Supporting Information

Assembly of the MHC I peptide-loading complex determined by a conserved ionic lockswitch

Running title: PLC assembly by a conserved salt bridge

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Fig. S1. Charge exchange restores the TAP-tapasin interaction. Interaction (*A-B*) rat and (*C*) chicken $TAP1^{D-to-K}$ and $TAP2^{D-to-K}$ with tapasin, can be rescued by co-expressing tapasin^{*K428D*} in HeLa cells. By tandem affinity purification, TAP complexes were selectively purified and subsequently evaluated by SDS-PAGE followed by immunoblotting. Whole cell extract (WCE) and input represent 1/20 aliquot of the precipitate.



Fig. S2. Surface accessible residues in TMD0^{TAP1} and the effect of tapasin on dimer formation. Membranes were prepared from transiently transfected M553 cells, and treated with CuP (+), PBS (-) or DTT, and subsequently subjected to to non-reducing SDS-PAGE and subsequent immunoblotting. An empty lane separates the oxidative and reduced samples.

| | TM1 | TM2 |
|---------------------------|---|--|
| A | BASLAWLKSYXSLLLADXALLRLASSED | ESLEXELASELASEWERS |
| Gorilla Q28433 17-79 | ZU ZZ ZZ GASLAW – LGTVLEF HADWVLLRTALPRII | ⁴⁸ FS∎LVPTALP■LRVMAAV€LSRWAVDMLGACGVLRAT |
| Chimpanzee H2R2Q4 17-79 | GASLAW-LGTVLEFMADWVLLRTALPRII | 4°° γ FS∎LVPTALP■LRVMAAV€LSRWAV∎WLGACGVLRAT |
| Orangutan H2PL26 17-79 | GASLAW-LGTVLLLADWVLLRTALPRII | FSILVPTALPILRVMAVCLSRWAVIMLGACGVLKAT |
| Gibbon G1R4V2 17-79 | GASLAW-LGTVLLLADWVLLRTALPRII | 48 FSILVPTALPILRVΩGVCLSRWALINGARGVLRAT |
| Macaque F6UP20 17-79 | GASLAW-LGTVLLLTDWLLLRTALPRII | FSULVPTALPULQVMAVCLSRWAVIMLGACGVLRAT |
| Horse F6TVN9 17-79 | RASLAW-LGATL LLAD CV LL RPALPRII | FSILVPAALPILRVMAAVELSRWAVIMLGARRVLRAT |
| Panda G1L5G1 17-79 | 20 28 38 RASLAW-LGITL ILIAD WV LI REALPGIC | 48 /9 CSVLVPTALPILRVXVVCLSRWALIXULGARGILRAT |
| Dog F1PTN57 17-78 | 20 28 38 RARLAW – LGVAL ILIAD WA LI RCS – PAV(| 48 |
| B Gorilla Q28437 5-57 | 6 12 22 D L R P W T S 5 5 5 7 V D A A 5 5 W L L Q G P | 29 57 MGTLLPQGMPGMMTECTIRLGGTMGLMKLRG |
| Chimpanzee K7C7N8 5-57 | 6 12 22 D L R P W T S L L V D A A L W L L Q G P | 29 39 49 57 IGTLLPQGIPGIMLECTLRLGGIMGLIKLRG |
| Orangutan H2PL24 5-57 | 6 12 22 D L R P W T S L L V D A A L L W L L Q G P - · | 19 49 57 19 TLLPQG 19 G 10 LECTLRLGG 10 GLIKLRG |
| Gibbon G1R4S3 5-57 | 6 12 22 D L R P W T S L L V D A A L W L L Q G P - · | 19 49 57 19 TLLPQGQPG11 18 ECTLRLGG110GL11 KLSG |
| Macaque F6UWB4 5-57 | 6 12 22 D L R P W T F I I V D A A I I W L L Q G P | 29 49 57 - – IGTLLPQGIPGINLECTLRLGGING- – – LIKLRG |
| Horse F7DH87 6-57 | 7 13 23 D L R P W A S ILLAD LA LL WLLQGT | 30 40 50 58 IIG D L L P P G II P G II W L E C T L R L G G I W W L I K L G G |
| Panda G1L5N9 5-57 | 6 12 22 DLRPWAF IIIAD SV II WLIQGT | 29 49 57 - – IGALLPPGIPGINLECTLRLGGIWW – – – LIKVGG |
| Dog F1PTP4 6-78 | 7 13 23 D L R P W A F L L A D W A L L W L L Q G T - · | 30 40 50 - – UGARLPRGUPGUWLECTLRLGGUWW – – – LUKVGG |
| TM | | |
| C | LEVAGLSGPSLEDsyGLFLSAFLLLGLFK | LGWAAXYL <mark>STSKDSK</mark> KKog |
| Gorilla G3QUP3 400-448 | 400 420 LEVAGLS GPSLEDS VGLFLSAFLLLGLFK | 450 459 4440 ALGWAAVYLSTCKDSKKVQC |
| Chimpanzee H2QST3 400-448 | 400 410 420 LEVAGLSCPSLEDSVGLFLSAFLLLGLFK | 430 439 448 ALGWAAVYLSTCKDSKKKAE |
| Organgutan H2PL58 400-448 | 400 420 LEVAGLSGPSLEDSVGLFLSAFLLLGLFK | 430 439 448 ALGWAAVYLSTCKDSKKKAE |
| Gibbon G1R571 400-448 | 400 420 420 420 420 420 420 420 420 420 | 430 439 448 V LGWAAVYL STCKDSKKVQC |
| Macaque F7H7T7 400-448 | 410 	420 	420 	420 	420 	420 	420 	420 	42 | 430 439 448 ALGWAAVYLSTCKDSKKKAE 420 449 |
| Horse F6T753 400-448 | $4 \downarrow 0 \qquad 4 \downarrow $ | 430 		 439 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 		 448 |
| Panda G1L3Z3 400-448 | 4 U 4 Z 4 Z 4 Z 4 Z 4 Z 4 Z 4 Z 4 Z 4 Z | 430 439 440 ALGWAAAYKSTWKESKEKVT |
| Dog Q5TJE4 400-448 | ⁴ γυ 4 ² υ LEVAGLSCPSLEDSVGLFLSAFLLLGLIK | 490 499 498 ALGWVAASRSKOPKEKKA |

Fig. S3. TAP1, TAP2 and tapasin show conservation of charged residues in their TMs. Multiple sequence alignments (MAFFT) for TAP1 *(A)*, TAP2 *(B)* and tapasin *(C)*. Eight representative species were shown for each protein. Conserved Asp and Arg in TAP1/2 are marked in red, and conserved Lys in tapasin is marked green. TM helices for TAP1/2 and tapasin are indicated.



Fig. S4: Control simulations of alternative, lower scoring models. Cα-RMSD time series (TM helices only) with respect to starting structure during MD initiated from (i) model belonging to cluster 1 and matching nine of the thirteen measured cross-links (1, green curve), (ii) cluster 3 model matching eight cross-links (2, grey curve), and (iii) cluster 3 model matching thirteen cross-links (3, red curve). For comparison, the RMSD from Figure 5 (for the top-scoring model) is also shown (blue curve). The structures after 250 ns (red) are superimposed on the starting structure (grey). Side chains of D32 and R64 are shown as sticks.



Fig. S5: MD simulation of the charge exchange complex (double mutant TMD0 D32K and tapasin K428D). (A) C α -RMSD of the TM helices with respect to starting structure. (B) Minimal residue-residue distance shows the stability of the salt bridge on the 250 ns time scale. Grey and red curves show the data for the charge exchange and wild-type systems, respectively.



Fig. S6: Charge exchange of the intra-molecular salt bridge leads to stabilization of TMD0^{TAP1}. Expression of D32R, R64D and D32R/R64D reveals that the disruption of the intra-molecular salt bridge leads to destabilization of TMD0^{TAP1} (D32R or R64D of human TAP1), whereas charge exchange restores stabilizing effect (D32R/R64D). HeLa cells were transiently transfected

with $TMD0^{TAP1}$ mutants indicated above, and $1x10^{6}$ cells were loaded per lane. Expression levels were evaluated by SDS-PAGE followed by immunoblotting. Control lane (ctr.) represents untransfected cells

Movie 1: MD simulation of the TAP1-tapasin interaction. The starting structure represents an intermediate state characterized by hydrogen bonds between D32 and both R64 and K428. In the final dimer state, the side-chain of R64 is oriented towards the lipid