

name	sequence	hydro. (TM tend.)	charge $\Delta(C-N)$
$\beta_1$ AR	<u>QVSA<b>E</b>LLSQQ<b>W</b><b>E</b>AGMSLLMALVLLIVAGNVLVIAAIGSTQ<b>R</b>LQTLT</u>	26.3	+3
1A	<u>QVSA<b>E</b>LLSQQ<b>W</b><b>E</b>AGMSLLMA<b>A</b>VLLIVAGNVLVIAAIGSTQ<b>R</b>LQTLT</u>	24.9	+3
2A	<u>QVSA<b>E</b>LLSQQ<b>W</b><b>E</b>AGMSLLMA<b>A</b>V<b>V</b>ALIVAGNVLVIAAIGSTQ<b>R</b>LQTLT</u>	23.4	+3
1L	<u>QVSA<b>E</b>LLSQQ<b>W</b><b>E</b>AGMSLLMALVLLIV<b>L</b>GNVLVIAAIGSTQ<b>R</b>LQTLT</u>	27.7	+3
2L	<u>QVSA<b>E</b>LLSQQ<b>W</b><b>E</b>AGMSLLMALVLLIV<b>LL</b>NVLVIAAIGSTQ<b>R</b>LQTLT</u>	29.8	+3
3L	<u>QVSA<b>E</b>LLSQQ<b>W</b><b>E</b>AGMSLLMALVLLIV<b>LLL</b>VLVIAAIGSTQ<b>R</b>LQTLT</u>	33.2	+3
0	<u>QVSA<b>Q</b>LLSQQ<b>W</b><b>Q</b>AGMSLLMALVLLIVAGNVLVIAAIGSTQ<b>Q</b>LQTLT</u>	26.3	0
+1	<u>QVSA<b>Q</b>LLSQQ<b>W</b><b>Q</b>AGMSLLMALVLLIVAGNVLVIAAIGSTQ<b>R</b>LQTLT</u>	26.3	+1
+2	<u>QVSA<b>E</b>LLSQQ<b>W</b><b>Q</b>AGMSLLMALVLLIVAGNVLVIAAIGSTQ<b>R</b>LQTLT</u>	26.3	+2
+4	<u>QVSA<b>E</b>LLSQQ<b>W</b><b>E</b>AGMSLLMALVLLIVAGNVLVIAAIG<b>R</b>TQ<b>R</b>LQTLT</u>	26.3	+4
+5	<u>QVSA<b>E</b>LLSQQ<b>W</b><b>E</b>AGMSLLMALVLLIVAGNVLVIAAIG<b>R</b>TQ<b>R</b>L<b>K</b>TLT</u>	26.3	+5
+6	<u>QVSA<b>E</b>LL<b>S</b>E<b>Q</b>W<b>E</b>AGMSLLMALVLLIVAGNVLVIAAIG<b>R</b>TQ<b>R</b>L<b>K</b>TLT</u>	26.3	+6
$\Delta 1$	<u>QVSA<b>E</b>LLSQQ<b>W</b><b>E</b>AGMSLLMALVLLIVAG-VLVIAAIGSTQ<b>R</b>LQTLT</u>	27.9	+3
$\Delta 2$	<u>QVSA<b>E</b>LLSQQ<b>W</b><b>E</b>AGMSLLMALVLLIVA--VLVIAAIGSTQ<b>R</b>LQTLT</u>	28.1	+3
$\Delta 3$	<u>QVSA<b>E</b>LLSQQ<b>W</b><b>E</b>AGMSLLMALVLLIV---VLVIAAIGSTQ<b>R</b>LQTLT</u>	27.7	+3
LepB	<u>ANMFALILVIATLVTGILWS<b>V</b>D<b>K</b>FFFAP<b>KRRER</b>QAAAQ</u>	21.9	+3
LepB-Ext	<u>ANMFAL<b>S</b>ILVGIATLVTGIL<b>T</b>WS<b>V</b>D<b>K</b>FFFAP<b>KRRER</b>QAAAQ</u>	20.8	+3

**Table S2 - Sequences of TMD mutants analyzed in this study (related to Fig. 4).** The  $\beta_1$ AR-TMD1 and LepB constructs were mutated as indicated (green residues indicate changes). The calculated TM tendency score and charge difference are indicated for each TMD region. The TMD is underlined. Note that the assignment of the TMD for  $\beta_1$ AR is different from that indicated in Uniprot (Table S1) and is based on the known structure of  $\beta_1$ AR. Although not shown here, we have verified that the effect of 3L and  $\Delta 3$  are due to the increase in hydrophobicity and decrease in TMD length, respectively, and not to the specific residues that are mutated. This was done by mutating or deleting three other residues in the TMD to achieve the same approximate hydrophobicity and length.