A new model for CD8⁺ T cell memory inflation based upon a recombinant adenoviral vector

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Supporting Information
**Figure S1. βgal96-specific CD8+ T cells show an effector memory phenotype.** (A) Peripheral blood from C57BL/6 (B6) mice immunized i.v. with 2x10^9 pfu Ad-LacZ was collected at different time points and stained with βgal-specific tetramers and antibodies specific for CD8 and the indicated cell surface molecules. βgal96 CD8+ T cells (black squares), βgal497 CD8+ T cells (white circles), and total CD8+ T cells (grey triangles) are displayed. (B) Peripheral blood from d100 B6 mice was stained with the βgal96 tetramer and antibodies specific for CD8, CD122, NKG2A and NKG2D. The plots shown are gated on tetramer+ CD8+ T cells (black) or tetramer- CD8+ T cells (grey) from the same sample. (C, D, E, F) Staining for CD122, NKG2A and NKG2D on different time points in blood (C), spleen (D), liver (E) and lung (F). Mean percentage or MFI respectively of surface molecule positive cells within the tetramer-positive CD8+ T cell compartment is indicated (±SEM; blood d21 n=6-13, d50 n= 6-12, d100 n=5-7, d200 n=3-6; spleen d21 n=12-16, d50 n=6-11, d100 n=8, d200 n=6; liver and lung d21 n=6-9, d50 n=5-7, d100 n=6, d200 n=3-6; range
indicates the different markers assessed; Each marker was at least measured in two independently performed experiments).
Figure S2. The effector-memory phenotype of βgal96-specific CD8+ T cells is not restricted to the route of immunization. (A) B6 mice were immunized i.v. (black circles), i.m. (black squares, dashed-dotted line) and s.c. (black triangles, dashed line) with 2×10⁹ pfu Ad-LacZ and expansion of βgal96- and βgal497-tetramer+ CD8+ T cells was measured. Significantly reduced expansion of βgal96-specific CD8+ T cells after i.m. (d21*; d50**; d100***) and s.c. (d21**; d50***; d100***) injection, and no memory inflation after s.c. immunization. Mean percentage of tetramer-positive cells within the CD8 compartment is indicated (±SEM; s.c. (data from two independently performed experiments) d21 n=7, d50 n=7, d100 n=7; i.m. (data from a single experiment) d21=3, d50=3, d100=3). (B) The inflationary potential expressed by the ratio of percentage of tetramer-positive CD8+ T cells from day 100 to day 21 in B6 mice after i.v., i.m. and s.c. immunization for both βgal epitopes is shown. (C) Expression of CD44, CD62L, CD127, KLRG-1 and CD27 in βgal96-positive CD8+ T cells in blood at different time points after i.v. (black circles), s.c. (black
squares, dashed line), i.d. (black triangles, dotted line) and i.m. (black triangles, dotted-dashed line) immunization. Mean percentage or MFI respectively of surface molecule positive cells within the tetramer-positive CD8⁺ T cell compartment is indicated (±SEM; d21 n=3, d50 n= 3).
Figure S3. Long-term low-level antigen persistence after i.v. immunization with Ad-LacZ.

(A) CFSE-labeled, βgal96-specific, Ly5.1⁺ TCR-transgenic CD8⁺ T cells from Bg1 mice transferred on day 50 and day 100 after Ad-LacZ immunization proliferated in spleen, liver, hepatic LNs and lung 3 days after transfer. The numbers indicate the percentage of proliferated Ly5.1⁺ CD8⁺ T cells that are donor derived (±SEM, spleen n=4, liver n=4, hLN n=4 lung n=4; two independently performed experiments for day 50 and day 100 immune mice). (B) CFSE-labeled, βgal96-specific, Ly5.1⁺ TCR-transgenic CD8⁺ T cells transferred on day 100 after MCMV-LacZ infection proliferated in spleen 3 days after transfer. The numbers indicate the percentage of proliferated Ly5.1⁺ CD8⁺ T cells that are donor derived (±SEM, spleen n=3). (C) CFSE-labeled, βgal96-specific, Ly5.1⁺ TCR-transgenic CD8⁺ T cells transferred on day 21 after Vacc-LacZ infection did not proliferate (in spleen 3 days after transfer). The numbers indicate the percentage of proliferated Ly5.1⁺ CD8⁺ T cells that are donor derived (±SEM, spleen n=3).
Figure S4. CD8⁺ T cell memory inflation is spleen-independent. B6 mice (black circles) and splenectomized B6 mice (black squares, dashed line) were immunized intravenously with 2x10⁹ pfu Ad-LacZ and expansion of βgal₉₆-tetramer⁺ CD8⁺ T cells was measured with flowcytometry. Mean percentages of tetramer-positive cells within the CD8 compartment are indicated (±SEM; d8 n=6, d14 n=6, d21 n= 3, d50 n=3, d100 n=3, d440 n=3).