SUPPLEMENTAL MATERIALS AND METHODS

Luciferase Assays

Cells were plated in poly-L-lysine-coated 96-well plates. Once adhered, cells were co-transfected with a pNF-κB-luciferase reporter plasmid (Stratagene) and *Renilla* luciferase plasmid (pRL-TK) using FuGENE HD transfection reagent (Promega, E2311) per the manufacturer's instructions. Cells were incubated for 8-16 hours and then the medium was aspirated, fresh medium added, and siRNA was transfected. After 48-72 hours SULT2B1b siRNA transfection (indicated in Figure Legends), cell lysates were tested for Firefly and *Renilla* luciferase activity using the Dual Luciferase Reporter Assay kit (Promega, E1910).[18] Relative luciferase activity (RLU=Firefly/*Renilla*) of the NF-κB reporter construct is shown as the mean of the fold change (over samples without siRNA transfection) +/- SEM from at least three independent experiments performed in triplicate.

C1 Loading

Viable cells were spun down and concentrated to 300-400 cells/μL in serum-free RPMI and mixed at a 3:2 ratio of cell suspension:C1 suspension reagent in preparation for loading into the C1 Single-Cell Auto Prep System (Fluidigm).

Priming of the 17-25 μm (large) IFC plate (Fluidigm, 100-5761) was completed according to the manufacturer's instructions. Following priming, 15 μL of cell mix was added to the IFC plate along with LIVE/DEAD cell staining solution containing 4 μM ethidium homodimer-1 and calcein AM (Life Technologies, L-3224). Cells were isolated and stained within the C1 system and then manually observed on an inverted fluorescence microscope with FITC and Texas Red filters (Olympus IX51). Capture sites containing 0 or >1 cell were excluded, and cells with positive ethidium homodimer-1 staining were also excluded. Cell lysis, reverse transcription, and cDNA amplification of single cells were conducted following the protocol for mRNA sequencing (Fluidigm). cDNA was produced in this system using SMARTer chemistry (Clontech).

Library Preparation

The Purdue Genomics Facility prepared libraries using the Nextera® XT DNA Sample Preparation Kit and Index Kit (Illumina, FC-131-1096 and FC-131-1002) according to the Fluidgm C1 mRNA sequencing protocol. Libraries were separated into pools and cleaned using Agencourt AMPure® XP beads (Agencourt BioScience Co., A63880). Cells from each condition within Batch 1 were divided into five pools (generated by combining 2 µL from each sample) and clean-up was performed using a 0.8:1.0 bead to sample ratio. Subsequent batches combined 6 µL from each sample into 10 total pools and used an AMPure® cleanup ratio of 0.7:1. The library pool quality was assessed using the Agilent Bioanalyzer with the high Sensitivity DNA Chip (Agilent Technologies, 5067-4626). Library pools were quantified using the Library Quantification kit – Illumina/Universal (Kapa Biosystems Inc., KK4824). Equimolar volumes of pools were combined, then rechecked using the Kapa kit.