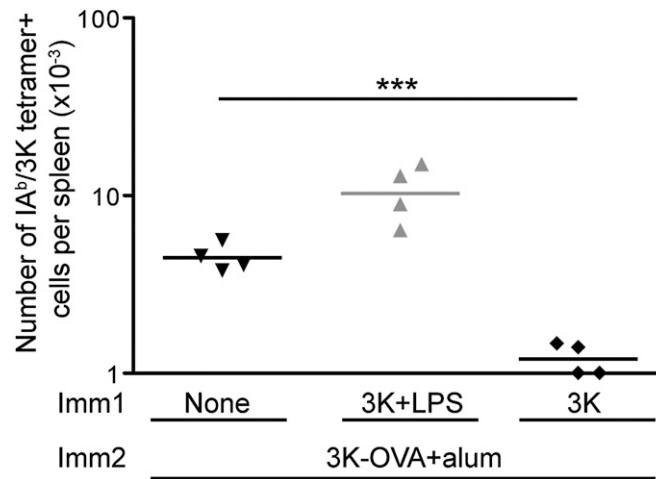
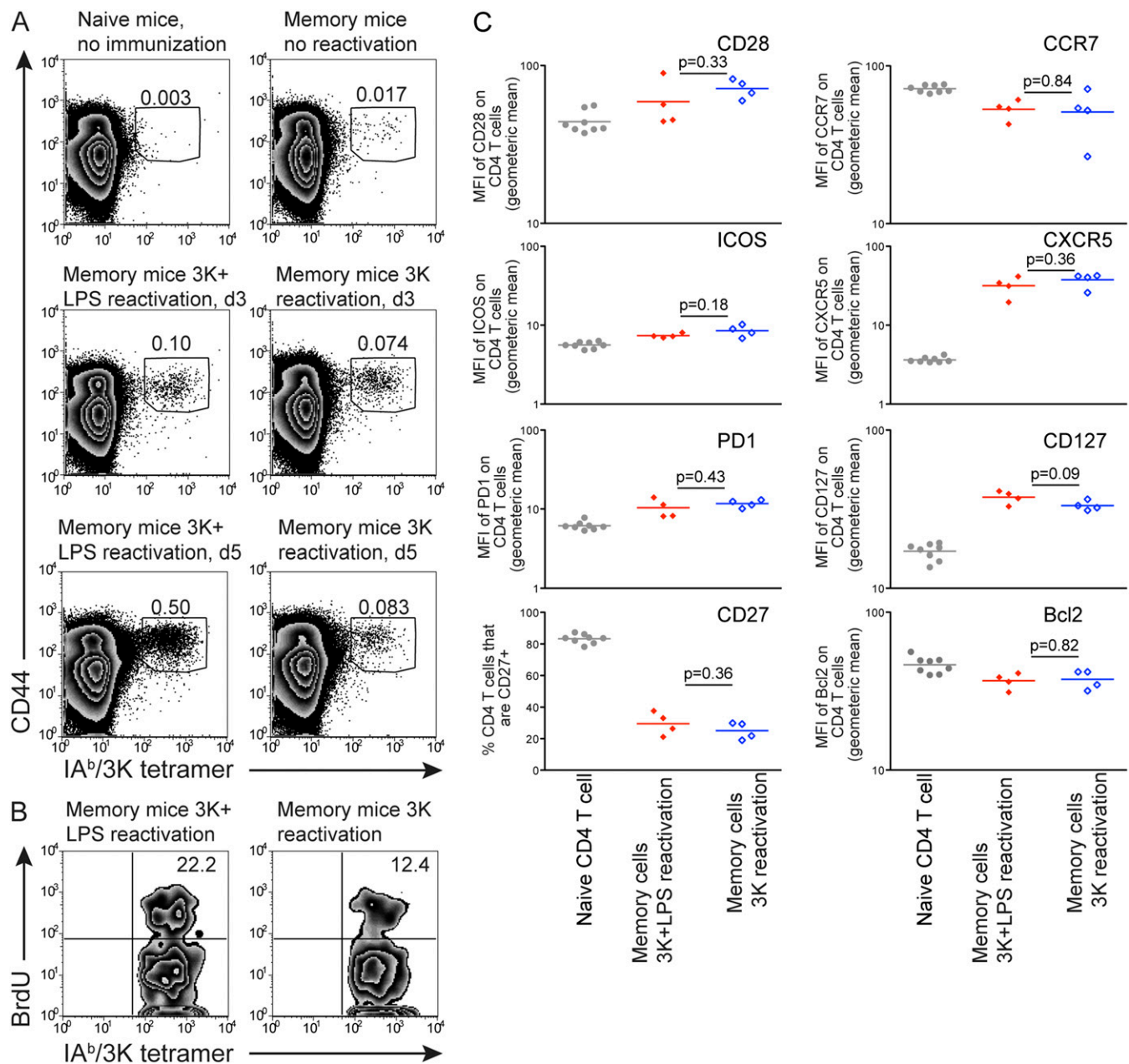


# Supporting Information

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**Fig. S1.** Naive mice injected with soluble 3K peptide fail to respond to further reactivation. 40 d after naive B6 mice had been injected with 3K peptide or 3K peptide and lipopolysaccharide (LPS) i.v. these and naive mice were immunized with 3K-ovalbumin (OVA) and alum i.p. After an additional 6 d, the numbers of IA<sup>b</sup>/3K tetramer<sup>+</sup> CD44<sup>hi</sup> in the spleens of these and naive age-matched mice were examined. Each symbol represents one mouse and the line shows the mean of the group. The x axis is set at the limit of detection as determined by the number of IA<sup>b</sup>/3K tetramer<sup>+</sup> CD44<sup>hi</sup> cells in naive animals. These data are from one of two experiments with four mice per group, \*\*\**P* < 0.001.



**Fig. S2.** IA<sup>b</sup>/3K-specific memory CD4 T cells proliferate in response to 3K peptide delivered with or without LPS and return to the memory pool following reactivation. B6 mice immunized with 3K peptide and LPS i.v 40 d previously were injected with 3K + LPS or 3K without adjuvant. The percentages of CD4<sup>+</sup> cells that were CD44<sup>hi</sup> IA<sup>b</sup>/3K tetramer<sup>+</sup> were examined before or 3 and 5 d after the second immunization (A). These animals were injected with bromodeoxyuridine (BrdU) 15 h before the percentages of CD44<sup>hi</sup> IA<sup>b</sup>/3K tetramer<sup>+</sup> that were BrdU<sup>+</sup> were examined on day 3 after second immunization (Imm2) (B). In A, cells are gated on CD4<sup>+</sup> dump negative live cells, and in B, on the CD44<sup>hi</sup> IA<sup>b</sup>/3K tetramer<sup>+</sup> cells. The numbers in the plots show the percentages of the cells within the indicated gate or quadrant. These data are representative FACS plots from two experiments with three to four mice per group. Alternatively, these mice were rested for an additional 40 d following the second immunization and their expression of various cell-surface and intracellular molecules examined. Cells were either gated on naive CD4 T cells (CD4<sup>+</sup> CD44<sup>low</sup>, gray symbols) or on memory CD44<sup>hi</sup> IA<sup>b</sup>/3K tetramer<sup>+</sup> cells from mice immunized twice with 3K + LPS (red symbols) or immunized once with 3K + LPS and reactivated with 3K alone (blue symbols) (C). In each graph, the symbols represent individual mice (naive CD4 T cells from the two immunized groups are combined as no differences were expected). The horizontal line in each group represents the mean for that group. The data are from one of two representative experiments with three to four mice per group.



