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Supplemental information

Functional diagnostics using fresh uncultured lung tumor cells to guide personalized treatments

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Supplemental Information

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Supplementary Figures

Figure S1

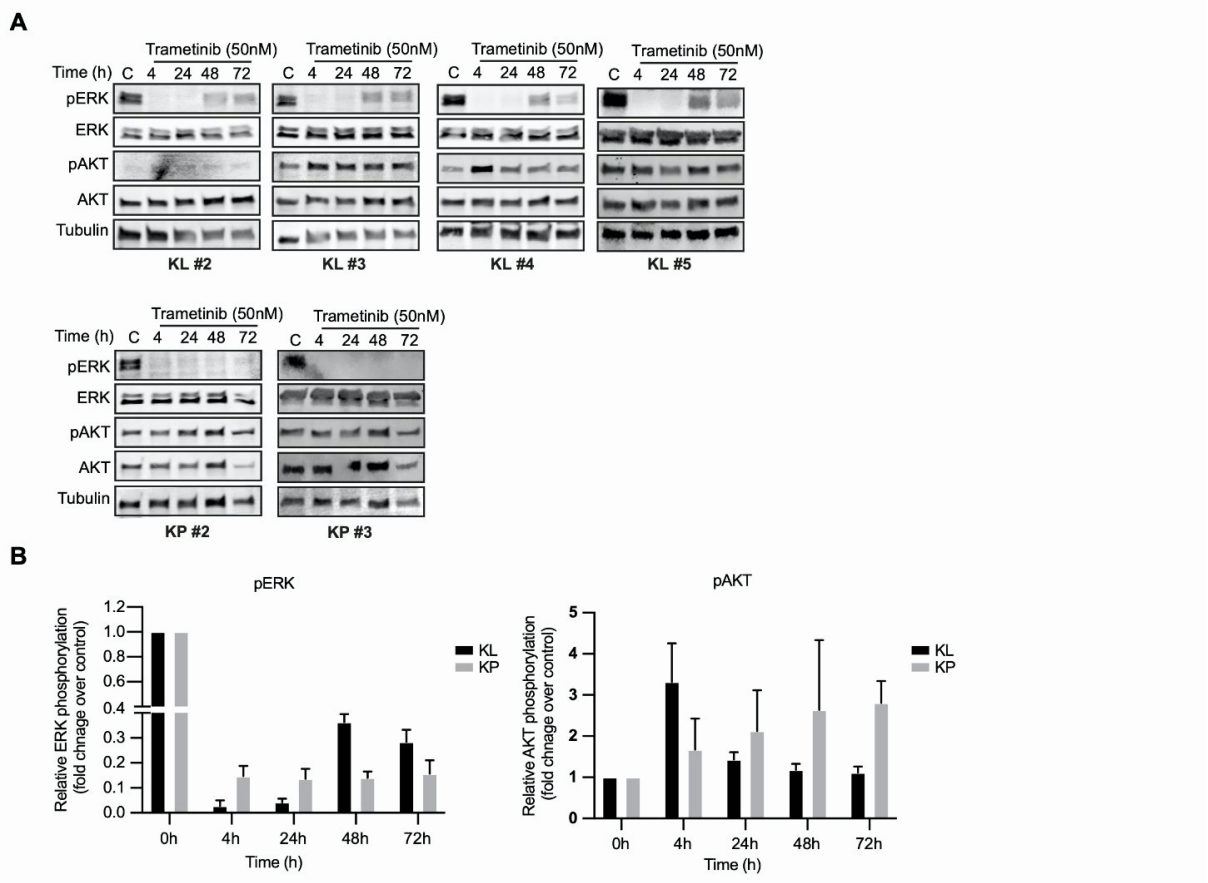

Figure S1. Analysis of trametinib-induced signaling rewiring. Related to Figure 2. (A) Immunoblots of KL and KP FUTCs treated with vehicle (C; DMSO) and or treated with 50 nM TR for various time points (4, 24, 48, and 72 h), and probed with indicated antibodies. (B) Relative quantification of pERK and pAKT signals on immunoblots presented in Figure 2D and S1A. Error bars represent ± SEM.

FIGURE S2

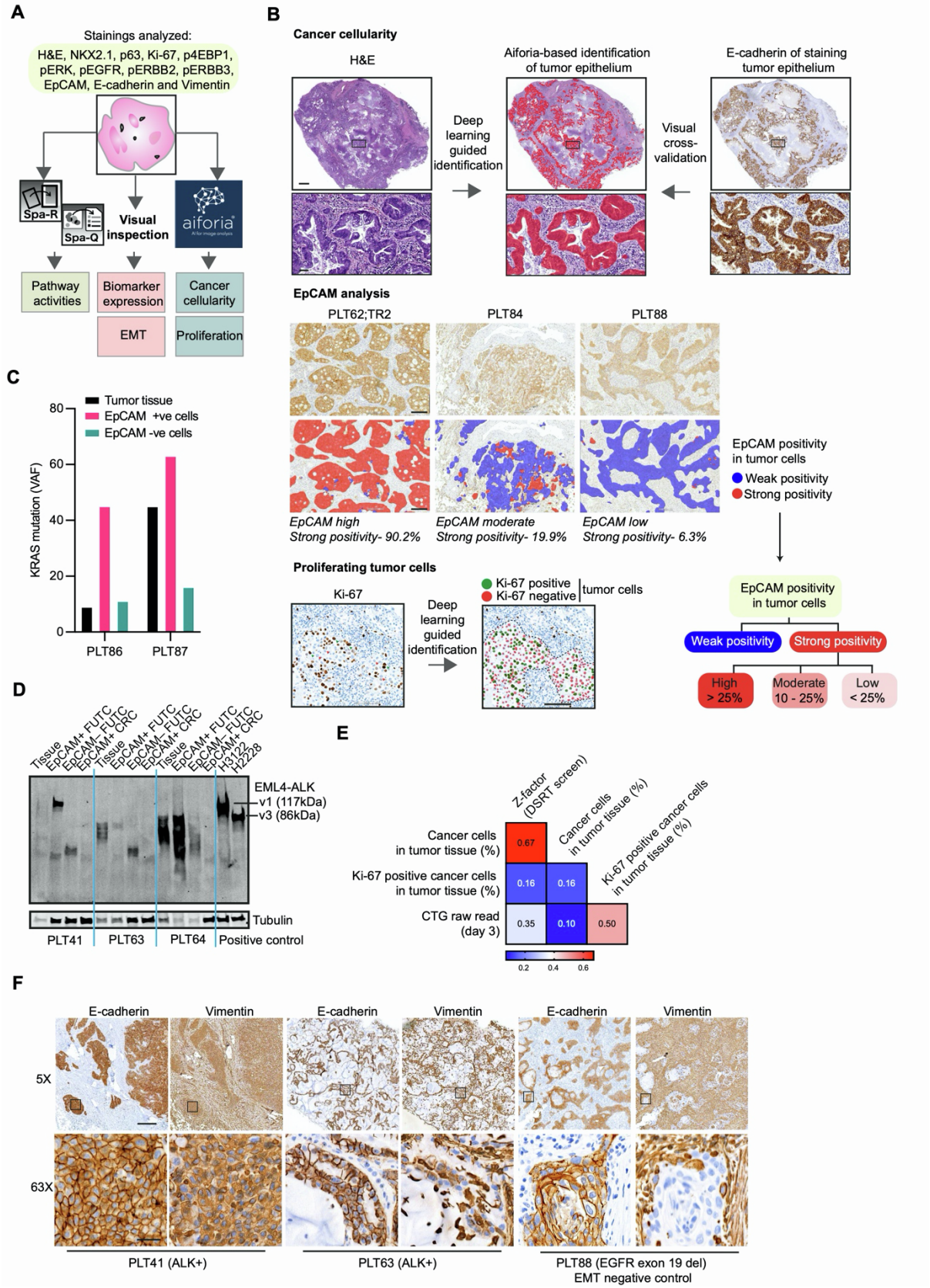

Figure S2. Tumor tissue phenotyping and biochemical analysis of clinical samples. Related to Figure 3 and 4. (A) The image analysis software package Spa-RQ[5] was used for quantification of signaling activities in tumor regions. To identify cancer cellularity, percentage of EpCAM positive cancer cells, and percentages of

Ki-67 positive proliferating cancer cells, deep learning-based Aiforia software was used. Biomarker expression and EMT status was visually assessed for each tumor sample. (B) Top panel: representative images demonstrating H&E staining (left), tumor epithelium identified using Aiforia (middle), and the validation via epithelial staining of an adjacent tissue section stained with E-cadherin (right). Scale bars correspond to 1 mm or 50 μm for low or high magnifications, respectively. Middle panel: representative EpCAM stained images and Aiforia-based detection of tumor cells exhibiting strong (red) and weak (blue) EpCAM positivity. Based on the percentage of cancer cells exhibiting strong EpCAM positivity, samples were further subclassified into high (> 25%), moderate (10 - 25%), or low (< 10%). Scale bars correspond to 200 μm. Bottom panel: representative Ki-67 stained images and Aiforia-based detection of Ki-67 positive (green) and negative (red) tumor cells. The black dotted line indicates the edge of the tumor epithelium region identified by Aiforia in a separate analysis. Scale bars correspond to 100 μm. (C) KRAS variant allele frequencies in tumor tissue and tumor-derived EpCAM+ and EpCAM– cells. (D) Immunoblot of EML4-ALK+ patient samples analyzed to verify the expression of ALK fusion protein in patient-matched tumor tissue, tumor-derived EpCAM+ and EpCAM– cells, or CR cultures (passage #4). Samples from H3122 and H2228 cells (EML4-ALK+ cell lines) were used as positive controls for v1 (117 kDa) and v3 (86 kDa) variants of EML4-ALK fusion proteins. (E) Heatmap displaying Pearson correlation coefficient values between various factors associated with FUTC-based drug testing. (F) Representative IHC images of E-cadherin and vimentin staining in patient-derived EML4-ALK+ lung tumor tissues. Scale bars correspond to 200 μm or 20 μm for low or high magnifications, respectively. Boxes indicate areas depicted at higher magnification in the bottom row.

FIGURE S3

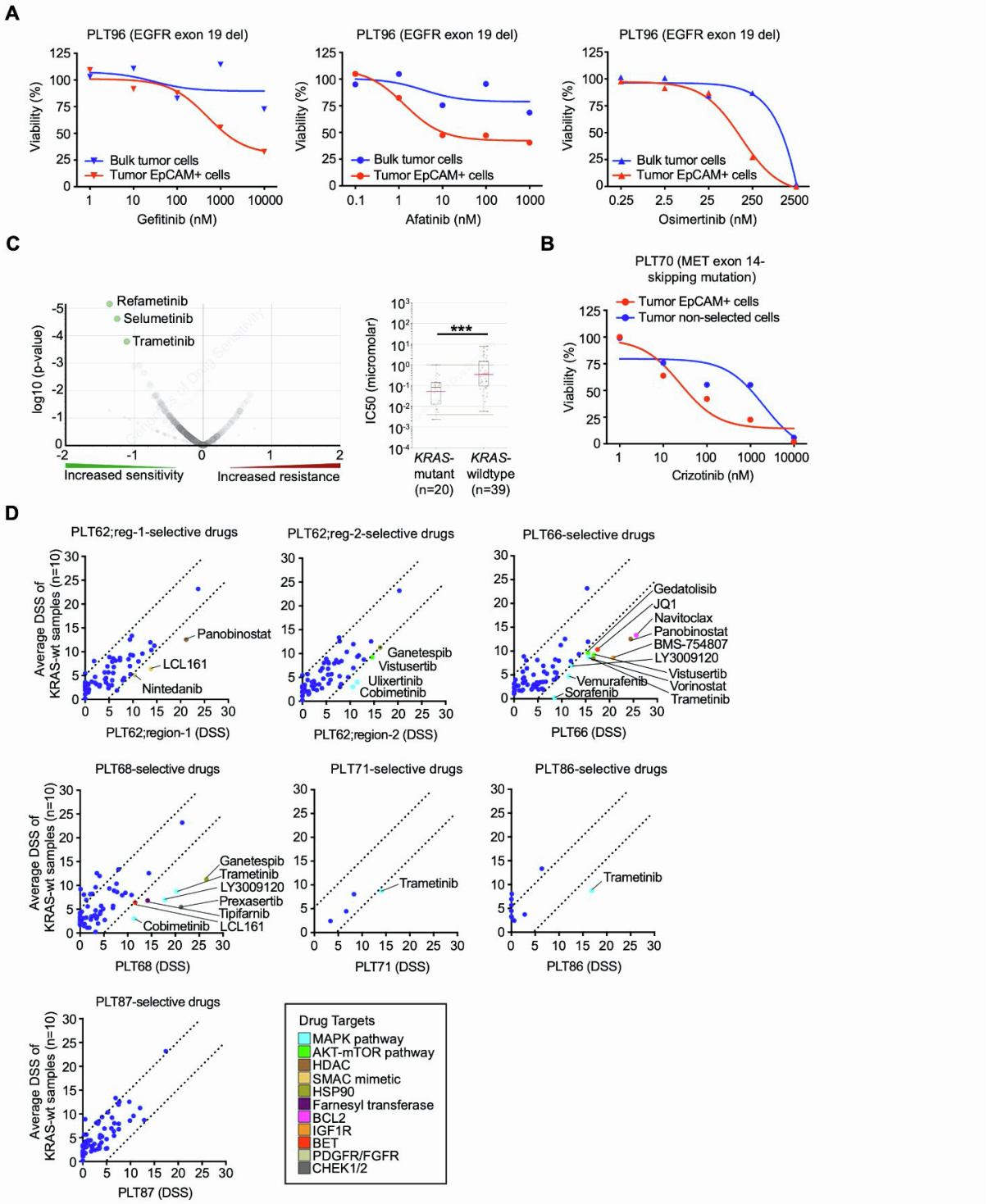

Figure S3. Drug response correlation to identify *KRAS* mutant-selective drug sensitivities. Related to Figure 4 and 5. (A) Dose-response curves of gefitinib, osimertinib, and afatinib in patient-matched tumor-derived bulk cells and EpCAM+ cells. (B) Dose-response curves of crizotinib in patient-matched tumor-derived bulk cells and EpCAM+ cells. (C) A volcano plot (left) and box plot (right) representation adapted from Genomics of Drug Sensitivity in Cancer (GDSC1) database, showing association between MEK inhibitor sensitivity and KRAS mutation in lung adenocarcinoma cells. (D) Identification of patient-selective drug vulnerabilities by comparison of DSSs of individual *KRAS* mutant samples to average DSSs of *KRAS* wildtype samples (n=10). Drugs showing *KRAS* mutant sample-selective drug sensitivities (DSS difference >5) are color coded based on their target.

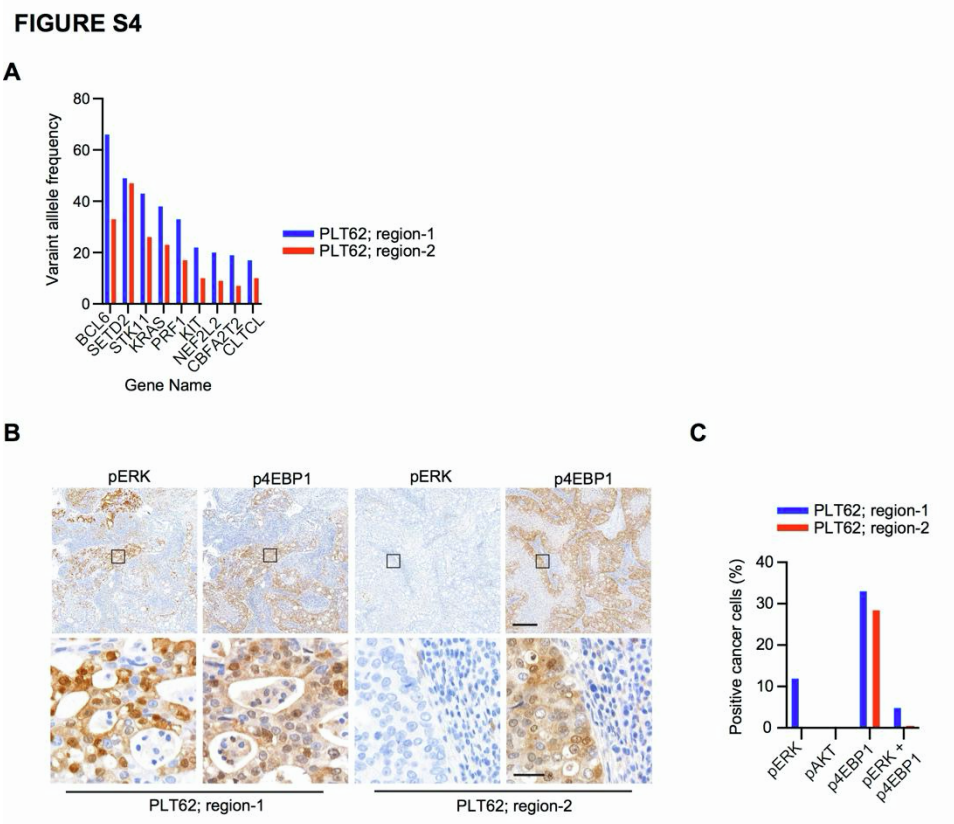

Figure S4. Analysis of intratumoral genomic and phenotypic heterogeneity in a *KRAS* mutant lung cancer specimens. Related to Figure 5. (A) Bar graph displaying the variant allele frequencies of mutations normalized to percentages of cancer cells in their respective tumor region. (B) Representative IHC images of pERK, pAKT, p4EBP1 IHC staining in different regions of the PLT62 tissue sample. Scale bars correspond to 200 μm or 20 μm for low or high magnifications, respectively. (C) Bar graph displaying levels of pERK (Thr202/Tyr204), pAKT (Ser473), p4EBP1 (Thr37/46) and overlapping phosphorylation of ERK and 4EBP1 levels in cancer cells quantified using the IHC image registration and quantification tool, Spa-RQ.

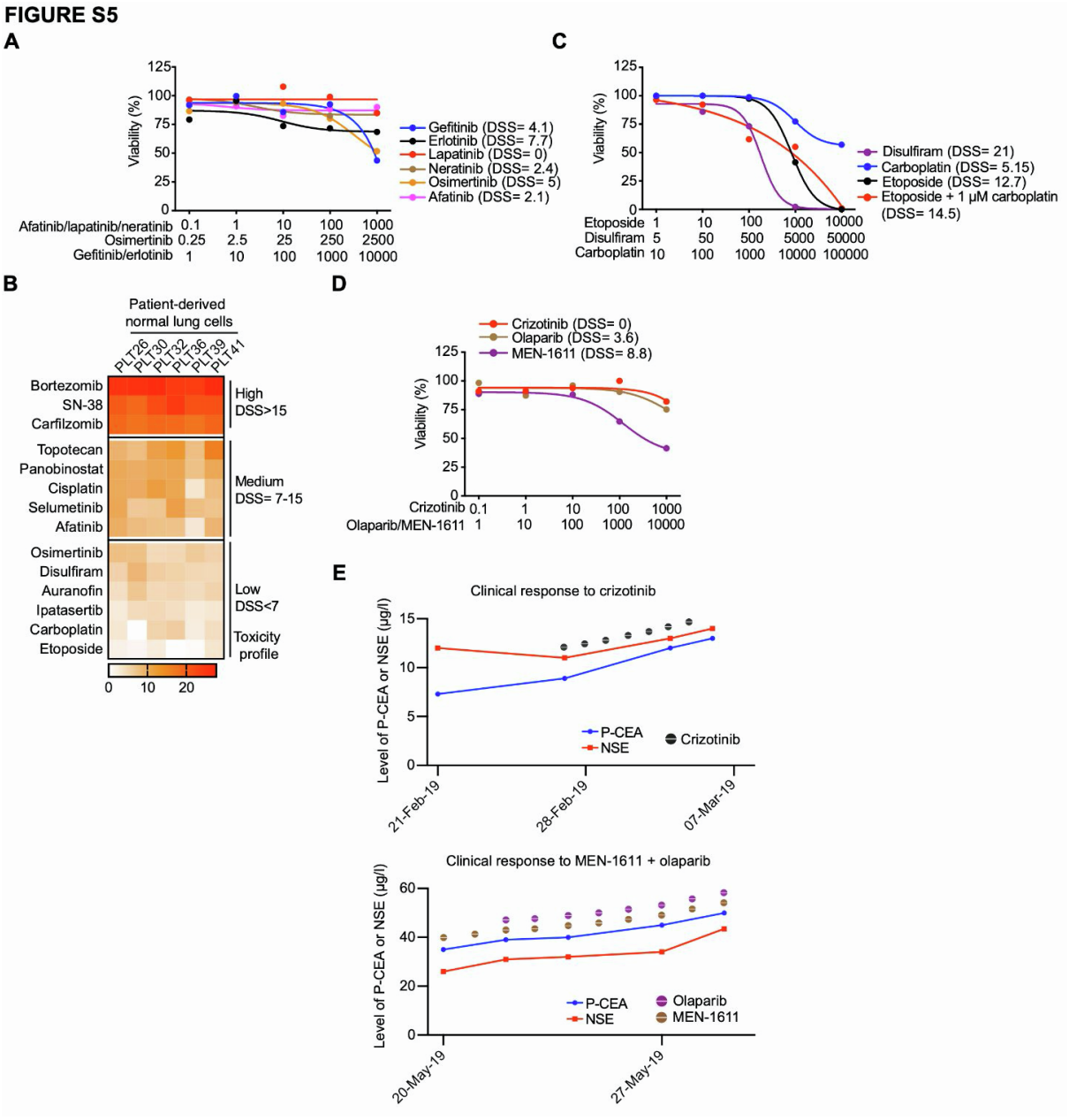

Figure S5. FUTC-based drug profiling identifies patient-selective drug sensitivities. Related to Figure 6. (A) Dose-response curves of different EGFR inhibitors in patient-derived FUTCs. (B) Toxicity profiles of top hits were assessed by testing their sensitivity in CR cultures established from healthy lung tissue of lung cancer patients. Drugs with average DSS >15 or DSS >7 are denoted as highly or mildly toxic, respectively. Drugs with average DSS <7 in the control cells were considered as putative lower toxicity compounds for clinical treatment. (C) Dose-response curves of patient-derived FUTCs treated with five doses of disulfiram, carboplatin, etoposide, and combination treatment. For the combination screen, 1 µM of carboplatin was used together with a dose series of etoposide. (D) Dose-response curves of patient-derived FUTCs treated with five doses of crizotinib, olaparib, and MEN-1611. (E) Changes in the level of CEA and NSE after treatment with crizotinib and MEN-1611 plus olaparib treatments.

FIGURE S6

A

Tumor burden before and after carboplatin plus etoposide treatment			
Body part	**Metastsis ID**	**Before treatment (11 Dec 2018)**	**After treatment (17 Jan 2019)**
Collum	Lymph node met-1	29 x 21 mm	12 x 7 mm
Collum	Lymph node met-2	12 mm	6 mm
Thorax	Lung met-1	5 x 4 mm	3 x 1
Thorax	Lung met-2	6 mm	1 mm
Thorax	Lung met-3	8 x 5 mm	4 x 3 mm
Thorax	Lung met-4	MNA	Decrease
Thorax	Lung met-5	MNA	No change
Thorax	Lung met-6	MNA	No change
Thorax	Lymph node met-3	MNA	Decreased
Thorax	Lymph node met-4	18 x 22 mm	15 x 15 mm
Thorax	Lymph node met-5	10 mm	5 mm
Thorax	Lymph node met-6	9 mm	6 mm
Abdomen/pelvis	Liver met-1	11 x 9 mm	3 x 3 mm
Abdomen/pelvis	Liver met-2	14 x 14 mm	7 x 6 mm
Abdomen/pelvis	Liver met-3	6 x 4 mm	Not found
Abdomen/pelvis	Liver met-4	10 x 7 mm	Not found
MNA: measurements not avaialable			

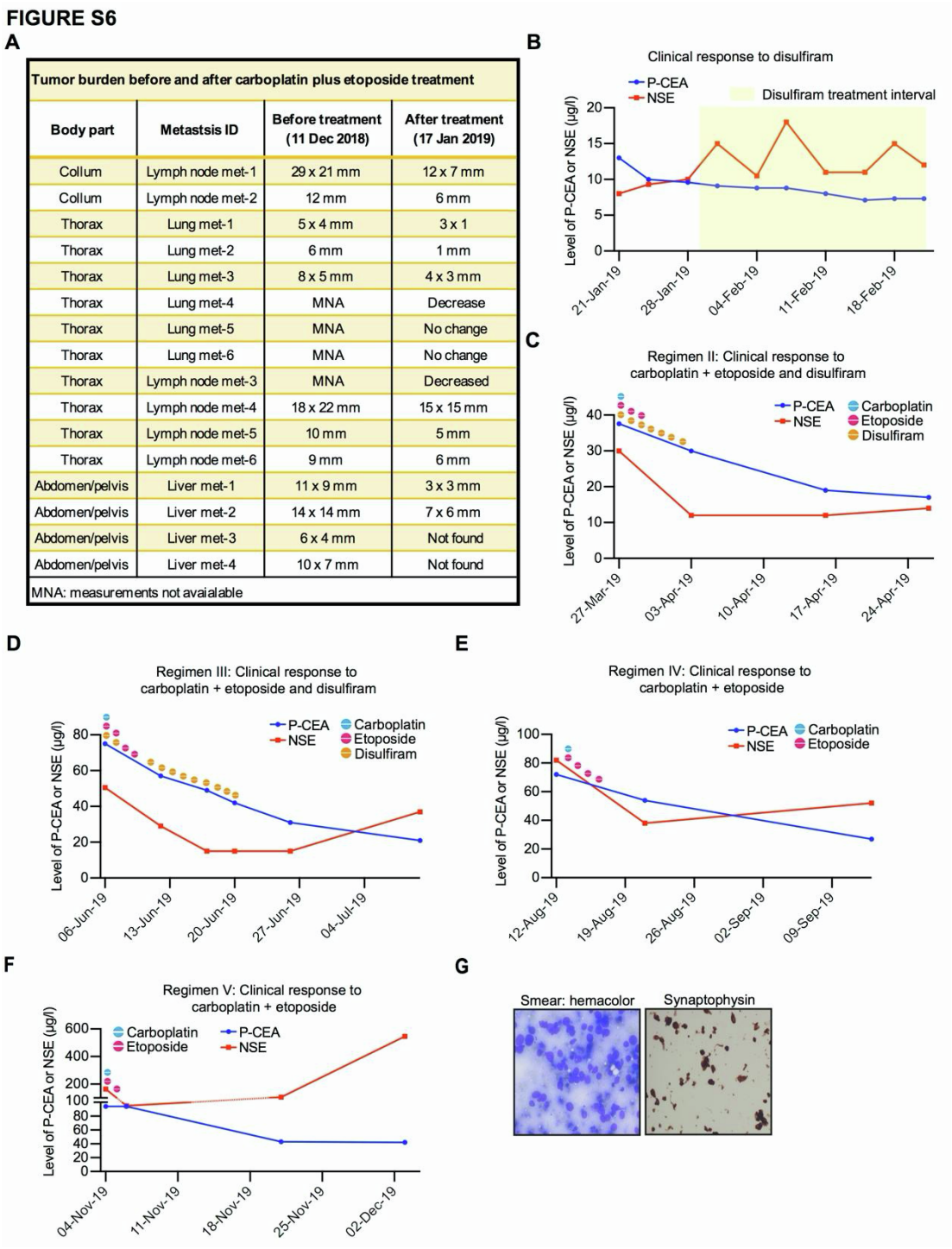

Figure S6. Clinical response to carboplatin plus etoposide treatments. Related to Figure 6. (A) Effect of disulfiram and carboplatin plus etoposide treatment on size of metastatic lesions in lymph nodes, lung, and liver. (B) Changes in the level of CEA and NSE after disulfiram treatments. (C-F) Changes in the level of CEA and NSE after carboplatin plus etoposide treatments with or without disulfiram. (G) Hemacolor stained (left) and synaptophysin (right) stains of cell smear obtained from a fine needle aspiration from a lymph node biopsy (collected in Jan 2020).

Table S1

Table S1 Clinical information of samples used for FUTC-based drug testing. Related to Figure 3.

Sample ID	Gender/age	Lung cancer	TNM stage	Oncogenic driver	Treatment	Smoking history	Tumor location
PLT37	F/73	SCC	T1bN0M0	nd	surgery	2	Left lower lobe
PLT38	M/57	AC	T3N1M0	uod	surgery	0	Left upper lobe
PLT39	M/66	MAC	results not available	uod	surgery	0	Right upper lobe
PLT41	M/56	AC	solitary recurr in mediastinum	ALK fusion +	surgery	0	Mediastinum
PLT62	F/50	AC	T2aN1M0	KRAS G13D	surgery + chemotherapy	2	Right lower lobe
PLT63	F/68	AC	T3N0M0	ALK fusion +	surgery	1	Left upper lobe
PLT64	F/25	AC	T2aN1M0	ALK fusion +	surgery + chemotherapy	0	Left lower lobe
PLT66	M/71	AC	T3N0M0	KRAS G12C	surgery	2	Right lower lobe
PLT68	F/68	AC	T1cN0M0	KRAS G12S	surgery	1	Right lower lobe
PLT70	M/82	AC	T1cN0M0	MET exon 14 skipping mutation	surgery	0	Right upper lobe
PLT71	F/58	AC	T2N0M0	KRAS G12V	surgery	2	Right lower lobe
PLT72	M/70	AC	T2bN0M0	EGFR L858R	surgery	1	Right upper lobe
PLT84	M/60	AC	T2N2M0	EGFR exon 19 del	surgery + adjuv. chemotherapy	0	Right upper lobe
PLT85	F/76	AC	T2aN0M0	EGFR L858R	surgery	0	Right upper lobe
PLT86	F/68	AC	T2N0M0	KRAS G12V	surgery + adjuv. chemotherapy	0	Right upper lobe
PLT87	F/61	AC	T3N0M0	KRAS G12C	surgery	2	Right lower lobe
PLT88	F/43	ASC	T2bN1M0	EGFR exon 19 del and EGFR amp	surgery + postop. chemoradiotherapy	0	Right upper lobe

SCC: squamous cell carcinoma, AC: adenocarcinoma, MAC: mucinous adenocarcinoma, ASC: adenosquamous carcinoma, nd: not done, uod: unknown oncogenic driver, never smoker: 0, ex-smoker: 1, current smoker: 2

Table S2

Table S2 Immunohistochemical and phenotypic characterization of clinical lung tumor tissues. Related to Figure 3, Figure 4 and Figure 5.

Sample ID	Lung cancer type	Tumor epithelial region (%)	EpCAM+ cells normalized to total cancer cells (%): strong positivity	EpCAM+ cells normalized to total cancer cells (%): weak positivity	Ki-67+ cells normalized to total cancer cells (%)	p4EBP1+ cells normalized to total cancer cells (%)	pERK+ cells normalized to total cancer cells (%)	pERK and p4EBP1 double positive cells normalized to total cancer	pEGFR positive cancer cells	pERBB2 positive cancer cells	pERBB3 positive cancer cells	E-cadherin and vimentin double positive cells
PLT37	SCC	28.15	26.35	73.65	39.82	10.25	0.00	0.00	–	–	–	–
PLT38	AC	14.3	46.85	53.15	39.32	13.58	1.47	1.03	–	–	–	–
PLT39	MAC	4.754	29.79	70.21	15.85	1.46	3.42	0.09	–	–	–	–
PLT41	AC	46.56	6.71	93.29	10.85	0.33	0.00	0.00	nd	nd	nd	+
PLT62; region1	AC	42.33	77.29	22.71	29.91	33.31	12.21	5.10	+	–	–	–
PLT62; region2	AC	49.69	90.23	7.77	32.31	28.69	0.00	0.00	+	–	–	–
PLT63	AC	21.8	18.05	81.95	9.77	9.30	5.91	0.65	–	–		+
PLT64	AC	47.3	2.795	97.205	16.26	3.51	0.00	0.00	–	–	–	+
PLT66	AC	16.68	54.6	45.4	23.71	1.19	47.56	0.47	–	–	–	–
PLT68	AC	38.07	63.61	36.39	24.69	0.09	27.13	0.01	–	–	–	–
PLT70	AC	21.14	32.53	67.47	4.28	0.30	0.00	0.00	–	–	–	–
PLT71	AC	10.84	29.43	70.57	49.58	2.47	7.17	0.18	+	–	–	–
PLT72	AC	22.51	1.67	98.33	11.11	3.46	0.00	0.88	–	–	–	+
PLT84	AC	17.73	19.97	80.03	5.82	13.85	42.23	6.32	+	+	+	+
PLT85	AC	2.574	45.09	54.01	4.39	13.96	69.05	7.79	+	+	+	–
PLT86	AC	12.99	70.57	29.43	17.45	45.18	71.58	6.46	–	–	–	–
PLT87	AC	61.15	86.99	13.01	65.42	7.44	23.24	2.41	+	+	–	–
PLT88	ASC	22.55	6.39	93.61	27.94	0.08	0.00	0.00	+	+	+	+
PLT 96	AC	12.39	30.64	69.36	10.89	nd	nd	nd	nd	nd	nd	nd

SCC: squamous cell carcinoma, AC: adenocarcinoma, MAC: mucinous adenocarcinoma, ASC: adenosquamous carcinoma, + : present, – : absent, nd: not done

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Table S3 DSS comparison between KRAS mutant cells and KRAS wildtype cells for 66 oncology drugs . Related to Figure 5.

KRAS-selective hit ID	Drug Name	Mean Diff	Median Diff	p.val	adjusted Pval	t.test	t.adjust
1	Trametinib	8.060	7.850	0.004	0.262	0.001	0.072
2	Panobinostat	4.633	1.350	0.268	0.937	0.245	0.737
3	LY3009120	4.227	2.600	0.149	0.937	0.114	0.737
4	Ganetespib	3.884	2.300	0.745	0.958	0.424	0.737
5	Cobimetinib	3.103	0.100	0.329	0.937	0.199	0.737
6	Ulixertinib	2.573	4.400	0.191	0.937	0.370	0.737
7	Sorafenib	2.083	0.000	0.353	0.937	0.280	0.737
8	Ravoxertinib	1.577	1.500	0.254	0.937	0.349	0.737
9	Olaparib	1.504	1.400	0.074	0.809	0.188	0.737
10	Gandotinib	1.366	3.000	0.415	0.937	0.376	0.737
11	Prexasertib	1.340	2.850	1.000	1.000	0.771	0.950
12	Nintedanib	1.217	1.750	0.432	0.937	0.610	0.895
13	Afatinib	1.005	5.900	0.356	0.937	0.673	0.907
14	Tipifarnib	0.996	1.150	0.745	0.958	0.652	0.906
15	LCL161	0.963	3.600	0.569	0.937	0.801	0.950
16	Vorinostat	0.821	0.700	0.876	1.000	0.761	0.950
17	Vismodegib	0.707	0.950	0.501	0.937	0.727	0.941
18	Gedatolisib	0.683	1.900	0.755	0.958	0.837	0.950
19	BMS-754807	0.669	0.050	0.935	1.000	0.848	0.950
20	JQ1	0.363	-1.300	1.000	1.000	0.914	0.955
21	Vemurafenib	0.057	-1.200	1.000	1.000	0.982	0.982
22	Dabrafenib	-0.151	0.700	0.563	0.937	0.926	0.955
23	Doxorubicin	-0.244	3.000	0.639	0.937	0.946	0.961
24	AZD4547	-0.257	0.400	1.000	1.000	0.860	0.950
25	Carboplatin	-0.297	-0.100	1.000	1.000	0.856	0.950
26	Dasatinib	-0.314	-0.050	1.000	1.000	0.896	0.955
27	Roxadustat	-0.324	-0.200	1.000	1.000	0.864	0.950
28	Vistusertib	-0.417	-2.000	1.000	1.000	0.912	0.955
29	Chloroquine	-0.500	-1.550	1.000	1.000	0.808	0.950
30	PAC-1	-0.531	-0.400	0.807	0.987	0.659	0.906
31	TAK-715	-0.796	-2.150	0.508	0.937	0.574	0.876
32	Sonidegib	-0.881	0.000	0.440	0.937	0.315	0.737
33	Perifosine	-0.884	-0.750	0.456	0.937	0.296	0.737
34	Alpelisib	-0.920	-1.200	0.394	0.937	0.391	0.737
35	Metformin	-0.923	-0.500	0.238	0.937	0.183	0.737
36	Foretinib	-1.003	-1.450	0.608	0.937	0.447	0.737
37	Docetaxel	-1.027	1.550	0.684	0.958	0.647	0.906
38	KU-60019	-1.030	-0.100	0.522	0.937	0.284	0.737
39	Volasertib	-1.126	-1.100	0.102	0.937	0.062	0.737
40	Cabozantinib	-1.130	0.600	0.934	1.000	0.416	0.737
41	Ipatasertib	-1.176	-2.600	0.755	0.958	0.704	0.929
42	Ponatinib	-1.287	-0.475	0.628	0.937	0.444	0.737
43	Palbociclib	-1.303	-1.300	0.515	0.937	0.472	0.760
44	Veliparib	-1.320	-0.700	0.463	0.937	0.264	0.737
45	Osimertinib	-1.428	-0.950	0.518	0.937	0.446	0.737
46	Dexamethasone	-1.487	0.000	0.717	0.958	0.370	0.737
47	Birabresib	-1.664	-2.000	0.639	0.937	0.584	0.876
48	Gefitinib	-1.677	1.300	0.792	0.987	0.389	0.737
49	Dovitinib	-1.711	-0.200	0.741	0.958	0.389	0.737
50	Lapatinib	-1.743	-0.100	0.066	0.809	0.250	0.737
51	NVP-LGK974	-1.813	-1.550	0.623	0.937	0.217	0.737
52	Gemcitabine	-1.888	-2.275	0.429	0.937	0.186	0.737
53	Crizotinib	-1.902	-2.700	0.136	0.937	0.069	0.737
54	Ralimetinib	-1.971	-1.550	0.515	0.937	0.255	0.737
55	VE-821	-2.069	-1.400	0.260	0.937	0.070	0.737
56	Ruxolitinib	-2.171	-1.100	0.624	0.937	0.330	0.737
57	Ceritinib	-2.492	-1.725	0.228	0.937	0.201	0.737
58	Pomalidomide	-2.869	-2.000	0.116	0.937	0.182	0.737
59	Vincristine	-2.994	-1.700	0.326	0.937	0.155	0.737
60	Azacitidine	-3.344	-3.650	0.073	0.809	0.069	0.737
61	Navitoclax	-3.365	-5.325	0.628	0.937	0.486	0.764
62	Dinaciclib	-3.503	-7.100	0.432	0.937	0.438	0.737
63	Everolimus	-3.991	-4.000	0.074	0.809	0.061	0.737
64	Pictilisib	-4.691	0.000	0.569	0.937	0.155	0.737
65	Idasanutlin	-4.938	-7.300	0.202	0.937	0.082	0.737
66	Cisplatin	-5.811	-1.300	0.074	0.809	0.117	0.737

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References

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