Supplemental Video 1:

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 Experimental Procedures

Apoptosis Assay

HmyA2GFP APCs or mel526 cells were co-cultured with either high, low, or both high and low CTLs for 2 hours at room temperature, followed by staining with anti-CD8 surface marker for 15 minutes in order to distinguish the CTLs from the target cells. The cells were then incubated with 5µl Annexin V APC and 5µl 7-aminactinomycin D (7-AAD) in Annexin-binding buffer at room temperature for 15 minutes in the dark and immediately followed by FACS analysis. To quantify apoptotic target cells, the percentage of CD8-negative and Annexin V/7AAD double-positive cells was calculated.

Staining of mel526 cell line with calcein AM intracellular dye

To assess the viability of mel526 cell line before and after addition of high or low avidity CTL clones, we employed a calcein AM fluorescent detection viability assay (Wartenberg and Acker, 1995). In live cells the non-fluorescent calcein AM (BD Biosciences) is converted to a green-fluorescent calcein after hydrolysis by intracellular esterases, and diminishes in fluorescent intensity after cellular death. Briefly, calcein AM at a final concentration of 2µM was incubated with $1.0x10^6$ mel526 cells in 1mL 1xPBS for 30 minutes as described previously (Zhang et al., 2005). Cells were washed 3 times with 1xPBS to remove excess stain and were ready for use.

Generation of 3D mel526 tumor spheroids and quantification of spheroid cellular viability

To mimic the 3D environment which tumor cells interact with their surroundings, specifically as it pertains to G209n-specific high and low avidity CTLs, we employed a hanging drop 3D spheroid formation method using mel526 cells. Spheroid cellular death was quantified by a fluorescent detection viability assay. Formation of the 3D spheres was performed as described previously (Tung et al., 2011). Briefly, $1.0x10^5$ mel526 cells previously stained with calcein AM, as described earlier, were seeded into a single well of the Perfecta3D 96 well hanging drop plate

(3DBiomatrix, Ann Arbor, MI) at a final volume of 50µl D10 medium. Drop plates are incubated at 37 degrees for overnight and cells naturally form spheroids by the force of gravity. Once spheroids were formed, the initial fluorescence was measured using a Wallac Victor2 multilabel counter (Perkin Elmer) for green fluorescent protein like emission. Subsequently, G209n specific high avidity CTLs, low avidity CTLs, or combination of high plus low were added to the spheroid inside the hanging drop at a ratio of 1:1 (Target cell: CTL). Fluorescent readings were taken again after 24 hours to determine cellular spheroid viability by percent reduction of initial reading for each mel526 spheroid/CTL co-culture.

Supplemental References:

Tung, Y.C., Hsiao, A.Y., Allen, S.G., Torisawa, Y.S., Ho, M., and Takayama, S. (2011). High-throughput 3D spheroid culture and drug testing using a 384 hanging drop array. The Analyst *136*, 473-478.

Wartenberg, M., and Acker, H. (1995). Quantitative recording of vitality patterns in living multicellular spheroids by confocal microscopy. Micron (Oxford, England : 1993) *26*, 395-404.

Zhang, X., Wang, W., Yu, W., Xie, Y., Zhang, X., Zhang, Y., and Ma, X. (2005). Development of an in vitro multicellular tumor spheroid model using microencapsulation and its application in anticancer drug screening and testing. Biotechnology progress *21*, 1289-1296.