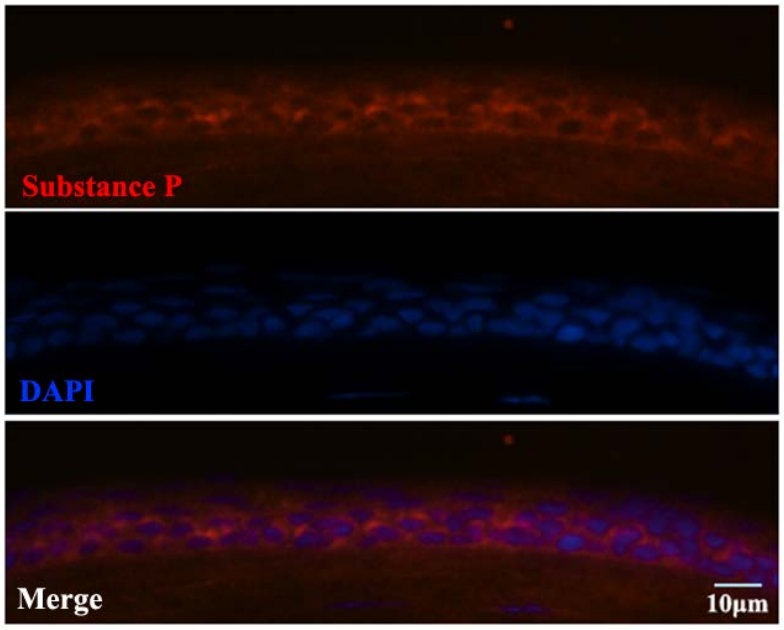
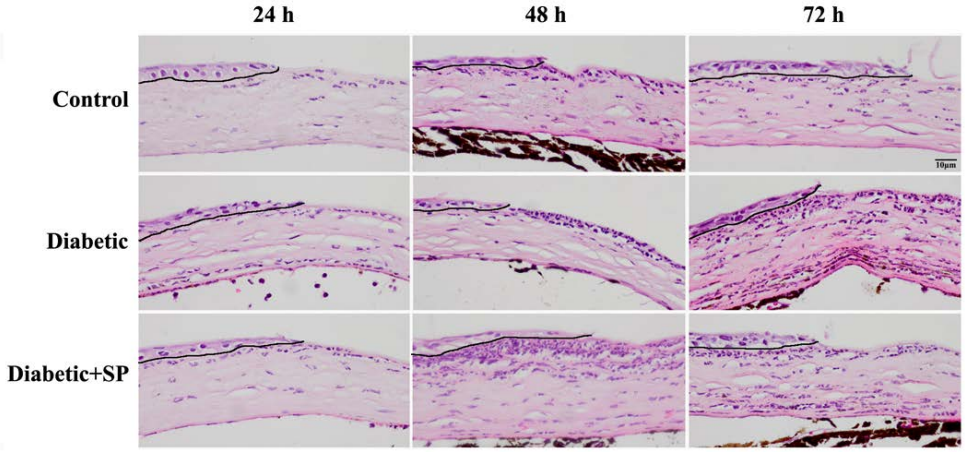
SUPPLEMENTARY DATA

Supplementary Figure 1. Topically applied substance P penetrates into the corneal epithelium in diabetic mice. SP was detected in the cytoplasm of corneal epithelial cells by immunofluorescence staining, which suggests that topically applied SP can penetrate into the corneal epithelium in diabetic mice.

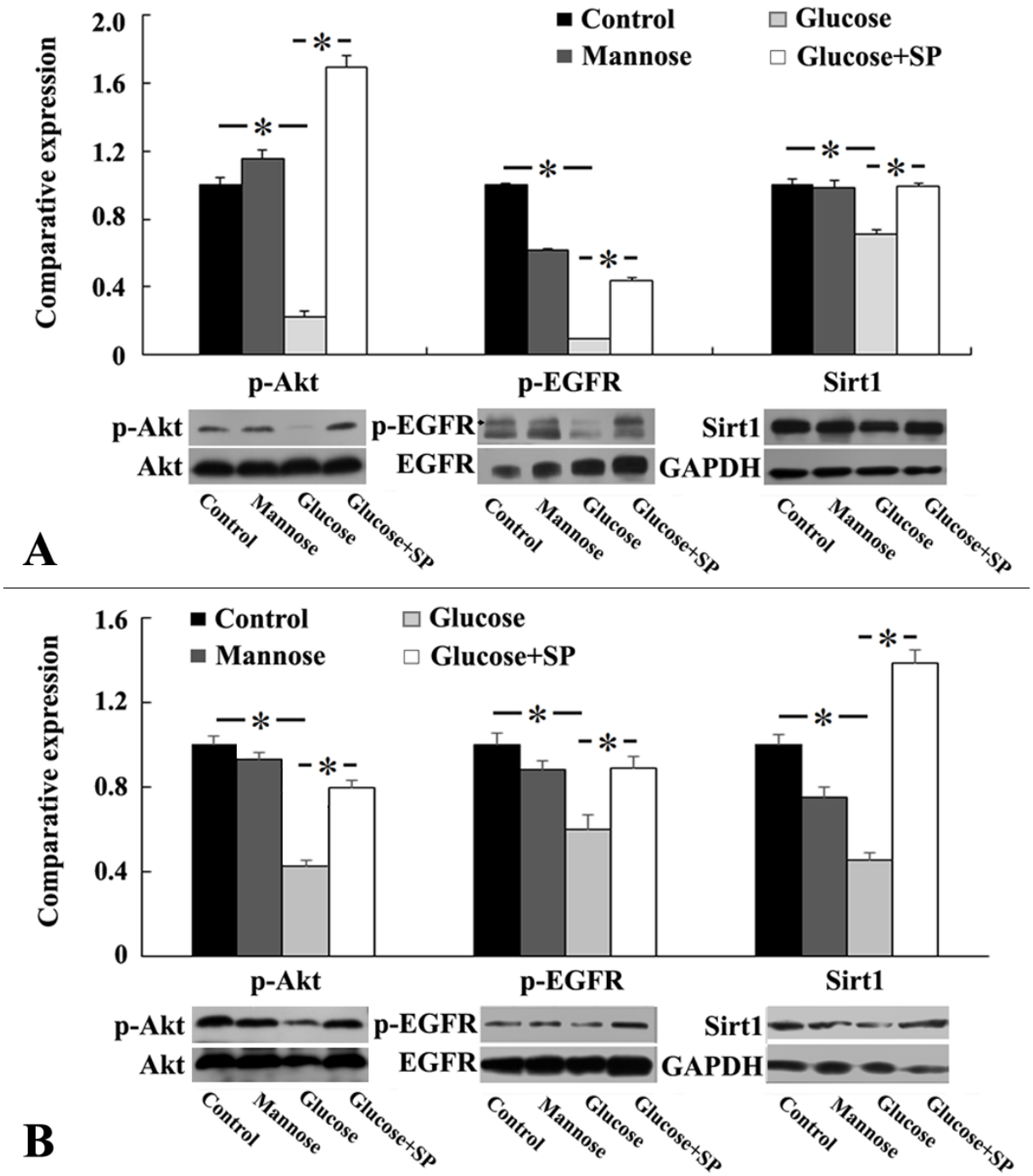


Supplementary Figure 2. Substance P promotes the early inflammatory and resolution response in diabetic cornea. At 48 h after corneal epithelium scrape, more inflammatory cell infiltration was found underneath the corneal epithelium margin of SP-treated diabetic mice as compared to untreated diabetic mice, whereas this response was reduced at 72 h when compared with diabetic mice (black lines represent the basal of regenerating corneal epithelium).



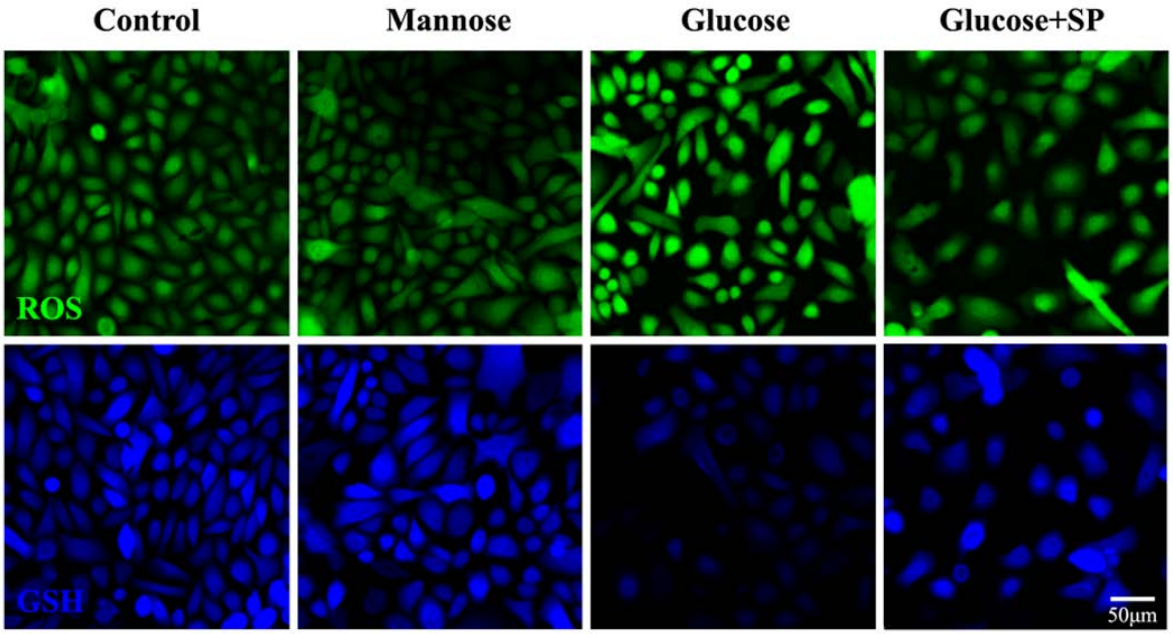
SUPPLEMENTARY DATA

Supplementary Figure 3. Substance P recovers p-Akt, p-EGFR and Sirt1 levels impaired by high glucose in cultured corneal epithelial cells. Mouse TKE2 (A) cells or human primary corneal epithelial cells (B) were treated with high glucose in the absence or presence of SP for 3 days, with mannose as osmotic control. SP recovered the expression levels of p-Akt, p-EGFR and Sirt1 that was impaired by high-glucose treatment in both mouse TKE2 cells (A, n=4 per group) and primary human corneal epithelial cells (B, n=3 per group). * $p < 0.05$, ns: no significance.



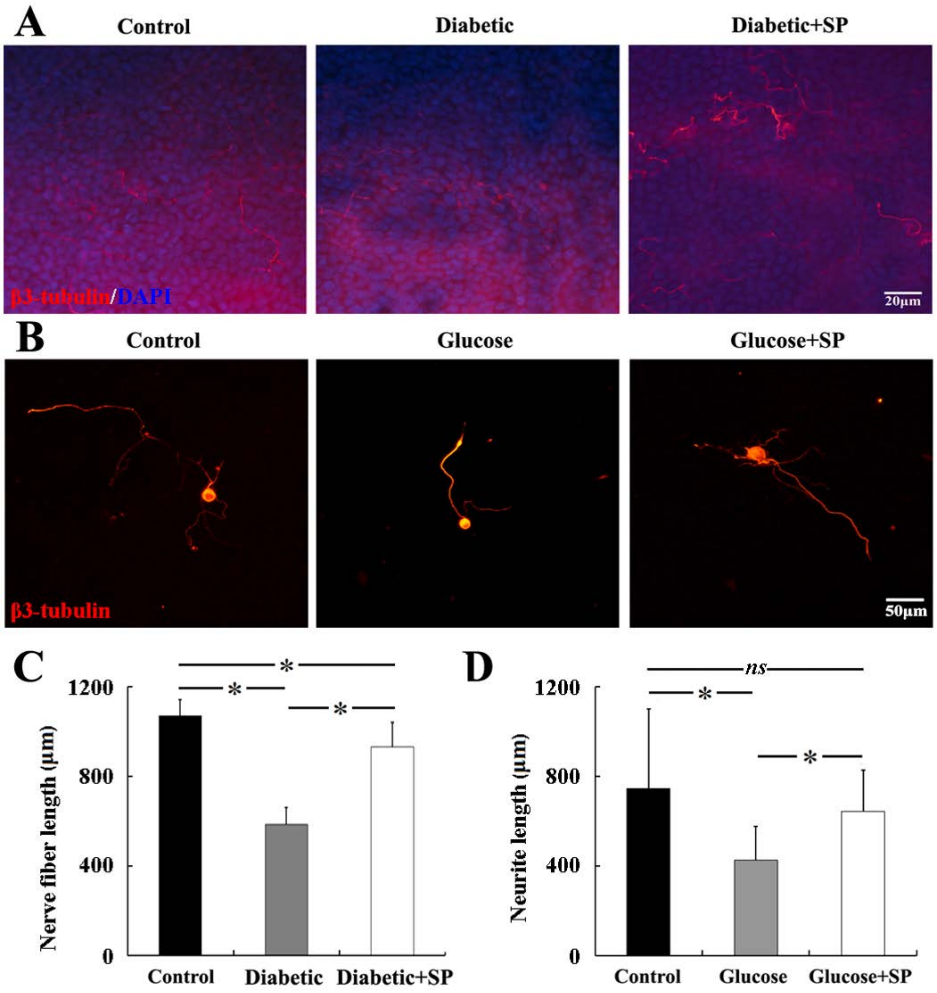
SUPPLEMENTARY DATA

Supplementary Figure 4. Substance P attenuates oxidative stress of primary human corneal epithelial cells induced by high glucose. Human primary corneal epithelial cells were treated with 30 mM glucose or mannose for 3 days in the presence or absence of SP. SP recovered the staining density of intracellular ROS and glutathione (GSH).



SUPPLEMENTARY DATA

Supplementary Figure 5. Substance P promotes the regeneration of diabetic corneal nerve fibers in vivo and the growth of high glucose-treated trigeminal ganglion cells in vitro. To explore the promotion of SP on corneal nerve fiber regeneration in diabetic mice, central corneal epithelium (marked with 2 mm trephine) was scraped after 6 times of topical SP application per day for 4 days. The SP application was continued with 6 times per day for another 3-4 days until the completion of corneal epithelial wound healing, subsequently with 1-2 times per day for 2 weeks until sample collection. The whole-mounted corneal staining with β3-tubulin antibody demonstrated that SP application significantly promoted the regeneration of nerve fibers in diabetic corneal epithelium (A, C, n=3 per group). In vitro, mouse trigeminal nerves were isolated and incubated in Neurobasal A medium as previous descriptions [1]. The fresh-isolated trigeminal neuronal cells were treated with 30 mM glucose for 3 days with or without 1 μM SP. The results demonstrated that SP accelerated neuronal growth that was impaired by high glucose, as showed by the immunofluorescence staining with β3-tubulin antibody (B) and the counting of total nerve fiber length (D, n=6 per group). * $p < 0.05$, ns: no significance.



References

[1] Malin SA, Davis BM, Molliver DC. Production of dissociated sensory neuron cultures and considerations for their use in studying neuronal function and plasticity. Nat Protoc. 2007;2:152–160.

SUPPLEMENTARY DATA

Supplementary Table 1. Primary antibodies for immunofluorescent staining and Western blots

Primary antibody	Residue	Supplier	Code
Substance P		Santa cruz	sc9758
p-AKT	pS473	Epitomics	2118-1
AKT	-	Epitomics	1085-1
p-EGFR	pY1068	Epitomics	1727-1
EGFR	-	Epitomics	1902-1
Sirt1	-	AbCam	Ab12193
NQO1	C-terminus	AbCam	Ab34173
Catalase	-	AbCam	Ab16731
MnSOD	-	AbCam	Ab13533
Ki-67		Abcam	Ab15580
β3-tubulin		R&D	NL1195R
Alexa Fluor 488 donkey anti-rabbit IgG		Life technologies	A21206
Alexa Fluor 594 donkey anti-rabbit IgG		Life technologies	A21207
Donkey anti-goat IgG-CFL 488		Santa cruz	sc362255

Supplementary Table 2. Primer sequences for qRT-PCR

Gene	Accession number	Forward primer	Reverse primer
MnSOD	NM_013671.3	GTGGAGAACCCAAAGGAGAG	AACCTTGGACTCCCACAGAC
Catalase	NM_009804.2	GTCTTCGTCCCGAGTCTCTC	CTGCCTCTCCATCTGCATTA
NQO1	NM_008706.5	GGCCCATTCAGAGAAGACAT	TTCGAGTACCTCCCATCCTC
GCLC	NM_010295.2	CAGCACGTTGCTCATCTCTT	TTTGGAGGAGGAGGCTTAAA
TXN	NM_011660.3	TGGTGGACTTCTCTGCTACG	CTTCACAGTCTGCAGCAACA
Hmox1	NM_010442.2	GCACTAGCTCATCCCAGACA	CATGGCATAAATTCCCACTG
GLRX	NM_053108.4	CCTCAGTCAACTGCCTTTCA	CTCCGGTGAGCTGTTGTAAA