Supplementary Information

Supplementary Figures S1-S17

NVL-655 Is a Selective and Brain-Penetrant Inhibitor of Diverse ALK Mutant Oncoproteins, Including Lorlatinib-Resistant Compound Mutations

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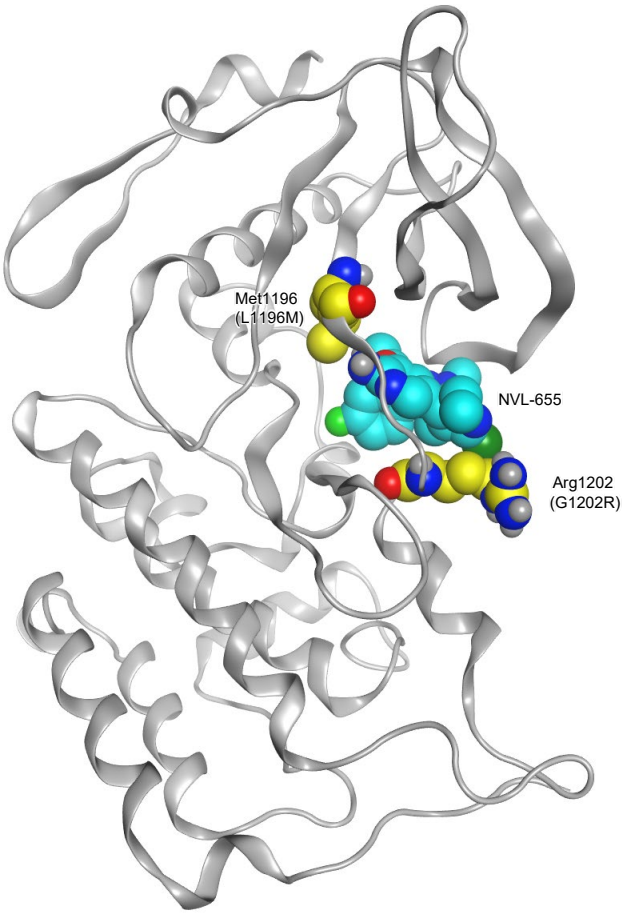
A

Ligand	NVL-655
X-ray source	PXII/X10SA (SLS)
Wavelength [Å]	1.0000
Detector	EIGER
Temperature [K]	100
Space group	P 2_1 2_1 2_1
Cell: a; b; c; [Å]	51.52; 57.20; 105.23
α; β; γ; [°]	90.0; 90.0; 90.0
Resolution [Å]	1.58 (1.61-1.58)
Unique reflections	43503 (2140)
Multiplicity	7.3 (6.9)
Completeness [%]	99.9 (100.0)
R_{pim} [%]	2.1 (53.4)
R_{sym} [%]	5.3 (131.9)
R_{meas} [%]	5.7 (142.5)
CC1/2 [%]	99.90 (75.10)
Mean(I)/sd	14.6 (1.3)
Refinement	
Resolution [Å]	52.61 - 1.58
Number of reflections (working /test)	39564 / 3937
Rcryst [%]	18.3
Rfree[%]	22.4
Total number of atoms:	
Protein	2539
Water	211
Ligand	32
Deviation from ideal geometry:	
Bond lengths [Å]	0.006
Bond angles [°]	1.455
Ramachandran plot	
Most favoured regions [%]	92.2%

B



C

Properties of NVL-655

Chemical formula	$C_{23}H_{22}ClFN_6O$
Molecular weight	452.921 g/mol
Calculated $logD_{7.4}$	4.4
Topological polar surface area	83.8 $Å^2$
Predicted basic pK_a	5.7
Number of H-bond donors	2
Number of H-bond acceptors	7
Number of rotatable bonds	1

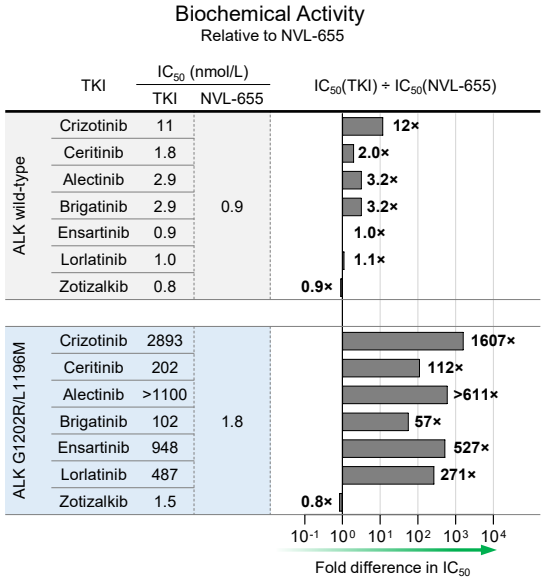
Supplementary Figure S1. Chemistry/X-ray A, Data collection and refinement statistics for structure determination of ALK G1202R/L1196M in complex with NVL-655. **B**, Same as Fig. 1B but shown as space-filling model for NVL-655 (cyan) and the mutated residues L1196M and G1202R (yellow). **C,** Properties of NVL-655. Calculator Plugins were used for structure property prediction and calculation, Marvin 19.20.0, 2019, ChemAxon (http://www.chemaxon.com): logD7.4, topological polar surface area, and predicted basic pKa.

A

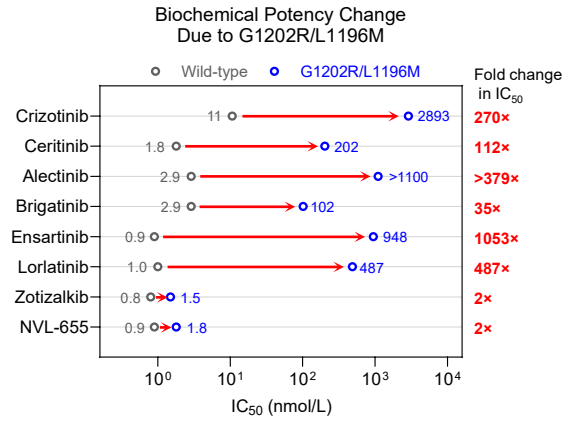
Biochemical Activity

	Crizotinib	Ceritinib	Alectinib	Brigatinib	Ensartinib	Lorlatinib	Zotizalkib	NVL-655
IC_{50} ALK wild-type	11 nM ×/÷ 1.6 (n=6)	1.8 nM ×/÷ 1.4 (n=5)	2.9 nM ×/÷ 2.1 (n=8)	2.9 nM ×/÷ 2.9 (n=8)	0.9 nM ×/÷ 1.5 (n=4)	1.0 nM ×/÷ 1.7 (n=7)	0.8 nM ×/÷ 2.0 (n=5)	0.9 nM ×/÷ 1.7 (n=5)
IC_{50} ALK G1202R/L1196M	2893 nM ×/÷ 1.3 (n=5)	202 nM ×/÷ 1.7 (n=5)	> 1100 nM (n=6)	102 nM ×/÷ 1.5 (n=6)	948 nM ×/÷ 1.9 (n=7)	487 nM ×/÷ 1.5 (n=11)	1.5 nM ×/÷ 1.3 (n=4)	1.8 nM ×/÷ 1.6 (n=7)

B



D



C

Biochemical Potency

ALK with single amino acid mutation (substitution or insertion)

IC_{50} (nM)	Crizotinib	Ceritinib	Alectinib	Brigatinib	Ensartinib	Lorlatinib	Zotizalkib	NVL-655
ALK T1151insT	ND	ND	6.9 (n=1)	ND	ND	1.6 ×/÷ 3.1 (n=3)	ND	1.5 ×/÷ 2.0 (n=3)
ALK T1151M	18 ×/÷ 1.4 (n=2)	3.6 ×/÷ 2.0 (n=2)	6.8 ×/÷ 1.5 (n=2)	4.1 ×/÷ 1.2 (n=2)	2.1 ×/÷ 1.2 (n=2)	2.4 ×/÷ 1.3 (n=4)	3.5 ×/÷ 1.3 (n=2)	1.5 ×/÷ 1.2 (n=4)
ALK L1152R	ND	ND	ND	ND	ND	4.7 ×/÷ 1.2 (n=2)	ND	2.5 ×/÷ 1.1 (n=2)
ALK C1156Y	ND	ND	5.8 (n=1)	ND	ND	0.7 ×/÷ 2.8 (n=3)	ND	1.0 ×/÷ 1.8 (n=3)
ALK I1171N	ND	ND	51 ×/÷ 1.1 (n=2)	27 ×/÷ 1.2 (n=2)	ND	18 ×/÷ 1.6 (n=2)	ND	3.4 ×/÷ 1.7 (n=2)
ALK I1171S	ND	ND	31 ×/÷ 1.0 (n=2)	21 ×/÷ 1.1 (n=2)	ND	18 ×/÷ 1.1 (n=2)	ND	2.3 ×/÷ 1.1 (n=2)
ALK I1171T	ND	ND	21 ×/÷ 1.1 (n=2)	1.8 ×/÷ 1.1 (n=2)	ND	16 ×/÷ 1.1 (n=2)	ND	3.6 ×/÷ 1.1 (n=2)
ALK F1174L	44 ×/÷ 1.1 (n=2)	4.2 ×/÷ 1.3 (n=2)	21 ×/÷ 1.7 (n=3)	11 ×/÷ 2.2 (n=3)	17 ×/÷ 1.0 (n=2)	2.3 ×/÷ 2.9 (n=6)	33 ×/÷ 1.6 (n=2)	1.1 ×/÷ 1.6 (n=5)
ALK F1174S	34 ×/÷ 1.2 (n=2)	8.9 ×/÷ 1.9 (n=2)	53 ×/÷ 1.3 (n=2)	5.2 ×/÷ 1.2 (n=2)	12 ×/÷ 1.3 (n=2)	3.8 ×/÷ 1.0 (n=2)	38 ×/÷ 1.3 (n=2)	1.1 ×/÷ 1.2 (n=2)
ALK V1180L	ND	ND	60 ×/÷ 1.0 (n=2)	0.7 ×/÷ 1.1 (n=2)	ND	2.6 ×/÷ 1.1 (n=2)	ND	0.87 ×/÷ 1.2 (n=2)
ALK L1196M	210 ×/÷ 1.1 (n=2)	1.5 ×/÷ 1.4 (n=2)	26 ×/÷ 1.1 (n=3)	11 ×/÷ 1.6 (n=2)	ND	20 ×/÷ 1.3 (n=2)	7.0 ×/÷ 1.0 (n=2)	11 ×/÷ 2.0 (n=3)
ALK L1198F	ND	ND	24 ×/÷ 1.5 (n=2)	64 ×/÷ 1.2 (n=2)	ND	32 ×/÷ 1.1 (n=2)	ND	0.91 ×/÷ 1.4 (n=4)
ALK G1202R	351 ×/÷ 1.2 (n=2)	110 ×/÷ 1.1 (n=2)	724 ×/÷ 1.1 (n=2)	137 ×/÷ 1.4 (n=2)	674 ×/÷ 1.1 (n=2)	51 ×/÷ 1.2 (n=5)	6.2 ×/÷ 1.4 (n=2)	0.9 ×/÷ 1.5 (n=3)
ALK D1203N	ND	ND	15 ×/÷ 1.1 (n=2)	13 ×/÷ 1.1 (n=2)	ND	15 ×/÷ 1.4 (n=2)	ND	6.8 ×/÷ 1.3 (n=2)
ALK S1206R	ND	ND	29 (n=1)	ND	ND	1.4 ×/÷ 2.1 (n=3)	ND	1.8 ×/÷ 1.8 (n=3)
ALK G1269A	ND	ND	27 (n=1)	ND	ND	7.8 ×/÷ 2.0 (n=3)	ND	16 ×/÷ 1.6 (n=3)
ALK G1269S	ND	ND	46 (n=1)	ND	ND	67 ×/÷ 2.5 (n=3)	ND	79 ×/÷ 1.2 (n=3)
ALK R1275Q	17 ×/÷ 1.0 (n=2)	1.2 ×/÷ 1.1 (n=2)	25 ×/÷ 1.1 (n=2)	8.0 ×/÷ 2.0 (n=2)	3.1 ×/÷ 1.2 (n=2)	1.8 ×/÷ 1.3 (n=2)	7.3 ×/÷ 1.1 (n=2)	0.8 ×/÷ 1.5 (n=2)

Supplementary Figure S2. Biochemical activity of ALK TKIs. A, Biochemical activity against ALK and ALK G1202R/L1196M. Geometric mean ×/÷ standard deviation (number of repeats) is shown. **B,** Potency of 7 TKIs relative to NVL-655, calculated by dividing IC_{50}(TKI) by IC_{50}(NVL-655) in each row. IC_{50} values are from Panel A. "Relative potency >" is treated as "relative potency =" for graph plotting. **C,** Activity against ALK containing single amino acid substitution or insertion, shown as geometric mean ×/÷ standard deviation (number of repeats). ND, not determined. **D,** Plot showing the IC_{50} of each TKI against ALK (gray) and ALK G1202R/L1196M (blue) taken from Panel A, along with the associated fold change in IC_{50}. Red indicates an increase in IC_{50}. "IC_{50} >" is treated as "IC_{50} =" for plotting.

A

335 Kinases in the Kinome Panel

ABL1	CDK1/CycE1	CSK	FGR	MAP3K10	NEK2	PKMzeta	STK17A
ABL2	CDK12/CycK	DAPK1	FLT3	MAP3K11	NEK3	PKN3	STK23
ACK1	CDK13/CycK	DAPK2	FRK	MAP3K7/MAP3K7IP1	NEK4	PLK1	STK25
ACVR1	CDK16/CycY	DAPK3	FYN	MAP3K9	NEK6	PLK3	STK33
ACVR1B	CDK17/p35NCK	DCAMKL2	GRK2	MAP4K2	NEK7	PRK1	STK39
ACVR2A	CDK18/CycY	DDR2	GRK3	MAP4K4	NEK9	PRK2	SYK
ACVR2B	CDK19/CycC	DMPK	GRK4	MAP4K5	NIK	PRKD2	TAOK2
ACVRL1	CDK2/CycA2	DNAPK	GRK5	MAPKAPK2	NLK	PRKG1	TAOK3
AKT1	CDK2/CycD1	DYRK1A	GRK6	MAPKAPK3	p38alpha	PRKG2	TBK1
AKT2	CDK2/CycE1	DYRK1B	GRK7	MAPKAPK5	p38beta	PRKX	TEC
AKT3	CDK20/CycH	DYRK2	GSG2	MARK1	p38delta	PYK2	TGFBR1
ALK	CDK20/CycT1	DYRK3	GSK3alpha	MARK2	p38gamma	RAF1 YDYD	TGFBR2
AMPKalpha1	CDK3/CycC	DYRK4	GSK3beta	MARK3	PAK1	RET	TIE2
ARK5	CDK3/CycE1	EEF2K	HCK	MARK4	PAK2	RIPK2	TLK1
ASK1	CDK4/CycD1	EGFR	HIPK1	MASTL	PAK3	RIPK4	TLK2
AuroraA	CDK4/CycD2	EIF2AK2	HIPK2	MATK	PAK4	RIPK5	TNK1
AuroraB	CDK4/CycD3	EIF2AK3	HIPK3	MEK1	PAK6	ROCK1	TRKA
AuroraC	CDK5/p25NCK	EPHA1	HIPK4	MEK2	PAK7	ROCK2	TRKB
AXL	CDK5/p35NCK	EPHA2	HRI	MEK5	PASK	RON	TRKC
BLK	CDK6/CycD1	EPHA3	IGF1R	MEKK2	PBK	ROS	TSF1
BMPR1A	CDK6/CycD2	EPHA4	IKKalpha	MEKK3	PDGFRalpha	RPS6KA1	TSK2
BMPR1B	CDK6/CycD3	EPHA5	IKKbeta	MELK	PDGFRbeta	RPS6KA2	TSSK1
BMX	CDK7/CycH/MAT1	EPHA6	IKKepsilon	MERTK	PDK1	RPS6KA3	TTBK1
BRAF	CDK8/CycC	EPHA7	INSR	MET	PHKG1	RPS6KA4	TTBK2
BRK	CDK9/CycK	EPHA8	INSRR	MINK1	PHKG2	RPS6KA5	TTK
BRSK1	CDK9/CycT1	EPHB1	IRAK1	MKK4	PIM1	RPS6KA6	TXK
BRSK2	CHK1	EPHB2	IRAK4	MKK6 SDTD	PIM2	S6K	TYK2
BTK	CHK2	EPHB3	ITK	MKK7	PIM3	S6Kbeta	TYRO3
BUB1B	CK1alpha1	EPHB4	JAK1	MKNK1	PKA	SAK	ULK2
CAMK1D	CK1delta	ERBB2	JAK2	MKNK2	PKCalpha	SGK1	VEGFR1
CAMK2A	CK1epsilon	ERBB4	JAK3	MLK4	PKCbeta1	SGK2	VEGFR2
CAMK2B	CK1gamma1	ERK1	JNK1	MST1	PKCbeta2	SGK3	VEGFR3
CAMK2D	CK1gamma2	ERK2	JNK2	MST2	PKCdelta	SIK1	VRK1
CAMK2G	CK1gamma3	ERK5	JNK3	MST3	PKCepsilon	SIK2	VRK2
CAMK4	CK2alpha1	ERK7	KIT	MST4	PKCeta	SIK3	WEE1
CAMKK1	CK2alpha2	FAK	LCK	MTOR	PKCgamma	SLK	WNK1
CAMKK2	CLK1	FER	LIMK1	MUSK	PKCiota	SNARK	WNK2
CDC42BPA	CLK2	FES	LIMK2	MYLK	PKCmu	SNK	WNK3
CDC42BPB	CLK3	FGFR1	LRRK2	MYLK2	PKCnu	SRC	YES
CDC7/DBF4	CLK4	FGFR2	LTK	MYLK3	PKCtheta	SRMS	ZAK
CDK1/CycA2	COT	FGFR3	LYN	NEK1	PKCzeta	SRPK1	ZAP70
CDK1/CycB1	CSF1R	FGFR4	MAP3K1	NEK11	PKMYT1	SRPK2	

B

Top 31 Hits from the Screen

Kinase	IC_{50} (nM)	Selectivity window	
ALK	2.8	*1-fold (baseline)*	≤ 1-fold
ROS1	1.2	0.41-fold	
LTK	9.0	3.2-fold	1-fold to 10-fold
PYK2	14	4.8-fold	
TRKB	18	6.3-fold	
FAK	21	7.2-fold	
SLK	44	15-fold	10-fold to 50-fold
TRKA	53	19-fold	
FER	58	20-fold	
MUSK	90	32-fold	
EPHA6	115	40-fold	
TRKC	138	48-fold	
ACK1	170	60-fold	
INSR	190	67-fold	
BRK	235	83-fold	
RIPK5	250	88-fold	
FES	415	146-fold	
IGF1R	455	160-fold	
CSF1R	460	162-fold	
TIE2	460	162-fold	
EPHA7	475	167-fold	
EPHB1	485	171-fold	
EPHA4	510	180-fold	
EPHA2	525	185-fold	
SAK	565	199-fold	
EPHA5	635	224-fold	
EPHA1	640	225-fold	
INSRR	655	231-fold	
MAP4K5	795	280-fold	
FRK	895	315-fold	
IRAK1	1025	361-fold	
304 kinases	> 1000	> 352-fold	

Supplementary Figure S3. Kinome profiling of NVL-655. A, Table listing 335 kinases evaluated in this screen (Reaction Biology, Germany). The screen was performed at ATP concentrations near the K_m of each kinase. **B,** Selectivity window for NVL-655 (= IC_{50} for each kinase ÷ IC_{50} for ALK) of the 31 most strongly inhibited kinases from the screen.

Potency (IC_{50}, nmol/L) in Cell Viability Assay

Cell Name	Fusion	Mutation	Crizotinib	Ceritinib	Alectinib	Brigatinib	Ensartinib	Lorlatinib	Zotizalkib	NVL-655
MGH048-1	EML4-ALK v1	—	187.8 ×/÷ 1.1 (n=3)	34.6 ×/÷ 1.3 (n=3)	32.5 ×/÷ 1.3 (n=3)	10.9 ×/÷ 1.7 (n=3)	ND	2.2 ×/÷ 1.3 (n=3)	ND	0.8 ×/÷ 1.2 (n=3)
NCI-H3122	EML4-ALK v1	—	177 ×/÷ 1.24 (n=5)	35.3 ×/÷ 1.67 (n=6)	23.2 ×/÷ 1.41 (n=4)	21.3 ×/÷ 1.19 (n=4)	21.5 ×/÷ 1.56 (n=5)	3.78 ×/÷ 1.33 (n=7)	128 ×/÷ 1.17 (n=2)	2.25 ×/÷ 1.34 (n=6)
Ba/F3	EML4-ALK v1	—	217 ×/÷ 1.79 (n=6)	77.4 ×/÷ 1.37 (n=5)	24.8 ×/÷ 2.09 (n=7)	36.4 ×/÷ 1.51 (n=10)	17.8 ×/÷ 1.76 (n=4)	< 3.64 ×/÷ 1.74 (n=24)	19.2 ×/÷ 1.66 (n=3)	1.62 ×/÷ 1.90 (n=3)
MGH064-1	EML4-ALK v2	—	24.8 ×/÷ 1.4 (n=3)	3.4 ×/÷ 1.2 (n=3)	4.7 ×/÷ 1.2 (n=3)	2.1 ×/÷ 1.3 (n=3)	ND	0.6 ×/÷ 1.1 (n=3)	ND	0.3 ×/÷ 1.3 (n=3)
MGH026-1	EML4-ALK v3	—	318.5 ×/÷ 1.5 (n=3)	79.7 ×/÷ 1.2 (n=3)	79.9 ×/÷ 1.3 (n=3)	12.1 ×/÷ 1.2 (n=3)	ND	2.9 ×/÷ 1.2 (n=3)	ND	1.6 ×/÷ 1.2 (n=3)
NCI-H2228	EML4-ALK v3	—	90.0 ×/÷ 1.11 (n=3)	52.0 ×/÷ 1.33 (n=4)	13.4 ×/÷ 1.71 (n=3)	12.5 ×/÷ 1.45 (n=7)	8.77 ×/÷ 1.76 (n=3)	< 1.02 ×/÷ 1.51 (n=11)	5.48 ×/÷ 1.24 (n=2)	< 0.645 ×/÷ 1.21 (n=6)
Ba/F3	EML4-ALK v3	—	22.4 ×/÷ 1.3 (n=3)	10.7 ×/÷ 1.3 (n=3)	19.8 ×/÷ 1.4 (n=3)	7 ×/÷ 1.4 (n=3)	ND	2 ×/÷ 1.3 (n=4)	ND	1.1 ×/÷ 1.2 (n=4)
Karpas299	NPM1-ALK	—	56.9 ×/÷ 1.28 (n=4)	25.2 ×/÷ 1.28 (n=3)	19.6 ×/÷ 1.43 (n=5)	8.25 ×/÷ 1.74 (n=5)	10.5 ×/÷ 1.28 (n=4)	3.27 ×/÷ 1.23 (n=5)	9.85 ×/÷ 1.25 (n=2)	1.95 ×/÷ 1.20 (n=2)
Average potency, wild-type ALK fusion (8 cell lines)			94.8 ×/÷ 2.7 (n=8)	27.8 ×/÷ 2.9 (n=8)	20.9 ×/÷ 2.2 (n=8)	10.6 ×/÷ 2.3 (n=8)	ND	< 2.1 ×/÷ 1.9 (n=8)	ND	< 1.1 ×/÷ 2 (n=8)
Ba/F3	EML4-ALK v1	G1202R	694 ×/÷ 1.52 (n=6)	607 ×/÷ 1.26 (n=6)	893 ×/÷ 1.76 (n=8)	333 ×/÷ 1.51 (n=8)	619 ×/÷ 2.48 (n=5)	54.2 ×/÷ 2.00 (n=44)	9.45 ×/÷ 1.55 (n=3)	< 0.729 ×/÷ 1.61 (n=3)
MGH953-4	EML4-ALK v3	G1202R	1872.2 ×/÷ 1.4 (n=3)	316.7 ×/÷ 2.1 (n=3)	1235.4 ×/÷ 2 (n=3)	58.2 ×/÷ 2.7 (n=3)	ND	56.6 ×/÷ 1.9 (n=3)	ND	0.8 ×/÷ 1.6 (n=3)
MGH9037-2	EML4-ALK v3	G1202R	331.4 ×/÷ 2 (n=3)	71.1 ×/÷ 1.6 (n=3)	384.5 ×/÷ 3 (n=3)	34.9 ×/÷ 1.2 (n=3)	ND	15.5 ×/÷ 1.6 (n=3)	ND	0.1 ×/÷ 1.3 (n=3)
YU-1077	EML4-ALK v3	G1202R	362.9 ×/÷ 1 (n=2)	170.4 ×/÷ 1 (n=2)	377.6 ×/÷ 1 (n=2)	69.1 ×/÷ 1.2 (n=2)	231.1 ×/÷ 1.1 (n=2)	9.2 ×/÷ 1.3 (n=2)	ND	0.2 ×/÷ 1 (n=2)
Ba/F3	EML4-ALK v3	G1202R	134.8 ×/÷ 1.1 (n=3)	137.8 ×/÷ 1.2 (n=3)	384.4 ×/÷ 1.1 (n=3)	100.2 ×/÷ 1.2 (n=3)	ND	53 ×/÷ 1.2 (n=5)	ND	0.4 ×/÷ 1.1 (n=5)
Average potency, ALK G1202R single mutation (5 cell lines)			462.1 ×/÷ 2.7 (n=5)	200.1 ×/÷ 2.3 (n=5)	572.6 ×/÷ 1.8 (n=5)	85.9 ×/÷ 2.3 (n=5)	ND	29.7 ×/÷ 2.3 (n=5)	ND	< 0.3 ×/÷ 2.4 (n=5)
Ba/F3	EML4-ALK v3	T1151M	19.4 ×/÷ 1.2 (n=3)	12.7 ×/÷ 1.2 (n=3)	11.2 ×/÷ 1.1 (n=3)	3.7 ×/÷ 1 (n=3)	ND	1.8 ×/÷ 1.1 (n=4)	ND	1.6 ×/÷ 1.2 (n=4)
Ba/F3	EML4-ALK v1	T1151insT	571 ×/÷ 1.37 (n=3)	267 ×/÷ 1.60 (n=3)	132 ×/÷ 1.03 (n=2)	109 ×/÷ 1.16 (n=2)	112 ×/÷ 1.18 (n=3)	19.8 ×/÷ 1.19 (n=2)	52.4 ×/÷ 1.14 (n=2)	6.68 ×/÷ 1.22 (n=2)
Ba/F3	EML4-ALK v1	C1156Y	231 ×/÷ 1.06 (n=2)	177 ×/÷ 1.38 (n=3)	48.3 ×/÷ 1.42 (n=2)	83.6 ×/÷ 1.09 (n=2)	21.7 ×/÷ 1.36 (n=2)	6.29 ×/÷ 1.34 (n=3)	11.7 ×/÷ 1.04 (n=2)	4.45 ×/÷ 1.11 (n=3)
Ba/F3	EML4-ALK v1	I1171N	365 ×/÷ 1.34 (n=4)	135 ×/÷ 2.23 (n=5)	602 ×/÷ 1.58 (n=5)	80.9 ×/÷ 1.33 (n=4)	40.9 ×/÷ 1.80 (n=3)	53.1 ×/÷ 1.57 (n=61)	303 ×/÷ 1.17 (n=3)	26.7 ×/÷ 1.66 (n=4)
Ba/F3	EML4-ALK v1	I1171S	459 ×/÷ 1.59 (n=3)	102 ×/÷ 1.68 (n=3)	281 ×/÷ 1.76 (n=3)	29.8 ×/÷ 2.48 (n=3)	60.4 ×/÷ 1.25 (n=2)	56.1 ×/÷ 1.37 (n=11)	251 ×/÷ 1.31 (n=2)	28.5 ×/÷ 1.30 (n=2)
Ba/F3	EML4-ALK v1	I1171T	559 ×/÷ 1.80 (n=3)	106 ×/÷ 1.71 (n=3)	191 ×/÷ 1.67 (n=3)	24.1 ×/÷ 2.17 (n=3)	85.9 ×/÷ 1.26 (n=2)	54.8 ×/÷ 1.53 (n=14)	316 ×/÷ 1.01 (n=2)	35.3 ×/÷ 1.23 (n=2)
Ba/F3	EML4-ALK v3	F1174L	24.4 ×/÷ 1.2 (n=3)	13.8 ×/÷ 1.3 (n=3)	18.3 ×/÷ 1.4 (n=3)	9.2 ×/÷ 1.3 (n=3)	ND	2.6 ×/÷ 1.2 (n=4)	ND	1.3 ×/÷ 1.2 (n=4)
Ba/F3	EML4-ALK v1	V1180L	99.1 ×/÷ 1.03 (n=2)	39.8 ×/÷ 1.29 (n=2)	602 ×/÷ 1.17 (n=2)	12.7 ×/÷ 1.15 (n=2)	6.60 ×/÷ 1.02 (n=2)	1.78 ×/÷ 1.01 (n=2)	59.5 ×/÷ 1.06 (n=2)	0.868 ×/÷ 1.04 (n=2)
MGH045-1	EML4-ALK v1	L1196M	1024.1 ×/÷ 1.9 (n=3)	26.8 ×/÷ 1.8 (n=3)	121.3 ×/÷ 1.8 (n=3)	28 ×/÷ 1.6 (n=3)	ND	41.8 ×/÷ 1.2 (n=3)	ND	24.7 ×/÷ 1.4 (n=3)
Ba/F3	EML4-ALK v1	L1196M	1090 ×/÷ 1.21 (n=3)	79.1 ×/÷ 1.61 (n=3)	118 ×/÷ 1.16 (n=3)	99.7 ×/÷ 1.57 (n=5)	132 ×/÷ 1.03 (n=2)	68.6 ×/÷ 2.06 (n=20)	19.2 ×/÷ 1.28 (n=2)	29.3 ×/÷ 1.09 (n=3)
Ba/F3	EML4-ALK v1	L1196Q	432 ×/÷ 1.08 (n=2)	150 ×/÷ 1.55 (n=3)	880 ×/÷ 1.01 (n=2)	33.7 ×/÷ 1.00 (n=2)	21.6 ×/÷ 1.57 (n=3)	19.2 ×/÷ 1.05 (n=2)	14.1 ×/÷ 1.21 (n=3)	9.75 ×/÷ 1.12 (n=2)
Ba/F3	EML4-ALK v1	L1198F	11.9 ×/÷ 1.22 (n=2)	> 1000 ×/÷ 1.00 (n=2)	137 ×/÷ 1.42 (n=3)	97.1 ×/÷ 1.59 (n=4)	2.11 (n=1)	13.9 ×/÷ 1.35 (n=3)	3.21 ×/÷ 1.41 (n=3)	1.89 ×/÷ 1.51 (n=2)
Ba/F3	EML4-ALK v1	G1202del	95.5 ×/÷ 1.17 (n=2)	273 ×/÷ 1.76 (n=3)	186 ×/÷ 1.36 (n=3)	122 ×/÷ 1.23 (n=3)	79.4 ×/÷ 1.34 (n=2)	3.89 ×/÷ 1.45 (n=2)	13.5 ×/÷ 1.04 (n=2)	4.24 ×/÷ 1.20 (n=2)
Ba/F3	EML4-ALK v1	D1203N	414 ×/÷ 1.00 (n=2)	310 ×/÷ 1.16 (n=2)	73.4 ×/÷ 1.18 (n=3)	76.3 ×/÷ 1.41 (n=2)	29.5 ×/÷ 2.14 (n=3)	17.6 ×/÷ 1.63 (n=13)	59.6 ×/÷ 1.65 (n=2)	22.9 ×/÷ 1.33 (n=4)
Ba/F3	EML4-ALK v1	S1206F	102 ×/÷ 1.03 (n=3)	67.0 ×/÷ 1.39 (n=3)	35.6 ×/÷ 1.89 (n=3)	90.5 ×/÷ 1.01 (n=2)	22.2 ×/÷ 1.12 (n=2)	1.61 ×/÷ 1.15 (n=2)	12.3 ×/÷ 1.11 (n=2)	1.28 ×/÷ 1.15 (n=2)
Ba/F3	EML4-ALK v1	S1206Y	102 ×/÷ 1.12 (n=2)	70.9 ×/÷ 1.65 (n=3)	36.3 ×/÷ 1.61 (n=3)	88.7 ×/÷ 1.00 (n=2)	29.4 ×/÷ 1.47 (n=3)	2.20 ×/÷ 1.16 (n=2)	14.4 ×/÷ 1.08 (n=2)	2.27 ×/÷ 1.75 (n=3)
Ba/F3	EML4-ALK v1	E1210K	113 ×/÷ 1.08 (n=3)	108 ×/÷ 1.30 (n=3)	66.7 ×/÷ 1.60 (n=2)	191 ×/÷ 1.09 (n=2)	64.2 ×/÷ 1.19 (n=3)	1.02 ×/÷ 1.28 (n=2)	7.41 ×/÷ 1.01 (n=2)	0.625 ×/÷ 1.01 (n=2)
Ba/F3	EML4-ALK v1	G1269A	408 ×/÷ 1.06 (n=3)	70.5 ×/÷ 1.27 (n=3)	114 ×/÷ 1.33 (n=3)	31.2 ×/÷ 1.11 (n=2)	66.0 ×/÷ 1.20 (n=3)	21.0 ×/÷ 1.02 (n=2)	22.8 ×/÷ 1.15 (n=2)	15.9 ×/÷ 1.02 (n=2)
MR448re	EML4-ALK v3	G1202R/T1151M	727.4 ×/÷ 1.4 (n=2)	688.7 ×/÷ 1.2 (n=2)	815.4 ×/÷ 1.3 (n=2)	276.9 ×/÷ 1.6 (n=2)	ND	369.5 ×/÷ 2.4 (n=2)	ND	0.1 ×/÷ 1.9 (n=2)
Ba/F3	EML4-ALK v3	G1202R/T1151M	223 ×/÷ 1.4 (n=4)	309.3 ×/÷ 1.4 (n=4)	563.6 ×/÷ 1.1 (n=4)	124.6 ×/÷ 1.5 (n=4)	ND	183.8 ×/÷ 1.5 (n=7)	ND	2.1 ×/÷ 1.9 (n=7)
Ba/F3	EML4-ALK v3	G1202R/F1174L	149.1 ×/÷ 1.1 (n=3)	238.2 ×/÷ 1.1 (n=3)	461 ×/÷ 1.2 (n=3)	332.5 ×/÷ 1.7 (n=3)	ND	101.5 ×/÷ 1.2 (n=4)	ND	0.5 ×/÷ 1.4 (n=4)
NCI-H3122	EML4-ALK v1	G1202R/L1196M	1600 ×/÷ 1.50 (n=2)	198 ×/÷ 2.72 (n=2)	1130 ×/÷ 3.40 (n=2)	126 ×/÷ 2.01 (n=2)	2160 ×/÷ 1.81 (n=2)	1300 ×/÷ 1.30 (n=2)	ND	5.68 ×/÷ 1.35 (n=2)
Ba/F3	EML4-ALK v1	G1202R/L1196M	1330 ×/÷ 1.23 (n=5)	1370 ×/÷ 1.11 (n=5)	> 2340 ×/÷ 2.73 (n=6)	811 ×/÷ 1.82 (n=42)	2700 ×/÷ 1.19 (n=2)	3530 ×/÷ 1.51 (n=50)	11.6 ×/÷ 1.76 (n=2)	7.25 ×/÷ 1.27 (n=8)
MGH953-7	EML4-ALK v3	G1202R/L1196M	3715.3 ×/÷ 1.2 (n=3)	365.2 ×/÷ 1.4 (n=3)	1565 ×/÷ 1.5 (n=3)	100.5 ×/÷ 2.1 (n=3)	ND	344.1 ×/÷ 2.8 (n=3)	ND	1.8 ×/÷ 1.5 (n=3)
Ba/F3	EML4-ALK v1	G1202R/L1198F	166 ×/÷ 1.22 (n=2)	1290 ×/÷ 1.06 (n=2)	2150 ×/÷ 1.21 (n=2)	474 ×/÷ 1.16 (n=2)	202 ×/÷ 1.60 (n=2)	580 ×/÷ 1.46 (n=5)	2.78 ×/÷ 1.06 (n=2)	2.00 ×/÷ 1.29 (n=3)
Ba/F3	EML4-ALK v1	G1202R/G1269A	1050 ×/÷ 1.07 (n=3)	352 ×/÷ 1.22 (n=3)	1340 ×/÷ 1.47 (n=3)	241 ×/÷ 1.38 (n=6)	1670 ×/÷ 1.27 (n=2)	899 ×/÷ 1.41 (n=10)	28.2 ×/÷ 1.09 (n=2)	3.01 ×/÷ 1.56 (n=3)
Average potency, ALK G1202R compound mutation (8 cell lines)			653.7 ×/÷ 3.3 (n=8)	467.5 ×/÷ 2.1 (n=8)	>1122.8 ×/÷ 1.8 (n=8)	246.1 ×/÷ 2.1 (n=8)	ND	523.9 ×/÷ 3.1 (n=8)	ND	1.6 ×/÷ 4 (n=8)
Ba/F3	EML4-ALK v1	I1171N/L1198F	30.8 ×/÷ 1.40 (n=2)	1100 ×/÷ 1.12 (n=2)	199 ×/÷ 1.62 (n=2)	29.5 ×/÷ 1.23 (n=2)	2.99 ×/÷ 1.52 (n=2)	183 ×/÷ 1.37 (n=30)	30.1 ×/÷ 1.30 (n=2)	15.7 ×/÷ 1.36 (n=3)
Ba/F3	EML4-ALK v1	I1171N/D1203N	759 ×/÷ 1.65 (n=5)	695 ×/÷ 1.82 (n=5)	2110 ×/÷ 1.57 (n=5)	1310 ×/÷ 1.42 (n=7)	81.8 ×/÷ 1.92 (n=3)	599 ×/÷ 1.58 (n=99)	1590 ×/÷ 1.44 (n=7)	341 ×/÷ 1.35 (n=7)
Kelly	—	F1174L	98.0 ×/÷ 1.22 (n=2)	64.8 ×/÷ 1.38 (n=4)	68.5 ×/÷ 1.41 (n=3)	34.8 ×/÷ 1.10 (n=2)	26.4 ×/÷ 1.39 (n=3)	8.15 ×/÷ 1.40 (n=5)	9.47 ×/÷ 1.79 (n=2)	9.11 ×/÷ 1.28 (n=5)
SH-SY5Y	—	F1174L	226 ×/÷ 1.47 (n=2)	124 ×/÷ 1.05 (n=2)	190 ×/÷ 1.33 (n=2)	262 ×/÷ 1.03 (n=2)	78.9 ×/÷ 1.20 (n=2)	31.4 ×/÷ 1.82 (n=3)	23.6 ×/÷ 1.67 (n=2)	19.0 ×/÷ 2.07 (n=3)
NB-1	—	Amp, Ex2–3del	22.0 ×/÷ 1.24 (n=2)	12.3 ×/÷ 1.20 (n=2)	11.4 ×/÷ 1.13 (n=2)	6.92 ×/÷ 1.14 (n=2)	5.06 ×/÷ 1.01 (n=2)	2.62 ×/÷ 1.22 (n=3)	5.74 ×/÷ 1.03 (n=2)	1.99 ×/÷ 1.27 (n=3)
Aska-SS	—	Ex2–17del	43.7 ×/÷ 1.54 (n=3)	29.1 ×/÷ 1.01 (n=2)	26.8 ×/÷ 1.84 (n=3)	12.7 ×/÷ 1.12 (n=2)	16.2 ×/÷ 1.47 (n=3)	6.73 ×/÷ 1.08 (n=3)	34.7 ×/÷ 1.38 (n=2)	6.96 ×/÷ 1.32 (n=3)

Supplementary Figure S4. Potency in cell viability assay. Cell viability IC_{50} (nmol/L) is shown as geometric mean ×/÷ standard deviation (number of repeats). Average potencies, highlighted in blue, reflect the geometric mean across the cell lines within each category (wild-type kinase domain, G1202R single mutants, or G1202R compound mutants). ND, not determined.

Maximal Effect (E_{max}, %) in Cell Viability Assay

Cell Name	Fusion	Mutation	Crizotinib	Ceritinib	Alectinib	Brigatinib	Ensartinib	Lorlatinib	Zotizalkib	NVL-655
MGH048-1	EML4-ALK v1	—	105.4 [99.54 to 111.6]	106.9 [99.40 to ???]	108.7 [101.2 to 117.0]	104.5 [94.97 to 114.4]	ND	100.9 [92.78 to 109.2]	ND	105.7 [97.57 to 115.1]
NCI-H3122	EML4-ALK v1	—	70.5 ×/÷ 1.13 (n=5)	78.1 ×/÷ 1.10 (n=6)	70.6 ×/÷ 1.09 (n=4)	75.1 ×/÷ 1.11 (n=4)	67.0 ×/÷ 1.06 (n=5)	71.2 ×/÷ 1.11 (n=7)	82.9 ×/÷ 1.03 (n=2)	68.9 ×/÷ 1.15 (n=6)
Ba/F3	EML4-ALK v1	—	99.9 ×/÷ 1.00 (n=6)	100 ×/÷ 1.00 (n=5)	99.6 ×/÷ 1.01 (n=7)	99.8 ×/÷ 1.00 (n=10)	99.5 ×/÷ 1.01 (n=4)	99.8 ×/÷ 1.00 (n=24)	99.9 ×/÷ 1.00 (n=3)	99.3 ×/÷ 1.01 (n=3)
MGH064-1	EML4-ALK v2	—	100.2 [96.35 to 104.2]	98.09 [94.03 to 102.4]	96.99 [94.01 to 100.1]	97.78 [93.05 to 103.0]	ND	100.9 [97.50 to 104.6]	ND	97.02 [91.72 to 102.8]
MGH026-1	EML4-ALK v3	—	100.7 [96.15 to 105.5]	99.18 [93.99 to 104.8]	101.2 [96.69 to 106.1]	96.86 [94.13 to 99.74]	ND	103.2 [98.26 to 108.5]	ND	103.7 [97.75 to 110.3]
NCI-H2228	EML4-ALK v3	—	32.4 ×/÷ 1.75 (n=4)	30.3 ×/÷ 1.47 (n=5)	32.5 ×/÷ 1.73 (n=4)	38.4 ×/÷ 1.68 (n=8)	27.6 ×/÷ 1.68 (n=4)	33.2 ×/÷ 1.44 (n=12)	30.1 ×/÷ 1.30 (n=2)	29.4 ×/÷ 1.73 (n=6)
Ba/F3	EML4-ALK v3	—	100.3 ×/÷ 1 (n=3)	99.5 ×/÷ 1 (n=3)	100.4 ×/÷ 1 (n=3)	98.8 ×/÷ 1 (n=3)	ND	99.5 ×/÷ 1 (n=4)	ND	99.7 ×/÷ 1 (n=4)
Karpas299	NPM1-ALK	—	82.6 ×/÷ 1.06 (n=4)	78.6 ×/÷ 1.07 (n=3)	78.2 ×/÷ 1.11 (n=5)	76.2 ×/÷ 1.08 (n=5)	80.0 ×/÷ 1.09 (n=4)	76.9 ×/÷ 1.08 (n=5)	73.4 ×/÷ 1.09 (n=2)	80.5 ×/÷ 1.06 (n=2)
Ba/F3	EML4-ALK v1	G1202R	99.8 ×/÷ 1.00 (n=6)	99.9 ×/÷ 1.00 (n=6)	91.8 ×/÷ 1.16 (n=8)	100 ×/÷ 1.00 (n=8)	99.9 ×/÷ 1.00 (n=5)	99.8 ×/÷ 1.01 (n=44)	100 ×/÷ 1.00 (n=3)	99.7 ×/÷ 1.00 (n=3)
MGH953-4	EML4-ALK v3	G1202R	100.1 [95.65 to 104.4]	94.83 [??? to 102.3]	98.24 [91.90 to 105.4]	93.14 [82.33 to 103.9]	ND	96.7 [88.72 to 106.2]	ND	97.29 [89.44 to 106.8]
MGH9037-2	EML4-ALK v3	G1202R	98.9 [91.36 to 105.8]	93.48 [87.99 to 99.29]	100.7 [90.98 to 109.9]	94.84 [90.93 to 99.05]	ND	98.15 [91.71 to 105.3]	ND	100.3 [94.98 to 106.0]
YU-1077	EML4-ALK v3	G1202R	50 ×/÷ 1 (n=2)	50 ×/÷ 1 (n=2)	50 ×/÷ 1 (n=2)	50 ×/÷ 1 (n=2)	50 ×/÷ 1 (n=2)	50 ×/÷ 1 (n=2)	ND	50 ×/÷ 1 (n=2)
Ba/F3	EML4-ALK v3	G1202R	100.3 ×/÷ 1 (n=3)	99.7 ×/÷ 1 (n=3)	102.8 ×/÷ 1 (n=3)	100.4 ×/÷ 1 (n=3)	ND	100.6 ×/÷ 1 (n=5)	ND	99.6 ×/÷ 1 (n=5)
Ba/F3	EML4-ALK v3	T1151M	100.5 ×/÷ 1 (n=3)	99.6 ×/÷ 1 (n=3)	100.4 ×/÷ 1 (n=3)	100.1 ×/÷ 1 (n=3)	ND	99.9 ×/÷ 1 (n=4)	ND	100.1 ×/÷ 1 (n=4)
Ba/F3	EML4-ALK v1	T1151insT	99.9 ×/÷ 1.00 (n=3)	100 ×/÷ 1.00 (n=3)	99.7 ×/÷ 1.00 (n=2)	100 ×/÷ 1.00 (n=2)	99.3 ×/÷ 1.01 (n=3)	100 ×/÷ 1.00 (n=2)	99.4 ×/÷ 1.01 (n=2)	99.3 ×/÷ 1.01 (n=2)
Ba/F3	EML4-ALK v1	C1156Y	100 ×/÷ 1.00 (n=2)	99.9 ×/÷ 1.00 (n=3)	99.4 ×/÷ 1.00 (n=2)	99.5 ×/÷ 1.01 (n=2)	99.8 ×/÷ 1.00 (n=2)	99.4 ×/÷ 1.01 (n=3)	98.6 ×/÷ 1.00 (n=2)	99.1 ×/÷ 1.01 (n=3)
Ba/F3	EML4-ALK v1	I1171N	100 ×/÷ 1.00 (n=4)	100 ×/÷ 1.00 (n=5)	99.0 ×/÷ 1.02 (n=5)	100 ×/÷ 1.00 (n=4)	100 ×/÷ 1.00 (n=3)	99.8 ×/÷ 1.00 (n=61)	99.5 ×/÷ 1.01 (n=3)	100 ×/÷ 1.00 (n=4)
Ba/F3	EML4-ALK v1	I1171S	99.8 ×/÷ 1.00 (n=3)	99.8 ×/÷ 1.00 (n=3)	99.9 ×/÷ 1.00 (n=3)	99.4 ×/÷ 1.01 (n=3)	100 ×/÷ 1.00 (n=2)	99.8 ×/÷ 1.00 (n=11)	100 ×/÷ 1.00 (n=2)	100 ×/÷ 1.00 (n=2)
Ba/F3	EML4-ALK v1	I1171T	99.9 ×/÷ 1.00 (n=3)	100 ×/÷ 1.00 (n=3)	100 ×/÷ 1.00 (n=3)	100 ×/÷ 1.00 (n=3)	100 ×/÷ 1.00 (n=2)	99.8 ×/÷ 1.00 (n=14)	100 ×/÷ 1.00 (n=2)	99.8 ×/÷ 1.00 (n=2)
Ba/F3	EML4-ALK v3	F1174L	100.3 ×/÷ 1 (n=3)	99.7 ×/÷ 1 (n=3)	100.6 ×/÷ 1 (n=3)	99.6 ×/÷ 1 (n=3)	ND	99.8 ×/÷ 1 (n=4)	ND	100.1 ×/÷ 1 (n=4)
Ba/F3	EML4-ALK v1	V1180L	100 ×/÷ 1.00 (n=2)	100 ×/÷ 1.00 (n=2)	100 ×/÷ 1.00 (n=2)	100 ×/÷ 1.00 (n=2)	100 ×/÷ 1.00 (n=2)	100 ×/÷ 1.00 (n=2)	100 ×/÷ 1.00 (n=2)	100 ×/÷ 1.00 (n=2)
MGH045-1	EML4-ALK v1	L1196M	101.8 [94.15 to 109.0]	104.2 [96.71 to 112.6]	103.3 [96.57 to 111.0]	106.6 [99.93 to 113.8]	ND	105.4 [99.56 to 111.7]	ND	103.5 [96.70 to 110.9]
Ba/F3	EML4-ALK v1	L1196M	100 ×/÷ 1.00 (n=3)	100 ×/÷ 1.00 (n=3)	99.9 ×/÷ 1.00 (n=3)	100 ×/÷ 1.00 (n=5)	100 ×/÷ 1.00 (n=2)	99.8 ×/÷ 1.01 (n=20)	100 ×/÷ 1.00 (n=2)	100 ×/÷ 1.00 (n=3)
Ba/F3	EML4-ALK v1	L1196Q	100 ×/÷ 1.00 (n=2)	100 ×/÷ 1.00 (n=3)	100 ×/÷ 1.00 (n=2)	100 ×/÷ 1.00 (n=2)	100 ×/÷ 1.00 (n=3)	100 ×/÷ 1.00 (n=2)	98.7 ×/÷ 1.01 (n=3)	99.9 ×/÷ 1.00 (n=2)
Ba/F3	EML4-ALK v1	L1198F	100 ×/÷ 1.00 (n=2)	100 ×/÷ 1.00 (n=2)	100 ×/÷ 1.00 (n=3)	100 ×/÷ 1.00 (n=4)	100 (n=1)	99.9 ×/÷ 1.00 (n=3)	100 ×/÷ 1.00 (n=3)	100 ×/÷ 1.00 (n=2)
Ba/F3	EML4-ALK v1	G1202del	99.5 ×/÷ 1.01 (n=2)	99.7 ×/÷ 1.01 (n=3)	99.2 ×/÷ 1.01 (n=3)	99.4 ×/÷ 1.01 (n=3)	99.3 ×/÷ 1.00 (n=2)	99.6 ×/÷ 1.01 (n=2)	98.1 ×/÷ 1.02 (n=2)	98.9 ×/÷ 1.00 (n=2)
Ba/F3	EML4-ALK v1	D1203N	100 ×/÷ 1.00 (n=2)	100 ×/÷ 1.00 (n=2)	100 ×/÷ 1.00 (n=3)	100 ×/÷ 1.00 (n=2)	99.8 ×/÷ 1.00 (n=3)	99.8 ×/÷ 1.00 (n=14)	99.9 ×/÷ 1.00 (n=3)	100 ×/÷ 1.00 (n=4)
Ba/F3	EML4-ALK v1	S1206F	99.4 ×/÷ 1.01 (n=3)	100 ×/÷ 1.00 (n=3)	100 ×/÷ 1.00 (n=3)	100 ×/÷ 1.00 (n=2)	100 ×/÷ 1.00 (n=2)	100 ×/÷ 1.00 (n=2)	100 ×/÷ 1.00 (n=2)	100 ×/÷ 1.00 (n=2)
Ba/F3	EML4-ALK v1	S1206Y	100 ×/÷ 1.00 (n=2)	100 ×/÷ 1.00 (n=3)	100 ×/÷ 1.00 (n=3)	100 ×/÷ 1.00 (n=2)	100 ×/÷ 1.00 (n=3)	100 ×/÷ 1.00 (n=2)	98.8 ×/÷ 1.00 (n=2)	99.8 ×/÷ 1.00 (n=3)
Ba/F3	EML4-ALK v1	E1210K	100 ×/÷ 1.00 (n=3)	99.9 ×/÷ 1.00 (n=3)	99.6 ×/÷ 1.00 (n=2)	99.8 ×/÷ 1.00 (n=2)	99.8 ×/÷ 1.00 (n=3)	100 ×/÷ 1.00 (n=2)	99.6 ×/÷ 1.00 (n=2)	99.5 ×/÷ 1.00 (n=2)
Ba/F3	EML4-ALK v1	G1269A	100 ×/÷ 1.00 (n=3)	100 ×/÷ 1.00 (n=3)	98.9 ×/÷ 1.01 (n=3)	99.9 ×/÷ 1.00 (n=2)	100 ×/÷ 1.00 (n=3)	99.9 ×/÷ 1.00 (n=2)	100 ×/÷ 1.00 (n=2)	100 ×/÷ 1.00 (n=2)
MR448re	EML4-ALK v3	G1202R/T1151M	120.7 ×/÷ 1.2 (n=2)	122.3 ×/÷ 1.1 (n=2)	115.6 ×/÷ 1.1 (n=2)	111 ×/÷ 1 (n=2)	ND	84 ×/÷ 1.1 (n=2)	ND	70.8 ×/÷ 1 (n=2)
Ba/F3	EML4-ALK v3	G1202R/T1151M	99.4 ×/÷ 1 (n=4)	101.3 ×/÷ 1 (n=4)	98.6 ×/÷ 1 (n=4)	100.1 ×/÷ 1 (n=4)	ND	95.4 ×/÷ 1.1 (n=7)	ND	85.1 ×/÷ 1.3 (n=7)
Ba/F3	EML4-ALK v3	G1202R/F1174L	99.8 ×/÷ 1 (n=3)	100.4 ×/÷ 1 (n=3)	99.3 ×/÷ 1 (n=3)	100.8 ×/÷ 1 (n=3)	ND	99.5 ×/÷ 1 (n=4)	ND	99.4 ×/÷ 1 (n=4)
NCI-H3122	EML4-ALK v1	G1202R/L1196M	75.0 ×/÷ 1.00 (n=2)	75.0 ×/÷ 1.00 (n=2)	75.0 ×/÷ 1.00 (n=2)	75.0 ×/÷ 1.00 (n=2)	75.0 ×/÷ 1.00 (n=2)	75.0 ×/÷ 1.00 (n=2)	ND	74.1 ×/÷ 1.05 (n=2)
Ba/F3	EML4-ALK v1	G1202R/L1196M	97.9 ×/÷ 1.03 (n=5)	99.8 ×/÷ 1.00 (n=5)	112 ×/÷ 3.13 (n=6)	99.9 ×/÷ 1.00 (n=42)	98.6 ×/÷ 1.02 (n=2)	97.6 ×/÷ 1.05 (n=51)	100 ×/÷ 1.00 (n=2)	99.9 ×/÷ 1.00 (n=8)
MGH953-7	EML4-ALK v3	G1202R/L1196M	96.87 [92.17 to 101.1]	91.84 [87.11 to 96.45]	94.66 [90.62 to 98.89]	94.08 [86.17 to 102.7]	ND	94.95 [86.81 to 104.3]	ND	92.03 [85.74 to 100.1]
Ba/F3	EML4-ALK v1	G1202R/L1198F	100 ×/÷ 1.00 (n=2)	100 ×/÷ 1.00 (n=2)	100 ×/÷ 1.00 (n=2)	100 ×/÷ 1.00 (n=2)	100 ×/÷ 1.00 (n=2)	100 ×/÷ 1.00 (n=5)	100 ×/÷ 1.00 (n=2)	100 ×/÷ 1.00 (n=3)
Ba/F3	EML4-ALK v1	G1202R/G1269A	98.2 ×/÷ 1.03 (n=3)	99.9 ×/÷ 1.00 (n=3)	100 ×/÷ 1.00 (n=3)	99.9 ×/÷ 1.00 (n=6)	99.7 ×/÷ 1.00 (n=2)	100 ×/÷ 1.00 (n=10)	100 ×/÷ 1.00 (n=2)	99.8 ×/÷ 1.00 (n=3)
Ba/F3	EML4-ALK v1	I1171N/L1198F	100 ×/÷ 1.00 (n=2)	100 ×/÷ 1.00 (n=2)	100 ×/÷ 1.00 (n=2)	100 ×/÷ 1.00 (n=2)	100 ×/÷ 1.00 (n=2)	100 ×/÷ 1.00 (n=30)	100 ×/÷ 1.00 (n=2)	100 ×/÷ 1.00 (n=3)
Ba/F3	EML4-ALK v1	I1171N/D1203N	99.9 ×/÷ 1.00 (n=5)	100 ×/÷ 1.00 (n=5)	97.0 ×/÷ 1.07 (n=5)	100 ×/÷ 1.00 (n=7)	100 ×/÷ 1.00 (n=3)	99.1 ×/÷ 1.05 (n=99)	99.9 ×/÷ 1.00 (n=7)	99.9 ×/÷ 1.00 (n=7)
Kelly	—	F1174L	74.3 ×/÷ 1.02 (n=2)	69.9 ×/÷ 1.13 (n=4)	66.1 ×/÷ 1.06 (n=3)	68.3 ×/÷ 1.02 (n=2)	65.0 ×/÷ 1.03 (n=3)	61.4 ×/÷ 1.05 (n=5)	58.4 ×/÷ 1.04 (n=2)	63.9 ×/÷ 1.06 (n=5)
SH-SY5Y	—	F1174L	44.7 ×/÷ 1.17 (n=2)	44.7 ×/÷ 1.17 (n=2)	45.8 ×/÷ 1.21 (n=2)	44.7 ×/÷ 1.17 (n=2)	41.9 ×/÷ 1.13 (n=2)	47.0 ×/÷ 1.02 (n=3)	39.1 ×/÷ 1.03 (n=2)	45.5 ×/÷ 1.16 (n=3)
NB-1	—	Amp, Ex2–3del	82.5 ×/÷ 1.04 (n=2)	76.4 ×/÷ 1.23 (n=2)	79.2 ×/÷ 1.06 (n=2)	79.0 ×/÷ 1.05 (n=2)	81.7 ×/÷ 1.04 (n=2)	80.2 ×/÷ 1.03 (n=3)	82.7 ×/÷ 1.03 (n=2)	80.5 ×/÷ 1.04 (n=3)
Aska-SS	—	Ex2–17del	78.3 ×/÷ 1.04 (n=3)	81.2 ×/÷ 1.01 (n=2)	79.2 ×/÷ 1.05 (n=3)	80.0 ×/÷ 1.00 (n=2)	80.8 ×/÷ 1.03 (n=3)	77.7 ×/÷ 1.01 (n=3)	76.5 ×/÷ 1.07 (n=2)	78.7 ×/÷ 1.05 (n=3)

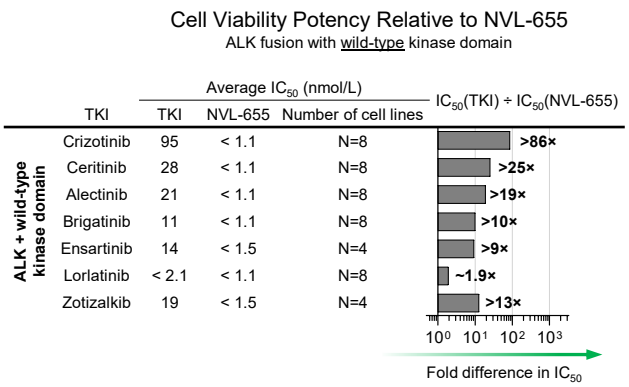
Supplementary Figure S5. Maximal effect (E_{max}) in cell viability assay. Cell viability E_{max} (%) is shown in either of the following two formats. The first format is based on analysis of individual experimental repeats plotted separately and then averaged to obtain "geometric mean ×/÷ geometric standard deviation (number of repeats)". The second format is based on composite analysis of all repeats plotted together and then fitted to obtain "best-fit value [95% confidence interval] (number of repeats)". ND, not determined.

A

Treatment History and Cell Viability Potency of Patient-Derived Cell Lines

Patient-derived cell line	EML4-ALK	Prior TKI: Crizotinib	Prior TKI: Alectinib	Prior TKI: Brigatinib	Prior TKI: Lorlatinib	IC_{50} (nmol/L): Crizotinib	Ceritinib	Alectinib	Brigatinib	Lorlatinib	NVL-655
MGH048-1	v1					188	35	33	11	2.2	0.8
MGH064-1	v2					25	3.4	4.7	2.1	0.6	0.3
MGH026-1	v3					319	80	80	12	2.9	1.6
MGH953-4	v3 G1202R	●	●			1872	317	1235	58	57	0.8
MGH9037-2	v3 G1202R			●		331	71	385	35	16	0.1
YU-1077	v3 G1202R		●			363	170	378	69	9.2	0.2
MR448re	v3 G1202R/T1151M	●	●	●	●	727	689	815	277	370	0.1
MGH953-7	v3 G1202R/L1196M	●	●		●	3715	365	1565	101	344	1.8
MGH045-1	v1 L1196M	●				1024	27	121	28	42	25

B



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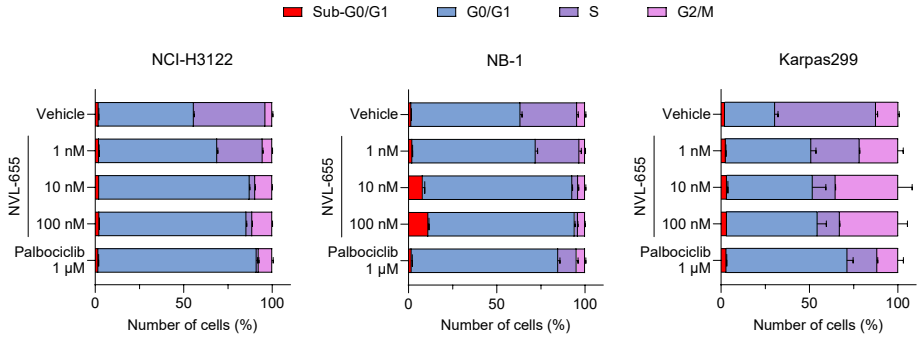

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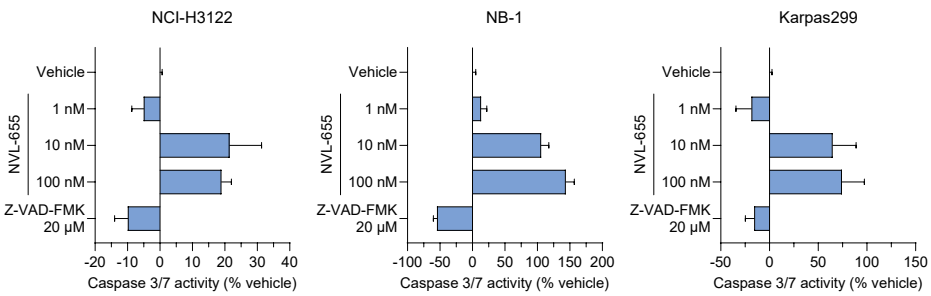

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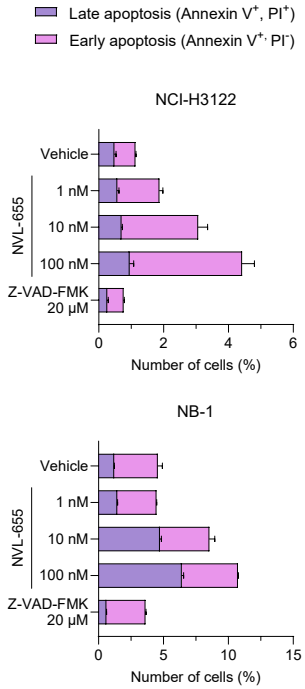

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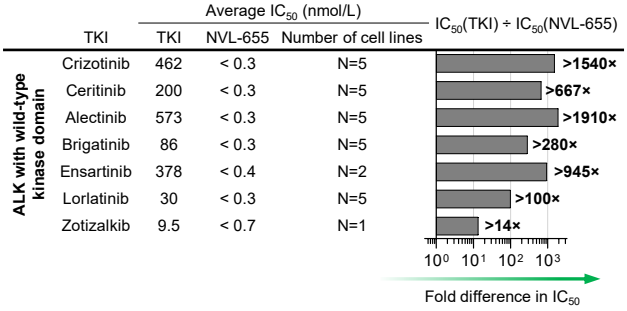

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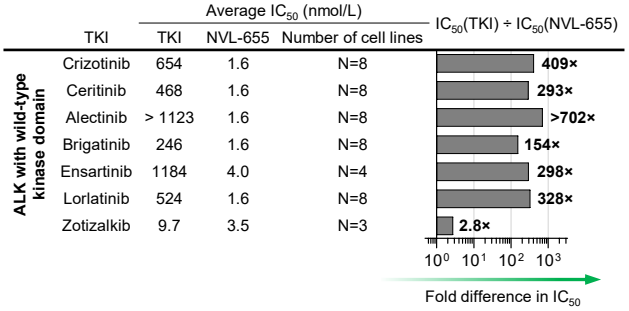
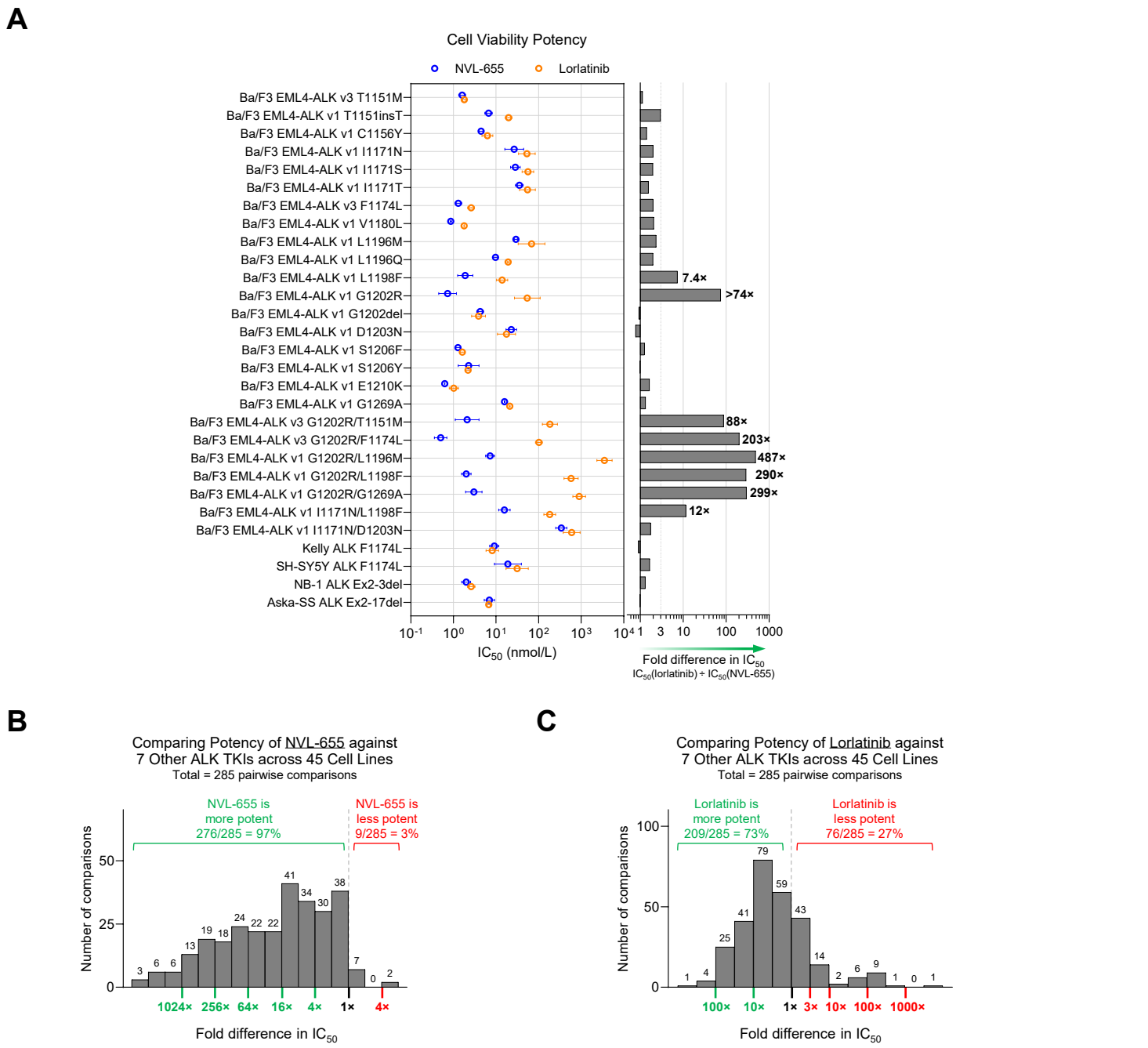


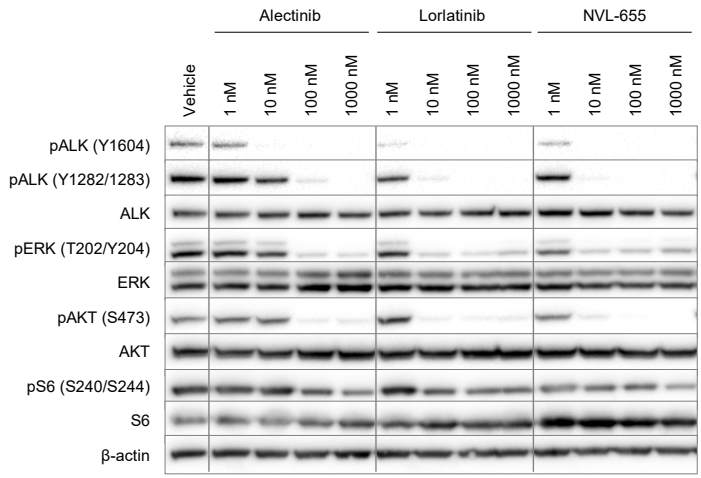
Supplementary Figure S6. Cellular activity of NVL-655. A, Table showing prior TKI treatment (dots) for the 9 patient-derived cell lines used in this report. YU-1077 was collected while patient was on ceritinib treatment. **B,** ALK wild-type potency of 7 TKIs relative to NVL-655, calculated by dividing IC_{50}(TKI) by IC_{50}(NVL-655) in each row. IC_{50} indicates geometric mean IC_{50} across individual cell lines. For ensartinib and zotizalkib, which were not tested in all cell lines, only the matched cell lines were used to calculate IC_{50}. "Relative potency >" is treated as "relative potency =" for graph plotting. **C**, Cell cycle analysis with FITC BrdU and 7-AAD after 24-hour treatment, with palbociclib (an inhibitor of CDK4/6 causing G1 cell cycle arrest) serving as a control. Mean ± SD (2 technical replicates). **D**, Caspase activation analysis using the Caspase-Glo assay after 24-hour treatment, with Z-VAD-FMK (a pan-caspase inhibitor) serving as a control. Mean ± SD (2 technical replicates). **E**, Apoptosis analysis using Annexin V and propidium iodide (PI) staining after 24-hour treatment, with Z-VAD-FMK serving as a control. Mean ± SD (2 technical replicates). **F–G,** Same as panel B but for ALK G1202R (**F**) or ALK G1202R/L1196M (**G**).



Supplementary Figure S7. Comparing NVL-655 and lorlatinib potency. A, Plot showing geometric mean and standard deviation of IC_{50} for NVL-655 (blue) and lorlatinib (orange) against cell lines harboring various ALK mutations. Graph indicates fold difference in IC_{50} compared to NVL-655. **B,** Histogram showing the fold-difference in IC_{50} between NVL-655 and the 7 other ALK TKIs across 45 cell lines shown in Supplementary Fig. S4. Theoretically, there are 7 × 45 = 315 total combinations, but only 285 are shown due to 30 datapoints being "not determined". Green text indicates that NVL-655 was more potent, whereas red text indicates that NVL-655 was less potent. **C,** Same as panel B, except for lorlatinib instead of NVL-655.

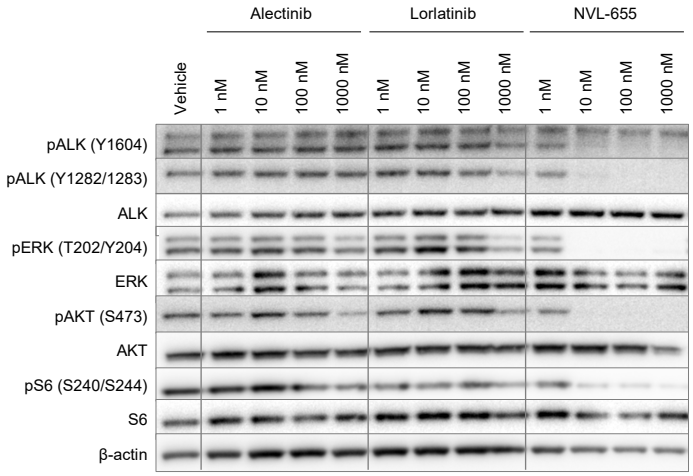
ALK with no resistance mutations

NCI-H3122
EML4-ALK v1



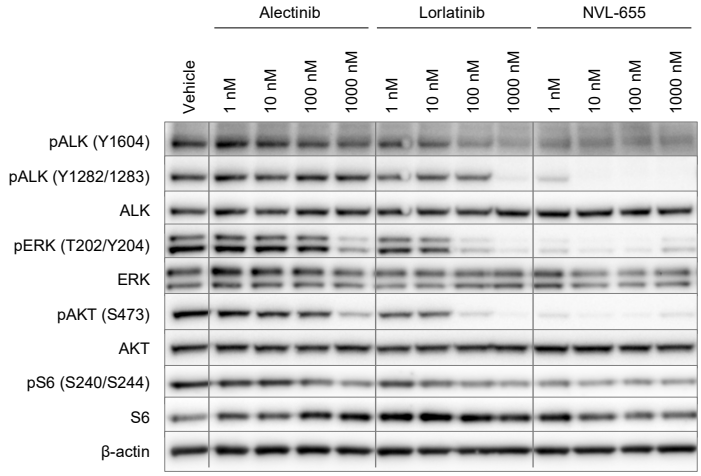
ALK G1202R compound mutation

MGH953-7
EML4-ALK v3 G1202R/L1196M

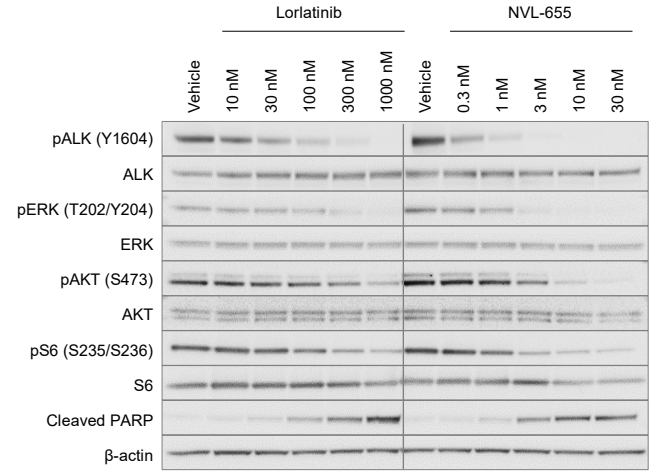


ALK G1202R single mutation

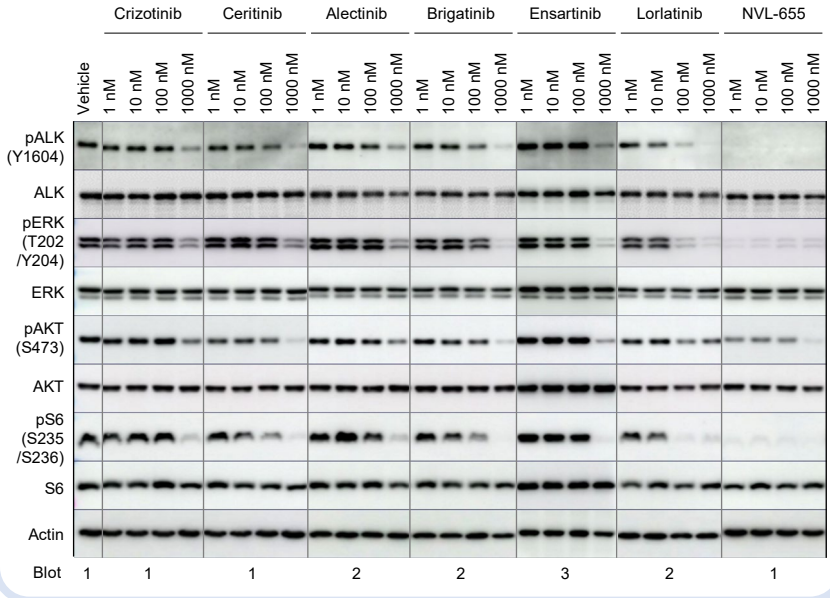
MGH953-4
EML4-ALK v3 G1202R



MR448re
EML4-ALK v3 G1202R/T1151M

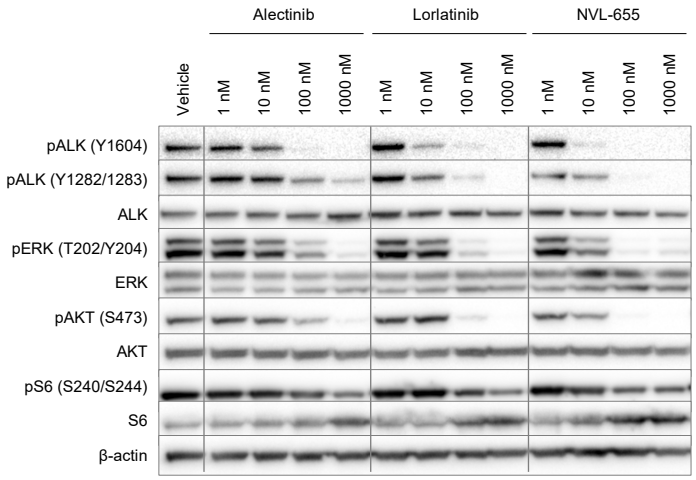


YU-1077
EML4-ALK v3 G1202R



ALK non-G1202R mutation

MGH045-1
EML4-ALK v1 L1196M



Supplementary Figure S8. Pathway analysis in cell lines. For YU-1077, the experiment was performed across 3 blot papers and was reordered based on ALK TKI generations, with the matching blot paper numbers indicated at the bottom.

Cell Viability Potency

Cell line	Genetics or condition	Lorlatinib IC_{50} (nmol/L)	NVL-655 IC_{50} (nmol/L)
Ba/F3	EML4-ALK v1 wild-type	< 3.6 ×/÷ 1.7 (n=24)	1.6 ×/÷ 1.9 (n=3)
Ba/F3	CD74-ROS1 wild-type	1.4 ×/÷ 1.9 (n=17)	0.7 ×/÷ 1.5 (n=3)
Ba/F3	CD74-ROS1 G2032R	326 ×/÷ 1.4 (n=28)	1.1 ×/÷ 1.6 (n=8)
Ba/F3	CLIP1-LTK	1.3 ×/÷ 1.4 (n=3)	1.9 ×/÷ 1.4 (n=5)
Ba/F3	Parental + IL3	> 10000 ×/÷ 1.0 (n=7)	> 10000 (n=1)
A431	*EGFR*-amplified	> 10000 (n=1)	3650 (n=1)
A549	KRAS G12S	> 8410 ×/÷ 1.4 (n=15)	> 9570 ×/÷ 1.1 (n=6)

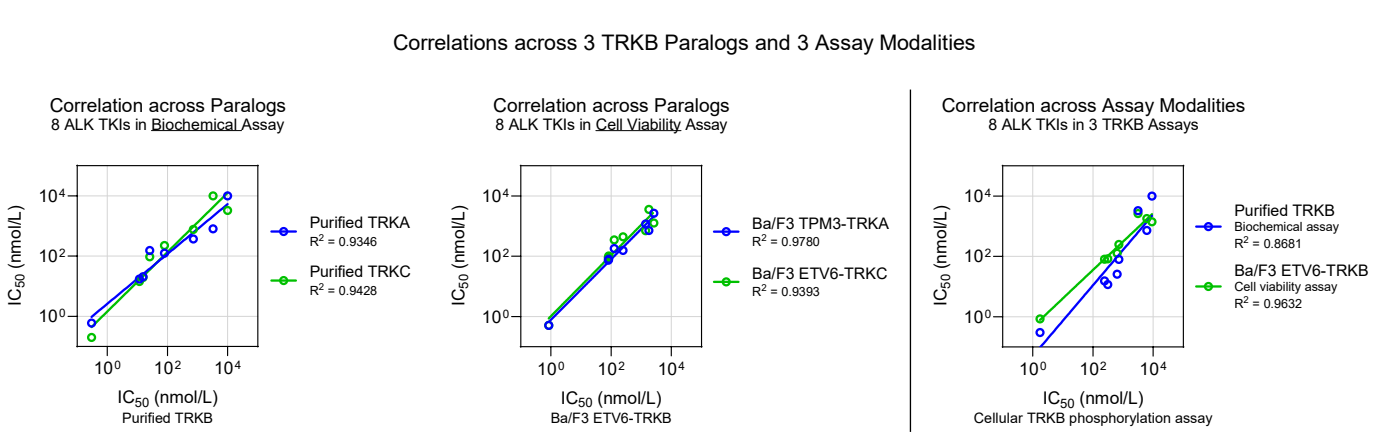
Supplementary Figure S9. Further evidence for on-target activity of NVL-655. Cell viability IC_{50} shown as geometric mean ×/÷ standard deviation (number of repeats). IL3, interleukin 3.

A

Potency against TRK

Assay Modality		Crizotinib	Ceritinib	Alectinib	Brigatinib	Ensartinib	Lorlatinib	Zotizalkib	NVL-655
Biochemical	TRKA IC_{50}	18 nM ×/÷ 1.5 (n=5)	> 3300 nM (n=4)	371 nM ×/÷ 1.7 (n=5)	800 nM ×/÷ 2.0 (n=6)	21 nM ×/÷ 2.0 (n=6)	126 nM ×/÷ 1.5 (n=11)	0.6 nM ×/÷ 1.0 (n=2)	155 nM ×/÷ 1.3 (n=4)
	TRKB IC_{50}	12 nM ×/÷ 1.5 (n=7)	> 3300 nM (n=4)	> 724 nM ×/÷ 1.7 (n=4)	> 3300 nM (n=4)	15 nM ×/÷ 1.4 (n=5)	80 nM ×/÷ 1.6 (n=41)	0.3 nM ×/÷ 4.0 (n=4)	26 nM ×/÷ 1.8 (n=7)
	TRKC IC_{50}	14 nM ×/÷ 1.4 (n=4)	> 1100 nM (n=3)	> 765 nM ×/÷ 1.9 (n=3)	> 3300 nM (n=5)	20 nM ×/÷ 1.7 (n=4)	226 nM ×/÷ 1.6 (n=5)	0.2 nM ×/÷ 2.1 (n=4)	96 nM ×/÷ 1.4 (n=3)
Cell Viability	Ba/F3 TPM3-TRKA IC_{50}	80 nM ×/÷ 2.2 (n=5)	1180 nM ×/÷ 1.7 (n=3)	718 nM ×/÷ 3.0 (n=4)	2690 nM ×/÷ 1.5 (n=36)	74 nM ×/÷ 2.5 (n=3)	156 nM ×/÷ 1.4 (n=64)	< 0.508 nM ×/÷ 1.0 (n=2)	181 nM ×/÷ 1.8 (n=6)
	Ba/F3 ETV6-TRKB IC_{50}	84 nM ×/÷ 1.7 (n=5)	1410 nM ×/÷ 1.1 (n=3)	1800 nM ×/÷ 1.4 (n=3)	2640 nM ×/÷ 2.5 (n=4)	81 nM ×/÷ 1.7 (n=3)	248 nM ×/÷ 1.4 (n=81)	0.9 nM ×/÷ 1.2 (n=5)	128 nM ×/÷ 1.5 (n=5)
	Ba/F3 ETV6-TRKC IC_{50}	102 nM ×/÷ 1.5 (n=4)	706 nM ×/÷ 2.3 (n=2)	3560 nM ×/÷ 1.5 (n=5)	1260 nM ×/÷ 3.3 (n=2)	91 nM ×/÷ 1.7 (n=4)	439 nM ×/÷ 1.7 (n=4)	< 0.5 nM (n=4)	350 nM ×/÷ 1.4 (n=4)
Cellular phosphorylation	Ba/F3 TRKB IC_{50}	312 nM ×/÷ 1.2 (n=4)	> 9190 nM ×/÷ 1.2 (n=4)	6180 nM ×/÷ 1.3 (n=4)	3150 nM ×/÷ 2.0 (n=3)	241 nM ×/÷ 1.1 (n=3)	727 nM ×/÷ 1.5 (n=10)	1.8 nM ×/÷ 1.3 (n=2)	637 nM ×/÷ 1.1 (n=4)

B



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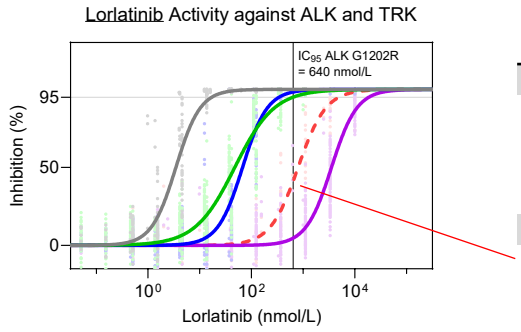
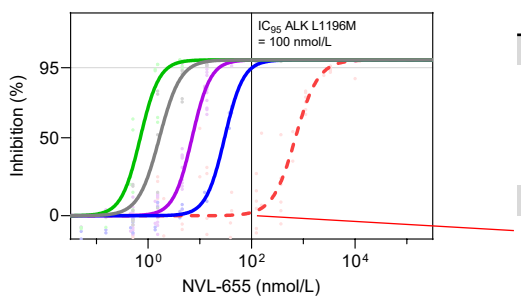


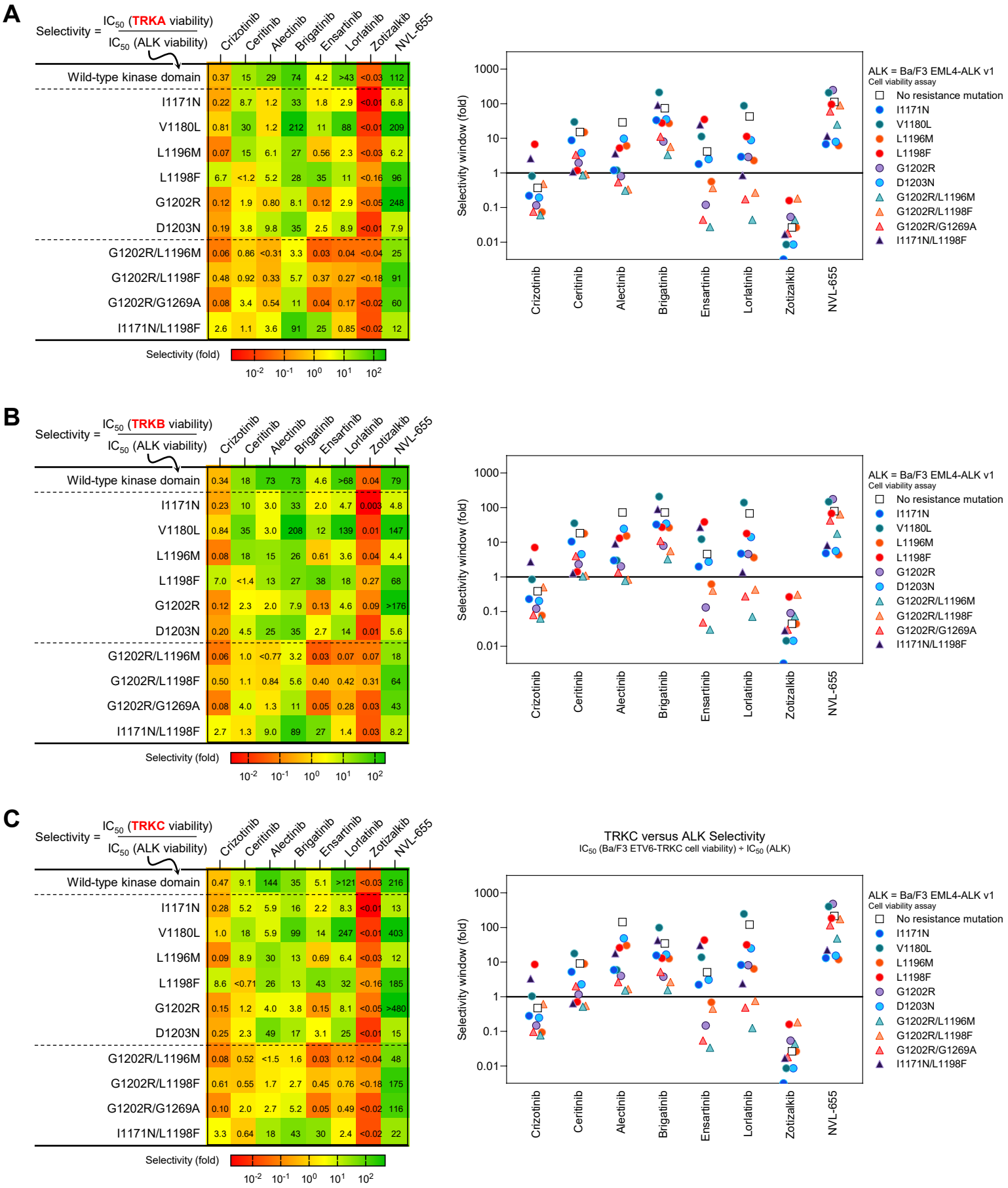
	Inflection (nmol/L)	Hill slope	Top	Bottom	Inhibition at 640 nmol/L
Cell viability assay (Ba/F3 EML4-ALK v1)					
No mutations	3.5	1.84	100%	0%	**100%**
G1202R	48	1.13	100%	0%	**95%**
L1196M	67	1.81	100%	0%	**98%**
G1202R/L1196M	3542	1.77	100%	0%	**5%**
Cellular phosphorylation assay (Ba/F3 TRKB)					
TRKB	800	1.61	100%	0%	**41%**

D

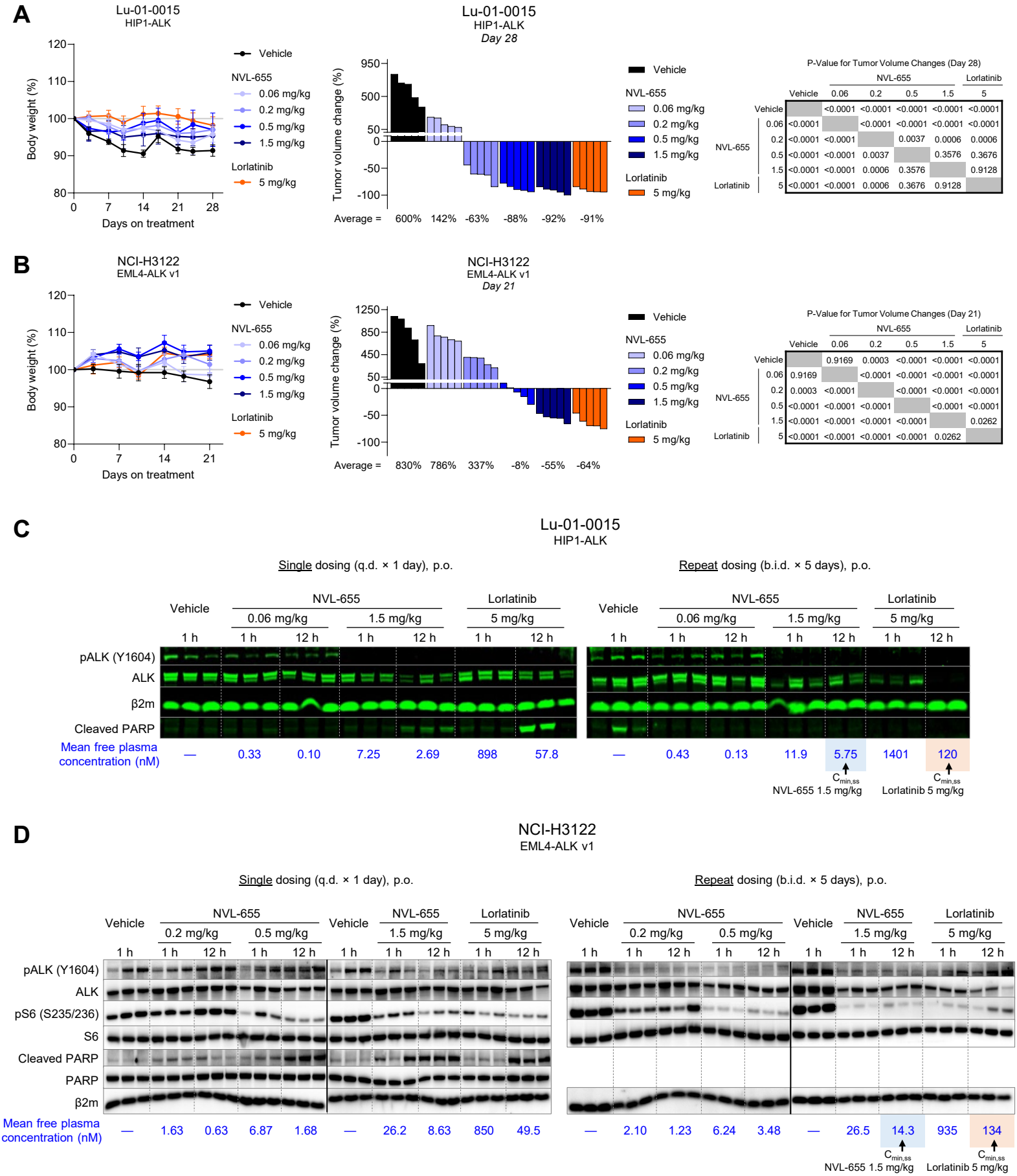


	Inflection (nmol/L)	Hill slope	Top	Bottom	Inhibition at 100 nmol/L
Cell viability assay (Ba/F3 EML4-ALK v1)					
No mutations	1.7	2.13	100%	0%	**100%**
G1202R	0.7	2.36	100%	0%	**100%**
L1196M	30	2.39	100%	0%	**95%**
G1202R/L1196M	7.1	2.43	100%	0%	**100%**
Cellular phosphorylation assay (Ba/F3 TRKB)					
TRKB	704	1.98	100%	0%	**2%**

Supplementary Figure S10. TRK activity. A, IC_{50} (nmol/L) against TRKA, TRKB, and/or TRKC in 3 assay modalities (biochemical, cell viability, and cellular phosphorylation assays) is shown as geometric mean ×/÷ standard deviation (number of repeats). **B,** IC_{50} correlations between 3 paralogs in the biochemical assay (left panel), between 3 paralogs in the cell viability assay (middle panel), and between 3 TRKB assays (right panel). Each dot represents a compound. Correlation coefficients (R^2) are indicated. **C,** Dose-response plots of lorlatinib in ALK cell viability assays and TRKB cellular phosphorylation assays. All experimental repeats are plotted, and curves are fitted to the plotted data. Vertical line indicates IC_{95} for ALK G1202R. The table lists the parameters for logistic regression and the percent inhibition of various targets at IC_{95} ALK G1202R. **D,** Same as panel C but for NVL-655 with IC_{95} for ALK L1196M.



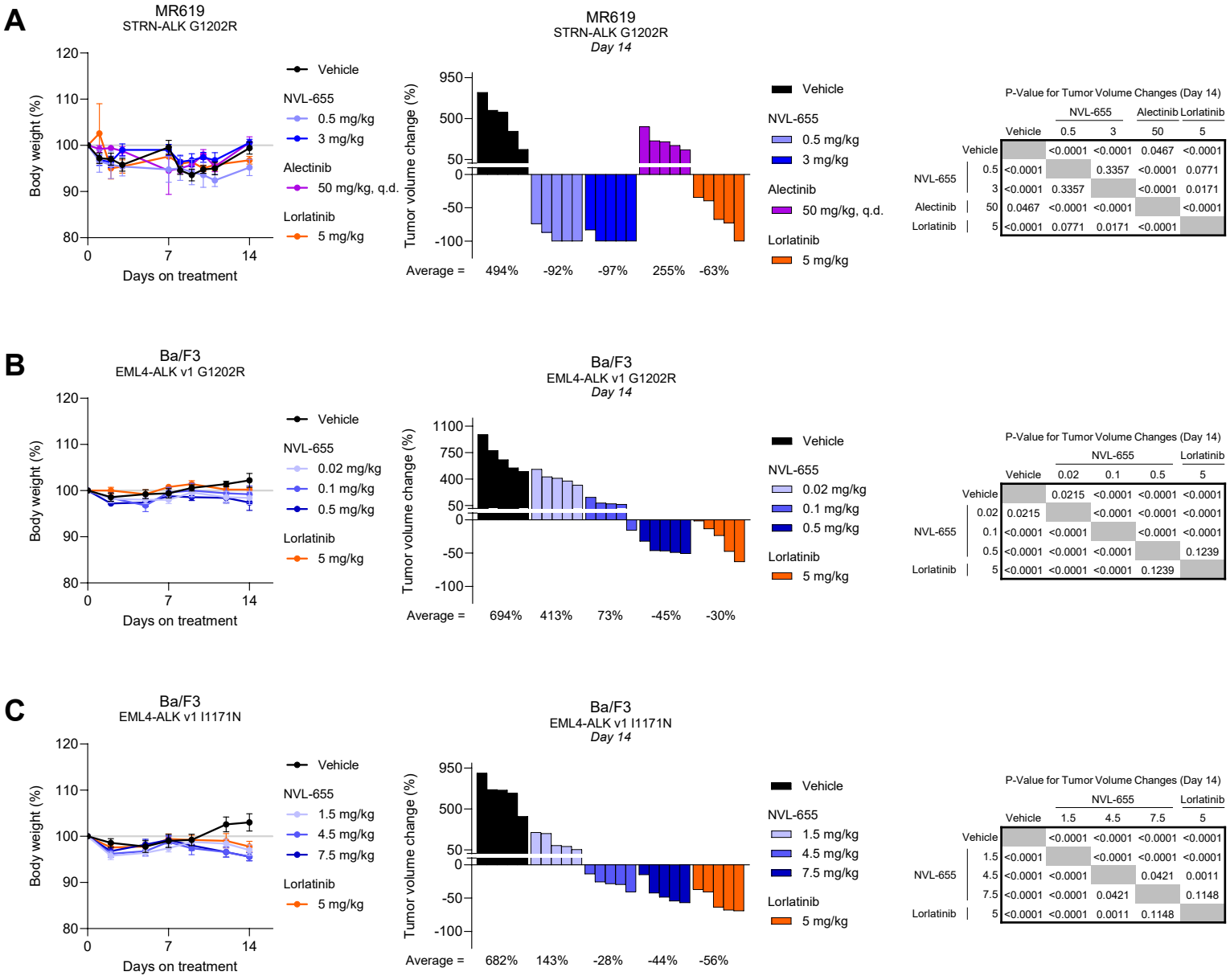
Supplementary Figure S11. Selectivity for ALK versus TRK using additional assays. A, (Left panel) Heat map showing the selectivity window calculated from Ba/F3 TPM3-TRKA IC_{50} (Supplementary Fig. S10A) divided by the Ba/F3 EML4-ALK v1 IC_{50} (Supplementary Fig. S4) in the cell viability assay. "Selectivity <" and "selectivity >" are treated as "selectivity =" for heat map coloring. (Right panel) Graphical representation of the heatmap. "Selectivity <" and "selectivity >" are treated as "selectivity =" for plotting. Horizontal line indicates ALK-TRK equipotency. **B–C,** Same as panel A, except with ETV6-TRKB (**B**) and ETV6-TRKC (**C**) instead of TPM3-TRKA.



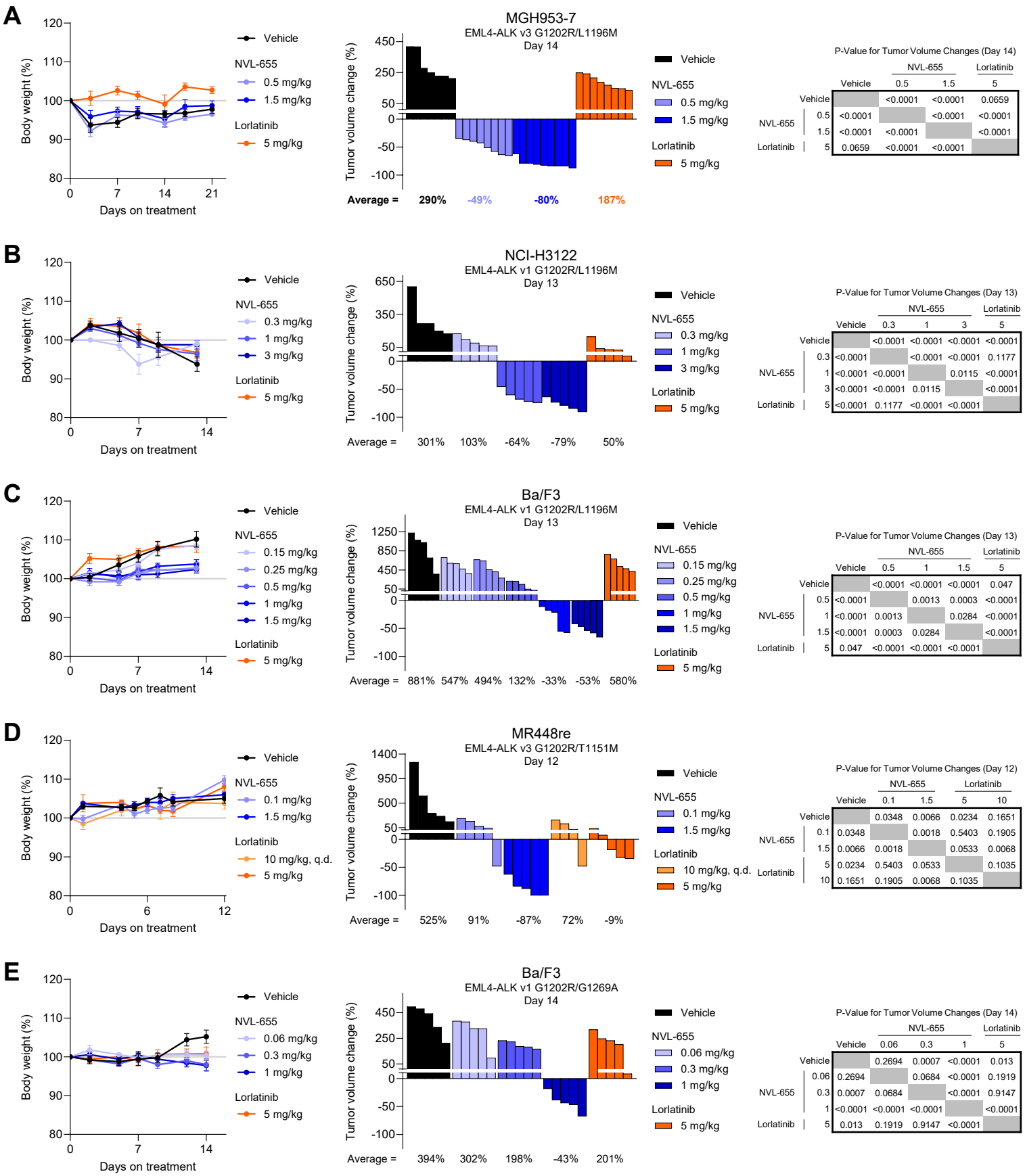
P-Value for Tumor Volume Changes (Day 28)	Vehicle	NVL-655 0.06	NVL-655 0.2	NVL-655 0.5	NVL-655 1.5	Lorlatinib 5
Vehicle		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
NVL-655 0.06	<0.0001		<0.0001	<0.0001	<0.0001	<0.0001
NVL-655 0.2	<0.0001	<0.0001		0.0037	0.0006	0.0006
NVL-655 0.5	<0.0001	<0.0001	0.0037		0.3576	0.3676
NVL-655 1.5	<0.0001	<0.0001	0.0006	0.3576		0.9128
Lorlatinib 5	<0.0001	<0.0001	0.0006	0.3676	0.9128	

P-Value for Tumor Volume Changes (Day 21)	Vehicle	NVL-655 0.06	NVL-655 0.2	NVL-655 0.5	NVL-655 1.5	Lorlatinib 5
Vehicle		0.9169	0.0003	<0.0001	<0.0001	<0.0001
NVL-655 0.06	0.9169		<0.0001	<0.0001	<0.0001	<0.0001
NVL-655 0.2	0.0003	<0.0001		<0.0001	<0.0001	<0.0001
NVL-655 0.5	<0.0001	<0.0001	<0.0001		<0.0001	<0.0001
NVL-655 1.5	<0.0001	<0.0001	<0.0001	<0.0001		0.0262
Lorlatinib 5	<0.0001	<0.0001	<0.0001	<0.0001	0.0262	

Supplementary Figure S12. In vivo studies for ALK fusion with a wild-type kinase domain. A–B, (Left) Body weight plotted as mean ± SEM. Horizontal gray line denotes the initial body weight (100%). (Middle) Waterfall plot showing tumor volume changes from day 0 to the final time point indicated. Average tumor volume changes are provided underneath. (Right) P-values for pairwise comparisons of tumor volume changes between the first day of treatment and the final timepoint indicated. Data for Lu-01-0015 (**A**) and NCI-H3122 (**B**) models. **C–D,** Western blot showing pharmacodynamic modulation in Lu-01-0015 (**C**) and NCI-H3122 (**D**) models. Compounds were administered as a single dose (q.d. × 1 day) or as short-term repeat doses (b.i.d. × 5 days). Plasma samples were collected at the indicated timepoints after the final dose to determine free drug concentrations indicated underneath. β2m serves as a loading control. Q.d., once per day; b.i.d., twice per day; p.o., orally administered; h, hour.

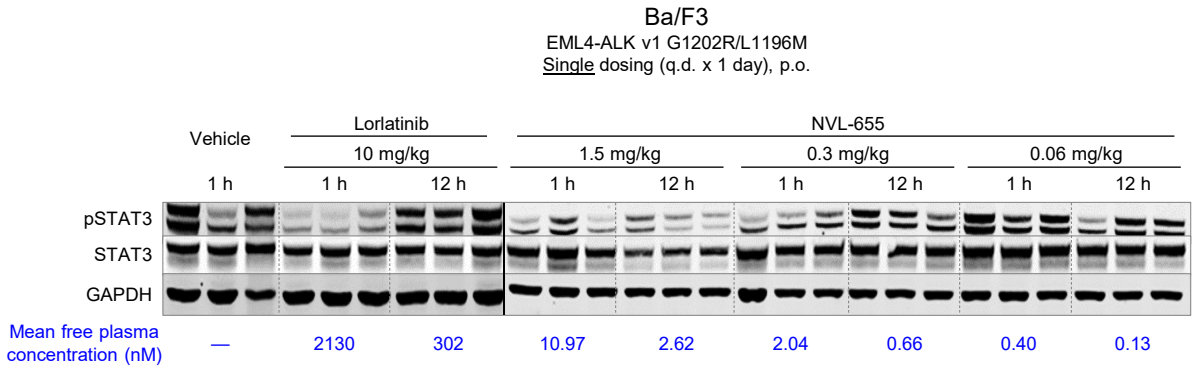


Supplementary Figure S13. In vivo studies for ALK fusion with G1202R or I1171N single mutations. A–C, (Left) Body weight plotted as mean ± SEM. Horizontal gray line denotes the initial body weight (100%). (Middle) Waterfall plot showing tumor volume changes from day 0 to the final time point indicated. Average tumor volume changes are provided underneath. (Right) P-values for pairwise comparisons of tumor volume changes between the first day of treatment and the final timepoint indicated. Data for MR619 (**A**), Ba/F3 EML4-ALK v1 G1202R (**B**), and Ba/F3 EML4-ALK v1 I1171N (**C**) models.

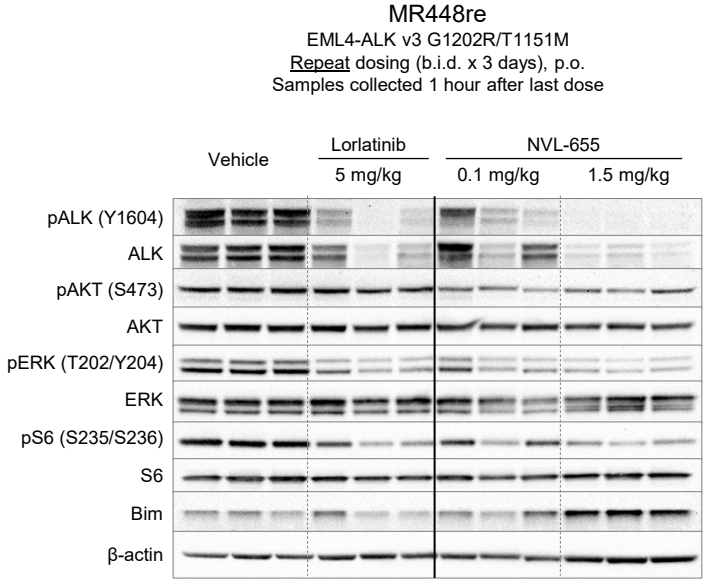


Supplementary Figure S14. In vivo studies for ALK fusion with G1202R compound mutations. A–E, (Left) Body weight plotted as mean ± SEM. Horizontal gray line denotes the initial body weight (100%). (Middle) Waterfall plot showing tumor volume changes from day 0 to the final time point indicated. Average tumor volume changes are provided underneath. (Right) P-values for pairwise comparisons of tumor volume changes between the first day of treatment and the final timepoint indicated. Data for MGH953-7 (**A**), NCI-H3122 EML4-ALK v1 G1202R/L1196M (**B**), Ba/F3 EML4-ALK v1 G1202R/L1196M (**C**), MR448re (**D**), and Ba/F3 EML4-ALK v1 G1202R/G1269A (**E**) models.

A

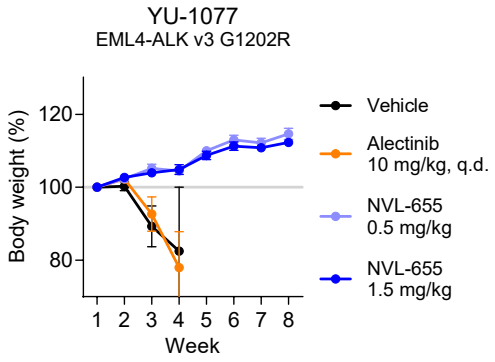


B

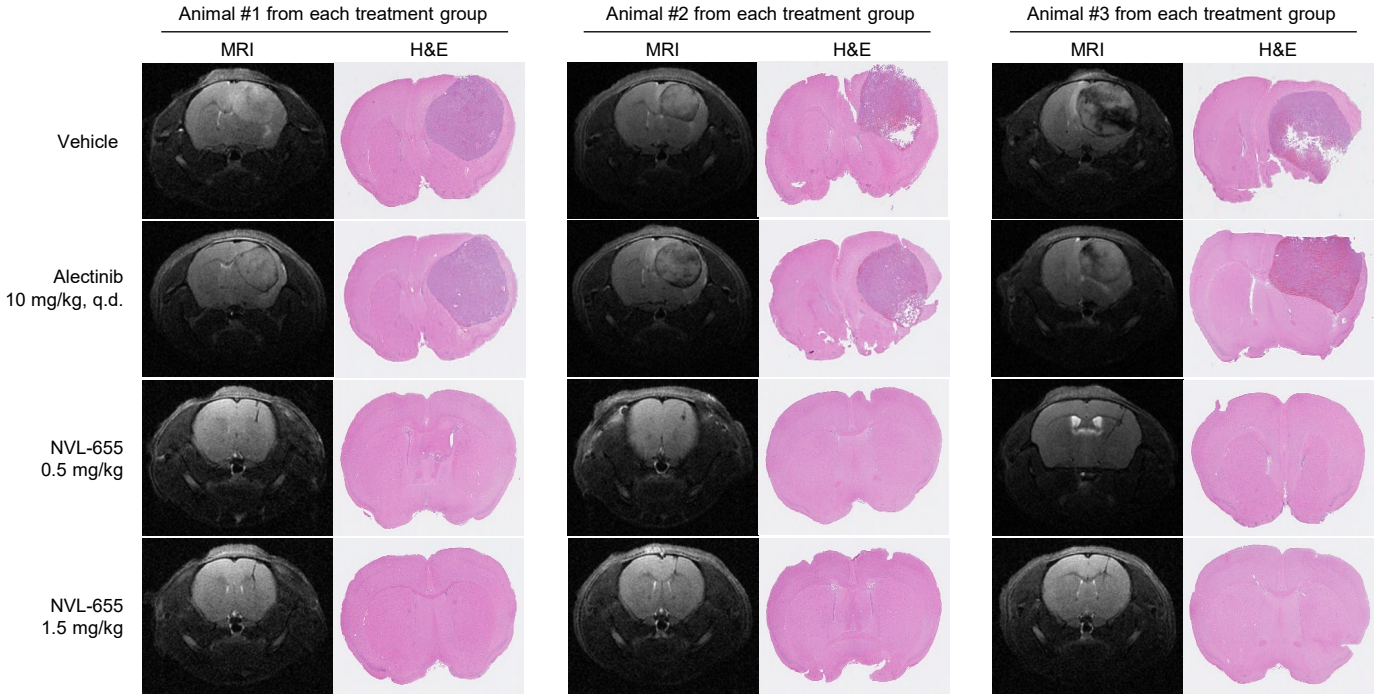


Supplementary Figure S15. Pharmacodynamic analysis in G1202R compound mutation models. A–B, Western blot showing pharmacodynamic modulation in Ba/F3 EML4-ALK v1 G1202R/L1196M (**A**) and MR448re (**B**) models. Compounds were administered as a single dose (q.d. × 1 day) or as short-term repeat doses (b.i.d. × 3 days). Plasma samples were collected at the indicated timepoints after the final dose to determine free drug concentrations indicated underneath. GAPDH and β-actin serve as loading controls. Q.d., once per day; b.i.d., twice per day; p.o., orally administered; h, hour.

A

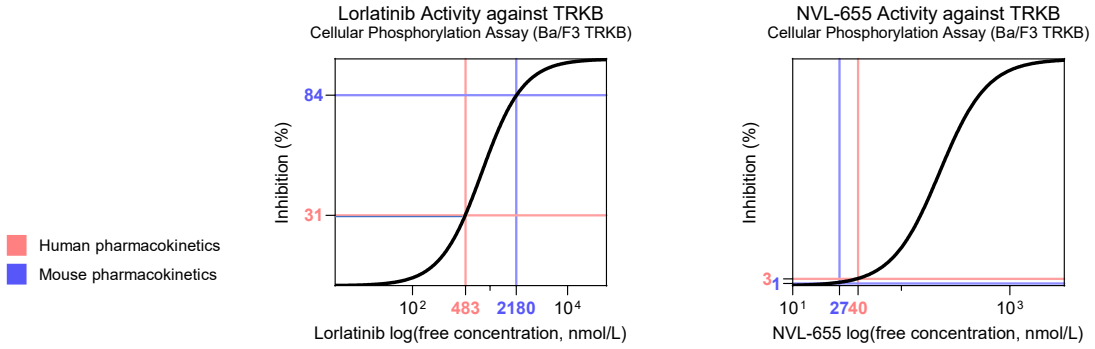


B



Supplementary Figure S16. Body weight and H&E staining for the YU-1077 intracranial study. A, Body weight plotted as mean ± SEM. Horizontal gray line denotes the initial body weight (100%). **B,** Brain MRI and H&E staining analyses of mice treated for 3 weeks (n=3 per treatment group).

Parameter	Lorlatinib	NVL-655
Cellular phosphorylation assay (Ba/F3 TRKB)		
Inflection	800 nmol/L	704 nmol/L
Fraction unbound in 10% fetal bovine serum	0.98	0.32
Free inflection	800 × 0.98 = 784 nmol/L	704 × 0.32 = 225 nmol/L
Hill slope	1.61	1.98
Top	100%	100%
Bottom	0%	0%
Human pharmacokinetics		
Maximum plasma drug concentration	577 ng/mL = 1420 nmol/L	—
Fraction unbound in human plasma	0.34	—
Maximum plasma free drug concentration	1420 × 0.34 = 483 nmol/L	40 nmol/L
Estimated TRKB inhibition	**31%**	**3%**
Mouse pharmacokinetics (1 h, 10 mg/kg orally, 1 dose)		
Free plasma drug concentration	2180 ± 189 nmol/L	27 ± 2.7 nmol/L
Estimated TRKB inhibition	**84%**	**1%**



Supplementary Figure S17. Estimating TRKB inhibition in humans and mice. Four-parameter logistic equation was used to estimate TRKB inhibition using mouse and human pharmacokinetics data. The dose-response curves are identical to Supplementary Fig. S11C–D except that the concentration was adjusted by fraction unbound in 10% fetal bovine serum. Pharmacokinetics data represent the plasma free drug concentration at 1 hour after a single 10 mg/kg oral administration in mice or the maximum plasma free drug concentration in humans[23,66].