Supporting Information

Chan et al. 10.1073/pnas.0809918106

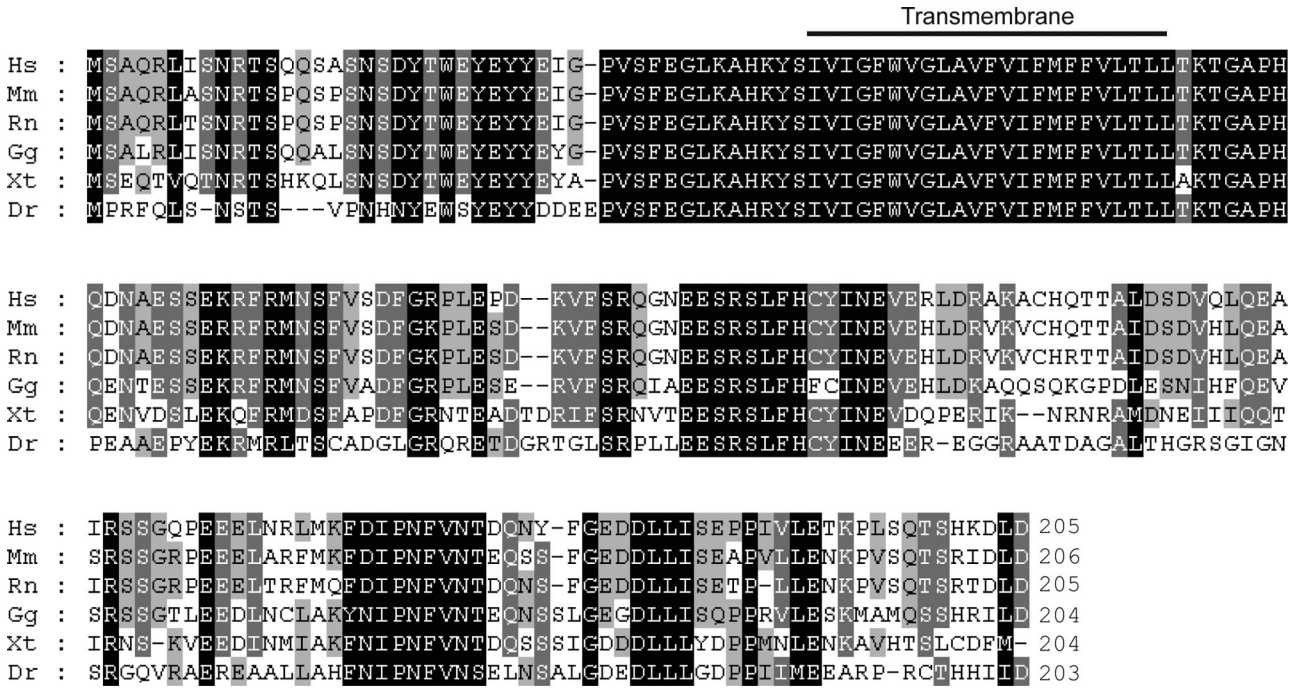


Fig. S1. Protein alignment of MRAP2 orthologues, showing conservation of MRAP2 across vertebrates. Hs, *homo sapiens*; Mm, *Mus musculus*; Rn, *Rattus norvegicus*; Gg, *Gallus gallus*; Xt, *Xenopus tropicalis*; Dr, *Danio rerio*.

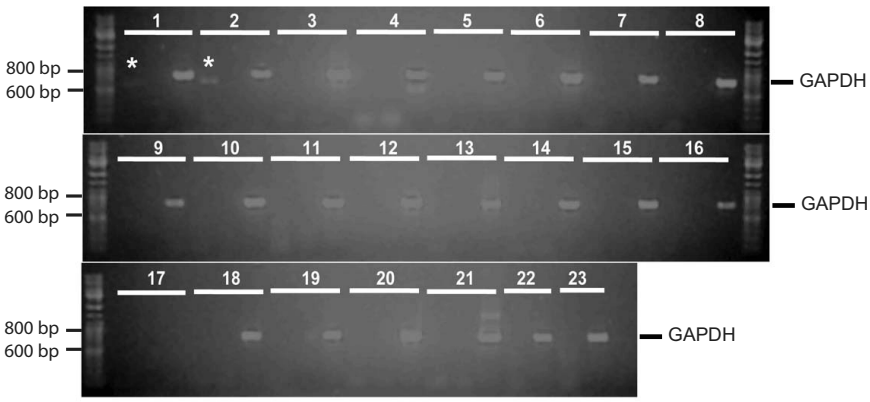
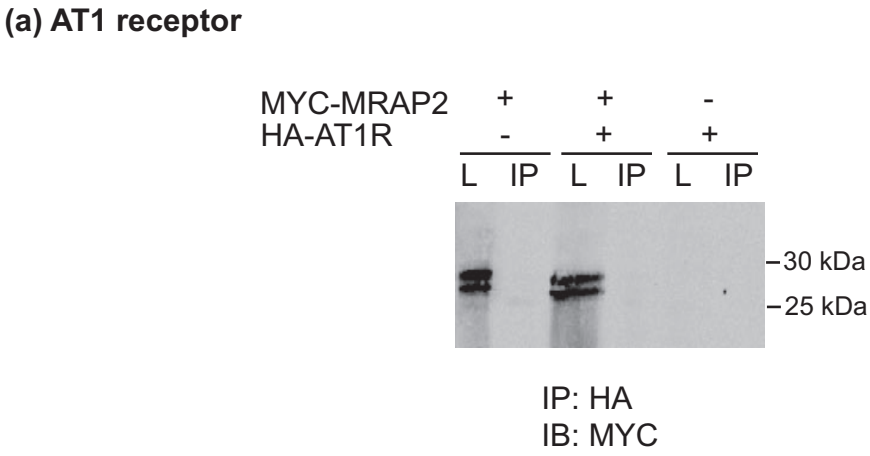
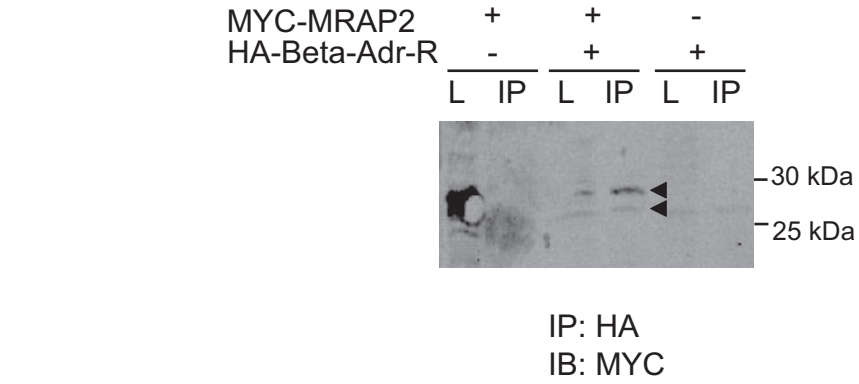


Fig. S2. MRAP2 mRNA expression in human tissues was determined by RT-PCR analysis. Full-length MRAP2 mRNA (618 bp, indicated by asterisk) was only detected in human adrenal and brain. PCR products were sequenced to confirm MRAP2 transcript.





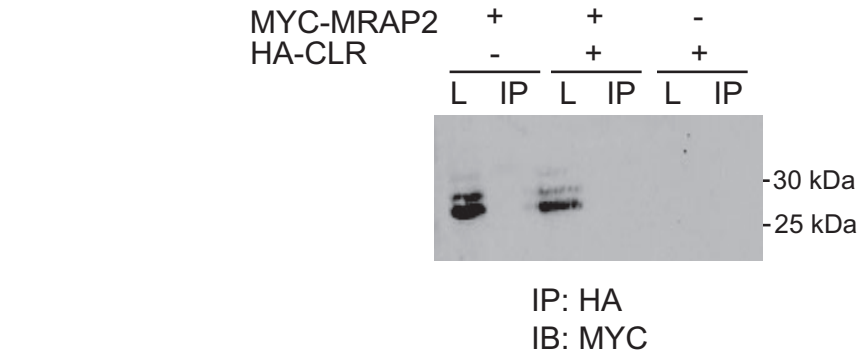


Fig. S3. Specificity of the interaction between MRAP2 and other G protein-coupled receptors. The HA-tagged AT1R, the BAR, or the CLR were cotransfected into CHO cells with the MYC-tagged MRAP2 as described for the MCRs, followed by Co-IP as shown. MRAP2 coprecipitated with the BAR (arrowheads) but not with the other receptors. IB, immunoblotting.

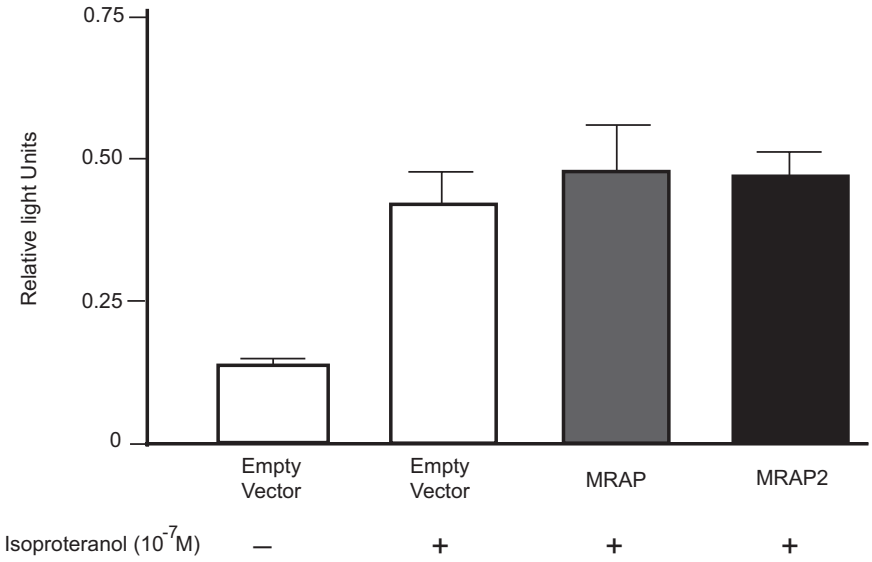


Fig. S4. Influence of MRAP or MRAP2 on cAMP generation by the BAR in response to isoproterenol (10−7M). CHO cells were transfected with the BAR expression vector and either empty vector, MRAP, or MRAP2 expression vectors. cAMP signal transduction was measured using a cAMP luciferase reporter construct as described elsewhere (1, 2). 1. Cooray SN, et al. (2008) The melanocortin 2 receptor accessory protein exists as a homodimer and is essential for the function of the melanocortin 2 receptor in the mouse y1 cell line. *Endocrinology* 149:1935–1941. 2. Webb TR, et al. (2009) Distinct melanocortin 2 receptor accessory protein domains are required for melanocortin 2 receptor interaction and promotion of receptor trafficking. *Endocrinology* 150:720–726.

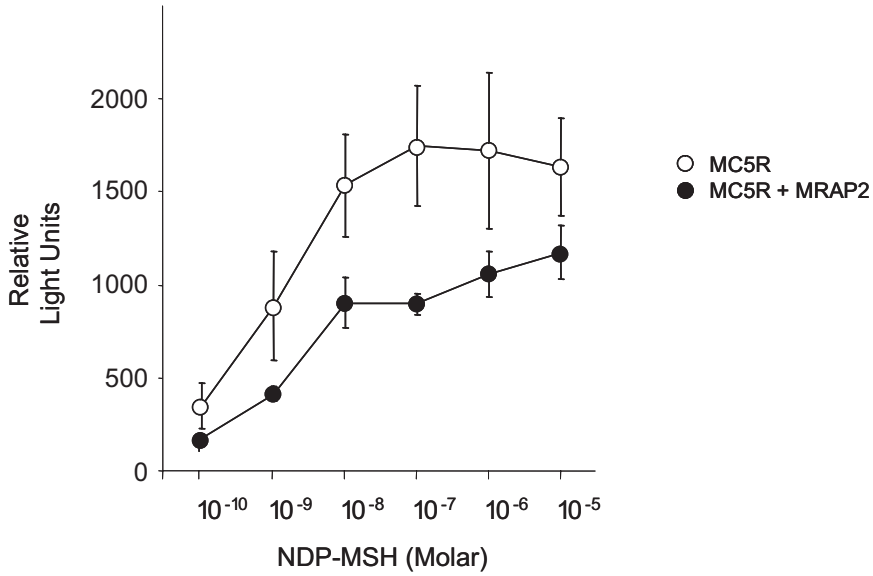


Fig. S5. MC5R dose-response curve in the presence or absence of MRAP2. CHO cells were transfected with the MC5R with or without the MRAP2 expression vector. After 24 h cells were stimulated with various concentrations of NDP-MSH. cAMP signal generation was measured using a cAMP luciferase reporter assay as described previously (1, 2). Results are the mean ± SD of 2 independent experiments. 1. Cooray SN, et al. (2008) The melanocortin 2 receptor accessory protein exists as a homodimer and is essential for the function of the melanocortin 2 receptor in the mouse y1 cell line. *Endocrinology* 149:1935–1941. 2. Webb TR, et al. (2009) Distinct melanocortin 2 receptor accessory protein domains are required for melanocortin 2 receptor interaction and promotion of receptor trafficking. *Endocrinology* 150:720–726.

Table S1. Primers used in study

Primer	Forward	Reverse
MRAP2	5′ ATGTCCGCCCAGAGGTTA 3′	5′ TCAATCCAGGTCTTTGTG 3′
MRAP2-FLAG	5′atcggaattcatgtccgcccagaggtta 3′	5′ aatctcgagtcacttgtcatcgtcgtccttgtagtcatccaggtctttgtgtga 3′
MYC-MRAP2	5′ atcggaattcatggagcagaaactcatctctgaagaggatctgatgtccgcccagaggtta 3′	5′ aatctcgagtcaatccaggtctttgtgtga 3′
N9Q-FLAG	5′ ctcacacaaagacctggattagtacaaggacgacgatgac 3′	3′ gagtgtgtttctggacctaatcatgttcctgctgctactg 5′
MRAP-FLAG	5′ ACGCTGAAAGCTTAGTG CCACAGACATG 3′	5′ ACCAGTTGAA TTCGCTATGGCCACGAT 3′