

## **Supplementary Materials and Methods**

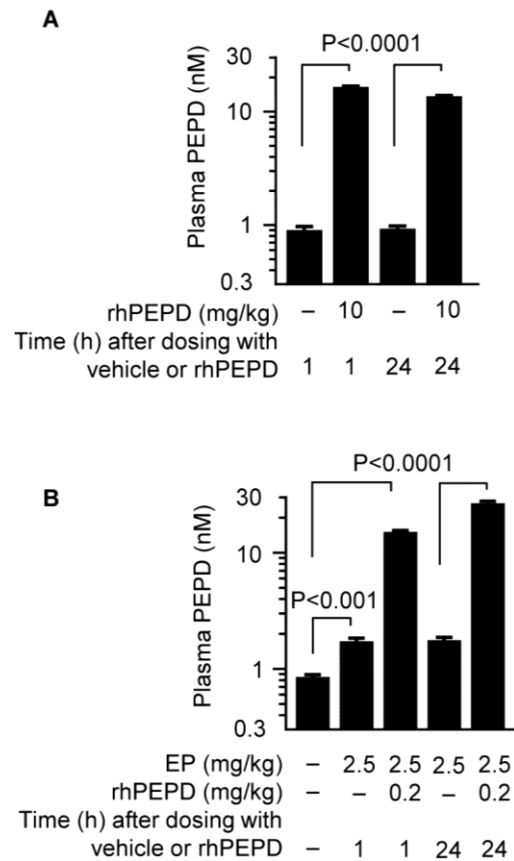
### **Animal study**

The animal study was performed in accordance with a protocol approved by the Institutional Animal Care and Use Committee at RPCI. C57BL/6 mice (male, 7-8 weeks age, bred at RPCI Animal Facility) were used. Mice were given a single i.p. dose of vehicle or rhPEPD, followed by collection of blood samples from the mice at 1 or 24 h post dosing. In a separate experiment, mice were given EP i.p. once daily; 1 h after the fourth EP dose, the mice were given rhPEPD or vehicle, followed by collection of blood samples from the mice at 1 or 24 h post dosing. All blood samples were promptly processed into plasma samples for measurement of levels of endogenous mouse PEPD and rhPEPD as described below. EP and rhPEPD were given to the mice in PBS.

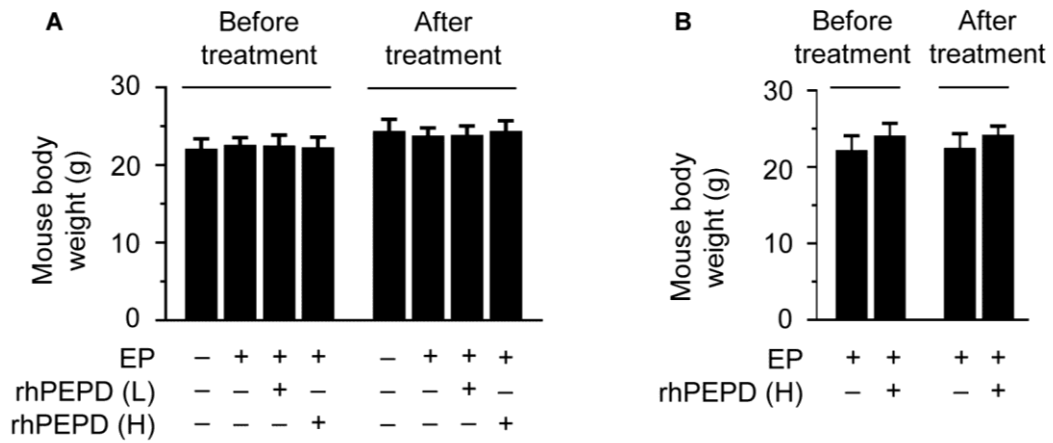
### **Histology**

Tissue samples were fixed in 10% buffered formalin, embedded in paraffin and sectioned at 5 microns. Sections were deparaffinized and rehydrated through xylene and graduated alcohol series to water and stained with hematoxylin and eosin (H/E). The slides were examined using a Nikon 50i light microscope.

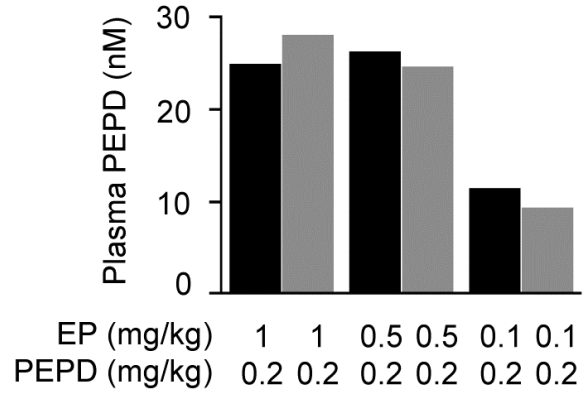
## Supplementary Data



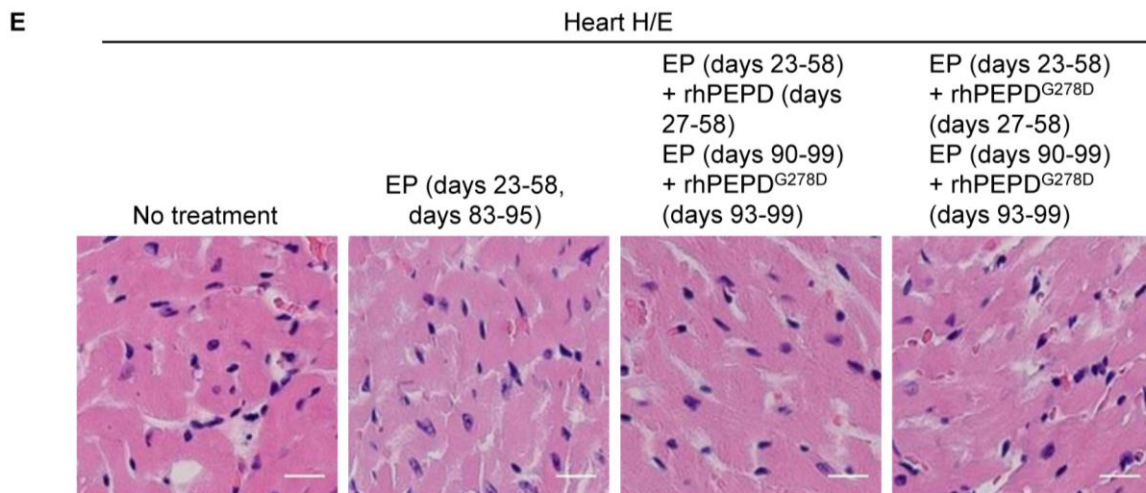
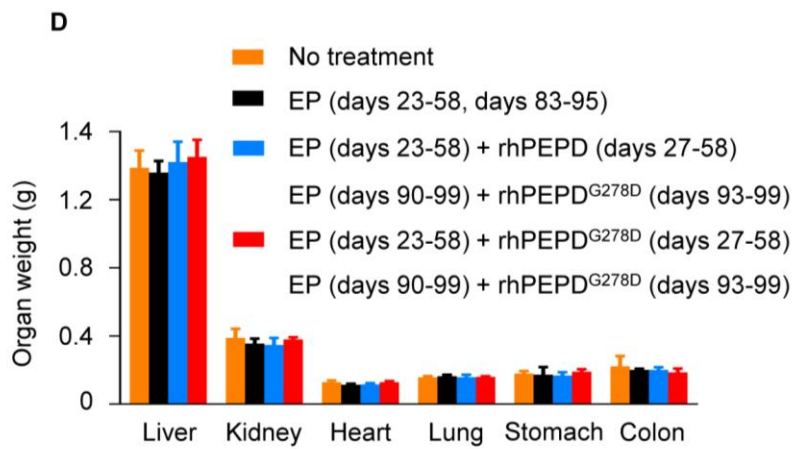
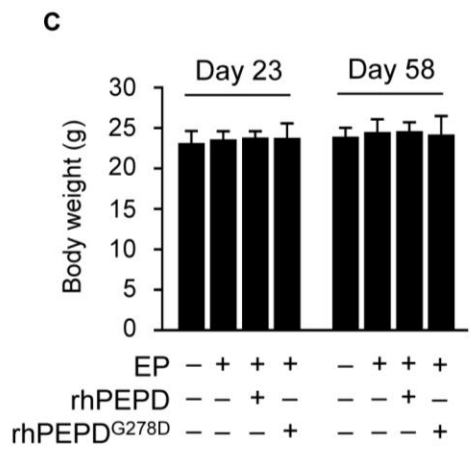
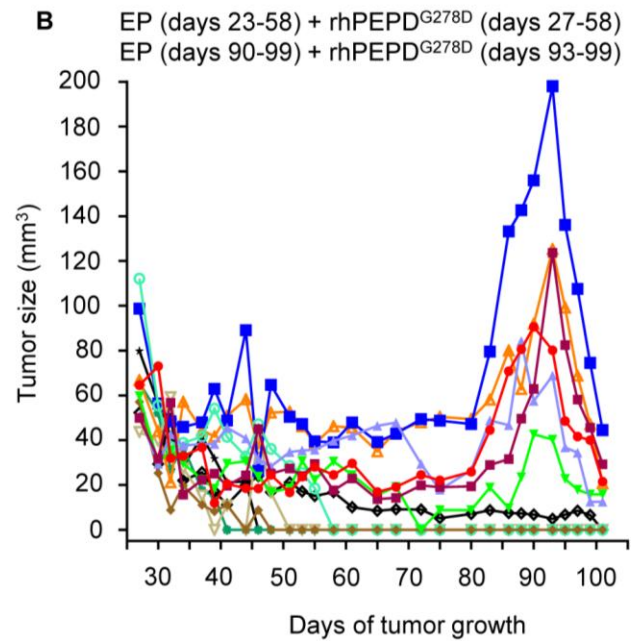
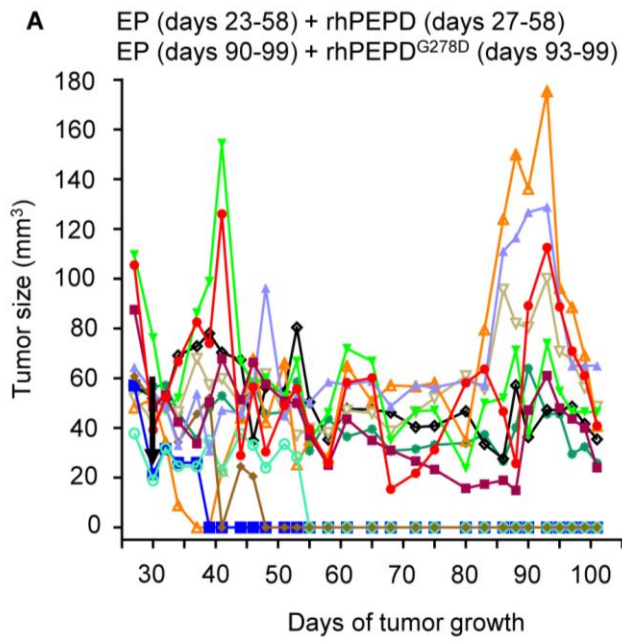
**Supplementary Fig. 1.** EP enables marked reduction of rhPEPD dose. [A] Plasma concentrations of endogenous PEPD and rhPEPD in mice given a single i.p. dose of vehicle or rhPEPD. [B] Mice were either untreated or treated with EP i.p. once daily for 4 days; 1 h after the last EP dose, the EP-treated mice were given an i.p. dose of vehicle or rhPEPD, followed by measurement of plasma concentrations of endogenous PEPD and rhPEPD. Error bars are SD (n = 3).



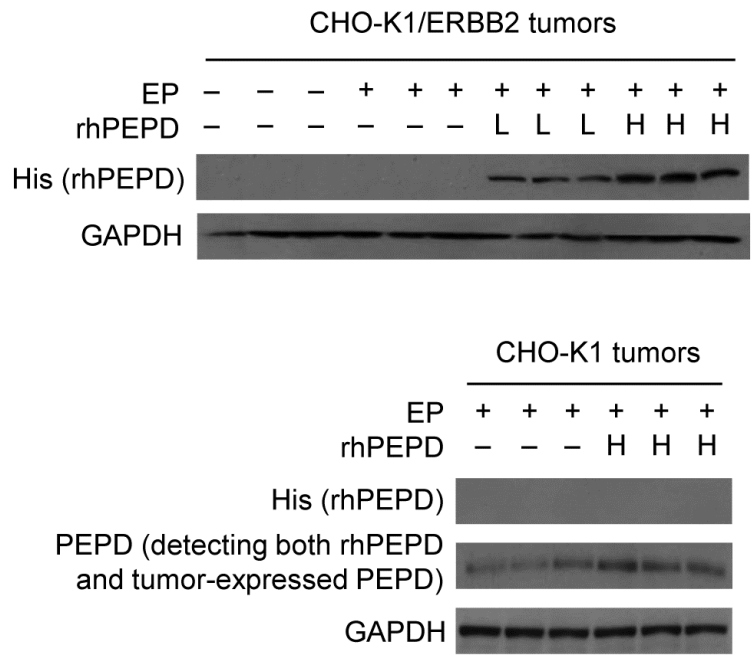
**Supplementary Fig. 2.** The effect of EP and rhPEPD on mouse body weight. [A] Mice bearing subcutaneous CHO-K1/ERBB2 tumors were treated with vehicle, EP (2.5 mg/kg) daily, and EP (2.5 mg/kg daily) plus rhPEPD at 0.02 mg/kg (L) or 0.2 mg/kg (H) thrice weekly, as detailed in Fig. 1A legend. [B] Mice bearing subcutaneous CHO-K1 tumors were treated with EP (2.5 mg/kg) daily, or EP (2.5 mg/kg daily) plus rhPEPD at 0.2 mg/kg (H) thrice weekly, as detailed in Fig. 1C legend. Error bars indicate SD.



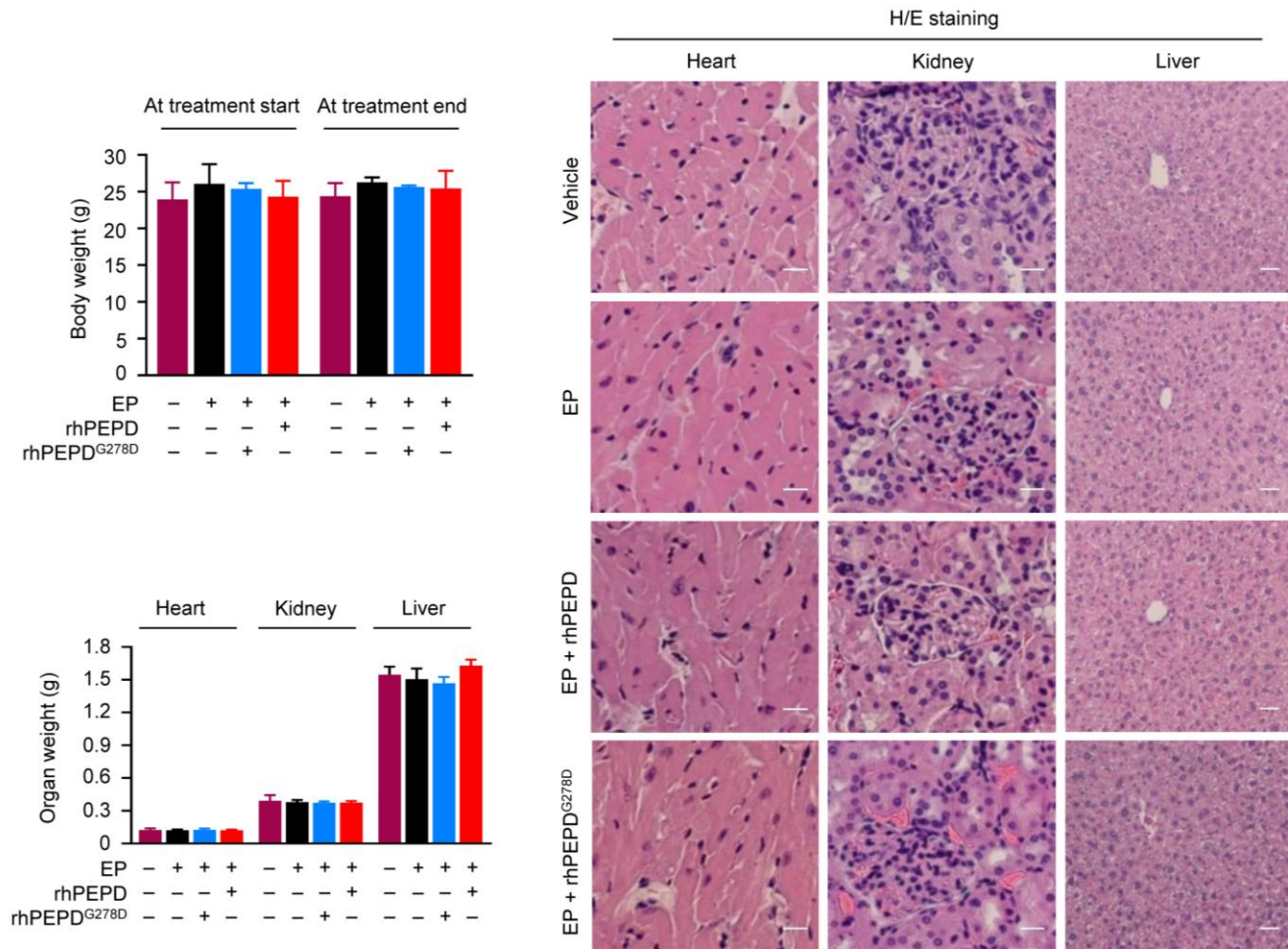
**Supplementary Fig. 3.** The effect of EP on plasma PEPD concentration. EP was administered to mice i.p. once daily. One hour after the fourth EP dose, the mice were injected i.p. with vehicle or rhPEPD. Plasma PEPD levels (endogenous mouse PEPD plus rhPEPD) were measured at 1 and 24 h after giving vehicle or rhPEPD. Each bar represents one sample (2 mice per group).



**Supplementary Fig. 4.** Individual tumor size, mouse body weight, mouse organ weight, and mouse heart histology after experimental antitumor treatments. [A & B] Individual tumor size in mice bearing orthotopic BT-474 tumors, upon treatment with EP plus rhPEPD or EP plus rhPEPD<sup>G278D</sup>, during treatment pause, and upon retreatment with EP plus rhPEPD<sup>G278D</sup> (see Fig. 2A legend for treatment detail). The treatment days are counted from the day of cancer cell inoculation. [C] Body weights of mice bearing orthotopic BT-474 tumors, at the beginning and end of the initial phase of experimental treatments (see Fig. 2A legend for treatment detail). Day 23 and Day 58 are relative to the day of cancer cell inoculation. Error bars indicate SD. [D & E] Organ weight and heart histology of mice bearing orthotopic BT-474 tumors with or without experimental treatments. Details of experimental treatments and sample size are provided in Fig. 2A legend. Mice were killed 2 days after the final experimental treatments. Organ weights are shown as mean  $\pm$  SD. The H/E images are representative of at least three mice in each group; scale bar: 10  $\mu$ m.

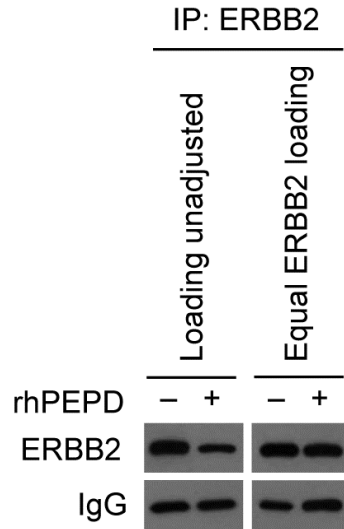


**Supplementary Fig. 5.** ERBB2-mediated rhPEPD internalization into tumor cells and tissues. Mice bearing CHO-K1/ERBB2 tumors or CHO-K1 tumors were treated with vehicle, EP (2.5 mg/kg), EP (2.5 mg/kg) plus rhPEPD at 0.02 mg/kg (L) or 0.2 mg/kg (H) as described in Figure 1 legend. Tumor tissue homogenates were measured by immunoblotting for rhPEPD (measuring the His tag) or using an antibody that binds to both rhPEPD and tumor-expressed endogenous PEPD. The results show that rhPEPD is accumulated in tumor tissues in an ERBB2-dependent manner. rhPEPD may slightly up regulate the endogenous, tumor-expressed PEPD.



**Supplementary Fig. 6.** Body weight, organ weight and organ histology of mice after experimental treatments. Mice bearing BT-474 tumors were treated with vehicle, EP, EP plus rhPEPD, or EP plus rhPEPD<sup>G278D</sup>. The tumor data and sample size are shown in Fig. 3. EP was administered to the mice i.p. at 0.5 mg per kg body weight daily, started 4 days before rhPEPD or rhPEPD<sup>G278D</sup>. rhPEPD or rhPEPD<sup>G278D</sup> was administered to the mice i.p. at 2 mg per kg body weight twice, separated by 2 days. The mice were killed at 24 h after the last dose. Body weight and organ weight are shown as mean  $\pm$  SD. The H/E images are representative of at least three mice in each group; scale bars of H/E images; scale bar: 10  $\mu$ m (heart and kidney) or 20  $\mu$ m (liver).





**Supplementary Fig. 7.** Immunoblotting with and without ERBB2 loading adjustment. CHO-K1/ERBB2 cells were treated with rhPEPD at 5 nM for 1 h. Cell lysates were incubated with an ERBB2 antibody, and the immunocomplexes were pulled down with protein G-agarose, followed by immunoblotting for ERBB2. Sample loading was either adjusted to contain an equal amount of ERBB2 or not adjusted.