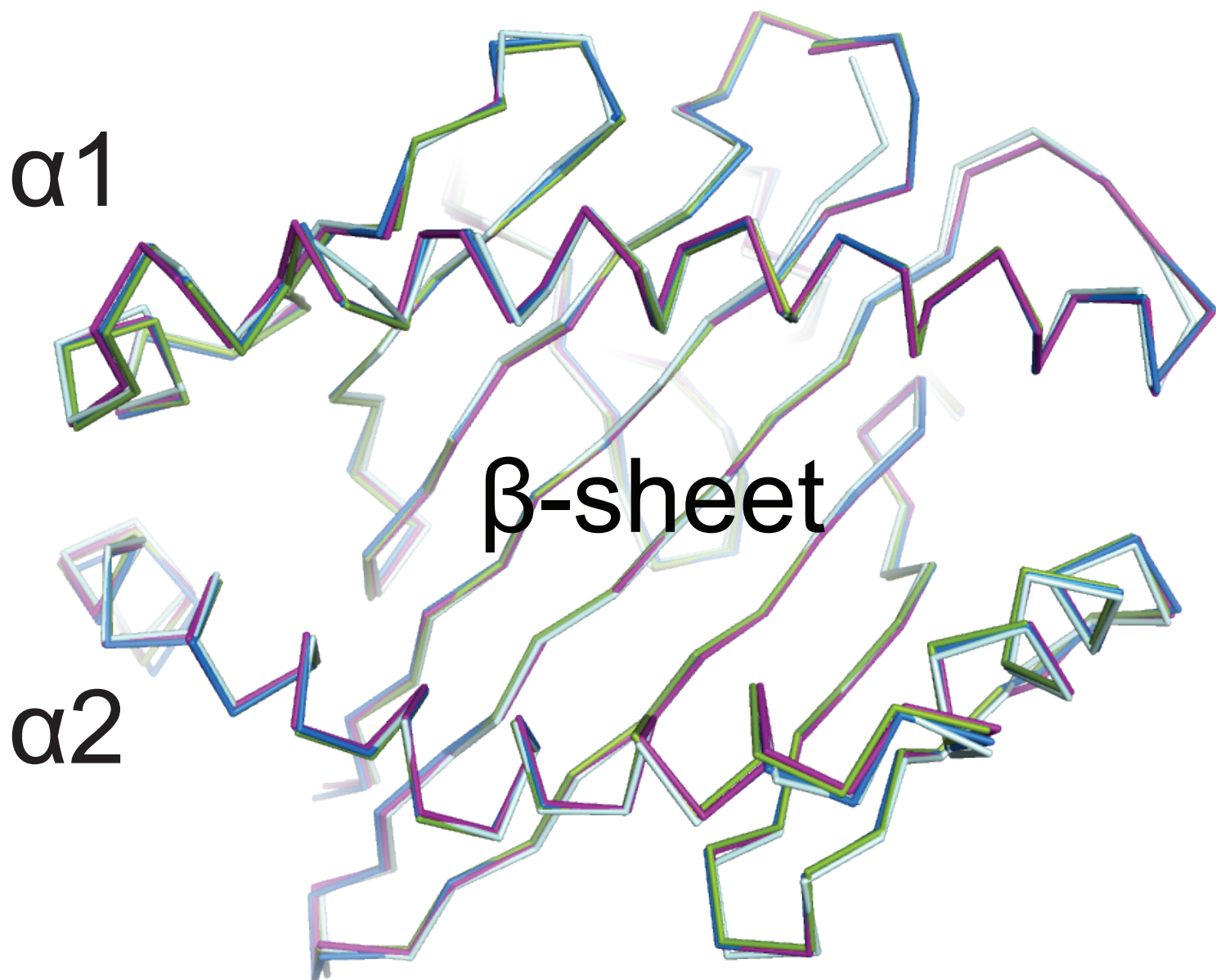


Human Va7.2Ja33 MAIT TCR/hbMR1/r_RL

Human Va7.2Ja33 MAIT TCR/bMR1

Unliganded human Va7.2Ja33 MAIT TCR

Human Va7.2Ja33 MAIT TCR/hMR1/RL_Me



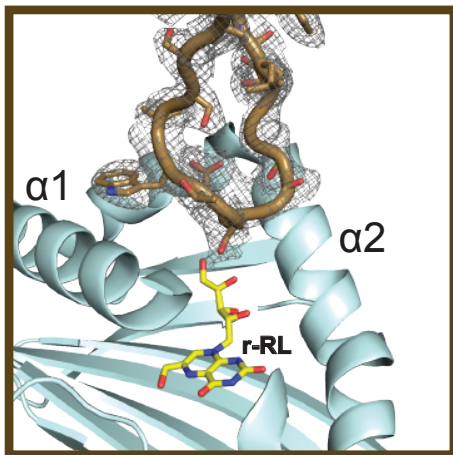
F7 MAIT TCR/hbMR1

G2 MAIT TCR/bMR1

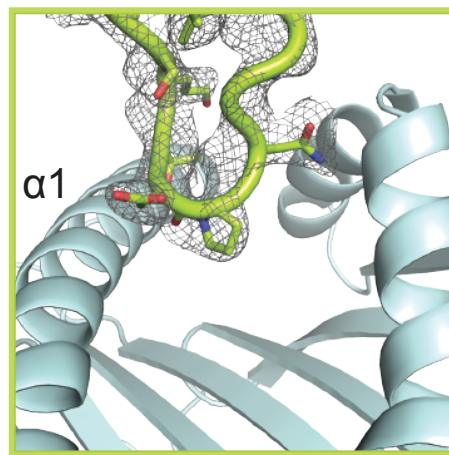
F7 MAIT TCR/bMR1

AE6 MAIT TCR/bMR1

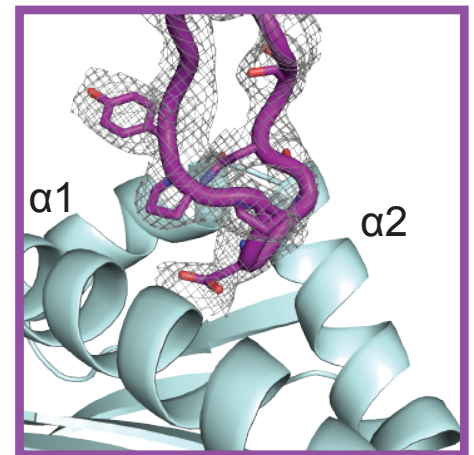
Supplementary Figure 4



F7 CDR3 β
TRBV6-1/TRBJ2.2



G2 CDR3 β
TRBV6-1/TRBJ2.2



AE6 CDR3 β
TRBV6-2/TRBJ1.3

Supplementary Figure 1. Detail of residues that are different in human and bovine MR1 that mediate MAIT TCR xenoreactivity. Our previous complex structure of a human MAIT TCR in complex with bovine MR1 (PDB ID: 4IIQ) allowed us to determine the residues in bovine MR1 that mediate the xeno-reactivity. These positions and their corresponding residues in bovine and human MR1 molecules are A72M, R147Q and Q151L. Displayed in this figure is a comparison of the positioning of these residues in both complexes with bovine and the hbMR1.

Supplementary Figure 2. The MAIT TCR CDR3 α loop and Tyr95, which contacts the stimulatory ligand rRL-6-CH₂OH, adopt a rigid and conserved conformation that is independent of the ligand. Shown for comparison are the CDR3 α loops in cartoon representation and Tyr95, in sticks, of an unliganded MAIT TCR α chain (orange, PDB ID: 4DZB), and those of the human F7 MAIT TCR bound to hbMR1/rRL-6-CH₂OH (brown) and bovine MR1 (blue) as well as the human MAIT TCR bound to human MR1 with RL-6-Me-7-OH (RL_Me) (mauve, PDB ID:4L4V). In all four cases, the MAIT TCR α chain variable domains are encoded by the V α 7.2 and J α 33 gene segments. The unliganded TCR is aligned to the others via the V domain, the others are all aligned via the MR1 molecules to which they are complexed.

Supplementary Figure 3. Superposition of bovine and hbMR1 structures in the different complexes. Displayed in ribbons are the α 1, α 2 and β -sheet platforms of the

hbMR1 from the complex with the F7 MAIT TCR (cyan, PDB ID: 4IIQ) and bovine MR1 from the complexes with F7 (marine), G2 (green) and AE6 (purple) MAIT TCRs.

Supplementary Figure 4. Electron density maps for the MAIT TCR CDR3 β loops. The CDR3 β loops were omitted for the calculation of the initial phases and built along the refinement processes and according to positive electron density maps. Shown are the final $2F_o - F_c$ electron density maps for the CDR3 β loops for the human F7 MAIT TCR/hbMR1/rRL-6-CH₂OH complex (left), human G2 MAIT TCR/bovine MR1 complex (center) and the human AE6 MAIT TCR/bovine MR1 complex (right).