Supplementary Figures and Figure Legends

Supplementary Figure 1. Representative fluorescent microscopic images of TUNEL in 6-week SD rat retinas. Intravitreal injection of EPO protected retinal neurons from cell death. Compared with that in normal control, more TUNEL positive cells were detected in the 6-week diabetic rat retinas, which were decreased after intravitreal injection of EPO (16 mU/eye). The exposure time is 400 ms (TUNEL staining, green) and 30 ms (DAPI staining, blue); and the magnification is 200 x. NC: negative control; PC: positive control; N: normal 6-week retina; D6w: 6-week diabetic retina; E: EPO-treated 6-week retina.
Supplementary Figure 2. The immunostaining of PAR polymer in 4 and 6 weeks SD rat retinas. EPO reduced PAR polymer levels in diabetic rat retina. PAR polymer expressions were increased in 4 and 6 weeks diabetic rat retinas, which were decreased by EPO. The exposure time is 400 ms (PAR polymer staining, red); and the magnification is 200 x. N4w: normal 4-week retina; D4w: 6-week diabetic retina; E4w: EPO-treated 4-week retina; N6w: normal 6-week retina; D6w: 6-week diabetic retina; E6w: EPO-treated 6-week retina.
Supplementary Figure 3. Western blot result of GLAST and GS protein expression with the progression of diabetes. (A) The time-dependent decreased expression of GLAST with the progression of diabetes from 2-week to 6-month. (B) The time-dependent decreased expression of GS with the progression of diabetes from 2-week to 6-month. Data were expressed as mean ± SE (n = 6), β-actin was used as the internal control. * means P < 0.05 when compared with normal control group. N: normal control; D2w: 2-week diabetic retina; D1m: 1-month diabetic retina; D2m: 2-month diabetic retina; D4m: 4-month diabetic retina; D6m: 6-month diabetic retina.
Supplementary Figure 4. Western blot result of NR1 protein expression in 4-month normal (N) and 4-month diabetic retinas (D4m). Compared with that in normal control, the protein level of NR1 in D4m was increased. Data were expressed as mean ± SE (n = 8), β-actin was used as the internal control. * means P < 0.05 when compared with normal control group.
Supplementary Figure 5. Immunostaining of NR1 in the 6-week retinas. Compared with that in normal control, the protein levels of NR1 in 6-week diabetic rat retinas were increased, which were decreased after intravitreal injection of EPO (16 mU/eye). The exposure time is 200 ms (NR1, green) and 30 ms (DAPI, blue), respectively; and the magnification is 200 x. NC: negative control; N: normal retina; D6w: 6-week diabetic retina; E: EPO-treated retina; GCL: ganglion cell layer; INL: inner nuclear layer; ONL: outer nuclear layer.
Supplementary Figure 6. Western blot and immunostaining of caspase-3 in retinas. Compared with that in normal control, the protein levels of caspase-3 in diabetic and EPO-treated group had no significantly changes. (A and B) The protein changes of caspase-3 in the 2 and 4 weeks retinas. Data were expressed as mean ± SE (n = 10), β-actin was used as the internal control. (C) Immunostaining of caspase-3 in the 2-week retinas. The exposure time is 300 ms (caspase-3, green), and 30 ms (DAPI, blue), respectively; and the magnification is 200 x. NC: negative control; N: normal retina; D2w: 2-week diabetic retina; E: EPO-treated retina; GCL: ganglion cell layer; INL: inner nuclear layer; ONL: outer nuclear layer. (D) Immunostaining of caspase-3 in the 2, 4 and 6-week retinas. The exposure time is 300 ms (caspase-3, green), and 30 ms (DAPI, blue), respectively; and the magnification is 200 x. NC: negative control; N4w: normal 4-week retina; D2w: 2-week diabetic retina; D4w: 6-week diabetic retina; D6w: 6-week diabetic retina.
Supplementary Figure 7. EPO protection of retinal neurons from cell death (A), and reduction of the retinal glutamate levels (B) in 2-week diabetic rats. Data were expressed as mean ± SE (n = 8), *P < 0.05 when compared to 2-week diabetic rats (D2w). N, Normal control; D2w, 2-week diabetic rats; EPO, EPO-treated diabetic rats; GCL, ganglion cell layer; INL, inner nuclear layer; ONL, outer nuclear layer. Magnification: X200.
Supplementary Figure 8. (A) Glutamate effects on R28 cell viability. R28 cells were treated with different concentrations of glutamate for 24 hours, and cell viability was measured with MTT assay. (B) EPO protection on R28 cells treated with glutamate (10 mM). R28 cells incubated with 10 mM glutamate were treated with different concentrations of EPO for 24 hours, and the cell viability was measured with MTT assay. Each point was expressed as mean ± SE. (n=6). *P<0.05 when compared to the cells incubated with glutamate only. (C) The morphology of R28 cells treated with or without EPO. Cont, normal control; Glu, glutamate (10 mM) treatment for 24 hours; Glu+EPO, glutamate (10 mM) and EPO (0.2 U/mL) treatment for 24 hours. (D) EPO (0.2 U/mL) protection of R28 cells from cell death detected with TUNEL assay (same condition as in Fig. C). Magnification: X200.
Supplementary Figure 9. The changes of GS (A, C) and GLAST (B, D) in diabetic rat retinas treated with or without exogenous EPO. N, Normal control; D2w, 2-week diabetic rats; EPO, EPO-treated diabetic rats. Data were expressed as mean ± SE. (n = 6), *P<0.05 when compared to D2w. Magnification: X200.
Supplementary Figure 10. The mRNA changes of Glutamate receptors in diabetic rat retinas treated with or without exogenous EPO. Glutamate receptors, such as KA1 (A), KA2 (B), NR1 (C) and other iGluRs (D), i.e., GluR1-4, NR 2A-C, NR-2D, GluR 5, GluR 6, GluR 7 were amplified with PCR. N, Normal control; D2w, 2-week diabetic rats; EPO, EPO-treated diabetic rats. Data were expressed as mean ± SE. (n = 6), *P<0 .05 when compared to D2w.
Supplementary Figure 11. The changes of KA1 (A, C) and NR1 (B, D) in diabetic rat retinas treated with or without exogenous EPO. N, Normal control; D2w, 2-week diabetic rats; EPO, EPO-treated diabetic rats. Data were expressed as mean ± SE. (n = 6), *P<0.05 when compared to D2w. Magnification: X200.
Supplementary Figure 12. The changes of glutamate receptors KA1 (A, C) and NR1 (B, D) in R28 cells treated with or without EPO. Cont, Normal control; Glu, glutamate (10 mM) incubation for 24 hours; Glu+EPO, glutamate (10 mM) and EPO (0.2 U/mL) incubation for 24 hours. Data were expressed as mean ± SE. (n = 6), *P<0.05 when compared to cells treated with glutamate only. Magnification: X630.