SUPPLEMENTAL FIGURE LEGENDS:

Supplemental Figure 1. MET is inducible in MET, TD/MET, and TL/MET models

(A) Western blots of lung lysates from two different founders of CCSP-rtTA/MET bitransgenic mice, CCSP-rtTA/EGFR L858R/T790M (TL)/MET tri-transgenic mice, and CCSP-rtTA/EGFR deletion/T790M (TD)/MET tri-transgenic mice fed with diets without (-) or with (+) doxycycline, demonstrating MET expression in TD/MET and TL/MET mice. Cell lysate from gefinitinib-resistant, MET-amplified HCC827GR6-GFP cells (1) was used as a positive control for hMET expression. (B) Western blot showing phosphorylation of hMET upon doxycycline treatment in MET and TD/MET mice. TD, MET (founder#16 and founder#31), and TD/MET transgenic mice were administered a diet without (-) or with doxycycline (+) for 12-16 weeks. TD/MET mice with similar tumor burden verified by MRI were subjected to treatment with vehicle or crizotinib for 2 weeks as indicated. Two hours after the last dose, mice were sacrificed and tumor lysate were prepared. 350µg per lane of lysates were resolved on SDS-PAGE and Western blot was performed with the indicated antibodies.

Supplemental Figure 2. Quantification of hMET expression in mouse models

Quantitative RT-PCR analysis of total human MET transcript in lung or tumor from transgenic mice maintained on diets without (-) or with (+) doxycycline. Each sample was amplified in triplicate for quantification of total MET and β-actin transcripts. Primers and probes for human MET were purchased from Life Technologies (Assay ID: Hs01565584_m1). Mean expression level of human MET from CCSP-rtTA/MET bi-transgenic mice on food without doxycycline was arbitrarily designated as 1. Data were analyzed by relative quantitation using the ΔΔCt method with normalization to β-actin. Error bars, SEM.
Supplemental Figure 3. Comparison of the tumor counts in mutant EGFR lung tumors with or without expression of hMET

EGFR L858R/T790M (TL) mice or EGFR TL + MET overexpression (TL/MET) mice were sacrificed after 12 weeks of doxycycline treatment for pathological analysis. The ratio of tumor area/total lung area was measured using ImageJ software. For the measurement of tumor area and total lung area, microscopic fields were chosen under low magnification (x20), and saved on a computer as JPEG files. Areas of interest were drawn manually as appropriate to calculate the ratio of tumor area/total lung area. There was no significant difference in the ratio of tumor area/total lung area between TL and TL/MET mice.

Supplemental Figure 4. Representative MRI images prior to and after treatment in TL and TL/MET mice.

Tumor-bearing mice were subjected to MRI imaging prior to and after two weeks of treatment with the indicated drugs. Representative MRI images show tumor regression in the mice with EGFR L858R + T790M mutation (EGFR TL) but not in EGFR TL + MET overexpression (EGFR TL/MET) after 2 weeks of WZ4002 treatment. Tumor also does not respond to treatment with single agent of crizotinib or 17-DMAG in either TL or TL/MET mice. However, combination therapy with WZ4002/crizotinib or WZ4002/17-DMAG results in substantial tumor regression.

Supplemental Figure 5. Xenografts of HCC827GR6 cells ectopically expressing EGFR E746_A750del/T790M are sensitive to the combination of WZ4002 and 17-DMAG.

Xenografts in nul/nu mice were established using gefitinib-resistant, MET-amplified HCC827GR6 cells ectopically expressing E746_A750del/T790M. WZ4002 was administrated at 50mg/kg daily by oral gavage, and 17-DMAG was administrated at 20mg/kg daily by intraperitoneal injection. Tumors were measured twice weekly. Mean tumor volumes are shown. Bars, + SD.
Supplemental Figure 6. The WZ4002/crizotinib or WZ4002/17-DMAG combinations suppress phosphorylation of EGFR, Akt, and Erk in TD and TD/MET to a greater extent than individual treatments.

Tumor nodules from TD and TD/MET mice treated with two doses of vehicle, WZ4002 alone, crizotinib alone, crizotinib/WZ4002 or WZ4002/17-DMAG 2 hours prior to sacrifice were subjected to Western blot with the indicated antibodies. p-EGFR; anti phospho-EGFR (Y1068), p-Akt: anti phospho-Akt (S473), pErk: anti-phospho-Erk (T202/Y204).
REFERENCES