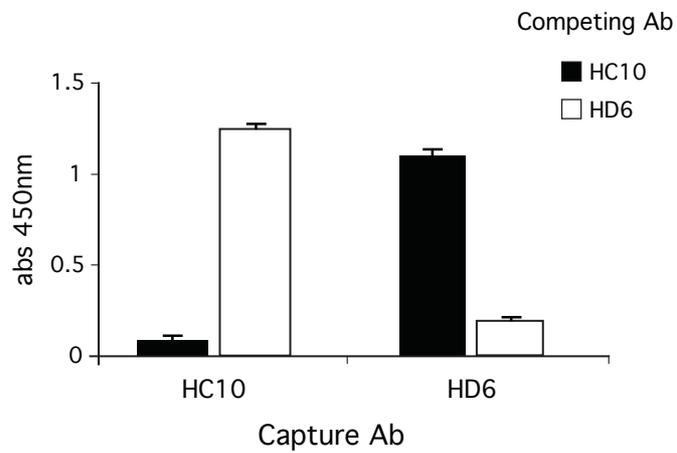


Supplementary Figure 1. Characterisation of the phage-derived HLA-B27₂-specific antibody HD6.

A. ELISA showing binding to B27₂ but not B27 heterotrimer of 4 phage-derived antibodies (HD4-6 and one non-binding phage (IRR)), HC10 and W6/32. Note B27 homodimers obtained using either of the B27-binding peptides were recognized by HD6. Representative of 3 independent experiments. **B.** Capture ELISA confirming the chimeric phenotype of HD6. Anti-mouse Fc capture antibody was used as coating antibody, followed by anti-human FAB-HRP as detection antibody. Anti-human Fc antibody, mouse and human antibodies served as controls. Representative of n=3 experiments. **C.** Direct ELISA against HLA-G and HLA-B27₂ homodimers (n=4) using HD6, HD10 and W6/32 antibodies. **D.** Western blot analysis of recombinant proteins (HLA- B8, B27 & B27₂) after reducing disulphide bonds using irreversible reducing agent TCEP 5mM. The upper of the HLA-B27 bands may represent a C-terminally truncated form of the recombinant protein.

Supplementary figure 2



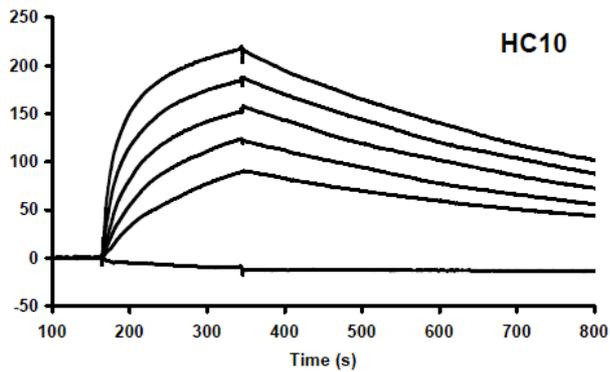
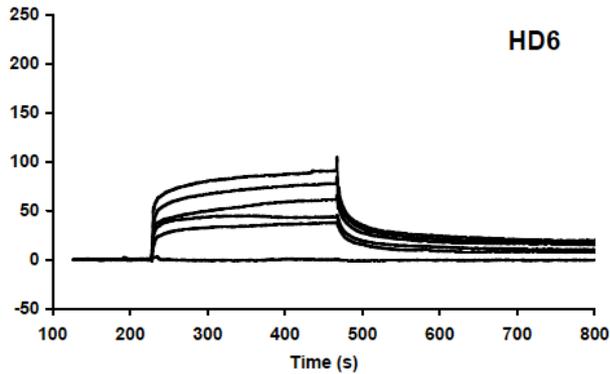
Supplementary Figure 2. HD6 has a different binding specificity for B27₂ compared to HC10

Competition between HD6 and HC10 binding for B27₂ in ELISA. B27 homodimer was pre-incubated either with HD6 or HC10 in excess amounts and the complex was allowed for binding on HD6 or HC10 coated wells. Experiments were performed in triplicates and representative of three independent experiments are shown.

Supplementary figure 3

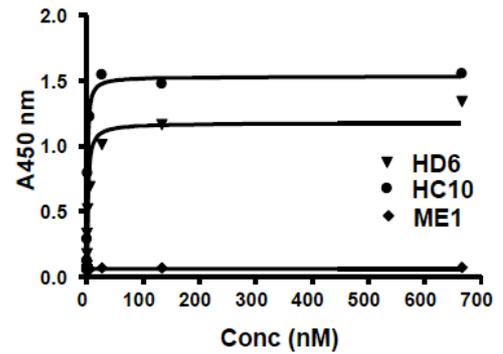
A

Fab binding to B27₂ in SPR



B

IgG binding to B27₂ in ELISA

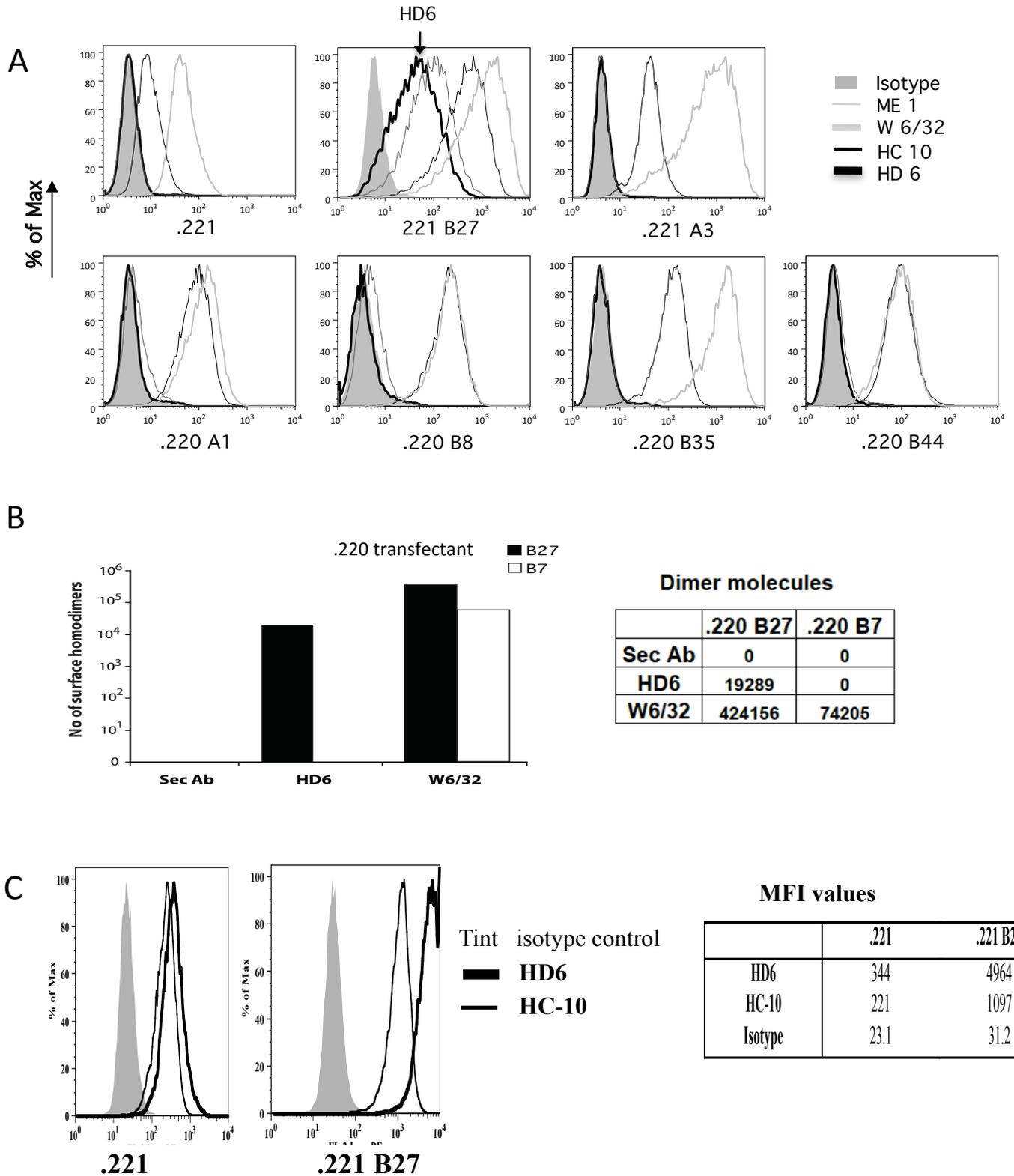


KD (nM)	HD6	HC10	ME1
Fab	270	220	----
IgG	1.77	1.00	1.3e-005

Supplementary Figure 3. HD6 and HC10 have comparable affinity and avidity for B27₂

A. Dissociation constant (Kd) was determined using Fab fragments of HD6 (upper panel) and HC10 (lower panel) over immobilized B27₂ in surface Plasmon resonance. Concentrations used were, from upper to lower traces, 8 μ M, 4 μ M, 2 μ M, 1 μ M, 500nM and 0 moles for HD6, and 6.8 μ M, 3.4 μ M, 1.7 μ M, 0.85 μ M, 0.45 μ M and 0 moles for HC10. Representative of three independent experiments are indicated. B. IgG affinity for homodimer was determined using serially 5 fold diluted HD6 or HC10 at 100 μ g/ml–1pg/ml (666 μ M–0.0006nM) on 1 μ g/L of immobilized HLA-B27₂. Representative of three independent experiments were shown. Estimated affinity constants of HD6 and HC10 FAbs are indicated in the table. ME1 (IgG1) served as irrelevant control.

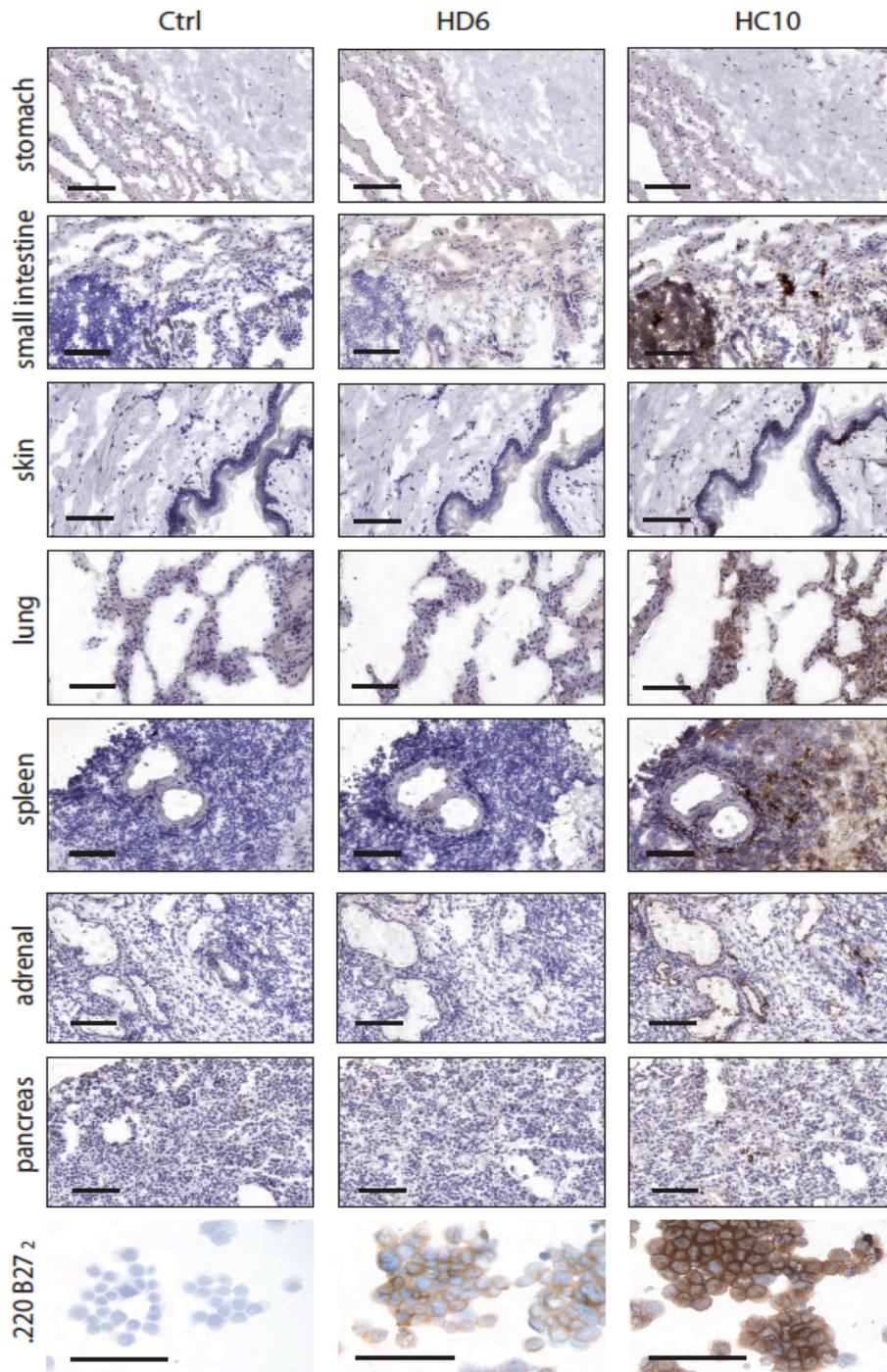
Supplementary figure 4



Supplementary Figure 4. Further characterisation of HD6 binding specificity

A. Representative staining of LBL721.220 and LBL721.221 cells expressing different HLA molecules with ME1, W6/32, HC10 and HD6 antibodies. Representative staining from three independent experiments. **B.** Semi-quantitative measurement of HD6 staining to LBL721.220 B27 cells was performed using Quantibrite beads as described. LBL721.220 B7 cells and W6/32 antibody served as controls. **C.** Intracellular staining of permeabilized (Cytoperm™ BD UK) .221 and .221B27 cells with HD6 and HC10.

Supplementary figure 5



Supplementary Figure 5. HD6 does not stain tissues from healthy humans.

Representative DAB stained sections from tissue arrays obtained from human healthy patients (Biochain). Isotype control antibody (left panel), HD6 antibody (central panel) and control HC10 (right panel) were tested. Magnification 20x, scale bar 40 μm . LBL721.220 and LBL721.220 B27 cells were used as positive controls (bottom panels). Magnification 40x, scale bar 50 μm .