Supplemental information

Unbiased modifier screen reveals that signal strength determines the regulatory role murine TLR9 plays in autoantibody production

Robyn E. Mills, Viola C. Lam, Allison Tan, Nicole Cresalia, Nir Oksenberg, Julie Zikherman, Mark Anderson, Arthur Weiss, and Michelle L. Hermiston

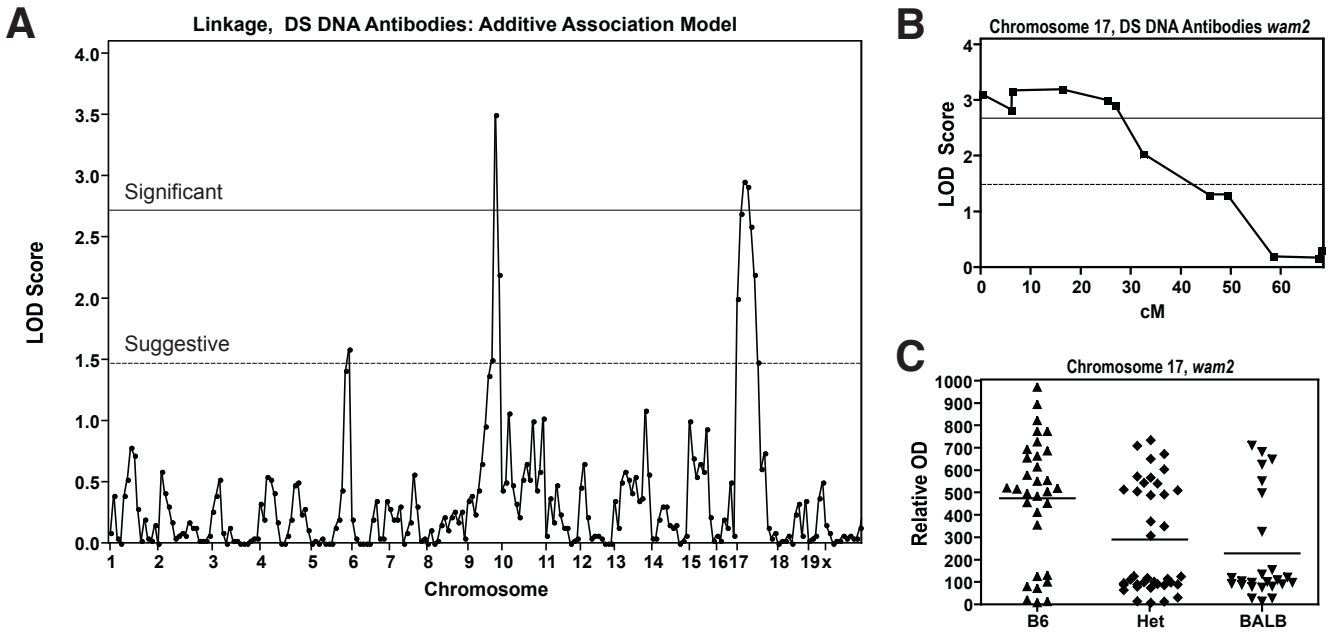

Figure S1. Additive QTL analysis of (B6 x BALB/c) CD45E613R F2 mice and susceptibility loci for ANA.
(A) Additive linkage analysis for anti-dsDNA IgG was performed on 94 F2 (B6 x BALB/c) CD45E613R mice. The solid line indicates the threshold for a significant LOD score and the dashed line indicates the threshold for a suggestive LOD score as based on permutation testing (1000 permutations) for each model. (B) Interval mapping of locus *Wam2* on chromosome 17. (C) Anti-dsDNA IgG relative OD of cohort segregated by *Wam2* genotype.

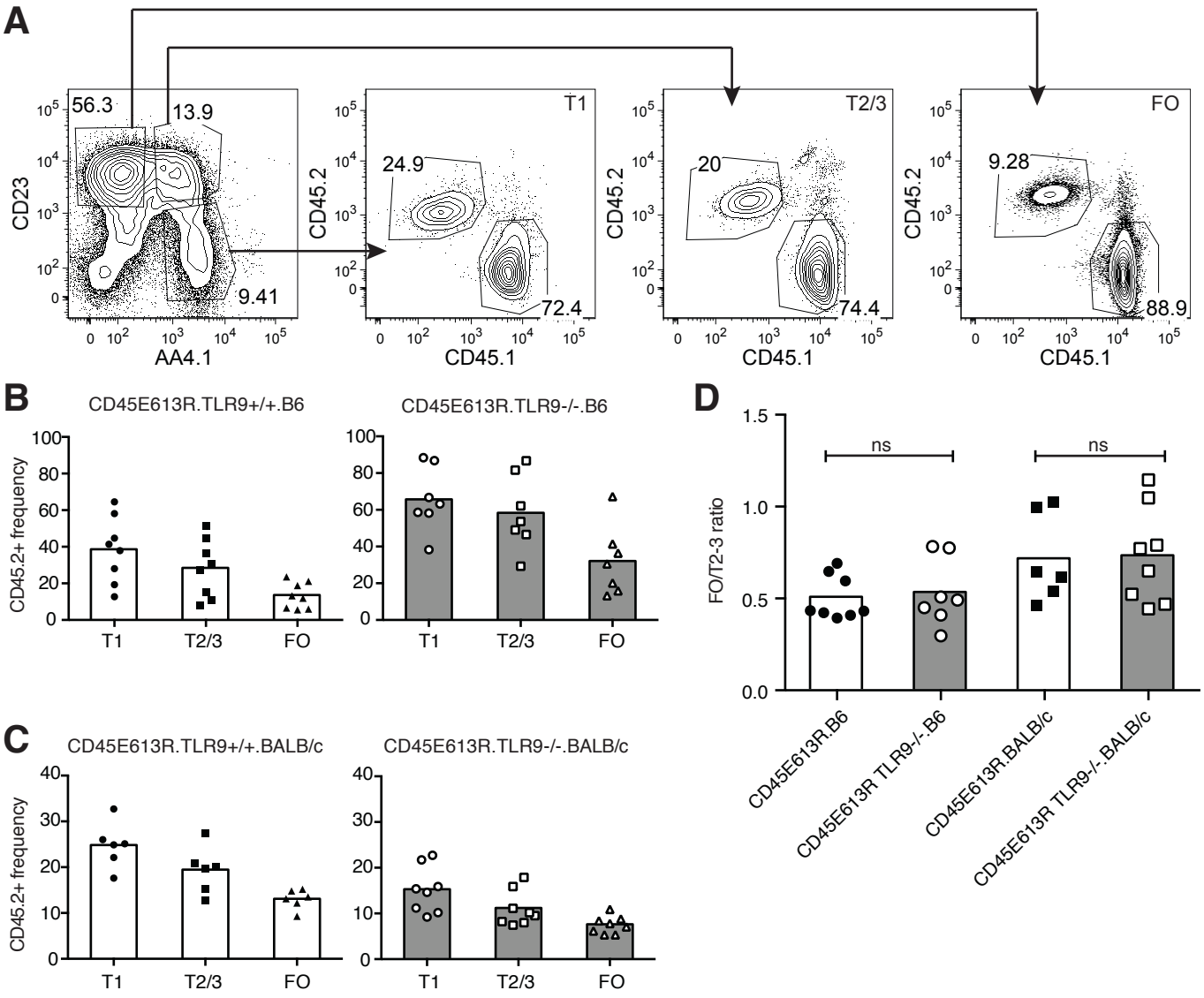

Figure S2. TLR9-independent negative regulation of CD45E613R B cell development in a competitive splenic microenvironment. Bone marrow chimeras were generated as described in Fig 7. (A) The CD45E613R proportion of developing B cell subsets in spleen was determined by CD45.2+ frequency of cells in the indicated compartments as identified by IgM/IgD staining of cells in the CD19+ gate in the spleen according to gating strategy shown. (B) CD45.2+ frequency of the indicated B cell compartment in spleens of chimeras of the indicated genotypes on the B6 background. (C) CD45.2+ frequency of indicated B cell compartment in spleen of chimeras of the indicated genotypes on the BALB/c background. (D) CD45.2+ frequency of follicular (FO) compartment was divided by CD45.2+ frequency of the T2/3 compartment for each chimera and plotted as ratio. Data in (B) are compiled from three independent experiments, and in (C) from three independent experiments.

Supplemental Table 1*
Genes of Immunologic Interest in *Wam1* on Chromosome 9

Gene	Location (bp)	KO phenotype**
Bcl12a1a	88754739	Abnormal neutrophil morphology/development, increased apoptosis
Plscr1 (phospholipid scramblase 1)	92054019	Decreased neutrophils, GCSF responsiveness, altered myeloid maturation
Chst2 (carbohydrate sulfotransferase 2)	95213460	Decreased lymphocyte homing
Rbp1 (retinol binding protein 1)	98232313	Marked expansion neutrophils
Pik3cb (PI3 kinase β)	98847754	Increased TNFα response to LPS
Rab6b	102970101	Ras family member; numerous immunologic abnormalities
Nphp3	103860949	Focal interstitial inflammatory infiltrates kidneys; anemia
TLR9	106080699	Decreased NK cells and total B cells; increased B1 cells; abnormal macrophages hyporesponsive to CpG; altered sensitivity to infection; altered cytokine production
Mapkapk3	107113028	Decreased TNF cytokine production with LPS stimulation
Cacna2d	107257713	Voltage dependent calcium channel subunit; neurologic abnormalities; altered thymic output
Rassf1	107409698	Ras family protein; propensity to lymphomas
Gnai2	107472231	GTP binding protein; Colitis, increased cytokines, increased neutrophils, IgG, IgM, background dependent phenotype
Mst1r (Friend virus susceptibility 2)	107764990	Also called c-met related tyrosine kinase and macrophage stimulating R1; *Known polymorphism:* Sensitive allele in BALB/c, resistant allele in B6. Hyperactive macrophages; increased acute inflammation.
Gpx1 (glutathione peroxidase 1)	108197368	Increased inflammatory response, decreased body wt, abnormal platelets, colitis
Lamb2 (laminin β2)	108338048	Postnatal lethal, glomerularnephritis
Impdh2	108418594	Embryonic lethal; impaired T cell dev, proliferation, and cytotoxic T lymphocyte responses
Cdc25a	109732985	Abnormal erythropoiesis, increased hematopoietic cell proliferation, *spontaneous mutation in BALB/c*
CCR4	114339014	Decreased TNFα production, IL-1β production; altered macrophage function
Tgfβr2	115932995	Abnormal erythropoiesis, multiple inflammatory infiltrates; increased B1 cells, increased B cell proliferation, increased IgG and M

*Searched for genes of hematopoeitic and/or immunologic relevance between the SNPs flanking *Wam1* (Chromosome 9 bp 8721984-117595153; peak LOD for *Wam1* at SNP rs3088463, Chromosome 9 bp 103464705) using EnsEMBL (http://www.ensembl.org/Mus_musculus/index.html).
**See www.informatics.jax.org for summary of phenotypes and references.

Supplemental Table 2*
Select Genes of Immunologic Interest in *Wam2* on Chromosome 17 (in addition to MHC)

Gene	Location bp	KO phenotype
Ube2i (ubiquitin-conjugating enzyme E2I)	12221966	Conjunctivitis, colitis
NKx2-5 (NK2 transcription)	14403747	Decreased macrophage recruitment; acute and chronic lung inflammation
Bak1 (BCL2-antagonist/killer 1)	15072317	Abnormal homeostasis, splenic architecture; absent marginal zone B cells
Ppard	24277550	Peroxisome proliferators activator receptor δ; Renal and bile duct inflammation
Mapk14	24988111	Altered hematopoietic cell development and homeostasis
Cdkn1a (Cyclin-dependant kinase inhibitor)	26566254	Similar to human DiGeorge syndrome; thymic aplasia; abnormal hematopoiesis, anemia
Pim1 (Proviral integration site 1)	26747399	Abnormal B cell development; increased bone marrow cell number, WBC count, splenomegally; anti-DS DNA antibodies; arthritis, glomerulonephritis, autoimmunity susceptibility
Ubash3a (ubiquitin associated and SH3 domain containing protein A)	27935419	Homolog associated with human SLE, abnormal effector T cell function, LPD, increased immunoglobulins, abnorm cytokine secretion, increased ANAs
Cbs (cystathionine beta-synthase	27960392	Increased inflammatory response
Abcg1 (ATP-binding cassette, sub-family G)	28821439	Susceptibility gene in human SLE; increased T cell proliferation; increased IgG and IgM; increased autoantibody level (dsDNA, anti-histone, ssDNA),
Tff2 (trefoil factor 2 (spasmolytic protein 1)	30777799	Abnormal macrophage physiology
Tapbp (TAP binding protein)	30852327	Colitis
Rxrb (retinoid X receptor beta)	30868074	Abnormal adaptive immunity, abnormal innate immunity
Psmb9	30935131	LPD; hyperresponsive T, B cells; increased susceptibility to EAE; increased cytokine secretion
Psmb8	31341351	Extramedullary hematopoiesis, abnormal TNF physiology, autoimmune hepatitis
Lta (lymphotoxin A)	33529932	Abnormal T, dendritic cell function with abnormal antigen presentation via MHC class I
TNF	33642306	Increased immunoglobulins, WBC counts, LPD; Dermatitis
Ltb (lymphotoxin B)	33702886	Abnormal antigen presentation via MHC class II
Pram1 (PML-RAR alpha-regulated adaptor molecule 1)	33798022	Defective T cell development, CTL function; abnormal dendritic cell antigen presentation due to defective intracellular transport of class I molecules
H2-M3	34205918	Histocompatibility 2, M region locus 3; Abnormal cytokine secretion
MOG (Myelin oligodendrocye glycoprotien)	34224720	Extramedullary hematopoiesis, LPD
Ubd (ubiquitin D)	34464437	Abnormal complement cascade, impaired complement alternative pathway activation
MAP3K	34802574	Increased WBC, IgM, LPD
Sod2 (Superoxide dismutase 2)	34807442	Susceptibility gene in human IBD, rheumatoid arthritis; Increased WBC count, enlarged spleen, abnormal T, B, and myeloid function.
Dll1 (Delta-like 1)	34811218	Increased WBC count, enlarged Spleen, absent LNs, increased IgM

*Searched for genes of hematopoeitic and/or immunologic relevance between the SNPs flanking *Wam2* (Chromosome 17 bp 12070979-36878315; peak LOD for *Wam2* at SNPs CEL-17-12736614 (bp 22133279) and rs3703275 (bp 36702283) as described for Supplemental Table 1.