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Supplemental Material to:

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Lysosomal-mediated waste clearance in retinal pigment epithelial cells is regulated by CRYBA1/βA3/A1-crystallin via V-ATPase-MTORC1 signaling

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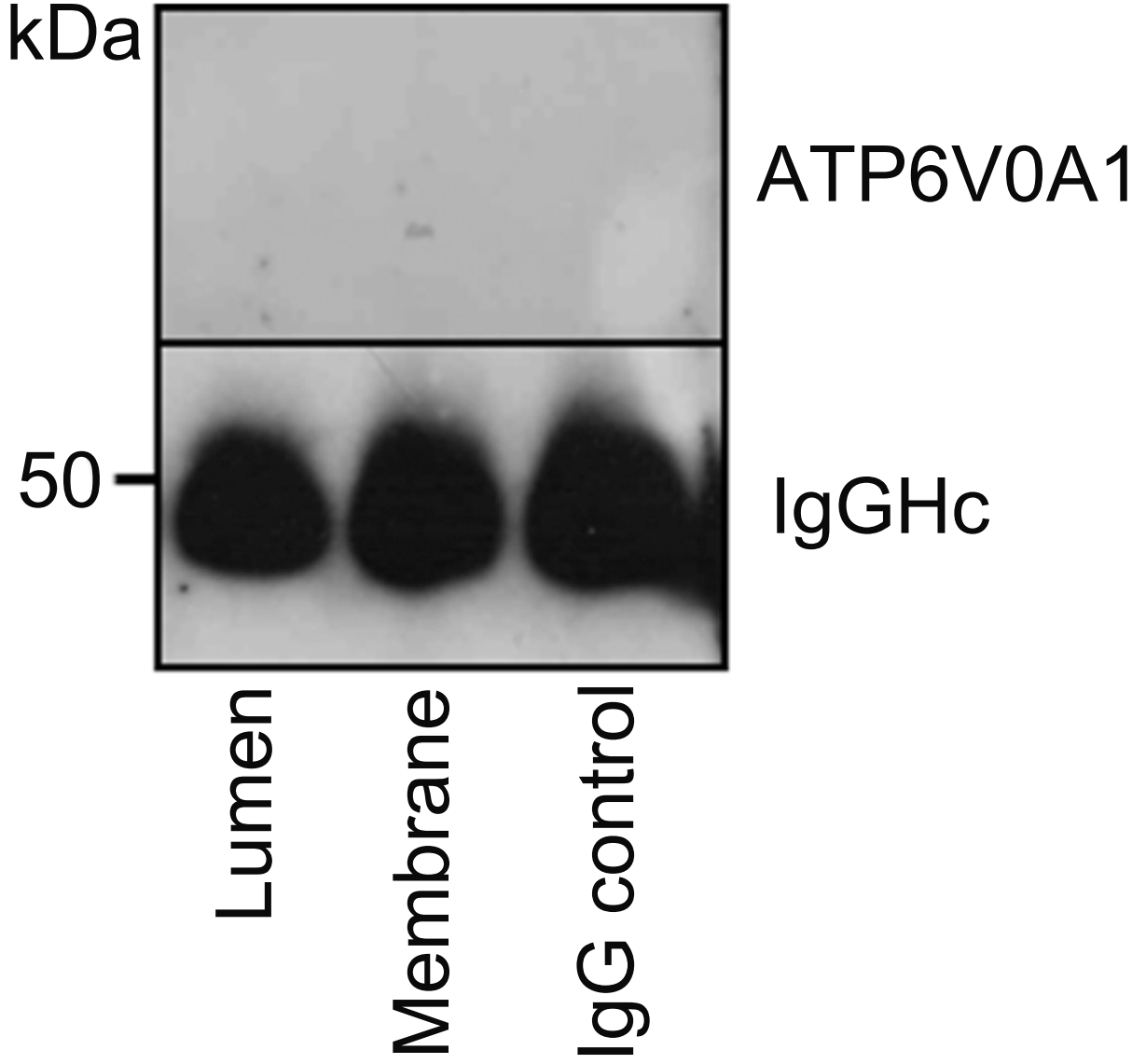
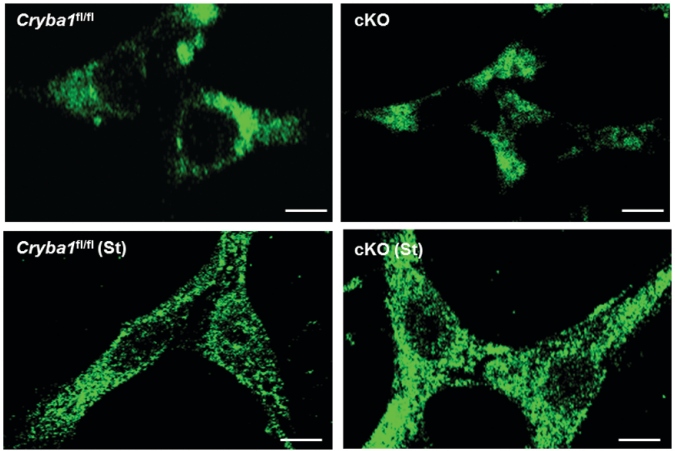

kDa
ATP6V0A1
50
IgGHc
Lumen
Membrane
IgG control

Figure S1. CRYBA1 antibody does not precipitate detectable ATP6V0A1 from cKO RPE. Coimmunoprecipitation of lysosomal lumen and membrane extracts from *cryba1* cKO cells with CRYBA1 antibody and immunoblotting with V_0-ATPase ATP6V0A1 antibody, demonstrating that the crystallin antibody does not pull down V_0-ATPase ATP6V0A1 as there is no detectable band on the gel. Non-specific IgG was used as negative control.


Cryba1fl/fl
cKO
Cryba1fl/fl (St)
cKO (St)

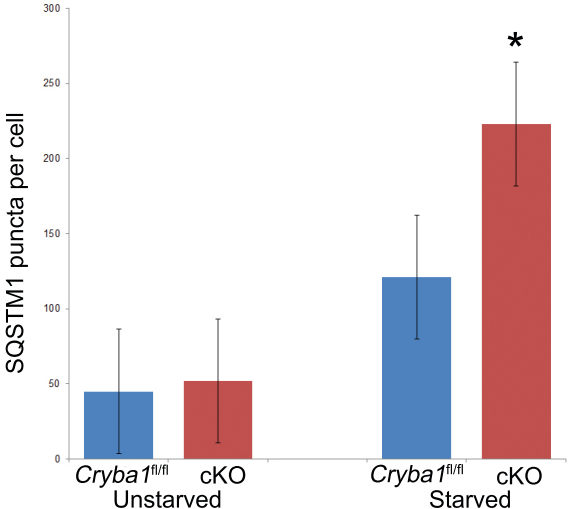

SQSTM1 puncta per cell
300
250
200
150
100
50
0
*
Cryba1fl/fl
cKO
Unstarved
Cryba1fl/fl
cKO
Starved

Figure S2. SQSTM1 is increased in cKO RPE following starvation. Levels of SQSTM1, an autophagy substrate, were assessed in primary cultures of *Cryba1*$^{fl/fl}$ and *cryba1* cKO RPE cells by immunofluorescence using a specific antibody. Fluorescence was increased in both cell types following autophagy induction (st), but there was increased accumulation of SQSTM1 in *cryba1* cKO cells relative to *Cryba1*$^{fl/fl}$ cells after autophagy induction (bottom panel). The number of SQSTM1 puncta was calculated from at least 30 cells per group. Data is represented as mean ± S.E.M. **P*<0.05.