

## SUPPLEMENTARY MATERIALS

The L730V/I RET roof mutations display different activities towards pralsetinib and selpercatinib

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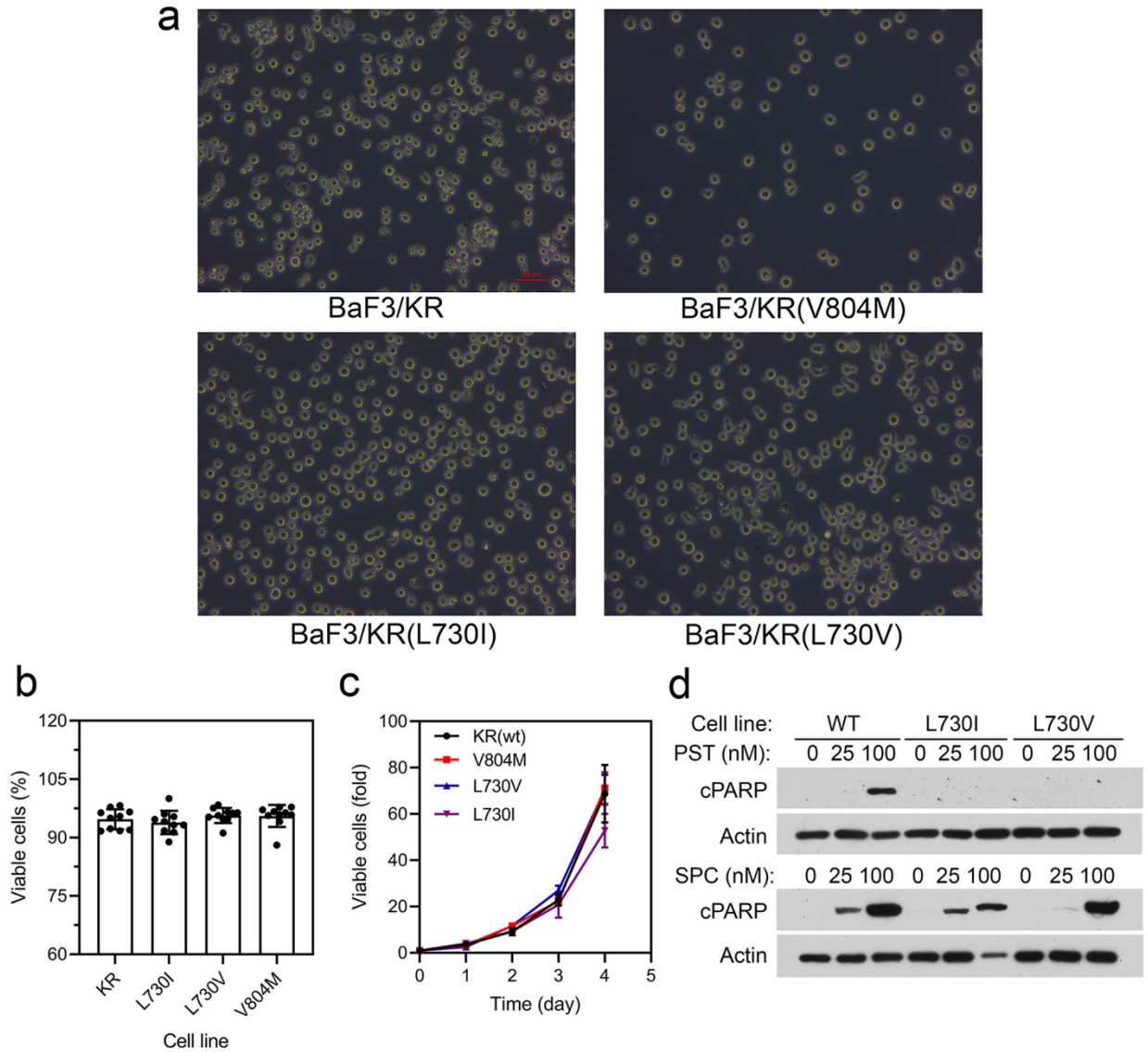
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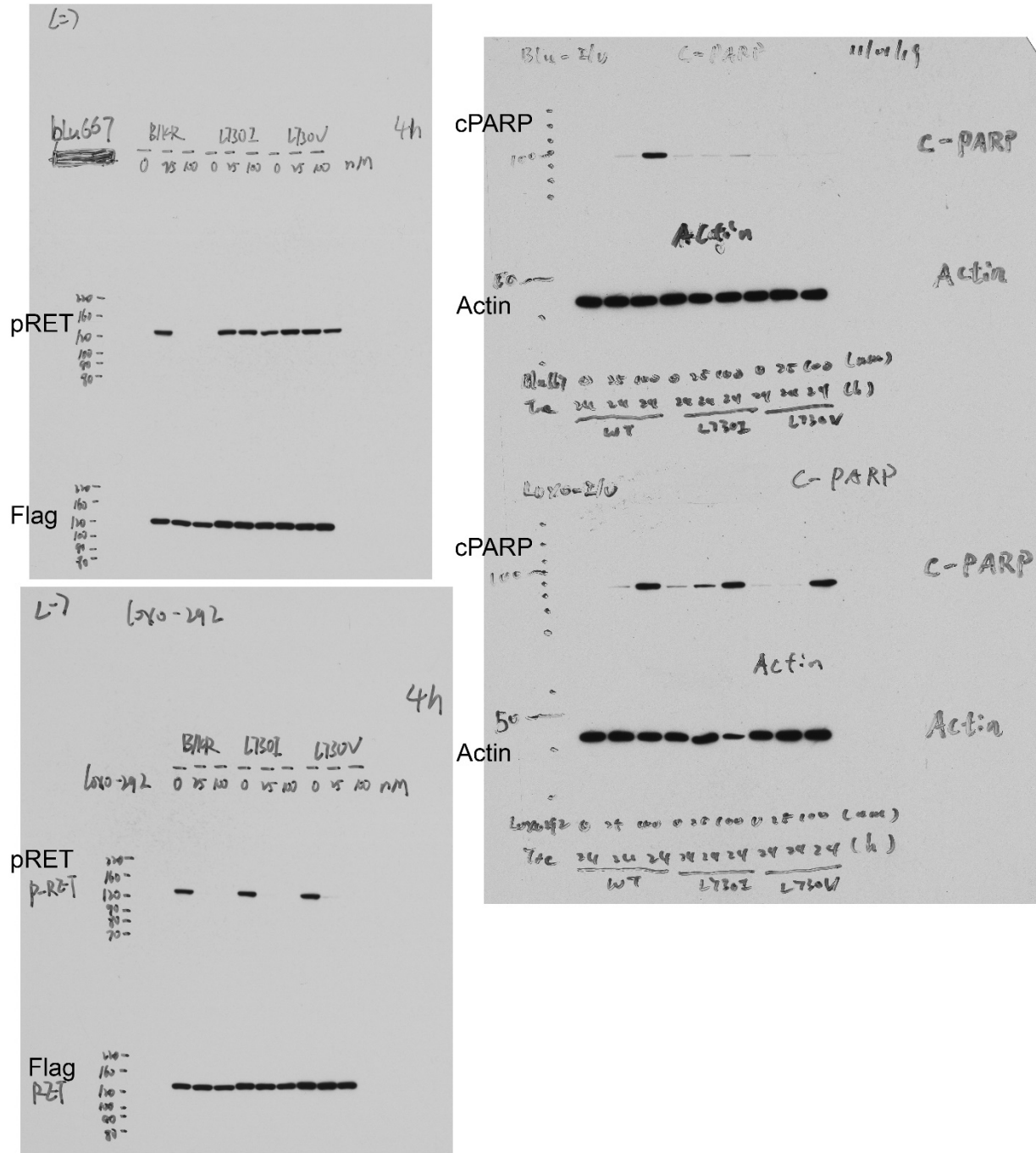
+These authors contributed equally

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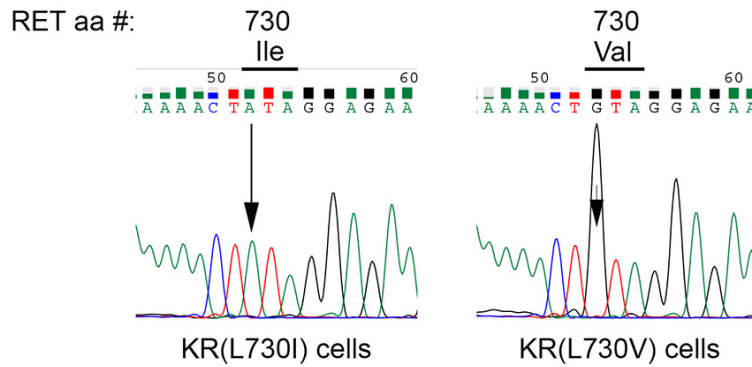
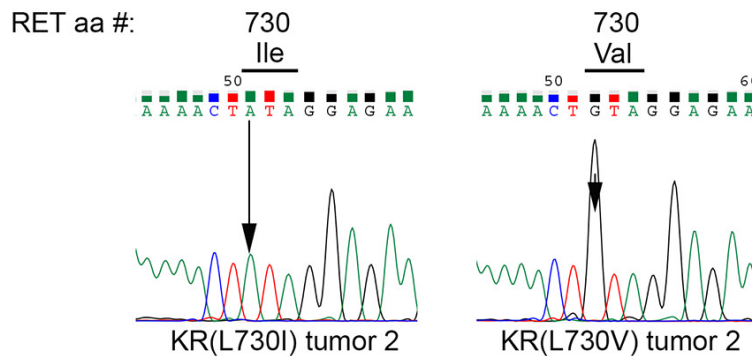
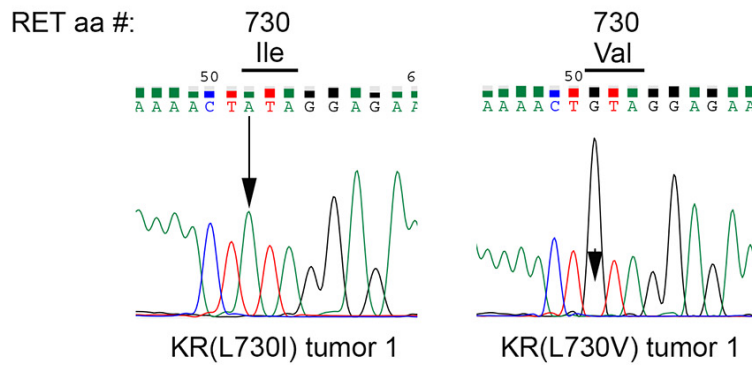
**SUPPLEMENTARY FIGURES**



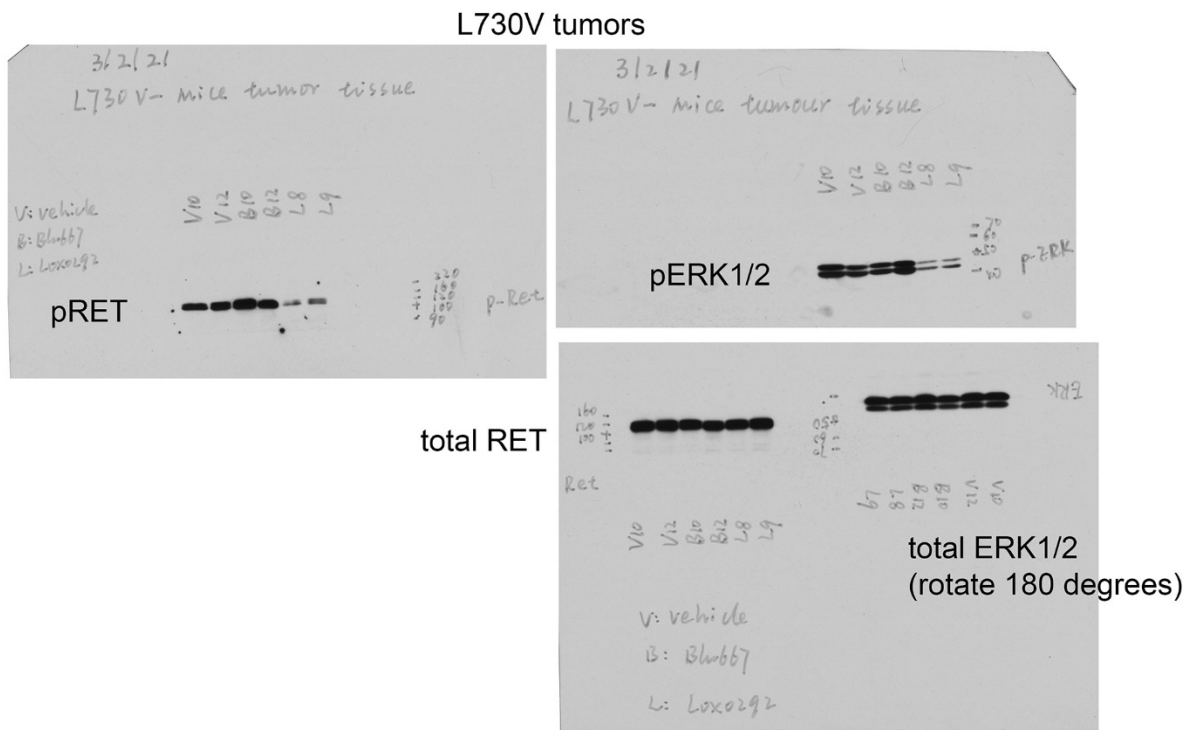
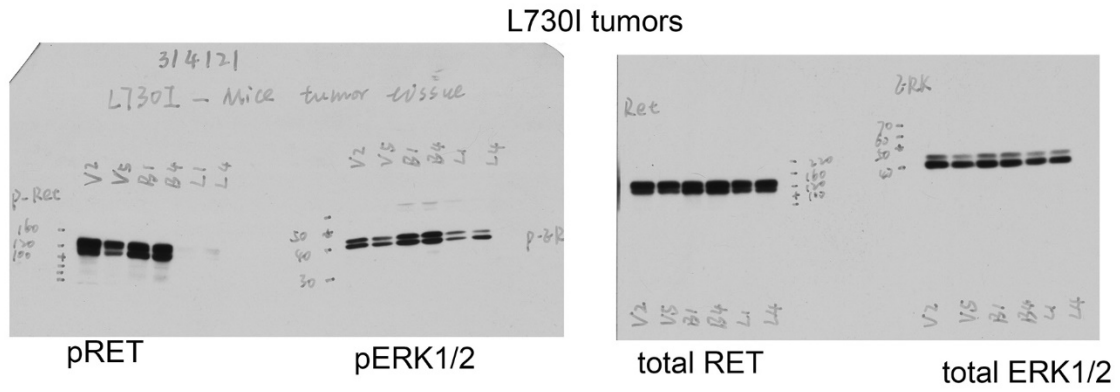
**Supplementary Figure 1. Properties of BaF3 cells expressing KIF5B-RET and KIF5B-RET with V804M, L730I, and L730V mutants.** **a** Morphology of cell lines in cell culture plates at 200x magnification. **b** Cell viability determined by Trypan blue exclusion assay. The data were from 3 independent assays. **c** Comparison of cell growth rates. Cells (250 cells/each) were plated in 96-well plates (n=6) and cultured for 0-4 days. Viable cells were measured using the Celltiter glo reagent (Promega). The data were from two independent experiments. On Day 4, the relative number of viable L730I cells was 75% of that of L730V ( $p < 0.05$ ), whereas there was not significant difference in the growth rates among the wildtype KIF5B-RET, the V804M, and L730V mutant cell lines. **d** A repeated experiment of immunoblotting analysis of cleaved PARP as that shown in Fig. 1b.



**Supplementary Figure 2. Original X-ray films of immunoblots for Fig. 1b.** The original scan images of the X-ray films of immunoblots are shown.

**a****b**

**Supplementary Figure 3. DNA sequencing of BaF3/KIF5B-RET(L730I) and BaF3/KIF5B-RET(L730V) cells and tumors.** Traces of Sanger DNA sequencing across the L730I or L730V region of each sample are shown. Arrow indicates the mutated residue. **a** DNA samples from BaF3/KIF5B-RET(L730I) cells (left) or BaF3/KIF5B-RET(L730V) cells (right). **b** DNA samples from two BaF3/KIF5B-RET(L730I) tumors (left) or two BaF3/KIF5B-RET(L730V) tumors (right). The tumors were from vehicle-treated groups.



**Supplementary Figure. 4. Original X-ray films of immunoblots for Fig. 1g, k.** The original scan images of the X-ray films of immunoblots are shown. The samples were renamed as X1 and X2 (X = V, B, or L) in Fig. 1g, k.