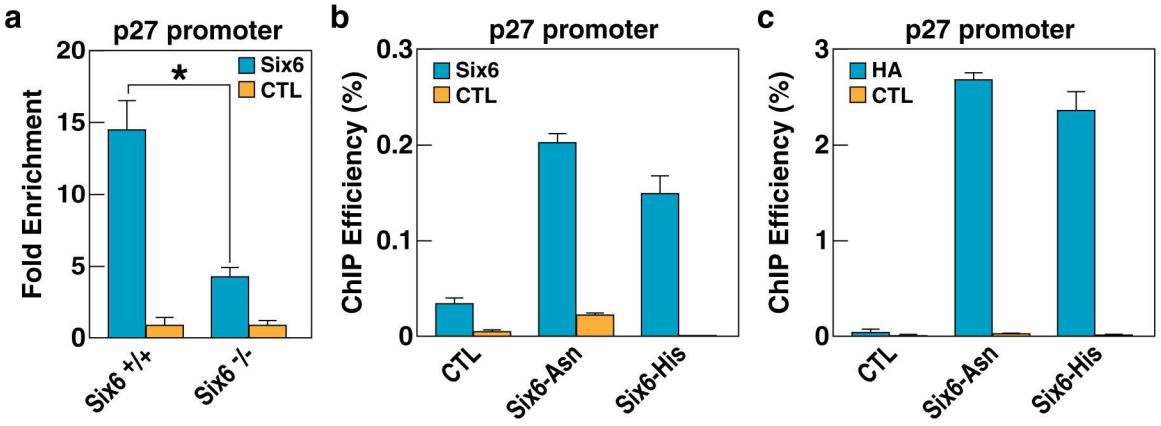
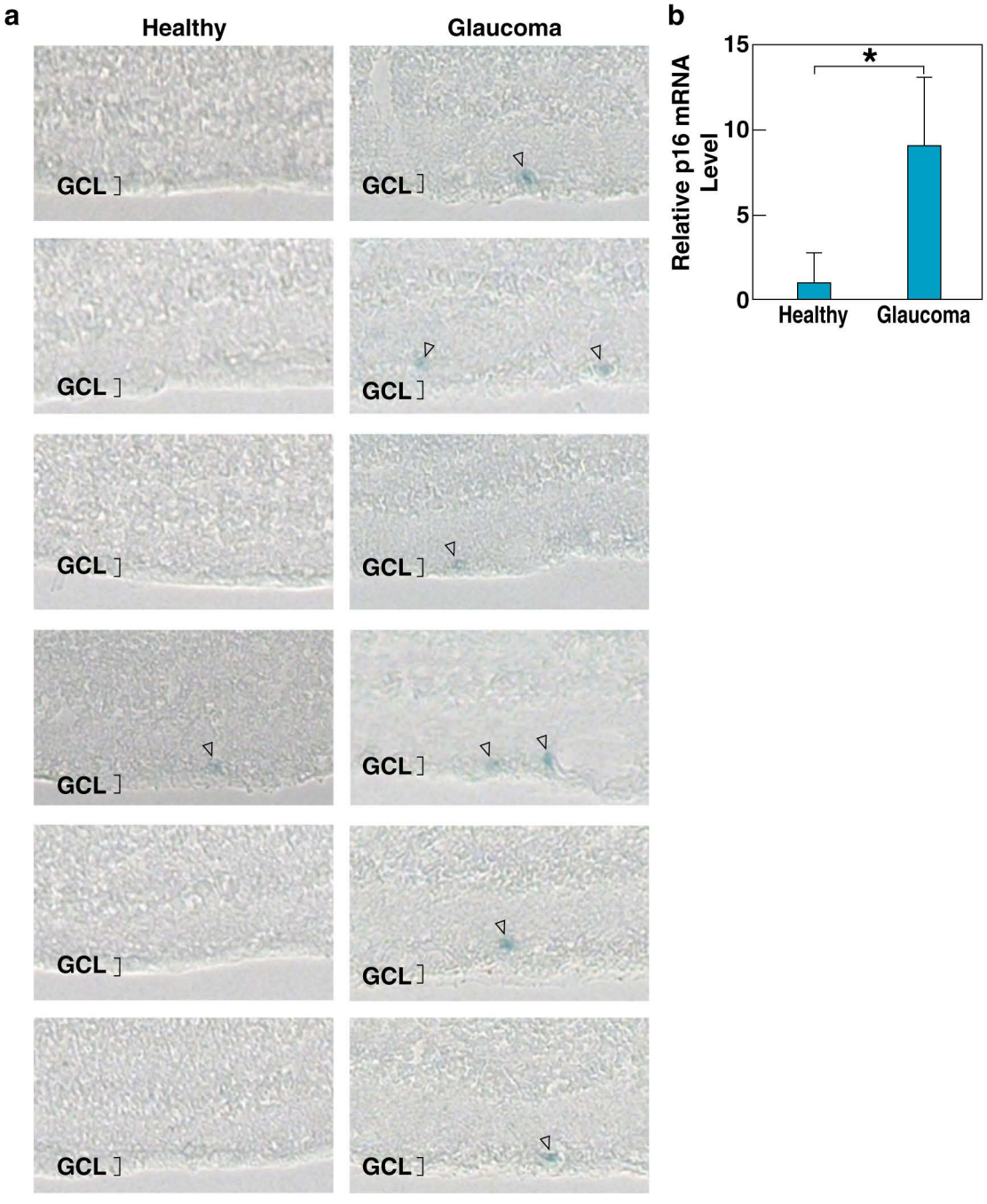
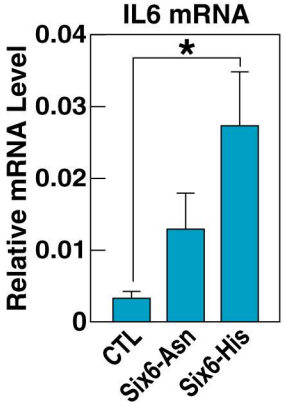
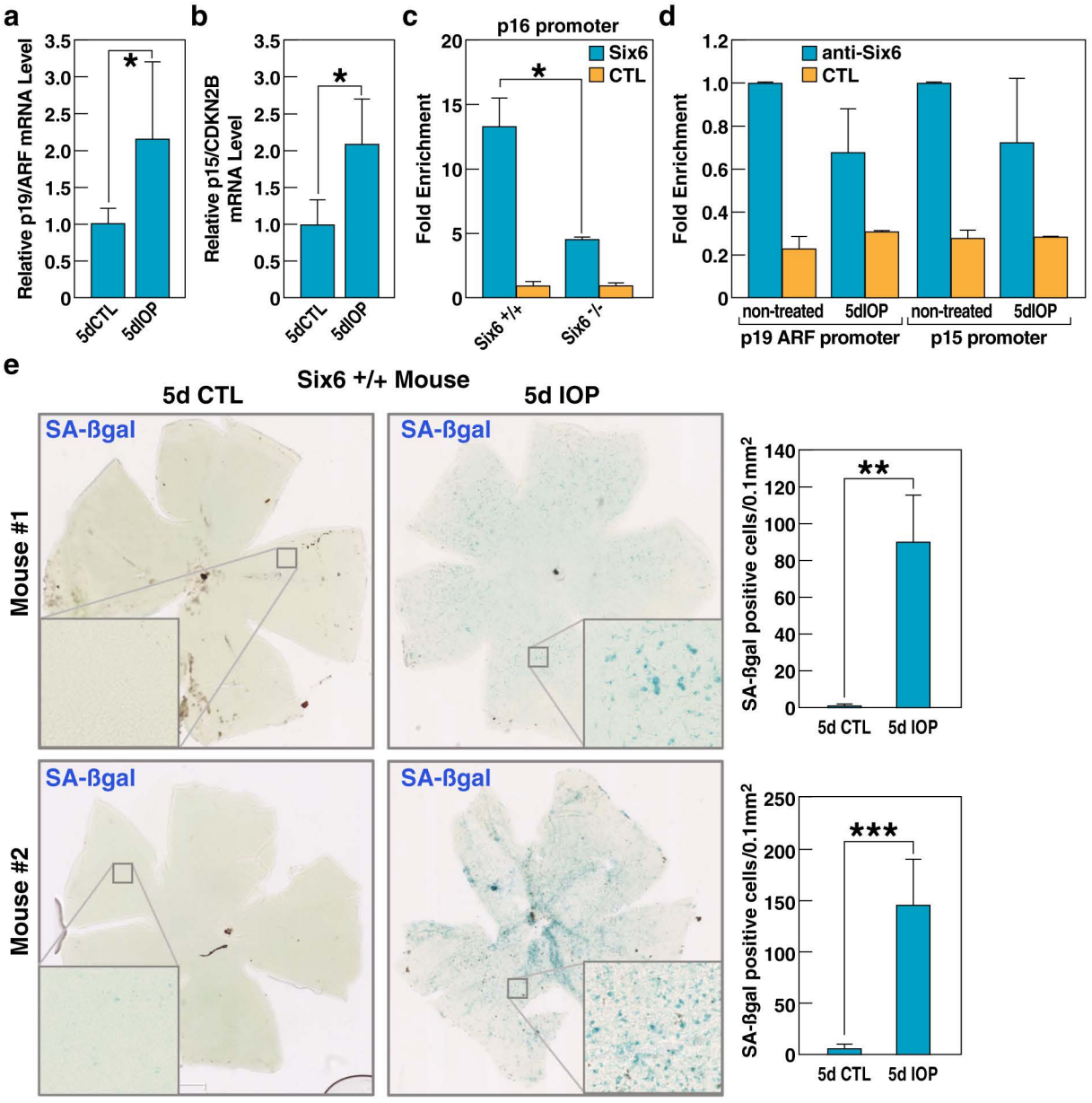
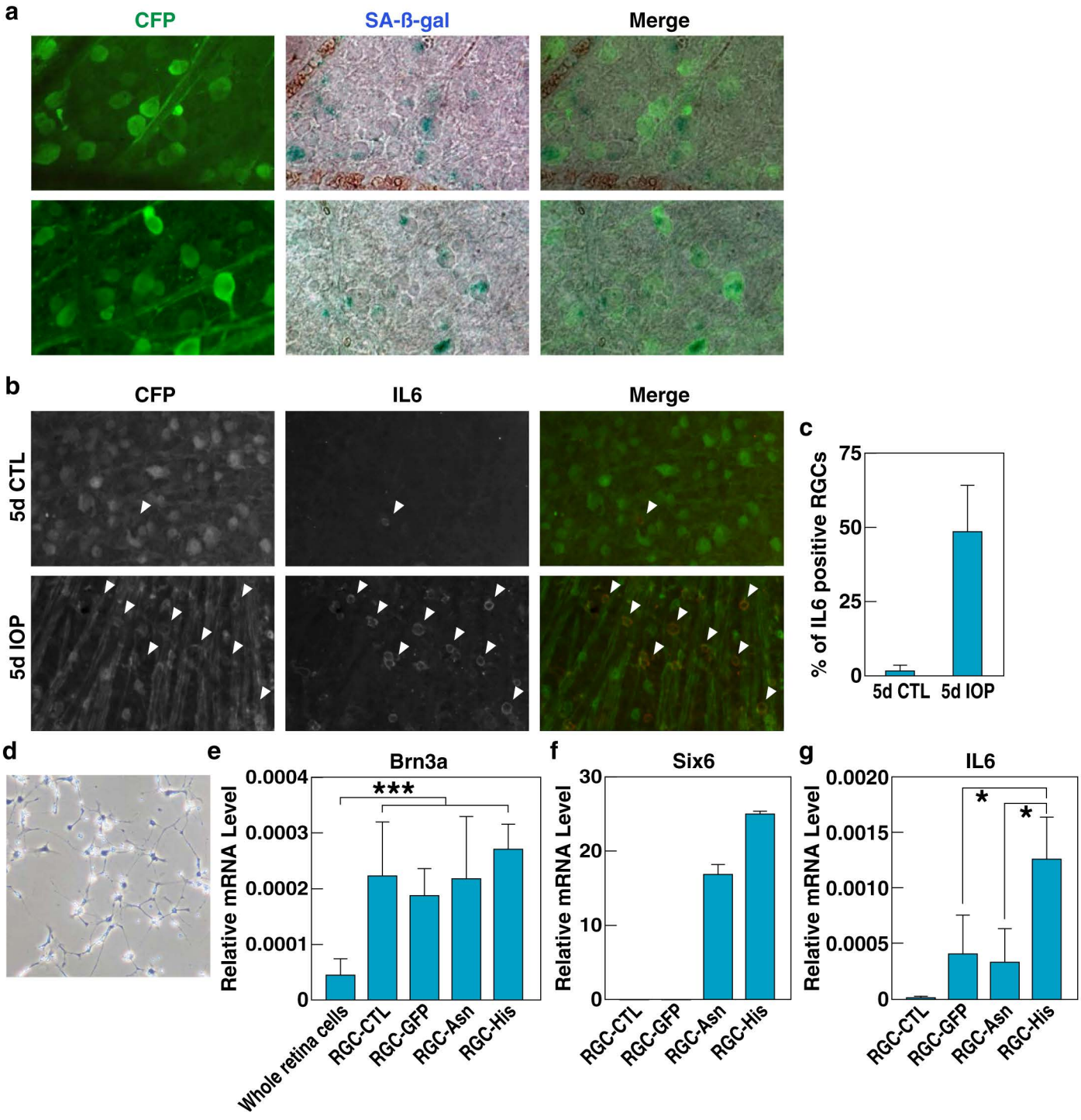

a
p27 promoter
Fold Enrichment
20
15
10
5
0
*
Six6
CTL
Six6 +/+
Six6 -/-
b
p27 promoter
ChIP Efficiency (%)
0.3
0.2
0.1
0
Six6
CTL
CTL
Six6-Asn
Six6-His
c
p27 promoter
ChIP Efficiency (%)
3
2
1
0
HA
CTL
CTL
Six6-Asn
Six6-His

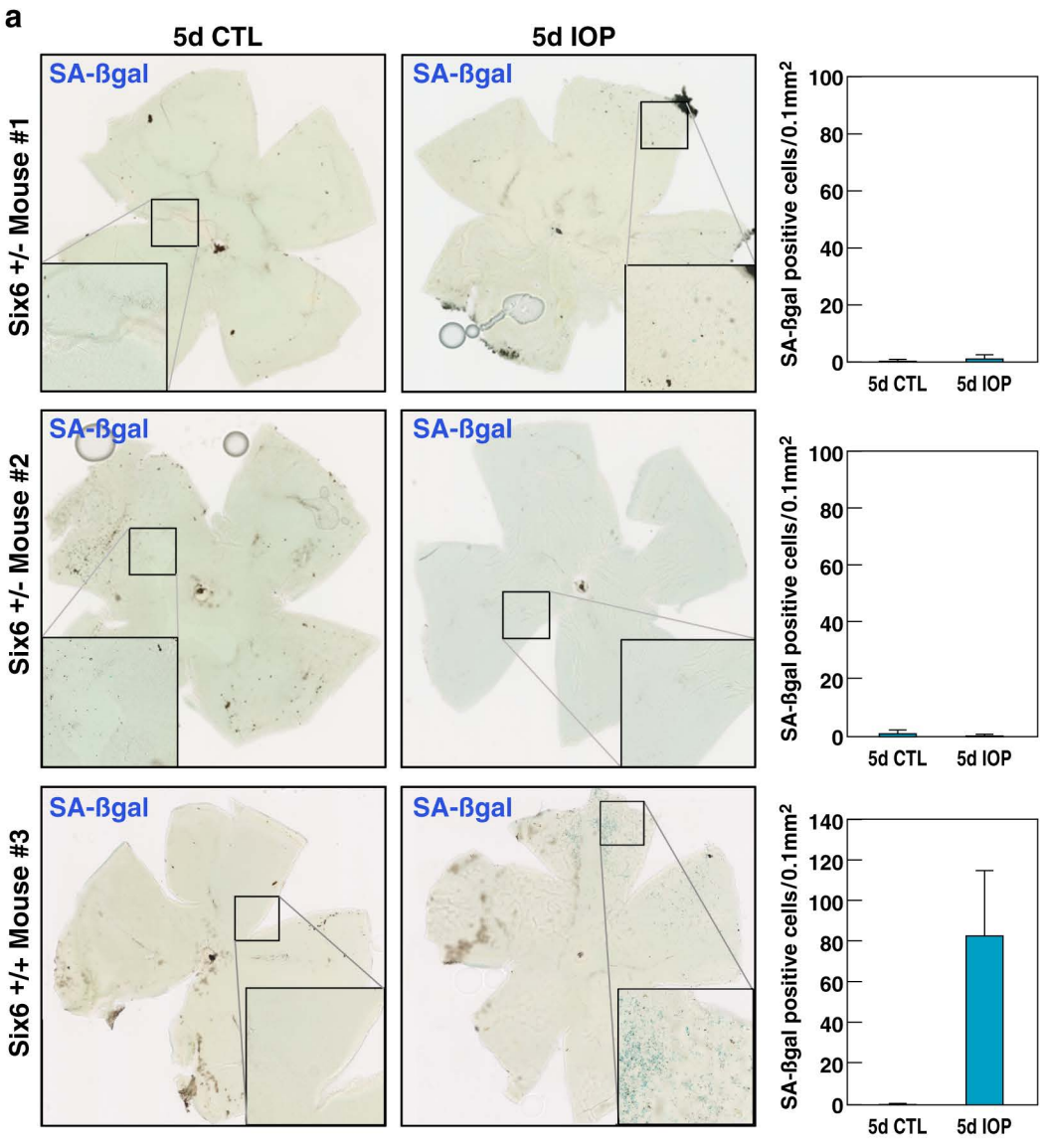

a
Healthy
Glaucoma
GCL
GCL
GCL
GCL
GCL
GCL
GCL
GCL
GCL
GCL
GCL
GCL
b
Relative p16 mRNA Level
15
10
5
0
*
Healthy
Glaucoma


IL6 mRNA
Relative mRNA Level
0.04
0.03
0.02
0.01
0
*
CTL
Six6-Asn
Six6-His


a
Relative p19/ARF mRNA Level
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
*
5dCTL
5dIOP
b
Relative p15/CDKN2B mRNA Level
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
*
5dCTL
5dIOP
c
p16 promoter
Fold Enrichment
20
15
10
5
0
*
Six6
CTL
Six6 +/+
Six6 -/-
d
Fold Enrichment
1.2
1.0
0.8
0.6
0.4
0.2
0
anti-Six6
CTL
non-treated
5dIOP
non-treated
5dIOP
p19 ARF promoter
p15 promoter
e
Six6 +/+ Mouse
5d CTL
5d IOP
Mouse #1
Mouse #2
SA-ßgal
SA-ßgal
SA-ßgal
SA-ßgal
SA-ßgal positive cells/0.1mm2
140
120
100
80
60
40
20
0
**
5d CTL
5d IOP
SA-ßgal positive cells/0.1mm2
250
200
150
100
50
0
***
5d CTL
5d IOP


a
CFP
SA-ß-gal
Merge
b
CFP
IL6
Merge
5d CTL
5d IOP
c
% of IL6 positive RGCs
75
50
25
0
5d CTL
5d IOP
d
e
Brn3a
Relative mRNA Level
0.0004
0.0003
0.0002
0.0001
0
***
Whole retina cells
RGC-CTL
RGC-GFP
RGC-Asn
RGC-His
f
Six6
Relative mRNA Level
30
20
10
0
RGC-CTL
RGC-GFP
RGC-Asn
RGC-His
g
IL6
Relative mRNA Level
0.0020
0.0015
0.0010
0.0005
0
*
*
RGC-CTL
RGC-GFP
RGC-Asn
RGC-His

a



b

Association of P53-rs1042522 (Pro72Arg) Risk Allele and POAG

SNP	Risk Allele	Case (n=833)	Control (n=763)	P Value (allelic)	OR (95% CI)
rs1042522	C	0.304	0.271	0.040	1.17 (1.00-1.37)

Figure S1 (related to Figure 1). Six6 protein residue 141 variants bind to DNA with similar efficiency. a, Validation of SIX6 antibody specificity by qPCR analysis of ChIP signals on known target. p27 regulatory region shows highly reduced signal in *Six6*$^{-/-}$ retinas. Experiments repeated 3 times, p-values calculated using a two-tailed Student's t-test (+/- SD; *p<0.05). CTL, negative control. **b, c,** ChIP-qPCR experiments using either SIX6 antibody (**b**) or HA antibody (**c**) after overexpression of HA-tagged SIX6 protein variants show that both forms bind efficiently to the *p27* regulatory region. Experiments repeated 3 times, p-values calculated using a two-tailed Student's t-test. +/- SD. CTL, negative control.

Figure S2 (related to Figure 2). Increased senescence in human glaucoma retinas. a, Several examples of SA-βgal analysis of the human retina showing higher numbers of senescent cells in retinas with POAG. **b,** RT-qPCR analysis of expression of p16/INK4A mRNA in four healthy and four POAG human retinas showing significant upregulation of the p16/INK4A transcript in the diseased retinas. p-values were calculated using a two-tailed Student's t-test (+/- SD; *p<0.05).

Figure S3 (related to Figure 3). Induction of IL6, a senescence associated secretory phenotype marker, in immunopanned RGCs upon Six6 protein overexpression. Experiments were repeated 3 times, p-values were calculated using a two-tailed student's t-test. (+/- SD; *p<0.05). CTL, negative control.

Figure S4 (related to Figure 4). Higher expression of Six6 protein and induction of senescence in retinas upon IOP elevation. **a,b,** RT-qPCR analysis of p19ARF (**a**) and p15/CDKN2B (**b**) gene expression in retinas 5 days after IOP-elevation (5d IOP) as compared to non-treated retinas (5d CTL). p-values were calculated using a two-tailed student's t-test (+/- SD; *p<0.05). **c,** ChIP-qPCR analysis of WT and *Six6*$^{-/-}$ retinas showed enrichment of SIX6 protein on *p16* regulatory elements. Experiments were repeated 3 times, p-values were calculated using a two-tailed student's t-test (+/- SD; *p<0.05). CTL, negative control. **d**, ChIP-qPCR analysis of the recruitment of Six6 protein to the p19ARF and p15/CDKN2B promoters after IOP-elevation (5d IOP) as compared to no-treated retinas (5d CTL). Experiment performed 3 times (+/- SD). CTL, negative control. **e,** SA-βgal staining of flat-mounted retinas isolated from treated (5d IOP) and non-treated (5d CTL)

eyes shows a high number of senescent cells in treated tissue. *Right panels:* quantification of number of SA-βgal positive cells in presented retinas. p-values were calculated using a two-tailed Student's t-test (+/- SD; **p<0.01, ***p<0.001).

Figure S5 (related to Figure 5). IOP-elevation affects retinal ganglion cells. a, Several examples of double, SA-βgal and CFP positive cells in IOP-treated Thy1-CFP retinas. **b,** Immunostaining of IOP-treated (5d IOP) and untreated (5d CTL) Thy1-CFP retinas using anti-GFP and anti-IL6 antibodies. The number of double-positive cells is specifically increased in IOP-treated tissue. **c,** Quantification of IL6/CFP double-positive cells in IOP-treated retinas in 3 randomly selected fields (+/- SD). **d,** RGC purified by immunopanning after 2 days in culture. **e,** Expression of Brn3a in whole retina cells and immunopanned cells was measured by RT-qPCR. p-values were calculated using a two-tailed Student's t-test, (+/- SD; ***p<0.001). **f,** Overexpression levels of the His and Asn variants of Six6 in transfected cells was measured using RT-qPCR. +/- SD. **g,** RT-qPCR analysis of IL6 expression in RGCs upon Six6 variants or GFP (RGC-GFP) overexpression as compared to non-transfected cells (RGC-CTL). p-values were calculated using a two-tailed Student's t-test (+/- SD; *p<0.05).

Figure S6 (related to Figure 6). Lack of Six6 protects RGCs against senescence. a, SA-βgal staining of flat-mounted mouse retinas isolated from IOP treated (5d IOP) and non-treated (5d CTL) *Six6*$^{+/-}$ eyes shows no increase in the number of senescent cells upon IOP elevation, as compared to the Six6$^{+/+}$ eyes (*bottom panels*) *Right panels:* quantification of SA-βgal positive cells in presented retinas, +/- SD; **b,** Association of p53-rs1042522 (Pro72Arg) amino acid variant with POAG.

Table S1 (related to Figure 1). Meta-analysis results for SNP rs33912345 in POAG

Gene/SNP	Risk Allele	Cohorts	Sample size (n)		Risk allele frequency		P value(Allelic)	OR (95% CI)	P_{het}
			Case	Control	Case	Control			
SIX6/rs33912345	C	Caucasian cohort	1,130	4,036	0.46	0.38	4.49E-12	1.39 (1.27-1.53)	
		Mexican cohort	105	188	0.41	0.31	1.10E-02	1.57 (1.09-2.27)	
		Cohort (Carnes et al., 2014)	482	433	0.47	0.38	1.05E-04	1.41 (1.16-1.70)	
		All combined	1,717	4,657	0.46	0.38	4.84E-16	1.40 (1.29-1.52)	0.818

Meta-analysis was performed using inverse-variance method. CI, confidence interval; OR, odds ratio; P_{het}, *P*-value of heterogeneity.

Carnes, M.U., Liu, Y.P., Allingham, R.R., Whigham, B.T., Havens, S., Garrett, M.E., Qiao, C., Katsanis, N., Wiggs, J.L., Pasquale, L.R., *et al.* (2014). Discovery and functional annotation of SIX6 variants in primary open-angle glaucoma. PLoS genetics *10*, e1004372.

Table S2. Primer sequence information

Primer name	Sequence (5'-3')
Genotyping	
rs33912345	
rs33912345-Forward	GTGGCCTTTCACGGTGGCAACT
rs33912345-Reverse	GTTGCCCACCTGCGTAGGGGT
rs33912345-Extension	GGTTAGGGTATGGATCCTGCAGGTACCACTCGCGTAGCAGGT
rs1042522	
rs1042522-Forward	GATGCTGTCCCCGGACGATAT
rs1042522-Reverse	GCCCAGACGGAAACCGTAGCT
rs1042522-Extension	CGGTGTAGGAGCTGCTGGTGCAGGGGCCACG
rs3731239	
rs3731239-Forward	GTAAGATGTGCTGGGACTACT
rs3731239-Reverse	CGAACTCCCGACCTCAGGTGAT
rs3731239-Extension	CTGTGGTGTATGTTGGAATAAATATCGAATA
qPCR	
Human GAPDH-Forward	GAGTCAACGGATTTGGTCGT
Human GAPDH-Reverse	GACAAGCTTCCCGTTCTCAG
Human P16 -Forward	GAGCAGCATGGAGCCTTC
Human P16 -Reverse	CCTCCGACCGTAACTATTCG
Human SIX6-Forward	AGAATGAGTCGGTGCTACGC
Human SIX6-Reverse	GCCTCCTGGTAGTTGTGCTTC
Human IL6 - Forward	ACTCACCTCTTCAGAACGAATTG
Human IL6 - Reverse	CCATCTTTGGAAGGTTCAGGTTG
Mouse GAPDH-Forward	GTCAAGGCCGAGAATGGGAA
Mouse GAPDH-Reverse	TTGGCTCCACCCTTCAAGTG
Mouse p16/INK4a-Forward	GCGGACTCCATGCTGCTC
Mouse p16/INK4a-Reverse	CACGACTGGGCGATTGGG
Mouse SIX6-Forward	ACTCCAGCAGCAGGTTCTGT
Mouse SIX6-Reverse	AGATGTCGCACTCACTGTCG
Mouse p19/ARF-Forward	CGCAGGTTCTTGGTCACTGT
Mouse p19/ARF-Reverse	TGTTCACGAAAGCCAGAGCG
Mouse p15/cdkn2b-Forward	TTGCGGAAGGCGGAGGGAAC
Mouse p15/cdkn2b-Reverse	AAGAGCAGGGCCACCGTGAC
Rat GAPDH-Forward	Qiagen cat no. QT0099633
Rat GAPDH-Reverse	
Rat P16-Forward	CGATCCGGAGCAGCATGGAGTC
Rat P16-Reverse	TTCCAGCAGTGCCCGCACCTCG
Rat IL6 - Forward	GCCTTCTTGGGACTGATG
Rat IL6 - Reverse	TGTGGGTGGTATCCTCTG
Rat Brn3a - Forward	CAGGAGTCCCATGTAAGA

Rat Brn3a - Reverse	ACAGGGAAACACTTCTGC

ChIP

Human P16 promoter-Forward	ACCCTGTCCCTCAAATCC
Human P16 promoter-Reverse	GGTGCCACATTCGCTAAG
Human P27 promoter-Forward	CAATATGGCGGTGGAAGG
Human P27 promoter-Reverse	CCGCAACCAATGGATCTC
Mouse P16 promoter-Forward	ATGGAGCCCGGACTACAG
Mouse P16 promoter-Reverse	GGTGTTAGCGTGGGTAGC
Mouse p19/ARF promoter-Forward	CACTGTGACAAGCGAGGTGAG
Mouse p19/ARF promoter-Reverse	GATGGGCGTGGAGCAAAGATG
Mouse p15 promoter-Forward	AAGTTGTGCCTCTGCACTC
Mouse p15 promoter-Reverse	GCGATTGATGCCTCCAAAG