SUPPLEMENTAL MATERIAL

Supplementary Materials and Methods

Intra-vital imaging. Fluorescence intra-vital microscopy of the liver was conducted as previously described (1). Briefly, following general anesthesia, the left carotid artery was cannulated for tumor cell injection. The abdomen was opened and the left lobe of the liver was positioned on a glass cover slip over the microscope objective. The exposed liver lobe was continuously perfused with warm saline and animals were maintained at 37°C with an infrared heat lamp throughout the experiment. The anterior surface of the liver was captured with an inverted microscope (Nikon TE300) equipped with a 20x objective lens and a video camera (Panasonic Digital KR222). CFDA SE-labelled (Invitrogen) tumor cells were visualized using epi-fluorescence. In each experiment, $1x10^6$ of the indicated CFDA SE-labelled cells were injected intra-arterially into the left heart via the carotid artery cannula, and the livers were visualized by fluorescence intra-vital microscopy 30, 45 and 60 minutes post-injection.

Short term splenic injection assay. $1x10^6$ of the indicated CFDA SE-labelled cells were subjected to splenic injection as previously described (2). After 3, 27 and 51 hours post-injection, five mice per cohort were sacrificed, livers collected and whole-mounted. The number of CFDA SE-labelled breast cancer cells per field was assessed using an inverted microscope (Zeiss Axiovert 135) equipped with a 20x objective lens and a video camera (Qimaging Retiga 1300).

Supplementary References

1. **McDonald, B., J. Spicer, B. Giannais, L. Fallavollita, P. Brodt, and L. E. Ferri.** 2009. Systemic inflammation increases cancer cell adhesion to hepatic sinusoids by neutrophil mediated mechanisms. Int J Cancer **125:**1298-1305.

2. **Tabariès, S., Z. Dong, M. G. Annis, A. Omeroglu, F. Pepin, V. Ouellet, C. Russo, M. Hassanain, P. Metrakos, Z. Diaz, M. Basik, N. Bertos, M. Park, C. Guettier, R. Adam, M. Hallett, and P. M. Siegel.** 2011. Claudin-2 is selectively enriched in and promotes the formation of breast cancer liver metastases through engagement of integrin complexes. Oncogene **30:**1318-1328.

Supplementary Figure legends

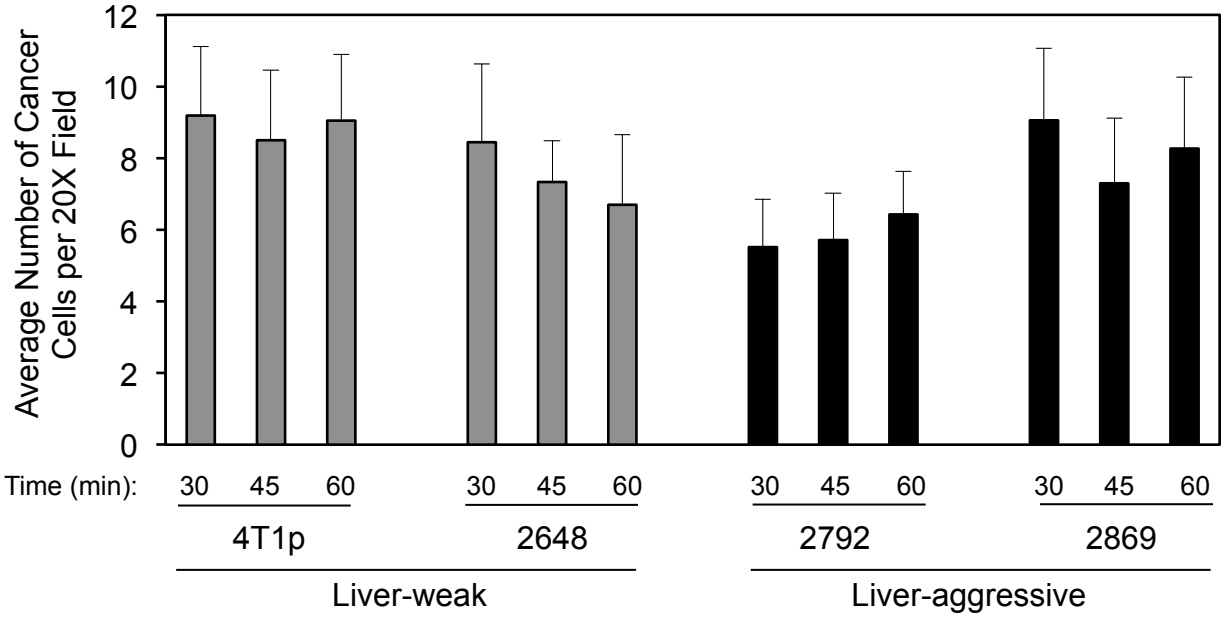
Figure S1. Liver-aggressive breast cancer cells do not preferentially adhere to liver sinusoidal endothelium. 4T1-derived cells, fluorescently tagged with CFDA SE, were injected in the carotid arteries of mice and cells that arrested in the liver sinusoids were counted after 30, 45 or 60 minutes. Representative images of the cells that have arrested in the liver sinusoids (white arrows) are shown for mice injected with one weakly metastatic (2648) or one aggressive metastatic cell population (2792). No significant differences were observed between the two groups. Scale bar (*right panel*) represents 50 μm.

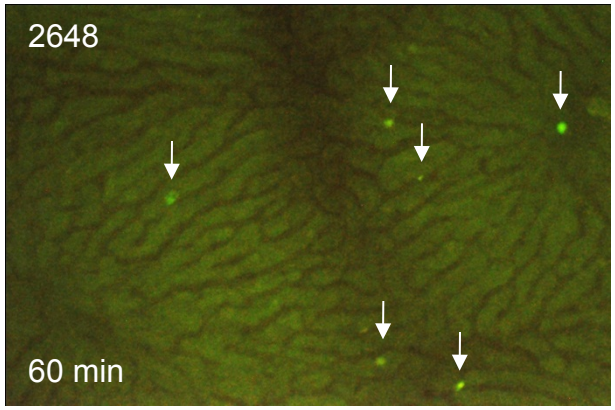
Figure S2. Reduced claudin-2 expression results in diminished cell survival in the liver. Quantification of CFDA SE-labelled cancer cells in whole-mounted livers following splenic injection with the 2776 liver-aggressive cells harboring a claudin-2 knockdown (KD1, KD2) and the corresponding empty vector (EV) controls. A significant decrease in cell number was observed in the cell populations with claudin-2 knockdown at 27 and 51 hours post-injection. *, $P < 0.02$; **, $P < 0.006$; *** $P < 0.01$.

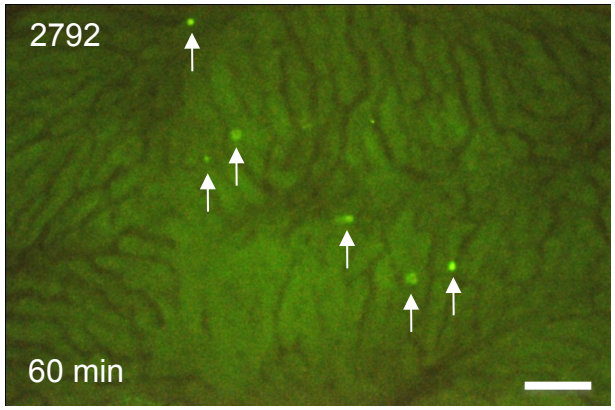
Figure S3. The first extracellular loop of claudin-2 is sufficient to promote breast cancer/hepatocyte adhesion. **(*A*)** Immunoblot analysis of claudin-2, claudin-4 or the indicated chimeric proteins demonstrates equivalent expression levels in cells infected with a *Cldn2* shRNA expression vector (KD) or in cells harbouring the empty vector (EV). As a loading

control, total cell lysates were blotted for α-tubulin. The gray lines in the claudin schematics indicate the extracellular loops derived from claudin-2 within a claudin-4 backbone (solid line). **(*B*)** The number of breast cancer cells that adhered to a monolayer of primary hepatocytes was quantified. Diminished adhesion was observed in cells with reduced claudin-2 levels in which an empty vector, claudin-4 or the C4(C4/C2) chimera was expressed. *, $P < 0.005$. However, a complete rescue of the adhesive phenotype was observed when the C4(/C2/C2) or C4(C2/C4) chimera was expressed.

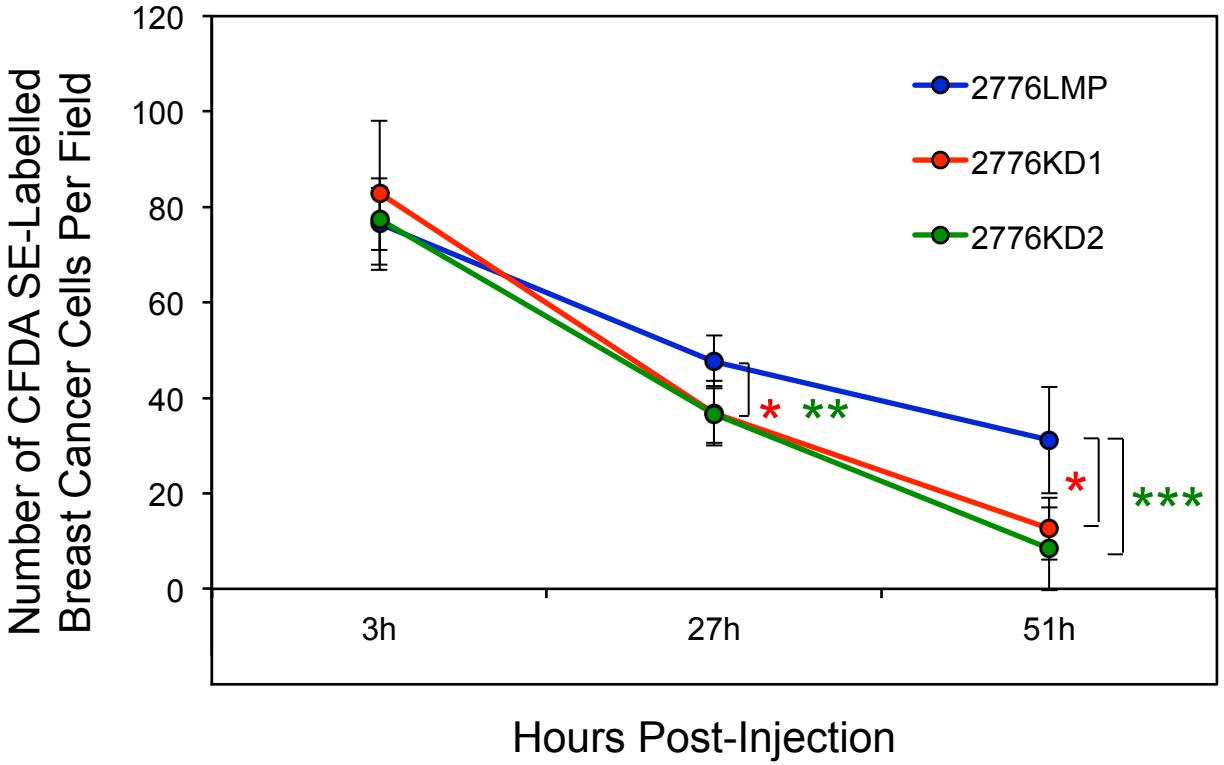
Figure S4. First extracellular loop of claudin-2 is required to promote the breast cancer liver metastatic phenotype. Quantification of the tumor burden (tumor area/tissue area) within the cardiac liver lobe following splenic injection of the indicated cell populations is shown. The number of mice analyzed in each cohort is indicated in brackets. Decreases in liver metastatic burden were observed when empty vector, claudin-4 or C4(C4/C2) expressing cells, in the context of diminished claudin-2, were injected into the spleen. *, $P < 0.02$; **, $P < 0.05$. However, the liver metastatic phenotype is rescued when the C4(C2/C2) or the C4(C2/C4) chimera was expressed. H&E images of the cardiac liver lobe are shown for mice injected with each of the cell populations. Dotted lines circumscribe breast cancer metastatic lesions within the liver. Scale bar represents 2mm.



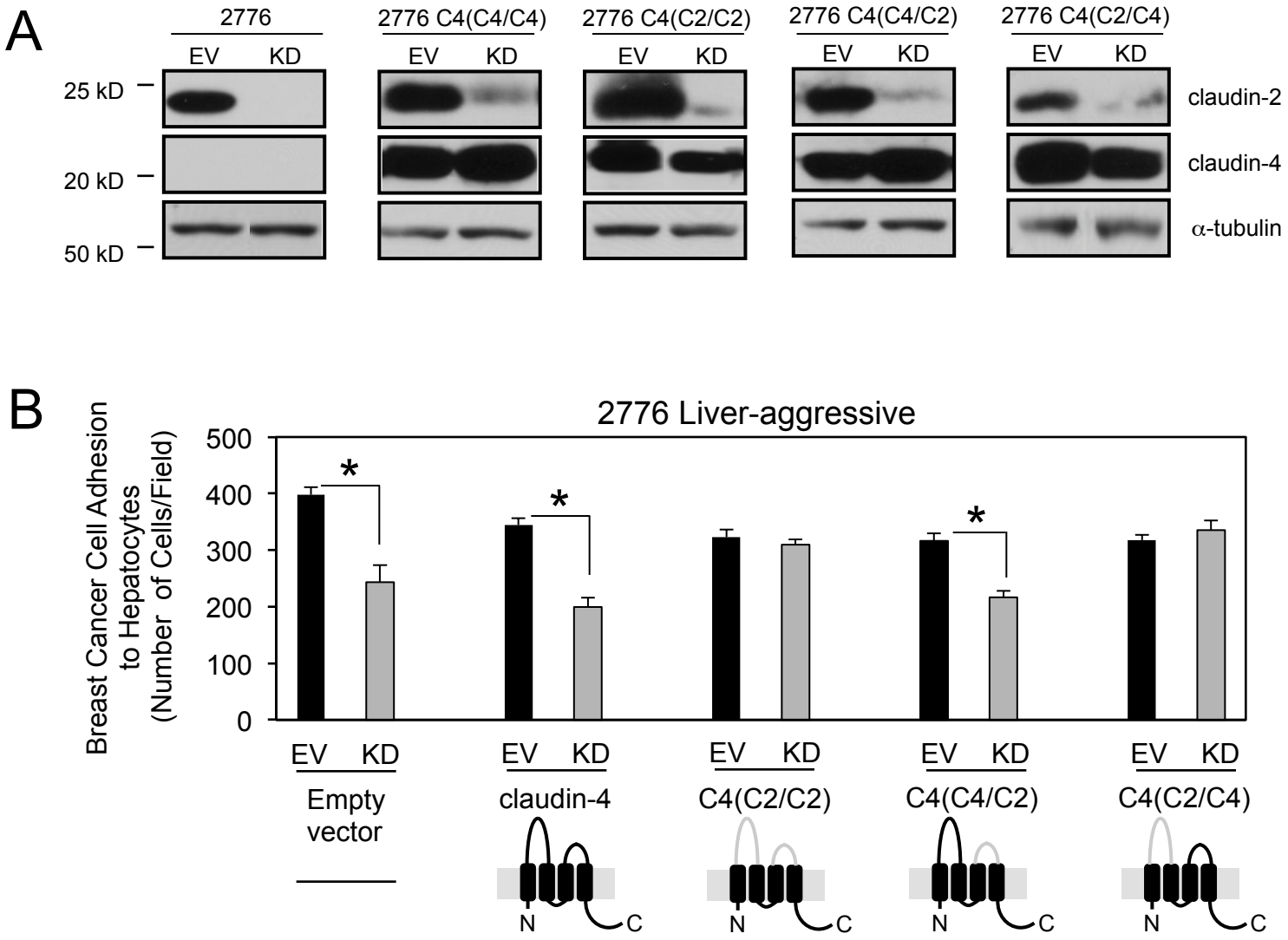




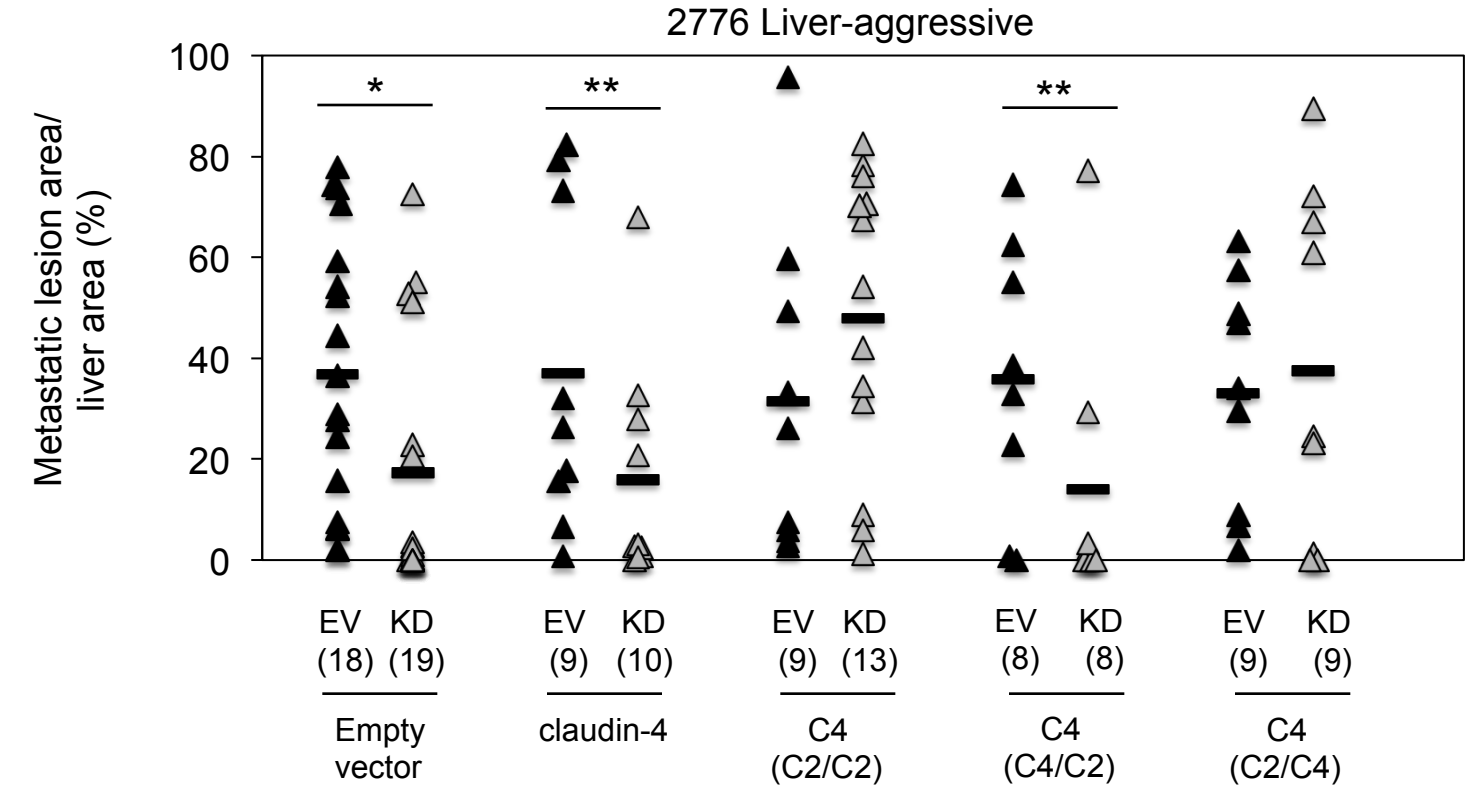
Tabariès *et al*., Supplementary Figure S1

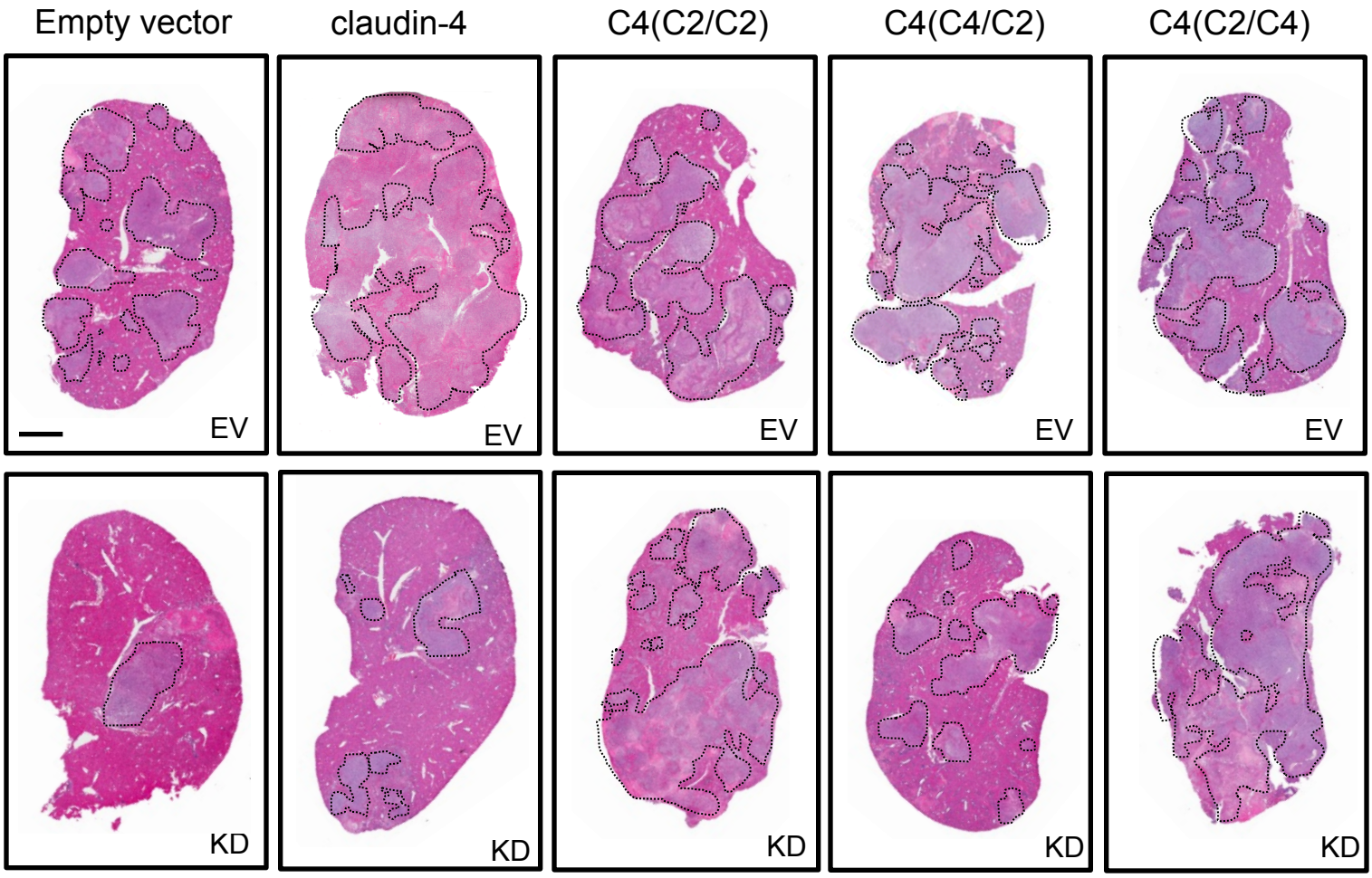


Tabariès *et al*., Supplementary Figure S2



Tabariès *et al.*, Supplementary Figure 3





Tabariès *et al.*, Supplementary Figure S4