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	C106 resistant	C106 resistant	# of genotyped cancer cells	# of mutated cancer cells (total)	# of mutated cancer cells (all variants covered)	Tumor MAF (%)
NRAS G12D	69%	100%	3756/3949 (95%)	3751/3756 (100%)	1714/1714 (100%)	75%
KRAS G12C	48%	100%	3793/3949 (96%)	3644/3793 (96%)	1714/1714 (100%)	50%
APC H1490Lfs*17	49%	100%	3187/3949 (81%)	3149/3187 (99%)	1714/1714 (100%)	42%
ERBB3 V104M	51%	100%	3788/3949 (96%)	3504/3788 (93%)	1714/1714 (100%)	51%
APC Q879*	0.7%	0.5%	3922/3949 (96%)	6/3922 (0.2%)	5/1714 (0.3%)	0.2%

Bulk sequencing

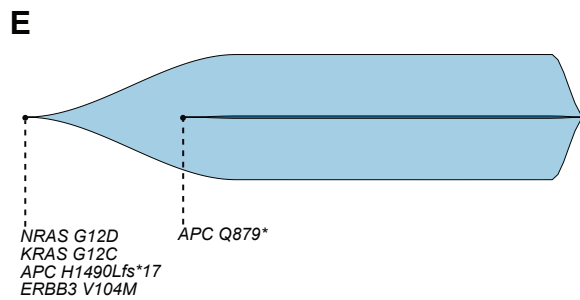
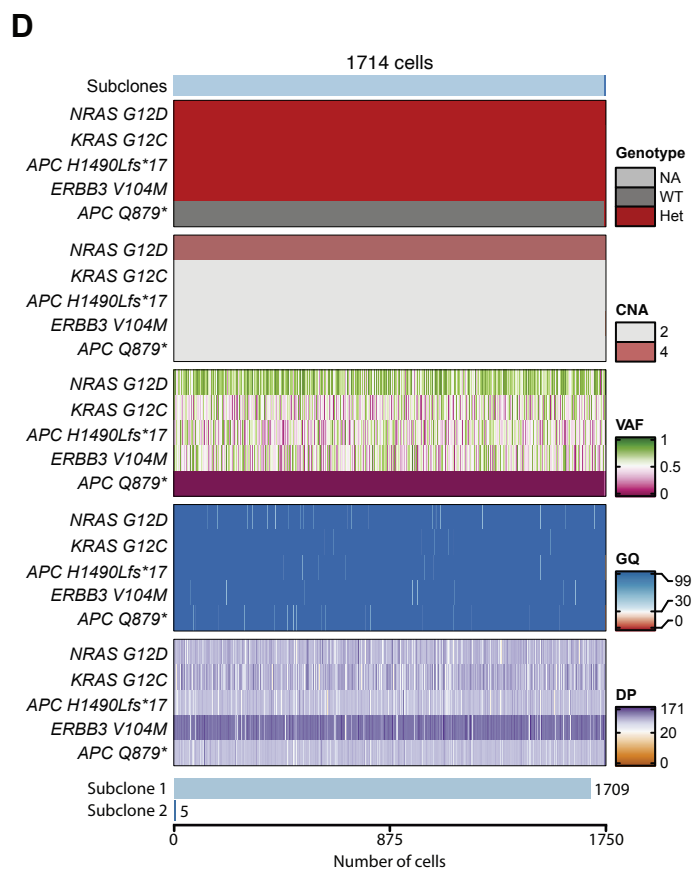
SC-sequencing

Mutation type

- Hotspot Mutation (red)
- Frame-shift indel (blue)
- Truncating SNV (purple)

Cancer cell fraction

- 0% < CCF <= 5% (light blue)
- 80% < CCF < 100% (dark blue)



Supplementary Figure 2. A. Nonsynonymous somatic mutations identified by MSK-IMPACT in the parental and resistant C106 cancer cell line (top). Mutation types (left) and cancer cell fraction (CCF) of mutations identified (right) are color coded according to the legend. The length of the trunk and branches of the phylogenetic trees (bottom) is proportional to the number of shared and private mutations identified in the parental and resistant cell line. The somatic hotspot mutations, as well as the number of nonsynonymous mutations, are shown along their corresponding branches. B. Copy-number alterations (CNAs) of the parental and resistant C106 cancer cell line (top). Copy-number log₂ ratios are shown on the y-axis according to the chromosomes on the x-axis. The arrows show the chromosome positions of the genes included in the customized gene panel used for single-cell DNA targeted sequencing. Phylogenetic trees based on CNAs are shown (bottom). The numbers alongside the branches represent gains and losses shown in red and in blue, respectively. C. Nonsynonymous somatic mutations identified by MSK-IMPACT in the resistant C106 cancer cell line subjected to single-cell sequencing. Mutation types (left), variant allele frequency and CCFs of mutations identified (right) are color coded according to the legend. Number of genotyped and mutated cancer cells of the same mutations detected in the matched single-cell sequencing data, number of mutated cancer cells after single-cell genotyping analysis of the union of all variants, and median of the variant allele frequency (VAF) of each mutation (right). D. Single-cell genotyping analysis of each mutation detected in the single-cell data, including the genotypes detected (top), the allele specific copy number estimation of each mutation per cell (second from top), VAF determined for each variant per cell (middle), as well as the genotype quality (second from bottom) and read depth of each variant (bottom) per cell. Cells are shown in columns and variants in rows. Bar plot depicting the frequency of each subclone determined in the clonal architecture analysis. E. Fish plot showing the emergence of the subclones in tumor evolution. The dotted lines depict the type of mutations that constitutes each of the subclones.