

Supplementary Table 2. Organotypic *in vitro* systems for the study of metastatic dormancy.

| Description | Stromal cells | Breast cancer cells tested | Validation strategies | Refs. |
|--|--|--|--|--|
| <i>Bone</i> | | | | |
| 3D-collagen biomatrix seeded with human bone marrow stromal cells. Matrixes with defined stromal composition provided reversible growth arrest signals <i>in vitro</i> and upon implantation <i>in vivo</i> (inhibitory niche). Specific inhibition of RTK, Alk5, and p38 signaling in the inhibitory niche results in increased proliferation of BCCs without affecting stromal cells. | Supportive niche: Primary human mesenchymal stem cells Inhibitory niche: hFOBs (human foetal osteoblasts) HS-5 (human mesenchymal cells of bone marrow origin) HUVEC (human umbilical cord endothelial cells) | SUM149, SUM159, MDA-MB-231, BT474, MCF7, T47D, ZR75-1 | Reversible growth arrest, Ki67 staining, cell cycle arrest markers (p21, p27), response to signals | Marlow et al., 2013; McGrath et al., 2019 |
| BCCs are added together with laminin-rich ECM to human microvasculature niche. Within this system, BCCs show heterogeneous behavior, with cells adjacent to mature capillaries remaining dormant (via TSP-1), and cells growing closer to neovascular tips due to expression of POSTN and TGFβ1. This <i>in vitro</i> model has been further utilized to dissect signals that sustain chemoresistance of dormant BCCs <i>in vivo</i> . | HUVEC (human umbilical cord endothelial cells) Primary human mesenchymal stem cells | HMT-3522-T4-2, MCF7, MDA-MB-231 | Reversible growth arrest, Ki67 staining, cell cycle arrest markers, response to signals | Ghajar et al., 2013; Carlson et al., 2019 |
| Osteoblasts can be cultivated for several weeks in a bioreactor forming a multilayered bone-like structure as substrate for BCCs. Bone remodeling cytokines have been shown to drive proliferation of quiescent BCCs in this organotypic model. | MC3T3-E1 (mouse calvariaosteoblasts) NH0st (human osteoblasts) hFOBs (human foetal osteoblasts) | MDA-MB-231 BRMS1, MCF7 | Reversible growth arrest, Ki67, response to signals | Dhurjati et al., 2006; Sosnoski et al., 2015 |
| <i>Liver</i> | | | | |
| Polystyrene (or hydrogel) scaffold and coated with collagen I, then primary hepatocytes and non-hepatocytes liver stromal cells are added and allowed to form the organ-like system. The system has been validated by testing proliferative signals (such as LPS) and exploited to uncover potential biomarkers released from the stroma in response to disseminated BCCs. | Primary human hepatocytes and non-hepatocytes | MDA-MB-231, MCF7 | Reversible growth arrest, Ki67/EdU, cell shape, response to signals | Wheeler et al., 2014; Clark et al., 2016, 2018 |
| <i>Lung</i> | | | | |
| Alveolar type1-like cells, alveolar type2-like cells and lung fibroblasts are cultured with mitogen and nutrient low medium on an air-permeable surface to recreate the gas flow through the alveolar wall. | TT1 (human type-1 pneumocytes) H441 cells (human lung adenocarcinoma type-2 pneumocytes) Human lung fibroblasts | D2.OR, D2.A1, MCF7, T47D-DBM, 4T07 | Reversible growth arrest, Ki67 staining, cell shape, gene expression, response to signals | Montagner et al., 2020 |