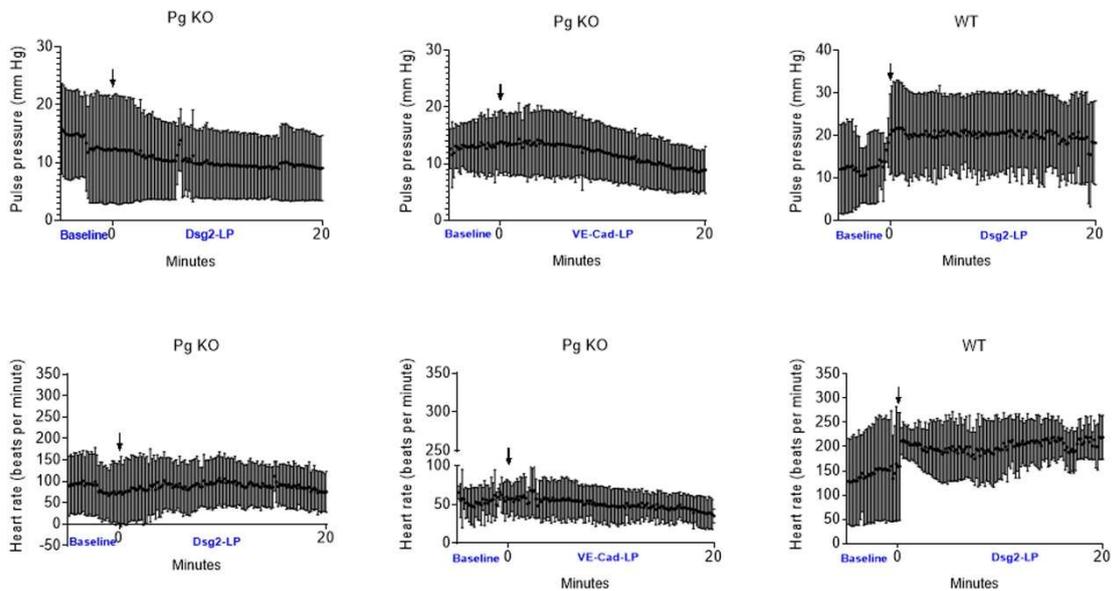


1 Supplementary Figures



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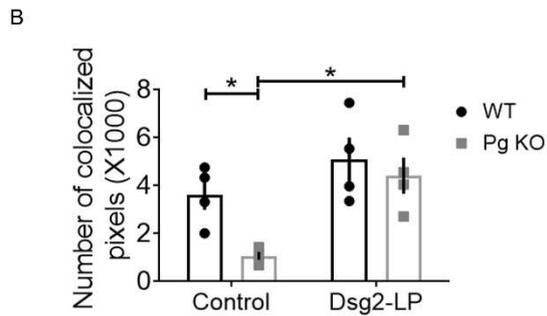
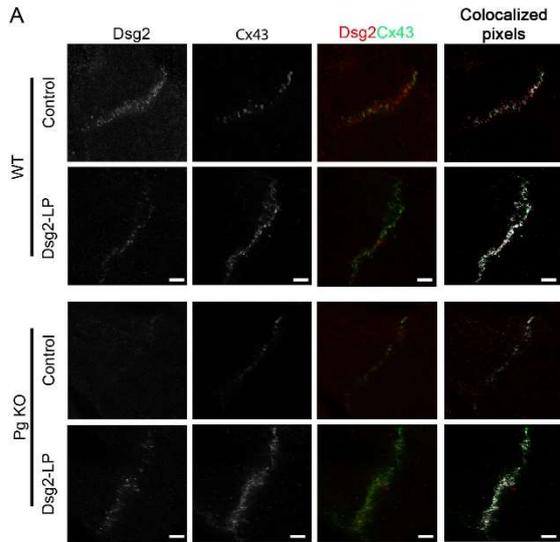
3 Supplementary figure 1. Pulse pressure and heart rates measured in Langendorff 4 perfused Pg WT and KO hearts.

5 Time course of pulse pressure (upper panel) and heart rate (lowe panel) of perfused hearts,
6 from the same mice shown in figure 1, under baseline condition and after addition of Dsg2-LP.
7 VE-Cad-LP served as peptide control. (n=5 mice for Dsg-LP and Pg KO and WT; n=3 mice for
8 VE-Cad-LP). Arrow indicates the time point of addition of Dsg2-LP or VE-Cad-LP. Average of
9 baseline values and 10 minutes of Dsg2-LP were compared. Two-tailed paired Student's t-test
10 with 95 % confidence levels was performed and no significant changes were observed.

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2 **Supplementary figure 2. Dsg2 and Cx43 membrane localization analyzed by STED**
 3 **microscopy in WT and Pg KO ventricular cardiac slices.**

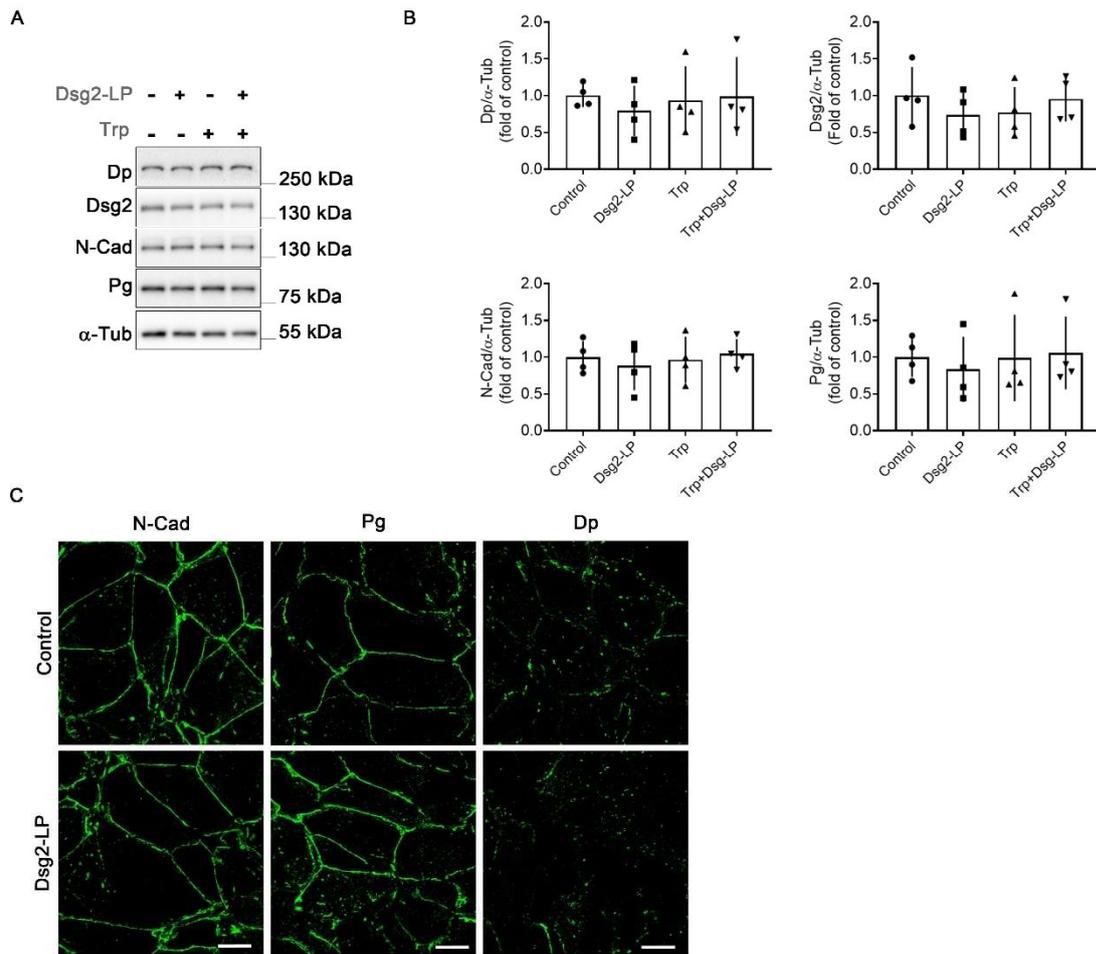
4 **A**, STED images of ICDs of WT and Pg KO mice treated with Dsg2-LP for 1 hour and stained
 5 for Dsg2 (red) and Cx43 (green) (four ICDs per mouse were analyzed for quantification, n=4
 6 mice per condition). Scale bar: 2µm. Colocalization of both proteins was analyzed and
 7 colocalized pixels are visualized in white. **B**, Quantification of colocalized pixels. **P*<0.05.
 8 Repeated measures two-way ANOVA with Sidak post-hoc test was performed.

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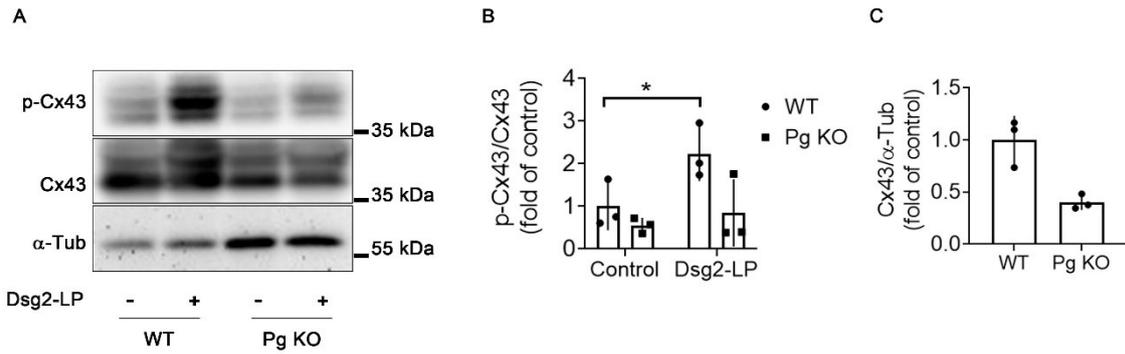
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2 **Supplementary figure 3. Dsg2-LP did not alter desmosomal cadherin expression or**
 3 **localization in HL-1 cells.**

4 **A**, Western blot analysis of desmoplakin (Dp), desmoglein 2 (Dsg2), N-cadherin (N-Cad) or
 5 plakoglobin (Pg) after 24 h treatment with Dsg2-LP. α -tubulin (α -tub) was used as a loading
 6 control **B**, Quantitative analysis of Western blots performed in **A** (n=4). One-way ANOVA with
 7 Sidak post-hoc test was performed and no significant changes were observed. **C**,
 8 Representative immunostaining of HL-1 cells stained for N-Cad, Pg and Dp treated with Dsg2-
 9 LP for 24h (n=3), scale bar: 10 μ m.

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2 **Supplementary figure 4. Dsg2-LP mediated phosphorylation of Cx43 in WT and Pg KO**
 3 **ventricular cardiac slices.**

4 **A**, Western blot analysis of Cx43 phosphorylation at serine S368 (p-Cx43) and total Cx43 after
 5 1 h treatment. α -tubulin (α -tub) was used as a loading control. **B**, **C**, Quantitative analysis of
 6 Western blots performed in **A** (n=3 mice per group). Two-way ANOVA with repeated measures
 7 and matching both factors with Sidak post-hoc test was performed for **B**. Two-tailed unpaired
 8 Student's t-test with 95 % confidence levels was performed for **C**.

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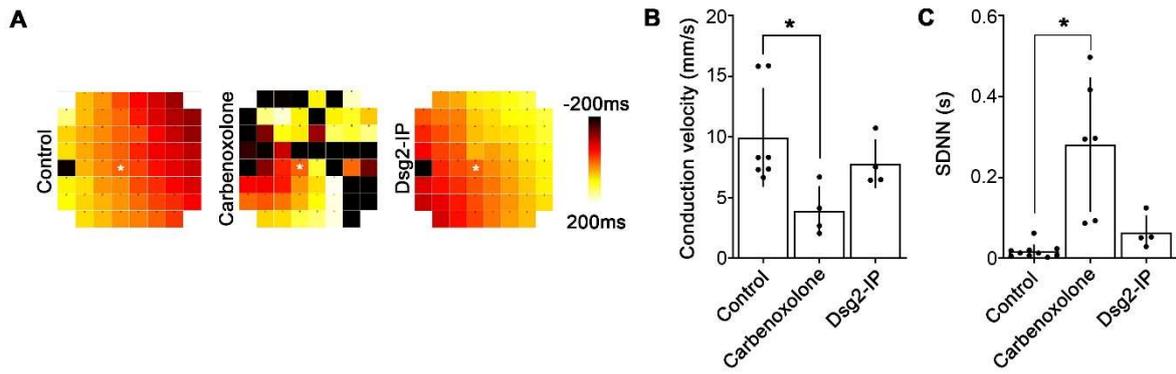
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2 **Supplementary Figure 5. MEA of HL-1 cardiomyocyte treated with carbenoxolone or**
 3 **Dsg2-IP.**

4 **A**, Representative MEA heat maps of HL-1 cardiomyocytes treated with the GJ inhibitor
 5 carbenoxolone or the inhibitory peptide Dsg2-IP. Reference electrode is marked by (*). **B**, **C**,
 6 Corresponding analysis of conduction velocity and standard deviation of beat-to-beat-intervals
 7 (SDNN) of MEA experiments described in **A** (n=3). * $P < 0.05$. One-way ANOVA with Dunnett
 8 post-hoc test.

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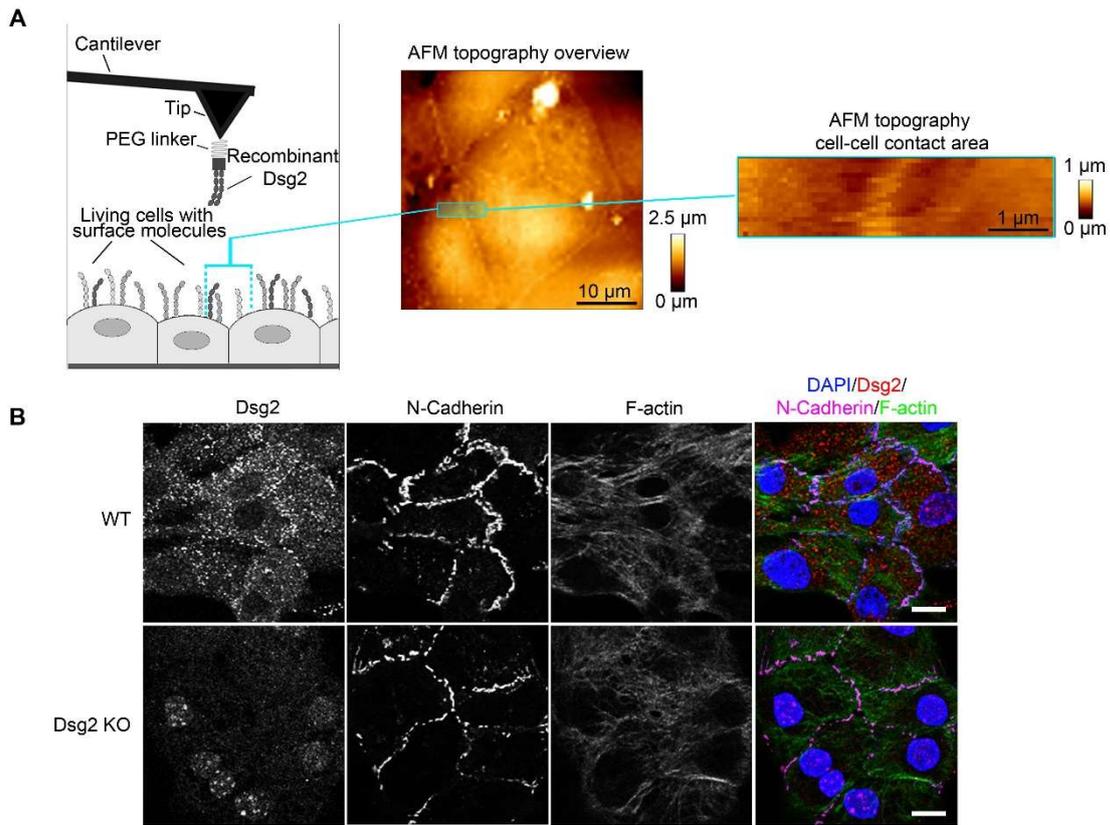
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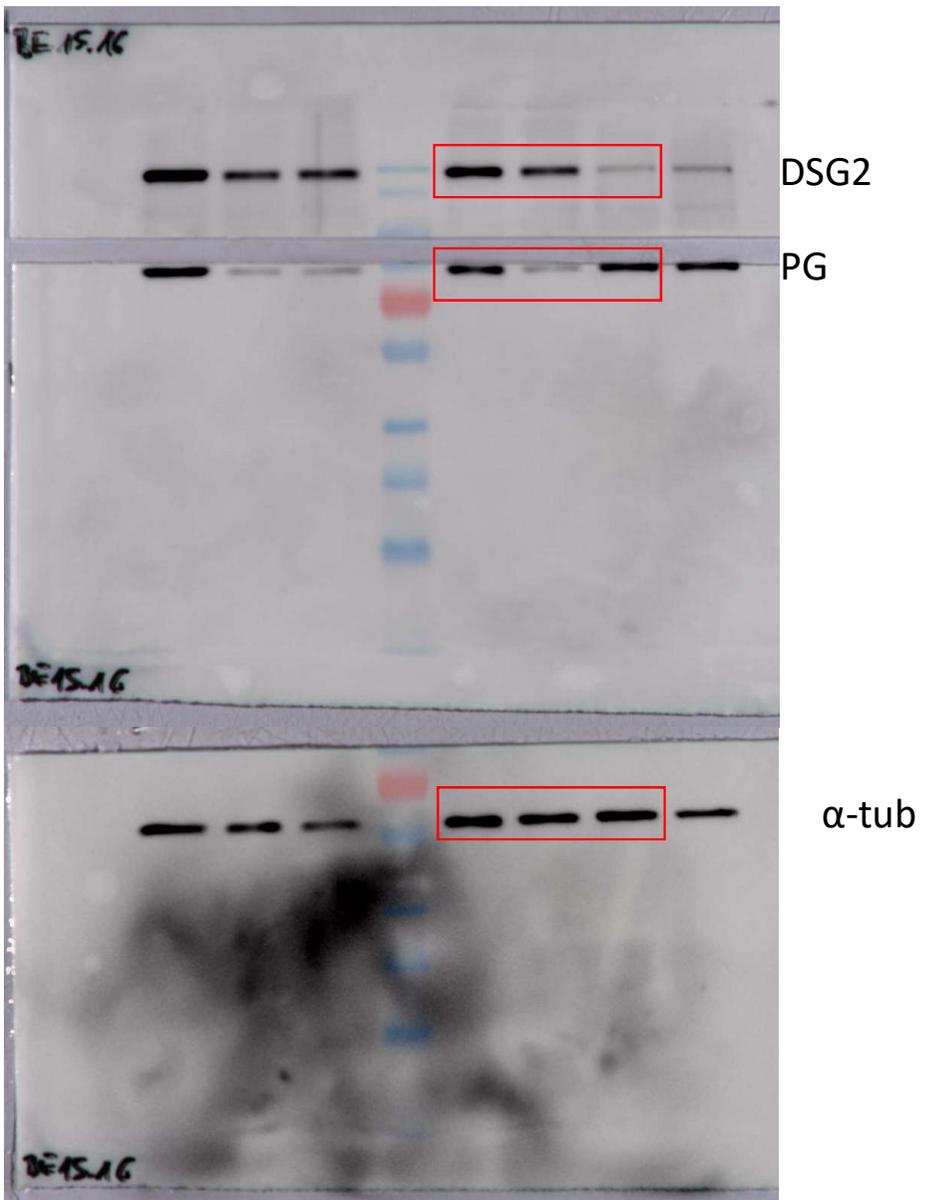
2 **Supplementary Figure 6. AFM force mapping on living cardiomyocytes.**

3 **A**, Schematic of AFM set-up on living cells. An area spanning the cell-cell border (marked in
 4 cyan) was selected for force spectroscopy as shown in the overview topography image. Scale
 5 bar overview: 10 μm ; insert: 1 μm . **B**, Representative immunostaining of cardiomyocytes
 6 isolated from neonatal Dsg2 KO mice and WT littermates stained for Dsg2 (red), N-cadherin
 7 (magenta), F-actin (green) and the nucleus (DAPI, blue) paralleled to AFM experiments shown
 8 in Fig. 5 (n=7 mice per phenotype). Scale bar: 10 μm .

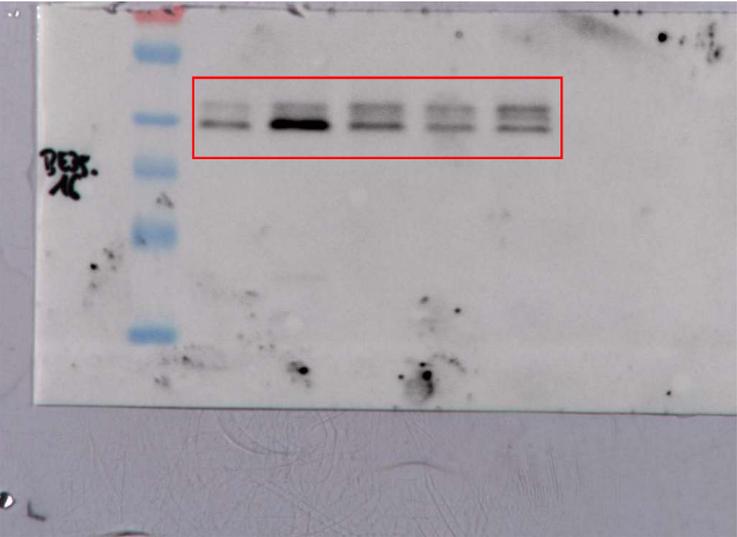
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Full Unedited gel for Figure 3B



Full Unedited gel for Figure 4I



pCx43 S368

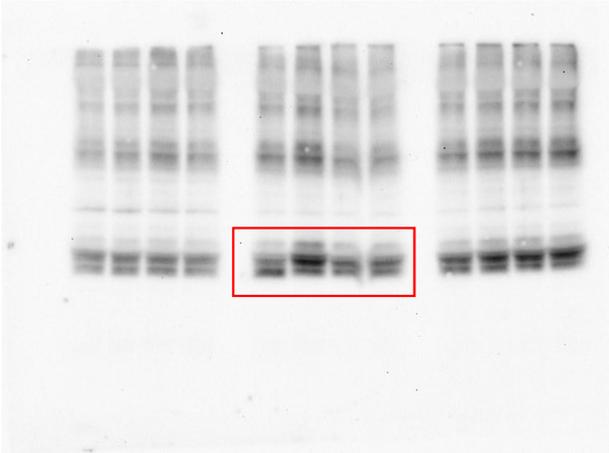


α -tub



Total Cx43

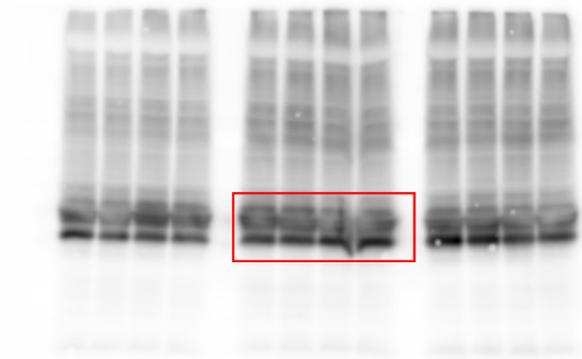
Full Unedited gel for Figure 4J



pCx43 S368

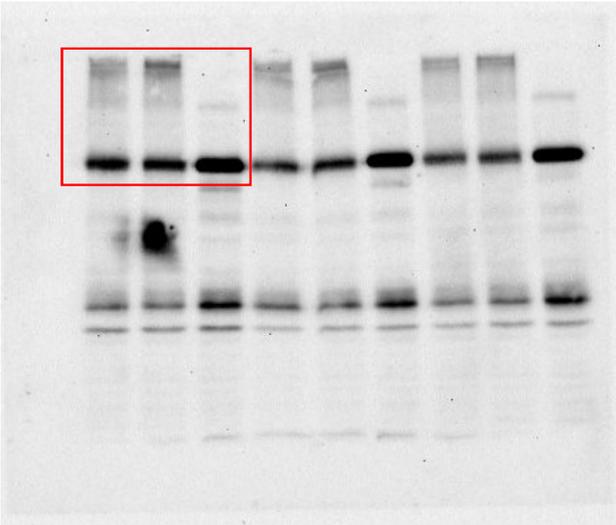


α -tub

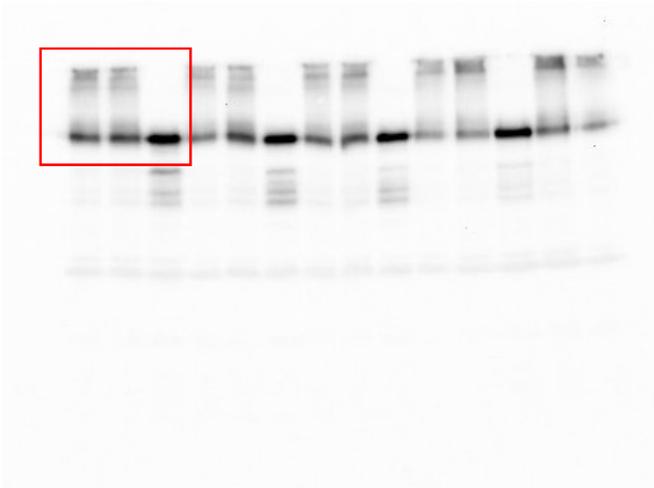


Total Cx43

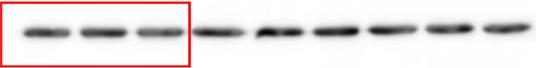
Full Unedited gel for Figure 7A



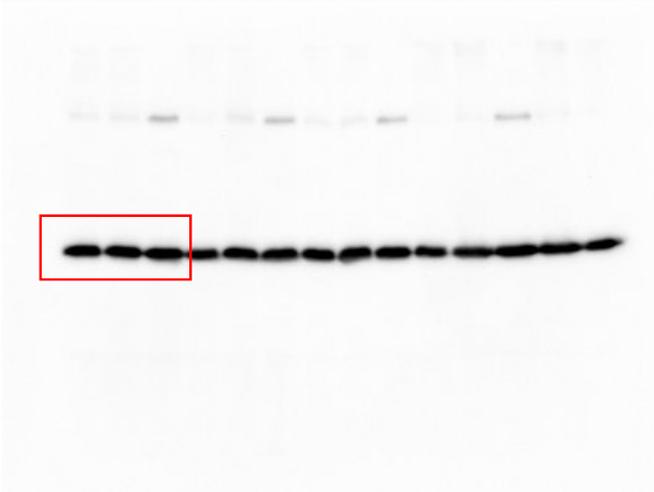
Dsg2



N-Cad

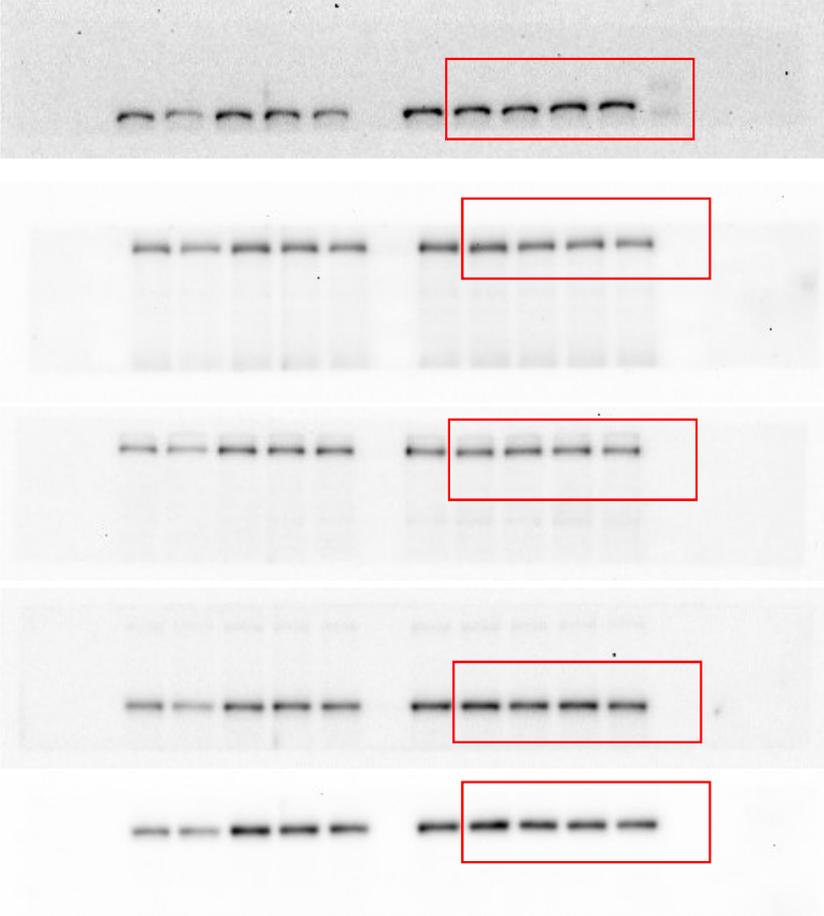


α -tub



α -tub

Full Unedited gel for Supplementary figure 3A



Dp

Dsg2

N-Cad

Pg

α -tub

Full Unedited gel for Supplementary figure 4A

