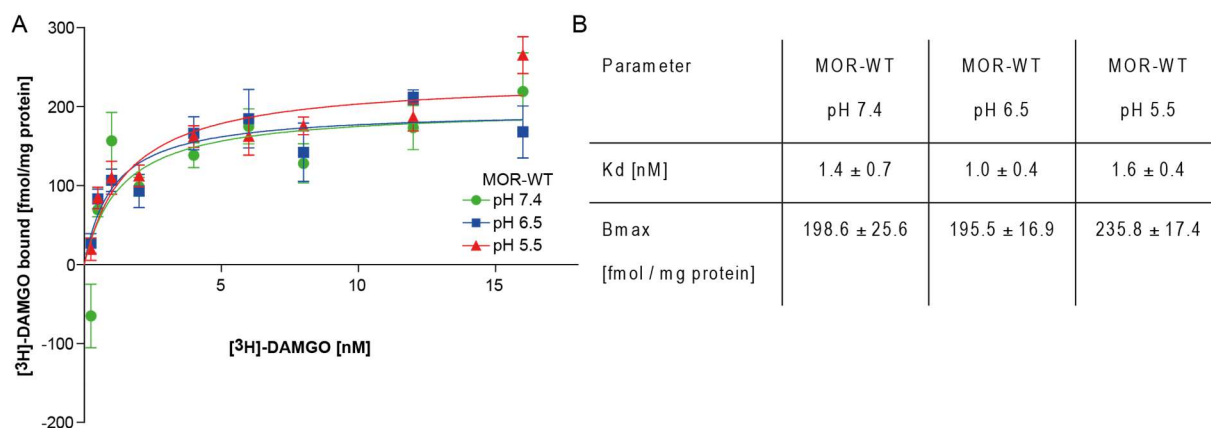
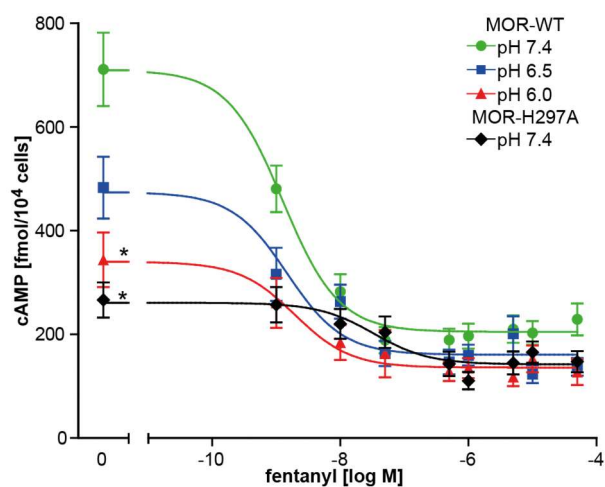


Supporting Information to manuscript “Modulation of mu-opioid receptor activation by acidic pH is dependent on ligand structure and an ionizable amino acid residue”



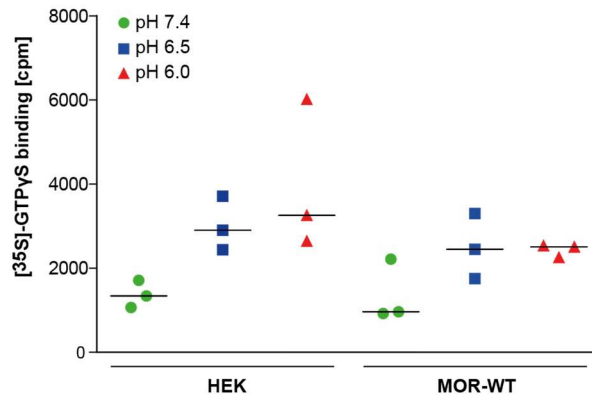
Supporting Figure S1. [³H]-DAMGO binding after pre-incubation at low pH

[³H]-DAMGO saturation binding to membrane fractions of HEK MOR-WT at pH 7.4, following pre-incubation for 20 min at different pH values. Data represent means ± SEM of fmol of bound [³H]-DAMGO per mg of total protein with nonlinear fit. **(B)** Parameters derived from curve fits in A. K_d and B_{max} were each compared by One-way ANOVA, no significant differences were found, *n* = 6 per experiment.



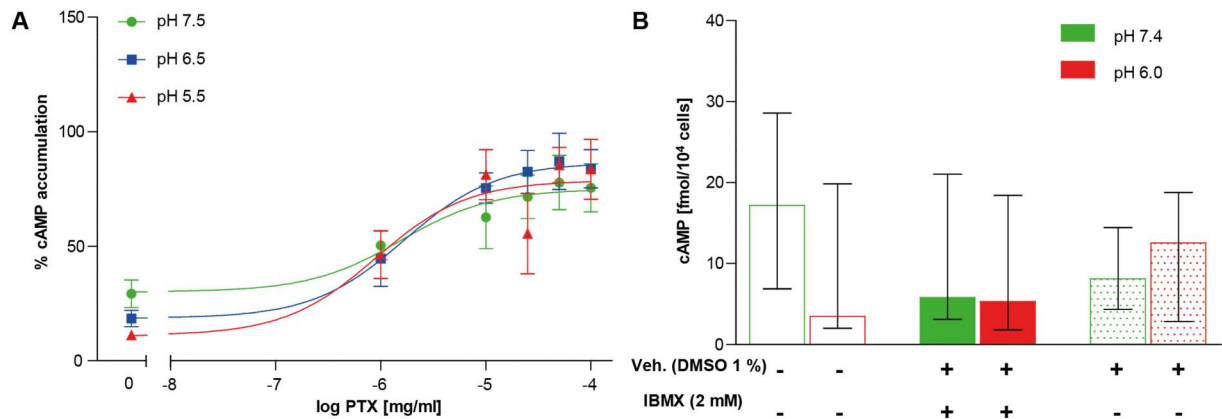
Supporting Figure S2. Fentanyl effects on cAMP content in HEK MOR-WT and MOR-H297^{6.52}A.

Fentanyl-induced cAMP reduction in HEK MOR-WT and HEK MOR-H297^{6.52}A. Data are expressed as cAMP per 10⁴ cells and represent mean ± SEM with nonlinear fit. Baselines in absence of fentanyl were analyzed by One-way ANOVA with Dunnett's Multiple Comparison Test (control group = MOR-WT pH 7.4), * $P < 0.05$, n= 6 per curve.



Supporting Figure S3. Basal [³⁵S]-GTPγS binding at physiological and low pH

Basal [³⁵S]-GTPγS binding at pH 7.4, 6.5 and 6.0 in membrane fractions of HEK WT and HEK MOR-WT in absence of opioid ligands. Data represent specific [³⁵S]-GTPγS bound in cpm by experiment (dots) with mean (lines), *n* = 3 per condition.



Supporting Figure S4. Effects of pertussis toxin on cAMP accumulation and basal cAMP concentrations in intact HEK MOR-WT cells at different pH values

(A) Reversal of fentanyl-induced cAMP reduction by PTX in HEK MOR-WT cells. Data are normalized to cAMP levels in absence of fentanyl and PTX at the respective pH and represent mean \pm SEM with nonlinear fit. Comparison of fits with extra sum of squares F test (Top, logEC₅₀), ns, one curve for all datasets, n = 5 per curve. (B) cAMP accumulation in HEK MOR-WT at extracellular pH 7.4 or 6.0, in absence or presence of dimethyl sulfoxide (DMSO) and/or 3-isobutyl-1-methylxanthine (IBMX). Data represent median with interquartile range of cAMP in fmol/ 10⁴ cells, Friedman test, ns, n = 6 per condition.