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Supplementary Information for

Pejvakin-mediated pexophagy protects auditory hair cells against noise-induced damage

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This PDF file includes:

Figs. S1 to S5

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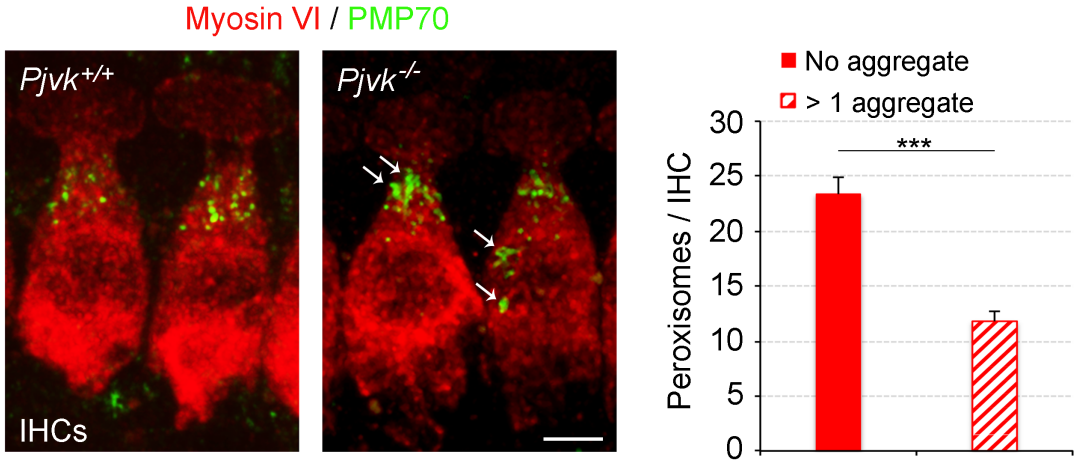

Fig. S1. Peroxisomes cluster together after sound exposure in *Pjvk*$^{-/-}$ IHCs. Peroxisomes were counted 24 hours after sound exposure (5-40 kHz, 105 dB SPL, for 1 hour). IHCs and peroxisomes were immunostained with anti-myosin VI, and anti-PMP70 antibodies, respectively. The decrease in peroxisome number was correlated with the formation of aggregates (indicated by arrows) in *Pjvk*$^{-/-}$ IHCs. The bar charts compare the numbers of peroxisomes in IHCs containing at least one aggregate with the number of those containing no aggregates (n = 40 IHCs from 4 mice). The number of peroxisomes is significantly lower in *Pjvk*$^{-/-}$ IHCs containing aggregates. Scale bar, 5 µm. Mean ± SEM; ***$P < 0.001$ (unpaired Student's t-test).

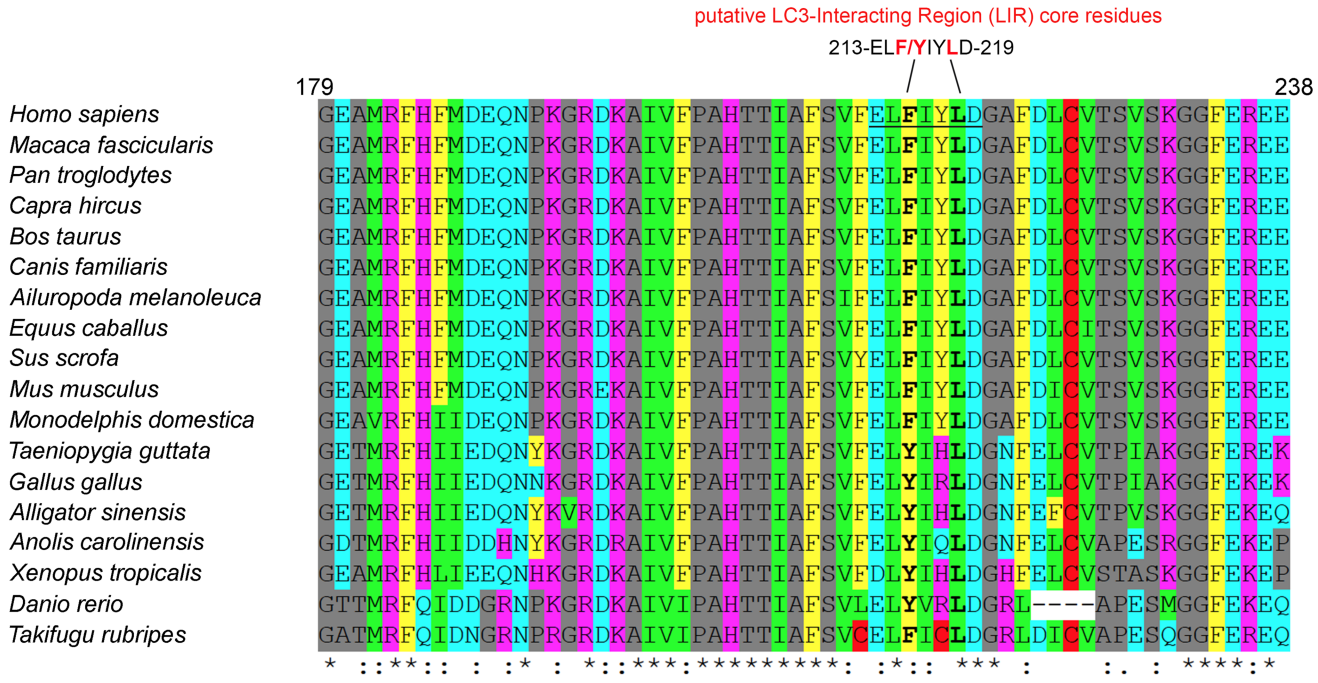

Fig. S2. Comparison of the putative LIR sequence within the chaperone domain of pejvakin between vertebrates. The amino-acid residues of the LIR motif of pejvakin are underlined in black. The core residues of LIR, F/Y215 and L218, are highly conserved throughout evolution.

A

Pejvakin sequence (*Mus musculus*)

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  1 MFAAATKSFVKQVGDGGRLVPVPSLSEADKYQPLSLVVKKKRCFLFPRCKFTSTPFTLKD  60
 61 ILLGDREISAGISSYQLLNYEDESDVSLYGRRSNHIVNDVGINVTGSDSIAVKASFGVVT 120
                                    predicted chaperone domain (aa 154 - 227)
121 KHEVEVSTLLKEITARKINFDHSLIRQSRSSRKAVLCVVMESIRTTRQCSLSVHAGIRGE 180
181 AMRFHFMDEQNPKGREKAIVFPAHTTIAFSVFELFIYLDGAFDICVTSVSKGGFEREETT 240
241 TFAMFYRLRNILFERNRRVMDAISRSQLYLDDLFSDFYDKPLSMTDISLKEGTHIRVNLL 300
301 NHNIPKGPCILCGMGNLKRETVYGCFQCSVDGVKYVRLHAVPCFDIWHKRMK 352
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putative LC3-Interacting Region (LIR) motif

B

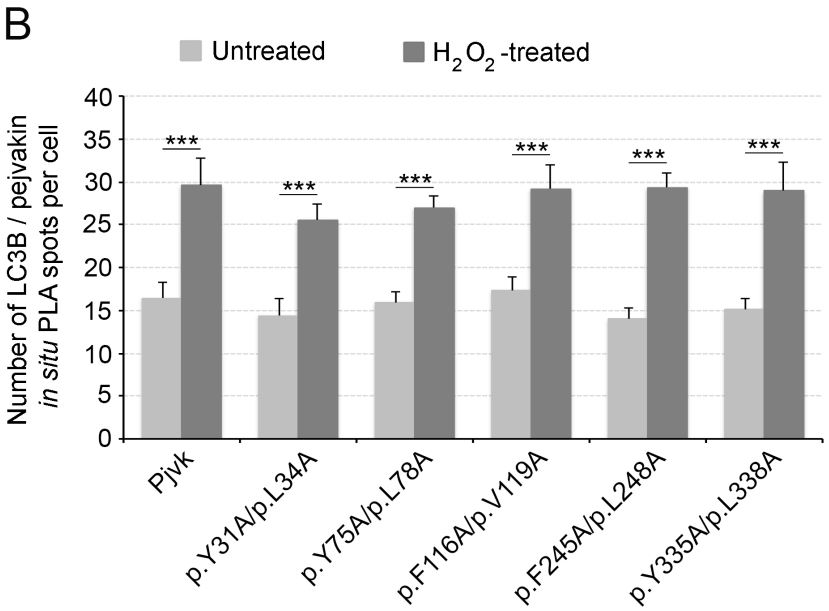

Fig. S3. Mutations in putative LIR motifs outside the chaperone domain of pejvakin do not impair the recruitment of LC3B by pejvakin in response to oxidative stress. (*A*) Schematic localization of putative LIR motifs in the pejvakin sequence (in red, with a core consensus sequence [W/F/Y]X_1X_2[L/I/V]) outside the chaperone domain (highlighted in blue). (*B*) The number of LC3B/pejvakin *in situ* PLA spots is significantly increased by H_2O_2 treatment in transfected HeLa cells producing EGFP and wild-type pejvakin (Pjvk) or EGFP and the p.Y31A/p.L34A, p.Y75A/p.L78A, p.F116A/p.V119A, p.F245A/p.L248A or p.Y335A/p.L338A mutated forms of pejvakin (n = 20 cells per condition). Mean ± SEM; ***P < 0.001 (unpaired Student's t-test).

Fig. S4. Pejvakin contains a predicted protein-binding pocket including functional LIR residues. Pocket detection in the pejvakin sequence, with the Fpocket tool. The predicted chaperone domain is boxed in blue and the predicted pocket amino-acid residues are highlighted in red. The amino-acid residues of the functional LIR motif are underlined in black within the predicted pocket.

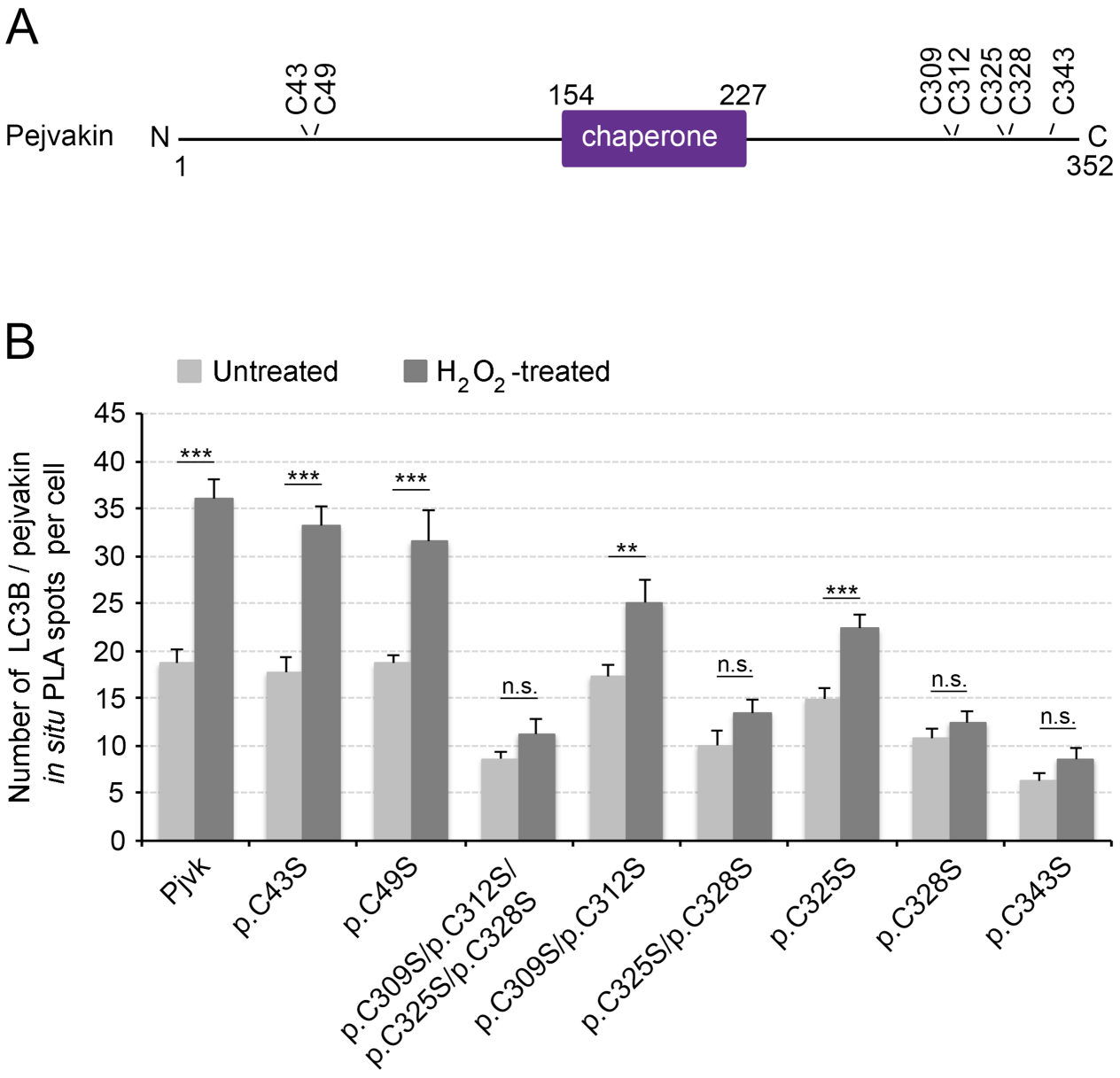

Fig. S5. C-terminal cysteines are required for the oxidative stress-induced binding of pejvakin to LC3B whereas N-terminal cysteines are not. (*A*) Schematic representation of cysteines N- or C-terminal to the predicted chaperone domain of pejvakin. (*B*) The number of LC3B/pejvakin *in situ* PLA spots was significantly increased by H_2O_2 treatment in transfected HeLa cells producing EGFP and wild-type pejvakin (Pjvk) or EGFP and the p.C43S, p.C49S, p.C309S/p.C312S or p.C325S mutated forms of pejvakin, but not in transfected HeLa cells producing EGFP and the p.C325S/p.C328S, p.C309S/p.C312S/p.C325S/p.328S, p.C328S or p.C343S mutated forms of pejvakin (n = 20 cells per condition). Mean ± SEM; $^{**}P < 0.01$, $^{***}P < 0.001$, n.s., not significant (unpaired Student's t-test).