

Intestinal serotonin and fluoxetine exposure modulate bacterial colonization in the gut

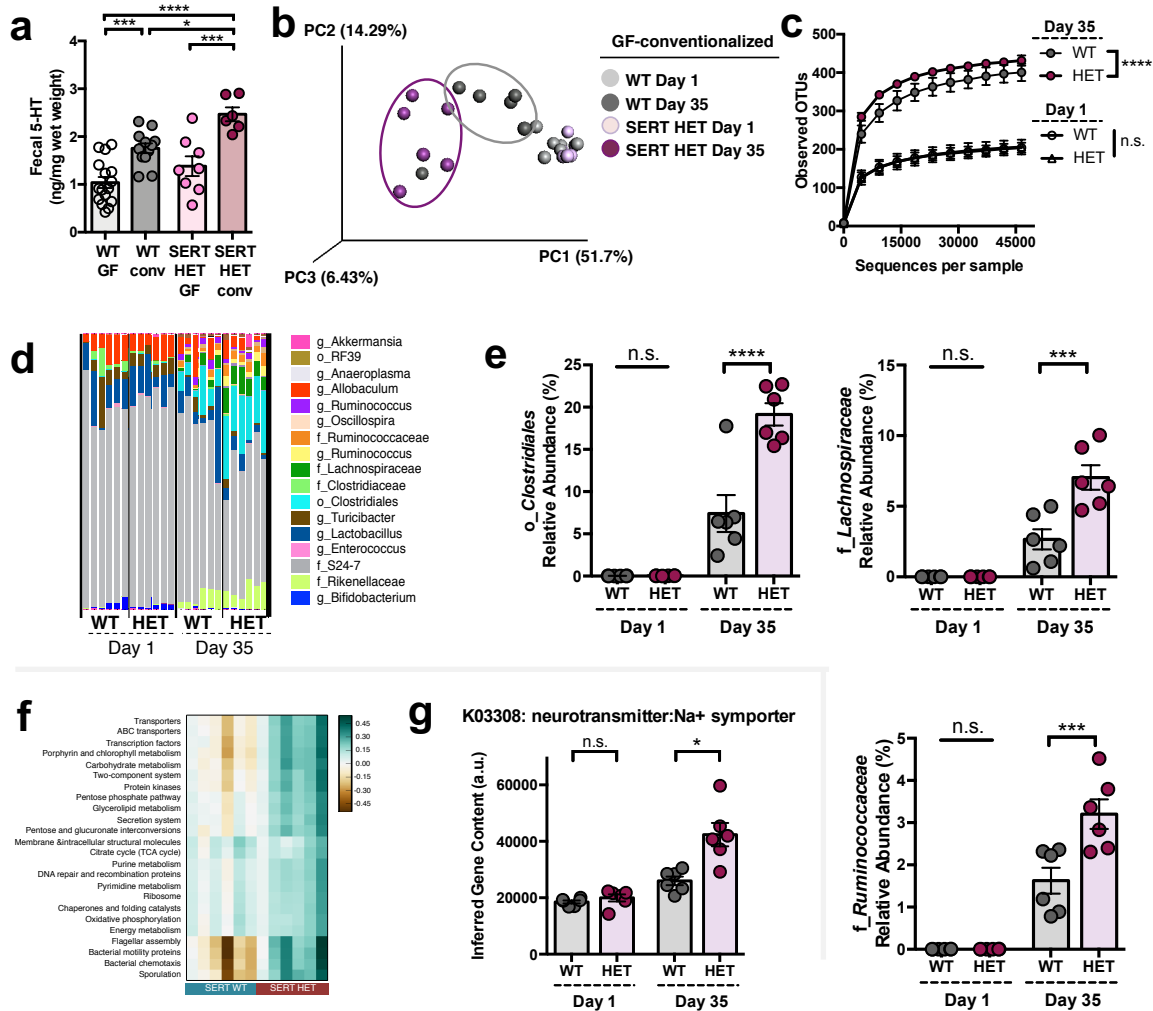
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Supplementary Materials:

- Supplementary Data Figures 1-19
- Supplementary Tables 1-11 (Excel document)
- Supplementary Dataset 1
- Supplementary Discussion

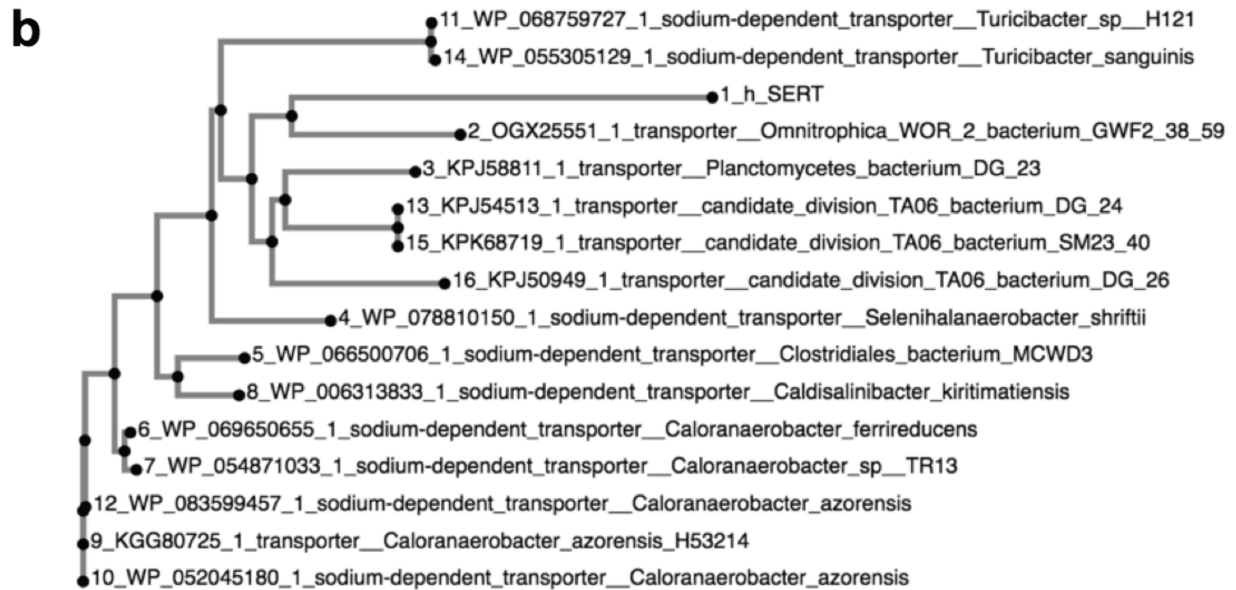


Supplementary Data Fig. 1: Conventionalization of GF SERT^{+/-} mice results in enrichment of spore-forming bacteria of the gut microbiota. **a**, Fecal 5-HT levels from conventionalized (conv) WT vs. SERT^{+/-} (HET) mice relative to GF controls (one-way ANOVA with Bonferroni, n=16, 12, 8, 6 mice). **b**, Principal coordinate analysis of 16S rDNA sequencing data of feces from WT vs. SERT HET mice at 1 day and 35 days after conventionalization (n=6 cages). **c**, Alpha-diversity of OTUs from 16S rDNA sequencing of feces from WT vs SERT HET mice at 1 day and 35 days after conventionalization (two-way ANOVA with Bonferroni, n=6 cages). **d**, Taxonomic diversity of the fecal microbiota from WT vs SERT HET mice at 1 day and 35 days after conventionalization (n=6 cages). **e**, Relative abundance of bacterial taxa in fecal microbiota from WT vs SERT HET mice at 1 day and 35 days after conventionalization (one-way ANOVA with Kruskal-Wallis, n=6 cages). **f**, Pathway analysis of inferred metagenomes generated by PICRUSt analysis of 16S rDNA sequencing data from WT vs SERT HET mice at 35 days after conventionalization (n=6 cages). **g**, Inferred gene content for neurotransmitter sodium symporter (NSS) related proteins calculated by PICRUSt analysis of 16S rDNA sequencing data from WT vs SERT HET mice at 35 days after conventionalization (one-way ANOVA with Kruskal-Wallis,

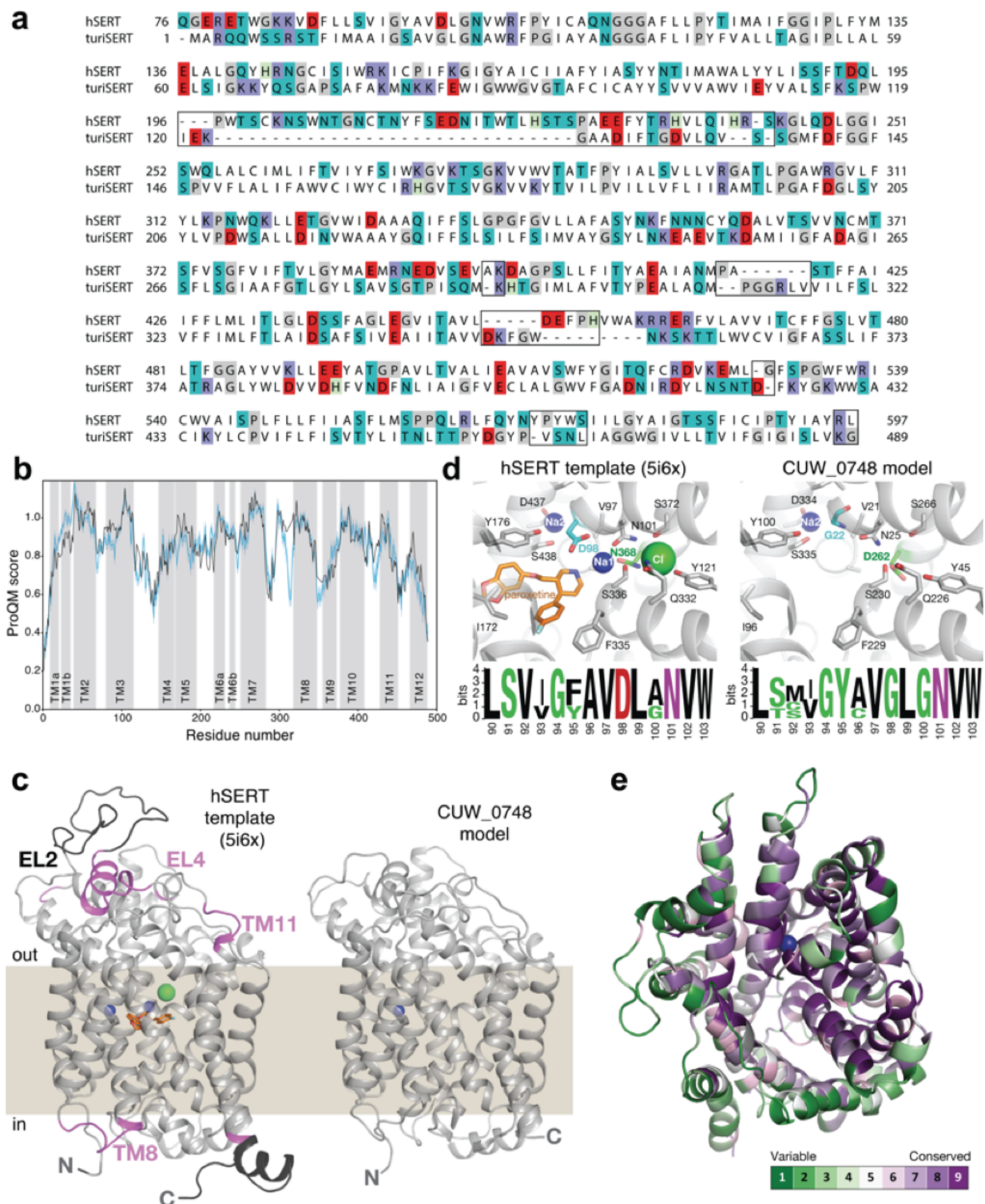
n=6 cages). (Mean \pm SEM, * $p < 0.05$, *** $p < 0.001$, **** $p < 0.0001$, n.s. = not statistically significant; refer to Supplementary Table 11 for detailed statistical information)

a

Accession	Organism	Protein	Identity (%)	Coverage (%)	Max Score	Length (bp)	E-value
AAW80933	<i>Homo sapiens</i>	serotonin transporter (<i>SERT</i>)				630	
NP_034614.2	<i>Mus musculus</i>	serotonin transporter (<i>SERT</i>)	93	100	1204	630	0.E+00
CAA62566.1	<i>Homo sapiens</i>	norepinephrine transporter (<i>NET</i>)	53	86	607	617	0.E+00
AAC50179.2	<i>Homo sapiens</i>	dopamine transporter (<i>DAT</i>)	50	90	598	620	0.E+00
N/A	<i>Turicibacter sanguinis</i> MOL361	putative serotonin transporter	34	76	264	498	2.E-85
ZP_08167800.1	<i>Turicibacter</i> sp. HGF1	putative sodium-dependent serotonin transporter	34	76	264	498	2.E-85
ZP_06621923.1	<i>Turicibacter sanguinis</i> PC909	sodium:neurotransmitter symporter family protein	34	76	268	498	9.E-87
WP_068759727.1	<i>Turicibacter</i> H121	sodium-dependent transporter	35	76	279	503	2.E-83
WP_055305129.1	<i>Turicibacter sanguinis</i>	sodium-dependent transporter	35	76	278	503	8.E-83
2A65_A	<i>Aquifex aeolicus</i> VF5	leucine transporter (<i>LeuT</i>)	26	71	106	519	2.E-28

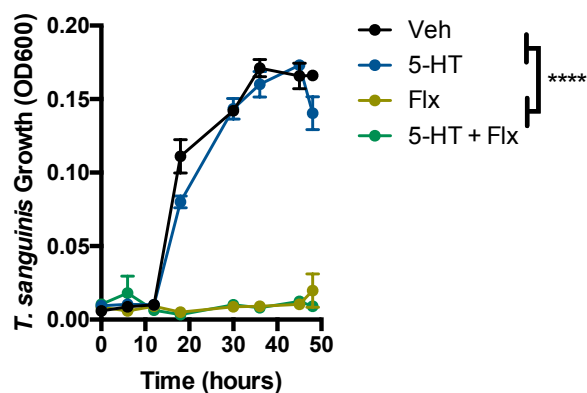


Supplementary Data Fig. 2: Homology of *Turicibacter sanguinis* putative serotonin transporter to human SERT. a, BLASTP alignment scores between human (h) SERT with other mammalian biogenic amine transporters, *Turicibacter* orthologs and bacterial amino acid transporter, LeuT. **b**, Unrooted phylogenetic tree of top 15 bacterial orthologs to hSERT.

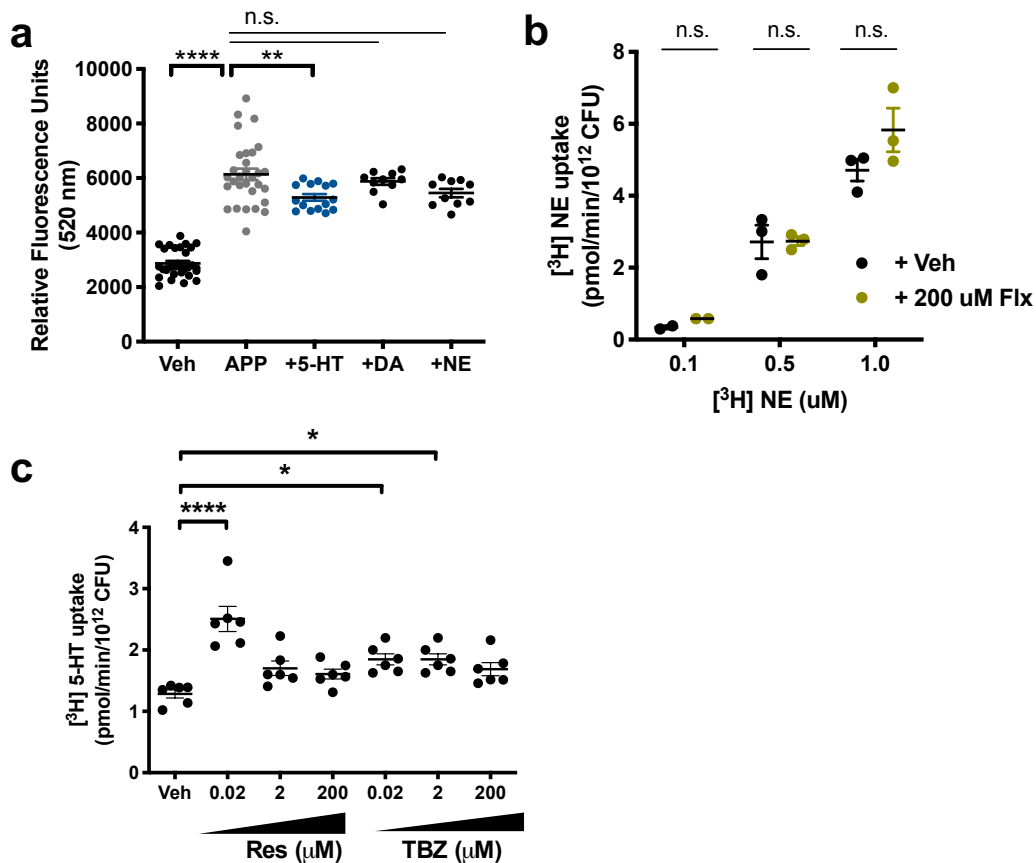


Supplementary Data Fig. 3: Structural modeling of CUW_0748 from *Turicibacter sanguinis* strain MOL361. **a**, Sequence alignment of CUW_0748 (turiSERT) and the template, thermostabilized human (h) SERT (PDB code 5i6x). Black rectangles indicate areas where refinements were made relative to the initial AlignMe PST pair-wise alignment. Background colors indicate residue type: acidic (red), basic (dark blue), polar (cyan), hydrophobic or aromatic

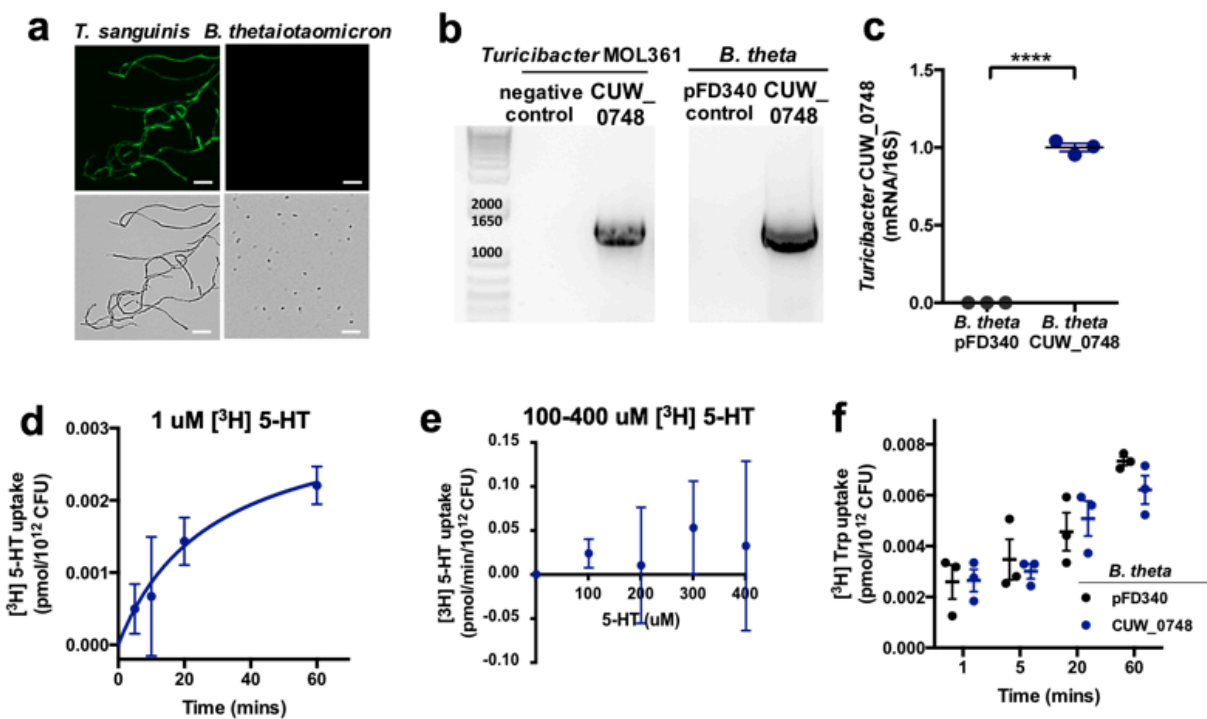
(white), glycine and proline (gray), and histidine (light green). **b**, Average per-residue ProQM scores of 500 models obtained after refining the sequence alignment (cyan line) and their standard deviation (blue shaded region). Global ProQM score before refinement: 0.83, after refinement: 0.86; ProQM score of truncated X-ray structure template (5i6x): 0.83 (black line). MolProbity score of final model: 1.45 Å, on par with the score of the template (5i6x chain A; 2.2 Å), and there were no residues in the generously-allowed or disallowed residues of the Ramachandran plot. **c**, Template and structural model viewed from within plane of the membrane, highlighting segments that are only present in template (dark gray), and segments where alignment was refined (pink). These adjustments were only found to be necessary in loop regions or at the ends of transmembrane helices. Bound ligands include paroxetine (orange sticks), Na⁺ (blue) and Cl⁻ ions (green). The ion at the Na2 site is taken from another structure of hSERT, 5i71, and its position was obtained by superposing the backbone of the Na2-site residues onto the equivalent region in 5i6x. **d**, Central binding pocket in outward-open hSERT (left) compared to the equivalent region in the refined model of CUW_0748 (right) highlighting the substitution of hSERT residue D98 by G22 (cyan) in the amine binding region, as well as the substitution of hSERT residue N368 by D262 in the Cl⁻ binding region (green). Ligands are shown as in panel c. Weblogos show conservation at position 98 of the SLC6 family of human NSS transporters, i.e. Asp at position 98 in monoamine transporters (left) and Gly in amino-acid transporters (right). **e**, ConSurf analysis using the ConSurf server, based on a HMMER search with 1 iteration and E-value cut-off of 0.0001 against the Uniref-90 database on 11 Dec 2018, illustrating the location of conserved and variable residues in the CUW_0748 model. The protein is viewed down the extracellular pathway.



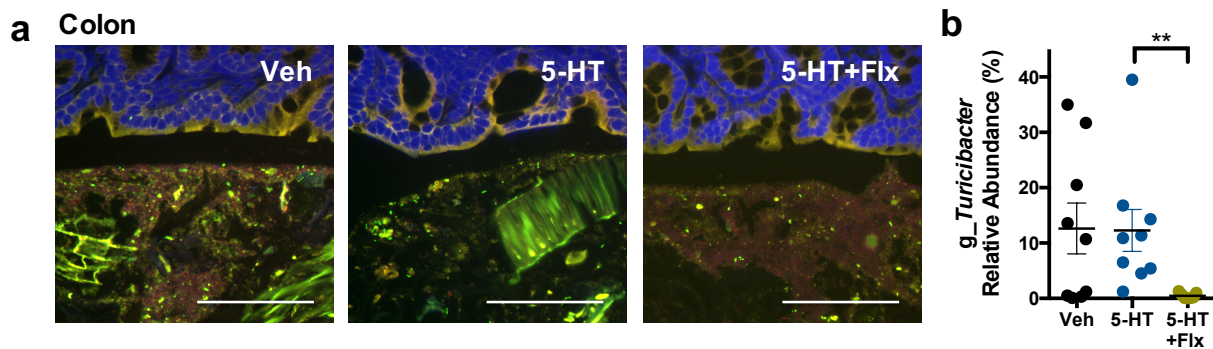
Supplementary Data Fig. 4: Growth curve of *Turicibacter sanguinis* MOL361 in Schaedler broth supplemented with 5-HT and/or fluoxetine. Cell density of *T. sanguinis* at 6, 12, 18, 30, 36, 45 and 48 hours of growth in the presence of 200 uM 5-HT, fluoxetine (Flx) or both 5-HT and Flx. Treatments were added at the start of culture (e.g. t = 0 h). (two-way ANOVA with Bonferroni, n=3 cultures) (Mean \pm SEM, **** p < 0.0001; refer to Supplementary Table 11 for detailed statistical information)



Supplementary Data Fig. 5: Neurotransmitter uptake in *Turicibacter sanguinis* MOL361 in response to unlabeled biogenic amines and VMAT inhibitors. **a**, APP+ uptake by *T. sanguinis* exposed to vehicle (Veh) or APP+, either alone (APP), or with 30 min pre-treatment with 200 uM unlabeled 5-HT (+5-HT), dopamine (+DA) or norepinephrine (+NE) (one-way ANOVA with Bonferroni, n=33, 33, 18, 10, 10 cultures; n.s. (APP vs. +DA): p= >0.9999, n.s. (APP vs +NE): p=0.1525). **b**, [³H]NE uptake in counts per min (CPM) by *T. sanguinis* after 30 min pre-treatment with vehicle (Veh) or 200 uM fluoxetine (+ Flx) (two-way ANOVA with Bonferroni, n=3 cultures). **c**, [³H]5-HT uptake in counts per min (CPM) by *T. sanguinis* after 30 min pre-treatment with vehicle (Veh), reserpine (+ Res) or tetrabenazine (TBZ) (one-way ANOVA with Bonferroni, n=6 cultures). (Mean ± SEM, * p < 0.05, ** p < 0.01, **** p < 0.0001, n.s. = not statistically significant; refer to Supplementary Table 11 for detailed statistical information)

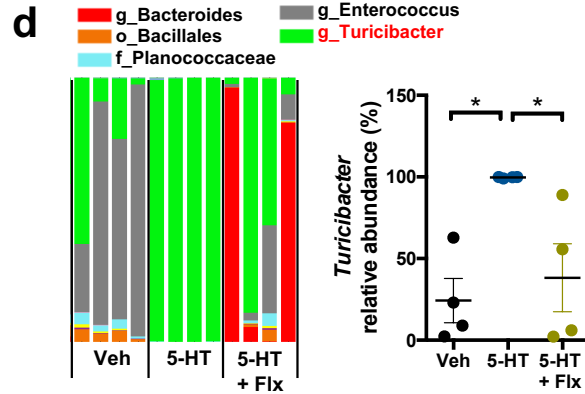
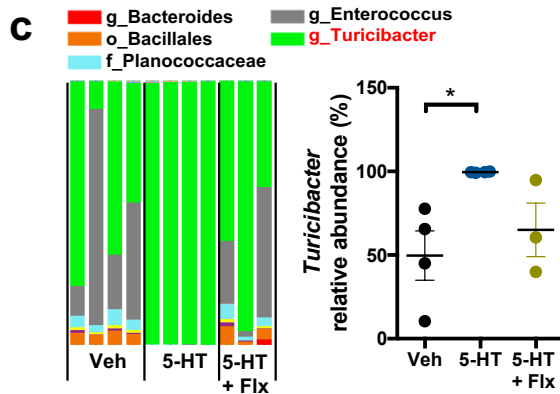
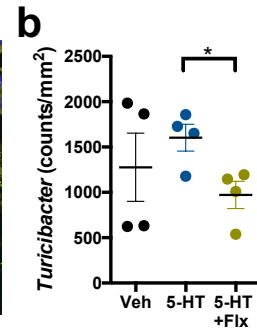
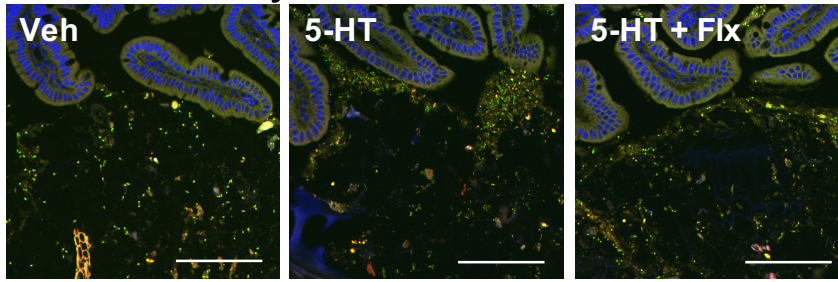


Supplementary Data Fig. 6: Heterologous expression of CUW_0748 from *Turicibacter sanguinis* in *Bacteroides thetaiotaomicron*. **a**, Representative image of imported APP+ (green) in wildtype *T. sanguinis* versus *B. thetaiotaomicron* (*B. theta*) as measured by the APP+ uptake assay (representative of at least 3 experiments). **b**, PCR amplification of the CUW_0748 gene in *B. theta* clones harboring the CUW_0748 insert compared to vector control (pFD340) (representative of at least 3 experiments). **c**, qPCR for CUW_0748 mRNA expression in *B. theta* clones harboring the CUW_0748 insert compared to the vector control (pFD340) (two-tailed unpaired Student's t-test, n=3 cultures). **d**, Kinetics of [³H]5-HT uptake by CUW_0748, as measured as the difference in [³H]5-HT import between *B. theta* expressing CUW_0748 and the vector control (pFD340) at 5, 10, 20 and 60 min of incubation with 1 uM [³H]5-HT. n=4, 10, 7, 13, 3 biologically independent samples per time point. **e**, Dose-dependent [³H]5-HT uptake by CUW_0748, as measured as the difference in [³H]5-HT import between *B. theta* expressing CUW_0748 and the vector control (pFD340) at 1-400 uM [³H]5-HT. n=8, 9, 9, 9, 9 biologically independent samples per time point. **f**, [³H]Trp uptake in counts per min (CPM) by *B. theta* expressing CUW_0748 compared to the vector control (pFD340) after 1, 5, 20 and 60 min of incubation with 1 uM [³H]Trp (two-way ANOVA with Bonferroni, n=3 biologically independent samples per time point). (Mean ± SEM, **** p < 0.0001, n.s. = not statistically significant; refer to Supplementary Table 11 for detailed statistical information).



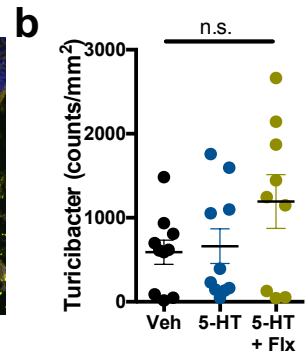
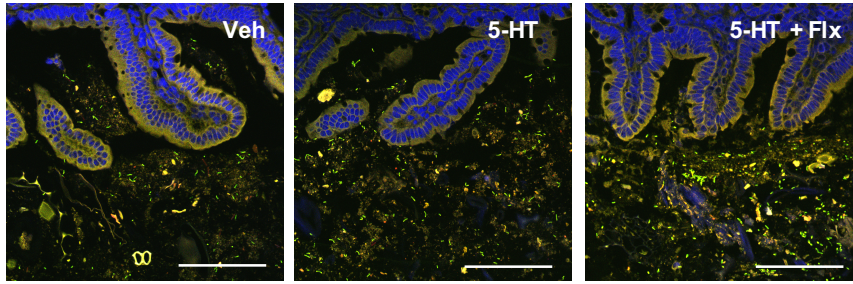
Supplementary Data Fig. 7: Effects of 5-HT and fluoxetine on *T. sanguinis* colonization in the colon from antibiotic-treated mice. a, Representative FISH images of *T. sanguinis* (green), intestinal epithelial cells (DAPI, blue) and bacteria stained with the eubacterial probe EUB338 (red) in colons from antibiotic-treated mice gavaged with *T. sanguinis* pre-treated for 4 hr with vehicle, 200 uM 5-HT, or 200 uM 5-HT with Flx (n=9). Scale bar denotes 100 um. **b**, Relative abundance of *Turicibacter* measured by 16S rDNA sequencing of colonic luminal contents harvested from antibiotic-treated mice colonized with *T. sanguinis* pre-treated for 4 hr with vehicle, 200 uM 5-HT, or 200 uM 5-HT with Flx (one-way ANOVA with Bonferroni, n=9). (Mean \pm SEM, ** p < 0.01; refer to Supplementary Table 11 for detailed statistical information)

a Pre-treatment only

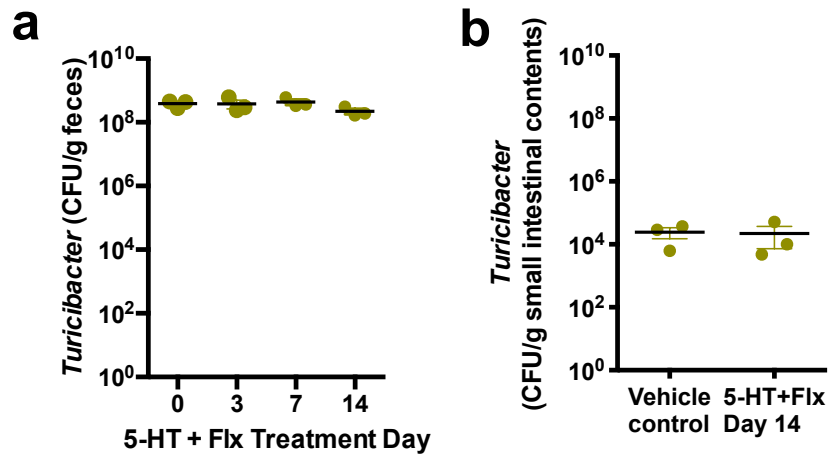


Supplementary Data Fig. 8: Effects of 5-HT and fluoxetine bacterial pre-treatment only on *Turicibacter sanguinis* colonization in the small intestine of antibiotic-treated mice. **a**, Representative FISH images of *T. sanguinis* (green), intestinal epithelial cells (DAPI, blue) and bacteria stained with the eubacterial probe EUB338 (red) in small intestines from antibiotic-treated mice gavaged with *T. sanguinis* pre-treated for 4 hr with vehicle, 200 uM 5-HT, or 200 uM 5-HT with Flx (representing n=4 mice). Scale bar denotes 100 um. **b**, *T. sanguinis* cell counts from FISH images of small intestines from antibiotic-treated mice gavaged with *T. sanguinis* pre-treated for 4 hr with vehicle, 200 uM 5-HT, or 200 uM 5-HT with Flx (one-way ANOVA with Bonferroni, n=4 mice). **c**, *Turicibacter* relative abundances based on 16S rDNA sequencing of small intestinal luminal contents from antibiotic-treated mice gavaged with *T. sanguinis* pre-treated for 4 hr with vehicle, 200 uM 5-HT, or 200 uM 5-HT with Flx (n=4, 4, 3 cages). **d**, *Turicibacter* relative abundances based on 16S rDNA sequencing of colonic luminal contents from antibiotic-treated mice gavaged with *T. sanguinis* pre-treated for 4 hr with vehicle, 200 uM 5-HT, or 200 uM 5-HT with Flx (n=4 cages). See supplementary discussion for details. (Mean \pm SEM, * p < 0.05; refer to Supplementary Table 11 for detailed statistical information).

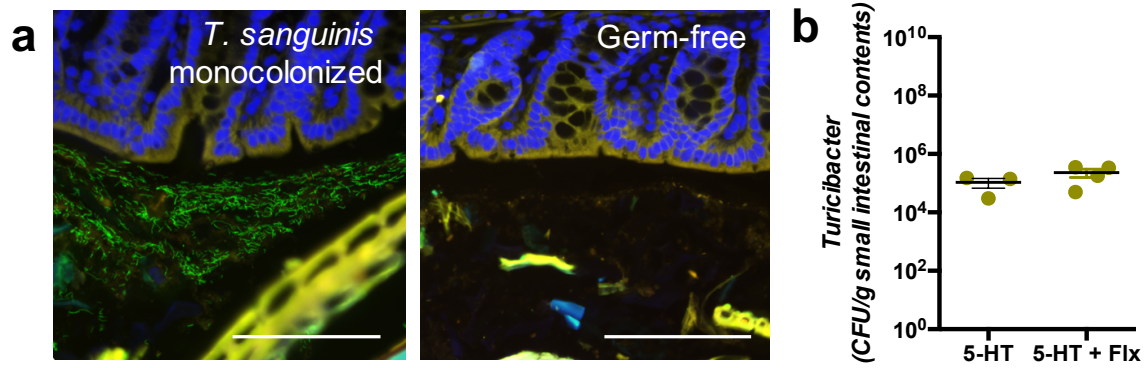
a Drinking water only



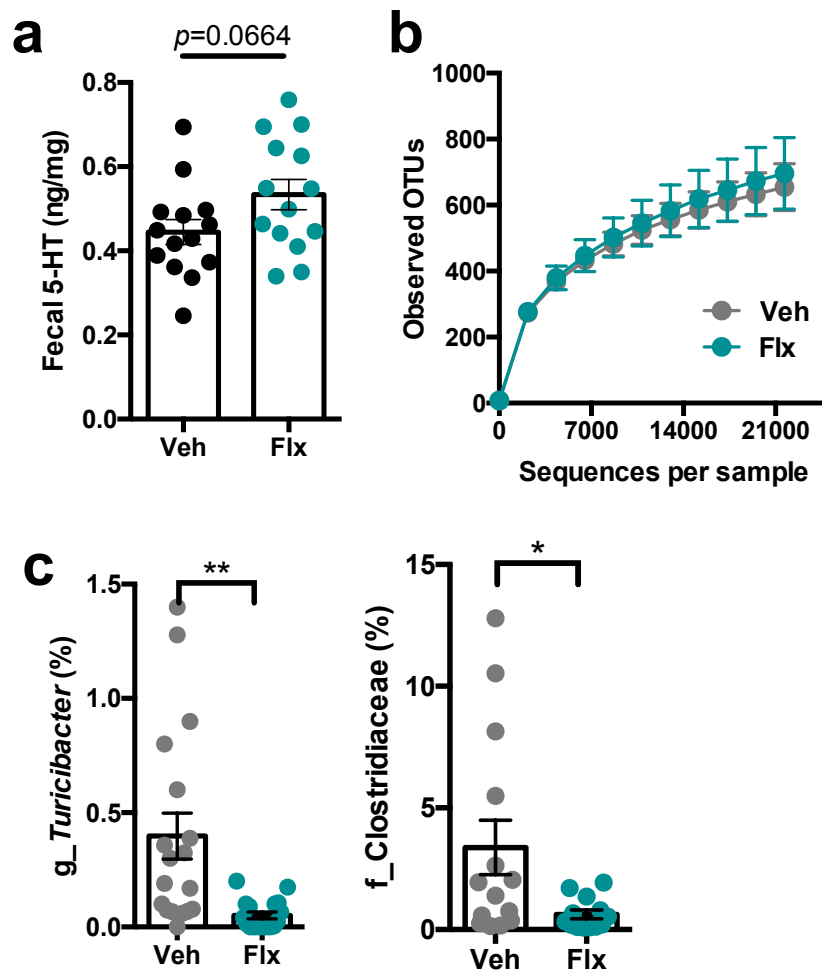
Supplementary Data Fig. 9: Effects of 5-HT and fluoxetine administration in drinking water only on *Turicibacter sanguinis* colonization in the small intestine of antibiotic-treated mice. **a**, Representative FISH images of *T. sanguinis* (green), intestinal epithelial cells (DAPI, blue) and bacteria stained with the eubacterial probe EUB338 (red) in small intestines from antibiotic-treated mice gavaged with wildtype *T. sanguinis* once daily for 5 days and administered vehicle, 5-HT (24 $\mu\text{g}/\text{ml}$), or 5-HT (24 $\mu\text{g}/\text{ml}$) and Flx (40 $\mu\text{g}/\text{ml}$) in the drinking water (representing n=9, 10, 10 mice) **b**, *T. sanguinis* cell counts from FISH images of small intestines (one way ANOVA with Bonferroni, n=9, 10, 10 mice; n.s.: p=0.2254). (n.s. = not statistically significant)



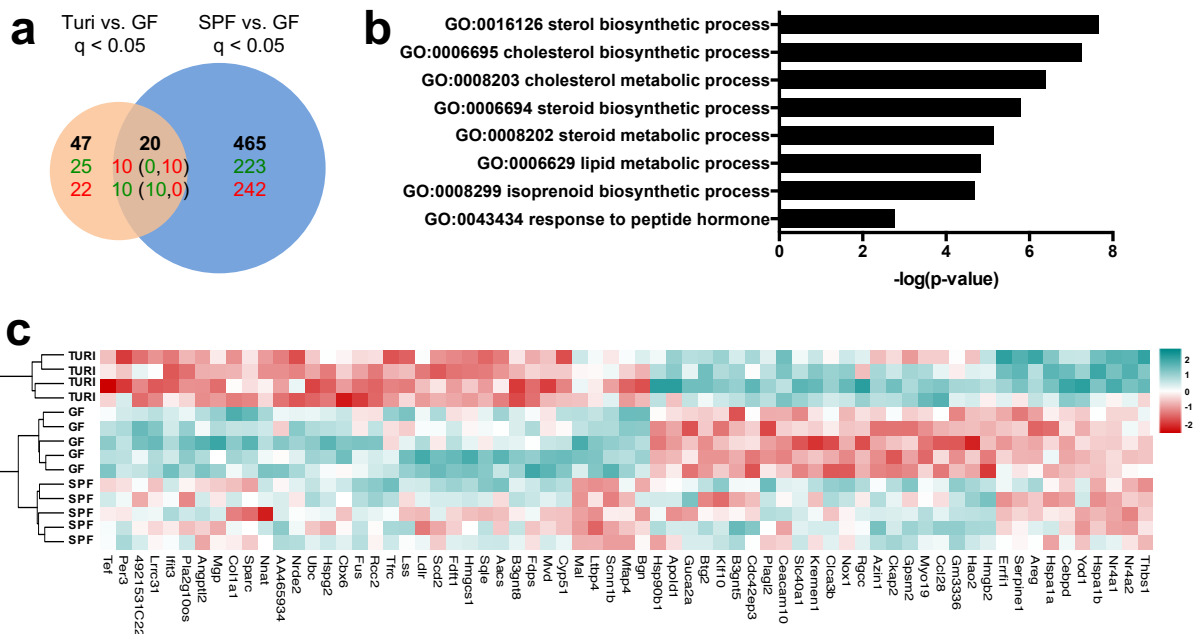
Supplementary Data Fig. 10: Effects of 5-HT and fluoxetine on *Turicibacter sanguinis* loads in monocolonized mice. **a**, Fecal loads of *T. sanguinis* MOL361 at 0, 3, 7 and 14 days after host treatment with 5-HT (24 μ g/ml) and Flx (40 μ g/ml) in drinking water (one way ANOVA with Bonferroni, n=3 mice). **b**, Small intestinal fecal loads of *T. sanguinis* at 14 days after host treatment with 5-HT (24 μ g/ml) and Flx (40 μ g/ml) in drinking water (two-tailed unpaired t-test, n=3 mice). (n.s. = not statistically significant)



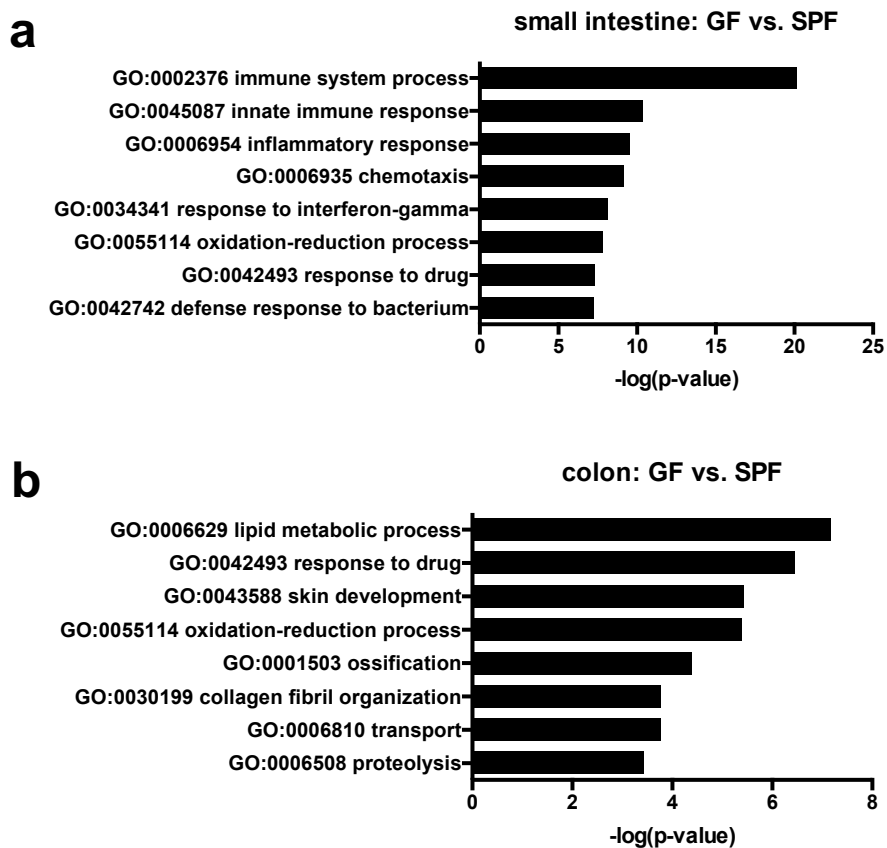
Supplementary Data Fig. 11: Effects of 5-HT and fluoxetine bacterial pre-treatment on *T. sanguinis* colonization of GF-mice. **a**, Representative images of FISH staining for *T. sanguinis* (green) and intestinal epithelial cells (DAPI, blue) in colons from *T. sanguinis* monocolonized mice versus GF controls. Scale bar denotes 100 μ m (n=3). **b**, Fecal loads of *T. sanguinis* at 14 days after gavage with *T. sanguinis* pre-treated for 4 hours with 200 μ M 5-HT or 5-HT with Flx (n=3, 4 mice).



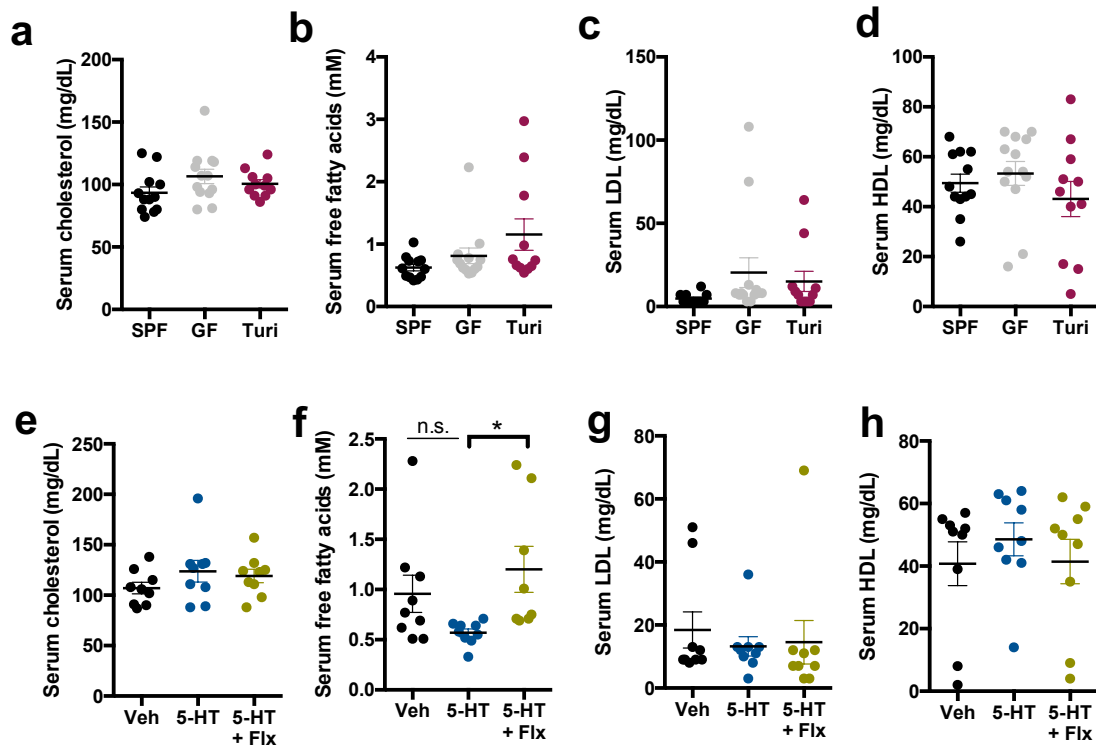
Supplementary Data Fig. 12: Effects of fluoxetine treatment on the fecal microbiota of SPF mice. **a**, Fecal levels of 5-HT after oral gavage with 10 mg/kg fluoxetine (Flx) daily for 7 days or fluoxetine treatment in drinking water (40 μ g/ml) for 2 weeks (two-tailed unpaired Student's t-test, $n=14$ mice). **b**, Alpha-diversity of the fecal microbiota after Flx vs. vehicle (veh) treatment ($n=18$ cages). **c**, Relative abundance of significantly altered bacterial taxa (two-way ANOVA with Kruskal Wallis, $n=18$ cages). (Mean \pm SEM, * $p < 0.05$, ** $p < 0.01$; refer to Supplementary Table 11 for detailed statistical information).



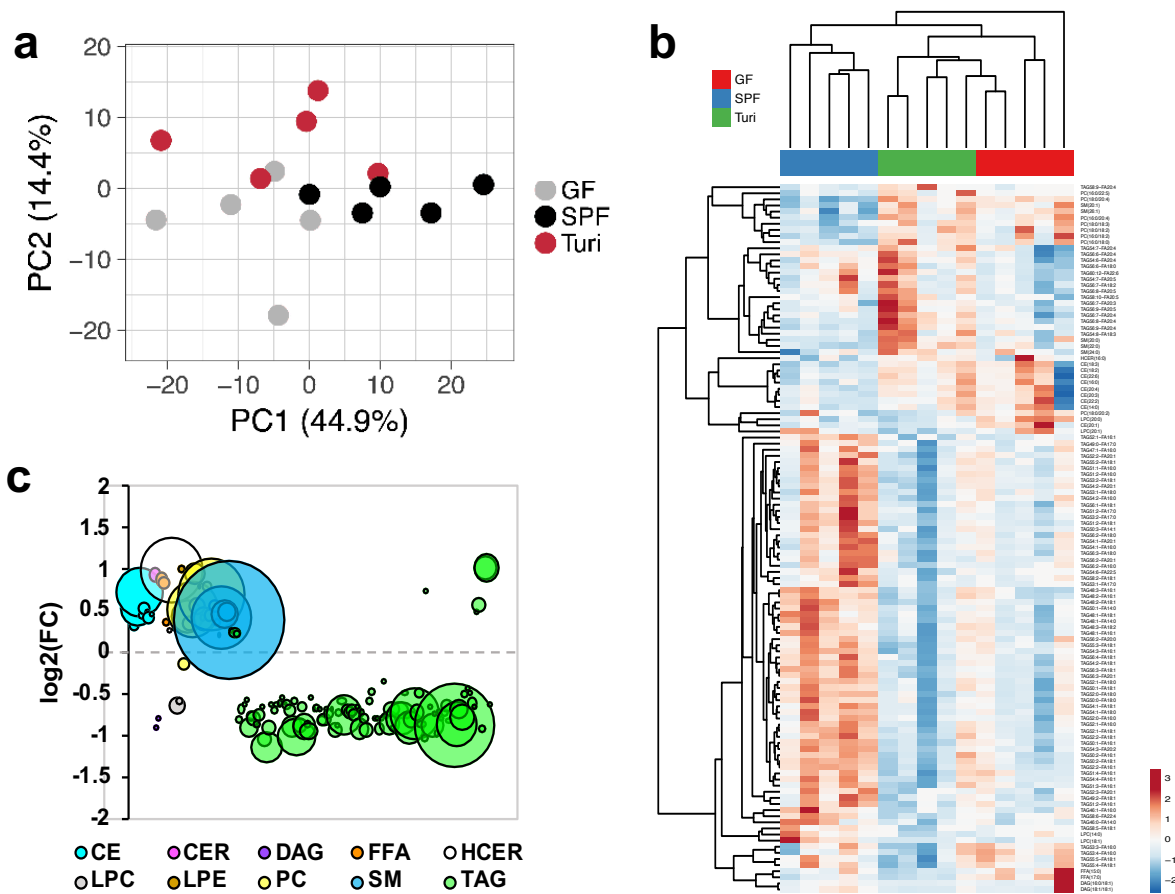
Supplementary Data Fig. 13: Effects of *Turicibacter sanguinis* MOL361 on colon gene expression. **a**, Differentially expressed genes ($q < 0.05$) in colons from *T. sanguinis* monocolonized, GF or SPF mice (Wald test, $n=5, 5, 4$ mice). Bolded numbers represent total differentially expressed genes. Numbers in green denote upregulated genes; numbers in red denote downregulated genes. Numbers in parentheses denote subsets of *T. sanguinis*-regulated genes that were further differentially expressed by SPF ($n=5, 5, 4$ mice). **b**, GO term enrichment analysis of genes differentially expressed in colon in response to *T. sanguinis* relative to GF vs SPF controls (Fisher exact, $n=5, 5, 4$). **c**, Heatmap of the 87 genes that are differentially expressed genes by colon in response to *T. sanguinis* colonization ($n=5, 5, 4$).



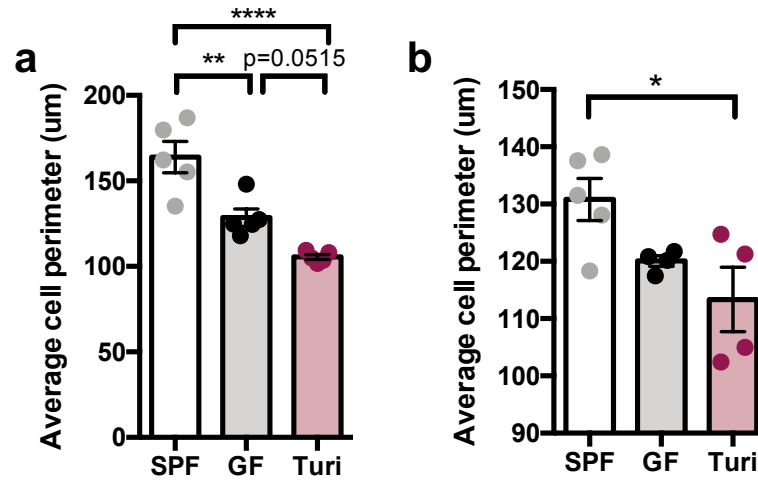
Supplementary Data Fig. 14: Gene ontology pathway analysis of colon and small intestine genes differentially regulated in GF mice relative to SPF controls. **a**, Top 8 pathways from GO-term enrichment analysis of genes differentially regulated in GF vs SPF small intestine (Fisher exact, n=5 mice). **b**, Top 8 pathways from go-term enrichment analysis of genes differentially regulated in GF vs SPF colon (Fisher exact, n=5 mice).



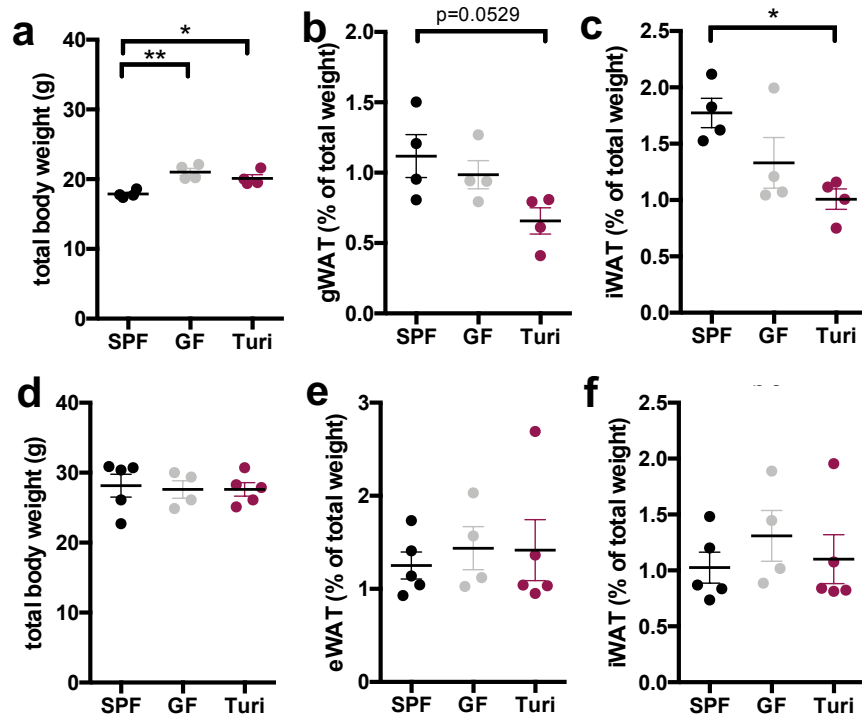
Supplementary Data Fig. 15: There is no effect of *T. sanguinis* colonization on total serum cholesterol, free fatty acids or lipoprotein levels. a-d, Serum lipid levels in mice reared GF, SPF, or monocolonized with *T. sanguinis*. (one-way ANOVA with Bonferroni, n=12, 13, 11 mice). e-h, Serum lipid levels in antibiotic-treated mice gavaged with *T. sanguinis* pre-treated for 4 hr with vehicle, 200 μ M 5-HT, or 200 μ M 5-HT with Flx (one-way ANOVA with Bonferroni, n=9 mice; n.s.: p=0.3156) (Mean \pm SEM, * p < 0.05; refer to Supplementary Table 11 for detailed statistical information).



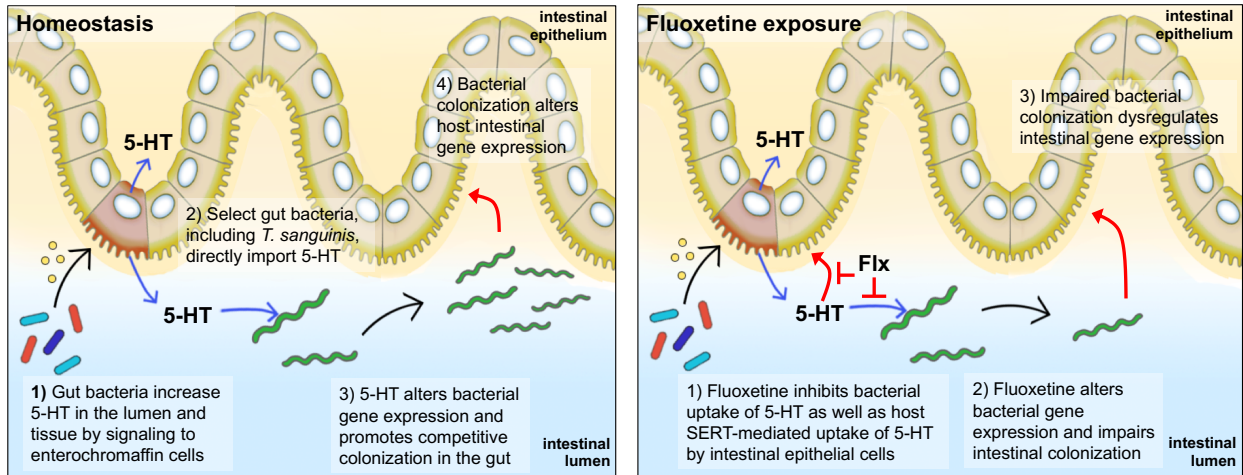
Supplementary Data Fig. 16: Lipidomic analysis of serum from GF, SPF and *T. sanguinis*-monocolonized mice. **a**, Principal components analysis of lipidomic data for all 1100 lipid species detected in serum from GF, SPF and *T. sanguinis*-monocolonized mice (n=5 mice). **b**, Heatmap of all serum lipid species differentially-regulated ($p < 0.05$) by *T. sanguinis* colonization relative to either GF or SPF controls (n=5 mice). **c**, Average fold change (FC) of serum lipid species ($p < 0.05$) differentially regulated by *T. sanguinis* compared to SPF controls. Largest circle = $p < 0.001$, smallest circle = $p < 0.05$. CE=cholesterol esters, CER=ceramides, DAG=diacylglycerols, FFA=free fatty acids, HCER=hexosyl ceramides, LPC=lysophosphatidylcholines, LPE=lysophosphatidylethanolamine, PC=phosphatidylcholines, SM=sphingomyelins, TAG=triacylglycerides. (n=5 mice).



Supplementary Data Fig. 17: Adipocyte perimeter in inguinal white adipose tissue from SPF, GF, or *T. sanguinis*-monocolonized mice. **a**, Adult female mice after >2 weeks of *T. sanguinis* colonization (one-way ANOVA with Bonferroni, n=5 mice). **b**, Adult male mice after >2 weeks of *T. sanguinis* colonization (one-way ANOVA with Bonferroni, n=5, 4, 4 mice). (Mean ± SEM, * p < 0.05, ** p < 0.01, **** p < 0.0001; refer to Supplementary Table 11 for detailed statistical information).



Supplementary Data Fig. 18: Host body weight, gonadal white adipose tissue (gWAT), inguinal white adipose tissue (iWAT) mass and epididymal white adipose tissue (eWAT) in SPF, GF, or *T. sanguinis*-monocolonized mice. a-c, Adult female mice after 2 weeks of *T. sanguinis* colonization (one-way ANOVA with Bonferroni, n=4 mice) d-f, Adult male mice after 2 weeks of *T. sanguinis* colonization (one-way ANOVA with Bonferroni, n=5, 4, 5 mice). (Mean \pm SEM, * p < 0.05, ** p < 0.01, n.s. = not statistically significant; refer to Supplementary Table 11 for detailed statistical information).



Supplementary Data Fig. 19: Model of interactions between the gut microbiota, intestinal 5-HT and fluoxetine.

Supplementary Table and Dataset Legends:

Supplementary Table 1: Taxonomic distribution of fecal microbiota from SPF mice supplemented with 5-HT vs vehicle

Supplementary Table 2: Taxonomic distribution of fecal microbiota from SERT deficient mice

Supplementary Table 3: Taxonomic distribution of fecal microbiota on Day 1 vs 35 after inoculation of WT vs SERT+/- GF mice with SPF microbiota

Supplementary Table 4: Bacterial sequence homologs for CUW_0748 from *Turicibacter sanguinis* MOL361

Supplementary Table 5: Taxonomic distribution of fecal microbiota from mice colonized with mouse-derived (m) or human-derived (h) spore-formers (Sp)

Supplementary Table 6: RNAseq of *Turicibacter sanguinis* MOL361 after exposure to 5-HT, fluoxetine, both 5-HT and fluoxetine, or vehicle

Supplementary Table 7: Differentially expressed genes in *Turicibacter sanguinis* MOL361 induced by fluoxetine in the absence of 5-HT. Mean reads per kilobase of transcript per million mapped reads (RPKM) for differentially expressed genes ($p < 0.05$) in *T. sanguinis* treated for 4 hr with 200 μ M fluoxetine vs vehicle control.

Supplementary Table 8: Normalized counts of genes differentially expressed in small intestine by monocolonization with *T. sanguinis*

Supplementary Table 9: Normalized counts of genes differentially expressed in colon by monocolonization with *T. sanguinis*

Supplementary Table 10: Serum lipid concentrations (nmol/ml) in response to colonization with *Turicibacter sanguinis* MOL361

Supplementary Table 11: Statistical Data

Supplementary Data Set 1: Protein structural model of CUW_0748 from *Turicibacter sanguinis* MOL361, based on available structure of human SERT (PDB entry 5i6x chain A)

Supplementary Discussion:

We identify and characterize the function of a putative bacterial neurotransmitter sodium symporter (NSS) expressed by *Turicibacter sanguinis*, which displays considerable sequence and structural homology to the mammalian serotonin transporter (SERT). Consistent with the role of SERT, *T. sanguinis* uptakes 5-HT, which is partially inhibited by the selective serotonin reuptake inhibitor (SSRI) fluoxetine. Uptake of 5-HT is associated with reduced expression of genes encoding membrane solute transporters and sporulation factors. Treatment of *T. sanguinis* with fluoxetine reduces 5-HT uptake, reverses a subset of 5-HT-induced gene expression changes and significantly limits intestinal colonization. Recent microbiota profiling studies in human cohorts demonstrated that psychotropic drugs significantly alter the composition of the gut microbiota⁶³⁻⁶⁵. However, whether these compounds mediate microbiota changes through direct interactions with bacteria or indirect host responses remains unknown. In our study, we interrogate and identify direct effects of fluoxetine on the physiology of a gut bacterium and its ability to colonize the host gastrointestinal tract.

We find that 5-HT and fluoxetine regulate bacterial 5-HT import, gene expression and competitive colonization of *T. sanguinis*. While *T. sanguinis* treated with 5-HT is able to persistently colonize intestinal tracts of antibiotic-treated mice, co-treatment of *T. sanguinis* with fluoxetine reduced levels of colonized bacteria detected 1-7 days after initial colonization. Notably, we observed that the severity of fluoxetine-induced reductions in *T. sanguinis* colonization may vary depending on the competing bacterial taxon that enriches most highly after colonization. In 4 out of 6 competitive colonization experiments, *T. sanguinis* treated with fluoxetine exhibited substantially reduced competitive colonization ability when the dominant colonizing taxon was *Akkermansia*, *Blautia*,

Enterococcus or *Lactobacillus*; however in 2 experiments, *T. sanguinis* treated with fluoxetine maintained colonization ability when the second dominant taxon was *Clostridiaceae*. This is interesting in light of our findings that relative abundances of both *T. sanguinis* and *Clostridiaceae* co-vary and increase in response to elevations in host 5-HT. Additional studies are needed to fully examine the mechanisms underlying fluoxetine-induced impairments in *T. sanguinis* colonization, and to further explore the nature of microbe-microbe interactions between *T. sanguinis* and different bacterial species from the gut microbiome.

Previous reports revealed species- and dose-dependent antimicrobial effects of some SSRIs including fluoxetine, paroxetine and sertraline⁶⁶. Possible mechanisms of SSRI antimicrobial activity are largely unclear but may involve inhibition of bacterial multidrug efflux pumps⁶⁷. Fluoxetine inhibits the growth of *T. sanguinis* but does not significantly alter bacterial viability when added to a grown batch culture or when administered to monocolonized mice. In addition, fluoxetine has little effect on *T. sanguinis* gene expression in the absence 5-HT, and fluoxetine impairs the ability of *T. sanguinis* to competitively colonize in microbiota-depleted mice, but not its ability to monocolonize germ-free mice. Therefore, we believe there are direct effects of fluoxetine on *Turicibacter* that are mediated by 5-HT modulation, rather than any cytostatic, antimicrobial or off-target effects, but we do not exclude the possibility there may be additional effects of varied fluoxetine concentrations or treatment regimens on *Turicibacter* that modulate 5-HT uptake and intestinal colonization. Notably, paroxetine also inhibited growth of *T. sanguinis* and had no effect on viability when added to grown batch cultures. However, in a pilot experiment, pre-treatment of *T. sanguinis* with paroxetine, instead of fluoxetine, had no effect on its ability to colonize antibiotic-treated mice when examined 3 days later. This suggests that the

in vitro growth effects of SSRIs on select gut microbes can be decoupled from its *in vivo* effects on microbial colonization, and is consistent with literature indicating that different SSRIs exhibit disparate interactions with mammalian SERT in terms of binding site and specificity, allosteric modulation, duration of binding, and clinical effects. Notably, our results with fluoxetine were recently corroborated by microbiota profiling of a large (>2700) cohort of UK twins, which identified *Turicibacteraceae* as the only significantly altered taxon negatively associated with SSRI use³². While the study did not stratify by specific type of SSRI, the findings support the hypothesis that SSRIs can modulate particular bacteria indigenous to the gut microbiota. Given the prevalence of SSRI use worldwide, microbiota associations with major depressive disorder, and neuromodulatory effects of gut microbes, further research defining specific interactions between SSRIs and the gut microbiota is warranted.