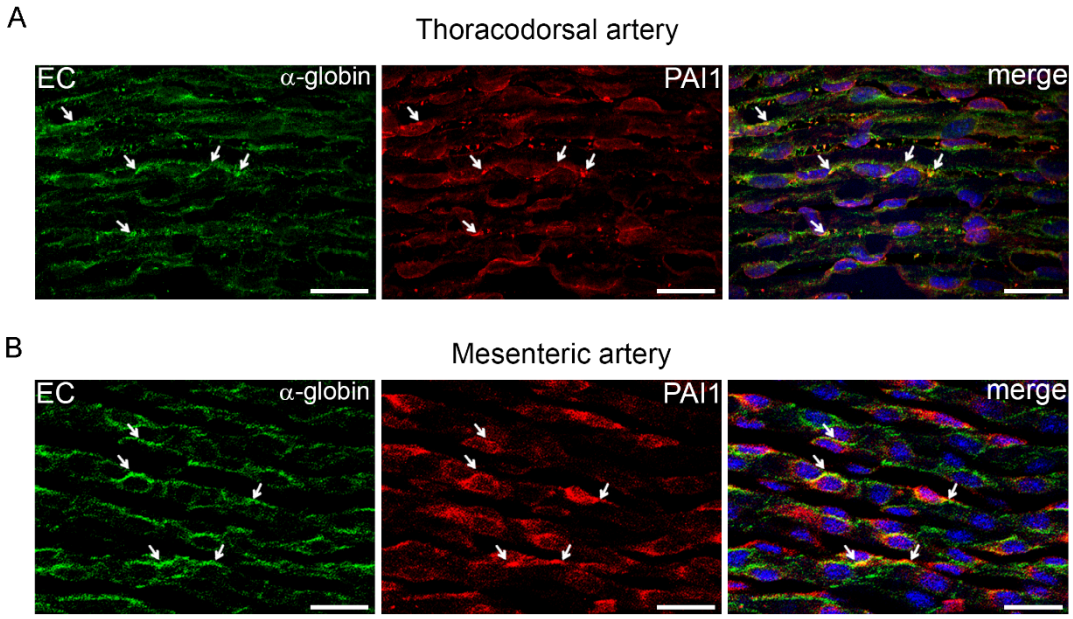
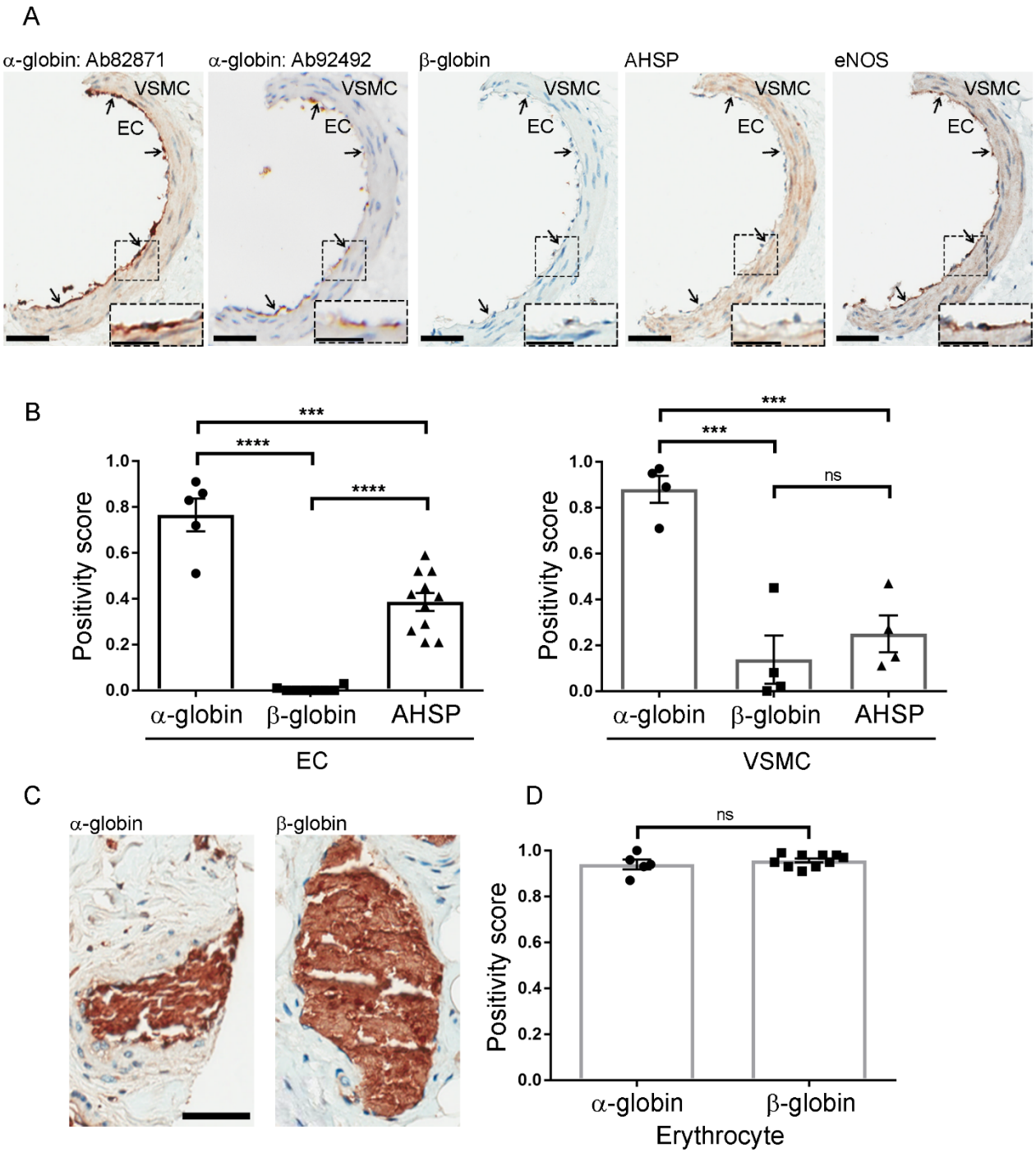
Supplemental Figures

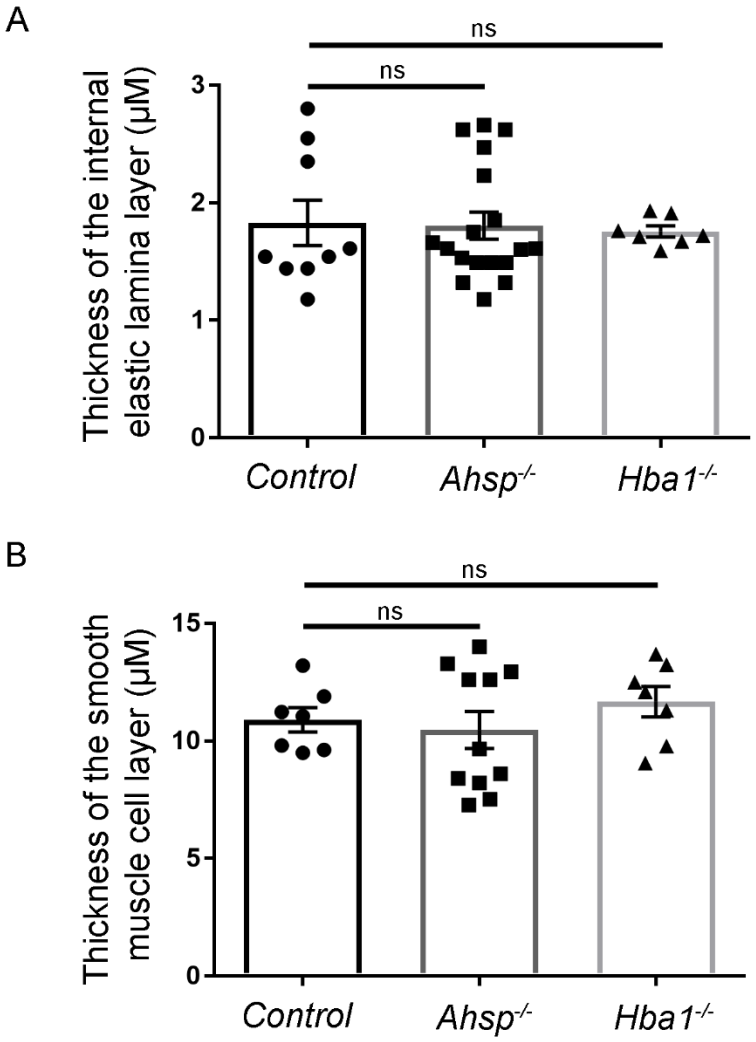


Supplemental Figure 1. Indirect immunofluorescence staining for α-globin and PAI1 (a myoendothelial junction marker) in flat-mount thoracodorsal (**A**) and mesenteric (**B**) artery preparations from 6-month–old wild-type mice. DAPI-stained nuclei appear blue. Images are shown at the same magnification with scale bars equivalent to 25 μm.

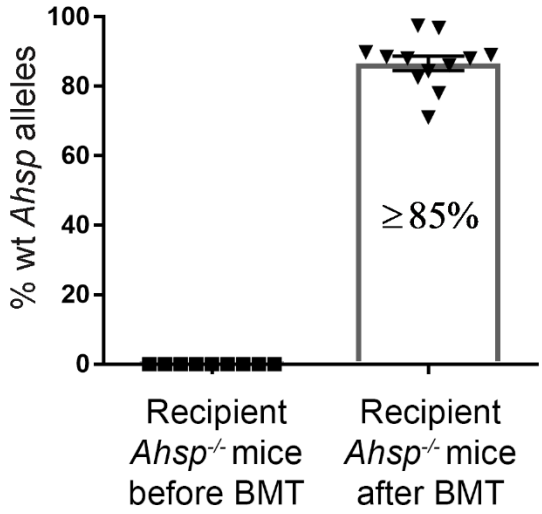


Supplemental Figure 2. Immunohistochemical analysis of human coronary arteries. (**A**) Expression of α-globin (detected by two different antibodies), β-globin, AHSP, and eNOS in adjacent cross-sections. Note that α-globin and AHSP (indicated by arrows), but not β-globin, are expressed in endothelial cells (ECs) and vascular smooth muscle cells

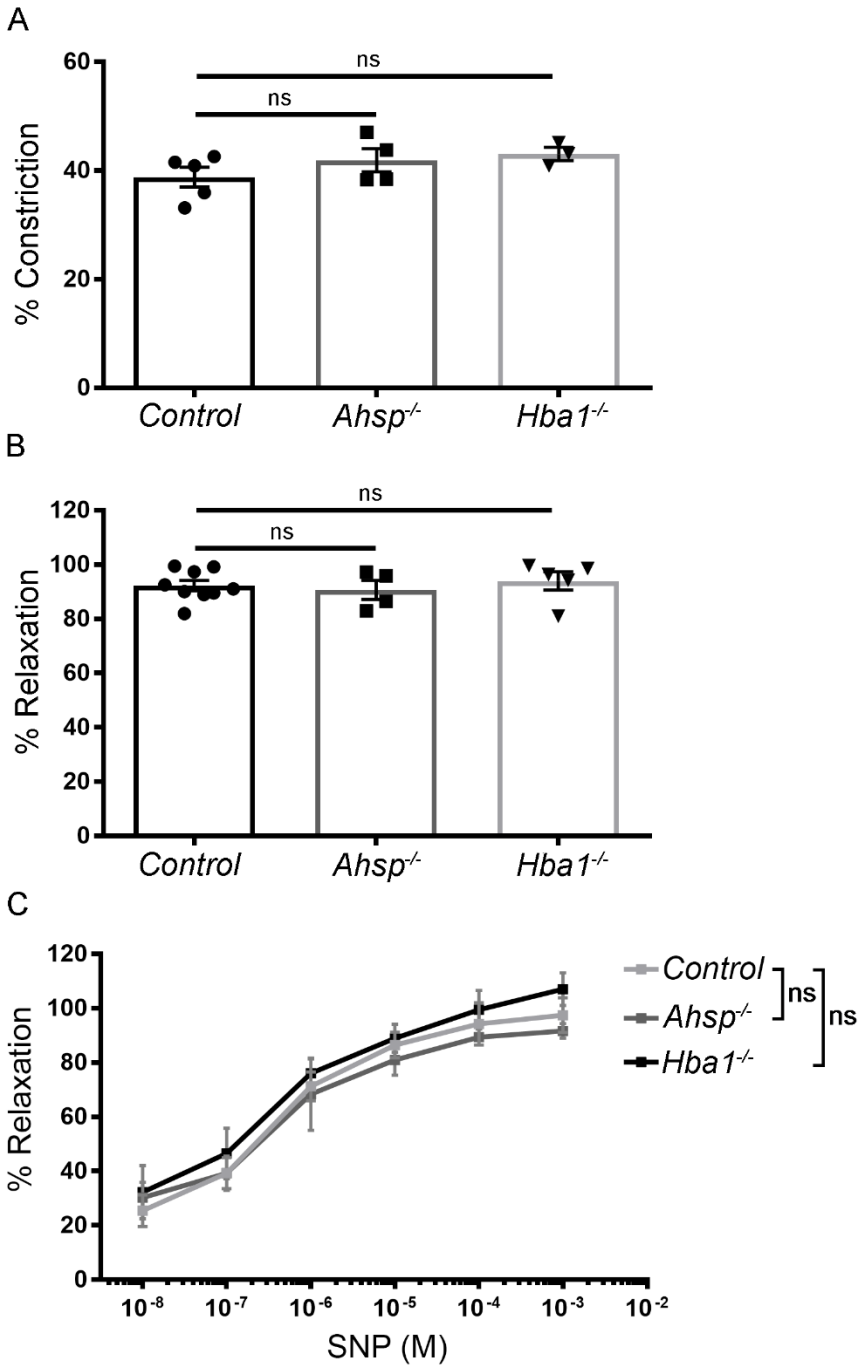
(VSMC). Images are shown at the same magnification with scale bars equivalent to 50 μm (main images) and 25 μm (inset rectangles). (**B**) Positivity scores for multiple experiments performed as in panel A, with each symbol representing a sample from a different individual (n = 4–6). (**C**) Expression of α-globin and β-globin proteins in luminal red blood cells. Images are shown at the same magnification with scale bars equivalent to 50 μm. (**D**) Scatter plot summarizing the positivity scores for multiple experiments performed as in panel C, with each symbol representing a sample from a different individual (n = 4–6). Statistical significance was determined using an unpaired *t*-test. ***$P < 0.005$; ****$P < 0.001$; ns: not significant.



Supplemental Figure 3. Thicknesses of the internal elastic lamina (**A**) and the vascular smooth muscle layer (**B**) of TDAs from control (n = 7), *Ahsp*$^{-/-}$ (n = 11), and *Hba1*$^{-/-}$ (n = 7) mice. Measurements were taken at three separate locations for each artery. Statistical significance was determined using an unpaired *t*-test. ns: not significant.

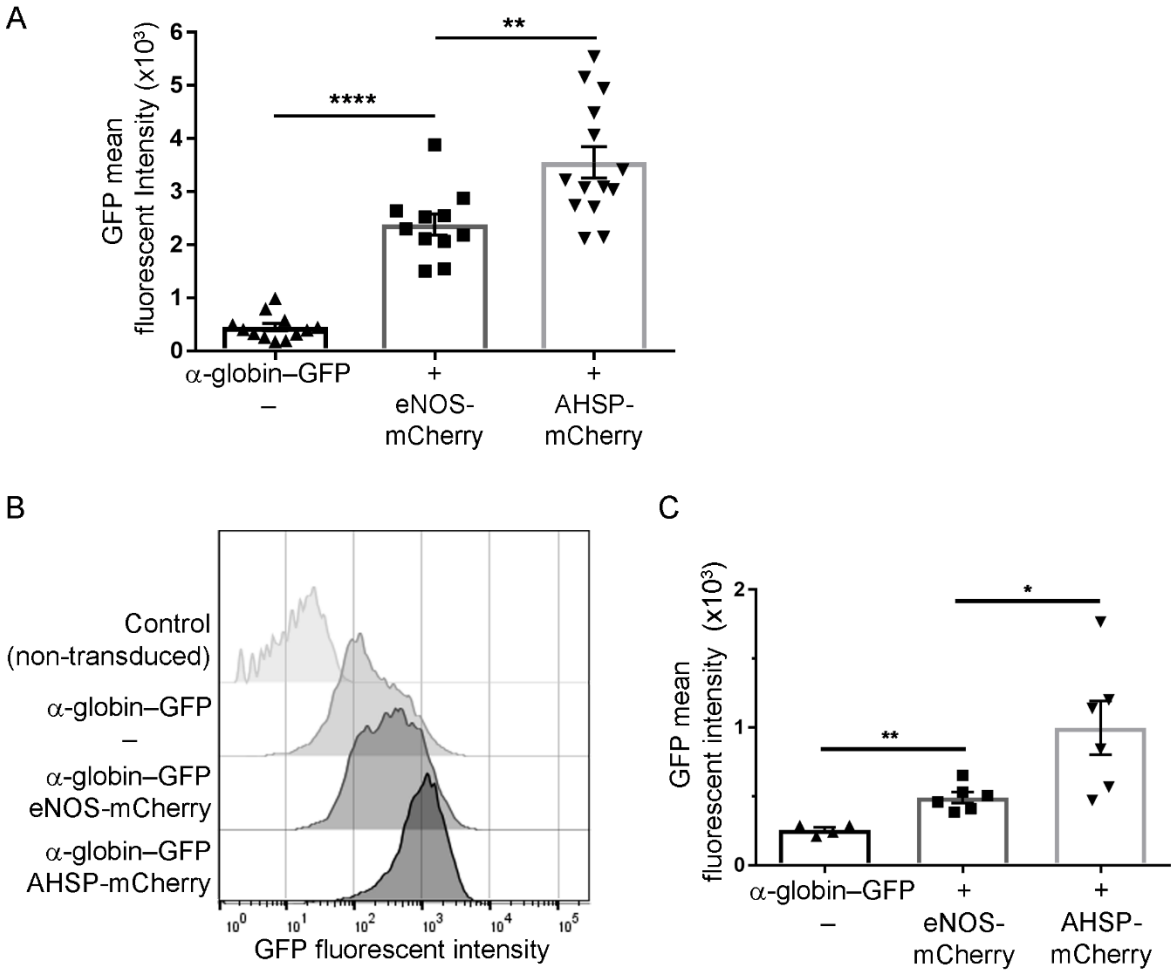


Supplemental Figure 4. Hematopoietic reconstitution after bone marrow transplant (BMT). Wild-type bone marrow cells were transplanted into lethally irradiated *Ahsp*$^{-/-}$ mice. Four weeks later, donor reconstitution was measured by PCR analysis of circulating mononuclear cells for the presence of WT and mutant *Ahsp* alleles (n = 12) (1).

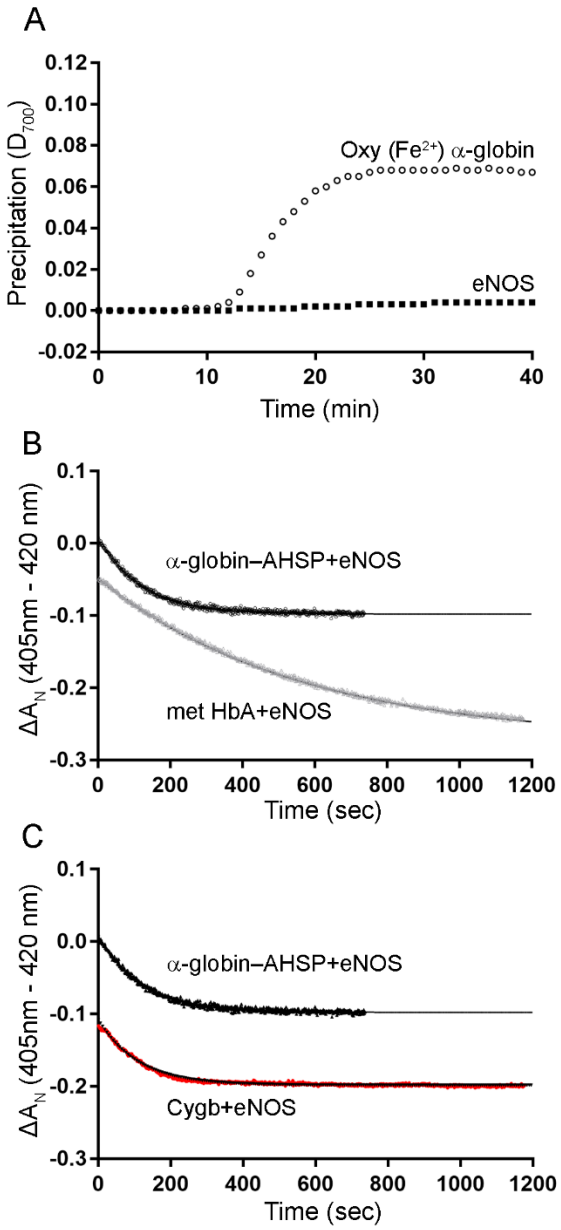


Supplemental Figure 5. Smooth muscle contractility, coupling with ECs, and sensitivity to NO are not altered by *Ahsp* or *Hba1* gene disruption. (**A**) Vasoconstriction of thoracodorsal arteries (TDAs) from control (n = 5) and mutant (*Ahsp*$^{-/-}$ [n = 4] and *Hba1*$^{-/-}$ [n = 3]) mice after treatment with 40 mM KCl. (**B**) Vasodilation of TDAs from control (n = 9) and mutant (*Ahsp*$^{-/-}$ [n = 4] and *Hba1*$^{-/-}$ [n = 5]) mice after treatment with the intermediate

and small potassium (IK/SK) channel activator NS309 (1 μM). (**C**) Vasodilation of TDAs from control (n = 9) and mutant (*Ahsp*$^{-/-}$ [n = 4] and *Hba1*$^{-/-}$ [n = 5]) mice after treatment with escalating doses of the NO donor sodium nitroprusside (SNP). Statistical significance was determined using an unpaired *t*-test. ns: not significant.

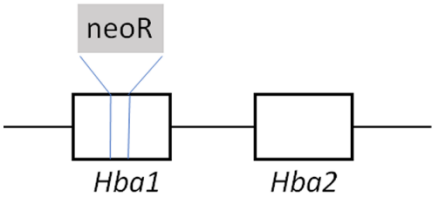


Supplemental Figure 6. AHSP or eNOS stabilize α-globin in ECs. Coronary artery ECs in culture were transduced with lentiviral vectors encoding α-globin–GFP, AHSP-mCherry, or eNOS-mCherry (see also Figure 5 in the main paper). (**A**) The mean fluorescence intensities of α-globin–GFP were determined by immunofluorescence microscopy using Nikon NIS-Elements software, version 4.50. The bar chart depicts the mean fluorescence intensity of α-globin–GFP when expressed alone, with AHSP-mCherry, or with eNOS-mCherry (9–10 fields were assessed for each condition, with approximately 10 cells per field). (**B**) The mean fluorescence intensities of α-globin–GFP expressed alone, with AHSP-mCherry, or with eNOS-mCherry, as quantified by flow cytometry. (**C**) Summary of the results of multiple flow-cytometry experiments. Statistical significance was determined using an unpaired *t*-test. **P* < 0.05; ***P* < 0.01; *****P* < 0.001.

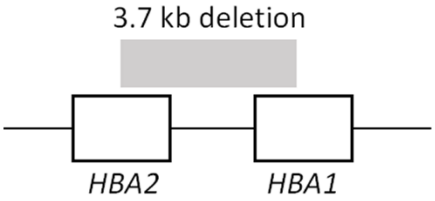


Supplemental Figure 7. (**A**) Oxygenated (Fe^{2+}) α-globin (15 μM) or full-length recombinant eNOS (15 μM) were incubated with potassium ferricyanide (50 μM final concentration), and protein precipitation was monitored by light absorbance at 700 nm. (**B**) Reduction of (Fe^{3+}) α-globin-AHSP (0.5 μM) or metHbA (1.5 μM) by recombinant human eNOS (1 μM). (**C**) Reduction of (Fe^{3+}) α-globin–AHSP (0.5 μM) or cytoglobin (Cygb, 0.5 μM) by recombinant human eNOS (1 μM). Reactions for (**B**) and (**C**) were performed at 25°C in PBS under anaerobic conditions with 50 μM NADPH and 250 U/mL catalase.

A Disruption of the mouse α-globin gene locus by homologous recombination



B Human common deletions in the α-globin gene cluster



Supplemental Figure 8. α-Globin gene disruptions in the *Hba1*$^{-/-}$ mice used in this study (2) (A) and in Kenyan individuals with α-thalassemia trait examined by Etyang et al. (3) (B). The diagram in panel B is modified from that of Shaji et al. (4).

References

1. Kihm AJ, Kong Y, Hong W, Russell JE, Rouda S, Adachi K, Simon MC, Blobel GA, and Weiss MJ. An abundant erythroid protein that stabilizes free alpha-haemoglobin. *Nature.* 2002;417(6890):758-63.
2. Chang J, Lu RH, Xu SM, Meneses J, Chan K, Pedersen R, and Kan YW. Inactivation of mouse alpha-globin gene by homologous recombination: mouse model of hemoglobin H disease. *Blood.* 1996;88(5):1846-51.
3. Etyang AO, Khayeka-Wandabwa C, Kapesa S, Muthumbi E, Odipo E, Wamukoya M, Ngomi N, Haregu T, Kyobutungi C, Tendwa M, et al. Blood Pressure and Arterial Stiffness in Kenyan Adolescents With alpha+Thalassemia. *J Am Heart Assoc.* 2017;6(4).
4. Shaji RV, Srivastava A, Chandy M, and Krishnamoorthy R. A single tube multiplex PCR method to detect the common alpha+ thalassemia alleles. *Blood.* 2000;95(5):1879-80.

Supplementary Table 1. Antibodies used in this study.

Antibody	Type	Concentration	Supplier, reference
α-globin	Polyclonal	2.5 µg/mL (histology, mouse)	Abcam, Ab102758
α-globin	Polyclonal	10 µg/mL (histology, mouse and human)	Abcam, Ab92492
α-globin	Polyclonal	10 µg/mL (histology, human)	Abcam, Ab82871
α-globin	Polyclonal	(Western blot)	Home-made
β-globin	Polyclonal	10 µg/mL (histology, human)	Novus Biologics NBP2-14081
β-globin	Polyclonal	0.2 µg/mL (histology)	RayBiotech 119-14366
β-globin	Polyclonal	0.2 µg/mL (Western blot)	Santa Cruz SC-31116
AHSP	Polyclonal	5–10 µg/mL (histology, mouse and human)	Rockland 100-401-E79S
AHSP	Polyclonal	10 µg/mL (histology, mouse and human)	Abcam, Ab180861
AHSP	Polyclonal	(Western blot)	Home-made
eNOS/NOSIII	Monoclonal	1 µg/mL (histology, mouse) 0.2 µg/mL (Western blot)	BD 610297
eNOS/NOSIII	Polyclonal	2.5 µg/mL (histology, human)	Abcam, Ab5589
PAI 1	Monoclonal	1 µg/mL (histology, mouse) 0.2 µg/mL (Western blot)	Abcam, Ab125687
Alexa 488	Rabbit	4 µg/mL	Invitrogen, Life Technologies, A11008
Alexa 594	Mouse	4 µg/mL	Invitrogen, Life Technologies, A11005
Fibronectin	Polyclonal	200 µg/mL	Santa Cruz, SC-6952
Laminin	Rabbit	4100 µg/mL	DAKO, Z0097
Goat anti-rabbit IgG	HRP conjugate	0.05 µg/mL	Jackson ImmunoResearch Laboratories, 111-035-003
Donkey anti-chicken IgG	HRP conjugate	0.05 µg/mL	Jackson ImmunoResearch Laboratories, 703-035-155

Supplementary Table 2. Red blood cell indices of *Ahsp*$^{-/-}$ and *Hba1*$^{-/-}$ mice at baseline and six to eight weeks post bone marrow transplant (BMT) with wild-type donor hematopoietic stem and progenitor cells. RDW: red cell distribution width. One-way ANOVA statistical analysis. Statistical significance was determined using unpaired t test. *$P < 0.05$; ****$P < 0.001$; ns: not significant.

Genotype:	**Wild-type**	***Ahsp*$^{-/-}$**	***Ahsp*$^{-/-}$ post-BMT**	***Hba1*$^{-/-}$**	***Hba1*$^{-/-}$ post-BMT**
n	6	6	6	6	4
Hemoglobin (g/dL)	13.02 ± 0.10	12.27 ± 0.34 ns	13.65 ± 0.22 ns	12.32± 0.35 ns	13.3 ± 0.52 ns
RDW (%)	13.55 ± 0.20	14.63 ± 0.45 ns	13.83 ± 0.49 ns	25.75 ± 1.28 ****	14.4 ± 0.89 ns
Reticulocyte (%)	2.38 ± 0.47	5.07 ± 1.15 *	2.85 ± 1.27 ns	8.17 ± 0.74 ****	2.62 ± 0.12 ns

Supplementary Table 3. Oligonucleotide primers used for quantitative RT-qPCR.

GENE	FORWARD PRIMER 5′→ 3′	REVERSE PRIMER 5′→ 3′
HBA	GCTCTCTGGGGAAGACAAAA	GCCGTGGCTTACATCAAAGT
AHSP	GGATCAGCAGGTCTTTGATGA	TTGCTGGAATTCTGTCATGG
HBB	GCTGGTTGTCTACCCTTGGA	GGCTGTCCAAGTGATTCAGG
ENOS	GCACCCAGAGCTTTTCTTTG	GTCAACCGAACGAAGTGACA
GAPDH	GTGTTCCTACCCCCAATGTG	AGGAGACAACCTGGTCCTCA
ACTB	CCATCTACGAGGGCTATGCT	TTTGATGTCACGCACGATTT