ELECTRONIC SUPPLEMENTARY MATERIAL

METHODS

Islet isolation, treatments and adenoviral infection. Mouse islets were isolated (Lee 2009) and all treatments were performed in 22 mmol/l glucose. *Cholesterol loading*: islets were incubated in the presence of 2 mmol/l cholesterol in culture media for 8 days (Sigma, Oakville, ON). Media was refreshed every 48 h and fresh cholesterol was added. *miR-33a and ABCA1 overexpression*: islets were infected with empty vector control or GFP control or miR-33a (ABM, Richmond, BC) or ABCA1 adenovirus (Wellington 2003) at 100 multiplicity of infection (MOI) for 24 h in culture media, after which the virus was removed and fresh media was added and cultured for further 7 days with every 48 h media changes (Wijesekara 2012). *Palmitate treatment*: islets were treated with vehicle or 0.1 mmol/l palmitate (Sigma, Oakville, ON) in 0.5% BSA (Sigma, Oakville, ON)) for 48 h and subsequently infected with ABCA1 adenovirus (Wellington 2003) at 100 MOI for 24 h in culture media, after which, the virus was removed and fresh media was added in the presence of palmitate or 10 μmol/l mevastatin (Wijesekara 2012) (Sigma, Oakville, ON) was added on day 3 for up to 8 days in the presence of palmitate. Media was refreshed every 48 h and fresh mevastatin and palmitate were added.

Thioflavin S staining and confocal microscopy. Islets were labelled using anti-insulin (Dako, Burlington, ON) primary (1:100) and 0.5% thioflavin S (Sigma, Oakville, ON) or anti-guinea pig secondary (1:100). Images were acquired using an Olympus SP5 confocal microscope. 6-10 islets were randomly imaged and quantified per mouse/condition per experiment. All quantifications were performed using NIH Image J software. Intensities were calculated as integrated density –

(area of selected cell x mean fluorescence of background reading) and normalized to total islet area.

Western blotting. Total islet lysates were prepared as previously described (Wijesekara 2010). Lysates were resolved by 7.5% SDS-PAGE and immunoblotted with anti-ABCA1 (1:500, (Wellington 2002)) or anti-β-tubulin (1:1000; Sigma, Oakville, ON) antibodies. Immunoblots were scanned within the linear range of intensity and quantified using NIH Image J software.

Cholesterol measurement. Neutral sterol isolation and total cholesterol measurement were performed as described (Wijesekara 2012). Total protein was measured using DC Biorad Protein Assay Kit (Biorad, Mississauga, ON).

Glucose tolerance and plasma insulin and proinsulin measurement. Following a 4 h fast, glucose (1.5 g/kg body weight) was given by intraperitoneal injection and glucose measured from tail vein blood was measured at 0, 15, 30, 60, and 120 min using a glucometer. Blood was collected in EDTA-coated microvettes (Sarstedt, Numbrecht, Germany) at 0 and 15 min and plasma was isolated for insulin measurement using ultrasensitive insulin ELISA kit from ALPCO Diagnostics, Salem, NH, USA. Blood was collected at sacrifice and plasma insulin and proinsulin were measured using insulin and proinsulin ELISA kits from ALPCO Diagnostics, Salem, NH, USA.

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