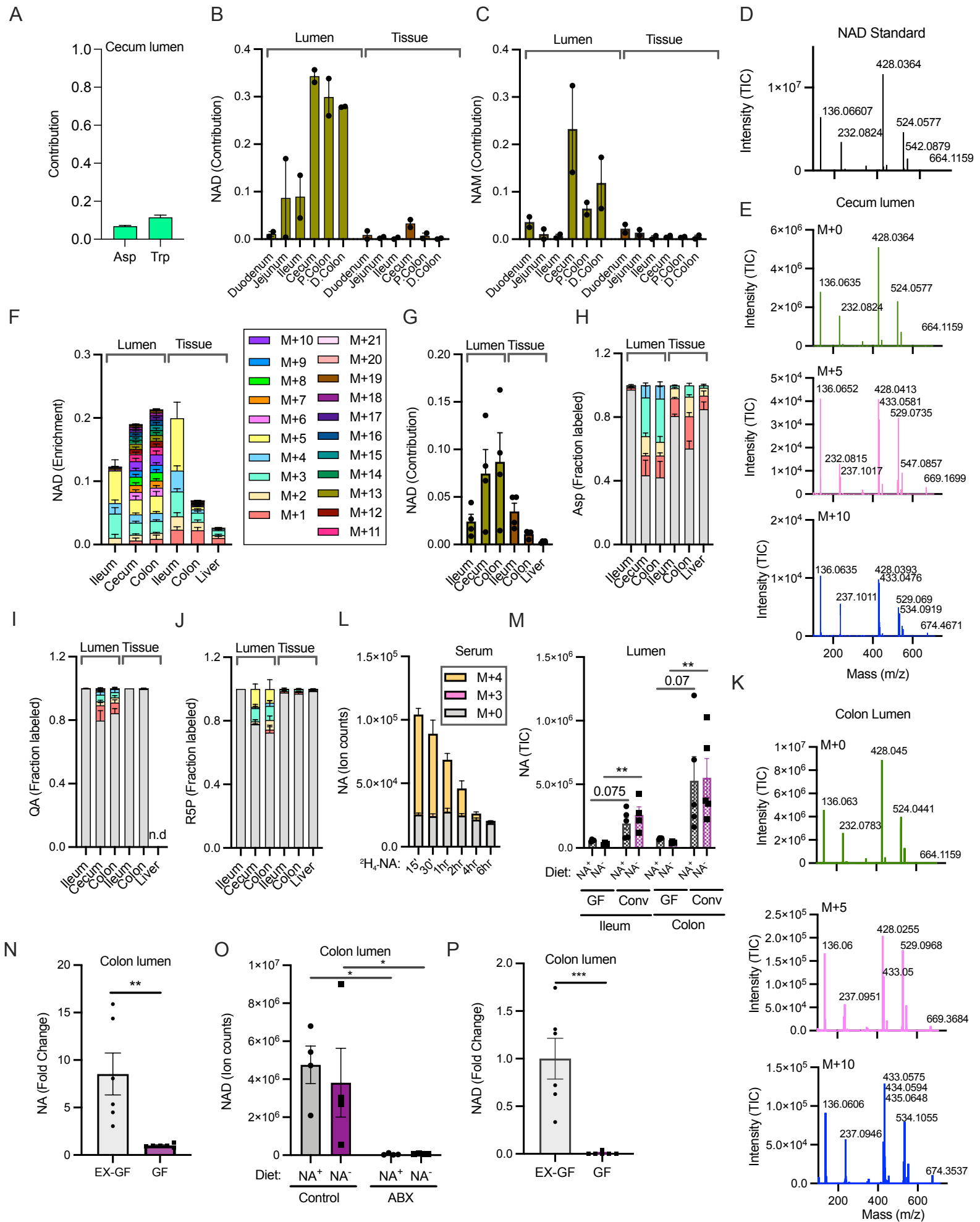


## SUPPLEMENTAL INFORMATION

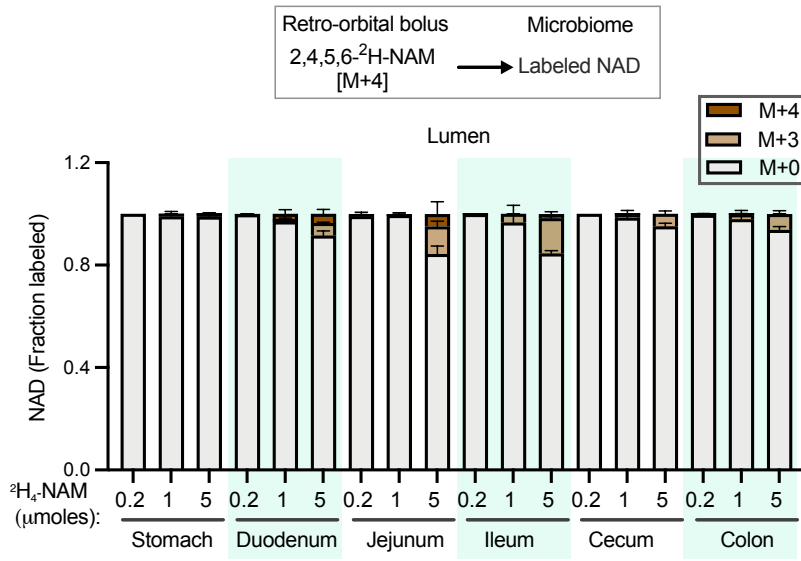
### **NAD precursors cycle between host tissues and the gut microbiome**

Karthikeyani Chellappa, Melanie R. McReynolds, Wenyun Lu, Xianfeng Zeng, Mikhail Makarov, Faisal Hayat, Sarmistha Mukherjee, Yashaswini R. Bhat, Siddharth R. Lingala, Rafaella T. Shima, H el ene C. Descamps, Timothy Cox, Lixin Ji, Connor Jankowski, Qingwei Chu, Shawn M. Davidson, Christoph A. Thaiss, Marie E. Migaud, Joshua D. Rabinowitz and Joseph A. Baur

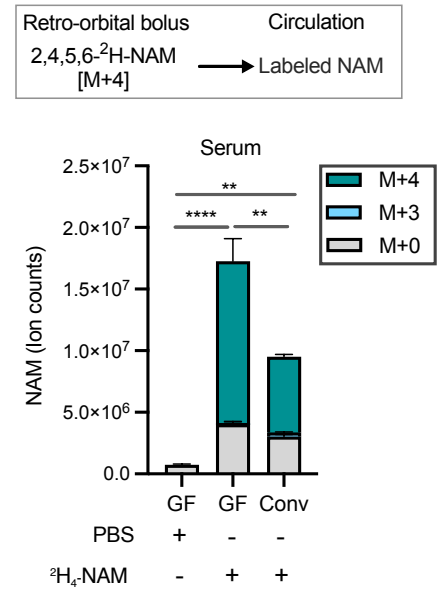


**Supplementary Figure S1. Site-specific use of dietary NAD precursors by microbes along the GI tract, Related to Figure 1.** (A) Contribution of U-<sup>13</sup>C-protein to aspartate (asp) and tryptophan (trp) levels in the cecum lumen of mice fed with labeled protein diet for 24h; (n=3 mice). (B) Contribution of inulin to NAD synthesis in different parts of GI tract of mice fed with labeled U-<sup>13</sup>C-inulin for 24h; (n=2 mice). (C) Contribution of U-<sup>13</sup>C-inulin to NAM in the gut of mice treated as in B; (n=2 mice). (D) MS/MS fragments of NAD standard. (E) MS/MS fragments of NAD detected in the cecum lumen of mice fed with U-<sup>13</sup>C-inulin as in B. (F-G) Enrichment of NAD labeling (F) and contribution to NAD synthesis (G) in mice orally gavaged with U-<sup>13</sup>C-fructose for 2h at a dose of 2g/kg; (n=4 mice). (H-J) Enrichment of aspartate (H), quinolinic acid (I), and ribose phosphate (J) labeling in mice treated as in F. (K) MS/MS fragments of NAD detected in the colonic lumen of mice treated as in F. (L) Labeled and unlabeled NA in the serum after oral gavage of 1.96 μmoles of [2,4,5,6-<sup>2</sup>H]-NA. (n=2-6 mice per time point). (M) NA content in the lumen of germ free (GF) and conventional (Conv) mice fed diet with (NA<sup>+</sup>) or without nicotinic acid (NA<sup>-</sup>); (n=4-5 mice per group; Sidak's multiple comparison test following two-way ANOVA, statistical analysis was performed independently for each luminal site \* = p < 0.05, \*\* = p < 0.01). (N) Abundance of NA in the colonic lumen of germ-free (GF) and Ex-germ free (Ex-GF) mice colonized with microbiota from SPF mice; (n=6 per group; two-tailed unpaired t-test, \*\* = p < 0.01). (O) NAD content in the colonic lumen of conventional mice fed diet with (NA<sup>+</sup>) or without nicotinic acid (NA<sup>-</sup>) that were on drinking water or antibiotics cocktail (ABX); (n=4 per group; Holm-Sidak test following two-way ANOVA, \* = p < 0.05). (P) Abundance of NAD in the colonic lumen of germ-free (GF) and Ex-germ free (Ex-GF) mice colonized with microbiota from SPF mice; (n=6 per group; two-tailed unpaired t-test, \*\*\* = p < 0.001).

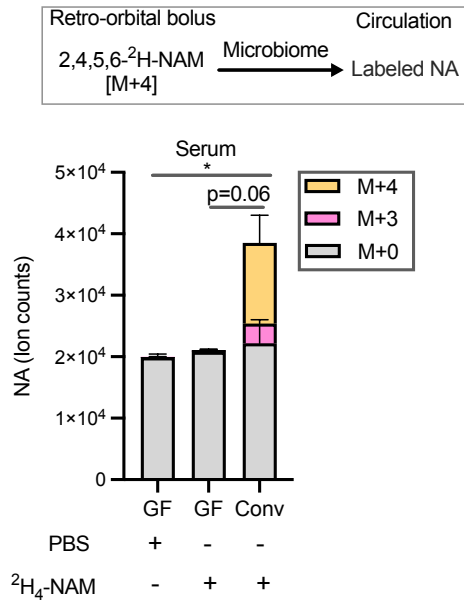
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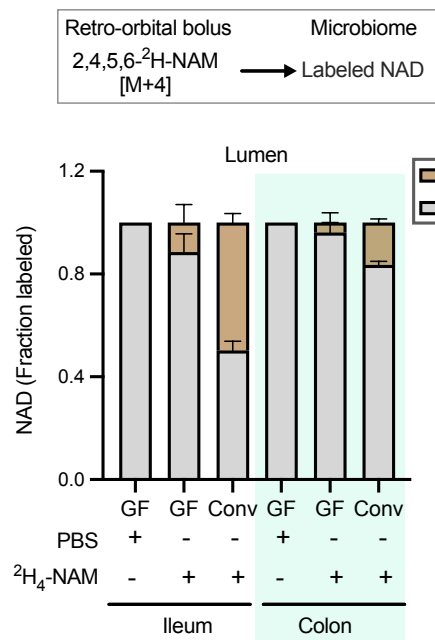
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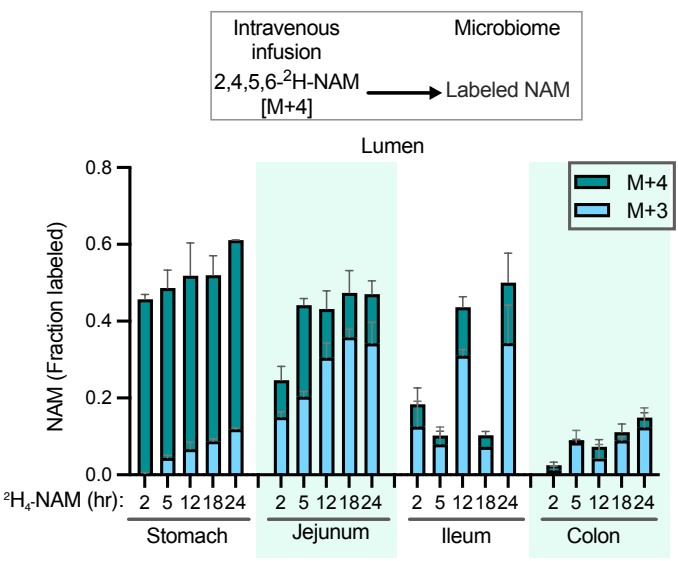
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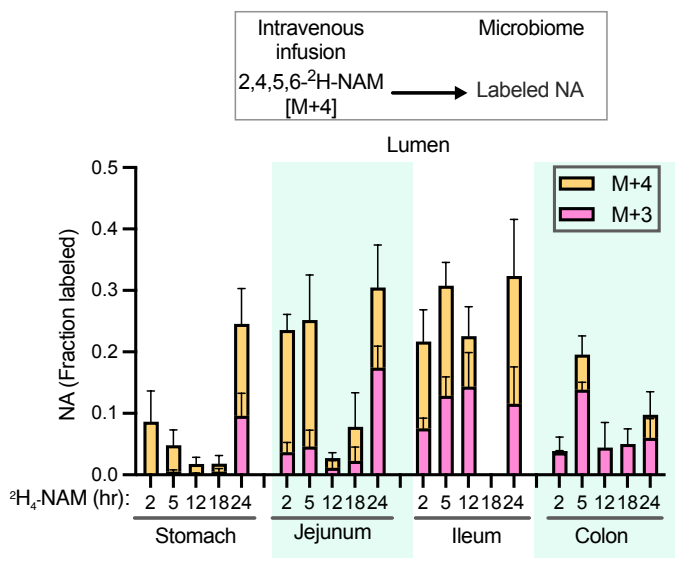
### Supplementary Figure S2. Circulating NAM labels luminal NAD, Related to Figure 2. (A)

Fraction labeled NAD in luminal contents collected from mice retro-orbitally injected with 5 μmoles [2,4,5,6-<sup>2</sup>H]-NAM and sacrificed after 15 min. n=2-3 mice per group. (B) NAM levels in the serum samples collected from germ-free (GF) and conventional (Conv) mice 2h after retro-orbitally injection of either PBS or 5 μmoles of [2,4,5,6-<sup>2</sup>H]-NAM; (n=4 per group; Tukey's multiple comparison test following one-way ANOVA, \*\* = p < 0.01, \*\*\*\* = p < 0.0001). (C) Serum NA labelling in mice treated as in B; (n=4 per group; Tukey's multiple comparison test following one-way ANOVA, \* = p < 0.05). (D) Fraction labeled NAD in the luminal samples collected from mice treated as in B.

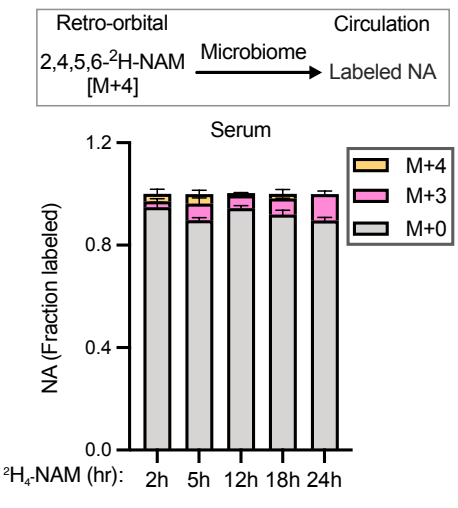
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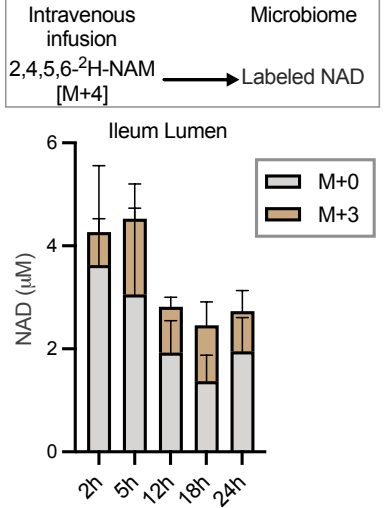
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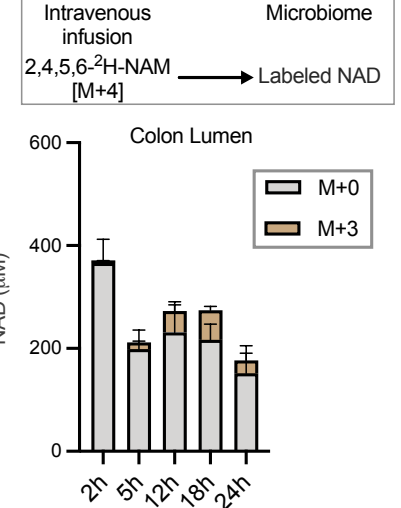
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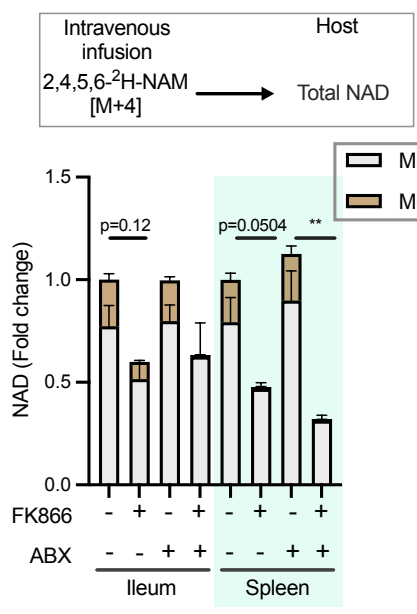
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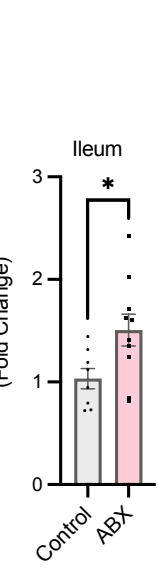
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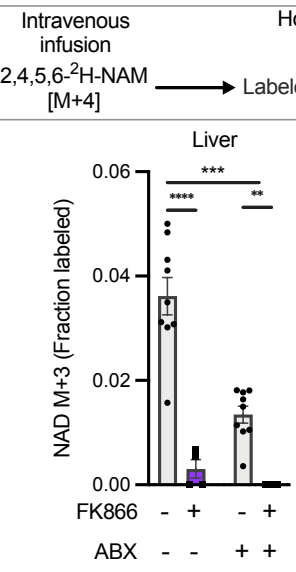
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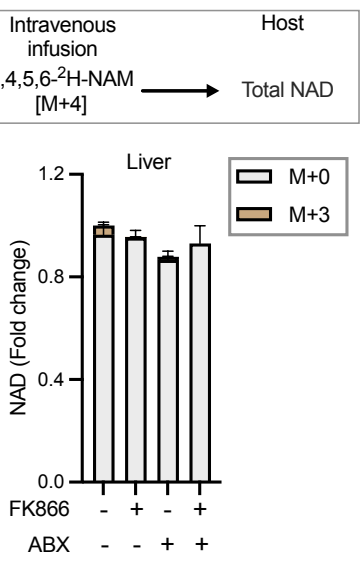
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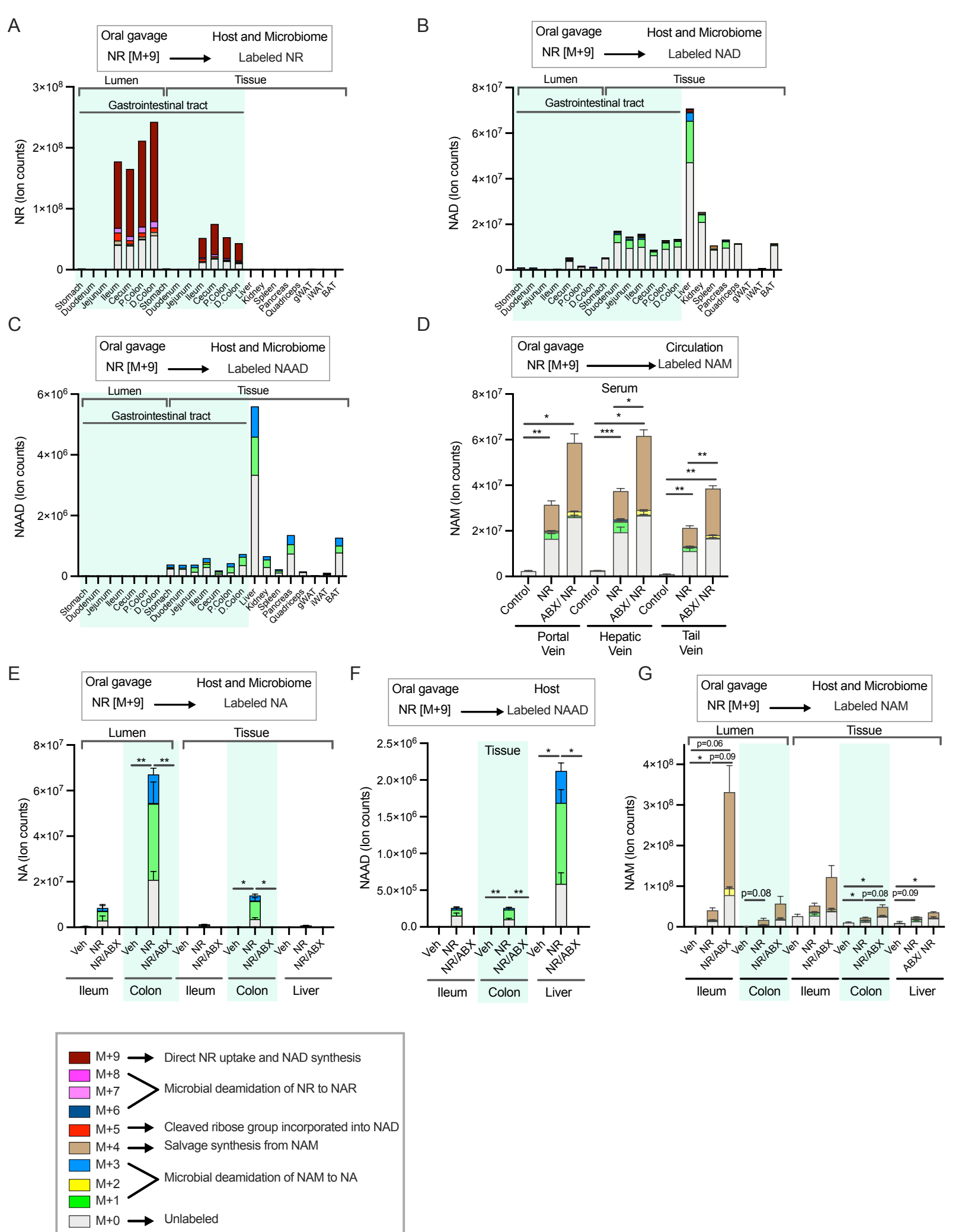
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I



**Supplementary Figure S3. The gut microbiome provides metabolic flexibility to bypass salvage synthesis in liver, Related to Figure 3.** (A) Fraction isotope labeling of NAM in the lumen of mice intravenously infused with 4mM [2,4,5,6-<sup>2</sup>H]-NAM; (n=2-4 mice per group). (B) Fraction isotope labeling of NA in the lumen of mice treated as in A; (n=2-4 mice per group). (C) Labeling of serum NA in mice infused as in A; (n=2-4 mice per group). (D) NAD concentration in the ileum lumen of mice infused as in A; (n=2-4 mice per group). (E) NAD concentration in the colon lumen of mice infused as in A; (n=2-4 mice per group). (F) Relative levels of labeled and unlabeled NAD in mice intraperitoneally injected with vehicle or FK866 and infused with [2,4,5,6-<sup>2</sup>H]-NAM for 5h; (n=5-9 mice per group; Sidak's multiple comparison test following two-way ANOVA, statistical analysis was performed independently for each tissue \*\* = p < 0.01). (G) Relative expression of *Nampt* in the ileum of control and antibiotics treated mice (n=8-10 mice per group; two-tailed unpaired t-test, \* = p < 0.05). (H) Fraction labeled NAD in the liver from control and antibiotics (ABX) treated mice intraperitoneally injected with vehicle or FK866 and infused with [2,4,5,6-<sup>2</sup>H]-NAM for 5h; (n=4-9 mice per group; Sidak's multiple comparison test following two-way ANOVA, \*\* = p < 0.01, \*\*\*\* = p < 0.0001). (I) Relative levels of labeled and unlabeled NAD in the liver of mice intraperitoneally injected with vehicle or FK866 and infused with [2,4,5,6-<sup>2</sup>H]-NAM for 5h (n=4-9 mice per group).



**Supplementary Figure S4. The gut microbiome is required to generate deamidated precursors from orally delivered NR, Related to Figure 4.** (A-C) NR (A), NAD (B), and NAAD (C) labeling in the lumen and tissues of a mouse orally gavaged with NR (600 mg/kg body weight) for 3h. (D) Serum NAM levels in mice 3h after oral gavage with NR (600 mg/kg body weight); (n=3-5 per group; Tukey's multiple comparison test following two-way ANOVA, \* =  $p < 0.05$ , \*\* =  $p < 0.01$ ). (E-G) NA (E), NAAD (F), and NAM (G), labeling in the lumen and tissues of mice orally gavaged with mixture of unlabeled NR at a dose of 600 mg/kg body weight for 3h; (n=3-5 mice per group; Tukey's multiple comparison test following two-way ANOVA, \* =  $p < 0.05$ ).