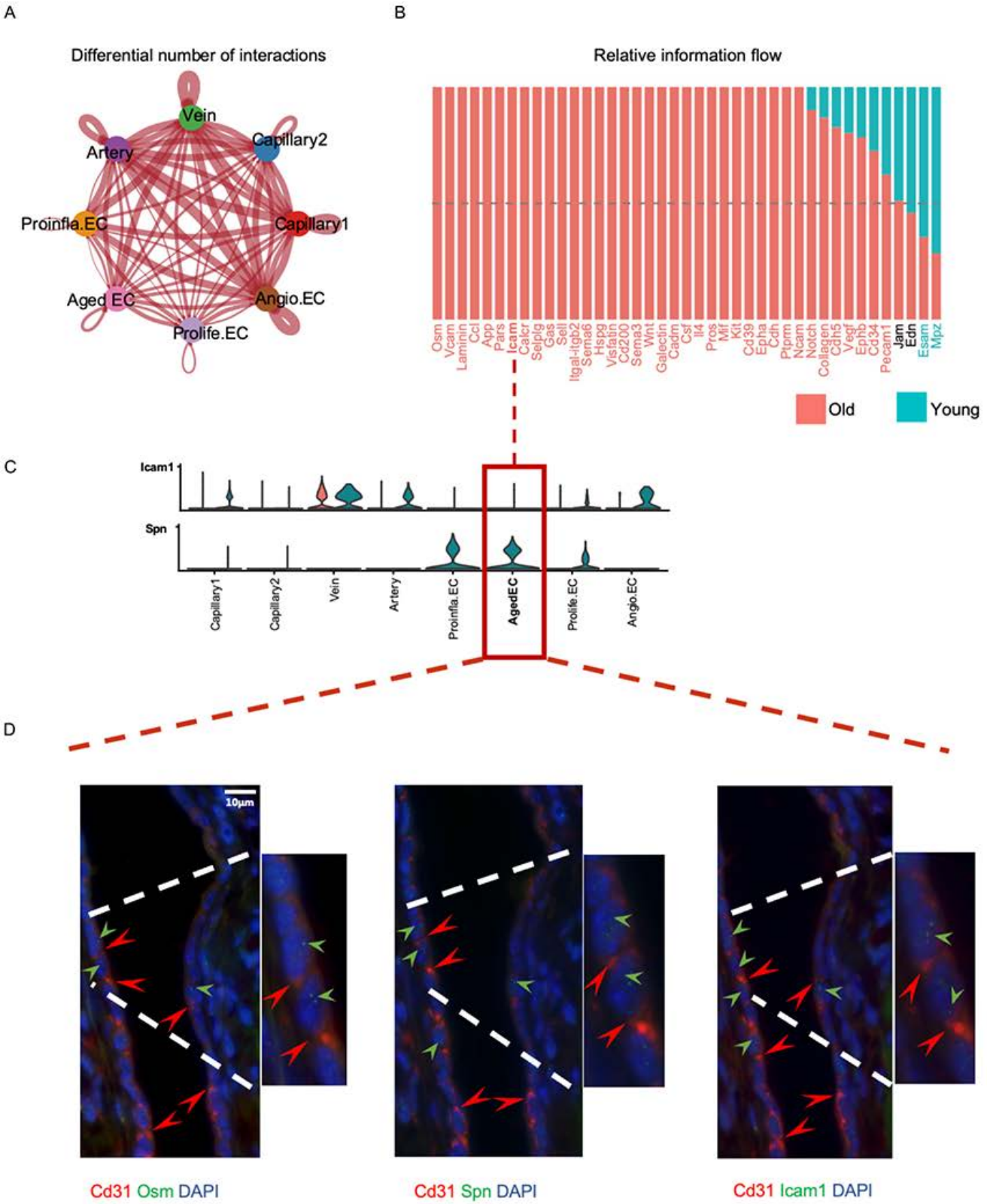
A
Differential number of interactions
Vein
Capillary2
Artery
Proinfla.EC
Capillary1
Aged EC
Angio.EC
Prolife.EC
B
Relative information flow
Osm Vcam Laminin Ccl App Pars Icam Calcr Selplg Gas Sell Itgal-itgb2 Sema6 Hspg Visfatin Cd200 Sema3 Wnt Galectin Cadm Csf Il4 Pros Mif Kit Cd39 Epha Cdh Ptprm Ncam Notch Collagen Cdh5 Vegf Ephb Cd34 Pecam1 Jam Edn Esam Mpz
Old
Young
C
Icam1
Spn
Capillary1
Capillary2
Vein
Artery
Proinfla.EC
AgedEC
Prolife.EC
Angio.EC
D
10μm
Cd31 Osm DAPI
Cd31 Spn DAPI
Cd31 Icam1 DAPI

Figure S5

E

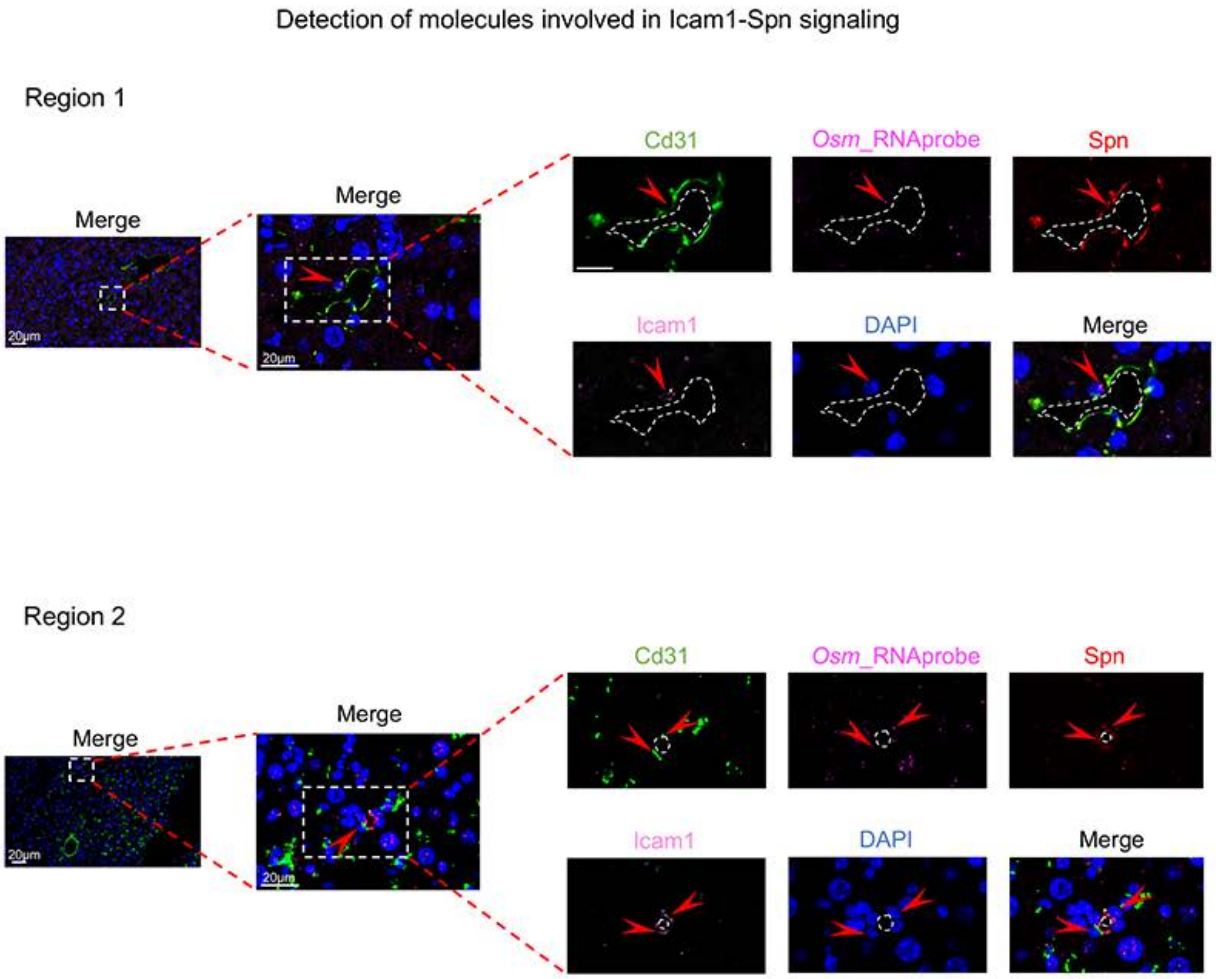
Detection of molecules involved in Icam1-Spn signaling
Region 1
Merge
Merge
20μm
20μm
Cd31
Osm_RNAprobe
Spn
Icam1
DAPI
Merge
Region 2
Merge
Merge
20μm
20μm
Cd31
Osm_RNAprobe
Spn
Icam1
DAPI
Merge

Figure S5

Figure S5. Ligand-receptor pair, Icam1-Spn, may enhance cell-cell interaction between ECs and immune cells in the old hepatic vascular niche.

(A) Network plot showing changes in ligand-receptor interaction events between different types of ECs in old and young mouse livers. Cell-cell communication is indicated by connected lines. The thickness of the lines is positively correlated with the number of ligand-receptor interaction events.

(B) All significant signaling pathways were ranked based on their differences of overall information flow within the inferred networks between the young and old. The top signaling pathways colored red are more enriched in the old, the middle ones colored black are equally enriched in two groups, and the bottom ones colored green are more enriched in the young.

(C) Violin plots showing different signaling gene expression distribution between young and old liver ECs (Yellow: young, Green: old) in *Icam* pathway.

(D) RNAscope in situ hybridization showing representative micrographs of *Icam1* transcripts and *Spn* transcripts in old liver sections. *Osm* transcripts, *Icam1* transcripts, and *Spn* transcripts are indicated by green arrowheads. Immunostaining for Cd31 protein was used to visualize ECs (indicated by red arrowheads). Nuclei are counterstained by DAPI. Images show the red, green, and blue channel images. Scale bars, 10 μm.

(E) Immunofluorescence staining and RNAscope experiments confirmed Icam1 protein/Spn protein/Cd31 protein/*Osm* transcripts coimmunostaining for Aged EC in liver sinusoidal region from old and young mice. Scale bar, 20 μm.