

## Supplementary Tables

**Table S1.** Gene usages of humanized 44H10 candidates generated by IMGT CDR-grafting.

| VERSION # | CDR DEFINITION | IGHV | IGKV |
|-----------|----------------|------|------|
| 1         | IMGT           | 4-59 | 1-16 |
| 2         | IMGT           | 4-59 | 1-39 |
| 3         | IMGT           | 4-59 | 1-6  |
| 4         | IMGT           | 2-26 | 1-16 |
| 5         | IMGT           | 2-26 | 1-39 |
| 6         | IMGT           | 2-26 | 1-6  |
| 7         | IMGT           | 4-4  | 1-16 |
| 8         | IMGT           | 4-4  | 1-39 |
| 9         | IMGT           | 4-4  | 1-6  |

**Table S2.** Gene usages of humanized 44H10 candidates generated by Kabat CDR-grafting.

| VERSION # | CDR DEFINITION | IGHV | IGKV |
|-----------|----------------|------|------|
| 10        | Kabat          | 4-59 | 1-6  |
| 11        | Kabat          | 4-59 | 1-16 |
| 12        | Kabat          | 4-59 | 1-16 |
| 13        | Kabat          | 4-59 | 1-6  |
| 14        | Kabat          | 4-59 | 1-16 |
| 15        | Kabat          | 4-59 | 1-16 |
| 16        | Kabat          | 2-26 | 1-6  |
| 17        | Kabat          | 2-26 | 1-16 |
| 18        | Kabat          | 2-26 | 1-16 |

**Table S3.** X-ray crystallography data collection and refinement statistics for structures reported in this work (provided in separate file because of size considerations).

**Table S4.** Binding affinity and kinetics of humanized 44H10 antibodies to recombinant HLA-DR measured using biolayer interferometry. Measured on and off rates, as well as calculated dissociation constants are given, along with the standard error values.

|        | Parental IgG                                                | V17 IgG  | V21 IgG | V22 IgG  |
|--------|-------------------------------------------------------------|----------|---------|----------|
| HLA-DR | K <sub>D</sub> apparent (M)                                 | 2.23E-09 | N/A     | 9.46E-08 |
|        | K <sub>D</sub> apparent Error                               | 3.91E-11 | N/A     | 1.82E-09 |
|        | k <sub>on</sub> apparent (M <sup>-1</sup> s <sup>-1</sup> ) | 6.07E+05 | N/A     | 1.59E+05 |
|        | k <sub>on</sub> apparent Error                              | 5.92E+03 | N/A     | 2.98E+03 |
|        | k <sub>off</sub> apparent (s <sup>-1</sup> )                | 1.35E-03 | N/A     | 1.50E-02 |
|        | k <sub>off</sub> apparent Error                             | 1.97E-05 | N/A     | 6.73E-05 |

**Table S5.** Binding affinity and kinetics of wild-type (WT) and mutant V14 and V21 Fabs to recombinant HLA-DR measured using biolayer interferometry. Measured on and off rates, as well as calculated dissociation constants are given, along with the standard error values.

|        |                                                      | V14 Fabs |      |      |             | V21 Fabs |      |      |             |
|--------|------------------------------------------------------|----------|------|------|-------------|----------|------|------|-------------|
|        |                                                      | WT       | V71K | F78V | V71K + F78V | WT       | K71V | V78F | K71V + V78F |
| HLA-DR | <b>K<sub>D</sub> (M)</b>                             | N/A      | N/A  | N/A  | 2.06E-07    | 1.57E-07 | N/A  | N/A  | N/A         |
|        | <b>K<sub>D</sub> Error</b>                           | N/A      | N/A  | N/A  | 1.90E-09    | 1.49E-09 | N/A  | N/A  | N/A         |
|        | <b>k<sub>on</sub> (M<sup>-1</sup>s<sup>-1</sup>)</b> | N/A      | N/A  | N/A  | 1.99E+04    | 4.02E+04 | N/A  | N/A  | N/A         |
|        | <b>k<sub>on</sub> Error</b>                          | N/A      | N/A  | N/A  | 1.72E+02    | 3.58E+02 | N/A  | N/A  | N/A         |
|        | <b>k<sub>off</sub> (s<sup>-1</sup>)</b>              | N/A      | N/A  | N/A  | 4.10E-03    | 6.32E-03 | N/A  | N/A  | N/A         |
|        | <b>k<sub>off</sub> Error</b>                         | N/A      | N/A  | N/A  | 1.32E-05    | 2.06E-05 | N/A  | N/A  | N/A         |

**Table S6.** Binding affinity and kinetics of parental and humanized (V22) 44H10 IgGs to recombinant HLA-DR measured using biolayer interferometry. Measured on and off rates, as well as calculated dissociation constants are given, along with the standard error values.

|                     |       | Storage Temp. | Timepoint | K <sub>D</sub> (M) | K <sub>D</sub> Error | k <sub>on</sub> (M <sup>-1</sup> s <sup>-1</sup> ) | k <sub>on</sub> Error | k <sub>off</sub> (1/s) | k <sub>off</sub> Error |
|---------------------|-------|---------------|-----------|--------------------|----------------------|----------------------------------------------------|-----------------------|------------------------|------------------------|
| Parental IgG        | -20°C | Baseline      | Week 1    | 2.27E-07           | 7.79E-09             | 3.97E+05                                           | 1.30E+04              | 9.01E-02               | 9.45E-04               |
|                     |       |               | Week 2    | 4.13E-07           | 8.01E-09             | 3.01E+05                                           | 5.65E+03              | 1.24E-01               | 5.98E-04               |
|                     |       |               | Week 3    | 3.29E-07           | 1.06E-08             | 3.82E+05                                           | 1.18E+04              | 1.26E-01               | 1.09E-03               |
|                     |       |               | Week 4    | 2.96E-07           | 1.12E-08             | 4.09E+05                                           | 1.49E+04              | 1.21E-01               | 1.28E-03               |
|                     |       |               | Week 4    | 3.33E-07           | 9.92E-09             | 3.54E+05                                           | 1.01E+04              | 1.18E-01               | 9.46E-04               |
|                     | 4°C   | Baseline      | Week 1    | 2.45E-07           | 4.84E-09             | 3.85E+05                                           | 7.25E+03              | 9.44E-02               | 5.63E-04               |
|                     |       |               | Week 2    | 2.07E-07           | 7.76E-09             | 4.85E+05                                           | 1.73E+04              | 1.00E-01               | 1.18E-03               |
|                     |       |               | Week 3    | 1.91E-07           | 7.40E-09             | 5.06E+05                                           | 1.86E+04              | 9.67E-02               | 1.18E-03               |
|                     |       |               | Week 4    | 2.03E-07           | 6.80E-09             | 4.63E+05                                           | 1.47E+04              | 9.38E-02               | 9.88E-04               |
|                     | 40°C  | Baseline      | Week 1    | 2.97E-07           | 1.02E-08             | 3.75E+05                                           | 1.24E+04              | 1.12E-01               | 1.07E-03               |
|                     |       |               | Week 2    | 2.91E-07           | 1.26E-08             | 4.00E+05                                           | 1.67E+04              | 1.16E-01               | 1.41E-03               |
|                     |       |               | Week 3    | 2.75E-07           | 9.40E-09             | 3.85E+05                                           | 1.26E+04              | 1.06E-01               | 1.02E-03               |
|                     |       |               | Week 4    | 2.89E-07           | 8.72E-09             | 3.47E+05                                           | 1.01E+04              | 1.00E-01               | 8.43E-04               |
| Humanized (V22) IgG | -20°C | Baseline      | Week 1    | 2.85E-07           | 1.97E-08             | 4.49E+05                                           | 2.95E+04              | 1.28E-01               | 2.76E-03               |
|                     |       |               | Week 2    | 4.32E-07           | 5.57E-08             | 3.80E+05                                           | 4.74E+04              | 1.64E-01               | 5.52E-03               |
|                     |       |               | Week 3    | 3.25E-07           | 4.49E-08             | 4.23E+05                                           | 5.60E+04              | 1.37E-01               | 5.40E-03               |
|                     |       |               | Week 4    | 4.14E-07           | 4.75E-08             | 3.24E+05                                           | 3.58E+04              | 1.34E-01               | 4.17E-03               |
|                     |       |               | Week 4    | 3.93E-07           | 5.02E-08             | 3.34E+05                                           | 4.11E+04              | 1.31E-01               | 4.53E-03               |
|                     | 4°C   | Baseline      | Week 1    | 3.01E-07           | 2.83E-08             | 4.08E+05                                           | 3.64E+04              | 1.23E-01               | 3.57E-03               |
|                     |       |               | Week 2    | 2.92E-07           | 3.05E-08             | 4.68E+05                                           | 4.65E+04              | 1.37E-01               | 4.45E-03               |
|                     |       |               | Week 3    | 2.62E-07           | 1.86E-08             | 4.33E+05                                           | 2.92E+04              | 1.14E-01               | 2.54E-03               |
|                     |       |               | Week 4    | 2.12E-07           | 1.95E-08             | 4.70E+05                                           | 4.10E+04              | 9.96E-02               | 2.98E-03               |
|                     | 40°C  | Baseline      | Week 1    | 7.44E-07           | 2.66E-08             | 2.31E+05                                           | 8.08E+03              | 1.72E-01               | 1.31E-03               |
|                     |       |               | Week 2    | 3.27E-07           | 5.35E-08             | 4.31E+05                                           | 6.77E+04              | 1.41E-01               | 6.55E-03               |
|                     |       |               | Week 3    | 2.60E-07           | 2.54E-08             | 4.17E+05                                           | 3.87E+04              | 1.08E-01               | 3.36E-03               |
|                     |       |               | Week 4    | 4.03E-07           | 5.28E-08             | 3.45E+05                                           | 4.35E+04              | 1.39E-01               | 5.05E-03               |

**Table S7.** Table of contacts between the V22 Fab and HLA-DR.

| V22 RESIDUE | HYDROPHOBIC INTERACTIONS  | HYDROGEN BONDS                                 |
|-------------|---------------------------|------------------------------------------------|
| H-TYR96     | $\alpha$ -Glu88           | $\alpha$ -Val89 (x2)                           |
| H-TYR99     | $\alpha$ -Asp171          | $\alpha$ -Asp171                               |
| H-HIS100A   | $\alpha$ -Pro86           |                                                |
| H-TYR100B   | $\alpha$ -Pro86, A-Leu170 | $\alpha$ -Pro87                                |
| L-TYR32     | $\alpha$ -Thr83           | $\alpha$ -Asn84 (x2)                           |
| L-TYR49     |                           | $\alpha$ -Asp142                               |
| L-ALA50     | $\alpha$ -Val85           |                                                |
| L-SER52     |                           | $\beta$ -Glu63                                 |
| L-THR53     | $\alpha$ -Leu144          |                                                |
| L-LYS60     |                           | $\beta$ -Arg58, $\beta$ -Glu65, $\beta$ -Arg68 |
| L-SER65     |                           | $\beta$ -Glu81                                 |

Supplementary Figures

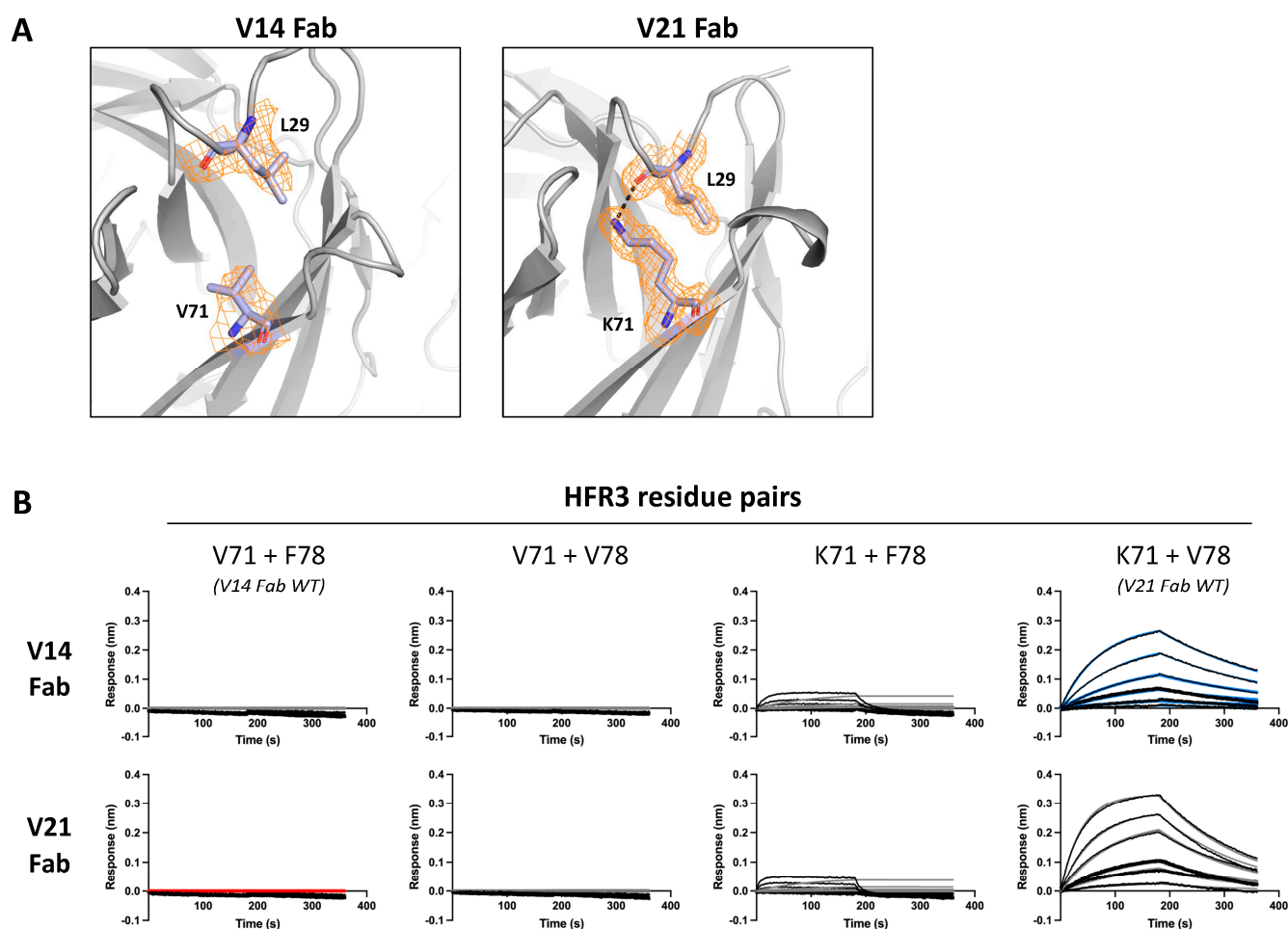


**Figure S1.** HFR3 residues 71 and 78 mediate humanized 44H10 binding to HLA-DR. (A) Composite omit map electron densities associated with residues at heavy chain positions 29 and 71 are shown as orange mesh and contoured at 1.0  $\sigma$ . The hydrogen bond formed by K71 and L29 in the V21 Fab is indicated by a black dotted line. (B) BLI binding profiles of V14 and V21 wild-type, single mutant and double mutant Fabs to recombinant HLA-DR, where black lines represent measured binding and colored curves correspond to the data fitted to a 1:1 binding model.

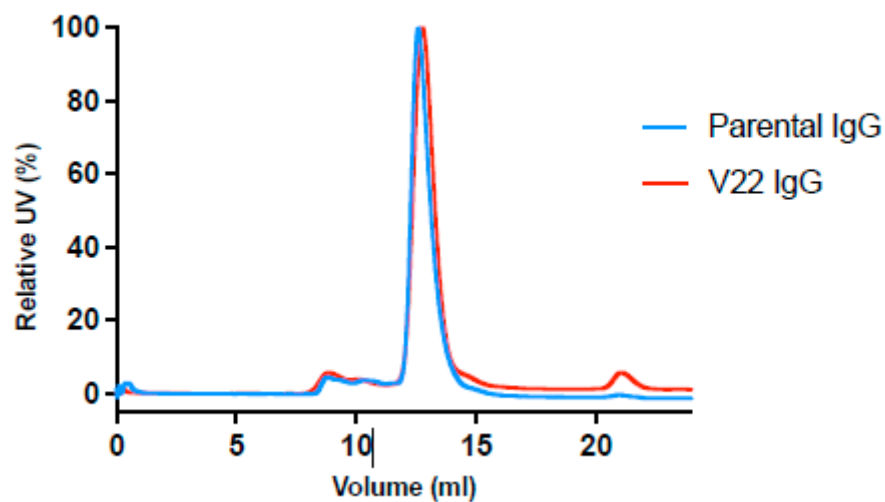


**Figure S2. Purified parental and V22 IgGs are highly monodisperse.** Size exclusion chromatography profiles (Superdex200) of parental 44H10 and V22 IgGs, revealing high monodispersity for both antibodies.

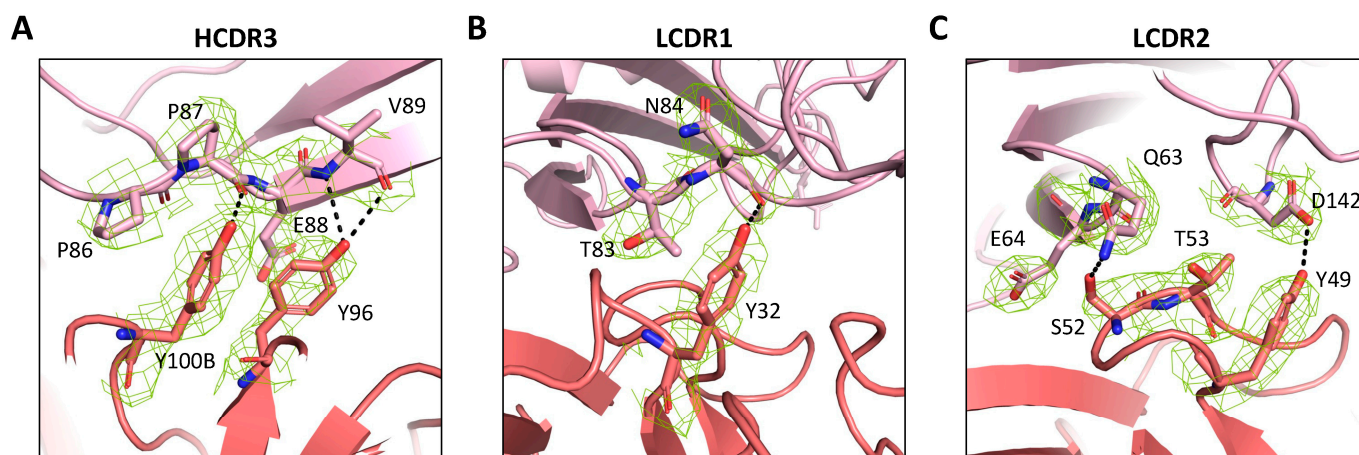


**Figure S3. V22 Fab replicates key antigen contacts mediated by 44H10.** (A-C) Composite omit map electron densities associated with residues in the V22 Fab HCDR3 (A), LCDR1 (B) or LCDR2 (C) that mediate key contacts with HLA-DR, shown as green mesh and contoured at 1.0  $\sigma$ . Hydrogen bonds are indicated by black dotted lines.