Appendix S1

Dissociation of human renal tissue. The cortical tissue farthest from the tumor was selected and put in ice-cold Dulbecco's modified Eagle's medium (Invitrogen, Carlsbad, CA) supplemented with 10% fetal calf serum and 1% penicillin/streptomycin. Samples were rinsed, minced, and subjected to overnight collagenase treatment at 37°C in a processing medium consisting of Ham's F-12/Dulbecco's modified Eagle's medium [1:1 (v/v); Invitrogen], supplemented with 5% fetal calf serum, 1% penicillin/streptomycin, collagenase IV at a final concentration of 300 U/ml (Invitrogen), and deoxyribonuclease I type II at a final concentration of 200 U/ml (Sigma-Aldrich, St. Louis, MO). After trituration by slow repeated pipetting through a 10-ml pipette, the resulting tissue suspension was serially passed through tissue strainers with mesh sizes of 100 and 70 μm, respectively, thereby excluding glomeruli from the preparation. The suspension was treated with 1X trypsin–EDTA for 5 minutes and then was passed through a 20-μm strainer, which resulted in single cells.

Figure S1. Optimisation of downstream processing of ClearCell FX output. (A) Representative image of Total Eukaryote RNA Pico Chip electropherogram and RIN values for RNA extracted from ClearCell FX output containing 27 and 8 SNU-349 cells amongst background of leucocytes. (B) qPCR monitoring of pre-amplification cycles using SYBR green for 500pg and 50pg total RNA input from SNU-349 cells. Vertical red line represents selected number of cycles for pre-amplification of future pre-amplification reactions. (C) cDNA fragment analysis of pre-amplified cDNA compared to non-pre-amplified cDNA from SNU-349 cells. nt, nucleotides; rRNA, ribosomal RNA.

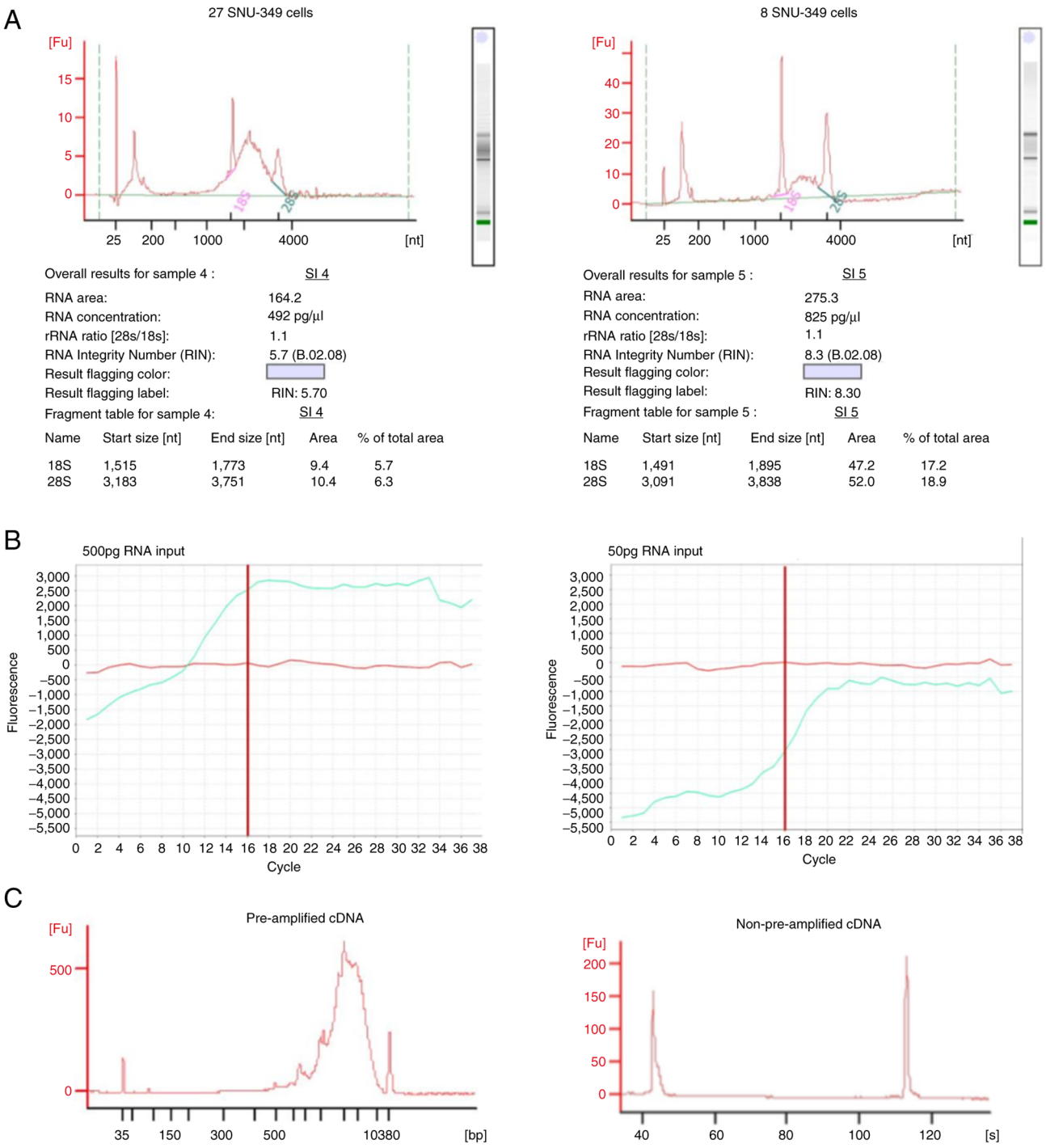

Figure S2. Expression of all subtype specific markers in 6 healthy blood samples enriched for CTCs.