

Table S1

VARIANT				GENOTYPE				VAF				ANNOTATION						
CHROM	POS	REF	ALT	S2	S3	A10	A13	S2	S3	A10	A13	ALL.CODE	C.CODE	P.CODE	GENE	FUNCTION	EXON	TOT_EXON
<u>Chr1</u>	<u>36932346</u>	<u>G</u>	<u>C</u>	<u>0/1</u>	<u>0/0</u>	<u>./.</u>	<u>./.</u>	<u>0.1237</u>	.	.	.	<u>NM_156039.3:c.2204C&gt;G;NP_724781.1:p.(Pro735Arg)</u>	<u>NM_156039.3:c.2204C&gt;G</u>	<u>NP_724781.1:p.(Pro735Arg)</u>	<u>CSF3R</u>	<u>missense_variant</u>	<u>17</u>	<u>17</u>
Chr1	158656281	T	C	./.	./.	1/.	./.	.	.	0.9985	.	NM_003126.2:c.24+3A>G	.	.	SPTA1	splice_region_variant:intron_variant	.	.
Chr1	206669442	T	C	./.	1/.	./.	./.	.	0.9919	.	.	NM_014002.3:c.2118-3T>C	.	.	IKBKE	splice_region_variant:intron_variant	.	.
<u>Chr2</u>	<u>48027259</u>	<u>G</u>	<u>C</u>	<u>0/1</u>	<u>0/1</u>	<u>0/1</u>	<u>0/0</u>	<u>0.1408</u>	<u>0.1344</u>	<u>0.0637</u>	.	<u>NM_000179.2:c.2137G&gt;C;NP_000170.1:p.(Asp713His)</u>	<u>NM_000179.2:c.2137G&gt;C</u>	<u>NP_000170.1:p.(Asp713His)</u>	<u>MSH6</u>	<u>missense_variant</u>	<u>4</u>	<u>10</u>
<u>Chr2</u>	<u>209110106</u>	<u>C</u>	<u>G</u>	<u>0/0</u>	<u>0/0</u>	<u>0/1</u>	<u>0/0</u>	.	.	<u>0.1304</u>	.	<u>NM_005896.3:c.457G&gt;C;NP_005887.2:p.(Glu153Gln)</u>	<u>NM_005896.3:c.457G&gt;C</u>	<u>NP_005887.2:p.(Glu153Gln)</u>	<u>IDH1</u>	<u>missense_variant</u>	<u>5</u>	<u>10</u>
Chr3	12666367	A	G	./.	1/1	1/1	1/1	1	1	1	1	NM_002880.3:c.-26-6121T>C	.	.	RAF1	intron_variant	.	.
Chr6	20407314	C	T	0/0	0/0	0/1	0/0	.	.	0.0688	.	NM_001949.4:c.393+4458C>T	.	.	E2F3	intron_variant	.	.
Chr6	30673403	A	G	./.	0/1	./.	0/1	.	0.0158	.	0.0219	NM_014641.2:c.3557T>C;NP_055456.2:p.(Val1186Ala)	NM_014641.2:c.3557T>C	NP_055456.2:p.(Val1186Ala)	MDC1	missense_variant	10	15
<u>Chr6</u>	<u>112382221</u>	<u>C</u>	<u>T</u>	<u>0/0</u>	<u>0/1</u>	<u>0/0</u>	<u>0/0</u>	.	<u>0.0544</u>	.	.	<u>NM_198239.1:c.130C&gt;T;NP_937882.1:p.(Pro44Ser)</u>	<u>NM_198239.1:c.130C&gt;T</u>	<u>NP_937882.1:p.(Pro44Ser)</u>	<u>WISP3</u>	<u>missense_variant</u>	<u>2</u>	<u>5</u>
Chr6	162992294	T	A	./.	./.	1/1	./.	1	1	1	1	NM_004562.2:c.8-127789A>T	.	.	PRKN	intron_variant	.	.
<u>Chr8</u>	<u>117866550</u>	<u>C</u>	<u>G</u>	<u>0/1</u>	<u>0/0</u>	<u>0/0</u>	<u>0/0</u>	<u>0.1678</u>	.	.	.	<u>NM_006265.2:c.1095G&gt;C;NP_006256.1:p.(Glu365Asp)</u>	<u>NM_006265.2:c.1095G&gt;C</u>	<u>NP_006256.1:p.(Glu365Asp)</u>	<u>RAD21</u>	<u>missense_variant</u>	<u>9</u>	<u>14</u>
Chr9	133762667	G	A	0/0	0/1	./.	0/0	.	0.3204	.	.	NM_007313.2:c.*1597G>A	.	.	ABL1	3_prime_UTR_variant	11	11
Chr11	69629199	C	G	0/1	./.	./.	./.	0.3196	.	.	.	NM_005247.2:c.324+1889G>C	.	.	FGF3	intron_variant	.	.
Chr12	4488996	C	A	0/0	0/0	0/1	0/1	.	.	0.1566	0.0376	-	-	-	FGF23	upstream_gene_variant	.	.
Chr13	22253094	C	T	0/0	0/1	0/.	0/0	.	0.4983	.	.	NM_002010.2:c.278-2087C>T	.	.	FGF9	intron_variant	.	.
Chr13	22258282	C	T	0/1	0/0	./.	0/0	0.0194	.	.	.	NM_002010.2:c.381+2998C>T	.	.	FGF9	intron_variant	.	.
Chr13	28608241	A	G	0/1	0/0	0/0	0/0	0.0175	.	.	.	NM_004119.2:c.1815T>C(p.(Phe605=))	NM_004119.2:c.1815T>C	.	FLT3	synonymous_variant	14	24
<u>Chr16</u>	<u>3640521</u>	<u>G</u>	<u>A</u>	<u>./.</u>	<u>0/0</u>	<u>0/1</u>	<u>0/0</u>	.	.	<u>0.2686</u>	.	<u>NM_032444.3:c.3118C&gt;T;NP_115820.2:p.(Pro1040Ser)</u>	<u>NM_032444.3:c.3118C&gt;T</u>	<u>NP_115820.2:p.(Pro1040Ser)</u>	<u>SLX4</u>	<u>missense_variant</u>	<u>12</u>	<u>15</u>
Chr16	67645327	C	T	0/0	0/0	0/1	0/0	.	.	0.0103	.	NM_006565.3:c.592C>T;NP_006556.1:p.(Gln198Ter)	NM_006565.3:c.592C>T	NP_006556.1:p.(Gln198Ter)	CTCF	stop_gained	3	12
<u>Chr17</u>	<u>37650854</u>	<u>A</u>	<u>G</u>	<u>0/0</u>	<u>0/0</u>	<u>0/0</u>	<u>0/1</u>	.	.	.	<u>0.3354</u>	<u>NM_016507.2:c.2326A&gt;G;NP_057591.2:p.(Lys776Glu)</u>	<u>NM_016507.2:c.2326A&gt;G</u>	<u>NP_057591.2:p.(Lys776Glu)</u>	<u>CDK12</u>	<u>missense_variant</u>	<u>5</u>	<u>14</u>
Chr20	15124864	A	G	0/1	./.	./.	./.	0.5227	.	.	.	-	-	-	-	NA	.	.
ChrX	66871495	A	C	0/0	0/1	0/0	0/0	.	0.2755	.	.	NM_002010.2:c.278-2087C>T	.	.	AR	intron_variant	.	.
ChrX	100611036	C	G	1/.	./.	./.	1/.	0.9973	.	.	0.9991	NM_000061.2:c.1566+4G>C	.	.	BTK	splice_region_variant:intron_variant	.	.

Genetic analysis of variants detected in sotorasib and adagrasib resistant clones by TSO500 analysis. Underlined variants represent selected missense variants.

Table S2

**S1-S2**

Gene	FI
<i>COL4A2</i>	10.6294365
<b><i>CXCL1</i></b>	16.098223
<i>IGFBP3</i>	7.9592023
<i>FOSL1</i>	4.919283
<i>NAV3</i>	4.552033
<i>TMEM40</i>	3.807875
<b><i>HGF</i></b>	3.6571987
<i>PLAU</i>	3.401096
<i>FHL2</i>	3.2563412
<i>EFNB2</i>	3.0197997
<i>MICAL2</i>	2.805881
<i>DUSP7</i>	2.6566582
<i>UAP1</i>	2.5481768
<i>KRT18</i>	2.3635592
<i>EPHA2</i>	2.237792
<i>ETV4</i>	2.2210042
<i>KRT8</i>	2.2122033
<i>NRG1</i>	2.2048469
<i>RIPK4</i>	2.1392994
<i>PFKFB3</i>	2.1130006
<i>PLEK2</i>	2.1083176
<i>PFKP</i>	2.0840902
<i>ETS1</i>	2.080492
<i>PNP</i>	2.0468736

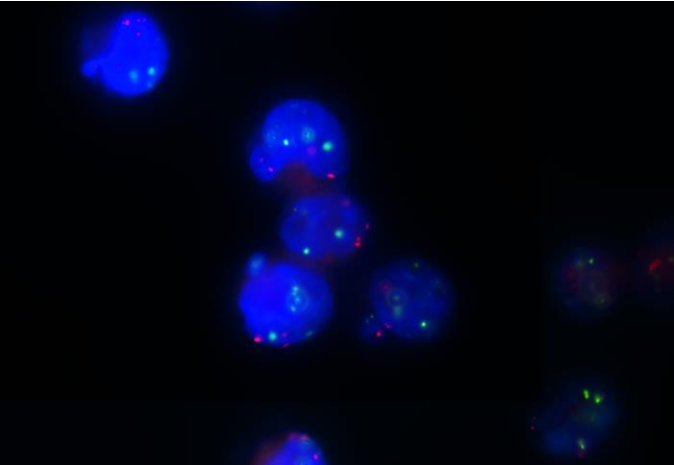
**A1-A2**

Gene	FI
<i>COL4A2</i>	16.53918
<b><i>CXCL1</i></b>	13.208717
<i>IGFBP3</i>	8.8488245
<i>FOSL1</i>	7.985571
<b><i>HGF</i></b>	4.8262115
<i>NAV3</i>	4.0730076
<i>PLEK2</i>	3.7022858
<i>MICAL2</i>	3.4434016
<i>PLAU</i>	3.3647099
<i>PFKP</i>	3.1797469
<i>FHL2</i>	3.0443323
<i>UAP1</i>	3.028848
<i>KRT18</i>	2.97067
<i>KRT8</i>	2.9701595
<i>TMEM40</i>	2.8658571
<i>EPHA2</i>	2.7494767
<i>RIPK4</i>	2.697443
<i>ETV4</i>	2.6436524
<i>DUSP7</i>	2.3766265
<i>ETS1</i>	2.3434048
<i>EFNB2</i>	2.3199584
<i>PFKFB3</i>	2.3026052
<i>NRG1</i>	2.1181934
<i>PNP</i>	2.1169662

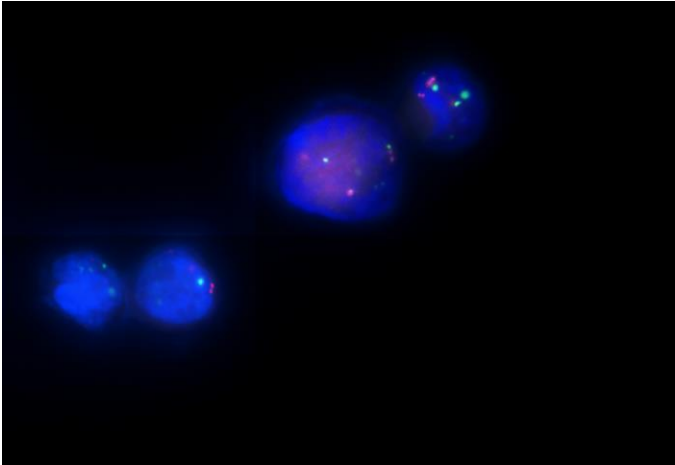
Common upregulated genes in sotorasib (left) and adagrasib (right) resistant clones and their fold increase vs treated parental cells.

Figure S1

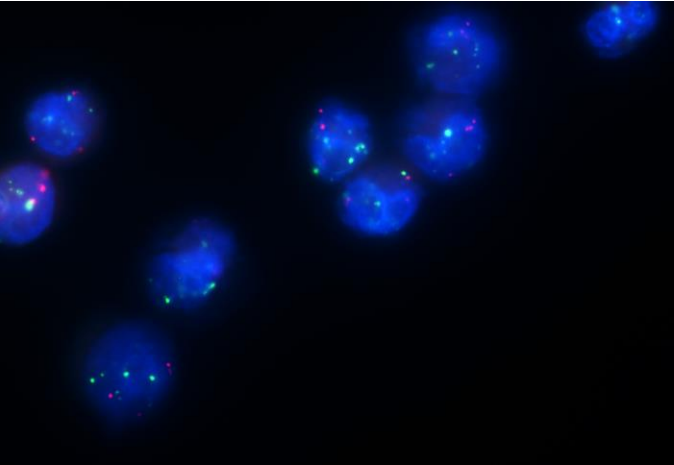
H23



S2

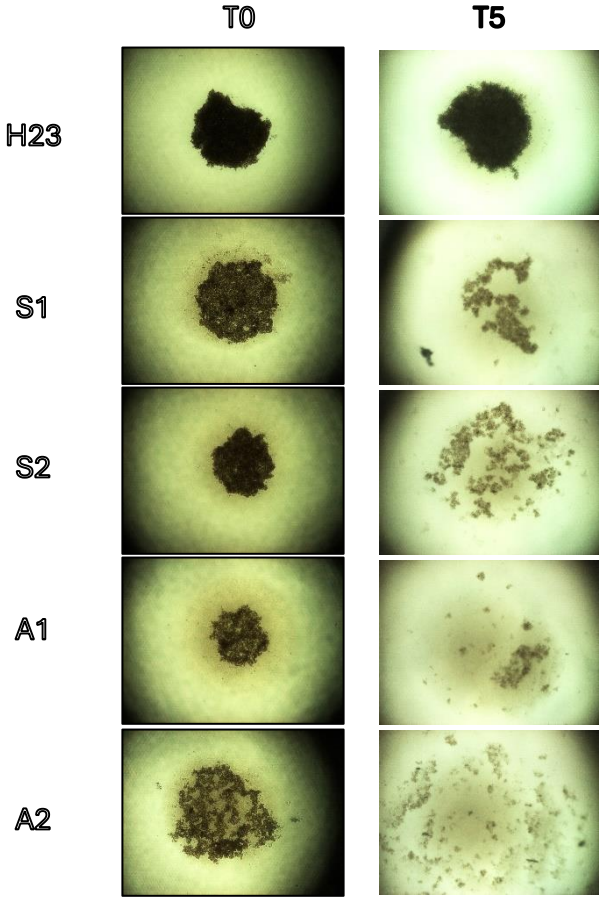


A2



FISH analysis of representative clones resistant to sotorasib (S2) and adagrasib (A2) vs H23 parental cells.

Figure S2



Generation of tumor spheroids from H23, S1, S2, A1, and A2 cells. T0: images of cancer cells after 4 days from cell seeding. T5: images of cancer cells after 5 days from the first acquisition (T0).

Figure S3

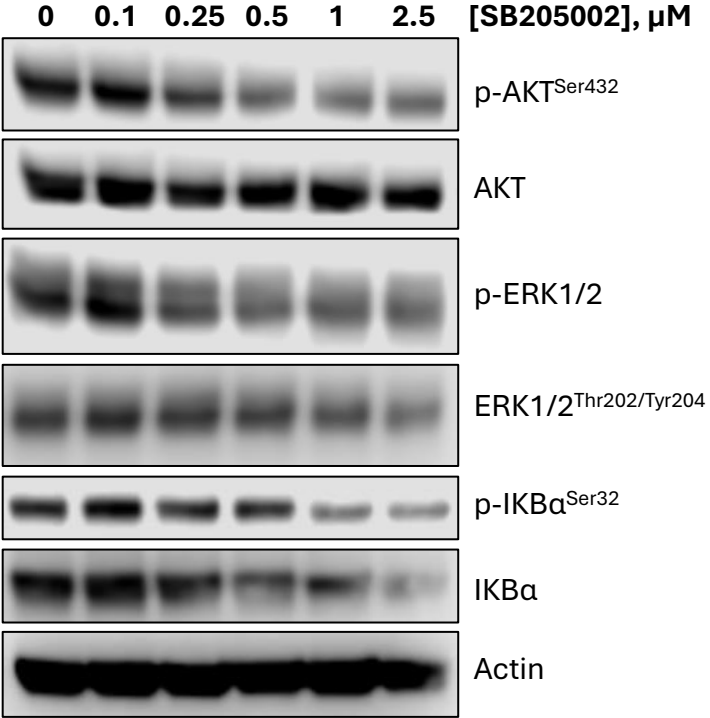
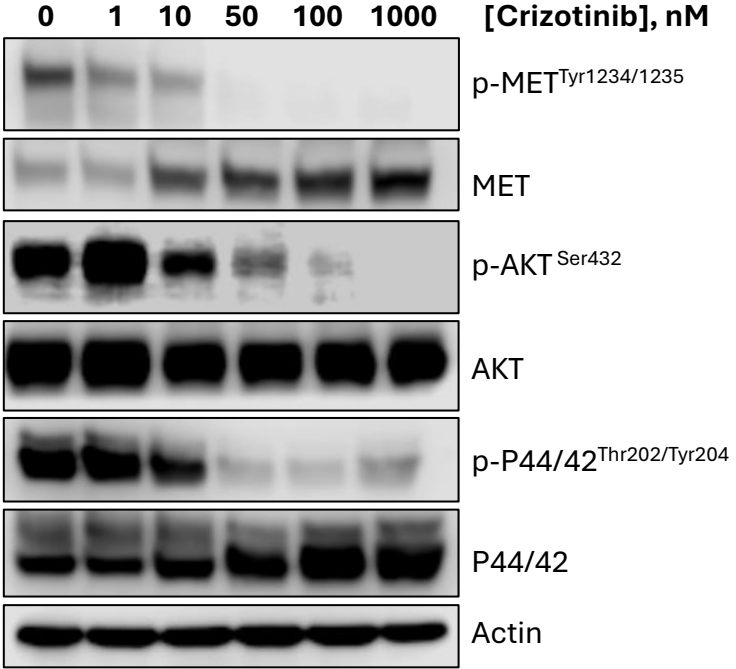
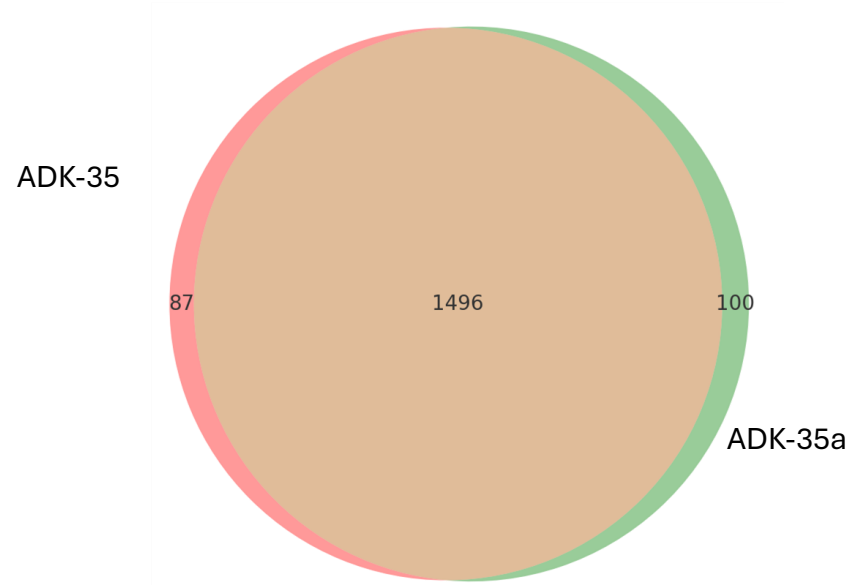


Figure S4

A

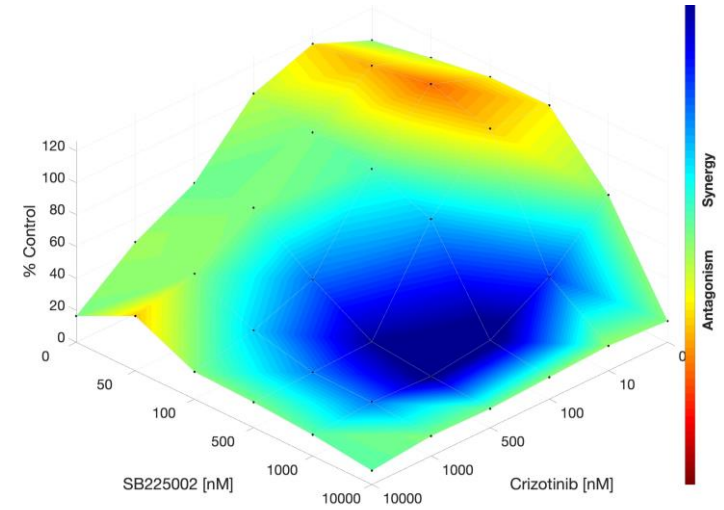
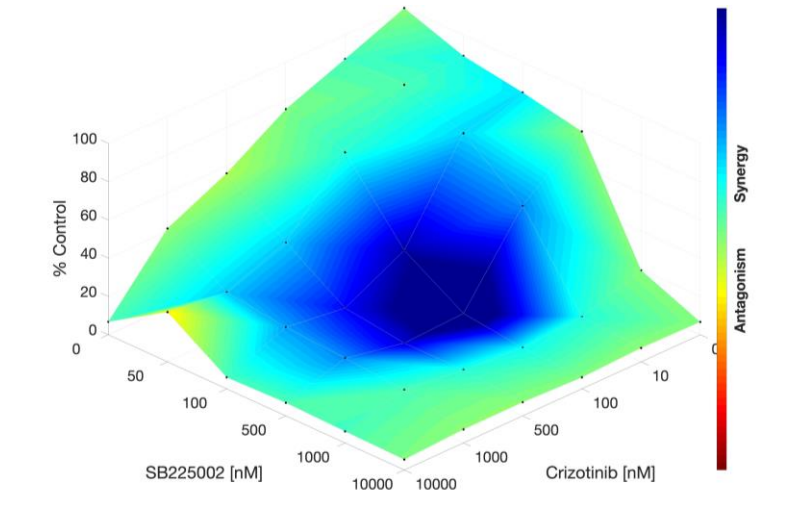


B



(A) Venn diagram on the most relevant common genetic alterations in ADK-35 and ADK-35a cells. (B) Network map of shared and unique mutations with pathway outcomes

Figure S5



ADK-35 (A) and ADK-35a (B) cells were treated with increasing doses of crizotinib and SB225002 and after 72h, cell proliferation was assessed by SRB assay, and the effect of this combination was evaluated by Combeneft software (HSA model)