Supporting Information

Wei et al. 10.1073/pnas.1305394110

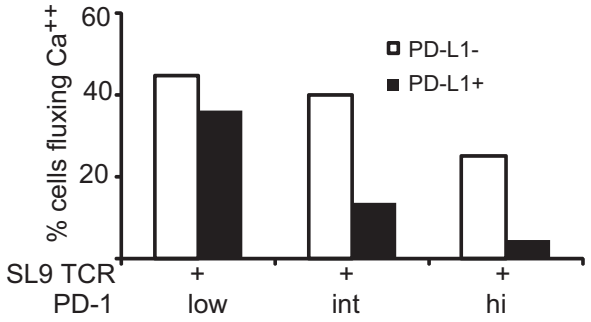


Fig. S1. High levels of programmed death 1 (PD-1) can inhibit Ca^{++} flux. RNA (10 μg) encoding both chains of the A2-SL9–specific T-cell receptor (TCR) was mixed with 0, 0.1 (low), 1 μg (int), or 10 μg (hi) RNA encoding PD-1 and was transfected into resting primary human CD8 T cells. T cells pulsed with the calcium-sensitive dye Fura-2 were injected into the chamber containing poly-L-lysine–anchored K.A2 DsRed SL9.PD-L1 artificial antigen-presenting cells (aAPCs), and the 510-nm emissions excited by 340 nm and 380 nm were captured immediately for 45 min at 5-s intervals. The plot shows the number of T cells that flux Ca^{2+} at least once during the 45-min experiment as a function of PD-1 expression. An increase in the ratio of 510-nm emission excited by 340 nm to that excited by 380 nm indicated increased intracellular calcium level. T cells with a ratio more than two times higher than before stimulation were considered to have fluxed calcium. Data are representative of three individual experiments.

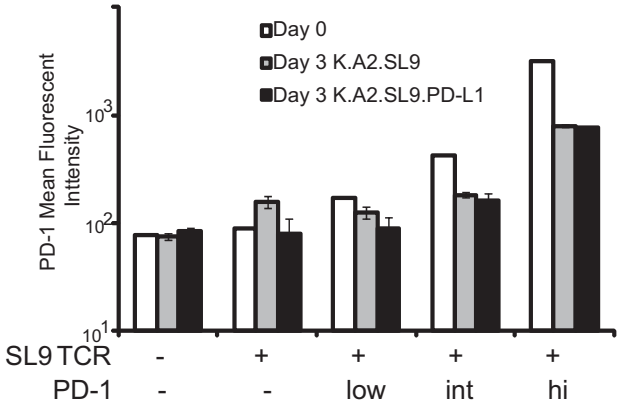


Fig. S2. Time course of PD-1 expression after RNA transfection. RNA (10 μg) encoding the A2-SL9–specific TCR was mixed with 0, 0.125, 1, or 10 μg RNA encoding PD-1 and was transfected into equally mixed resting primary human CD8 and CD4 T cells. After overnight culture, PD-1 expression was measured by flow cytometry (day 0, white bars). T cells then were stimulated with either K.A2.SL9 (gray bars) or K.A2.SL9 PD-L1 (black bars) aAPCs for 3 d, and PD-1 expression was measured again (day 3). Data are representative of three individual experiments.

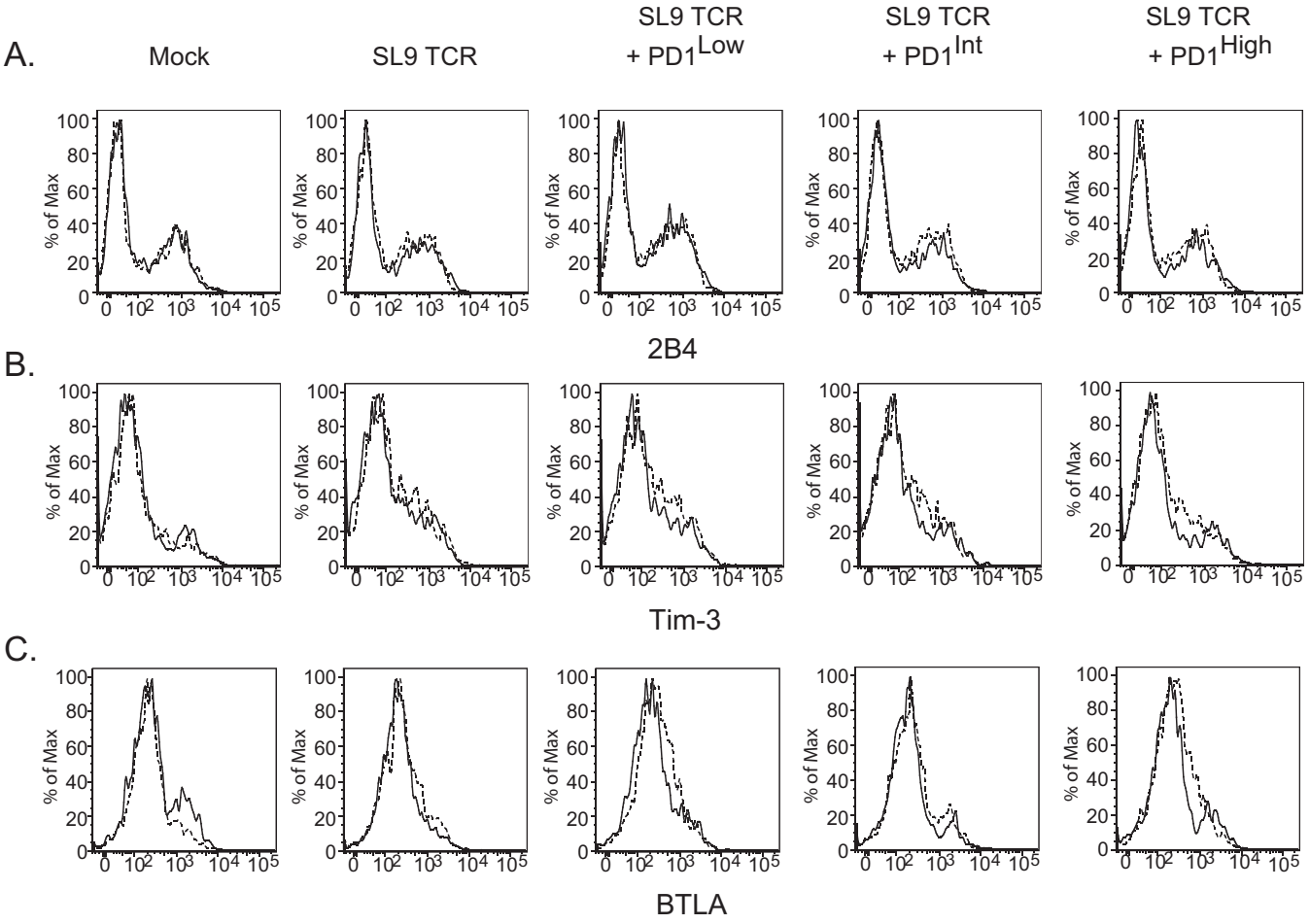
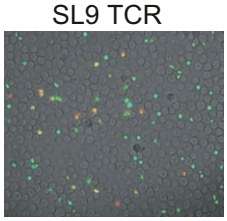


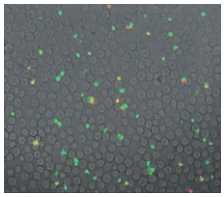
Fig. S3. Time course of PD-1 expression after RNA transfection. RNA (10 μg) encoding the A2-SL9–specific TCR was mixed with 0, 0.125 μg (Low), 1 μg (Int), or 10 μg (High) RNA encoding PD-1 and was transfected into equally mixed resting primary human CD8 and CD4 T cells. Then 2B4 (CD244) (*A*), T-cell immunoglobulin domain and mucin domain 3 (Tim-3) (*B*), and B- and T-lymphocyte attenuator (BTLA, CD272) (*C*) expression was measured 3 d after stimulation with K.A2.SL9 (solid lines) or K.A2.SL9.PD-L1 (long dashed lines).



Movie S1. Primary data from which the still image in Fig. 2*C* was collected showing T cells that were not transfected with additional PD-1. Movies are run at 20× faster than real time.

Movie S1

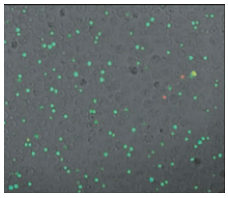
SL9 TCR + PD-1$_{LOW}$



Movie S2. Primary data from which the still image in Fig. 2*C* was collected showing T cells that were transfected with a low amount (0.1 μg) of PD-1. Movies are run at 20× faster than real time.

Movie S2

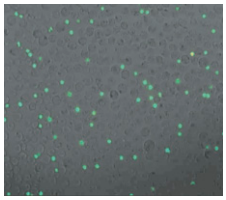
SL9 TCR + PD-1$_{INT}$



Movie S3. Primary data from which the still image in Fig. 2*C* was collected showing T cells that were transfected with an intermediate amount (1 μg) of PD-1. Movies are run at 20× faster than real time.

Movie S3

SL9 TCR + PD-1$_{HIGH}$



Movie S4. Primary data from which the still image in Fig. 2*C* was collected showing T cells that were transfected with a high amount (10 μg) of PD-1. Movies are run at 20× faster than real time.

Movie S4