

Phosphorylation of Connexin36 near the C-terminus switches binding affinities for PDZ-domain and 14-3-3 proteins *in vitro*

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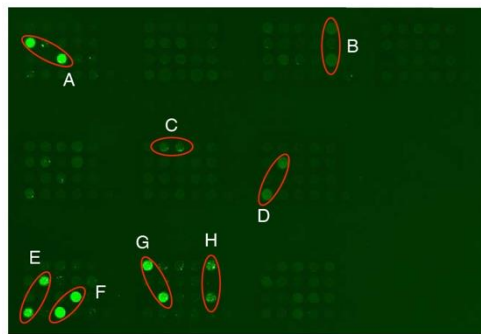
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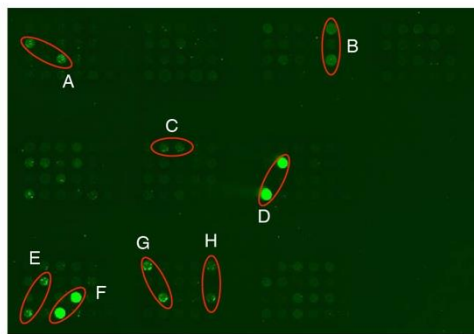
C-Terminal Domain Readers Array

PDZ	PDZ	PDZ	14-3-3 / 14-3-3 like
A1) α-1-syntrophin (1/1)/Q61234 A2) β1-syntrophin (1/1)/Q99L88 A3) γ1-syntrophin (1/1)/Q925E1 A4) γ2-syntrophin (1/1)/Q925E0 A5) Chapsyn-110 (2/3)/Q91XM9 A6) Chapsyn-110 (3/3)/Q91XM9 A7) Dlg3 (1/1)/Q6XE40 A8) Dvl1 (1/1)/Q60838 A9) Dvl2 (1/1) A10) Dvl3 (1/1)	B1) Cipp (3/10)/Q63ZW7 B2) Cipp (5/10)/Q63ZW7 B3) Cipp (8/10)/Q63ZW7 B4) Cipp (9/10)/Q63ZW7 B5) Cipp (10/10)/Q63ZW7 B6) Radil (1/1)/Q69Z89 B7) Erbin (1/1)/Q80TH2 B8) GRASP55 (1/1)/Q99JX3 B9) Grip1 (6/7)/Q925T6 B10) Grip2 (5/7)/E0CX54	C1) Harmonin (2/3)/Q9ES64 C2) HtrA1 (1/1)/Q9QZK6 C3) HtrA3 (1/1)/Q9D236 C4) Interleukin 16 (1/4)/Q9QZP6 C5) LARG (1/1)/Q8R4H2 C6) LIN-7A (1/1)/Q8JZS0 C7) Lin7c (1/1)/Q88952 C8) Lnx1 (2/4)/O70263 C9) Lnx1 (3/4)/O70263 C10) Lrrc7 (1/1)/Q80TE7	D1) sigma/NP_006133 D2) beta/alpha/NP_647539.1 D3) epsilon/NP_006752 D4) gamma/CAG46702 D5) eta/CAG30498 D6) theta/NP_006817 D7) zeta/delta/NP_663723 D8) SMG5/NP_056142 D9) SMG7/NP_963862
E1) Magi-1 (2/6)/Q6RHR9 E2) Magi-1 (4/6)/Q6RHR9 E3) Magi-1 (6/6)/Q6RHR9 E4) Magi-2 (2/6)/Q9WVQ1 E5) Magi-2 (5/6)/Q9WVQ1 E6) Magi-2 (6/6)/Q9WVQ1 E7) Magi-3 (5/6)/Q9EQJ9 E8) Mais2 (1/1)/lin-7/Q9HAP6 E9) Magi-3 (1/6)/Q9EQJ9 E10) Sempcap3 (1/2)/Q69ZS0	F1) Mpp7 (1/1)/Q8BVD5 F2) MUPP1 (5/13)/Q8VBX6 F3) MUPP1 (10/13)/Q8VBX6 F4) MUPP1 (11/13)/Q8VBX6 F5) MUPP1 (12/13)/Q8VBX6 F6) MUPP1 (13/13)/Q8VBX6 F7) nNOS (1/1)/Q9Z0J4 F8) OMP25 (1/1)/Q8K4F3 F9) PAR-3 (3/3)/Q99NH2 F10) Shank3 (1/1)/Q4ACU6	G1) NHERF-1 (1/2)/P70441 G2) NHERF-1 (2/2)/P70441 G3) NHERF-1 FL/P70441 * G4) NHERF-2 (1/2)/Q9JHL1 G5) NHERF-2 (2/2)/Q9JHL1 G6) NHERF-2 FL/Q9JHL1 * G7) Pdzk1 (1/4)/NHERF-3/Q9JIL4 G8) Pdzk1 (3/4)/NHERF-3/Q9JIL4 G9) Pdzk3 (1/4)/NHERF-4/Q99MJ6 G10) Pdzk3 (3/4)/NHERF-4/Q99MJ6	
H1) PAR6B (1/1)/Q9JK83 H2) Pdlim5 (1/1)/Q3UGD0 H3) Pdzk11 (1/1)/Q9CGZ9 H4) PDZ-RGS3 (1/1)/Q9DC04 H5) PSD95 (1/3)/Q62108 H6) PSD95 (2/3)/Q62108 H7) PSD95 (3/3)/Q62108 H8) PTP-BL (2/5)/Q64512 H9) PAR3B (1/3)/Q5SV53 H10) TIP-1 (1/1)/Q9DBG9	I1) SAP102 (2/3)/P70175 I2) SAP102 (3/3)/P70175 I3) SAP97 (1/3)/Q811D0 I4) SAP97 (2/3)/Q811D0 I5) SAP97 (3/3)/Q811D0 I6) Scrbl (3/4)/Q80U72 I7) Shank1 (1/1)/D3YZU1 I8) Pdzk3 (1/6)/E9Q1M1 I9) Pdzk3 (2/6)/E9Q1M1	J1) Shroom (1/1)/Q9QXN0 J2) SLIM (1/1)/Q8R1G6 J3) Tiam2 (1/1)/Q6ZPF3 J4) Whirlin (3/3)/Q5MLF8 J5) ZO-1 (1/3)/P39447 J6) ZO-1 (2/3)/P39447 J7) ZO-2 (1/3)/Q9Z0U1 J8) ZO-3 (1/3)/Q9QXY1 J9) Scrbl (1/4)/Q80U72 J10) Scrbl (2/4)/Q80U72	

*= Non-Codon Optimized Construct

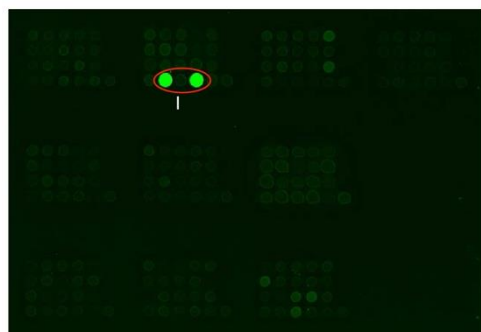


Cx36 CT tip - 100 μg
Biotin-VSPNFGRTQSSDSAYV-COOH



Cx36 phospho-CT tip - 100 μg
Biotin-VSPNFGRTQ[ps]SDSAYV-COOH

- A. Chapsyn-110 (2/3) PDZ
- B. Interleukin 16 (1/4) PDZ
- C. MUPP1 (5/13) PDZ
- D. NHERF-2 FL
- E. PSD95 (2/3) PDZ
- F. PTP-BL (2/5) PDZ
- G. SAP102 (2/3) PDZ
- H. SAP97 (2/3) PDZ
- I. Grip1 (6/7) PDZ

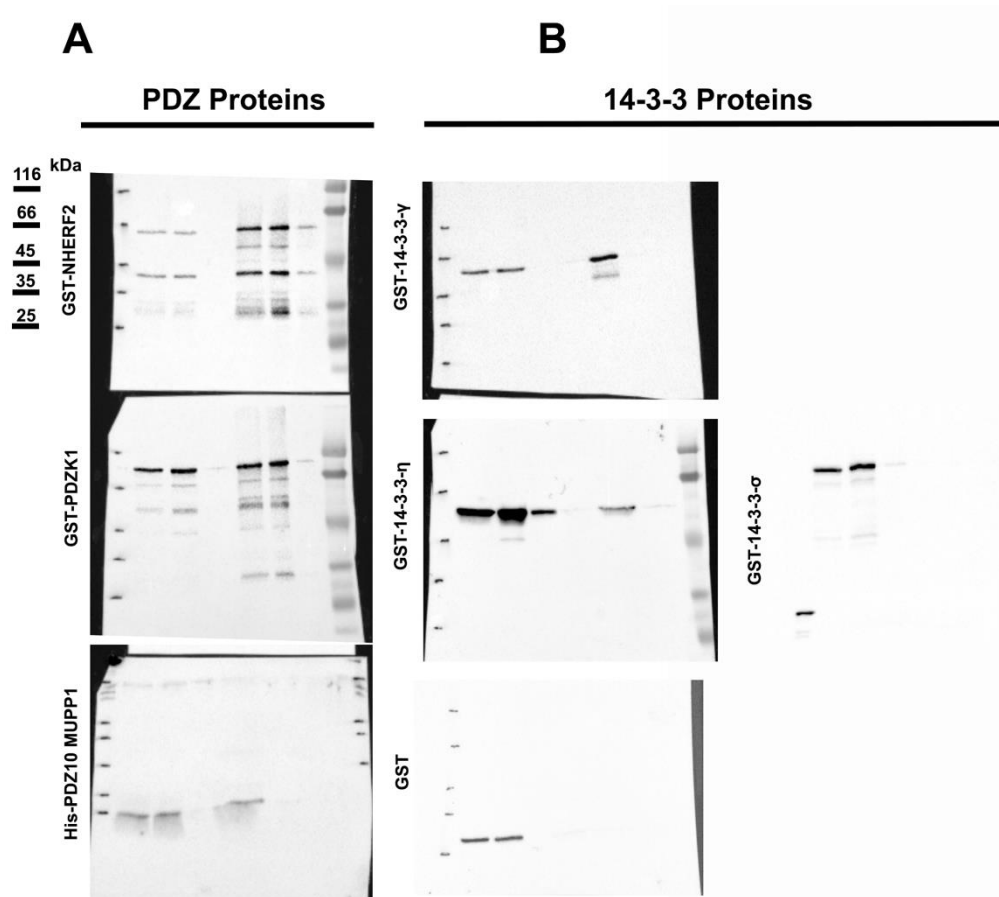


Scrambled - 927206 - 100 μg
Biotin-QRTDNKSRALSGYRVEV-COOH

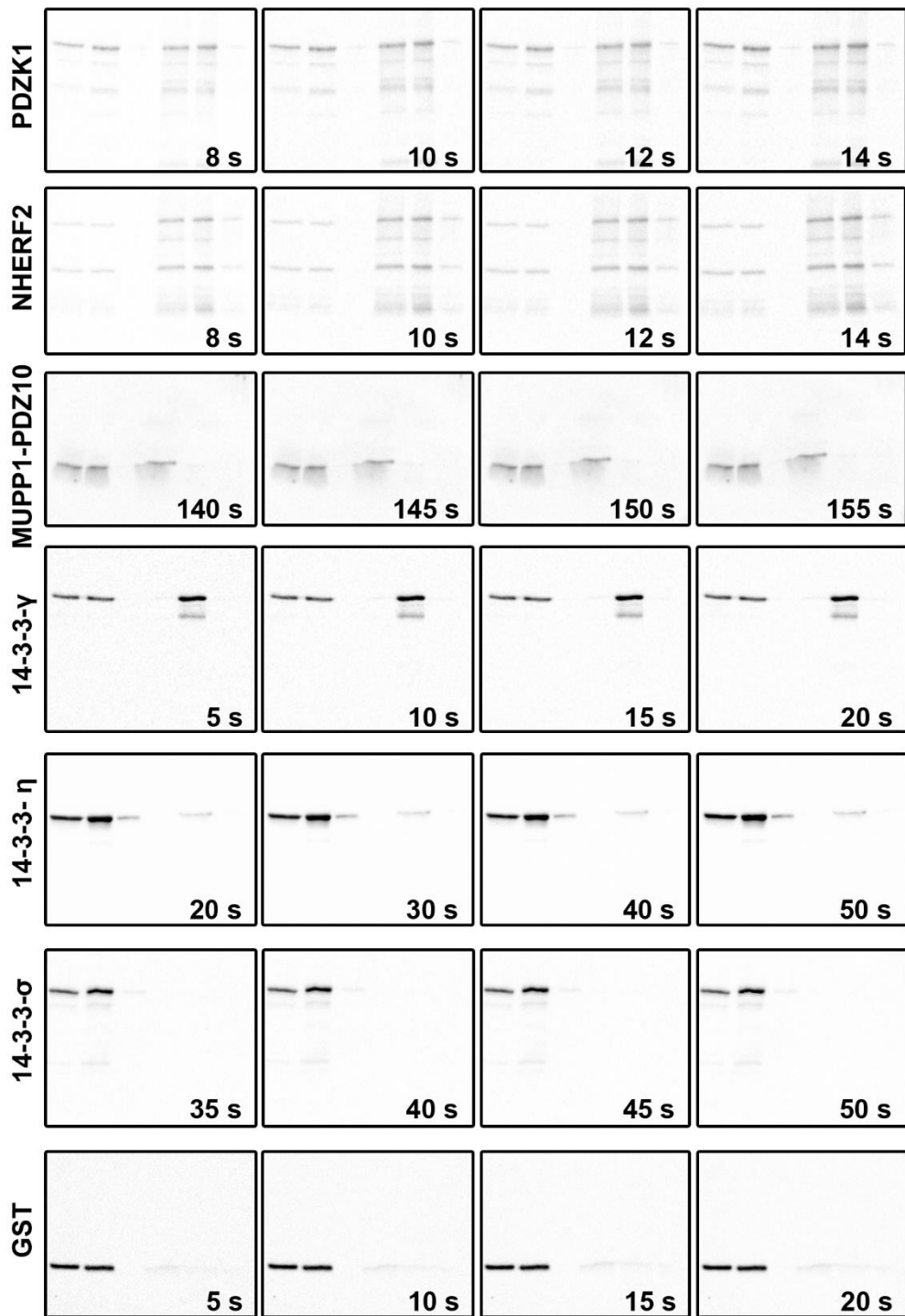


αGST

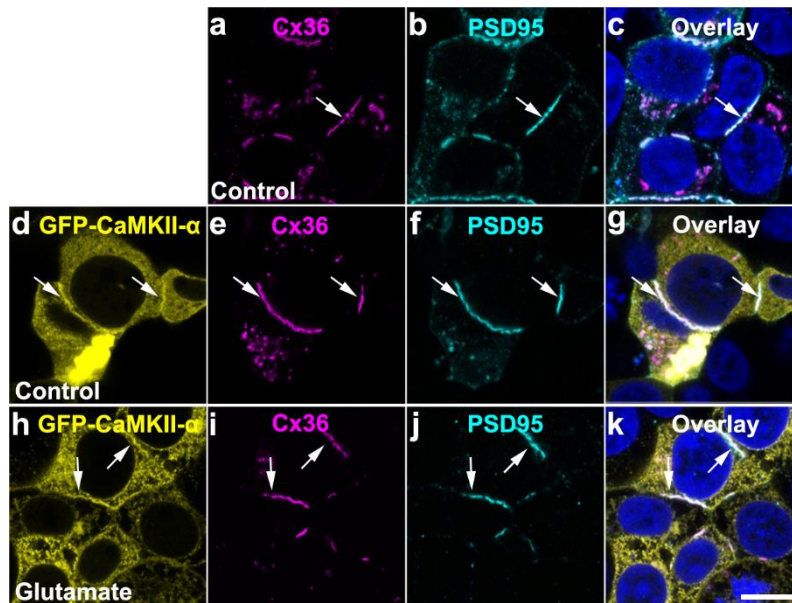
Supplementary Figure 1. C-terminal domain readers microarray probed with mmCx36 peptides. Note that although the scrambled peptide sequence was generated by reassigning residues of Cx36 peptides with a random number generator, a PDZ-binding site was generated that binds to Grip1.



Supplementary Figure 2. Original blots of pull-down experiments. **(A)** Original blots of pull-down experiments with PDZ proteins. **(B)** Original blots of pull-down experiments with GST-tagged 14-3-3 proteins.



Supplementary Figure 3. Blots of pull-down experiments with variable exposure times.



Supplementary Figure 4. Activation of co-expressed CaMKII did not cause any apparent changes in Cx36/PSD95 association. (a-c) Colocalization of Cx36 and PSD95 at gap junctions (arrow) in co-transfected HEK293 cells. **(d-g)** Co-expression of CaMKII- α with Cx36 and PSD95 in HEK293 cells. **(h-k)** Activation of co-transfected CaMKII by treatment with glutamate (100 μ M) did not affect the localization of PSD95 at Cx36-containing gap junctions. Arrows in a-k point to gap junctions between two adjacent cells. Scale: 10 μ m.