Supplementary Information

Caspase-8 regulates endoplasmic reticulum stress-induced necroptosis independent of the apoptosis pathway in auditory cells

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Supplementary Figure S1a

Representative transmission electron microscopy photomicrographs of low magnifying scales in HEI-OC1 cells treated with tunicamycin (50 μ g/ml for 24 h). Tunicamycin-treated cells showed morphological characteristics of apoptosis (arrow) or necroptosis (arrowhead) (left and middle panel: 2,000×, and right panel: 2,500× magnifications).

Supplementary Figures S1-6.

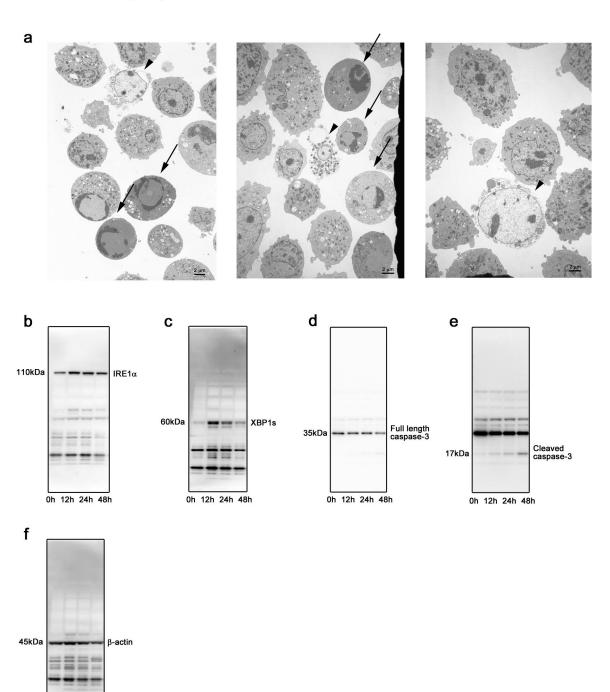
Uncropped version of blots displayed in main figure 1f (Figures S1 b–f), 2c (Figures S2 a and b), 2f (Figures S2 c–e), 3b (Figures S3 a–d), 4a (Figures S4 a–d), 4d (Figures S4 e–i), 5a (Figures S5 a–c) and 5c (Figures S5 d–h).

Supplementary Figure S4j.

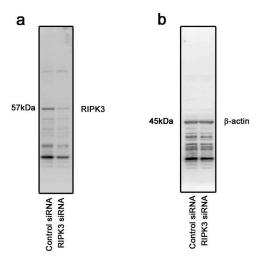
Representative western blots showing the expressions of caspase-8 and MLKL in tunicamycin-treated caspase-8 KD cells (50 μ g/ml for 48 h). β -actin was included as the loading control. The expressions of caspase-8 and MLKL were detected, and the means \pm S.D. (fold changes compared to the control group) of three or more independent studies were presented.

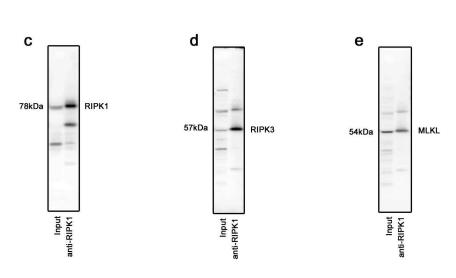
Supplementary Figure 1.

0h 12h 24h 48h

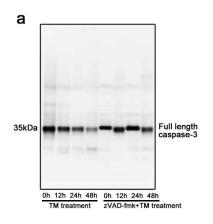


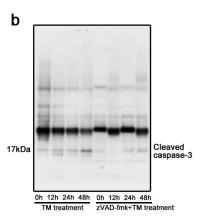
Supplementary Figure 2.

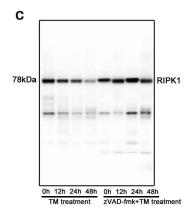


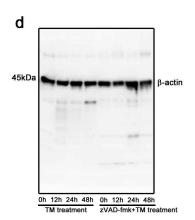


Supplementary Figure 3.









Supplementary Figure 4. d a С 78kDa RIPK1 Full length caspase-8 57kDa 45kDa β-actin Cleaved 10kDa caspase-8 0h 12h 24h 48h 0h 12h 24h 48h 0h 12h 24h 48h 0h 12h 24h 48h е g Full length caspase-8 57kDa Full length caspase-3 35kDa Cleaved 17kDa caspase-3 0h 12h 24h 48h 0h 12h 24h 48h Caspase-8 siRNA 0h 12h 24h 48h 0h 12h 24h 48h 0h 12h 24h 48h 0h 12h 24h 48h Control siRNA Caspase-8 siRNA Control siRNA Caspase-8 siRNA h TM treatment Control siRNA 12h 24h 48h 0h 12h 24h 48h Caspase-8 78kDa RIPK1 (kDa) MLKL 45kDa β-actin ■ Control siRNA ■ Caspase-8 siRNA 1.2 Relative level 9.0 8.0 9.0

Oh 12h 24h 48h Oh 12h 24h 48h

Control siRNA Caspase-8 siRNA

0h 12h 24h 48h0h 12h 24h 48h

Control siRNA Caspase-8 siRNA

0.4

0.2

Supplementary Figure 5. a С Full length caspase-9 49kDa 45kDa β-actin 37kDa Cleaved caspase-9 0h 12h 24h 48h 0h 12h 24h 48h 0h 12h 24h 48h d е Full length caspase-9 49kDa Full length caspase-3 35kDa Cleaved caspase-3 17kDa 0h 12h 24h 48h 0h 12h 24h 48h Caspase-9 siRNA 0h 12h 24h 48h 0h 12h 24h 48h 0h 12h 24h 48h 0h 12h 24h 48h Control siRNA Caspase-9 siRNA Control siRNA Caspase-9 siRNA h g

