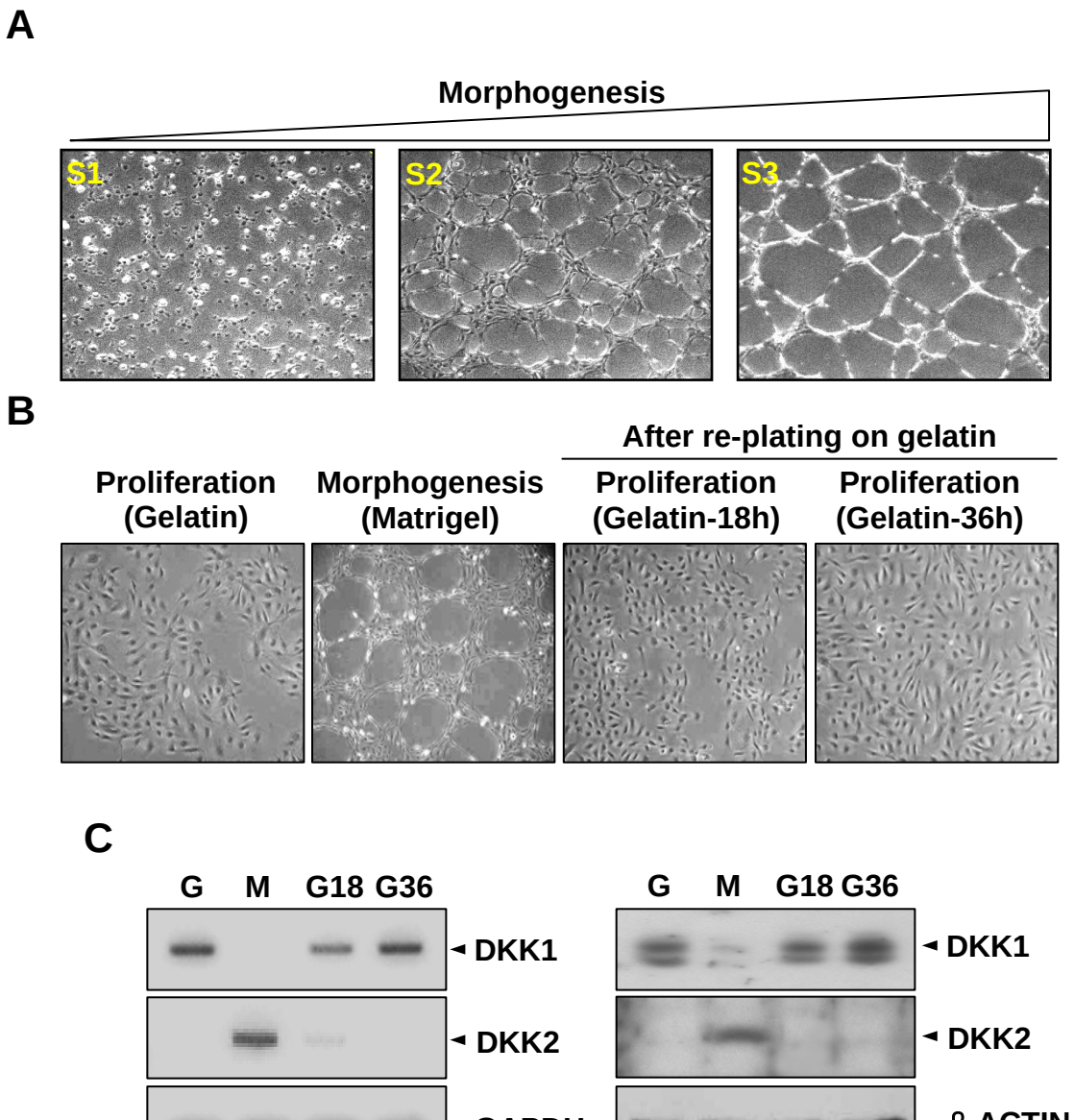
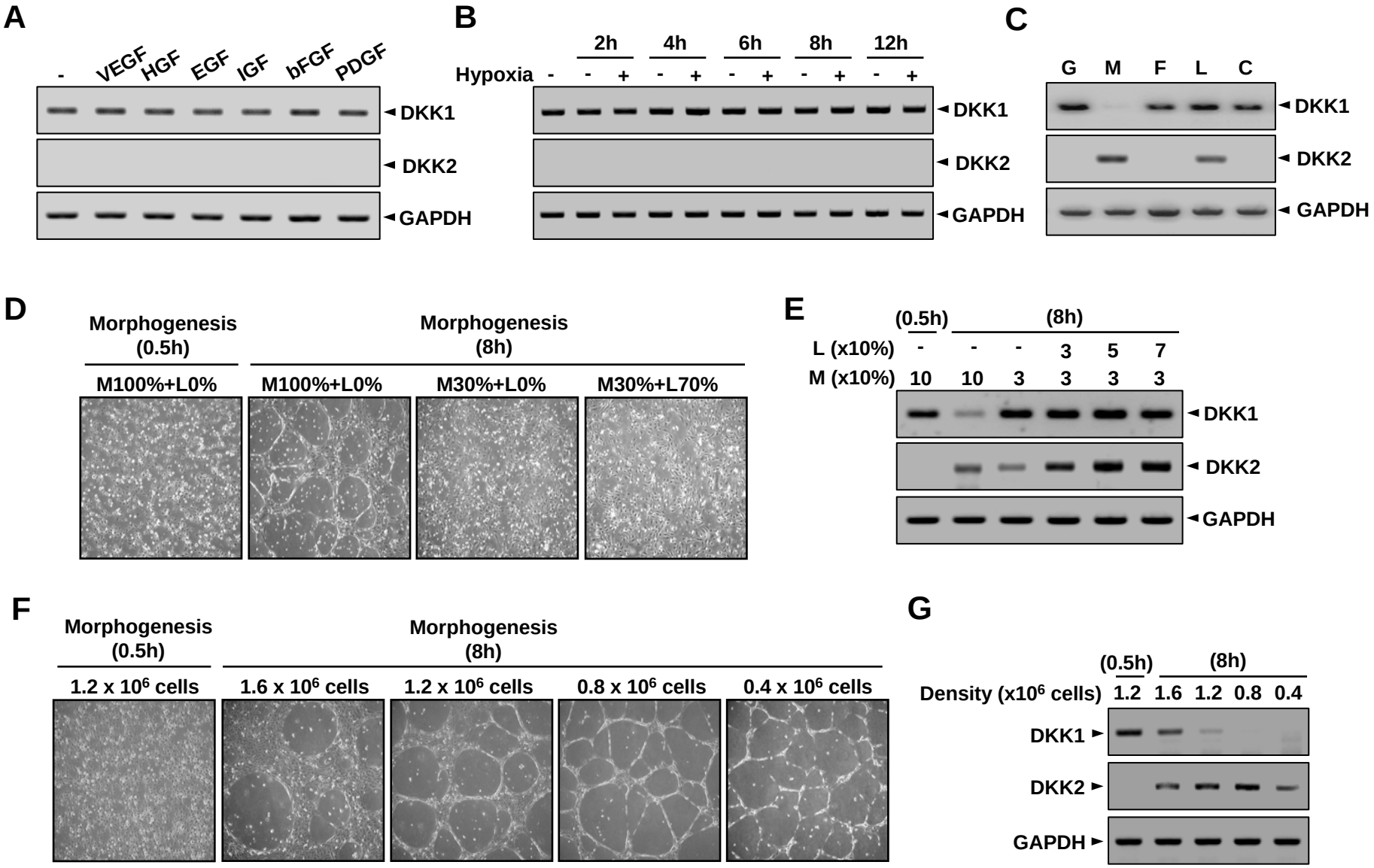
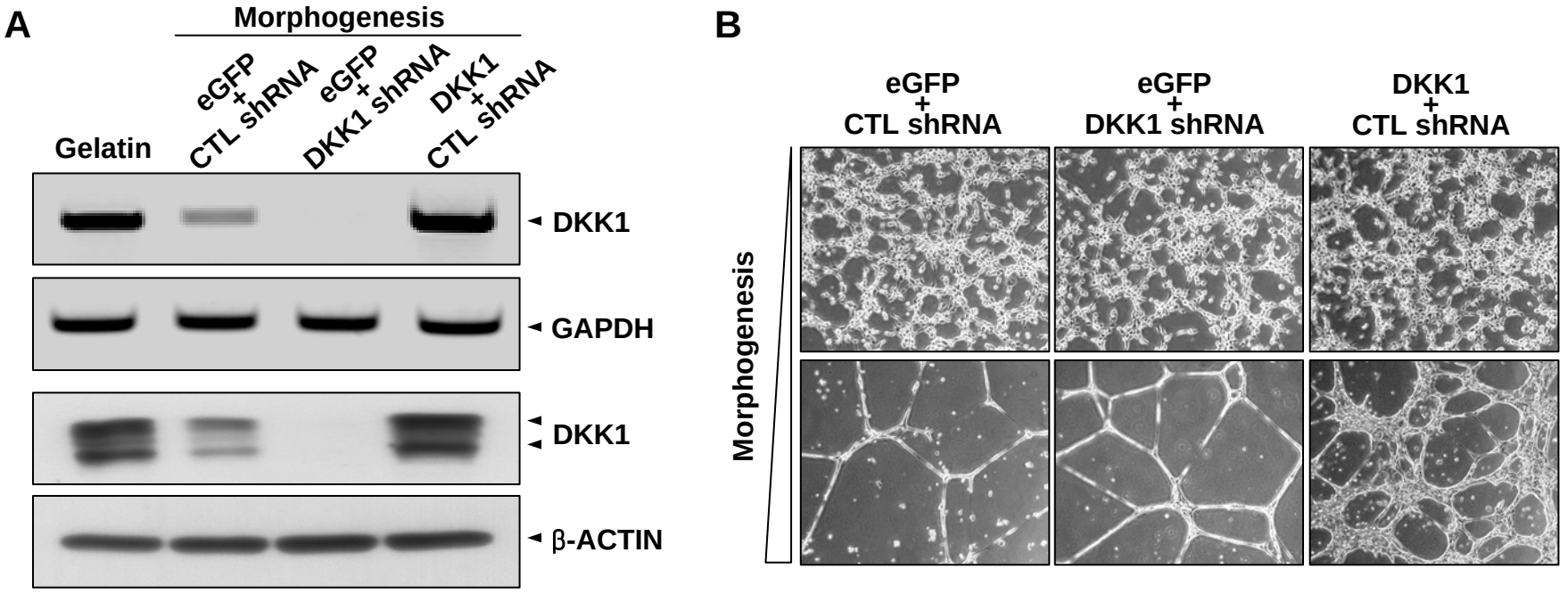
Supplemental Figure 1



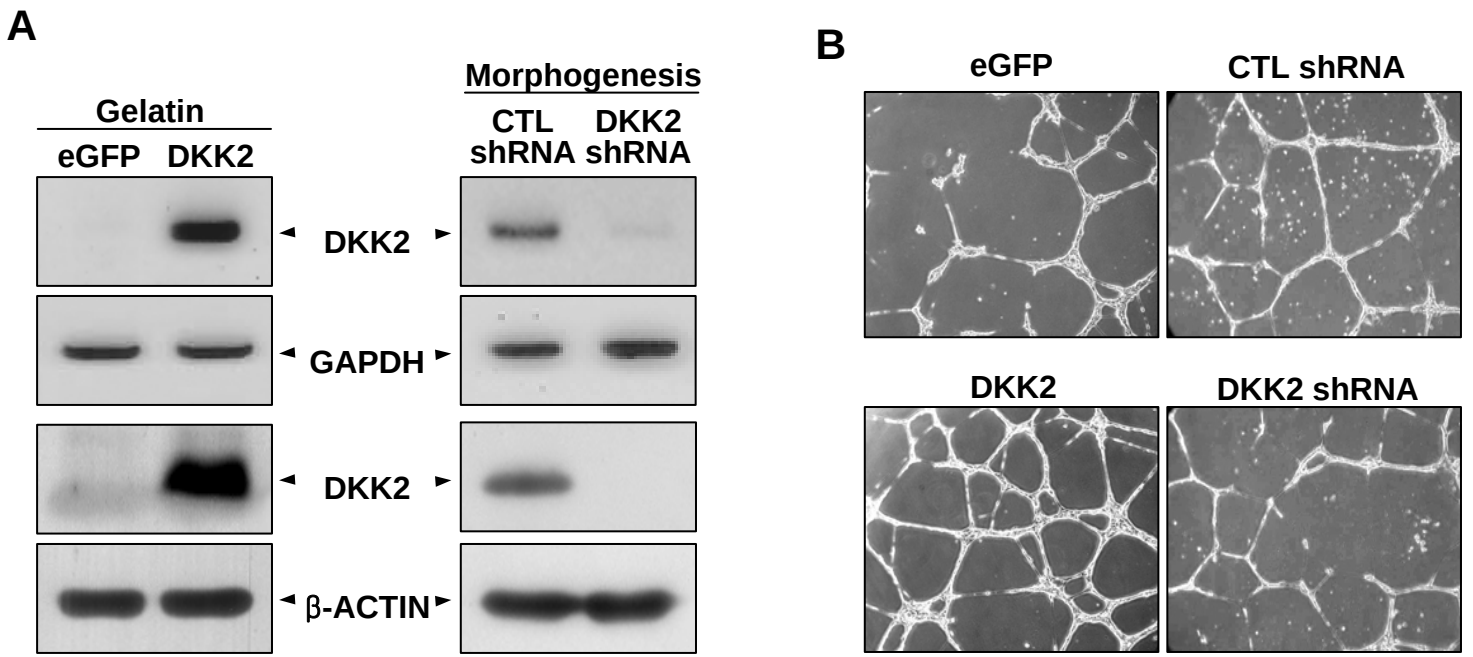
Supplemental Figure 2



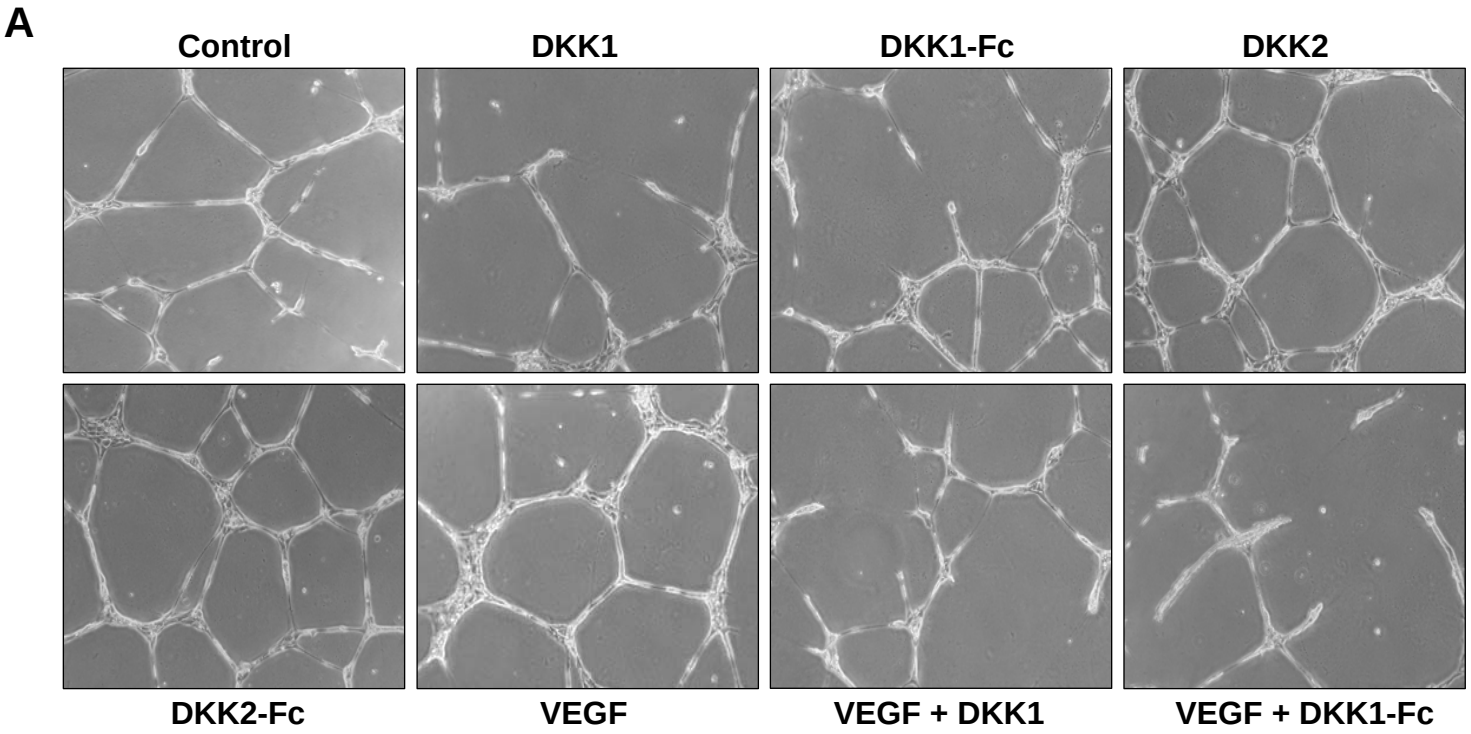
Supplemental Figure 3

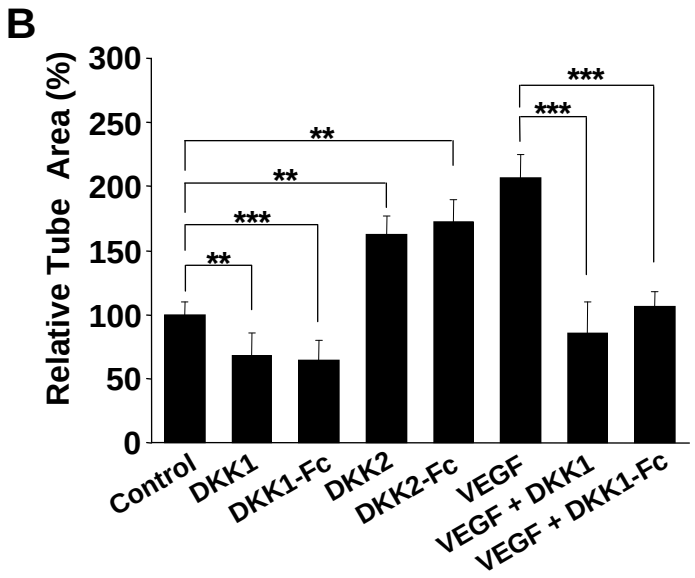


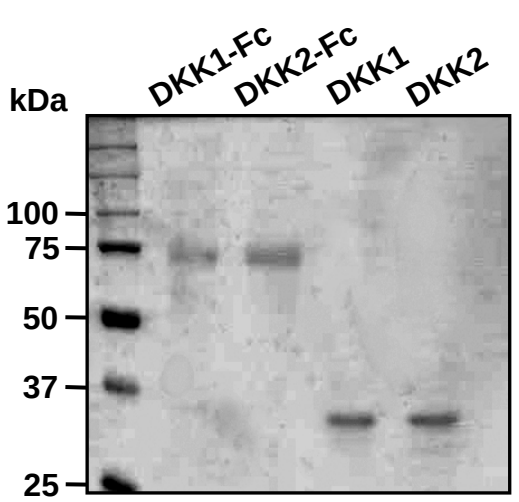
Supplemental Figure 4



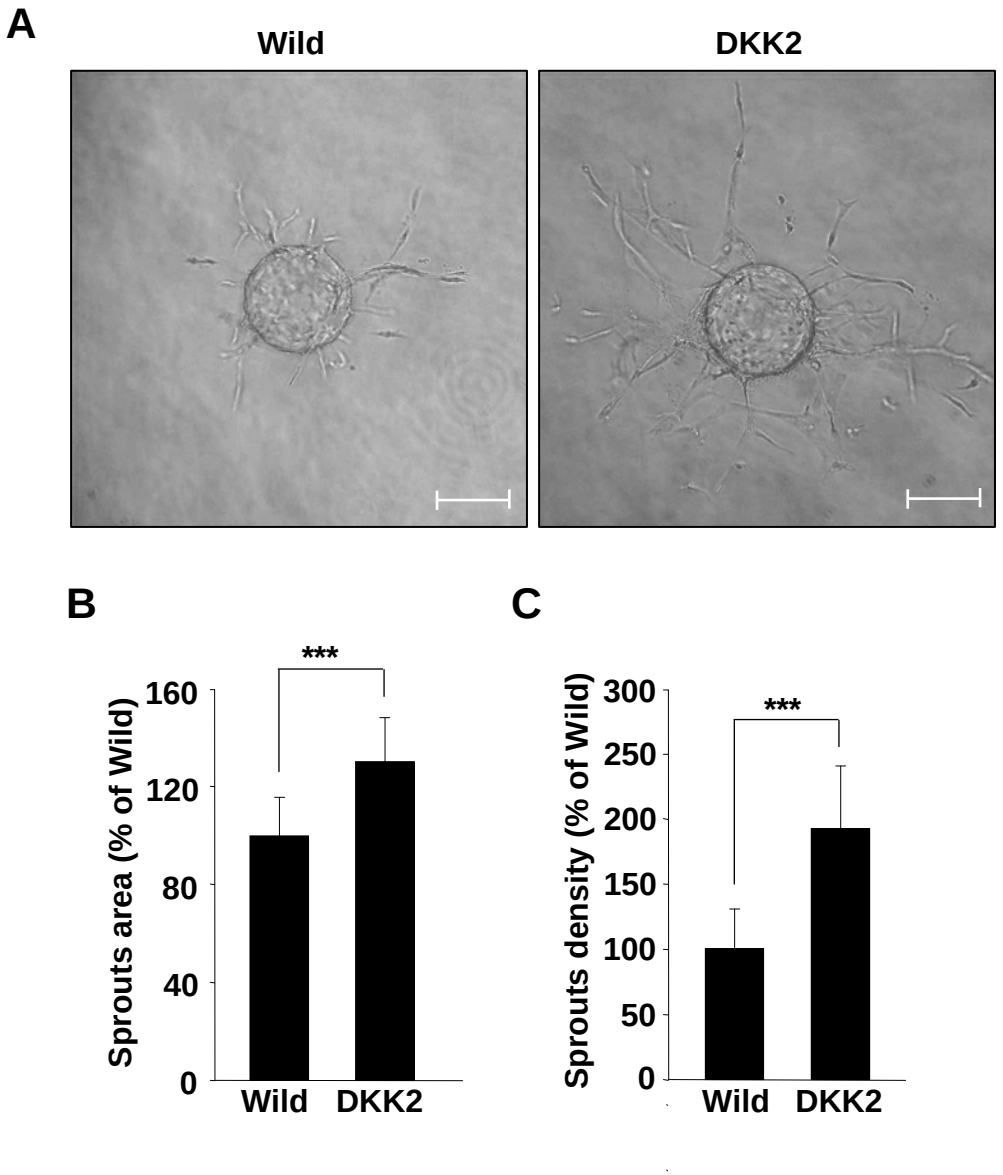
Supplemental Figure 5



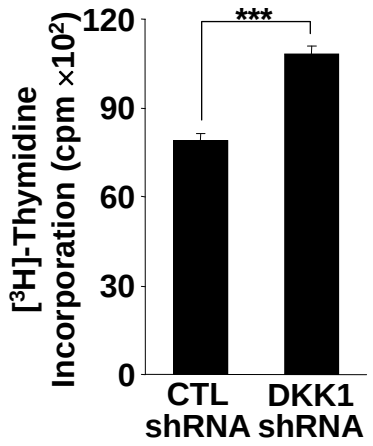




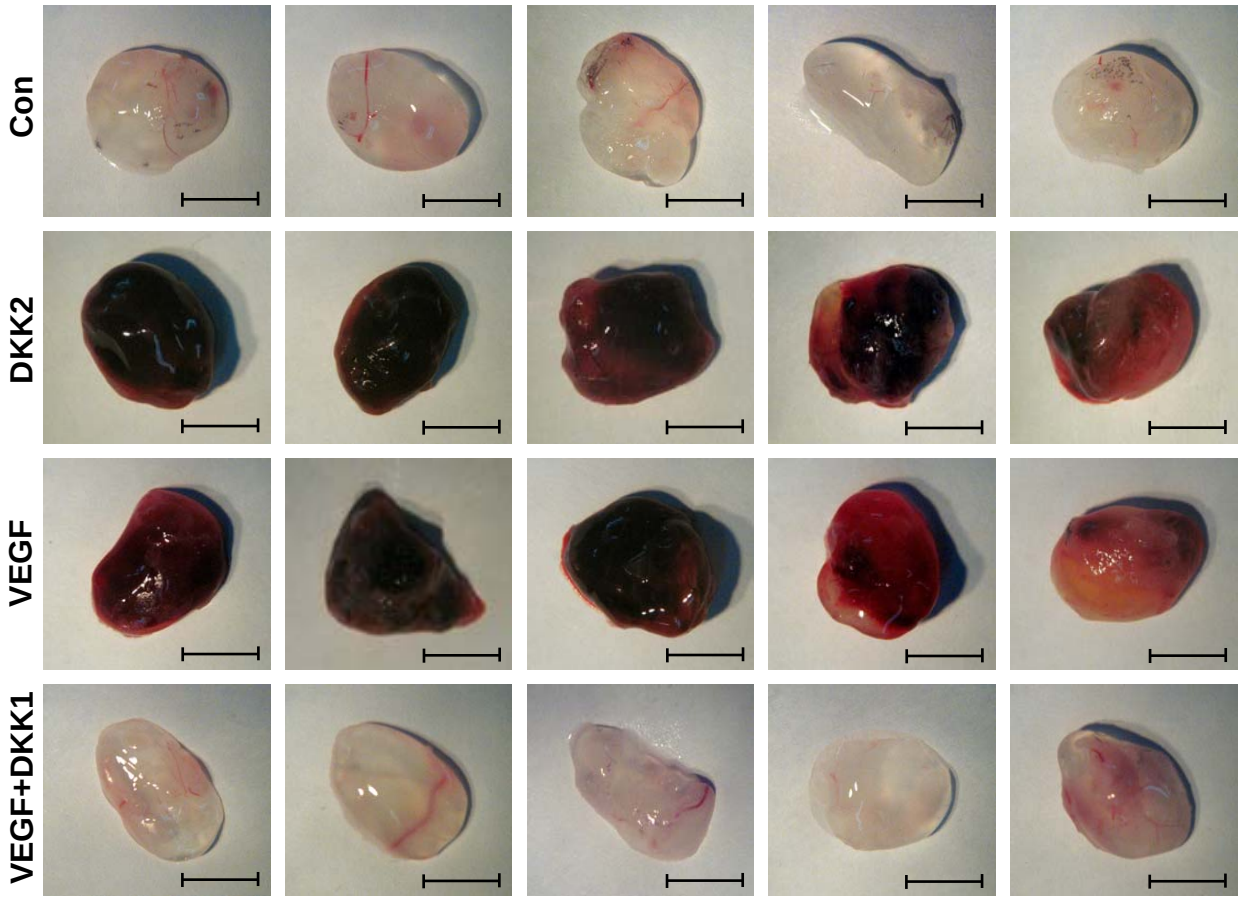
Supplemental Figure 6



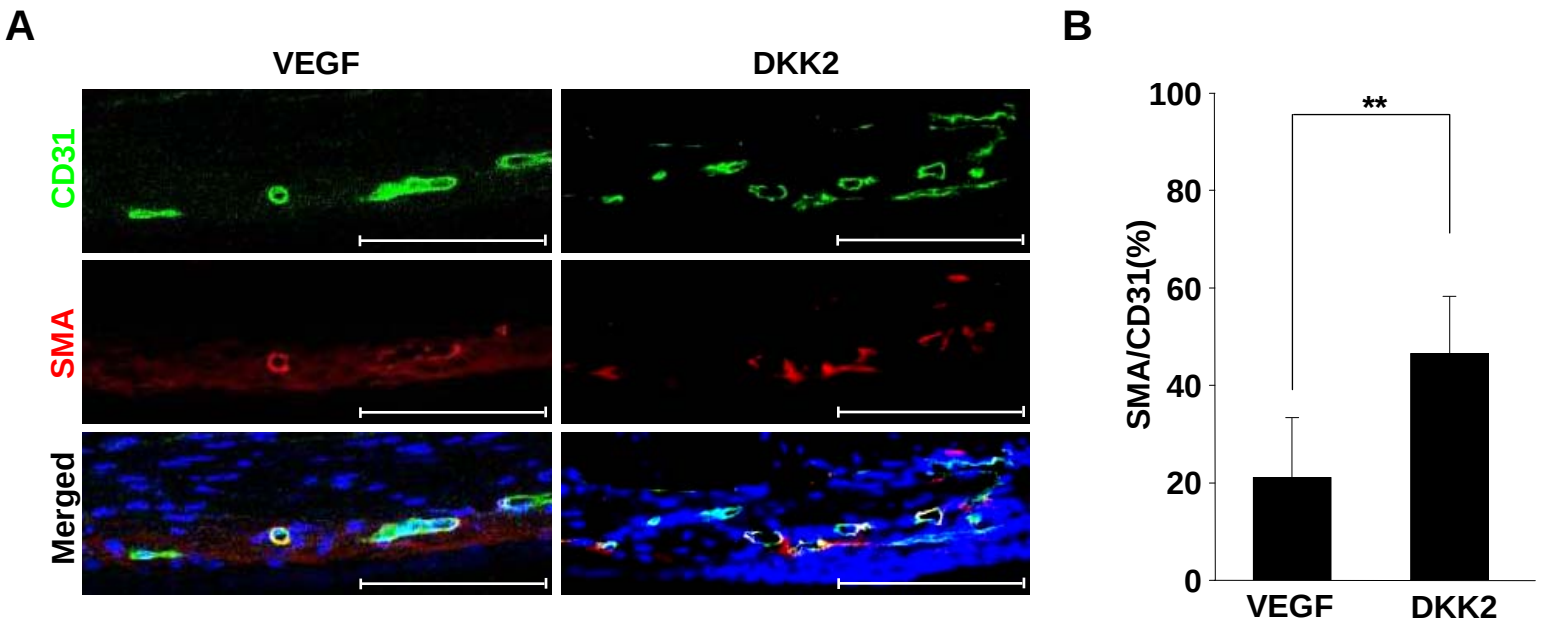
Supplemental Figure 7



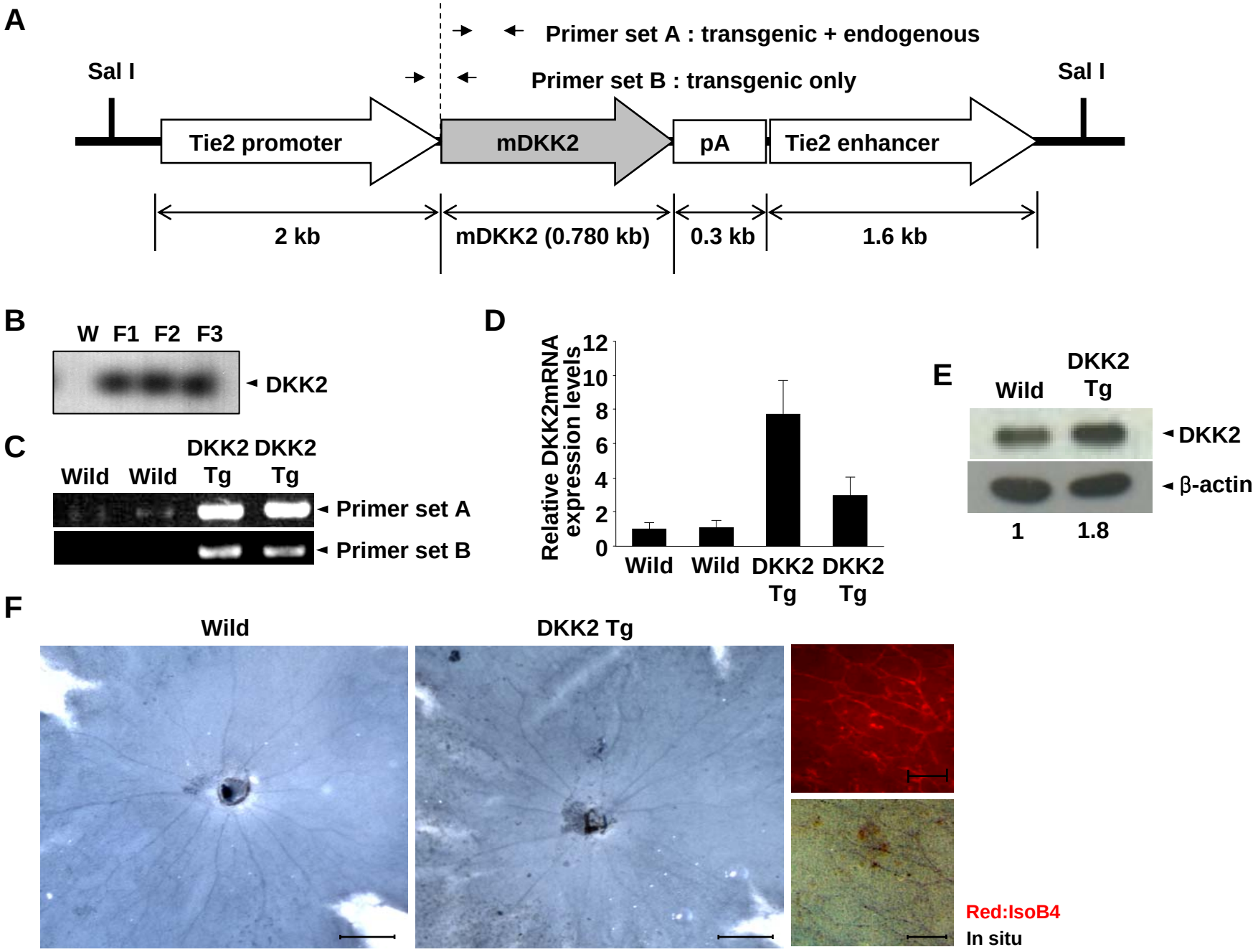
Supplemental Figure 8



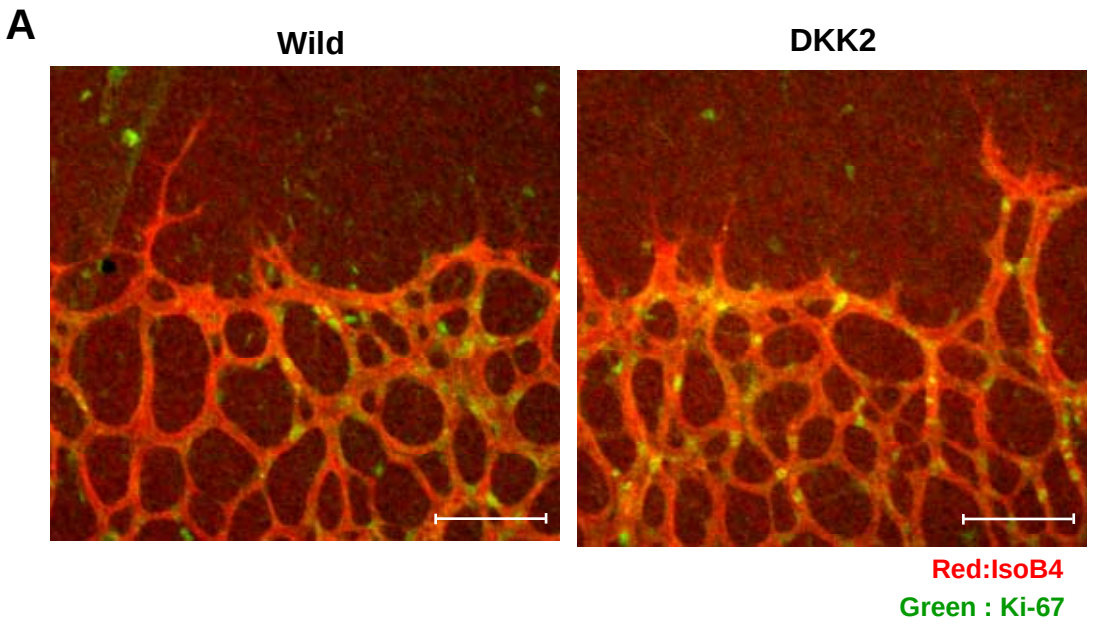
Supplemental Figure 9



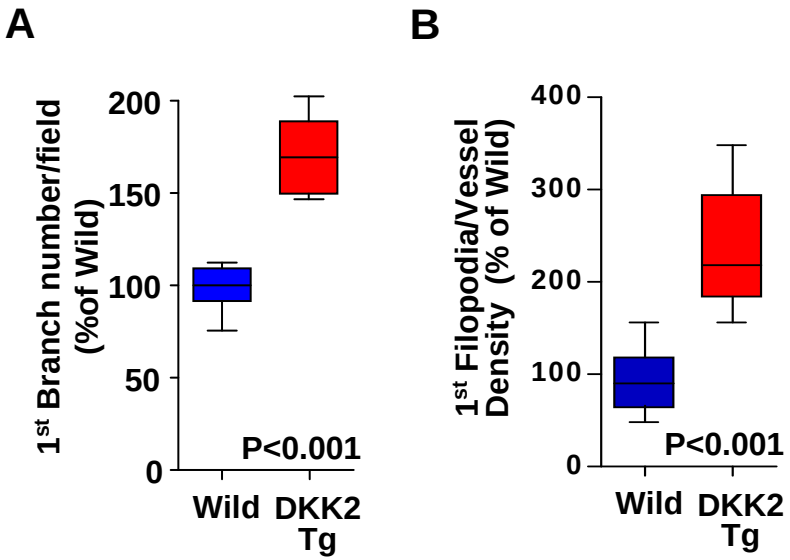
Supplemental Figure 10



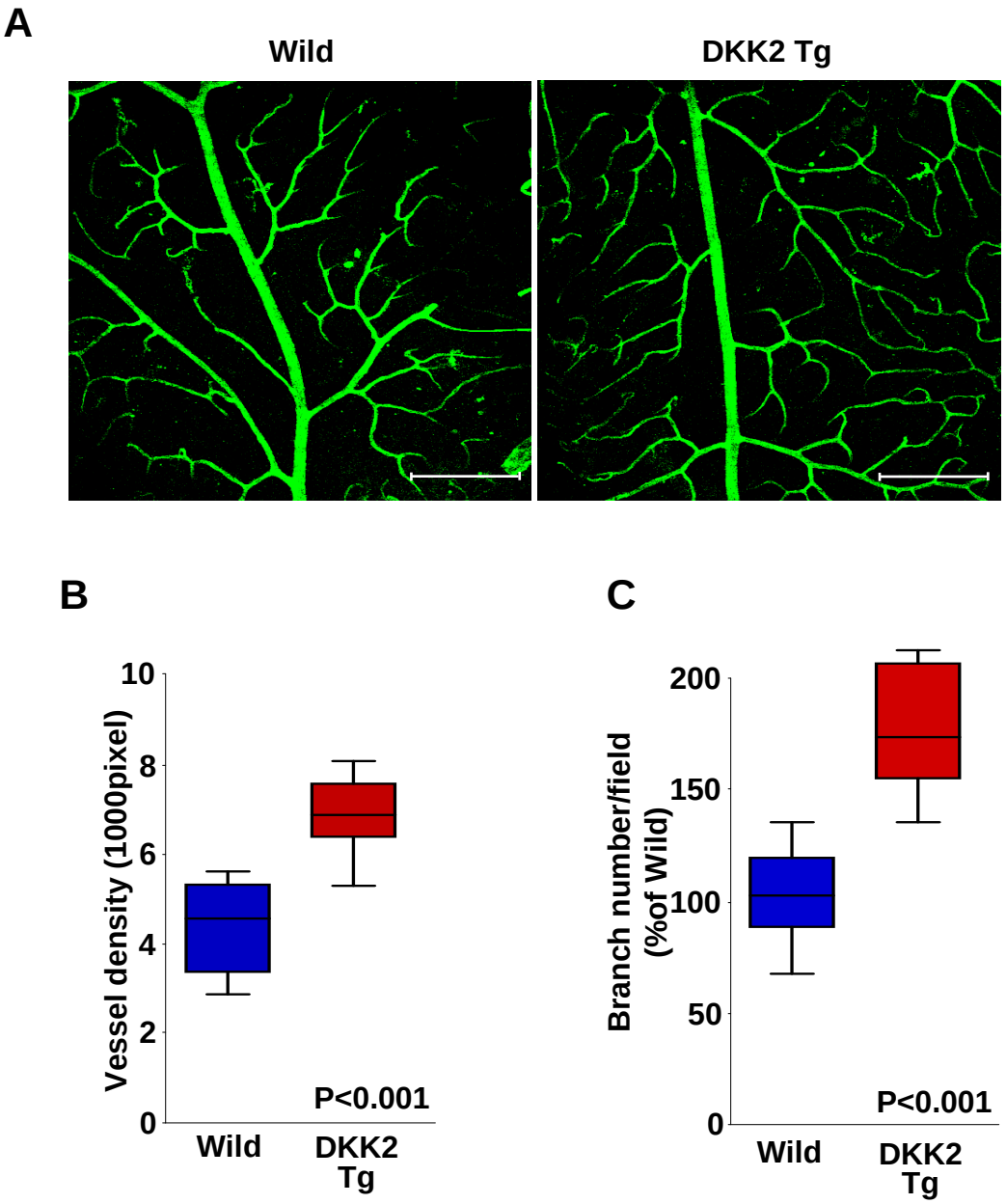
Supplemental Figure 11



Supplemental Figure 12

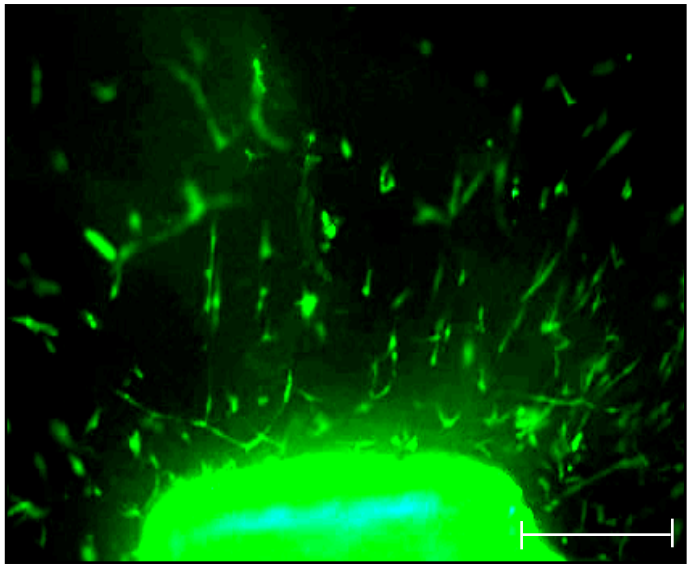


Supplemental Figure 13

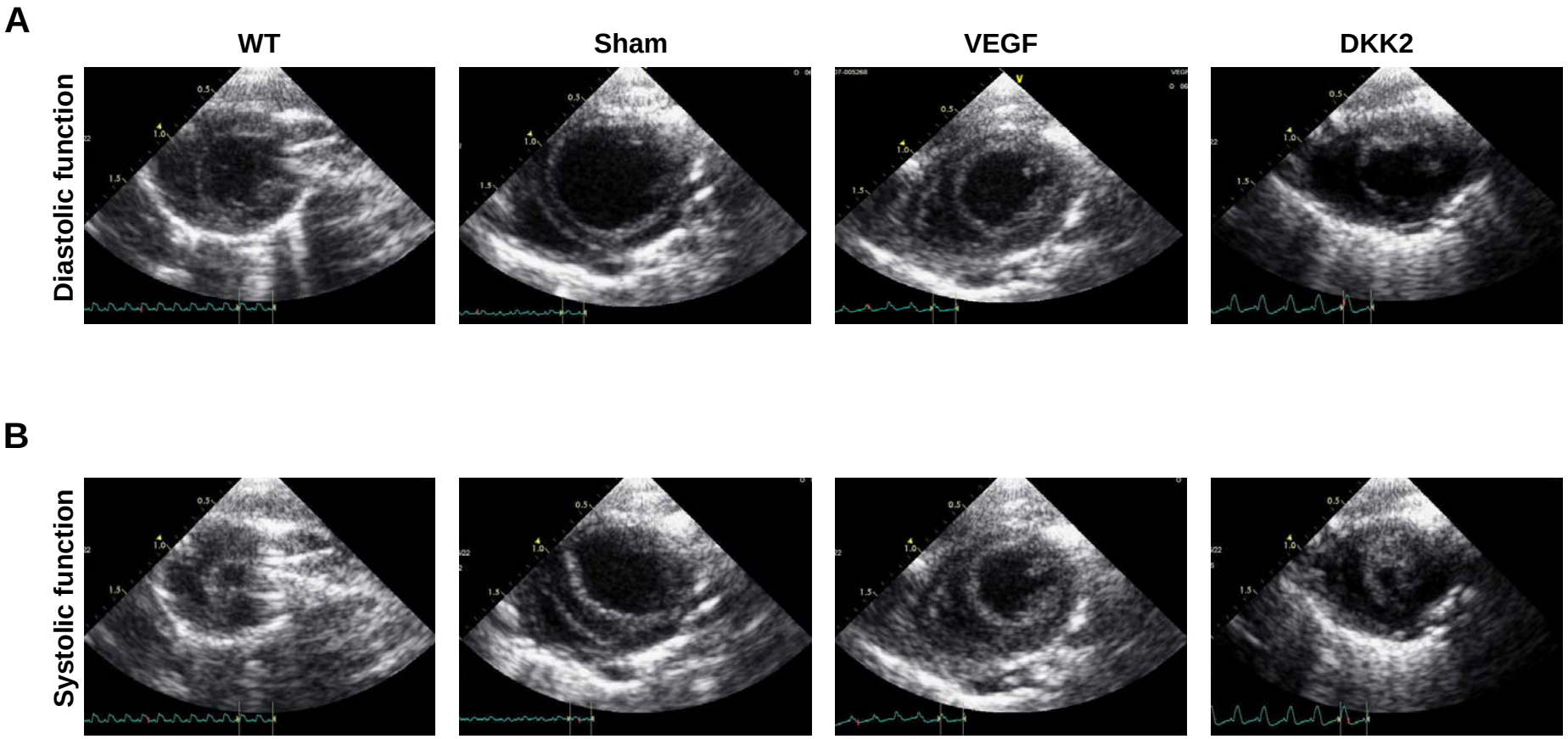


Supplemental Figure 14

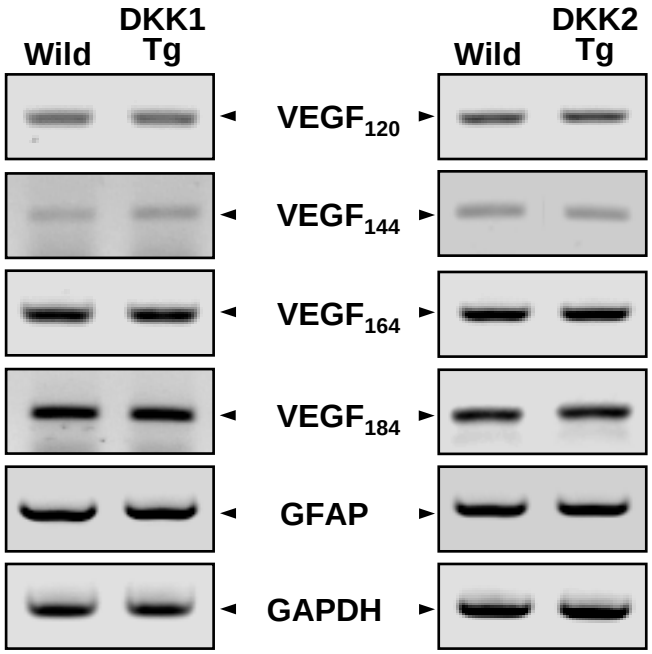
Isolectin B4



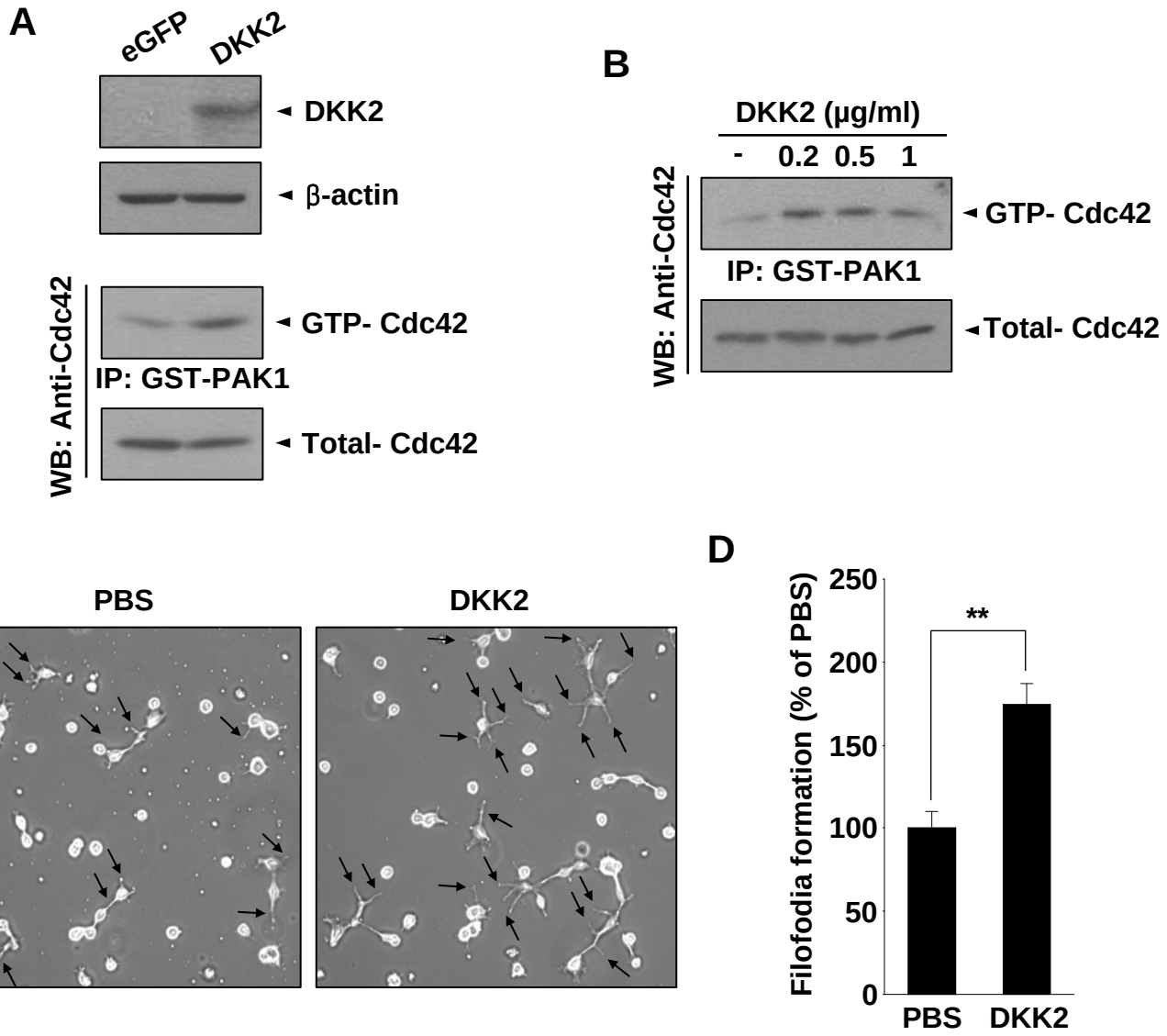
Supplemental Figure 15



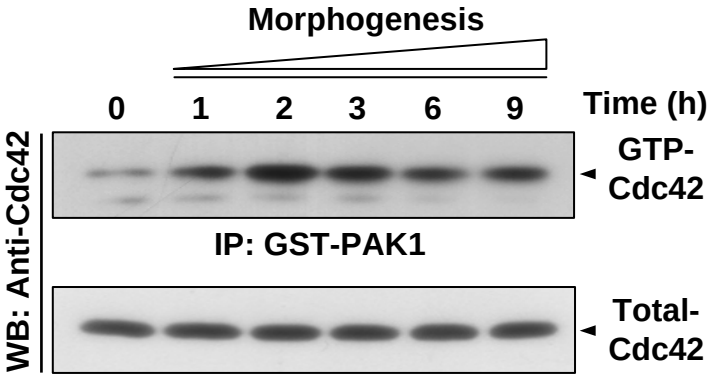
Supplemental Figure 16



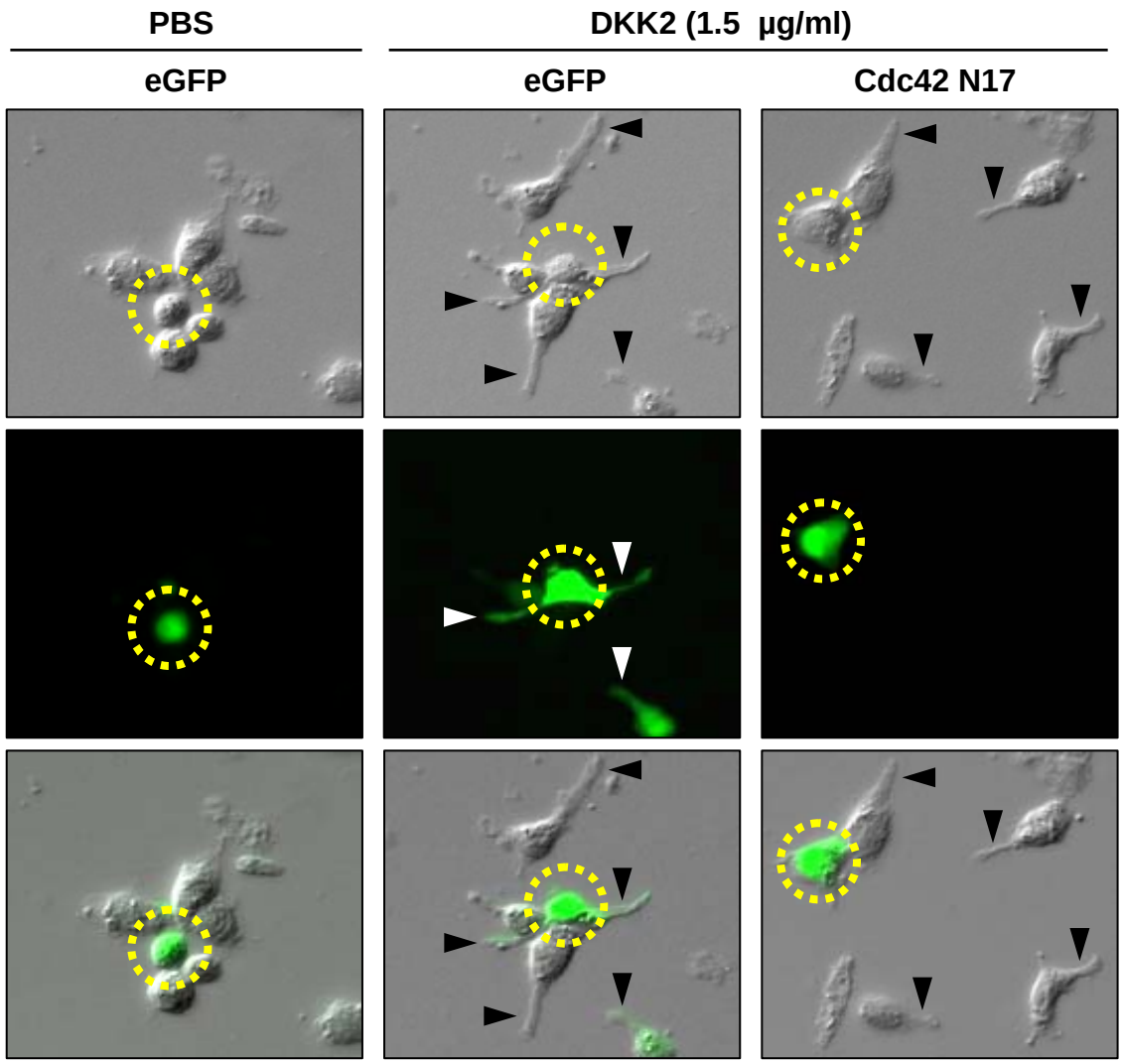
Supplemental Figure 17



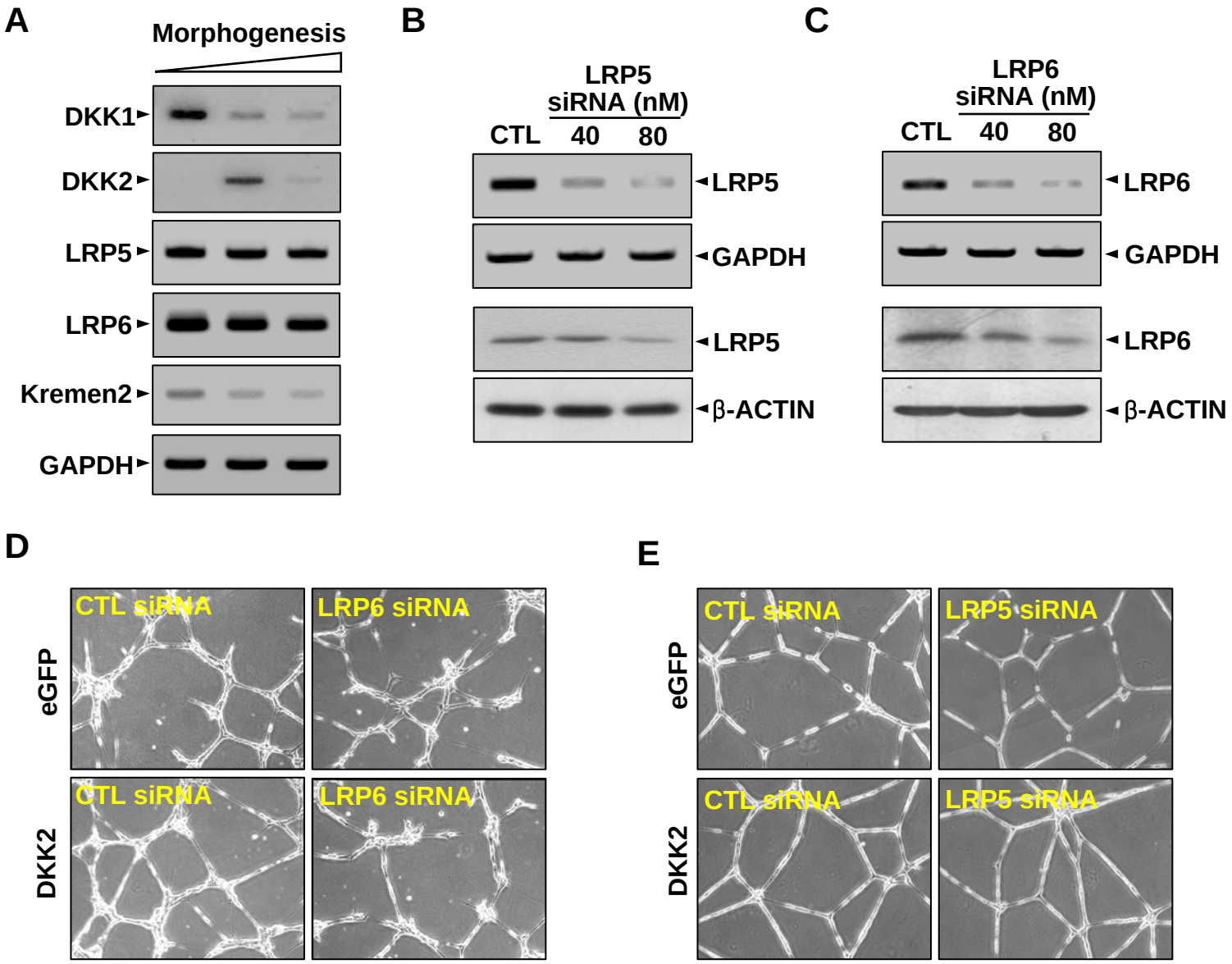
Supplemental Figure 18



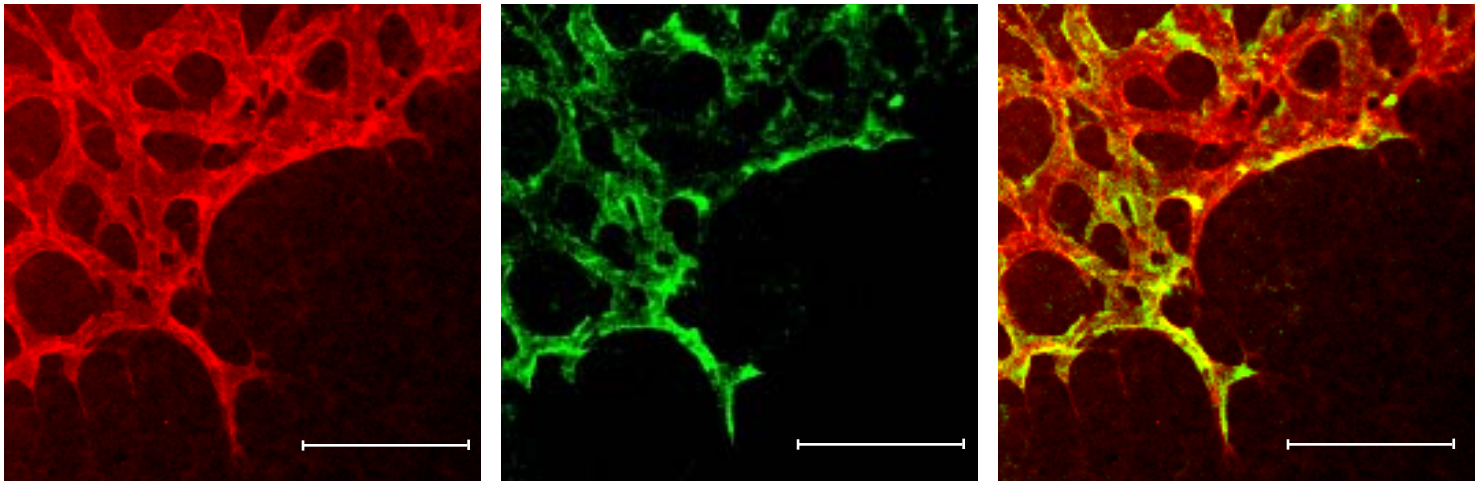
Supplemental Figure 19



Supplemental Figure 20

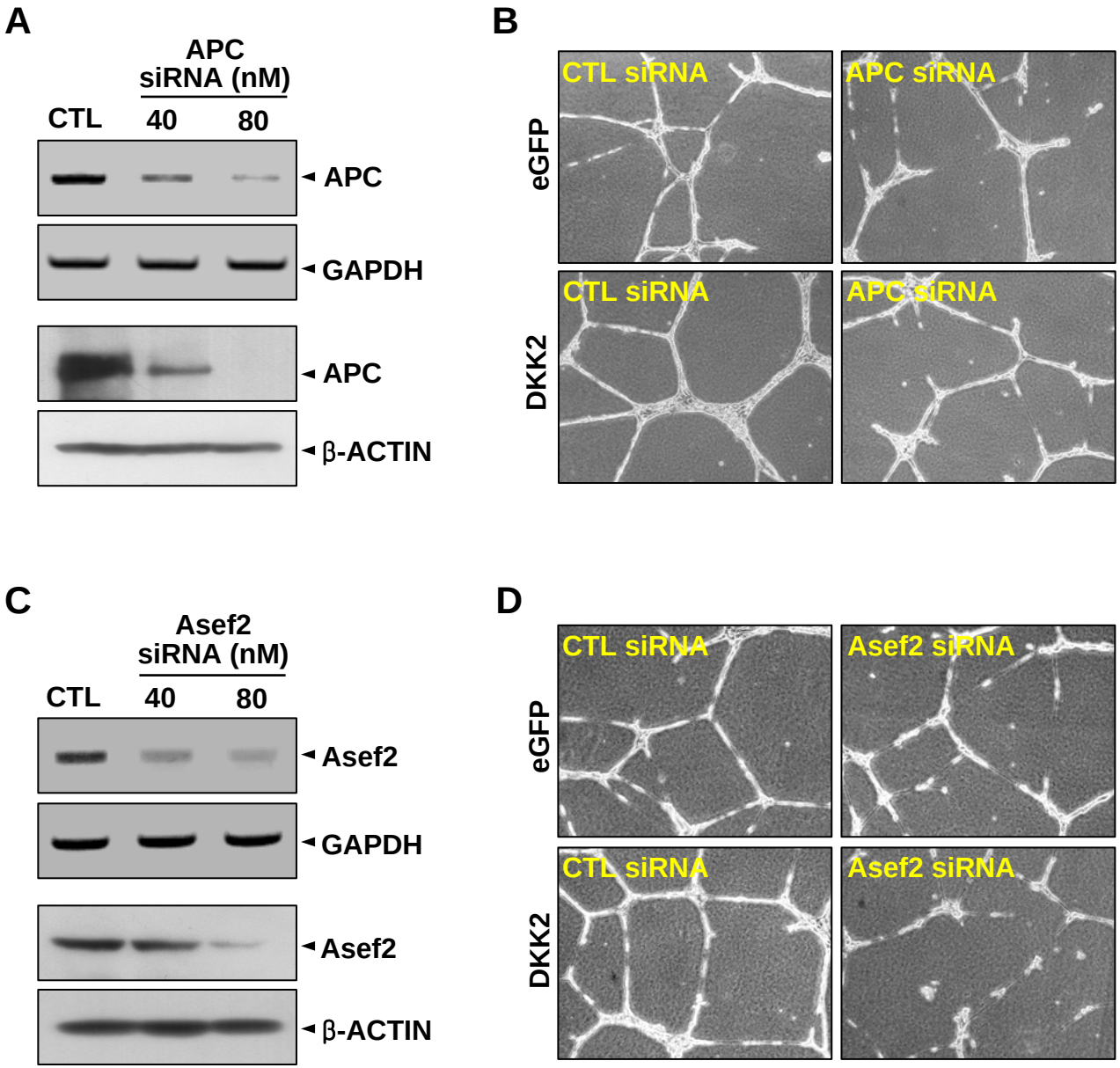


Supplemental Figure 21

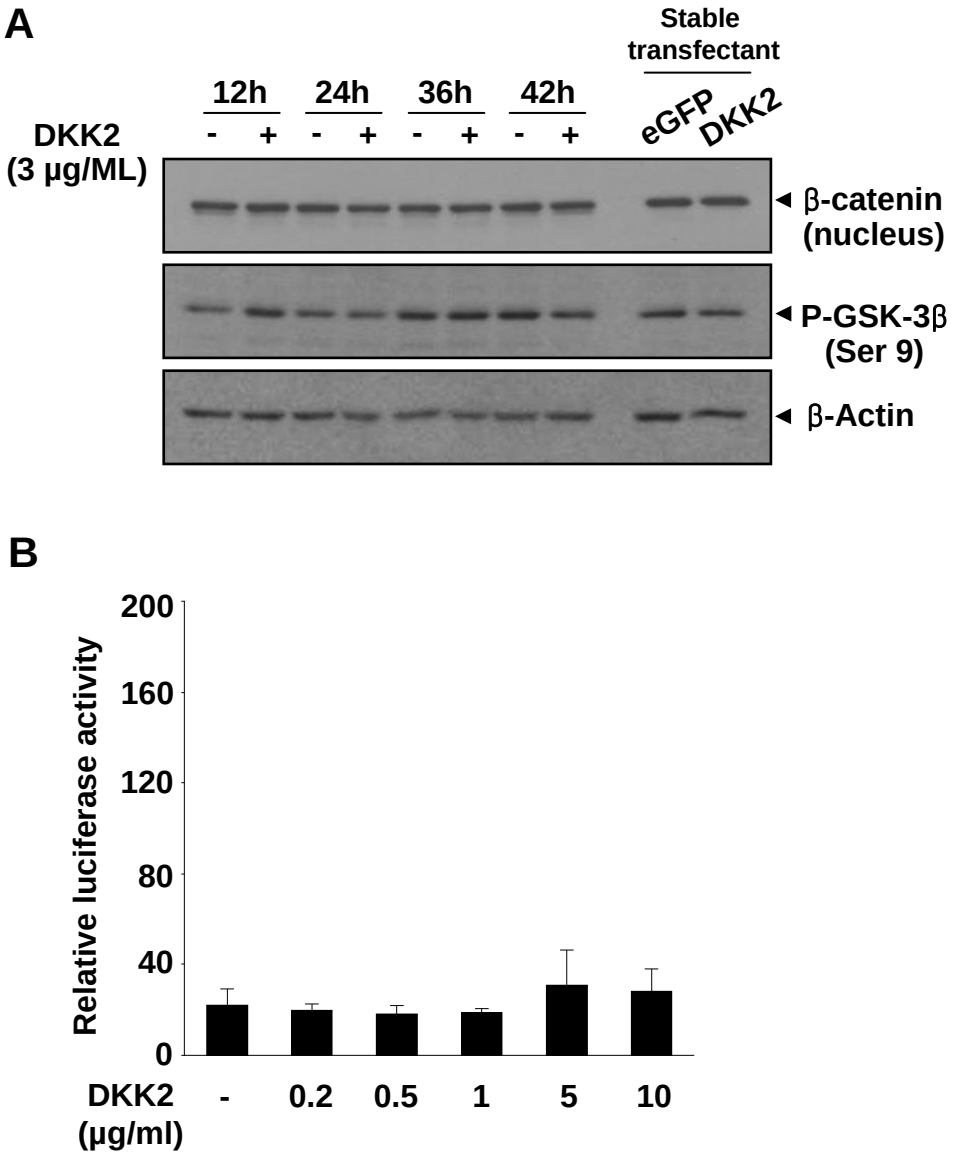


Green: LRP6
Red: CD31

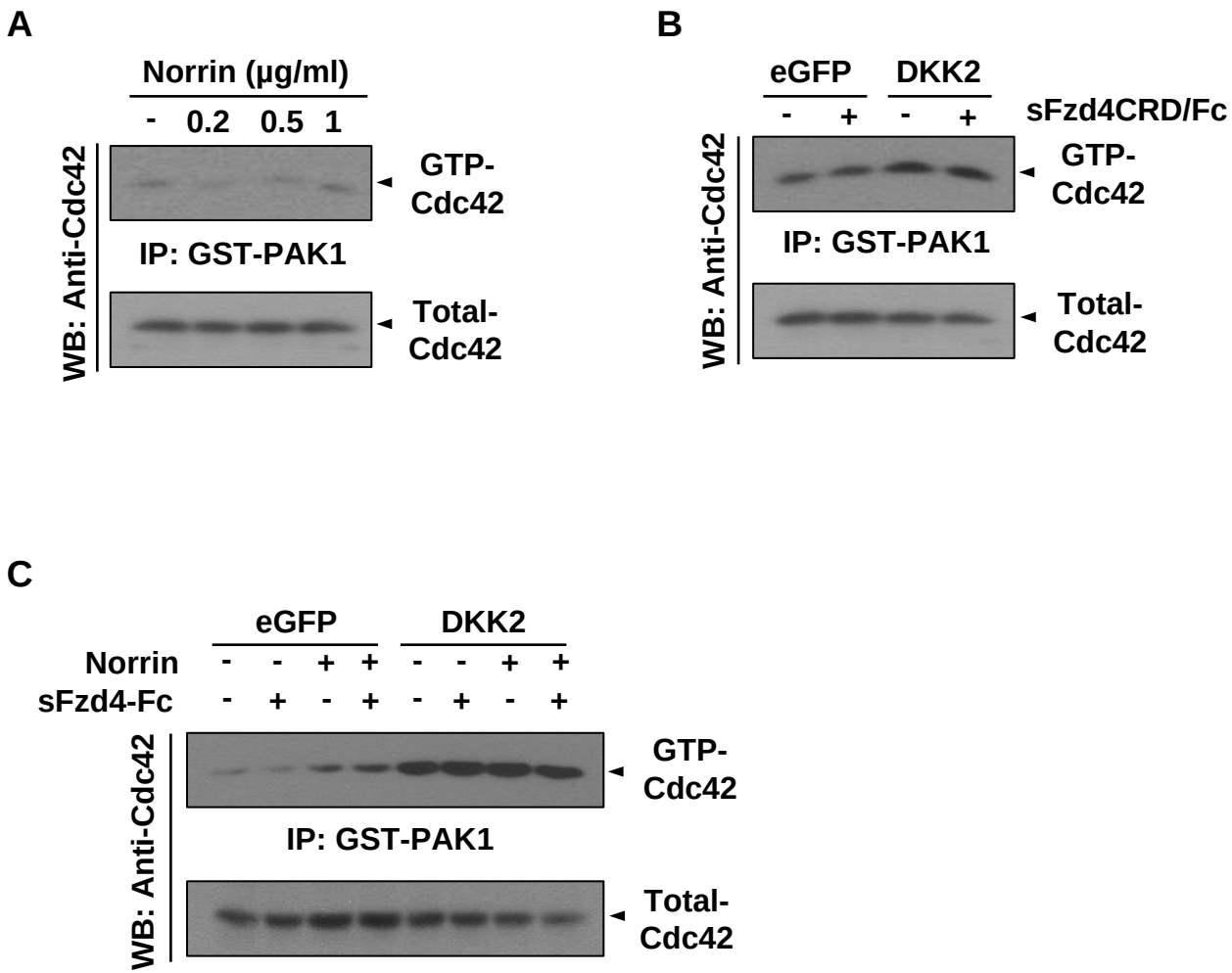
Supplemental Figure 22



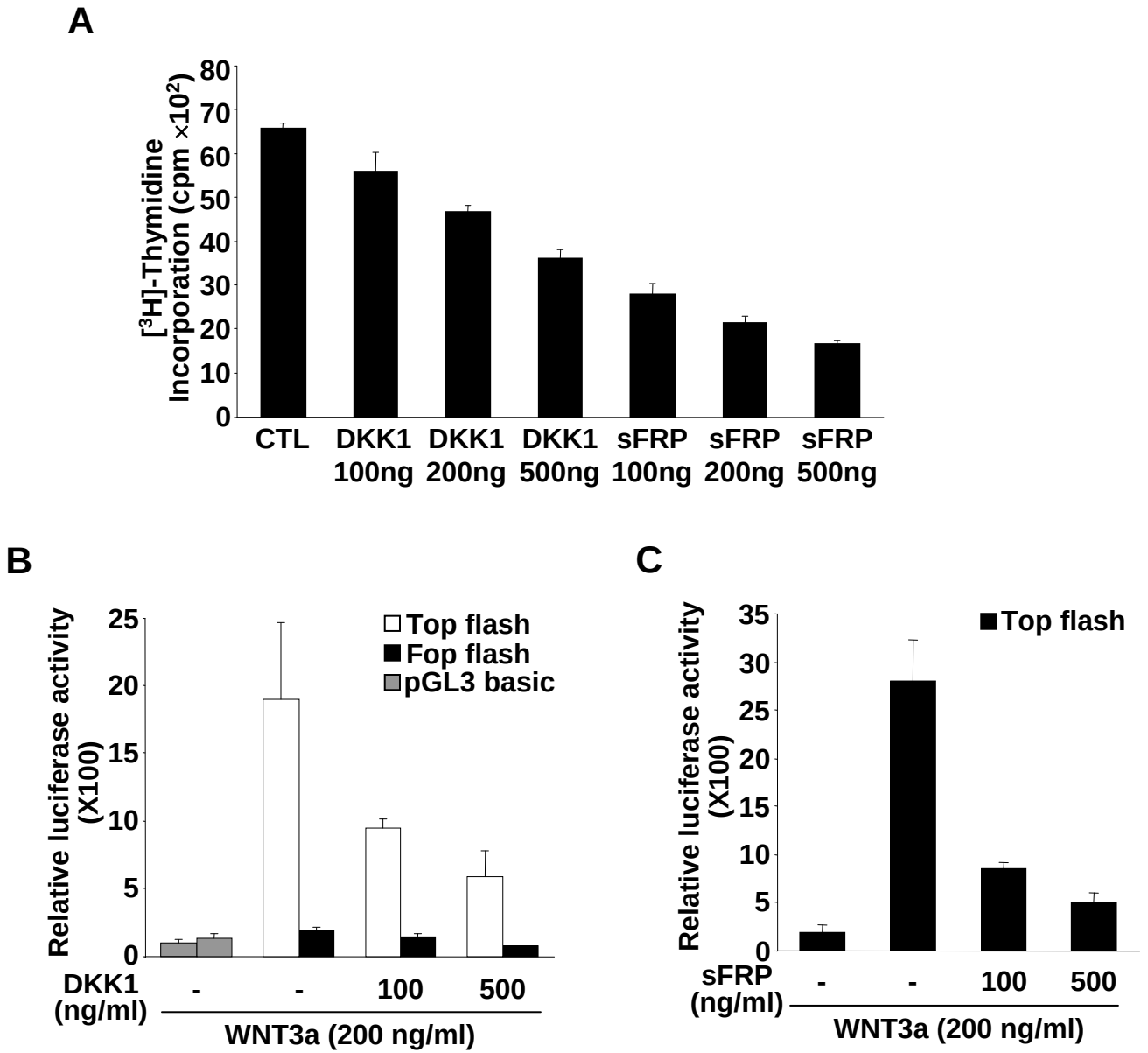
Supplemental Figure 23



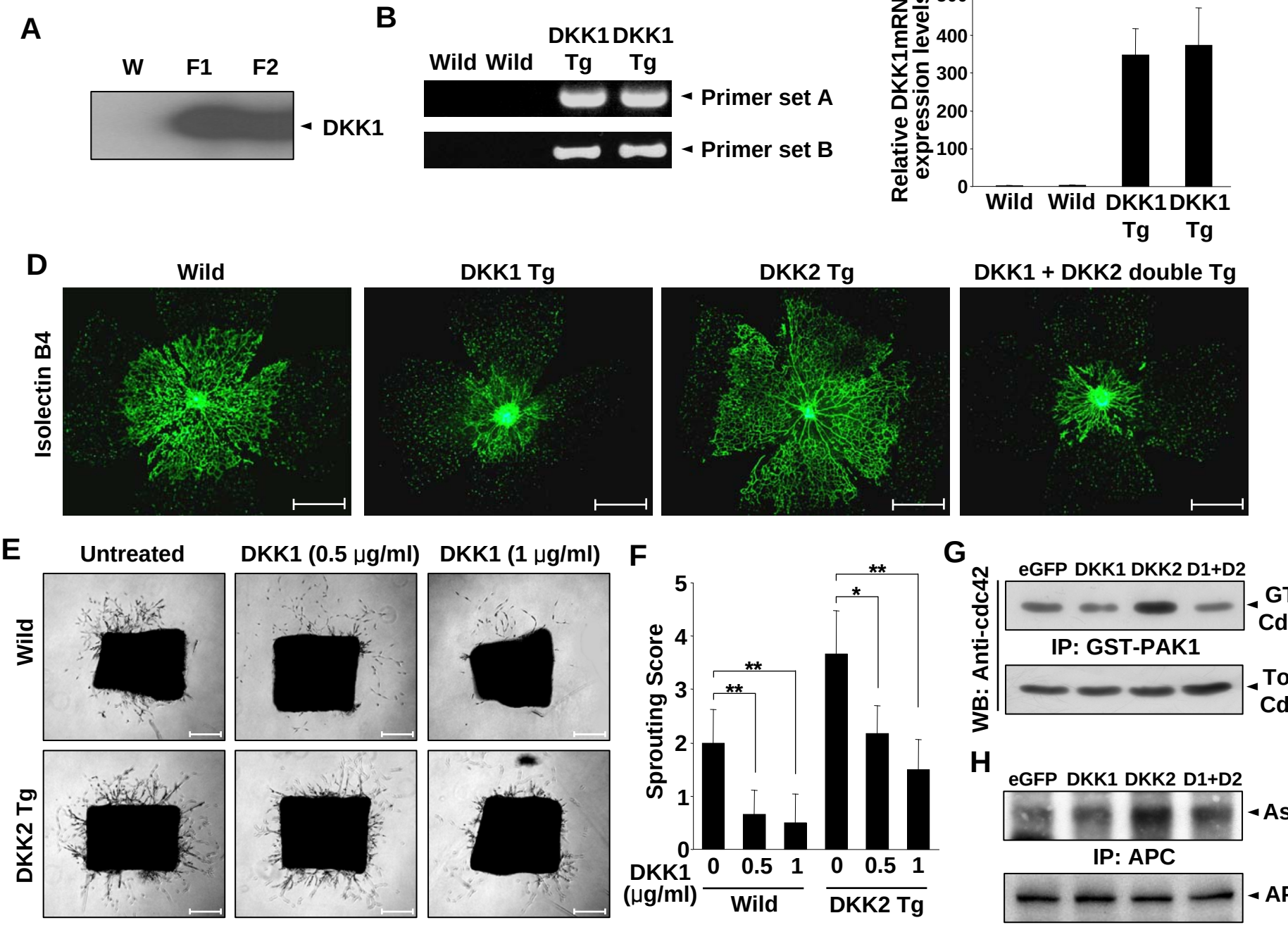
Supplemental Figure 24



Supplemental Figure 25



Supplemental Figure 26



Supplementary information

Supplementary Figure Legends

Figure 1 Reversible expression of DKK1 and DKK2 during in vitro morphogenesis (**A**) In vitro process of endothelial morphogenesis of HUVECs in 2D Matrigel. Microphotographs of HUVECs were taken at different time points (S1; 0.5, S2; 8, and S3; 18 hrs). (**B**) Proliferating HUVECs on gelatin were plated on Matrigel-coated plates and incubated for 8 hrs. Then, the cells were replated on gelatin-coated plates and allowed to proliferate for the indicated times (18 hrs and 36 hrs). (**C**) mRNA and protein levels of DKK1 and DKK2 were measured by RT-PCR (left) and Western blotting (right). G; Gelatin, M; Matrigel, G18; 18hrs after replated on gelatin, G36; 36hrs after replated on gelatin

Figure 2 Effects of angiogenic mediators and microenvironments on the DKK1 and DKK2 expression. (**A**) HUVECs were stimulated with VEGF (20 ng/ml), HGF (20 ng/ml), EGF (20 ng/ml), IGF (50 ng/ml), bFGF (20 ng/ml), or PDGF (50 ng/ml) for 8 hrs. (**B**) HUVECs were incubated for the indicated times under hypoxia or normoxia conditions. (**C**) HUVECs were plated on gelatin (*G*)-, Matrigel (*M*)-, fibronectin (*F*)-, laminin (*L*)-, or collagen (*C*)-coated plates and incubated for 8 hrs. mRNA levels of DKK1 and DKK2 were measured by RT-PCR. (**D**) and (**E**) HUVECs were plated on Matrigel (M)- or Matrigel plus laminin (L)- mixtures-coated plates at a density of 1 x 10^6 cells/well and incubated for 0.5 or 8 hrs. (**D**) Microphotographs were taken. (**E**) mRNA levels of DKK1 and DKK2 were measured by RT-PCR. (**F, G**) HUVECs were plated on Matrigel-coated plates at the indicated densities and incubated for 0.5 or 8 hrs. (**F**) Microphotographs were taken. (**G**) mRNA levels of DKK1 and DKK2 were measured by RT-PCR.

Figure 3 HUVECs stably expressing eGFP plus control (CTL) shRNA, eGFP plus DKK1 shRNA, or DKK1 plus control (CTL) shRNA were established with lentivirus. (**A**) DKK1 mRNA and protein levels were measured with RT-PCR (top) and western blotting (bottom). (**B**) Stable transfectants were plated on Matrigel-coated plates at a density of 1.5 x 10^5 cells/well and incubated for 18 hrs. Microphotographs were taken.

Figure 4 HUVECs stably expressing eGFP, DKK2, control shRNA, or DKK2 shRNA with lentivirus. (**A**) mRNA and protein levels were measured with RT-PCR (top) and western blotting (bottom). (**B**) Stable transfectants were plated on Matrigel-coated plates at a density of 1.5 x 10^5 cells/well and incubated for 18 hrs. Microphotographs were taken.

Figure 5 Effects of recombinant DKK1 and DKK2 on the formation of EC tube-like structure. (**A, B**) HUVECs (5 X 10^5) were incubated with recombinant native (DKK1) (100 ng/ml), Fc-fusion (DKK1-Fc) (100 ng/ml), recombinant native (DKK2) (100 ng/ml), Fc-fusion (DKK2-Fc) (100 ng/ml) or DKK1(100 ng/ml) in the presence of VEGF (20 ng/ml) on Matrigel. (**A**) After 18 hrs, microphotographs were taken and representative endothelial tubes were shown. (**B**) Capillary-like networks which completely differentiated into tube-like structure were quantified with Image-Pro Plus software. Data are mean ± SD; **p < 0.01 or ***p < 0.001. (**C**) Recombinant native (nDKK1, -2) or Fc-fusion (DKK1-Fc,-2-Fc) were separated by SDS-PAGE and stained with Coomassie blue.

Figure 6 The effect of DKK2 protein on endothelial cell sprouting in fibrin-gel bead assay. (**A-C**) HUVEC coated beads were embedded in fibrin gel and cultured in fibroblast cell conditioned media in the presence or absence of 50 ng/ml DKK2 for 5 days. Beads pictures

were taken (**A**) and analyzed for sprouts area (**B**) and sprouts density (**C**). Number of DKK2 treated beads = 28 ; control beads =32. Data are mean ± SD (***p < 0.001). Scale bar : 50 μm.

Figure 7 Proliferative indices of HUVECs stably transfected with control or DKK1-specific shRNA were assessed by [^{3}H] Thymidine incorporation assay.

Figure 8 The effect of DKK2 recombinant protein on vessel recruitment in Matrigel plugs. C57BL/6 mice were injected with 0.6 ml of Matrigel containing VEGF (200 ng) and DKK2 (1 μg) as indicated (n = 5 per group). After 5 d, mice were killed and Matrigel plugs were excised. Photographs of the plugs were taken. Scale bars : 10 mm.

Figure 9 CD31 and SMA staining of pellet implanted corneas. Cryosection staining of corneas (**A**). Scale bars: 100 μm. SMC coverage was calculated as a ratio of SMA to CD31 staining area (**B**). Green; CD31-positive, Red; SMA-positive, Blue; DAPI. Data are presented as mean ± S.D. (***p < 0.001).

Figure 10 Construction and generation of EC-specific DKK2 Tg mice. (**A**) The transgene construct. (**B**) Three (DKK2) founder mice per group were identified. Confirmation of the presence of the transgene was done with Southern blotting. (**C**) Total RNA was extracted from P12 mouse retinas, and Tg-specific DKK2 expression was confirmed with RT-PCR using primer set A (DKK2 cDNA-specific primer set) and B (Tg-specific primer set). (**D**) Real-time RT-PCR using primer set A was performed. (**E**) DKK2 protein overexpression was confirmed by western blot of whole retina. (**F**) DKK2 expression pattern in retina was shown by in situ

hybridization and costaining with Isolectin B4. Scale bars : 200 μm.

Figure 11 Ki-67 staining of whole-mount P4 retinas. (**A**) Staining with Isolectin B4 and Ki-67. (**B**) Quantification of Ki-67 positive endothelial cells per field. Scale bars : 100 μm.

Figure 12 Isolectin B4 staining of whole-mount P12 retinas. (**A**) Quantification of vessel branching point in the ganglion layer(1^{st}). (**B**) Internal filopodia per vessel in the ganglion layer (1^{st}).

Figure 13 Retina of 10 weeks of DKK2 Tg mice. (**A**) Retina vessel staining of 10 weeks old wild (n=15) and DKK2 (n=9) Tg mice. Flat mounted retinas were analyzed by confocal fluorescence microscopy (LSM 510 META). Scale bars: 200 μm. (**B, C**) Retative vessel density (**B**), branch number (**C**) of Tg retina compared to wild. Density and branch number are measured by Multi Guage V2.2 . Data are mean ± SD (***$p < 0.001$).

Figure 14 Staining of sprouted aorta with Isolectin B4 (green). Scale bars : 200 μm.

Figure 15 Effective improvement of cardiac function following DKK2 injection. The image represents diastolic function (**A**) and systolic function (**B**). Cardiac functions were measured with two-dimensional conventional parameters: fractional shortening (FS) and LV ejection fraction (EF) 3 weeks after injection of DKK2 into MI rats.

Figure 16 Effects of DKK1 and DKK2 on expression of VEGF isoforms and GFAP. RT-PCR analysis detects VEGF isoforms and GFAP in the retina at P4.

Figure 17 DKK2 stimulates Cdc42 activation and increases filopodial protrusions in human retinal endithelial cells (HRECs). (**A**) HRECs stably expressing eGFP or DKK2 were analyzed for Cdc42 activities. (**B**) HRECs were treated with various concentration (0.2, 0.5, or 1 μg/ml) of DKK2 for 20 min and Cdc42 activities were measured. (**C**, **D**) HRECs were plated on Matrigel-coated plates and incubated with PBS or DKK2 (0.5 μg/ml) for 2 hrs. Then, microphotographs were taken (**C**) and filopodia number was quantified (**D**). Arrowheads indicate filopodial extension. Data are mean ± SD (**p < 0.01).

Figure 18 HUVECs were cultured on Matrigel-coated plate (*morphogenesis*) for the indicated times. Cdc42 activities were measured.

Figure 19 HUVECs were transiently transfected with expression plasmids encoding GFP-control or the GFP-dominant negative mutant of Cdc42 (Cdc42 N17). After 24 hrs, cells were plated on Matrigel-coated plates and incubated with PBS or DKK2 (1.5 μg/ml) for 2 hrs. Then, microphotographs were taken. White and black arrows indicate filopodial extension. Yellow broken circles indicate transfected cell.

Figure 20 Expression of putative DKK2 receptors in HUVECs. (**A**) During EC morphogenesis, mRNA levels of DKK1, DKK2, LRP5, and LRP6 were measured with RT-PCR. (**B, C**) HUVECs were transiently transfected with control siRNA (CTL), LRP5-, or LRP6-specific siRNA. After 40 (for mRNA) or 60 (for protein) hrs, levels of mRNA and protein were measured with RT-PCR (top) and western blotting (bottom). (**D**) HUVECs stably expressing eGFP or DKK2 were transiently transfected with LRP5 specific siRNA. The cells were plated on Matrigel-coated plates at a density of 1.5 x 10^5 cells/well and incubated for 18

hrs. Microphotographs were taken.

Figure 21 Staining of LRP6 with CD31 in retinal vessel (green). Scale bars : 100 μm

Figure 22 Effects of APC and Asef2 on DKK2-induced EC morphogenesis. (**A, C**) HUVECs were transiently transfected with control siRNA (CTL), APC-, or Asef2-specific siRNA. After 40 (for mRNA) or 80 (for protein) hrs, mRNA and protein levels were measured with RT-PCR (top) and western blotting (bottom). (**B, D**) HUVECs stably expressing eGFP or DKK2 were transiently transfected with control siRNA (CTL), APC-, or Asef2-specific siRNA. After 60 hrs, cells were plated on Matrigel-coated plates at a density of 1.5×10^5 cells/well and incubated for 18 hrs. Microphotographs were taken.

Figure 23 DKK2 does not affect canonical WNT signaling (**A**) HUVECs were incubated with 3 μg/mL of DKK2 for the indicated times. Western blots were probed with anti-β-catenin and anti-phospho-GSK-3β antibodies, and reprobed with anti- β-actin antibody to verify equal loading of protein. (**B**) HUVECs were transiently transfected with 1 μg of Topflash reporter plasmid and incubated with various concentrations (0.2, 0.5, 1, 5, or 25 μg/ml) of DKK2 for 24 hrs. Data are mean ± SD of triplicate experiments relative to the luciferase light units.

Figure 24 DKK2 stimulates Cdc42 activation independent of WNT or Norrin/Fzd signaling in HRECs. (**A**) HRECs were treated with various concentration (0.2, 0.5, or 1 μg/ml) of Norrin for 20 min and Cdc42 activity was measured. (**B**) HRECs stably expressing eGFP or DKK2 were incubated with sFzd4CRD/Fc (0.5 μg/ml) for 24 hrs, and Cdc42 activity was measured. (**C**) HRECs stably expressing eGFP or DKK2 were incubated with sFzd4CRD/Fc

(0.5 μg/ml), Norrin (0.5 μg/ml), or sFzd4CRD/Fc (0.5 μg/ml) plus Norrin (0.5 μg/ml) for 24 hrs, and Cdc42 activity was measured.

Figure 25 Antagonistic effects of DKK1 or sFRP on WNT/β-catenin signaling in ECs. (**A**) HUVECs were incubated with DKK1 or sFRP for 72 hrs. Proliferative indices were assessed by [^{3}H] Thymidine incorporation assay. (**B, C**) HUVECs were transiently transfected with control pGL-basic, Top, or Fop flash plasmids and then treated with Wnt3a in the presence or absence of DKK1 (**B**) or sFRP (**C**) for 24 hrs. Luciferase activities were measured using the luciferase reporter assay system (Promega)

.

Figure 26 DKK1 antagonizes DKK2 function in the vasculature. (**A**) Two (DKK1) founder mice per group were identified. Confirmation of the presence of the transgene was done with Southern blotting. (**B**) Total RNA was extracted from P12 mouse retinas, and Tg-specific DKK2 expression was confirmed with RT-PCR using primer set A (DKK2 cDNA-specific primer set) and B (Tg-specific primer set). (**C**) Real-time RT-PCR using primer set A was performed. (**D**) Isolectin B4 staining of whole-mounted P4 retinas. Scale bars: 500 μm. (**E**), (**F**) Aortic segments were harvested from wild and DKK2 Tg mice (n = 7 per group). Aortas in Matrigel were treated with DKK1 (0.5 or 1 μg/ml) for 4 days. (**E**) Representative aortic rings were photographed. Scale bars : 200μm. (**F**) Sprouting scores were classified from 0 (least positive) to 5 (most positive). Data are mean ± SD (*$p < 0.05$, **$p < 0.01$). (**G, H**) Stable transfectants expressing eGFP, DKK1, DKK2, or DKK1 plus DKK2. Cdc42 activity (**G**). Coimmunoprecipitation of APC with Asef2 (**H**).

Supplemental Table

	WT	Sham	VEGF	DKK2
LVEDD(mm)	6.24±0.41	7.87±0.50	6.95±0.38	6.38±0.33
LVESD(mm)	3.15±0.11	5.88±0.42	4.83±0.49	3.39±0.23
FS(%)	49.15±1.83	25.31±0.56	30.62±3.20	46.89±0.84
LVEF(%)	79.61±1.55	48.31±1.15	56.68±4.96	77.28±1.06

Table 1 Effective improvement of cardiac function with DKK injection.

Supplementary Video Legend

Video 1 Organ culture model of vessel sprouting adapted from the aortic ring model. Time-lapse movie of filopodial extension at tip cells. Aortic segments were harvested from wild-type (*left*) and DKK2 (*right*) Tg mice. Endothelial-cell sprouts forming branching cords from the margins of vessel segments taken from mice were photographed with a real-time microscope for 9 hrs.