**Figure 3-source data 1**

**(Figure 3-source data 1, *Panel D*). Levels of significance for ligand-dependent changes in the FRET efficiency of FlAsH-reacted mCh-M2-FCM and mCh-M2(D103A)-FCM.** The sensor (mCh-M2-FCM), the binding-deficient mutant (mCh-M2(D103A)-FCM), and the mutant plus the wild-type receptor (M2) were expressed or co-expressed in CHO cells, and the FRET efficiency (*E*app) was measured in the absence and presence of NMS (1**M) as described in the legend to Figure 3. The mean changes in the FRET efficiency (∆*E*app ± S.D.) were compared by means of the *t*-statistic to obtain the corresponding levels of significance (*P*) listed in the table. The number of cells is shown in parentheses. The level of significance for the difference in *E*app between the vacant and NMS-liganded receptor was < 0.001 throughout.

|  |  |  |  |
| --- | --- | --- | --- |
|  |  | Level of significance for differences in ∆*E*app (*P*) | |
|  | ∆*E*app (*%*) | mCh-M2(D103A)-  FCM + M2 | mCh-M2(D103A)-FCM |
|  |  |  |  |
| mCh-M2-FCM (26) | 20.2 ± 5.3 | < 0.001 | < 0.001 |
| mCh-M2(D103A)-FCM (18) | 0.5 ± 0.6 | < 0.001 |  |
| mCh-M2(D103A)-FCM + M2 (42) | 9.5 ± 1.8 |  |  |
|  |  |  |  |