

Characterizing Autophagy in the Cold Ischemic Injury of Small Bowel Grafts: Evidence from Rat Jejunum.

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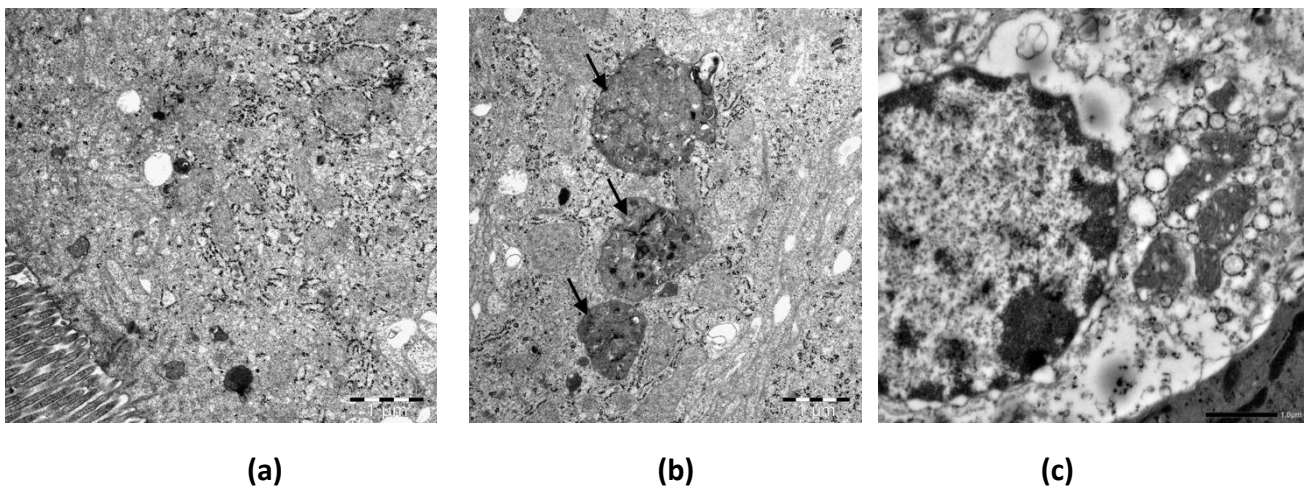
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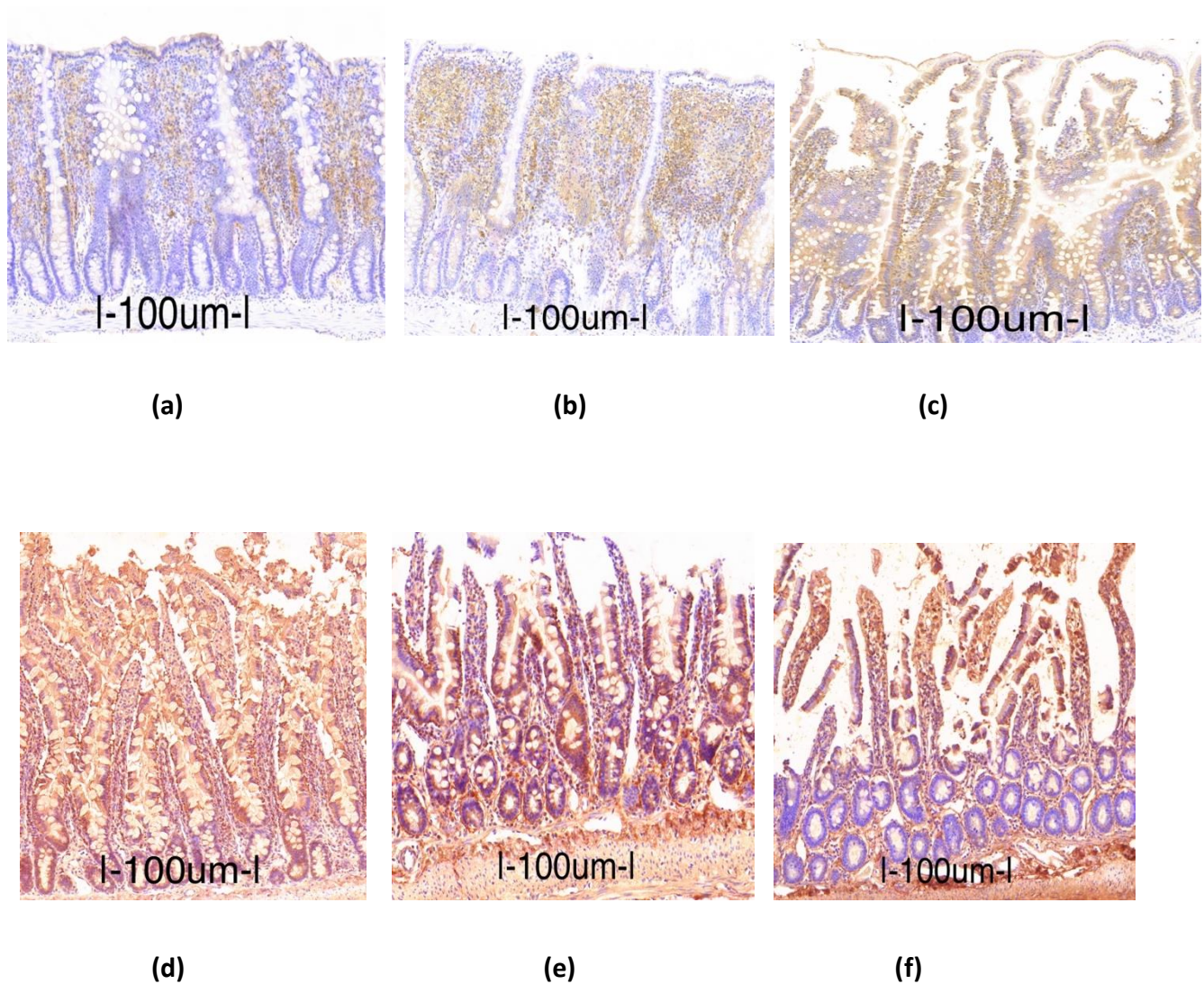
Supplementary Materials

S. 1: Transmission Electron Microscope photographs.

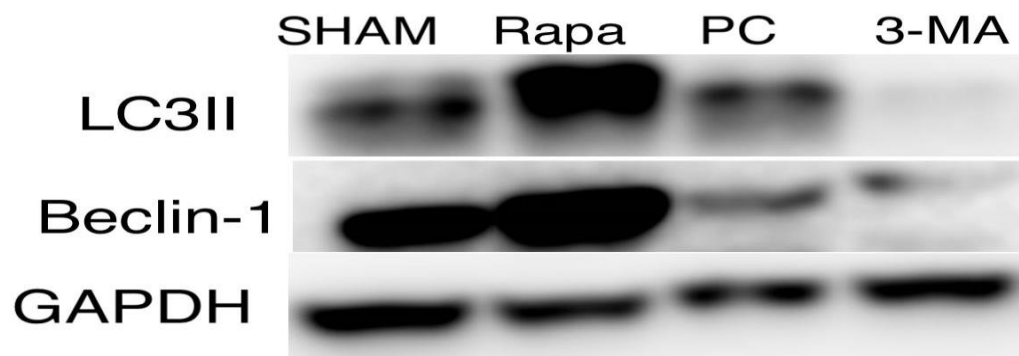


S. 1 – Representative TEM images from the cytoplasm of intestinal villi epithelial cells. Scale bar: 1.0μm. Panels a represents the 0hr group; Panel b represents the 6hr and Panels c represents 12hr group. In the 6hr group, the presence of autophagosomes (membrane bound structure containing cytoplasmic structures: black arrows) are clearly visible.

S. 2: Changes in autophagy after pharmacological manipulation using rapamycin and 3-methyladenine.



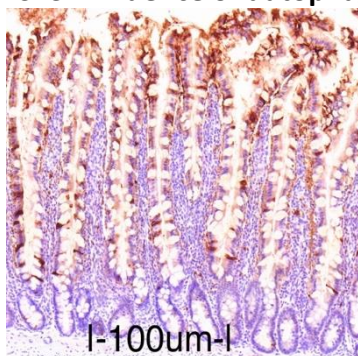
S. 2.1 – Immunostaining for p62 and Beclin-1. Panel a-c represent changes in the immunostaining for p62 protein after 6 hours of preservation. Panel a represents the Rapa group; Panel b represents the PC group; Panel c represents the 3-MA group. Panel d-f represent changes in immunostaining for Beclin-1 at the end of preservation (12 hours). Panel d represents the Rapa group; Panel e represents the PC group; Panel f represents the 3-MA group. Both proteins stain the cytoplasm of the intestinal epithelial cells brown. Scale bar: 100um.



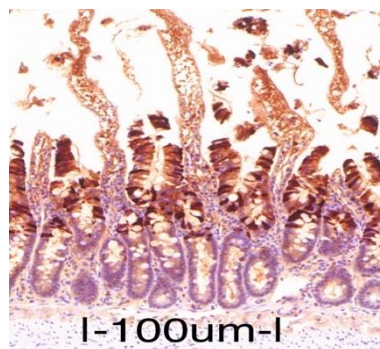
(a)

S2.2 – Panel a show the changes in electrophoresis pattern for LC3II and Beclin-1 at the end of the preservation period.

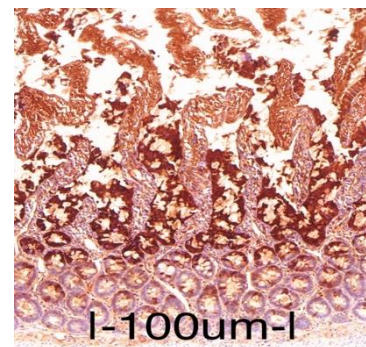
S. 3: Influence of autophagy on apoptosis



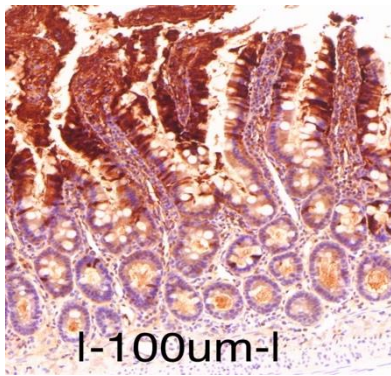
(a)



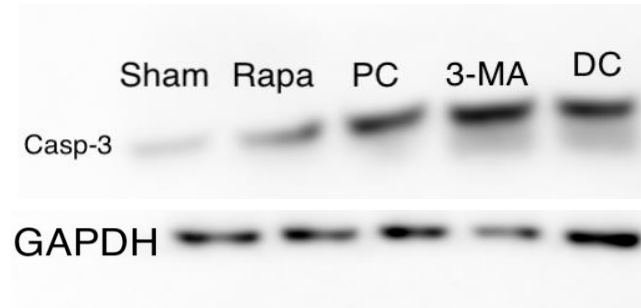
(b)



(c)



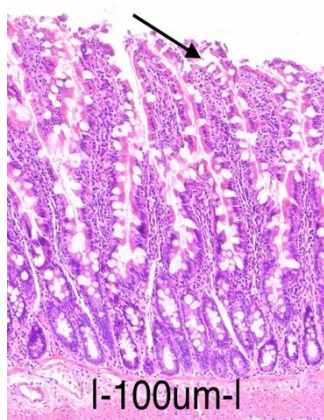
(d)



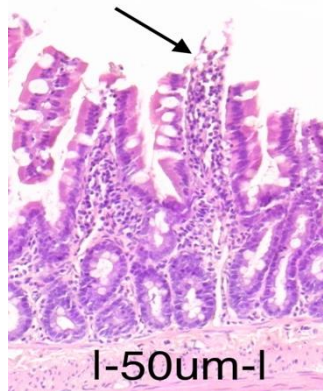
(e)

S. 3 – Effect of autophagy on apoptosis after 12 hours of preservation. Panel a-d are picture representation of immunostaining for active caspase-3. Dark brown staining of the cytoplasm is considered a positive stain. The Rapa group is represented in Panel a showing few positive stains. Panel b represents the PC group, showing more positive cells than the Rapa group. 3-MA group is represented by Panel C, here there is a strong positivity for caspase-3. The DC group (Panel D) also shows positive cytoplasmic stain. When compared the Rapa group has the least positivity among the groups. Panel e shows the representative electrophoresis pattern for the cleaved caspase-3.

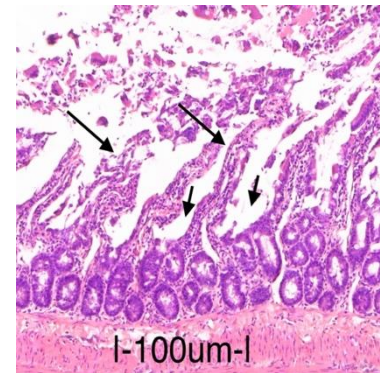
S. 4: Influence of autophagy on preservation injury



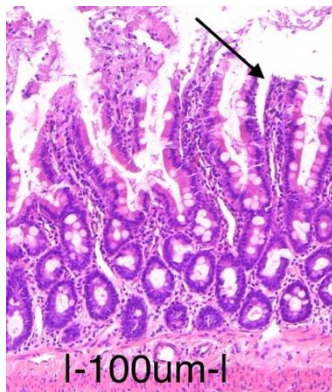
(a)



(b)



(c)



(d)

S. 4.1 – effect of autophagy on the intestinal mucosa. Panel a-d represent pictures from HE staining of the small bowel graft after 12 hours of preservation. Panel a is from the Rapa group, the arrow points to extended epithelial space at the tip of the villus (Park/Chiu score 2) which was characteristic for mucosa of this group. Panel b is from the PC group, arrow points to the characteristic incomplete denudation of villi with some loss of villous tissue (Park/Chiu score 4-5). Panel c is from the 3-MA group which shows complete villous denudation (long arrow) and some loss of villous tissue (short arrow) (Park/Chiu =5). Panel d is from the DC group, the epithelium also shows regions of villous tissue loss and incomplete denudation (arrow) (Park/Chiu score 4-5).

Groups	Sham	Rapa	PC	3-MA	DC
LDH (IU/L)	25.61±1.49	425.61±11.79#	593.30±22.68	685.40±40.32 #	558.00±20.16 ^
Lactate (mmol/l)	0.06±0.01	1.06±0.11#	1.55±0.06	1.83±0.04#	1.53±0.14^.

S. 4.2 – Table showing changes in Ldh and lactate levels at the end of the 12-hour long cold storage. Data are mean \pm SEM. #P < 0.05 versus PC. ^P < 0.05 DC versus Rapa group.

S.5: Reperfusion Injury

Groups	Sham	Rapa	PC	3-MA
Park/Chiu scores	0.94 \pm 2.75	2.86 \pm 0.11#	5.04 \pm 0.11	5.76 \pm 0.12
LDH (IU/L) - 1 min	72.71 \pm 9.66	103.680 \pm 6.01#	145.20 \pm 10.38	183.30 \pm 12.64#
LDH (IU/L) - 60min	210.80 \pm 16.45	332.47 \pm 198.17#	433.23 \pm 20.46	530.20 \pm 16.53#

S.5 – Table showing changes in Park/Chiu scores and Ldh levels corresponding to the reperfusion injury. Data are mean \pm SEM. #P < 0.05 versus PC. group.