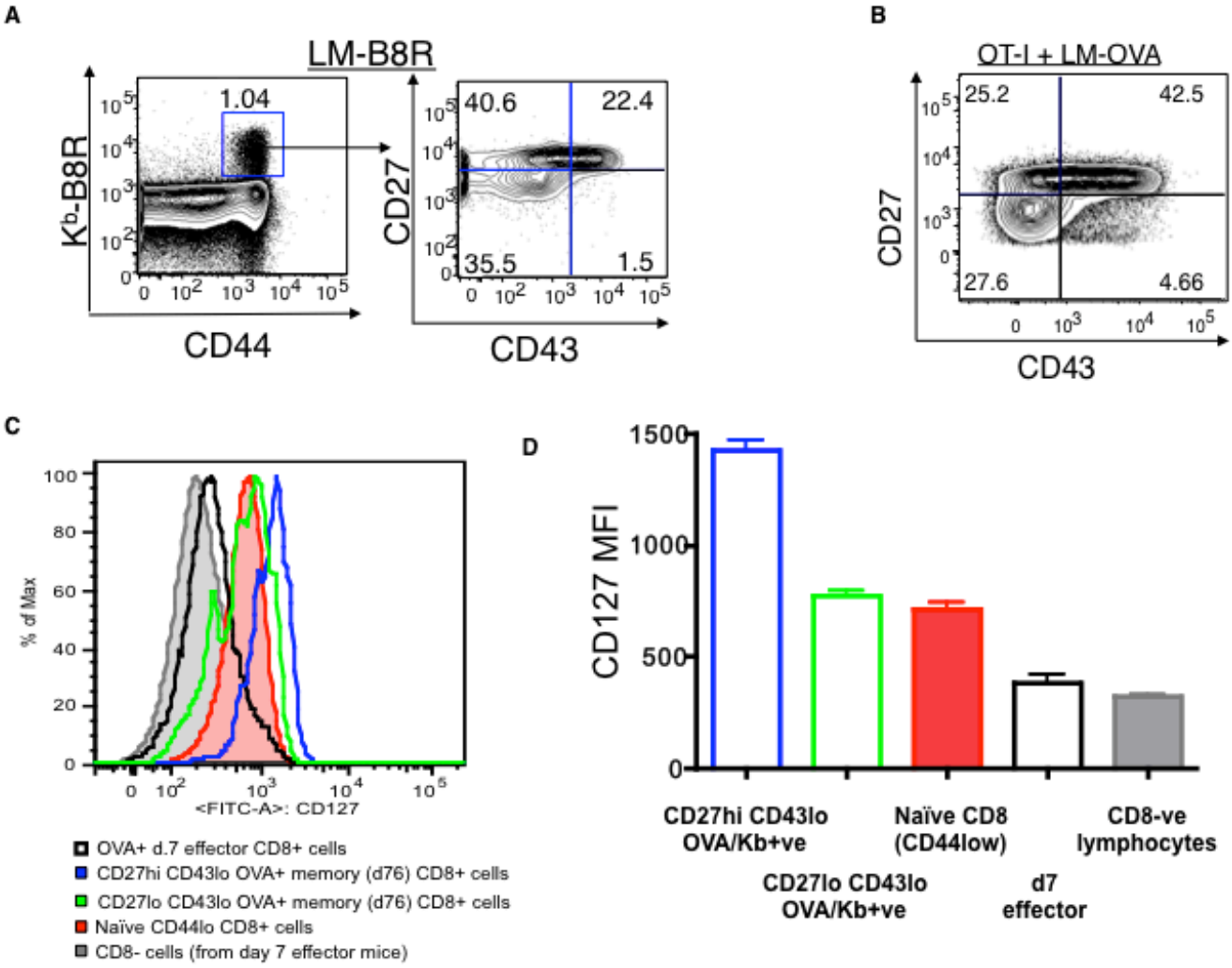
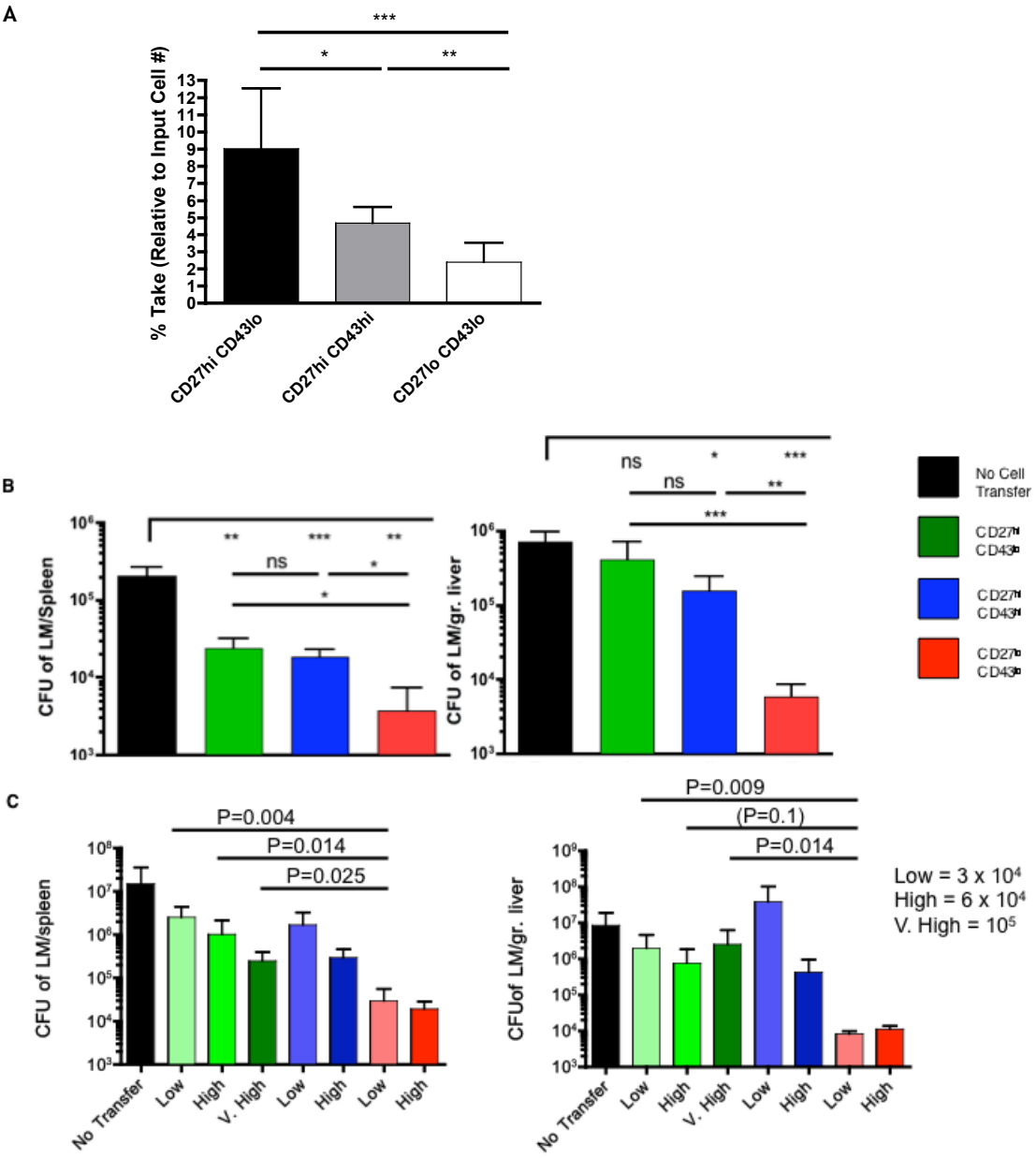
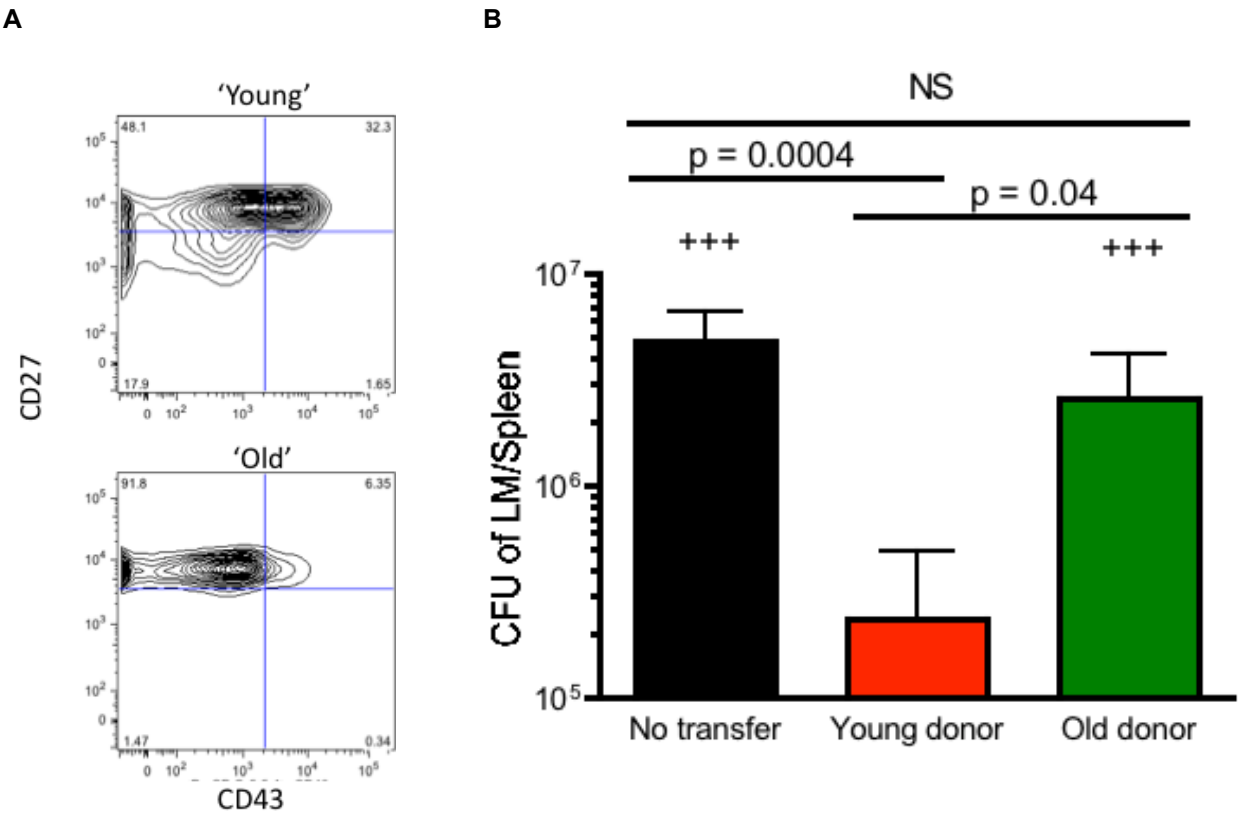


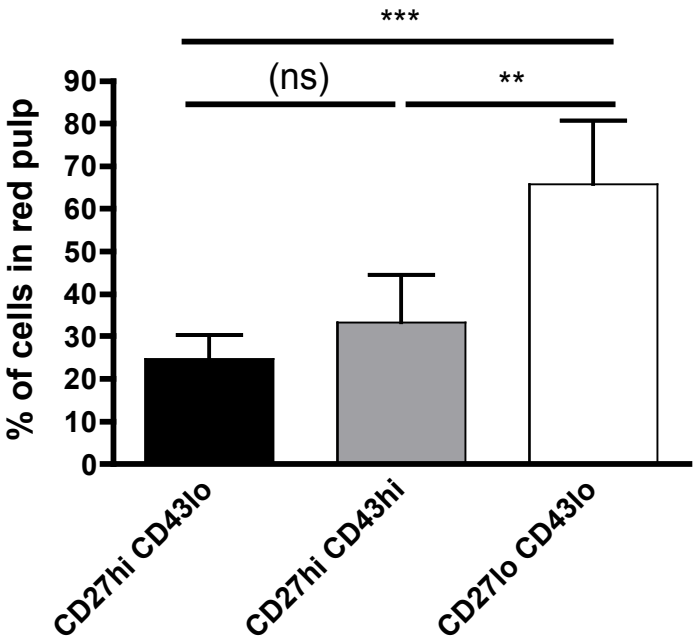
Supplemental Figure 1. *Induction and characterization of CD27^{lo} CD43^{lo} memory cells.* (A, B) CD27/CD43 defined subsets are generated following Listeria infection. (A) Expression of CD27 and CD43 on splenic B8R/K^{b} – tetramer +ve cells isolated 1 month after LM-B8R infection. (B) Expression of CD27 and CD43 on splenic OT-I CD8 T cells one month following adoptive transfer and priming with *ActA* LM-OVA. Data are representative of 2 independent experiments. (C,D) CD27^{lo} CD43^{lo} cells express intermediate levels of CD127. (C) CD127 expression level on OVA/K^{b} tetramer-binding CD8 T cells isolated at an effector timepoint (d.7) or CD27^{hi} CD43^{lo} and CD27^{lo} CD43^{lo} cells isolated at a memory timepoint (d.76), from an *ActA* LM-OVA infected mouse. Naïve CD44^{lo} and CD8-ve lymphocytes are shown as controls. (D) CD127 MFI for populations defined in (C). Data are representative of 6 animals from two distinct experiments and shown as mean +/- SD. Related to Figure 1.



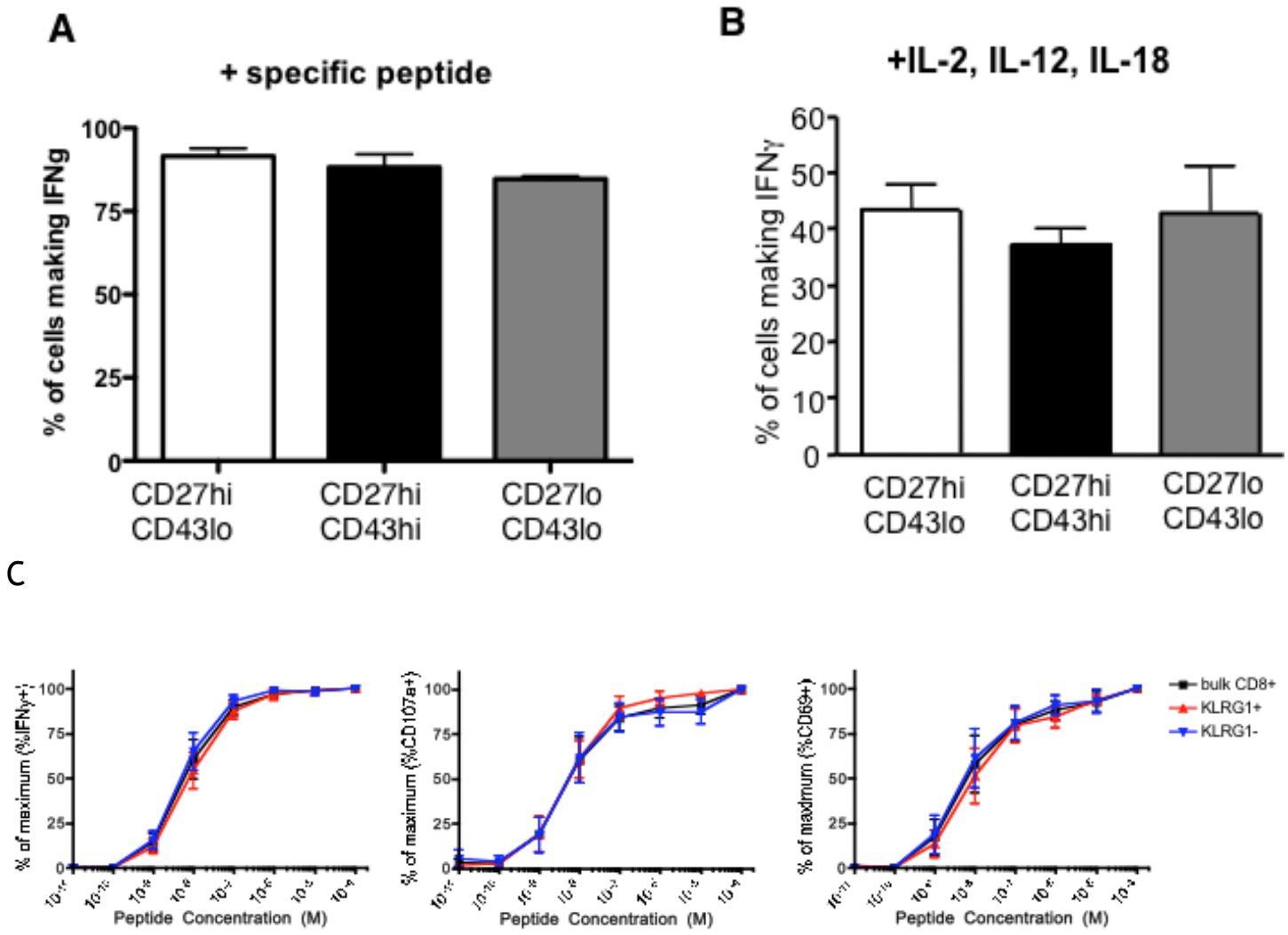
Supplemental Figure 2. *Optimal protection by CD27^{lo} CD43^{lo} CD8 T cells.* (A) Less efficient adoptive transfer of the CD27^{lo}CD43^{lo} subset. Indicated memory subsets were sorted from mice immunized with *ActA* LM-OVA at least one month earlier, and 1.6-3x10^5 cells transferred to recipient mice. The "take" (percentage of input cells) recovered from the spleen was determined 1 day later (combined from 3 experiments). (B) Naïve OT-I CD8 T cells were adoptively transferred and primed with *ActA* LM-OVA. One month later, memory OT-I subsets were isolated and transferred into host mice that were challenged with LM-OVA one day later. At 5 days post infection, the CFU of LM in the spleen and liver was determined (n=3). (C) Memory subsets were sorted and transferred as in A, but with the indicated numbers of transferred cells. Recipient mice were challenged the next day with LM-OVA and CFU in the spleen (left) and liver (right) were quantified at day 5. (n=3). All data show mean +/- SD. P-values are represented as follows: ***, p<0.001; **, p<0.01; *, p<0.05. Related to Figure 2.



Supplemental Figure 3. *Per-cell protective immunity wanes with age of the memory pool, correlating with a loss of CD27^{lo} CD43^{lo} CD8 memory T cells.* P14 TCR transgenic T cells (Thy-1.1 congenic) were adoptively transferred into B6 hosts which were then infected with LCMV. One month later ("young") or 10-13 months later ("old") the P14 population was (A) phenotyped for expression of CD27 and CD43. At the same time (B) young and old memory P14 cells were enriched in bulk, and 10^3 cells were adoptively transferred into normal recipient mice, which were then infected with 2-4 x 10^4 CFU LM-gp33. Protection was assessed in the spleen at day 5. The "+" symbol indicates animals that died prior to harvest for CFU. Data show mean +/- SD. The data in A are representative of 3 experiments, the data in B are compiled from 2 experiments, with similar outcomes from a 3rd experiment using a higher LM-gp33 infectious dose (6 x 10^4 CFU). Expansion of donor P14 cells during the challenge response was variable but similar for both young and old donor groups (data not shown). Related to Figure 3.



Supplemental Figure 4. After adoptive transfer, CD27^{lo}CD43^{lo} memory cells preferentially migrate to the red pulp in the spleen. Cells were sorted and adoptive transfers performed as described for Figure S2A. Distribution of donor cells in the white and red pulp of the spleen was determined using in vivo anti-CD8 staining (as in Figure 5). Data show mean +/- SD. P-values are represented as follows: ***, $p<0.001$; **, $p<0.01$. Related to Figure 5.



Supplemental Figure 5. *Long-lived effector cells exhibit similar cytokine secretion and functional avidity compared to other memory populations.* (A) OT-I and P14 memory CD8 T cells were generated (via adoptive transfer, followed by host infection with *ActA* LM-OVA or LCMV, respectively) and sort purified based on CD27 and CD43 expression. Cells were then incubated for 5-6 hours in vitro with splenocytes coated with the relevant peptide (OVAp or gp33, respectively). Surface staining for Thy-1.1 (the TCR transgenic T cell congenic marker) and intracellular staining for IFN-γ was then performed. Data are compiled from 2 independent experiments. (B) One month after infection of B6 mice with attenuated LM-B8R, memory CD8+ T cells were sorted, based on CD27 and CD43 expression. Cells were then incubated in vitro with a cocktail of IL-2, IL-12, and IL-18 for 18-20 hours. Surface staining for phenotypic markers was performed, followed by intracellular staining for IFN-γ (n=2). The same experiment was also performed after sorting of P14 memory cells with similar results (data not shown). In (C), mice were primed with LCMV, either with or without prior adoptive transfer of naïve P14 CD8 T cells. At >1 month, splenocytes were isolated and challenged in vitro with titrated gp33 peptide for 5 hours. The response of P14 cells (gated on congenic Thy-1.1 marker) or bulk CD8 T cells (for animals which did not receive P14 transfer) was assessed by measuring induction of IFN-γ, CD107a (a measure of degranulation) or CD69 expression. Data are gated on KLRG1+ve (corresponding to CD27lo CD43lo cells) versus KLRG1-ve (corresponding to CD27hi subsets), since CD27 (but not KLRG1) expression changes during in vitro activation (not shown). Data are compiled from 4 experiments (2 with P14 adoptive transfer, 2 measuring polyclonal B6 responder cells). All data show mean +/- SD. Related to Figure 6.