Das, et al., Supplemental Figure S1

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  1 MAAAVPRAAF LSPLLPLLLG FLLLSAPHGG SGLHTKPVAT MVSKGEELFT
 51 GVVPILVELD GDVNGHKFSV SGEGEGDATY GKLTLKFICT TGKLPVPWPT
101 LVTTLTYGVQ CFSRYPDHMK QHDFFKSAMP EGYVQERTIF FKDDGNYKTR
151 AEVKFEGDTL VNRIELKGID FKEDGNILGH KLEYNYNSHN VYIMADKQKN
201 GIKVNFKIRH NIEDGSVQLA DHYQQNTPIG DGPVLLPDNH YLSTQSALSK
251 DPNEKRDHMV LLEFVTAAGI TLGMDELYKY SDLELKLAAL HTKGALPLDT
301 VTFYKVIPKS KFVLVKFDTQ YPYGEKQDEF KRLAENSASS DDLLVAEVGI
351 SDYGDKLNME LSEKYKLDKE SYPVFYLFRD GDFENPVPYT GAVKVGAIQR
401 WLKGQGVYLG MPGCLPVYDA LAGEFIRASG VEARQALLKQ GQDNLSSVKE
451 TQKKWAEQYL KIMGKILDQG EDFPASEMTR IARLIEKNKM SDGKKEELQK
501 SLNILTAFQK KGAEKEEL

aas    1-50 – signal sequence of ERp29
aas  51-279 – EGFP
aas 280-411 – ERp29 N-terminal domain dimerization motif
aas 412-514 – ERp29 C-terminal domain ligand binding motif
aas 515-518 – KEEL ER retention motif
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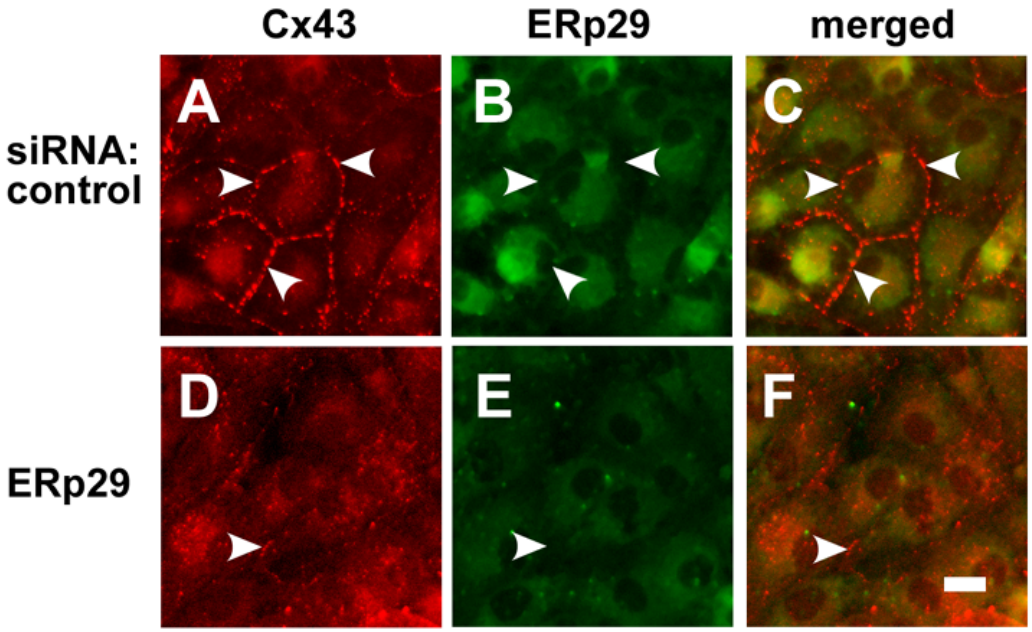
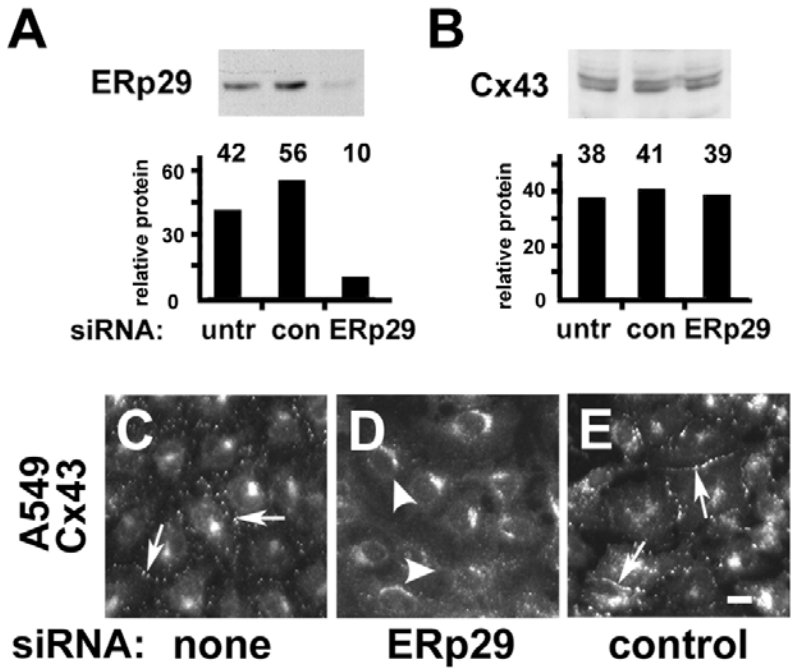
Amino acid sequence of EGFP-ERp29. Shown is the amino acid sequence for the EGFP-ERp29 construct. Amino acids 1-50 correspond to the signal recognition sequence, amino acids 51-279 correspond to EGFP, amino acids 280-518 represent the remainder of ERp29, including the N-terminal dimerization motif (amino acids 280-411) and C-terminal ligand binding motif (amino acids 412-514). In the EGFP-ERp29-N construct, amino acids 412-514 were deleted, retaining the KEEL ER-retention motif.

Das, et al., Supplemental Figure S2



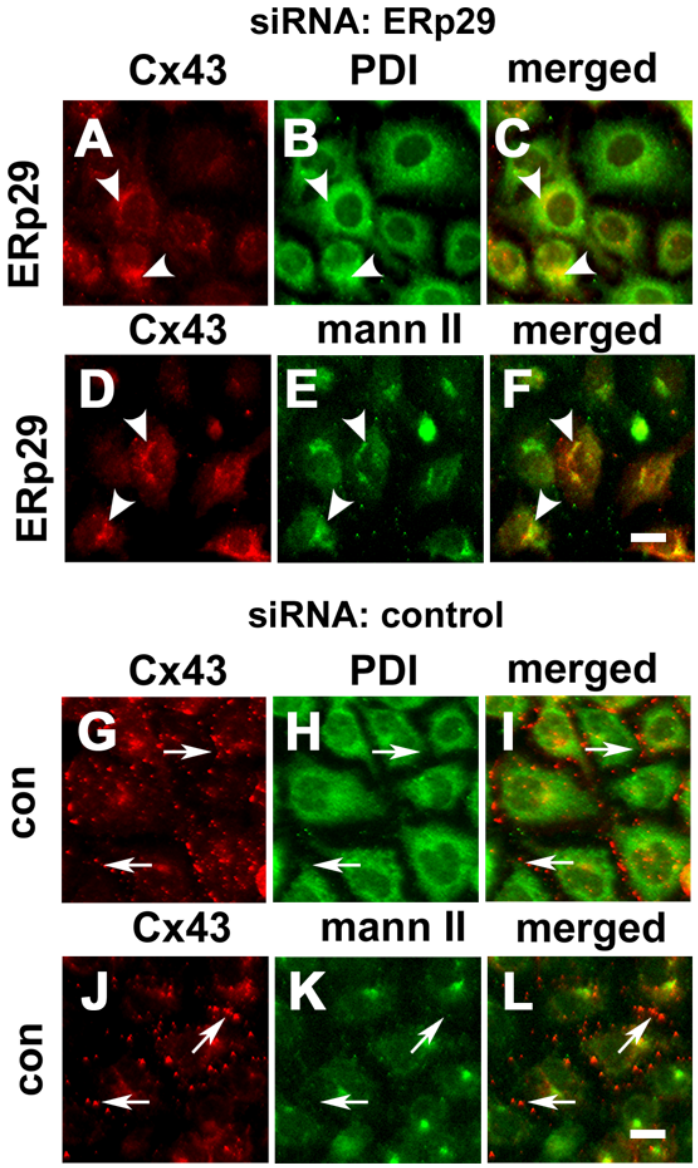
ERp29 depletion interferes with Cx43 trafficking in ROS cells. ROS cells were transfected with either control siRNA (A-C) or siRNA specific for ERp29 (D-F). After 48 h, the cells were fixed and double label immunostained for Cx43 (A,D) and ERp29 (B, E). In identically processed images, ERp29 immunofluorescence was reduced by specific siRNA, as compared to control-treated cells. Cells expressing higher levels of ERp29 showed more Cx43 assembled into gap junction plaques (arrowheads), consistent with a role for ERp29 in regulating Cx43 trafficking. Bar – 10 μ.

Das, et al., Supplemental Figure S3



ERp29 depletion interferes with Cx43 trafficking to the plasma membrane of A549 cells. A549 cells were either untreated, or transfected with control or ERp29 siRNA. After 48 h, cells were harvested and ERp29 (A), and Cx43 (B) content was assessed by immunoblot. Graphs show densitometric analysis of the individual immunoblots in A,B. C-E. A549 cells untreated (C), transfected with ERp29 siRNA (D) or with control siRNA (E) and further incubated for 48 h. The cells were then fixed and immunostained for Cx43. Both untreated and control cells showed Cx43 present at cell-cell interfaces with a distribution consistent with gap junction plaque formation (arrows). Consistent with Figure 2, surface labeling of Cx43 was diminished by ERp29 depletion and instead accumulated in the perinuclear region of the cell (arrowheads). Bar – 10 μ.

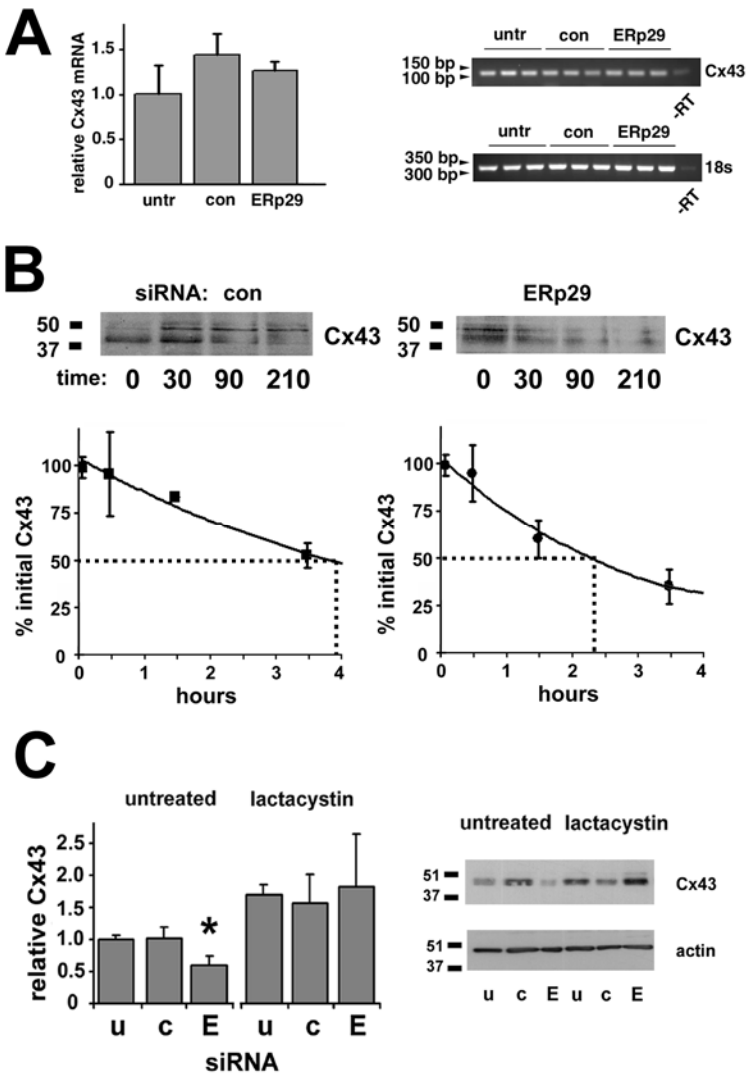
Das, et al., Supplemental Figure S4



Intracellular Cx43 localized predominantly to the Golgi apparatus in ERp29-depleted A549 cells. A549 cells were transfected with either ERp29 siRNA (A-F) or with control siRNA (G-L) and further incubated for 48 h. The cells were then fixed and immunostained for Cx43 (A,D,G,J) and an ER marker (protein disulfide isomerase, PDI) (B,H) or a Golgi marker (mannosidease II, mann II) (E,K). Merged images are in

C,F,I,L. In cells treated with ERp29 siRNA, Cx43 was retained in an intracellular compartment (arrowheads) that co-localized with the Golgi apparatus marker, mann II (F,L). To a lesser extent, Cx43 also colocalized with an ER marker, PDI, in cells treated with ERp29 siRNA (C). In cells treated with control siRNA, Cx43 was transported to the plasma membrane and assembled into gap junctions (I,L). Bar – 10 μ.

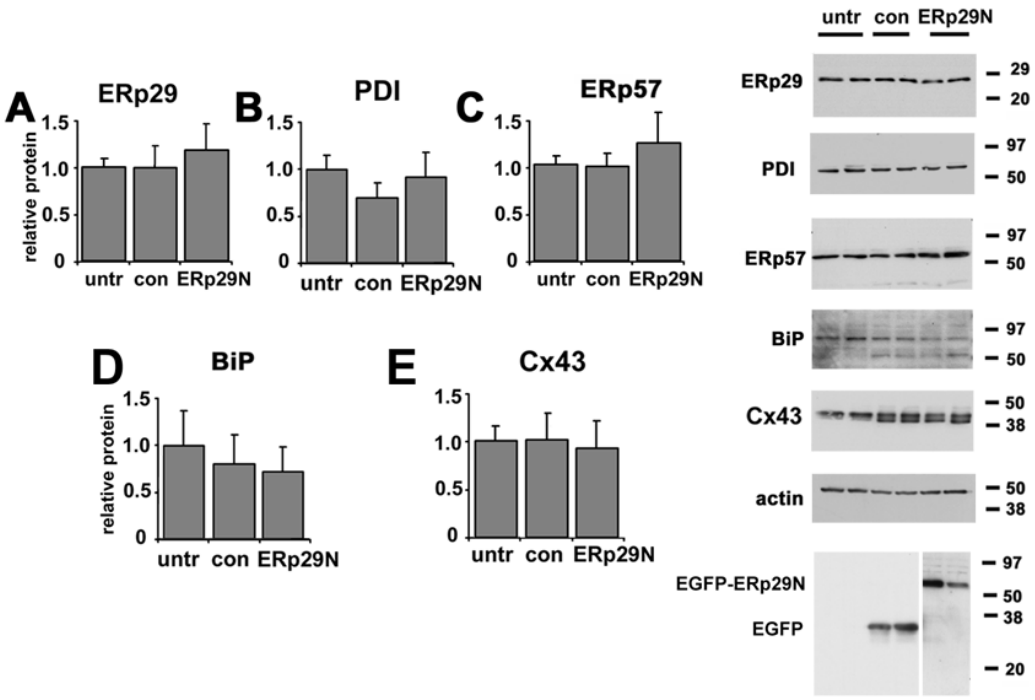
Das, et al., Supplemental Figure S5



Increased rate of Cx43 turnover in ERp29-depleted HeLa/Cx43 cells. A. HeLa/Cx43 cells were either untreated (untr), treated with control siRNA (con) or siRNA specific for ERp29. Two days after transfection, the cells were harvested and the level of Cx43 mRNA was measured by quantitative RT-PCR using a Cepheid SmartCycler and normalizing the signals to 18 S RNA. PCR products were resolved by agarose gel electrophoresis to confirm product size. B. Pulse chase analysis. HeLa/Cx43 cells were

treated with either control or ERp29 siRNA, then 2 days later were metabolically labeled with a pulse of ^{35}S, chased with cold medium for varying amounts of time, processed and immunoprecipitated with anti-Cx43. Cx43 turnover was ~50% more rapid for ERp29 depleted HeLa/Cx43 cells than controls. C. Treatment of cells for 4h with 10 μM lactacystin treatment increased the Cx43 content of HeLa/Cx43 cells, regardless of whether they were untreated (u), treated with control siRNA (c) or ERp29 siRNA (E).

Das, et al., Supplemental Figure S6



Effect of EGFP-ERp29-N expression by HeLa/Cx43 cells on ERp29, PDI, ERp57, BiP, and Cx43. HeLa/Cx43 cells were either untransfected (untr) or transfected with either EGFP-ERp29 (con) or the N-terminal fragment of EGFP-ERp29 (ERp29N), then harvested and analyzed 48 h after transfection. Shown are duplicate lanes for each treatment analyzed for ERp29 (A), PDI (B), ERp57 (C), BiP (D), Cx43 (E) and actin by immunoblot. The average ratio of protein expression was normalized to actin expression and plotted as average $\pm$ SE . EGFP-ERp29-N overexpression caused a significant increase in ERp29 and BiP expression (* $p < 0.05$, n=7), however, expression of the other proteins was unaffected by EGFP-ERp29 or EGFP-ERp29-N.