Identification of novel predictive factors for post surgical corneal haze

Nimisha R. Kumar^{1,3}, Pooja Khamar², Rohit Shetty^{2,5}, Ankit Sharma⁴, Naren Shetty², Natasha Pahuja², Valsala Gopalakrishnan Abilash³, Vishal Jhanji⁹, Anuprita Ghosh¹, Rajiv R Mohan^{6,7,8}, Rajani Kanth Vangala⁴ and Arkasubhra Ghosh^{1,5,*}

*Corresponding author:

Arkasubhra Ghosh, MSc, PhD GROW Research Laboratory

Narayana Nethralaya Foundation, Narayana Health City,

258/A, Bommasandra, Hosur Road, Bangalore - 560 099 - INDIA.

Email: arkasubhra@narayananethralaya.com

Disclosure: The authors have no financial disclosures or conflicts for interest to declare.

¹GROW Research Laboratory, Narayana Nethralaya Foundation, Bangalore, India

²Cornea and Refractive Surgery Division, Narayana Nethralaya, Bangalore, India

³Department of Biomedical Sciences, School of Bio Sciences and Technology, VIT, Vellore, India

⁴Thrombosis Research Institute, Bangalore, India

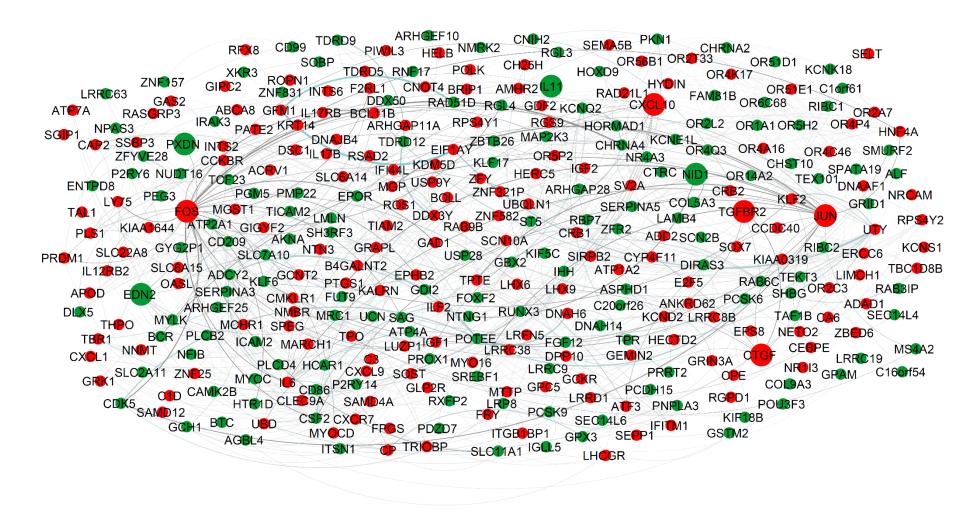
⁵Singapore Eye Research Institute, Singapore

⁶Department of Veterinary Medicine and Surgery, University of Missouri, Columbia, MO, 65211 USA

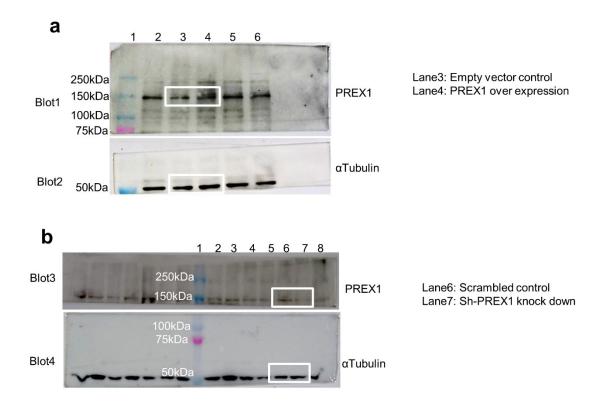
⁷Mason Eye Institute, School of Medicine, University of Missouri, Columbia, MO 65212 USA

⁸Harry S Truman Veterans' Memorial Hospital, Columbia, MO 65201

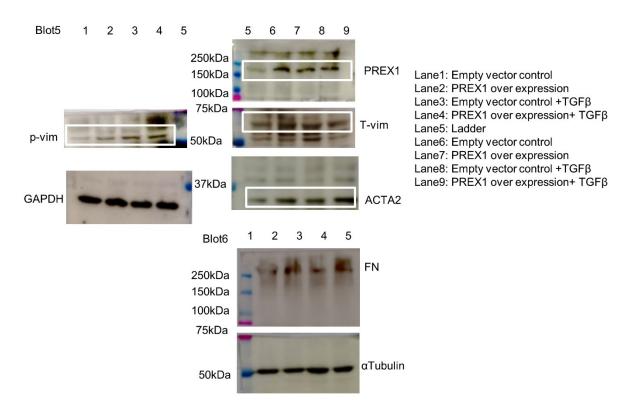
⁹Department of Ophthalmology & Visual Sciences, The Chinese University of Hong Kong, Hong Kong, China; Department of Ophthalmology, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA.



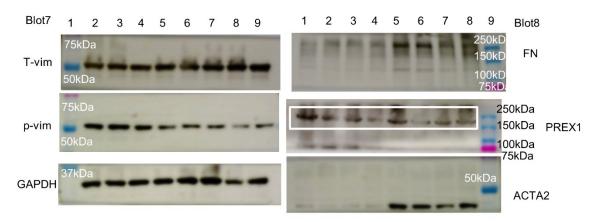
Supplementary figure1: Represents the network analysis showing 327 differentially expressed genes cut off > ±2 fold change (p-value<0.05) in microarray and their protein- protein interactions. The network analysis suggested that c-FOS, IL6, c-JUN, CXCL10, EDN2, IL1 and IL11 with node degrees of 34, 26, 23, 13, 9, 8 and 5 forms the backbone of the network architecture. The size of the circle (node) represents the fold change between control and haze predisposition (red color signifies down regulation and green color signifies up regulation).



Supplementary figure2: Represents unedited western blot images used in the figure 4c and 5c. Panel (a) consists of blot1 representing PREX1 protein expression from empty vector transfected control (lane3) and PREX1 over expression plasmid transfected (lane4) and corresponding α-tubulin in blot2 marked within white square box, shown in figure4c. Panel (b) consists of blot3 representing PREX1 protein in scrambled control (lane 6) and sh-PREX1 knock down (lane 7) with their corresponding α-tubulin in blot4, delineated with white box and shown in figure5c.



d



Lane1: Ladder

Lane2: Scrambled control
Lane3: Sh-PREX1 knock down
Lane4: : Sh-PREX1 knock down
Lane5: : Sh-PREX1 knock down
Lane6: Scrambled control + TGFβ
Lane7: Sh-PREX1 knock down +TGFβ

Lane8: Sh-PREX1 knock down +TGF β Lane9: Sh-PREX1 knock down +TGF β

Lane1: Scrambled control

Lane2: Sh-PREX1 knock down
Lane3: : Sh-PREX1 knock down
Lane4: : Sh-PREX1 knock down
Lane5: Scrambled control + TGFβ
Lane6: Sh-PREX1 knock down +TGFβ
Lane7: Sh-PREX1 knock down +TGFβ
Lane8: Sh-PREX1 knock down +TGFβ

Lane9: Ladder

Supplementary figure 2: Represents unedited western blot images used in the figure 4h and 5h. Panel (c) consists of blot 5 representing PREX1 overexpression with empty controls in human corneal epithelial cells. Lane 1-9 represents the sample conditions loaded for protein analysis of p-vim, GAPDH, FN, PREX1, T-vim and ACTA2 marked within white square box, shown in figure 4h. Blot 6 represents FN and its corresponding loading control tubulin. Panel (d) consists of blot 7 representing PREX1 knockdowns with scrambled control in human corneal epithelial cells. Lane 1-9 represents the sample conditions loaded for protein analysis of T-vim, p-vim and GAPDH. Blot 8, lane 1-9 represents the sample conditions loaded for protein analysis of FN, PREX1 and ACTA2 marked within white square box, shown in figure 5h.