Supplemental Figure Legends

Supplemental Figure S1. Schematic representation of components of IGF-1R/ErbB3 merged mechanistic signaling model. See main text and Supplementary File 1 for more information on model.

Supplemental Table S1. Mean ligand-stimulated pAKT (S473) levels (A) or simulated levels (B) in BxPC-3, AdRr and MCF7 cells that were used to generate 3D contour plots in Figure 1A.

Supplemental Figure S2. Western blots used to generate bar graphs in Figures 1, 3, & 4. (A) Caki-1 xenograft lysates (n=5 per group) used to generate bar graphs in Figures 1E & 1F. (B) BxPC-3 xenograft lysates (n=5 per group) used to generate bar graphs in Figures 3D-3F. (C) BxPC-3 xenograft lysates (n=4 per group) used to generate bar graphs in Figures 4A & 4B. (D) DU145 xenograft lysates (n = 4 per group) used to generate bar graphs in Figures 4D & 4E. (E) BxPC-3 *in vitro* lysates (representative of 2 independent experiments) used to generate bar graphs in Figure 2D.

Supplemental Figure S3. MM-141 is selective for IGF-1R and ErbB3, and is cross-reactive to relevant toxicology species. ELISA binding assays were performed and indicate that MM-141 binds to IGF-1R (A) and ErbB3 (B) but not to related receptors within the same family. ELISA binding assays were performed and indicate that MM-141 is cross-reactive to human, cynomolgus monkey, mouse, and rat IGF-1R (C) and ErbB3 (D). MM-141 blocks HRG binding to ErbB3 (E).

Supplemental Table S2. MM-141 is more potent than monospecific antibodies at inhibition of single ligand-induced pAKT (Ser473) signaling. BxPC-3 cells were pretreated for 1 hour with a full dose response of antibody, then stimulated for 15 minutes with the indicated concentrations of IGF-1 or HRG1b1-ECD. IC50 values were determined using the formula in the Supplemental Methods.

Supplemental Figure S4. MM-141 does not promote basal signaling. A549 (A,B) or BxPC-3 (C,D) cells were serum starved and treated with a full dose response of MM-141 OR individual ligands for 15 minutes (A,C) or 24 hours (B,D). Phosphorylation of AKT (Ser473) was assessed by ELISA. While ligands induce phosphorylation of AKT (predominantly at the 15 minute time point), MM-141 did not stimulate the activation of AKT at any time point or dose tested.

Supplemental Figure S5. Generation of isogenic BxPC-3 cell lines with stable knockdown of IGF-1R or ErbB3. (A) BxPC-3 cells were infected with lentivirus targeting IGF-1R or ErbB3. Cells were selected with Puromycin, and single cell clones were isolated. (B) Knockdown of IGF-1R or ErbB3 was determined by FACS analysis. IGF-1R:ErbB3 receptor ratio was 1:1 for Clone 2, 1:2 for Clone 11 and 1:4 for Clone 7 and Clone 9. (C) Mechanistic model was able to recapitulate the binding of MM-141 to engineered BxPC-3 cells. 2:1 (parental) line is shown in red, 1:1 (50% IGF-1R knockdown) is shown in blue, and 4:1 (50% ErbB3 knockdown) is shown in black.

Supplemental Table S3. Mean pAKT (S473) levels in engineered BxPC-3 cells that were used to generate 3D contour plots in Figure 3A.

Supplemental Figure S6. MM-141 maintains activity over a broad range of receptor profiles. (A-C) Isogenic BxPC-3 cell lines were serum starved, pretreated with a dose response of MM-141 or monospecific antibodies, and then stimulated with IGF-1 and HRG1b1-ECD as described. Phosphorylation of AKT (Ser473) was determined by ELISA analysis. (D) IGF-1R:ErbB3 ratio across isogenic cell lines.

Supplemental Figure S7. Mechanistic model can recapitulate signal inhibition properties of MM-141. (A) Engineered BxPC-3 cells were stimulated with increasing doses of IGF-1 in combination with HRG. The computational model can fit the signaling data across cell lines. (B-D) Engineered cell lines were pretreated for 1 hour with MM-141, then stimulated for 15 minutes with IGF-1 + HRG. pAKT (Ser473) phosphorylation was assessed by ELISA. Blue dots are experimental data, and red line is simulation using merged mechanistic model.

Supplemental Figure S8. Pharmacokinetic properties of MM-141. (A-B) Pharmacokinetic properties of MM-141 in mice (A) or cynomolgus monkeys (B). Squares represent the 25mg/kg dose, and diamonds represent the 5mg/kg dose. The solid lines represent the fits of the curves using Phoenix NLME.

Supplemental Figure S9. MM-141 controls gemcitabine-induced network adaptation. BxPC-3 cells were treated for 8 hours with control, MM-141, gemcitabine, or the combination. Lysates were prepared and Western blots performed for: (A) total IGF-1R or ErbB3; (B) pAKT (S473), pAKT (T308), or pFoxO1/FoxO3a. Signals were normalized to b-Actin, and then normalized to the signal of the control group. Bar graphs were generated from signal normalized to β-Actin and are representative mean and standard deviation from technical triplicate samples.

Supplemental Figure S10. MM-141 controls expression of IGF-1R and ErbB3-interacting RTKs. (A,B) In BxPC-3 xenograft (n=4) PD studies, the combination of MM-141 + Gemcitabine caused decreases in InsR and EGFR. (C,D) In CAKI-1 xenograft (n=4) PD

studies, MM-141 displayed greater control of InsR and EGFR relative to a combination of anti-IGF-1R and anti-ErbB3 IgGs.