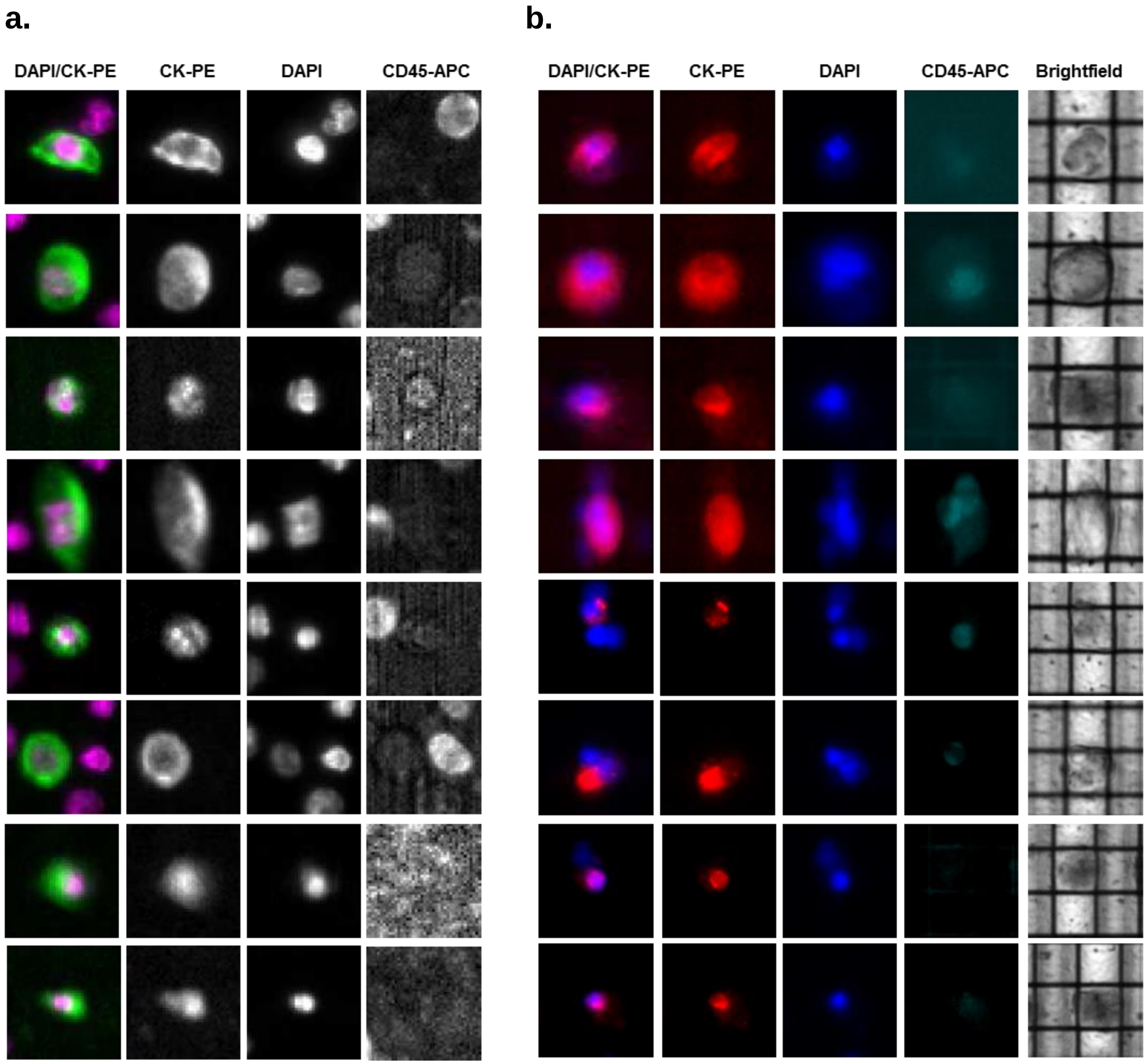
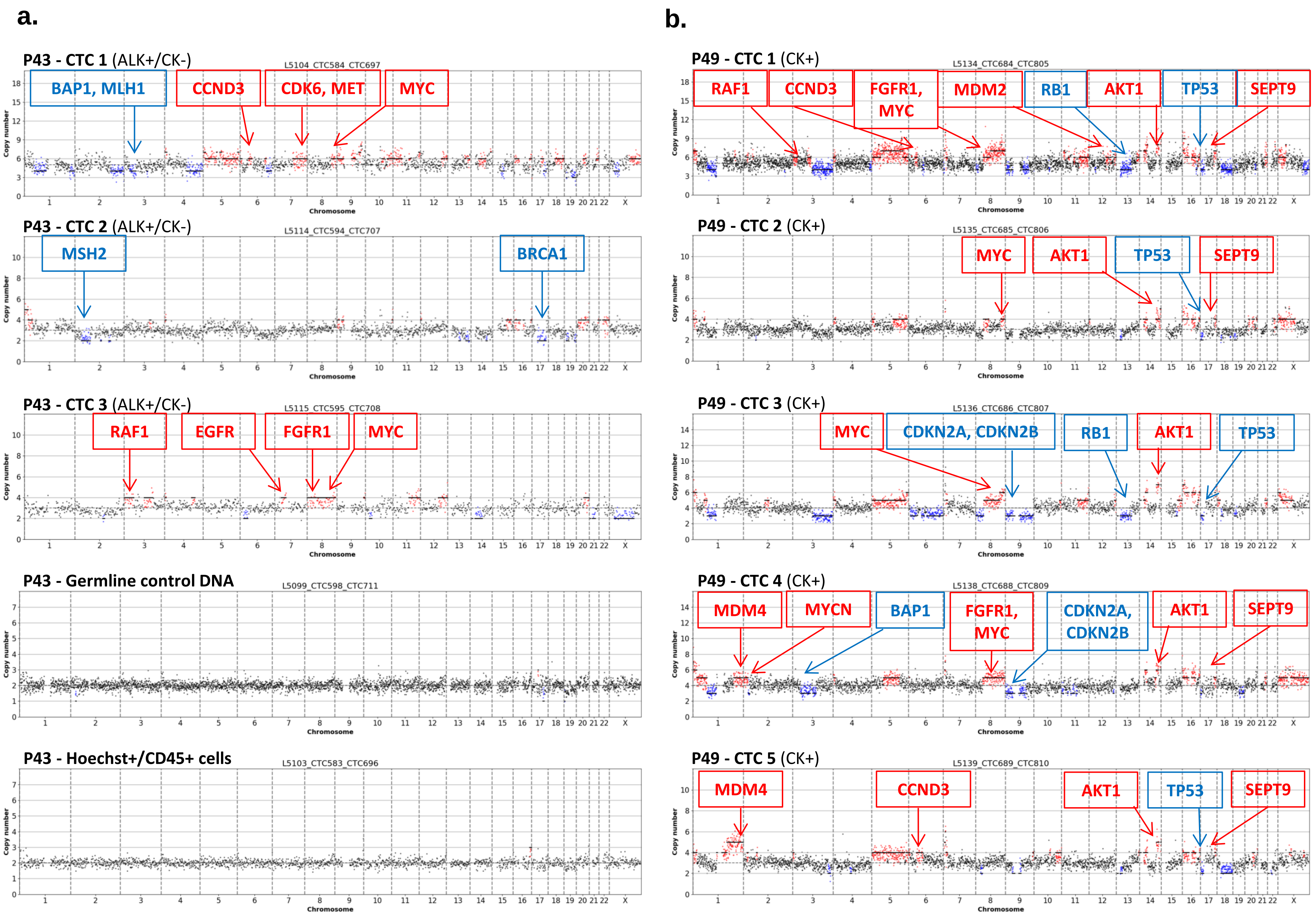
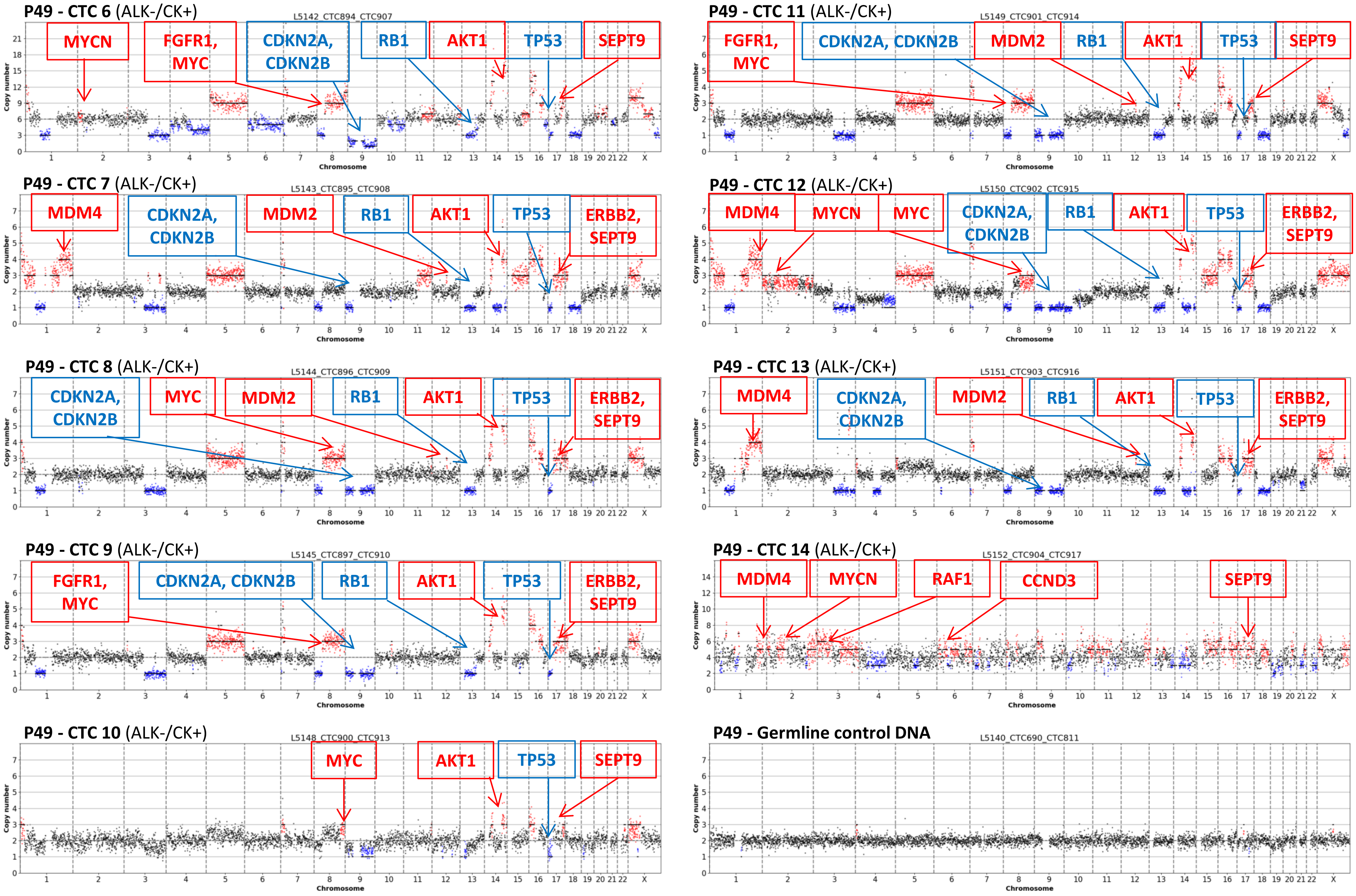


Supplementary Figure 1. Isolated CTCs by DEPArray. (**a**) Enrichment and detection of CTCs by the CellSearch. (**b**) Isolation of single CTCs using the DEPArray. The *VHL*^{R161} mutation was previously identified in one P49 CK^{+} CTC (27).


a.
P43 - CTC 1 (ALK+/CK-)
L5104_CTC584_CTC697
BAP1, MLH1
CCND3
CDK6, MET
MYC
P43 - CTC 2 (ALK+/CK-)
L5114_CTC594_CTC707
MSH2
BRCA1
P43 - CTC 3 (ALK+/CK-)
L5115_CTC595_CTC708
RAF1
EGFR
FGFR1
MYC
P43 - Germline control DNA
L5099_CTC598_CTC711
P43 - Hoechst+/CD45+ cells
L5103_CTC583_CTC696
b.
P49 - CTC 1 (CK+)
L5134_CTC684_CTC805
RAF1
CCND3
FGFR1, MYC
MDM2
RB1
AKT1
TP53
SEPT9
P49 - CTC 2 (CK+)
L5135_CTC685_CTC806
MYC
AKT1
TP53
SEPT9
P49 - CTC 3 (CK+)
L5136_CTC686_CTC807
MYC
CDKN2A, CDKN2B
RB1
AKT1
TP53
P49 - CTC 4 (CK+)
L5138_CTC688_CTC809
MDM4
MYCN
BAP1
FGFR1, MYC
CDKN2A, CDKN2B
AKT1
SEPT9
P49 - CTC 5 (CK+)
L5139_CTC689_CTC810
MDM4
CCND3
AKT1
TP53
SEPT9
Copy number
Chromosome
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 X

b.



P49 - CTC 6 (ALK-/CK+)

P49 - CTC 11 (ALK-/CK+)

P49 - CTC 7 (ALK-/CK+)

P49 - CTC 12 (ALK-/CK+)

P49 - CTC 8 (ALK-/CK+)

P49 - CTC 13 (ALK-/CK+)

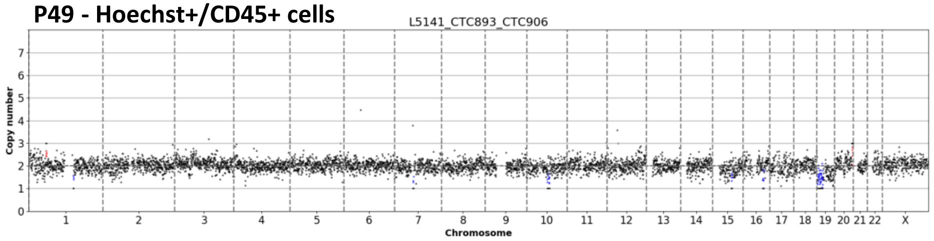
P49 - CTC 9 (ALK-/CK+)

P49 - CTC 14 (ALK-/CK+)

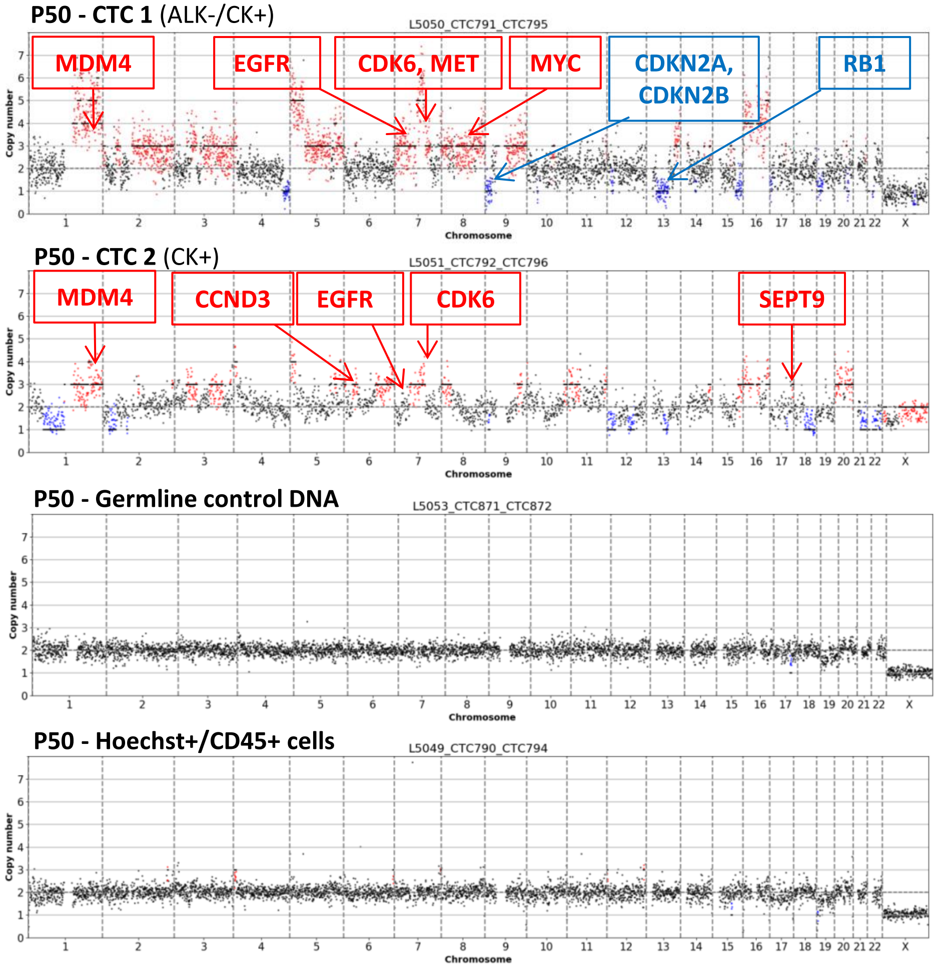
P49 - CTC 10 (ALK-/CK+)

P49 - Germline control DNA

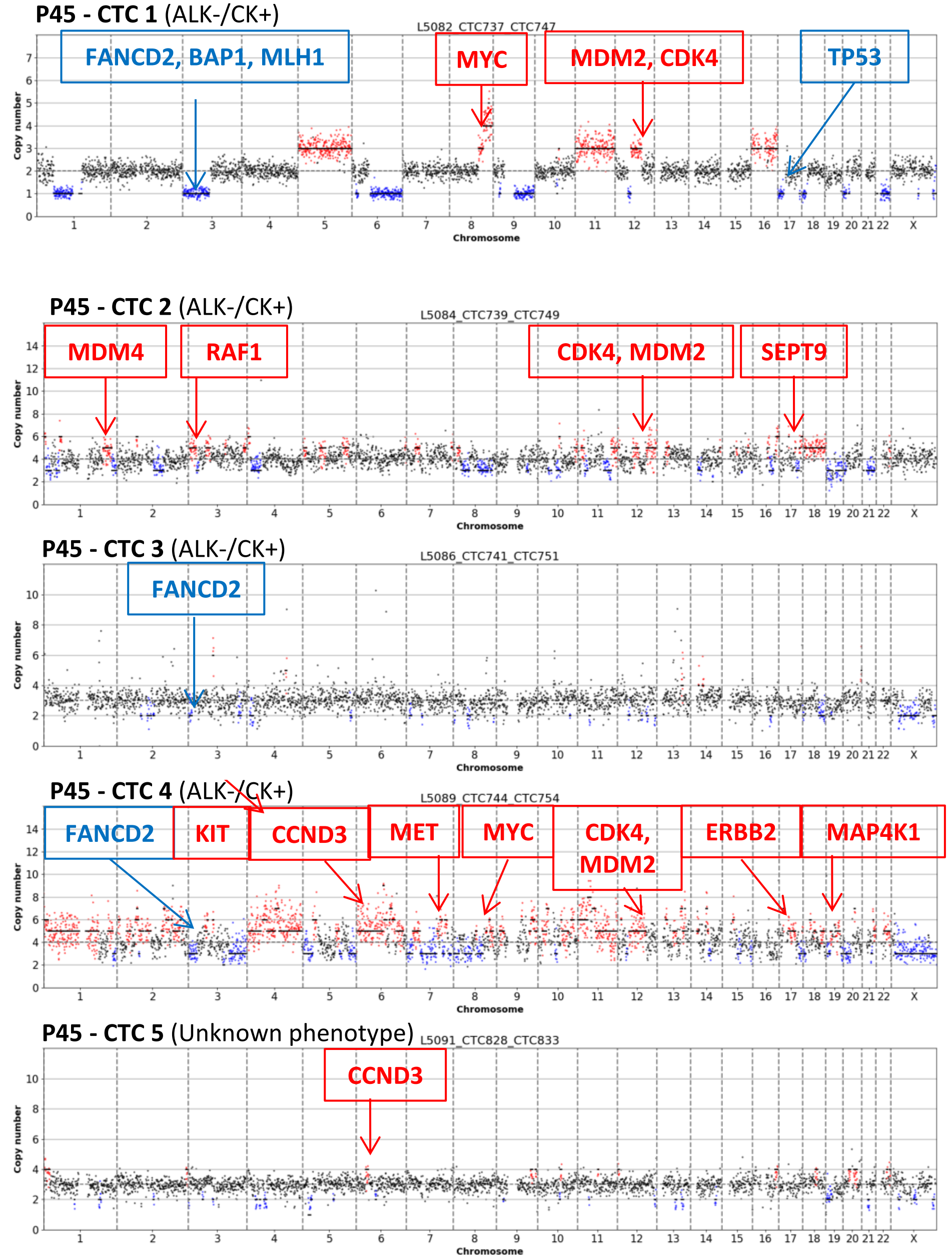
b.

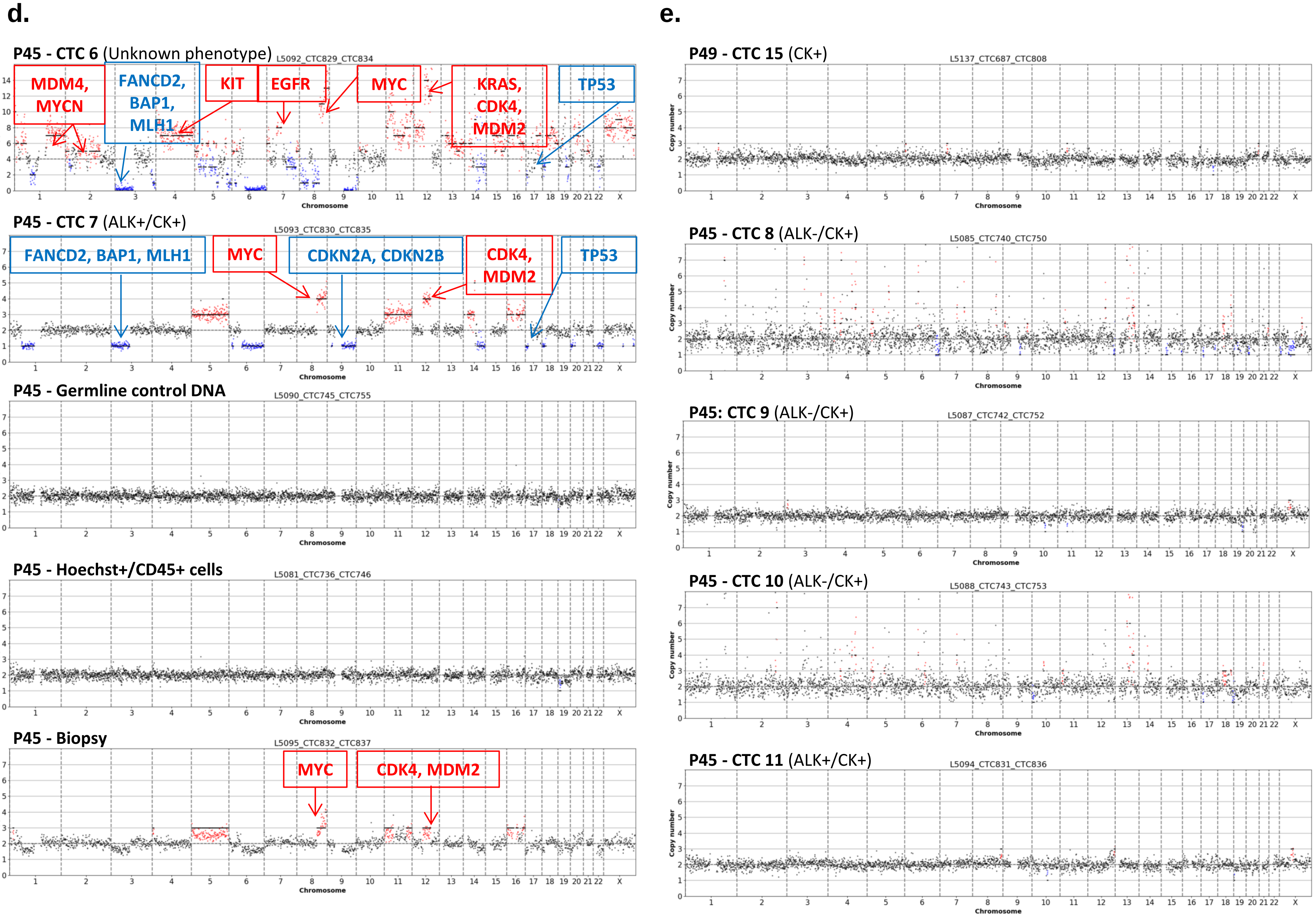

P49 - Hoechst+/CD45+ cells
L5141_CTC893_CTC906
Copy number
Chromosome
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 X

c.


P50 - CTC 1 (ALK-/CK+)
L5050_CTC791_CTC795
MDM4
EGFR
CDK6, MET
MYC
CDKN2A, CDKN2B
RB1
P50 - CTC 2 (CK+)
L5051_CTC792_CTC796
MDM4
CCND3
EGFR
CDK6
SEPT9
P50 - Germline control DNA
L5053_CTC871_CTC872
P50 - Hoechst+/CD45+ cells
L5049_CTC790_CTC794
Copy number
Chromosome
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 X

d.


P45 - CTC 1 (ALK-/CK+)
L5082_CTC737_CTC747
FANCD2, BAP1, MLH1
MYC
MDM2, CDK4
TP53
P45 - CTC 2 (ALK-/CK+)
L5084_CTC739_CTC749
MDM4
RAF1
CDK4, MDM2
SEPT9
P45 - CTC 3 (ALK-/CK+)
L5086_CTC741_CTC751
FANCD2
P45 - CTC 4 (ALK-/CK+)
L5089_CTC744_CTC754
FANCD2
KIT
CCND3
MET
MYC
CDK4, MDM2
ERBB2
MAP4K1
P45 - CTC 5 (Unknown phenotype)
L5091_CTC828_CTC833
CCND3
Copy number
Chromosome
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 X



P45 - CTC 6 (Unknown phenotype)

P45 - CTC 7 (ALK+/CK+)

P45 - Germline control DNA

P45 - Hoechst+/CD45+ cells

P45 - Biopsy

P49 - CTC 15 (CK+)

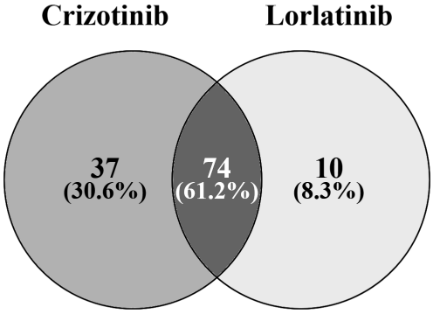
P45 - CTC 8 (ALK-/CK+)

P45: CTC 9 (ALK-/CK+)

P45 - CTC 10 (ALK-/CK+)

P45 - CTC 11 (ALK+/CK+)

Supplementary Figure 2. Low-pass whole-genome CNA profiles of CTC samples from *ALK*-positive patients at resistance to ALK-TKIs. CNAs in predominant pathways are annotated (gain in red, loss in blue). (**a**) CNA profiles of CTCs, corresponding leucocytes and germline DNA from patient P43 resistant to crizotinib. (**b**) CNA profiles of CTCs, corresponding leucocytes and germline DNA from patient P49 resistant to crizotinib. (**c**) CNA profiles of CTCs, corresponding leucocytes and germline DNA from patient P50 resistant to crizotinib. (**d**) CNA profiles of CTCs, corresponding tumor biopsy, leucocytes and germline DNA from patient P45 resistant to lorlatinib. (**e**) Flat CNA profiles of CK^+ cells from P49 and P45.



Supplementary Figure 3. Numbers and percentages of CNA drivers identified in crizotinib-resistant patients only, in lorlatinib-resistant patients only and in both.

Supplementary Table 1. Detailed list of CNAs identified in tumor samples (38 CTC samples and one tumor biopsy) of *ALK*-positive patients at resistance to ALK-TKIs.

ALK TKI	Patient	Sample ID	WGA QC GII (/4)	LowPass Genome QC			
				Read counts	DLRS	LMAD	R50
Crizotinib	P43	CTC 1	3	718,457	0,25	0,23	45
Crizotinib	P43	CTC 2	3	638,892	0,24	0,23	45
Crizotinib	P43	CTC 3	3	399,283	0,24	0,23	45
Crizotinib	P46	CTC 1	4	771,976	0,25	0,25	46
Crizotinib	P46	CTC 2	4	851,956	0,28	0,28	46
Crizotinib	P46	CTC 3	4	855,924	0,24	0,24	46
Crizotinib	P46	CTC 4	4	876,131	0,22	0,21	46
Crizotinib	P46	CTC 5	4	838,436	0,25	0,24	46
Crizotinib	P46	CTC 6	3	1,030,049	0,28	0,30	46
Crizotinib	P46	CTC 7	3	1,122,952	0,31	0,32	46
Crizotinib	P46	CTC 8	4	737,347	0,24	0,26	46
Crizotinib	P46	CTC 9	4	835,369	0,26	0,26	45
Crizotinib	P46	CTC 10	4	957,580	0,27	0,26	45
Crizotinib	P46	CTC 11	4	1,026,747	0,32	0,33	46
Crizotinib	P49	CTC 1	4	1,696,671	0,23	0,64	46
Crizotinib	P49	CTC 2	4	1,029,086	0,22	0,22	45
Crizotinib	P49	CTC 3	3	868,550	0,23	0,67	45
Crizotinib	P49	CTC 4	4	1,125,220	0,22	0,42	46
Crizotinib	P49	CTC 5	4	1,050,782	0,22	0,22	45
Crizotinib	P49	CTC 6	3	989,475	0,27	0,64	44
Crizotinib	P49	CTC 7	3	1,452,899	0,25	0,22	45
Crizotinib	P49	CTC 8	3	1,445,407	0,23	0,21	44
Crizotinib	P49	CTC 9	4	1,319,753	0,24	0,20	46
Crizotinib	P49	CTC 10	4	1,411,964	0,23	0,59	46
Crizotinib	P49	CTC 11	3	1,579,673	0,24	0,20	44
Crizotinib	P49	CTC 12	4	1,661,213	0,26	0,42	47
Crizotinib	P49	CTC 13	4	1,600,028	0,25	0,41	46
Crizotinib	P49	CTC 14	3	1,390,551	0,29	0,91	46
Crizotinib	P49	CTC 15	3	1,092,214	0,22	0,21	46
Crizotinib	P50	CTC 1	3	1,675,152	0,31	0,32	45
Crizotinib	P50	CTC 2	2*	934,641	0,27	0,55	44
Lorlatinib	P41	CTC 1	1*	1,279,226	0,33	0,38	46
Lorlatinib	P45	CTC 1	4	1,363,737	0,23	0,20	46
Lorlatinib	P45	CTC 2	4	1,207,893	0,24	0,24	46
Lorlatinib	P45	CTC 3	4	1,108,999	0,27	0,25	47
Lorlatinib	P45	CTC 4	4	1,137,825	0,29	0,78	46
Lorlatinib	P45	CTC 5	3	1,097,406	0,23	0,21	47
Lorlatinib	P45	CTC 6	3	805,981	0,51	0,71	45
Lorlatinib	P45	CTC 7	2*	782,591	0,23	0,19	47
Lorlatinib	P45	CTC 8	4	1,239,079	0,31	0,44	48
Lorlatinib	P45	CTC 9	4	1,139,258	0,21	0,19	46
Lorlatinib	P45	CTC 10	4	1,027,209	0,31	0,41	47
Lorlatinib	P45	CTC 11	4	948,745	0,22	0,19	47
Lorlatinib	P45	Biopsy	4	656,066	0,21	0,41	46

Abbreviations: DLRS, derivative log ratio spread is a measure of point-to-point consistency or noisiness in copy number along the genome (expected = 0,2-0,3); LMAD, absolute deviation from local median copy number (expected = 0,2-0,3); R50, percentage of target (total bp of genome covered) covered by 50% of reads. Low values (<20%) indicate low WGA/library complexity.
DLRS and LMAD are calculated with a subsampled number of 200,000 reads.
* The three CTC samples with lower Genome Integrity Index (GII) passed sequencing QC and 43 CTC samples in total were analyzed by the LowPass WGS.

Supplementary Table 2. Whole Genome Amplification and LowPass Genome quality controls