

Supplementary Movie Legends

Suppl. Movie 1 – Deep-tissue, in vivo visualization of normal 3D islet vasculature

Rotating 3D isosurfacing rendition of a normal pancreatic islet (green) from a RIP-LCMV.GP/MIP-GFP animal after injection of 70kDa Dextran-TxRed vascular dye (red). Note the dense, tortuous network of capillaries that perfuse the islet and drain in larger adjacent venules. Corresponds with Figure 2C,D. Image dimensions: $w/h=1.52/d=5/z=32$

Suppl. Movie 2 – CTL vascular arrest occurs primarily in larger pancreatic venules

Image series consisting of maximum intensity projection images (Image dimensions: $w/h=0.56, d=4/z=25/t=30s$). Inset represents a subregion captured at high frame rate (5f/s) within a single z-plane ($w/h=0.51$). Red arrows indicate immobile, round-shaped cells in contact with the vascular wall (brownish, 70kDa Dextran-TxRed). Fast-moving white dots in the inset are from injected fluorescent beads and show blood flow directionality. Corresponds with Figure 2E,F.

Suppl. Movie 3 – CTL vascular arrest occurs primarily in larger pancreatic venules

Image series consisting of maximum intensity projection images (Image dimensions: $w/h=1.52, d=5/z=21/t=30s$) Inset represents a subregion captured at high frame rate (5f/s) within a single z-plane ($w/h=0.51$). Green arrows indicate immobile, round-shaped cells in contact with the vascular wall (red, 70kDa Dextran-TxRed). Cyan arrow shows CTL crawling against the vascular wall. Yellow arrow indicates freely flowing CTL. White arrow marks an unlabelled cell in stable contact with the vascular wall. Red arrow shows leakage of vascular dye. Corresponds with Figure 2G.

Suppl. Movie 4 – Leaky venule phenotype in the prediabetic RIP-LCMV.GP pancreas

Isosurfacing rendition of vascular leakage in the prediabetic pancreas. Dextran-TxRed vascular dye (red) was injected at $T=0$. Corresponds with Figure 3A,B and compound isosurfaced volume was used to generate Figure 3E. Image dimensions: $w/h=1.52/d=5/z=26/t=60s$

Suppl. Movie 5 - Intact vascular integrity in the naive RIP-LCMV.GP pancreas

Isosurfacing rendition of intact vascular integrity (red) and islet structure (green) in the naive pancreas. Dextran-TxRed vascular dye was injected at T=0. Corresponds with Figure 3C,D and compound isosurfaced volume was used to generate Figure 3E. Image dimensions: w/h=1.52/d=5/z=33/t=60s

Suppl. Movie 6 – 3D tracking of diabetogenic CTL within acinar tissue

Random motility of CTL within acinar tissue adjacent to a pancreatic islet. Magenta dots are single CTL subjected to tracking and are associated with color-coded 'dragontails' that depict the last five positions of the cell. Corresponds with Figure 4 C. . Image dimensions: w/h=0.52/d=6/z=17/t=30s

Suppl. Movie 7 – High-resolution visualization of random CTL motility in the exocrine pancreas

Highly detailed image sequence with CTL in green and isosurfaced vascular network (red). Note that all CTL are located in the tissue and not the vasculature. Corresponds with Figure 4E. Image dimensions: w/h=0.27/d=5/z=24/t=30s

Suppl. Movie 8 – Random motility associated with peptide/adjuvant-activated CTL

Identical experimental setup as in Movies 6 and 7 except for the use of non-infectious CTL activation instead of LCMV infection. Note the formation of an inflammatory CTL 'continuum' between the adjacent islets that are imaged. Corresponds with Figure 4F. Image dimensions: w/h=0.69/d=6/z=14/t=30s

Suppl. Movie 9 – Inflammatory CTL 'continuum' between anatomically adjacent islets

CTL (motile green cells) can be seen to form a continuum between adjacent islets (green masses left and right). Corresponds with Figure 5B. Image dimensions: w/h=0.70/d=6/z=22/t=30s

Suppl. Movie 10 – Random CTL motility between anatomically adjacent islets

Example of CTL behavior and tracking in the 3D region between anatomically nearby islets (large green masses). The magenta dots represent single CTL and the color-coded 'dragontail' tracks depict the last five positions of the cell. Corresponds with Figure 5D. Image dimensions: w/h=1.11/d=6/z=16/t=30s

Suppl. Movie 11 – Constrained CTL motility and arrest within the islet milieu.

CTL (CFP, magenta) and beta cells (GFP, green) imaged at cellular resolution. Corresponds with Figure 6A. Image dimensions: $w/h=0.79/d=5/z=22/t=60s$

Suppl. Movie 12 – CTL arrest and scanning behavior within pancreatic islets

Magnified 3D region corresponding with Figure 6A showing CTL-beta cell contacts during the entire imaging time (red arrows) along with numerous motile CTL migrating through the field of view. Image dimensions and time resolution: see Suppl. Movie 11.

Suppl. Movie 13 - CTL arrest and scanning behavior within pancreatic islets

Magnified 3D region corresponding with Figure 6B showing stable CTL-beta cell contacts during the entire (red arrow) or partial (blue arrow) imaging time along with numerous motile CTL migrating through the field of view. Image dimensions of original sequence: $w/h=0.88/d=5/z=20/t=60s$

Suppl. Movie 14 – In vivo detection of beta cell death

Red arrow indicates loss of GFP signal associated with beta cell death. Corresponds with Figure 7A. Image dimensions: $w/h=0.58/d=6/z=21/t=30s$

Suppl. Movie 15 – Association of beta cell death (green; red arrow) with arrested CTL (magenta; white arrow).

Corresponds with Figure 7E. Image dimensions: $w/h=0.86/d=5/z=22/t=60s$

Suppl. Movie 16 – Detail from Suppl. Movie 15

Image dimensions: $w/h=0.06/d=5/z=22/t=60$

Suppl. Movie 17 – Lack of CTL confinement and arrest in the full-blown diabetic pancreas

As single beta cell (large, immobile green) can be observed in the middle of the sequence, with CTL in red. Small motile green cells are likely phagocytic cells that have phagocytized beta cell-derived GFP (phenomenon described previously by Tang, Q., Adams, J.Y., Tooley, A.J., Bi, M., Fife, B.T., Serra, P.,

Santamaria, P., Locksley, R.M., Krummel, M.F., and Bluestone, J.A. 2006. Visualizing regulatory T cell control of autoimmune responses in nonobese diabetic mice. *Nat Immunol* 7:83-92). Image is a magnified region from Figure 7E. Image dimensions of original sequence: w/h=1.52/d=5/z=20/t=60s

Suppl. Movie 18 – Rapid disappearance of CTL upon antigen depletion

A minor population of slowly meandering CTL (small green) are seen in proximity to a remnant islet (larger yellowish/green cluster) in this highly diabetic animal. Vasculature is stained with Dextran-TxRed. Image corresponds with Figure 7G after injection of vascular dye. Image dimensions: w/h=1.52/d=5/z=18/t=30s