

**Supplementary Figure 1. Additional analyses of the relationship between mTORC1 signaling and osteoclast differentiation.**

(a) Representative images of TRAP-stained bone marrow osteoclast differentiation cultures.

(b) Expression of osteoclast markers in bone marrow osteoclast differentiation cultures (n=5).

(c) Serum CTX-1 bone resorption marker (n=7).

(d) Serum P1NP bone formation marker (n=7).

(e) Representative  $\mu$ CT images of the trabecular bone of the tibial metaphysis (top) and the entire proximal tibia (bottom).

(f-i) Quantification of trabecular bone volume and architecture in proximal tibiae by  $\mu$ CT (n=7). (f) BV/TV, bone volume/tissue volume ratio; (g) BS, bone surface; (h) Tb.Th, trabecular thickness; (i) Tb.N, trabecular number; (j) Tb.Sp, trabecular separation; (k) BS/BV, bone surface/bone volume ratio; (L) SMI, structure model index.

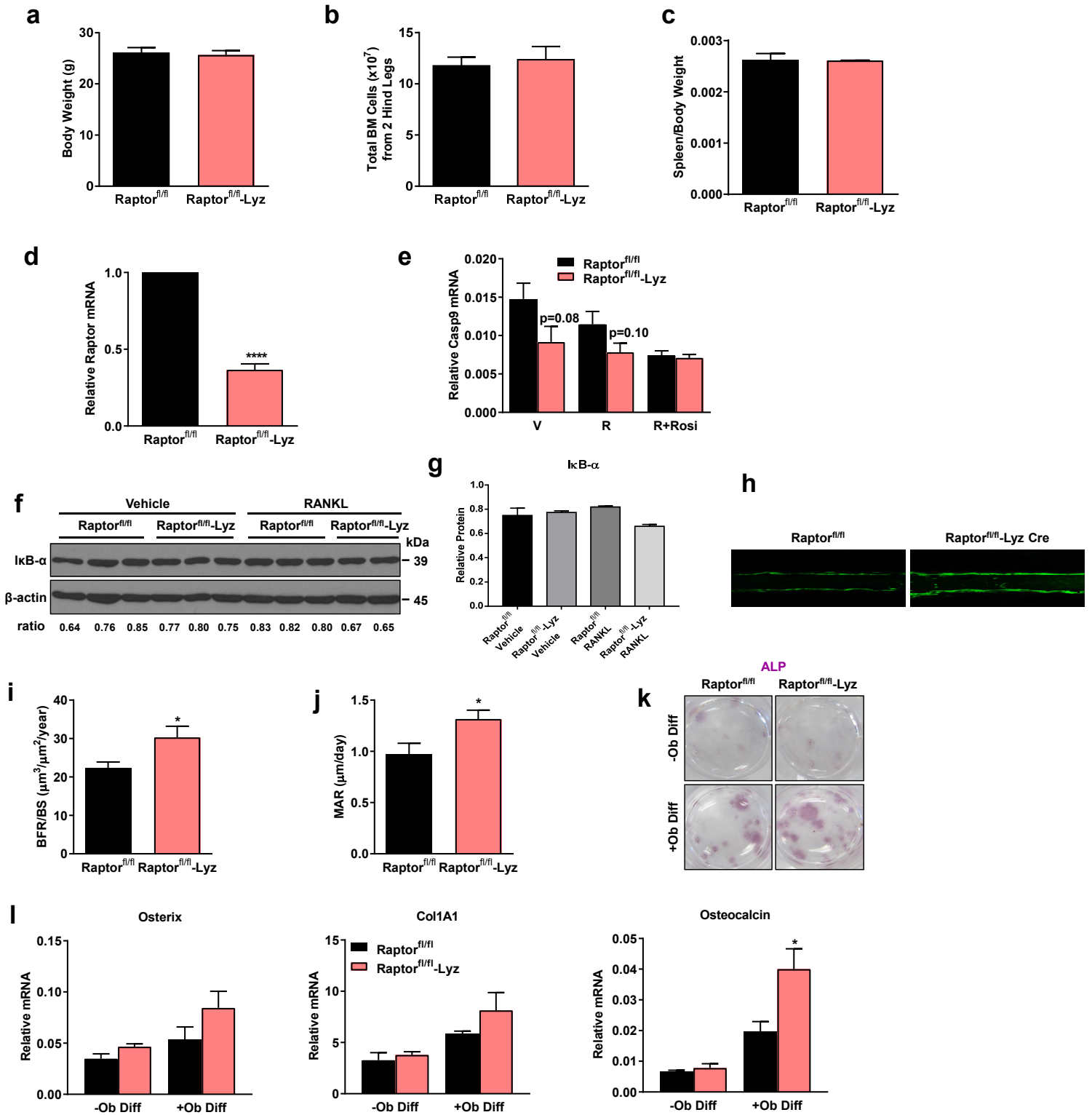
(m-n) Expression of TRAP on day 6 of bone marrow osteoclast differentiation cultures treated with rapamycin at indicated dose and time (n=9-11).

(o-n) NFATc1 mobility shifts in bone marrow osteoclast differentiation cultures from VLDLR<sup>-/-</sup> mice or WT littermate controls 24 hr after RANKL treatment with or without rosiglitazone.

(p) mTORC1 signaling in bone marrow osteoclast differentiation cultures from VLDLR<sup>-/-</sup> mice or WT littermate controls 24 hr after RANKL treatment with or without rosiglitazone, measured by S6K1 phosphorylation.

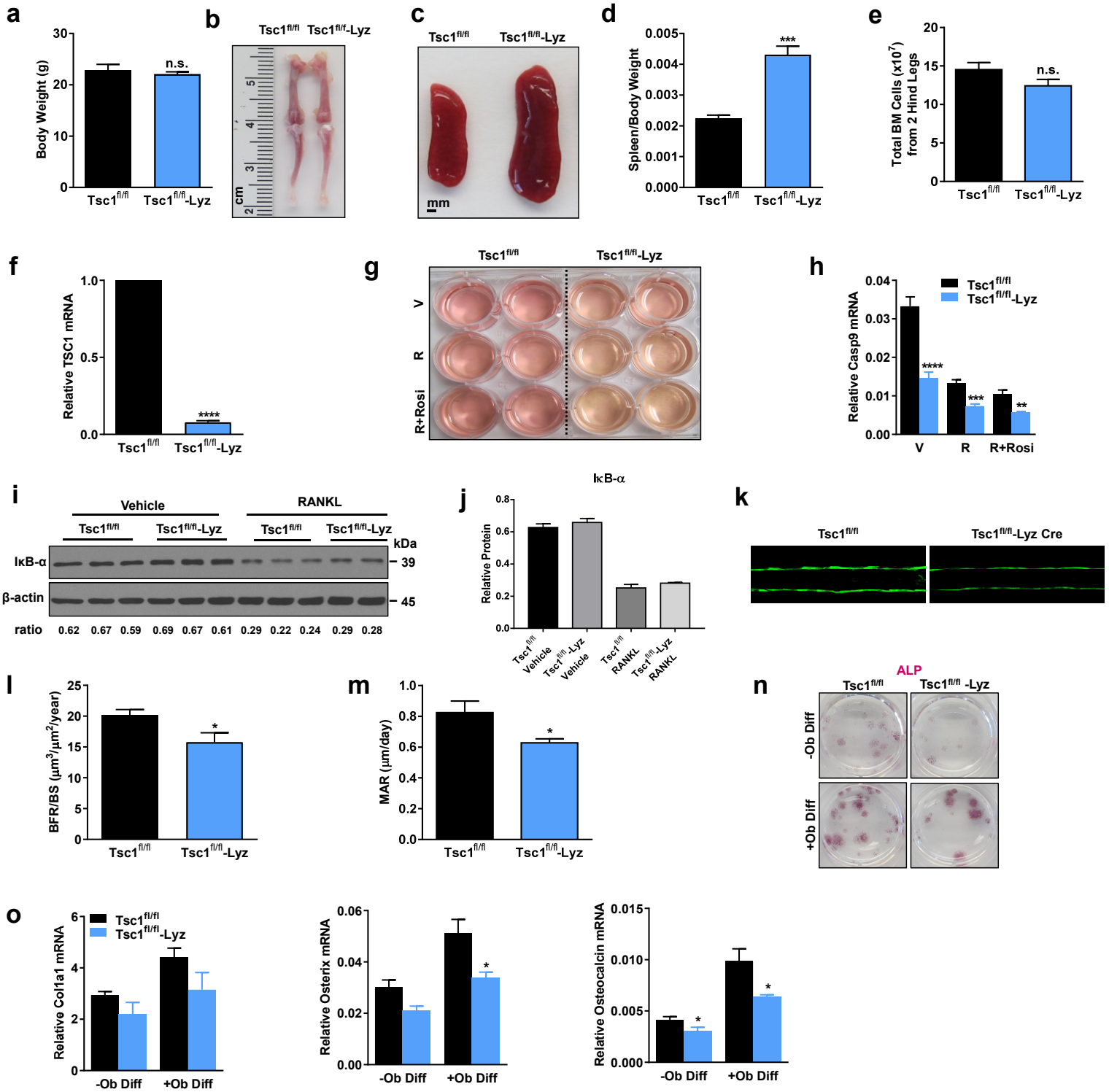
(q) mTORC1 signaling in bone marrow osteoclast differentiation cultures from VLDLR<sup>-/-</sup> mice or WT littermate controls 72 hr after RANKL treatment with or without rosiglitazone, measured by S6K1 phosphorylation.

Error bars, SEM; \*, p<0.05; \*\*, p<0.01; \*\*\*, p<0.005; \*\*\*\*, p<0.001; n.s. non-significant.



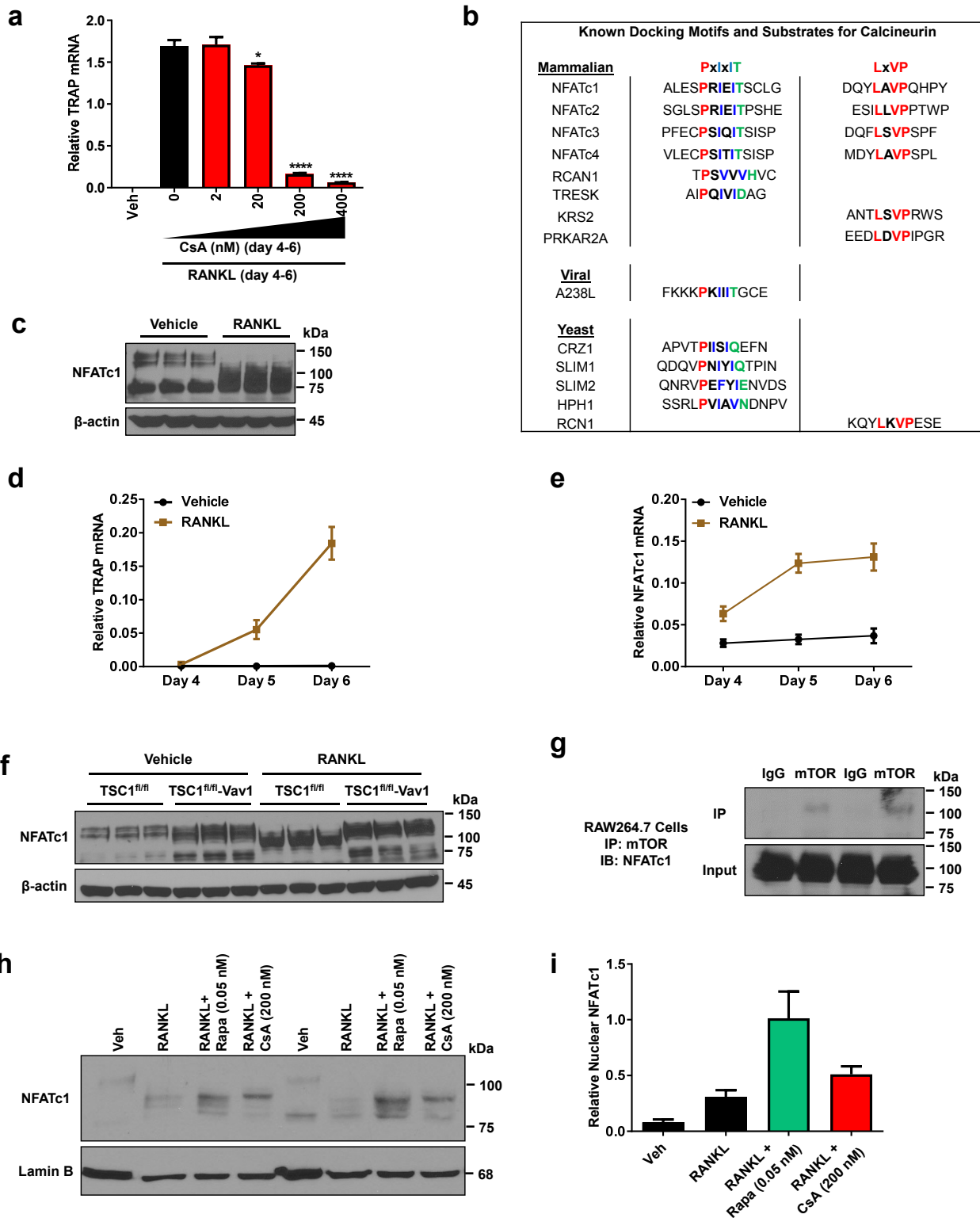
**Supplementary Figure 2. Additional analyses of Raptor<sup>fl/fl</sup>-Lyz cKO mice.**

(a) Pup body weight at 3 months old (n=9-12).  
 (b) Total bone marrow cells in 3 months old pups (n=6).  
 (c) Quantification of spleen/body weight ratio at 6 weeks of age (n=5).  
 (d) Raptor expression in bone marrow osteoclast differentiation cultures (n=6).  
 (e) Caspase 9 expression in bone marrow osteoclast differentiation cultures (n=6).  
 (f-g) NF-κB activity measured by western blot of IκB-α at the same time point as Fig. 3f-g (50 hrs after RANKL treatment). Ratios of IκB-α/β-actin are shown for individual samples (f) and averages (g).  
 (h-j) Dynamic histomorphometry by double calcein labeling (n=6).  
 (h) Representative images.  
 (i) Bone formation rate.  
 (j) Mineral apposition rate.  
 (k) Representative images of alkaline phosphatase staining of bone marrow osteoblast differentiation cultures.  
 (l) Expression of osteoblast differentiation markers (n=6).  
 Error bars, SEM; \*, p<0.05; \*\*, p<0.01; \*\*\*, p<0.005; \*\*\*\*, p<0.001; n.s. non-significant.



**Supplementary Figure 3. Additional analyses of Tsc1<sup>fl/fl</sup>-Lyz cKO mice.**

- (a) Pup body weight at 2 months old (n=5).  
 (b) Representative image of bone length at 2 months old pups.  
 (c) Representative image of spleen at 2 months old.  
 (d) Quantification of spleen/body weight ratio (n=5).  
 (e) Total bone marrow cells in 2 months old pups (n=5).  
 (f) Tsc1 expression in bone marrow osteoclast differentiation cultures (n=6).  
 (g) Representative image of media color changes in Tsc1<sup>fl/fl</sup>-Lyz cKO bone marrow cultures.  
 (h) Caspase 9 expression in bone marrow osteoclast differentiation cultures (n=6).  
 (i-j) NF-κB activity measured by western blot of IκB-α at the same time point as Fig. 5f-g (60 hrs after RANKL treatment). Ratios of IκB-α/β-actin are shown for individual samples (i) and averages (j).  
 (k-m) Dynamic histomorphometry by double calcein labeling (n=6).  
 (k) Representative images.  
 (l) Bone formation rate.  
 (m) Mineral apposition rate.  
 (n) Representative image of alkaline phosphatase staining of bone marrow osteoblast differentiation cultures.  
 (o) Expression of osteoblast differentiation markers (n=6).  
 Error bars, SEM; \*, p<0.05; \*\*, p<0.01; \*\*\*, p<0.005; \*\*\*\*, p<0.001; n.s. non-significant.



**Supplementary Figure 4. Additional analyses of the calcineurin  $\rightarrow$  mTORC1  $\rightarrow$  NFATc1 pathway in osteoclastogenesis.**

- (a) Expression of TRAP on day 6 in bone marrow osteoclast differentiation cultures treated with CsA at indicated dose and time (n=6).
- (b) PxxIT and LxVP docking motifs in known substrates for calcineurin.
- (c) Western blot showing NFATc1 mobility shift in bone marrow osteoclast differentiation cultures 3 days after RANKL treatment.
- (d-e) Expression of osteoclast markers (d) and osteoclastogenic transcription factors (e) in a time course of bone marrow osteoclast differentiation (n=9).
- (f) Western blot showing NFATc1 mobility shift in bone marrow osteoclast differentiation cultures from *Tsc1<sup>fl/fl</sup>-Vav1-iCre* cKO mice 60 hr after RANKL treatment.
- (g) Western blot of NFATc1 from mTOR immunoprecipitates in RAW264.7 macrophages.
- (h) Western blot of nuclear protein lysates from bone marrow osteoclast differentiation cultures 48 hr after RANKL treatment with or without rapamycin or CsA.
- (i) Quantification of nuclear NFATc1/lamin B ratio (n=2).

**a** Examples of Known mTOR Phosphorylated Motifs (Hsu et al, Science 2011)

Protein	Site	-	5	4	3	2	1	⊖	1	2	3	4	+
4E-BP1	T37		G	D	Y	S	T		T	P	G	G	T
	T46		T	L	F	D	T		P	G	G	T	
	S65		M	E	C	R	N		S	P	V	T	K
	T70		S	P	V	T	K		T	P	P	R	D
S6K1	T389		V		L	G	F		T	Y	V	A	P
	AKT1	S473		H		P	Q		S	Y	S	A	S
	SGK1	S422		A		L	G		F	Y	A	P	P

Proline-directed sites  
Hydrophobic motifs

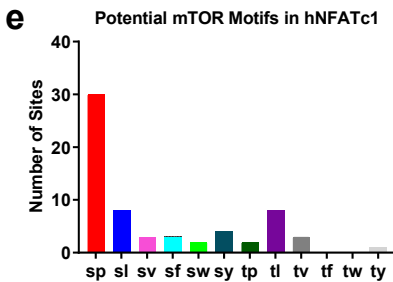
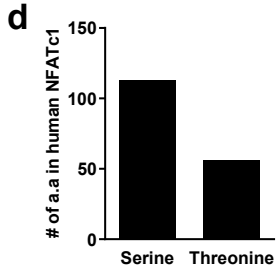
**b** Phosphopeptide detected by MS in HEK-293E (Hsu et al, Science 2011)

Protein	Protein ID	Peptide
NFATC1	NP_006153	GLGACTLLGSPR

**c** Human NFATC1

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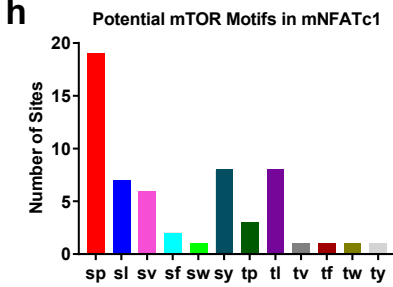
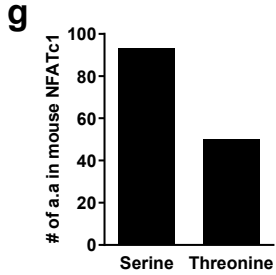
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541 rnsdielrkgetdigrkntvrllvfrvhvppqngfsvqvasnpiecsqrsaqelplve
601 kqstdcpviggkkmvlsgnflqdksvifvekpadghhvemeaktddrdlckpnsveve
661 ippfrnqrirtsqavsfyvcngkrkrsyqrflylpangnsvflsseselelrggfy
    
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**f** Mouse NFATC1

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1 mpntsfpvpskflgppaaavcgsgegpapppsggtmkaeeehygsststslplpt
61 ahsalpaachdlqtsfgisavpsanhppvvgavdsgpsgyflssgntprngapleesc
121 rieitvlgllhggqffhdvevedvlpssckrstahlpveayrdpsclspasfss
181 srscnseasfvesnyfpyeagqtswgscvckttddpeegfprlgachllgchxst
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601 kqstdcpviggkkmvlsgnflqdksvifvekpadghhvemeaktddrdlckpnsveve
661 ippfrnqrirtsqavsfyvcngkrkrsyqrflylpangnsvflsseselelrggfy
    
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**i** NFATC1

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Human 1 MPSTSPVPSKFLGPPAAAVFGRGEGPAPRAGGTMKSAAEEHYGYASS
Mouse 1 MPNTSPVPSKFLGPPAAVCGSGEGRPAPPSSGTTMKAEEHYGYVSP

Human 51 NVSPALPLPTAHSTLPAACHDLQTSGLI--IPPADHPSPGYGAALDGGPA
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Human 99 GYFLSSGHTRPDGAPELSEIRIETSCGLYHNNQFFHDVEVEDVLPSS
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Human 149 KRSTSTALPQLPTEAYRDFSCLEASLSSRSCNSEASSESNYSYPYA
Mouse 151 KRSTSTALPQLPTEAYRDFSCLEASLSSRSCNSEASSESNYSYPYA

Human 199 RSTQTSWQSCVCKTTDPEEGFPRGLGACTLLGSRHSESTSRASVTE
Mouse 201 RSTQTSWQSCVCKTTDPEEGFPRSLGACHLLGSRHSESTSRASITE

Human 249 ELGARSRRPACNKRKNGRQPPYSHHSGSRVSVTDD
Mouse 251 ELGARSRRPACNKRKNGRQPPYSHHSGSRVSVTDD

Human 299 SWLGNTTQYTSSAIVAAINALTTDSSLDLGDGVPVKSRTTLEQPPVAL
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Human 349 KVEPVGDELGSPPPADFAPEDYSSFQHIRKGGFCQYLAVPQHPYQWAK
Mouse 351 KVEPAGEDLGTTPPTSDFPPEEYTFQHLRKGAFCEQYLSVQASQWAK

Human 399 PKPLSTSYMSTLPAALDQWLPSSHSGPYELRIEVQPKSHHRAHYETEGSR
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Human 449 GAVKASAGGHPVQLHGYLENEPLMLQLFIGTADDRLLRPHAFYQVHRIT
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Human 499 GKWTSTTSHEAILSNTKVLLEIPLPENMRAIDCAGILKLNDSIELRK
Mouse 500 GKWTSTTSHEAILSNTKVLLEIPLPENMRAIDCAGILKLNDSIELRK

Human 549 GETDIGRKNTRVRLVFRVHVPPQSGRFSVQVASNPIECQRSAQELPLV
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Human 599 EKQSTDSPVVGKMMVLSGNFLQDKSVIFVEKAPDGHVWEMEAKTDR
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Human 649 DLCKPNSVVEIPFRNQRITVHVSFVYVCNGKRKRSYQRFVLYLPANV
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Mouse 699 -----GNS-----

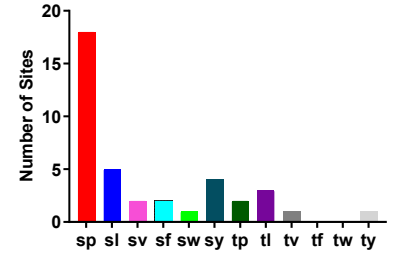
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Mouse 702 -----

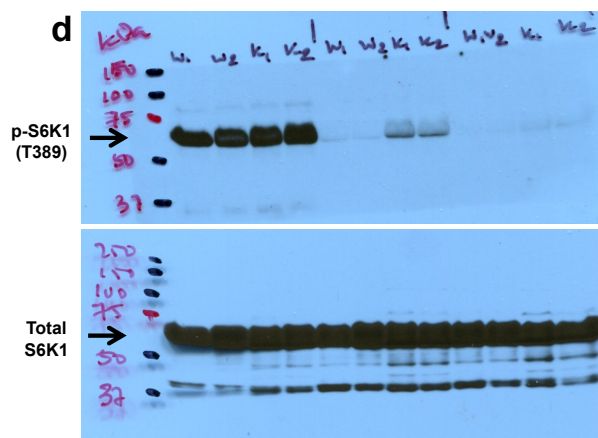
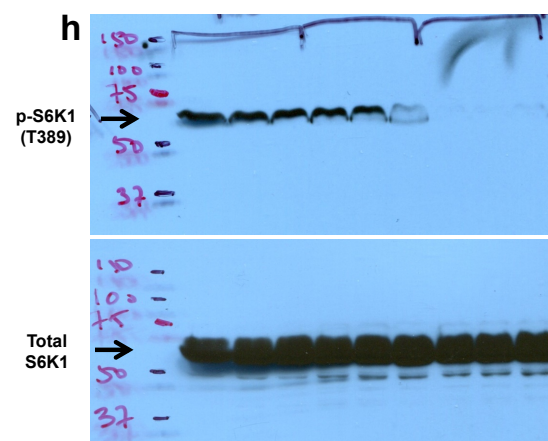
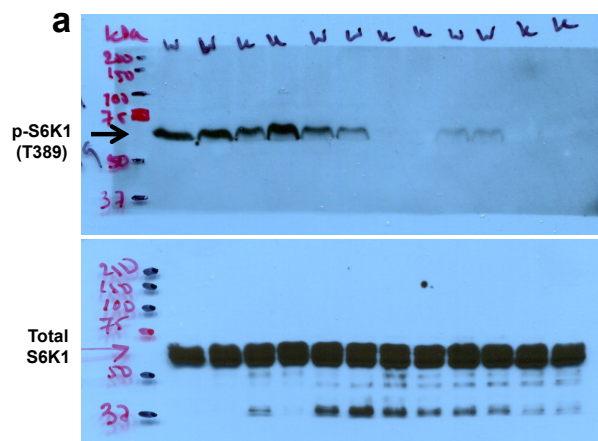
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Mouse 702 -----

Human 899 HEDGSPNLAPIVTVKREPEELDQLYLDVNEIIRNDSLSTSTHS
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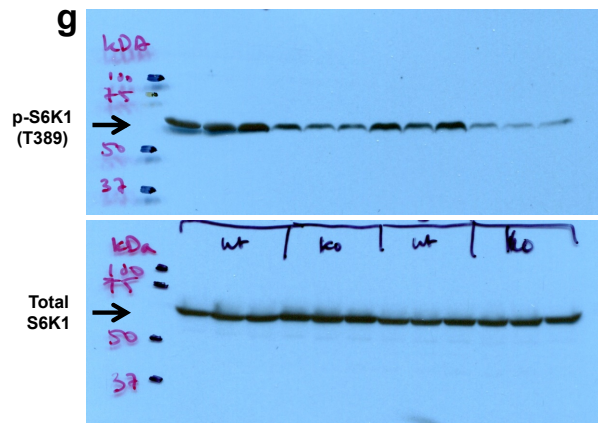
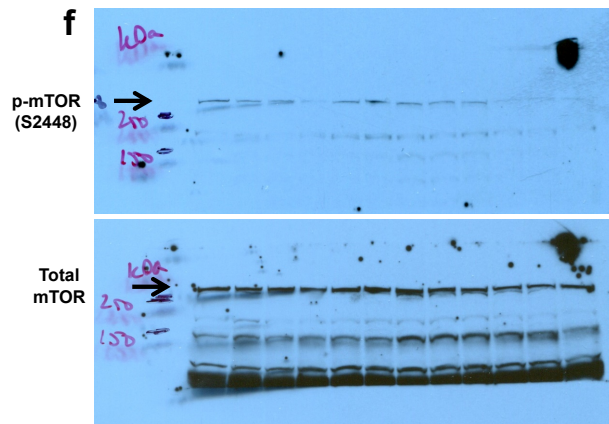
**j** Potential mTOR Motifs in NFATC1 Share by Human and Mouse





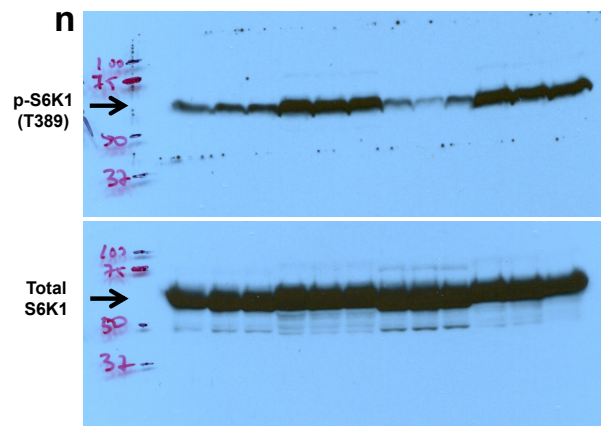
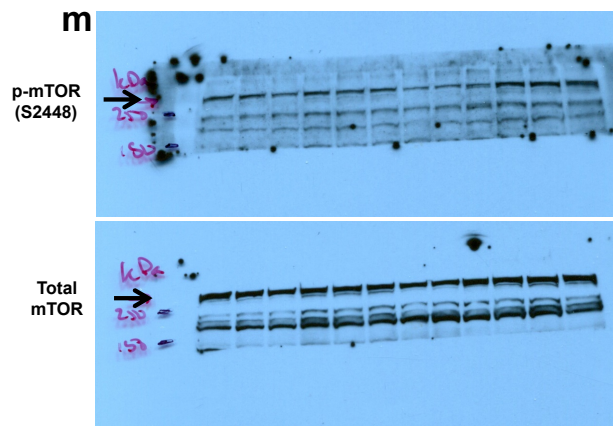


Supplementary Figure 6. Full size scans of immunoblots in Figure 1. Panel labels correspond to panels in Figure 1.

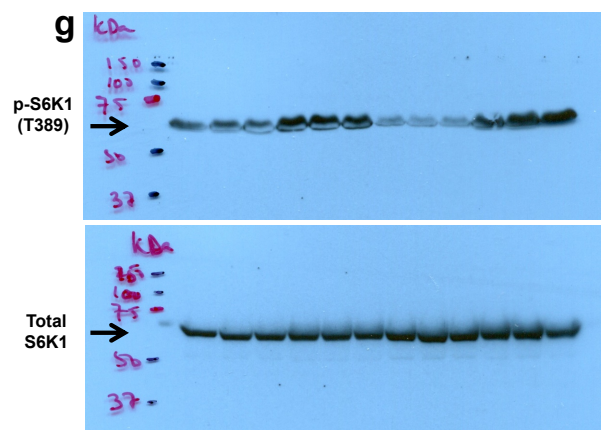
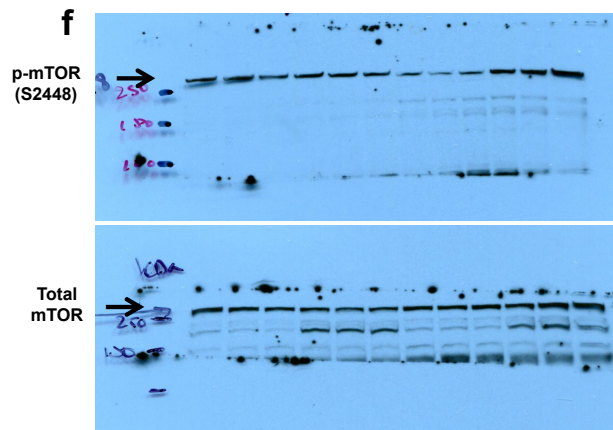


Supplementary Figure 7. Full size scans of immunoblots in Figure 3.  
 Panel labels correspond to panels in Figure 3.

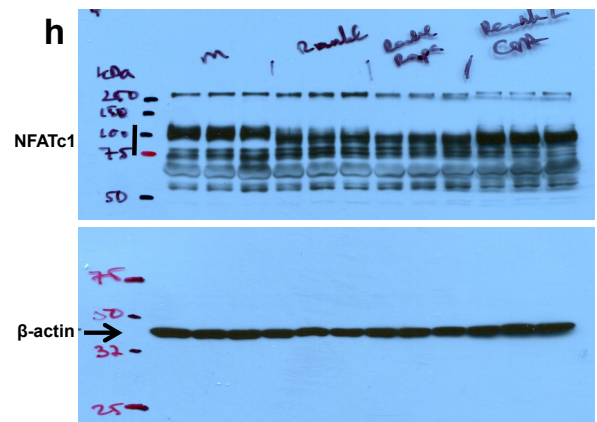
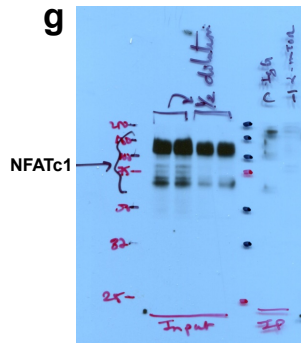
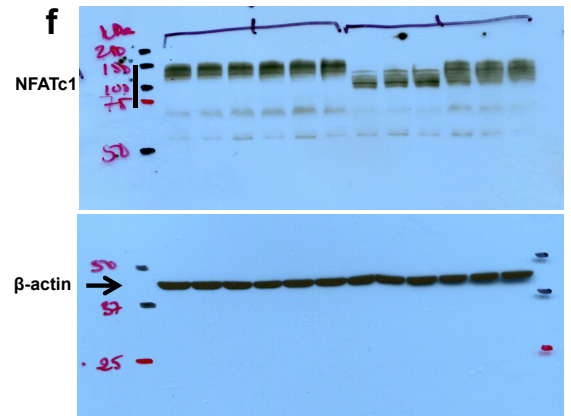
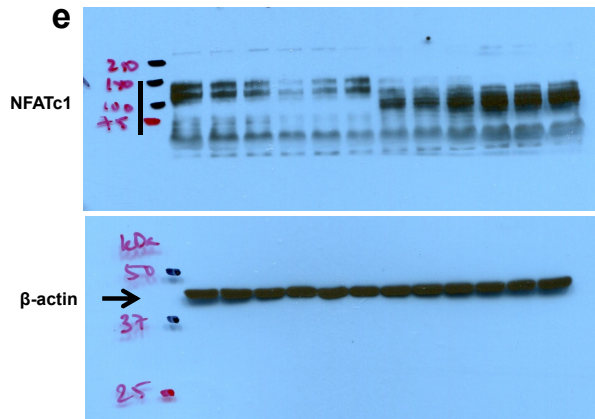
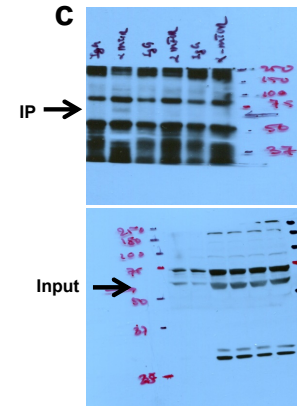
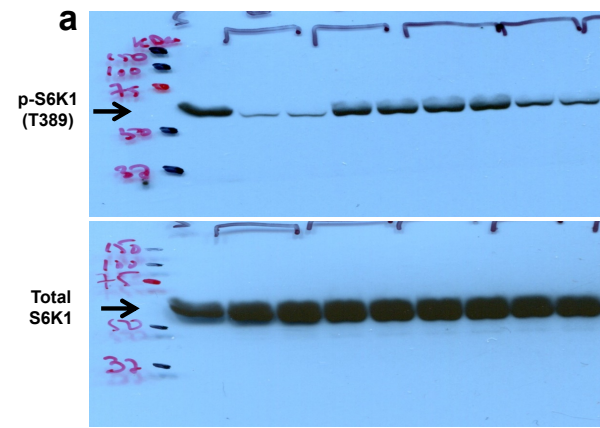




**Supplementary Figure 8. Full size scans of immunoblots in Figure 4.**  
Panel labels correspond to panels in Figure 4.



**Supplementary Figure 9. Full size scans of immunoblots in Figure 5.**  
Panel labels correspond to panels in Figure 5.



Supplementary Figure 10. Full size scans of immunoblots in Figure 6. Panel labels correspond to panels in Figure 6.

**Supplementary Table 1. Bone Marrow Cellularity of Raptor<sup>fl/fl</sup>;Vav1-iCre Mice (n=2)**

<u>Surface Marker</u>	<u>Raptor<sup>fl/fl</sup></u>	<u>Raptor<sup>fl/fl</sup>+Vav1i</u>
Mac-1 <sup>+</sup> (%)	42.20 ± 2.97	55.05 ± 3.18
Gr-1 <sup>+</sup> (%)	43.05 ± 2.33	42.10 ± 2.26
B220 <sup>+</sup> (%)	27.9 ± 2.0	22.8 ± 1.6
Thy1.2 <sup>+</sup> (%)	1.42 ± 0.16	1.73 ± 0.24
Ter119 <sup>+</sup> (%)	24.45 ± 1.06	12.35 ± 0.78

**Supplementary Table 2. Bone Marrow Cellularity of Raptor<sup>fl/fl</sup>-Lyz-Cre Mice (n=5)**

<u>Surface Marker</u>	<u>Raptor<sup>fl/fl</sup></u>	<u>Raptor<sup>fl/fl</sup>-Lyz</u>
Mac-1 <sup>+</sup> (%)	45.33 ± 8.52	50.73 ± 0.72
Gr-1 <sup>+</sup> (%)	44.53 ± 8.69	48.67 ± 0.32
B220 <sup>+</sup> (%)	18.63 ± 3.72	17.40 ± 0.85
Thy1.2 <sup>+</sup> (%)	0.68 ± 0.96	0.65 ± 0.98
Ter119 <sup>+</sup> (%)	32.0 ± 6.24	27.23 ± 2.36

**Supplementary Table 3. Bone Marrow Cellularity of Tsc1<sup>fl/fl</sup>-Vav1-iCre Mice (n=3)**

<u>Surface Marker</u>	<u>Tsc1<sup>fl/fl</sup></u>	<u>Tsc1<sup>fl/fl</sup>-Vav1i</u>
Mac-1 <sup>+</sup> (%)	35.81 ± 11.61	63.73 ± 7.64*
Gr-1 <sup>+</sup> (%)	35.56 ± 11.17	61.50 ± 7.61*
B220 <sup>+</sup> (%)	27.43 ± 2.82	14.33 ± 3.94**
Thy1.2 <sup>+</sup> (%)	1.37 ± 0.68	1.99 ± 1.19
Ter119 <sup>+</sup> (%)	28.77 ± 6.33	14.97 ± 4.12*

**Supplementary Table 4. Bone Marrow Cellularity of Tsc1<sup>fl/fl</sup>-Lyz-Cre Mice (n=5)**

<u>Surface Marker</u>	<u>Tsc1<sup>fl/fl</sup></u>	<u>Tsc1<sup>fl/fl</sup>-Lyz</u>
Mac-1 <sup>+</sup> (%)	36.203 ± 4.47	39.22 ± 5.75
Gr-1 <sup>+</sup> (%)	37.07 ± 5.08	38.88 ± 5.67
B220 <sup>+</sup> (%)	25.50 ± 4.09	29.34 ± 2.08
Thy1.2 <sup>+</sup> (%)	1.75 ± 0.24	1.34 ± 0.15*
Ter119 <sup>+</sup> (%)	34.47 ± 4.10	26.40 ± 3.39**