**Title**: Topical application of calcitonin gene-related peptide as a regenerative, antifibrotic, and immunomodulatory therapy for corneal injury

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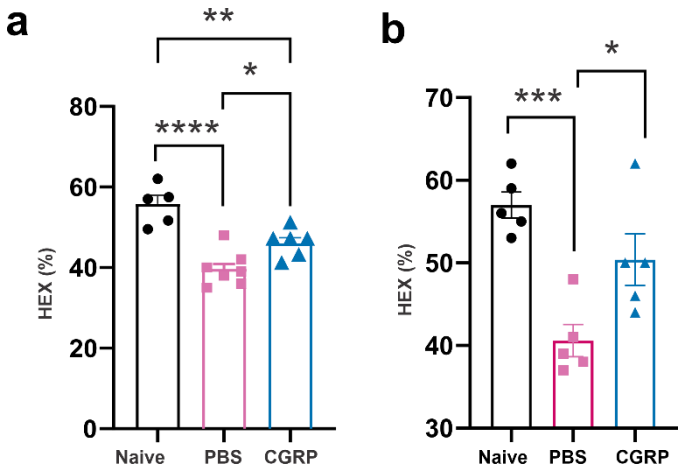
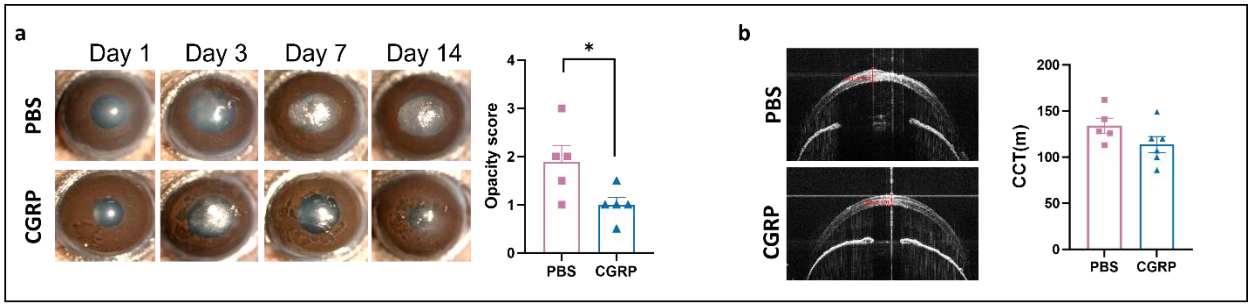
**Keywords:** Corneal wound healing; corneal injury; corneal opacity; ocular trauma; CGRP, calcitonin gene-related peptide

**Supplementary Figure 1. CGRP preserved normal corneal endothelial cell (CEnC) morphology.**



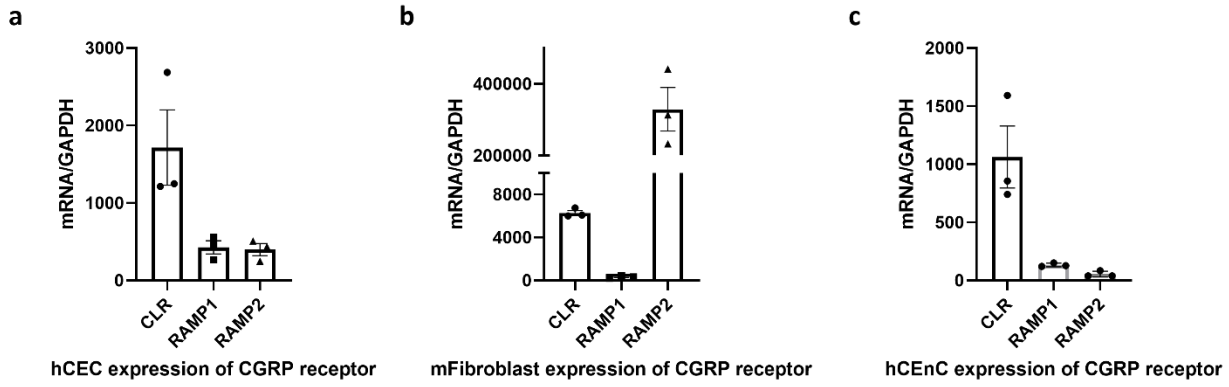

**a** CEnC pleomorphism, or the percentage of normal hexagonal shaped cells over total cells (HEX %), was analyzed using in vivo confocal microscopy images of corneal endothelial cells. While pleomorphism in the injured PBS treated corneas was increased, CGRP treatment preserved the normal morphology. **b** CEnC pleomorphism was analyzed using images of corneal endothelium whole mount stained by ZO-1. Results were similar to Figure A. N=5 mice per group. The data are presented as mean ± standard error of mean and was determined by one-way ANOVA test with pairwise comparison. *p<0.05, ***p<0.001, ****p<0.0001.

**Supplementary Figure 2. Short term application of CGRP alleviates corneal opacity but does not impact thickness.**



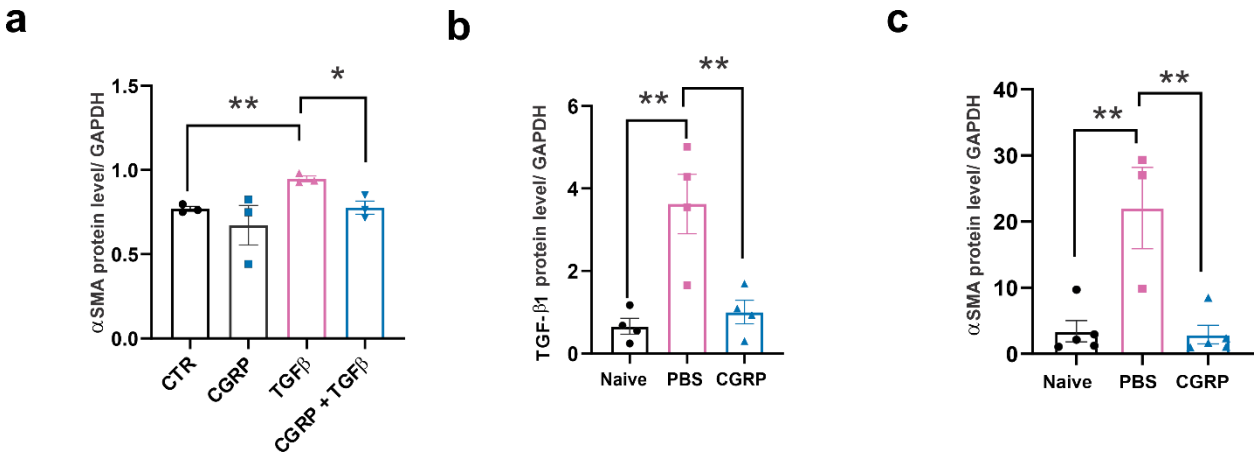

**a** Representative slit lamp photographs of PBS and CGRP-treated eyes up to 5 days after injury. The corneas of the PBS-treated controls showed progressive stromal opacification, whereas CGRP treatment showed significantly lower corneal opacity. **b**. Representative AS-OCT images and analysis showed significant increase in central corneal thickness (CCT) and stromal hyperreflectivity in PBS-treated mice, whereas they were comparable to CGRP treated group on days 14 post-injury. (n=5 per group). The data are represented as mean±SEM. The statistical significance was determined by unpaired t-test, * $p<0.05$.

**Supplementary Figure 3. Expression of CGRP receptors by corneal cells.**



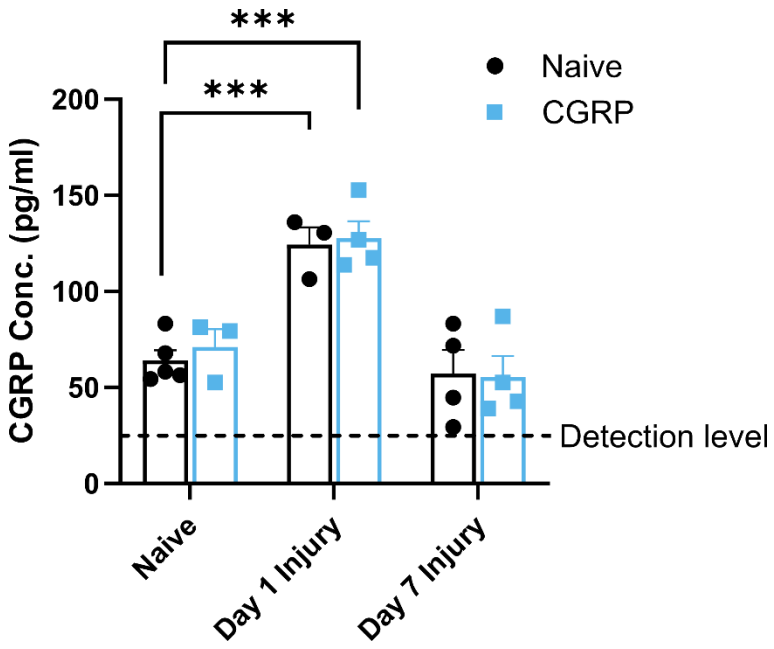

The RT-PCR analysis shows that corneal epithelial cells (hCEC, **a**), corneal fibroblast (**b**) and corneal endothelial cells **(c)** express the CGRP receptors components CLR, RAMP1 and RAMP2.

**Supplementary Figure 4. Analysis of western blot on TGF-β1 expression in vivo and αSMA expression in vivo and in vitro.**



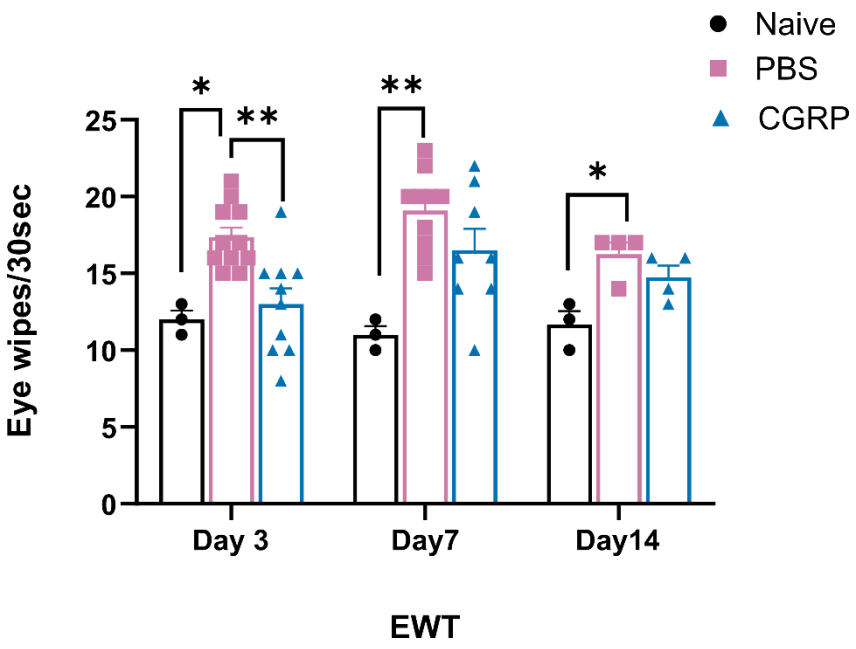

**a** CGRP significantly suppressed TGF-β1-induced αSMA expression in vitro. **b** CGRP significantly decreased TGF-β1 expression in corneal tissues on day 5 after injury compared to the PBS control. **C.** CGRP significantly decreased αSMA expression in corneal tissues on day 5 after injury compared to the PBS control. n=4 per group. The data are presented as mean ± SEM and was determined by one-way ANOVA test with pairwise comparison. *p<0.05, **p<0.01.

**Supplementary Figure 5. CGRP topical application failed to increase the CGRP concentration in the Aqueous humor.**




Aqueous humor was collected from naïve, one day and seven days post-injury mice and CGRP concentration was determined using CGRP ELISA Kit. Surprisingly, we noticed a significant increase in CGRP conc. On day 1 post-injury compared to naïve mice. By day 7- post injury, the CGRP concentration was back to normal. Then, CGRP was applied topically for 30 minutes before aqueous humor collection in either naïve mice, or injured mice on day 1 or day 7. No significant difference in the AH concentration was noticed in response to the CGRP application. (Two eyes were pooled together to generate enough volume/sample, n=4/group). The data are represented as mean±SEM. The statistical significance was determined by unpaired t-test, $p<0.001$.

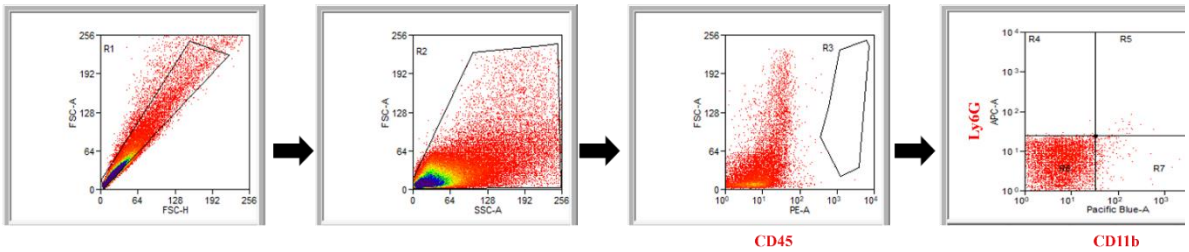
**Supplementary Figure 6. CGRP topical application suppresses ocular hyperalgesia (eye-wiping test) on day 3 post-injury.**




Ocular hyperalgesia was assessed using the eye-wiping test [1,2]. Briefly, a single drop of 2M NaCl was instilled into the animal's eye, and the count of eye wipes with the ipsilateral forelimb was recorded over a 30-second period.

Mechanical injury induced increased eye wipes number up to day 14. CGRP topical administration significantly suppressed the eye wipes on day 3 post-injury. The data are presented as mean ±SEM and was determined by one-way ANOVA test with pairwise comparison, *$p<0.05$, **$p<0.01$.

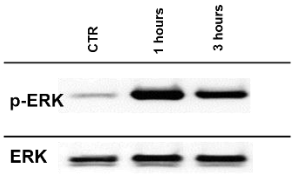
**Supplementary Figure 7. The flow cytometry gating strategy.**




The figure illustrates the gating strategy utilized for identifying inflammatory cells (CD45+) within the cornea, as well as distinguishing the subpopulations of neutrophils (CD11b+Ly6G+) and macrophages (CD11b+Ly6G-).

**Supplementary Figure 8. Uncropped blot images.**

Fig. 4g



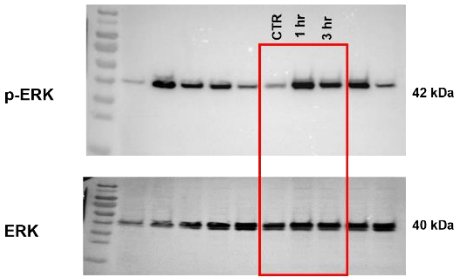
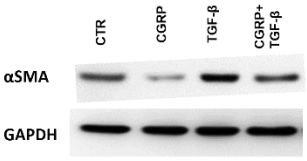




Fig. 5b



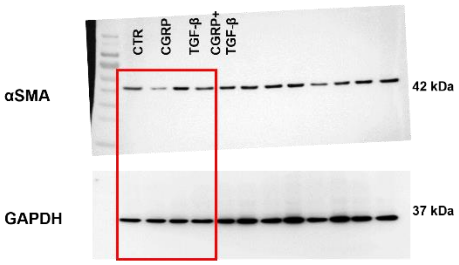
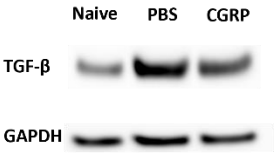




Fig. 5e



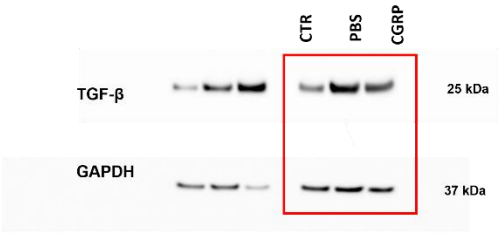
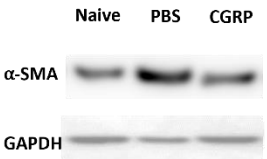
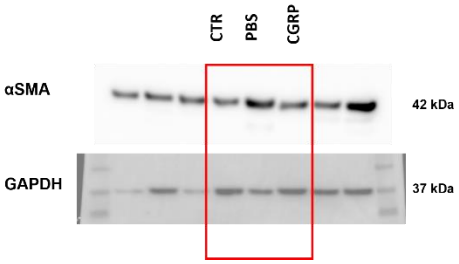




Fig. 5h



**Supplementary Table 1: The primers used in the RT-qPCR analysis.**

| Name | Gene name | Primer |
|---|---|---|
| GAPDH | *GAPDH* | Mm99999915_g1 |
| CLR | *Calcr* | Mm00432282_m1 |
| RAMP1 | *RAMP1* | Mm00489796_m1 |
| RAMP2 | *RAMP2* | Mm00490256_g1 |
| Laminin | *LAMA3* | Hs00165042_m1 |
| Alpha SMA | *Acta-2* | Mm00725412_s1 |
| TGF-beta1 | *Tgfb1* | Mm01178820_m1 |
| Na/K ATPase α1 | *ATP1A1* | Mm00523255_m1 |
| Na/K ATPase α3 | *ATP1A3* | Mm00523430_m1 |
| Caspase 3 (human) | *CAS3* | Hs00234387_m1 |
| BAX (human) | *BAX* | Hs00180269_m1 |
| GAPDH (human) | *GAPDH* | Hs02786624_g1 |
| CXCL1 | *CXCL1* | Mm00434772_m1 |
| TNFα1 | *TNFA* | Mm00443258_m1 |
| MMP9 | *MMP9* | Mm00442991_m1 |
| IL-1beta | *Il1b* | Mm00434228_m1 |

All the listed Taqman primers were purchased from Thermo-Fischer Scientific Company.

**Supplementary Table 2: The antibodies used in the immunohistochemistry (IHC) and western blot (WB).**

| Antibody | Manufacturer | Cat# |
|---|---|---|
| **Primary Antibodies (IHC/WB)** | | |
| Ki-67 Monoclonal Antibody (SolA15), FITC, | Invitrogen | 11-5698-82 |
| p-ERK Antibody | Thermofisher | 14-9109-82 |
| ERK1/ERK2 Antibody | Thermofisher | 82380 |
| Laminin Antibody | Thermofisher | PA1-16730 |
| Alpha-Smooth Muscle Actin Antibody | Thermofisher | 14-9760-82 |
| TGF beta-1 Monoclonal Antibody | Thermofisher | MA1-21595 |
| Propidium iodide | Sigma-Aldrich | P4864 |
| Annexin V-FITC | Biolegend | 640906 |
| Sodium Potassium ATPase Recombinant Rabbit Monoclonal Antibody (ST0533 | Thermofisher | MA5-32184 |
| Zonula Occludens Antibody | Thermofisher | 33-9100 |
| **Secondary Antibodies (IHC)** | | |
| Anti-Mouse IgG1 Nano Recombinant Secondary Antibody, Alexa Fluor™ 488 | Thermofisher | SA5-10329 |
| Goat anti-Rabbit IgG Fc, Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 488 | Thermofisher | A78953 |
| Goat anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor™ Plus 594 | Thermofisher | A32740 |
| Goat anti-Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 594 | Thermofisher | A-11005 |
| **Secondary Antibodies (WB)** | | |
| Goat anti-Rabbit IgG (H+L) secondary antibody, HRP | Thermofisher | 32460 |
| Goat anti-Mouse IgG (H+L) Secondary Antibody, HRP | Thermofisher | 31430 |
| GADPH loading control antibody-HRP | Thermofisher | MA5-15738-HRP |

**Supplementary References:**


1 Farazifard, R., Safarpour, F., Sheibani, V. & Javan, M. Eye-wiping test: a sensitive animal model for acute trigeminal pain studies. *Brain Res Brain Res Protoc* **16**, 44-49 (2005). https://doi.org:10.1016/j.brainresprot.2005.10.003

2 Nazeri, M., Zarei, M. R., Pourzare, A. R., Ghahreh-Chahi, H. R., Abareghi, F. & Shabani, M. Evidence of Altered Trigeminal Nociception in an Animal Model of Fibromyalgia. *Pain Med* **19**, 328-335 (2018). https://doi.org:10.1093/pm/pnx114