

Supporting Information

Smoum et al. 10.1073/pnas.0912479107

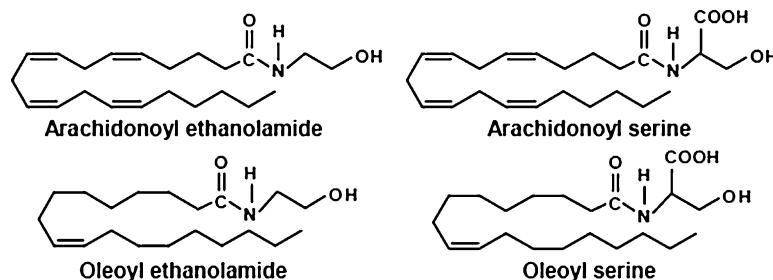


Fig. S1. Structures of oleoyl serine and related compounds.

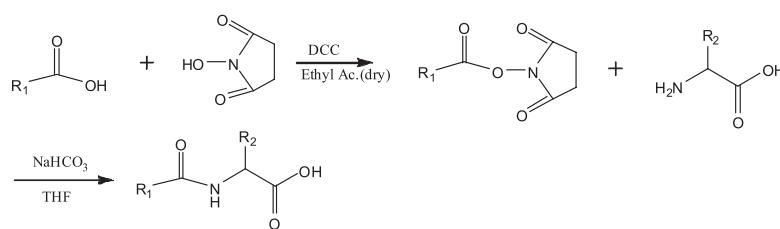


Fig. S2. Synthesis of *N*-acyl amino acids. R₁, acyl chain of fatty acid; R₂, amino acid moiety.

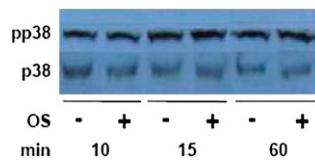


Fig. S3. *N*-oleoyl-L-serine (OS), at 10⁻¹¹ M does not affect p38 phosphorylation in MC3T3 E1 cell. Western blot analysis.

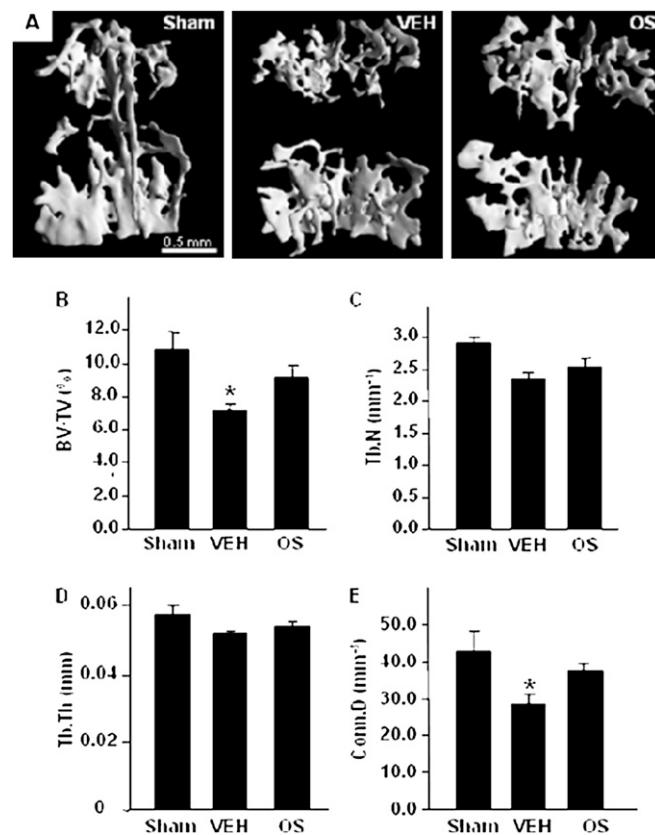


Fig. S4. OS regulates bone mass in L3 vertebral bodies of ovariectomized (OVX) mice. (A) Specimens from mice with median trabecular bone volume density. (B) Trabecular bone volume density (BV/TV). (C) Trabecular number (Tb.N). (D) Trabecular thickness (Tb.Th). (E) Connectivity density (Conn.D). Data are mean \pm SE obtained in six to eight mice per condition. *Versus Sham and VEH, $P < 0.05$.

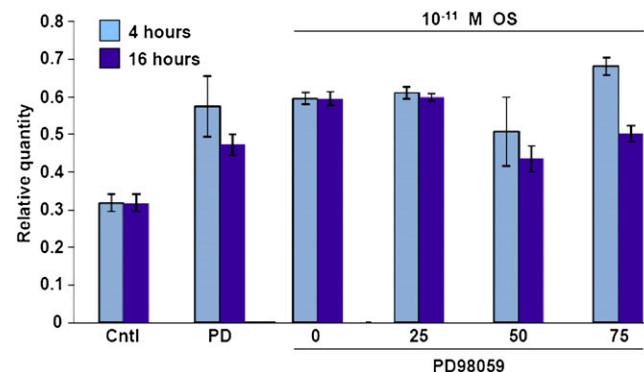


Fig. S5. MC3T3 E1 Mapkapk2 mRNA is not affected by the MEK/Erk1/2 inhibitor PD98059. Cntl, OS-, and PD98059-free control culture; PD, 100 μ M PD98059. Real-time RT-PCR analysis was carried out 4 and 16 h after OS or PD98059 supplementation. Data are mean \pm SE obtained in three culture dishes per condition.

Table S1. Measured masses, mass errors, and proposed formulae of theoretically justified peaks in product ion scan of partially purified mouse trabecular bone extract shown in Fig. 1A

Peaks <i>m/z</i> (ppm error)	Proposed formulae	Comments
368.2783 (-6.3)	$C_{21}H_{38}NO_4^-$	$[M-H]^-$
338.2734 (9.8)	$C_{20}H_{36}NO_3^-$	$[M-H]^- - CH_2O$
320.2630 (10.9)	$C_{20}H_{34}NO_2^-$	$[M-H]^- - CH_2O - H_2O$
306.2824 (7)	$C_{20}H_{36}NO^-$	$[M-H]^- - CO_2 - H_2O$
294.2816 (4.6)	$C_{19}H_{36}NO^-$	$[M-H]^- - C_2H_2O_3$
280.2580 (-23.5)	$C_{18}H_{34}NO^-$	$[M-H]^- - C_3H_4O_3$
263.2394 (5.1)	$C_{18}H_{31}O^-$	$[M-H]^- - C_3H_7NO_3$ (Ser)
104.0337 (-15.5)	$C_6H_6NO_3^-$	$[M-H]^- - C_{18}H_{32}O$, or $[Ser - H]^-$
74.0253 (7.4)	$C_2H_4NO_2^-$	$[M-H]^- - C_{18}H_{32}O - CH_2O$

Table S2. Tissue and cell culture levels of OS

Tissue/culture	OS content (pmol/g wet tissue)
Distal femoral metaphysis	3.20 ± 0.25
Whole brain	32.32 ± 3.40
Spleen	0.32 ± 0.09
Plasma	Undetectable
MC3T3 E1 osteoblastic cells	$1,350 \pm 199$
Newborn calvarial osteoblasts	$1,390 \pm 451$

Data are mean \pm SE obtained in seven mice or 12 culture dishes for each cell type.

Table S3. Trabecular bone parameters measures in 3D micro-computed tomographic (μ CT) images of L3 vertebrae from intact mice

Treatment parameter	Intact/VEH	Intact/OS
BV/TV (%)	12.58 ± 0.29	11.98 ± 0.65
Tb.N (mm^{-1})	3.59 ± 0.07	3.51 ± 0.14
Tb.Th (mm)	0.057 ± 0.001	0.055 ± 0.001
Conn. D (mm^{-3})	51.4 ± 2.8	50.7 ± 3.5

Data are mean \pm SE obtain in same animals reported in Fig. 5.

Table S4. Cortical, middiaphyseal parameters measures in 3D μ CT images of femora from intact mice

Treatment parameter	Intact/VEH	Intact/OS
Dia.Dia (mm)	1.48 ± 0.01	1.46 ± 0.02
Med.Dia (mm)	1.24 ± 0.01	1.21 ± 0.02
Cort.Th (μm)	133 ± 5	133 ± 3

Dia.Dia, diaphyseal diameter; Med.Dia, medullary cavity diameter; Cort. Th, cortical thickness. Data are mean \pm SE obtain in same animals reported in Fig. 5.

Table S5. Cortical, middiaphyseal parameters measures in 3D μ CT images of femora from sham-OVX and OVX mice

Treatment parameter	Sham-OVX	OVX/VEH	OVX/OS
Dia.Dia (mm)	1.41 ± 0.01	1.41 ± 0.01	1.41 ± 0.01
Med.Dia (mm)	1.04 ± 0.01	$1.09 \pm 0.01^*$	$1.08 \pm 0.01^*$
Cort.Th (μm)	197 ± 3	$170 \pm 4^*$	$176 \pm 10^*$

Data are mean \pm SE obtain in same animals reported in Fig. 6.

*Versus sham-OVX, $P < 0.05$.