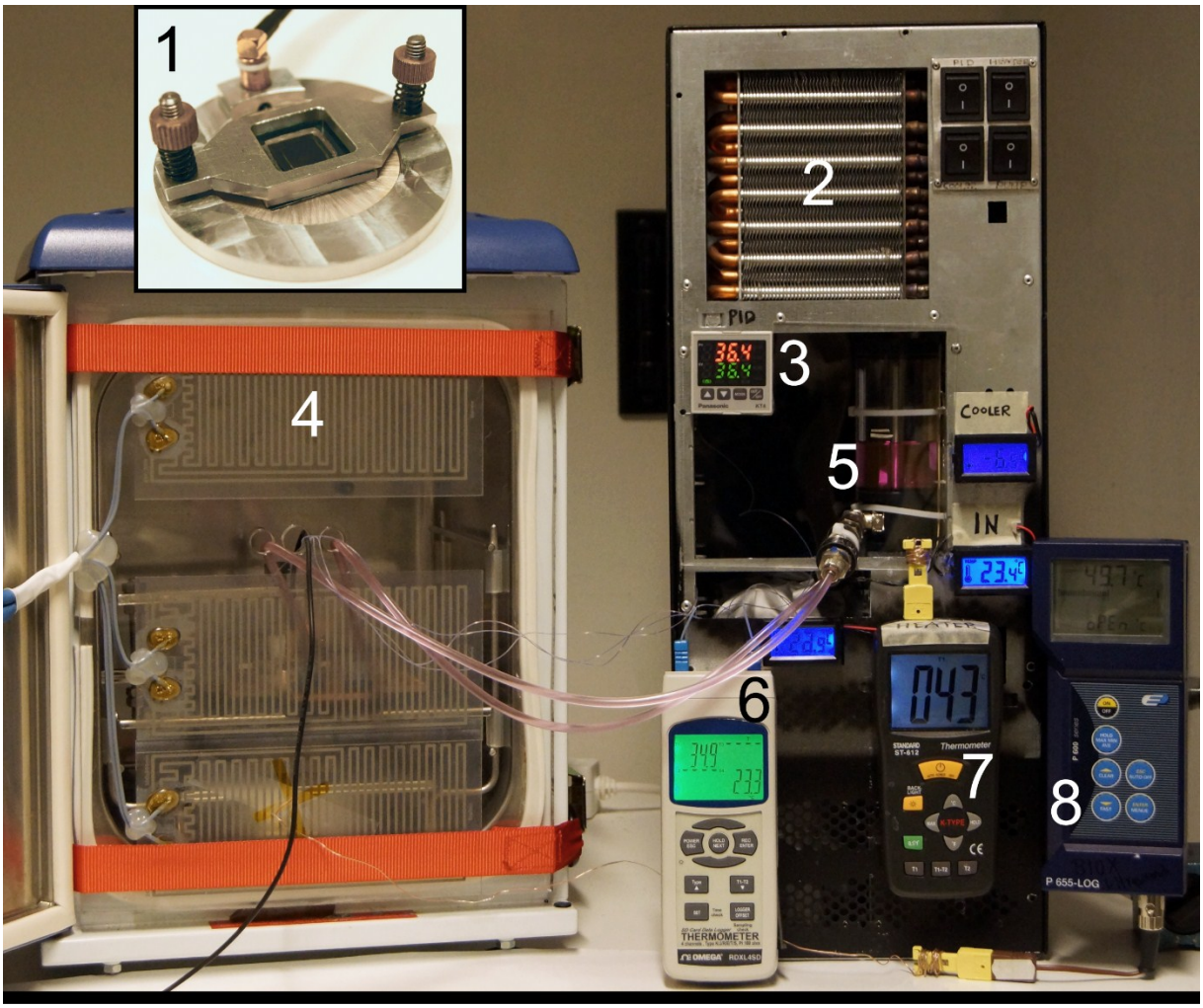
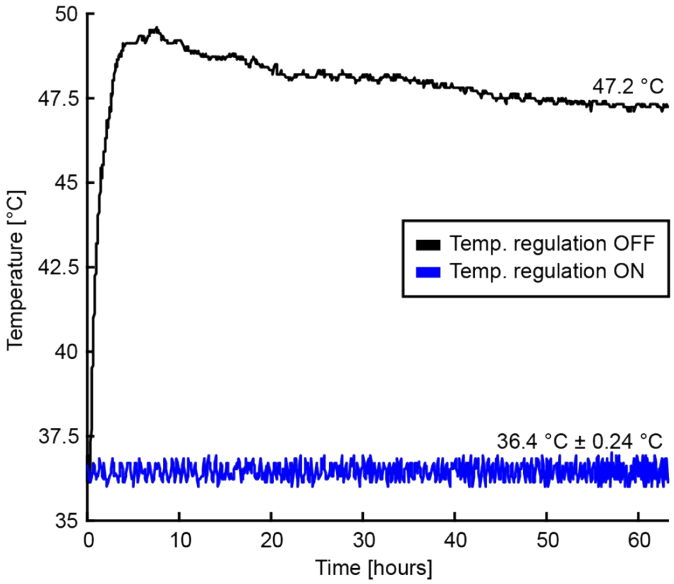
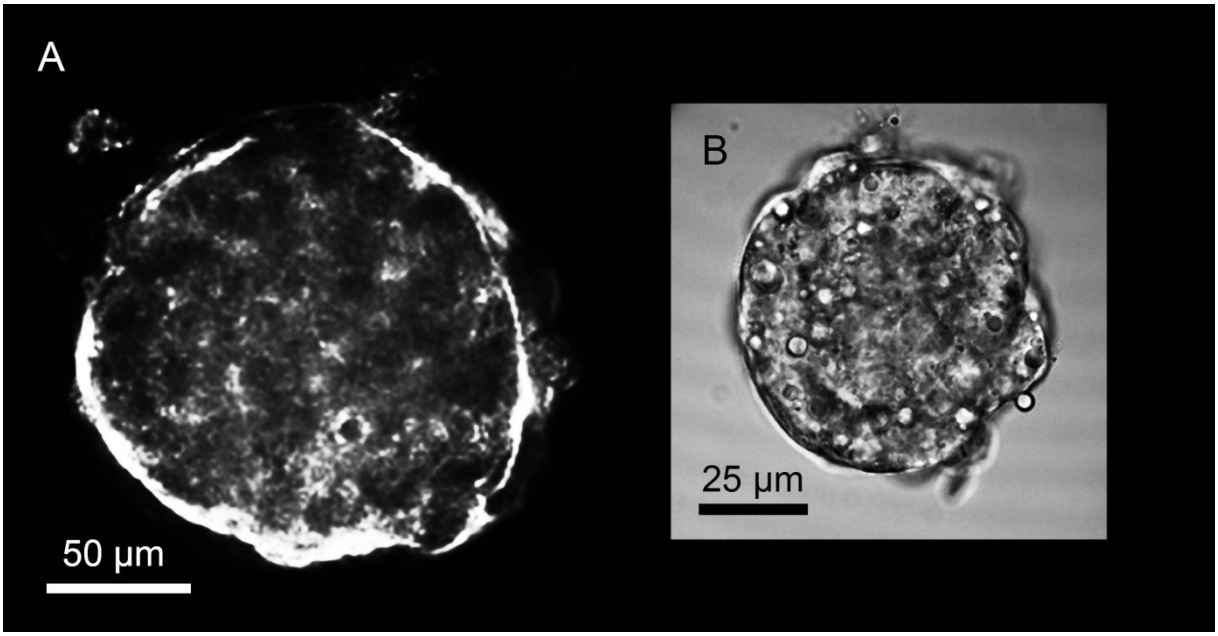
Electronic Supplementary Material (ESI) for Lab on a Chip.



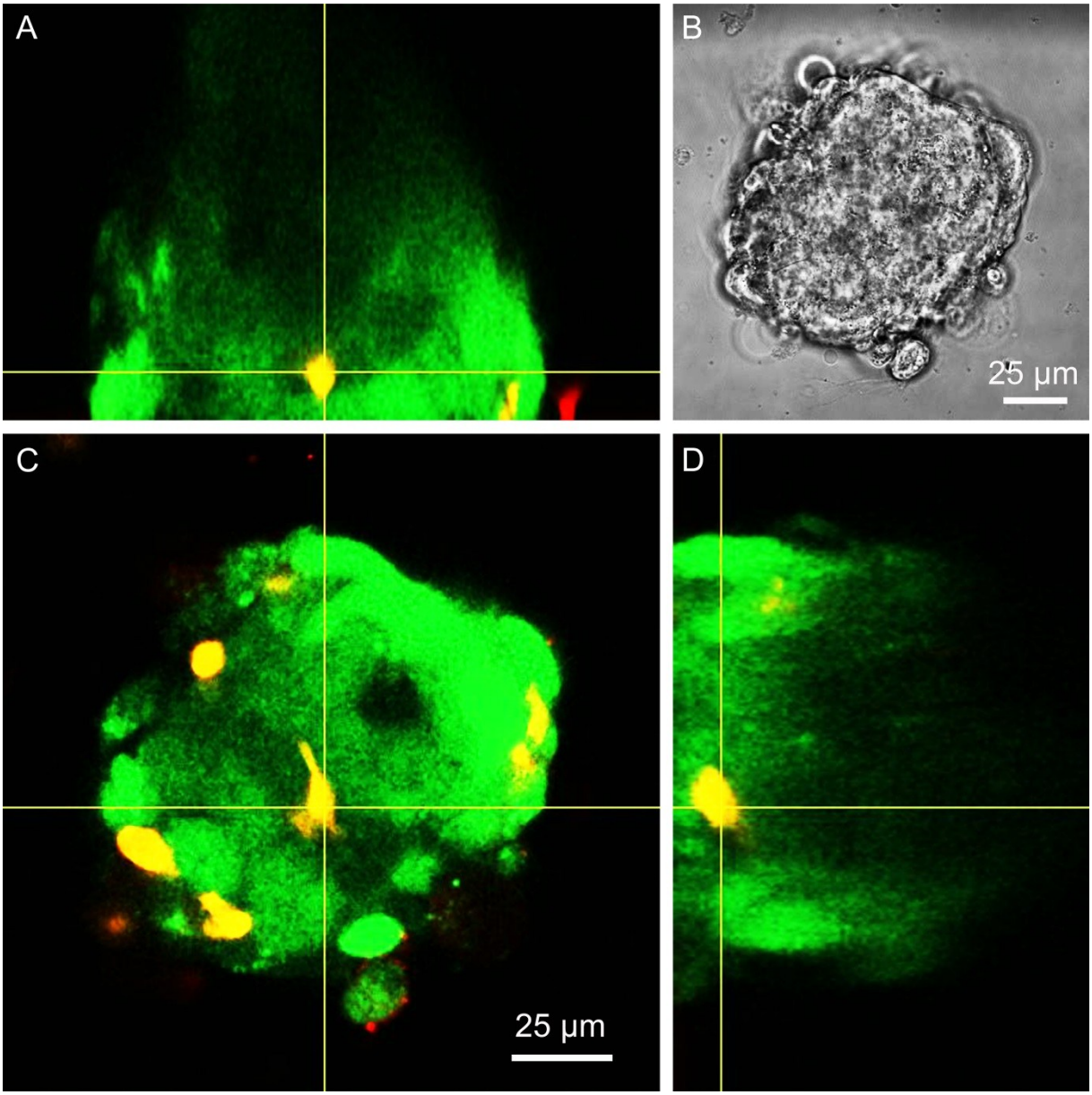
S1: The temperature regulating system with the cell incubator to the left and the external temperature control unit to the right. The inset (1) shows a photo of the multi-well microdevice located inside the cell incubator (4). The in-house built temperature control unit (2) equipped with a water heater and cooler is mounted in a computer-rack. A PID controller (3) (KT4, Panasonic, Japan) regulates the temperature of the water based on feedback from the multi-well microplate. Tubing connects the water block situated inside the cell incubator (4) with the water pump (5). Temperatures are registered on several thermocouple logging units: Temperature on the multi-well microplate and on the water block is registered on temperature logger (6) (RDXL4SD, OMEGA, USA). Temperature of the water heating unit is registered on temperature logger (7) (ST-612, Clas Ohlson, Sweden), and the temperature on the cell incubator's Plexiglas window is registered on temperature logger (8) (P655-LOG, Dostmann electronic GmbH, Germany).



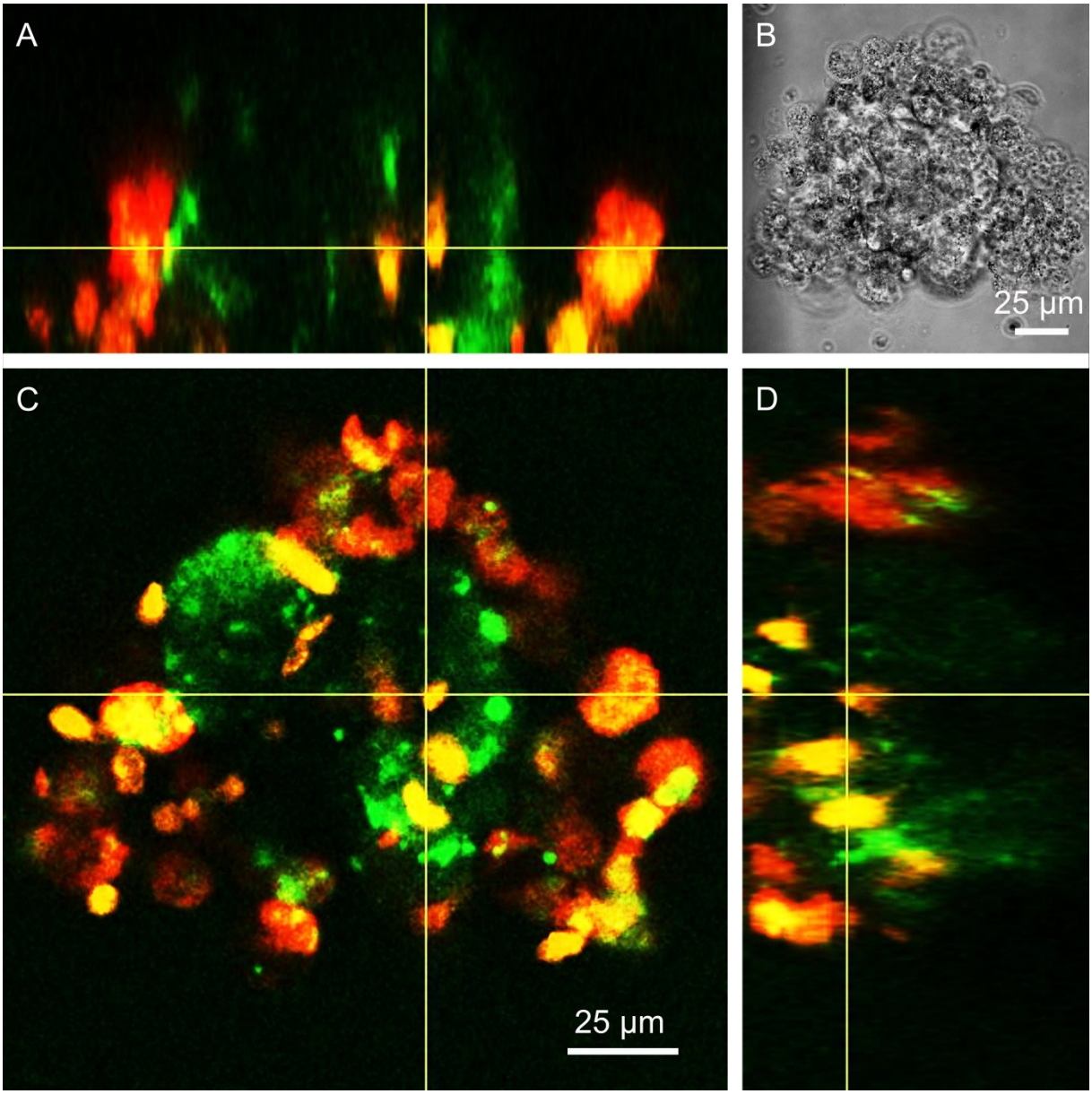
S2: Temperature regulation with (blue) and without (black) the external temperature control unit (cf. Fig. S1). In both experiments, the cell incubator's own temperature control system is active; however, as seen in the diagram, regulation is not sufficient without the external unit. The temperature on the microplate is measured next to the wells by a thermocouple probe (cf. Fig. 1) during continuous ultrasound actuation inside the cell incubator.



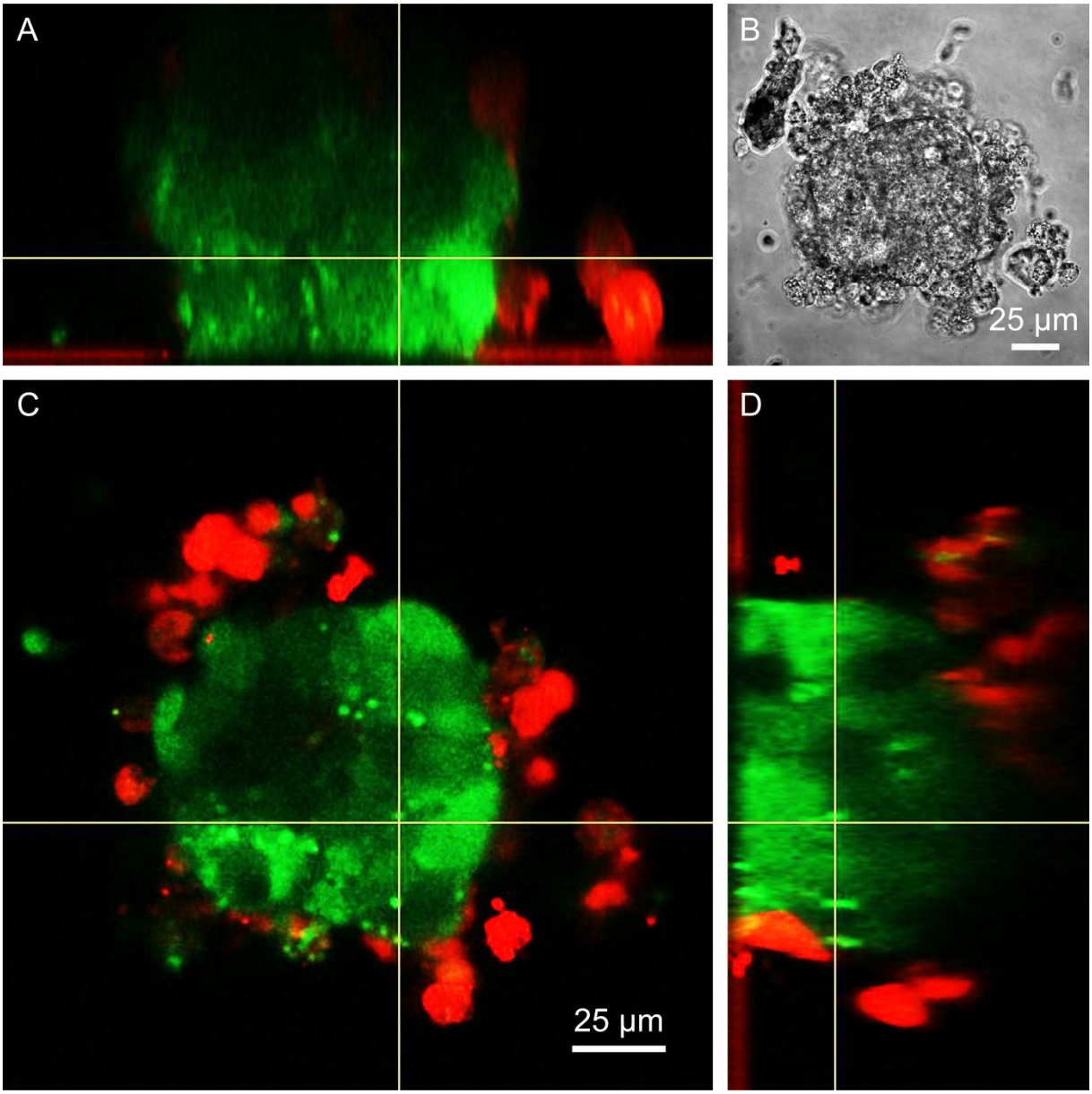
S3: Characteristic morphology of HepG2 tumors. (A) Dark field image obtained by a 10×/0.3 objective. (B) High-resolution bright field image of a relatively small tumor. The image was acquired with a 40×/1.3 oil objective and the image plane is located approximately in the center of the tumor.



S4: Solid tumor cultured with NK cells for 2 hours. In the fluorescent images NK cells are shown in orange (calcein red-orange AM) and the tumor is labelled in both green (calcein green AM) and red (far red DDAO-SE). (A) A vertical XZ cross-section of the tumor, (B) a bright field image of the tumor showing that the tumor is mainly intact although NK cell mediated lysis has been initiated in the periphery. (C) Horizontal (XY) cross section of the tumor and (D) a vertical (YZ) cross section of the tumor. The combination of the three different planes validates the presence of the NK cells in the interior of the tumor.



S5: Follow up image of the tumor presented in S4. An overview of the tumor 20 hours after NK cells were seeded in the chip clearly showed cell lysis in the periphery of the tumor (dead cells are shown in red) and some NK cells (shown in orange) that had penetrated into the tumor mass (A, C and D tumor cross sections as described previously). (B) The bright field image of the tumor also indicates tumor cell death in the periphery and dead cell disaggregation.



S6: Solid tumor cross sections of high-resolution confocal images of the solid tumor presented in Fig. 7A at time point 51 hours. The cells that are alive are shown in green and dead cells in red (A) a XZ cross section, (B) bright field image of the tumor, (C) the horizontal plane XY approximately 25 µm from the well bottom and (D) a YZ cross section.

Movie S1: Calcein green AM-stained 3D solid tumor co-cultured with calcein red-orange AM-stained NK cells. One of the NK cells extends a projection into the interior of the tumor at a distance of 17 μm from the glass bottom of a total tumor height of 77 μm.

Movie S2: Time-lapse sequence of the interaction between NK cells and a solid tumor. The image sequence is presenting the same well as in Figure 7A and 7B at approx. time points t = 6 hours to 11 hours. Movie shows an overlay between bright filed and fluorescence channels. HepG2 cells are stained with calcein green AM (indicating viable cells) and far red DDAO-SE (indicating dead cells) and NK cells with calcein red-orange AM. NK cells are observed to migrate across the tumor surface, at the interface between glass and tumor and inside the tumor. Occasionally NK cells stop and form stable contacts with tumor target cells, some leading to cell lysis apparent as a shift from green to red.

Movie S3: Confocal Z-scan of the well presented in Fig. 7A, here at time 127 hours. Before imaging all cells in the well were stained by adding calcein green AM to the microplate. NK cells are distinguishable from the dead HepG2 cells from the morphology shown in bright field in combination with the red color (far red-DDAO-SE) that is present only on dead HepG2 cells.