

## SUPPLEMENTAL FIGURE LEGENDS

SUPPLEMENTAL FIGURE 1. **MRAP and RAMP constructs.** Shown are the sequences of MRAP and RAMP constructs used in the experiments described here.

SUPPLEMENTAL FIGURE 2. **Specificity of bimolecular fluorescence complementation.** CHO cells were transfected with V5-MRAP-YFP-F2 and either YFP-F1-V5-RAMP3 or YFP-F1-V5-MRAP, or with YFP-F1-V5-MRAP and V5-RAMP3-YFP-F2. Live cells were stained for surface V5 epitope and YFP fluorescence was imaged. Panel A shows identically processed fluorescent images. B. YFP fluorescence was quantified in all cells staining for V5 in randomly selected fields of blinded slides using Metamorph software from Universal Imaging. The bargraph presents mean and SE for the number of cells shown. \*= P<0.001.

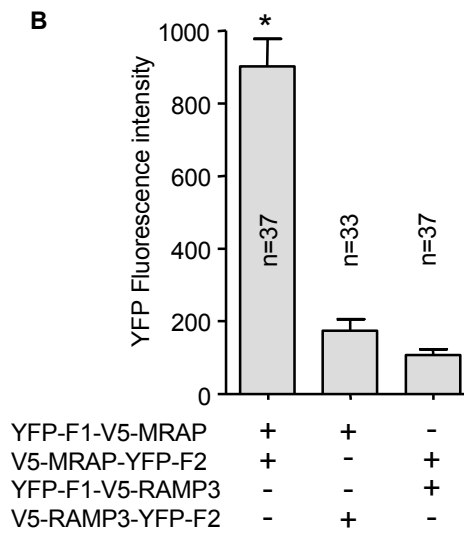
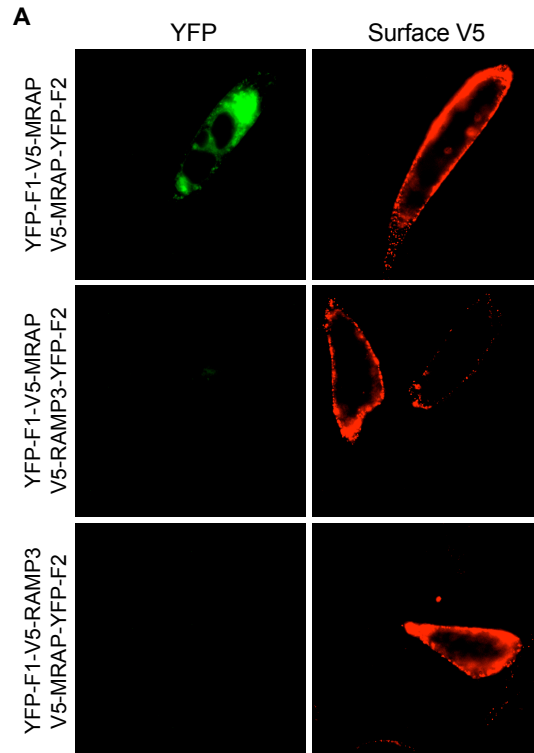
SUPPLEMENTAL FIGURE 3. **Total MC2 receptor expression with MRAP mutants.** CHO cells transfected with HA-MC2 receptor and RAMPs, MRAP or MRAP mutants were treated with vehicle or 20  $\mu$ M MG132 for 6 hours, fixed and permeabilized. Total HA-MC2 receptor expression was measured using anti-HA antibody and is normalized to the value with MRAP.

SUPPLEMENTAL FIGURE 4. **MC2 receptor agonist specificity.** CHO cells were either mock-transfected or transfected with HA-MC2 receptor and MRAP or MRAP(18-21A). Cells were incubated for 20 min with 0.1 mM isobutylmethylxanthine and 100 nM ACTH(1-24), ACTH(1-18) or NDP- $\alpha$ -MSH before measurement of cAMP.

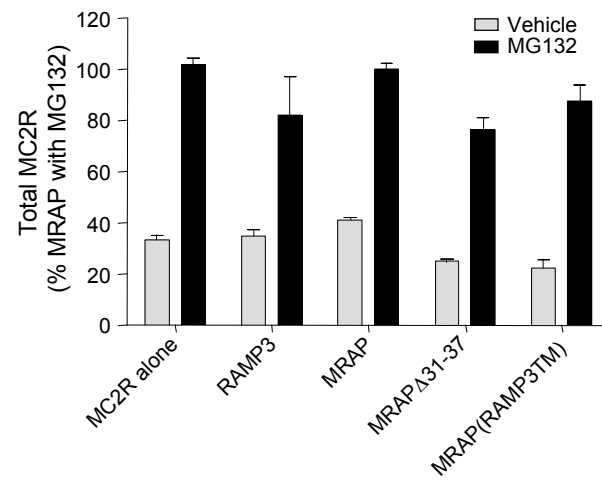
Supplemental Figure 1

NAME	SEQUENCE	MRAP DELETION MUTANTS
	1            10            20            30            40            50            60            127	
MRAP	MANGTDASVPLTSY EYYLDYIDLIPVDEK K LKANKHSIVIALWLSLATFV VLLFLILLYMSWSGS...PLALPGKPIPNPLLGLDST	
MRAPΔ4-8	MAN-----VPLTSY EYYLDYIDLIPVDEK K LKANKHSIVIALWLSLATFV VLLFLILLYMSWSGS...PLALPDYKDDDDK	
MRAPΔ10-20	MANGTDASV-----IDLIPVDEK K LKANKHSIVIALWLSLATFV VLLFLILLYMSWSGS...PLALPGKPIPNPLLGLDST	
MRAPΔ21-30	MANGTDASVPLTSY EYYLDY-----LKANKHSIVIALWLSLATFV VLLFLILLYMSWSGS...PLALPDYKDDDDK	
MRAPΔ31-37	MGKPIPNPLLGLDSTANGTDASVPLTSY EYYLDYIDLIPVDEK K-----IVIALWLSLATFV VLLFLILLYMSWSGS...PLALPTRDYKDHGDYKDHDIDYKDDDDK	
MRAPΔ1-30	MGKPIPNPLLGLDST-----LKANKHSIVIALWLSLATFV VLLFLILLYMSWSGS...PLALPTRDYKDHGDYKDHDIDYKDDDDK	
		MRAP SUBSTITUTION MUTANTS
	1            10            20            30            40            50            60            127	
MRAP	MANGTDASVPLTSY EYYLDYIDLIPVDEK K LKANKHSIVIALWLSLATFV VLLFLILLYMSWSGS...PLALPGKPIPNPLLGLDST	
MRAP 10-13A	MANGTDASVAAAAEYYLDYIDLIPVDEK K LKANKHSIVIALWLSLATFV VLLFLILLYMSWSGS...PLALPGKPIPNPLLGLDST	
MRAP 13-17A	MANGTDASVPLTSAAAALDYIDLIPVDEK K LKANKHSIVIALWLSLATFV VLLFLILLYMSWSGS...PLALPGKPIPNPLLGLDST	
MRAP 18-21A	MANGTDASVPLTSY EYAAAADLIPVDEK K LKANKHSIVIALWLSLATFV VLLFLILLYMSWSGS...PLALPGKPIPNPLLGLDST	
MRAP L18A	MANGTDASVPLTSY EYYADYIDLIPVDEK K LKANKHSIVIALWLSLATFV VLLFLILLYMSWSGS...PLALPGKPIPNPLLGLDST	
MRAP D19A	MANGTDASVPLTSY EYYLAYIDLIPVDEK K LKANKHSIVIALWLSLATFV VLLFLILLYMSWSGS...PLALPGKPIPNPLLGLDST	
MRAP Y20A	MANGTDASVPLTSY EYYLDAIDLIPVDEK K LKANKHSIVIALWLSLATFV VLLFLILLYMSWSGS...PLALPGKPIPNPLLGLDST	
MRAP I21A	MANGTDASVPLTSY EYYLDYADLIPVDEK K LKANKHSIVIALWLSLATFV VLLFLILLYMSWSGS...PLALPGKPIPNPLLGLDST	
		RAMP3/MRAP CHIMERAS CONSTRUCTS
RAMP3	METGALRRPQLLPLLLLLCGGCPRAGGCNETGML...LEDPPDEVLIPLIVIPVLTVMAGLVVWRSKRTDTLL	
RAMP3 (no SP)	MGKPIPNPLLGLDSTCNETGML...LEDPPDEVLIPLIVIPVLTVMAGLVVWRSKRTDTLLTRDYKDHGDYKDHDIDYKDDDDK	
RAMP3 (MRAP 29-37)	MGKPIPNPLLGLDSTCNETGML...LEDPPDEK LKANKHSIVLIPLIVIPVLTVMAGLVVWRSKRTDTLLTRDYKDHGDYKDHDIDYKDDDDK	
MRAP (RAMP3-TM)	MGKPIPNPLLGLDSTANGTDASVPL...IDLIPVDEK K LKANKHVLIVIPVLTVMAGLVVWRMSWS...ALPTRDYKDHGDYKDHDIDYKDDDDK	
		BIMOLECULAR FLUORESCENCE COMPLEMENTATION CONSTRUCTS
YFP-F1	MVSKGEELFTGVVPIVVELDGDVNGHKFSVSGEGDATYKGLTLKFICTTGKLPVWPVTLVTTFGYGLMCFARYPDHMKQHDFFK SAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLVNRIELKGIKDFKEDGNILGHKLEYNYNSHNVYIMADKQRS	
YFP-F2	KNGIKVNFKIRHNIEDGSVQLADHYQQNTPIGDGPVLLPDNHYLSYQSALS KDPNEKRDMVLLFVTAAGITLGMDELYK	
YFP-F1-V5-MRAP-3F	(YFP-F1) ATMGKPIPNPLLGLDSTANGTDASVPLTSY...PLALPTRDYKDHGDYKDHDIDYKDDDDK	
V5-MRAP-YFP-F2	MGKPIPNPLLGLDSTANGTDASVPLTSY...PLALPTR (YFP-F2)	
V5-MRAP-YFP-F1	MGKPIPNPLLGLDSTANGTDASVPLTSY...PLALPTR (YFP-F1)	
YFP-F1-V5-MRAPΔ31-37-3F	(YFP-F1) ATMGKPIPNPLLGLDSTANGTD...IPVDEK K-----IVIALWLSLATFV VLLFLILLYM...LALPTRDYKDHGDYKDHDIDYKDDDDK	
V5-MRAPΔ31-37-YFP-F2	ATMGKPIPNPLLGLDSTANGTD...IPVDEK K-----IVIALWLSLATFV VLLFLILLYM...PLALPTR (YFP-F2)	
YFP-F1-V5-RAMP3-3F	(YFP-F1) ATMGKPIPNPLLGLDSTCNETGML...RSKRTDTLLTRDYKDHGDYKDHDIDYKDDDDK	
V5-RAMP3-YFP-F2	MGKPIPNPLLGLDSTCNETGML...RSKRTDTLLTR (YFP-F2)	
		V5 epitope (red) / Flag epitope (blue) / Signal peptide (green)

Supplemental Figure 2



Supplemental Figure 3



Supplemental figure 4

