# Supplemental Materials

Detecting Insulitis in Type 1 Diabetes with Ultrasound Phase-change Contrast Agents.

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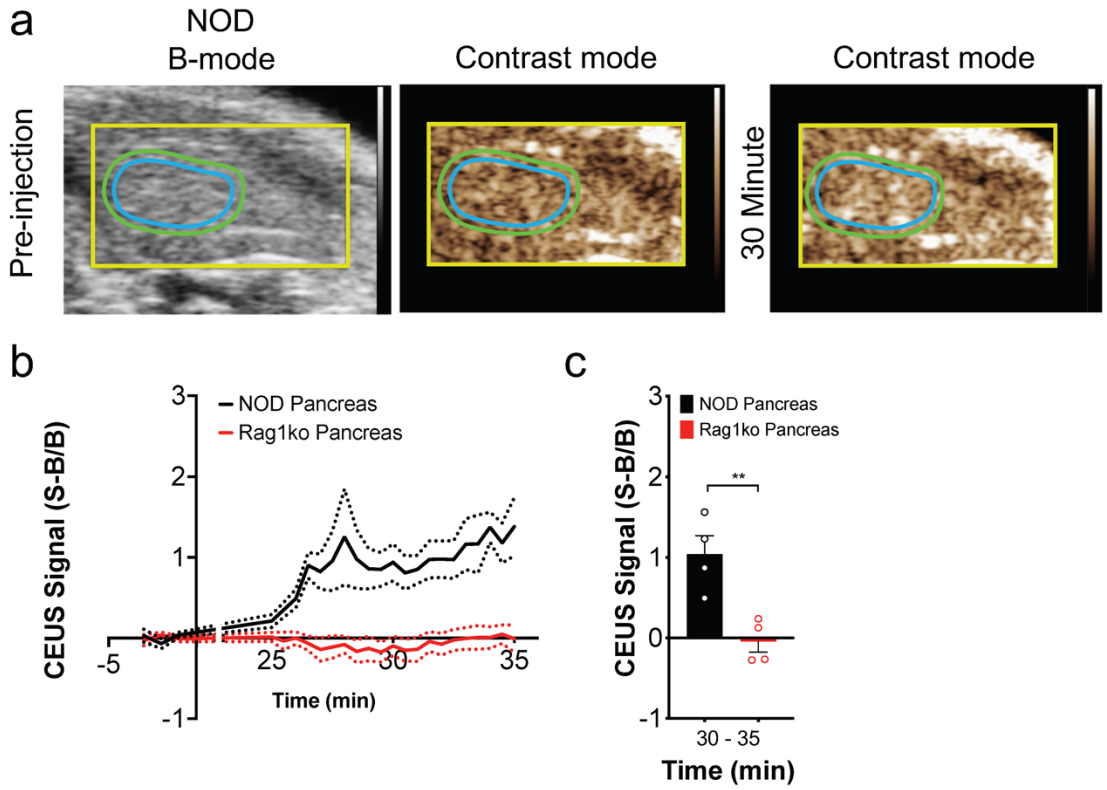
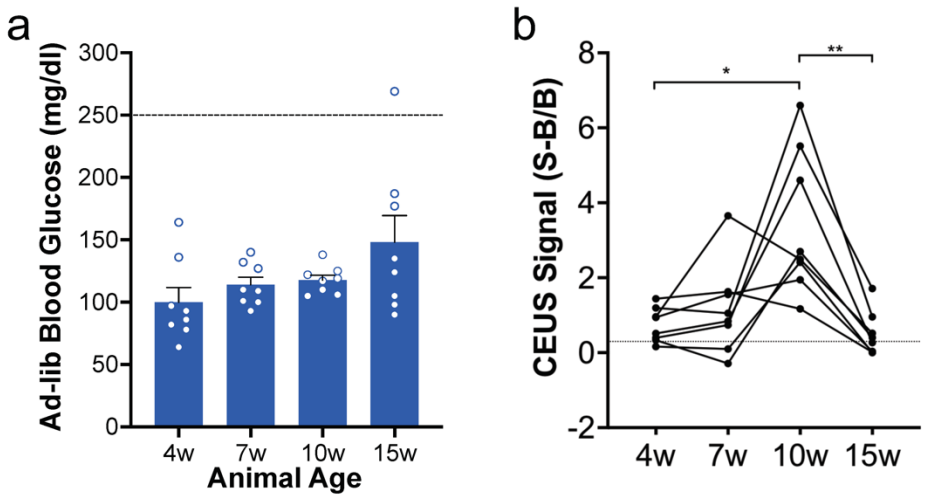
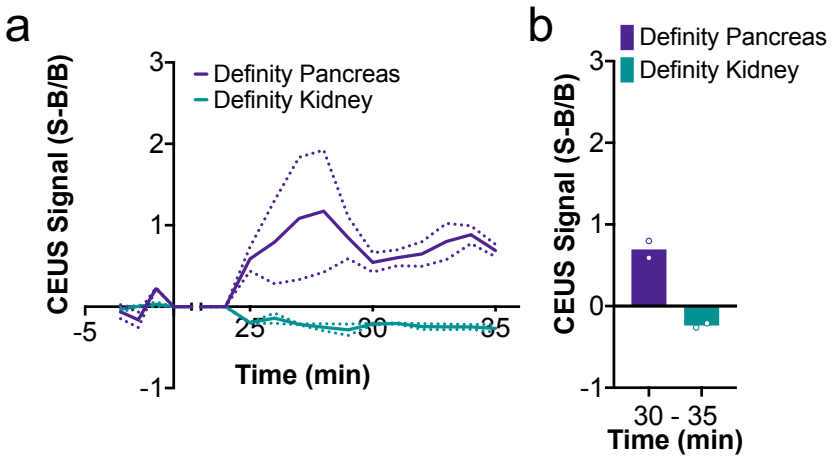


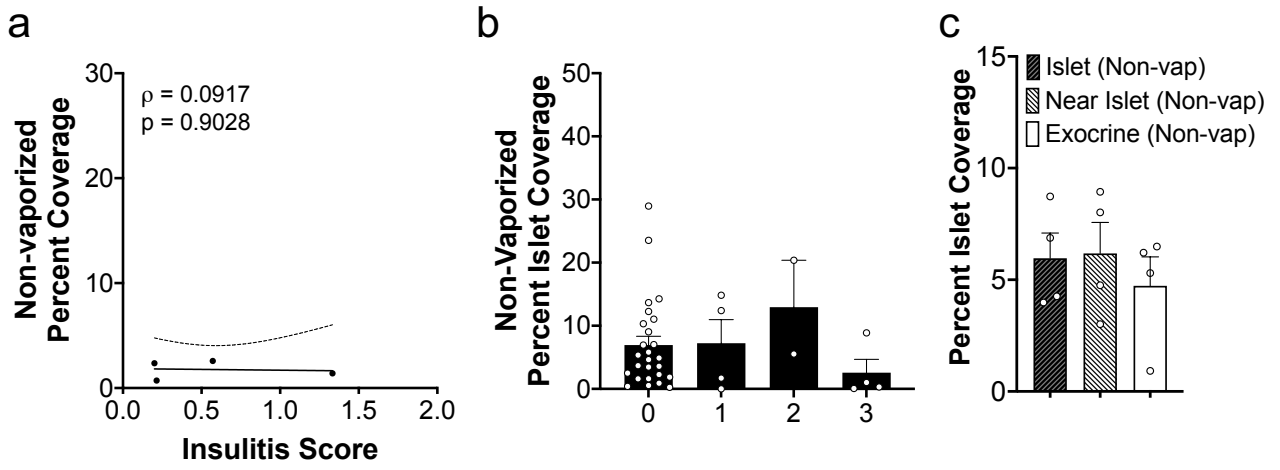

*Supplemental Figure S1. More stringent inclusion of pancreas region does not impact findings.* (A) Representative B-mode and sub-harmonic contrast images of the pancreas (green/blue) before and 30 min. after nanodroplet infusion in 10-week-old female NOD mice. Blue represents more stringent ROI taken to cover the pancreas. (B) Time-course of mean contrast signal, normalized to background, in the pancreas of 10-week-old female NOD mice versus 10-week-old female Ragk1o mice following nanodroplet infusion and vaporization. Data calculated using more stringent ROI. (C) Mean contrast signal averaged between 30-35 minutes following nanodroplet infusion. Dashed lines in B represent 95% CI, error bars in D represent s.e.m. Data in B,C representative of n=4 mice (NOD) and n=4 mice (Rag1ko). The p-value in D is 0.0083 (**p<0.01) comparing groups indicated (unpaired Student's t-test).




*Supplemental Figure S2. Nanodroplet contrast elevation for NOD mice measured at 15 weeks age. (*A) mean ad-lib blood glucose levels at 4 weeks, 7 weeks, 10 weeks and 15 weeks age prior to mice developing diabetes, for those mice measured at 15 weeks. (B) Mean contrast elevation (averaged between 30-35 minutes following nanodroplet infusion) within the pancreas of NOD mice at 4 weeks, 7 weeks 10 weeks and 15 weeks age prior to mice developing diabetes. Data in A,B represents n=8 NOD mice (mice that had already developed diabetes by 15 weeks age were excluded from this analysis). In B * represents $p<0.05$ (0.0376), and ** represents $p<0.01$ (0.0013), comparing conditions indicated (ANOVA)




*Supplemental Figure S3. Polydisperse DEFINITY-like nanodroplets show increased contrast within the pancreas prior to T1D.* (A) Mean time-course of contrast signal in the pancreas of 10 week old female NOD mice with NDs made from poly-dispersed DEFINITY-like microbubbles. (B) Mean contrast signal averaged between 30-35 minutes following ND infusion with NDs made from poly-dispersed DEFINITY-like microbubbles. Dashed lines in A represent 95% CI.




*Supplemental Figure S4. Histological assessment of non-vaporized DiO labelled nanodroplets in the islets in T1D.* (A) Scatterplot of nanodroplet-DiO fluorescent coverage of islets within the pancreas against the mean insulitis score within the pancreas, for NOD mice without ultrasonic vaporization. (B) Mean nanodroplet-DiO fluorescent coverage of non-vaporized islets within the pancreas that show insulitis scores of 0, 1, 2, or 3. (C) Mean nanodroplet-DiO fluorescent coverage in the islet, near islet regions and exocrine tissue in 10-week-old female NOD mice where nanodroplets were not vaporized. Error bars in B,C represent s.e.m. Trend line in A indicates linear regression with 95% confidence intervals. ANOVA was performed in B. A mixed-effects model was used to assess the statistical significance and generate the regression in A. Data in B represents 36 islets from 4 NOD mice.