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Supplemental information

GABA_A receptor subunit M2-M3 linkers have asymmetric roles in pore gating and diazepam modulation

Joseph W. Nors, Zachary Endres, and Marcel P. Goldschen-Ohm

Supplemental Material for: GABA_A receptor subunit M2-M3 linkers have asymmetric roles in pore gating and diazepam modulation

Joseph W. Nors^{1,2,†}, Zachary Endres^{1,†}, and Marcel P. Goldschen-Ohm^{1,*}

¹Department of Neuroscience, University of Texas at Austin, Austin, TX, USA

²Department of Molecular and Cellular Physiology, Stanford University, Stanford, CA, USA

[†]Co-first author

*Corresponding author

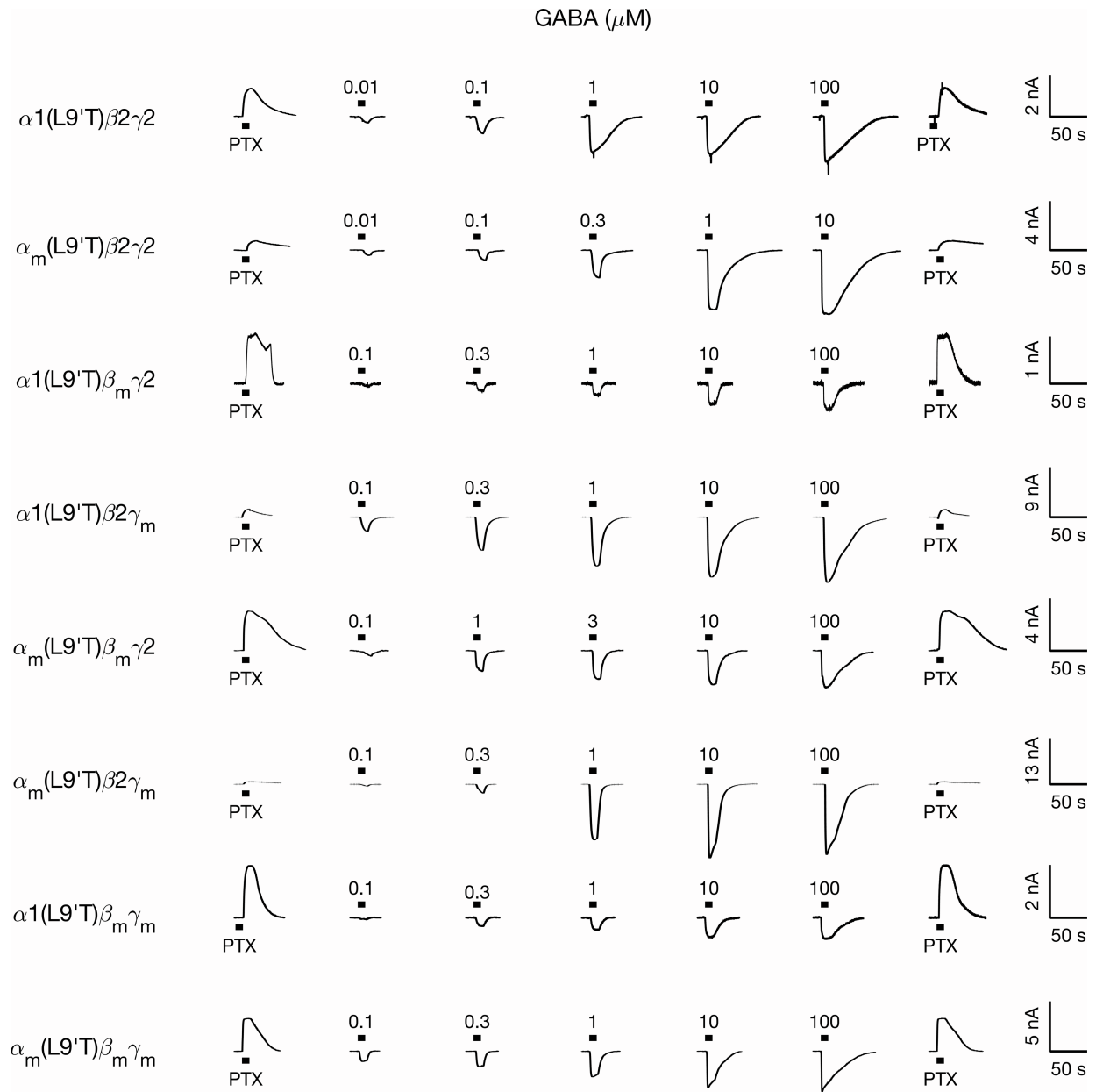


Figure S1. Example GABA-evoked currents from receptors with M2-M3 linker mutations α_m , β_m , and/or γ_m in the gain-of-function $\alpha 1(\text{L9'T})\beta 2\gamma 2$ background. Currents are in response to 10 second pulses (black bars) of either 1 mM PTX or a series of increasing concentrations of GABA (concentration in micromolar above respective black bar). Summaries of concentration-response curves across oocytes are shown in **Figure S4**. Responses to GABA were bookended by the pore blocker PTX to assess the amount of spontaneous unliganded activity and identify the zero-current baseline.



Figure S2. Example DZ-evoked currents from receptors with M2-M3 linker mutations α_m , β_m , and/or γ_m in the gain-of-function $\alpha 1(\text{L9'T})\beta 2\gamma 2$ background. Currents are in response to 10 second pulses (black bars) of either 1 mM PTX or a series of increasing concentrations of DZ (concentration in micromolar above respective black bar). Summaries of concentration-response curves across oocytes are shown in **Figure S5**. Responses to DZ were bookended by the pore blocker PTX to assess the amount of spontaneous unliganded activity and identify the zero-current baseline.

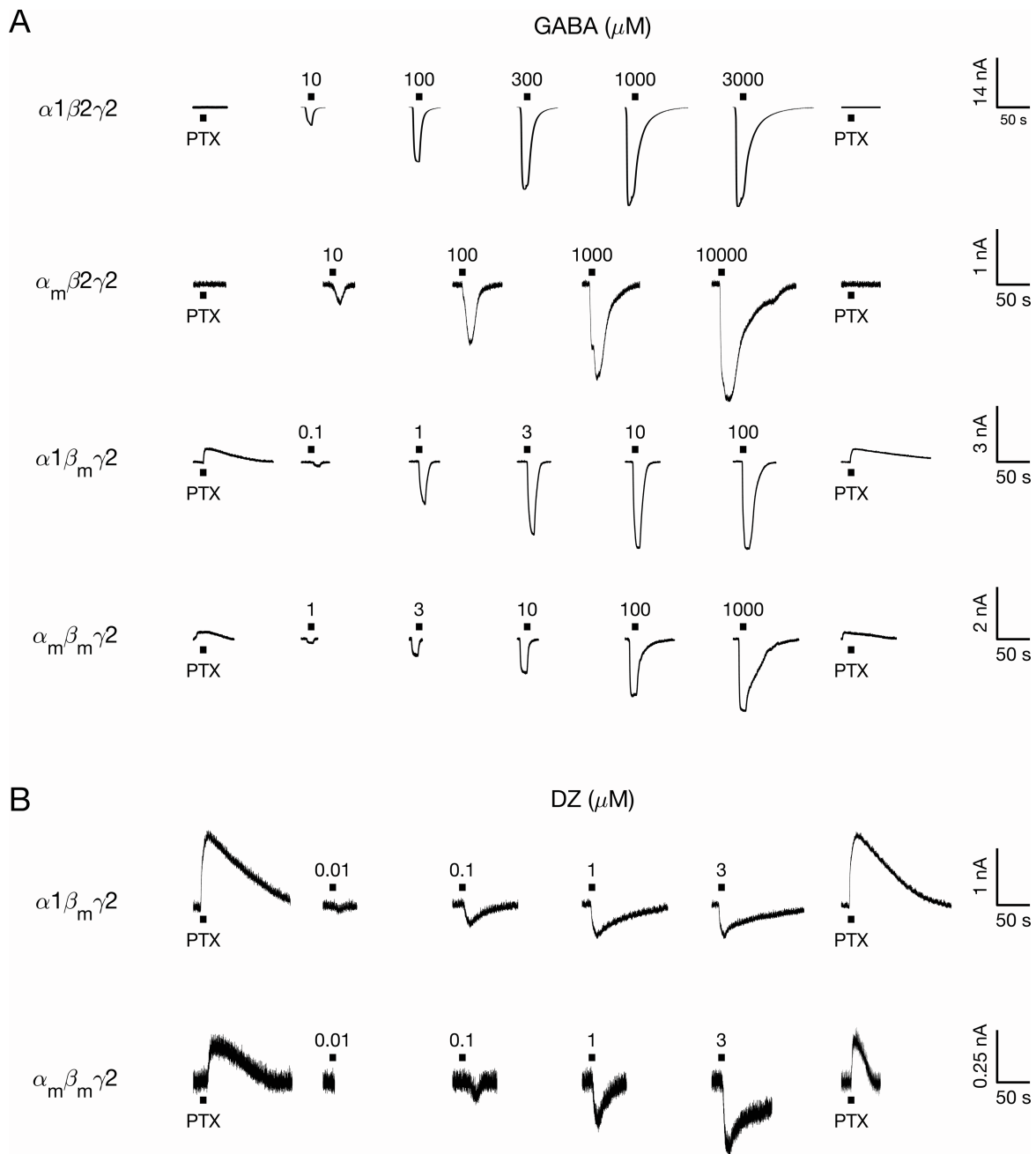


Figure S3. Example GABA- or DZ-evoked currents from receptors with M2-M3 linker mutations α_m , β_m , and/or γ_m in a wild type $\alpha 1 \beta 2 \gamma 2$ background. (A) Currents are in response to 10 second pulses (black bars) of either 1 mM PTX or a series of increasing concentrations of GABA (concentration in micromolar above respective black bar). Summaries of concentration-response curves across oocytes are shown in **Figure S6. Responses to GABA were bookended by the pore blocker PTX to assess the amount of spontaneous unliganded activity and identify the zero-current baseline. (B) Same as in (A) except for currents evoked with DZ instead of GABA.**

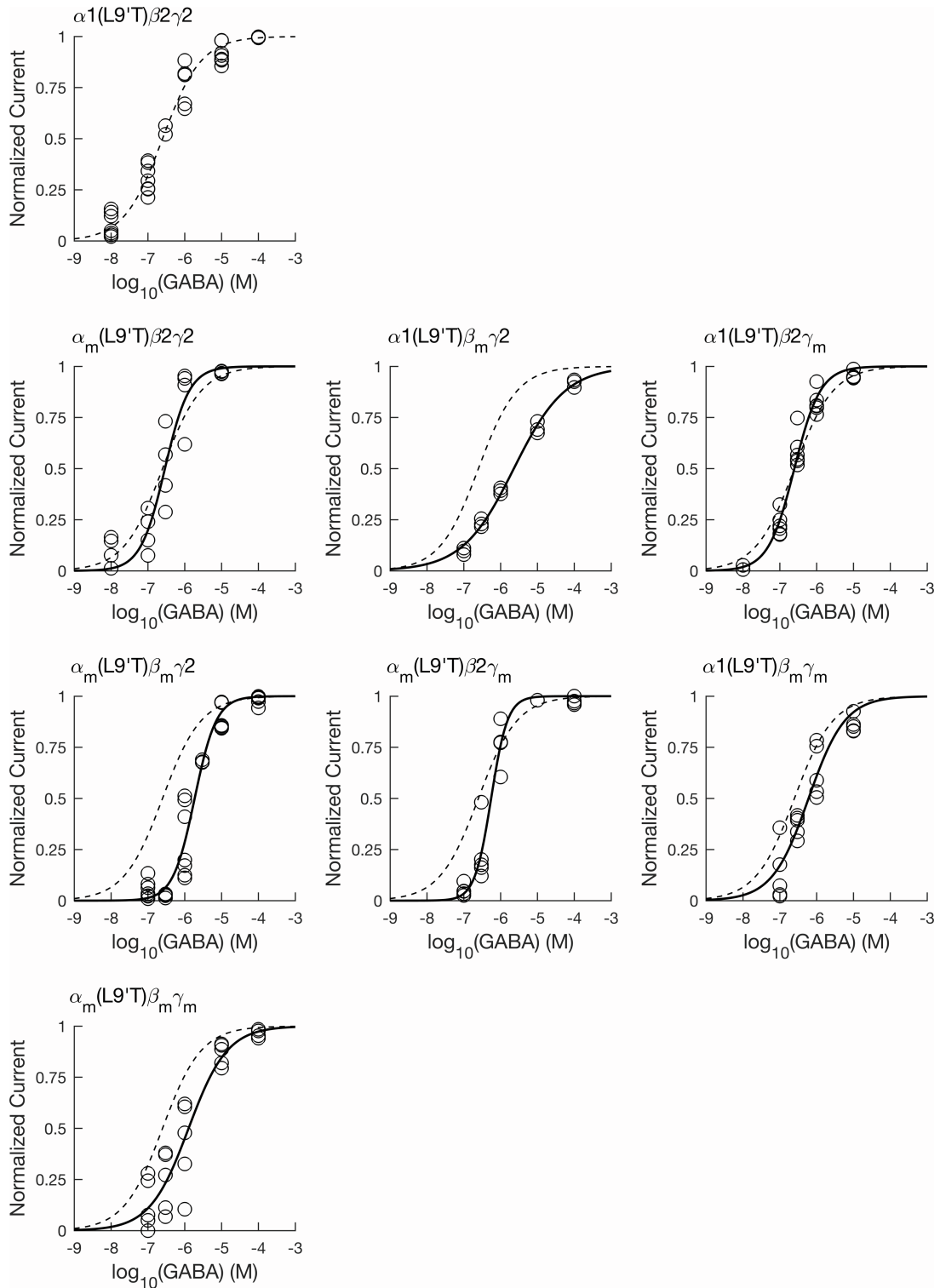


Figure S4. GABA-evoked concentration-response relationships for gain-of-function $\alpha 1(L9'T)\beta 2\gamma 2$ receptors without and with M2-M3 linker mutations α_m , β_m , and/or γ_m . For each oocyte, GABA-evoked current amplitudes from the unliganded baseline as shown in **Figure S1** versus GABA concentration were fit to the Hill equation (**Equation 1**). Parameters for individual fits are shown in **Figure S7**. Data points for each oocyte were normalized to their Hill fit maximum, and the

combined normalized data from all oocytes (circles) was fit to the Hill equation with $I_{max} = 1$ (solid lines, dashed line is fit for $\alpha_1(L9'T)\beta_2\gamma_2$ receptors). Combined fit parameters (EC_{50} , Hill coefficient h) and number of oocytes (n) for each construct: $\alpha_1(L9'T)\beta_2\gamma_2$: $EC_{50} = 0.25 \mu M$, $h = 0.83$, $n = 7$, $\alpha_m(L9'T)\beta_2\gamma_2$: $EC_{50} = 0.29 \mu M$, $h = 1.31$, $n = 4$, $\alpha_1(L9'T)\beta_m\gamma_2$: $EC_{50} = 2.28 \mu M$, $h = 0.62$, $n = 3$, $\alpha_1(L9'T)\beta_2\gamma_m$: $EC_{50} = 0.25 \mu M$, $h = 1.23$, $n = 6$, $\alpha_m(L9'T)\beta_m\gamma_2$: $EC_{50} = 1.86 \mu M$, $h = 1.47$, $n = 7$, $\alpha_m(L9'T)\beta_2\gamma_m$: $EC_{50} = 0.53 \mu M$, $h = 1.95$, $n = 5$, $\alpha_1(L9'T)\beta_m\gamma_m$: $EC_{50} = 0.6 \mu M$, $h = 0.88$, $n = 5$, $\alpha_m(L9'T)\beta_m\gamma_m$: $EC_{50} = 1.27 \mu M$, $h = 0.83$, $n = 5$.

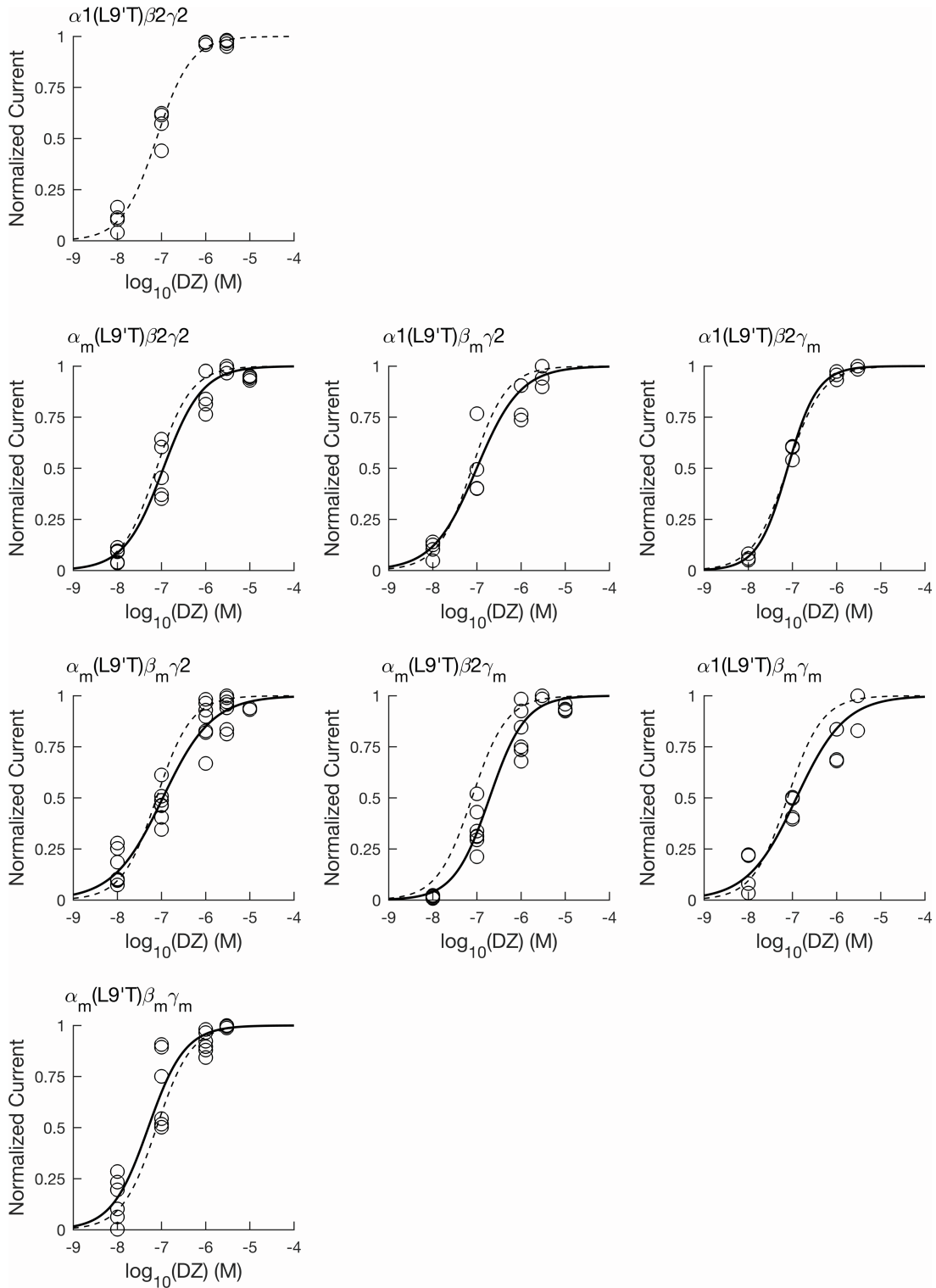


Figure S5. DZ-evoked concentration-response relationships for gain-of-function $\alpha1(L9'T)\beta2\gamma2$ receptors without and with M2-M3 linker mutations α_m , β_m , and/or γ_m . For each oocyte, DZ-evoked current amplitudes from the unliganded baseline as shown in **Figure S2** versus DZ concentration were fit to the Hill equation (**Equation 1**). Parameters for individual fits are shown in **Figure S8**. Data points for each oocyte were normalized to their Hill fit maximum, and the combined

normalized data from all oocytes (circles) was fit to the Hill equation with $I_{max} = 1$ (solid lines, dashed line is fit for $\alpha_1(L9'T)\beta_2\gamma_2$ receptors). Combined fit parameters (EC_{50} , Hill coefficient h) and number of oocytes (n) for each construct: $\alpha_1(L9'T)\beta_2\gamma_2$: $EC_{50} = 0.08 \mu\text{M}$, $h = 1.10$, $n = 4$, $\alpha_m(L9'T)\beta_2\gamma_2$: $EC_{50} = 0.11 \mu\text{M}$, $h = 0.98$, $n = 5$, $\alpha_1(L9'T)\beta_m\gamma_2$: $EC_{50} = 0.1 \mu\text{M}$, $h = 0.88$, $n = 4$, $\alpha_1(L9'T)\beta_2\gamma_m$: $EC_{50} = 0.08 \mu\text{M}$, $h = 1.28$, $n = 3$, $\alpha_m(L9'T)\beta_m\gamma_2$: $EC_{50} = 0.11 \mu\text{M}$, $h = 0.77$, $n = 7$, $\alpha_m(L9'T)\beta_2\gamma_m$: $EC_{50} = 0.19 \mu\text{M}$, $h = 1.07$, $n = 7$, $\alpha_1(L9'T)\beta_m\gamma_m$: $EC_{50} = 0.12 \mu\text{M}$, $h = 0.79$, $n = 4$, $\alpha_m(L9'T)\beta_m\gamma_m$: $EC_{50} = 0.05 \mu\text{M}$, $h = 1.05$, $n = 6$.

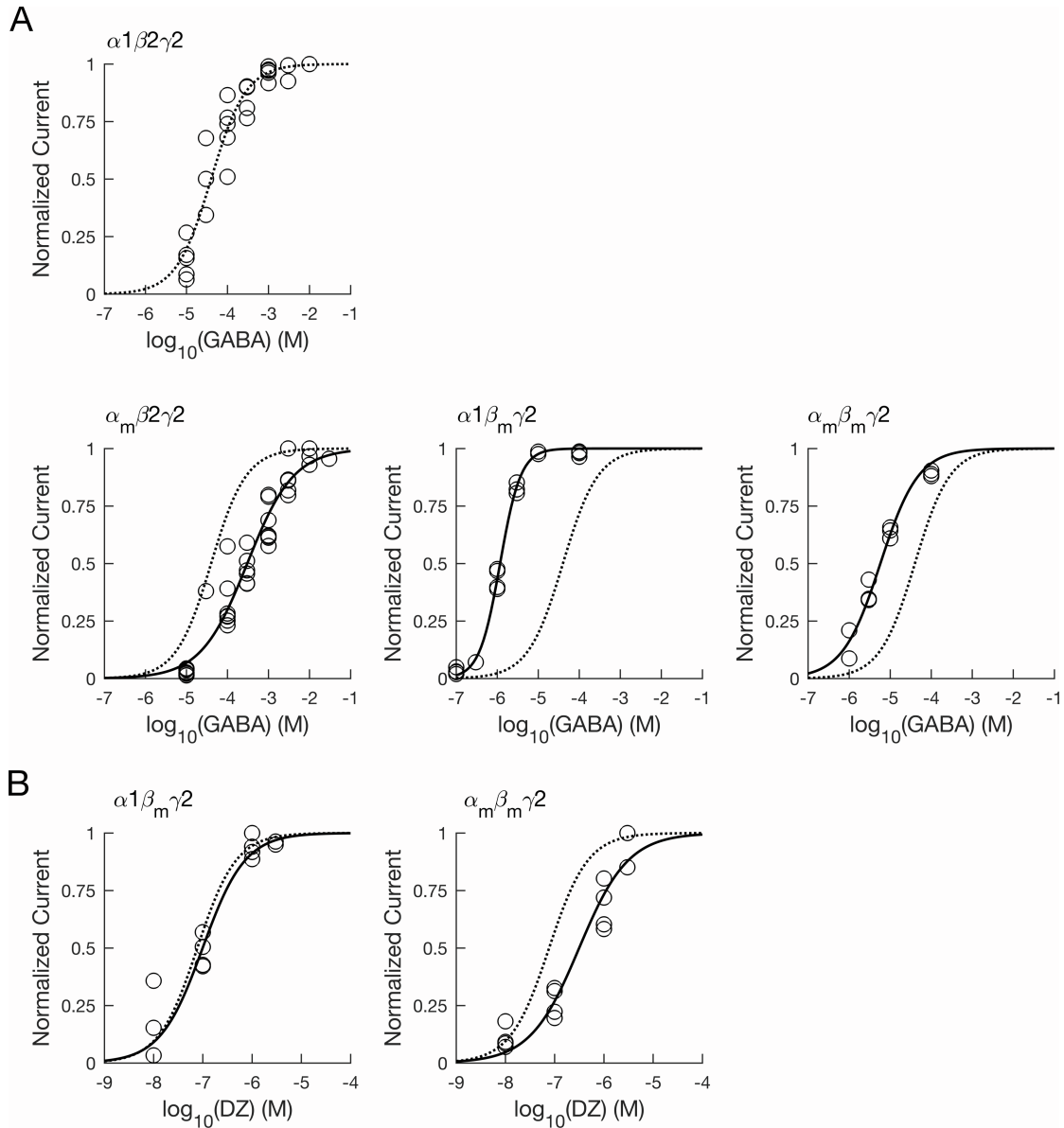


Figure S6. GABA- and DZ-evoked concentration-response relationships for wild type $\alpha 1\beta 2\gamma 2$ receptors without and with M2-M3 linker mutations α_m , β_m , and/or γ_m . For each oocyte, GABA- (A) or DZ-evoked (B) current amplitudes from the unliganded baseline as shown in Figure S3 versus GABA or DZ concentration, respectively, were fit to the Hill equation (Equation 1). Parameters for individual fits are shown in Figures S7-S8. Data points for each oocyte were normalized to their Hill fit maximum, and the combined normalized data from all oocytes (circles) was fit to the Hill equation with $I_{max} = 1$ (solid lines, dashed line is fit for $\alpha 1\beta 2\gamma 2$ receptors). GABA-evoked (A) combined fit parameters (EC_{50} , Hill coefficient h) and number of oocytes (n) for each construct: $\alpha 1\beta 2\gamma 2$: $EC_{50} = 39.94 \mu M$, $h = 1.02$, $n = 5$, $\alpha_m\beta 2\gamma 2$: $EC_{50} = 303.37 \mu M$, $h = 0.77$, $n = 7$, $\alpha 1\beta_m\gamma 2$: $EC_{50} = 1.17 \mu M$, $h = 1.73$, $n = 4$, $\alpha_m\beta_m\gamma 2$: $EC_{50} = 5.76 \mu M$, $h = 0.94$, $n = 3$. DZ-evoked (B) combined fit parameters (EC_{50} , Hill coefficient h) and number of oocytes (n) for each construct: $\alpha 1\beta_m\gamma 2$: $EC_{50} = 0.1 \mu M$, $h = 1.01$, $n = 4$, $\alpha_m\beta_m\gamma 2$: $EC_{50} = 0.31 \mu M$, $h = 0.87$, $n = 4$.

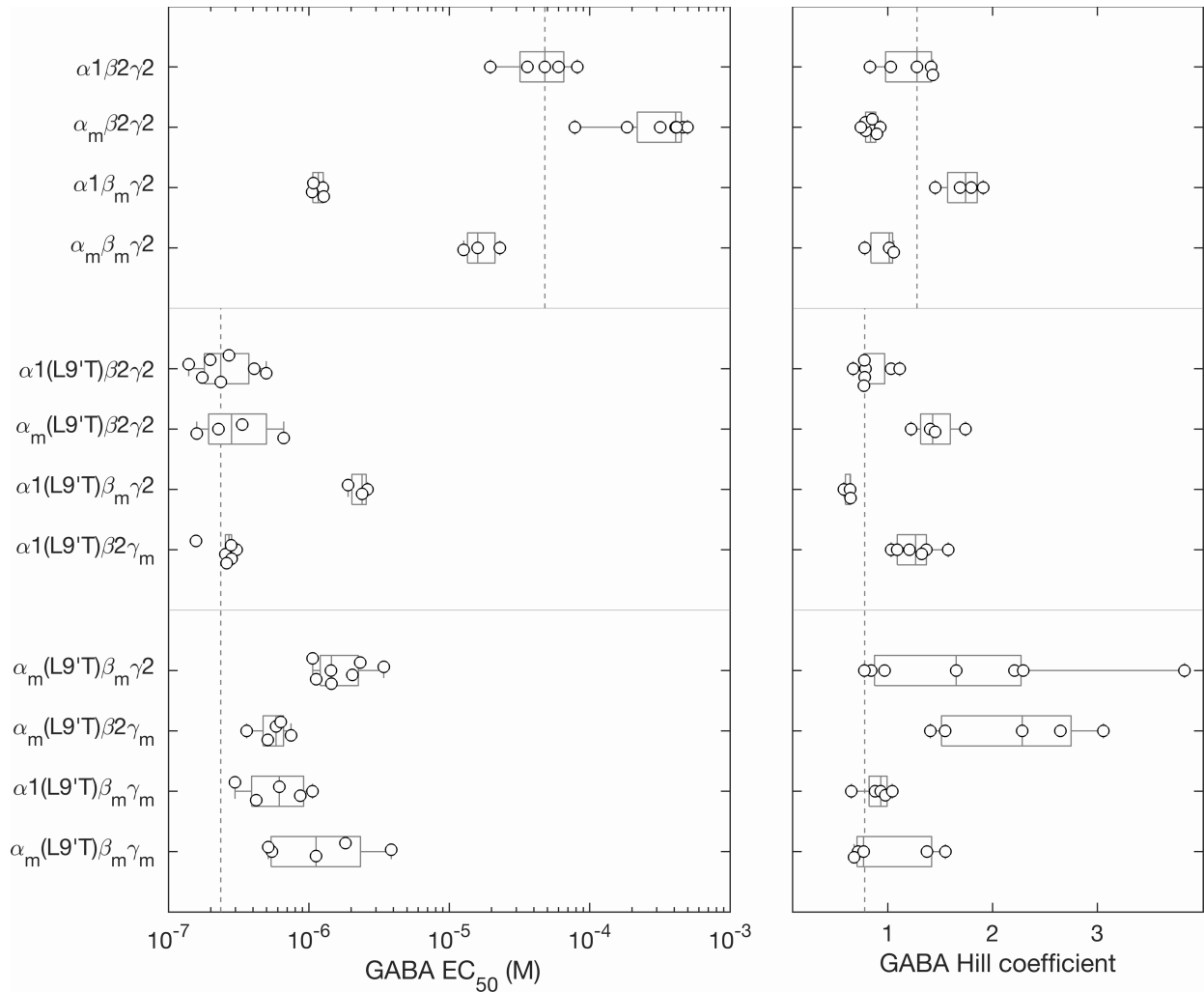


Figure S7. Summary of GABA concentration-response relation Hill fit parameters for individual oocytes. Data points are for individual oocytes ($\alpha_1\beta_2\gamma_2$: $n = 5$; $\alpha_m\beta_2\gamma_2$: $n = 7$; $\alpha_1\beta_m\gamma_2$: $n = 4$; $\alpha_m\beta_m\gamma_2$: $n = 3$; $\alpha_1(L9'T)\beta_2\gamma_2$: $n = 7$; $\alpha_m(L9'T)\beta_2\gamma_2$: $n = 4$; $\alpha_1(L9'T)\beta_m\gamma_2$: $n = 3$; $\alpha_1(L9'T)\beta_2\gamma_m$: $n = 6$; $\alpha_m(L9'T)\beta_m\gamma_2$: $n = 7$; $\alpha_m(L9'T)\beta_2\gamma_m$: $n = 5$; $\alpha_1(L9'T)\beta_m\gamma_m$: $n = 5$; $\alpha_m(L9'T)\beta_m\gamma_m$: $n = 5$). Box plots indicate quartiles, and the upper and lower vertical dashed lines are the median for the $\alpha_1(L9'T)\beta_2\gamma_2$ and $\alpha_1\beta_2\gamma_2$ backgrounds, respectively.

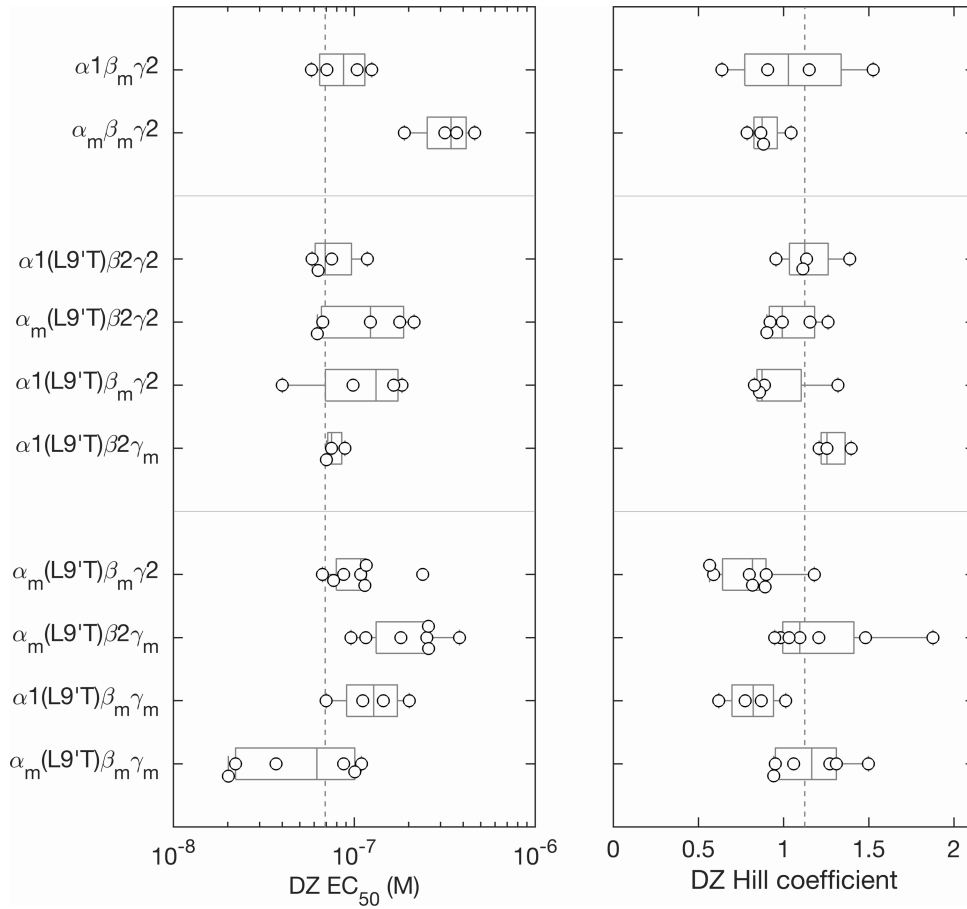


Figure S8. Summary of DZ concentration-response relation Hill fit parameters for individual oocytes. Data points are for individual oocytes ($\alpha_1\beta_m\gamma_2$: n = 4; $\alpha_m\beta_m\gamma_2$: n = 4; $\alpha_1(L9'T)\beta_2\gamma_2$: n = 4; $\alpha_m(L9'T)\beta_2\gamma_2$: n = 5; $\alpha_1(L9'T)\beta_m\gamma_2$: n = 4; $\alpha_1(L9'T)\beta_2\gamma_m$: n = 3; $\alpha_m(L9'T)\beta_m\gamma_2$: n = 7; $\alpha_m(L9'T)\beta_2\gamma_m$: n = 7; $\alpha_1(L9'T)\beta_m\gamma_m$: n = 4; $\alpha_m(L9'T)\beta_m\gamma_m$: n = 6). Box plots indicate quartiles, and the vertical dashed line is the median for the $\alpha_1(L9'T)\beta_2\gamma_2$ background.

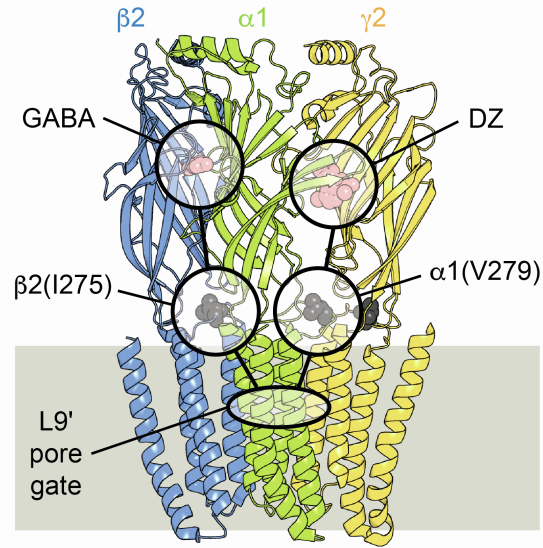


Figure S9. Functional asymmetry in M2-M3 linkers may correspond to the ligand binding interface in which the linker is located. Side-on view from the plane of the membrane omitting back two subunits for clarity. Subunits $\alpha 1$, $\beta 2$, and $\gamma 2$ are shown in green, blue, and yellow, respectively. The relative locations of bound GABA or DZ and M2-M3 linker residues $\beta 2(I275)$ or $\alpha 1(V279)$ at β/α or α/γ intersubunit interfaces, respectively, and the 9' leucine pore gate are highlighted.