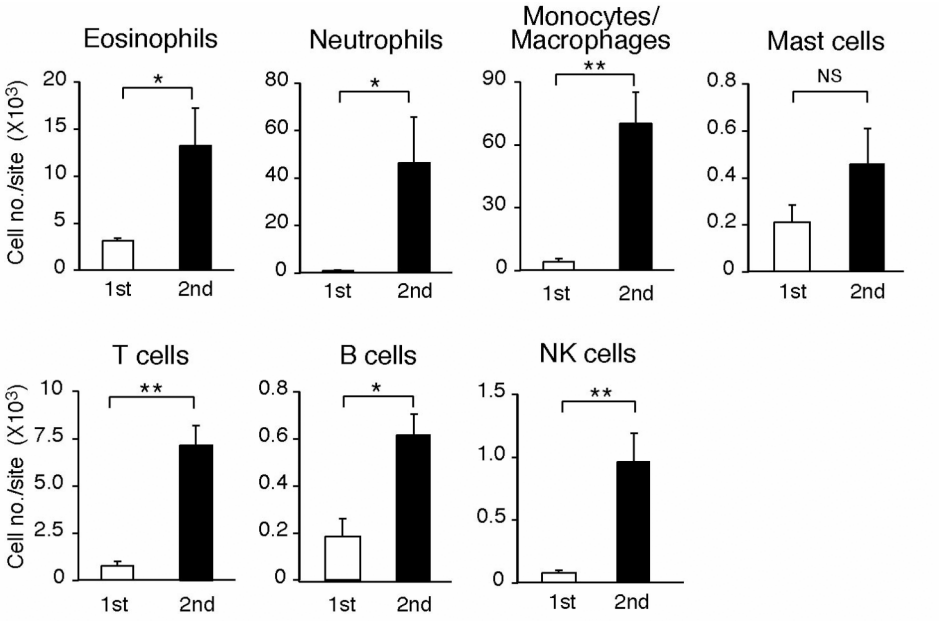
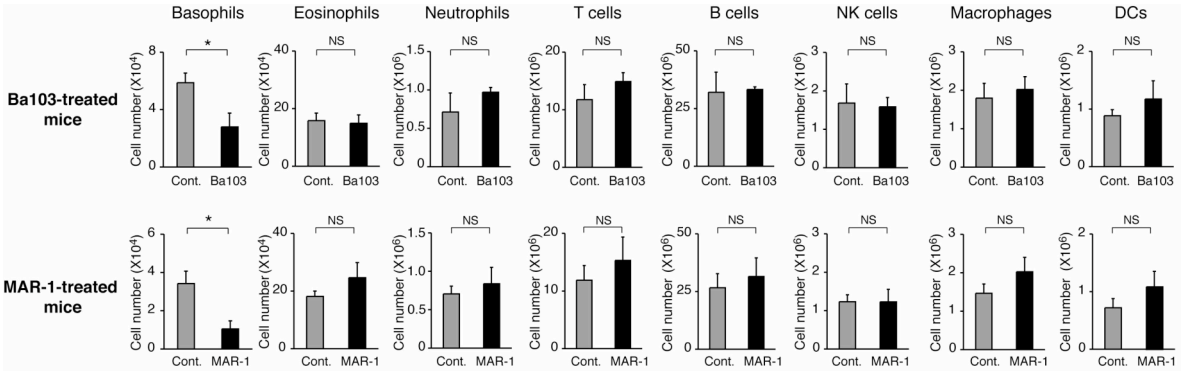
Supplemental Table 1. Both the number and body weight of engorged ticks are reduced in the second infestation compared to the first one.

Mice	Infestation	Engorged ticks	
		Number	Body weight (μg)
C57BL/6 (*n*=4)	First	35.0±0.5	784±7
C57BL/6 (*n*=4)	Second	27.5±1.2**	692±19**

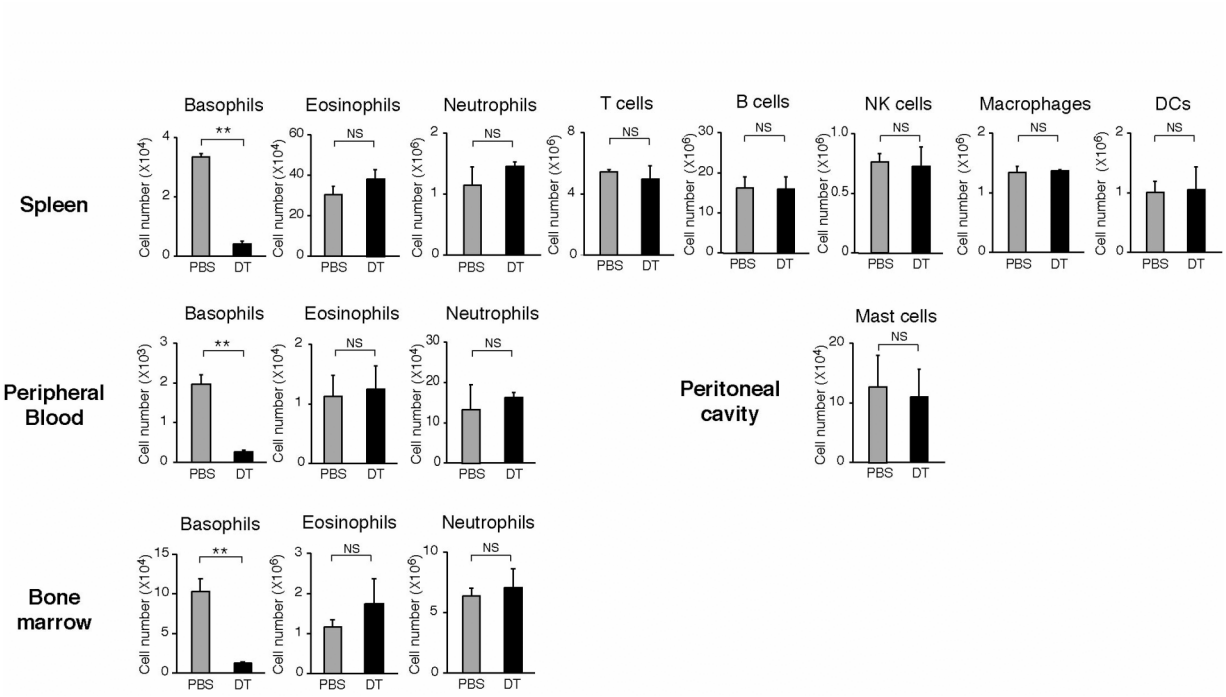
Mice were infested once or twice with ticks (40 larvae/mouse/infestation) as in Figure 1A. The number and mean body weight of engorged ticks (as defined in Methods) are shown (mean ± SEM, *n*=4 each). **, *P*<0.01, when compared to the first infestation.



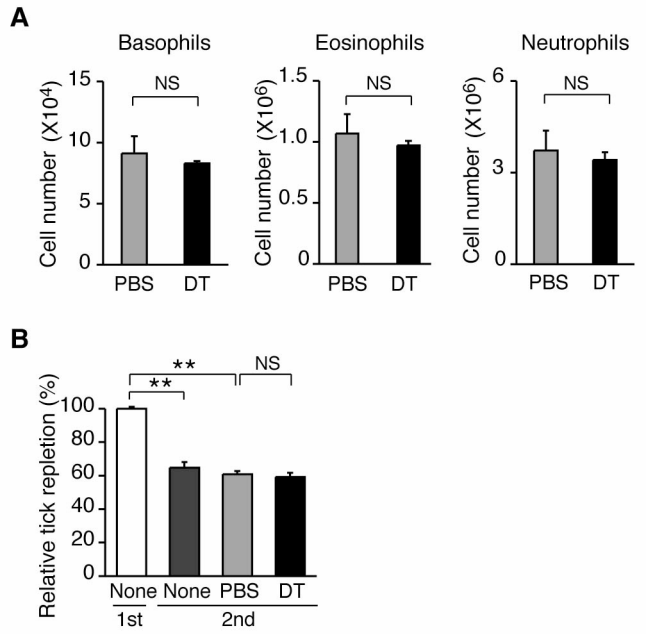
Supplemental Figure 1. Flow cytometric analysis of cells in the tick feeding sites. Skin lesions caused by tick feedings were isolated 18 h after the initiation of the first (open bar) or second infestation (filled bar), and subjected to flow cytometric analysis. The cell numbers of indicated cell types are shown (mean ± SEM, n=3 each), as representative of three repeated experiments. **, P<0.01; *, P<0.05; NS, not significant.



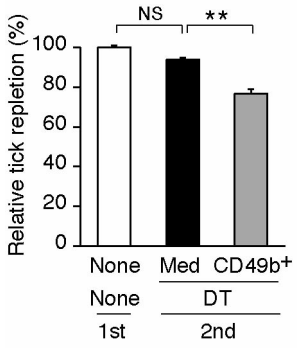
Supplemental Figure 2. Flow cytometric analysis in the Ba103- or MAR-1-treated mice. C57BL/6 mice were infested twice, treated with Ba103, MAR-1 or an isotype-matched control antibody (Cont.) before the second infestation as in Figure 3, and subjected to flow cytometric analysis 2 days after the initiation of the second infestation. The cell numbers of indicated cell types in the spleen are shown (mean ± SEM, n=3 each), as representative of three repeated experiments. *, P<0.05; NS, not significant.



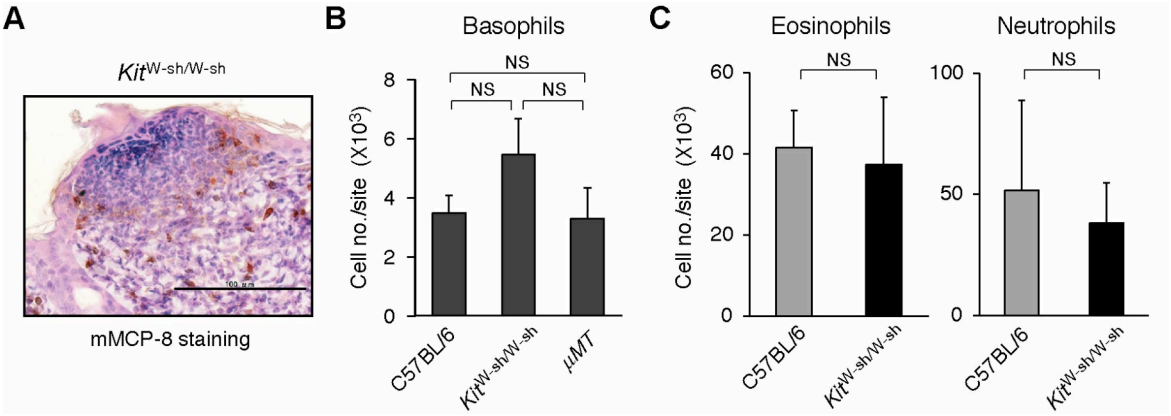
Supplemental Figure 3. DT-mediated, basophil-specific ablation in *Mcpt8*^{DTR} mice. *Mcpt8*^{DTR} mice were treated with DT or PBS as in Figure 4. The cell numbers of indicated cell types in the spleen, peripheral blood, peritoneal cavity, and bone marrow 3 days after the injection are shown (mean ± SEM, n=3 each), as representative of three repeated experiments. **, P<0.01; NS, not significant.



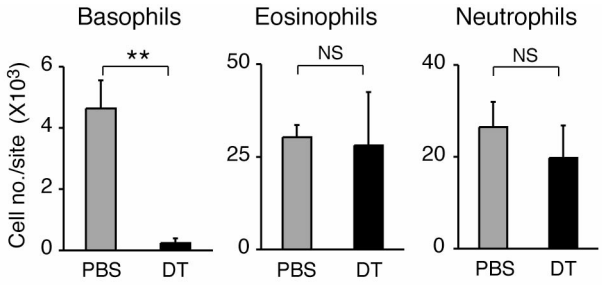
Supplemental Figure 4. DT-treatment of control littermates of *Mcpt8*^{DTR} mice shows no significant effect on the number of basophils or the acquired tick resistance. (**A**) Control littermates of *Mcpt8*^{DTR} mice were treated with DT (750ng/20g body weight) or vehicle (PBS). The numbers of basophils, eosinophils and neutrophils in the bone marrow 3 days after the injection are shown (mean ± SEM, n=3 each). (**B**) Control littermate mice were infested once or twice with ticks as in Figure 1. DT or PBS was administered twice, 2 days before and 2 days after the initiation of the second infestation. The relative tick repletion for each group is shown (mean ± SEM, n=3 each). Data shown in A and B are representative of at least three repeated experiments. **, P<0.01; NS, not significant.



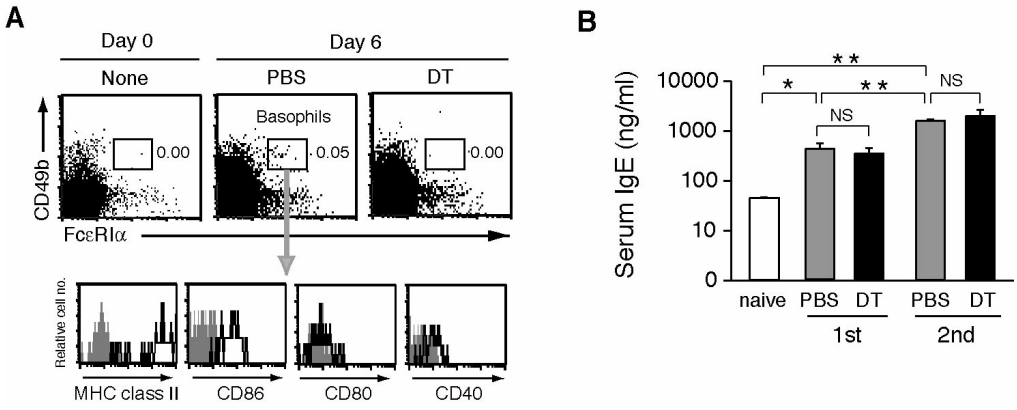
Supplemental Figure 5. Adoptive transfer of basophils from control littermates reconstitutes the tick resistance in DT-treated *Mcpt8*^{DTR} mice. *Mcpt8*^{DTR} mice were infested once or twice with ticks, and DT was administered twice, 2 days before and 2 days after the initiation of the second infestation, as in Figure 5. Two h before the initiation of the second infestation, the CD49b^{+} basophil-enriched fraction of splenocytes from uninfested, control littermate mice (or control medium) was adoptively transferred into the mice. The relative tick repletion in each group is shown (mean ± SEM., n=3 each), in that the value in the first infestation of DT-untreated *Mcpt8*^{DTR} mice was defined as 100%, as representative of two duplicate experiments. **, P<0.01; NS, not significant.



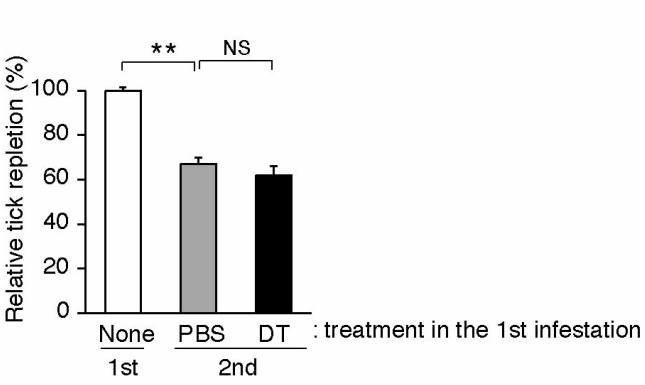
Supplemental Figure 6. Mast cells and antibodies are dispensable for the recruitment of basophils to the tick feeding sites during the second infestation. Indicated mouse strains were infested twice with ticks, and 18 h after the initiation of the second infestation, the tick feeding sites were subjected to the immunohistochemical analysis with mMCP-8 staining (A) as in Figure 2C, and the flow cytometric analysis for the recruitment of granulocytes (B and C). Bar in A indicates 100 μm. Data shown in A-C are representative of at least three repeated experiments. NS, not significant.



Supplemental Figure 7. DT-mediated basophil ablation shows no significant effect on the recruitment of eosinophils and neutrophils to the tick feeding sites. *Mcpt8*^{DTR} mice were infested twice with ticks, and treated with DT or PBS as in Figure 5. The cell numbers of indicated cell types in the tick feeding sites 6 days after the initiation of the second infestation are shown (mean ± SEM, n=3 each), as representative of three repeated experiments. **, P<0.01; NS, not significant.



Supplemental Figure 8. DT-mediated basophil ablation in the first or second infestation with ticks shows no significant effect on serum IgE levels. (**A**) *Mcpt8*^{DTR} mice were infested once with ticks. DT or PBS was administered twice, 2 days before and 2 days after the initiation of infestation. Cells were isolated from bronchial and axillary lymph nodes at the indicated time points, and subjected to flow cytometric analysis for the expression of indicated cell surface markers on basophils. The number shown in each of upper panels is the frequency (%) of basophils, and shaded histograms in lower panels show the control staining with isotype-matched antibodies. (**B**) *Mcpt8*^{DTR} mice were infested once (1st) or twice (2nd) or left uninfested (naïve) with ticks. DT or PBS was administered twice, 2 days before and 2 days after the initiation of the first (1st) or the second (2nd) infestation. Two weeks after the initiation of the first (1st) or the second (2nd) infestation, serum IgE levels (mean ± SEM., n=3 each) were determined by using Mouse IgE ELISA Set (BD OptEIA). Data shown in A and B are representative of two duplicate experiments. **, P<0.01; *, P<0.05; NS, not significant.



Supplemental Figure 9. DT-mediated basophil depletion in the first infestation shows no significant effect on the manifestation of acquired tick resistance in the second infestation. *Mcpt8*^{DTR} mice were infested once or twice with ticks as in Figure 5. DT or PBS was administered twice, 2 days before and 2 days after the initiation of the first but not second infestation. In the DT-treated mice, basophils were depleted in the peripheral blood for 7 days after the initiation of the first infestation, and their number returned to the normal level by the initiation of the second infestation (data not shown). The relative tick repletion for each group is shown (mean ± SEM., n=4 each), in that the value in the first infestation of untreated *Mcpt8*^{DTR} mice was defined as 100%. Data shown are representative of two duplicate experiments. **, P<0.01; NS, not significant.