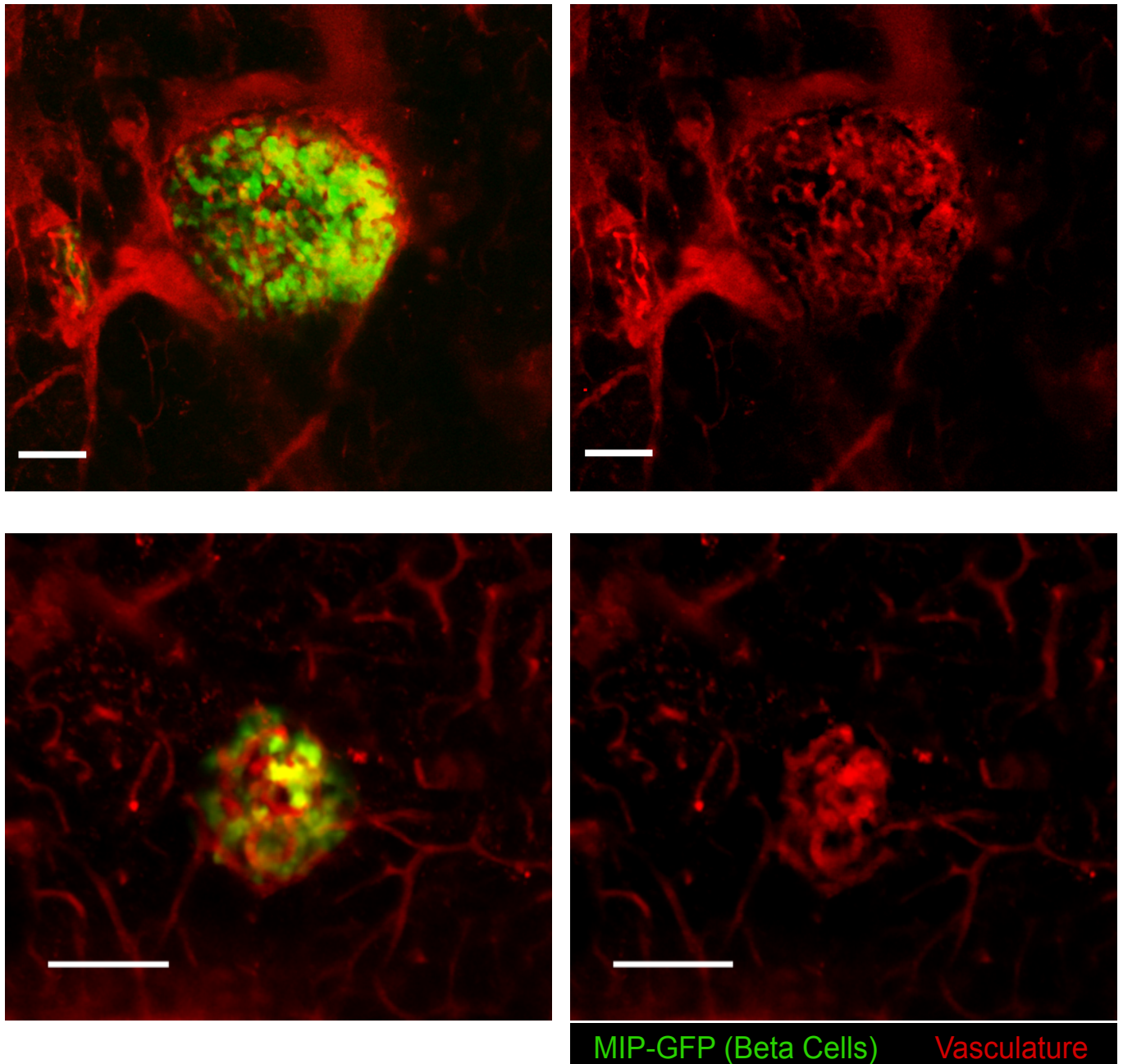
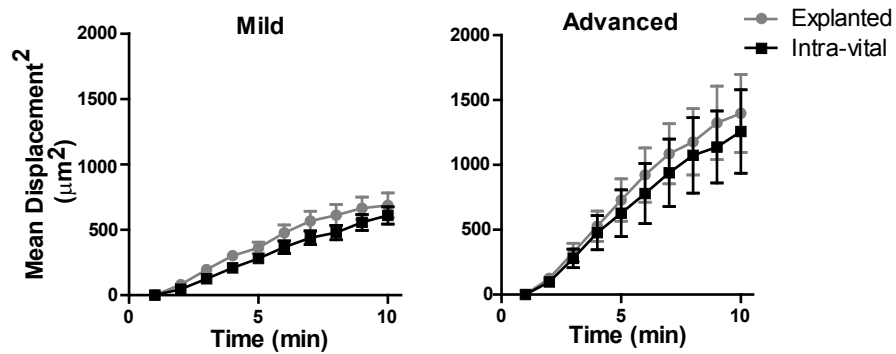


## Supplemental Figure 1: Distinct vasculature identifies islets



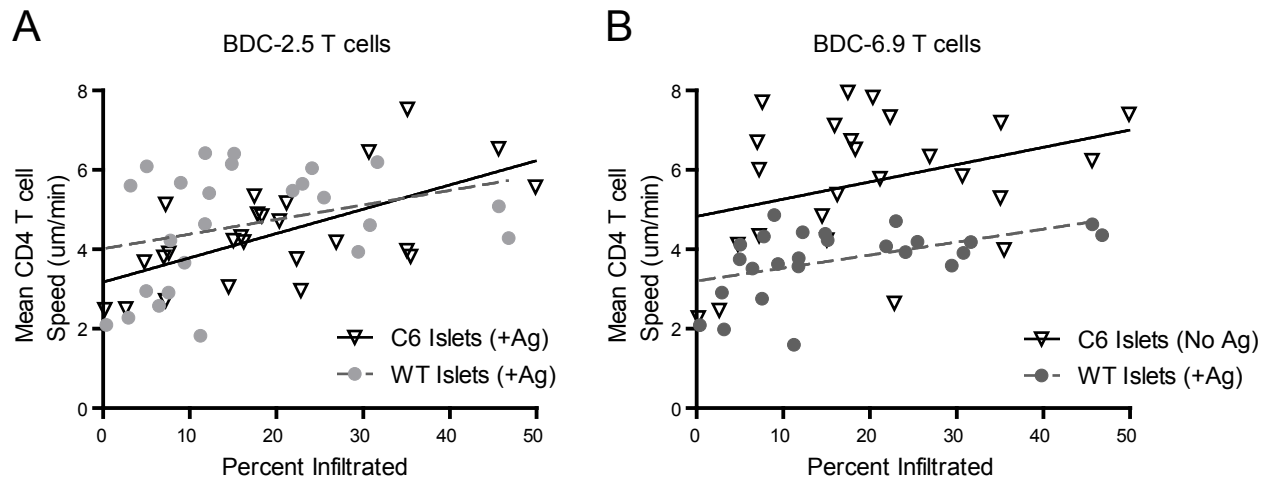
**Supplemental Figure 1: Distinct vasculature identifies islets.** Example maximum intensity projection images of 2 islets in B6.MIP-GFP mice. The beta cells of the pancreatic islets are labeled with GFP. The vasculature space is labeled with Evans blue, and demonstrates the distinctive shape of the islet vasculature, which is highlighted in the panels on the right.

## Supplemental Figure 2: Motility is similar using both explanted and intra-vital imaging methods



**Supplemental Figure 2: Motility is similar using both explanted and intra-vital imaging methods.** See figure 4 for experimental conditions. Comparison of mean squared displacement ( $\mu\text{m}^2$ ) for explanted and intra-vital imaging in islets with mild and advanced infiltration.

### Supplemental Figure 3: BDC-6.9 T cell motility is selectively increased in C6 Islets



**Supplemental Figure 3: BDC-6.9 T cell motility is selectively increased in C6 islets.** See figure 5 for experimental conditions. **A)** Comparison of BDC-2.5 T cells in WT (+Ag) or NOD.C6(+Ag)islets. BDC2.5 T cells have similar motility in both types of islets at similar infiltration levels. **B)** Comparison of B]DC-6.9 T cells in WT (+Ag) or NOD.C6 (No Ag) islets. BDC-6.9 T cells move faster when their antigen is absent in the NOD.C6 islets.

**Supplemental Video 1: Neutrophils do not accumulate at the imaging site.** Video of Figure 1D. Representative maximum intensity projection of an intra-vital islet. 3x3 fields of view around the islet were stitched together to give a large field of view. Images were collected every 2 minutes for 2.5 hours. CMTMR-labeled neutrophils (red) were located in the vasculature (red-purple) and tracked at every time point.

**Supplemental Video 2: Bead motility through the islet vasculature at the imaging site.** Video of Figure 1E. A representative single Z-plane in the center of an islet one hour after surgery. Evans blue (red) labels the vasculature. Trailing lines represent tracks of i.v. injected beads (blue). Images were continuously collected at 30 frames per second for 19 seconds. This procedure was repeated at three Z-depths per islet for three islets.

**Supplemental Video 3: T cell motility in an explanted islet with mild infiltration.** Video of Figure 3A. Activated BDC-2.5 T cells (green) were fluorescently labeled and transferred 24h prior to imaging. Representative maximum intensity projection images from explanted islets captured using 2-photon microscopy. Green tracks represent the previous 10 minutes of motion of transferred BDC-2.5 T cells (green). Scale bar=50  $\mu\text{m}$ ; timestamp is in min:sec.

**Supplemental Video 4: T cell motility in an explanted islet with advanced infiltration.** Video of Figure 3A. Activated BDC-2.5 T cells (green) were fluorescently labeled and transferred 24h prior to imaging. Representative maximum intensity

projection images from explanted islets captured using 2-photon microscopy. Green tracks represent the previous 10 minutes of motion of transferred BDC-2.5 T cells (green). Scale bar=50  $\mu\text{m}$ ; timestamp is in min:sec.

**Supplemental Video 5: T cell motility in an intra-vitally imaged islet with mild**

**infiltration.** Video of Figure 3B. Activated BDC-2.5 T cells (green) were fluorescently labeled and transferred 24h prior to imaging. Representative maximum intensity projection images from intra-vital islets captured using 2-photon microscopy. Green tracks represent the previous 10 minutes of motion of transferred BDC-2.5 T cells (green). 70kD Dextran-Rhodamine labels the vascular space (red). Leakage from the vasculature can be observed surrounding, but not inside of the islet. Scale bar=50  $\mu\text{m}$ ; timestamp is in min:sec.

**Supplemental Video 6: T cell motility in an intra-vitally imaged islet with advanced**

**infiltration.** Video of Figure 3B. Activated BDC-2.5 T cells (green) were fluorescently labeled and transferred 24h prior to imaging. Representative maximum intensity projection images from intra-vital islets captured using 2-photon microscopy. Green tracks represent the previous 10 minutes of motion of transferred BDC-2.5 T cells (green). 70kD Dextran-Rhodamine labels the vascular space (red). Scale bar=50  $\mu\text{m}$ ; timestamp is in min:sec.

**Supplemental Video 7: Sustained T cell-APC interaction within an islet with mild**

**infiltration.** Video of Figure 6A. Fluorescently labeled BDC-2.5 T cells were transferred

into CD11c-YFP hosts 24 hours prior to islet isolation and imaging. Maximum intensity projection images showing BDC-2.5 T cells (red) and CD11c<sup>+</sup> APCs (green) within pancreatic islets. A BDC-2.5 T cell (red) interacts with a CD11c<sup>+</sup> APC (green) in an explanted NOD.CD11c-YFP islet with mild infiltration. The T cell remains in contact with the CD11c<sup>+</sup> APC for the duration of the video (30 min). Scale bar= 10  $\mu$ m; timestamp is in min:sec.

**Supplemental Video 8: Transient T cell-APC interactions within an islet with advanced infiltration.** Video of Figure 6B. Fluorescently labeled BDC-2.5 T cells were transferred into CD11c-YFP hosts 24 hours prior to islet isolation and imaging. Maximum intensity projection images showing BDC-2.5 T cells (red) and CD11c<sup>+</sup> APCs (green) within pancreatic islets. A BDC-2.5 T cell transiently contacts multiple CD11c<sup>+</sup> APCs in an explanted NOD.CD11c-YFP islet with advanced infiltration. The white line represents a 20 min path of motion of the T cell. Yellow ovals mark T cell-APC contacts. Scale bar= 30  $\mu$ m; timestamp is in min:sec.