SUPPLEMENTAL MATERIAL

Supplemental Materials and Methods

Ethics Statement.

All animal experiments were carried out at the Stanford RAF under the supervision of the Veterinary Service Center at the Department of Comparative Medicine at Stanford, which is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC). All experiments were approved by the Administrative Panel of Laboratory Animal Care (APLAC) and are consistent with federal, state and local guidelines for laboratory animal care.

Mouse strains and transgenic line design.

Congenic B10.S.Tmevp3$^{SJL/J}$ and SJL/J.Tmevp3$^{B10.S}$ mice were imported from the Pasteur Institute animal facility and colonies were established. B10.S and SJL/J mice were obtained from the Jackson Laboratory (Bar Harbor, ME). (Balb/c x 129) F1 pseudopregnant female mice were provided by Drs. Hugh McDevitt and Grete Sonderstrup (Stanford, CA). All mice were bred at the Stanford Research Animal Facility (RAF) except for SJLJ/J mice, which were purchased at 4 weeks old and housed in the Stanford RAF for at least 6 weeks prior to all experiments.

B10.S mice that express NeST RNA transgenically were developed by pronuclear microinjections. NeST cDNA was cloned into the unique *Sal I* site of the p428 expression vector, placing it downstream of a $CD4^+$ and $CD8^+$ T cell-specific promoter and upstream of an SV40 polyadenylation signal. The p428 plasmid contains a mouse *Cd4* promoter with a 428bp silencer deletion, which allows specific expression in both $CD4^+$ and $CD8^+$ T cells (Sawada et al., 1994). The T cell promoter-NeST transgene was released from the vector backbone using *Not I*. The transgene was gel purified and injected into fertilized B10.S oocytes obtained via natural estrous cycle. Estrous cycle determination, recovery of single cell embryos, microinjection procedures and transfer to pseudopregnant females has been previously described (Singer et al., 1998). Transgenic founders were identified by quantitative PCR (qPCR) (Transnetyx, TN) and mated to B10.S wild-type mice. Approximate copy numbers of integrated transgenes were calculated by qPCR analysis of over 100 mice. All experiments included littermate controls.

Inoculations.

Four- or five-week-old mice were inoculated with Theiler's virus as previously described (Bureau et al., 1992) with the following modifications. Prior to intracerebral infections, mice were anesthetized with 2,2,2-tribromoethanol/2-methyl-2-butanol (Sigma-Aldrich, St. Louis, MO). A solution that contained 125 mg of this compound dissolved in 0.25 ml of 2-methyl-2-butanol was added to 10 mls of sterile distilled water. The solution was filter sterilized and kept at 4^{o}C in the dark for a maximum of 10 days. Each mouse received 20ml/gram of Anesthetic by intraperitoneal injection. Mice were inoculated with Theiler's virus by injecting 40ml of viral suspension in the left hemisphere. Mice were dissected at the indicated day after inoculation. To avoid contamination with peripheral blood, mice were perfused with PBS prior to brain and spinal cord dissections. Homogenized tissues were assayed for viral titers by plaque assay.

Mice inoculated with *Salmonella enterica* Typhimurium were 10-12 weeks old; the lethality of bacterial infection was found to be very sensitive to animal age. All experiments reported here include concurrent controls. For oral *Salmonella* infections, mice were denied food for 12 hours and then provided with bread containing the indicated bacterial inoculum (Broz et al.). For intraperitoneal inoculations, mice were injected with live bacteria in 100ml of PBS. Bacteria were grown for 10 hours aerobically at 37^{o}C. Colony-forming units (CFU) were determined by plating after inoculation. Tissues were collected at the indicated day after inoculation, weighed and

homogenized in PBS. Dilutions were plated on LB plates supplemented with 100mg/ml of streptomycin to determine CFUs. Mice used for lipopolysaccharide (LPS) injections were 10-12 weeks old. 100mg of LPS (Sigma- Aldrich, St. Louis, MO) was delivered by intraperitoneal injection in 100ml of sterile PBS. Mice were monitored for mortality.

Macrophage culture and infection.

Macrophage culture and infection has been previously described (Arpaia et al., 2011; Martinat et al., 2002). Briefly, tibia and femur were dissected and bone marrow was flushed with 10mls of DMEM (Invitrogen). The recovered cells were cultured in DMEM supplemented with 10% (v/v) FBS (Omega Scientific) and 10% (v/v) of mouse L-cell conditioned medium as a source of macrophage colony stimulating factor at a density of 5×10^6 cells per plate. Three days after culturing, 4 ml of additional medium was added to each plate. On day six, cultured medium was removed and cells were incubated in PBS (without calcium and magnesium) for 20 min at 4^0C. Macrophages were then detached from the plate by scraping.

For infections, macrophages were seeded at 2×10^5 cells per well and infected at an MOI of 5 CFU/cell with bacteria in DMEM. Infection was carried out at 37^0C for 30min. After infection, cells were washed thoroughly with DMEM containing 100mg/mL gentamicin and cultured in the presence of the antibiotic. Cells were harvested at 2, 4, 6, 8, and 24 hr after infection to measure intracellular bacteria.

T cell culture and stimulation.

Spleens were dissected and passed through a 70-micron cell strainer (BD Falcon, Franklin Lakes, NJ) to dissociate splenocytes. Red blood cells were lysed in Gey solution (Sigma-Aldrich, St. Louis, MO), Remaining splenocytes were magnetically labeled using either $CD3^+$ T cell, $CD4^+$ T cell or $CD8^+$ T cell Isolation Kits (Miltenyi Biotec, Auburn, CA). Cells were passed through a magnetic column and unretained T cells were collected. Nuclei were enriched as previously described (Huarte et al., 2010).

For T-cell stimulation assays, cells were cultured in RPMI 1640 (Invitrogen) supplemented with 10% (v/v) fetal bovine serum (Omega Scientific), 50mMb-mercaptoethanol and 1% (v/v) Pen Strep (Invitrogen). Cells were cultured for 10 hours prior to stimulation with 50 ng/ml phorbol 12-myristate 13-acetate and 1.5mM ionomycin (Sigma-Aldrich). Cell pellets and supernatant were harvested at indicated times post stimulation and stored at -80^oC.

WDR5 chromatin immunoprecipitation.

$NeST^{B10.S}$ and $NeST^{SJL/J}$ were cloned into KpnI- and XhoI-digested pcDNA3.1+ for eukaryotic gene expression. HEK 293T cells (ATCC) were cotransfected using Lipofectamine 2000 (Invitrogen) with pcDNA3.1+FlagWDR5 and RNA expression plasmid (pcDNA3.1+HOTTIP, pcDNA3.1+$NeST^{B10.S}$, or pcDNA3.1+$NeST^{SJL/J}$). After 48-72 hours, cells were harvested by scraping into cold PBS, spun down and snap frozen in liquid nitrogen. Cells were lysed and immunoprecipitated as previously described, with modifications (Wang et al., 2011). Coimmunoprecipitated RNA was extracted using TRIzol LS (Invitrogen) and RNeasy Mini Kits (Qiagen), treated with TurboDNAFree (Ambion), and analyzed by SYBR Green Brilliant II qRT-PCR (Agilent). Controls that lacked reverse transcriptase demonstrated that no contaminating DNA was present (data not shown).

RNA and cytokine quantitation.

Protein quantitation was performed using commercially available ELISA kits (R&D Systems, Inc., Minneapolis MN) or Luminex (Affymetrix, Inc,. Santa Clara CA) according to manufacturer's instructions. For quantitative RT-PCR, total RNA from cells or tissue of interest

was extracted with TRIzol Reagent (Invitrogen) according to the manufacturer's specifications and stored at -80^{o}C. Standard curves were prepared using serial dilutions of known quantities of RNA. A plasmid that encodes *NeST* RNA from C57BL/6 mice was purchased from Invitrogen (IMAGE clone 599035). The three B10.S and 19 SJL/J polymorphisms (Supplemental Table 1) were introduced by site-directed mutagenesis. Non-polymorphic fragments of *Ifng*, *Il-22*, and *Actin* cDNAs were obtained from B10.S and SJL/J splenic RNA by reverse transcription and PCR; the PCR products were cloned using TOPO TA (Invitrogen) according to the manufacturer's instruction. Viral RNA was obtained by *in vitro* transcription of the PTMDA plasmid. Quantitative measurements of RNA prepared from cells were done by real time RT-PCR using the 7300 Time PCR System (Applied Biosystems, Carlsbad CA) and QuantiTect Sybr Green RT-PCR (Qiagen cat. # 204243). The primers for quantitation were: HOTTIP-F 5'-CAAACTCCGTCCTCCAAAAC-3', HOTTIP-R 5'-CAGTGAAGAGCGATCAGTGG-3', U1-F 5'- ATACTTACCTGGCAGGGGAG-3' U1-R 5'-CAGGGGGAAAGCGCGAACGCA-3', GAPDH-F 5'-AGGTGGAGGAGTGGGTGTCGCTGTT-3', GAPDH-R 5'- CCGGGAAACTGTGGCGTGATGG-3', NeST-F: 5'-CAACGTACGCTGCCTCCCGATG-3', NeST-R: 5'-CTATTTGGTCGAGTCTGACAGAG-3', Ifng-F: 5'-CCTGTTACTACCTGACACATTC-3', Ifng-R: 5'- CCTTTACTTCACTGACCAATAAG-3', Il-22-F: 5'-AGAACGTCTTCCAGGGTGAA-3', Il-22-R: 5'- GCTACCTGATGAAAGCAGG-3', Actin-F: 5'-GCCTCGTCACCCACATAGGA-3', Actin-R: 5'-AGGTGTGATGGTGGGAATGG-3', TMEV-F: 5'- CCCAGTCCTCAGGAAATGAAGG and TMEV-R: 5'-TCCAAAAGGAGAGGTGCCATAG (Jin et al., 2007).

Quantitative PCR of genome segments following chromatin IP:

Chromatin IP and quantitative PCR was carried out following the Farnham protocol (O'Geen et al., 2011). Briefly, approximately 5x10^{6} CD8^{+} T cells were purified and crosslinked with 1% formaldehyde. Chromatin was isolated using Nuclear Lysis Buffer (50mM Tris-Cl pH 8.0, 10mM EDTA, 1 % SDS, PMSF, PI) and sonicated to reduce the size to approximately 1000 base pairs per fragment. Chromatin was incubated with 2mg of H3K4me3 Antibody (Abcam #ab8580) overnight at 4ºC. Staph A cells were pre-blocked via inclubation with 10mg/mL BSA, then added for 15min at room temperature. The Staph A cells were washed twice with dialysis buffer (2 mM EDTA, 50 mM Tris-Cl pH 8.0, 0.2% Sarkosyl, PMSF) and twice with washing buffer (100 mM Tris-Cl pH 9.0, 500 mM LiCl, 1% Igepal, 1% Deoxycholic Acid, PMSF). Material was eluted from precipitates and input controls using elution buffer (50 mM Tris-Cl pH 8.0, 10 mM EDTA, 1% SDS, PMSF) and vortexing at room temperature for 30 minutes. Crosslinking was released by overnight incubation at 67ºC followed by RNAse A treatment at 37ºC for 30 minutes. The isolated DNA was purified using Qiagen columns. For qPCR analysis of the eluted DNA was performed using Roche's Lightcycler. The primers for quantitation were: Ifng1-F 5'-CCATCGGCTGACCTAGAGAA-3' ; Ifng1-Reverse 5'- ATGAGGAAGAGCTGCAAAGC-3', Ifng2-F 5'-ACCAAAACTACGCAGGGAAA-3', Ifng2-R 5'- GCTGGCTTTGATTCGATTGT-3', Ifng3-F 5'-TCAGAGGCCTGGACCATAAG-3', Ifng3-R 5'-GAAACTGCAAGGCCACAAAT-3', Ifng4-F 5'-ATTTGTGGCCTTGCAGTTTC-3'; Ifng4-R 5'-GGGCCCTTCCACTTACTTCT-3'**.**

Supplemental Figure Legends

Figure S1. Lethal inflammatory phenotype linked to the *Tmevp3* locus, related to Figure 2. In two independent experiments, strains B10.S and B10.S.Tmevp3$^{SJL/J}$ were injected intraperitoneally injection with 100mg of lipopolysaccharides (LPS) and mortality was monitored. The SJL/J allele of the *Tmevp3* locus reduced mortality. Statistical significance was determined by the logrank test.

Figure S2. Cytokine and NeST RNA expression in CD4^{+} and CD8^{+} T cells, related to Figure 5. Splenic **(A)** CD4^{+} and **(B)** CD8^{+} T cells were isolated from three B10.S (black circles) and three

B10.S.Tmevp3$^{SJL/J}$ (white circles) mice and stimulated *ex vivo* with PMA and ionomycin. The abundance of IFN-g and NeST RNA per cell was determined using quantitative RT-PCR. Means and standard error are indicated for each time point. Statistical significance was determined using a two-way ANOVA test; asterisks denote those values that differ significantly between T cells derived from B10.S and T cells derived from B10.S.Tmevp3$^{SJL/J}$ mice. **(C)** CD8^{+} T cells from three B10.S (black) and two B10.S.Tmevp3$^{SJL/J}$ (white) mice were stimulated with PMA and ionomycin *ex vivo*. The abundance of 27 different cytokines was assayed 24 hours later with the Luminex assay. Significant differences in expression were observed for IFN-g (p = 0.0322), IL-2 (p = 0.0265), IL-13 (p = 0.0314), IL-17 (p = 0.0469), RANTES (p = 0.0417), and TNF-a (p = 0.0058).

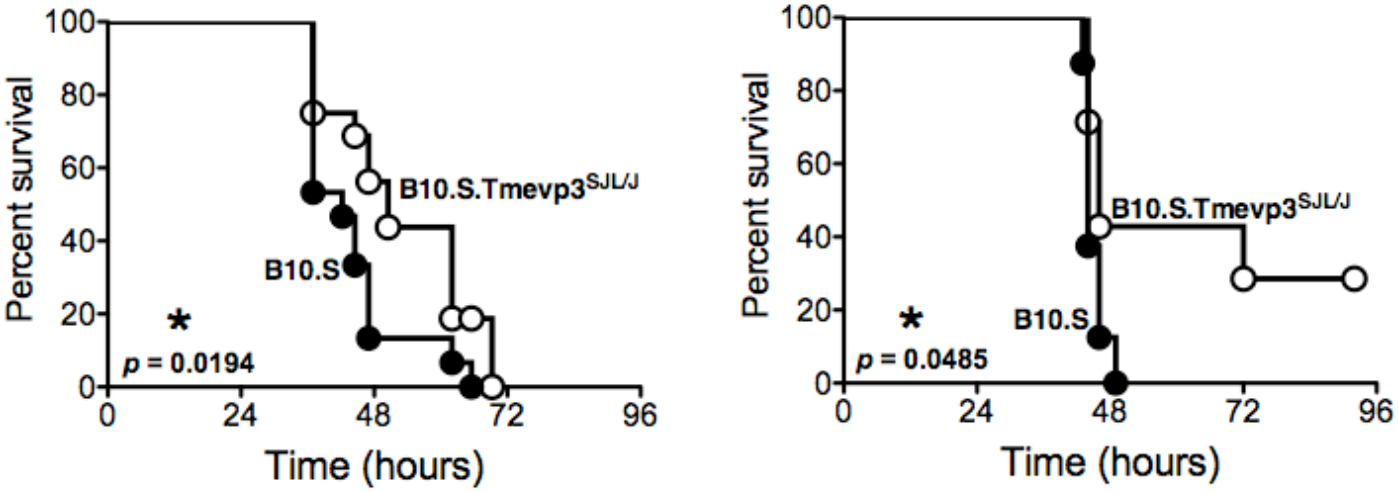
Supplemental Table 1, *Tmevp3* polymorphisms, related to Figure 1. List of single-nucleotide polymorphisms between B10.S and SJL/J in the *Tmevp3* locus of mouse chromosome 10. Included as an Excel file.

Supplemental Reference

Jin, Y.H., Mohindru, M., Kang, M.H., Fuller, A.C., Kang, B., Gallo, D., and Kim, B.S. (2007). Differential virus replication, cytokine production, and antigen-presenting function by microglia from susceptible and resistant mice infected with Theiler's virus. J Virol 81, 11690-11702.

Supplemental Figure

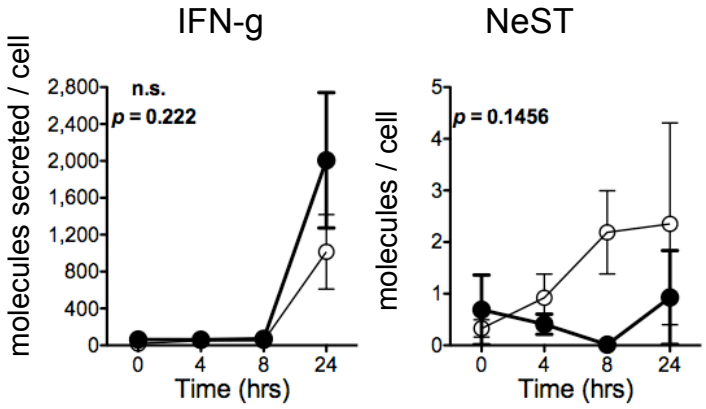
Figure S1. Lethal inflammatory phenotype linked to the *Tmevp3* locus, related to Figure 2



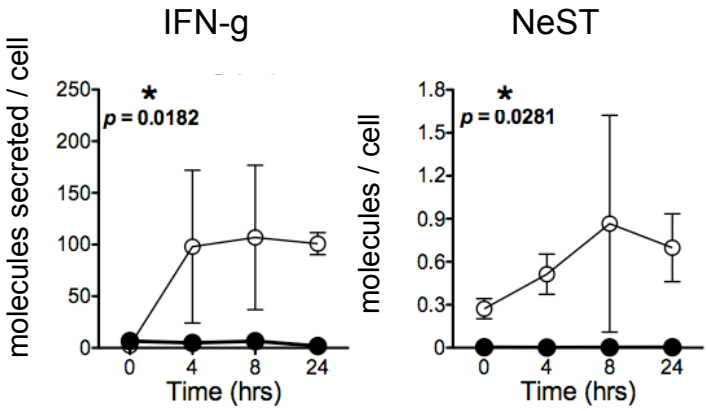
Supplemental Figure 2

Figure 2S. IFN-g and NeST RNA expression in CD4+ and CD8+ T cells, related to Figure 5

A. IFN-g secreted and RNA abundance in CD4+ T cells



B. IFN-g secreted and RNA abundance in CD8+ T cells



C. Cytokine expression in CD8+ T cells