Table S2. ACTH $_{(1\text{-}39)}$-induced cAMP responses at MC2R, MRAP1 or MRAP2 mutants. cAMP accumulation was normalized to the maximum response of wild-type (WT). Dose–response curves were analyzed using a three-parameter logistic equation to obtain pEC_{50} values. Cell surface expression was assessed by FACS. Values were normalized to the wild-type construct (WT, shown as percentage). All the mutant constructs were modified by single-point mutation in the setting of the WT construct. The experiments were carried out independently at least three times. Data shown are means ± S.E.M. One-way ANOVA were used to determine statistical difference. N.D., values that could not be determined due to incomplete curve fits. *P< 0.05, **P< 0.01, ***P< 0.001.

Mutants	pEC_{50} ± S.E.M.	E_{max} ± S.E.M. (% WT)	Cell surface expression (% MC2R (WT) + MRAPs (WT))
MC2R (WT) + MRAP (WT)	7.46 ± 0.03	100.00 ± 0.76	100.00
MRAP1 (WT) + MC2R (mutants)			
S19A	6.37 ± 0.05***	100.10 ± 2.14	134.36 ± 14.69
D20A	7.40 ± 0.07	98.90 ± 1.87	105.55 ± 4.57
R23A	7.37 ± 0.08	100.51 ± 2.40	110.88 ± 23.88
R23E	8.17 ± 0.07**	97.46 ± 1.59	119.74 ± 7.87
E28A	6.57 ± 0.10**	102.87 ± 3.37	94.38 ± 11.26
F31A	6.37 ± 0.09***	100.31 ± 3.32	111.63 ± 15.48
Y76A	6.62 ± 0.08**	99.80 ± 2.87	120.93 ± 12.78
K77A	5.69 ± 0.06***	97.58 ± 2.41	128.40 ± 19.40
E80A	N.D.	N.D.	89.97 ± 13.87
N81A	6.11 ± 0.06***	99.42 ± 2.44	97.85 ± 10.03
I84A	5.21 ± 0.19***	48.17 ± 4.43	112.18 ± 15.16
R87A	7.57 ± 0.07	99.97 ± 1.99	146.72 ± 9.23
E99A	6.83 ± 0.10	92.01 ± 3.12	94.41 ± 31.09
T100A	7.42 ± 0.06	98.29 ± 1.70	120.05 ± 4.39
D103A	5.45 ± 0.11***	89.29 ± 4.66	158.94 ± 15.51
D104A	5.53 ± 0.08***	100.36 ± 3.67	92.75 ± 7.24
D107A	N.D.	N.D.	113.91 ± 14.75
F110A	4.56 ± 0.16***	45.46 ± 4.53	140.89 ± 20.20
F110I	N.D.	N.D.	115.08 ± 15.25
F110A/G262L	5.44 ± 0.08***	78.89 ± 2.71	117.59 ± 3.00
F110I/G262L	N.D.	N.D.	98.60 ± 13.18
V111A	6.45 ± 0.06***	103.97 ± 2.28	125.23 ± 6.44
M165A	6.39 ± 0.06***	98.33 ± 2.16	119.58 ± 11.94
V166A	6.61 ± 0.08**	102.55 ± 2.68	97.12 ± 7.30
S169A	5.66 ± 0.07***	104.62 ± 3.17	91.19 ± 4.55
H170A	5.53 ± 0.09***	101.69 ± 4.07	133.08 ± 11.42
F178A	5.13 ± 0.07***	93.39 ± 3.50	131.71 ± 20.49

F197A	5.70 ± 0.07***	96.04 ± 2.98	133.85 ± 1.40
R201A	7.32 ± 0.06	97.95 ± 1.65	116.43 ± 17.80
R205A	7.20 ± 0.08	100.02 ± 2.62	105.42 ± 22.80
N214A	6.52 ± 0.07	98.33 ± 2.28	114.20 ± 15.39
M215A	7.51 ± 0.07	103.64 ± 2.17	134.72 ± 20.33
L221A	7.16 ± 0.05	103.64 ± 2.17	122.50 ± 22.30
F230A	7.49 ± 0.07	102.04 ± 2.12	123.88 ± 8.56
P234A	6.52 ± 0.07*	97.87 ± 2.47	158.06 ± 17.38
F235A	4.55 ± 0.17***	75.50 ± 8.07	143.69 ± 9.20
L237A	6.93 ± 0.06	101.81 ± 1.94	95.10 ± 16.08
H238A	6.44 ± 0.06***	99.06 ± 2.18	152.51 ± 12.19
V239A	6.52 ± 0.06**	101.89 ± 2.29	140.15 ± 7.48
L241A	6.91 ± 0.06***	102.34 ± 2.23	102.42 ± 9.32
M242A	6.41 ± 0.07***	101.81 ± 2.62	161.76 ± 13.09
Y250A	5.65 ± 0.09***	92.25 ± 3.73	101.53 ± 9.93
C251A	6.35 ± 0.08***	100.65 ± 2.76	112.06 ± 9.91
Y254A	6.25 ± 0.08***	98.01 ± 2.94	111.67 ± 8.74
M255A	6.38 ± 0.06***	98.03 ± 2.13	129.79 ± 10.91
L257A	6.72 ± 0.07	101.88 ± 2.34	103.24 ± 1.57
F258A	5.01 ± 0.06***	84.02 ± 2.91	148.80 ± 6.40
G262L	N.D.	N.D.	137.01 ± 9.34
L264A	6.49 ± 0.09**	97.71 ± 3.33	132.37 ± 18.94
M266A	5.98 ± 0.06***	107.77 ± 2.47	131.17 ± 17.36
F274A	6.79 ± 0.09	88.48 ± 3.04	113.13 ± 4.46
D285A	7.61 ±0.04	99.25 ± 1.14	127.04 ± 17.48
MC2R (WT) + MRAP1 (mutants)			
E15A	6.46 ± 0.07***	102.91 ± 2.50	46.61 ± 8.39
Y16A	6.28 ± 0.08***	102.98 ± 3.47	54.85 ± 9.24
Y17A	6.40 ± 0.08***	101.73 ± 2.62	53.72 ± 5.70
L18A	6.60 ± 0.06**	101.83 ± 2.24	68.09 ± 8.30
Y20A	N.D.	N.D.	70.46 ± 9.45
L21A	6.43 ± 0.07***	102.12 ± 2.78	65.89 ± 19.25
L23A	7.34 ± 0.07***	99.52 ± 1.99	12.80 ± 4.23
I24A	7.46 ± 0.08	100.03 ± 2.14	29.56 ± 6.93
K35A	6.94 ± 0.04	100.03 ± 1.50	131.59 ± 4.87
H36A	6.95 ± 0,05	99.94 ± 1.61	126.26 ± 7.86
I38A	6.97 ± 0,07	98.29 ± 2.33	66.60 ± 7.13
W43A	7.22 ± 0.07	100.03 ± 2.10	57.40 ± 2.97
F54A	6.81 ± 0.06	100.75 ± 2.16	146.37 ± 25.33
L57A	5.64 ± 0.05***	95.67 ± 2.20	129.23 ± 6.73
L58A	7.07 ± 0.08	101.02 ± 2.69	116.35 ± 6.28
S61A	6.83 ± 0.08	99.99 ± 2.71	172.00 ± 13.24

MRAP1 (14-172)	6.25 ± 0.06***	100.41 ± 2.61	16.32 ± 1.40
MRAP1 (31-172)	N.D.	N.D.	14.52 ± 1.50
MRAP1 (1-62)	7.28 ± 0.05	99.16 ± 1.46	6.89 ± 0.88
MC2R (WT) + MRAP2 (mutants)			
MRAP2 (WT)	N.D.	N.D.	42.69 ± 9.04
MRAP2-LDYL	6.00 ± 0.13***	76.95 ± 3.72	64.73 ± 30.59