

1 **TITLE**

2 **Identification of ADAMTS19 as a novel retinal factor involved in ocular growth**  
3 **regulation**

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25 **ABSTRACT**

26 Refractive errors are the most common ocular disorders and are a leading cause of  
27 visual impairment worldwide. Although ocular axial length is well established to be a  
28 major determinant of refractive errors, the molecular and cellular processes regulating  
29 ocular axial growth are poorly understood. Mutations in genes encoding the PRSS56  
30 and MFRP are a major cause of nanophthalmos. Accordingly, mouse models with  
31 mutations in the genes encoding the retinal factor PRSS56 or MFRP, a gene  
32 predominantly localized in the retinal pigment epithelial (RPE) exhibit ocular axial length  
33 reduction and extreme hyperopia. However, the precise mechanisms underlying  
34 PRSS56- and MFRP-mediated ocular axial growth remain elusive. Here, we show that  
35 *Adamts19* expression is significantly upregulated in retina of mice lacking either *Prss56*  
36 or *Mfrp*. Using a combination of genetic approaches and mouse models, we show that  
37 while ADAMTS19 is not required for ocular growth during normal development, its  
38 inactivation exacerbates ocular axial length reduction in both *Prss56* or *Mfrp* mutant  
39 mice. These results suggest that the upregulation of retinal *Adamts19* expression is part  
40 of an adaptive molecular response to counteract impaired ocular growth. Using a  
41 complementary genetic approach. We further demonstrate that loss of PRSS56 or  
42 MFRP function prevents excessive ocular axial growth in a mouse model of  
43 developmental myopia caused by a null mutation in *Irbp*, demonstrating that ocular axial  
44 elongation in *Irbp*<sup>-/-</sup> mice is fully dependent on PRSS56 and MFRP functions.  
45 Collectively, our findings provide insight into the molecular network involved in ocular  
46 axial growth regulation and refractive development and support the notion that relay of

47 the signal between the retina and RPE could be critical for promoting ocular axial  
48 elongation.

49

## 50 **INTRODUCTION**

51 Nanophthalmos is a rare developmental disorder characterized by significantly smaller  
52 but structurally normal eyes and extreme hyperopia resulting from compromised ocular  
53 growth [1]. Also, nanophthalmic individuals are highly susceptible to developing blinding  
54 conditions including secondary angle-closure glaucoma, spontaneous choroidal  
55 effusions, cataracts, and retinal detachment [1]. Both sporadic and familial forms of  
56 nanophthalmos with autosomal dominant or recessive inheritance have been reported  
57 [2]. To date, six genes (*PRSS56*, *MFRP*, *TMEM98*, *CRB1*, *BEST1*, and *MYRF*) have  
58 been implicated in familial forms of nanophthalmos, with *PRSS56* and *MFRP* mutations  
59 accounting for the most frequent causes among multiple cohorts [1, 3-10]. Furthermore,  
60 the eyes of nanophthalmic individuals with biallelic mutations in *PRSS56* or *MFRP* were  
61 found to be significantly smaller compared to those carrying dominant mutations in  
62 *TMEM98* or *MYRF*. Interestingly, common variants of *PRSS56* and *MFRP* have also  
63 been found to be associated with myopia, a condition phenotypically opposite to  
64 nanophthalmos that is characterized by increased ocular elongation [11]. Together,  
65 these findings underscore the importance of *PRSS56* and *MFRP* in ocular size  
66 regulation[2].

67 Ocular growth can be broadly divided into two distinct phases that take place pre- and  
68 postnatally[12]. Prenatal ocular growth occurs in the absence of visual stimulation and is  
69 primarily dictated by genetic factors [13]. In contrast, postnatal ocular growth also

70 referred to as emmetropization, is a vision-guided process modulated by the refractive  
71 status of the eye to ensure that the axial length matches the optical power of the eye to  
72 achieve optimal focus and clear vision. Abnormal postnatal ocular axial growth leading  
73 the increased axial length constitutes a major cause of myopia, a condition  
74 characterized by blurred vision caused by focused images falling in front of the retina  
75 [12, 14, 15]. Nanophthalmos is generally attributed to impaired prenatal ocular growth  
76 as individuals with this condition are born hyperopic [1, 16]. Interestingly, in addition to  
77 being responsible for nanophthalmos, common variants of *MFRP* and *PRSS56* have  
78 also been found to be associated with myopia in the general population (an opposite  
79 condition) that primarily results from alterations in postnatal ocular axial growth [11, 17].  
80 Thus, the association of *PRSS56* and *MFRP* with nanophthalmos and myopia support a  
81 role for these factors in the regulation of embryonic and postnatal ocular growth  
82 development and suggest that the molecular mechanisms underlying pre- and postnatal  
83 ocular growth are shared.

84 It is generally accepted that postnatal ocular growth is regulated by a cascade of  
85 signaling events by which information is relayed from the retina to the sclera to induce  
86 scleral extracellular matrix (ECM) remodeling to promote ocular axial elongation and  
87 [14, 18]. Notably, *PRSS56* expression is specifically detected in the retina [13], which is  
88 consistent with a central role for the retina in ocular growth regulation. *MFRP* is  
89 predominantly expressed in the retinal pigment epithelium (RPE) and ciliary  
90 epithelium[16] and is implicated in the transmission of molecular cues between retina  
91 and sclera during ocular growth. Using a genetic mouse model, we have recently  
92 demonstrated that the genetic ablation of *Prss56* from retinal Müller glia leads to a

93 significant reduction in ocular axial length and hyperopia[13]. Similarly, mice and  
94 zebrafish lacking MFRP exhibit ocular axial length reduction, and *MFRP* variants in  
95 humans are associated with myopia[19-21].

96 Although current shreds of evidence support a key role of MFRP and PRSS56 in ocular  
97 axial length determination, the underlying mechanisms remain elusive. In this study, we  
98 use *Prss56* and *Mfrp* mutant mouse model in combination with complementary genetic  
99 approaches to gain insights into the molecular network involved in ocular size  
100 regulation. Importantly, we identified characteristic changes in retinal gene expression in  
101 response to impaired ocular growth. Specifically, we show that *Adamts19* mRNA levels  
102 are significantly increased in the retina of *Prss56* and *Mfrp* mutant mice and provide  
103 evidence that the upregulation of retinal *Adamts19* expression is part of an adaptive  
104 molecular response to impaired ocular growth. Furthermore, we demonstrate that loss  
105 of PRSS56 or MFRP function prevents excessive ocular axial elongation in a mouse  
106 model of early-onset myopia caused by a mutation in *Irbp*. Collectively, our finding hints  
107 at a potential molecular link between Müller glia and RPE involved in ocular axial growth  
108 regulation.

109

## 110 RESULTS

111 ***Adamts19* expression is upregulated in the retina of *Prss56* mutant mice.** To begin  
112 addressing the molecular processes underlying PRSS56-mediated ocular size  
113 regulation, we performed RNA-Seq analysis on the retina from *Prss56<sup>gclr4</sup>* mutant mice  
114 and their wild-type littermates. We recently demonstrated that the ocular size reduction  
115 that we originally described in mice with a *Prss56<sup>gclr4</sup>* mutation (causing PRSS56 protein

116 truncation) result from a loss of function mechanism, hence, *Prss56*<sup>gclr4/gclr4</sup> mice will be  
117 referred to as *Prss56*<sup>-/-</sup> throughout the manuscript for simplicity [13]. Our transcriptome  
118 analysis identified *Prss56* and *Adamts19* as the top two differentially expressed genes  
119 between *Prss56* mutant (*Prss56*<sup>-/-</sup>) and control (*Prss56*<sup>+/-</sup>) retina. Consistent with the  
120 RNA-Seq data, the qPCR analysis revealed that *Prss56* and *Adamts19* mRNA levels  
121 were significantly upregulated in the retina of *Prss56* mutant mice (*Prss56*<sup>-/-</sup>) compared  
122 to their *Prss56*<sup>+/-</sup> and *Prss56*<sup>+/+</sup> littermates at both ages examined (postnatal day (P) 15  
123 and P30) (Fig. 1A-B). Importantly, *Prss56* and *Adamts19* retinal expression levels in  
124 heterozygous *Prss56*<sup>+/-</sup> mice were comparable to those detected in *Prss56*<sup>+/+</sup> mice,  
125 which is consistent with the absence of an ocular phenotype in *Prss56*<sup>+/-</sup> mice [13].  
126 *Prss56*<sup>+/-</sup> mice were therefore used as controls for all experiments presented in this  
127 study. As described previously [13], we detected a progressive upregulation of *Prss56*  
128 mRNA levels in *Prss56*<sup>-/-</sup> retina from P15 to P60 (Fig. S1). The increase in *Adamts19*  
129 retinal expression of was found to precede that of *Prss56* in *Prss56*<sup>-/-</sup> retina, and was  
130 detected as early as P10 and gradually increased to reach peak expression levels by  
131 P30 (Fig. S1). Notably, qPCR-Ct values suggested that the expression of *Adamts19*  
132 was minimal or negligible in *Prss56*<sup>+/+</sup> and *Prss56*<sup>+/-</sup> retina. Furthermore, the  
133 upregulation of retinal *Prss56* and *Adamts19* expression was also observed in mice  
134 carrying a null allele of *Prss56* (*Prss56*<sup>Cre</sup>), which we had described previously [13].  
135 Thus, confirming that the increase in *Prss56* and *Adamts19* expression results from a  
136 loss of PRSS56 function (Fig. 1C). To determine the spatial distribution of *Adamts19*  
137 mRNA, we next performed *in situ* hybridization on ocular sections from *Prss56*<sup>-/-</sup> and  
138 *Prss56*<sup>+/-</sup> mice. Despite using the highly sensitive QuantiGene View RNA *in situ*

139 hybridization method, *Adamts19* expression was only detected in *Prss56*<sup>-/-</sup> retina,  
140 indicating that *Adamts19* expression was below the threshold of detection in control  
141 *Prss56*<sup>+/-</sup> retina (Fig. 1D). In *Prss56* mutant retina, *Adamts19* expression was  
142 predominantly observed in the inner nuclear layer (INL), a region containing the cell  
143 bodies of Müller glia, a cell type in which *Prss56* is normally expressed. Collectively,  
144 these findings demonstrate that in addition to causing ocular size reduction, loss of  
145 PRSS56 function leads to alterations in retinal gene expression marked by increased  
146 *Prss56* and *Adamst19* mRNA levels.

147 ***Retinal Prss56 and Adamts19 mRNA levels are upregulated in response to ocular***  
148 ***size reduction in Prss56 mutant mice***

149 To determine whether the upregulation in retinal *Adamts19* and *Prss56* mRNA levels  
150 correlates with ocular size reduction in *Prss56* mutant mice, we took advantage of the  
151 *Egr1*; *Prss56* double mutant mouse model (*Egr1*<sup>-/-</sup>; *Prss56*<sup>-/-</sup>) that we described  
152 previously [13]. EGR1 (early growth response1) is a major regulator of ocular growth  
153 and *Egr1*<sup>-/-</sup> mice exhibit increased ocular axial length[13, 22]. We have previously shown  
154 that *Egr1* inactivation rescues the reduction in ocular axial length and vitreous chamber  
155 depth (VCD) in *Prss56* mutant mice as the ocular size of *Egr1*<sup>-/-</sup>;*Prss56*<sup>-/-</sup> mice is  
156 comparable to that of control *Egr1*<sup>+/-</sup>;*Prss56*<sup>+/-</sup> mice [12]. Using qPCR analysis, we show  
157 that in addition to rescuing ocular axial elongation, *Egr1* inactivation also prevented the  
158 increase in retinal expression of *Prss56* and *Adamts19* in *Prss56* mutant mice (compare  
159 *Egr1*<sup>-/-</sup>; *Prss56*<sup>-/-</sup> to *Egr1*<sup>+/-</sup>; *Prss56*<sup>-/-</sup> in Fig. 2). These findings suggest that the  
160 upregulation of retinal *Prss56* and *Adamts19* does not result from loss of PRSS56  
161 function *per se*, but rather from its effect on ocular size.

162 **ADAMTS19 is not required for ocular growth during normal development**

163 PRSS56 and ADAMTS19 are both secreted serine proteases, raising the possibility that  
164 they might have overlapping functions in ocular growth regulation. To test this  
165 possibility, we first generated *Adamts19* knockout mice by crossing a conditional  
166 *Adamts19* mutant mouse line with the ubiquitous  $\beta$ -actin-*Cre* line (Fig. S2). To  
167 determine if the loss of ADAMTS19 function lead to ocular defects, we performed  
168 optical coherence tomography (OCT) to assess various ocular biometric parameters.  
169 We found that all the ocular parameters examined, including ocular axial length, VCD,  
170 and retinal thickness were indistinguishable between *Adamts19*<sup>+/+</sup>, *Adamts19*<sup>+/-</sup> and  
171 *Adamts19*<sup>-/-</sup> mice (Fig. 3 and Fig. S3). These findings demonstrate that ADAMTS19 is  
172 not required for ocular growth during normal development.

173

174 **Loss of ADAMTS19 function exacerbates ocular axial length reduction in *Prss56*<sup>-/-</sup>**  
175 **mice**

176 In light of our findings, we hypothesized that the upregulation of retinal *Adamts19*  
177 expression might be part of an adaptive molecular response to compensate for the loss  
178 of PRSS56 function and promote ocular axial growth. To this end, we tested the effect  
179 of *Adamts19* inactivation in *Prss56*<sup>-/-</sup> mice by crossing *Prss56* mutant mice to the  
180 *Adamts19* mutant line to generate *Prss56*<sup>-/-</sup> mice that are wild-type, heterozygous or  
181 homozygous for the *Adamts19* null allele (*Adamts19*<sup>+/+</sup>, *Adamts19*<sup>+/-</sup> or *Adamts19*<sup>-/-</sup>).  
182 Notably, since all ocular biometric parameters of *Adamts19*<sup>+/-</sup>; *Prss56*<sup>+/-</sup> mice were  
183 comparable to those of wild-type (*Adamts19*<sup>+/+</sup>; *Prss56*<sup>+/+</sup>) littermates (Fig. S4A),  
184 *Adamts19*<sup>+/-</sup>; *Prss56*<sup>+/-</sup> mice were used as controls. As expected, axial length and VCD



185 were significantly reduced in all three groups of mice lacking *Prss56* (*Prss56*<sup>-/-</sup>)  
186 compared to the control mice (Fig. 4). However, the axial length and VCD were  
187 significantly reduced in *Adamts19*;*Prss56* double mutant mice (*Adamts19*<sup>-/-</sup>;*Prss56*<sup>-/-</sup>)  
188 compared to *Prss56* single mutants (*Adamts19*<sup>+/-</sup>;*Prss56*<sup>-/-</sup> or *Adamts19*<sup>+/+</sup>;*Prss56*<sup>-/-</sup>) at  
189 both age examined (P18 and P30) (Fig. 4). As reported previously [13], ocular axial  
190 length reduction in *Prss56*<sup>-/-</sup> mice was associated with an increase in retinal thickness  
191 (Fig. S5). Notably, a modest but significant increase in retinal thickness was observed in  
192 *Adamts19*<sup>-/-</sup>;*Prss56*<sup>-/-</sup> mice compared to *Prss56* mutant mice (*Adamts19*<sup>+/-</sup>;*Prss56*<sup>-/-</sup> and  
193 *Adamts19*<sup>+/+</sup>;*Prss56*<sup>-/-</sup>) at P18 (Fig. S5).

194 Besides, we found that *Adamts19* expression was significantly increased in the retina  
195 from both *Adamts19*<sup>+/+</sup>;*Prss56*<sup>-/-</sup> and *Adamts19*<sup>+/-</sup>;*Prss56*<sup>-/-</sup> mice compared to that of  
196 *Adamts19*<sup>+/-</sup>;*Prss56*<sup>+/-</sup> control mice, which is consistent with the observation that  
197 exacerbation of the ocular axial length reduction in *Prss56* mutant mice is only observed  
198 when *Adamts19* is completely knocked out (Fig. S5C). Together, these results  
199 demonstrate that *Adamts19* inactivation exacerbates ocular size reduction in *Prss56*<sup>-/-</sup>  
200 mice and is consistent with the upregulation of retinal *Adamts19* expression being part  
201 of an adaptive molecular response triggered by impaired ocular growth in *Prss56*  
202 mutant mice.

203

#### 204 ***Adamts19* inactivation exacerbates ocular axial length reduction in *Mfrp* mutant** 205 **mice**

206 Interestingly, elevated retinal levels of *Prss56* expression has recently been reported in  
207 another mouse model of nanophthalmos caused by a mutation in the gene coding for

208 membrane frizzed related-protein (*Mfrp*) [23]. Increased *Adamts19* expression was also  
209 observed in *Mfrp*<sup>-/-</sup> eyes but the specific ocular tissue/cell type in which *Adamts19* was  
210 expressed was not addressed [23]. Since *Adamts19* expression was specifically  
211 detected in the retina of the *Prss56*<sup>-/-</sup> mice, we performed a qPCR analysis to confirm  
212 that the levels of *Prss56* and *Adamts19* were upregulated in the retina of *Mfrp*<sup>-/-</sup> mice  
213 compared to control *Mfrp*<sup>+/-</sup> littermates (Fig. 5A). To determine if *Adamts19* inactivation  
214 also exacerbates the ocular size reduction caused by *Mfrp* deficiency, we crossed *Mfrp*  
215 mutant mice with the *Adamts19* mutant line and conducted OCT analyses on the  
216 progeny. Since the ocular biometric parameters of *Adamts19*<sup>+/-</sup>;*Mfrp*<sup>+/-</sup> were comparable  
217 to those of wild-type (*Adamts19*<sup>+/+</sup>;*Mfrp*<sup>+/+</sup>), they were used as controls (Fig. S6). As  
218 expected, *Mfrp* mutant mice (*Adamts19*<sup>+/-</sup>;*Mfrp*<sup>-/-</sup>) exhibited reduced ocular axial length  
219 and VCD compared to *Adamts19*<sup>+/-</sup>;*Mfrp*<sup>+/-</sup> control mice (Fig. 5C-E). Importantly, the  
220 ocular axial length and VCD of *Adamts19*<sup>-/-</sup>;*Mfrp*<sup>-/-</sup> mice were significantly reduced  
221 compared to *Mfrp* mutant mice (*Adamts19*<sup>+/-</sup>;*Mfrp*<sup>-/-</sup>) (Fig. 5C-E). In addition, retinal  
222 thickness was increased in *Adamts19*<sup>+/-</sup>;*Mfrp*<sup>-/-</sup> and *Adamts19*<sup>-/-</sup>;*Mfrp*<sup>-/-</sup> mice compared to  
223 control *Adamts19*<sup>+/-</sup>;*Mfrp*<sup>+/-</sup> mice (Fig. S7). These findings further support a role for the  
224 upregulation of retinal *Adamts19* expression being part of a compensatory mechanism  
225 triggered by impaired ocular axial growth.

226

### 227 **Inactivation of *Prss56* or *Mfrp* prevents excessive ocular axial elongation in *Irbp*** 228 **mutant mice**

229 To further establish the role of PRSS56 and MFRP in ocular elongation, we tested the  
230 effects of *Prss56* and *Mfrp* inactivation in a mouse model of early-onset developmental

231 myopia associated with excessive ocular axial growth caused by a null mutation in the  
232 gene coding for IRBP (Interphotoreceptor retinoid-binding protein)[24]. To this end, each  
233 of the *Prss56* and *Mfrp* mutant lines were crossed to *Irbp* mutant mice and biometric  
234 ocular assessment was conducted on their progeny. As expected, OCT analyses  
235 revealed that ocular axial length and VCD were significantly increased in *Irbp* single  
236 mutant mice (*Irbp*<sup>-/-</sup>;*Prss56*<sup>+/-</sup> or *Irbp*<sup>-/-</sup>;*Mfrp*<sup>+/-</sup>) and significantly reduced in *Prss56* or *Mfrp*  
237 single mutant mice (*Irbp*<sup>+/-</sup>;*Prss56*<sup>-/-</sup> or *Irbp*<sup>+/-</sup>;*Mfrp*<sup>-/-</sup>;) compared to their respective  
238 controls (*Irbp*<sup>+/-</sup>;*Prss56*<sup>+/-</sup> and *Irbp*<sup>+/-</sup>;*Mfrp*<sup>+/-</sup> mice) (Fig. 6A-D). Inactivation of either  
239 *Prss56* or *Mfrp* prevented ocular axial elongation in *Irbp* mutant mice (*Irbp*<sup>-/-</sup>;*Prss56*<sup>-/-</sup>  
240 and *Irbp*<sup>-/-</sup>;*Mfrp*<sup>-/-</sup>, respectively) (Fig. 6A-D). Notably, ocular axial length and VCD were  
241 significantly reduced in both double mutant lines (*Irbp*<sup>-/-</sup>;*Prss56*<sup>-/-</sup> and *Irbp*<sup>-/-</sup>;*Mfrp*<sup>-/-</sup>)  
242 compared to their respective control littermates (*Irbp*<sup>+/-</sup>;*Prss56*<sup>+/-</sup> and *Irbp*<sup>+/-</sup>;*Mfrp*<sup>+/-</sup>,  
243 respectively) and were comparable to those observed in *Prss56* and *Mfrp* single mutant  
244 mice (*Irbp*<sup>+/-</sup>;*Prss56*<sup>-/-</sup> and *Irbp*<sup>+/-</sup>;*Mfrp*<sup>-/-</sup>, respectively) (Fig. 6A-D and Fig. S8 A, C). In  
245 addition, while retinal thickness was increased in both *Prss56* and *Mfrp* single mutant  
246 mice (*Irbp*<sup>+/-</sup>;*Prss56*<sup>-/-</sup> and *Irbp*<sup>+/-</sup>;*Mfrp*<sup>-/-</sup>), it was significantly reduced in *Irbp* single mutant  
247 mice (*Irbp*<sup>-/-</sup>;*Prss56*<sup>+/-</sup> or *Irbp*<sup>-/-</sup>;*Mfrp*<sup>+/-</sup>) compared to control littermates (*Irbp*<sup>+/-</sup>;*Prss56*<sup>+/-</sup>  
248 and *Irbp*<sup>+/-</sup>;*Mfrp*<sup>+/-</sup>, respectively) (Fig. S8B, D). Interestingly, the retinal thickness of *Irbp*<sup>-/-</sup>  
249 ;*Prss56*<sup>-/-</sup> and *Irbp*<sup>-/-</sup>;*Mfrp*<sup>-/-</sup> mice was comparable to that of their *Prss56* and *Mfrp* single  
250 mutant littermates (*Irbp*<sup>+/-</sup>;*Prss56*<sup>-/-</sup> and *Irbp*<sup>+/-</sup>;*Mfrp*<sup>-/-</sup>, respectively) (Fig. S8B, D).  
251 Together, these findings demonstrate that the excessive ocular elongation observed in  
252 *Irbp*<sup>-/-</sup> mice is dependent on PRSS56 and MFRP functions.  
253

254 **DISCUSSION**

255 The molecular and cellular mechanisms involved in ocular axial growth and  
256 emmetropization are poorly understood. Previous studies have identified *PRSS56* and  
257 *MFRP* mutations as a major cause of nanophthalmos, a condition characterized by  
258 severe ocular size reduction and extreme hyperopia, suggesting that these factors play  
259 a critical role in ocular axial growth[3-6]. Consistent with this, *Prss56* and *Mfrp* mutant  
260 mice recapitulate the characteristic pathophysiological features of nanophthalmos, i.e.  
261 exhibit reduced ocular axial length and hyperopia[3, 13, 21]. Here, we use  
262 complementary genetic approaches in *Prss56* and *Mfrp* mutant mouse models as a first  
263 step to elucidate the molecular and cellular factors playing a role in the ocular size  
264 regulation. Notably, we identified ADAMTS19 as a novel factor involved in ocular size  
265 regulation and demonstrate that the upregulation of retinal *Adamts19* expression is part  
266 of a protective molecular response to impaired ocular growth. Also, we use a  
267 complementary strategy to show that inactivation of *Prss56* or *Mfrp* prevents excessive  
268 ocular elongation in a mouse model of early-onset developmental myopia caused by a  
269 null mutation in *lrpb*. Overall, our findings suggest that PRSS56 and MFRP are not only  
270 necessary for supporting ocular axial elongation under normal conditions, but also in the  
271 context of childhood-onset high myopia.

272 Gene expression profiling led us to the identification of PRSS56 and ADAMTS19 two  
273 secreted serine proteases, whose expression is altered in the retina of mouse model  
274 recapitulating features of nanophthalmos. We have previously reported that increased  
275 retinal expression of *Prss56* was a key molecular feature of *Prss56* mutant mice  
276 exhibiting a reduction in ocular axial length [13]. Here, we show that *Adamts19*

277 expression is also upregulated in the retina of *Prss56* mutant mice. Importantly, taking  
278 advantage of the *Egr1;Prss56* double mutant mouse model in which *Egr1* inactivation  
279 rescues the ocular size reduction caused by loss of PRSS56 function, we demonstrate  
280 that the increased expression of retinal *Adamts19* results from ocular size reduction and  
281 is not a direct consequence of *Prss56* mutation per se. Further to support this finding,  
282 we show that retinal *Prss56* and *Adamts19* mRNA levels are also upregulated in an  
283 independent mouse model of nanophthalmos caused by a null *Mfrp* mutation.  
284 Importantly, we show that ADAMTS19 is not required for ocular axial growth during  
285 normal development, however, *Adamts19* inactivation exacerbates the reduction in  
286 ocular axial length and VCD in both *Prss56* and *Mfrp* mutant mouse models.  
287 Collectively, these findings indicate that the upregulation of retinal *Prss56* and  
288 *Adamst19* expression constitutes a protective response to overcome impaired ocular  
289 axial growth in two distinct mouse models of nanophthalmos. Since both PRSS56 and  
290 ADAMTS19 belong to the family of secreted serine-protease, it raises the possibility that  
291 they likely have overlapping or redundant function(s) and share the same substrate(s),  
292 which might explain the compensatory effect of ADAMTS19 on ocular elongation in  
293 mutant mice lacking PRSS56. Interestingly a recent study has found an association  
294 between genetic variant near *Adamts19* and ocular axial length, making our findings in  
295 mice relevant to human ocular size regulation [25].  
296 Using a complementary genetic approach, we demonstrate that *Prss56* and *Mfrp*  
297 inactivation prevents the excessive ocular axial growth observed in a mouse model of  
298 early-onset high myopia caused by a null mutation in *Irbp* [24]. In the currently  
299 accepted model of ocular axial elongation, signals originating from the retina must first

300 be relayed to the RPE before being transmitted to the choroid and subsequently to the  
301 sclera to induce scleral ECM remodeling and ocular axial growth [14, 18]. Importantly,  
302 the expression pattern of *Prss56* and *Adamts19* in the retina and that of *Mfrp* in the RPE  
303 are consistent with a central role for the retina and RPE in promoting ocular axial growth  
304 [13, 16]. More specifically, the cellular localization of *Prss56* and *Adamts19* highlights  
305 the importance of Müller glia in mediating crosstalk between the retina and RPE.  
306 Interestingly, IRBP is localized in the interphotoreceptor matrix, a layer occupying the  
307 subretinal space juxtaposing the retinal photoreceptor cells and RPE. Thus, IRBP along  
308 with PRSS56 and MFRP may be part of a signaling network that not only connects the  
309 retina and RPE but also facilitates the flow of information, which are integral to ocular  
310 growth regulation. The increased expression of retinal *Prss56* in *Mfrp* mutant mice  
311 further lends support to the existence of potential crosstalk between Müller glia and  
312 RPE in the regulation of ocular axial growth. Overall, our findings suggest that PRSS56  
313 and MFRP are critical for ocular axial growth during early developmental stages.  
314 Furthermore, as PRSS56 and MFRP are localized in the Müller glia and RPE  
315 respectively, they point towards a role for the interplay between Müller glia and RPE in  
316 ocular axial growth regulation.

317

318 We have shown previously that the ocular expression of *Prss56* is restricted to the  
319 retina, and predominantly observed in Müller glia [13]. Also, *Prss56* upregulation was  
320 seen in retinal Müller glia of *Mfrp* mutant mice [23]. Here, we show that *Adamst19*  
321 expression is specifically detected in the INL of the *Prss56* mutant retina, a region  
322 where the cell body of Müller glia soma is found. These findings suggest that impaired

323 ocular growth triggers the activation of a transcriptional program in retinal Müller glia  
324 leading to increased expression of *Prss56* and *Adamts19*, two genes encoding secreted  
325 serine proteases. Müller cells have been postulated to play a role in the detection of  
326 subtle changes in retinal structure due to mechanical stretching of their long processes  
327 or side branches[26, 27]. Reduction in ocular size may alter the structural and  
328 mechanical properties of the retina that are sensed by Müller glia triggering  
329 transcriptional activation of factors participating in the regulation of ocular axial growth  
330 [27].

331 Since genetic variants of PRSS56 and MFRP are also associated with common forms  
332 of myopia[11, 17], it raises the possibility of whether a nexus between Müller glia and  
333 RPE may have a broader role that contributes to vision-guided postnatal ocular growth.  
334 Also, given that loss of PRSS56 and MFRP function causes a reduction in ocular  
335 length[6, 13], it is plausible their noncoding variants associated with myopia may cause  
336 an increase in gene expression and thus, act via gain of function mechanism thereby  
337 contributing to an opposite phenotype characterized by an increase in ocular axial  
338 length. Furthermore, supporting the role of PRSS56 in myopia pathogenesis, a recent  
339 study in marmoset has shown an increase in retinal expression of *Prss56* in response to  
340 minus lens-induced axial elongation/myopic compared to the control eyes[28]. Future  
341 efforts will focus on determining the cellular and molecular basis of the potential  
342 crosstalk between Müller glia and RPE in the regulation of ocular axial growth and their  
343 relevance to axial elongation in the context of myopia.

344 In summary, we identify ADAMTS19 as a novel factor involved in ocular size regulation  
345 and use a distinct mouse model of hyperopia/reduced ocular size and myopia/

346 excessive ocular growth to describe a regulatory genetic network playing a central role  
347 in regulating eye growth during development and disease. Collectively, these findings  
348 raise the possibility that modulation of *Adamts19* expression could be part of the  
349 general adaptive mechanism needed for regulating ocular axial growth. Furthermore,  
350 they suggest that PRSS56 and MFRP are indispensable for normal and aberrant ocular  
351 axial growth as a consequence of mutation in *Irbp* and point towards *Prss56* and *Mfrp*  
352 likely being part of a sequential pathway necessary for supporting ocular elongation.

353

#### 354 **CONFLICT OF INTEREST STATEMENT**

355 All authors declare for no conflict of interests in the study

356

#### 357 **AUTHOR CONTRIBUTIONS**

358 SK and KSN conceived and designed the study. SK, CL-D, SP, and YZ performed  
359 experiments. SK, CL-D, YZ, and KSN interpreted the results of analyses on mouse  
360 study. SK, CL-D, KSN, contributed to the drafting of the original manuscript. SK, CL-D,  
361 and KSN critically reviewed the manuscript.

362

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371 with mouse genotyping assay and general laboratory care.

372

## 373 **MATERIALS AND METHODS**

### 374 **Animals**

375 All experiments were conducted in compliance with protocols approved by the  
376 Institutional Animal Care and Use Committee at University of California San Francisco  
377 (IACUC) (Protocols # AN181358-01D) and following the guidelines from the Association  
378 for Research in Vision and Ophthalmology's statement on the use of animals in  
379 ophthalmic research. Animals were given access to food and water ad libitum and  
380 housed under controlled conditions including 12-h light/dark cycle per the National  
381 Institutes of Health guidelines. Both male and female mice were used in all experiments  
382 and no differences were observed between sexes, all comparisons were made between  
383 littermates to minimize variability.

384

### 385 **Mouse lines**

- 386 1. ***Prss56*<sup>-/-</sup>** : (C57BL/6, Cg-*Prss56*<sup>glcr4</sup>/SjJ) – Mice carrying ENU induced mutation in  
387 *Prss56* causing truncation of PRSS56 protein at its C-terminal region [3].
- 388 2. ***Prss56*<sup>cre/cre</sup>** : (C57BL/6.Cg-*Prss56*tm(cre)) – Mice carrying a null allele of *Prss56* in  
389 which the exon1 of *Prss56* is replaced by CRE recombinase sequence[13, 29].

- 390 3. ***Egr1*<sup>-/-</sup>** :(C57BL/6. *Egr1*<sup>tm1Jmi/J</sup>) - *Egr1* mutant mice: C57BL/6. *Egr1*<sup>tm1Jmi/J</sup>, the  
391 targeted mutation by insertion of a PGK-neo cassette introduces stop codon  
392 resulting in protein truncation upstream of the DNA-binding domain[30].
- 393 4. ***Adamts19*<sup>-/-</sup>** :(*Adamts19*<sup>tm4a(EUCOMM)Wtsi</sup>) - A conditional *Adamts19* knockout mouse  
394 with *LoxP* sites flanking exon3. Excision of the *LoxP* sites by ubiquitously expressed  
395 CRE recombinase driven by beta-actin promoter leads to the generation of a  
396 knockout allele of *Adamts19* (Supplementary Fig.2 A&B).
- 397 5. ***Mfrp*<sup>-/-</sup>** :(B6.C3Ga-*Mfrp*<sup>rd6/J</sup>): The mouse strain is homozygous for rd6 exhibiting  
398 retinal degeneration around four weeks during retinal developmental phase [23].
- 399 6. ***Irbp*<sup>-/-</sup>** :(B6.129P2-*Rbp3*<sup>tm1Gil/J</sup>): A knockout mouse model *Irbp*(Interstitial retinal  
400 binding protein 3) gene. This mouse line carries a targeted mutation for the *Rbp3*  
401 gene where the promoter and Exon 1 have been replaced by a NEO selection  
402 cassette rendering *Irbp* protein inactive.

403 PCR genotyping of all mouse strains was performed on genomic DNA obtained from tail  
404 biopsies digested with Proteinase K (Sigma, St. Louis, MO, USA) using primers listed  
405 in Table S1.

406

### 407 **Ocular Biometry**

408 Ocular biometry was performed using Envisu R4300 spectral-domain optical coherence  
409 tomography (SD-OCT, Leica/Bioptigen Inc., Research Triangle Park, NC, USA).  
410 Measurements of various ocular parameters including axial length, vitreous chamber  
411 depth (VCD), anterior chamber depth (ACD), lens diameter and retinal thickness were

412 performed on mice anesthetized with ketamine/xylazine (100 mg/kg and 5mg/kg,  
413 respectively; intraperitoneal (IP)) following pupil dilation as described previously[13].

414

#### 415 **Quantitative polymerase chain reaction (qPCR)**

416 For qPCR analysis of gene expression, eyes were enucleated and retinas were  
417 immediately dissected and total RNA was extracted from mouse retinal tissue using  
418 Qiagen RNeasy Mini Kit as per manufacturers protocol (Qiagen, Valencia, CA, USA)  
419 and reverse-transcribed using iScript cDNA Synthesis Kit (Bio-Rad, Hercules, CA, USA)  
420 and primer sets listed in Table S2. qPCR was performed on Bio-Rad C1000 Thermal  
421 Cyclor/CF96 Real-Time System using SSOAdvanced™ SYBR Green® Supermix (Bio-  
422 Rad, Hercules, CA, USA). Briefly, 100ng of cDNA and 10uM primers were used per  
423 reaction in a final volume of 20ul. Each cycle consisted of denaturation at 95°C for 15s,  
424 followed by annealing at 60°C for 15s, extension 72°C for 30s for a total of 39 cycles. All  
425 the experiments were run as technical duplicates and a minimum of three biological  
426 replicates were used per group. The relative expression level of each gene was  
427 normalized to housekeeping genes (*Actinβ* and *Mapk1*) and analyzed using the CFX  
428 Maestro software (Bio-Rad, Hercules, CA, USA).

429

#### 430 ***In situ* hybridization**

431 Mice were transcardially perfused with ice-cold RNase-free PBS followed by 4% PFA (in  
432 RNase-free PBS). Eyes were enucleated post-fixed in RNase-free 4% PFA,  
433 cryoprotected in 20% sucrose, and embedded in OCT and sectioned within 24 hours for  
434 *in situ* hybridization. QuantiGene View RNA (Affymetrix, Santa Clara, CA, USA). *In situ*

435 hybridization was performed according to the manufacturer protocol. Briefly, 12 $\mu$ m  
436 cryosections were fixed overnight in 4% PFA, dehydrated through a graded series of  
437 ethanol, were subjected to 2X protease digestion for 10 minutes, fixed and hybridized  
438 with probe sets against *Adamts19* (NM\_175506 (*Adamts19*), TYPE1, high sensitivity  
439 with 40~50 bp DNAs) for 3 hours at 40°C using a ThermoBrite system (Abbott  
440 Molecular, Des Plaines, IL, USA). Cryosections were then washed and subject to signal  
441 amplification and detection using a fast red substrate, counterstained and mounted for  
442 subsequent imaging. Fluorescent images were acquired using an AxioImager M1  
443 microscope equipped with an MRm digital camera and AxioVision software, with an  
444 LSM700 confocal microscope and Zen software (Carl Zeiss Microscopy, LLC,  
445 Germany).

446

#### 447 **Statistical analyses**

448 Statistical comparisons between mutant and control groups or between multiple  
449 experimental groups at a given age were performed using two-tailed unpaired Student's  
450 t-test and one-way ANOVA, respectively, using Prism statistical software (version 6.02,  
451 GraphPad Software, San Diego, CA). A p-value of < (0.05, 0.01 and 0.001) was  
452 considered significant.

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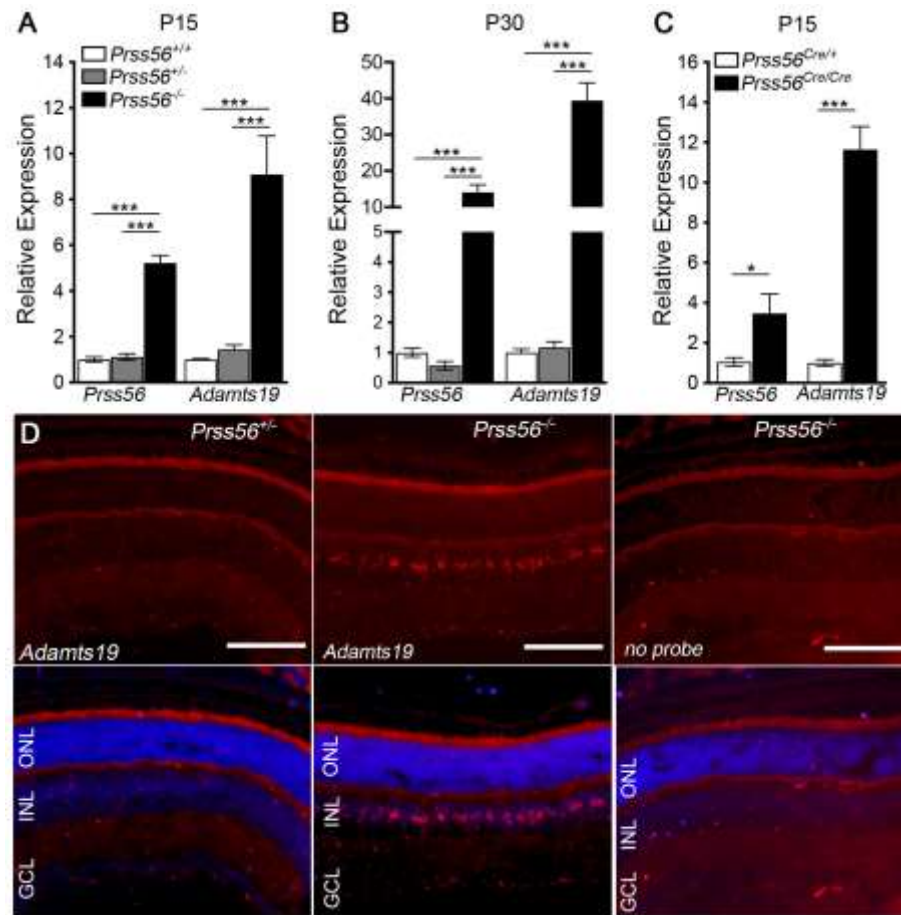
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**FIGURE AND LEGEND**



583

584 **Figure 1. *Adamts19* expression is upregulated in the retina of *Prss56* mutant**  
 585 **mice.**

586 (A-C) Graph showing quantification of *Prss56* and *Adamts19* mRNA levels using qPCR  
 587 in P15 (A and C) and P30 (B) retina from *Prss56*<sup>gcr4</sup> (A and B) and *Prss56*<sup>Cre</sup> (C) mutant  
 588 strains. While no difference was observed between *Prss56*<sup>+/+</sup> and *Prss56*<sup>gcr4/+</sup> (*Prss56*<sup>+/-</sup>  
 589 ) retina, a significant increase in *Prss56* and *Adamts19* mRNA levels was detected in  
 590 *Prss56*<sup>gcr4/gcr4</sup> (*Prss56*<sup>-/-</sup>) retina compared to *Prss56*<sup>+/+</sup> and *Prss56*<sup>gcr4/+</sup> retina at both  
 591 ages examined (A and B). Similarly, significant increases in *Prss56* and *Adamts19*  
 592 mRNA levels were detected in *Prss56*<sup>Cre/Cre</sup> retina compared to the control *Prss56*<sup>Cre/+</sup>  
 593 retina. *Prss56* and *Adamts19* expression were normalized to the expression of three  
 594 housekeeping genes (*Hprt1*, *Actb1*, and *Mapk1*). Data are presented as fold expression  
 595 relative to wild-type (mean ± SEM), N= 4 to 6 retina/group, data are presented as



596 mean $\pm$  SEM, \* $p < 0.05$ ; \*\*\* $p < 0.001$ , t-test. (D) QuantiGene View RNA *in situ*  
597 hybridization revealed that *Adamts19* expression was below the threshold level of  
598 detection in control *Prss56<sup>gclr4/+</sup>* retina and was only detectable in *Prss56<sup>gclr4/gclr4</sup>* retina at  
599 P16. *Adamts19* expression (red) was predominantly observed in the INL of *Prss56*  
600 mutant retina with low levels also detected in the GCL. Scale bars: 100 $\mu$ m

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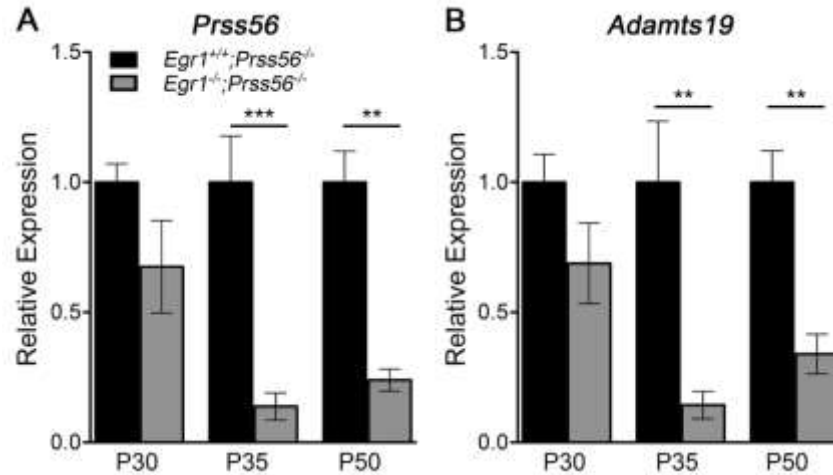
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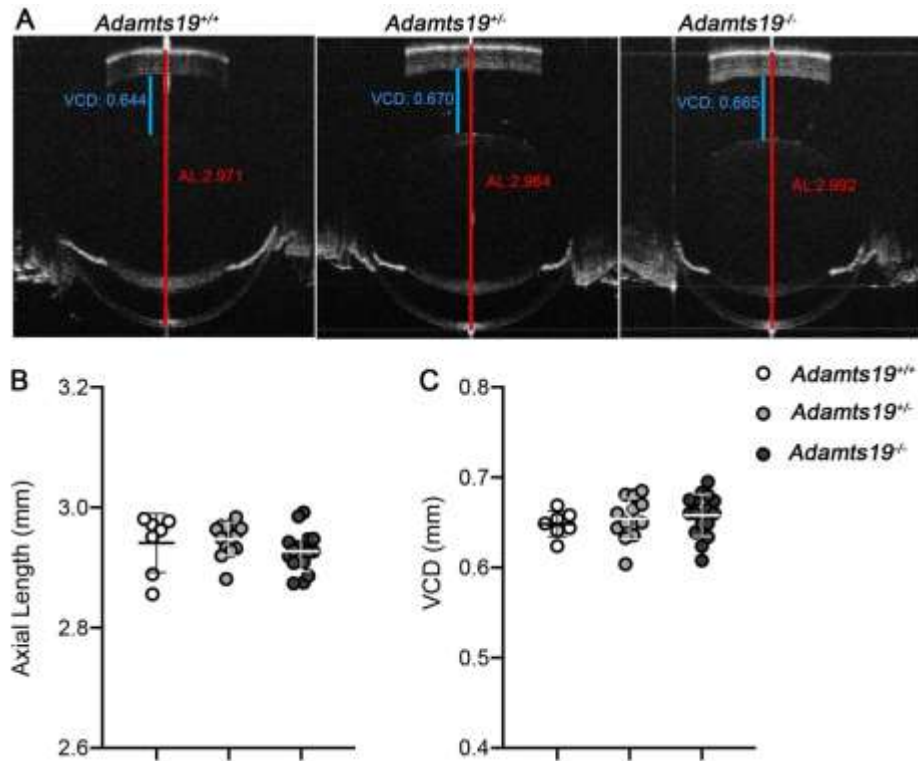
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**Figure 2. *Egr1* inactivation prevents the upregulation of retinal *Prss56* and *Adamts19* expression in *Prss56* mutant mice. (A-B) Graphs showing quantification of *Prss56* (A), *Adamts19* (B) mRNA levels using qPCR in *Prss56* mutant (*Egr1*<sup>+/+</sup>;*Prss56*<sup>-/-</sup>) and *Prss56*;*Egr1* double mutant (*Egr1*<sup>-/-</sup>;*Prss56*<sup>-/-</sup>) retina at different developmental stages. *Egr1* inactivation reduced retinal *Prss56* (A) and *Adamts19* (B) mRNA levels in *Prss56* mutant mice (compare *Egr1*<sup>+/+</sup>;*Prss56*<sup>-/-</sup> to *Egr1*<sup>-/-</sup>;*Prss56*<sup>-/-</sup>). Data are presented as fold expression relative to wild-type (mean ± SEM), N=4 to 6/group. \*\*p<0.01; \*\*\*p<0.001, t-test.**



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650 **Figure 3. ADAMTS19 is not required for axial growth during normal ocular development. (A)**

651 Representative OCT images showing that ocular axial length (quantified in **B**) and VCD (quantified in **C**)

652 are indistinguishable between *Adamts19*<sup>-/-</sup>, *Adamts19*<sup>+/-</sup> and control *Adamts19*<sup>+/+</sup> mice at P18. Data are

653 presented as mean ± SD, N>7/group.

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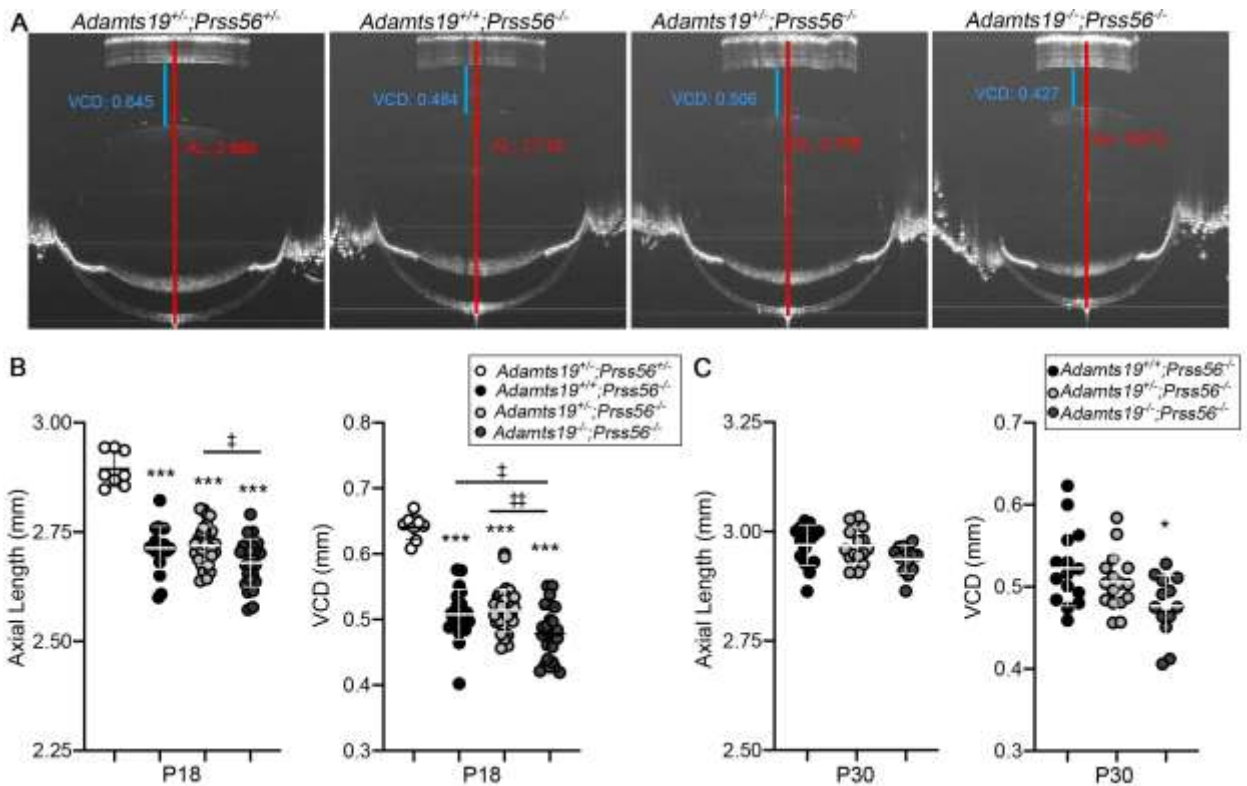
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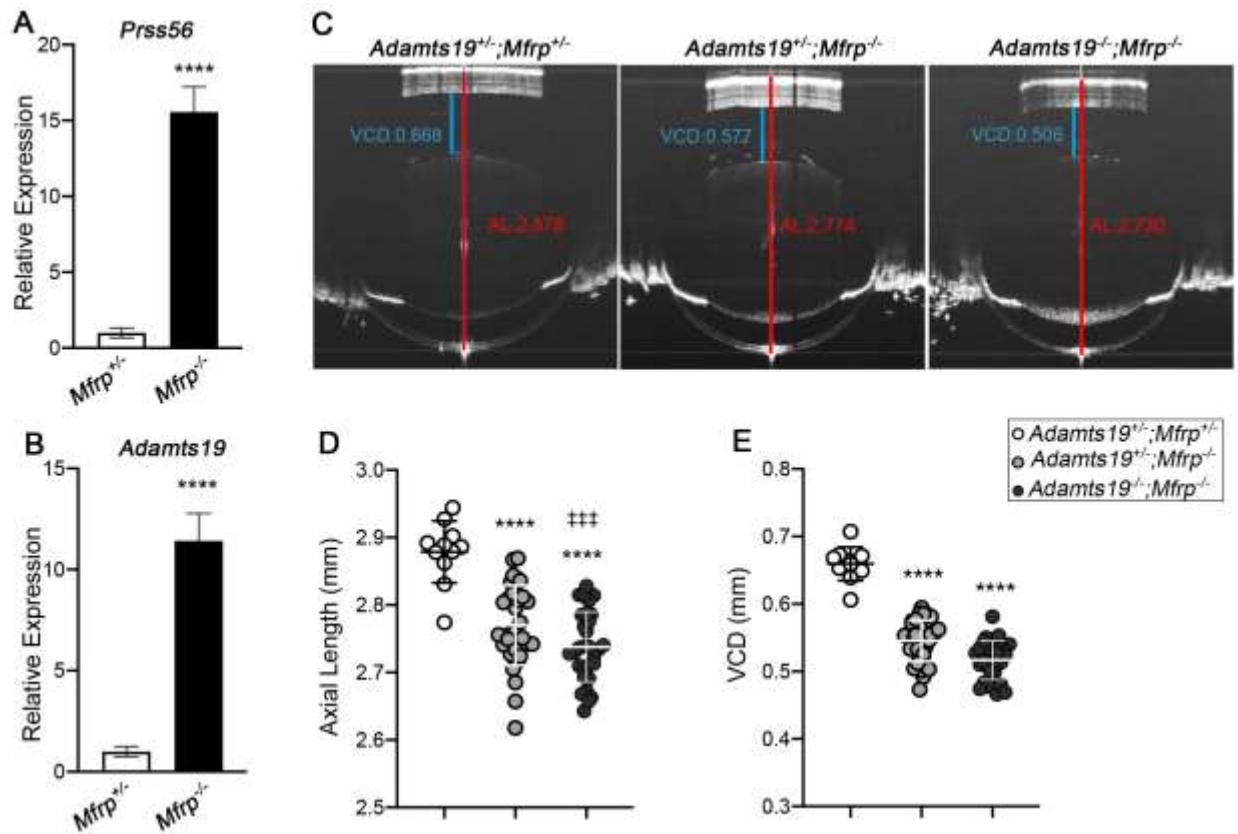
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**Figure 4. *Adamts19* inactivation exacerbates the ocular axial length reduction in *Prss56* mutant mice.** (A) Representative OCT images showing reduced ocular axial length and VCD (quantified in B and C) in mice carrying a *Prss56* mutation (*Adamts19*<sup>+/+</sup>;*Prss56*<sup>-/-</sup>;*Adamts19*<sup>+/+</sup>;*Prss56*<sup>-/-</sup> and *Adamts19*<sup>-/-</sup>;*Prss56*<sup>-/-</sup>) compared to control *Adamts19*<sup>+/+</sup>;*Prss56*<sup>+/+</sup> mice. Importantly, the *Adamts19*;*Prss56* double mutant (*Adamts19*<sup>-/-</sup>;*Prss56*<sup>-/-</sup>) mice show a modest but consistent reduction in ocular axial length and VCD compared to *Prss56* single mutant (*Adamts19*<sup>+/+</sup>;*Prss56*<sup>-/-</sup> or *Adamts19*<sup>+/+</sup>;*Prss56*<sup>-/-</sup>) mice at both ages examined (P18 and P30). Data are presented as mean ± SD, N>13/group. \*p<0.05; \*\*p<0.01, One-way ANOVA.

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688 **Figure 5. *Mfrp* mutant mice induced ocular size reduction is exacerbated by**

689 ***Adamts19* inactivation (A-B)** Histogram showing relative *Prss56* (A) and

690 *Adamts19* (B) mRNA levels in *Mfrp*<sup>+/+</sup> and *Mfrp*<sup>-/-</sup> retina at P15. A significant

691 increase in *Prss56* (A) and *Adamts19* (B) was detected in *Mfrp*<sup>-/-</sup> compared to

692 *Mfrp*<sup>+/+</sup> retina, N>4/group. (C) Representative OCT images showing that ocular

693 axial length (quantified in D) and VCD (quantified in E) are reduced in *Mfrp* single

694 mutant (*Adamts19*<sup>+/+</sup>; *Mfrp*<sup>-/-</sup>) and *Adamts19*; *Mfrp* double mutant (*Adamts19*<sup>-/-</sup>; *Mfrp*<sup>-/-</sup>)

695 mice compared to control (*Adamts19*<sup>+/+</sup>; *Mfrp*<sup>+/+</sup>) eyes at P18, N>15 groups. Of

696 note, the ocular axial length was significantly more reduced in *Adamts19*<sup>-/-</sup>;*Mfrp*<sup>-/-</sup>

697 than *Adamts19*<sup>+/+</sup>;*Mfrp*<sup>-/-</sup> mice. Data are presented as mean ± SD. \*\*\*\*p<0.0001

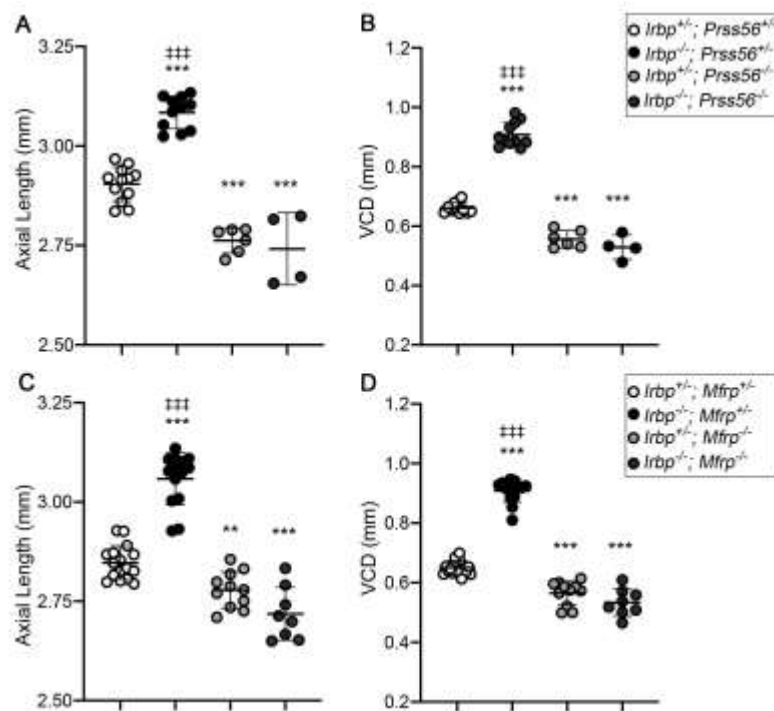
698 (compared to controls); ###p<0.001 (compared to *Mfrp* single mutant), One way

699 ANOVA.

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703 **Figure 6. Ocular axial length elongation in *Irbp* mutant mice is dependent on**

704 **PRSS56 or MFRP. (A-D) Scatter plot showing ocular axial length (A and C) and**

705 **vitreous chamber depth (VCD) (B and D) values in *Irbp* mutant or control mice carrying**

706 **a heterozygous or recessive mutation in *Prss56* or *Mfrp*: *Irbp* single mutants (*Irbp*<sup>-/-</sup>;**

707 ***Prss56*<sup>+/+</sup> in A and B, and *Irbp*<sup>-/-</sup>;*Mfrp*<sup>+/+</sup> in C and D), *Irbp*;*Prss56* double mutant mice**

708 **(*Irbp*<sup>-/-</sup>;*Prss56*<sup>-/-</sup> in A and B), *Irbp*;*Mfrp* double mutant mice (*Irbp*<sup>-/-</sup>;*Mfrp*<sup>-/-</sup> in C and D)**

709 **and *Prss56* and *Mfrp* single mutant mice (*Irbp*<sup>+/+</sup>;*Prss56*<sup>-/-</sup> in A and C and *Irbp*<sup>+/+</sup>;**

710 ***Mfrp*<sup>-/-</sup> in B and D respectively). Biometric analyses revealed that significant ocular axial**

711 **elongation in *Irbp* single mutant mice (*Irbp*<sup>-/-</sup>;*Prss56*<sup>+/+</sup> or *Irbp*<sup>-/-</sup>;*Mfrp*<sup>+/+</sup>) compared to**

712 **control mice (*Irbp*<sup>+/+</sup>;*Prss56*<sup>+/+</sup> or *Irbp*<sup>+/+</sup>;*Mfrp*<sup>+/+</sup>, respectively) that contrasts with the ocular**

713 **axial length reduction observed in *Prss56* and *Mfrp* single mutant mice (*Irbp*<sup>+/+</sup>;*Prss56*<sup>-/-</sup>**

714 **and *Irbp*<sup>+/+</sup>;*Mfrp*<sup>-/-</sup>, respectively). Notably, *Prss56* and *Mfrp* inactivation prevented the**

715 **ocular axial length elongation observed in *Irbp* mutant mice, as the ocular biometry of**

716 ***Irbp*<sup>-/-</sup>;*Prss56*<sup>-/-</sup> and *Irbp*<sup>-/-</sup>;*Mfrp*<sup>-/-</sup> are comparable to *Prss56* or *Mfrp* single mutants,**

717 **respectively (*Irbp*<sup>+/+</sup>;*Prss56*<sup>-/-</sup> and *Irbp*<sup>+/+</sup>;*Mfrp*<sup>-/-</sup>, respectively). Overall, they suggest that**

718 **PRSS56 and MFRP functions are required to induce ocular axial length elongation in**

719 ***Irbp* mutant mice. Data are presented as mean ± SD (A-D) and as fold expression**

720 relative to wild-type (mean  $\pm$  SEM), N=2 to 10/group (**E-F**). \*\*p<0.01; \*\*\*p<0.0001  
721 (compared to controls); ##p<0.001 (compared to double mutant *Irpb*<sup>-/-</sup>;*Prss56*<sup>-/-</sup> and *Irpb*<sup>-/-</sup>;  
722 *Mfrp*<sup>-/-</sup> mice), One way Anova.

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