Blood parameters of roGFP2 transgenic mice

Parameters	RoGFP2 Tg mice	WT	*P* value	Normal range
RBC (M/uL)	9.07 ± 0.23	9.34±0.09	n.s	6.36-9.42
Hgb (g/dL)	13.73 ±30.34	13.35±0.15	n.s	11-15.1
HCT (%)	43.30 ± 1.04	42.33±0.28	n.s	35-45.4
MCV (fL)	47.75 ± 0.45	45.28±0.20	0.0026	45.4-60.3
RDW (%)	17.43 ±70.21	17.43±0.13	n.s	12.4-27
WBC (K/uL)	6.47 ±.0.56	6.76±0.54	n.s	1.8-10.7
PLT(K/uL)	773.8 ± 26.65	786±26.65	n.s	592-2972

Data are shown in Mean ± SEM

Table S1. Complete Blood Count with focus on Red Cell Indices of roGFP2 transgenic mice
Complete blood count was performed using an automated analyzer (Hemavet 950, Drew Scientific, Waterbury, CT). Results for WT (n = 4) and roGFP2 transgenic mice (n = 4) are shown as mean ± SEM. Statistical comparisons between WT and roGFP2 transgenic mice were performed by student *t*-test. RBC, red blood cell count; Hgb, hemoglobin; Hct, hematocrit; MCH, mean corpuscular hemoglobin; MCHC, MCH concentration, MCV, mean corpuscular volume; and RDW, red cell distribution width. WBC, white blood cell count; PLT, platelet count.

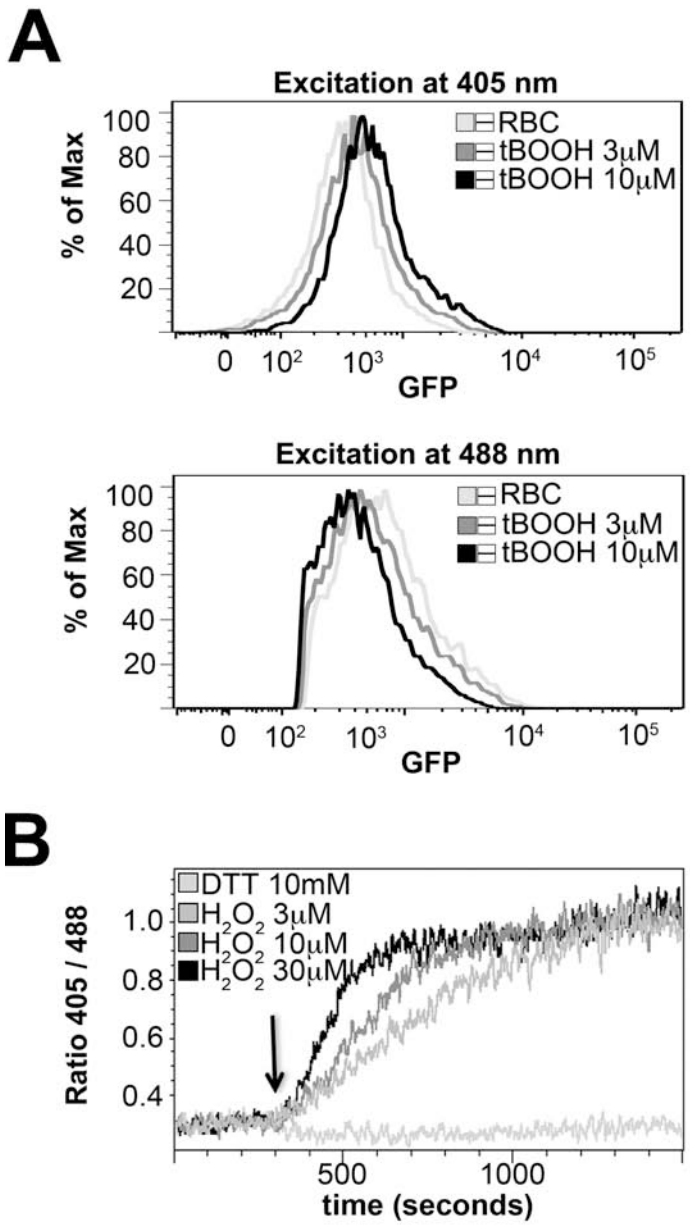

Figure S1

(A). Fluorescence signal from roGFP2 expressing RBC upon t-butyl hydroperoxide (t-BOOH) stimulus. Representative histograms for GFP fluorescence are shown for both excitation wavelengths 405 and 488 nm. Treatment with oxidant increases intensity of emission after stimulation at 405 nm, and decreases intensity of emission after stimulation at 488 nm.

(B). Kinetics of change in roGFP2 ratio (405/488) upon stimulus with indicated amount of H_2O_2 or DTT versus time. Panel B shows that after adding 3, 10, or 30 μM H_2O_2, RBC reach the same plateau after approximately 20 minutes. However, the slope of the curves is dose-dependent, indicating that kinetic analysis and end-point analysis of roGFP2 ratio are both useful approaches for evaluation of changing redox status.

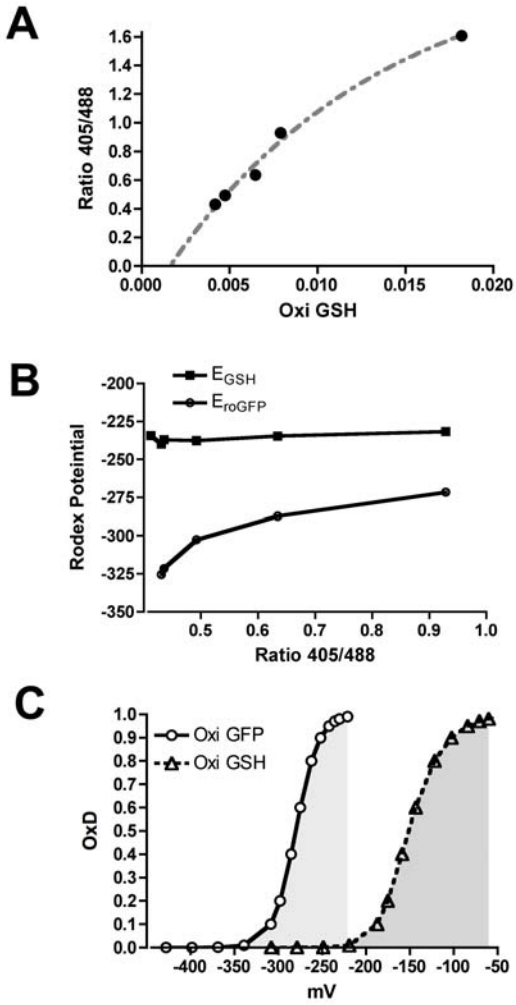

Figure S2. Correlation of Redox potential of GSH and roGFP2

(A) roGFP2 transgenic RBCs were treated with tBOOH. GSSG and GSH concentration were measured with GSH/GSSG ratio assay kit (EMD Chemicals). The degree of oxidized GSH fraction ($OxiD_{GSH}$) was defined as $2[GSSG]/[GSH]_{total}$. The $OxiD_{GSH}$ was plotted versus the ratio of 405/488 stimulated emission determined by FACS.

(B) Based on the experiments in (A), the redox potential of GSH was calculated according to the Nernst equation as: $E_{GSH} = E'_{GSH} - RT*\ln(2GSH_{total}*(1-OxD_{GSH})^2/OxD_{GSH})/(z*F)$. The redox potential of roGFP2 was calculated according to equation 2. The measured E_{GSH} and E_{roGFP2} was compared in Fig S2B.

(C) Theoretical sensitivity range in detecting cellular redox potential using GSH or roGFP2 as a probe. The redox potential was calculated according to the Nernst Equation. In this figure, the concentration of total GSH was set as 2mM. The dynamic range of roGFP2 is -340 to -240mV, while that of GSH is -227 to -95mV.