

**Table S1. Effect of Itk PH domain point mutations on protein abundance, PI(3,4,5)P<sub>3</sub> binding, and CaM binding.** Protein abundance was determined for PH domain-YFP fusion protein by Western blot with antibodies recognizing GFP and YFP (JL8). In vitro PI(3,4,5)P<sub>3</sub> binding was determined by precipitation of PI(3,4,5)P<sub>3</sub>-coated beads. CaM binding was determined by coimmunoprecipitation in the presence of Ca<sup>2+</sup>. —, undetectable

|           | <b>Protein abundance</b> | <b>In vitro PI(3,4,5)P<sub>3</sub> binding</b> | <b>CaM binding</b> |
|-----------|--------------------------|--|--------------------|
| WT        | ++++                     | ++++   | ++++               |
| L41Y      | +                        | ++++++   | +++                |
| K35R      | ++++                     | ++++   | +++++              |
| K35D      | ++++                     | +  | ++                 |
| K35A      | ++++                     | +  | ++++               |
| K35G      | ++++                     | +  | ++++               |
| K35V      | ++++                     | +  | ++++               |
| K48A      | ++++                     | ++++   | ++++               |
| K48E      | ++++                     | ++++   | ++++               |
| L51Q      | ++++                     | ++++   | ++++               |
| L51R      | ++++                     | ++++   | ++                 |
| L51A      | ++++                     | ++   | ++++               |
| L51D      | ++++                     | +  | ++++               |
| L51E      | ++++                     | ++   | ++++               |
| L51M      | ++++                     | ++++   | +++                |
| L51G      | +++                      | —  | +                  |
| L51T      | +++                      | +  | ++++               |
| L51K      | +++                      | ++++   | +++                |
| L51V      | ++                       | +  | +                  |
| K48E R49E | ++                       | +  | +                  |
| F41D L51M | ++++                     | +  | ++++               |
| F41D L51R | +                        | —  | ++                 |
| F41D L51V | +                        | —  | +                  |
| F41V L51M | +                        | —  | +                  |
| F41A L51A | ++                       | +  | +++                |

**Table S2. Oligo primers for cloning PH domains and YFP.** The red sequences represent the linkers added between the PH domain and YFP.

| Primer          | Primer sequence  |
|-----------------|--|
| Ipcef1 PH F     | 5'-TAAGCTAGCCGCCACCATGGCTAGTCCGAGAAGGATATCCTGTAAA-3'           |
| Ipcef1 PH R     | 5'-TAA <b>GGATCCGCCTCCACC</b> GGTCACAGCAAATCCAAGTTTGTTTAACC-3' |
| Veph1 PH F      | 5'-<br>TAAGCTAGCCGCCACCATGGCTAATCAAGATGGCCAGCCTCTCATCGA-3'     |
| Veph1 PH R      | 5'-TAA <b>GGATCCGCCTCCACC</b> CTCCTTTGCCTGGGCCAGCGCCACGTTG-3'  |
| Plekha8 PH F    | 5'-TAAGCTAGCCGCCACCATGGAGGGCGTGCTGTACAAGTGGACC-3'              |
| Plekha8 PH R    | 5'-TAA <b>GGATCCGCCTCCACC</b> CAGGCAGGCCTTGGCAGATCCCAGAGC-3'   |
| Plek2 PH F      | 5'-TAAGCTAGCCGCCACCATGGAGGACGGCGTGCTCAAGGAAGGC-3'              |
| Plek2 PH R      | 5'-TAA <b>GGATCCGCCTCCACC</b> CTTCCCTGGCTGCCCTGCATGGATAGC-3'   |
| Arhgap12 PH F   | 5'-TAAGCTAGCCGCCACCATGGGTGAGGAGAAATATGGACTGTAAAT-3'            |
| Arhgap12 PH R   | 5'-TAA <b>GGATCCGCCTCCACC</b> AGTACTACTAAGAAGTTTAAACCAATC-3'   |
| Acap1 PH F      | 5'-<br>TAAGCTAGCCGCCACCATGGAGGGGCCTAGTGGCTTGGTAATGGAAG-3'      |
| Acap1 PH R      | 5'-TAA <b>GGATCCGCCTCCACC</b> GAAGGCTGAGGCAATGCTGCTCTGGAC-3'   |
| Phlpp2 PH F     | 5'-TAAGCTAGCCGCCACCATGGATCATCTGGATCGAATCCTACTG-3'              |
| Phlpp2 PH R     | 5'-TAA <b>GGATCCGCCTCCACC</b> CCGCTGCGACACCACCTTAGACGCCTG-3'   |
| Cadps2 PH F     | 5'-<br>TATGCTAGCGCCGCCACCATGGCTCCAGCACACATGAAGCATAGTGGG-<br>3' |
| Cadps2 PH R     | 5'-ATC <b>GGATCCGCCTCCACC</b> TTGGACTGCAGGAAGTGGTTTGTAAAGAC-3' |
| Cdc42bpa PH F   | 5'-TATTCTAGACGCCACCATGGCTAAGGGAGTTGGGACGGCGTATG-3'             |
| Cdc42bpa PH R   | 5'-ATA <b>AGATCTGCCTCCACC</b> TAGCGTGCTGTCGTACGCCT-3'          |
| Plch1 PH F      | 5'-CTGGCTAGCCGCCACCATGGCAGACCTTGAAGTGTATAAA-3'                 |
| Plch1 PH R      | 5'-ATA <b>GGATCCGCCTCCGCC</b> AGCCATCAGATACTTGAGG-3'           |
| Vav1 PH F       | 5'-TATGCTAGCCGCCACCATGGCTAACTATGGCCGGCCCAAGA-3'                |
| Vav1 PH R       | 5'-TAT <b>GGATCCGCCTCCACC</b> TGGGTAAATGTTGGAGATGGCC-3'        |
| Arhgef7 PH F    | 5'-ACTGCTAGCCGCCACCATGGATGACATAAAGACCCTGGGCAG-3'               |
| Arhgef7 PH R    | 5'-TAT <b>GGATCCGCCTCCACC</b> CTTCGTCTGCTTCTGCAGG-3'           |
| Itk PH F        | 5'-TATGCTAGCGCCGCCACCATGGCTATGAACAATTCATCCT-3'                 |
| Itk PH R        | 5'-TAT <b>GGATCCGCCTCCACC</b> GGATGGGTCGTAGGGAGCACAG-3'        |
| YFP F           | 5'-TAT <b>GGATCCGGTGGCGGAGGTTCT</b> ATGGTGAGCAAGGGCGAGGA-3'    |
| YFP R           | 5'-TATGGGCCCTTACTTGTACAGCTCGTCCATG-3'                          |
| Itk LS bridge F | 5'-TATGAATATGACTTTGAACGCGGGAAGAAGCGCACGT-3'                    |
| Itk LS bridge R | 5'-AAGTCATATTCATAGTAGGCCAGGCTGGCTTTCGTT-3'                     |