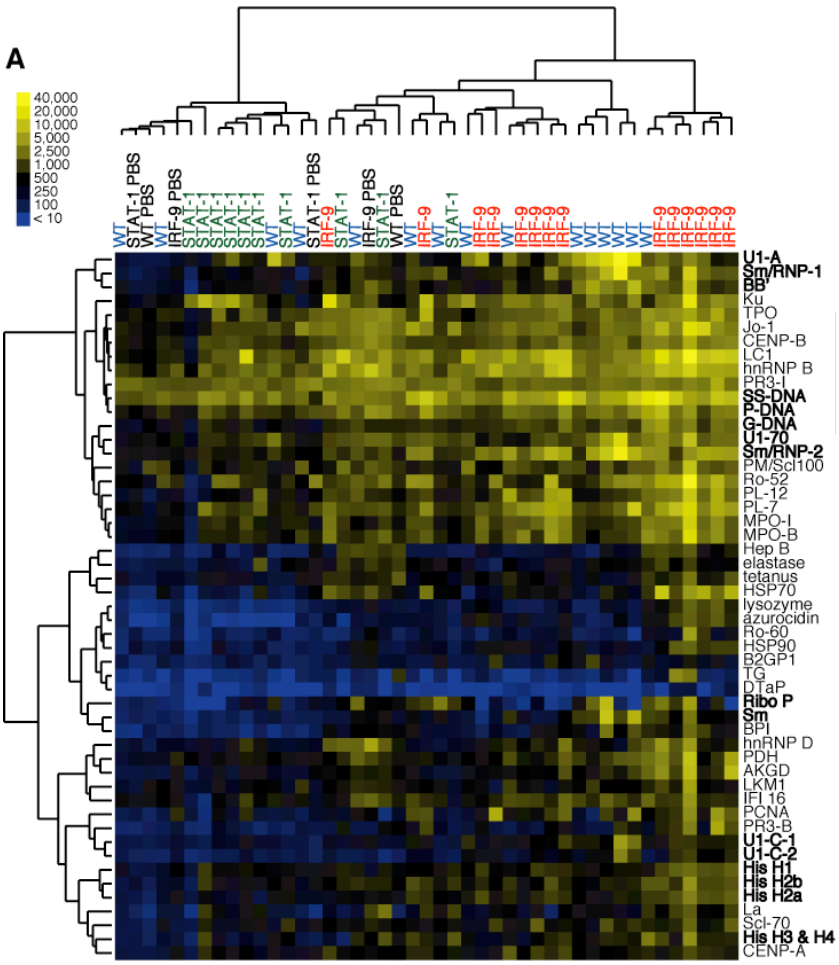
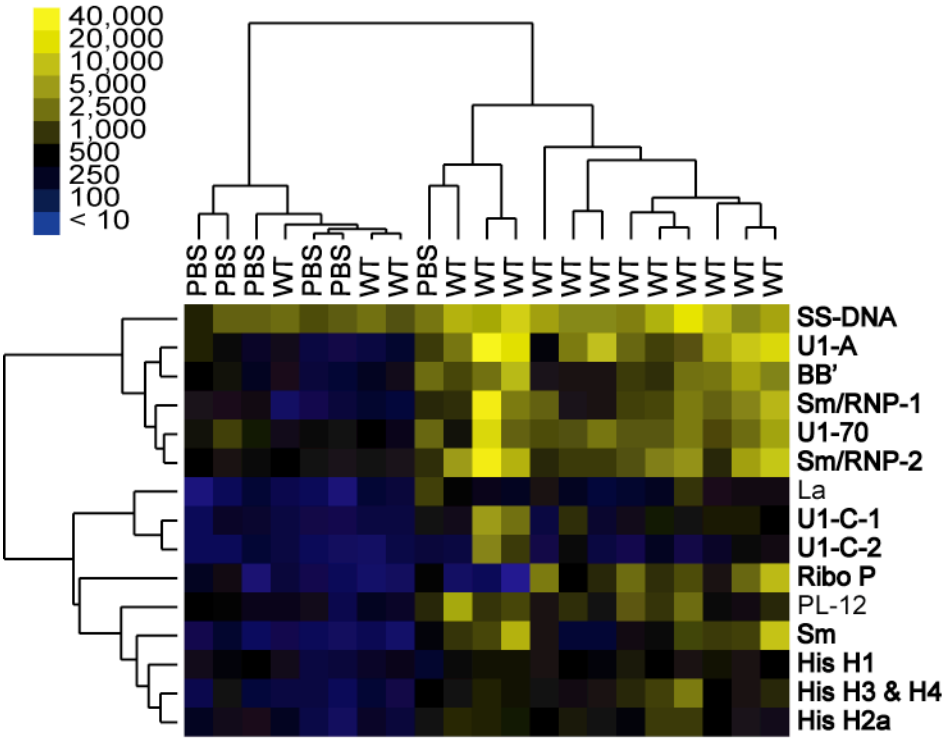
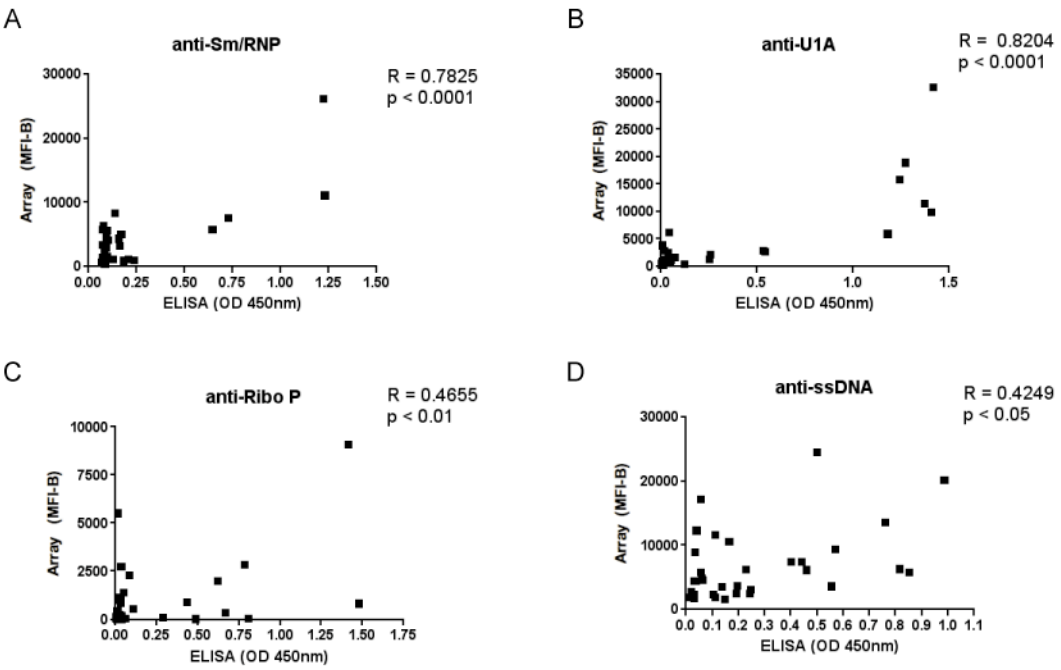
SUPPLEMENTAL DATA



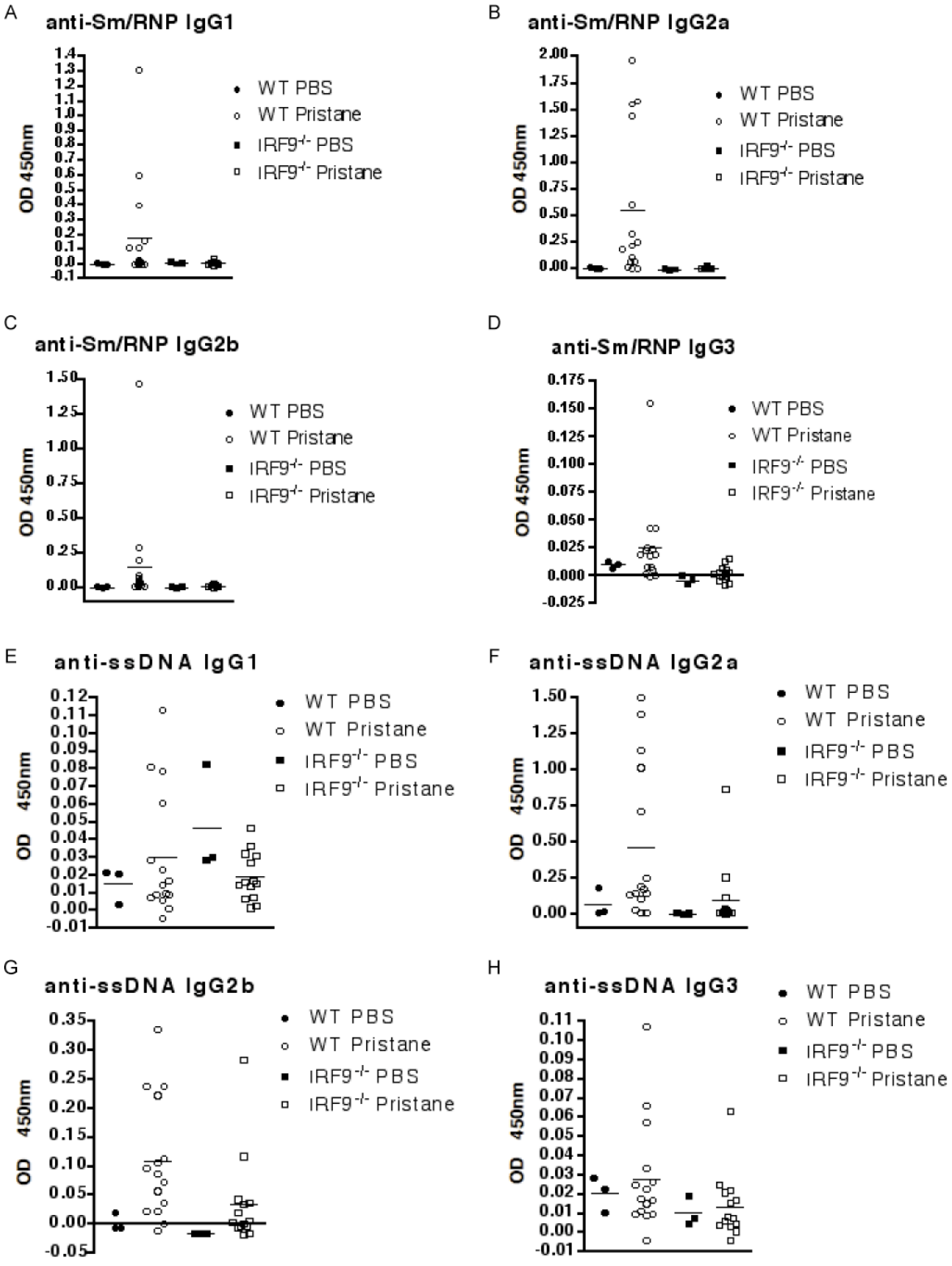
Supplemental Figure 1. Autoantibody profiling in pristane-treated mice. Individual arrays were incubated with 1:300 dilutions of serum obtained from WT, IRF9$^{-/-}$, and STAT1$^{-/-}$ mice 6 months after treatment with pristane (WT, IRF9, STAT1) or PBS (WT PBS, IRF9 PBS, STAT1 PBS). A hierarchical clustering algorithm using uncentered correlation similarity metric and complete linkage method was applied to order mice into cluster relationships based on similarity in array reactivity (top dendogram), and to order antigen features into cluster relationships based on similarities in reactivities in the mice studied (left dendogram). Results of cluster analysis are depicted as a heatmap and dendogram generated using Java Treeview software.



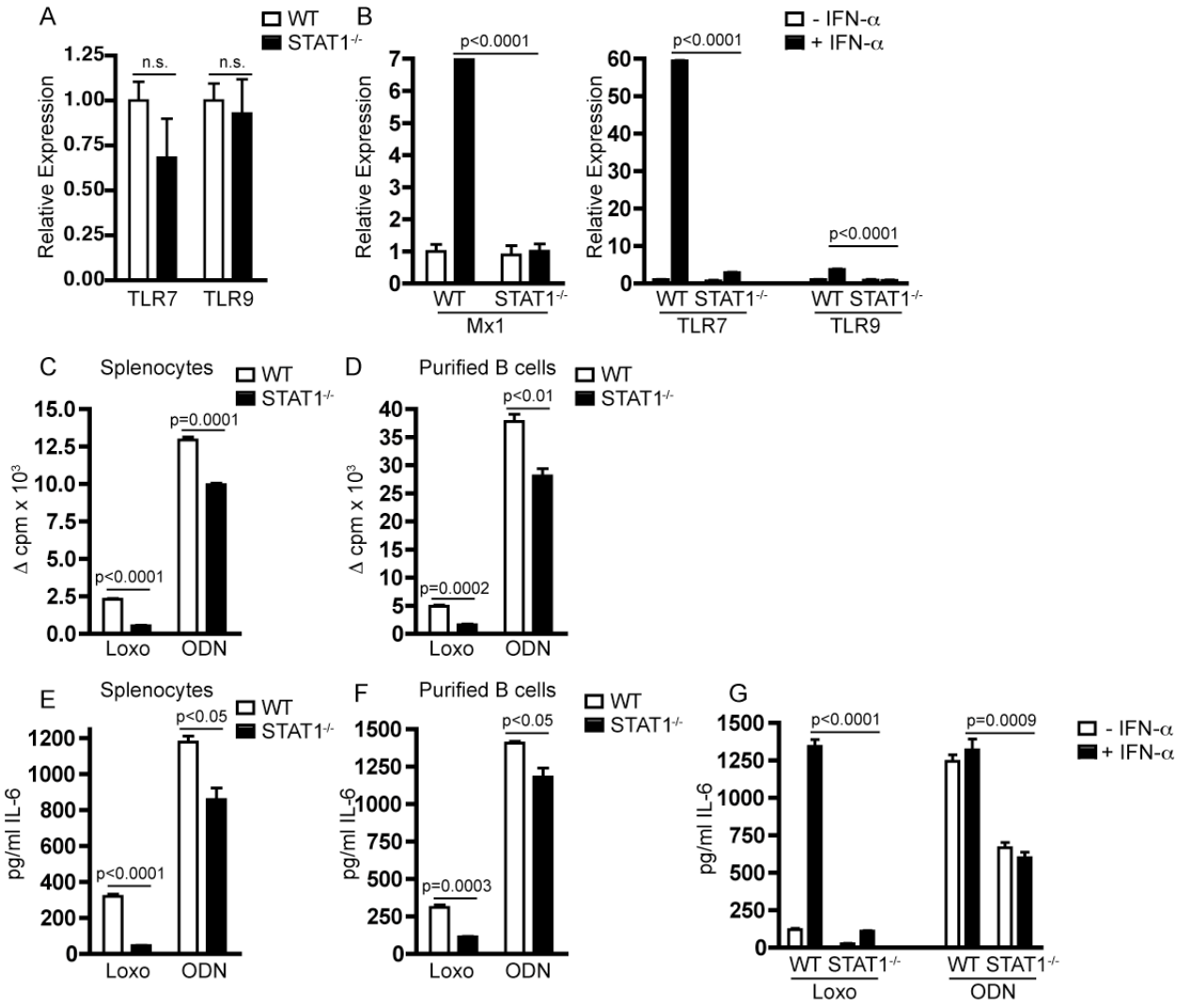
Supplemental Figure 2. Autoantigen Microarrays Identify Known Pristane Autoantigens. Pairwise significance analysis of microarrays (SAM) was used to determine antigen features with statistically significant differences in array reactivity between pristane-treated and PBS-treated WT mice. Hierarchical clustering of SAM-identified antigens (FDR < 0.05, fold change > 3) was displayed as a heatmap and dendogram.



Supplemental Figure 3. Correlation Plots for ELISA values versus Array values. Correlation plots were constructed for Sm/RNP (A), U1A (B) RiboP (C) and ssDNA (D) by plotting ELISA values (OD 450 nm) on the x axis and Array values (median fluorescence intensity minus background (MFI-B)) on the y axis. Pearson correlation values (R) and p values are displayed on each graph.

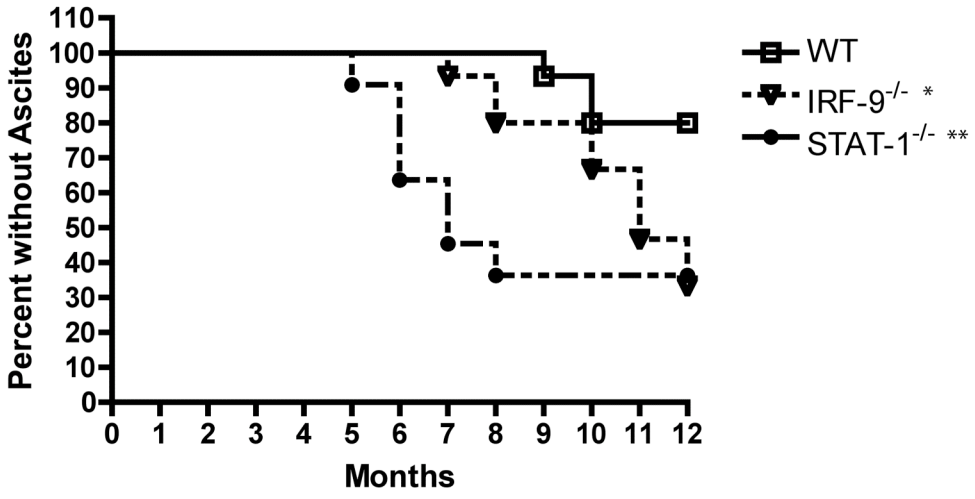

A
anti-Sm/RNP IgG1
OD 450nm
WT PBS
WT Pristane
IRF9-/- PBS
IRF9-/- Pristane
B
anti-Sm/RNP IgG2a
C
anti-Sm/RNP IgG2b
D
anti-Sm/RNP IgG3
E
anti-ssDNA IgG1
F
anti-ssDNA IgG2a
G
anti-ssDNA IgG2b
H
anti-ssDNA IgG3

Supplemental Figure 4. IgG Isotype-Specific Autoantibody Production. Sera obtained from WT and IRF9$^{-/-}$ mice 6 months after treatment with PBS or pristane were analyzed for levels of IgG isotype specific anti-Sm/RNP (A-D) or anti-ssDNA (E-H) autoantibodies by ELISA. Data are plotted as absorbance values for individual mice minus background.

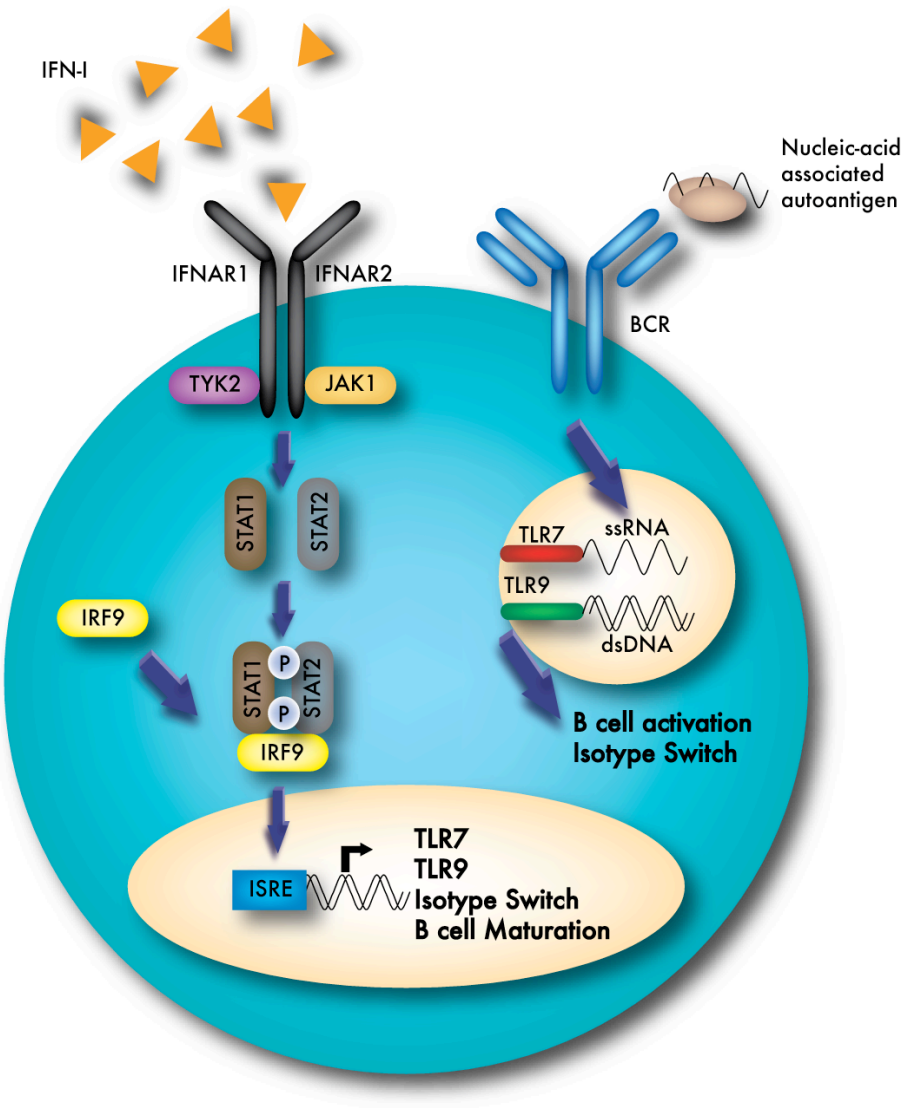


Supplemental Figure 5. STAT1 is required for the expression of, and activation through, TLRs in B cells. (A) B cells were purified from WT or STAT1$^{-/-}$ mice using magnetic beads. RNA was extracted and the relative expression of TLR7 and TLR9 normalized to GAPDH was measured by real-time quantitative PCR using the $2^{-\Delta\Delta Ct}$ method. (B) Purified B cells were cultured in the presence or absence of 1000 IU/ml IFN-α for 4 hours. RNA was extracted and the relative expression of Mx1, TLR7, and TLR9 normalized to GAPDH was measured as in (A). (C-D) Whole splenocytes (C) or splenic B cells purified using magnetic beads (D) were cultured with 1mM Loxoribine (Loxo) or 1 μM ODN1826 (ODN). Cells were pulsed with [^{3}H]TdR at 16

hours, harvested at 24 hours, and incorporated radioactivity was measured. Data are represented as the difference in mean cpm of stimulated and unstimulated triplicate wells (Δ cpm) + SEM. (E-F) Whole splenocytes (E) or purified B cells (F) were cultured as above. Cells were harvested at 24 hours following stimulation, and the concentration of IL-6 in the supernatant was measured by ELISA. (G) Purified B cells were pretreated with 10,000 IU/ml IFN-α for 24 hours before treatment with Loxoribine or ODN1826. The concentration of IL-6 in the supernatant was measured by ELISA after 24 hours in culture with TLR ligands. p values were determined using the student's t test and are displayed above each graph.



Supplemental Figure 6. Pristane induces plasmacytoma formation in IRF9$^{-/-}$ and STAT1$^{-/-}$ mice. The percentage of mice that did not develop ascites following pristane immunization in each group of mice was plotted according to the method of Kaplan and Meier and compared by the log-rank test. * $p < 0.05$ IRF9$^{-/-}$ mice versus WT. ** $p < 0.01$ STAT1$^{-/-}$ mice versus WT.



Supplemental Figure 7. Role of IFN-I in B cell activation by nucleic acid-associated autoantigens. IFN-I binds to the IFNAR, resulting in activation of ISGF3, which is composed of STAT1, STAT2, and IRF9. ISGF3 translocates to the nucleus, and induces the transcription of IFN-I inducible genes, including TLR7 and TLR9. Following upregulation of TLRs, an autoreactive B cell may become activated by a nucleic acid-associated autoantigen through TLR7 or TLR9. IFN-I signaling also promotes B cell maturation to plasma cells and isotype switching to IgG, resulting in the production of SLE-associated IgG autoantibodies.

Supplemental Table 1

	glomerular sclerosis	tubular dilation	perivascular lymphocytic infiltration	NIH acute scores: cellular crescents	hyaline deposits	leukocyte exudates	interstitial inflammation	necrosis/ karyorrhexis	proliferation	sum	Activity Score (sum div by 6)	avg	sd	n	NIH chronic scores: glomerulo sclerosis	tubular atrophy	Interstitial fibrosis	fibrous crescents	sum	Notes
BALB/c PBS	0	0	0	0	0	0	0	0	0	0	0	0	0	6	0	0	0	0	0	
BALB/c PBS	0	0	0	0	0	0	0	0	0	0	0				0	0	0	0	0	Mild mesangial deposits
BALB/c PBS	0	0	0	0	0	0	0	0	0	0	0				0	0	0	0	0	
BALB/c PBS	0	0	0	0	0	0	0	0	0	0	0				0	0	0	0	0	
BALB/c PBS	0	0	0	0	0	0	0	0	0	0	0				0	0	0	0	0	
BALB/c PBS	0	0	0	0	0	0	0	0	0	0	0				0	0	0	0	0	
BALB/c Pri	0	0	0	0	0	0	0	0	0	0	0	0.2	0.4	28	0	0	0	0	0	
BALB/c Pri	0	0	0	0	1	0	0	2	1	4	0.666666667				0	0	0	0	0	
BALB/c Pri	0	0	0	0	0	0	0	0	0	0	0				0	0	0	0	0	
BALB/c Pri	0	1	1	2	2	0	0	4	3	11	1.833333333				0	0	0	0	0	
WT Pris	0	0	0	0	0	0	0	0	0	0	0				0	0	0	0	0	
WT Pris	0	0	0	0	1	0	0	0	1	2	0.333333333				0	0	0	0	0	
WT Pris	0	0	0	0	0	0	0	0	0	0	0				0	0	0	0	0	
WT Pris	0	0	0	0	0	0	0	0	1	1	0.166666667				0	0	0	0	0	
WT Pris	0	0	0	0	0	0	0	0	1	1	0.166666667	1.4	2.7	27	0	0	0	0	0	
WT Pris	0	0	0	0	1	0	0	0	1	2	0.333333333				0	0	0	0	0	
WT Pris	0	0	0	0	0	0	0	0	2	2	0.333333333				0	0	0	0	0	
WT Pris	0	0	0	0	0	0	0	0	0	0	0				0	0	0	0	0	
WT Pris	0	0	1	0	0	0	0	0	1	1	0.166666667				0	0	0	0	0	
WT Pris	1	0	1	2	1	0	1	2	3	9	1.5				1	1	1	0	3	
WT Pris	0	0	0	0	0	0	0	0	0	0	0				0	0	0	0	0	
WT Pris	0	0	1	0	0	0	0	0	1	1	0.166666667				0	0	0	0	0	
WT Pris	0	0	0	0	0	0	0	0	0	0	0				0	0	0	0	0	
WT Pris	0	0	0	0	0	0	0	0	0	0	0				0	0	0	0	0	
WT Pris	0	0	0	0	0	0	0	0	0	0	0				0	0	0	0	0	
WT Pris	0	0	0	0	0	0	0	0	0	0	0				0	0	0	0	0	
WT Pris	0	0	0	0	0	0	0	0	0	0	0				0	0	0	0	0	
WT Pris	0	0	0	0	0	0	0	0	1	1	0.166666667				0	0	0	0	0	
WT Pris	0	0	0	0	0	0	0	0	2	2	0.333333333				0	0	0	0	0	
WT Pris	High	grade	sarcoma								0								0	
WT Pris	0	0	0	0	0	0	0	0	2	2	0.333333333				0	0	0	0	0	
WT Pris	0	0	0	0	0	0	0	0	0	0	0				0	0	0	0	0	
WT Pris	0	0	0	0	0	0	0	0	0	0	0				0	0	0	0	0	
WT Pris	0	0	0	0	0	0	0	0	0	0	0				0	0	0	0	0	
IRF9 PBS	0	0	0	0	0	0	0	0	0	0	0	0.1	0.2	5	0	0	0	0	0	
IRF9 PBS	0	0	0	0	1	0	0	0	0	1	0.166666667				0	0	0	0	0	
IRF9 PBS	0	0	0	0	0	0	0	0	0	0	0				0	0	0	0	0	
IRF9 PBS	0	0	0	0	3	0	0	0	0	3	0.5				0	0	0	0	0	Strange glomerulopathy with intracapillary accumulations
IRF9 PBS	0	0	0	0	0	0	0	0	0	0	0				0	0	0	0	0	
IRF9 Pris	0	0	0	0	0	0	0	0	0	0	0	0	0	6	0	0	0	0	0	
IRF9 Pris	0	0	0	0	0	0	0	0	0	0	0				0	0	0	0	0	
IRF9 Pris	0	0	0	0	0	0	0	0	0	0	0				0	0	0	0	0	
IRF9 Pris	0	0	0	0	0	0	0	0	0	0	0				0	0	0	0	0	
IRF9 Pris	0	0	0	0	0	0	0	0	0	0	0				0	0	0	0	0	
IRF9 Pris	0	0	0	0	0	0	0	0	0	0	0				0	0	0	0	0	
STAT1 PBS	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	
STAT1 PBS	0	0	0	0	0	0	0	0	0	0	0				0	0	0	0	0	
STAT1 PBS	0	0	0	0	0	0	0	0	0	0	0				0	0	0	0	0	
STAT1 Pris	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0	0	0	
STAT1 Pris	0	0	0	0	0	0	0	0	0	0	0				0	0	0	0	0	favor involvement by leukemia/lymphoma
STAT1 Pris	0	0	0	0	0	0	0	0	0	0	0				0	0	0	0	0	
STAT1 Pris	0	0	0	0	0	0	0	0	0	0	0				0	0	0	0	0	Coverslip coming off/section on bottom
STAT1 Pris	0	0	0	0	0	0	0	0	0	0	0				0	0	0	0	0	