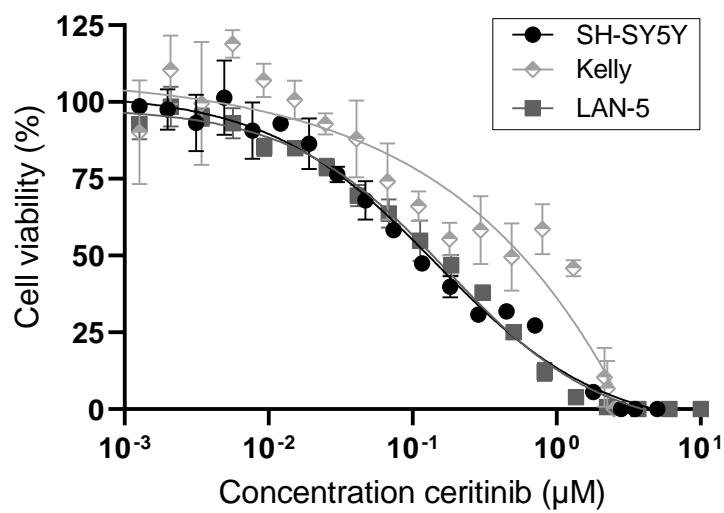
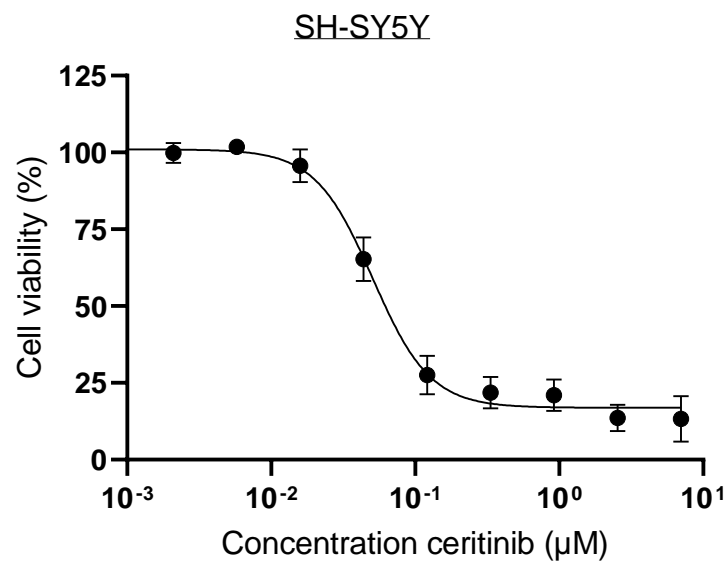


Figure S1

a



b



c

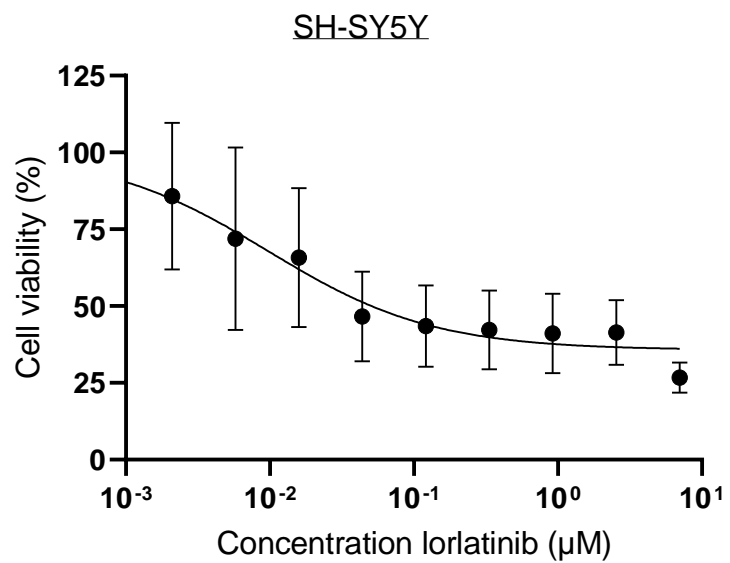
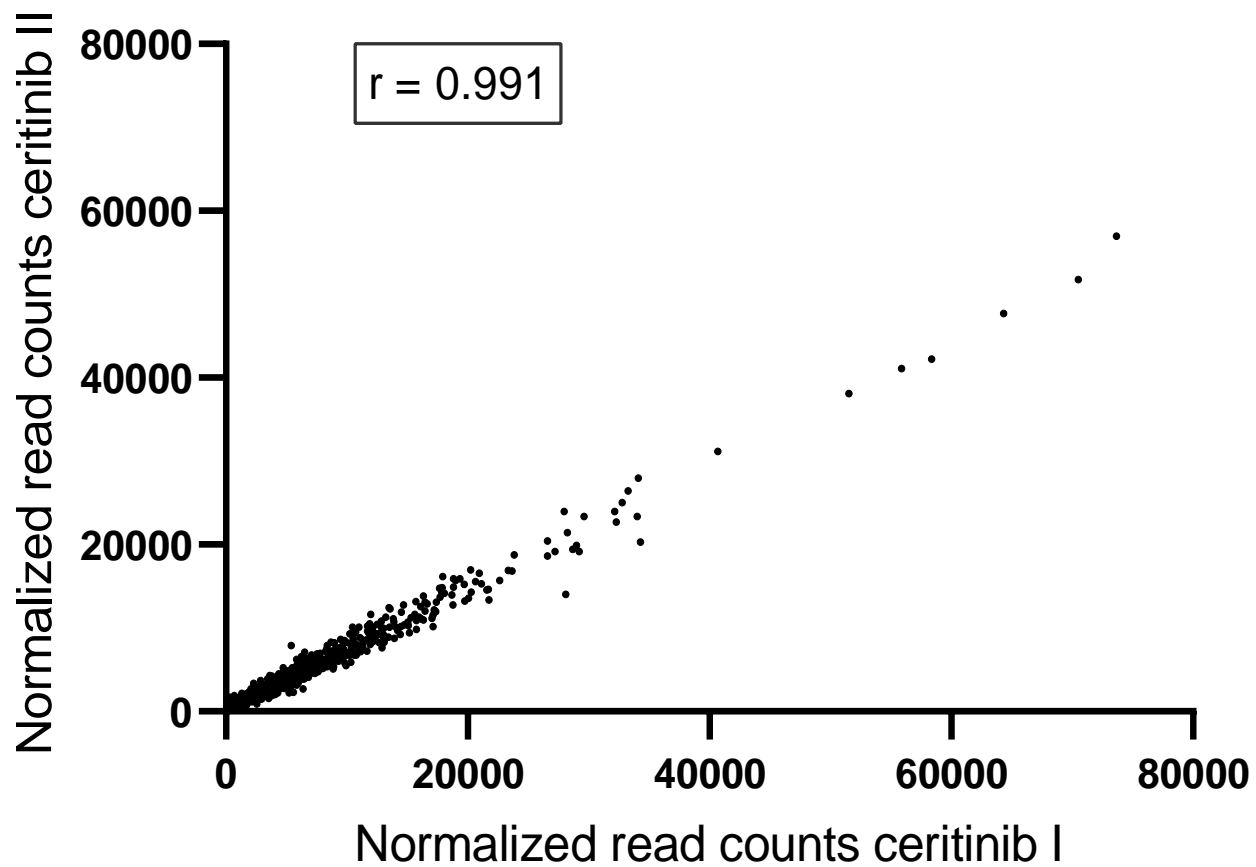
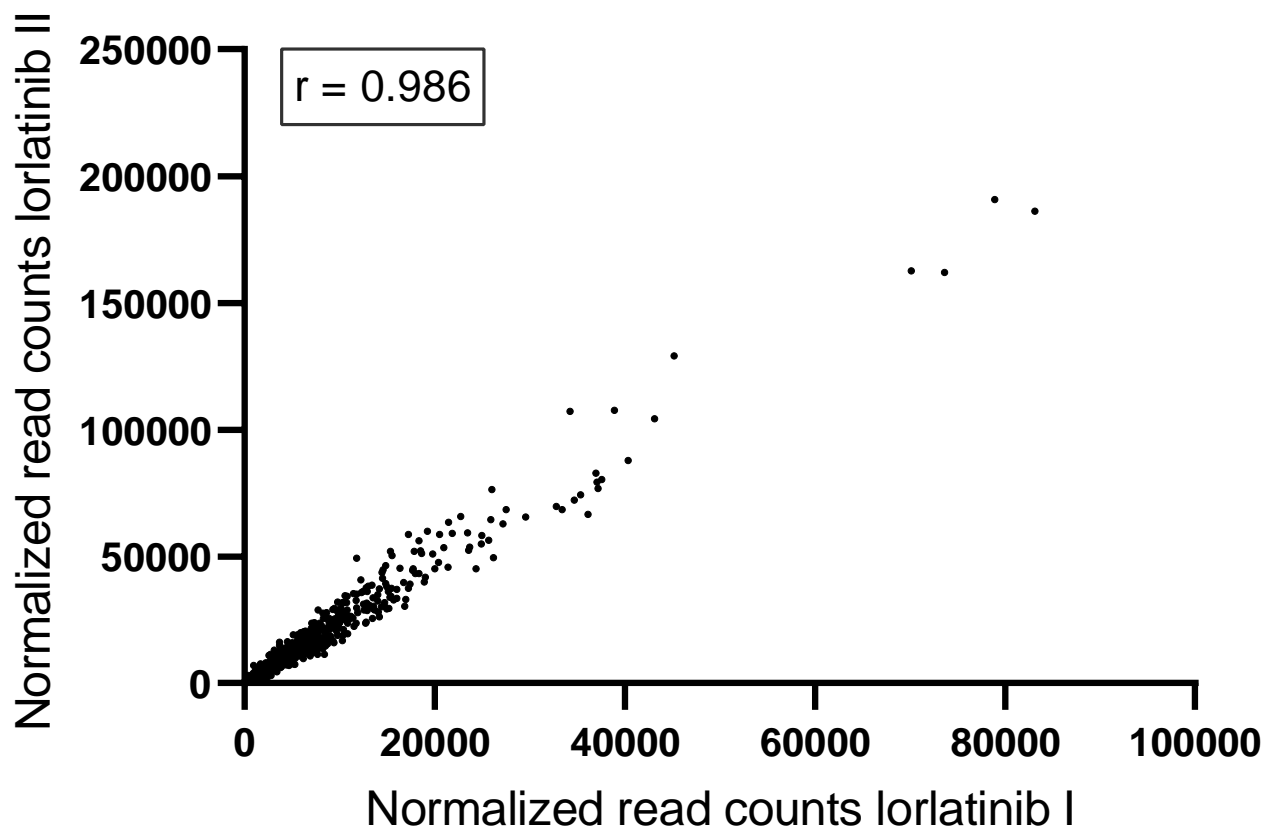


Figure S2

a

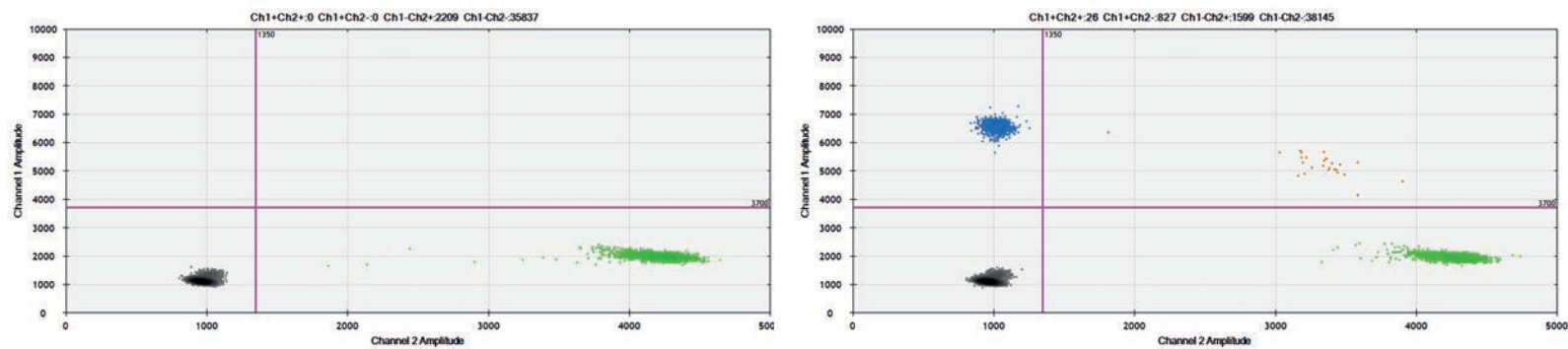


b

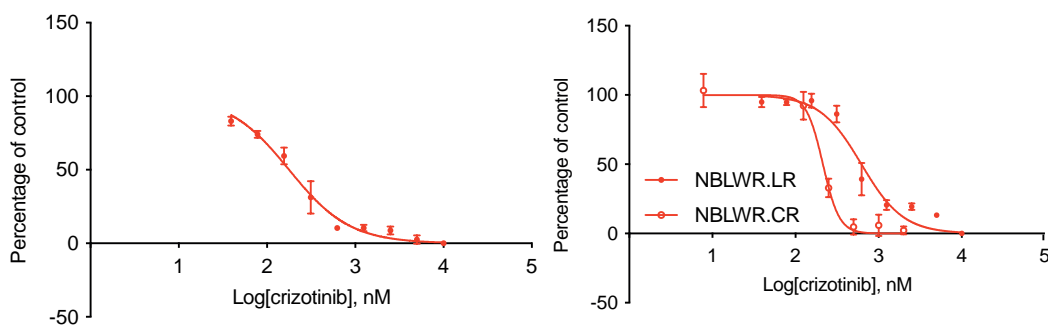


# Figure S3

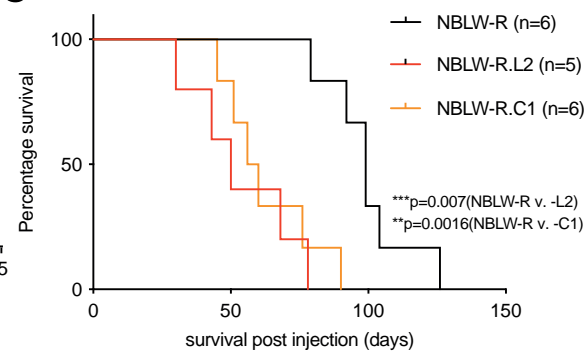
**a**



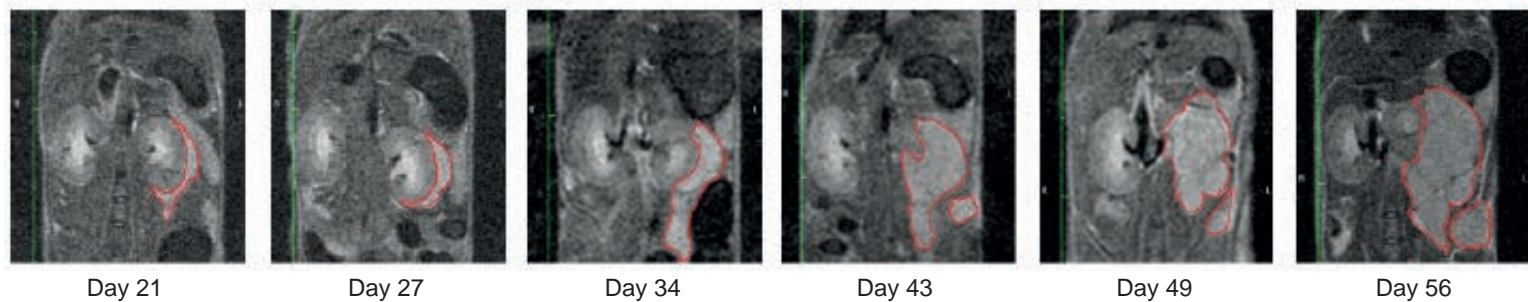
**b**



**c**



**d**



**e**

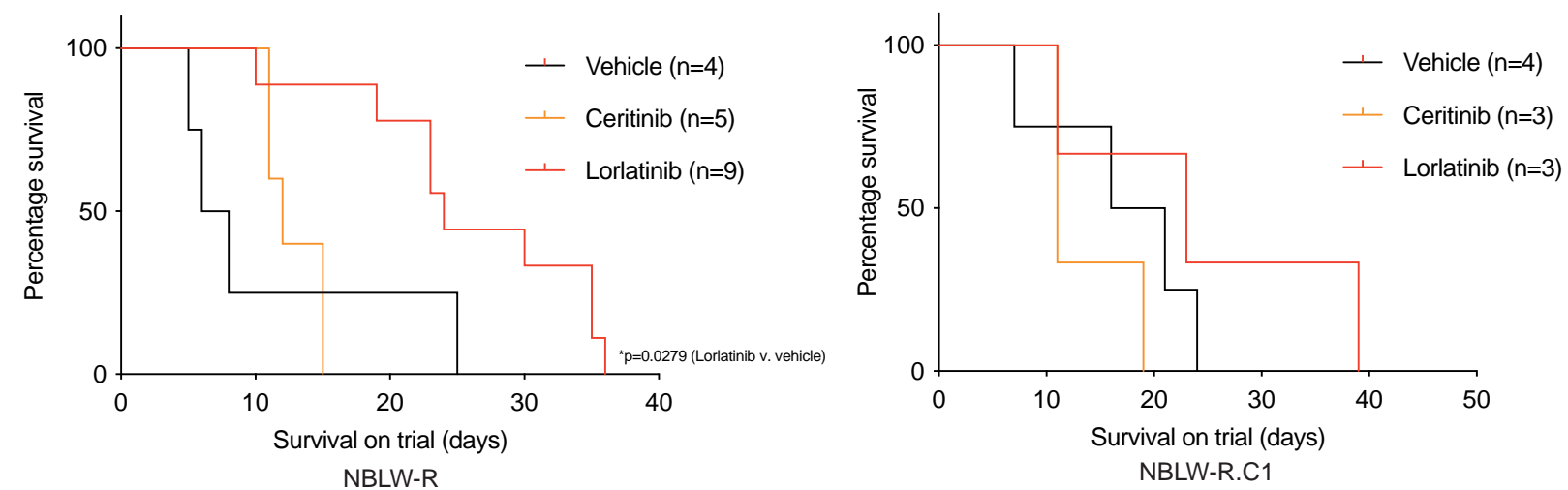


Figure S4

a

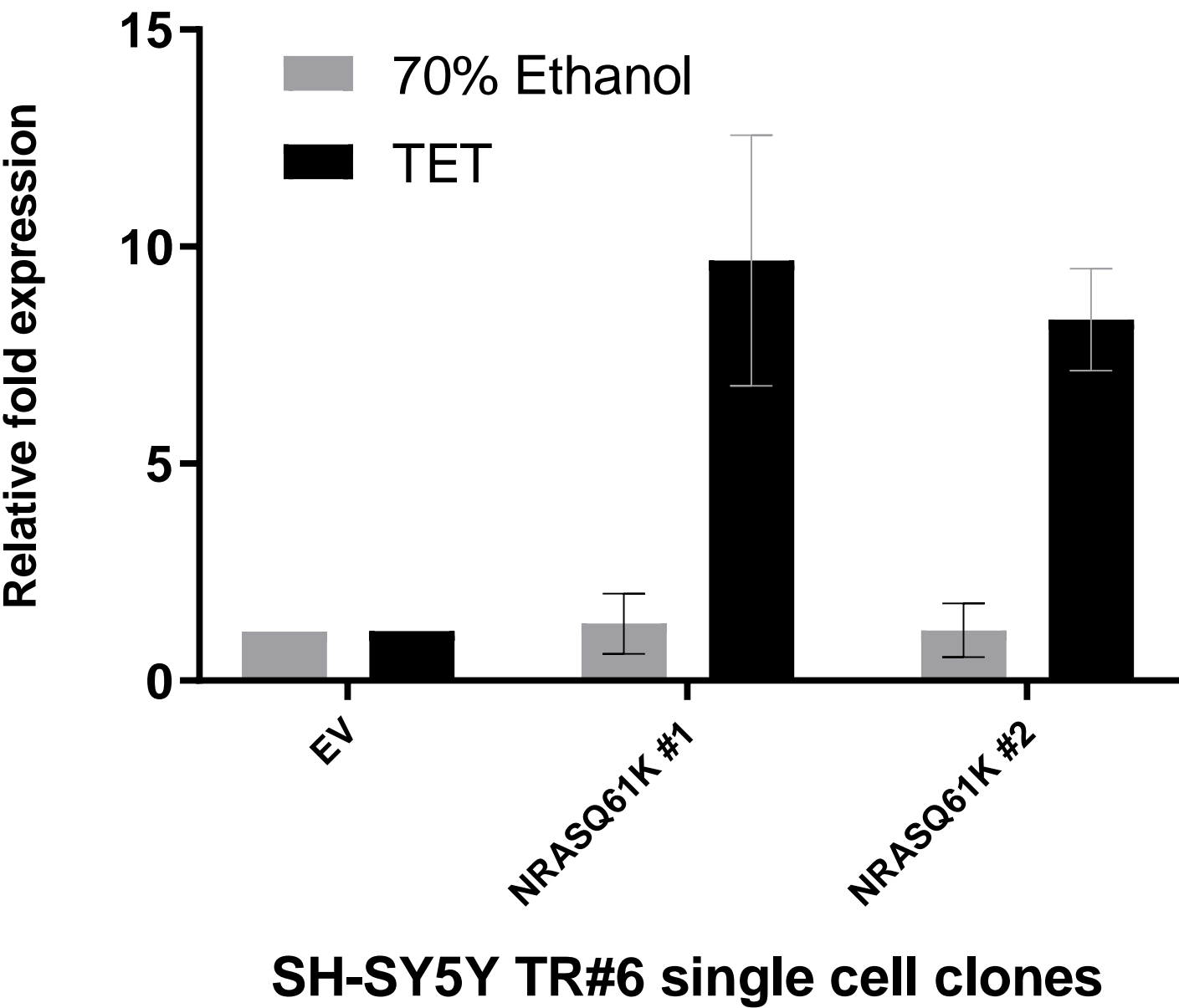
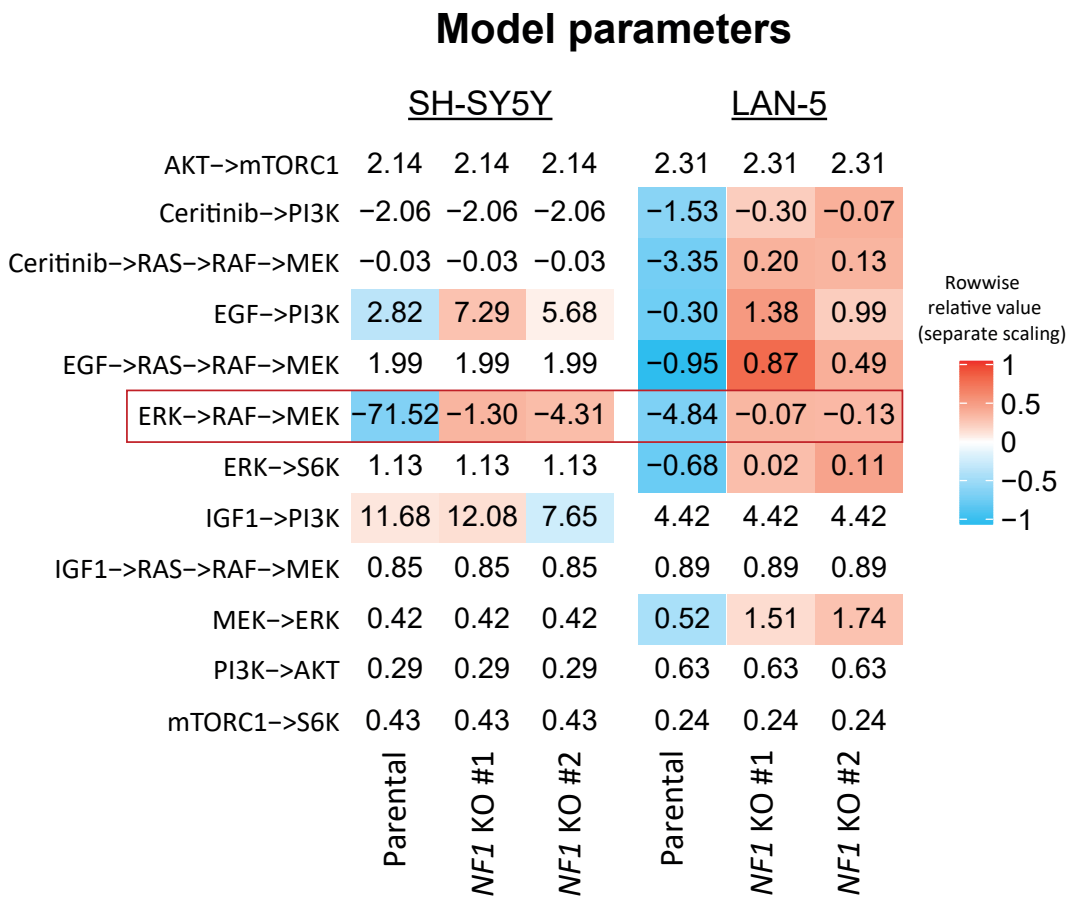
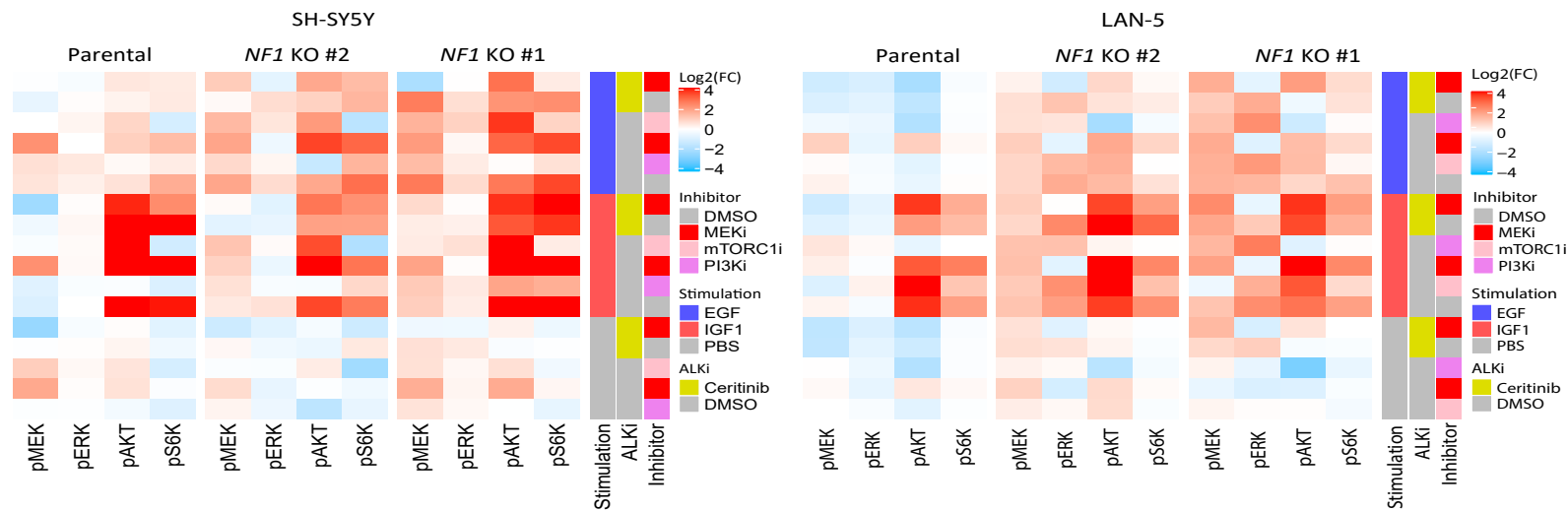


Fig. S5

a



b



c

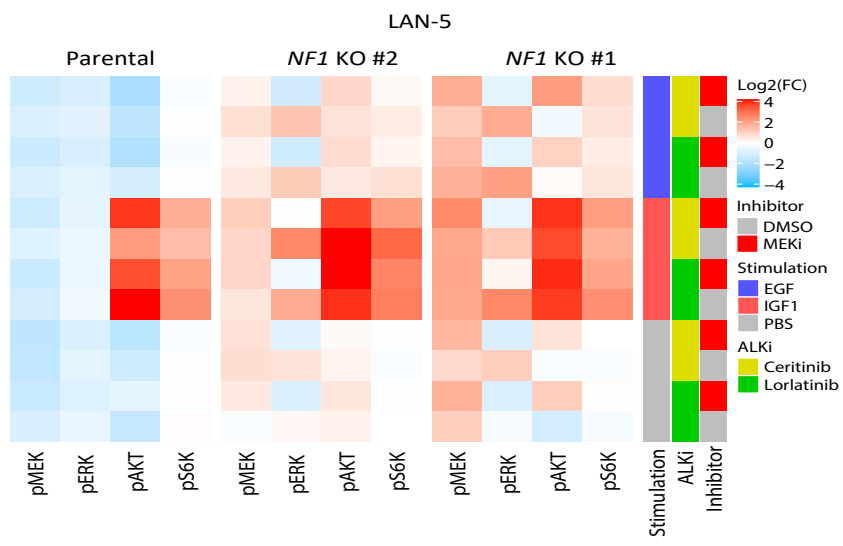
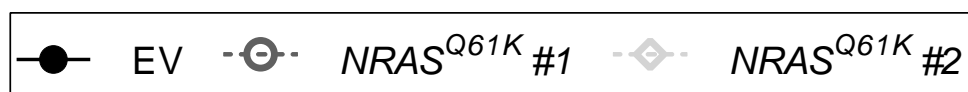
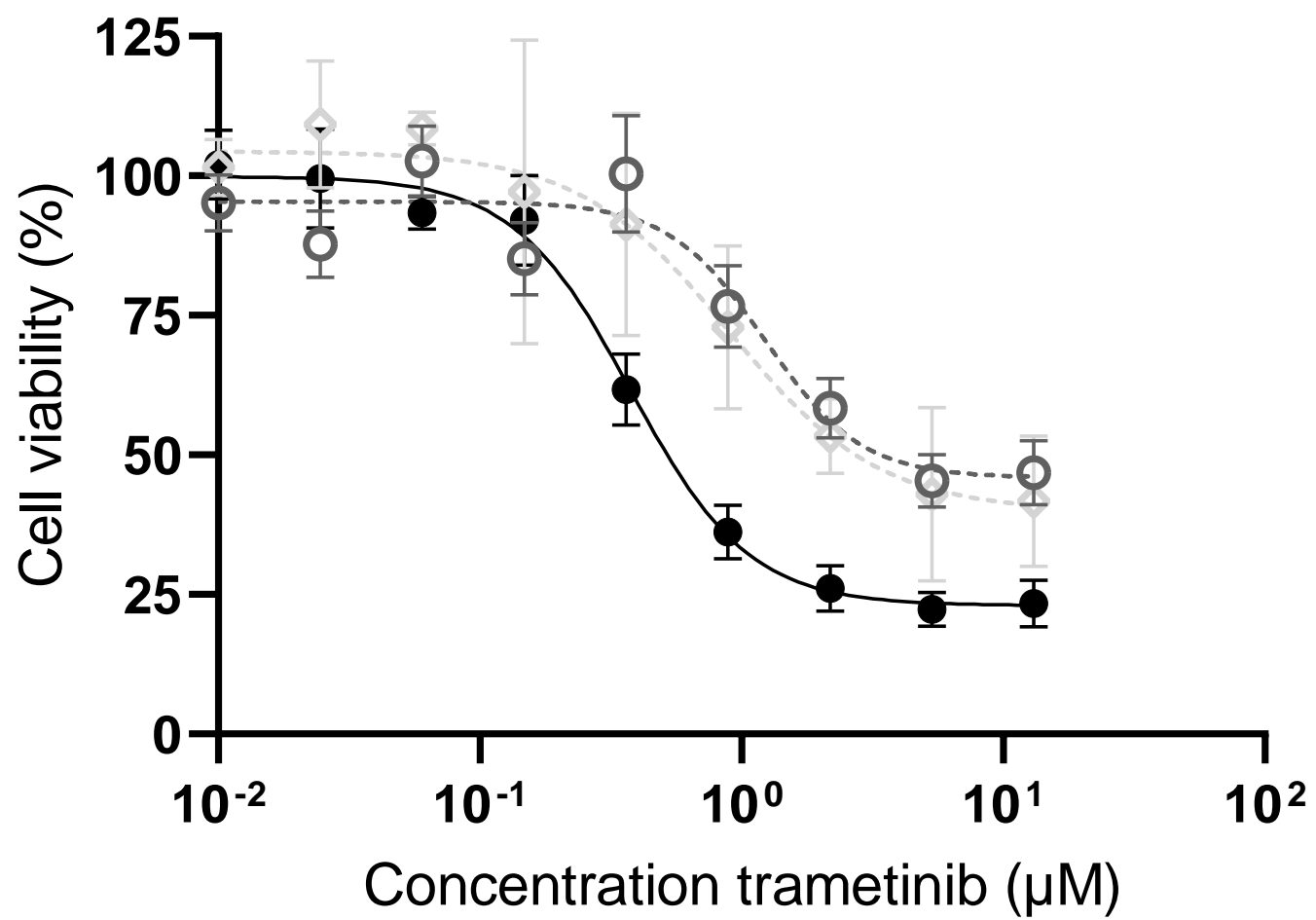


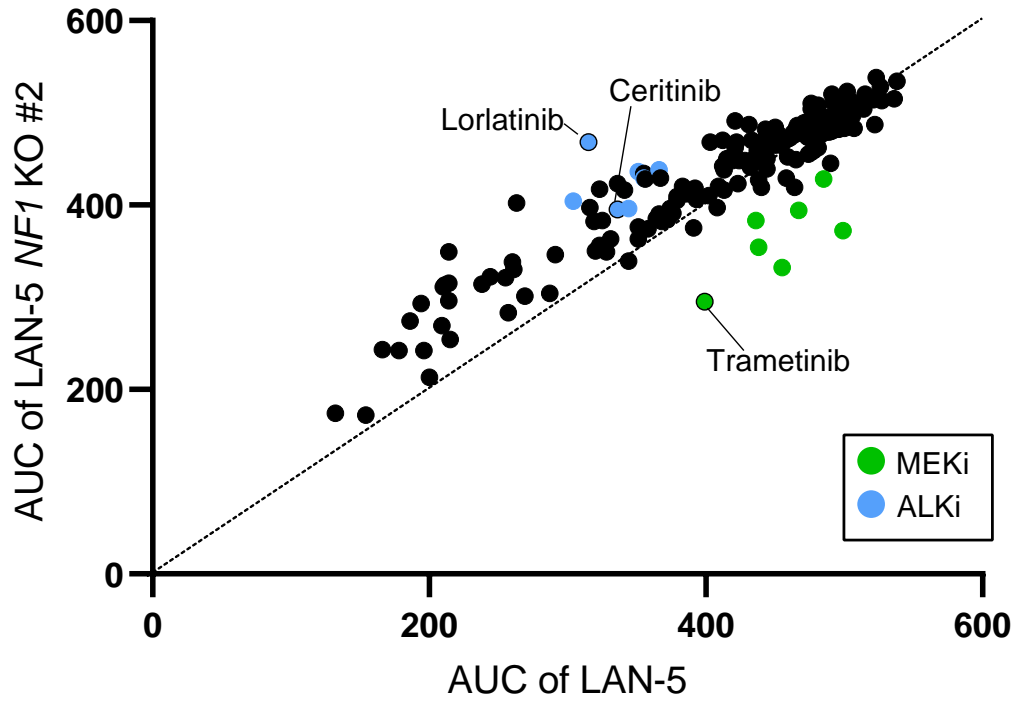
Figure S6

a

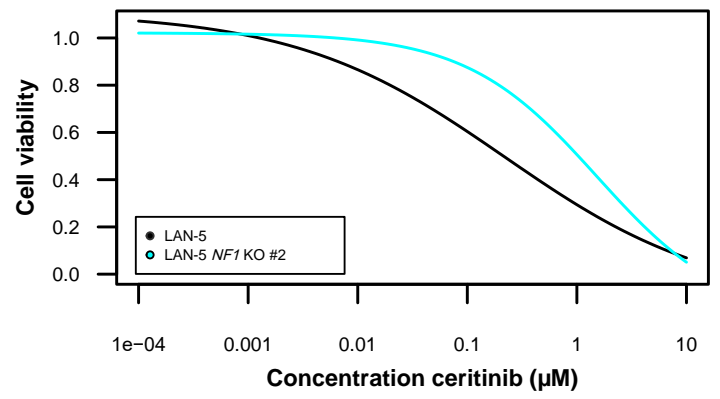
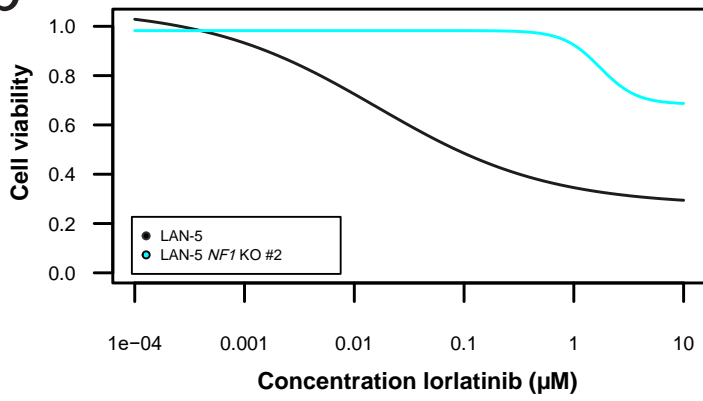


# Figure S7

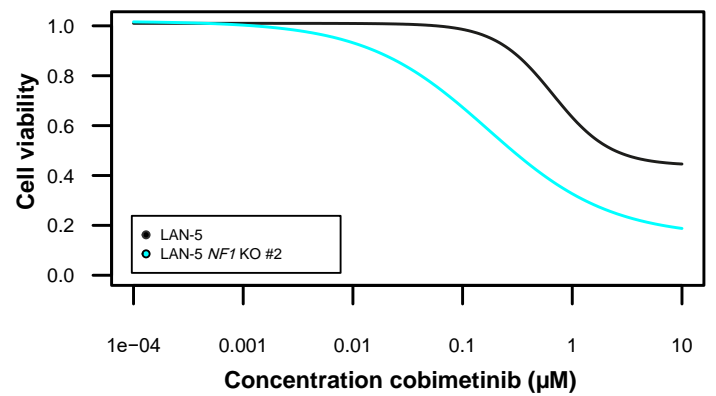
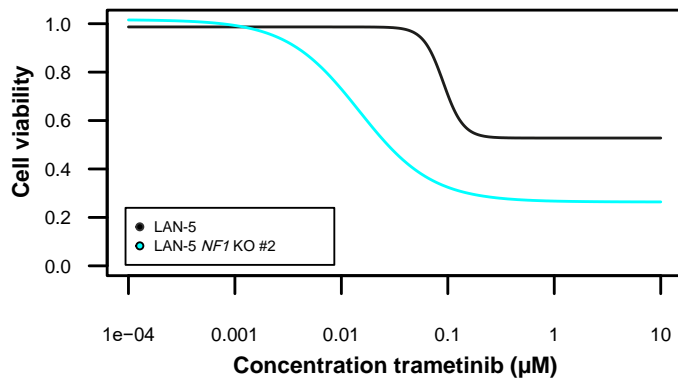
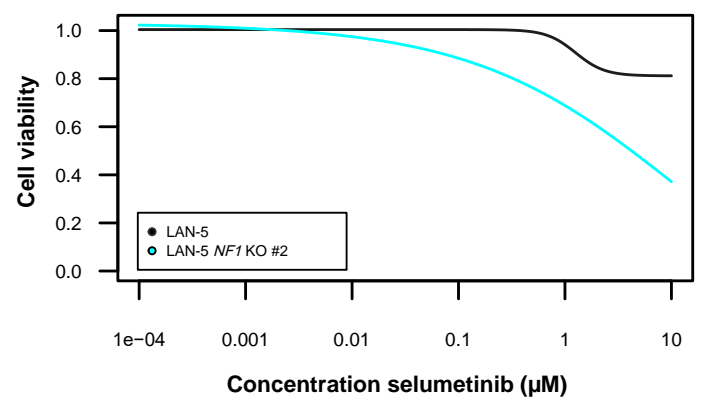
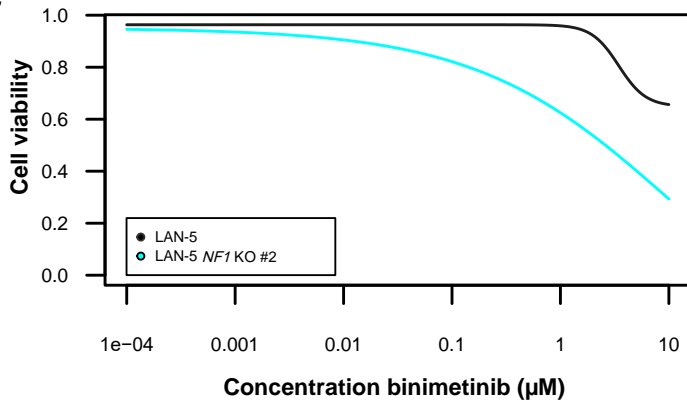
a



b



c



## SUPPLEMENTAL ITEMS TITLES AND LEGENDS

### Figure S1: ALK inhibitor treatment of *ALK*-mutated neuroblastoma cell lines for optimized screening conditions (related to Figure 1).

(a) Cell viabilities of different *ALK*-mutated neuroblastoma cell lines were assessed during a 72 hours ALK inhibitor treatment with ceritinib or lorlatinib to investigate its sensitivity to ALK inhibition; values represent mean  $\pm$  SD, n=3.

(b and c) Cell viability of the neuroblastoma cell line SH-SY5Y was determined during a 72 hours ALK inhibitor treatment with ceritinib or lorlatinib using an Incucyte® live-cell imaging system; values represent mean  $\pm$  SD, n=3.

### Figure S2: Quality control of CRISPR/Cas9 knockout screen (related to Figure 1).

(a and b) Correlation of normalized read counts per sgRNA between the two technical screen replicates for each ALKi respectively. Correlation coefficients (*r*) were calculated using Pearson correlation and tested using a two-tailed t-test; *p*<0.0001.

### Figure S3: Lorlatinib- and Ceritinib- resistant NBLW-R neuroblastoma cells grow as aggressive tumors in the kidney capsule of nude mice (related to Figure 3).

(a) ddPCR for NRAS c.181C>A in NBLW-R bulk population (left panel) and NBLW-R.L3 bulk population (right panel). Negative represented by black droplets; wild-type NRAS represented by green droplets; NRAS c.181C>A represented by blue droplets; double positive (mutant and wild-type) represented by orange droplets. Fractional abundance of NRAS c.181C>A = 35.5 % in NBLW-R.L3 cells.

(b) 10-day GI<sub>50</sub> of crizotinib in NBLW-R (178 nM) (left) and 5-day GI<sub>50</sub> of crizotinib in NBLW-R.LR (621.6 nM) and NBLW-R.CR (213.9 nM) (right).

(c) Survival curve of NBLW-R (n=6) kidney capsule tumor model versus NBLW-R.L2 (n=5) and NBLW-R.C1 (n=6), following injection of 1 million cells per animal at day 1. Survival NBLW-R versus NBLW-R.L2 *p*=0.0007\*\*\*, and NBLW-R versus NBLW-R.C1 *p*=0.0016\*\* according to log-rank (Mantel-Cox) test.

(d) Series of representative MR images of an untreated NBLW-R.C1 tumor (red outline).

(e) Survival curves of NBLW-R and NBLW-R.C1 kidney capsule tumor models treated with either ceritinib (50 mg/kg), lorlatinib (10 mg/kg) or vehicle control. NBLW-R lorlatinib versus vehicle *p*=0.0279\* according to log-rank (Mantel-Cox) test.

### Figure S4: Tetracycline induced NRAS<sup>Q61K</sup> expression (related to Figure 4).

(a) Tetracycline induced NRAS<sup>Q61K</sup> expression measured using qPCR after 72 hours of tetracycline exposure (2  $\mu$ g/ml). This qPCR was performed with an NRAS primer that detects wildtype NRAS and NRAS<sup>Q61K</sup>, values represent mean  $\pm$  SD, n=2, except for EV n=1.

### Figure S5: Computational modeling of ALK downstream signaling using STASNet (related to Figure 6).

(a) STASNet computational modeling of ALK downstream signaling. Shown are relative values of the path with separate scaling for LAN-5 and SH-SY5Y cell lines. Negative feedback is indicated in blue. *NF1* knockout models show a weaker ERK-RAF inhibitory feedback in comparison to the respective parental cell line.

(b) Measurement and quantification of 4 phosphoproteins after perturbation with ceritinib (ALKi), trametinib (MEKi), rapamycin (mTORi), pictilisib (PI3Ki) or DMSO and subsequent stimulation with EGF, IGF or PBS (carrier) of *NF1* knockout models and respective parental lines. Values are shown as log<sub>2</sub>(fold change) to PBS+DMSO control. *NF1* knockout cell lines show increased RAS-MAPK signaling in comparison to the respective parental cell line.

(c) Measurement and quantification of 4 phosphoproteins after perturbation with ceritinib (ALKi), lorlatinib (ALKi), trametinib (MEKi), a combination of ALKi and MEKi or DMSO and subsequent stimulation with EGF, IGF or PBS (carrier) of LAN-5 *NF1* knockout models and respective parental line. Values are shown as log<sub>2</sub>(fold change) to PBS+DMSO control. Cell lines show a similar response to different ALK inhibitors.



**Figure S6: MEK inhibitor treatment of ectopic NRAS<sup>Q61K</sup> expression models (related to Figure 7).**

(a) Cell viabilities of ectopic NRAS<sup>Q61K</sup> expression models were assessed after 72 hours of MEK inhibitor exposure with trametinib using an Incucyte® live-cell imaging system to investigate sensitivities to MEK inhibition; values represent mean  $\pm$  SD, n=3

**Figure S7: High-throughput drug screening of LAN-5 and LAN-5 *NF1* KO#2 clone (related to Figure 7)**

(a) Comparison of area under the curve (AUC) values derived from a high-throughput drug screen of the parental LAN-5 cell line and the LAN-5 *NF1* KO #2 clone using a compound library composed of 197 drugs. ALK inhibitors are highlighted in blue and MEK inhibitors are colored in green. A higher AUC value describes a less sensitive phenotype.

(b) Cell viabilities of parental LAN-5 and the LAN-5 *NF1* KO #2 clone were assessed after a 72 hour drug exposure to ALK inhibitors using the MTT assay as part of a high-throughput drug screen to investigate new collateral sensitivities of *NF1* KO cell lines.

(c) Cell viabilities of parental LAN-5 and the LAN-5 *NF1* KO #2 clone were assessed after a 72 hour drug exposure to MEK inhibitors using the MTT assay as part of a high-throughput drug screen to investigate new collateral sensitivities of *NF1* KO cell line

**Table S1. | Full clinical data on neuroblastoma patients**

	Patient #1	Patient #2	Patient #3	Patient #4
<b>Diagnosis</b>	stage 4 high risk NB	stage 4 high risk NB	stage 4 high risk NB	stage 4 high risk NB
<b>Primary therapy</b>	according to NB 2004 HR	according to NB 2004 HR	according to SIOPEN HR NBL	according to NB 2004 HR
<b>First relapse</b>	<p><i>15 months after diagnosis</i></p> <ul style="list-style-type: none"> <li>disseminated disease</li> </ul>	<p><i>63 months after diagnosis</i></p> <ul style="list-style-type: none"> <li>bone marrow metastasis</li> <li>Bone metastasis right Ulna</li> </ul>	<p><i>9 months after diagnosis</i></p> <ul style="list-style-type: none"> <li>metastatic skull site</li> </ul>	<p><i>18 months after diagnosis</i></p>
<b>Timepoint of first relapse biopsy</b>	<p><i>15 months after diagnosis</i></p> <ul style="list-style-type: none"> <li>Biopsy of first relapse in right adrenal gland</li> <li>panel sequencing</li> <li>– ALK p.F1174L (c.3522C&gt;A)</li> </ul>	<p><i>63 months after diagnosis</i></p> <ul style="list-style-type: none"> <li>panel sequencing</li> <li>– ALK p.R1275Q (c.3823C&gt;T)</li> <li>– MYCN p.P44L</li> </ul>	<p><i>9 months after diagnosis</i></p> <ul style="list-style-type: none"> <li>Biopsy: ALK p.R1275Q (c.3823C&gt;T)</li> </ul> <p><i>15 months after diagnosis</i></p> <p><i>Biopsy before Lorlatinib therapy</i></p> <ul style="list-style-type: none"> <li>Liquid biopsy on ctDNA</li> <li>– ALK p.R1275Q (c.3823C&gt;T)</li> </ul>	<p><i>58 months after diagnosis</i></p> <p>Tumor biopsy</p> <p>Analysed using whole-exome sequencing:</p> <ul style="list-style-type: none"> <li>ALK p.R1275Q (c.3823C&gt;T)</li> </ul>
<b>Salvage therapy before ALK targeted therapy</b>	<ul style="list-style-type: none"> <li>Anti-GD2 antibody therapy and chemotherapy</li> </ul>	<ul style="list-style-type: none"> <li>Therapy according to RIST protocol</li> </ul>	<p>Cyclophosphamide and topotecan</p> <p><i>stable disease</i></p>	<ul style="list-style-type: none"> <li>Therapy according to RIST protocol</li> <li>radiation 39.6Gy, Carboplatin, <sup>131</sup>I-mIBG therapy, High-dose chemotherapy regimes BuMel, autologous stem cell transplantation, anti-GD2 antibody (Dinutuximab) + interleukin 2 (IL2)+ 13-cis retinoic acid</li> <li>Metronomic therapy</li> <li>Metronomic therapy, radiation</li> </ul>

<b>ALK targeted therapy</b>	Ceritinib	Ceritinib	<p>12 months after diagnosis</p> <p>Ceritinib initial visible shrinkage</p> <p>15 months after diagnosis</p> <p>Lorlatinib evidence of tumour growth</p>	<p>41 months after diagnosis</p> <p>Ceritinib</p> <p>44 months after diagnosis</p> <ul style="list-style-type: none"> <li>Ribociclib, Ceritinib</li> <li>Ceritinib, Haplo stem cell transplantation, anti-GD2 antibody (Dinutuximab) + IT</li> </ul> <p>58 months after diagnosis</p> <ul style="list-style-type: none"> <li>Lorlatinib, Ribociclib, Temodal</li> <li>Lorlatinib, Temodal, MIBG</li> </ul>
<b>Timepoint of biopsy after ALK targeted therapy</b>	<p>22 months after diagnosis</p> <ul style="list-style-type: none"> <li>panel sequencing <ul style="list-style-type: none"> <li>ALK p.F1174L (c.3522C&gt;A)</li> <li>NF1 p.A320fs (c.960_961delTG)</li> <li>NF1 p.F1593S (c.4778T&gt;C)</li> </ul> </li> </ul>	<p>77 months after diagnosis</p> <ul style="list-style-type: none"> <li>panel sequencing <ul style="list-style-type: none"> <li>ALK p.R1275Q (c.3823C&gt;T)</li> <li>NF1 p.R1276*</li> <li>-MYCN p.P44L</li> </ul> </li> </ul>	<p>20 months after diagnosis</p> <ul style="list-style-type: none"> <li>Liquid biopsy on ctDNA <ul style="list-style-type: none"> <li>NRAS p.Q61K</li> <li>ALK p.R1275Q (c.3823C&gt;T)</li> </ul> </li> </ul>	<p>63 months after diagnosis</p> <p>Tumor biopsy:</p> <ul style="list-style-type: none"> <li>Analysed using whole-exome sequencing <ul style="list-style-type: none"> <li>ALK p.R1275Q (c.3823C&gt;T)</li> <li>HRAS p.Q61K</li> </ul> </li> </ul>
<b>Best response to ALK targeted therapy</b>	partial response	partial response	partial response	partial response
<b>Further therapy</b>	Therapy according to RIST protocol	Anti-GD2 antibody therapy and chemotehrapy	Trametinib and Debrafinib therapy	
<b>Further course of disease</b>	<p>27 months after diagnosis</p> <p>death</p>	<p>80 months after diagnosis</p> <p>death</p>	<p>22 months after diagnosis</p> <p>death</p>	<p>64 months after diagnosis</p> <p>death</p>