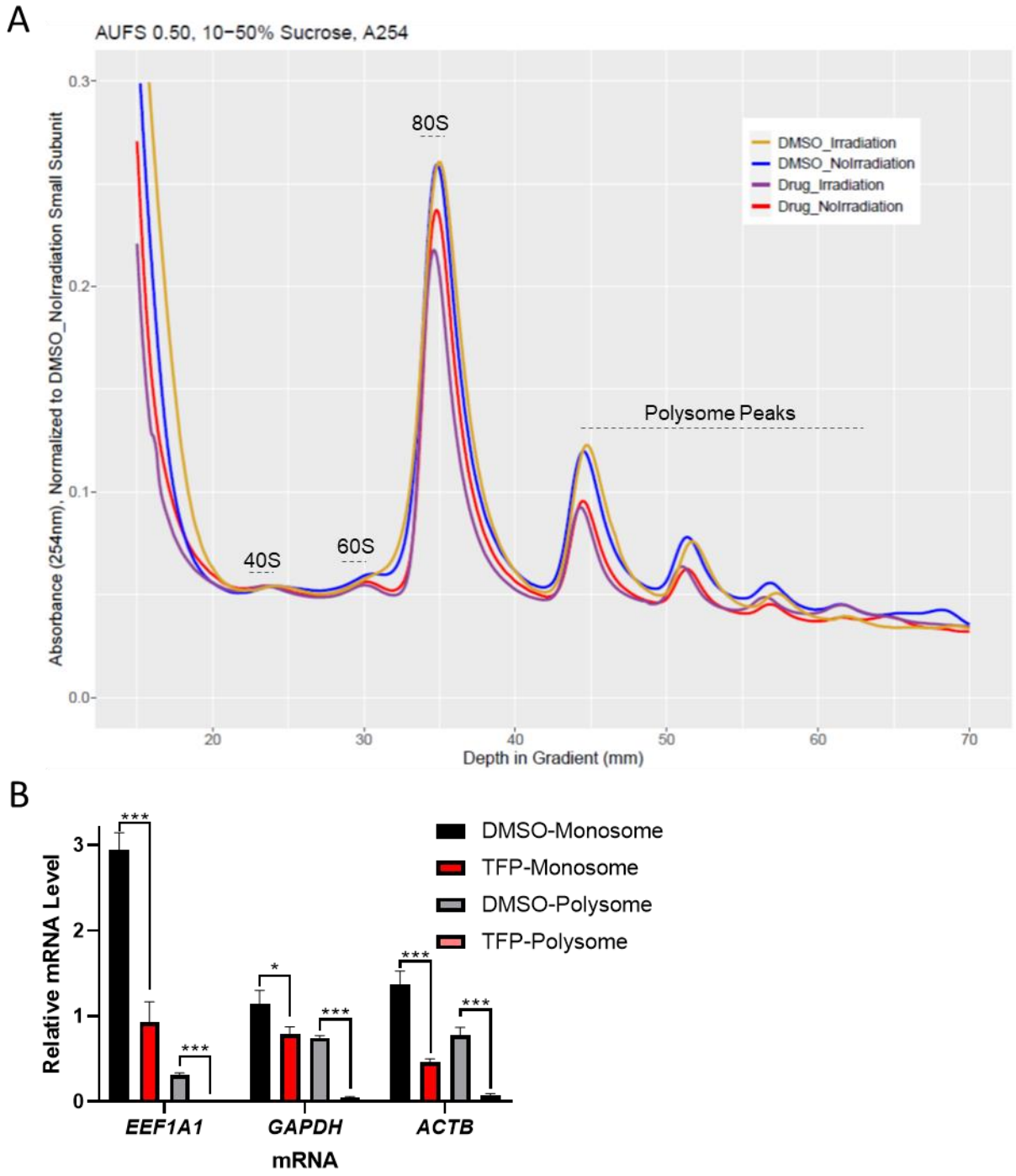

Fig. S4. TFP treatment leads to decreased mRNAs in monosome and polysome fractions.

A. Embryos at 50% epiboly were treated with DMSO or 50 μM TFP before 10 Gy irradiation at 24 hpf and collected for preparation of polysome fractionation at 25 hpf. Y-axis is

absorbance, x-axis is sedimentation fraction. Polysome profiles are normalized to the 40s ribosomal subunit peak.

B. RNA was isolated from monosome and polysome fractions of treated cells, and amounts of mRNAs *EEF1A1*, *GAPDH*, and *ACTβ* were measured by qPCR. Relative mRNA quantity represents Ct values normalized to each sample's pool of all polysome fractions. Student's t-test, *$p < 0.05$, *** $p < 0.001$.

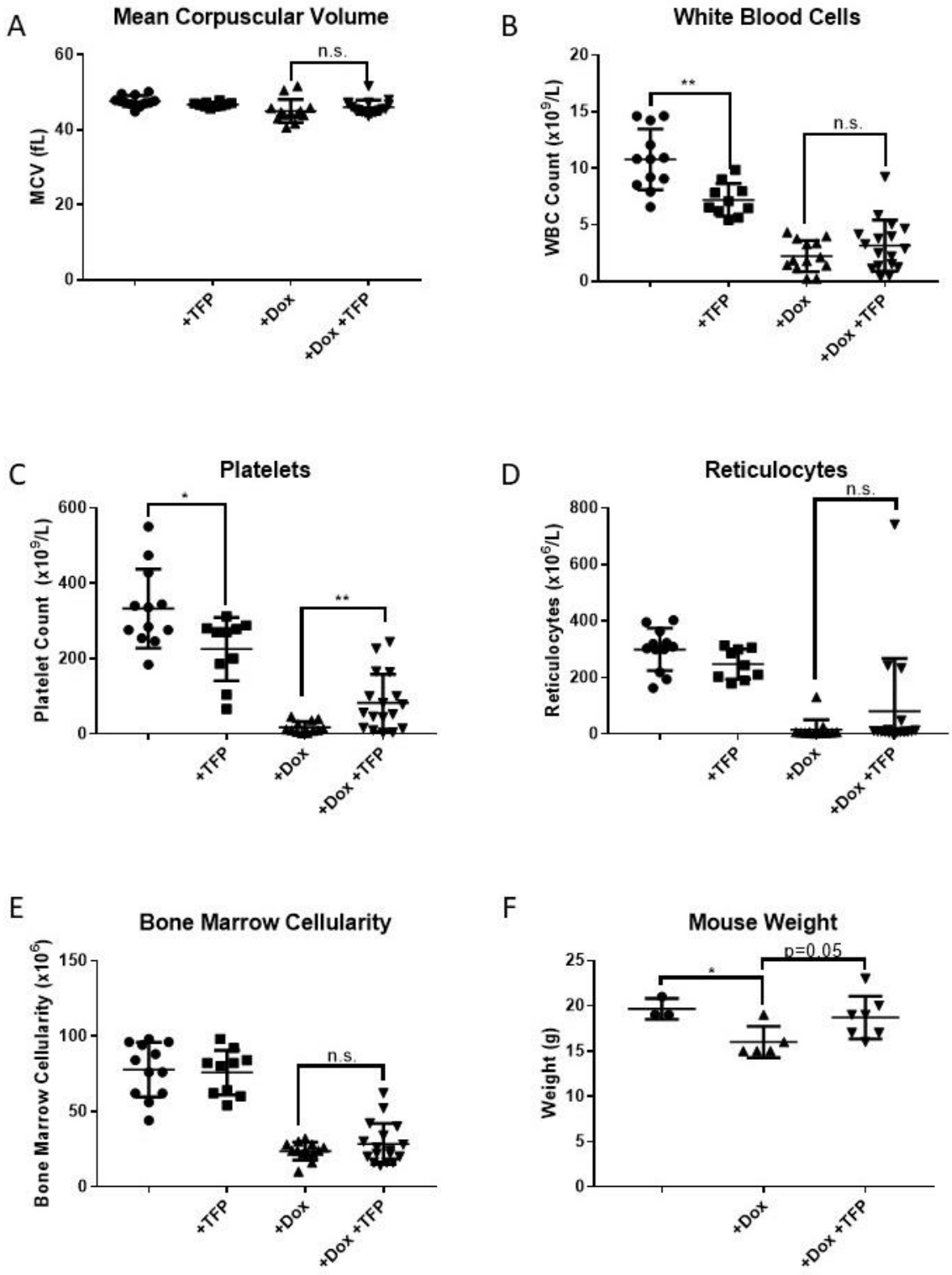

Fig. S5. TFP improves platelet counts and mouse weight in a mouse model of Diamond-Blackfan anemia.

Unfractionated bone marrow from inducible Rps19 shRNA donor mice was transplanted into irradiated wildtype recipients. After engraftment, hairpin expression was induced with doxycycline and mice were treated with TFP or vehicle for two weeks. The following were measured: (A) mean corpuscular volume (MCV), (B) white blood cells (WBC), (C) platelets, (D) reticulocytes, (E) bone marrow cellularity, and (F) mouse weight. Student's t-test, *$p < 0.05$, ** $p < 0.01$.

+TFP = TFP-treated without dox, RPS19 is wildtype

+Dox = doxycycline-treated without TFP, RPS19 is knocked down by shRNA

+Dox+TFP = both doxycycline- and TFP-treated, RPS19 is knocked down by shRNA

Calmodulin/Calcium Channel Antagonists	***Flk* Rescue?**	**Head Rescue?**
W-7	Yes	No
A-3	No	Yes
YS-035	Yes	No
Nimodipine	Yes	No
Other Ion Channel Inhibitors	***Flk* Rescue?**	**Head Rescue?**
5-hydroxydecanoate	Yes	No
Procainamide	Yes	No
Fipronil	Yes	No
Bioactive Lipids	***Flk* Rescue?**	**Head Rescue?**
Leukotriene D4	Yes	No
Eicosatrieonic acid (20:3 n-3)	Yes	No
Nitric Oxide Pathway	***Flk* Rescue?**	**Head Rescue?**
L-SNAP	Yes	No
Aminoguanidine hemisulfate	Yes	No
NF-KB Inhibitors	***Flk* Rescue?**	**Head Rescue?**
Bay 11-7082	Yes	No
Vinpocetine	Yes	No
DNA synthesis Inhibitors	***Flk* Rescue?**	**Head Rescue?**
Mitomycin C	Yes	No
Aphidicolin	Yes	No
Other	***Flk* Rescue?**	**Head Rescue?**
Capsaicin (E)	Yes	No
Pregnenonlone-16-alpha-carbonitrile	Yes	No
Betaxolol-HCL	Yes	No

Table S1. Summary of validated hits for screen phenotypes.

Chemicals are grouped by mechanism of action.

Flk1 Staining in _rps29_ Mutants

Vehicle - 46 out of 53 embryos have low ISV staining

Drug	Embryos Tested	Embryos with Wildtype ISV Staining	P-value (binomial test)
W-7	76	32	3.89197E-10
A-7	31	13	5.99465E-05
W-5	13	7	0.000514238
Trifluoperazine	24	8	0.007060806
CGS 9343B	29	14	4.55377E-06
Nimodipine	17	7	0.003307119
YS-035	12	5	0.011808909

Head Morphology in _rps29_ Mutants

Vehicle - 34 out of 37 embryos have increased cell death in the head

Drug	Embryos Tested	Embryos without Cell Death in the Head	P-value (binomial test)
A-3	14	8	3.37734E-06

Benzidine Staining in _rps29_ Mutants

Vehicle - 34 out of 40 embryos have fewer hemoglobinized cells

Drug	Embryos Tested	Embryos with Higher Benzidine Staining	P-value (binomial test)
A-3	22	10	2.87865E-06
W-7	36	24	2.95725E-18

Phospho-H3 Staining in Irradiated Embryos

Vehicle - 10 out of 10 embryos have few phospho-H3 positive cells

Drug	Embryos Tested	Embryos with Higher Number of Phospho-H3 Positive Cells	P-value (binomial test)
A-3	8	5	0
Trifluoperazine	10	6	0

Table S2. Numbers of zebrafish embryos that respond to drug treatment, with statistical analysis. Embryo counts for *flk1* staining, head morphology, benzidine staining, and phospho-H3 staining.