Supplemental Information

Oral administration of miR-30d from feces of MS patients suppresses MS-like symptoms in mice by expanding *Akkermansia muciniphila*

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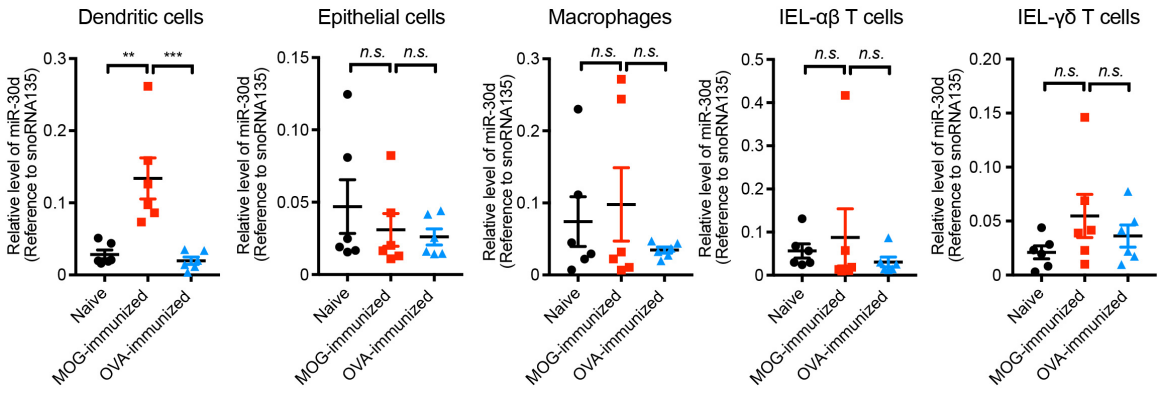
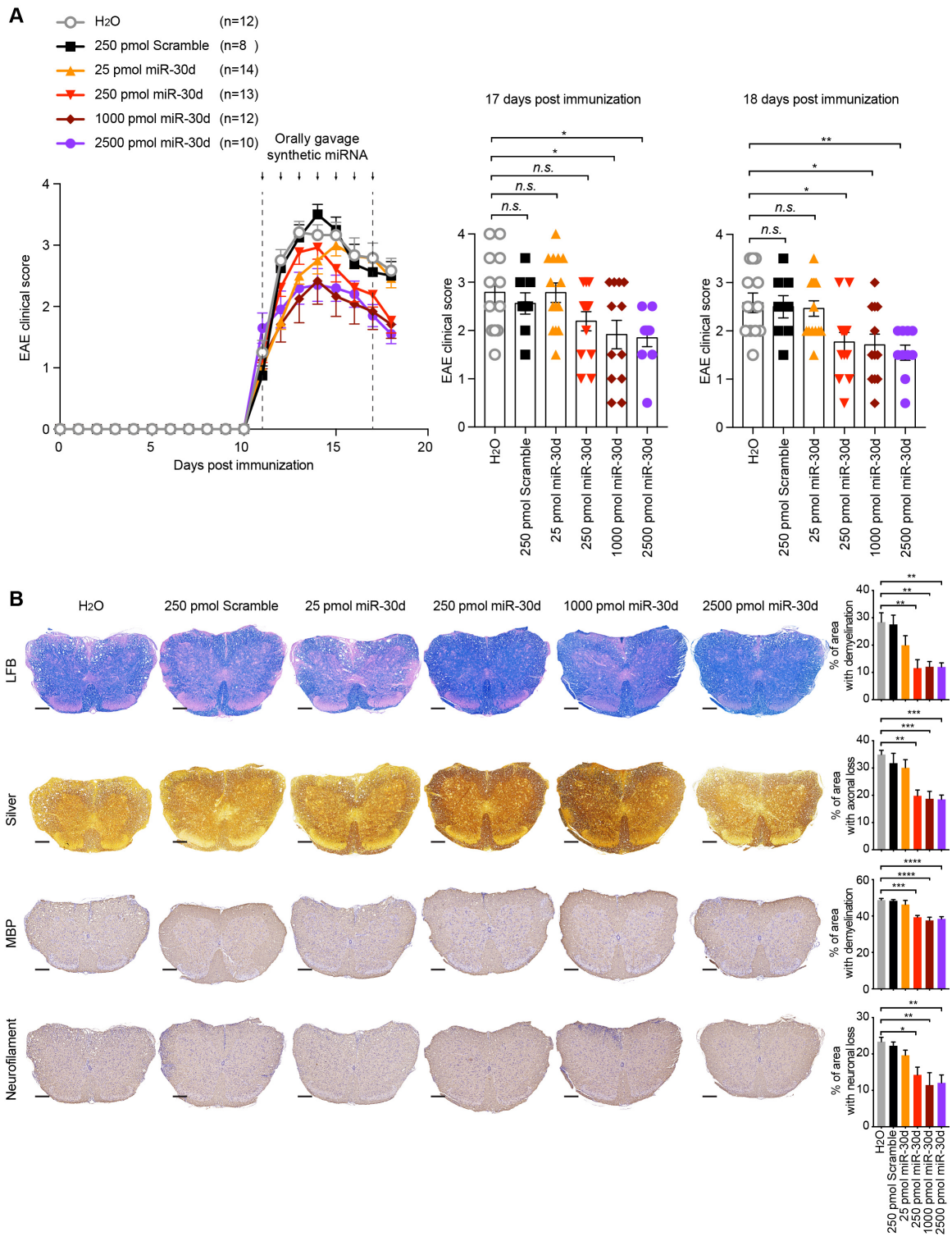


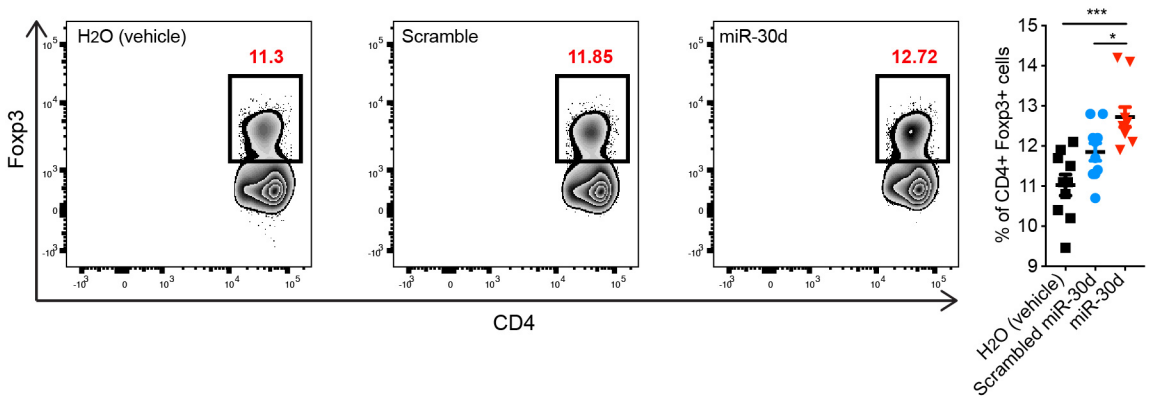
Supplementary Figure 1. Intestinal Dendritic Cells Are Responsible for the Generation of miR-30d Specifically upon MOG-Immunization, Related to Figure 3.

Mice were immunized with MOG or OVA/CFA. 10 days post immunization, dendritic cells, epithelial cells, macrophages, TCRαβ^{+} and TCRγδ^{+} intraepithelial lymphocytes (IEL) in the colon were sorted. The expression of miR-30d in these cells was determined by qPCR. n=6, Error bars denote mean ± SEM, one-way ANOVA Dunnett's multiple comparisons test. *n.s.*= not significant, ** P<0.01, *** P<0.001.



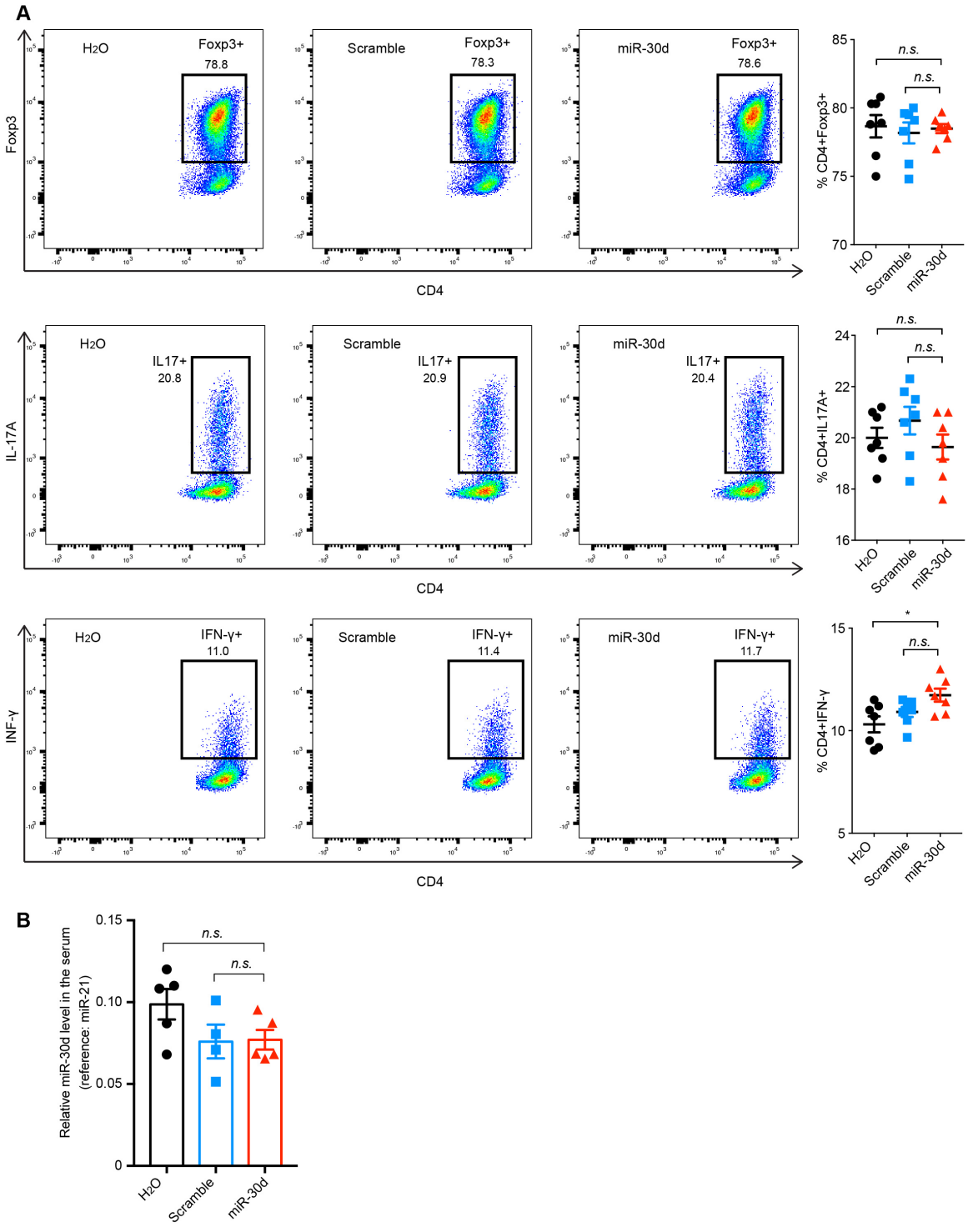
Supplementary Figure 2. The Dose Response of Oral Administration of Synthetic miR-30d in Ameliorating EAE, Related to Figure 4.

The indicated dose of synthetic miR-30d, scramble control, or H_2O were orally administered to MOG/CFA-induced EAE mice starting from when the mice were scored 1, for 7 consecutive days. (**A**) Clinical score curves of EAE (left) and tested clinical scores at the end of treatment (17 d.p.i) (middle) and one day after the treatment (18 d.p.i) (right). Sample size of each group is indicated, Error bars denote mean ± SEM, Kruskal-Wallis test with Dunn's multiple comparisons as compared with H_2O-administered mice. (**B**) Quantification of demyelination (LFB and MBP) and neuronal/axonal loss (Silver and Neurofilament) for individual mice were determined by histological stains. Scale bar: 200 μm; n=6 mice/group; Error bars denote mean ± SEM, one-way ANOVA Dunnett's multiple comparisons as compared with H_2O-administered mice. All panels: * P<0.05, ** P<0.01, *** P<0.001, **** P<0.001, *n.s.* = not significant.



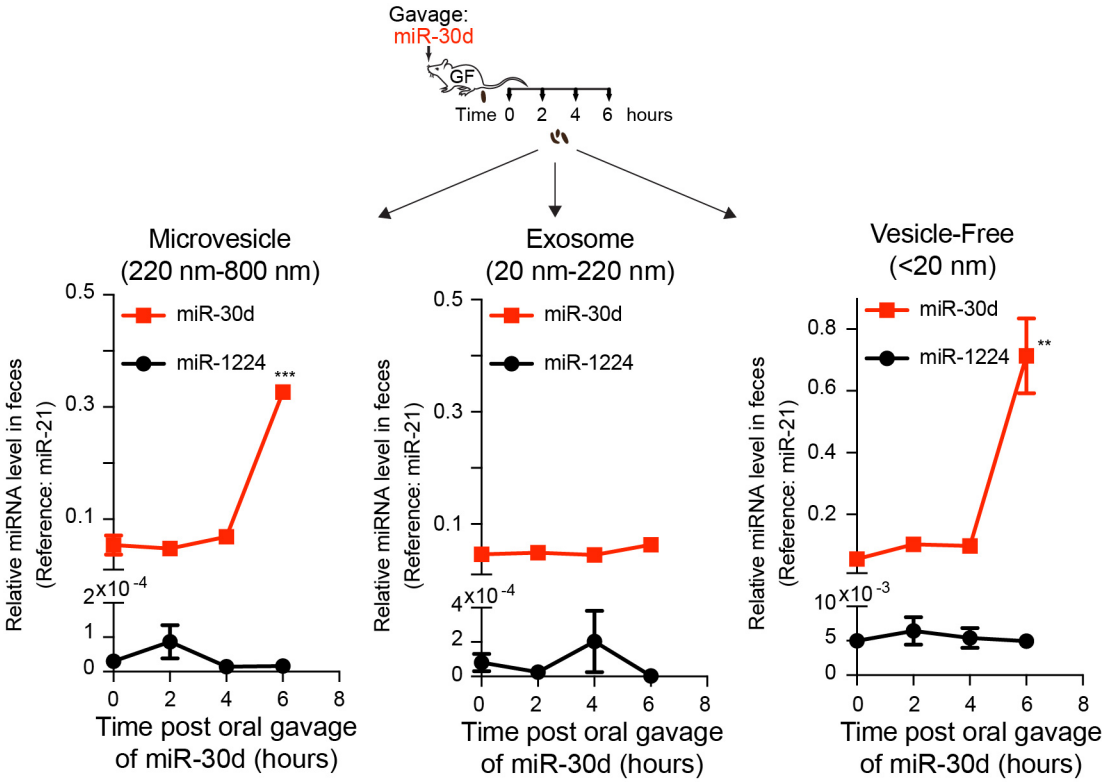
Supplementary Figure 3. Oral Administration of Synthetic miR-30d Increases Foxp3$^+$ Regulatory T Cells, Related to Figure 4.

Mice were immunized with MOG and orally administered synthetic miR-30d or scramble control daily at the dose of 250 pmol for 7 consecutive days. Foxp3$^+$ T cells in the total CD4$^+$ T cell population in the spleen were analyzed by FACS. Left panel: Representative FACS plots of CD4$^+$ Foxp3$^+$ T cells; Right panel: % of CD4$^+$ Foxp3$^+$ T cells in individual animals (n=10 per group). Error bars denote mean ± SEM; one-way ANOVA Tukey's multiple comparisons test. * P<0.05, *** P<0.001.



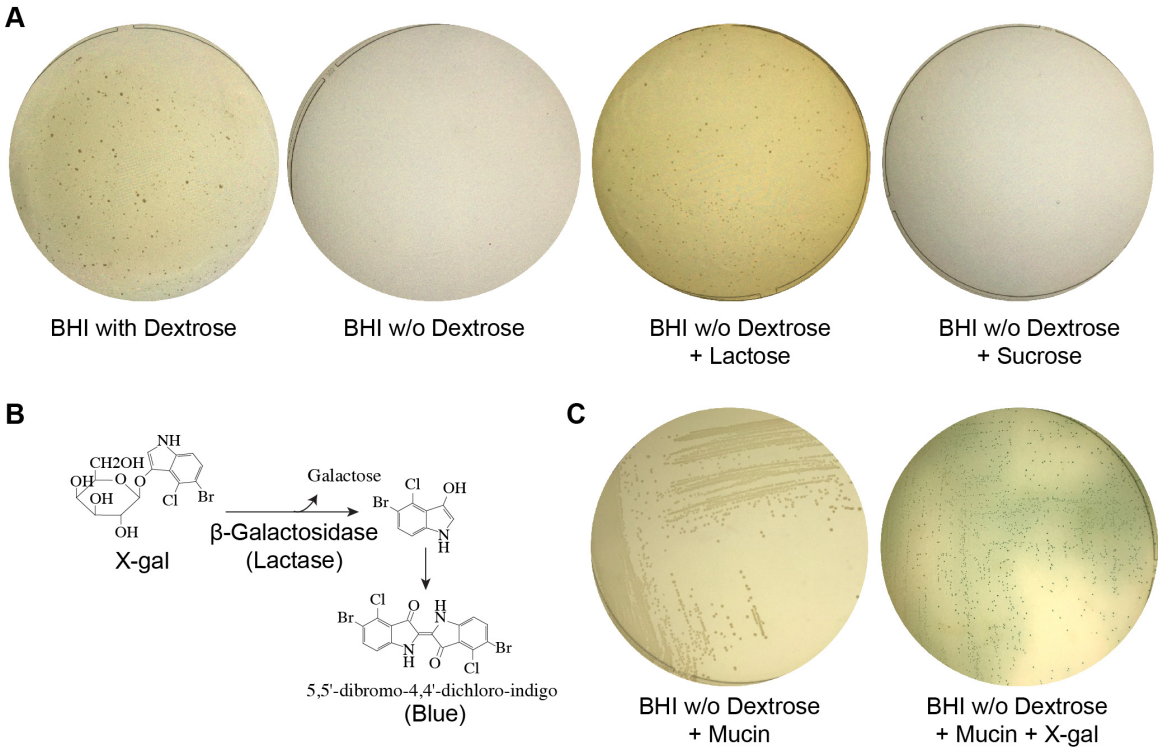
Supplementary Figure 4. Treg-Promoting Effect of Oral MiR-30d Administration is not Caused by Acting Directly on T Cell Differentiation, Related to Figure 4.

(**A**) Naïve $CD4^+$ T cells from C57BL/6 spleen were differentiated into Treg ($Foxp3^+$), Th17 ($IL\text{-}17A^+$) and Th1 ($IFN\text{-}\gamma^+$) cells by plate bound anti-CD3 and anti-CD28 in the presence of polarizing cytokines. The direct effect of miR-30d on T cell differentiation was examined by supplying synthetic miR-30d to the culture. T cell subsets were analyzed by FACS. Left panel: Representative FACS plots of T cell subsets; Right panel: Bar graph of percentage of T cell subsets individual culture. Error bars denote mean ± SEM; one-way ANOVA Tukey's multiple comparisons test. * $P<0.05$, *n.s.*= not significant. (**B**) Mice were immunized with MOG and orally administered synthetic miR-30d or scramble control daily at a dose of 1000 pmol for 7 consecutive days. miR-30d level in the serum specimen were quantified by qPCR. n=5 per group, Error bars denote mean ± SEM; one-way ANOVA Tukey's multiple comparisons test. *n.s.* = not significant.



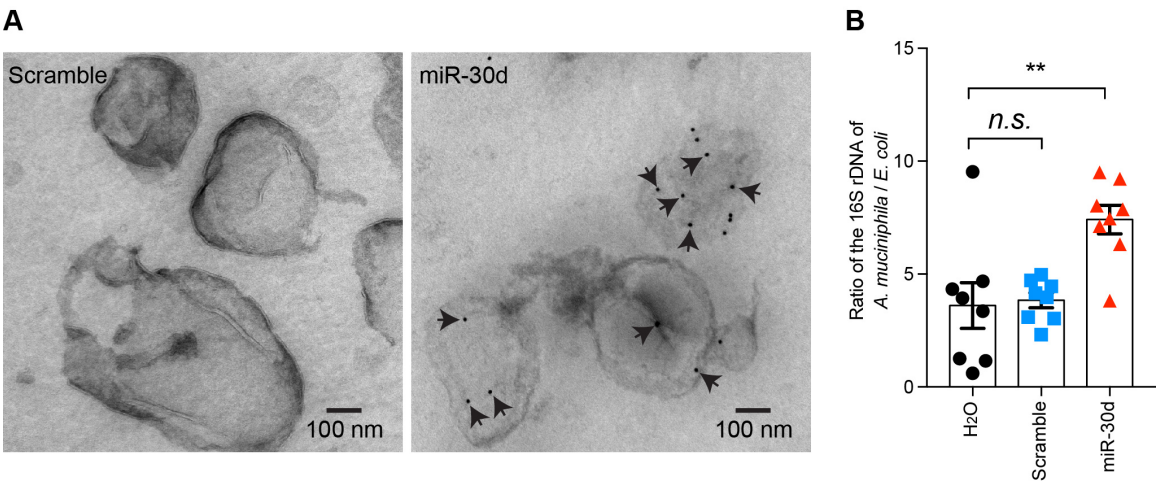
Supplementary Figure 5. Orally Administered miR-30d Keeps Intact to the Gut in Forms of Microvesicle and Vesicle-free, Related to Figure 4 and Figure 5.

Germ-free (GF) mice were orally administered 1000 pmol synthetic miR-30d in 200 µl water. Fecal specimen was collected every two hours for six hours. Microvesicle (220 nm-800 nm) fractions, Exosome (20 nm-220 nm) fractions and Vesicle-free (<20 nm) fractions of the feces were separated by size filtration. RNA was isolated and miR-30d, and as control, miR-1224 levels in the specimen were quantified by qPCR. n=2 per group, Error bars denote mean ± SEM; One-way ANOVA Tukey's multiple comparisons as compared with time 0 levels; ** P<0.01, *** P<0.001.



Supplementary Figure 6. Detection of β-galactosidase (lactase) Activity in *A. muciniphila* which Hydrolyzes Lactose into Dextrose (glucose) that is Essential for *A. muciniphila*, Related to Figure 5.

(**A**) Growth of *A. muciniphila* on different agar: *A. muciniphila* were spread over the surface of Brain heart infusion (BHI) agar (with 0.2% dextrose), BHI agar without (w/o) dextrose, BHI agar w/o dextrose plus 0.2% lactose, and BHI agar w/o dextrose plus 0.2% sucrose and incubated at 37ºC anaerobically for 5 days. (**B-C**) β-galactosidase activity of *A. muciniphila* on mucin containing agar was tested. (**B**) scheme of β-galactosidase activity test with X-gal. (**C**) *A. muciniphila* were streaked over the surface of BHI agar w/o dextrose plus 0.2% mucin with (right panel) or without X-gal (left panel) and incubated at 37ºC anaerobically for 5 days. Images are representative of at least three independent experiments.



Supplementary Figure 7. MiR-30d Enters *A. muciniphila* and Promotes the Growth of *A. muciniphila in vitro*, Related to Figure 5.

(**A**) In situ hybridization (ISH) mapping of miR-30d internalized in *A. muciniphila* using a miR-30d probe. *A. muciniphila* was cultured in the presence of 5 μM synthetic miR-30d or scramble control for 18 hours to an exponential phase. miR-30d in *A. muciniphila* was determined by ISH using a 5'-DIG and 3'-DIG dual labeled probe for miR-30d and 10 nm immuno gold-conjugated anti-Digoxigenin antibody, and imaged with transmission electron microscopy. Arrowheads: miR-30d (10 nm gold positive) located in the *A. muciniphila*. (**B**) 5 μM of synthetic miR-30d or scramble control were supplied in a mixed culture of *A. muciniphila* and *E. coli* for 18 hours. The relative abundance of *A. muciniphila* and *E. coli* was determined by qPCR detecting 16S rDNA of *A. muciniphila* and *E. coli*. n=8 per group, Error bars denote mean ± SEM; one-way ANOVA Tukey's multiple comparisons test. ** $P<0.01$, *n.s.* = not significant.