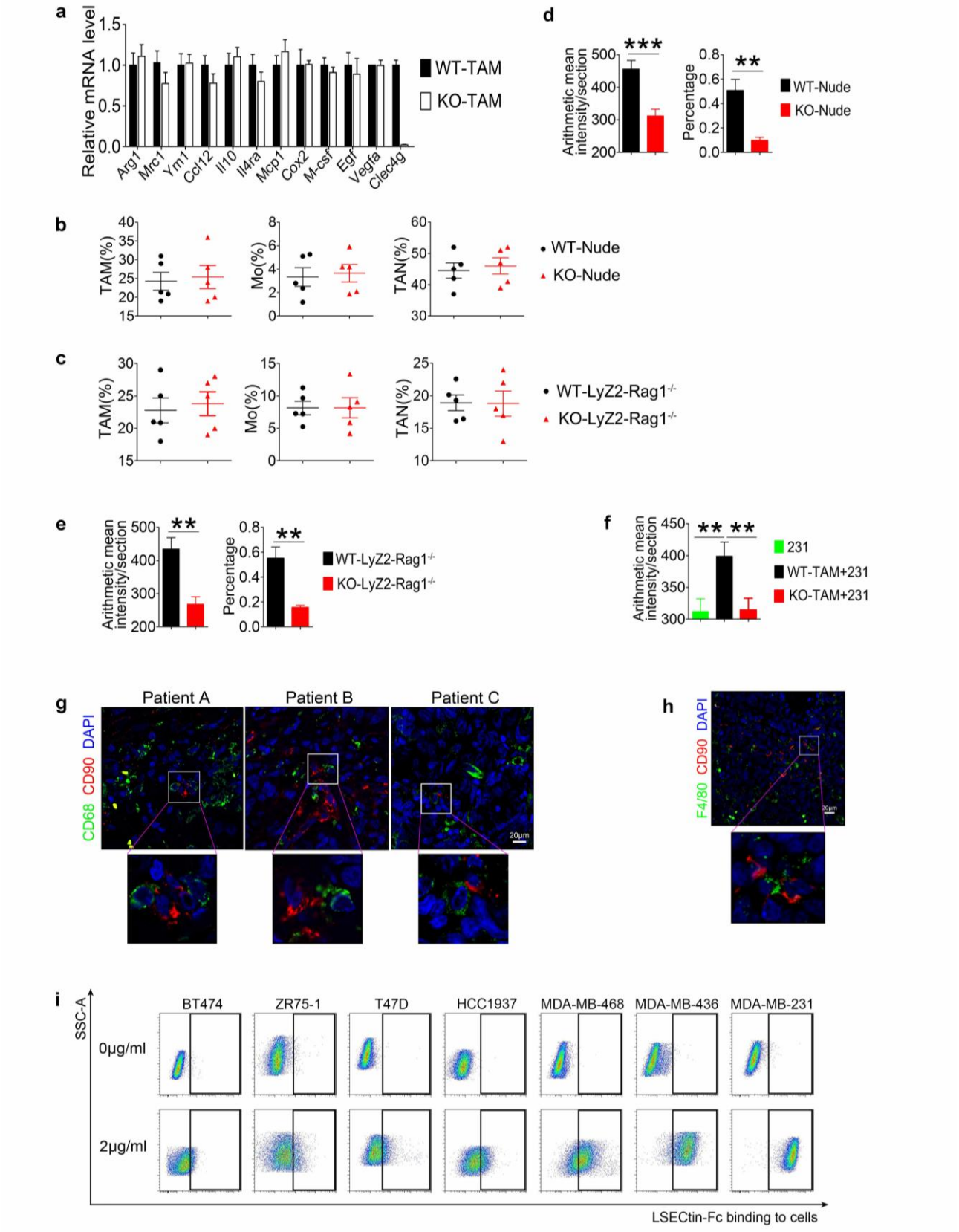
Supplementary information, Fig. S2



Supplementary information, Fig. S2. Macrophage-expressed LSECtin promotes breast cancer stemness in a contact-dependent manner

(a) Real-time PCR analysis showing the expression of TAM indicators relative to that of the housekeeping gene *β-actin* in primary WT-TAMs or KO-TAMs in human xenograft tumors. One of three experiments is shown.

(b,c) Monitoring of immune cells was performed using flow cytometry. The composition of different intra-tumor immune cell subsets within $CD45^+$ cells in human xenograft tumors in KO-Nude mice or littermate controls (b); in KO-LyZ2-$Rag1^{-/-}$ or littermate controls (c) are shown. One of two experiments is shown.

(d,e) Immunofluorescent staining of tumor tissues for human CD90 (red) in the human xenografts constructed with KO-Nude mice or littermate controls (d); with KO-LyZ2-$Rag1^{-/-}$ or littermate controls (e). The anti-human CD90 antibody without reactivity with mouse CD90 were used in the immunofluorescence analysis or flow cytometric analysis. The arithmetic mean (left) or the percentage (right) of CD90 expression are shown.

(f) Immunofluorescent staining of tumor tissues for human CD90 (red) in human xenograft tumors constructed in the KO-Nude mice with co-injection of 231 cells and WT-TAMs or KO-TAMs. The anti-human CD90 antibody without reactivity with mouse CD90 were used in the immunofluorescence analysis. The arithmetic mean of CD90 expression are shown.

(g) Confocal images of primary patient-derived breast tumor sections co-stained for $CD68^+$ TAMs (green) and $CD90^+$ CSCs (red) showed CD68-CD90 mediated juxtaposed TAM-CSC pairs in the patient. Nuclei were counterstained with DAPI (blue) (scale bar=20 μm). Details regarding the tumor pathology are presented in Supplementary information, Table S2.

(h) Confocal images of tumor tissues for TAM-CSC pairs mediated by $F4/80^+$ TAMs (green) and $CD90^+$ CSCs (red) in human xenograft tumors. The anti-human CD90 antibody without reactivity with mouse CD90 were used in the immunofluorescence analysis. Nuclei were counterstained with DAPI (blue) (scale bar=20 μm).

(i) Binding of LSECtin-Fc recombinant protein at 2 μg/ml to different breast cancer cell lines. One of two experiments is shown.

Data are presented as the mean ± SD. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ in unpaired Student's *t* test (d, e, f)