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Supplementary information

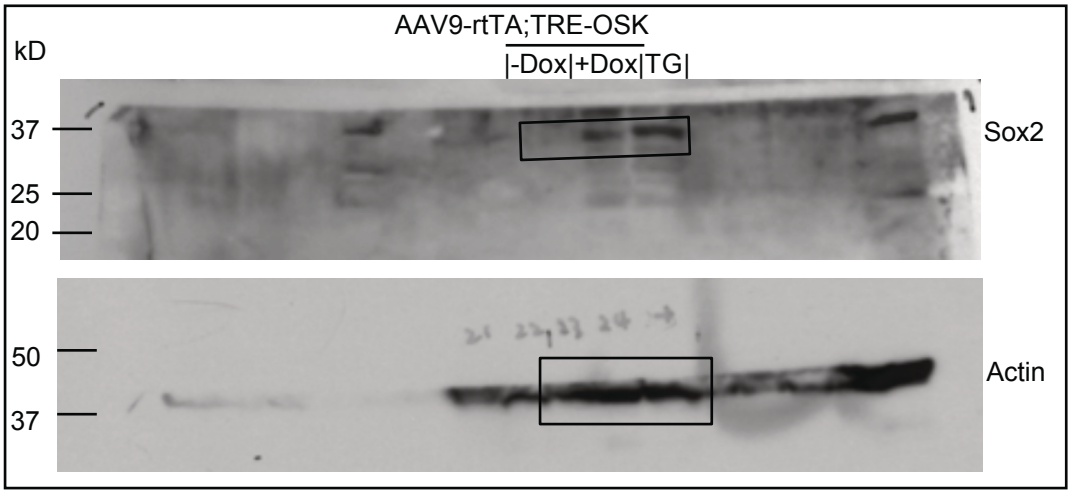
Reprogramming to recover youthful epigenetic information and restore vision

In the format provided by the authors and unedited

Supplementary Figure 1

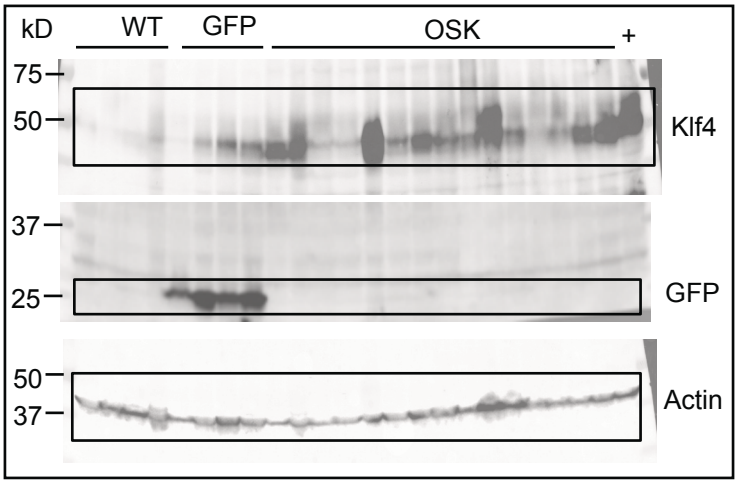
Original western blot scans with size marker indications. Boxes indicate cropped area.

Extended Data Fig. 1h



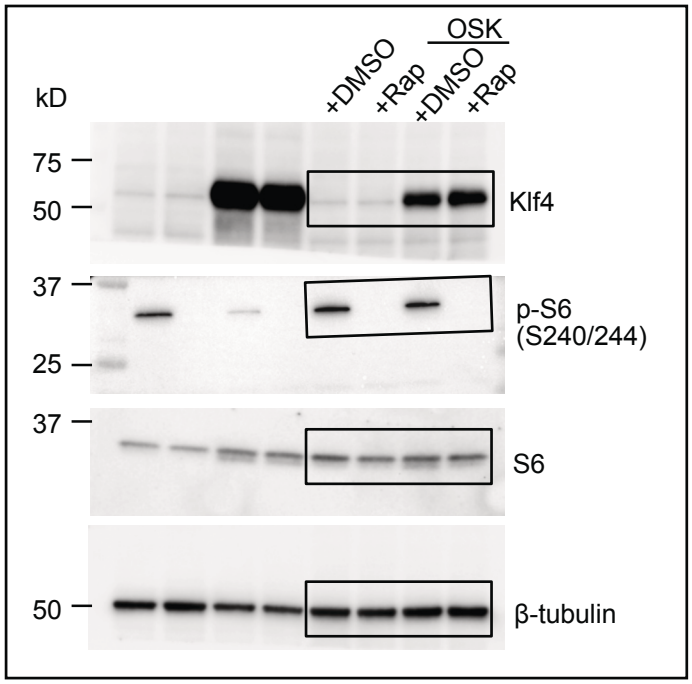
Beta-actin was run on a separate gel as sample processing control, due to the similar size of Sox2 and Actin.

Extended Data Fig. 1k



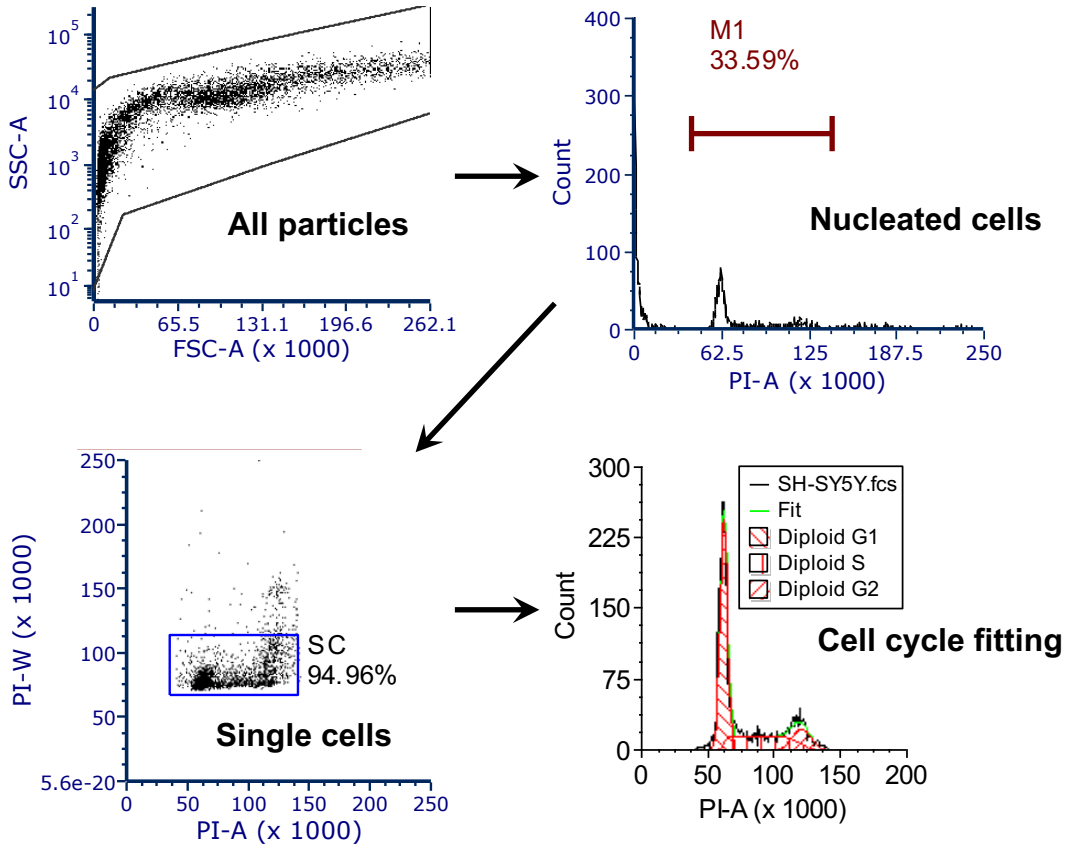
Beta-actin was reblotted after striping on the same gel as loading control

Extended Data Fig. 8s



Beta-tubulin was run with S6 on the same gel as loading control

Supplementary Figure 2



Gating strategies for cell cycle analysis. A representative flow cytometry analysis of cells stained with propidium iodide in Extended Data Fig. 8c is shown.

Supplementary Discussion

Mouse models of glaucoma and the restoration of vision

There have been numerous approaches to treating glaucoma in rodent models, including those involved in inflammation[1-8], apoptosis[9-11], pro-survival[12-16], and metabolic pathways.[17,18] The majority of these studies have initiated treatment prior to, or during the initial stages of glaucoma when intraocular pressure is increasing, but no axonal damage or decreased visual function has yet occurred. While these experiments demonstrate the ability to prevent neuronal damage, their mode of action may limit their window of efficacy to earlier stages of the disease. Some studies have intervened when the loss of axons and a decline in visual function has already begun. For example, one study[18] used D2 mice with chronic age-related inherited glaucoma in which there is prolonged axon degeneration that accumulates with age. When nicotinamide, an NAD precursor, was given to mice orally at an intermediate stage of glaucoma, disease progression halted and visual function was preserved. However, the study was not designed to assess restorative effects on already damaged axons. In this paper, we used the microbead model that is a more acute than a chronic model of glaucoma. Intraocular pressure was elevated for four weeks and, by then, the loss of RGC, axons and visual function was complete. OSK expression from AAV started at a time point when glaucomatous damage was already established, and even under these conditions, visual function was improved.

1 Anderson, M. G., Libby, R. T., Gould, D. B., Smith, R. S. & John, S. W. M. High-dose radiation with bone marrow transfer prevents neurodegeneration in an inherited glaucoma. *P Natl Acad Sci Usa* **102**, 4566-4571, doi:10.1073/pnas.0407357102 PMID - 15758074 (2005).

2 Howell, G. R. *et al.* Radiation treatment inhibits monocyte entry into the optic nerve head and prevents neuronal damage in a mouse model of glaucoma. *J Clin Investigation* **122**, 1246-1261, doi:10.1172/jci61135 PMID - 22426214 (2012).

3 Williams, P. A. *et al.* Inhibition of the classical pathway of the complement cascade prevents early dendritic and synaptic degeneration in glaucoma. *Mol Neurodegener* **11**, 26, doi:10.1186/s13024-016-0091-6 PMID - 27048300 (2016).

4 Harder, J. M. *et al.* Early immune responses are independent of RGC dysfunction in glaucoma with complement component C3 being protective. *P Natl Acad Sci Usa* **114**, E3839-E3848, doi:10.1073/pnas.1608769114 PMID - 28446616 (2017).

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10 Krishnan, A., Kocab, A. J., Zacks, D. N., Marshak-Rothstein, A. & Gregory-Ksander, M. A small peptide antagonist of the Fas receptor inhibits neuroinflammation and prevents axon degeneration and retinal ganglion cell death in an inducible mouse model of glaucoma. *J Neuroinflamm* **16**, 184, doi:10.1186/s12974-019-1576-3 PMID - 31570110 (2019).
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Supplementary Code

The R code for developing and analyzing the DNA methylation ageing signature. Supplementary Data DNAm.csv should be imported into R. The numbers in the code c(#) correspond to the row #s in DNAm.csv, not counting first name row.

```
library(WGCNA)

#Non-linear Age Transformation
trafo= function(x,adult.age=6) { x=(x+1)/(1+adult.age); y=ifelse(x<=1, log( x),x-1);y }
anti.trafo= function(x,adult.age=6) { ifelse(x<0, (1+adult.age)*exp(x)-1,
(1+adult.age)*x+adult.age) }
TransAge=trafo(c(rep(1,6),rep(12,2),rep(30,6)))

#CpG Correlations with Age (2 batches), Injury, OSK
Cors=matrix(ncol = 4,nrow = ncol(DNAm))
Cors[,1]=bicor(DNAm[c(1:7,56,8:15),],c(rep(0,8),rep(1,8)))
Cors[,2]=bicor(DNAm[c(21,30,37,40,46,55,41,42,16:20,22),],TransAge)
Cors[,3]=bicor(DNAm[c(21,30,37,40,46,55,24,25,28,29),],c(rep(0,6),rep(1,4)))
Cors[,4]=bicor(DNAm[c(50,51,43,44),],c(rep(0,2),rep(1,2)))
colnames(Cors)=c("6m vs. 18m","1m vs. 30m","1m vs. Injury","12m vs. 12mOSK")

#CpG preselection based on DNAm correlations for age/injury/OSK
DNAm=DNAm[,which((Cors[,1]>quantile(abs(Cors[,1]), probs = seq(0, 1, .1),na.rm = T)[8] &
is.na(Cors[,1])==FALSE &
                 Cors[,2]>quantile(abs(Cors[,2]), probs = seq(0, 1, .1),na.rm = T)[8] &
is.na(Cors[,2])==FALSE &
                 Cors[,3]>quantile(abs(Cors[,3]), probs = seq(0, 1, .1),na.rm = T)[8] &
is.na(Cors[,3])==FALSE &
                 Cors[,4]>quantile(abs(Cors[,4]), probs = seq(0, 1, .1),na.rm = T)[8] &
is.na(Cors[,4])==FALSE ) |
                (Cors[,1]< -quantile(abs(Cors[,1]), probs = seq(0, 1, .1),na.rm = T)[8] &
is.na(Cors[,1])==FALSE &
                  Cors[,2]< -quantile(abs(Cors[,2]), probs = seq(0, 1, .1),na.rm = T)[8] &
is.na(Cors[,2])==FALSE &
                  Cors[,3]< -quantile(abs(Cors[,3]), probs = seq(0, 1, .1),na.rm = T)[8] &
is.na(Cors[,3])==FALSE &
                  Cors[,4]< -quantile(abs(Cors[,4]), probs = seq(0, 1, .1),na.rm = T)[8] &
is.na(Cors[,4])==FALSE)) ]

#PCA in the age samples (batch 2)
PCA=prcomp(DNAm[c(21,30,37,40,46,55,41,42,16:20,22),],scale. = F)

#Estimation of PC1 for all samples
PC1.DNAmAge=predict(PCA,DNAm)
```

Supplementary Table 1

List of axon regeneration-related genes and their changes by OSK

+OSK/ -OSK	logFC	Q Value
Stat3	1.14	5.84E-13
RhoA	0.52	1.46E-05
CREB1	0.64	1.94E-05
SRF	0.71	0.002762
ASCL1	-1.45	0.003949
KLF7	-0.56	0.01106
KLF6	0.46	0.022004
SOCS3	0.61	0.090817
SOX11	-1.00	0.150632
PTEN	-0.20	0.205178
c-JUN	-0.23	0.498078
HIF1a	0.14	0.617879
SMAD1	-0.14	0.646251
ATF3	0.14	0.917888

q values were given by the exact test of the edgeR Bioconductor package

Supplementary Table 2

List of genes differentially expressed during RGC ageing

Genes down during ageing			
1700031P21Rik	Gm11264	Gm42895	Olfr127
1810053B23Rik	Gm11337	Gm42994	Olfr1291-ps1
2900045O20Rik	Gm11368	Gm43027	Olfr1406
2900060B14Rik	Gm11485	Gm43158	Olfr1469
4921504E06Rik	Gm11693	Gm43288	Olfr215
4930402F11Rik	Gm12793	Gm43366	Olfr273
4930453C13Rik	Gm13050	Gm44044	Olfr328
4930455B14Rik	Gm13066	Gm44081	Olfr355
4930500H12Rik	Gm13323	Gm44187	Olfr372
4930549P19Rik	Gm13339	Gm44280	Olfr390
4930555B11Rik	Gm13346	Gm44535	Olfr427
4930556J02Rik	Gm13857	Gm45338	Olfr456
4932442E05Rik	Gm14387	Gm45644	Olfr466
4933431K23Rik	Gm14770	Gm45740	Olfr481
4933438K21Rik	Gm15638	Gm46555	Olfr522
6720475M21Rik	Gm16072	Gm46565	Olfr6
9830132P13Rik	Gm16161	Gm4742	Olfr601
A430010J10Rik	Gm16181	Gm47485	Olfr603
A530064D06Rik	Gm17200	Gm47853	Olfr706
A530065N20Rik	Gm17791	Gm47992	Olfr727
Abcb5	Gm18025	Gm48225	Olfr728
Abhd17c	Gm18757	Gm48314	Olfr741
AC116759.2	Gm18795	Gm48383	Olfr801
AC131705.1	Gm18848	Gm48673	Olfr812
AC166779.3	Gm19719	Gm48804	Olfr816
Acot12	Gm20121	Gm48832	Olfr822
Adig	Gm20356	Gm4994	Olfr860
Akr1cl	Gm2093	Gm5487	Olfr890
Ankrd1	Gm21738	Gm5724	Olfr923
Asb15	Gm21940	Gm595	Olfr943
Atp2c2	Gm22933	Gm6012	Otogl
AU018091	Gm24000	Gm6024	Pi15
AW822073	Gm24119	Gm7669	Pkhd1
Btnl10	Gm25394	Gm7730	Pkhd1l1
C130093G08Rik	Gm26555	Gm8043	Platr6
C730027H18Rik	Gm27047	Gm8953	Pou3f4
Ccdc162	Gm28262	Gm9348	Prr9
Chil6	Gm28530	Gm9369	Pvalb

Col26a1	Gm29295	Gm9495	Rhag
Corin	Gm29825	H2al2a	Sav1
Crls1	Gm29844	Ido2	Serpinb9b
Cybrd1	Gm3081	Igfbp1	Skint1
Cyp2d12	Gm32051	Kif7	Skint3
Cyp7a1	Gm32122	Klhl31	Skint5
D830005E20Rik	Gm33056	Lrrc31	Slc10a5
Dlx3	Gm33680	Mc5r	Slc6a4
Dnah14	Gm34354	Mgam	Smok2a
Dsc3	Gm34643	Msh4	Tcaf3
Dthd1	Gm3551	Mucl2	Tomm20l
Eid2	Gm36660	Mug1	Trcg1
Eps8l1	Gm36948	Mybl2	Trdn
EU599041	Gm37052	Myh15	Ugt1a6a
Fam90a1a	Gm37142	Nek10	Usp17la
Fancf	Gm37262	Neurod6	Vmn1r178
Fau-ps2	Gm37535	Nr1h5	Vmn1r179
Fezf1	Gm37569	Olfr1042	Vmn1r33
Gja5	Gm37589	Olfr1043	Vmn1r74
Gm10248	Gm37647	Olfr1082	Vmn1r87
Gm10513	Gm37648	Olfr1090	Vmn2r102
Gm10635	Gm37762	Olfr1124	Vmn2r113
Gm10638	Gm38058	Olfr1167	Vmn2r17
Gm10718	Gm38069	Olfr1205	Vmn2r52
Gm10722	Gm38137	Olfr1206	Vmn2r66
Gm10800	Gm38218	Olfr1223	Vmn2r68
Gm10801	Gm39139	Olfr1263	Vmn2r76
Gm11228	Gm42535	Olfr1264	Vmn2r78
Gm11251	Gm42680	Olfr1269	Wnt16

Genes up during ageing			
0610040J01Rik	Fbxw21	Gm9796	Plxna4
1700080N15Rik	Ffar4	Gm9954	Prickle2
2900064F13Rik	Flt4	Gpr75	Qsox1
4833417C18Rik	Fmod	Gprc5c	Rapgef4os2
4921522P10Rik	Foxp4	Grid2ip	Rbp4
4930447C04Rik	Fzd7	Gsg1l2	Rcn3
4930488N15Rik	Gabrd	Hapln4	Sec14l5
Ace	Galnt15	Hcn3	Sel1l3
Ackr1	Galnt18	Hcn4	Serpinh1

Acot10	Gfra2	Hhatl	Sgpp2
Acvr1	Ggt1	Hs6st2	Shisa6
Adamts17	Gm10416	Htr3a	Siah3
Adra1b	Gm14964	Il1rap	Siglech
AI504432	Gm17634	Il1rapl2	Slc12a4
Best3	Gm2065	Inka1	Slc24a2
Boc	Gm32352	Kbtbd12	Slc2a5
Cadm3	Gm33172	Kcnj11	Slc4a4
Cand2	Gm34280	Kcnk4	Slitrk3
Ccl9	Gm35853	Kdelc2	Smagp
Cd14	Gm36298	Klhl33	Smoc2
Cd36	Gm36356	Lamc3	Speer4b
Cfh	Gm36937	Lilra5	Spon2
Chrm3	Gm3898	Lman1l	Sstr2
Chrna4	Gm42303	Lrfn2	Sstr3
Cntn4	Gm42484	Lrrc38	St3gal3
Cracr2b	Gm42537	Lrrn4cl	Stc1
Cryaa	Gm42743	Ltc4s	Stc2
CT573017.2	Gm43151	Mansc1	Syndig1
Cyp26a1	Gm43843	Mir344c	Syt10
Cyp27a1	Gm44545	Msr1	Thsd7a
D330050G23Rik	Gm44722	Mycbpap	Tlr8
D930007P13Rik	Gm45516	Myoc	Tmem132a
Ddo	Gm45532	Ngfr	Tmem132d
Dgkg	Gm47494	Nipal2	Tmem200a
Dlk2	Gm47982	Olfr1372-ps1	Tmem44
Dnaja1-ps	Gm47989	Otop3	Trpc4
Drd2	Gm48398	P2rx5	Trpv4
Dsel	Gm48495	P2ry12	Unc5b
Dytn	Gm48593	P4ha2	Vgf
Ecscr	Gm48958	Pcdha12	Vmn1r90
Edn1	Gm49089	Pcdha2	Vwc2l
Ednrb	Gm49326	Pcdhac2	Wfikkn2
Efemp1	Gm49331	Pcdhb18	Wnt11
Elfn2	Gm49760	Pcdhb5	Wnt6
Epha10	Gm5796	Pcsk2os1	Zeb2os
Ephx1	Gm6374	Pcsk6	Zfp608
Erbb4	Gm7276	Perp	Zfp976
Fam20a	Gm8237	Pkp1	

Supplementary Table 3

1,226 signature CpG sites

Hypomethylated during ageing						
Chromosome	Start	End		Chromosome	Start	End
chr1	106172796	106172797		chr2	121008460	121008461
chr1	120628817	120628818		chr2	121867699	121867700
chr1	132191568	132191569		chr2	124255188	124255189
chr1	132881477	132881478		chr2	132144264	132144265
chr1	134835808	134835809		chr2	132578277	132578278
chr1	138848376	138848377		chr2	136059473	136059474
chr1	143777539	143777540		chr2	13793496	13793497
chr1	153486854	153486855		chr2	139678900	139678901
chr1	159983064	159983065		chr2	147178897	147178898
chr1	16172622	16172623		chr2	148395311	148395312
chr1	167284368	167284369		chr2	157261911	157261912
chr1	172532410	172532411		chr2	162659215	162659216
chr1	18016416	18016417		chr2	166154529	166154530
chr1	180794381	180794382		chr2	168702224	168702225
chr1	180941962	180941963		chr2	168765809	168765810
chr1	18472998	18472999		chr2	168766854	168766855
chr1	189728345	189728346		chr2	24585995	24585996
chr1	191049127	191049128		chr2	24607620	24607621
chr1	24586843	24586844		chr2	26021740	26021741
chr1	36068191	36068192		chr2	27540317	27540318
chr1	3671142	3671143		chr2	27740102	27740103
chr1	36785077	36785078		chr2	30093464	30093465
chr1	38129823	38129824		chr2	32570804	32570805
chr1	38626922	38626923		chr2	38287476	38287477
chr1	38626946	38626947		chr2	50878043	50878044
chr1	39368373	39368374		chr2	56455001	56455002
chr1	39900661	39900662		chr2	62356970	62356971
chr1	51088748	51088749		chr2	69585929	69585930
chr1	51747646	51747647		chr2	74698349	74698350
chr1	55773436	55773437		chr2	74720240	74720241
chr1	57862286	57862287		chr2	79428747	79428748
chr1	59912574	59912575		chr2	82467524	82467525
chr1	61143337	61143338		chr2	93045793	93045794
chr1	62703430	62703431		chr2	96317911	96317912

chr1	64328635	64328636		chr2	96317974	96317975
chr1	64736784	64736785		chr3	101068590	101068591
chr1	75219562	75219563		chr3	103171796	103171797
chr1	75309052	75309053		chr3	106625184	106625185
chr1	75484467	75484468		chr3	121262334	121262335
chr1	89931151	89931152		chr3	121262376	121262377
chr1	93235920	93235921		chr3	121292116	121292117
chr1	98542840	98542841		chr3	122729089	122729090
chr10	11080560	11080561		chr3	13170993	13170994
chr10	121365243	121365244		chr3	134331701	134331702
chr10	127311600	127311601		chr3	141465913	141465914
chr10	13008920	13008921		chr3	38484650	38484651
chr10	13966354	13966355		chr3	52662147	52662148
chr10	17634248	17634249		chr3	81323271	81323272
chr10	17722975	17722976		chr3	88621702	88621703
chr10	17723003	17723004		chr3	89332650	89332651
chr10	17723029	17723030		chr3	90476384	90476385
chr10	20149647	20149648		chr3	96390686	96390687
chr10	36507483	36507484		chr4	102087688	102087689
chr10	3741083	3741084		chr4	106102567	106102568
chr10	39731615	39731616		chr4	106911206	106911207
chr10	40025145	40025146		chr4	109201780	109201781
chr10	40300908	40300909		chr4	118290709	118290710
chr10	46973032	46973033		chr4	120268393	120268394
chr10	47940338	47940339		chr4	120287191	120287192
chr10	57291024	57291025		chr4	120855020	120855021
chr10	67096387	67096388		chr4	124575814	124575815
chr10	67285330	67285331		chr4	124659264	124659265
chr10	70440783	70440784		chr4	124895005	124895006
chr10	79766739	79766740		chr4	128883722	128883723
chr10	80330317	80330318		chr4	129121398	129121399
chr10	80506395	80506396		chr4	131462269	131462270
chr10	80656357	80656358		chr4	132048694	132048695
chr10	86498584	86498585		chr4	134343074	134343075
chr10	93889808	93889809		chr4	134944323	134944324
chr11	109721709	109721710		chr4	136291228	136291229
chr11	113683952	113683953		chr4	136424102	136424103
chr11	115708061	115708062		chr4	136463048	136463049
chr11	117408744	117408745		chr4	136755780	136755781

chr11	119086644	119086645		chr4	137278030	137278031
chr11	11926475	11926476		chr4	138216631	138216632
chr11	119491634	119491635		chr4	138325983	138325984
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Hypermethylated during ageing						
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chr12	69371913	69371914		chr5	130024653	130024654
chr12	69724996	69724997		chr5	134315249	134315250
chr12	71309875	71309876		chr5	134555577	134555578
chr12	72236690	72236691		chr5	135251132	135251133
chr12	72937998	72937999		chr5	136565872	136565873
chr12	72944989	72944990		chr5	136882944	136882945
chr12	73286862	73286863		chr5	139735931	139735932
chr12	78912104	78912105		chr5	140784842	140784843
chr12	84222801	84222802		chr5	142468866	142468867
chr12	85746356	85746357		chr5	142819636	142819637
chr12	86883272	86883273		chr5	143731734	143731735
chr12	87057150	87057151		chr5	145218598	145218599
chr13	101767395	101767396		chr5	147430107	147430108
chr13	101767429	101767430		chr5	147430113	147430114
chr13	107888548	107888549		chr5	150593875	150593876
chr13	111489385	111489386		chr5	150594040	150594041
chr13	112101023	112101024		chr5	21784580	21784581
chr13	112993978	112993979		chr5	24474524	24474525
chr13	119488800	119488801		chr5	24474833	24474834
chr13	17695729	17695730		chr5	24842209	24842210
chr13	17805478	17805479		chr5	29474073	29474074
chr13	21363853	21363854		chr5	30588864	30588865
chr13	29698853	29698854		chr5	30945379	30945380
chr13	34345147	34345148		chr5	34549951	34549952
chr13	42052217	42052218		chr5	36397817	36397818
chr13	48731712	48731713		chr5	36868746	36868747
chr13	48870734	48870735		chr5	66260035	66260036
chr13	54303437	54303438		chr5	67098768	67098769
chr13	54687642	54687643		chr5	76304826	76304827
chr13	55600053	55600054		chr5	76595010	76595011
chr13	57948326	57948327		chr5	76975034	76975035
chr13	58273864	58273865		chr5	8038597	8038598
chr13	59832812	59832813		chr5	90629683	90629684
chr13	63919076	63919077		chr5	93093130	93093131
chr13	74167071	74167072		chr5	95856674	95856675
chr13	95891000	95891001		chr5	98031229	98031230

chr13	95891025	95891026		chr5	99252615	99252616
chr14	118132885	118132886		chr6	106800119	106800120
chr14	118235730	118235731		chr6	113326968	113326969
chr14	119138446	119138447		chr6	115135038	115135039
chr14	122482982	122482983		chr6	115808351	115808352
chr14	15960731	15960732		chr6	117061236	117061237
chr14	18242868	18242869		chr6	117871640	117871641
chr14	18531391	18531392		chr6	120463711	120463712
chr14	20083217	20083218		chr6	124917667	124917668
chr14	20083251	20083252		chr6	133262305	133262306
chr14	20083258	20083259		chr6	134034940	134034941
chr14	20324434	20324435		chr6	134792820	134792821
chr14	21983531	21983532		chr6	135065805	135065806
chr14	25800357	25800358		chr6	137169650	137169651
chr14	33189994	33189995		chr6	137169690	137169691
chr14	34614852	34614853		chr6	137252565	137252566
chr14	46788194	46788195		chr6	143806755	143806756
chr14	46788248	46788249		chr6	145291665	145291666
chr14	54417604	54417605		chr6	20551420	20551421
chr14	54876492	54876493		chr6	24665226	24665227
chr14	55592440	55592441		chr6	29496187	29496188
chr14	55895057	55895058		chr6	30624676	30624677
chr14	56885619	56885620		chr6	47778272	47778273
chr14	61121710	61121711		chr6	47953600	47953601
chr14	63500237	63500238		chr6	47953622	47953623
chr14	63743550	63743551		chr6	47953635	47953636
chr14	69927581	69927582		chr6	48627289	48627290
chr14	70126623	70126624		chr6	48627297	48627298
chr14	70458011	70458012		chr6	48627311	48627312
chr14	70554999	70555000		chr6	49218095	49218096
chr14	93935093	93935094		chr6	52713995	52713996
chr15	102987643	102987644		chr6	54681883	54681884
chr15	10715385	10715386		chr6	56797509	56797510
chr15	27509258	27509259		chr6	67592657	67592658
chr15	32707004	32707005		chr6	71494336	71494337
chr15	39989179	39989180		chr6	72414282	72414283
chr15	41869470	41869471		chr6	72439002	72439003
chr15	44787733	44787734		chr6	82688016	82688017
chr15	53341401	53341402		chr6	83109189	83109190

chr15	56988056	56988057		chr6	86733368	86733369
chr15	73061061	73061062		chr6	97211441	97211442
chr15	73645723	73645724		chr7	101451316	101451317
chr15	75709134	75709135		chr7	110768851	110768852
chr15	76454348	76454349		chr7	110787462	110787463
chr15	76818043	76818044		chr7	127717059	127717060
chr15	77956013	77956014		chr7	127876800	127876801
chr15	78947969	78947970		chr7	12803710	12803711
chr15	79075379	79075380		chr7	128418555	128418556
chr15	79119615	79119616		chr7	130692528	130692529
chr15	79131755	79131756		chr7	139582787	139582788
chr15	80288243	80288244		chr7	141464900	141464901
chr15	81235690	81235691		chr7	142082370	142082371
chr15	81235827	81235828		chr7	24036799	24036800
chr15	83381861	83381862		chr7	24082170	24082171
chr15	84681535	84681536		chr7	24884713	24884714
chr15	96736441	96736442		chr7	28544242	28544243
chr15	98092927	98092928		chr7	28986689	28986690
chr15	98092938	98092939		chr7	29621815	29621816
chr15	98092940	98092941		chr7	30515429	30515430
chr15	98504957	98504958		chr7	34753199	34753200
chr15	99030262	99030263		chr7	35535978	35535979
chr15	99055631	99055632		chr7	44086473	44086474
chr15	99420867	99420868		chr7	44587371	44587372
chr15	99506138	99506139		chr7	46002661	46002662
chr16	17646885	17646886		chr7	4922823	4922824
chr16	17722787	17722788		chr7	4922829	4922830
chr16	21246296	21246297		chr7	5080225	5080226
chr16	28929150	28929151		chr7	6383113	6383114
chr16	36530881	36530882		chr7	64392661	64392662
chr16	43979789	43979790		chr7	65693418	65693419
chr16	55054573	55054574		chr7	65693421	65693422
chr16	55895081	55895082		chr7	66110071	66110072
chr16	55974630	55974631		chr7	68318655	68318656
chr16	57391451	57391452		chr7	68350229	68350230
chr16	8783711	8783712		chr7	79392138	79392139
chr16	91830393	91830394		chr7	79535946	79535947
chr17	12507390	12507391		chr7	79535954	79535955
chr17	17005020	17005021		chr7	79660651	79660652

chr17	23605792	23605793		chr7	80326200	80326201
chr17	25222645	25222646		chr7	80876110	80876111
chr17	26716019	26716020		chr7	86249898	86249899
chr17	27436074	27436075		chr7	96210416	96210417
chr17	28052226	28052227		chr7	99353092	99353093
chr17	30247950	30247951		chr7	99468358	99468359
chr17	31381523	31381524		chr7	99468383	99468384
chr17	31855698	31855699		chr7	99468467	99468468
chr17	32113756	32113757		chr7	99468480	99468481
chr17	36231467	36231468		chr7	99643376	99643377
chr17	39843859	39843860		chr8	105686165	105686166
chr17	39843860	39843861		chr8	105965686	105965687
chr17	39843873	39843874		chr8	109337853	109337854
chr17	39843878	39843879		chr8	117256555	117256556
chr17	39844955	39844956		chr8	117537157	117537158
chr17	39845245	39845246		chr8	119446806	119446807
chr17	39845252	39845253		chr8	119558641	119558642
chr17	39845253	39845254		chr8	120229794	120229795
chr17	39845260	39845261		chr8	12757232	12757233
chr17	39845942	39845943		chr8	13159250	13159251
chr17	39848432	39848433		chr8	28200305	28200306
chr17	39848437	39848438		chr8	31168730	31168731
chr17	42876402	42876403		chr8	3588148	3588149
chr17	45549465	45549466		chr8	46636208	46636209
chr17	45549487	45549488		chr8	47674176	47674177
chr17	46628944	46628945		chr8	50016595	50016596
chr17	46858633	46858634		chr8	62825273	62825274
chr17	47705378	47705379		chr8	70166120	70166121
chr17	47792713	47792714		chr8	71463332	71463333
chr17	56510513	56510514		chr8	78820899	78820900
chr17	67354140	67354141		chr8	83417756	83417757
chr17	69439166	69439167		chr8	83608759	83608760
chr17	70809039	70809040		chr8	84638972	84638973
chr17	75292829	75292830		chr8	84702812	84702813
chr17	80303690	80303691		chr8	84786520	84786521
chr17	80373160	80373161		chr8	84893455	84893456
chr17	83223791	83223792		chr8	87472861	87472862
chr17	83706082	83706083		chr8	88361730	88361731
chr17	84364908	84364909		chr8	91803115	91803116

chr17	85691967	85691968		chr8	94012621	94012622
chr18	10725753	10725754		chr8	94012626	94012627
chr18	10725756	10725757		chr8	94447353	94447354
chr18	11840162	11840163		chr8	94548379	94548380
chr18	12236630	12236631		chr8	95142471	95142472
chr18	12941700	12941701		chr9	101046068	101046069
chr18	20896143	20896144		chr9	107551698	107551699
chr18	21002289	21002290		chr9	107551734	107551735
chr18	21002305	21002306		chr9	111439305	111439306
chr18	24205712	24205713		chr9	122233568	122233569
chr18	24603316	24603317		chr9	14276183	14276184
chr18	24603345	24603346		chr9	20898313	20898314
chr18	24708763	24708764		chr9	20976776	20976777
chr18	24709394	24709395		chr9	31030991	31030992
chr18	31702247	31702248		chr9	36917173	36917174
chr18	35652722	35652723		chr9	44084747	44084748
chr18	35702749	35702750		chr9	44084775	44084776
chr18	35858538	35858539		chr9	44084825	44084826
chr18	35930265	35930266		chr9	45299380	45299381
chr18	36753560	36753561		chr9	46329046	46329047
chr18	37748291	37748292		chr9	48985146	48985147
chr18	37955846	37955847		chr9	51103333	51103334
chr18	38186147	38186148		chr9	56041974	56041975
chr18	70568977	70568978		chr9	57261624	57261625
chr18	80986798	80986799		chr9	59486050	59486051
chr18	84851399	84851400		chr9	60172789	60172790
chr18	88894035	88894036		chr9	63953730	63953731
chr18	89769452	89769453		chr9	65346626	65346627
chr19	10041378	10041379		chr9	65909402	65909403
chr19	10240643	10240644		chr9	68268229	68268230
chr19	16133309	16133310		chr9	69453662	69453663
chr19	23141532	23141533		chr9	69453784	69453785
chr19	24477158	24477159		chr9	72858842	72858843
chr19	36057284	36057285		chr9	85919008	85919009
chr19	36349259	36349260		chr9	87610544	87610545
chr19	43601105	43601106		chr9	95407002	95407003
chr19	43753277	43753278		chrX	102157068	102157069
chr19	43879741	43879742		chrX	102966911	102966912
chr19	4439561	4439562		chrX	103322419	103322420

chr19	4439565	4439566		chrX	114656674	114656675
chr19	45036276	45036277		chrX	12000959	12000960
chr19	45329601	45329602		chrX	121055679	121055680
chr19	45815824	45815825		chrX	12128745	12128746
chr19	46573855	46573856		chrX	12158332	12158333
chr19	46620552	46620553		chrX	124123284	124123285
chr19	47253404	47253405		chrX	134601343	134601344
chr19	5051204	5051205		chrX	139610897	139610898
chr19	53404872	53404873		chrX	140600455	140600456
chr19	53529676	53529677		chrX	150588067	150588068
chr19	5437350	5437351		chrX	154937719	154937720
chr19	56293985	56293986		chrX	155623036	155623037
chr19	56909032	56909033		chrX	164484840	164484841
chr19	60790600	60790601		chrX	20548356	20548357
chr19	6987434	6987435		chrX	23692953	23692954
chr2	104712365	104712366		chrX	24345972	24345973
chr2	105017193	105017194		chrX	38121152	38121153
chr2	105182331	105182332		chrX	42149737	42149738
chr2	118728456	118728457		chrX	47913013	47913014
chr2	122302014	122302015		chrX	48344004	48344005
chr2	131171357	131171358		chrX	56732100	56732101
chr2	13491841	13491842		chrX	57503519	57503520
chr2	13793519	13793520		chrX	71557185	71557186
chr2	145032739	145032740		chrX	74393141	74393142
chr2	147178790	147178791		chrX	75514618	75514619
chr2	150470230	150470231		chrX	75673388	75673389
chr2	153814593	153814594		chrX	7810391	7810392
chr2	155605328	155605329		chrX	7841960	7841961
chr2	160733370	160733371		chrX	78716610	78716611
chr2	163658647	163658648		chrX	7878266	7878267
chr2	166620078	166620079		chrX	7922420	7922421
chr2	167189840	167189841		chrX	8252860	8252861
chr2	167503669	167503670		chrX	9272963	9272964
chr2	168206546	168206547		chrX	95967786	95967787
chr2	168206552	168206553		chrX	99152835	99152836
chr2	168206562	168206563		chrX	99586611	99586612
chr2	168206564	168206565				

Supplementary Table 4

AAV vectors used in this study

Vector	qPCR Primer for measuring titer	Source
pAAV-TRE-Oct4	TRE3G	This paper
pAAV-TRE-Sox2	TRE3G	This paper
pAAV-TRE-Klf4	TRE3G	This paper
pAAV-TRE-Oct4-Sox2	TRE3G	This paper
pAAV-TRE-OSK	TRE3G	This paper
pAAV-TRE-d2EGFP	TRE3G	This paper
pAAV-TRE-Luc	TRE3G	This paper
pAAV-CMV-rtTAV16	WPRE	This paper
pAAV-UBC-rtTA4	WPRE	This paper
pAAV-CAG-tTA	hGH	This paper
pAAV-sh-Scr-YFP	WPRE	Addgene #85741
pAAV-Sh-Tet1-YFP	WPRE	Addgene #85742
pAAV-sh-Tet2-YFP	WPRE	Addgene #85743
pAAV-EF1a-HA-hTet1CD-WPRE-PolyA	WPRE	Addgene #39454
pAAV-EF1a-HA-hTet1CDmu-WPRE-PolyA	WPRE	Addgene #39455
pAAV-CAG-FLEX2-tTA2-WPRE-bGHpA	WPRE	Addgene #65458
pAAV-sh-Scr-H2BGFP	hGH	Weng et al., 2017, Neuron
pAAV-sh-TDG-H2BGFP	hGH	Weng et al., 2017, Neuron

Primer name	Sequence
TRE3G F	AACGTATCTACAGTTTACTCCCTATC
TRE3G R	GGTAGGAAGTGGTACGGAAAG
WPRE F	CACTGACAATTCCGTGGTGT
WPRE R	GAGATCCGACTCGTCTGAGG
hGH F	TGGGAAGACAACCTGTAGGG
hGH R	TGAAACCCCGTCTCTACCAA

Supplementary Table 5

Batches of RGC samples used for DNA methylation analyses

Batch 1	Treatment
6 mo	6-month-old
18 mo	18-month-old
Batch 2	Treatment
1 mo	1-month-old 18 days
12 mo	12-month-old
30 mo	30-month-old
1 mo GFP	AAV2-tTA;TRE-d2EGFP infection for 18 days from 1-month-old
1 mo GFP_inj	AAV2-tTA;TRE-d2EGFP infection for 18 days (4 day post nerve crush) from 1-month-old
1 mo OSK_inj	AAV2-tTA;TRE-OSK infection for 18 days (4 day post nerve crush) from 1-month-old
12 mo GFP	AAV2-tTA;TRE-d2EGFP infection for 4 weeks from 11-month-old
12 mo OSK	AAV2-tTA;TRE-OSK infection for 4 weeks from 11-month-old
Batch 3	Treatment
12 mo OSKshScr	AAV2-tTA;TRE-OSK;sh-Scr infection for 4 weeks from 11-month-old
12 mo OSKshTet1	AAV2-tTA;TRE-OSK;sh-Tet1 infection for 4 weeks (Tet1 knockdown) from 11-month-old
12 mo OSKshTet2	AAV2-tTA;TRE-OSK;sh-Tet2 infection for 4 weeks (Tet2 knockdown) from 11-month-old

Note: Batch 3 was not used for the development of DNAm ageing signature due to the lack of intact ageing samples to normalize batch effect.

Supplementary Table 6

Metadata for Supplementary Data

SampleID	Type
10	6 mo
11	6 mo
12	6 mo
13	6 mo
14	6 mo
15	6 mo
16	6 mo
17	18 mo
18	18 mo
19	18 mo
20	18 mo
21	18 mo
22	18 mo
23	18 mo
24	18 mo
25	30 mo
26	30 mo
27	30 mo
28	30 mo
29	30 mo
3	1 mo
30	30 mo
33	1 mo GFP
34	1 mo GFP_inj
35	1 mo GFP_inj
36	1 mo GFP
37	1 mo GFP
38	1 mo GFP_inj
39	1 mo GFP_inj
4	1 mo
40	1 mo GFP_inj
41	1 mo GFP_inj
42	1 mo GFP_inj

43	1 mo GFP_inj
48	1 mo OSK_inj
49	1 mo OSK_inj
5	1 mo
50	1 mo OSK_inj
51	1 mo OSK_inj
6	1 mo
64	12 mo
65	12 mo
67	12 mo GFP
68	12 mo GFP
69	12 mo GFP
7	1 mo
70	12 mo GFP
71	12 mo GFP
72	12 mo GFP
73	12 mo OSK
74	12 mo OSK
76	12 mo OSK
77	12 mo OSK
78	12 mo OSK
8	1 mo
9	6 mo
31	1 mo GFP
44	1 mo OSK_inj
45	1 mo OSK_inj
46	1 mo OSK_inj
47	1 mo OSK_inj

Supplementary Table 7

Primers used for RT-PCR

Gene	Primer sequence
mOct4 F	ACA TCG CCA ATC AGC TTG G
mOct4 R	AGA ACC ATA CTC GAA CCA CAT CC
mSox2 F	ACA GAT GCA ACC GAT GCA CC
mSox2 R	TGG AGT TGT ACT GCA GGG CG
mKlf4 F	GTGCCCCGACTAACCGTTG
mKlf4 R	GTCGTTGAACTCCTCGGTCT
mMyc F	ATGCCCCTCAACGTGAACTTC
mMyc R	CGCAACATAGGATGGAGAGCA
mHist1 h2a F	GCG ACA ACA AGA AGA CGC GCA T
mHist1 h2a R	CTG GAT GTT GGG CAG GAC GCC
mHist1 h2b F	AAG AAG GAC GGC AAG AAG CGC A
mHist1 h2b R	CGC TCG AAG ATG TCG TTC ACG A
mHIST1 H3.1/H3.2 F	GAA GAA GCC TCA CCG CTA CCG
mHIST1 H3.1/H3.2 R	GGT TGG TGT CCT CAA ACA GAC CC
mHist1 h4 F	AAC ATC CAG GGC ATC ACC AAG C
mHist1 h4 R	GTT CTC CAG GAA CAC CTT CAG C
mLmnb1 F	CCG GCC TCA AGG CTC TCT A
mLmnb1 R	TGC CGC CTC ATA CTC TCG AA
mActb F	AGT GTG ACG TTG ACA TCC GT
mActb R	TGC TAG GAG CCA GAG CAG TA
mNanog F	TCTTCCTGGTCCCCACAGTTT
mNanog R	GCAAGAATAGTTCTCGGGATGAA
mChaf1a R	GTG TCT TCC TCA ACT TTC TCC TTG G
mChaf1a F	CGC GGA CAG CCG CGG CCG TGG ATT GC
mChaf1b R	GGC TCC TTG CTG TCA TTC ATC TTC CAC
mChaf1b F	CAC CGC CGT CAG GAT CTG GAA GTT GG
mLmnb1 F	CCG GCC TCA AGG CTC TCT A
mLmnb1 R	TGC CGC CTC ATA CTC TCG AA
mp16 (Cdkn2a) F	ACA TCA AGA CAT CGT GCG ATA TT
mp16 (Cdkn2a) R	CCA GCG GTA CAC AAA GAC CA
mApob F	AAG CAC CTC CGA AAG TAC GTG
mApob R	CTC CAG CTC TAC CTT ACA GTT GA
hTet2 F	GATAGAACCAACCATGTTGAGGG

hTet2 R	TGGAGCTTTGTAGCCAGAGGT
hActb F	CACCATTGGCAATGAGCGGTTC
hActb R	AGGTCTTTGCGGATGTCCACGT
mTet1 F	TCAAGCAATGGACCACTGGG
mTet1 R	TCTCCATGAGCTCCCTGACA
mTet2 F	ACT CCT GGT GAA CAA AGT CAG A
mTet2 R	CAT CCC TGA GAG CTC TTG CC
mTet3-F	CCGGATTGAGAAGGTCATCTAC
mTet3-R	AAGATAACAATCACGGCGTTCT
mGAPDH F	CCA ATG TGT CCG TCG TGG ATC T
mGAPDH R	GTT GAA GTC GCA GGA GAC AAC C
mStat3-F	CAATACCATTGACCTGCCGAT
mStat3-R	GAGCGACTCAAACTGCCCT
mKlf6-F	GTTTCTGCTCGGACTCCTGAT
mKlf6-R	TTCCTGGAAGATGCTACACATTG
Efemp1-F	GCGCTGGTCAAGTCACAGTA
Efemp1-R	AAGCATCTGGGACAATGTCAC