Induction of islet autophagy in response to IFN-alpha

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Introduction

- Type 1 diabetes (T1D) is a multifactorial disease involving genetic and environmental factors. One of the factors implicated in disease pathogenesis is early life viral infection.
- A typical immune response to viral infection includes the production of type 1 interferons (IFN), such as IFN-alpha, which can induce stress in the pancreatic β-cells.
- Intrinsic cellular stress response mechanisms exist, including autophagy, a process that serves to degrade and recycle cellular components to promote homeostasis, such as during the response to infection.
- We recently discovered that autophagy is impaired in the residual β-cells of human organ donors with T1D as well as in islets of the diabetic non-obese diabetic (NOD) mouse model of autoimmune diabetes. Additionally, we observed a significant accumulation of autophagosomes and defective lysosomes in the β-cells of autoantibody-positive donors, suggesting that autophagy is perturbed prior to diabetes onset (Muralidharan *et al., Diabetologia,* 2021).
- There is currently no literature linking IFN-alpha to autophagy in the β-cell, so we set out to determine if β-cell autophagy is modulated by acute IFN-alpha exposure *in vitro* and *in vivo*.

Hypothesis: Ineffective autophagy in the beta cells in response to elevated circulating IFN-alpha may play a role in T1D pathogenesis

Methods

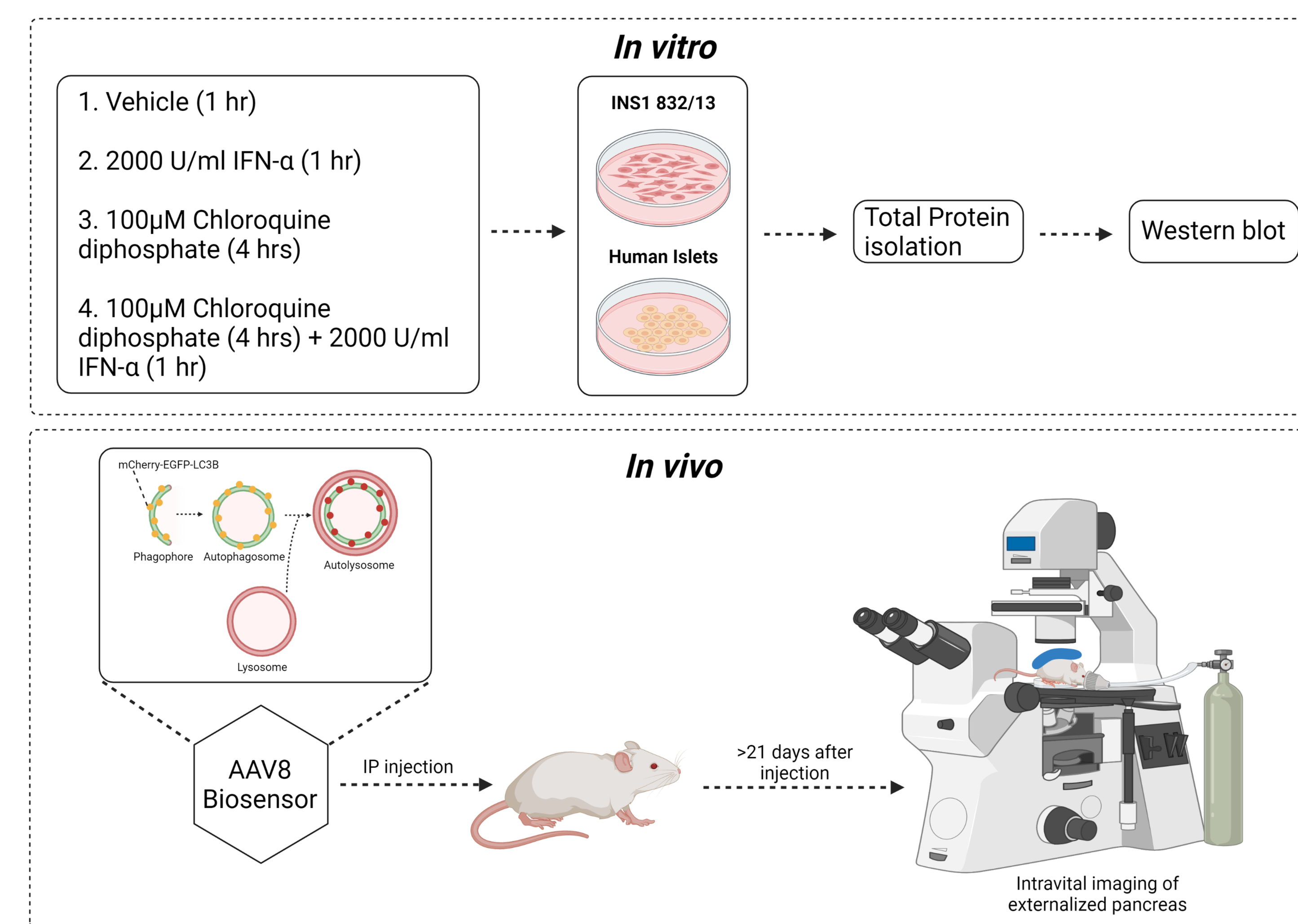

Figure 1. Methodology. Figure created using Biorender.com. INS1 832/13 cells, human islets obtained from IIDP or the University of Alberta, and wildtype C57Bl/6J mice were used to assess autophagy induction upon IFN-alpha exposure. All samples were evaluated for stimulation of autophagy after treatment with IFN-alpha, in the presence/absence of chloroquine-diphosphate to inhibit autophagic flux. *In vitro* experiments using INS1 832/13 cells and Human islets involved treatment with different conditions to assess autophagy induction using immunoblotting analysis. For *in vivo* imaging experiments, 7-week-old wild type C57Bl/6J mice were intraperitoneally injected with approximately $3.44{*}10^{12}$ genomic copies of a custom β-cell-selective fluorescent autophagy biosensor (AAV8-INS-mCherry-EGFP-LC3B). Three weeks later, animals were anesthetized, and the pancreas was externalized for live imaging. Islets were identified based on GFP+mCherry fluorescence, then imaged before and after IV/IP injections as indicated using a LEICA SP8 DIVE two-photon microscope fitted with a 40x/1.1NA water objective.

Results

Interferon alpha stimulates autophagy in INS1 832/13 cells

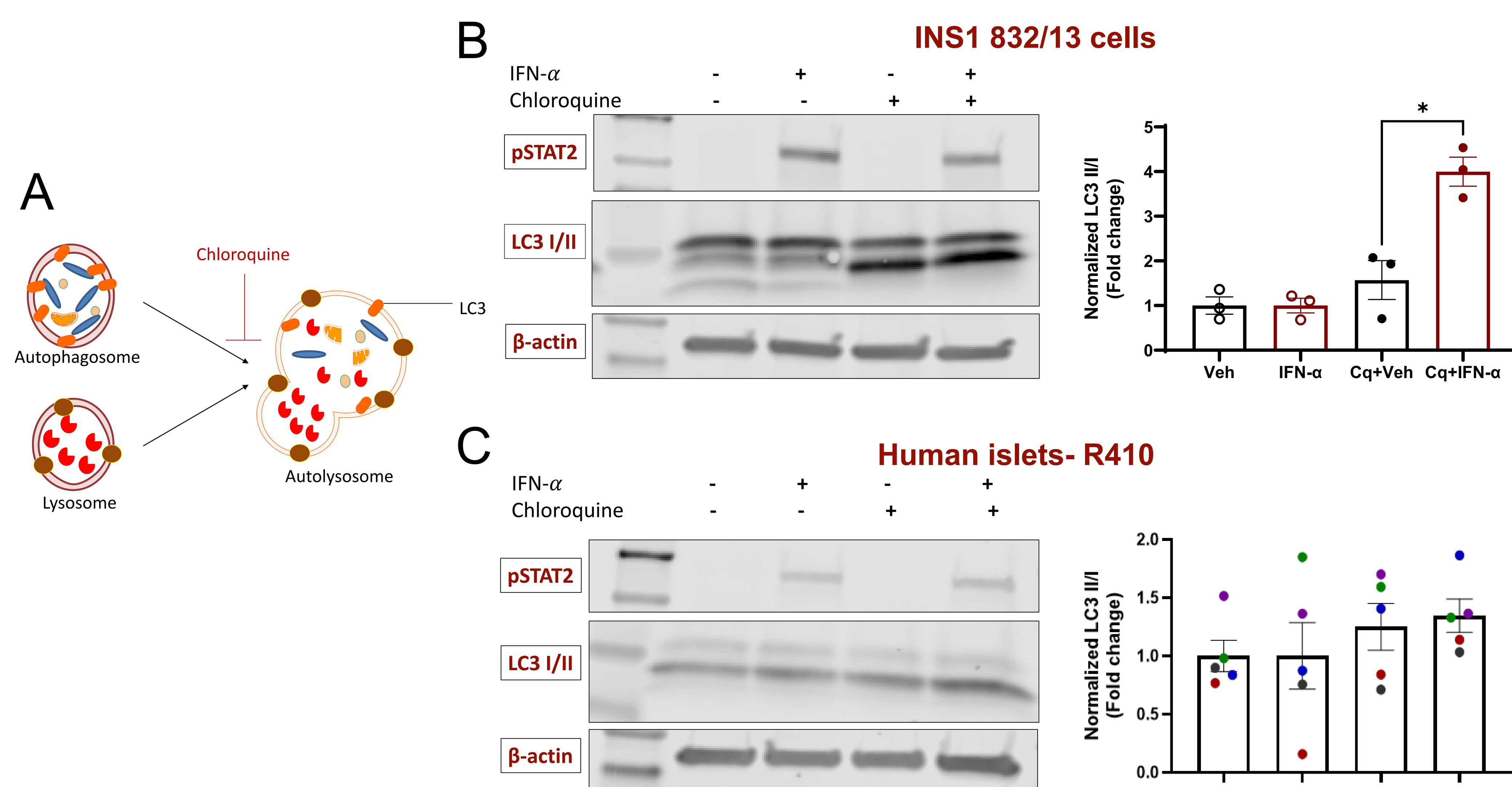

Figure 2. IFN-alpha stimulates autophagy in INS1 832/13 cells but is variable in human islets. (A) Image showing inhibition of autophagosome-lysosome fusion event by Chloroquine. Protein LC3 is used as a marker to identify autophagosomes. (B&C) INS1 832/13 cells or human islets were split into four groups with an approximately equal number of cells or islets. Two groups were pre-treated with 100uM chloroquine-diphosphate (Cq) for 3 hours followed by vehicle or 2000 U/mL IFN-alpha for 1 hour. In the end, all chloroquine treatments were for 4 hours. Two other groups were treated with either vehicle or 2000 U/mL IFN-alpha for 1 hour without pre-treatment. Total protein was assessed for the IFN-alpha second messenger, pSTAT2, and the autophagosome marker LC3. Quantification of normalized LC3 II/I is depicted, and statistics was performed using unpaired students t-test for chloroquine treated vehicle vs IFN-alpha. For human islets, each color represents a donor.

Autophagy is stimulated *in vivo* in C57Bl/6J mice islets, in response to IFN-alpha

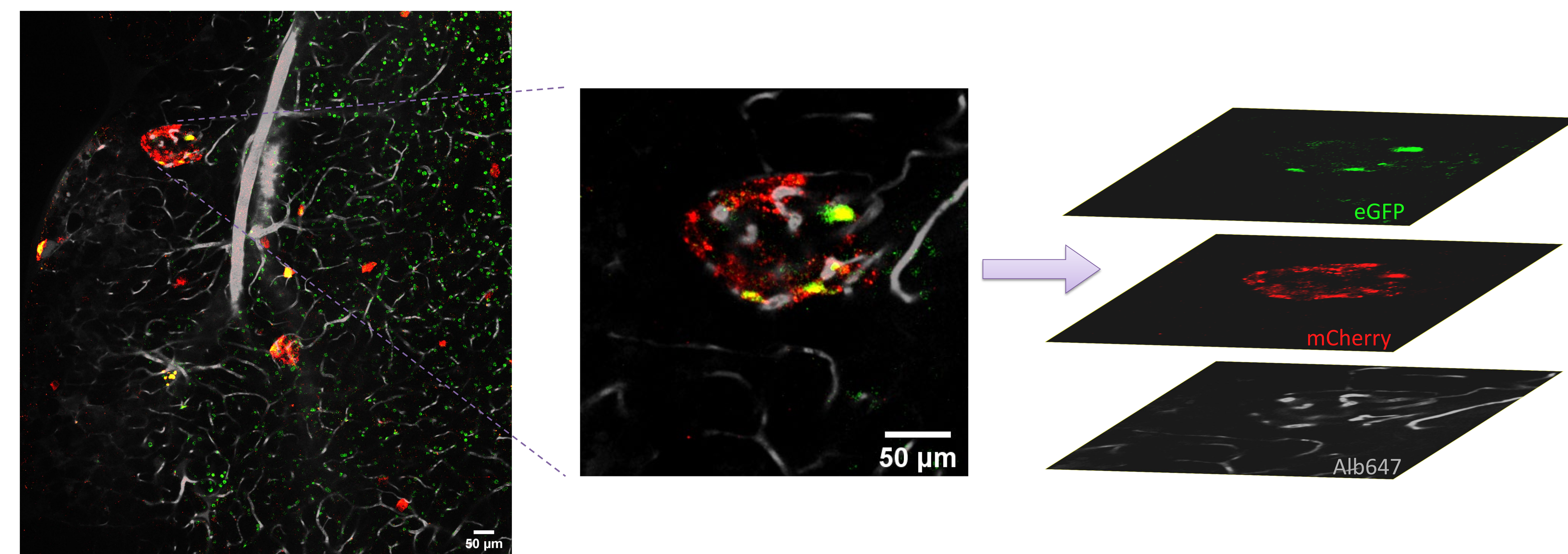

Figure 3A. Intravital image of C57BL/6J mouse pancreas with islet identified. Each image contains eGFP signal, mCherry signal and Albumin 647 signal at different depth of pancreas tissue.

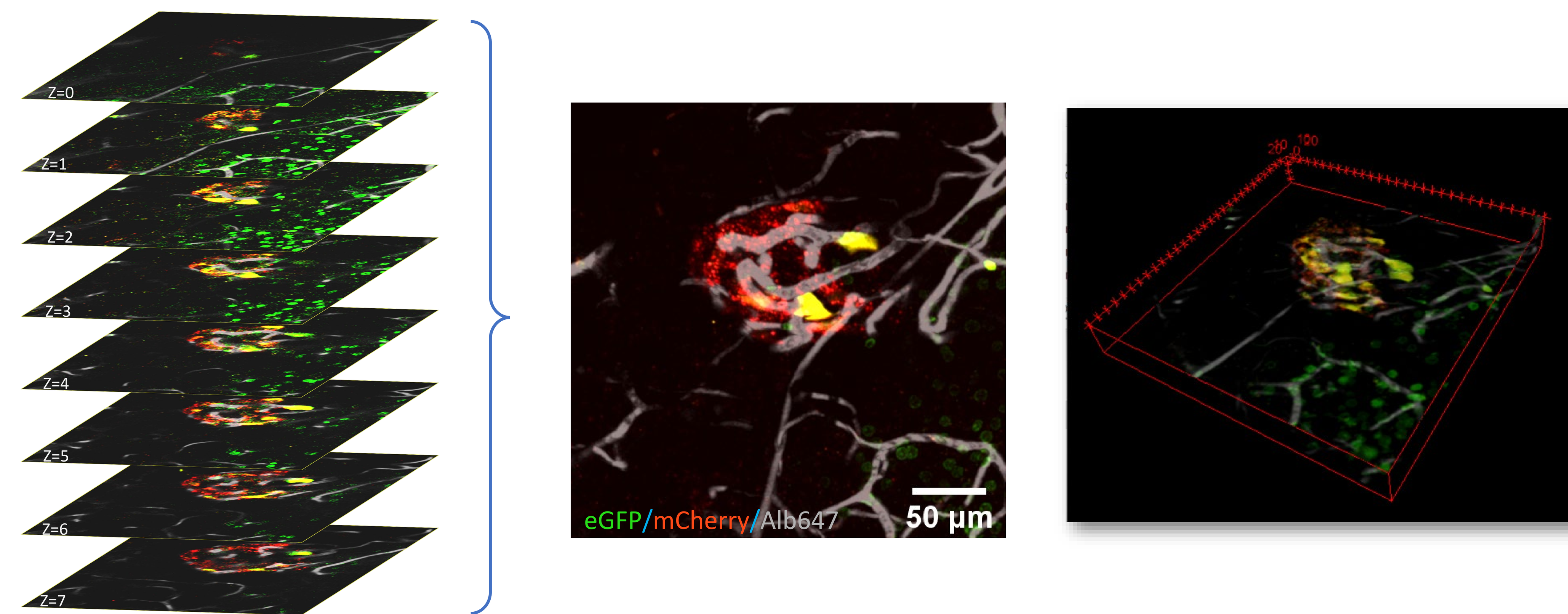

Figure 3B. Intravital image of C57BL/6J mouse pancreas islet with multiple fluorescent proteins collected at different depth of the pancreas. For data analysis images were combined for 3D reconstruction of the islets.

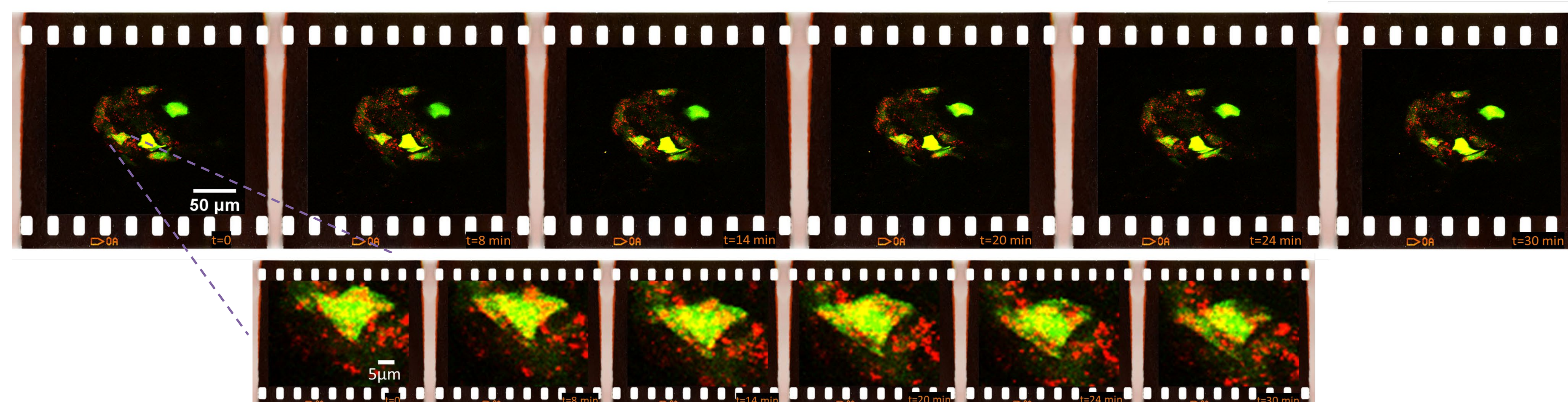

Figure 3C. Movie reconstructions from intravital images of C57BL/6J mouse pancreas islet behavior with IP injected saline at t=0 over 30 min imaging period.

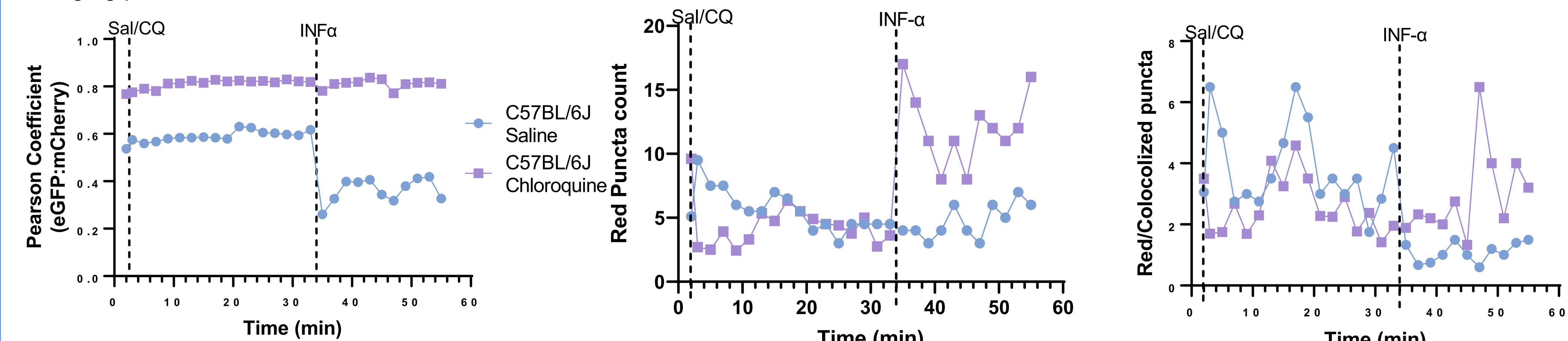

Figure 3D. Data analysis of the mCherry:eGFP puncta behavior with different treatments of C57BL/6J mice over imagine time period. eGFP:mCherry signal was colocalized for autophagosomes detection and observation in time.

Conclusions and Future Directions

- We observed stimulation of pancreatic β-cell autophagy in response to IFN-alpha exposure both *in vitro* and *in vivo*.
- Novel beta-cell-selective autophagy biosensor technology (AAV8-INS-mCherry-EGFP-LC3B) was utilized to observe autophagic flux *in vivo*. Wild type mouse data show clear evidence of autophagic flux alteration in the presence of IFN-alpha. Autophagy inhibitor (chloroquine) was used for verification of active autophagy.
- These data support our hypothesis that loss of autophagic response to IFN-alpha-induced cellular stress could promote β-cell dysfunction and death in T1D pathogenesis.
- Further studies are planned for *in vivo* settings: utilizing autoimmune diabetic mouse models as well as human donor islet transplants in immune compromised mice to determine both the mechanism and timing of the autophagic decline in T1D.

Acknowledgements

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