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Supplemental information

Inhibition of PHLPP1/2 phosphatases rescues pancreatic β-cells in diabetes

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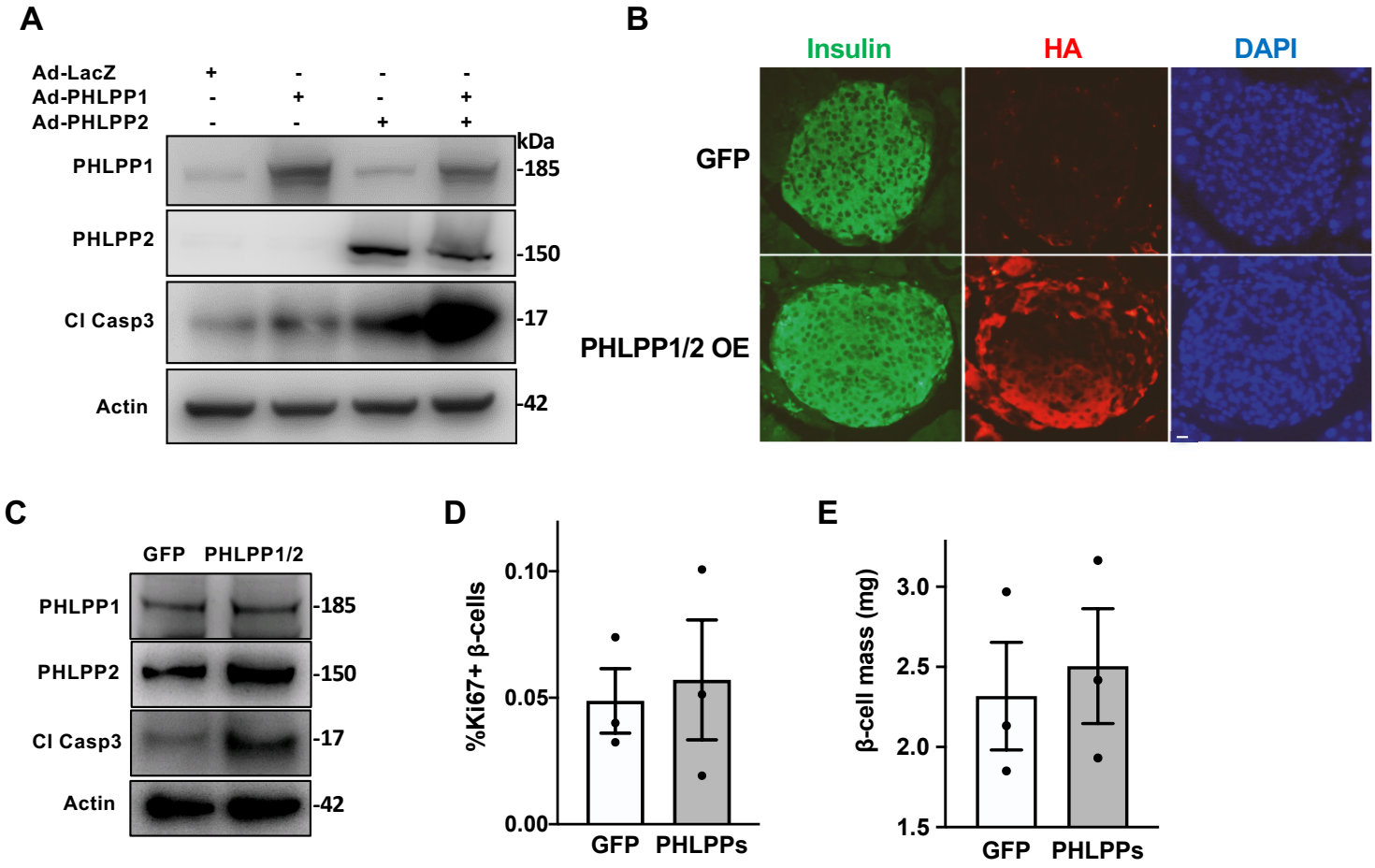

Figure S1. PHLPP1/2 overexpression induces β-cell apoptosis. Related to Figure 1. (A) Representative Western blots of INS-1E β-cells transduced with LacZ control or PHLPP1 and/or PHLPP2 adenoviruses for 48h (n=3). (B) Representative images of triple immunostaining for HA in red, insulin in green and DAPI in blue in pancreatic sections (n=3) (scale bar depicts 50µm), (C) representative Western blots of isolated islets (n=1 independent experiments; each pooled from 3 mice/ condition) and (D) analysis of Ki67+/insulin+ β-cells and (E) β-cell mass from *in vivo* plasmid/jetPEI GFP- or PHLPP1/2-transfected mice over 10 days (n=3). Data are expressed as means ± SEM

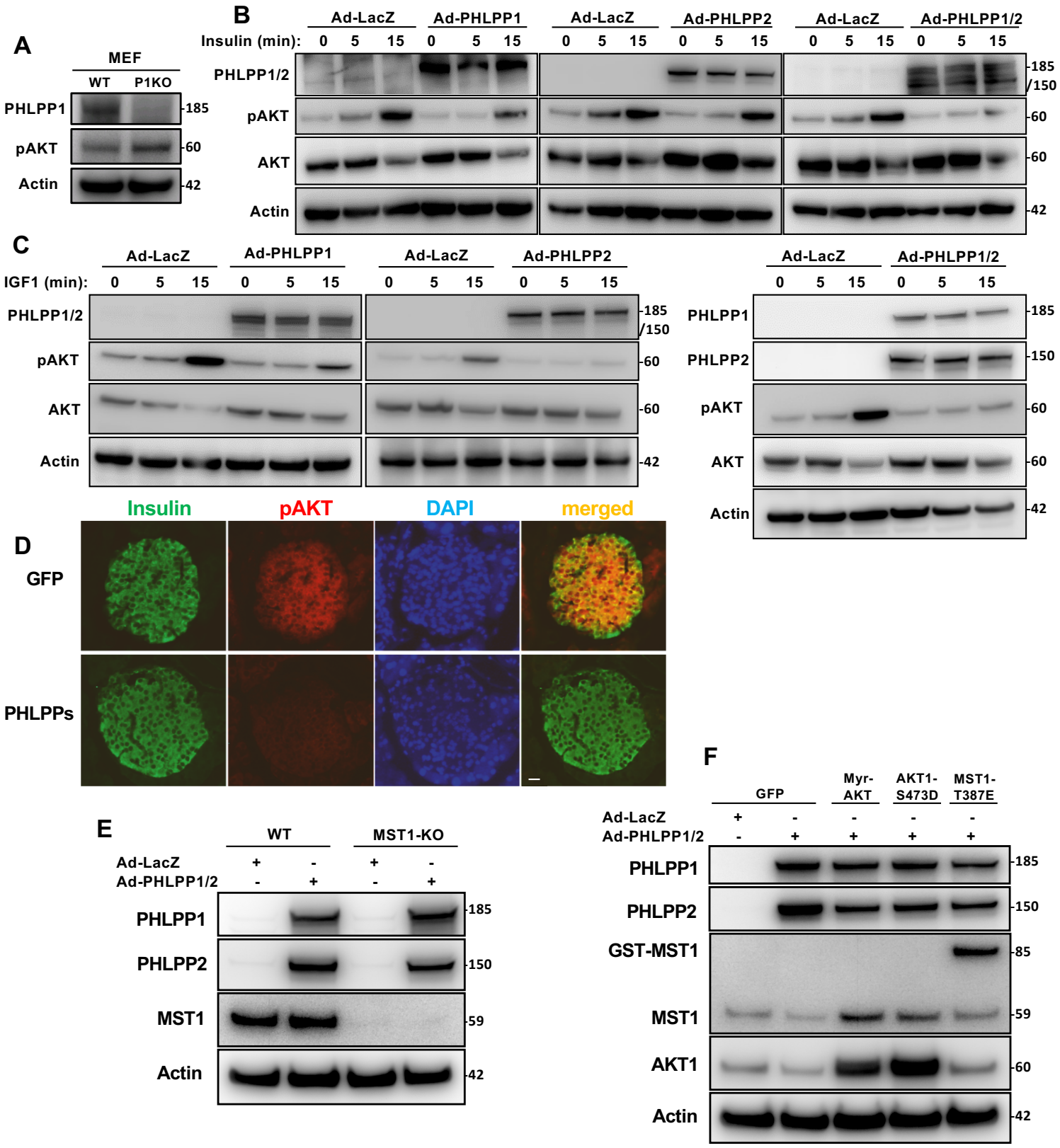

Figure S2. PHLPP-AKT-MST1 crosstalk. Related to Figure 2. (A) Western blots of WT and PHLPP1-KO MEF cells (n=5). (B,C) Representative Western blots of INS-1E β-cells transduced with LacZ control or PHLPP1 and/or PHLPP2 adenoviruses and then subjected to (B) insulin (100nM) or (C) IGF-I (100 μg/ml) stimulation (n=2-3). (D) Representative images of triple immunostaining for pAKT in red, insulin in green and DAPI in blue in pancreatic sections from GFP- or PHLPP1/2-transfected mice; scale bar depicts 50μm (n=3). (E) Representative Western blots of isolated islets from MST1-KO mice and their WT littermates after transduction with adenoviruses for LacZ (control) or PHLPP1/2 (n=1 independent experiments; each pooled from 4 mice/ condition). (F) Representative Western blots of isolated human islets overexpressed with adenoviruses for LacZ (control) or PHLPP1/2 and transfected with GFP (control) or Myr-AKT1 or HA-tagged AKT-S473D, or MST1-T387E plasmids (n=1).

Figure S2

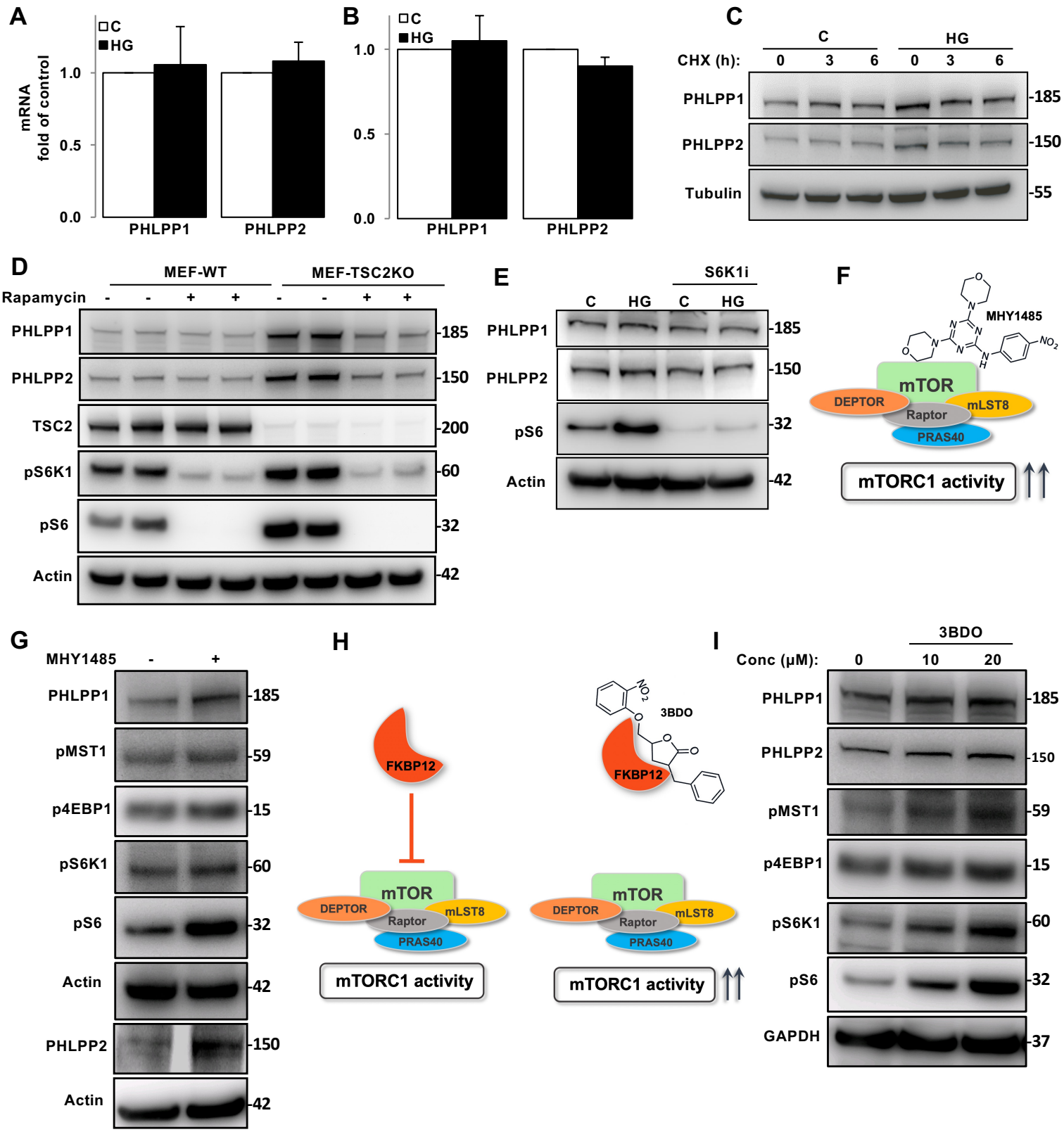

Figure S3. mTORC1 induces PHLPPs. Related to Figure 3. (A,B) qPCR for PHLPP1 or PHLPP2 mRNA expression in INS-1E β-cells (A; n=4) or isolated human islets (B; n=3) treated with high glucose (22mM) for 1 or 3 days respectively. Data are expressed as means ± SEM. (C) Representative Western blots of INS-1E β-cells treated with high glucose (22mM) for 2 days and then treated with 50 μg/ml cycloheximide (CHX) for different time points (n=1). (D) Representative Western blots of WT and TSC2KO-MEF cells left untreated or treated with 100 nM rapamycin for 1 day (WT-MEF: n=2; TSC2-KO-MEF: n=8). (E) Representative Western blots of INS-1E β-cells pre-treated with S6K1 inhibitor (10 μM) and cultured with 22.2 mM glucose for 2 days (n=3). (F) Scheme of MHY1485 action. (G) Representative Western blots of INS-1E β-cells treated with MHY1485 (25 μM) for 3 hours (n=2). (H) Scheme of 3BDO action. (I) Representative Western blots of INS-1E β-cells treated with 3BDO (10 or 20 μM) for 3 hours (n=1).

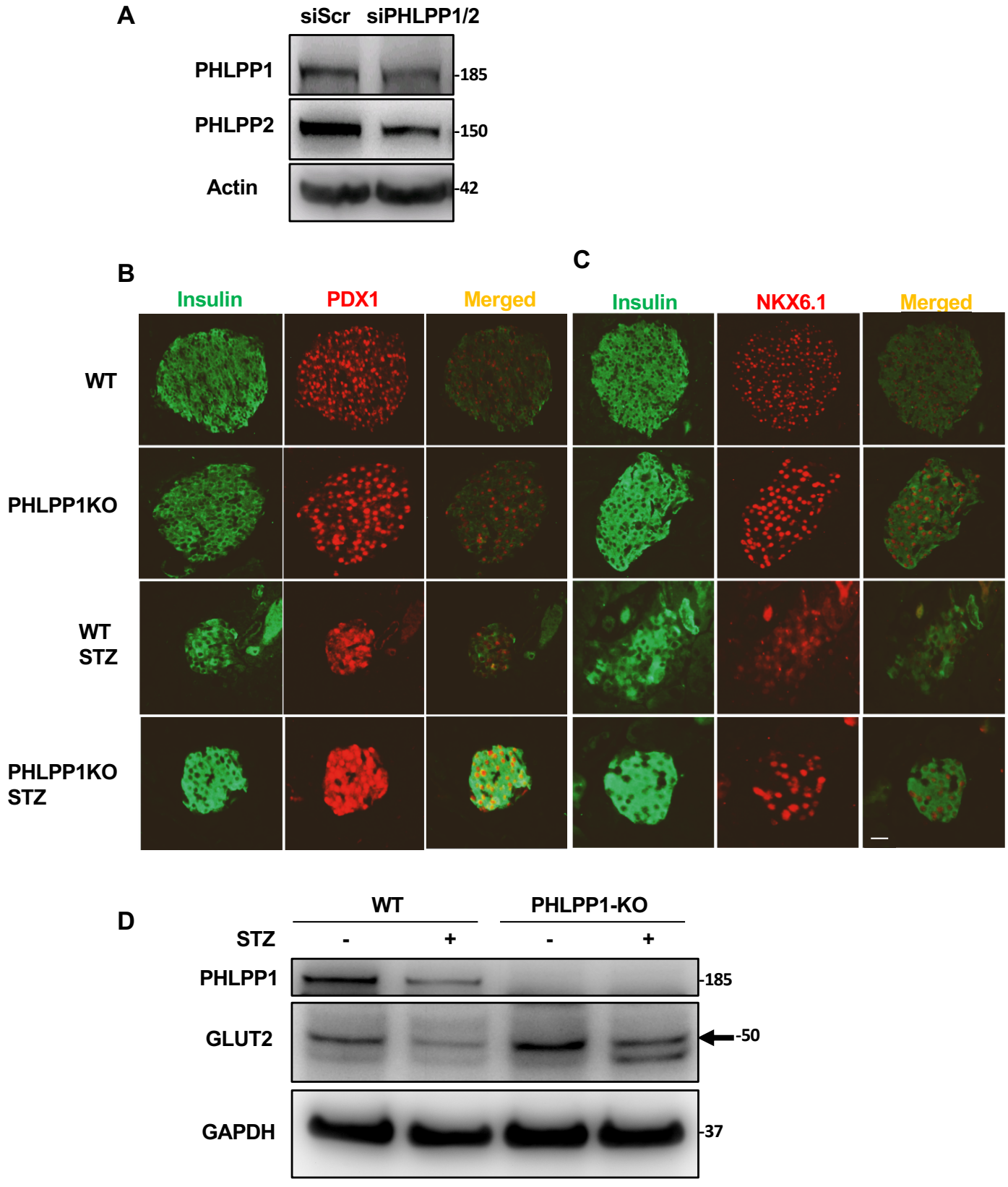

Figure S4. PHLPP1 deficiency restores PDX1, NKX6.1 and GLUT2 expression. Related to Figure 4. (A) Representative Western blot of isolated human islets transfected with PHLPP1 and PHLPP2 siRNA or control siScr for 3 days (n=4). (B,C) Representative double-stainings for PDX1 (red, B), or NKX6.1 (red, C), and insulin (green) are shown from PHLPP1-KO and WT controls mice injected with streptozotocin (STZ) or saline (n=6-7). Scale bar depicts 50μm. (D) Representative Western blot of isolated islets from PHLPP1-KO and WT control mice exposed to 1mM STZ in culture for 6h (n=2 independent experiments; each pooled from 3 mice/condition).

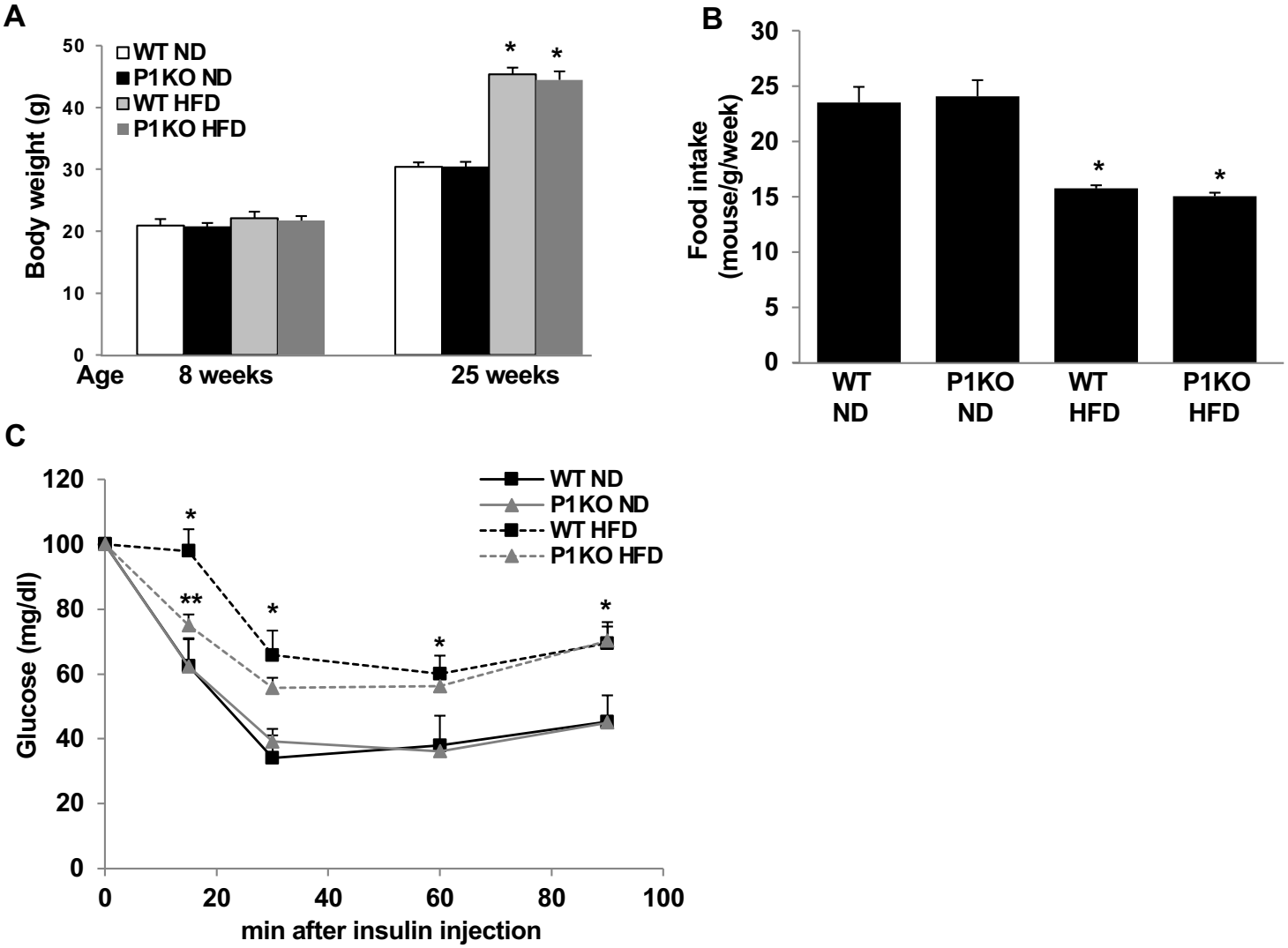

Figure S5. Characterization of ND- or HFD-fed PHLPP1-KO mice. Related to Figure 5. (A-C) PHLPP1-KO and WT controls mice were fed a normal (ND) or high fat/ high sucrose diet ("Surwit"; HFD) for 17 weeks. (A,B) Body weight and average weekly food intake/mouse (n=7-15). (C) Intraperitoneal insulin tolerance tests (ipITT) with 0.75IU/kg BW insulin (n=7-22). Data are expressed as means ± SEM. *p<0.05 WT- or PHLPP1KO-HFD compared to WT- or PHLPP1KO-ND mice. **p<0.05 PHLPP1-KO-HFD compared to WT-HFD mice.

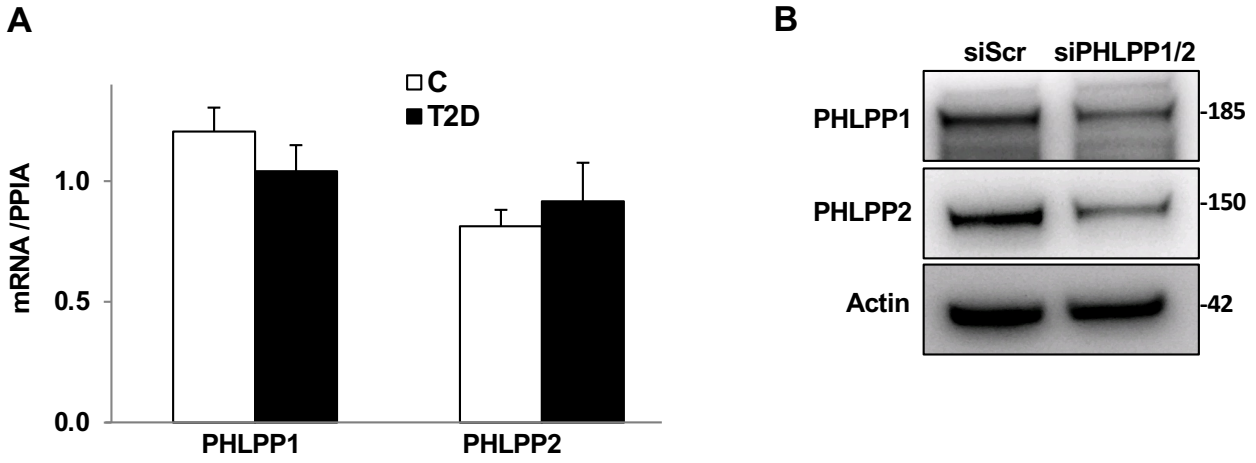

Figure S6. PHLPP1/2 expression and silencing in isolated human T2D islets. Related to Figure 6. (A) qPCR for PHLPP1 or PHLPP2 mRNA expression in human islets isolated from nondiabetic (n=24) or individuals with T2D (n=7) normalized to cyclophilin. Data are expressed as means ± SEM (B) Representative Western blots of isolated human islets from patients with T2D transfected with PHLPP1 and PHLPP2 siRNA or control siScr for 2 days (n=2).