



Poster # B0268

Efficacy of Complement C3 Inhibition in Preclinical Models of Wet Age-related Macular Degeneration (AMD)

Wei-Sheng Chen, Benbo Song, Mary-Kamala Isoka, Maria Bogacheck, Betty Li, Kalyani Mondal, Yan Wang, Serena Leong, Darrin Lindhout, Bin Fan, Raj Haldankar, Jie Tang, David Shen, Hui Tian, Zhonghao Liu and Alexander Loktev

NGM Biopharmaceuticals, South San Francisco, CA, USA

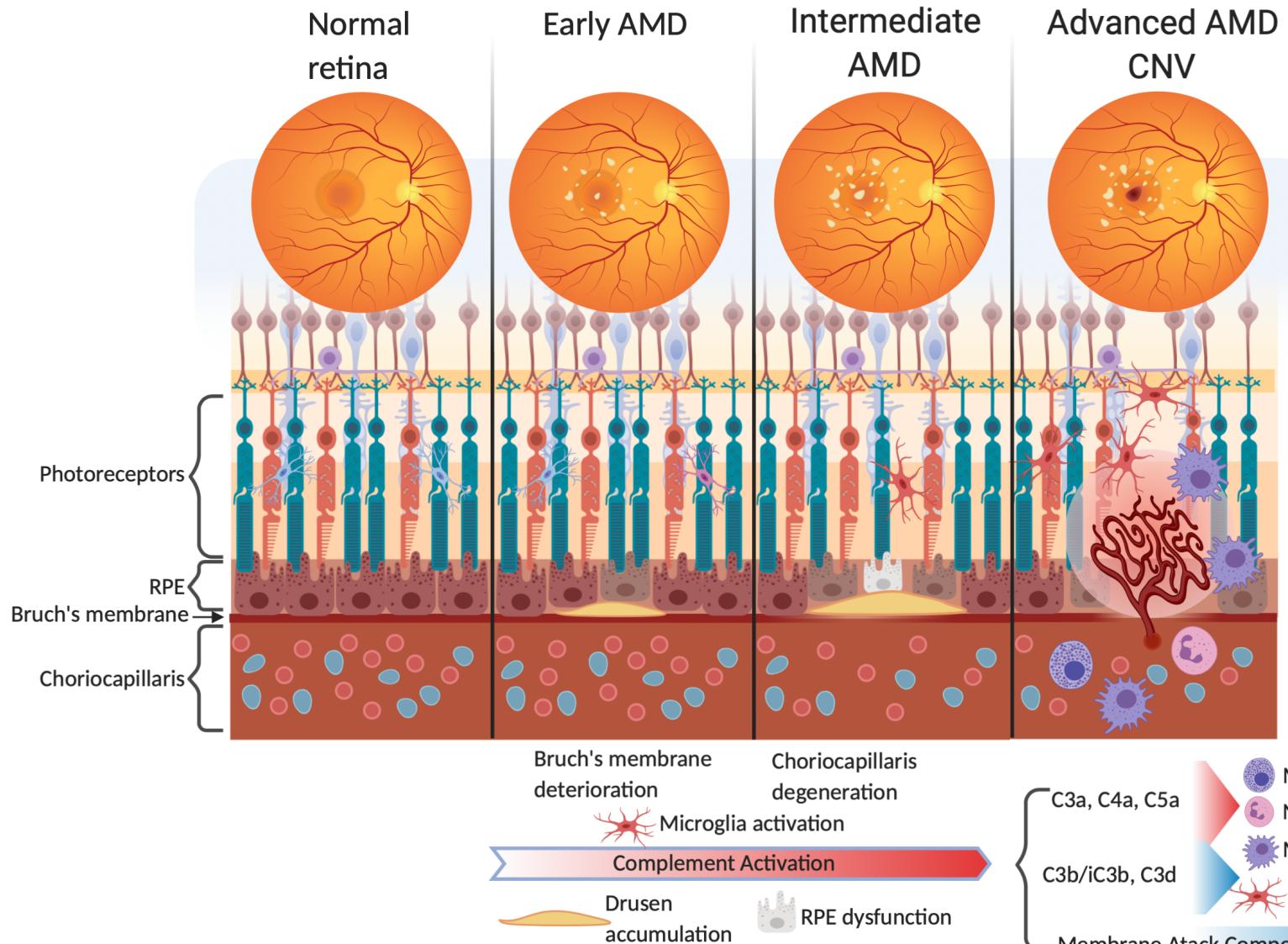


Poster # B0268

Financial Disclosures:

All authors are employees of NGM Biopharmaceuticals, South San Francisco, CA, USA

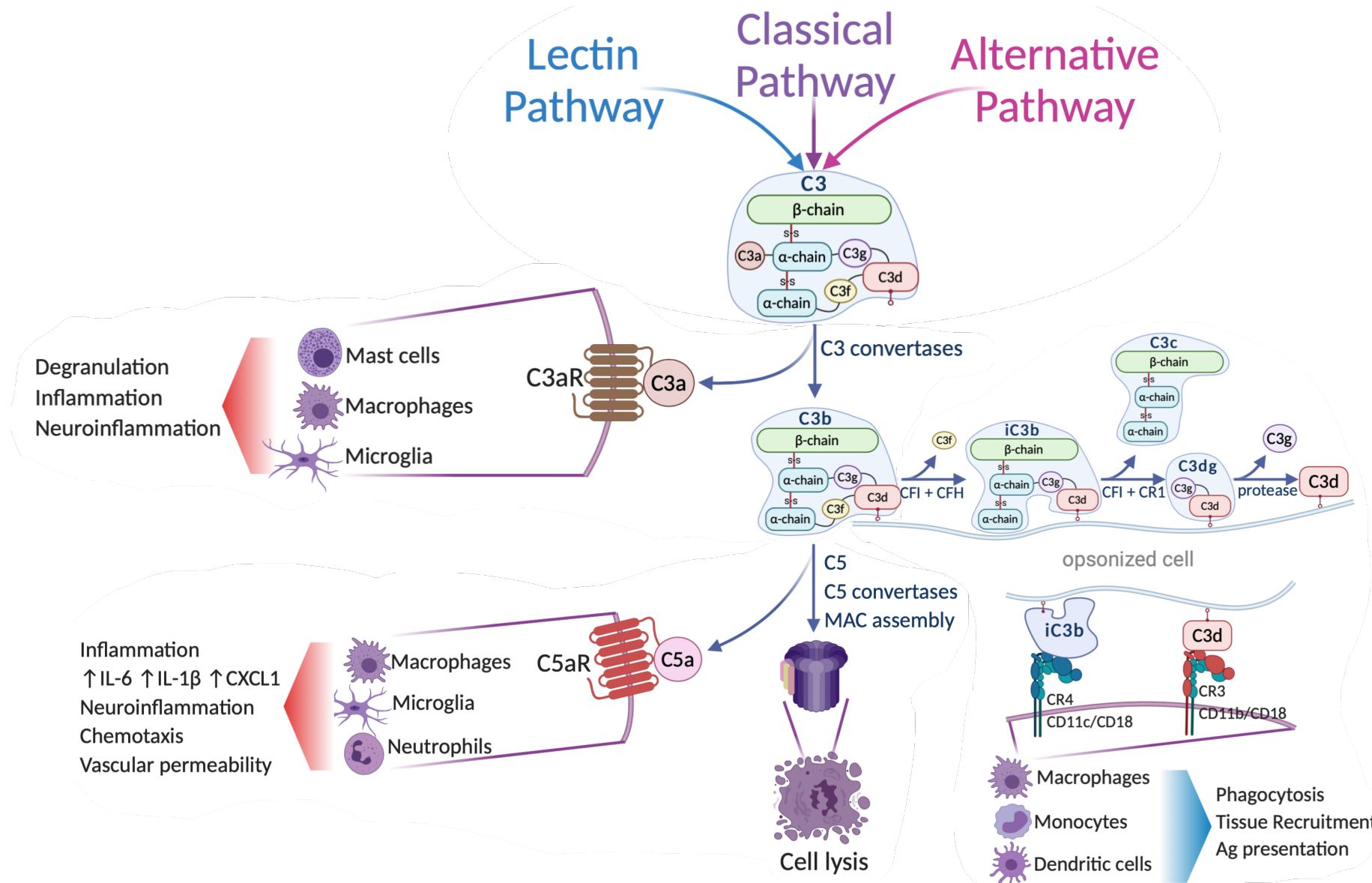
Dysregulation of Complement Contributes to Development and Progression of Advanced Age-related Macular Degeneration (AMD)



- AMD is the leading cause of blindness in the US and the developed world
- Human genetics and other evidence implicate dysregulation of complement system in pathogenesis of both forms of advanced AMD – Geographic Atrophy and Choroidal Neovascularization (CNV)

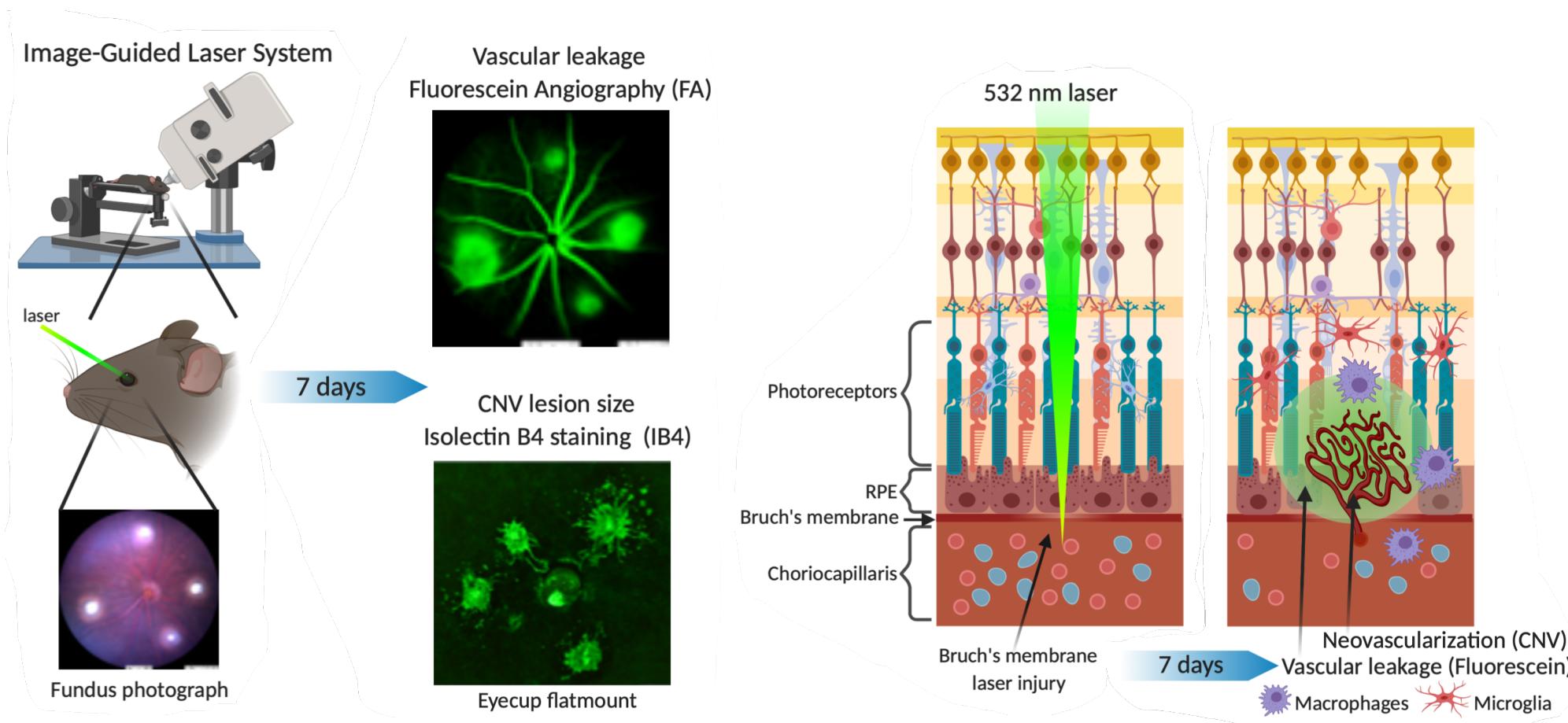
CNV lesion: the abnormal new vessel growth from the choriocapillaris that extends through Bruch's membrane into the sub-RPE and/or subretinal space

Complement Activation Pathways Converge on Complement C3



Laser-induced CNV Model Mechanistically Recapitulates Wet AMD

Laser-induced CNV model: one of the most widely used models that recapitulates the VEGF dependent angiogenic aspect of wet AMD, including activation of microglia and recruitment of myeloid immune cells



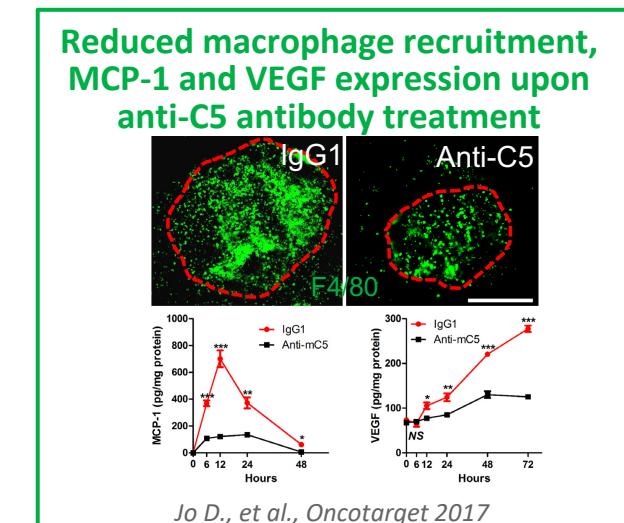
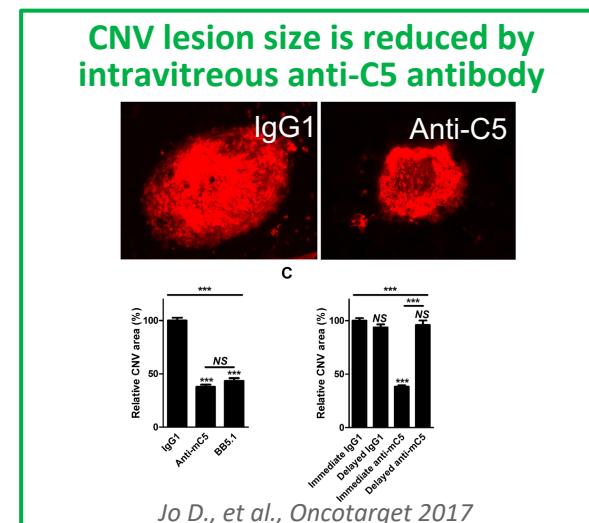
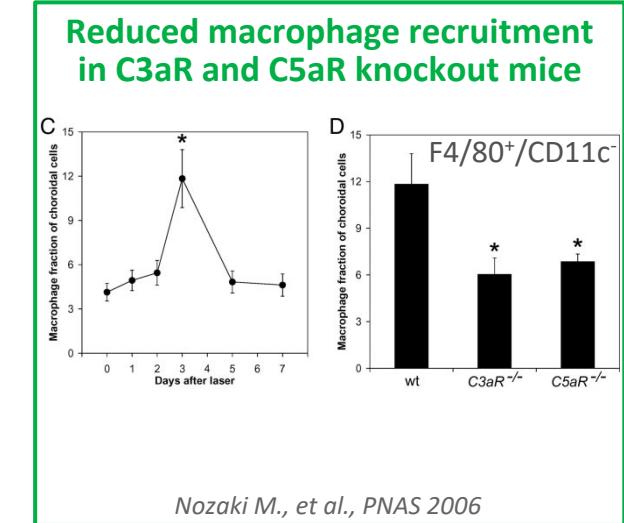
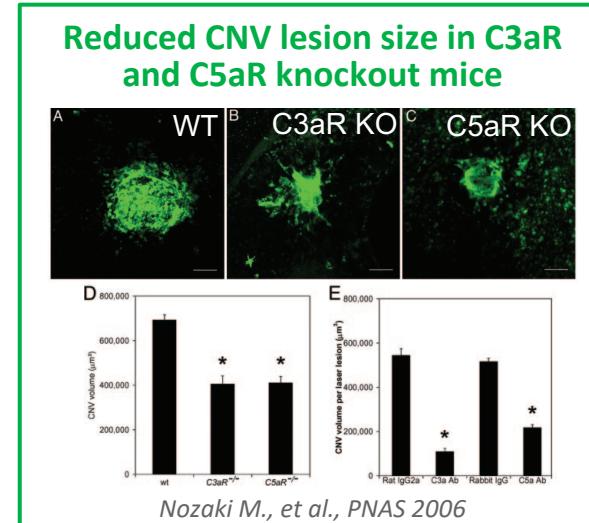
Complement Inhibition Ameliorates Laser-induced CNV in Mice

Inactivation of C3a or C5a function is protective in mouse laser-induced CNV model (Nozaki M., et al., PNAS 2006)

- CNV lesion size is reduced in C3aR and C5aR knockout mice or by treatment with anti-C3a, anti-C5a antibodies and with C3aR antagonist
- Recruitment of neutrophils and macrophages is diminished
- Concentration of VEGF is reduced in C3aR or C5a knockout mice
- C3a and C5a activate RPE to secrete VEGF and MCP-1

Blocking of complement C5 activation by genetic knockout or by blocking antibody is protective in mouse laser-induced CNV model (Bora N., et al., J Immunol 2006; Jo D., et al., Oncotarget 2017)

- CNV lesion size is reduced by treatment with anti-C5a antibodies
- Recruitment of macrophages (F4/80+) to CNV lesions is reduced
- Concentrations of MCP-1 and VEGF in choroid/ sclera is reduced



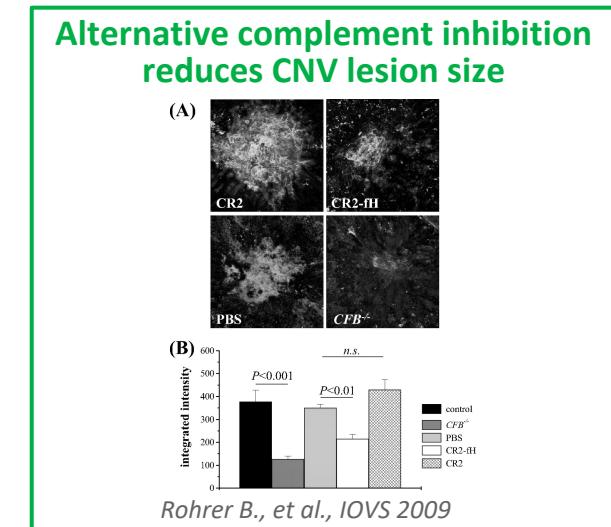
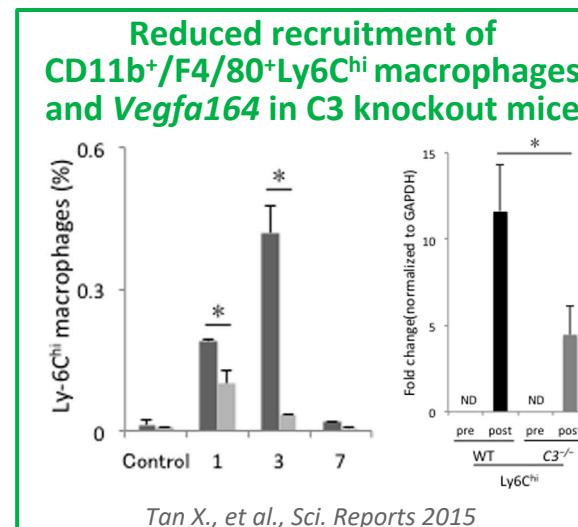
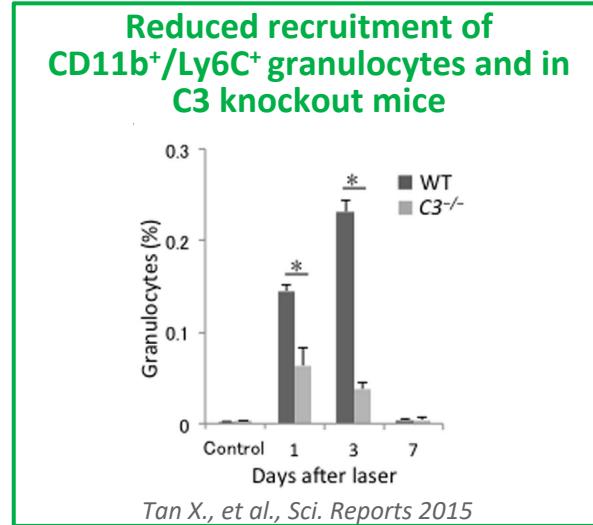
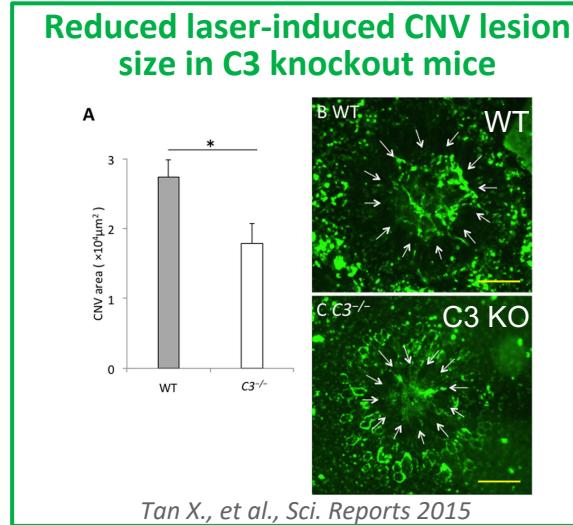
Genetic Inactivation of Complement C3 in Mice Ameliorates CNV Phenotype in Laser-induced Model



Genetic inactivation of complement C3 is protective in mouse laser-induced CNV model (*Tan X., et al., Sci. Reports 2015; Poor S., et al., IOVS 2014; Bora P., et al., J Immunol 2005*)

- Laser-induced CNV lesions were significantly smaller in C3 knockout (C3 KO) mice than in wild-type mice
- Reduced intraocular granulocytes, macrophage/monocyte subsets in C3 KO mice at days 1-3 after laser injury.
- Expression of Vegfa164 was reduced in intraocular inflammatory Ly6C^{hi} macrophages/monocytes of C3 KO mice

Multiple studies demonstrate reduction in CNV phenotype upon complement inhibition through modulation of downstream inflammatory signaling

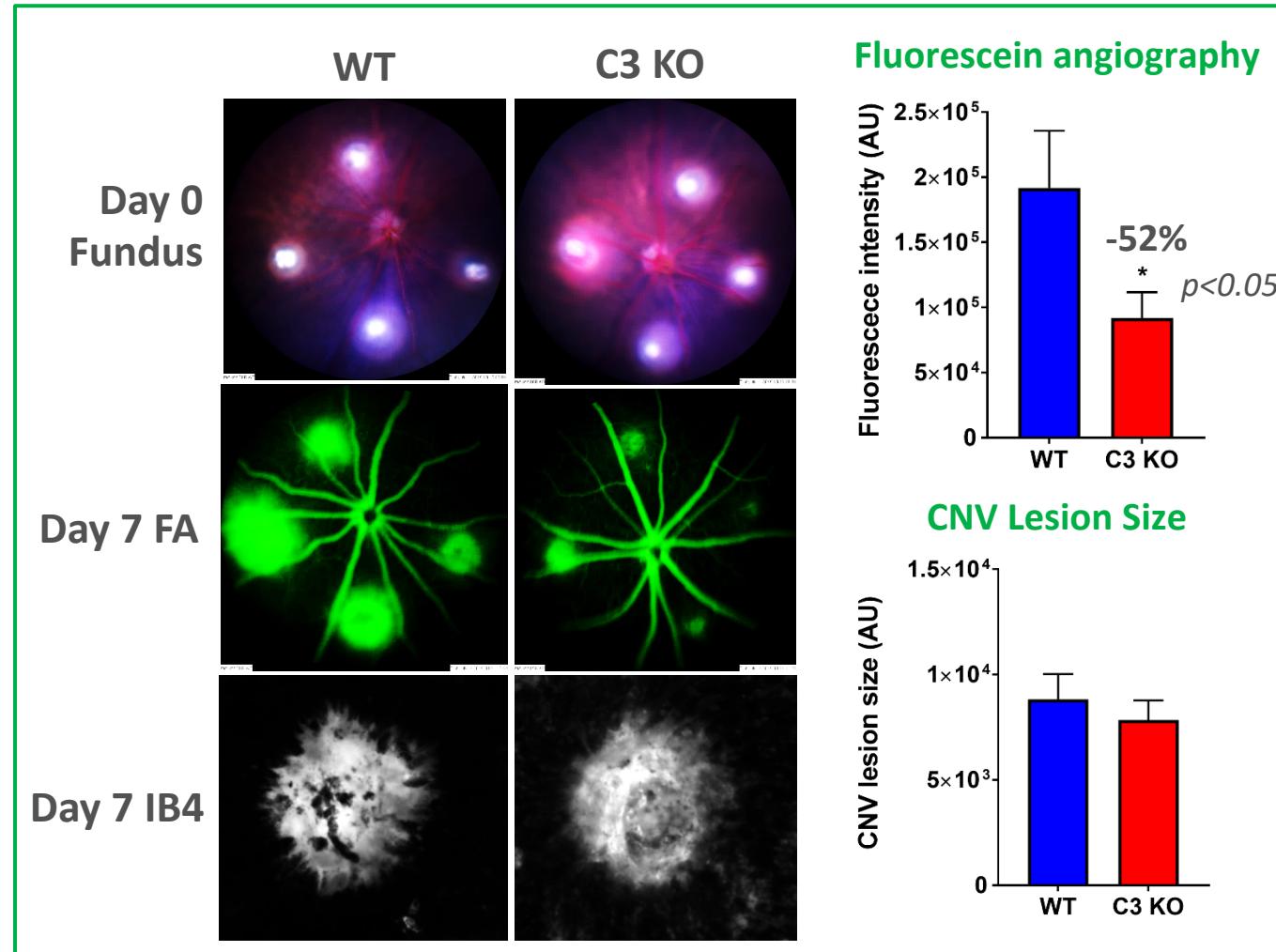


Genetic Deletion of Complement C3 Reduces Vascular Leakage in Mouse Laser-induced CNV Model



Animals: C3 KO mice (JAX #32042, backcrossed to C57BL/6, N2) males, 8 week-old (WT: N=10, KO: N=11)

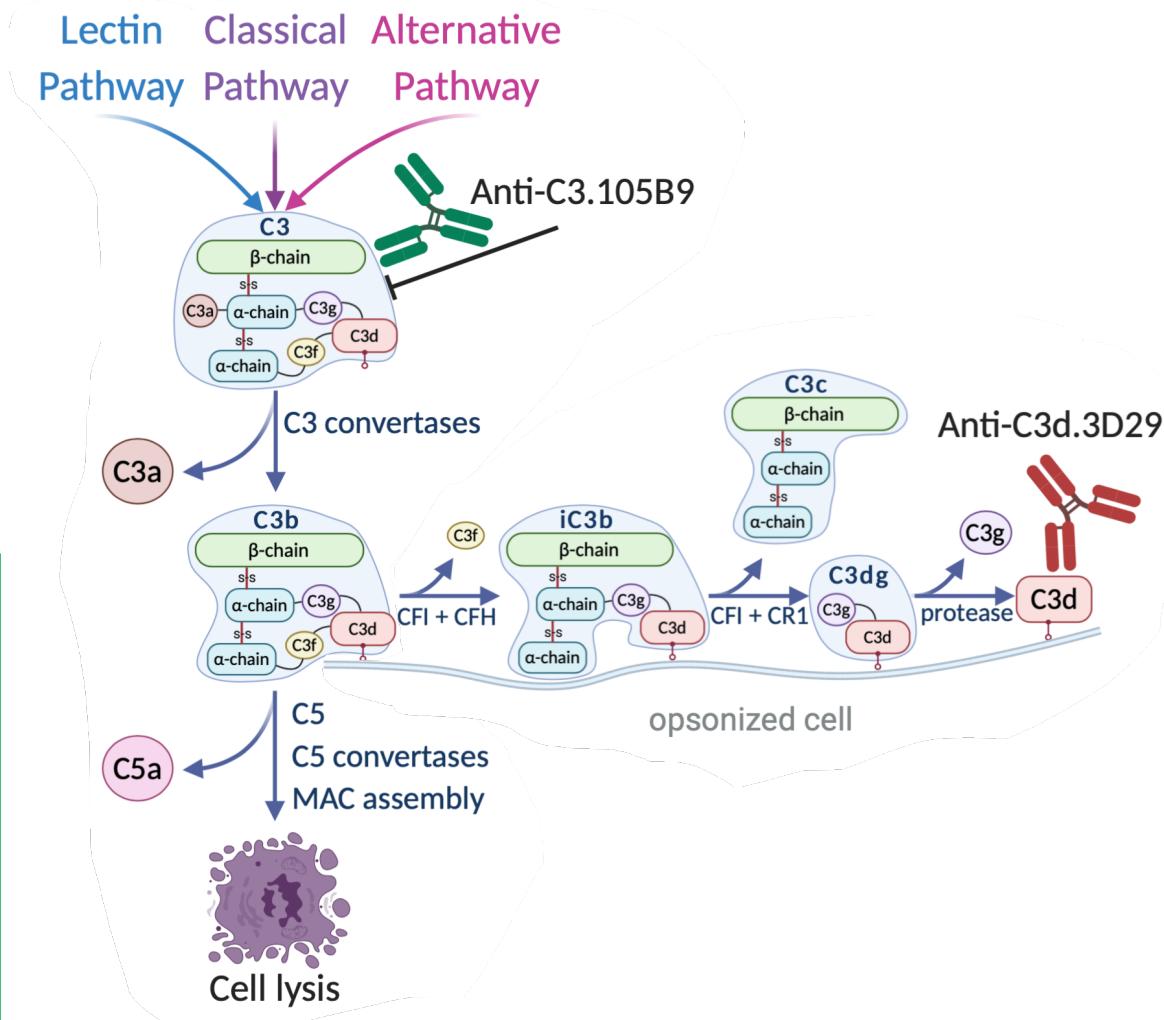
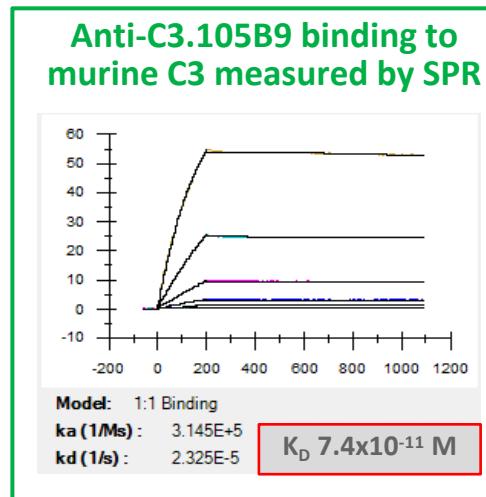
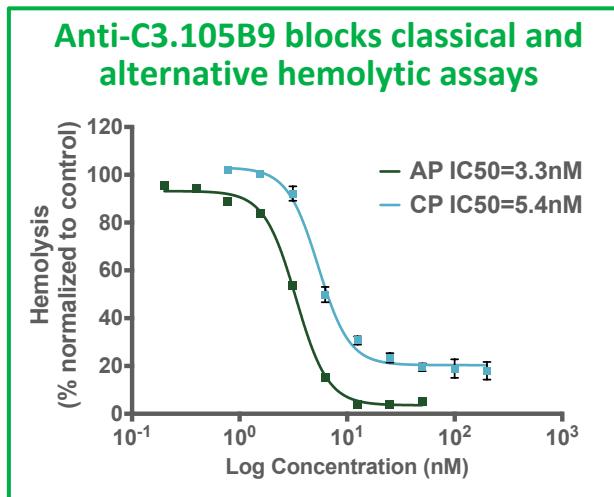
- Vascular leakage, as determined by FA, was reduced by 52% in C3 KO mice compared to WT littermate controls
- A trend of decreased CNV size in C3 KO mice was observed but did not reach statistical significance
- Mixed genetic background may contribute to insignificant reduction in CNV



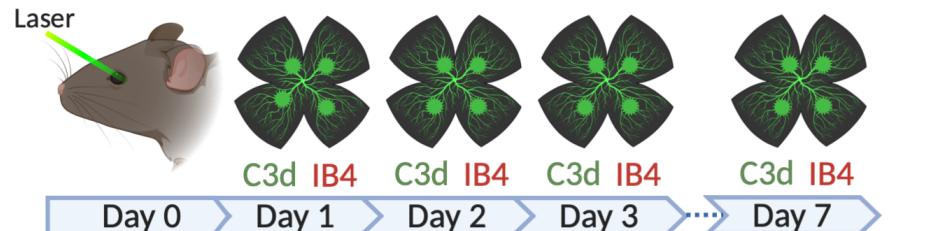
Anti-Complement C3 Antibodies to Interrogate Complement Biology in Rodents



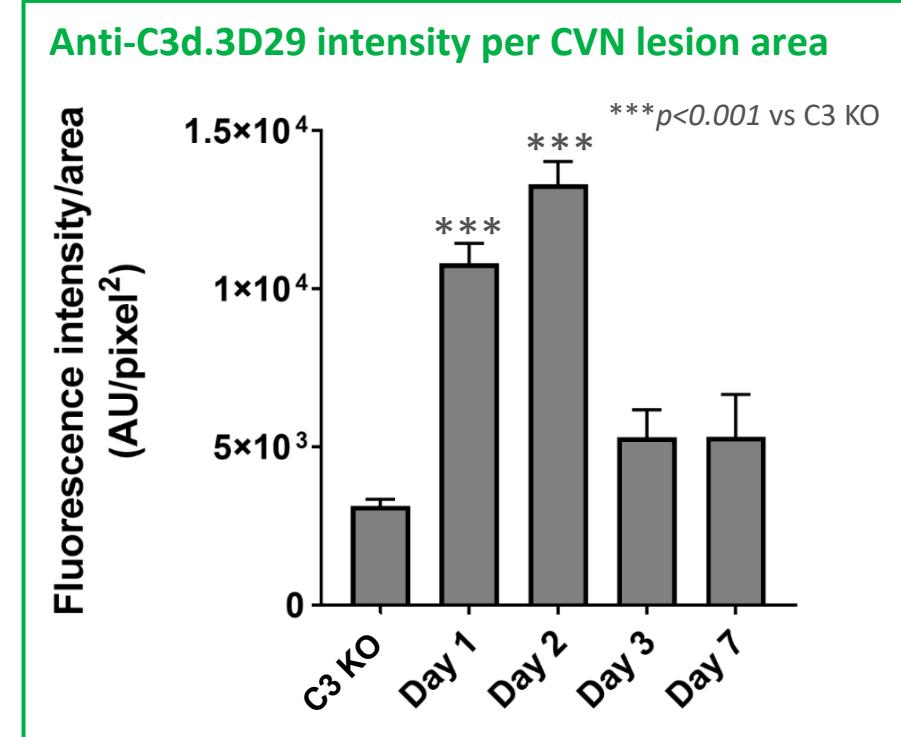
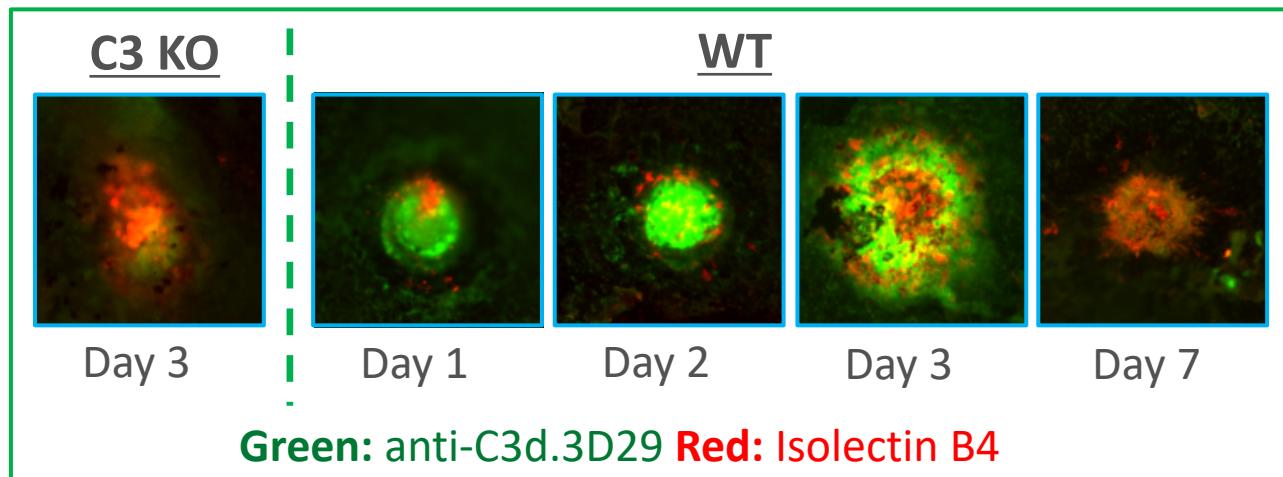
- We generated a murine complement C3 specific inhibitory antibody anti-C3.105B9
 - Binds to intact complement C3 with high affinity $K_D=74$ pM
 - Blocks complement activation by classical and alternative pathways with IC_{50} 5.4 nM and 3.3 nM respectively
- Anti-C3d.3D29 antibody specific to C3d can be used to detect complement activation *in vivo*, including marking laser-induced lesions in the retina (Thurman J., et al., JCI 2013)



Complement Activation and C3d Deposition in the Retina Peaks at Day 2 after Laser-induced Injury

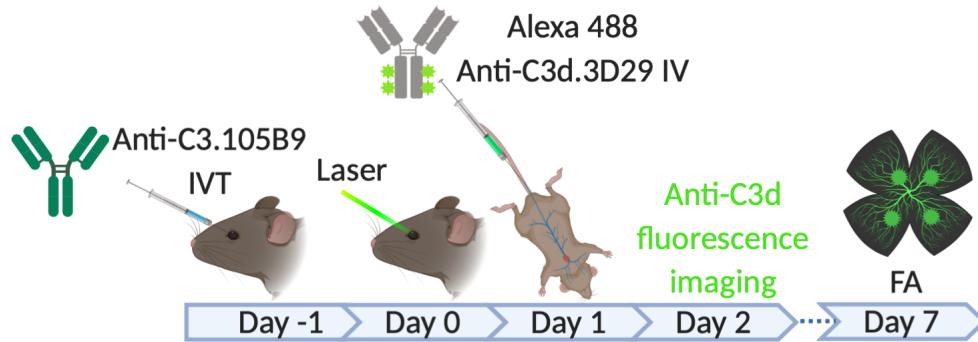


Animals: C57BL/6 and C3 KO mice 2-3 per group/time point



- Complement activation in the retina, measured by staining for deposition of C3d with anti-C3d.3D29, peaks at Day 2 after laser injury
- C3d deposition *in vivo* can be detected by intravenous administration of Alexa 488 labeled anti-C3d.3D29 at Day 1 followed by live fluorescence imaging

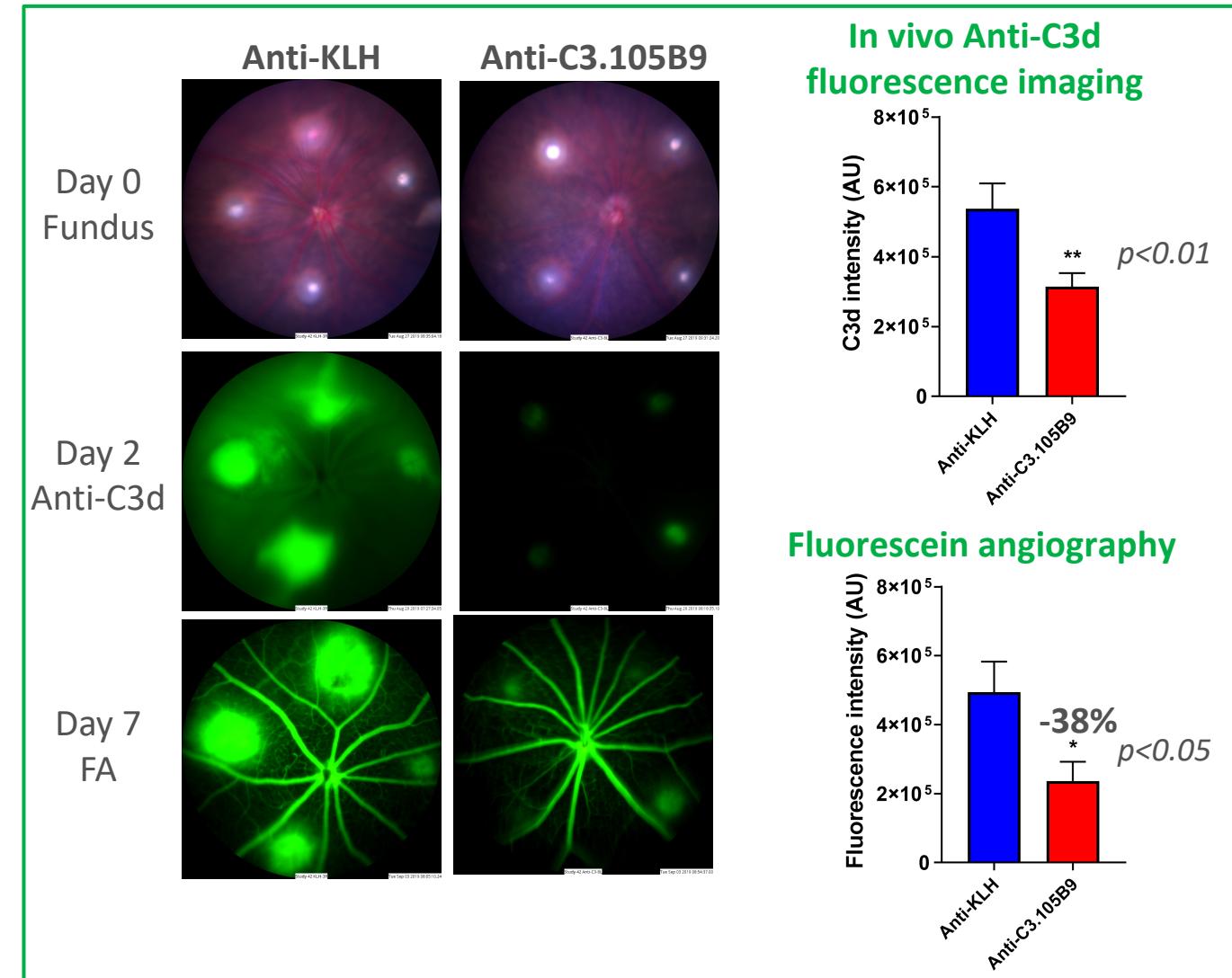
Pharmacological Inhibition of C3 in the Eye Results in Reduced Complement Activation and Vascular Leakage



Animals: C57BL/6 males, 8 weeks-old, n=8 per arm

Treatment:

- Arm 1: anti-KLH control (100 ug/eye) intravitreal (IVT)
 - Arm 2: anti-C3.105B9 (100 ug/eye) IVT
-
- C3 inhibition with anti-C3.105B9 antibody reduced complement activation at day 2 after laser injury, which was demonstrated by in vivo imaging of C3d deposition in the retinal lesions
 - Intravitreally administered anti-C3.105B9 antibody reduced vascular leakage by 38% at day 7 post-laser injury, compared to anti-KLH control



Conclusions: Inhibition of C3 in the Eye Reduces in Vascular Leakage in Mouse Laser-Induced CNV Model of Wet AMD



- We demonstrate decreased vascular leakage upon genetic or pharmacological inhibition of complement C3 in mouse laser-induced CNV model of wet AMD
 - Pharmacological inhibition by intravitreal administration of the mouse specific anti-C3 antibody reduced C3d deposition and vascular leakage
 - Genetic ablation of C3 in mice ameliorated CNV phenotype
- Our data in conjunction with published studies suggest the therapeutic potential for C3 inhibition for neovascular AMD
- We continue to investigate the mechanism through which complement inhibition affects the progression of angiogenesis in response to acute injury
- NGM Biopharmaceuticals is developing anti-C3 antibody NGM621 for the treatment of geographic atrophy – see **ARVO Abstract/Video Presentation # B0267**



Thank you!

Contact information:

Zhonghao Liu: zliu@ngmbio.com

**Novel Biology.
Powerful Medicines.
Transformative Impact.**



Poster # B0268