Supplemental Materials

Supplemental Methods

Reagents and Antibodies

Reagent	Company	Catalog #
Concanamycin A	Sigma	C9705
Intracellular pH Calibration Buffer kit	Thermo Fisher Scientific	P35379
Live/Dead Fixable Near IR (780) Viability Kit	Thermo Fisher Scientific	L34994
LysoSensor Yellow/Blue DND-160	Thermo Fisher Scientific	L7545
Lysosomal Intracellular Activity Assay kit	Abcam	Ab234622
Phenol-red free High Glucose DMEM	Gibco	21063029
Phenol-red free RPMI	Gibco	11835030
Polymorphprep	Fisher scientific	NC0863559
Tamoxifen	Selleckchem	S1238
3AC	Echelon Biosciences	B-0341
NSC87877	Selleckchem	S8182
PIK-75 HCl	Selleckchem	S1205
TGX-221	Selleckchem	S1169
IC-87114	Selleckchem	S1268
CellROX Deep Red Flow Cytometry Assay kit	Thermo Fisher Scientific	C10491
Cholera Toxin Subunit B AlexaFluor 594 conjugated	Thermo Fisher Scientific	C34777

Antibody Target	Fluorochrome	Company	Catalog #	RRID
CD19	PE	Biolegend	392506	AB_2750097
CD3	BV650	Biolegend	300468	AB_2629574
CD3	PE-Dazzle 594	Biolegend	300336	AB_2632652
CD14	AF 488	Biolegend	301811	AB_493159
CD14	APC-Cy7	Biolegend	301820	AB_493695
CD16	PECy7	Biolegend	302016	AB_314216
CD11c	PE-CF 594	BD Biosciences	562393	AB_11153662
CD11c	APC	BD Biosciences	559877	AB_398680
HLA-DR	BV421	Biolegend	307636	AB_2561831
HLA-DR	AF 488	Biolegend	307620	AB_493175
HLA-DR	BUV395	BD Biosciences	564040	AB_2738558
CD66b	PerCP-Cy5.5	Biolegend	396914	AB_2820061
CD56	PerCP-Cy5.5	Biolegend	392420	AB_2734444
CD24	Superbright 600	Thermo Fisher Scientific	63-0247-42	AB_2717025
CD21	PECy7	BD Biosciences	561374	AB_10681717
IgD	APC-H7	Biolegend	348218	AB_11203722
CXCR5	AF 488	R and D Systems	FAB190G-100UG	AB_3647470
CD38	PerCP-Cy5.5	BD Biosciences	551400	AB_394184
CD27	BV510	BD Biosciences	563092	AB_2313577
CD64	AF 647	Biolegend	305012	AB_528867
CD16	AF 647	Biolegend	302020	AB_492976
CD32A	AF 647	Novus	NBP2-47830AF647	AB_3312796
CD32B/C	APC	Biolegend	398304	AB_2860997
CD15	BV605	Biolegend	323031	AB_2562131
CD45	PE	Biolegend	304058	AB_2564156
CD32A (clone IV.3)	Unconjugated	Stemcell Technologies	60012	AB_2925215
Ly6G	BUV563	Thermo Fisher Scientific	365-5931-82	AB_2925400
Ly6G	AF 488	Biolegend	127625	AB_2561339
CD11b	Spark Plus UV385	Biolegend	101240	AB_3097646
CD11b	APC	BIolegend	101212	AB_312795
F4/80	eFluor 450	Thermo Fisher Scientific	48-4801-82	AB_1548747
CD19	BUV737	Thermo Fisher Scientific	367-0193-82	AB_2895945
CD3	BV785	Biolegend	100232	AB_2562554
SHP-1	-	Cell Signaling Technology	3759S	AB_2173694
pSHP-1 (Tyr564)	-	Cell Signaling Technology	8849S	AB_11141050
SHIP-1	-	Cell Signaling Technology	2728S	AB_2126244
pSHIP-1 (Tyr1022)	-	Cell Signaling Technology	3941S	AB_2296062
Anti-Rabbit IgG	AF 647	Thermo Fisher Scientific	A-21244	AB_2535812
PIP3	Fluorescein	Echelon Biosciences	Z-G345	AB_427218
PI(3,4)P2	-	Echelon Biosciences	Z-P034	Unavailable

PCR

B6.SHP-1$^{fl/fl}$ x B6.Rosa26-CreERT2 were genotyped for CreERT2 using primers for Cre negative forward 5' CTG GCT TCT GAG GAC CG 3', Cre negative reverse 5' CCG AAA ATC TGT GGG AAG TC 3', Cre positive forward 5' CGT GAT CTG CAA CTC CAG TC 3', Cre positive reverse 5' AGG CAA ATT TTG GTG TAC GG 3'.

Cell markers for flow cytometry

Mouse cell markers

Cell Types	Markers
B cells	$CD3^{neg}CD19^{pos}$
T cells	$CD19^{neg}CD3^{pos}$
CD11b myeloid cells	$CD19^{neg}CD3^{neg}CD11b^{pos}$
CD11c+ DCs	$CD19^{neg}CD3^{neg}CD11c^{high}$
Neutrophils	$CD19^{neg}CD3^{neg}CD11b^{pos}Ly6G^{pos}$
BMMφs	$CD11b^{pos}F4/80^{pos}$

neg = negative, pos = positive

Human cell markers

Cell Types	Markers
Monocyte	$CD19^{neg}CD3^{neg}CD66b^{neg}CD56^{neg}CD14^{pos}$HLA-$DR^{pos}$
DCs	$CD19^{neg}CD3^{neg}CD66b^{neg}CD56^{neg}CD14^{neg}CD11c^{pos}$
B cells	$CD66b^{neg}CD56^{neg}CD3^{neg}CD19^{pos}$
T cells	$CD66b^{neg}CD56^{neg}CD19^{neg}CD3^{pos}$
rNAV	$CD19^{pos}CD27^{neg}IgD^{pos}CD24^{neg}CD38^{neg}CD21^{pos}CD11c^{neg}$
aNAV	$CD19^{pos}CD27^{neg}IgD^{pos}CD24^{neg}CD38^{neg}CD21^{neg}CD11c^{pos}$
DN1	$CD19^{pos}CD27^{neg}IgD^{neg}CD21^{pos}CD11c^{neg}$
DN2	$CD19^{pos}CD27^{neg}IgD^{neg}CD21^{neg}CD11c^{pos}$

neg = negative; pos = positive

Flow cytometry

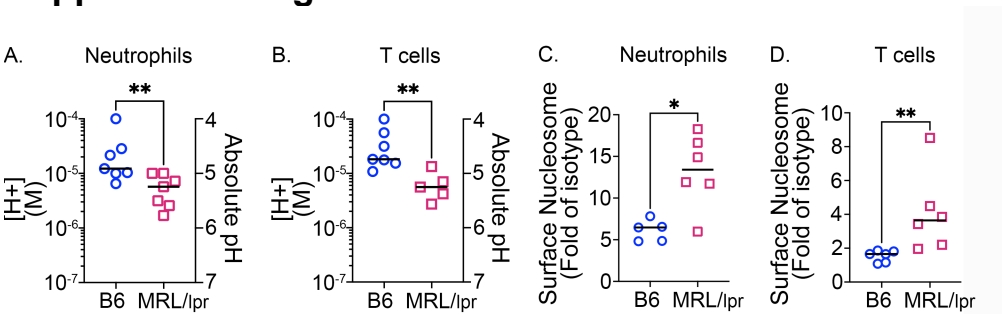
Blood cells were collected after RBC lysis with ACK buffer (0.15 M NH_4Cl, 1 M $KHCO_3$, 0.1 mM Na_2EDTA, pH 7.2-7.4), or for mouse splenocytes after RBC lysis with NH_4Cl_2 (0.154 M, pH 7.2). Cells were washed twice with PBS, filtered through 40μm strainer, and resuspended in complete media. BMMφs from 8-10 wk old mice were derived as previously described (15). In short, bone marrow cells from tibias and femurs were grown in complete media with 3-5% L-cell supernatant for 7 days with media replenishment on day 4. Day 7 BMMφs (95-98% $CD11b^{+}F4/80^{+}$) were collected and rested for 2hrs at 37°C before treatment.

Human unfractionated blood cells ($1x10^6$) or mouse splenocytes/BMMφs ($1x10^6$) were blocked with 30% human AB serum or 200 µg/ml of 2.4G2 (anti-FcγRIIB/III) for 15 min on ice. Cells were washed in FACS buffer (1x PBS with 2% FBS, 0.2% NaN_3) and stained for surface bound nucleosome (PL2-3, mouse IgG2a, conjugated with Alexafluor 647), dsDNA (33H11, human IgG1, conjugated with Alexafluor 647), FcγRI, FcγRIIa, FcγRIIb, and FcγRIII in the presence of antibodies to various cell markers, then fixed with 2% paraformaldehyde (PFA) in FACS buffer. For intracellular levels of p-SHIP-1^{Y1022}, SHIP-1, p-SHP-1^{Y564}, SHP-1, PIP_3, and $PI(_{3,4})P_2$, cells were stained for cell markers, fixed in 3% PFA, permeabilized with 0.2% saponin in FACS buffer, then stained for intracellular antigens. All samples were stained with Live/Dead Fixable Viability Kit. Data are presented as mean fluorescence intensity (MFI) of sample/MFI of isotype antibody.

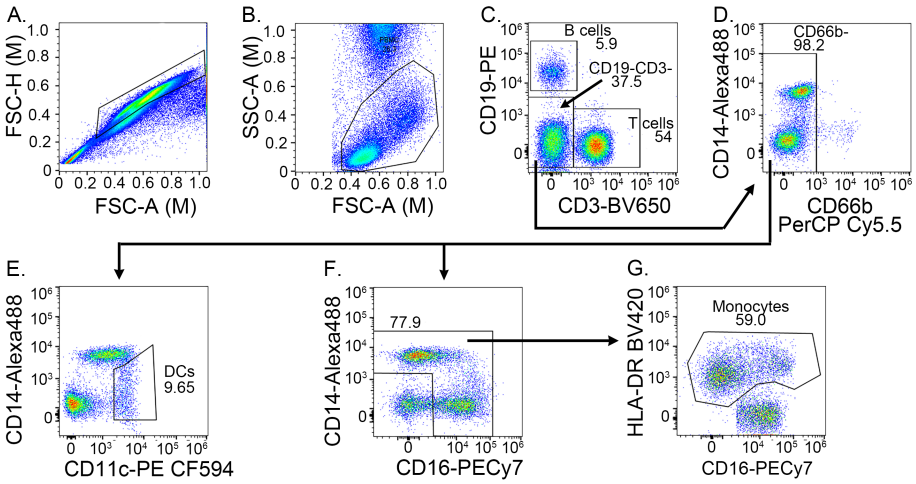
Confocal microscopy

BMMφs were treated with fluorochrome-labeled IgG-ICs (30 min, 37°C), then stained (10 min, 4°C) with cholera toxin B subunit conjugated with AF594 (CTx) to identify membrane lipid rafts. Cells were fixed in 2% PFA in PBS, then permeabilized with 0.2% saponin in FACS buffer. Cells were blocked with 5% rat serum in FACS buffer, then stained for intracellular p-SHIP-1^{Y1022} or p-SHP-1^{Y564}. Cells were resuspended in FluorSave and loaded onto coverslips for imaging. Images were acquired using Zeiss 710 confocal microscope with a 63x 1.4 N.A. (oil) PLAN APO lens and Zeiss Zen software. Cells were imaged from many fields randomly selected across the coverslip. Cell clumps or poorly stained cells were excluded. Images were processed using Image J. Colocalization of p-SHIP-1^{Y1022} or p-SHP-1^{Y564} with CTx positive lipid rafts were quantified using the Mander's coefficient of colocalization (colocalized pixels/total fluorescent pixels within region of interest).

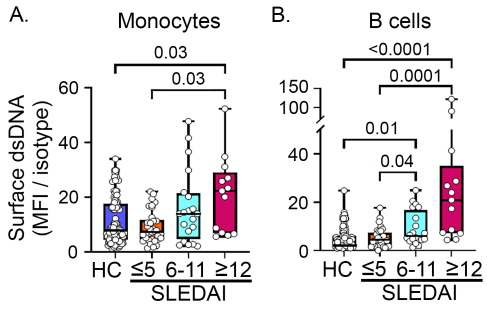
Supplemental Figures



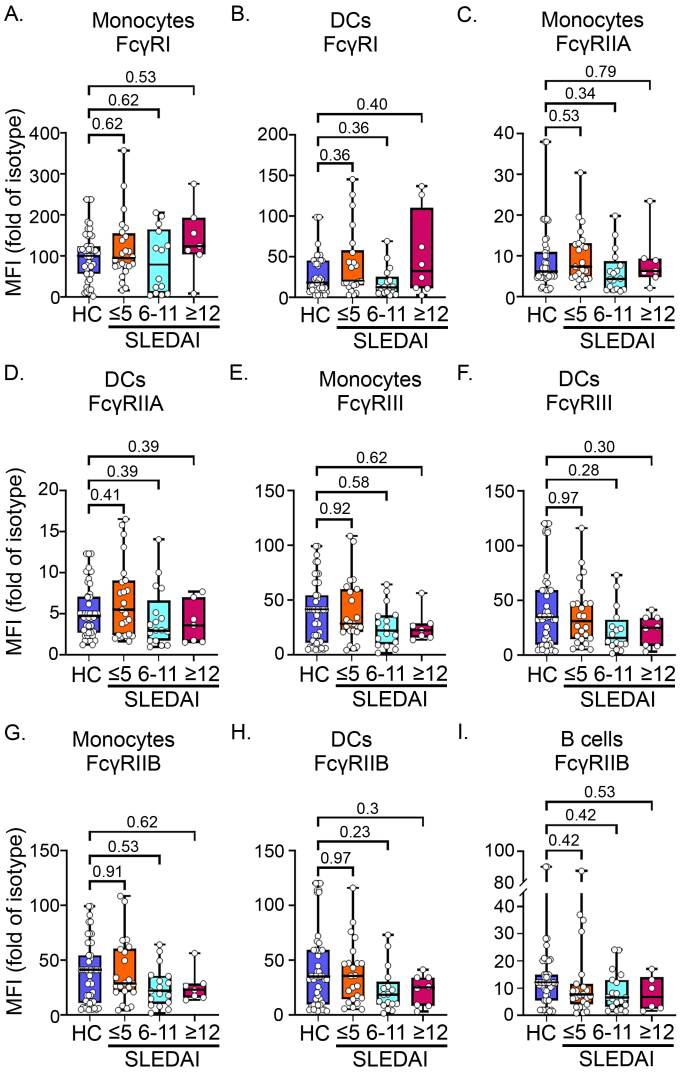
Supplemental Figure 1. T cells and neutrophils from MRL/*lpr* mice show diminished [H+] and elevated surface nucleosome. LEL [H+] (**A**, **B**) and the levels of surface nucleosome (**C**, **D**) were analyzed on splenic neutrophils ($CD45^+CD19^-CD3^-CD11b^+Ly6G^+$, **A, C**) and T cells ($CD45^+CD19^-CD3^+$, **B, D**). Splenocytes were incubated at 37°C in the presence (for neutrophils, **A**, **C**) or absence (for T cells, **B**, **D**) of IgG-ICs (30μl of IgG-ICs per $0.25x10^6$ cells) for 30min and LEL pH was measured by flow cytometry. Absolute pH was calculated using a standard curve, then converted to [H+] using pH= $-\log_{10}[H+]$ **(A, B)**. N = 5-7, 3 experiments. Statistical analysis used Mann-Whitney test. *p<0.05, **p<0.01, ***p<0.001. Bar = median.



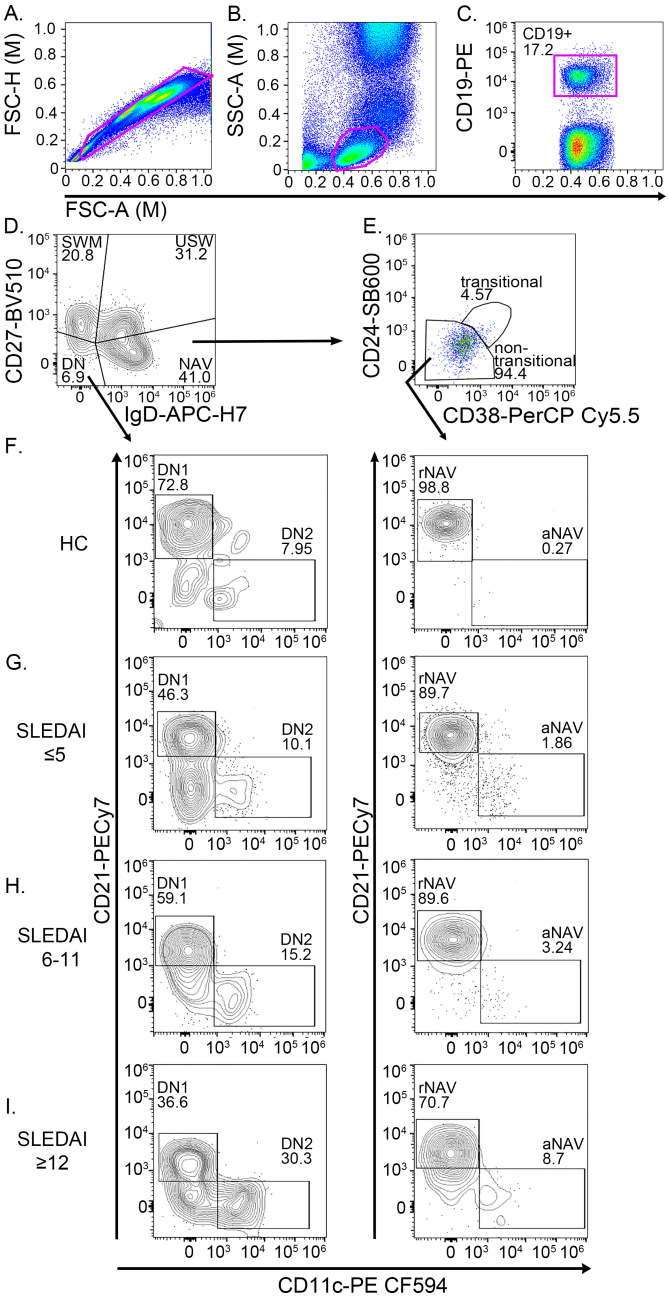
Supplemental Figure 2. Flow cytometry gating of human blood hematopoietic cells. Unfractionated blood cells were stained with antibodies specific for CD19, CD3, CD14, CD66b, CD11c, CD16, and HLA-DR. Singlets **(A),** were further gated for lymphocytes plus monocytes (lymph-mono gate) **(B)**, then subsequently gated for B and T cells using CD19 and CD3 **(C)**. $CD19^{neg}CD3^{neg}$ cells were further gated for CD66b to exclude residual granulocytes **(D)** that might have been inadvertently included in lymph-mono gate. $CD66b^{neg}$ cells were then gated for $CD14^{neg}CD11c^{pos}$ DCs **(E)**. $CD66b^{neg}$ cells were gated with CD14 and CD16 to exclude $CD16^{neg}CD14^{neg}$ cells **(F)**, and then further gated for $HLA\text{-}DR^{pos}$ to identify monocytes **(G)**. Dot plots were generated from representative HC sample using FlowJo. Plotted numbers depict frequency of cells from immediate parent gates. The gating strategy was used for Figures 3 and 4, and Supplemental Figures 3, 4, and 6.



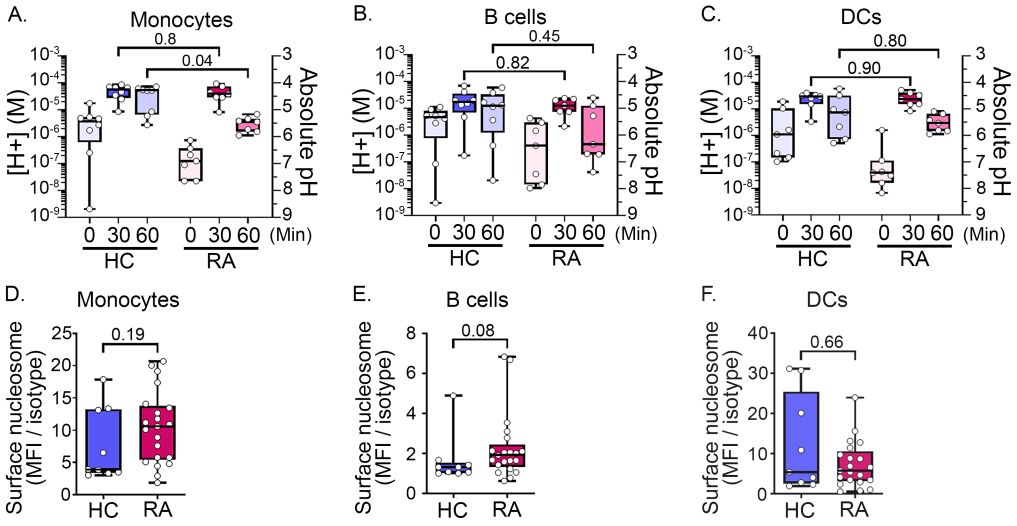
Supplemental Figure 3. Accumulation of nuclear self-antigen on the surface of hematopoietic cells is not unique to nucleosome. Unfractionated blood cells from HC (N= 101) or SLE patients (N=67) were stained with anti-dsDNA (33H11) or isotype control antibody, then analyzed by flow cytometry. N=13-101 patients per disease group, >10 experiments. Statistical analysis used Kruskal-Wallis. Adjusted *p* values with significance are shown. Bar = median. Box= 25th-75th percentiles. Whiskers= minimum and maximum values.



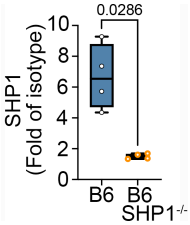
Supplemental Figure 4. Levels of surface FcγRs are not different in patients, regardless of SLEDAI group. (A-I). The levels of FcγRI, FcγRIIA, or FcγRIII on monocytes **(A, C, E)** and DCs **(B, D, F)** and FcγRIIb on monocytes **(G),** DCs **(H),** and B cells **(I)** were quantified by flow cytometry from unfractionated blood cells of HC or SLE patients. N = 6-48 patients per disease group, >6 experiments. Statistical analysis used Kruskal-Wallis. Adjusted *p* values are shown. Bar = median. Box= 25th-75th percentiles. Whiskers= minimum and maximum values.



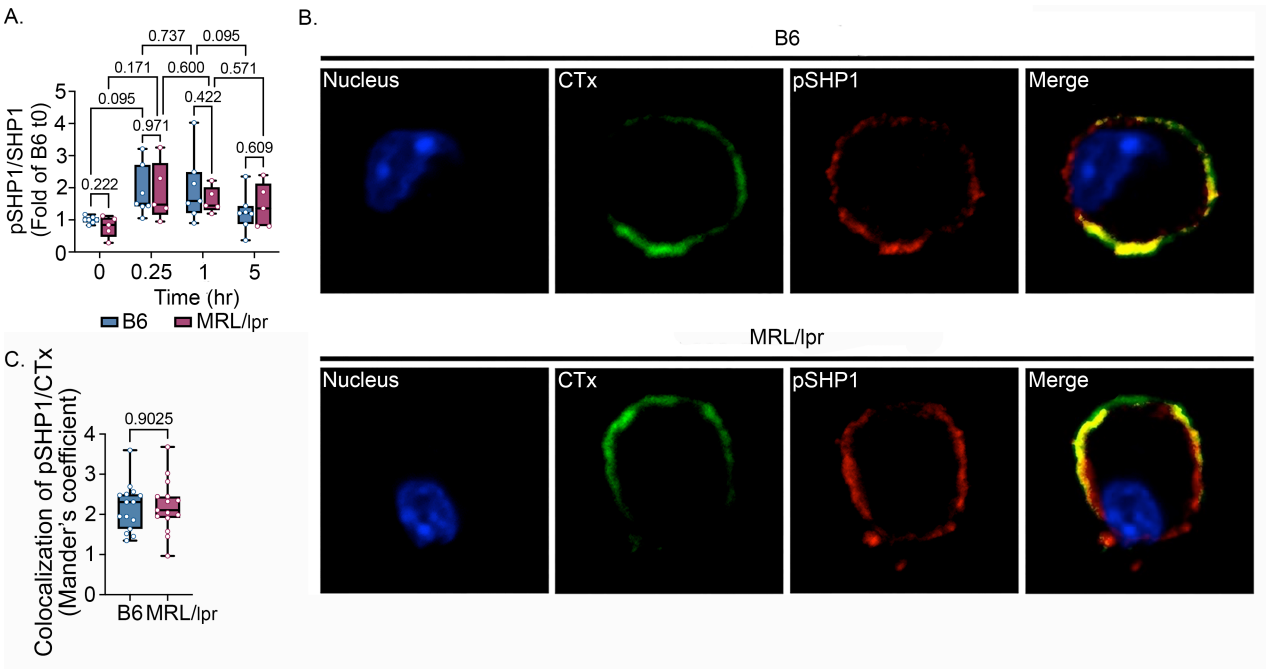
Supplemental Figure 5. Flow cytometry gating for B cell subsets. Unfractionated blood cells **(A)** were gated for lymphocytes **(B)**, then CD19+ B cells **(C)**. B cells were gated for switched memory (SWM), unswitched (USW), double-negative (DN), and naïve (NAV) cells using CD27 and IgD **(D)**. Naïve cells were sub-gated for transitional and non-transitional cells using CD24 and CD38 **(E)**. Non-transitional cells were further gated for resting naïve (rNAV) and activated naïve (aNAV) cells using CD21 and CD11c **(right panels in F-I)**. DN cells **(D)** were further gated for DN1 and DN2 cells using CD21 and CD11c **(left panels in F-I)**. Representative contour plots for DN1/DN2 and rNAV/aNAV cells from HC **(F)**, inactive **(G)**, moderately active **(H)**, and highly active **(I)** SLE are shown. Plots were generated with FlowJo. Gating strategy was used for Figure 4 **(D-G)**.



Supplemental Figure 6. Late endosome/lysosome (LEL) dysfunction is not evident in rheumatoid arthritis (RA) patients. LEL pH was measured in unfractionated blood cells from HC or active RA patients. Cells were stimulated with IgG-ICs (30 μl of IgG-ICs per $0.25x10^6$ cells), then pH was measured by flow cytometry at designated time points **(A-C)**. To inhibit vATPase activity in unstimulated samples (t_0), cells were treated with Concanamycin A (20 ng/ml). Absolute pH was calculated using a standard curve, then converted to [H+] (pH = $-\log_{10}$ [H+]). Levels of surface nucleosome were quantified by flow cytometry **(D-F)**. N= 8 HC, 7 RA patients, 6 experiments **(A-C)**, N= 9 HC, 21 RA patients, >8 experiments **(D-F)**. Statistical analysis used Kruskal-Wallis **(A-C)** and Mann-Whitney **(D-F)**. Adjusted *p* values with significance are shown. Bar = median. Box= 25^{th}-75^{th} percentiles. Whiskers= minimum and maximum values.



Supplemental Figure 7. Tamoxifen treatment markedly reduced SHP-1 in an inducible knockout model. B6.SHP-1$^{fl/fl}$ x B6. Rosa26-CreERT2 (B6.SHP-1$^{-/-}$) mice were treated with 2mg of tamoxifen in 100 ul of corn oil per mouse (i.p.) for 4 consecutive days. Bone marrow cells were collected at day 17 post injection and cultured for 7 days to derive BMMφs. SHP-1 levels in day 7 BMMφs from B6 and B6.SHP-1$^{-/-}$ were measured by flow cytometry. N = 4, 2 experiments. Statistical analysis used Mann-Whitney test. Adjusted *p* values with significance are shown. Bar = median. Box= 25th-75th percentiles. Whiskers= minimum and maximum values.



Supplemental Figure 8. B6 and MRL/*lpr* mice showed comparable levels of p-SHP-1^{Y564}, and localization of p-SHP-1 to lipid raft. BMMφs were treated with IgG-ICs (25μl of IgG-ICs per 0.25x10^6 cells) and at designated times, analyzed for intracellular p-SHP-1^{Y564} levels by flow cytometry **(A)**. Fold of B6 t_0 was graphed. N= 5-7, 4 separate experiments. The localization of p-SHP-1^{Y564} to plasma membrane lipid raft was assessed by confocal microscopy **(B)** and quantitated using Mander's coefficient **(C)**. BMMφs were treated with IgG-ICs (25μl of IgG-ICs per 0.25x10^6 cells) and stained for intracellular p-SHP-1^{Y564} (red), lipid raft (cholera toxin, CTx, green), and cell nucleus (blue) **(B)**. Images were processed using Image J and representative images are shown. Yellow in merged images depicts colocalized p-SHP-1^{Y564} and CTx. N=15 cells from 2 mice, 2 separate experiments. Statistical analysis used 2-way ANOVA with multiple comparisons **(A)** and Mann-Whitney test **(C)**. Adjusted *p* values with significance are shown. Bar = median. Box= 25th-75th percentiles. Whiskers= minimum and maximum values.

Supplemental Tables

Supplemental Table 1. Median LEL pH and [H+] of each cell type in B6 and MRL/*lpr* mice of different ages.

	B6 (pH[B])	MRL/lpr (pH)	B6 ([H+] (M)[C])	MRL/lpr ([H+] (M))	Fold reduction over B6 ([H+] (M))
CD11b+ myeloid cells[A]					
Age 9-10wks old	4.5	4.7	2.84E-05	1.91E-05	1.5
Age 12-13wks old	4.4	5.1	4.26E-05	7.18E-06	5.9
Age 15-16wks old	4.3	5.4	4.48E-05	3.56E-06	12.6
Age >18wks old	4.7	5.7	1.97E-05	2.04E-06	9.7
B cells					
Age 9-10wks old	5.2	5.4	6.65E-06	4.46E-06	1.5
Age 12-13wks old	5.1	5.4	8.49E-06	3.83E-06	2.2
Age 15-16wks old	5.4	5.8	3.83E-06	1.66E-06	2.3
Age >18wks old	5.4	5.7	4.20E-06	2.03E-06	2.1
DCs					
Age 9-10wks old	5.1	5	7.67E-06	1.03E-05	0.7
Age 12-13wks old	5	5.5	1.12E-05	3.39E-06	3.3
Age 15-16wks old	5.4	5.5	4.32E-06	3.10E-06	1.4
Age >18wks old	5.2	5.6	6.75E-06	2.24E-06	3

[A]CD11b+ myeloid cells ($CD19^{neg}CD3^{neg}CD11b^{pos}CD11c^{neg/lo}$). [B] pH is calculated using a standard curve from cells stimulated with IgG-ICs for 30 min. [C] [H+] (M) is derived from absolute pH values using $[H^+] = 10^{-pH}$.

Supplemental Table 2. Demographic and clinical characteristics of participating SLE patients.

	Overall
N	81
SLEDAI categories	
arthritis [N (%)]	19 (23)
rash [N (%)]	26 (32)
renal[A] [N (%)]	24 (32)
vasculitis [N (%)]	1 (1)
serositis[B] [N (%)]	2 (3)
alopecia [N (%)]	11 (13)
neuropsych[C] [N (%)]	0
ulcers [N (%)]	5 (6)
hematologic[D] [N (%)]	12 (15)
taking HCQ [N (%)]	65 (80)
taking steroids [N (%)]	33 (41)
taking DMARDs[E] [N (%)]	46 (62)
Demographics	
age (mean ± SD[F])	40.4 ± 14
Black [N (%)]	50 (65)
White [N (%)]	27 (33)
Female [N (%)]	72 (89)
Ethnicity	
Hispanic [N (%)]	7 (9)
SLE History	
mean length of disease (yr) ± SD)	11 ± 9.5
historical renal disease[G] [N (%)]	45 (56)

[A]Renal: any SLEDAI renal parameter including active SLEDAI hematuria, pyuria, urinary casts, or proteinuria due to SLE. [B]Serositis: positive for pleurisy or pericarditis. [C]Neuropsych: positive for seizure, psychosis, organic brain syndrome, visual disturbance, cranial nerve disorder, lupus headache, or cerebrovascular accident. [D]Hematologic: positive for SLEDAI leukopenia or, thrombocytopenia. [E]DMARDS include Mycophenolic acid, Mycophenolate mofetil, Azathioprine, Methotrexate, and Tacrolimus. [F]SD = standard deviation. [G]Historic renal disease: ever had manifestation of nephritis based on ACR, SLICC, ACR/EULAR or renal SLEDAI.

Supplemental Table 3. Median LEL pH and [H+] in blood hematopoietic cells from HCs and SLE patients.

	Mo	B cells	DCs
pH[A]			
HC	4.8	5.3	4.8
SLEDAI ≤ 5	4.8	5.4	4.7
SLEDAI 6-11	5.4	5.8	5.5
SLEDAI ≥12	5.6	5.9	5.7
[H+] (M)[B]			
HC	1.74E-05	5.62E-06	1.67E-05
SLEDAI ≤ 5	1.80E-05	3.65E-06	2.19E-05
SLEDAI 6-11	4.13E-06	1.76E-06	2.88E-06
SLEDAI ≥12	2.66E-06	1.22E-06	2.01E-06
[H+] (M) fold reduction/HC			
SLEDAI ≤ 5	1	1.5	0.8
SLEDAI 6-11	4.2	3.2	5.8
SLEDAI ≥12	6.5	4.6	8.3

[A]pH is calculated using a standard curve from cells stimulated with IgG-ICs for 30 min. [B][H+] (M) is derived from absolute pH values using $[H^+] = 10^{-pH}$.

Supplemental Table 4. Proportions of patients in each SLEDAI category showing non-acidic late endosomes/lysosomes (LELs), high surface nucleosomes, or high CIC levels for each cell type.

	arthritis	rash	renal[D]	alopecia	hematol[E]	HCQ	steroids	DMARDs[F]
N	19	26	24	11	12	33	46	12
Low [H+] (Non-acidic) [N(%)][A]								
Mo	13 (68)	19 (73)	22 (92)	7 (64)	9 (75)	23 (70)	32 (70)	9 (75)
B cells	12 (63)	19 (73)	22 (92)	8 (73)	9 (75)	24 (73)	34 (74)	9 (75)
DCs	12 (63)	17 (65)	20 (83)	8 (73)	9 (75)	23 (70)	30 (65)	9 (75)
High surface nucleosome [N(%)][B]								
Mo	1 (6)	6 (29)	5 (23)	1 (14)	3 (27)	6 (22)	7 (18)	3 (27)
B cells	2 (12)	8 (38)	11 (50)	3 (43)	2 (18)	10 (37)	14 (34)	2 (18)
DCs	1 (6)	3 (14)	4 (18)	2 (29)	3 (27)	5 (19)	9 (22)	3 (27)
High CIC [N(%)][C]								
CIC	2 (50)	9 (82)	11 (85)	3 (75)	4 (50)	12 (86)	16 (76)	4 (50)

[A]Patients with “non-acidic” LELs have [H+] lower than the cut-off. For each cell type, the non-acidic cutoff was established at 1.8-fold above the mean [H+] of the HC. [B]Patients with “high surface nucleosome” have cell surface nucleosome levels above the cut-off. For each cell type, the cut-off was established at 1.8-fold above the mean surface nucleosome level of the HC. [C]Patients with “high CIC” show plasma CIC levels higher than the cut-off. The cut-off was established at 1.5-fold above the mean CIC level of the HC. [D]Renal – any SLEDAI renal parameter including active SLEDAI hematuria, pyuria, urinary casts, or proteinuria due to SLE. [E]Hematologic - positive for SLEDAI leukopenia or, thrombocytopenia. [F]DMARDS include Mycophenolic acid, Mycophenolate mofetil, Azathioprine, Methotrexate, and Tacrolimus. SLEDAI categories of vasculitis, serositis, and ulcers were not included because of few patients (1 vasculitis, 2 serositis, 5 ulcers).

Supplemental Table 5. Demographic and clinical characteristics of all participants with SLE and those with non-acidic late endosomes/lysosomes (LELs) for each cell type.

	Overall	Non-acidic[H] Monocytes	Non-acidic B cells	Non-acidic DCs
N (%)	81	53 (65)	54 (67)	46 (57)
SLEDAI categories				
arthritis [N (%)]	19 (23)	13 (24)	12 (22)	12 (26)
rash [N (%)]	26 (32)	19 (36)	19 (35)	17 (37)
renal [N (%)][A]	24 (32)	22 (46)	22 (44)	20 (47)
vasculitis [N (%)]	1 (1)	1 (2)	1 (2)	1 (2)
serositis [N (%)][B]	2 (3)	2 (4)	2 (4)	1 (2)
alopecia [N (%)]	11 (13)	7 (13)	8 (15)	8 (17)
neuropsych [N (%)][C]	0	0	0	0
ulcers [N (%)]	5 (6)	2 (4)	2 (4)	3 (7)
hematologic [N (%)][D]	12 (15)	9 (17)	9 (17)	9 (20)
taking HCQ [N (%)]	65 (80)	42 (79)	45 (83)	36 (78)
taking steroids [N (%)]	33 (41)	23 (43)	24 (44)	23 (50)
taking DMARDs [N (%)][E]	46 (62)	32 (67)	34 (72)	30 (71)
Demographics				
age (mean ± SD[F])	40.4 ± 14	38.2 ± 13.5	38.1 ± 13.5	44.9 ± 13
Black [N (%)]	50 (65)	34 (64)	34 (68)	28 (67)
White [N (%)]	27 (33)	15 (31)	16 (32)	14 (33)
Female [N (%)]	72 (89)	47 (89)	47 (87)	39 (85)
Ethnicity				
Hispanic [N (%)]	7 (9)	6 (12)	7 (14)	5 (12)
SLE History				
mean length of disease (yr) ± SD)	11 ± 9.5	9 ± 7.3	10.9 ± 9	9.4 ± 7.9
historical renal disease [N (%)][G]	45 (56)	33 (62)	33 (61)	29 (63)

[A]Renal – any SLEDAI renal parameter including active SLEDAI hematuria, pyuria, urinary casts, or, proteinuria due to SLE. [B]Serositis - positive for pleurisy or pericarditis. [C]Neuropsych - positive for seizure, psychosis, organic brain syndrome, visual disturbance, cranial nerve disorder, lupus headache, or cerebrovascular accident. [D]Hematologic - positive for SLEDAI leukopenia or, thrombocytopenia. [E]DMARDS include Mycophenolic acid, Mycophenolate mofetil, Azathioprine, Methotrexate, and Tacrolimus. [F]SD = standard deviation. [G]Historic renal disease – ever had manifestation of nephritis based on ACR, SLICC, ACR/EULAR or renal SLEDAI. [H]Patients with "non-acidic" lysosomes have [H+] lower than the cut-off. For each cell type, the non-acidic cut-off was established at 1.8-fold above the mean [H+] of the HC.

Supplemental Table 6. Demographics of SLE patients analyzed for surface DNA.

	Overall	SLEDAI ≤5	SLEDAI 6-11	SLEDAI ≥12
N	69	35	21	13
Demographics				
Age (mean ± SD[A])	42 ± 13	43 ± 13	44 ± 14	34 ± 8
Black N (%)	34 (49)	21 (60)	5 (24)	8 (62)
White N (%)	24 (35)	11 (31)	9 (43)	4 (31)
Female N (%)	61 (88)	32 (92)	20 (95)	9 (69)
Ethnicity				
Hispanic N (%)	8 (11)	2 (6)	5 (24)	1 (8)

[A]SD = standard deviation. Missing data: race-1; ethnicity-4

Supplemental Table 7. Correlation between SLEDAI and molecular events associated with late endosome/lysosome (LEL) dysfunction.

	SLEDAI ≤5	SLEDAI 6-11	SLEDAI ≥12
LEL [H+] Monocytes			
N	44	24	13
[H+] mean (SD[A])	2.7E-5 (2.8E-5)	1.4E-5 (2.3E-5)	8.6E-6 (1.3E-5)
Spearman's p-value	0.84	0.69	0.56
Correlation coefficient[B]	-0.03	-0.09	-0.18
LEL [H+] B cells			
N	44	24	13
[H+] mean (SD)	9.6E-6 (1.0E-6)	7.1E-6 (1.0E-5)	1.5E-6 (1.0E-6)
Spearman's p-value	0.67	0.77	0.92
Correlation coefficient	-0.07	0.06	-0.03
LEL [H+] DCs			
N	44	24	13
[H+] mean (SD)	2.8E-5 (2.9E-5)	9.7E-6 (1.5E-5)	1.4E-5 (2.7E-5)
Spearman's p-value	0.85	0.87	0.69
Correlation coefficient	0.03	0.03	-0.12
LEL hydrolase activity Monocytes			
N	22	12	8
MFI - mean (SD)[C]	84.2 (67.7)	53.3 (39.8)	34.5(19.0)
Spearman's p-value	0.75	0.4	0.91
Correlation coefficient	0.07	0.27	-0.05
LEL hydrolase activity B cells			
N	20	11	8
MFI - mean (SD)	24.3 (29.7)	12.3 (9.3)	14.5 (11.8)
Spearman's p-value	0.06	0.49	0.71
Correlation coefficient	0.49	-0.23	-0.16
LEL hydrolase activity DCs			
N	22	10	8
MFI - mean (SD)	43.3 (43.5)	21.9 (14.2)	21.2 (15.2)
Spearman's p-value	0.54	0.7	0.98
Correlation coefficient	0.14	-0.14	-0.01
Surface nucleosome Monocytes			
N	37	24	10
MFI - mean (SD)	11.7 (10.2)	12.8 (11.1)	22.6 (15.8)
Spearman's p-value	0.6	0.74	0.19
Correlation coefficient	0.09	0.07	0.45
Surface nucleosome B cells			
N	38	24	10
MFI - mean (SD)	7.8 (11.1)	10.4 (10.4)	32.5 (23.4)
Spearman's p-value	0.55	0.26	**0.004**
Correlation coefficient	0.1	0.24	**0.81**
Surface nucleosome DCs			
N	38	24	10
MFI - mean (SD	8.3 (7.6)	8.8 (7.2)	12.4 (5.0)
Spearman's p-value	0.29	0.91	0.36
Correlation coefficient	-0.18	-0.02	0.32
Circulating immune complexes (CIC)			
N	41	24	11
mean (SD)	7.1 (9.7)	9.9 (9.4)	22.4 (20.3)
Spearman's p-value	0.68	0.42	0.68
Correlation coefficient	0.07	0.17	0.14

[A]SD = standard deviation. [B]Spearman's correlation coefficient and p-value. [C]MFI = mean fluorescence intensity

Supplemental Table 8. Demographics and clinical characteristics of SLE patients with high levels of CIC.

	Overall	high CIC[H]
N (%)	76	37 (49)
SLEDAI categories		
arthritis [N (%)]	18 (24)	9 (24)
rash [N (%)]	24 (32)	13 (35)
renal [N (%)][A]	22 (32)	12 (36)
vasculitis [N (%)]	1 (1)	1 (3)
serositis [N (%)][B]	1 (1)	1 (3)
alopecia [N (%)]	11 (15)	6 (16)
neuropsych [N (%)][C]	0	0
ulcers [N (%)]	5 (7)	3 (8)
hematologic [N (%)][D]	11 (15)	6 (16)
plaquenil [N (%)]	60 (79)	30 (81)
steroids [N (%)]	30 (40)	16 (43)
DMARDs [N (%)][E]	43 (61)	20 (61)
Demographics		
Age (mean ± SD[F])	41 ± 14	37 ± 13
Black [N (%)]	46 (64)	22 (65)
White [N (%)]	26 (36)	12 (35)
Female [N (%)]	68 (90)	32 (87)
Ethnicity		
Hispanic [N (%)]	7 (10)	7 (20)
SLE History		
duration of disease in years (mean ± SD)	10.5 ± 8.7	7.8 ± 4.9
historical renal disease [N (%)][G]	44 (58)	23 (62)

[A]Renal –any SLEDAI renal parameter including active SLEDAI hematuria, pyuria, urinary casts, or proteinuria due to SLE. [B]Serositis - positive for pleurisy or pericarditis. [C]Seuropsych - positive for seizure, psychosis, organic brain syndrome, visual disturbance, cranial nerve disorder, lupus headache, or cerebrovascular accident. [D]Hematologic - positive for SLEDAI leukopenia or, thrombocytopenia. [E]DMARDS include Mycophenolic acid, Mycophenolate mofetil, Azathioprine, Methotrexate, and Tacrolimus. [F]SD = standard deviation. [G]Historic renal disease – ever had manifestation of nephritis based on ACR, SLICC, ACR/EULAR criteria or renal SELENA-SLEDAI. [H]Patients with “high CIC” show plasma CIC levels higher than the cut-off. The cut-off was established at 1.5-fold above the mean CIC level of the HC.

Supplemental Table 9. Demographics of rheumatoid arthritis patients.

	Overall
N	23
Demographics	
Age (mean ± SD[A])	53 ± 10
Black N (%)	7 (30)
White N (%)	13 (57)
Other[B]	3 (13)
Female N (%)	18 (78)
Ethnicity	
Hispanic N (%)	3 (13)

[A]SD = standard deviation. [B]Other: Asian, Pacific Islander and not identified.

Supplemental Table 10. Proportion of patients in each renal disease activity group showing low [H+] in late endosomes/lysosomes (LELs) or high surface nucleosome.

	Mo with non-acidic LEL[A]	B cells with non-acidic LEL	DCs with non-acidic LEL	Mo with high surface nucleosome[B]	B cells with high surface nucleosome	DCs with high surface nucleosome
Active Nephritis						
N total	25	25	25	23	23)	23
N positive (%)	23 (92)	22 (88)	20 (80)	5 (22)	11(48)	4 (17)
Remission Nephritis						
N total	20	20	20	15	15	15
N positive (%)	10 (50)	11 (55)	9 (45)	3 (20)	4 (27)	2 (13)
Never Nephritis						
N total	36	36	36	33	33	33
N positive (%)	20 (54)	21 (57)	18 (49)	3 (9)	3 (9)	6 (18)

[A]Patients with “non-acidic” LELs have [H+] lower than the cut-off. For each cell type, the cut-off was established at 1.8-fold above the mean [H+] of the HC. [B]Patients with “high surface nucleosome” have cell surface nucleosome levels above the cut-off. For each cell type, the cut-off was established at 1.8-fold above the mean surface nucleosome level of the HC.