SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure 1. Stable knock-down of PAR1 expression from high PAR1-expressing OVCAR4 cells.

A, Puromycin-selected stable PAR1-shRNAi OVCAR-4 clones including OVCAR4-A3 were tested 4 weeks after initial transfection for PAR1 expression using Q-PCR. PAR1 mRNA levels were normalized to GAPDH and expressed as fold relative to parent OVCAR-4 cell line.

B, Flow cytometry was carried out on OVCAR-4A3 and parental OVCAR-4 using the PAR1 polyclonal SFLLR-Ab. Data from the OVCAR-4A3 clone showed a 63% loss in migratory potential (data not shown) as compared to native OVCAR-4 in correlation with a 75% loss in PAR1 surface expression and an 80% loss in mRNA expression.

Supplementary Figure 2. ELISA analysis was used to measure IL-8 (A), GRO-α (B), and VEGF-A-165 (C) levels in CM from three ovarian carcinoma cell lines (OVCAR-4, IGROV-1, OVCAR-3) that were stimulated with 1 nM thrombin in the presence or absence of the PAR1 antagonist pepducin P1pal-7 (3 μM), the small molecule PAR1 antagonist RWJ-56110 (5 μM), or buffer control as indicated. Data (mean ± SE) are representative of at least 2 or more experiments performed in duplicate. $P<0.05$

Supplementary Figure 3. IL-8 and GRO-α induced HUVEC wound-healing migration is inhibited by the CXCR1/2 pepducin X1/2pal-i3. 0.2 million HUVEC cells were plated in 6 well plates and grown in EBM2 with bullet kit media until almost confluent. Wells were scratched and media changed to EBM2, 0.5% BSA supplemented with IL-8 (6 nM) + GRO-α (6 nM) in

the presence or absence of X1/2pal-i3 (0.3 μM). Digital photographs at 40x magnification were acquired at 0 h and 24 h to record migration of cells.

Supplementary Figure 4. HUVECs were added to matrigel and stimulated with VEGF-A-165 (200 pg/ml) either in presence of X1/2pal-i3 (300 nM), Avastin (0.25 mg/ml), or PBS buffer control (—) and incubated overnight prior to assessment of tube formation. Experiments (mean ± SE) shown are representative of 2 or more experiments performed in triplicate. *P<0.05,

Supplementary Figure 5. HUVECs were added to matrigel-coated MatTek wells and stimulated with IL-8 (30 nM) in the presence of CXCR1 and CXCR2 neutralizing antibodies or IgG control and incubated overnight. Tube formation was quantified by ImageJ software in a blinded manner to assess tubal length and branch point complexity.

Supplementary Figure 6. The CXCR1/2 pepducin X1/2pal-i3 blocks MMP1-induced angiogenesis in mice. MMP-1 or vehicle-treated OVCAR-4 CM was concentrated 6-fold and mixed with matrigel and HUVECs as in Figure 5 before injecting into the flanks of NCR Nu/Nu mice. Mice were treated subcutaneously with X1/2pal-i3 (5 mg/kg/d x 7 days) or vehicle (15% DMSO) as indicated. After 7 days, matrigel plugs were harvested, sectioned and blood vessels stained with a Factor VIII-von Willebrand Factor antibody.