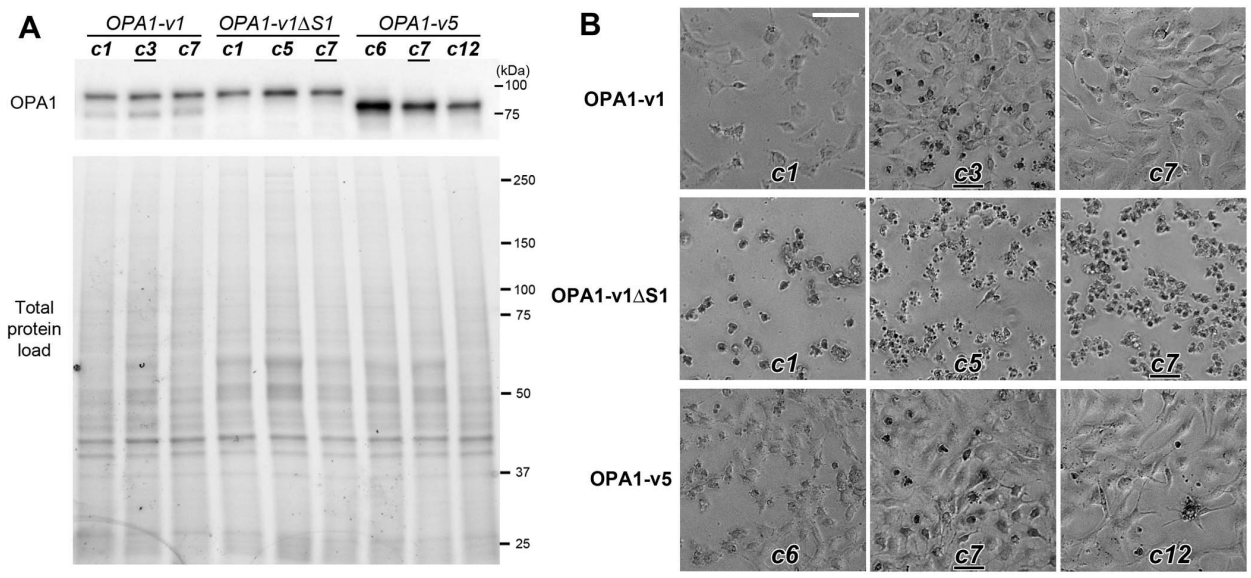
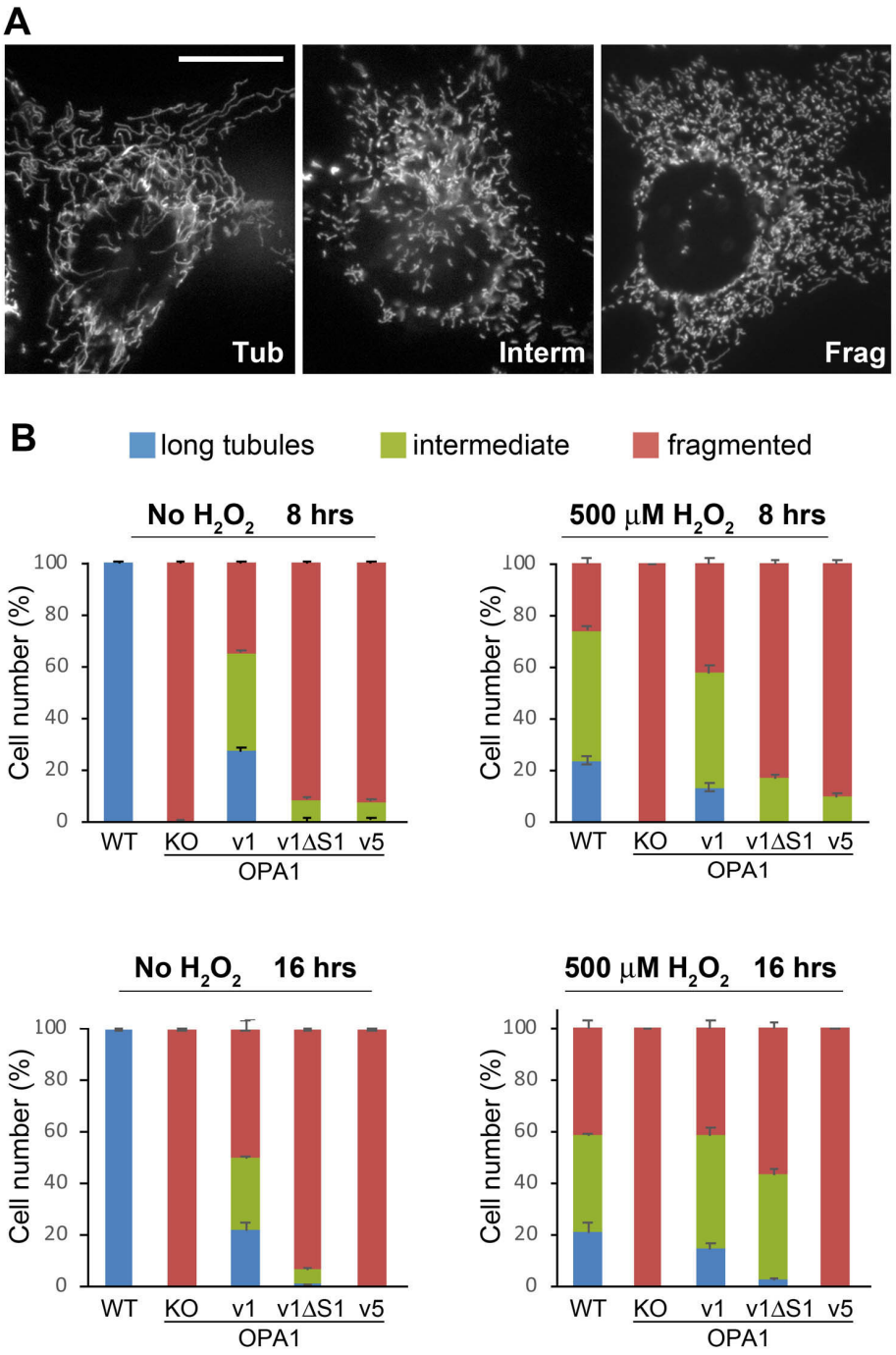
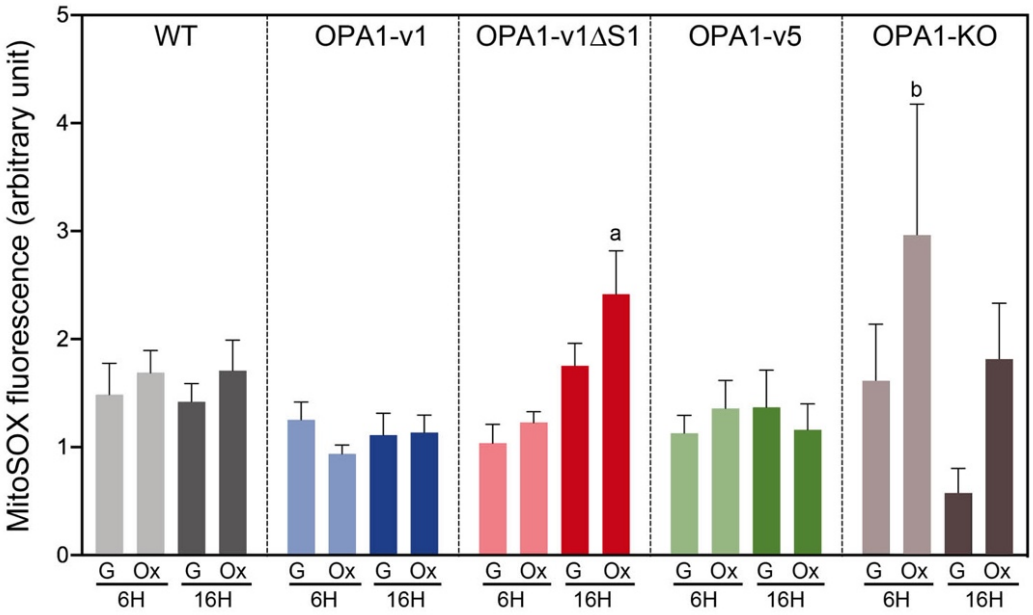


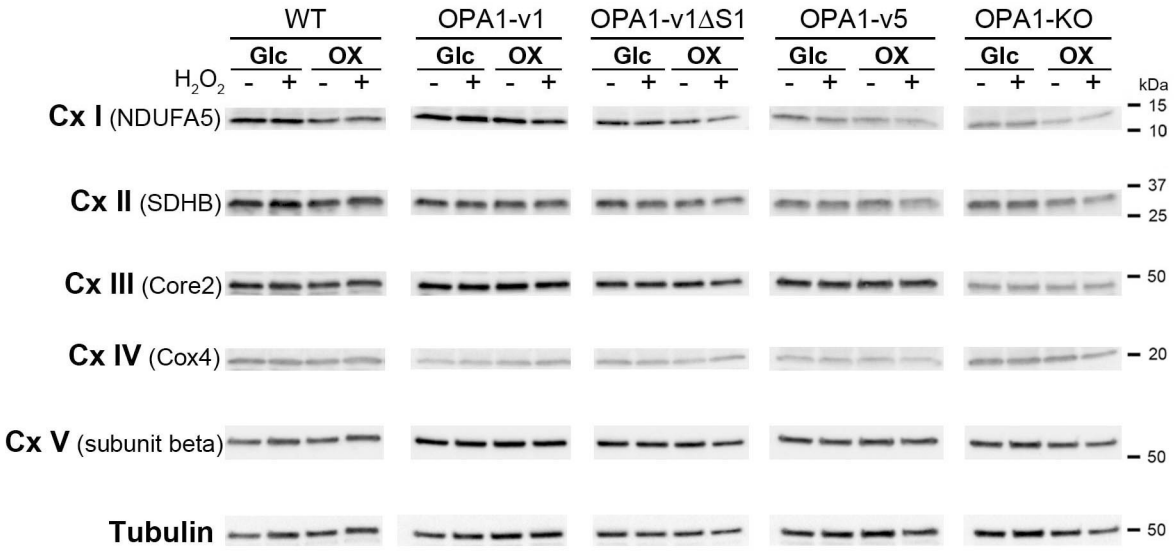
Supp Fig. S1. Expression levels and sensitivity to H_2O_2 in different clones of OPA1 variants. Three different clones of OPA1-v1 (c1, c3, and c7), OPA1-v1ΔS1 (c1, c5, and c7), and OPA1-v5 (c6, c7, and c12) were tested. (A) OPA1-v1-c3, OPA1-v1ΔS1-c7, and OPA1-v5-c7 were selected based on their expression levels similar to the overall OPA1 levels in WT MEFs. (B) Bright field images of different OPA1 variant clones incubated in 500 μM H_2O_2 for 24 hours. All three clones of OPA1-v1ΔS1 were shrunk and detached, whereas clones of OPA1-v1 and OPA1-v5 were mostly well spread and attached. Scale bar: 100 μm.



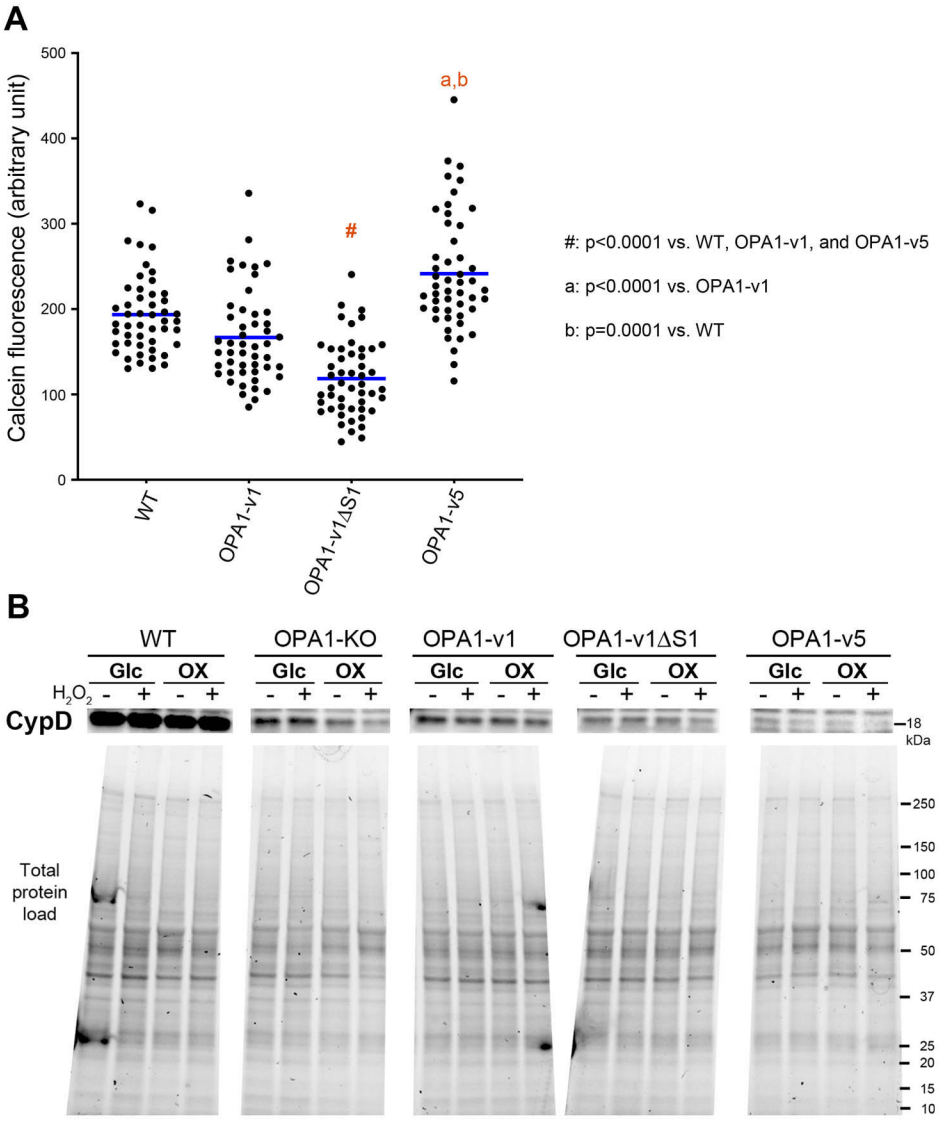
Supp Fig. S2. Mitochondrial morphology of OPA1 variant cells in H_2O_2 treatment under OXPHOS conditions. (A) Representative tubular, intermediate, and fragmented mitochondrial morphologies. Scale bar: 20 μm. (B) Mitochondrial morphology quantification. WT cells show increase of mitochondrial shortening and fragmentation with H_2O_2 treatment, while other cells mostly maintain original morphologies.



Supp Fig. S3. MitoSOX assays. OPA1 variant cells, WT, and OPA1-KO cells were incubated glucose-containing (G) or glucose-free OXPHOS (Ox) media, and MitoSOX fluorescence was evaluated at 6 and 16 hours. One-way ANOVA, Turkey's multiple comparisons. Error bars, SEM.



Supp Fig. S4. Immunoblots for respiratory complex proteins. Immunoblots for subunits of each respiratory complex in OPA1 variant cells treated with and without 500 μM H_2O_2 for 16 hours in glucose and OXPHOS conditions.



Supp Fig. S5. Mitochondrial calcein fluorescence and CypD immunoblot. (A) OPA1 variant cells along with WT cells were treated with 500 μM H_2O_2 in OXPHOS conditions, and mitochondrial calcein fluorescence was evaluated after 16 hours. OPA1-v5 cells retained higher calcein fluorescence than other cells, indicating OPA1-v5 rendering reduced sensitivity to MPT. n = 49 – 50. One-way ANOVA with Turkey's multiple comparisons. Horizontal bars, mean. (B) CypD immunoblots of OPA1 variant cells treated with and without 500 μM H_2O_2 for 16 hours in glucose and OXPHOS conditions.