Supplemental Information

Supplemental Figure Legends:

Supplemental Figure S1 related to Figure 1: Cytolytic ability of high and low avidity CTL clones at varying levels of peptide concentration.

Peptide pulsed T2 cells from 100ng/ml down to 0.0001ng/ml (100fg/ml) are analyzed for percent specific release (51-chromium assay data), when incubated with either high avidity clone 476.139 or low avidity clone 476.105. The plot of percent specific release versus peptide concentration for high and low avidity clones used in our experiments indicates the limit for an effective cytolytic response. The peptide concentration limit for killing effect in high avidity clones was measured to be between 0.0001ng/ml to 0.001ng/ml (100fg/ml to 1pg/ml). Whereas similar percent specific release is observed in low avidity clone starting at peptide concentrations as high as 1ng/ml. Increasing peptide concentrations compensate for diminished ability of low avidity CTLs to lyse target cells to similar levels of high avidity counterparts.

Supplemental Figure S2 related to Figure 2: Inhibition of high avidity CTL-mediated lysis of different melanoma cell lines by low avidity CTL and effect of various preincubation times with low avidity CTL on inhibition of high avidity CTL-mediated lysis of melanoma cells.

^{51}Cr-labeled melanoma cell lines Mel526, Malme-3M and MeWo were preincubated one hour with, or without, low avidity gp100-specific or MART-specific low avidity CTL before adding high avidity CTL of similar specificity for an additional 1.5 hours incubation. Percent inhibition of lysis was calculated as described in the methods section. Black bars: melanoma cells combined with low and high avidity gp100 specific CTL. Grey bars: melanoma cells combined with low and high avidity MART-specific CTL. Assays were performed in triplicates and error bars reflect standard deviation between these. ^{51}Cr-labeled Mel526 melanoma cells were preincubated 0.5, 1.0, 1.5, 2.0, 2.5 or 3.0 hours with, or without, low avidity gp100-specific low avidity CTL before adding high avidity CTL of similar specificity for additional 1.5 hours

incubation. For the "0 hour" experiment, low and high avidity CTL were added simultaneously to melanoma cells and incubated 1.5 hours. Percent inhibition of lysis was calculated as described in the methods section. Assays were performed in triplicates and error bars reflect standard deviation between these.

Supplemental Figure S3 related to Figure 2: Antigen-specific inhibition of high avidity CTL mediated lysis by multiple different low avidity gp100-specific and MART-specific CTL.

^{51}Cr-labeled mel526 melanoma cells were preincubated 1.5 hours with, or without, 3 different gp100-specific and 7 different MART-specific low avidity, and one viral antigen-specific, CTL before adding high avidity gp100- and MART-specific CTL of similar or different specificity, for additional 1.5 hours incubation. Percent inhibition of lysis was calculated as described in the methods section. The antigen-specific nature of inhibition by low avidity clones is displayed as the ratio of percent inhibition of gp100- vs. MART-specific high avidity CTL: Negative value bars indicate specific inhibition of high avidity MART-specific CTL-mediated melanoma cell lysis, and positive value bars indicate specific inhibition of high avidity gp100-specific CTL-mediated melanoma cell lysis. Assays were performed in triplicates. Error bars represent standard deviation between assays (each in triplicate).

Supplemental Figure S4 related to Figure 3: Inhibition of mel526 cell viability by antigen-specific low avidity CTL in three-dimensional (3D) tumor spheroid culture system.

Calcein AM stained Mel526 melanoma cell spheroids were incubated with the presence of high avidity CTLs, low avidity CTLs, or high plus low avidity CTLs for 24 hrs. Following incubation, samples were excited at 488 nm, and green fluorescence emission for calcein (ie, 530/30 bandpass) was measured. Reduced levels of green fluorescence represent dead cells. Assays were performed in duplicates. Error bars represent standard deviation between assays. Error bars represent standard deviation between assays (High vs. Low; $p \leq 0.01$, High vs. Low + High; $p \leq 0.01$).

Supplemental Figure S5 related to Figure 6: Increasing peptide concentration enhance killing by low avidity CTL

Changes in expression of apoptotic markers 7AAD and Annexin V on G209n peptide-pulsed HmyA2GFP Target Cells: at 1pg/ml and 10pg/ml in the presence of low avidity CTLs, high avidity CTLs, and the combination of high and low avidity CTLs. In this assay CD8 cells were gated out and only the target cells were analyzed for 7AAD and Annexin V expression.

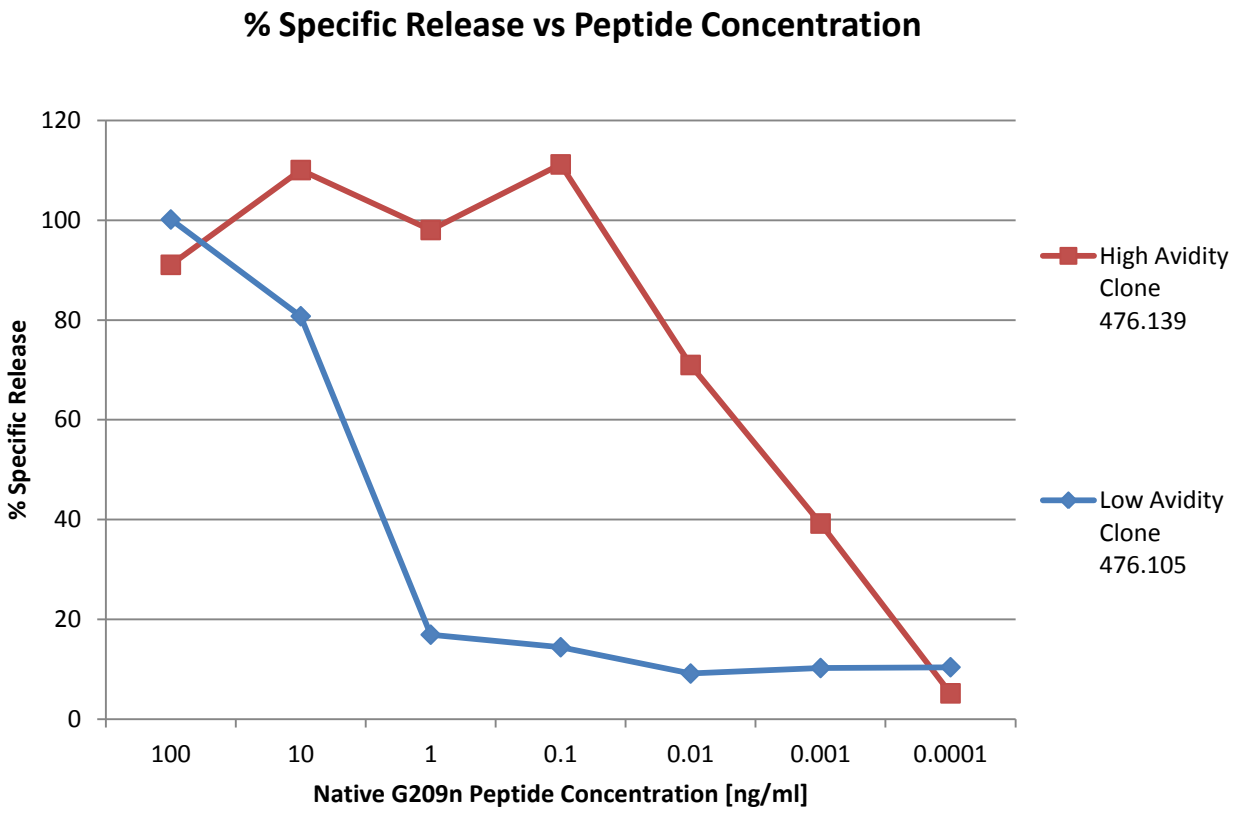
Supplemental Figure S6 related to Figure 1 and 5: Peptide concentration dependent cytotoxicity and trogocytosis of G209n specific high and low avidity CTL clones.

Combined representation of data collected indicating the threshold G209n peptide concentrations required for either cytotoxicity or trogocytosis in the G209n specific low and high avidity CTL clones.

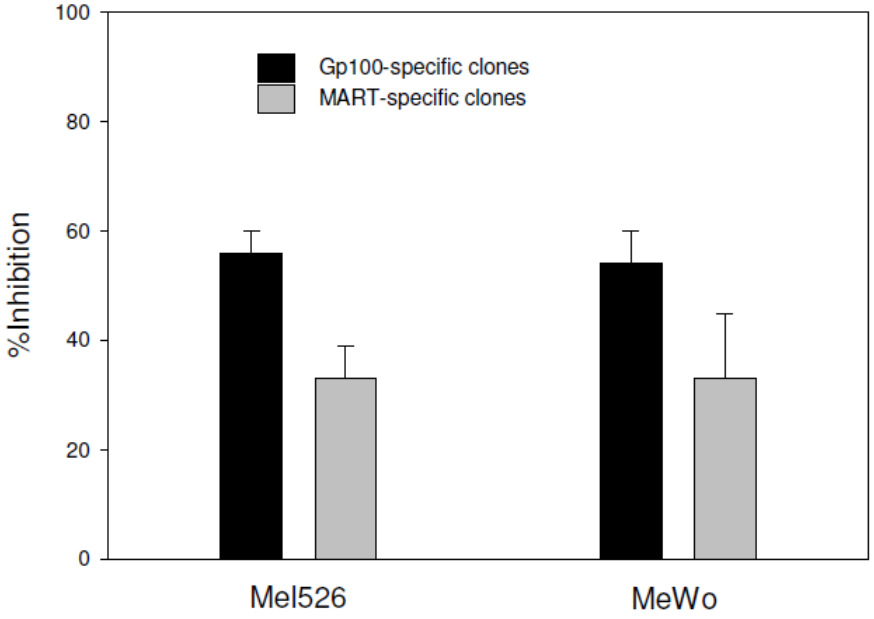
Supplemental Video 1 related to Figure 4: Live cell video of interactions between the T cells and HmyA2GFP cells during trogocytosis.

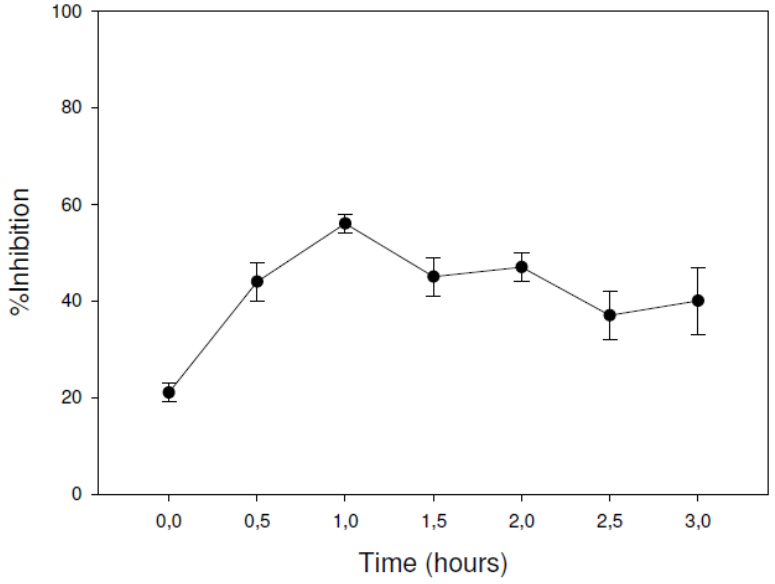
Target cells are G209n peptide pulsed HmyA2GFP cells (green) and the G209 CTLs are stained with anti-CD8 (blue). Within 5 minutes, capturing of HLA-GFP clusters by T cells can be visualized.

Supplemental Figure S1.



Supplemental Figure S2.





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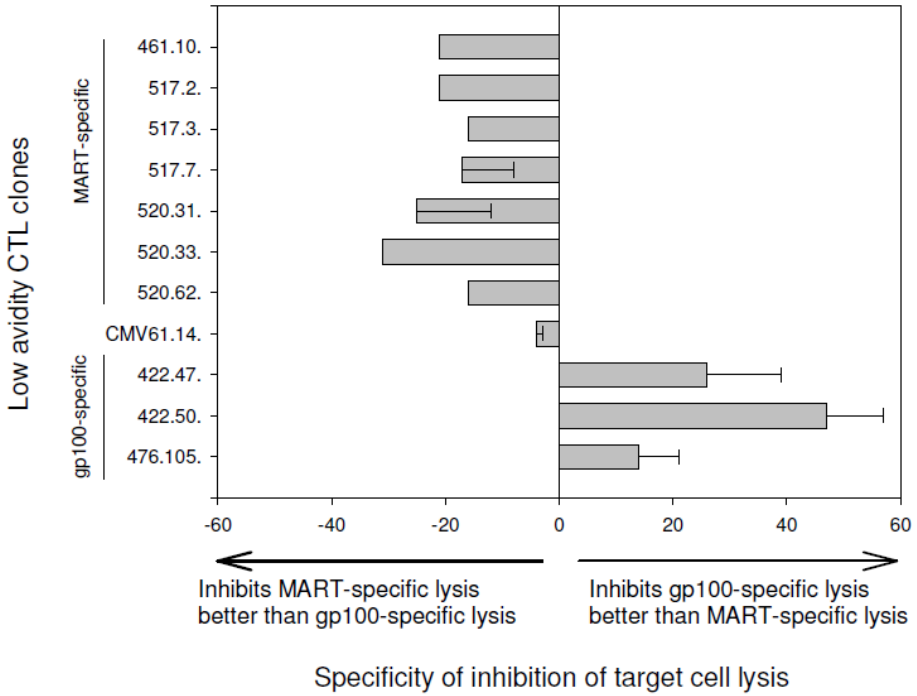

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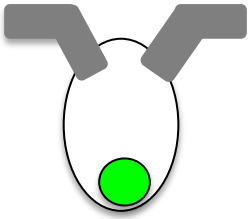
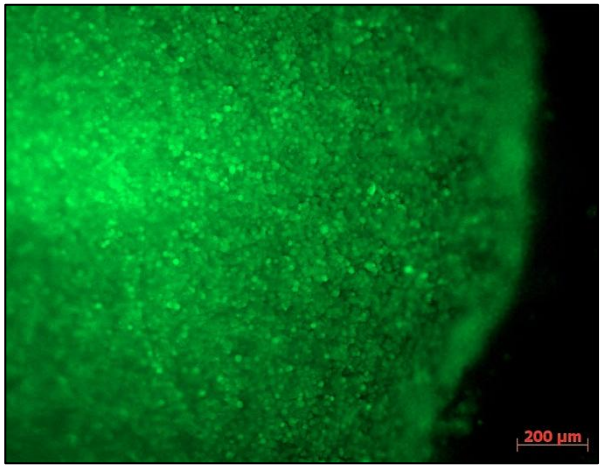
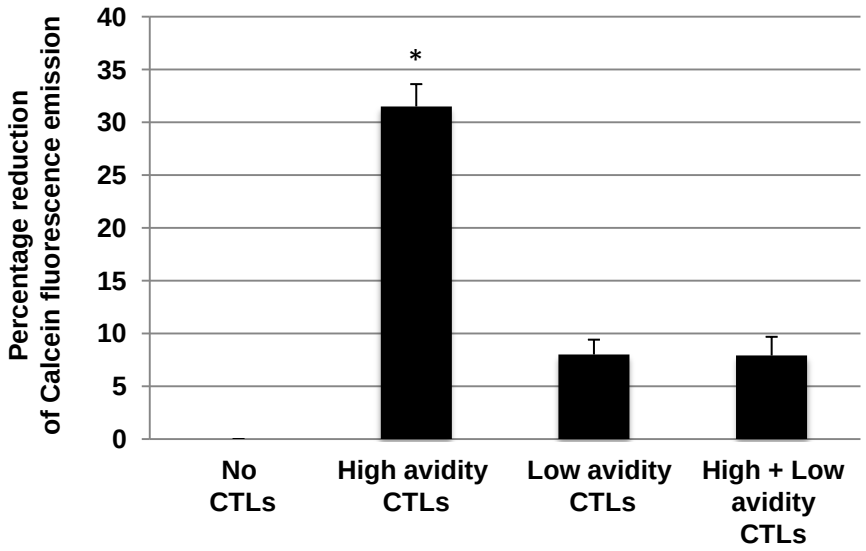


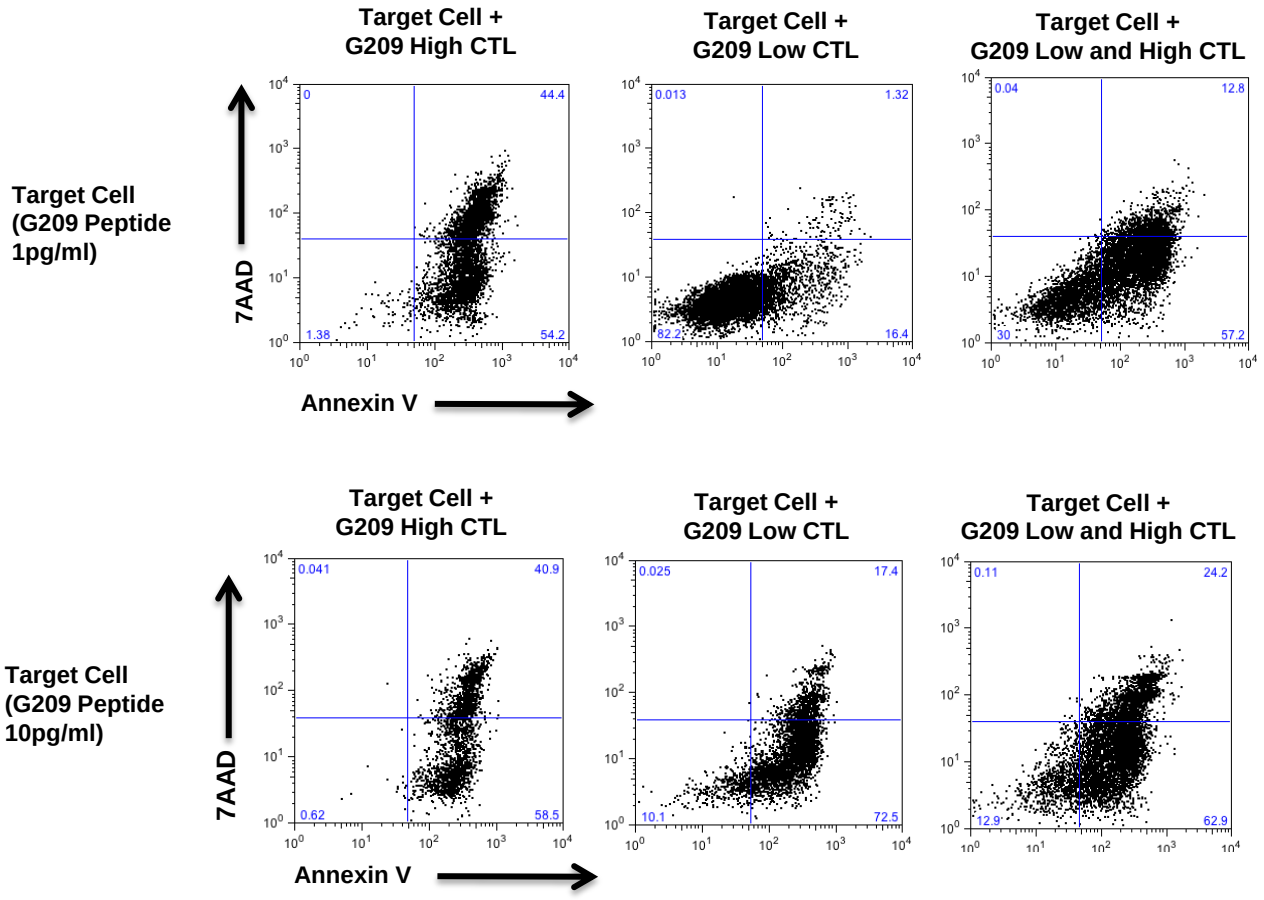
Spheroid formation in hanging drop culture

Calcein stained Mel526 spheroid





Supplemental Figure S5.



Supplemental Figure S6.