Supplemental Table 1.

Expression level of genes involved in response to oxidative stress in response to GAA and/or H_2O_2 treatment.

Supplemental Table 2.

Primers used for qRT-PCR.

Supplemental Figure legend

Luciferase reporter assay

ARPE-19 cells were transfected with the pCMV β-galactoside reporter plasmid (0.1 μg) and a luciferase reporter plasmid (0.1 μg) using Lipofectamine LTX (Life Technologies) transfection reagent. At 24 h post‐transfection, cells were treated with GAA for 24 hours, and then with/without H_2O_2 for 30 minutes, before harvest for luciferase assays using Luciferase Assay System (Promega). Luciferase activity was normalized by β-galactosidase activity in the same assay.

Supplemental Fig. 1

Ability of GAA to directly scavenge ROS. GAA (5μM) was added to the medium at the time of inducing oxidative stress with 300μM H_2O_2. Cell survival was measured 24h later with MTT.

Supplemental Fig. 2

p21-luciferase assay in RPE cells upon H_2O_2 and/or GAA treatment.

Supplemental Fig. 3

Expression of FoxO1, FoxO3 and FoxO4 gene expression in RPE cells after normalized to level of Cyclophilin A gene.

Supplemental Fig. 4

(A) FoxO1 downregulation analyzed by measuring mRNA level with qRT-PCR. (B) FoxO1 downregulation measured by Western Blot using 25μg of total protein extract. (C) *FoxO1* knockdown did not affect *SESN2* upregulation by pretreatment with 5μM GAA measured by qRT-PCR. (D) *FoxO1* knockdown reduced RPE cell viability at the baseline and abolished the ability of 5μM GAA to protect RPE cells from H_2O_2-induced necrosis. (E) FoxO4 knockdown measured by mRNA level with qRT-PCR. (F) *FoxO4* downregulation reduced *SESN2* upregulation induced by pretreatment with 5μM GAA measured by qRT-PCR. (G) *FoxO4* downregulation reduced RPE cell viability at the base line and decreased the ability of 5μM GAA pretreatment to protect RPE cells from 300μM H2O2-induced necrosis.

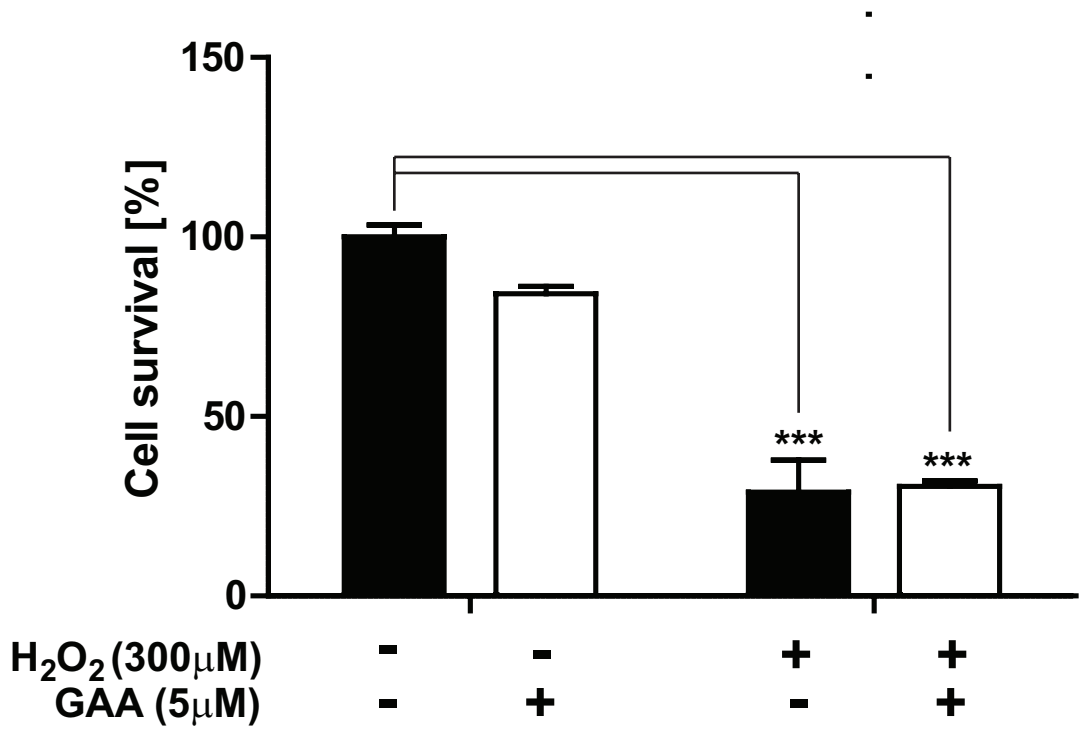
Supplemental Table 1

	control		5μM GAA		300μM H_2O_2		5μM GAA 300μM H_2O_2	
		Expression SEM	*Expression*	*Expression SEM*	*Expression*	*Expression SEM*	*Expression*	*Expression SEM*
ALOX	1.00	*0.31*	1.56	*0.27*	0.24	*0.30*	2.19	*0.43*
CAT	1.00	*0.09*	0.98	*0.13*	1.00	*0.11*	1.23	*0.19*
GCLC	1.00	*0.15*	0.67	*0.11*	0.67	*0.08*	0.67	0.09
GPX1	1.00	*0.08*	1.18	*0.23*	1.09	*0.20*	1.50	0.29
GPX3	1.00	*0.11*	1.16	*0.21*	1.41	*0.20*	1.72	0.35
GSTA2	1.00	*0.41*	0.00	*0.00*	0.96	*0.21*	0.53	0.12
HMOX-1	1.00	*0.10*	1.88	*0.25*	1.28	*0.17*	1.84	0.31
NQO-1	1.00	*0.09*	0.93	*0.12*	1.26	*0.15*	1.10	0.17
SESN2	1.00	*0.19*	1.77	*0.46*	1.34	*0.20*	4.07	0.90

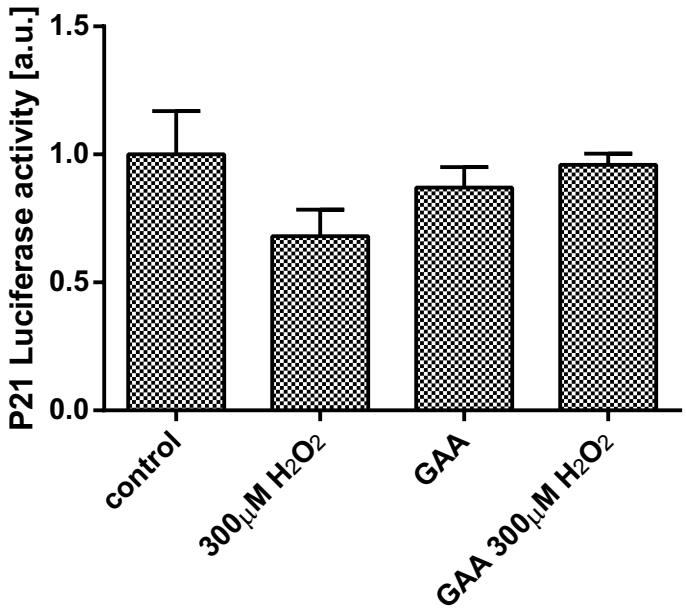
Supplemental Table 2

GeneID	Description	Forward Primer	Revers Primer
ALOX5	Arachidonate 5-lipoxygenase	CCTCCCCT TTTCTTGCCTAC	ACCTCAGCCATCAAGTGTCC
CAT	Catalase	CGTGCTGAATGAGGAACAGA	AGTCAGGGTGGACCTCAGTG
FOXO1	Forkhead box O1	TGGAGTACATTTCGCCCTCG	AGTAGAGGCCATCTTTGCGG
FOXO3	Forkhead box O3	TGTGTCTGCCCAGAATTCCC	CTGGTGGTGGAGCAAGTTCT
FOXO4	Forkhead box O4	CCCACCCTCAATGAAGGTCT	TGCTGTAGGTGTGTAAGGGG
FTH	Ferritin, heavy polypeptide 1	ATGAGCAGGTGAAAGCCATC	CAGGGTGTGCTTGTCAAAGA
FTL	Ferritin, light polypeptide	AGGCCCTTTTGGATCTTCAT	CAGGTGGTCACCCATCTTCT
GCLC	Glutamate-cysteine ligase, catalytic subunit	GTGGATGTGGACACCAGATG	GCGATAAACTCCCTCATCCA
GPX1	Glutathione peroxidase 1	CCAAGCTCATCACCTGGTCT	TCGATGTCAATGGTCTGGAA
GPX3	Glutathione peroxidase 3	ATGCTGGCAAATACGTCCTC	AGAATGACCAGACCGAATGG
GSTA2	Glutathione S-transferase alpha 2	CTCCAATATACGGGGCAGAA	GCACTTGCTGGAACATCAAA
HMOX-1	Heme oxygenase 1	AACTTTCAGAAGGGCCAGGT	GTAGACAGGGGCGAAGACTG
NQO1	NAD(P)H dehydrogenase, quinone 1	GCACTGATCGTACTGGCTCA	CATGGCATAGAGGTCCGACT
NRF2	Nf-E2 related factor 2	CCTGGGAACAGAACAGGAAA	TTCTGTGCAACCAGTTCAGG
SESN1	Sestrin 1	ATTCGGCTGTGGAATCAGTC	TCCACACTGTGATTGCCATT
SESN2	Sestrin 2	GCTCTCCTCCTTCGTGTTTG	GGGCTGCTCTGTTCACTAGG
SESN3	Sestrin 3	ATGCTTTGGCAAGCTTTGTT	GCAAGATCACAAACGCAGAA

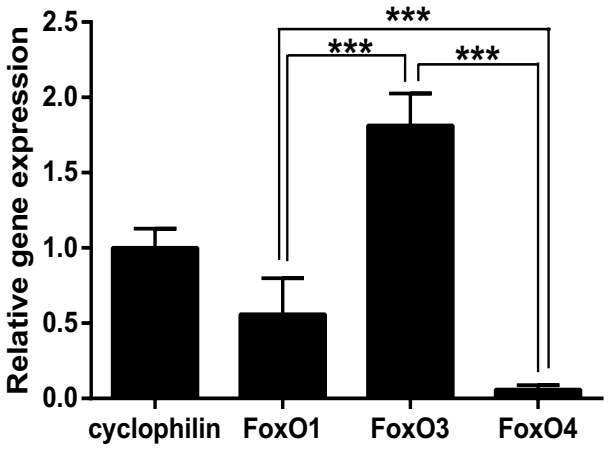
Supplemental Fig. 1



Supplemental Fig. 2



Supplemental Fig. 3



Supplemental Fig. 4