**Supplementary Table 1.** **Chimeric ACTH/MC4 receptors and their functional properties**

|  |  |  |  |
| --- | --- | --- | --- |
| Receptor | -MRAP | +MRAP | Reference |
| Surface | ACTH | MSH | Surface | ACTH | MSH |
| MC4R |  | **++** | 1 | 1 | **+** | 1 | 1 |  |
| ACTHR |  | **-** | 0 | 0 | **++** | 1 | 0 |  |
| Ch1 |  | **+** | EC50×38.1MC4R | EC50×7.0MC4RKi×1.2MC4R |  |  |  | [1; 2] |
| Ch2 |  | **-** | EC50ND | EC50ND | **+** | EC50×129.1ACTHREC50×8.0MC4R | ND | [1] |
| 2C2a |  | **-** |  |  | **+** | RlucMax×5.4ACTHRRlucMax×5.0MC4R | RlucMax×130.0MC4R | [3] |
| 2C1c |  | **+** |  |  | **++** | RlucMax×0.9ACTHR |  | [3] |
| Ch3 |  | **+** | EC50ND | EC50ND |  |  |  | [1] |
| 2C1 |  | **+** | RlucMax×ND | **++** | RlucMax×18.2ACTHRRlucMax×23.6MC4R | [3] |
| 2C1a |  | **+** |  |  | **++** | RlucMax×1.0ACTHR |  | [3] |
| 2C1b |  | **+** |  |  | **++** | RlucMax×0.8ACTHR |  | [3] |
| Ch4 |  | **-** | EC50ND | EC50ND |  |  |  | [1] |
| Ch5 |  | **++** | EC50ND | EC50ND |  |  |  | [1] |
| 2C6 |  | **-** | RlucMax×18.2MC4R | **-** | RlucMax×1.8ACTHRRlucMax×2.4MC4R | [3] |
| Ch6 |  | **++** | EC50NDKi×11.1MC4R | EC50NDKi×0.2MC4R |  |  |  | [1] |
| 4C4 |  | **-** | RlucMax×12.6MC4R | **-** | RlucMax×2.2ACTHRRlucMax×2.8MC4R | [3] |
| Ch7 |  | **+** | EC50×62.3MC4RKi×1.8MC4R | EC50×37.9MC4RKi×0.7MC4R |  |  |  | [1] |
| Ch8 |  | **+** | EC50ND | EC50ND |  |  |  | [1] |
| hMC4R/TM2 hMC2R |  |  | EC50×0.04ACTHR+MRAPKi×0.3ACTHR+MRAPEC50×1.9MC4RKi×2.2MC4R |  | **+** |  | EC50×1.4MC4RKi×2.0MC4R | [4; 5] |
| Ch9 |  | **+** | EC50ND | EC50ND |  |  |  | [1] |
| hMC4R/TM3 hMC2R |  |  | EC50×0.84ACTHR+MRAPKi×1.2ACTHR+MRAPEC50×35.6MC4RKi×9.6MC4R |  | **+** |  | EC50×40.0MC4RKi×12.7MC4R | [4; 5] |
| hMC4R/TM4 hMC2R |  |  | EC50×0.04ACTHR+MRAPKi×0.2ACTHR+MRAPEC50×1.7MC4RKi×1.6MC4R |  | **+** |  | EC50×2.1MC4RKi×1.9MC4R | [4; 5] |
| hMC4R/TM5 hMC2R |  |  | EC50×0.05ACTHR+MRAPKi×0.2ACTHR+MRAPEC50×2.0MC4RKi×1.3MC4R |  | **+** |  | EC50×1.8MC4RKi×1.4MC4R | [4; 5] |
| Ch10 |  | **++** | EC50×124.3MC4RKi×12.7MC4R | EC50×137.0MC4RKi×3.1MC4R |  |  |  | [1] |
| hMC4R/TM6 hMC2R |  |  | EC50×0.04ACTHR+MRAPKi×0.2ACTHR+MRAPEC50×1.7MC4RKi×1.4MC4R |  | **+** |  | EC50×1.6MC4RKi×1.7MC4R | [4; 5] |
| 4C2 |  | **++** | RlucMax×ND | **+** | RlucMax×ND | [3] |
| Ch11 |  | **++** | EC50NDKi×2.5MC4R | EC50NDKi×0.5MC4R |  |  |  | [1] |
| 4C3 |  | **++** | RlucMax×1.4MC4R | **+** | RlucMax×0.9ACTHRRlucMax×1.2MC4R | [3] |
| 4C1 |  | **-** | RlucMax×7.8MC4R | **-** | RlucMax×4.8ACTHRRlucMax×6.2MC4R | [3] |
| Ch12 |  | **+** | EC50ND | EC50ND |  |  |  | [1] |
| Ch14 |  | **++** | EC50NDKi×0.5MC4R | EC50NDKi×1.0MC4R |  |  |  | [1] |
| 4C6 |  | **+** | RlucMax×ND | **-** | RlucMax×18.4ACTHRRlucMax×23.9MC4R | [3] |
| Ch13 |  | **-** | EC50ND | EC50ND |  |  |  | [1] |
| Ch15 |  | **+** | EC50×4.0MC4RK×i0.3MC4R | EC50×1.7MC4RKi×0.3MC4R |  |  |  | [1] |
| 2C4 |  | **+** | RlucMax×ND | **+** | RlucMax×ND | [3] |
| 4C5 |  | **-** | RlucMax×25.1MC4R | **+** | RlucMax×ND | [3] |
| 2C2 |  | **-** | RlucMax×4.0MC4R | **+** | RlucMax×2.1ACTHRRlucMax×1.9MC4R | RlucMax×1.9MC4R | [3] |
| RlucMax×1.7ACTHRRlucMax×2.3MC4R |
| Ch16 |  | **+** | EC50ND | EC50ND | **-** | EC50×4.5ACTHREC50×0.3MC4R | EC50ND | [2] |
| Ch19 |  | **++** | EC50ND | EC50ND | **++** | EC50×239.3ACTHREC50×14.9MC4R | EC50ND | [2] |
| 2C3 |  | **+** | RlucMaxND | **++** | RlucMax×1.0ACTHRRlucMax×1.3MC4R | [3] |
| hMC2R/TM4 hMC4R |  | **-** | EC50ND | EC50ND | **-** | EC50ND | EC50ND | [5] |
| Ch20 |  | **++** | EC50ND | EC50ND | **++** | EC50×16.2ACTHREC50×1.0MC4R | EC50ND | [2] |
| Ch21 |  | **++** | EC50ND | EC50ND | **-** | EC50ND | EC50ND | [2] |
| Ch17 |  | **++** | EC50ND | EC50ND | **+** | EC50×5.3ACTHREC50×0.3MC4R | EC50ND | [2] |
| Ch18 |  | **-** | EC50ND | EC50ND | **-** | EC50ND | EC50ND | [2] |
| hMC2R/TM5 hMC4R |  | **-** | EC50ND | EC50ND | **-** | EC50ND | EC50ND | [5] |
| hMC2R/TM6 hMC4R |  | **-** | EC50ND | EC50ND | **-** | EC50ND | EC50ND | [5] |
| Ch22 |  | **-** | EC50ND | EC50ND | **++** | EC50ND | EC50ND | [2] |
| hMC2R/TM3 hMC4R |  | **-** | EC50ND | EC50ND | **-** | EC50ND | EC50ND | [5] |
| 2C2c |  | **-** |  |  | **++** | RlucMax×4.7ACTHRRlucMax×4.4MC4R | RlucMax×43.3MC4R | [3] |
| hMC2R/TM2 hMC4R |  | **-** | EC50ND | EC50ND | **-** | EC50ND | EC50ND | [5] |
| 2C2b |  | **-** |  |  | **-** | RlucMax×7.3ACTHRRlucMax×6.7MC4R | RlucMax×130.0MC4R | [3] |
| 2C5 |  | **-** | RlucMax×6.6MC4R | **-** | RlucMax×3.6ACTHRRlucMax×4.7MC4R | [3] |

× , fold difference; Superscript, compared values; Subscript, wild type receptor to which measurement was compared;
ND, not determined; Regions from the MC4R are shaded grey; Regions from the ACTHR are shaded black; empty field, no data available.
++, high membrane transportation; +, low membrane transportation; , no membrane transportation;
Approximate RlucMax values from Hinkle et al. [3] were acquired through digitalization of the included bar graphs.

[1] D. Fridmanis, R. Petrovska, I. Kalnina, M. Slaidina, R. Peculis, H.B. Schioth, and J. Klovins, Identification of domains responsible for specific membrane transport and ligand specificity of the ACTH receptor (MC2R). Mol Cell Endocrinol 321 (2010) 175-83.

[2] D. Fridmanis, R. Petrovska, D. Pjanova, H.B. Schioth, and J. Klovins, Replacement of short segments within transmembrane domains of MC2R disrupts retention signal. J Mol Endocrinol 53 (2014) 201-15.

[3] P.M. Hinkle, M.N. Serasinghe, A. Jakabowski, J.A. Sebag, K.R. Wilson, and C. Haskell-Luevano, Use of chimeric melanocortin-2 and -4 receptors to identify regions responsible for ligand specificity and dependence on melanocortin 2 receptor accessory protein. Eur J Pharmacol 660 (2011) 94-102.

[4] M. Chen, M. Cai, C.J. Aprahamian, K.E. Georgeson, V. Hruby, C.M. Harmon, and Y. Yang, Contribution of the conserved amino acids of the melanocortin-4 receptor in [corrected] [Nle4,D-Phe7]-alpha-melanocyte-stimulating [corrected] hormone binding and signaling. J Biol Chem 282 (2007) 21712-9.

[5] Y. Yang, V. Mishra, C.J. Crasto, M. Chen, R. Dimmitt, and C.M. Harmon, Third transmembrane domain of the adrenocorticotropic receptor is critical for ligand selectivity and potency. J Biol Chem 290 (2015) 7685-92.