A

<table>
<thead>
<tr>
<th></th>
<th>myc</th>
<th>Mull-myc</th>
<th>MARCH5-myC</th>
<th>Parkin-myc</th>
<th>MARCH5-CS-myc</th>
<th>flag</th>
<th>RNF185-flag</th>
<th>Keap1-flag</th>
<th>Smurfl-flag</th>
<th>Smurfl-CA-flag</th>
</tr>
</thead>
<tbody>
<tr>
<td>FUNDC1-myc</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

**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.
Hypoxic stress promotes disassembly of MARCH5 homo-oligomer.

HeLa cells expressing MARCH5-flag were exposed to 1% O₂ 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.

---

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% O₂ 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% O2 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.