Supplementary Information

Drug resistance profiles of mutations in the RET kinase domain

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Supplementary figure legends

Figure s1

Comparison of RET mutants expressed in stable BaF3 derived cell lines. Cell lysates of indicated cell lines were prepared and equal amounts of protein were analyzed by immunoblotting with antibodies to the Flag-tag or actin.

Figure s2

Comparison of sensitivities to vandetanib between BaF3/RET(G810S) and two vandetanib-resistant BaF3/RET(G810S/G949R) cell lines. Experiments were performed as described in the Methods section of the main text.

Figure s3

Comparison of inhibiting RET, RET(V804L), and RET(V804M) kinase activity by nintedanib *in vitro*. The *in vitro* kinase assay was performed as a commercial service by Reaction Biology (Malvern, PA) in parallel 10-dose IC50 singlet assay, with 3-fold serial dilution starting at 10 μM. Curve fitting was as described (Scott et al., 2011).

Figure s4

Immunoblot analysis of pRET in BaF3/KR, BaF3/KR(V871I), and BaF3/KR(F998V) cells after TKI treatment. (A) BaF3/KR and BaF3/KR(V871I) cells were treated with cabozantinib, lenvatinib, or vandetanib at the indicated concentrations for 4 h. Cleared cell lysates were analyzed by immunoblotting with indicated antibodies. (B) BaF3/KR and BaF3/KR(F998V) cells were treated and analyzed as in (A).

Figure s5

Inhibition of KR and KR(M918T) kinase activities in BaF3/KR and BaF3/KR(M918T) cells by cabozantinib, lenvatinib, vandetanib, and nintedanib. Cells were treated with 0, 0.1, or 0.25 μM of each TKI for 4 h (A) or with the indicated concentration of vandetanib for 4 h (B). Cell lysates were analyzed by immunoblotting with antibodies to pRET or flag-tag.

Supplementary Table 1

KIF5B-RET mutations in nintedanib-resistant cell lines derived from BaF3/KR cells*

[Nintedanib] (μM)	Cell line and mutation									
	1	2	3	4	5	6	7	8	9	10
0.2	/	/	/	/	/	/	/	/	/	/
0.4	nm	nm	nm	nm	nm	nm	nm	nm	nm	die
0.8	L730V	L730V	L730V	L730V	L730V	L730V	/	/	/	
1.6	/	L730V	/	die	/	/	L730V/V804M	L730V	L730V	
2.4	/	die	/		die	die	L730V/V804M	L730V/V804M	L730V/V804M	
3.2	die	die	die				L730V/V804M	L730V/V804M	/	

*/, DNA not analyzed; nm, no mutation found

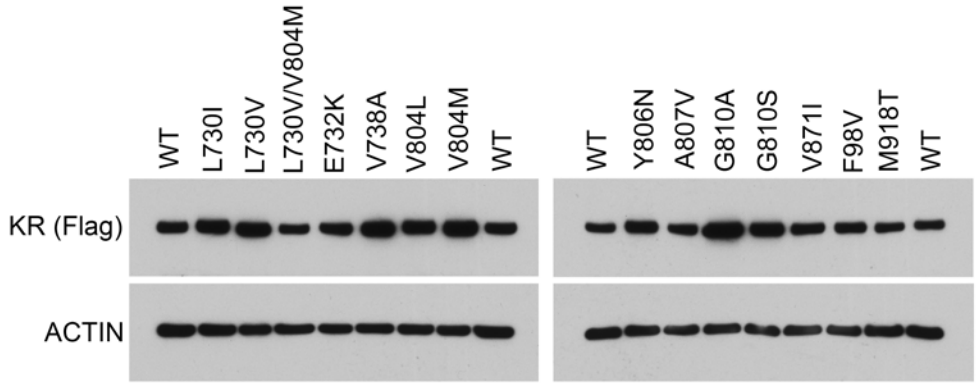

WT
L730I
L730V
L730V/V804M
E732K
V738A
V804L
V804M
WT
WT
Y806N
A807V
G810A
G810S
V871I
F98V
M918T
WT
KR (Flag)
ACTIN

Fig. s1

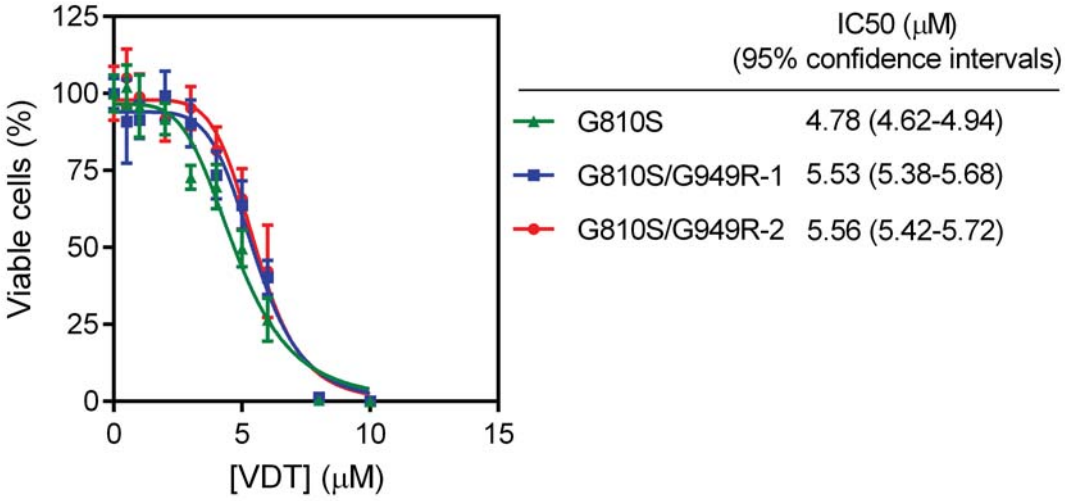
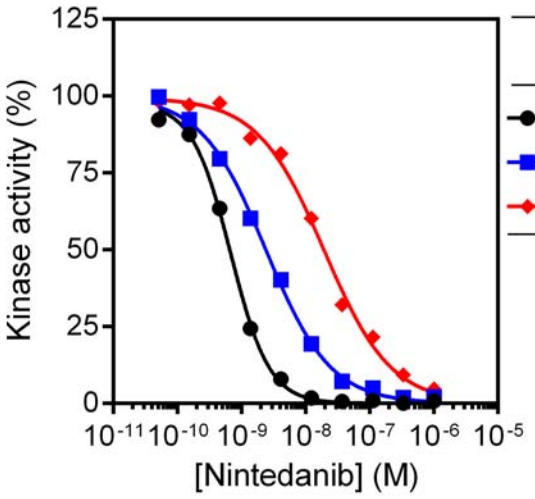

125
100
75
50
25
0
Viable cells (%)
0
5
10
15
[VDT] (μM)
IC50 (μM)
(95% confidence intervals)
G810S 4.78 (4.62-4.94)
G810S/G949R-1 5.53 (5.38-5.68)
G810S/G949R-2 5.56 (5.42-5.72)

Fig. s2



	IC_{50} (nM)	Fold change
RET	0.67	1.0
V804L	2.40	3.6
V804M	19.5	29.9

Fig. s3

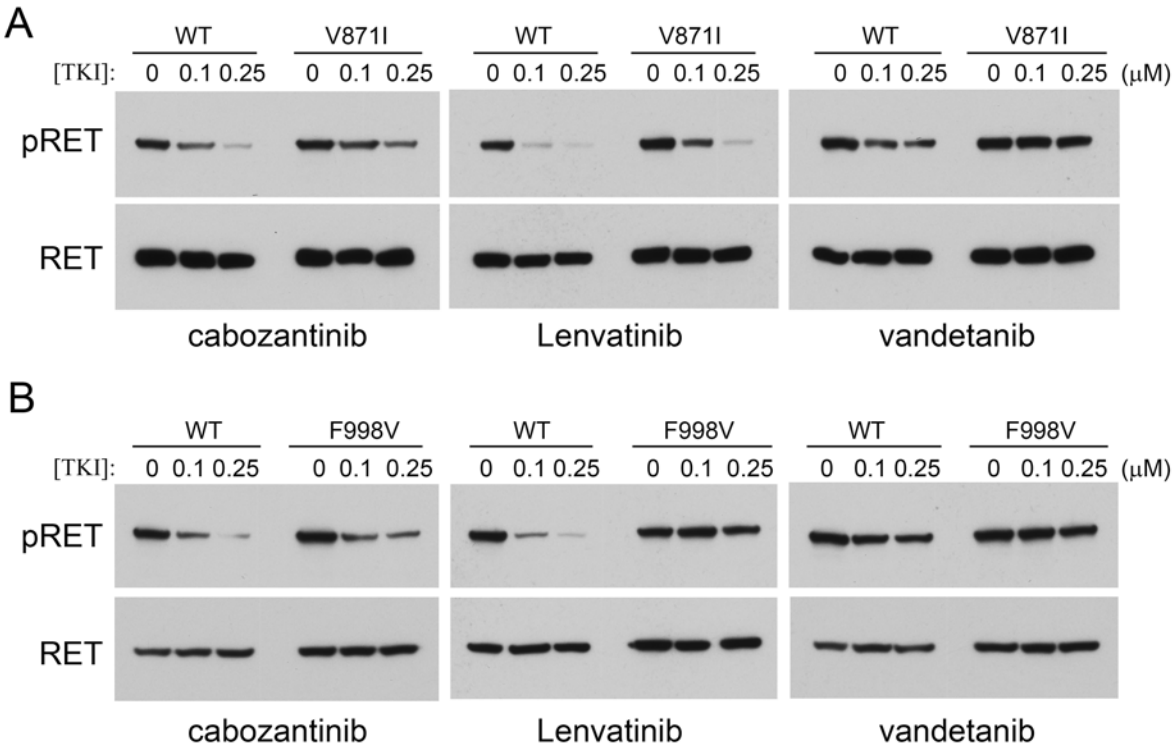

A
WT
V871I
WT
V871I
WT
V871I
[TKI]:
0 0.1 0.25 0 0.1 0.25
0 0.1 0.25 0 0.1 0.25
0 0.1 0.25 0 0.1 0.25
(μM)
pRET
RET
cabozantinib
Lenvatinib
vandetanib
B
WT
F998V
WT
F998V
WT
F998V
[TKI]:
0 0.1 0.25 0 0.1 0.25
0 0.1 0.25 0 0.1 0.25
0 0.1 0.25 0 0.1 0.25
(μM)
pRET
RET
cabozantinib
Lenvatinib
vandetanib

Fig. s4

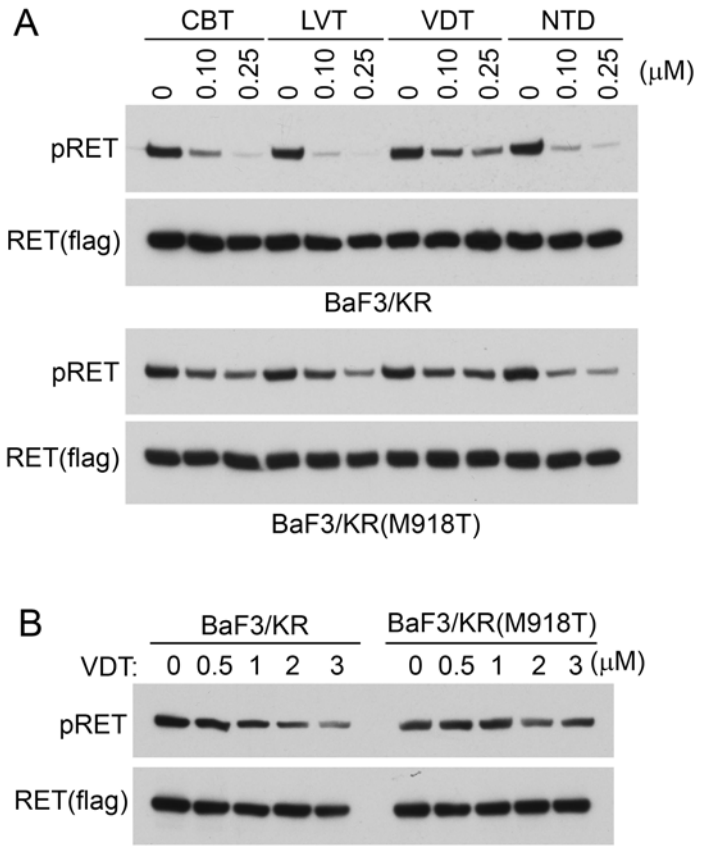

A
CBT
LVT
VDT
NTD
0
0.10
0.25
(μM)
pRET
RET(flag)
BaF3/KR
pRET
RET(flag)
BaF3/KR(M918T)
B
BaF3/KR
BaF3/KR(M918T)
VDT: 0 0.5 1 2 3 0 0.5 1 2 3 (μM)
pRET
RET(flag)

Fig. s5