

Supplemental File

Supplementary Figure Legends

Supplementary Figure 1. Calculation of Xist RNA localization scores. Xist RNA localization patterns in ≥ 100 lymphocyte nuclei per biological replicate were quantified as Types 1, 2, 3, or 4, reflecting Xist RNA transcripts that are completely dispersed from or tightly localized to the inactive X chromosome (Xi), respectively. Each “Type” pattern is depicted adjacent to the chart. The calculated Xist RNA localization score is a continuous variable spanning from 1 to 4, where 1.0 indicates that 100% of all nuclei have a Type 1 pattern where Xist RNA is completely dispersed or absent, and a score of 4.0 indicates that 100% of all nuclei show a Type 4 pattern where Xist RNA is tightly localized to the Xi, as described previously (referenced in the main text).

Supplementary Figure 2. Quantitation of serum anti-dsDNA autoantibody concentrations from MRL/lpr and NZM2328 mice. A. Median [interquartile range] serum anti-dsDNA concentration of female MRL/lpr mice stratified by mouse age: 8-10 weeks (n=6), 18-22 weeks (n=9), ≥ 23 weeks (n=9). Concentration values are expressed relative to the standard curve on an arbitrary scale. Serum from an aged female NZB/W F1 mouse was used as a positive control. Serum from an mb1cre⁺ homozygous female mouse (B6 background) that lacks B cells was used as a negative control. All samples were evaluated on a single plate. B. Median [interquartile range] serum anti-dsDNA concentration of female NZM2328 mice stratified by mouse age: 8-10 weeks (n=6), 18-22 weeks (n=5), ≥ 23 weeks (n=11). Concentration values are expressed relative to the standard curve on an arbitrary scale. The concentration value from n=1 mouse ≥ 23 weeks of age exceeded the maximum value of the standard curve. Serum from another aged female NZB/W F1 mouse was used as a positive control. Serum from another mb1cre⁺ homozygous female mouse (B6 background) which lacks B cells was used as a negative control. All samples

were evaluated on a single plate. * = $p < 0.05$; ** = $p < 0.01$ by a nonparametric ANOVA amongst the three age groups, with adjustment for multiple comparisons testing using Dunn's multiple comparisons test.

Supplementary Figure 3. A. Linear regression of mouse age (weeks) versus Xist RNA localization score for non-activated (open) and activated (filled) CD23+ cells from each female B6 (green), MRL/lpr (orange), and NZM2328 (red) mouse, shown with the 95% confidence interval. B. Linear regression of mouse age (weeks) versus the percentage of nuclei with H3K27me3 foci for non-activated (open) and activated (filled) CD23+ cells from each female B6 (green), MRL/lpr (orange), and NZM2328 (red) mouse, shown with the 95% confidence interval. Statistical testing indicates the probability of a non-zero slope for each linear model by pure chance.

Supplementary Figure 4. List of differentially upregulated and differentially downregulated X-linked genes between *in vitro* activated T cells from female NZM2328 mice and those from both female B6 and male NZM2328 mice. Genes in bold have immune functions.