PNAS

Supplementary Information for

Yoda1's energetic footprint on Piezo1 channels and its modulation by voltage and temperature.

Tharaka D. Wijerathne, Alper D. Ozkan, and Jérôme J. Lacroix

Jérôme J. Lacroix
Email: jlacroix@westernu.edu

This PDF file includes:

Figures S1 to S6
Tables S1 to S2

Fig. S1. Shut and open dwell time distributions of individual patches measured at 23 °C in the presence of DMSO (grey), Yoda1 (green) and Dooku1 (fuchsia).

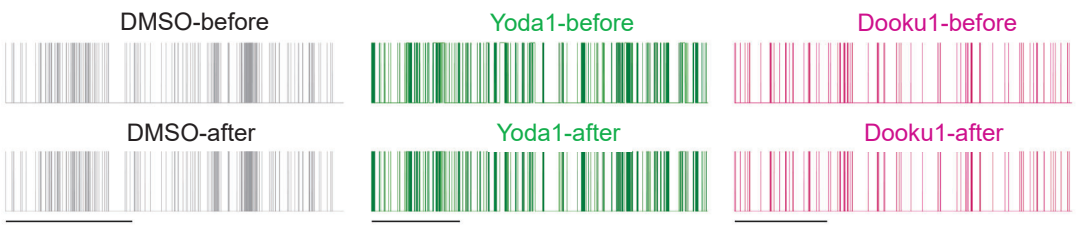

Fig. S2. Idealized single channel trajectories are not modified by subsequent QuB idealization. The figure shows exemplar idealized current traces before and after QuB idealization for different treatments. Scale bar = 10 s.

Fig. S3. Shut and open dwell time distributions of individual patches measured at 29 °C in the presence of DMSO (grey), Yoda1 (green) and Dooku1 (fuchsia).

Fig. S4. Shut and open dwell time distributions of individual patches measured at 35 °C in the presence of DMSO (grey), Yoda1 (green) and Dooku1 (fuchsia).

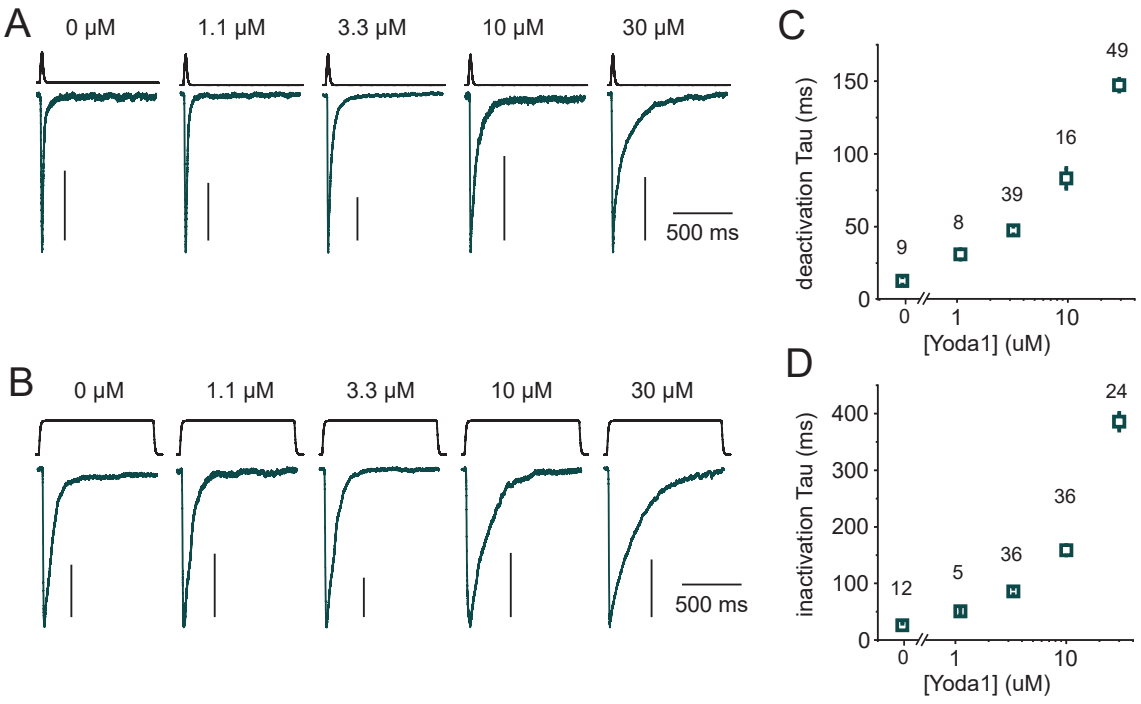

Fig. S5. Yoda1 slows down Piezo1 inactivation and deactivation in a concentration-dependent manner. The figure displays representative current traces of Piezo1 relaxation kinetics evoked using 40 ms (**A**) or 1 s (**B**) poking stimuli (3 µm) and in the presence of the indicated bath concentration of Yoda1 (V = -90 mV, T = 25 °C, vertical scale bar = 400 pA). DMSO was added in each condition to maintain [DMSO] = 0.075% in all experimental conditions. The time constant of deactivation (**C**) and inactivation (**D**) are plotted as a function of Yoda1 concentration. Numbers above plots indicate n values. Error bars = s.e.m.

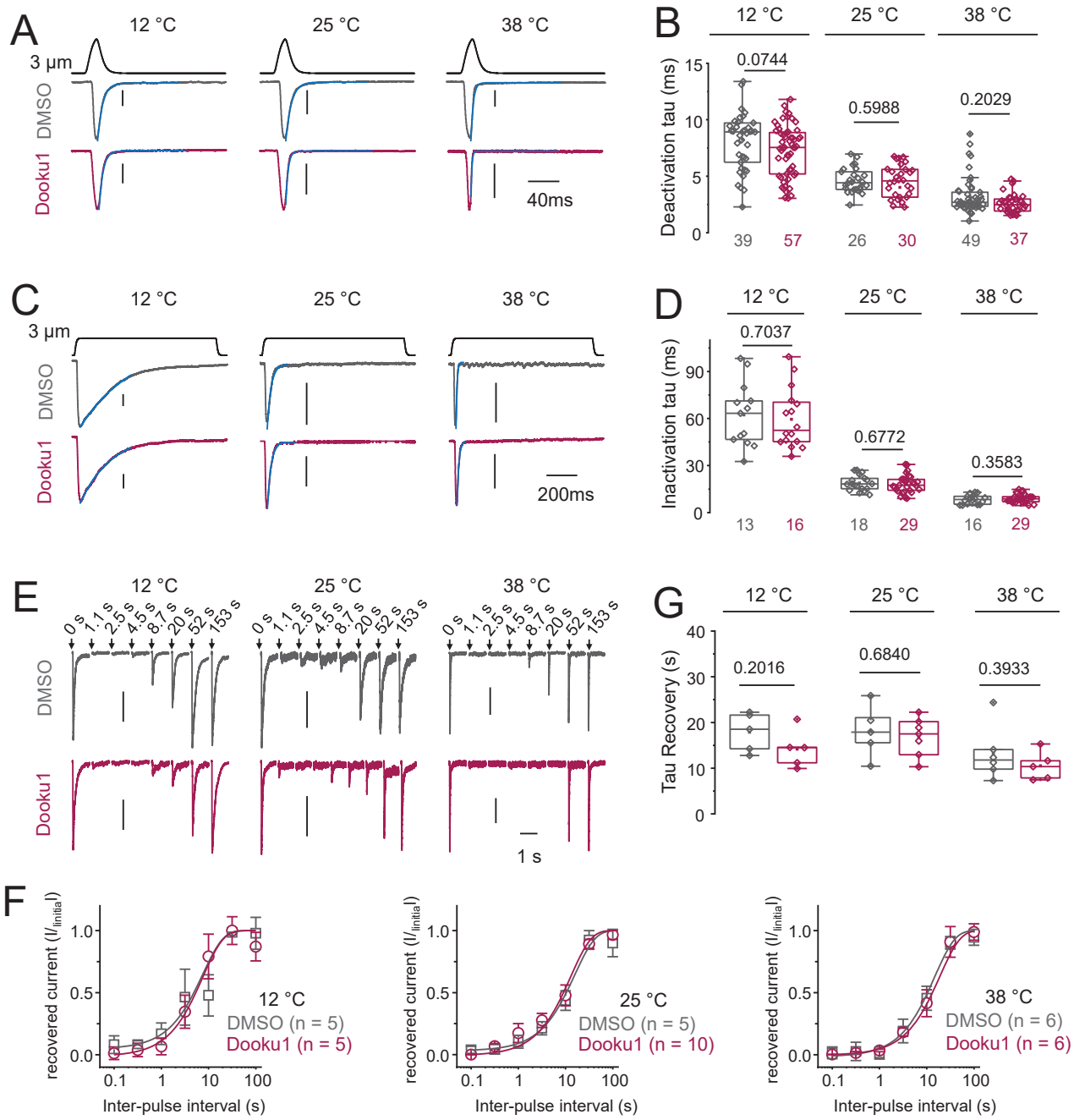

Fig. S6. Dooku1 does not appreciably modulate macroscopic Piezo1 currents kinetics. (**A**) Representative relaxation Piezo1 current traces evoked using 40 ms poking stimuli (3 µm) in the presence of 30 µM Dooku1 (fuchsia) or 0.3% DMSO control (grey) at the indicated temperature (V = -90 mV). (**B**) Scatter plots showing deactivation time-constant values obtained by exponential fit (blue lines) of individual traces shown in (A). Numbers underneath plots indicate n values. (**C**) Representative relaxation Piezo1 current traces obtained as in (A) but using 1 s long poking stimuli instead of 40 ms. (**D**) Scatter plots showing inactivation time-constant values obtained by exponential fit (blue lines) of individual traces shown in (C). Numbers underneath plots indicate n values. (**E**) Representative relaxation Piezo1 current traces evoked by repetitive 1 s poking stimuli obtained at the indicated time relative to the first stimulus and in the presence of 30 µM Dooku1 or DMSO control (V = -90 mV). (**F**) The relative fraction of recovered peak current from data shown in (E) is plotted as a function of the inter-pulse time interval for each experimental condition. Lines are exponential fit from aggregated data. (**G**) Scatter plots of individually fitted time-constants from data shown in (E). In panels B, D, F, and G: error bars = s.e.m. In panels A, C, and E: vertical scale bars = 300 pA. In panels B, D, and G: numbers indicate exact p-values from Students' T-tests.

Table S1. Microscopic rate constant parameters fitted using the unconstrained Markov model in QuB.

transitions	DMSO	Yoda1	Dooku1
S1→S2	$2.1 \times 10^{-5} \pm 3.1 \times 10^{-9}$	$1.7 \times 10^{-5} \pm 2.7 \times 10^{-3}$	$2.6 \times 10^{-10} \pm 6.4 \times 10^{-21}$
S2→S1	$1.3 \times 10^{-3} \pm 7.3 \times 10^{-7}$	$4.3 \times 10^{-7} \pm 1.6 \times 10^{-3}$	$7.4 \times 10^{-7} \pm 2.5 \times 10^{-21}$
S1→O1	839.7 ± 0.1	963.2 ± 0.036	$665 \pm 1.3 \times 10^{-6}$
O1→S1	1279 ± 0.01	994.1 ± 0.069	$1498 \pm 2.0 \times 10^{-7}$
S1→O2	75.1 ± 0.04	$74.3 \pm 2.3 \times 10^{-3}$	$49.7 \pm 1.3 \times 10^{-7}$
O2→S1	55.4 ± 0.02	$16.3 \pm 6.2 \times 10^{-4}$	$95.4 \pm 1.8 \times 10^{-6}$
S2→O1	7.8 ± 0.001	$7.3 \pm 8.1 \times 10^{-6}$	$5.1 \pm 4.7 \times 10^{-10}$
O1→S2	285.1 ± 0.1	$151.6 \pm 1.1 \times 10^{-2}$	$162.4 \pm 7.9 \times 10^{-9}$
S2→O2	$9.6 \times 10^{-14} \pm 1.4 \times 10^{-17}$	$1.2 \times 10^{-13} \pm 7.2 \times 10^{-11}$	$5.8 \times 10^{-7} \pm 6 \times 10^{-10}$
O2→S2	$1.3 \times 10^{-7} \pm 1.7 \times 10^{-11}$	$1.2 \times 10^{-7} \pm 2.1 \times 10^{-5}$	$1.6 \times 10^{-5} \pm 2.1 \times 10^{-10}$
O1→O2	$2.1 \times 10^{-13} \pm 9.8 \times 10^{-4}$	$5.9 \times 10^{-13} \pm 3.4 \times 10^{-10}$	$2.6 \times 10^{-6} \pm 5 \times 10^{-10}$
O2→O1	$8.7 \times 10^{-4} \pm 4.7 \times 10^{-7}$	$5.3 \times 10^{-7} \pm 2.3 \times 10^{-3}$	$3.2 \times 10^{-6} \pm 4.9 \times 10^{-3}$

Values are in s^{-1}; errors = standard deviations.

Table S2. Quality control comparison between Markov-chain models.

Convergence parameter	Unconstrained model			Minimal model		
	DMSO	Yoda1	Dooku1	DMSO	Yoda1	Dooku1
Log-Likelihood	91,824	179,700	95,020	91,824	179,700	95,020
Akaike's Information criterion (AIC)	-183,600	-359,300	-190,000	-183,600	-359,300	-190,000
Bayesian Information criterion (BIC)	-183,500	-359,200	-189,900	-183,600	-359,200	-190,000