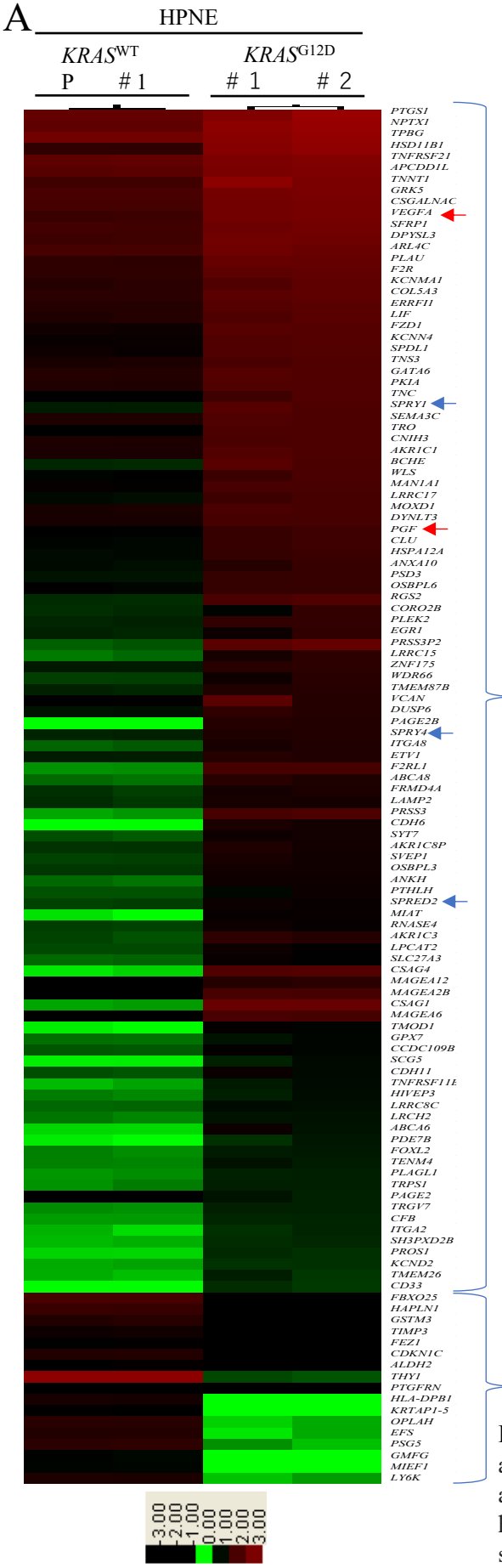
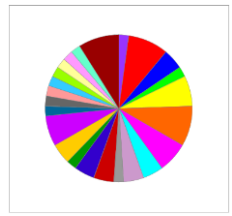
Supplementary Figure

Manuscript Title:
Nintedanib inhibits the VEGFR-ERK signaling pathway in human *KRAS*-mutated cancer cells

•Supplementary information includes 7 supplementary figures

•All the full and uncropped version of the western blot images used in the manuscript has been included in this file (Fig. S5~Fig. S7)





B

PANTHER Pathway

- Alzheimer disease-presenilin
- Angiogenesis
- ngiotensin II-stimulated signaling through G proteins and beta-arrestin
- Axon guidance mediated by semaphorins
- Blood coagulation
- CCKR signaling map
- Cadherin signaling
- EGF receptor signaling
- FGF signaling
- Gonadotropin-releasing hormone receptor pathway
- Heterotrimeric G-protein signaling pathway-Gi alpha and Gs alpha mediated
- Heterotrimeric G-protein signaling pathway-Gq alpha and Go alpha mediated
- Huntington disease
- Inflammation mediated by chemokine and cytokine signaling
- Integrin signalling
- Muscarinic acetylcholine receptor 1 and 3 signaling
- Muscarinic acetylcholine receptor 2 and 4 signaling
- Nicotinic acetylcholine receptor signaling
- Oxidative stress response
- Plasminogen activating cascade
- Pyrimidine Metabolism
- Synaptic vesicle trafficking
- VEGF signaling
- Wnt signaling

Fig. S1B. Pathway analysis of upregulated 113 genes by using Panther Classification Analysis.

C

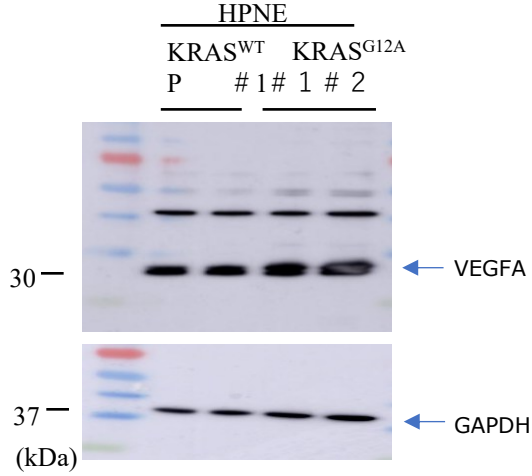


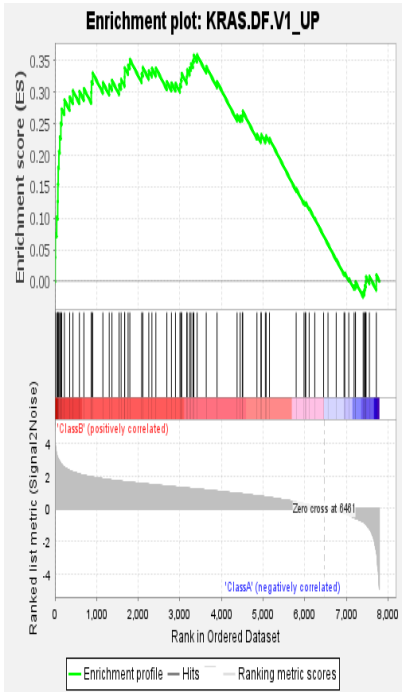
Fig. S1C. The expression of the VEGFA protein was assessed via Western blot analysis, with GAPDH used as the internal control in KRAS^{G12D} cell clones derived from HPNE.

Fig. S1. **A.** Heat map analysis with upregulated (113 genes; FC >5) and downregulated (13 genes, FC <0.2) genes in *KRAS*^{G12D} cells (#1 and #2) compared with parental (P) and *KRAS*^{WT} (Ctrl) cells. The heatmap was constructed based on the normalized values of all samples using TreeView (Cluster 3.0) http://jtreeview (http://jtreeview.sourceforge.net). The corresponding upregulated or downregulated genes in the heatmap are shown on the right side. FC, fold change. Red arrow indicates genes increase VEGF signaling; Blue arrow indicates genes which are classic negative feedback regulators hyperactive RAS-MAPK signaling.

Fig.S2

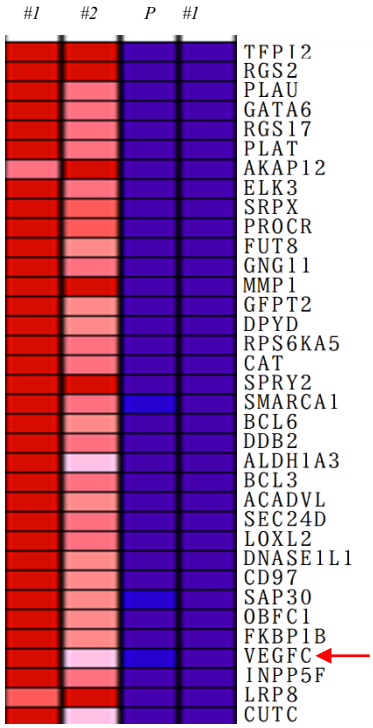
A

GSEA analysis with Gene ontology gene sets (C6 Oncogenic Signature)



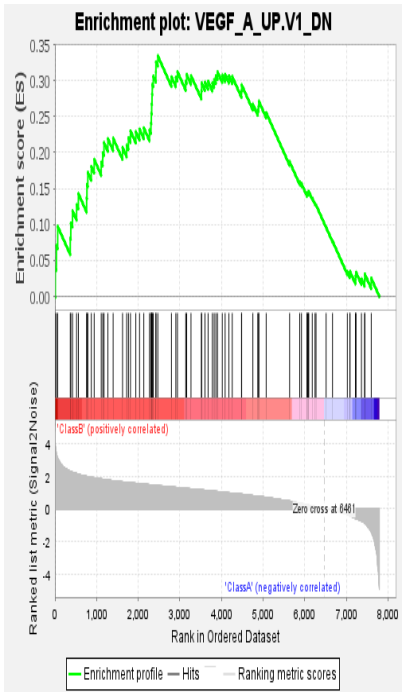
HPNE

KRAS-G12D *KRAS-WT*



B

GSEA analysis with Gene ontology gene sets (C6 Oncogenic Signature)



HPNE

KRAS-G12D *KRAS-WT*

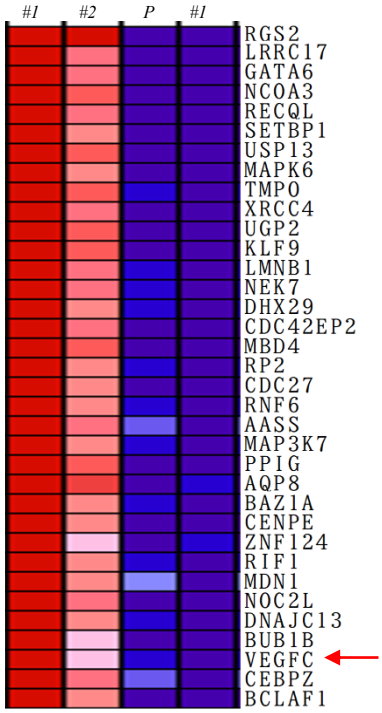


Fig. S2. GSEA in *KRAS*^{G12D} HPNE cells. GSEA were conducted using GSEA v4.33 software and the Molecular Signatures Database (Broad Institute). All the raw data were formatted and applied to **A.** Gene ontology gene sets (c6, Oncogenic Signature). **B.** Curated gene sets (C6 , Oncogenic Signature). Representative enrichment plots and corresponding heatmap image of the indicated gene sets in the KRAS-G12D and KRAS-WT HPNE cells are shown. Genes contributing to enrichment are shown in rows. Expression level is represented as a gradient from high (red) to low (blue). normalized enrichment score.

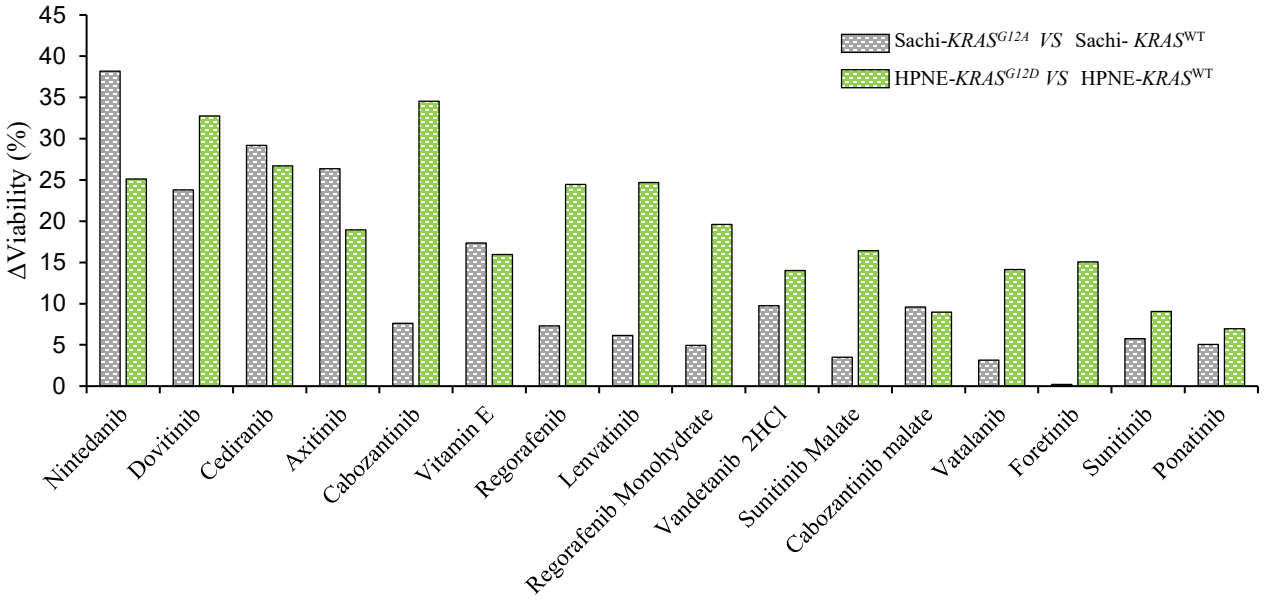


Fig. S3. Identification of VEGFR inhibitors that target KRAS-mutant cell lines. The impact of 16 VEGFR inhibitors in reduction of viability in KRAS mutant cells compared with KRAS wild type cells.

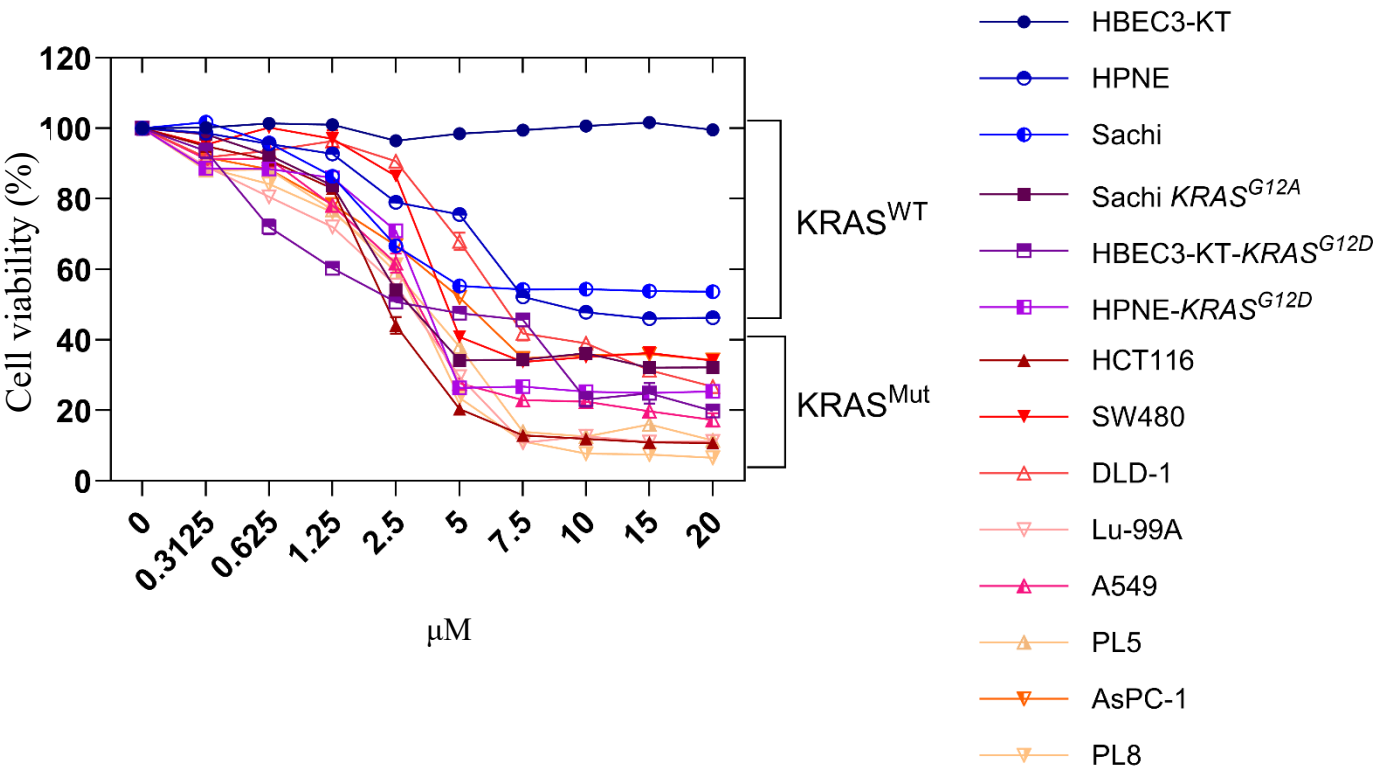


Fig. S4. Nintedanib Selectively Suppresses the Proliferation of KRAS-Mutant Cells. To evaluate the impact of Nintedanib on the proliferation of *KRAS* mutant cell lines, we conducted MTT assays using various *KRAS* mutant cell lines. These included HBEC3-KT, HPNE, and Sachi (*KRAS* wild-type cell lines; black lines) as well as Sachi-*KRAS*^{G12D}, HBEC3-KT-*KRAS*^{G12D}, HCT116, SW480, DLD1, Lu-99A, A549, PL5, AsPC-1, and PL8 (KRAS mutant cell lines; red lines). Interestingly, the average IC50 values in the *KRAS* mutant cell lines ranged from 2μM to 7.5 μM, which is lower than the IC_{50} value in the *KRAS* wild-type cell lines (approximately 20 μM).

Fig.S5

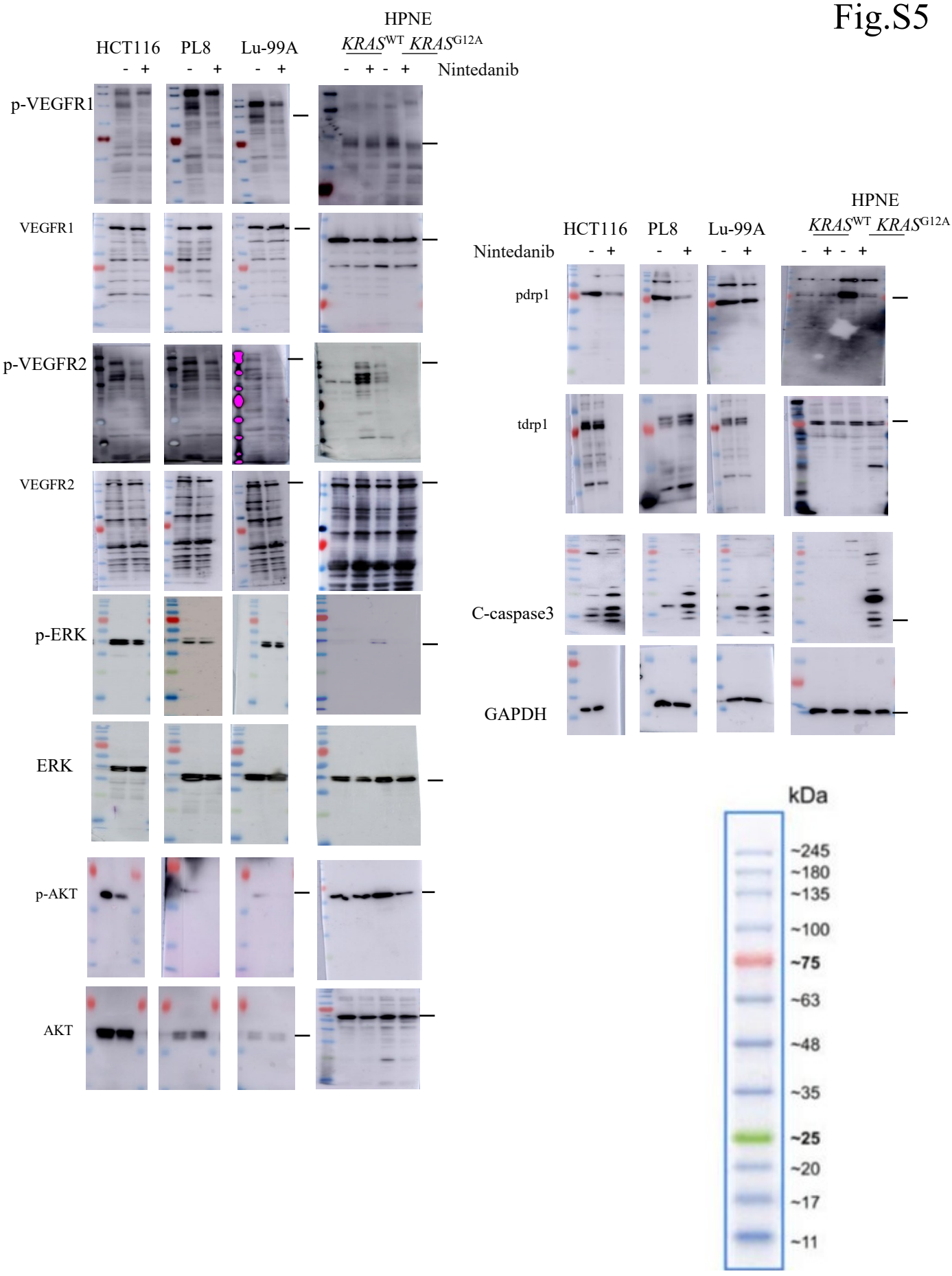


Fig. S5. Complete and unaltered Western blot image corresponding to Figure 3B

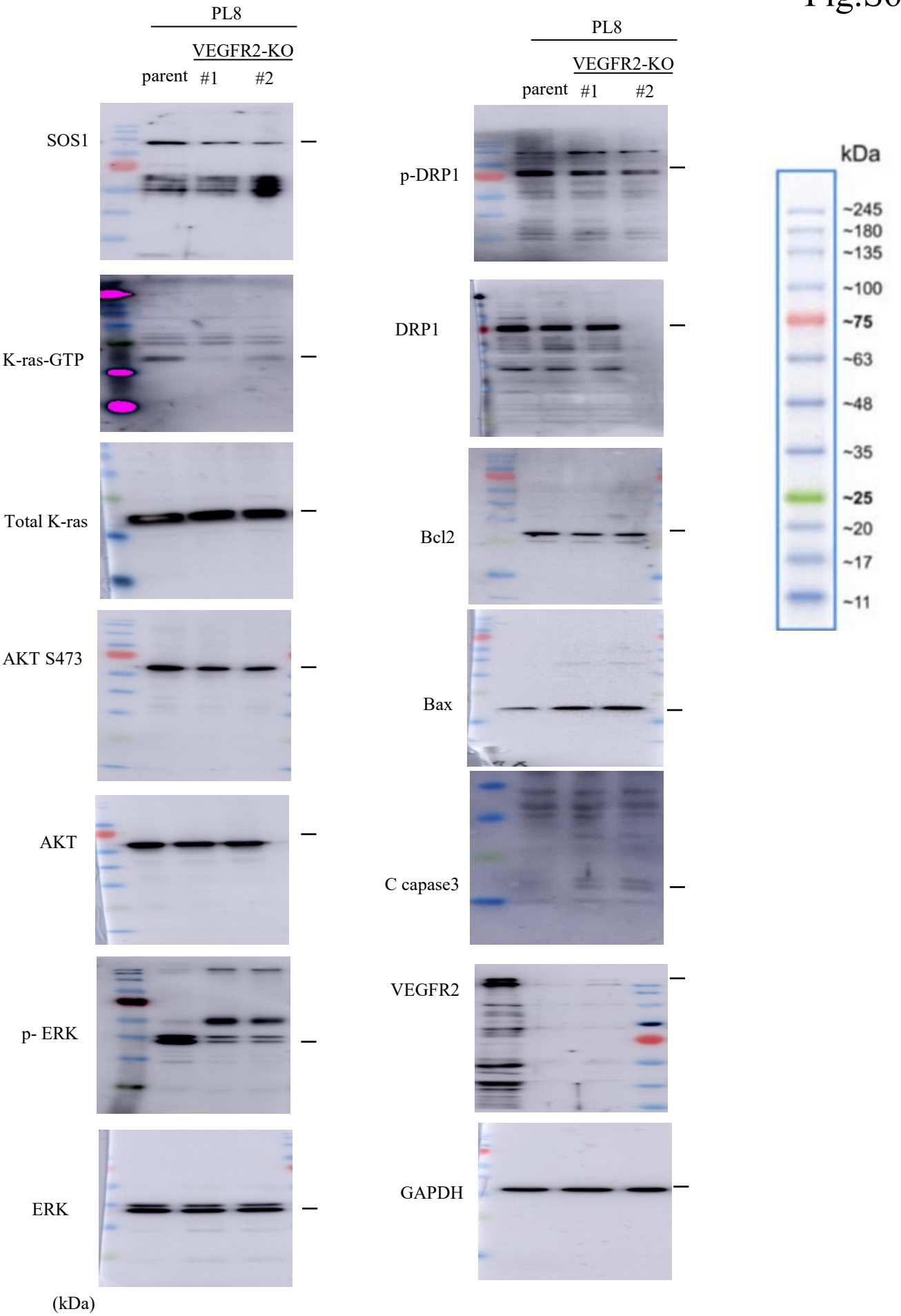


Fig. S6. Complete and unaltered Western blot image corresponding to Figure 4C

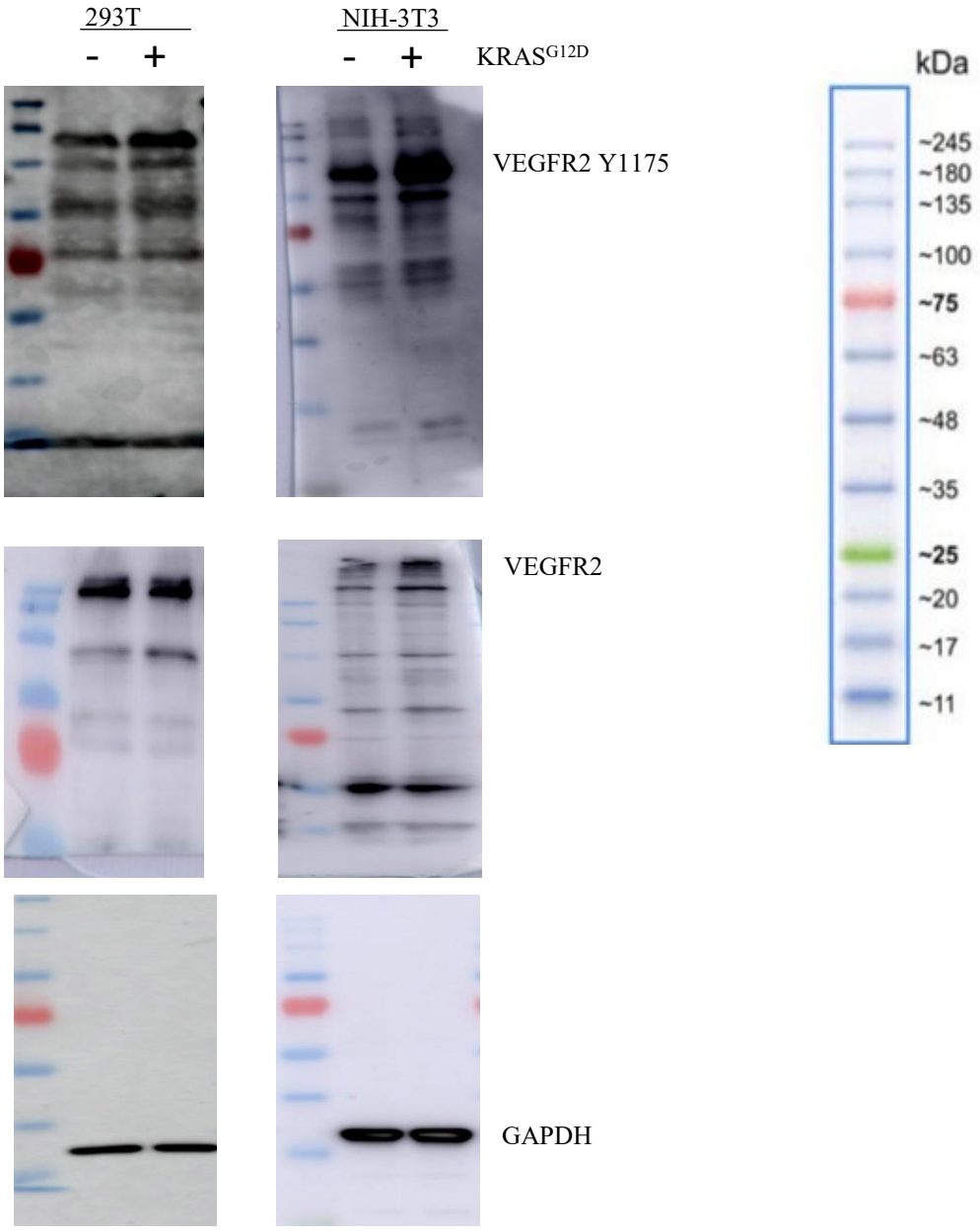


Fig. S7. Complete and unaltered Western blot image corresponding to Figure 4E