

Supporting Information

Table S1. Clinical characteristics of patients in the CTC study

Index	Age	Gender	Histology	Stage	Differentiation
P1	66	m	Squamous	IB	Poor
P2	91	f	Adenocarcinoma	IB	Moderate
P3	81	f	Adenocarcinoma	IIIA	Moderate
P4	92	m	Squamous	IA	Moderate
P5	52	f	Adenocarcinoma	IIIA	Poor
P6	57	m	Small cell	IIB	High grade
P7	70	f	Adenocarcinoma	IIA	Moderate
P8	85	f	Adenocarcinoma	IIB	Moderate
P9	86	m	Adenocarcinoma	IA	Well
P10	77	m	Squamous	IIIA	Poor
P11	62	m	Squamous	IB	Poor
P12	73	f	Adenocarcinoma	IIIA	Poor
P13	65	m	Squamous	IA	Moderate
P14	75	f	Adenocarcinoma In Situ	IB	Well
P15	60	m	Adenocarcinoma	IB	Moderate
P16	70	m	Squamous	IIA	Moderate
P17	58	f	Squamous	IB	Moderate
P18	71	m	Adenocarcinoma	IA	Moderate
P19	82	f	Adenocarcinoma	IA	Moderate
P20	57	m	Squamous	IB	Poor
P21	72	f	Adenocarcinoma	IB	Well
P22	54	m	Squamous	IIIB	Poor
P23	73	f	Adenocarcinoma	IA	Moderate
P24	66	f	Adenocarcinoma	IIIA	Poor
P25	58	f	Adenocarcinoma	IB	Moderate
P26	73	m	Squamous	IB	Moderate
P27	68	m	Squamous	IA	Poor
P28	77	m	Squamous	IIB	Clear cell features
P29	79	f	Adenocarcinoma	IIB	Poor
P30	72	f	Squamous	IV	Moderate
P31	53	f	Adenocarcinoma	IA	Moderate
P32	58	f	Squamous	IIIA	Poor
P33	67	f	Adenocarcinoma	IA	Moderate
P34	74	f	Adenocarcinoma	IA	Well
P35	67	f	Squamous	IIB	Poor
P36	60	f	Adenocarcinoma	IIIB	Poor

Figure S1. (A). Image of the microfluidic device, the OncoBean Chip, (B). Scanning electron microscope image of microstructures in the device.

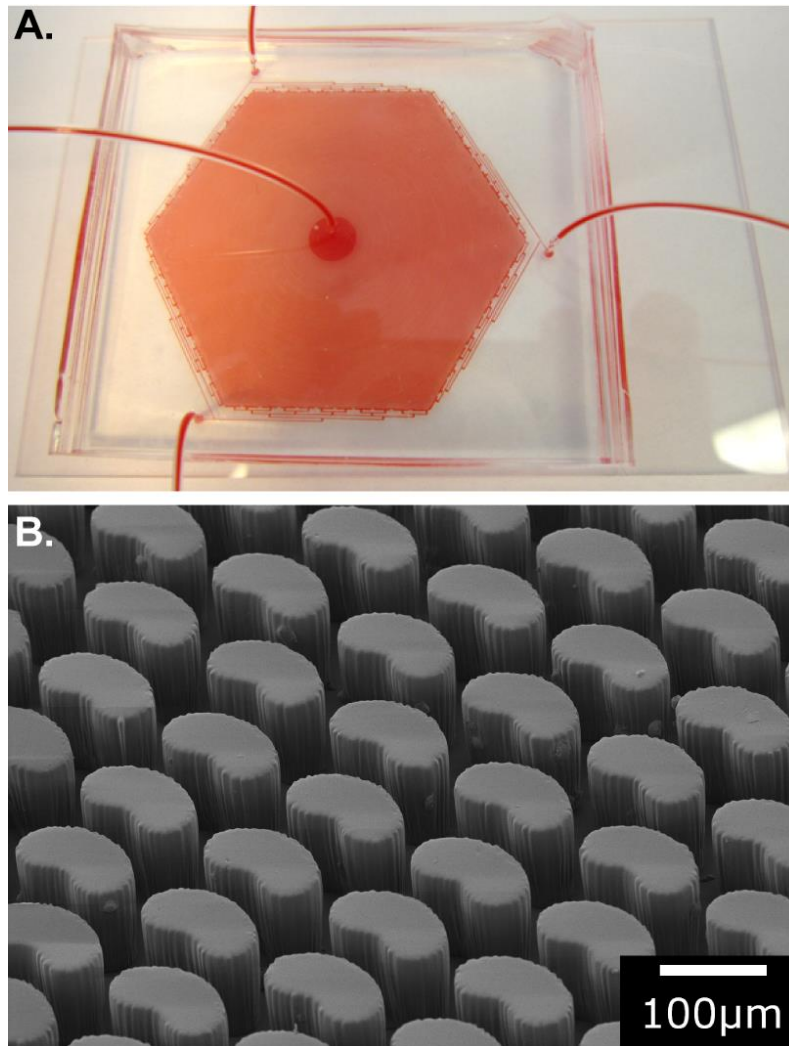


Figure S2. Range of CTCs per 3 ml in the healthy volunteer samples.

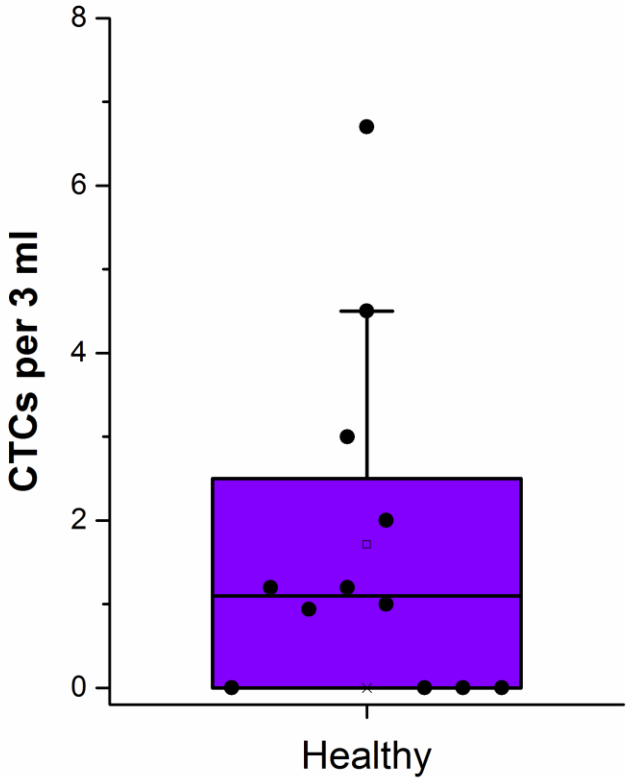


Figure S3. Trend showing increase in average number of clusters containing 5 or 10 CTCs (obtained from any vein) with increasing stage.

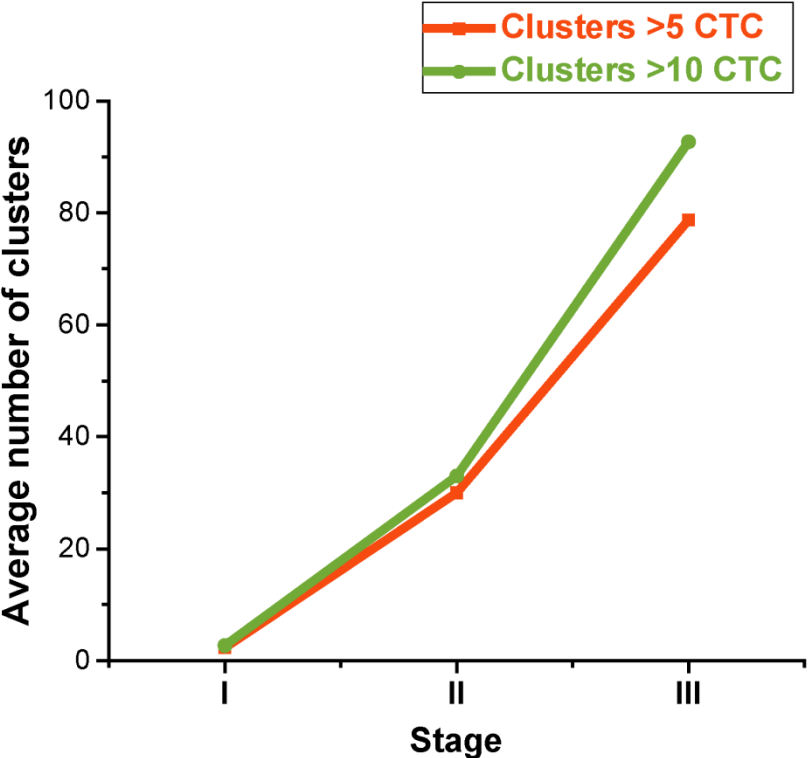


Figure S4. Gene expression levels (Ct values) of housekeeping genes GAPDH and RAB7A among the initial set of 26 samples analyzed for mRNA expression.

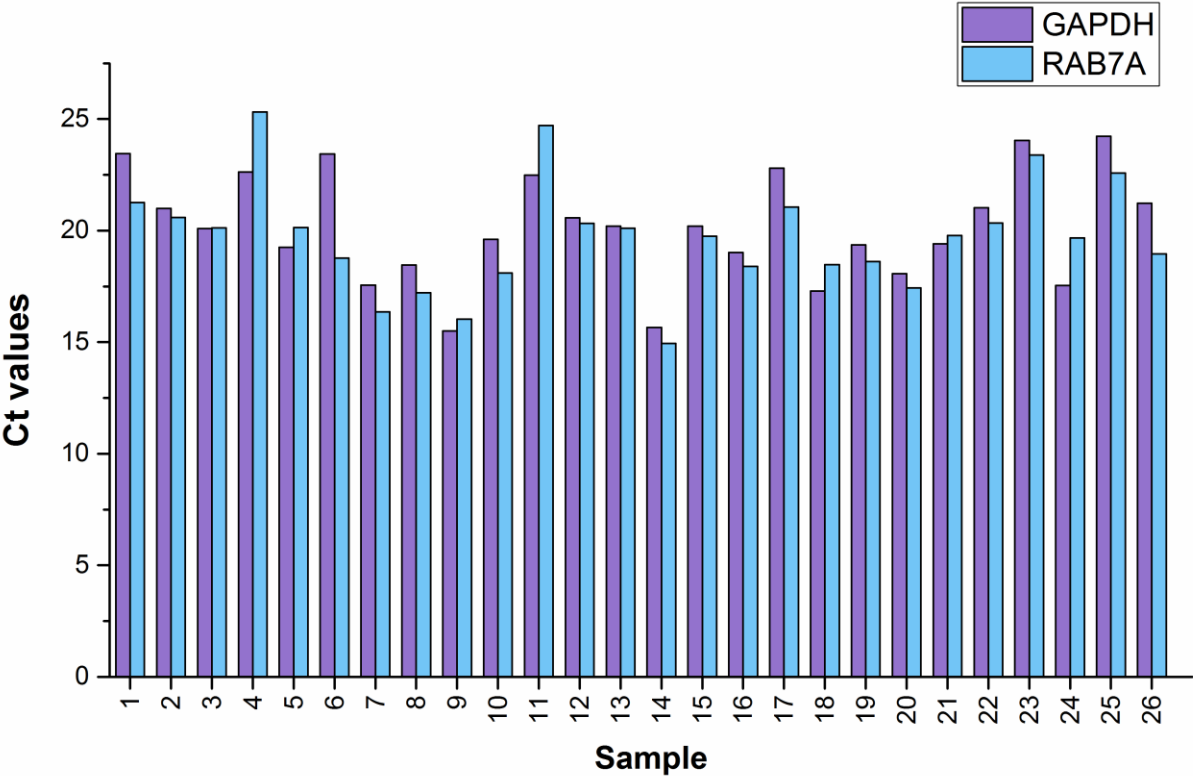


Figure S5. Gene expression $2^{-\Delta Ct}$ of all genes normalized to GAPDH showing expression range among pulmonary vein (PV) and peripheral (Pe) samples.

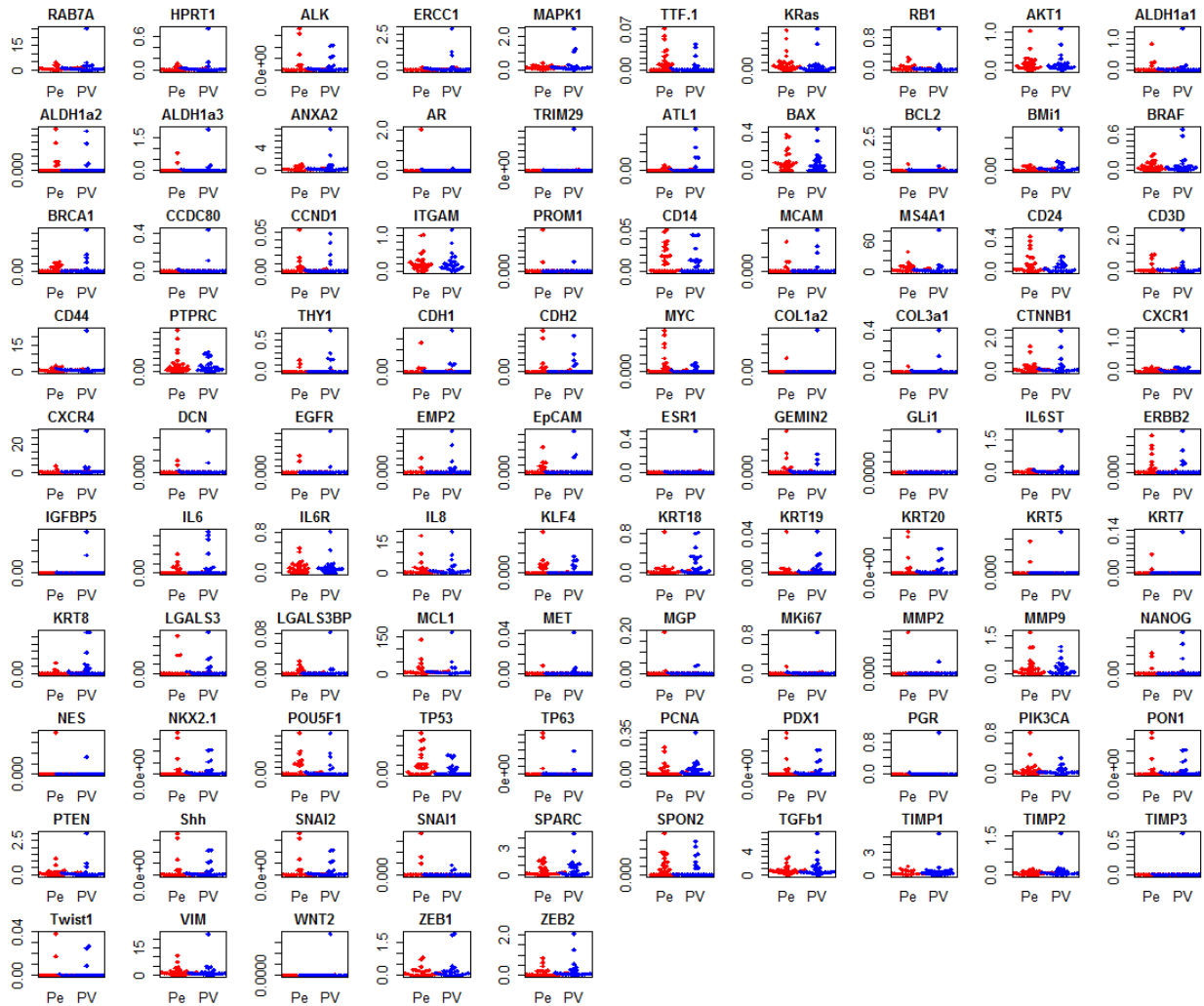


Figure S6. \log_2FC plot showing differentially expressed genes ($p < 0.05$) among 51 PV and Pe samples.

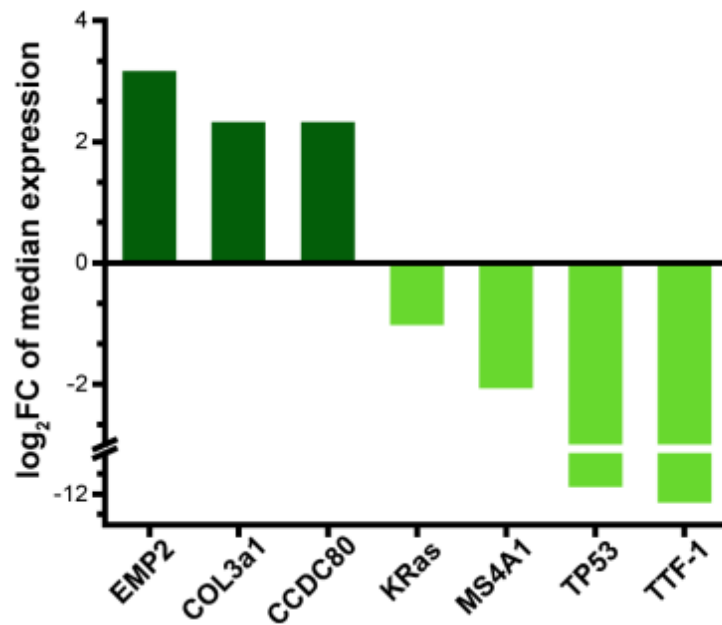


Table S2. Top pathways enriched in gene expression comparison of PV versus Pe, with differentially expressed genes having \log_2FC cut-off of 2 and p-value cut-off of 0.05.

Pathway Name	p-value
Thyroid hormone synthesis	0.000359
p53 signaling pathway	0.001171
Longevity regulating pathway	0.001398
Cell cycle	0.001607
Glioma	0.002309
Chronic myeloid leukemia	0.002309
Epstein-Barr virus infection	0.074775
Apoptosis	0.080062

Table S3. Top GO terms enriched in gene expression comparison of PV versus Pe, with differentially expressed genes having log₂FC cut-off of 2 and p-value cut-off of 0.05.

GO term	P-value
positive regulation of cell-substrate adhesion	0.0098
cell-substrate adhesion	0.0104
integrin-mediated signaling pathway	0.019
regulation of cell-substrate adhesion	0.0308
negative regulation of DNA metabolic process	0.0308
Ras protein signal transduction	0.0448
cell-matrix adhesion	0.0609
small GTPase mediated signal transduction	0.0609
DNA strand renaturation	0.0632
positive regulation of cell-matrix adhesion	0.0632

Figure S7. Immunohistochemistry of primary tumors. (A). Representative H&E staining images of adenocarcinoma and squamous cell carcinoma of lung cancer patients in the study, (B). p53 negative, p53 low and p53 high primary tumors (by IHC) and their corresponding CTC RNA status (orange font), (C). Ki67 negative, Ki67 low and Ki67 high primary tumors (by IHC) and their corresponding CTC RNA status (orange font).

FIGURE 7

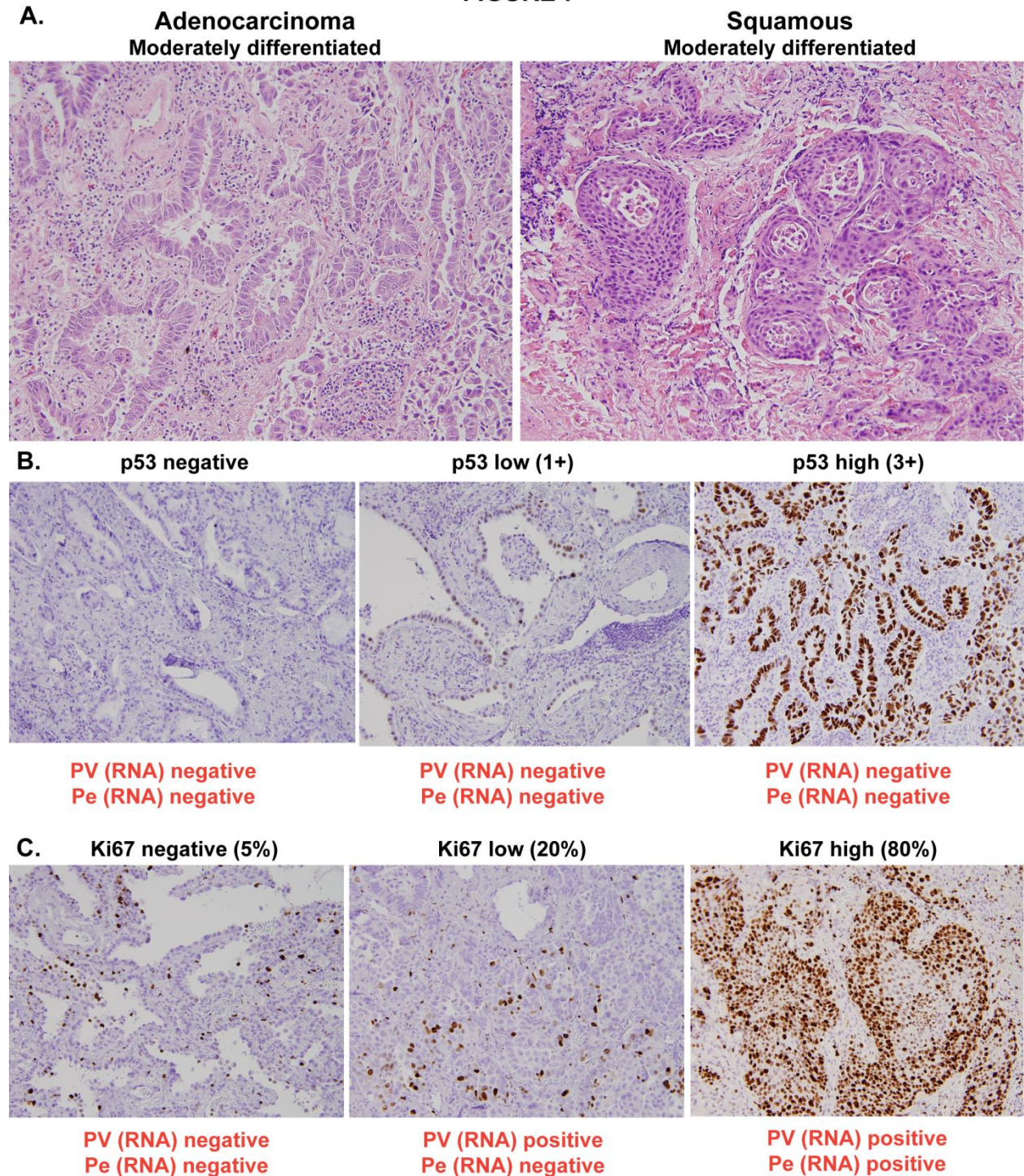


Figure S8. CD44 immunostaining of CTCs showing percentage of CD44+ CTCs in patient samples. Panel below shows immunofluorescence images of a CD44- and a CD44+ CTC cluster.

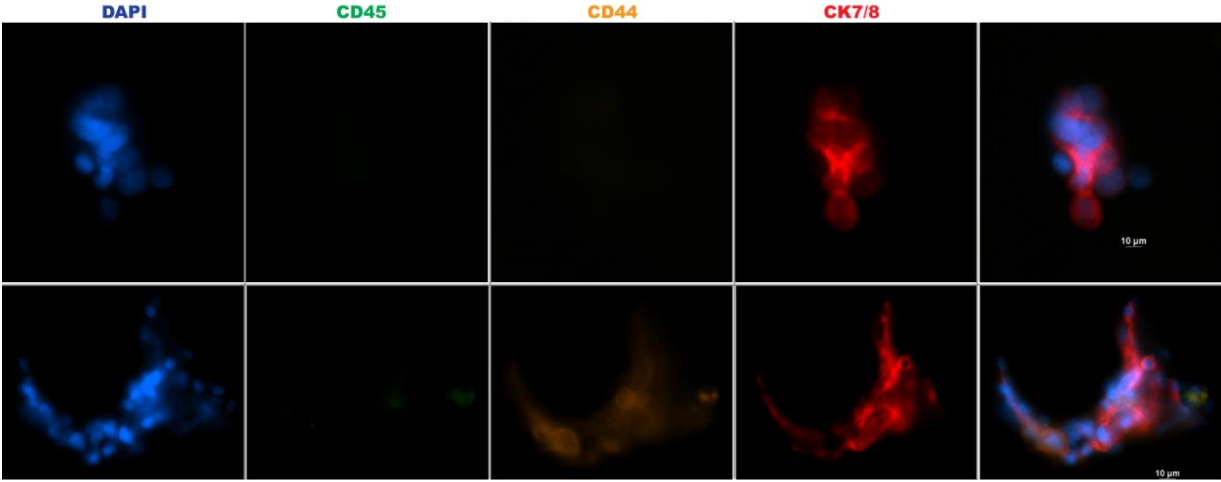
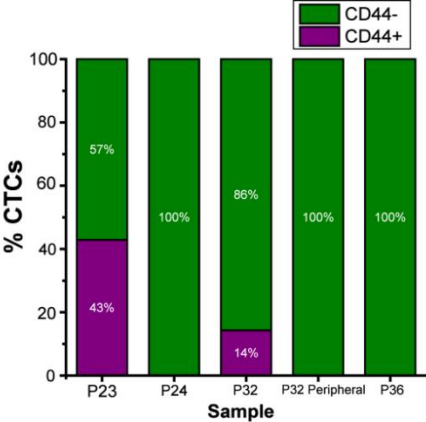


Figure S9. Volcano plot showing criteria for differential gene expression of CTC clusters and single CTCs for pathway analysis (\log_2FC of 0.3, p-value of 0.05).

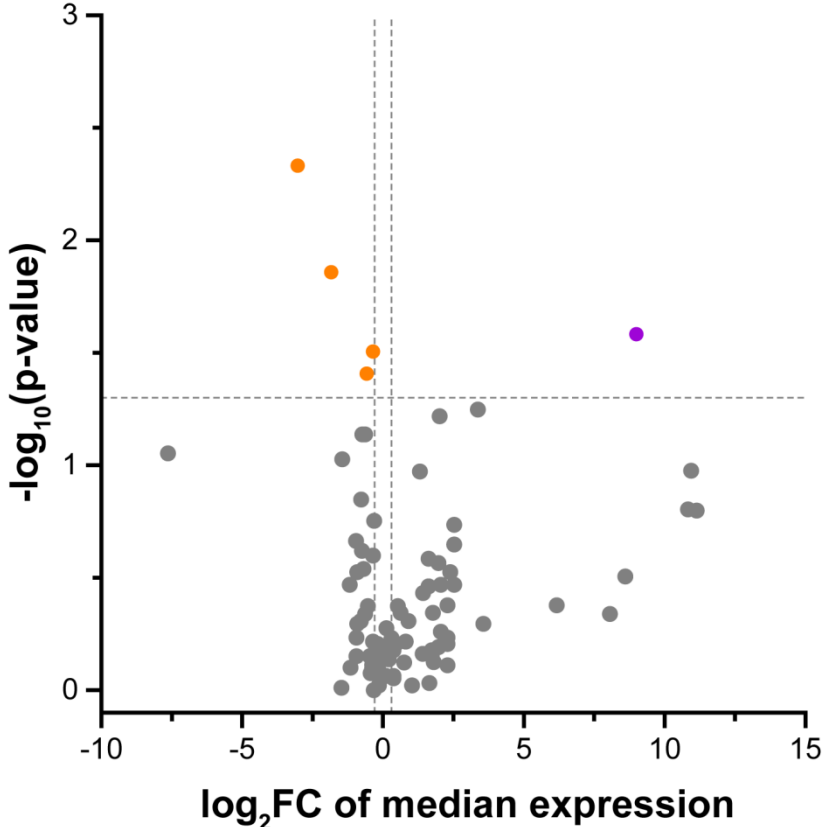


Figure S10. Gene expression of Ki67 normalized to GAPDH among PV and Pe samples.

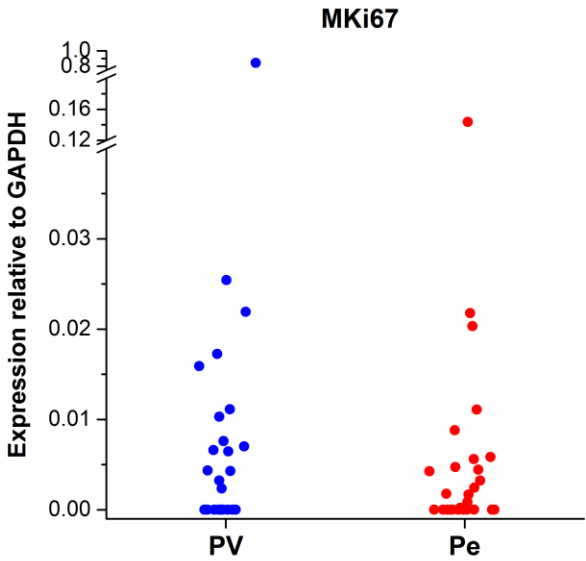


Figure S11. Gene expression of Ki67 (normalized to GAPDH) among single and clustered CTCs in PV samples.

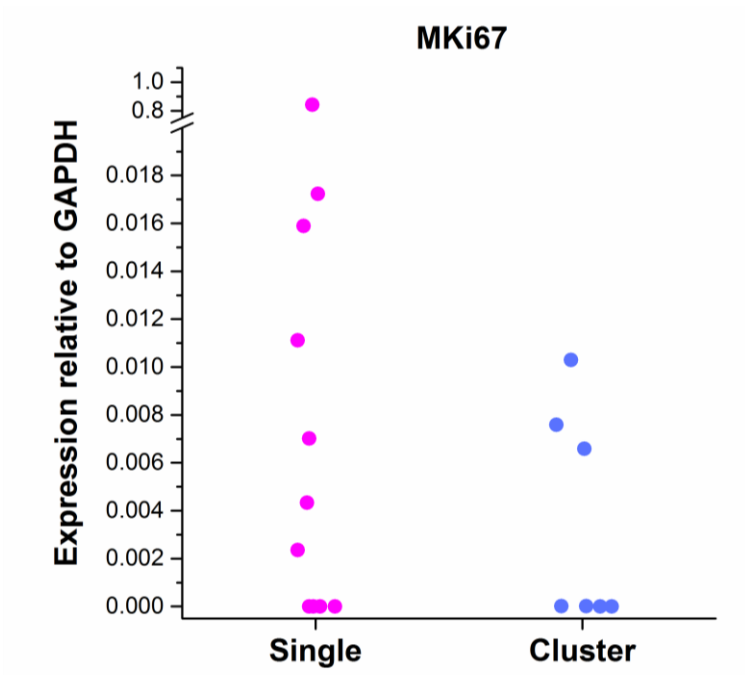


Table S4. List of mutations detected in patient samples using the qBiomarker Somatic Mutation PCR Array: Human Lung Cancer (Qiagen).

			Genotype Call					
			Samples					
Symbol	nt change	AA change	M1 Peripheral	M2 Peripheral	M2 Pulmonary	M3 Peripheral	M3 Pulmonary	M4 Pulmonary
AKT1	c.49G>A	p.E17K	-	-	-	-	-	-
BRAF	c.1397G>T	p.G466V	-	-	-	-	-	-
BRAF	c.1406G>C	p.G469A	-	-	-	-	-	-
BRAF	c.1789C>G	p.L597V	-	-	-	-	-	-
BRAF	c.1799T>A	p.V600E	-	-	-	-	-	-
CTNNB1	c.110C>G	p.S37C	-	-	-	-	+	-
CTNNB1	c.110C>T	p.S37F	-	-	-	-	-	-
EGFR	c.2125G>A	p.E709K	-	-	-	-	-	-
EGFR	c.2126A>C	p.E709A	-	-	-	-	-	-
EGFR	c.2155G>A	p.G719S	-	-	-	-	-	-
EGFR	c.2155G>T	p.G719C	-	-	-	-	-	-
EGFR	c.2156G>C	p.G719A	-	-	-	-	-	-
EGFR	c.2235_2249del15	p.E746_A750del	-	-	-	-	-	-
EGFR	c.2236_2250del15	p.E746_A750del	-	-	-	-	-	-
EGFR	c.2237_2251del15	p.E746_T751>A	-	-	-	-	-	-
EGFR	c.2237_2255>T	p.E746_S752>	-	-	-	-	-	-

		V						
EGFR	c.2239_2247del9	p.L747_E749del	-	-	-	-	-	-
EGFR	c.2239_2248TTAAGAGAAG>C	p.L747_A750>P	-	-	-	-	-	-
EGFR	c.2239_2251>C	p.L747_T751>P	-	-	-	-	-	-
EGFR	c.2239_2253del15	p.L747_T751del	-	-	-	-	-	-
EGFR	c.2239_2256del18	p.L747_S752del	-	-	-	-	-	-
EGFR	c.2240_2254del15	p.L747_T751del	-	-	-	-	-	-
EGFR	c.2240_2257del18	p.L747_P753>S	-	-	-	-	-	-
EGFR	c.2303G>T	p.S768I	-	-	+	-	-	-
EGFR	c.2307_2308insGCCAGCGTG	p.V769_D770insASV	-	-	-	-	-	-
EGFR	c.2369C>T	p.T790M	-	-	-	-	-	-
EGFR	c.2497T>G	p.L833V	-	-	-	-	-	-
EGFR	c.2573T>G	p.L858R	-	-	-	-	-	-
EGFR	c.2582T>A	p.L861Q	-	-	-	-	-	-
ERBB2	c.2322_2323insGCATACGTGATG	p.M774_A775insAYVM	-	-	-	-	-	-
ERBB2	c.2325_2326insTACGTGATGGCT	p.A775_G776insYVMA	-	-	-	-	-	-
HRAS	c.182A>T	p.Q61L	-	-	-	-	-	-
KRAS	c.182A>G	p.Q61R	-	-	-	-	-	-
KRAS	c.182A>T	p.Q61L	-	-	-	-	-	-

KRAS	c.183A>C	p.Q61H	-	-	-	-	-	-
KRAS	c.183A>T	p.Q61H	-	-	+	-	+	-
KRAS	c.34_35GG>TT	p.G12F	-	-	-	-	-	-
KRAS	c.34G>A	p.G12S	-	-	-	-	-	-
KRAS	c.34G>C	p.G12R	-	-	-	-	-	-
KRAS	c.34G>T	p.G12C	-	-	-	-	-	-
KRAS	c.35G>A	p.G12D	-	-	-	-	-	-
KRAS	c.35G>C	p.G12A	-	-	-	-	-	-
KRAS	c.35G>T	p.G12V	-	-	-	-	+	-
KRAS	c.37G>T	p.G13C	-	+	-	-	-	-
KRAS	c.38G>A	p.G13D	-	-	-	-	-	-
NRAS	c.181C>A	p.Q61K	-	-	-	-	-	-
PIK3CA	c.1633G>A	p.E545K	-	-	+	+	+	-
PIK3CA	c.3140A>G	p.H1047R	-	-	-	-	-	-
STK11	c.1062C>G	p.F354L	-	-	-	-	-	-
STK11	c.109C>T	p.Q37*	-	-	-	-	-	-
TP53	c.461G>T	p.G154V	-	-	-	-	-	-
TP53	c.469G>T	p.V157F	-	-	-	-	-	-
TP53	c.473G>T	p.R158L	-	+	-	-	-	-
TP53	c.488A>G	p.Y163C	-	+	+	-	-	-
TP53	c.517G>T	p.V173L	-	-	-	-	-	-
TP53	c.524G>A	p.R175H	-	-	-	-	-	-

TP53	c.527G>A	p.C176Y	-	-	+/-	+	-	-
TP53	c.527G>T	p.C176F	-	-	-	-	-	-
TP53	c.536A>G	p.H179R	-	+	-	-	-	-
TP53	c.574C>T	p.Q192*	-	-	-	+	-	-
TP53	c.578A>G	p.H193R	-	-	-	-	-	-
TP53	c.614A>G	p.Y205C	-	+	-	-	-	-
TP53	c.637C>T	p.R213*	-	-	-	-	-	-
TP53	c.641A>G	p.H214R	-	-	-	-	-	-
TP53	c.659A>G	p.Y220C	-	-	-	-	-	-
TP53	c.701A>G	p.Y234C	-	-	-	-	-	-
TP53	c.711G>T	p.M237I	-	-	-	-	-	-
TP53	c.713G>T	p.C238F	-	-	-	-	-	-
TP53	c.725G>T	p.C242F	-	-	-	-	-	-
TP53	c.733G>T	p.G245C	-	-	-	-	-	+
TP53	c.734G>T	p.G245V	-	-	-	-	-	-
TP53	c.742C>T	p.R248W	-	-	-	+	+	-
TP53	c.743G>A	p.R248Q	-	-	-	-	-	-
TP53	c.743G>T	p.R248L	-	-	-	-	-	-
TP53	c.745A>T	p.R249W	-	-	-	-	-	-
TP53	c.746G>T	p.R249M	-	-	-	-	-	-
TP53	c.747G>T	p.R249S	-	-	-	-	-	-
TP53	c.817C>T	p.R273C	-	-	-	-	-	-

TP53	c.818G>A	p.R273H	-	-	-	-	-	-
TP53	c.818G>T	p.R273L	-	-	-	-	-	-
TP53	c.844C>T	p.R282W	-	-	-	-	-	-
TP53	c.856G>A	p.E286K	-	-	-	-	-	-
TP53	c.892G>T	p.E298*	-	+	-	+	+	+