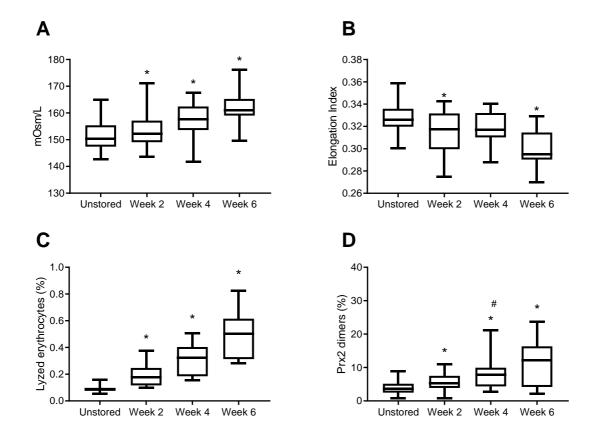
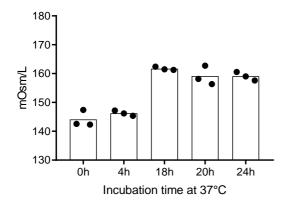
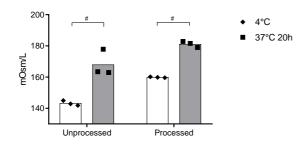
## **Supplemental Data**



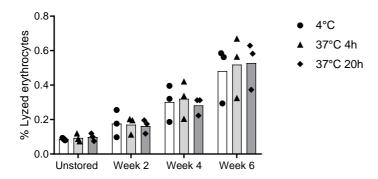
Supplemental Figure S1. Effects of storage at 4°C on physical properties and Prx2 dimerization in RBCs. (A) Osmotic fragility based on solution osmolality leading to 50% hemolysis [n=36], (B) elongation index [n=21], (C) percentage lysed erythrocytes [n=18], and (D) percentage Prx2 present as dimer [n=33] at 0 (unstored), 2, 4 and 6 weeks of storage at 4°C. Boxplots show median, 25th and 75th percentile (box) and min/max values (whiskers). \*  $p \le 0.05$  in repeated measures 1-way ANOVA in comparison to unstored RBCs. #  $p \le 0.05$  in deviation from Gaussian distribution (D'Agostino-Pearson normality test).



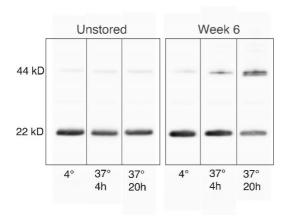
**Supplemental Figure S2. Effect of incubation time at 37°C on osmotic fragility.** Osmotic fragility based on solution osmolality leading to 50% hemolysis in fresh RBC samples stored at 37°C for 0, 4, 18, 20, or 24 h.



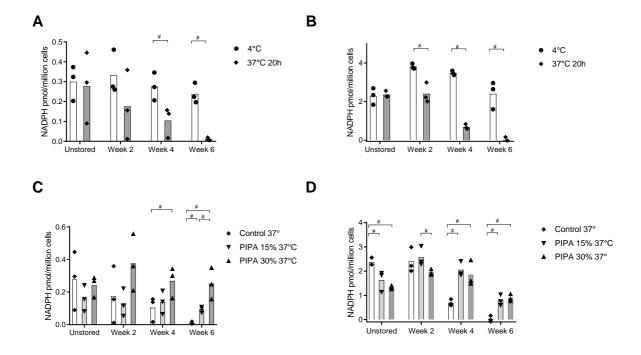
Supplemental Figure S3. Effect of incubation at 37°C on osmotic fragility of RBCs before and after processing of erythrocyte concentrates. Osmotic fragility based on solution osmolality leading to 50% hemolysis in fresh blood samples unprocessed (whole blood in CPD analyzed within 1 h after collection) or processed (erythrocytes in SAGM after centrifugation and leukoreduction, analyzed 36h after collection), before ( $\blacklozenge$ ) or after warming to 37°C for 20 h ( $\blacksquare$ ) [n=3]. # p≤0.05 in Mann-Whitney non-parametric test.



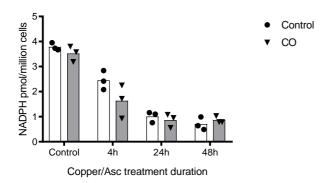
Supplemental Figure S4. Effect of incubation at 37°C on hemolysis of RBCs stored at 4°C. Hemolysis at 0, 2, 4 and 6 weeks of storage at 4°C before ( $\bullet$ ) and after warming to 37°C for 4 h ( $\blacktriangle$ ) or 20 h ( $\bullet$ ) [n=3].



Supplemental Figure S5. Representative Western Blots of Prx2 showing the effect of incubation at 37°C on Prx2 dimerization. Representative Western blot images for blood unstored or stored RBCs for 6 weeks at 4°C, followed by 4 or 20 h incubation at 37°C. Corresponding results are shown in Figure 1D.



Supplemental Figure S6. Effects of post-storage incubation at 37°C on NADPH content in RBCs measured by HPLC or by enzymatic cycling. NADPH content measured (A) by HPLC, and (B) by enzymatic cycling at 0, 2, 4 and 6 weeks of storage at 4°C before (•) and after (•) warming to 37°C for 20 h [n=3]. NADPH content measured (C) by HPLC, and (D) by enzymatic cycling in untreated blood samples (•), or samples treated with 15% ( $\blacktriangledown$ ) or 30% ( $\blacktriangle$ ) PIPA solution, and stored at 4°C for 0, 2, 4 and 6 weeks followed by 20 h at 37°C [n=3]. Same samples used for analysis in (A) and (B) and for analysis in (C) and (D). # p≤0.05 in Mann-Whitney non-parametric test.



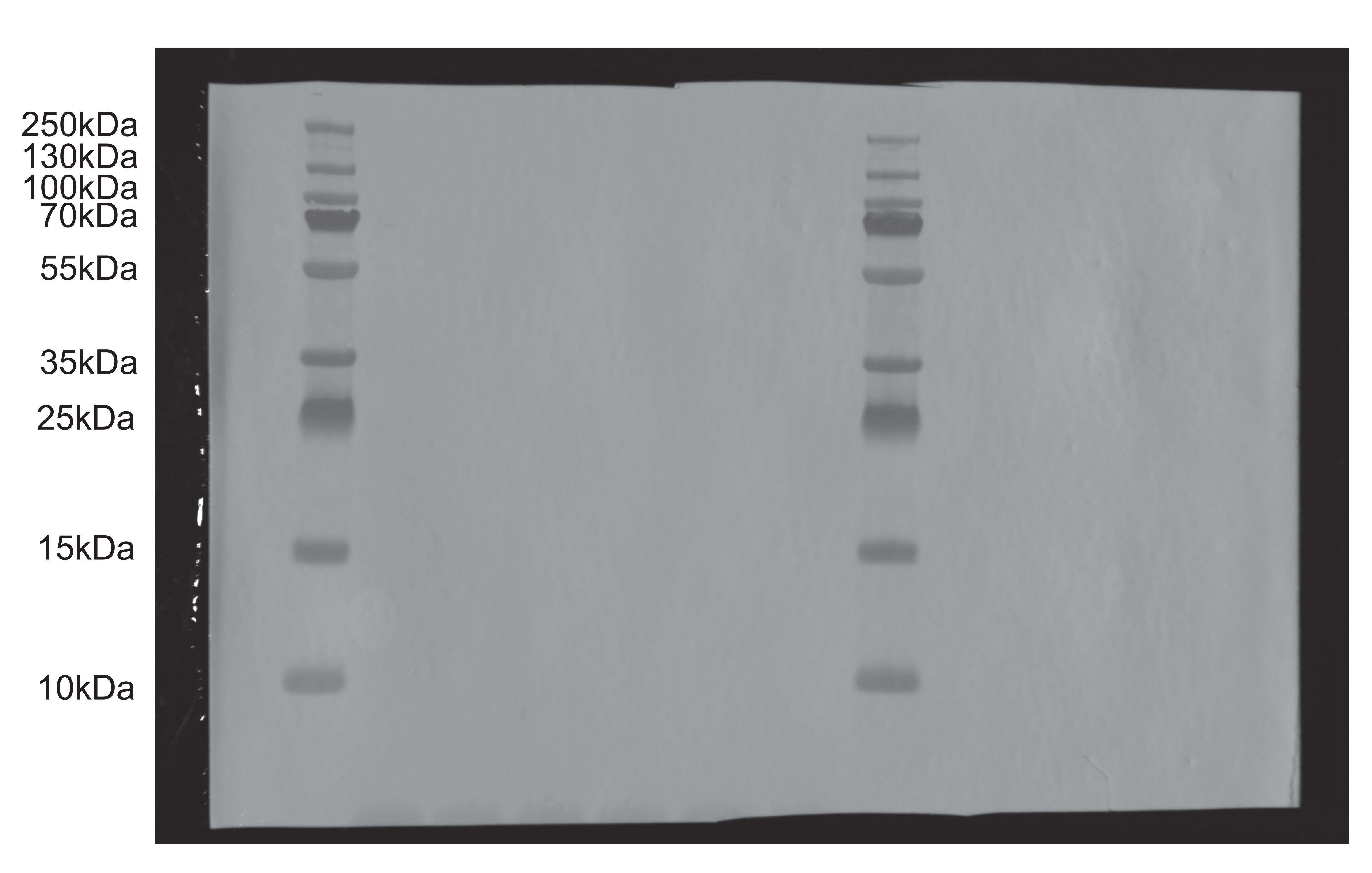
Supplemental Figure S7. Effects of oxygen removal by CO purging on total NADP(H) and NADPH content in RBCs exposed to copper/ascorbate-induced oxidative stress. (A) Total NADP(H) and (B) NADPH content determined by enzymatic cycling in fresh RBC samples in untreated ( $\bullet$ ) or CO-treated ( $\blacktriangledown$ ) samples, followed by exposure to copper/ascorbate for 0 (ctrl), 1, 4, 24, or 48 h at 4°C [n=3].

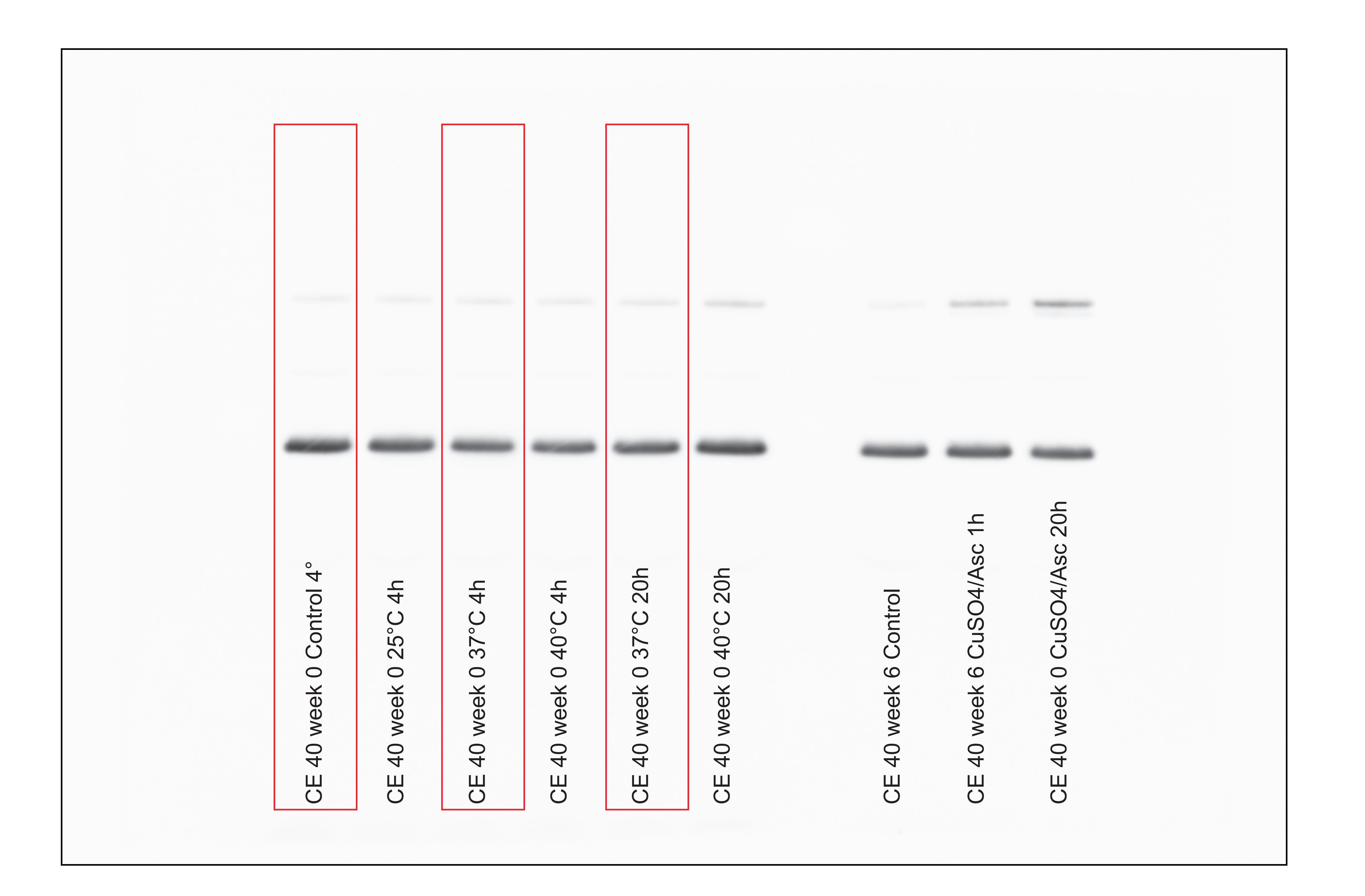
## Full unedited images for western blot shown in Supplementary Figure S5

Lanes shown in the figure are highlighted in red

Antibodies used: anti-Prx2 (R8656 Sigma) and anti-rabbit horseradish peroxidase (Jackson)

Gel of Week 0





Gel of Week 6

