Supplementary Material

Supplementary Materials and Methods:

Gene expression analysis: For gene expression studies in mice, total RNA was extracted from tumor samples using TRIZOL according to the manufacturer's instructions (Invitrogen, Carlsbad, CA). RNA was then purified using Qiagen RNeasy miniprep cleanup kit per manufacturer instructions (Qiagen, Valencia, CA). cDNA was generated using oligo-dT primers as previously described (1). Real time PCR (RT-PCR) was then performed on an ABI Prism 7500 Fast Real-Time PCR system using SYBR Green PCR Master mix according to the manufacturer's recommendations (Applied Biosystems, Foster City, CA). The following primers were used: mouse *Igf2-r* (5'-CTGGAGGTGATGAGTGTAGCTCTGGC-3'and 5'-GAGTGACGAGCCAACACAGACAGGTC-3'), mouse *Igf1* (5'-GGACCAGAGACCCTTTGCGGGG-3'and 5'-GGCTGCTTTTGTAGGCTTCAGTGG-3'), mouse *Igf1r* (5'-GCTTCGTTATCCACGACGATG-3' and 5'-GAATGGCGGATCTTCACGTAG-3'), mouse *Igf2* (5'-GTCGATGTTGGTGCTTCTCATC-3' and 5'-GGGTATCTGGGGAAGTCGT-3'), mouse *IR-A* and *IR-B* isoforms (5'-CAGAAGCACAATCAGAGTGAG-3' and 5'-GTGTGGTGGCTGTCACATTC-3'). Expression of gene products was normalized to levels of *GAPDH*. Primer sequences for mouse *GAPDH* were: 5'-TGCACCACCAACTGCTTAG-3'and 5'-GGATGCAGGGATGATGTTC-3'. For human samples, RT-PCR was performed by using TaqMan probes as previously described (2). Expression levels in human tumors were reported previously (2) but comparison to skeletal muscle expression is newly reported here.

RNA-interference: Unpooled mouse *Igf1r* siRNA (Cat# J-056843-06; Dharmacon, Lafayette, CO) was transfected into mouse tumor cells using Lipofectamine-2000 (Invitrogen). The cells were incubated with *Igf1r* siRNA for 6 days, after which the cells were lysed and the lysate was used for western blot analysis. Scrambled siRNA (Dharmacon) was used a control.

Supplementary Figure Legends:

Figure S1. Chemical structures for compounds in this study (a) NVP-AEW541 (b) Lapatinib.

Figure S2. Characterization of CAM assay properties. (a) Standard curve showing a concomitant increase in bioluminescence signal with the increase in the number of tumor cells growing on a quail CAM. (b) Average weight of

quail embryos three days after tumor cells on a 3D scaffold were placed on quail CAMs and treated with vehicle (DMSO) or drugs (Imatinib, NVP-AEW541).

Figure S3: Quantitative RT-PCR showing increased mRNA levels of *Igf1r* in human and mouse alveolar rhabdomyosarcoma**.** (a) *Igf1r* and *Igfr2* along with their ligand *Igf2* are over expressed in human ARMS and ERMS compared to normal skeletal muscle [top panel]. (b) *Igf1r*, *Igf2 and Igf2r* are highly expressed in the primary tumors compared to the normal skeletal muscle in the mouse model of alveolar rhabdomyosarcoma. The *Igf1 receptor* not only is highly expressed in the primary tumors but also in metastatic tumors [bottom panel].

Figure S4: *Igf1r* siRNA treatment inhibits cell proliferation and phosphorylation of Igf1r and downstream mediators. (a) Cell viability assay performed on naive rhabdomyosarcoma primary cell cultures (U21089) treated with *Igf1r* siRNA. (b) Immunoblots showing that treatment of mouse rhabdomyosarcoma cell line (U21089) with 100nm *Igf1r* siRNA causes a reduction in the phosphorylation of Igf1r, Mapk, Akt, IRS1 and P70 S6 kinase.

Supplementary References:

1. Nishijo K, Chen QR, Zhang L, McCleish AT, Rodriguez A, Cho MJ, et al. Credentialing a preclinical mouse model of alveolar rhabdomyosarcoma. Cancer Res. 2009;69:2902-11.
2. Blandford MC, Barr FG, Lynch JC, Randall RL, Qualman SJ, Keller C. Rhabdomyosarcomas utilize developmental, myogenic growth factors for disease advantage: a report from the Children's Oncology Group. Pediatr Blood Cancer. 2006;46:329-38.