Supplementary Material from *Interacting network of the gap junction protein connexin43 (Cx43) is modulated by ischemia and reperfusion in the heart*

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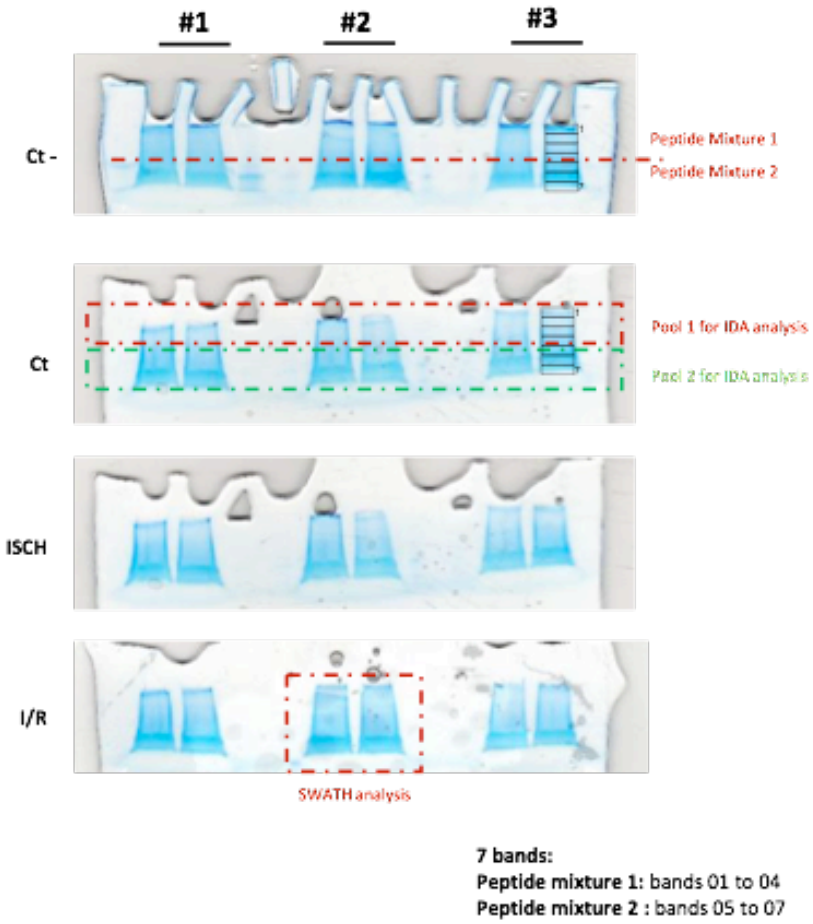
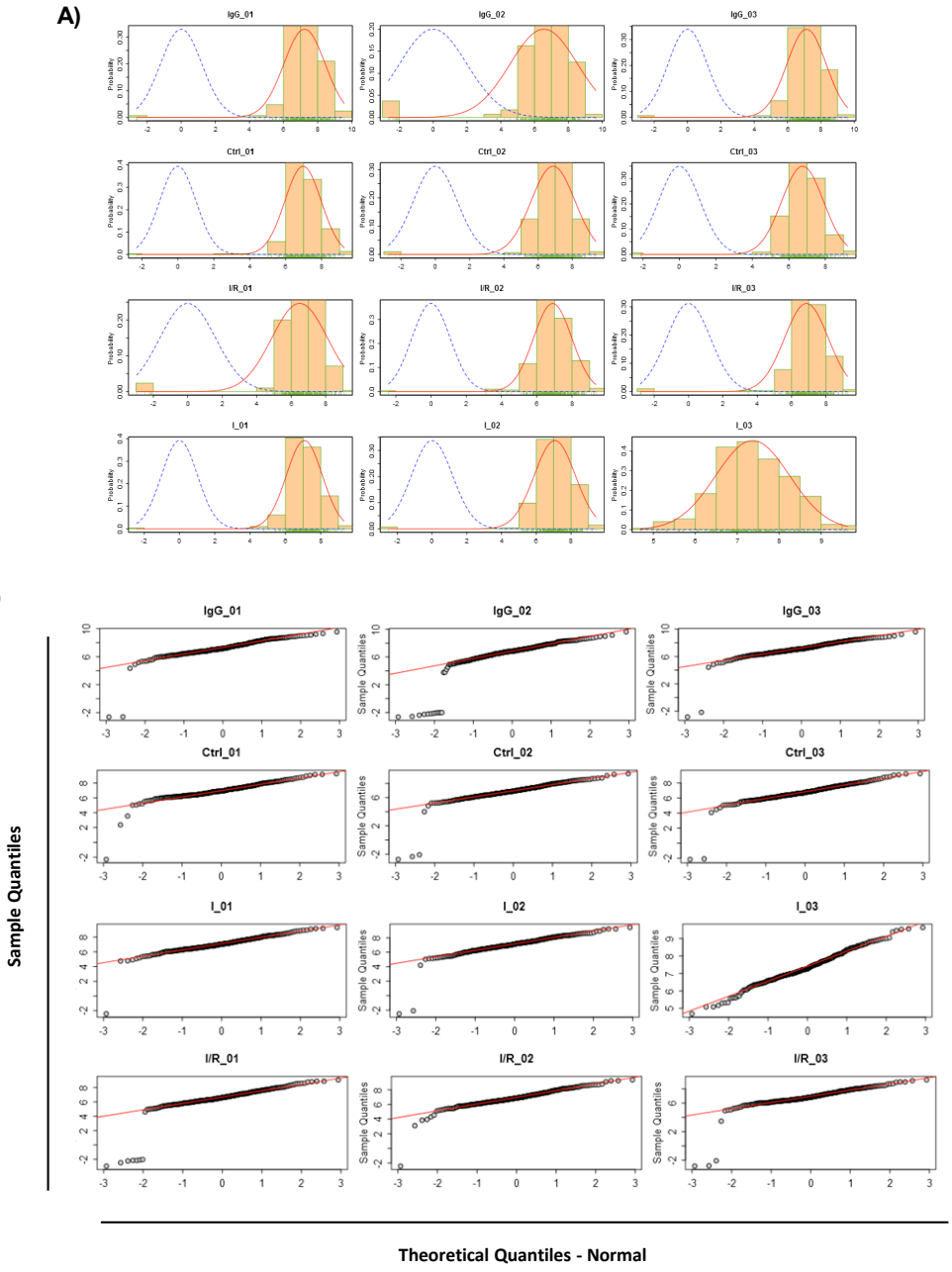
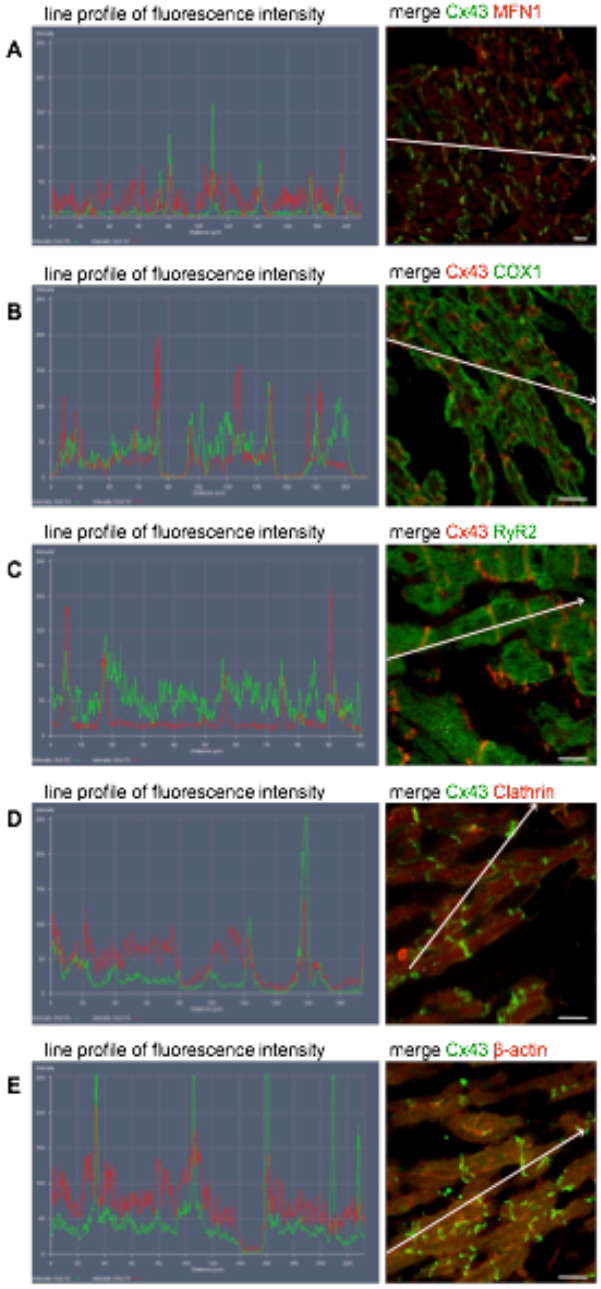
Supplementary Figures



Supplementary figure S1 – Short-GeLC approach used in sample preparation. A partial electrophoretic separation of the immunopurified samples was visualized by staining with Colloidal Coomassie Blue. Gel lanes were sliced into 7 bands of equal size (as indicated by the grid-cutter), and further sliced into small pieces, for independent processing. Peptides extracted from different bands were pooled together in two-peptide mixtures (mixture 1 and mixture 2) per sample, for subsequent liquid chromatography (LC)-MS/MS analysis. For IDA experiments a pool of all the biological replicates was done, and for SWATH analysis the two peptide mixture of each sample were combined.

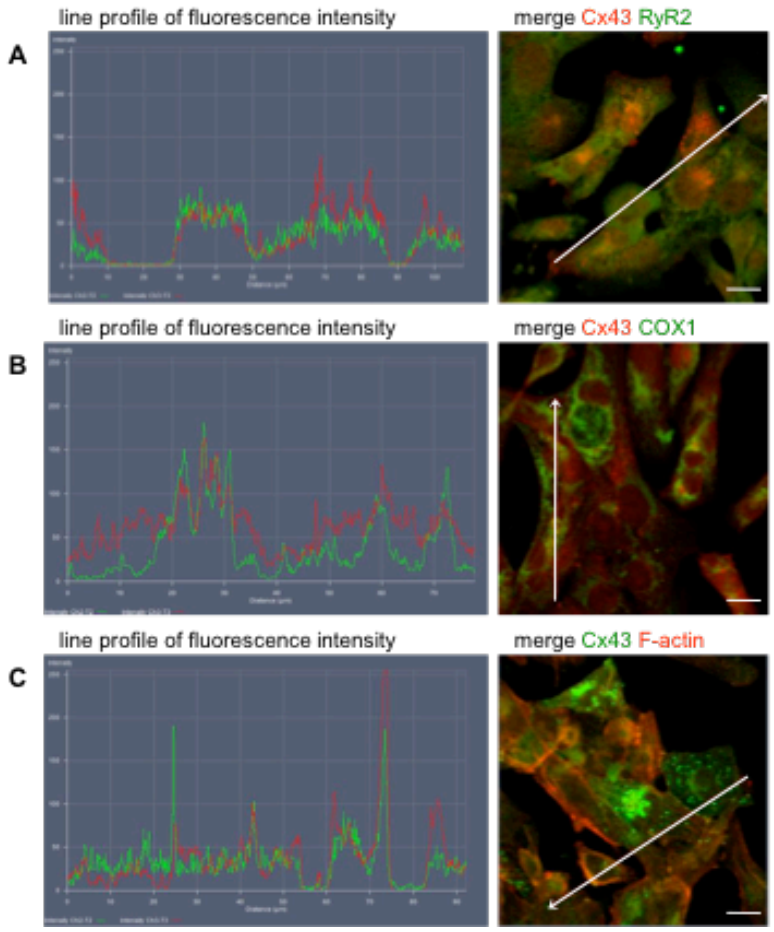


Supplementary figure S2 – Evaluation of the normality of the SWATH data. Normality was indirectly accessed by parallel histogram (A) q-q plots (B) analyses. The logarithmized quantitative data follows a normal distribution, therefore it can be compared by parametric statistical methods.

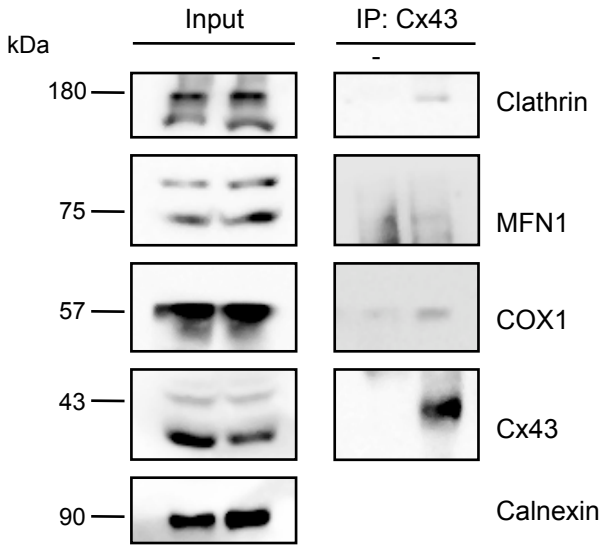


Supplementary Figure S3 – RyR2, Mfn1 and COX1 co-localize with Cx43 in the rat heart. Hearts from 10-week-old Wistar rats were maintained using a Langendorff apparatus for 10 min, followed by 20 min of perfusion (CT). Cryosections of control or ischemic hearts were immunostained using antibodies against **A.** Cx43 (Sc9059) and RyR2 **B.** Cx43 (610062) and Mfn1 **C.** Cx43 (Sc9059) and COX1 **D.** Cx43 (Sc9059) and Clathrin heavy chain **E.** Cx43 (Sc9059) and ß-actin. Scale bars, 25 µm. Co-localization graphs show the fluorescence intensity profile from a line (white arrows, right panels) crossing through

merged images (right panel). The coincidence of fluorescence intensity peaks (green and red) represents the co-localization between two proteins.



Supplementary Figure S4 – RyR2 and COX1 co-localize with Cx43 in HL-1 cells. Immunostaining for **A.** Cx43 (Sc9059) and RyR2 **B.** Cx43 (Sc9059) and COX1 **C.** Cx43 (610062) and F-actin (Phalloidin), in HL-1 cells. Scale bars, 20 μm. Co-localization graphs show the fluorescence intensity profile from a line (white arrows, right panels) crossing through merged images (right panel). The coincidence of fluorescence intensity peaks (green and red) represents the co-localization between two proteins.



Supplementary Figure S5 – Clathrin, Mfn1 and COX1 interact with Cx43 in the rat heart. Hearts from 10-week-old Wistar rats were maintained using a Langendorff apparatus for 10 min, followed by 20 min of perfusion. Heart lysates (Input) were immunoprecipitated (IP) using goat polyclonal antibodies directed against Cx43 (AB0016, Sicgen). Goat non-specific polyclonal antibodies (anti-GFP; AB0020, Sicgen) were used for control IP. WB analysis of the immunoprecipitates was performed using antibodies against Clathrin heavy chain, Mfn1 and COX1. Calnexin was used as loading control.

Supplementary Tables

Legends

Supplementary Table S1 – List of all the proteins identified in our study.

Supplementary Table S2 – List of all the proteins quantified by SWATH-MS.

Supplementary Table S3 – Multiple t-test evaluation for each pair of experimental conditions.

Supplementary Table S4 – Cx43-interacting proteins identified in the both the present study and in the Gago-fuentes et al. study, 2015

Supplementary Table S5 – List of the 236 putative Cx43 interactors distributed by each respective cluster/profile of interaction.

Supplementary Table S6 – ISCH-enriched Cx43-interacting partners. List of proteins which interaction with Cx43 is enhanced during ischemia.

Supplementary Table S7 – I/R-enriched Cx43-interacting partners. List of Cx43-interacting partners whose interaction is enhanced in I/R.