

## 1 **Supplementary Figure Legends**

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3 **Figure S1.** A) Dissection and mounting procedure. Panels 1-6 show the sequential cuts  
4 that prepare the eye for mounting on the system (dotted lines depict the cuts). We  
5 routinely make the cuts on the superior side of the eye. Brighter dots represent the new  
6 cuts at each step. Note that two cuts are made at step 4, at each end of the very original  
7 cut to make a V at each end. Panel 6 shows an eye just prior to mounting - after  
8 removal of the lens and iris and with the inferior limbus on top. Panel 7 shows the eye  
9 mounted on the frustum. During mounting, the flaps generated by cutting are first eased  
10 apart to widen the opening in the globe. Approximate position of the optic nerve (ON)  
11 stub is indicated. The eye is then maneuvered carefully and gently over the frustum and  
12 flaps uncurled so that it sits snugly and uniformly. The flaps are lightly glued using  
13 tissue glue to stabilize the eye while positioning the O-ring and washer.

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15 **Figure S2.** Photographs showing eye dissection and an eye mounted on the frustum of  
16 a perfusion device. A) The sequential cuts that prepare the eye for mounting on the  
17 system run from left to right. Top row shows cartoons from Figure S1, middle row shows  
18 photographs of an eye undergoing dissection where at each step a new cut is marked  
19 using a red dotted line and existing cut with yellow dotted lines. Bottom row shows the  
20 same photographs without the dotted lines. In the photographs, some single cuts have  
21 allowed tissue to gape or separate around the cut and in these cases the dotted lines  
22 mark each edge of cut tissue. (In contrast, the same cuts are shown as single lines in  
23 the cartoons). B) A cartoon of a dissected eye in the process of being mounted on the  
24 frustum of a perfusion device (left), and photograph of an eye being mounted on the  
25 device frustum (right). In the photograph, the flaps made in the eye tissue during  
26 dissection have curled up (especially on the right side). These flaps are straightened (as

1 in cartoon) prior to securing the eye on the frustum with glue and the O-ring. ON, optic  
2 nerve. C) Photograph of a perfusion device (version 2).

3  
4 **Figure S3. Prototype perfusion system.** A) The device consists of a base (note base  
5 is different from that in Figure 1) with central frustum onto which the eye is mounted.  
6 The frustum has two fluid ports for addition and removal of fluids. The frustum is  
7 functionally identical to that shown in Figure 1. The eye is held in place using an O-ring  
8 that seals the eye against the frustum. A mounting washer and 4 screws hold the O-  
9 ring in place creating a tight seal. B) A cross sectional view of the system. C) The  
10 mounted eye is orientated so that the majority of the drainage angle is clear of where  
11 the O-ring clamps the tissue. Approximate position of the optic nerve (ON) stub is  
12 indicated. Images not to scale.

13  
14 **Figure S4. Scatter plots of outflow facility using different versions of the**  
15 **perfusion apparatus.** All eyes were perfused using Protocol A. The C values were:  
16 Version 1)  $4.34 \pm 1.4$  nl/min/mmHg (whole eye C =  $9.548 \pm 3.08$  nl/min/mmHg), and  
17 Version 2)  $4.7 \pm 1.2$  nl/min/mmHg (whole eye C =  $10.4 \pm 2.64$  nl/min/mmHg). Blue dots  
18 indicate mean. No significant difference in C was observed between the apparatus  
19 styles.

20  
21 **Figure S5.** Major flow is restricted to the TM/SC region. Dextran tracer detected no flow  
22 through the RPE and underlying sclera. A and C) Images of a compressed Z projection  
23 of limbal tissue from a B6.*Prox1-GFP* mouse that was perfused with 3-kDa Dextran  
24 Texas Red (TXR). The SC and lymphatics appear in green, the perfused dextran in red,  
25 and nuclei in blue (shows the size of the limbal strip). The location of the cornea and  
26 sclera are indicated. In C the nuclei are not shown, to reveal the SC, TM, lymphatics

1 and the perfused dextran. Dotted lines show the corneal and scleral edges of the limbal  
2 strip (as matches A), B and D) Images show the XZ projection (Y compressed) of the  
3 regions shown in A and C respectively. Zoomed in regions indicated by yellow boxes in  
4 upper panels of D are shown in the lower panels. Note that there is no dextran tracer in  
5 the region marked sclera in A and C or above the plane of the SC in D indicating  
6 dextran is not leaking through the sclera. Although only the limbal region containing the  
7 SC and TM is show, all tissue of the mounted eye was analyzed for tracer. E) A  
8 representative region of the retinal pigmented epithelium (RPE) labeled with ZO1 (cyan)  
9 shows no dextran tracer fluorescence. In E, a Z projection is shown. RPE is in the  
10 forefront. Note the lack of any red fluorescence from tracer. F) An XZ projection of the  
11 same region shown in E reveals no red fluorescence throughout the thickness of the  
12 tissue indicating that the tracer has not passed through the RPE into the sclera (blue,  
13 nuclei; cyan, ZO1). Scales: A-D, 200  $\mu\text{m}$ ; E and F, 30  $\mu\text{m}$

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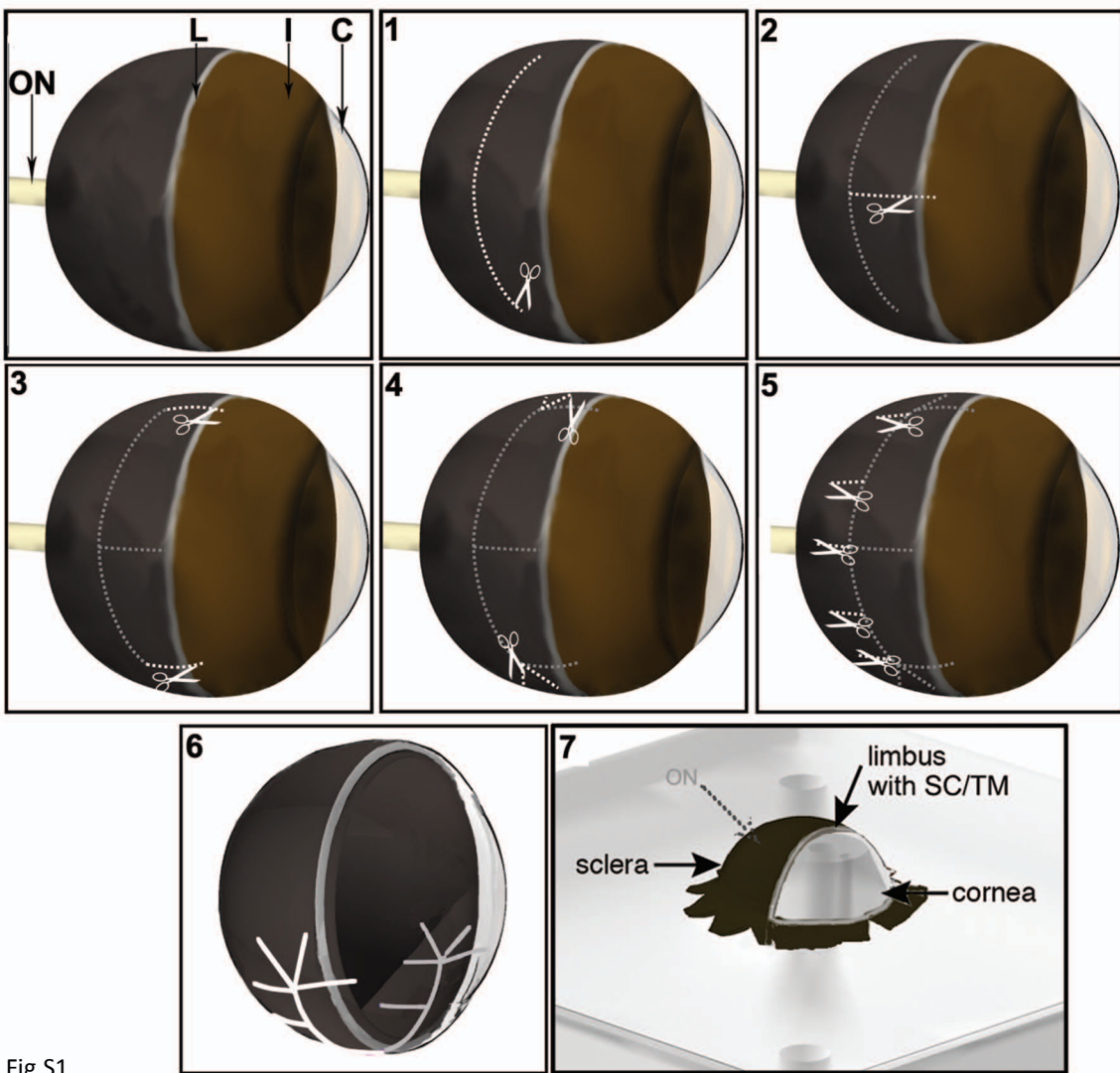


Fig.S1

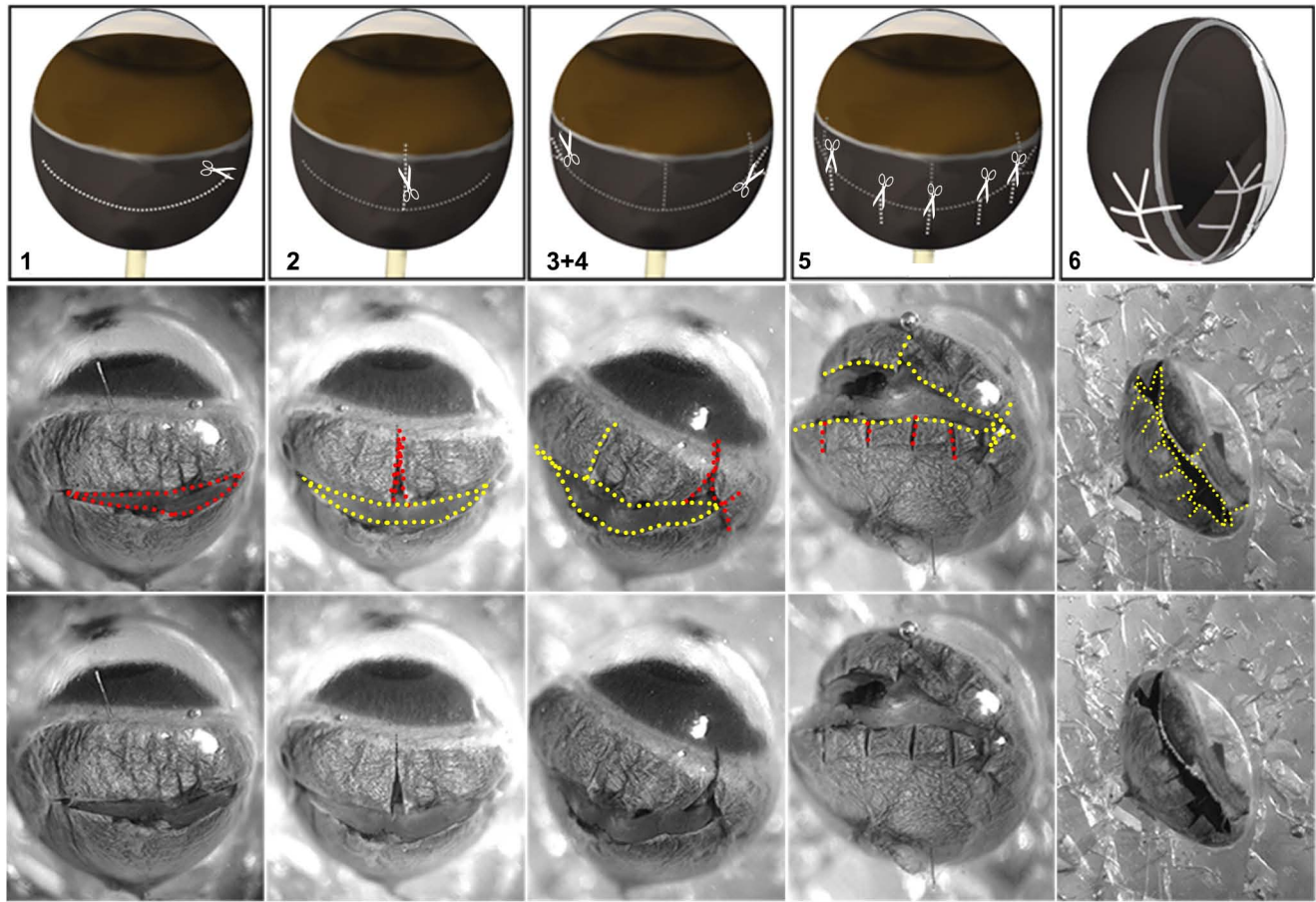
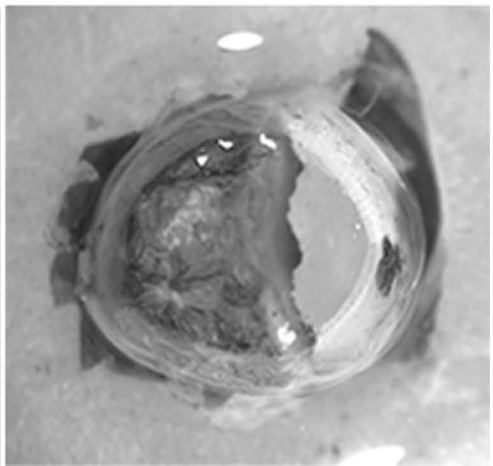
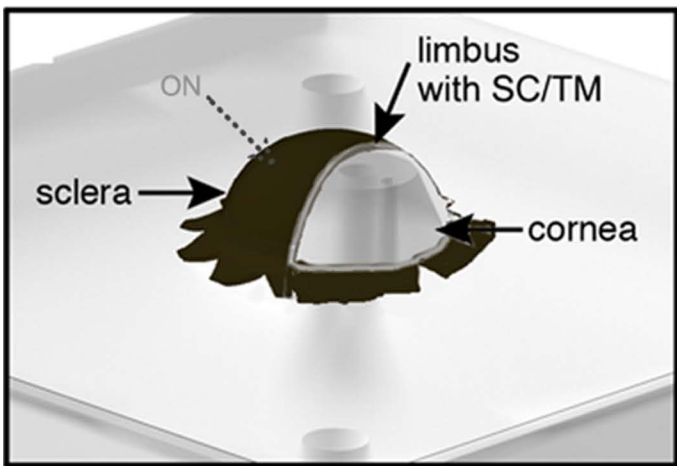
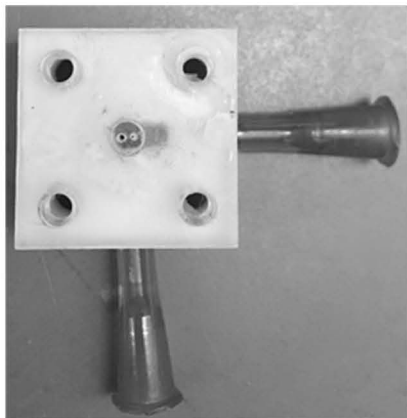
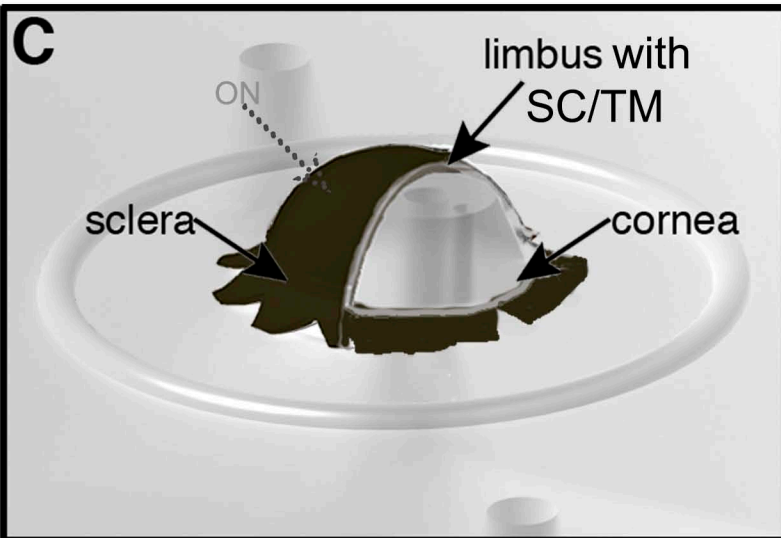
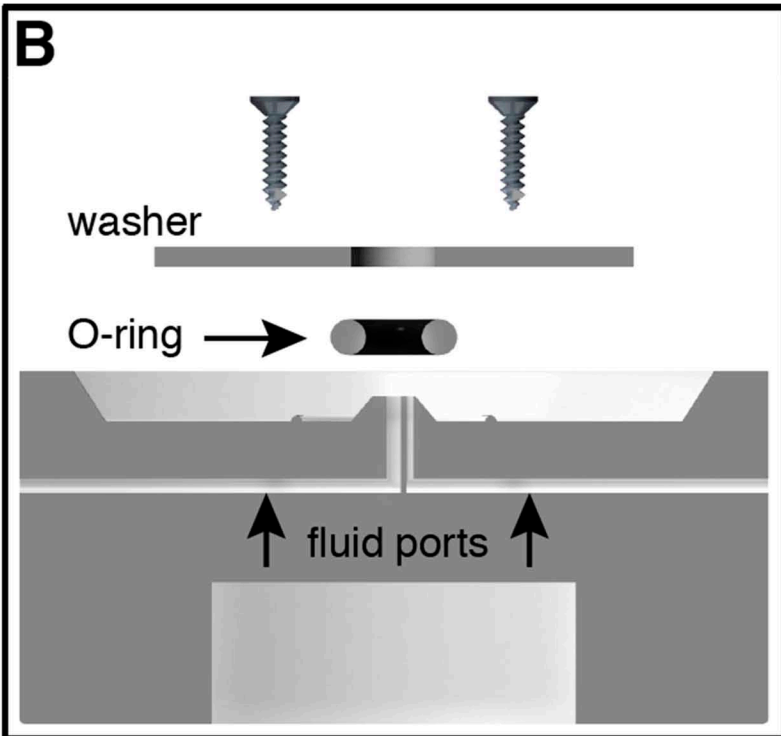
**A****B****C**

Fig.S2



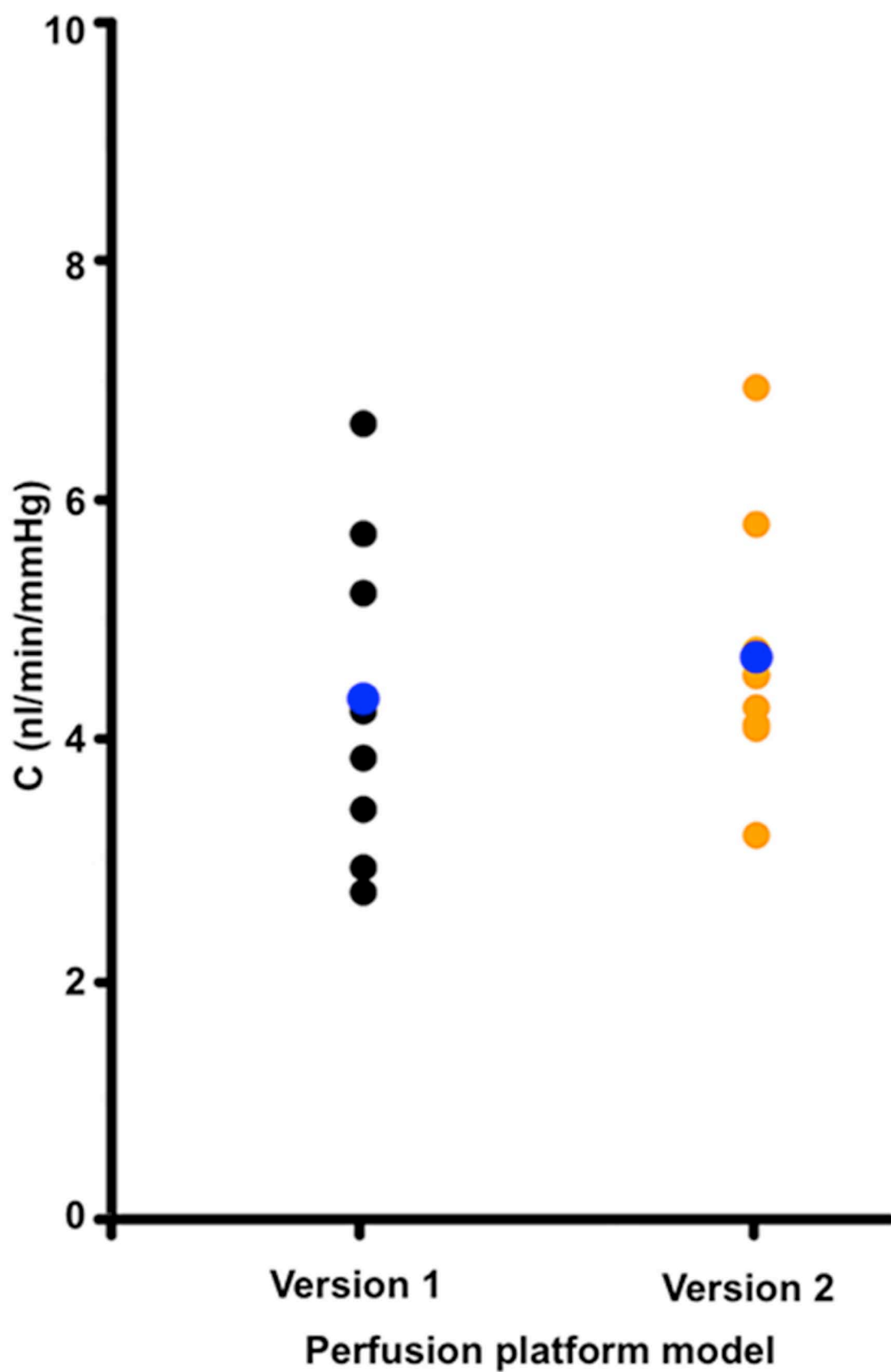


Fig.S4



