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**Figure S1. TLR stimulation at the time of T-cell activation with lower affinity epitopes *in vitro* affects expression of PD-1.** OT-1 splenocytes were stimulated with either the moderate affinity SIINTEKL (panel A) or the low affinity SIINFEKP (panel B) peptides, alone or in combination with the designated TLR agonists. At daily intervals up to 4 days, the expression of PD-1, LAG3, and 4-1BB was assessed by flow cytometry. Asterisks indicate p<0.01. Results are from one experiment, with samples assessed in triplicate, and are representative of two independent experiments.



**Figure S2. TLR stimulation at the time of T-cell activation *in vitro* does not reduce expression of other T-cell checkpoint molecules.** OT-1 splenocytes were stimulated *in vitro* with SIINFEKL peptide alone or in combination with the designated TLR agonists. At daily intervals up to 4 days, the expression of additional immune checkpoints (CTLA-4, TIM3, VISTA, CD244.2, TIGIT, and CD160) was assessed by flow cytometry. Asterisks indicate p<0.01. Results are from one experiment, with samples assessed in triplicate, and are representative of two independent experiments.



**Figure S3. TLR stimulation at the time of T-cell activation *in vitro* affects expression of PD-1 that is not affected by blockade of CD80, CD86, or OX40L.** Splenocytes were prepared from the spleens of OT-1 mice and stimulated *in vitro* with the SIINFEKL (OVA) peptide in the presence or absence of specific TLR agonists (TLR 1/2 (Pam3CSK4) or TLR 9 (ODN1826), or blocking antibodies for OX40L (0.05 µg/mL), CD80 (0.6 µg/mL), or CD86 (5 µg/mL). Shown is the MFI of PD-1 (panel A) and 4-1BB (panel B) on CD3+CD4-CD8+ T-cells collected daily for 4 days. Asterisks indicate p<0.01. Results are from one experiment, with samples assessed in triplicate, and are representative of two similar independent experiments.



**Figure S4. TLR1/2 or TLR7 ligand adjuvants alone do not have significant anti-tumor efficacy in the absence of antigen-specific T-cell activation**: Ovalbumin-expressing E.G7 cells were implanted in C57BL/6 mice and permitted to grow until palpable (~14 days). OT-1 T cells were then adoptively transferred and mice were immunized the following day with SIINFEKL (OVA) or non-specific (NS) peptide, alone or with TLR1/2 (Pam3) or TLR7 (Gard) agonist. Shown are the tumor growth curves (median +/- standard error, n=5 animals per group). Asterisks indicate P<0.001 Results are from one experiment, with N=6 mice/group.