Supporting Information

Agonist and antagonist diverted twisting motions of single TRPV1 channel

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Supporting information

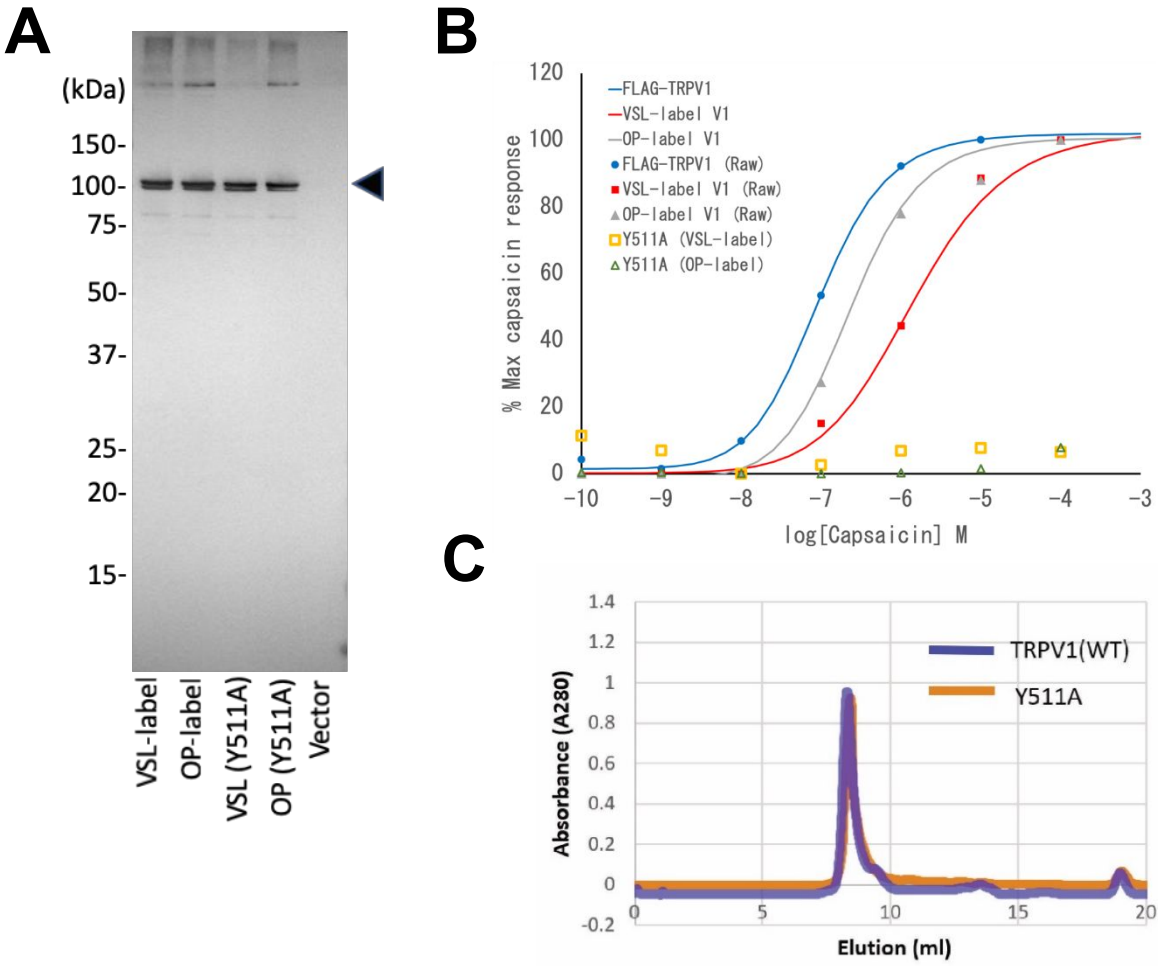

Figure S1 | Expression and function of TRPV1 receptors used in this experiment.

(A) Expression of VSL-label TRPV1, OP-label TRPV1, VSL-label Y511A mutant, and OP-label Y511A mutant on the HEK293 cells were confirmed by the Western blotting of the anti-FLAG antibodies. The right-end lane is a control transfection by the vector only. The arrowhead shows the position of TRPV1 receptors. **(B)** Responses to the capsaicin were tested by the calcium influx assay using the FlexStation 3 system with the FLIPR Calcium 6 reagent (Molecular Devices Inc.). Mean values of

2-4 independent measurements were plotted. EC_{50} was 0.088 μM (FLAG-TRPV1), 1.32 μM (VSL-label TRPV1) and 0.245 μM (OP-label TRPV1), respectively. **(C)** Gel-filtration profiles (Superdex 200 column) of TRPV1 purification. Buffer composition was; 20 mM Tris-HCl, pH 7.4, 150 mM NaCl, 50 mM $MgCl_2$, 1 mM n-Decyl-β-D-maltoside).

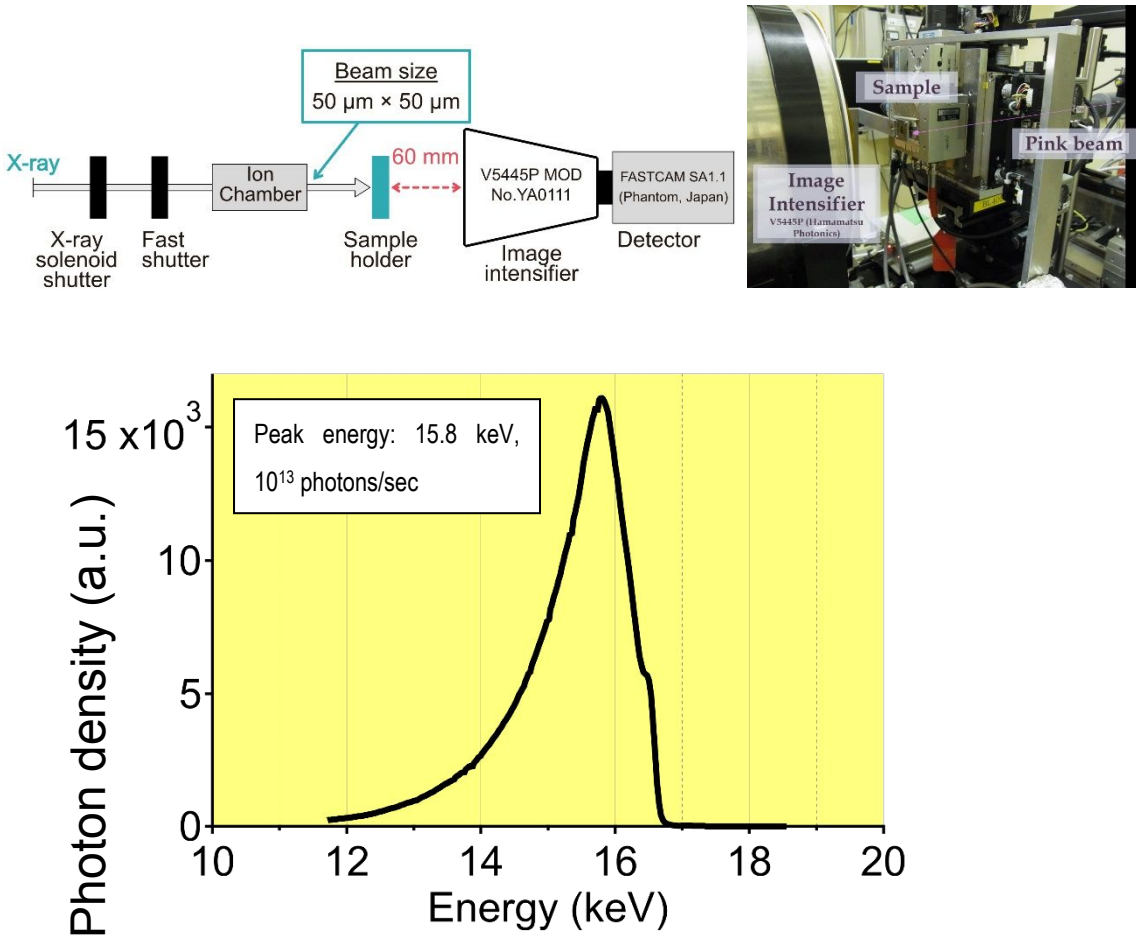

Figure S2 | DXT measurement system (upper panels) and the energy profile of incident X-ray for the DXT measurement (lower panel) X-rays from the beamline (BL40XU, SPring-8, Japan) with energy widths from 14.0-16.5 keV (undulator gap = 31.0 mm) were used for DXT measurements. The sample-to-detector distance was set 60 mm in our DXT measurement.

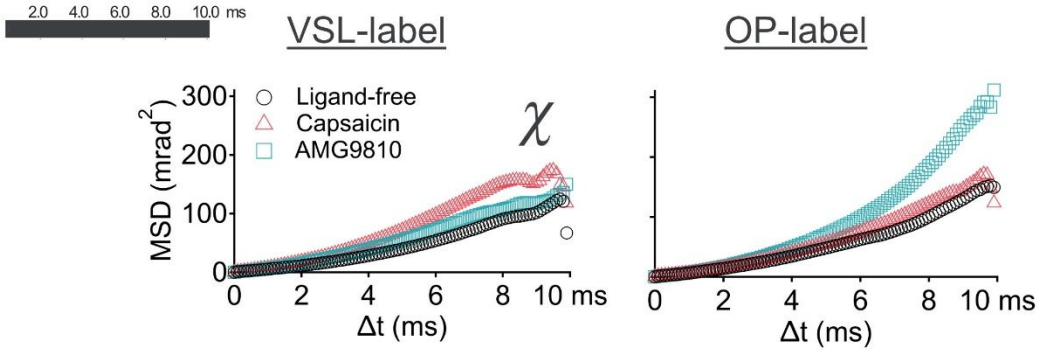

Figure S3 | MSD curves of TRPV1 motion for the χ axis up to 10 ms.

The slopes were increased by capsaicin and AMG9810.

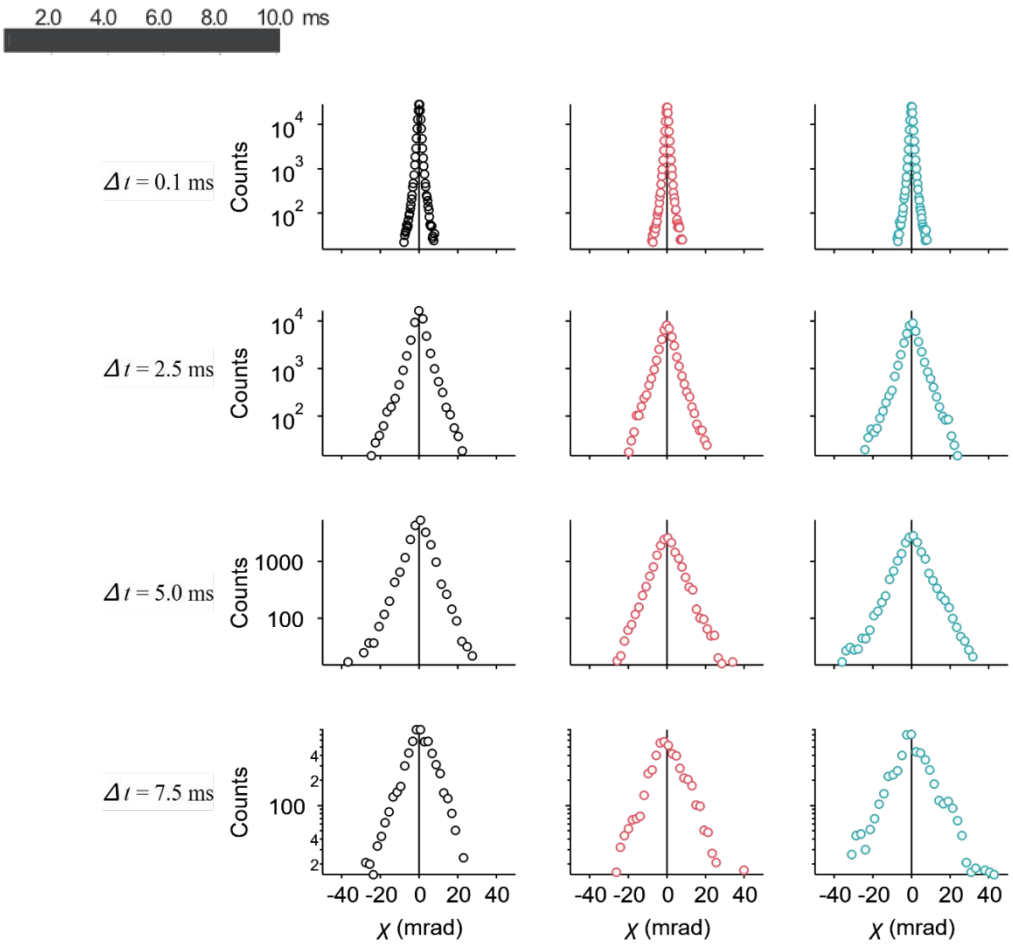

Figure S4 | The VHC provide symmetric distribution for OP-label.

Before classification of lifetime, the distribution were agreed well by Gaussian function at short time intervals (= 0.1 ms) to large time intervals (= 7.5 ms).

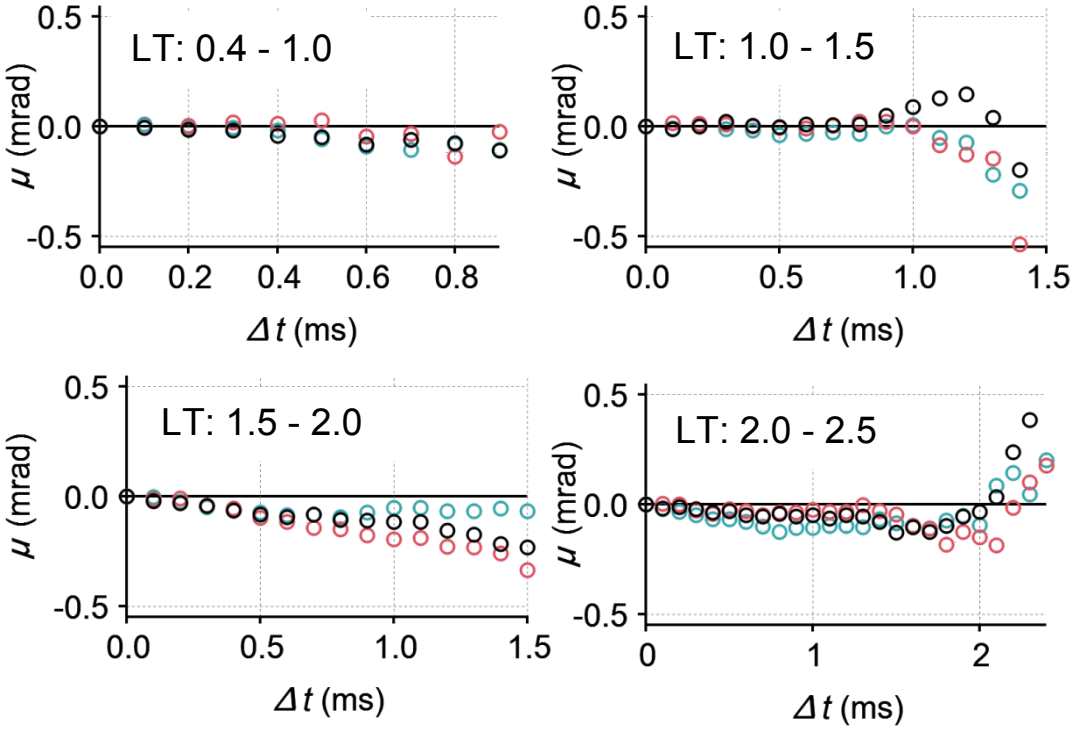

Figure S5 | Mean-plot of the VSL-label for the further classified short (the lifetime (LT) < 2.5 ms) lifetime group.

Different length of lifetime filtering was further applied to the short lifetime group (LT < 2.5 ms), and the mean-plots of subgroups were calculated. Rotational motion of the subgroups was also confirmed to be non-biased as shown in the result from the short lifetime group (Fig. 3A). Applied lifetime filtering is shown at upper left of each panel.

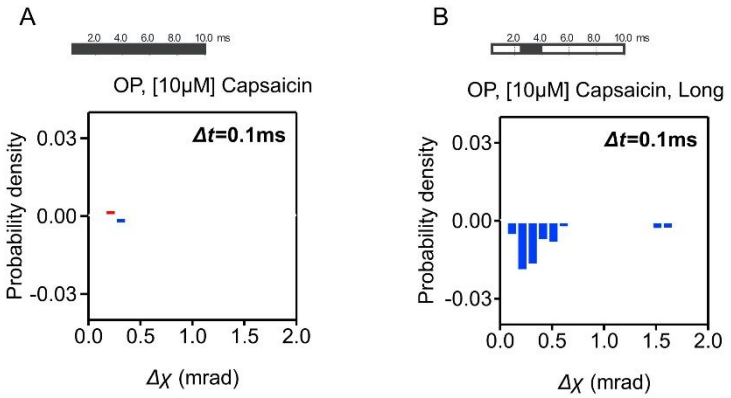

Figure S6 | Subtraction in the probability density between the positive value and the negative value for OP-label at Δt = 0.1 ms.

(A) Subtraction of the probability density for all detected trajectories group (0.4 ≤ lifetime (LT) < 10 ms) with capsaicin. **(B)** Subtraction of the probability density for long (2.5 ≤ LT < 4 ms) with capsaicin. Comparing with long LT group which shows significant negative difference, all detected trajectories group didn't show any obvious rotational bias.

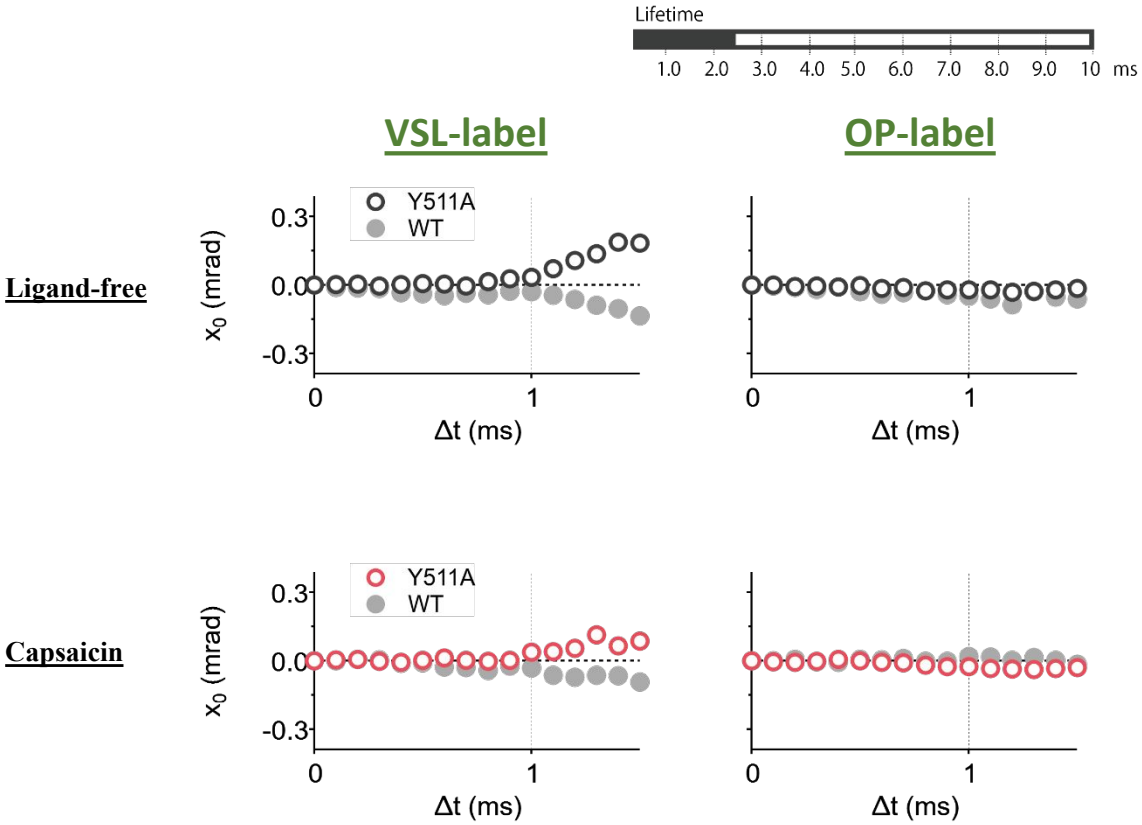

Figure S7 | The mean plots of the WT and Y511A against capsaicin for the short lifetime group (LT < 2.0 ms).

Like the motion observed in the WT (Fig. 3A), Y511A in the short lifetime groups also did not show any specific rotational bias. Dark-filled area of the top right bar shows the applied lifetime periods.

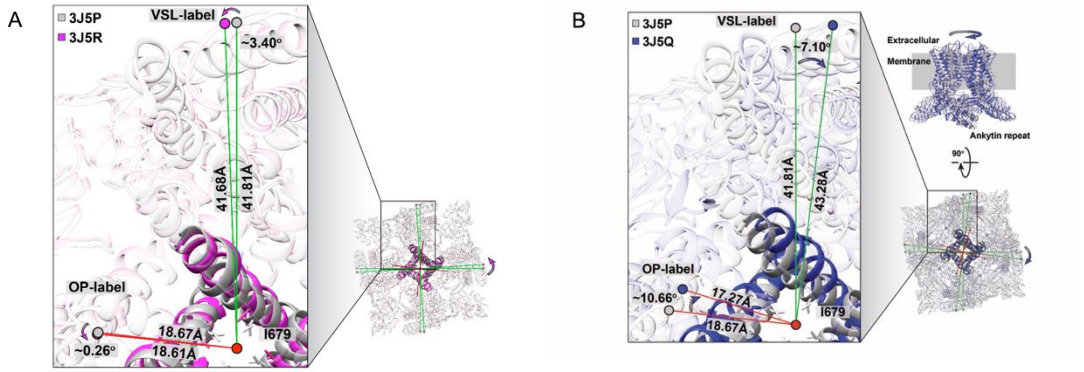

Figure S8 | Superimposed views of closed and open-form of TRPV1.

(A) The ankyrin repeats domain at residues 111-143 was held as a fulcrum, and the structures were viewed and compared from the extracellular side. Apo (PDB: 3J5P, gray) and capsaicin-bound partially open (3J5R, magenta) structures were superimposed. (B) Apo- (PDB: 3J5P, grey) and DkTx/RTX-bound full-open (3J5Q, blue) structures were superimposed (reposted from the Fig. 5B). No significant conformational change was shown both at the OP- and VSL- positions by capsaicin, while the DkTx/RTX, which induces full-open structure, generated the CW rotation biases. Positions of OP (Gly 602)- and VSL (Tyr 463)- labels were indicated by circles.

VSL-label

	Ligand-free (10,465)	Capsaicin (8,026)	AMG9810 (10,578)
D_α (mrad²/ms)	4.03	5.88	5.68
α	1.37	1.51	1.38
β^2 (mrad²)	0.59	0.86	0.53

OP-label

	Ligand-free (12,108)	Capsaicin (10,660)	AMG9810 (11,090)
D_α (mrad²/ms)	4.30	4.39	4.61
α	1.49	1.57	1.68
β^2 (mrad²)	0.75	0.83	0.85

Table S1| Parameter of MSD for wild type of TRPV1

The MSD curve were fitted by the following function: $\delta^2(t) = D_\alpha t^\alpha + 2\beta^2$ on the range of 0−4 ms.

D_α is the anomalous diffusion constant, a non-linear relationship to time, α called subdiffusion ($1 > \alpha > 0$) or superdiffusion ($\alpha > 1$) and β called measurement error.

	short LF:0.4-2.5 ms	middle LF:2.6-4.0 ms	long LF:8.0-10 ms
D_α (mrad^2/ms)	9.87	7.30	5.04
α	1.27	1.40	1.39
β^2 (mrad^2)	0.59	0.46	0.29

Table S2| Parameter of MSD for each Lifetime group in OP-label with capsaicin

The MSD curve were fitted by the following function: $\delta^2(t) = D_\alpha t^\alpha + 2\beta^2$ on the range of 0−2 ms.

D_α is the anomalous diffusion constant, a non-linear relationship to time, α called subdiffusion ($1 > \alpha > 0$) or superdiffusion ($\alpha > 1$) and β called measurement error.