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Fig. S1. Nucleic acid (top) and amino acids (bottom) sequences of iGlow.

Black = GPR68; purple = linkers; green = cpGFP; red = Helix 8. Brackets indicate the C-terminal fragment eliminated in H8Del.

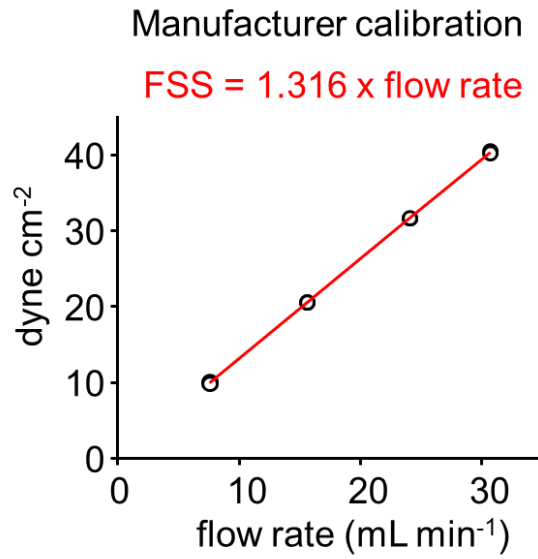


Fig. S2. Shear stress calibration. Shear stress applied through our flow chamber was calculated using the manufacturer's calibration.

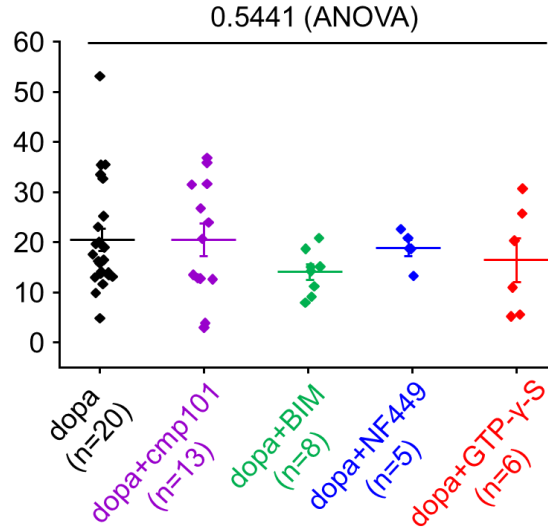


Fig. S3. Dopamine sensitivity of dLight1.2 is not eliminated by pharmacological modulation of G protein signaling. The scatter plots show max $\Delta F/F_0$ values obtained upon acute perfusion with 10 μ M dopamine in cells pre-treated with CMPD101 (purple dots, n = 13), BIM-46187 (BIM, green dots, n = 8), NF-449 (blue dots, n = 5), GTP- γ -S (red dots, n = 6), or a vehicle control (black dots, n = 20). The number above the graph indicates the exact p-value from a one-way ANOVA. Error bars = s.e.m.

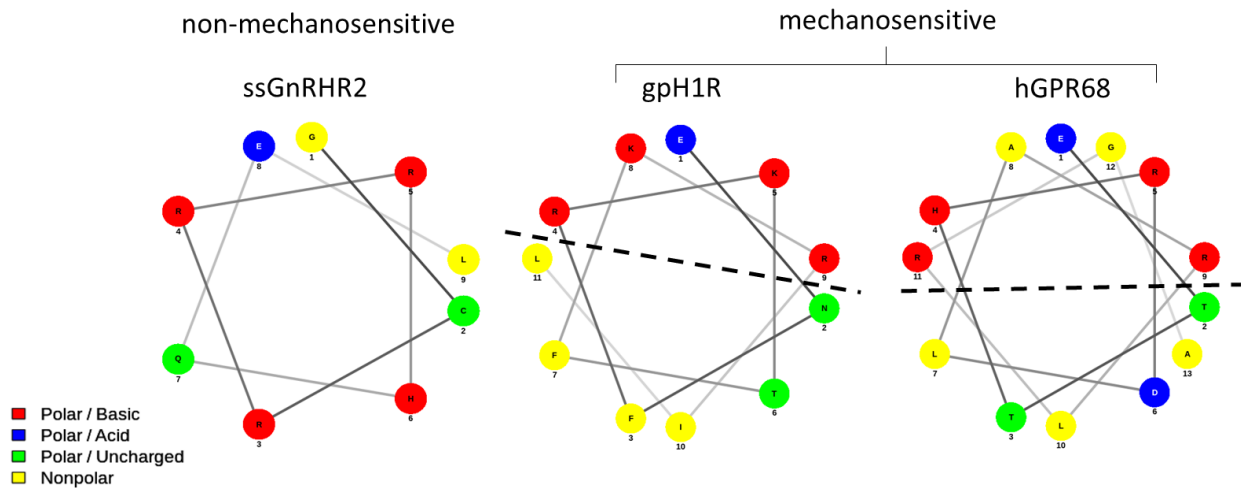


Fig. S4. Prediction of an amphipathic Helix 8 in GPR68 using the online predictor NetWheels. Helical wheel plot of Helix 8 in the long isoform of the swine gonadotropin-releasing hormone receptor (ssGnRHR2), the guinea pig histamine H1 receptor (gpH1R) and human GPR68 (hGPR68). The dotted line indicates the separation between the polar vs. apolar interfaces of the Helix.

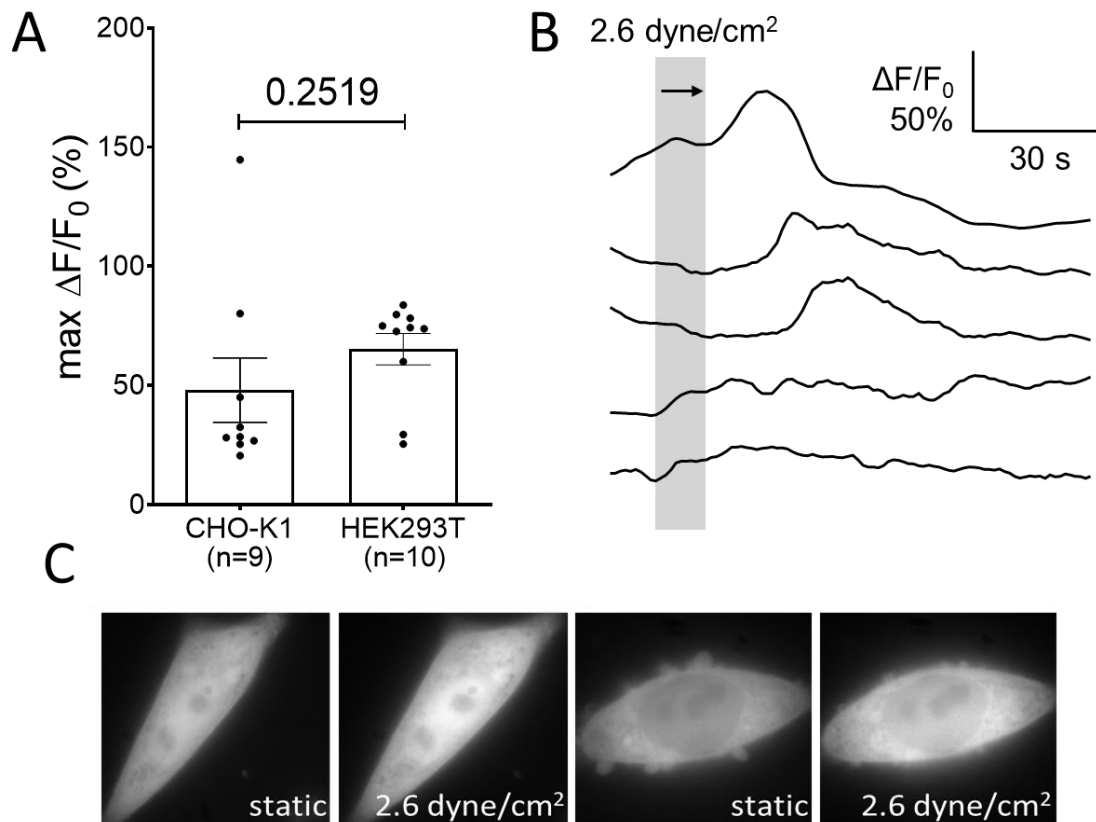


Fig. S5. H8Del is functionally expressed in CHO-K1 cells. (A) Scatter plots showing maximum $\Delta F/F_0$ in CHO-K1 and HEK293- $\Delta Pz1$ cells transfected with H8Del and exposed to 2.6 dyne cm⁻² of shear stress for 10 sec. (B) Representative time-course traces of individual cells. The duration of shear stimulus is indicated in grey. (C) Epifluorescence images of CHO-K1 cells before and after shear stimulus.

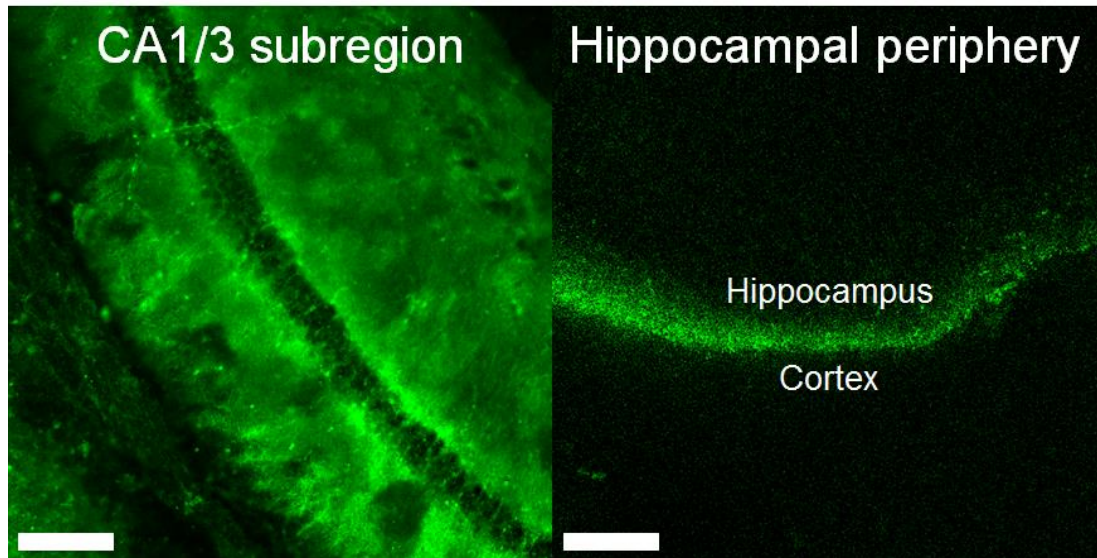


Fig. S6. AAV-mediated expression of iGlow in mouse hippocampal CA3 region. Confocal microscopy images at the site of injection (CA1/3 subregion) and its surrounding (Hippocampal periphery). The faint peripheral signal is produced by background autofluorescence. Scale bars = 150 μ M.

Table S1. Primers used to insert cpGFP into GPR68 using High-Fidelity DNA assembly.

primers	Sequences (5'→3')
GPR68 Fwd (backbone)	aatcatgaccaactgagccgcaaggaccagatccagcgg
GPR68 Rev (backbone)	aatgagtgagctcaggctccggcgcacggcgcg
cpGFP with linkers Fwd	gcgccggagcctgagctcactcattaacgtctatatcaaggcc
cpGFP with linkers Rev	ccttgccggctcagttggtcatgattggttactccagcttg