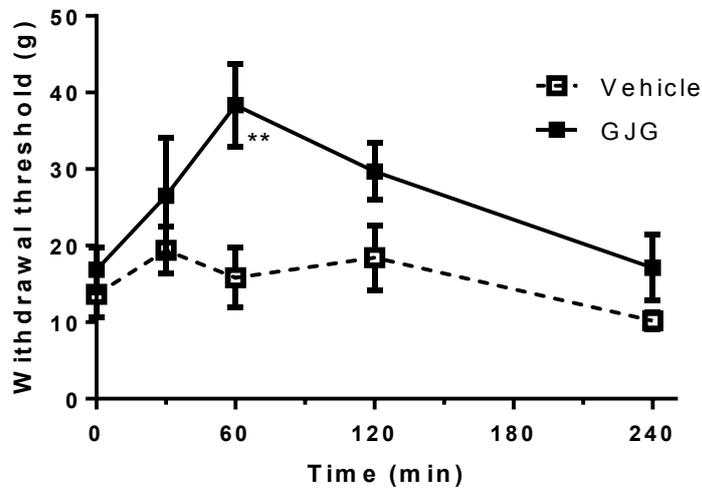


Supplementary Figures

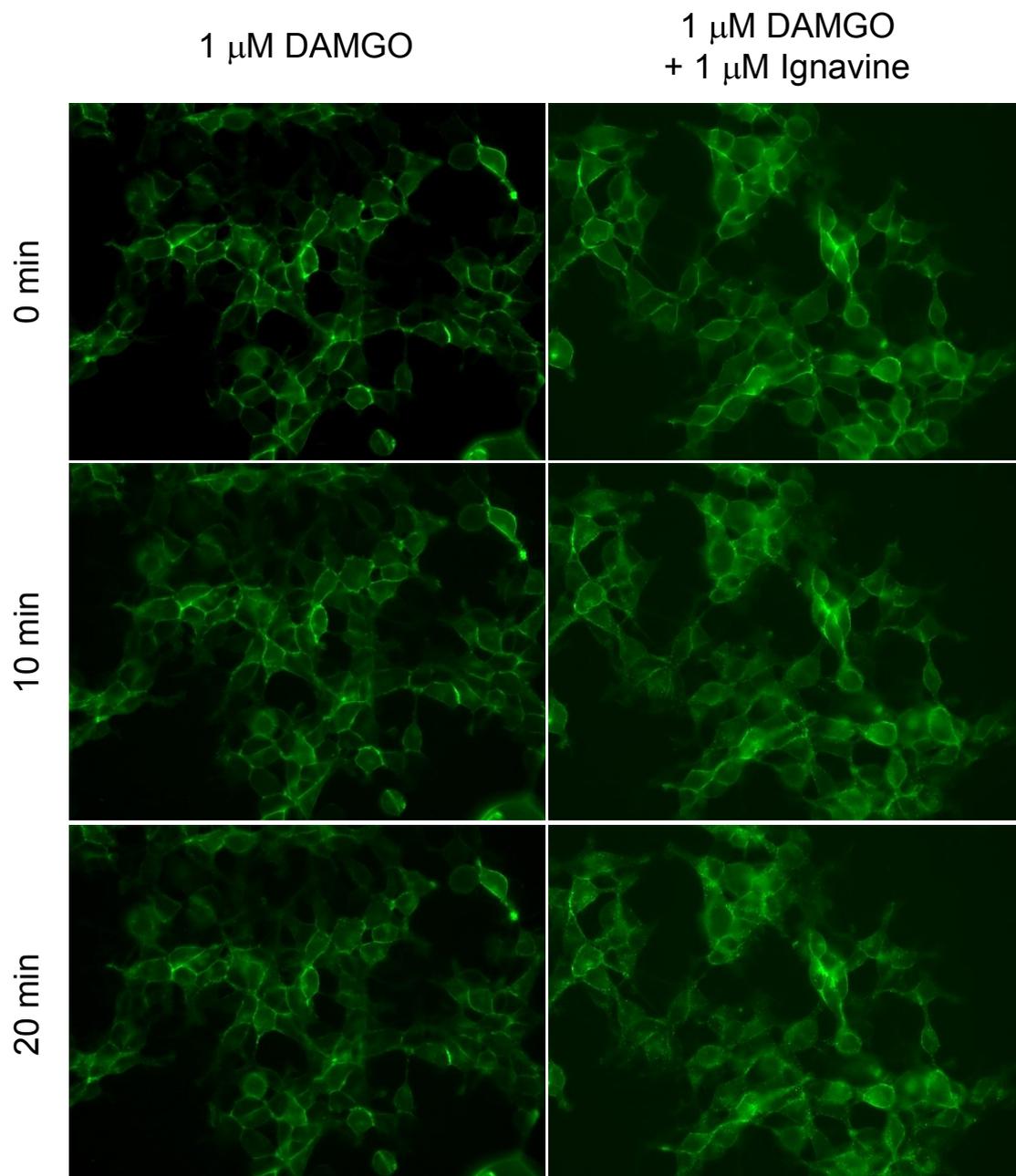
Ignavine: a novel allosteric modulator of the μ opioid receptor

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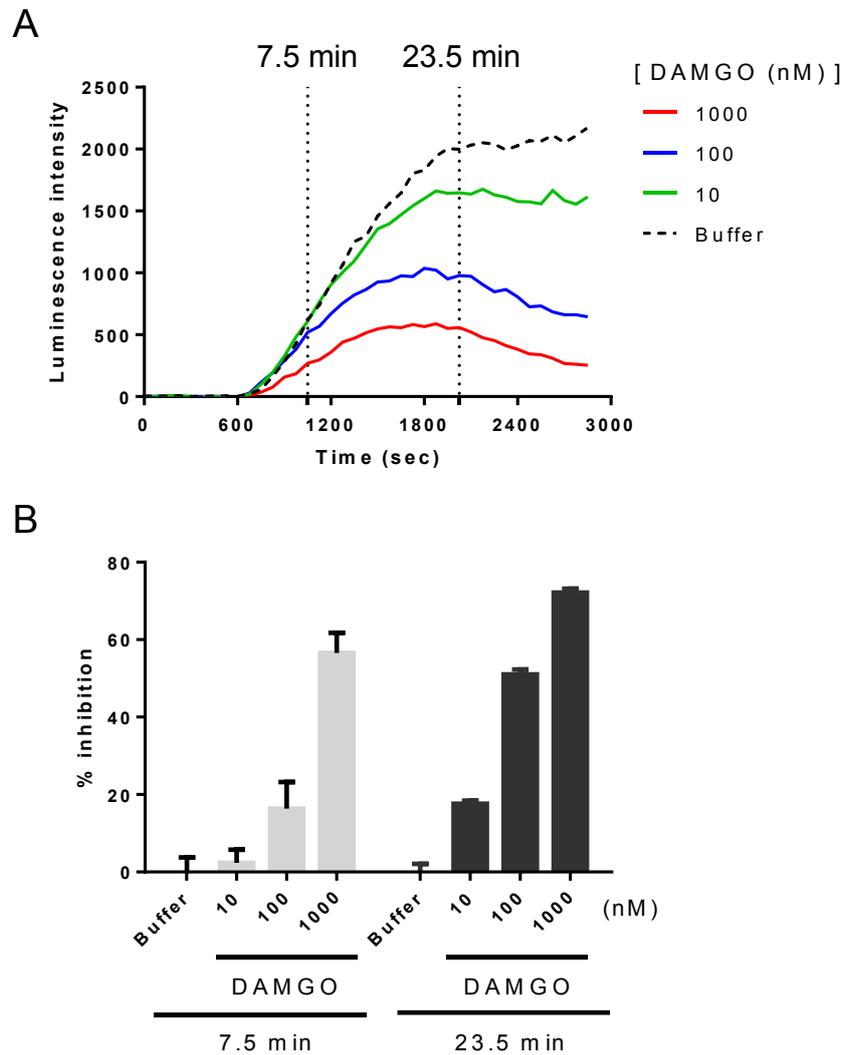
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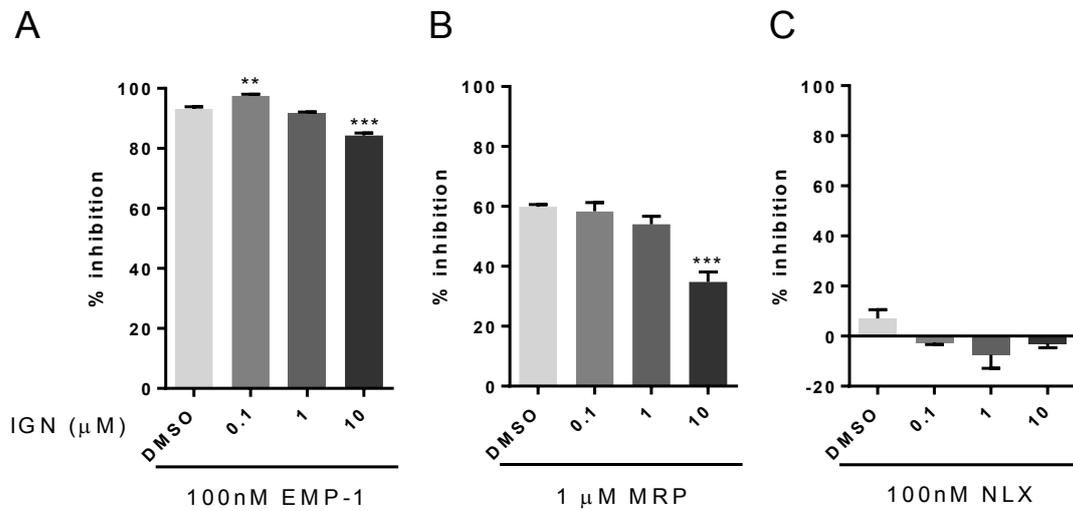
Supplemental Figure S1. Amelioration of mechanical allodynia by GJG in a rat CCI-neuropathic pain model. Fourteen days after CCI in the left hind paw, 2 g/kg GJG or its vehicle were orally administered and the pressure threshold of paw withdrawal was measured at each time-point. GJG significantly increased the withdrawal threshold 60 min after administration. Data shown represent mean \pm SEM (n = 4 in vehicle, n = 5 in GJG). ** $P < 0.01$; by two-way ANOVA with Bonferroni's correction.



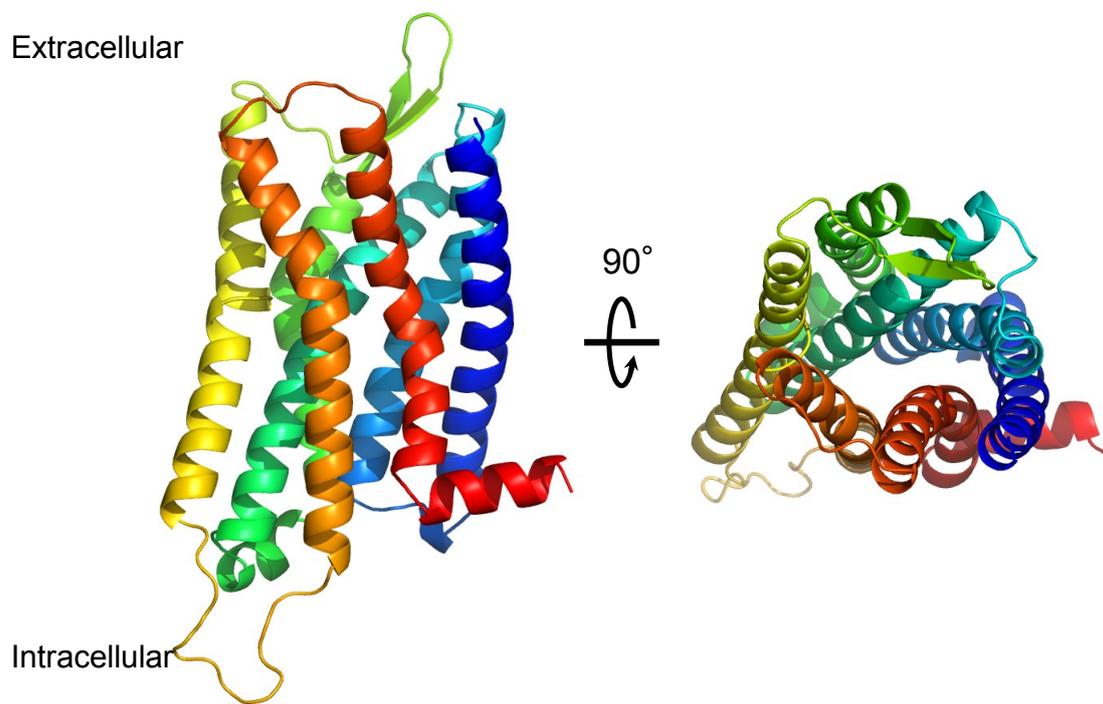
Supplemental Figure S2. All field views in receptor internalization assay. 1 μ M DAMGO induced receptor internalization 20 min after application. Receptor internalization was observed 10 min after application of 1 μ M DAMGO with 1 μ M ignavine.



Supplementary Figure S3. Intracellular cAMP assay using recombinant human MOR and a GloSensor™ protein-expressing cell line. The cells were treated with DAMGO at 0 sec. Then, forskolin was added at 10 min (600 sec). **A** The amount of intracellular cAMP was traced by luminescence intensity. DAMGO inhibited the increase in intracellular cAMP in a dose-dependent manner. **B** % inhibition was calculated at 7.5 and 23.5 min after forskolin stimulation. Data shown represent mean (n = 3).



Supplementary Figure S4. Intracellular cAMP assay. The effect of ignavine on MOR expressing cell line in the presence of 100 nM endomorphin-1 (EMP-1, **A**), 1 μM morphine (MRP, **B**) and 100 nM naloxone (NLX, **C**) was evaluated. % inhibition was calculated from the data obtained at 23.5 min after forskolin application. Data represent the mean \pm SEM (n = 3). ** $P < 0.01$; *** $P < 0.001$ by Dunnett's test vs. vehicle.



Supplementary Figure S5. The 3D structure of the complete human MOR homology model. The structure of the complete human MOR viewed from a position parallel to the membrane (left) and from the extracellular side (right). Each of the transmembrane helices are represented in different colors.