Engineered Colorectal Cancer Tissue Recapitulates Key Attributes of a Patient-derived Xenograft Tumor Line

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Supplemental Methods

CRC-PDX Tumor Dissociation and Cell Isolation

Using an optimized method adapted from previous publications [1-5], a portion of the excised tumor was dissociated to isolate the cells for subsequent *in vitro* culture as 2D-CRC-PDX cells and 3D-eCRC-PDX tissues. A dissociation solution with a final concentration of 250 U/mL collagenase IV (Worthington) and 100 U/mL deoxyribonuclease I (DNase I) (Worthington) in Hank's Buffered Saline Solution (HBSS) (Lonza) was prepared with gentle mixing and sterile-filtered using a 0.2 μm syringe filter (VWR). Fresh CRC PDX tissues were collected and placed on a sterile Petri dish. The tissues were washed with 1X phosphate-buffered saline (PBS) (Lonza), and 10 mL of dissociation solution per 1 g of tissue was added to the Petri dish. The tumor was minced into small pieces between 2-4 mm using sterile surgical scissors. Minced CRC-PDX tumor (500 mg) was transferred into a 50 mL centrifuge tube, along with 5 mL of fresh dissociation solution, and incubated at 37 °C. The tube was manually swirled every 5 minutes to resuspend settled tissue fragments. The supernatant containing the dissociated cells was collected in a 50 mL centrifuge tube every 10 minutes, and 5 mL of fresh, pre-warmed (37 °C) dissociation solution was added to the minced tissue. When all tissue segments were dissociated (approximately 1 hour

of incubation), the total collected supernatant containing CRC PDX cells was passed through a 40-μm cell strainer (Corning) and the strainer was rinsed with 20 mL of HBSS. The cell suspension was centrifuged at 300 ×g for 5 min without the brake, the supernatant was removed, and the cell pellets were resuspended in 10 mL of cell culture media. Media for all experiments was composed of DMEM (Lonza) containing 10% (v/v) fetal bovine serum (FBS) (Atlanta Biologicals™), 1% (v/v) penicillin:streptomycin solution (pen/strep) (HyClone™), and 2 mM glutagro™ Supplement (Corning). The centrifugation was repeated for a total of six times to remove dead cells and extracellular matrix (ECM) debris from the cell suspension as much as possible. Finally, the viable cells were labeled with trypan blue (Lonza) and manually counted using a hemocytometer.

Cell Viability Assay and Image Analysis

A cell viability assay was performed using the LIVE/DEAD® Viability/Cytotoxicity Kit (ThermoFisher Scientific) according to the manufacturer's protocol. Briefly, the optimal dye concentration was determined and accordingly, a viability solution was prepared by mixing the 4 mM calcein AM stock solution (live dye) and 2 mM ethidium homodimer-1 (EthD-1) stock solution (dead dye) at ratios of 1:2000 and 1:500 in PBS, respectively. To quantify the percentage of live cells, a viability working solution was made by adding Hoechst 33342 (Calbiochem) (20 μg/mL) to the viability solution to counterstain the cell nuclei. The 3D-eCRC-PDX tissues were washed thoroughly with PBS (3 times over 15 minutes) and incubated in the viability working solution at room temperature for 30 minutes in the dark. For the 2D-CRC-PDX cell viability assay on Day 1, the cells previously cultured on a 24-well plate were washed thoroughly with PBS three times prior to adding the working solution. Since most of the cell colonies did not adhere to the tissue culture surface, the cell culture media containing these non-adherent cell colonies was also

collected, placed into a centrifuge tube, centrifuged at 300 ×g for 5 minutes, and then washed three times with PBS. After the final washing step, the cell colonies were resuspended in the viability assay working solution and was added to the adherent 2D-CRC-PDX cells. The confocal viability images presented in Fig. 1B were processed using ImageJ software (version: 2.0.0-rc-68/1.52e) as Z-Projection, meaning that the captured Z-stack of images for each 3D-eCRC-PDX tissue was flatted into one layer of image.

Flow Cytometry

Here, we present a comprehensive protocol for cell immunolabeling and flow cytometry analysis of the cells from the CRC-PDX tumors, 3D-eCRC-PDX tissues, and 2D-CRC-PDX cultures. It must be noted that the centrifuge brake was turned off throughout the entire process to minimize cell loss. Moreover, centrifugation was performed at 300 ×g for 5 minutes throughout the process unless otherwise stated. Cell preparation, antibody-Zenon™ labeling conjugate preparation, cell labeling, compensation controls, unstained controls, viability controls, and flow cytometer and data analysis are described.

Cell Preparation: For the CRC-PDX tumors, the cells were cultured on T-75 standard tissue culture flasks in CRC-PDX media and maintained in a standard incubator with a temperature of 37 °C and 5% CO2. Cells were given 12 hours to retrieve surface proteins that may have been damaged during the process of dissociation. After 12-hours of surface protein retrieval, the media containing non-adherent cells (mostly cell colonies) was collected into a centrifuge tube, centrifuged, and washed twice with PBS (the adherent cells were washed with PBS twice as well). After the final washing step, the cells were resuspended in Accumax (Innovative Cell Technology, Inc.) and

placed back to the culture flask alongside the adherent cells. All cells were then incubated in a total amount of 3 mL Accumax at room temperature. The cell suspension in the flask was mixed using a pipette every 5 minutes to help dissociate the cells and examined under a microscope to check for single cells. When single cells were achieved (typically after 30 minutes of incubation), the cell suspension was collected into a centrifuge tube and washed with PBS once to remove Accumax from the suspension, and the cells were resuspended in PBS for labeling.

To begin 3D-eCRC-PDX cell preparation for flow cytometry, a dissociation solution was prepared by gently mixing 250 U/mL collagenase IV in PBS, followed by filter sterilization. The desired number of 3D-eCRC-PDX tissues (typically 3 to 5) were placed in a 1.5 mL centrifuge tube and washed with PBS twice over 10 minutes. The PBS was removed, and the dissociation solution was added to the 3D-eCRC-PDX tissues. The 3D-eCRC-PDX tissues were then incubated for 30 minutes at 37 °C, and the suspension was mixed every 10 minutes using a micropipette. The cell suspension was then centrifuged, and the cells were resuspended in Accumax and incubated at room temperature with mixing every 5 minutes until single cells were achieved (typically for 30 minutes). The dissociated single cells were centrifuged to remove Accumax and resuspended in PBS. The dissociated single cells from 3D-eCRC-PDX tissues were ready for labeling at this point.

For 2D-CRC-PDX cell preparation for flow cytometry, the process of cell preparation was similar to that of the isolated cells from CRC-PDX tumors, however, the 12-hour surface protein retrieval was not necessary because the 2D-CRC-PDX cells had been maintained in culture for 7 days.

Antibody-Zenon™ Labeling Conjugate Preparation: To label the cells, β-2-microglobulin (B2M) mouse IgG2a mAb, MHC Class I H-2 Db antibody (H2Db) mouse IgG2a mAb, cytokeratin 20

(CK20) rabbit IgG mAb, and Ki-67 rabbit IgG pAb were used to mark human-derived cells, mouse cells, CRC cells, and proliferative cells, respectively. All primary antibodies were conjugated to Zenon™ Labeling Kits. B2M mouse IgG2a mAb, H2Db mouse IgG2a mAb, CK20 rabbit IgG mAb, and Ki-67 rabbit IgG pAb were conjugated to Zenon™ R-phycoerythrin (PE) mouse IgG2a, Zenon™ R-phycoerythrin (PE) mouse IgG2a, Zenon™ Alexa Fluor™ 647 rabbit IgG, and Zenon™ Alexa Fluor™ 647 rabbit IgG Labeling Kits, respectively, according to the manufacturer protocol. As such, a required amount (calculated based on the number of samples) of primary antibody solution with a concentration of 50 μg/mL in PBS (the corresponding volume of the primary antibody was calculated based on the antibody concentration) was prepared in a microcentrifuge tube. One volume of Zenon™ labeling reagent (Component A) was added to 4 volumes of primary antibody solution, mixed gently, and incubated for 5 minutes at room temperature, protected from light. Then, an equal amount of Zenon™ blocking reagent (Component B) was added to the mixture, mixed gently, and incubated for 5 minutes at room temperature, protected from light. The antibody-Zenon™ labeling conjugate was ready at this point and applied to the samples within 30 minutes.

To distinguish nonspecific background signal from a particular antibody, isotype controls that matched the class and type of the primary antibodies were used. Mouse IgG2a isotype control (ThermoFisher Scientific) and rabbit IgG isotype control (ThermoFisher Scientific) with sat the same concentration as the corresponding primary antibodies were conjugated to Zenon™ R-phycoerythrin (PE) mouse IgG2a and Zenon™ Alexa Fluor™ 647 rabbit IgG labeling kit according to the above protocol.

Cell Labeling: After the cell preparation step, the cells were transferred to 1.5 mL centrifuge tubes, washed with cold protein-free PBS, and centrifuged. To exclude dead cells and debris from the

analysis, 100 µL viability solution was prepared for each sample tube by mixing Zombie Green™ dye in PBS at a ratio of 1:1000 (based on titration). The viability solution was added to the cells, and cells were incubated at 4 °C for 30 minutes in the dark. The cells were then washed by adding 1.3 mL of cold blocking buffer (0.5 % (w/v) BSA and 10 % (v/v) FBS in 1X PBS) to the sample tube and mixing gently, centrifuged, and the supernatant was decanted. To block nonspecific binding on the cell surface, 90 µL of cold blocking buffer containing Human BD Fc Block™ (12 mg/L) and Mouse BD Fc Block™ (5 mg/L) was added to the cells, and the cells were vortexed briefly and incubated at 4 °C for 30 minutes, protected from light. For surface staining, B2M antibody conjugated to Zenon™ PE was added directly to cells with a final primary antibody (B2M) concentration of 0.4 mg/L. The cells were incubated at 4 °C for 30 minutes in the dark. After the incubation, the cells were fixed and permeabilized by adding 0.5 mL of cold Foxp3 Fixation/Permeabilization (eBioscience™) working solution (prepared according to manufacturer protocol by mixing 1 part of Foxp3 Fixation/Permeabilization Concentrate with 3 parts of Foxp3 Fixation/Permeabilization Diluent) to each sample tube, pulse vortexing, and incubating at 4 °C for 30 minutes in the dark. The cells were then centrifuged to remove the Foxp3 Fixation/Permeabilization solution and washed with 1.3 mL of 1X Permeabilization Buffer (eBioscience™) once. To block nonspecific binding for intracellular staining, 90 µL of FACS buffer (0.5 % (w/v) BSA and 10 % (v/v) FBS in 1X Permeabilization Buffer) was added to the cells, and the cells were vortexed briefly and incubated at room temperature for 30 minutes in the dark. For intracellular staining, the conjugated antibodies were directly added to the cells, and the cells were vortexed briefly and incubated at room temperature for 30 minutes in the dark. It should be noted that we had to use separate samples for each primary antibody-Zenon™ conjugate as a similar Zenon™ labeling kit (Zenon™ Alexa Fluor™ 647) was used to create CK20-Zenon™

conjugate and Ki-67-Zenon™ conjugate. After a 30-minute incubation, 1.3 mL of 1X Permeabilization Buffer was added to each tube and the cells were incubated for 10 minutes at room temperature in the dark and centrifuged to remove unbound antibodies. The washing step was repeated once more, and the cells were resuspended in the blocking buffer and filtered using 40 µm Scienceware® Flowmi™ Cell Strainers before analyzing via a BD Accuri C6 cytometer (BD Biosciences).

Compensation Controls: Compensation beads (ThermoFisher Scientific) were used according to the manufacturer protocol to ensure that the fluorescent signal measured by each flow cytometer filter is merely due to the fluorochrome of interest and to accurately set the compensation if there was any overlap between the fluorochromes. Briefly, AbCTM Total Compensation capture beads (Component A) and negative beads (Component B) were resuspended thoroughly by vortexing for 10 seconds. One drop of Component A was added to a 1.5 mL centrifuge tube for each fluorochrome-conjugated antibody. Each antibody-Zenon™ labeling conjugate was added to the designated tube, mixed, and incubated for 15 minutes at room temperature in the dark. PBS (1.3 mL) was then added to the beads, and the beads were centrifuged and resuspended by adding 0.5 mL of PBS. One drop of Component B was then added to the tubes, and the beads were mixed thoroughly and vortexed before analyzing by flow cytometry.

Unstained Controls: To check for cell autofluorescence, unstained cells were used. The unstained cells underwent the entire flow cytometry protocol with the exception of adding Zombie Green™ and the antibody-Zenon™ labeling conjugates.

Viability Controls: To set an accurate compensation for Zombie Green™ dye, some cells were killed using pure ethanol. Briefly, 2×10^5 of prepared cells in PBS were transferred in a 1.5 mL centrifuge tube and centrifuged at 200 ×g for 5 minutes. The supernatant was then removed, and 1 mL of pure ethanol was added to the cells. The cells were incubated at room temperature for 5 minutes and immediately centrifuged at 200 ×g for 5 minutes. After centrifugation, the ethanol was removed thoroughly, and the cells were washed once with PBS, resuspended in 100 µL viability solution, and incubated at 4 °C for 20 minutes in the dark. The cells were washed by adding 1.3 mL of cold blocking buffer to the sample tube and mixing gently, centrifuged at 200 ×g for 5 minutes, resuspended in the blocking buffer and filtered using 40 µm Scienceware® Flowmi™ Cell Strainers before analyzing via BD Accuri C6 cytometer.

Flow Cytometer and Data Analysis: A BD Accuri C6 cytometer was used to analyze the labeled cells. The cytometer was equipped with two excitation lasers, 488nm (blue) and 635nm (red), and four filters, FL1 533/30, FL2 585/40, FL3 670/LP, and FL4 675/25. The Zombie Green™ dye and Zenon™ PE were excited by the blue laser and captured by FL1 and FL2, respectively, and the Zenon™ Alexa Fluor™ 647 was excited by the red laser and captured by FL4. Twenty thousand events were counted for compensation, unstained, and viability controls. For the labeled cells, twenty thousand viable cells were counted from each sample tube.

FlowJo software (version 10.0.7) was employed for flow cytometry data analysis. Briefly, a compensation matrix was created based on compensation beads and dead cell control. Small debris and particles were gated out using the side scattering-area (SSC-A) vs forward scattering-area (FSC-A) graph. By excluding the small debris and particles and only looking at the cells, the singlets were gated on using forward scattering-height (FSC-H) vs FSC-A graph. Then the dead

cells were gated out from the singlets using FSC-H vs Compensated FL1-Height (Comp-FL1-H) (Zombie Green™ dye). By looking at the viable singlets, the Comp-FL4-H vs Comp-FL3-H graph was used to analyzed $B2M^{+/-}$ and $CK20^{+/-}$ cells or the $B2M^{+/-}$ and $Ki\text{-}67^{+/-}$ cells. Furthermore, the Comp-FL4-H vs Comp-FL3-H graph was used to check whether unstained cells were autofluorescent and whether there was nonspecific binding on isotype control.

Immunostaining for Examination of Cell Morphology

To study the cell morphology within both 3D-eCRC-PDX tissues and the 2D-CRC-PDX cultures, the cells were labeled with Hoechst 33342, B2M, Phalloidin, and CK20 to observe the cell nuclei, human-derived cells, f-Actin filaments, and CRC cells, respectively. Briefly, the 3D-eCRC-PDX tissues and 2D-CRC-PDX cells were separately washed 3 times with 1X PBS over a period of 10 minutes. The PBS was then removed, and the cells were fixed using 4% paraformaldehyde (Electron Microscopy Sciences, 15710) (diluted in PBS) for 1 hour at room temperature. Next, samples were rinsed with PBS 2 times over a period of 10 minutes and subsequently permeabilized using sterile-filtered PBS-T (1% w/v BSA and 0.2% v/v TritonX (Sigma-Aldrich) in PBS) for 30 minutes on an Orbitron. The PBS-T was removed, and the samples were incubated in blocking buffer (0.5 % (w/v) BSA and 10 % (v/v) FBS in 1X PBS) for 2 hours at room temperature. The blocking buffer was discarded and a solution of B2M primary antibody in blocking buffer (5 µg/mL) was added to the cells and the cells were again incubated at room temperature for 2 hours. Next, cells were washed with PBS-T 3 times over a period of 15 minutes; PBS-T was removed, a blocking buffer solution containing goat anti-mouse IgG Alexa Fluor™ 488 (ThermoFisher Scientific) (1:100 ratio), Alexa Fluor™ 568 Phalloidin (ThermoFisher Scientific) (1:100 ratio), and CK20 rabbit IgG mAb conjugated to Zenon™ Alexa Fluor™ 647

rabbit IgG (1.25 µg/mL) was added and samples were then incubated for 2 hours at room temperature. Finally, cells were washed with PBS-T 3 times over a period of 15 minutes, counterstained using Hoechst 33342 (20 µg/mL) for 30 minutes.

Histological Examination of CRC-PDX Tumors and 3D-eCRC-PDX Tissues

To stain the samples, the CRC-PDX tumors and 3D-eCRC-PDX tissues were washed with 1X PBS, fixed with 4% paraformaldehyde, dehydrated with increasing grades of ethanol (70% to 100%), cleared in Hemo-De, soaked in wax, embedded in paraffin, and sectioned. The sections (6 µm) were cleared using Hemo-De (3 washes; 8 minutes each), rehydrated with decreasing grades of ethanol (100% to 80%) and rinsed with distilled water, stained with Mayers Hematoxylin for 8 minutes, rinsed with tap water for 20 minutes and 80% ethanol for 2 minutes, stained with eosin-phloxine B solution for 2 minutes, dehydrated by increasing grades of ethanol (70% to 100%), and finally cleared using Hemo-De (3 washes; 8 minutes each). A thin layer of Eukitt™ mounting medium was applied and the slides were covered with clean coverslips. The stained sections were scanned using an Aperio CS2 scanner (Leica Biosystems) at 40x magnification. The digital images were viewed using Aperio ImageScope (Ver. 12.3.2.8013).

Proteomics Analysis

Sample Preparation: CRC-PDX tumors and 3D-eCRC-PDX tissues were processed via Millipore Compartmental Protein Extraction Kit following the manufacturer's instruction. Of note, tissues were weighed prior to processing, and buffer volume was adjusted appropriately based on the sample weight. Following sequential extraction of cytoplasmic, nuclear, membrane, and cytoskeletal proteins via the kit [6, 7], the remaining pellet was washed once with 500 µL of PBS

and reconstituted in 2x Novex NuPAGE LDS sample buffer (Invitrogen) supplemented with 2x NuPAGE sample reducing agent (Invitrogen). ECM-enriched fractions were then sonicated in an ultrasonic water bath for 20min. at ambient temperature. Debris was removed by centrifugation for 20min. at 16,000×g, 4°C. Proteomics analysis was carried out as previously referenced with minor changes [8]. The ECM-enriched fraction was quantified using an EZQ Protein Quantitation Kit (Invitrogen). Twenty micrograms of protein per sample were then diluted to 35 µL using NuPAGE LDS sample buffer (1x final conc.) and denatured at 70°C for 10min. prior to loading onto a Novex NuPAGE 10% Bis-Tris Protein gel (Invitrogen) and separated for its full-length (35min. at 200 constant V). The gels were stained overnight with Novex Colloidal Blue Staining Kit (Invitrogen). Following de-staining, each lane was cut into four MW fractions and equilibrated in 100 mM ammonium bicarbonate (AmBc). Each gel plug was then digested overnight with Trypsin Gold, Mass Spectrometry Grade (Promega) following manufacturer's instruction. Peptide extracts were reconstituted in 0.1% Formic Acid/ ddH_2O at 0.1 µg/µL.

Mass Spectrometry: Peptide digests (8 µL each) were injected onto a 1260 Infinity nHPLC stack (Agilent Technologies), and separated using a 75 micron I.D. x 15 cm pulled tip C-18 column (Jupiter C-18 300 Å, 5 microns, Phenomenex). This system runs in-line with a Thermo Orbitrap Velos Pro hybrid mass spectrometer, equipped with a nano-electrospray source (Thermo Fisher Scientific), and all data were collected in CID mode. The nHPLC was configured with binary mobile phases that included solvent A (0.1%FA in ddH_2O), and solvent B (0.1%FA in 15% ddH_2O / 85% ACN), programmed as follows; 10min @ 5%B (2 µL/min, load), 90min @ 5%-40%B (linear: 0.5 nL/min, analyze), 5min @ 70%B (2 µL/min, wash), 10min @ 0%B (2 µL/min, equilibrate). Following each parent ion scan (300-1200 m/z @ 60k resolution), fragmentation data

(MS2) was collected on the top most intense 15 ions. For data-dependent scans, charge state screening and dynamic exclusion were enabled with a repeat count of 2, repeat duration of 30s, and exclusion duration of 90s.

MS Data Conversion and Searches: The XCalibur RAW files were collected in profile mode, centroided, and converted to MzXML using ReAdW v. 3.5.1. The mgf files were then be created using MzXML2Search (included in TPP v. 3.5) for all scans. The data were searched using SEQUEST, which was set for two maximum missed cleavages, a precursor mass window of 20 ppm, trypsin digestion, variable modification C @ 57.0293, and M @ 15.9949. Searches were performed with species-specific subsets of the UniProtKB database (ex: Homo sapiens, Mus musculus).

Peptide Filtering, Grouping, and Quantification: The list of peptide IDs generated based on SEQUEST (Thermo Fisher Scientific) search results were filtered using Scaffold (Protein Sciences, Portland Oregon). Scaffold filters and groups all peptides to generate and retain only high confidence IDs while also generating normalized spectral counts (N-SC's) across all samples for the purpose of relative quantification. The filter cut-off values were set with minimum peptide length of >5 AA's, with no MH+1 charge states, with peptide probabilities of >80% C.I., and with the number of peptides per protein ≥2. The protein probabilities were then set to a >99.0% C.I., and an FDR<1.0. Scaffold incorporates the two most common methods for statistical validation of large proteome datasets, the false discovery rate (FDR) and protein probability [9-11]. Relative quantifications across experiments were then performed via spectral counting [12, 13], and when relevant, spectral count abundances were then normalized between samples [14].

Reagent Table

REAGENT OR RESOUCE	SOURCE	IDENTIFIER
Matrix High Concentration, Phenol-Red free Matrigel	Corning	cat. #354262
NOD.Cg-Prkdc^{scid} Il2rg^{tm1Wjl}/SzJ (NOD-SCID) mouse	The Jackson Laboratory	cat. #005557
Collagenase IV	Worthington	cat. #LS004188
Deoxyribonuclease I (DNase I)	Worthington	cat. #LS002139
Hank's Buffered Saline Solution (HBSS)	Lonza	cat. #10-508F
Syringe Filter	VWR	cat. #28145-501
Phosphate-buffered Saline (PBS)	Lonza	cat. #17-517Q
40-µm Cell Strainer	Corning	cat. #352340
DMEM	Lonza	cat. #12-614Q
Fetal Bovine Serum	Atlanta Biologicals™	cat. #S10250
Penicillin:Streptomycin Solution	HyClone™	cat. #16777-164
Glutagro™ Supplement	Corning	REF #25-015-CI
Trypan Blue	Lonza	cat. #17-942E
1-Vinyl-2-pyrrolidinone	Sigma-Aldrich	SKU #V3409
0.25% Trypsin-EDTA	Corning	REF #25-053-CI
LIVE/DEAD® Viability/Cytotoxicity Kit	ThermoFisher Scientific	cat. #L3224
Hoechst 33342	Calbiochem	cat. #382065
Beta-2-microglobulin (B2M) mouse IgG2a mAb	OriGene Technologies	cat. #BM604S
Zenon™ R-phycoerythrin (PE) mouse IgG2a	ThermoFisher Scientific	cat. #Z25155
MHC Class I H-2 Db antibody (H2Db) mouse IgG2a mAb	OriGene Technologies	cat. #AM08082PU-N
Zenon™ PE mouse IgG2a, cytokeratin 20 (CK20) rabbit IgG mAb	Cell Signaling Technology	cat. #13063S
Zenon™ Alexa Fluor™ 647 rabbit IgG	ThermoFisher Scientific	cat. #Z25308
Ki-67 mouse IgG1 mAb	abcam	cat. #ab15580
Zombie Green™ Fixable Viability Kit, dye	BioLegend	cat. #423111
Bovine Serum Albumin (BSA)	Sigma-Aldrich	SKU #A2153
Human BD Fc Block™	BD Biosciences	cat. #564220
Mouse BD Fc Block™	BD Biosciences	cat. #553141
Foxp3	eBioscience™	cat. #00-5523-00
AbC™ Total Antibody Compensation Bead Kit	ThermoFisher Scientific	REF #A10513
B2M mouse IgG2a mAb with goat anti-mouse IgG Alexa Fluor™ 488	ThermoFisher Scientific	cat. #A-11001

Alexa Fluor™ 568 Phalloidin	ThermoFisher Scientific	cat. # A12380
RNeasy Plus Micro Kit	Qiagen	cat. #74034
SuperScript™ IV First-Strand Synthesis System	Invitrogen	cat. #18091050
RT2 SYBR Green qPCR Mastermix	Qiagen	cat. #330503
Poly(ethylene glycol) (PEG)	Sigma-Aldrich	SKU #81280
Acryloyl chloride	Alfa Aesar	STK #L10363-09
Anhydrous dichloromethane	Honeywell Research Chemicals	cat. #AS299-1
Triethylamine (TEA)	Sigma-Aldrich	SKU #471283
NaCl	Sigma-Aldrich	SKU #S5886
KCl	Sigma-Aldrich	SKU #P5405
Na_2HPO_4	Sigma-Aldrich	SKU #71643
KH_2PO_4	Sigma-Aldrich	SKU P5655
Bovine fibrinogen	Sigma-Aldrich	SKU #F8630
Urea	Sigma-Aldrich	SKU #51456
Acetone	VWR	cat. #BDH1101-4LP
Eosin Y	Fisher Scientific	cat. #E511-25
Pierce™ BCA Protein Assay Kit	ThermoFisher Scientific	cat. #23225
SYLGARD® 184 Silicone Elastomer Kit	Dow Corning	SKU #4019862
Accumax	Innovative Cell Technology, Inc.	cat. #AM105-500
Triton™ X-100	Sigma-Aldrich	SKU # X100-500ML
Safety scalpel	Bard-Parker®	REF #372611
Compartmental Protein Extraction Kit	Millipore	cat. #2145
NuPAGE™ LDS Sample Buffer	Invitrogen	cat. #NP0007
NuPAGE™ Sample Reducing Agent	Invitrogen	cat. #NP0009
EZQ™ Protein Quantitation Kit	Invitrogen	cat. #R33200
NuPAGE™ 10% Bis-Tris Protein Gels	Invitrogen	cat. #NP0315BOX
Colloidal Blue Staining Kit	Invitrogen	cat. #LC6025
Trypsin Gold, Mass Spectrometry Grade	Promega	cat. #V5280

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