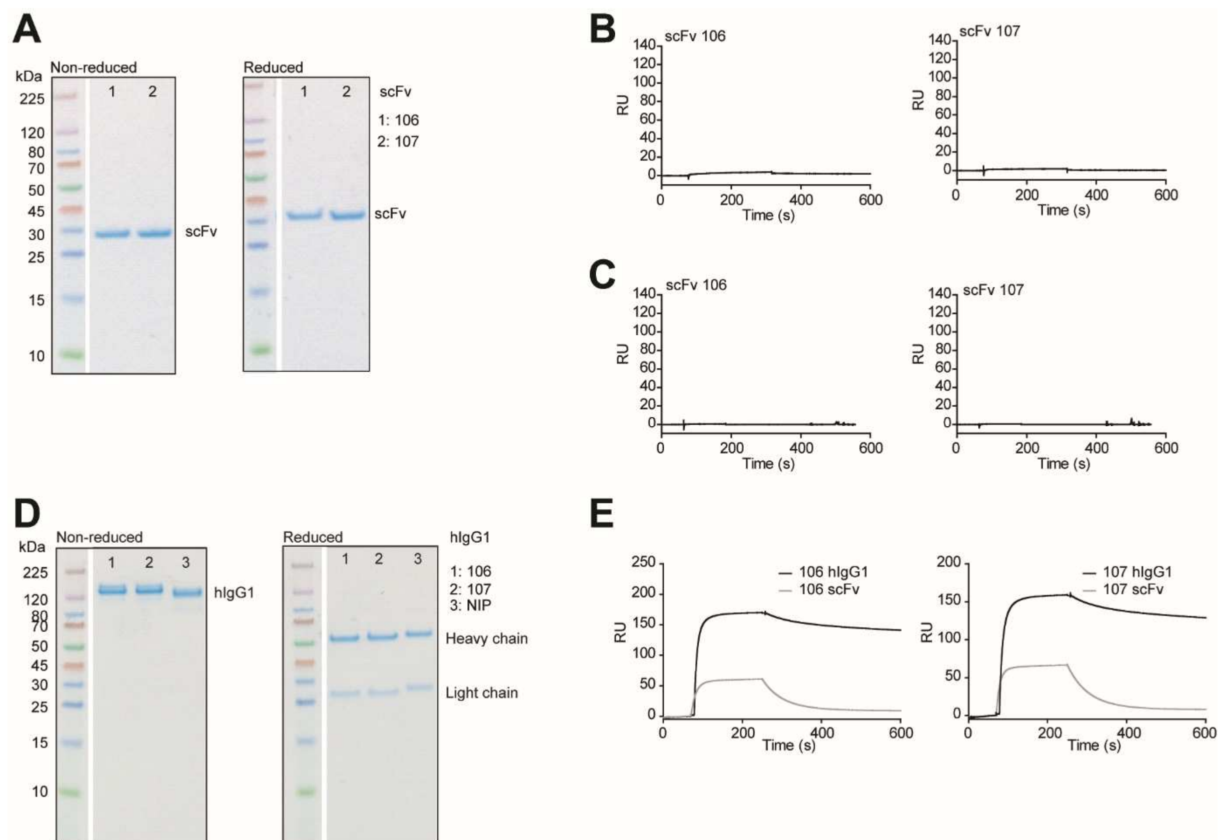
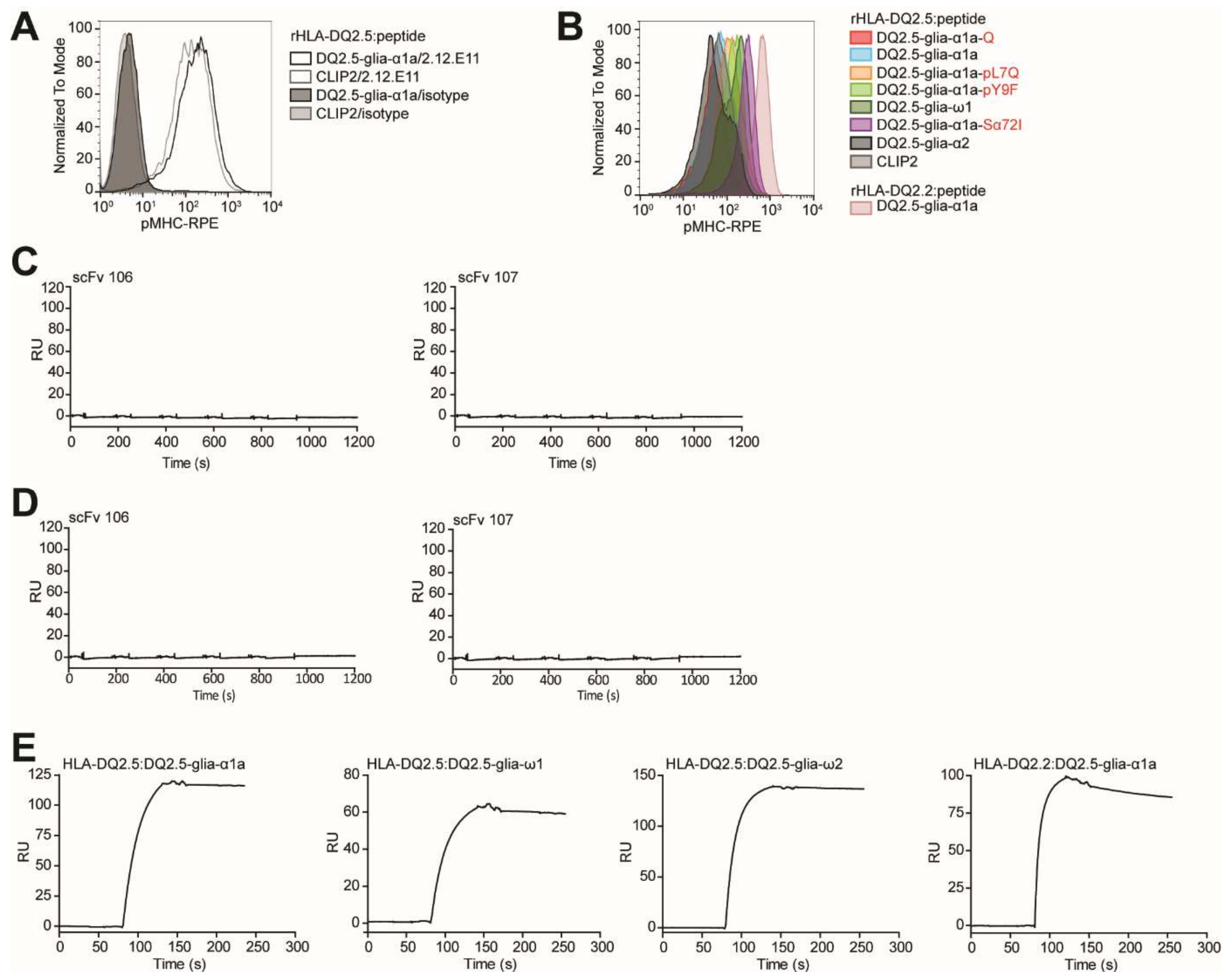
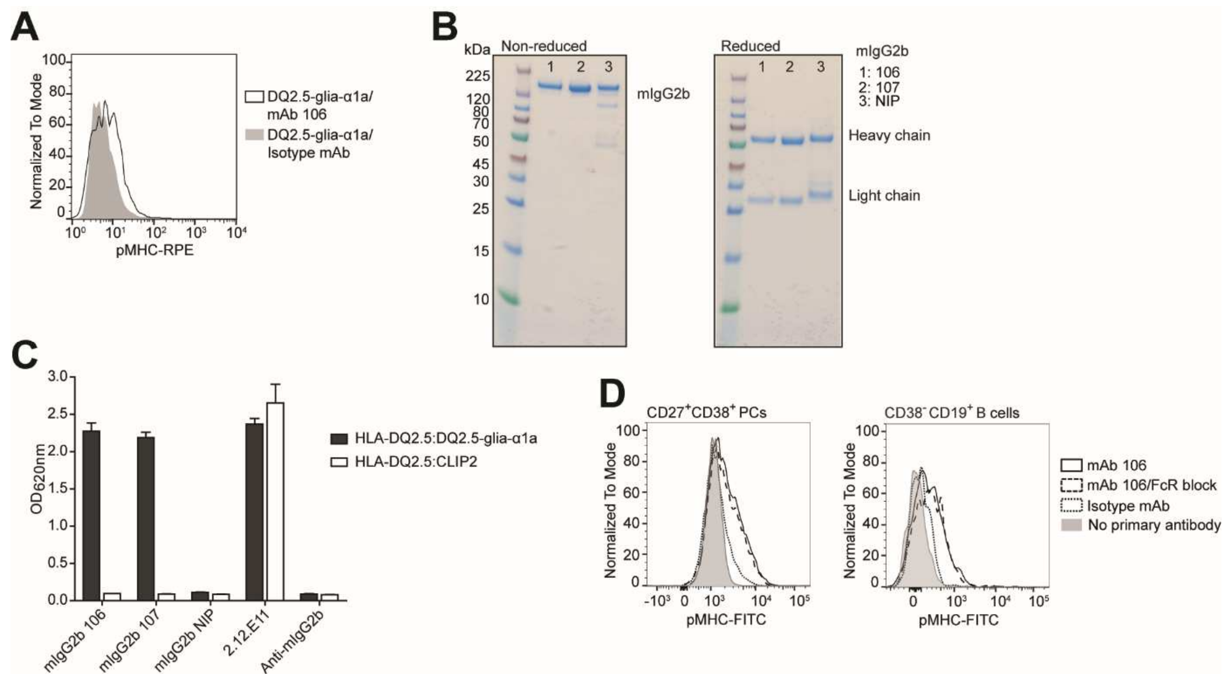


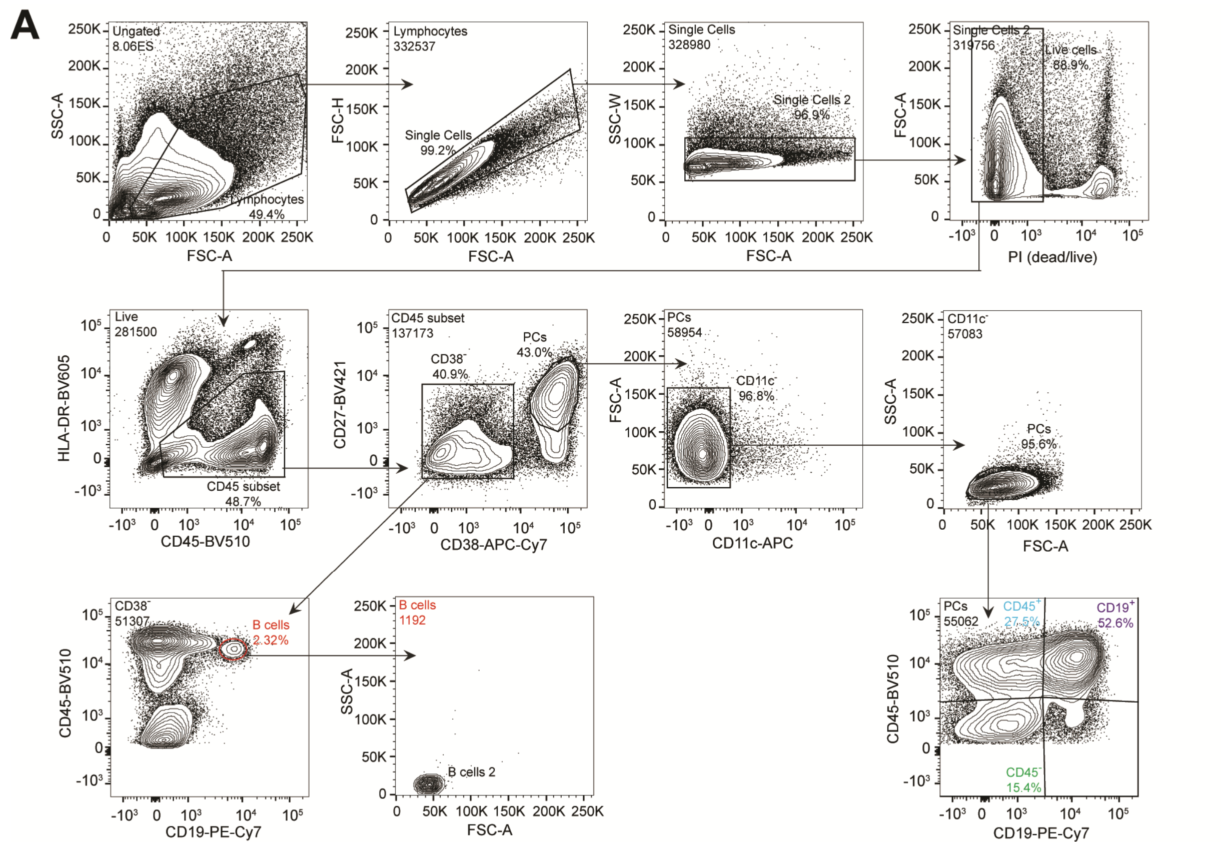
Supplementary Figure 1. Expression and binding of scFv clones and hIgG1 mAbs. (*A-C*) The lead scFv clones were expressed and purified from *E. coli* periplasmic fractions. (*A*) SDS-PAGE gel analysis of the scFv clones that specifically bound HLA-DQ2.5:DQ2.5-glia-α1a. Gels were run after purification by IMAC and size exclusion chromatography under non-reducing and reducing conditions. scFv size of approx. 30 kDa is indicated. (*B,C*) Representative SPR sensorgrams of the scFv clones for binding to (*B*) HLA-DQ2.5:CLIP2 (n=2) and (*C*) HLA-DQ2.5:DQ2.5-glia-α2 (n=2). (*D,E*) The lead scFv clones and isotype control scFv (anti-NIP) were reformatted to hIgG1 mAbs, expressed by transient transfection of HEK293E cells and purified from supernatants. (*D*) SDS-PAGE gel of purified hIgG1 mAbs run under non-reducing and reducing conditions. Appropriate-size bands at about 150 kDa for full-length hIgG1 and bands at 50 kDa and 23 kDa (reduced heavy and light chains, respectively) are indicated. (*E*) SPR sensorgrams of hIgG1 mAbs and the corresponding scFv clones binding to HLA-DQ2.5:DQ2.5-glia-α1a to validate gain in functional affinity after reformatting to full-length hIgG1 mAbs.

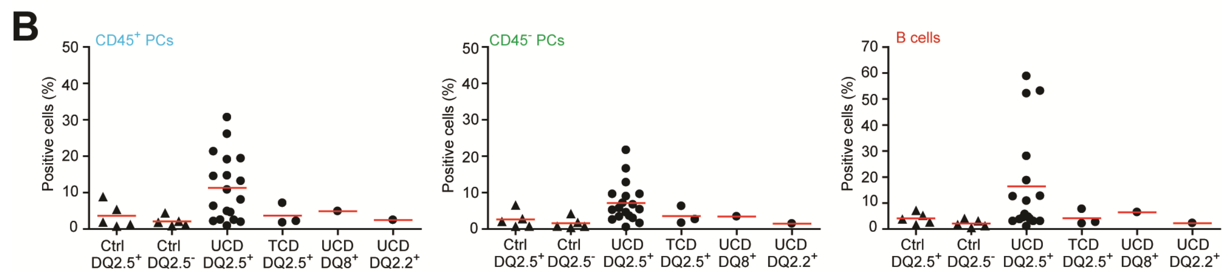


Supplementary Figure 2. Fine-specificity assessment. (*A*) Flow cytometric analysis of A20 B cells transduced to express HLA-DQ2.5 with covalently bound DQ2.5-glia-α1a or CLIP2 peptides stained with biotinylated mAb 2.12.E11 or isotype control mAb, followed by RPE-conjugated streptavidin (n=2). (*B*) Flow cytometric assessment of the pMHC expression level of the panel of A20 B cells transduced with either HLA-DQ2.5 or HLA-DQ2.2 with covalently bound peptide. Q indicates native (glutamine) DQ2.5-glia-α1a epitope. Unless specified, all epitopes are in the deamidated form. All cells were stained with biotinylated mAb 2.12.E11 followed by streptavidin-RPE (n=2). (*C,D*) Representative SPR sensorgrams showing binding to (*C*) HLA-DQ2.5:DQ2.5-glia-ω1 (n=2) and (*D*) HLA-DQ2.2:DQ2.5-glia-α1a (n=1) after capture of pMHC on sensor chips and injection of scFv clones as indicated. (*E*) SPR binding analysis of the HLA-DQ conformational-specific mAb SPV-L3 to evaluate the conformational integrity of HLA-DQ2.5:DQ2.5-glia-α1a, HLA-DQ2.5:DQ2.5-glia-ω1, HLA-DQ2.5:DQ2.5-glia-ω2, and HLA-DQ2.2:DQ2.5-glia-α1a as indicated after scFv binding experiments.

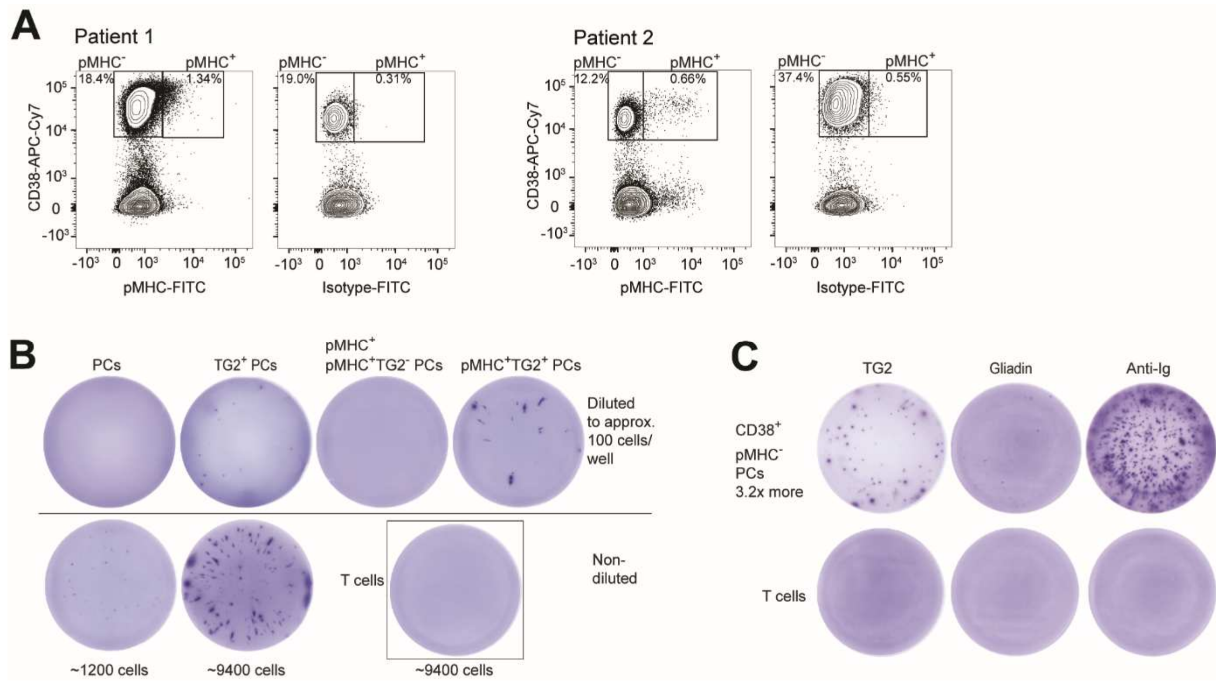


Supplementary Figure 3. Construction of mIgG2b mAbs and flow cytometric analysis of peptide-loaded cells and single-cell suspensions from celiac patient biopsies. (*A*) Monocyte-derived DCs were loaded with peptide and stained with hIgG1 mAb 106 or isotype control mAb before flow cytometric analysis (n=1). (*B*) SDS-PAGE gels of the mAbs 106 and 107 and isotype control mAb after reformatting to mIgG2b and purification from supernatants of transfected HEK293E cells. Full-length mIgG2b of approx. 150 kDa run under non-reducing conditions and separated heavy and light chains at approx. 50 kDa and 23 kDa run under reducing conditions are indicated. (*C*) Representative ELISA showing retained specificity of mIgG2b mAbs 106 and 107 after reformatting (n=2). mAb 2.12.E11 was included to control pMHC capture levels. (*D*) The figure is based on Fig. 3A showing detection of HLA-DQ2.5:DQ2.5-glia-α1a using mAb 106 with or without use of FcR block. Single-cell suspensions of intestinal biopsies from 3 patients all being HLA-DQ2.5$^+$ with Marsh 3B/C were run in parallel.

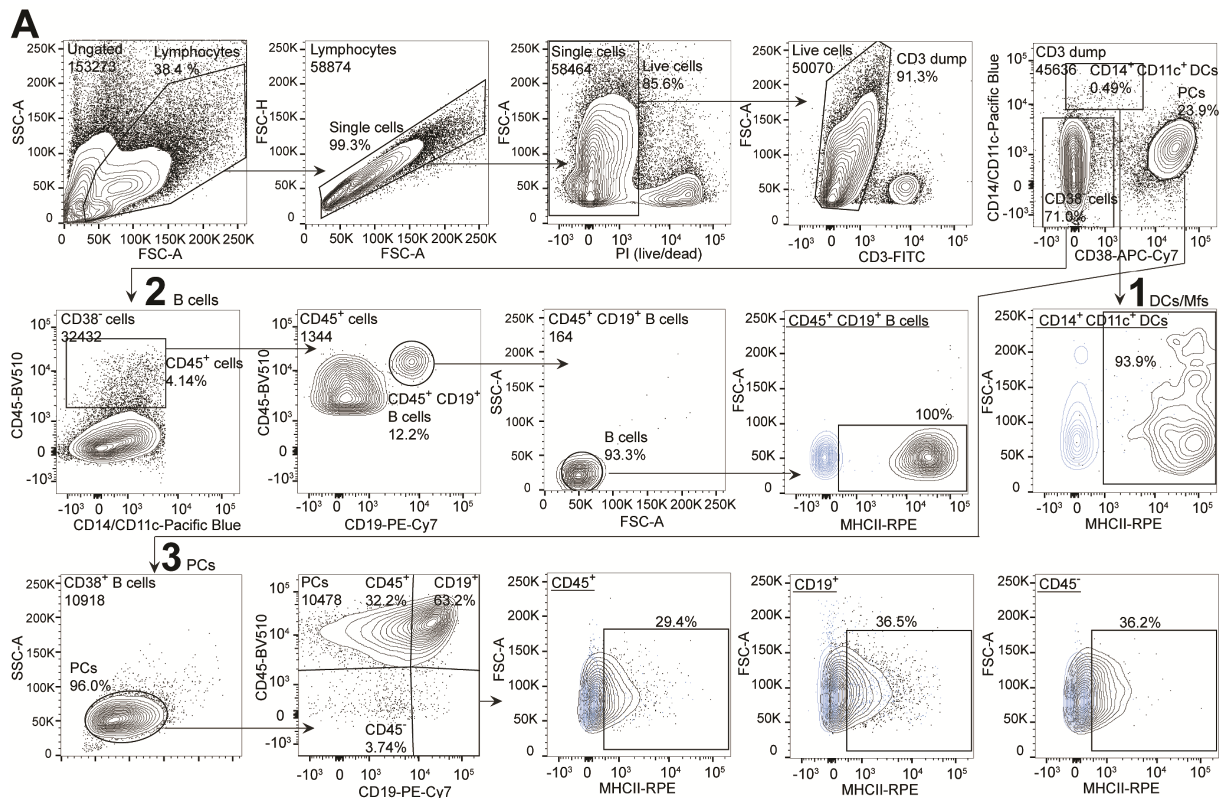




Supplementary Figure 4. Flow cytometric gating strategy and analysis of plasma cells and B cells presenting DQ2.5-glia-α1a peptide. Single-cell suspensions were prepared from intestinal biopsies, cells were stained with the indicated antibodies and immediately analyzed by flow cytometry. (*A*) Representative gating strategy for detection of gluten peptide presentation is shown. FSC-A, FSC-H and SSC-W were used to gate out doublet cells. (*B*) Stratification of the control patients among the $CD45^+$ plasma cells, $CD45^-$ plasma cells and B cells from Fig. 4B. Ctrl HLA-$DQ2.5^+$ (n=5), Ctrl HLA-$DQ2.5^-$ (n=5), UCD HLA-$DQ2.5^+$ (n=18), TCD HLA-$DQ2.5^+$ (n=3), UCD HLA-$DQ8^+$ (n=1), and UCD HLA-$DQ2.2^+$ (n=1). mIgG2b mAb 106 or 107 were used for detection and percent positive cells was determined relative to use of secondary antibody alone. Each data point represents an individual subject; non-celiac ctrl patients did not have mucosal alterations; red bars indicate mean percentage. (*A,B*) PCs, plasma cells.



Supplementary Figure 5. FACS sorting of plasma cells for ELISPOT analyses. (*A*) pMHC$^+$ and pMHC$^-$ CD38$^+$ plasma cell populations (gated as large viable, CD38$^+$, CD11c$^-$, CD14$^-$, CD4$^-$ cells) that were assessed for IgA and IgM expression in Fig. 5A. Notably, the two populations were FACS sorted and used for the TG2 and gliadin ELISPOT shown in Fig. 5C and panel C of this figure. (*B*) Representative TG2-specific ELISPOT using the sorted plasma cell subsets as indicated (n=3). TG2-specific IgA autoantibodies were captured onto TG2-coated plates and detected using AP-conjugated anti-IgA Ab. T cells were used as negative control. (*C*) TG2 and gliadin ELISPOT. Upper panel; CD38$^+$ pMHC$^-$ plasma cells were used in approximately 3.2x higher numbers than as shown in Fig. 5C, resulting in increased spot frequency and also visible gliadin spots in this group. Lower panel; T cells were used as negative control. Derived from the same ELISPOT experiment as shown in Fig. 5C. (*B,C*) PCs, plasma cells.



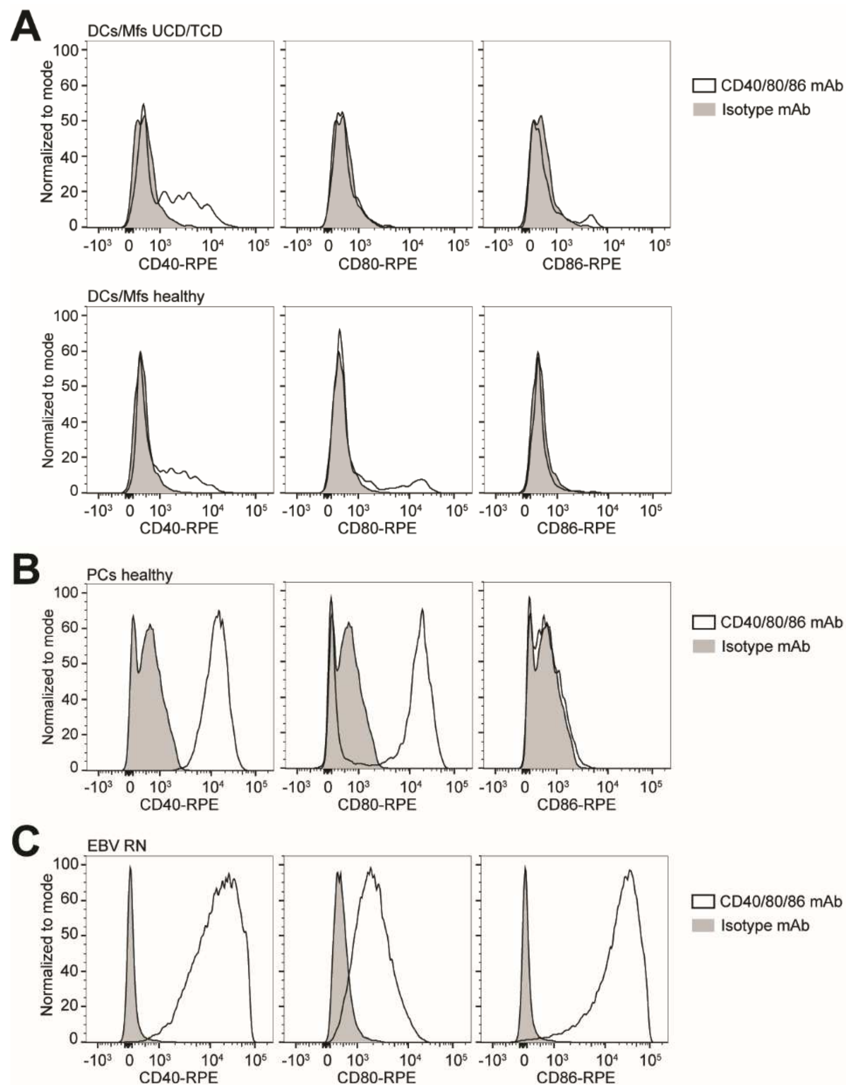
B

MHC class II (%)

60
50
40
30
20
10
0

MHCII Iso. HLA-DQ Iso.

Supplementary Figure 6. (*A*) Gating strategy for detection of MHCII on APC subsets. Single-cell suspensions prepared from intestinal biopsies were stained with the indicated antibodies and immediately analyzed by flow cytometry. Representative gating strategy for detection of MHCII on bulk DCs and Mfs (1), B cells (2) and plasma cells (3) are shown. MHCII staining (black) is overlaid isotype control staining (blue). FSC-A and FSC-H were used to gate out doublet cells. PCs, plasma cells. (B) Percentage MHCII expression on bulk plasma cell subsets (large, viable, $CD19^{+/-}CD45^{+/-}CD38^{+}$) stained with either a pan-MHCII mAb or an HLA-DQ2 mAb. Material was obtained from patients undergoing Whipple procedure and confirmed to have a normal intestinal histology (n=3). Each data point represents an individual subject.



Supplementary Figure 7. (*A,B*) Representative staining for costimulatory markers CD40, CD80 and CD86. (*A*) Costimulatory marker expression profile on DCs/Mfs from UCD or TCD patients (n=4, 3 HLA-DQ2.5^{+} UCD patients with Marsh 3A/B and 1 TCD patient with Marsh 0) and from healthy, non-inflamed gut samples (n=3, all confirmed non-inflamed duodenum). (*B*) Expression profile on plasma cells from the same healthy, non-inflamed gut samples as above. PCs, plasma cells. (*C*) Staining of the EBV-B cell line RN (CD114) was included as a positive control.

Supplementary Table 1. Kinetics of the scFv-HLA-DQ2.5:DQ2.5-glia-α1a interaction.

	Single cycle kinetics[a]				Steady state[b]	
Clone	k_{on} ($M^{-1}s^{-1}$)	k_{off} (s^{-1})	K_D (M)	SE K_D (M)	K_D (M)	SE K_D (M)
scFv 106	$1.29x10^5$	0.01262	$9.79x10^{-8}$	$5.63x10^{-8}$	$1.42x10^{-7}$	$2.20x10^{-8}$
scFv 107	$2.89x10^5$	0.02151	$7.43x10^{-8}$	$7.44x10^{-8}$	$6.70x10^{-8}$	$1.30x10^{-8}$

[a] Kinetics were determined by fitting data to a 1:1 Langmuir binding model.

[b] Steady state K_D was derived from the single cycle kinetics runs.

Supplementary Table 2. Recombinant pMHCs and soluble peptides.

Recombinant pMHC	Peptide amino acids
HLA-DQ2.5:DQ2.5-glia-α1a[a]	QLQPFPQPELPY
HLA-DQ2.5:DQ2.5-glia-α1a-Q	QLQPFPQPQLPY
HLA-DQ2.5:DQ2.5-glia-α1a-pL7Q	QLQPFPQPEQPY
HLA-DQ2.5:DQ2.5-glia-α1a-pY9F	QLQPFPQPELPF
HLA-DQ2.5:DQ2.5-glia-α1a-Sα72I	QLQPFPQPELPY
HLA-DQ2.5:DQ2.5-glia-α2	PQPELPYPQPE
HLA-DQ2.5:DQ2.5-glia-ω1	QQPFPQPEQPFP
HLA-DQ2.5:DQ2.5-glia-ω2	FPQPEQPFPWQP
HLA-DQ2.5:DQ2.5-glia-γ1	PEQPQQSFPEQERP
HLA-DQ2.5:DQ2.5-glia-γ2	QGIIQPEQPAQL
HLA-DQ2.5:DQ2.5-glia-γ3	TEQPEQPYPQP
HLA-DQ2.5:DQ2.5-glia-γ4c	TEQPEQPFPQP
HLA-DQ2.5:CLIP2	MATPLLMQALPMGAL
Soluble peptides	**Peptide amino acids**
DQ2.5-glia-α1a	QLQPFPQPELPY
DQ2.5-glia-α2	PQPELPYPQPQL
33mer	LQLQPFPQPELPYPQPELPYPQPELPYPQPQPF

The 9mer core sequence constituting the T-cell epitope is underlined and all peptides are in their deamidated form unless otherwise specified (native=Q). Soluble, recombinant pMHCs were generated as previously described[1]

[a] Or HLA-DQ2.2.

Supplementary Table 3. Antibodies used for ELISA.

Antigen	Conjugate	Clone	Supplier	Dilution
NeutrAvidin	-	-	Avidity	10 µg/ml
M13	HRP	Monoclonal	GE Healthcare	1:5000
His-tag	HRP	AD1.1.10	AbD Serotech	1:5000
Human IgG Fc	AP	Polyclonal	Sigma	1:2000
HLA-DQ	-	2.12.E11	Diatech[2]	1 µg/ml
Mouse IgG Fc	AP	Polyclonal	Sigma	1:2000

Normalized amounts of soluble, recombinant pMHC were captured onto the NeutrAvidin coated plates, before addition of phage/periplasmic fractions/0.5 µg/ml hIgG1. Samples were detected with anti-M13-HRP/anti-His-tag-HRP/anti-human IgG Fc-AP, respectively; mAb 2.12.E11 detected using anti-mouse IgG Fc-AP; 0.1 µg/ml hIgG1 was pre-incubated 30 min with non-biotinylated pMHC/peptides 2-fold diluted from 1 µM for competition assays (peptides specified in Supplementary Table 2). PBS supplemented with 4% (w/v) non-fat skim milk powder was used to block plates, and PBS supplemented with 0.05% (v/v) Tween-20 was used as buffer for dilution of samples and for washing the plates between each layer.

Supplementary Table 4. Antibodies used for flow cytometry.

Antigen	Conjugate	Clone	Supplier	Dilution
Staining of A20 B cells and peptide-loaded monocyte-derived DCs[c]				
hIgG1 106/107	-	106, 107	In-house	10 µg/ml
hIgG (F(ab')2)[a]	Biotin	Polyclonal	Southern Biotech	2 µg/ml
HLA-DQ2[a]	Biotin	2.12.E.11	Diatech[2]	10 µg/ml
Isotype control	Biotin	15H6	Southern Biotech	10 µg/ml
Staining of pMHC on DC, Mfs, plasma cells and B cells[d]				
mIgG2b 106/107	-	106, 107	In-house	10 µg/ml
Isotype control	-	NIP, MPC-11	In-house, Sigma	10 µg/ml
mIgG2b	FITC	Polyclonal	Southern Biotech	1 µg/ml
CD3	APC	OKT3	eBioscience	1:20
CD11c	APC	S-HCl-3	BD Biosciences	1:20
CD14	APC	HCD14	Biolegend	1:20
CD14	APC-Cy7	HCD14	Biolegend	1:20
HLA-DR	BV605	L243	Biolegend	1:20
CD45	BV510	H130	Biolegend	1:20
CD19	PE-Cy7	HIB19	Biolegend	1:20
CD38	APC-Cy7	HIT2	Biolegend	1:20
CD27	BV421	O323	Biolegend	1:20
Staining of MHCII on DC, Mfs, plasma cells and B cells[d]				
HLA-DP/DR/DQ/Dx	-	CR3/43	Santa Cruz Biotechnology	10 µg/ml
Isotype ctrl.	-	MOPC-21	BD Pharmingen	10 µg/ml
mIgG1	PE	A85-1	BD Biosciences	1 µg/ml
HLA-DQ	-	SPV-L3	Diatech	10 µg/ml
Isotype ctrl.	-	HOPC-1	Southern Biotech	10 µg/ml
mIgG2a	PE	RMG2a-62	Biolegend	1 µg/ml
CD3	FITC	OKT3	Biolegend	1:50
CD11c	BV450	B-Ly6	BD Horizon	1:20
CD14	Pacific Blue	M5E2	BD Pharmingen	1:20
CD45	BV510	H130	Biolegend	1:20
CD19	PE-Cy7	HIB19	Biolegend	1:20
CD38	APC-Cy7	HIT2	Biolegend	1:20
TG2 multimers[b]	APC	-	In-house	
Staining of co-stimulatory molecules[d]				
CD3	FITC	OKT3	Biolegend	1:50
CD11c	APC	S-HCl-3	BD Biosciences	1:20
CD14	APC	HCD14	Biolegend	1:20
CD45	BV510	H130	Biolegend	1:20
CD19	PE-Cy7	HIB19	Biolegend	1:20
CD38	APC-Cy7	HIT2	Biolegend	1:20
CD27	BV421	O323	Biolegend	1:20
CD40	PE	HI40a	ImmunoTools	1:20
CD80	PE	MEM-233	SeroTech	1:20
CD86	PE	2331 (FUN-1)	BD Pharmingen	1:20
Isotype ctrl.	PE	P3.6.2.8.1	eBioscience	1:20

1x PBS supplemented with 5% FCS and 0.1% NaN_3 was used as staining buffer and all stainings were performed on ice and with centrifugations at 4°C.

[a] Detected with streptavidin RPE (Invitrogen).

[b] Prepared by preincubation of biotinylated TG2 with Strep-tactin-APC (iba solutions) as described[3].

[c] Dead cells were excluded using 7-AAD and samples were immediate analysis on FACSCalibur (BD).

[d] Propidium iodide exclusion of dead cells and human FcR Blocking Reagent (Miltenyi Biotec) was included. Cells were immediately acquired on LSR Fortessa cytometer (BD).

Supplementary Table 5. Antibodies used for FACS sorting.

Antigen	Conjugate	Clone	Supplier	Dilution
Sorting of plasma cells, panel 1				
hIgG1 106	Alexa-488	106	In-house	10 µg/ml
hIgG1 RSV	Alexa-488	RSV	In-house	10 µg/ml
CD4	Pacific Blue	SK3	Biolegend	1:33
CD8	Pacific Blue	SK1	Biolegend	1:33
CD14	Pacific Blue	M5E2	Biolegend	1:33
CD11c	BV450	B-Ly6	BD Horizon	1:33
CD27	PE-Cy7	LG.7F9	eBioscience	1:500
IgA	PE	Polyclonal	Southern Biotech	1:2000
TG2 multimers[a]	APC	-	In-house	
Sorting of plasma cells, panel 2				
mIgG2b 107	-	107	In-house	10 µg/ml
Isotype control	-	NIP, OMV	In-house[4]	10 µg/ml
mIgG2b	FITC	Polyclonal	Southern Biotech	1 µg/ml
CD4	Pacific Blue	SK3	Biolegend	1:20
CD14	Pacific Blue	M5E2	Biolegend	1:20
CD11c	BV450	B-Ly6	BD Horizon	1:20
CD38	APC-Cy7	HIT2	Biolegend	1:20
IgA	APC	REA1014	MACS	1:20
IgM	PE	SA-DA4	eBioscience	1:20

1x PBS supplemented with 5% FCS and 0.1% NaN_3 was used as staining buffer and all stainings were performed on ice and with centrifugations at 4°C. Samples were sorted on FACSAriaII (BD) with a 100 µM nozzle.

[a] Prepared by preincubation of biotinylated TG2 with Strep-tactin-APC (iba solutions) as described[3].

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4. Høydahl LS, Nilssen NR, Gunnarsen KS, et al. Multivalent pIX phage display selects for distinct and improved antibody properties. Sci Rep 2016;6:39066.