Supporting Information for:

Protective Polymer Coatings for High-Throughput, High-Purity Cellular Isolation

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MATERIALS AND METHODS

UV Degradable Monomer Synthesis. A poly(ethylene glycol) diacrylatemacromer (M_n ~ 3500 Da) incorporating an *o*-nitrobenzyl functionality was synthesized according to a published protocol by Kloxin. Structure and purity was confirmed by H-NMR.[1]

PEG-diacrylate Monomer Synthesis. A poly(ethylene glycol) diacrylatemacromer was synthesized with a similar molecular length as UV degradable macromers. Poly(ethylene glycol) (PEG, M_n ~3350, Sigma Aldrich) was added to the bottom of an additional funnel flask at a 10 gram batch size and purged with ultra-pure N_2 for 10 minutes. Anhydrous DCM (30mL) was added to the flask, and the PEG was allowed to dissolve under magnetic stirring for 5 minutes. The flask was kept in a stirred room temperature water bath (>500mL) to serve as a heat sink for the exothermic reaction. For acrylation of the PEG macromer, a molar ratio of 1:4:4 PEG:acryloyl chloride (AC, Sigma Aldrich):triethylamine (TEA, Acros) was used. The calculated amount of TEA was added directly to the bottom of the flask under continuous stirring. The calculated amount of AC was mixed with 5mL of DCM and injected through a septum into the top of the addition funnel, and the entire apparatus was then purged with N_2 for 15 minutes. The AC/DCM solution was then added dropwise (~1 drop every 5 secs) with the addition funnel under continuous N_2 purge and stirring. When the entire amount of AC/DCM solution was added to the PEG/TEA/DCM solution, purging was stopped and the apparatus was sealed, covered with aluminum foil and reacted overnight. The resulting pale yellow mixture was then filtered through a Buchner funnel and washed with excess DCM to remove the bulk of the TEA salts that formed during the reaction. Next, 10-fold molar excess sodium carbonate was added to the solution, and the slurry was stirred vigorously for 1 hour. The mixture was then again passed through a Buchner filter funnel and washed with DCM to remove the insoluble

sodium carbonate. A packed bed of alumina (~3cm thick) was then prepared in a glass frit column, and the solution was passed through the bed and washed with DCM. The DCM was then evaporated off so that the solution volume was ~40mL, and cold diethyl ether (400mL) was added to precipitate the PEG diacrylate product. The mixture was kept at 4˚C for at least 1 hour to allow full precipitation. The white precipitate was then collected with a Buchner filter funnel and allowed to dry overnight in the dark. This process of dissolving in ~40mL of DCM and then precipitating the PEG diacrylate product in cold ether and filtering was repeated to further remove any impurities. Structure and extent of acrylation (90%) were confirmed with H-NMR.

Photopolymerization of degradable coatings on cultured Jurkat cells in suspension. Jurkat cells (ATCC) were cultured to ~60% confluency in RPMI-1640 (Cellgro) supplemented with 10% fetal bovine serum (FBS, Gibco) and 100U Penicillin, 10 mg/mL Streptomycin (Gibco). For each polymerization trial, 1.5 x 10^6Jurkat cells were collected in culture media. In a 15 mL conical tube, the cells were centrifuged at 300*xg* for 5 minutes at 4 °C. The culture media was removed, and the cell pellet was resuspended by gentle vortexing in 115 µL of DPBS (Gibco) with 3% FBS and 35 µLbiotinylated mouse anti-human CD45 (BD Biosciences), and incubated for 40 minutes at 4 °C. Following incubation with primary antibody, the cell suspension was rinsed twice by centrifuging at 300x*g* for 5 minutes and then resuspending with cold DPBS with 3% FBS followed by gentle vortexing. After the final rinse, the cell pellet was resuspended in 1mL of cold PBS with 3% FBS with 25 µg/mL streptavidin-eosin isothiocyanate (SA-EITC), gently vortexed and incubated at 4 °C for 30 minutes. Streptavidin (Thermo Scientific) and eosin-5-isothiocyanate (Sigma Aldrich) were reacted and purified in-house by the method described by Hansen *et al* (2008), The cell suspension was then rinsed once, as before, with cold PBS with 3% FBS and then once with cold DPBS. The monomer mixture was then prepared as

follows: 25 wt% UV cleavable PEG diacrylate,[1] 21 mMtriethanol amine, 35 mM 1-vinyl-2-pyrrolidinone, and 0.05 wt% nile red 20 nanometer fluorescent nanoparticles (Fluospheres, Invitrogen) in 1x phosphate buffer. The solution was bubbled with humidified ultra-pure N_2 for approximately 3 minutes to remove dissolved oxygen and reduce turbidity. The cell pellet was resuspended in 300 μL monomer solution, gently vortexed, and placed in pre-cooled Chip-Clip well (Whatman) with a standard microscopy slide (Fisherbrand). The Chip-Clip was then placed in a sealed clear plastic bad and purged with humidified N_2 for 3 minutes. While continuing to purge, the reaction was initiated by turning on an LED lamp (Thor Labs) emitting 530 nm light at 30 mW/cm^2 (**Figure S7**) and centering the irradiation area on the well containing the cell suspension. The photopolymerization was allowed to proceed for 10 minutes, at which time the Chip-Clip was removed from the bag and the cell suspension was removed rinsed 3 times with room temperature DPBS.

Photopolymerization of degradable coatings on cultured A549 cells in suspension. Before a coating experiment, A549 cells (ATCC) were cultured to ~80% confluency in RPMI-1640 with 10% FBS and 100U Penicillin, 10 mg mL^{-1} Streptomycin. The culture flask was then rinsed with warm PBS 1x to remove media, and then trypsin/EDTA solution was added for ~5 minutes to detach the adherent cells. After detachment, cells were washed with 1 part trypsin neutralizing solution, pelleted and resuspended in culture media. For each polymerization trial, 1.5 x 10^6 A549 cells in 1 mL of media were used. Throughout the protocol, a rinsing solution consisting of DPBS 1x (free of calcium and magnesium), 5mM EDTA, and 3% FBS was used to minimize A549 attachment during immunolabeling and polymerization. The cells in media from culture were centrifuged at 300*xg* for 5 mins at 4 °C and resuspended in 100 μL rinsing solution and 1 μL of stock mouse anti-human EpCAM (BioLegend) by gentle vortexing. The cell suspension

was incubated in primary antibody for 40 minutes at 4 °C. At the end of the incubation the sample was centrifuged at 300x*g* for 5 mins at 4 °C and resuspended in 1 mL rinsing solution with 5 μL stock biotinylated goat anti-mouse IgG, gently vortexed and incubated at 4 °C for 40 minutes. The cells were then again centrifuged as before, rinsed twice by centrifuging and resuspending with rinsing solution. After the final rinse, the cell pellet was resuspended in 1 mL of cold rinsing solution with 25 μg/mL streptavidin-eosin isothiocyanate (SA-EITC), gently vortexed and incubated at 4 °C for 30 minutes. The cell suspension was then rinsed twice, as before, with cold rinsing solution. The monomer mixture was then prepared as described previously for Jurkat coating: 25 wt% UV cleavable PEG diacrylate,[1] 21 mMtriethanol amine, 35 mM 1-vinyl-2-pyrrolidinone, and 0.05 wt% nile red fluorescent nanoparticles in 1x phosphate buffer. The solution was bubbled with humidified ultra-pure N_2 for approximately 3 minutes to remove dissolved oxygen and reduce turbidity. The cell pellet was resuspended in 300 μL monomer solution, gently vortexed, and placed in a pre-cooled Chip-Clip well (Whatman) with a standard microscopy slide (Fisherbrand). The Chip-Clip was then placed in a sealed clear plastic bag and purged with humidified N_2 for 3 minutes. While continuing to purge, the reaction was initiated by turning on an LED lamp (Thor Labs) emitting 530 nm light at 30 mW/cm^2 and centering the irradiation area on the well containing the cell suspension. The photopolymerization was allowed to proceed for 10 minutes, at which time the Chip-Clip was removed from the bag and the cell suspension was removed and rinsed 3 times with room temperature DPBS.

Polymerizing cell mixtures. In experiments with mixtures of Jurkat and A549 cells, each respective cell type was cultured and collected as previously described and then combined at varying ratios. The polymerization protocol used depended on the cell type targeted for isolation.

Because A549 cells were present in these experiments, 5 mM EDTA was included in all rinsing solutions to prevent cell attachment.

Removal of antigen-negative cells by surfactant lysis. Following photopolymerization and subsequent rinsing, cell mixtures were resuspended in 500 μL PBS 1x and gently vortexed. To the cell suspension was added 500 μL of 10% sodium dodecyl sulfate (SDS) in PBS 1x and gently mixed by pipette to get final concentration of 5% SDS in PBS 1x. The suspension was then immediately centrifuged at 300*g* for 5 minutes to collect the polymer coated, antigen-positive cells. The surfactant solution was removed by pipette, and the pellet was rinsed twice more with cold DPBS.

Coating removal by UV degradation. Polymer coated cells were suspended in 300 μL of 10 mM EDTA gently vortexed and pipetted into a Chip Clip well. A UV LED lamp emitting 365 nanometer light was set up to irradiate the entire well at 10 mW/cm^2 (Figure S8) for 15 minutes. The cell solution was then removed from the well, and the well was rinsed twice and combined with the removed solution. The cells were then pelleted by centrifugation at 300*g* for 5 minutes at 4 °C, and then resuspended in PBS 1x. The cells were rinsed twice more in DPBS 1x similarly by pelleting and resuspending.

Cell Identity Assays. For cytometry experiments, an Accuri C6 flow cytometer was used. Gating between coated and uncoated cells is based on nanoparticle fluorescence (535/575nm) and forward scatter. Each experiment was set to count 100,000 events per run and each run was recorded using identical measurement parameters. Each condition tested was replicated five times per experiment, and each experiment was repeated at least 3 times on different days. To support the cell identity analyses above, a cytometry experiment was designed to ASL sort GFP-

transfected A549 cells (Cell Biolabs), where GFP signal serves as a strong biochemical reporter for A549 identity. GFP positive A549 and Jurkats were cultured in RPMI-1640 and collected as previously described above, and mixed at a ratio of approximately 10% GFP positive A549 into 90% Jurkats (Figure S2A), where gating was set on FL-1 vs forward scatter based on single cell population controls. GFP-A549 cells were targeted with anti-EpCAM, and coated by photopolymerization of 25 wt% PEG-diacrylatemacromer similarly to the coating protocol detailed previously. After SDS lysis and rinsing with DPBS/FBS, cells were suspended in culture media and incubated overnight to allow recovery of GFP signal that is partially photobleached during photopolymerization. Cells were then resuspended in DPBS/FBS and analyzed again by cytometry (Figure S2B). The experiment was repeated 3 times on different days with 1,000 events per replicate.

Viability assays. Cell viability was typically determined by MTT, calcein, caspase-3/7, and SYTOX assays. Measurements were taken after antibody incubations, polymerization, surfactant lysis, and UV degradation (Figure S9). After the final rinse with cold PBS 1x at each step, cells were centrifuged at 300*g* for 5 minutes and resuspended in 50 nM of calcein AM (Invitrogen) in PBS and incubated for 15 minutes at 4 °C. Flow cytometric analysis was then performed to measure calcein fluorescence. First, a threshold of fluorescence was generated using uncoated and cells without exposure to calcein as control samples. All events corresponding to uncoated cells without calcein were located at intensities below this threshold for both green and red fluorescence, while events corresponding to polymer coated cells without calcein were located at intensities below the threshold for the green fluorescence channel. Each experiment was set to count 100,000 events per run and each run was recorded using identical measurement parameters. Each condition tested was replicated five times and each experiment was repeated at

least 3 times in different days. The variation between each replicate was always less than 5% and between experiments less than 10% in cell mixtures.

Cell viability after immunostaining, polymerization, lysis and polymer degradation was also studied with the MTT assay. After each step, 100,000 cells in 200 μL of culture media were seeded into each well of 96-well plates. Thiazolyl Blue Tetrazolium Bromide (Sigma Aldrich) was dissolved in PBS 1x at a concentration of 5 mg/mL.Then, 20 μL of MTT solution was added into each well and incubated for 3 hours. The absorbance was measured by means of a plate reader at 570 nm.

To further assay viability, a flow cytometry based caspase-3/7+SYTOX reporter kit (Invitrogen) was used. 10^6Jurkat cells were obtained from culture, coated with polymer via anti-CD45, exposed to SDS lysis, then to degradation conditions of UV light in 10mM EDTA as described above**.** Apoptosis and membrane integrity were probed after polymerization and degradation steps by incubating samples with both 500nM caspase reporter reagent and 1μM SYTOX reagent for 60 minutes at room temperature in the dark. For analysis, flow cytometry gating was set from Jurkat controls to 1,000 events per replicate. Fluorescence analysis was performed by excitation with a 488 laser with a 530/30 BP filter for the caspase channel and a 675/25 BP filter for SYTOX. A dead control consisted of 70% ethanol fixed Jurkats, and an apoptotic control was induced with a 3 hour incubation of Jurkat cells at 37 C with 10μM camptothecin in RPMI-1640 culture media.

Proliferation assay. Cell proliferation after polymer degradation was studied with the MTT assay. 5,000 cells in 200 μL of culture media were plated into each well of 96-well plate. During 5 days of coincubation, 20 μL of MTT solution (5 mg/mL in PBS 1x) was added into each well

of the plates and incubated for 3 h. The absorbance was measured by means of a plate reader at 570 nm.

ɣH2AX Foci quantitation. A549 cells were plated on 12 mm coverslips and incubated in DMEM 10% FBS + 1% streptomycin& penicillin + 1 % L-Gln for 16 h. Then the coverslips were soaked in 2 ml PBS in 6 cm dishes, and exposed to 10 mW/cm^2, 365 nm irradiation for 10 or 20 min, followed by incubation in a regular medium for 30 min, 1 h, or 3 h. Cells were then fixed in 50% methanol followed by 100 % methanol, and then stained with anti-ɣH2AX (Millipore) and probed with anti-mouse IgG-Alexa Fluor 488. Cells with foci were counted to calculate mean %cells with foci from 10 independent observations. Foci formation induced by 5 minutes of 0.2 mM H_2O_2 and measured after 30 minutes in regular medium post exposure treatment is shown as a ɣH2AX positive control.

FIGURES

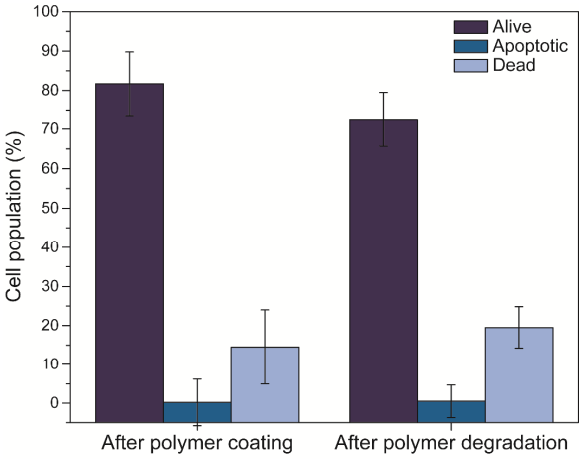

Figure S1. Viability of Jurkat cells before and after polymer degradation measured by Caspase and SYTOX assay. Data are mean ± s.d.

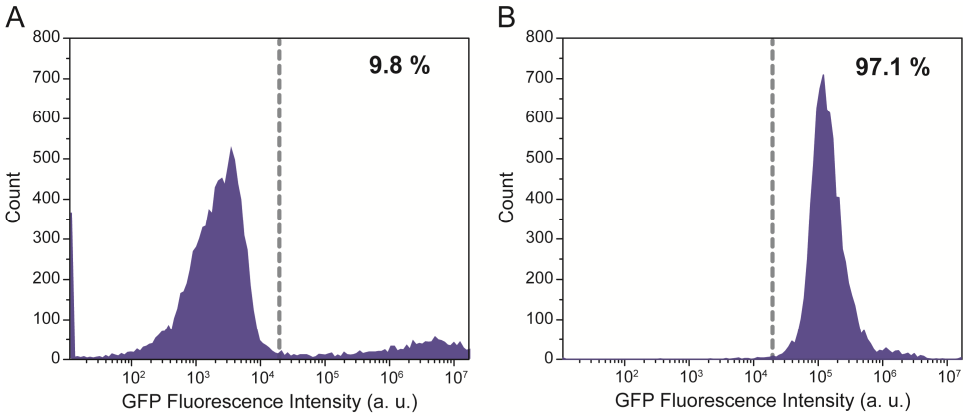

Figure S2. Antigen Specific Lysis purity from a cell mixture composed by 90% Jurkat cells + 10% GFP-positive A549 cells. A) Flow cytometric distribution from the cell mixture before ASL and B) Flow cytometric data of GFP-positive cells after ASL.

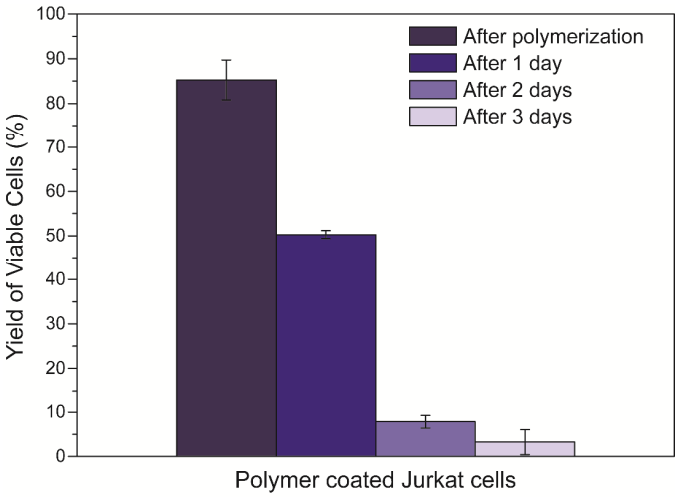

Figure S3. Survivial of PEG-diacrylate coated Jurkat cells in media at 37C in 5% CO_2. 10^6 Jurkat cells were coated with a non-degradable PEG diacrylate (Mn 575) polymer as described previously. After coating, the cells were resuspended in RPMI 1640 media and held at 37C in

5% CO_2. After 1, 2, and 3 days, cells were removed and the viability of the removed cells was evaluated by the calcein assay. Data are mean ± s.d.

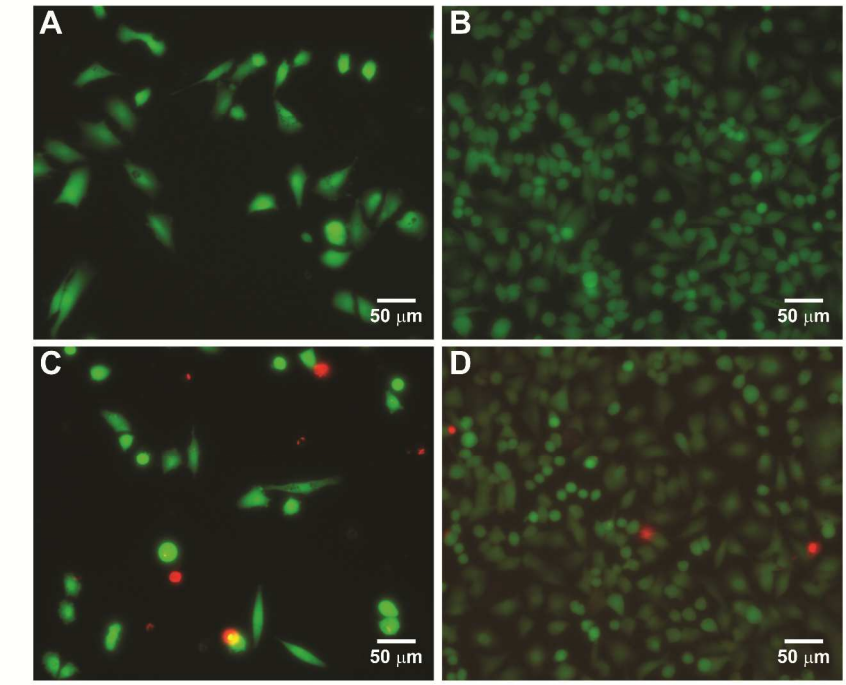

Figure S4. Calcein staining for control A549 cells after 1 and 4 days of culturing (A and B), and released A549 cells after polymer degradation after 1 and 4 days of culturing (C and D). Degradation of the coating by 15 minute exposure to 365 nm light at 10 mw/cm^2, A549 cells were replated in standard polystyrene 96 well plates, and incubated at 37 C, in culture media. At the designated time, the media was removed and the cells were rinsed in cold PBS, then 50 nM of calcein AM (Invitrogen) in PBS was added. The cells were incubated for 15 minutes at 4 °C, and then imaged in the FITC channel (capturing intracellular esterase activity) and the red channel (capturing residual polymer fluorescence). Released A549 cells show appropriate spreading and esterase activity. The presence of red fluorescence indicates regions of residual red fluorescent nanoparticle-loaded polymer.

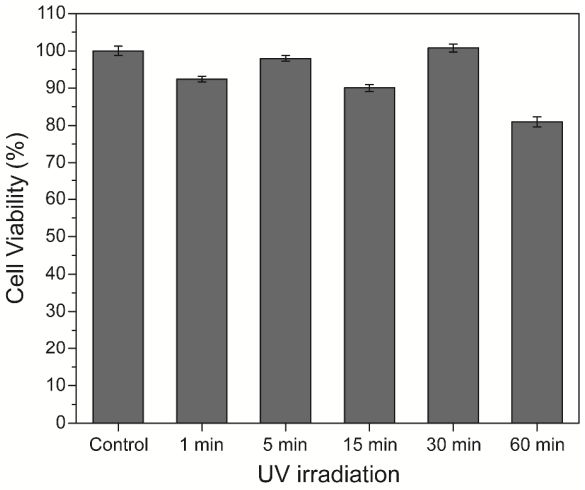

Figure S5. Cell viability under 365 nm, 10 mW/cm^2 light for indicated duration (determined by MTT assay). Data are mean ± s.d.

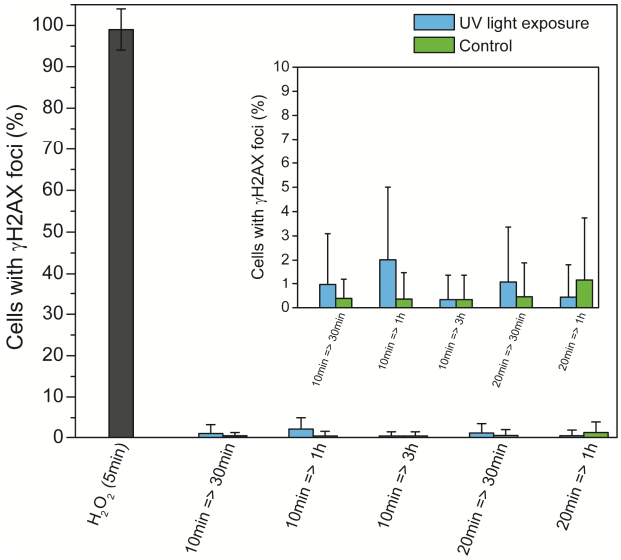

Figure S6. DNA repair activity in A549 cells after UV irradiation (365 nm, 10 mW/cm^2). Activity correlated to fraction of cells displaying ɣH2AX foci. X-axis indicates "[UV exposure

time] => [recovery time prior to analysis]". Inset provides an expanded y-axis. H_2O_2 condition was 5 minutes of 0.2 mM H_2O_2 and measured 30 minutes post exposure. Data are mean ± s.d.

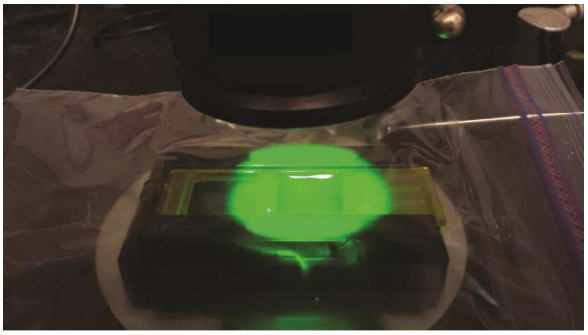

Figure S7. Photograph of the photopolymerization apparatus. 530 nm light with a ChipClip / FAST-slide assembly inside a plastic bag with humidified nitrogen flow delivered by needle.

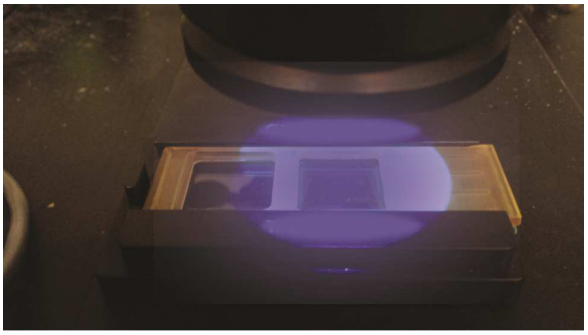

Figure S8. Photograph of the photodegradation apparatus. 365 nm light with a ChipClip / FAST-slide assembly.

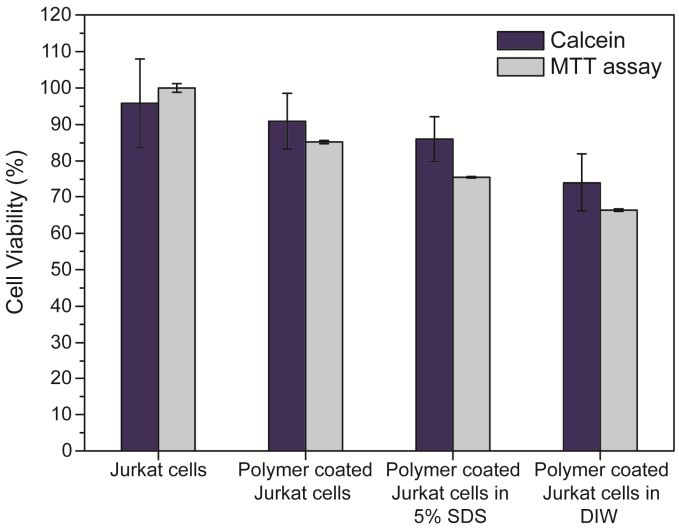

Figure S9. Agreement of MTT and calcein viability assays at critical points in the Antigen Specific Lysis process. Data are mean ± s.d.

REFERENCES

1. Kloxin, A. M.; Kasko, A. M.; Salinas, C. N.; Anseth, K. S., Photodegradable Hydrogels for Dynamic Tuning of Physical and Chemical Properties. *Science* **2009,** *324*.