Supplementary Data

Manuscript title: "Phosphomimetic mutants of pigment epithelium-derived factor with enhanced antiangiogenic activity as potent anticancer agents"

Alexander Konson, Sunila Pradeep and Rony Seger

Department of Biological Regulation

The Weizmann Institute of Science

76100 Rehovot, Israel

Corresponding author:

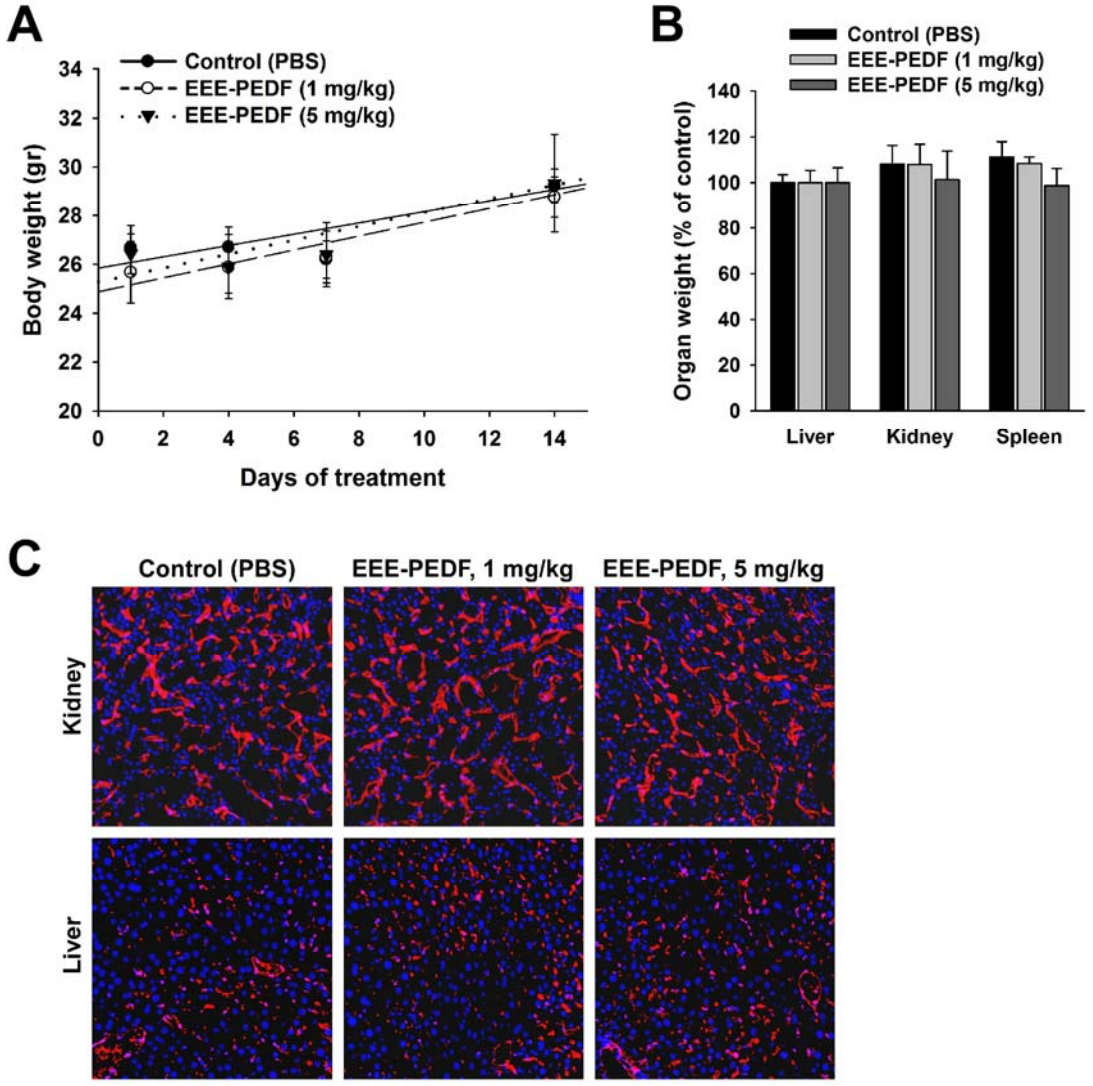
Prof. Rony Seger, Department of Biological Regulation, The Weizmann Institute of Science, POB 26, Herzel Str., 76100 Rehovot, Israel, E-mail: rony.seger@weizmann.ac.il

Supplementary Table 1

	Treatment				
	Control (PBS)	**WT-PEDF** 1 mg/kg×3/week	**EEA-PEDF** 1 mg/kg×3/week	**EEE-PEDF** 1 mg/kg×3/week	**Avastin** 5 mg/kg×5/week
	mean TTE (± SEM), days				
MDA-MB-231	**21.18** ± 0.88	**24.45** ± 0.43*	**27.46** ± 1.77*	**31.66** ± 2.13*,#	**29.84** ± 2.33*
U87-MG	**14.91** ± 0.33	**16.92** ± 0.26*	**18.28** ± 0.56*	**21.52** ± 1.26*,#	**25.53** ± 6.06*
HCT116	**15.61** ± 0.21	**19.01** ± 0.77*	**19.93** ± 0.28*	**25.20** ± 1.66*,#	**25.82** ± 1.46*

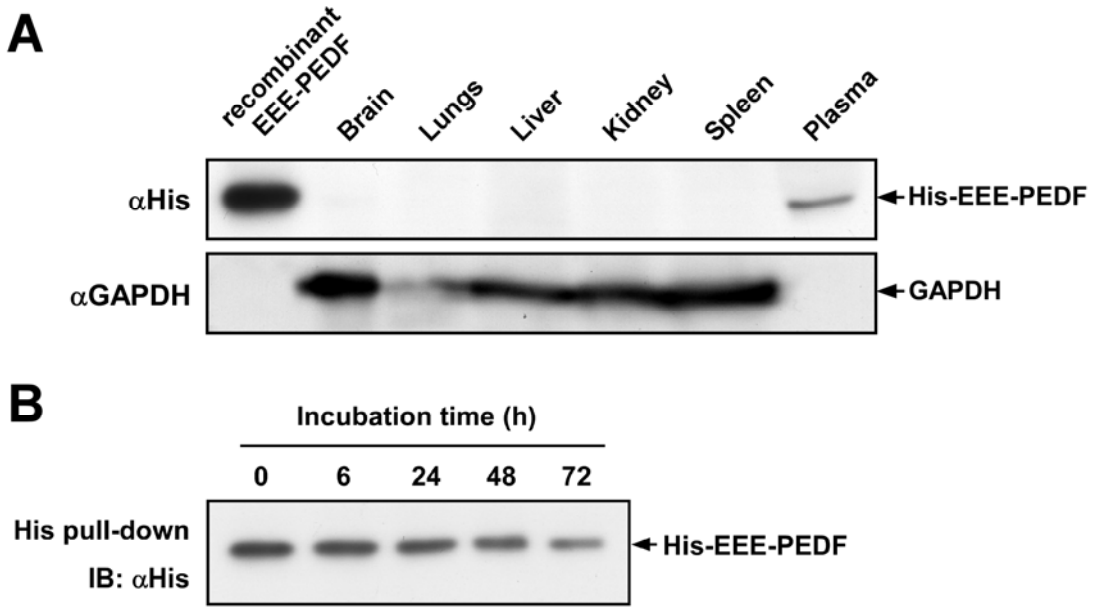
Supplementary Table 1. Mean tumor volume quadrupling times of MDA-MB-231, HCT116 and U87-MG xenografts treated with WT-PEDF, its phosphomimetic mutants or Avastin. CD-1 nude mice were inoculated subcutaneously with tumorigenic dose of MDA-MB-231, HCT116 or U87-MG cells and, upon the establishment of tumors, treated intravenously with WT-PEDF, EEE-PEDF, EEA-PEDF or Avastin at indicated dosage regimens. To test for differences between treatments' efficacies, tumor quadrupling times were chosen as time-to-endpoint (TTE) and were calculated using a non-linear regression analysis for exponential growth of each individual tumor, based on its volume at the beginning of treatment. Tereafter, differences between TTE of treated and control groups were analyzed by Log-rank test using GraphPad Prism software (GraphPad Software, La Jolla, CA), where values of $P < 0.05$ were considered statistically significant.

Supplementary Figure S1



Supplementary Figure S1. Assessment of systemic toxicity following intravenous administration of the phosphomimetic PEDF. CD-1 nude mice were injected intravenously with either EEE-PEDF at indicated dosages or the corresponding volume of vehicle (PBS) 3 times/week (n = 3 in each group). A, changes in body weight were monitored over treatment period of 2 weeks. B, weight of selected major organs was evaluated at experimental endpoint (2 weeks of treatment). Data shown are mean ± SEM, n = 3. C, vascularization of selected major organs was assessed at the endpoint by immunohistochemistry with anti CD31 as described in Materials and Methods. Shown are representative CD31-stained sections of livers and kidneys excised from animals in each experimental group. Pictures were taken at X10 magnification. Red, CD34; blue, DAPI.

Supplementary Figure S2



Supplementary Figure S2. Organ distribution and in vitro plasma stability of the phosphomimetic PEDF. A, organ distribution of the phosphomimetic PEDF was assessed following intravenous injection of recombinant His-tagged EEE-PEDF (1 mg/kg BW) into the tail vein of CD-1 nude mice. Thirty six hours after injection mice were sacrificed and tissue homogenates of selected major organs as well as plasma samples were analyzed for the presence of EEE-PEDF by immunoblotting with anti His-tag antibody. B, in vitro plasma stability of the phosphomimetic PEDF. Five micrograms of recombinant His-tagged EEE-PEDF were mixed with freshly drawn mouse plasma (1:10 v/v) and incubated for indicated time intervals at 37°C. After incubation, plasma samples were subjected to His pull-down assay using Ni-NTA agarose followed by Western blotting with anti His-tag antibody.