

Supplementary information

Title: Specific targeting of PDGFR β in the stroma inhibits growth and angiogenesis in tumors with high PDGF-BB expression

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The supplementary file contains:

-Supplementary tables (Table S1-S3)

-Supplementary figures and figure legends (Figures S1-S4)

Gene	Primer (Forward)	Primer (Reverse)
<i>Cspg4</i>	TTGGTCTACACCATCGAGCA	AGGGCTCCTCTGTGTGAGAA
<i>Xiap</i>	CGAGCTGGGTTTCTTTATACCG	GCAATTTGGGGATATTCTCCTGT
<i>Vegf-a</i>	CAGGCTGCTGTAACGATGAA	TATGTGCTGGCTTTGGTGAT

Table S1: List of primer sequences used in this present study.

Antibody	Assay	Dilution	Distributor
CD31	IF	1:100	MEC13.3; BD
podocalyxin	IF	1:200	AF1556; R&D Systems
PDGFRβ	IF	1:100	#3169; Cell Signaling
α-smooth muscle actin	IF	1:400	C6198; Sigma
NG2	IF	1:200	ab5320; Millipore
PDGF-BB	IF	1:100	#07-1437; Millipore
cleaved caspase 3	IF	1:400	#9664; Cell Signaling
phosphorylated Erk1/2	WB	1:1000	#9101, Cell Signaling
pS473 Akt	WB	1:1000	#4060, Cell Signaling
pY857 PDGFRβ	WB	1:1000	#3170, Cell Signaling
NG2	WB	1:1000	ab5320; Millipore
PDGFRβ	WB	1:1000	#3169; Cell Signaling
α-smooth muscle actin	WB	1:100	sc-53015; SantaCruz
Alix	WB	1:1000	home-made; Lennartsson et al., 2006
PDGFRα	WB	1:1000	home-made

Table S2: List of antibodies being used in this present study.

Inhibitors	Final concentration (μ M)	Distributor
1-NaPPI	1	Taconic Artemis
Imatinib	3	Novartis Pharma AG
CI-1040	3	Sigma
AG1296	10	Calbiochem

Table S3: List of low molecular weight inhibitors used for cell culture experiments in this present study.

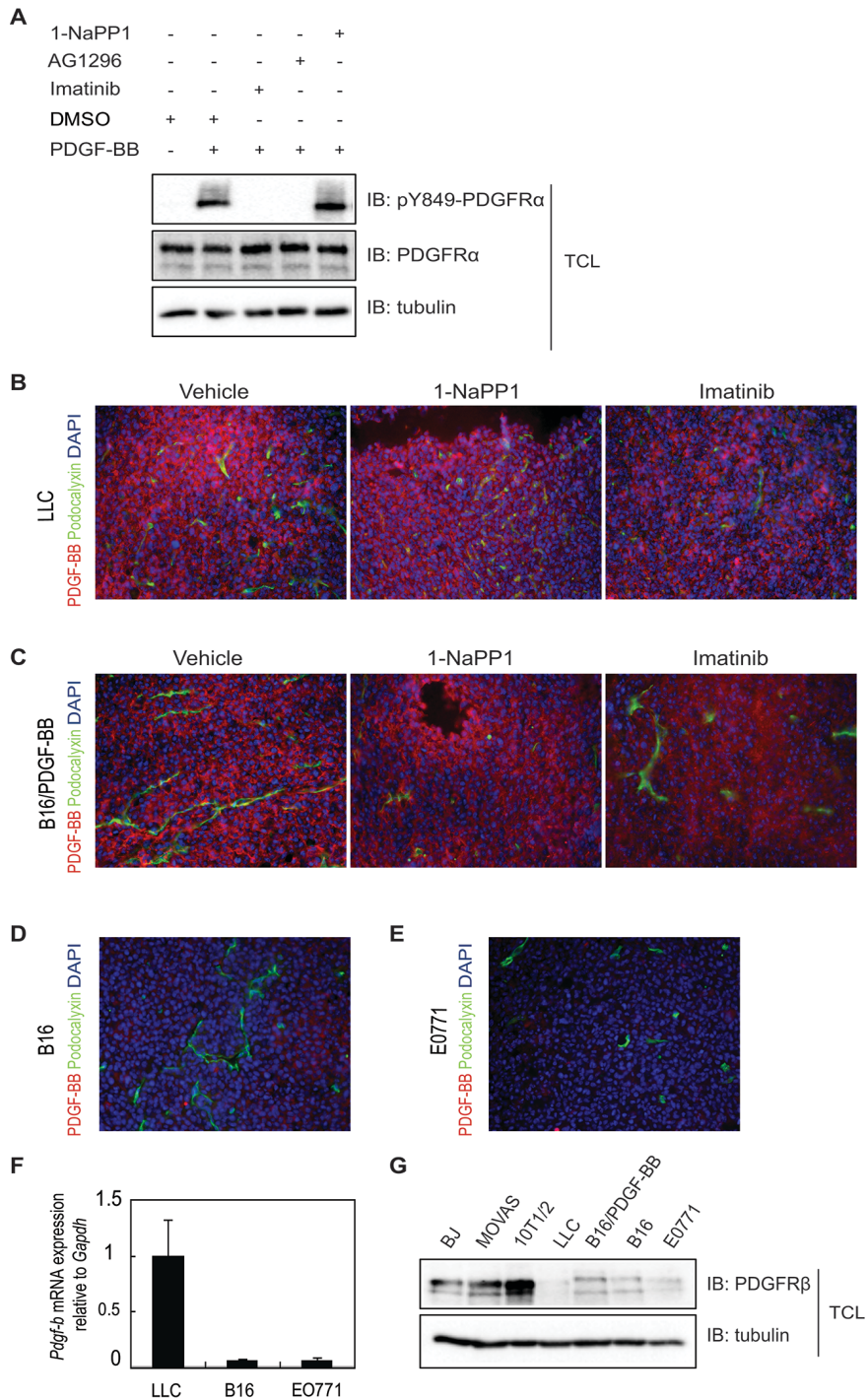


Figure S1: (A) 1-NaPP1 does not inhibit the kinase activity of PDGFR α . PAE-PDGFR α were serum-starved overnight, pre-treated with 1-NaPP1, imatinib or another PDGFR inhibitor, AG1296, for 1 h at 37°C, and then stimulated with 20 ng/mL PDGF-BB for 10 min. Total cell lysates (TCL) were collected and PDGFR α kinase activity was evaluated by immunoblotting (IB) using a pY849 PDGFR α antibody. α -tubulin was used as a loading control. (B-E) Co-immunostaining of PDGF-BB and podocalyxin in LLC, B16, B16/PDGF-BB and E0771 tumors (PDGF-BB, red; podocalyxin, green; DAPI, blue). (F) Analysis of *Pdgf-b* mRNA levels in B16, E0771 and LLC cells by quantitative PCR. Expression levels of *Pdgf-b* were quantified using *Gapdh* as an endogenous loading control. (G) Immunoblotting of multiple cell lines for PDGFR β ; IB for α -tubulin was used as a loading control.

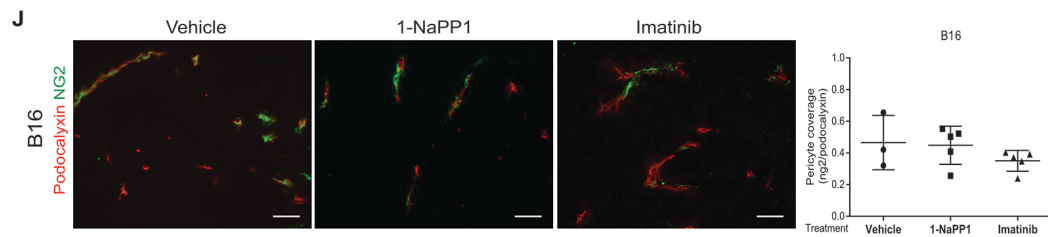
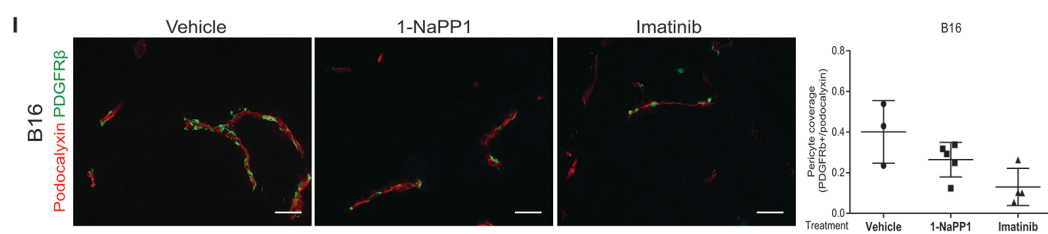
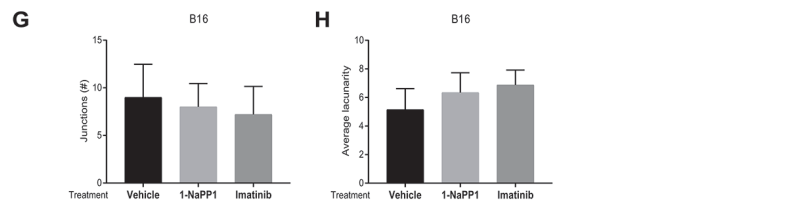
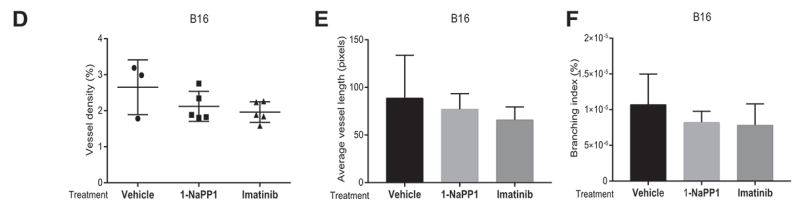
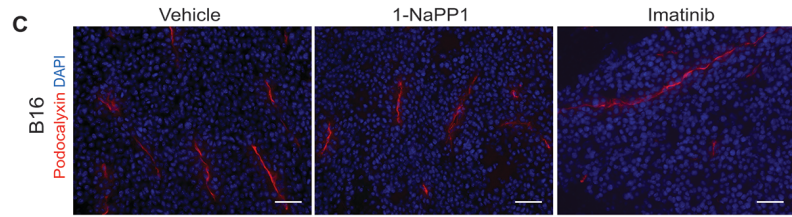
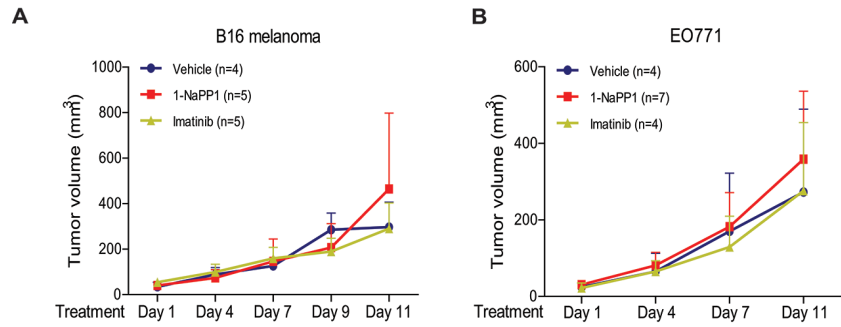


Figure S2: Low PDGF-BB expressing B16 melanoma (**A**) and EO771 breast carcinoma (**B**) murine tumors are insensitive to treatment with 1-NaPP1 or imatinib in the ASKA mice (n= as stated in the figure). Representative images of podocalyxin-stained vessels (**C**) and analysis of vessel parameters; vessel density (**D**), average vessel length (**E**), branching (**F**), number of junctions (**G**) and average lacunarity (**H**) in B16 tumors treated with vehicle, 1-NaPP1 and imatinib daily for 10 consecutive days. PDGFR β + (**I**) and NG2+ (**J**) pericyte coverage quantification in B16 tumors treated with vehicle and 1-NaPP1 and imatinib daily for 10 consecutive days.

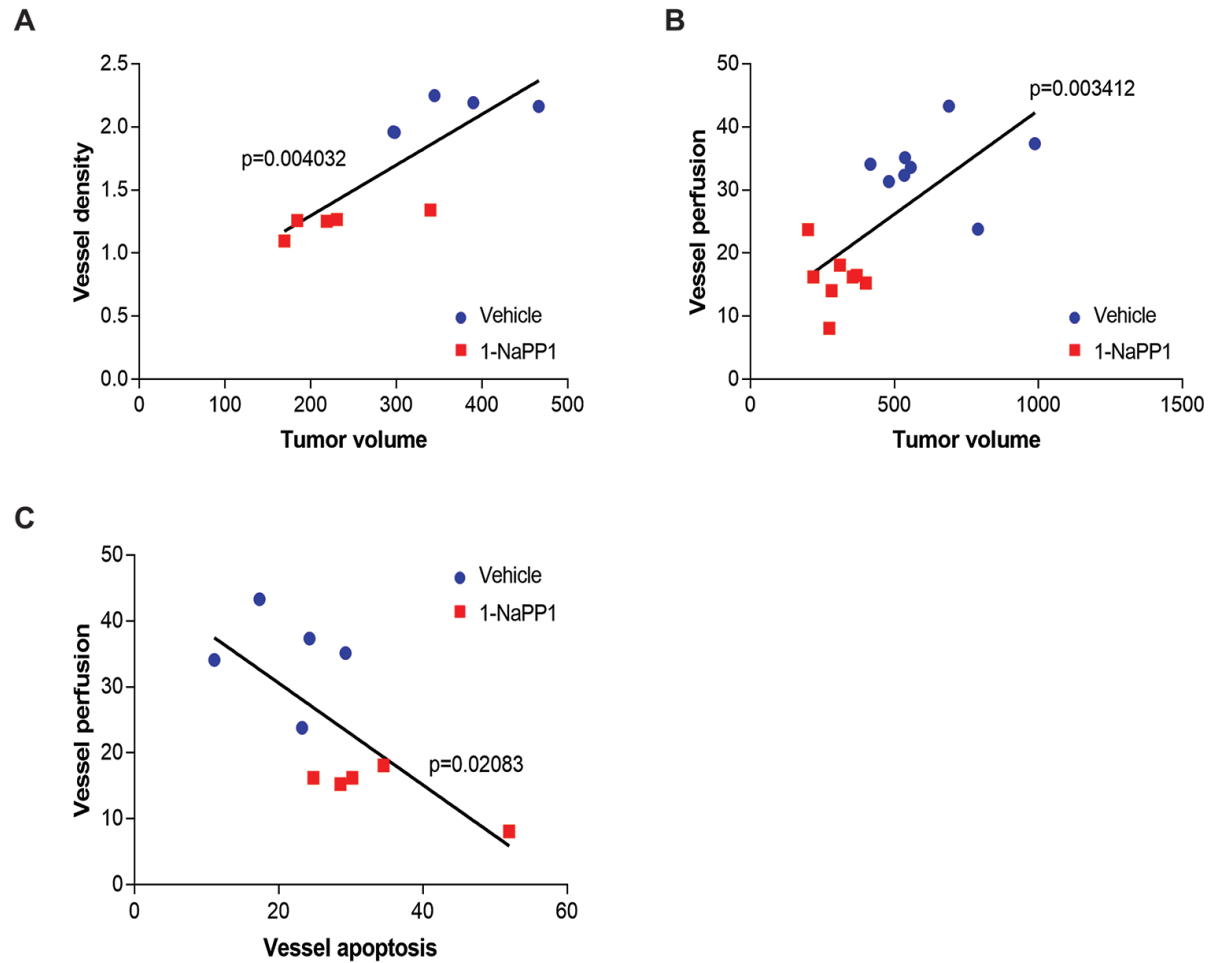


Figure S3: Tumor analysis of control and treated mice after 10 days displayed a positive correlation between tumor volume and vessel density as well as vessel perfusion, as shown from the scatterplots of tumor volume versus vessel density (**A**) and tumor volume versus vessel perfusion (**B**). Scatterplot of vessel perfusion against endothelial cell apoptosis showed a negative correlation (**C**).

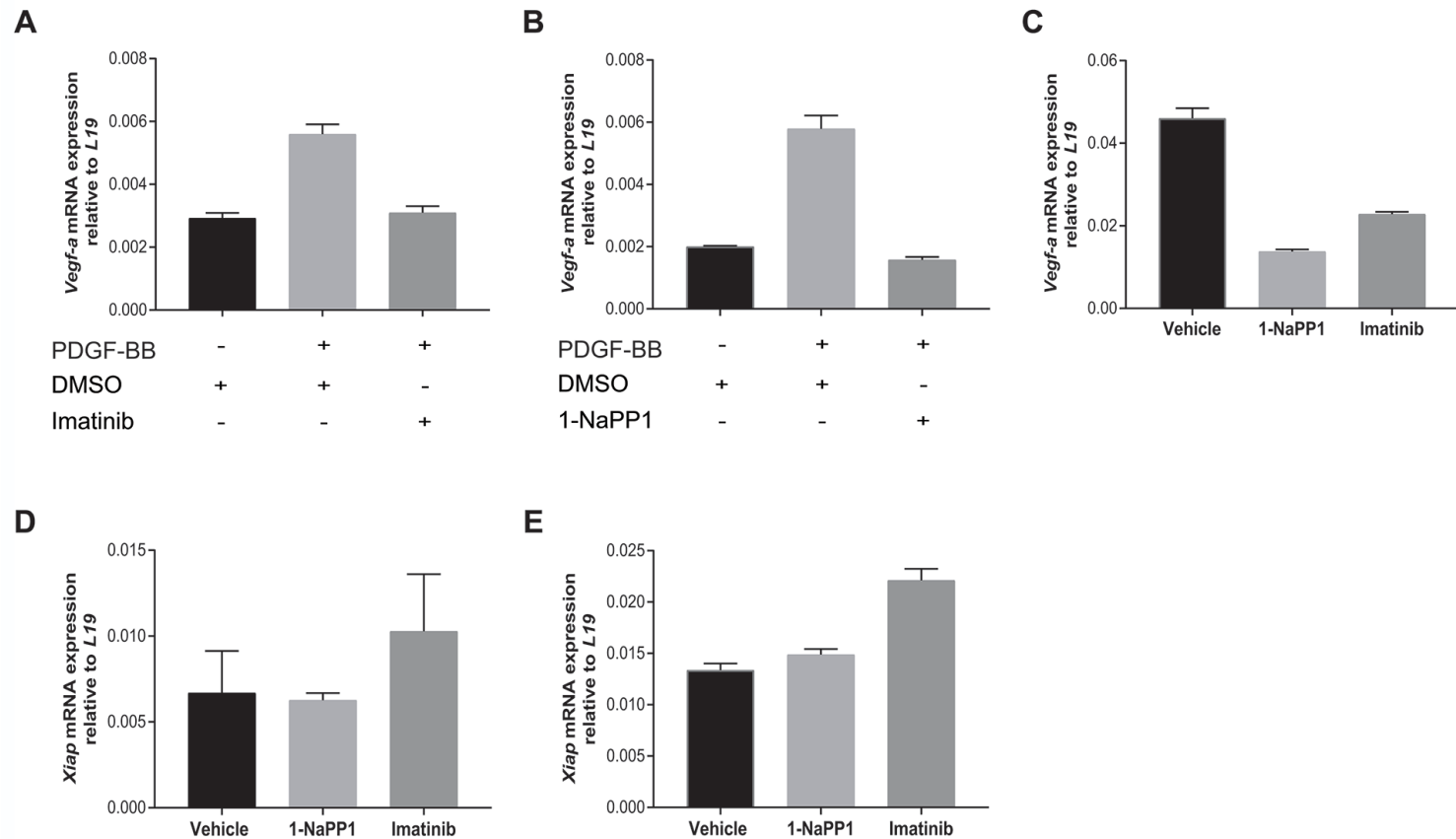


Figure S4: Expression of *Vegf-a* and *Xiap* in cultured cells and tumors treated with 1-NaPP1 or imatinib. To measure *Vegf-a* mRNA expression in 10T1/2 cells (**A**) and ASKA-PDGFR β MEFs (**B**), serum-starved and treated with vehicle (DMSO), imatinib (3 μ M) or 1-NaPP1 (1 μ M) in the presence or absence of 20 ng/ml PDGF-BB. After 24 h, mRNA was prepared and quantitative real-time PCR was performed. Error bars indicate standard deviation from triplicate samples. All mRNA expression is relative to *L19* ribosomal gene expression. Two independent experiments were performed. (**C**) PDGFR β + cells were isolated from B16/PDGF-BB tumors and *Vegf-a* mRNA expression was measured by qPCR. A representative experiment out of two replicates is shown. To measure *Xiap* mRNA expression in LLC tumors, we performed qPCR of mRNA prepared from isolated PDGFR β + cells (**D**) as well as the remaining cell population (**E**). Error bars indicate standard deviation. All mRNA expression is relative to *L19* ribosomal gene expression. Representative data from at least two mice per experimental condition is shown.