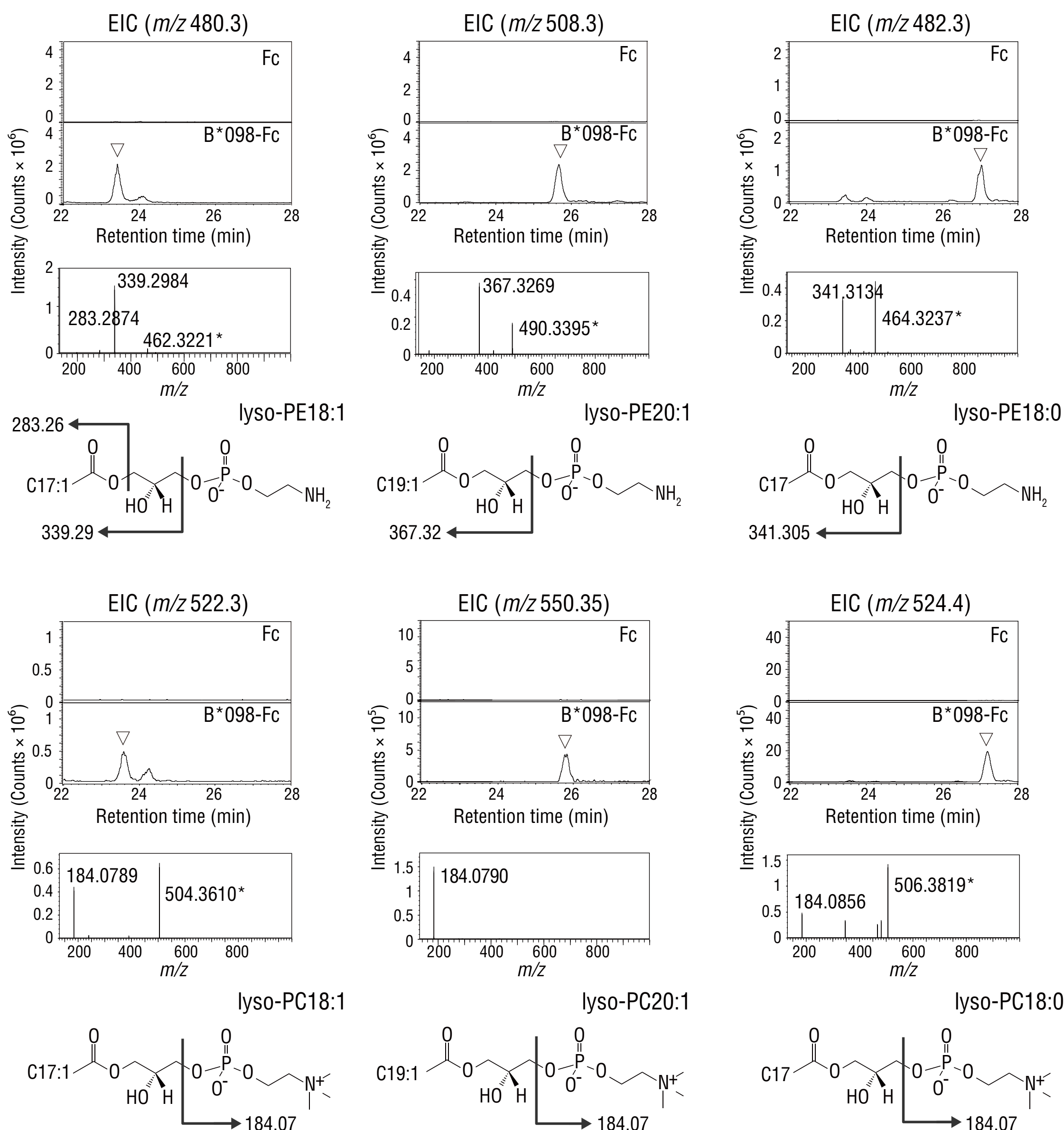

Figure S1. LC-MS/MS analysis of ions associated with soluble Mamu-B*098 proteins

Samples extracted from Fc protein or B*098-Fc proteins were analyzed by reversed-phase LC-MS/MS, as shown in Figure 1. The extracted ion chromatograms (EIC) are shown at the *m/z* values of ion pairs found in peaks 2, 3, and 4 in Figure 1*B* (each top panel), and the MS/MS spectra at the retention times indicated by open triangles are shown (each middle panel). The structures and MS/MS fragmentation of the corresponding lysophospholipids are also shown (each bottom panel). Asterisks indicate dehydrated ions.

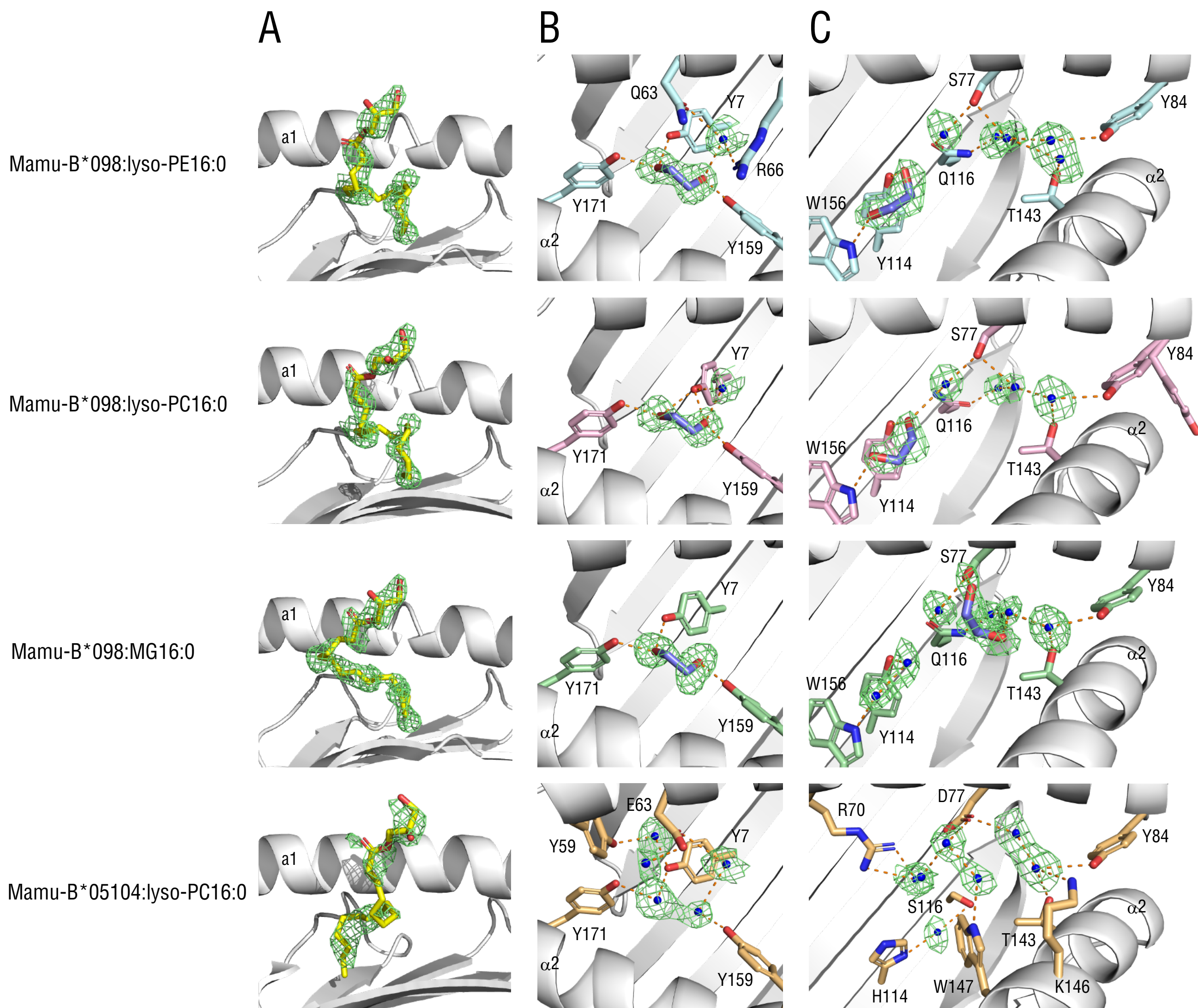

Figure S2. Omit maps for bound ligands in the Ag-binding groove of Mamu-B*098 and Mamu-B*05104

Side views of the B and D pockets (*A*), A pockets (*B*), and C, E, and F pockets (*C*) of Mamu-B*098 complexed with lyso-PE16:0, lyso-PC16:0, and MG16:0 (left three panels) and Mamu-B*05104 complexed with lyso-PC16:0 (right panels) are displayed with ribbon models of heavy chains. Fo-Fc omit maps (contoured at 2.4σ) for MG16:0 (yellow sticks), water molecules (blue sphere), and ethylene glycol (blue sticks) are also shown.

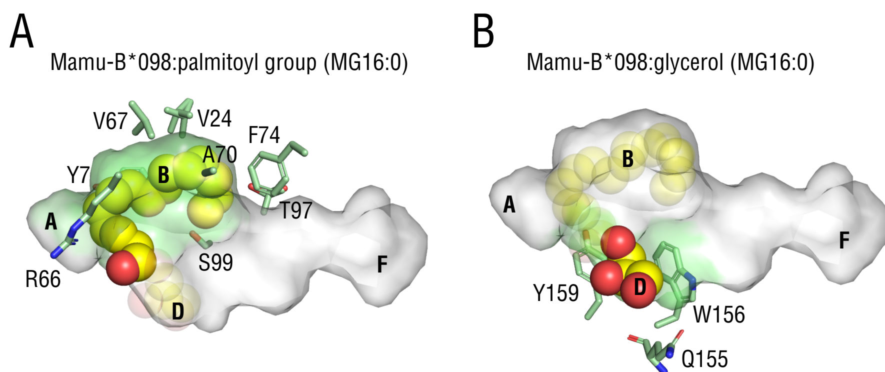

Figure S3. Interactions with MG16:0 and Mamu-B*098

A, B, Top view images of the palmitoyl group (*A*) and glycerol moiety (*B*) of bound ligands (yellow sphere) are displayed with the semi-transparent antigen-binding groove of Mamu-B*098 complexed with MG16:0. The side chains of amino acid residues surrounding the palmitoyl group in the colored B pocket (*A*) and surrounding the glycerol moiety in the colored D pocket (*B*) are shown.

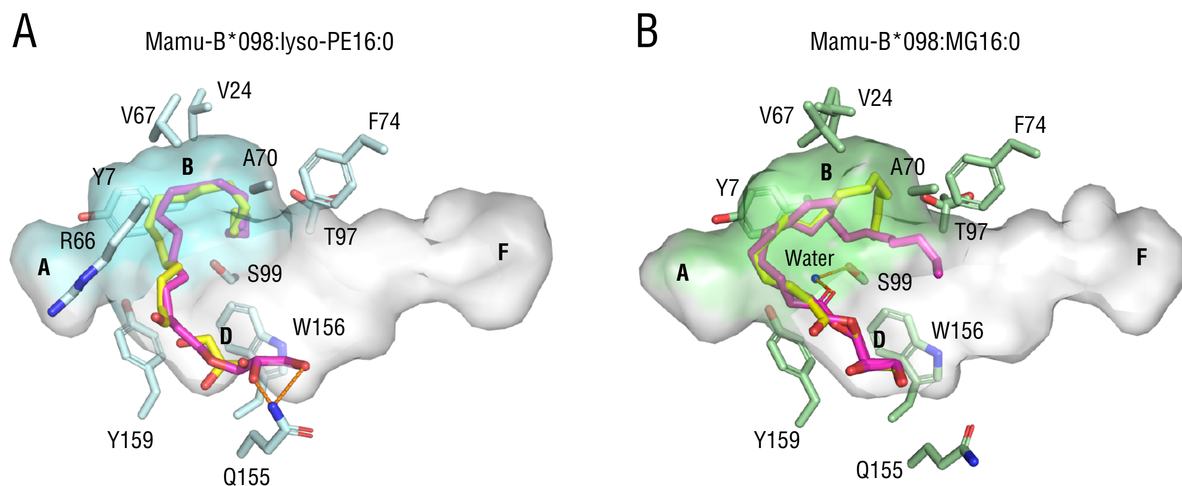

Figure S4. Alternate conformations of bound ligands

A, B, Top view images of Mamu-B*098:lyso-PE16:0 (chain C-D, *A*) and Mamu-B*098:MG16:0 (chain A-B, *B*). Bound ligands with an alternate conformation are displayed as magenta sticks. Ligands with a conformation as in Fig. 3 (lyso-PE16:0) and Fig. S3 (MG16:0) are also shown as a comparison (yellow sticks). Hydrogen bonds are displayed as dashed orange lines.

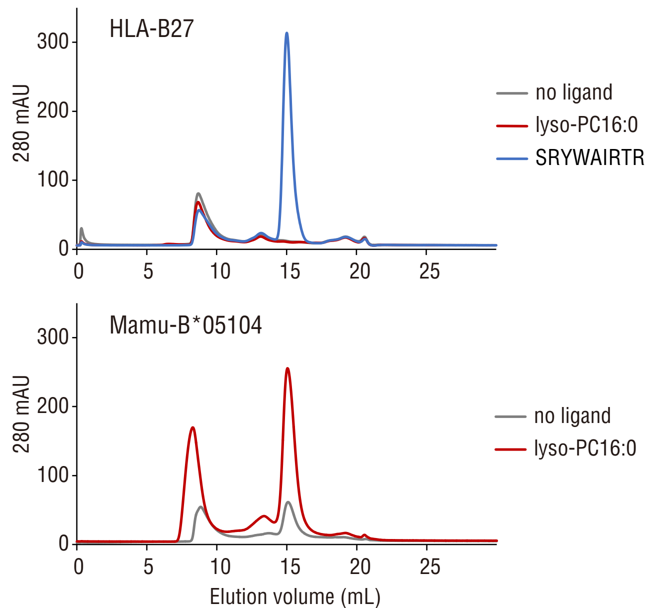

Figure S5. Lysophosholipids failed to induce a refolding of the recombinant HLA-B27

The recombinant HLA-B27 heavy chain (upper panel) was refolded with β2m in a buffer system in the presence or absence of indicated ligands (lyso-PC16:0 or SRYWAIRTR; the influenza A virus NP peptide), and the refolded samples were analyzed by size-exclusion chromatography (GE healthcare, Superdex 200 Increase 10/300GL). The formation of the heavy chain:β2m:ligand trimer complex was monitored by an increase of A280 values at an elution volume of 15 ml. The recombinant Mamu-B*05104 heavy chain (bottom) was also analyzed as a positive control to indicate the lyso-PC16:0-dependent protein refolding.