

# Neutrophil elastase inhibition improves intestinal mucosal damage and gut microbiota in a mouse model of 5-fluorouracil-induced intestinal mucositis

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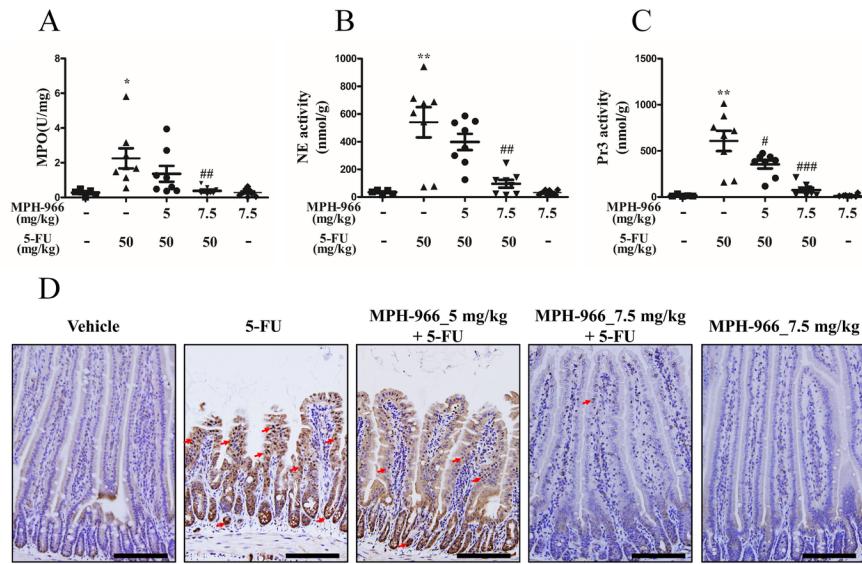
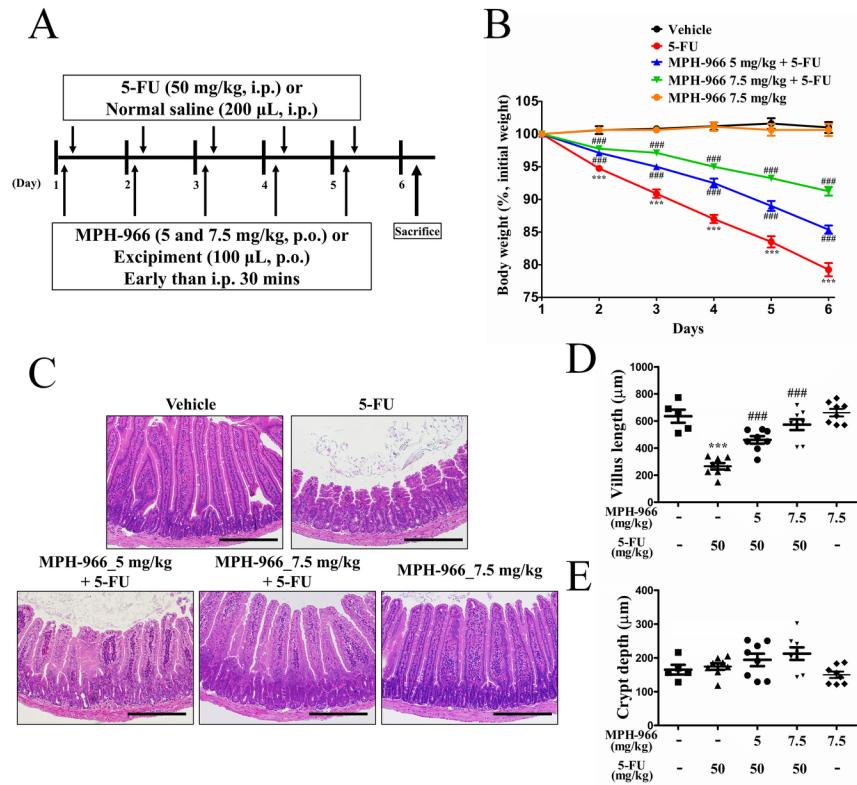
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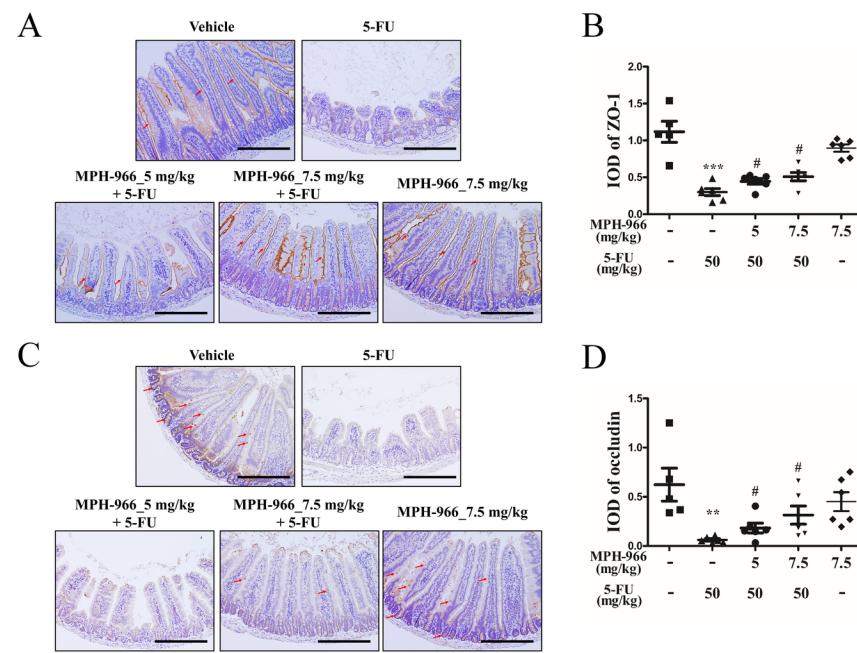
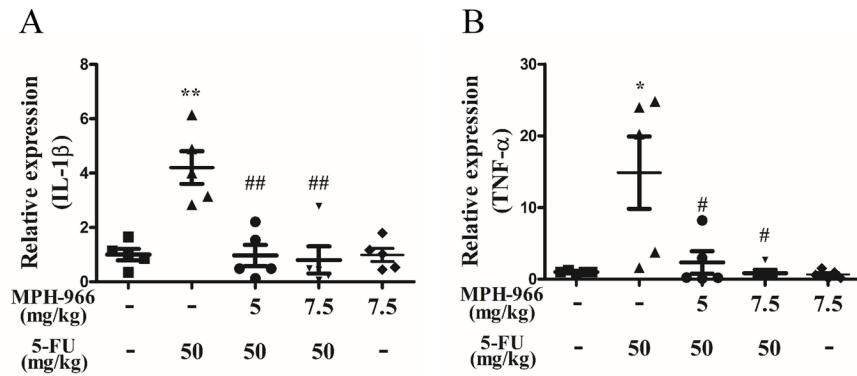
## Abstract

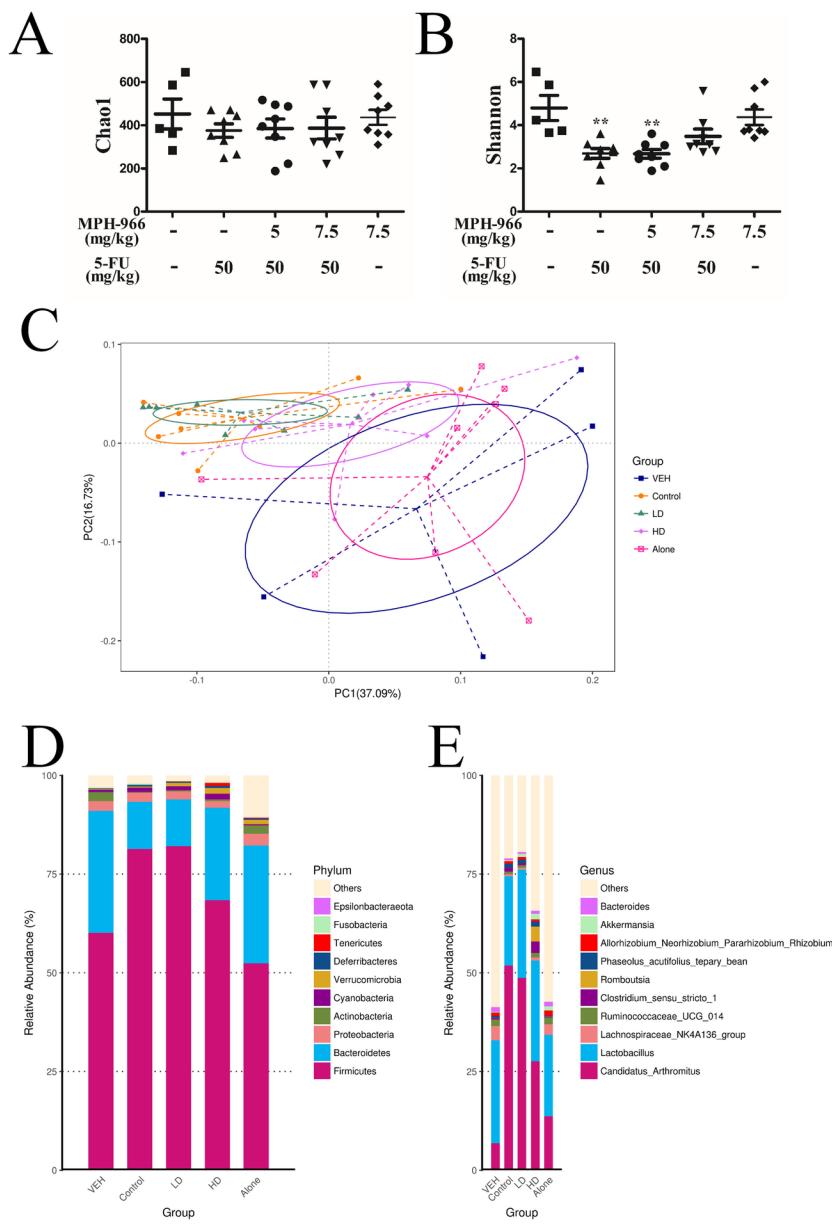
**Background and Purpose:** 5-Fluorouracil (5-FU)-based chemotherapy is the first-line chemotherapeutic agent for colorectal cancer. However, 5-FU-induced intestinal mucositis (FUIIM) is a common adverse effect that severely impairs drug tolerance and results in poor patient health. **Experimental Approach:** Neutrophil elastase (NE) overexpression contributes to FUIIM via abnormal inflammatory responses, microbiota imbalance, and tissue damage. Therefore, restoring NE homeostasis could prevent or improve FUIIM. **Key Results:** This study shows that treatment with the specific NE inhibitor MPH966 (7.5 mg/kg; p.o.) significantly reversed 5-FU-induced losses in body weight; reversed villus atrophy; significantly suppressed myeloperoxidase, NE, and proteinase 3 activity; and reduced pro-inflammatory cytokine levels in a mouse model of FUIIM. In addition, MPH966 prevented 5-FU-induced intestinal barrier dysfunction, as was indicated by modulated expression of the tight junction proteins zonula occludin-1 and occludin. MPH966 also reversed 5-FU-induced changes in gut microbiota diversity and abundances, specifically the Firmicutes-to-Bacteroidetes ratio; Muribaculaceae, Ruminococcaceae, and Eggerthellaceae abundances at the family level; and *Candidatus Arthromitus* abundance at the genus level. **Conclusion and Implications:** These data indicate that NE inhibitors are a potential treatment candidate to alleviate FUIIM by regulating abnormal inflammatory responses, intestinal barrier dysfunction, and gut microbiota imbalance.

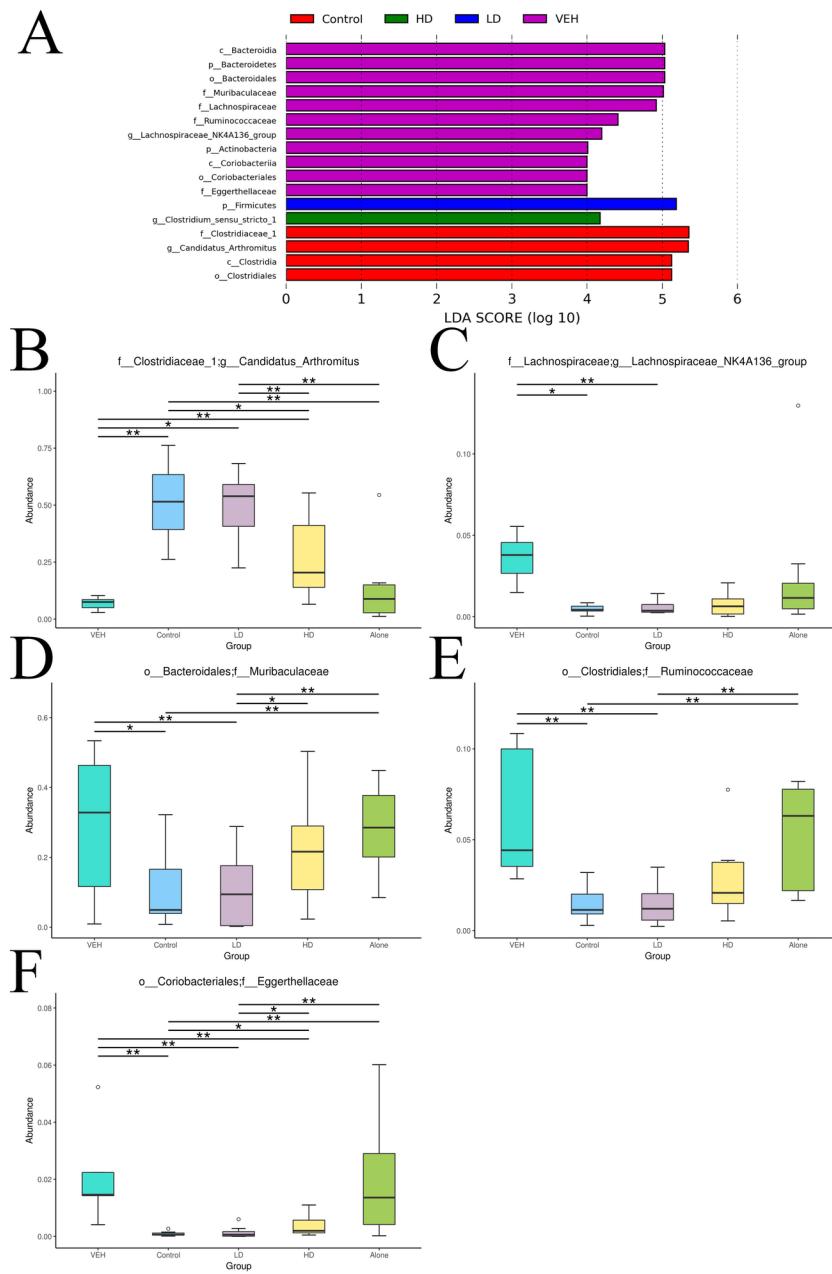
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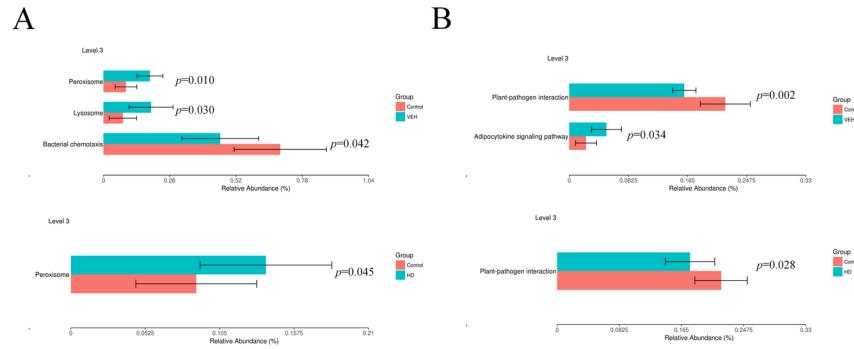
Main Text.pdf available at <https://authorea.com/users/307244/articles/438205-neutrophil-elastase-inhibition-improves-intestinal-mucosal-damage-and-gut-microbiota-in-a-mouse-model-of-5-fluorouracil-induced-intestinal-mucositis>











**Table 1. Primers sequences**

Gene	Direction and sequence
IL-1 $\beta$	F: 5'-CAGCAGCACATCAACAAGAG-3' R: 5'-GTCACACACCAGCAGGTTAT-3'
TNF- $\alpha$	F: 5'-TGCTCTGTGAAGGGAATGG-3' R: 5'-GGCTCTGAGGAGTAGACAATAAAG-3'
GAPDH	F: 5'-AGCTTGTATCACCGGAAG-3' R: 5'-TTGATGTTAGTGGGTCTG-3'

**Table 2.** The relative abundance of top 10 gut microbiota distributed at the phylum level.

Phyla Relative abundance (%)	Vehicle	5-FU	MPH-966 (5 mg/kg) + 5-FU	MPH-966 (7.5 mg/kg) + 5-FU	MPH-966 (7.5 mg/kg)
Firmicutes	60.09 ± 9.08	81.32 ± 3.70	82.07 ± 3.76	68.41 ± 5.53	52.37 ± 3.74
Bacteroidetes	30.90 ± 9.92	12.00 ± 3.92	11.83 ± 3.83	23.39 ± 5.41	29.90 ± 4.39
Proteobacteria	2.47 ± 0.72	2.30 ± 0.45	2.07 ± 0.46	1.66 ± 0.31	2.93 ± 0.95
Actinobacteria	2.28 ± 0.80	0.17 ± 0.04	0.27 ± 0.07	0.47 ± 0.13	2.13 ± 0.72
Cyanobacteria	0.60 ± 0.24	1.12 ± 0.32	1.05 ± 0.39	1.44 ± 0.35	0.32 ± 0.10
Verrucomicrobia	0.18 ± 0.12	0.25 ± 0.11	0.82 ± 0.34	1.46 ± 1.10	1.10 ± 0.66
Deferribacteres	0.17 ± 0.04	0.43 ± 0.29	0.31 ± 0.10	0.56 ± 0.34	0.34 ± 0.19
Tenericutes	0.03 ± 0.02	0.05 ± 0.03	0.04 ± 0.01	0.70 ± 0.67	0.17 ± 0.13
Fusobacteria	0.17 ± 0.09	0.18 ± 0.09	0.11 ± 0.05	0.12 ± 0.04	0.14 ± 0.09
Epsilonbacteraeota	0.04 ± 0.02	0.03 ± 0.02	0.03 ± 0.01	0.02 ± 0.01	0.07 ± 0.05
Firmicutes / Bacteroidetes (F/B)	1.94	6.78	6.94	2.93	1.75

Values were represented as the mean ± SEM. (n=5 for vehicle group, n=8 for other each groups)

**Table 3.** The relative abundance of top 10 gut microbiota distributed at the genus level.

Genera Relative abundance (%)	Vehicle	5-FU	MPH-966 (5 mg/kg) + 5-FU	MPH-966 (7.5 mg/kg) + 5-FU	MPH-966 (7.5 mg/kg)
<i>Candidatus_Arhromitus</i>	6.86 ± 1.30	51.84 ± 6.52	48.69 ± 5.72	27.60 ± 6.77	13.65 ± 6.25
<i>Lactobacillus</i>	26.07 ± 8.57	22.63 ± 4.44	27.41 ± 3.56	25.53 ± 3.74	20.62 ± 5.54
<i>Lachnospiraceae_NK44136_group</i>	3.61 ± 0.71	0.47 ± 0.10	0.55 ± 0.14	0.79 ± 0.28	2.66 ± 1.51
<i>Ruminococcaceae_UCG_014</i>	1.68 ± 0.66	0.68 ± 0.25	0.57 ± 0.21	1.11 ± 0.34	1.71 ± 0.46
<i>Clostridium_sensu_stricto_1</i>	0.35 ± 0.04	0.99 ± 0.25	0.44 ± 0.09	2.91 ± 2.43	0.17 ± 0.04
<i>Romboutsia</i>	0.05 ± 0.03	0.02 ± 0.01	0.03 ± 0.01	3.76 ± 3.60	0.06 ± 0.04
<i>Phaseolus_acutifolius_teprary_beans</i>	0.42 ± 0.19	0.99 ± 0.28	0.94 ± 0.36	1.28 ± 0.30	0.28 ± 0.10
<i>Allorhizobium_Neorrhizobium_Pararhizobium_Rhizobium</i>	0.91 ± 0.27	0.64 ± 0.15	0.68 ± 0.21	0.55 ± 0.18	1.31 ± 0.56
<i>Akkermansia</i>	0.17 ± 0.12	0.25 ± 0.11	0.82 ± 0.34	1.46 ± 1.10	1.09 ± 0.66
<i>Bacteroides</i>	1.18 ± 0.23	0.46 ± 0.07	0.43 ± 0.09	0.75 ± 0.16	1.15 ± 0.20

Values were represented as the mean ± SEM. (n=5 for vehicle group, n=8 for other each groups)