**Supplementary Material**

**Supplementary Table S1**

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| **Antibodies used in immunohistochemical studies** |
| **Primary antibodies** |
| **Antibody** | **Company/Code/Lot Number** | **Working dilution** |
| Nitrotyrosine (NT) | EMD Millipore/ 06-284/ 3199176 | 1/1000 |
| Caspase-3 | Cell Signaling/ 9661S/ No 47 | 1/300 |
| Neurofilament (NFL) | EMD Millipore/ MAB1615/ 2736736 | 1/500 |
| Anti-Brain Nitric Oxide Synthetase (bNOS) | Sigma/ N7280/048M4805V  | 1/2000 |
| Anti-Glial Fibrillary Acidic Protein (GFAP) | Sigma/ G3893/ 083M4785 | 1/2000 |
| Anti-Ionizing Calcium Binding Adaptor Molecule 1 (Iba-1) | Wako Chemicals/ 019-19741 | 1/2500 |
| **Secondary Antibodies** |
| CF543 goat anti-rabbit IgG | Biotium/ 20309/ 12C0213 | 1/1000 |
| CF488A goat anti-mouse IgG | Biotium/ 20010/ 13C0619 | 1/400 |
| **Antibodies used in Western blot analysis** |
| **Primary antibodies** |
| **Antibody** | **Company/Code/Lot Number** | **Working dilution** |
| Vascular Endothelial Growth Factor (VEGF) | Santa Cruz Biotechnology/ sc-7269/ L1020 | 1/100 |
| Anti-Glial Fibrillary Acidic Protein (GFAP) | Sigma/ G3893/ 083M4785 | 1/1000 |
| GAPDH  | Cell Signaling/ 2118/ 10 | 1/1000 |
| **Secondary antibody** |
| **Antibody** | **Company/Code/Lot Number** | **Working dilution** |
| HRP-goat anti- mouse IgG  | EMD Merck Millipore/ AP124P/ 3255010 | 1/10000 |
| HRP-goat anti-rabbit IgG | Invitrogen/ 656120/ 14286894 | 1/5000 |

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| **Primer sequences used in quantitative real-time PCR analysis** |
| **Gene** | **Forward primer** | **Reverse primer** |
| IL-1β | CCTCAAGGGGAAGAATCTAT | GAGGTGCTGATGTACCAGTT |
| IL-6 | GGGACTGATGTTGTTGACAG | TGTTCTTCACAAACTCCAGG |
| VEGF164 | GCACATAGGAGAGATGAGCT | TGCAGCATAGCAGATGTGAATGCAGACC |
| NOX4 | CAGTCAAACAGATGGGATACAGA | ATAGAACTGGGTCCACAGCAGA |
| β-actin | CTAAGGCCAACCGTGAAAAG | TACATGGCTGGGGTGTTGA |

**Figure Legends**

**Supplementary Figure 1.** Effect of GLX7013114 on the oxidative damage in the five-wk model of DR. *A:* Representative images of ΝΤ-IR in control, diabetic non treated and diabetic treated [GLX7013114 (10mg/ml, 20μl/eye)] rat retinas. DAPI and Merge are also presented in each case. Magnification x20. Scale bar 20μm. *B:* Quantification study of the immunohistochemical images of NT-IR in control, diabetic non treated and diabetic treated rat retinas. NOX4 inhibition by GLX7013114 attenuated the number of NT positive cells observed in the diabetic retina, 5 weeks post STZ injections (control: n= 3, diabetic non treated: n=3, diabetic treated: n=3, \*\*\*p <0.001 compared to control, ###p <0.001 compared to diabetic non treated).

**Supplementary Figure 2.** Effect of GLX7013114 on the expression of caspase-3 in the five-wk model of DR. Representative images of caspase-3-IR in control, diabetic non treated and diabetic treated [GLX7013114 (10mg/ml, 20μl/eye)] rat retinas, 5 weeks after the STZ administration. Magnification x20. Scale bar 20μm.

**Supplementary Figure 3.** Evaluation of the effect of GLX7013114 on bNOS positive amacrine cells in the DR models. *A, B:* Representative images of bNOS immunohistochemical images in control, diabetic non treated and diabetic treated [GLX7013114 (10mg/ml, 20μl/eye)] rat retinas in the 2-wk and the 5-wk DR model, respectively. Magnification x40. Scale bar 20μm. Arrows depict bNOS positive cells. *C, D:* Quantification of bNOS-IR in control, diabetic non treated and diabetic treated rat retinas in the 2-wk and the 5-wk model of DR, respectively. In both models, diabetes caused a significant reduction in the number of bNOS positive cells in the retina [(2-wk model: control: n= 8, diabetic non treated: n=8) (5-wk model: control= 4, diabetic non treated=4), \*\*p <0.01, \*\*\*p <0.001 compared to control)], effect that was attenuated by GLX7013114 [(2-wk model: diabetic treated: n=9) (5-wk model: diabetic treated: n=4), ##p <0.05, ##p <0.01 compared to diabetic non treated)].

**Supplementary Figure 4.** Effect of GLX7013114 on microglia in the 5-wk DR model. Representative images of Iba-1-IR in control, diabetic non treated and diabetic treated [GLX7013114 (10mg/ml, 20μl/eye)] rat retinas. DAPI and Merge are also presented in each case. Magnification x20. Scale bar 20μm. Arrows depict activated Iba-1 positive cells. \*Depicts artifact.

**Supplementary Figure 5.** Evaluation of the effect of GLX7013114 on macroglia in the 5-wk model of DR. Representative images of GFAP immunohistochemical staining in control, diabetic non treated and diabetic treated [GLX7013114 (10mg/ml, 20μl/eye)] rat retinas. Magnification x20. Scale bar 20 μm.

**Supplementary Figure 6.** Pharmacokinetic study of GLX7013114 in rat retina. GLX7013114 (10mg/ml, 20μl/eye) was administered in healthy rats and its concentration was determined in 7 different time-points: 0.25h, 0.5h, 1h, 2h, 4h, 8h and 24h (n=4). The normalized proportion of the GLX7013114 concentration to mg of retinal tissue (pmol/mg) was expressed as a function of time. The highest concentration in the retina was detected 30 min after administration, while a significant reduction was observed in the time-points that follow (\*\*p < 0.01 compared to the concentration at 30m).