Electronic Supplementary Material (ESI) for Integrative Biology

Supporting Information

Live Cell Imaging in a Micro-Array of Acoustic Traps Facilitate Quantification of Natural Killer Cell Heterogeneity

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Supporting figures

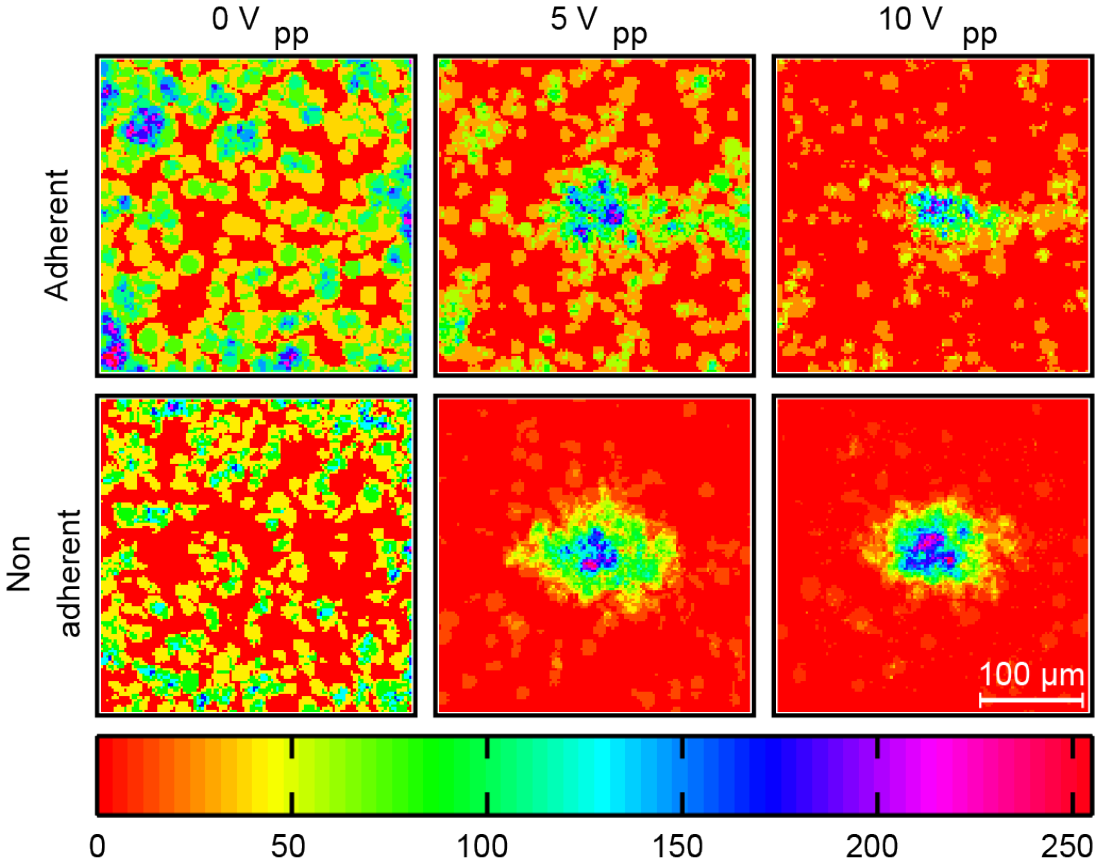

Figure S1. Non-adherent cells are more efficiently trapped by the ultra sound compared to adherent cells. Superposition colormap of 100 wells for adherent 293T (top) and non-adherent 221 cells (bottom) at different actuation voltages: 0 V_{pp}, 5 V_{pp} and 10 V_{pp}. Cells were seeded prior to ultrasonic exposure (0 V_{pp}) becoming randomly distributed in the wells. The fraction of firmly adhered 293T cells was not trapped by the ultrasound.

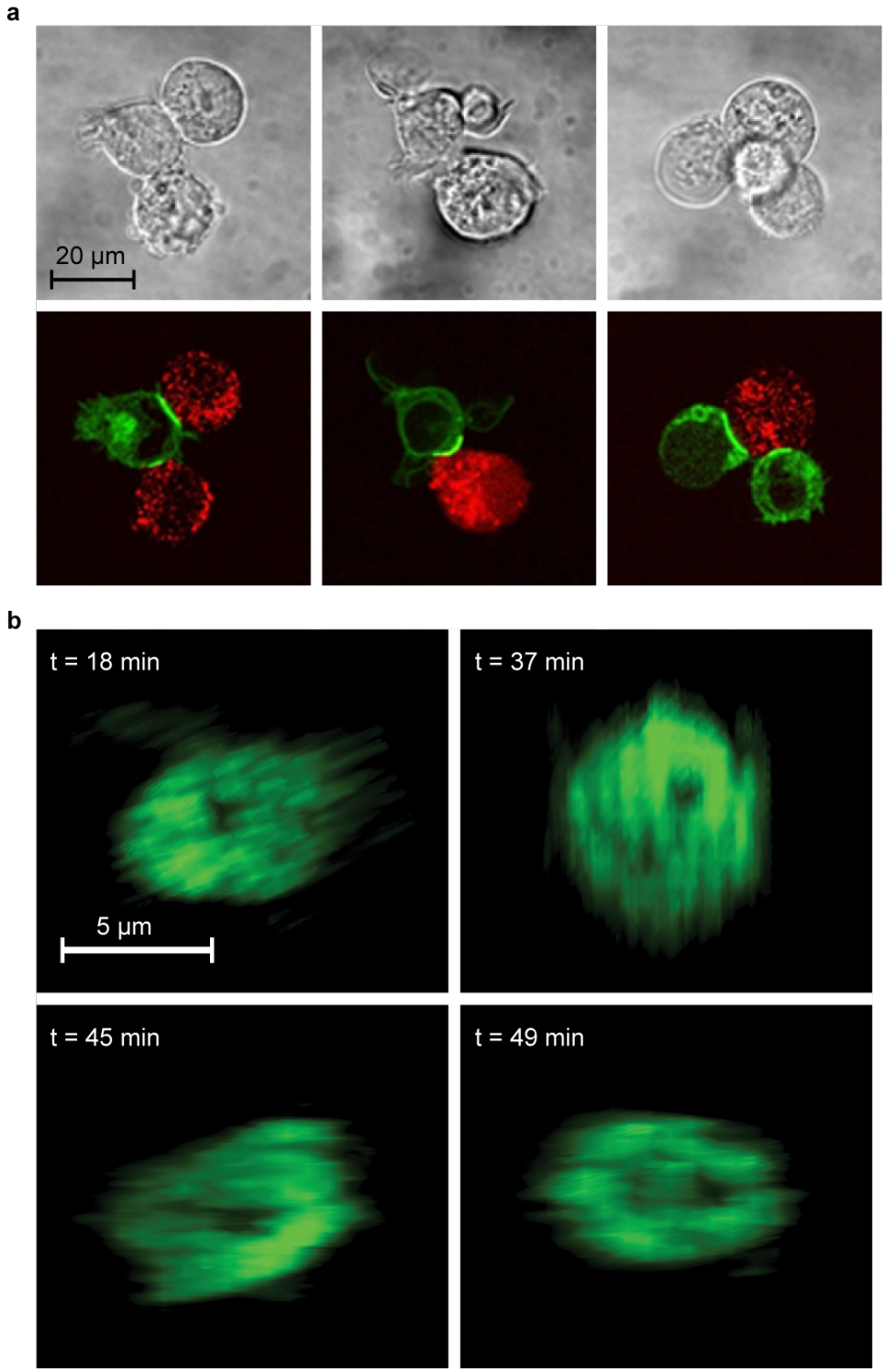

Figure S2. Ultrasound induces cell-cell contact leading to formation of immune synapses. (a) High-resolution bright-field and confocal fluorescence images of the inhibitory interactions between YTS/KIR1 stained with DDAO (red) and 221/Cw6-GFP showing HLA-Cw6-GFP (green) clustering at the immune synapse. Two NK cells form two synapses with a single target (left panel), single NK cell engaged in a synapse with a single target cell (middle panel), and one NK cell forms two synapses with two separate target cells (right panel). (b) Reconstructed, en face, spatio-temporal accumulation of HLA-Cw6-GFP molecules at the immune synapse.

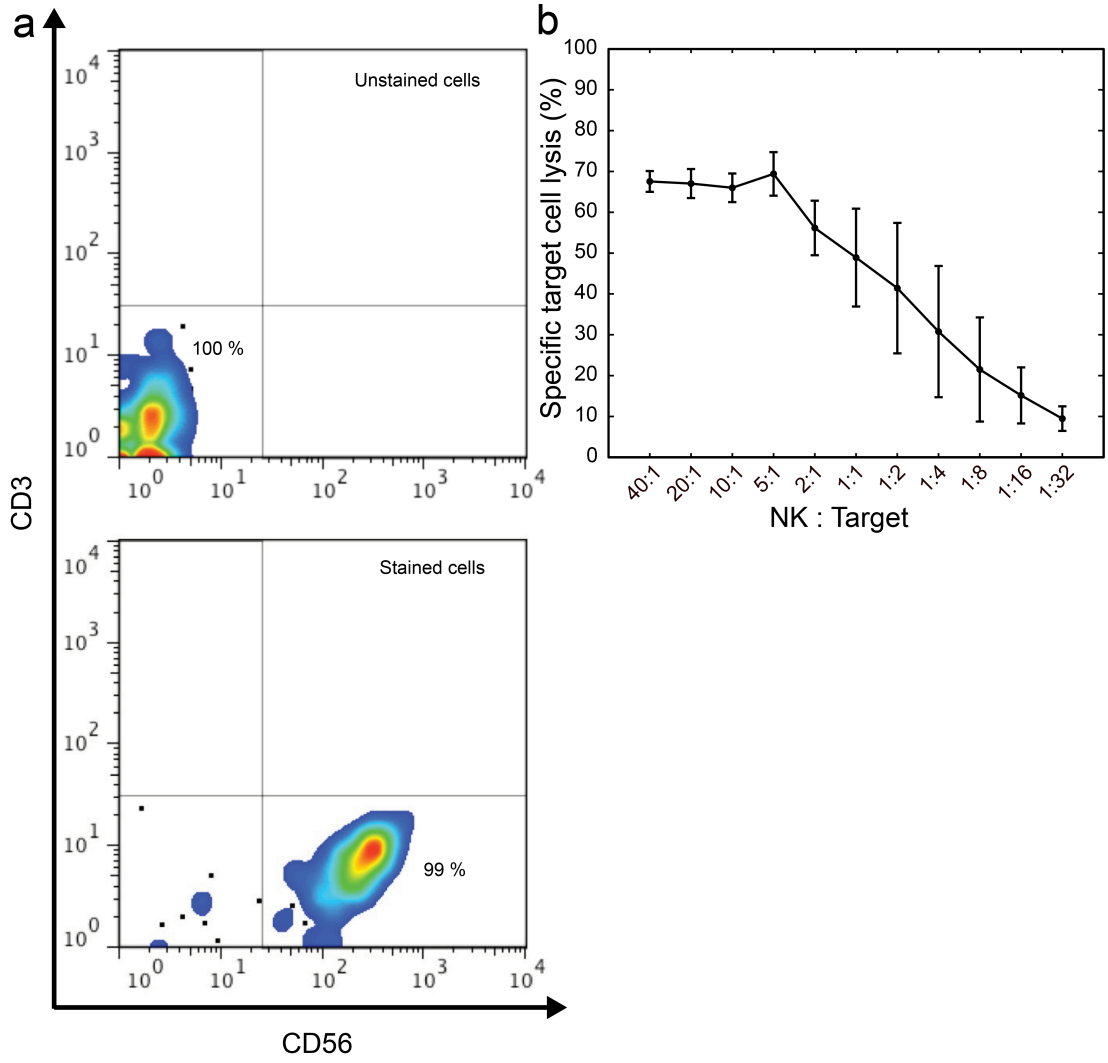

Figure S3. Purity and cytotoxicity of the human peripheral blood NK cells used in the experiments. (a) The purity of the NK cell populations used were ≈99% based on $CD3^-/CD56^+$ expression. (b) Percent specific target cell (221) lysis at different NK:target cell ratios.

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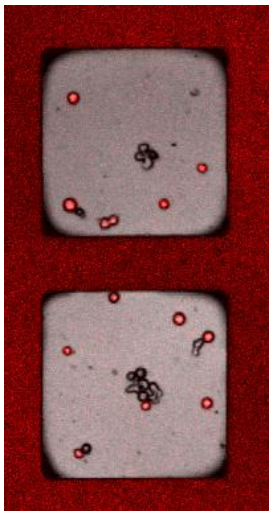
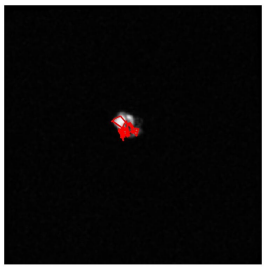
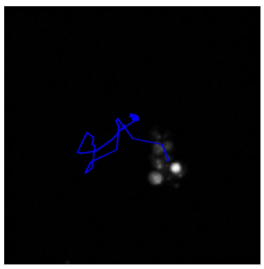
Descriptions of supporting movies



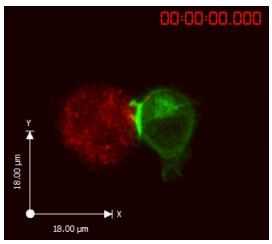
Movie 1. Time-lapse movie of two wells containing adherent 293T cells stained with calcein green (shown in red) and non-adherent 221 cells (unstained) exposed to ultrasonic actuation. The movie corresponds to the data presented in figure 2b.



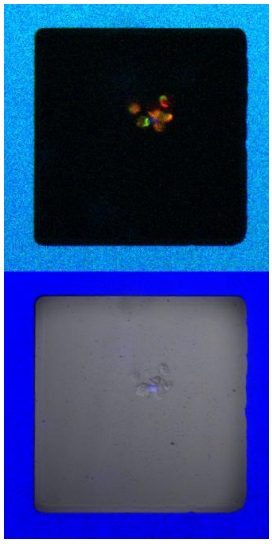
Movie 2. Time-lapse imaging and tracking of a cell aggregate imaged for ≈17 hours under continuous exposure of ultrasound. The cell aggregate is kept close to the center for the duration of the movie.



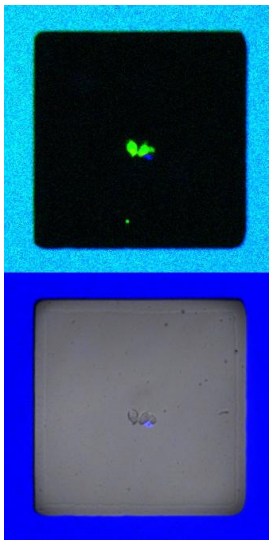
Movie 3. Time-lapse imaging and tracking of a cell aggregate initially formed by ultrasound and then imaged for ≈17 hours with the ultrasound off. At the beginning of the movie the aggregate remains close to the center (even when the ultrasound was turned off) but at later times it starts to move away from the center in a random fashion.



Movie 4. Time-lapse 3D rendered confocal imaging data of an inhibitory immune synapse between NK (YTS/KIR1) and target cell (221/Cw6-GFP). The MHC Class 1 protein is observed to accumulate at the interface between the cells.



Movie 5. Time-lapse imaging of a serial killer eliminating six target cells in a 4 hour long assay. The movie corresponds to the data presented in Figure 6c.



Movie 6. Time-lapse imaging of an inactive NK cell, unable to induce killing any of the surrounding target cells in a 4 hour long assay. The movie corresponds to the data presented in Figure 6d.