Supplemental Table 1

Ascorbic acid in reduced and non-reduced RBC samples

Mouse #	RBC-ascorbate without TCEP (µM)	RBC-ascorbate with TCEP (µM)
1	24.0 ± 0.5	25.2 ± 0.7
2	25.4 ± 0.9	23.4 ± 0.7
3	21.2 ± 0.7	22.5 ± 0.7
4	21.5 ± 0.8	20.4 ± 0.9
5	22.5 ± 0.7	22.8 ± 1.1
6	22.5 ± 1.0	21.6 ± 1.0

Data are displayed in Supplemental Figure 1

10% RBC lysates from C57/BL6 wild type mice were measured with/without 20 fold excess tris (2-carboxyethyl) phosphine (TCEP).

Supplemental table 2

Recovery of dehydroascorbic acid (DHA) spiked in PBS containing 10% RBCs

Type of RBC	Added DHA (µM)	Measured after addition (µM)	Recovery (%)
C57 (gulo -/-)	2	1.7 ± 0.1	87.2 ± 0.1
	5	4.7 ± 0.5	94.8 ± 0.1
	10	9.0 ± 0.3	89.9 ± 0.0
C57 (wild type)	2	1.6 ± 0.0	80.5 ± 0.0
	5	4.6 ± 0.2	92.6 ± 0.0
	10	8.5 ± 0.1	85.3 ± 0.0

20 µl, 50 µl, or 100 µl dehydroascorbic acid (0.1 mM) was added to 1ml PBS containing 10% RBCs. Immediately after addition, the mixture was centrifuged at 1000 x g for 2 minutes and supernatant was withdrawn. 50 µl 1mM TCEP was added to 50 µl supernatant , the mixture was vortexed , 100 µl 90% Methanol/EDTA was added, the solution centrifuged at 25,000 x g for 10 min., and the supernatant analyzed by HPLC.

Supplemental Figure 1

Dehydroascorbic acid in RBCs.

After blood from wildtype mice (n=6) was isolated and washed three times , 40 µl packed RBCs were lysed either with 160 µl water or 140 µl water + 20 µl 0.5 mM tris(2-carboxyethyl)phosphine (TCEP). Samples were processed by centrifugal ultrafiltration and analyzed as described in Methods. Dehydroascorbic acid is the difference between non-reduced and tris (2-carboxyethyl)phosphine-reduced samples.