Supplementary information for:

The predicted binding site and dynamics of peptide antagonists to the Methuselah GPCR from *Drosophila melanogaster*

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Table S1 Kinetics analysis of peptide binding by surface plasmon resonance. Rate constants are given as averages (± s.d.) where more than one independent sensor chip surface was used. K_D is calculated from the on and off rates (k_d/k_a).

	k_a	k_d	K_D
	$M^{-1}s^{-1}$ ($\times 10^6$)	s-1 ($\times 10^{-2}$)	nM
WT	2.4 ± 1.3	4.4 ± 2.3	18.5
L11Q	3.2 ± 0.8	7.8 ± 2.0	24.5
Y9F	2.0 ± 0.7	9.9 ± 3.8	49.5
R15Q	2.8 ± 0.6	10.4 ± 2.4	37.7
W5F	0.13	154	12000
W5K	No binding	No binding	No binding

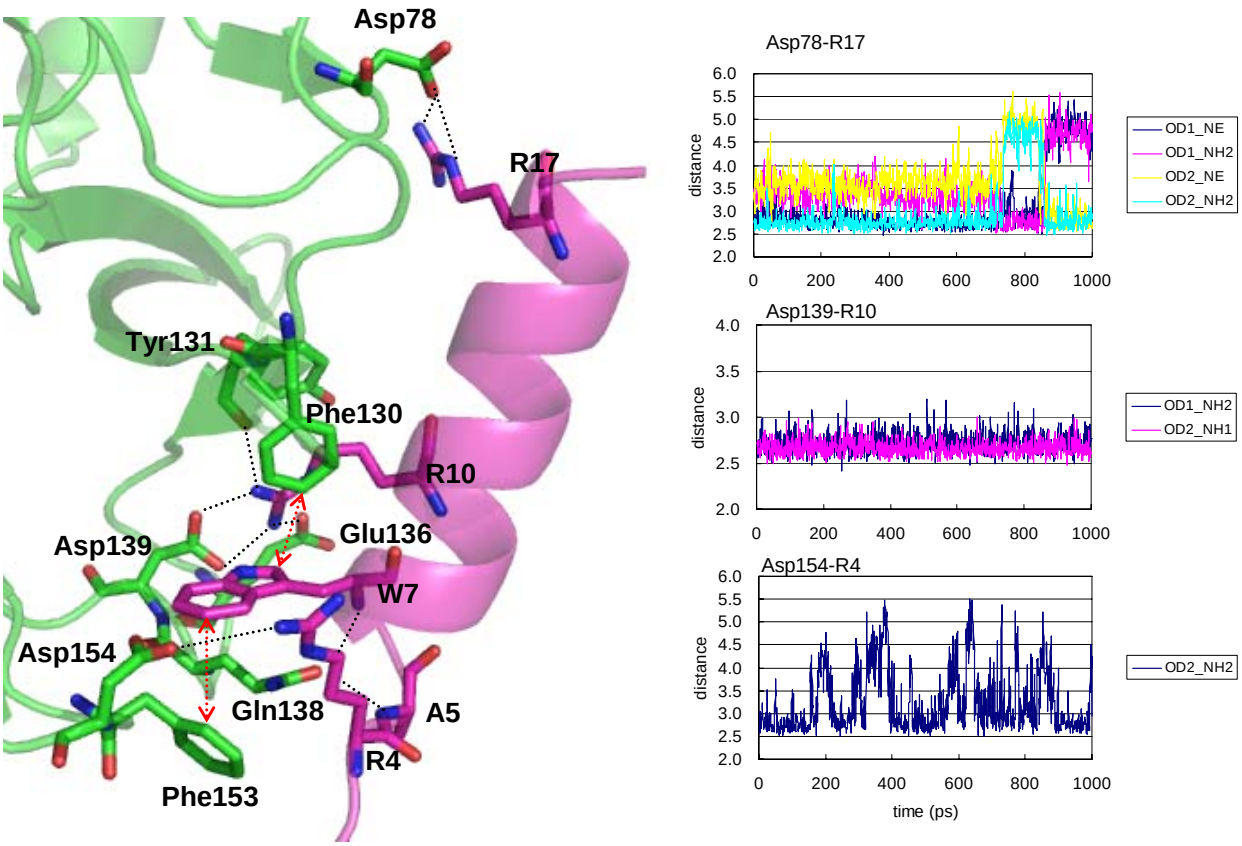

Figure S1 Binding of LR2 ligand into the Mth ectodomain. The residues from the receptor are in three-letter code and those from the ligand are in single-letter code. The hydrogen bond and the aromatic interaction are specified with the dotted line and arrow, respectively. The distance profiles for three salt-bridge pairs are shown on the right.

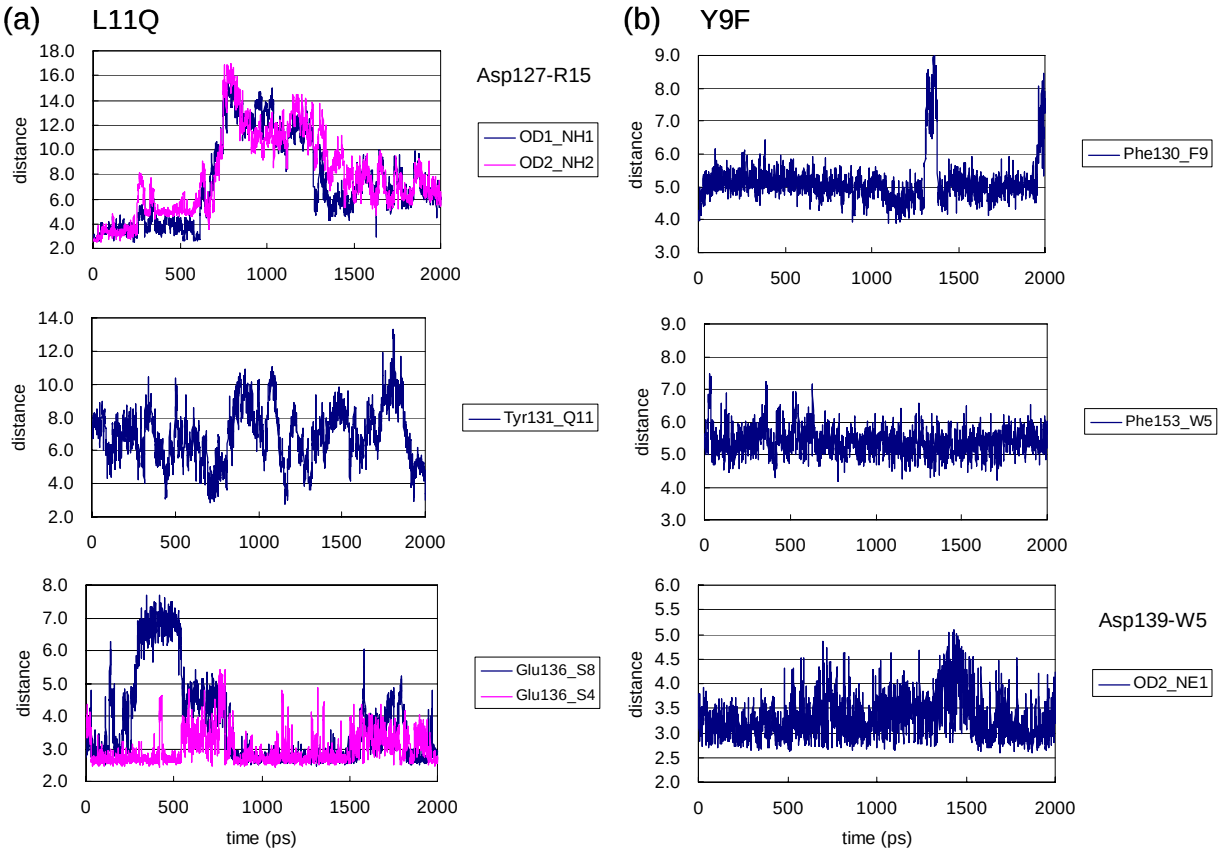

Figure S2 Changes during 2 ns MD simulation of key intermolecular interactions in mutant complexes. Shown are distances (in Å) between sidechain heavy atoms. (a) L11Q, (b) Y9F, (c) W5K, (d) R15Q

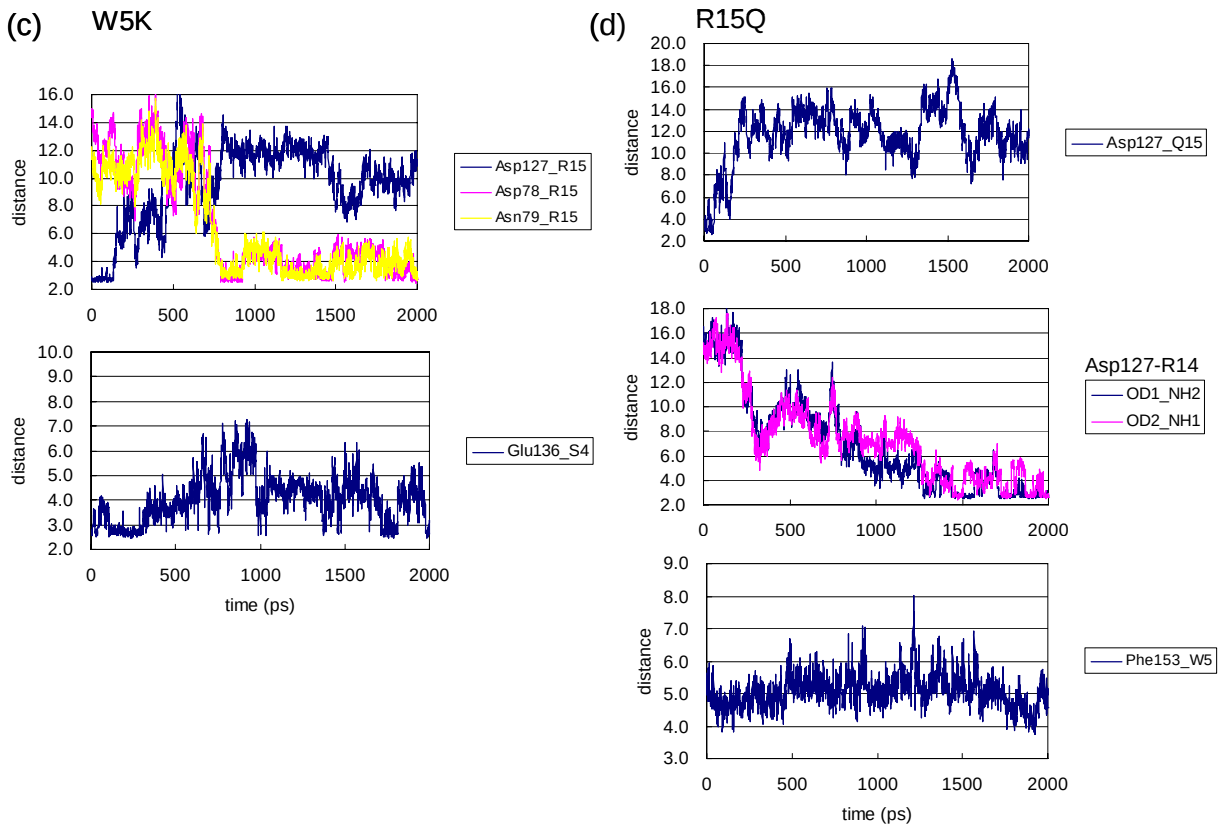

Figure S2 – *continued*

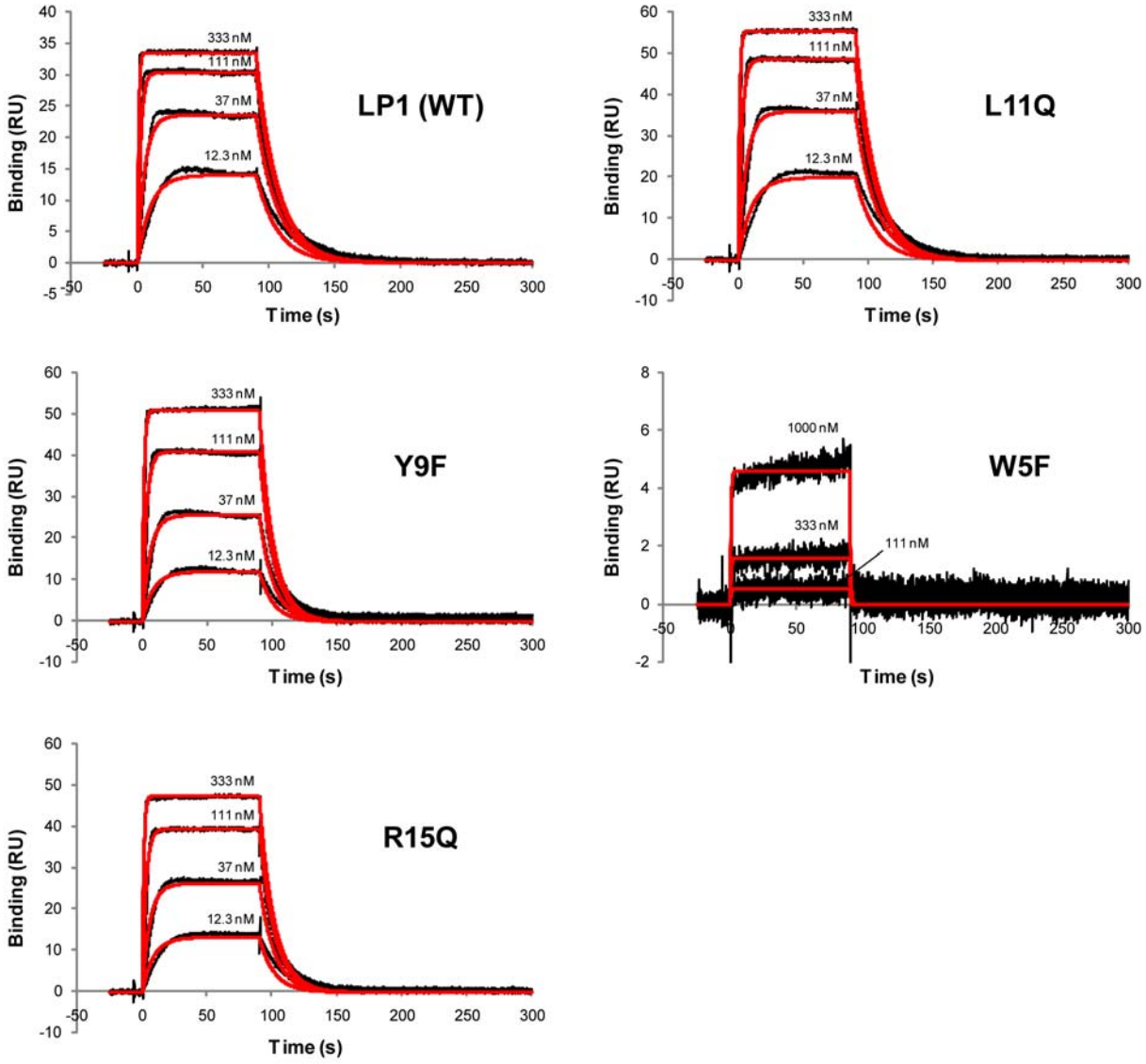

Figure S3 Binding interactions of LP1 wild-type (WT) and mutant peptides with the Mth ectodomain. Surface plasmon resonance sensorgrams were generated by immobilizing the Mth ectodomain on the surface of a flow cell. A peptide concentration series of the indicated peptide was injected (150 μL at 0 s, with a 100 μL/min flow rate) and binding response was measured in real-time as resonance units (RU). The global kinetic fits (red) are overlaid on the original sensorgrams (black). Duplicate injections of the 111 nM concentration for each peptide were performed and sensorgrams for both are overlaid. Sensorgrams have been double-referenced from response curves generated by a flow cell without Mth and averaged buffer blank injections. The derived kinetic parameters are shown in Table 2.