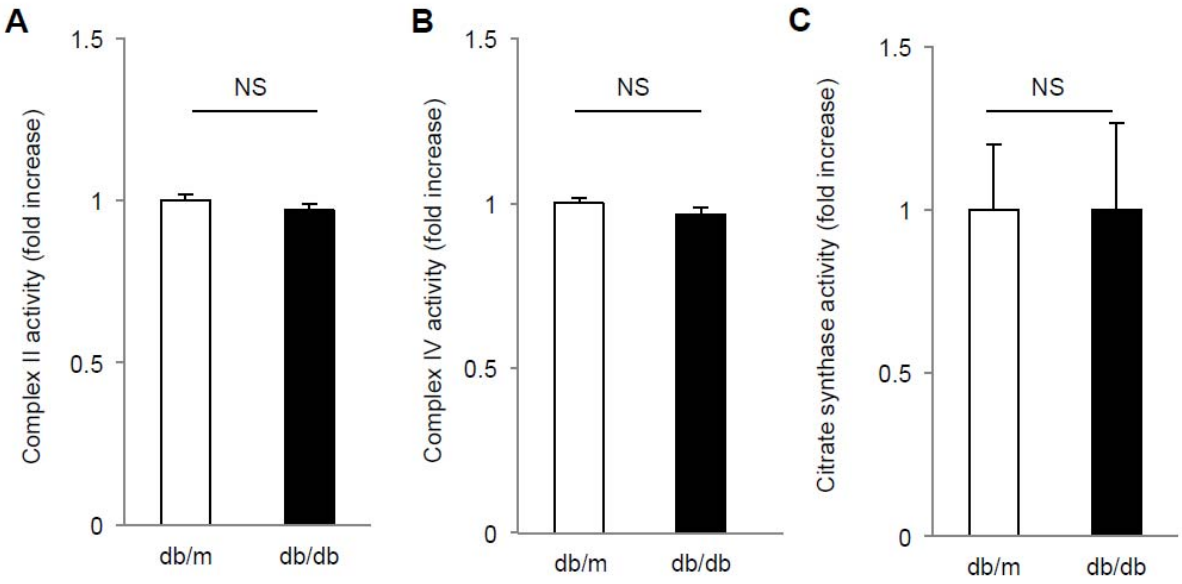
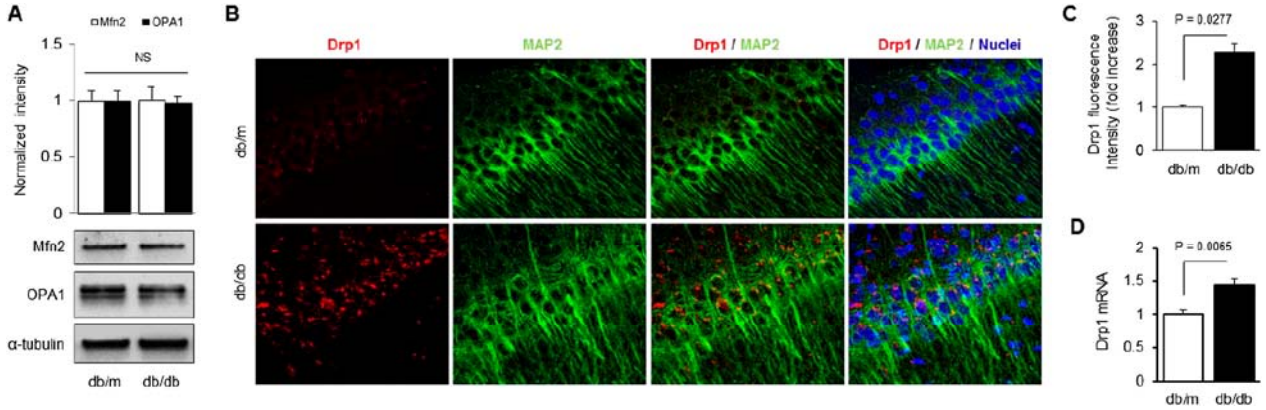
SUPPLEMENTARY DATA

Supplementary Figure 1. Complex II (**A**), Complex IV (**B**), and citrate synthase activity (**C**) were measured in hippocampal lysates from db/m and db/db mice. There were no significant differences in enzymatic activity between db/db and db/m control hippocampus. NS = non-significance, P > 0.05. N = 10 animals per group.

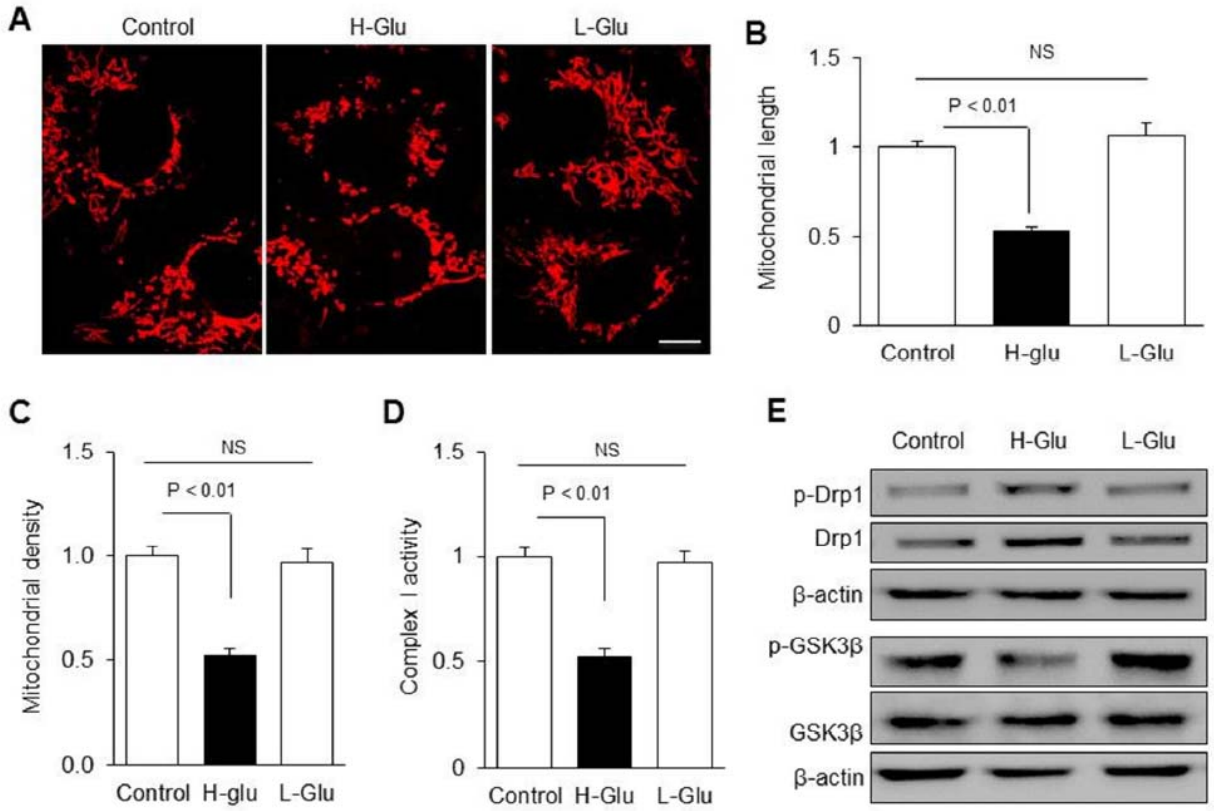


Supplementary Figure 2. Diabetes-induced changes in proteins associated with mitochondrial dynamics. (**A**) Western blots showed no significant alterations in Mfn2 and OPA1 levels in diabetes-affected hippocampus when compared to that of db/m control mice. (**B**) Immunostaining for Drp1 (red), MAP2 (green), and nuclei (blue) in db/m and db/db hippocampal sections. (**C**) Quantification of Drp1 immunofluorescence intensity in hippocampal CA1 region demonstrated an increase in Drp1 in diabetes-affected hippocampus. (D) Quantitative real-time PCR revealed increased Drp1 mRNA levels in db/db mice compared to that in db/m control mice. Scale bar = 50 μm. N = 4-6 animals per group.



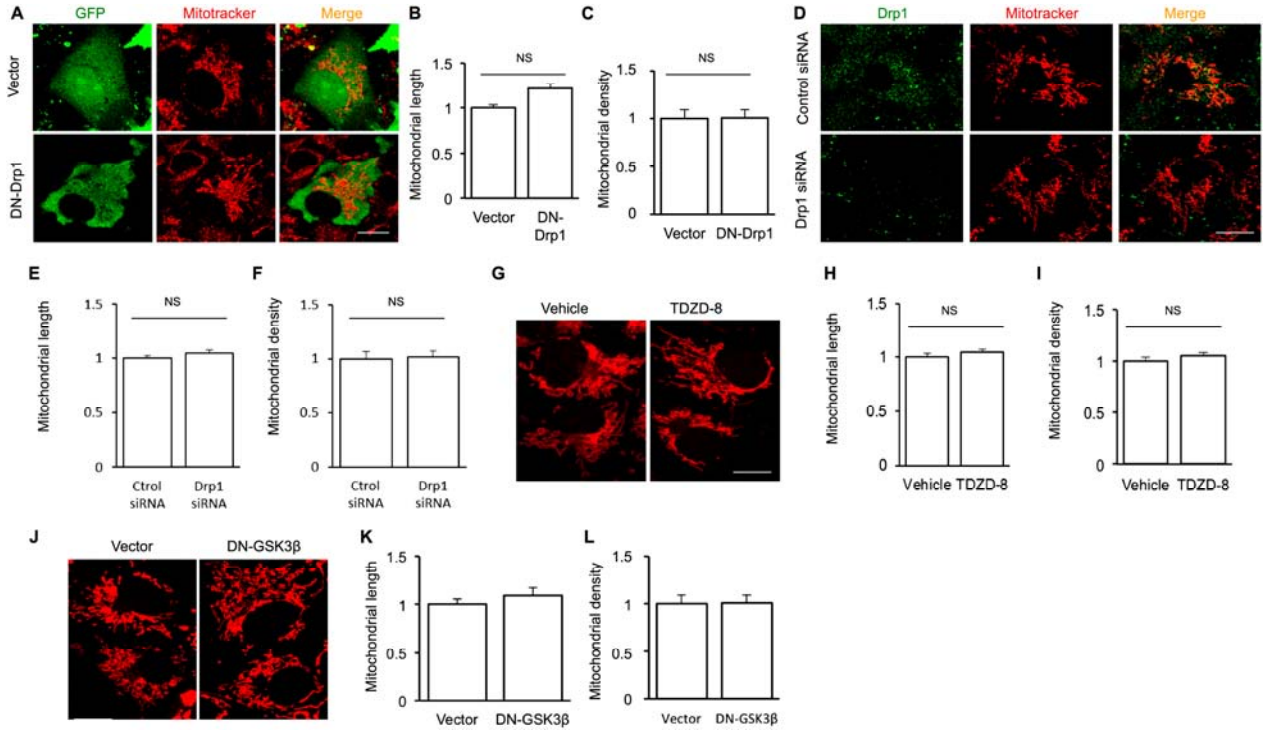
SUPPLEMENTARY DATA

Supplementary Figure 3. Non-metabolizable sugar L-glucose did not change mitochondrial morphology and function as well as GSK/Drp1 signaling. Effect of normal glucose (5.5 mM; control), high glucose (50 mM, H-Glu) or normal glucose (5.5 mM) plus L-glucose (same osmolality level as 50 mM glucose) exposure on mitochondrial morphology (**A**),length (**B**), density (**C**), and complex I activity (**D**) were measured on SK cells. **E**) Densitometry of immunoreactive bands of the indicated groups for p-Drp1, Drp1, p-GSK3β, and GSK3β. Scale bar = 10 μm. H-Glu: high glucose. L-Glu: L-glucose. NS: non-significance. N = 4 wells from 2 independent experiments.



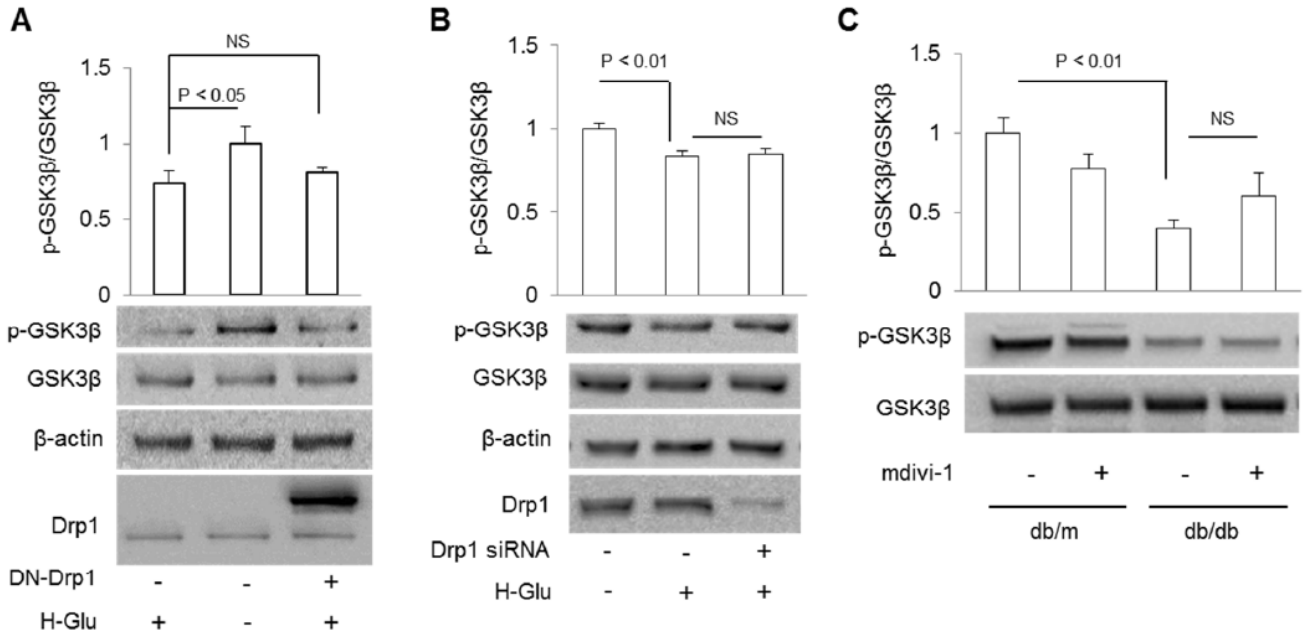
SUPPLEMENTARY DATA

Supplementary Figure 4. Modulations of GSK3β/Drp1 signaling have no significant effects on mitochondria morphology in SK cultured cells under normal glucose condition. **A-C**) Representative images (**A**) for mitotracker red staining of vector (up panel) or DN-Drp1 plasmids (bottom panel), and Measurement of mitochondrial length (**B**) and density (**C**) in the indicated groups of cells. **D-F**) Representative images for Drp1 immunostaining (left, green), mitotracker red (middle, red), and merged (right) from control siRNA (upper panel) or Drp1 siRNA (lower panel) transfected SK cells (**D**). Mitochondrial length (**E**) and density (**F**) were measured in cells transfected with the control siRNA or Drp1 siRNA. **G-I**) Treatment of TDZD8 (5 μM for 1 h) did not affect mitochondrial morphology. Representative images of mitotracker red (**G**), Measurement of mitochondrial length (**H**) and density (**I**) are shown in the indicated groups of cells. **J-L**) Transfection with vector or DN-GSK3β plasmids had no effect on mitochondrial morphology. Representative images of mitotracker red (**J**), and Measurement of mitochondrial length (**K**) and density (**L**) are shown in the indicated groups of cells. Scale bar = 10 μm. N ≥ 20 cells per group from 2 independent experiments. NS: non-significance.



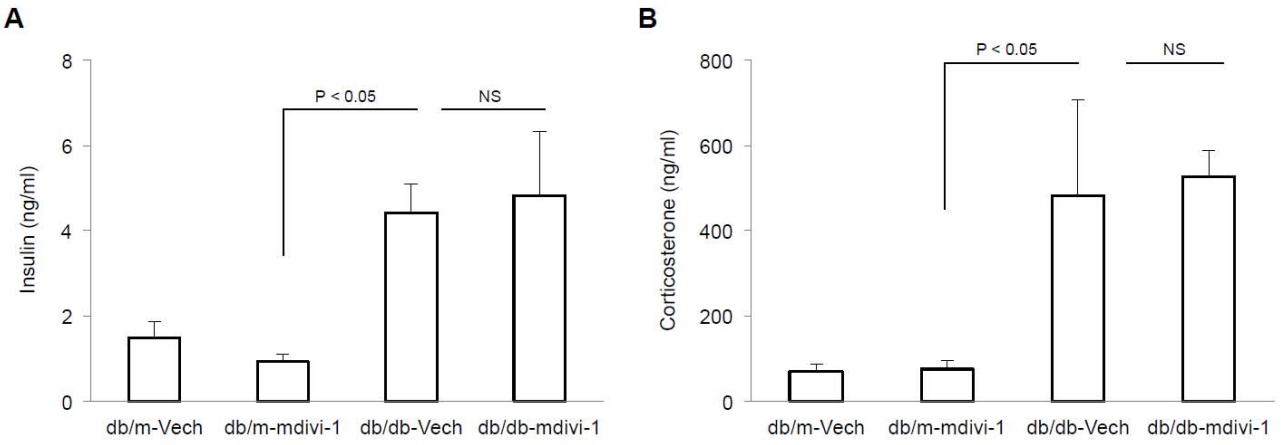
SUPPLEMENTARY DATA

Supplementary Figure 5. Immunoblots for GSK3β and phospho-GSK3β from *in vitro* and *in vivo* samples. **A-B**) Densitometry of immunoreactive bands for GSK3β and p-ser9 GSK3β. SK cells transfected with dominant negative Drp1 plasmid (DN-Drp1, +), vector alone (-) (A), control siRNA (-) (B), (**A**) or Drp1 siRNA (+) (**B**) had no significant effects on GSK3β phosphorylation/expression under diabetic condition. **C**) Immunoblots indicated that administration of mdivi-1 (+) failed to alter levels of p-ser9 GSK3β/GSK3β in hippocampus of the indicated groups of animals compared to vehicle (-) treated group. NS: non-significance. N = 6 wells from 3 independent experiments or N = 6 animals per group for immunoblots.



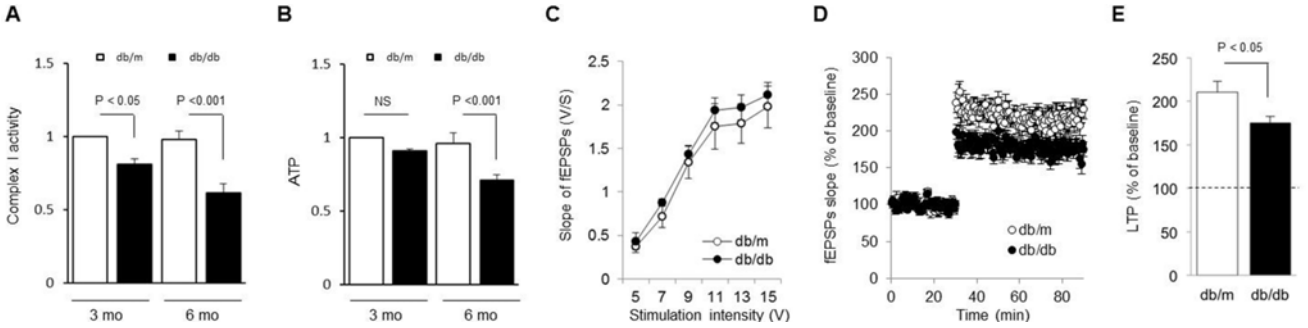
SUPPLEMENTARY DATA

Supplementary Figure 6. Serum insulin and corticosterone levels after mdivi-1 administration. There were significant increases of insulin (**A**) and corticosterone (**B**) within db/db serum compared to db/m controls. Treatment with Drp1 inhibitor (mdivi-1,25 mg/kg for 2 weeks daily) did not significantly affect insulin and corticosterne concentration in serum. NS = non-significance, P > 0.05. N = 7-8 animals per group. Vech: vehicle



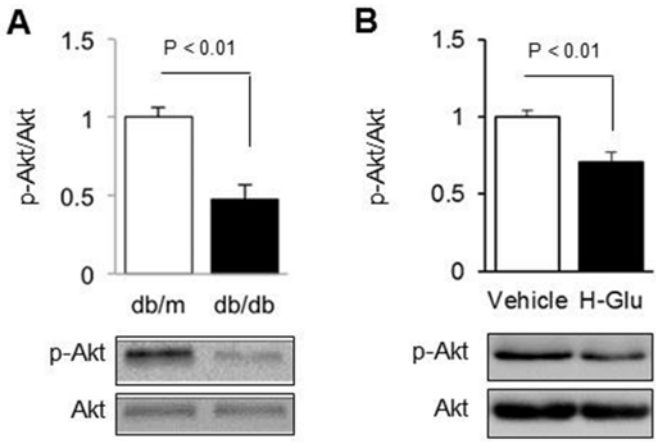
SUPPLEMENTARY DATA

Supplementary Figure 7. Mitochondrial defects and hippocampal LTP at 3 months and 6 months of age of diabetic mice. Complex I (**A**) and ATP content (**B**) were measured in hippocampal lysates from db/m and db/db mice at 3 months or 6 months of age. N = 6-10 animals per group. **C**) The basal synaptic transmission was illustrated as the indicated groups of animals. (**D**) Normalized slope of fEPSPs in the indicated groups of animals at 3 months of age. (**E**) Residual potentiation from the normalized fEPSP slopes occurring over the last 10 min of LTP recordings. N= 9-11 slices from 4 mice of each groups. NS = non-significance, P > 0.05.



SUPPLEMENTARY DATA

Supplementary Figure 8. Dephosphorylation of Akt at Ser473 in diabetic conditions both *in vivo* and *in vitro*. **A-B**) Densitometry of immunoreactive phosphorylation of Akt (p-Akt) bands relative to the total Akt in diabetes-affected hippocampus (**A**) or high glucose exposed SK cultures (**B**). The lower panels of A-B are representative immnoblots for p-Akt and Akt in hippocampal tissues (**A**) or SK cells treated with vehicle (5.5 mM D-glucose + 44.5 mM L-glucose) or high glucose (50 mM for one hour, **B**). N = 6-8 animals per group or 4 wells from 2 independent experiments.



SUPPLEMENTARY DATA

Supplementary Table 1. Effects of Diabetes and mdivi-1 treatment on body weight and blood glucose levels.

	db/m-vehicle		db/m-Mdivi-1		db/db-vehicle		db/db-Mdivi-1	
	Body weight (g)	Blood glucose (mg/dl)	Body weight (g)	Blood glucose (mg/dl)	Body weight (g)	Blood glucose (mg/dl)	Body weight (g)	Blood glucose (mg/dl)
	26.2	132	23.5	135	49.4	600	54.1	450
	28.7	158	27.9	150	37.5	550	43.6	564
	30.5	150	27.3	112	46.5	600	50.6	600
	25.3	138	29.2	172	46.8	600	39.1	600
	27.3	162	30.9	158	51.1	600	42.1	600
	30.6	156	31.5	158	43.3	600	47.5	600
	31.6	151	30.9	189	42.3	600	46.8	600
	30.2	172	30.5	190				
Mean ± SEM	28.8 ± 0.8	152.4 ± 4.2	29.0 ± 0.9	158 ± 8.7	45.3 ± 2.8 *	592.9 ± 6.6 #	46.3 ± 1.8 *	573.4 ± 19.6 #

* $P < 0.01$ in bodyweight (mdivi-1-treated db/m mice vs. vehicle-treated db/m mice, or mdivi-1-treated db/db mice vs. vehicle-treated db/db mice). # $P < 0.01$ in blood glucose (mdivi-1-treated db/m mice vs. vehicle-treated db/m mice, or mdivi-1-treated db/db mice vs. vehicle-treated db/db mice). No significance in either body weight or glucose levels were detected between vehicle-treated and mdivi-1-treated db/db mice.