Supporting Information

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SI Methods

HLA-DQ2.5 Testing. Subjects were tested for HLA-DQ2.5 by PCR using the following primers: DQA 5′-TCTTATGGTGTAAACTTGTACCAGTC-3′; DQA 3′-ATCAGACTGTTCAAGTTATGTTTTAGG-5′; DQB 5′-GCGTGCGTCTTGTGAGCAGAAG-3′; and DQB 3′-CCTGTCCACCGCCGCCCGTTT-5′.

Intestinal Biopsy Preparation. Three to four intestinal biopsy fragments were incubated in Roswell Park Memorial Institute medium (RPMI) with 5% FCS containing 0.5 mg/ml of Type 4 collagenase (Worthington). Cells were periodically disrupted during incubation by passing through a syringe topped with a blunt-ended 16-gauge needle. Lymphocytes were enriched through Percoll (GE Healthcare) gradient centrifugation. CyTOF staining was performed on freshly isolated lymphocytes.

Flow Cytometry. The following antibody clones were used for flow cytometry: anti-CD3 (SK7; Biolegend), anti-CD4 (RPA-T4; Biolegend), anti-CD8 (OKT8; eBioscience), anti–γδ-T-cell receptor (TCR) (MHGD04; Invitrogen), anti-CD38 (HIT2; Biolegend), anti–integrin-β7 (FIB504; eBioscience), anti-CD103 (Ber-ACT8; Biolegend), anti-CD27 (O323; eBioscience), and anti-NKG2D (1D11; Biolegend). Dead cells were excluded using a LIVE/DEAD Fixable Dead Cell Stain Kit (Invitrogen).

Time-of-Flight Mass Cytometry Staining. Time-of-flight mass cytometry (CyTOF) and data acquisition were performed as described (1). Antibodies used for mass cytometry included: anti–integrin-β7 (FIB504; Biolegend), anti-CD103 (B-Ly7; Biolegend), anti-γδTCR [using anti-Phycoerythrin (PE) (PE001; Biolegend) as secondary, with PE-labeled γδTCR (SA6.E9; Invitrogen) as primary], anti–IL-17 (BL168; Biolegend), anti–IL-4 (8D4-8; Becton Dickinson), anti–IL-10 [using a biotinylated primary antibody (JES3-12G8; Biolegend), and heavy metal-conjugated streptavidin as secondary as described (1)], anti-CD5 (UCHT2; Biolegend), anti-CD25 (M-A251; Becton Dickinson), anti-CD95 (DX2; Biolegend), and anti–CTLA-4 (BNI3; Becton Dickinson). All other antibody clones used for CyTOF can be found in Newell et al. (1). Cryopreserved peripheral blood mononuclear cells (or freshly isolated intestinal lymphocytes) were thawed and washed with complete RPMI before overnight recovery at 37 °C. Cells were transferred to 96-well plates (or tubes), washed, and resuspended in cytometry buffer [PBS, 0.05% sodium azide, 2 mM EDTA, 2% (vol/vol) FCS] for staining as previously described (1). For stimulation, all cells were cultured for 3 h at ∼15×10^6 per mL in complete RPMI [10% (vol/vol) FCS] plus 1× brefeldin A (eBioscience), 1× monensin (eBioscience), 2.5 μg/mL anti-CD107a, 1.25 μg/mL anti-CD107b, and 10 μM TAPI-2 (VWR International). For phorbol-12-myristate-13-acetate+ionomycin stimulation, 150 ng/mL PMA + 1 μM ionomycin was added to the cells.

At the end of the 3-h stimulation, cells were pipetted vigorously to remove adherent cells from the plate and transferred to 96-well plates (or tubes), washed, and resuspended in cytometry buffer. The cells were incubated for 30 min on ice with a prepared mixture of metal-conjugated surface-marker antibodies at concentrations found to be effective in prior antibody tests. After surface staining, cells were washed once and resuspended in 20 μM ^{115}In-loaded maleimido-monoamine-DOTA (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid) in PBS [a sulfhydryl-reactive trivalent cation-chelating bifunctional ligand; Macrocyclics B-272; mixed with a 0.5 molar ratio of ^{115}InCl and stock solution dissolved in L-buffer (DVS Sciences) at 1 mM, stable at 4 °C, and working much like commercially available amine-reactive LIVE/DEAD staining reagents (Invitrogen)]. Cell fixation using paraformaldehyde (Electron Microscopy Sciences), DNA labeling with an iridium-containing DNA intercalator (DVS Sciences), intracellular staining, washing, and data acquisition was performed as described (1).

TCR Sequencing. A series of three nested PCRs was performed on single sorted T cells. For the first reaction, reverse transcription and preamplification were performed with a One-Step RT-PCR Kit (Qiagen) using multiplex PCR with multiple Vβ or Vδ region primers and a Cβ or Cδ region primer. When necessary, base degeneracy was incorporated into the primers to account for TCR polymorphism and ensure amplification of all known functional TCRVβ or TCRVδ and TCRCβ and Cδ regions identified in the international ImMunoGeneTics information system (IMGT) database (www.imgt.org). Next, an aliquot of the first reaction was used as a template for the second PCR using a set of multiple internally nested TCRVβ or TCRVδ primers and an internally nested Cβ or Cδ primer with a HotStarTaq DNA Polymerase Kit (Qiagen). The second set of TCRV region primers also incorporated base degeneracy when needed and contained a common 23-base sequence at the 5′ end to enable further amplification with a common 23-base primer. The third and final PCR was performed on an aliquot of the second reaction using a primer containing the common 23-base sequence (incorporated into the second set of Vβ primers) and a third internally nested Cβ or Cδ primer using HiFi Hotstar DNA polymerase (Qiagen). Amplified PCR products were treated with ExoSAP-IT (Affymetrix) and sequenced using primers from the final PCR from both ends. Primer sequences for TCRβ sequencing can be found in Su et al. (2). Primer sequences for TCRδ can be found in Table S4.

TCR Sequence Analysis. TCR sequence analysis was performed with VDJFasta (3). Segment classification was performed to reference segment databases from the IMGT. CDR3 from all domains was extracted and translated using TCR-specific profile hidden Markov models, constructed from 95% nonredundant concatenations of IMGT V, D, and J segments. TCR junctional analysis was performed using IMGT/V-Quest (www.imgt.org/). A dataset of 165,291 naive $CD8^+$ TCRβ sequences (4) was used as a control for CDR3β convergence. To generate TCRδ reference sequences, between 10^5 and 10^6 TCR$\gamma\delta^+$ T cells from the peripheral blood (PB) of eight different individuals and intraepithelial lymphocytes (IELs) from one individual were sorted by flow cytometry. RNA was extracted using an RNeasy RNA extraction kit (Qiagen). RNA from each of these samples was amplified and sequenced using the primers described above. Sequencing was performed by using an Illumina MiSeq platform after incorporation of Illumina paired-end adapters through PCR. As a control for TCRδ convergence, 18,579 total unique TCRδ sequences using TRDV1 were used. Motif enrichment was evaluated by comparing the observed vs. expected frequency of 2-mer and 3-mer motifs within CDR3β or CDR3δ clones using the same V region. Enrichment is represented as the odds of encountering enrichment of the motif in the reference dataset to the degree observed in the selected set. The significance of motif enrichment was evaluated by using the Fisher's exact test with the Bonferroni

correction such that P values <0.05/<howmany> = 1e−4 were considered to be statistically significant. Analysis was performed in R version 2.11.1. The most statistically significant examples are illustrated.

1. Newell EW, Sigal N, Bendall SC, Nolan GP, Davis MM (2012) Cytometry by time-of-flight shows combinatorial cytokine expression and virus-specific cell niches within a continuum of CD8(+) T cell phenotypes. *Immunity* 36(1):142–152.
2. Su LF, Kidd BA, Han A, Kotzin JJ, Davis MM (2013) Virus-specific CD4(+) memory-phenotype T cells are abundant in unexposed adults. *Immunity* 38(2):373–383.
3. Glanville J, et al. (2011) Naive antibody gene-segment frequencies are heritable and unaltered by chronic lymphocyte ablation. *Proc Natl Acad Sci USA* 108(50):20066–20071.
4. Warren RL, et al. (2011) Exhaustive T-cell repertoire sequencing of human peripheral blood samples reveals signatures of antigen selection and a directly measured repertoire size of at least 1 million clonotypes. *Genome Res* 21(5):790–797.

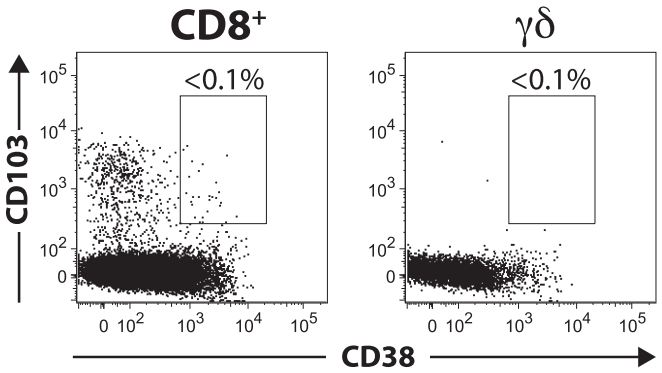


Fig. S1. Patients with active celiac disease (CD), as determined by ongoing symptoms and positive autoantibody titers, have αEβ7$^+$CD38$^+$CD8$^+$ and γδ T-cell proportions below background levels of 0.05% and 0.01%, respectively. Representative FACS analysis of CD8$^+$ αβ and γδ T cells with respect to CD103 (αE integrin) and CD38.

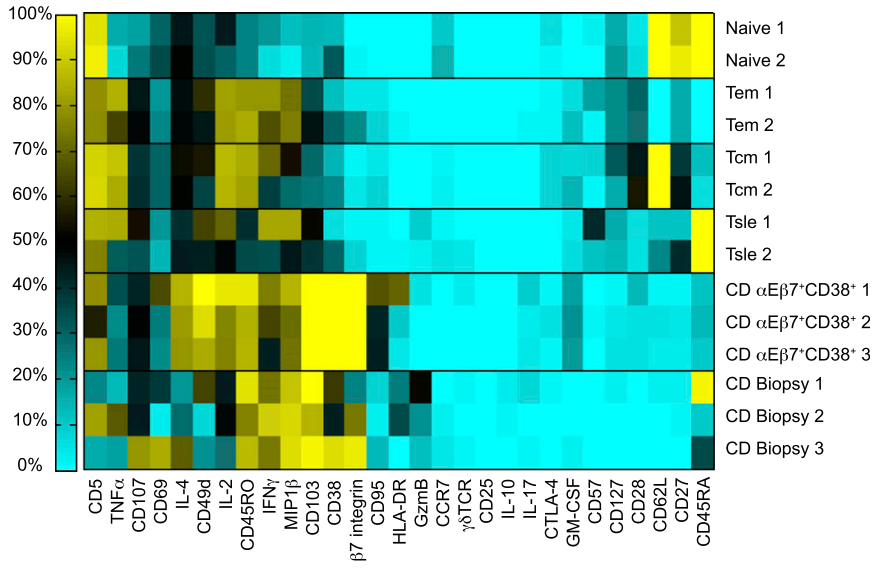


Fig. S2. Phenotype and functional capacity of αEβ7$^+$CD38$^+$CD8$^+$ T cells resemble effector memory cells and CD8$^+$ IELs. Functional capacities of the indicated cell types with respect to the indicated markers are plotted as a heat plot. The color scale is shown (*Left*) and indicates the percentage of cells expressing the indicated marker. Cells were stimulated with PMA and ionomycin and analyzed for the indicated cell-surface or intracellular markers. Cells were segregated based on stringent criteria based on the gates indicated: naive (CD45RA$^+$CD27$^+$CD62L$^+$CCR7$^+$), effector memory (Tem; CD45RA$^-$CD27$^-$CD62L$^-$CCR7$^-$), central memory (Tcm; CD45RA$^-$CD27$^+$CD62L$^+$CCR7$^+$), short-lived effector (Tsle; CD45RA$^+$CD27$^-$CD62L$^-$CD28$^-$), celiac PB (CD3$^+$αEβ7$^+$CD38$^+$CD8$^+$), and celiac biopsy (CD3$^+$CD8$^+$). All blood samples analyzed are from celiac patients on day 6 following gluten challenge. Biopsy samples are from different celiac patients with active celiac disease including villous blunting and IEL expansion by histologic examination.

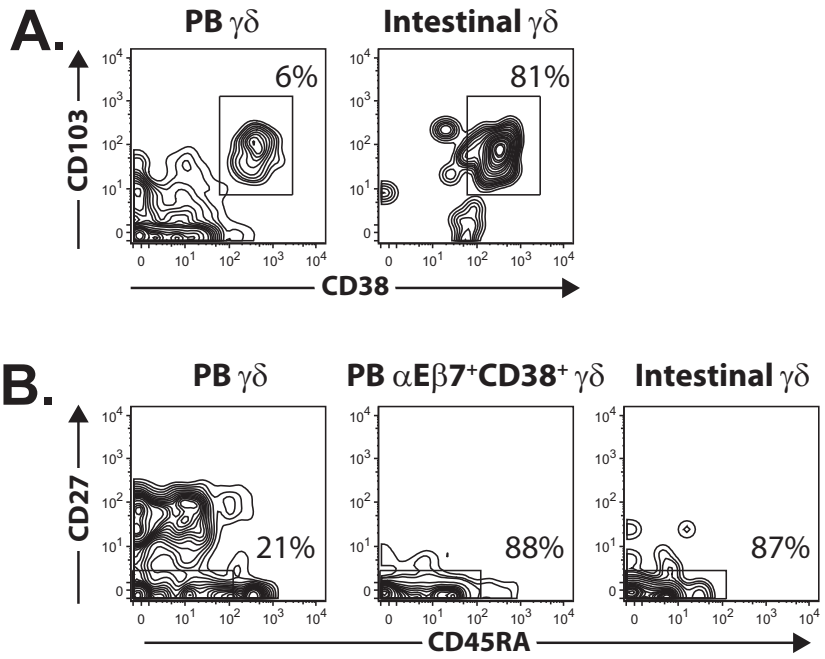


Fig. S3. Peripheral blood αEβ7$^+$CD38$^+$ γδ T cells induced by oral gluten challenge express surface markers of memory cells and resemble intestinal epithelial γδ T lymphocytes from celiac mucosal biopsies. (*A*) CyTOF analysis of total peripheral blood γδ and total intestinal γδ T cells with respect to CD103 and CD38 expression. (*B*) CyTOF analysis of total peripheral blood γδ, αEβ7$^+$CD38$^+$ γδ, and total celiac intestinal γδ with respect to CD27 and CD45RA expression. αEβ7$^+$ CD38$^+$ γδ and celiac intestinal γδ T cells are predominantly CD27$^-$ and CD45RA$^-$, consistent with a memory phenotype.

A.

Patient 1 αEβ7$^+$CD38$^+$ CD8	TCRβ CDR3	Freq 1 (98)	Freq 2 (94)
	CASSPGTDTQYF	9	20
	CSVEMNTEAFF	2	13
	CASSFGGELFF	5	1
	CASSAGHPEQFF	3	3
	CASSNLRQGAAGNTIYF	3	3
	CASSQEEQGAFYEQFF	3	3
	CASTEGQAEAFF	3	2
	CASSLINTEAFF	3	1
	CSVDGNYLTDTQYF	3	1
	CAWSVKTLRRADTQYF	1	3
	CASRIQGEGSPLHF	2	1
	CASSLASVGSTEAFF	1	2
	CASEMDANTGELFF	1	1
	CASSPFSGDYYEQYF	1	1

B.

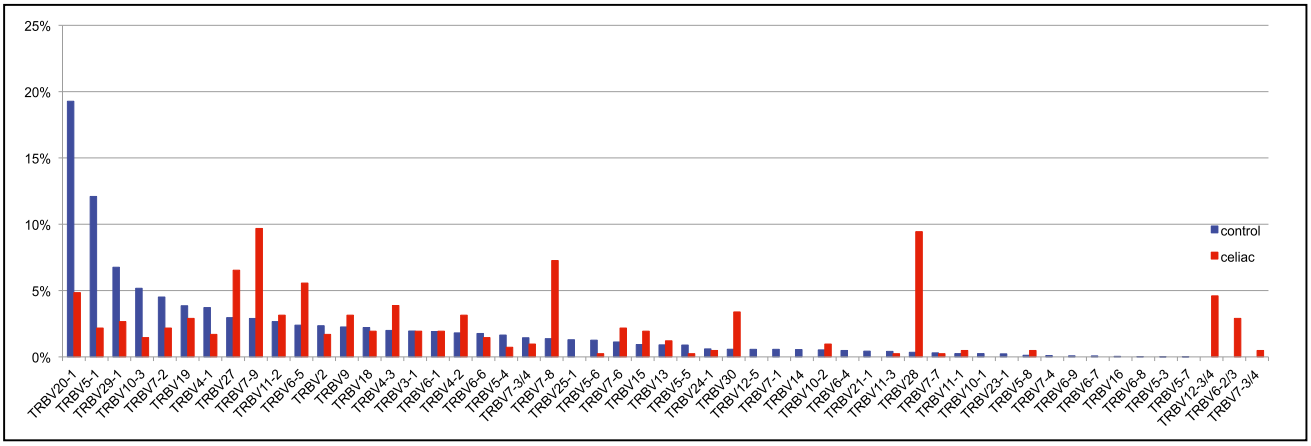
Patient 2 αEβ7$^+$CD38$^+$ CD8	TCRβ CDR3	Freq 1 (127)	Freq 2 (75)
	CASSYDVRSGNYEQYF	5	1
	CASSVGGVQPQHF	4	2
	CASNLAGGSNEQFF	2	1
	CASSKLDSGYTF	1	1
	CASSLGRVEAFF	1	1
	CASSLSQGGHNEQFF	1	1
	CASSPTSGRTTSYEQYF	1	1
	CASSQDGGTYNEQFF	1	1
	CASSSTPGGLWYGYTF	1	1
	CASTAGFNQPQHF	1	1

C.

Patient 1 αEβ7$^+$CD38$^+$ γδ	TCRδ CDR3	Freq 1 (96)	Freq 2 (56)
	CALGGLPTLGDTPTDKLIF	59	17
	CALCLLADWGYTDKLIF	5	1
	CALGELRSLLHLHWGIRTDKLIF	1	4
	CALGDGGGFYTSRVLGGYAFVTTDKLIF	2	2
	CALGELPYWALRGADKLIF	2	1
	CALGGSGISYVGILGKLIF	1	2
	CALGEFFPRYWGTTYTDKLIF	1	1
	CALGELQPRYWGRRFDKTKLFF	1	1
	CALGFPPVLGDPYTDKLIF	1	1

Fig. S4. Identical αEβ7$^+$CD38$^+$CD8$^+$ and αEβ7$^+$CD38$^+$ γδ TCR clones reappear in celiac patients upon repeat gluten challenge. (*A* and *B*) Identical αEβ7$^+$CD38$^+$ CD8$^+$ TCRβ clones are reencountered upon repeat gluten challenge within the same patient. All TCRβ sequences reoccurring in the same individual upon repeat challenge are shown. CDR3β motif and frequency are indicated. (*C*) Identical αEβ7$^+$CD38$^+$ TCRδ clones are reencountered upon repeat gluten challenge within the same patient. All TCRδ sequences reoccurring in the same individual upon repeat challenge are shown. CDR3δ motif and frequency are indicated.

A.



B.

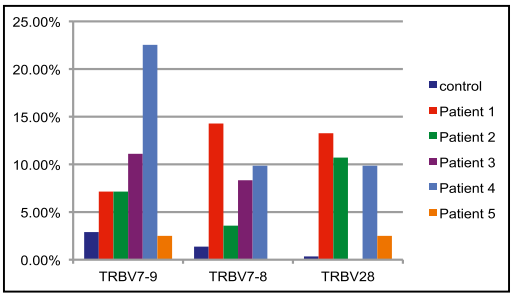


Fig. S5. Certain TRBV genes are overrepresented in a nonredundant sampling of $\alpha E\beta 7^{+}CD38^{+}CD8^{+}$ T cells in some celiac patients compared with controls. (*A*) Relative frequency of TRBV gene use in unique (nonredundant) TCR clones in celiac patients compared with a reference database of sequences shows overrepresentation of TRBV7-9, TRBV7-8, and TRBV28 in celiac patients vs. controls. (*B*) Relative frequency of TRBV7-9, TRBV7-8, and TRBV28 use in unique TCR clones in individual celiac patients compared with controls.

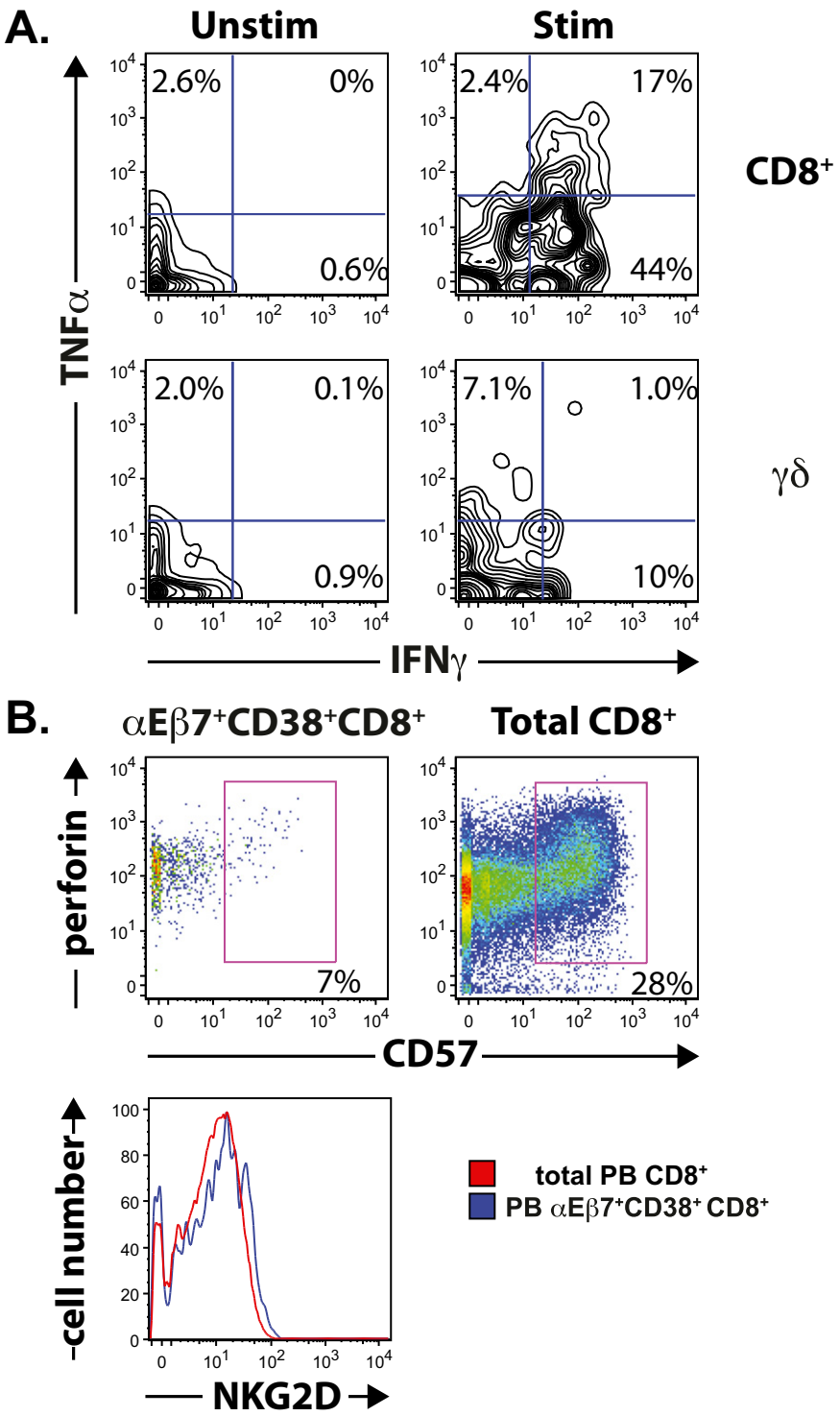


Fig. S6. αEβ7$^+$CD38$^+$CD8$^+$ T cells can produce IFNγ but do not express higher levels of perforin or NKG2D relative to total blood CD8$^+$ T cells. (*A*) Stimulated αEβ7$^+$CD38$^+$CD8$^+$ T cells but not αEβ7$^+$CD38$^+$CD8$^+$ γδ T cells are able to produce TNFα and IFNγ in response to stimulation with PMA and ionomycin. (*B*) αEβ7$^+$ CD38$^+$CD8$^+$ T cells do not express higher levels of perforin or NKG2D than total CD8$^+$ T cells.

Table S1. Quantification of peripheral blood $\alpha E\beta7^+CD38^+CD8^+$ and $\alpha E\beta7^+CD38^+$ $\gamma\delta$ T cells in celiac patients and control individuals following gluten challenge

Table S1

All six celiac patients but none of the controls exhibit induction of peripheral blood $\alpha E\beta7^+CD38^+CD8^+$ and $\alpha E\beta7^+CD38^+$ $\gamma\delta$ T cells on day 6 following oral gluten challenge. Numbers indicate $\alpha E\beta7^+CD38^+CD8^+$ or $\alpha E\beta7^+CD38^+$ $\gamma\delta$ T cells as a percentage of total blood $CD8^+$ or $\gamma\delta$ T cells.

Table S2. Single-cell TCR sequencing of α-II-gliadin tetramer-positive T cells shows most clones use TRBV7-2 and share a consensus arginine at position 5

Table S2

CDR3β sequences from two patients with indicated V and J use and frequency. Use of TRBV7-2 and consensus arginine are highlighted in bold.

Table S3. Summary of single-cell TCRβ and TCRδ sequencing

Table S3

CDR3β and CDR3δ sequences from all patients tested with indicated V and J use and frequency.

Table S4. Primers used for TCRδ sequencing

Table S4

TCRδ was amplified using a series of nested PCRs as described (*SI Methods*) (1).

1. Su LF, Kidd BA, Han A, Kotzin JJ, Davis MM (2013) Virus-specific CD4(+) memory-phenotype T cells are abundant in unexposed adults. *Immunity* 38(2):373–383.