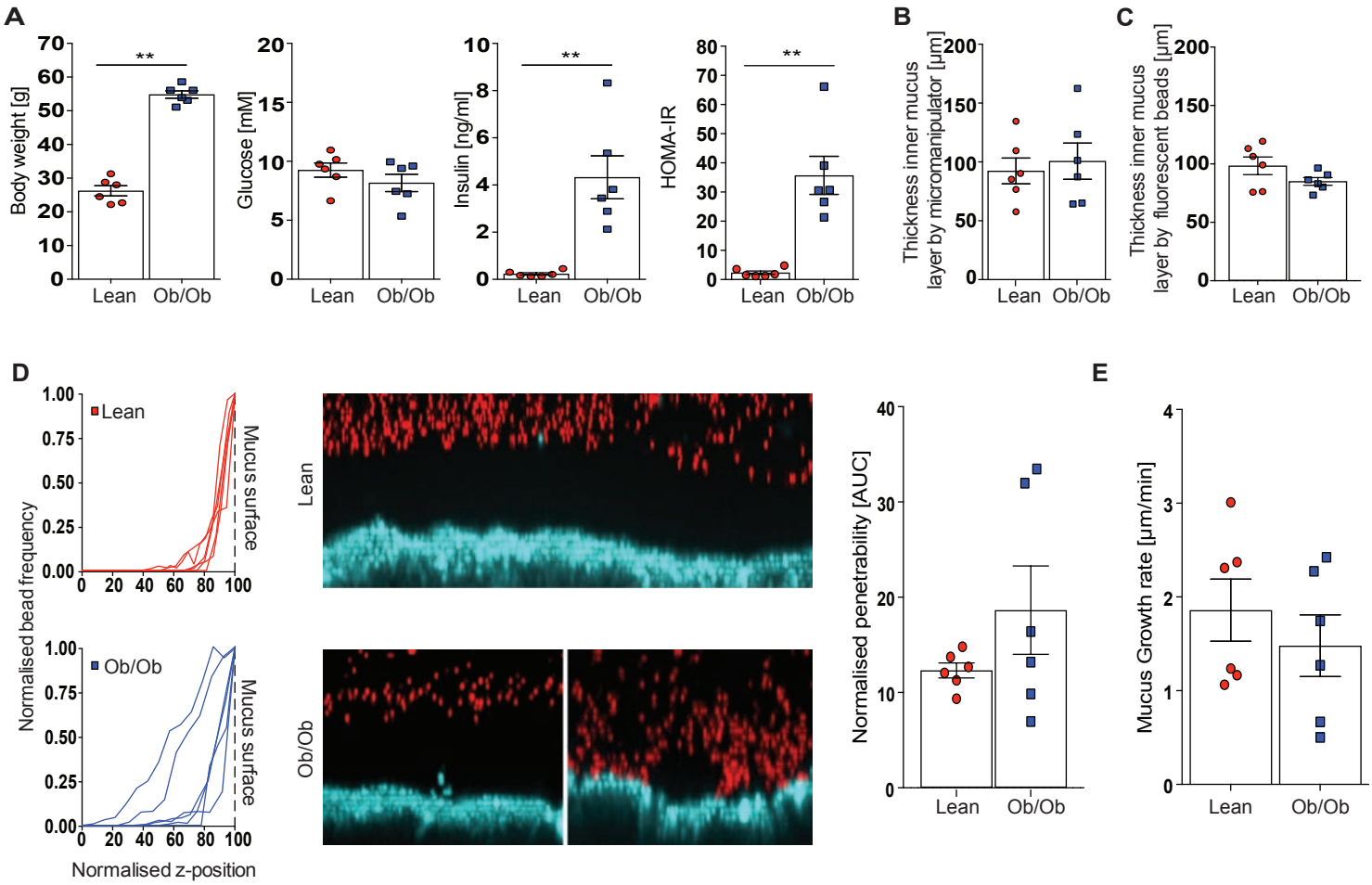
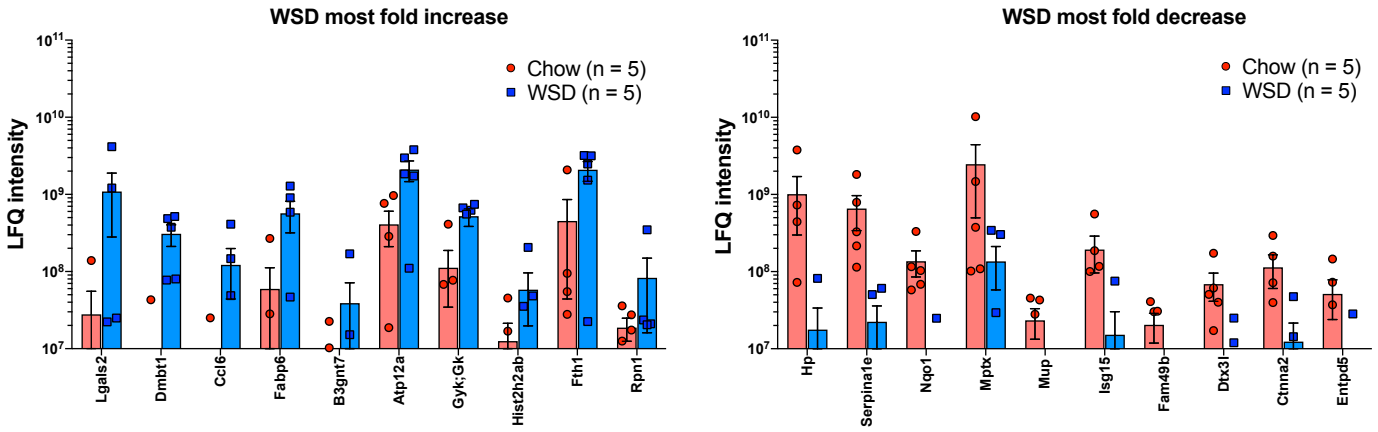
Figure S1



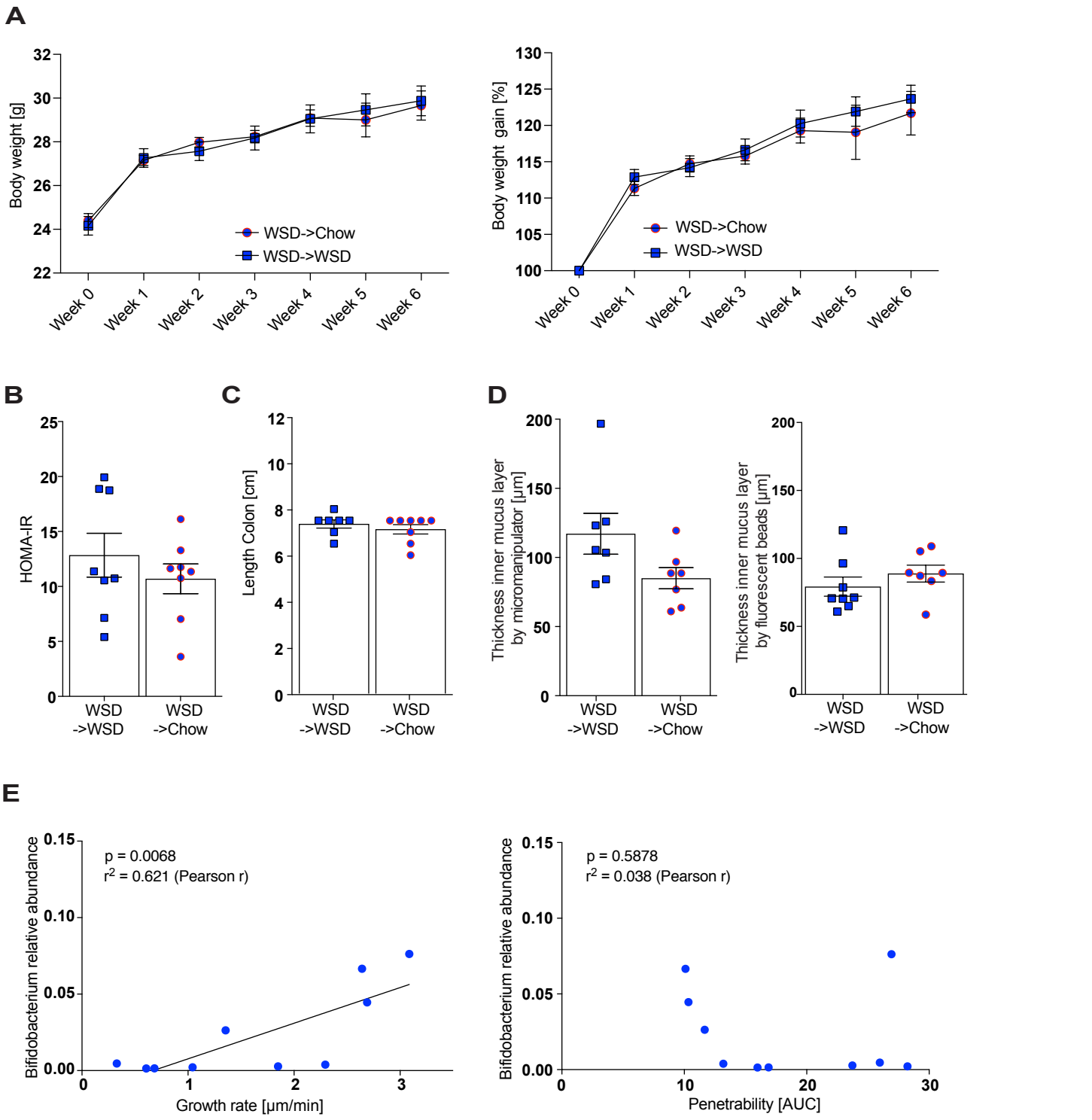
Genetic obesity does not affect the inner colonic mucus layer in mice. Related to Figure 1. Body weight, fasting blood glucose, insulin concentration and HOMA-IR were determined from littermate controlled, co-housed genetically obese and lean mice (6 mice/group) (A). Thickness of the inner colonic mucus layer was measured *ex vivo* by measuring the distance between black 10 µm beads and the epithelium by a micromanipulator (B) or by measuring the distance between 1 µm fluorescent beads and the stained epithelial surface with a confocal microscope (C). (D) Confocal z-stacks (left) calculated from the position of 1 µm fluorescent beads (center) were used to determine penetrability of the inner colonic mucus layer (right). The median z-stack is shown for each mouse. Turquoise: colonic tissue; red: bacteria-sized beads. (E) Growth rate of the colonic mucus. Data are presented as mean ± SEM. Statistical significance was determined by Mann-Whitney U test with (*) = $p < 0.05$ and (**) = $p < 0.01$.

Figure S2



Western diet feeding affects distinct proteins in the inner colonic mucus. Related to Figure 2. Label-free quantification (LFQ) of mucus proteins that were altered between chow- and WSD-fed mice (n = 5 mice/group) and detected in at least 3 samples of the group with higher concentration. Data are presented as mean ± SEM and missing values indicate that peptide was not detected in sample. Lgals2: Galectin-2, Dmbt1: Deleted in malignant brain tumors 1 protein (data for Dmbt1 are also plotted in Figure 2C and included here for comparison), Ccl6: C-C motif chemokine 6; CCL6(22-95);CCL6(23-95), Fabp6: Gastrotropin, B3gnt7: UDP-GlcNAc:betaGal beta-1,3-N-acetyl-glucosaminyl-transferase 7, Atp12a: Potassium-transporting ATPase alpha chain 2, Gyk;Gk: Glycerol kinase, Hist2h2ab: Histone H2A type 2-B, Fth1: Ferritin heavy chain, Rpn1: Dolichyl-diphosphooligosaccharide--protein glycosyl-transferase subunit 1. Hp: Haptoglobin;Haptoglobin alpha chain;Haptoglobin beta chain, Serpina1e: Alpha-1-antitrypsin 1-5, Nqo1: NAD(P)H dehydro-genase [quinone] 1, Mptx: Mucosal pentraxin, Mup: Major urinary proteins, Isg15: Ubiquitin-like protein ISG15, Fam49b: Protein FAM49B, Dtx3l: E3 ubiquitin-protein ligase DTX3L, Ctnna2: Catenin alpha-2, Entpd5: Ectonucleoside triphosphate diphosphohydrolase 5.

Figure S3



Microbial transplant does not affect host metabolism or mucus thickness while mucus growth rate correlates with Bifidobacterium abundance. Related to Figure 5.
Mice (n = 7 - 8/ group) received a weekly microbiota transplant from WSD-fed donor mice (WSD->WSD) or chow fed donor mice (WSD->Chow). (A) Body weight and body weight gain was followed over the course of the experiment. After six weeks of WSD feeding HOMA-IR (B) and length of the colon (C) were determined. (D) Thickness of the inner colonic mucus layer was measured *ex vivo* with a micromanipulator by measuring the distance between black 10 µm beads and the epithelial surface or with a confocal microscope by measuring the distance of fluorescent 1 µm (bacteria sized) beads and the stained epithelium. A-D: Data are presented as mean ± SEM and statistical significance was determined by unpaired t-test with (*) = $p < 0.05$. (E) Correlation between relative abundance of Bifidobacterium and mucus growth rate (left) and mucus penetrability (right). A linear regression line in the figure indicates a significant Pearson correlation with $p < 0.05$.