

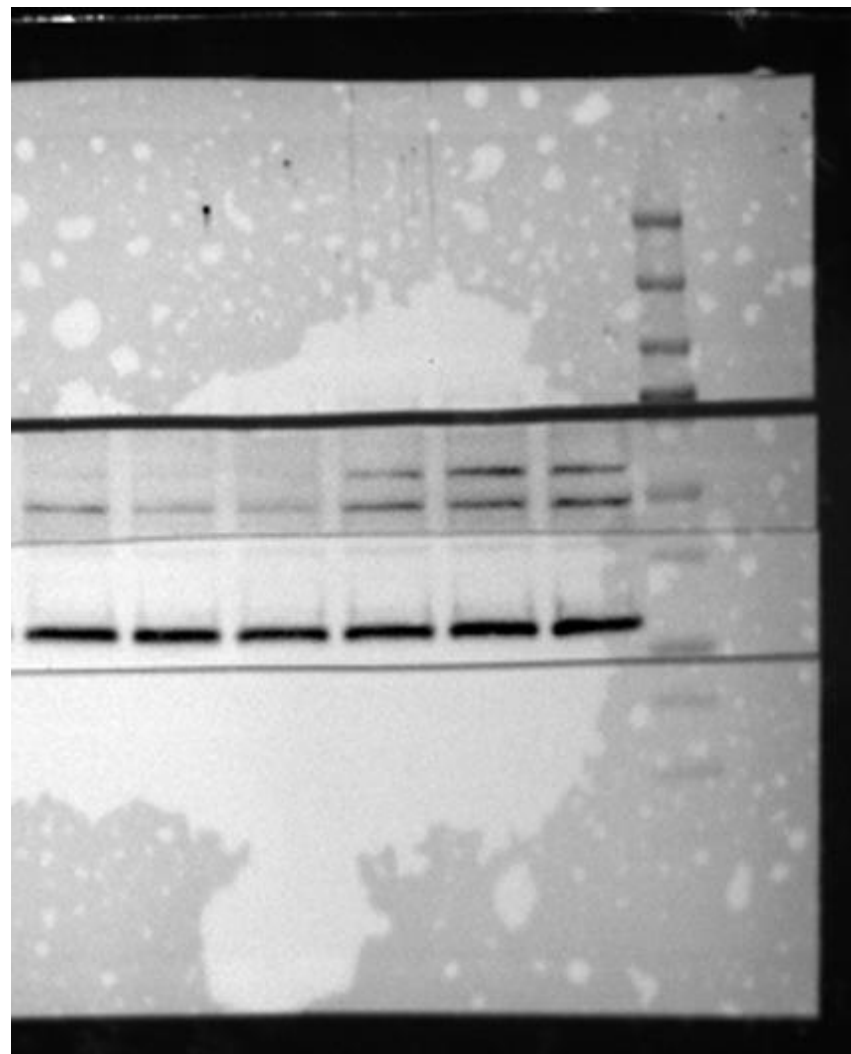
Supplementary Information

Palmitate and Western diet induce oxidative damage and impairment of mitophagy in hepatocytes - reversal by a neurotrophic factor

Simon Musyoka Mwangi^{1,2}; Ge Li^{1,2}; Arun Balasubramaniam^{1,2}; Didier Merlin^{2,5};

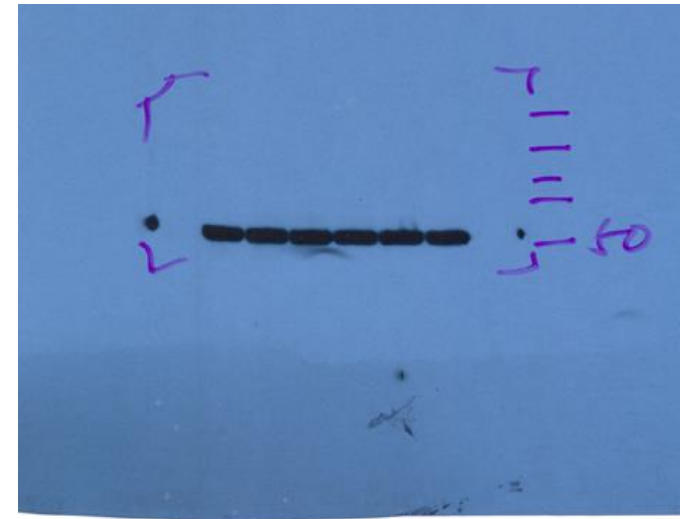
Paul A. Dawson⁴; Young C. Jang⁶; C. Michael Hart^{2,3}; Mark J. Czaja¹; Shanthi

Srinivasan^{1,2*}.

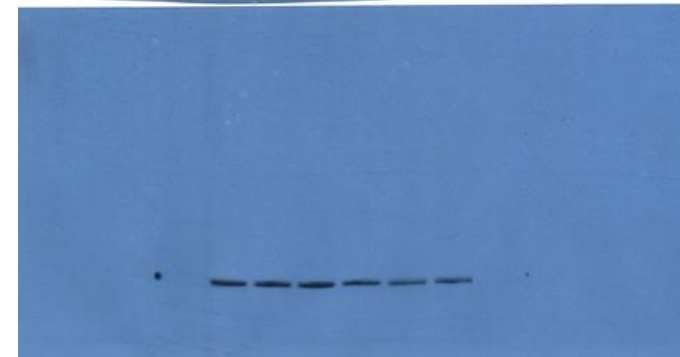


} PINK1

} SIRT3

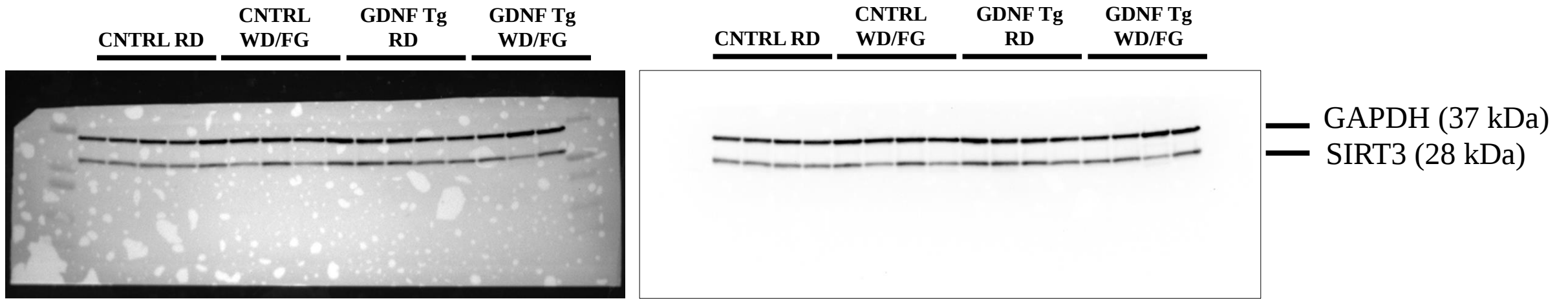


} α -tubulin

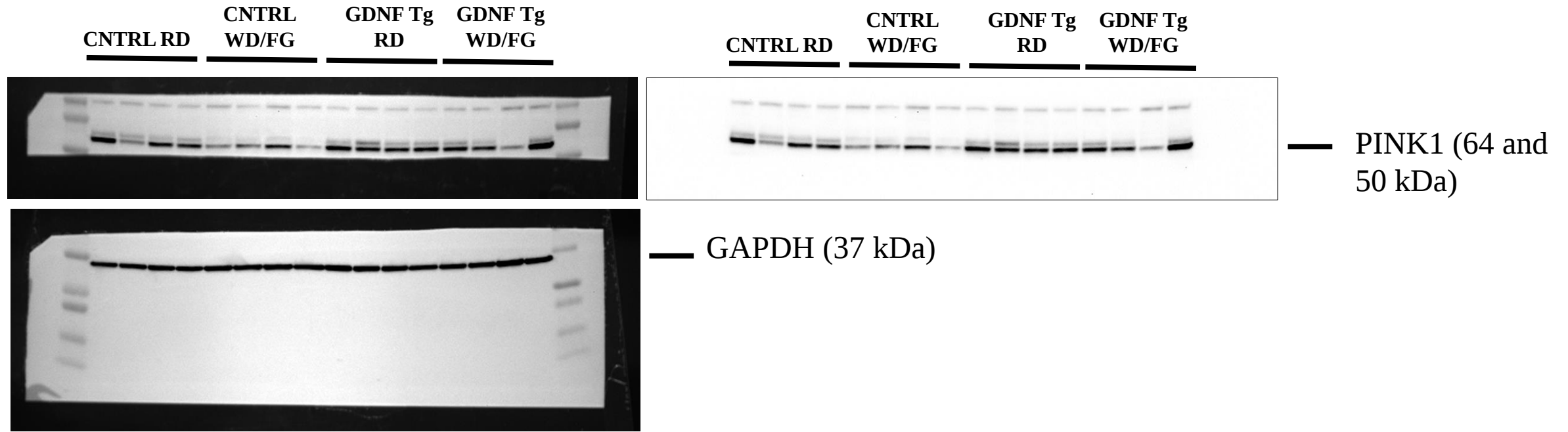


} α -tubulin

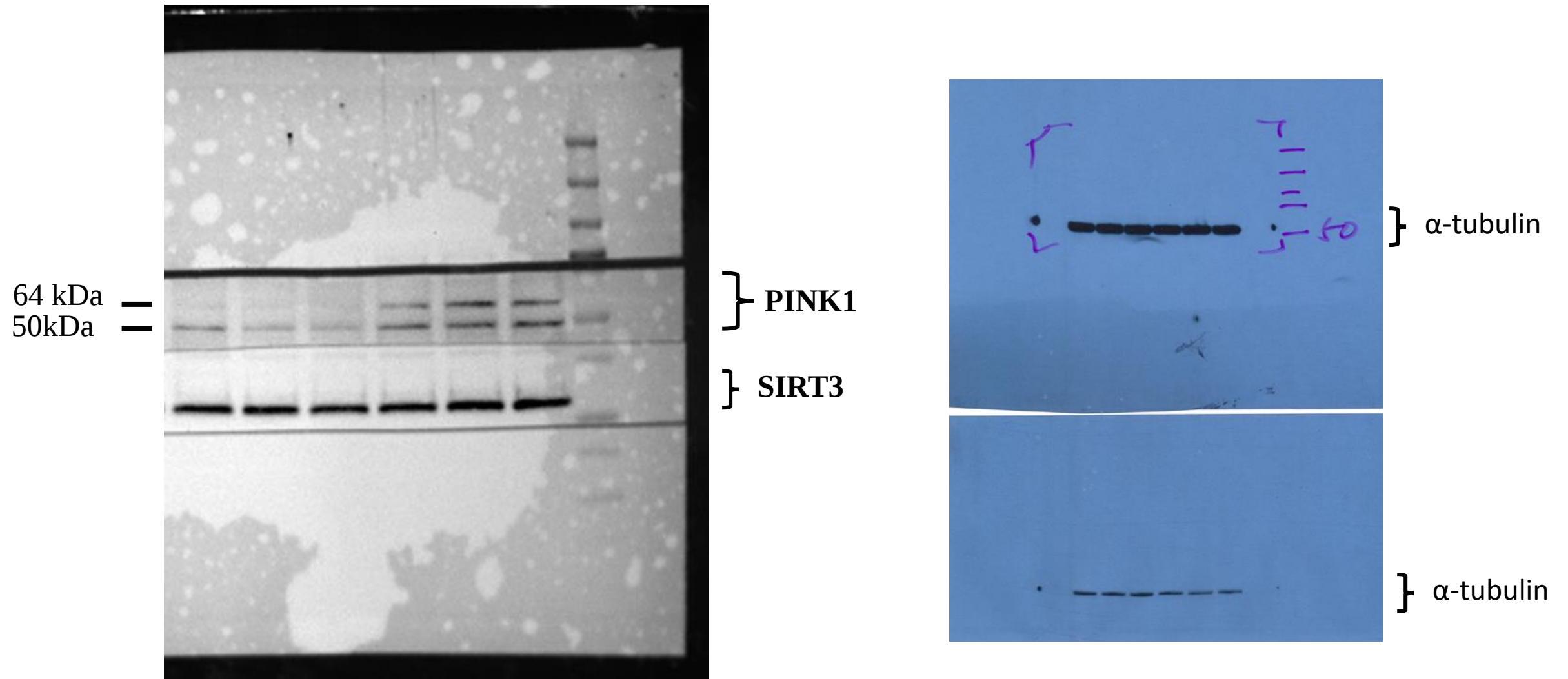
S1. Western blot analysis of SIRT3 and α -tubulin (loading control) protein levels in primary human hepatocytes cultured for 24h in the presence and absence of palmitate (PA) and GDNF.



S2. Western blot analysis of hepatic SIRT3 and GAPDH (loading control) protein levels in control (CNTRL) and GDNF transgenic (GDNF Tg) mice fed regular diet (RD) or a Western diet with fructose and glucose in drinking water (WD/FG) for 25 weeks.



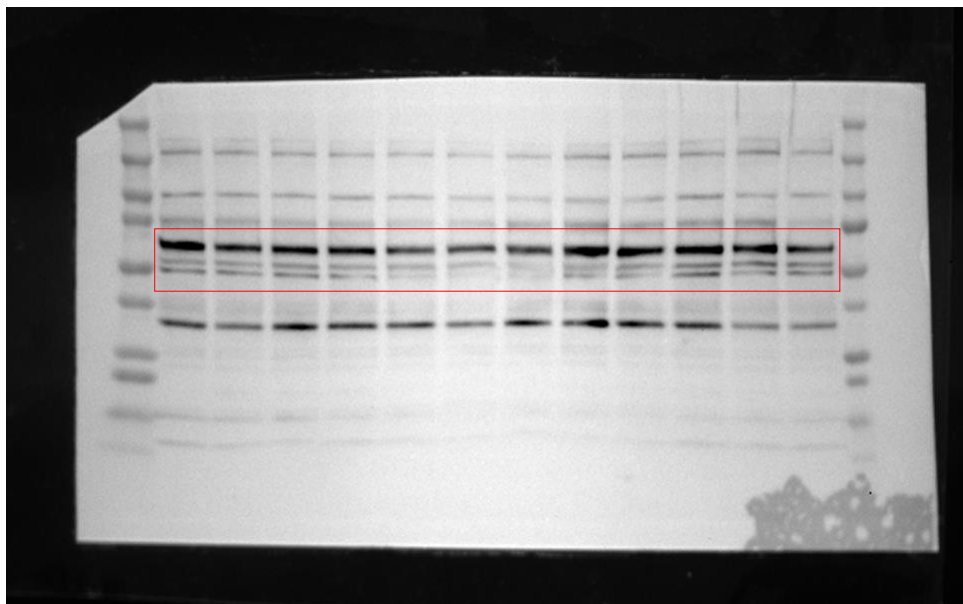
S3. Western blot analysis of hepatic PINK1 and GAPDH (loading control) protein levels in control (CNTRL) and GDNF transgenic (GDNF Tg) mice fed regular diet (RD) or a Western diet with fructose and glucose in drinking water (WD/FG) for 25 weeks.



S4. Western blot analysis of PINK1 and α -tubulin (loading control) protein levels in primary human hepatocytes cultured for 24h in the presence and absence of palmitate (PA) and GDNF.

-	-	-	0.2	0.2	0.2	-	-	-	0.2	0.2	0.2
-	-	-	-	-	-	+	+	+	+	+	+

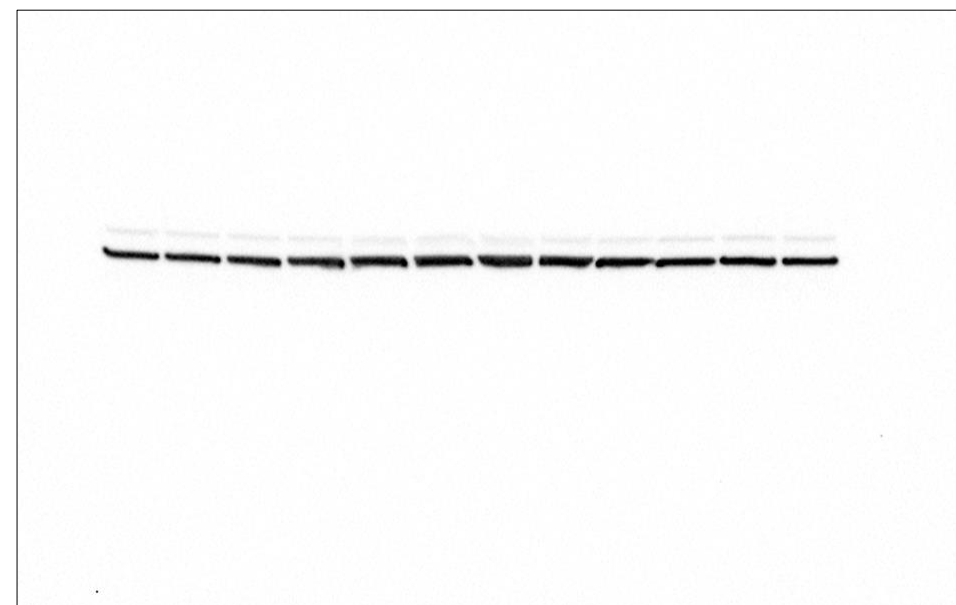
Palmitate (mM)
GDNF (100 ng/ml)



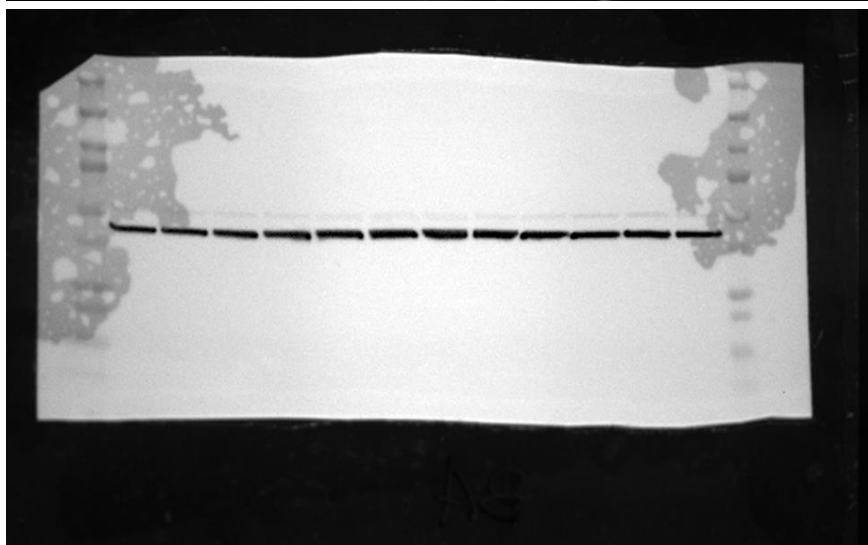
— PINK1 (64 kDa)
— PINK1 (50 kDa)

-	-	-	0.2	0.2	0.2	-	-	-	0.2	0.2	0.2
-	-	-	-	-	-	+	+	+	+	+	+

Palmitate (mM)
GDNF (100 ng/ml)



— β -actin
(45 kDa)

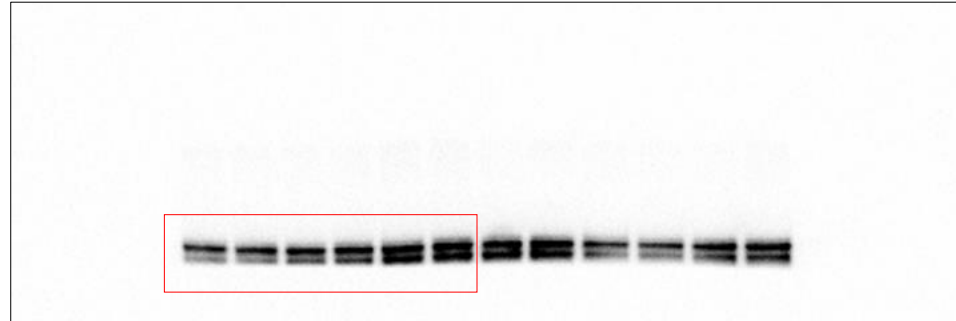
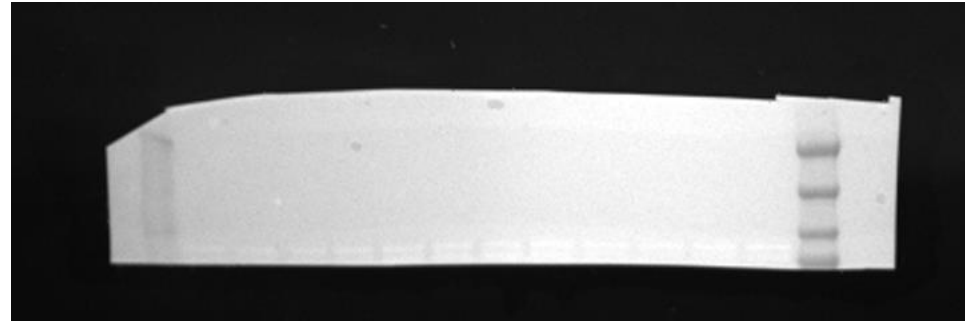


— β -actin
(45 kDa)

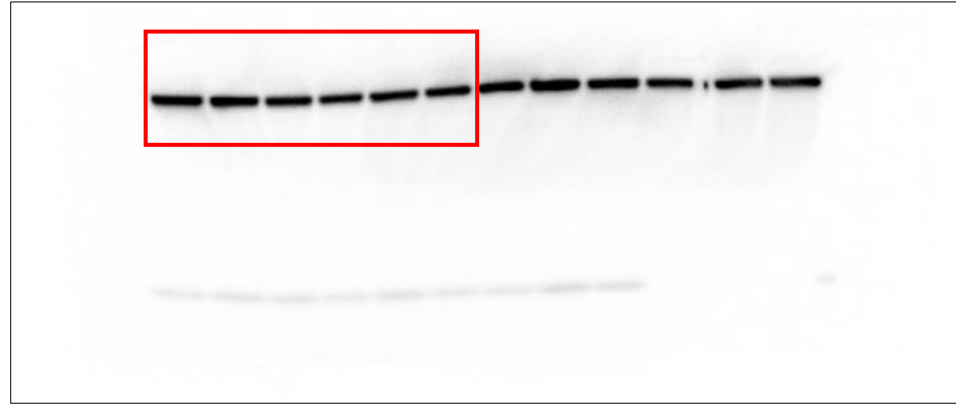
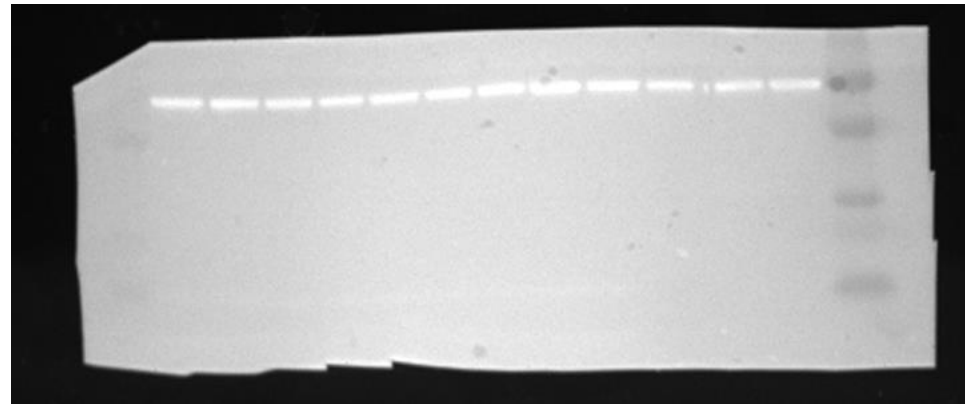
S5. Western blot analysis of PINK1 and β -actin (loading control) protein levels in rat hepatocytes exposed to palmitate (PA) and GDNF for 24h.

- 0.1 0.15 - 0.1 0.15
- - - + + +

Palmitate (mM)
GDNF (100 ng/ml)



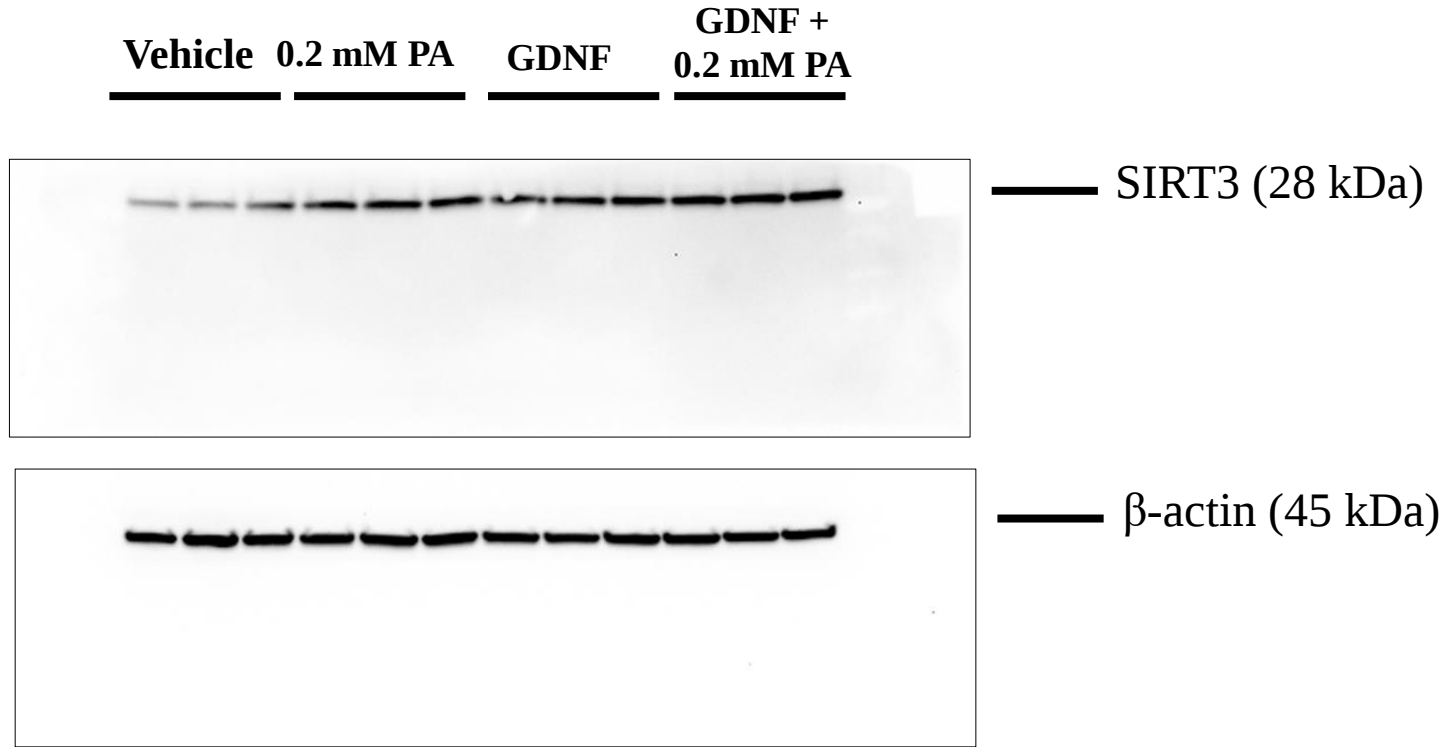
— OPA1 (80-100 kDa)



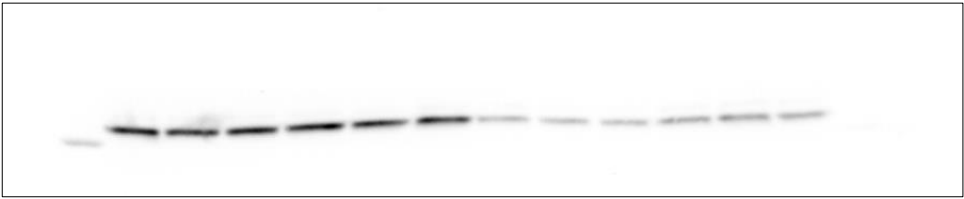
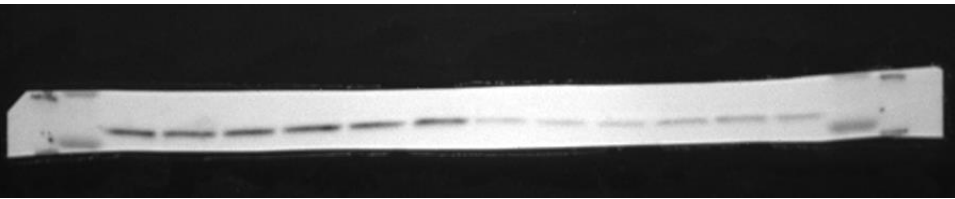
— α -tubulin (52 kDa)

S6. Western blot analysis of OPA1 and α -tubulin (loading control) protein levels in rat hepatocytes exposed to Palmitate (PA) and GDNF for 24h.

ORIGINAL WESTERN BLOTS



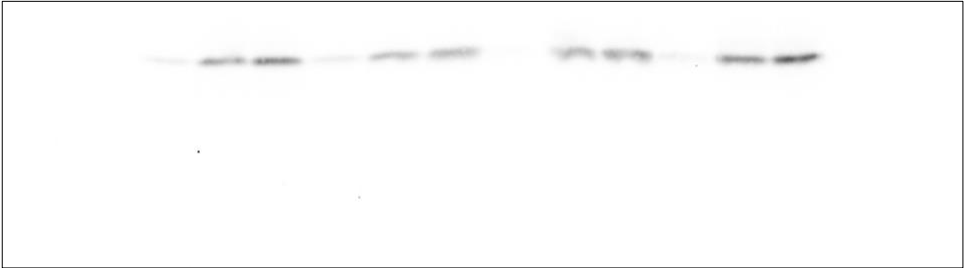
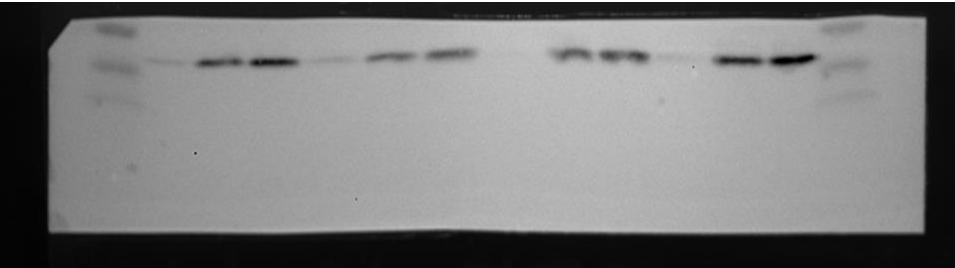
S7. Western blot analysis of SIRT3 and β -actin (loading control) protein levels in rat hepatocytes cultured for 24h in the presence and absence of palmitate (PA) and GDNF.



-	0.2	0.3	-	0.2	0.3	-	0.2	0.3	-	0.2	0.3
-	-	-	+	+	+	-	-	-	+	+	+

Palmitate (mM)
GDNF (100 ng/ml)

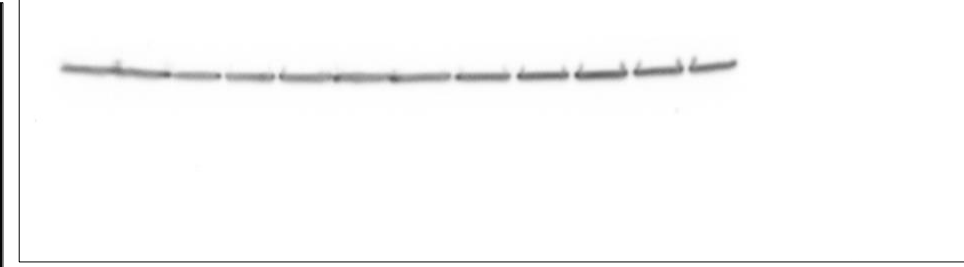
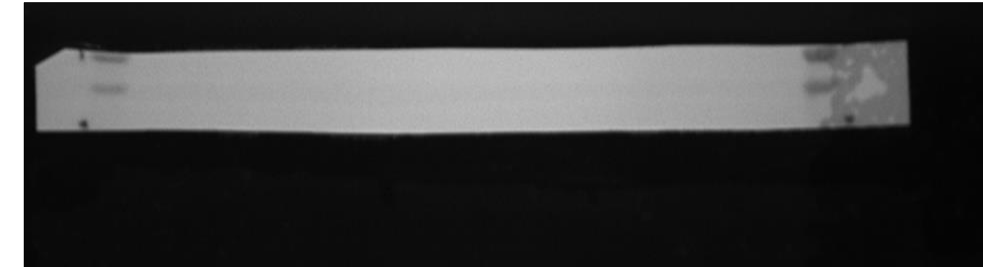
— SIRT3 (28 kDa)



-	0.2	0.3	-	0.2	0.3	-	0.2	0.3	-	0.2	0.3
-	-	-	+	+	+	-	-	-	+	+	+

Palmitate (mM)
GDNF (100 ng/ml)

— Cleaved caspase-3
(17 kDa)



-	0.2	0.3	-	0.2	0.3	-	0.2	0.3	-	0.2	0.3
-	-	-	+	+	+	-	-	-	+	+	+

Palmitate (mM)
GDNF (100 ng/ml)

— α-tubulin (52 kDa)

S8. Western blot analyses of SIRT3, cleaved caspase-3 and α-tubulin (loading control) levels in control and SIRT3 siRNA-transfected rat hepatocytes after 24h exposure to palmitate (PA) and GDNF.