Materials and Methods

Animal care and experimentation

All animal experiments and animal care were conducted in accordance with the *Guides for the Care and Use of Laboratory Animals* (Washington, DC, National Academy press, 1996). All protocols used in this study were approved by the laboratory Animal Care Committee of Medicine, National Taiwan University.

General surgical procedures

Rats were anesthetized with sodium pentobarbital (35 mg/kg, i.p). The trachea was intubated to keep the airway patent, and the right jugular vein was catheterized for infusion of saline at a rate of 1.2 ml h\(^{-1}\). The left carotid artery was catheterized for continuous measurement of systemic blood pressure. The mean blood pressure (MABP) was continuously displayed on a polygraph (Gould, Quincy, MA).

Measurement of oxidative stress after intestinal I/R injury

In order to quantitate the lipid peroxidation in oxidative stress, we measured the amount of malondialdehyde (MDA), a product of lipid peroxidation, in the intestinal mucosa tissue after intestinal I/R injury [1]. The procedure was performed using commercial kit (Biovision, CA). The mucosa of distal ileum
Supplemental Digital Content (SDC)

was procured and homogenized. The MDA in the sample reacted with the added thiobarbituric acid (TBA) to form MAD-TBA adduct which was quantified by colorimetry ($\lambda = 532$ nm). The results of analysis were expressed per milligram of proteins measured in the homogenates.

SDC references:

## SDC Table S1. The height of crypt, villi and C/V ratio after intestinal I/R injury

<table>
<thead>
<tr>
<th>Groups</th>
<th>Crypt height (µm)</th>
<th>Villus height (µm)</th>
<th>C/V ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>143.28 ± 12.39</td>
<td>360.15 ± 37.26</td>
<td>0.38 ± 0.03</td>
</tr>
<tr>
<td>I/R</td>
<td>140.09 ± 18.17</td>
<td>202.32 ± 34.34</td>
<td>0.73 ± 0.12</td>
</tr>
<tr>
<td>IPoC</td>
<td>152.47 ± 5.35</td>
<td>389.18 ± 22.09</td>
<td>0.40 ± 0.03*</td>
</tr>
<tr>
<td>I/R+NIM811</td>
<td>141.01 ± 16.49</td>
<td>323.19 ± 33.75</td>
<td>0.37 ± 0.02*</td>
</tr>
<tr>
<td>IPoC+ CATR</td>
<td>151.12 ± 19.84</td>
<td>180.12 ± 12.62</td>
<td>0.80 ± 0.08**</td>
</tr>
</tbody>
</table>

(C: crypt; V: villi; * < 0.05 when compared with I/R group; ** < 0.05 when compared with IPoC group)
Figure S1. Experimental protocol.

Rats were randomized into five groups (n= 6 in each group). Sham group receive laparotomy only; I/R group received 30 minutes’ ischemia and 60 minutes’ reperfusion. Ischemic postconditioning (IPoC) group received 3 cycles of 30 seconds’ reperfusion followed by 30 seconds’ ischemia at the end of ischemia; Carboxyatractyloside (CATR, 10 mg kg\(^{-1}\)) was injected 5 minutes before intestinal ischemia in IPoC+CATR group; NIM811 (10 mg kg\(^{-1}\)) was infused in peritoneal cavity 15 minutes before onset of intestinal reperfusion in control +NIM 811 group. (w= with; w/o =without)
Figure S2. The expression of malondialdehyde (MDA) in small intestine after I/R injury.

Significant elevation of MDA was noted in the I/R group after 60 minutes' reperfusion. IPoC and NIM811 showed less increase of MDA level when compared with I/R group ($p<0.05$). The treatment of CATR, a mPTP opener, negated the protection offered by IPoC.