THE LANCET

Supplementary appendix

This appendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

Supplement to: Lai Z-W, Kelly R, Winans T, et al. Sirolimus in patients with clinically active systemic lupus erythematosus resistant to, or intolerant of, conventional medications: a single-arm, open-label, phase 1/2 trial. *Lancet* 2018; published online March 15. http://dx.doi.org/10.1016/S0140-6736(18)30485-9.

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LIST OF ABBREVIATIONS

$\Delta\Psi_m$, mitochondrial transmembrane potential
Annexin V, AnnV
BILAG, British Isles Lupus Assessment Group
Ca^{2+}, calcium
CMT, central memory T-cell
DAF-FM, 4-amino-5-methylamino-2',7'-difluoroflourescein diacetate; nitric oxide indicator
DAR-4M, Diaminorhodamine-4M; peroxynitrite indicator
DCF-DA, dichlorofluorescein diacetate, H_2O_2 sensor
$DiOC_6$, 3,3'-dihexyloxacarbocyanine iodide; mitochondrial potential indicator
DN T, $CD3^+CD4^-CD8^-$ double-negative T cell
EMT, effector memory T-cell
HE, hydroethidine; ROI sensor
IRB, Institutional Review Board
MFI, mean fluorescence intensity
MHP, mitochondrial hyperpolarization
MTG, MitoTracker Green, mitochondrial mass sensor
mTOR, mechanistic (formerly mammalian) target of rapamycin
NAC, N-acetylcysteine
NAO, nonyl acridine orange, mitochondrial mass sensor
NO, nitric oxide
PBL, peripheral blood lymphocytes
PBS, phosphate buffered saline
PLS-DA, partial least square-discriminant analysis
PI, propidium iodide
ROI, reactive oxygen intermediates
SLE, systemic lupus erythematosus
SLEDAI, systemic lupus erythematosus disease activity index
TMRM, tetramethylrhodamine methyl ester
Treg, regulatory T cell; $CD3^+CD4^+CD25^+Foxp3^+$ T cell

SUPPLEMENTARY METHODS

Human Subjects. 40 SLE patients were enrolled in a prospective treatment trial with sirolimus (FDA approval, IND No: 101566; clinicaltrials.gov identifier: NCT00779194). The mean (± SD) age of patients was 45.4 (± 14.3) years, ranging between 18-71 years (**Table S1**). 38 patients were females including 35 Caucasians, three African-Americans. 2 patients were Caucasian males. Three consented patients (Rapa-38, Rapa-39, Rapa-42) failed screening. Baseline clinical characteristics of all enrolled patients, including age, gender, ethnicity, SLEDAI, BILAG, prednisone dosage, and medication use are shown in **Table S2**. 56 healthy subjects were individually matched for each patient blood donation for age within ten years, gender, and ethnic background, and freshly isolated cells were used in parallel as controls for immunological studies (**Table S3**). The mean (± SD) age of controls was 45.4 (± 12.7) years, ranging between 20-65 years. 51 controls were females including 45 Caucasians, five African-Americans, and one Hispanic. 5 controls were Caucasian males.

We estimated that the drop-out rate would be 25% or less, due to intolerance, side-effects, or relocation. Patients were recruited by physicians of the Division of Rheumatology at the State University of New York in Syracuse, NY. Informed consent was obtained from each patient and matched healthy control using forms approved by the Institutional Review Board (IRB). Patients were given 2 mg sirolimus as a starting dose and adjusted to tolerance and trough levels of 6-15 ng/ml. Clinical and laboratory assessments were performed on day 0 (prior to initiation of first sirolimus dose), and 1 month, 3 months, 6 months, 9 months, and 12 months after initiation of sirolimus. Sirolimus trough levels and complete blood counts (CBC) alone were performed on day 15 and day 60. During the study, prednisone dose was titrated to control disease activity. In other words, prednisone was dosed to control diseases activity in the patients' interest.

Inclusion criteria: age > 18 year, male or female, SLE with $\geq$ 4 of eleven diagnostic criteria approved by the American College of Rheumatology (1;2). We anticipated that most patients enrolled in the study would have active disease (SLEDAI $\geq$ 4) and receive at least 10 mg/day prednisone as well.

Exclusion criteria: Patients with allergy or intolerance to sirolimus were excluded. Patients with life-threatening manifestations of SLE, e.g. cerebritis substantiated by inflammatory MRI lesions, catastrophic anti-phospholipid antibody syndrome, rapid progressive glomerulonephritis requiring intravenous cyclophosphamide, GFR < 40 ml/min were not to be entered into the study. Patients with proteinuria exceeding 500 mg/24 h or urine protein/creatinine ratio > 0.5 were excluded. Patients with anemia (hemoglobin < 10 g/dl), leukopenia (WBC < 3,000/µl) and thrombocytopenia (platelets < 100,000/µl) were excluded. Patients with WBC between 3,000-3,500/µl, hemoglobin between 10-12 g/dl and platelet counts between 100,000-150,000/µl were monitored weekly for 1 month. If WBC and platelet counts were sustained or improved, patients were followed according to standard protocol. If WBC and platelet counts were reduced at any weekly follow-up, patients were removed from the study. Patients with a fasting lipid profile that included total cholesterol > 300 mg/dl or triglyceride > 400 mg/dl were excluded. Patients who were pregnant were excluded and use of contraceptives was required in potentially fertile female patients. Patients developing pneumonitis confirmed by high-resolution computer tomography (3), were excluded. Patient with acute infection requiring

antibiotics were not to be entered into the study. Patients on sirolimus who developed infections and required intravenous antibiotics and failed to show clinical improvement in 5 days were to be discharged from the study.

Medication use. Steroid dosage was fully adjustable throughout the trial. Hydroxychloroquine (HCQ) and existing immunosuppressive medications, such as mycophenolate mofetil (MMF), could be continued, adjusted for dosage or discontinued during the trial. HCQ, or new immunosuppressive mediation, such as MMF, was not started during the trial.

Study Procedures

1) Patient consenting and screening. Each patient was fully informed about the potential risks and benefits of sirolimus administration. This included a detailed discussion of other therapeutic options and their risks and benefits. Informed consents were obtained and kept within the Rheumatology office. All study procedures were approved by the SUNY Upstate Medical University Institutional Review Board.

2) Clinical examinations. Prior to enrollment and upon each visit, a complete physical examination and the following routine laboratory tests were performed: complete blood count with differential, comprehensive metabolic panel including serum creatinine, SGOT, SGPT, routine urine analysis, 24 h urinary protein excretion or urine protein/creatinine ratio, fasting lipid profile, anti-DNA, C3, C4, and sirolimus serum level. SLEDAI (4) and BILAG scores were recorded (5).

Enrollment: In accordance with the principles of this study to address unmet medical need in SLE, 40 patients who were unable to tolerate or failed to respond to other immunosuppressants or biologicals were preferentially enrolled. 43 patients signed informed consent. The first subject was consented on March 9, 2009, while the last subject was consented on 12/8/2014. The last study visit occurred on 12/7/2015. Data from two of the enrolled patients were excluded from analysis due to protocol non-compliance. If a subject could not tolerate or refused to continue taking study medication, we continued to follow and evaluate that subject if he/she was willing.

Study materials: SLE patients were prescribed 2 mg/day sirolimus (Rapamune) upon enrollment. If prescription coverage was denied by insurance carrier, medication was provided by the sponsor. Compliance was assessed by monitoring of sirolimus blood levels.

*Study visit*s: Routine and lupus-specific clinical and laboratory data were acquired during six visits. Visit 1: baseline assessment before first sirolimus dose; provision of prescription or study drug. Visit 2: after one-month treatment, provision of prescription or study drug. Visit 3: after three-month treatment, provision of prescription or study drug. Visit 4: after six-month treatment, provision of prescription or study drug. Visit 5: after nine-month treatment, provision of prescription or study drug. Visit 6: after 12-month treatment. Two additional visits occurred 15 days and 60 days after enrollment which involved assessment of sirolimus blood levels.

Clinical outcomes and assessments

1. Tolerance: common side effects (nausea, headache, mouth sores) seen in prior trials were specifically asked for at each visit and reviewed by the Data Safety and Monitoring Board (DSMB) bi-annually. Hyperlipidemia, thrombocytopenia, mucositis, edema, and proteinuria, which have been commonly noted in renal transplant patients (6), were also monitored as safety outcomes.

2. Clinical efficacy assessments: A complete physical examination was performed before enrollment. A directed physical examination of the cardiovascular, respiratory, gastrointestinal, musculoskeletal, neurological systems, skin, head, neck, sinuses, nasal and oral cavities were performed at each visit. SLE disease activity was assessed by using the British Isles Lupus Assessment Group (BILAG) (25) and SLE Disease Activity Index (SLEDAI) (23). Concurrent use and dosage of other medications were documented. Improvement in SLEDAI and BILAG disease score was considered as primary clinical efficacy outcome.

Due to the evolution of clinical assessment of therapeutic response in SLE, we also determined the impact of sirolimus on the SLE Responder Index (SRI), which is mainly driven by $\geq$ 4-point drop of SLEDAI and absence of BILAG A or two BILAG B scores (7). Physicians Global Assessment (PGA) scores were not recorded according to the trial design, as the original SLEDAI scoring system was used (4). Therefore, PGA scores were not used in generating SRI in this study.

3. Routine blood tests included complete blood count, liver and kidney function test, fasting lipid profile, urinalysis and lupus-relevant laboratory tests, such as anti-double-stranded DNA, C3, and C4.

6. Compliance with study procedures is described in the Supplemental Materials section.

Immunobiological outcomes and assessments

For each patient visit, we obtained blood from healthy donors matched for age (within one decade), gender, and ethnicity, to be used as control for flow cytometry measurement of mitochondrial function, T-cell activation and death pathway selection, Ca^{2+} flux, production of nitric oxide (NO) and reactive oxygen intermediates (ROI), intracellular IL-4, IL-17, and IFN-γ production, distribution of effector and memory CD4 and CD8 T-cell compartments , and overall monitoring of pro- and anti-inflammatory specification within the immune system. We have recorded 677 flow cytometry data points for each of the six patient visits, both for the patients and the matching controls. DNA, RNA, and protein lysates have been saved and catalogued for each visit. Individual controls gave blood on multiple occasions.

Assessment of metabolic biomarkers in live cells by flow cytometry

We examined unstimulated cells and cells stimulated with CD3/CD28 for 16 h (8). T-cell subsets were analyzed by staining with antibodies to CD4, CD8, CD25, CD27, CD197, CD98, CD45RA, CD45, and CD62L. The memory panel comprised of CD45RA, CD45RO, CD62L and CD197 was introduced from September 20, 2010 and involved subjects Rapa-15 through Rapa-40. Complete study set was available for 22 of these patients, which are shown in Figure 5. B cells were identified by CD19 staining. Cell death pathway selection was monitored with annexin V-FITC, annexin V-PE, or annexin V-AlexaFluor-647 matched with emission spectra of propidium iodide (PI) to detect Annexin V+/PI- apoptotic cells and PI+ necrotic cells (9). Mitochondrial transmembrane potential ($\Delta\psi m$) was assessed with positively charged cationic dyes ($DiOC_6$, 40 nM, excitation: 488 nm, emission: 525 nm recorded in FL-1; TMRM, 100 nM, excitation: 543 nm, emission: 567 nm recorded in FL-2). Mitochondrial mass was evaluated with potential-insensitive mitochondrial dyes MitoTracker Green-FM (MTG, 100 nM; excitation: 490 nm,

emission: 516 nm recorded in FL-1) or nonyl acridine orange (NAO, 50 nM; excitation: 490 nm, emission: 540 nm recorded in FL-1). Reactive oxygen intermediates (ROI) were assessed with superoxide-sensing hydroethidine (HE, 1 μM) and H_2O_2-sensing dichlorofluorescein diacetate (DCF-DA, 1 μM), nitric oxide (NO) sensor 4-amino-5-methylamino-2',7'-difluoroflourescein diacetate (DAF-FM, 1 μM, excitation: 495, emission: 515 nm recorded in FL-1). Cytosolic $Ca^{2+}$was assessed with Fluo-3 (1 μM, excitation: 506 nm, emission: 526 nm recorded in FL-1) and mitochondrial Ca^{2+} was assessed with Rhod-2 (1 μM; excitation: 552 nm, emission: 581 nm recorded in FL-2), respectively. All metabolic and mitochondrial sensor dyes were obtained from Invitrogen (Carlsbad, CA) and used as earlier described (8;10-12). We recorded up to 12 parameters simultaneously using a Becton Dickinson LSRII flow cytometer equipped with 20 mW solid-state Nd-YAG (emission at 355 nm), 20 mW argon (emission at 488 nm), 10 mW diode pumped solid state yellow-green (emission 561 nm) and 16 mW helium-neon lasers (emission at 634 nm).

For detection of mTOR activity and FoxP3 expression, cells were permeabilized with Cytofix/CytopermPlus (eBiosciences) and stained with AlexaFluor-488 or AlexaFluor-647-conjugated antibody to pS6RP (Cell Signaling; Beverly, MA; Cat. No. 4851) and AlexaFluor-647-conjugated antibody to FoxP3 (BioLegend, San Diego, CA; Cat No 320014), as earlier described (13). Intracellular cytokine production was measured after additional in vitro stimulation for 3 h with 50 ng/ml phorbol myristyl acetate (PMA) and 1 μg/ml ionomycin in the presence of 10 μg/ml Brefeldin-A. PMA, ionomycin and Brefeldin-A were purchased from Sigma-Aldrich (St. Louis, MO), followed by fixation and permeabilization and staining with antibodies from BD Biosciences: FITC-conjugated anti-IFN-γ (Cat.No. 554700), APC-conjugated anti-IL-4 (Cat.No. 560671), and PE-conjugated anti-IL-17a (Cat.No. 560436). Each patient's cells were freshly isolated, stained and analyzed in parallel with a matched control. Mean channel fluorescence (MFI) values of patient samples were normalized to controls set at 1.0 for each analysis and expressed as fold changes. Frequencies of cell populations were compared as absolute values. Relative fluorescence intensity (RFI) was calculated by comparison of MFI values of patients' cells to healthy subjects' cells, which were analyzed in parallel and normalized to 1.0.

Statistics

Power and sample size requirements for this study were based on a type I error rate of 0.05, two-tailed testing, and a minimal power level of .80, using Sample Power software (SPSS Chicago, Ill). Estimates of effect size were based on our preliminary data (14) and the relevant literature to compare mean values of SLEDAI and BILAG after a meaningful length of intervention, such as 12 months. As indicated in each figure legend, repeated measures mixed model logistic regression analysis, chi-square testing, and two-tailed paired t-test were used to assess the effects of sirolimus on clinical indices and biomarkers recorded on visits 2-6 relative to visit 1; $p < 0.05$ was considered significant. Patients and controls were compared with mixed models and two-tailed unpaired t-test. Two-tailed chi-square and Fisher's exact tests were used to compare categorical parameters with GraphPad Prism version 5.0 software (San Diego, CA). To analyze the data taking into account all repeated measures from each patient, we employed a mixed model approach with study visit as a fixed effect and subject ID number as a random effect, using Stata 15.0 software (College Station, TX). This model uses all available data points and assumes that missing values are missing at random. For group comparisons, we included the main effect of group and the group by visit interaction as fixed effects. The interaction tests whether the change across visits differs between groups. The group comparison models used matched pairs of patients; pairs were included as a random effect. We used a Gaussian model, except for the case of ordinal variables, in which case we used ordinal logistic regression. All

dependent variables that were percentages were transformed into logits (i.e., logit(x) = ln(x/(1-x)). All significant p values have been provided as exact values with 4 decimal places. When no z value is reported, no mixed model would not converge and yield analysis report. The Stata software package reports changes as z-values with two decimal places and p-values up to three decimal places. Thus, if the p-value from Stata was generated as p=0.000, we reported it as $p \leq 0.0009$. Area under the receiver operating characteristic (ROC) curve (AUC) logistic regression analysis was performed with Metaboanalyst 3.0 (15).

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Table S1. Demographics of SLE patients enrolled in the prospective treatment trial with sirolimus (IND No: 101566; clinicaltrials.gov identifier: NCT00779194). The mean (± SD) age of patients was 45.4 (± 14.3) years, ranging between 18-71 years. 11/40 enrolled subjects dropped out of the study due to intolerance (2 subjects) or noncompliance with protocol (9 subjects). Intolerance was due to non-healing oral ulcers (subject Rapa-2; 6 weeks post enrollment) and new onset headache (subject Rapa-12; 12 weeks post enrollment). Rapa-38, Rapa-41, and Rapa-42 were consented but not enrolled. F, female; M, male; C, Caucasian; AA, African-American.

Study number	Age	Gender	Ethnicity	Side effect	Non-compliance
Rapa-001	47	F	C	0	0
Rapa-002	48	F	C	1	0
Rapa-003	47	F	C	0	0
Rapa-004	22	F	C	0	1
Rapa-005	48	F	C	0	1
Rapa-006	57	F	C	0	0
Rapa-007	65	F	C	0	0
Rapa-008	35	F	C	0	0
Rapa-009	21	F	C	0	0
Rapa-010	48	F	C	0	0
Rapa-011	52	F	C	0	1
Rapa-012	55	F	C	1	0
Rapa-013	25	F	C	0	1
Rapa-014	65	F	C	0	1
Rapa-015	35	M	C	0	0
Rapa-016	18	F	C	0	1
Rapa-017	26	F	AA	0	1
Rapa-018	24	F	C	0	1
Rapa-019	56	F	C	0	0
Rapa-020	55	F	C	0	0
Rapa-021	23	F	C	0	0
Rapa-022	60	F	AA	0	0
Rapa-023	53	M	C	0	0
Rapa-024	26	F	AA	0	0
Rapa-025	56	F	C	0	0
Rapa-026	63	F	C	0	0
Rapa-027	51	F	C	0	0
Rapa-028	21	F	C	0	0
Rapa-029	47	F	C	0	1
Rapa-030	47	F	C	0	0
Rapa-031	45	F	C	0	0
Rapa-032	71	F	C	0	0
Rapa-033	49	F	C	0	0
Rapa-034	53	F	C	0	0
Rapa-035	53	F	C	0	0
Rapa-036	34	F	C	0	0
Rapa-037	51	F	C	0	0
Rapa-038	62	F	C	-	-
Rapa-039	62	F	C	0	0
Rapa-040	57	F	C	0	0
Rapa-041	23	F	C	-	-
Rapa-042	64	F	C	-	-
Rapa-043	39	F	C	0	0

Table S2. Matching of healthy subjects for gender, ethnicity, and age within ten years for mechanistic immunobiological studies through visits 1-6 (V1-V6) of the clinical trial.

Demographics of SLE Patients				**Age of Healthy Subjects Matched for Gender and Ethnicity**					
Patient	Age	Gender	Ethnicity	V1	V2	V3	V4	V5	V6
Rapa-001	47	F	C	49		39	49	49	47
Rapa-002	48	F	C	54		45			
Rapa-003	47	F	C	38	49	25	25	49	54
Rapa-004	22	F	C	38		25	25		
Rapa-005	48	F	C	48		46	49		
Rapa-006	57	F	C	50		50	47	50	45
Rapa-007	65	F	C	49			58	47	58
Rapa-008	35	F	C	49		41	26	47	44
Rapa-009	21	F	C	25		26	24	27	45
Rapa-010	48	F	C	50		54		44	48
Rapa-011	52	F	C	50					
Rapa-012	55	F	C	62		50	50		
Rapa-013	25	F	C	26		50			
Rapa-014	65	F	C	63				64	
Rapa-015	35	M	C	31		24	24	25	24
Rapa-016	18	F	C	50			25	20	22
Rapa-017	26	F	AA	25		26	25		
Rapa-018	24	F	C	20	20				
Rapa-019	56	F	C	25	58	59	59	56	59
Rapa-020	55	F	C	51	56	59	50	53	60
Rapa-021	23	F	C	22	27	24	23	23	23
Rapa-022	60	F	AA	59	50	63	59	60	54
Rapa-023	53	M	C	49	49	49	47	47	48
Rapa-024	26	F	AA	27	26	30		32	33
Rapa-025	56	F	C	57	60	51	60	52	61
Rapa-026	63	F	C	60	64		65	56	53
Rapa-027	51	F	C	60		60	55	49	60
Rapa-028	21	F	C	23	28	30	29	30	29
Rapa-029	47	F	C	38	50				
Rapa-030	47	F	C	52	49	52	48	51	52
Rapa-031	45	F	C	61	53	52	53	51	40
Rapa-032	71	F	C	60	61	61	62	61	61
Rapa-033	49	F	C	52	48	51	48	53	44
Rapa-034	53	F	C	59	50	54	60	53	52
Rapa-035	53	F	C	59	54	52	54	54	49
Rapa-036	34	F	C	30	31	40	35	32	32
Rapa-037	51	F	C	52	52	51	52	53	49
Rapa-038		F	C	54					
Rapa-039	62	F	C	54	44	52	49	53	55
Rapa-040	57	F	C	53	40	52	40	49	52
Rapa-041		F	C	22					
Rapa-042		F	C	60					
Rapa-043	39	F	C	30	30	35	44	49	32
Average	45.4			45.3	46.3	45.1	44.4	46.3	46.7

LEGENDS TO SUPPLEMENTARY FIGURES

Figure S1. Monitoring of fasting lipid profile during 12-month sirolimus treatment of 40 patients with SLE. Effect of sirolimus was assessed by 2-tailed paired t-test relative to visit 1. *, $p<0.05$; **, $p< 0.01$; ***, $p<0.001$.

Figure S2. Effect of sirolimus treatment on all BILAG organ domain scores. Effects of sirolimus were assessed by 2-tailed paired t-test relative to visit 1. *, $p<0.05$; **, $p< 0.01$; ***, $p<0.001$. Overall distribution of subjects with ≥3 organ domain scores were also assessed by 2-tailed chi-square ($\chi 2$) test.

Figure S3. Expansion of CD8 (panel A) and CD4 memory T cells is confined to SRI-responsive patients (with ≥4 SLEDAI drop) upon treatment with sirolimus for 12 months (panel B). Effects of sirolimus were assessed by 2-tailed unpaired t-test relative to matched HC subjects (*, $p<0.05$; **, $p< 0.01$) and by 2-tailed paired t-test relative to visit 1 in each patient (#, $p<0.05$; ##, $p< 0.01$; ###, $p<0.001$).

Figure S4. Expansion of naïve and depletion of memory CD8 T cells in SLE patients are progressively corrected by sirolimus treatment over 12-month treatment in vivo. These findings are shown in naïve (panel **A**) and memory CD8 T cells following CD3/CD28 stimulation *in vitro* (panels **B**). Effects of sirolimus were assessed by 2-tailed unpaired t-test relative to matched HC subjects (*, $p<0.05$; **, $p< 0.01$; ***, $p<0.001$) and by 2-tailed paired t-test relative to visit 1 in each patient (#, $p<0.05$).

Figure S5. Depletion of FoxP3$^+$ Tregs in SLE patients is responsive to treatment with sirolimus *in vivo*. Representative dot plots of FoxP3$^+$ Tregs of SLE patient Rapa-32 and matched HC subject are shown upon enrollment at visit 1 and after 12-month treatment at visit 6. SLEDAI and BILAG scores are also indicated.

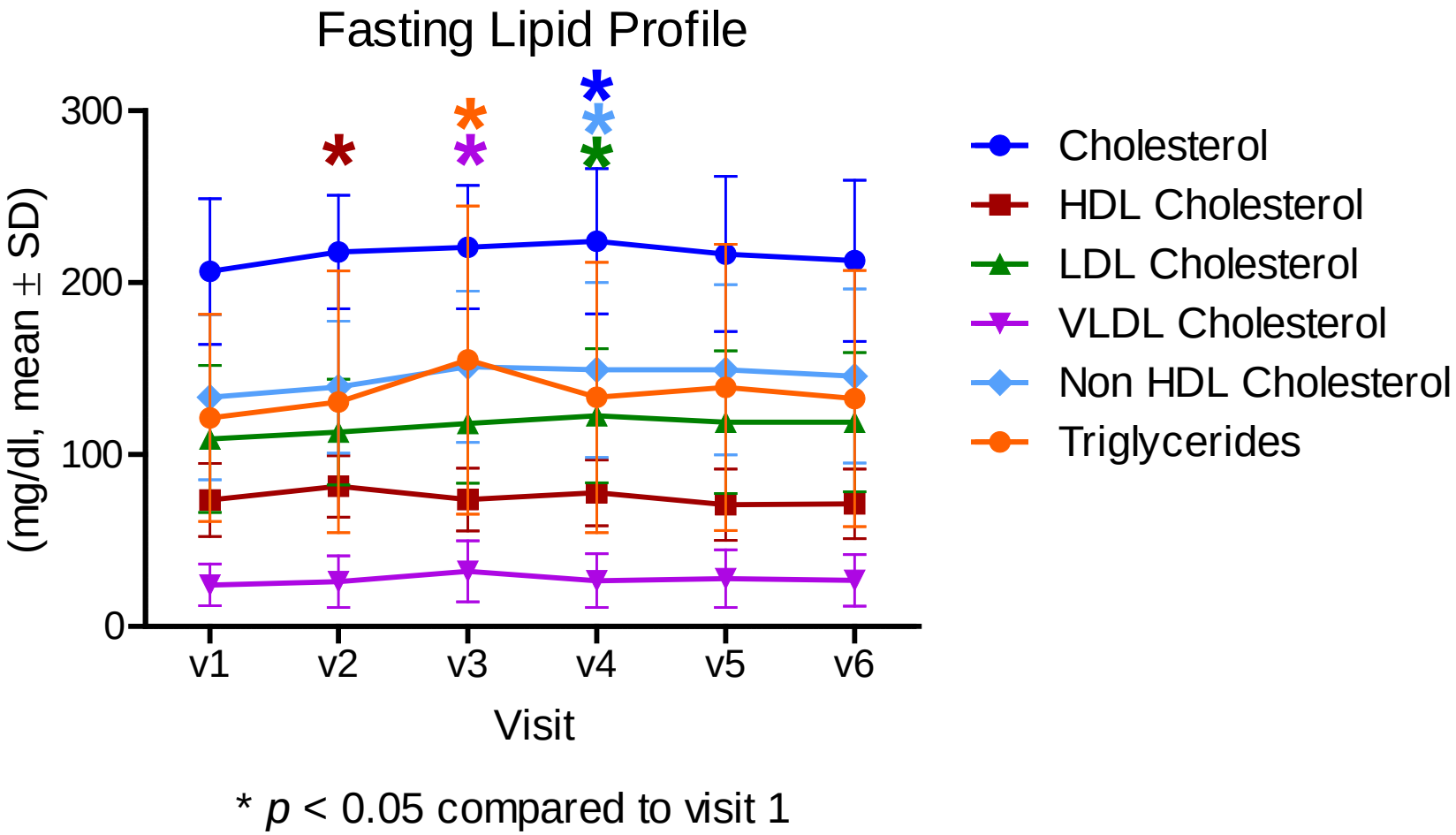
Figure S6. Cumulative analysis of FoxP3$^+$ Treg depletion in SLE patients and its responsiveness to 12-month sirolimus treatment. Effects of sirolimus were assessed by 2-tailed unpaired t-test relative to matched HC subjects (*, $p<0.05$) and by 2-tailed paired t-test relative to visit 1 in each patient (#, $p<0.05$).

Figure S7. Effect of sirolimus treatment on intracellular production of IL-4, IL-17, and IFNγ in CD4, CD8, and CD4$^-$CD8$^-$ double-negative (DN) T cells with and without CD3/CD28 co-stimulation. Effects of sirolimus were assessed by 2-tailed unpaired t-test relative to matched HC subjects (*, $p<0.05$; **, $p< 0.01$; ***, $p<0.001$) and by 2-tailed paired t-test relative to visit 1 in each patient (#, $p<0.05$; ##, $p< 0.01$; ###, $p<0.001$).

Figure S8. Sirolimus treatment reduced mitochondrial mass in DN T cells. Effects of sirolimus were assessed by 2-tailed unpaired t-test relative to matched HC subjects (*, $p<0.05$; **, $p<0.01$) and by 2-tailed paired t-test relative to visit 1 in each patient (#, $p<0.05$).

Figure S9. Effect of sirolimus treatment on levels of IgM and IgA anti-β_2 glycoprotein I (anti-β_2GPI) and anti-cardiolipin antibodies (ACLA) in SLE patients during 12-month intervention. Antibody levels during treatment (visits 2-6) were compared to baseline (visit 1) normalized at 1.0 for each reactivity. Effect of sirolimus was assessed by 2-tailed paired t-test relative to visit 1 (*, $p<0.05$).

Figure S1



* $p < 0.05$ compared to visit 1

Figure S2

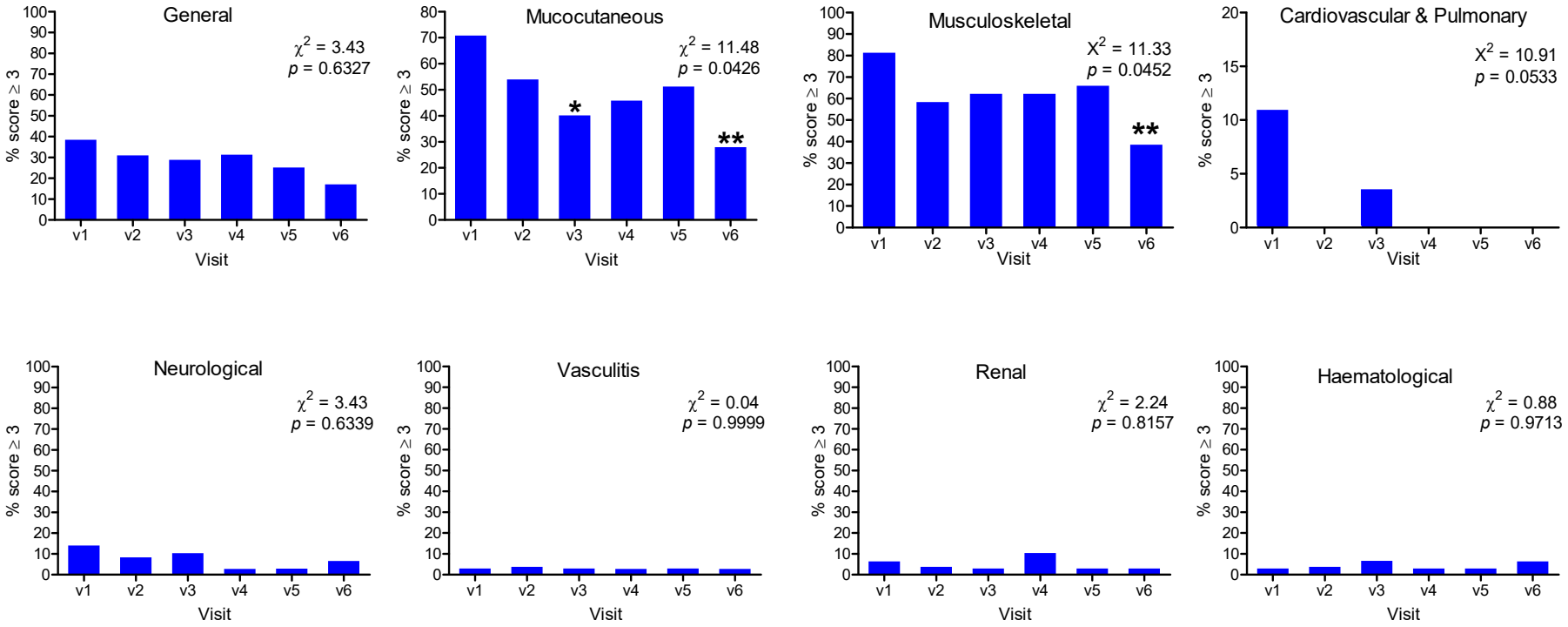
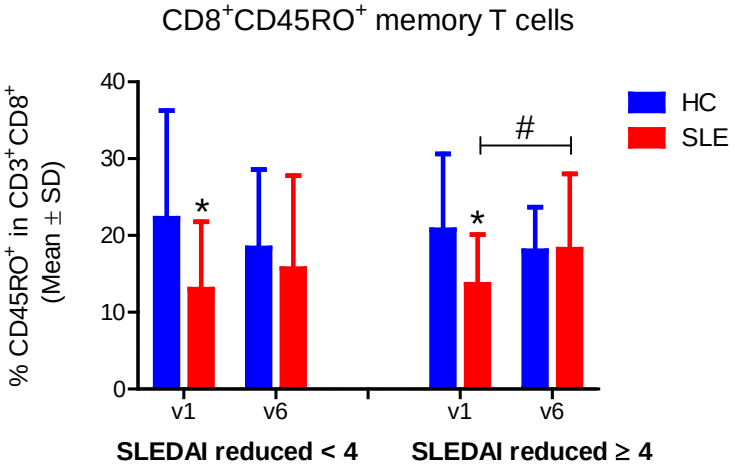

General
χ^2 = 3.43
p = 0.6327
Mucocutaneous
χ^2 = 11.48
p = 0.0426
*
**
Musculoskeletal
X^2 = 11.33
p = 0.0452
**
Cardiovascular & Pulmonary
X^2 = 10.91
p = 0.0533
Neurological
χ^2 = 3.43
p = 0.6339
Vasculitis
χ^2 = 0.04
p = 0.9999
Renal
χ^2 = 2.24
p = 0.8157
Haematological
χ^2 = 0.88
p = 0.9713
% score ≥ 3
Visit
v1
v2
v3
v4
v5
v6

Figure S3

A


CD8+CD45RO+ memory T cells
% CD45RO+ in CD3+CD8+ (Mean ± SD)
40
30
20
10
0
*
*
#
HC
SLE
v1
v6
v1
v6
SLEDAI reduced < 4
SLEDAI reduced ≥ 4

B

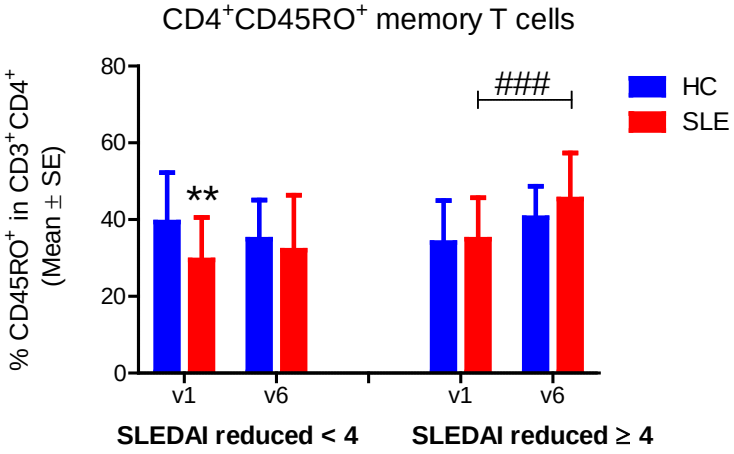

CD4+CD45RO+ memory T cells
% CD45RO+ in CD3+CD4+ (Mean ± SE)
80
60
40
20
0
**
###
HC
SLE
v1
v6
v1
v6
SLEDAI reduced < 4
SLEDAI reduced ≥ 4

Figure S4

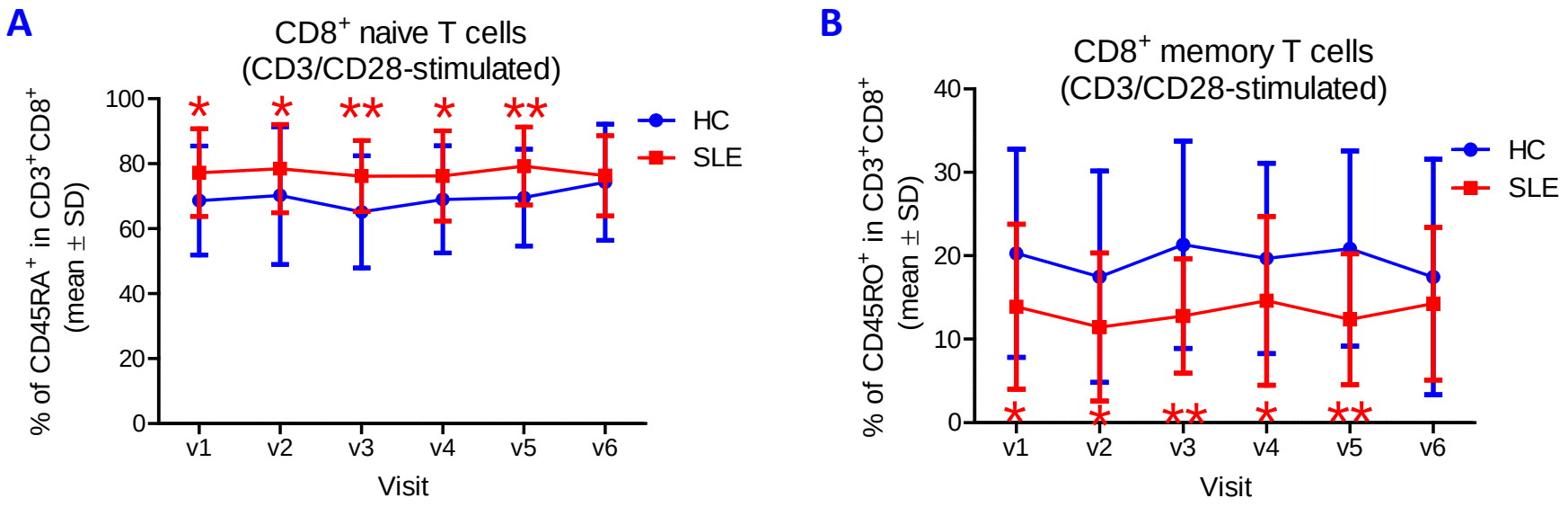

A
CD8+ naive T cells
(CD3/CD28-stimulated)
% of CD45RA+ in CD3+CD8+
(mean ± SD)
100
80
60
40
20
0
v1
v2
v3
v4
v5
v6
Visit
HC
SLE
*
*
**
*
**
B
CD8+ memory T cells
(CD3/CD28-stimulated)
% of CD45RO+ in CD3+CD8+
(mean ± SD)
40
30
20
10
0
v1
v2
v3
v4
v5
v6
Visit
HC
SLE
*
*
**
*
**

Figure S5

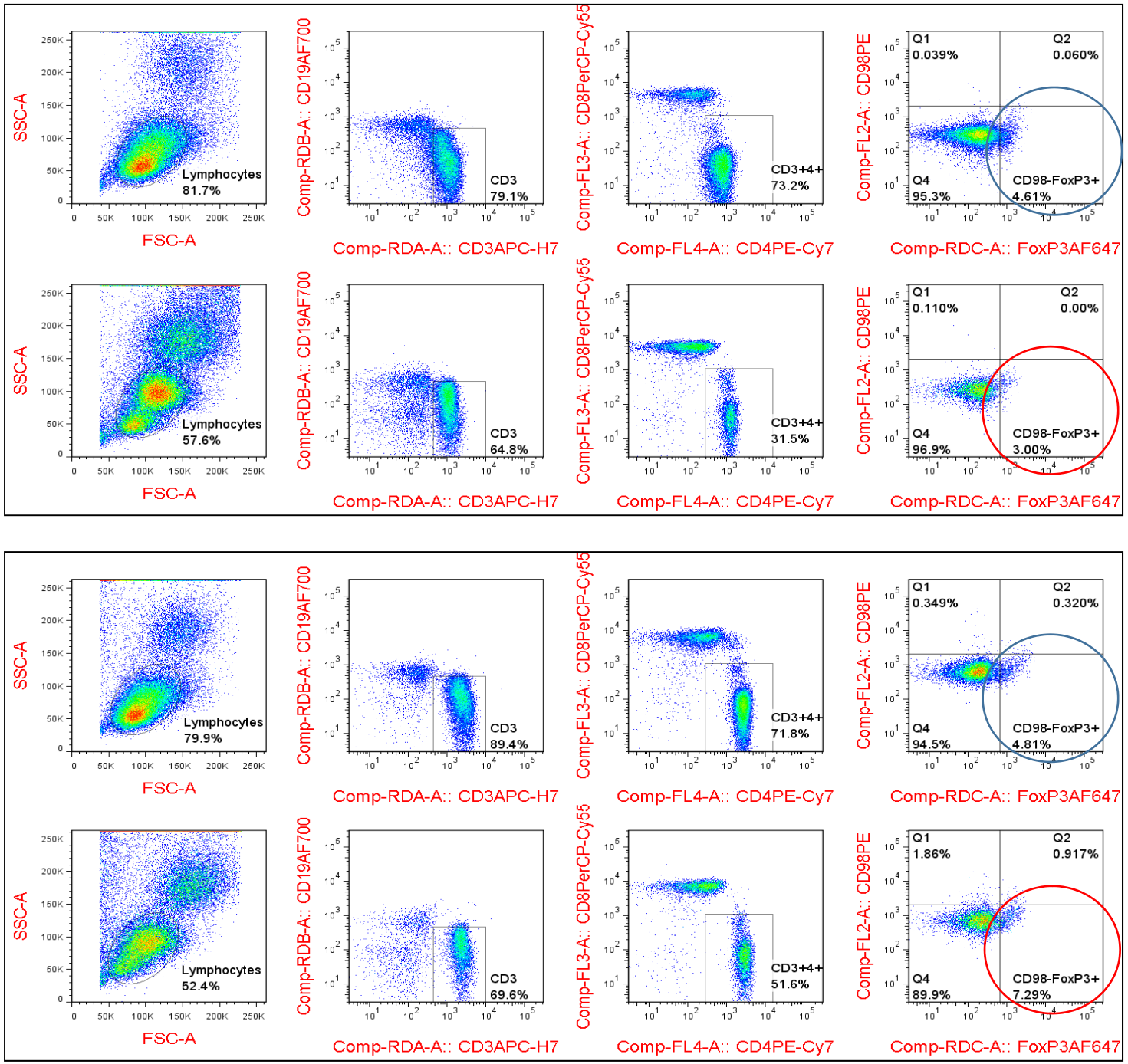
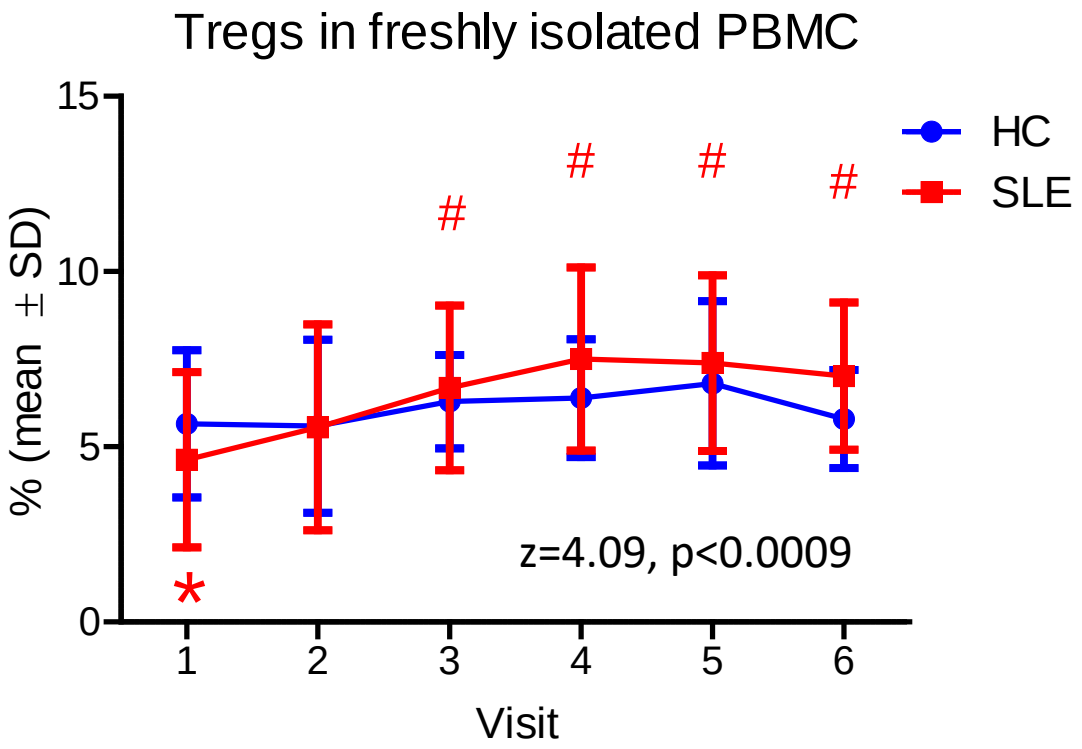

HC
Visit-1
(1/28/2014)
SLE # Rapa-32
SLEDAI = 4
BILAG = 15
SSC-A
FSC-A
Lymphocytes
81.7%
Comp-RDB-A:: CD19AF700
Comp-RDA-A:: CD3APC-H7
CD3
79.1%
Comp-FL3-A:: CD8PerCP-Cy55
Comp-FL4-A:: CD4PE-Cy7
CD3+4+
73.2%
Comp-FL2-A:: CD98PE
Comp-RDC-A:: FoxP3AF647
Q1
0.039%
Q2
0.060%
Q4
95.3%
CD98-FoxP3+
4.61%
Lymphocytes
57.6%
CD3
64.8%
CD3+4+
31.5%
Q1
0.110%
Q2
0.00%
Q4
96.9%
CD98-FoxP3+
3.00%
HC
Visit-6
(2/24/2015)
SLE # Rapa 32
SLEDAI = 0
BILAG = 4
Lymphocytes
79.9%
CD3
89.4%
CD3+4+
71.8%
Q1
0.349%
Q2
0.320%
Q4
94.5%
CD98-FoxP3+
4.81%
Lymphocytes
52.4%
CD3
69.6%
CD3+4+
51.6%
Q1
1.86%
Q2
0.917%
Q4
89.9%
CD98-FoxP3+
7.29%

Figure S6

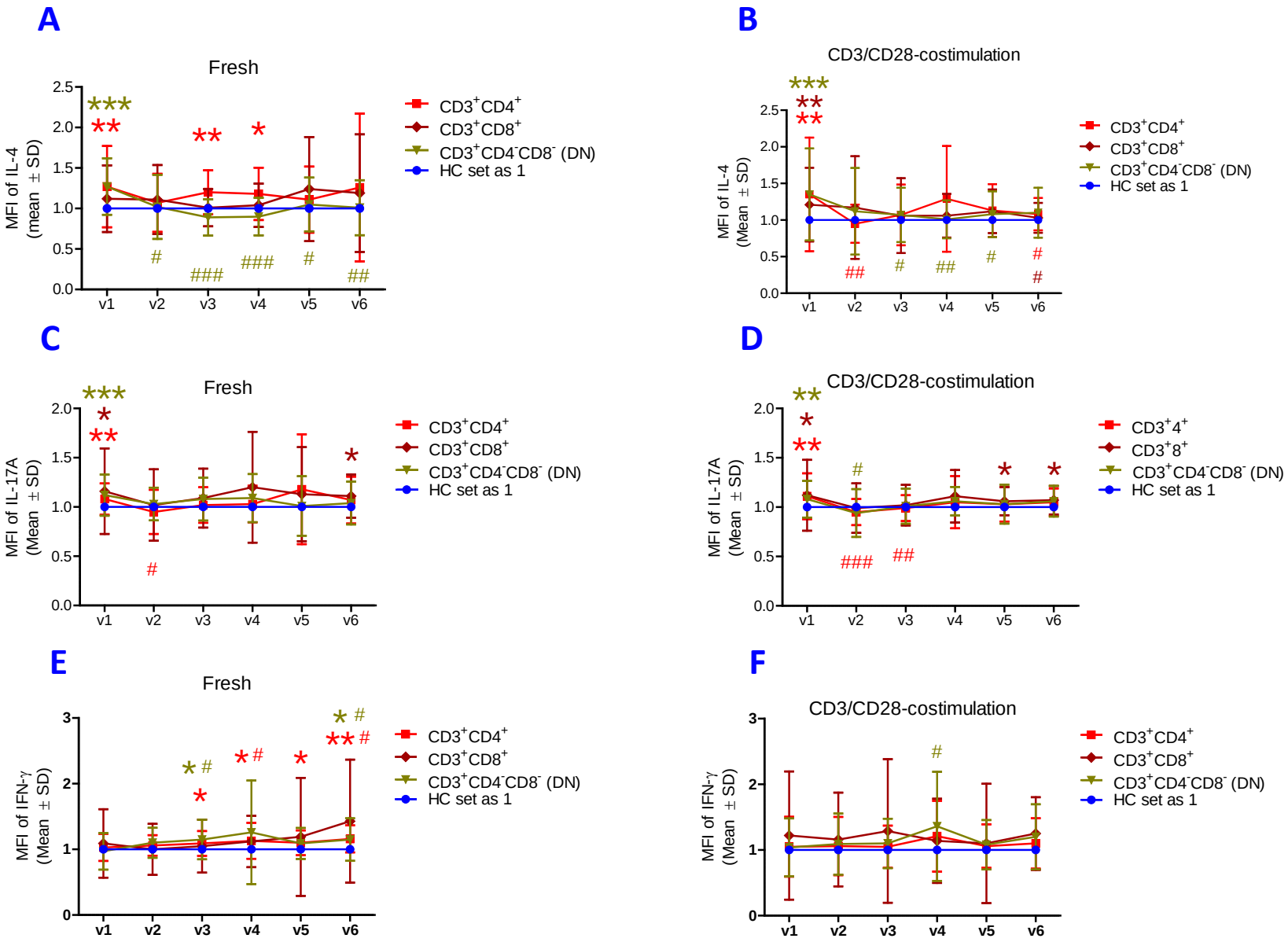


Visit 1: Baseline
Visit 2: Month 1
Visit 3: Month 3
Visit 4: Month 6
Visit 5: Month 9
Visit 6: Month 12

*, $p < 0.05$ compared to control (HC)

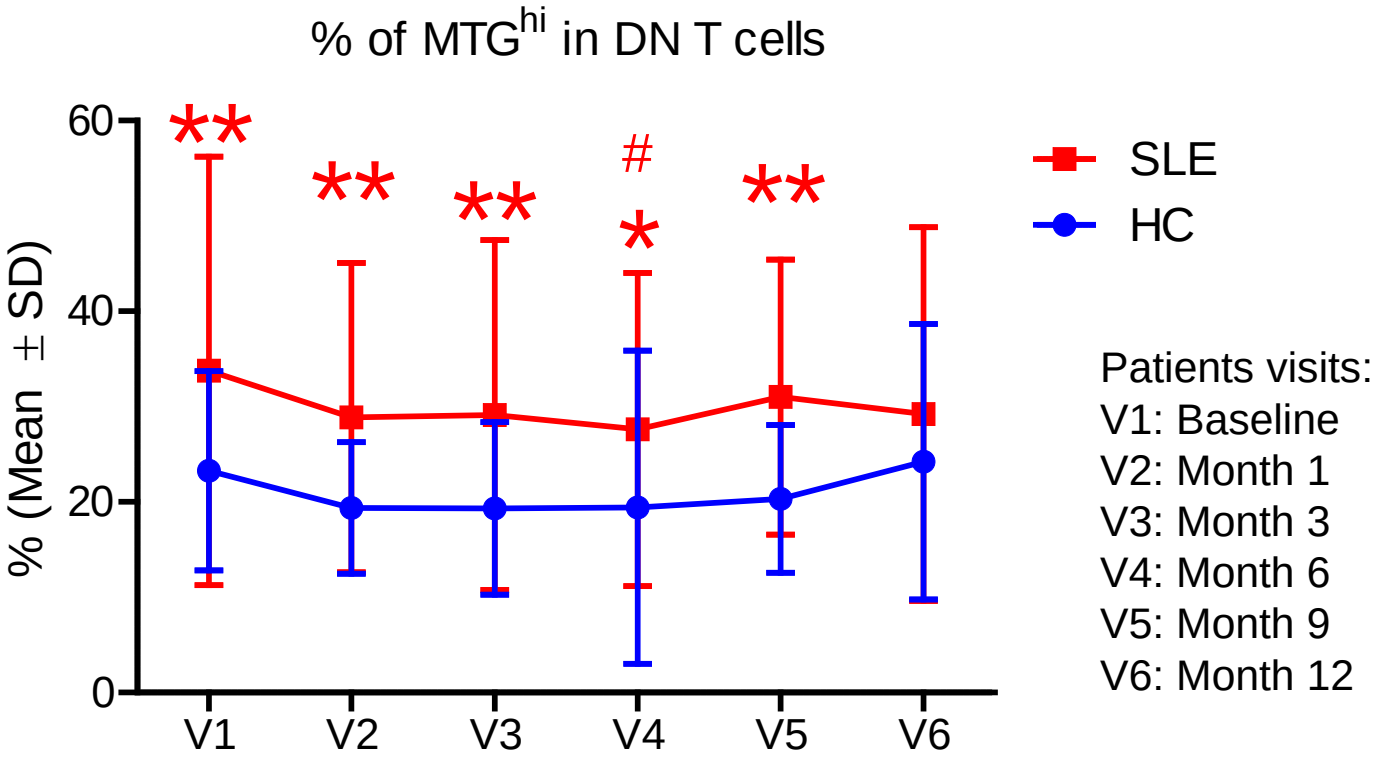
#, $p < 0.05$ compared to baseline (visit 1)

Figure S7



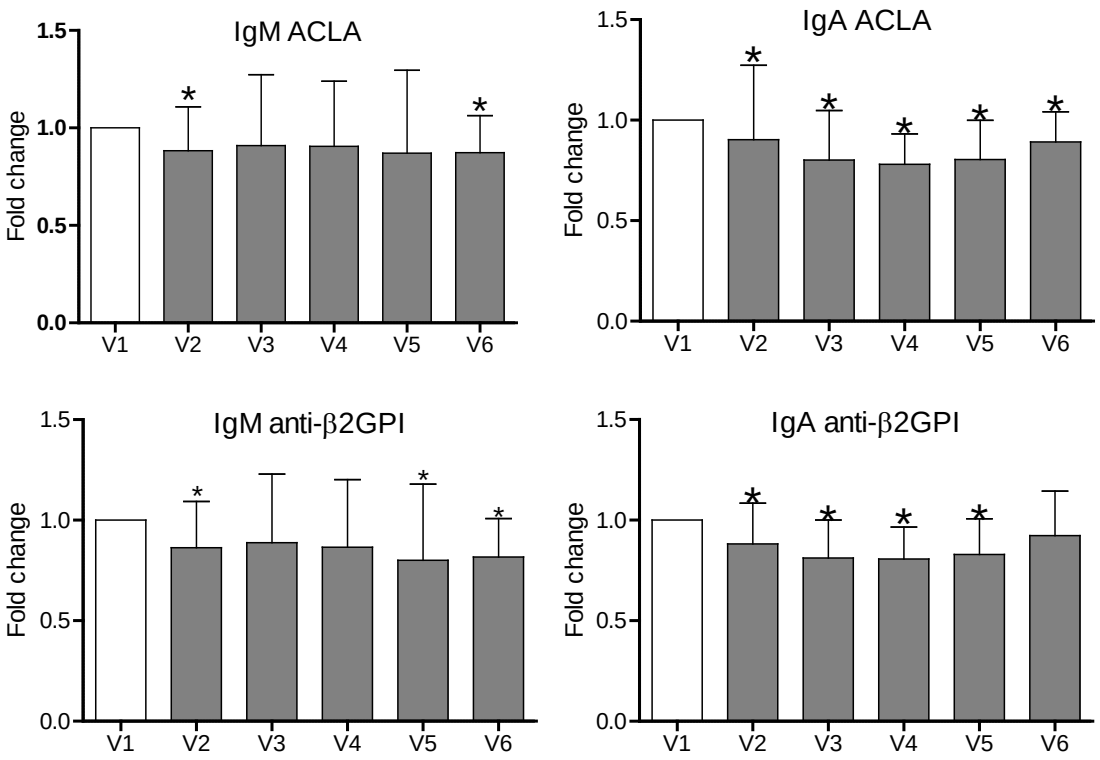
* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared to control; # $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$ compared to baseline (visit 1)

Figure S8



- $p < 0.05$, ** $p < 0.01$, compared to control (HC);
- # $p < 0.05$, compared to baseline (V1)

Figure S9



Patients visits:
V1: Baseline
V2: Month 1
V3: Month 3
V4: Month 6
V5: Month 9
V6: Month 12