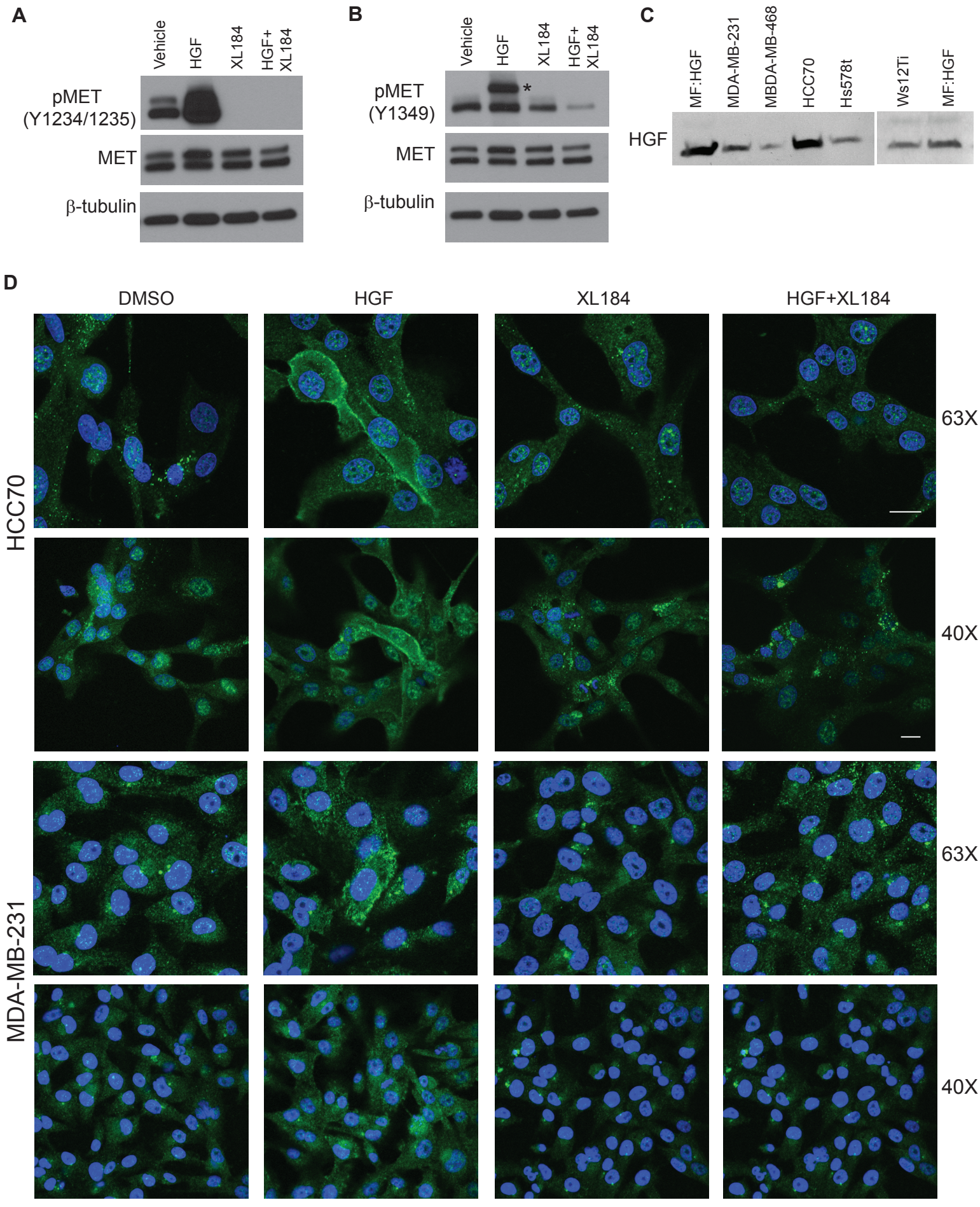
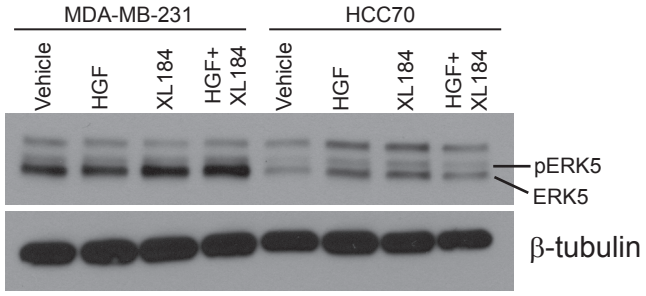
Supplementary Methods

MAME Cultures of TNBC Cells ± MF:HGF or CAFs

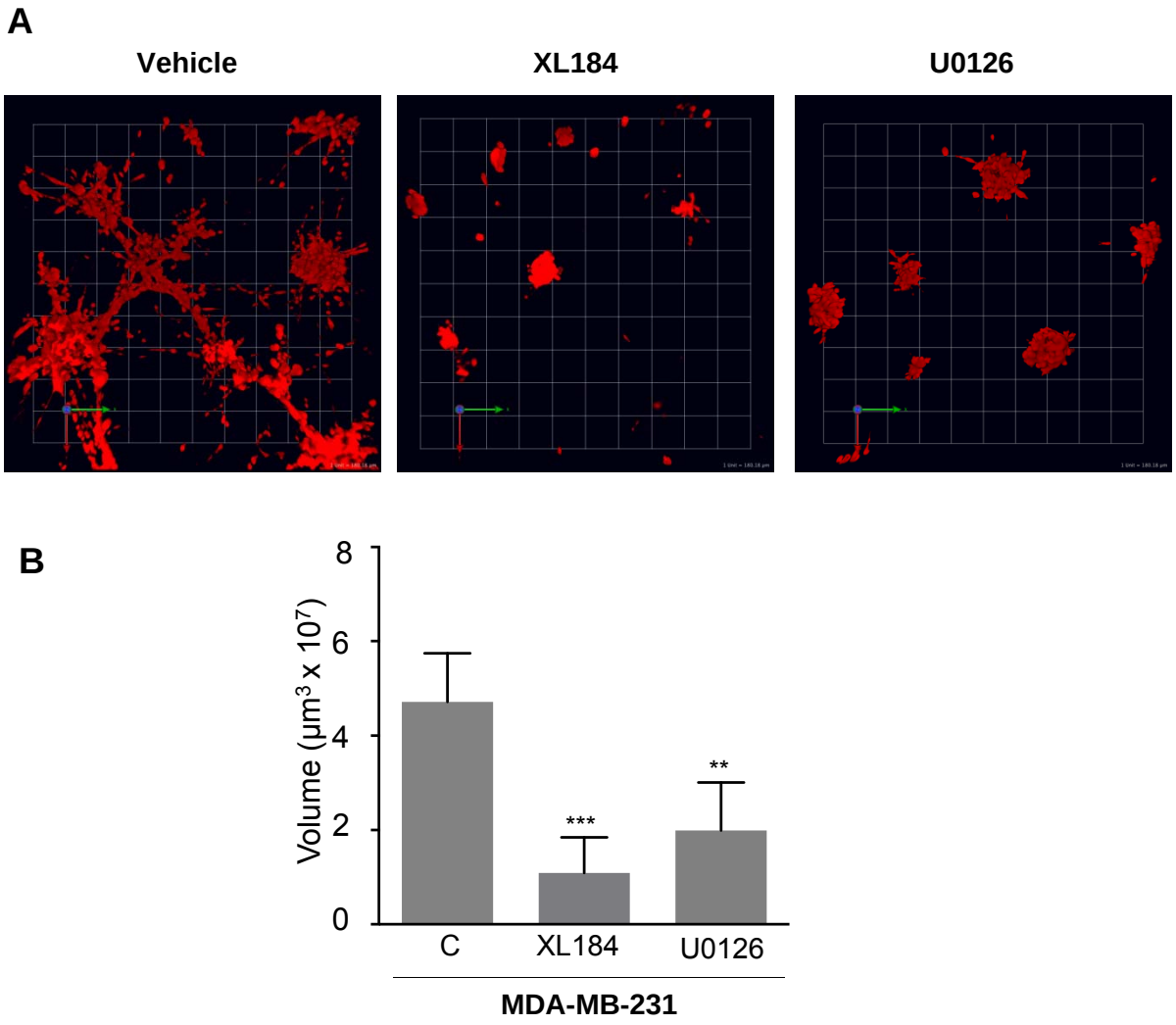
MDA-MB-231 and HCC70 cells were grown as mono-cultures or in co-cultures with CAF or MF:HGF cells. Details for establishing and analyzing MAME cultures are described in detail in text and video (34). For co-culture a ratio of 5:1 of tumor cells and fibroblasts, respectively, was used. Cells were seeded on glass coverslips coated with 50 µl rBM, overlaid with 2% rBM and grown for periods up to 10 days. Cells were treated with XL184 starting at the day of seeding or 4 days after seeding; XL184 was replaced every 2 days. Cells were grown for 6 days after the initial addition of XL184 and imaged live on a Zeiss LSM 510 Meta NLO microscope with a water-dipping objective. Z-stack images were acquired; Volocity software (Perkin Elmer) was used to generate 3-D reconstructions of the Z-stack images, quantify volumes and segment 3D structures into central cores and invasive outgrowths before quantifying volumes.


A
Vehicle
HGF
XL184
HGF+ XL184
pMET (Y1234/1235)
MET
β-tubulin
B
Vehicle
HGF
XL184
HGF+ XL184
pMET (Y1349)
*
MET
β-tubulin
C
MF:HGF
MDA-MB-231
MBDA-MB-468
HCC70
Hs578t
Ws12Ti
MF:HGF
HGF
D
DMSO
HGF
XL184
HGF+XL184
HCC70
MDA-MB-231
63X
40X
63X
40X

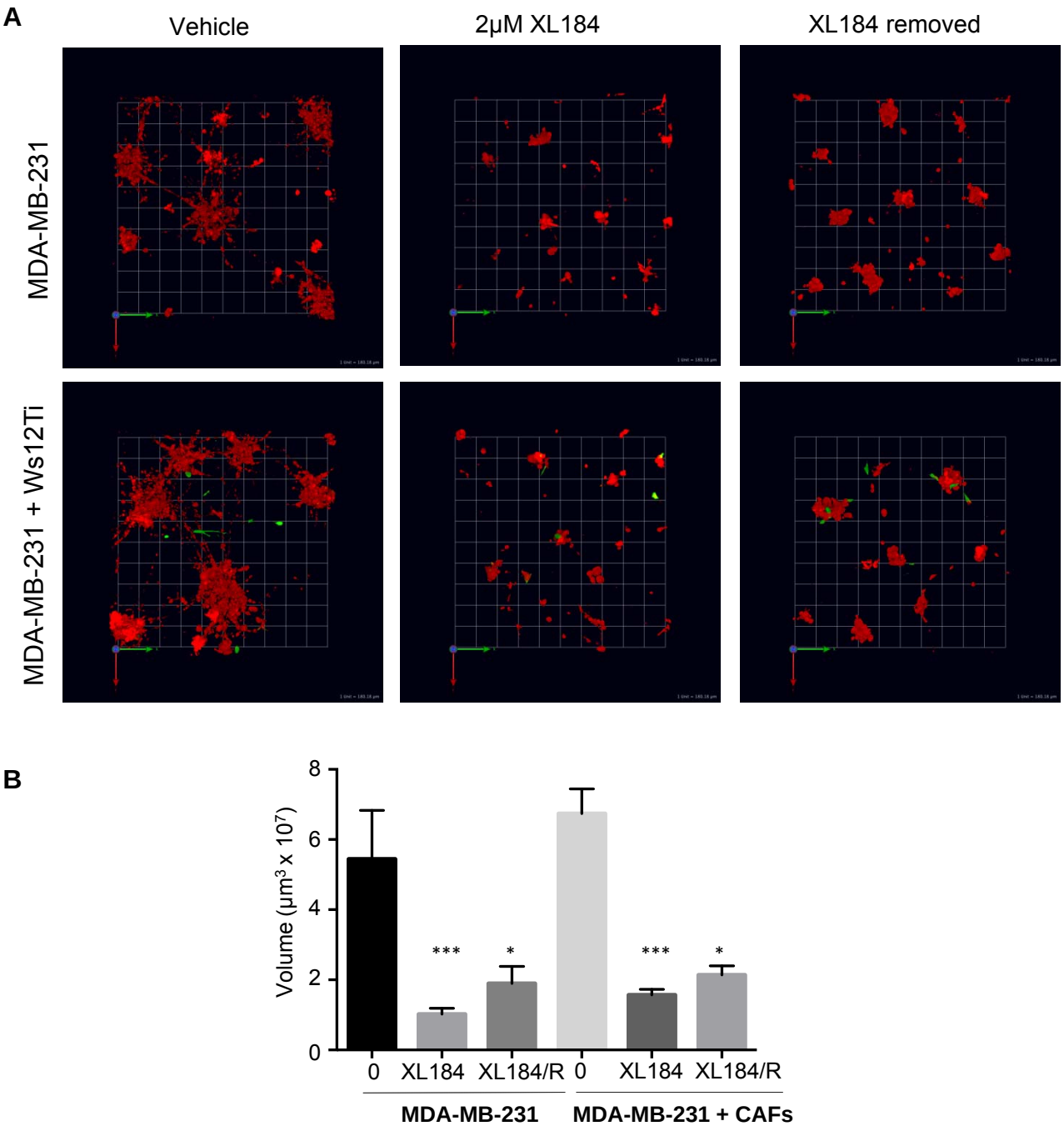
Supplemental Figure 1: Immunoblot and immunofluorescence analysis of MET and HGF in TNBC and fibroblast cell lines. A) HGF induces high levels of MET Y1234/1235 phosphorylation in HCC70 cells which is completely inhibited in the presence of XL184. B) HGF induction of MET Y1349 phosphorylation was observed (upper band as noted by *) which is inhibited by XL184 treatment. C) HGF expression was examined in the conditioned media of TNBC cell lines, normal mammary fibroblasts (MF:HGF) and CAF cells Ws12Ti. D) Confocal immunofluorescence analysis of pMET (Y1234/Y1235) in HCC70 and MDA-MB-231 cells is shown at 40X and 63X. Cells were treated with DMSO, XL184 (2μ uM), HGF(100 ng/mL), or XL184 + HGF. Blue pseudocolor represents DAPI nuclear stain. Scale = 20 μm.



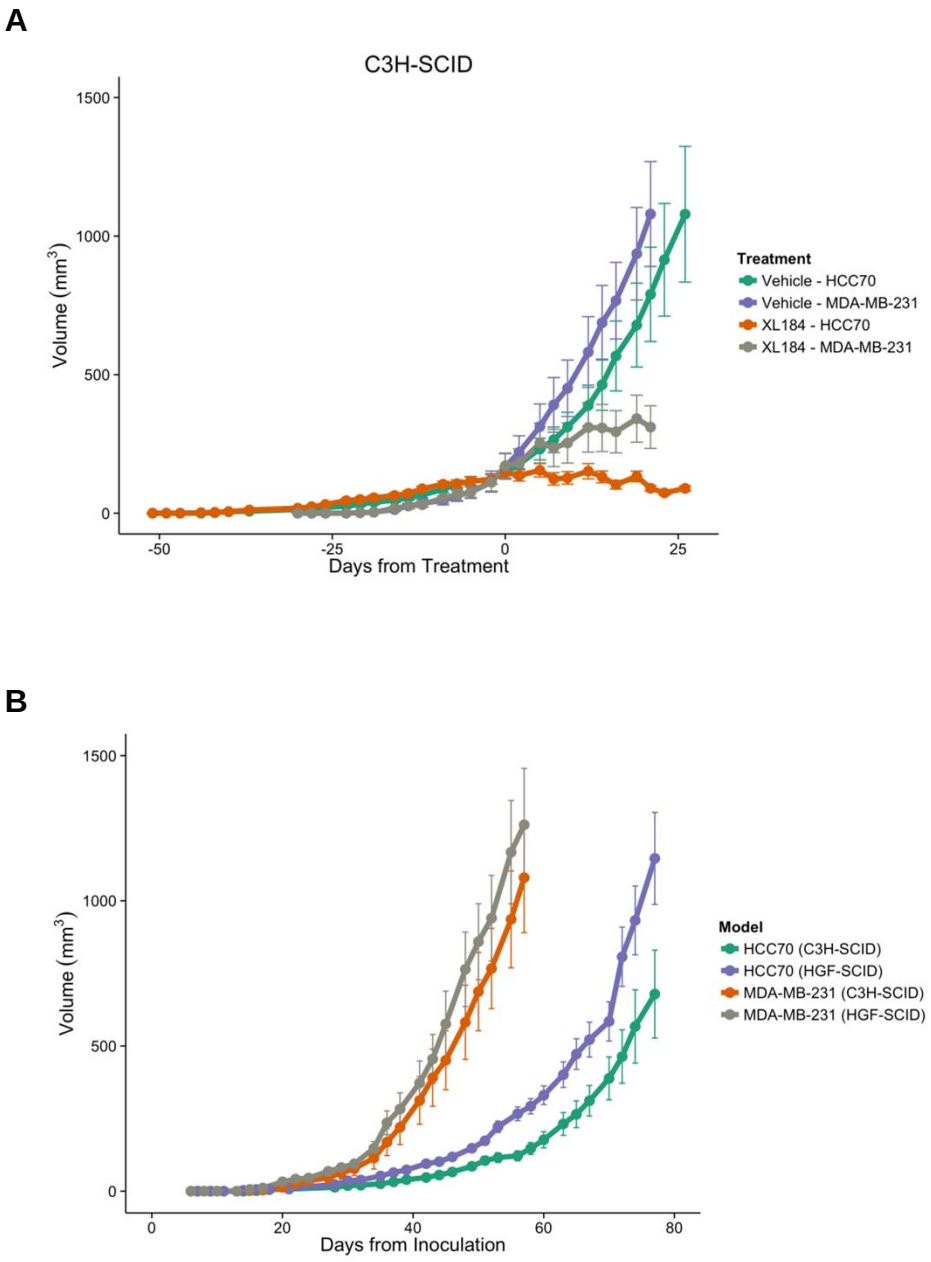
Supplemental Figure 2 – Met inhibition has minimal effect on ERK5 signaling in human TNBC cells. Immunoblot analysis was used to evaluate the effect of XL184 treatment on ERK5 signaling in MDA-MB-231 and HCC70 TNBC cells diverse TNBC cell lines. Cells were serum starved, treated with HGF or HGF + XL184 and then harvested 24 hrs later. Total ERK5 expression was slighltly upregulated in MDA-MB-231 cells treated with XL184. However no difference in pERK5 (see upshifted band) was observed with HGF or HGF+XL184 treatment.



Supplemental Figure 3 – XL184 and U0126 significantly reduce volume and outgrowths of MDA-MB-231 structures. MDA-MB-231- lenti RFP (red) were seeded on rBM overly cultures, drugs were added at day 4 (2 µM XL-184,10 µM U0126), and were imaged at day 10. (A) Representative 3D reconstructions were used to illustrate volume of MDA-MD-231 structures; one grid unit = 180 µm. (B) Volumes of MDA-MD-231 structures were quantified in 64 fields (16 fields/experiment and four independent experiments) and p-values were calculated by Student's t-tests (** $p < 0.01$, *** $p \leq 0.0005$).



Supplemental Figure 4 – Minimal growth recovery is observed after XL184 removal.
A) MDA-MB-231- lenti RFP (red) alone or together with Ws12Ti cells (green) were seeded on rBM overlay cultures. Vehicle control or 2 μM XL184 were added at day 0 and replaced every other day. XL184 was removed at day 6 and all structures were imaged at day 12. Panels on left are 3D reconstructions of Z-stacks of MDA-MB-231 and MDA-MB-231+CAFs with vehicle control; panels in middle (one grid unit = 180 μm). B) Volumes of structures were quantified in 64 fields (16 fields/experiment ; four independent experiments) and p-values were calculated by Student's t-tests (** $p < 0.01$, *** $p \leq 0.0005$).



Supplemental Figure 5: MET inhibition in C3H-SCID mice. A) Growth of orthotopic MDA-MB-231 and HCC70 tumors in C3H-SCID mice was significantly inhibited by 30 mg/kg XL184 treatment (MDA-MB-231, $p < 0.035$; HCC70 $p < 0.0001$). B) Growth of orthotopic MDA-MB-231 and HCC70 tumors in HGF-SCID and C3H-SCID mice is shown from time of inoculation. Tumor measurement started 7 days after inoculation. A significant increase in growth rate was observed for HCC70 cells in HGF-SCID mice ($p < 0.036$). Linear mixed-effects modeling was used test for significant differences in tumor growth.