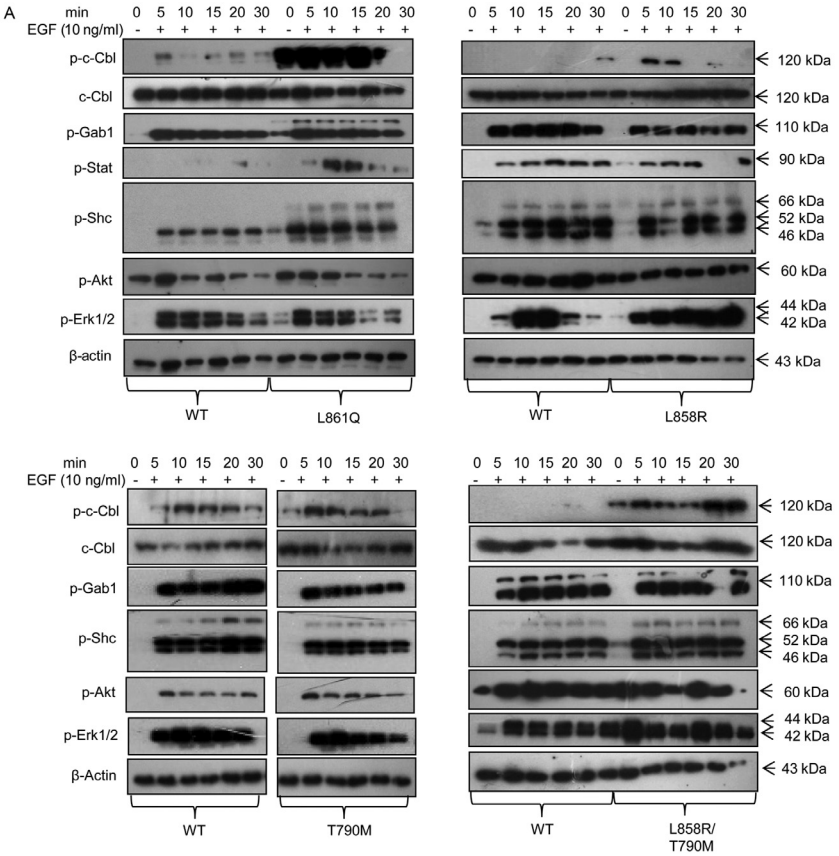
Figure S1. Increased phosphorylation of signaling proteins. The experiment was performed as described in Fig. 1. (A) Protein lysates recovered were subjected to SDS-PAGE followed by western blot analysis with phsopho-specific antibodies to c-Cbl, Gab1, Stat, Shc, Akt, Erk1/2 and β-actin. Increased phosphorylation of signaling proteins was observed in cells expressing receptor mutants L861Q, L858R, double mutant L858R/T790M as compared to Wt/T790M receptors. (B) Densitometric analysis of (A). Densitometric analysis was performed to quantify the expression levels of proteins shown in (A). Bar graphs represent the expression of p-c-Cbl, p-Shc, p-Gab, p-Akt and p-Erk1/2 vs. β-actin.



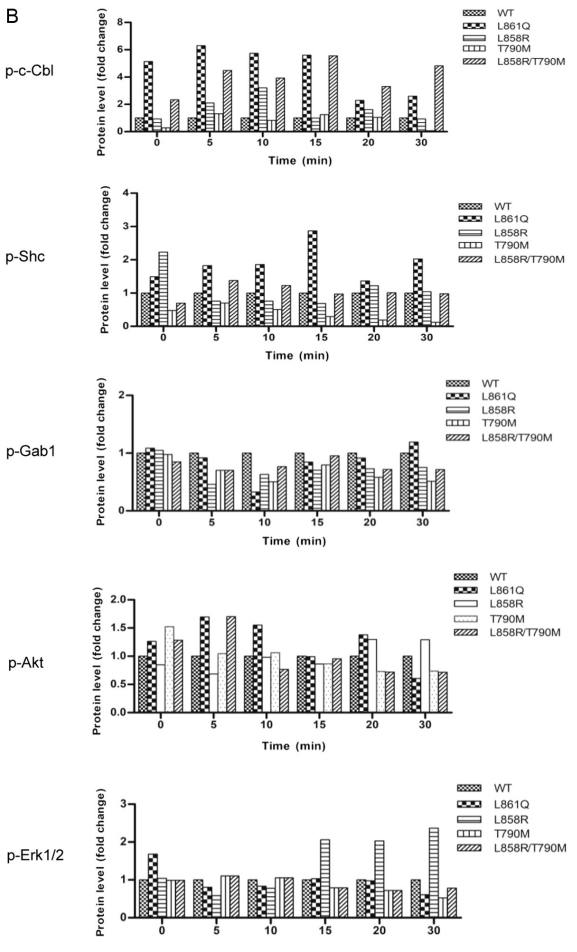


Figure S2. Migration of cells expressing Wt receptor. The experiment was performed as described in Fig. 4. Wound closure was measured at 0 and 24 h post-treatment.

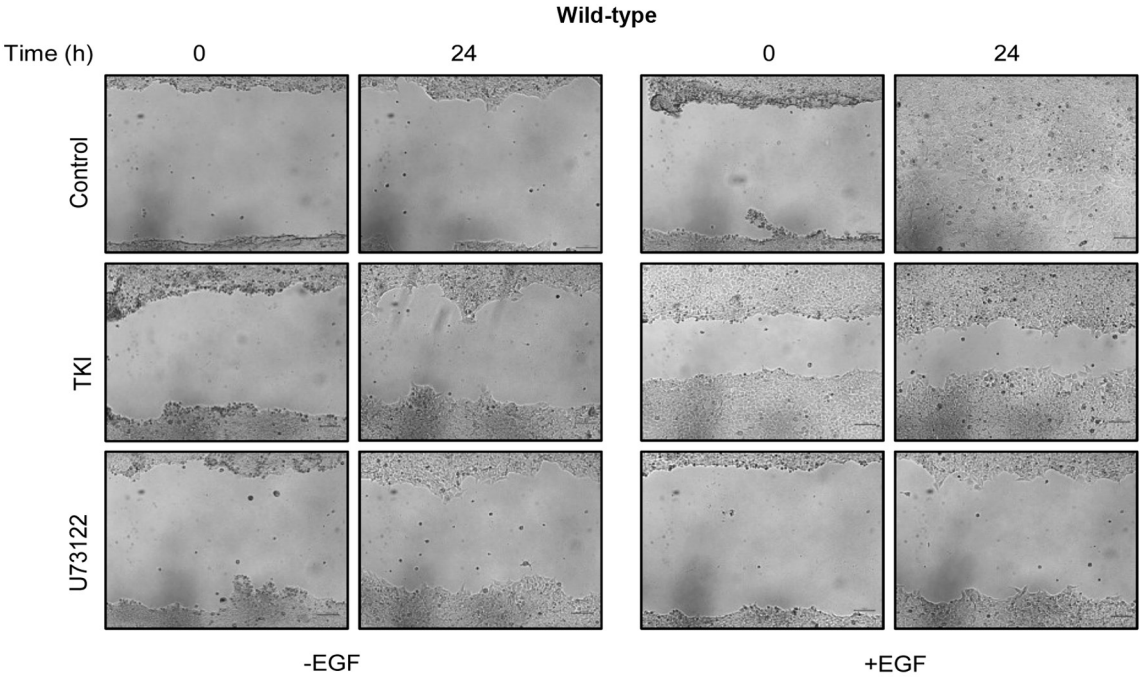


Figure S3. Migration of cells expressing L861Q receptor. The experiment was performed as described in Fig. 4. Wound closure was measured at 0 and 24 h post-treatment.

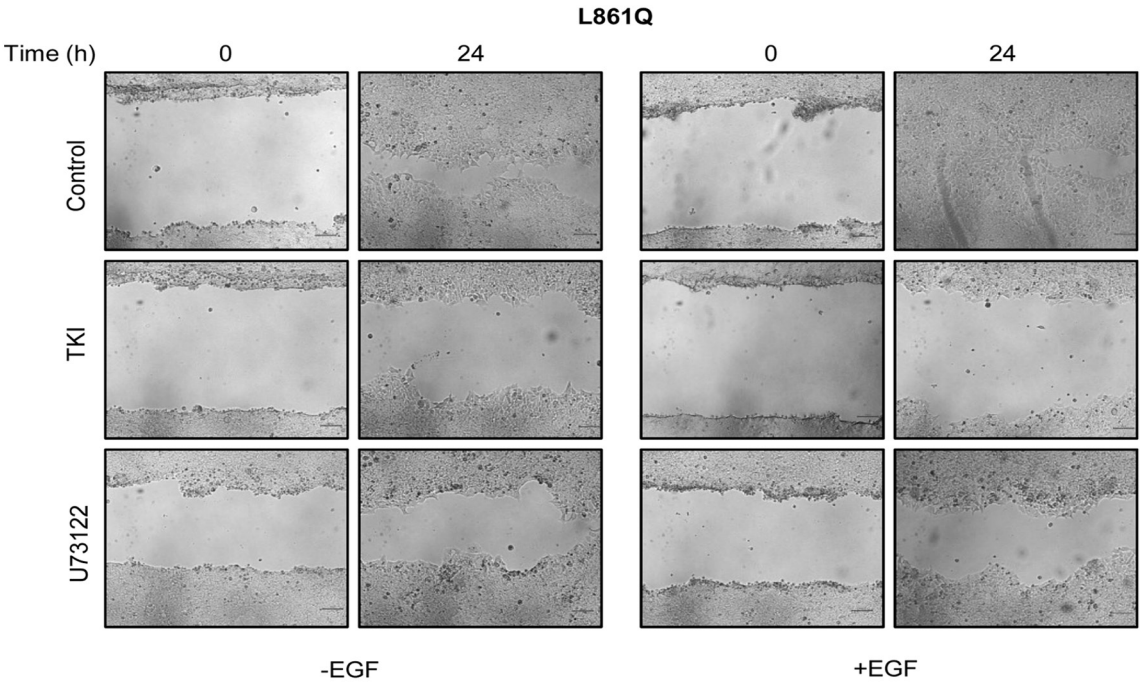


Figure S4. Migration of cells expressing L858R receptor. The experiment was performed as described in Fig. 4. Wound closure was measured at 0 and 24 h post-treatment.

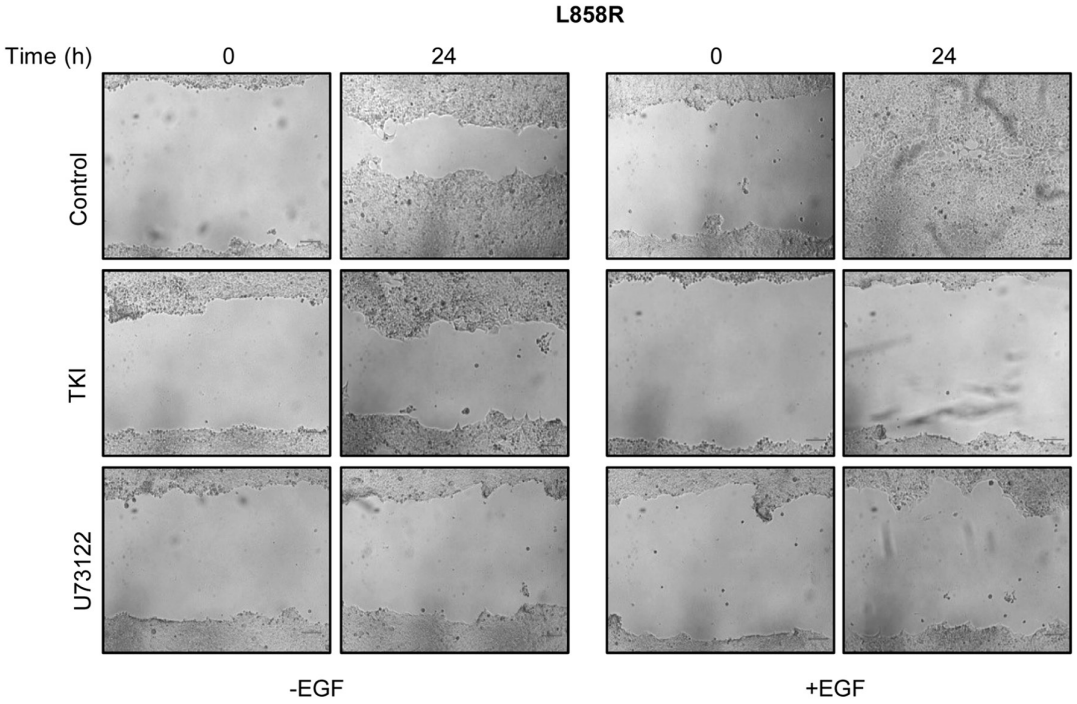


Figure S5. Migration of cells expressing T790M receptor. The experiment was performed as described in Fig. 4. Wound closure was measured at 0 and 24 h post-treatment.

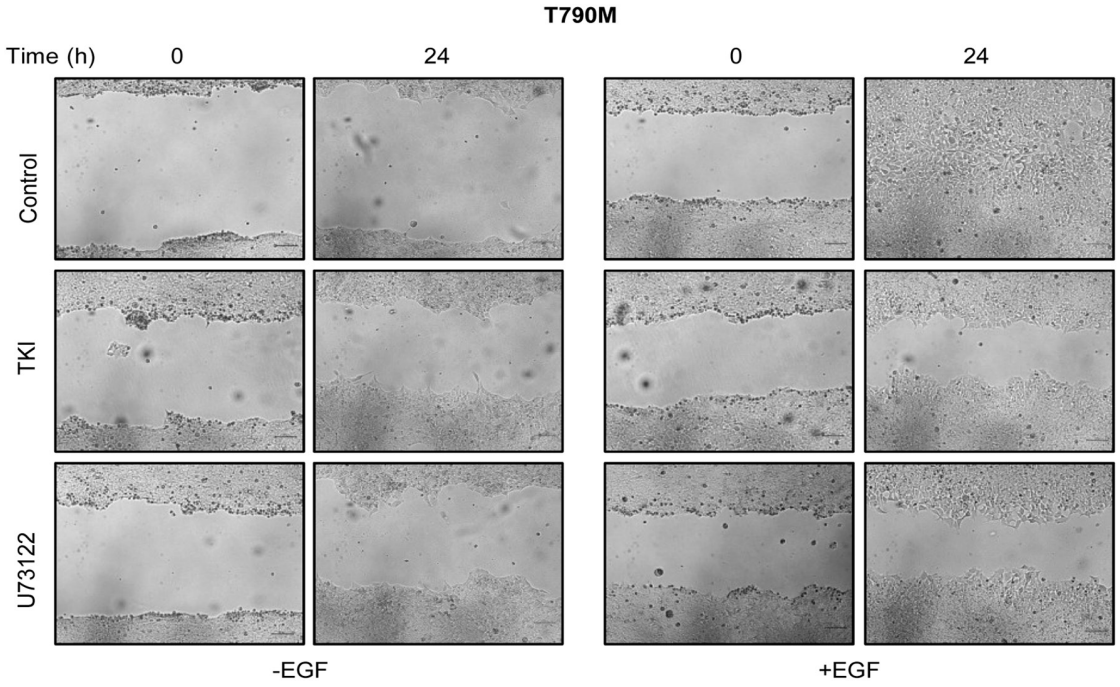


Figure S6. Migration of cells expressing L858R/T790M receptor. The experiment was performed as described in Fig. 4. Wound closure was measured at 0 and 24 h post-treatment.

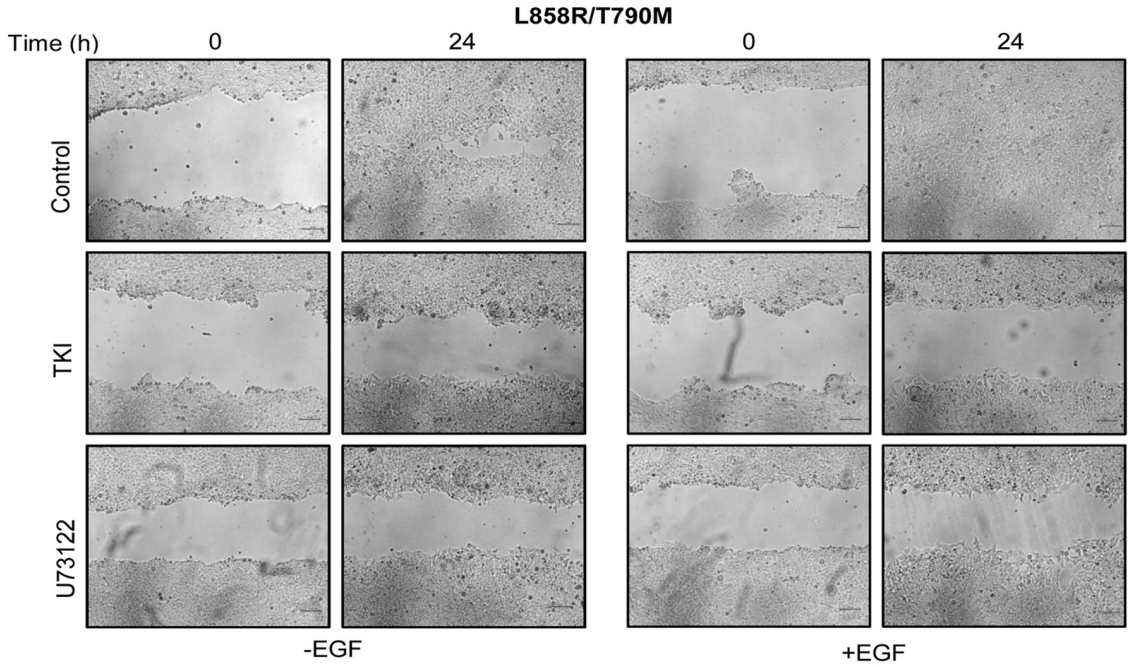


Figure S7. PLC inhibitor does not inhibit the proliferation of cells expressing mutant receptors. Approximately 5,000 cells expressing Wt or mutant receptors were mixed with indicated concentration of U73122 in 0.4% low melting agarose and overlaid on bottom layer of 0.8% soft agar. After 10 days, colonies were stained with 0.005% crystal violet for 1 h. (A) Colonies obtained in soft agar assay, (B) graph representing the number of colonies counted using ImageJ software. PLC, phospholipase.

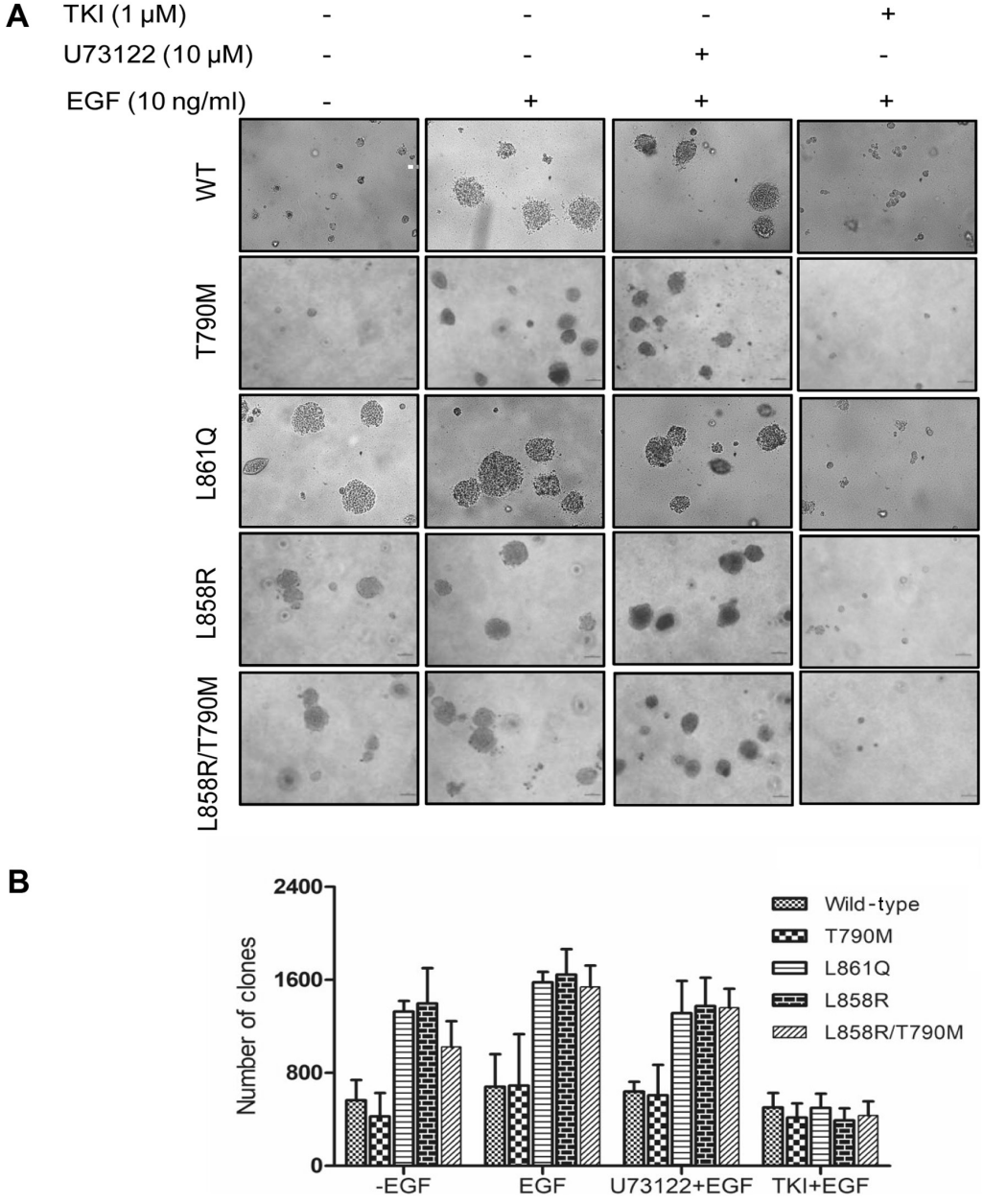


Figure S8. Regulation of Akt and Erk1/2 signaling following inhibition of PLC activity. Protein lysates were prepared from cells expressing Wt and mutants were serum-starved and treated with various concentrations of the PLC inhibitor, U73122, for 48 h. Lysates were subjected to SDS-PAGE followed by western blot analysis with phosphor-specific and total antibodies. (A) Expression of p-EGFR, EGFR p-PLCγ1, PLCγ1, p-Akt, Akt, p-Erk1/2, Erk1/2 and β-actin, (B) quantification of phospho vs. total proteins. PLC, phospholipase.