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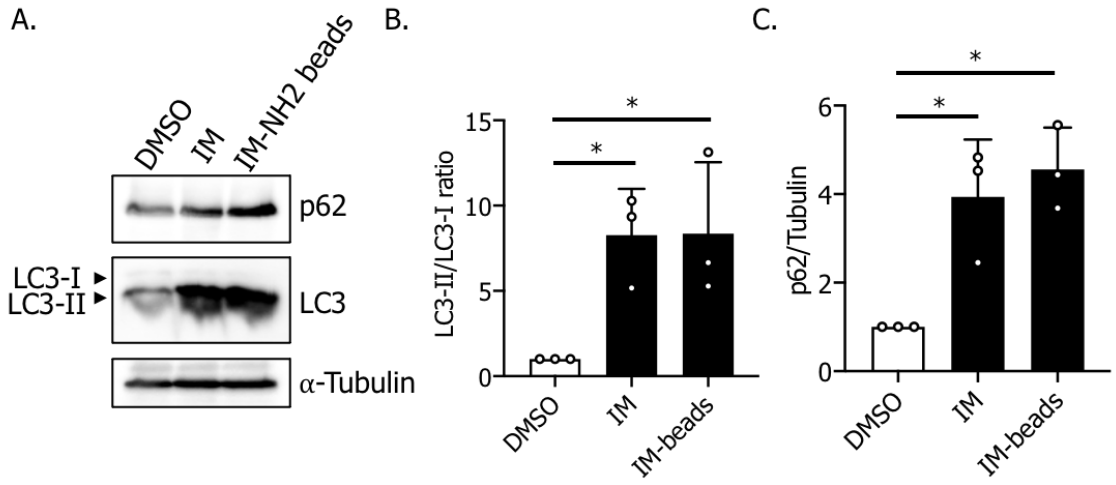
Rapamycin and Starvation Mitigate Indomethacin-Induced Intestinal Damage through Preservation of Lysosomal Vacuolar ATPase Integrity

Makoto Shirakawa[†], Shunichi Yokoe[†*], Takatoshi Nakagawa, Kazumasa Moriwaki, Toshihisa Takeuchi, and Michio Asahi[*]

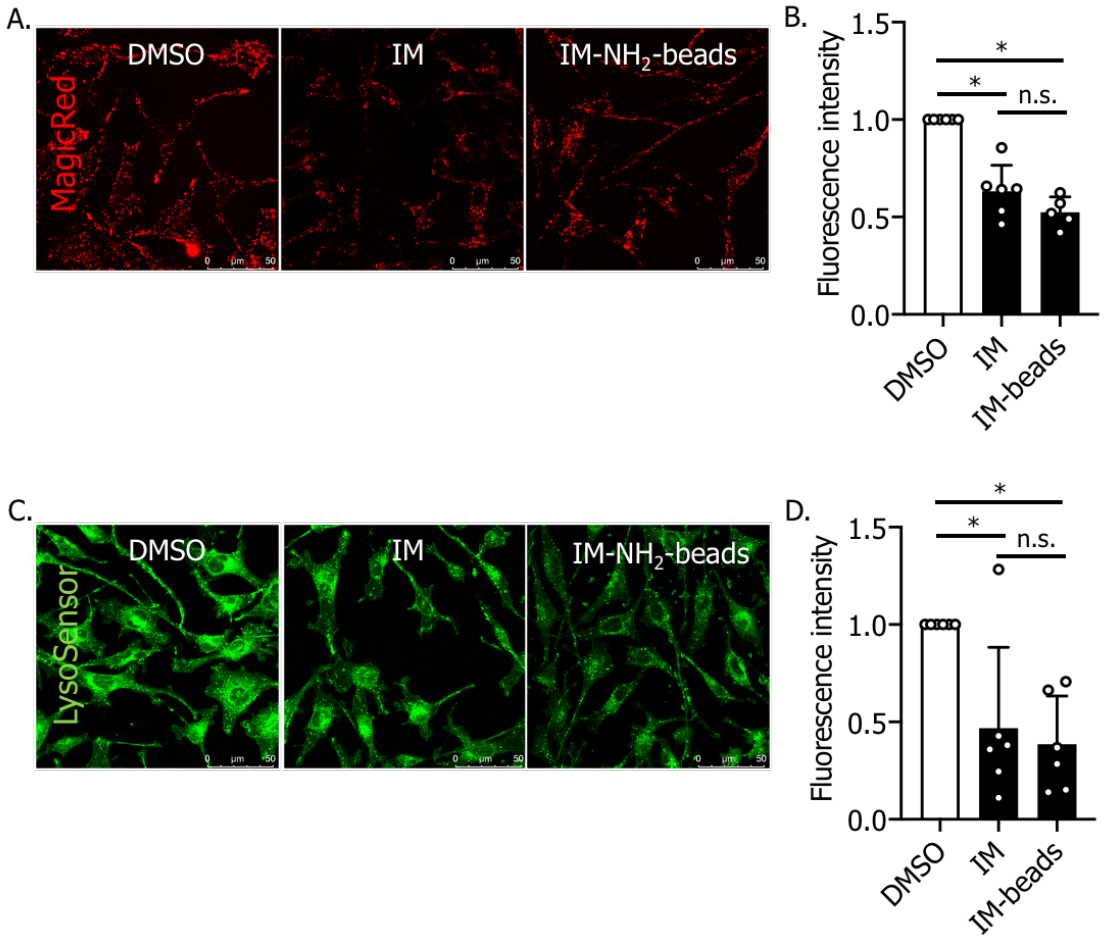
Department of Pharmacology, Faculty of Medicine, Osaka Medical and Pharmaceutical University, 2-7, Daigaku-machi, Takatsuki, Osaka 569-8686, Japan (M.S., S.Y., K.M., M.A.); Department of Regenerative Dermatology, Graduate School of Medicine, Osaka University, Osaka 565-0871, Japan (T.N.); The Second Department of Internal Medicine, Osaka Medical and Pharmaceutical University, 2-7, Daigaku-machi, Takatsuki, Osaka 569-8686, Japan (T.T).

[†]These authors contributed equally to this work.

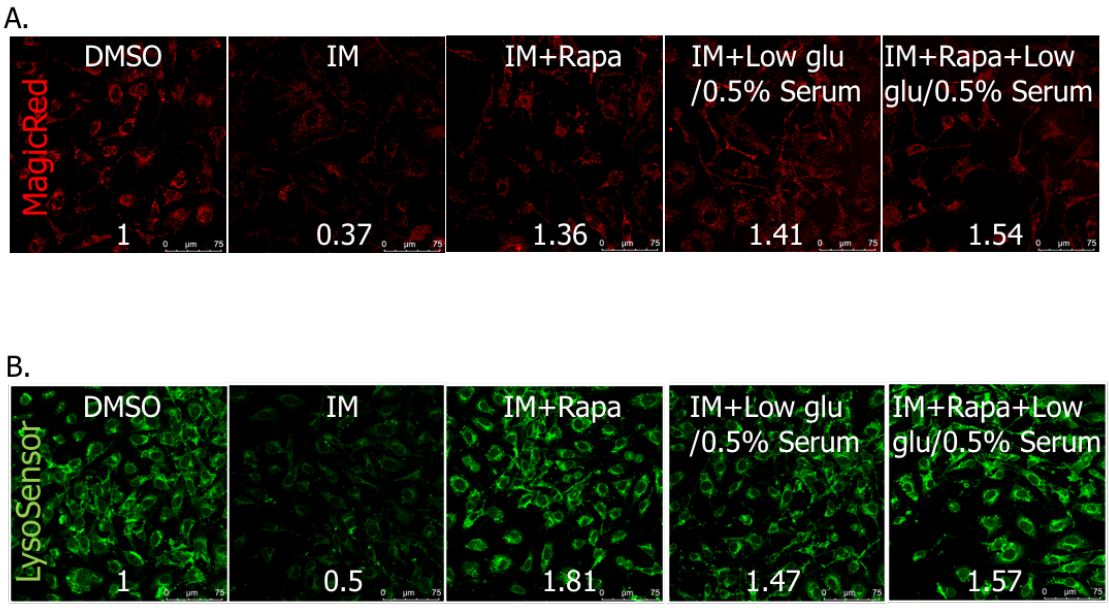


Supplemental Figure 1. IM-immobilized beads disrupt autophagy flux in IEC6 cells without altering the bioactivity of IM.

(A) IEC6 cells were exposed to either IM or IM-immobilized beads. Subsequently, LC3 and p62 expression levels were assessed using western blot analysis. (B–C) Quantitative analyses of the LC3-II/LC3-I ratio (B) and p62 expression (C) as depicted in (A). Values are presented as the mean ± SD (n = 3) (*$p < 0.05$).



Supplemental Figure 2. IM-immobilized beads decrease both the activity and acidification of lysosomes in IEC6 cells without altering the bioactivity of IM.
(A) IEC6 cells were exposed to either indomethacin (IM) or IM-immobilized beads and subsequently stained with Magic Red dye to evaluate lysosomal activity. (B) Quantitative analysis of the Magic Red fluorescence intensity per cell shown in (A). Values are presented as mean ± SD (n=5-6) (*$p < 0.05$). (C) IEC6 cells were treated with either IM or IM-immobilized beads and then stained with LysoSensor dye to determine lysosomal pH. (D) Quantitative assessment of LysoSensor fluorescence intensity per cell as depicted in (C). Values are denoted as mean ± SD (n = 5–6) (*$p < 0.05$).



Supplemental Figure 3. IM reduces lysosomal activity and acidification, but these effects are mitigated by rapamycin (Rapa) treatment, low glucose conditions, and with a combination of both Rapa and low glucose conditions in IEC6 cells.
(A) IEC6 cells pretreated with Rapa alone or low glucose/0.5% serum or a combination of Rapa and low glucose/0.5% serum were exposed to 200 μM IM and subsequently stained with Magic Red dye to assess lysosomal activity. (B) Quantitative analysis of the Magic Red fluorescence intensity per cell shown in (A). (C) IEC6 cells pretreated with Rapa alone or low glucose/0.5% serum or a combination of Rapa and low glucose/0.5% serum were exposed to 200 μM IM and subsequently stained with LysoSensor dye to measure lysosomal pH. (D) Quantitative evaluation of LysoSensor fluorescence intensity per cell as depicted in (C).