Supplementary Information

Sustained cellular effects of MET inhibitors like tepotinib are driven by prolonged target engagement and lysosomal retention

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Supplementary Figure S1

EBC-1 cells were treated with selected MET inhibitors for 1 hour, before media were replaced three times to remove the compound from the supernatant, and 72-hour viability was measured. Dose–response curve for crizotinib (A) and savolitinib (B) show activity after washout compared to non-washed control treatment. MET phosphorylation 24 hours after washout for crizotinib (C) and savolitinib (D) was measured with 0.5 µM tepotinib as inhibitor control and DMSO as vehicle. DMSO: dimethyl sulfoxide; MET: mesenchymal-epithelial transition factor; NSCLC: non-small cell lung cancer; wt: wild type.
Supplementary Figure S2

EBC-1 cells were treated with selected MET inhibitors for 1 hour, before media were replaced three times to remove the compound from the supernatant, and 72-hour viability was measured. For the unspecific binding control (“plastic binding control”), plates without cells were processed like washout samples. Cells were added after the washing steps to check the effect of residual compound in a plate. MET: mesenchymal-epithelial transition factor; NSCLC: non-small cell lung cancer; wt: wild type.
Supplementary Figure S3

Cellular target engagement of MET was determined by NanoBRET assay. Cells were treated for 1 hour with the respective MET inhibitors. For washout samples, IC$_{50}$ determination was performed after 2 and 4 hours of incubation following washout: media were replaced three times to remove the compound from the supernatant.
DMSO and tracer were used as 100% occupancy control. Shift factors were calculated using the ratio of washout and control IC<sub>50</sub> values. DMSO: dimethyl sulfoxide; MET: mesenchymal-epithelial transition factor.
Cellular target engagement of MET was determined by NanoBRET assay. Cells were treated for 1 hour with the respective MET inhibitors. For washout samples, IC$_{50}$ determination was performed after 4 hours of incubation following washout: media were replaced three times to remove the compound from the supernatant. DMSO and tracer were used as 100% occupancy control. Mean IC$_{50}$ and standard deviation are based on 3–6 independent experiments and analyzed by multiple unpaired Student $t$-tests. **$P < 0.01$. DMSO: dimethyl sulfoxide; MET: mesenchymal-epithelial transition factor.
EBC-1 cells were co-treated with selected MET inhibitors and chloroquine for 1 hour, before media were replaced three times to remove the compound from the supernatant, and MET phosphorylation was measured after 24 hours. pMET levels after co-treatments compared to mono-treatments for crizotinib (A), capmatinib (B), and savolitinib (C). Mean ± SEM; N = 3; two-way ANOVA; *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001. ANOVA: analysis of variance; pMET: phosphorylated mesenchymal-epithelial transition factor; SEM: standard error of the mean.
Supplementary Figure S6

LysoTracker staining (green) and nuclear counterstain using DAPI (blue). Imaging was performed using the high content imager ImageXpressULTRA (Molecular Devices) and a 20x objective. Scale bars indicate 50 µm. (A) EBC-1 cells treated for 1 hour with 10 µM tepotinib and 0.5% DMSO (control). (B) A549 cells treated for 1 hour with 10 µM tepotinib and 0.5% DMSO (control) and immunofluorescent co-staining of lysosomes using LAMP1 (red). (C) A549 cells treated for 1 hour with 50 µM chloroquine and images quantified counting vesicles using MetaXpress software (version 6.6.1.42). Two-point normalization using 0.5% DMSO (100%) and 50 µM chloroquine (0%). DAPI: 4′,6-diamidino-2-phenylindole; DMSO: dimethyl sulfoxide; LAMP1: lysosomal-associated membrane protein 1.