**APPENDIX**

**CMx Microfluidic Chip Design and Cell Capture**

Briefly, the CMx microfluidic chip (similar in size to a standard microscope slide) consists of a top PMMA [poly-(methyl-methacrylate)] layer and a bottom glass coverslip assembled together with 3M adhesive which defines the microfluidic channel patterns and height. The ceiling of the microfluidic channel contains micro-patterns etched into the PMMA layer to create flow patterns that promote frequent contact of cells with the capture antibody as blood is transported through the fluidic channel.

The entire inner surface of microfluidic channel is coated with anti-fouling biomimetic lipid layers to reduce non-specific adhesion by blood components. Circulating epithelial cells (simply referred as CTCs) are captured by specific recognition of EpCAM using an anti-EpCAM antibody implanted on the fluidic lipid layers. The fluidic lipid layers allow lateral movement which facilitates clustering of antibodies and collaborate binding to EpCAM antigens on target cells, thus increasing capture sensitivity. The inlet port on one side of the PMMA layer provides an entrance for whole blood and reagents to enter the chip. The outlet port on the other side of the chip allows processed blood and reagents to leave the chip. A 2.5 mL sample (2mL blood with 0.5mL preservative) is pulled through the chip by a syringe pump connected to the outlet port.

After cell capture is completed, gentle flushes of the microfluidic channel at low flow rate (3 mL/hour) with phosphate buffer saline (PBS) remove unwanted red and white blood cells and debris. This biomimetic lipid-coated surface enables 3X greater capture efficiency and 6X better purity compared to a conventional silane-treated surface.23 Fine air bubbles (air foam) created with cell culture medium and fetal bovine serum (FBS) are protein-rich and possess a hydrophilic surface and hydrophobic core (properties that match lipid layers); upon contact they can lift off the lipid layer on which the capture antibody, anti-EpCAM, is implanted. This allows the release of captured cells without breaking the strong antibody-antigen hydrogen bonds and reduces damage to the captured cells.24 Injection of air foam via the outlet port allows most cells captured near the inlet to traverse the shortest path into the Eppendorf collection tube.

Released cells are subsequently pipetted onto a special “membrane chip” containing a 10-mm diameter 3M membrane filter with 2-micron pores. The membrane filter serves as the substrate for immunofluorescent staining (IF) and washing. Cells are safely retained during incubations and reagents efficiently absorbed by the pads placed underneath the membrane.