Supplemental Data

The Lupus-Related *Lmb3* Locus Contains a Disease-Suppressing Coronin-1A Gene Mutation

M. Katarina Haraldsson, Christine A. Louis-Dit-Sully, Brian R. Lawson, Gabriel Sternik, Marie-Laure Santiago-Raber, Nicholas R. J. Gascoigne, Argyrios N. Theofilopoulos, and Dwight H. Kono

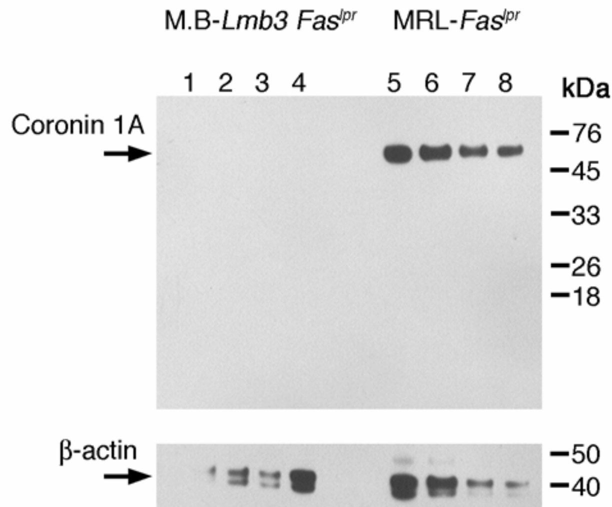


Figure S1. Immunoblot of Coronin 1A Protein in MRL.B6-*Lmb3 Fas*^{lpr} Mice
Total splenic protein was probed with antibodies to either Coronin 1A or β-actin. Lanes 1-4 are individual MRL.B6-*Lmb3 Fas*^{lpr} (M.B-*Lmb3 Fas*^{lpr}) mice and lanes 5-8 individual MRL-*Fas*^{lpr} mice. Total protein, extracted from unfractionated splenocytes per manufacturer (Pierce Biotechnology, Rockford, IL), was separated on a 4-12% SDS polyacrylamide gel, then transferred to a polyvinylidene difluoride membrane (Invitrogen). After blocking with 5% nonfat dry milk (wt/vol) in 0.5% Tween-20 Tris-buffered saline, the membrane was probed with a hamster anti-Coronin 1A antibody (kindly provided by Dr. A. Chan, Genentech) followed by a goat anti-hamster IgG-HRP antibody conjugate (Invitrogen) and chemiluminescence detection (SuperSignal Substrate System, Pierce Biotechnology). The membrane was then stripped and reprobed with a rabbit anti-β-actin antibody followed by a donkey anti-rabbit-HRP antibody conjugate (antibodies from BioLegend).

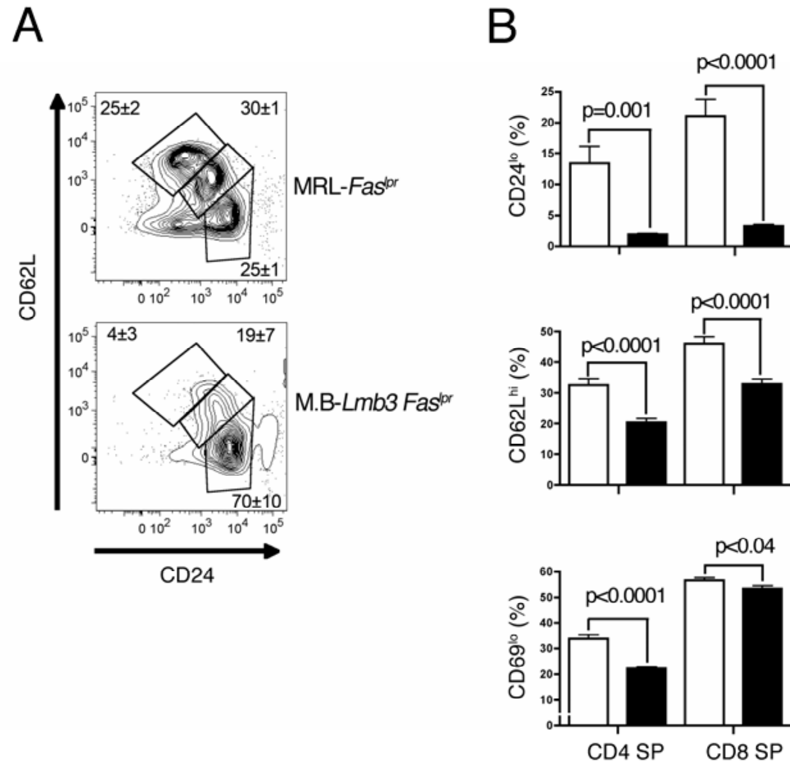


Figure S2. SP Thymocyte Subsets in MRL.B6-*Lmb3 Fas^lpr* Mice
(A) Representative plot of $CD8^+$ single positive thymocyte subsets. Mean±SEM from 4-5 mice/group.
(B) Mean±SEM percentages of SP thymocyte subsets expressing CD24, CD62L, and CD69 from 9-15 mice/group.

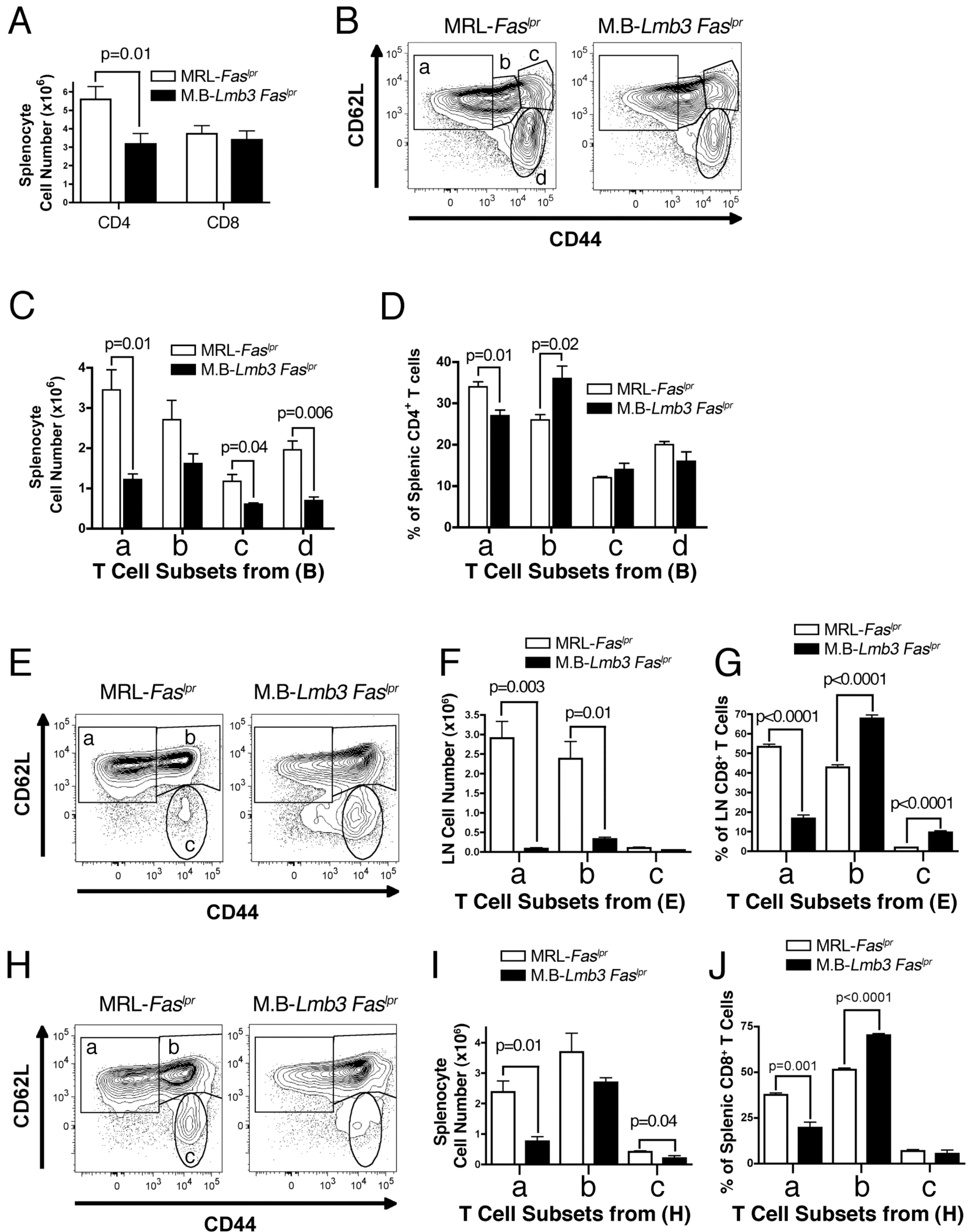


Figure S3. Spleen and LN T Cell Populations in MRL.B6-*Lmb3 Fas*^{lpr} Mice

(A) Reduced numbers of splenic CD4^{+} T cells. n=8-15/group.

(B) CD44/CD62L-defined subsets of splenic $CD4^+$ T cells: $CD44^{lo}CD62L^{hi}$ (a, naïve), $CD44^{int}CD62L^{hi}$ (b), $CD44^{hi}CD62L^{hi}$ (c), $CD44^{hi}CD62L^{lo}$ (d, effector)
(C-D) Cell numbers and percentages for subsets defined in panel B.
(E) CD44/CD62L-defined subsets LN $CD8^+$ T cell subsets
(F-G) Cell numbers and percentages for subsets defined in panel E.
(H) CD44/CD62L-defined splenic $CD8^+$ T cell subsets
(I-J) Cell numbers and percentages for subsets (a-c) defined in panel H. Data for B-J from 3-4 mice/group.

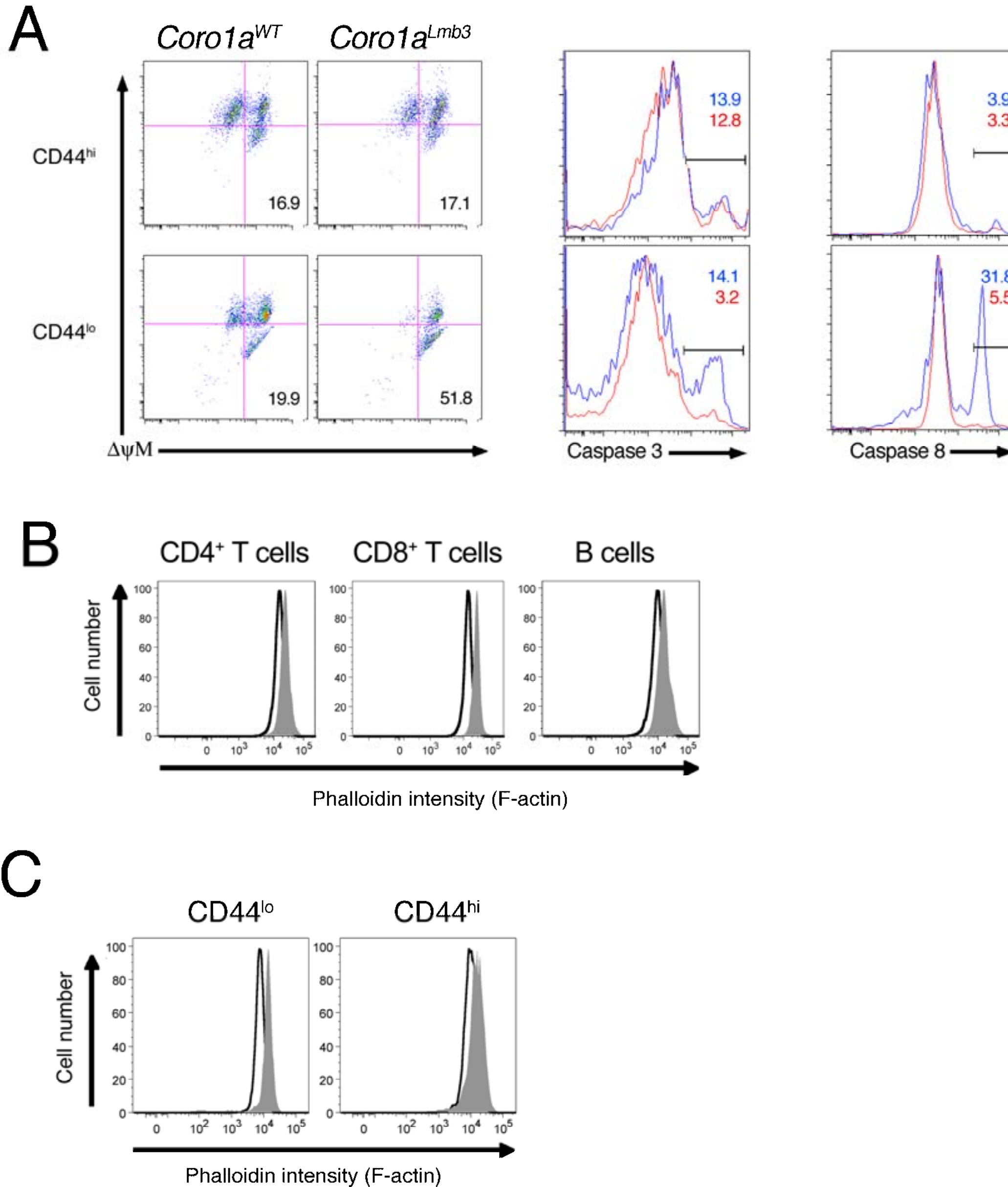
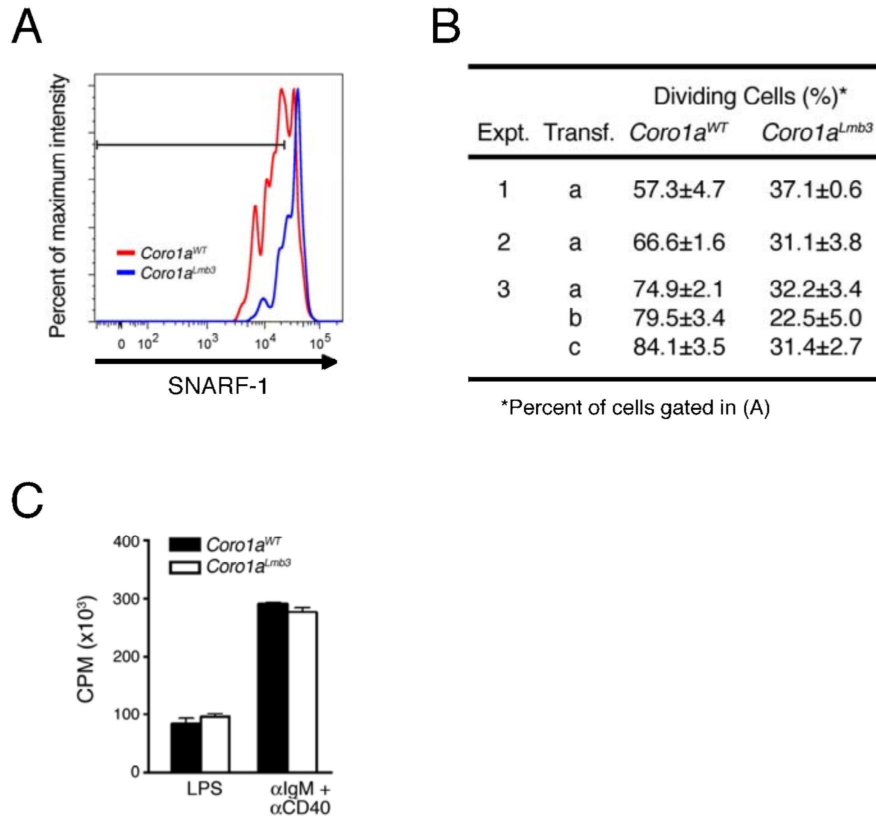


Figure S4. Spontaneous Apoptosis of T Cells and Amounts of Cellular F-Actin
(A) *Ex vivo* spontaneous apoptosis in naïve ($CD44^{lo}$) and memory-effector ($CD44^{hi}$) $CD4^+$ T cells. Purified T cells from MRL-*Fas^{lpr}* (*$Coro1a^{wt}$*, red lines) or MRL.B6-*Lmb*3 *Fas^{lpr}* (*$Coro1a^{Lmb3}$*, blue lines) mice were cultured for 5 h then assessed for ΔΨM potential and active Caspases 3 and 8. Percentages of cells with depolarized mitochondria (lower right quadrant) or positive for active caspases are shown. Data are representative of two independent experiments.
(B) Representative histograms of F-actin amounts in $CD4^+$ and $CD8^+$ T cells and B cells. Splenocytes from MRL-*Fas^{lpr}* and MRL.B6-*Lmb3 Fas^{lpr}* mice were stained with fluorescent dye-labeled Phalloidin, anti- CD4, anti-CD8, and anti-CD19. B6 *Coro1a* mutant (filled-in histogram) and WT cells (line histogram). 3 mice/group.

(C) F-actin in CD44^{lo} and CD44^{hi} subsets of splenic CD4^{+} T cells from B6 WT and B6-*Coro1a*^{Lmb3} mice. Mean fluorescent intensities were increased by 61±7% for the CD44^{lo} subset (p=0.0003, paired t test) and 63±3% for the CD44^{hi}subset (p=0.0007). 6 mice/group.



Expt.	Transf.	Dividing Cells (%)* Coro1a^WT	Coro1a^Lmb3
1	a	57.3±4.7	37.1±0.6
2	a	66.6±1.6	31.1±3.8
3	a	74.9±2.1	32.2±3.4
	b	79.5±3.4	22.5±5.0
	c	84.1±3.5	31.4±2.7

*Percent of cells gated in (A)

Figure S5. Proliferation of *Coro1a*^{Lmb3} T Cells Transfected with WT or Mutant *Coro1a*-Expressing Plasmids and Ex Vivo Activation of B Cells

(A-B) Representative cell division analysis of B6-*Coro1a*^{Lmb3} CD4^{+} T cells transfected with either *Coro1a*^{WT} (red line)- or *Coro1a*^{Lmb3} (blue line)-expressing plasmids (panel A). Percent dividing cells based on gate shown in panel A (panel B). Mean±SD of duplicate proliferations are shown. Three independent experiments (Expt.) using pooled T cells from 3-4 mice involving 1-3 transfections (Transf.) were performed. $p<0.0001$ for the 5 transfections.

Splenic T cells from B6-*Coro1a*^{Lmb3} mice, isolated by negative selection (Miltenyi Biotec), were transfected (Mouse T Cell Nucleofectin Kit, Amaxa, Gaithersburgh, MD) with aforementioned plasmid vectors expressing either *Coro1a*^{WT} or *Coro1a*^{Lmb3} C-terminal GFP fusion mRNAs. After 18 h of culture, cells were stained with SNARF-1 (Invitrogen) and then stimulated with precoated plate-bound anti-CD3 (BD Biosicences) and 10 µg/ml soluble anti-CD28 antibodies (Biolegend). Three days later, cells were stained with fluorescent labelled anti-CD4 and CD3 antibodies and cell divisions identified by flow cytometery.

(C) Mean±SEM thymidine incorporation by ex vivo stimulated B cells. Isolated B cells were stimulated with either LPS or anti-CD40 plus anti-IgM. Data representative of 3 independent experiments. Purified B cells from wild-type MRL-*Fas*^{lpr} or MRL.B6-*Lmb3 Fas*^{lpr} mice (3 mice/group) were either stimulated with LPS (20 µg/ml) or anti-CD40 (0.5 µg/ml) plus anti-IgM (10 µg/ml) for 3 d. [^{3}H]-TdR was added 16 h prior to harvesting and incorporation was measured by liquid scintillation.

Table S1. Polymorphic Microsatellite Markers Used to Map the *Lmb3* Interval

Name	Mb*	L primer	R primer
D7Mit98	114.71	CGCCATAGAACAGATTTGATACC	ATGGGTCTCAGATATCCCACC
D7Tsri108	122.60	ACCTGCATACCCTCCATCCTC	TGTAATCAAACCTGAGTCCATGTTG
D7Tsri109	122.72	CAAATTATCCCTTCTCCTAATGCAA	TTGGAGTTTCTGTTGGTAGACAGC
D7Tsri84	123.60	CAGCACTCAATGAAGCTCTGGT	TGCACAAACACTCAGGTGCATA
D7Tsri308	124.41	TGGTTACTGTTCACAGCATAGATCA	TGATGATGTACTTATGGCAGAGGAC
D7Tsri309	124.54	GGCATGTGTTCCAGCTTTCTTTAG	AGATATGAATTCCCTCTCCATCCA
D7Tsri283	125.04	CCCACAAACTGCAGTATCTCCAT	CAAGGGCACTTTCTGCTACACA
D7Mit101	125.42	TACAGTGTGAACATGTAGGGGTG	TCCCAACATGGATGTGCTAA
D7Tsri316	125.55	CCTGGGCTACATGAAACCTTGT	AAGGGAAAGGGAGAGTTCTGCT
D7Tsri317	**125.71**	**TCACAGAGCATCTGCCTTGTTT**	**GTCACTCAGGCTTGACGGATTT**
D7Tsri321	**126.36**	**TGGCTATACCAAGACAAAACCAAA**	**AGGGGTTCACAGTACTGGCTATTC**
D7Tsri322	**126.37**	**CCCAAGTGTGTGTCTCTCTCTCTC**	**GGACTCAACAGAGTGATGTGCTGT**
D7Tsri324	**126.48**	**TTGACACAAGAGGGAACCTCAA**	**GGAAAATGTTCTACAGGCTTGC**
D7Tsri87	**126.50**	**TGGAACCCACATGGTAGAAAGA**	**ACGGGGAACACGAACACCTAC**
D7Tsri88	**126.62**	**AGTTGGGCTAGGCACCAATTTA**	**GCCTGACGCTATGTGAATGACT**
D7Tsri49	127.22	CCACATGTATAACATGCACTCTCG	TGTCCATTTCTTTCTTTTAAATTTCC
D7Tsri55	127.44	CCAGTTCCAGGGGATCTAATGT	CACCATGCCAGGGTAGATTTATT
D7Tsri56	127.48	GATCTCTACTTGTATACTGTGGCATGG	TCTTAGTCAGGCAAGGAAGTCAGA
D7Tsri128	127.69	AGGCTGAAGCAGCAGAGATAGC	AGCAGAGGGACAGAGAAGTGCT
D7Tsri125	128.71	GGGGAGTGGAAGCAGCTATTTA	CCTGATCTGGCTTTTGCACTTA
D7Tsri65	130.09	AGGGTGTGTGTGTGAGTGTGTG	GGAAGAGAGCAGATTCCACGAT
D7Tsri200	131.00	GGTTACACAGAGAAACCTGTCTCAAA	CTAAGTCATCTCTCCAGCCCAAA
D7Tsri103	132.24	TCCTTCTGGTCATGATTCCTGTT	ATGAGGGGTATCCGTGAGAGAA
D7Tsri104	133.28	GGGCAGCTATTCCTAAACTGAATG	ACTGAGCTGTCTCCACAGATGC
D7Mit109	136.35	TCAACACCAGGAAGTCTCTTCA	CCTCCATCTCCCATCCAATA

*Chromosomal locations are based on Ensembl NCBI m36 assembly. The final 0.9 Mb *Lmb3* interval is indicated in bold type with the flanking markers at 125.7 Mb (D7Tsri317) and 126.6 Mb (D7Tsri88). D7Tsri markers were obtained by screening dinucleotide repeats for length polymorphisms between MRL-Fas^{lpr} and B6-Fas^{lpr} strains using primers generated by the web-based Primer3 program (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi).