

Figure S8
Flow cytometric analyses of vaccine-primed gp70:Kb/p15E-dimer+ PD-1+ LAG-3+ CD8 T cells

## Figure S8 continued

(a) B6 mice were untreated (ctrl, n=4), injected with 2.5 x 10<sup>5</sup> irradiated KPC7735 cells (KPC-ir; n=9) or IFN-γ-treated KPC7735 cells irradiated at d3 post IFN-γ treatment start (KPC-Id3-ir; n=8). On day 12 post immunization, Cr-1:Kb/cr<sub>16-24</sub>- and gp70:Kb/p15E-specific CD8 T-cell frequencies were determined in the spleen. Graphs show the actual proportions (%) of Kb/cr<sub>16-24</sub>-dimer+ and K<sup>b</sup>/p15E-dimer<sup>+</sup> cells among all CD8 T cells ± SD and combine data out of two independent experiments. For statistical evaluation, groups were compared using one-way ANOVA (with 95% CI) followed by Tukey's multiple comparisons test (p < 0.05 (\*), p<0.0001 (\*\*\*\*), ns, not significant). (b) Expression of PD-1 and LAG-3 was assessed on the Kb/p15E-dimer+ CD8 T-cell populations in the spleens of B6 mice vaccinated with KPC-ir cells (n=5, data from one experiment) and KPC-Id3-ir cells (n=8, combined data from the two independent experiments also shown in (a)). Graphs show the frequencies (%) of PD-1+, LAG-3+ and PD-1/LAG-3 double positive cells among all Kb/p15E-dimer+ CD8 T cells  $\pm$  SD. Groups were compared using unpaired student's t test (p < 0.05 (\*)). (c) One representative dot blot per group is shown.