

# Supplementary Table S3

## Cell Surface ELIZA of Single Alanine Mutants of hMC2R

			One-Way ANOVA
<u>TM4 (A)</u>	<u>Average</u>	<u>S.E.M (n=3)</u>	<u>p Value</u>
Negative	0.162	$\pm$ 0.001	< 0.0001
Positive	0.792	$\pm$ 0.091	-
G162	1.420	$\pm$ 0.081	< 0.0001
<b>I163</b>	<b>0.380</b>	<b><math>\pm</math> 0.037</b>	<b>0.0016</b>
T164	0.705	$\pm$ 0.011	0.666
<u>TM4 (B)</u>	<u>Average</u>	<u>S.E.M (n=3)</u>	<u>p Value</u>
Negative	0.236	$\pm$ 0.016	< 0.0001
Positive	0.598	$\pm$ 0.091	-
<b>M165</b>	<b>0.198</b>	<b><math>\pm</math> 0.016</b>	<b>&lt; 0.0001</b>
<b>V166</b>	<b>0.354</b>	<b><math>\pm</math> 0.018</b>	<b>&lt; 0.0001</b>
I167	0.530	$\pm$ 0.035	0.1275
<u>TM5 (C)</u>	<u>Average</u>	<u>S.E.M (n=3)</u>	<u>p Value</u>
Negative	0.125	$\pm$ 0.033	0.004
Positive	0.423	$\pm$ 0.027	-
T177	0.721	$\pm$ 0.043	0.0004
<b>F178</b>	<b>0.212</b>	<b><math>\pm</math> 0.032</b>	<b>0.0053</b>
T179	0.419	$\pm$ 0.037	0.9954
<u>TM5 (D)</u>	<u>Average</u>	<u>S.E.M (n=3)</u>	<u>p Value</u>
Negative	0.182	$\pm$ 0.031	0.0447
Positive	0.496	$\pm$ 0.061	-
S180	1.496	$\pm$ 0.076	< 0.0001
L181	0.518	$\pm$ 0.126	0.9997
F182	0.461	$\pm$ 0.861	0.9983
P183	0.861	$\pm$ 0.100	0.443
<u>EC2 (E)</u>	<u>Average</u>	<u>S.E.M (n=3)</u>	<u>p Value</u>
Negative	0.219	$\pm$ 0.003	< 0.0001
Positive	1.364	$\pm$ 0.035	-
<b>F168</b>	<b>0.413</b>	<b><math>\pm</math> 0.272</b>	<b>0.0001</b>
H170	1.171	$\pm$ 0.157	0.3123

Negative Control - hMC2R expressed alone in CHO cells

Positive Control - hMC2R co-expressed with mMrp1 in CHO cells

All mutants were co-expressed with mMrp1 Mutants in highlighted in **red** resulted in a statistically significant drop in trafficking