

# Supplementary Table 1

# of read	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Actinobacteri	46487	58727	68004	76041	71873	30029	10813	30414	19197	44524	5072	4558	400	1667	2376	943	3790	2089	2655	958	1013
Aquificae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Armatimonac	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bacteroidetes	32484	27880	41671	20485	43612	112728	81001	50156	79354	43452	0	168	0	63	976	0	72	0	0	910	1296
Chlamydiae <sup>Δ</sup>	3646	17874	8620	13540	12917	3926	2062	7051	3802	16135	0	0	0	0	0	0	0	0	0	0	0
Chloroflexi	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Chrysiogenet	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cyanobacteri	0	0	0	0	0	74	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Deferribacter	493	0	358	230	273	1089	430	2639	314	910	0	0	0	0	0	0	0	0	0	0	0
Deinococcus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Elusimicrobi	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fibrobacteres	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Firmicutes	265377	235344	254929	225792	208753	153093	137944	162255	140183	146490	268520	271752	318929	324485	294735	215371	237872	266226	307564	262171	267497
Fusobacteria	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Gemmatimon	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nitrospirae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Planctomycet	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Proteobacteri	1927	4047	3088	5638	5138	9758	6488	9328	5235	11448	47730	50285	42244	27454	40993	9641	29060	45341	44358	14526	15316
Spirochaetes	332	445	1275	1537	1537	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Synergistetes	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tenericutes	1096	2072	600	1661	2338	342	92	0	0	65	0	0	0	0	86	0	0	0	0	0	54
Thermotogae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total read	351842	346389	37701	344662	346441	311039	238830	261843	248085	263024	321322	326763	361573	353669	339166	225955	270794	313656	354577	278565	285176

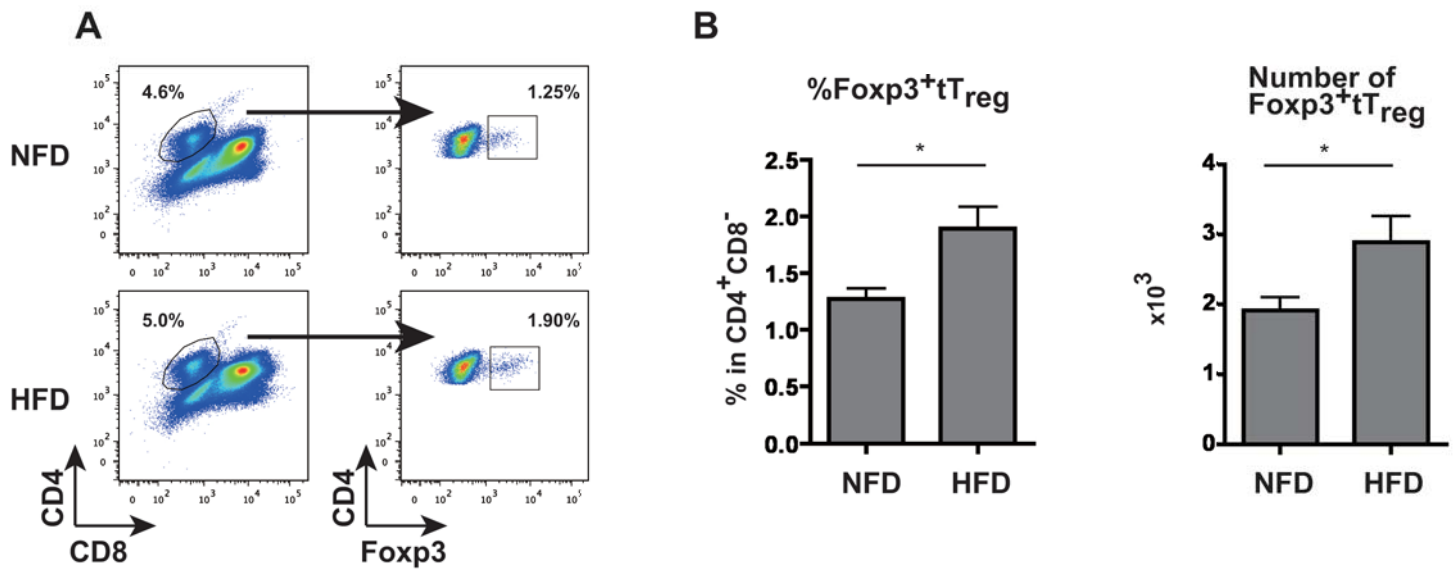
  

relative abundance	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Actinobacteri	0.13212465	0.1695406	0.18004718	0.22062484	0.20746101	0.09654416	0.04527488	0.11615357	0.07738074	0.16927733	0.01578479	0.01394895	0.00110628	0.00471345	0.00700542	0.0041734	0.01399588	0.00666016	0.0074878	0.00343905	0.00355219
Aquificae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Armatimonac	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bacteroidetes	0.09232553	0.08048754	0.11032801	0.05943504	0.12588579	0.362424	0.33915756	0.1915499	0.31986617	0.16520165	0.000051413	0.000017813	0.00287765	0.000026588	0.000026588	0.000026588	0.000026588	0.000026588	0.000026588	0.000026588	0.000026588
Chlamydiae <sup>Δ</sup>	0.01036261	0.05160095	0.02282229	0.03928486	0.03728485	0.01262221	0.00863376	0.02692835	0.01532539	0.06134421	0	0	0	0	0	0	0	0	0	0	0
Chloroflexi	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Chrysiogenet	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cyanobacteri	0	0	0	0	0	0.00023791	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Deferribacter	0.0014012	0	0.00094784	0.00066732	0.00078801	0.00350117	0.00180044	0.01007856	0.0012657	0.00345976	0	0	0	0	0	0	0	0	0	0	0
Deinococcus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Elusimicrobi	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fibrobacteres	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Firmicutes	0.75425049	0.67942111	0.67494923	0.65511138	0.60256436	0.49219873	0.57758238	0.61966522	0.55600636	0.55694537	0.83567263	0.83164863	0.88205978	0.91748217	0.86899925	0.95315881	0.87842419	0.84878338	0.86741103	0.94114839	0.9380067
Fusobacteria	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Gemmatimon	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nitrospirae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Planctomycet	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Proteobacteri	0.00547689	0.0116834	0.00812283	0.01635806	0.01483081	0.03137227	0.02716577	0.0356244	0.02110164	0.04352455	0.14854258	0.15388829	0.11683395	0.07762626	0.12086412	0.04266779	0.10731405	0.14455646	0.12510118	0.05214582	0.05370718
Spirochaetes	0.00094361	0.00128468	0.00119407	0.00369928	0.00443654	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Synergistetes	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tenericutes	0.00311503	0.00598171	0.00158856	0.00481921	0.00674862	0.00109954	0.00038521	0	0	0.00024713	0	0	0	0	0.00025356	0	0	0	0	0	0.00018936
Thermotogae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

log10 (relative abundance)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Actinobacteri	-0.8790162	-0.7707263	-0.7446137	-0.6563456	-0.6830635	-1.015274	-1.3441427	-0.9349674	-1.1113671	-0.7714012	-1.8017612	-1.8554586	-2.956136	-2.3266614	-2.1545659	-2.3795103	-1.8539998	-2.1765152	-2.125646	-2.465561	-2.4495035
Aquificae	<-4	<-4	<-4	<-4	<-4	<-4	<-4	<-4	<-4	<-4	<-4	<-4	<-4	<-4	<-4	<-4	<-4	<-4	<-4	<-4	<-4
Armatimonac	<-4	<-4	<-4	<-4	<-4	<-4	<-4	<-4	<-4	<-4	<-4	<-4	<-4	<-4	<-4	<-4	<-4	<-4	<-4	<-4	<-4
Bacteroidetes	-1.0346782	-1.0942713	-0.9573142	-1.2259574	-0.9000233	-0.440783	-0.4695985	-0.7177181	-0.4950317	-0.7819856	<-4	-3.2889236	<-4	-3.7492564	-2.5409625	<-4	-3.5753065	<-4	<-4	-2.4858852	-2.342508
Chlamydiae <sup>Δ</sup>	-1.984531	-1.2873423	-1.6416409	-1.4057747	-1.4284676	-1.8988646	-2.0638002	-1.5697903	-1.8145884	-1.2122264	<-4	<-4	<-4	<-4	<-4	<-4	<-4	<-4	<-4	<-4	<-4
Chloroflexi	<-4	<-4	<-4	<-4	<-4	<-4	<-4	<-4	<-4	<-4	<-4	<-4	<-4	<-4	<-4	<-4	<-4	<-4	<-4	<-4	<-4
Chrysiogenet	<-4	<-4	<-4	<-4	<-4	<-4	<-4	<-4	<-4	<-4	<-4	<-4	<-4	<-4	<-4	<-4	<-4	<-4	<-4	<-4	<-4
Cyanobacteri	<-4	<-4	<-4	<-4	<-4	<-4	<-4	<-4	<-4	<-4	<-4	<-4	<-4	<-4	<-4	<-4	<-4	<-4	<-4	<-4	<-4
Deferribacter	-2.8535008	<-4	-3.0232651	-3.1756656	-3.1034666	-2.455787	-2.7446204	-1.9966016	-2.8976709	-2.460954											

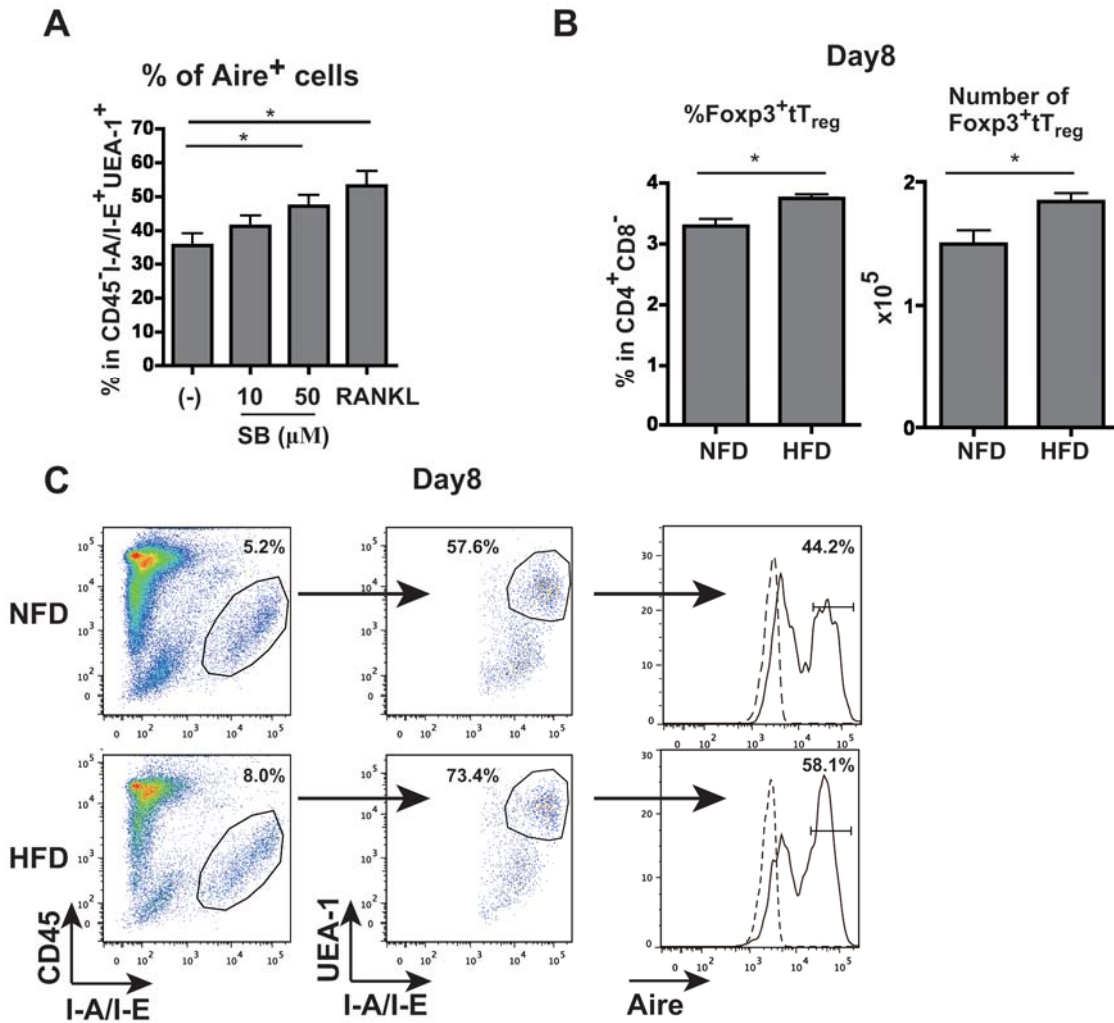
## Supplementary Figure 1



### Supplementary Figure 1. GPR41-induced elevation of tT<sub>reg</sub>.

(A) Flowcytometric analysis of Foxp3<sup>+</sup> tT<sub>reg</sub> cells in the thymus. Total thymocytes of offspring from NFD-fed and HFD-fed mice in GPR41-deficient mice at Day 3 were stained with anti-CD4 and anti-CD8 antibodies, then fixed and stained with anti-Foxp3 antibody. Foxp3<sup>+</sup> tT<sub>reg</sub> cells (gated on right panel) were analyzed on gated CD4<sup>+</sup> CD8<sup>-</sup> cells (left panel). The frequency of Foxp3<sup>+</sup> cells gated on CD4<sup>+</sup> CD8<sup>-</sup> cells was analyzed by FACS Verse. (B) The frequency and number of Foxp3<sup>+</sup> tT<sub>reg</sub> cells of offspring born from NFD-fed (N = 7) and HFD-fed mice (N = 11) in GPR41-deficient mice at Day 3 after birth were estimated from total thymic cell numbers. Data are presented as the mean ± SD. \* P < 0.05.

## Supplementary Figure 2



### Supplementary Figure 2. Aire expression of the offspring from NFD- and HFD-fed mice.

(A) Fetal thymic lobes were stimulated with SB (10 μM and 10 μM) or RANKL (10 μg/ml) for 3 days in FTOC. The frequency of Aire<sup>+</sup> cells was shown. FACS analysis were shown in Figure 5B. The graph was compiled from three independent experiments. Data are presented as the mean ± SD. \* P < 0.05. (B) The frequency and number of Foxp3<sup>+</sup> tT<sub>reg</sub> cells of the offspring from NFD- (N = 5) and HFD-fed mice (N = 6) in wild-type mice at Day 8 after birth were estimated from total thymic cell numbers. Data are presented as the mean ± SD. \* P < 0.05. (C) TECs were sorted individually from the offspring at Day 8 born from HFD-fed (N = 5) and HFD-fed mice (N = 6). CD45<sup>+</sup> cells of whole thymi were depleted by anti-CD45 microbeads. CD45<sup>-</sup> cells were counted and further stained with antibodies against I-A/I-E, UEA-1 and Aire. Histograms of Aire expression (right panel) were analyzed on gated CD45<sup>-</sup> I-A/I-E<sup>+</sup> cells (left panel) and UEA-1<sup>+</sup> I-A/I-E<sup>+</sup> mTEC cells (middle panel) by flow cytometry. The representative FACS plots were shown. The black dotted lines represent the isotype control.