

Supplementary Materials: Phosphorylation-Dependent Intra-Domain Interaction of the Cx37 Carboxyl-Terminus Controls Cell Survival

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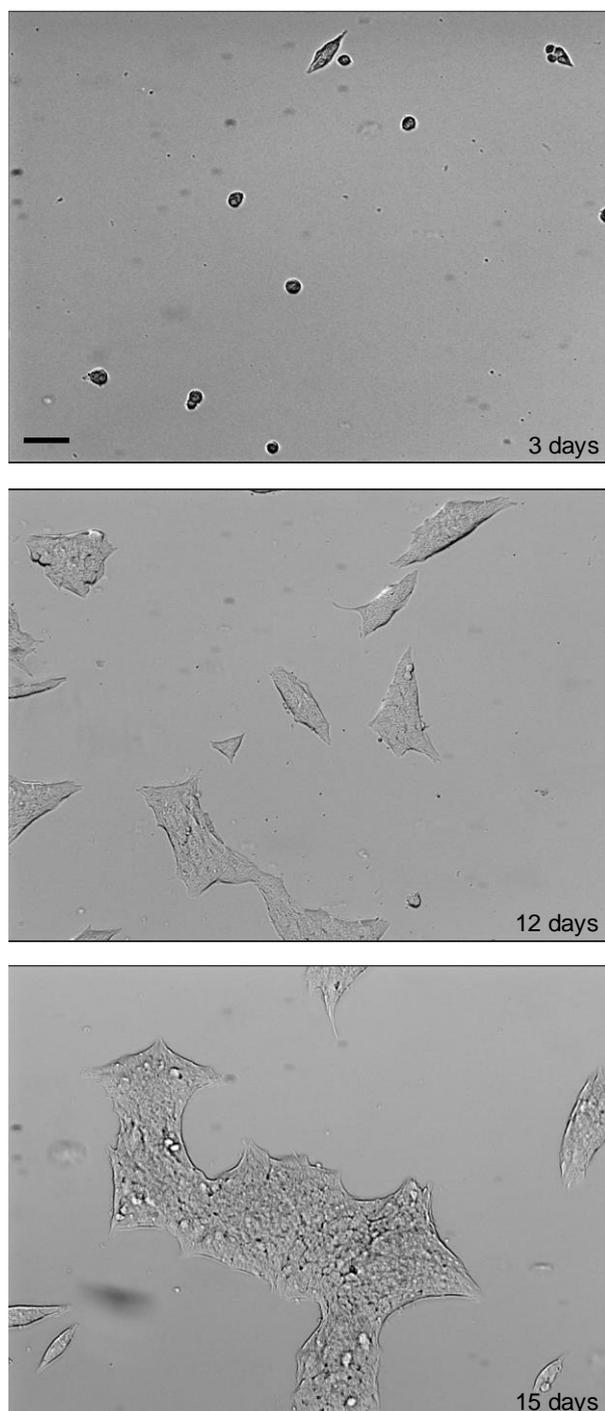


Figure S1. Cell density and cell-cell contact increase as a function of time post-plating in the absence of Cx37 expression (dox -). iRin37 cells plated at an initial density of 3,125 cells/cm² imaged at 3, 12 and 15 days. Scale bar applies to all images: 30 μ m.

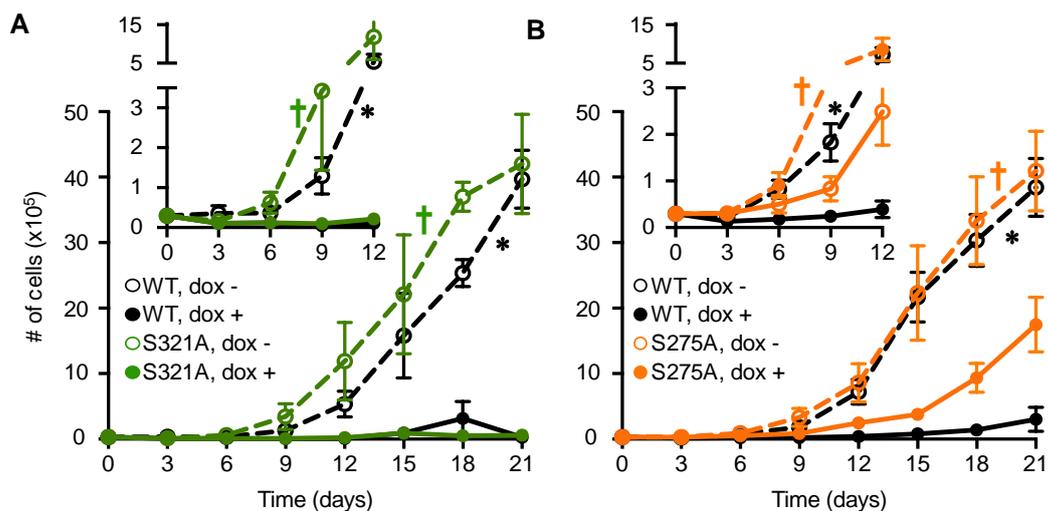


Figure S2. Cx37-S321A and Cx37-S275A do not induce death when expressed in iRin cells. **(A)** Expression of Cx37-S321A induces potent growth arrest at low density. **(B)** iRin37-S275A cells proliferate after a short period of growth arrest (days 0-3), little or no cell death occurs.

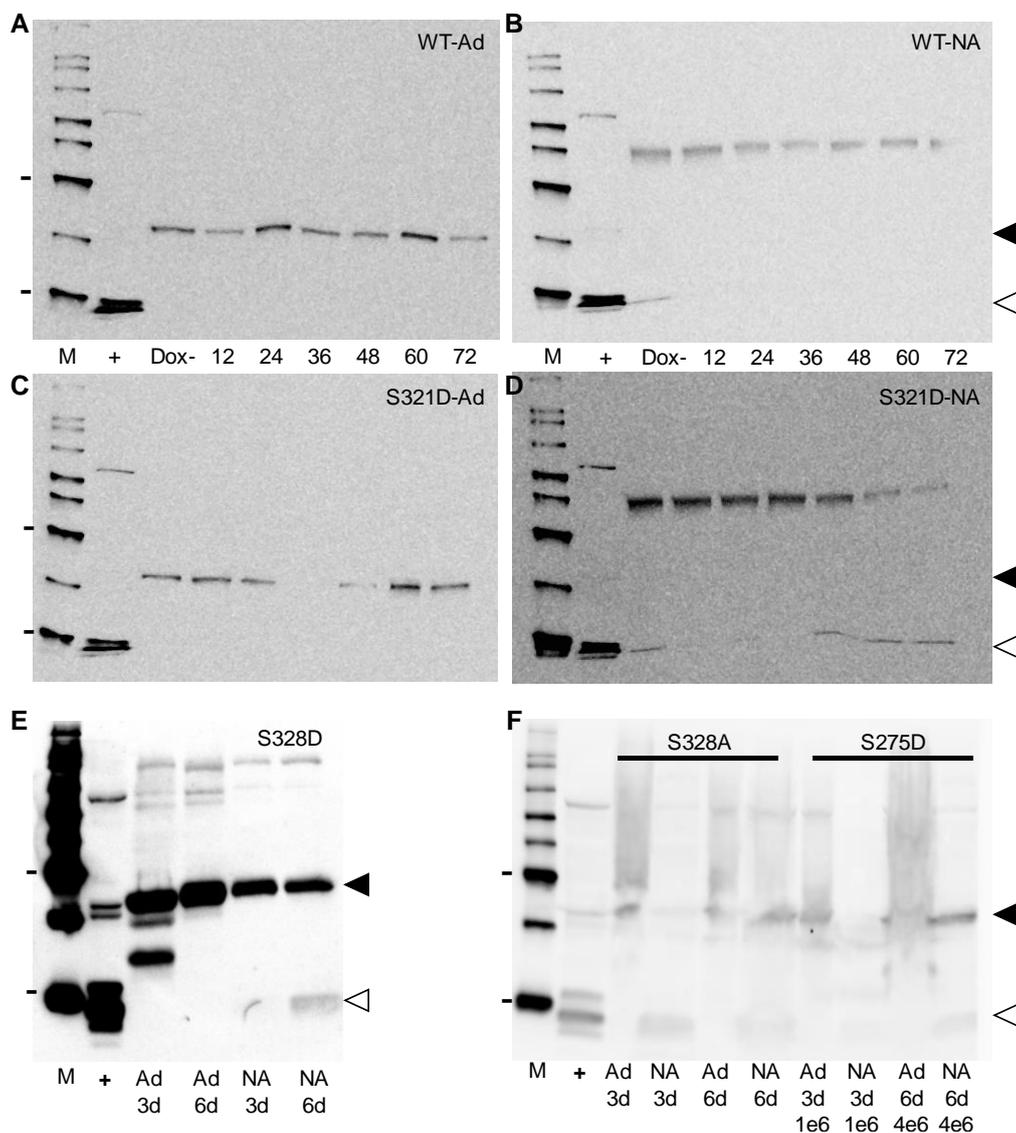


Figure S3. Activated caspase 3 is detected in non-adherent, dying cells expressing Cx37-S321D, -S328D, -S328A or -S275D. **(A,B)** Activated caspase 3 was not detected in the adherent

(Ad; still living) or non-adherent (NA; dying) Cx37-WT expressing cells. (C,D) Activated caspase 3 was detected in dying, NA cells but not in the adherent cells. In the Cx37-WT and -S321D blots adherent (Ad) and non-adherent (NA) cells were collected from non-induced cells (dox-) and cells induced with dox for 12, 24, 36, 48, 60 and 72 hours. (E,F) In S328D, S328A, S275D blots, Ad and NA cells were collected from cells induced for 3 or 6 days. For all blots, the left lane shows molecular mass markers (M) with the 40 and 20 kDa bands marked; the second lane (+) contains the positive control for activated caspase 3. Solid triangle shows the position of uncleaved caspase 3 and the open triangle shows the position of activated (cleaved) caspase 3.

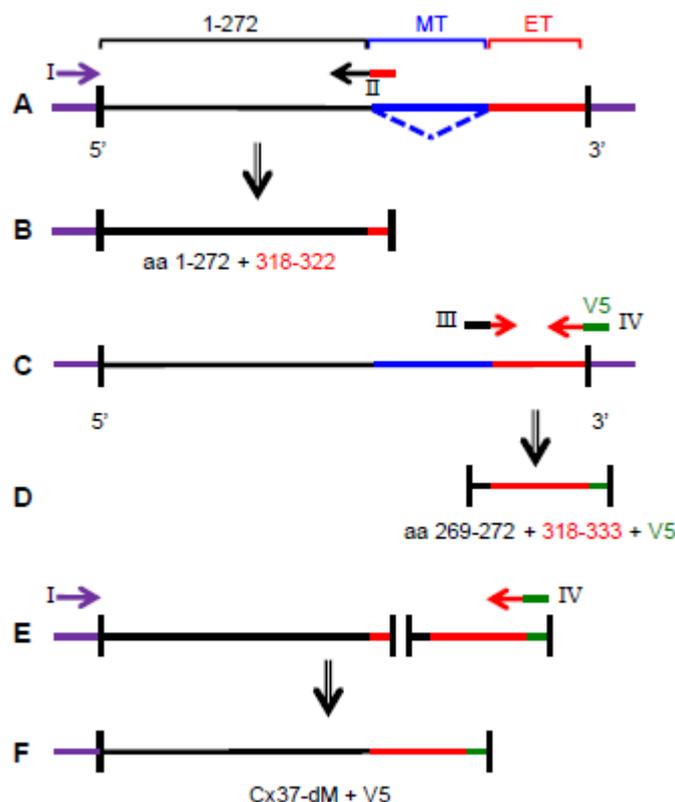


Figure 4. Schematic of strategy used to generate the Cx37-dM construct. The plasmid containing the mid-tail (aa 273-317) deletion was made in a 3-step amplification process. (A,B) aa 1-272 and 318-323 were linked with primers I & II and full length Cx37 in pTre2-Hygro plasmid. (C,D) aa 269-272 were linked to aa 318-333 plus V5 tag and *NheI* restriction site were linked with primers III & IV, (E,F) PCR products from steps 1 and 2 were combined using primers I & IV (E) to make the contiguous Cx37-dM (F; containing aa 1-272 + 318-333) + V5 and the 5' *BamHI* and 3' *NheI* restriction sites. The resulting plasmid was directionally cloned into *BamHI* and *NheI* sites in pTRE2h.

Table S1. Significance of differences in HCh P_o between Cx37-WT and (de)phospho-mimicking mutants. P values for the comparison of P_o for the isoform in the left column to remaining columns for each group; e.g. closed state probability of WT HChs is not different from S321D but significantly greater than the closed state probability of S328A. * indicates $p < 0.0001$; ns indicates not significant.

| WT | S321D | S328D | S328A | S275D |
|-----------------|-----------|-------|----------|-------|
| Closed | ns | ns | >, * | ns |
| All open states | ns | ns | <, * | ns |
| 100-300 pS | ns | ns | <, * | ns |
| 300-500 pS | <, 0.0054 | ns | <, 0.045 | ns |
| 500-800 pS | >, 0.0025 | ns | ns | ns |
| >800 pS | ns | <, * | <, * | ns |

| S321D | S328D | S328A | S275D |
|-----------------|-------|-------|-----------|
| Closed | ns | >, * | ns |
| All open states | ns | <, * | <, * |
| 100-300 pS | ns | <, * | >, 0.0034 |
| 300-500 pS | ns | ns | >, 0.0233 |
| 500-800 pS | ns | <, * | <, 0.0004 |
| >800 pS | <, * | >, * | ns |

| S328D | S328A | S275D |
|-----------------|-----------|-------|
| Closed | >, * | ns |
| All open states | <, * | ns |
| 100-300 pS | <, * | ns |
| 300-500 pS | <, 0.0424 | ns |
| 500-800 pS | ns | ns |
| >800 pS | ns | >, * |

| S328A | S275D |
|-----------------|-----------|
| Closed | <, * |
| All open states | >, * |
| 100-300 pS | >, * |
| 300-500 pS | >, 0.0164 |
| 500-800 pS | ns |
| >800 pS | >, * |

Table S2. List of primers used to generate Cx37 mutants.

| Construct/mutation | Primer sequence 5' to 3' |
|------------------------|---|
| 1-272 + 318-323 | F (I): CGCCTGGAGACGCCATTCC (plasmid sequence) |
| | R (II): GCTAGGGGACTTTCCTCGCCCATGGGGAG |
| 269-272 + 318-333 + V5 | F (III): ATGGGCGAGGGAAGTCCCCTAGCCGCCCC |
| | R (IV): CTAGCTAGCCTACGTAGAATCGAGACCGAGGAGAGGGTTAGGGATAGGCTTACC CACATACTGCTTCTT (stop codon/restriction site) |
| dM +V5 | F (I): CGCCTGGAGACGCCATTCC |
| | R (IV): CTAGCTAGCCTACGTAGAATCGAGACCGAGGAGAGGGTTAGGGATAGGCTTACC CACATACTGCTTCTT |
| dE +V5 | F (I): CGCCTGGAGACGCCATTCC |
| | R: CTAGCTAGCCTACGTAGAATCGAGACCGAGGAGAGGGTTAGGGATAGGCTTACC CTTTCGGCCACCCTG |
| S319A, S321A | F: CCATGGGCGAGGAAAGGCACCAGCCCCGCCCCAACAGCTCTGC R: GCAGAGCTGTTGGGGCGGTCTGGTGCCTTTCCTCGCCCATGG |
| S325A, S328A | F: CGAGGAAAGGCCCTGCCGCCCAACGCCTCTGCAGCCAAGAAGCA R: TGCTTCTTGGCTGCAGAGCGCTTGGGGCGGGCAGGGCCTTTCCTCG |
| S319D, S321D | F: CCATGGGCGAGGAAAGGATCCAGACCGCCCCAACAGCTCTGC R: GCAGAGCTGTTGGGGCGGTCTGGATCCTTTCCTCGCCCATGGG |
| S325D, S328D | F: CGAGGAAAGGACCCTGACCGCCCCAACGACTCTGCAGACAAGAAGCA |

| | | |
|-------|----------|--|
| | R: | TGCTTCTTGTCTGCAGAGTCGTTGGGGCGGTCAGGGTCCTTCCCTCG |
| S275A | F: R: | GGGACCTCTGCCCCACCGTGTC GACACGGTGGGGCAGAGGGTCCC |
| S302A | F: R: | GAGAGACTGACCGCTTCCAGACTCCC GGGAGGTCTGGAAGCGGTCAGTCTCTC |
| S285A | F: R: | CCTACAACGGGCTCGCTTCCACTGAGCAGAAC GTTCTGCTCAGTGGAAAGCGAGCCC GTTGTAGG |
| S321A | F: R: | GAAAGTCCCCTGCACGCCCAACAG CTGTTGGGGCGTGCAGGGGACTTTC |
| S275D | F: R: | CATGGGCGAGGGACCCTCTGATCCACCGTGTCCCACCTAC GTAGGTGGGACACGGTGGATCAGAGGGTCCCTCGCCCATG |
| S302D | F: R: | CACAGAGGAGAGACTGACCGACTCCAGACCTCCCCATTG CAAATGGGGGAGGTCTGGAGTCGGTCAGTCTCTCCICTGTG |
| S285D | F: R: | CCTACAACGGGCTCGACTCCACTGAGCAGAAC GTTCTGCTCAGTGGAGTCGAGCCC GTTGTAGG |
| S321D | F: R: | GCCGAAAGTCCCCTGATCGCCCCAACAGCTC GAGCTGTTGGGGCGATCAGGGGACTTTCGGC |