

# **SUPPLEMENTAL APPLIEDIX FOR THE STUDY:**

PTEN-induced kinase 1 exerts protective effects in diabetic kidney disease by attenuating mitochondrial dysfunction and  
necroptosis

**Table S1. Primer sequences used in this study**

<b>Gene</b>	<b>Primer sequence</b>
<b>Human primer</b>	
<b><math>\alpha</math>-SMA</b>	F: 5'-AAAAGACAGCTACGTGGGTGA-3' R: 5'-GCCATGTTCTATCGGGTACTTC-3'
<b>Fibronectin</b>	F: 5'-GCGAGAGTGCCCCTACTACA-3' R: 5'-GTTGGTGAATCGCAGGTCA-3'
<b>E-cadherin</b>	F: 5'-CGAGAGCTACACGTTCACGG-3' R: 5'-GGGTGTCGAGGGAAAAATAGG-3'
<b>TGF-<math>\beta</math>1</b>	F: 5'-CAATTCCTGGCGATACCTCAG-3' R: 5'-GCACAACCTCCGGTGACATCAA -3'
<b>PINK1</b>	F: 5'-CCCAAGCAACTAGCCCCTC-3' R: 5'-GGCAGCACATCAGGGTAGTC-3'
<b>RIP1</b>	F: 5'-TGGGCTTCACACAGTCTCAG-3' R: 5'-CCACTTTTGGAGCATCTGGT-3'
<b>RIP3</b>	F: 5'-TAGGAAGTGGGGCTACGATG-3' R: 5'-TGGTCCCAGTTCACCTTCTC-3'
<b>MLKL</b>	F: 5'-CATCCACAAACGGTGTGAAG-3' R: 5'-GCTCCTCTTTCCTTGGTCCT-3'
<b>GAPDH</b>	F: 5'-CGACCACTTTGTCAAGCTCA-3' R: 5'-CCCTGTTGCTGTAGCCAAAT-3'

<b>Gene</b>	<b>Primer sequence</b>
<b>Mouse primer</b>	
<b><math>\alpha</math>-SMA</b>	F: 5'-GATGAAGCCCAGAGCAAGAG-3' R: 5'-CTTTTCCATGTCTCGTCCCAGT-3'
<b>Fibronectin</b>	F: 5'-ACAAGGTTTCGGGAAGAGGTT-3' R: 5'-CCGTGTAAGGGTCAAAGCAT-3'
<b>E-cadherin</b>	F: 5'-TCGGAAGACTCCCGATTCAA-3' R: 5'-CGGACGAGGAACTGGTCTC -3'
<b>TGF-<math>\beta</math>1</b>	F: 5'-CCACCTGCAAGACCATCGAC-3' R: 5'-CTGGCGAGCCTTAGTTTGGAC-3'
<b>PINK1</b>	F: 5'-TTCTTCCGCCAGTCGGTAG-3' R: 5'-CTGCTTCTCCTCGATCAGCC-3'
<b>GAPDH</b>	F: 5'-ACTCCCACTCTTCCACCTTC-3' R: 5'-TCCAGGGTTTCTTACTCCTT-3'

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**Primer for human mitochondrial DNA**


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<b>mtDNA 4,977-bp deletion</b>	F: 5'-CCTTACACTATTCTCATCACC-3' R: 5'-TGTGGTCTTTGGAGTAGAAACC-3'
<b>COX I</b>	F: 5'-TTCGCCGACCGTTGACTATTCTCT-3' R: 5'-AAGATTATTACAAATGCATGGGC-3'
<b>Nuclear <math>\beta</math>-actin</b>	F: 5'-ACCCACACTGTGCCCATCTAC-3'

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**Primer for mouse mitochondrial DNA**


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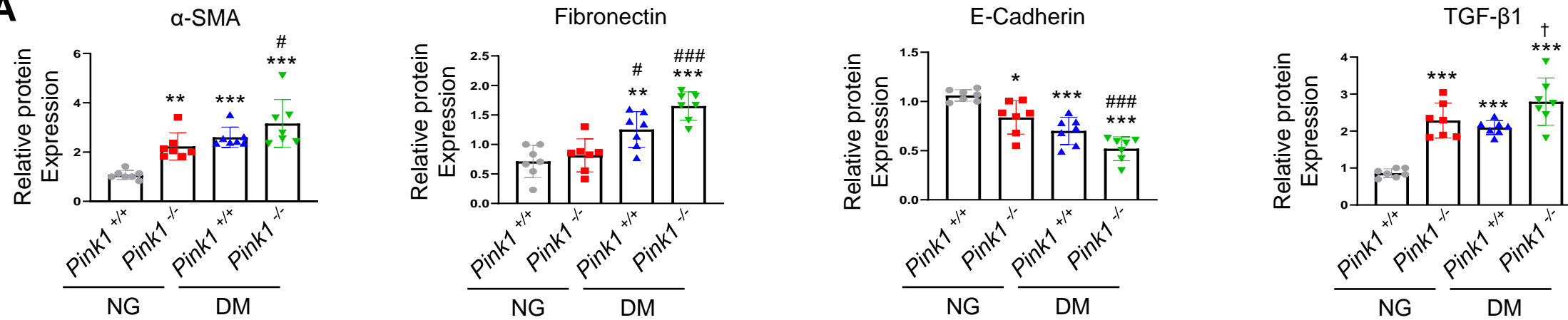
<b>mtDNA 3,860-bp deletion</b>	F: 5'-TCATTCTAGCCTCGTACCAACA-3' R: 5'-GAGGTCTGGGTCATTTTCGTTA-3'
<b>12r</b>	F: 5'-ACCGCGGTCATACGATTAAC-3' R: 5'-CCCAGTTTGGGTCTTAGCTG-3'

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**Abbreviation:**  $\alpha$ -SMA, alpha smooth muscle actin; TGF- $\beta$ 1, transforming growth factor beta 1; PINK1, PTEN-induced kinase 1; RIP, receptor-interacting serine/threonine-protein kinase; MLKL, mixed lineage kinase domain like pseudokinase; GAPDH, glyceraldehyde 3-phosphate dehydrogenase.

**Figure S1. Quantitative analysis of western blotting (Figure 2)**

**A**

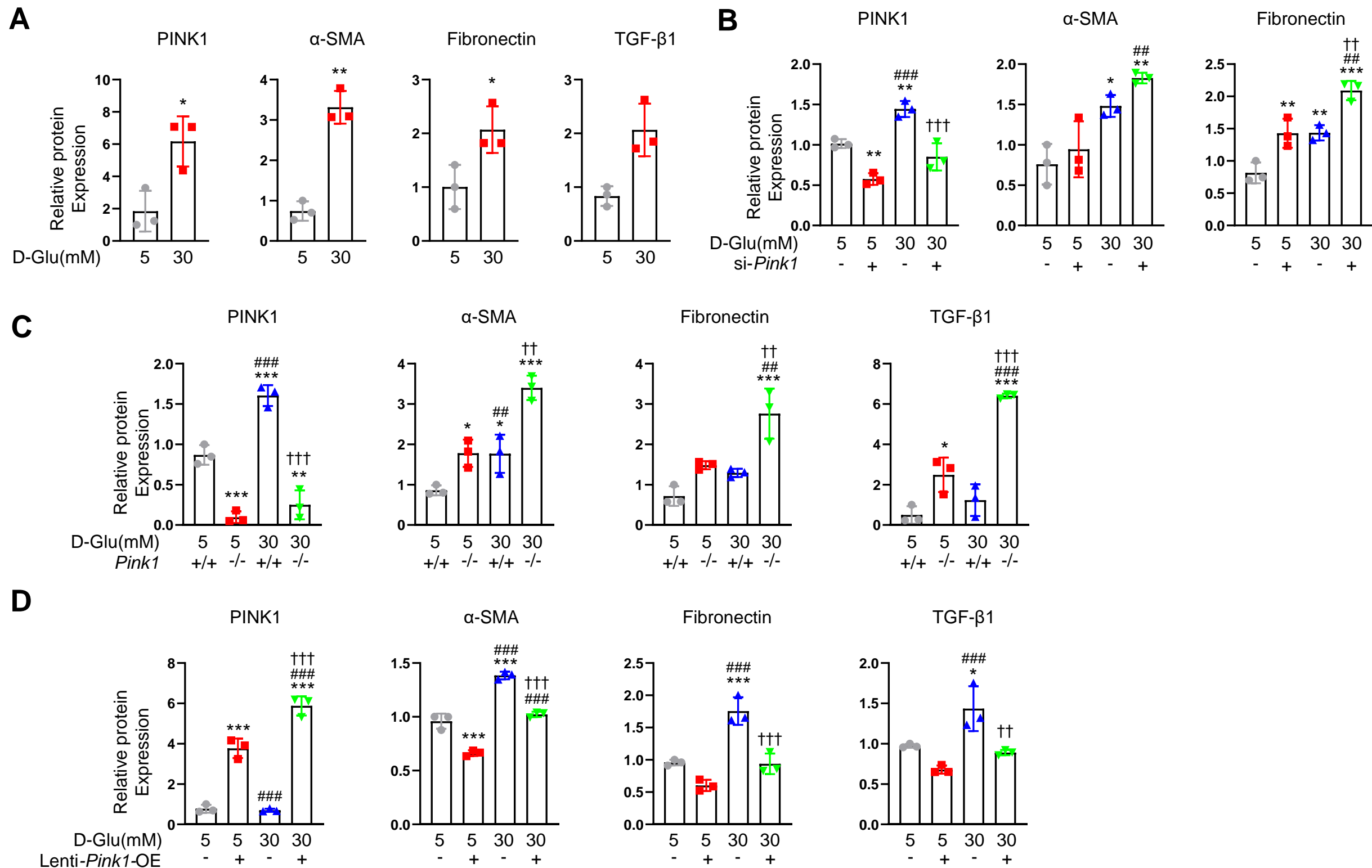


Shown are the quantitative analysis of western blotting in **Figure 2B**.

Data are expressed as means  $\pm$  standard error. \* $p$  < 0.05, \*\* $p$  < 0.01, \*\*\* $p$  < 0.001 vs *Pink1*<sup>+/+</sup> NG, # $p$  < 0.05, ### $p$  < 0.001 vs *Pink1*<sup>-/-</sup> NG, † $p$  < 0.05 vs *Pink1*<sup>+/+</sup> DM

Abbreviations: PINK1, PTEN-induced serine/threonine kinase;  $\alpha$ -SMA, alpha-smooth muscle actin; TGF- $\beta$ 1, transforming growth factor- $\beta$ 1; NG, normal glucose; DM, diabetes mellitus.

**Figure S2. Quantitative analysis of western blotting (Figure 3)**



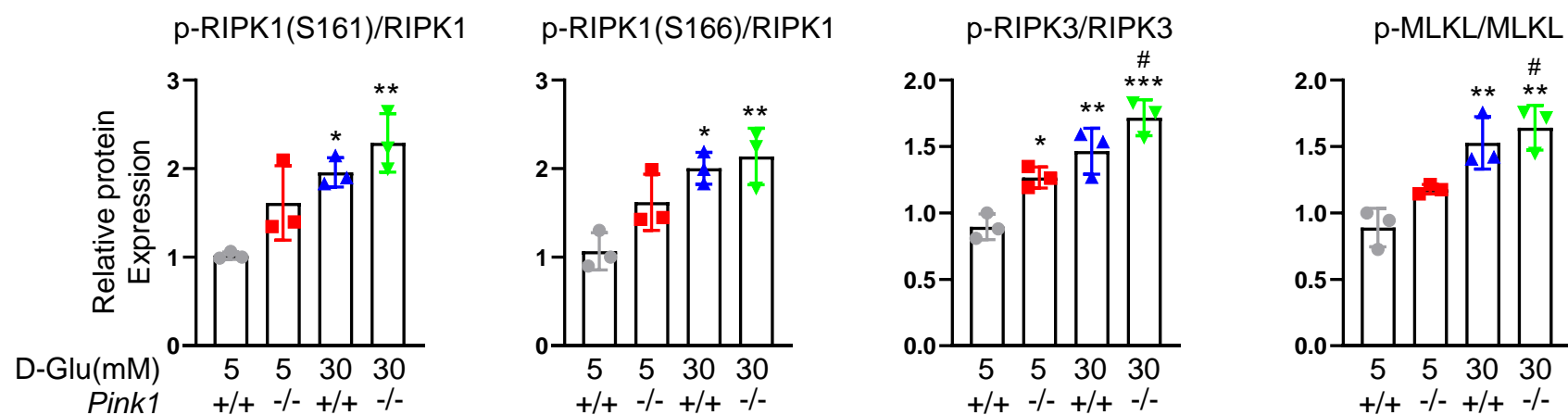
Shown are the quantitative analysis of western blotting in **Figure 3**. Panels **A**, **B**, **C**, and **D** correspond to the relative protein expressions of **Figure 3B**, **3D**, **3F**, and **3H**, respectively.

Data are expressed as means  $\pm$  standard error. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$  vs *Pink1*<sup>+/+</sup> NG, ## $p < 0.01$ , ### $p < 0.001$  vs *Pink1*<sup>-/-</sup> NG, †† $p < 0.01$ , ††† $p < 0.001$  vs *Pink1*<sup>+/+</sup> DM

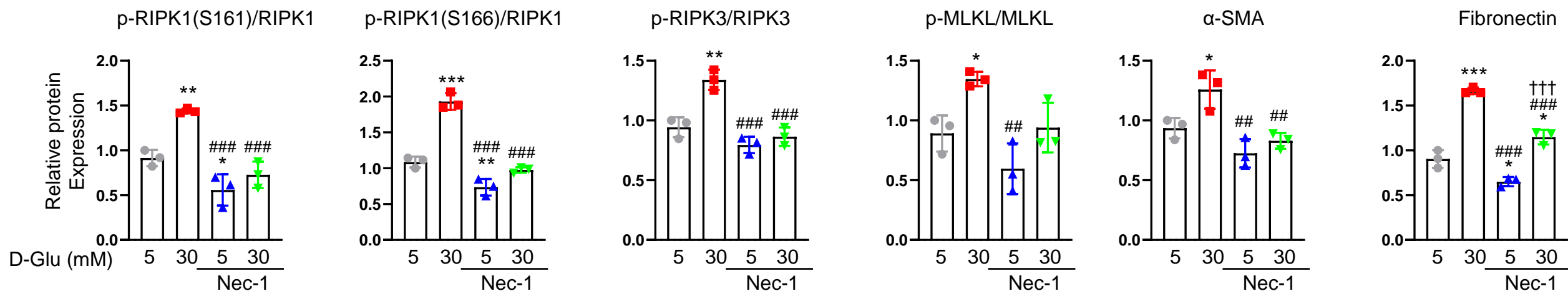
Abbreviations: PINK1, PTEN-induced serine/threonine kinase; α-SMA, alpha-smooth muscle actin; TGF-β1, transforming growth factor-β1; NG, normal glucose; DM, diabetes mellitus; OE, overexpression.

**Figure S3. Quantitative analysis of western blotting (Figure 7)**

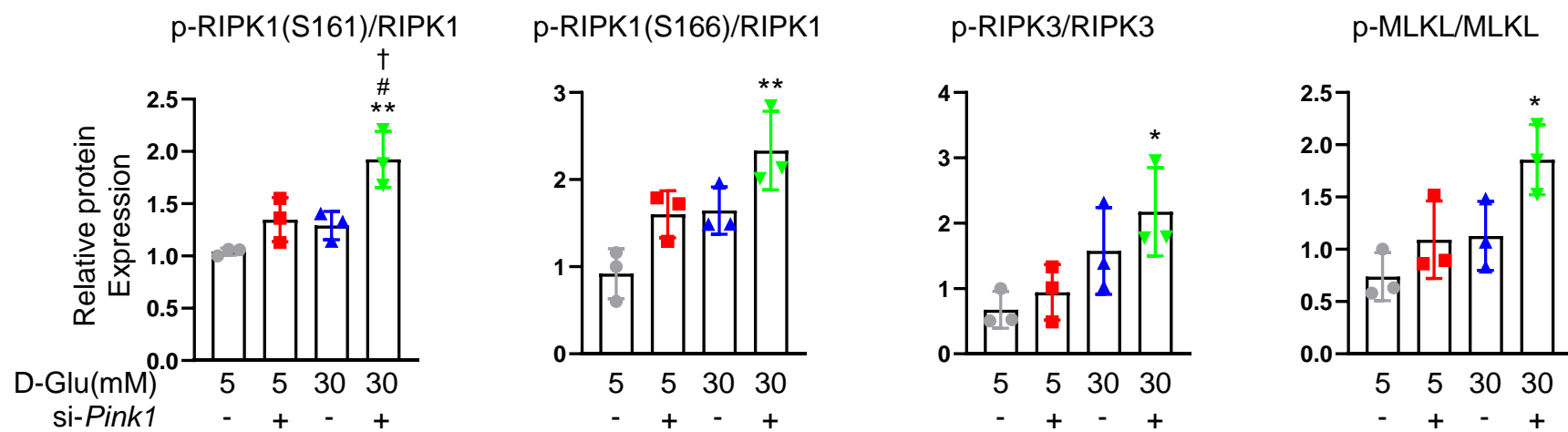
**A**



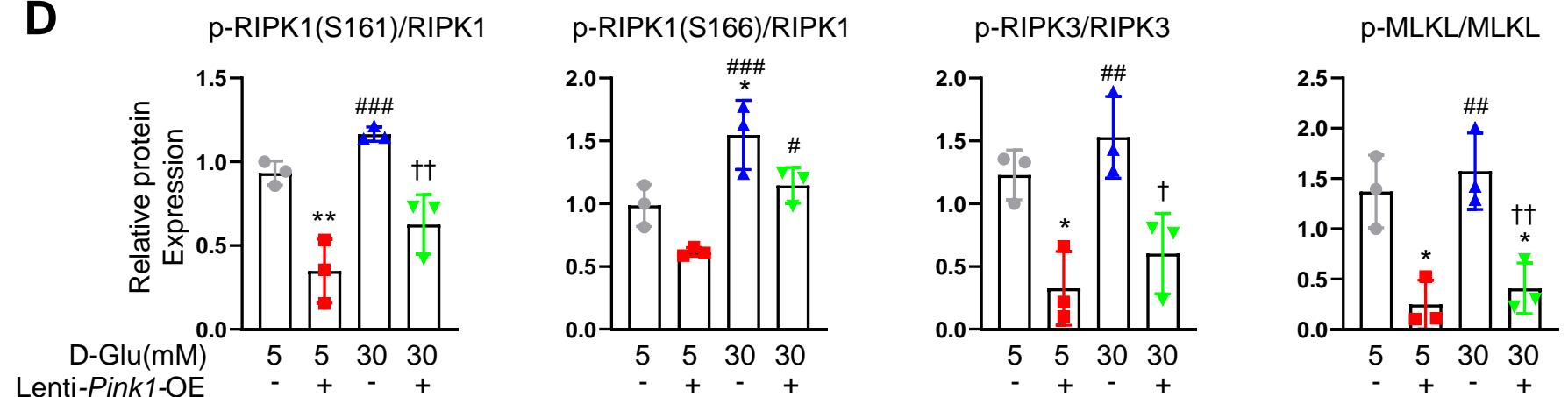
**B**



**C**



**D**



Shown are the quantitative analysis of western blotting in **Figure 7**. Panels **A**, **B**, **C**, and **D** correspond to the relative protein expressions of **Figure 7B**, **7C**, **7E**, and **7G**, respectively.

Data are expressed as means  $\pm$  standard error. \* $p < 0.05$ , \*\* $p < 0.01$  vs *Pink1*<sup>+/+</sup> NG, # $p < 0.05$ , ## $p < 0.01$ , ### $p < 0.001$  vs *Pink1*<sup>-/-</sup> NG, †† $p < 0.01$  vs *Pink1*<sup>+/+</sup> DM

Abbreviations: PINK1, PTEN-induced serine/threonine kinase 1; RIPK, receptor-interacting protein kinase; TNF $\alpha$ , tumor necrosis factor  $\alpha$ ; MLKL, mixed lineage kinase domain-like pseudokinase;  $\alpha$ -SMA, alpha-smooth muscle actin; OE, overexpression.