The interleukin-like epithelial–mesenchymal transition inducer ILEI exhibits a non-interleukin-like fold and is active as a domain-swapped dimer

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SUPPLEMENTAL DATA

FIGURES S1-S7

SUPPLEMENTAL REFERENCES

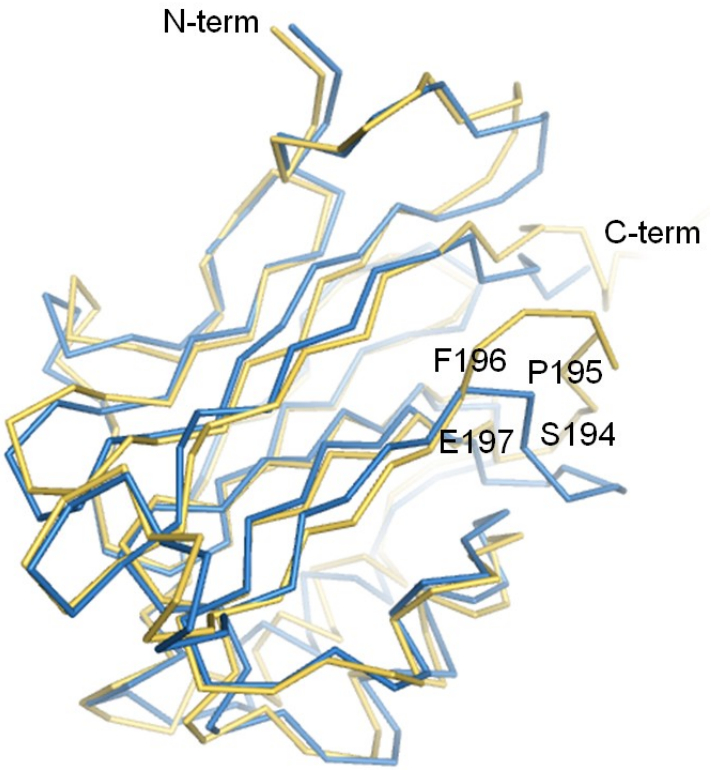

FIGURE S1. Superposition of FAM3B PANDER and FAM3C ILEI. FAM3B PANDER (PDB id 2YOP) in yellow and human FAM3C ILEI monomer in blue. The Ser194-Glu197 stretch forming a sharp kink in the ILEI monomer and dimer structures is labeled.

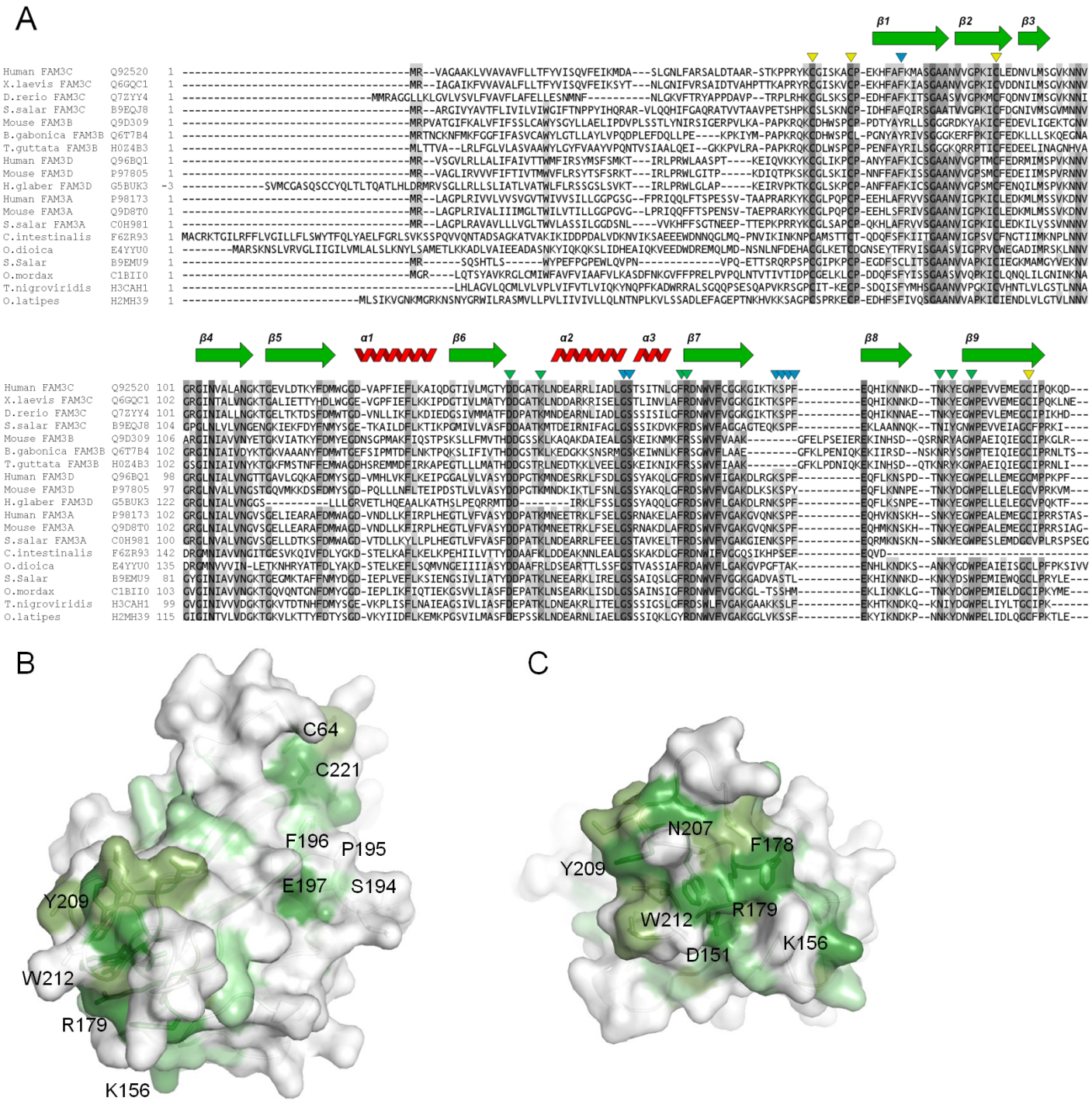

FIGURE S2. Conservation across FAM3. A) Structure-based alignment of a number of classified and unclassified FAM3A, B, C and D members. Darker color corresponds to higher level of conservation. The two ILEI cysteine bridges are indicated by yellow triangles. Residues involved in dimer formation (Figure 4C) are marked by blue triangles and the highly conserved surface residues by green triangles. B,C) The FAM3 conservation mapped onto the human ILEI surface. Darker green corresponds to higher level of conservation. The conserved cluster Tyr209, Asp151, Lys156, Phe178, Arg179, Asn207 and Trp212 is located around the water filled pocket while the partially conserved S194-E197 patch forms the β7-β8 kink.

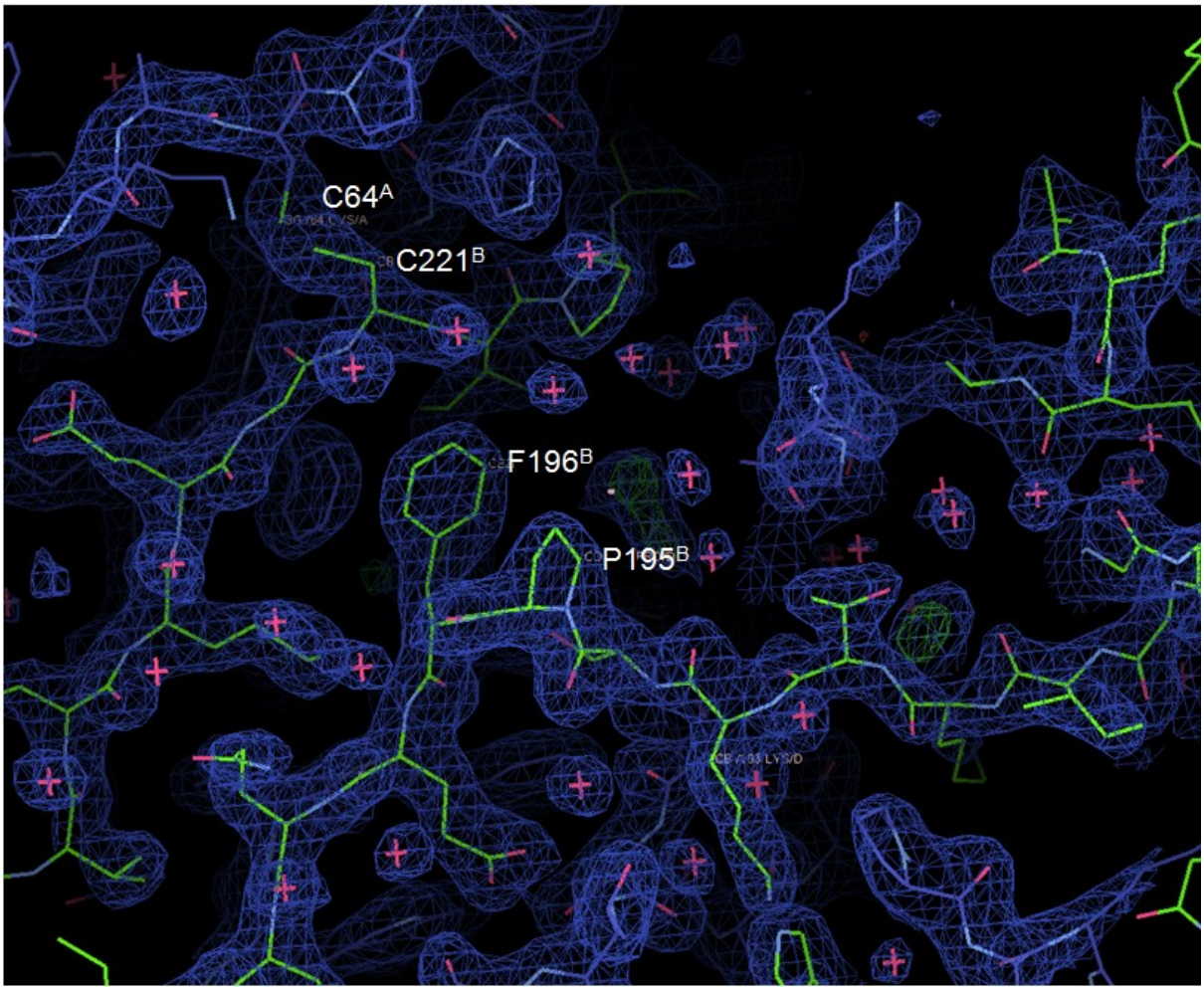

FIGURE S3. The ILEI dimer bridge and the intramolecular cysteine link. The loop after strand β7 extends over to the other dimer chain where it forms a kink adjacent to strand β8, resulting in the domain swap. β8 and β9 are stabilized by the *trans*-linked cysteine bridge between Cys64 of one molecule (blue) and Cys221 of the other (green). 2Fo-Fc density map contoured at 1.2σ.

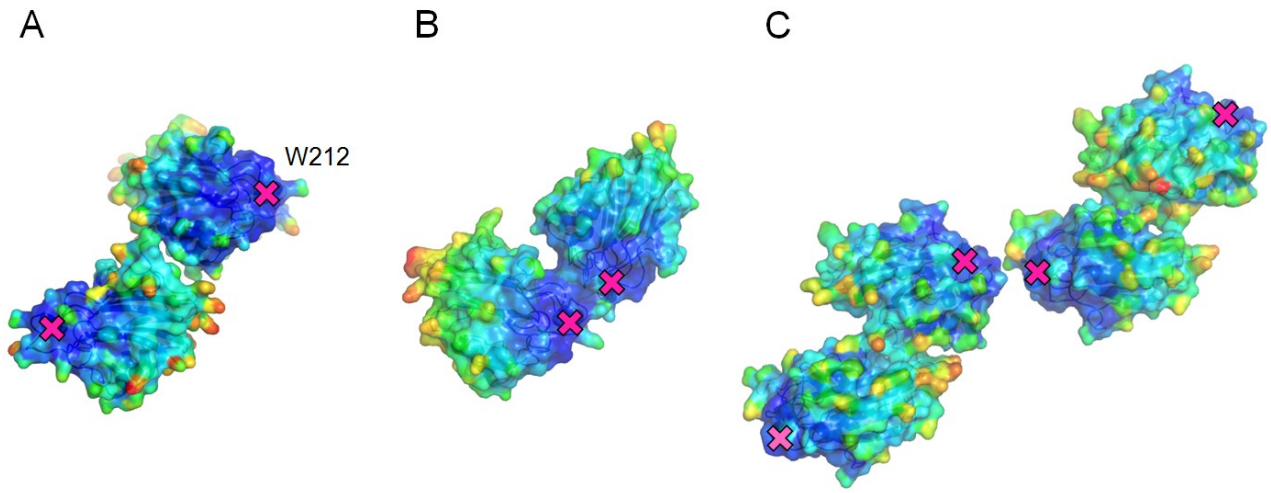

FIGURE S4. B-factor distribution of the eight independent chains in the ILEI monomer and dimer structures. A) B-factors mapped onto the surface of the two molecules of the human monomer structure from blue (low) to red (high). Trp212 forming one side of the water filled pocket is indicated by a pink cross. B) B-factor distribution of the two molecules of the mouse monomer structure. C) B-factor distribution of the four molecules of the mouse ILEI dimer structure.

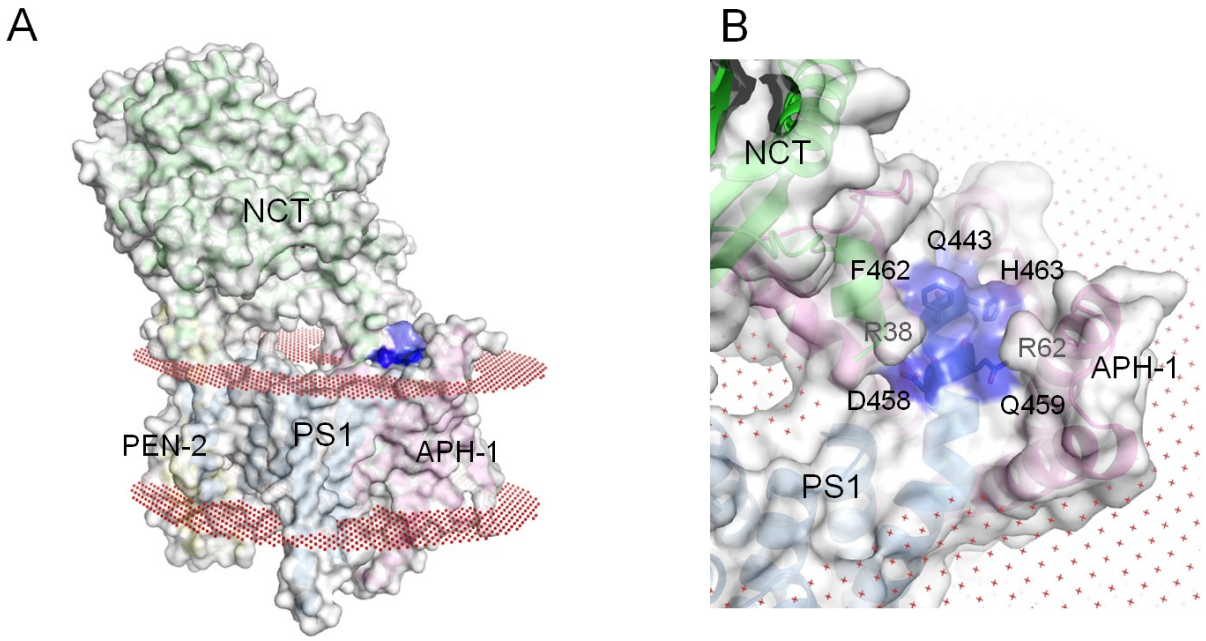

FIGURE S5. Potential mode-of-action of FAM3C ILEI on the γ-secretase complex based on the mutational studies of Hasegawa et al. (16). A) Surface of the γ-secretase protease (PDB id 5FN3). B) The ILEI non-binding mutants F462, H463, Q464, D458, Q459 and L460 in the C-terminal portion of the PS1 domain highlighted in blue. The lipid bilayer limits were calculated using a combined anisotropic solvent PPM model (1,2).

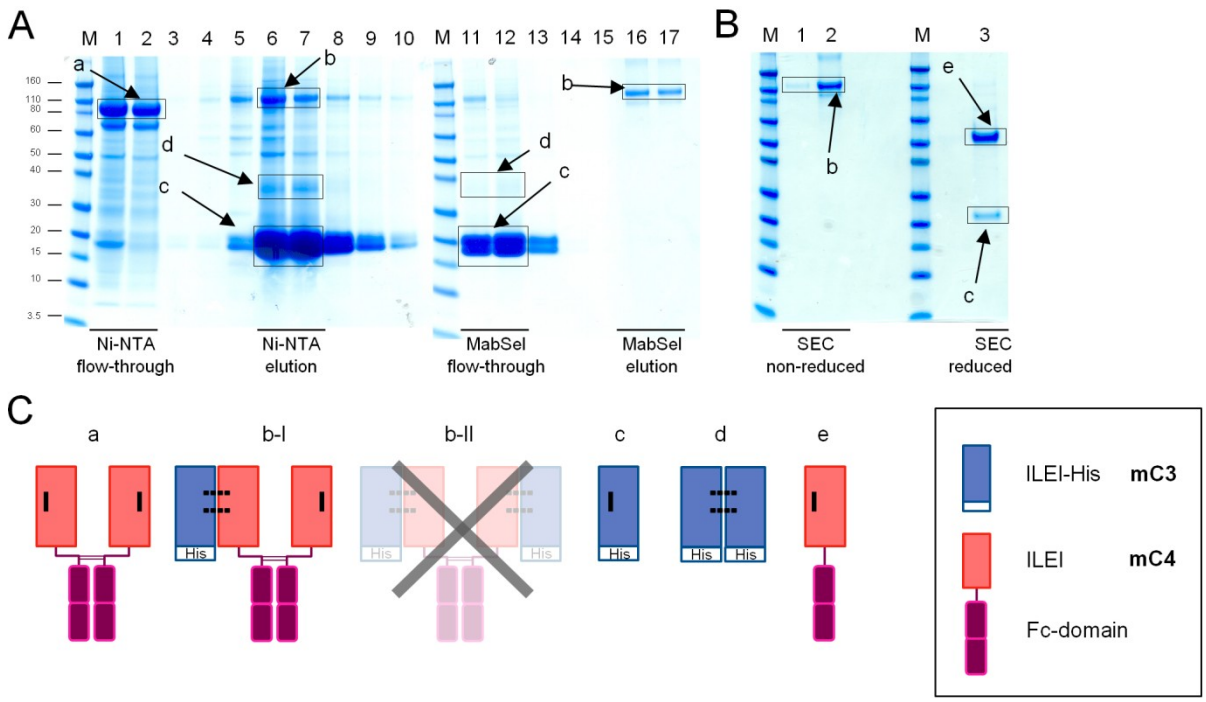

FIGURE S6. SDS-page analysis of the different fractions captured after C3 and C4 co-expression. The His-tagged mC3 construct is colored in blue while the Fc-tagged mC4 construct is colored in red. A) Population (a) in the Ni-NTA flow-through represents mC4 Fc-ILEI dimers where the Fc-domains have dimerized and ILEI is in monomer form. The theoretical molecular weight of this complex is 93.7 kDa. In the Ni-NTA elution one high-molecular weight population (b) was captured together with mC3 His-tagged ILEI monomer (c) and dimer (d). The two mC3 His-ILEI populations are removed in the MabSelect step. In the elution from the MabSelect resin we only find bands from population (b), a mC3:mC4 complex consisting of a mC4 Fc-ILEI dimer where either one or two of the ILEI molecules form dimers with mC3 His-ILEI corresponding to a mC3:mC4 heterodimer. This mC3:mC4 complex, including only one His-ILEI molecule would have a molecular weight of 115kDa. B) The material from A) lanes 16 and 17 was run on size exclusion chromatography (SEC) (lane 1) and subsequently concentrated (lane 2). When analyzing this sample under reducing conditions it separates into the two bands seen in lane 3, corresponding to mC4 Fc-ILEI and mC3 His-ILEI at a ratio of 2:1 as judged by intensities on the gel. C) A schematic illustration of the various populations of mC3 His-ILEI and mC4 Fc-ILEI that were identified during the purification.

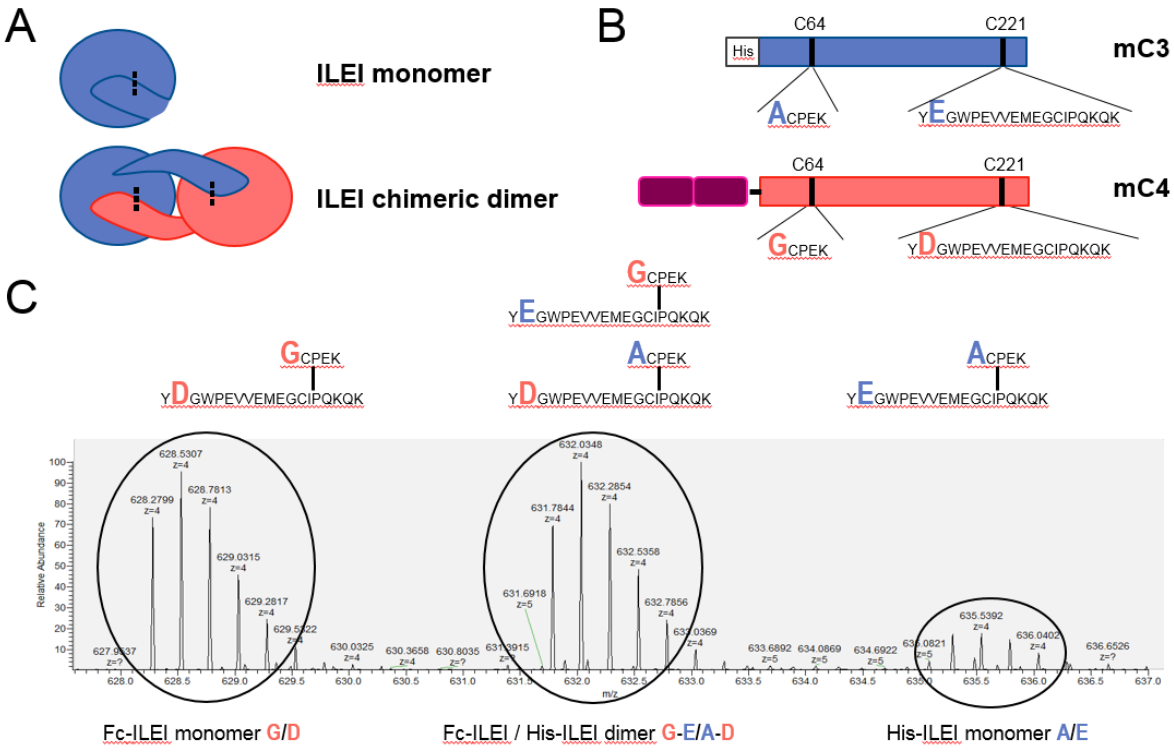

FIGURE S7. MS analysis of the chimeric ILEI dimer. A) Schematic illustration of the ILEI monomer and dimer with dashed lines representing the Cys64-Cys221 disulfide bridges. B) ILEI constructs mC3 (ILEI in blue) and mC4 (ILEI in red) including the expected peptides after trypsin cleavage. C) Two quadruply charged peaks (MH_4^{4+}=626.28 and MH_4^{4+}=631.75) for the covalently linked peptides were observed in the MS spectrum of the non-reduced sample after several capture and purification steps (Figure S6B, lane 2). The left peak represents the internal cysteine bridge within an Fc-ILEI chain. The middle peak represents the intermolecular cysteine bridge between Fc-ILEI and His-ILEI and thus confirms the strand swap. The small peak to the right represents the internal Cys58-Cys86 cysteine bridge in the mC3 His-ILEI monomer. Part of the Fc-ILEI monomer peak and the small His-ILEI monomer peak are both a result from partial separation of the chimeric mC3:mC4 dimer during the MS experiment. All peaks were verified by MS/MS.

SUPPLEMENTAL REFERENCES

1. Lomize, A. L., Pogozheva, I. D., Lomize, M. a., and Mosberg, H. I. (2006) Positioning of proteins in membranes: A computational approach. *Protein Science* **15**, 1318-1333
2. Lomize, A. L., Pogozheva, I. D., and Mosberg, H. I. (2011) Anisotropic solvent model of the lipid bilayer. 2. Energetics of insertion of small molecules, peptides, and proteins in membranes. *Journal of Chemical Information and Modeling* **51**, 930-946