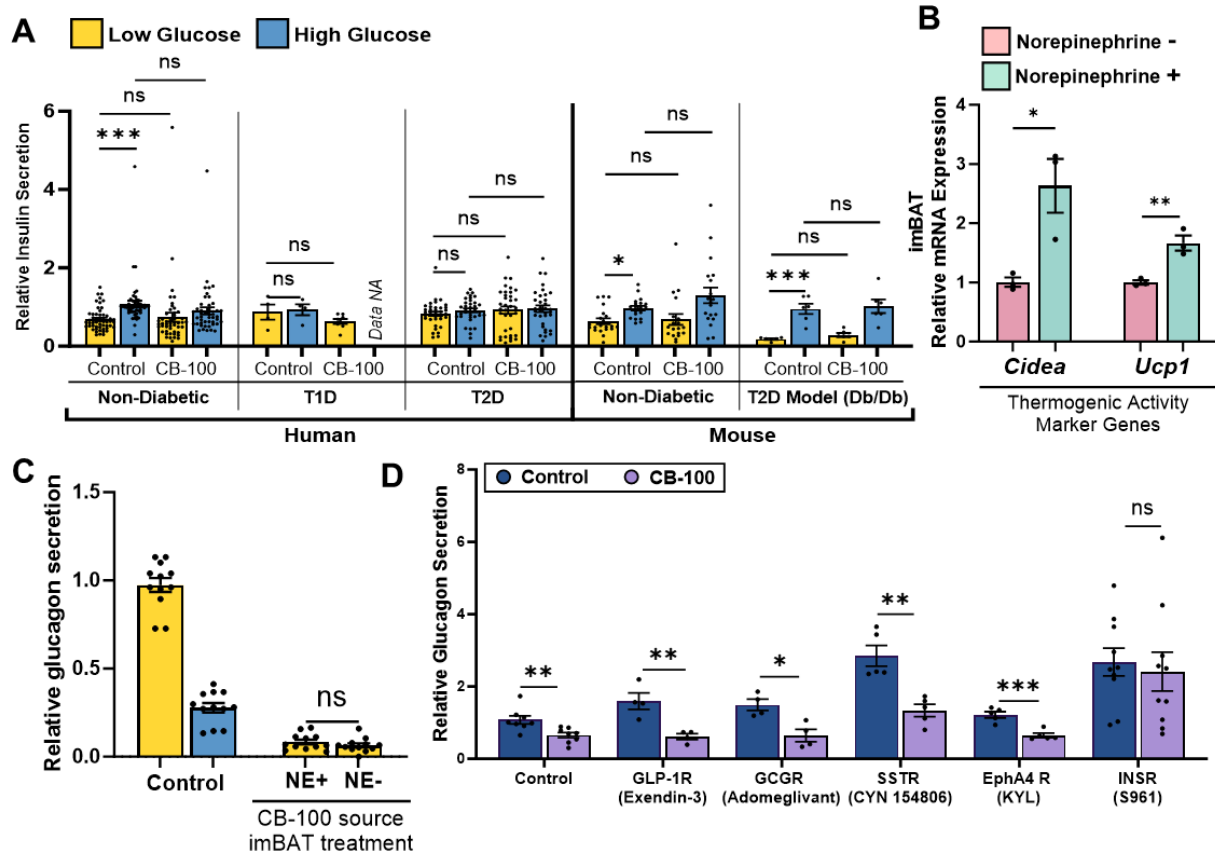


## **Supplementary Figures S1-S9**



**Supplementary Figure S1. Further characterization of large brown adipocyte-secreted proteins (CB-100). Related to Figure 1 and Figure 6.**

**(A)** Relative insulin secretion measured in low (yellow, 1mM) and high (teal, 11mM) glucose conditions in both human (non-diabetic n=16, T1D n=1, T2D n=7, see Supplementary Table S1 for more detail) and mouse (non-diabetic n=30, T1D model n=5, T2D model n=3) pancreatic islets.

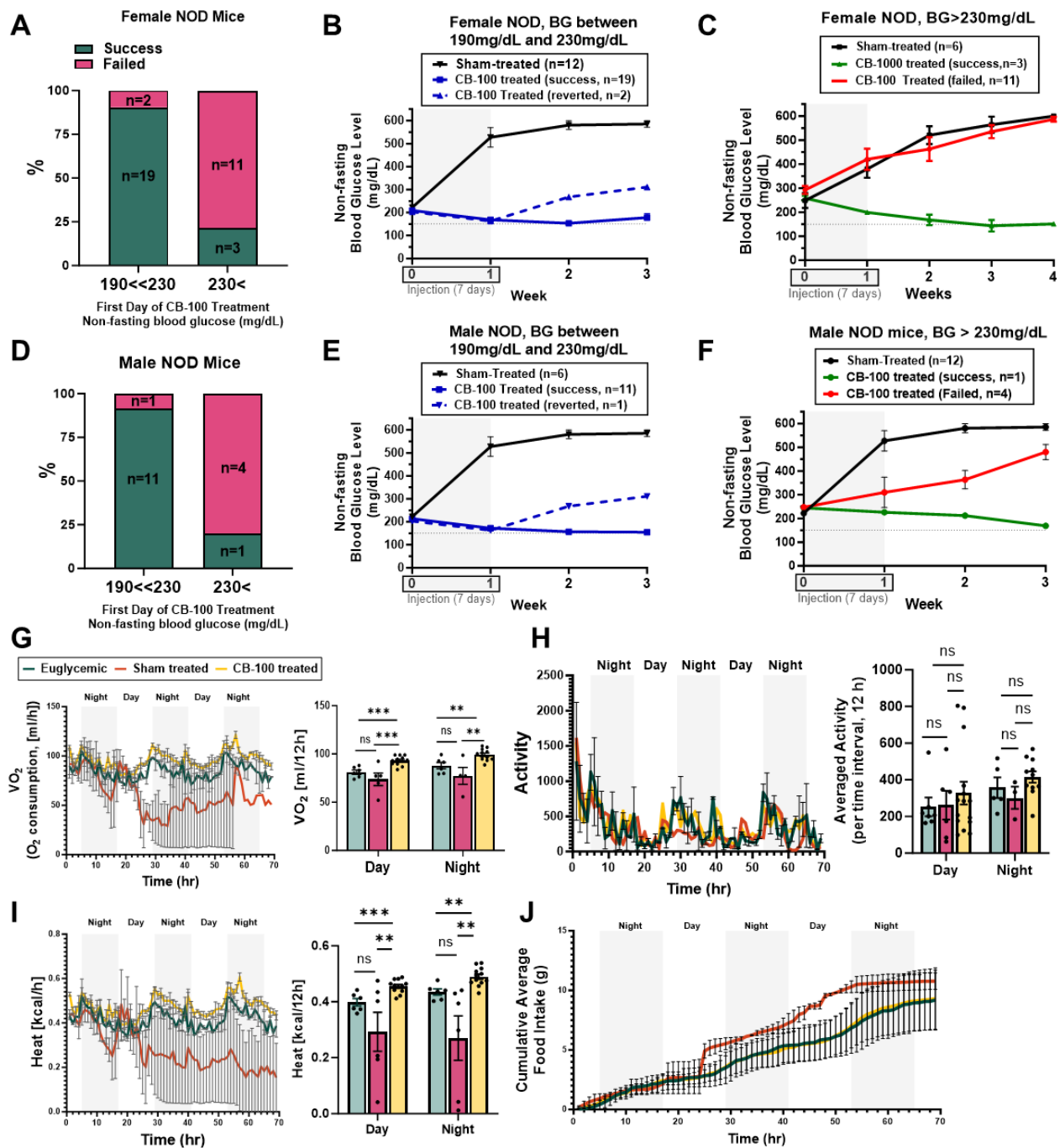
**(B)** Relative mRNA expression of *Cidea* and *Ucp1* of imBAT without (pink) or with (green) norepinephrine (NE) stimulation (10μM, 16 hours).

(C) Relative glucagon secretion in low (yellow, 1mM) and high (teal, 11mM) glucose conditions in pancreatic islets from non-diabetic mice (n=4) with CB-100 (26.5μg/mL) collected from imBAT without (NE-) or with (NE+) norepinephrine stimulation (10μM, 16 hours).

**(D)** Relative glucagon secretion measured without (navy blue) or with CB-100 (purple, 5 $\mu$ g/mL) in low (1mM) glucose condition with GLP-1 receptor (GLP-1R) antagonist (Exendin-3, 50nM) or glucagon receptor (GCGR) antagonist (Adomeglivant, 1.5 $\mu$ M) or Somatostatin receptor (SSTR) antagonist (CYN154806, 25nM) or Ephrin A4 receptor (EphA4R) antagonist (KYL, 50 $\mu$ M), insulin receptor (INSR) antagonist (S961, 1 $\mu$ M) in non-diabetic mouse pancreatic islets (n=3). Related to Figure 6B.

Data are presented as Mean  $\pm$  SEM. Statistical significance was determined using an unpaired t-test.

Groups compared for statistical analysis are indicated by the line. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , ns ( $p > 0.05$ ).



**Supplementary Figure S2. Detailed characterization of CB-100 treated NOD mice. Related to Figure 2 and Figure 4**

**(A&D)** Comparison of CB-100 treatment success (green) and failed (red) percentage of early (female: n=21, male: n=12, blood glucose (BG) between 190mg/dL and 230mg/dL) and late-stage (female: n=14, male: n=5, >230mg/dL) of hyperglycemia in **(A)** female and **(D)** male NOD mice. Related to Figure 2B.

**(B)** Weekly non-fasting blood glucose levels following one round of seven days injection of CB-100 (subcutaneous, 1.5 mg/kg BW; blue, success n=19 & reverted n=2) and sham (KRBH, black, n = 12) female NOD mice in late hyperglycemia (18-22 weeks old, BG between 190mg/dL and 230mg/dL). Related to Figure 2B.

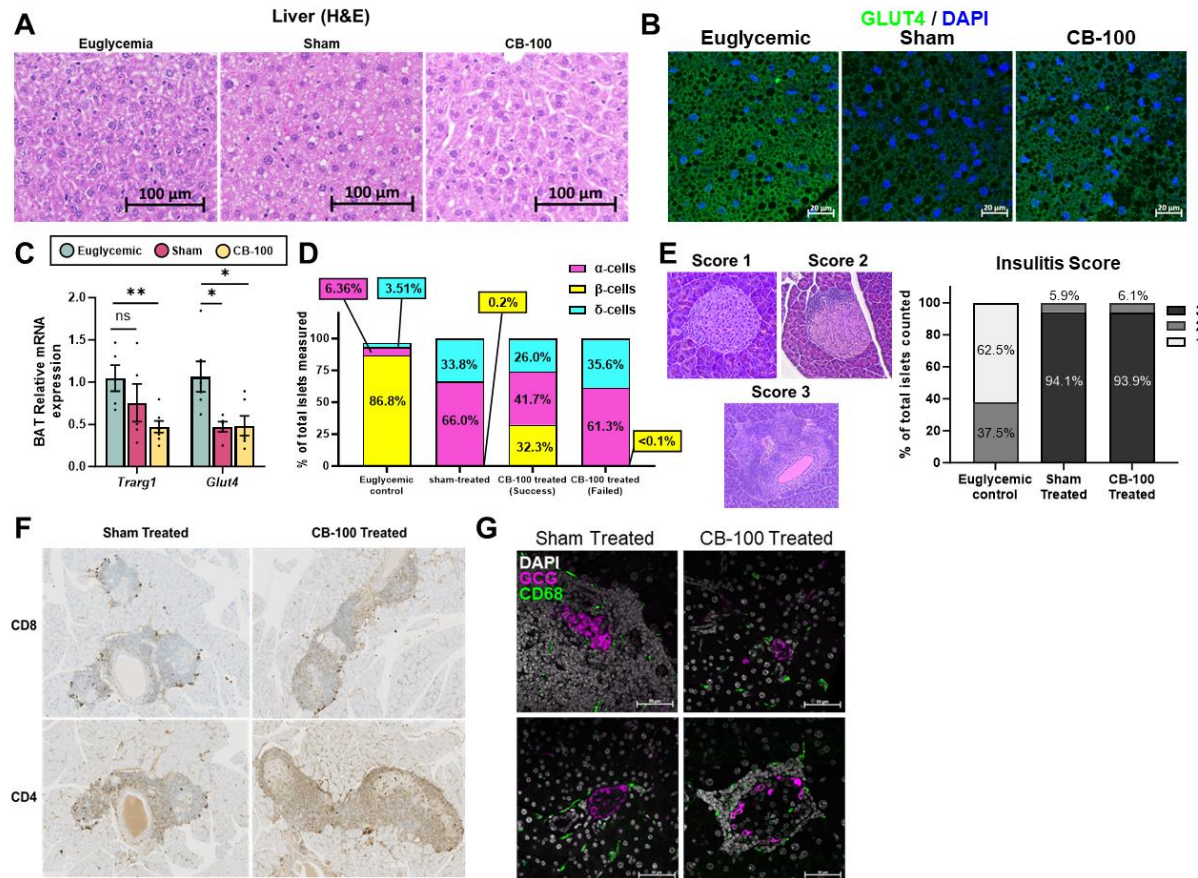
**(C)** Weekly non-fasting blood glucose levels following one round of seven days injection of CB-100 (subcutaneous, 1.5 mg/kg BW; Success, green, n = 3 & failed, red, n=11) and sham (KRBH, black, n = 6) female NOD mice in late hyperglycemia (18-22 weeks old, BG >230mg/dL). Related to Figure 2B.

**(E)** Weekly non-fasting blood glucose levels following one round of seven days injection of CB-100 (subcutaneous, 1.5 mg/kg BW; blue, success n=11 & reverted n=1) and sham (KRBH, black, n = 6) male NOD mice in late hyperglycemia (18-22 weeks old, BG between 190mg/dL and 230mg/dL). Related to Figure 2B.

**(F)** Weekly non-fasting blood glucose levels following one round of seven days injection of CB-100 (subcutaneous, 1.5 mg/kg BW; green, success n=1 & failed, red, n=4) and sham (KRBH, black, n = 12) male NOD mice in late hyperglycemia (18-22 weeks old, BG >230mg/dL). Related to Figure 2B.

**(G-J)** Metabolic cages measured and averaged **(G)** VO<sub>2</sub> consumption (ml/h), **(H)** activity, **(I)** heat (kcal/h), and **(J)** cumulative food intake (g) from euglycemic control (yellow, n = 2), sham (red, n = 12), and CB-100 treated (brown, n = 4) NOD mice. Related to Figure 2C.

Data are presented as Mean ± SEM. Statistical significance was determined using an unpaired t-test. Groups compared for statistical analysis are indicated by the line. \*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001, ns (p > 0.05).



**Supplementary Figure S3. Detailed analysis of *in vivo* tissues from CB-100 treated NOD mice. Related to Figure 2 and Figure 4.**

(A) H&E Staining of liver sections from euglycemic control, sham treated, and CB-100 treated NOD mice. Scale bar: 100  $\mu$ m. Related to Figure 4.

(B) Immunofluorescence of GLUT4 (green) and nuclei (blue) in brown adipose tissue of euglycemic control, sham treated, and CB-100 treated NOD mice. Scale bar: 100  $\mu$ m. Related to Figure 4.

(C) Relative expression levels of trafficking Regulator of GLUT4 1 (*Trarg1*) and glucose transporter type 4 (*Glut4*) in BAT of euglycemic control (green, n=5), sham treated (red, n=5) and CB-100 treated (yellow, n=7) NOD mice. Related to Figure 4.

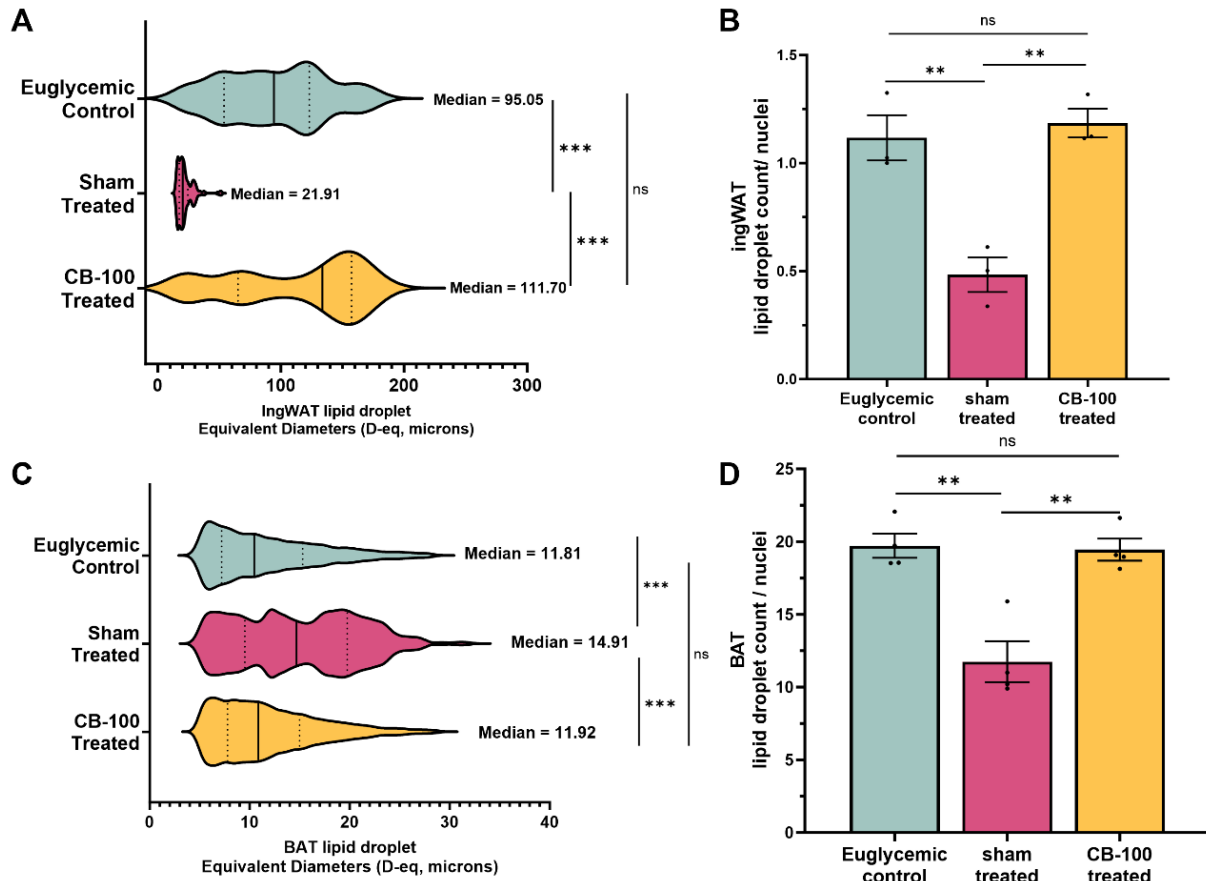
(D) Quantification of immunofluorescent stained insulin (yellow), glucagon (magenta), and somatostatin (cyan) area over whole islet area in euglycemic control (n=3, 12 islets), sham treated (n=4, 5 islets), Successful CB-100 treated (n=4, 10 islets), Failed CB-100 treated (n=2, 5 islets) NOD mice. Related to Figure 2J.

(E) Insulinitis score in euglycemic control (n=3, 19 islets), sham treated (n=2, 8 islets), CB-100 treated (1.5mg/kg BW, n=4, 33 islets) NOD mice. Representative image for insulitis score; 1: peri-insulitis (light grey), 2: <50% of infiltrative insulitis (grey), 3: >50% infiltrative insulitis (dark grey), with percentage of each insulitis score of total islets scored.

(F) Representative pancreatic immunohistochemistry staining of regulatory T-cells (CD8 and CD4) in sham treated and CB-100 treated NOD mice.

(G) Representative pancreatic immunofluorescent staining of nuclei (white, DAPI), glucagon (magenta), and macrophages (green, CD68) in sham treated and CB-100 treated NOD mice. Scale bar 50  $\mu$ m.

Data are presented as Mean  $\pm$  SEM. Statistical significance was determined using an unpaired t-test. Groups compared for statistical analysis are indicated by the line. \*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001, ns (p > 0.05).

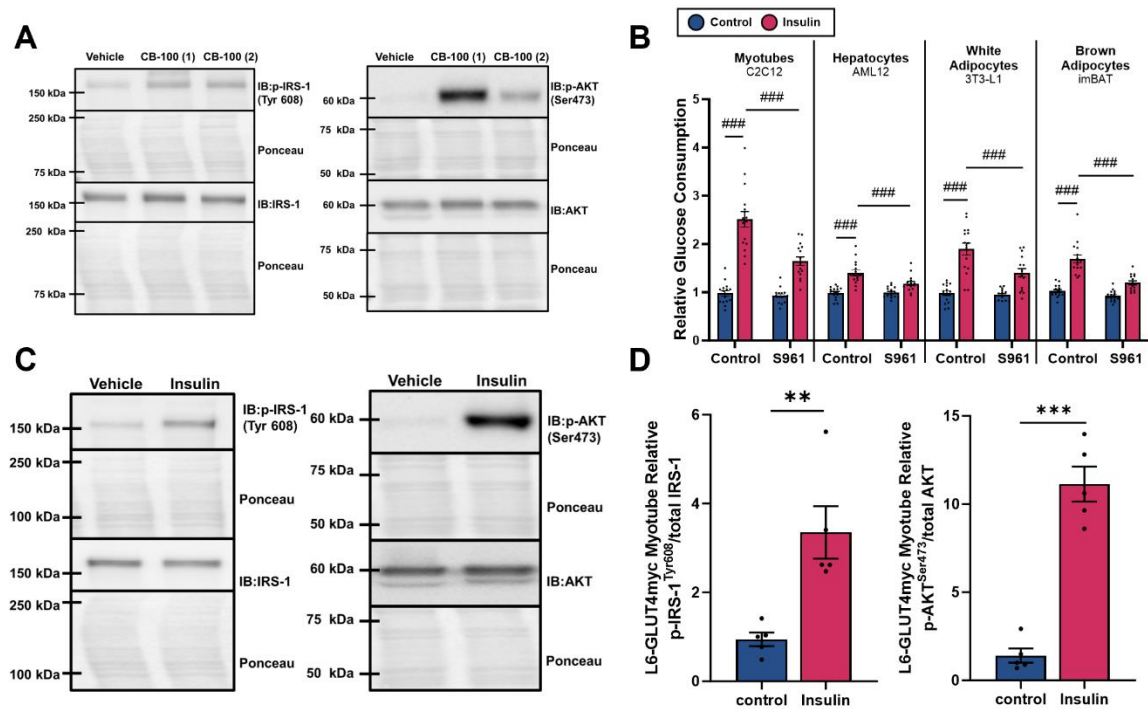


**Supplementary Figure S4. Detailed adipose tissue lipid droplet analysis. Related to Figure 3H and 3N.**

(A&C) Equivalent diameter (D-eq, microns) of lipid droplet in (A) ingWAT and (C) BAT in euglycemic control (green, n=4), sham treated (red, n=3), and CB-100 treated (yellow, n=4) female NOD mice.

(B&D) Number of lipid droplet, normalized to cell count (nuclei count), in (B) ingWAT and (D) BAT in euglycemic control (green, n=4), sham treated (red, n=3), and CB-100 treated (yellow, n=4) female NOD mice.

Data are presented as Mean  $\pm$  SEM. Statistical significance was determined using an unpaired t-test. Groups compared for statistical analysis are indicated by the line. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , ns ( $p > 0.05$ ).



**Supplementary Figure S5. Validation of insulin-responsiveness of the myotubes (L6.GLUT4myc and C2C12), hepatocytes, and adipocytes. Related to Figure 4 G.**

**(A)** Immunoblot (IB) for p-IRS-1(Tyr<sup>612</sup>) and total IRS-1, and p-AKT(Ser<sup>463</sup>) and total AKT normalized with ponceau staining in fully differentiated L6-GLUT4myc myotube cells untreated (Vehicle) and treated with CB-100 (10μg/mL, 16 hours) and spiked in insulin (10nM, 5minutes).

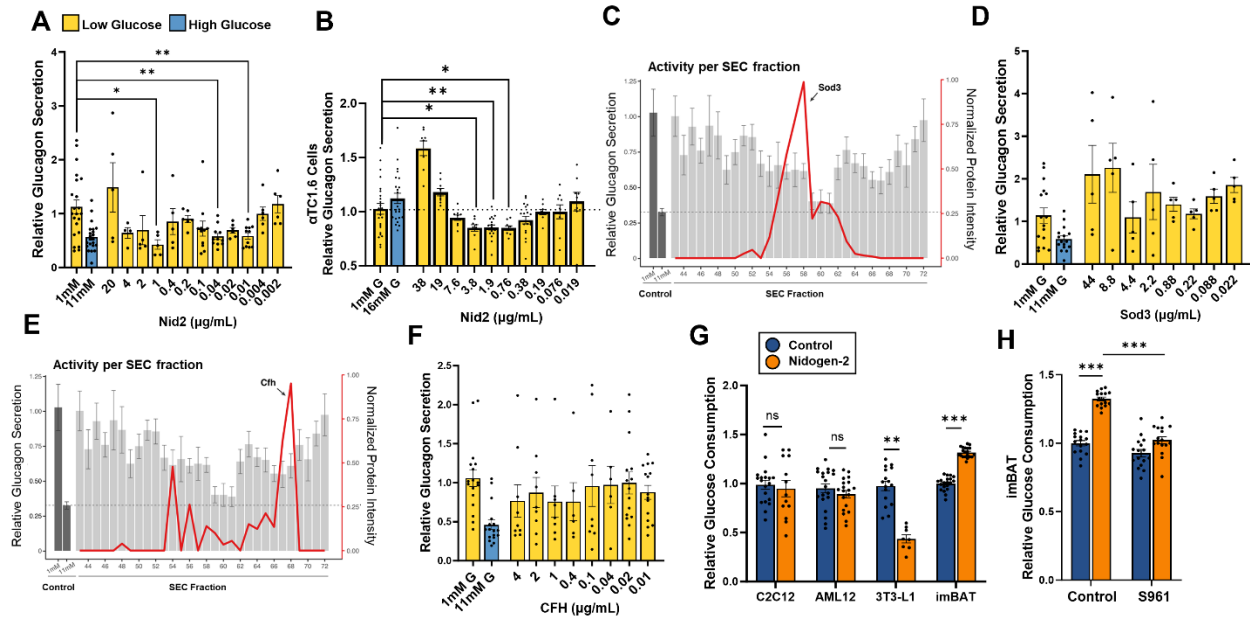
**(B)** Relative glucose consumption in myotubes (C2C12), hepatocytes (AML12), white adipocytes (3T3-L1), and brown adipocytes (imBAT) treated without (control, navy blue) or with insulin (red, 10nM) and in the absence (control) or presence of S961 (200 nM) for 24 hours.

**(C)** IB for p-IRS-1(Tyr<sup>612</sup>) and total IRS-1, and p-AKT(Ser<sup>463</sup>) and total AKT normalized with Ponceau staining in fully differentiated L6-GLUT4myc myotube cells untreated (Vehicle) and treated with insulin (10nM, 16~18 hours), spiked in insulin (10nM, 5 minutes).

**(D)** IB analysis of p-IRS-1<sup>Tyr606</sup>/total-IRS-1 and p-AKT<sup>Ser473</sup>/total-AKT from L6-GLUT4myc myotube untreated (navy blue, control) or treated with insulin (red, 10nM, 16~18 hours) then spiked in insulin (10nM, 5minutes).

Data are presented as mean ± SEM. Statistical significance was assessed using an unpaired t-test (indicated with \*) or two-way ANOVA (indicated with #; Figure S5B), with significance denoted as follows: \* or # p < 0.05, \*\* or ## p < 0.01, \*\*\* or ### p < 0.001, ns (p > 0.05). The groups compared for statistical analysis are connected by lines.





**Supplementary Figure S6. Candidate protein testing and further investigation of the effects of nidogen-2 on islets secretion and *in vitro* glucose uptake. Related to Figure 5 and 6.**

**(A, D & F)** Relative glucagon secretion measured in low (yellow, 1mM) and high (teal, 11mM) glucose conditions in non-diabetic mice (n=6) without or with **(A)** nidogen-2 (Nid2) **(D)** superoxide dismutase 3 (Sod3) or **(F)** complement factor H (CFH). Related to Figure 5 B.

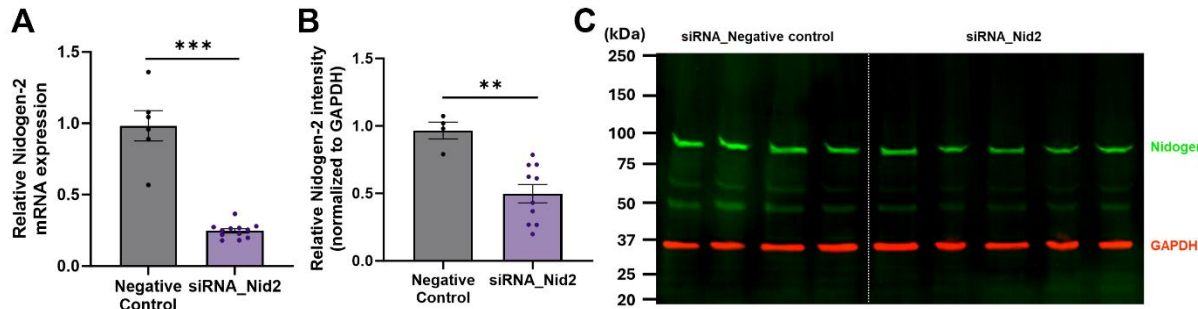
**(B)** Relative glucagon secretion measured in low (yellow, 1mM) and high (teal, 11mM) glucose conditions in αTC1.6 cells without or with nidogen-2 (Nid2).

**(C&E)** Glucagon secretion level at low glucose (1mM) condition (grey bar) plotting with protein intensity (red) of **(C)** superoxide dismutase 3 (Sod3) and **(E)** complement factor H (CFH) in each SEC fraction. Glucagon secretion of a control (1mM and 11mM) is indicated with the left two dark grey bars. The expected lowest glucagon secretion was determined with the glucagon secretion level at high glucose condition (11mM) and marked with the dotted line. Related to Figure 5B.

**(G)** Relative glucose consumption measured in C2C12, AML12, 3T3-L1, and imBAT cell line without (control, navy blue) or with nidogen-2 (orange, 1μg/mL) (16 hours). Related to Figure 5.

**(H)** Relative glucose consumption in imBAT treated without (control, navy blue) or with nidogen-2 (orange, 1μg/mL) and in the absence (control) or presence of S961 (200 nM) for 24 hours. Related to Figure 5 and 6.

Data are presented as Mean ± SEM. Statistical significance was determined using an unpaired t-test. Groups compared for statistical analysis are indicated by the line. \*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001, ns (p > 0.05).



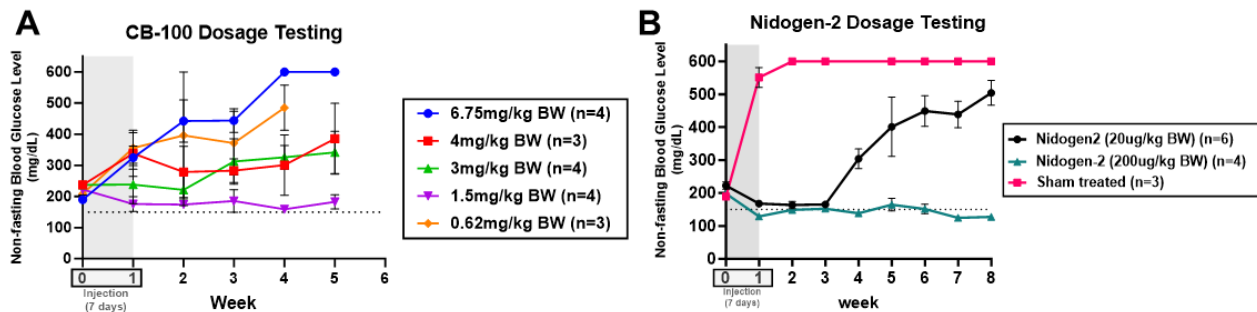
**Supplementary Figure S7. Validation of nidogen-2 knocked down in imBAT with siRNA. Related to Figure 5I.**

(A) Relative mRNA expression of nidogen-2 in imBAT with scramble siRNA negative control (grey) or siRNA nidogen-2 gene knock down (purple).

(B) Relative western blot analysis of nidogen-2 normalized with GAPDH in imBAT cell lysate with scramble siRNA negative control (grey) or siRNA nidogen-2 gene knock down (purple).

(C) Immunoblot for nidogen-2 (green) and GAPDH (red) in scramble siRNA negative control and nidogen-2 siRNA gene knocked down imBAT cell lysate.

Data are presented as Mean  $\pm$  SEM. Statistical significance was determined using an unpaired t-test. Groups compared for statistical analysis are indicated by the line. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , ns ( $p > 0.05$ ).



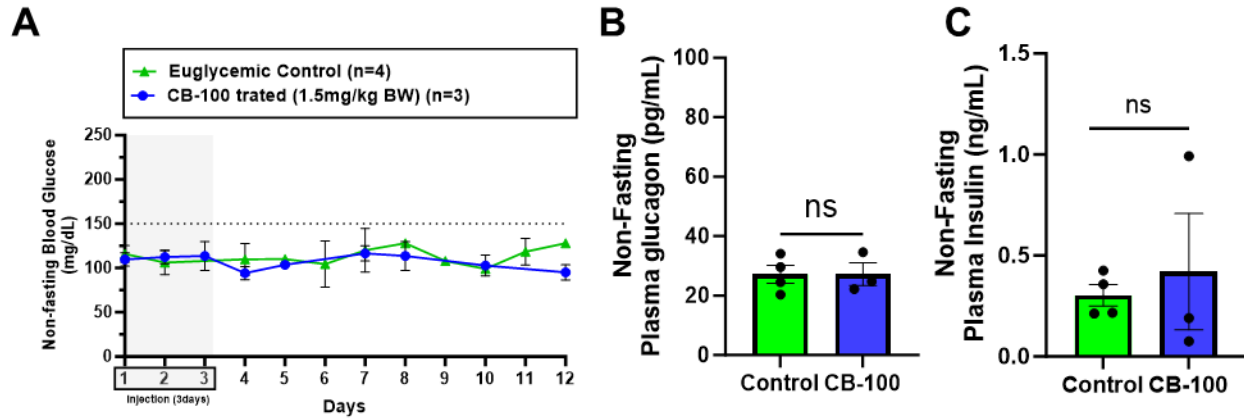
**Supplementary Figure S8. Optimizing CB-100 and Nidogen-2 dosages for Injection in NOD Mice. Related to Figure 2B and 5J.**

(A) Weekly non-fasting blood glucose monitoring of NOD mice with one round of 7 days subcutaneous CB-100 dosage injections (blue: 6.75mg/kg BW (n=4), red: 4mg/kg BW (n=3), green: 3mg/kg BW (n=4), purple: 1.5mg/kg BW (n=4), orange: 0.62mg/kg BW (n=3)). Normal non-fasting blood glucose level for NOD mice was indicated with a dotted line (150mg/dL).

(B) Weekly non-fasting blood glucose monitoring of NOD mice with one round of 7 days subcutaneous nidogen-2 injection (black: 20 $\mu$ g/kg BW (n=6), green: 200 $\mu$ g/kg BW (n=4)) or sham (KRBH, red, n=3). Normal non-fasting blood glucose level for NOD mice was indicated with a dotted line (150mg/dL).

Data are presented as Mean  $\pm$  SEM.





**Supplementary Figure S9. Effect of CB-100 injection to euglycemic NOD mice.**

(A) Daily blood glucose monitoring of euglycemic NOD mice in euglycemia (18-22 weeks old, BG below 150 mg/dL) without (green, n=4) or with 3 days of CB-100 injection (blue, subcutaneous, 1.5mg/kg BW, n=3).

(B&C) Non-fasting blood plasma (B) glucagon and (C) insulin measured in euglycemic control (green, n = 4) and CB-100 treated (blue, 1.5mg/kg BW, n=3) euglycemic NOD mice (female, 18-22 weeks old).

Data are presented as Mean  $\pm$  SEM. Statistical significance was determined using an unpaired t-test. Groups compared for statistical analysis are indicated by the line. \*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001, ns (p > 0.05).