

Figure S1.

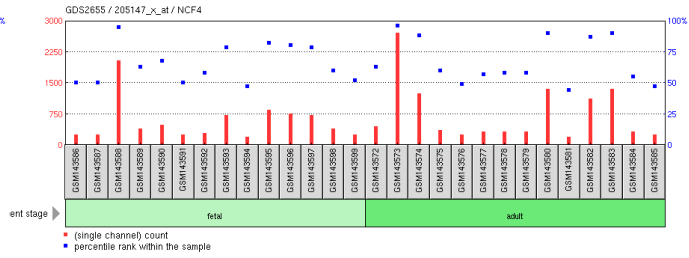
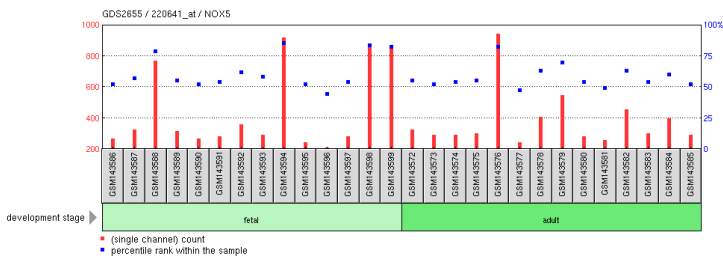
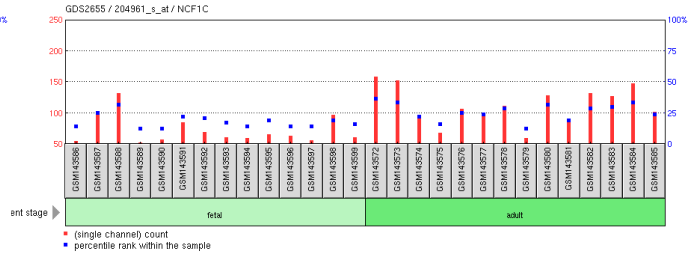
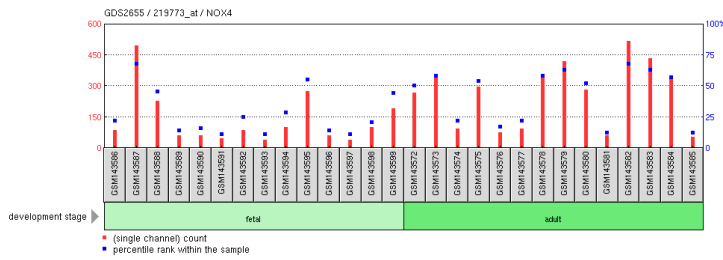
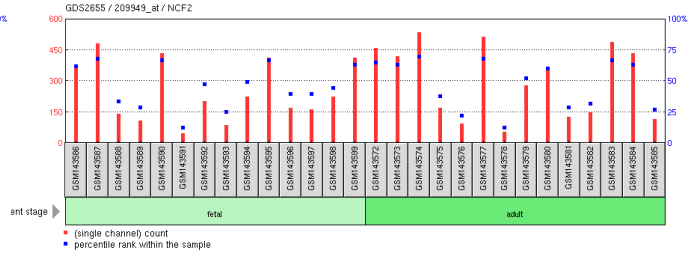
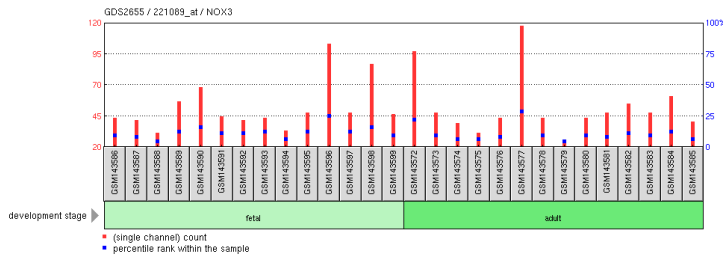
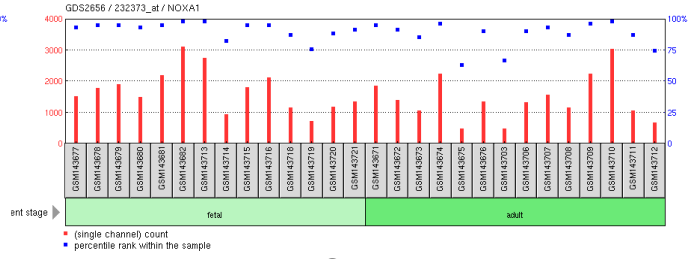
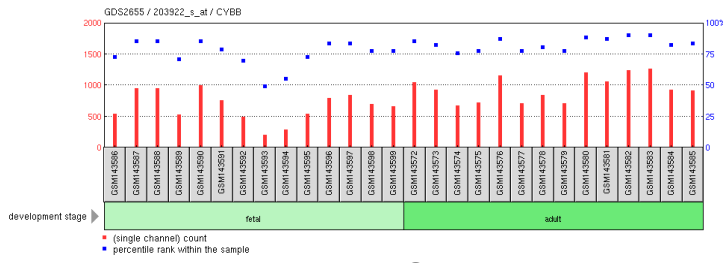
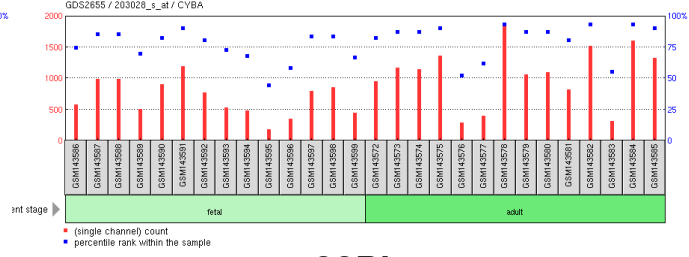
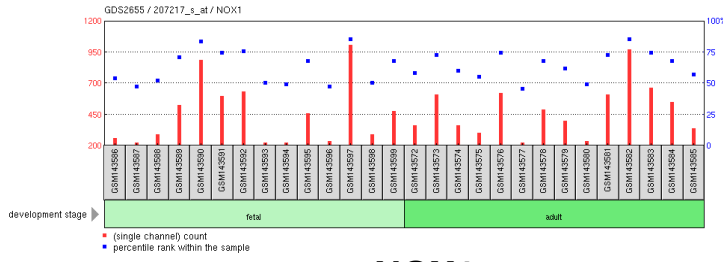
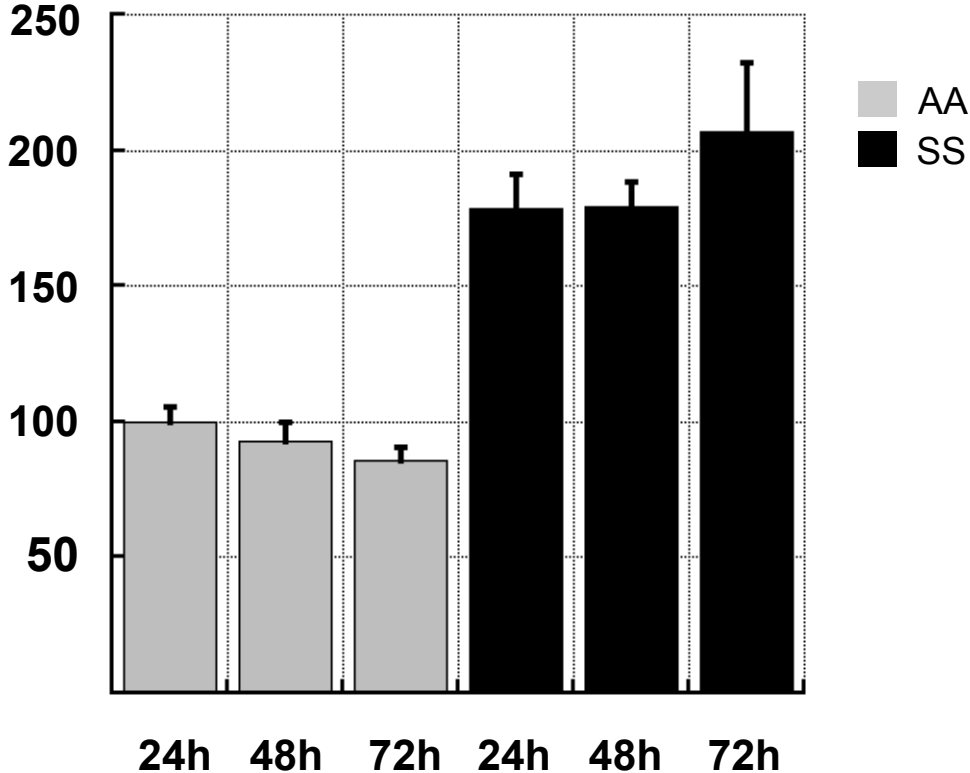


Figure S2.

A.



B.

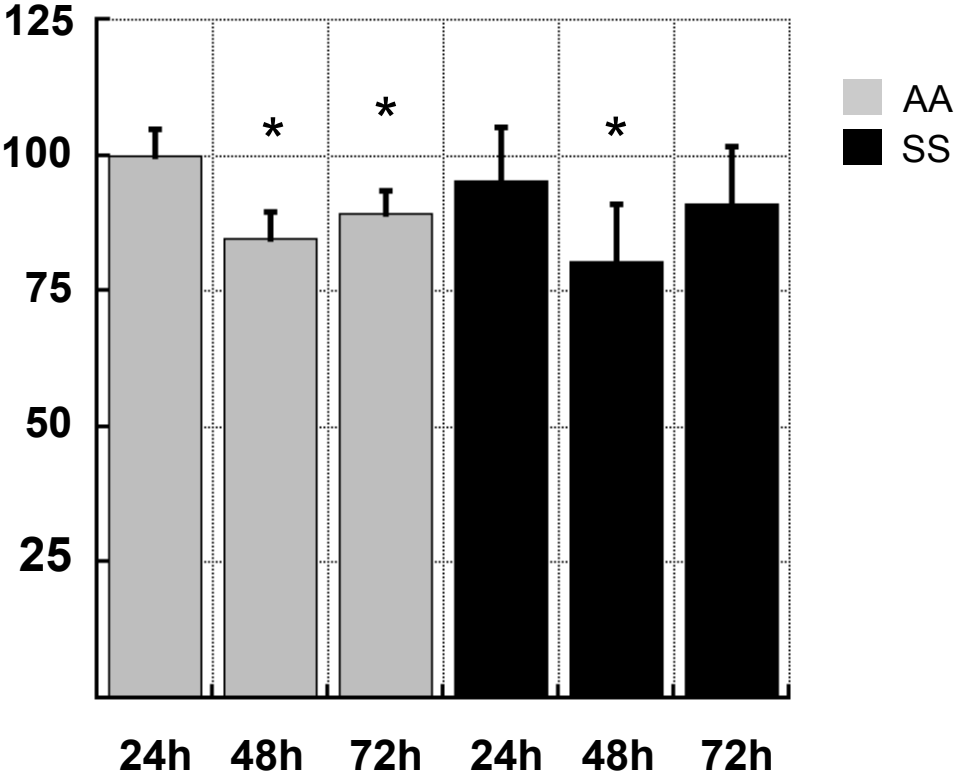
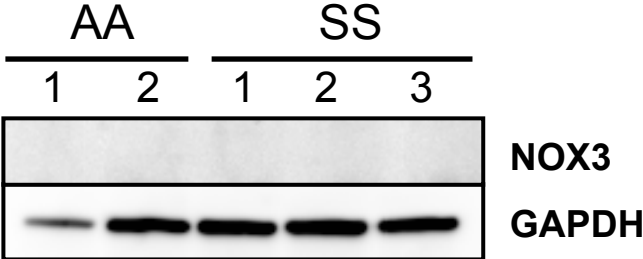


Figure S3.



Supplementary Figure Legends:

Figure S1: Transcriptome data on the expression of NADPH oxidase subunits in human reticulocytes. Goh *et al*¹ have processed and analyzed mRNA from human adult and cord blood reticulocytes to provide a database of genes transcribed at the final stages of erythroblast maturation, and have made available the human reticulocyte transcriptome data in searchable format at <http://www.ncbi.nlm.nih.gov/gds/> (GDS records 2655 and 2656). This database was searched for expression of the catalytic subunits of NADPH oxidases 1-5 (designated NOX1 to NOX5), the p22Phox membrane subunit that is a component of NADPH oxidases 1-4, the p67, p47, and p40 cytosolic subunits that are a component of the active NADPH oxidase type 2, and the NOXA1 subunit that is a homologue of p67 and is a component of NADPH oxidase type 1.² The height of the red bars indicates the single channel count for transcript in each sample (scale on left of panel), while the position of the blue dot indicates the percentile rank of the transcript signal within the sample (scale on right of panel). While there is considerable inter-sample variability, these data indicate that NOX1, NOX2, and NOX5 are expressed at 50th-90th percentile levels, while NOX3 is expressed at less than 25th percentile and NOX4 is expressed at 10th-75th percentile levels. This expression pattern is consistent with our Western blot data showing protein levels of the various NOX isoforms in circulating AA and SS RBC (Figure 3A). Of note, NOX1 and NOX5 appear to have a reciprocally inverse pattern of expression in the reticulocyte transcriptome, as is also suggested by our Western blot data for RBC.

Figure S2: Stability of ROS signal over 72h in RBC and WBC. The whole blood samples from AA and SS subjects used for the experiment described in figure 2D were stored at 4°C in the original EDTA collection tubes for 72 hours and assayed for ROS signal at the 24h, 48h, and 72h timepoints. Aliquots of whole blood were co-labeled with

anti-CD45, anti-GPA, and CM-H2-DCFDA to permit the identification of WBC (CD45⁺;GPA⁻) and RBC (CD45⁻;GPA⁺) populations and the quantitation of ROS signal from each population. (A). AA and SS RBC ROS signal at the above timepoints. There was no statistically significant change in signal over the time period studied. (B) AA and SS WBC ROS signal. In the AA samples, there was a statistically significant decline in ROS signal of ~20% from the 24h timepoint to the 48h one, but no further decline from 48h to 72h. In the SS samples, there was a statistically significant decline in signal intensity, also ~20%, from the 24h to the 48h timepoint, but signal intensity increased from the 48h to the 72h timepoint. n = 10 for AA samples and 7 for SS samples.

Figure S3: Absence of NOX3 signal in RBC lysate. Whole AA and SS RBC lysates were subjected to Western blotting for NOX3 and GAPDH as described in the Methods section. NOX3 signal was not detectable in any RBC sample. NOX3 has a very limited expression pattern and no positive control was readily available to us to confirm antibody sensitivity, but the protein absence matches the low expression indicated by the human reticulocyte transcriptome data (Figure S1).

1. Goh SH, Josleyn M, Lee YT, et al. The human reticulocyte transcriptome. *Physiol Genomics* 2007;30:172-8.
2. Bedard K, Krause KH. The NOX family of ROS-generating NADPH oxidases: physiology and pathophysiology. *Physiol Rev* 2007;87:245-313.