Mitochondrial oxidative phosphorylation complex regulates NLRP3 inflammasome activation and predicts patient survival in nasopharyngeal carcinoma

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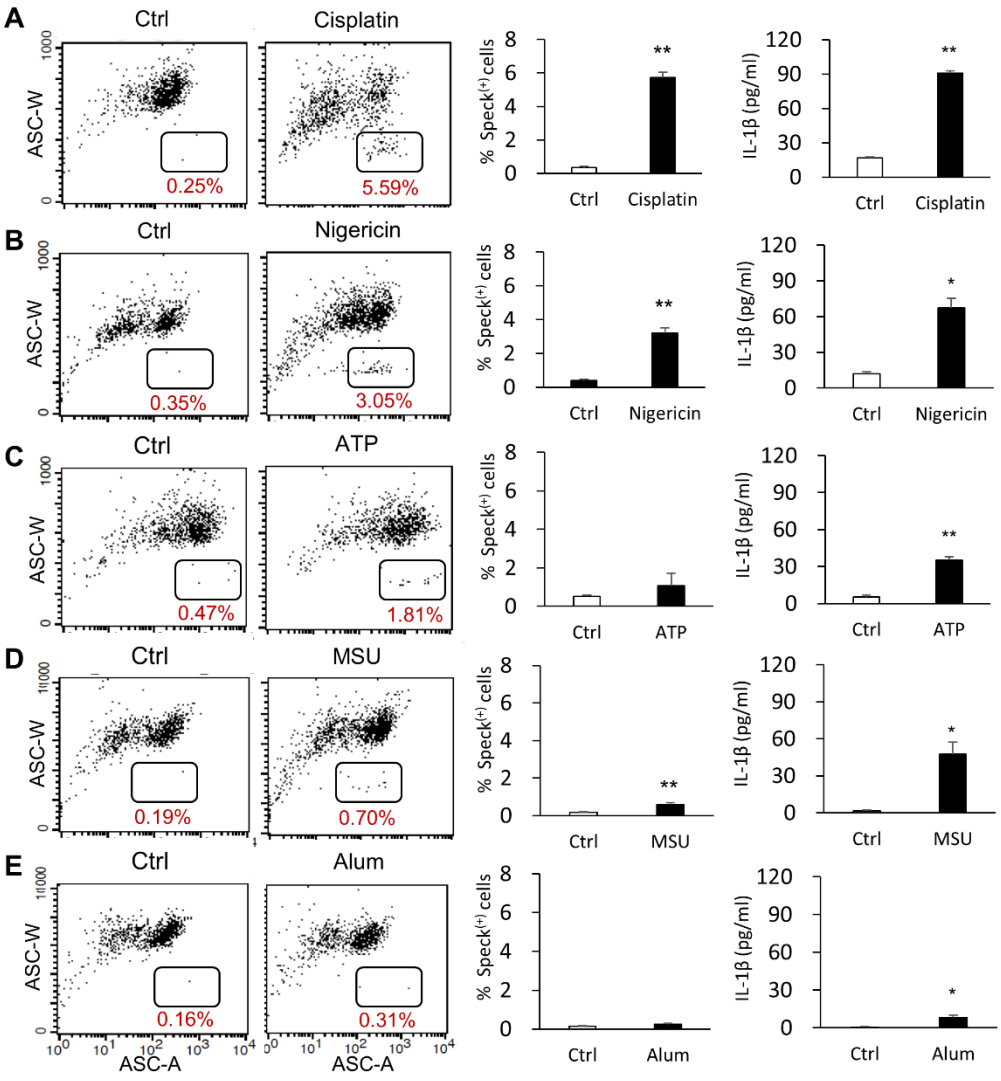
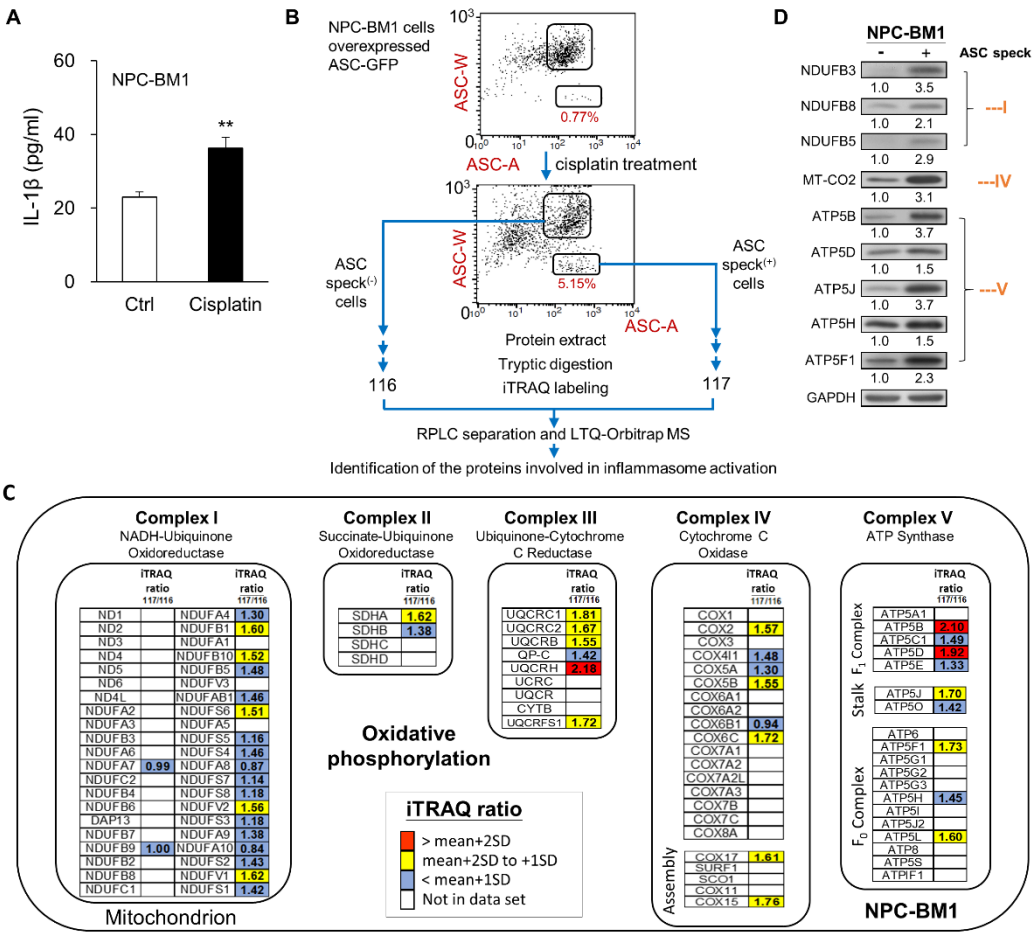
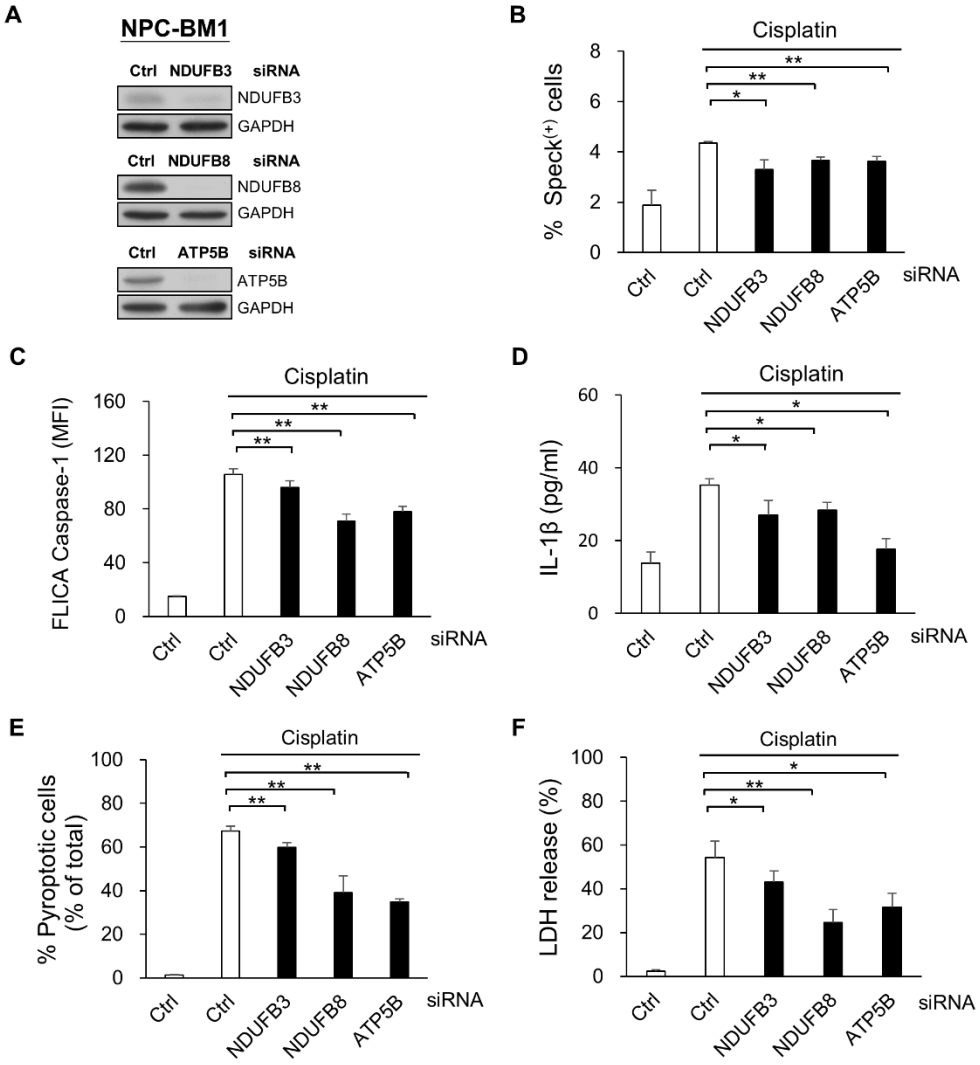


Supplemental Figure S1. ASC speck formation and IL-1β secretion in NPC-HK1-ASC-GFP cells after NLRP3 inflammasome activation. NPC-HK1-ASC-GFP cells were treated with cisplatin (40 μM) (A), nigericin (10 μM) (B), ATP (5 mM) (C), MSU (200 μM) (D) or Alum (200 μM) (E) for 24hr. The percentages of ASC speck$^{(+)}$ cells were quantified by flow cytometry. The concentrations of IL-1β were measured in the cultured medium of cells by ELISA. Symbols: *P < 0.05; **P < 0.01.

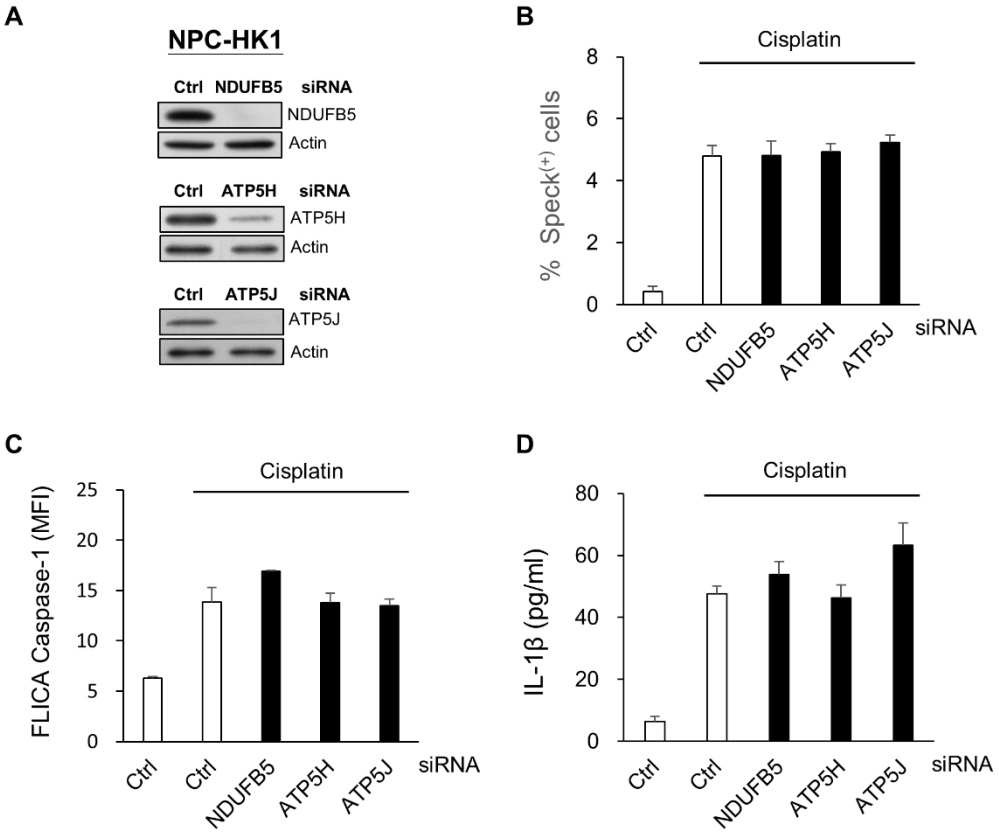


Supplemental Figure S2. Mitochondrial OxPhos components are enriched in ASC speck$^{(+)}$ NPC-BM1 cells. A, NPC-BM1 cells were treated with cisplatin (40 μM) for 30 hr, and the concentrations of IL-1β were measured in the cultured medium of cells by ELISA. Symbols: *P < 0.05; **P < 0.01. B, Flow chart of the iTRAQ-based proteomics approach. NPC-BM1-ASC-GFP cells were treated with cisplatin (40 μM) for 30 hr, ASC speck-containing cells were isolated by flow cytometry (low and high ASC-W:ASC-A profiles were taken as indicating ASC speck$^{(+)}$ cells and ASC speck$^{(-)}$ cells, respectively), and iTRAQ- based quantitative proteomic analysis was performed. C, List of OxPhos protein subunits identified by iTRAQ labeling and LC-MS/MS analysis. The iTRAQ ratios of 117/116 represent the quantitative ratio between ASC speck$^{(+)}$ (labeled with 117 iTRAQ reagents) and speck$^{(-)}$ (labeled with 116 iTRAQ reagents) cells. Red boxes indicate proteins with iTRAQ ratios of at least 2SD above the mean. Yellow boxes indicate proteins with iTRAQ ratios between 1 and 2 SD above the mean. Blue boxes indicate proteins that did not show a change in iTRAQ

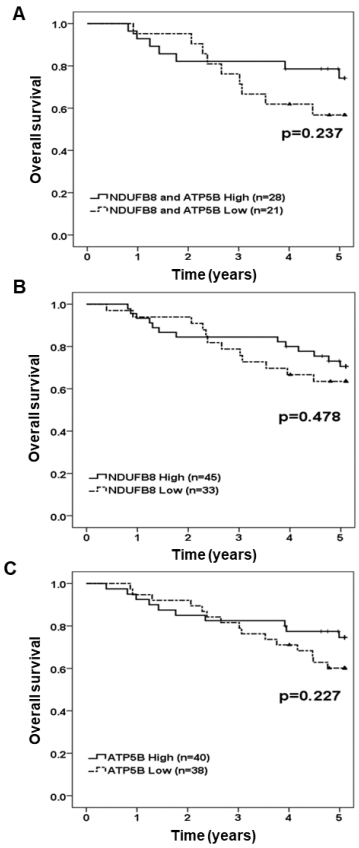
ratio. White boxes indicate proteins that were not identified in the proteome. Figure adapted from https://www.wikipathways.org/index.php/ Pathway:WP111. D, Cell lysates from ASC speck$^{(+)}$ and ASC speck$^{(-)}$ NPC-BM1-ASC-GFP cells were used for Western blot analysis. The ratio of OxPhos proteins were calculated by normalization with GAPDH, with the values from the ASC speck$^{(-)}$ cells set as 1.0.



Supplemental Figure S3. NDUFB3, NDUFB8 and ATP5B are involved in NLRP3 inflammasome activation and cell pyroptosis. NPC-BM1 cells were transfected with NDUFB3-, NDUFB8-, ATP5B-, or control (Ctrl)- siRNA for 48 hr, treated with cisplatin (40 μM) for 30 hr and analyzed for ASC speck formation, caspase-1 activity, IL-1β release and cell pyroptosis. A, Lysates were collected from knockdown cells and used for Western blot analysis. B, The percentages of ASC speck$^{(+)}$ cells were quantified from NPC-BM1-ASC-GFP cells by flow cytometry. C, Activated caspase-1 was determined by staining with YVAD-FLICA followed by flow cytometry. D, The levels of IL-1β in cell culture supernatants were measured by ELISA. E, The percentages of pyroptotic cells (FLICA-positive/PI-positive cells) were detected by flow cytometry. F, Supernatant LDH activity was assessed using an LDH Cytotoxicity Assay Kit and the percentages of LDH release are presented in a histogram. Symbols: *P < 0.05; **P < 0.01.



Supplemental Figure S4. Effects of the OxPhos proteins, NDUFB5, ATP5H and ATP5J, on NLRP3 inflammasome activation in NPC-HK1 cells. Cells were transfected with NDUFB5-, ATP5H-, ATP5J-, or control (Ctrl)- siRNA for 48 hr, treated with cisplatin (40 μM) for 24 hr and analyzed for ASC speck formation, caspase-1 activity and IL-1β release. A, Lysates from knockdown cells were collected for Western blot analysis. B, The percentages of ASC speck$^{(+)}$ cells were quantified from NPC-HK1-ASC-GFP cells by flow cytometry. C, Activated caspase-1 was determined by staining with YVAD-FLICA and measured by flow cytometry. D, The levels of IL-1β in cell culture supernatants was measured by ELISA.



D

Multivariate analysis of the association between NDUFB8 and ATP5B expression with overall survival of NPC patients

Characteristics	Hazards Ratio (95% CI)	*P-value*
Patients (n=49)		
T stage (3-4 vs. 1-2)	0.753 (0.278-2.041)	0.577
N stage (2-3 vs. 0-1)	1.417 (0.508-3.951)	0.505
NDUFB8+ATP5B (High vs. Low)	0.571 (0.208-1.567)	0.277
Patients (n=78)		
T stage (3-4 vs. 1-2)	1.388 (0.631-3.054)	0.415
N stage (2-3 vs. 0-1)	2.620 (1.081-6.349)	0.033*
NDUFB8 (High vs. Low)	0.892 (0.401-1.983)	0.780
Patients (n=78)		
T stage (3-4 vs. 1-2)	1.314 (0.592-2.913)	0.502
N stage (2-3 vs. 0-1)	2.576 (1.072-6.194)	0.034*
ATP5B (High vs. Low)	0.675 (0.299-1.525)	0.345

The Cox proportional hazards model was applied for multivariate analysis to determine the independence of each prognostic factor.

Supplemental Figure S5. Association of NDUFB8 and ATP5B with overall survival in NPC patients. Kaplan-Meier survival analysis of overall survival curves for NPC patients with combine expression of NDUFB8 and ATP5B (A), NDUFB8 expression (B) or ATP5B expression (C). D, Multivariate analysis of the association between NDUFB8 and ATP5B expression with overall survival of NPC patients Symbols: *P < 0.05.