Supplementary Figure Legends

Figure S1. *MAPK1* copy number as determined by quantitative PCR (QPCR). The *EGFR* mutant MA1 cells are used as a negative control. The PC9 GR4 cells contain 2 copies of MAPK1 while the WZR clones contain between 10 and 14 copies of MAPK1. Each column represents the average of 3 independent experiments. Error bars indicate standard deviation.

Figure S2. Quantitative PCR for *EGFR*, *MAPK1* and *MAPK3* expression from WZ4002 sensitive and resistant cells. **A.** PC9 GR and WZR. **B.** H1975 and H1975 WZR. The data are normalized to the parental cells and represent mean values experiments performed in triplicate. Error bars denote standard deviation.

Figure S3. The MEK inhibitor GSK1120212 reverses resistance to WZ4002. Cells were treated with WZ4002 at the indicated concentrations or in combination with GSK1120212 (WZR10 only). Viable cells were measured after 72 hours of treatment and plotted relative to untreated controls.

Figure S4. Cell viability assays from PC9, PC9 GR4 and WZR 10 cells following exposure to control (DMSO), gefitinib (1 μM), WZ4002 (100 nM), CI-1040 (3 μM) or Cmpd 11e (1 μM) or their combinations for 72 hours. The mean values and SEM from 3 independent experiments is shown.

Figure S5. Downregulation of ERK 1/2 detected by Western blotting following infection with control (NT) or 3 different *ERK2* (*MAPK1*) specific shRNAs. Cell extracts were immunoblotted to detect the indicated proteins.

Figure S6. The PI3K or AKT inhibition does not restore sensitivity to WZ4002 in PC9 WZR or H1975 WZR 6 cells. **A.** PC9GR4 or WZR12 cells were treated with WZ4002 alone at the indicated concentrations or in combination with PI-103 (1 μM). Viable cells were measured after 72 hours of treatment and plotted relative to untreated controls. **B.** PC9GR4 or WZR12 cells were treated with WZ4002 alone at indicated concentrations or with PI-103 (1 μM) for 6 hours. Cell extracts were immunoblotted to detect the indicated proteins. **C.** PC9GR4 or WZR10 cells were treated with WZ4002 alone at the indicated concentrations or in combination with the AKT inhibitor MK-2206 (1 μM). MK-2206 effectively inhibited AKT in both cells (right). **D.** H1975 or WZR6 cells were treated with WZ4002 alone at the indicated concentrations or in combination with PI-103 (1 μM). Viable cells were measured after 72 hours of treatment and plotted relative to untreated controls.

Figure S7. Activated *MEK1* allele causes resistance to EGFR kinase inhibitors. **A.** PC9 GR4 cells expressing either green fluorescent protein (GFP) or *MEK 1* K57N were treated with WZ4002 at indicated concentrations. Viable cells were measured after 72 hours of treatment and plotted relative to untreated controls. **B.** Cells from A were treated with increasing concentrations of WZ4002 for 6 hours. Cell extracts were immunoblotted to detect the indicated proteins. **C.** PC9 cells expressing either GFP or *MEK1* K57N were treated with WZ4002 at indicated concentrations. Viable cells were measured after 72 hours of treatment and plotted

relative to untreated controls (left). MEK1 K57N expression and increased ERK 1/2 phosphorylation is confirmed by Western blotting (right). **D.** H1975 cells expressing either GFP or *MEK1* K57N were treated with WZ4002 at indicated concentrations. Viable cells were measured after 72 hours of treatment and plotted relative to untreated controls (left). MEK1 K57N expression and increased ERK 1/2 phosphorylation is confirmed by Western blotting.

Figure S8. A. Quantitative PCR of genes in MEK/ERK transcriptional output in PC9 GR4 and WZR cells. The data are normalized to the PC9 GR4 cells. The data represent mean values from experiment performed in triplicate. Error bars denote standard deviation. **B.** PC9 cells were treated with gefitinib (1 μM) or WZ4002 (100 nM) following transfection with control (NT) or *DUSP6* siRNA and viable cells were measured after 72 hours of treatment and plotted (mean +/- SD) relative to untreated controls. *; $p < 0.05$ DUSP6 vs. NT

Figure S9. Evolution of WZ4002 resistance *in vivo*. **A.** Change in tumor volume over time in *EGFR* DelE746_A750/T790M mice (n = 3) treated with WZ4002. Each curve represents and individual mouse. **B.** Mean (+/- SD) changes in tumor volume over time following WZ4002 treatment of *EGFR* DelE746_A750/T790M (n = 3) or L858R/T790M (n = 5) mice.

Figure S10. Low power view of Immunohistochemical analyses using indicated antibodies of tumors from lungs of *EGFR* L858R/T790M untreated and WZ4002 treated (10 weeks) mice. EGFR phosphorylation but not ERK 1/2 phosphorylation is inhibited in the tumor nodules. H&E; hematoxylin and eosin

Figure S11. Evaluation of WZ4002 resistant tumors from *EGFR* DelE746_A750/T790M or L858R/T790M mice. **A.** FISH analyses for *Mapk1* from control and WZ4002 drug resistant tumors from EGFR L858R/T790M mice. There is no evidence of amplification. *Mapk1* (red); reference probe RP23-122A24 (green). **B.** Evaluation for changes in NF1 protein from sensitive and resistant mouse tumors. Tumor extracts were immunoblotted to detect the indicated proteins. **C.** Quantitative PCR for *Nf1* expression from WZ4002 sensitive and resistant tumors. *Nf1* expression is normalized to *Gapdh*.

Figure S12. Quantitative PCR of genes in MEK/ERK transcriptional output in *EGFR* DelE746_A750/T790M (left) and L858R/T790M (right) drug sensitive (Sens) and resistant tumors. The expression values are normalized to 1 control tumor for each genotype. Decreased *Dusp6* expression is observed in mouse 122, 2092, 2093 and 2129 compared with untreated controls.