Expanded View Figures

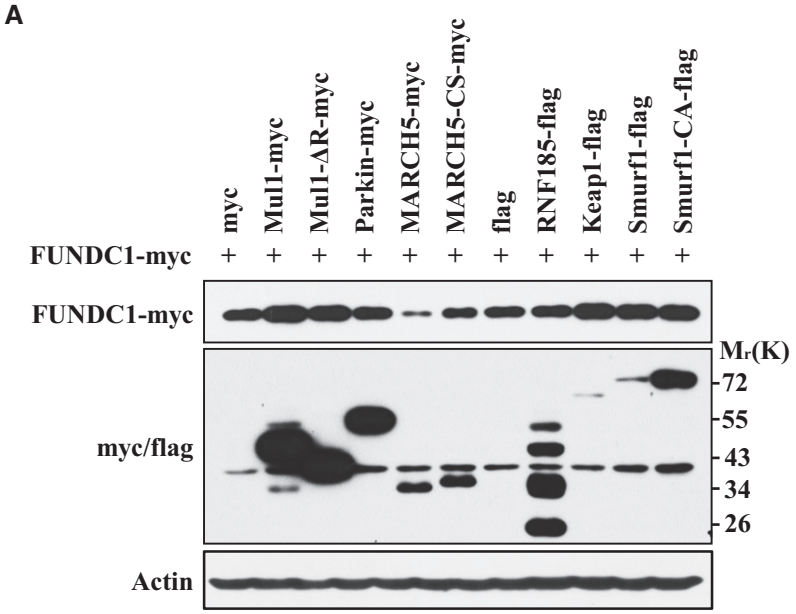
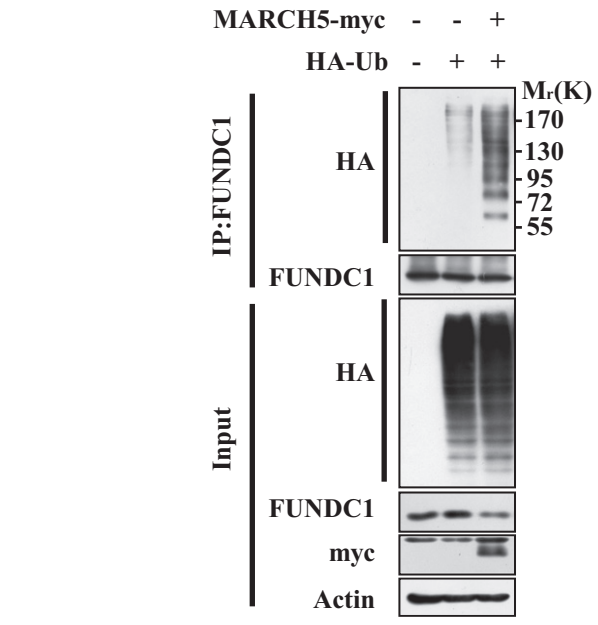


Figure EV1. Identification of MARCH5 as the E3 ligase for FUNDC1 degradation.

A *FUNDC1*-knockdown HeLa cells were transfected with FUNDC1-myc for 24 h, and then with plasmids expressing the indicated mitochondria-associated E3 ligases. FUNDC1-myc protein level was detected by Western blotting.

B HeLa cells were transfected with MARCH5-myc or the empty myc-vector together with HA-Ub for 24 h. Ubiquitylation assays were performed as described in Materials and Methods, and ubiquitylated FUNDC1 was detected using an anti-HA antibody. FUNDC1 and MARCH5-myc expression was detected by Western blotting.

Source data are available online for this figure.



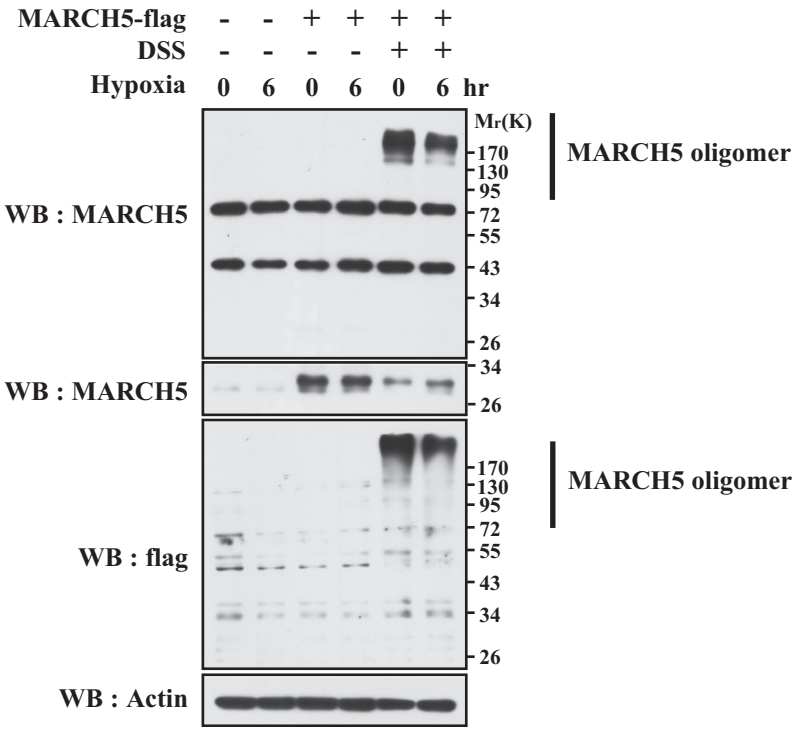


Figure EV2. Hypoxic stress promotes disassembly of MARCH5 homo-oligomer.

HeLa cells expressing MARCH5-flag were exposed to 1% O_2 for the indicated time, and then isolated cells were treated with DSS (1 mM) for 10 min. MARCH5 polymerization was detected by Western blotting analysis with anti-flag and anti-MARCH5 antibodies.

Source data are available online for this figure.

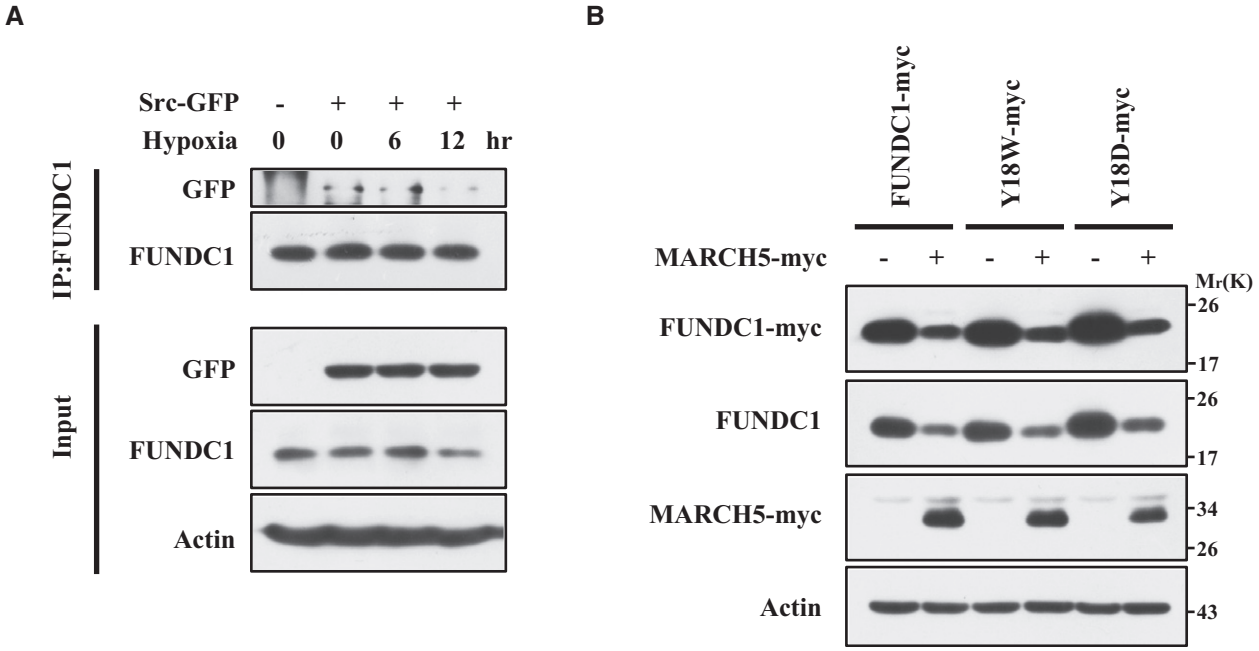


Figure EV3. The interaction between FUNDC1 and Src is significantly decreased upon prolonged hypoxic stress.

A HeLa cells were transfected with Src-GFP for 24 h and then exposed to 1% O_2 for the indicated time. Immunoprecipitation was performed with an anti-FUNDC1 antibody. Co-immunoprecipitated Src-GFP and FUNDC1 were detected by Western blotting with anti-GFP and anti-FUNDC1 antibodies, respectively.

B *FUNDC1*-knockdown HeLa cells were transfected with FUNDC1-myc, FUNDC1-Y18W-myc, and FUNDC1-Y18D-myc together with MARCH5-myc for 24 h, and then FUNDC1-myc protein level was detected by Western blotting.

Source data are available online for this figure.

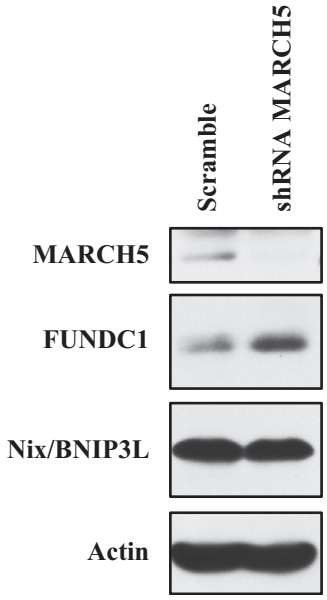


Figure EV4. MARCH5 is not responsible for Nix/BNIP3L degradation.
HeLa cells stably expressing MARCH5 shRNA or scramble plasmid were harvested and lysed for Western blotting. MARCH5, FUNDC1, and Nix/BNIP3L were detected using anti-MARCH5, anti-FUNDC1, and anti-Nix/BNIP3L antibodies, respectively.

Source data are available online for this figure.

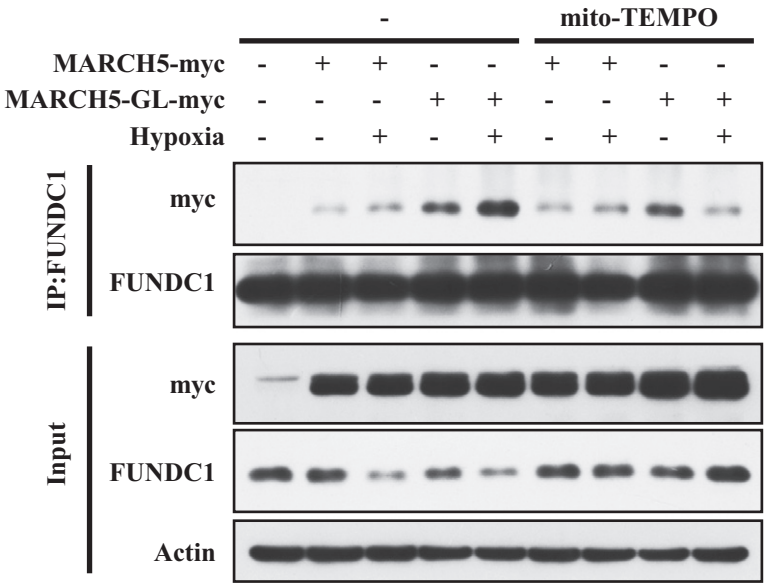


Figure EV5. Hypoxia-induced ROS generation promotes MARCH5–FUNDC1 interaction.
HeLa cells were transfected with MARCH5-myc or the MARCH5-GL-myc mutant for 24 h and then exposed to 1% O_2 for 12 h, with or without mito-TEMPO (10 μM). Immunoprecipitation was performed with an anti-FUNDC1 antibody. Co-immunoprecipitated MARCH5-myc and FUNDC1 were detected by Western blotting with anti-myc and anti-FUNDC1 antibodies, respectively.

Source data are available online for this figure.