**Figure 2–figure supplement 2**



**Modulatory effect of gallamine on the binding of [3H]NMS to mCh-M2-FCM**. Membranes were prepared from CHO cells expressing M2 receptor bearing mCherry at the *N*-terminus and the FlAsH-reactive sequence FCM in the second extracellular loop. The binding of [3H]NMS was measured at the concentrations of gallamine (M) shown on the *x*-axis. Both ligands were added simultaneously, and the reaction mixture was incubated for 16 h at 25 ºC. The line depicts the best fit of Equation 2 to all of the data taken in concert. The values of log *Kj* were common to all of the data, and the fitted estimates are as follows: log *K*1 = −6.39 ± 0.10, log *K*2 = −3.15 ± 0.63, log *K*3 = −2.60 ± 0.59. The values of *n*H(*j*) were indistinguishable from 1, either individually or collectively (*P* ≥ 0.13), and were fixed accordingly. The mean concentration of [3H]NMS was 3.08 ± 0.05 nM. Points at the left- and right-hand ends of the abscissa (F) depict binding in the absence of unlabeled ligand and in the presence of 3 **M atropine.