SUPPLEMENTARY FIGURE LEGENDS

Figure S1. NT compounds are allosteric inhibitors of the full-length IGF1R, and induce translocation of IRS1 to the cytoplasm

(A) IC$_{50}$ values of IGF1R inhibition by the NT compounds. The ability of the NT compounds to inhibit the phosphorylation of poly(Glu,Tyr) (PGT) by IGF1R was tested in a cell free assay using an intact IGF1R. (B) NT compounds are ATP non-competitive and peptide-substrate non-competitive inhibitors of IGF1R. Cell-free kinase assay on full-length IGF1R resulted in Lineweaver-Burk plots showing that: (i) NT75 is non-competitive with ATP. (ii) NT157 is non-competitive with ATP. (iii) NT75 is non-competitive with a peptide substrate, PGT. (iv) NT157 is non-competitive with PGT. Similar results were obtained for NT52 and NT205 (not shown). (C) NT157 induces translocation of IRS1 to the cytoplasm within 30 min in the absence of IGF1 stimulation. Serum-starved A375 cells were treated as indicated, lysed, and fractionated by differential centrifugation.

Figure S2. The anti-proliferative effects of the NT inhibitors are maintained long after the inhibitors are washed out

(A,B) The anti-proliferative effect of the NT compounds is governed by the first exposure to the inhibitor. A375 cells were treated with NT75 or NT157 (+). 24 or 48 hrs later, either the medium was replaced with fresh medium containing inhibitor (+) or the medium was not replaced (-). (C, D) The anti-proliferative effect of the NT compounds is maintained following removal of the inhibitor. As in (A,B), except that (-) represents replacement of the medium with inhibitor-free medium. Cells were grown and treated in complete medium, and 72 hrs following the first addition of inhibitor survival was quantified by methylene blue staining.

Figure S3. NT157 inhibits IGF2-induced and insulin-induced PKB activation, without inhibiting the autophosphorylation of IR and IGF1R.

(A) PKB activation stimulated by IGF1, IGF2, or insulin is effectively inhibited by NT157. Serum-starved A375 cells were treated with NT157 for 4 hrs, and then stimulated with IGF1 (50 ng/ml), IGF2 (50 ng/ml) or insulin (100 nM) for 5 min. The cells were then lysed and analysed
by western blot. (B,C) **NT157 does not inhibit the autophosphorylation of IGF1R and IR.** Serum-starved A375 cells were treated with NT157 as indicated, and lysed. IGF1R or IR were immunoprecipitated and their phosphorylation status was analyzed by western blot.

**Figure S4. The NT-induced Ser-phosphorylation occurs in an IGF1-independent manner.**

(A) **Time-dependent activation of the ERKMAPK pathway in the absence of IGF1 stimulation.** Serum-starved A375 cells were treated with NT157 and lysed. (B) **mTOR and PI3K inhibition displays no effect on the NT157-induced Ser-phosphorylation of IRS1/2.** Serum-starved A375 cells were treated with inhibitor of mTOR (rapamycin) or with a dual inhibitor of mTOR and PI3K (PI-103) as indicated for 30 min, and then 3 μM NT157 was added for 4 hrs. The cells were then stimulated with IGF1 for 5 min and lysed. (C) **NT157 promotes recruitment of Shc to IGF1R in the absence of IGF1 stimulation.** Serum-starved A375 cells were treated with NT157 and lysed. 0.5 mg total protein was immunoprecipitated using 2 μg anti-IGF1R. (D) **The ERKMAPK pathway mediates NT157-induced Ser-phosphorylation of IRS1 in the absence of IGF1 stimulation.** Serum-starved A375 cells were treated with an inhibitor of mutated B-RAF (PLX4720) or an inhibitor of MEK1/2 (PD184352) for 30 min, NT157 was added for 4 hrs, and the cells were lysed. (E) **The NT157-induced C-RAF activation and IRS1/2 Ser-phosphorylation are Ras-dependent.** Serum-starved A375 cells were treated with a Ras inhibitor (FTS) for 30 min, then NT157 were added for 4 hrs, and the cells were lysed. (F) **The effects of NT157 are independent of IGF1R kinase activity.** Serum-starved A375 cells were treated with an ATP-competitive inhibitor of IGF1R (NVP-AEW541) in the indicated concentration for 30 min, and then NT157 was added for 4 hrs. The cells were lysed following 5 min stimulation with IGF1, and analyzed by western blot.

**Figure S5. NT compounds inhibit human ovarian cancer and hormone-refractory prostate cancer in xenograft models in mice.**

(A) **Increased survival of NT157-treated nude mice in a model of i.p. administered human ovary cancer.** 2.0x10^6 A2780 cells were injected i.p. into female nude mice (8 mice/group) and developed highly vascular and aggressive tumors in the peritoneum. Treatments with NT157 (100 mg/kg, i.v.,3 times a week, every other day) or vehicle (i.v.,3 times a week, every other day) were initiated seven days later for 3 weeks only.
(B) *Growth inhibition of ovarian cancer tumors in mice by NT75.* 2.0x10⁶ A2780 cells were injected s.c. into female nude mice (5 mice/group). When tumor volumes reached 25 mm³, administrations of NT75 (daily for 11 days, 25 mg/kg, i.p.), CDDP (twice a week, 8 mg/kg), or vehicle were initiated. (C) *Growth inhibition of human refractory prostate cancer tumors in male mice by NT52.* 2.0x10⁶ PC3 cells were injected s.c. into male nude mice. When tumor volumes reached 25 mm³, daily i.p. administrations of vehicle or NT52 (50 mg/kg) were initiated. In both s.c. xenograft models tumor dimensions were measured twice a week. Results are presented with standard errors.

**Figure S6. Combined treatment with NT157 and PLX4032 induces a synergistic cytotoxic effect in 451-Lu melanoma cells, and NT157 reverses the over-expression of IRS1/2 in Vemurafenib-resistant Mel1617-BR cells.**

(A) **Synergistic cytotoxic effect of NT157 and PLX4032 in PLX4032-sensitive 451-Lu cells.** In the PLX4032-sensitive (Parental) 451-Lu cells, PLX4032 inhibits the ERKMAPK pathway, whereas NT157 inhibits the IGF1R/IRS axis, and thus the combined effect is synergistic. Cells were treated with the indicated concentration of NT157, and PLX4032 (0.1 µM) was added 4 hour later. Surviving cells were quantified by methylene blue 72 hrs later, indicating synergism.

Right: Additive cytotoxic effect of NT157 and PLX4032 in resistant 451-Lu-BR cells. In the PLX4032-resistant 451-Lu-BR cells, PLX4032 has no effect on the signaling through ERKMAPK, and thus combination with NT157 yields an additive effect. Cells were treated with the indicated concentration of NT157, and PLX4032 (9 µM) was added 4 hrs later. Methylene blue staining 72 hrs later indicated additivity. (B) **Treatment with PLX4032 induces up-regulation of IRS1/2 in the parental Mel1617 cells, resulting in activation of IGF1-induced signaling to PKB.** Serum-starved Mel1617 cells were treated with 1 µM PLX4032 for 20 hrs, stimulated with IGF1 and lysed. (C) **IRS1 and IRS2 levels are increased in PLX4032-resistant human melanoma Mel1617-BR cells, as compared to the parental PLX4032-sensitive human melanoma Mel1617 cells.** Serum-starved Mel1617-BR cells (R) and Mel1617 cells (P) were stimulated with IGF1 and lysed. (D) **NT157 induces Ser-phosphorylation and degradation of IRS proteins in Mel1617-BR cells.** Serum-starved cells were treated with 1 µM PLX4032 for 1 hour or with 3 µM NT157 for 4 hrs, stimulated with IGF1 and lysed. (E) **NT157 effectively inhibits the proliferation of Mel1617 and Mel1617-BR cells.** Mel1617 and Mel1617-BR cells were grown in complete
medium for 24 hrs and then treated with different concentrations of NT157 for 72 hrs. The amount of surviving cells was quantified by methylene blue staining and IC_{50} values were derived.