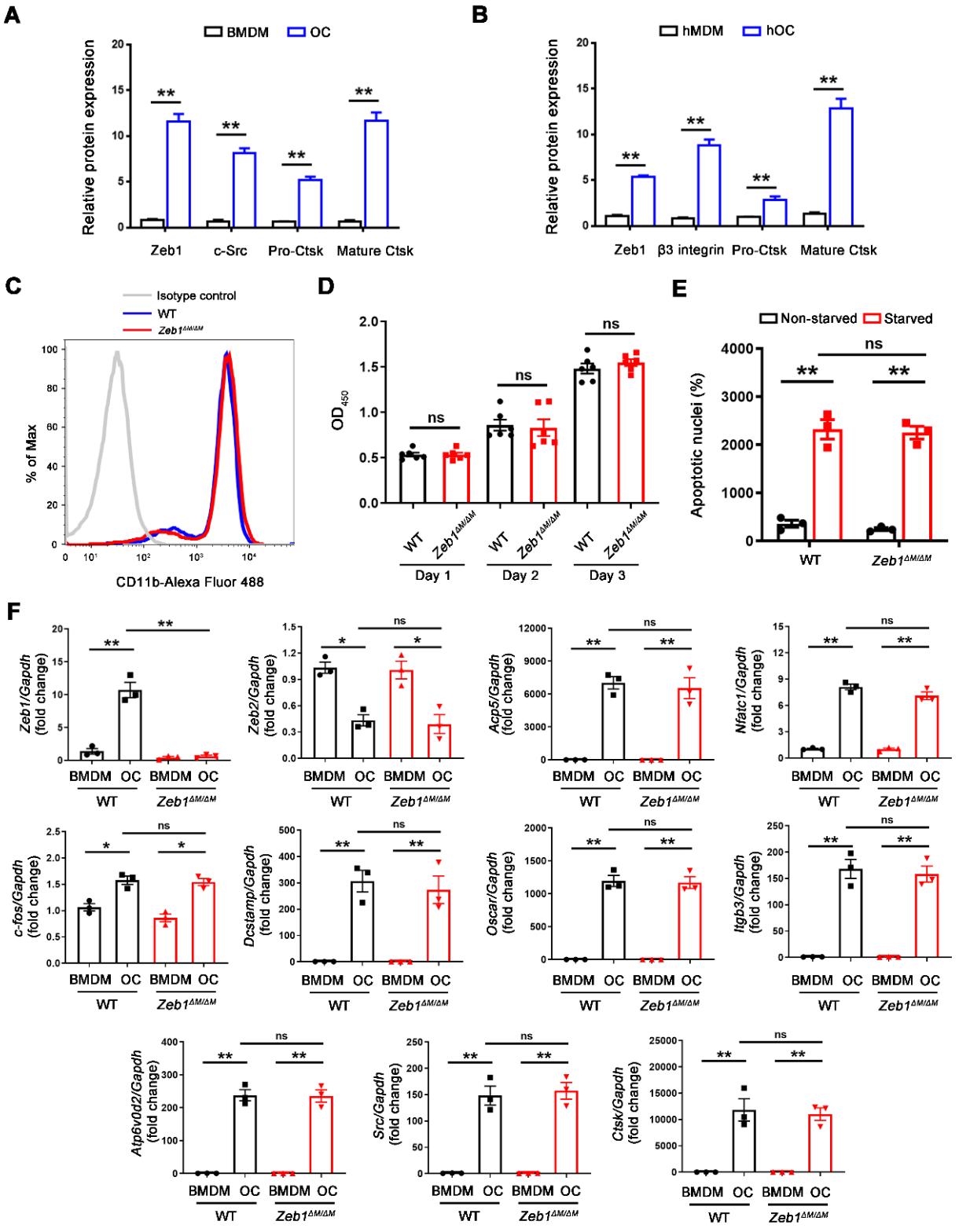
APPENDIX

A Zeb1/Mitochondrial Creatine Kinase Metabolic Axis Controls Osteoclast Activation and Skeletal Remodeling

Lingxin Zhu1,2,3*, Yi Tang2,3, Xiao-Yan Li2,3, Samuel A. Kerk4,5,6, Costas A. Lyssiotis4,6,11, Wenqing Feng3, Xiaoyue Sun1, Geoffrey E. Hespe2,3,7, Zijun Wang1, Marc P. Stemmler9, Simone Brabletz9, Thomas Brabletz9, Evan T. Keller10, Jun Ma2,3, Jung-Sun Cho2,3, Jingwen Yang1,8, and Stephen J Weiss2,3,12*

Table of Contents


A
BMDM
OC
Relative protein expression
Zeb1
c-Src
Pro-Ctsk
Mature Ctsk
B
hMDM
hOC
Relative protein expression
Zeb1
β3 integrin
Pro-Ctsk
Mature Ctsk
C
Isotype control
WT
Zeb1ΔM/ΔM
% of Max
CD11b-Alexa Fluor 488
D
OD450
ns
WT
Zeb1ΔM/ΔM
Day 1
Day 2
Day 3
E
Non-starved
Starved
Apoptotic nuclei (%)
WT
Zeb1ΔM/ΔM
F
Zeb1/Gapdh (fold change)
Zeb2/Gapdh (fold change)
Acp5/Gapdh (fold change)
Nfatc1/Gapdh (fold change)
c-fos/Gapdh (fold change)
Dcstamp/Gapdh (fold change)
Oscar/Gapdh (fold change)
Itgb3/Gapdh (fold change)
Atp6v0d2/Gapdh (fold change)
Src/Gapdh (fold change)
Ctsk/Gapdh (fold change)
BMDM
OC
WT
Zeb1ΔM/ΔM

Appendix Figure S1. Characterization of *Zeb1* null osteoclast precursors and normal osteoclast differentiation-related gene expression in *Zeb1*-deleted osteoclasts.

A Western blot quantification of Zeb1, c-Src, pro-Ctsk, and mature Ctsk expression in BMDMs and mature osteoclasts as shown in Fig 1D (n = 3).

B Western blot quantification of ZEB1, β3 INTEGRIN, and CTSK protein expression in hMDMs and mature hOCs as shown in Fig EV1D (n = 3).

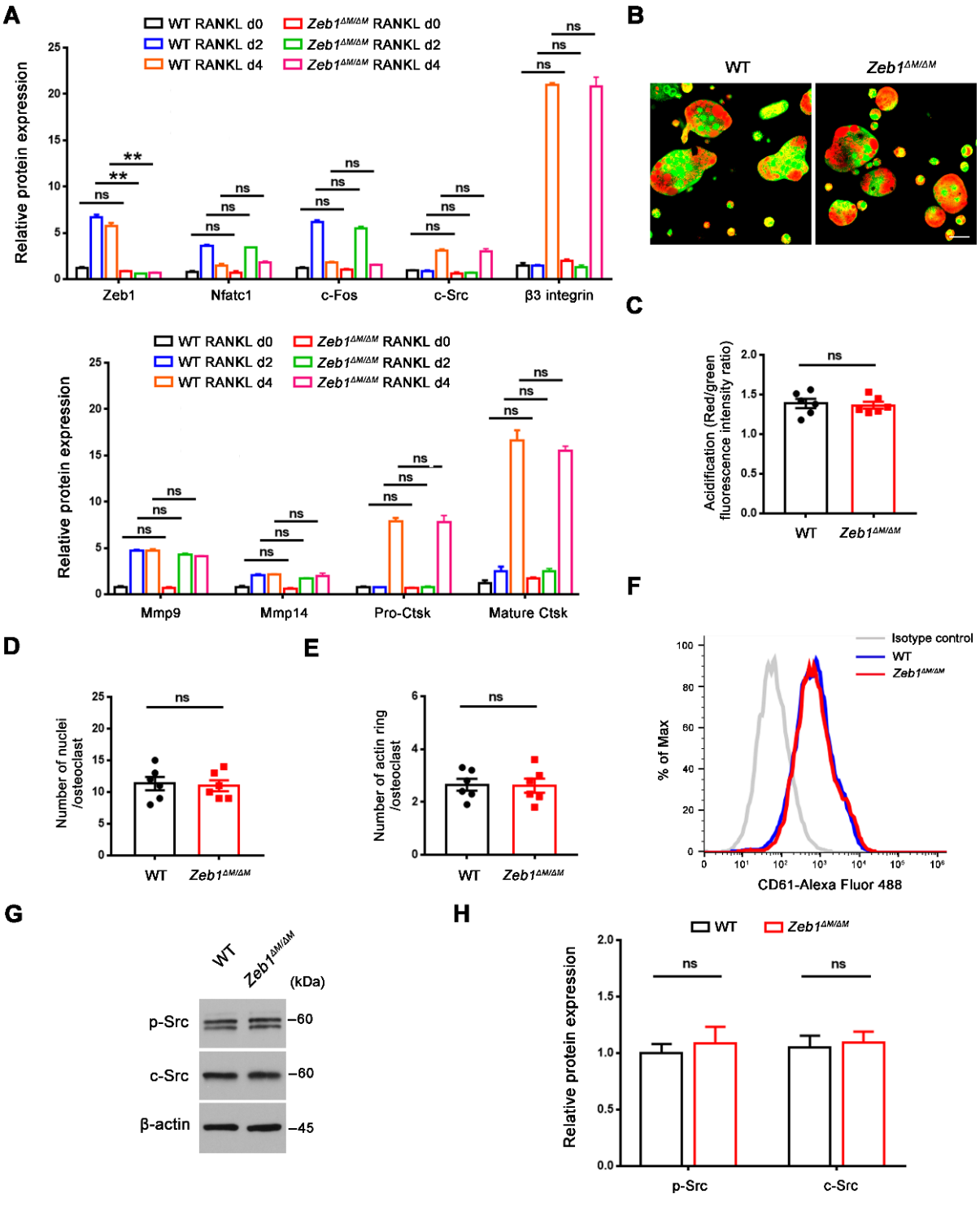
C CD11b expression in wild-type or $Zeb1^{\Delta M/\Delta M}$ osteoclast precursor cells as determined by flow cytometry (n = 3).

D Proliferation of wild-type and $Zeb1^{\Delta M/\Delta M}$ BMDMs by WST-1 assay (n = 6).

E Wild-type and $Zeb1^{\Delta M/\Delta M}$ osteoclasts cultured atop plastic substrata were non-starved or starved for 12h. TUNEL staining was then performed and apoptotic nuclei quantitated (n = 3).

F Relative mRNA expression of *Zeb1*, *Zeb2*, *Acp5*, *Nfatc1*, *c-fos*, *Dcstamp*, *Oscar*, *Itgb3*, *Atp6v0d2*, *Src*, and *Ctsk* in BMDMs and osteoclasts generated from wild-type or $Zeb1^{\Delta M/\Delta M}$ mice (n = 3).

Data information: Bars and error bars represent mean ± SEM. Data are representative of at least 3 independent experiments with biological replicates. Data were analyzed using unpaired Student's t test (A, B, D) or one-way ANOVA with Bonferroni correction (E, F). ns, not significant; *P < 0.05, **P < 0.01.


A
WT RANKL d0
WT RANKL d2
WT RANKL d4
Zeb1ΔM/ΔM RANKL d0
Zeb1ΔM/ΔM RANKL d2
Zeb1ΔM/ΔM RANKL d4
Relative protein expression
Zeb1
Nfatc1
c-Fos
c-Src
β3 integrin
B
WT
Zeb1ΔM/ΔM
C
Acidification (Red/green fluorescence intensity ratio)
Mmp9
Mmp14
Pro-Ctsk
Mature Ctsk
D
Number of nuclei /osteoclast
E
Number of actin ring /osteoclast
F
Isotype control
% of Max
CD61-Alexa Fluor 488
G
(kDa)
p-Src
c-Src
β-actin
H
p-Src
c-Src
ns

Appendix Figure S2. Normal osteoclast differentiation-related protein expression, unaltered surface β3 integrin expression and β3 integrin downstream activation in *Zeb1*-deleted osteoclasts.

A Western blot quantification of Zeb1, Nfatc1, c-Fos, c-Src, β3 integrin, Mmp9, Mmp14, and Ctsk expression in wild-type and $Zeb1^{\Delta M/\Delta M}$ BMDMs during osteoclast differentiation as shown in Fig 3B (n = 3).

B, C BMDMs were isolated from wild-type or $Zeb1^{\Delta M/\Delta M}$ mice and cultured atop bone slices with M-CSF and RANKL for 6 d. Acidification of osteoclast lacunar zones was visualized by acridine orange staining (B) and quantified as the ratio of red versus green fluorescence intensity (C). Scale bar, 20 μm.

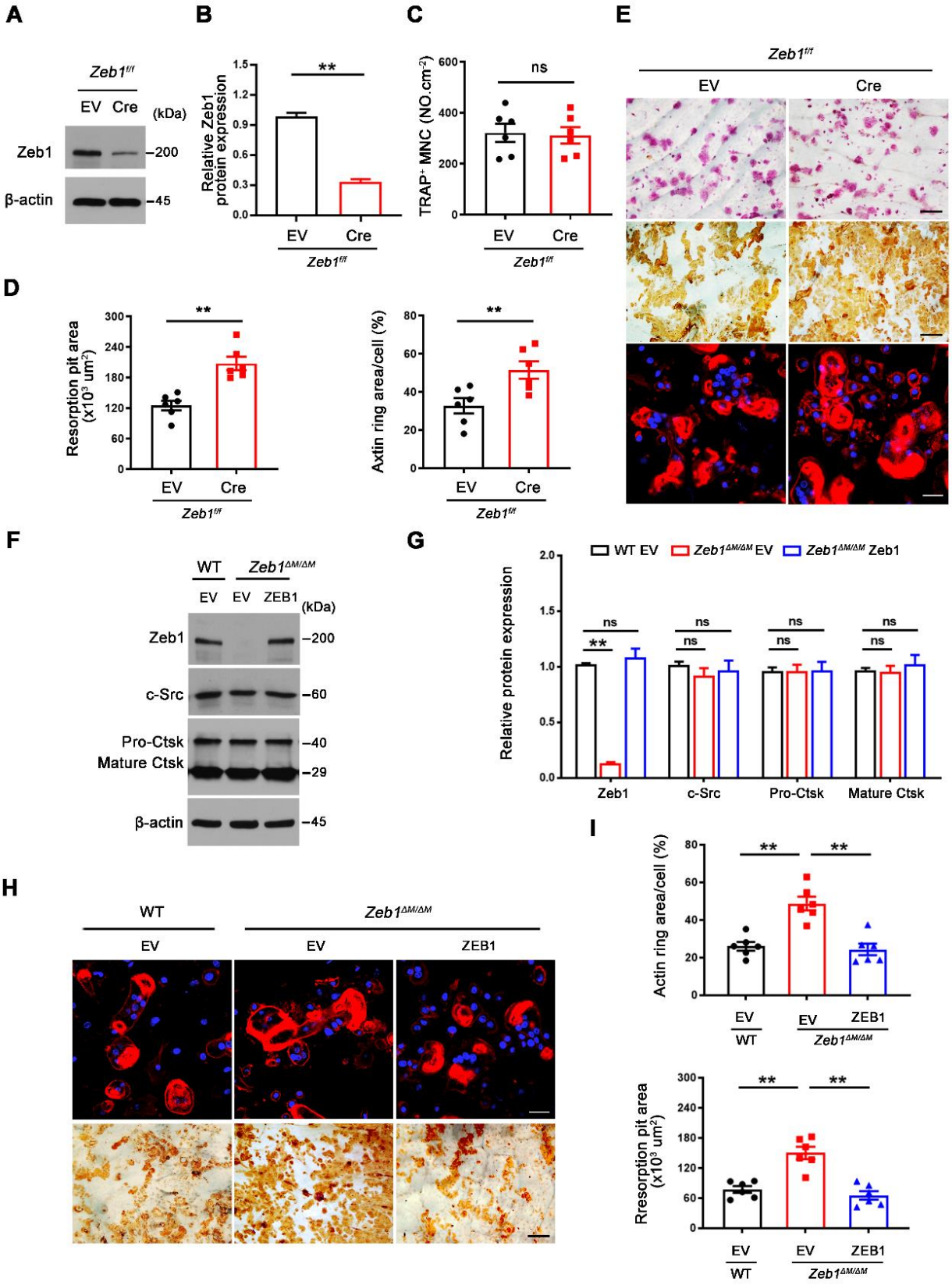
D, E After a 6-day culture atop bone slice, phalloidin staining was performed in wild-type versus $Zeb1^{\Delta M/\Delta M}$ osteoclasts, then the number of nuclei per osteoclast (D) and the number of actin ring per osteoclast (E) quantified (n = 6).

F Measurements of surface β3 integrin (CD61) expression in osteoclasts generated from wild-type or $Zeb1^{\Delta M/\Delta M}$ mice with flow cytometry (n = 3).

G, H Phospho-Src and c-Src expression as assessed by Western blot in wild-type and $Zeb1^{\Delta M/\Delta M}$ osteoclasts attached on vitronectin-pretreated plate for 30 min (G), and quantified (H; n = 3).

Data information: Bars and error bars represent mean ± SEM. Data are representative of at least 3 independent experiments with biological replicates. Data were analyzed using one-way ANOVA with Bonferroni correction (A, H) or unpaired Student's t test (C-E). ns, not significant; $^{**}P < 0.01$.

Source data are available online for this figure.


A
Zeb1f/f
EV Cre
(kDa)
Zeb1
-200
β-actin
-45
B
Relative Zeb1 protein expression
**
EV Cre
Zeb1f/f
C
TRAP+ MNC (NO.cm-2)
ns
EV Cre
Zeb1f/f
E
Zeb1f/f
EV Cre
D
Resorption pit area (x10³ um²)
**
EV Cre
Zeb1f/f
Axtin ring area/cell (%)
**
EV Cre
Zeb1f/f
F
WT Zeb1ΔM/ΔM
EV EV ZEB1
(kDa)
Zeb1
-200
c-Src
-60
Pro-Ctsk
Mature Ctsk
-40
-29
β-actin
-45
G
WT EV Zeb1ΔM/ΔM EV Zeb1ΔM/ΔM Zeb1
Relative protein expression
ns
**
Zeb1
c-Src
Pro-Ctsk
Mature Ctsk
H
WT Zeb1ΔM/ΔM
EV EV ZEB1
I
Actin ring area/cell (%)
**
EV EV ZEB1
WT Zeb1ΔM/ΔM
Rresorption pit area (x10³ um²)
**
EV EV ZEB1
WT Zeb1ΔM/ΔM

Appendix Figure S3. Temporal requirements for Zeb1 in orchestrating osteoclast function.

A, B *Zeb1*$^{\Delta M/\Delta M}$ BMDMs were transduced with a lentiviral Cre or an empty expression vector (EV), differentiated into osteoclasts, and cell lysates collected for Zeb1 immunoblotting (A) and quantified (B; *n* = 3).

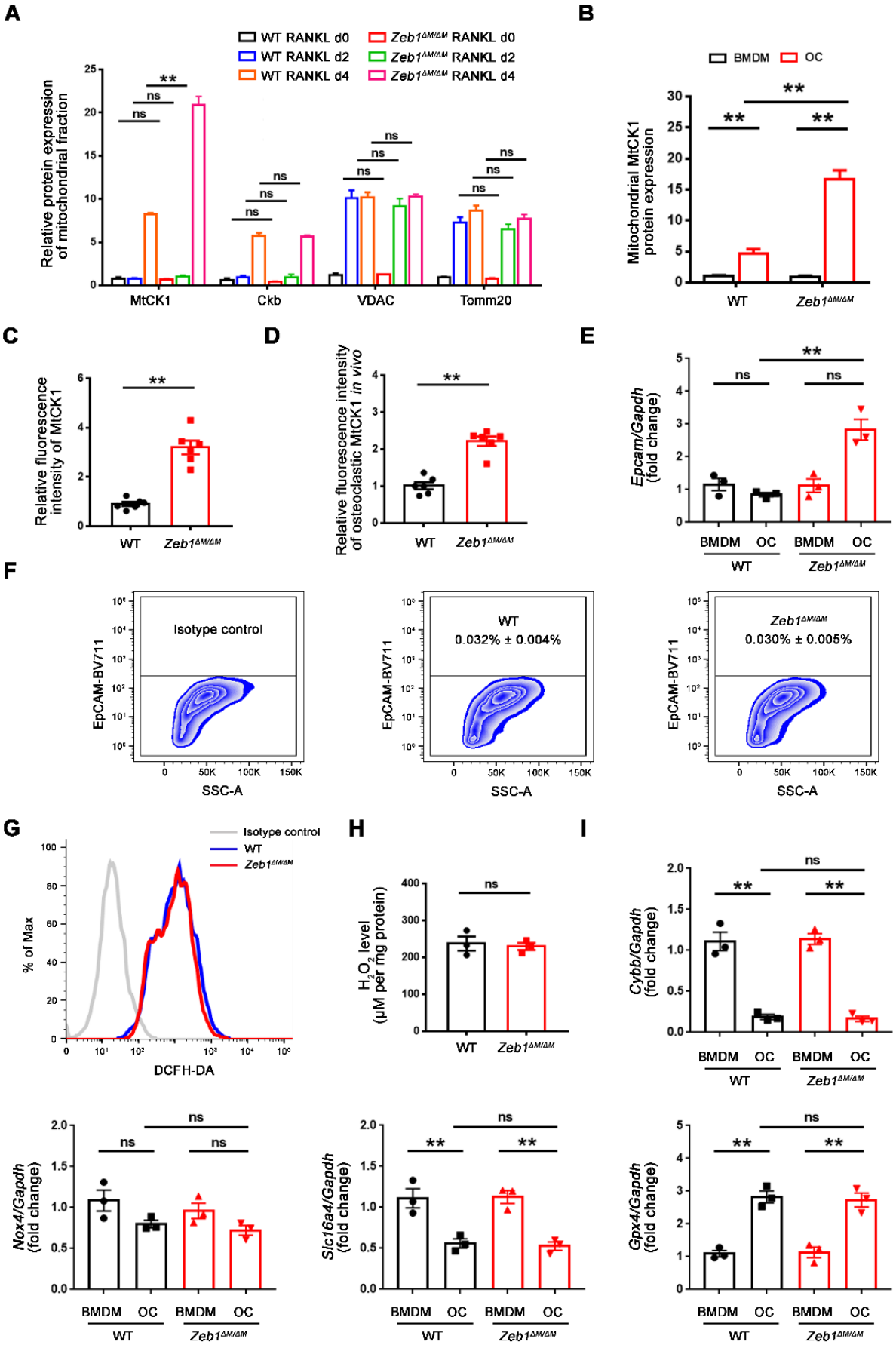
C-E Lentiviral Cre or EV-transduced *Zeb1*$^{\Delta M/\Delta M}$ BMDMs were induced into osteoclasts, cultured atop bone slices for 3 days and cells stained with phalloidin (red) or TRAP. Following osteoclast removal, resorption pits were visualized by WGA-DAB staining (E). Scale bar, upper and middle 100 μm, lower 20 μm. Quantification of TRAP (C), actin ring area per cell, and WGA staining (D; *n* = 6).

F, G An empty control vector (EV)-transduced wild-type BMDMs, and EV- or ZEB1-transduced *Zeb1*$^{\Delta M/\Delta M}$ BMDMs, were differentiated into osteoclasts, and cell lysates collected for Zeb1, c-Src, and Ctsk immunoblotting (F) and quantified (G; *n* = 3).

H, I An empty control vector (EV)-transduced wild-type pre-osteoclasts, and EV- or ZEB1-transduced *Zeb1*$^{\Delta M/\Delta M}$ pre-osteoclasts were cultured atop bone slices for 3 days, stained with phalloidin (red). Osteoclasts were removed and resorption pits visualized by WGA-DAB staining (H). The actin ring area per cell and resorption pit area were quantified (I; *n* = 6).

Data information: Bars and error bars represent mean ± SEM. Data are representative of at least 3 independent experiments with biological replicates. Data were analyzed using unpaired Student's *t* test (B-D) or one-way ANOVA with Bonferroni correction (G, I). ns, not significant; ** $P < 0.01$.

Source data are available online for this figure.


A
WT RANKL d0
WT RANKL d2
WT RANKL d4
Zeb1ΔM/ΔM RANKL d0
Zeb1ΔM/ΔM RANKL d2
Zeb1ΔM/ΔM RANKL d4
Relative protein expression of mitochondrial fraction
MtCK1
Ckb
VDAC
Tomm20
B
BMDM
OC
Mitochondrial MtCK1 protein expression
WT
Zeb1ΔM/ΔM
C
Relative fluorescence intensity of MtCK1
D
Relative fluorescence intensity of osteoclastic MtCK1 in vivo
E
Epcam/Gapdh (fold change)
F
Isotype control
WT
0.032% ± 0.004%
Zeb1ΔM/ΔM
0.030% ± 0.005%
EpCAM-BV711
SSC-A
G
Isotype control
WT
Zeb1ΔM/ΔM
% of Max
DCFH-DA
H
H2O2 level (μM per mg protein)
I
Cybb/Gapdh (fold change)
Nox4/Gapdh (fold change)
Slc16a4/Gapdh (fold change)
Gpx4/Gapdh (fold change)

Appendix Figure S4. Zeb1 regulates osteoclastic MtCK1 expression while the intracellular ROS level and H_2O_2 production remain unaltered in *Zeb1*-deleted osteoclasts.

A Western blot quantification of MtCK1, Ckb, VDAC, and Tomm20 expression in BMDMs and osteoclasts generated from wild-type or *Zeb1*$^{\Delta M/\Delta M}$ mice as shown in Fig 4F (*n* = 3).

B Western blot quantification of MtCK1 expression in the mitochondrial fraction in BMDMs and osteoclasts generated from wild-type or *Zeb1*$^{\Delta M/\Delta M}$ mice as shown in Fig 4G (*n* = 3).

C Quantification of MtCK1 immunofluorescence intensity of wild-type and *Zeb1*$^{\Delta M/\Delta M}$ osteoclasts *in vitro* as shown in Fig 4H (*n* = 6).

D Quantification of MtCK1 immunofluorescence intensity of the TRAP$^+$ wild-type and *Zeb1*$^{\Delta M/\Delta M}$ osteoclasts of a femur section from wild-type and *Zeb1*$^{\Delta M/\Delta M}$ mice as shown in Fig EV3D (*n* = 6).

E Relative mRNA expression of *Epcam* in BMDMs and osteoclasts generated from wild-type or *Zeb1*$^{\Delta M/\Delta M}$ mice (*n* = 3).

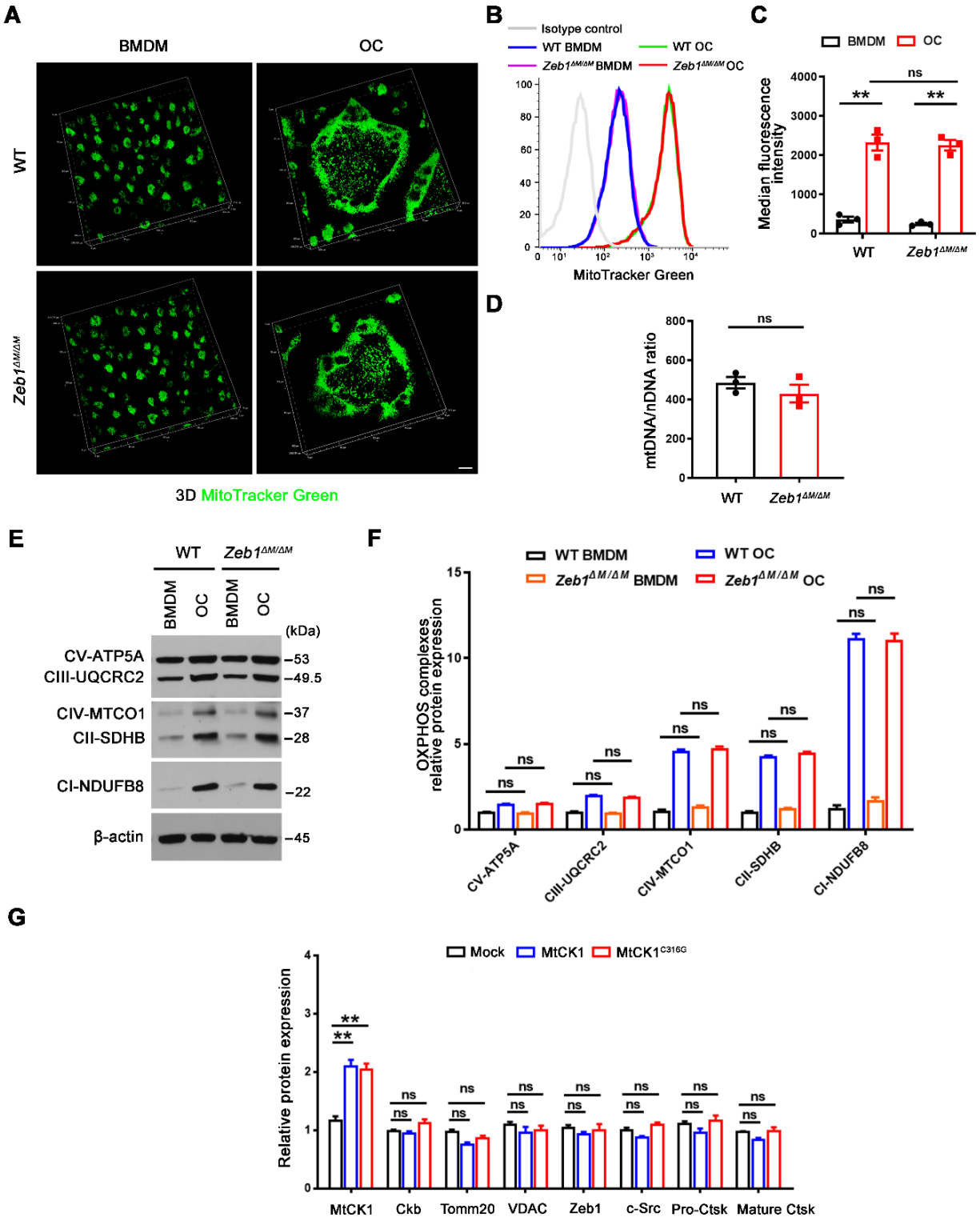
F Representative FACS plot analysis of EpCAM expression in osteoclasts generated from wild-type or *Zeb1*$^{\Delta M/\Delta M}$ mice with flow cytometry (*n* = 3).

G Measurements of intracellular ROS level by the DCFH-DA probe in osteoclasts generated from wild-type or *Zeb1*$^{\Delta M/\Delta M}$ mice with flow cytometry (*n* = 3).

H Measurements of H_2O_2 level in wild-type or *Zeb1*$^{\Delta M/\Delta M}$ osteoclasts *in vitro* (*n* = 3).

I Relative mRNA expression of *Cybb*, *Nox4*, *Slc16a4*, and *Gpx4* in BMDMs and osteoclasts generated from wild-type or *Zeb1*$^{\Delta M/\Delta M}$ mice (*n* = 3).

Data information: Bars and error bars represent mean ± SEM. Data are representative of at least 3 independent experiments with biological replicates. Data were analyzed using one-way ANOVA with Bonferroni correction (A), two-way ANOVA with Bonferroni correction (B, E, I), or unpaired Student's *t* test (C, D, H). ns, not significant; $^{**}P < 0.01$.


A
BMDM
OC
WT
Zeb1ΔM/ΔM
3D MitoTracker Green
B
Isotype control
WT BMDM
WT OC
Zeb1ΔM/ΔM BMDM
Zeb1ΔM/ΔM OC
MitoTracker Green
C
BMDM
OC
Median fluorescence intensity
ns
**
**
WT
Zeb1ΔM/ΔM
D
mtDNA/nDNA ratio
ns
WT
Zeb1ΔM/ΔM
E
WT
Zeb1ΔM/ΔM
BMDM
OC
BMDM
OC
(kDa)
CV-ATP5A
CIII-UQCRC2
CIV-MTCO1
CII-SDHB
CI-NDUFB8
β-actin
53
49.5
37
28
22
45
F
WT BMDM
WT OC
Zeb1ΔM/ΔM BMDM
Zeb1ΔM/ΔM OC
OXPHOS complexes relative protein expression
CV-ATP5A
CIII-UQCRC2
CIV-MTCO1
CII-SDHB
CI-NDUFB8
G
Mock
MtCK1
MtCK1C316G
Relative protein expression
MtCK1
Ckb
Tomm20
VDAC
Zeb1
c-Src
Pro-Ctsk
Mature Ctsk

Appendix Figure S5. *Zeb1*$^{\Delta M/\Delta M}$ osteoclasts display normal mitochondria abundance and mitochondrial complex expression.

A 3D reconstruction of MitoTracker Green immunofluorescence of wild-type or *Zeb1*$^{\Delta M/\Delta M}$ osteoclasts. Scale bar, 20 μm.

B, C Measurements of mitochondrial mass of BMDMs and osteoclasts generated from wild-type or *Zeb1*$^{\Delta M/\Delta M}$ mice using MitoTracker Green with flow cytometry (B) and quantified (C; $n = 3$).

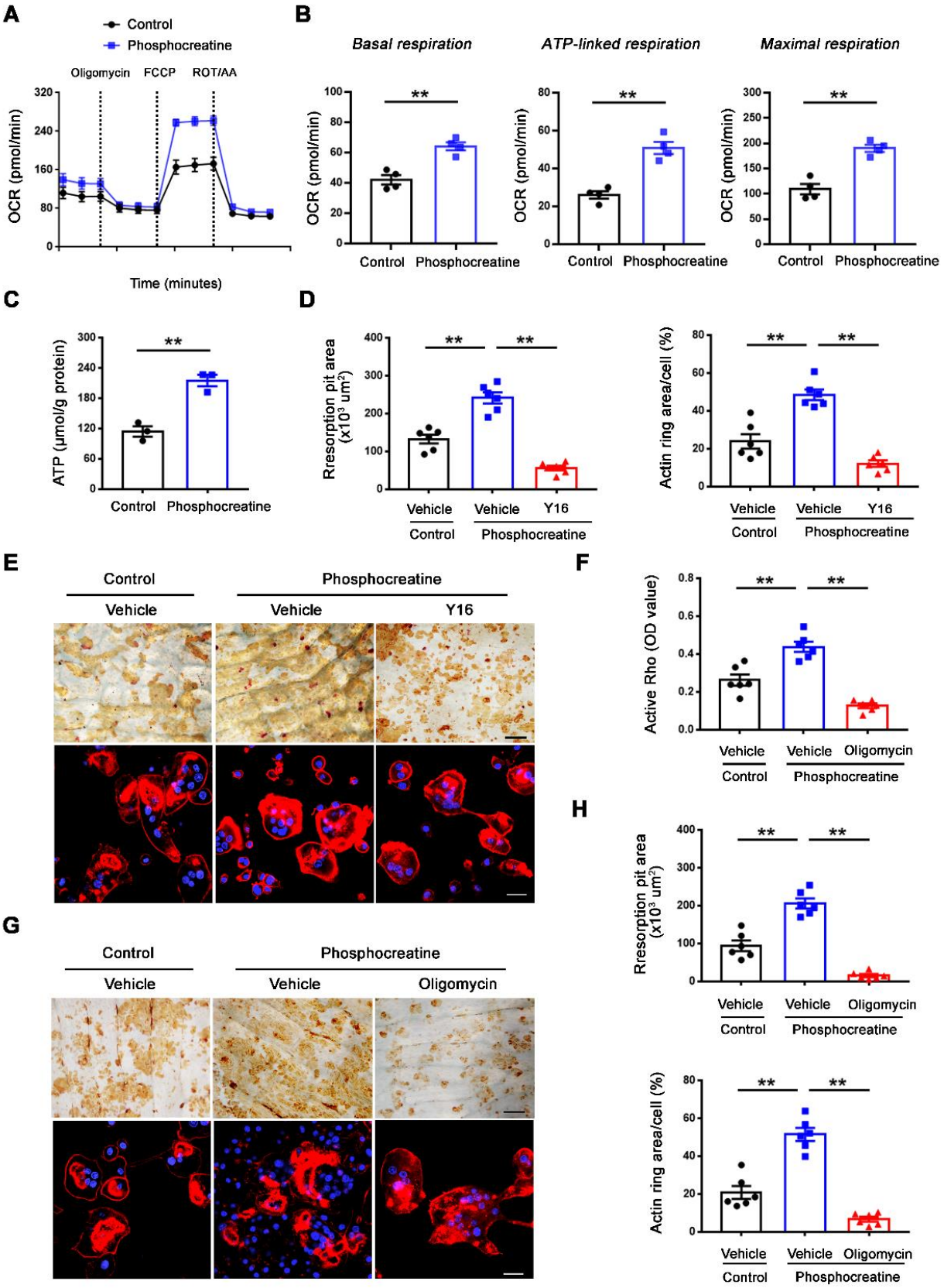
D Relative mtDNA copy number per nuclear genome in osteoclasts generated from wild-type or *Zeb1*$^{\Delta M/\Delta M}$ mice ($n = 3$).

E, F OXPHOS protein expression as assessed by Western blot (E) in osteoclasts generated from wild-type or *Zeb1*$^{\Delta M/\Delta M}$ mice and quantified (F; $n = 3$).

G Western blot quantification of MtCK1, Ckb, Tomm20, VDAC, Zeb1, c-Src, Pro-Ctsk, and mature Ctsk expression in osteoclasts differentiated from the mock vector, wild-type human MtCK1, or a catalytically-inactive MtCK1^{C316G} mutant expression vector-transduced wild-type BMDMs ($n = 3$).

Data information: Bars and error bars represent mean ± SEM. Data are representative of at least 3 independent experiments with biological replicates. Data were analyzed using two-way ANOVA with Bonferroni correction (C), unpaired Student's *t* test (D), or one-way ANOVA with Bonferroni correction (F, G). ns, not significant; $^{**}P < 0.01$.

Source data are available online for this figure.


A
Control
Phosphocreatine
Oligomycin
FCCP
ROT/AA
OCR (pmol/min)
Time (minutes)
B
Basal respiration
ATP-linked respiration
Maximal respiration
OCR (pmol/min)
Control
Phosphocreatine
C
ATP (μmol/g protein)
Control
Phosphocreatine
D
Rresorption pit area (x10³ um²)
Actin ring area/cell (%)
Vehicle
Y16
Control
Phosphocreatine
E
Control
Phosphocreatine
Vehicle
Vehicle
Y16
F
Active Rho (OD value)
Vehicle
Oligomycin
G
Control
Phosphocreatine
Vehicle
Vehicle
Oligomycin
H
Rresorption pit area (x10³ um²)
Actin ring area/cell (%)

Appendix Figure S6. Phosphocreatine potentiates mitochondrial bioenergetics and osteoclast activation *in vitro*.

A, B Oxygen consumption rate (OCR) profile plot (A) and mitochondrial function parameters (B) analyzed by XF Cell Mito Stress Assay in phosphocreatine-treated wild-type osteoclasts after sequential treatment of compounds modulating mitochondrial function (n = 4).

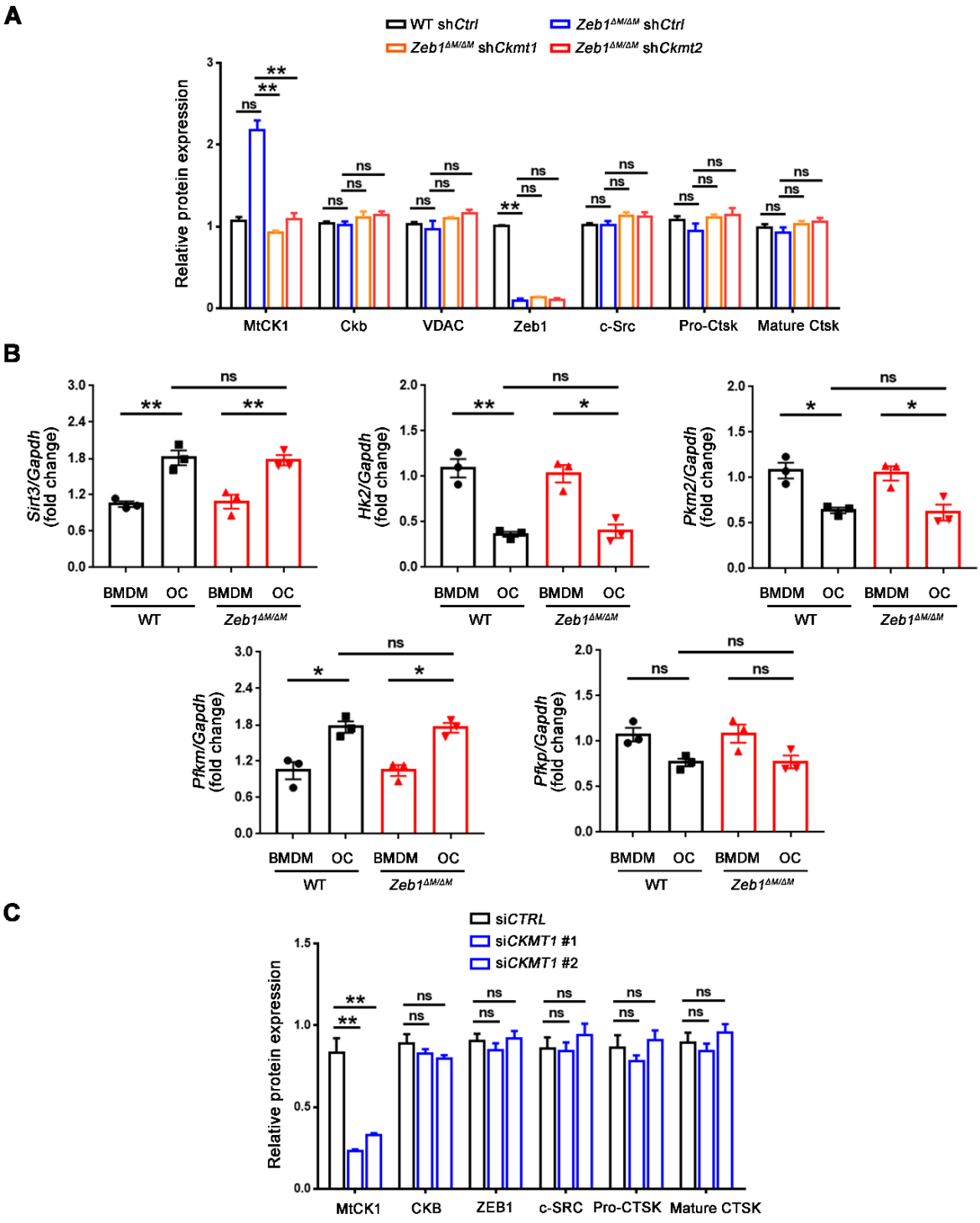
C ATP levels of phosphocreatine-treated wild-type osteoclasts (n = 3).

D, E Resorption pits visualized with WGA-DAB staining and phalloidin staining (E) in phosphocreatine-treated wild-type osteoclasts in the presence or absence of 50 μM Y16 cultured on bone slices with the resorption pit area and actin ring area per cell quantified (D; n = 6).

F RhoA activity of phosphocreatine-treated wild-type osteoclasts in the presence or absence of 10 nM oligomycin (n = 6).

G, H Resorption pits visualized with WGA-DAB staining and phalloidin staining (G) in phosphocreatine-treated wild-type osteoclasts in the presence or absence of 10 nM oligomycin cultured on bone slices with the resorption pit area and actin ring area per cell quantified (H; n = 6).

Data information: Bars and error bars represent mean ± SEM. Data are representative of at least 3 independent experiments with biological replicates. Data were analyzed using unpaired Student's t test (B, C) or one-way ANOVA with Bonferroni correction (D, F, H). $^{**}P < 0.01$.


A
WT shCtrl
Zeb1ΔM/ΔM shCtrl
Zeb1ΔM/ΔM shCkmt1
Zeb1ΔM/ΔM shCkmt2
Relative protein expression
MtCK1
Ckb
VDAC
Zeb1
c-Src
Pro-Ctsk
Mature Ctsk
B
Sirt3/Gapdh (fold change)
Hk2/Gapdh (fold change)
Pkm2/Gapdh (fold change)
Pfkm/Gapdh (fold change)
Pfkp/Gapdh (fold change)
BMDM
OC
WT
Zeb1ΔM/ΔM
C
siCTRL
siCKMT1 #1
siCKMT1 #2
Relative protein expression
MtCK1
CKB
ZEB1
c-SRC
Pro-CTSK
Mature CTSK

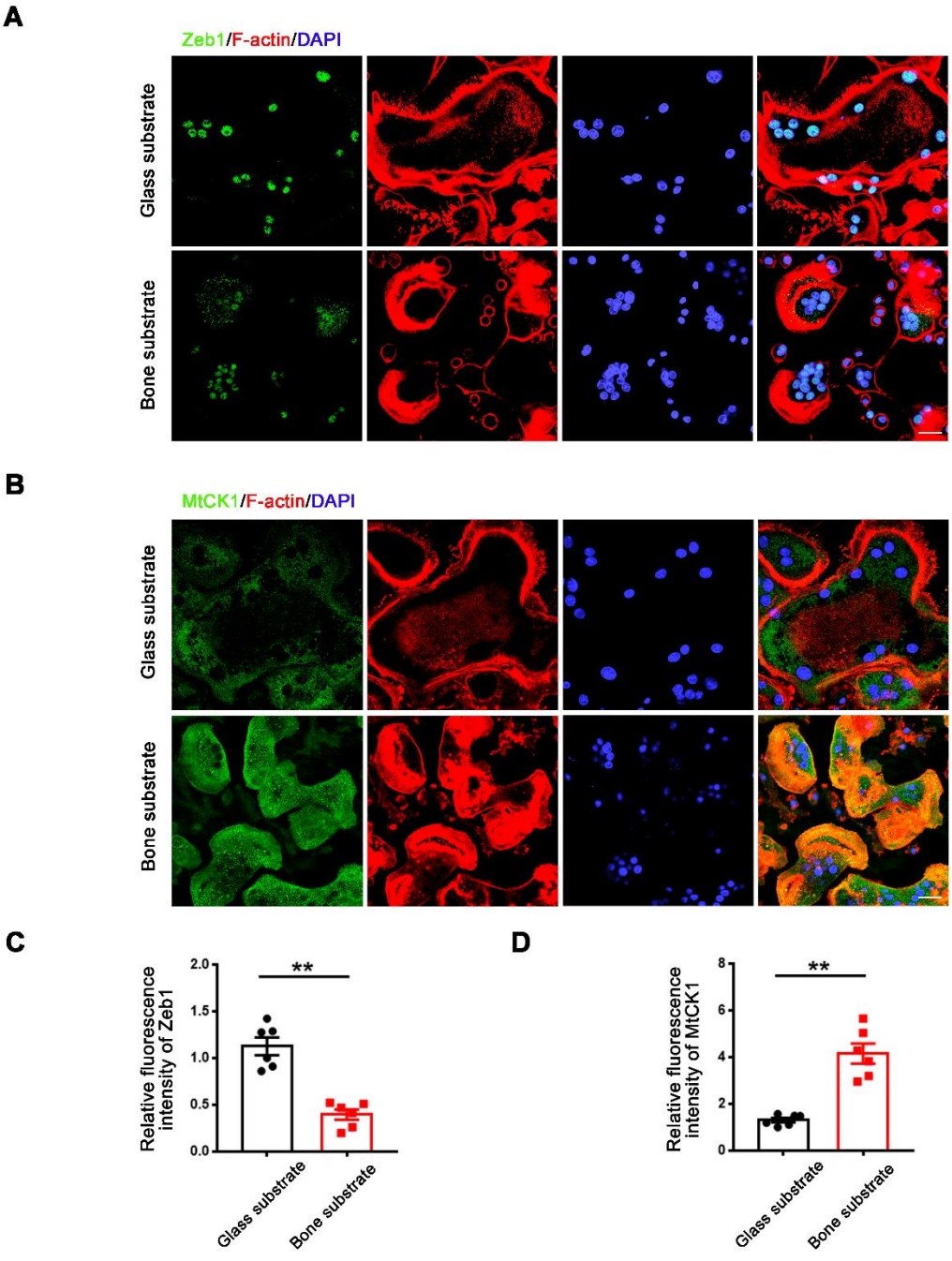
Appendix Figure S7. The alternative involvement of other key cellular metabolism-related genes is excluded during Zeb1-regulated osteoclast activity.

A Western blot quantification of MtCK1, Ckb, VDAC, Zeb1, c-Src, pro-Ctsk, and mature Ctsk expression in osteoclasts differentiated from sh*Ctrl*-transduced wild-type BMDMs, and sh*Ctrl*- or sh*Ckmt1*-transduced *Zeb1*$^{\Delta M/\Delta M}$ BMDMs as shown in Fig EV4E (*n* = 3).

B Relative mRNA expression of *Sirt3*, *Hk2*, *Pkm2*, *Pfkm*, and *Pfkp* in BMDMs and osteoclasts generated from wild-type or *Zeb1*$^{\Delta M/\Delta M}$ mice (*n* = 3).

C Western blot quantification of MtCK1, CKB, ZEB1, c-SRC, pro-CTSK, and mature CTSK expression in human osteoclasts differentiated from si*CTRL*- or si*CKMT1*-transfected hMDMs as shown in Fig EV5A (*n* = 3).

Data information: Bars and error bars represent mean ± SEM. Data are representative of at least 3 independent experiments with biological replicates. Data were analyzed using one-way ANOVA with Bonferroni correction (A, C) or two-way ANOVA with Bonferroni correction (B). ns, not significant; **P* < 0.05, ***P* < 0.01.


A
Zeb1/F-actin/DAPI
Glass substrate
Bone substrate
B
MtCK1/F-actin/DAPI
Glass substrate
Bone substrate
C
Relative fluorescence intensity of Zeb1
2.0
1.5
1.0
0.5
0.0
**
Glass substrate
Bone substrate
D
Relative fluorescence intensity of MtCK1
8
6
4
2
0
**
Glass substrate
Bone substrate

Appendix Figure S8. Differentially regulated Zeb1 and MtCK1 level in osteoclasts when cultured on bone substrate.

A Zeb1 (green) and F-actin (red) immunofluorescence of wild-type osteoclasts cultured on glass and bone substrate. Scale bar, 20 μm.

B MtCK1 (green) and F-actin (red) immunofluorescence of wild-type osteoclasts cultured on glass and bone substrate. Scale bar, 20 μm.

C Quantification of Zeb1 immunofluorescence intensity of wild-type osteoclasts cultured on glass and bone substrate as shown in Appendix Fig S8A (n = 6).

D Quantification of MtCK1 immunofluorescence intensity of wild-type osteoclasts cultured on glass and bone substrate as shown in Appendix Fig S8B (n = 6).

Data information: Bars and error bars represent mean ± SEM. Data are representative of at least 3 independent experiments with biological replicates. Data were analyzed using unpaired Student's t test (C, D). $^{**}P < 0.01$.

Appendix Table S1. Genotyping PCR primers.

Allele	Sequence (5' to 3')
Zeb1^{flox}_fwd	CGTGATGGAGCCAGAATCTGACCCC
Zeb1^{flox}_rev	GCCCTGTCTTTCTCAGCAGTGTGG
Zeb1^{del}_rev	GCCATCTCACCAGCCCTTACTGTGC
Csf1r-Cre_ fwd	ACAACTACCTGTTCTGCCG
Csf1r-Cre_ rev	GCCTCAAAGATCCCTTCCAG

Appendix Table S2. Quantitative real-time PCR primers.

Gene	Forward (5’ to 3’)	Reverse (5’ to 3’)
Mouse Zeb1	GCTGGCAAGACAACGTGAAAG	GCCTCAGGATAAATGACGGC
Mouse Zeb2	ATTGCACATCAGACTTTGAGGAA	ATAATGGCCGTGTCGCTTCG
Mouse Acp5	CACTCCCACCCTGAGATTTGT	CCCCAGAGACATGATGAAGTCA
Mouse Nfatc1	GACCCGGAGTTCGACTTCG	TGACACTAGGGGACACATAACTG
Mouse c-fos	CGGGTTTCAACGCCGACTA	TTGGCACTAGAGACGGACAGA
Mouse Dcstamp	GGGGACTTATGTGTTTCCACG	ACAAAGCAACAGACTCCCAAAT
Mouse Oscar	CCTAGCCTCATACCCCCAG	CGTTGATCCCAGGAGTCACAA
Mouse Itgb3	CCACACGAGGCGTGAACTC	CTTCAGGTTACATCGGGGTGA
Mouse Src	GAACCCGAGAGGGACCTTC	GAGGCAGTAGGCACCTTTTGT
Mouse Atp6v0d2	CAGAGCTGTACTTCAATGTGGAC	AGGTCTCACACTGCACTAGGT
Mouse Ctsk	AGGTCGGTGTGAACGGATTTG	TGTAGACCATGTAGTTGAGGTCA
Mouse Slc6a8	GCAGGGTGTGCATATCTCCAA	TACCCCCACTCACATCAGTCA
Mouse Slc6a12	TGCTTCCTTGTTACCATCTGC	CATGCTGTTTGGGAGTAATCCT
Mouse Gatm	GCTTCCTCCCGAAATTCCTGT	CCTCTAAAGGGTCCCATTCGT
Mouse Gamt	CACGCACCTGCAAATCCTG	TACCGAAGCCCACTTCCAAGA
Mouse Ckmt1	TGTCTTCAAGAGTCAGAACTGGC	AGCATCCACCACAACACGTT
Mouse MtCK2	ACACCCAGTGGCTATACCCTG	CCGTAGGATGCTTCATCACCC
Mouse Ckb	AGTTCCCTGATCTGAGCAGC	GAATGGCGTCGTCCAAAGTAA
Mouse Ckm	CTGACCCCTGACCTCTACAAT	CATGGCGGTCCTGGATGAT
Mouse Gapdh	AGGTCGGTGTGAACGGATTTG	AGGTCGGTGTGAACGGATTTG
Human ZEB1	CAGCTTGATACCTGTGAATGGG	TATCTGTGGTCGTGTGGGACT
Human CKMT1	TGAGGAGACCTATGAGGTATTTGC	CTCATCAAAGTAGCCAGAACGG
Human CTSK	ACACCCACTGGGAGCTATG	GACAGGGGTACTTTGAGTCCA
Human	GGAGCGAGATCCCTCCAAAAT	GGCTGTTGTCATACTTCTCATGG

GAPDH		
Mouse Epcam	GCGGCTCAGAGAGACTGTG	CCAAGCATTTAGACGCCAGTTT
Mouse Cybb	AGTGCGTGTTGCTCGACAA	GCGGTGTGCAGTGCTATCAT
Mouse Nox4	GAAGGGGTTAAACACCTCTGC	ATGCTCTGCTTAAACACAATCCT
Mouse Slc16a4	AAAACGCCCTCCCCTTACAC	CCAATTTGCTCTGAAGTGCCT
Mouse Gpx4	GATGGAGCCCATTCCTGAACC	CCCTGTACTTATCCAGGCAGA
Mouse cytochrome c oxidase I	GCCCCAGATATAGCATTCCC	GTTCATCCTGTTCCTGCTCC
18s ribosomal RNA	TAGAGGGACAAGTGGCGTTC	CGCTGAGCCAGTCAGTGT
Mouse Ppargc1b	CCCTGTACTTATCCAGGCAGA	GAAAGCTCGTCCACGTCAGAC
Mouse Sirt3	ATCCCGGACTTCAGATCCCC	CAACATGAAAAAGGGCTTGGG
Mouse Hk2	TGATCGCCTGCTTATTCACGG	AACCGCCTAGAAATCTCCAGA
Mouse Pkm2	GCCGCCTGGACATTGACTC	CCATGAGAGAAATTCAGCCGAG
Mouse Pfkm	TGTGGTCCGAGTTGGTATCTT	GCACTTCCAATCACTGTGCC
Mouse Pfkp	GAAACATGAGGCGTTCTGTGT	CCCGGCACATTGTTGGAGA