SUPPLEMENTARY INFORMATION
Supplementary Figures 1-10

Microbiota depletion promotes browning of white adipose tissue and reduces obesity

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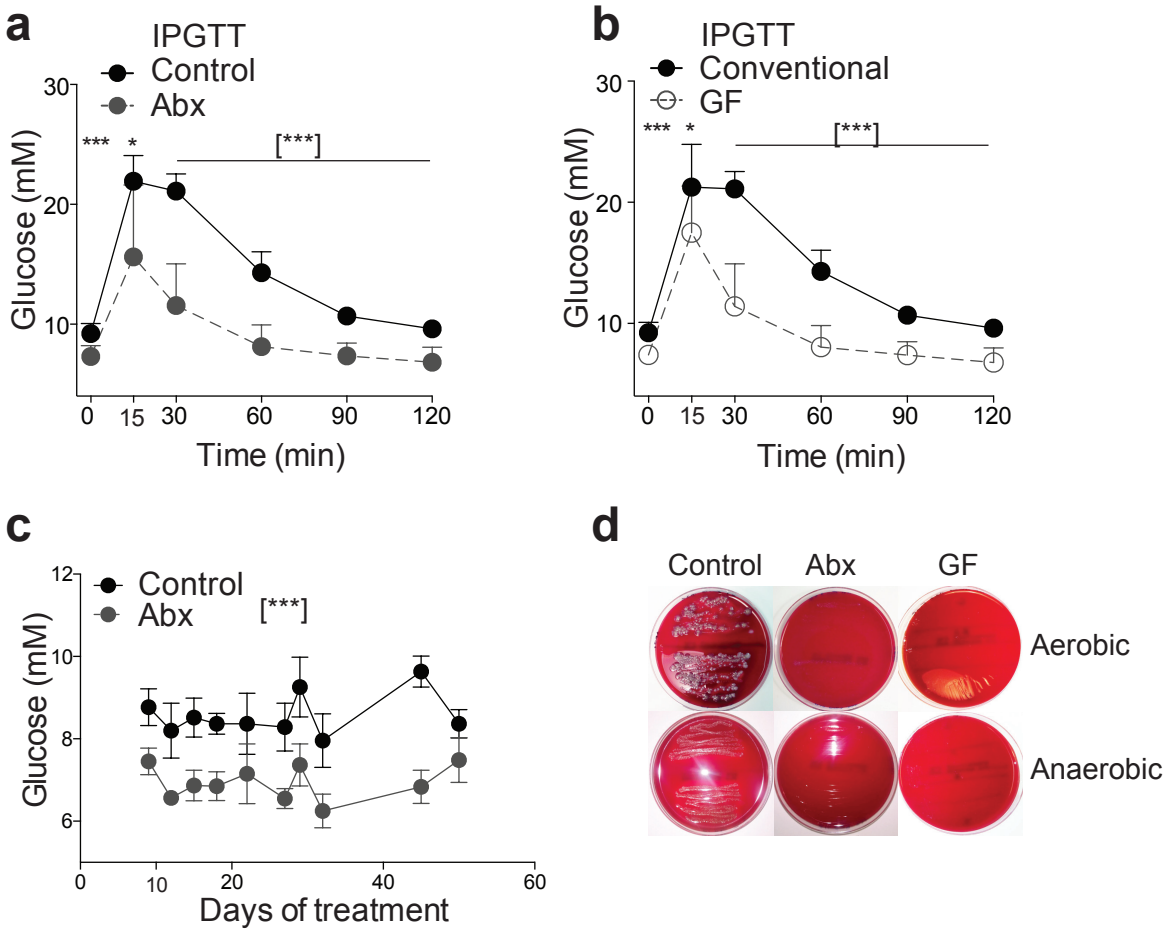
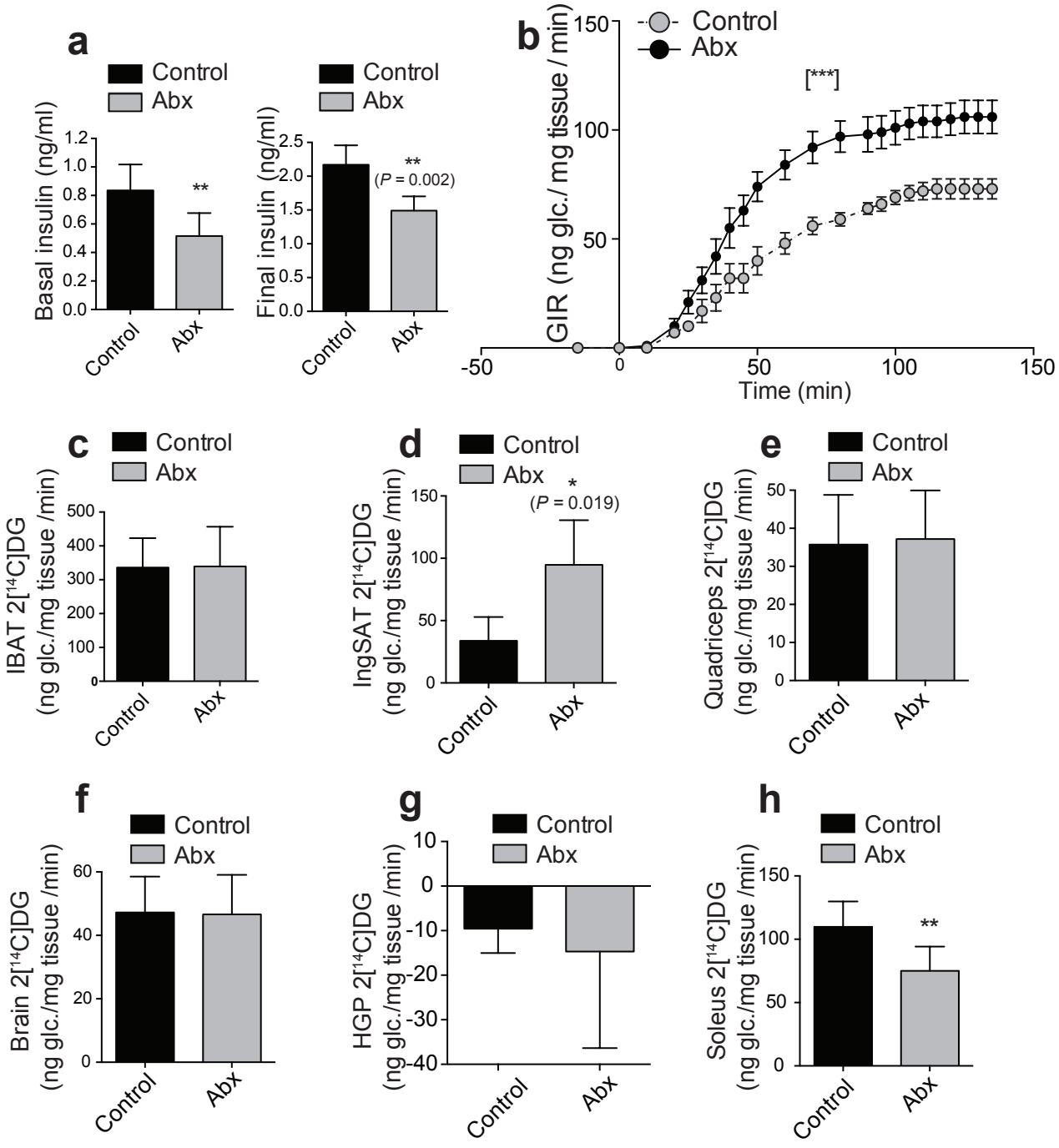
Supplementary Figure 1



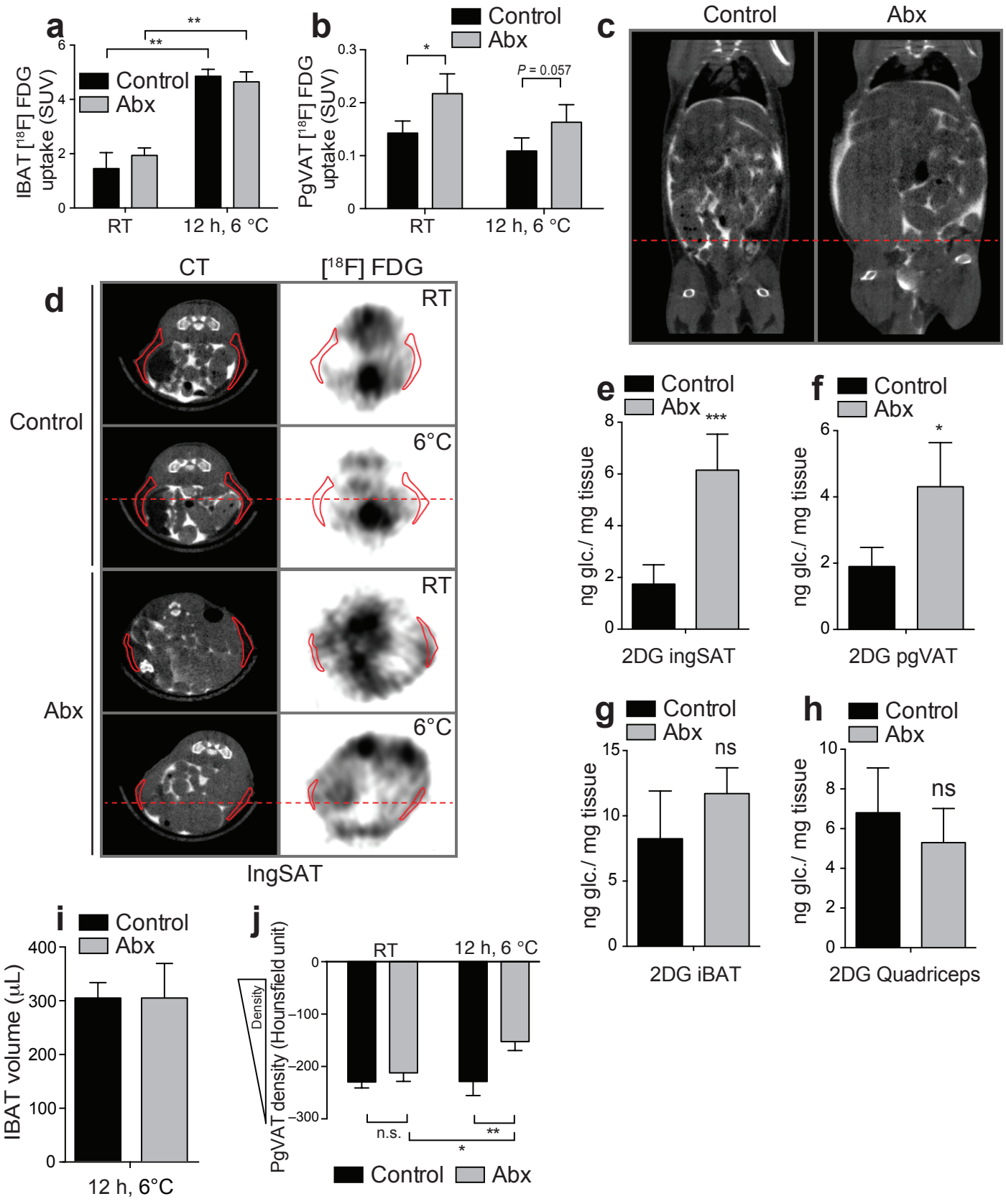
Supplementary Figure 1. Microbiota depletion improves glucose homeostasis. (**a**,**b**) Intraperitoneal glucose tolerance tests (IPGTT) on Abx (**a**), or GF mice (**b**) compared to the respective controls. (**c**) Random blood glucose levels during the antibiotic treatment as in (a). (**d**) Fecal content cultured on 5% sheep blood agar plates from mice as in (a) in anaerobic and aerobic conditions. All values show mean ± sd (n = 8 per group). Significance was calculated using non-paired two tailed Students t-test.*: $P \leq 0.05$, **: $P \leq 0.01$, ***: $P \leq 0.001$.

Supplementary Figure 2



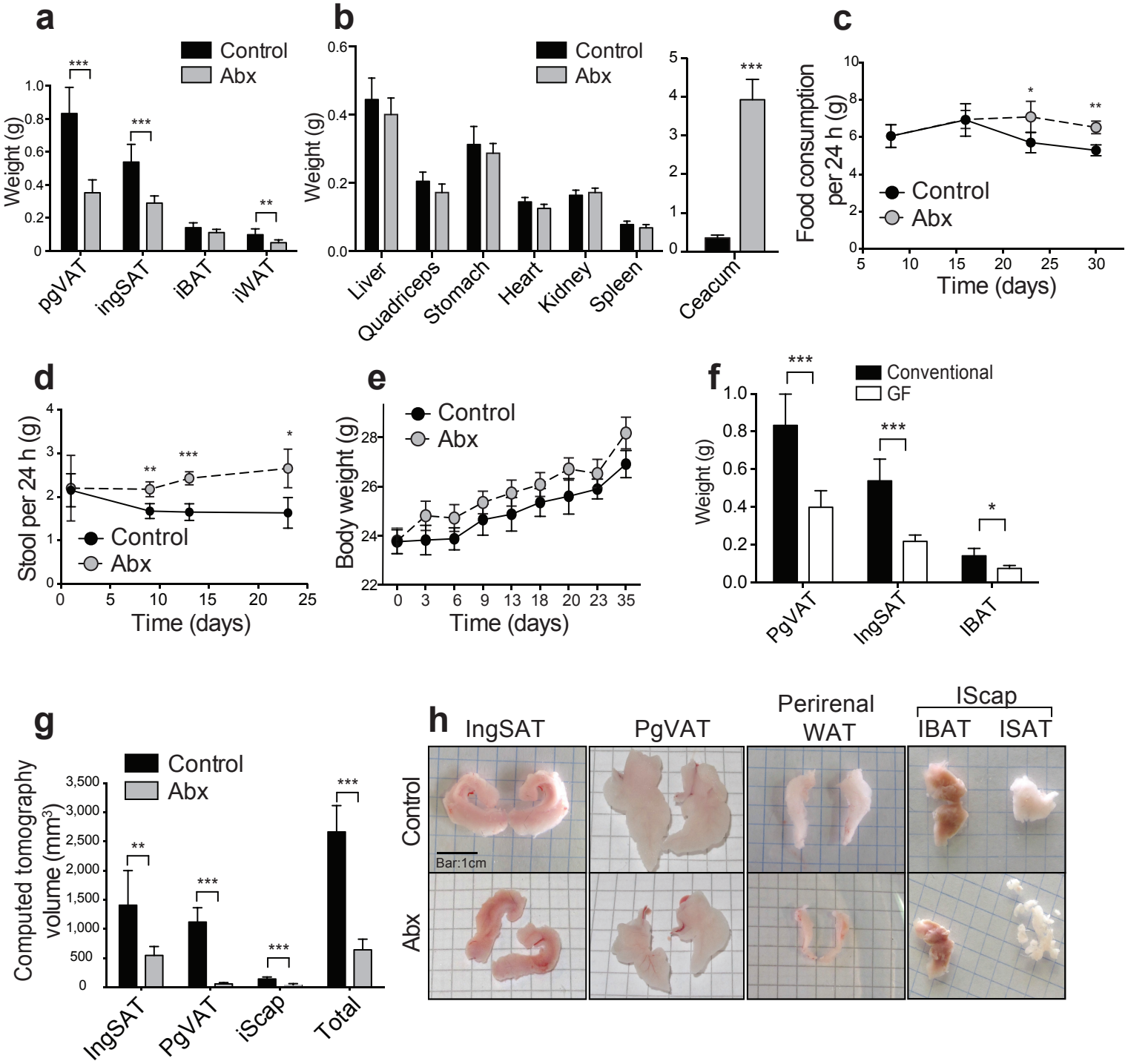
Supplementary Figure 2. Microbiota depletion promotes glucose uptake preferentially in white fat under hyperinsulinemic conditions. (**a**,**b**) Basal and final insulin levels (**a**) and glucose infusion rates (GIR, **b**) during hyperinsulinemic-euglycemic clamp in awake and unrestrained C57Bl6/J mice as in Fig. 1e. (**c**–**h**) Assessment of the tissue-specific glucose uptake 2[14C]de-oxyglucose (2[^{14}C]DG) in iBAT (**c**), ingSAT (2nd depot) (**d**), quadriceps (**e**), brain (**f**), and soleus (**h**); or hepatic glucose production (HGP) (**g**) during hyperinsulinemic-euglycemic clamp in awake C57Bl6/J mice as in Fig. 1e. All bars show mean ± sd ($n = 6$ per group). Significance was calculated using non-paired two tailed Students t-test.*: $P \leq 0.05$, **: $P \leq 0.01$, ***: $P \leq 0.001$.

Supplementary Figure 3



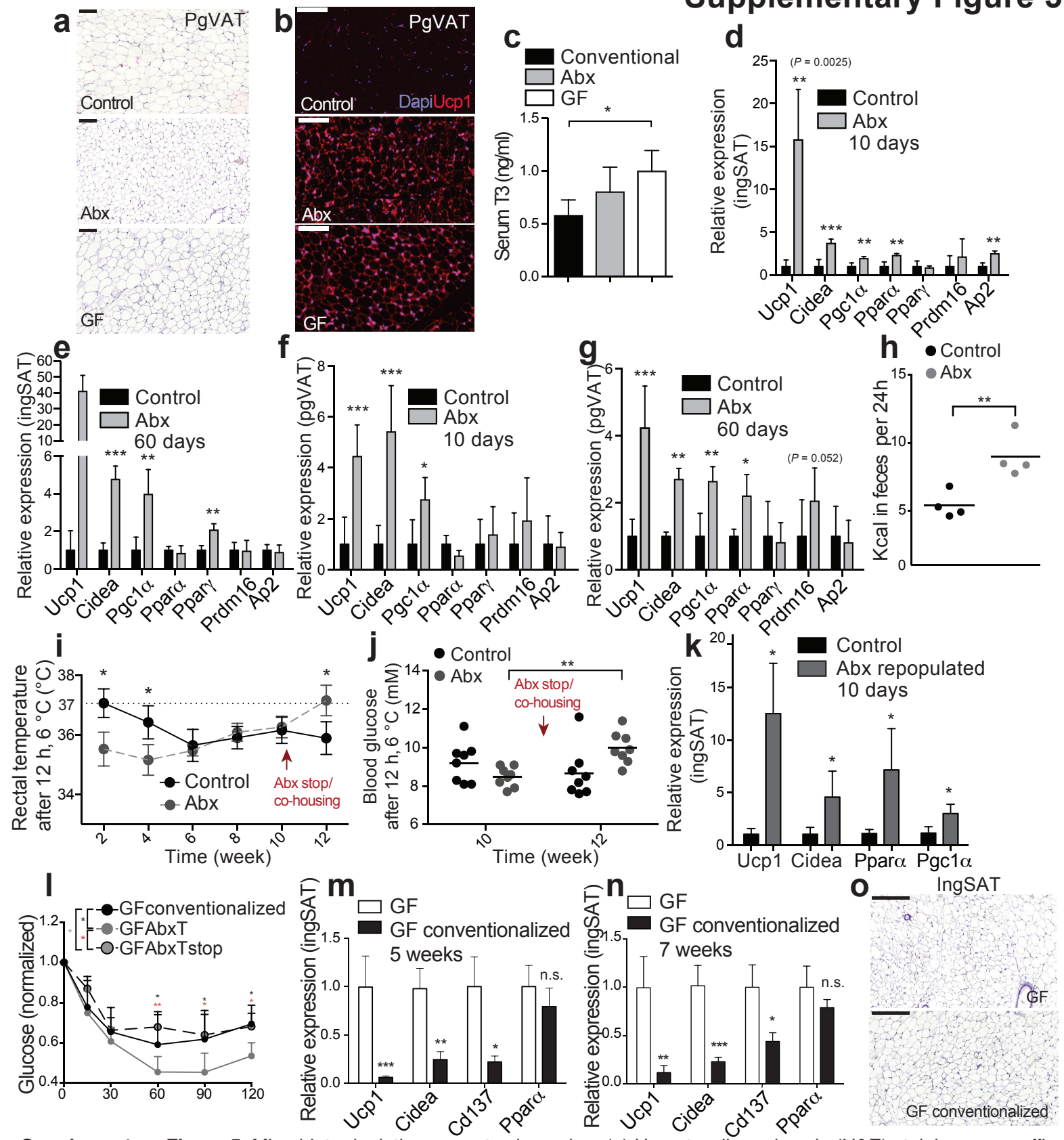
Supplementary Figure 3. Increased white fat glucose uptake after microbiota depletion at basal conditions. (**a**,**b**) Standardized Uptake Values (SUVs) of the radiolabeled tracer 2-deoxy-2-[^{18}F]-fluoro-D-glucose ([^{18}F]FDG) from the MicroPET-CT in iBAT (**a**) and pgVAT (**b**), in mice kept at room temperature, or exposed to 6 °C for 12 h as in Fig. 1h. (**c**) Coronal view of the CT in Control and Abx mice. (**d**) Transversal [^{18}F]FDG PET-CT images of ingSAT in Control and Abx mice at RT and after 12 h exposure to 6 °C. (**e**–**h**) 2-[1-^{3}H]deoxyglucose (2DG) uptake during IPGTT in ingSAT (**e**), pgVAT (**f**), iBAT (**g**), and quadriceps (**h**) in 11 weeks old Abx and Control C57Bl6/J. (**i**) PET-CT measurement of activated iBAT volume in 12 weeks old Abx and Control C57Bl6/J mice exposed to cold for 12 h as in Fig. 1h. (**j**) Density of the pgVAT represented in Hounsfield units. All bars show mean ± sd ($n = 6$ per group). Significance was calculated using non-paired two tailed Students t-test.*: $P \leq 0.05$, **: $P \leq 0.01$, ***: $P \leq 0.001$.

Supplementary Figure 4



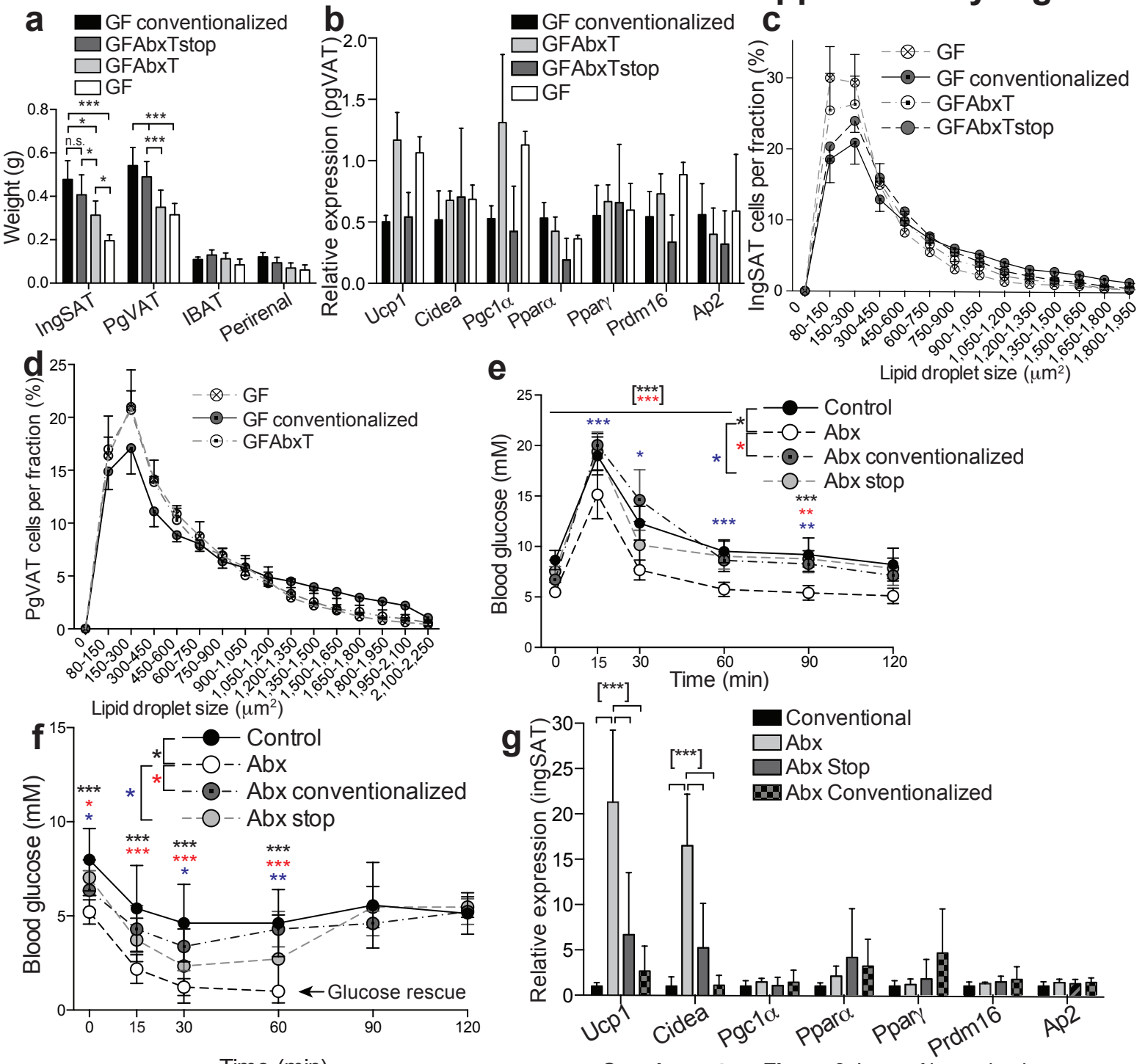
Supplementary Figure 4. Microbiota depleted mice have reduced body weight and fat mass. (**a**,**b**) Excised fat pads (**a**) and organ (**b**) weights after sacrifice of 12 weeks old Abx and Control C57Bl6/J mice kept at RT ($n = 8$ per group). (**c**,**d**) Food intake (**c**) and stool output (**d**) per 24 h of Control, or Abx mice. Mice ($n = 8$ per group) were kept 2 mice per cage. Each cage was considered and is shown as one pooled sample. (**e**) Body weight in the course of the antibiotic treatment experiment of mice as in (a). (**f**) Excised adipose tissue weights after sacrifice of GF or control mice ($n = 6$ per group). (**g**) Multidetector computed tomography (CT) measurements of different fat depots. (**h**) Representative ingSAT, pgVAT, perirenalWAT, iBAT, and interscapular SAT (iSAT) images from mice as in (a). All bars show mean ± sd. Significance was calculated using non-paired two tailed Students t-test.*: $P \leq 0.05$, **: $P \leq 0.01$, ***: $P \leq 0.001$.

Supplementary Figure 5



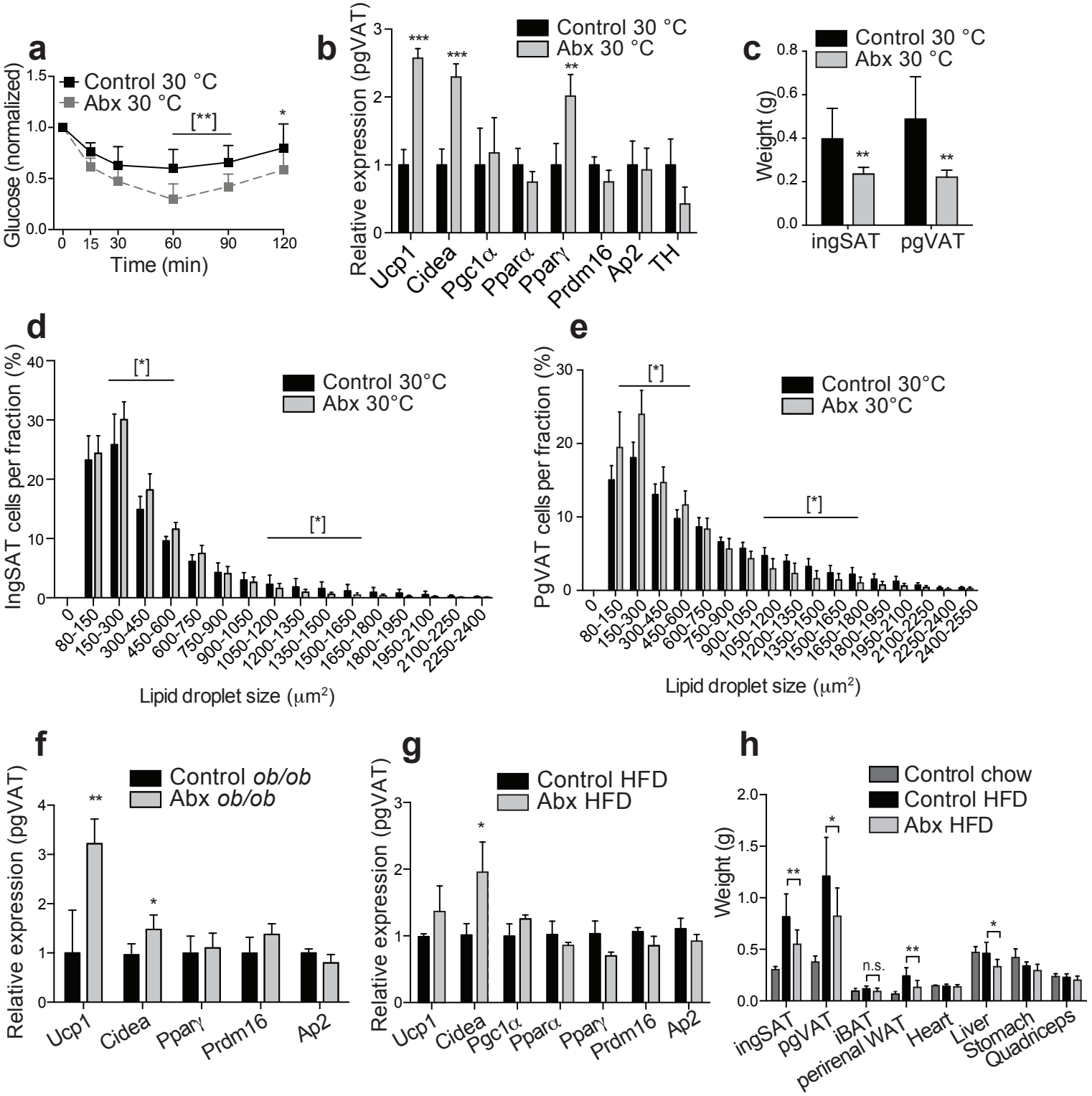
Supplementary Figure 5. Microbiota depletion promotes browning. (**a**) Hematoxylin and eosin (H&E) staining on paraffin sections from pgVAT of mice at 14 weeks of age. (**b**) Immunohistochemistry of paraffin sections from pgVAT of mice at 14 weeks of age. (**c**) Serum 3,5,3'-Triiodothyronine (T3) levels in Control, Abx, or GF mice at 14 weeks of age. (**d**–**g**) Relative mRNA expression in ingSAT (**d**,**e**), or pgVAT (**f**,**g**) of Abx mice and respective controls after 10 days (**d**,**f**), and 60 days (**e**, **g**) of antibiotic treatment, quantified by real-time PCR and normalized to the house keeping beta-2-microglobulin (*B2m*). (*n* = 6 per group). (**h**) Bomb calorimetric measurements of 24 h fecal calories content of 12 weeks old Control, or Abx mice. Mice (*n* = 8 per group) were kept 2 mice per cage. Each cage was considered as one pooled sample. (**i**,**j**) Rectal temperature (**i**) and blood glucose levels (**j**) following cold exposure for 12 h (*n* = 8 per group). Lines show mean. (**k**,**m**,**n**) Relative mRNA expression in ingSAT of Control wt, or Abx mice 10 days after repopulation (Abx repopulated) (**k**); or of GF mice without, or 5 and 7 weeks after transplantation (GF conventionalized) (**m**,**n**), quantified by real-time PCR and normalized to *B2m*. (*n* = 4-6 per group) (**l**) Normalized insulin tolerance tests (ITT) of GF mice transplanted with microbiota from control wt (GF conventionalized), or Abx mice. The antibiotic treatment of the recipient GF mice getting the Abx microbiota was continued or not (GFAbxT, or GFAbxTstop, respectively). (*n* = 4-5 per group) (**o**) H&E staining of paraffin sections from ingSAT of GF, or transplanted GF mice as in k). Scale bars represent 200 µm. All bars show mean ± sd. Significance was calculated using non-paired two tailed Students t-test. *: $P \leq 0.05$, **: $P \leq 0.01$, ***: $P \leq 0.001$.

Supplementary Figure 6



Supplementary Figure 6. Loss of browning in conventionalized GF and Abx mice. (**a**) Excised organ weights after sacrifice of GF mice transplanted with microbiota from control wt (GF conventionalized), or Abx mice. The antibiotic treatment of the recipient GF mice transplanted with the residual Abx microbiota was continued or not (GFAbxT, or GFAbxTstop, respectively). All values show mean ± sd ($n = 6$ per group). (**b**) Relative mRNA expression in ingSAT of GF mice as in (a), quantified by real-time PCR and normalized to *B2m*. All values show mean ± sd ($n = 6$ per group). (**c**,**d**) Cell size profiling of adipocytes in ingSAT (c), or pgVAT (d) of mice as in (a). The values show % from the total number of analyzed cells. Bars show mean of the pooled corresponding fractions from each animal ± sd ($n = 6$ per group). (**e**) Oral glucose tolerance tests (OGTT) of Control and 12 weeks antibiotic treated mice conventionalized or not with microbiota from control wt after 8 weeks of treatment (Abx conventionalized); or in which the antibiotic treatment was stopped at the 8th week and allowed repopulation to occur from the residual microbiota (Abx stop) ($n = 6$ per group). (**f**) Insulin tolerance tests (ITT) of mice as in (e). (**g**,**h**) Relative mRNA expression in ingSAT (**g**), or pgVAT (**h**) of mice as in (e) 12 weeks after antibiotics initiation. Repopulation was done for four weeks. ($n = 4$ per group). Values show mean ± sd. Significance was calculated using two way Anova. *: $P \leq 0.05$, **: $P \leq 0.01$, ***: $P \leq 0.001$.

Supplementary Figure 7



Supplementary Figure 7. The metabolic improvements are maintained at thermoneutrality and in obese mice. (**a**) Insulin tolerance tests (ITT) of Abx, or control mice kept at thermoneutrality after 40 days of treatment. Starting values are normalized to 1. (**b**) Relative mRNA expression in pgVAT of mice as in a), quantified by real-time PCR and normalized to *B2m*. (**c**) Weight of fat tissues of mice as in (a). All values in (a-c) show mean ± sd ($n = 6$ per group). (**d**,**e**) Cell size profiling of adipocytes in ingSAT (**d**) and pgVAT (**e**) of mice as in (a). The values show % from the total number of analyzed cells. Bars show mean of the pooled corresponding fractions from each animal ± sd ($n = 6$ for each panel). (**f**,**g**) Relative mRNA expression in pgVAT of ob/ob (**f**), or HFD fed (**g**) Control or Abx mice quantified by real-time PCR and normalized to *B2m*. (**h**) Organ weights of chow fed, or HFD mice treated or not with antibiotics for 40 days. ($n = 6$ per group). Significance was calculated using non-paired two tailed Students t-test. *: $P \leq 0.05$, **: $P \leq 0.01$, ***: $P \leq 0.001$.

Supplementary Figure 8

a

IngSAT cytokines (pg/ml)

	Control (pg/ml)	Abx (pg/ml)	Control sd	Abx sd	p-value
IL-1a	26.33	36.58	22.166	11.199	0.3830
IL-1b	269.321667	376.65	41.601	21.923	0.0006
IL-2	87.78	126.022	52.038	21.648	0.1677
IL-3	7.37	10.344	2.120	2.145	0.0466
IL-4	4.522	7.286	0.852	0.661	0.0004
IL-5	7.06166667	9.956	1.742	2.035	0.0314
IL-6	16.63	28.032	5.290	15.233	0.1525
IL-9	318.705	511.086	71.513	56.320	0.0009
IL-10	36.8016667	60.056	7.438	9.362	0.0013
IL-12(p40)	20.346	38.246	17.274	12.443	0.0969
IL-12(p70)	131.775	208.644	46.827	54.684	0.0330
IL-13	211.266	362.168	54.742	26.318	0.0005
IL-17	19.0883333	37.6	6.212	21.687	0.0749
Eotaxin	706.023333	823.68	122.339	69.640	0.0900
G-CSF	6.185	7.742	2.021	0.951	0.1501
GM-CSF	74.2483333	90.73	17.522	14.299	0.1266
IFN-g	19.0616667	27.012	2.932	5.308	0.0116
KC	9.22	16.64	2.870	3.823	0.0051
MCP-1	129.288333	176.99	12.244	19.225	0.0007
MIP-1a	7.50833333	12.94	3.327	2.244	0.0128
MIP-1b	79.6366667	96.454	18.651	20.266	0.1858
RANTES	15.3666667	22.624	9.226	1.089	0.1171
TNF-a	588.811667	788.51	200.519	54.785	0.0607

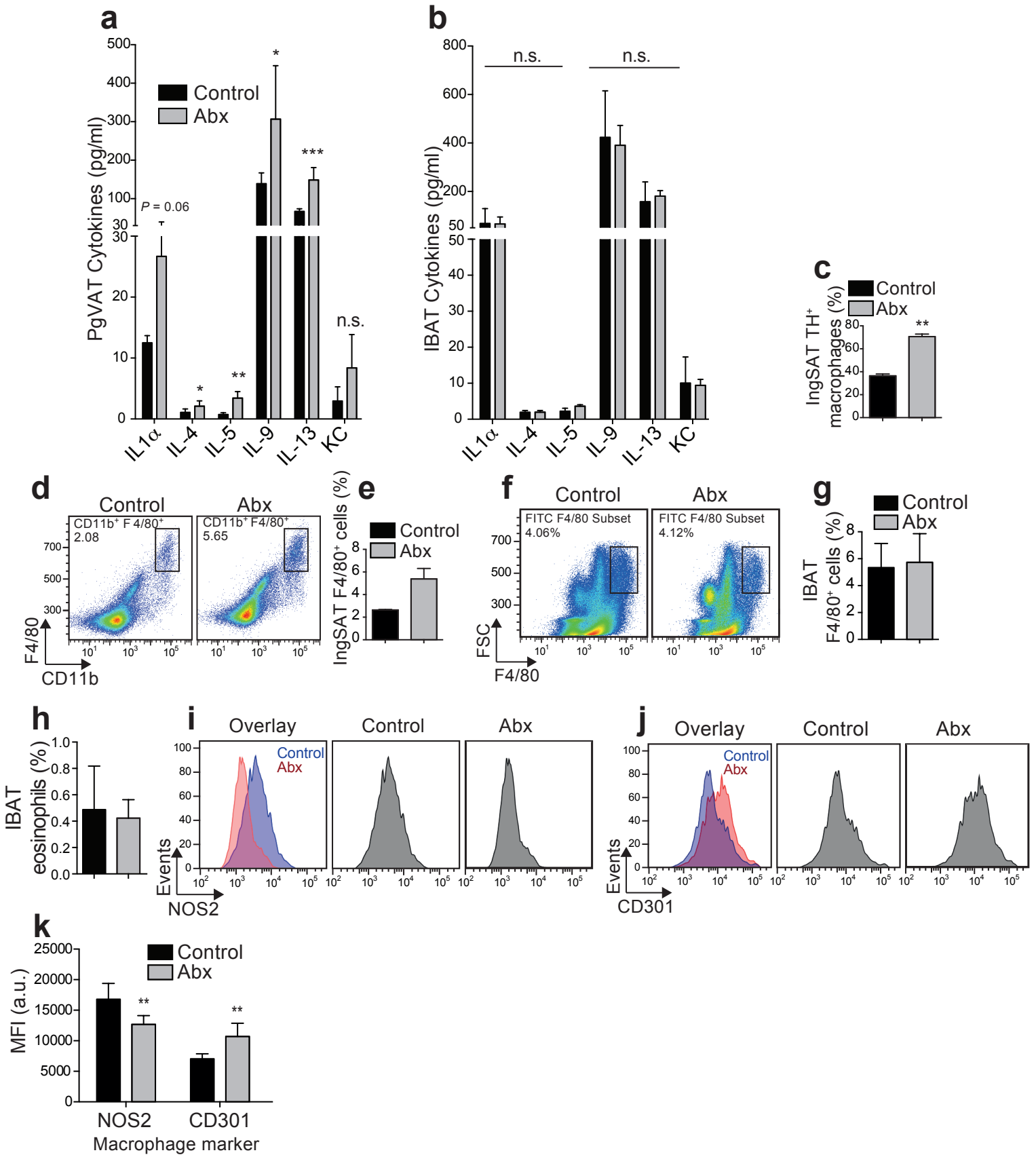
b

Jejunum cytokines (pg/ml)

	Control (pg/ml)	Abx (pg/ml)	Control sd	Abx sd	p-value
IL-1a	93.78	57.49	3.656	25.995	0.1593
IL-1b	298.33	281.67	10.373	12.080	0.2119
IL-2	60.72	100.66	0.750	31.561	0.1881
IL-3	16.20	16.76	3.642	1.615	0.8181
IL-4	4.79	4.66	0.601	0.199	0.7508
IL-5	6.94	11.32	0.000	4.686	0.2984
IL-6	36.69	38.48	1.188	1.923	0.3354
IL-9	254.77	396.50	26.311	99.210	0.1561
IL-10	29.91	30.61	3.797	0.665	0.7560
IL-12(p40)	33.74	32.75	6.286	3.601	0.8327
IL-12(p70)	812.65	812.62	7.997	82.848	0.9997
IL-13	252.02	253.18	5.190	14.823	0.9250
IL-17	17.11	18.54	3.889	0.699	0.5465
Eotaxin	539.55	550.62	13.336	16.603	0.4935
G-CSF	11.19	11.45	4.306	0.800	0.9162
GM-CSF	84.36	80.20	1.011	6.817	0.4756
IFN-g	32.08	40.34	1.082	7.879	0.2556
KC	6.20	7.27	0.594	1.266	0.3606
MCP-1	113.16	129.34	5.134	9.156	0.1147
MIP-1a	13.25	13.08	0.290	1.549	0.8942
MIP-1b	98.72	100.32	15.889	9.841	0.8945
RANTES	26.28	29.46	1.280	4.153	0.3888
TNF-a	1033.01	1082.85	156.348	70.317	0.6450

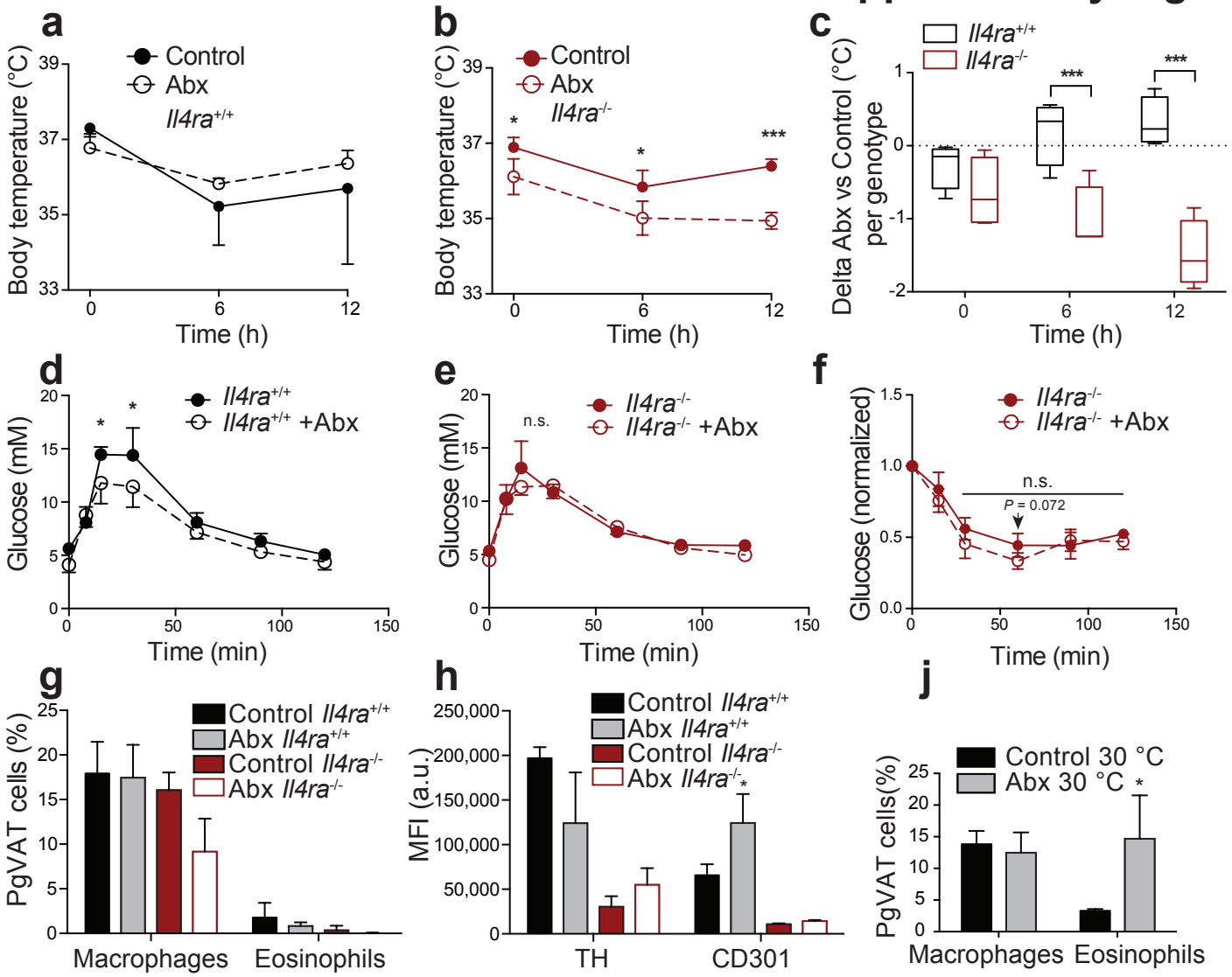
Supplementary Figure 8. Increased cytokine levels in white adipose tissue of Abx mice. (**a**,**b**) Tables representing ingSAT (**a**), or jejunal (**b**) cytokine levels measured in 12 weeks old Abx, or control mice (n = 8 per group). Significance was calculated using non-paired two tailed Students t-test.

Supplementary Figure 9



Supplementary Figure 9. Microbiota depletion increases type 2 cytokine signaling in white fat. (**a**,**b**) Cytokine levels measured in pgVAT (**a**), or iBAT (**b**) of 12 weeks old Abx, or control mice. Bars show mean ± sd ($n = 8$ per group). (**c**–**e**) Frequency of TH$^+$ macrophages (**c**) and CD11b$^+$ F4/80$^+$ cells (**d**–**e**) in ingSAT SVF of Abx, or control mice. (f, g) F4/80$^+$ cells abundance in iBAT of Abx, or control mice. FSC: forward-scattered light. (**h**) Eosinophils abundance in iBAT of mice as in (f). (**i**–**k**) Mean Fluorescent intensity (MFI) of NOS2 (M1), or CD301 (M2) labeled macrophages shown as arbitrary units (a.u.) in ingSAT SVF of 40 days treated Abx and control mice. Bars show mean of two pooled groups with four mice each (**g**) or eight mice analyzed separately (**c**,**e**,**h**,**k**) ± sd. Y axis in (**i**,**j**) shows number of events normalized to the mode. *: $P \leq 0.05$, **: $P \leq 0.01$, ***: $P \leq 0.001$.

Supplementary Figure 10



Supplementary Figure 10. Loss of type 2 signaling impairs browning of Abx mice. (**a**,**b**) Eye body temperature of control Il4ra$^{+/+}$ (n = 6 per group) (**a**) or Il4ra$^{-/-}$ (n = 4 per group) (**b**) mice, following antibiotic treatment during cold exposure of 12 h. (**c**) Temperature change of Abx vs. control mice in Il4ra$^{+/+}$ and Il4ra$^{-/-}$ during cold exposure of 12 hrs. (**d**,**e**) Oral glucose tolerance tests (OGTT) of control Il4ra$^{+/+}$ (d) or Il4ra$^{-/-}$ (e) mice as in (a, b), following antibiotic treatment compared to the respective controls. (**f**) Insulin tolerance tests (ITT) of mice as in (e). (**g**) Macrophage and eosinophil abundance in pgVAT of Control Il4ra$^{+/+}$ or Il4ra$^{-/-}$ mice Abx or control mice after 40 days of treatment. (**h**) Mean Fluorescent intensity (MFI) of TH, or CD301 labeled macrophages shown as arbitrary units (a.u.) in pgVAT tissue of control Il4ra$^{+/+}$ or Il4ra$^{-/-}$ mice following 40 days antibiotic treatment compared to respective controls. (**i**) Relative mRNA expression in ingSAT and pgVAT of Il4ra$^{-/-}$ mice with or without 40 days antibiotic treatment quantified by real-time PCR and normalized to *B2m* (n = 4 per group). (**j**,**k**) Macrophage and eosinophil abundance in ingSAT (**j**), and pgVAT (**k**) SVF of control or Abx mice kept at thermoneutrality. (**l**) Mean Fluorescent intensity (MFI) of TH, or CD301 labeled cells shown as arbitrary units (a.u.) in pgVAT tissue of mice as in (k). All values show mean ± sd. (n = 4-6 per group). Significance was calculated using non-paired two tailed Students t-test. *: $P \leq 0.05$, **: $P \leq 0.01$, ***: $P \leq 0.001$.