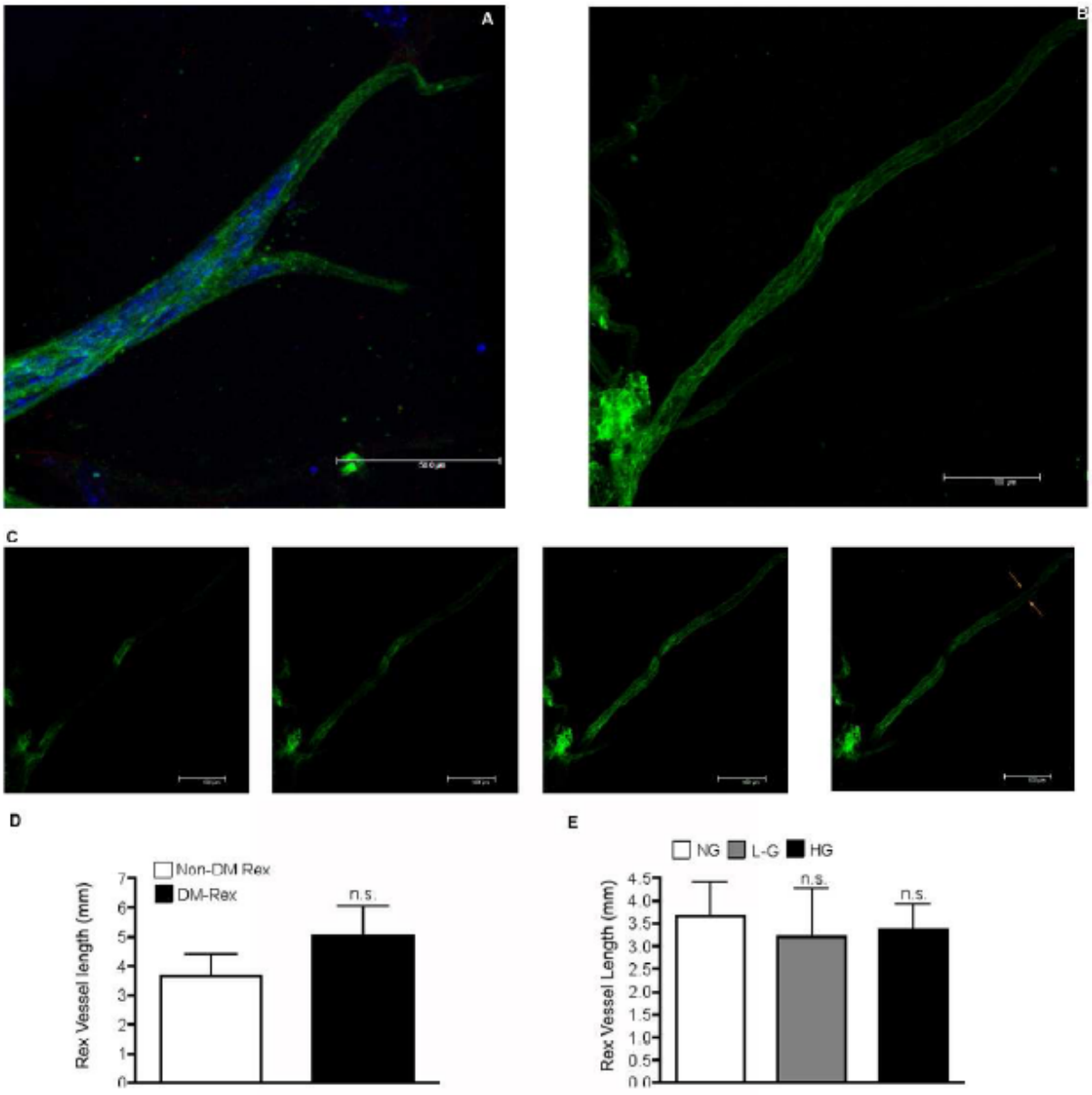
SUPPLEMENTARY DATA

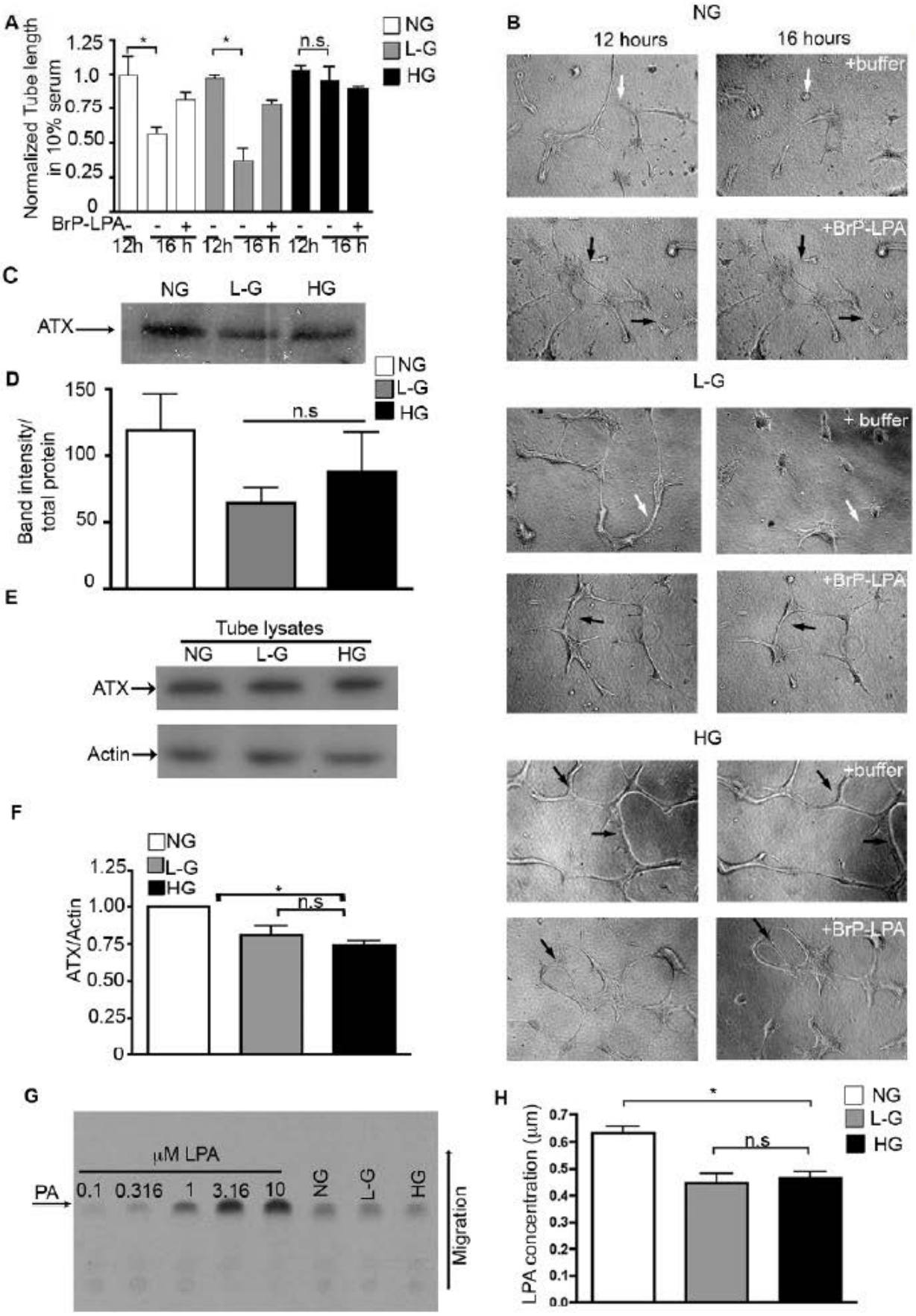
Supplementary Figure 1. Characterization of Rex vessels.
Retinal explants were cultured between two layers of collagen and overlaid with EBM supplemented with VEGF-A (25 ng/ml) for 2-3 weeks. The tissue was fixed and stained with the endothelial marker isolectin B4 (IB4, green) (A). All outgrowths were positive for this marker. No cells were detected with anti-NG-2, which recognizes pericytes (results not shown). The absence of DAPI-stained nuclei (blue) adjacent to the neo-vessels further supported the conclusion that these vessels did not contain pericytes. (B) Complete Z-stack of a vessel. (C) Subsets of the Z-stack shown in panel B that indicate lumenized segments of the vessel (orange arrows). The absence of blood flow in this ex vivo setting may be the reason why the entire vessel does not have a lumen. (D/E) Quantification of Rex vessel length. While the DM group showed a tendency to form more vessels, the difference did not reach statistical significance. (n.s. $p>0.05$). Note that "Non-DM Rex" is the same as "NG Rex". Data shown are + SEM of five experiments.



SUPPLEMENTARY DATA

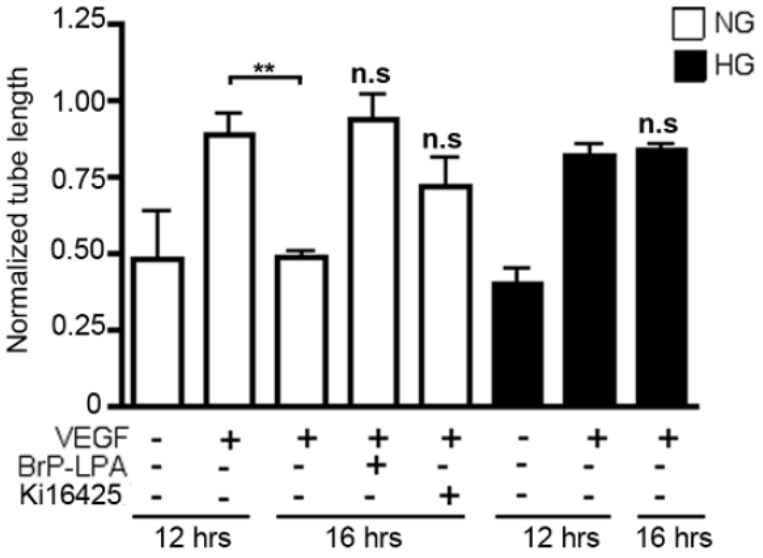
Supplementary Figure 2. Hyperglycemia induced resistance to endogenously produced LPA, and did not interfere with its production.
(A) Quantification of four independent tube assays in the presence of 10% serum. Bovine retinal endothelial cells (BREC) were pre-treated with normal glucose (5 mM, NG, white bars), L-glucose (25 mM, L-G, grey bars) or high glucose (25 mM, HG, black bars). As previously reported (12) tubes in the NG group spontaneously regressed, and an LPA receptor inhibitor (BrP-LPA) blocked this event. L-G tubes behaved in the same fashion. In contrast HG tubes failed to regress, and were not affected by the presence of BrP-LPA. (B) Representative photographs of tube that organized from NG, L-G and HG BRECs; white arrows and black arrows point to tubes that did or did not, respectively, regress. (C) Representative ATX Western blot of conditioned media collected from a tube assay performed in low serum (0.5%) to minimize the contribution of ATX from the serum. Conditioned medium containing the same amount of total protein was analyzed. (D) Results from 4 independent experiments were quantified and are presented. (E) Same type of experiment as in panel C, expect the cell lysate was analyzed. (F) Quantification of replicate experiments indicated that intracellular ATX was significantly lower in HG and L-G tubes compared to the NG group. However, no significant difference was found in HG vs. L-G. (G) Representative thin-layer chromatography determination of LPA content in tube assay. (H) Quantification of four independent LPA assays. HG tubes had significantly less LPA than the NG group. No significant differences were detected when HG and LG groups were compared. All bars are +SEM. *$p<0.05$, "n.s." no significant difference.

SUPPLEMENTARY DATA

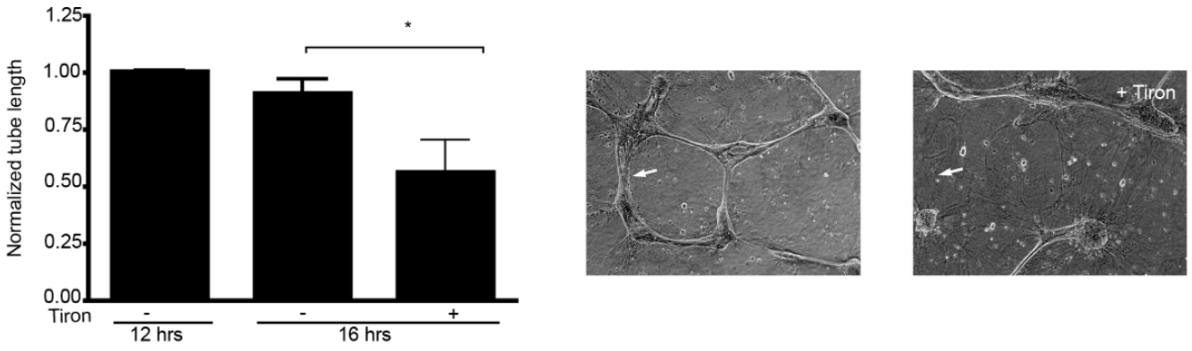


SUPPLEMENTARY DATA

Supplementary Figure 3. Hyperglycemia blunted LPA-responsiveness in tubes organized from human retinal endothelial cells.
Human retinal endothelial cells were pre-treated with 5 mM (NG, white bars), or 25 mM D-glucose, (HG, black bars) for 10 days and subjected to a tube assay under conditions that promote spontaneous regression (10% serum). Tubes formed by NG-HREC spontaneously regressed, whereas HG-HREC tubes did not. Addition of the LPA receptor inhibitor BrP-LPA (10 μM), or Ki16425 (5 μM) after tubes had formed prevented regression. All bars are + SEM. The experiment was repeated three times, * $p<0.05$.



Supplementary Figure 4. Antioxidant treatment re-established LPA sensitivity in HG-BREC.
Tubes formed by HG-BRECs were subjected to a tube assay, and treated with the antioxidant Tiron. (A) Quantification of four independent experiments showed a significant regression in the presence of Tiron. (B) Representative photographs of tubes treated with Tiron or buffer.



SUPPLEMENTARY DATA

Supplementary Figure 5. MLC2 phosphorylation was reduced in HG-tubes.
The phosphorylation of myosin light chain 2 (MLC2) was evaluated by Western blot in lysates from tubes organized under conditions that were permissive for spontaneous regression (10% serum). Densitometric analysis of three independent experiments revealed a significant decrease in MLC2 phosphorylation in HG tubes compared to NG and LG structures.