Supplementary Fig. 6

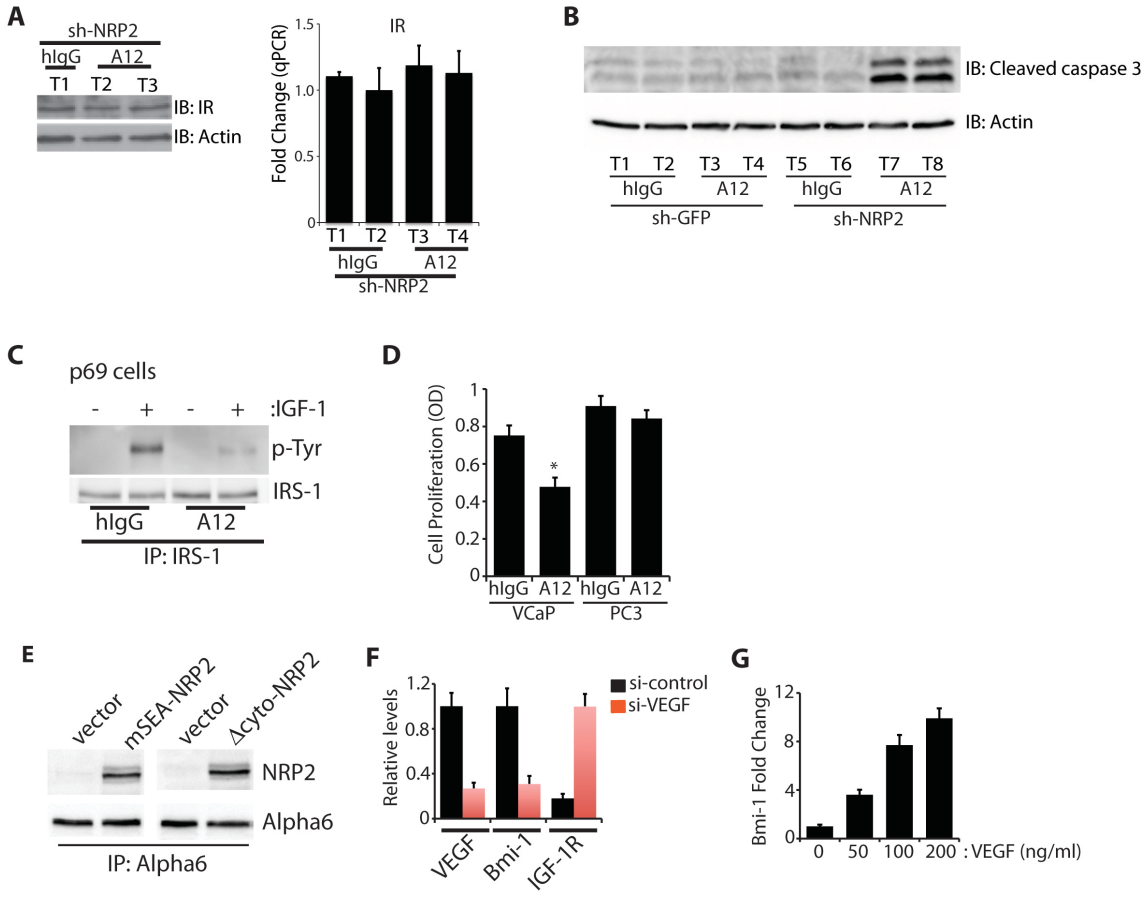

Figure S6. (A) Extracts from the xenograft tumors (sh-NRP2, hIgG or sh-NRP2, A12) were immunoblotted for the insulin receptor (IR) and actin (left blot). In addition, expression of IR mRNA isolated from the xenograft tumors (sh-NRP2+ hIgG or sh-NRP2 + A12) was quantified by qPCR (right graph). (B) Extracts from the xenograft tumors described in Figure 4, were immunoblotted for cleaved caspase 3 and actin. (C) Functional validation of A12 was done using p69 cells. p69 cells were serum-deprived for 12 hours in the presence of either A12 or hIgG and stimulated with IGF-1 (50 ng/ml) for 10 minutes. Cell extracts were used to immunoprecipitate IRS-1 and immunoblotted for phospho-tyrosine (p-Tyr) or IRS-1. (D) VCaP and PC3 cells were plated on 96-well plate (5,000 cells per well) and cells were cultured for 72 hours with either A12 or hIgG (10 μg/ml). Cell proliferation was measured using the MTT assay. (E) p69 prostate carcinoma cells, which lack NRP2 expression, were infected with lentivirus particles expressing NRP2 constructs lacking either the PDZ-binding domain [i.e., three terminal amino acids in the cytoplasmic domain (SEA)] domain or the entire cytoplasmic domain

(Δcyto). Extracts were used to immunoprecipitate the α6 integrin. Note that the association between NRP2 and α6 integrin is not affected by deletion of SEA (last three amino acid) or the cytoplasmic domain. (F) Graph represents densitometric analysis of band intensity for Fig 7A. Results are presented from three independent experiments. (G) PC3 cells expressing sh-VEGF were incubated with different concentrations of VEGF. Cells were analyzed for expression of Bmi-1 by qRT-PCR.