Science Translational Medicine
AAAS

www.sciencetranslationalmedicine.org/cgi/content/full/10/434/eaan2306/DC1

Supplementary Materials for

Commensal orthologs of the human autoantigen Ro60 as triggers of autoimmunity in lupus

Teri M. Greiling, Carina Dehner, Xinguo Chen, Kevin Hughes, Alonso J. Iñiguez, Marco Boccitto, Daniel Zegarra Ruiz, Stephen C. Renfroe, Silvio M. Vieira, William E. Ruff, Soyeong Sim, Christina Kriegel, Julia Glanternik, Xindi Chen, Michael Girardi, Patrick Degnan, Karen H. Costenbader, Andrew L. Goodman, Sandra L. Wolin,* Martin A. Kriegel*

*Corresponding author. Email: martin.kriegel@yale.edu (M.A.K.); sandra.wolin@nih.gov (S.L.W.)

Published 28 March 2018, *Sci. Transl. Med.* **10**, eaan2306 (2018)
DOI: 10.1126/scitranslmed.aan2306

The PDF file includes:

Materials and Methods
Fig. S1. Sequence alignment of full-length hRo60 and selected commensal orthologs.
Fig. S2. Coimmunoprecipitation of lupus study subject sera confirmed anti-Ro60 antibody status.
Fig. S3. α-Diversity represented by box plots of the Shannon-Weiner diversity index.
Fig. S4. SCLE skin eruption.
Fig. S5. TT-reactive CD4$^+$ T cell clone from a healthy donor generated by a CD4 T cell library assay.
Fig. S6. Ro60-negative SLE patient CD4$^+$ T cells lack reactivity to hRo60 protein.
Fig. S7. Cytokine concentrations (pg/ml) of supernatants from the cross-reactive T cell clone from Fig. 5 measured using a bead-based immunoassay.
Fig. S8. Anti-Ro60 antibody status of Harvard cohort lupus subjects.
Fig. S9. YrlA RNA is not detected in immunoprecipitates from *B. theta* using human lupus sera.
Fig. S10. *B. theta* monocolonization of GF mice induces gut and systemic immune changes.
Fig. S11. Schematic of proposed mechanism of how Ro60 bacteria trigger and sustain autoimmunity.
Table S1. Commensal bacterial Ro60 orthologs identified by in silico methods.

Table S2. Lupus study subject clinical data.
Table S3. Healthy control study subject clinical data.
Table S4. Efficiency and specificity of bacterial Ro60 qPCR primers.
References (*63–77*)

Other Supplementary Material for this manuscript includes the following:
(available at
www.sciencetranslationalmedicine.org/cgi/content/full/10/434/eaan2306/DC1)

Table S5 (Microsoft Excel format). Primary data.

Supplementary Materials and Methods

Identification of Ro60 orthologs and YrlA RNAs *in silico*

An initial list of Ro60 orthologs was identified by searching a custom database of human gut microbes (*63*). A more comprehensive list was generated using an NCBI query for proteins annotated as containing a TROVE domain (*64*), using the constraints of bacteria as the organism, 400 to 650 amino acids as the length, and GenBank as the source database. This resulted in a list of 1,943 bacterial TROVE proteins. The bacterial species were cross-referenced with the Pathosystems Resource Integration Center (PATRIC) (*21*) and the Human Microbiome Project (*22*) for previous identification as a human commensal. Commensal protein sequences were aligned using Clustal Omega and a phylogenetic tree was constructed using the online tool iTOL (*65*). The Ro60 structure (protein data bank code 1YVR, *Xenopus laevis*) image in Fig. 1B was prepared using PyMOL (*66*).

For each commensal bacterium possessing a Ro60 ortholog, the genome sequence was collected from GenBank and searched for YrlA RNA using a covariance model built with Infernal (version 1.1) (*67*) as described (*14*).

HLA typing of human subjects

HLA typing was performed as previously published (*68*) for *HLA-DR3* (*DRB1*0301*, *DRB1*0301-0302*) and *HLA-DR15* (*DRB1*1502*).

Microbiome collection and sequencing

Oral and skin microbiota samples were collected using sterile gloves with sterile Catch-All Swabs (EpiCentre Biotech) rubbed on the bilateral buccal mucosa for 60 seconds (sec), or pre-moistened in EpiCentre Yeast Cell Lysis Buffer and rubbed vigorously on a 2 cm^2 area of skin for 60 sec. Swabs were stored in Yeast Cell Lysis Buffer at -80°C until DNA was extracted. For DNA extraction, swabs were incubated at 37°C for 1 hour (hr) with shaking. Catch-all swabs were removed from the tube, placed in basket columns (Promega) and spun down to extract all remaining liquid. Samples were bead-beaten using a BioSpec Mini-Beadbeater-16 with 0.1 mm glass beads (MP Bio) for 2 minutes (min) and then incubated for 30 min at 65°C. Samples were cooled on ice for 5 min and then 250 µl of MPC Reagent (EpiCentre) was added to precipitate protein. Samples were centrifuged for 10 min and the supernatant was transferred to a new sterile tube. An equal volume of 100% ethanol was added and then the standard protocol for the Invitrogen PureLink genomic DNA mini kit was followed. DNA quantification and quality were measured via A_{260} and A_{280} on a NanoDrop 2000 spectrophotometer.

Stool samples were collected by subjects at home in sterile containers and shipped overnight on ice to the laboratory, at which time they were aliquoted and stored at -80°C. 100 to 300 mg of human stool was combined with 1 ml MoBio Bead Solution and 1 mm ceramic beads (BioSpec) and were subjected to bead beating twice for 1 min with a 2-min rest on ice in the middle. Samples were centrifuged and the supernatant was

transferred to a MoBio Garnet Bead tube, heated for 10 min at 65°C, then 10 min for 95°C, then processed per the MoBio Power Soil DNA Isolation Kit protocol.

DNA isolation from microbiota samples was performed as above. The V4 region of the 16S rDNA was PCR-amplified, normalized, pooled, and sequenced using the Illumina MiSeq with 2 x 250bp paired-end reads as described (*69*). Analysis of 16S sequencing reads was performed as described (*70*) with the following minor modifications: QIIME (*71*) analysis was performed with version 1.8 and a quality score cutoff of 30. Filtered operational taxonomic units (OTUs) were rarefied to a depth of 10,000 sequences per sample and OTUs representing less than 0.01% of total abundance were excluded from further analysis.

Bacterial culture and preparation

Anaerobic bacteria were grown in an anaerobic chamber filled with 82% N_2, 15% CO_2, 3% H_2 by volume. Liquid cultures were quantified using OD_{600} values. All cultures were confirmed by DNA extraction using the Qiagen DNeasy kit for gram-positive bacteria per the manufacturer's instructions and PCR amplification of the 16S rDNA region (forward primer AGAGTTTGATCCTGGCTCAG, reverse primer GACGGGCGGTGWGTRCA, 95°C for 5 min, 30 cycles of 95°C for 10 sec, 60°C for 20 sec, 72°C for 15 sec, 72°C for 10 min), followed by Sanger sequencing.

To prepare whole heat-killed bacteria, frozen stocks were cultured overnight in the appropriate broth at 37°C under anaerobic conditions, then cultured to an optical density at 600 nm [OD_{600}] of 1.0. To prepare inactivated bacterial suspensions, bacteria were harvested by centrifugation (8,000 × *g* for 10 min at 4°C) and washed three times with phosphate-buffered saline (PBS, pH 7.2) to remove secreted proteins, and then re-suspended in PBS. Lastly, bacterial suspensions were heat inactivated at 65°C for 10 min.

For Western blotting experiments, lysates were prepared from pelleted monocultures of bacteria, washed three times with PBS, subject to bead-beating using 0.1 mm glass beads for 2 min, spun down at 10,000 x *g* for 5 min, and quantified by bicinchoninic acid (BCA) using a BSA standard curve (Thermo Fisher).

Detection of Ro60-containing commensals in patient microbiomes

Quantitative real-time PCR of bacterial Ro60 was performed on a QuantStudio 6 (Applied Biosystems). *B. theta* Ro60 (GenBank EOS03901.1) and total 16S load (*72*) were measured using 20 ng of stool or control *B. theta* DNA, 250 nM forward and reverse primers (*B. theta* Ro60 forward CCTGCTTGCAACGTGACTTC, reverse TTGGCTGCTTACCGTGAGTT, product length 244 nt; 16S rDNA forward CGGCAACGAGCGCAACCC, reverse CCATTGTAGCACGTGTGTAGCC, product length 146 nt) and Power SYBR green PCR Master Mix (Applied Biosystems) in a total reaction volume of 25 µl, in triplicate. Samples were heated at 50°C for 2 min followed by 40 cycles of 95°C for 10 min, 95°C for 15 sec, 60°C for 1 min.

P. prop Ro60 (GenBank CP002734.1, TaqMan custom proprietary primers/probe AICSXLT), *C. amycolatum* Ro60 (GenBank NZ_ABZU01000011.1, CDS 51027 - 52661, TaqMan custom proprietary primers/probe "CamyRo60"), *A. massiliensis* Ro60

(GenBank AKFT01000221.1, TaqMan custom proprietary primers/probe AID1VR1), and total 16S load (custom degenerate primers and probe as published previously (*73*)) were measured from 100 ng of oral or skin swab or control bacterial DNA using 10 µl TaqMan Multiplex Master Mix (Applied Biosystems), 1 µl each of custom TaqMan primers/probes in a 20 µl reaction volume, in duplicate. Samples were heated at 50°C for 2 min followed by 50 cycles of 95°C for 10 min, 95°C for 10 sec, 60°C for 1 min.

All commensal ortholog-specific primers and primer/probe sets were validated by calculating the efficiency (Table S4) when used to amplify a range of 10-fold dilutions from 20 ng to 2 pg of the intended template bacterial DNA extracted from single bacterial culture as above. Specificity was tested by using primers to amplify 20 ng of off-target bacteria with (*B. theta*, *P. prop*, *A. mass*, *C. amyc*) and without (*P. acnes*, *R. intes* (*R. intestinalis*)) Ro60 orthologs (Table S4). Some custom proprietary TaqMan primer/probe sets amplified a gene product from off-target bacteria but usually this was at a concentration that would require at least a 100-fold higher amount of off-target bacteria than the intended template.

Genomic DNA from the species-specific Ro60 genes were used to represent the species-specific bacterial load and compared with the total 16S load to normalize between samples. Replicates were averaged and bacterial load was quantified using the delta-delta-Ct method per the formula: 2 ^ -((C_t of sample Ro60 – C_t of sample 16S) – (C_t of control bacteria Ro60 – C_t of control bacteria 16S)). If replicates were not concordant, i.e., only one well failed to amplify, the assay was repeated with a new sample.

Purification of recombinant human and bacterial Ro60 proteins

Recombinant human Ro60 was made using both an insect cell and a mammalian system. Human Ro60 cDNA (*74*) was inserted into the BamHI/HindIII sites in pFastbac1 (Invitrogen), expressed in High Five insect cells and purified as described (*75*) except that purified protein was stored in 5 mM DTT. Human Ro60 cDNA was also inserted into the BamHI/Not1 sites in pcDNA3.1 (Thermo Fisher Scientific), containing a 12xHis-tag vector. Expi293 cells (Thermo Fisher Scientific) were transfected using Expifectamine (Thermo Fisher Scientific). 72 hr post-transfection, cells were lysed by sonication on ice, using a mix of lysis buffer and protease inhibitors (25 mM HEPES, 2 mM EDTA, 25 mM NaF, 0.01% sodium dodecyl sulfate (pH 7.4), protease inhibitor mixture (Roche)). For purification, the lysate was added to the Ni-NTA resin (Qiagen), washed in 50 mM NaH_2PO_4, 300 mM NaCl, 20 mM imidazole (pH 8.0), and eluted in 250 mM imidazole of this buffer. The protein was dialyzed overnight and protein purity was confirmed via Coomassie staining. This protein was used in Fig. 3C and F, while baculovirus-expressed hRo60 was used in all other figures.

DNAs encoding bacterial Ro60 orthologs were amplified from genomic DNA and the encoded proteins expressed in *Escherichia coli* BL21(DE3) cells. The *B. theta* Ro60 coding sequence was inserted into the EcoRI/NotI sites of pETDuet-1 (Novagen) while the *P. prop* Ro60 sequence was cloned into the BamHI/NotI sites of the same vector. To purify *B. theta* Ro60, BL21(DE3) cells harboring pETDuet-1-His-Ro60 were grown to

OD_{600} = 0.5, incubated on ice for 20 min and IPTG added to 0.1 mM. After 18 hr at 25°C, cells were resuspended in lysis buffer B (50 mM NaH_2PO_4, 300 mM NaCl, 10 mM imidazole pH 8.0, 2 mg/ml lysozyme, 1X protease inhibitor cocktail), incubated on ice for 30 min and lysed by sonication. Ro60 was purified using Ni-NTA resin (Qiagen) and Heparin Sepharose CL-6B (Pharmacia). Briefly, after applying the lysate to Ni-NTA resin, and washing with 50 mM NaH_2PO_4, 300 mM NaCl, 20 mM imidazole (pH 8.0), Ro60 was eluted with 250 mM imidazole in the same buffer. Eluates were pooled and applied to a Heparin Hitrap column (GE Healthcare), washed with 25 mM Tris-Cl pH 8.0, 0.3 M NaCl, 3mM $MgCl_2$, 0.1 mM EDTA, 2.5 mM β-mercaptoethanol, and eluted with 25 mM Tris-Cl (pH 8.0), 0.6 M NaCl, 3 mM $MgCl_2$, 0.1 mM EDTA, 5 mM DTT. Purification of *P. prop* Ro60 was similar except that 0.2 mM IPTG was used to induce expression and the cells were incubated afterwards for 5 hr at 25°C.

Immunoassays

Enzyme-linked immunosorbent assay (ELISA) for anti-Ro60 IgG was performed with 96-well polystyrene flat-bottom high-binding plates (Corning) coated with 1 µg/ml recombinant Ro60 protein in 0.05 M carbonate-bicarbonate buffer pH 9.2 for 2 hr, washed three times with PBS with 0.05% tween-20, blocked with PBS tween-20 with 4% bovine serum albumin (BSA, Sigma) for 2 hr, and incubated with serum overnight at 4˚C. Wells were washed three times with PBS tween-20 and incubated with horseradish peroxidase (HRP) conjugated secondary antibody (sheep anti-human IgG 1:6000 or rabbit anti-mouse IgG 1:1000 (ThermoFisher Scientific)) for 30 min, followed by another 6 washes with PBS tween-20 and colorimetric development with TMB Substrate Buffer (Thermo Scientific) stopped after 15 min with 2M H_2SO_4. The optical density (OD) was measured at 450 nm and 650 nm. Human Ro60 IgG samples were also tested with the Fooke Ro60 ELISA (Mikrogen Diagnostik) in duplicate using the manufacturer's protocol. Both methods rendered the same results.

Cytokine concentrations from supernatants of memory $CD4^+$ T cell clones were measured with the bead-based immunoassay LEGENDplex (Biolegend, human Tfh and Th panels). Supernatants of activated T cell clones stimulated as detailed in the figure legends were collected run according to the manufacturer's protocol.

T cell cloning, tetramers, and proliferation assay

PBMCs were isolated from whole blood by Lymphoprep (STEMCELL Technologies) gradient centrifugation. PBMCs were immunomagnetically separated using the following kits (STEMCELL Technologies) per manufacturers' instructions: B lymphocytes using the EasySep Human CD19 Positive Selection Kit, monocytes using the EasySep Human CD14 Positive Selection kit, and $CD4^+$ T cells using the EasySep Human $CD4^+$ T Cell Isolation Kit. Selected cells were cooled in 90% human AB serum with 10% dimethyl sulfoxide to -80°C at -1°C/min and transferred to liquid nitrogen within 24 hr. Monocytes were used as antigen-presenting cells for the T cell library assay. Viable $CCR6^-$ memory ($CD45RA^-CD45RO^+CD25^-CCR6^-$) $CD4^+$ T cells and $CCR6^+$ memory

($CD45RA^{-}CD45RO^{+}CD25^{-}CCR6^{+}$) $CD4^{+}$ T cells were sorted to at least 97% purity (antibodies from Biolegend) on a FACSAria machine (BD Biosciences).

The DR0301* soluble class II molecules were generated at the Tetramer Core Laboratory of the Benaroya Research Institute (W. Kwok) as described (*76*). The Ro60 peptide p370-384 was synthesized to load HLA-DR0301*0401 to generate MHC class II tetramers. Phycoerythrin (BioSource International)–conjugated streptavidin was used for cross-linking of peptide–MHC II monomers.

The T cell library assays were performed as previously described (*32*). After sorting, $CCR6^{-}$ memory and $CCR6^{+}$ memory $CD4^{+}$ T cells from SLE patients or healthy controls were sorted and cultured in 96-well round-bottom plates (Corning) at 2000 cells per well in X-Vivo Media (Lonza), and stimulated with phytohemagglutinin (PHA, 1 mg/ml) (Roche) and IL-2 (30 U/ml) (Invitrogen) in the presence of irradiated (45 Gy) allogeneic PBMCs as feeder cells (25000 per well). In the case of tetramer-positive cells, T cells were single-cell sorted directly into plates containing the feeder cells. IL-2 was added on days 3, 6, and 10. After 14 days of maintenance and expansion, T cell cultures were washed and split equally into two "mirror" 96-well plates. Library screening was carried out by stimulation of 250,000 T cells per well with irradiated (45 Gy) autologous monocytes (~25,000 per well). The monocytes were then pulsed for 3 hr with 100 μg/ml recombinant mouse or human Ro60 protein or tetanus toxoid (TT) (1 ug/ml). Positive control wells were expanded and then re-stimulated at a ratio of 1:10 with irradiated autologous monocytes that were pulsed with either whole heat-killed bacteria or commensal mimic peptides. Negative control wells contained monocytes alone to assess any background signal. After 64 hr, culture supernatants were removed for cytokine measurement using a bead-based immunoassay (Legendplex, Biolegend). Cell proliferation was measured either by measuring [^{3}H]-thymidine incorporation on a scintillation β-counter (Perkin Elmer) or alternatively non-radioactive ATP measurement using the ATP lite kit (Perkin Elmer) as indicated in the figure legends.

TCR Sequencing

Individual T cell clone total cDNA was obtained after lysis (0.2% Triton, 2.5U RNAse Inhibitor) of 10^{3}– 5×10^{3} cells/reaction. The reverse transcription was performed using oligo dT(18) primers (Life Technologies) and Superscript III (Life Technologies), in a reaction mix containing DTT, dNTPs, and 5X Buffer. Reactions were run with the following conditions: 25°C x 10 min, 50°C x 1 hr, 94°C x 5 min. TCR sequences from T cells were identified from cDNA. Three μl of cDNA were used for PCR (final volume 25 μl) containing HotStart DNA Polymerase (Qiagen). Sequences were amplified using designed TCR Vβ-specific forward primer pools and TBC-rev reverse primers pairing to C1-C2 β chain constant region, respectively (*34*). PCR reactions were performed with the following conditions: 95°C x 1 min; (95°C x 20 sec; 50°C x 20 sec; 72°C x 30 sec) x 45 cycles; 72°C x 3 min. Amplified fragments were sequenced through Sanger method using TBC-rev primer.

Immunoprecipitation of RNPs from human cell lysates

Five µl of each patient serum was coupled to SureBeads protein G magnetic beads (Bio-Rad) in PBS [137 mM NaCl, 2.7 mM KCl, 18 mM Na_2HPO_4, 2 mM KH_2PO_4 (pH 7.4)] at room temperature for 30 min. Beads were washed once in PBS and then resuspended in NET-2 lysis buffer (50 mM Tris-HCl pH 7.4, 150 mM NaCl, 1 mM $MgCl_2$, 0.5% Nonidet P-40). After lysing HEK293T cells by sonicating in NET-2 containing 1X complete protease inhibitors (Roche Applied Science), 100 uM PMSF, 200 U/mL RNase OUT (Invitrogen), lysates were added to the beads and incubated for 30 min at room temperature. The beads were washed three times in 750 µl of NET-2, transferred to new microcentrifuge tubes, and washed an additional three times. After resuspending beads in 350 µl NET-2 containing 2 µl glycogen, 0.25% SDS and 0.3 M NaOAc, RNAs were extracted with phenol:chloroform:isoamyl alcohol (25:24:1, v/v), and precipitated with 2.5 volumes ethanol. RNAs were labeled with [Υ^{32}P]pCp and fractionated in 5% polyacrylamide, 7 M urea gels.

Immunoprecipitation and Northern blotting

After growing *P. prop* in liquid medium, bacteria were pelleted, washed in 1× Dulbecco's phosphate-buffered saline (PBS) (Gibco), and resuspended in ice-cold lysis buffer A (20 mM Tris-HCl pH 7.5, 100 mM NaCl, 5% glycerol, 0.1% NP-40, 1 mM $MgCl_2$, 1 mM PMSF, 2.5 mM vanadyl ribonucleoside complexes and 1x protease inhibitor cocktail). Droplets of bacterial cells were flash-frozen in liquid nitrogen and lysed by cryogenic grinding with a planetary ball mill (Retsch). After resuspending 1 gram of the powder in 3.5 ml lysis buffer A and removing debris by sedimenting twice at 15,000 x *g*, proteins that bind non-specifically were removed by incubating the lysates for 20 min with protein A Sepharose CL-4B (Pharmacia) that had been pre-swollen in lysis buffer A. Afterwards, supernatants were aliquoted and incubated with 20 µl human sera for 1 hr at 4°C, followed by incubating with 15 µl protein A Sepharose 4B for 1 hr at 4°C. After washing the beads 4 times with lysis buffer A, RNA was extracted using phenol/chloroform/isoamyl alcohol (50:50:1), fractionated in 6% polyacrylamide/8 M urea gels, transferred to Hybond (Amersham) and RNA crosslinked to the membrane as described (*60*). Hybridization with [Υ^{32}P]ATP-labeled oligonucleotides was as described (*61*). Oligonucleotides were: *P. prop* YrlA RNA: 5'-ATCCCTGATAACCGATCCCCTGCGG-3' and 5'-CAACCTCCTGATCCCTGATAAC -3'; *P. prop* $tRNA^{Pro}$: 5'-TTGTCGGGCTGACAGGATTTG-3'.

Immunoprecipitation of *B. theta* RNPs was performed similarly, except that after harvesting, cells were lysed by passing through a French press (Thermo IEC) at 10,000 psi. For detecting *B. theta* YrlA RNA, asymmetric PCR products used as probes were prepared as described (*77*) with the following modifications. Briefly, 250 ng of template genomic DNA extracted from cultured *B. theta* was used for asymmetric PCR amplification of the YrlA RNA using 0.01 µmol of forward primer (TGTCGTAGAGAAGAGTTACTTCG) and 0.2 µmol of reverse primer (ACAAGGTAACAAACGAAAAGAGAC), incorporating [Υ^{32}P]-dCTP. Cycling parameters were 94°C for 1 min followed by 60 cycles of 94°C for 30 sec, 55°C for 30

sec, 72°C for 30 sec with a second addition of Taq DNA Polymerase after the 30th cycle, then a final 72°C for 3 min and 4°C hold.

16s rRNA FISH on human skin tissue

The 16S rRNA-targeted oligonucleotide probes used in this study were a previously published *P. prop*-specific probe (*29*) and a previously published eubacterial probe EUB338 (*28*). For *in situ* hybridization, the probes were labeled with either FITC or Cy3. Formalin-fixed, paraffin embedded SCLE skin biopsies were obtained from the Dermatopathology laboratory at Yale and were de-paraffinized in xylene and absolute ethanol.

Hybridizations were performed at 46°C for 2 hr with hybridization buffer (0.9 M NaCl, 20 mM Tris-HCl [pH 7.5], 0.05% sodium dodecyl sulfate, 20% formamide) containing 0.5 ng/µl of each labeled probe. A washing step was done at 46°C for 10 min with washing buffer (0.215 M NaCl, 20 mM Tris-HCl [pH 7.5], 0.05% sodium dodecyl sulfate, 0.025 EDTA). Slides were air-dried and then mounted using Antifade Mounting Media (Invitrogen) with DAPI. Finally, the slides were visualized with a Leica Confocal Microscopy.

Western Blotting

15 µg of bacterial lysates, 4 µg of recombinant *B. theta* Ro60 protein, and 0.35 µg of human Ro60 protein were loaded on a 4-12% gradient SDS gel. Human sera were incubated overnight as the primary antibody in 5% BSA in PBS Tween-20 at a dilution of 1:1000. Anti-human IgG as secondary antibody was used at a dilution of 1:10000 in PBS and blots were developed using enhanced chemiluminscence substrate (Pierce).

Mice

GF C57Bl/6 mice were housed in gnotobiotic isolators at the Yale Gnotobiotic Animal Facility. Four males and twelve females at 6 weeks of age were orally gavaged with 0.2 ml of thawed *B. theta* at ~1.3 x 10^9 cells/ml in 20% glycerol. Colonization was confirmed by DNA extraction from the fecal pellet 2 weeks after colonization, PCR amplification of the 16S region, and Sanger sequencing. One animal was found dead 5 months after gavage but animals otherwise appeared healthy. After 3 months (n = 7) or 5 months (n = 8), mice were anesthetized with isoflurane for terminal blood collection and sacrificed with CO_2 asphyxiation for organ collection.

To see if irritants would augment the adaptive immune response to Ro60, subsets of mice were treated with 1-2% DSS (MP Bio) and/or oral imiquimod (0.1% w/v, Sigma) in the drinking water. DSS therapy consisted of 3 cycles of 7 days DSS followed by 7 days of water at 3-4 months of age. Imiquimod therapy was continuous from 5 months of age until sacrifice. Of the four cages of mice, one was treated with DSS + imiquimod (n = 4), one with imiquimod alone (n = 4), one with DSS alone (n = 4), and one was not treated (n = 3). No differences between cohorts in B or T cell reactivity, no organ damage, and no

skin lesions were observed, so all animals were analyzed as one cohort (n = 15, Fig. 8A-C).

A second group of C57Bl/6 GF mice were treated with topical imiquimod three times weekly from age 8 to 16 weeks (Fig. 8G-K) to mirror an inducible lupus model as previously described under SPF conditions (*44*). 14 C57Bl/6 mice remained GF and 6 mice were monocolonized with *B. theta* as above. Half of each group was treated with topical imiquimod.

GF non-obese diabetic (NOD) mice were received from L. Wen (Yale) and housed in gnotobiotic isolators. Three 11-week old males and three 6-week old females were orally gavaged as above. Mice were sacrificed after 2 weeks for blood and organ collection (Fig. 8D-F).

Lymph node and spleen proliferation assay

Mouse mesenteric lymph nodes and spleen were harvested in RPMI 1640 medium (Life Technologies) supplemented with 2 mM L-glutamine, 2 mM HEPES, 0.1 mM nonessential amino acids, 1 mM sodium pyruvate, penicillin (50 U/ml), streptomycin (50 U/ml, Lonza), and 10% fetal calf serum. Tissue was ground with the rubber stopper of a 3-ml syringe over a 70-µm cell strainer and washed into RPMI. For spleens, red cells were lysed with 1 ml of red blood cell lysing buffer (Sigma) for 3 min, then washed three times with PBS. Cells were plated in 96-well plates at 100,000 cells per well in 100 µl of RPMI. Another 100 µl of stimulus was added and cells were incubated at 37°C for 72 hr. Stimuli included *B. theta* lysate from cultured bacteria (bacteria were washed three times with PBS, bead-beaten using 0.1 mm glass beads for 2 min, spun down at 10,000 x *g* for 10 min); recombinant *B. theta* Ro60, recombinant human Ro60; anti-CD3-epsilon 0.5 mg/ml (BioLegend) and anti-CD28 0.5 mg/ml (BioLegend); bovine serum albumin (Sigma); and human serum purified β_2-glycoprotein-I (Haematologic Technologies). After 72 hr, cells were transferred to white opaque 96 well plates (Greiner). Proliferation was measured using the CellTiter-Glo Luminscent Cell Viability Assay (Promega) or the Perkin Elmer ATPlite 1 step as per the manufacturer's protocol.

Kidney immunofluorescence

Mice were anesthetized with ketamine and perfused with 15 ml of Dulbecco's PBS. Kidneys were fixed in PLP (4% paraformaldehyde, 0.2 M L-Lysine, 0.2% metaperiodate in phosphate buffer pH 7.4) overnight. They were then washed in PBS, dehydrated in 20% sucrose for at least 4 hr and frozen in OCT compound (Sakura). 10-µm cryosections were rehydrated, blocked with 0.1M Tris-HCl pH 7.4, 2% fetal bovine serum, 0.3% Triton X-100 for 30 min and stained with the following primary antibodies overnight at 4°C: FITC-coupled rabbit anti-mouse IgG, IgA, IgM (1:100, Abcam), anti-mouse C1q (1:100, Abcam), anti-rat C3 (1:100, Abcam). Slides were rinsed 3 times with PBS for 5 min before incubating with secondary antibodies for 2 hr at room temperature: donkey anti-rat Cy5 (1:300, Jackson Laboratories) and goat anti-mouse Cy3 (1:600, Jackson Laboratories). Slides were counter-stained with 4',6-diamidin-2-fenilindolo (DAPI). Finally, slides were mounted with Prolong AntiFade mounting medium (Invitrogen).

Confocal microscopy was performed on a Leica TCS SP5 laser confocal scanner mounted on a Leica DMI 6000B inverted microscope equipped with motorized stage. All images were acquired with a HCX PL APO 40X (NA 1.25) oil immersion objective. Leica LAS AF was used for all acquisitions. ImageJ software package or Imaris (Bitplane) were used for image analysis and fluorescence quantification.

Intracellular FOXP3+ staining and flow cytometry

Cells isolated from spleen or small intestinal lamina propria were surface-stained in FACS buffer (PBS, 2% bovine serum albumin) for 30 min in the dark at room temperature with fluorescently conjugated antibodies specific to CD3ε, CD4, CD8, CD45, CD45R (B220), PDCA-1, CD11c, CD11b, and MHCII. For intracellular staining, cells were fixed overnight and stained with fluorescently conjugated antibody specific for FoxP3 (BD Biosciences) for 45 min in the dark at room temperature using a Mouse FOXP3 Buffer Set (BD Biosciences). Cell viability was assessed with a Fixable Viability Dye (eBioscience). Samples were collected by an LSR II flow cytometer (Becton Dickinson). All flow cytometry data were analyzed by FlowJo version 10 (Tree Star).

```
H. sapiens          -------------------MEESVNQMQPLNEKQIANSQDGYVWQVTDMNRLHRFLCFGSEGGTYYIKEQKLGLENAEAL 61
M. musculus         -------------------MEGSANQLQPLSETQVVNSEGGCVWQVTDMNRLRRFLCFGSEGGTYYIKEQKLGLENAEAL 61
P. propionicum      MVANTNRKGGNTMDVLREINLRSTPQSKPADERQVQNSAGGHTFQLDDAARLRRFLTLGVDAGTYYASAKELAIDNVEVL 80
C. amycolatum       ------------MDSLHNFSFENTPQQVRASEKQVQNSAGGFTFELDDKARLRRFLILGVEGGTFYANARHLAFDNVQIL 68
A. massiliensis     ------------MDILSDISTRLTPQSQRAAANQEPNSAGGYTFTLDDAARLRRFLILGVDGGTYYTSAQDLAIDNLEVL 68
B. thetaiotaomicron ---------------------MMKFNWINKGMNKVLNKESAPAYRLTPEWELYTSVVTTSLNNSFYEQEE----ERIERV 55
                                         **        *

H. sapiens          IRLIEDGRGCEVIQEIKSFSQEGRTTKQEPMLFALAICSQCSDISTKQAAFKAVSEVCRIPTHLFTFIQFKKDLKESMKC 141
M. musculus         IRLIEDGRGCEVIQEIKSFSQEGRTAKQEPLLFALAVCSQCADINTKQAAFKAVPEVCRIPTHLFTFIQFKKDLKESMKC 141
P. propionicum      KRMAASEP-ETLVATIVDVSVRGAAPRQNPVLFALAYAASMP--ASAPLALAALPKVARTGTHLFTFADYVQQFRG---- 153
C. amycolatum       QRMAVNDP-VTLVDTIVDVSVSGAAPKQQPALFALAFAASVP--QSSQAALAALPRVARTGSALLQFVSYVEKFRG---- 141
A. massiliensis     KRMAVEAP-RTLVDMIVEVSTSGAAPRQNPALFALAYATSVP--QTREAALAALPKVARTGSHLFTFAGYTEQFRG---- 141
B. thetaiotaomicron RTLIGKCN-PLFVAQLAAYARETMNLRSIPLVMAVELARIHQGDNLVKRVTARTVRRADEITELLACYQQANRRTG---T 131

H. sapiens          GMWGRALRKAIADWYNEKGGMALALAVTKYKQRNGWSHKDLLRLSHLKPSSEGLAIVTKYITKGWKEVHELYKEKALSVE 221
M. musculus         GMWGRALRKAVADWYNEKGGMAVALVVTKYKQRNGWSHKDLLRLSHLKPSSEGLAIVTKYITKGWKEVHEEYKEKALSVE 221
P. propionicum      --WGRGLRRAVGNWYTGRRADDLAHQAVKYRQRSGWTHRDLLRLSHPVTTVPELRALFEWIVRG--------------SL 217
C. amycolatum       --WGRGLRRAVGNWYSTKNSDDLAYQVVKYRNRGGWSHRDLLRLAHPSTSDESLRATFDWIVRGS--GSASISDSAGDTS 217
A. massiliensis     --WGRGLRRAVGSWYTSRTADRLAYQAVKYRQREGWTHRDLLRLAHPRTDDAGLGATFDWIAHG--------------AV 205
B. thetaiotaomicron KKLNR-LSKQLQAGLQDAFNTFDAYQFAKYNRSAEVCLKDALFLVHPKAKDERQQEIFNGIVND---------------N 195

H. sapiens          TEKLLKYLEAVEKVKRTKDELEVIHLIEEHRLVREHLLTNHLKSKEVWKALLQE-MPLTALLRNLGKMTANSVLEPGNSE 300
M. musculus         AEKLLKYLEAVEKVKRTKDDLEVIHLIEEHQLVREHLLTNHLKSKEVWKALLQE-MPLTALLRNLGKMTANSVLEPGNSE 300
P. propionicum      GEDTPELVRAFLAAQEATTVAAWVALVREHRLAWEMLPDAALREPEVWEALLDAGIPQTALMRQLPRLTGLGLLDDLSAR 297
C. amycolatum       SENIPTIIEGFTKASHATTSSQWAALIRGYGLSWEMLPDAALGEPEVWDALLETGVPQTAVIRQLPRLTRLGLLPGLGGR 297
A. massiliensis     GEATPSLIEGFIKAQEATIARSWAQIVRDYRLTWEMLPDAALARPEVWDALLDVGVPMTALMRQLPRLTRLGMLPAIGGR 285
B. thetaiotaomicron -LPVPYTWETELSALGQRTFATEEERRKAFRAKWEELIDSGK---LGYMALLRN-------------LRNILEAEVSADH 258

H. sapiens          VSLVCEKLCNEKLLKKARIHPFHILIALETYKTGHGLRGKLKWRPDEEILKALDAAFYKTFKTVE--PTGKRFLLAVDVS 378
M. musculus         VSLICEKLSNEKLLKKARIHPFHVLIALETYRAGHGLRGKLKWIPDKDILQALDAAFYTTFKTVE--PTGKRFLLAVDVS 378
P. propionicum      TEQVCAQLTDPDRLRRARVHPVNVLVAGRTYASGRSTRGSSTWQPSTKVIDALDAAFYAAFGAVT--PSGKRTMLALDVS 375
C. amycolatum       TSDVVSQITNAERLRNARIHPVSVLAAQRTYAKGRSFHGMTEWEPTARISDALDEAFYLSFGAVK--PANKRTLLSLDVS 375
A. massiliensis     TREVCAQLTDADRLRKARVHPVSVLVAQRTYAGGASHRGTAQWEPTTKVADALDAAFYAAFGAVE--PSGRRTMLAVDVS 363
B. thetaiotaomicron ILTVGKRLSSEKAVENSRQLPFRFLAAYRELSKTPSLYAT-------MLMTALERAVQVSARNITGFDESTRVLIACDVS 331

H. sapiens          ASMNQRVLGSILNASTVAAMCMVVTRTEKDSYVVAFSDE----------MVPCPVTTDMTLQQVLMAMSQIPAGGTDCS 448
M. musculus         ASMNQRALGSVLNASTVAAMCMVVTRTEKESSVVAFACD----------MVPFPVTTDMTLQQVLTAMNKVPAGNTDCS 448
P. propionicum      GSMCSHIAGLPITAREASAALALVQLATEPVSAVVGFTSG----------LVPLDLSPRQRLDDALHRIGNLPFGGTDCA 445
C. amycolatum       ASMHWPLGDTPLTARDASAALSLVTLATESESMVLGFTTNSLTKGFLRDVVTPLDISPRQRLDDVLDYIDGLPFGGTDCS 455
A. massiliensis     GSMTMPISGMAITAREASAALALVQLATEPDAEAYGFSSAGGWY---KPALTPLGISPRRRLDDALAAVSSIPMGGTDCA 440
B. thetaiotaomicron GSMQCPVSAKSKVLYYDIGLLLGMLLKSRCKQVMTGIFGDR-------WKIINLPDTGILSNVDAFYKREGEVGYSTNGY 404

H. sapiens          LPMIWAQKTNTPADVFIVFTDNETFAGGVHPAIA-LREYRKKMDIPAKLIVCGMTSNGFTIADPDDRGMLDMCGFDTGAL 527
M. musculus         LPMIWAQKTDTAADVFVVFTDNETFAGQVHPAVA-LREYRKKMDIPAKLIVCGMTSNGFTIADPDDRGMLDMCGFDTAAL 527
P. propionicum      QPMLHALKRRLEVDTFVVYTDNETWCGRIHPHQA-LVRYRRETGIPAKLVVVGMTSTGFSIADPDDAGMLDVAGFDHAVP 524
C. amycolatum       LPMLYALENSLEVDTFVIYTDNETWAGKMHPHQA-LQRYRKESGIDAKLVVAGMTATKFSIANPDDAGMLDVVGFDAAVP 534
A. massiliensis     LPMLHAIEQGLEVDTFVIYTDNETWYGKVHPHQA-LRRYRECSGIDARLIVVGMTSTGFSIADPDDPGMLDVVGFDAAVP 519
B. thetaiotaomicron LVIKDLIDRKAQMDKIMMFTDCQLWNSHSDLQITDLWRKYKKICPAAKLYLFDLSGYGNTPLDITRDDVFLIAGWSDKIF 484

H. sapiens          DVIRNFTLDMI                                                                      538
M. musculus         DVIRNFTLDVI                                                                      538
P. propionicum      NLISEFSRGF                                                                       534
C. amycolatum       NLISEFSRGF                                                                       544
A. massiliensis     SLITEFARGF                                                                       529
B. thetaiotaomicron DILSAIDKGNDALQEIKKIVV                                                            505
```

Fig. S1. Sequence alignment of full-length hRo60 and selected commensal orthologs. GenBank protein accession numbers for Ro60 sequences: H. sapiens, NP_001166995.1; M. musculus, NP_038863.1; *P. propionicum*, AFN45864.1; *C. amycolatum*, EEB62751.1; *A. massiliensis*, EJF36407.1; *B. thetaiotaomicron*, ALJ40817.1. Black highlight indicates identical amino acids. Blue highlight indicates similar amino acids. The three possible start sites for *B. theta* Ro60 are indicated by asterisks.

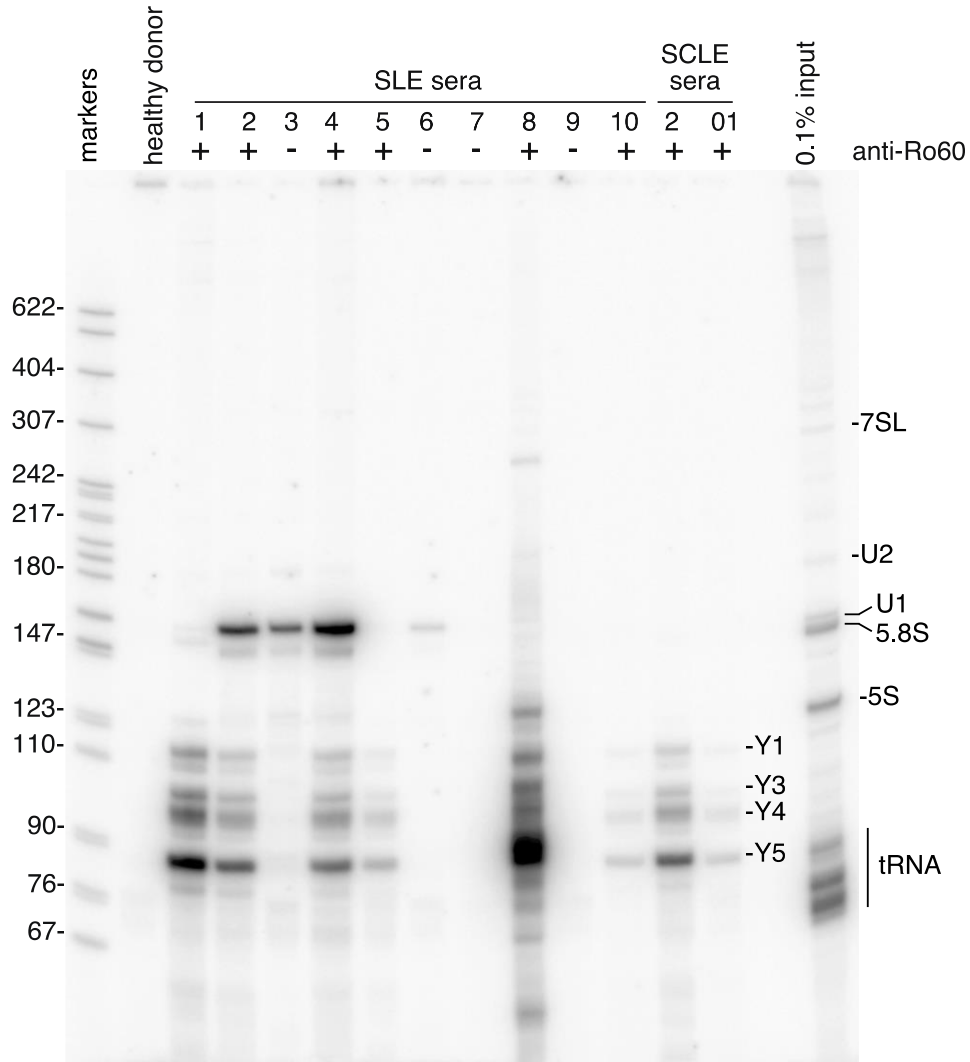

Fig. S2. Coimmunoprecipitation of lupus study subject sera confirmed anti-Ro60 antibody status. Lupus sera were incubated with HEK293T cell lysate and the resulting extracted RNA was labeled with ^{32}P-pCp. Markers with nucleotide size are shown in lane one and a negative healthy control in lane two. Ten SLE sera and two SCLE sera are shown in the following lanes, with + or – indicating anti-Ro60 IgG positivity by ELISA. The RNAs extracted from the total HEK293T cell lysate are shown in the last lane.

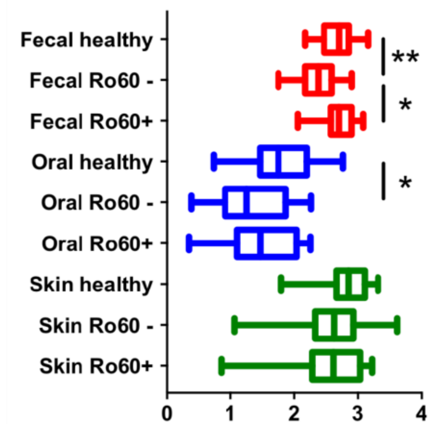

Fig. S3. α-Diversity represented by box plots of the Shannon-Weiner diversity index. Healthy (n=7) and anti-Ro60+ lupus patient (n=15) fecal (red), oral (blue) or skin (green) microbiomes. Black bars show significance between groups measured by t-test (*p<0.05, **p<0.01).

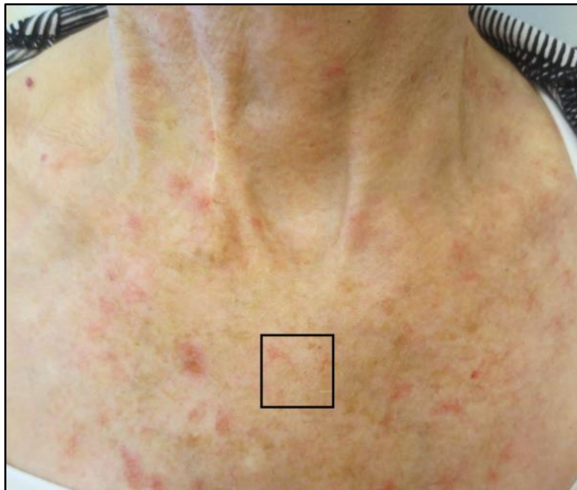

Fig. S4. SCLE skin eruption. The chest skin swab (boxed region) from patient SCLE01 was collected from an active eruption of SCLE for microbiome analysis (photo published with written consent).

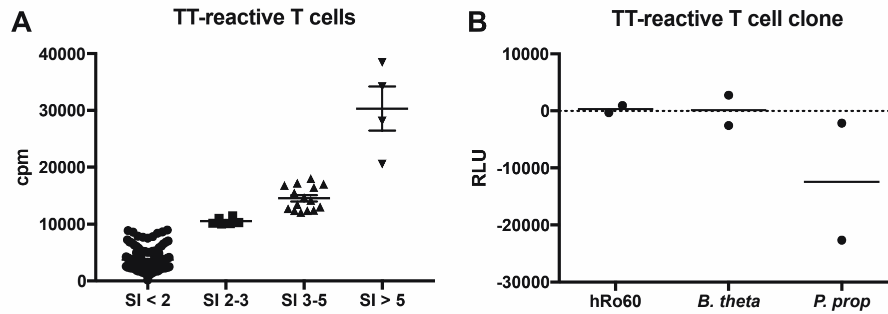

Fig. S5. TT-reactive CD4$^+$ T cell clone from a healthy donor generated by a CD4 T cell library assay. (**A**) TT-specific T cell clones were generated as described for Ro60-reactive T cell clones. X-axis indicated stimulation index (SI) and y-axis indicated proliferation measured by tritiated thymidine as counts per minute (cpm). Each point on the graph represents one clone. (**B**) One of the four clones with SI > 5 was re-stimulated with hRo60 or Ro60 commensal bacteria as indicated (*B. theta*, *P. prop*). Proliferation shown on the y-axis was measured with an ATP release assay as reactive light units (RLU).

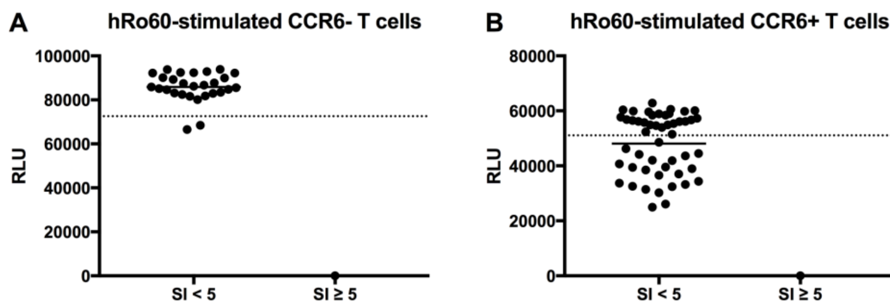

Fig. S6. Ro60-negative SLE patient CD4$^+$ T cells lack reactivity to hRo60 protein. T cells from one Ro60-negative SLE patient were expanded with hRo60 using a T cell library assay as above. T cell activation shown on the y-axis was measured with an ATP release assay as reactive light units (RLU). Dotted line represents background of monocyte-T cell co-cultures without the hRo60 protein. CCR6$^-$ (**A**) or CCR6$^+$ (**B**) T cell subsets separated by stimulation index (SI), with SI ≥ 5 indicating proliferation. No CCR6$^-$ or CCR6$^+$ clones were identified with SI ≥ 5.

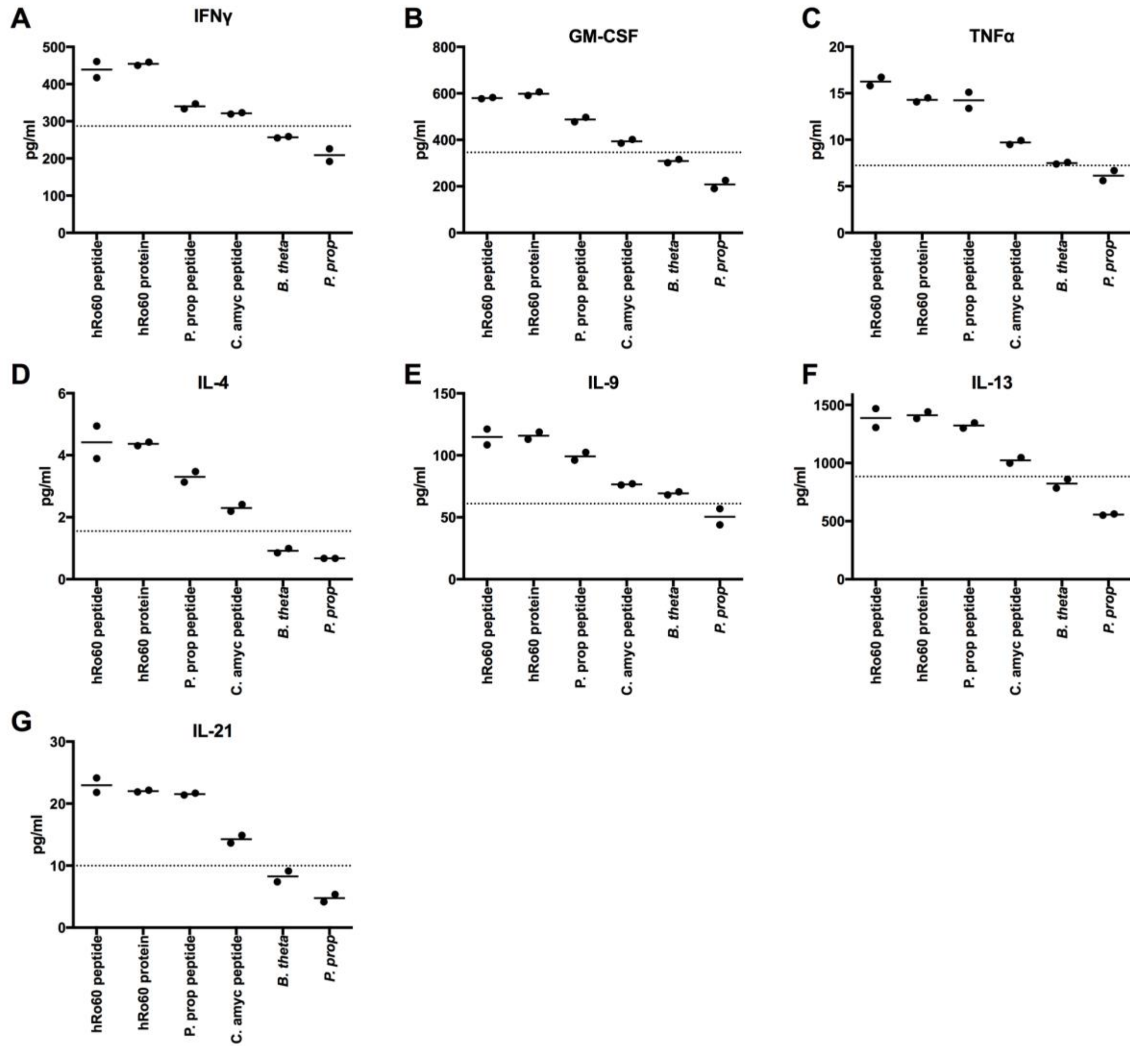

Fig. S7. Cytokine concentrations (pg/ml) of supernatants from the cross-reactive T cell clone from Fig. 5 measured using a bead-based immunoassay. Monocyte control baseline is shown as a dotted line in each graph.

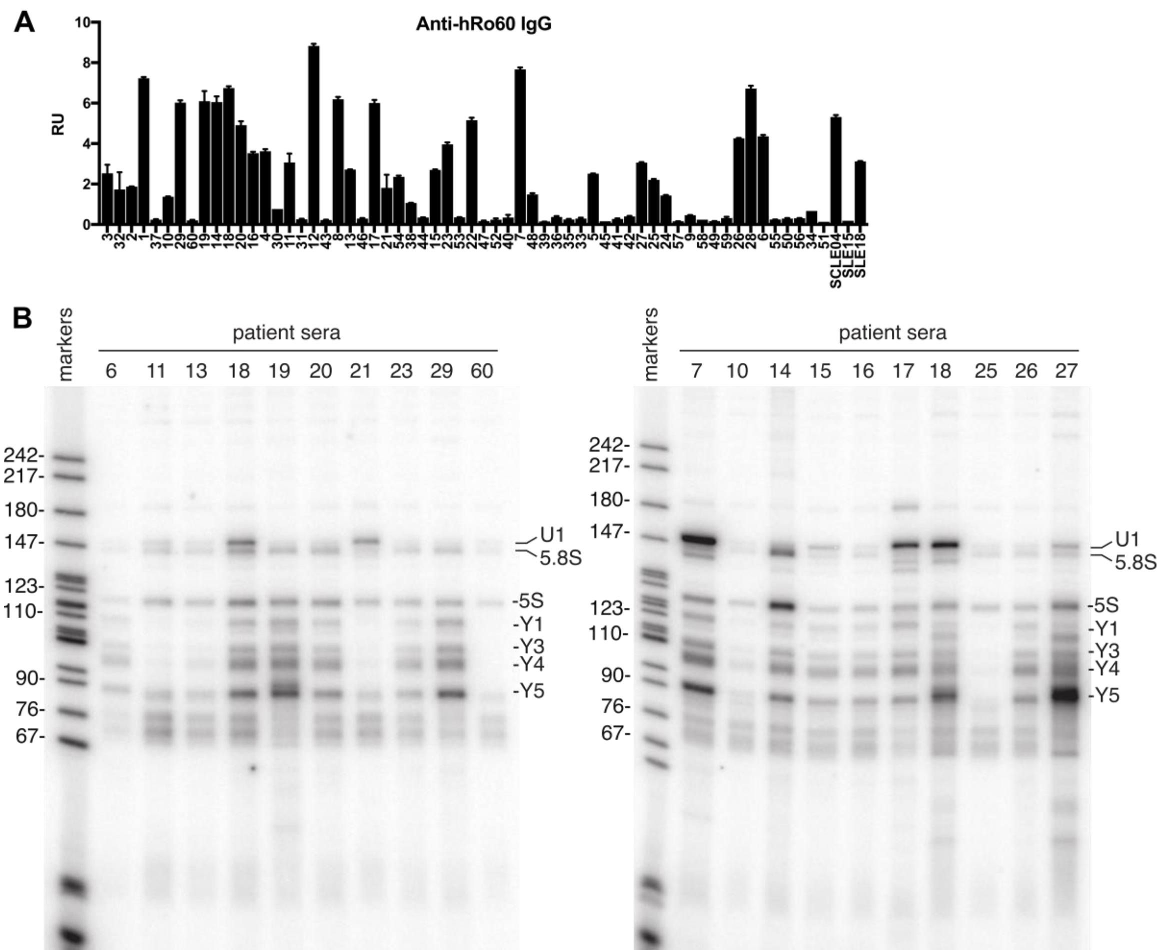

Fig. S8. Anti-Ro60 antibody status of Harvard cohort lupus subjects. (**A**) ELISA of anti-human Ro60 IgG autoantibodies using Harvard lupus sera, listed by identification number. Relative units (RU) shown on y-axis. (**B**) Immunoprecipitation of human Ro60-Y RNA complexes from HEK293T cell lysate using Harvard lupus sera (as described in Fig. S2).

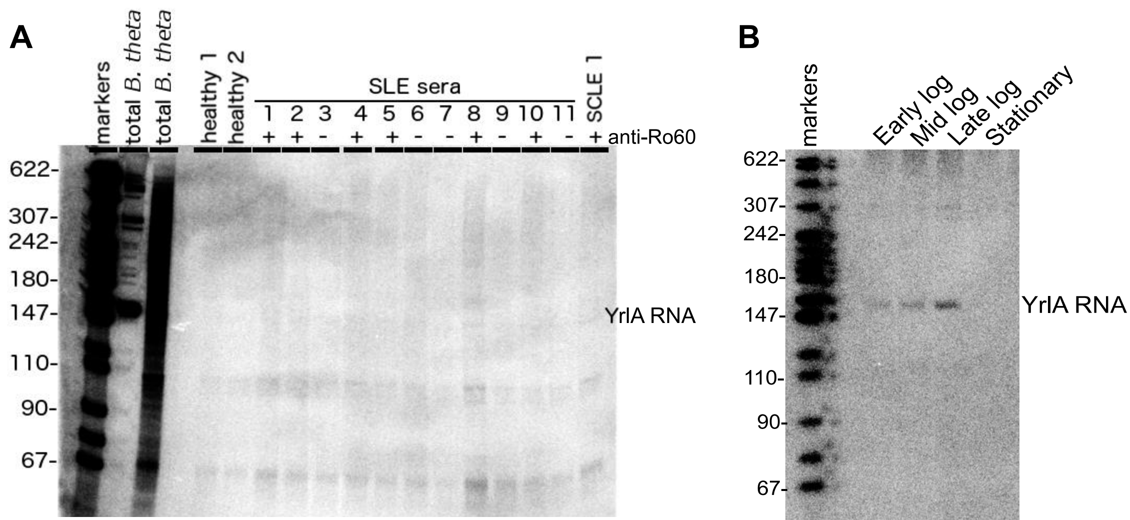

Fig. S9. YrlA RNA is not detected in immunoprecipitates from *B. theta* using human lupus sera. (**A**) Human lupus sera did not immunoprecipitate YrlA RNA-containing RNPs from *B. theta* lysates. First lane, molecular size markers (nt). Total *B. theta*, total RNA extracted directly from the input cells (first total lane) or from the subsequent lysate (second total lane). The degradation of rRNAs in the lysate resulted in significant background hybridization. The RNAs present in immunoprecipitates using sera from healthy donors are shown in the next two lanes. Eleven SLE sera and one SCLE serum are labeled with + or – representing the anti-Ro60 IgG antibody status by ELISA. (**B**) The presence of YrlA in RNA extracted from *B. theta* cells was detected by Northern blotting using a ^{32}P-labeled full-length probe. First lane, molecular markers (nt). Early log, *B. theta* grown to an optical density at 600 nm (OD_{600}) of 0.7. Mid log, whole *B. theta* lysates at OD_{600} of 1.2. Late log, whole *B. theta* lysates at OD_{600} of 1.8. Stationary, whole *B. theta* lysates collected 24 hours after maximum OD_{600} (~2.2) was reached.

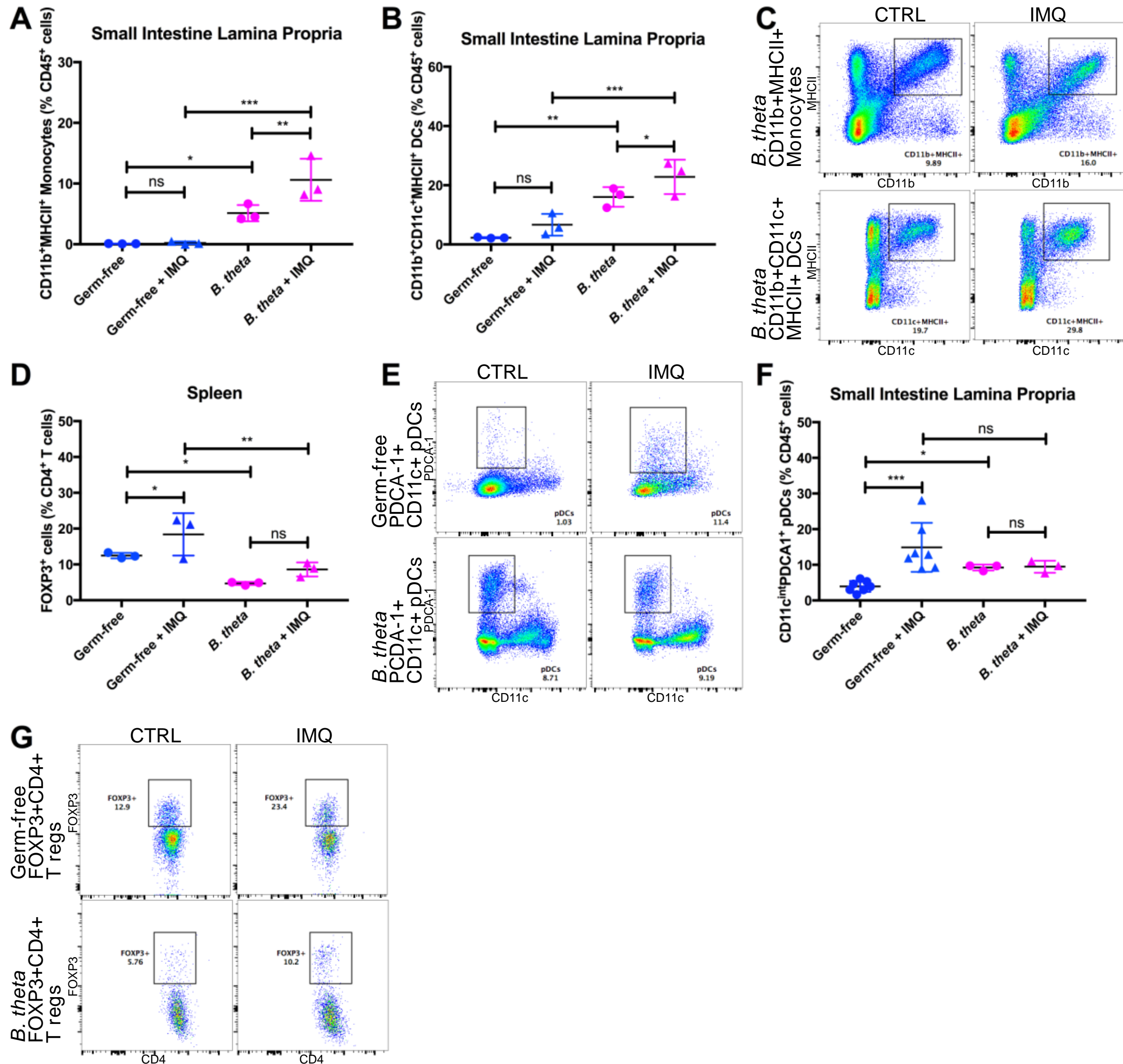

Fig. S10. ***B. theta*** **monocolonization of GF mice induces gut and systemic immune changes.** (**A**) FACS analysis of small intestine lamina propria (SI-LP) CD11b$^+$MHCII$^+$ monocytes and (**B**) CD11b$^+$CD11c$^+$MHCII$^+$ dendritic cells (DCs) compared to GF age- and sex-matched control mice. Mice received imiquimod (IMQ) topically 3 times per week for a total of 8 weeks to induce a SLE-like phenotype. Each dot represents one mouse, n = 3 mice per group. Black bars indicate significance by t-test; ns, not significant, *p<0.05, **p<0.01, ***p<0.001. (**C**) Representative plots of CD11b$^+$MHCII$^+$ monocytes and CD11b$^+$CD11c$^+$MHCII$^+$ DCs in SI-LP. **(D)** *B. theta* monocolonization of GF mice increases gut plasmacytoid dendritic cells (pDCs). FACS analysis of SI-LP CD11c^{int}PDCA-1+ pDCs compared to GF age- and sex-matched control mice. Each dot represents one mouse, n = 3 mice per group. Black bars indicate significance by t-test. (**E**) Representative plots of CD11c^{int}PDCA-1$^+$ pDCs in SI-LP. (**F**) FACS analysis of spleen FOXP3$^+$ T cells. Each dot represents one mouse, n = 7 in each GF group, n = 3 in each B. theta group. Black bars indicate significance by t-test. (**G**) Representative plots of FOXP3$^+$ T cells in spleen.

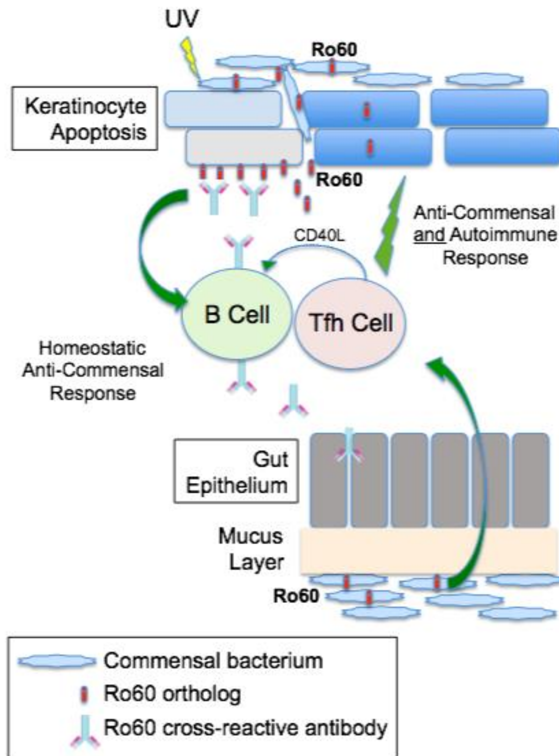

Fig. S11. Schematic of proposed mechanism of how Ro60 bacteria trigger and sustain autoimmunity. Human commensal Ro60 orthologs could drive both cutaneous and systemic lupus erythematosus. The cartoon shows the skin and gut barrier epithelia colonized by commensal bacteria containing Ro60 orthologs (red barrels). Besides homeostatic responses of the innate and adaptive immune system to commensal bacteria at these barrier sites, breach of the epithelial barriers, for example during keratinocyte apoptosis after ultraviolet light exposure (yellow lightning bolt) or gut mucosal irritation by dietary or environmental factors, leads to Ro60 ortholog antigen presentation to T follicular helper cells (Tfh) and activation of B cells by Ro60 and co-stimulation (e.g., CD40 ligand, CD40L) provided by Tfh. These events result in the production of anti-Ro60 antibodies and T cells cross-reactive (green lightning bolt) to both human and bacterial Ro60 due to the high sequence similarity at autoepitopes followed by subsequent tissue damage. Cross-reactive antibodies could thus drive both anti-commensal and autoimmune responses in genetically predisposed individuals.

Table S1. Commensal bacterial Ro60 orthologs identified by in silico methods.*

Phylum	Species	Genebank Accession no.	Locus Tag	Length	Commensal Niche	Genome sequence Genebank Accession no.	Ro60 Coordinate			Distance between Ro60 and YrlA	Putative YrlA Coordinate			E-value
							from	to	strand		from	to	strand	
Actinobacteria	Actinomyces massiliensis F0489	EJF36407.1	HMPREF1318_2237	529	Oral	AKFT01000221.1	20694	22283	+	447	20114	20247	+	6.60E-11
	Actinomyces sp. Oral taxon 414	ALC98376.1	AM609_00765	528	Oral	CP012590.1	203197	204783	+					
	Corynebacterium amycolatum SK46	EEB62751.1	CORAM0001_1910	544	Skin	ABZU01000011.1	51027	52661	+					
	Mycobacterium smegmatis (strain ATCC 700084 / mc(2)155)	ABK75237.1	MSMEG_1193	564	Urogenital	CP000480.1	1259580	1261274	+	164	1259281	1259416	+	3.70E-18
	Parascardovia denticolens IPLA 20019	EIT87591.1	A200_07914	472	Oral, breast milk	AKII01000025.1	49615	51033	-	2062	53276	53095	-	0.00082
										1271	52428	52304	-	0.0028
	Propionibacterium prop F0230a	AFN45864.1	HMPREF9154_0590	534	Oral	CP002734.1	618204	619808	+	17	618059	618187	+	3.40E-10
	Segniliparus rugosus ATCC BAA-974	EFV12828.2	HMPREF9336_02315	538	Airway	ACZI02000002.1	656886	658502	+	518	656228	656368	+	2.80E-10
	Streptomyces sp. HGB0020	EPD57925.1	HMPREF1211_06263	527	GI	AGER01000027.1	358449	360032	+	199	358113	358250	+	5.20E-11
Bacteroidetes	Bacteroides theta 7330	ALJ40817.1	Btheta7330_01248	504	Gut	CP012937.1	1628304	1629818	+	47	1629865	1630002	+	6.20E-07
	Chryseobacterium	EFK36987.1	HMPREF0204_10833	505	Urogenital	ACKQ02000003.1	6745	8262	-	2293	4317	4452	-	4.00E-

	gleum ATCC 35910													08
	Prevotella sp. CAG:1092	CCZ13284.1	BN465_02442	570	Gut	CAZL010000441.1	26326	28038	+					
Firmicutes	Clostridium sp. BL8	EQB87098.1	M918_10850	487	Gut	AUPA01000204.1	6895	8358	+	254	6505	6641	+	1.20E-07
	Paenibacillus sp. HGF7	EGL16326.1	HMPREF9413_2200	501	Gut	AFDH01000102.1	40356	41861	+	282	39937	40074	+	4.40E-13
	Paenibacillus sp. HGH0039	EPD88908.1	HMPREF1207_01859	501	GI	AGEN01000024.1	53351	54856	-	282	55275	55138	-	4.40E-13
beta-Proteobacteria	Eikenella corrodens ATCC 23834	EEG25246.1	EIKCOROL_00065	522	Oral	ACEA01000002.1	41672	43240	+	1402	40137	40270	+	4.70E-14
										1162	40391	40510	+	2.50E-08
	Kingella oralis ATCC 51147	EEP66649.1	GCWU000324_03050	524	Oral	ACJW02000008.1	80514	82088	-	1413	83501	83634	-	8.90E-11
										1132	83220	83353	-	4.60E-08
	Neisseria elongata subsp. glycolytica ATCC 29315	EFE48372.1	NEIELOOT_02899	521	Nasopharyngeal	ADBF01000255.1	30060	31625	-	998	32623	32765	-	1.40E-12
										203	31828	31951	-	2.40E-11
										351	31976	32115	-	5.30E-09
										766	32391	32510	-	4.50E-07
	Neisseria flavescens strain CD-NF1	KZC75466.1	TV01_0020	524	Skin	LAEH01000038.1	8407	9981	-	239	10362	10220	-	3.90E-14

											10656	10516	-	3.10E-09
	Neisseria macacae ATCC 33926	EGQ76909.1	HMPREF9418_1500	524	Gut	AFQE01000071.1	5272	6846	-	1623	8469	8612	-	1.60E-13
										1384	8230	8352	-	4.50E-07
										260	7106	7238	-	4.60E-06
	Neisseria mucosa strain C6A	KGJ32323.1	ES17_04055	524	Nasopharyngeal	JQHF01000006.1	64599	66173	+	246	64217	64353	+	4.20E-16
										533	63926	64066	+	1.50E-09
	Neisseria shayeganii 871	EGY53080.1	HMPREF9371_0707	545	Airway	AGAY01000023.1	12515	14152	-	265	14417	14562	-	1.50E-11
										85	14237	14348	-	1.30E-06
	Neisseria sicca ATCC 29256	EET43536.1	NEISICOT_02760	523	Nasopharyngeal	ACKO02000019.1	47989	49560	+	1263	46586	46726	+	8.50E-14
										1022	46845	46967	+	3.90E-07
	Neisseria sp. GT4A_CT1	EGY60774.1	HMPREF1028_01178	521	Oral	ACWS01000057.1	17480	19045	+	264	17073	17216	+	2.80E-14
	Neisseria sp. oral taxon 020 str. F0370	EKY03172.1	HMPREF9120_02756	522	Oral	AMER01000212.1	8150	9718	+	206	7810	7944	+	7.20E-15
										344	7670	7806	+	8.80E-10
	Neisseria subflava NJ9703	EFC52289.1	NEISUBOT_04391	524	Oral	ACEO02000005.1	134838	136412	-	239	136651	136793	-	1.90E-15

										534	136946	137086	-	1.30E-10
gamma-Proteobacteria	Cardiobacterium valvarum F0432	EHM52985.1	HMPREF9080_02018	306 (partial)	Oral	AGCM01000115.1	<1	919	-	364	1418	1283	-	3.10E-14
										525	1571	1444	-	1.20E-08
										200	1255	1119	-	8.40E-08
Synergistes	Synergistes sp. 3_1_syn1	EHL69418.1	HMPREF1006_01942	511	Gut	ACUH01000014.1	3108	4643	-					

*Ro60 orthologs were identified in GenBank and cross-referenced with public databases from the Pathosystems Resource Integration Center (PATRIC) and the Human Microbiome Project to identify human commensal species. Bacterial genomes were searched for the presence of YrlA RNA as described (*12*).

Table S2. Lupus study subject clinical data.*

Subject	SLE01	SLE02	SLE03	SLE04	SLE05	SLE06	SLE07	SLE08	SLE09
Number visits completed	3	3	3	3	3	2	3	1	1
Age	40	47	49	29	49	40	31	52	34
Sex	F	F	F	F	F	F	F	F	F
Race	White	White	Black	Black, American Indian, White	White	Black	White	Black	White
Ethnicity	Latina	Not Hispanic	Not Hispanic	Not Hispanic	Not Hispanic	Not Hispanic	Latina	Not Hispanic	Latina
Weight (kg)	59	87.5	62.6	47.6	72.6	65.3	87.1	54.4	68.6
Height (inches)	62.75	62	64.75	66.5	66.5	59	65	63	60.5
BMI	23.2	35.3	23.1	16.7	24.4	29.1	31.9	21.3	29.5
Lupus Diagnosis	SLE	SLE	SLE	SLE	SLE	SLE	SLE	SLE	SLE
Other autoimmune diagnoses	MCTD	Sjogren’s	MCTD	Sjogren’s, MCTD	None	DLE	None	None	None
Year of diagnosis	2003	1984	1985	2006	2013	1997	2014	unknown	2011
ANA	P	P	P	P	P	P	P	P	P
Ro/SSA	P	P	P	P	P	N	N	P	N
La/SSB	P	N	N	N	N	N	N	P	N
dsDNA	P	P	N	P	P	P	N	N	N
Sm/U1RNP	P	P	P	P	N	P	N	-	N
WBC (1000 cells/ml)	10.7	9.6	3.0	3.8	3.8	2.7	7.5	5.6	6.7
Hemoglobin (g/dl)	13.5	11.8	11.8	12.8	13.2	12.4	12.8	12.5	14
Platelets (cells/ml)	185,000	207,000	165,000	194,000	274,000	98,000	376,000	135,000	234,000
Creatinine (mg/dl)	0.6	2.4	0.7	0.6	0.7	1	0.5	0.8	0.76
CRP (mg/l)	6.6	8.0	0.4	-	-	0.5	7.3	1.1	0.5
C3 (mg/dl)	72	109	97	95	86	69	109	122	88

C4 (mg/dl)	14	27	23	26	14	21	28	28	16
Flare	N	Y (mild)	N	Possibly	N	N	N	N	N
General	Fatigue, occasional sweats	Sweats related to menopause; insomnia	Fatigue	Fatigue	No, prior fatigue and fevers	Fatigue, 6 lb weight loss	Fatigue	Fatigue, 4 pound weight loss	Fatigue
Photosensitivity	Y	Y	N	Y	Y	N	N	N	Y
Other rashes	N	Malar cheeks	N	Malar rash with flares	N	Extensive DLE on legs, arms, scalp > back	Previous malar erythema	N	Malar rash at diagnosis
Oral and nasal ulcers	N	Y	N	N	Y	N	N	N	N
Raynaud’s	N	N	Y	Y	Y	Y	N	N	N
Neurologic disorders	N	Depression	Prior facial and arm unilateral numbness and weakness, no CVA ever diagnosed	Bipolar disorder	Impaired balance	Prior cerebritis	Migraines, forgetfulness	N	N
Joint pains	Y	Y	Y	Y	Y	Y	Y	Y	Y
Cardiovascular/ Respespiratory disorders	N	Prior pericarditis and pleurisy; hypertension	Prior pleuritis	Interstitial lung disease	Prior frequent chest pressure and SOB	Prior pericarditis	Palpitations and chest pains	N	N
Vascular disorders	N	5 pregnancy losses	N	N	N	Prior blood clot with negative APS	Pulmonary embolus	N	N
Renal disorders	N	CKD stage III	CKD Stage I, proteinuria	N	N	Lupus nephritis class II	Kidney stones	N	Microscopic hematuria
GI disorders	N	Gastroparesis	N	Acute and chronic pancreatitis	N	N	Galllstones	N	N
Ro60 ELISA (Dr. Fooke)	Positive (RU 4.3)	Positive (RU 4.6)	Negative (RU 0.76)	Positive (RU 5.8)	Low Positive (RU 1.7)	Negative (RU 0.4)	Negative (RU 0.63)	Positive (RU 2.2)	Negative (RU 0.9)
HLA DRB1*0301 (PCR)	N	**P**	N	N	N	N	N	N	**P**
HLA DRB1*0301-0302 (PCR)	N	N	N	N	N	N	N	N	**P**

HLA DR15 (PCR)	N	N	N	N	**P**	**P**	N	N	N
HLA DR53 (PCR)	**P**	N	**P**	N	N	N	N	**P**	N
VISIT 1	Y	Y	Y	Y	Y	Y	Y	Y	Y
Hydroxychloroquine	N	N	200 mg daily	discontinued against medical advice 2 months prior to first visit	200 mg twice daily	200 mg twice daily	200 mg twice daily	200 mg twice daily	200 mg twice daily
Immunomodulatory medications	Azathioprine 50 mg twice daily	Rituximab 1 month prior to first visit	Mycophenolate mofetile 750 mg twice daily	discontinued against medical advice 1 month prior to first visit	N	Mycophenolate mofetil 1500 mg twice daily	N	N	N
Prednisone	N	5 mg daily	7.5 mg daily	discontinued against medical advice 1 month prior to first visit	1 mg daily	5 mg daily	tapered off 1 month prior to first visit	N	N
Analgesics	Naproxen	Acetaminophen	N	N	Aspirin, naproxen	N	N	N	N
Anticoagulants	N	N	N	N	N	Warfarin	Warfarin	N	N
Antihypertensives	N	Metoprolol, enalapril	Lisinopril	N	N	Carvedilol, losartan	N	N	N
Proton pump inhibitor	Pantoprazole	Esomeprazole	Esomeprazole	N	N	N	N	N	N
Other prescription medications	Valacyclovir	Diazepam, nortriptyline, prochlorperazin, sumatriptan, venlafaxine	Simvastatin	N	N	Simvastatin, hydroxyzine	N	N	N
Vitamins, minerals, supplements	N	B-complex vitamin, vitamin D3, cranberry, iron, thiamine	Calcium, iron, multivitamin, biotin, black seed oil	N	Multivitamin, fish oil	Multivitamin, folic acid	Iron	N	Vitamin C, vitamin B12, iron
Topical medications	N	Ciclopirox solution to left great toenail	N	N	N	Clobetasol	N	N	N
Breakfast	Coffee, bite of turkey egg sandwich	Raisin bagel, coffee	Orange	None	Toast with butter, egg, bacon	Oatmeal, hashbrowns, coffee	Corn muffin with butter, coffee with sugar and cream	Grits, whole milk, butter, coffee, cream, sugar	Oatmeal maple brown sugar, powdered peanut butter, coffee

									with cream and Coffee Mate caramel flavor
Lunch	Chicken wings	None	Gritts, eggs, bacon, sausage	Rice and peas with roasted chicken and pork	Apple, pasta with tomato sauce and parmesan	Package of cheese and crackers, flavored water	Rice, beans, chicken, tortilla, mild sauce, avocado, Pepsi	Popcorn, salt, butter	Strawberry yogurt, plum
Dinner	None	Beef and dumpling stew	Fried fish, hush puppies, salad	None	Turkey, stuffing, potatoes, asparagus, apple cider	Chicken wings, half cheeseburger, potato chips	Pepperoni and cheese pizza, carbonated soft drink	Steamed broccoli, cauliflower, carrots, butter	Chinese rice, chicken, crab rangoon, watermelon, pineapple, cantaloupe
Major dietary changes in last 2 weeks	N	N	N	N	Usually vegetarian based diet, had meat twice in last day	N	N	N	N
Bristol stool scale	type 1	type 2	type 3	type 3	type 3	type 3	type 3	Not recorded	type 4
VISIT 2	Y	Y	Y	Y	Y	Y	Y	Y	N
Change in health	N	N	N	N	N	N	Pregnant		
Protocol deviations	N	N	N	N	N	N	N	Took antibiotics, cancelled visit	
Change in medication	N	N	N	N	Prednisone decreased to 0.5 mg daily	N	Stopped warfarin, started rivaroxaban		
Changes in diet	N	N	N	N	N	N	N		
Breakfast	Coffee	Coffee with cream and Equal, raisin bagel with margarine	None	None	Whole grain toast, cereal, fried egg, tea, honey, milk	Hash browns, coffee with cream and sugar	Belvita biscuits, coffee, light cream, sugar		
Lunch	Granola bar	None	Crackers and cheese	Turkey sandwich, grape juice	Chickpea stew with tomatoes and basmati rice, baked butternut squash with apples and raisins, decaf	Sandwich of turkey, lettuce, tomato, salt, pepper, oil, vinegar, mayonnaise, onions, american	Chicken nuggets, french fries, ginger ale		

					coffee, half and half, sugar	cheese, hardroll; flavored water			
Dinner	Rice, beef, cauliflower with white sauce	Baked chicken	Baked macaroni and cheese, stewed pig feet, mixed collard greens, ginger ale	Fried popcorn chicken, Pepsi	Corn chips, hummus, sesame seeds, olives, apple cider herb tea, biscuit cracker with chocolate	Cake and ice cream	Mashed potatoes with butter and milk, boneless pork rib, cranberry peach soda		
Bristol stool scale	type 1	type 6	type 3	type 1	type 4	not received	type 1		
VISIT 3	Y	Y	Y	Y	Y	N	Y	N	N
Change in health	N	Pleurisy	N	N	N		Pregnant, thrombophilia		
Protocol deviations	N	N	N	N	N		N		
Change in medication	N	Prednisone 5 mg twic e daily for 4 days	N	Started acetaminophen/oxycodone as needed for pain	Stopped prednisone		Stopped rivaroxaban, started enoxaparin, hydroxychloroquine, prenatal vitamin, vitamin D		
Changes in diet	Added protein shakes, changed from white bread to wheat, smaller portions and exercise	Phosphate restriction	N	N	N		N		
Breakfast	White bread with cream cheese, orange juice, coffee with milk and sugar	Coffee, cream, aspartame sweetener	Grits, eggs, bacon, flavored water	Ginger ale	2 banana oatmeal cookies, black tea with milk and maple syrup		Banana, biscuit, coffee, cream, sugar		
Lunch	Grilled chicken, brown rice, corn, peas, broccoli	None	Potato chips, cheese-flavored crackers	Cheese grits, chocolate chip cookies, Min Maid fruit punch	Drunken noodles with chicken, peppers, onions, broccoli		White rice, grilled chicken, pineapple, soy sauce, ginger ale		

Dinner	Beef with onions and mushrooms, white rice, salad with red wine vinegar	Bagel with butter, American cheese, Egg Beaters, butter, bread	Lasagne, bread, flavored, Adirondack soda	Ice cream dulce de leche, Slim Jims, cheese and crackers, C&C black cherry soda	Cottage cheese with pineapple, fritata with kale, chard, sweet potato, herbal tea; snack of banana oatmeal cookies		Yogurt, string cheese		
Bristol stool scale	type 4	not received	type 1	not received	not recorded		type 2		

*Abbreviations: F, female; BMI, body mass index, SLE, systemic lupus erythematosus; SCLE, subacute lupus erythematosus; DLE, discoid lupus erythematosus; MCTD, mixed connective tissue disease; PBC, primary biliary cirrhosis; P, positive; N, negative/no; Y, yes; -, not available; RU, relative units.

Table S2. Lupus study subject clinical data. continued*

Subject	**SLE10**	**SLE11**	**SLE12**	**SLE13**	**SLE14**	**SLE15**	**SLE18**	**SCLE01**	**SCLE04**
Number visits completed	3	3	3	1	3	3	2	1	2
Age	48	31	54	26	32	44	33	75	72
Sex	F	F	F	F	F	F	F	F	M
Race	White	White	Black	Black	Black	Portuguese/Native American	Black	White	White
Ethnicity	Not Hispanic	Not Hispanic	Not Hispanic	Not Hispanic	Not hispanic	Hispanic	Not Hispanic	Not Hispanic	Not Hispanic
Weight (kg)	52.8	69.6	72	63.6	85.7	87.8	95.3	51.7	81.4
Height (inches)	68.5	67	62.75	62.5	64	59.25	63.5	64	65
BMI	17.4	24.0	28.3	25.2	32.4	38.8	36.5	19.6	29.8
Lupus Diagnosis	SLE	SLE	DLE	SLE	SLE	SLE/Discoid Lupus	SLE	SCLE	SCLE
Other autoimmune diagnoses	None	None	None	Sjogren's	None	None	None	PBC/autoimmune hepatitis	None
Year of diagnosis	1982	2015	1990	2015	2016	1993	2012	unknown	~1997
ANA	P	P	N	P	P	P	P	N	P
Ro/SSA	-	N	-	P	N	P	P	P	Y
La/SSB	-	N	-	4.1	N	N	P	N	N

dsDNA	-	P	-	Y	N	P	P	-	-
Sm/U1RNP	-	N	-	-	P	-	P	-	-
WBC (1000 cells/ml)	11.6	4.1	4.9	11.5	-	6.8	4.2	4.9	4.5
Hemoglobin (g/dl)	11.4	12.2	12.7	9.1	-	13	12.2	12.5	12.6
Platelets (cells/ml)	158,000	218,000	168,000	351,000	-	237,000	229,000	205,000	37,400
Creatinine (mg/dl)	0.95	1.0	0.6	1	-	0.9	0.74	0.8	1.37
CRP (mg/l)	3.37	<0.1	-	2.7	-	10.9	5.1	-	-
C3 (mg/dl)	114	84	-	58	-	151	132	-	-
C4 (mg/dl)	24	12	-	7	-	42	29	-	-
Flare	N	N	N	Y	N	N	-	Y	Y
General	N	Fatigue	Chronic pain	Fatigue, fevers 1 - 2x/wk, weight loss	Fatigue	Fatigue, body aches, weight gain	N	Fatigue	Fatigue
Photosensitivity	N	Y	N	Y	Y	Y	N	Y	Y
Other rashes	N	N	Vertex scalp DLE	Pink pruritic papular eruption on the face, chest, back, arms, legs	N	Stress-induced rash on face, arms, hands, legs	Malar rash with flares	Annular eruption on chest and back	Eruption on neck, arms, chins
Oral and nasal ulcers	N	N	N	Y	N	Y	N	N	N
Raynaud's	N	N	N	Y	-	-	-	N	N
Neurologic disorders	Seizures in 1983 from uncontrolled hypertension	Seizures, stroke, varicella zoster CNS vasculitis	N	N	N	Migraines	N	N	N
Joint pains	N	N	Y	N	Y	Y	Y	N	Y
Cardiovascular/Respespiratory disorders	Hypertension	N	N	Prior pericarditis and pleuritis	N	Hypertension	Pericarditis and pleuritis	Myocardial infarction age 58	Chronic heart failure, chronic

									obstructive pulmonary disease
Vascular disorders	N	DVT right leg	N	N	N	DVT	N	N	N
Renal disorders	Lupus nephritis	Lupus nephritis	N	Lupus nephritis class IV	N	N	N	N	N
GI disorders	N	Vomiting	N	Diarrhea and vomiting	Prior esophageal dilation	Constipation	N	N	N
Ro60 ELISA (Dr. Fooke)	Positive (RU 3.4)	Negative (RU 0.3)	Negative (RU 0.7)	Positive (RU 12.1)	Negative (RU 0.1)	Negative (RU 0.5)	Positive (RU 3.1)	Positive (RU 2.9)	Positive (RU 5.3)
HLA DRB1*0301 (PCR)	P	P	P	P	N	N	N	P	N
HLA DRB1*0301-0302 (PCR)	P	P	N	P	N	N	N	P	N
HLA DR15 (PCR)	P	P	N	N	N	P	N	N	N
HLA DR53 (PCR)	N	N	N	P	-	-	-	N	-
VISIT 1	Y	Y	Y	Y	Y	Y	Y	Y	
Hydroxychloroquine	200 mg daily	200 mg twice daily	200 mg daily	200 mg twice daily	200 mg twice daily	200 mg twice daily	N	200 mg twice daily	N
Immunomodulatory medications	N	Mycophenolic acid 360 mg twice daily	N	Mycophenolic acid 1080 mg twice daily, Methotrexate 25 mg weekly, Benlysta	N	N	N	Azathioprine 50 mg daily	N
Prednisone	N	10 mg daily	N	7.5 mg daily	N	7 mg daily	N	N	N
Analgesics	N	N	Oxycodone, morphine	Oxycodone/acetaminophen, fentanyl transdermal patch	N	Oxycodone	N	N	N
Anticoagulants	Aspirin	Warfarin	N	N	N	Enoxaparin	N	Aspirin	Prasugrel, aspirin

Antihypertensives	Nifedipine, enalapril	N	N	Amlodipine, carvedilol	N	Lisinopril	N	Amlodipine	Metoprolol
Proton pump inhibitor	N	N	Esomeprazole	Pantoprazole	Lansoprazole	N	N	Omeprazole	N
Other prescription medications	N	Gabapentin, valacyclovir, lorazepam	Gabapentin	Trazodone	Linaclotide, Mometasone furoate inhaled, albuterol	Zolpidem, albuterol, tiotropium, botulinum toxin, metoclopramide, senna, marijuana, polyethylene glycol	Etonorgestrel implant	Levothyroxine, Ursodiol, conjugated estrogen, progesterone, ranitidine	Tamsulosin, finasteride, alprazolam, atorvastatin, escitalopram, furosemide
Vitamins, minerals, supplements	Multivitamin	Vitamin D, vitamin B12, biotin	Folic acid	Folic acid	Vitamin D	Calcium, Vitamin D, multivitamin	Biotin, multivitamin	Vitamin D, vitamin B, calcium, fish oil	N
Topical medications	N	N	Clobetasol	Triamcinolone, hydrocortisone, pimecrolimus	Diclofenac 1% gel	N	N	Triamcinolone	Triamcinolone, betamethasone
Breakfast	Oatmeal with banana, cantaloupe, tea with sugar and cream	Gluten free banana bread	None	Eggs, grilled cheese, coffee, sugar, almond milk, iced tea	Peanut butter, white bread, orange-flavored drink	Coffee, cream, sugar	Granola bar, fruit-flavored cereal, milk	Cheese Danish, coffee, cream	Bialy bread, butter, coffee, cream
Lunch	Chicken soup, 2 slices rye toast with cream cheese	None	Steak and cheese with mushrooms and lettuce sandwich, tomatoes, low-calorie lemonade	Cannot remember	White rice, red beans, orange-flavored soda	N	Chicken wings, fried rice with pork	Apple	Ham and cheese sandwich
Dinner	Pork roast, fettucini alfredo, peas	Carrot, ginger, onion soup; chicken broth, gluten free	Steak, baked potato with swiss cheese, low-calorie	3 bowls sweetened oatmeal, bread with chocolate	More rice and beans, peanut butter + white bread	Macaroni with cheese, collard greens, ribs with barbeque	Grilled chicken, vegetables, cheese,	Chicken, spinach, cabbage-beet salad,	Chicken salad, potatoes, ice cream,

		bread, 2 chocolate chip cookies	lemonade	spread, juice, 2 sips wine		sauce, corn bread, butter	potatoes, red wine cocktail	chocolate chip cookies, milk	chamomile tea, 1 beer
Major dietary changes in last 2 weeks	N	N	N	N	N	N	N	N	N
Bristol stool scale	type 1	type 3	type 7	type 5	-	-	-	type 2	-
VISIT 2	Y	Y	Y	N	Y	Y	Y	N	Y
Change in health	N	N	N		Miscarriage	N	N		N
Protocol deviations	N	N	N		Antibiotic use 4 weeks prior to visit, brushed teeth prior to visit	N	N		N
Change in medication	N	Stopped warfarin, started folic acid	N		Stopped mometasone inhaler, started fluticasone inhaler	N	N		Stopped topical steroids, started hydroxychloroquine 200 mg daily
Changes in diet	N	Added green leafy vegetables after stopping warfarin	N		N	N	N		N
Breakfast	Maple and brown sugar oatmeal	Banana, coffee with cream	Blueberry muffin		Peanut butter, jelly, white bread	None	Bacon, eggs, toast, butter, jelly, coffee, cream, sugar		Bread, butter, banana, coffee, cream
Lunch	Taco Bell crunch wrap supreme	Salad with tomatoes, cucumbers, italian dressing; baked chicken, roasted	Salad with lettuce, Swiss cheese, onions, pastrami, broccoli, celery, mushrooms,		Peanut butter, jelly, white bread, cheese-flavored crackers, juice	Chili hot dog, cheese, chili cheese fries, cranberry juice, coffee, orange soda, lemon-lime	Fish sandwich on white roll, tartar sauce, red plum juice, chocolate frappe		Hamburger, bean, ice cream, melon

		potatoes	raspberry wine vinaigrette, 1/2 cup beer			soda			
Dinner	Roasted pork loin with roasted potatoes, pork gravy, corn with butter	Cod fish, broccoli and mushrooms with olive oil and balsamic vinegar	Pizza with mushrooms, pepperoni, sausage, cheese, garlic powder, fruit punch		Peanut butter, jelly, white bread, banana, juice	Tortilla chips, cheese, tomatoes, beef, beans, fried chicken, french fries, cranberry juice	None		Pasta with tomato sauce, chamomil e tea
Bristol stool scale	type 1	type 2	type 6		type 7	-	-		type 7
VISIT 3	Y	Y	Y	N	Y	Y	N	N	N
Change in health	N	N	N		N	N			
Protocol deviations	N	N	N		Brushed teeth	N			
Change in medication	N	Decreased prednisone to 7.5 mg daily	N		N	Decreased prednisone to 5 mg daily, started diazepam			
Changes in diet	N	N	N		N	N			
Breakfast	Oatmeal, fresh pineapple, almonds, black tea, sugar, nonfat creamer, banana	Banana, orange, coffee, cream	Pink grapefruit		N	Sausage, egg, cheese, roll			
Lunch	Tortilla chips, guacamole, cheddar and bean dip, lettuce, carrots, breaded chicken, croutons, blue cheese dressing, 1/2	Corn taco with beef, cheddar cheese, tomatoes, sour cream, salsa	Pork chop, gravy, 1 beer		Pizza with sausage and pepperoni, turkey sandwich, lettuce, tomato, mayonnaise, onion	Hotdog, hotdog bun, fruit-flavored drink			

	grilled cheese sandwich, hot chocolate, mashmallows									
Dinner	Pasta, green beans, black cherry seltzer, swiss roll	Veggie burger with hummus, broccoli, red pepper, onion	Sausage with green peppers and onions on a potato roll, low-calorie lemonade, 1 beer		Pierogi, noodle soup, flavored water, lemon-lime soda, chocolate candy	Cheese sandwich, cola, fruit juice, cheese-flavored chips, pretzels				
Bristol stool scale	type 3	type 1	type 5		-	-				

*Abbreviations: F, female; BMI, body mass index, SLE, systemic lupus erythematosus; SCLE, subacute lupus erythematosus; DLE, discoid lupus erythematosus; MCTD, mixed connective tissue disease; PBC, primary biliary cirrhosis; P, positive; N, negative/no; Y, yes; -, not available; RU, relative units.

Table S3. Healthy control study subject clinical data.*

Subject	**NOR01**	**NOR02**	**NOR04**	**NOR05**	**NOR06**	**NOR07**	**NOR08**	**NOR09**	**NOR10**	**NOR11**	**NOR12**
Visits completed	3	1	3	1	3	3	3	3	2	3	3
Age	55	32	29	36	29	47	55	21	38	40	-
Sex	F	F	F	F	F	F	F	F	F	F	-
Race	White	Mixed	White	Costa Rican	White	White	White	Black	White	White	White
Ethnicity	Not Hispanic	Not Hispanic	Not Hispanic	Hispanic/Latina	Not Hispanic	Hispanic/Latina	Not Hispanic	Hispanic	Hispanic	Not Hispanic	Not Hispanic
Weight (kg)	74.8	89.8	94	71	92.2	82.1	63.8	53.2	88.2	77.4	58.9
Height (inches)	67	67	67	58	70	61	62.5	63	64	69.5	63.75
BMI	25.8	31	32.4	32.7	29.1	34.2	25.3	20.8	33.4	24.8	22.5
Medical diagnoses	Schatzki ring	N	Alpha thalassemia trait	Hypothyroidism, hypertension, seasonal allergies	N	Migraine	Hypothyroidism	Asthma	Anxiety, prior kidney stones	N	N
Ro60 ELISA (Dr. Fooke)	Negative (RU 0.29)	Negative (RU 0.34)	Negative (RU 0.10)	Negative (RU 0.51)	Negative (RU 0.67)	Negative (RU 0.61)	Negative (RU 0.20)	-	-	-	-

HLA DRB1*0301 (PCR)	N	N	N	N	N	N	N	-	-	-	-
HLA DRB1*0301-0302 (PCR)	N	N	N	N	N	N	N	-	-	-	-
HLA DR15 (PCR)	N	N	N	N	N	N	N	-	-	-	-
HLA DR53 (PCR)	N	P	P	P	P	N	N	-	-	-	-
VISIT 1	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Prescription medications	N	N	N	Levothyroxine, hydrochlorothiazide	N	Sumatriptan	Levothyroxine	Albuterol	Clonazepam, gabapentin, nortriptyline, oral contraceptive	N	Sertraline
Vitamins, minerals, supplements	Multivitamin, omega 3, fiber, melatonin, calcium, glucosamine and chondroitin	N	Ibuprofen	N	Multivitamin	Vitamin C, vitamin D	N	N	N	N	N
Breakfast	Banana, milk	Tea, milk, sugar, orange juice	English muffin, low-fat peanut butter, sugar, coffee, skim milk	None	Chocolate protein shake, banana, spinach, dry peanut butter, coffee	Coffee, whole milk, sugar, white bread, butter	Rye bread with mayonnaise, ham, roasted red peppers; black coffee with coconut oil	Donut, coffee with sugar, cream, chocolate	Coffee with cream and sugar, biscuit	Milk, banana, grapes	Bagel, cream cheese, coffee, cream, sugar
Lunch	Salad, diet carbonated soda, cake, cheese	Egg and cheese sandwich on wheat bread, tomato, coffee, cream,	Turkey sandwich on wheat roll with mustard, clementine, wheat thins	Cannot recall	Peanuts	Chicken nuggets, hamburger, French fries	Rye bread with red peppers	Cheese flavored corn puffs, chocolate and cookie bar	N	Rice, chicken, cabbage, carrots, yogurt	Cheese pizza

		sugar									
Dinner	Linguini, clam sauce	Chicken wing, vegetable soup, 2 small spring rolls	Grilled chicken breast, temaki sauce, roasted potatoes, broccoli, beer	Cactus, octopus, shrimp, crab, tomatoes, onions, cilantro fried chicken, lemon juice, wine	Grilled chicken, onions, green pepper, wheat tortilla wrap, guacamole, tomato salsa, cheese mix, Zero calorie carbonated soda	Beef stew with potatoes, white rice	Vegetable and chicken soup over spaetzle noodles, mushrooms, carrots, parsnips, pepper, celery and celery root	Rice, beans, broccoli, asparagus, blueberry juice with mango	Coconut shrimp, stuffed clams, roll, butter, lemon-lime soda, cola, vanilla ice cream, white wine	Green tea, tomato, cheese, bread, sunflower seeds, almonds, walnuts, dried fruit	Pork meatballs, cranberry and beet sauce, brown rice, sour cream, sports beverage
Major dietary changes in last 2 weeks	N	N	N	Not answered	Not answered	N	N	N	N	Ate Indian food	N
Bristol stool scale	type 3	type 3	type 4	type 7	type 2	type 4	type 4	type 5	type 1	-	type 3.5
VISIT 2	Y	N	Y	N	Y	Y	Y	Y	Y	Y	Y
Change in health	Endoscopy with dilation of Schatzki ring		N		N	N	Syncopal episode	N	Sinusitis	N	N
Protocol deviations	N		N		N	N	N	N	Antibiotic use 4 weeks prior	N	N
Change in medication	N		N		Started metformin	N	N	N	N	N	N
Changes in diet	N		N		N	N	N	N	N	N	N
Breakfast	Banana, milk		Wheat toast with peanut butter, coffee, skim milk		Chocolate protein shake with banana, peanut butter, coconut milk, coffee, cream	Eggs, vegetables, onion, American cheese, butter, coffee, whole milk, sugar	Coffee with coconut oil	Egg, cheese bread, orange juice	Cereal, milk, bagel, butter, coffee, cream, sugar	N	Bagel, cream cheese, coffee, cream, sugar
Lunch	Salad with cranberries, blue cheese,		Beef and potato stew, apple, grapes,		Lasagne soup with chicken, sausage,	Chicken broth, chicken	Salad with spinach, eggs, barbecue	Rice, beans, salad of	Hot dog, bun, lemon-lime	Bacon, lettuce, tomato,	Cheese pizza

	spinach, raspberry dresssing, walnuts, diet carbonated soft drink		yogurt		marinara sauce, chicken broth, whole wheat pasta, vanilla greek yogurt	breast	spare ribs, cranberries, sunflower seeds, chia seeds, flax seeds, bell pepper, roasted pepper, avocado	cucumbers, lettuce, cheese, olive oil, lemon, orange juice	soda	bread, orange juice	
Dinner	Onion soup, milk		Chicken, broccoli, rice, quinoa		Lasagne soup with chicken, sausage, marinara sauce, chicken broth, whole wheat pasta, Italian multigrain bread, butter, lemonade fruit punch, beer	Pizza, broccoli, Coke	Salad with spinach, eggs, barbecue spare ribs, cranberries, sunflower seeds, chia seeds, flax seeds, bell pepper, roasted pepper, avocado	Rice, beans, salad of cucumbers, lettuce, cheese, olive oil, lemon	Tacos with beef, cabbage, ketchup, mayonnaise	Spaghetti, tomato sauce, pickles, salad of lettuce, cucumber, onions, celery, olive oil, lemon juice	Turkey, cranberry sauce, potato, Brussels sprouts
Bristol stool scale	type 5		type 2		type 2	type 2	type 4	-	-	type 2	type 6
VISIT 3	Y	N	Y	N	Y	Y	Y	Y	N	Y	Y
Change in health	N		N		N	N	N	N		N	N
Protocol deviations	N		N		N	N	N	N		N	N
Change in medication	N		N		N	N	N	Started eye health vitamin		N	N
Changes in diet	N		N		N	N	Started drinking smoothies 4 times per week with kale, spinach,	N		N	N

							mango, strawberry, coconut water				
Breakfast	Apple cinnamon instant oatmeal, milk		Wheat bread, peanut butter, nectarine, coffee, milk		Chocolate protein shake with powdered peanut butter, banana, coconut milk, coffee with creamer; snack of maple brown sugar oatmeal	Boiled egg, hash browns, coffee, milk, sugar, banana muffin	Wrap of guacamole, spinach, ham, mango, cheddar cheese, water with cucumber, mint, lemon	Mango and kiwi smoothie, cream cheese and cheddar sandwich		N	Bagel, cream cheese, coffee, milk, sugar
Lunch	Green salad, pear, dried cranberries, walnuts, French dressing, blue cheese, diet carbonated soda		Garden salad with cucumbers, carrots, red peppers, walnuts, dried cranberries, lettuce, and balsamic vinaigrette, broccoli and cheddar soup, crackers, croutons		Egg salad sandwich with lettuce on multigrain bread, potato chips	Garden salad with sunflower seeds, croutons, French dressing, turkey, strawberries	Salad of mixed greens, chopped vegetables, shredded parmesan, water with cucumber mint and lemon	Broiled tilapia with salt, rice and beans, cucumber, mango and kiwi smoothie		Hamburger, greens, bread, plain yogurt	Pad Thai noodles, chicken, lemon-lime soda
Dinner	Pasta, cream sauce, parmesan cheese, raspberry sweetened iced tea		Spaghetti with tomato sauce, meatball, grilled shrimp, beer		Buffalo wings with cheese, bacon, and parmesan, mozzarella sticks with marinara sauce, 2 beers, hamburger with provolone	Vegetables, pasta, cheese, Ding Dong chocolate cake	Salad of mixed greens and vegetables, shredded parmesan, cherries, water	Strawberries with chocolate-hazelnut spread		Salad of tomatoes, beans, chicken, mixed greens, Italian dressing, biscuits, tea	Pasta, marinara sauce, cranberry juice

					cheese, onions, mushrooms, tater tots						
Bristol stool scale	type 3	-	type 5	-	type 2	-	type 5	type 2		type 2	type 2

*Note: NOR5/7/8/10 are subjects enrolled initially as healthy but with very common illnesses that carry no significant morbidity when treated. The medications taken by these individuals are not immunomodulatory. Abbreviations: F, female; BMI, body mass index, Y, yes; N, negative/no.

Table S4. Efficiency and specificity of bacterial Ro60 qPCR primers.*

Primers	*B. theta*	*P. prop*	*A. mass*	*C. amyc*	*P. acnes*	*R. intes*	NTC
B. theta SYBR	102.7% efficiency	-	-	-	-	-	-
P. prop TaqMan	-	89.7% efficiency	-	-	-	-	-
A. mass TaqMan	400x	69x	105.2% efficiency	-	-	138x	-
C. amyc TaqMan	>3000x	-	200x	99.8% efficiency	-	-	-

*First column indicates intended primer target. Additional columns indicate the results when DNA from single-species bacterial cultures was used as the template; NTC, no template control. - indicates no amplification. Numbers indicate the fold increase in off-target amplification. For example, if *A. mass* primers were used to amplify pure *B. theta* DNA, 400-times the amount *B. theta* DNA would be needed to obtain an equivalent C_t value compared to *A. mass* DNA used as the template. *R. intes*, *R. intestinalis*.