Supplemental Material

Islet Isolation and Insulin Secretion Assay

Islets were isolated following ductal perfusion of collagenase and handpicked into RPMI media (10% FBS, 5 mM glucose) for overnight culture before experimental use. For the secretion assay, 3x10 islet aliquots per mouse were sequentially incubated in Krebs buffer at low glucose (2 mM, LG, 30 min), HG (high glucose, 16.7 mM, 30 min) and 30 mM KCl (15 min). Islet were collected into RIPA buffer (Cell Signaling Technology (CST)) with protease inhibitor cocktail (CST). Insulin secretion is presented as % of post-secretion islet insulin content. Total islet insulin content was calculated as the sum of secreted insulin + insulin content of the remaining islets, normalized to DNA.

Western Blot

25-80 ug of protein lysates in RIPA buffer (+ 1% SDS (Biorad) + protease/phosphatase inhibitor cocktails (CST)), as determined by Pierce BCA protein quantitation (ThermoScientific), were resolved by SDS-PAGE, transferred to PVDF membrane, blocked with 5% non-fat dry milk, and probed with primary antibodies, followed by HRP-conjugated secondary antibodies (Supplemental Table1). The blot was visualized with the SuperSignal West Pico PLUS (ThermoScientific), per manufacturer's protocol. Densitometry analysis was performed with NIH ImageJ software.

EM Imaging

For EM, the day after islet isolation, the islets were fixed in primary fixative (2% glutaraldehyde and 2.5% paraformaldehyde in 0.1M NaCac buffer) then embedded into Histogel, then fixed with secondary fixative (1% osmium tetroxide in 0.1M NaCac buffer), dehydrated, infiltrated with Embed-812 resin, then sectioned at thickness of 70-100 microns, stained with 3% aqueous uranyl

acetate and Sato's lead citrate stain. The images are observed on Philips CM-12 transmission electron microscope at 60kV. Imaging of EM were performed by UMN Imaging Center.

Rotarod Testing

Motor coordination of the eIF4G1f/f mice was assessed using a mouse rota-rod (Linton Instrumentation), provided by the UMN Mouse Behavior Core. Over the 5-min test, rod-speed was increased from 4 to 40 rpm and the latency of each mouse to fall was recorded. The procedure was performed for 4 days with 3 replicate runs per individual mouse each day. Inter-replicate interval was 30 min.