**Bertheleme et al,**

**Supplementary Information**

**Table S1** Oligos used to generate the mutant receptor constructs

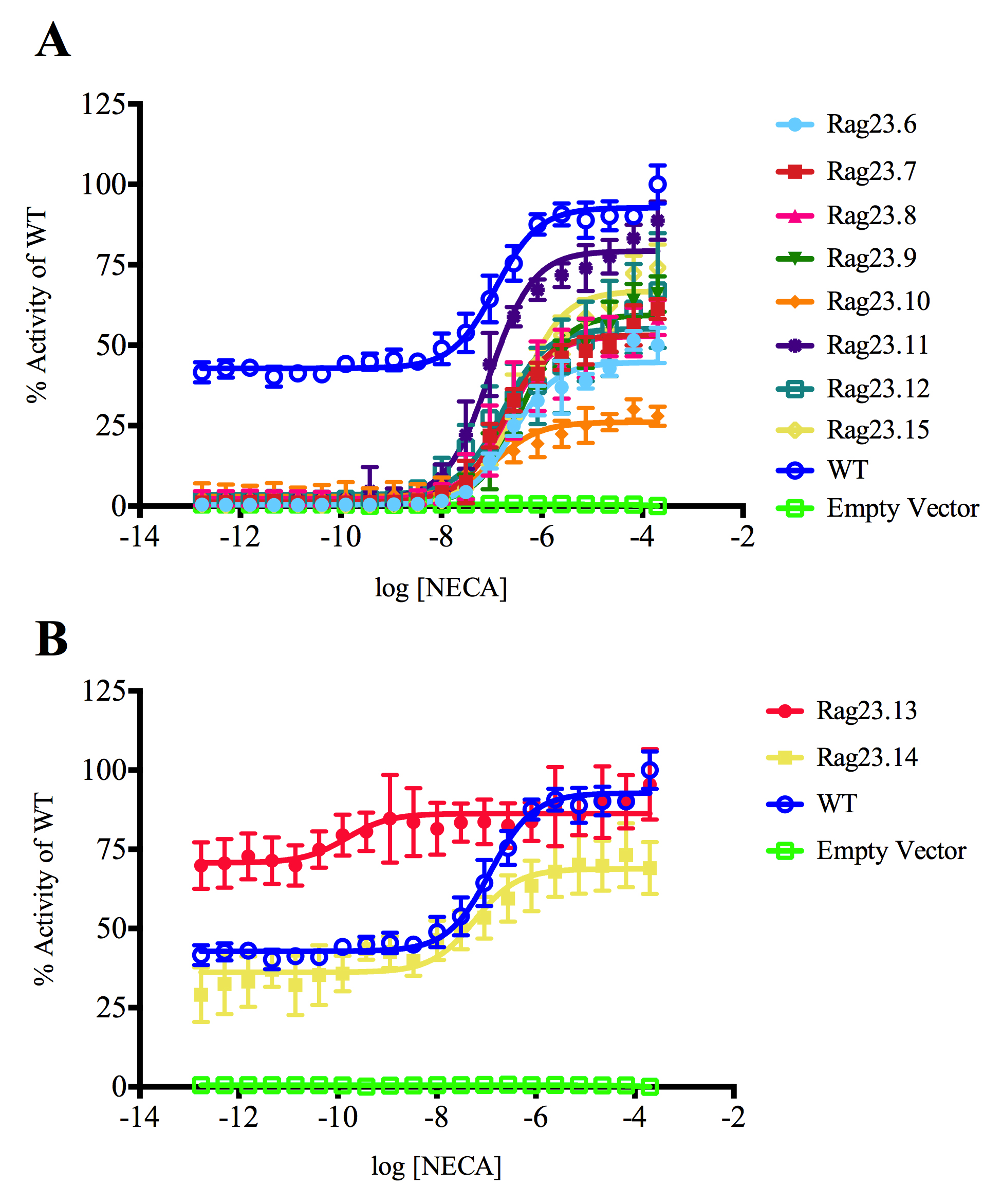
|  |  |
| --- | --- |
| **Primer** | **Sequence (5' to 3')** |
| A79F\_forward | gatgttgggtgtttacttgcgtattttcttggctgccaga |
| A79F\_reverse | taccaaaacgaagcaagcaataaacaaacaaccatgacaagcagc |
| L184A\_forward | catggtctacttcaatttcttcgcgtgtgttttggtccctttgttg |
| L184A\_reverse | ggagaagatagaagattgagtcaaaaccaaaacgaagcaag |
| A199R\_forward | ctaagtccttggctattatagttggtttgttcgctttgtgttg |
| A199R\_reverse | gcaatgatgcccgcagccctcgtgccggtca |
| A208L\_forward | ctattttcttggctgccagaagacaactaaagcaaatggaatctcaacc |
| A208L\_reverse | ggttgagattccatttgctttagttgtcttctggcagccaagaaaatag |
| A272L\_forward | tcatgctccattgtggttgatgtatctagctatagttttgtcccacac |
| A272L\_reverse | gtgtgggacaaaactatagctagatacatcaaccacaatggagcatga |

**Table S2** Expression levels of the thirty mutants and the wild-type calculated using the eGFP fluorescence as described by Drew *et al.* (2008, Nature Protocols, 3: 784-798).

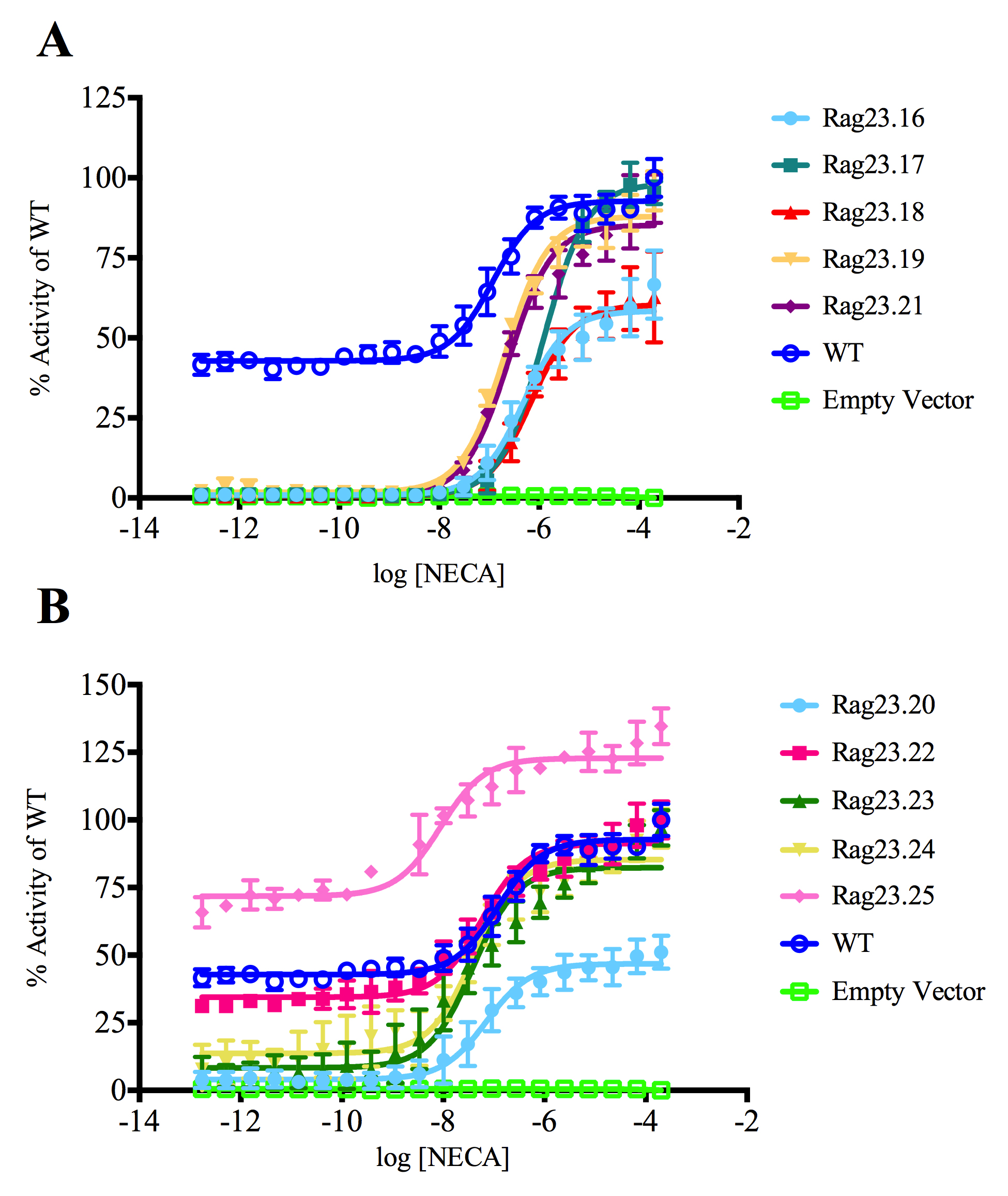
|  |  |  |
| --- | --- | --- |
|  | **RFU** | **Level of Expression (mg/L)** |
| **Rag 23.1** | 16,843 | 0.6 |
| **Rag 23.2** | 20,715 | 1.1 |
| **Rag 23.3** | 22,439 | 1.2 |
| **Rag 23.4** | 19,046 | 0.9 |
| **Rag 23.5** | 16,389 | 0.6 |
|  |  |  |
| **Rag 23.6** | 20,479 | 1.0 |
| **Rag 23.7** | 23,799 | 1.4 |
| **Rag 23.8** | 14,547 | 0.4 |
| **Rag 23.9** | 19,162 | 0.9 |
| **Rag 23.10** | 17,677 | 0.7 |
| **Rag 23.11** | 18,500 | 0.8 |
| **Rag 23.12** | 19,021 | 0.9 |
| **Rag 23.13** | 20,392 | 1.0 |
| **Rag 23.14** | 18,314 | 0.8 |
| **Rag 23.15** | 18,906 | 0.9 |
|  |  |  |
| **Rag 23.16** | 21,423 | 1.2 |
| **Rag 23.17** | 20,992 | 1.1 |
| **Rag 23.18** | 21,782 | 1.2 |
| **Rag 23.19** | 23,018 | 1.3 |
| **Rag 23.20** | 22,388 | 1.2 |
| **Rag 23.21** | 16,312 | 0.6 |
| **Rag 23.22** | 14,803 | 0.4 |
| **Rag 23.23** | 15,874 | 0.5 |
| **Rag 23.24** | 19,569 | 0.9 |
| **Rag 23.25** | 14,356 | 0.4 |
|  |  |  |
| **Rag 23.26** | 15,904 | 0.6 |
| **Rag 23.27** | 16,984 | 0.7 |
| **Rag 23.28** | 19,843 | 1.0 |
| **Rag 23.29** | 20,120 | 1.0 |
| **Rag 23.30** | 18,013 | 0.8 |
|  |  |  |
| **WT** | 19,954 | 1.0 |



**Figure S1** NECA-inducedactivity of the WT A2AR, Rag23 and the quadruple mutants intermediate between the WT and Rag23. See Table 1 for the precise details of each mutant. The receptor constructs were expressed in the MMY24 *S. cerevisiae* strain using the p306GPD vector. The activity of cells containing empty vector is shown as a control.

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**Figure S2A and B** NECA-inducedactivity of the WT A2AR and the triple mutants intermediate between the WT and Rag23. See Table 1 for the precise details of each mutant. The receptor constructs were expressed in the MMY24 *S. cerevisiae* strain using the p306GPD vector. The activity of cells containing empty vector is shown as a control.

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**Figure S3A and B** NECA-inducedactivity of the WT A2AR and the double mutants intermediate between the WT and Rag23. See Table 1 for the precise details of each mutant. The receptor constructs were expressed in the MMY24 *S. cerevisiae* strain using the p306GPD vector. The activity of cells containing empty vector is shown as a control.



**Figure S4** NECA-inducedactivity of the WT A2AR and the single mutants intermediate between the WT and Rag23. See Table 1 for the precise details of each mutant. The receptor constructs were expressed in the MMY24 *S. cerevisiae* strain using the p306GPD vector. The activity of cells containing empty vector is shown as a control.