Supplementary Materials

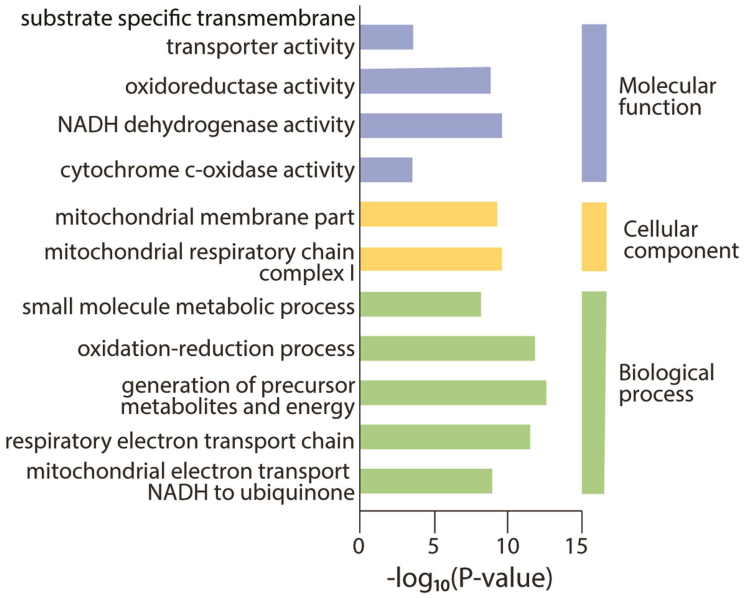
Molecular Biology of the Cell

Chojnacka *et al.*

SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure S1
Chojnacka, Elancheliyan, et al.

Supplementary Figure S1: OCIAD2 is a metazoan specific protein. Alignment of the human (H. sapiens) OCIAD2 amino acid sequence to its metazoan homologs using ClustalW. Dark gray shading indicates identical amino acids, whereas medium or light gray shading depicts similar amino acids: 80% or 60% similarity, respectively.



Supplementary Figure S2
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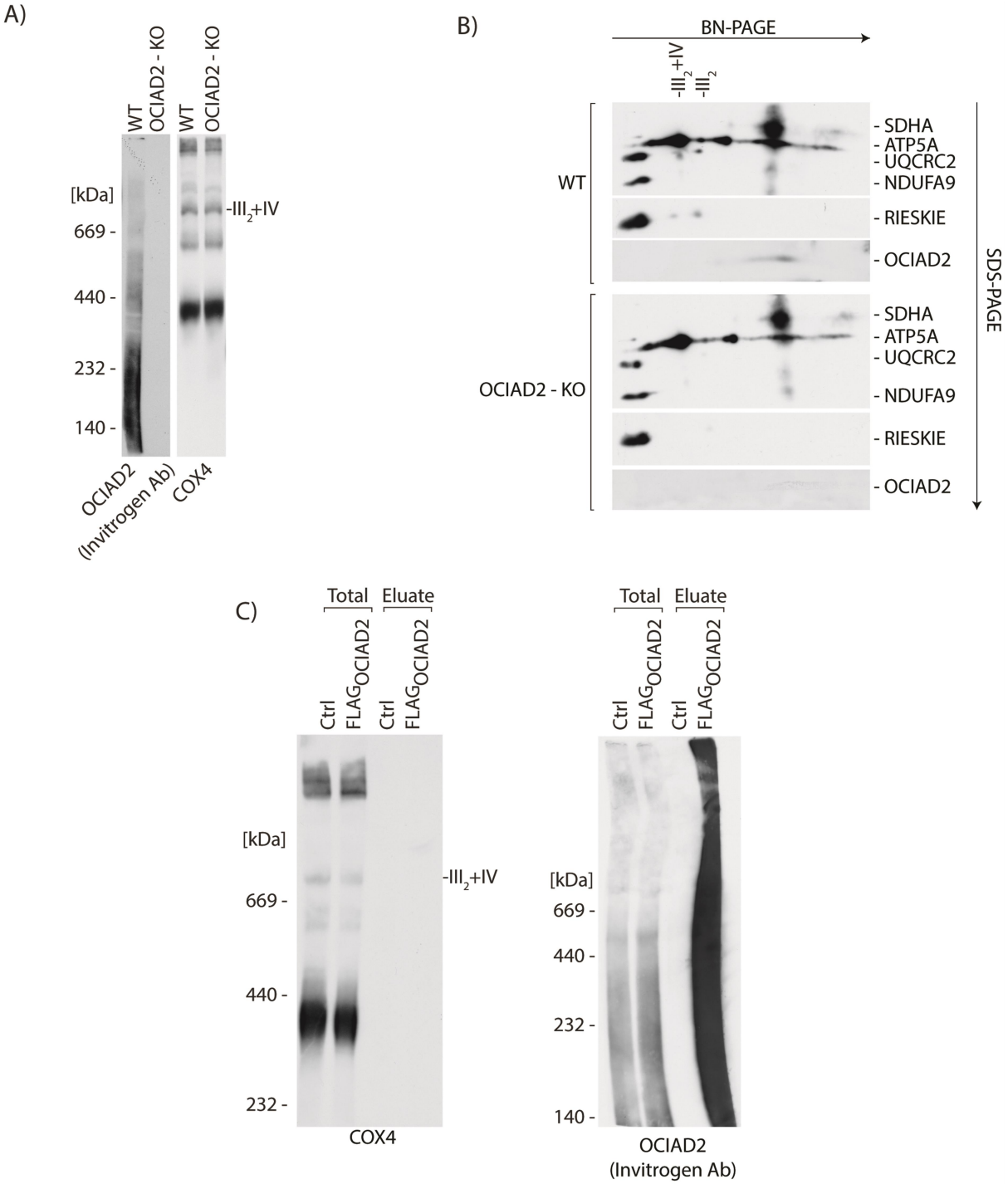
Supplementary Figure S2: OCIAD2 interacts with electron transport chain proteins. GO terms significantly enriched among the mitochondrial proteins specifically bound to ^{FLAG}OCIAD2 obtained in Fisher test's for 48 proteins groups with mean fold enrichment >2 in ^{FLAG}OCIAD2 vs Ctrl samples compared to all IMPI listed protein groups (bait was excluded) identified in the co-IP experiment (FDR = 0.01)



Supplementary Figure S3
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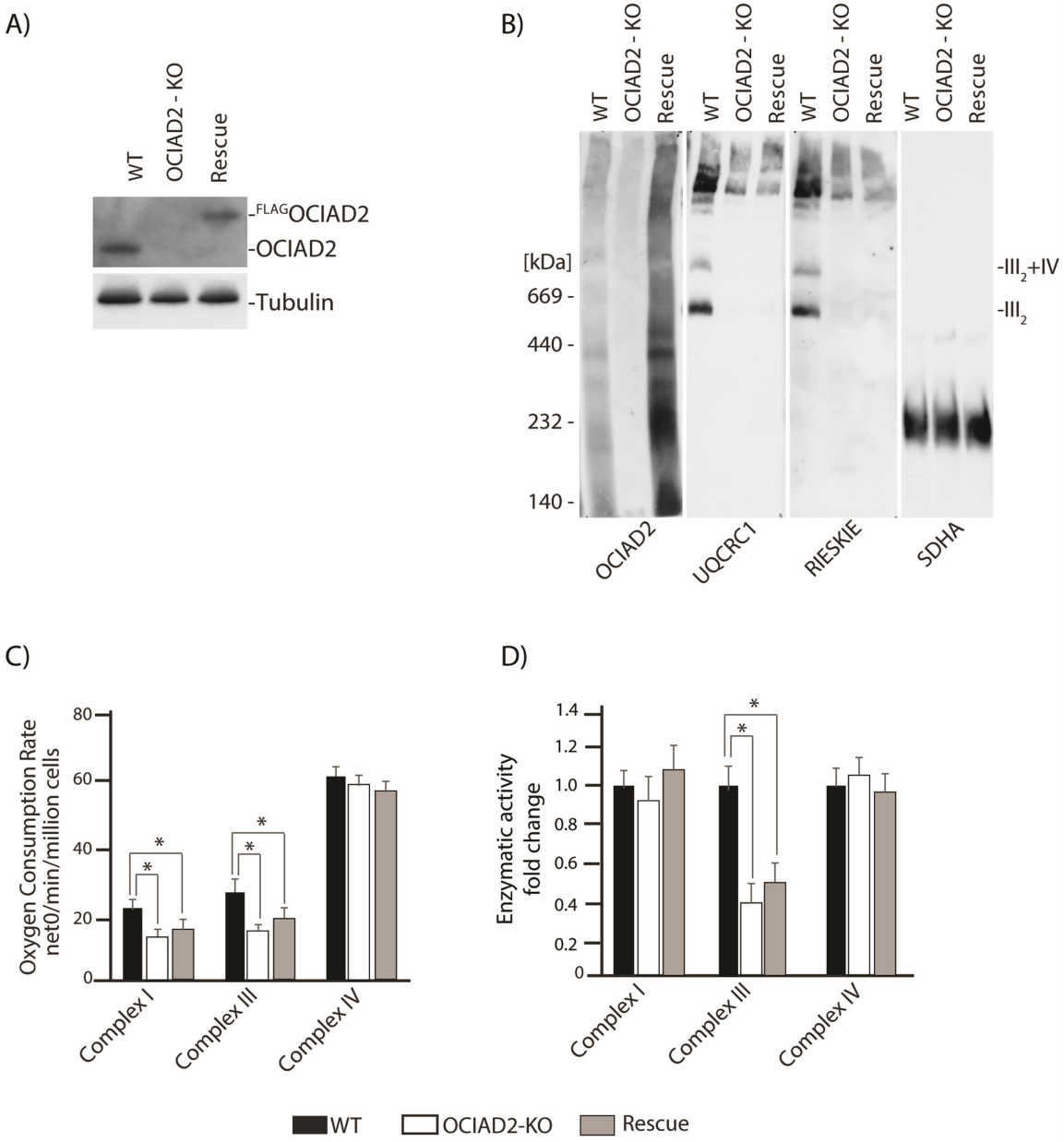
Supplementary Figure S3: Analysis of isolated mitochondria from WT and OCIAD2-KO cells. (A) Isolated mitochondria were solubilized by 1% digitonin buffer and resolved using BN-PAGE gels. Followed by western blotting and immunodetection using indicated antibodies. (B) 2D gel analysis of isolated mitochondria of WT and OCIAD2-KO cells. Solublized

mitochondria resolved using BN-PAGE gels. The complex in the gels are disrupted and resolved again in SDS-PAGE followed by western blotting and immunodetection using indicated antibodies. (C) ^{FLAG}OCIAD2 was immunopurified and elute was resolved using BN-PAGE gels.



Supplementary Figure S4
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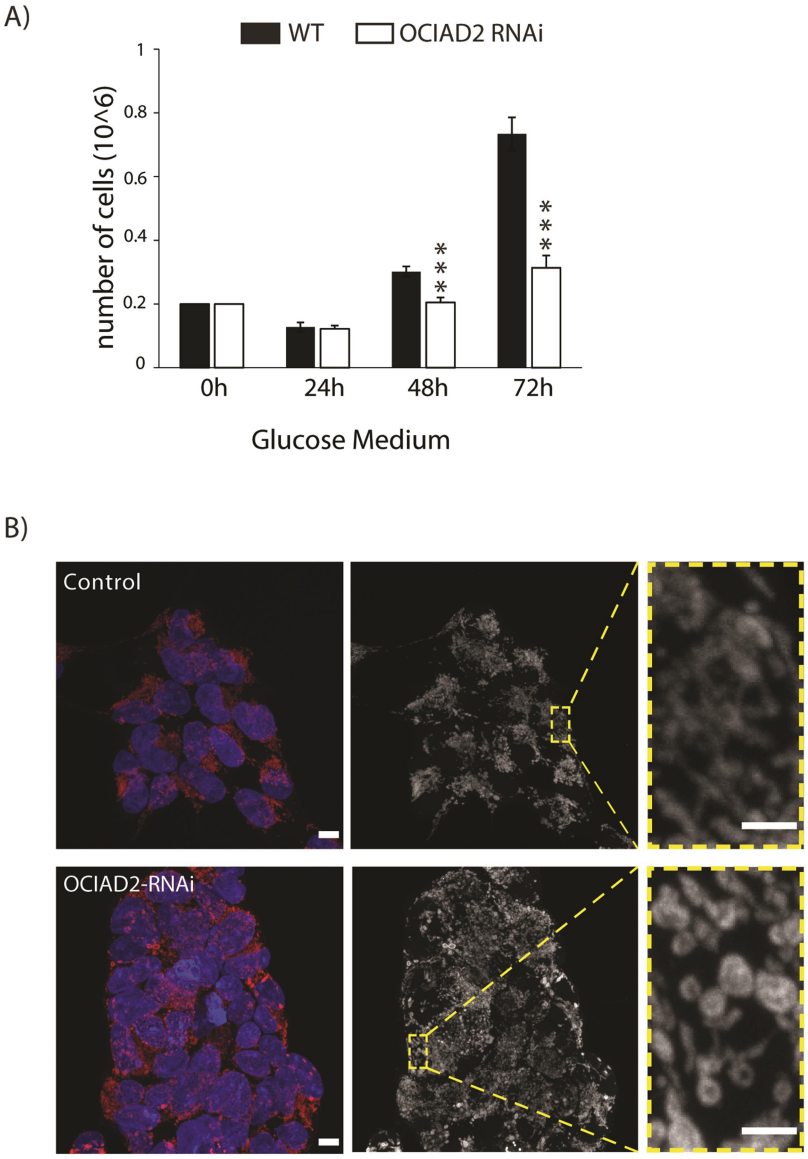
Supplementary Figure S4: Overexpression of ^{FLAG}OCIAD2 in OCIAD2-KO cells. (A) Expression of ^{FLAG}OCIAD2 was analyzed by western blotting. (B) Isolated mitochondria from WT, OCIAD-KO, ^{FLAG}OCIAD2 overexpressed in OCIAD2-KO cells were analyzed by BN-PAGE and western

blotting. (C) Respiration of digitonin-permeabilized cells to assess isolated complex I, complex III and complex IV activity in control, OCIAD2-KO and OCIAD2-KO overexpressing ^{FLAG}OCIAD2. (D) Complex I, complex III and complex IV activity assessed by enzymatic assay in control, OCIAD2-KO and OCIAD2-KO overexpressing ^{FLAG}OCIAD2. mean ± SD (n = 3). *p < 0.05 (two-tailed Student's t-test).



Supplementary Figure S5
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Supplementary Figure S5: Replication of OCIAD2-KO cells phenotype by silencing OCIAD2.

(A) Proliferation assay of OCIAD2 RNAi treated cells. 0.2x106 cells/well were seeded and reverse transfected with OCIAD2 RNAi duplex in growth medium. After indicated times, cells were harvested by trypsinization and counted. Data are shown as the mean ± SD (n = 5) ***p<0.01(two-tailed Student's t-test). (B) Control and OCIAD2 RNAi duplex treated cells were immunolabelled using antibody CyclophilinD to visualize mitochondria. Scale bar 16 μm.