Non-hematopoietic Nrf2 dominantly impedes adult-progression of sickle cell anemia in mice

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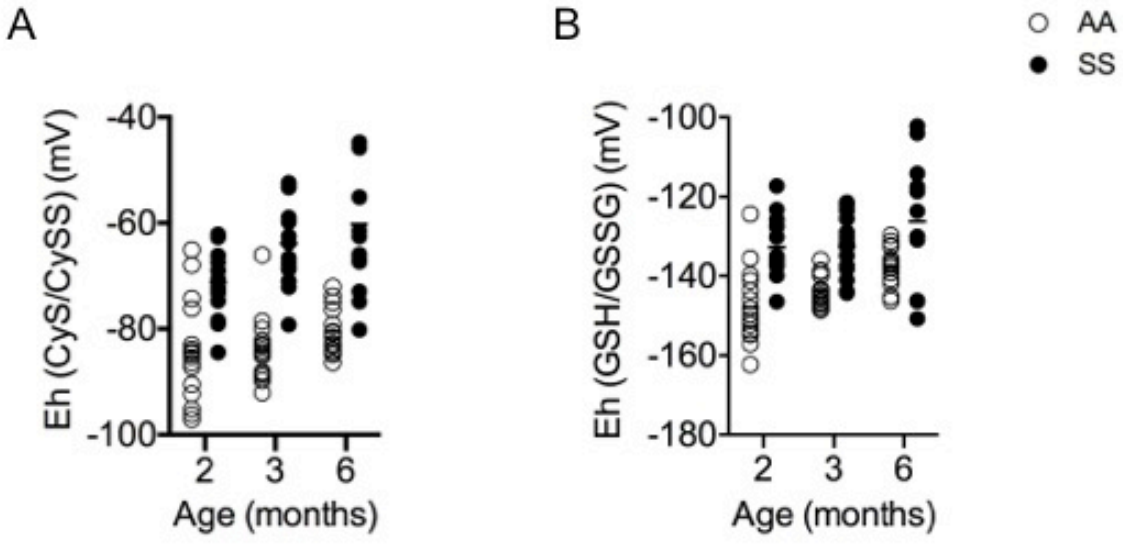
Conflict of Interest:

The authors have declared that no conflict of interest exists

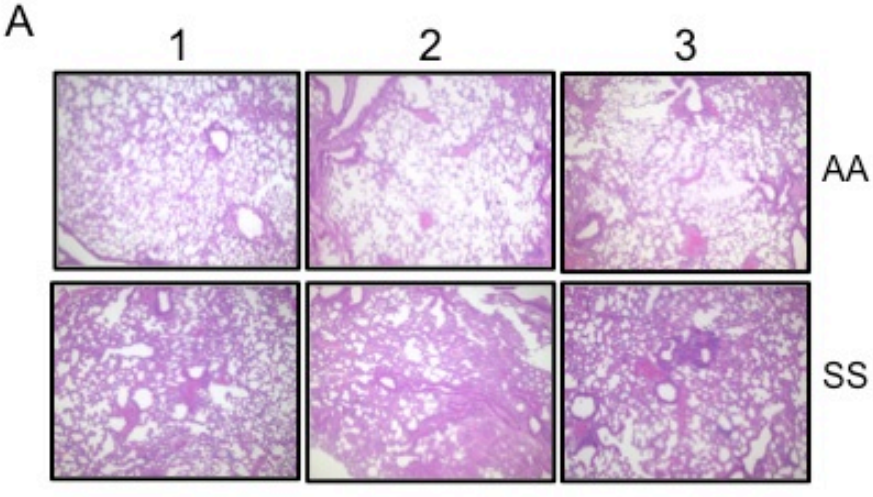
Supplementary Table S1. Hematological parameters in chimeric mice and in donor and recipient littermates.

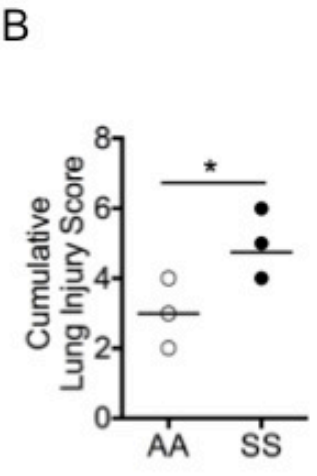
	$SS^{NHNrf2-/-}$	$SS^{Nrf2+/+(B6)}$	$SS^{WT(DL)}$	$Nrf2^{-/-(RL)}$	$AA^{NHNrf2-/-}$
Total Hb (g/dl)	**8.57±0.3**	**7.28±0.3**	**7.48±0.2**	**15.7±0.18**	**12.24±0.27**
Retics (%)	**43.61±0.14**	**45.02±4.16**	**41.96±0.86**	**6.16±0.1**	**7.12±0.18**
WBC ($x10^3$/µl)	**24.83±1.05**	**23.04±3.38**	**30.26±3.6**	**5.7±0.73**	**18±2.1**
RBC ($x10^6$/µl)	**7.58±0.22**	**6.45±0.14**	**6.92±0.14**	**9.85±0.74**	**11.16±0.26**
PLT ($x10^3$/µl)	**492.25±15.1**	**523±34.9**	**605±44.4**	**340±47.8**	**783±39.4**
HCT (%)	**32.12±1.11**	**27.24±0.76**	**30.38±0.68**	**45.7±3.8**	**37.3±1.05**



Supplementary Figure 1

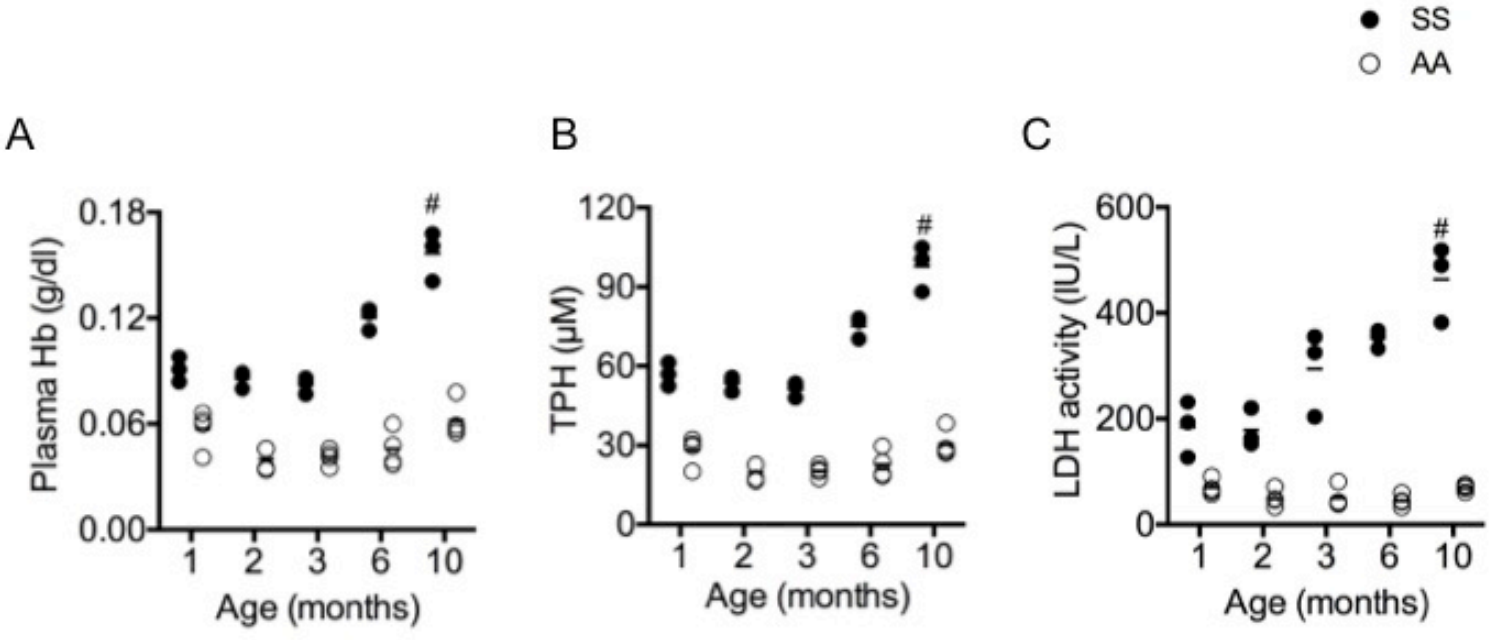
Supplementary Figure 1. Plasma amino thiols are oxidized at an early maturation stage in SS but not in the AA mice. **(A)** CyS/CySS redox potential (E_h Cys/CySS) was no different at the three maturation stages in the AA mice while it increased at every stage in the SS mice. **(B)** The GSH/GSSG redox potential (E_h GSH/GSSG) in the 2-month old SS mice was as oxidized as in the 6-month old AA mice (n=8).





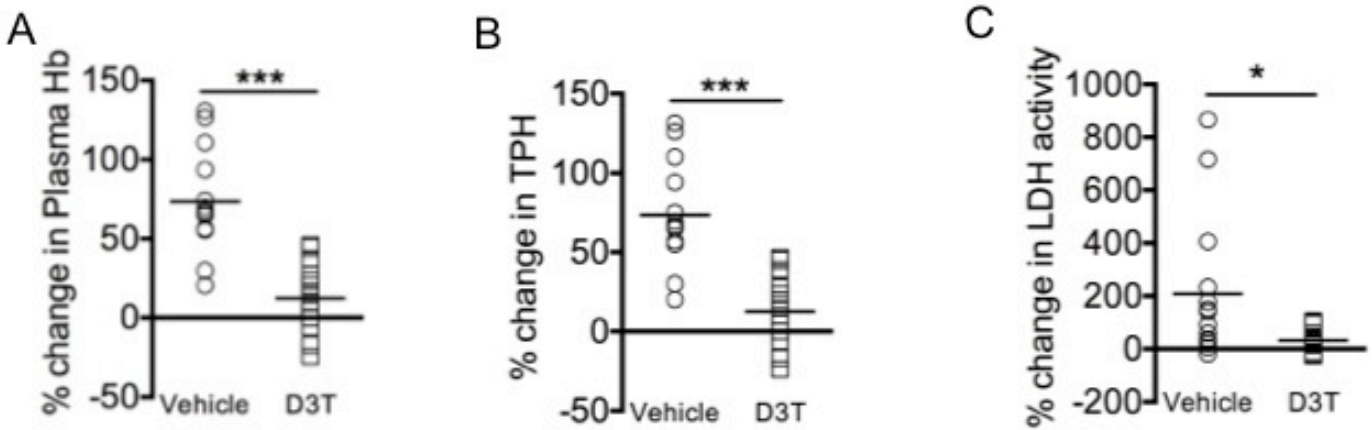
Supplementary Figure 2

Supplementary Figure 2. Severe lung damage in mature SS mice. (A) Representative H&E stained lung sections of 10-month old 3 SS and 3 AA mice show severe congestion in the SS mice. Original magnification 100X. **(B)** Cumulative histological injury score derived from congestion and alveolar wall thickness from H&E stained lung sections of 10-month old SS and AA mice (n=5; *$p<0.05$).



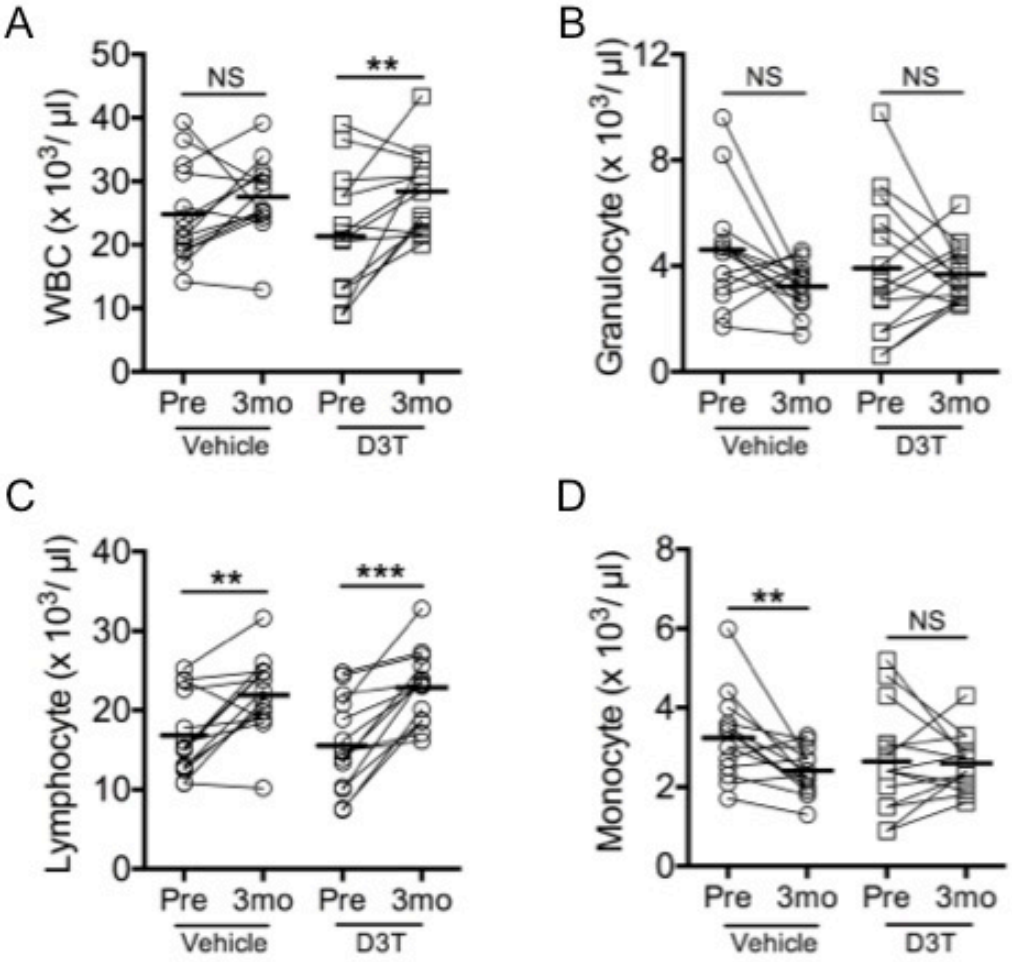
Supplementary Figure 3

Supplementary Figure 3. Intravascular hemolysis in random cross-section of SS mice. (A-C) Plasma was collected from a random selection of sickle (SS) and control (AA) mice aged 1-10 months (n=3). Age related deterioration of intravascular hemolysis defined by elevated levels of plasma Hb, TPH and LDH activity in the cross-sectional cohort of sickle mice. # signifies increase ($p<0.001$; ANOVA) in indicated hemolytic parameters with age within SS group of mice.



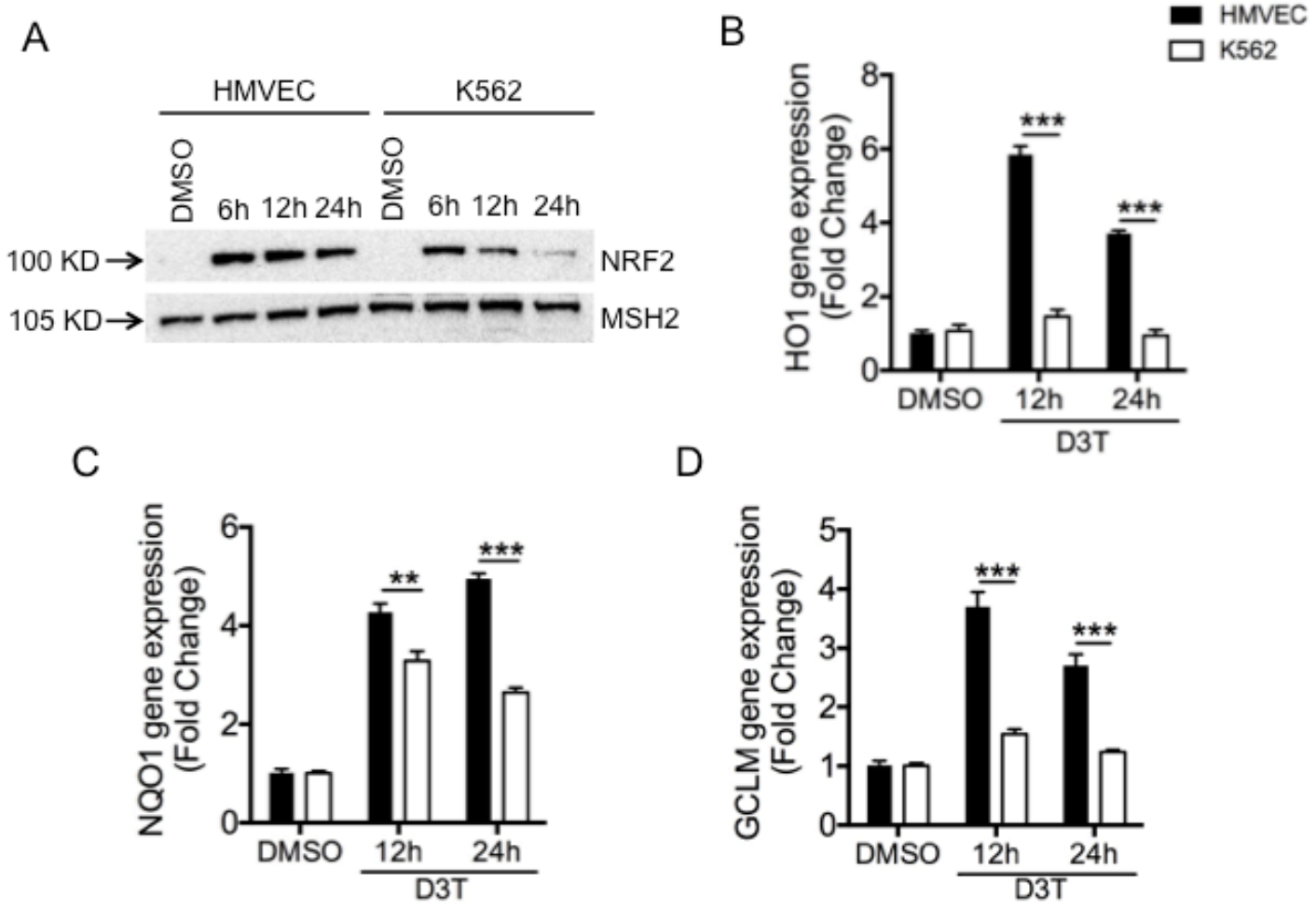
Supplementary Figure 4

Supplementary Figure 4. Nrf2 activation stabilizes intravascular hemolysis in sickle mice. **(A-C)** Percent change in plasma Hb, TPH and LDH activity following 3 months treatment with D3T or vehicle (n=14-15).



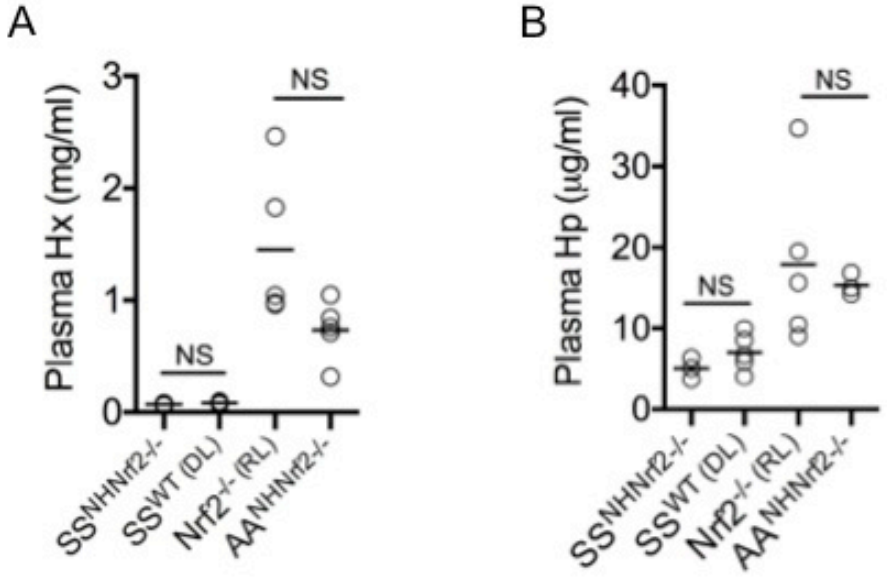
Supplementary Figure 5

Supplementary Figure 5. Effect of D3T treatment on white cell counts in sickle mice. **(A-D)** Automated white cell count with a 3-part differential at the start of treatment (Pre) and after 3-months (3 mo) of treatment.



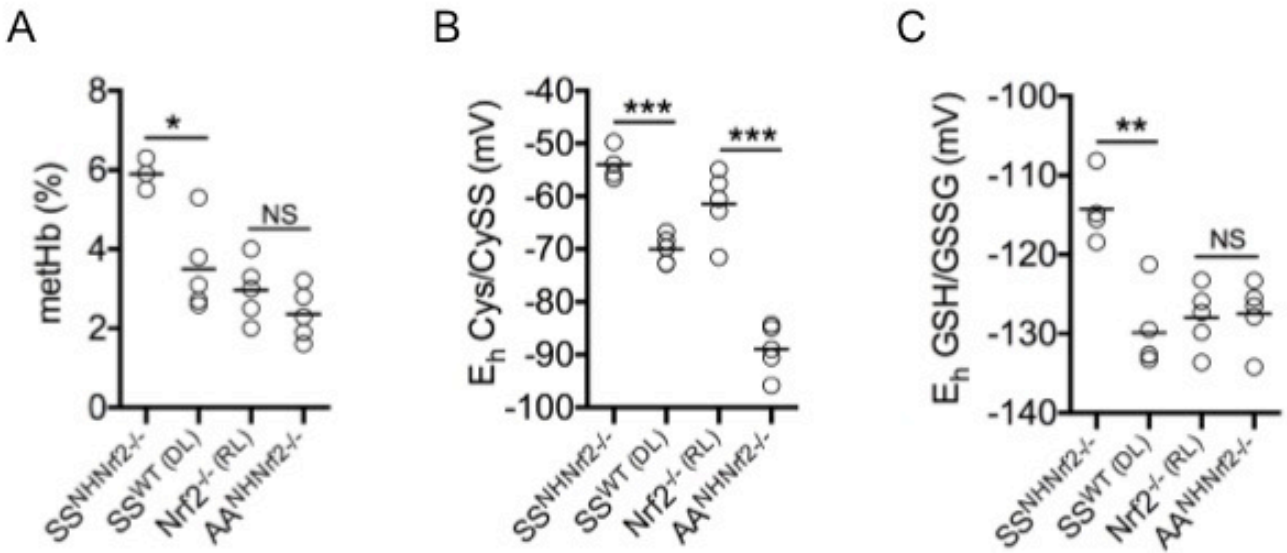
Supplementary Figure 6

Supplementary Figure 6. Heterogeneity of Nrf2 activation in endothelial and hematopoietic cells. (A) Western blot analysis of nuclear fractions from HMVECs and K562 cells showing translocation of NRF2 following D3T treatment. **(B-D)** Relative mRNA expression of three NRF2 regulated genes - HO-1, NQO1 and GCLM in HMVECs and K562 cells treated with 50 μM D3T.



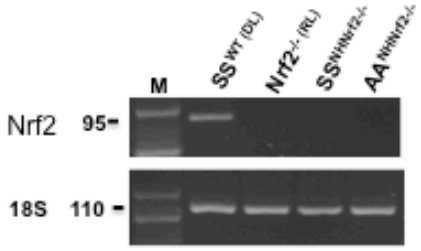
Supplementary Figure 7

Supplementary Figure 7. (A) Plasma hemopexin and **(B)** Plasma haptoglobin level in $SS^{NHNrf2-/-}$ mice (~4 months after acquiring SCD), and control mice. NS, non-significant.



Supplementary Figure 8

Supplementary Figure 8. Nrf2 deficiency in non-hematopoietic tissues exacerbates peripheral oxidative stress in SS mice. **(A)** Percent MetHb is significantly higher in the $SS^{NHNrf2-/-}$ with shorter duration of sickle phenotype than in the $SS^{WT(DL)}$ mice with relatively longer exposure to SCD. $AA^{NHNrf2-/-}$ mice expressing normal human hemoglobin had the lowest mean percent metHb, which was significantly lower than the mean value in the $SS^{WT(DL)}$ mice (n=5). **(B, C)** Compared to the $SS^{WT(DL)}$ mice the redox potential of CyS/CySS and GSH/GSSG was more positive indicative of higher oxidative stress in the $SS^{NHNrf2-/-}$ mice. The CyS/CySS but not the GSH/GSSG redox potential was normalized by transplanting the $Nrf2^{-/-}$ mice with normal AA bone marrow (n=9-11). *p<0.05, p**<0.01, ***p<0.001 and NS, non-significant.



Supplementary Figure 9

Supplementary Figure 9. Nrf2 expression in mutant mice. RT-PCR analysis of Nrf2 and 18S transcripts in total RNA extracted from the lungs of the mice indicated.