Supplementary Figure legends

Supplementary Figure S1. Alignment of myofibroblasts and periostin deposition parallels collagen organization. PDAC tissues were immunolabeled for αSMA (brown in A,B) or periostin (brown in C) followed by trichrome staining (labels collagen-I blue with cells counterstained pink). In areas of invasion where collagen fibers are thinner and more disorganized, myofibroblast orientation is similarly disorganized (A). Well-organized, parallel bundles of collagen frequently encapsulate tumor areas (right side of B and C) and this organization is reflected by parallel alignment of myofibroblasts along these bundles (B) and parallel distribution of periostin (C). Size bars, 50 μm.

Supplemental Figure S2. Macrophages are not uniformly distributed in PDAC. A-C. Macrophages (brown, CD68+) are abundant around PanIN1 (A), PanIN2 (B), and PanIN3 (C) lesions. Note that some brown labeling occurs non-specifically at the luminal surface of the epithelium, commonly a tissue edge effect. D-F. Tissues were immunolabeled with CD68 (dark brown) and trichrome staining for collagen fibers (blue). In areas lacking invading epithelium (D), the well-organized, parallel bundles of collagen contain fewer macrophages than the spaces between these bundles. Surrounding areas of active invasion (arrows in E, F) CD68+ cells are found in abundance. G-I. Triple immunofluorescence for macrophages (CD68, red), myofibroblasts (αSMA, green) and tumor epithelium (CK19, blue) reflects the non-random distribution of macrophages, with fewer macrophages in areas of well-organized, parallel-oriented myofibroblasts containing lower numbers of macrophages (top right of G, bottom right of H)

while disorganized myofibroblasts around invading epithelium (top left of H) contain higher numbers of macrophages. Note that tumor-associated macrophages (arrowheads) label less brightly than infiltrating macrophages (asterisks). Individual panels for each fluorophore are shown in Supplemental Figure S3. Size bars, 50 μm.

Supplemental Figure S3. Single channel immunofluorescence for Supplemental Figure S2 G-I. G1, H1, I1, CD68 immunofluorescence alone (IF). G2, H2, I2, αSMA IF alone. G3, H3, I3, CK19 IF alone.

Supplemental Figure S4. The majority of CD68-positive cells are also CD163 positive. A. PDAC tissue was labeled with the CD68 antibody (red), CD163 antibody (green) and counterstained with Toto3 (blue) to label nuclei. The majority of CD68+ cells were also CD163+ (arrows), although rare CD68+, CD163- cells could be found (arrowheads). Inset: A large blood vessel in the nearby duodenal muscularis can be seen to carry many CD68+, CD163- cells. B. CD68 labeling alone. C. CD163 labeling alone.

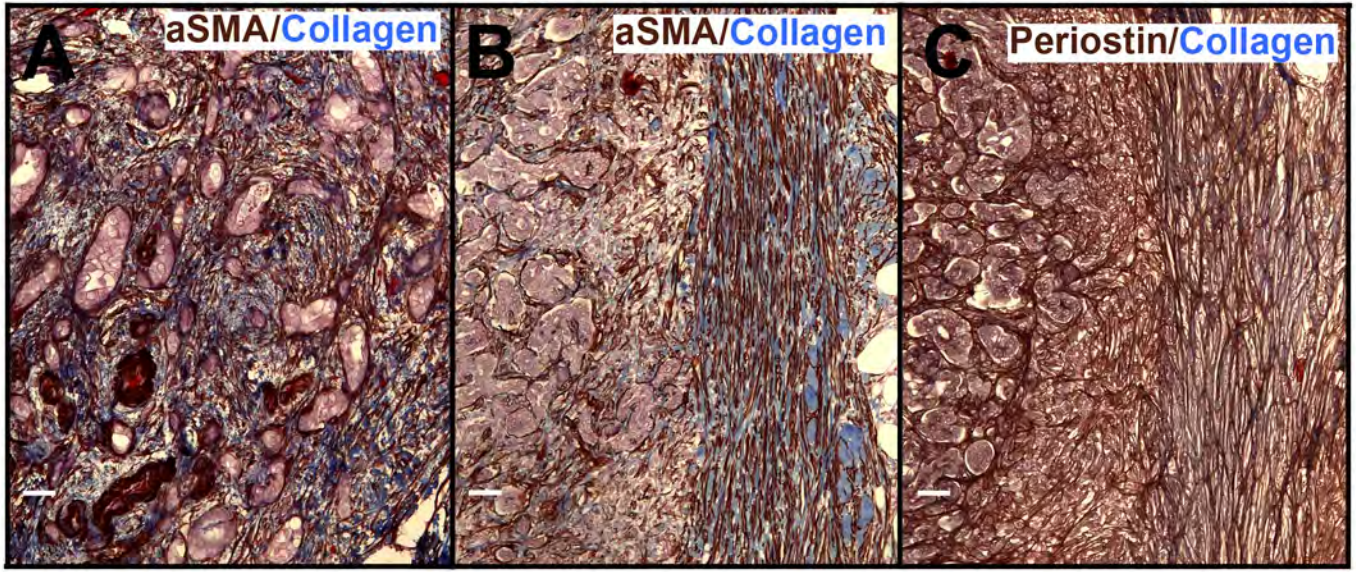
Supplemental Figure S5. Murine PanIN and PDAC have similar microenvironments to that in humans. Immunohistochemistry (brown color) for αSMA (A,D,G), periostin (B,E,H) or F4/80 (I) and trichrome histological staining for collagen-I fibers (blue, C and F). A-C. In a mouse model that expresses the $Kras^{G12D}$ oncogene, ADMs first appear around 5 weeks of age and are uniformly surrounded by myofibroblasts, periostin and thin collagen fibers. D-F. In a 6-month old $Kras^{G12D}$ mouse, many PanIN1-like lesions are observed that

vary considerably in their microenvironment. Many are similar to human PanIN1 with only a thin layer of myofibroblasts surrounding them and little or no periostin. However, some PanIN1-like lesions have a microenvironment more similar to ADM lesions with more abundant myofibroblasts and periostin. G, H. In mouse PDAC, αSMA and periostin are both abundant as in human PDAC. I. Macrophages, labeled by F4/80 immunohistochemistry, are present throughout the tissue and are highly concentrated at invasive fronts as in human disease. Size bars, 50 μm.

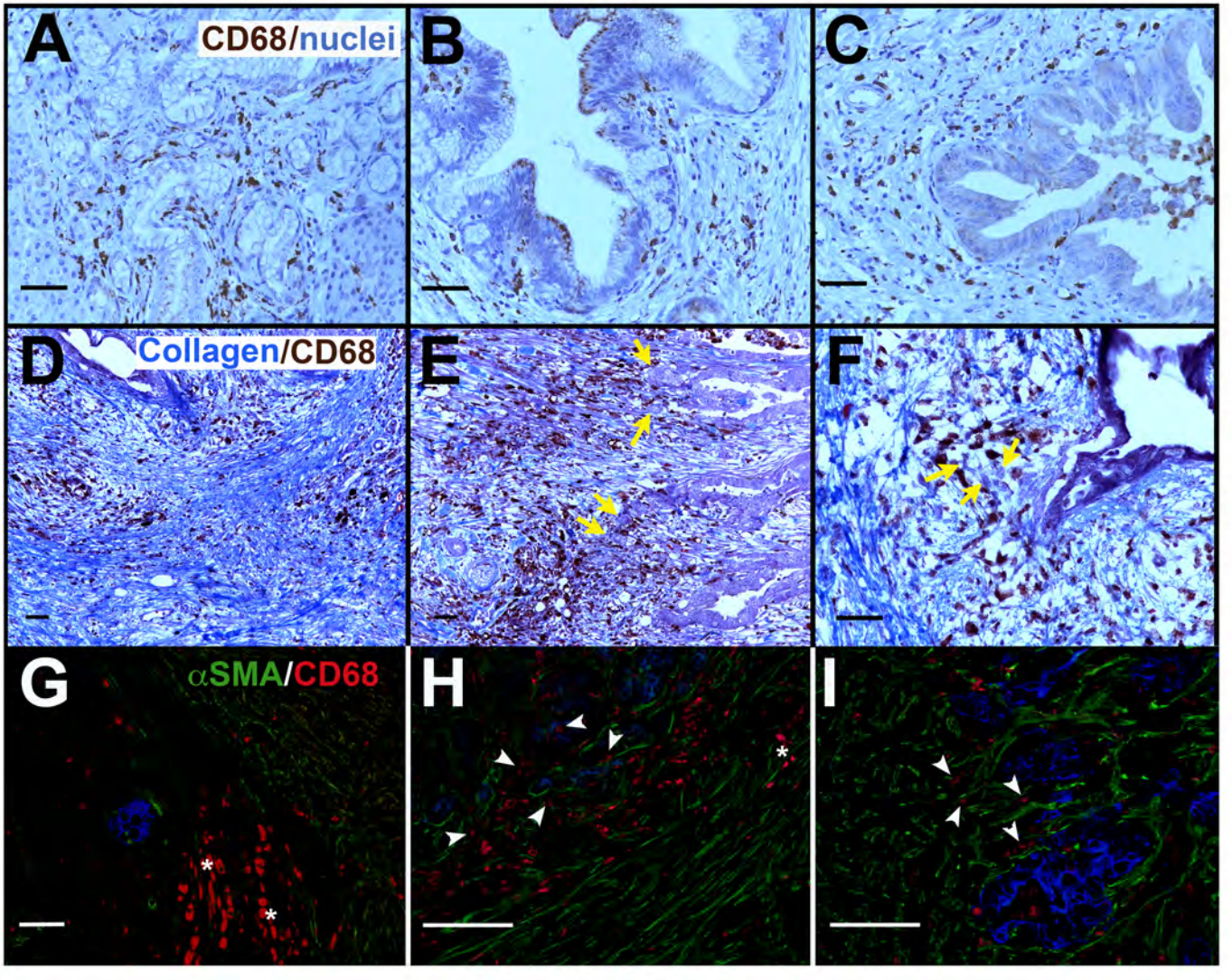
Supplemental Figure S6. IPS-1 cells remain quiescent when cultured on matrigel then transferred to a 3-dimensional collagen matrix. A. When cultured on matrigel and then moved to a 3-dimensional collagen matrix, IPS-1 cells formed tight clusters of cells. B, C. When these cell clusters were removed from collagen and stained with oil red O, lipid droplets were evident. Size bars, 50 μm.

Supplemental Figure S7. Immortalized BMDM cells also activate IPS-1 cells. IPS-1 cells were labeled in green and BMDM cells labeled in red as described in Figure 4. IPS-1 cells were cultured on matrigel for 3 days then transferred to 3-dimensional collagen matrix with or without BMDM and/or rHB-EGF and cultured for a further 3 days. A. IPS-1 cells alone. B. IPS-1 + BMDM. C. IPS-1 + BMDM + rHB-EGF. A'-C' correspond to IPS-1 fluorescence alone from panels A-C. Size bars, 50 μm.

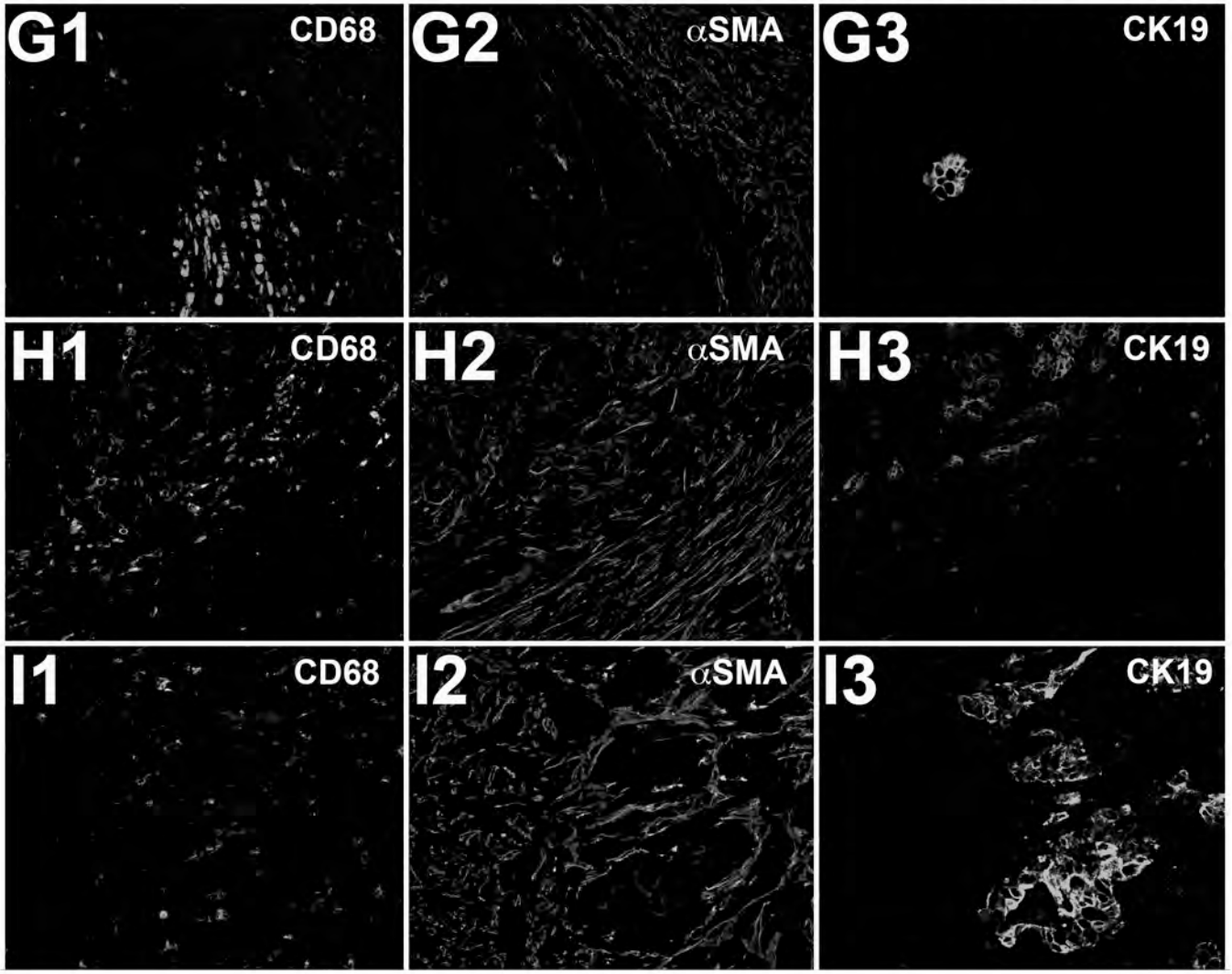
Supplemental Figure S1



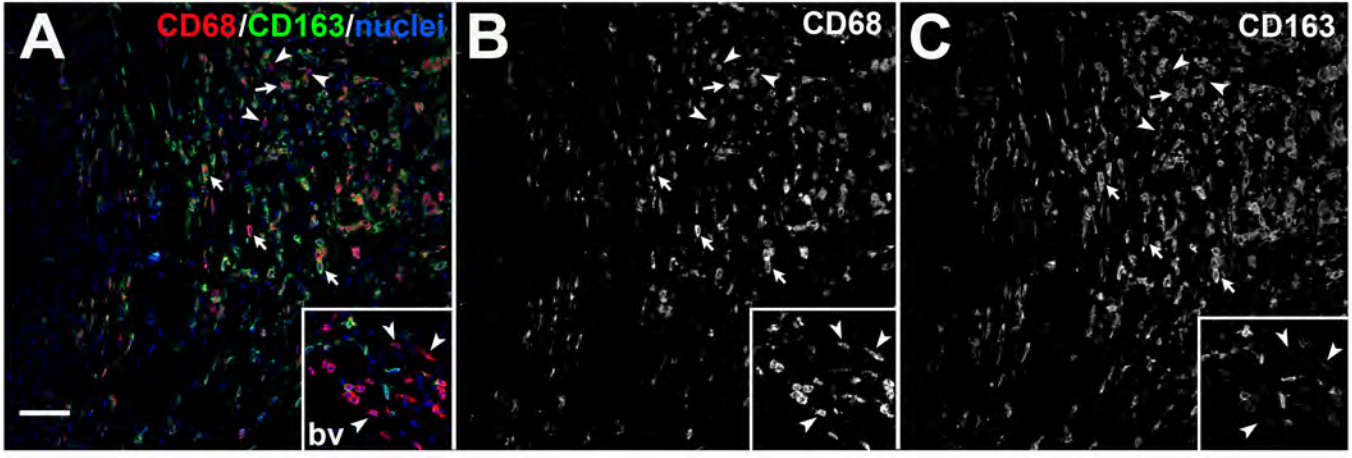
Supplemental Figure S2



Supplemental figure S3



Supplemental Figure S4



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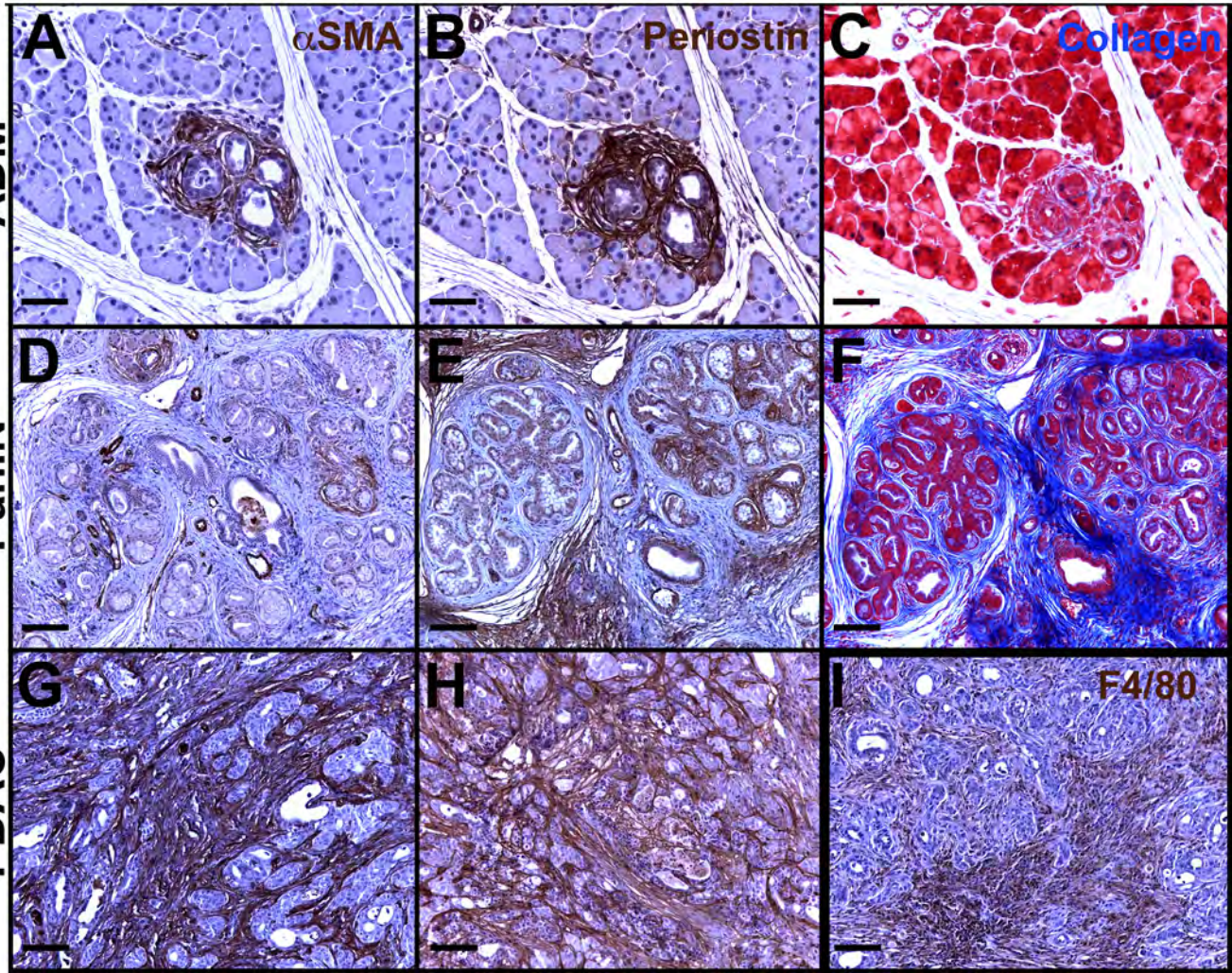
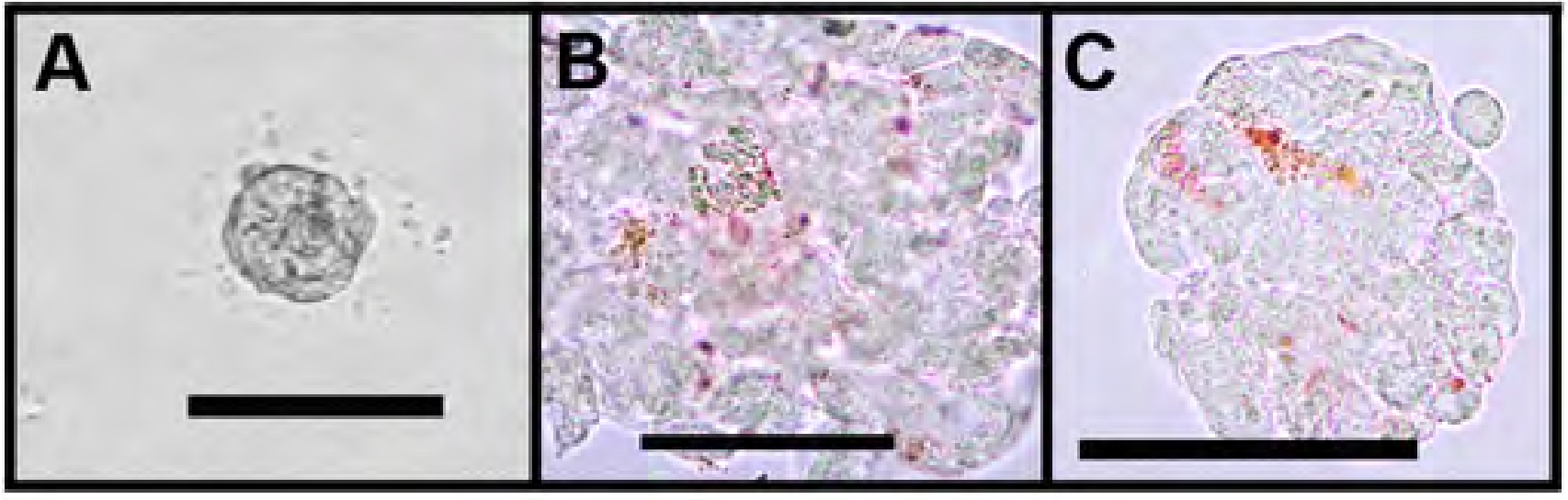


Supplemental Figure S6



Supplemental Figure S7