Supplemental material

Supplemental Figure 1. Priming with DC-Tag induces a long-lasting antigen-specific immune response. WT mice were primed by i.d. injection of DC-Tag and groups of animals were killed 1, 10, 16 and 22 weeks after priming to conduct immunological analyses. (A) Schematic representation of the experiment. (B) The effector function of Tag-specific CTLs was assessed *ex vivo* by 24 h ICP assay. Results are reported as percentage ± SD of IFN-γ-producing cells after gating on $CD8^+CD44^+$ T cells. Background release was subtracted. Data are from at least 3 independent experiments involving six to twelve animals per group. Statistical analyses were done using the ANOVA, and NewmanKeuls tests: ***$p < 0.001$.

Supplemental Figure 2. Boosting increases the absolute number of rapidly responding Ag-specific $CD8^+$ splenocytes. (A) Schematic representation of the experiment. WT mice were primed by DC-Tag. One group of primed mice was killed one week later (Priming), while the remaining mice were boosted (Boost) or not (No Boost) 4 weeks later, and sacrificed 10 weeks after priming. Splenocytes were assessed *ex vivo* for IFN-γ-producing by 24 h ICP assay. (B) Data are reported as absolute number of IFN-γ-producing cells following electronic gating on $CD8^+CD44^+$ T cells. Background release was subtracted. Data from at least 3 independent experiments were aggregated. Statistical analyses were conducted using Student's *t*-tests: Priming versus Boost or No Boost, $p < 0.0001$; **$0.001 < p < 0.01$.

Supplemental Figure 3. Boosting does not increase the cytolytic activity of Tag-specific $CD8^+$ T cells. WT mice were primed with DC-Tag, either boosted (Boost) or not (No Boost) 4 weeks later, and sacrificed 10 weeks after priming. Splenocytes were stimulated *in vitro* with the relevant Ag and tested 5 days later for cytolytic activity by standard 4 h ^{51}Cr release assays; unpulsed (squares) or Tag-pulsed (diamonds) RMA cells and B6/K-0 cells (triangles) were used as targets at the

indicated effector: target (E:T) ratio. (B) Quantification (percentage) of lysis against Tag-pulsed RMA cells at 50:1 E:T ratio (lysis against RMA was subtracted). Data are from at least 3 independent experiments.

Supplemental Figure 4. Repeated DC-Tag boosting helps maintaining the pool of Ag-specific CD8$^+$ T_{CM} cells. (A) Schematic representation of the experiment. WT mice were primed with DC-Tag and either boosted (Boost) or not (No Boost) at week 4, 10 and 16 after priming. (B) Groups of animals were killed 6 weeks after each boost and their splenocytes were assessed for IFN-γ-production *ex vivo* by 24h ICP assays. Data are reported as absolute number of IFN-γ-producing cells from individual mice after gating on CD8$^+$CD44$^+$ cells. Background release was subtracted. (C) Other mice were primed with DC-Tag and boosted once with Tag emulsified in CFA and twice with Tag emulsified in IFA every 6 weeks. Groups of animals were killed 6 weeks after each boost, and their splenocytes were assessed *ex vivo* for IFN-γ-production by 24 h ICP assays (left panel). Data are reported as absolute number of IFN-γ-producing cells after gating on CD8$^+$CD44$^+$ cells from individual mice. Background release was subtracted. Splenocytes after 5 days of *in vitro* restimulation were assessed for cytolitic activity by standard 4 h ^{51}Cr release assays. Data are reported as specific lysis at 50:1 E.T ratio against Tag-pulsed RMA (lysis against RMA was subtracted) (C, right panel). Data from at least 4 independent experiments were aggregated. Statistical analyses were done using the Student's *t*-tests: **$0.001 < p < 0.01$.

Supplemental Figure 5. Different vaccination schedules do not impact on the pool of Ag-specific CD8$^+$ T_{CM} cells. (A) Mice were primed with DC-Tag and treated as depicted in the schematic representation of the experiment. (B) All mice were killed 22 weeks after priming and their splenocytes were assessed *ex vivo* for IFN-γ-production by 24 h ICP assays. Data are reported as percentage (left panel) and

absolute number (right panel) of IFN-γ-producing cells after gating on $CD8^+CD44^+$ cells (background release was subtracted) from individual mice.

Supplemental Figure 6. A larger pool of Ag-specific $CD8^+$ T_{CM} cells ensures better protection against tumor challenge. Mice were treated as described in the legend to Figure 5. The survival of mice in the different experimental groups is reported in Kaplan-Meyer plots. Statistical comparisons, conducted by Long-Rank test, of survival curves gave the following results: Loose/Tight Boost/No Boost vs PBS, $p < 0.001$; Loose vs Tight/No Boost, $p < 0.05$; Tight vs No Boost, $p > 0.05$.

Supplemental Figure 7. Donor-derived $CD8^+$ T cells lead the Tag specific immune response in transplanted TRAMP mice. Seventeen-18 week-old WT and TRAMP mice (CD45.2) were transplanted with $CD45.1^+CD45.2^+$ F1 HSCT and $CD45.1^+$ DLI from female donor pre-sensitized against male Ags (pDLI), and recipients were then vaccinated with DC-Tag and either boosted or not. All animals were sacrificed 12 weeks after DC priming. Donor and host spleen-derived cells were identified by flow cytometry by the expression of congenic markers. (A) Representative dot plot. (B) Data are reported as percentage ± SD of $CD45.1^+$ DLI-derived (blue), $CD45.1^+CD45.2^+$ HSCT-derived (red) of $CD45.2^+$ host cells (black) within $CD8^+$ splenocytes. (C) Splenocytes were assessed *ex vivo* for IFN-γ-production by 24h ICP assays. Data are reported as percentage of IFN-γ^+ cells following electronic gating on $CD8^+CD44^+$ cells within the populations of DLI-, HSCT-derived and host cells. Data are from at least 2 independent experiments. Statistical analyses were done using the Student's *t*-tests: $^{**}0.001 < p < 0.01$.