Gut Microbiota Orchestrates Energy Homeostasis during Cold

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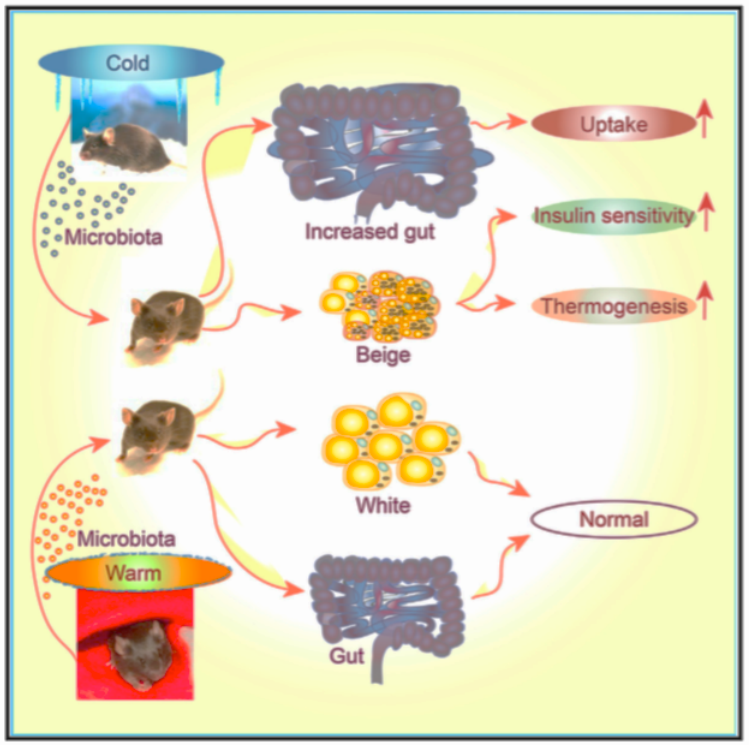
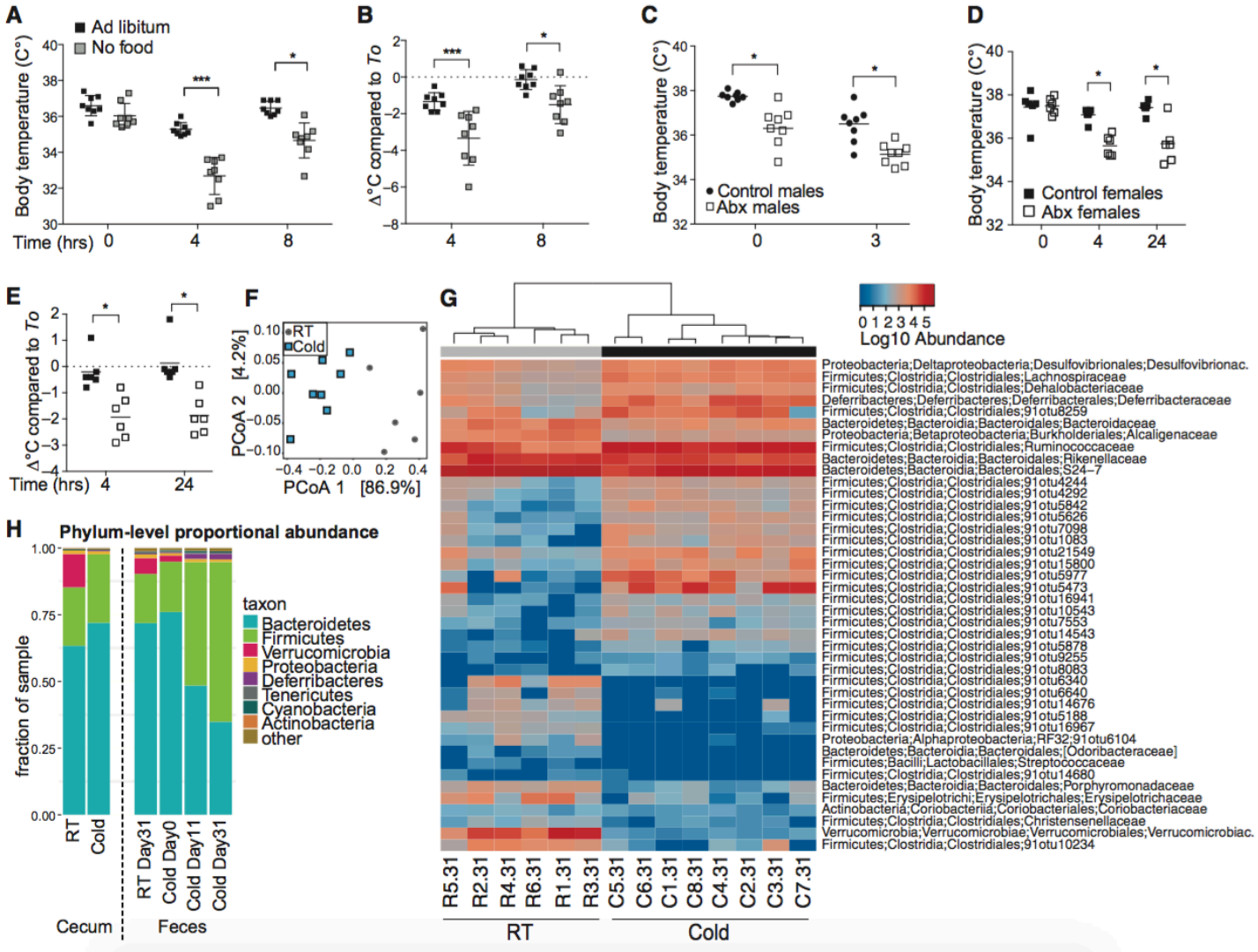
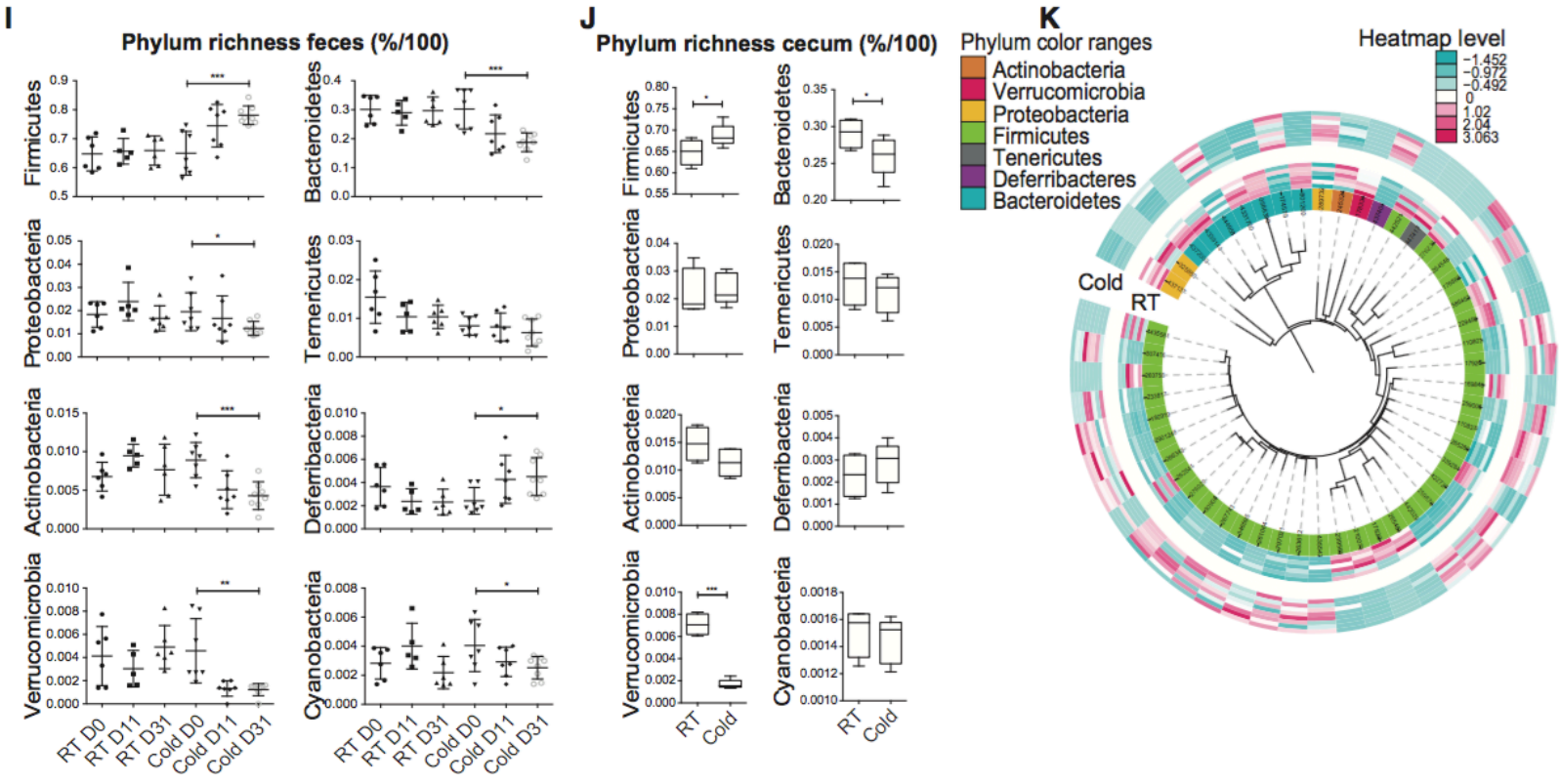
Microbial functions in the host physiology are a result of the microbiota-host co-evolution. **We show that cold exposure leads to marked shift of the microbiota composition, referred to as cold microbiota.** Transplantation of the cold microbiota to germ-free mice is sufficient to increase insulin sensitivity of the host and enable tolerance to cold partly by promoting the white fat browning, leading to increased energy expenditure and fat loss. During prolonged cold, however, the body weight loss is attenuated, caused by adaptive mechanisms maximizing caloric uptake and increasing intestinal, villi, and microvilli lengths. This increased absorptive surface is transferable with the cold microbiota, leading to altered intestinal gene expression promoting tissue remodeling and suppression of apoptosis—the effect diminished by co-transplanting the most cold-down- regulated strain *Akkermansia muciniphila* during the cold microbiota transfer. Our results demonstrate the microbiota as a key factor orchestrating the overall energy homeostasis during increased demand.

Graphical Abstract



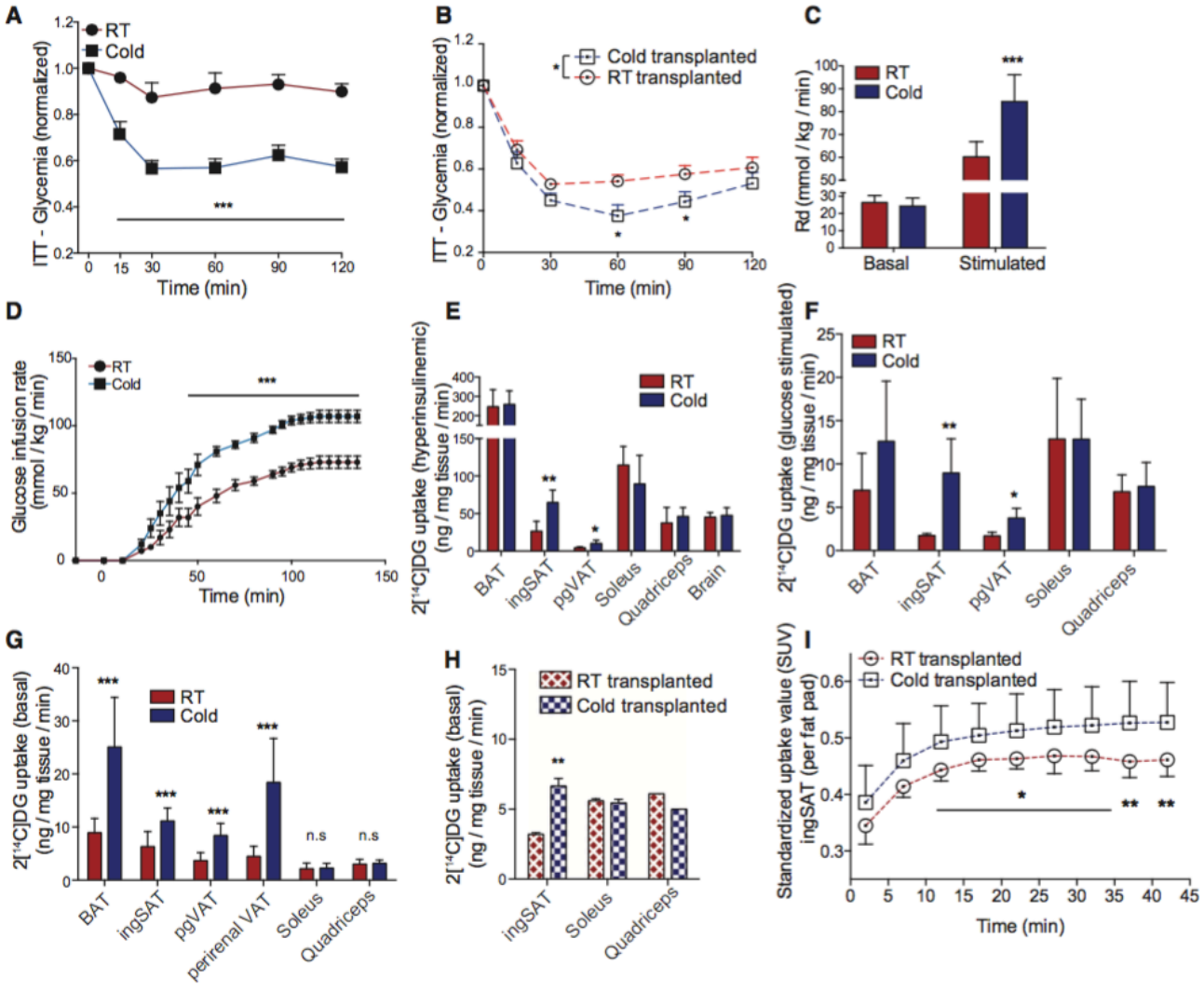


Cold exposure 6 °C



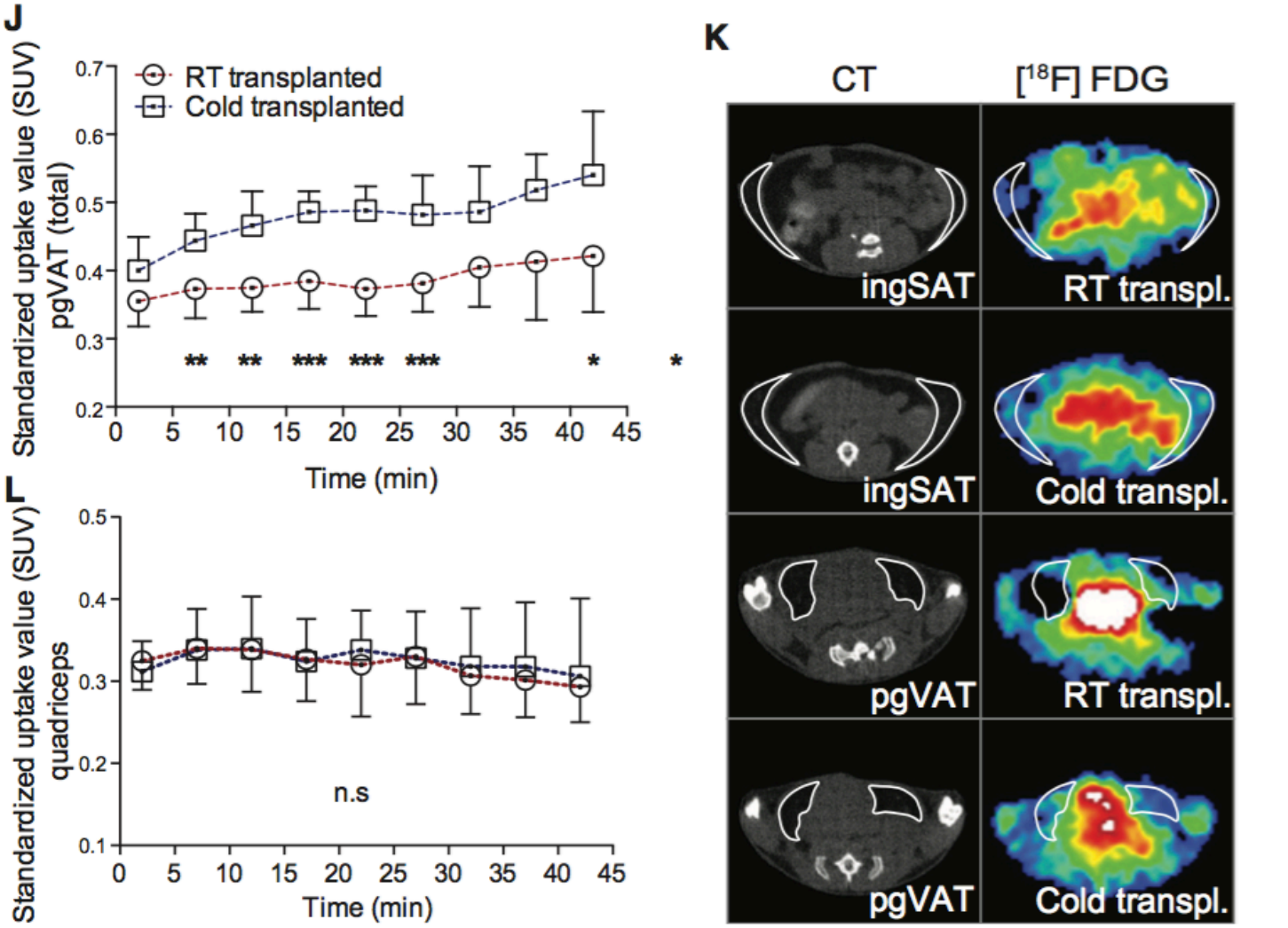
Cold exposure 6 °C

Cold exposure changes the gut microbiota composition. A) Rectal body temperature (BT) of food restricted or ad libitum-fed C57Bl6J mice after 4 and 8 hr (hr) of cold exposure (n = 8 per group). (B) Change in BT compared to initial as in (A).
(C) Rectal BT after 3 hr of cold exposure of male mice treated or not treated with antibiotics (n = 8 per group).
(D) Rectal BT after 4 hr and 24 hr of cold exposure in antibiotics-treated or control female mice (n = 6 per group).
(E) Change in BT compared to initial as in (D).
(F) Principal coordinates analysis (PCoA) based on weighted UniFrac analysis of OTUs. Each symbol represents a single sample of feces after 31 days of cold- exposed (n = 8) or RT controls (n = 6 per group).
(G) Hierarchical clustering diagram using the average-neighbor (HC-AN) method comparing feces of 31 days cold-exposed mice (n = 8) and their RT controls (n = 6). Associated heatmap shows the relative abundance of representative OTUs selected for $p < 0.05$, obtained with a Welch t test comparison of the two groups and then grouped into families. One representative OTU with the greatest difference between the two group means from each family is selected for inclusion in the heatmap diagram. OTUs are shown as: Phylum, Class, Order, Family, Genus, and Species. R, RT; C, cold-exposed.
(H) Comparison of phylum-level proportional abundance of cecum and feces of up to 31 days cold-exposed or RT control mice.
(I and J) Richness represented as the proportions of OTUs classified at the phylum rank. (I) Feces. (J) Cecum. In (H)–(J) n = 5 + 6 (cecum) or 6 + 8 (feces).
(K) Heatmap tree comparing selected OTUs abundance from feces of RT controls (n = 6, inner rings) and 31 days cold-exposed mice (n = 8, outer rings) and their phylogenic relationships. The OTUs representative of differentially abundant families are selected as described in (H).



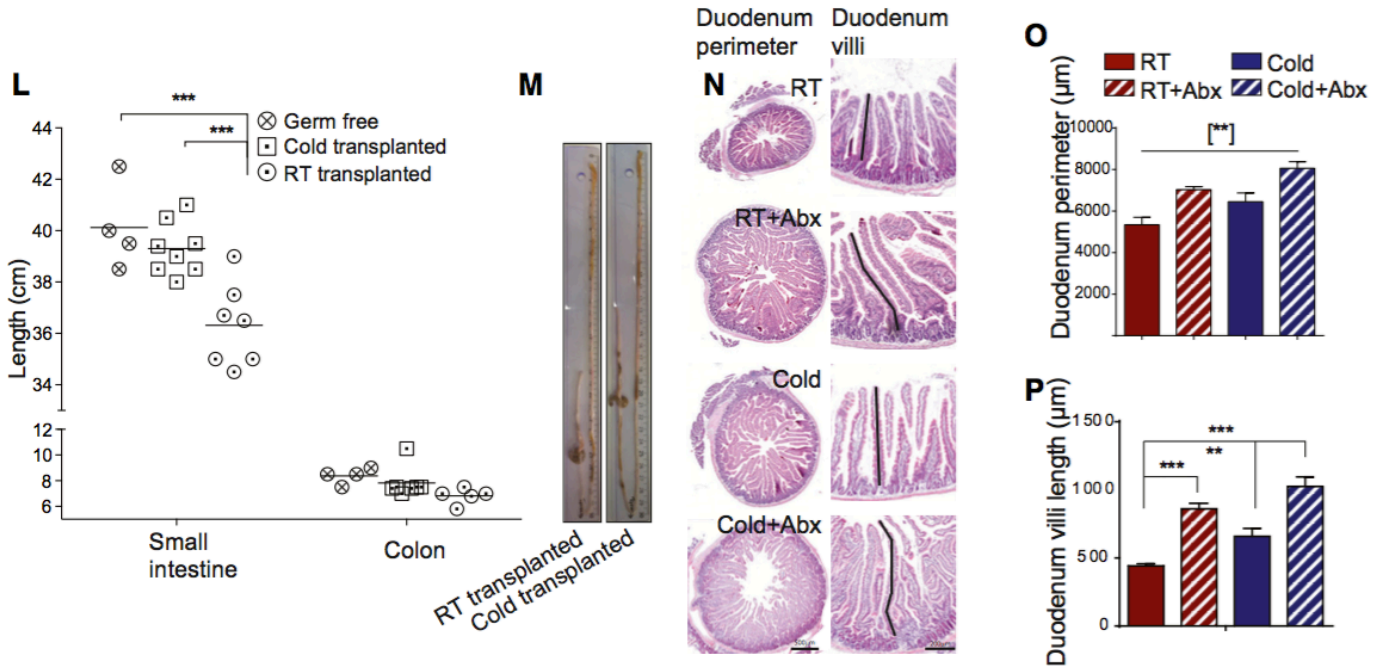
2-[14C]deoxyglucose

[18F]fluorodeoxyglucose



Cold microbiota transplantation increases insulin sensitivity and WAT (white adipose tissue) glucose uptake

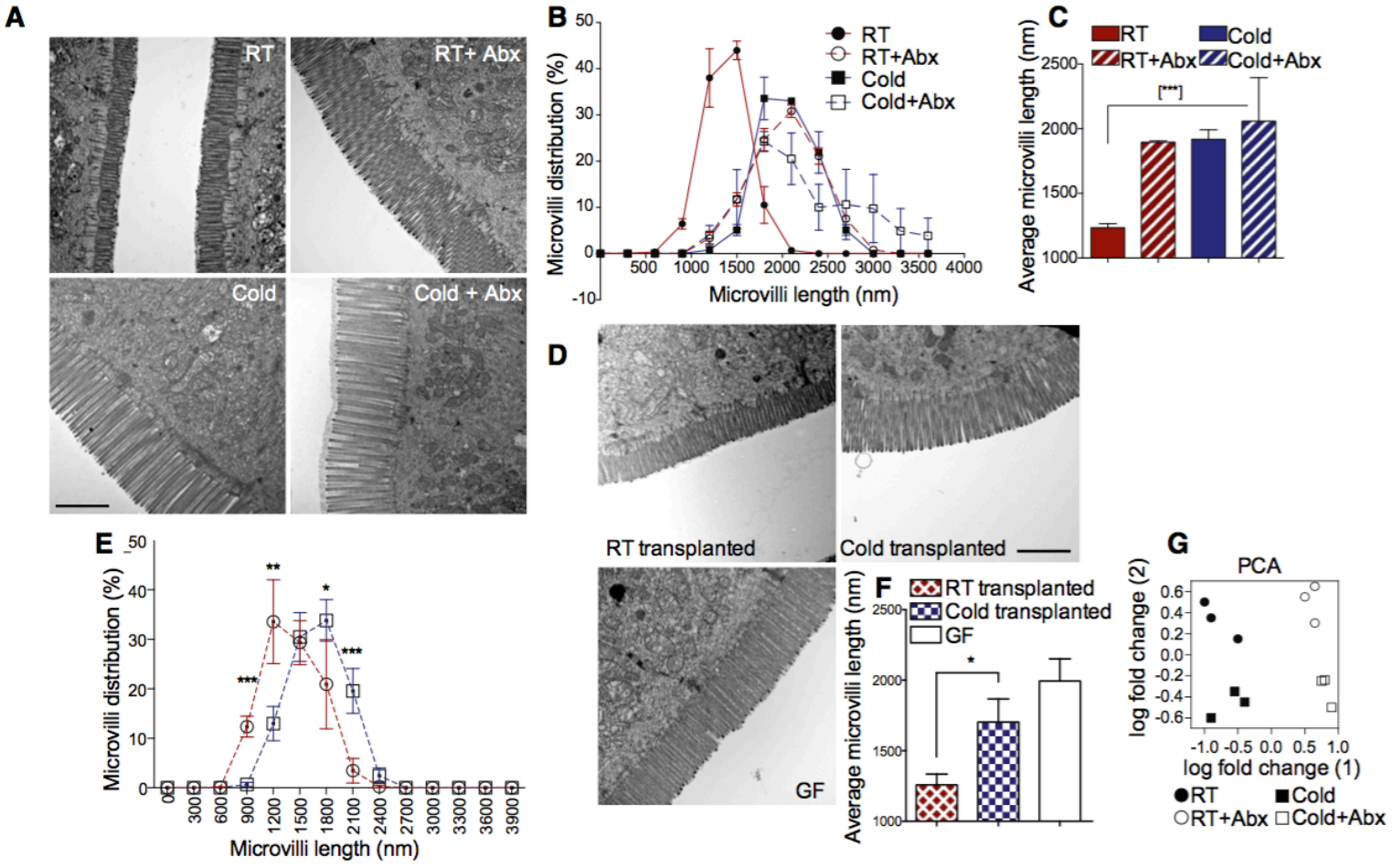
(A and B) Intraperitoneal insulin tolerance test (ITT) in RT and 25 days cold-exposed mice (A), or RT- and cold microbiota-transplanted mice (B) relative to initial blood glucose, (n = 8 per group).
(C–E) Euglycemic-hyperinsulinemic clamp of awake mice as in (A). Rate of disappearance of 3H-D-glucose (C). GIR time course during the hyperinsulinemic clamp (D). 2[14C]DG uptake in various tissues (E) (n = 6 + 6).
(F) 2[14C]DG tracer uptake in tissues 45 min after IP tracer and glucose (2 g/kg BW) administration in mice as in (A) (n = 6 per group).
(G and H) 2[14C]DG uptake in tissues 30 min after administration under basal conditions in anesthetized RT (n = 9) and cold (n = 10) (G); or RT- and cold- transplanted mice (n = 3) (H).
(I, J, and L) Positron emission tomography-computer tomography (microPET-CT) measurement of [18F]FDG uptake in ingSAT (I), pgVAT (J), or quadriceps muscle (L) in basal conditions of RT- and cold-transplanted mice as in (B) (n = 6 per group).
(K) Transversal [18F]FDG PET-CT images of ingSAT and pgVAT of mice as in (I) and (J).



Cold-Exposed and Cold Microbiota-Transplanted Mice Show Increased Intestinal Length and Caloric Uptake

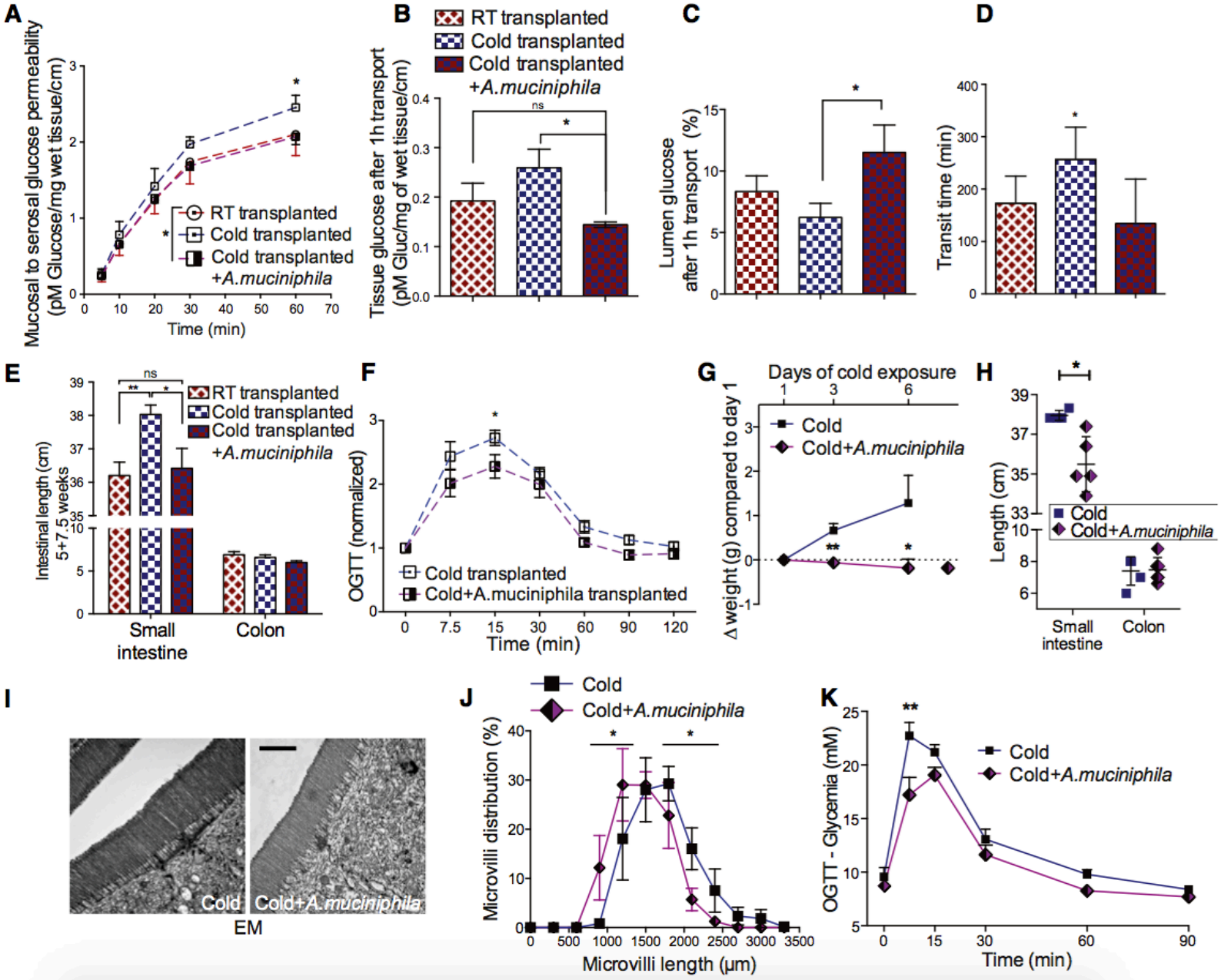
(L) Small intestine and colon lengths of RT- or cold microbiota-transplanted mice as in (B) (n = 8 per group), 21 days after transplantation, and GF controls (n = 4). (M) Representative images of cecum, small intestine, and colon of mice as in (L). (N–P) H&E staining of duodenum of cold-exposed mice with or without Abx treatment (N) and morphometric quantifications of duodenal perimeter (O) and villi length (P) (n = 8 per group in triplicates, data show mean ± SEM).

Cold-Exposed and Cold Microbiota-Transplanted Mice Have Increased Intestinal Absorptive Surface



Since the increased in- testinal surface area was also present in the microbiota-depleted mice, we assumed that absence of certain bacterial strains, rather than increased abundance, could be responsible for the observed intestinal phenotype following the cold microbiota transplantation.

Akkermansia muciniphila (*A. muciniphila*) is a Gram-negative bacterium that commonly constitutes 3%–5% of the gut microbial community. *A. muciniphila* within the mucus layer is implicated in the control of host mucus turnover, which improves gut barrier function and is linked to obesity. Since *A. muciniphila* is the most abundant species of the Verrucomicrobia, the most negatively affected phylum in response to cold exposure, we investigated whether this strain alone could revert part of the transplanted phenotype.


A
Mucosal to serosal glucose permeability (pM Glucose/mg wet tissue/cm)
Time (min)
RT transplanted
Cold transplanted
Cold transplanted +A.muciniphila
B
Tissue glucose after 1h transport (pM Gluc/mg of wet tissue/cm)
ns
C
Lumen glucose after 1h transport (%)
D
Transit time (min)
E
Intestinal length (cm) 5+7.5 weeks
Small intestine
Colon
F
OGTT (normalized)
Cold transplanted
Cold+A.muciniphila transplanted
Time (min)
G
Days of cold exposure
Δ weight (g) compared to day 1
Cold
Cold+A.muciniphila
H
Length (cm)
I
Cold
Cold+A.muciniphila
EM
J
Microvilli distribution (%)
Microvilli length (µm)
K
OGTT - Glycemia (mM)
Time (min)