Placental growth factor negatively regulates endothelial cell barrier function through suppression of glucose-6-phosphate dehydrogenase and anti-oxidant defense systems

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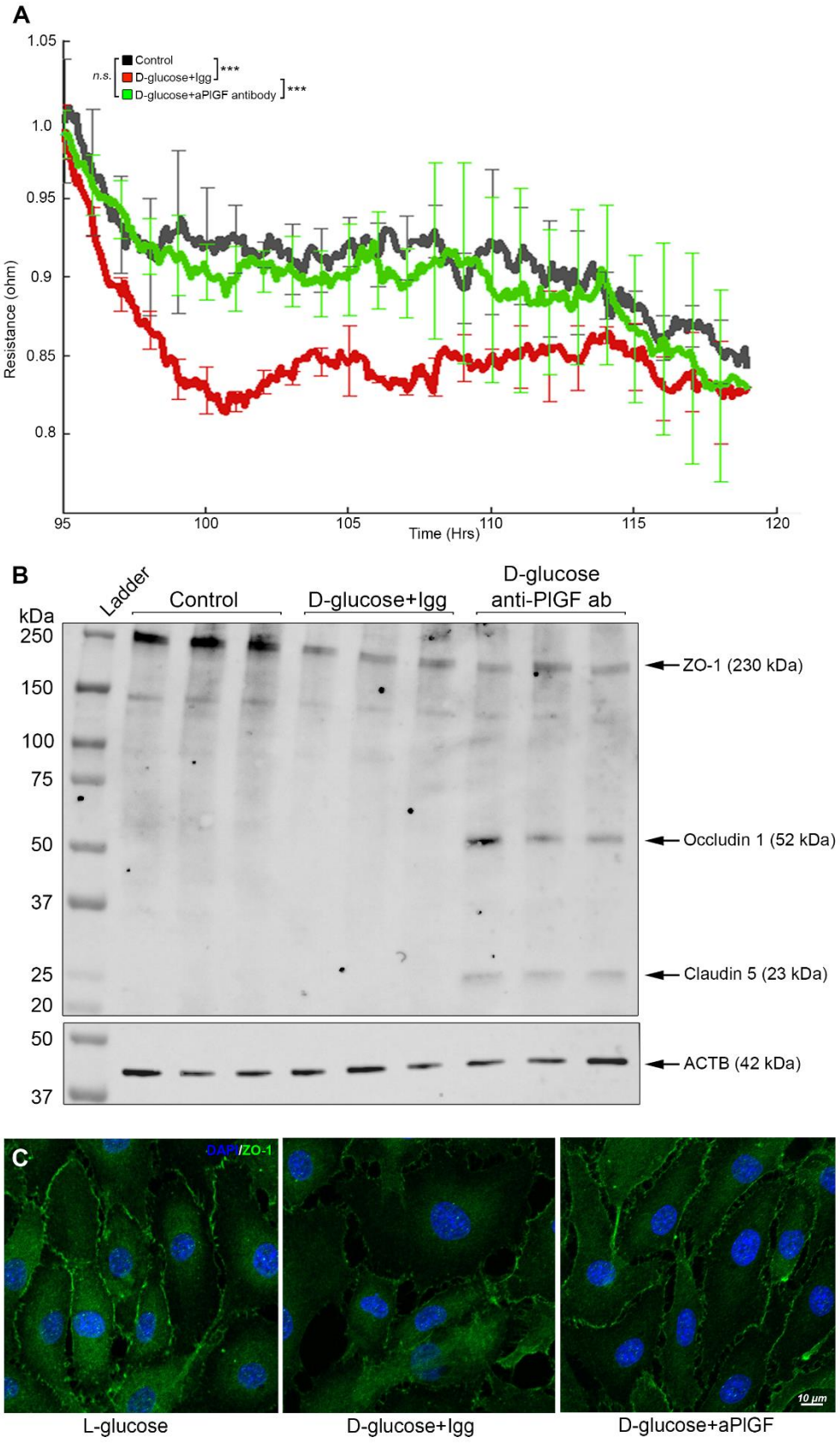
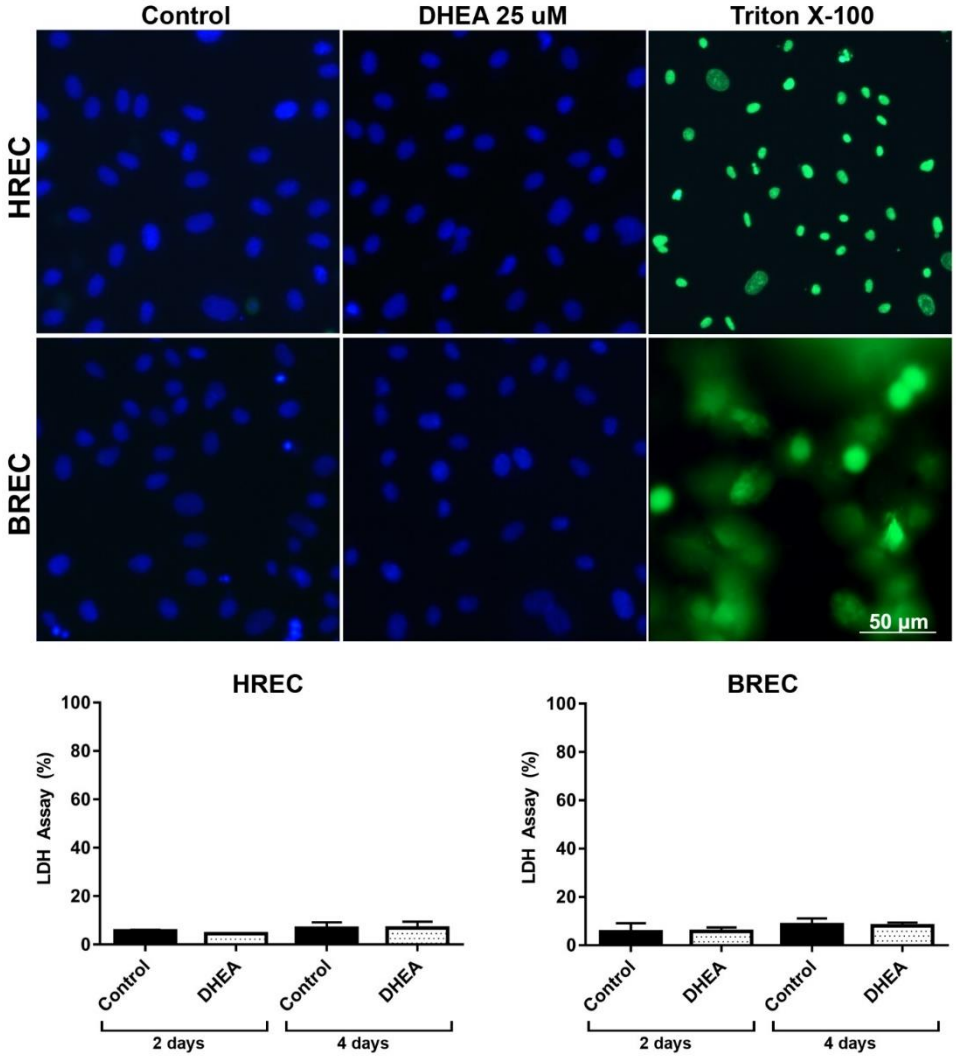
Supplementary Figures

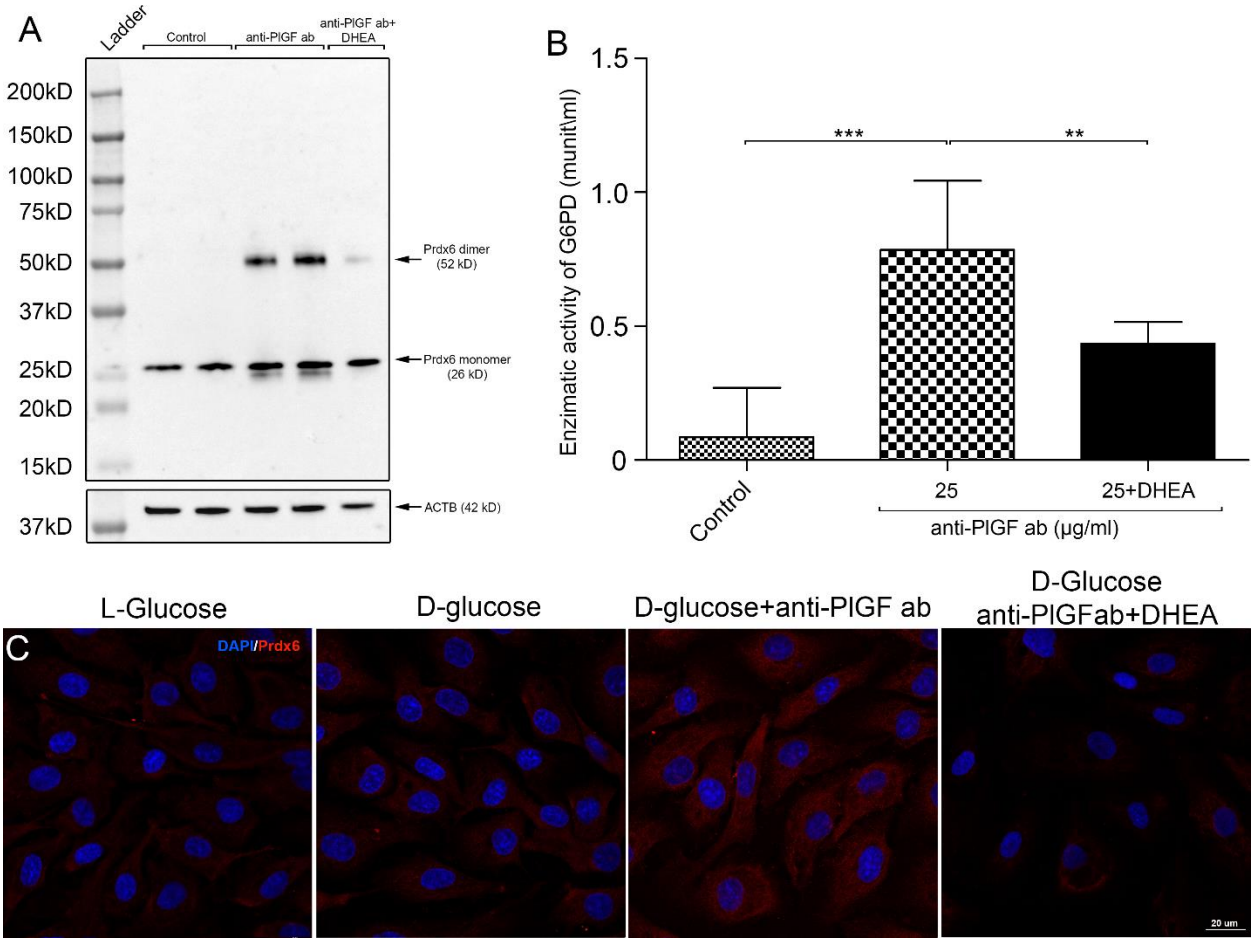
Supplementary Figure 1: PlGF neutralization prevents bovine retinal endothelial cell (BREC) barrier dysfunction by high glucose. (A) The TEER of BREC under conditions of high glucose: (HG: 25mM D-glucose + IgG control) (red), 25mM L-glucose + IgG control (gray), and 25mM D-glucose + αPlGF antibody (green). Note that TEER began dropping from HG at about 5 days (95 h). *** denotes $p < 0.001$; n.s. stands for no significance ($p > 0.05$). (B) The protein levels of ZO-1, Occludin-1, and Claudin-5 of BREC under normal glucose control, HG + Igg and HG + PlGF antibody. (C) Immunofluorescence staining results of ZO-1: Note that HG reduced ZO-1 protein disintegration in the plasma membrane, which however was prevented by PlGF antibody treatment.


A
1.05
1.0
0.95
0.9
0.85
0.8
Resistance (ohm)
n.s.
Control
D-glucose+Igg
***
D-glucose+aPlGF antibody
***
95
100
105
110
115
120
Time (Hrs)
B
Ladder
Control
D-glucose+Igg
D-glucose
anti-PlGF ab
kDa
250
150
100
75
50
37
25
20
50
37
ZO-1 (230 kDa)
Occludin 1 (52 kDa)
Claudin 5 (23 kDa)
ACTB (42 kDa)
C
DAPI/ZO-1
10 μm
L-glucose
D-glucose+Igg
D-glucose+aPlGF

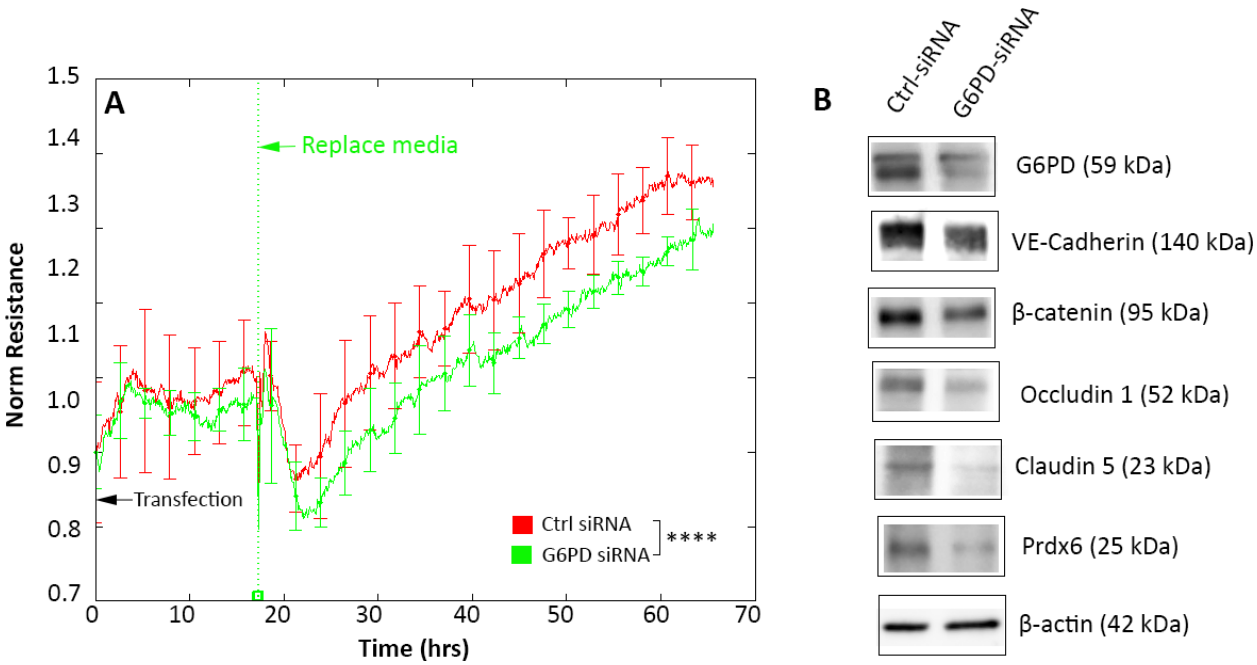
Supplementary Figure 2. The minimal cytotoxic effect of DHEA on the cells. Confluent human and bovine retinal endothelial cell (HREC and BREC) were treated with PBS DMSO control, DHEA (25 µM), and Triton X-100. Cytotoxic assay was performed as described in methods. (A) The results of fluorescent probe-based cell viability assay. Blue fluorescent staining by live regent showed the live cell numbers. Green fluorescent staining by dead reagent indicates dead cells. (B and C) The cell viability demonstrated by LDH activity in the cell culture media. The results were expressed as the mean cell viability percentage relative to controls. The experiments were repeated three times.



Supplementary Figure 3. G6PD inhibition (DHEA) diminishes elevated Prdx6 protein expression and G6PD activity in bovine retinal endothelial cells (BRECs). (A) Western blotting results of Prdx6: DHEA diminished the elevated level of PRDX6 protein expression (both monomer and dimer) due to PlGF antibody in BREC. (B) G6PD enzyme activity: DHEA diminished the elevated PRDX6 enzyme activity due to PGF antibody (25 µg/ml) in BREC. (C) Immunofluorescence staining of Prdx6: DHEA diminished the elevated Prdx6 protein expression through PGF inhibition in BREC.



Supplementary Figure 4. G6PD siRNA reduces HREC resistance and down-regulates barrier function protein expression levels. After HREC grew to about 80-90% confluence, transfection of control siRNA and G6PD siRNA was performed with lipofectamine. About 18 h after transfection, culture media were replaced with fresh media. Anti-PlGF antibody (100µg/ml) was supplemented to boost EC barrier function and junction formation. (A) HREC TEER was monitored with ECIS system in real time. Normalized resistances of control siRNA (red) and G6PD siRNA (green) are shown. N=4 for each condition. (B) G6PD siRNA effectively down-regulated the protein levels of G6PD, tight junction and adhesion proteins (VE-Cadherin, β-catenin, ZO-1, Claudin-5, Occludin-1), as well as PRDX6. β-Actin was used as protein loading control.



Supplementary Figure 5. Recombinant human (rh) PlGF down-regulates G6PD and PRDX6 in human retinal endothelial cells (HRECs). Confluent HRECs were treated with PBS control or rhPlGF protein for 48 h. (A) Double immunofluorescence staining of G6PD and PRDX6 in the PBS control condition: Note co-localization of the two proteins in cytoplasm (yellow). (B & C) Double immunostaining of G6PD and PRDX6 in the rhVEGF (B) and rhPlGF (C) treatment condition: Note the reduced staining signal in rhPlGF compared to PBS control and rhVEGF.