

SUPPLEMENTAL DATA

Supplementary Figure S1. DW14006 was a direct AMPK α activator.

(A) Direct AMPK activators were designed by retaining the key pharmacophores of A-769662 and compound 991 based on the principle of scaffold hopping and bioisosteric replacement. (B) DW14006 exhibited a good activity in activating AMPK α .

Supplementary Figure S2. Effects of DW14006 on body weight, blood glucose and lipid metabolism.

(A-F) No significant difference was found in blood glucose between diabetic mice and DW14006-treated diabetic mice. However, DW14006 treatment (15, 30 mg/kg) reduced body weight of *db/db* mice but had no impacts on STZ mice. (G and H) triglyceride (TG) and total cholesterol (TC) were measured in serum. DW14006 treatment reduced TC value in *db/db* mice and rendered no influence in STZ mice. Values were presented as mean \pm SEM, n = 12. *P<0.05; **P<0.01; ***P<0.001, STZ or *db/db* mice vs non-diabetic mice (Control, C57BL/6 or *db/m*) by Student's t-test. #P<0.05; ##P<0.01; ###P<0.001, DW14006-treated STZ or *db/db* mice vs diabetic mice (STZ induced or *db/db* mice) by one-way ANOVA with Dunnett's post-hoc test.

Supplementary Figure S3. DW14006 promoted neurite outgrowth in sensory neurons from normal mice by activating AMPK α .

(A) β -tubulin III-immunostained sensory neurons from normal mice (C57BL/6 mice) were untreated (DMSO) or treated with DW14006 (2, 5, 10 μ M) for 24 h. Scale bar = 100 μ m. (B) Quantitative analyses of total neurite outgrowth for (A). (C) β -tubulin III-immunostained sensory neurons from normal mice were untreated (DMSO) or treated

with DW14006 (10 μ M) and AMPK specific inhibitor compound C (CC, 3 μ M). Scale bar = 50 μ m. **(D)** Quantitative analyses for **(C)**. All data were presented as mean \pm SEM of n = 7 replicate cultures. * P < 0.05; **P < 0.01; ***P < 0.001 vs. Control by one-way ANOVA with Dunnett's post-hoc test. # P < 0.05; ## P < 0.01; ### P < 0.001 vs. DW14006 (10 μ M) by Student's t-test. β -tubulin III is a specific antibody against neuron.

Supplementary Figure S4. DW14006 upregulated expressions of NDUFS3 and COX IV by activating AMPK α in diabetic mice.

(A, B) Quantitative immunoblot analyses revealed that expressions of mitochondrial respiratory chain proteins (NDUFS 3 and COX IV) were reduced in STZ and *db/db* mice and upregulated in DW14006 (15, 30 mg/kg)-treated diabetic DPN mice (STZ+15, 30 mg/kg, *db/db*+15, 30 mg/kg) versus age-matched Control (C57BL/6 mice) or *db/m* mice. **(C, D)** Quantification of NDUFS3 and COX IV expressions normalized to total extracellular regulated protein kinase (T-ERK) level. T-ERK remained unchanged. Values were presented as mean \pm SD of n = 3. *P < 0.05; **P < 0.01; ***P < 0.001, STZ or *db/db* mice vs. Control or *db/m* mice by Student's t-test. # P < 0.05; ## P < 0.01; ### P < 0.001, DW14006-treated STZ or *db/db* mice vs. STZ or *db/db* mice by one-way ANOVA with Dunnett's post-hoc test. **(E, F)** DW14006 treatment gave no effects on the expressions of NDUFS3 and COX IV in AAV8-AMPK α -RNAi injected STZ mice (STZ+AAV8-AMPK α -RNAi). Values were presented as mean \pm SD of n = 3. **(G)** AMPK α expression in DRG tissue from diabetic mice was obviously decreased after injecting AAV8-AMPK α -RNAi into STZ mice 2 weeks later compared with AAV8-NC injected STZ mice (STZ+AAV8-NC). **(H)** Quantification of total AMPK α , normalized to total extracellular regulated protein kinase (T-ERK) level. T-ERK remained unchanged. Values were presented as mean \pm SD of n = 3. *P < 0.05; **P <

0.01; *** $P < 0.001$, STZ+AAV8-AMPK α -RNAi vs. STZ+AAV8-NC by Student's t-test.

NDUFS3 = NADH dehydrogenase (ubiquinone) iron-sulphur protein 3; COX IV = cytochrome c oxidase subunit IV.

Supplementary Figure S5. DW14006 treatment reduced oxidative stress level in diabetic mice by activating AMPK α .

(A-C) Representative immuno-fluorescent images showing the neuron-specific β -tubulin isotype III (neuron, green), 8-OHdG (oxidative stress, red) and Dapi (nucleus, blue) staining. (D, E) Quantification of 8-OHdG fluorescence intensity for (A-C). Bar = 50 μ M. (F, G) DW14006 treatment (15 or 30 mg/kg) increased SOD activity and GSH level, and decreased MDA level in serum from type 1 (STZ) and 2 (*db/db*) diabetic DPN mice. (H) DW14006 treatment (30 mg/kg) fail to change SOD activity, GSH or MDA levels in STZ+AAV8-AMPK α -RNAi mice. Values were presented as mean \pm SEM of $n = 8$ /group. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$, STZ or *db/db* mice vs. Control or *db/m* mice by Student's t-test. # $P < 0.05$; ## $P < 0.01$; ### $P < 0.001$, DW14006-treated STZ or *db/db* mice vs. STZ or *db/db* by one-way ANOVA with Dunnett's post-hoc test. 8-OHdG (8-hydroxy-2deoxyguanosine) was a biomarker of mitochondrial DNA (mtDNA) oxidative damage; SOD = superoxidase dismutase; GSH = glutathione; MDA = malondialdehyde.

Supplementary Figure S6. DW14006 treatment gave no impacts on neurovascular function in AMPK α knockdown diabetic mice.

(A) DW14006 (30 mg/kg) treatment had no effects on neurovascular function in AAV8-AMPK α -RNAi injected STZ mice. (B) Quantitative analysis for (A). All data were

normalized by the blood flow and perfusion areas in sciatic nerve and foot pad tissues from STZ+AAV8-NC mice presented as mean \pm SEM of n = 8. Lowest blood flow ratio was indicated in blue, maximum blood flow was in red, and intermediate grading was in green or yellow.

Supplementary Figure S7. DW14006 treatment improved mitochondrial inner membrane potential (MMP) in high glucose (HG)-treated primary DRG neurons.

(A) Relative level of MMP in primary DRG neurons measured by TMRM dye. Data were presented as mean \pm SD of n = 4. *P < 0.05; **P < 0.01; ***P < 0.001, HG vs. DMSO by Student's t-test. # P < 0.05; ## P < 0.01; ### P < 0.001, DW14006 or FCCP-treated HG groups vs. HG group by one-way ANOVA with Dunnett's post-hoc test. HG, 45 mM glucose; the uncoupling agent FCCP (2 μ M) induced partial depolarization.

Supplementary Figure S8. DW14006 treatment downregulated Akt/mTOR signaling in diabetic mice.

(A-B) Quantitative immunoblot analysis revealed that expressions of p-Akt (T308) and p-mTOR were increased in diabetic mice (STZ and *db/db*) and downregulated in DW14006-treated diabetic mice (STZ+15, 30 mg/kg and *db/db*+15, 30 mg/kg). (C) Quantitative analyses for (A). (D) Quantitative analyses for (B). T-ERK remained unchanged. Values were presented as mean \pm SD of n = 3. *P < 0.05; **P < 0.01; ***P < 0.001, STZ or *db/db* mice vs. Control or *db/m* mice by Student's t-test. # P < 0.05; ## P < 0.01; ### P < 0.001, DW14006-treated STZ or *db/db* mice vs. STZ or *db/db* mice by one-way ANOVA with Dunnett's post-hoc test.

Supplementary Figure S9. DW14006 treatment had no effect on neurological function in nondiabetic mice.

(A) Tactile allodynia, (B) radial heat plate test and (C) motor nerve conduction velocity (MNCV) were detected to evaluate neurological function in nondiabetic mice. All data were presented as mean \pm SEM (n = 8/group). Data were analyzed by two-way ANOVA with Tukey's test. There was no difference between groups with appropriate time-matched comparisons.