Supplementary Information

Evaluating the Role of IL-1β in Transmigration of Triple Negative Breast Cancer Cells Across the Brain Endothelium

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Supplementary Materials:

Supplementary Tables S1-S3

Supplementary Figures S1-S3

Table S1. List of the primers used for qRT-PCR assays

Gene Name	Unique ID or Forward and Reverse Sequence	Vendor
VWF	qHsaCED0043330	BioRad
OCLN	qHsaCED0038290	BioRad
TJP1	qHsaCID0018062	BioRad
CLDN5	qHsaCED0047644	BioRad
MMP2	qHsaCED0042560	BioRad
MMP9	qHsaCID0011597	BioRad
PECAM1	Fwd sequence: TGCCGTGGAAAGCAGATACT Rev sequence: TTCCAGGGATGTGCATCTGG	BioRad
GAPDH	qHsaCED0038674	BioRad
ACTA2	qHsaCID0013300	BioRad
TAGLN	qHsaCID0021424	BioRad

Table S2. List of the primary antibodies used for immunocytochemistry

Primary Antibody (Vendor, Clone or Product Number)	Dilution Factor
Mouse monoclonal Claudin-5 (Invitrogen, 4C3C2)	1:100
Rabbit polyclonal PECAM-1 (Lab Vision, RB10333P)	1:50
Rabbit polyclonal ZO-1 (Invitrogen, 40-2200)	1:100
Rabbit polyclonal SM-22α (Invitrogen PA527463)	1:200
Rabbit monoclonal GFAP (Abcam, EPR1034Y)	1:400
Goat polyclonal IL1RII (R&D systems, AF-263)	N/A
Goat polyclonal IL1RI (R&D systems, AF-269)	N/A
Mouse Monoclonal DARC (R&D systems, 358307)	1:200
Mouse Monoclonal IL-1β (R&D systems, 2805)	1:200

Table S3. List of the secondary antibodies used for immunocytochemistry

Secondary Antibody Conjugate (Vendor)	Dilution Factor
Goat anti mouse Alexa Fluor 488 (ThermoFisher)	1:200
Goat anti mouse Alexa Fluor 594 (ThermoFisher)	1:200
Goat anti rabbit Alexa Fluor 488 (ThermoFisher)	1:200
Goat anti rabbit Alexa Fluor 594 (ThermoFisher)	1:200

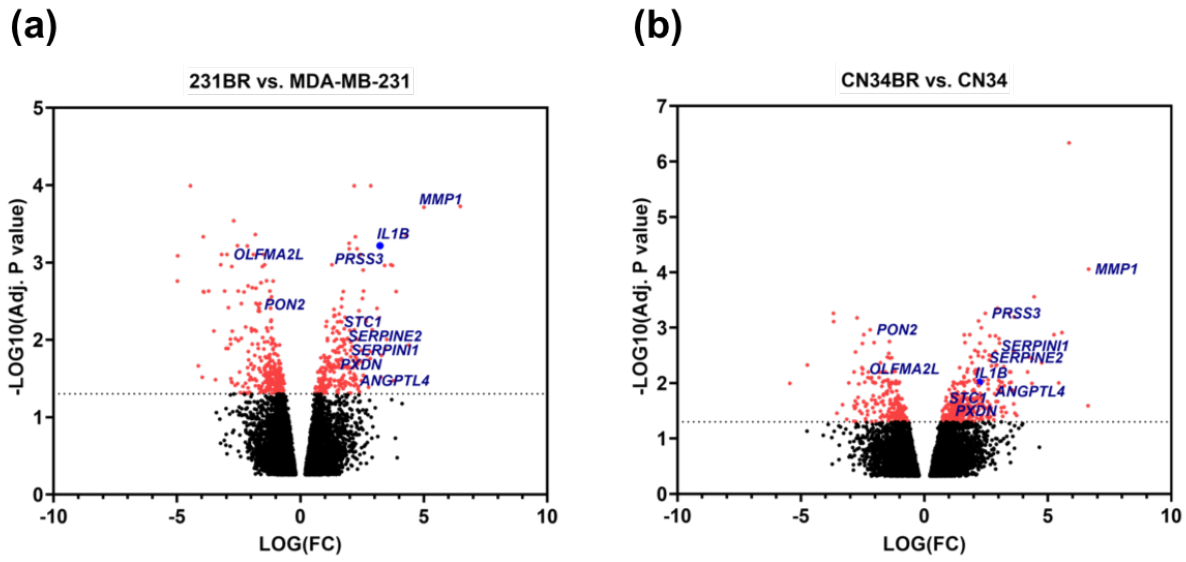

Figure S1. Expression of IL-1β is highly upregulated in brain-seeking triple negative breast cancer cells. Analysis was performed on the GSE12237 dataset. (a-b) Volcano plot analysis of differential gene expression in brain-seeking vs. parental MDA-MB-231 (a) and CN34 (b) cells. The dashed line shows adjusted P value of 0.05. *IL1B* data point is denoted by blue color. Other secreted proteins are indicated by their gene names on the plots.

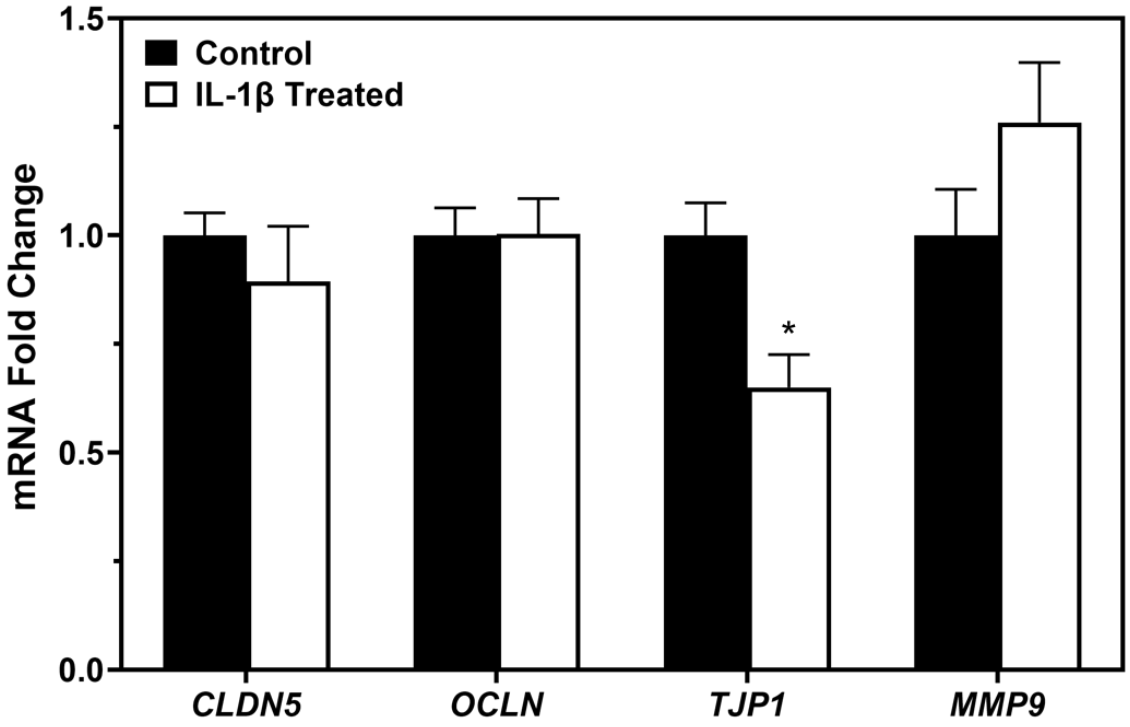

Figure S2. Expression of *MMP9* and *CLDN5* returns to normal 24 hours after IL-1β treatment. Gene expression analysis of iBMEC monoculture 24 hours after IL-1β was added to the culture. In IL-1β treated samples, *TJP1* is downregulated, whereas expression of *CLDN5*, *OCLN*, and *MMP9* is not altered. * indicates $P<0.05$ calculated by unpaired t-test.

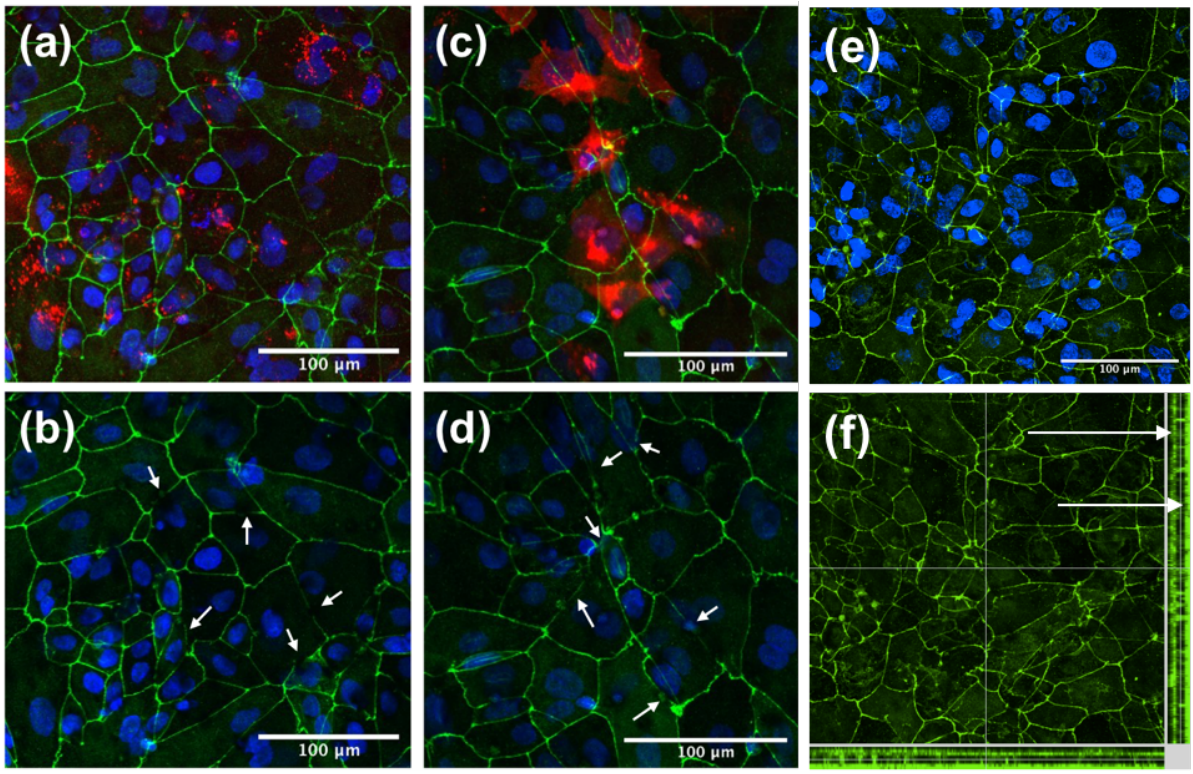

Figure S3. MDA-MB-231 and 231BR cells transmigrate across iBMECs in cell culture inserts. (a-d) Confocal images of cancer cells (red) and claudin-5 expression on iBMECs (green) showing transmigrated MDA-MB-231 (a) and 231BR (c) cells in red. (b) and (d) show the iBMEC monolayer above the transmigrated cancer cells in (a) and (c), respectively. There are some regions with tight junction discontinuity in (b) and (d), which are indicated by white arrows, possibly due to interactions with the cancer cells. (e) Confocal image of claudin-5 expression (green) in a region with a double layer iBMECs. (f) X- and y-axis projection of claudin-5 expression in the image in (e), showing the formation of a double layer of iBMECs on each side of the membrane, indicated by white arrows. Scale bars indicate 100 µm and images are maximum intensity projections of confocal z-stacks. Blue indicates cell nuclei.