Supplementary data

Video S1. Motility of ECC-1 cells in 3D monoculture. Time-lapse imaging was performed on ECC-1 3D monocultures. ECC-1 cells were labeled using RFP. Phase-contrast and fluorescence images were acquired every hour for 16 h using a 10X lens. Scale bar, 100 μm. Red represents RFP and grey represents phase-contrast. RFP, red fluorescence protein.

Video S2. Motility of EC48Fib in 3D monoculture. Time-lapse imaging was performed on EC48Fib 3D monocultures. EC48Fib cells were labeled using GFP. Phase-contrast and fluorescence images were acquired every hour for 16 h using a 10X lens. Scale bar, 100 μm. Green represents GFP and grey represents phase-contrast. GFP, green fluorescence protein.

Video S3. Motility of EC49Fib in 3D monoculture. Time-lapse imaging was performed on EC49Fib 3D monocultures. EC49Fib cells were labeled using GFP. Phase-contrast and fluorescence images were acquired every hour for 16 h using a 10X lens. Scale bar, 100 μm. Green represents GFP and grey represents phase-contrast. GFP, green fluorescence protein.

Video S4. Motility of EC50Fib in 3D monoculture. Time-lapse imaging was performed on EC50Fib 3D monocultures. EC49Fib cells were labeled using GFP. Phase-contrast and fluorescence images were acquired every hour for 16 h using a 10X lens. Scale bar, 100 μm. Green represents GFP and grey represents phase-contrast. GFP, green fluorescence protein.

Video S5. Effect of EC48Fib on ECC-1 cell motility in 3D co-culture. Time-lapse imaging was performed on ECC-1 and EC48Fib 3D co-culture models. ECC-1 cells were labeled using RFP and EC48Fib using GFP. Phase-contrast and fluorescence images were acquired every hour for 16 h using a 10X lens. Scale bar, 100 μm. Red represents RFP, green represents GFP and grey represents phase-contrast. RFP, red fluorescence protein; GFP, green fluorescence protein.

Video S6. Effect of EC49Fib on ECC-1 cells motility in 3D co-culture. Time-lapse imaging was performed on ECC-1 and EC49Fib 3D co-culture models. ECC-1 cells were labeled using RFP and EC49Fib using GFP. Phase-contrast and fluorescence images were acquired every hour for 16 h using a 10X lens. Scale bar, 100 μm. Red represents RFP, green represents GFP and grey represents phase-contrast. GFP, green fluorescence protein; RFP, red fluorescence protein.

Video S7. Effect of EC50Fib on ECC-1 cell motility in 3D co-culture. Time-lapse imaging was performed on ECC-1 and EC50Fib 3D co-culture models. ECC-1 cells were labeled using RFP and EC50Fib using GFP. Phase-contrast and fluorescence images were acquired every hour for 16 h using a 10X lens. Scale bar, 100 μm. Red represents RFP, green represents GFP and grey represents phase-contrast. GFP, green fluorescence protein; RFP, red fluorescence protein.

Video S8. Effect of EF2Fib on ECC-1 cell motility in 3D co-culture. Time-lapse imaging was performed on ECC-1 and EF2Fib 3D co-culture models. ECC-1 cells were labeled using RFP and EF2Fib using GFP. Phase-contrast and fluorescence images were acquired every hour for 16 h using a 10X lens. Scale bar, 100 μm. Red represents RFP, green represents GFP and grey represents phase-contrast. GFP, green fluorescence protein; RFP, red fluorescence protein.

Video S9. Effect of EH6Fib on ECC-1 cell motility in 3D co-culture. Time-lapse imaging was performed on ECC-1 and EH6Fib 3D co-culture models. ECC-1 cells were labeled using RFP and EH6Fib using GFP. Phase-contrast and fluorescence images were acquired every hour for 16 h using a 10X lens. Scale bar, 100 μm. Red represents RFP, green represents GFP and grey represents phase-contrast. GFP, green fluorescence protein; RFP, red fluorescence protein.

Video S10. Effect of NE14Fib on ECC-1 cell motility in 3D co-culture. Time-lapse imaging was performed on ECC-1 and NE14Fib 3D co-culture models. ECC-1 cells were labeled using RFP and NE14Fib using GFP. Phase-contrast and fluorescence images were acquired every hour for 16 h using a 10X lens. Scale bar, 100 μm. Red represents RFP, green represents GFP and grey represents phase-contrast. GFP, green fluorescence protein; RFP, red fluorescence protein.

Video S11. Motility of EF2Fib in 3D monoculture. Time-lapse imaging was performed on EF2Fib 3D monocultures. EF2Fib cells were labeled using GFP. Phase-contrast and fluorescence images were acquired every hour for 16 h using a 10X lens. Scale bar, 100 μm. Green represents GFP and grey represents phase-contrast. GFP, green fluorescence protein.

Video S12. Motility of EH6Fib in 3D monoculture. Time-lapse imaging was performed on EH6Fib 3D monocultures. EH6Fib cells were labeled using GFP. Phase-contrast and fluorescence images were acquired every hour for 16 h using a 10X lens. Scale bar, 100 μm. Green represents GFP and grey represents phase-contrast. GFP, green fluorescence protein.

Video S13. Motility of NE14Fib in 3D monoculture. Time-lapse imaging was performed on NE14Fib 3D monocultures. NE14Fib cells were labeled using GFP. Phase-contrast and fluorescence images were acquired every hour for 16 h using a 10X lens. Scale bar, 100 μm. Green represents GFP and grey represents phase-contrast. GFP, green fluorescence protein.

Video S14. Effect of the ROCK1 inhibitor, Y-27632, on EC48Fib motility in 3D monoculture. Time-lapse imaging was performed on EC48Fib 3D monocultures following treatment with 100 μM Y-27632. The velocity of the cells was assessed. EC48Fib were labeled using GFP. Phase-contrast and fluorescence images were acquired every hour for 16 h using a 10X lens. Scale bar, 100 μm. Green represents GFP and grey represents phase-contrast. GFP, green fluorescence protein; ROCK1, Rho-associated, coiled-coil containing protein kinase 1.

Video S15. Effect of the ROCK1 inhibitor, Y-27632, on EC49Fib motility in 3D monoculture. Time-lapse imaging was performed on EC49Fib 3D monocultures following treatment with 100 μM Y-27632. The velocity of the cells was assessed. EC49Fib were

labeled using GFP. Phase-contrast and fluorescence images were acquired every hour for 16 h using a 10X lens. Scale bar, 100 μm. Green represents GFP and grey represents phase-contrast. GFP, green fluorescence protein; ROCK1, Rho-associated, coiled-coil containing protein kinase 1.

Video S16. Effect of the ROCK1 inhibitor, Y-27632, on EC50Fib motility in 3D monoculture. Time-lapse imaging was performed on EC50Fib 3D monocultures following treatment with 100 μM Y-27632. The velocity of the cells was assessed. EC50Fib were labeled using GFP. Phase-contrast and fluorescence images were acquired every hour for 16 h using a 10X lens. Scale bar, 100 μm. Green represents GFP and grey represents phase-contrast. GFP, green fluorescence protein; ROCK1, Rho-associated, coiled-coil containing protein kinase 1.

Video S17. Effect of the ROCK1 inhibitor, Y-27632, on ECC-1 cell motility in 3D monoculture. Time-lapse imaging was performed on ECC-1 3D monocultures following treatment with 100 μM Y-27632. The velocity of the cells was assessed. ECC-1 were labeled using RFP. Phase-contrast and fluorescence images were acquired every hour for 16 h using a 10X lens. Scale bar, 100 μm. Red represents RFP and grey represents phase-contrast. ROCK1, Rho-associated, coiled-coil containing protein kinase 1.

Video S18. Effect of the ROCK1 inhibitor, Y-27632, on motility and spheroid formation in ECC-1 cells and EC48Fib co-cultures. Time-lapse imaging was performed on ECC-1 cells and EC48Fib 3D co-culture models following treatment with 100 μM Y-27632. The velocity and spheroid formation of the cells were assessed. ECC-1 cells were labeled using RFP, EC48Fib using GFP. Phase-contrast and fluorescence images were acquired every hour for 16 h using a 10X lens. Scale bar, 100 μm. Red represents RFP, green represents GFP and grey represents phase-contrast. GFP, green fluorescence protein; RFP, red fluorescence protein; ROCK1, Rho-associated, coiled-coil containing protein kinase1.

Video S19. Effect of the ROCK1 inhibitor, Y-27632, on motility and spheroid formation in ECC-1 cells and EC49Fib co-cultures. Time-lapse imaging was performed on ECC-1 cells and EC49Fib 3D co-culture models following treatment with 100 μM Y-27632. The velocity and spheroid formation of the cells were assessed. ECC-1 cells were labeled using RFP, EC49Fib using GFP. Phase-contrast and fluorescence images were acquired every hour for 16 h using a 10X lens. Scale bar, 100 μm. Red represents RFP, green represents GFP and grey represents phase-contrast. GFP, green fluorescence protein; RFP, red fluorescence protein; ROCK1, Rho-associated, coiled-coil containing protein kinase 1.

Video S20. Effect of the ROCK1 inhibitor, Y-27632, on motility and spheroid formation in ECC-1 cells and EC50Fib co-cultures. Time-lapse imaging was performed on ECC-1 cells and EC50Fib 3D co-culture models following treatment with 100 μM Y-27632. The velocity and spheroid formation of the cells were assessed. ECC-1 cells were labeled using RFP, EC50Fib using GFP. Phase-contrast and fluorescence images were acquired every hour for 16 h using a 10X lens. Scale bar, 100 μm. Red represents RFP, green represents GFP and grey represents phase-contrast. GFP, green fluorescence protein; RFP, red fluorescence protein; ROCK1, Rho-associated, coiled-coil containing protein kinase 1.

VideoS 21. Effect of the ROCK1 inhibitor, Y-27632, on EF2Fib motility in 3D monoculture. Time-lapse imaging was performed on EF2Fib 3D monocultures following treatment with 100 μM Y-27632. The velocity of the cells was assessed. EF2Fib were labeled using GFP. Phase-contrast and fluorescence images were acquired every hour for 16 h using a 10X lens. Scale bar, 100 μm. Green represents GFP and grey represents phase-contrast. GFP, green fluorescence protein; ROCK1, Rho-associated, coiled-coil containing protein kinase 1.

Video S22. Effect of the ROCK1 inhibitor, Y-27632, on EH6Fib motility in 3D monoculture. Time-lapse imaging was performed on EH6Fib 3D monocultures following treatment with 100 μM Y-27632. The velocity of the cells was assessed. EH6Fib were labeled using GFP. Phase-contrast and fluorescence images were acquired every hour for 16 h using a 10X lens. Scale bar, 100 μm. Green represents GFP and grey represents phase-contrast. GFP, green fluorescence protein; ROCK1, Rho-associated, coiled-coil containing protein kinase 1.

Video S23. Effect of the ROCK1 inhibitor, Y-27632, on NE14Fib motility in 3D monoculture. Time-lapse imaging was performed on NE14Fib 3D monocultures following treatment with 100 μM Y-27632. The velocity of the cells was assessed. NE14Fib were labeled using GFP. Phase-contrast and fluorescence images were acquired every hour for 16 h using a 10X lens. Scale bar, 100 μm. Green represents GFP and grey represents phase-contrast. GFP, green fluorescence protein; ROCK1, Rho-associated, coiled-coil containing protein kinase 1.

Video S24. Effect of the ROCK1 inhibitor, Y-27632, on motility and spheroid formation in ECC-1 cells and EF2Fib co-cultures. Time-lapse imaging was performed on ECC-1 cells and EF2Fib 3D co-culture models following treatment with 100 μM Y-27632. The velocity and spheroid formation of the cells were assessed. ECC-1 cells were labeled using RFP, EF2Fib using GFP. Phase-contrast and fluorescence images were acquired every hour for 16 h using a 10X lens. Scale bar, 100 μm. Red represents RFP, green represents GFP and grey represents phase-contrast. GFP, green fluorescence protein; RFP, red fluorescence protein; ROCK1, Rho-associated, coiled-coil containing protein kinase 1.

Video S25. Effect of the ROCK1 inhibitor, Y-27632, on motility and spheroid formation in ECC-1 cells and EH6Fib co-cultures. Time-lapse imaging was performed on ECC-1 cells and EH6Fib 3D co-culture models following treatment with 100 μM Y-27632. The velocity and spheroid formation of the cells were assessed. ECC-1 cells were labeled using RFP, EH6Fib using GFP. Phase-contrast and fluorescence images were acquired every hour for 16 h using a 10X lens. Scale bar, 100 μm. Red represents RFP, green represents GFP and grey represents phase-contrast. GFP, green fluorescence protein; RFP, red fluorescence protein; ROCK1, Rho-associated, coiled-coil containing protein kinase 1.

Video S26. Effect of the ROCK1 inhibitor, Y-27632, on motility and spheroid formation in ECC-1 cells and NE14Fib co-cultures. Time-lapse imaging was performed on ECC-1 cells and NE14Fib 3D co-culture models following treatment with 100 μM Y-27632. The velocity and spheroid formation of the cells were assessed. ECC-1 cells were labeled using RFP, NE14Fib using GFP. Phase-contrast and fluorescence images were acquired every hour for 16 h using a 10X lens. Scale bar, 100 μm. Red represents RFP, green represents GFP and grey represents phase-contrast. GFP, green fluorescence protein; RFP, red fluorescence protein; ROCK1, Rho-associated, coiled-coil containing protein kinase 1.

Figure S1. mRNA expression levels of fibroblast and epithelial markers in isolated CAFs and BAFs. Total RNA samples from (A) EC48Fib, (B) EC49Fib and (C) EC50Fib CAFs and from (D) EF2Fib, (E) EH6Fib and (F) NE14Fib BAFs were used forreverse transcription-quantitative PCR analysis of fibroblast (vimentin and α-SMA) and epithelial (EpCAM and E-cadherin) markers. The mRNA expression levels of fibroblast markers were normalized to those of ECC-1 epithelial cells. The mRNA expression leels of epithelial markers were normalized to those of THESC normal endometrium fibroblasts. GAPDH was used as house-keeping gene. The data are presented as the mean ± SEM of two independent experiments. No statistical analysis was performed. CAF, cancer-associated fibroblast; BAF, benign-associated fibroblast; ROCK1, Rho-associated, coiled-coil containing protein kinase 1; EpCAM, epithelial cell adhesion molecule; SMA, smooth muscle actin.

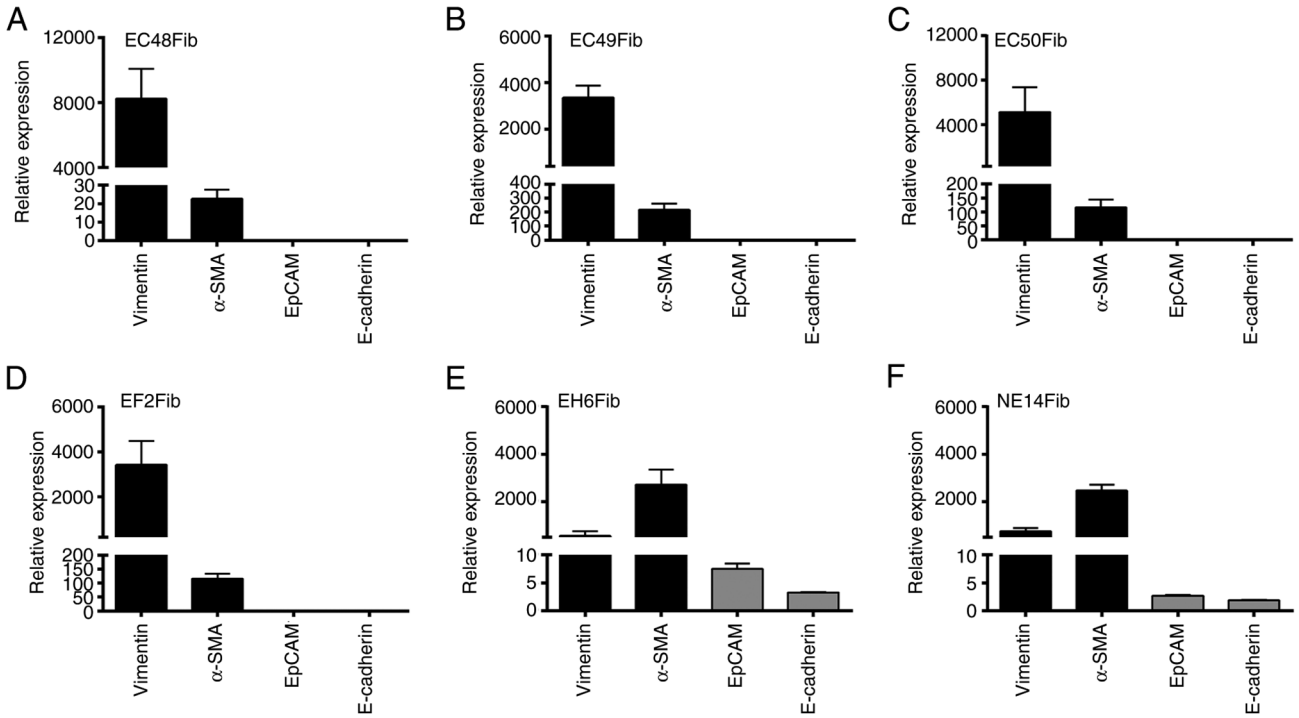

Figure S2. Effect of CAFs on ECC-1 cell motility. (A) ECC-1 cells were treated with CAF conditioned medium for 3 days. The distance travelled by the ECC-1 cells was measured and compared to 2% FBS on (B) day 1 (initial time point) and (C) day 3. Data are representative of two independent experiments and shown as the mean ± SEM. *P<0.05; unpaired Student's t-test. Scale bar, 100 μm, CAF, cancer-associated fibroblast; NS, not significant.

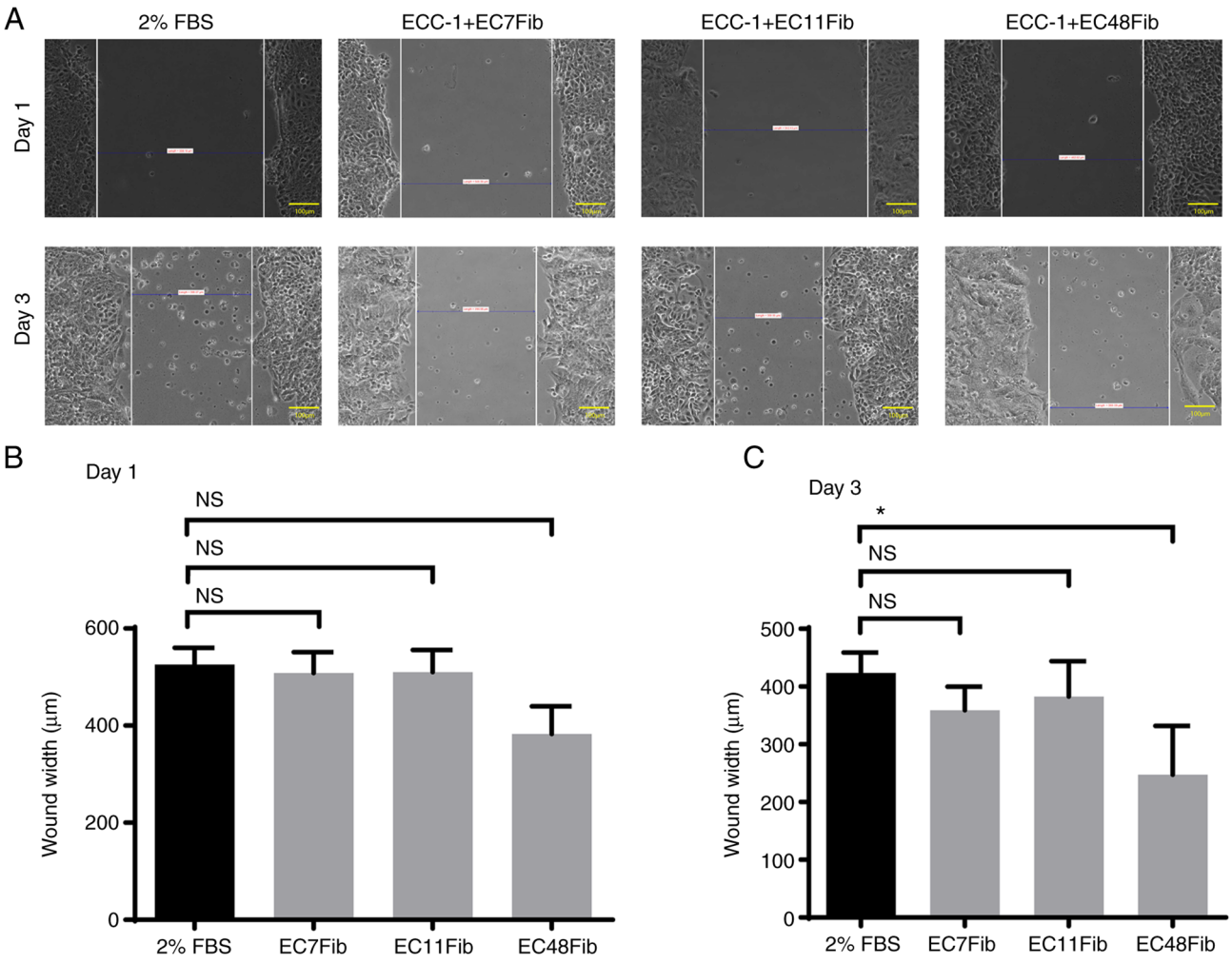

Figure S3. Effects of ECC-1 cells on CAF and BAF motility in a 3D environment. ECC-1 cells were co-cultured with (A) CAFs and (B) BAFs, respectively. CAF and BAF velocity was measured using live cell imaging over 16 h. Data are representative of two independent experiments and shown as the mean ± SEM. Each data point represents an individual cell. *P<0.05; unpaired Student's t-test. CAF, cancer-associated fibroblast; BAF, benign tissue-associated fibroblast; NS, not significant.

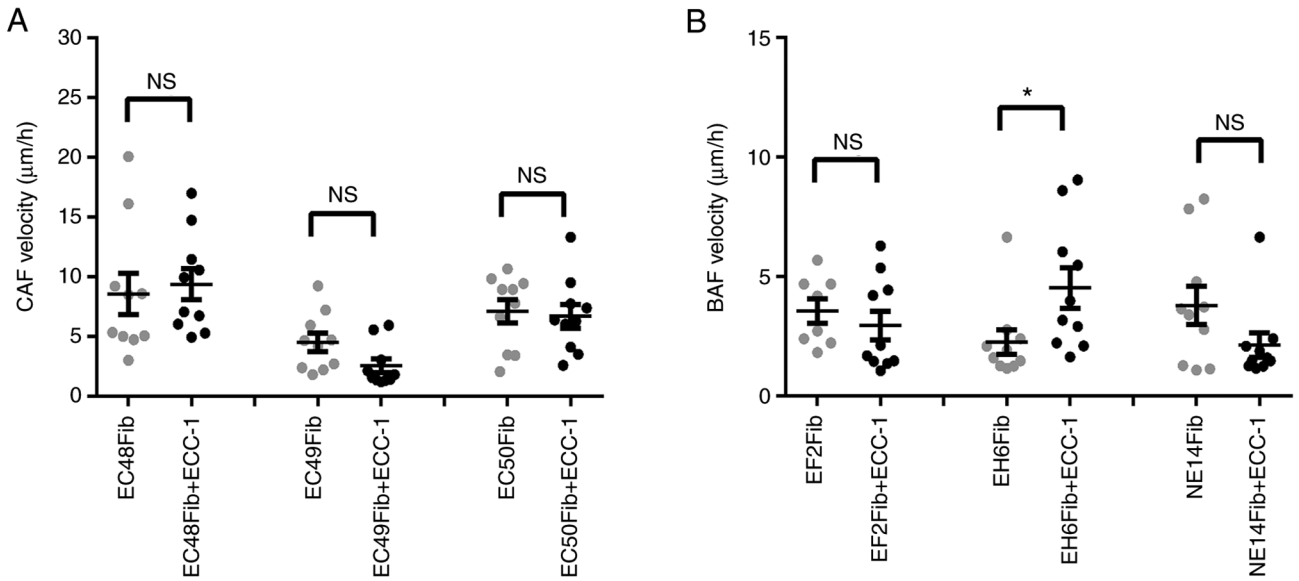

Figure S4. CAFs promote ECC-1 tumor cell spheroid formation in a 3D culture. CAFs were cultured separately or in combination with ECC-1 on Matrigel® for 14 days. (A) EC49Fib monoculture. (B) ECC-1 and EC49 co-culture. (C) EC50Fib monoculture. (D) ECC-1 and EC50Fib co-culture. The formation of spheroids was analyzed using confocal (Max. Pro, upper panel) and 3D reconstruction (Cross Sec, bottom panel) analyses. Scale bar, 200 and 250 μm. Data are representative of two independent experiments and shown as the mean ± SEM. CAF, cancer-associated fibroblast; Max pro, maximum projection; Cross Sec, cross section.

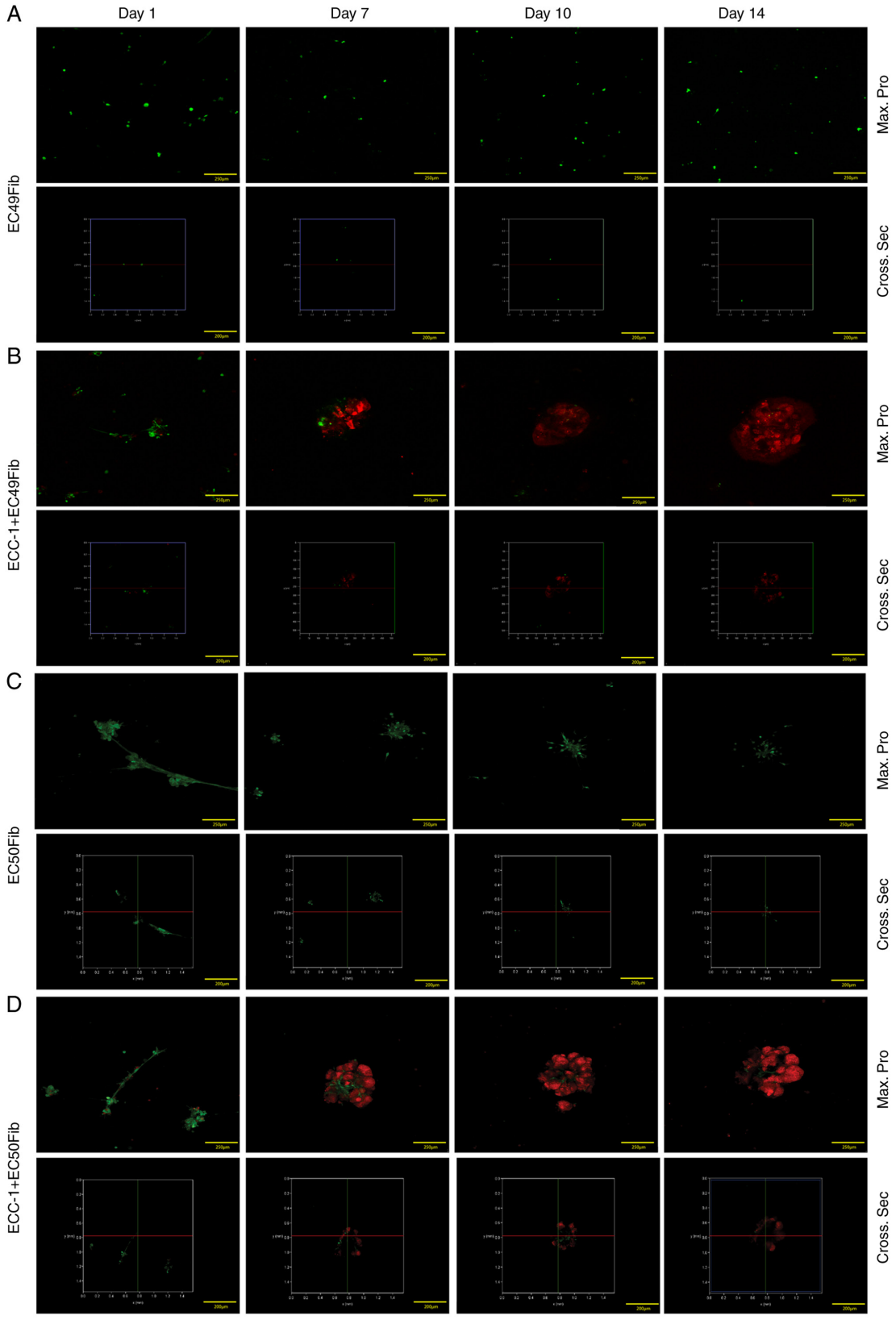

Figure S5. BAFs do not promote EC spheroid formation in 3D culture. BAFs were either cultured individually or with ECC-1 on Matrigel® for 14 days. (A) NE14Fib monoculture. (B) ECC-1 and NE14Fib co-culture. (C) EH6Fib monoculture. (D) ECC-1 and EH6Fib co-culture. Confocal (Max. Pro, upper panel) and 3D reconstruction (Cross Sec, bottom panel) analyses were performed over 14 days. Scale bar, 200 and 250 μm. Data are representative of two independent experiments and shown as the mean ± SEM. BAF, benign tissue-associated fibroblast; Max pro, maximum projection; Cross Sec, cross section.

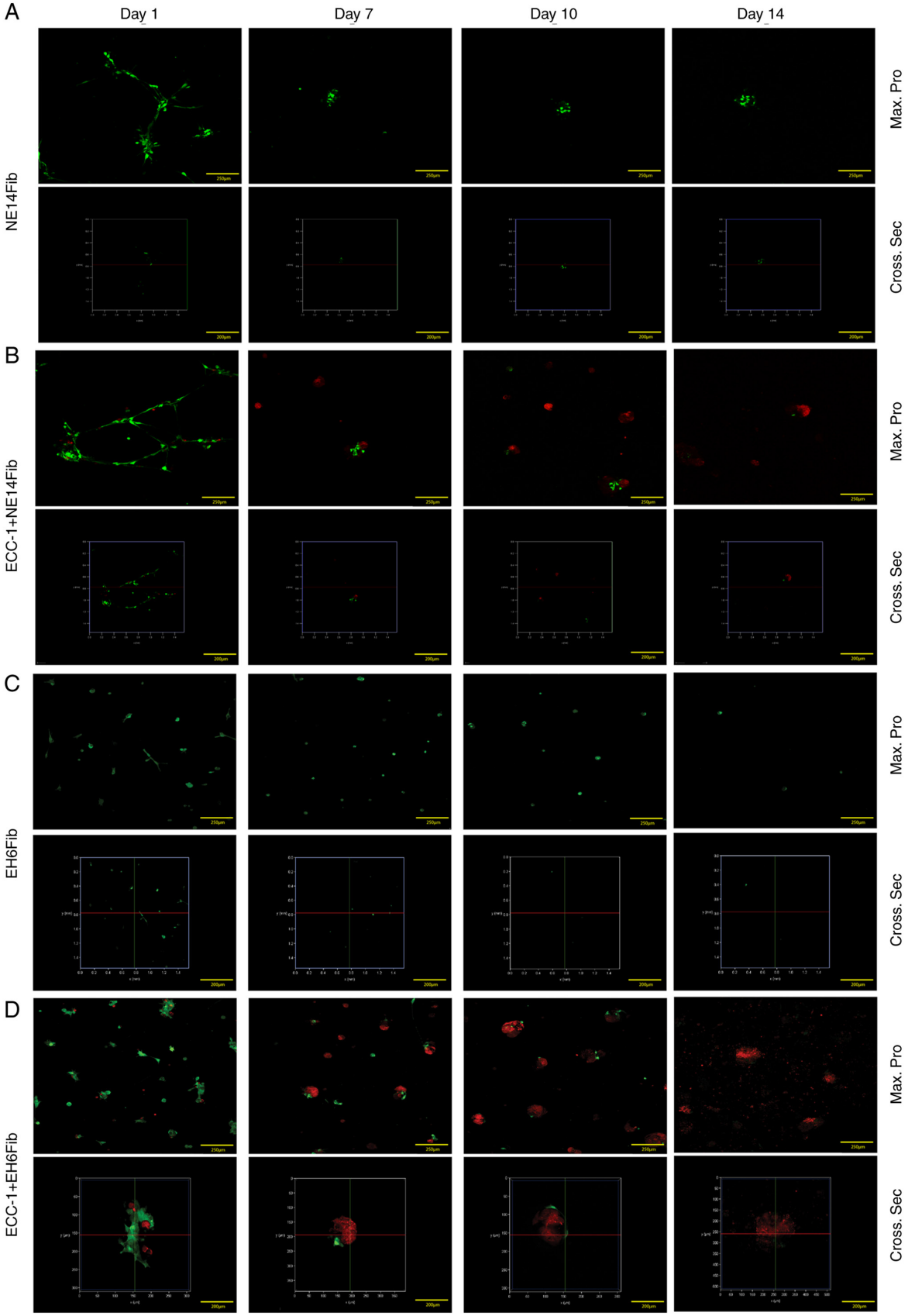

Figure S6. Effects of the ROCK 1 inhibitor Y-27632 on ECC-1 and fibroblast viability and stress fiber formation. (A) ECC-1, (B) CAFs and (C) BAFs were treated with the ROCK1 inhibitor Y-27632 at concentrations ranging from 0.2 to 100 μM for 24 h. Cell viability was examined using MTT assays and normalized to the vehicle-treated cells. Data are representative of two independent experiments and shown as the mean ± SEM. (D) ECC-1, (E-G) CAFs and (H-J) BAFs were treated with Y-27632 at 50, 75 and 100 μM for 24 h. The cells were stained with tetramethylrhodamine-conjugated phalloidin to examine stress fiber formation. The images were analyzed using NIS Element version 4.30. Scale bar, 100 μm. CAF, cancer-associated fibroblast; BAF, benign tissue-associated fibroblast; ROCK1, Rho-associated, coiled-coil containing protein kinase1.