**Time lapse live video (TLLV) microscopy**

Interactions between CTL and tumor cells were assessed by time lapse live microscopy, using a protocol previously published by our group ([1](#_ENREF_1)). Briefly, 24 hours prior to microscopy, tumor target cells were plated in an 8 well ibidi chamber slide (IBIDI, DKSH, Melbourne, Australia) at a concentration of 1x105 cells/ml. Two x 104 Fluo-4 labeled [labeled for 20 min with 1 μM Fluo-4 and 0.02 % (w/v) Pluronic F-127 carrier at 37°C/10% CO2) CTL were added to chamber slides containing targets in media containing 100 μM of PI. Chamber slides were mounted on a heated stage within a temperature controlled chamber maintained at 37°C and constant CO2 concentrations (5%) were infused using a gas incubation system with active gas mixer (‘The Brick’, ibidi, Germany). Optical sections were acquired through the center of the cells by sequential scans of Fluo-4 (excitation 488 nm) and Propidium Iodide (PI) (excitation 561 nm) or Brightfield/DIC on a TCS SP5 confocal microscope (Leica Microsystems, Deerfield, IL) using a 40x (NA 0.85) air objective and Leica LAS AF software. For the 488 and 561 channels, the pinhole was set to 4.2 AU, giving a section thickness of 5µM and XY pixel size of 378.8nM. Images were acquired at ~6-7 frames/min as described. Image analysis was performed using Leica LAS AF Lite software or MetaMorph Imaging Series 7 software (Universal Imaging, Downingtown, PA).

**References**

1. Lopez JA, Susanto O, Jenkins MR, Lukoyanova N, Sutton VR, Law RH, et al. Perforin forms transient pores on the target cell plasma membrane to facilitate rapid access of granzymes during killer cell attack. Blood. 2013;121:2659-68.