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## Supplementary Information for

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

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Figs. S1 to S5



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).

			213-EL <mark>F/Y</mark> IYLD-219	
	179			238
Homo sapiens	G <mark>EAMRFHFM</mark> DE	CQNP <mark>K</mark> GRD <mark>KAIVF</mark> PA <mark>H</mark> TT	IAFSVF <mark>ELF</mark> IY <b>LD</b> GAFDLC	VTSVS <mark>K</mark> GG <mark>FEREE</mark>
Macaca fascicularis	G <mark>EAMRFHFM</mark> DE	CQNP <mark>K</mark> GRDKAIVFPAHTI	IAFSVFEL <mark>F</mark> IY <b>L</b> DGAFDLC	VTSVS <mark>K</mark> GG <mark>F</mark> EREE
Pan troglodytes	G <mark>EAMRFHFM</mark> DE	CQNP <mark>K</mark> GRD <mark>K</mark> AIVFPAHTI	IAFSVFEL <mark>F</mark> IY <b>L</b> DGAFDLC	VTSVS <mark>K</mark> GG <mark>FEREE</mark>
Capra hircus	G <mark>EAMRFHFM</mark> DE	CQNP <mark>K</mark> GRDKAIVFPAHTI	IAFSVFEL <mark>F</mark> IY <b>L</b> DGAFDLC	VTSVS <mark>K</mark> GG <mark>F</mark> EREE
Bos taurus	G <mark>EAMRFHFM</mark> DE	CQNP <mark>K</mark> GRD <mark>K</mark> AIVFPAHTI	IAFSVFEL <mark>F</mark> IY <b>L</b> DGAFDLC	VTSVS <mark>K</mark> GG <mark>FEREE</mark>
Canis familiaris	G <mark>EAMRFHFM</mark> DE	CQNP <mark>K</mark> GRD <mark>KAIVF</mark> PAHTI	IAFSVFEL <b>F</b> IY <mark>L</mark> DGAFDLC	VTSVS <mark>K</mark> GG <mark>FEREE</mark>
Ailuropoda melanoleuca	G <mark>EAMRFHFM</mark> DF	QNP <mark>K</mark> GRDKAIVFPAHTI	'IAFSIFEL <mark>F</mark> IY <mark>L</mark> DGAFDL <mark>C</mark>	VTSVS <mark>K</mark> GG <mark>FE</mark> REE
Equus caballus	G <mark>EAMRFHFM</mark> DE	CQNP <mark>K</mark> GRDKAIVFPAHTI	'IAFSVFEL <mark>F</mark> IY <mark>L</mark> DGAFDL <mark>C</mark>	ITS <mark>V</mark> S <mark>K</mark> GG <mark>F</mark> EREE
Sus scrofa	G <mark>EAMRFHFM</mark> DE	CQNP <mark>K</mark> GRD <mark>K</mark> AIVFPAHTI	IAFSVYEL <mark>F</mark> IY <b>L</b> DGAFDLC	VTSVS <mark>K</mark> GG <mark>FEREE</mark>
Mus musculus	G <mark>EAMRFHFM</mark> DF	CQNP <mark>K</mark> GREKAIVFPAHTI	'IAFSVFEL <mark>F</mark> IY <b>L</b> DGAFDIC	VTSVS <mark>K</mark> GG <mark>FE</mark> REE
Monodelphis domestica	GEAVRFHIIDE	CQNP <mark>K</mark> GRD <mark>KAIVF</mark> PAHTI	IAFSVFEL <mark>F</mark> IY <mark>L</mark> DGAFDLC	VTSVS <mark>K</mark> GG <mark>FEREE</mark>
Taeniopygia guttata	GETM <mark>RFH</mark> IIEI	QN <mark>YK</mark> GRDKAIVFPAHTI	'IAFSVFEL <mark>Y</mark> I <mark>HL</mark> DGNFELC	VTPIAKGG <mark>FEREK</mark>
Gallus gallus	G <mark>ETMRFH</mark> IIEI	QNN <mark>K</mark> GRDKAIVFPAHTI	'IAFSVFEL <mark>Y</mark> IR <b>L</b> DGNFEL <mark>C</mark>	VTPIA <mark>K</mark> GG <mark>FE</mark> KEK
Alligator sinensis	GETM <mark>RFH</mark> IIEI	QN <mark>YKVR</mark> DKAIVFPAHTI	IAFSVFEL <b>Y</b> IH <mark>L</mark> DGNFEFC	VTPVS <mark>K</mark> GG <mark>FE</mark> KEQ
Anolis carolinensis	GDTMRFHIIDI	HNYKGRDRAIVFPAHTI	'IAFSVFEL <mark>Y</mark> IQ <b>L</b> DGNFELC	VAP <mark>E</mark> S <mark>R</mark> GG <mark>FE</mark> KEP
Xenopus tropicalis	G <mark>EAMRFH</mark> LIEF	QN <mark>HK</mark> GRDKAIVFPAHTI	'IAFSVFDL <mark>Y</mark> I <mark>HL</mark> DG <mark>HFELC</mark>	VSTAS <mark>K</mark> GG <mark>FE</mark> KEP
Danio rerio	GTTMRFQIDDO	RNPKGRDKAIVIPAHTI	'IAFSVLEL <mark>Y</mark> VR <b>L</b> DG <mark>R</mark> L	-AP <mark>ESM</mark> GG <mark>FE</mark> KEQ
Takifugu rubripes	GAT <mark>MRFQIDN</mark> G	G <mark>RNPR</mark> GRD <mark>K</mark> AIVIPAHTI	IAFSV <mark>C</mark> EL <mark>F</mark> ICLDGRLDIC	VAP <mark>E</mark> SQGG <mark>F</mark> EREQ
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putative LC3-Interacting Region (LIR) core residues

**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)

- 1 MFAAATKSFVKQVGDGGRLVPVPSLSEADKYQPLSLVVKKKRCFLFPRCKFTSTPFTLKD 60
- 61 ILLGDREISAGISS<u>YQLL</u>NYEDESDVSLYGRRSNHIVNDVGINVTGSDSIAVKASFGVVT 120 predicted chaperone domain (aa 154 - 227)
- 121 KHEVEVSTLLKEITARKINFDHSLIRQSRSSRKAVLCVVMESIRTTRQCSLSVHAGIRGE 180
- 181 AMRFHFMDEQNPKGREKAIVFPAHTTIAFSVFELFIYLDGAFDICVTSVSKGGFEREETT 240
- 241 TFAMFYRLRNILFERNRRVMDAISRSQLYLDDLFSDFYDKPLSMTDISLKEGTHIRVNLL 300
- 301 NHNIPKGPCILCGMGNLKRETVYGCFQCSVDGVK<u>YVRL</u>HAVPCFDIWHKRMK 352

putative LC3-Interacting Region (LIR) motif



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<sub>2</sub>O<sub>2</sub> 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).