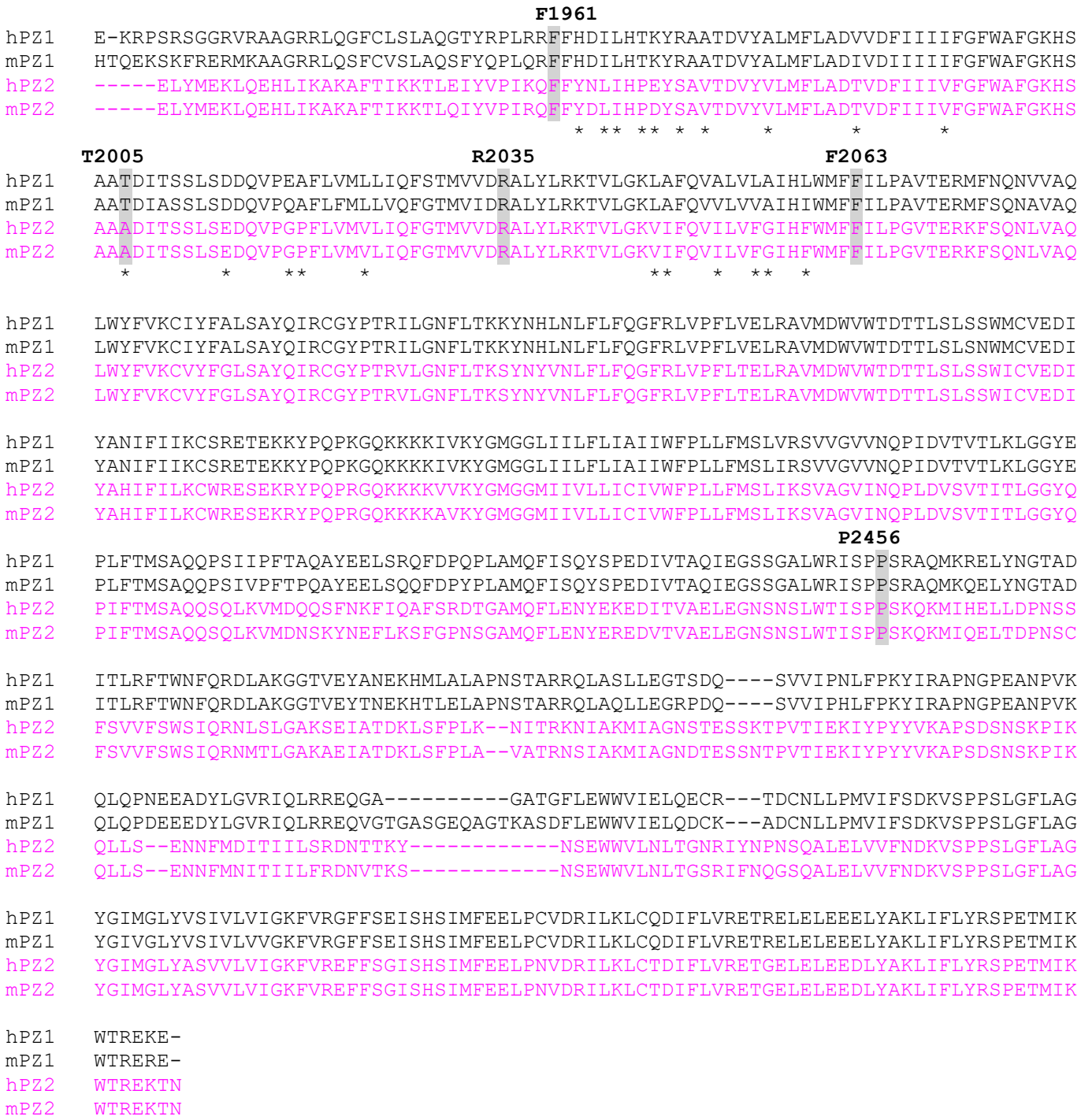
Probing the Gating Mechanism of the Mechanosensitive Channel Piezo1 with the Small Molecule Yoda1

Lacroix et al.

Supplementary Figures



Supplementary Figure 1: Sequence alignment (T-coffee) of the C-terminal region of mouse Piezo1 (mPZ1), Piezo2 (mPZ2) and human Piezo1 (hPZ1) and Piezo2 (hPZ2). The residues that are numbered and shaded correspond to positions used to create the chimeras and sub-domain chimeras. Asterisks indicate residues within the 1961-2063 region that are overall not conserved between Piezo1 and Piezo2.

Supplementary Tables

Supplementary Table 1: Fitted parameters for the I / Imax vs. Pressure plots using equation 1

	mPZ1	mPZ1 + 30 µM Yoda1	Chim	Chim + 30 µM Yoda1
k (mmHg)	9.46 ± 0.64	10.78 ± 1.51	5.73 ± 0.47	5.95 ± 0.48
P_{50} (mmHg)	-52 ± 1	-26 ± 1	-62 ± 1	-63 ± 1
R^2	0.988	0.952	0.980	0.979

Supplementary Table 2: Fitted parameters for different gating models

Number of activated subunits needed for pore opening		≥ 1	≥ 2	3
fitting equation from main text		2	3	4
Syeda et al.	B_{max}	2069.85 ± 100.69	1818.63 ± 48.79	1836.90 ± 55.18
	K_d (µM)	22.92 ± 2.95	10.83 ± 0.76	4.13 ± 0.32
	R^2	0.993	0.996	0.995
this study	B_{max}	1.67 ± 0.07	1.50 ± 0.08	1.49 ± 0.08
	K_d (µM)	10.92 ± 1.44	5.20 ± 0.85	1.89 ± 0.32
	R^2	0.980	0.954	0.948

Supplementary Table 3: Fitted parameters of I / I_{max} plots for WT and WT:Chim = 1:1 in presence of varying Yoda1 concentrations using equation 1.

	WT			WT:Chim = 1:1		
[Yoda1] (µM)	k (mmHg)	P_{50} (mmHg)	R^2	k (mmHg)	P_{50} (mmHg)	R^2
1	10.2 ± 0.7	-44.4 ± 0.8	0.996	9.3 ± 1.0	-40 ± 1.1	0.991
3	10.4 ± 0.7	-36.4 ± 0.8	0.988	9.9 ± 1.1	-36 ± 0.8	0.991
10	10.9 ± 0.7	-31.2 ± 0.8	0.988	9.6 ± 1.1	-27 ± 0.8	0.956
30	10.3 ± 0.7	-25.7 ± 0.7	0.985	11.3 ± 1.3	-20 ± 0.6	0.908
100	6.9 ± 0.6	-16.9 ± 0.6	0.976	13 ± 1.3	-18 ± 0.9	0.940

Supplementary Note 1

The measured fluorescence F is a function of the saturation function ν of the protein P by the agonist ligand L, of the background fluorescence q and of a constant b_{max}:

$$F = q + (b_{\max}\,\nu) \quad (1)$$

Before addition of the ligand (t = 0), $\nu = 0$ and thus $F_0 = q$. The relative change of fluorescence follows:

$$\frac{F - F_0}{F_0} = \frac{(q + (b_{\max}\,\nu)) - q}{q} = \frac{b_{\max}}{q}\ \nu = B_{\max}\,\nu \quad (2)$$

with $B_{max} = b_{max} / q$. In case of multiple binding sites per protein, the saturation function equals the total concentration of bound ligand ($[L]_{bound}$) over the total concentration of protein ($[P]_0$):

$$\nu = \frac{[L]_{\text{bound}}}{[P]_0} \quad (3)$$

Assuming each subunit of the trimeric channel interacts independently with the agonist:

$$\frac{[L]_{\text{bound}}}{[P]_0} = \frac{[PL] + 2[PL_2] + 3[PL_3]}{[P] + [PL] + [PL_2] + [PL_3]} \quad (4)$$

The different protein species are related by the corresponding macroscopic dissociation constant K_1, K_2 and K_3 corresponding to each binding step:

$$K_1 = \frac{[P][L]}{[PL]}\ ;\ K_2 = \frac{[PL][L]}{[PL_2]}\ ;\ K_3 = \frac{[PL_2][L]}{[PL_3]}$$

By replacing the macroscopic constants:

$$\nu\ = \frac{\frac{[L]}{K_1} + \frac{2[L]}{K_1K_2} + \frac{3[L]}{K_1K_2K_3}}{1 + \frac{[L]}{K_1} + \frac{[L]}{K_1K_2} + \frac{[L]}{K_1K_2K_3}} \quad (5)$$

The macroscopic constants are related to the microscopic dissociation constant K_d by the number of possible binding combinations $\Omega_{n,i}$ for each binding step i and for n binding sites:

$$K_i = \frac{\Omega_{n,i-1}}{\Omega_{n,i}} K_{\text{d}} = \frac{\binom{n}{i-1}}{\binom{n}{i}} = \frac{i}{n-i+1}\ K_{\text{d}}$$

Replacing the macroscopic constants by the K_d gives and using $n = 3$ for a trimeric channel:

$$\nu = \frac{\frac{3[L]}{K_d} + \frac{6[L]^2}{K_d{}^2} + \frac{3[L]^3}{K_d{}^3}}{1 + \frac{3[L]}{K_d} + \frac{3[L]^2}{K_d{}^2} + \frac{[L]^3}{K_d{}^3}} \quad (6)$$

Supplementary Equation 6 further simplifies by applying a binomial reduction:

$$\nu = \frac{3[L]}{K_d + [L]} \quad (7)$$

Assuming the activation of one or more subunit is sufficient for channel opening, every species in the numerator of Supplementary Equation 6 contribute to the observed fluorescence signal:

$$\frac{F - F_0}{F_0} = B_{max} \frac{3[L]}{K_d + [L]} \quad (8)$$

However, if the activation of two or three subunits is required for channel activation, the fraction of channel with a single activated subunit does not contribute to the signal. In this case, the fluorescence signal follows:

$$\frac{F - F_0}{F_0} = B_{max} \frac{\frac{6[L]^2}{K_d{}^2} + \frac{3[L]^3}{K_d{}^3}}{1 + \frac{3[L]}{K_d} + \frac{3[L]^2}{K_d{}^2} + \frac{[L]^3}{K_d{}^3}} \quad (9)$$

Similarly, if the activation of three subunits is necessary for channel activation, the fluorescence signal follows:

$$\frac{F - F_0}{F_0} = B_{max} \frac{\frac{3[L]^3}{K_d{}^3}}{1 + \frac{3[L]}{K_d} + \frac{3[L]^2}{K_d{}^2} + \frac{[L]^3}{K_d{}^3}} \quad (10)$$