Table1. Sequences of human primers used for RT–PCR

Gene	Forward sequence(5'-3')	Reverse sequence(5'-3')
GAPDH	ATGTTCGTCATGGGTGTGAA	GGTGCTAAGCAGTTGGTGGT
NLRP3	CGTGAGTCCCATTAAGATGGAGT	CCCGACAGTGGATATAGAACAGA
IL17A	TGTCACTGCTACTGCTGCTGAG	GGTGAGGTGGATCGGTTGTAGT
TNF-α	CTCAGCAAGGACAGCAGAGGAC	TGGAGCCGTGGGTCAGTATGT
Beclin1	TGTGTTGCTGCTCCATGCTCTG	GCCACTGCCTCCTGTGTCTTCA
P62	GAGCCTCATCTCCTCGGTGT	TTCTCAAGCCCCATGTTGCAC

Table2. Sequences of mouse primers used for RT–PCR

Gene	Forward sequence(5'-3')	Reverse sequence(5'-3')
GAPDH	TGTCCGTCGTGGATCTGAC	CCTGCTTCACCACCTTCTTG
ATG5	TGTGCTTCGAGATGTGTGGTT	GTCAAATAGCTGACTCTTGGCAA
Beclin1	TAAGGCGTCCAGCAGCACCAT	AGAGACACCATCCTGGCGAGTT
P62	CCAGCACAGGCACAGAAGACAA	CCCACCGACTCCAAGGCTATCT

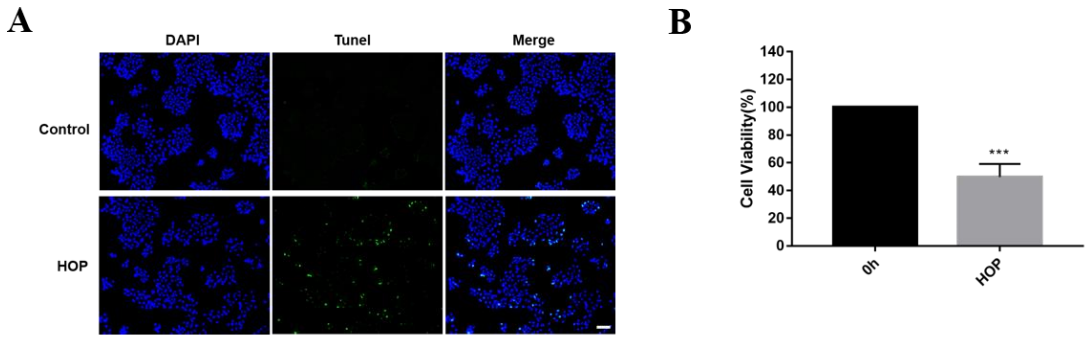

Figure S1. Hyperosmolarity induced cellular apoptosis and death in HCECs. (A) Tunel staining images in HCECs exposed to normal or hyperosmotic medium. Bar=50μm**. (B)** Changes to cell viability of HCECs by CCK8 assay. The results are shown as mean ± SD of three independent experiments. ***P < 0.001.

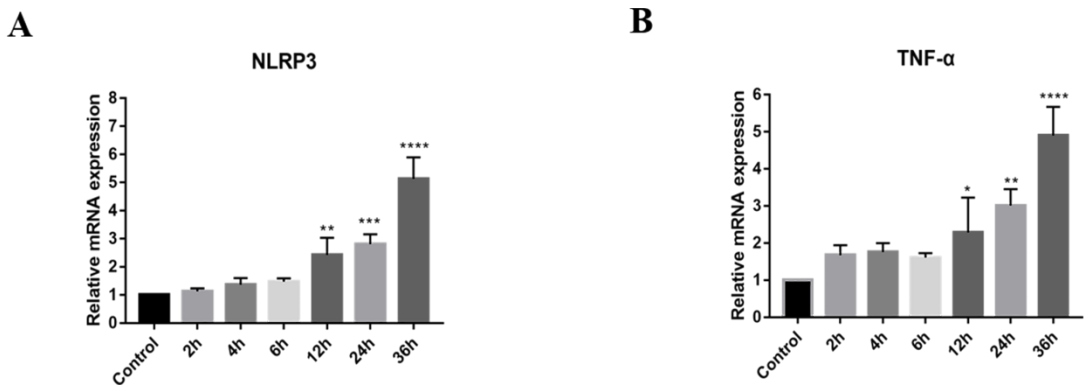

Figure S2. Hyperosmolarity stimulated inflammatory factors in HCECs. (A) Gene expression results of NLRP3. **(B)** Gene expression of TNFα. The results are shown as mean ± SD of three independent experiments. *P < 0.05, ** P < 0.01, ***P < 0.001, ****P < 0.0001.

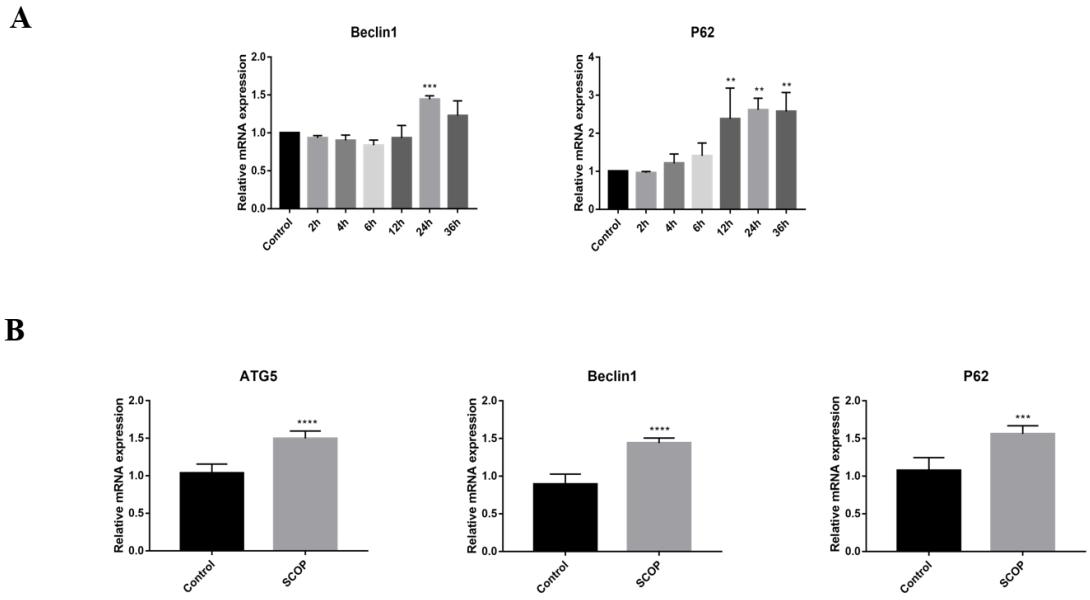

Figure S3. Expression of autophagy genes in dry eye. (A) RT-PCR results showing Beclin1 and P62 expression in untreated and HOP-stressed HCECs for indicated times (N=3). **(B)** Gene expression of ATG5, Beclin1 and P62 in normal and dry eye mice (N=5). The results are shown as mean ± SD. ** P < 0.01, ***P < 0.001, ****P < 0.0001.

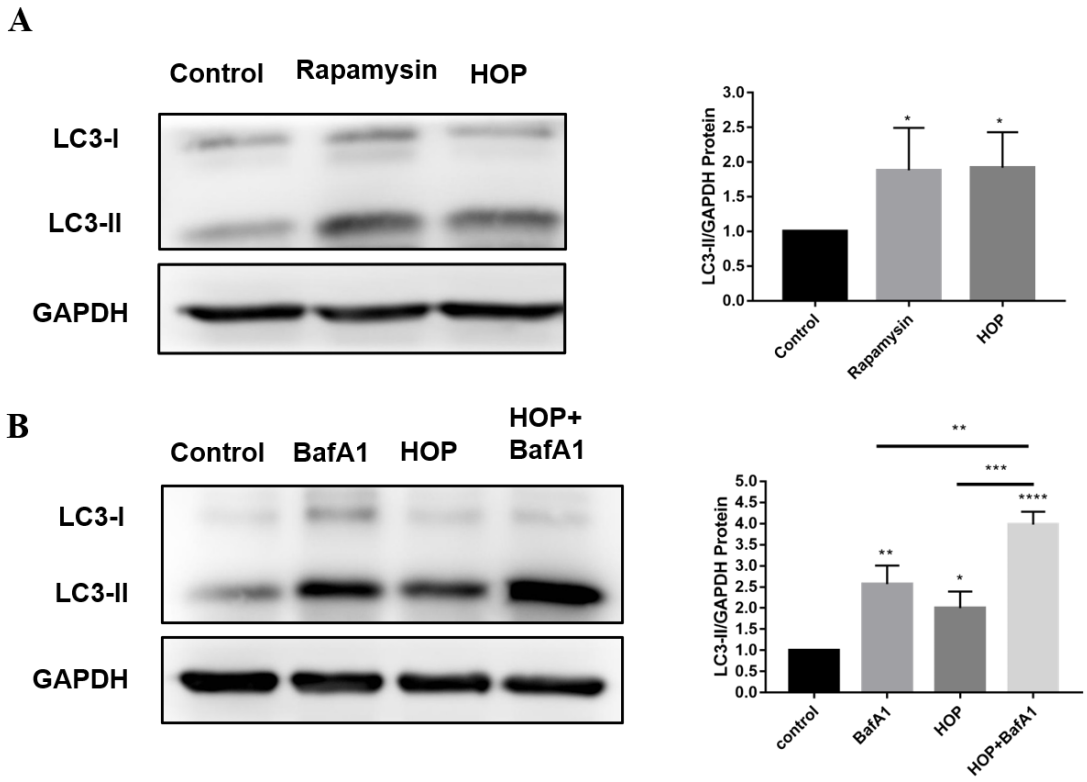

Figure S4. Hyperosmolarity induced increased autophagy flux in HCECs. (A) Western blot assays were performed to detect LC3 expression in HCECs exposed to Rapamycin (1μM) or HOP for 24 hours**. (B)** HCECs were exposed to HOP for 24 hours and then treated or not treated with bafilomycin A1 (100 nM, 4 hours). Changes to LC-3-II expression were detected by Western blot. The results are shown as mean ± SD of three or four independent experiments. *P < 0.05, ** P < 0.01, ** P < 0.001, ** P < 0.0001.

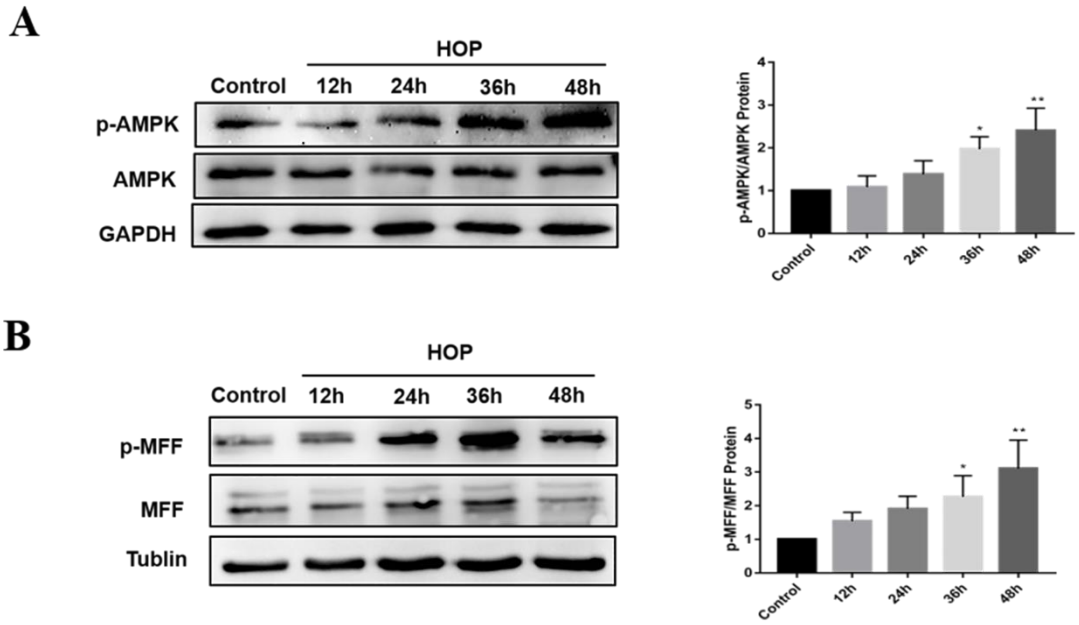

Figure S5. The AMPK/MFF/DRP1 pathway was activated in HOP-stressed HCECs. **(A)** Western Blot detected AMPK and p-AMPK expression in untreated and HOP-stressed HCECs for indicated times. **(B)** Western Blot results showing MFF and p-MFF expression in untreated and HOP-stressed HCECs for indicated times. The results are shown as mean ± SD of three independent experiments. *P < 0.05, ** P < 0.01.

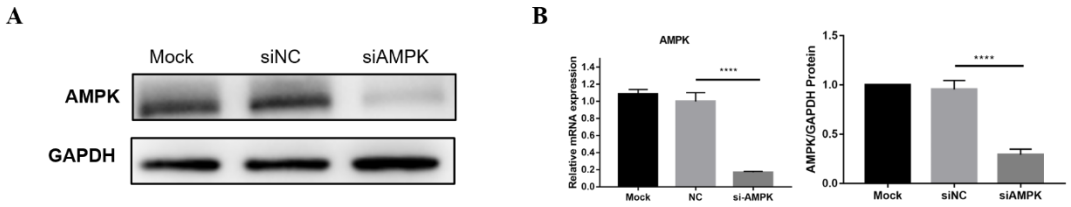

Figure S6. Validation of AMPK interference efficiency. (A) Western Blot showed AMPK expression after AMPK-specific siRNAs transfection in HCECs. **(B)** Quantitative analysis of the AMPK gene and protein expression after siAMPK transfection in HCECs. The results are shown as mean ± SD of three independent experiments. **** P < 0.0001.