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Appendix A. Supplementary materials

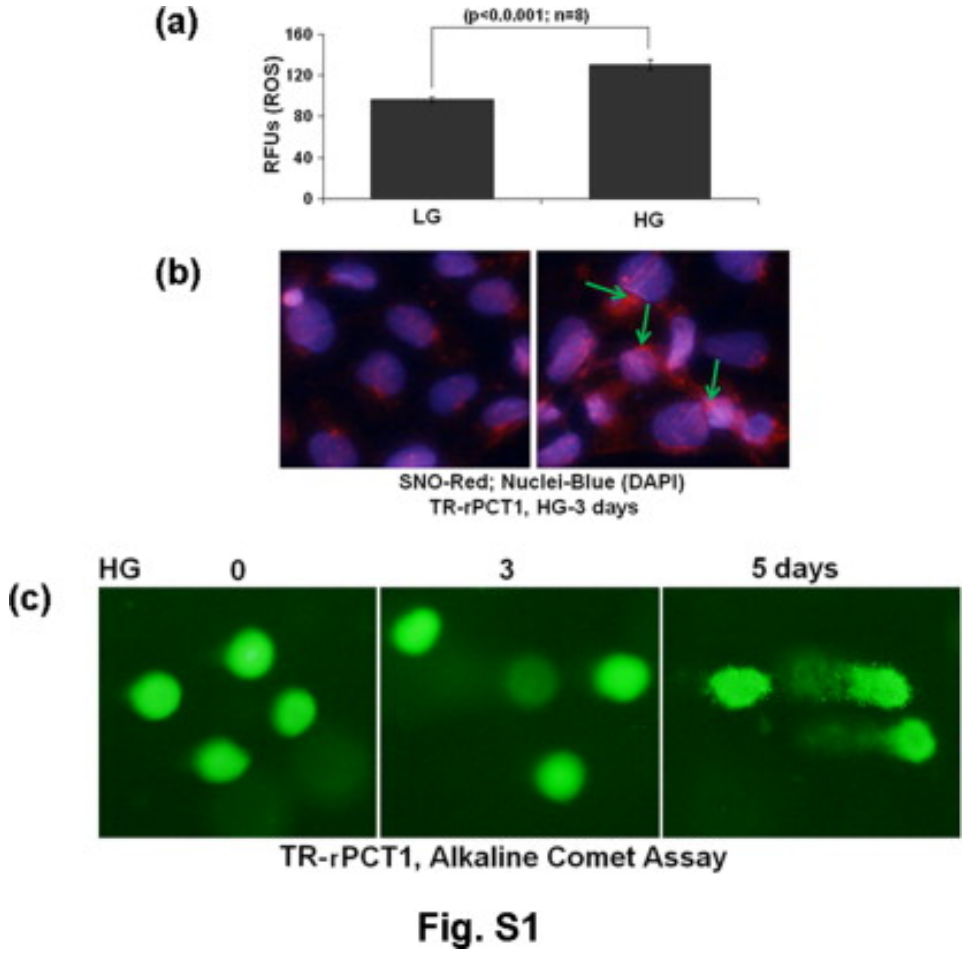

Fig. S1

Fig. S1 **HG induces ROS and RNS in retinal pericytes at day 3 but not chromatin breakage.** (a) ROS determination by CM-H2DCFDA. HG is able to increase ROS level in pericytes at day 3 (p<0.001; n=6 vs. LG). (b) Similarly, HG also increases protein-SNO at day 3 (arrows). A representative of n=3 is shown. (c). HG however does not induce chromatin damage in retinal pericytes at day 3 while chromatin break occurs at day 5. A representative n=3 is shown. We also observe TXNIP up regulation at day 3 as well (not shown).

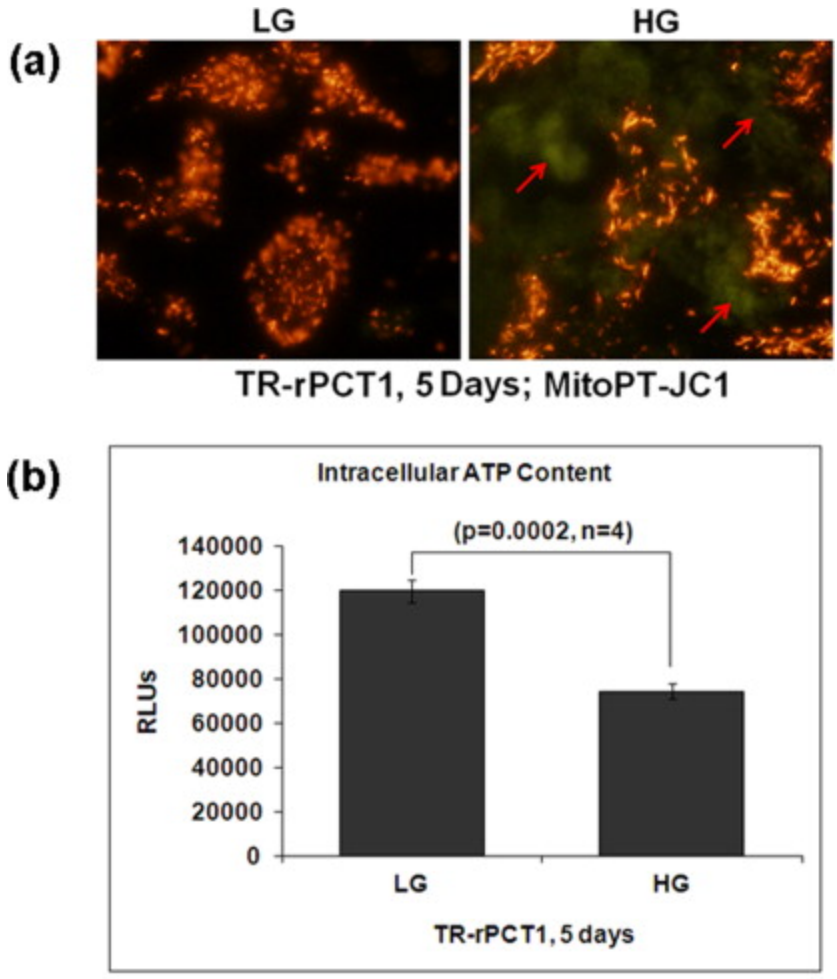

Fig. S2

Fig. S2 **HG induces mitochondrial membrane depolarization and ATP reduction in retinal pericytes**. (a) Mitochondrial membrane depolarization and damage was measured by MitoPT™ JC-1 (Immunochemistry Technologies). Non-apoptotic cells exhibit as orange-red stained mitochondria (larger arrows in LG) while apoptotic cells with mitochondrial membrane potential depolarization stains green (smaller arrows in HG). A representative of n=3 is shown here. (b) Intracellular ATP levels were measured by an ATP bioluminescence assay kit. TR-rPCT1 cells were incubated with HG or LG for 5 days in 24 well plates and intracellular ATP levels were determined by the boiling method as described in Materials and Methods. There is a significant decrease in cellular ATP levels in the presence of HG (~62% of LG; p<0.0002; n=8) when compared with that of LG.

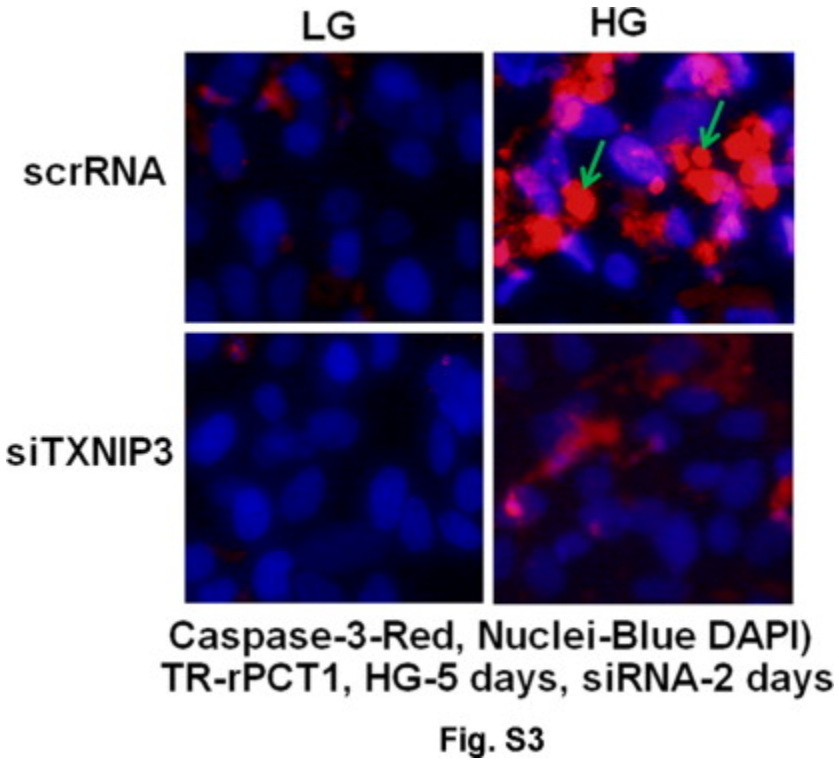

Fig. S3

Fig. S3 **TXNIP siRNA prevents caspase-3 activation.** HG activates caspase-3 in retinal pericytes transfected with srcRNA as revealed by enhanced staining of SR FLIVO (arrows); however, caspase-3 staining is reduced in siTXNIP3 transfected cells (right panels). Under LG, caspase-3 was not activated either in scrRNA or siTXNIP3 transfected cells (left panels). A representative of *n*=3 is shown.

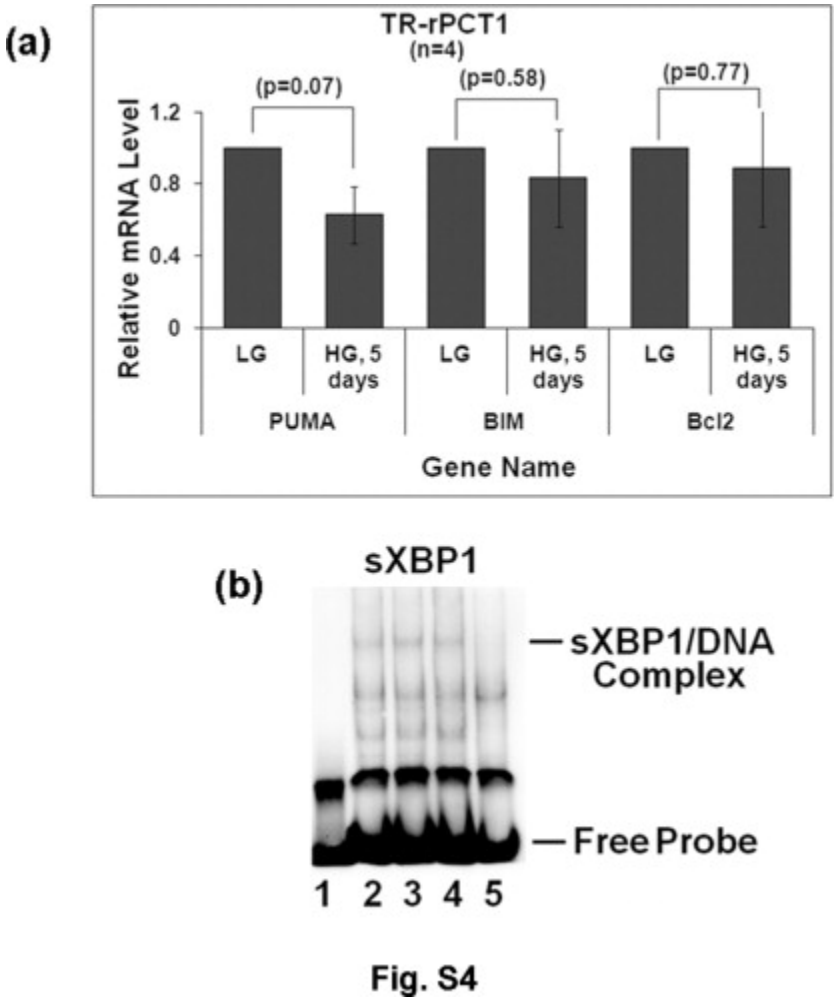

Fig. S4

Fig. S4 **HG does not increase pro-apoptotic Bim and PUMA mRNA expression and ER-stress/UPR mediator sXBP1 activity in pericytes**. (a) Quantitative RT-PCR for pro-apoptotic Bim, Puma and pro-survival Bcl2. No significant changes in Bim, PUMA, and Bcl2 mRNA levels were observed. (b) EMSA for spliced (active) XBP1. EMSA shows no change in the DNA binding activity of sXBP1 between LG and HG. Lane 1. Probe alone; Lane 2. LG; Lane 3. HG; Lane 4. Mannitol; and Lane 5. HG+cold probe (*n*=3).