

Light-Sheet Cable Assembly for Tiggered Acquisition I/O **Breakout Box** Computer В С D Branch 2 Ð \cap \mathbf{G} G Nidaq PCIe Card Port 0 Branch 1 Branch 5 PZ EXT In Port 1 Branch 1 Branch 2 Stage Controller Camera Link Branch 16 Timing out PCIe Card (Framegrabber) Camera Link interface Connector 1 Camera Link interface Connector 2 CMOS Camera

639

640 **Suppl. Fig. 1. Hardware wiring diagram of the light-sheet microscope.** Hardware integration (top 641 panel) for camera-triggered activation of the excitation lasers and piezo z-stage that limits 642 communication to a single instruction from the computer every 3 minutes. Wiring diagram (bottom 643 panel): two Nikon 'standard cables' connect to the NiDAQ card installed in the computer. These two 644 cables link to the laser control box, stage controller and camera. The images captured are received by 645 the computer through a camera link PCIe card. and



646

647 Suppl. Fig. 2. NIS-Elements software configuration. (A) Schematic for comparing continuous
648 acquisition and looped acquisition. The red box indicates the 3-minute window where storage speed is
649 higher than imaging speed. (B) Devices linked to NIS-Elements. (C) NIS-Elements JOBS module
650 configured for looped acquisition. (D) Optical configuration for simultaneous GCaMP6s/H2B-mCherry
651 excitation.

652



654 Suppl. Fig. 3. Location of early and late phase cells in an islet with stable wave axis. 3D 655 representation of the islet showing the location of early phase cells (blue) and late phase cells (red) over 656 three consecutive oscillations (top panel) and their corresponding Ca²⁺ traces (bottom panel).



657

653

658 **Suppl. Fig. 4. Effect of glycolytic activators on the β-cell network.** (A-C) Effect of vehicle, 659 glucokinase activator (GKa), and pyruvate kinase activator (PKa) on regional consistency of the β-cell 660 network (A), high degree cell retention (B), and low degree cells retention (C). Data are displayed as 661 mean \pm SEM.



662

663 **Suppl. Fig. 5. Effect of glycolytic activators on the β-cells that depolarize first.** (A-C) Effect of 664 vehicle, glucokinase activator (GKa), and pyruvate kinase activator (PKa) on the retention (A), regional 665 consistency (B), and wave axis change (C) of the β-cells that depolarize first (Early depolarizer). The 666 retention of β-cells that depolarize first (Early depolarizer) is similar to cells that depolarize and 667 repolarize first (Early phase). Data are displayed as mean ± SEM.