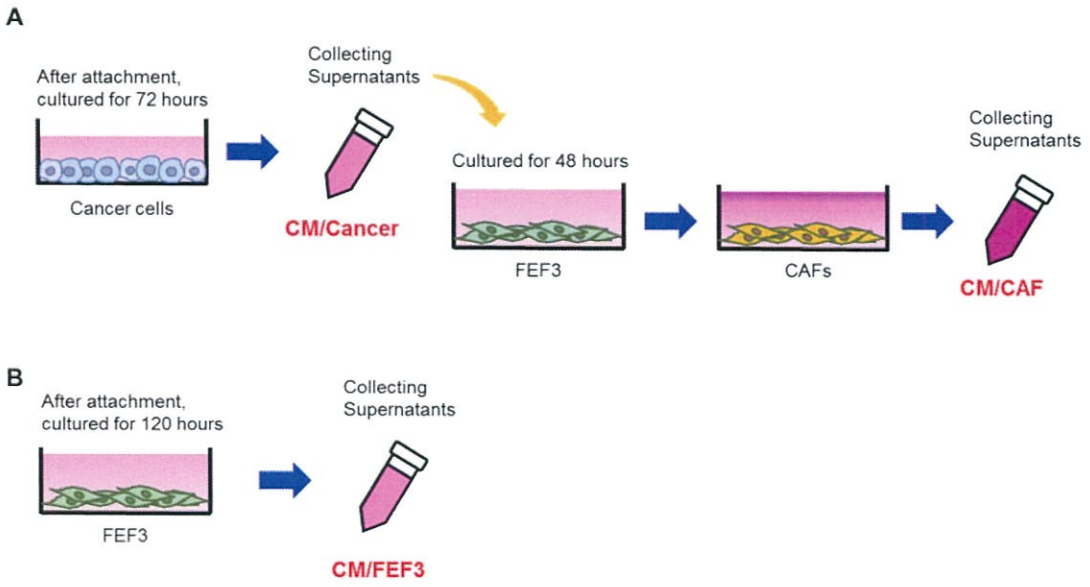
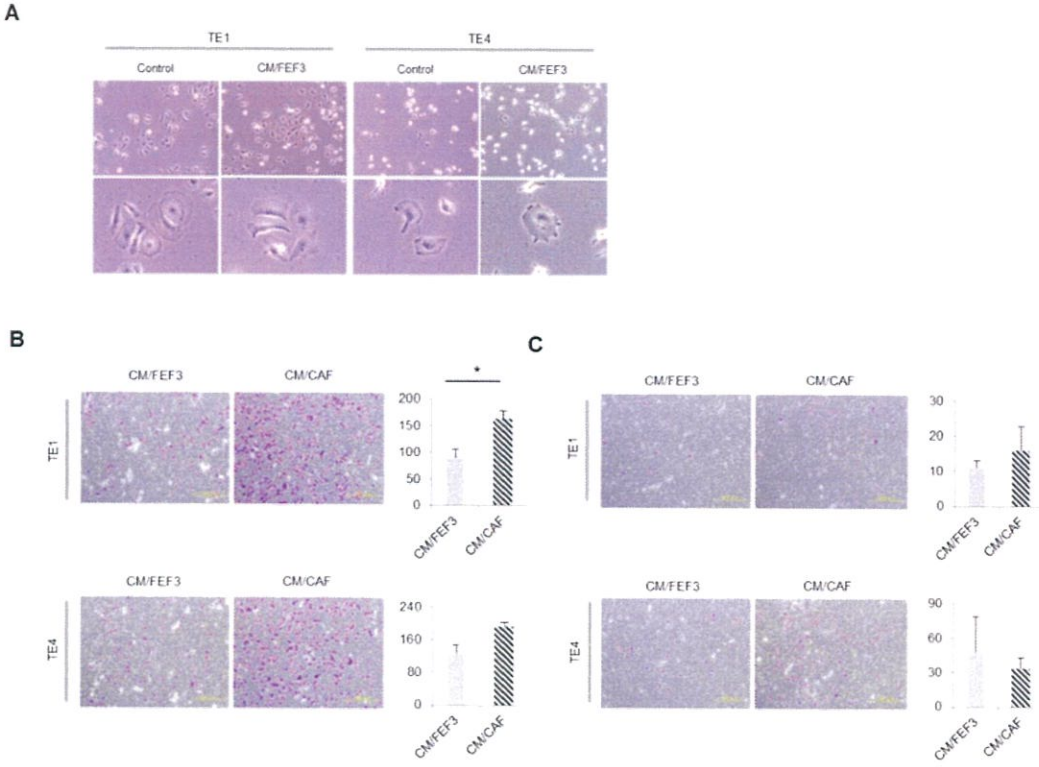
Supporting Information Figure 1



Supporting Information Figure S1. A schema of the protocol to make conditioned mediums.

(A)The protocol to make CM/Cancer (e.g.; CM/TE4) and CM/CAF is shown. Esophageal cells (2 x 10^6 cells /10cm^2 dish) were seeded. After confirmed the attachment of cancer cells to the dish, 10ml DMEM with 2% FBS was changed and cultured for 72 hours. CM/Cancer was collected and centrifuged. FEF3 was cultured with this CM/Cancer for 48 hours, which resulted in CAFs. The conditioned medium was collected and centrifuged at 1,000 rpm for 5 minutes, and defined as CM/CAF. (B) The protocol to make CM/FEF3 is shown. FEF3 (0.5 x 10^6 cells /10cm^2 dish) was seeded and cultured with 10ml DMEM with 2% FBS for 120 hours. After then, the supernatant was collected and centrifuged at 1,000 rpm for 5 minutes. This CM was defined as CM/FEF3.

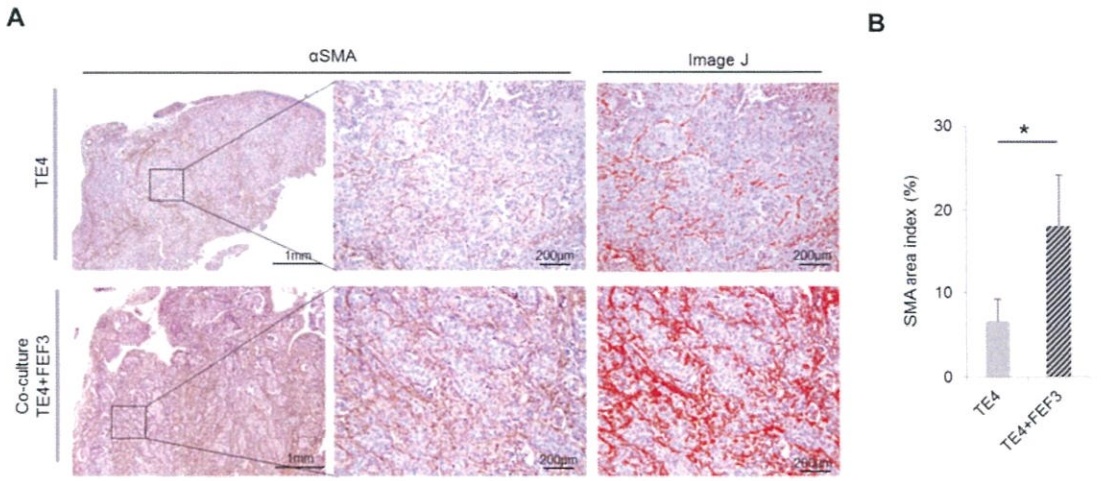
Supporting Information Figure 2



Supporting Information Figure S2. The impact of EC cells stimulated by CM/FEF3.

We investigated morphological changes in EC cells and their migratory and invasive abilities *in vitro* using CM/FEF3. (A) Observation morphological changes by microscopy. Neither loss of cell-cell adhesions nor acquisitions of a spindle cell-shaped morphology were detected in EC cells stimulated by CM/FEF3. (upper figures; x100 magnification, lower figures; x400 magnification) (B, C) Migration and invasion assay using EC cells stimulated by CM/FEF3 or CM/CAF, as described above. In migration assay (B), migratory abilities were increased by CM/CAF significantly (TE1; P=0.029, TE4; P=0.059). In invasion assay (C), no significant difference was found between CM/FEF3 and CM/CAF. (Scale bars: 500μm (x40). Data present mean ± SD. *p<0.05, t-test)

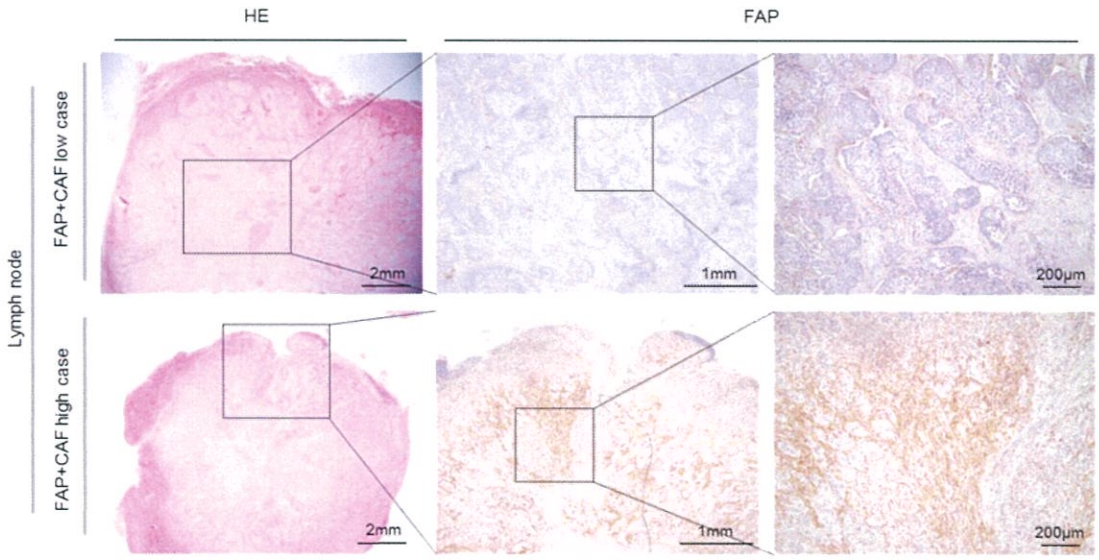
Supporting Information Figure 3



Supporting Information Figure S3. Quantification of SMA expression *in vivo* model.

αSMA scoring was evaluated using an "Area Index" calculated by Image J software (http://rsb.info.nih.gov/ij/). (A) Four fields (x 200 magnification) including stromal cells were carefully selected and calculated to evaluate CAFs. The mean value was defined as the αSMA area index. (B) The comparison about αSMA area index between TE4 and co-culture TE4+FEF3 group was analyzed. In co-culture TE4+FEF3 tumors, αSMA area index was significantly higher than that in TE4 tumors. (Data present mean ± SD. $*p<0.05$, *t*-test)

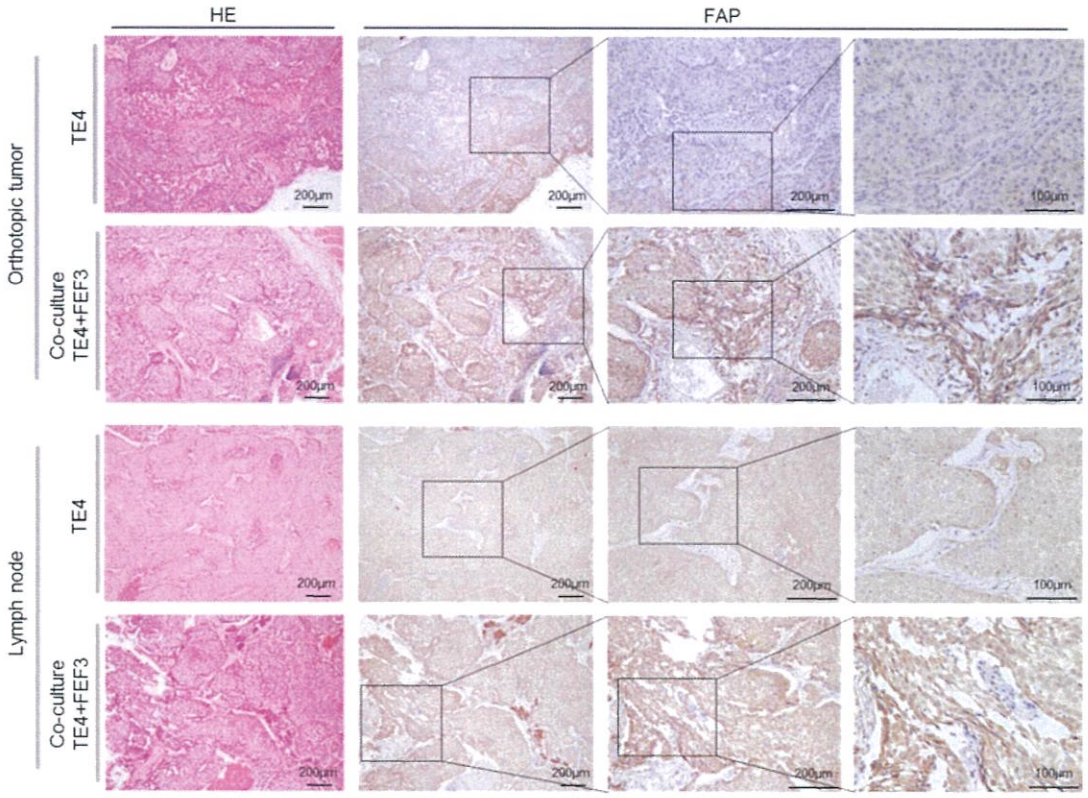
Supporting Information Figure 4



Supporting Information Figure S4. The evaluation of FAP$^+$ CAFs in metastatic lymph nodes in esophageal carcinomas.

HE and IHC of FAP expression in metastatic lymph nodes of esophageal carcinomas are shown. The difference in quantity of FAP$^+$ CAFs was detected for node-positive cases in clinical samples. (Scale bars: 2 mm (12.5x), 1 mm (40x), 200 μm (100x))

Supporting Information Figure 5



Supporting Information Figure S5. The evaluation of FAP$^+$ CAFs *in vivo* model.
HE and IHC of FAP expression *in vivo* model are shown. Harvested orthotopic tumors and metastatic lymph nodes were formalin-fixed and paraffin-embedded. Deparaffinization and antigen-retrieval were performed, as described above. We added and incubated primary antibodies against FAP (Abcam, ab53066, 1:50 dilution for overnight at 4°C). Appropriate secondary antibodies were added and incubated for 30 min at RT. Sections were visualized and counterstained, as described above. In co-culture TE4+FEF3 group, FAP$^+$ CAFs were detected more than those in TE4 group both in orthotopic tumors and metastatic lymph nodes. (Scale bars: HE; 200 μm (x100). FAP; 200 μm (x100), 200 μm (x200), 100 μm (x400), from left)