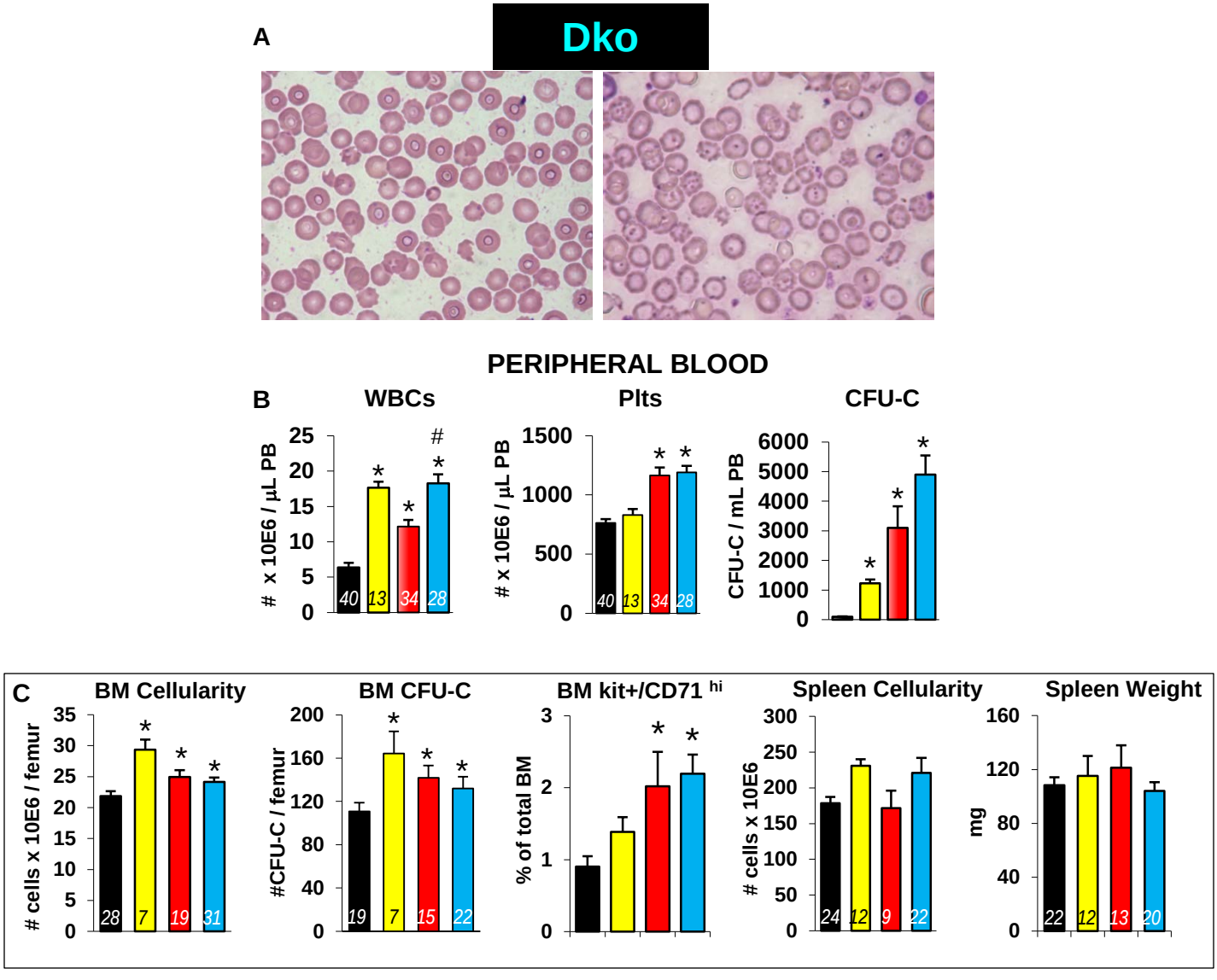
SUPPLEMENTAL FIGURE 1



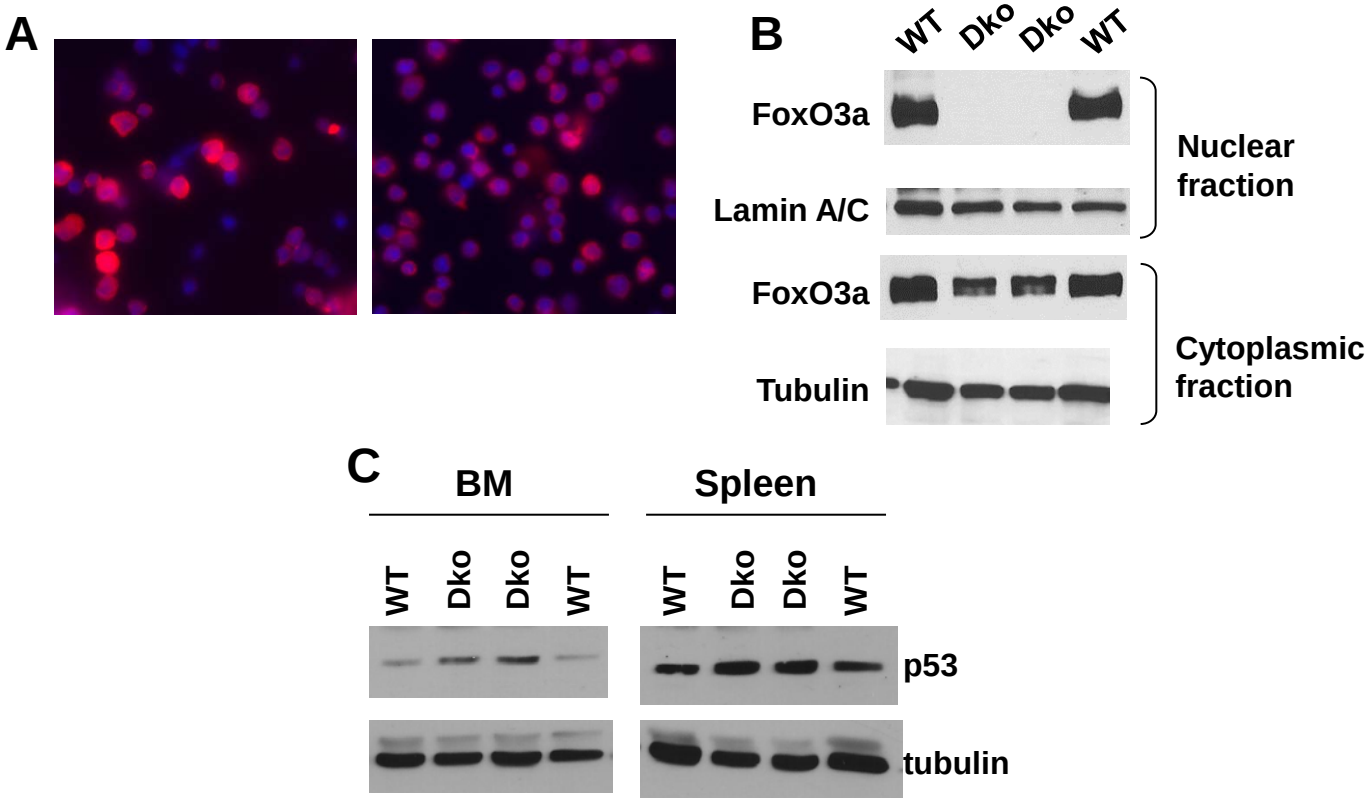
Supplemental Figure 1.

(A) Spectrum of RBC morphology seen in Dko mice with less or more anemia changes consistent with ineffective erythropoiesis are seen. PB smears were fixed and stained using PROTOCOL Hema 3 kit from Fisher Scientific. Photographs were taken of distilled water mounted slides at room temperature using a Nikon Coolpix 995 digital camera mounted to the ocular of an Olympus BHT-2 microscope at original magnification x40. Images were uploaded into a computer, and Adobe Photoshop was used to correct brightness and contrast.

(B) Levels of circulating white blood cells, platelets and hematopoietic progenitors in peripheral blood. A significant mobilization of myelo-erythroid progenitors was seen in peripheral blood (PB) of all mutant mice consistent with our previously published data,[23] but an additive or synergistic effect was seen in Dko mice. The number of platelets was also increased in $\beta1^{\Delta/\Delta}$ and Dko mice through an as yet unclear mechanism. These changes reflect the fact that integrins are deleted at the stem/progenitor level.

(C) Cellularity, total progenitor levels (CFU-C), and % of early erythroid cells ($kit^{+}/CD71^{hi}$) in BM. Spleen cellularity and splenic weight in control, $\alpha4^{-/-}$, $\beta1^{\Delta/\Delta}$ and Dko mice. Control mice (black bars), α4 mice (yellow bars), β1 mice (red bars), Dko mice (turquoise bars). *indicate $P<0.05$ difference from controls, Numbers within the bars indicate the number of mice tested.

SUPPLEMENTAL FIGURE 2

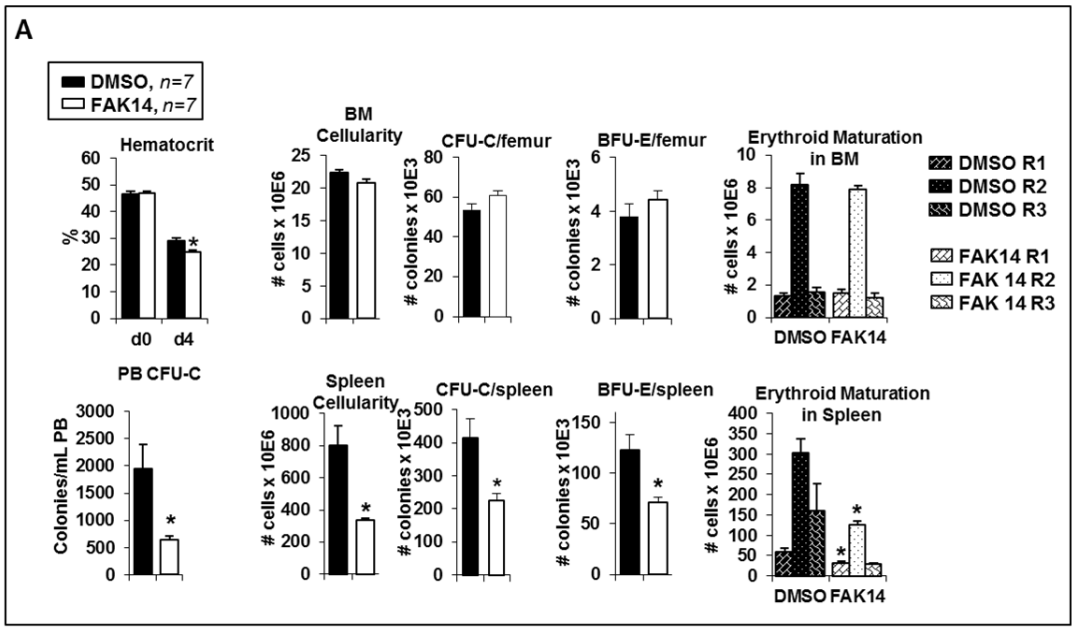


Supplemental Figure 2: (A) Anti-FoxO3 staining of TER119+ bead purified cells from controls and Dko mice 6 days after PHZ treatment. Proportions of cells with nuclear staining is higher in control cells. Cells in suspension were pre-fixed in 4% paraformaldehyde at 4º C for 10 min and after washing, stained with polyclonal FoxO3 Ab (1:100, Millipore) for 1 hr at 37º, followed by Alexa 594 goat anti-rabbit IgG secondary antibody (Molecular Probes). After washing, cells were again fixed with 4% paraformaldehyde, washed and mounted in Vectashield/DAPI (Vector Labs). Images were acquired at RT using a Leica DMLB fluorescence microscope (original magnification x 60) and a Spot RT Slider camera and SPOT Advanced software (Diagnostic Instruments Inc.).

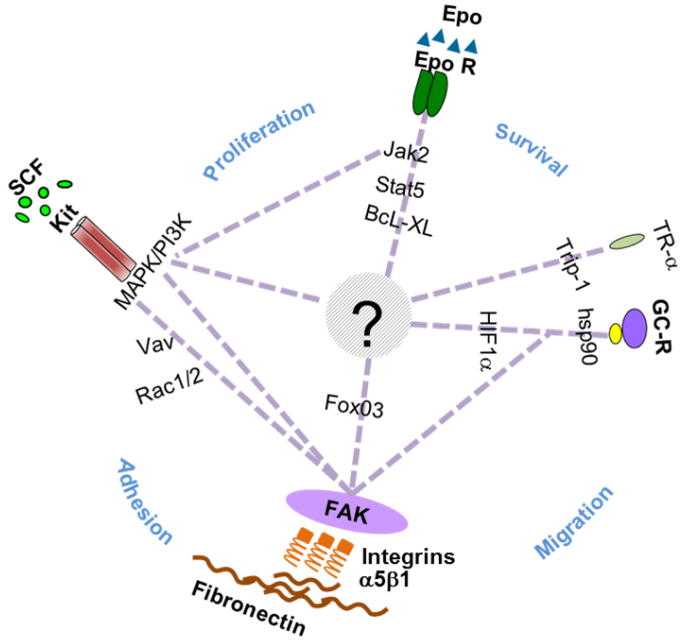
(B) Nuclear and cytoplasmic expression of FoxO3a in the spleens of Wild Type and Double knockout mice (Dko). Mice were treated with PHZ (100 mg/kg) on day 1 and sacrificed on day 4. CD71+ fraction was obtained by magnetic column enrichment using CD71-PE antibody (BD Bioscience) and anti-PE magnetic beads (Miltenyi Biotec). Nuclear and cytoplasmic fractions were obtained with NE-PER Nuclear and Cytoplasmic Extraction Reagent (Thermo Scientific) resolved by 7.5% PAGE and transferred on nitrocellulose membrane. Western blotting was performed using anti-FoxO3a or Lamin A/C, or α-Tubulin antibody (Cell Signaling), followed respectively by anti-Rabbit-HRP or anti-mouse-HRP antibody (Santa Cruz), and ECL detection (Thermo Scientific).

(C) Expression of p53 in BM and spleens of Wild Type and α4β1, double knockout mice. Mice were treated with PHZ (100 mg/kg) on day 1 and sacrificed on day 4. Ter119+ fraction and CD71+ fraction was obtained from BM and spleen respectively by magnetic column enrichment using Ter119-biotin or CD71-PE antibody (BD Bioscience) and anti-biotin or anti-PE magnetic beads (Miltenyi Biotec). Cytoplasmic fractions were obtained with NE-PER Nuclear and Cytoplasmic Extraction Reagent (Thermo Scientific) resolved by 7.5% PAGE and transferred on nitrocellulose membrane. Western blotting was performed using anti-p53 or anti-Tubulin antibody (Cell Signaling), followed by anti-mouse-HRP antibody, (Santa Cruz) and ECL detection (Thermo Scientific)

SUPPLEMENTAL FIGURE 3



B **Signaling Pathways Enhancing Erythroid Stress Proliferative Response in Spleen.**



Supplemental Figure 3A. Response of FAK14-treated normal mice to PHZ.
Control mice received daily injections of FAK inhibitor 14 (25 mg/kg, Tocris Bioscience) (n=7, experimental group, white bars) or vehicle (DMSO, n=7, control group, black bars) for 4 consecutive days (day 1 to day 4) and on day 2 received a single injection of PHZ (100 mg/kg). All animals were sacrificed on day 4. At sacrifice the following parameters were measured: Hematocrit, BM and spleen cellularity and progenitors (CFU-C and BFU-e) in peripheral blood, BM and spleen as well as erythroid maturation profile in BM and spleen. * indicates significant difference over control group, $P< 0.05$.

Supplemental Figure 3B. Important participating pathways leading to proliferative expansion of erythroid cells (mainly in the spleen) during stress. Following genetic impairment of several pathways (i.e., Kit, GC-R, TR-α, and β1 integrins in a cell intrinsic manner, see Suppl. Table 1 for references), proliferative erythroid expansion in the spleen is severely compromised even in the presence of adequate levels of Epo. It is possible that β1 integrins, (specifically α5β1) intercept at least some of these known pathways, but the molecular details and cooperating/interacting molecules are missing (illustrated by dashed lines). Interpretations of the influence of these pathways and their signaling intermediates are complicated by the fact that these pathways have not been selectively eliminated in erythroid cells. Consequently, it is not clear at what stage of erythroid differentiation the FAK- or Src-dependent signaling is exerted, early or late or both, and future studies should clarify these issues.

SUPPLEMENTAL TABLES

Supplemental Table 1. Mutant mice with impaired responses to PHZ-induced stress.

Erythroid Proliferative Expansion			Terminal Maturation	Refs.
BM		**SPLEEN**		
		Expansion impairment	*Impairment*	
NO	Stat5a,b–/–	NO	YES	6
NO	Gas6–/–	NO	YES	47
NO	Rb–/–	NO	YES	8, 48
YES	[Rac1/Rac2] –/–	NO	YES	
NO	FoxO3–/–	NO	YES	10
NO	Nrf2+Sel–/–	NO	YES	12, 13
NO	miR144/451–/–	NO	YES	
NO	SOD2–/–	NO	YES	5, 7, 9
NO	PRDX2–/–	NO	YES	11, 14
NO	HO-1–/–	NO	YES	
NO	Kit (W/W^v)	Delayed	NO	
NO	f/f (BMP4)	Delayed	NO	
YES	Spi2A–/–	Delayed	YES	49
NO	α4–/–	NO	YES	15
	Lyn–/–	NO	YES	16
	Kit PY567	YES	?	19
	TRα–/–	YES	?	50
NO	GC-R–/–	YES	NO	51
NO	FAK–/–	YES	?	44
NO	β1–/–	YES	YES	23

Signal transducer and activation of transcription5a,b (Stat5a,b); Growth arrest specific 6 (Gas6); retinoblastoma protein (Rb): Ras-related C3 botulinum toxin substrate (RAC); Forkhead box O3 (FoxO3); Nuclear factor (erythroid-derived 2)-like 2+ Selenoproteins (Nrf2+Sel)**;** microRNA (miR); Superoxide dismutase 2, mitochondrial (SOD2); Peroxiredoxin-2 (PRDX2); heme oxygenase-1 (HO-1); Bone morphogenetic protein 4 (BMP4); Glucocorticoid receptor **(**GC-R); focal adhesion kinase (FAK).

For references, see reference list in manuscript. These references are meant to be representative rather than all inclusive.

Supplemental Table 2. Red Cell Parameters in Control, $\alpha 4^{-/-}$, $\beta 1^{\Delta/\Delta}$, and Dko Mice in Standard International Units

	RBC	**Hb**	**HCT**	**MCV**	**MCH**	**MCHC**	**RDW**
	x 10^{12}/L	g/L	Prop. of 1.0	fL	fmol/cell	mmol/L	%
Ctrl	9.0 ± 0.1	141 ± 1.4	0.479 ± 0.17	53.0 ± 1.3	0.97 ± 0.01	18.3 ± 0.3	17.7 ± 0.3
n=17							
$\alpha 4^{-/-}$	9.1 ± 0.1	141 ± 1.3	0.453 ± 0.14	50.0 ± 1.2	0.97 ± 0.01	**19.5** ±0.5	17.9 ± 0.2
n=8						*	
$\beta 1^{\Delta/\Delta}$	**7.9** ± 0.3	**128** ± 5.3	**0.416** ± 0.02	53.6 ± 2.0	1.01 ± 0.02	19.0 ± 0.3	**23.6** ± 1.1
n=14	*	*	*				*
Dko	**6.1** ± 0.3	**113** ± 4.7	**0.388** ± 0.015	**64.9** ± 2.1	**1.17**± 0.03	18.1± 0.3	**25.8** ± 1.3
n=16	*	*	*	*	*		*

RBC, red blood cells; Hb, Hemoglobin; HCT, Hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; RDW, red cell distribution width. ± indicates standard error of the mean; ∗indicates $P<0.05$ calculated with Microsoft Excel.

Supplemental Table 3. Kit^{+} $\beta^{\Delta/\Delta}$ mRNA Arrays

Gene Abbreviation	**Gene Name**	**Fold decrease over controls**
GLRX1	Glutaredoxin	9.07
HMOX1	Heme oxygenase 1	3.53
CAT	Catalase	2.27
PRDX2	Peroxiredoxin 2	2.17