Supplement I. Supplemental Methods

Reagents and Antibodies

For flow cytometry or cell sorting, cells were incubated with the following primary antibodies: PE-conjugated anti-PTK7 (Miltenyi Biotec, Bergisch Gladbach, Germany), FITC-conjugated anti-CD11b, FITC-conjugated anti-CD45, FITC-conjugated anti-F4/80, APC-conjugated anti-F4/80 were purchased from eBioscience (San Diego, CA). APC-conjugated anti-VEGFR2 was purchased from Biolegend (San Diego, CA). Anti-CD13 (WM15) and anti-VEGFR1 blocking antibodies (AP-MAB0702) was purchased from Abcam (Cambridge, MA). Mouse angiopoietin-1 (AB3120) and -2 (AB3121) antibodies for ELISA, phospho-Tie2 (ABS219), and total Tie2 (AB3126) for western blot analysis were purchased from Merck Millipore (Darmstadt, Germany). Anti-mouse VEGFR2, - tubulin, and - -actin were purchased from Santa Cruz Biochemicals (Santa Cruz, CA). Anti-IκBα (#2859), phospho-VEGFR2 (Tyr951, 7H11, #2476), and anti-phospho-IκBα (#4812) were from Cell Signaling (Beverly, MA). For the immunofluorescence assay, anti-PTK7-biotin, anti-PTK7 (Miltenyl Biotec, Bergisch Gladbach, Germany), FITC-conjugated anti-CD31 (Santa Cruz, CA), streptavidin-Cy5 (Biolegend, San Diego, CA), and the TRITC-conjugated secondary antibodies (Jackson ImmunoResearch Laboratories, West Grove, PA) were used. Carrier free Mouse VEGF-A_{164} was purchased from Cell Signaling (Company information). LY294002, SB203580, PD98059, SN50, SN50M, Rapamycin, and DMSO were purchased from Sigma Aldrich (St. Louis, MO). RPMI 1640, Penicillin/Streptomycin, and fetal bovine serum were from GIBCO Life Technologies (Carlsbad, CA). RNAi products for ANG-1 (GS11600) and ANG-2 (GS11601) were from Qiagen (Limburg, Netherlands). siRNA for PTK7 and VEGFR2 was from Thermo Fisher Scientific Inc. (Waltham, MA) and Invitrogen (Carlsbad, CA), respectively.

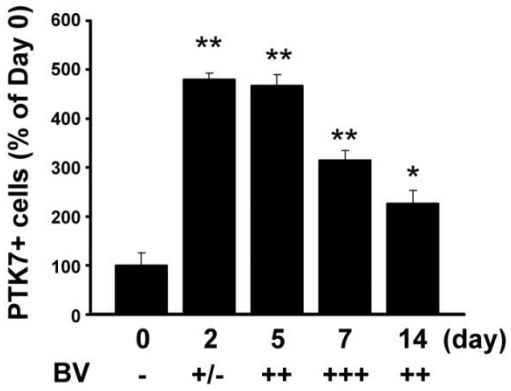
Supplement II. Primers used for quantitative real-time qPCR

Target gene	Sequence
vegfr2	Mm01222421_m1
dll4	Mm00444619_m1
tie2	Mm01256904_m1
ve-cadherin	Mm00486938_m1
ANG-2	Mm00516078_m1
Apelin	Mm00443562_m1
ephrin B2	Mm01215897_m1
vegfr1	Mm00438980_m1
unc5b	Mm00504054_m1
cxcr4	Mm01996749_s1
plxnd1	Mm01184367_m1
nrp1	Mm01253206_m1
nrp2	Mm00803099_m1
CD34	Mm00519283_m1
β-tubulin	Mm00727586_s1
ANG-1	Mm00456503_m1
PDGRF- β	Mm00435546_m1
Desmin	Mm00802455_m1
α-sma	Mm00516078_m1
Gapdh	Mm03302249_g1

*vegfr*2: vascular endothelial growth factor receptor 2, *dll4*: delta like ligand 4, *tie2*: tyrosine-protein kinase receptor tie2, *ve-cadherin*: vascular endothelium-cadherin, *ANG*: angiopoietin, *unc5b*: uncoordinated locomotion-5 homolog B, *plxnd1*: plexin D1, *nrp2*: neuropilin2, *α-sma:* alpha-smooth muscle actin.

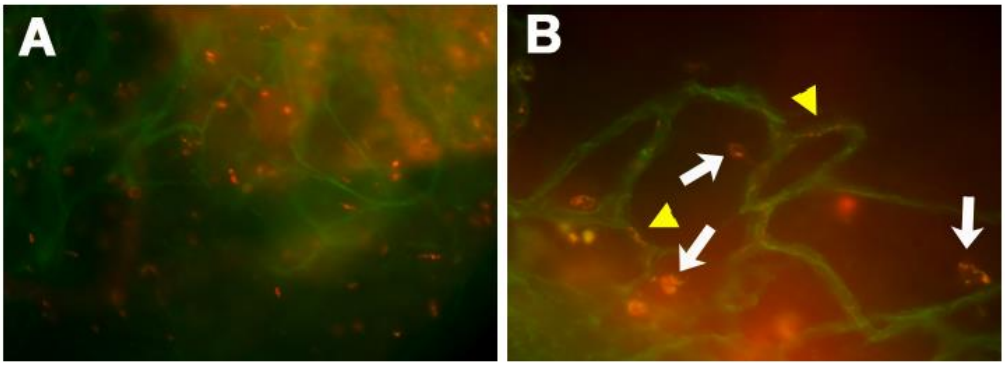
All primers described in the tables are from TaqMan® Gene Expression Assays (Applied Biosystems).

Supplement III. Frequencies of PTK7+ cells, budding vasculature, and opacity were increased in the cornea after micropellet implantation. The level of budding vasculature (BV) and cell frequencies of infiltrating PTK7+ cells were measured 0, 2, 5, 7, and 14 days after micropellet implantation (BV: +, vascular budding; ++, vascular growing not reaching the VEGF-A pellet; +++, mature BV reaching the pellet.) PTK7+ cells were counted by using epifluorescein microscopy. At least three mice eye were evaluated at each time point. Five random high power fields (HPF; × 400) were selected and summarized in each mouse. The graph represents the mean value of percent changes from day 0 at each experiments day.



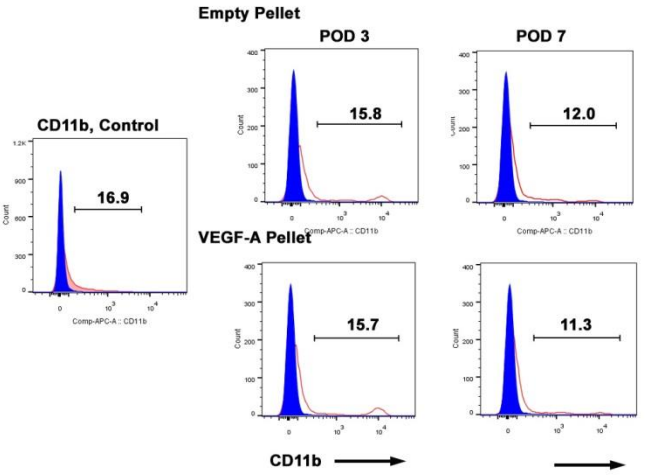
Supplement IV. Immunofluorescence co-staining of CD31+ blood vessels and PTK7+ cells.

A and B, Three days following VEGF-A pellet insertion, secured full-thickness corneal tissues were stained with anti-CD31 FITC and anti-PTK7 TRITC and observed by epifluorescence microscopy (Nikon, Eclipse TE200 instrument equipped with a Nikon digital camera, model DXM 1200). The white arrows indicate $PTK7^{+}$ cells that are attached to $CD31^{+}$ cells. Yellow arrowheads indicate overlapped PTK7+ with CD31 cells.

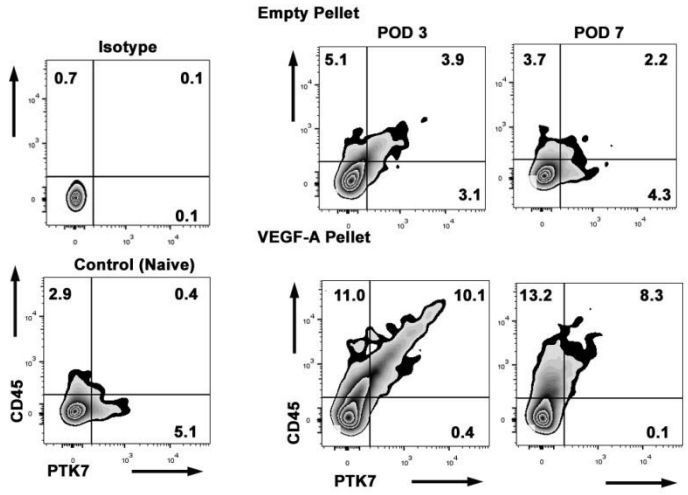


Supplement V. Video Clip showing that most PTK7+ cells are located near the angiogenic area, and do not express VEC marker, PECAM-1, and also do not incorporate into new vessels.

Supplement VI. Comparison of inflammatory cell changes in peripheral blood and cornea between vehicle and VEGF-A pellet inserted condition.
The upper histogram showed the changes of CD11b+ cells after corneal micropellet surgery. The lower panel showed the changes of PTK7+CD45+ cells between vehicle and VEGF-A pellet inserted condition at POD (postoperative days) 3 and 7 days.

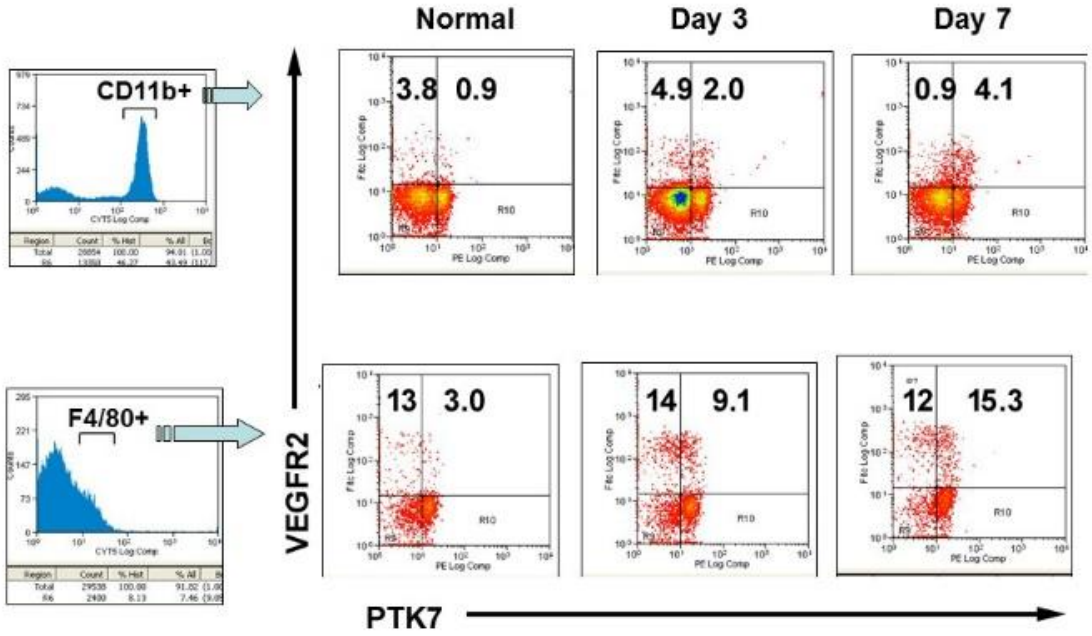


PBMC CD11b cells

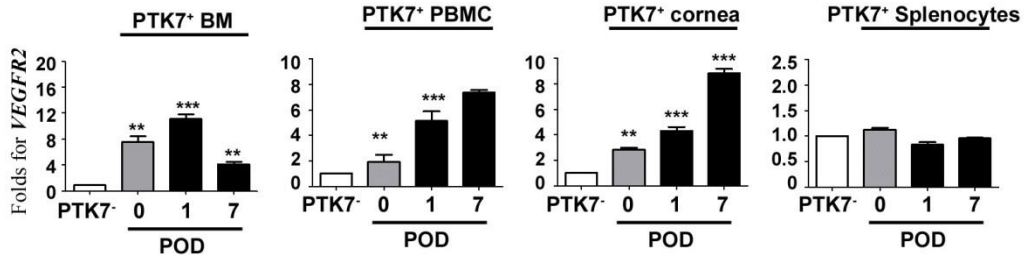


Cornea Pellet assay (Vehicle vs. VEGF-A 160 pellet)

Supplement VII. VEGF-A corneal pellets were implanted in 6-week-old Balb/c mice (n=7) under general anesthesia. PBMCs were stained with monoclonal anti-mouse PTK7-PE-, CD11b-FITC-, and VEGFR2-APC-conjugated antibodies. Frequencies of $VEGFR2^{+}PTK7^{+}$ were measured in $CD11b^{+}$ and $F4/80^{+}$ cells on postoperative day (POD) 0, 3, and 7 using flow cytometry.



Supplement VIII. Mononuclear cells were separated from the BM, blood, cornea, and spleen at postoperative day (POD) 1 and 7. Real-Time qPCR was performed for *VEFGR2* in $PTK7^{+}CD45^{+}$ and $PTK7^{-}CD45^{+}$ cells, which were separated by FACS Aria. Fold increases in non-operated control cells were also measured (*: $p<0.05$, **: $p<0.01$, ***: $p<0.001$, One-way ANOVA).



Supplement IX. Comparison of desmin mRNA expression between PTK7+CD11b+ and PTK7-CD11b+ cells in bone marrow (BM) and cornea after VEGF-A (160ng) micropellet implantation. Desmin mRNA levels of PTK7+ and PTK7- cells were normalized by the expression of GAPDH at each postoperative days (POD) (For each sample we analyzed triplicates in 3 independent experiments. Wilcoxon Rank Sum Test, **: $p<0.01$).