Supplementary material

Mutation	Protein change	Primers
c.677A>G	p.Y226C	5'-cggggtcaccacctgcgcagtgcac-3′ 5'-gtgcactgcgcaggtggtgaccccg-3'
c.325G>C	p.A109P	5'-ctatccgagcctttcctgagggctttctgg-3' 5'-ccagaaagccctcaggaaaggctcggatag-3'
c.38C>A	p.S13*	5'-gctcacctccttcttctagttcctgtatatggtag-3' 5'-ctaccatatacaggaactagaagaaggaggtgagc-3'

Table S1: Primers used for site directed mutagenesis. The mutated base pair is shown in red.

Gene	Forward primer	Reverse primer	Reference
Human			
ATF4	TCAAACCTCATGGGTTCTCC	GTGTCATCCAACGTGGTCAG	
CHOP	GACCTGCAAGAGGTCCTGTC	TGTGACCTCTGCTGGTTCTG	
sXBP1	CTGAGTCCGAATCAGGTGCAG	ATCCATGGGGAGATGTTCTGG	
GAPDH	ACAGTTGCCATGTAGACC	TTTTTGGTTGAGCACAGG	
NRF2	CAACTACTCCCAGGTTGCCC	AAGTGACTGAAACGTAGCCGA	
SOD2	GCTGGAAGCCATCAAACGTG	GCAGTGGAATAAGGCCTGTTG	
CAT	CTCCGGAACAACAGCCTTCT	GAATGCCCGCACCTGAGTAA	
GPX1	CCGGGACTACACCCAGATGA	TCTTGGCGTTCTCCTGATGC	
HO-1	CTCCTCTCGAGCGTCCTCAG	AAATCCTGGGGCATGCTGTC	
Zebrafish			
sod1	GGGCCAACCGATAGTGTGAG	TCATCCTCCTTCTCATGAATCACC	
sod2	CAAGGGACCACAGGTCTCATC	TGGAAACGCTCGCTGACATT	
cat	AGGGCAACTGGGATCTTACA	TTTATGGGACCAGACCTTG	42
gpx1	TGAGGCACAACAGTCAGGGA	CATTCTTGCAGTTCTCCTGGTGC	
hmox1a	CACAGAGACTGAGAGAGATTGGC	TGCCCACTCCTAATGCGAAC	
nrf2	ATGTCTAAAATGCAGCCAAGCC	CGGTAGCTGAAGTCGAACAC	43
atg5	CCCTACTATCTGCTCCTCCCAC	GGAGGTCGAACAACACACCA	
atg7	AGAGTCCAGTCCGATGTC	GAAGTAACAGCCGAGCG	
atg12	CCAGTTCATCTCACGCTTCCTC	TGCCGTCACTTCCGAAACAC	
p62	TTTGGCTCTTGTGAAGGATGAC	GAGGGCTAAAGTGAGGTGTAGTGA	
edem	CGTTTCGGTGCTCTTCCTGAG	GCGTAACCACACCTCACTTTG	44
bip	CAAGAAGAAGACGGGCAAAG	CTCCTCAAACTTGGCTCTGG	44
atf4	TGAGCACACTGAGGTTCCAG	GTCTTCACTCGGCCTTTGAG	44
atf6	TGATGAGGCACTGTCTCCAG	ATGGGTCTTTTTGCTGGTTG	44
chop	ATATACTGGGCTCCGACACG	GATGAGGTGTTCTCCGTGGT	44
xbp1-s	TGTTGCGAGACAAGACGA	CCTGCACCTGCTGCGGACT	45
actin	CGAGCTGTCTTCCCATCCA	TCACCAACGTAGCTGTCTTTCTG	

Table S2: RT-qPCR primer sequences.

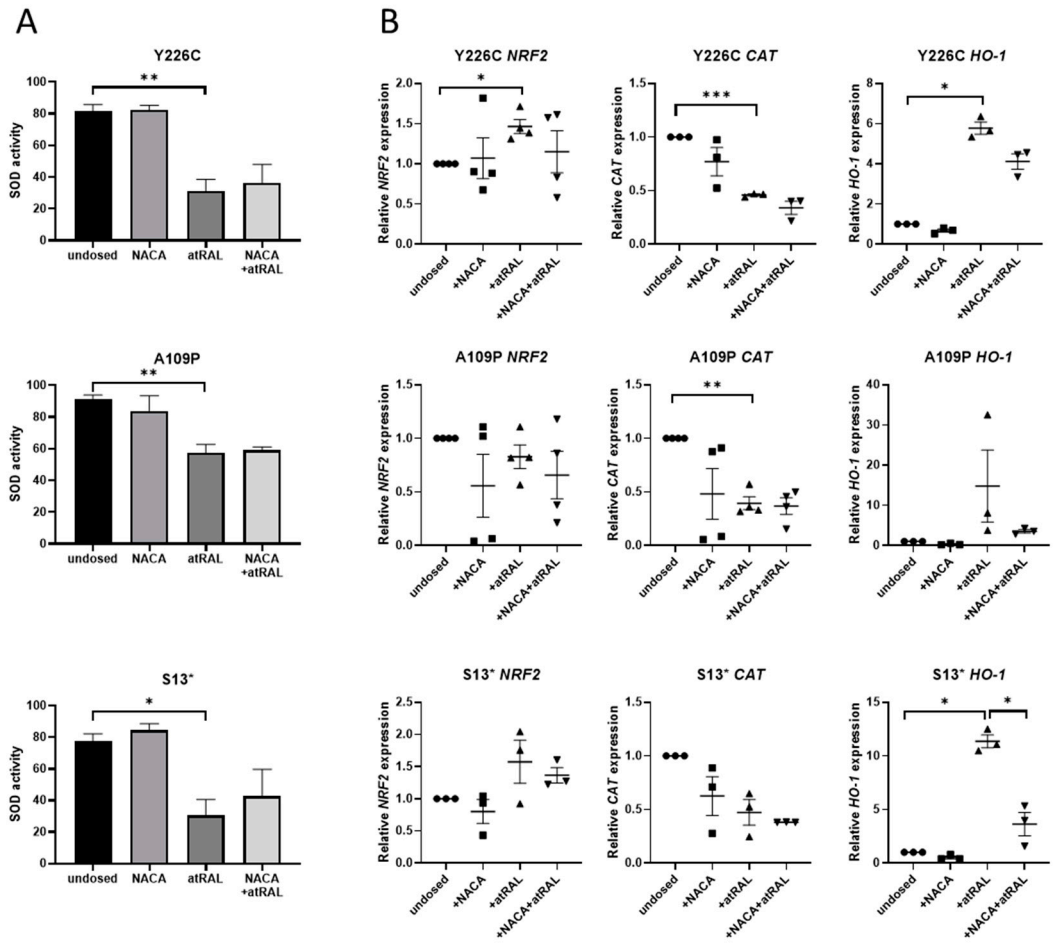

Figure S1: NACA does not attenuate atRAL induced oxidative stress. Cells were dosed with 50 μM atRAL, 750 μM NACA or both for 24 hours, then analysed by **(A)** SOD activity assay and **(B)** RT-qPCR of oxidative stress markers *NRF2*, *CAT* and *HO-1*. NACA reduced atRAL induced expression of HO-1 in the p.S13* line. Statistical significance was analysed using one-way ANOVA and Sidak's multiple comparison test. *p ≤0.05, **p ≤ 0.01, ***p ≤ 0.001.

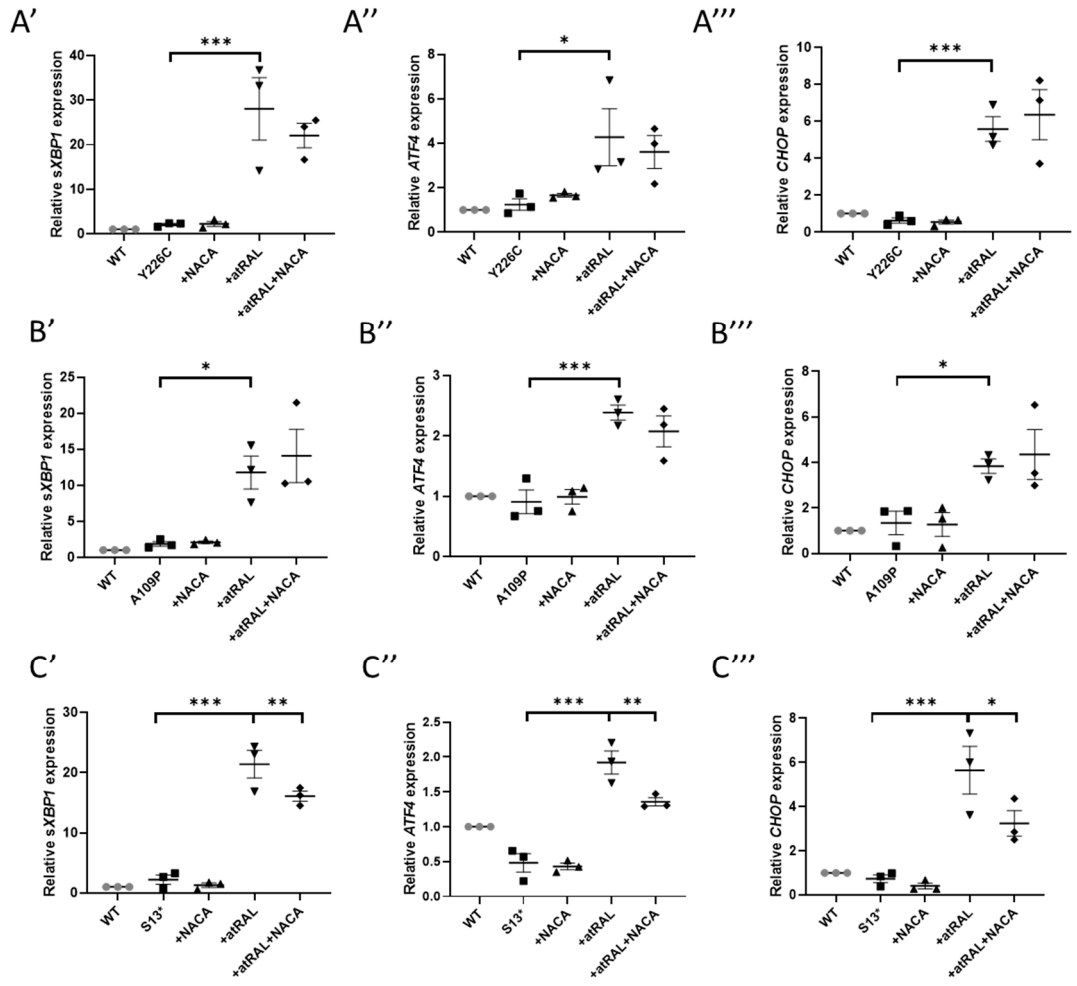

Figure S2: NACA reduces atRAL induced ER stress in the p.S13* cell line. Cells were dosed with 50 μM atRAL, 750 μM NACA or both for 24 hours. RT-qPCR was performed analysing the expression of ER stress markers. NACA did not significantly reduce expression of ER stress markers in p.Y226C or p.A109P cell lines. NACA significantly reduces expression of *XBP1*, *CHOP* and *ATF4* in p.S13* cell line. Statistical significance was analysed using one-way ANOVA and Sidak's multiple comparison test. *p ≤0.05, **p ≤ 0.01, ***p ≤ 0.001.

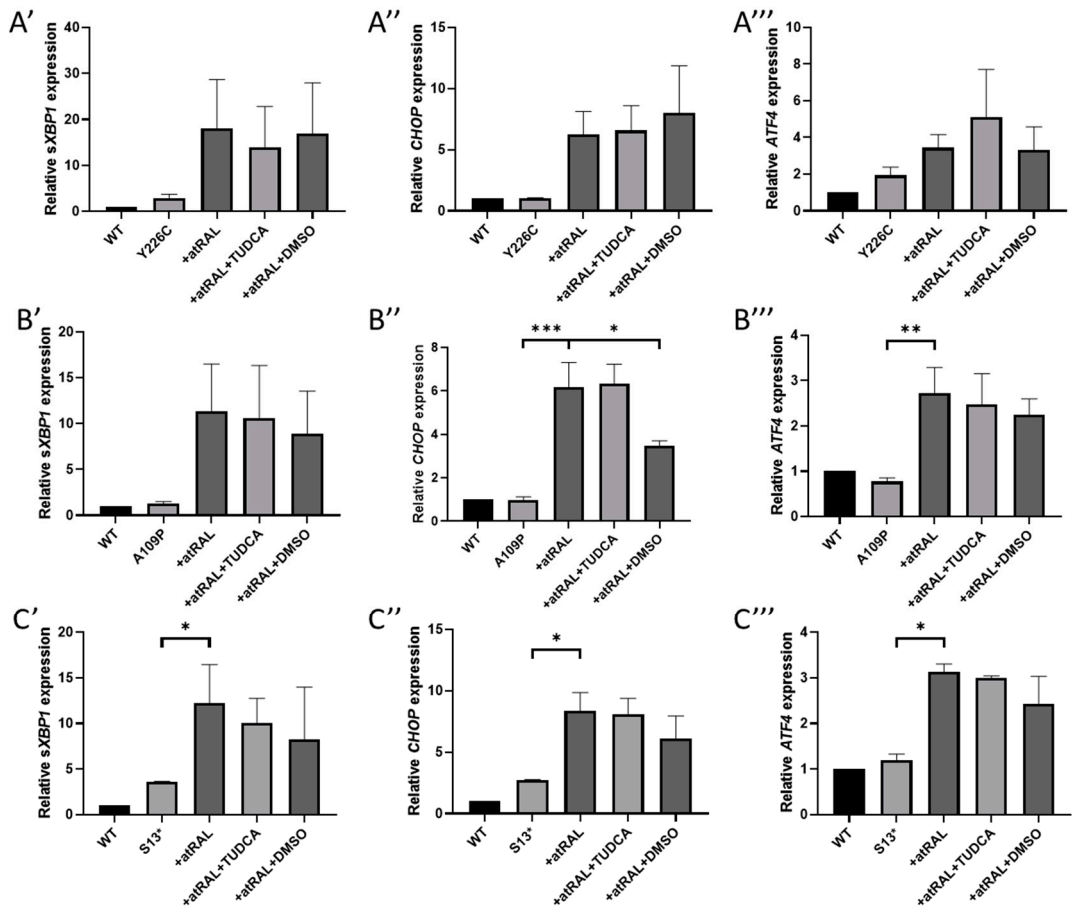

Figure S3: TUDCA does not reduce atRAL induced ER stress. TUDCA was prepared at a stock concentration of 20 mM in DMSO, and further diluted in culture media. Cells were dosed with 50 μM atRAL alone or together with 100 μM TUDCA or 0.5% DMSO for 24 hours. Expression of ER stress markers was analysed by RT-qPCR. Data are expressed as mean ± SEM. Statistical significance was analysed using one-way ANOVA and Sidak's multiple comparison test. *p ≤0.05, **p ≤ 0.01, ***p ≤ 0.001.

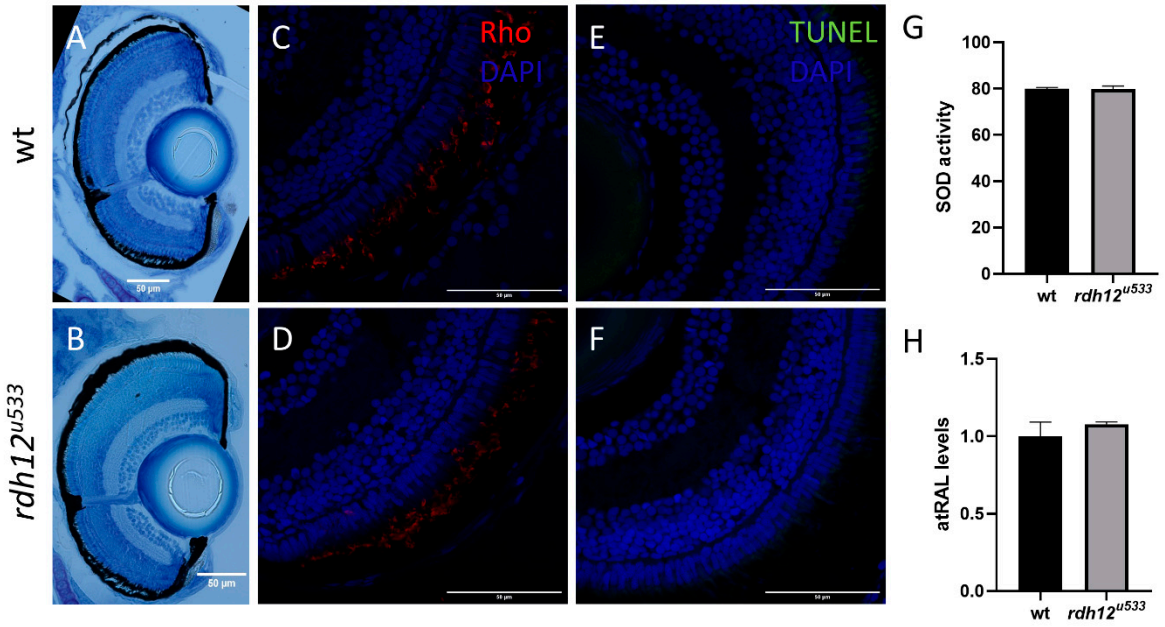

Figure S4: Characterisation of *rdh12*^{u533} zebrafish at 5 dpf. Retinal sections were stained with toluidine blue to assess retinal structure (A, B). Immunohistochemistry staining was used to detect rhodopsin (C, D). TUNEL assay was performed to detect cell death (E, F). Scale bar = 50 µM. (G) SOD activity was measured in wt and rdh12 fish at 5 dpf. (H) atRAL levels in wt and *rdh12*^{u533} fish were analysed using HPLC. No differences between wt and *rdh12*^{u533} fish were detected at 5 dpf.

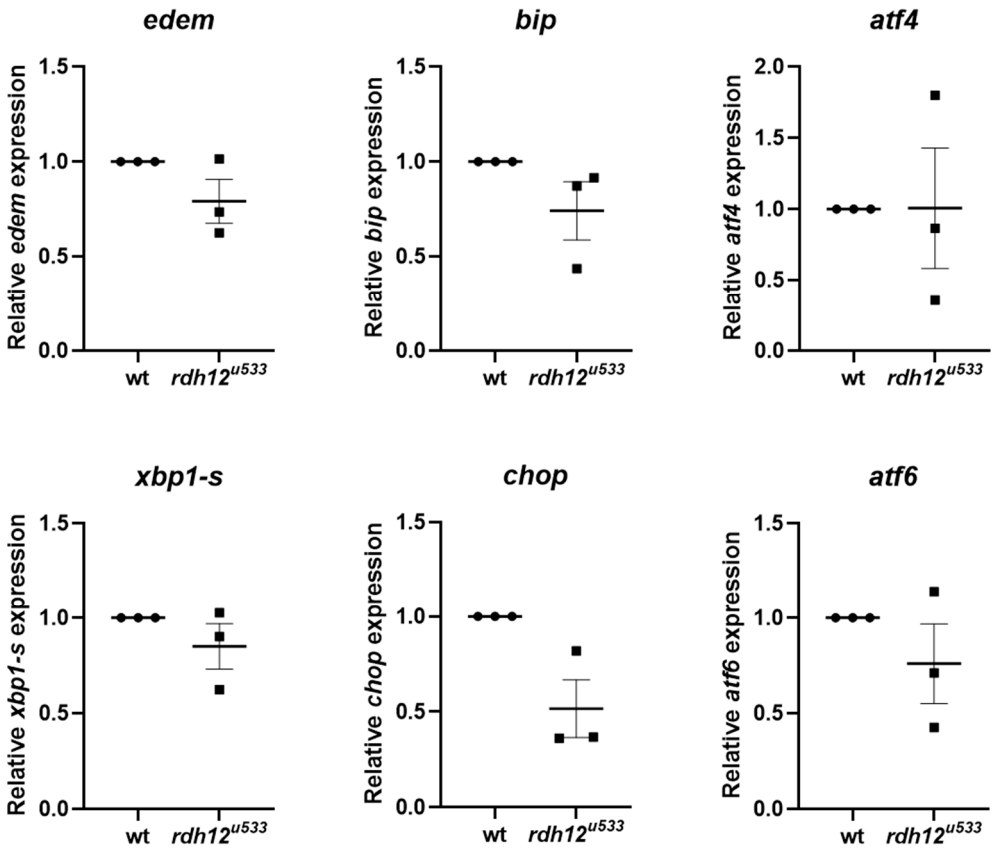

Figure S5: ER stress is not disrupted in *rdh12*^{u533} mutant fish. Expression of ER stress markers were analysed by RT-qPCR in 12 mpf retinas. No significant differences in expression between wt and *rdh12*^{u533} fish was seen. Data are expressed as mean ± SEM.

References

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