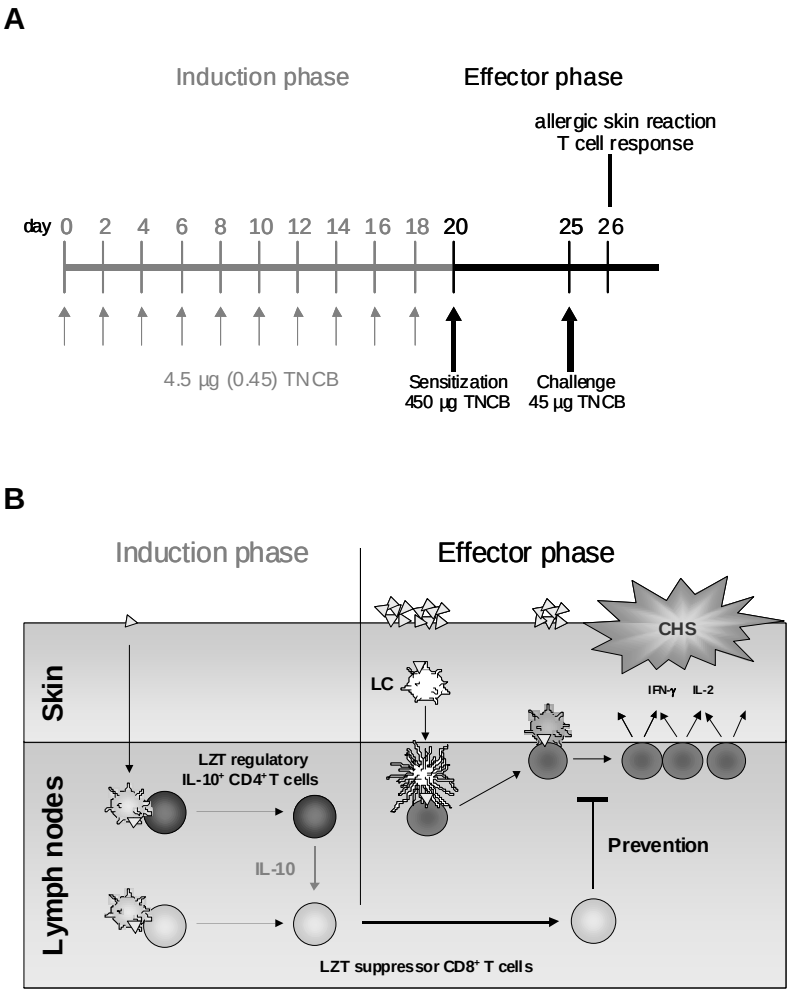
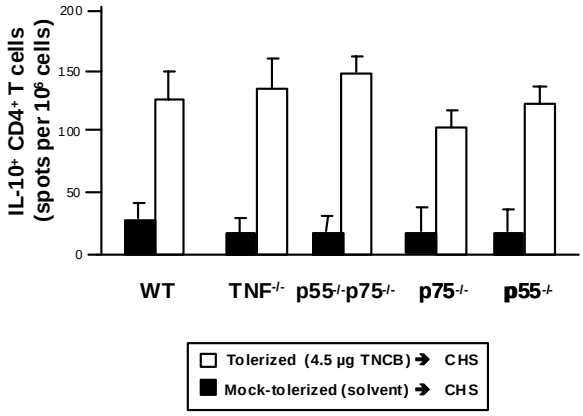
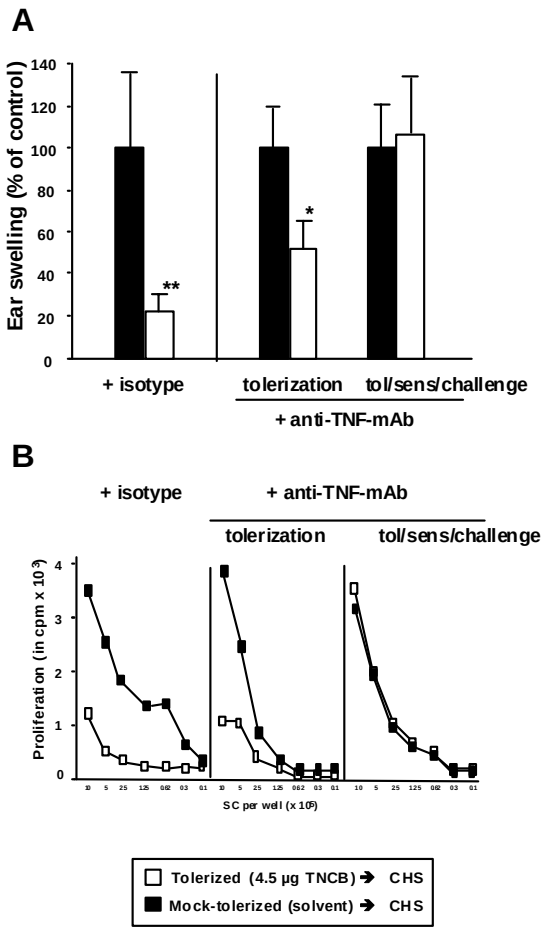
Supplemental Figures and Figure Legends



Supplemental Figure 1
Low zone tolerance
(A) Protocol of low zone tolerance (LZT) induction: Mice were tolerized by repeated treatment with 0.45 or 4.5 µg of TNCB or they were mock-tolerized by repeated treatment with solvent (AOO) before they were sensitized and then challenged to induce CHS responses. (B) LZT induction and effector phase: Repeated applications of low doses of contact allergens induce the activation of IL-10 producing $CD4^+$ regulatory T cells which results in the generation of $CD8^+$ suppressor T cells of LZT. These $CD8^+$ suppressor T cells efficiently inhibit the development of Tc1-mediated allergic skin immune responses in a hapten-specific manner.



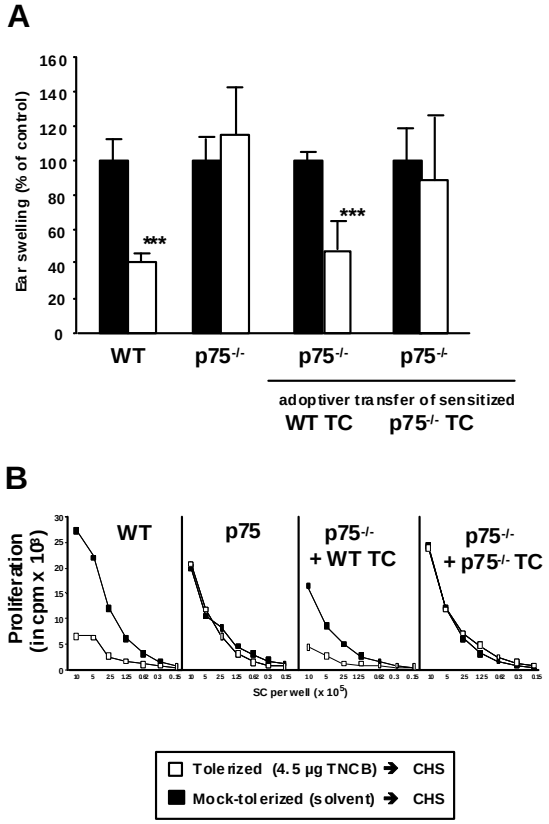
Supplemental Figure 2
$CD4^+$ regulatory T cells of LZT are induced in the absence of TNF.
Numbers of IL-10-releasing $CD4^+$ T cells as assessed by ELISPOT analysis after hapten-specific restimulation *in vitro* in lymph node cell populations obtained from tolerized (white bars) or mock-tolerized (solvent-treated) WT, $TNF^{-/-}$, $p55^{-/-}$, $p75^{-/-}$ or $p55^{-/-}/p75^{-/-}$ mice after sensitization and challenge to induce CHS. One of 3 independent experiments with similar results is shown (5 mice per group per experiment).



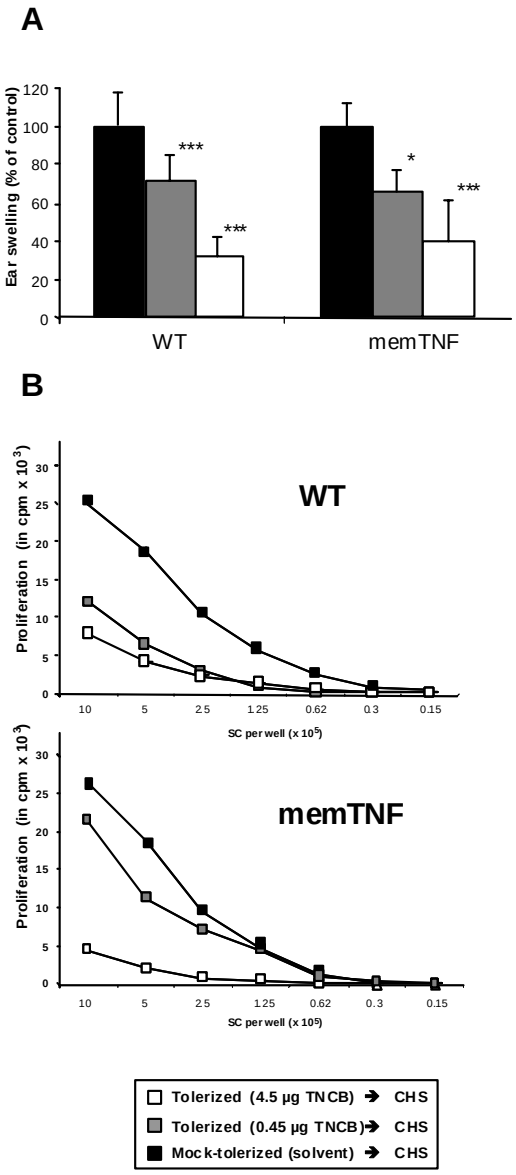
Supplemental Figure 3

Treatment with anti-TNF during the effector phase of LZT, but not during the induction phase of LZT, results in impaired LZT responses.

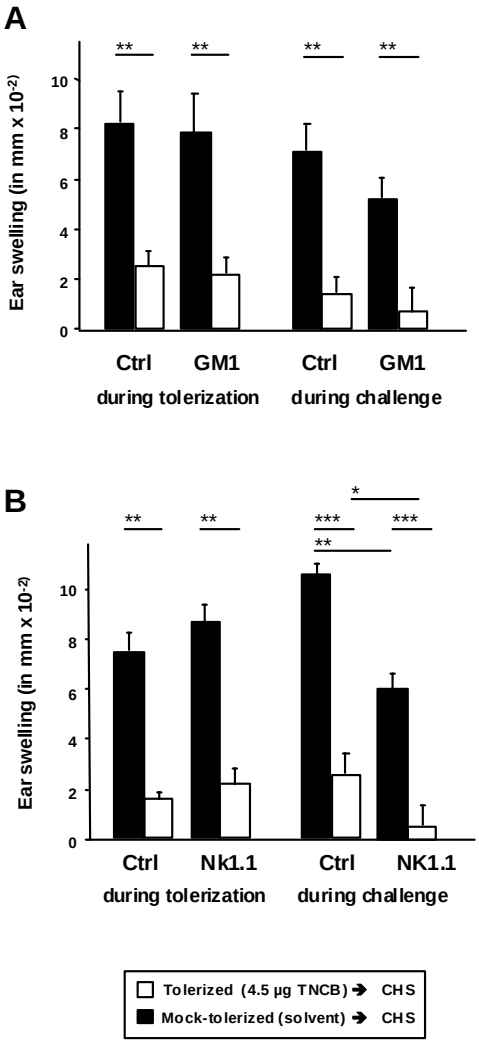
Efficacy of LZT as assessed by measuring inhibition of CHS responses (A) and T cell proliferation (B) after restimulation in tolerized (white bars and white symbols) or mock-tolerized (solvent-treated; black bars and black symbols), sensitized and challenged mice that were treated with isotype control antibody and in mice injected with anti-TNF (V1q) during tolerization (i.e. the induction phase of LZT) and in mice treated with anti-TNF during tolerization, sensitization and challenge (i.e. during the induction and the effector phase of LZT). One of two independent experiments with similar results is shown. SC = spleen cells. ** $p<0.01$; * $p<0.05$.



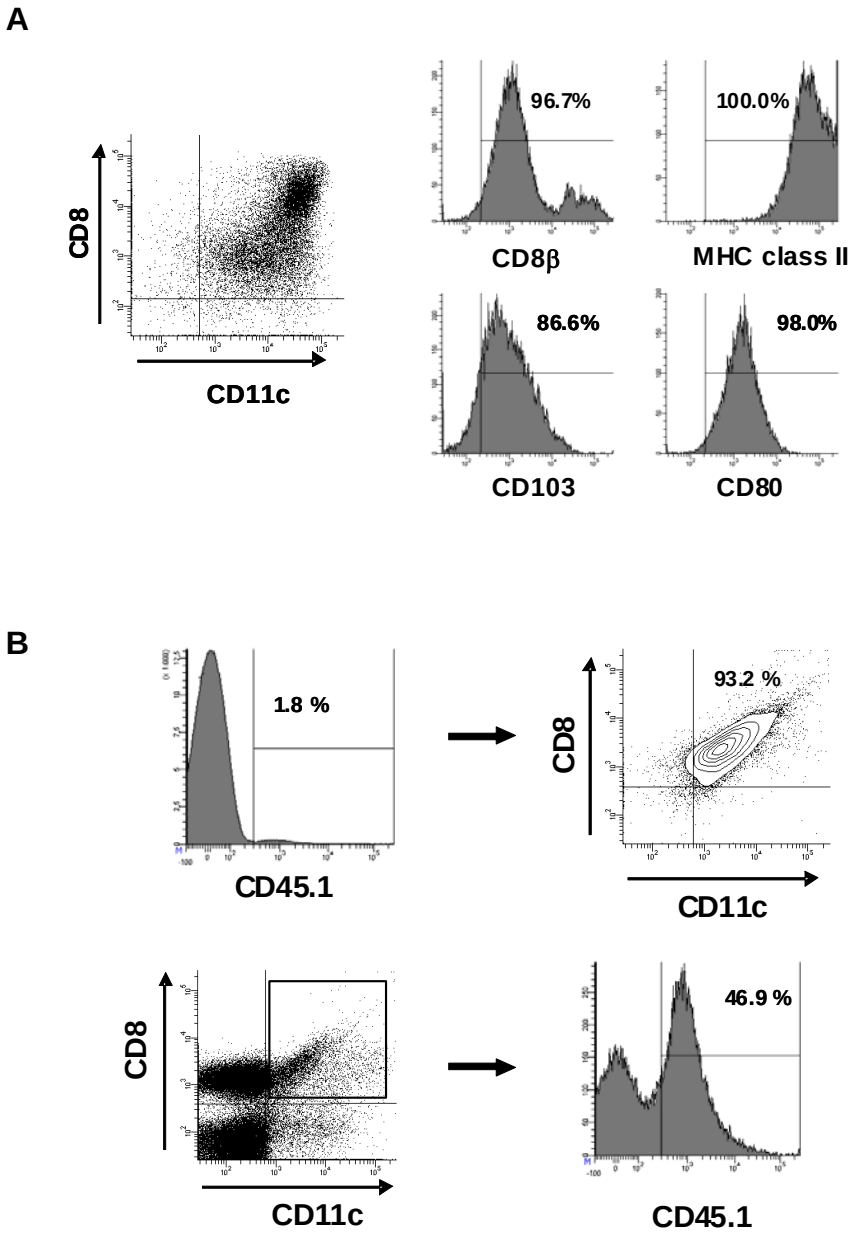
Supplemental Figure 4
p75-expressing T cells play a critical role in the effector phase of LZT.
Efficacy of LZT as determined by assessing inhibition of CHS responses (A) and T cell proliferation after restimulation (B) in tolerized (white bars and white symbols) or mock-tolerized (solvent-treated; black bars and black symbols), sensitized and challenged wild type mice (WT), p75-deficient mice ($p75^{-/-}$), and in $p75^{-/-}$ mice that were tolerized or mock-tolerized and then adoptively transferred with T cells obtained from sensitized WT mice ($p75^{-/-}$ + WT TC) or from sensitized $p75^{-/-}$ mice ($p75^{-/-}$ + $p75^{-/-}$ TC) and then challenged. Results from one of 3 independent experiments (5 – 6 mice per group per experiment) with similar results are shown. SC = spleen cells. *** $p < 0.001$.



Supplemental Figure 5
Membrane-bound TNF is sufficient for LZT development.
Efficacy of LZT as assessed by measuring inhibition of CHS responses (A) and T cell proliferation (B) after restimulation in tolerized (white and grey bars and white and grey symbols) or mock-tolerized (solvent-treated; black bars and black symbols), sensitized and challenged transgenic memTNF mice, which lack the soluble form of TNF, and corresponding wild type mice (WT). One of six independent experiments with similar results is shown (6 mice per group per experiment). *** $p< 0.001$; ** $p<0.01$; * $p<0.05$.



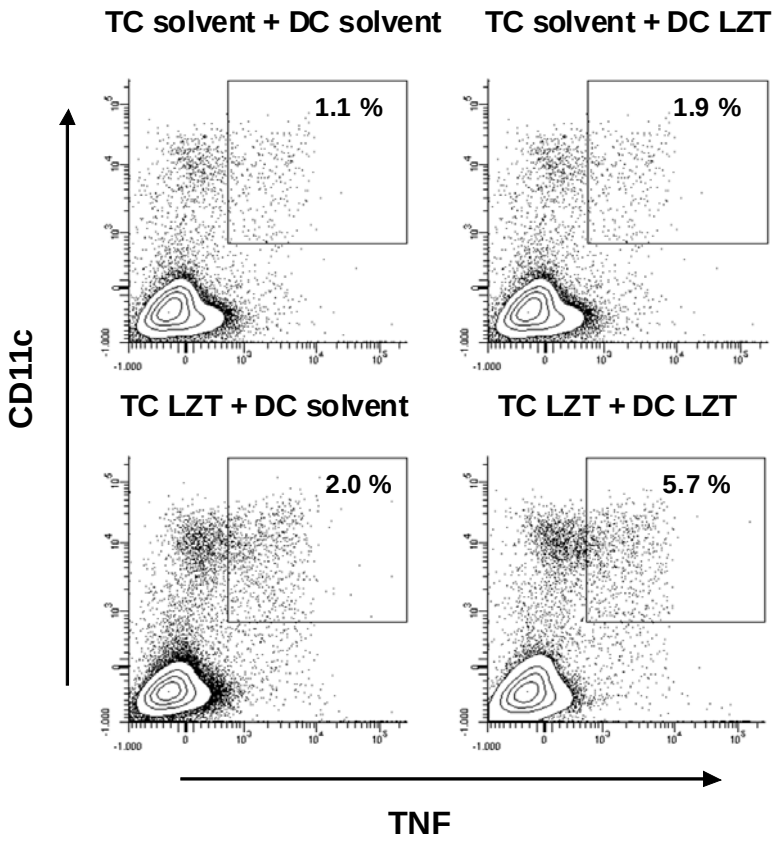
Supplemental Figure 6
NK cells are dispensable for LZT, but not for CHS responses.
Efficacy of LZT as determined by assessing inhibition of CHS responses in tolerized (white bars and white symbols) or mock-tolerized (solvent-treated; black bars and black symbols), sensitized and challenged mice treated with NK cell-depleting antibodies (A: anti-asialo-GM1; B: anti-NK1.1) or isotype control antibodies during tolerization or during challenge. One of two independent experiments with similar results is shown (6 mice per group per experiment). *** $p<0.001$; ** $p<0.01$; *$p<0.05$



Supplemental Figure 7

Phenotype and migration of donor $CD8^+CD11c^+$DCs.

(A) Expression of the surface molecules CD8ß, MHC II, CD103, and CD80 as assessed by flow cytometry in the $CD8^+CD11c^+$ DCs that were purified from lymph nodes of WT or CD45.1 mice and then used for adoptive transfer experiments. Data are derived from one of three experiments with similar results. (B) Percentage of $CD45.1^+CD8^+CD11c^+$ DCs in skin draining lymph nodes of $TNF^{-/-}$ CD45.2 mice that were tolerized, adoptively transferred with $CD45.1^+CD8^+CD11c^+$ DCs from naïve mice, sensitized and challenged. Top: gate set on CD45.1; Bottom: gate set on $CD8^+CD11c^+$ DCs. One of three independent experiments is shown. Analyses were performed with lymph node cells from single mice (three per group).



Supplemental Figure 8
LZT suppressor CD8$^+$ T cells can induce TNF-producing CD8$^+$CD11c$^+$ DCs *in vitro*.
Percentage of TNF-positive CD8$^+$CD11c$^+$ DCs was assessed by flow cytometry (gated for CD8$^+$) after coculture of CD8$^+$ T cells (1 x 10^5) obtained from lymph nodes of tolerized (TC LZT) or mock-tolerized (solvent-treated; TC solvent) control animals with CD8$^+$CD11c$^+$ DCs (1 x 10^4) obtained from tolerized (DC LZT) or mock-tolerized (DC solvent) control animals. One representative of two independent experiments (3 mice for T cell and 12 mice for DC isolation) per group and per experiment is shown.