Supporting Information

Kratky et al. 10.1073/pnas.1108945108

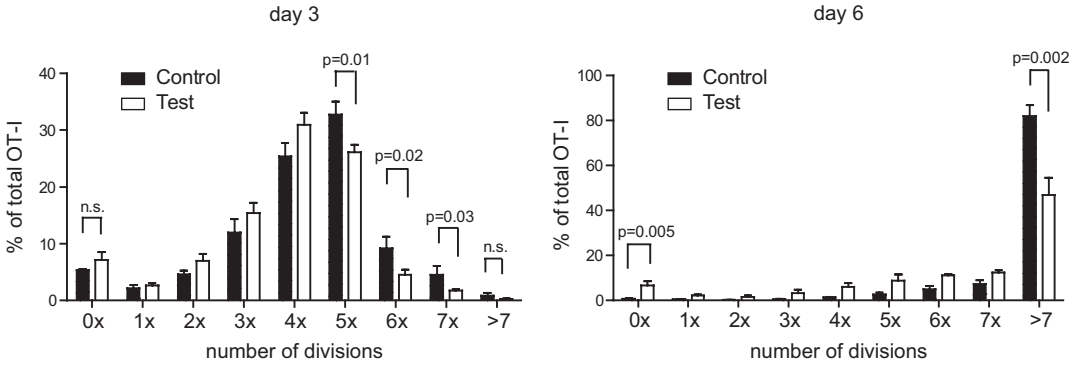

Fig. S1. Reduced relative frequencies of highly divided cells despite comparable generation number of CD8$^+$ T cells primed by indirectly activated antigen-presenting cells. As in Fig. 1*A*, carboxyfluorescein diacetate succinimidyl ester (CFSE)-labeled OT-I cells were primed in vitro under control and test conditions with pOVA/CpG. The abundance of individual generations (defined according to CFSE intensity) relative to the total number of OT-I cells was determined at day 3 (*Left*) and day 6 (*Right*) by flow cytometry. Error bars indicate the SD of technical triplicates. N.s., not significant. Data are representative of three independent experiments.

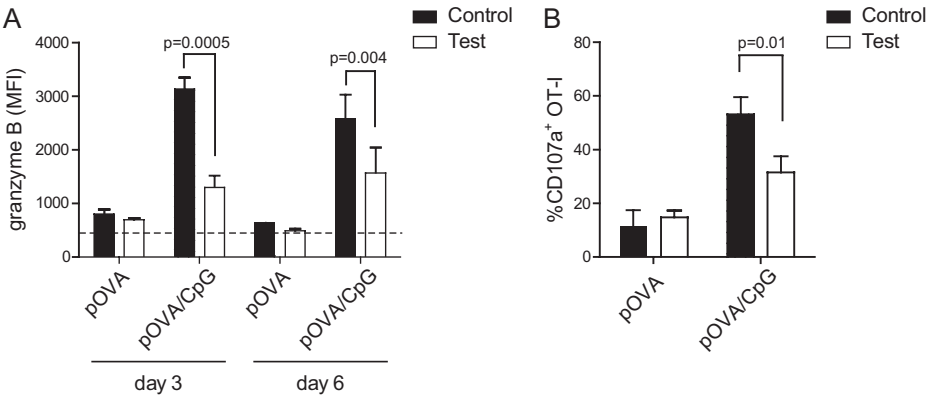

Fig. S2. CD8$^+$ T cells primed by indirectly activated antigen-presenting cells contain less granzyme B and degranulate poorly. OT-I cells were primed in vitro with pOVA or pOVA/CpG under test (*myd88*$^{+/+}$ *H2Kb*$^{-/-}$ + *myd88*$^{-/-}$ *H2Kb*$^{+/+}$) and control (*myd88*$^{+/+}$ *H2Kb*$^{-/-}$ + *myd88*$^{+/+}$ *H2Kb*$^{+/+}$) conditions. (*A*) After 3 and 6 days, the intracellular content of granzyme B in OT-I cells from control and test cultures was measured by flow cytometry. Granzyme B levels are expressed as the median fluorescence intensity. The dotted line indicates the background signal as assessed by staining with the respective isotype control. Error bars indicate the SD of technical replicates (n = 3–6). Pooled data from two independent experiments are shown. (*B*) Degranulation of OT-I cells upon restimulation after 5 d of culture. Percentages of OT-I cells staining positive for CD107a are shown. Error bars indicate the SD of technical triplicates. Data are representative of two independent experiments.

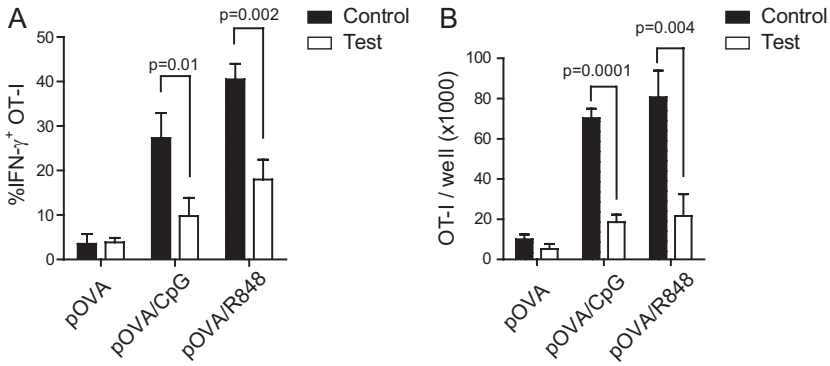

Fig. S3. Adujvanticity of TLR9 and TLR7 agonists is dependent on cognate recognition. OT-I cells were primed in vitro with pOVA, pOVA/CpG, or pOVA/R848 under test (*myd88*$^{+/+}$ *H2Kb*$^{-/-}$ + *myd88*$^{-/-}$ *H2Kb*$^{+/+}$) and control (*myd88*$^{+/+}$ *H2Kb*$^{-/-}$ + *myd88*$^{+/+}$ *H2Kb*$^{+/+}$) conditions. After 3 and 6 d, (*A*) antigen-induced IFN-γ production and (*B*) total number of viable OT-I cells were assessed by flow cytometry. Error bars indicate the SD of technical triplicates. Data are representative of two independent experiments.

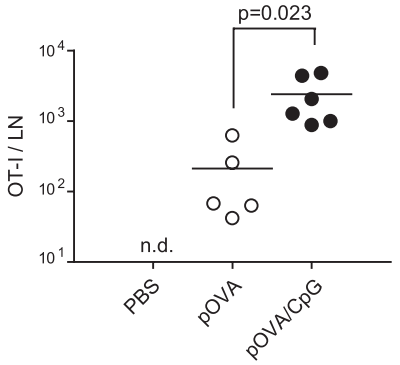

Fig. S4. Indirectly activated antigen-presenting cells promote the proliferation of CD8$^+$ T cells in vivo. Test chimeras were adoptively transferred with OT-I cells and subsequently immunized with pOVA (open circles), pOVA/CpG (filled circles), or injected with PBS. After 7 d, total numbers of OT-I cells in the popliteal lymph nodes were quantified. N.d., not detectable. Pooled data from two independent experiments are shown (n = 5–6).

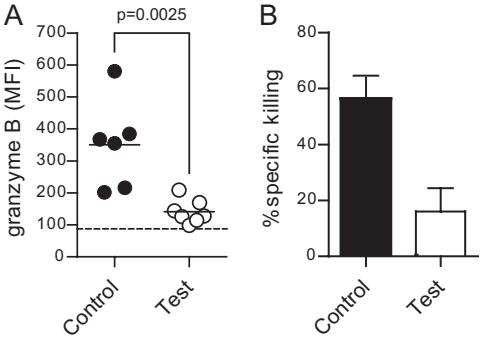

Fig. S5. Cognate pattern recognition is required for the acquisition of cytotoxic effector function in vivo. (*A*) As in Fig. 2, OT-I cells were primed in vivo in test (open circles) or control (filled circles) chimeras. Seven days later, the intracellular granzyme B content of OT-I cells from popliteal lymph nodes was measured by flow cytometry. Median fluorescence intensity values of granzyme B of CD8α$^+$ CD45.1$^+$ CD45.2$^-$ cells are shown. Data are representative of two independent experiments (n = 5). (*B*) P14 cells were primed in vivo in test (*H2Db*$^{-/-}$-B6 + *tlr7*$^{-/-}$-B6 → *tlr7*$^{-/-}$-B6, open bar) or control chimeras (*H2Db*$^{-/-}$-B6 + WT-B6 → WT-B6, filled bar) by injection of gp33/R848. Three days later, an in vivo cytotoxicity assay was performed as described in *Materials and Methods* (main text).