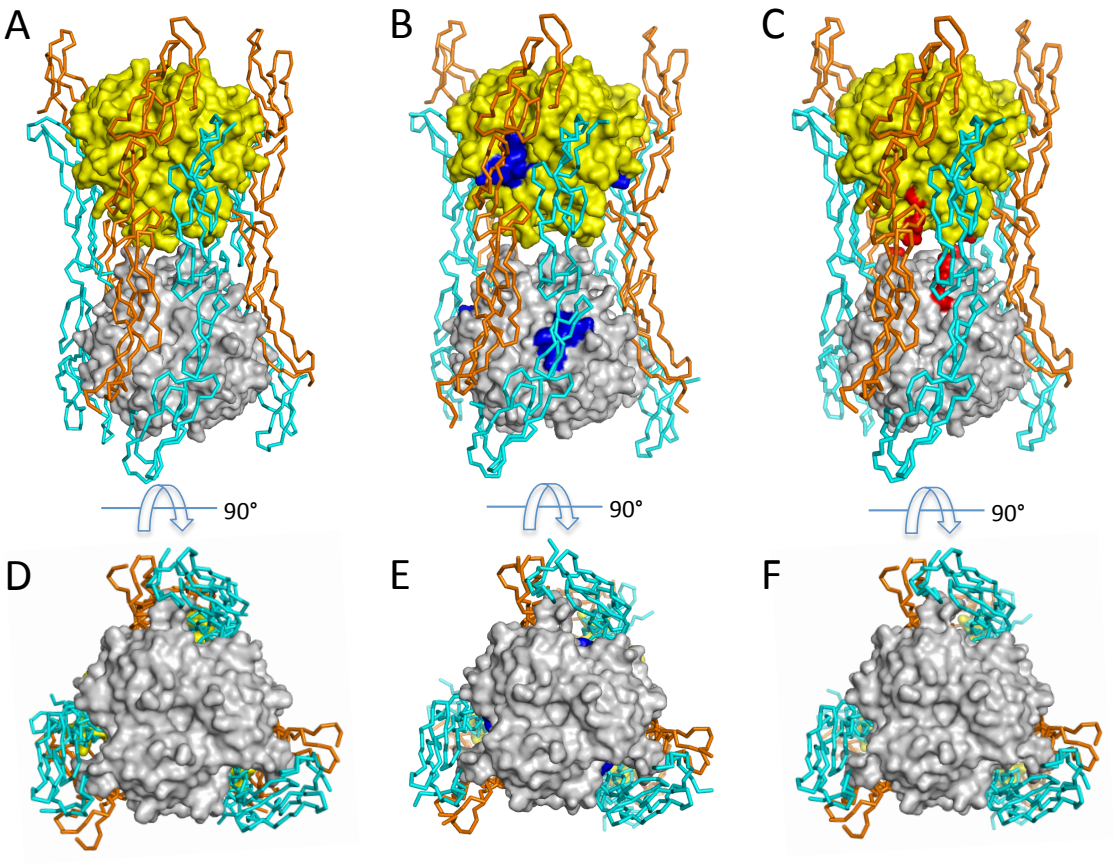

Figure S1, related to Figure 6. Comparison of wild type LIGHT:DcR3 complex with mutant LIGHT:DcR3 complexes.

LIGHT is shown in surface representation and the receptor DcR3 molecules are shown as ribbons. For clarity, one LIGHT:DcR3 hexamer is colored grey (LIGHT) and cyan (DcR3), and the other is colored yellow (LIGHT) and orange (DcR3). The transplanted loop on mutant 1 is shown as blue patches in B and E. Similarly, the transplanted loop of mutant 2 is denoted as red patches in C and F. (A) Side view of the LIGHT:DcR3 complex. (B) Side view of the mutant 1 LIGHT:DcR3 complex. (C) Side view of the mutant 2 LIGHT:DcR3 complex. (D) Bottom view of the interlocking hexamers of LIGHT:DcR3 complex. (E) Bottom view of the interlocking hexamers of mutant 1 LIGHT:DcR3 complex. (F) Bottom view of the interlocking hexamers of mutant 2 LIGHT:DcR3 complex.

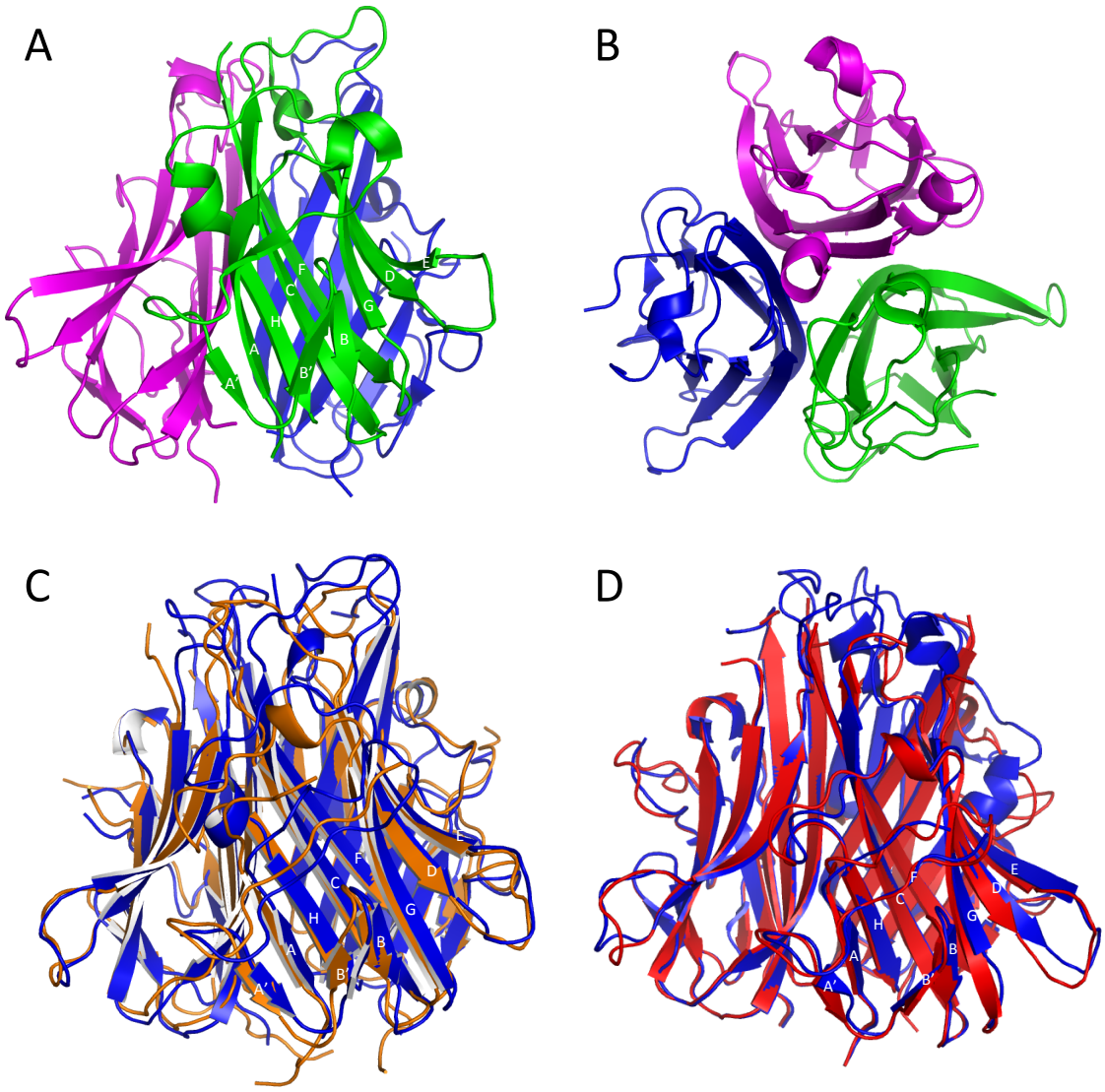

Figure S2, related to Figure 5. Overall structure of LIGHT and comparison with TL1A.

(A) Side view of the unbound trimeric LIGHT structure. Each protomer is represented in a different color. (B) Top view of the unbound LIGHT structure. (C) Superposition of unbound LIGHT (blue) with TL1A (orange, PDB entry 2QE3) shows similar overall structure. (D) Superposition of LIGHT unbound (blue) and bound with DcR3 (red) reveals slight conformational changes.

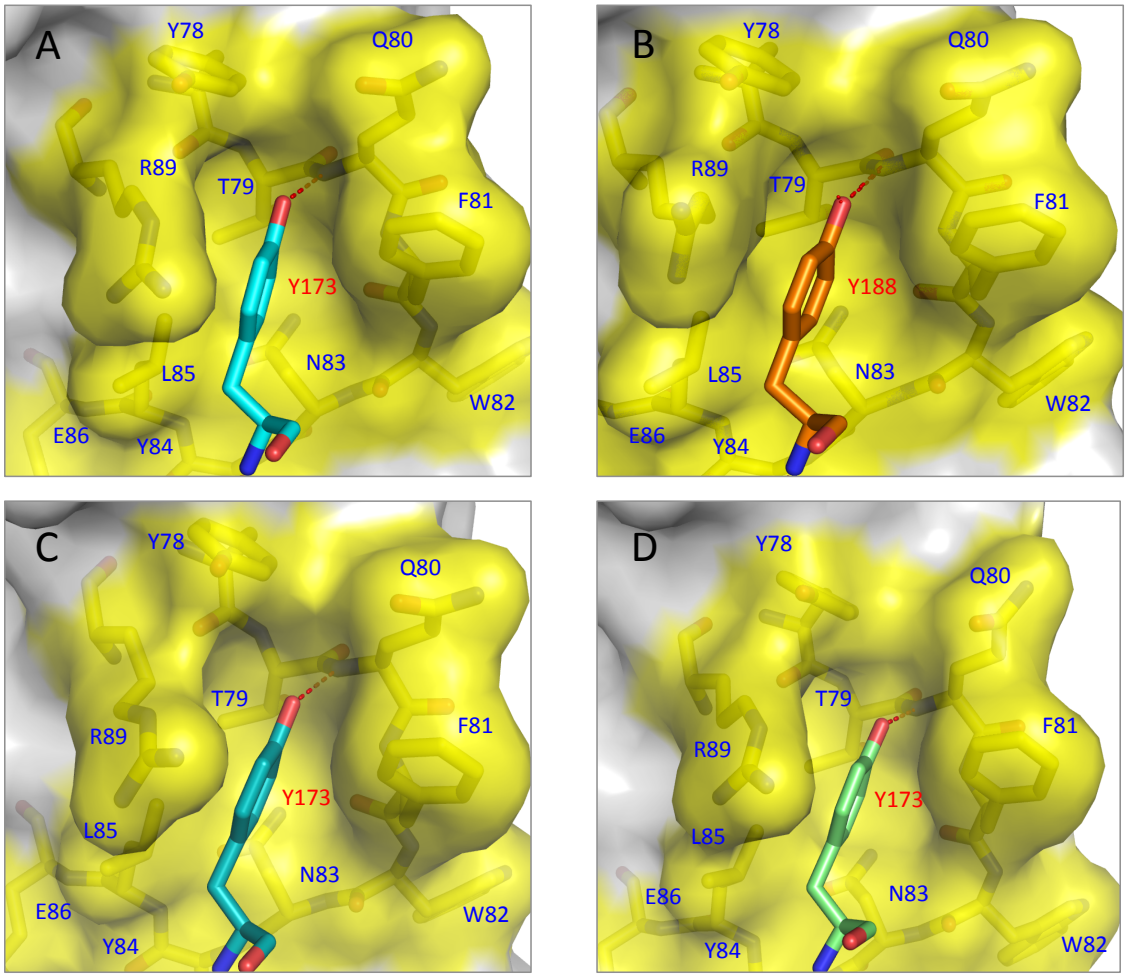

Figure S3, related to Figure 2. A common determinant of LIGHT and TL1A interacting with DcR3.

The conserved LIGHT Y173/TL1A Y188 both occupy the same pocket of DcR3 and make polar contacts with the main chain of DcR3 Q80. (A) Y173 (cyan) from wild type LIGHT has a polar contact with Q80 on DcR3 (yellow surface). (B) analogous Y188 (orange) from TL1A (PDB entry 3K51) forms a polar contact with Q80 on DcR3 (yellow surface). (C) Y173 (dark cyan) from mutant 1 forms a polar contact with Q80 on DcR3 (yellow surface). (D) Y173 (light green) from mutant 2 forms a polar contact with Q80 on DcR3 (yellow surface).

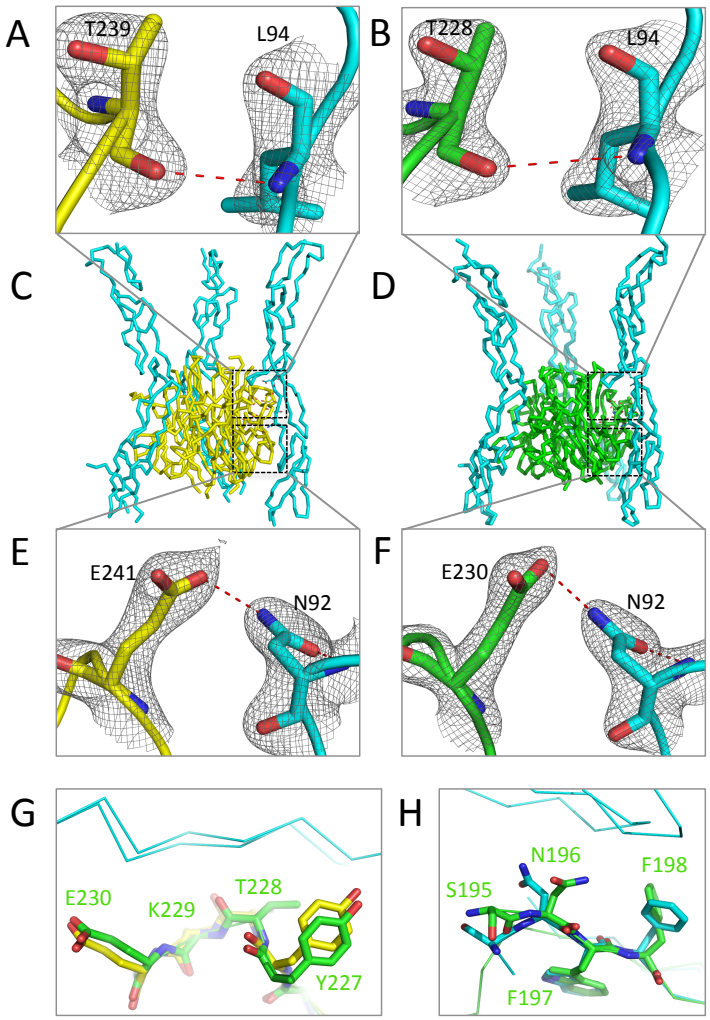

Figure S4, related to Figure 3. Comparison of TL1A:DcR3 (PDB entry 3K51) and mutant LIGHT:DcR3 structures.

TL1A is shown as yellow ribbons, LIGHT mutants are shown as green ribbons and DcR3 is shown as cyan ribbons. (A) and (E) show a magnified view of TLA:DcR3 complex. (B) and (F) show the magnified view of the transplanted loop on the mutant 1 LIGHT:DcR3 complex. *Fo-Fc* electron density map (contoured at 2 r.m.s.d.) of the binding sites is shown as grey mesh. (A) A magnified view of TL1A residue T239 making a polar contact with L94 on DcR3. (B) A magnified view of residue T228 of LIGHT mutant 1 making a polar contact with L94 on DcR3. (C) Overall structure of TL1A:DcR3 complex denoted as ribbons. (D) Overall structure of mutant 1 LIGHT:DcR3 complex denoted as ribbons. (E) A magnified view of TL1A residue T241 making a polar contact with N92 on DcR3. (F) A magnified view of residue E230 of LIGHT mutant 1 making a polar contact with N92 on DcR3. (G) Superposition of TL1A and LIGHT mutant 1 shows similar conformation of the transplanted loop. (H) Superposition of TL1A and LIGHT mutant 2 shows different conformation of the side chains in the transplanted loop and spatial organization of DcR3. Residues of LIGHT mutants are labeled.

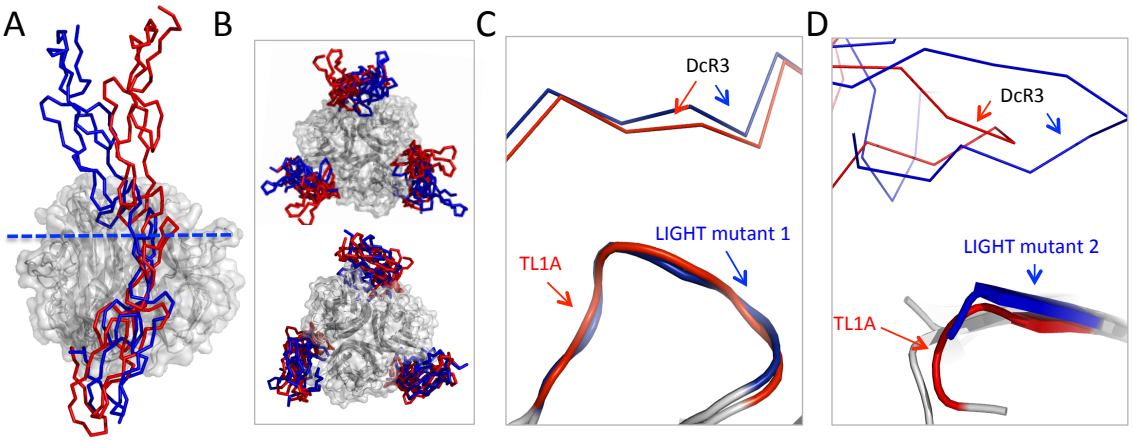

Figure S5, related to table 2. Structural organization of DcR3 in the lower and upper region of LIGHT:DcR3 and TL1A:DcR3 complexes.
DcR3 molecules bound to LIGHT or TL1A are shown as blue or red ribbons, respectively. The ligands LIGHT and TL1A are shown as transparent grey surfaces and grey ribbons. (A) Superposition of LIGHT:DcR3 and TL1A:DcR3 complexes by alignment of LIGHT and TL1A coordinates shows different orientations of CRDs 3 and 4 of DcR3 in the upper region. The upper and lower regions are separated by a blue dashed line. (B) Top view (upper panel) and bottom view (lower panel) of the superposed LIGHT:DcR3 and TL1A:DcR3 complexes. The top view reveals a weaker structural alignment of DcR3 than that observed in the bottom view. The transplanted loops on the LIGHT mutants are colored as blue and the corresponding sequences on TL1A are colored as red for (C) and (D). (C) The substituted loop of LIGHT mutant 1 and the proximal binding loops of DcR3 in the lower region show similar spatial organization as the corresponding region of the TL1A:DcR3 complex. (D) The substituted loop of LIGHT mutant 2 and the proximal binding loops of DcR3 in the upper region show spatial organization distinct from the corresponding region of the TL1A:DcR3 complex.

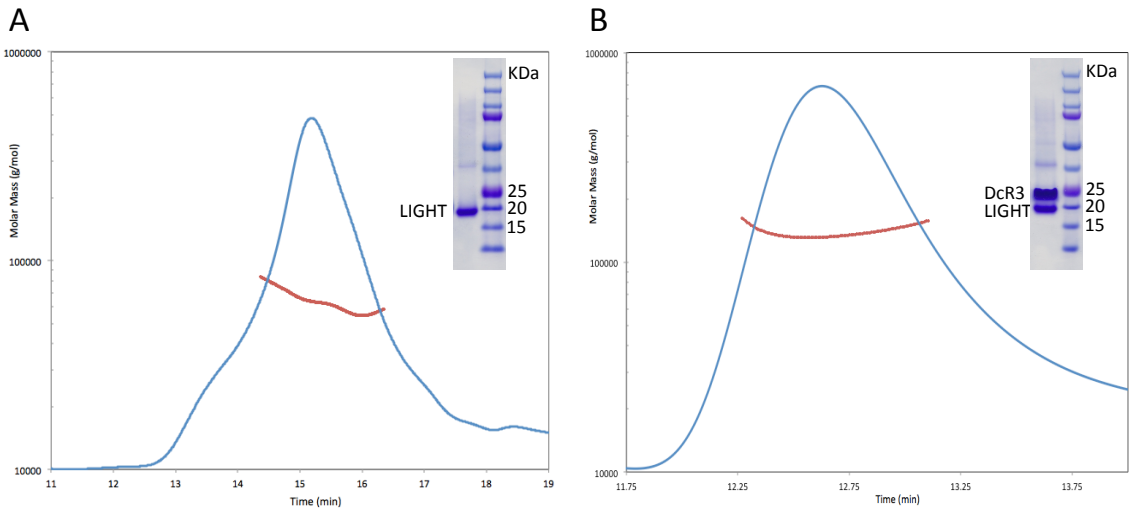

Figure S6, related to Figure 7. LIGHT and LIGHT:DcR3 complex are trimeric and hetero-hexameric, respectively, in solution.

The samples were also analyzed by SDS-PAGE as shown in the top right of each figure. (A) Representative light scattering data show that the ectodomain of LIGHT expressed in S2 cells is a trimer in solution with apparent molecular mass of $55.1x10^3$g/mol. (B) Representative light scattering data show that the LIGHT:DcR3 complex exists as a hetero-hexamer in solution with an apparent molecular mass of $138x10^3$ g/mol.

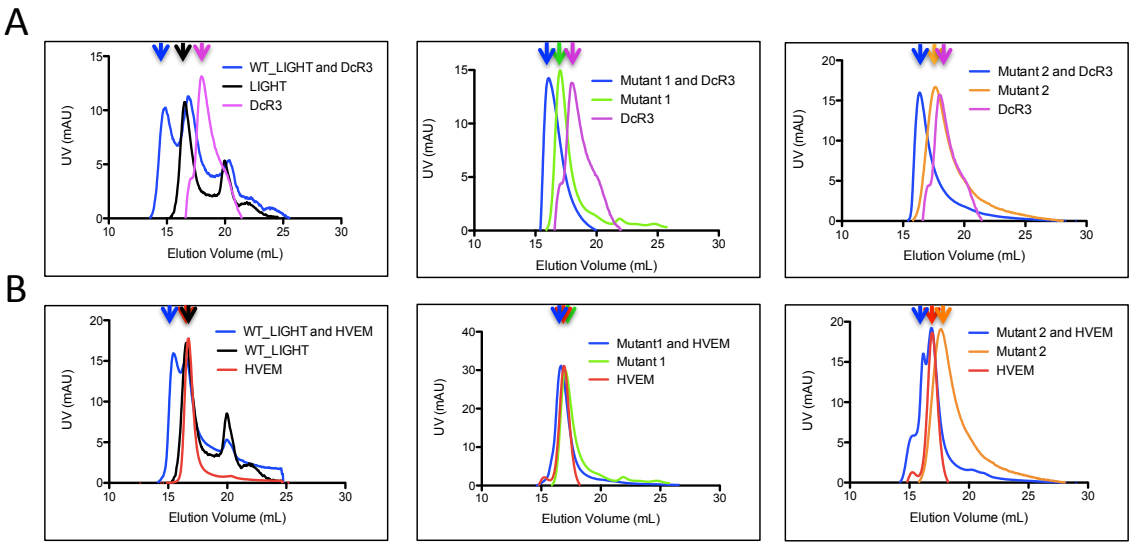

Figure S7, related to Figure 1. LIGHT mutants retain binding to DcR3 but have impaired binding to HVEM.

Each trace represents one independent experiment. (A) Left, size exclusion chromatography (SEC) traces for wild type LIGHT (black), DcR3 (magenta) and mixture of wild type LIGHT and DcR3 (blue). Middle, SEC traces for mutant 1 (green), DcR3 (magenta) and mixture of mutant 1 and DcR3 (blue). Right, SEC traces for mutant 2 (orange), DcR3 (magenta) and mixture of wild type LIGHT and DcR3 (blue). Shifts were observed in all the mixtures of wild type and mutation LIGHT with DcR3 (denoted by blue arrow), indicating that wild type and mutants were able to form complexes with DcR3. (B) Left, SEC traces for wild type LIGHT (black), HVEM (red) and mixture of wild type LIGHT and HVEM (blue). Middle, SEC traces for mutant 1 (green), HVEM (red) and mixture of mutant 1 and HVEM (blue). Right, SEC traces for mutant 2 (orange), HVEM (red) and mixture of wild type LIGHT and HVEM (blue). A shift was observed in the mixture of wild type LIGHT with HVEM (denoted by blue arrow), consistent with the formation of wild type LIGHT:HVEM complex. However, the mutants appear to have compromised binding with HVEM as indicated by the absence of an observed shift for mutant 1 and only a slight shift for mutant 2.

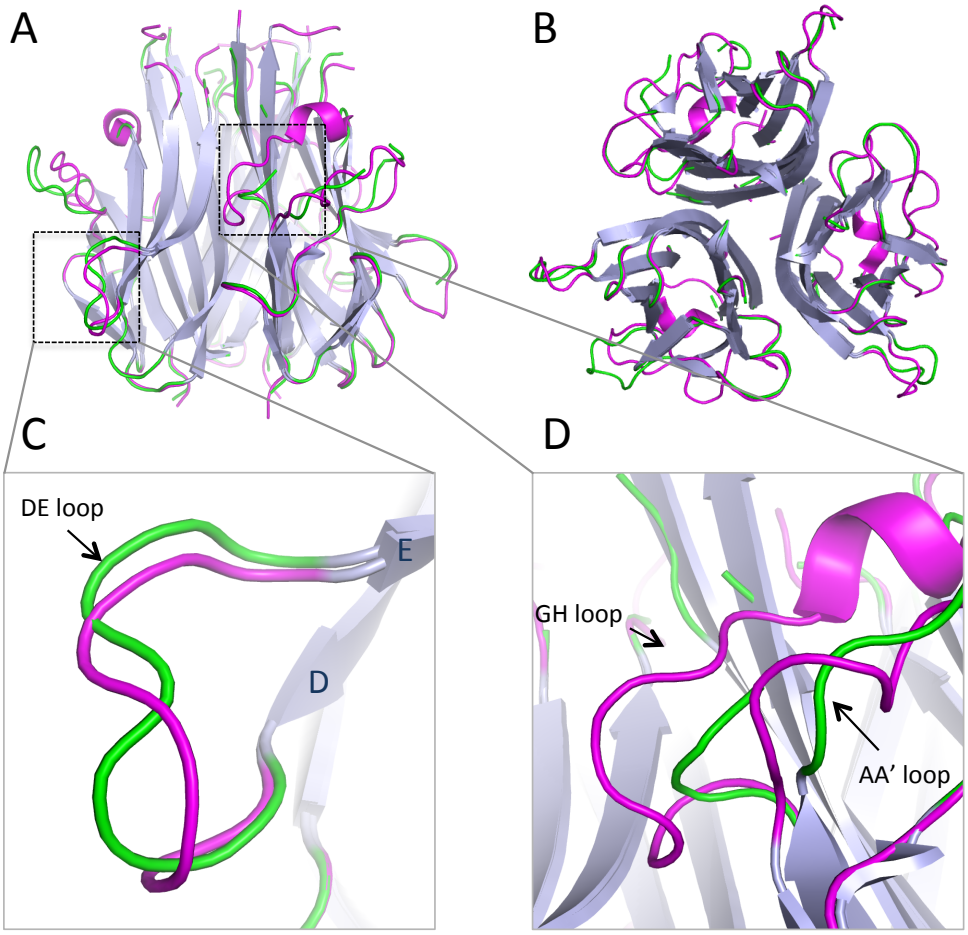

Figure S8, related to Figure 4. LIGHT mutant 2 undergoes DcR3-dependent conformational adjustments.

(A) Superposition of LIGHT mutant 2 unbound (green) or bound (magenta) with DcR3 shows minimal changes in the two parallel beta-sheets (light blue) but significant structural changes in the loops. (B) Bottom view of the superposed structures of LIGHT mutant 2 unbound (green) or bound (magenta) with DcR3. (C) A magnified view of DE loop shows a significant conformational alteration. (D) A magnified view of the AA' and GH loops shows significant conformational shifts.

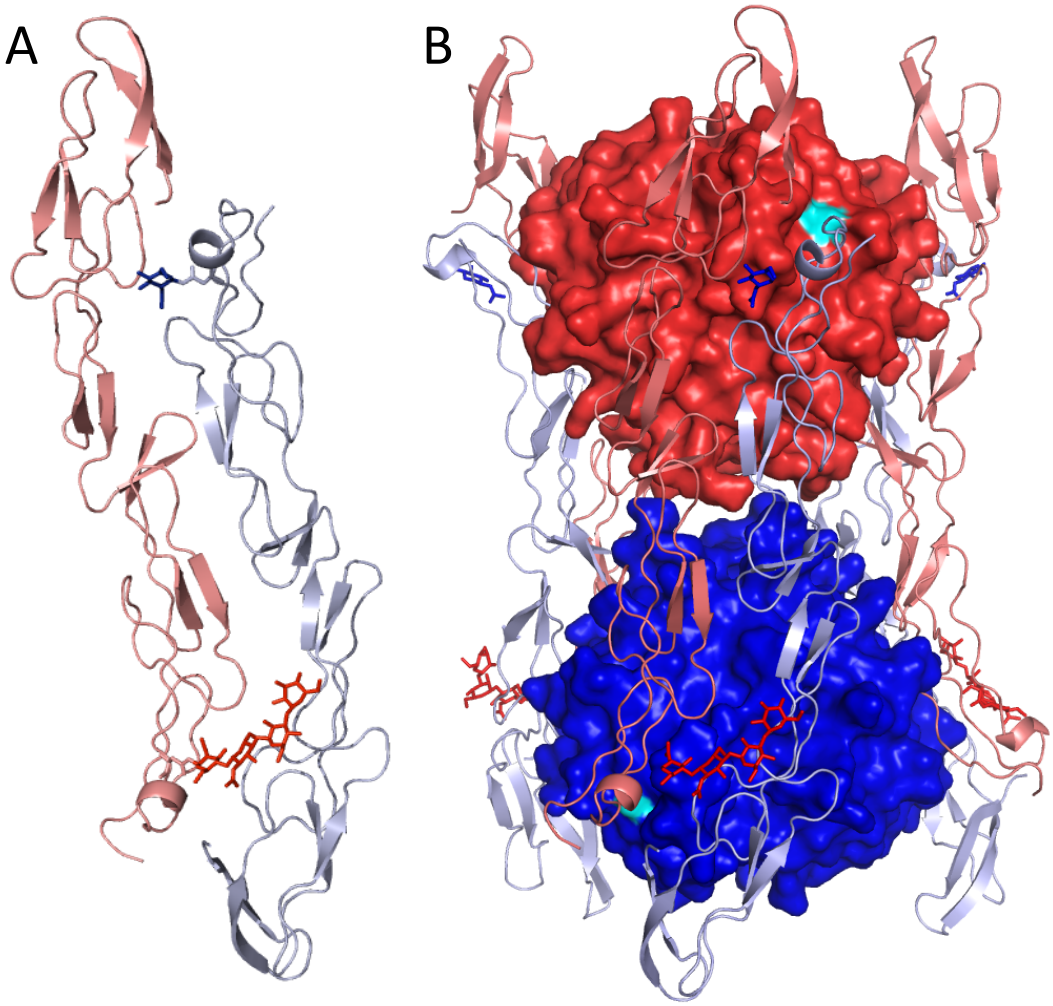

Figure S9, related to table 1. The interlocking hexamers of the LIGHT:DcR3 complex in the crystal.

(A) Sugars (shown as sticks) from one DcR3 (shown as cartoon) contact the anti-parallel DcR3 and stabilize the interlocking hexameric structure. (B) The overall structure of the interlocking hexamers in the crystal. LIGHT is shown in red and blue surface representation and DcR3 is shown as light blue and salmon cartoon. E214K mutant is shown as a cyan patch.