SDC, Materials and Methods

Immunoblotting

Selected proteins implicated by proteomic analysis of the 10-sample subset were analysed in the remaining 28 samples by immunoblotting. Samples containing 15 µg of protein were denatured at 95°C for 5 minutes in Laemmli buffer and loaded into 8–12% precast SDSPAGE gels (Bio-Rad, USA). Proteins separated by SDS-PAGE were transferred to hydrophobic PVDF membranes (Merck Millipore, USA) in transfer buffer (25 mM Tris, 192 mM glycine and 10% methanol) overnight. PVDF membranes were blocked for 1 hour in TBST buffer (25 mM Tris, pH 7.5, 0.15 M NaCl, 0.05% Tween 20) containing 5% milk. Membranes were incubated for overnight at 4 °C with anti TRX1 (ab26320, 1:1000), PRX3 (ab73349, 1:1000), Catalase (ab16731, 1:1000), GST (ab53940, 1:3000) (Abcam, UK), STAT-1 (mab14901, 1:1000), PDGFRα (af307na, 1:800) (R&D Systems, US). Rabbit monoclonal anti Beta-actin served as a loading control (ab8227, 1:5000) (Abcam, UK). Membranes were washed for 30 minutes with 5 changes of wash buffer and then incubated at room temperature for 1 hour in blocking buffer containing a 1:5000 dilution of Dye-800-conjugated anti-mouse, rabbit, or -goat secondary antibody (Li-Cor, Nebraska, USA), and visualization was performed with Odyssey CLx (Li-Cor Nebraska, USA). Detected signal was quantified and normalized to β-actin on the same blot.

Table S1: Remuzzi scoring

	Suboptimal Outcome (n=19)	Good Outcome (n=19)	*P* Value
Remuzzi score (%)			0.1
0- 3	13 (68)	15 (79)	
4-8	1 (5)	1 (5)	

Figure S1

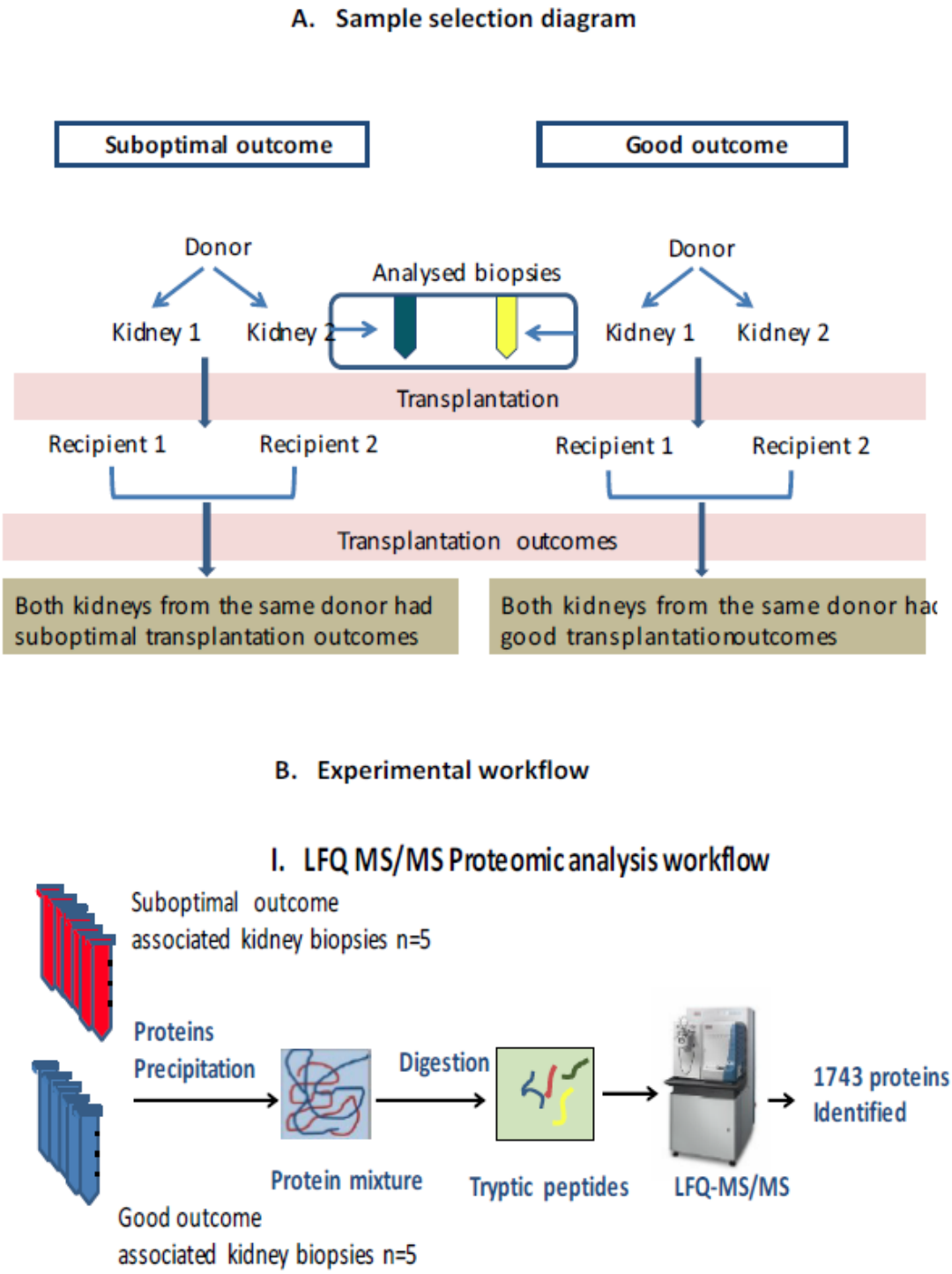
A. Sample selection diagram
Suboptimal outcome
Good outcome
Donor
Analysed biopsies
Donor
Kidney 1
Kidney 2
Kidney 1
Kidney 2
Transplantation
Recipient 1
Recipient 2
Recipient 1
Recipient 2
Transplantation outcomes
Both kidneys from the same donor had suboptimal transplantation outcomes
Both kidneys from the same donor had good transplantationoutcomes
B. Experimental workflow
I. LFQ MS/MS Proteomic analysis workflow
Suboptimal outcome associated kidney biopsies n=5
Proteins Precipitation
Digestion
1743 proteins Identified
Protein mixture
Tryptic peptides
LFQ-MS/MS
Good outcome associated kidney biopsies n=5
II. Immunoblotting analysis

Figure S1. ***Experimental design***

1A. The kidney biopsy sample selection diagram shows the selection of donors included in the study. Selected donors offered both kidneys as single transplants with both allografts giving rise to similar outcome suboptimal or good allogarfat function. GO donor kidneys had a mean 3-month eGFR= 65.2 +/- 8 ml/min/1.73 m^2 (25^{th} and 75^{th} percentiles for eGFR were 62 and 70.25 ml/min/1.73 m^2 respectively). SO donor kidneys had a mean 3-month eGFR= 29.8+/-7ml/min/1.73 m^2 (25^{th} and 75^{th} percentiles for eGFR were 37.2 and 24.8 ml/min/1.73 m^2 respectively). The difference of the posttransplant 3-month eGFR values between the 2 cohorts (SO vs GO) was significant different ($P<0.0001$; Mann-Whitney test).

1B. The experimental workflow contained 2 stages. (1) Initially, n=10 individual kidney biopsies (n=5 samples per transplantation outcome) were analysed by label-free quantitative MS. Biopsy sample homogenates were precipitated, proteins were enzymatically digested to tryptic peptides and analysed by tandem mass spectrometry. Bioinformatic analysis resulted in the identification of 1743 proteins (FDR<1%). (2). Western blot analysis was performed on the rest of the selected cohort of DBD kidney biopsies n=28 (n=14 biopsy samples per transplantation outcome). SO: suboptimal outcome, GO: good outcome.

Figure S2

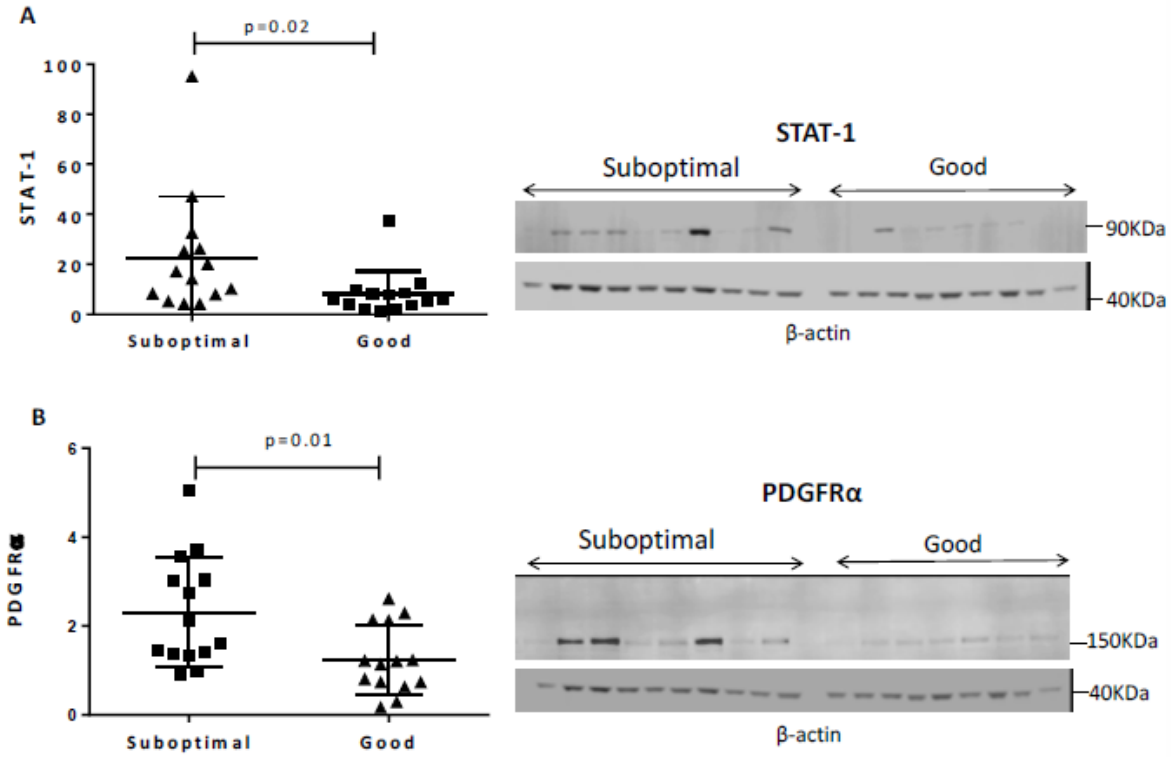

Figure S2. ***STAT-1 and PDGFRα are enriched in donor kidneys with SO***.

Western blot analysis of STAT-1 (**A**) and PDGF Rα (**B**) on the rest of the selected cohort of n= 28 biopsy samples (n=14 suboptimal and n=14 good outcome cohort). Normalised by β-actin, densitometry analysis shows significant increased levels of STAT-1 and PDGFRα in SO associated donor kidney biopises ($p \leq 0.05$).

Figure S3

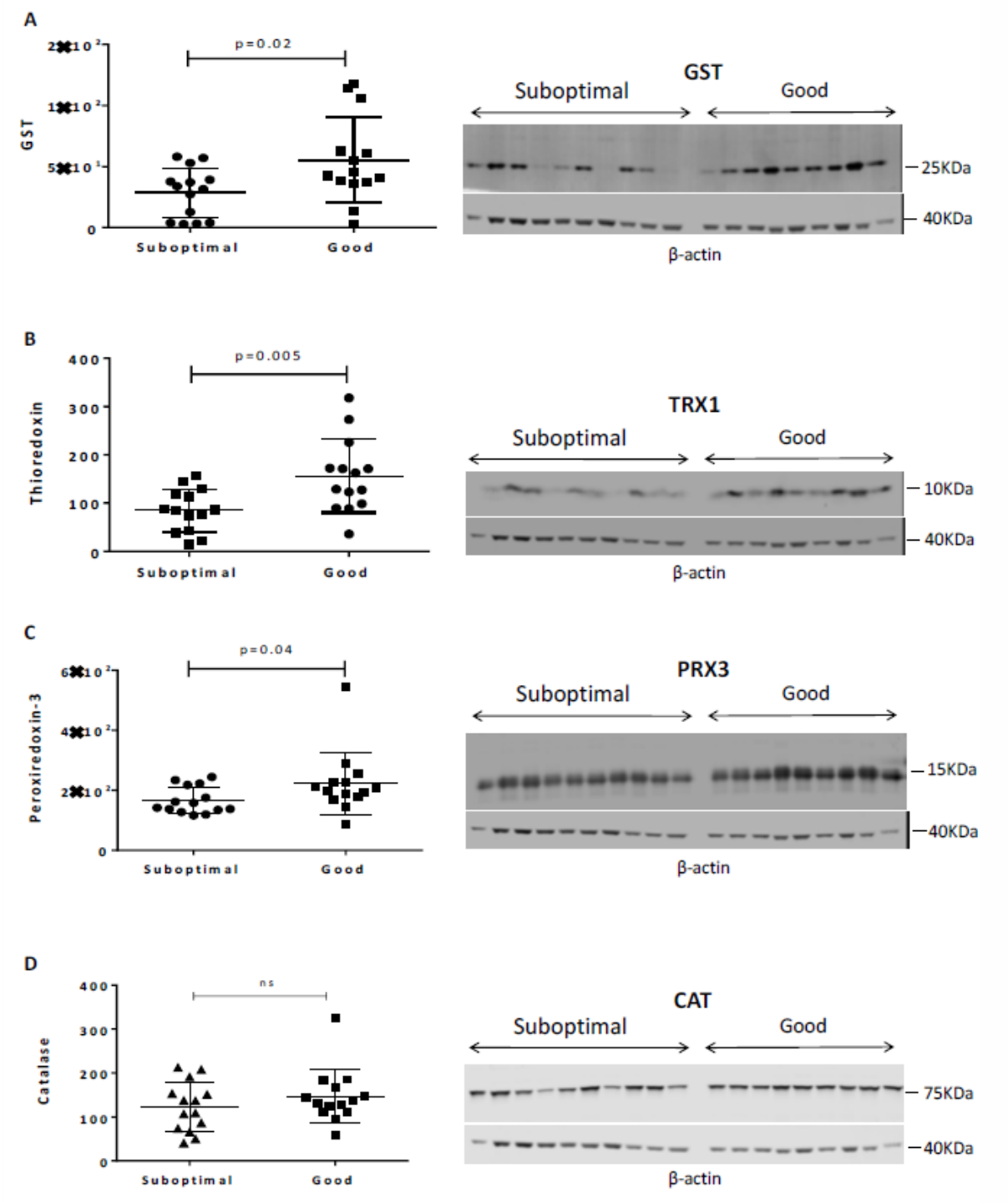

Figure S3. ***Antioxidant proteins are enriched in the donor kidneys with GO*****.**

Western blot analysis of GST (**A**), TRX1 (**B**), PRX3 (**C**) and CAT (**D**) on the rest of the selected cohort of n=28 DBD kidney biopsies (n=14 suboptimal and n=14 good outcome cohort).

Normalised by β-actin densidometric analysis shows significant increased levels of TRX1, GST, and PRX3 in GO associated donor kidney biopises ($p \leq 0.05$). CAT was not significantly altered.

Figure S4

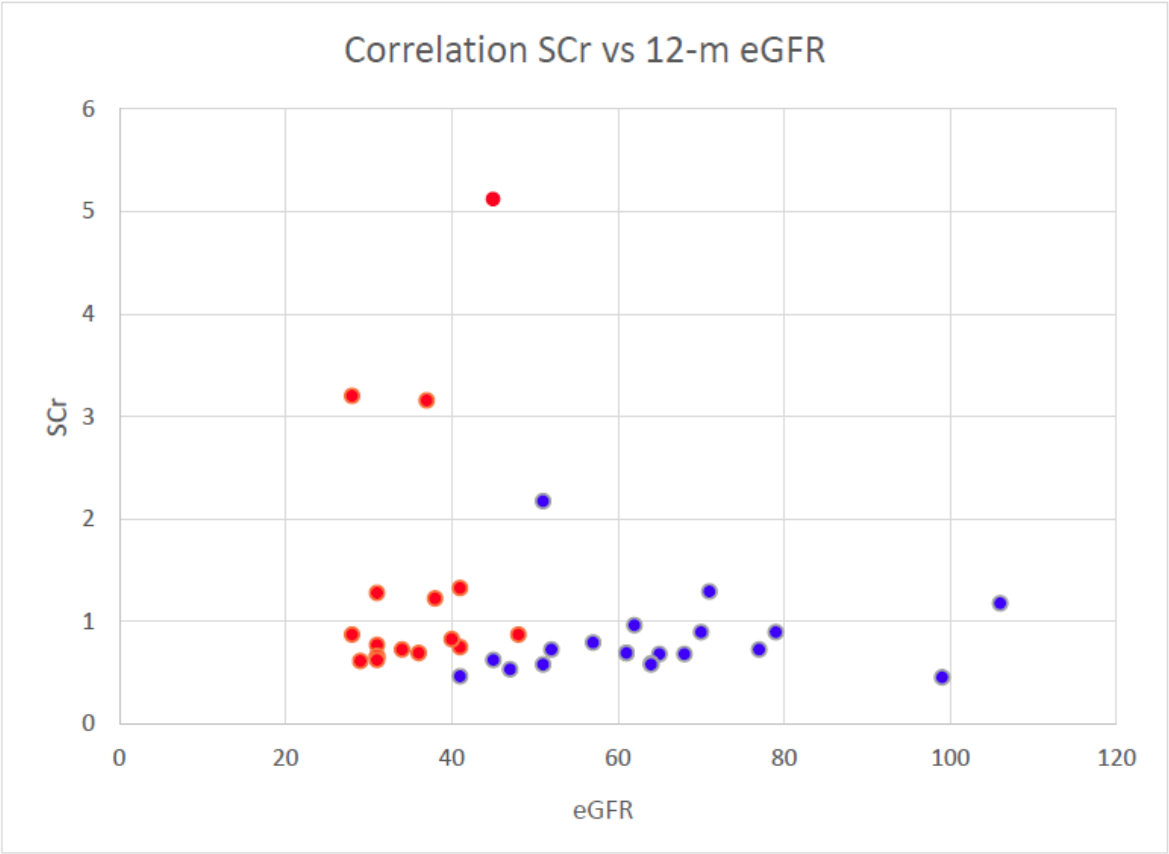

Figure S4. ***Correlation of Serum Creatinine versus 12-month eGFR***

A (nonstratified) comparison between donor serum creatinine and 12-mo eGFR did not find a strong correlation (Spearman Rho=-0.007; p<=0.65); plotting the data reveals that the observed mean difference is likely due to a few anomalous readings.

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