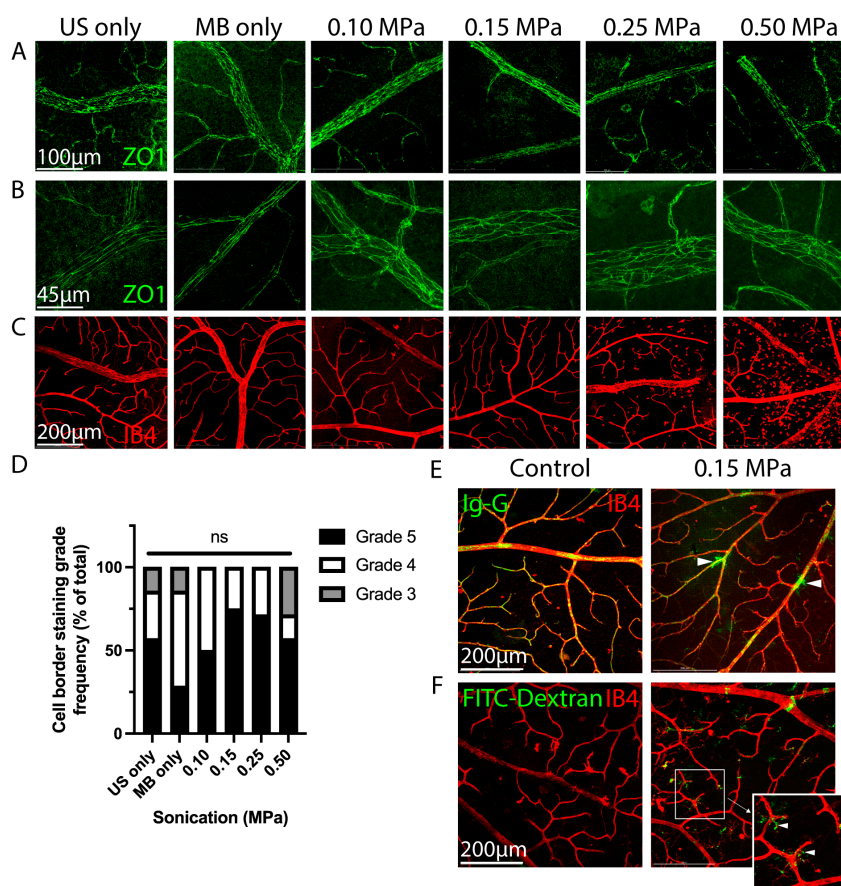


Article

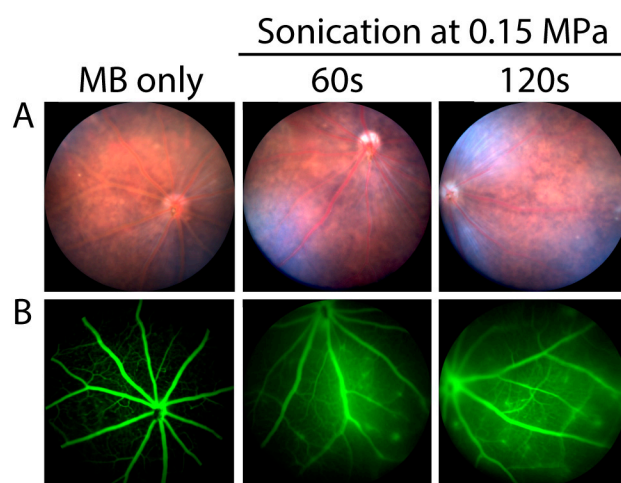
Efficacy and Safety of Low-Intensity Pulsed Ultrasound-Induced Blood–Retinal Barrier Opening in Mice

Alexandre Bourdin ^{1,†}, Manon Ortoli ^{1,†}, Remi Karadayi ¹, Lauriane Przegralka ¹, Florian Sennlaub ¹, Bahram Bodaghi ², Xavier Guillonnet ¹, Alexandre Carpentier ^{3,4,5,‡} and Sara Touhami ^{1,2,*,‡}

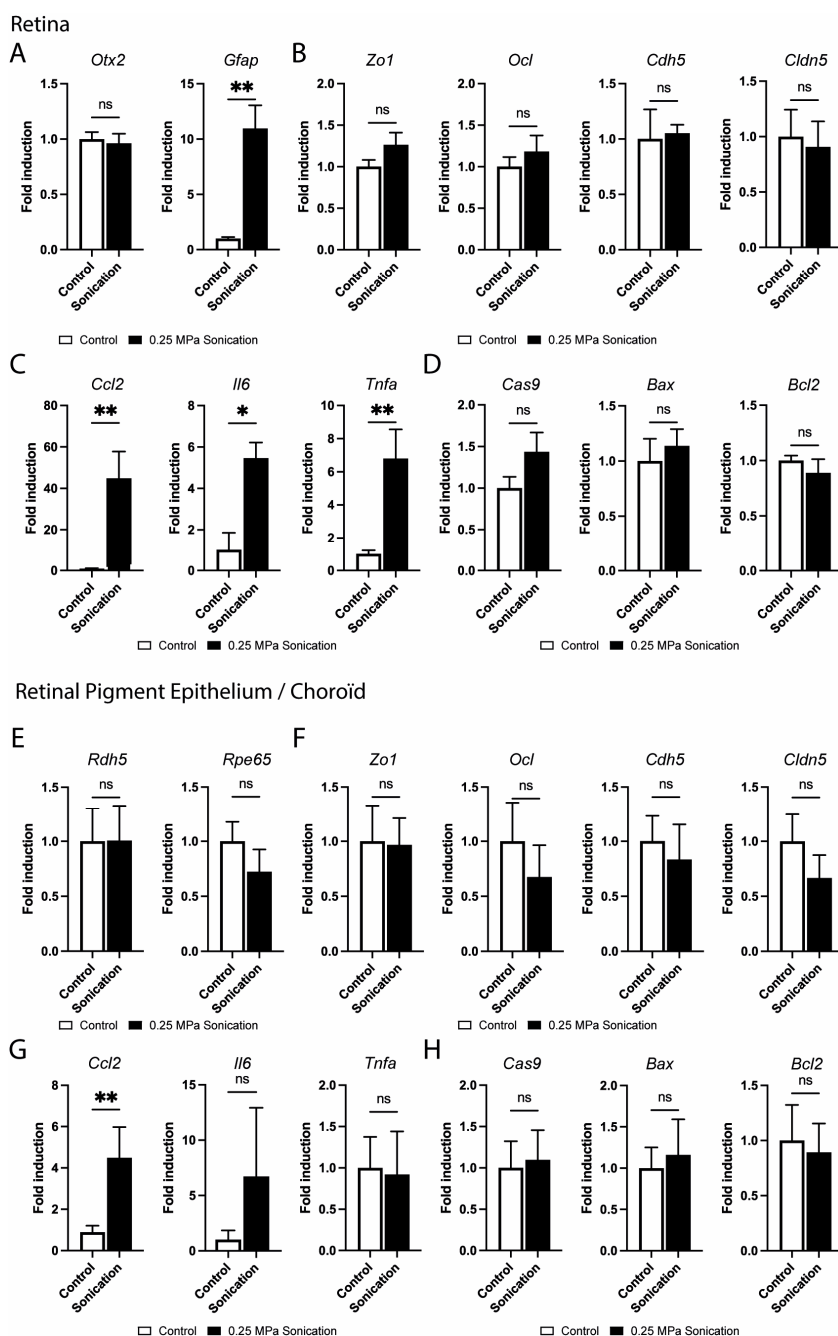
Supplementary Material



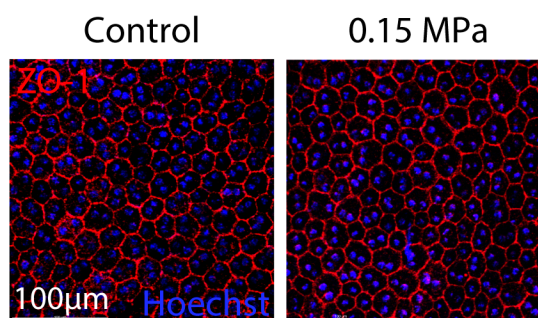
Supplementary Figure S1. Confocal microscopy of (A) ZO-1 (Zonula Occludens) immunostaining depicting vascular tight junctions on retinal flat mounts, (B) High magnification of the ZO-1 immunostaining and (C) Isolectin GS-IB4 immunostaining depicting retinal vessel architecture and presence of mononuclear phagocytes, after microbubble-assisted sonication using different acoustic pressures in C57/BL6J male mice. (D) Quantification of the ZO1 signal shown in (B) using Muthusamy et al. technique (see Materials and Methods), Fisher's exact test ($n = 8$ images/group). (E) Confocal microscopy of Immunoglobulin(Ig) G (green) and Isolectin GS-IB4 immunostaining (red) in C57/BL6J male mice showing extravasation (white arrow heads) of IgGs in eyes undergoing microbubble-assisted sonication at 0.15MPa versus controls. ($n = 5$ -8/group). (F) Confocal microscopy of 150 kDa FITC-dextran (green) injected before sonication showing extravasation at 0.15 MPa versus controls. The white square depicts a magnified region showing better the extravasation process (white arrowheads).



Supplementary Figure S2. Multimodal imaging of C57/BL6J male mice undergoing low-intensity pulsed ultrasound during 60 or 120 seconds at 0.15 MPa. Fundus retinophotograph, field of view 50°. (A) Fluorescein angiography (5min, late phase), field of view 50° (B). MB: microbubbles.



Supplementary Figure S3. RT-qPCR in retinal and RPE/choroid extracts of sonicated and unsonicated (contralateral) eyes at day(D) 1 after sonication at 0.25MPa. Relative expression of: (**A** retina and **E** RPE/choroids) genes implicated in tissue homeostasis: *Otx2*, *Gfap*, *Rdh5*, *Rpe65*, (**B** retina and **F** RPE/choroid) genes implicated in tight and adherens junctions: *Zo1*, *Ocl*, *Cdh5* and *Cldn5*, (**C** retina and **G** RPE/choroid) inflammation genes: *Ccl2*, *Il6* and *Tnfa*, and (**D** retina and **H** RPE/choroids) genes implicated in apoptosis: Caspase 9, Bax and Bcl. Gene expression was calculated relative to *Rps26* expression. (n=5-6/group, Mann-Whitney, comparison versus control: *P<0.05, **P<0.005, ns: not statistically significant). RPE: retinal pigment epithelium. *Rdh5*: 11-cis retinol dehydrogenase 5. *Rpe65*: retinal pigment epithelium-specific 65 kDa protein. *Otx2*: orthodenticle homeobox 2. *Gfap*: glial fibrillary acidic protein. *Ocl*: occludin. *Cdh5*: cadherin 5. *Cldn5*: claudin 5. *Ccl2*: chemokine (C-C motif) ligand 2. *Il6*: interleukin 6. *Tnfa*: tumor necrosis factor alpha. *Cas9*: caspase 9. *Bcl2*: B cell leukemia/lymphoma 2. *Bax*: BCL2-associated X protein.



Supplementary Figure S4. Confocal microscopy of ZO-1 (Zonula Occludens) immunostaining depicting vascular tight junctions on retinal pigment epithelium/choroid flatmounts.