Table S1 GJB2-MO, *GJB2* mRNA or *GJB2* c.109G>A mRNA injected into fertilized eggs in various doses to establish an optimal dose.

Concentration	6 hpf	24 hpf		48 hpf	
	mortality	mortality	Malformation rate	mortality	Malformation rate
GJB2 MO-0.1 mM	5.69%	16.11%	2.82%	17.06%	10.86%
GJB2 MO-0.3 mM	12.04%	19.91%	5.20%	25.00%	16.67%
GJB2 MO-1 mM	14.16%	28.33%	13.17%	30.47%	35.80%
GJB2 mRNA-100 ng/μL	2.59%	7.33%	0.00%	9.91%	3.35%
GJB2 mRNA-300 ng/μL	2.29%	8.02%	0.83%	12.21%	6.09%
GJB2 mRNA-600 ng/μL	10.00%	21.50%	2.55%	32.50%	17.78%
GJB2 c.109G>A mRNA-100 ng/μL	6.88%	14.29%	12.96%	16.40%	27.22%
GJB2 c.109G>A mRNA-300 ng/μL	11.06%	21.61%	14.10%	25.13%	31.54%
GJB2 c.109G>A mRNA-600 ng/μL	18.05%	47.80%	20.56%	58.05%	65.12%

Note: Based on the above results, the final optimal injection concentration for GJB2 mRNA or GJB2 c.109G>A mRNA was selected as 300 ng/uL, and the final optimal injection concentration for GJB2-MO was selected as 1 mM.

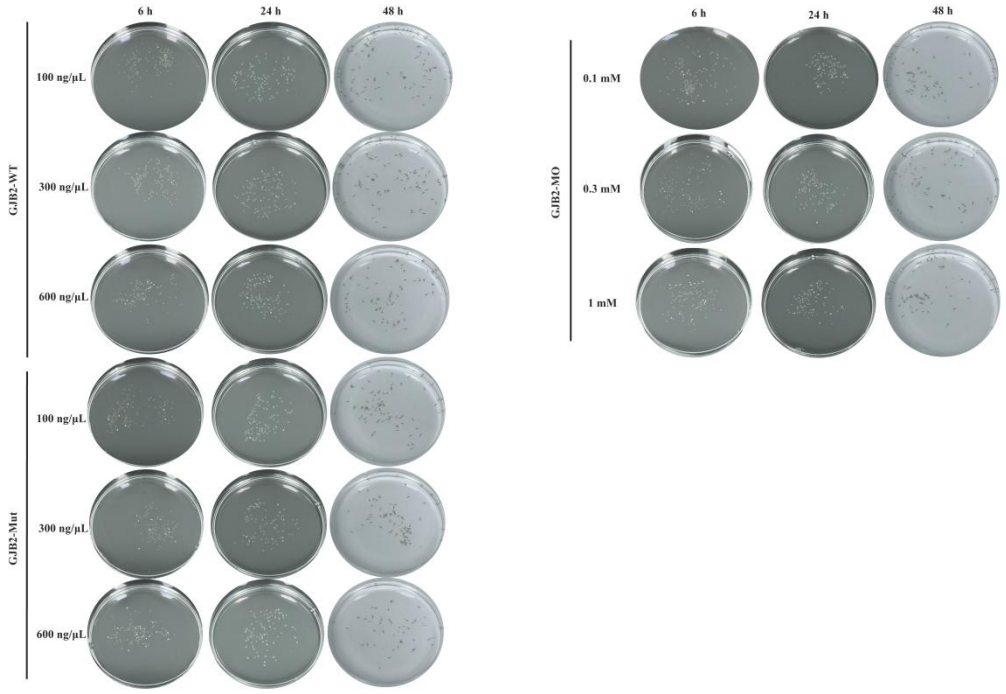

Figure S1 Photomicrographs of zebrafish embryos at 6 h, 24 h, and 48 h after fertilized eggs were injected with different concentrations of GJB2-MO, GJB2 mRNA or GJB2 c.109G>A mRNA. A transparent embryo represents a live embryo, while a white embryo represents a dead embryo.

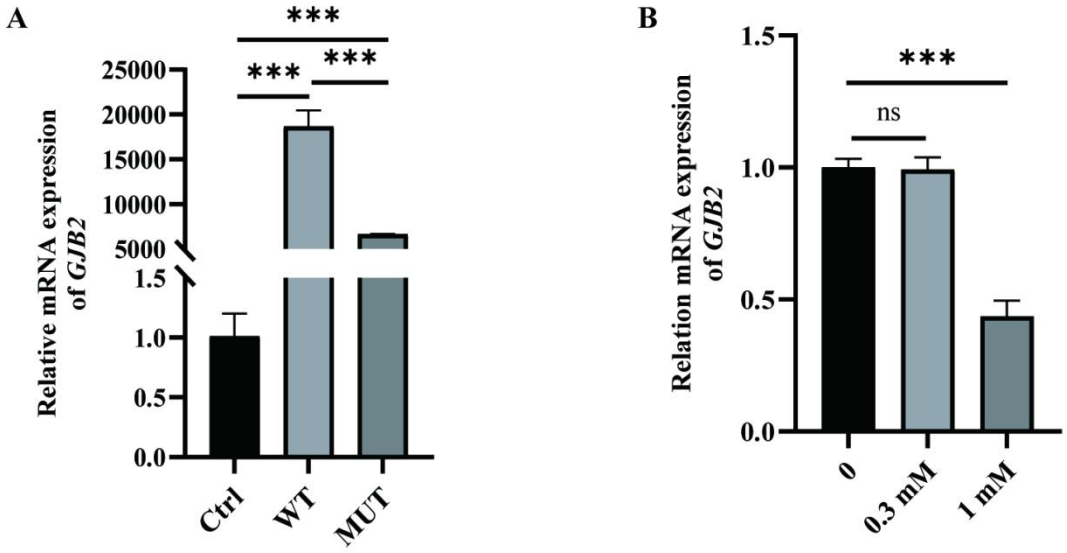

Figure S2 RT-qPCR validation of the efficiency of GJB2 overexpression and knockdown.The 24 hpi zebrafish embryos extract total RNA, RT-qPCR analyse the efficiency of (A) GJB2 mRNA, GJB2 c.109G>A mRNA or (B) GJB2-MO. The result

was calculated by the relative quantification method, and the expression level of the gene $F = 2^{-\triangle\triangle CT}$. All data analyses were using the one-way ANOVA method. ns to indicate no significant differences, asterisks were used to represent significant differences, ***P< 0.001 indicated extremely significant differences.

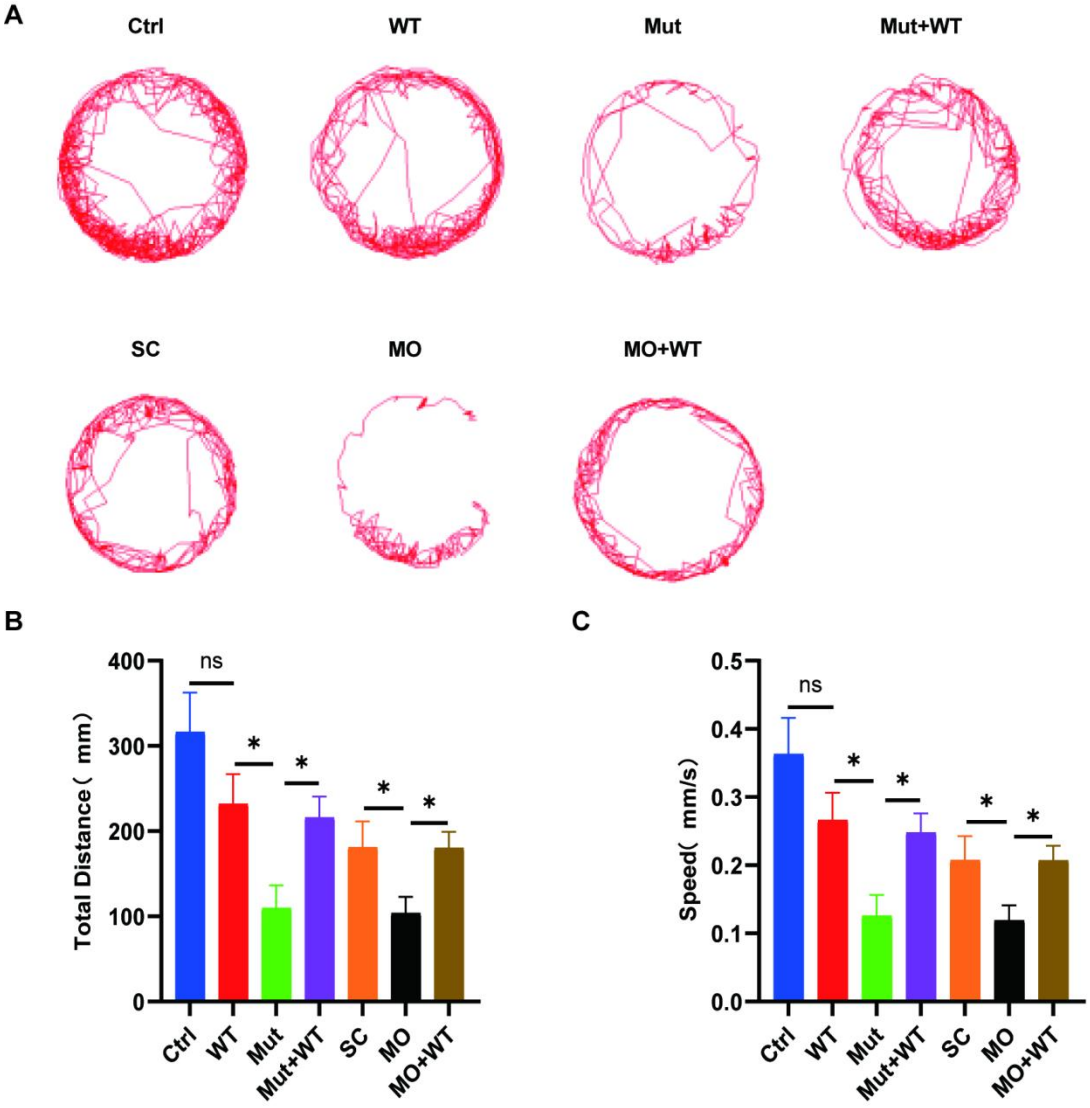

Figure S3 No sound stimulation behavioral trajectory of zebrafish after *GJB2 c.109G>A* mutation and knockdown. Videos were recorded using the DanioVision system recording software (total length 15 min), and the videos were analyzed using EthoVision XT 17 software, (A) Generating movement trajectories; Calculating the (B) total swimming distance and (C) speed value of zebrafish. n≥10. Each column and bar represent the mean ± SEM. All data were statistically analyzed by one-way ANOVA,

* p<0.05 indicates a significant difference, and *** p <0.001 indicates an extremely significant difference.

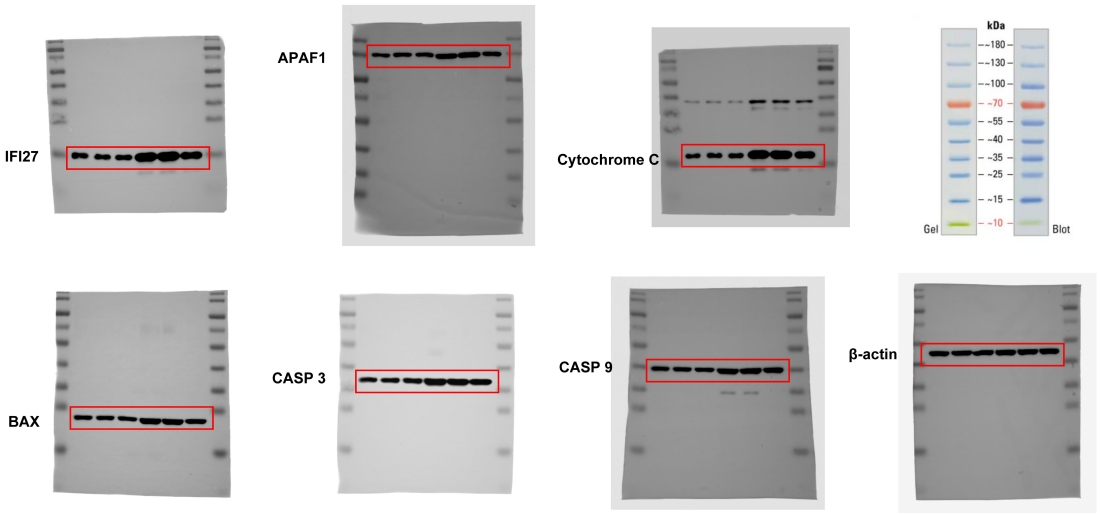

Figure S4 Original Western blot images for the proteins IFI27, Bax, Caspase-3, Caspase-9, and Apaf1. Each sample was loaded with 20 μg of protein.