**Supplementary Table S1, *Primers used for QuikChange mutagenesis***

| Construct | Primer sequence |
| --- | --- |
| 7/1F | 5'-CCCTGAGTGATTCTCTGCCTTTAAACGCCAACGTGCTCCTGTGGTACCTGG-3' |
| 7/1B | 5'-CCAGGTACCACAGGAGCACGTTGGCGTTTAAAGGCAGAGAATCACTCAGGG-3' |
| 7/2F | 5'-CCAACGTGCTCCTGCGAAGCTTGGTGAAGGTCCTATGTCTCTTGGGG-3' |
| 7/2B | 5'-CCCCAAGAGACATAGGACCTTCACCAAGCTTCGCAGGAGCACGTTGG-3' |
| TM7F | 5'-GCTTGGTGAAGGTCGTAGGCCTGTATGGCTTCATGCTCTGGGG-3' |
| TM7B | 5'-CCCCAGAGCATGAAGCCATACAGGCCTACGACCTTCACCAAGC-3' |
| 8/1F | 5'-CAGTGTCCCTCACCCTCCTTAGCCTGCTCCATATGCCTCTGCTTTTCC-3' |
| 8/1B | 5'-GGAAAAGCAGAGGCATATGGAGCAGGCTAAGGAGGGTGAGGGACACTG-3' |
| 8/2F | 5'-GCTCCATATGCCTTTCACAATTGCAGCGGAGAAGAAGGTGGGAAAATGG-3' |
| 8/2B | 5'-CCATTTTCCCACCTTCTTCTCCGCTGCAATTGTGAAAGGCATATGGAGC-3' |
| TM8F | 5'-GCAGCGGAGAAGGTTTATAACACCCGCCACCAGTTGCTGG-3' |
| TM8B | 5'-CCAGCAACTGGTGGCGGGTGTTATAAACCTTCTCCGCTGC-3' |
| 9/1F | 5'-GTGGCCTATGCACTCTACCTGCTCGTGAGGCGTATTTCAGGTATGCTGC-3' |
| 9/1B | 5'-GCAGCATACCTGAAATACGCCTCACGAGCAGGTAGAGTGCATAGGCCAC-3' |
| 9/2F | 5'-GCTCGTGAGGCGTGTGCTGCACTTGGGTGTACAGATGGGAATCCTCTAC-3' |
| 9/2B | 5'-GTAGAGGATTCCCATCTGTACACCCAAGTGCAGCACACGCCTCACGAGC-3' |
| TM9F | 5'-GGGTGTACAGATGCTGATGCTGAGCTGTGGGCTCCAGTTGGTGACCAGTGG-3' |
| TM9B | 5'-CCACTGGTCACCAACTGGAGCCCACAGCTCAGCATCAGCATCTGTACACCC-3' |
| 10/1F | 5'-GCAGTGGGAACCTTCTAAGCTTCATGATCTACCAGATGCAG-3' |
| 10/1B | 5'-CTGCATCTGGTAGATCATGAAGCTTAGAAGGTTCCCACTGC-3' |
| 10/2F | 5'-CATGATCTACCAGGAGAGCGTGGGGAGCTACGTAGAGGTACTGCTCTCC-3' |
| 10/2B | 5'-GGAGAGCAGTACCTCTACGTAGCTCCCCACGCTCTCCTGGTAGATCATG-3' |
| TM10F | 5'-GGGGAGCTACGTGCAGACCCTGGTATACATCTACCCCAG-3' |
| TM10B | 5'-CTGGGGTAGATGTATACCAGGGTCTGCACGTAGCTCCCC-3' |
| R$_1$QF | 5'-CAACTCCTGGACCAGAAGTATTTCCGGAATGCTGCTGCAAGTGGGAATCC-3' |
| R$_1$QB | 5'-GGATTCCCACTTGCAGCAGCATTCCGGAAATACTTCTGGTCCAGGAGTTG-3' |
| R$_2$QF | 5'-CCTGGACCACTAGAATTTCCGGAATGCTGCTGCAAGTGGGAATCC-3' |
| R$_2$QB | 5'-GGATTCCCACTTGCAGCAGCATTCCGGAAATTCTAGTGGTCCAGG-3' |
| RHQF | 5'-CCAGAAGTATTTCCCACATGCTGCTGCAAGTGG-3' |
| RHQB | 5'-CCACTTGCAGCAGCATGTGGGAAATACTTCTGG-3' |
| LYLLVRQF | 5'-GCTGTGGCCTATGCATTGTACCTGCTCGTCAGAAGTATTTCC-3' |
| LYLLVRQB | 5'-GGAAATACTTCTGACGAGCAGGTACAATGCATAGGCCACAGC-3' |
| YRHQF | 5'-GTGGCCTATGCAGTATACTCCTGGACCAG-3' |
| YRHQB | 5'-CTGGTCCAGGAGTATACTGCATAGGCCAC-3' |
| YLRHQ$_1$F | 5'-GTGGCCTATGCAGTATACTCCTTGACCAGAAGTATTTCC-3' |
| YLRHQ$_1$B | 5'-GGAAATACTTCTGGTCAAGGAGTATACTGCATAGGCCAC-3' |
| LRHQF | 5'-CCTATGCAGTCAACTCGTTAACCAGAAGTATTTCC-3' |
| LRHQB | 5'-GGAAATACTTCTGGTTAACGAGTTGACTGCATAGG-3' |
| YLRHQ$_2$F | 5'-GCCTATGCAGTATATCTCTGGACCAGAAG-3' |
| YLRHQ$_2$B | 5'-CTTCTGGTCCAGAGATATACTGCATAGGC-3' |
| YVRHQF | 5'-GTATACTCCTGGGTCCGGAGTATTTCCCACATGC-3' |
| YVRHQB | 5'-GCATGTGGGAAATACTCCGGACCCAGGAGTATAC-3' |
| YLVRHQF | 5'-GCCTATGCAGTATATCTCTGGGTCAGAAGTATTTCCC-3' |
| YLVRHQB | 5'-GGGAAATACTTCTGACCCAGAGATATACTGCATAGGC-3' |
| N411YF | 5'-GGCCTATGCAGTATACTCCTGGACCACTAG-3' |
| N411YB | 5'-CTAGTGGTCCAGGAGTATACTGCATAGGCC-3' |
| N411KF | 5'-GGCCTATGCAGTCAAATCCTGGACCACTAG-3' |
| N411KB | 5'-CTAGTGGTCCAGGATTTGACTGCATAGGCC-3' |
| N411AF | 5'-GGCCTATGCAGTCGCCTCCTGGACCACTAG-3' |
| N411AB | 5'-CTAGTGGTCCAGGAGGCGACTGCATAGGCC-3' |
| T415QF | 5'-CAACTCCTGGACCCAGAGTATTTCAGGTATGC-3' |
| T415QB | 5'-GCATACCTGAAATACTCTGGGTCCAGGAGTTG-3' |
| T415AF | 5'-CAACTCCTGGACCGCTAGTATTTCAGGTATGC-3' |

| | |
|---|---|
| T415AB | 5'-GCATACCTGAAATACTAGCGGTCCAGGAGTTG-3' |
| G419AF | 5'-GGACCACTAGTATTAGCGCTATGCTGCTGAAAGTGG-3' |
| G419AB | 5'-CCACTTTCAGCAGCATAGCGCTAATACTAGTGGTCC-3' |
| G419HF | 5'-CCACTAGTATTTCACATATGCTGCTGAAAGTGG-3' |
| G419HB | 5'-CCACTTTCAGCAGCATATGTGAAATACTAGTGG-3' |
| 3AQF | 5'-GCCTATGCAGTCGCCTCCTGGACCGCTAGTATTTCCGCAATGCTGCTGCAA-3' |
| 3AQB | 5'-TTGCAGCAGCATTGCGGAAATACTAGCGGTCCAGGAGGCGACTGCATAGGC-3' |
| 5AQF | 5'-GCCTATGCAGTCGCCGCCTGGGCCGCTAGTATTTCCGCAATGCTGCTGCAA-3' |
| 5AQB | 5'-TTGCAGCAGCATTGCGGAAATACTAGCGGCCCAGGCGGCGACTGCATAGGC-3' |
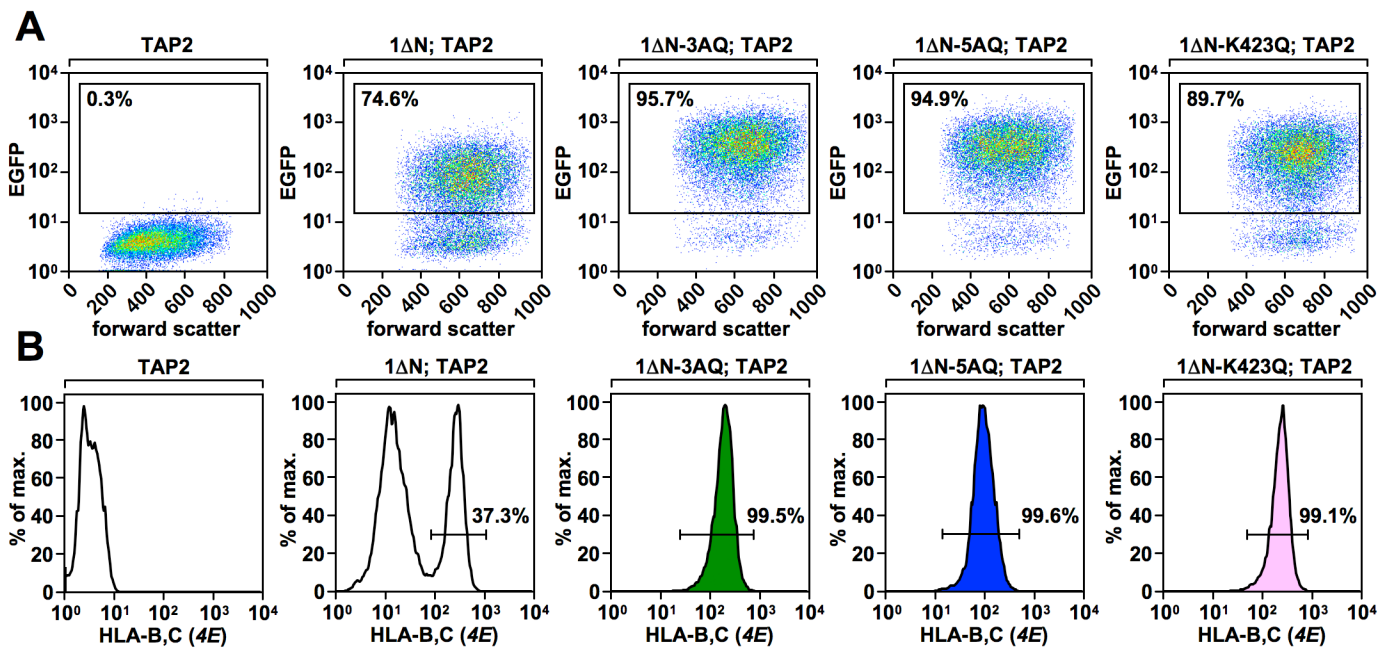
**Supplementary Figure S1, Flow cytometry analysis of T2 transfectants expressing TAP mutants. A)** Flow cytometry analysis showing EGFP expression versus forward scatter for the indicated T2 transfectants. The EGFP-positive population is shown in the gate. **B)** Flow cytometry analysis of surface-HLA-B,C expression using antibody 4E in the indicated T2 transfectants. Results were first gated on living cells (propidium iodide-negative), then on EGFP-positive cells using the gate shown in Fig. S1A. Cells expressing TAP2 alone (*first histogram*) were not gated on EGFP.
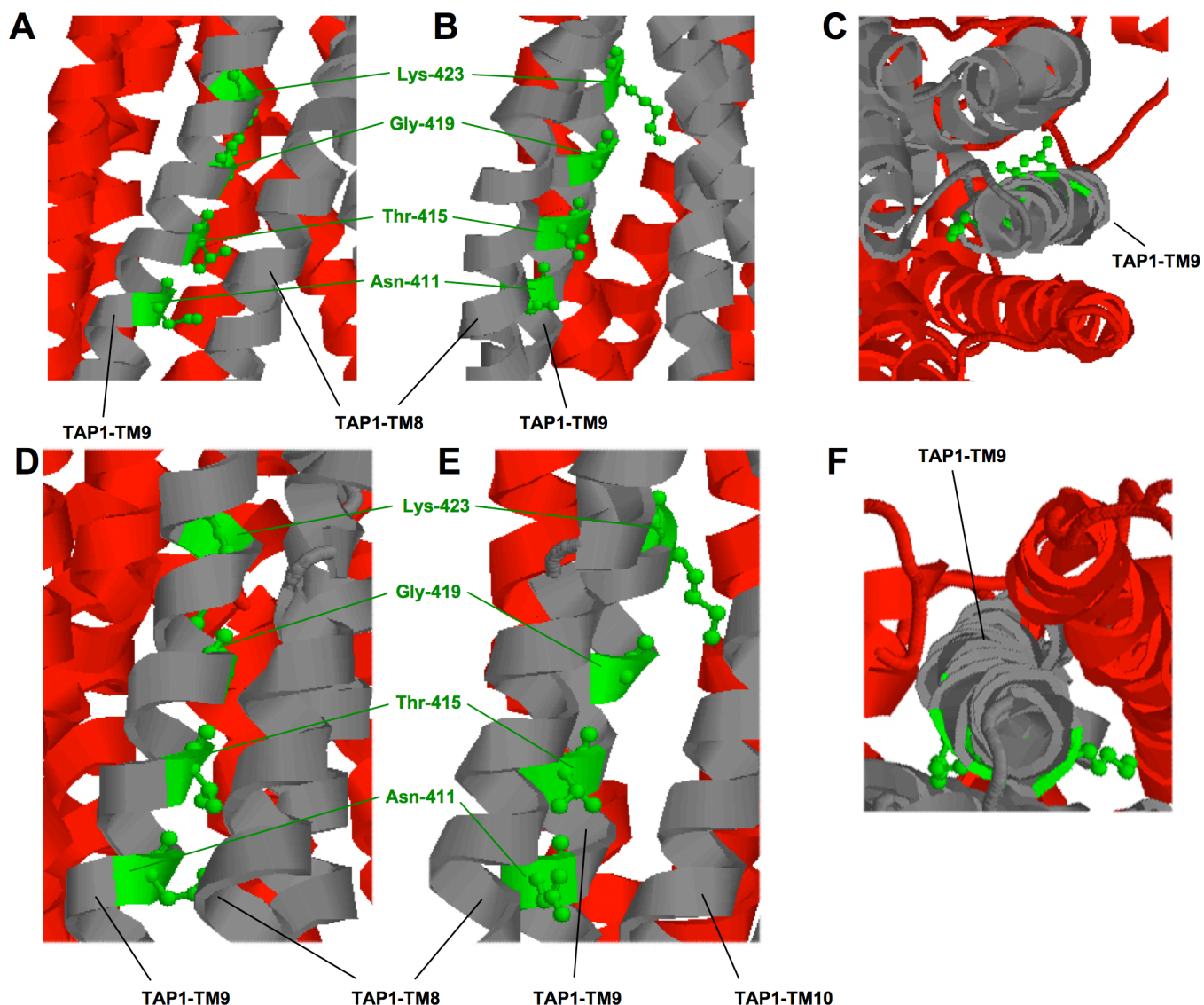
**Supplementary Figure S2, TAP homology models suggest that TM9 residues critical for tapasin binding do not face outwards of the assembled transporter.** Two published TAP homology models from Corradi et al, 2012 were used. TAP1 is shown in grey. TAP2 is shown in red. Residues Asn-411, Thr-415, Gly-419, and Lys-423, which are mutated in construct TAP1-3AQ, are displayed in green and in ball and stick representation. **A-C)** TAP model based on the crystal structure of the ABC transporter ABCB10 in an inward-facing conformation possibly representing the peptide binding-competent state. **D-F)** TAP model based on the crystal structure of the ABC transporter Sav1866 in an outward-facing conformation possibly representing the state when the transport cycle is finished and the translocated peptide has been released into the ER lumen. Panels S2A and S2D view at the front face of TM9 that is pointing outwards from the molecule. Note that the residues critical for tapasin binding (*green*) are pointing sideways towards TM8 or to the back into the inner of the pore. Panels S2B and S2E view at the inner pore region with TM8 on the left side in front of TM9. The surface of the TAP molecule facing outwards is to the left. Note that the residues critical for tapasin binding (*green*) are pointing towards the viewer in the direction of TM8 or sideways to the right into the inner of the pore (particularly Lys-423). Panels S2C and S2F view at the TAP molecule from the top. The molecular surface facing away from the pore is to the right in Fig. S2C and to the top in Fig. S2F. Note that the residues critical for tapasin binding (*green*) are not facing outwards. All images were prepared using RasMol 2.7.2.1.1.