**Figure 2–figure supplement 3**

**FRET between eGFP fused at the *N*-terminus and mCherry inserted after Val166.** CHO cells expressing the eGFP- and mCherry-tagged M2 receptor were excited at 473 nm at a power of 0.37 *µ*W. The emission spectrum was recorded from the region of the plasma membrane and analyzed to obtain the apparent FRET efficiency. (**A**) The distribution of FRET efficiencies from 33 cells. The dotted line depicts the best fit of a Gaussian distribution (** = 9.51, ** = 0.11). (**B**) The mean FRET efficiency (± S.D.) measured in the absence of ligand, in the presence of NMS (1 *µ*M) (9.1 ± 1.6, 20 cells), or in the presence NMS (1 *µ*M) plus gallamine (1 mM) (9.7 ± 1.0, 18 cells)

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