Supporting information for:

Transfer of *Akkermansia muciniphila* promotes Mucosal Homeostasis, induces Gut Microbiota Remodeling, and controls Islet-Autoimmunity in Non-Obese Diabetic Mice

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Supporting Discussion

The balance between inflammatory and regulatory T cells in the target organ plays a pivotal role in the development of many autoimmune diseases. *Clostridia* species which induce regulatory Foxp3 Treg cells in mice and down-regulate harmful immune responses [1-3], and segmented filamentous bacteria (SFB) which induce Th17 immune responses [4] were therefore gut symbionts to which we paid particular attention. We did not detect any *Clostridia* in either *C.leptum* or *C.coccoides* groups (clusters IV and XIVa), or any of the Treg-inducing species reported earlier [1-3]. The only exception was *Oscillibacter valericigenes*, which was recently identified as one human-derived Clostridial species, which induces Treg differentiation in mice [1]. It was present in both NOR/MrkTac and NODJax mice and did not provide an explanation to their difference in diabetes development. Consistent with the original finding regarding Taconic and Jackson mice [4], our NOD/MrkTac mice were colonized with SFB whereas NODJax mice were not. A difference in SFB abundance, as assessed from fecal samples of young donors of even the same colony of NOD mice, has been found to associate with the risk

of developing diabetes, indicating that SFB positivity predicts a low probability to develop diabetes [5]. In experiments with germ-free NOD mice, monocolonization with SFB was in turn not found to temper diabetes incidence in gnotobiotic female NOD mice [6]. Our microbiota transfer experiments appeared not to support any major role for SFB colonization in diabetes development. Although we cannot rule out that variation in SFB strains could be decisive in the protective role of SFB in autoimmune diabetes [6] it is important to note that SFB strains are found in mice but virtually not in human [7].

Supporting References

1. Atarashi K, Tanoue T, Oshima K et al. Treg induction by a rationally selected mixture of Clostridia strains from the human microbiota. *Nature* 2013;500(7461):232-6.
2. Atarashi K, Tanoue T, Shima T et al. Induction of colonic regulatory T cells by indigenous Clostridium species. *Science* 2011;331(6015):337-41.
3. Narushima S, Sugiura Y, Oshima K et al. Characterization of the 17 strains of regulatory T cell-inducing human-derived Clostridia. *Gut Microbes* 2014;5(3):333-9.
4. Ivanov, II, Frutos Rde L, Manel N et al. Specific microbiota direct the differentiation of IL-17-producing T-helper cells in the mucosa of the small intestine. *Cell Host Microbe* 2008;4(4):337-49.
5. Kriegel MA, Sefik E, Hill JA et al. Naturally transmitted segmented filamentous bacteria segregate with diabetes protection in nonobese diabetic mice. *Proc Natl Acad Sci U S A* 2011;108(28):11548-53.
6. Yurkovetskiy L, Burrows M, Khan AA et al. Gender bias in autoimmunity is influenced by microbiota. *Immunity* 2013;39(2):400-12.
7. Xiao L, Feng Q, Liang S et al. A catalog of the mouse gut metagenome. *Nat Biotechnol* 2015;33(10):1103-8.
8. Chassaing B, Koren O, Goodrich JK et al. Dietary emulsifiers impact the mouse gut microbiota promoting colitis and metabolic syndrome. *Nature* 2015;519(7541):92-6.

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Supporting Figure legends

Supporting Figure S1. (A) Genus-level identifications of microbial taxa showing lack of several genera in NOD/Jax mice, which are identified in NOD/MrkTac mice. Analysis of 16S rRNA sequencing data was performed using QIIME software and Greengenes database (2013 updated version). n= 10 mice/group.

Supporting Figure S2. Transfer of SFB (*C.arthromitus*) by oral gavage or co-housing of pregnant dams. (A) NOD/Jax mice do not have SFB, whereas NOD/MrkTac mice are colonized with SFB. Colonization of NOD/Jax mice with SFB following (B) co-housing and (C) oral microbiota transfer.

Supporting Figure S3. (A) *In situ* hybridization of Eubacteria in the gut of a mouse treated with *A.muciniphila*, and (B) mean distance of bacteria from epithelial surface calculated from 12-15 visual fields of *in situ* hybridization slides. Bars represent the minimum and maximum distance of bacteria in a visual field. Bacterial DNA was hybridized using a probe specific for all eubacteria (red), and mucus was stained with anti-muc2 antibody (green) according to a published method [8]. Cell nuclei were stained with DAPI (blue).

Supporting Figure S4. The 4-week treatment with oral *A.muc.* diminishes serum endotoxin levels and islet infiltration by mononuclear leukocytes also when *A.muc.* is administered at a lower dose ($2x10^6$ bacteria/dose).

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SupportingTable 1. Primers and probes used for quantitative PCR

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Gene	*Forward 5' → 3'*	*Reverse 5' → 3'*	*Probe*[a]	*Application*
Il10	AGGGCCCTTTGCTATGGTGT	TGGCCACAGTTTTCAGGGAT	N/A	SYBR Green
Bip	ATAAACCCCGATGAGGCTGT	CATCAAGCAGTACCAGATCACC	64	Taqman
Xbp1	CTGACGAGGTTCCAGAGGTG	GCAGAGGTGCACATAGTCTGAG	49	Taqman
Tgfß	GCAACATGTGGAACTCTACCAG	CAGCCACTCAGGCGTATCA	N/A	SYBR Green
Ym1	AAGAACACTGAGCTAAAAACTCTCCT	GAGACCATGGCACTGAACG	88	Taqman
ß-actin	CTAAGGCCAACCGTGAAAAG	ACCAGAGGCATACAGGGACA	64	Taqman/SYBR
Reg3g	TTCCTGTCCTCCATGATCAAA	CATCCACCTCTGTTGGGTTC	N/A	SYBR Green
Emr1	TGACAACCAGACGGCTTGTG	GCAGGCGAGGAAAAGATAGTGT	N/A	SYBR Green

[a] For probes, the Roche universal probe library number is given