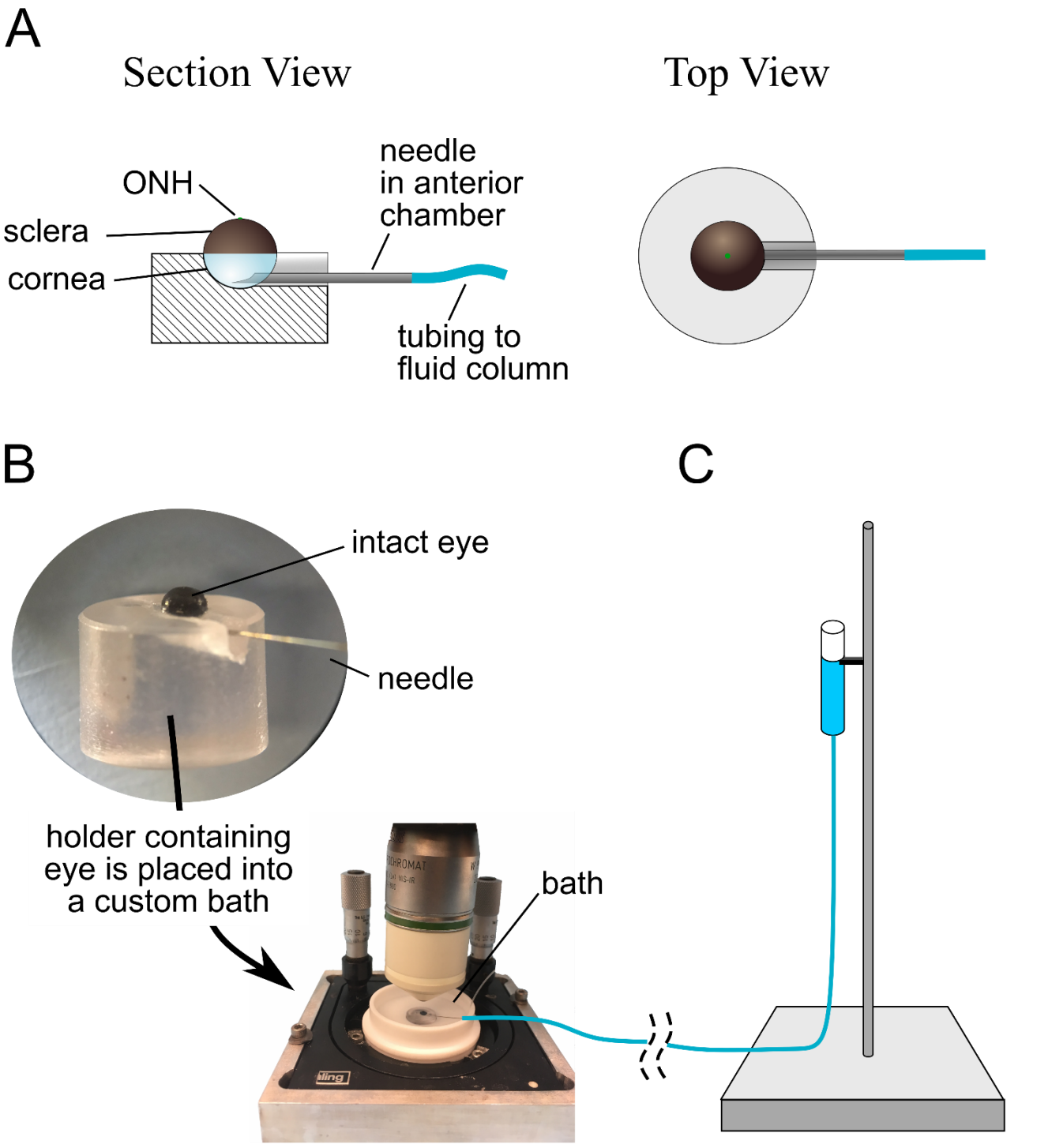

Figure S1. The experimental set-up for all eyes studied (including the treatment-only eyes). The eye globe remained intact without removal of any internal contents. The optic nerve was cut from the eye globe with a razor blade at the level of the posterior sclera to expose the optic nerve head (ONH). The ONH is marked by a dot in the Top View of (A). The cornea was glued to a crater in a holder, shown in schematic (A) and in the picture in (B). The anterior chamber was cannulated with a beveled 32-gauge needle. Tubing connected the needle to a fluid reservoir column regulating the intraocular pressure (IOP), shown in schematic (C). The height of the reservoir column was calibrated with an in-line pressure transducer. The eye globe was kept submerged in a custom bath filled with PBS shown in the bottom picture of (B). The treatment, buffer or TrypLE, and washes were performed in the custom bath. Only eyes subjected to ex vivo imaging were placed underneath the microscope objective shown in (B).

Table S1. Regional comparison of the deformation of the AL by treatment. Values are copied from Table 2, showing the expansion in the radial and circumferential directions due to buffer or TrypLE (n=8 per group), defined by E_{rr} and $E_{\theta\theta}$ calculated from DVC of image volumes taken at 10 mmHg before and after treatment. The values were calculated by averaging across the whole image volume, across the central region, or across a combined peripheral and rim region (c.f. Sect. 2.8 for methods). Radial expansion due to TrypLE was positive in the central and negative (i.e., contraction) in the combined peripheral and rim regions, (n=8, p=0.04). P-values are from Wilcoxon signed rank tests comparing the values in the central to the values in the combined peripheral region. Values are mean ± std.

	Region	Group	Expansion	*p-value*
Radial Expansion	Central	Buffer	0.0102 ± 0.0143	0.25
	Peri + Rim		0.0020 ± 0.0156	
	Central	TrypLE	**0.0015 ± 0.0209**	**0.04**
	Peri + Rim		**-0.0440 ± 0.0380**	
Circumferential Expansion	Central	Buffer	0.0158 ± 0.0169	0.31
	Peri + Rim		0.0120 ± 0.0111	
	Central	TrypLE	0.0120 ± 0.0104	0.11
	Peri + Rim		0.0023 ± 0.0233	

Table S2. Regional comparison of the width and area percentage of processes expressing GFP^{Astro}, after treatment, measured from slow scans acquired before and after treatment with buffer (n=8) or TrypLE (n=8). The values were calculated by averaging across the central, the peripheral, and the rim regions. The regions were based on the in-plane radial distance from the centroid to the boundary of the AL. The 0-50% of the radial distance defined the central region, the 50-90% defined the peripheral, and the 90-100% defined the rim (c.f. Sect. 2.8 for methods). Two-way ANOVA was performed with Greenhouse-Geisser correction. ANOVA tested the effect of the region (central, peripheral, or rim) and the effect of treatment (buffer or TrypLE). Repeated measures from the same sample were accounted for in the test. GraphPad Prism version 8 (GraphPad Software Inc., La Jolla, California) performed the test. P-values for the effect of region and the effect of treatment are listed below the table. P-values in the table are from multiple comparisons following the two-way ANOVA, with Sidak's correction. Values are mean ± std.

	Group	Region	After treatment	Buffer vs. TrypLE *p-value*	Regional multiple comparisons *p-value*
GFP^{Astro} area percentage	Buffer	Central	0.381 ± 0.070	Central p=0.97 Peripheral p=0.79 Rim p=0.21	Central vs. Peri p=0.07
		Peripheral	0.409 ± 0.051		Central vs. Rim p=0.85
		Rim	0.368 ± 0.035		**Peri vs. Rim p=0.04**
	TrypLE	Central	0.392 ± 0.038		**Central vs. Peri p=0.009**
		Peripheral	0.427 ± 0.028		Central vs. Rim p=0.59
		Rim	0.412 ± 0.054		Peri vs. Rim p=0.61
GFP^{Astro} process width (μm)	Buffer	Central	2.44 ± 0.13	Central p=1.00 Peripheral p=0.45 Rim p=0.96	Central vs. Peri p=0.74
		Peripheral	2.48 ± 0.10		Central vs. Rim p=0.85
		Rim	2.48 ± 0.14		Peri vs. Rim p=1.00
	TrypLE	Central	2.43 ± 0.14		Central vs. Peri p=1.00
		Peripheral	2.42 ± 0.06		Central vs. Rim p=0.98
		Rim	2.46 ± 0.14		Peri vs. Rim p=0.93

GFP^{Astro} area percentage results of Two-Way ANOVA
There was an effect of region, p-value = 0.01
No effect of treatment, p-value = 0.28

GFP^{Astro} process width results of Two-Way ANOVA
No effect of region, p-value = 0.84
No effect of treatment, p-value = 0.41

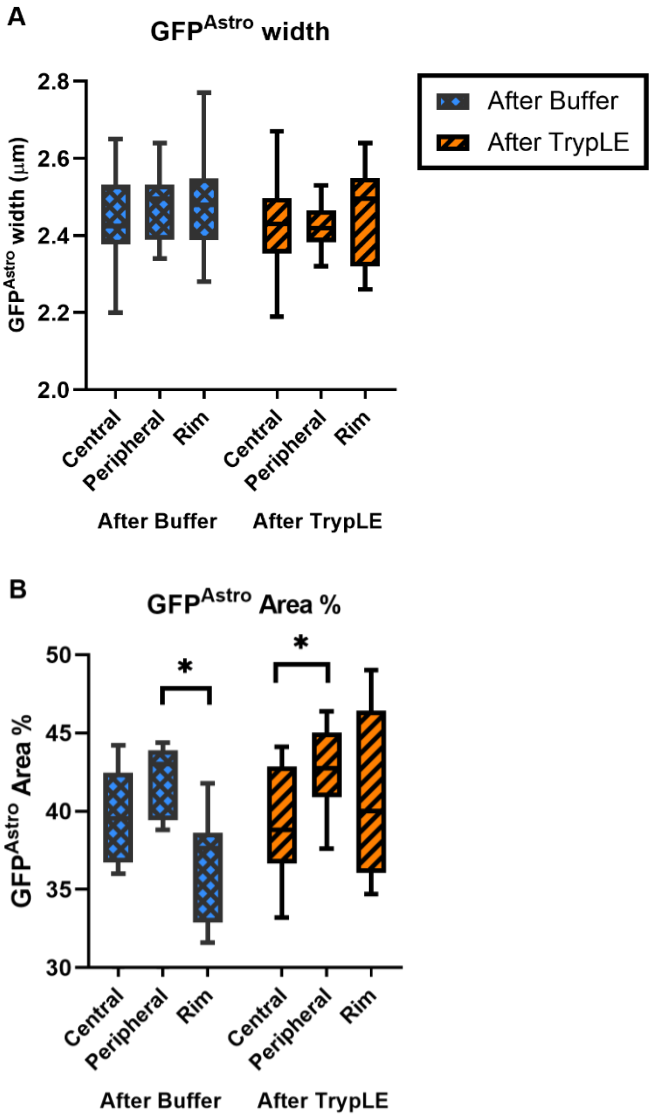

*Figure S2. Regional analyses of the processes expressing GFP^{Astro} in the AL after treatment with buffer or TrypLE. The values were calculated by averaging across the central, the peripheral, and the rim regions. The regions were based on the in-plane radial distance from the centroid to the boundary of the AL. The 0-50% of the radial distance defined the central region, the 50-90% defined the peripheral, and the 90-100% defined the rim (c.f. Sect. 2.8 for methods). (A) Boxplots of the GFP^{Astro} process width and (B) the area percentage of GFP^{Astro} processes. Boxplots: the interquartile range, the line marks the median, and whiskers are drawn by Tukey's method. Statistical significance is from multiple comparisons of two-way ANOVA tests described in the caption of Table S2. *indicates $p<0.05$ ($n=8$ buffer, $n=8$ TrypLE).*

Table S3 Regional comparison of the width and area percentage of F-actin processes. The values were calculated by averaging across the central, the peripheral, and the rim regions (c.f. Sect. 2.8 for methods). Values were from the network analysis algorithm performed on images from histochemistry labeling for F-actin. Histochemistry was performed on eyes after they were treated and fixed (n=9 buffer, n=9 TrypLE). Two-way ANOVA was performed with Greenhouse-Geisser correction. ANOVA tested the effect of the region (central, peripheral, or rim) and the effect of treatment (buffer or TrypLE). Repeated measures from the same sample were accounted for in the test. GraphPad Prism version 8 (GraphPad Software Inc., La Jolla, California) performed the test. P-values for the effect of region and the effect of treatment are listed below the table. P-values in the table are from multiple comparisons following the two-way ANOVA, with Sidak's correction. Values are mean ± std.

	Group	Region		Multiple comparisons, *p*-value from the comparison of treatment	Multiple comparisons, *p*-value from the comparison of regions
F-actin area percentage	Buffer	Central	67.4% ± 1.2%	Central *p*=0.84 Peripheral *p*=0.96 Rim *p*=1.00	Central vs. Peri *p*=0.95 **Central vs. Rim *p*=0.009** **Peri vs. Rim *p*=0.005**
		Peripheral	67.6% ± 0.7%		
		Rim	60.4% ± 4.3%		
	TrypLE	Central	66.8% ± 2.0%		Central vs. Peri *p*=0.98 **Central vs. Rim *p*=0.02** **Peri vs. Rim *p*=0.002**
		Peripheral	67.1% ± 3.5%		
		Rim	60.4% ± 5.4%		
F-actin process width (µm)	Buffer	Central	1.58 ± 0.14	Central *p*=0.39 Peripheral *p*=0.81 Rim *p*=0.80	**Central vs. Peri *p*=0.04** Central vs. Rim *p*=0.06 Peri vs. Rim *p*=0.17
		Peripheral	1.50 ± 0.10		
		Rim	1.40 ± 0.09		
	TrypLE	Central	1.47 ± 0.16		Central vs. Peri *p*=0.89 Central vs. Rim *p*=0.32 Peri vs. Rim *p*=0.25
		Peripheral	1.45 ± 0.14		
		Rim	1.44 ± 0.42		

F-actin area percentage results of Two-Way ANOVA
There was an effect of region, p-value = 0.0001
No effect of treatment, p-value = 0.74

F-actin process width results of Two-Way ANOVA
There was an effect of region, p-value = 0.003
No effect of treatment, p-value = 0.24

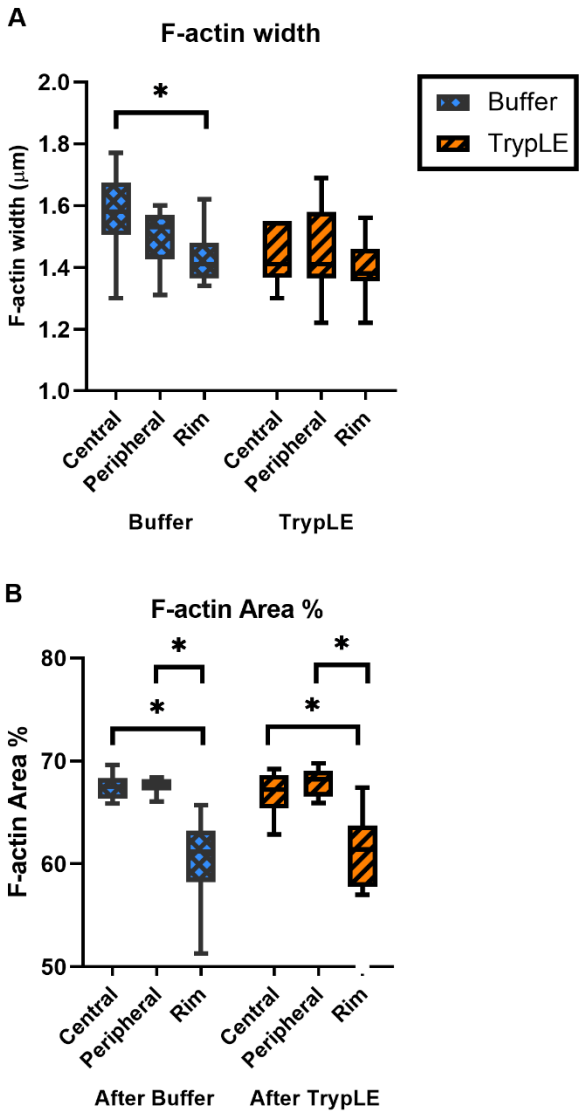

*Figure S3. Regional analyses of the processes with F-actin in the AL after treatment with buffer or TrypLE. The values were calculated by averaging across the central, the peripheral, and the rim regions (c.f. Sect. 2.8 for methods). (A) Boxplots of the F-actin process width and (B) the area percentage of F-actin processes. Statistical significance is from multiple comparisons of two-way ANOVA tests described in the caption of Table S3. *indicates $p<0.05$ ($n=9$ buffer, $n=9$ TrypLE).*

Table S4. Bias and uncertainty of the DVC measurement of strain in the AL, as previously described [1]. Pairs of two consecutive image volumes at 10mmHg from the same inflation test were used. One of the images was artificially warped by stretches of 1.02 in the x-direction, 1.02 in the y-directions, and 0.95 in the z-direction, and translated by 10 pixels in the x-direction, 10 pixels in the y-direction, and 3 pixels in the z-direction. This resulted in an applied strain, $E_{applied}$. Then, DVC was used to correlate the pair of image volumes and the strain between the images was calculated, $E_{calculated}$ in the same way as in Sect. 2.7. The strain error was calculated as abs($E_{applied}$ - $E_{calculated}$) at all points where abs takes the absolute value. The bias equals the mean and the uncertainty equals the std of the strain error. Values below are from n=16 samples from the inflation test before treatment with buffer (n=8) and before treatment with TrypLE (n=8). The bias and uncertainty of the DVC measurement were not affected by treatment with buffer or TrypLE in the AL nor in the PPS (data not shown). Values are mean ± std of n=16 samples.

	Bias	Uncertainty
AL		
E_{rr}	0.0041 ± 0.0015	0.0044 ± 0.0015
$E_{\theta\theta}$	0.0031 ± 0.0011	0.0035 ± 0.0013
$E_{r\theta}$	0.0024 ± 0.0008	0.0026 ± 0.0007
E_{xx}	0.0034 ± 0.0014	0.0040 ± 0.0016
E_{yy}	0.0038 ± 0.0015	0.0040 ± 0.0013
E_{xy}	0.0024 ± 0.0009	0.0025 ± 0.0008
E_{zz}	0.0283 ± 0.0136	0.0136 ± 0.0122
PPS		
E_{rr}	0.0027 ± 0.0005	0.0028 ± 0.0005
$E_{\theta\theta}$	0.0020 ± 0.0003	0.0021 ± 0.0003
$E_{r\theta}$	0.0017 ± 0.0003	0.0016 ± 0.0003
E_{xx}	0.0027 ± 0.0004	0.0029 ± 0.0005
E_{yy}	0.0022 ± 0.0004	0.0023 ± 0.0004
E_{xy}	0.0016 ± 0.0003	0.0015 ± 0.0003

[1] *A. Korneva, E.C. Kimball, J.L. Jefferys, H.A. Quigley, T.D. Nguyen, Biomechanics of the optic nerve head and peripapillary sclera in a mouse model of glaucoma, J. R. Soc. Interface. 17 (2020). https://doi.org/10.1098/rsif.2020.0708rsif20200708.*

Table S5. Comparison of in-plane strain outcomes for the AL of eyes before treatment. The strain outcomes from n=8 before treatment with buffer were combined with the n=8 before treatment with TrypLE. The values are means ± std of the strain outcomes from all 16 samples before treatment. Wilcoxon signed rank tests (two-tailed) were used to compare E_{xx} to E_{yy} and independently to compare $E_{\theta\theta}$ to E_{rr}. E_{xx} was greater than E_{yy} and $E_{\theta\theta}$ was greater than E_{rr} before treatment.

		p-value
E_{xx}	**0.0255 ± 0.0095**	**< 0.0001**
E_{yy}	**0.0108 ± 0.0065**	
$E_{\theta\theta}$	**0.0280 ± 0.0114**	**< 0.0001**
E_{rr}	**0.0083 ± 0.0066**	

Table S6. The differences in the strain response of the AL. The formula for the strain difference in E_{rr} is $\Delta E_{rr} = E_{rr,\,after} - E_{rr,before}$. The Mann-Whitney U tests (two tailed) were used to compare the strain differences due to buffer to those due to TrypLE. Strain increases in ΔE_{rr}, ΔE_{xx}, ΔE_{yy}, ΔE_{max}, and ΔE_{min} due to TrypLE were greater than the strain differences due to buffer. Values are mean ± std.

	Group		*p-value Buffer vs. TrypLE*
ΔE_{rr}	Buffer	**0.0035 ± 0.0038**	**0.001**
	TrypLE	**0.0212 ± 0.0089**	
$\Delta E_{\theta\theta}$	Buffer	-0.0027 ± 0.0095	0.06
	TrypLE	0.0059 ± 0.0099	
ΔE_{xx}	Buffer	**-0.0008 ± 0.0093**	**0.02**
	TrypLE	**0.0088 ± 0.0086**	
ΔE_{yy}	Buffer	**0.0016 ± 0.0024**	**0.0002**
	TrypLE	**0.0183 ± 0.0101**	
ΔE_{max}	Buffer	**-0.0020 ± 0.0116**	**0.02**
	TrypLE	**0.0127 ± 0.0132**	
ΔE_{min}	Buffer	**0.0028 ± 0.0059**	**0.005**
	TrypLE	**0.0144 ± 0.0073**	
$\Delta\gamma_{max}$	Buffer	-0.0024 ± 0.0075	0.74
	TrypLE	-0.0008 ± 0.0061	

Table S7. The strain increases due to TrypLE compared to the $\Delta E_{rr,}$ due to buffer. In Table S6, strain increases in ΔE_{rr}, ΔE_{xx}, ΔE_{yy}, ΔE_{max}, and ΔE_{min} due to TrypLE were greater than the strain differences due to buffer. The maximum increase due to buffer was in $\Delta E_{rr} = 0.0035 \pm 0.0038$ (n=8). The Mann-Whitney U tests (two tailed) were used to perform five pairwise comparisons between ΔE_{rr} due to buffer and a strain difference due to TrypLE. Strain increases in ΔE_{rr}, ΔE_{yy}, and ΔE_{min} due to TrypLE were greater than $\Delta E_{rr,}$ due to buffer ($p \leq 0.005$). Values below are copied from Table S6, they are mean ± std of the TrypLE group (n=8).

	Group		*p-value* ΔE_{rr} *Buffer vs.* $\Delta E\_\_$ *TrypLE*
ΔE_{rr}	TrypLE	**0.0212 ± 0.0089**	**0.001**
ΔE_{xx}	TrypLE	0.0088 ± 0.0086	0.28
ΔE_{yy}	TrypLE	**0.0183 ± 0.0101**	**0.0005**
ΔE_{max}	TrypLE	0.0127 ± 0.0132	0.13
ΔE_{min}	TrypLE	**0.0144 ± 0.0073**	**0.005**

Table S8. The strain outcomes were averaged within the central region and within the combined peripheral region; the latter combined the periphery and the rim of the AL. Values are mean ± std of strain outcomes in the AL of eyes before and after treatment with buffer (n=8) or TrypLE (n=8). Statistical analysis is in Table S9.

	Central Region		Peripheral + Rim	
	Before	After	Before	After
Buffer				
E_{rr}	0.0330 ± 0.0156	0.0309 ± 0.0122	-0.0014 ± 0.0091	0.0031 ± 0.0051
$E_{\theta\theta}$	0.0369 ± 0.0200	0.0313 ± 0.0131	0.0241 ± 0.0103	0.0223 ± 0.0064
$E_{r\theta}$	-0.003 ± 0.0014	0.0001 ± 0.0011	0.0007 ± 0.0017	0.0013 ± 0.0020
E_{xx}	0.0470 ± 0.0273	0.0432 ± 0.0180	0.0180 ± 0.0031	0.0175 ± 0.0057
E_{yy}	0.0229 ± 0.0106	0.0191 ± 0.0103	0.0046 ± 0.0066	0.0079 ± 0.0050
E_{xy}	0.0007 ± 0.0152	0.0036 ± 0.0108	0.0031 ± 0.0066	0.0031 ± 0.0077
E_{max}	0.0546 ± 0.0316	0.0498 ± 0.0184	0.0354 ± 0.0115	0.0339 ± 0.0071
E_{min}	0.0153 ± 0.0062	0.0125 ± 0.0093	-0.0127 ± 0.0100	-0.0085 ± 0.0064
γ_{max}	0.0196 ± 0.0143	0.0187 ± 0.0073	0.0241 ± 0.0100	0.0212 ± 0.0063
TrypLE				
E_{rr}	0.0314 ± 0.0101	0.0382 ± 0.0117	-0.0052 ± 0.0110	0.0212 ± 0.0110
$E_{\theta\theta}$	0.0359 ± 0.0135	0.0361 ± 0.0111	0.0236 ± 0.0077	0.0319 ± 0.0097
$E_{r\theta}$	0.0004 ± 0.0017	0.0002 ± 0.0014	0.0002 ± 0.0030	0.0002 ± 0.0028
E_{xx}	0.0408 ± 0.0138	0.0376 ± 0.0084	0.0146 ± 0.0072	0.0283 ± 0.0095
E_{yy}	0.0265 ± 0.0118	0.0367 ± 0.0156	0.0038 ± 0.0066	0.0248 ± 0.0072
E_{xy}	0.0075 ± 0.0069	0.0068 ± 0.0083	0.0022 ± 0.0063	0.0058 ± 0.0084
E_{max}	0.0491 ± 0.0155	0.0527 ± 0.0150	0.0328 ± 0.0070	0.0494 ± 0.0112
E_{min}	0.0182 ± 0.0097	0.0216 ± 0.0098	-0.0143 ± 0.0104	0.0037 ± 0.0085
γ_{max}	0.0155 ± 0.0056	0.0155 ± 0.0059	0.0236 ± 0.0065	0.0229 ± 0.0057

Table S9. Statistical comparison before to after treatment within regions of the AL. The strain outcomes were averaged within the central region and within the combined peripheral region; the latter combined the periphery and the rim of the AL. Values are reported in Table S8 for the buffer (n=8) or TrypLE (n=8) groups. Wilcoxon signed rank tests were used to compare the values before vs. after treatment. The tests were performed independently for each region and each treatment group. P-values are reported below.

	Buffer	*TrypLE*	*Buffer*	*TrypLE*
	Central *Before vs. After*	*Central* *Before vs. After*	*Peri + Rim* *Before vs. After*	*Peri + Rim* *Before vs. After*
E_{rr}	0.46	0.46	0.12	**0.01**
$E_{\theta\theta}$	0.23	0.84	0.46	**0.04**
E_{xx}	0.38	0.38	0.74	**0.02**
E_{yy}	0.15	0.31	0.08	**0.01**
E_{max}	0.38	0.74	0.64	**0.01**
E_{min}	0.46	0.55	0.15	**0.01**
γ_{max}	0.95	0.64	0.27	0.46

Table S10. The central to peripheral percent differences in the strain in the AL of eyes before and after treatment with TrypLE (n=8) or buffer (n=8). The regional differences in strain were computed by the equation $\frac{\textbf{Central}-\textbf{Combined}}{\textbf{abs}(\textbf{Combined})}$ *where abs takes the absolute value, and the combined refers to the strain in the combined peripheral and rim region. The Wilcoxon signed rank tests (two-tailed) were used to compare the values before treatment to those after treatment. TrypLE decreased the central to peripheral percent difference in* $E_{\theta\theta}$, E_{xx}, E_{max}, *and* E_{min} *(n=8,* $p \leq 0.03$*).*

$\frac{\textbf{Central}-\textbf{Combined}}{\textbf{abs}(\textbf{Combined})}$		Before	After	*p-value* *Before vs. After*
Difference for E_{rr}	Buffer	1700% ± 3500%	770% ± 640%	0.46
	TrypLE	710% ± 800%	200% ± 320%	0.15
Difference for $E_{\theta\theta}$	Buffer	50% ± 27%	38% ± 16%	0.30
	TrypLE	**50% ± 17%**	**14% ± 19%**	**0.02**
Difference for E_{xx}	Buffer	160% ± 120%	160% ± 89%	0.95
	TrypLE	**320% ± 430%**	**52% ± 74%**	**0.03**
Difference for E_{yy}	Buffer	3600% ± 6500%	290% ± 420%	0.31
	TrypLE	**650% ± 570%**	**49% ± 42%**	**0.01**
Difference for E_{max}	Buffer	49% ± 41%	44% ± 23%	0.66
	TrypLE	**48% ± 27%**	**7% ± 19%**	**0.01**
Difference for E_{min}	Buffer	530% ± 630%	590% ± 860%	0.74
	TrypLE	340% ± 300%	410% ± 400%	0.55
Difference for γ_{max}	Buffer	-23% ± 26%	-14% ± 20%	0.22
	TrypLE	-34% ± 17%	-33% ± 16%	0.95

Table S11. Comparison of strain outcomes for the PPS of eyes before treatment. The strain outcomes from n=8 before treatment with buffer were combined with the n=8 before treatment with TrypLE. The values are means ± std of the strain outcomes from all 16 samples before treatment. Wilcoxon signed rank tests (two-tailed) were used to compare E_{xx} to E_{yy} and independently to compare $E_{\theta\theta}$ to E_{rr}. E_{xx} was greater than E_{yy} and $E_{\theta\theta}$ was greater than E_{rr} before treatment.

		p-value
E_{xx}	**-0.0003 ± 0.0042**	**0.0005**
E_{yy}	**0.0047 ± 0.0030**	
$E_{\theta\theta}$	**0.0043 ± 0.0029**	**0.03**
E_{rr}	**0.0002 ± 0.0050**	

Table S12. The differences in the strain response of the PPS. The formula for the strain difference in E_{rr} is $\Delta E_{rr} = E_{rr,\,after} — E_{rr,before}$. The Mann-Whitney U tests (two tailed) were used to compare the strain differences due to buffer to those due to TrypLE. Strain differences due to TrypLE were not statistically different from those due to buffer. Values are mean ± std.

Post-Pre		*Δ = (Post)—(Pre)*	*p-value Buffer vs. TrypLE*
ΔE_{rr}	Buffer	-0.0006 ± 0.0023	0.59
	TrypLE	-0.0012 ± 0.0023	
$\Delta E_{\theta\theta}$	Buffer	0.0010 ± 0.0033	0.27
	TrypLE	0.0019 ± 0.0018	
ΔE_{xx}	Buffer	-0.0005 ± 0.0017	0.86
	TrypLE	0.0000 ± 0.0036	
ΔE_{yy}	Buffer	0.0009 ± 0.0037	0.59
	TrypLE	0.0007 ± 0.0017	
ΔE_{max}	Buffer	0.0004 ± 0.0067	0.14
	TrypLE	0.0043 ± 0.0039	
ΔE_{min}	Buffer	0.0000 ± 0.0079	0.27
	TrypLE	-0.0036 ± 0.0033	
$\Delta \gamma_{max}$	Buffer	0.0002 ± 0.0069	0.10
	TrypLE	0.0040 ± 0.0031	

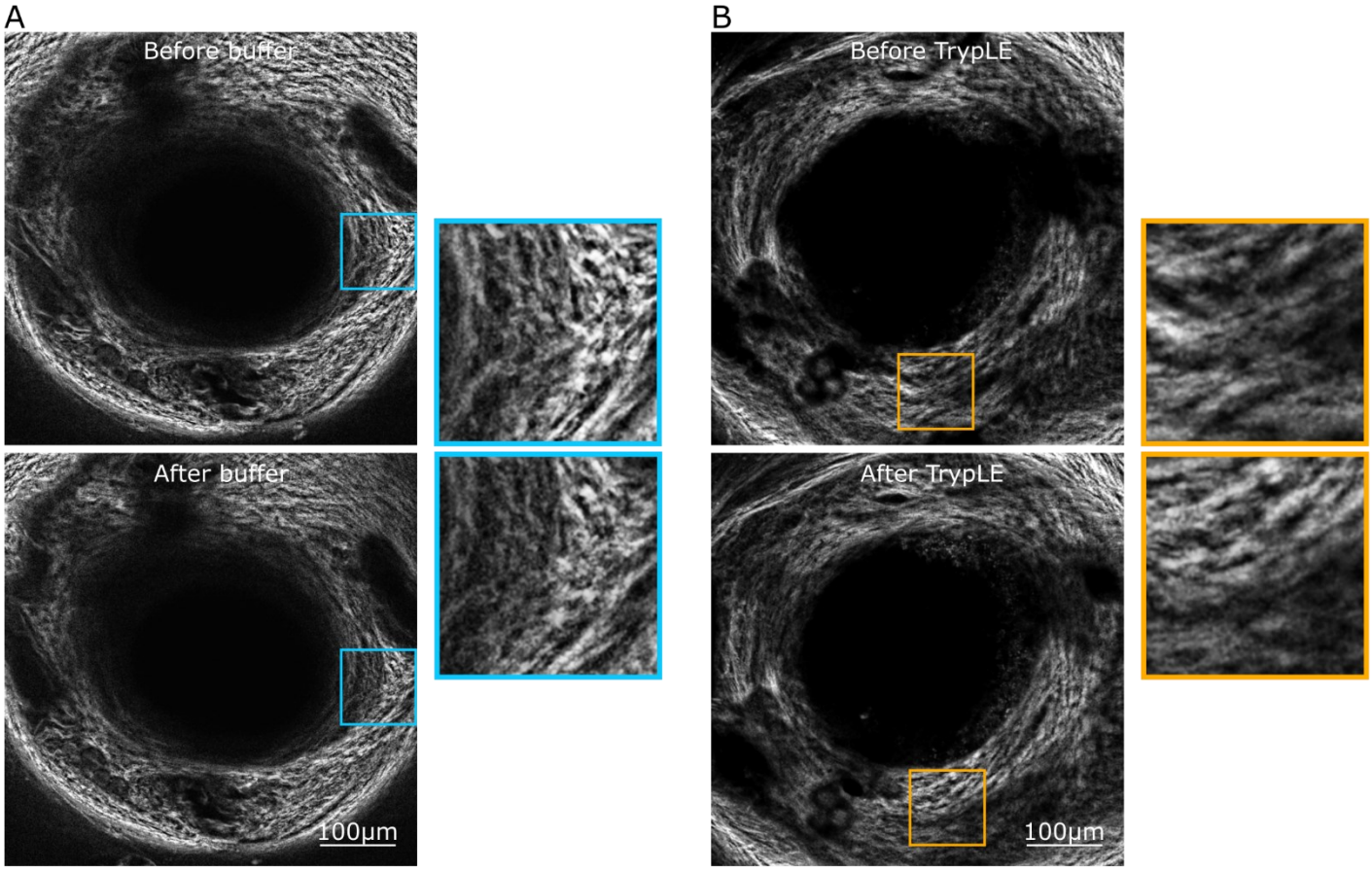

Figure S4. TrypLE and buffer did not produce visible alterations of collagen fibers. The z-stack was acquired by two-photon excitation at 780nm and collection of SHG and TPF signals. Image contrast was enhanced by deconvolution (Huygens Essential), and then by contrast-limited adaptive histogram equalization, cf. Section 2.7. The images were acquired at a resolution of 0.519 x 0.519 x 1 µm^3 with 20x objective, with 0.64µs pixel dwell time. Representative z-slices from fast scans taken before treatment (top row) and after treatment and wash steps (bottom row). (A) specimen from the buffer group. (B) specimen from the TrypLE group. Insets enlarge 100 x 100 µm^2 areas of each image to show that banded collagen fibers in white were unchanged in their orientation, assembly, or relative thickness. Note that the signal from the AL in the center of the image was acquired separately and is not shown. Scalebars are 100µm.