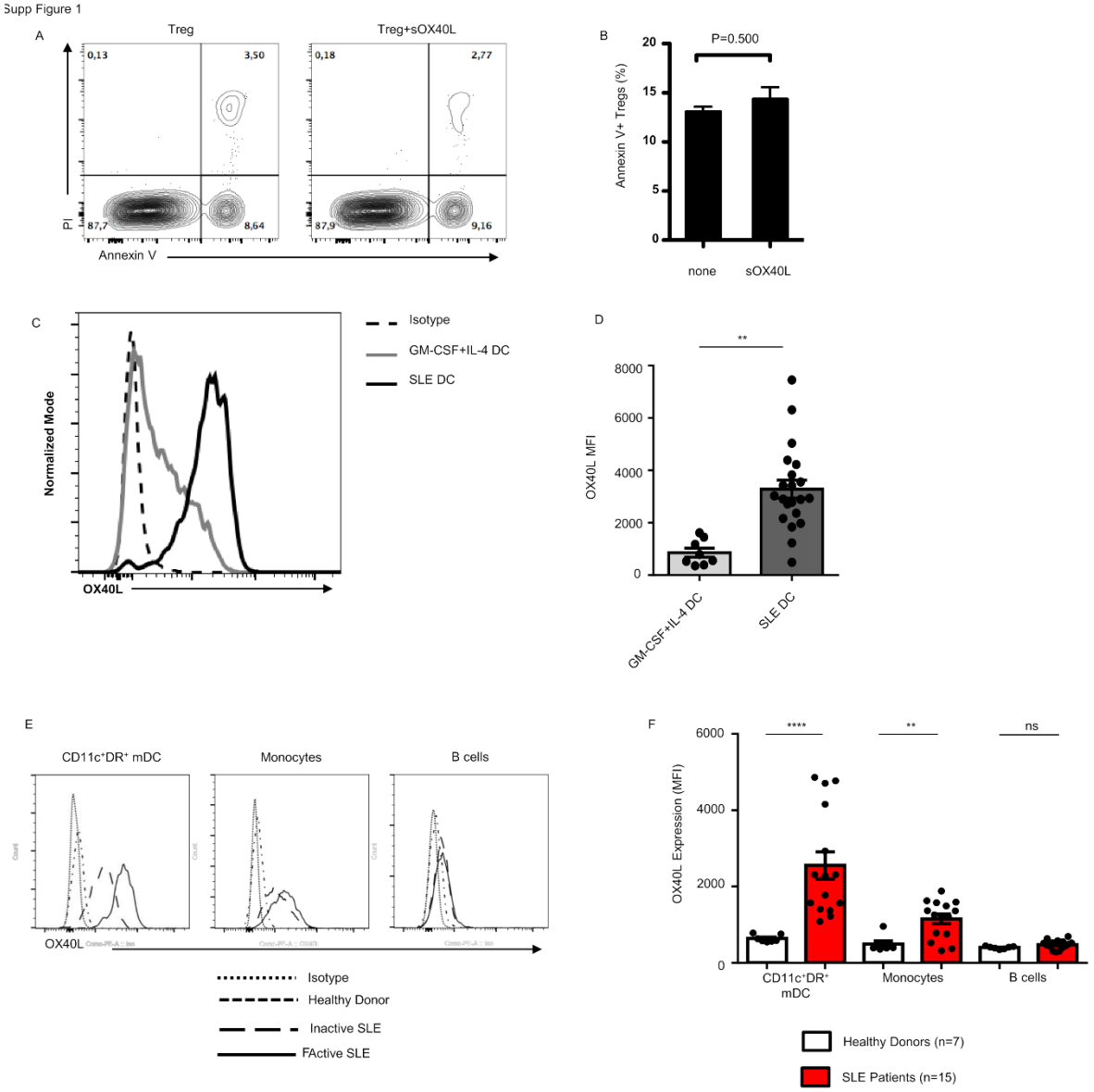


Figure S1: Recombinant sOX40L does not induce Treg cell death and Purified CD14+ monocytes cultured in the presence of SLE sera (SLE-DC) or ex-vivo SLE-mDC expressed OX40L.

(**A-B**), Sorted $CD4^+CD25^+CD127^-$ Tregs cells were cultured with or without sOX40L (100 ng/mL) and Annexin-V/PI staining was performed after 3 days of culture for the assessment of cell death. (**A**), Representative dot plot of Treg Annexin-V/PI staining after 3 days of culture. Annexin-V^+/PI^+ cells were considered as dead cells. (**B**), Cumulative data showing the percentage of dead cells after 3 days of Tregs culture. Statistical analyses were conducted using the two-tailed Mann-Whitney U-test. **(C-D)**, Purified $CD14^+$ monocytes from healthy donors were cultured with GM-CSF+IL-4 or SLE Sera (SLE-DC) for 4 days. OX40L expression was assessed by flow cytometry. (C) Representative staining for OX40L expression. (D). Cumulative results for OX40L-expression (as expressed by MFI) in both conditions. GM-CSF+IL-4 DC (n=8), SLE-DC (n=21). Cumulative data are shown with S.E.M and P value < 0.01 (**). **(E-F)** Ex-vivo OX40L expression level was assessed within circulating APCs such as $CD11c^+DR^+$ mDC, CD14+ monocytes and CD19+ B lymphocytes cells. 7 healthy donors and 15 SLE patients (including 9 active (SLEDAI ≥ 6 and 7 quiescent (SLEDAI < 6) SLE) APCs were analyzed by flow cytometry. The figure shows a representative staining and cumulative data represented as mean with S.E.M. Data are compared using non parametric Mann-Whitney test. ** P<0.01, ****P<0.001.

Supp Figure 2

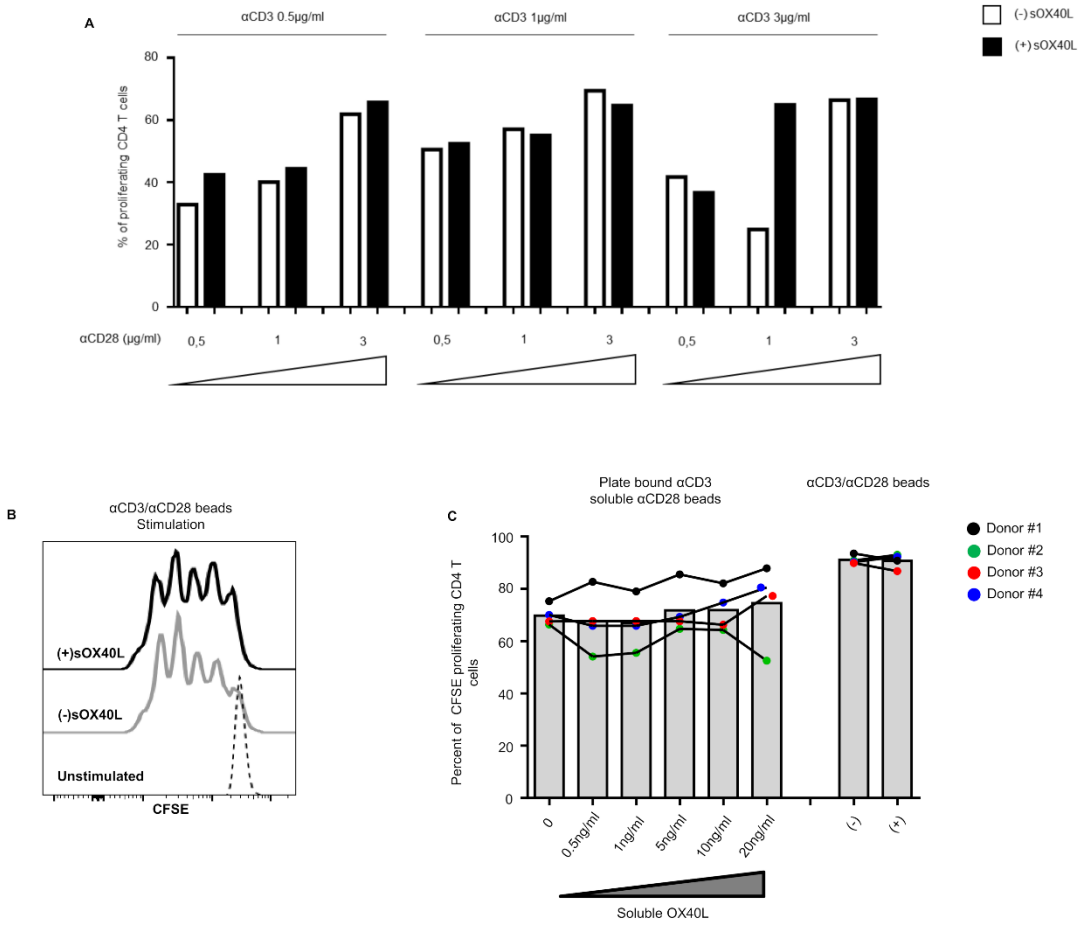


Figure S2: Effect of sOX40L on effector CD4 T cells proliferation alone

(**A**) Effect of sOX40L on the proliferative capacity of Effector CD4+ T cells following activation with anti-CD3 and anti-CD28 at different concentrations. Purified CD4 T cells were stained with CFSE and stimulated with or without agonist soluble OX40L (100ng/ml) for 4 days with different concentration of pre-coated anti-CD3 and soluble anti-CD28. The figure shows one of two independent experiments.
(**B**) Effect of soluble OX40L (100ng/ml) co-stimulation on effector CD4 T cells proliferation stimulated by anti-CD3 and anti-CD28 micro-beads. This figure is representative of 4 independent experiments.
(**C**) Dose effect of soluble OX40L co-stimulation (100ng/ml) on purified effector CD4 T cells stimulated by pre-coated anti-CD3 (1ug/ml) and soluble anti-CD28 (3ug/ml) or anti-CD3+anti-CD28 beads for 4 days. 4 independent experiments were realized using 4 different effector CD4 T cells donor (as show by color plots).

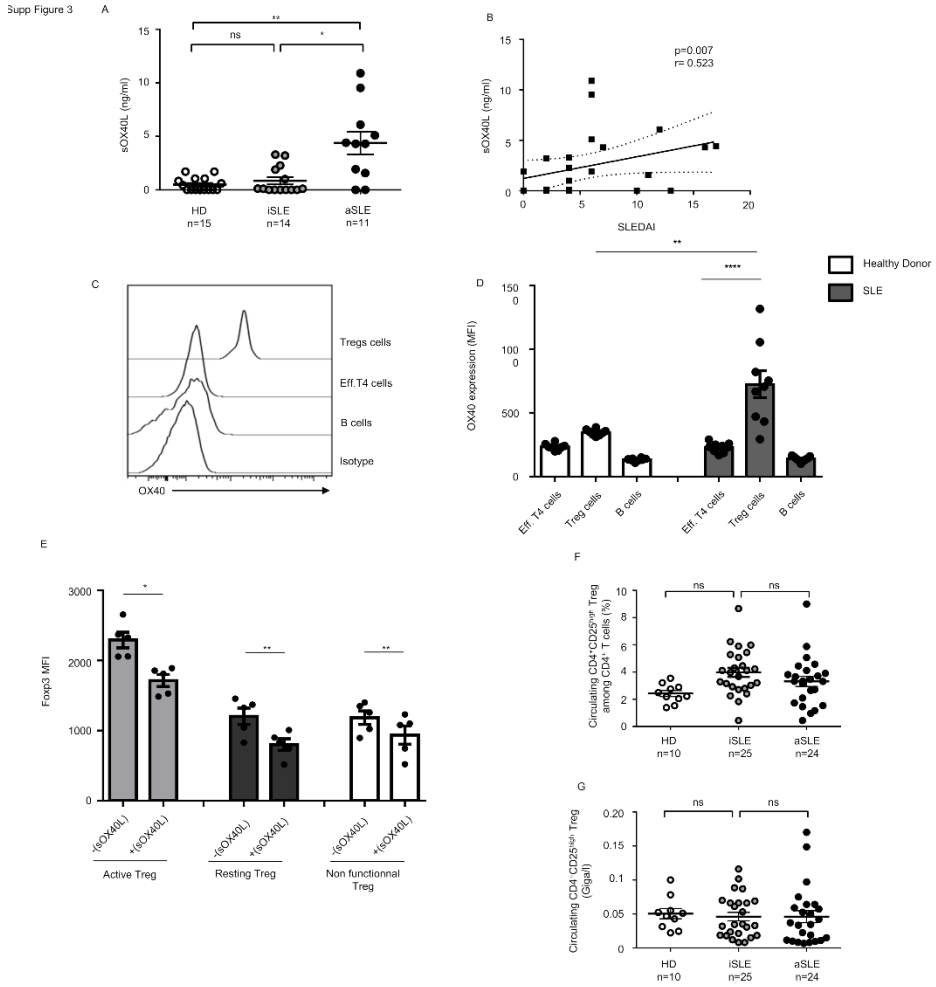
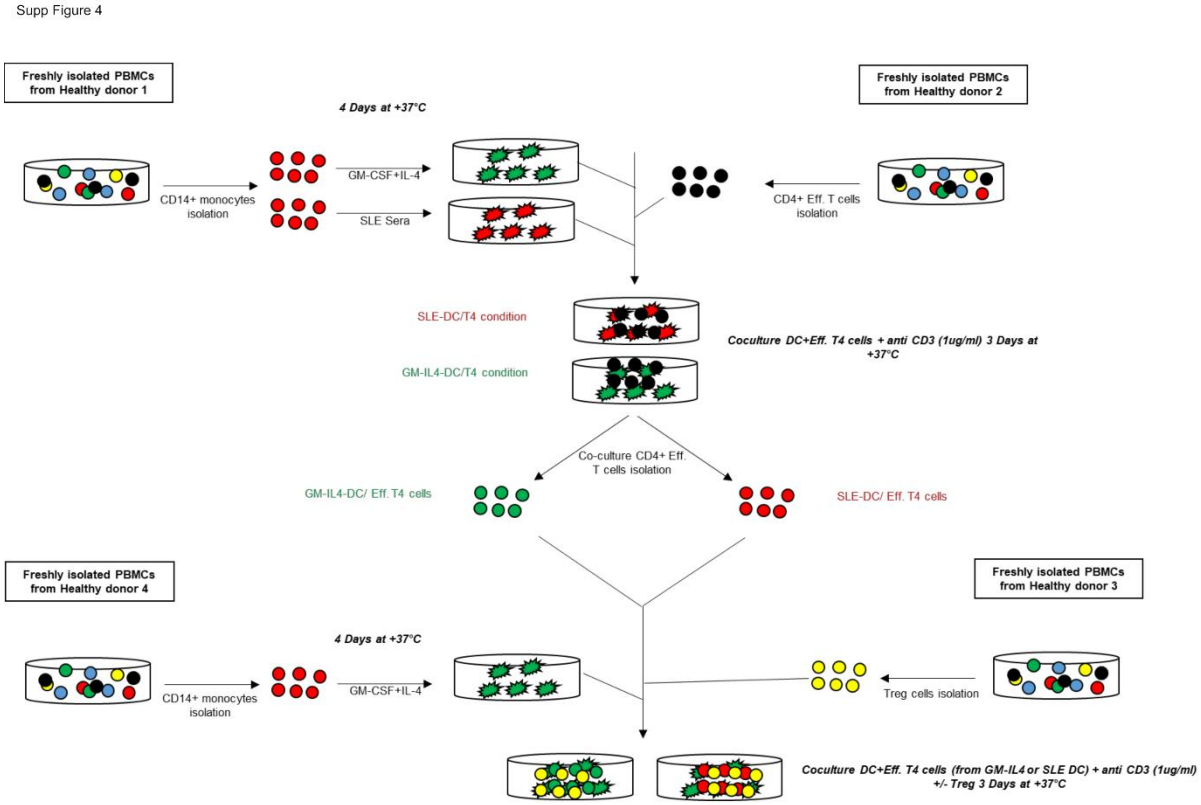


Figure S3: Blood CD4^{+}CD25^{high} Tregs and serum concentration of sOX40L in healthy donors and SLE patients.

(**A**) Serum concentration of sOX40L (in ng/ml) from HD (n=15), iSLE (n=14) and aSLE patients (n=11). Individual values are given with mean and S.E.M, and compared using non-parametric Kruskall-Wallis test with Dunn's correction for multiple comparisons. P-value < 0.05 (*), P-value < 0.01 (**).(**B**) Correlation between sOX40L concentration in serum and SLE activity (SLEDAI), using Spearman rank correlation test. (**C-D**)**,** Peripheral Effector CD4 T cells, Tregs, and B cells were investigated for OX40 expression by flow cytometry. PBMCs were isolated and stained for OX40 expression from Healthy donors (n=8) and SLE patients (n=9). **(C)** Representative staining and **(D)** OX40 MFI values with S.E.M are shown and compared using one-way ANOVA with Holm-Sidak's correction for multiple comparisons. P value <0.01 (**), p<0.0001 (****). **(E),** sOX40L impacts Foxp3 expression in different Treg subset. Purified Tregs with or without sOX40L (100ng/ml) pre-treatment were cultured in pre-coated anti CD3 (1ug/ml) supplemented by soluble anti-CD28 (3ug/ml) in 96 wells plate for 3 days. Intra-nuclear Foxp3 expression was assessed by flow cytometry on different Treg sub-population based on CD25 and CD45RA cell surface expression: active Tregs (CD45RA^{-}CD25^{high}), resting Tregs (CD45RA^{+}CD25^{+}) and non-functional Tregs (CD45RA^{-}CD25^{+}) subset. 5 different donors were studied in 2 independent experiments. Individual data are shown with mean and S.E.M, and compared using non-parametric two-tailed Mann-Whitney test, p<0.05 (*), p<0.01 (**). **(F-G)**, Frequency of blood CD4^{+}CD25^{high} Tregs in HD (n=10) and SLE patients with inactive (iSLE, n=25) and active (aSLE, n=24) disease. **(F)** Results are expressed as % of CD4^{+}CD25^{high} cells among CD4^{+} T cells, and are compared using non-parametric Kruskall-Wallis test with Dunn's correction for multiple comparisons. **(G)**, Absolute count of CD4^{+}CD25^{high} Tregs cell in blood of HD (n=10) and iSLE (n=25) and aSLE patients (n=24). Results are expressed in Giga/L and are compared using non-parametric Kruskall-Wallis test with Dunn's correction for multiple comparisons. iSLE, patients with inactive disease (SLEDAI<6); aSLE, SLE patients with active disease (SLEDAI≥6).

Figure S4: Schematic showing experimental design for experiment in Figure 3A.



Supp Figure 5

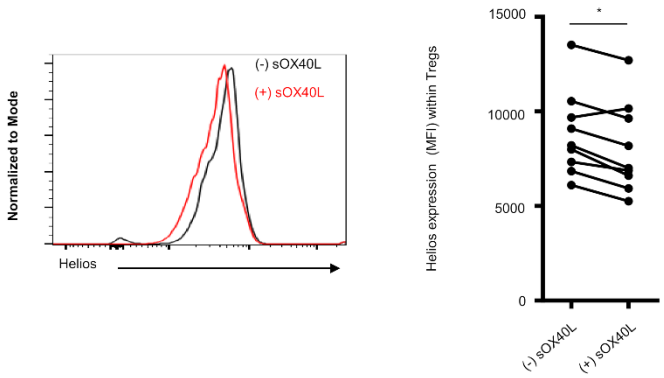


Figure S5: Tregs intranuclear Helios expression following OX40L co-stimulation

Isolated Tregs from healthy donors were cultured using pre-coated anti-CD3 (1ug/ml) 96 well plate with or without soluble OX40L (100ng/ml) for 48 hours. Intranuclear Helios expression level was evaluated by flow cytometry. 9 independents experiments using 9 different Tregs donors were realized. Data are shown as mean MFI and compared using two-tailed paired non-parametric Wilcoxon test. *, $p < 0.05$.

Supp Figure 6

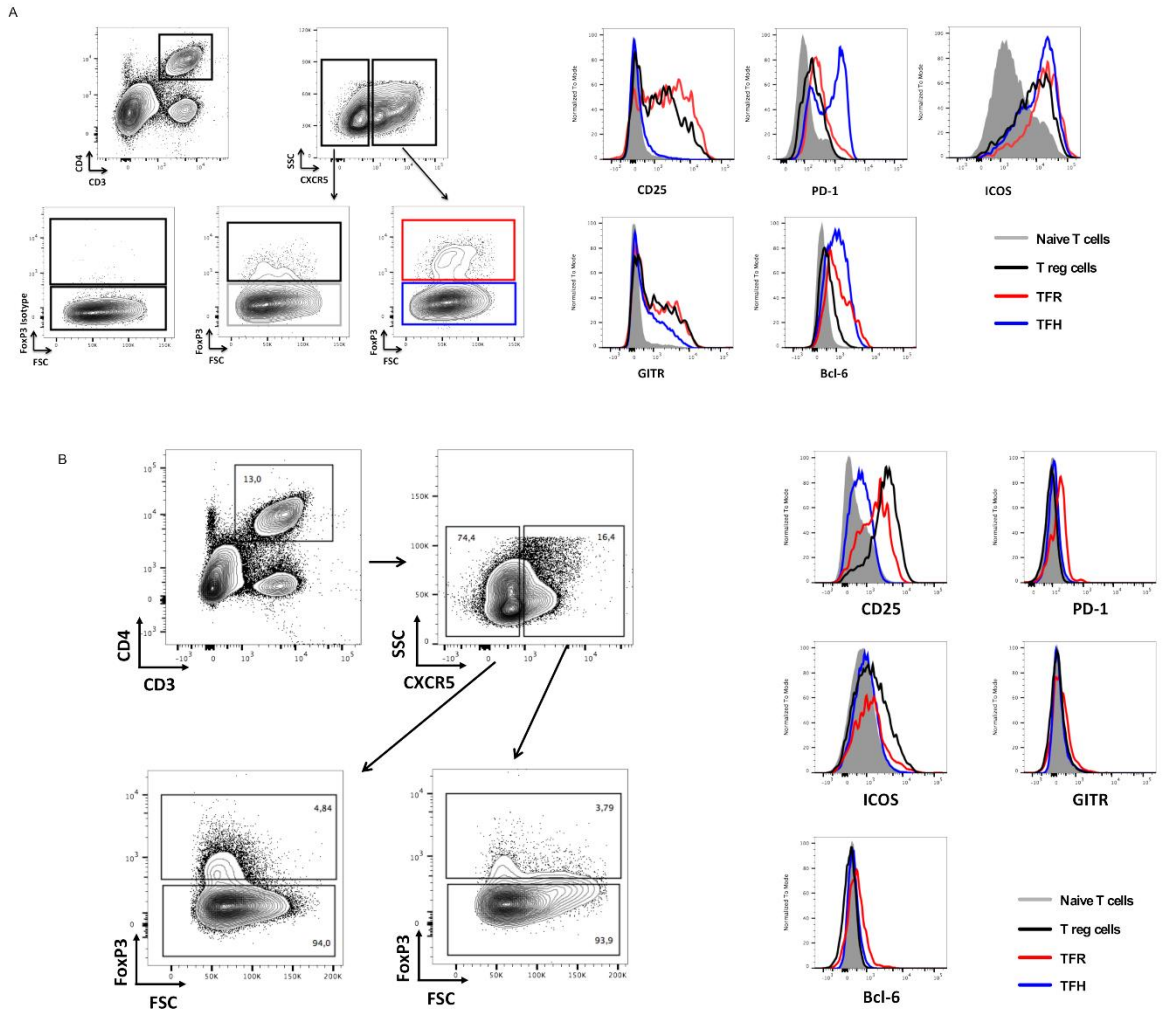


Figure S6: Cell surface expression of CD25, PD-1, ICOS, GITR and Bcl-6 of Tonsil and blood TFH, TFR, Tregs and naïve T cells in HD.

(**A, left and right panel**), cell surface expression of CD25, PD-1, ICOS, GITR and Bcl-6 in Tonsils TFH, TFR, Tregs and naïve T cells. (**A, left panel**), Representative dot plots showing $CD3^+CD4^+FoxP3^-CXCR5^+$ TFH cells (blue line), $CD3^+CD4^+FoxP3^+CXCR5^+$ TFR cells (red line), $CD3^+CD4^+FoxP3^+CXCR5^-$ Tregs cells (black line) and $CD3^+CD4^+FoxP3^-CXCR5^-$ naïve T cells (grey filled). (**A, right panel**), expression of cell surface markers according to cell subsets. Representative of one out of 2 experiments. (**B, left panel**), Representative dot plots showing $CD3^+CD4^+Foxp3^-CXCR5^+$ TFH cells (blue line), $CD3^+CD4^+Foxp3^+CXCR5^+$ TFR cells (red line), $CD3^+CD4^+Foxp3^+CXCR5^-$ Tregs cells (black line) and $CD3^+CD4^+Foxp3^-CXCR5^-$ naïve T cells (grey filled) in blood and (**B, right panel**) expression of cell surface markers according to cell subsets. Representative of one out of 2 experiments.

Supplementary Table 1: clinical and biological informations concerning SLE patients

Patient number	Age	Sex	Clinical failure	Fibre	Corticotherapy (dose, mg/day)	Associated treatment	SLEDAI
1	54	M	A, C, H, R			HCQ	2
2	27	F	A, C, H, N, PP, APS	A	10	MTX, RTX	8
3	35	F	A, H	A	20	HCQ	4
4	33	F	C, H, APS		10	HCQ, MMF	0
4	34	F	C, H, APS		10	HCQ, MMF	2
5	35	F	A, C, R		7	HCQ, AZA	2
6	35	F	A, C, H, R, APS		50	CYC	4
7	41	F	A, C, H, R	N, R	10	MPA	26
8	34	M	A, C, H, R	C, R, V			20
9	40	F	A, C, H			HCQ, MTX	0
10	32	F	A, C, H, R	A, C, R		HCQ	22
10	31	F	A, C, H, R		25	HCQ, CYC	4
10	31	F	A, C, H, R	R	25	HCQ, CYC	8
10	31	F	A, C, H, R	R	20	HCQ, CYC	8
11	41	F	A, H, APS		3	HCQ, MMF	4
12	72	M	C, H, N, PP, R	C, N, PP, R		HCQ	24
13	36	F	A, C, R		10	AZA	4
14	22	F	A, C, R			AZA	2
15	22	F	A, C, H, R	R	2,5	MMF	12
16	23	M	H, R	R		MMF	8
17	60	M	A, H, N, R	A, N, R	10	MMF	4
17	58	M	A, H, N, R		10	HCQ	19
17	59	M	A, H, N, R		5	HCQ	2
18	25	F	A, C, H, PP, APS		30	HCQ, RTX	4
18	25	F	A, C, H, PP, APS		25	HCQ, RTX	4
18	25	F	A, C, H, PP, APS	A	40	HCQ, RTX	8
19	18	F	A, C, H, R	A, C, N, R	20	HCQ, MMF	24
19	19	F	A, C, H, R		30	MPA	0
19	19	F	A, C, H, R		7	MPA	0
19	20	F	A, C, H, R	A	6	MPA	8
19	20	F	A, C, H, R		6	HCQ, MPA	2
20	40	F	A, C, R, APS		6	MMF	2
21	58	F	A, C, H		3	HCQ, MTX	4
22	42	F	A, H, R	R, V			33
23	44	M	A, H, PP, R		8	MMF	4
24	18	F	H				3
25	27	F	A, C, R	A	10	HCQ, MMF	8
26	48	F	D, H, M, PP, R	R	40	AZA	16
26	46	F	D, H, M, PP, R		20	MMF	4
26	46	F	D, H, M, PP, R		5	HCQ, MMF	4
27	39	F	A, H, N, R, APS		5	HCQ, AZA	2
28	35	F	A, C, H, N	A	10		8
28	35	F	A, C, H, N	A	15		8
29	58	F	A, H	A	15	HCQ, AZA	6
30	35	F	A, C, H, M, N, R	A, R	20	HCQ, MMF	16
31	58	F	A, C, H, M, N, R		17,5	HCQ, MMF	2
32	25	F	A, C		17,5	HCQ	2
32	25	F	A, H	A		MTX	4
33	38	F	A, H, R	R	10	MTX	8
34	39	F	A, H, R	R	8	MMF	16
34	17	M	A, C, PP, R	R	10	HCQ, MMF	2
35	21	F	A, C, APS			HCQ	4
35	21	F	A, C, APS			HCQ	4
36	19	F	A, C, R	R	5	HCQ, MMF	8
37	39	F	A, H, R		3	MMF, RTX	2
38	51	F	A, H, R	A	10	Abatacept	8
38	52	F	A, H, R	R	9	Abatacept	8
38	52	F	A, H, R	A	10		8
39	57	F	A, H, PP, R	R	5	HCQ	17
39	56	F	A, H, PP, R	R	20	HCQ	4
40	65	F	H, APS				0
41	38	F	A, C, PP	C	5	HCQ	6
42	34	F	A, C, H, N		10	HCQ, MMF	4
43	28	F	R, H, A	R	5	HCQ, MMF	9
44	63	F	C, A, R	H, A	30	HCQ, AZA	22
45	22	F	C, A	PP	30	HCQ, CYC	23
46	37	M	A, R, D		5	MMF	2
47	37	F	A		7	HCQ	2
48	57	F	A		5	HCQ	4
49	53	F	A		5	MTX	3
50	80	F	A, N, PP		9		4
51	20	F	R			HCQ, MTX	2
52	41	F	C, A, R				8
53	43	F	A	A	20		8
54	50	F	H			HCQ	4
55	55	F	A, C			MMF	4
56	27	F	A, R				16
57	25	F	A, R, H, PP				23
58	18	F	A, H	A	12,5	AZA	8
59	35	F	A		10	HCQ	4
60	37	F	A, C	A	10	HCQ	14
61	41	F	A, C, H	A, R	5	HCQ	25

Legends F: female, M: male, A: articular, C: cutaneous, D: digestive, H: haematologic, M: myocardic, N: neurologic, PP: pleuro-pericardic, R: renal, V: vascular, APS: anti-phospholipid syndrom.

HCQ: hydroxychloroquin, AZA: azathioprin, CYC: cyclophosphamide, MMF: mycophenolate mofetil, MPA: mycophenolicacid, MTX: methotrexate, RTX: rituximab

Supplementary Table 2: Antibodies and clones informations

Marker	Clone	Company
CD3 APC	UCHT1	Beckman Coulter
CD4 PC7	SFCI12T4D11	Beckman Coulter
CD8 FITC	B9.11	Beckman Coulter
CD16 FITC	3G8	BD Biosciences
CD11c APC	B-ly6	BD Biosciences
HLA-DR PC7	Immu-357	Beckman Coulter
CD14 PC5	RM052	Beckman Coulter
CD25 PC5	B1.49.9	Beckman Coulter
CD127 PE	R34.34	Beckman Coulter
TNFR2 PE	hTNFR-M1	BD Biosciences
CTLA-4 APC	BNI3	BD Biosciences
OX40L PE	ANC10G1	Ancell
CCR7 FITC	REA546	Miltenyi
CD56 PE	B159	BD Biosciences
GITR APC	DT5D3	Miltenyi
Foxp3 PE	236A/E7	eBioscience
CD45RA ECD	2H4LDH11LDB9	Beckman Coulter
CXCR5 AF647	RF8B2	BD Biosciences
PD-1 FITC	EH12.2H7	Biolegend
ICOS PC7	C398.4A	Biolegend
Bcl-6 AF647	K112-91	BD Biosciences
CD38 PC7	HIT2	Biolegend
CD27 PC5	O323	eBioscience
IgD FITC	IA6-2	BD Biosciences
CD19 PE	LT19	Miltenyi

928 **Supplementary Table 3: Clinical information concerning patients used in Tregs**
929 **experiment**
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Patient number	Clinical disease activity	Age	Sex	Flare	Current organ involvement	steroids (dose, mg/day)	Associated treatments	SLEDAI	%CD11cDR OX40L
#1	Active	65	F	yes	N	0	-	8	15
#2	Active	42	F	yes	R, C, H, V, PP	80	HCQ	29	5
#3	Active	41	F	-	A	3	HCQ	4	9,7
#4	Active	35	F	yes	R	60	HCQ - MMF	16	19
#5	Active	25	F	-	A	0	MTX	4	6
#6	Quiescent	60	F	-	-	5	MTX	1	1
#7	Quiescent	58	F	-	-	15	HCQ	4	0
#8	Quiescent	27	M	-	-	0	-	4	0
#9	Quiescent	19	F	-	-	7	MMF	0	3
#10	Quiescent	25	F	-	-	30	RTX	4	1

Legends:
F: female, M: male, A: articular, C: cutaneous, D: digestive, H: haematologic, M : myocardic, N : neurologic, PP: pleuro-pericardic, R: renal, V: vascular
HCQ: hydroxychloroquine, MMF: mycophenolate mofetil, MTX : methotrexate, RTX: rituximab
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