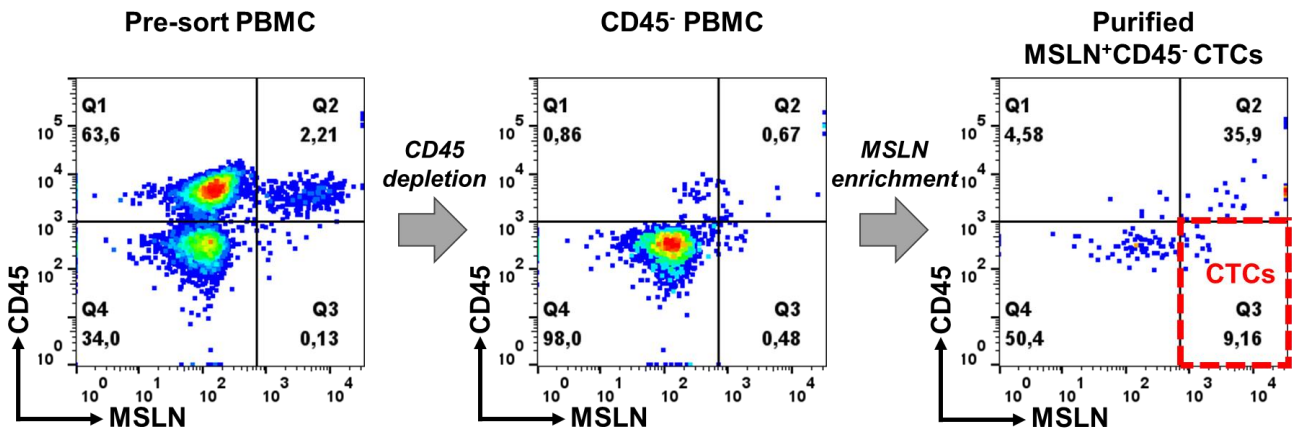


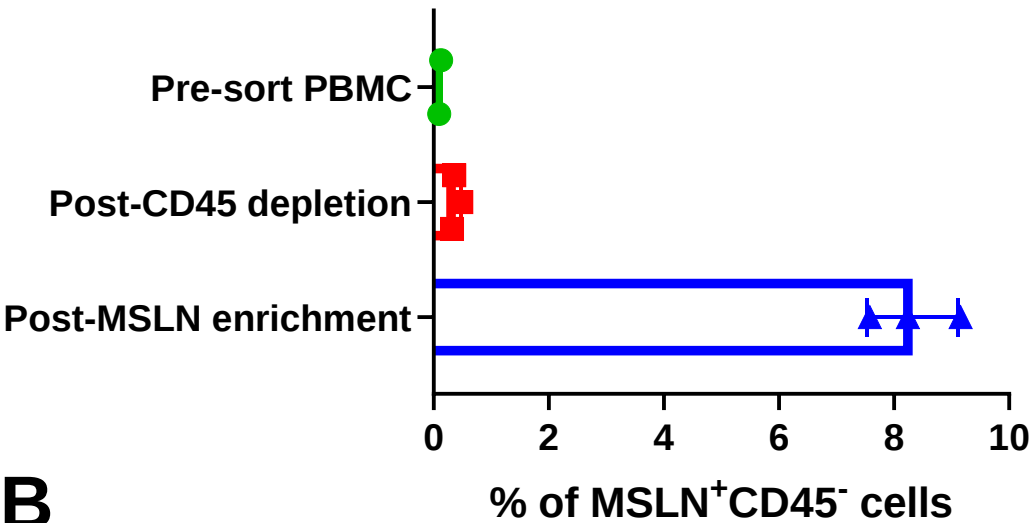
Figure 1S

Preparation of CTC from peripheral blood.

A) Gating strategy of identifying CTC by flow cytometry. B) Quantification of CTC pre- and post-sorting using a MACS column and a microfluidic chip using a protocol.



A



B

Six clusters of merged tumor cells were characterized in heat map illustrating top up- and down-regulated genes.

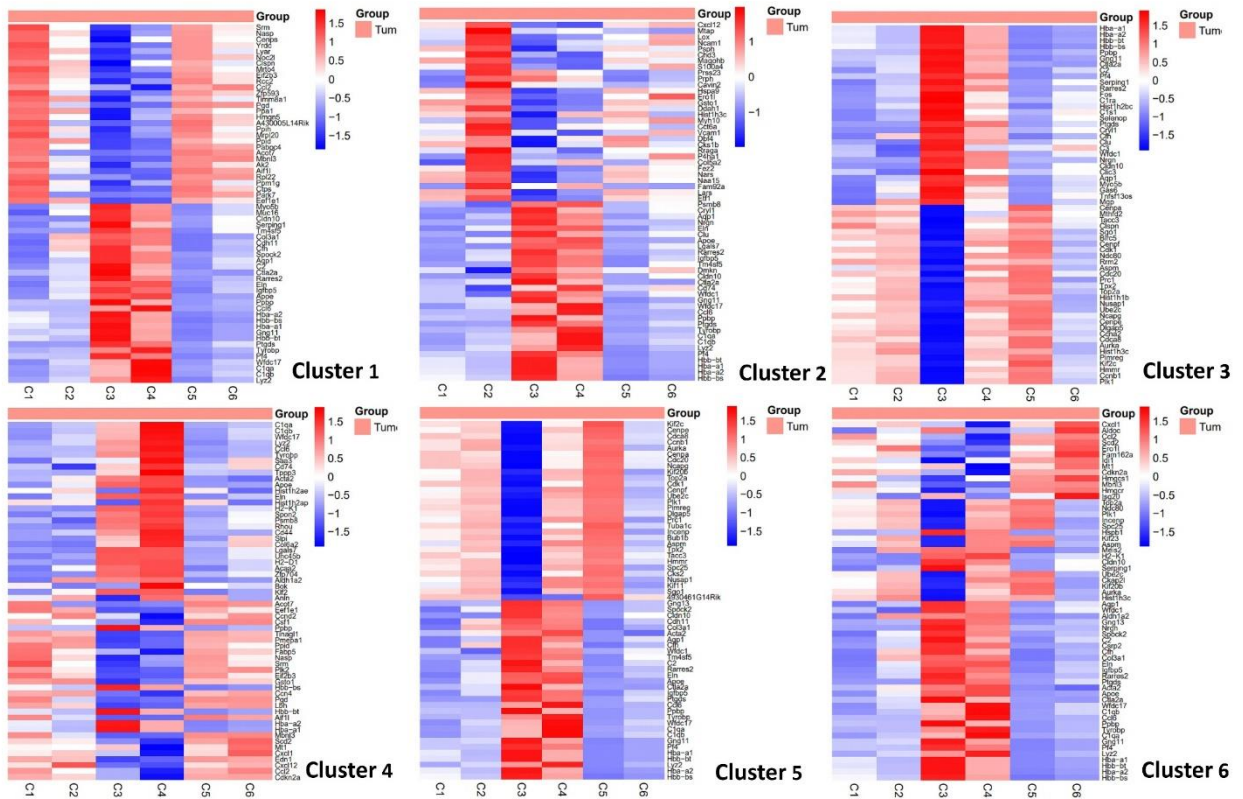


Figure 3S

Six clusters of merged tumor cells were characterized by volcano plots illustrating top up- and down-regulated genes.

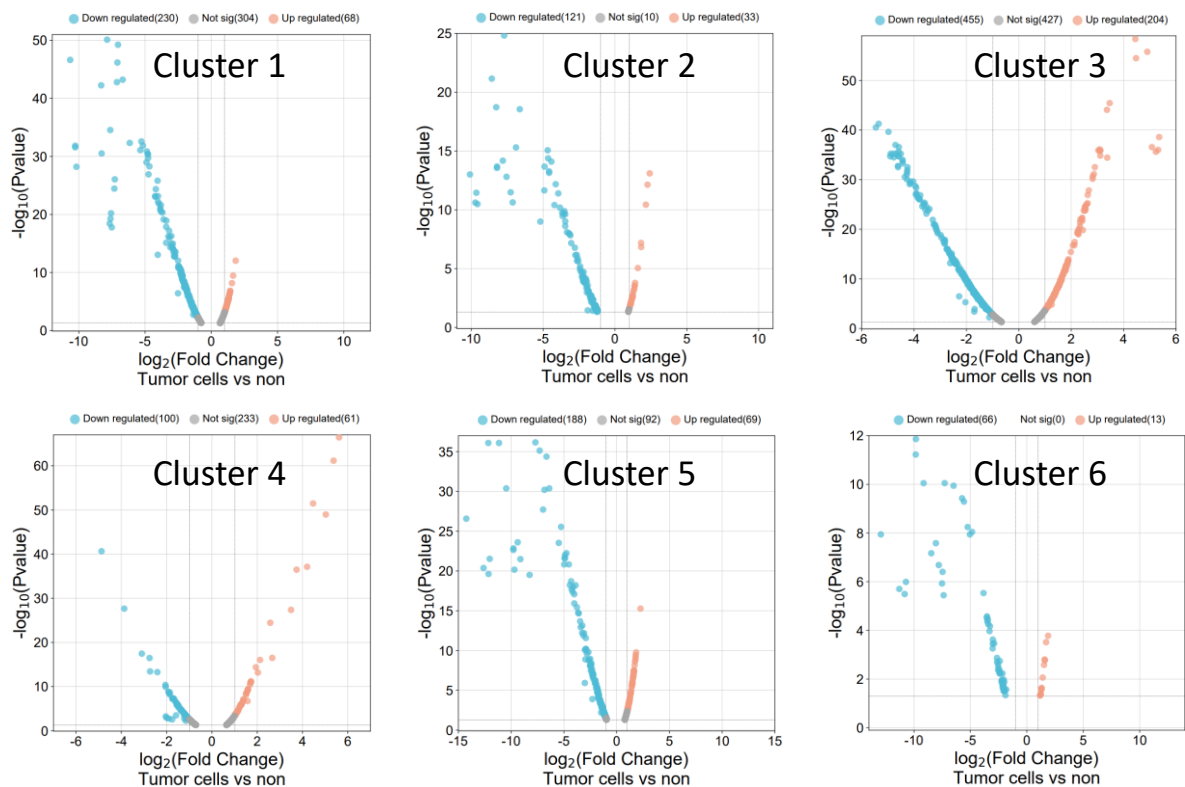


Figure 4S

Top Gene expression of top 30 up- and down-regulated genes in each group.

A) CC: cell culture RN5; B) CTC: circulating tumor cells; C) Lav: tumor cells in lavage from RN5-bearing mice at 4weeks ip.

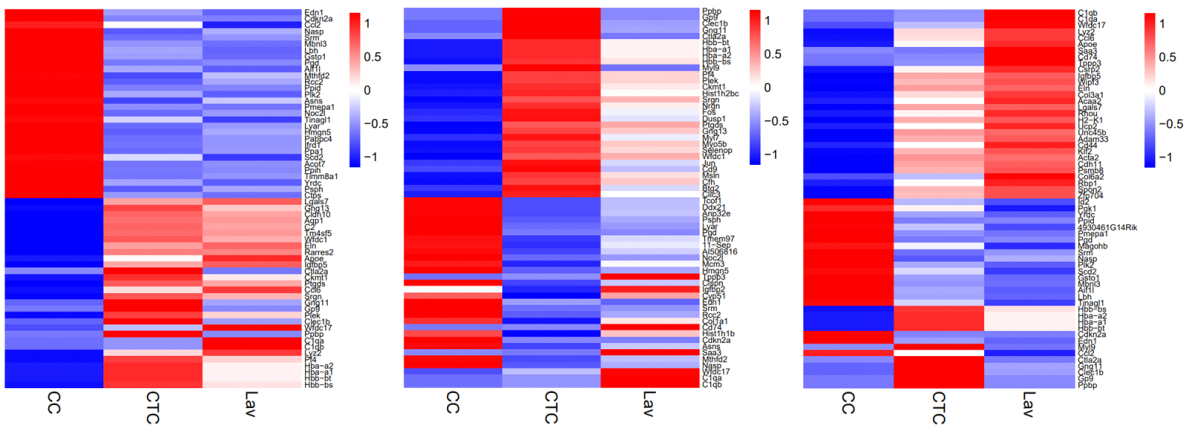


Figure 5S

All up- or down-regulated genes in tumor cells of each group after reclustering.

Three clusters of merged tumor cells based on sample ID (CC, CTC, Lav) were characterized by volcano plots illustrating top up- and down-regulated genes.

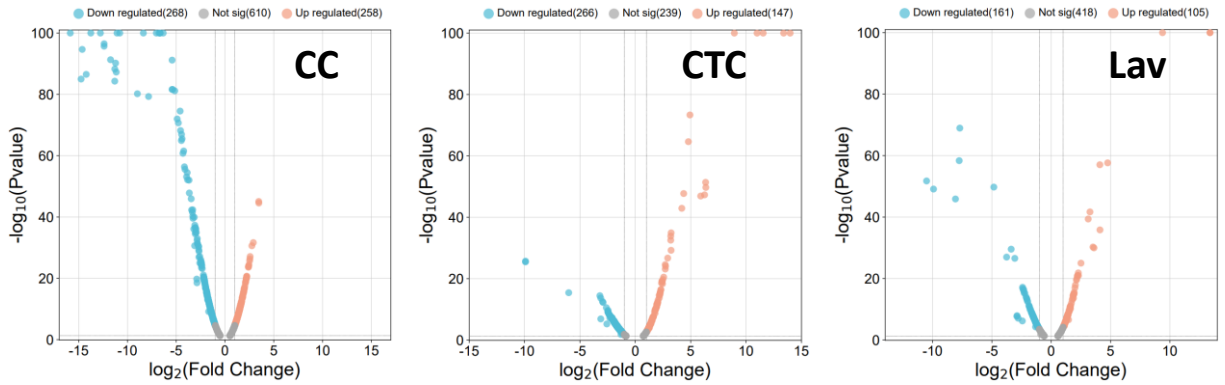


Figure 6S
Up-regulated genes in each group overlapped with hallmark gene sets contributed to cell proliferation.

A&B) Up-regulated genes in CC overlaps with MYC targets v1 16.67% (43/258) and v2 6.2% (16/258), respectively., while in CTC and Lav groups only had 1 gene or nothing overlapped.

C) Up-regulated genes in CC overlaps with E2F targets 11.24% (29/258), while the CTC and Lav groups had nothing overlapped.

D) Up-regulated genes in CC overlaps with MTORC1 signaling 10.50% (27/258), while the CTC and Lav groups had only a few genes overlapped, 3 and 2 genes, respectively.

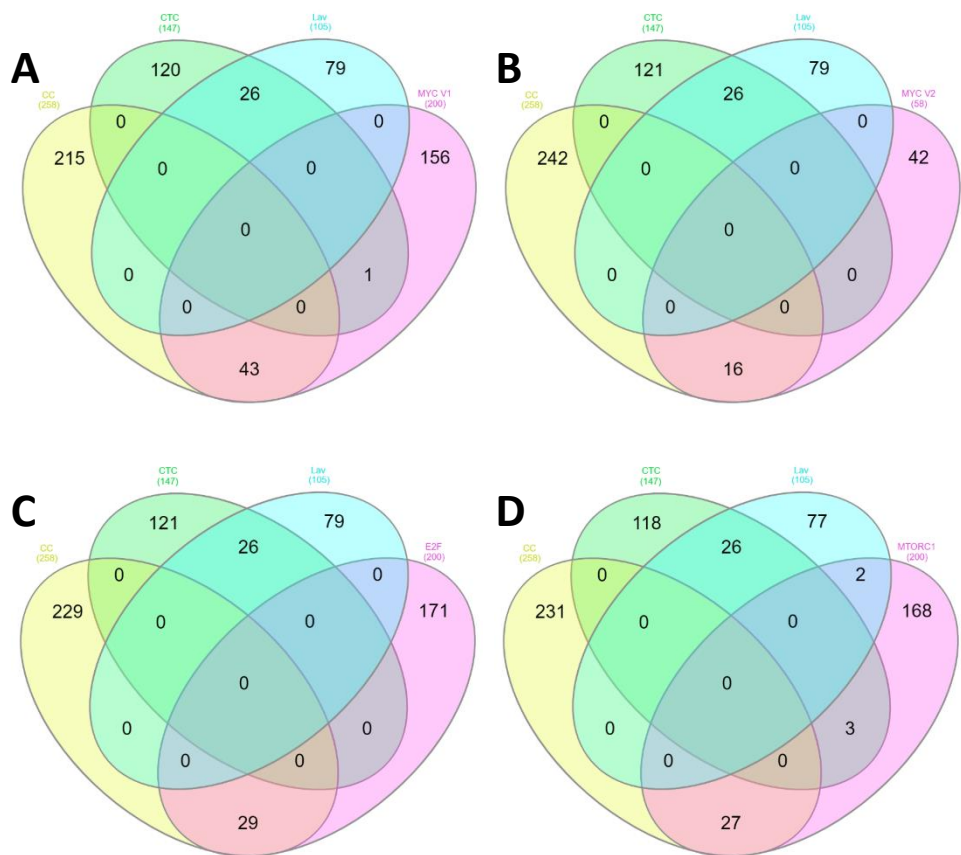


Figure 7S

Subclusters from each group were mapped onto the UMAP generated by velocity analysis.

A)CC, B)CTC, and C)LAV group were reclustered into 6, 4 and 5 subclusters, respectively. The arrows show the trend of pseudotime along the cell population. The pseudotime is normalized between 0 and 1, with 0 being the oldest part of the samples. The cells that are colored dark red are the more recent ones, and so the trend ends at them.

