Figure S1

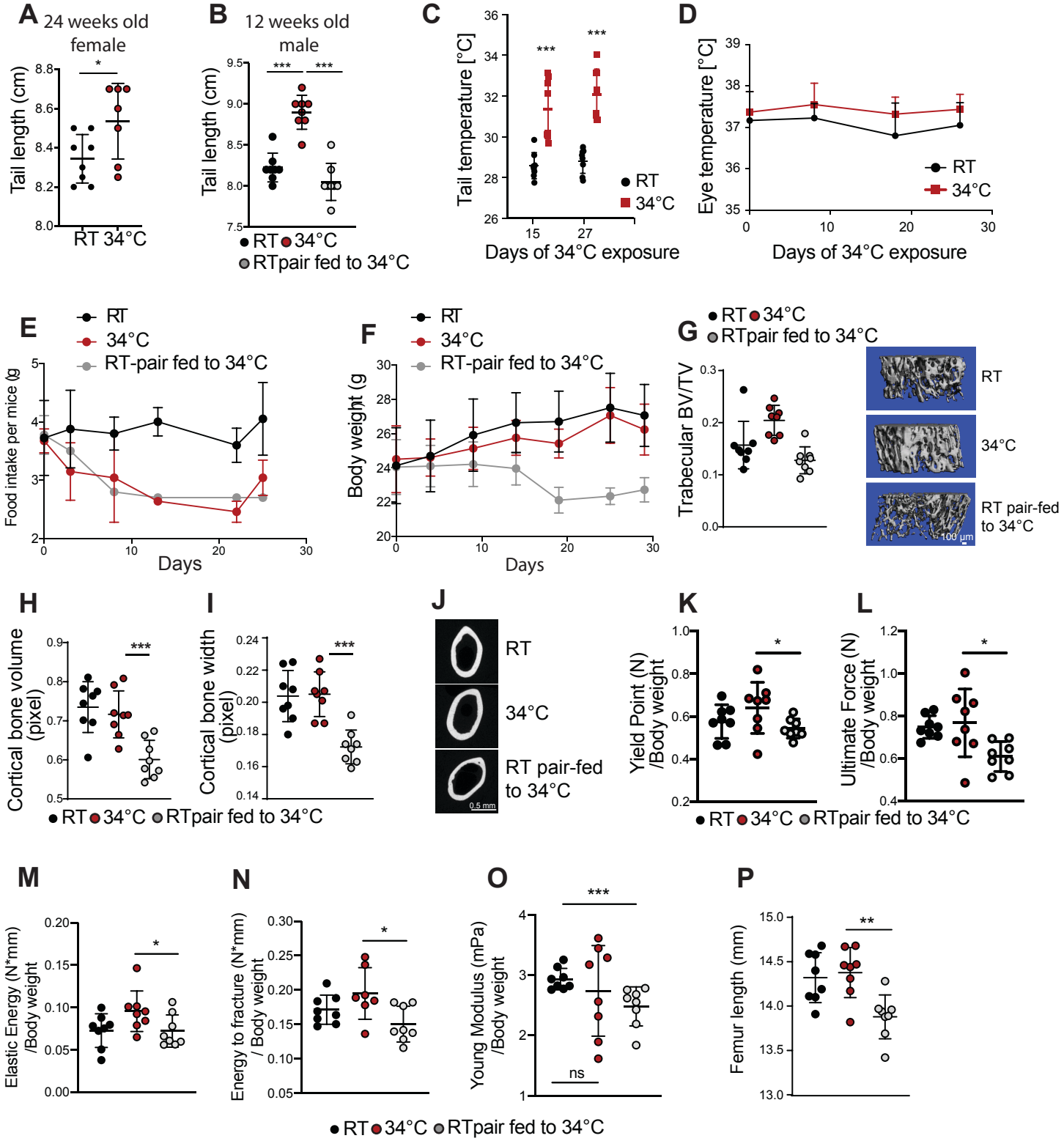

Figure S1. Warm Exposure Improves Bone Strength during Adulthood. Related to Figure 1

(A) Tail length of 24 weeks old female mice exposed to 34°C for 2 months and their RT controls.

(B) Tail length of 12 weeks old male mice exposed to 34°C for one month (34°C), or maintained at RT as control fed either ad libitum (RT) or pair–fed to the 34°C exposed mice (RT-pair-fed to 34°C).

(C and D) Tail temperature (C) and eye temperature (D) of mice as in (B).

(E and F) Food intake (E) and body weight (F) of mice as in (B)

(G) Trabecular bone volume/total volume (BV/TV) of femur from mice as in (B). (Right) representative reconstruction of trabecular bone (each consisting of 262 sections, *n* = 8 mice per group) used for the calculations. Scale = 100µm.

(H-J) Cortical bone volume (H) and width (I) measured in midshaft of femur from mice as in (B). (J) Representative cortical section (from 62 sections per bone of each mouse, *n* = 8 mice per group). Scale: 0.5mm.

(K-O). Biomechanical analysis of femur from mice as in (B) using a 3–point bending test. The measured parameters include yield point (K), ultimate stress (L), elastic energy (M), energy to fracture (N), and Young's modulus(O).

(P) Femur length of mice as in (B).

Data are shown as mean ±SD (*n* = 8 per group). Significance is calculated based on Mann-Whitney t-test: **P* < 0.05; ***P* < 0.01; ****P* < 0.001.

Figure S2

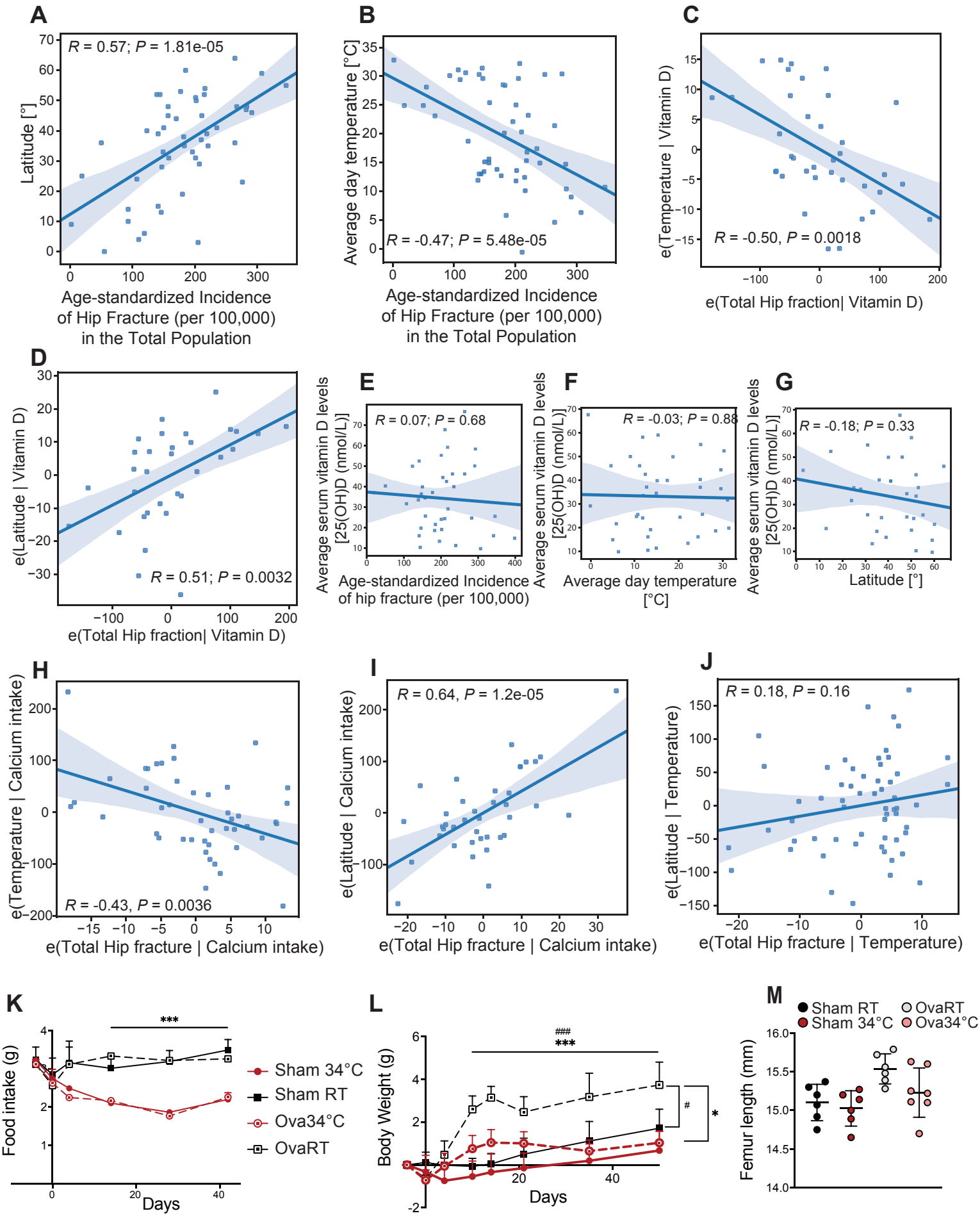
A
R = 0.57; P = 1.81e-05
Latitude [°]
Age-standardized Incidence of Hip Fracture (per 100,000) in the Total Population
B
R = -0.47; P = 5.48e-05
Average day temperature [°C]
Age-standardized Incidence of Hip Fracture (per 100,000) in the Total Population
C
R = -0.50, P = 0.0018
e(Temperature | Vitamin D)
e(Total Hip fraction| Vitamin D)
D
R = 0.51; P = 0.0032
e(Latitude | Vitamin D)
e(Total Hip fraction| Vitamin D)
E
R = 0.07; P = 0.68
Average serum vitamin D levels [25(OH)D (nmol/L)]
Age-standardized Incidence of hip fracture (per 100,000)
F
R = -0.03; P = 0.88
Average serum vitamin D levels [25(OH)D (nmol/L)]
Average day temperature [°C]
G
R = -0.18; P = 0.33
Average serum vitamin D levels [25(OH)D (nmol/L)]
Latitude [°]
H
R = -0.43, P = 0.0036
e(Temperature | Calcium intake)
e(Total Hip fracture | Calcium intake)
I
R = 0.64, P = 1.2e-05
e(Latitude | Calcium intake)
e(Total Hip fracture | Calcium intake)
J
R = 0.18, P = 0.16
e(Latitude | Temperature)
e(Total Hip fracture | Temperature)
K
***
Food intake (g)
Days
Sham 34°C
Sham RT
Ova34°C
OvaRT
L
###
***
#
*
Body Weight (g)
Days
M
Sham RT
Sham 34°C
OvaRT
Ova34°C
Femur length (mm)

Figure S2 (cont.)

Figure S2. Warm Temperature Negatively Corelates with Hip Fractures in Humans. Related to Figure 2

(A and B) Correlation of age–standardized hip fracture incidence in the total population per country with the latitude of their capitals (A), or with the country's average day temperature (B).
(C and D) Partial correlation as in (A, B) with effect of Vitamin D serum level per country removed.
(E-G) Correlation of Vitamin D serum levels per country either with age–standardized hip fracture incidence per country (E), with the latitude of their capitals (F), or with the country's average day temperature (G).
(H and I) Partial correlation as in (A, B) with effect of calcium intake per country removed.
(J) Partial correlation as in (A) with effect of temperature per country removed.
(K) Food intake of female mice that were ovariectomized, or sham-operated at 16 weeks of age, and then exposed to 34°C for 2 months (Ova34°C, or Sham34°C), or kept at RT (OvaRT, or ShamRT) (n = 8 per group).
(L) Body weight measurements of mice in (K)
(M) Femur length of mice as in (K) (n = 6 per group).
Data in (K-M) are shown as mean ± SD. Significance is calculated based on Mann-Whitney t-test: *P < 0.05; **P < 0.01; ***P < 0.001.

Figure S3

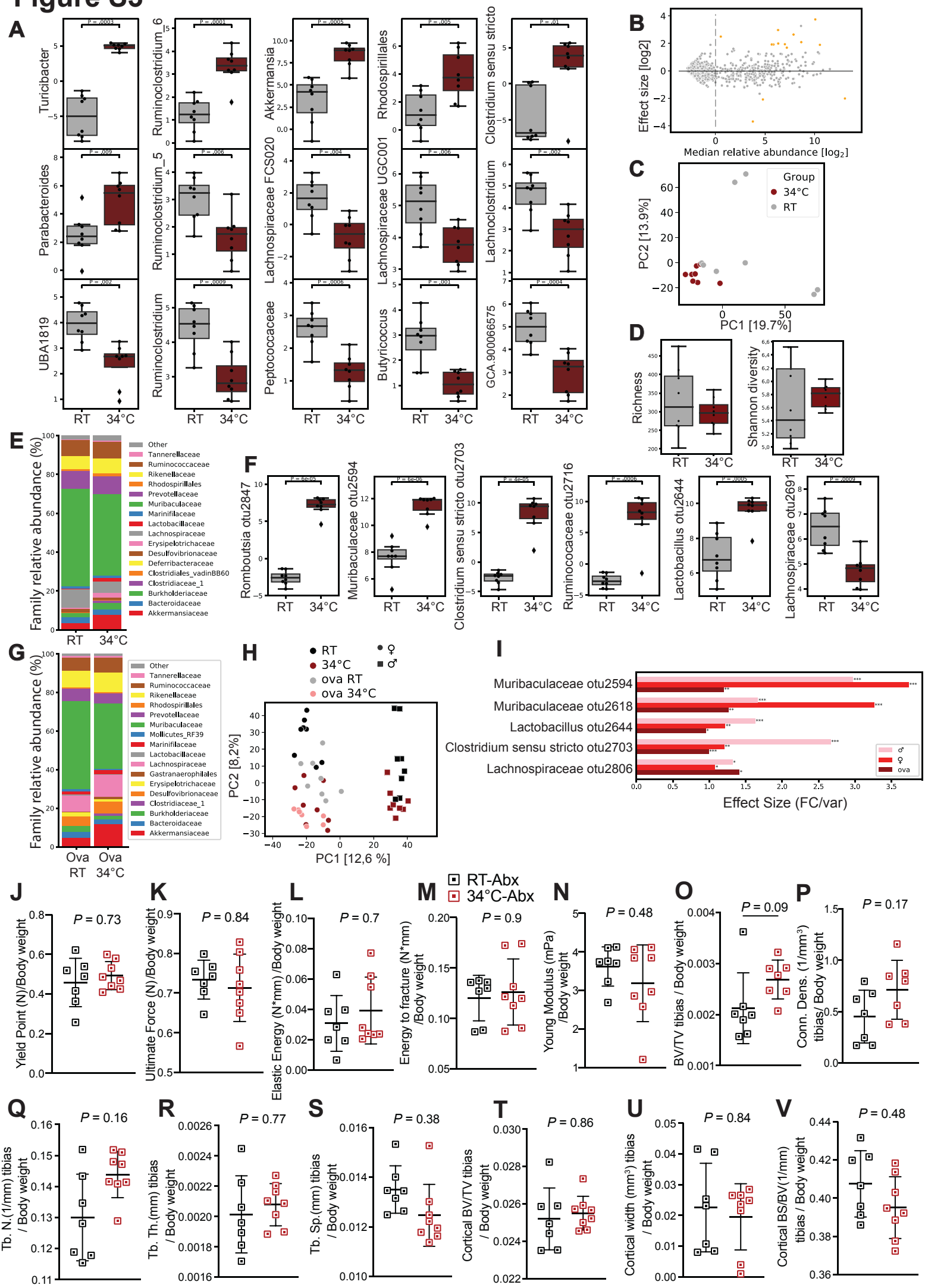
A
Turicibacter
Ruminoclostridium_6
Akkermansia
Rhodospirillales
Clostridium sensu stricto
Parabacteroides
Ruminoclostridium_5
Lachnospiraceae FCS020
Lachnospiraceae UGC001
Lachnoclostridium
UBA1819
Ruminoclostridium
Peptococcaceae
Butyricoccus
GCA.900066575
RT
34°C
B
Effect size [log2]
Median relative abundance [log2]
C
Group
34°C
RT
PC2 [13.9%]
PC1 [19.7%]
D
Richness
Shannon diversity
E
Family relative abundance (%)
Other
Tannerellaceae
Ruminococcaceae
Rikenellaceae
Rhodospirillales
Prevotellaceae
Muribaculaceae
Marinifilaceae
Lactobacillaceae
Lachnospiraceae
Erysipelotrichaceae
Desulfovibrionaceae
Deferribacteraceae
Clostridiales_vadinBB60
Clostridiaceae_1
Burkholderiaceae
Bacteroidaceae
Akkermansiaceae
F
Romboutsia otu2847
Muribaculaceae otu2594
Clostridium sensu stricto otu2703
Ruminococcaceae otu2716
Lactobacillus otu2644
Lachnospiraceae otu2691
G
Ova RT
Ova 34°C
Mollicutes_RF39
Gastranaerophilales
H
RT
34°C
ova RT
ova 34°C
♀
♂
PC2 [8,2%]
PC1 [12,6 %]
I
Muribaculaceae otu2594
Muribaculaceae otu2618
Lactobacillus otu2644
Clostridium sensu stricto otu2703
Lachnospiraceae otu2806
ova
Effect Size (FC/var)
J
Yield Point (N)/Body weight
P = 0.73
K
Ultimate Force (N)/Body weight
P = 0.84
L
Elastic Energy (N*mm) /Body weight
P = 0.7
M
RT-Abx
34°C-Abx
Energy to fracture (N*mm) /Body weight
P = 0.9
N
Young Modulus (mPa) /Body weight
P = 0.48
O
BV/TV tibias / Body weight
P = 0.09
P
Conn. Dens. (1/mm³) tibias/ Body weight
P = 0.17
Q
Tb. N.(1/mm) tibias / Body weight
P = 0.16
R
Tb. Th.(mm) tibias / Body weight
P = 0.77
S
Tb. Sp.(mm) tibias / Body weight
P = 0.38
T
Cortical BV/TV tibias / Body weight
P = 0.86
U
Cortical width (mm³) tibias / Body weight
P = 0.84
V
Cortical BS/BV(1/mm) tibias / Body weight
P = 0.48

Figure S3 (cont.)

Figure S3. Warm Exposure Changes the Microbiota Composition. Related to Figure 3
(A) Centric log2 ratio (CLR) of the changed genera after selection for false discovery rate (FDR) < 0.05 and groups' median CLR > 0 of fecal microbiota from 24 weeks old female mice that were ovariectomized at 16 weeks of age, and then exposed to 34°C for 2 months (Ova34°C), or kept at RT (OvaRT) (*n* = 8 per group).
(B) Scatter plot showing the selection criteria for the OTUs used in Figure 3E. Significantly different OTUs (FDR < 0.05 and median relative abundance > 0) are highlighted.
(C) Principal component analysis (PCA) of 16S ribosomal DNA (rDNA) sequencing of fecal microbiota from 12 weeks old male mice that were exposed to 34°C starting at 8 weeks of age, or kept at RT. Each dot represents fecal microbiota from a single mouse. The analysis is based on the centric log2 ratio (CLR).
(D) Estimated richness and Shannon diversity in fecal samples from mice as in (C).
(E) Bar chart of the relative abundance at family level in fecal microbiota from mice in (C).
(F) CLR of all significant OTUs selected for FDR < 0,1 and groups' median CLR > 0 in the fecal microbiota samples from mice as in (C).
(G) Bar chart of the relative abundance at family level in fecal microbiota from 24 weeks old female mice, ovariectomized at 16 weeks of age and exposed to 34°C for 2 months, and their ovariectomized RT controls (mice as in figure 2).
(H) PCA in fecal microbiota of 12 weeks old male mice that were exposed to 34°C starting at 8 weeks of age, or kept at RT; 24 weeks old female that were exposed to 34°C starting at 16 weeks of age, or kept at RT; and female mice that were ovariectomized at 16 weeks of age, and then exposed to 34°C for 2 months (Ova34°C), or kept at RT (OvaRT) (*n* = 8–10 per group). The analysis is based on the CLR. Each symbol represents a mouse's fecal microbiota.
(I) OTUs consistently changed in fecal microbiota of mice as in (H), selected with a p-value<0.05.
(J-N) 3-Point bending test analysis of femur from female mice that were treated with antibiotics and kept at either RT or 34°C starting at 16 weeks of age for 7 weeks (RT-Abx and 34°C-Abx, respectively). Biomechanical analysis shows yield point (J), ultimate force (K), elastic energy (L), energy to fracture (M) and young modulus (N) normalized to the body weight.
(O-S) Trabecular bone microarchitecture of tibias showing bone volume/total volume (BV/TV) (O), the connectivity density (Conn. Dens) (P), the number of trabeculae (Tb. N) (Q), the trabecular thickness (Tb.Th.) (R), and the trabecular separation (Tb.Sp) (S) of mice as in (J-N), normalized to the respective body weight at sacrifice.
(T-V) Cortical bone volume (T), width (U) and cortical bone surface to bone volume ratio (BS/BV) (V) of mice as in (J-N), measured in the midshaft of the tibias and normalized to the body weight.
Data are shown as mean ± SD. Significance in J-V is calculated using Mann-Whitney t-test. The boxplots in rest of the panels represent median and quantiles; the whiskers represent 1.5 inter quartile range and values outside of the whisker's box are represented as diamonds. The significance in all panels except J-V is calculated based on Welch t-test: **P* < 0.05; ***P* < 0.01; ****P* < 0.001 (*n* = 8 per group).

Figure S4

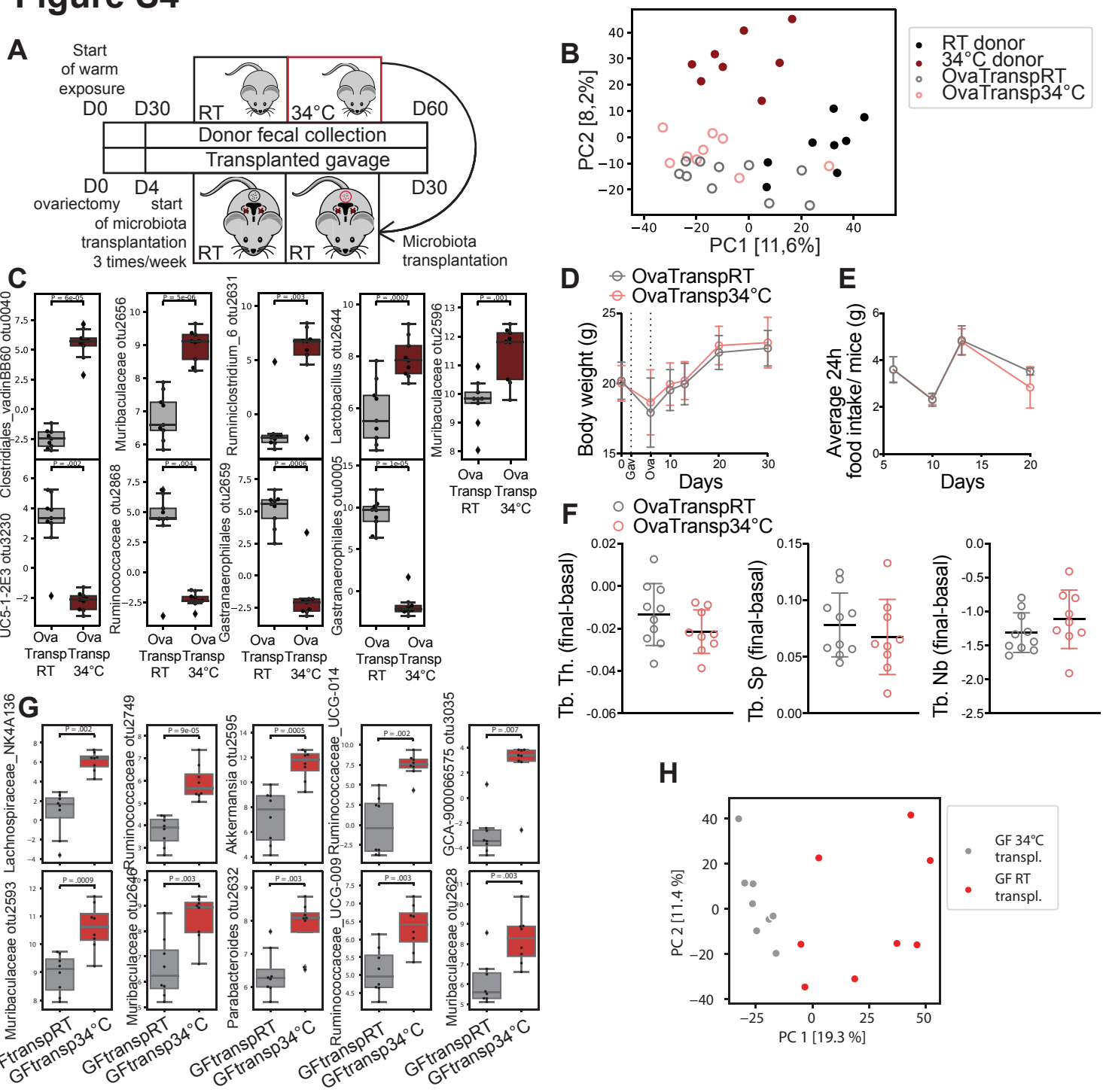
A
Start of warm exposure
D0
D30
RT
34°C
D60
Donor fecal collection
Transplanted gavage
D0 ovariectomy
D4 start of microbiota transplantation 3 times/week
D30
RT
RT
Microbiota transplantation
B
RT donor
34°C donor
OvaTranspRT
OvaTransp34°C
PC2 [8,2%]
PC1 [11,6%]
C
Clostridiales_vadinBB60 otu0040
Muribaculaceae otu2656
Ruminiclostridium_6 otu2631
Lactobacillus otu2644
Muribaculaceae otu2596
UC5-1-2E3 otu3230
Ruminococcaceae otu2868
Gastranaerophilales otu2659
Gastranaerophilales otu0005
Ova Transp RT
Ova Transp 34°C
D
OvaTranspRT
OvaTransp34°C
Body weight (g)
Gav
Ova
Days
E
Average 24h food intake/ mice (g)
Days
F
OvaTranspRT
OvaTransp34°C
Tb. Th. (final-basal)
Tb. Sp (final-basal)
Tb. Nb (final-basal)
G
Lachnospiraceae_NK4A136
Ruminococcaceae otu2749
Akkermansia otu2595
Ruminococcaceae_UCG-014
GCA-900066575 otu3035
Muribaculaceae otu2593
Muribaculaceae otu2640
Parabacteroides otu2632
Ruminococcaceae_UCG-009
Muribaculaceae otu2628
GFtranspRT
GFtransp34°C
H
GF 34°C transpl.
GF RT transpl.
PC 2 [11.4 %]
PC 1 [19.3 %]
I
GFtranspRT
GFtransp34°C
Trabecular bone volume (BV/TV)
P = 0.08
GFtranspRT
GFtransp34°C
100µm
J
GFtranspRT
GFtransp34°C
Cortical bone volume (pixel)
P = 0.06
Cortical bone width (pixel)
*
GFtranspRT
GFtransp34°C
1mm
K
GFtranspRT
GFtransp34°C
Young modulus (MPa)
Ultimate force (N)
Energy to fracture (N*mm)
Yield point (N)
Elastic energy (N*mm)
P = 0.05 microbiota effect
L
GFtranspRT
GFtransp34°C
Femur length (mm)

Figure S4 (cont.)

Figure S4. Warm–Microbiota Transplantation Prevents Osteoporosis. Related to Figure 4

(A) Schematic representation of the experimental plan. 16 weeks old female mice were exposed to 34°C for 1 month or kept at RT, and subsequently used as microbiota donors for transplanting ovariectomized 16 weeks old female recipients 3 times a week.

(B) Principal component analysis (PCA) of fecal microbiota from donor and recipient mice as in (A). Each symbol represents microbiota from individual mouse. The analysis is based on the centric log2 ratio (CLR).

(C) CLR of significant OTUs selected for false discovery rate (FDR) < 0.1 and groups' median CLR > 0 in fecal microbiota of mice as in (A).

(D and E) Body weight (D) and (E) food intake of mice as in (A).

(F) Trabecular Thickness (Tb. Th), Trabecular separation (Tb. Sp), and number of trabeculae (Tb.N) of tibias of mice as in (A).

(G and H) CLR of significant OTUs selected for false discovery rate (FDR) < 0.1 and groups' median CLR > 0 in fecal microbiota (G), or PCA of fecal microbiota of 11 weeks old germ-free mice transplanted at 8 weeks of age with cecal microbiota from 12 weeks old male donor mice that were exposed for one month at 34°C or kept at RT.

(I) Bone volume/tissue volume (BV/TV), measured proximally in trabecular bone and associated representative trabecular reconstruction (each consisting of 262 sections, n = 10 per group) in tibias of mice as in (G, H). Scale: 200μm. Bones of the recipients was collected 20 days after transplantation.

(J) Cortical bone volume and width measured in the midshaft, and associated representative images (from 62 sections per bone of each mouse of n = 10 per group) of the tibias from mice as in (G, H). Scale: 0.5mm

(K) Biomechanical analysis of femur from mice as in (G, H) using a 3–point bending test. The parameters show yield point, ultimate stress, elastic energy, energy to fracture and Young's modulus.

(L) Femur length of mice as in (G, H).

Data are shown as mean ±SD. Significance is calculated based on Mann-Whitney t-test, or Welch t-test for panel C: $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ (n = 10 per group for the transplanted, n = 8 per group for the donors).

Figure S5

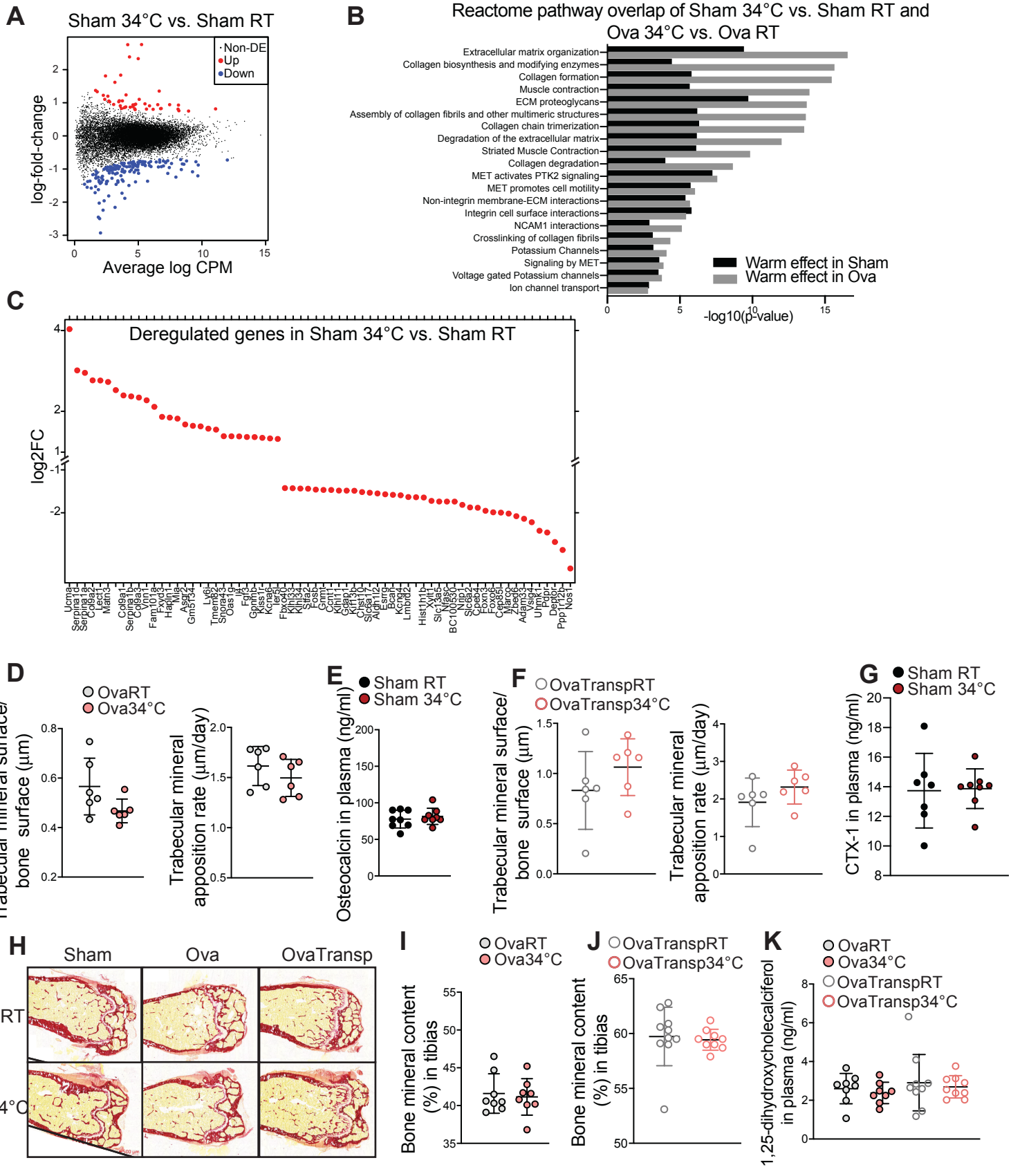
A
Sham 34°C vs. Sham RT
Non-DE
Up
Down
log-fold-change
Average log CPM
B
Reactome pathway overlap of Sham 34°C vs. Sham RT and Ova 34°C vs. Ova RT
Extracellular matrix organization
Collagen biosynthesis and modifying enzymes
Collagen formation
Muscle contraction
ECM proteoglycans
Assembly of collagen fibrils and other multimeric structures
Collagen chain trimerization
Degradation of the extracellular matrix
Striated Muscle Contraction
Collagen degradation
MET activates PTK2 signaling
MET promotes cell motility
Non-integrin membrane-ECM interactions
Integrin cell surface interactions
NCAM1 interactions
Crosslinking of collagen fibrils
Potassium Channels
Signaling by MET
Voltage gated Potassium channels
Ion channel transport
Warm effect in Sham
Warm effect in Ova
-log10(p-value)
C
Deregulated genes in Sham 34°C vs. Sham RT
log2FC
D
OvaRT
Ova34°C
Trabecular mineral surface/ bone surface (µm)
Trabecular mineral apposition rate (µm/day)
E
Sham RT
Sham 34°C
Osteocalcin in plasma (ng/ml)
F
OvaTranspRT
OvaTransp34°C
Trabecular mineral surface/ bone surface (µm)
Trabecular mineral apposition rate (µm/day)
G
Sham RT
Sham 34°C
CTX-1 in plasma (ng/ml)
H
Sham
Ova
OvaTransp
RT
34°C
I
OvaRT
Ova34°C
Bone mineral content (%) in tibias
J
OvaTranspRT
OvaTransp34°C
Bone mineral content (%) in tibias
K
OvaRT
Ova34°C
OvaTranspRT
OvaTransp34°C
1,25-dihydroxycholecalciferol in plasma (ng/ml)

Figure S5 (cont.)

Figure S5. Warm and Warm–Microbiota Transplantation Increase Periosteal Bone Formation. Related to Figures 5 and 6

(A) Mean-difference plot (MD-plot) of the log fold change of gene expression between tibias of 24 weeks old female mice that were sham-operated at 16 weeks of age, and then kept at RT or 34°C for two months (ShamRT and Sham34°C) shown as average count per million (CPM). Red dots show the increased and blue show the decreased genes selected for FDR<0.05.

(B) 20 commonly deregulated Reactome pathways between Ova34°C vs. Ova RT (Warm effect in Ova) and Sham 34°C vs. Sham RT (Warm effect in sham), and there associated -log10 (*P* value).

(C) Log2 fold changes of the deregulated genes in Sham 34°C vs Sham RT with a selective threshold of (|log2FC > 1 and $P < 0.05$).

(D) Trabecular mineralized surface and mineral apposition rate in femur of female mice that were ovariectomized, or sham-operated at 16 weeks of age, and then exposed to 34°C for 2 months (Ova 34°C, or Sham 34°C), or kept at RT (Ova RT, or Sham RT).

(E) Osteocalcin plasma concentration of Sham 34°C or Sham RT mice.

(F) Trabecular mineralized surface and mineral apposition rate in femur of 21 weeks old ovariectomized, microbiota recipient female mice. The recipient mice were ovariectomized at week 16, and repetitivelly transplanted with fecal microbiota from 34°C exposed, or RT-kept donors (OvaTransp34°C, or Ova-TranspRT, respectively). The 34°C treatment of the 16 weeks old female donor mice was initiated one month before the starting the transplantations, and lasted for the whole length of the experiment.

(G) CTX-1 levels in plasma as of Sham 34°C or Sham RT mice.

(H) Representative sirius red staining (of $n = 6$) of femur from mice as in (E), (D) and (F).

(I and J) Bone mineral content in tibias of mice as in (D) and (F).

(K) Concentration of Vitamin D (1,25-dihydroxycholecalciferol) in plasma of mice as in (D) and (F).

Data are displayed as mean ±SD ($n = 6$-10 per group). Significance is calculated based on Mann-Whitney t-test $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

Figure S6

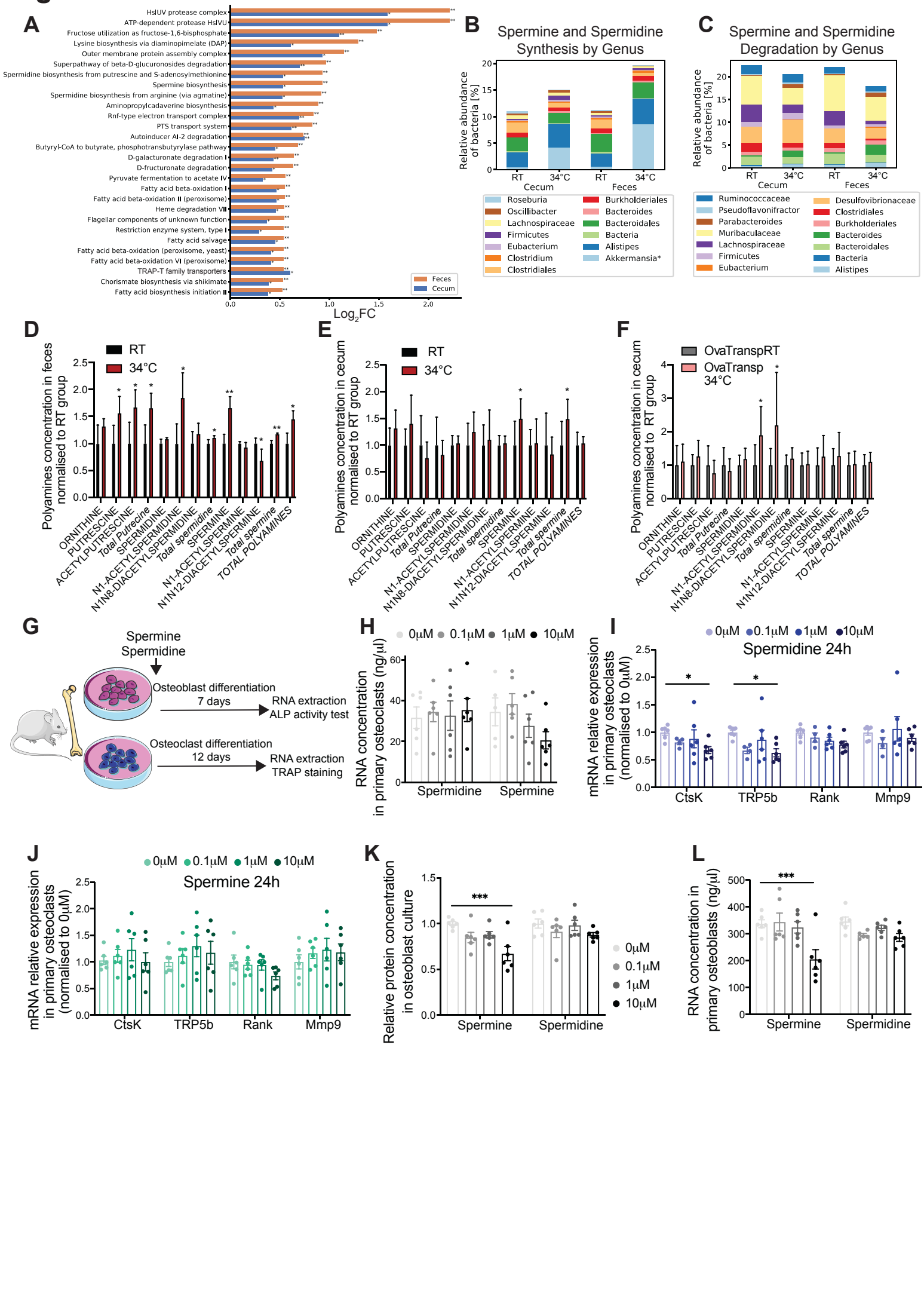
A
HslUV protease complex
ATP-dependent protease HslVU
Fructose utilization as fructose-1,6-bisphosphate
Lysine biosynthesis via diaminopimelate (DAP)
Outer membrane protein assembly complex
Superpathway of beta-D-glucuronosides degradation
Spermidine biosynthesis from putrescine and S-adenosylmethionine
Spermine biosynthesis
Spermidine biosynthesis from arginine (via agmatine)
Aminopropylcadaverine biosynthesis
Rnf-type electron transport complex
PTS transport system
Autoinducer AI-2 degradation
Butyryl-CoA to butyrate, phosphotransbutyrylase pathway
D-galacturonate degradation I
D-fructuronate degradation
Pyruvate fermentation to acetate IV
Fatty acid beta-oxidation I
Fatty acid beta-oxidation II (peroxisome)
Heme degradation VII
Flagellar components of unknown function
Restriction enzyme system, type I
Fatty acid salvage
Fatty acid beta-oxidation (peroxisome, yeast)
Fatty acid beta-oxidation VI (peroxisome)
TRAP-T family transporters
Chorismate biosynthesis via shikimate
Fatty acid biosynthesis initiation II
Feces
Cecum
Log2FC
B
Spermine and Spermidine Synthesis by Genus
Relative abundance of bacteria [%]
RT
34°C
Cecum
Feces
Roseburia
Oscillibacter
Lachnospiraceae
Firmicutes
Eubacterium
Clostridium
Clostridiales
Burkholderiales
Bacteroides
Bacteroidales
Bacteria
Alistipes
Akkermansia*
C
Spermine and Spermidine Degradation by Genus
Relative abundance of bacteria [%]
Ruminococcaceae
Pseudoflavonifractor
Parabacteroides
Muribaculaceae
Lachnospiraceae
Firmicutes
Eubacterium
Desulfovibrionaceae
Clostridiales
Burkholderiales
Bacteroides
Bacteroidales
Bacteria
Alistipes
D
Polyamines concentration in feces normalised to RT group
RT
34°C
ORNITHINE
PUTRESCINE
ACETYLPUTRESCINE
Total Putrecine
SPERMIDINE
N1-ACETYLSPERMIDINE
N1N8-DIACETYLSPERMIDINE
Total spermidine
SPERMINE
N1-ACETYLSPERMINE
N1N12-DIACETYLSPERMINE
Total spermine
TOTAL POLYAMINES
E
Polyamines concentration in cecum normalised to RT group
RT
34°C
F
Polyamines concentration in cecum normalised to RT group
OvaTranspRT
OvaTransp 34°C
G
Spermine
Spermidine
Osteoblast differentiation
7 days
RNA extraction
ALP activity test
Osteoclast differentiation
12 days
RNA extraction
TRAP staining
H
0μM 0.1μM 1μM 10μM
RNA concentration in primary osteoclasts (ng/μl)
Spermidine
Spermine
I
0μM 0.1μM 1μM 10μM
Spermidine 24h
mRNA relative expression in primary osteoclasts (normalised to 0μM)
CtsK
TRP5b
Rank
Mmp9
J
0μM 0.1μM 1μM 10μM
Spermine 24h
mRNA relative expression in primary osteoclasts (normalised to 0μM)
CtsK
TRP5b
Rank
Mmp9
K
Relative protein concentration in osteoblast culture
***
Spermine
Spermidine
0μM
0.1μM
1μM
10μM
L
RNA concentration in primary osteoblasts (ng/μl)
***
Spermine
Spermidine

Figure S6 (cont.)

Figure S6. Warm Exposure Increases Production of Polyamines that Affect Osteoblast Activity and Decrease Osteoclast Differentiation. Related to Figure 7

(A) Most upregulated pathways after metagenomics analysis of the bacteria present in both feces and cecum from 24 weeks old female mice that were exposed to 34°C starting at 16 weeks of age for 2 months, or kept at RT (FDR < 0.01, log2FC > 0.5, pathway relative abundance>10%)).

(B and C) Relative abundance of bacterial genera containing spermine and spermidine synthesis (B), or degradation pathways (C) in cecum and feces from mice as in (A).

(D and E) Polyamine concentration in feces (D), or cecum (E) from mice as in (A) (relative to RT controls).

(F) Polyamine concentration in cecum of 21 weeks old ovariectomized, microbiota recipient female mice. The recipient mice were ovariectomized at week 16, and repetitivelly transplanted with fecal microbiota from 34°C exposed, or RT-kept donors (OvaTransp34°C, or OvaTranspRT, respectively). The 34°C treatment of the 16 weeks old female donor mice was initiated one month before the starting the transplantations, and lasted for the whole length of the experiment.

(G) Schematic representation of the ex-vivo experiment used to address the direct effects of spermine and spermidine supplementation on osteoclasts or osteoblasts differentiation and function.

(H) RNA concentration in primary osteoclasts cell culture after spermine or spermidine administration for 12 days.

(I and J) Relative mRNA expression after 24h spermidine (I) or spermine (J) supplementation to primary osteoclasts following 12 days differentiation.

(K and L) Relative protein (K) and RNA concentration (L) of osteoblast culture after 7 days of spermine or spermidine supplementation at different concentrations.

Except for (A), significance was calculated using Mann-Whitney t-test: $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

Figure S7

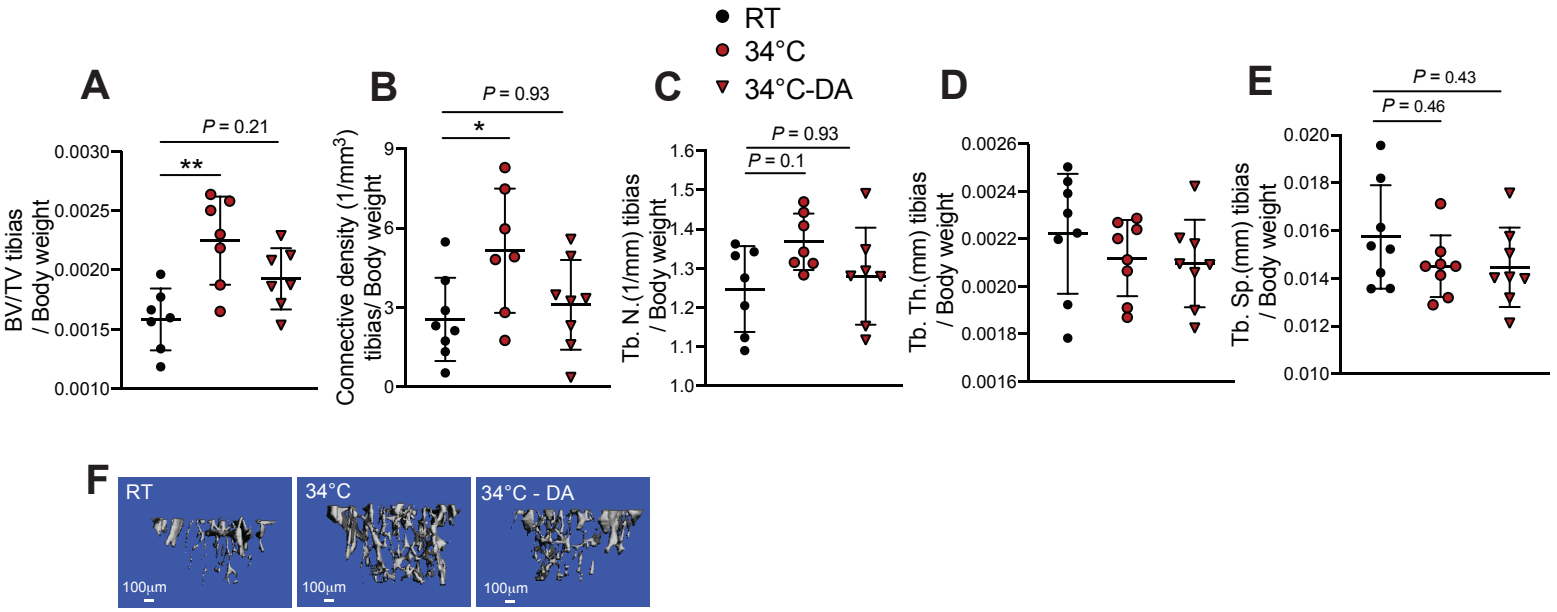

Figure S7. Inhibition of Polyamine Biosynthesis Limits the Warm Effects on the Bone. Related to Figure 7

(A-E) Trabecular bone microarchitecture of tibias showing bone volume/total volume (BV/TV), connectivity density (Conn. Dens) (B), number of trabeculae (Tb. N) (C), trabecular thickness (Tb.Th.) (D), and trabecular separation (Tb.Sp) (E) from 23 weeks old female mice that were either RT kept (RT); warm exposed (34°C); or provided with 50µm Diaminazene Acetureate (DA) and kept at 34°C (34°C-DA), starting at 16 weeks of age until sacrifice. DA wes supplemented in drinking water every second day. The measurements are normalized to the body weight.

(F) Representative trabecular reconstruction (each consisting of 262 sections, *n* = 7-8 mice per group) from the Micro-CT used in the analysis in (A-E). Scale: 100µm.

Data are shown as mean ±SD (*n* = 7-8 per group). Significance is calculated using One-Way ANOVA; **P* < 0.05; ***P* < 0.01; ****P* < 0.001.

Supplemental item

Table 1: List of primers used for qPCR. Specified in KRT and Related to Figure 7

Gene	Foward primer	Reverse primer
TATA Box binding Protein (*Tbp*)	GAAGCTGCGGTACAATTCCAG	CCCCTTGTACCCTTCACCAAT
Rank ligand (*Rankl*)	CCTGATGAAAGGAGGGAGCA	TGGAATTCAGAATTGCCCGA
Collagen type 1 (*Col1a*)	TGTCCCAACCCCCAAAGAC	CCCTCGACTCCTACATCTTCTGA
Dentin matrix protein1 (*Dpm1*)	TGTCATTCTCCTTGTGTTCCTTTG	AATCACCCGTCCTCTCTTCAGA
Osteopontin (*Opn*)	CCCATCTCAGAAGCAGAATCTCC	TTCATCCGAGTCCACAGAATCC
Osteocalcin (*Ocn*)	GGCCCTGAGTCTGACAAAGC	GCTCGTCACAAGCAGGGTTAA
Runt related transcription factor 2 (*Runx2*)	TACCAGCCACCGAGACCAA	AGAGGCTGTTTGACGCCATAG
Matrix metalloprotease 9 (*Mmp9*)	CAGCCGACTTTTGTGGTCTTC	GTACAAGTATGCCTCTGCCA.
Osteoprotegerin (*Opg*)	GACAACGTGTGTTCCGGAAA	GGTAGGAACAGCAAACCTGAAGA
Cathepsin K (*Ctsk*)	TGGGCCAGGATGAAAGTTG	CCCCACAGGAATCTCTCTGT
Acid phosphatase 5, tartrate resistant (*Trap5b*)	TGATCACCTTGGCAACGTCTCT	GGAATTTTGAAGCGCAAACG