Supporting Information

Kattah et al. 10.1073/pnas.1424796112

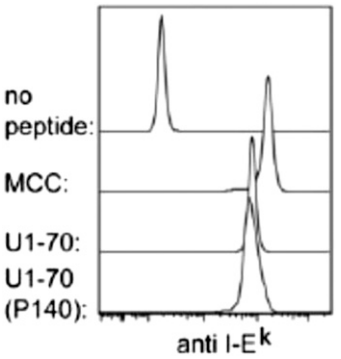

Fig. S1. U1-70 peptides form stable peptide:MHC complexes with I-E^k. Shown are histograms from flow cytometry staining of exchanged, biotinylated peptide:I-E^k complexes, incubated with streptavidin beads and stained with anti-I-E^k (PE), demonstrating that exchanged monomers are stable.

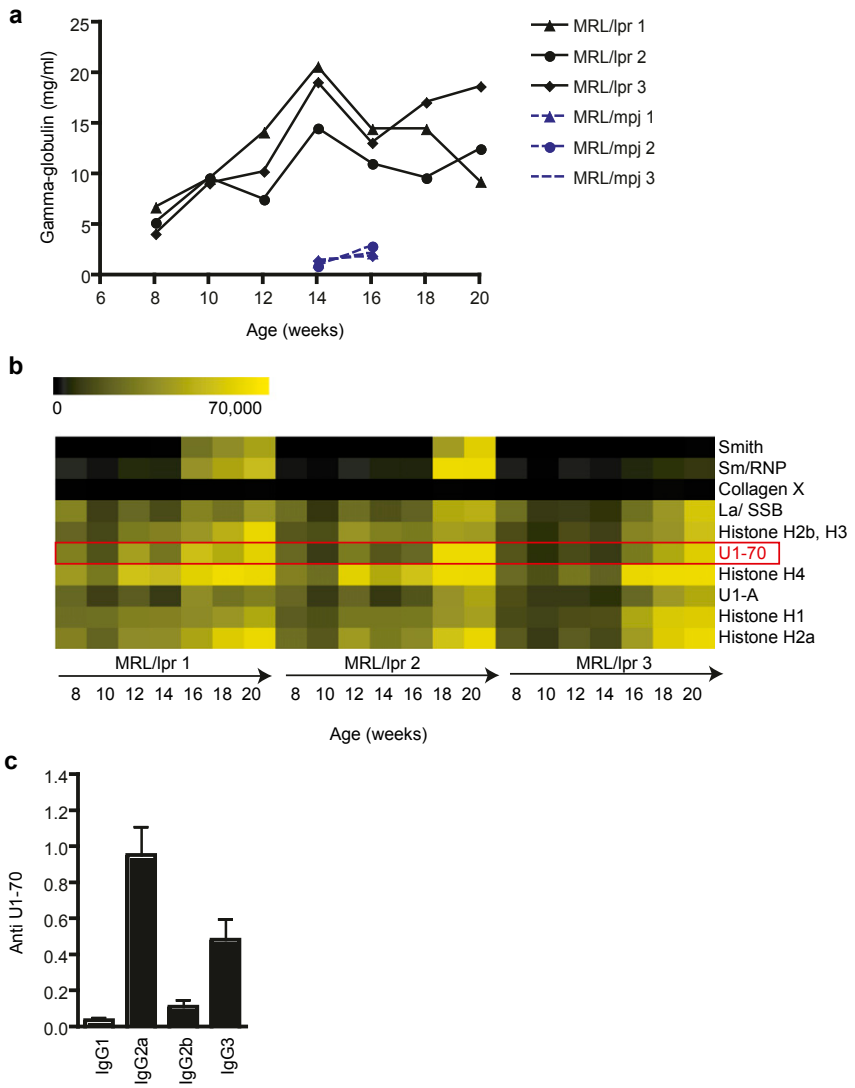

Fig. S2. Autoantibody production in MRL/lpr mice. (*A*) Levels of gamma globulin were measured in the serum of 3 MRL/*lpr* and 3 MRL/*mpj* mice, bled at 2-wk intervals for 12 wk, and detected by ELISA. (*B*) Heat map of antigens identified by SAM as having a statistically significant association with MRL/*lpr* serum from mice aged >15 wk vs. serum from mice aged <15 wk. Data are derived from autoantigen microarrays probed with individual mouse serum samples. The heat map represents a gradient from low (pseudocolored black) to high (pseudocolored yellow) IgG levels. (*C*) Levels of autoantibodies against U1-70 of various IgG isotypes, as measured by ELISA ($n = 3$ mice).

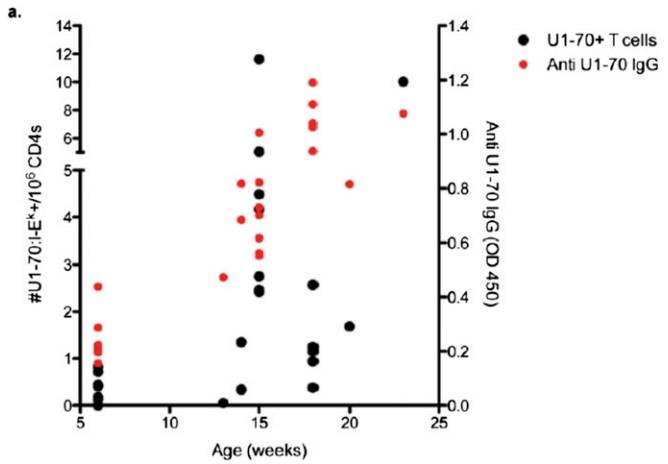

Fig. S3. Kinetics of U1-70:I-E^k–specific T cells and anti–U1-70 autoantibodies. Frequencies of U1-70:I-E^k–specific T cells (*Left y* axis) were determined based on the number of tetramer-positive cells detected following enrichment. The levels of IgG autoantibodies against the whole protein U1-70 in sera from MRL/*lpr* mice were measured by ELISA (*Right y* axis), and graphed alongside the frequency of U1-70:I-E^k–specific T cells.

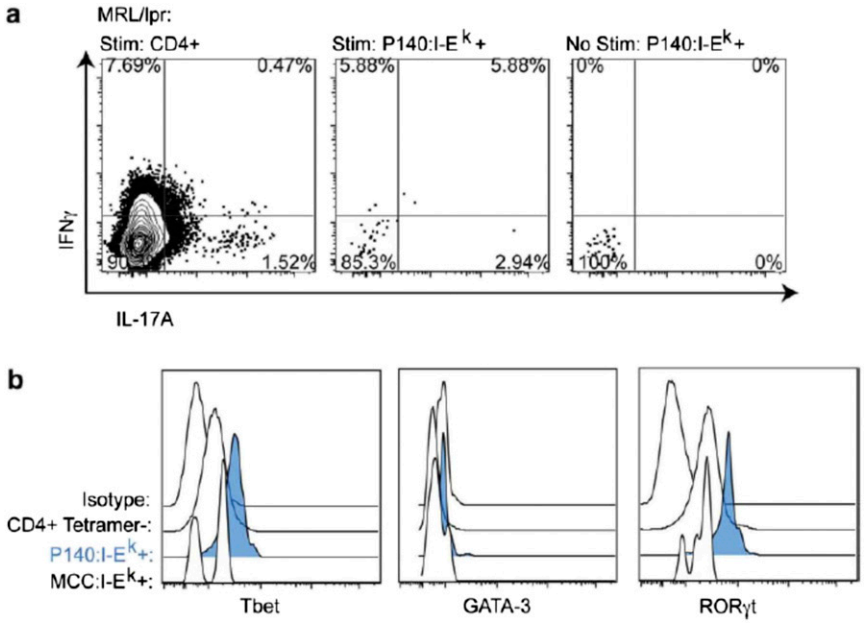

Fig. S4. P140:I-E^k–specific T-cell phenotype. (*A*) Flow cytometry plots showing intracellular IL-17 and IFNγ production by MRL/*lpr* CD4$^+$ T cells, stimulated with PMA/ionomycin for 3 h at 37 °C. The plots show the enrichment fraction and are gated on CD4$^+$ P140:I-E^k–negative cells (*Left*), CD4$^+$ P140:I-E^k–positive cells (*Middle*), and CD4$^+$ P140:I-E^k–positive cells that were not stimulated (*Right*). (*B*) Histograms of RORγt, Tbet, and GATA3 expression from tetramer-enriched MRL/*lpr* CD4$^+$ T cells, as measured by intracellular staining.

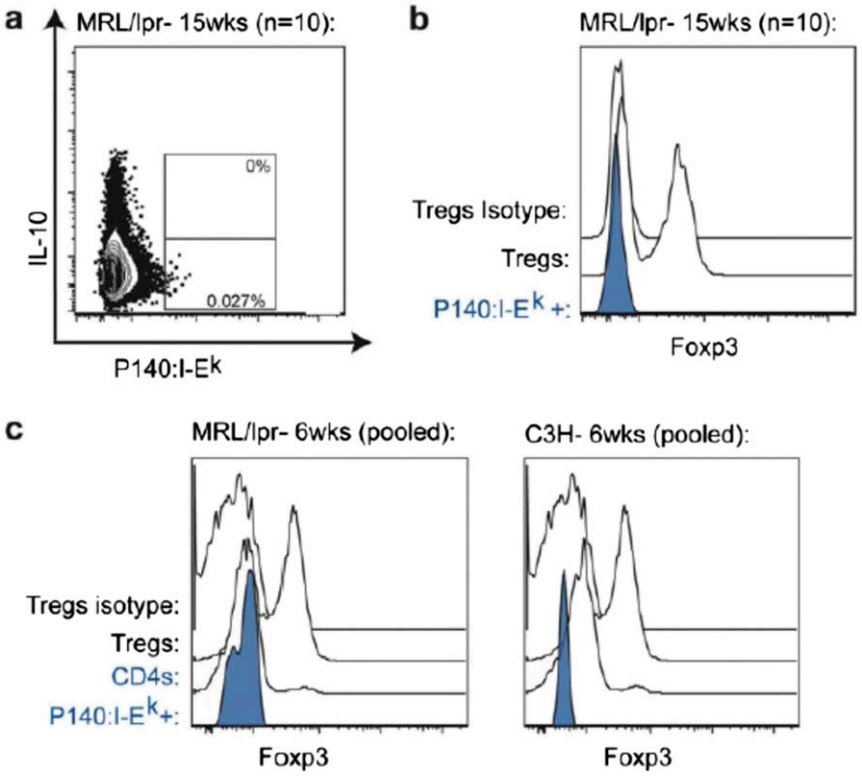

Fig. S5. P140:I-E^k–specific T cells do not express Treg markers. (*A* and *B*) Flow cytometry plots of CD4$^+$ T cells from MRL/*lpr* mice at age 15 wk, stimulated with PMA/ionomycin for 3 h at 37 °C, and stained for IL-10 (*A*) and Foxp3 (*B*). Plots are representative of 10 individual mice. (*C*) Foxp3 expression was measured in 6-wk-old MRL/*lpr* (*Left*) and C3H (*Right*) mice. Five mice were pooled for each sample. As a positive control, Foxp3 expression was detected in CD4$^+$ T cells from MRL/*lpr* mice that were purified by negative selection, cultured with TGFβ, and gated on CD25^{hi}, CD127^{lo}.

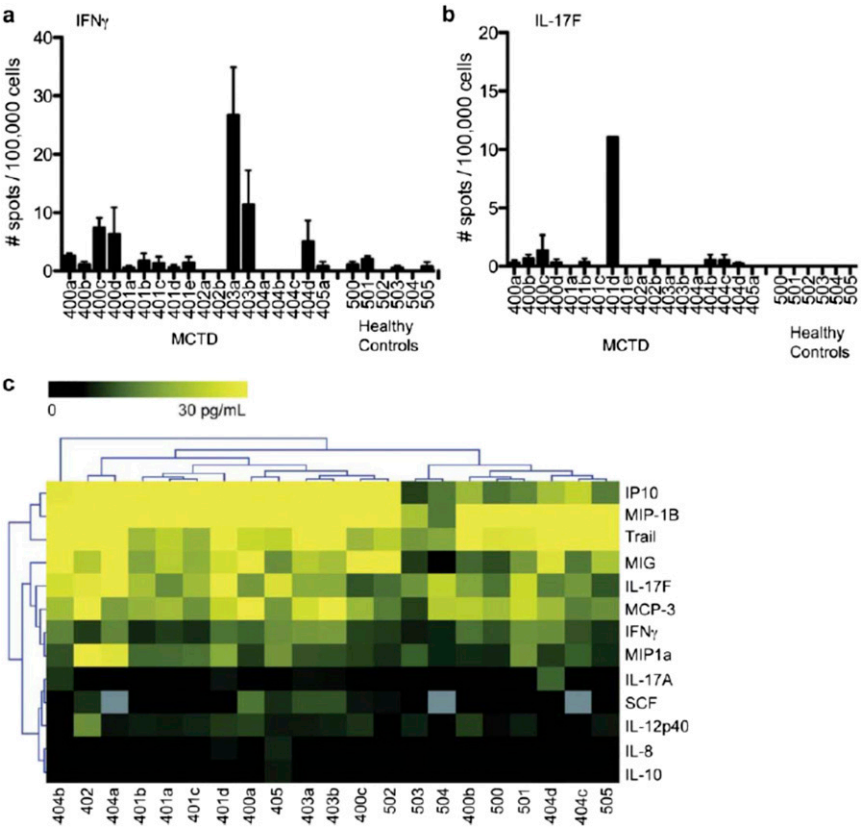

Fig. S6. Cytokine levels in MCTD patients. (*A* and *B*) Bar graphs showing IFNγ (*A*) and IL-17F (*B*) production from MCTD patient samples in response to the U1-70 peptide, as detected by ELISPOT assays. Error bars reflect triplicate wells. Age- and sex-matched healthy controls were included. (*C*) Heat map of unsupervised hierarchical clustering of soluble factors identified by SAM as having a statistically significant association with MCTD patient serum vs. healthy control serum. Data are derived from a Luminex assay and are the average values from triplicate wells.

Table S1. Candidate peptide panel for I-E^k tetramers

Test	Protein	Amino acids	Sequence	Source
*Δ◇	B2M	42–59	HPPHIEIQMLKNGKKIPK	(1)
*Δ◇	Histone H2A	84–99	HLQLAIRNDEELNKLLGKVT	(1)
*Δ	Histone H2A	84–99 E94N	HLQLAIRNDENLNKLLGKVT	Mutation made based on MHC prediction
Δ	Histone H3	20–40	RKSTFFKAPRKQLATKAARK	MHC prediction
Δ	Histone H4	60–80	VFLENVIRDAVTYTEHAKRK	MHC prediction
Δ	La	50–70	IMIKFNRLNRLTTDFNVIVE	MHC prediction
Δ	Ro52	1–20	MASAARLTMMWEEVTCPICL	MHC prediction
Δ	Ro52	210–230	LGEKEAKLAQQSQALQELIS	MHC prediction
*Δ	Ro60	272–287	LLQEMPLTALLRNLGK	MHC prediction
*Δ	Ro60	272–287 T297I	LLQEMPLIALLRNLGK	Mutation made based on MHC prediction
*Δ◇	Srp20	4–23	DSCPLDCKVYVGNLGNNGNK	(1)
*Δ	Srp20	4–23 N22K	DSCPLDCKVYVGNLGNNGKK	Mutation made based on MHC prediction
*Δ◇	U1-70	131–151	RIHMVYSKRSGKPRGYAFIE	(2)
*	U1-70	131–151 (P140)	RIHMVYSKRS(phospho) GKPRG YAFIE	(2)
Δ	U1-A	610–630	DIAFVEFDNEVQAGAARDAL	MHC prediction

Candidate peptides or whole antigens were tested for binding to I-E^k by competition assay (*), and/or for the ability to induce proliferation of CD4$^+$ T cells from MRL/*lpr* mice by ^{3}H thymidine incorporation (Δ), or the up-regulation of CD40L on CD4$^+$ T cells as measured by flow cytometry (◇).

1. Muller S, et al. (2008) Spliceosomal peptide P140 for immunotherapy of systemic lupus erythematosus: Results of an early phase II clinical trial. *Arthritis Rheum* 58(12):3873–3883.
2. Tusher VG, Tibshirani R, Chu G (2001) Significance analysis of microarrays applied to the ionizing radiation response. *Proc Natl Acad Sci USA* 98(9):5116–5121.