

**Figure S1. Localization of HA on paraffin-embedded tissues.** Hyaluronan (HA) is digested on control slides using hyaluronidase. Both control and experimental slides are treated with biotinylated hyaluronanbinding protein (HABP) followed by secondary streptavidin fluorophore. HA staining is visualized under a fluorescent microscope. Representative images show immunofluorescence microscopy for hyaluronic acid binding protein (HABP; green) and DAPI nuclear stain in a 5-week old (pubertal) murine mammary gland. As a control, each section was treated with hyaluronidase prior to staining (+Hyaluronidase). L.N. represents the lymph node, \*\* highlights the adipose-rich stroma, and the white box identifies two mammary epithelial buds. Each image was taken at 10× magnification. Images were acquired using the same settings (including exposure time and gain) and post-processing adjustments (including brightness and contrast). Scale bars represent 250 µm.



**Figure S2.** (**A**) Cell-specific isolation of EpCAM+ epithelial cells, CD45-/CD90.2+ fibroblasts, and F4/80+ macrophages from the mammary glands of 5- (pubertal) and 10- (adult) week old BALB/c mice via Miltenyi Biotec microbead kits. Image created with BioRender.com. (**B**) Gating strategy for flow cytometry was analyzed and (**C**) quantified via FlowJo, validating cell viability and enrichment.

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**Figure S3.** (**A**) Cell-specific isolation of EpCAM+ epithelial cells, CD45-/CD90.2+ fibroblasts, and F4/80+ macrophages from three murine models of breast cancer (4T1, HC11/R1, and HC11/R1-LM) via Miltenyi Biotec microbead kits. Image created with BioRender.com. (**B**) Gating strategy for flow cytometry was analyzed and (**C**) quantified via FlowJo, validating cell viability and enrichment.