**Supplementary Files for:**

**Conformational decoupling in acid-sensing ion channels uncovers mechanism and stoichiometry of PcTx1-mediated inhibition**

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# Contributed equally

**Contents:**

Supplementary Files 1a-i

**Supplementary File 1a.**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | **WT** | | | **F350L** | | |
| **PcTx1 (nM)** | **pH50** | **Hill** | **n** | **pH50** | **Hill** | **n** |
| **Activation** | | | | | | |
| 0 | 6.68 (6.64, 6.72) | 6.16 (2.74, 9.58) | 18 | 6.26 (6.17, 6.35) | 3.61 (2.75, 4.47) | 12 |
| 30 | 7.21 (7.12, 7.30) | 2.72 (2.08, 3.36) | 7 | 6.20 (6.06, 6.36) | 3.76 (3.02, 4.53) | 7 |
| **Steady-state desensitization** | | | | | | |
| 0 | 7.28 (7.22, 7.33) | 14.31 (6.20, 22.43) | 6 | 7.12 (7.09, 7.16) | 19.51 (12.79, 26.22) | 5 |
| 30 | 7.68 (7.63, 7.72) | 17.15 (7.04, 27.27) | 9 | 7.28 (7.21, 7.36) | 18.78 (5.36, 32.20) | 7 |

**Supplementary File 1b.**

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| --- | --- | --- | --- |
|  | **pH50** | **Hill** | **n** |
| **Steady-state desensitization** | | | |
| K105C\* | 7.06 (7.02, 7.09) | 13.96 (10.88, 17.03) | 4 |
| WT\*/WT\*/WT\* | 7.14 (7.12, 7.16) | 11.56 (7.87, 15.25) | 6 |
| WT\*/F350L\*/WT\* | 7.13 (7.07, 7.19) | 7.84 (4.73, 10.95) | 4 |
| WT\*/F350L\*/F350L\* | 7.06 (7.02, 7.10) | 15.04 (6.90, 23.18) | 4 |
| K105C\*F350L | 6.99 (6.96, 7.03) | 11.66 (7.34, 15.98) | 4 |
| V80C\* | 7.17 (7.13, 7.21) | 11.95 (5.34, 18.57) | 4 |
| V80C\*F350L | 7.31 (6.72, 7.89) | 8.56 (0.03, 17.15) | 4 |
| **Fluorescence signal** | | | |
| K105C\* | 7.11 (7.15, 7.06) | 7.24 (5.40, 9.07) | 5 |
| WT\*/WT\*/WT\* | 7.15 (7.18, 7.12) | 8.07 (5.54, 10.59) | 6 |
| WT\*/F350L\*/WT\* | 7.14 (7.11, 7.16) | 9.09 (5.06, 13.12) | 5 |
| WT\*/F350L\*/F350L\* | 7.07 (7.15, 7.00) | 7.72 (3.70, 11.74) | 4 |
| K105C\*F350L | 6.95 (6.86, 7.52) | 6.69 (3.91, 9.47) | 6 |
| V80C\* | 7.36 (7.30, 7.42) | 9.41 (6.62, 12.21) | 4 |
| V80C\*F350L | 7.16 (7.14, 7.18) | 13.87 (8.68, 19.06) | 5 |

**Supplementary File 1c.**

|  |  |  |  |
| --- | --- | --- | --- |
| **Construct** | **Application pH** | **ΔFPcTx1/ΔFpH 5.5** | **n** |
| K105C\* | 7.4 | 1.13 (1.09, 1.18) | 14 |
| WT\*/WT\*/WT\* | 7.4 | 1.25 (1.01, 1.50) | 6 |
| WT\*/F350L\*/WT\* | 7.4 | 0.99 (0.86, 1.12) | 8 |
| WT\*/F350L\*/F350L\* | 7.4 | 0.70 (0.46, 0.95) | 5 |
| K105C\*/F350L | 7.4 | 0.019 (0.001, 0,04) | 6 |
| K105C\*/F350L | 7.3 | 0.464 (0.24, 0.68) | 5 |
| V80C\* | 7.7 | -0.86 (-0.90, -0.81) | 13 |
| V80C\*/F350L | 7.4 | -0.70 (-0.87, -0.53) | 8 |

**Supplementary File 1d.**

|  |  |  |  |
| --- | --- | --- | --- |
| **Construct** | **Wash pH (3 min)** | **ΔF3min/ΔF PcTx1** | **n** |
| K105C\* | 7.4 | 0.68 (0.37, 0.98) | 6 |
| 8.4 | 0.28 (0.19, 0.37) | 4 |
| mix | 0.25 (0.08, 0.42) | 4 |
| WT\*/WT\*/WT\* | 7.4 | 0.83 (0.78, 0.88) | 6 |
| WT\*/F350L\*/WT\* | 7.4 | 0.49 (0.39, 0.60) | 8 |
| WT\*/F350L\*/F350L\* | 7.4 | 0.15 (0.33, 0.04) | 5 |
| K105C\*/F350L | 7.4 | 0.03 (-0.01, 0.08 ) | 5 |
| V80C\* | 7.7 | -0.70 (-0.96, -0.44) | 5 |
| 8.4 | -0.41 (-0.60, -0.23) | 4 |
| mix | -0.56 (-0.79, -0.33) | 4 |
| V80C\*/F350L | 7.4 | -0.059 (-0.13, 0.01) | 6 |

**Supplementary File 1e.**

|  |  |  |  |
| --- | --- | --- | --- |
| **Treatment** | **I7.4/IPcTx1** | **ΔF7.4/ ΔF PcTx1** | **n** |
| 3 min washout | 0.79 (0.69, 0.89) | 0.73 (0.49, 0.96) | 6 |
| Multiple activations | 0.83 (0.78, 0.88) | 0.68 (0.54, 0.82) | 4 |

**Supplementary File 1f.**

|  |  |  |
| --- | --- | --- |
| **Treatment** | **ΔF7.4/ΔF5.5** | **n** |
| PcTx1 at pH 8.0 | -0.08 (-0,14, -0.02) | 7 |
| 7.4 after 5.5 | 0.95 (0.81, 1.08) | 6 |
| 7.4 after 7.4 | 0.69 (0.60, 0.78) | 4 |
| 7.4 after 8.0 | 0.11 (0.01, 0.23) | 4 |

**Supplementary File 1g.**

|  |  |  |
| --- | --- | --- |
| **Treatment** | **ΔFpeptide/ΔFpH 5.5** | **n** |
| BigDyn (Control) | -0.84 (-1.05, -0.64) | 6 |
| BigDyn post PcTx1 | 0.50 (0.18, 0.83) | 6 |
| PcTx1 (Control) | 0.86 (0.67, 1.06) | 6 |
| PcTx1 post BigDyn | 0.77 (0.61, 0.93) | 4 |
| PcTx1 (Control) | 1.20 (1.05, 1.34) | 6 |
| PcTx1 post PcTx1 | 1.17 (1.06, 1.28) | 6 |

**Supplementary File 1h.**

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|  | **IC50** | **Hill** |  |
| **Construct** | **Mean (nM)** | **Mean** | **n** |
| WT | 0.6 (0.4, 0.9) | 0.9 (0.7, 1.2) | 9-14 |
| F350L | 977.2 (-, -) | 11.2 (- , -) | 4-7 |
| WT/WT/WT | 6.5 (5.2, 9.0) | 1.1 (0.9, 1.3) | 6-9 |
| F350L/WT/WT | 7.3 (5.9, 9.0) | 1.1 (0.9, 1.4) | 5-6 |
| WT/F350L/WT | 6.6 (5.7, 7.7) | 1.3 (1.0, 1.5) | 5-7 |
| WT/WT/F350L | 4.2 (3.2, 4.8) | 1.0 (0.9, 1.2) | 5-7 |
| F350L/F350L/WT | 94.6 (74.4, 120.1) | 0.9 (0.7, 1.1) | 4-6 |
| F350L/WT/F350L | 289.9 (218.1, 387.2) | 0.8 (0.7, 1.1) | 6-11 |
| WT/F350L/F350L | 108.0 (834, 141.8) | 0.8 (0.7, 1.0) | 4-7 |
| F350L/F350L/F350L | 797.1 (654.1, 962.4) | 2.6 (1.7, -) | 4-6 |

**Supplementary File 1i.**

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| **Construct** | **pH50** | **Hill** | **n** |
| **Activation** | | | |
| WT | 6.69 (6.65, 6.73) | 7.70 (5.88, 9.53) | 10 |
| WT/WT/WT | 6.72 (6.69, 6.75) | 6.31 (4.92, 7.71) | 10 |
| F350L/WT/WT | 6.59 (6.53, 6.64) | 5.80 (2.87, 8.74) | 8 |
| WT/F350L/WT | 6.62 (6.58, 6.65) | 8.82 (6.40, 11.24) | 13 |
| WT/WT/F350L | 6.62 (6.58, 6.66) | 7.72 (4.79, 10.66) | 10 |
| F350L/F350L/WT | 6.47 (6.44, 6.50) | 6.29 (4.26, 8.32) | 13 |
| F350L/WT/F350L | 6.43 (6.39, 6.46) | 5.34 (4.75, 5.92) | 8 |
| WT/F350L/F350L | 6.44 (6.37, 6.50) | 6.96 (4.15, 9.77) | 10 |
| F350L/F350L/F350L | 6.18 (6.11, 6.26) | 3.86 (2.56, 5.17) | 7 |
| F350L | 6.31 (6.18, 6.43) | 4.09 (2.87, 5.30) | 7 |
| **Steady-state desensitisation** | | | |
| WT | 7.27 (7.25, 7.30) | 14.15 (10.20,18.09) | 11 |
| WT/WT/WT | 7.24 (7.22, 7.25) | 12.11 (10.72, 13.50) | 5 |
| WT/F350L/WT | 7.24 (7.19, 7.29) | 13.79 (8.62, 18.97) | 5 |
| WT/F350L/F350L | 7.17 (7.14, 7.20) | 9.09 (5.77, 12.42) | 6 |
| F350L/F350L/F350L | 7.16 (7.11, 7.21) | 12.45 (4.60, 20.29) | 4 |
| F350L | 7.11 (7.09, 7.14) | 12.36 (8.96, 15.76) | 7 |