

## Supplemental materials

### Materials

Ocriplasmin batch P214-103865 was produced by Patheon (Swindon, UK) and supplied in 200 $\mu$ L vials at a concentration of 2.5mg/mL, stored at -70°C. Vehicle was freshly prepared by dissolving 5mM citric acid (sodium citrate, C0706, Sigma-Aldrich, Diegem, Belgium), 0.9% NaCl (S3014, Sigma, Diegem, Belgium) and 1.875mg/mL Mannitol (M8429, Sigma-Aldrich) in water and adjusting to pH 3.1.

Lipopolysaccharides (LPS suitable for cell culture, Sigma, L4391) was purchased from Sigma-Aldrich.

### Porcine posterior vitreous detachment model

Male farm pigs (*Sus scrofa domesticus*, Topig 20, cross-breeding of Yorkshire and Landrace, Diest, Belgium) of 5 to 7 week-old were anesthetized with an intramuscular injection of 2.2 mg/kg xylazin (Xyl-M® 2%; V.M.D.nv/sa, Arendonck, Belgium) and 4.4mg/kg tiletamin-zolazepam (Zoletil 100® ,Virbac, Carros, France) with 1.5%-2% isoflurane (IsoFlo®, Ecuphar, Oostkamp, Belgium). Animals were positioned on a surgical table, and 10mL retrobulbar anesthetic (xylazin 1%) was injected. Ocriplasmin or vehicle was injected mid-vitreally in 100 $\mu$ L. At the end of the experiment, the animals were either transferred to a recuperation pen for follow-up optical coherence tomography (OCT), or they were euthanized by an intravenous bolus of 20mL T61 (Intervet Int, Boxmeer, The Netherlands).

### OCT analysis

Eyes were examined by OCT (Heidelberg Spectralis HRA-OCT, Heidelberg Engineering, GmbH, Heidelberg, Germany), using a volume scan protocol using the “fast” preset in Automatic Real-Time mode, averaging 48 frames per image. Each volume covered 20°x25° and consisted of 31 B-scans, 260 $\mu$ m apart. Four volume scans were performed per eye, centered on, nasal, temporal and superior to the optic nerve head. OCT scans were analyzed manually according to following guidelines:

- An eye was scored positive for subretinal Fluid (SRF) an exudate between the choroid and the retina was observed on the scan.
- An eye was scored positive for high reflective spots (HRS) is any of its OCT scans contained highly reflective spots in the vitreous,

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- An eye was scored positive for posterior vitreous detachment (PVD) if a PVD line could be identified and correlated in at least 2 consecutive line scans in an OCT image. PVD was scored in a cumulative manner over time.

All scores are presented as incidence (number of eyes scored positive versus total number of eyes).

### **Histology**

Pan-laminin (NB600-680, Novus), fibronectin (ab6584, Abcam), collagen IV (1340-08, Sanbio), and Iba1<sup>+</sup> (019-19741, Wako) stains were performed using following a general immunohistochemical stain procedure. The staining was performed on 9-12 samples for ocriplasmin and CAM treated eyes and on 4 samples for LPS or non-treated eyes. Statistical differences were evaluated using Student's t-test.

### **Cytokine profiling**

Vitreous samples of 17 vehicle and 21 ocriplasmin treated eyes were collected as described by Ouchi *et al* (1) just before enucleation of the eyes at respectively week 1, 2, 4 and 6 after injection. Vitreous samples from 4 untreated eyes as well as 2 lipopolysaccharides treated eyes (LPS, 100 ng/eye) were included to mimic a strong acute inflammatory response (2). Detection of porcine CCL-2 was carried out using a custom generated Mesoscale Discovery ELISA assay using commercially available antibodies (Abcam). Multiplex detection of the remaining cytokines was performed at EVE technologies (Calgary, Canada) using a pig cytokine array based on MILLIPLEX® MAP technology (Millipore). Statistical differences between groups were evaluated using the Mann-Whitney test.

### Reference List

- (1) Ouchi M, West K, Crabb JW, Kinoshita S, Kamei M. Proteomic analysis of vitreous from diabetic macular edema. *Exp Eye Res* 2005 Aug;81(2):176-82.
- (2) Gilger BC, Abarca EM, Salmon JH, Patel S. Treatment of acute posterior uveitis in a porcine model by injection of triamcinolone acetonide into the suprachoroidal space using microneedles. *Invest Ophthalmol Vis Sci* 2013 Apr;54(4):2483-92.