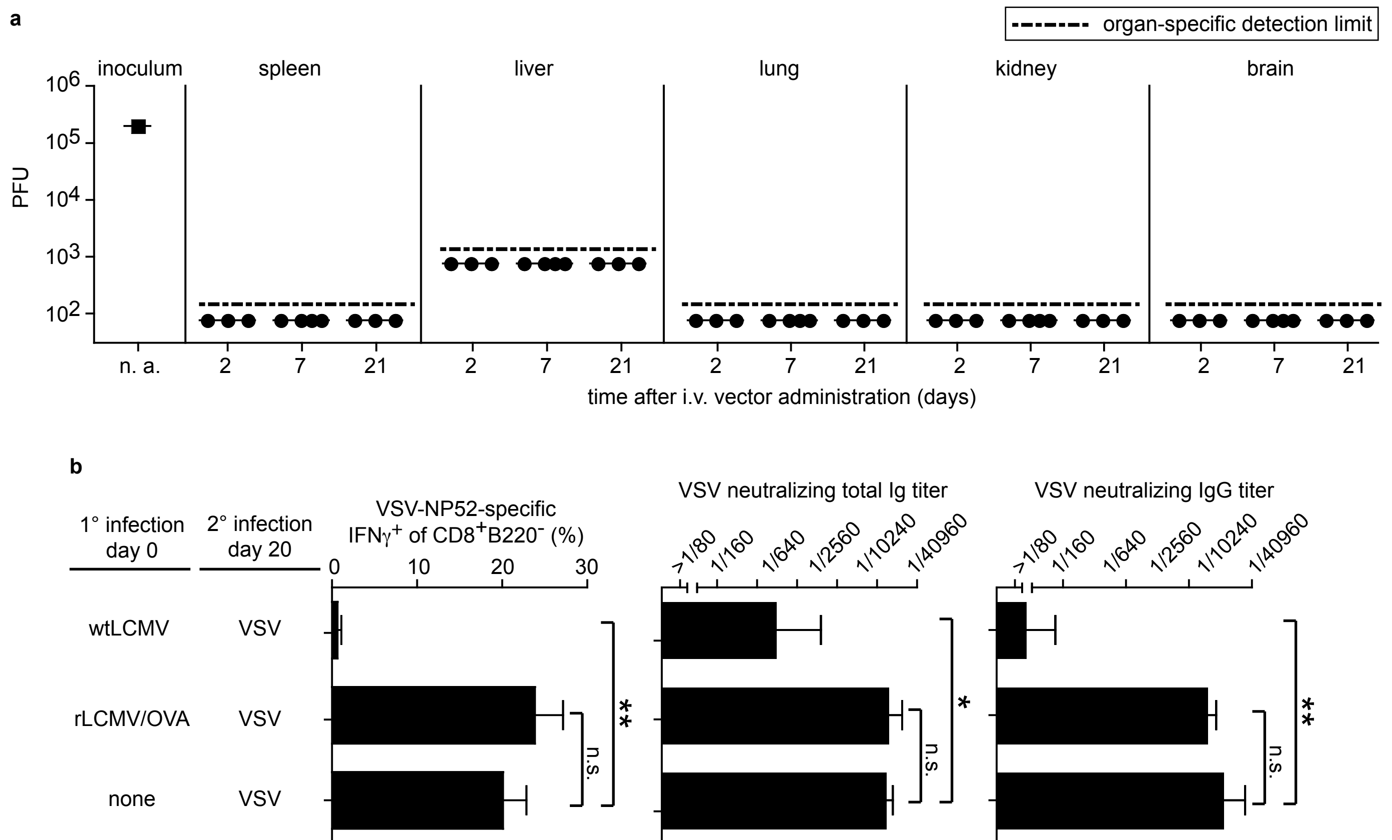
Supplementary figure 1



rLCMV vectors fail to replicate in interferon type I and type II receptor double-deficient mice and do not interfere with heterologous immune responses.

a: Interferon type I and type II receptor double-deficient mice (*ifnagr*$^{-/-}$) were inoculated with $2x10^5$ PFU of rLCMV/GFP. On day 2, 7 and 21 after vector administration, animals were sacrificed for collection of spleen, liver, lung, kidney and brain. Organ homogenates were tested for replicating vector in immunofocus assays on 293T/GP cells and did not yield any detectable infectivity. An aliquot of the vector inoculum was frozen back and was included as a positive control in the assay (square). Filled circles represent individual mice. The dotted dashed line indicates organ-specific detection limits of the immunofocus assay. **b**: C57BL/6 mice were infected i.v. on day 0 and on day 20 as indicated in the chart. VSV-NP52-specific $CD8^+$ T cell frequencies in blood, and VSV neutralizing total Ig and IgG titers in serum were measured on day 27. Bars indicate the mean±SEM of four mice.

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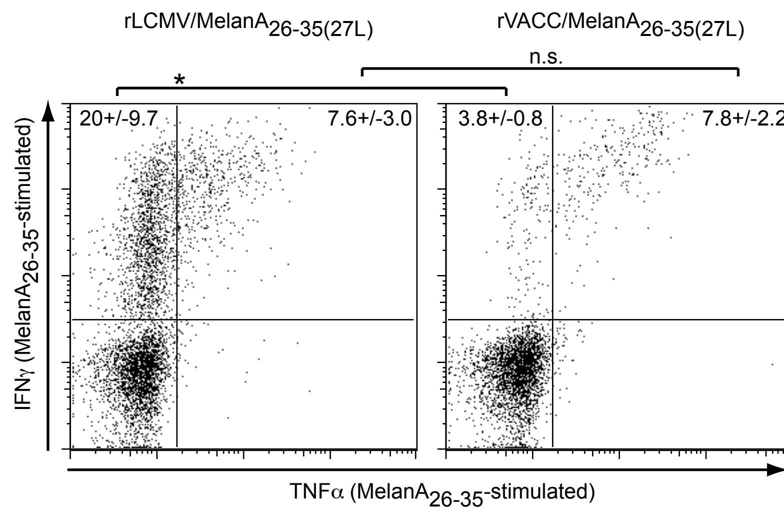
Supplementary figure 2

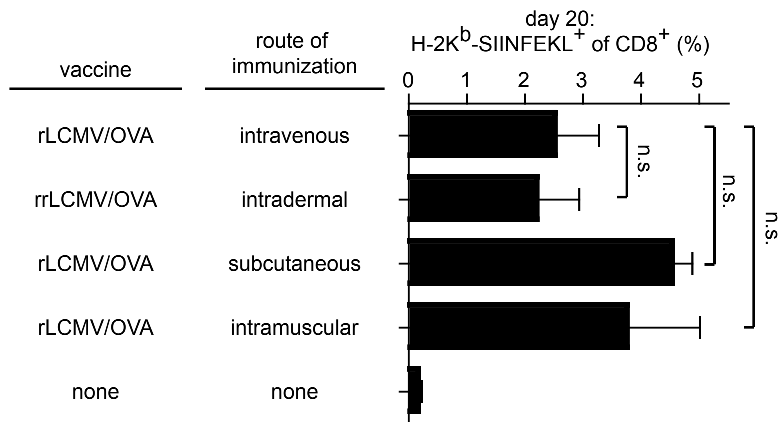


rLCMV vector induces potent MelanA-specific HLA-A2.1-restricted CD8^{+} T cell response.

HLA-A2.1-transgenic HHD mice[43] were vaccinated either with rLCMV/MelanA26-35(27L) or with a recombinant vaccinia virus vector containing the same fusion construct[44]. IFN-γ- and TNF-α-secreting MelanA26-35(27L)-specific CD8^{+} T cell frequencies in spleen were determined in an intracellular cytokine assay ten days later. Representative FACS plots are shown. Values indicate then mean±SD of three mice per group.

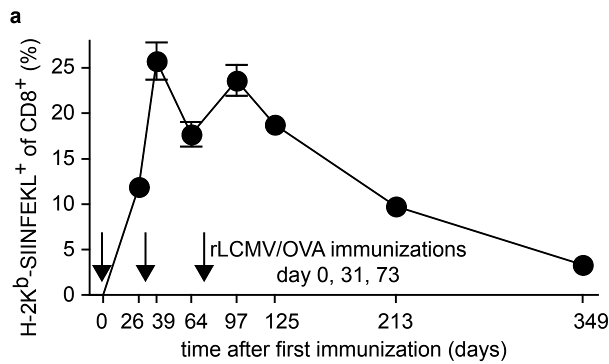
Nature Medicine: doi:10.1038/nm.2104

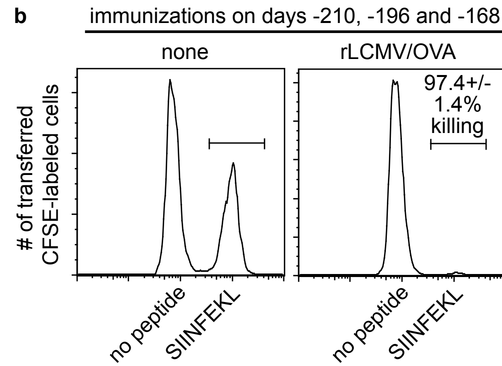
Supplementary figure 3

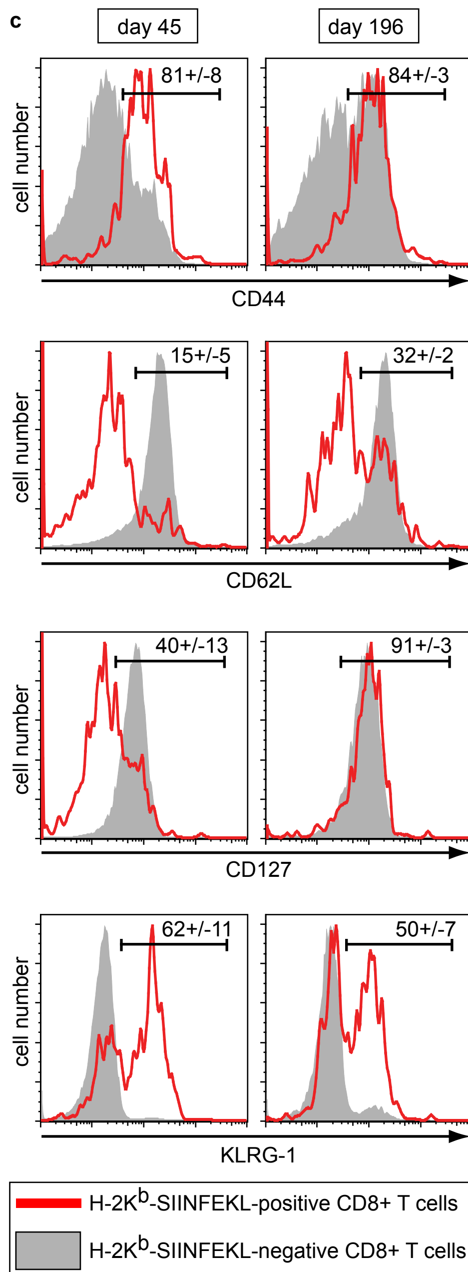


Potent CD8$^+$ T cell induction by rLCMV vectors is independent of the route of administration. C57BL/6 mice were immunized with $2x10^4$ PFU rLCMV/OVA via either the intravenous, intradermal, subcutaneous or intramuscular route. Controls were left without immunization ("none"). SIINFEKL-specific CD8$^+$ T cell frequencies in blood were analyzed ten days later.

Supplementary figure 4



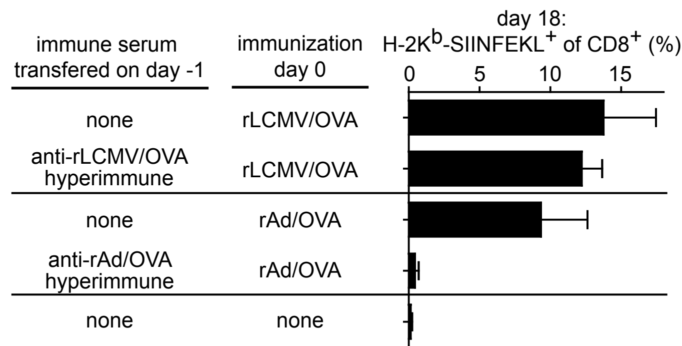




Long-lived and functional CD8$^+$ T cell memory after rLCMV/OVA prime-boost immunization and phenotypic characterization of CD8$^+$ memory T cells.

a: C57BL/6 mice were immunized with rLCMV/OVA on day 0, 31 and 73 and the frequency of SIINFEKL-specific CD8$^+$ T cells in blood was followed over time. Symbols represent the mean±SEM of five mice per group. **b**: C57BL/6 mice were immunized with rLCMV/OVA on day 0, 14 and 42. 168 days after the last immunization a SIINFEKL-specific *in vivo* CTL assay was performed as previously described [41]. In brief, syngeneic C57BL/6 splenocytes were labeled with the fluorescent dye carboxyfluorescein diacetate succinimidyl ester (CFSE) at two different concentrations (CFSE^{high} or CFSE^{low}). In addition, CFSE^{high} cells were pulsed with SIINFEKL peptide at 10^{-6} M concentration for epitope-specific recognition by vector-induced CTLs. $3x10^7$ cells of each population were co-transferred into vector-immunized recipient mice ("rLCMV/OVA"; right panel) and into naive C57BL/6 controls ("none"; left panel). 24 hours later, the percentage of CFSE^{high} and CFSE^{low} donor cells in peripheral blood mononuclear cells was determined by flow cytometry. Specific killing was calculated as: 100 - ([(% CFSE^{high} in vaccinated animal / % CFSE^{low} in vaccinated animal) / (% CFSE^{high} in naive / % CFSE^{low} in naive)] x 100). Killing is indicated as the mean± SD of three mice. **c**: OVA- (SIINFEKL)-specific CD8$^+$ T cells in peripheral blood were analyzed on day 45 after single immunization with rLCMV/OVA (left), or on day 196 after boost (day 234 after prime). Surface expression of CD44, CD62L, CD127 and KLRG-1 was detected as indicated. OVA tetramer-binding CD8$^+$ T cells (red line) are displayed in comparison to tetramer-negative CD8$^+$ T cells from the same mice (gray shaded). Values indicate the mean±SD of four to five mice.

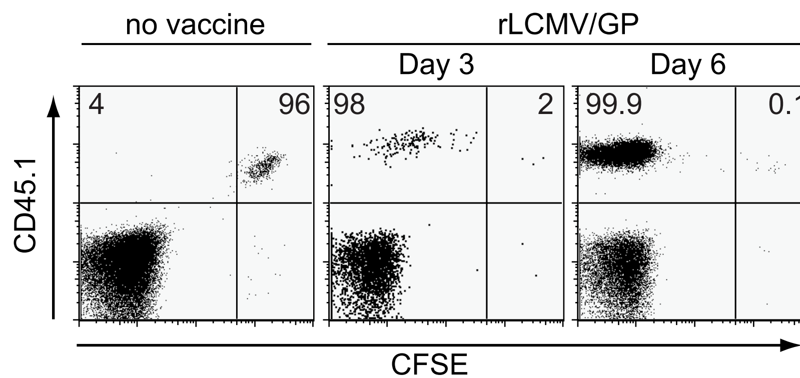
Supplementary figure 5



Transfer of rLCMV/OVA-hyperimmune serum fails to interfere with subsequent rLCMV/OVA-induced CD8^{+} T cell response.

C57BL/6 mice were vaccinated with rLCMV/OVA or rAd/OVA on day 0, 31 and 68. On day 168 (100 days after the last booster vaccination), serum was collected and was transferred (400 µl per recipient) to naive mice. One day later, both groups and controls without serum transfer were vaccinated with the same two vectors as indicated in the chart. SIINFEKL-specific CD8^{+} T cell frequencies in peripheral blood were determined 18 days later. Bars represent mean±SD of three to four mice per group.

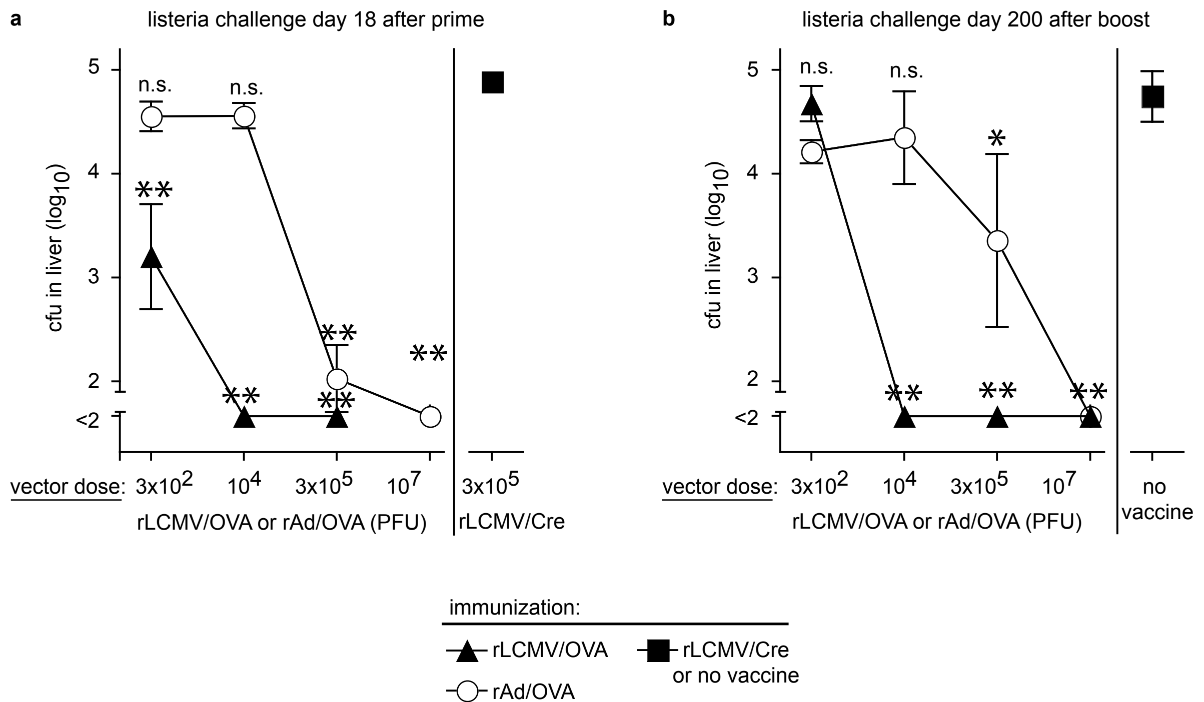
Supplementary figure 6



Rapid priming of CD8$^+$ T cells by rLCMV vector

Splenocytes from P14 transgenic mice[42] expressing a GP33-specific CD8 T cell receptor and carrying the CD45.1 congenic marker were labeled with CFSE and were adoptively transferred into naive C57BL/6 recipients. One day later (day 0), the transferred cells were activated by immunization with an rLCMV vector expressing their cognate antigen (center and right panel) or were left untreated ("no vaccine", left panel). Cell division as evident in CFSE dilution was monitored on day 3 and day 6. Plots are gated on CD8$^+$ T cells. One representative of five mice is shown.

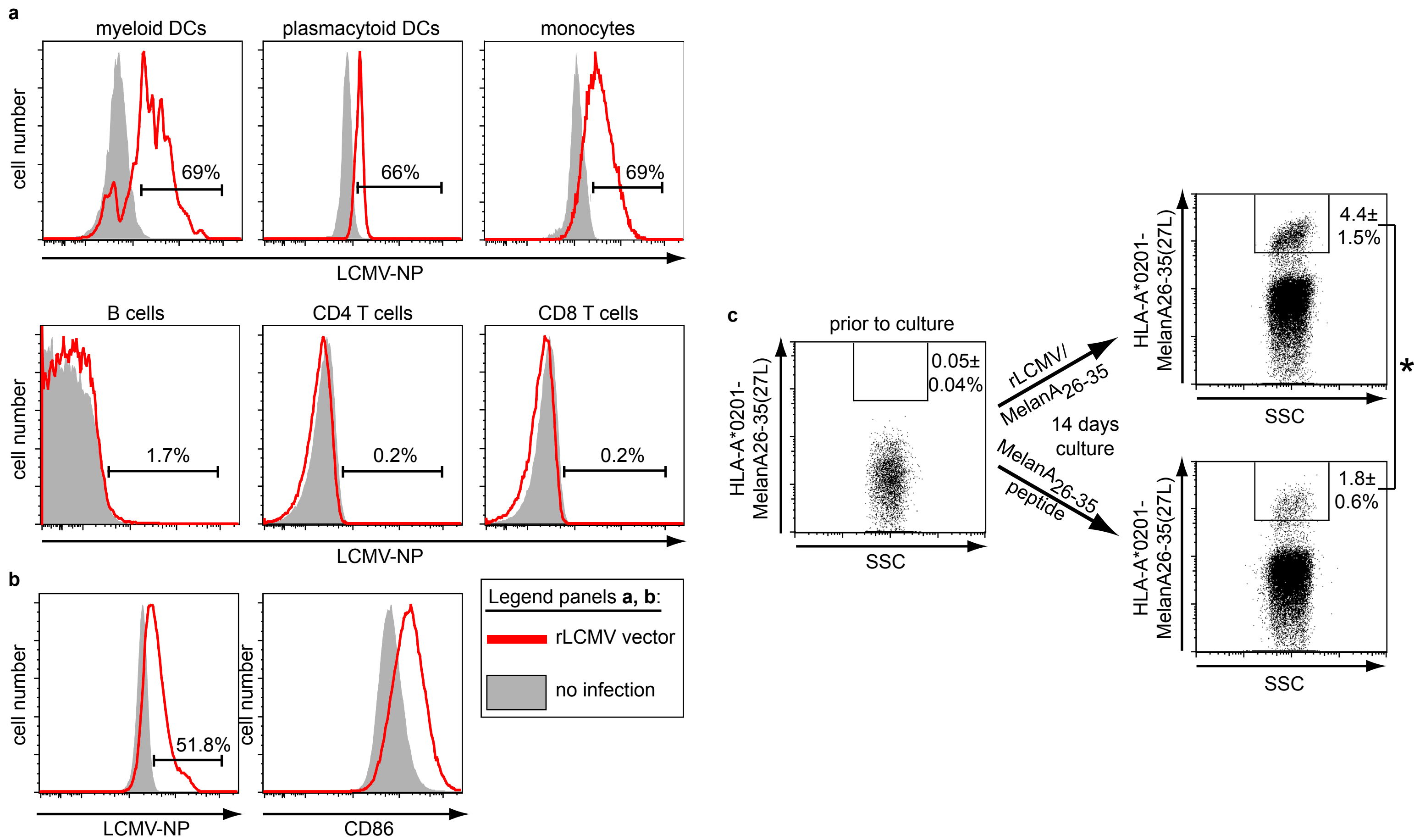
Supplementary figure 7



Dose response of rLCMV/OVA- and rAd/OVA-induced protection against recombinant listeria expressing OVA

Mice were vaccinated with various doses of rLCMV/OVA or rAd/OVA. Controls were given the irrelevant vector rLCMV/Cre or were left unvaccinated (no vaccine), as indicated. Challenge with recombinant Listeria monocytogenes expressing OVA was performed either 18 or 200 days later. Single immunizations were performed for challenge on day 18 (**a**), and prime-boost immunization (day 0 and day 38) was performed for challenge on day 200 after boost (**b**, same experiment as displayed in **Fig. 4a**). Bacterial titers in the liver were determined three days after challenge. Symbols represent the mean±SEM of three to six mice per group. Statistical analysis identified groups with bacterial titers that differed significantly from rLCMV/Cre- (**a**) or non-immunized controls (**b**), respectively.

Supplementary figure 8



Infection and maturation of human dendritic cells by rLCMV vector and induction of human HLA-A*0201-specific CD8 T cell response *in vitro*.

a, b: PBMCs (**a**) and purified myeloid dendritic cells (**b**) from healthy human donors were infected with rLCMV/MelanA26-35(27L) at multiplicity of infection (MOI) of 1 or were left uninfected. Infected cells (red line) are displayed in comparison to non-infected control cells from the same donor (gray shaded). Gates were set such that ≤1% of LCMV-NP$^+$ cells were recorded in non-infected control wells. Percentages indicate the proportion of LCMV-NP-positive cells in the infected culture. **a**: Two days after the addition of rLCMV/MelanA26-35(27L), infected myeloid dendritic cells (CD11c$^+$CD123$^-$CD14$^-$HLADR$^+$CD3$^-$CD20$^-$), plasmacytoid dendritic cells (CD123$^+$CD14$^-$CD11c$^-$HLADR$^+$CD3$^-$CD20$^-$), monocytes (CD14$^+$HLADR$^+$CD3$^-$CD20$^-$), B cells (CD20$^+$CD3$^-$lymphocytes), CD4$^+$ T cells (CD4$^+$CD8$^-$CD20$^-$ lymphocytes) and CD8 T cells (CD8$^+$CD4$^-$CD20$^-$ lymphocytes) were identified by intracellular staining for LCMV nucleoprotein (LCMV-NP). **b**: Two days after rLCMV/MelanA26-35(27L) infection of purified myeloid dendritic cells, infected cells were identified by intracellular staining for LCMV-NP and their activation state was assessed by surface staining of the CD86 marker. **c**: For induction of MelanA26-35(27L)-specific CD8$^+$ T cell responses, PBMCs from melanoma patients were incubated with rLCMV/MelanA26-35(27L) or with MelanA26-35 peptide for two weeks. MelanA26-35(27L)-specific CD8$^+$ T cells were identified by MHC class I tetramer staining prior to the addition of rLCMV/MelanA26-35(27L) or peptide, respectively, and 14 days later. One representative FACS plot of three individual patients in one experiment is shown, and numbers indicate the mean±SD of the three patients. One representative of two similar experiments is shown.