

## **Supplemental Information**

### **Data Availability Statement**

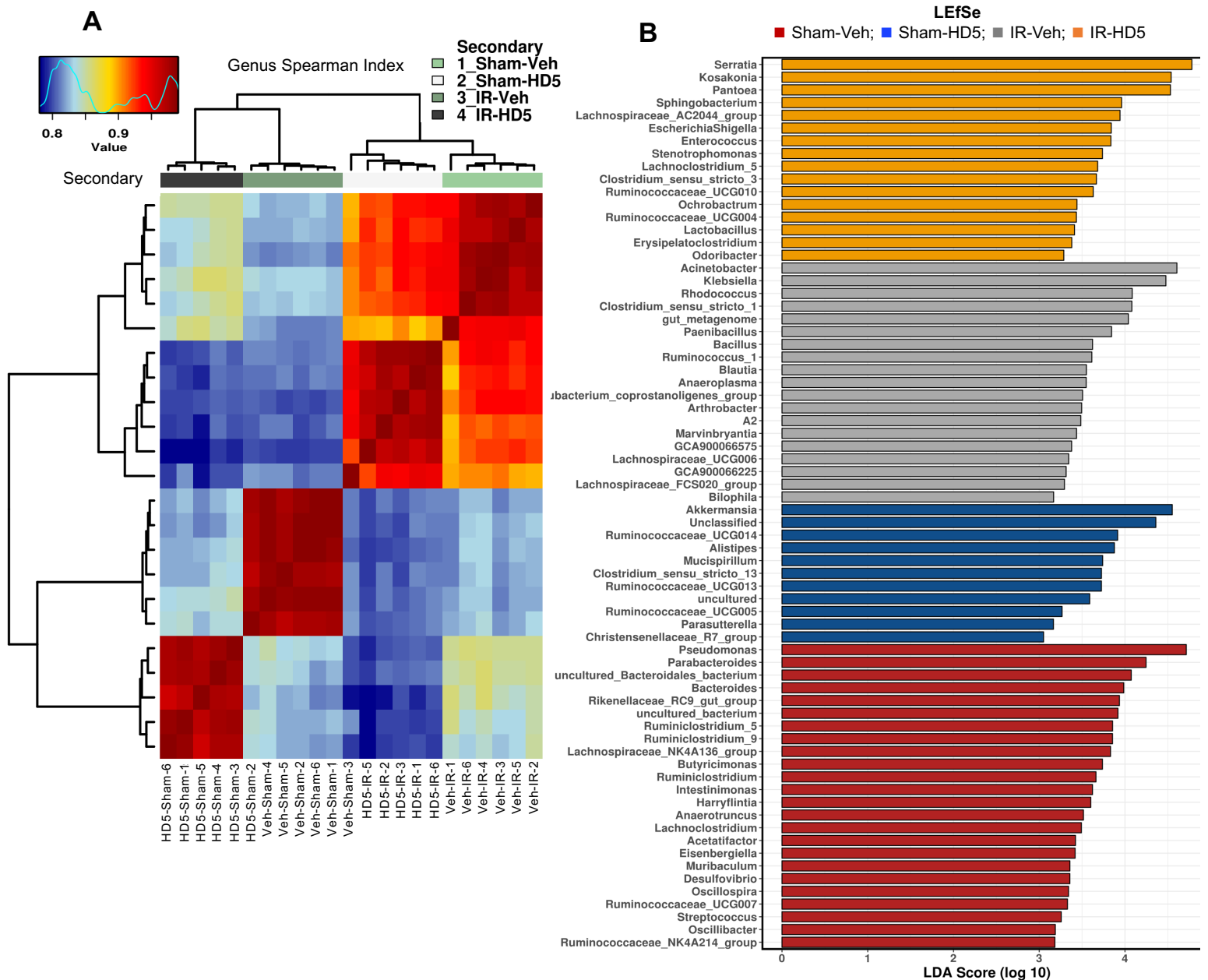
Microbiota metagenomic sequence data are deposited to NCBI BioProject database (NCBI [BioProject ID PRJNA839408](#))

<https://submit.ncbi.nlm.nih.gov/subs/bioproject/SUB11490178/overview>

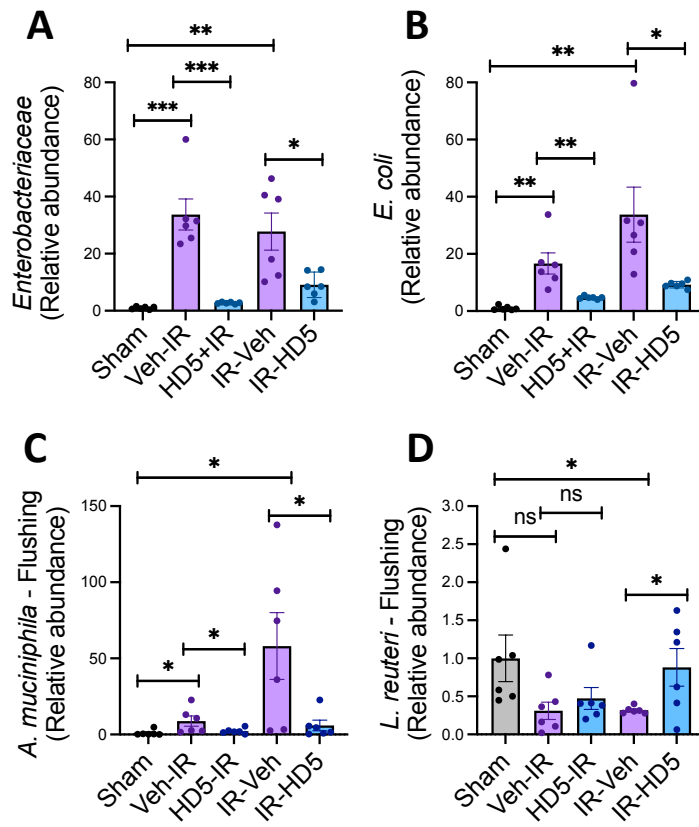
Individual values of data presented in different figures in the main article and the supplemental information are deposited to FigShare Data Repository  
DOI: [10.6084/m9.figshare.19758832](https://doi.org/10.6084/m9.figshare.19758832)

Table S1:	PCR primer sequences
<b>Gene</b>	<b>5'-3' Sequence</b>
23S rDNA ( <i>Enterobacteriaceae</i> ) <i>Enl</i> <i>lsu-1</i>	Forward: TGCCGTA ACTTCGGGAGAAGGCA Reverse: TCAAGGACCAGTG TTCAGTGTC
16S rDNA ( <i>E. coli</i> ) <i>Ecoli</i>	Forward: CATGCCGCGTGTATGAAGAA Reverse: CGGGTAACGTCAATGAGCAAA
<i>L. reuteri</i>	Forward: AGGGTGAAGTCGTAACAAGTAGCC Reverse: CCACCTTCCTCCGGTTTGTC
<i>A. muciniphila</i>	Forward: GAAGACGGAGGACGGAACT Reverse: GCGGATTGCTGACGAAGG
<i>Firmicutes</i>	Forward: TGAAACTYAAAGGAATTGACG Reverse: ACCATGCACCACCTGTC
<i>Bacteroidetes</i>	Forward: CRAACAGGATTAGATACCCT Reverse: GGTAAGGTTCCCTCGCGTAT
16S rDNA, (universal)	Forward: ACTCCTACGGGAGGCAGCAGT Reverse: ATTACCGCGGCTGCTGGC

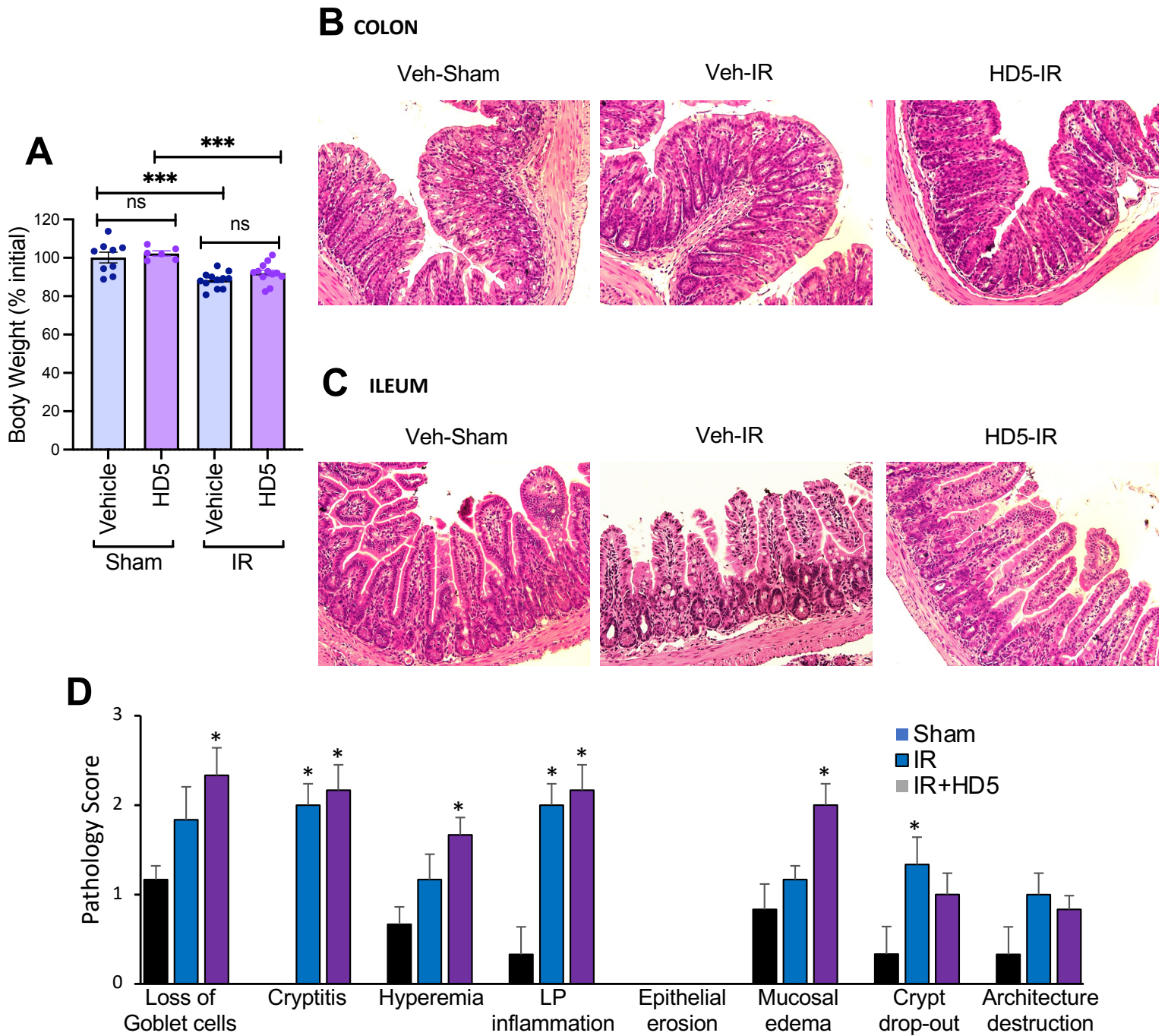
<b>Table S2:</b>	<b>PCR primer sequences</b>
<b>Gene</b>	<b>5'-3' Sequence</b>
<i>IL-1<math>\beta</math></i>	Forward: GCAACTGTTCTGAACTCAACT
	Reverse: ATCTTTTGGGGTCCGTCAACT
<i>IL-6</i>	Forward: TAGTCCTTCCTACCCCAATTCC
	Reverse: TTGGTCCTTAGCCACTCCTTC
<i>TNF-<math>\alpha</math></i>	Forward: CCCTCACACTCAGATCATCTTCT
	Reverse: GCTACGACGTGGGCTACAG
<i>MCP-1/CCL2</i>	Forward: TTAAAAACCTGGATCGGAACCAA
	Reverse: GCATTAGCTTCAGATTTACGGGT
<i>CXCL1</i>	Forward: CTGGGATTACCTCAAGAACATC
	Reverse: CAGGGTCAAGGCAAGCCTC
<i>CXCL2</i>	Forward: CCAACCACCAGGCTACAGG
	Reverse: GCGTCACACTCAAGCTCTG
<i>Defa5</i>	Forward: GCTCCTGCTCAACAATTCTCC
	Reverse: CAGCTGCAGCAGAATACGAA
<i>Defa6</i>	Forward: GACCAGGCTGTGTCTGTCTC
	Reverse: CCCTTTCTGCAGGTCCCATT
<i>GAPDH</i>	Forward: CTGCACCACCAACTGCTTAG
	Reverse: GGGCCATCCACAGTCTTCT



**Figure S1: Prophylactic HD5 treatment attenuates radiation-induced dysbiosis of intestinal microbiota.** Adult mice were fed a liquid diet with vehicle (Veh-Sham & Veh-IR) or HD5 (HD5-Sham & HD5-IR) for 24 hours before sham-treatment (Sham) or irradiated (IR). At 24 hours after irradiation, the microbiota composition in colonic flushing was analyzed by 16S rRNA-sequencing and metagenomics. **A:** Spearman's correlation of microbiota at the genus level in different experimental groups. **B:** Linear discriminate analysis of effect size (LefSe) was used to identify enriched taxa in different groups.

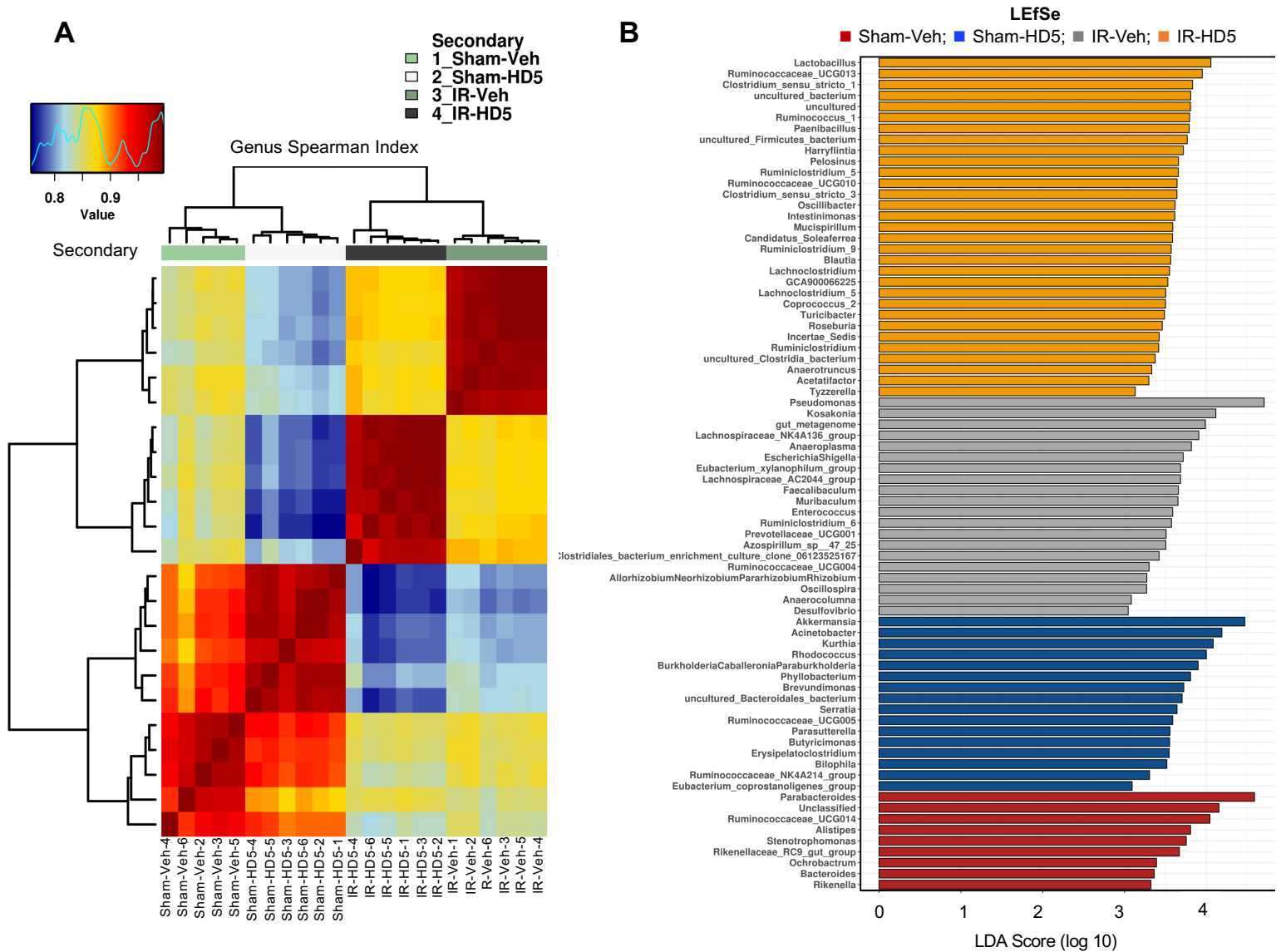


**Figure S2: HD5 prevents and mitigates radiation-induced gut microbiota composition.** Adult mice were subjected to sham treatment (Sham) or irradiation (IR). Some IR groups of mice were fed a liquid diet with vehicle or HD5 (Veh-IR & HD5-IR) for 24 hours before irradiation. Other IR groups of mice were fed a liquid diet with vehicle or HD5 (IR-Veh & IR-HD5) 24 hours after irradiation. DNA preparations from flushing samples were analyzed for the abundance of *Enterobacteriaceae* (A), *E. coli* (B), *A. muciniphila* (C), and *L. reuteri* (D) by RT-qPCR. Values are mean  $\pm$  sem (n = 6); \* =  $p < 0.05$ , \*\* =  $p < 0.01$ , \*\*\* =  $p < 0.001$  for significant difference between the indicated groups.



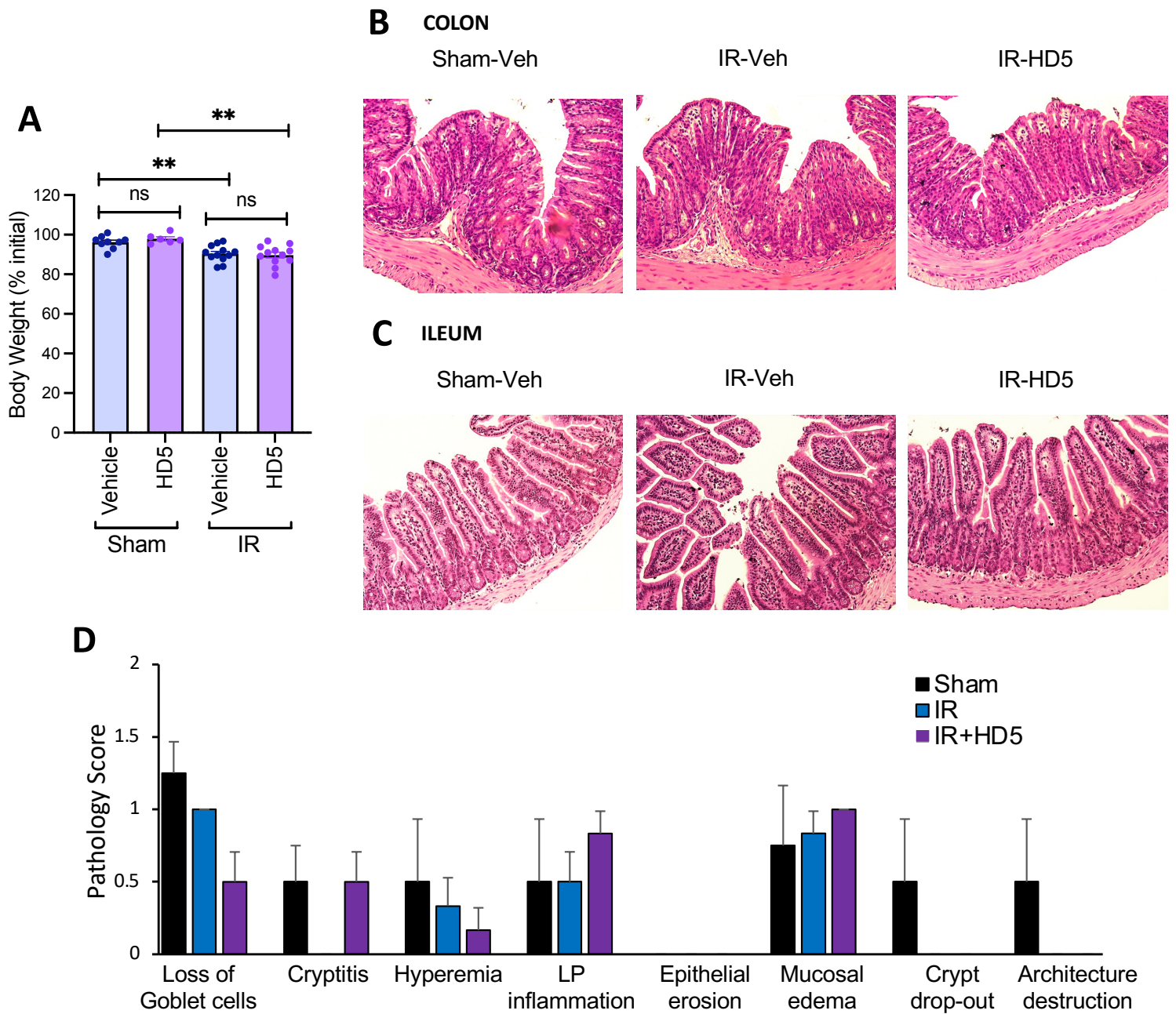
**Figure S3: Effects of radiation and prophylactic HD5 treatment on the intestine.**

Adult mice were subjected to sham treatment (Sham) or irradiation (IR). Some IR group of mice were fed a liquid diet with vehicle or HD5 (Veh-IR & HD5-IR) for 24 hours before irradiation. **A.** Body weight changes. **B & C.** Bright field light microscopic images of H & E-stained sections of colon (B) and ileum (C). **D.** Histopathology scores of H & E-stained sections. Values are mean  $\pm$  sem (n = 6); \* =  $p < 0.05$  for significant difference from corresponding Sham group values.



**Figure S4: HD5 at 24 hours after irradiation modulates altered gut microbiota composition.**

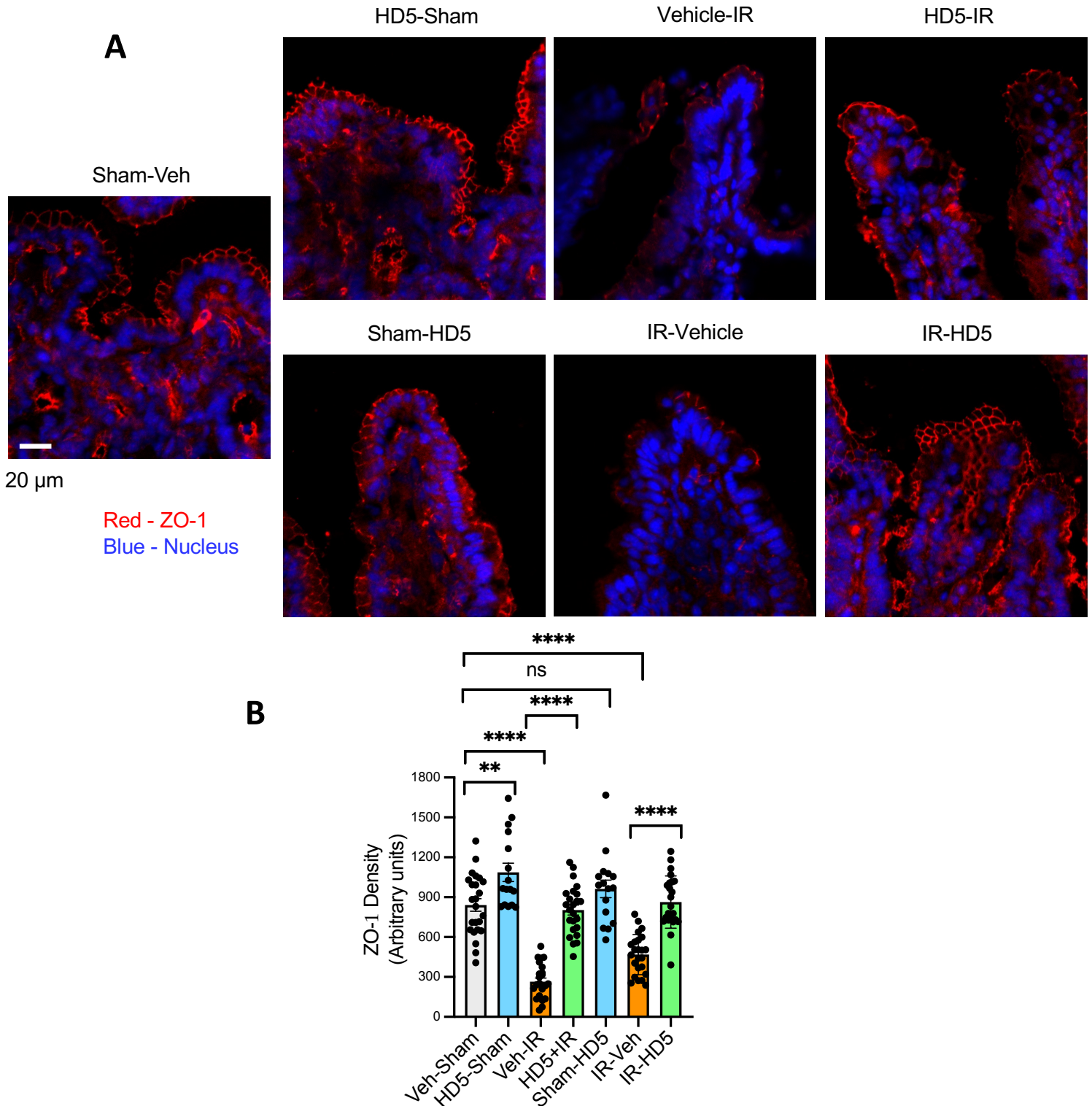
At 24 hours after sham-treatment (Sham) or irradiation (IR), mice were fed a liquid diet with vehicle (Sham-Veh & IR-Veh) or HD5 (Sham-HD5 & IR-HD5). After additional 24 hours, the microbiota composition in colonic flushing was analyzed by 16S rRNA-sequencing and metagenomics. **A:** Spearman's correlation of microbiota at the genus level in different experimental groups. **B:** Linear discriminate analysis of effect size (LefSe) was used to identify enriched taxa in different groups.



**Figure S5: Effects of radiation and therapeutic HD5 treatment on the intestine.**

Adult mice were subjected to sham treatment (Sham) or irradiation (IR). At 24 hours after irradiation, Sham and IR groups of mice were fed a liquid diet with vehicle or HD5 (Sham-Veh & Sham-HD5; IR-Veh & IR-HD5) for 24 hours. **A.** Body weight changes. **B & C.** Bright field light microscopic images of H & E-stained sections of colon (B) and ileum (C). **D.** Histopathology scores of H & E-stained sections. Values are mean  $\pm$  sem (n = 6).





**Figure S6: HD5 treatment reverses radiation-induced epithelial tight junction integrity in the small intestine.**

Adult mice were subjected to sham treatment (Sham) or irradiation (IR). Sham and IR groups of mice were administered Vehicle or HD5 either 24 hours before (HD5-Sham and HD5-IR) or 24 hours after (Sham-HD5 and IR-HD5) irradiation in a liquid diet. At 24 hours after irradiation (HD5-Sham and HD5-IR) or HD5 treatment (Sham-HD5 and IR-HD5) cryosections of ileum were stained for ZO-1 and nucleus. **A.** Representative images of ZO-1 (red) and nucleus (blue). **B.** Densitometric analyses of ZO-1 fluorescence. Values are mean  $\pm$  sem (n = 8 regions each from 3 mice per group).