Supplemental materials for:

B cells from African American Lupus patients exhibit an activated phenotype

- Supplemental Figure 1: Increased frequency of CD80+ and PD1+ B cells in African American SLE patients.
- Supplemental Figure 2: Expression of CD40L by T cells of African American and European American SLE patients.
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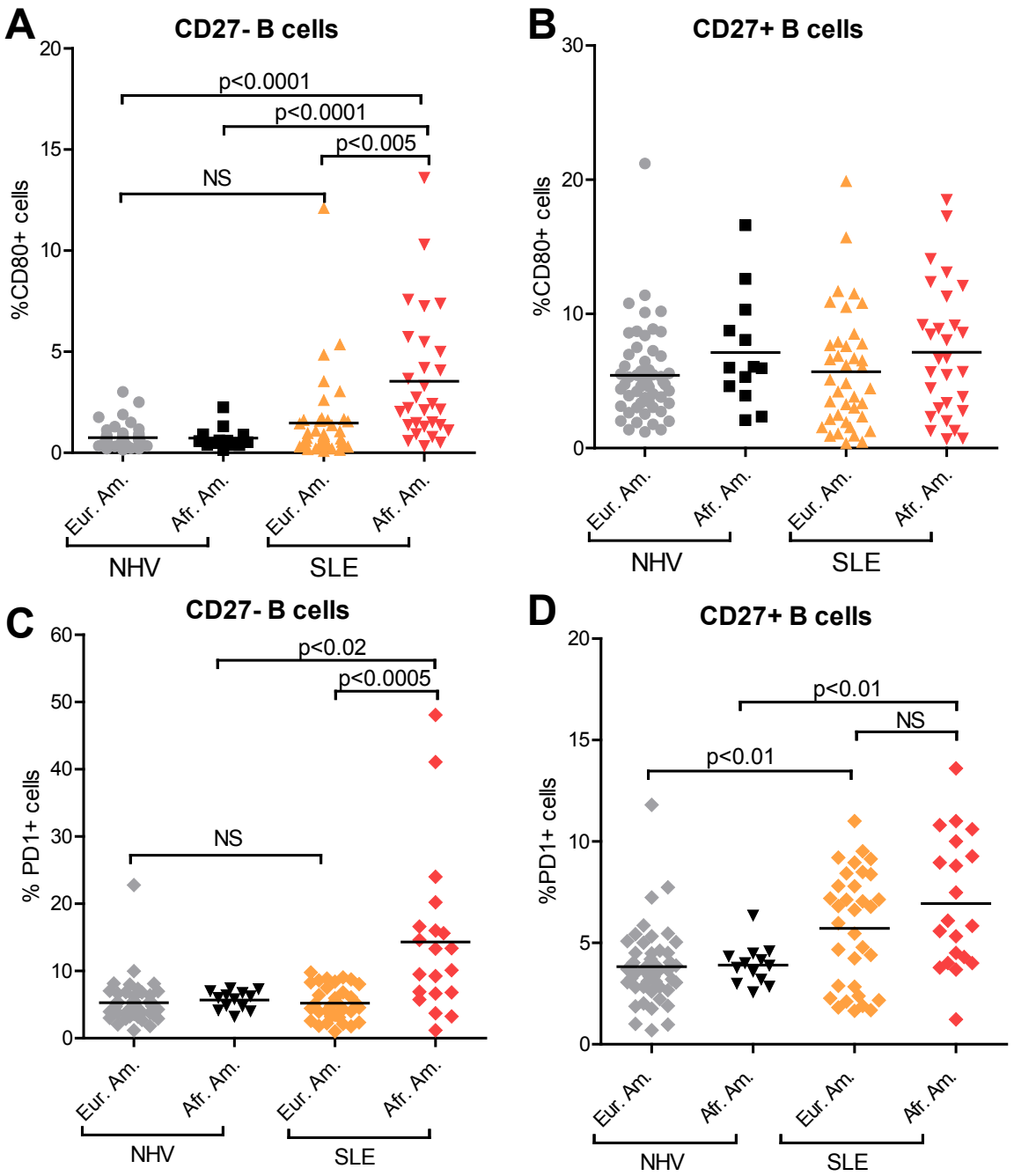
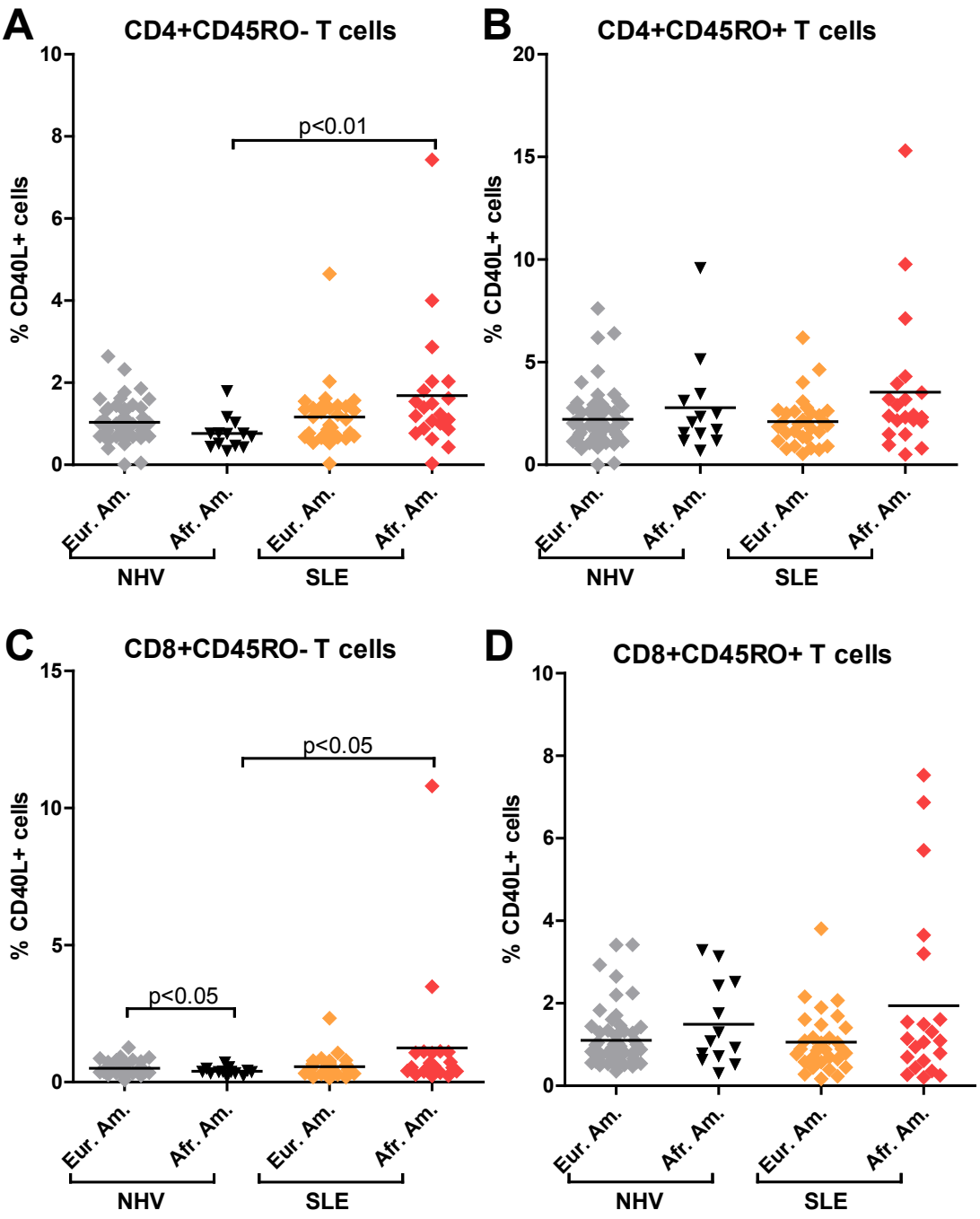
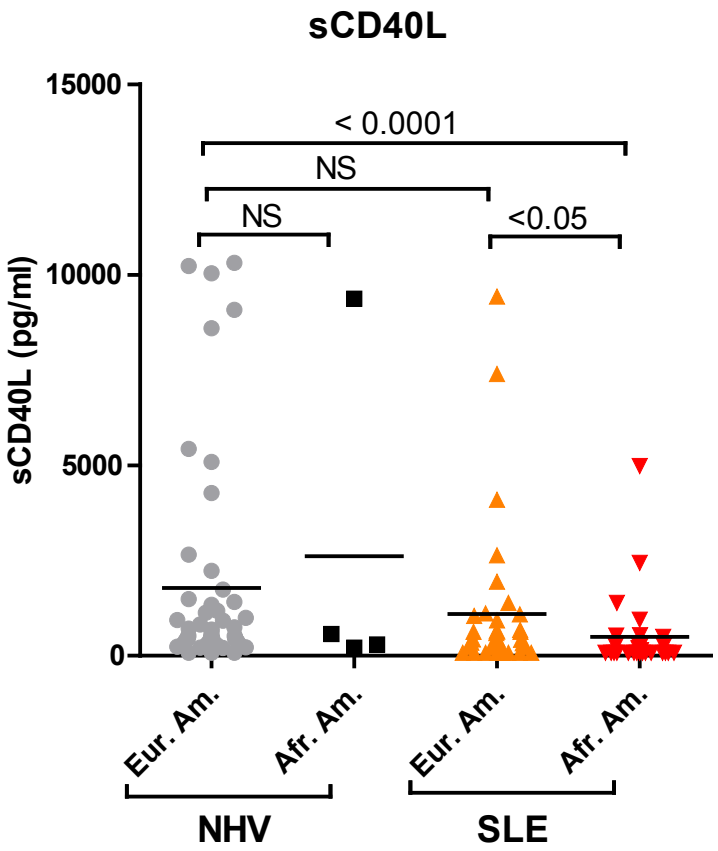


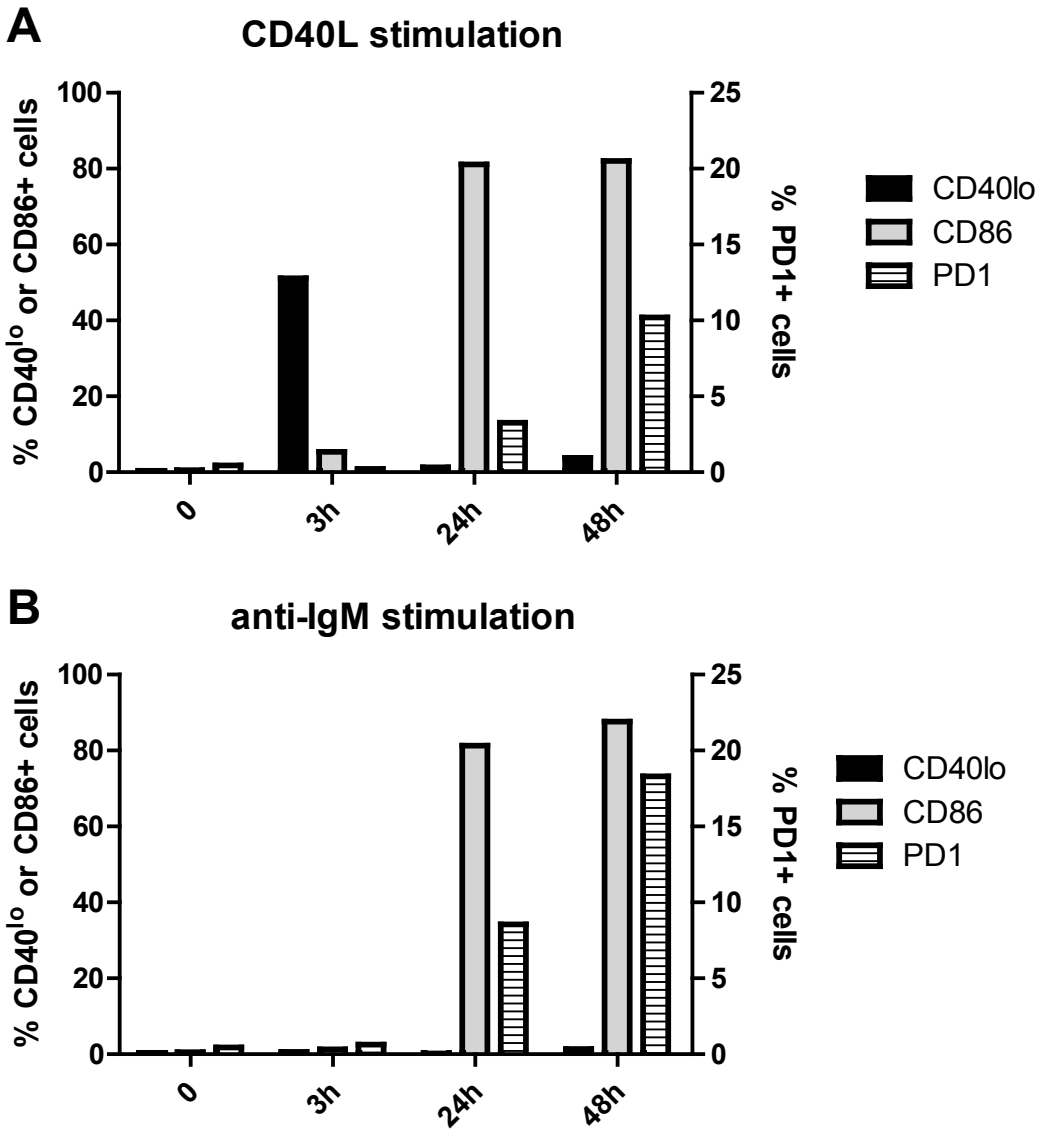
Supplemental Figure 1: Increased frequency of CD80+ and PD1+ B cells in African American systemic lupus erythematosus (SLE) patients. Frequencies of CD80+ CD19+ CD27– B cells **(A)**, CD80+ CD19+ CD27+ B cells **(B)**, PD1+ CD19+ CD27– B cells **(C)** and PD1+ CD19+ CD27+ B cells **(D)** in PBMC from African American (Afr. Am.) and European American (Eur. Am.) normal healthy volunteers (NHV) and SLE patients. 68 NHV and 68 SLE donors were used for CD80+ B cells frequencies and 62 NHV and 53 SLE donors for PD1+ B cell frequencies. P values are indicated (Mann Whitney test).



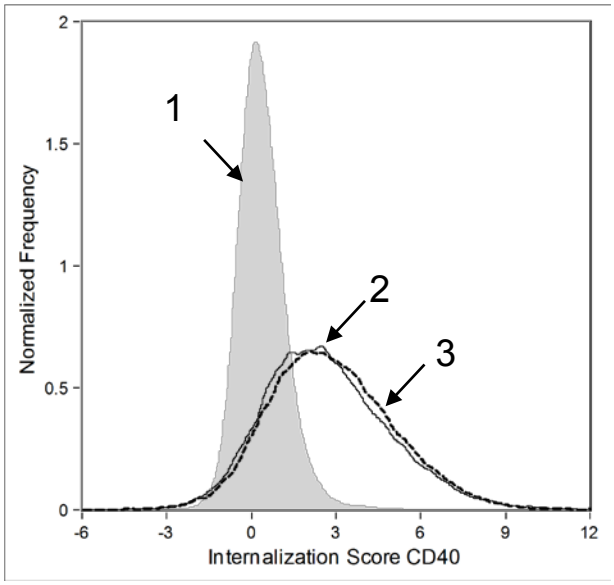
Supplemental Figure 2: Expression of CD40L by T cells of African American (Afr. Am.) and European American (Eur. Am.) systemic lupus erythematosus (SLE) patients. Summary of frequencies of CD40L+ CD4+ CD45RO- naïve T cells **(A)**, CD40L+ CD4+ CD45RO+ memory T cells **(B),** CD40L+ CD8+ CD45RO- naïve T cells **(C)** and CD40L+ CD8+ CD45RO+ memory T cells **(D)** in PBMC from 67 normal healthy volunteers (NHV) and 52 SLE patients**.** P values when statistically significant are indicated (Mann Whitney test).



Supplemental Figure 3: Plasma levels of soluble CD40L (sCD40L) in African American (Afr. Am.) and European American (Eur. Am.) normal healthy volunteers (NHV) and systemic lupus erythematosus (SLE) patients. sCD40L was measured by ELISA in plasma from 52 Eur. Am. and 4 Afr. Am NHV, and 36 Eur. Am. and 28 Afr. Am. SLE donors. P values when statistically significant are indicated (Mann Whitney test).

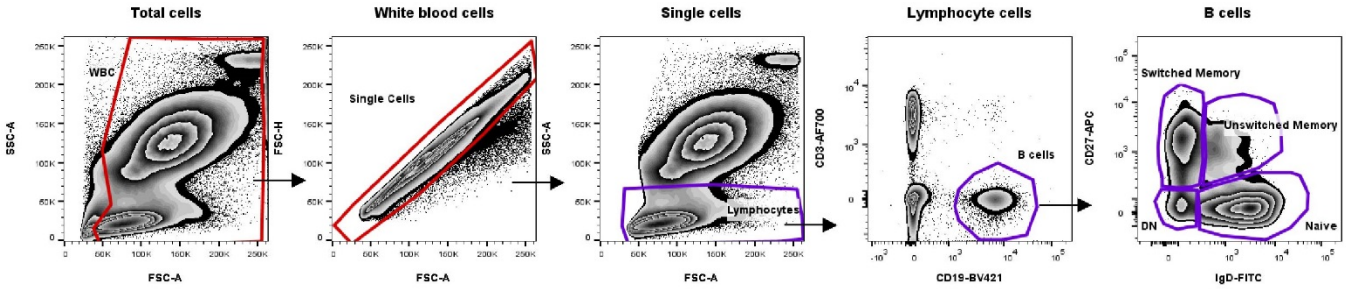


Supplemental Figure 4: Stimulation with CD40 induces CD40^{lo}, CD86+ and PD1+ CD27- B cells with different kinetics. Induction of CD40^{lo}, CD86+ and PD1+ CD27- B cells by CD40L-IZ **(A)** and by anti-IgM F(ab')$_2$ **(B)** stimulation at 3h, 24h, 48h.

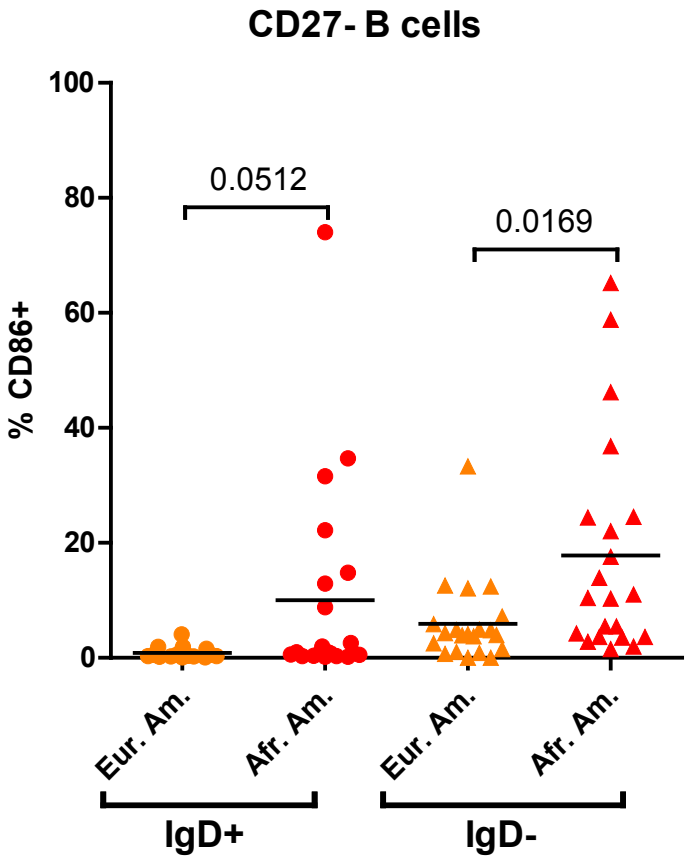


	Pre-incubation ($4C^0$)	Stimulation ($37C^0$)	Total Internalization Score (Median)	% Cells Internalized
1	Anti-CD40-PE	None	0.293	2%
2	Anti-CD40-PE	CD40L-IZ	2.471	49%
3	CD40L-IZ+anti- CD40-PE	CD40L-IZ	2.684	53%

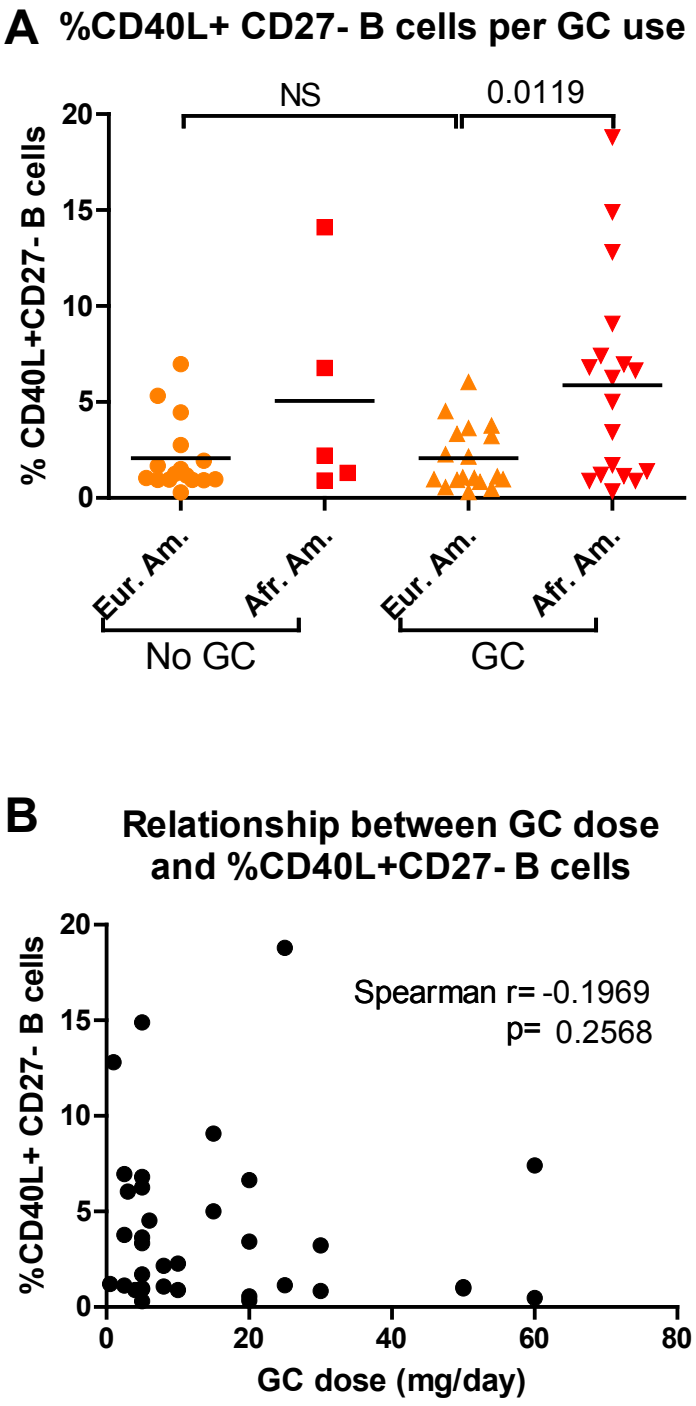
Supplemental Figure 5: CD40L-IZ does not prevent binding of anti-CD40-PE to CD40. Cells were stained at 4^0C with anti-CD40-PE without or with CD40L-IZ, washed and stimulated at 37^0C with CD40L. Internalization score and percentages of cells with high internalization of CD40 (score>2.5) were similar whether staining with CD40-PE antibody was performed with or without CD40L-IZ.



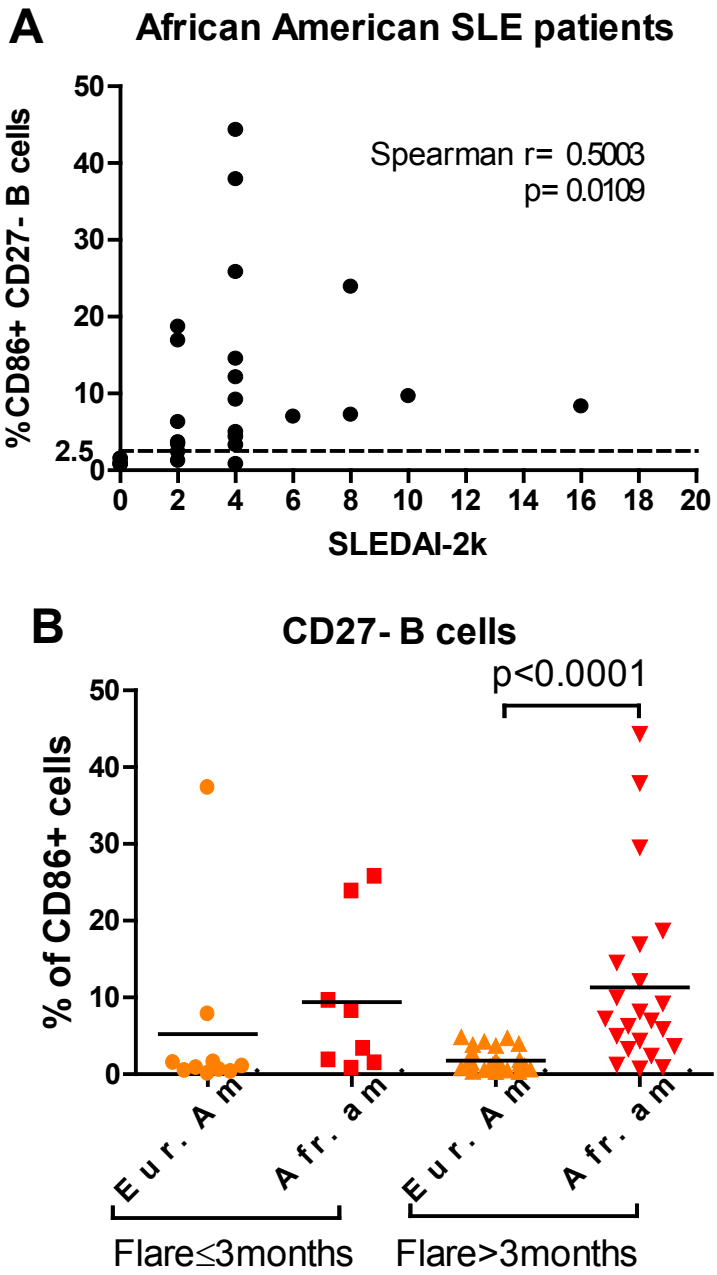
Supplemental Figure 6: Gating strategy for B cell subsets excludes doublets and CD3+ cells. Flow cytometry dot plots showing a representative gating strategy for whole blood B cell subsets. Single cells are selected, then CD3+ are excluded from the CD19+ gate. IgD and CD27 expressions are used to gate for naive, double negative (DN), switched and unswitched memory B cells in the CD19+ gate.



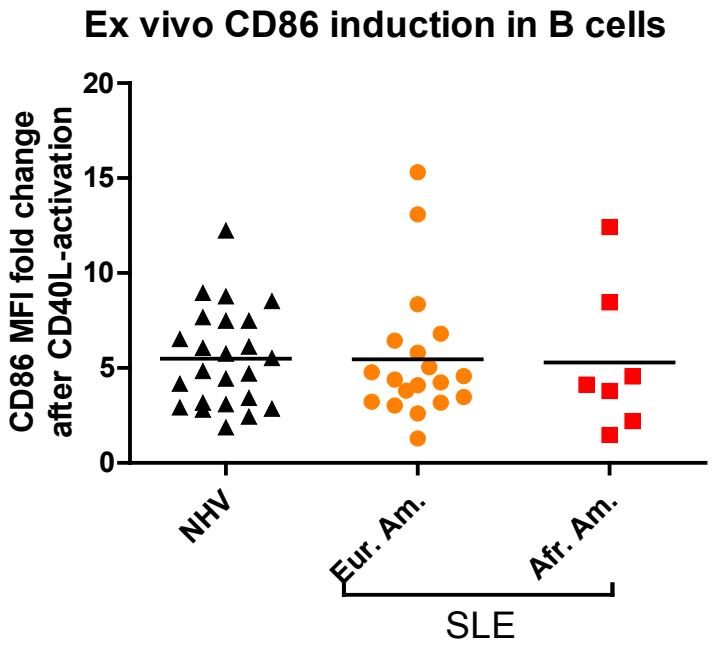
Supplemental Figure 7: Increased CD86 expression in both IgD+ and IgD- CD27- B cells in African American systemic lupus erythematosus (SLE) patients compared to patients of European descent. Summary of frequencies of CD86+ IgD+ CD27- (naïve) and CD86+ IgD-CD27- (DN) B cells in 21 African American (Afr. Am.) and 21 European American(Eur. Am.) SLE patients. p-values by Mann Whitney test are indicated.



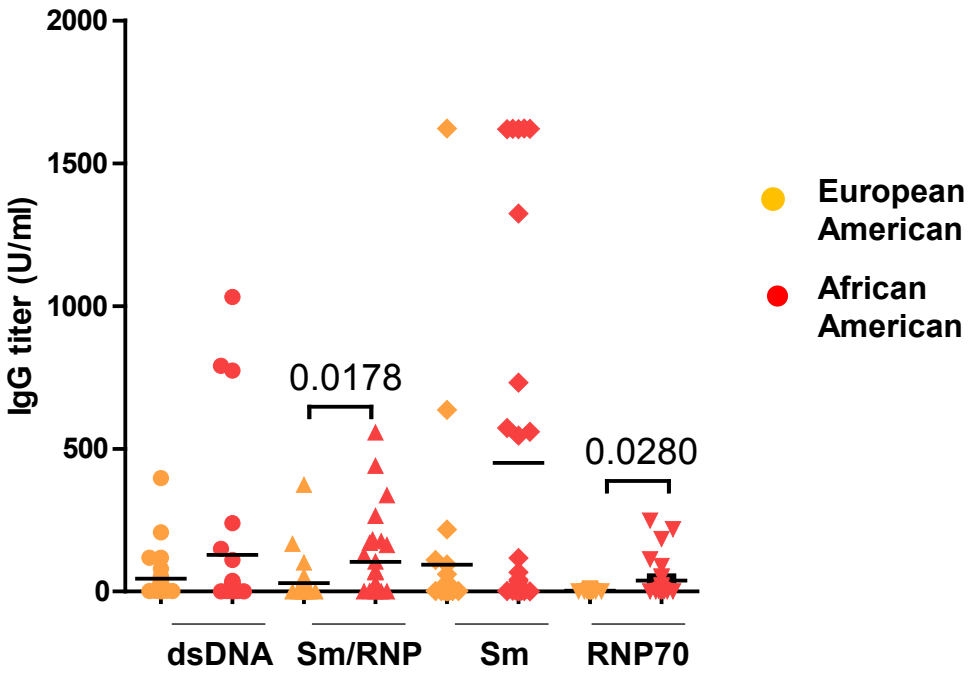
Supplemental figure 8. Glucocorticoid (GC) use is not associated with a higher frequency of CD40L+ CD27- B cells. A) Percentages of CD40L+CD27- B cells in 34 European American (Eur. Am.) and 23 African American (Afr. Am.) patients, treated (GC) or not treated (no GC) with glucocorticoids. B). Percentages of CD40L+CD27- B cells and GC dose (mg/day) in 36 treated patients show no significant correlation (Spearman correlation).



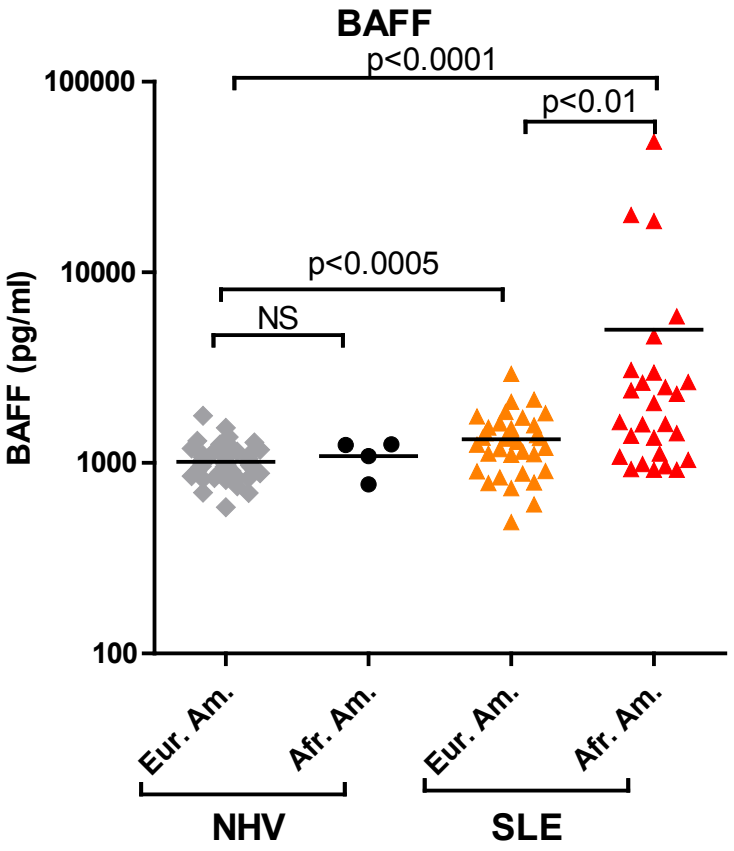
Supplemental figure 9: Recent flares do not account for the observed activated B cell phenotype. A) Spearman correlation of % CD86+CD27- B cells and SLEDAI-2k in 25 African American SLE patients. Spearman r and p-value are indicated on the plot. The dotted line represents the 2.5 % threshold. **B)** Percentages of CD86+ CD27- B cells in African American (Afr. Am.) or European American (Eur. Am.) patients who flared less (10 Eur. Am. and 8 Afr. Am.) or more (29 Eur. Am and 22 Afr. Am.) than 3 months ago.



Supplemental Figure 10: B cells from African American (Afr. Am.) and European American (Eur. Am.) systemic lupus erythematosus (SLE) patients and from normal healthy volunteers (NHV) respond similarly to CD40 ligand (CD40L) stimulation. CD86 median fluorescence intensity (MFI) was measured on B cells after overnight stimulation with CD40L isoleucine zipper of whole blood from 24 Eur. Am and Afr. Am. NHV, 19 Eur. Am. and 7 Afr. Am. SLE donors. Fold change of CD86 MFI in stimulated sample over non stimulated sample is represented.



Supplemental Figure 11: Higher anti-Sm/RNP and anti-RNP70 IgG titers in African American patients. Autoantibody plasma (IgG, U/ml) levels in 27 African American and 31 European American SLE patients. P-value for statistically significant differences are indicated (Mann Whitney).



Supplemental Figure 12: Plasma levels of BAFF in African American (Afr. Am.) and European American(Eur. Am.) NHV and SLE patients. BAFF was measured in plasma by ELISA in 32 Eur. Am. and 4 Afr. Am. NHV and 35 Eur. Am. and 27 Afr. Am. SLE donors. p-values when statistically significant are indicated (Mann Whitney test).

Supplemental table 1: Clinical data of sub-cohort described in Figure 7

	African Americans (n=21)	European Americans (n=21)
SLEDAI-2K, mean ± SD	4.2±4.0	3.3±1.6
Total ACR classification criteria, mean ± SD	5.3±1.4	5.2±1.3
Duration of disease (years), mean ± SD	13±7.9	17.3±15.5
Time since last flare (years), mean ± SD	2.6±3.5	3.3±5.1
Co-morbidities		
Nephritis, n(%)	9(43)	6(29)
Sjogren Syndrome, n(%)	0	3(14)
Antiphospholipid syndrome, n(%)	0	1(4.8)
Medications		
Hydroxychloroquine, n(%)	10(48)	9(43)
Mycophenolate mofetil, n(%)	6(29)	7(33)
Belimumab, n(%)	1(4.8)	1(4.8)
Glucocorticoids, n(%)	14(67)	9(43)

Supplemental Table 2. Average frequencies of immune cell subsets in SLE patients

	African Americans (n=21)	European Americans (n=21)	p-value
CD19+ B cells, % of WBC	3.1±3.7	3.2±2.4	p>0.05
IgD-CD27- (DN) B cells, % of CD19+ cells	20.2±15.6	7.4±6.1	**0.0012**
IgD+CD27- Naïve B cells, % of CD19+ cells	53±24.9	64.4±27	p>0.05
IgD-CD27+ switched memory B cells, % of CD19+ cells	19.4±12.9	12.5±9.9	p>0.05
IgD+CD27+ unswitched memory B cells, % of CD19+ cells	3.6±4	10.6±18.4	p>0.05
CD19+IgD-CD27^{hi}CD38^{hi} CD20^{lo} plasmablasts, % of CD19+ cells	0.24±0.45	0.21±0.53	p>0.05
CD19+IgD+CD27-CD24^{hi}CD38^{hi} transitional B cells, % of CD19+ cells	5.7±7.1	2.6±3.3	p>0.05
CD4 T cells, % of WBC	8.1±6.9	11.2±5.8	**0.0252**
CD8 T cells, % of WBC	5.6±4.0	9.5±13.9	p>0.05
CD4-CD8-DN T cells, % WBC	1.3±2.2	1.6±2.3	p>0.05
CD3+CD4+CD25+CD127^{lo}Treg, % of CD4+ T cells	9.6±6.7	8.7±7.9	p>0.05
CD3+CD56+ NKT, % of CD3+ T cells	9.4±11.3	3.6±2.9	p>0.05
CD3-CD19-CD20-CD14-CD56+ NK, % of WBC	1.7±1.4	1.8±1.2	p>0.05
CD3-CD19-CD20-CD14+ monocytes, % of WBC	5.3±4.1	6.1±4.7	p>0.05

Data are represented as mean ± SD. Adjusted p-value<0.05 (Mann Whitney) are indicated in bold. WBC: white blood cells; DN: double negative; Treg: regulatory T cells, NKT: natural killer T cells

Supplemental table 3: Comparison of patients' and controls' demographics

	SLE (n=68)	NHV(n=69)
Age(years), mean±SD	46±15	45±12
Female, n(%)	57(84)	53(77)
African American ethnicity, n (%):	29(43)	13(19)