

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 µCT 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 μ 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) NFATc1 mobility shifts in bone marrow osteoclast differentiation cultures from VLDLR-/- 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-/- 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-/- 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^{fl/fl}-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 IkB-α at the same time point as Fig. 3f-g (50 hrs after RANKL treatment). Ratios of IkB-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.

(I) 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.</p>



Supplementary Figure 3. Additional analyses of Tsc1^{fi/fi}-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^{fl/fl}-Lyz cKO bone marrow cultures.

(h) Caspase 9 expression in bone marrow osteoclast differentiation cultures (n=6).

(i) 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.

(I) 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 - mTORC1 - 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) PxlxIT 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^{fl/fl}-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).

а 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	Т	T	Ρ	G	G	Т		
	T46		Т	L	F	D	Т	Т	Ρ	G	G	Т	Proline-	directed
	S65		М	Е	С	R	Ν	S	Ρ	V	Т	Κ	sites	
	T70		S	Ρ	V	Т	Κ	Т	Ρ	Ρ	R	D		
S6K1	T389		V	F	L	G	F	Т	Υ	٧	А	Ρ	Hydrop	hobic
AKT1	S473		Н	F	Ρ	Q	F	S	Υ	S	А	S	motifs	
SGK1	S422		А	F	L	G	F	S	Υ	А	Ρ	Ρ		

b Phosphopeptide detected by MS in HEK-293E (Hsu e al, Science 2011) Protein Protein ID Peptide

NFATC1 NP_006153 GLGACTLLG<mark>SP</mark>R

С Human NFATC1





f Mouse NFATC1

> **40**· 20

> > 0

Serine Threonine

1 mpnt<mark>sf</mark>pvpskfplg<u>pp</u>aavcgsge<mark>t1</mark>rp<u>ap</u>psggtmkaaeeehy<mark>sy</mark>v 61 ahsalpaachdlqtstgisavpsanhpp<mark>sy</mark>ggavdsgpsgyflssgntrpngap lhlp<mark>sv</mark>eayrdpscl<mark>sp</mark>as 121 rieitsylglhhgsgqffhdvevedvlpsckrs sta sls 181 srscnseas<mark>sy</mark>esny<mark>sy</mark>pya<mark>sp</mark>qt<mark>sp</mark>wq<mark>sp</mark>cv kttdpeegfpr<mark>sl</mark>gachllg prh 241 st<mark>sp</mark>rasitee<mark>sw</mark>lgargsrpt<mark>sp</mark>cnkrky<mark>sl</mark>ngrqpsc phh phgsprvsvted 301 twlgnttqytssaivaainalttdstldlgdgvpiksrktalehap alkvepagedlg 361 t**up**ptsdfppeey<mark>tu</mark>qhlrkgafceqyl<mark>sv</mark>pqa<mark>sy</mark>qwakpk<mark>slsp</mark>t slpaldwgl 421 pshsgpyelrievqpkshhrahyetegsrgavkasagghpivqlhgylenepl $481 \ taddrllrphafyqvhritgktvsttsheiilsntkvleipllpennmraiidcagilkl$ 541 rnsdielrkgetdigrkntrvrlvfrvhipqpngr<mark>tlsl</mark>qvasnpiecsqrsaqelplve 601 kqstdsypviggkkmvlsghnflqdskvifvekapdghhvwemeaktdrdlckpnslvve 661 ippfrnqrit<mark>sp</mark>aqv<mark>sf</mark>yvcngkrkrsqyqrftylpangn<mark>sv</mark>fl**tl**sseselrggfy h g 100 # of a.a in mouse NFATc1 20 80-15 60·

n



sp sl sv sf sw sy tp tl tv tf tw ty

INFAIL	-1	
Human	1	MPST <mark>SF</mark> PVPSKFPLGPAAAVFGRGE T GPAPRAGGTMKSAEEEHYGYASS
Mouse	1	MPNT <mark>SF</mark> PVPSKFPLGPPAAVCGSGE
Human	51	NVSPALPLPTAHSTLPAPCHNLQTS GIIPPADHPSGYGAALDGGPJ :::
Mouse	51	SVTSTLPLPTAHSALPAACHDLQTS GISAVPSANHPPSYGGAVDSGP
Human	99	GYFLSSGHTRPDGAPALE RIEITSCLGLYHNNNQFFHDVEVEDVLPS
Mouse	101	GYFLSSGNTRPNGAPTLE RIEITSYLGLHHGSGQFFHDVEVEDVLPS(SRR1
Human	149	KRESTATISLPSTEAYRDPSCLETASSISSRSCNSEASSYESNYSYPY/
Mouse	151	KRESTSTATTIHLPSTEAYRDPSCLESTASSISSCNSEASSIS SP1
Human	199	TIPE CONTRACT
Mouse	201	SRR2 NLS SP3
Human	249	ESNLGARSSRPATEDNKRK <mark>(SI</mark> NGROPPY <mark>SE</mark> HH EFFSE HG <mark>E</mark> RV <mark>SV</mark> TDI
Mouse	251	E <mark>SW</mark> LGAR <mark>GSRPT<mark>SE</mark>CN<mark>KRK<mark>(SL</mark>NGRQPSC<mark>SE</mark>HH<mark>SPTPSE</mark>HG<mark>SE</mark>RV<mark>SV</mark>TEI</mark></mark>
Human	299	SWLGNTTQYTSSAIVAAINALTTDSSLDLGDGVPVKSRKTTLEQPP <mark>SV</mark> AI :////////////////////////////////////
Mouse	301	TWLGNTTQYTSSAIVAAINALTTDSTLDLGDGVPIKSRKTALEHAP <mark>SV</mark> AI
Human	349	KVEPVGEDLGSPPPPADFAPEDYSSFQHIRKGGFCDQYLAVPQHPYQWAH
Mouse	351	KVEPAGEDLGTTPPTSDFPPEEYT-FQHLRKGAFCEQYLSVPQASYQWAH
Human	399	PKPLOPTSYMETLPALDWQLPSHSGPYELRIEVQPKSHHRAHYETEGSI
Mouse	400	PKSL UPTSIMES SLPALDWQLPSHSGPYELRIEVQPKSHHRAHYETEGSI
Human	449	GAVKASAGGHPIVQLHGYLENEPLMLQLFIGTADDRLLRPHAFYQVHRI
Mouse	450	GAVKASAGGHPIVQLHGYLENEPLTLQLFIGTADDRLLRPHAFYQVHRI
Human	499	GKTVSTTSHEAILSNTKVLEIPLLPENSMRAVIDCAGILKLRNSDIELRI
Mouse	500	GKTVSTTSHEIILSNTKVLEIPLLPENNMRAIIDCAGILKLRNSDIELR
Human	549	GETDIGRKNTRVRLVFRVHVPQPSGR
Mouse	550	GETDIGRRNTRVRLVFRVHIPOPNGR <mark>HIST</mark> OVASNPIECSORSAQELPLV
Human	599	EKQSTDSTPVVGGRKMVLSGHNFLQDSKV1FVERAPDGHHVMEMEARTDI
Mouse	600	EKQSTDSTPVIGGRKMVLSGHNFLQDSKVIFVERAPDGHVWEMEARTDP
Human	649	
Mouse	650	
Human	699	PIIKTEPTDDYEPAPTCGPVSQGLSPLPRPYYSQQLAMPPDPSSCLVAGI
Mouse	740	
Mawaa	749	PPCPQK31LMPAAPGV3PKLHDL3PAA11KGVA3PGHCHLGLPQPAGEAI
Nouse	702	
Mourco	799	AVQUVEREVALTEGSEGGEFEALLEQQVSAFESSSCEEGLEDSLCESSE
Human	9102	
Moureo	702	
Human	202	
Moura	700	
nouse	102	vrLiLSSESELKGGfI



NEATO

i

NFATC1

IN IN	NFATC1		NFATC1
Pig Mouse Rat Monkey Human Chimpanzee	MTGLEEDQEFDFDFLFEFNQSDEGAAAAGATAERYSYATTGISSALPLP49 MPNTSPPVPSKFPLGPPAAVCGSGE GRPAPPSGGT-MKAAEEEHYSYXSPSVTSTLPL959 MSSTSPVPSKFPLGPPAAVCGSGE GRPAPLAGGT-MKAAEEEHYSYASSSVTSTLPL959 MTGLEDQEFDFEFLFEFNQR-DEG-AAAAPEHYGYASSNVSPALPL946 MPSTSPPVPSKFPLGPAAAVFGRGE GGPAPRAGGT-MKSAEEEHYGYASSNVSPALPL959 MPSTSPPVPSKFPLGPAAAVFGRGE GGPAPRAGGT-MKSAEEEHYGYASSNVSPALPL959 ** *:**:	Pig Mouse Rat Monkey Human Chimpanzee	IGTADDRLLRPHAFYQVHRITGKTVSTTSHEAVLSNTKVLEIPLLPENNMRAIIDCAGIL 527 IGTADDRLLRPHAFYQVHRITGKTVSTTSHEIILSNTKVLEIPLLPENNMRAIIDCAGIL 538 IGTADDRLLRPHAFYQVHRITGKTVSTTSHEAILSNTKVLEIPLLPENNMRAIIDCAGIL 534 IGTADDRLLRPHAFYQVHRITGKTVSTTSHEAILSNTKVLEIPLLPENSMRAVIDCAGIL 524 IGTADDRLLRPHAFYQVHRITGKTVSTTSHEAILSNTKVLEIPLLPENSMRAVIDCAGIL 537 IGTADDRLLRPHAFYQVHRITGKTVSTTSHEAILSNTKVLEIPLLPENSMRAVIDCAGIL 537
Pig Mouse Rat Monkey Human Chimpanzee	TAPPE PAPCHDQQASAAGISAVGSAGHPAGYAGAVDGGPSGYFLPSGGVRPNGAPALE 109 TAHSALPAACHDLQTS GISAVPSANHPPSYGGAVDSGPSGYFLSSGNTRPNGAPTLE 119 TAHSALPAACHDLQTS GISAVPSANHPSGYGGAVDSGPSGYFLSSGNTRPNGAPTLE 119 TAHSALPAACHDLQTS GIVQPADHPSGYGAALDGGPAGYFLSSGHARPDGAPALE 104 TAHSS PAPCHNLQTS GIIPPADHPSGYGAALDGGPAGYFLSSGHTRPDGAPALE 117 TAHSS PAPCHNLQTS GIIPPADHPSGYGAALDGGPAGYFLSSGHTRPDGAPALE 117 ** :*** **: ::: : : : : : : : : : : : :	Pig Mouse Rat Monkey Human Chimpanzee	KLRNSDIELRKGETDIGRKNTRVRLVFRVHIPQPNGROUS QVASNPIECSQRSAQELPL587 KLRNSDIELRKGETDIGRKNTRVRLVFRVHIPQPNGROUS QVASNPIECSQRSAQELPL598 KLRNSDIELRKGETDIGRKNTRVRLVFRVHIPQPSGROUS QVASNPIECSQRSAQELPL598 KLRNSDIELRKGETDIGRKNTRVRLVFRVHVPQPSGROUS QVASNPIECSQRSAQELPL597 KLRNSDIELRKGETDIGRKNTRVRLVFRVHVPQPSGROUS QVASNPIECSQRSAQELPL597 KLRNSDIELRKGETDIGRKNTRVRLVFRVHVPQPSGROUS QVASNPIECSQRSAQELPL597
Pig Mouse Rat Monkey Human Chimpanzee	RIEITSYLGLHHNNGQFFHDVAVEDVLPNPRRS STA 152 PNLEAYRD SCL 8AS 169 RIEITSYLGLHHGSGQFFHDVEVEDVLPSCKRS STA 11HPT EAYRD SCL 8AS 179 RIEITSYLGLHHGSNQFFHDVEVEDVLPSCKRS STA 11HPT EAYRD SCL 8AS 179 RIEITSYLGLHNNQFFHDVEVEDVLPSCKRS STA 11HPT EAYRD SCL 8AS 174 RIEITSCLGLYNNNQFFHDVEVEDVLPSSKRS STA 154 PN EAYRD SCL 8AS 177 RIEITSCLGLYNNNQFFHDVEVEDVLPSSKRS STA 154 PN EAYRD SCL 8AS 177 RIEITSCLGLYNNNQFFHDVEVEDVLPSSKRS STA 154 PN EAYRD SCL 8AS 177	Pig Mouse Rat Monkey Human Chimpanzee	VEKQSAASCPVLGGKRMVLTGHNFLQDSKVVFVEKAPDGHHIWEMEAKTDGDLCKPN V 647 VEKQSTDGTPVIGGKKMVLSGHNFLQDSKVIFVEKAPDGHHVWEMEAKTDRDLCKPN V 658 VEKQSTDSTPVIGGKKMVLSGHNFLQDSKVIFVEKAPDGHHVWEMEAKTDRDLCKPN V 644 VEKQSTDSTPVVGGKKMVLSGHNFLQDSKVIFVEKAPDGHHVWEMEAKTDRDLCKPN V 657 VEKQSTDSTPVVGGKKMVLSGHNFLQDSKVIFVEKAPDGHHVWEMEAKTDRDLCKPN V 657 VEKQSTDSTPVVGGKKMVLSGHNFLQDSKVIFVEKAPDGHHVWEMEAKTDRDLCKPN V 657 VEKQSTDSTPVVGGKKMVLSGHNFLQDSKVIFVEKAPDGHHVWEMEAKTDRDLCKPN V 657
Pig Mouse Rat Monkey Human Chimpanzee	SRR1 SP1 SP2 SSRSCNSEAST ESSF SSF TIMO CV0 KTDPEEGFPRSLGACHLIG RH 229 SSRSCNSEAST ESNY STATUTIONO CV0 KTDPEEGFPRSLGACHLIG RH 229 SSRSCNSEAST ESNY SPA H QT WQ CV0 KTDPEEGFPRSLGACHLIG RH 239 SSRSCNSEAST ESNY SPA H QT WQ CV0 KTDPEEGFPRGLGACTLIG RH 237 SSRSCNSEAST ESNY SPA H QT WQ CV0 KTDPEEGFPRGLGACTLIG RH 237 SSRSCNSEAST ESNY SPA H QT WQ CV0 KTDPEEGFPRGLGACTLIG RH 237 SSRSCNSEAST ESNY SPA H QT WQ CV0 KTDPEEGFPRGLGACTLIG RH 237	Pig Mouse Rat Monkey Human Chimpanzee	NLS VEIPPFRNQRIT VQVNFYVCNCKRRSQYQHFTVLPANAPVIKTEPSDDYEPALTCGP707 VEIPPFRNQRIT AQVSFYVCNCKRRSQYQHFTVLPANGNSVFLTLSSESE711 LEIPPFRNQRIT VQVSFYVCNCKRRSQYQHFTVLPANVPIIKTEPTDDFEPALTCGP718 VEIPPFRNQRIT VHVSFYVCNCKRRSQYQRFTVLPANVPIIKTEPTDDYEPAPNCGP704 VEIPPFRNQRIT VHVSFYVCNCKRRSQYQRFTVLPANVPIIKTEPTDDYEPAPTCGP717 VEIPPFRNQRIT VHVSFYVCNCKRRSQYQRFTVLPANGNAIFLTVSRHRAVCG-7-714
Pig Mouse Rat Monkey Human Chimpanzee	SRZ NLS SP3 STURTSVTEEDUGARTSRPSUCNKRKYGLORGQUFG WERAFT WERSTRVSVTD 289 STURASTTEEDUGARSRPTUCNKRKYGLORGQUSG WERBEVSUNG RVSVTD 299 STURASVTEEDUGARSRPTUCNKRKYGLORGQUPSCUENHEETSHGGRVSVTD 294 STURASVTEEDUGARSRPALCNKRKYGLORGQUPYGENHEFTSHGGRVSVTD 294 STURASVTEEDUGARSRPALCNKRKYGLORGQUPYGENHEFTSHGGRVSVTD 294 STURASVTEEDUGARSRPALCNKRKYGLORGQUPYGENHEFTSHGGRVSVTD 297 STURASVTEEDUGARSRPALCNKRKYGLORGQUPYGENHEFTSHGGRVSVTD 297 STURASVTEEDUGARSRPALCNKRKYGLORGQUPYGENHEFTSHGGRVSVTD 297	Pig Mouse Rat Monkey Human Chimpanzee	VSQGLNPLTKPCYGPPLALPPDPSSCLVAGFPPCPQRSAVMSPPPSA KLHDLSCAPYS 767 LRGGFY
Pig Mouse Rat Monkey Human Chimpanzee	DTWLGNTTQYTSSAIVAAINALSTDS DLGGGVPVKARKTALDHSPSLALKVEPAAEDL 349 DTWLGNTTQYTSSAIVAAINALTTDSTLDLGGGVPIKSRKTALEHAP <mark>SV</mark> ALKVEPAGEDL 359 DTWLGNTTQYTSSAIVAAINALTTDSTLDLGGGVSIKSRKTALEHVPSVALKVEPAGEDL 359 DSWLGNTTQYTSSAIVAAINALTTDS DLGGGVPVKSRKTTLEQPPSVALKVEPVGEDL 344 DSWLGNTTQYTSSAIVAAINALTTDS DLGGGVPVKSRKTTLEQPPSVALKVEPVGEDL 357 DSWLGNTTQYTSSAIVAAINALTTDS DLGGGVPVKSRKTTLEQPPSVALKVEPVGEDL 357 X:*****	Pig Mouse Rat Monkey Human Chimpanzee	KGMAGPGHLGLQRPAGGVLGGQEAPRPGGPHGAPQLHPLNLSQSIVTRLT-EPQP-822 717 KGLANPGHSGHLGLQPPASEAPTMQELPRPMAVQPNSPEQPTSVRLQPQ827 KGVTSPGHC-HLGLPQPAGEAPAVQDVPRPVATHPGSPGQPPPALLPQQVSVPPSSSCPP823 KGVASPGHC-HLGLPQPAGEAPAVQDVPRPVATHPGSPGQPPPALLPQQVSAPPSSSCPP836
Pig Mouse Rat Monkey Human Chimpanzee	GATPPTSDFPEEFPPFQHIRKGAFCDQYLSVPQHPYPWARPR	Pig Mouse Rat Monkey Human Chimpanzee	822 717 GLEHSLCPSSPSPPLPPAAQELTCLQPCSPACPPATGRPQHPPPTVRDESPAAQPRLLP 883 GLEHSLCPSSPSPPLPPATQEPTCLQPCSPACPPATGRPQHLPSTVRRDESPTAGPRLLP 896 716
Pig Mouse Rat Monkey Human Chimpanzee	QLPSHSGPYELRIEVQPKSHHRAHYETEGSRGAVKASAGGHPSVQLHGYVESEPLTLQLF467 QLPSHSGPYELRIEVQPKSHHRAHYETEGSRGAVKASAGGHPIVQLHGYLENEPLTLQLF478 QLPSHSGPYELRIEVQPKSHHRAHYETEGSRGAVKASAGGHPIVQLHGYLENEPLTLQLF478 QLPSHSGPYELRIEVQPKSHHRAHYETEGSRGAVKASAGGHPIVQLHGYLENEPLMLQLF477 QLPSHSGPYELRIEVQPKSHHRAHYETEGSRGAVKASAGGHPIVQLHGYLENEPLMLQLF477 VLPSHSGPYELRIEVQPKSHHRAHYETEGSRGAVKASAGGHPIVQLHGYLENEPLMLQLF477	Pig Mouse Rat Monkey Human Chimpanzee	822 717 EACEDSSPNLAPIPVTVKQEPEELDQLYLDDVNEIIRNDLSGTSTHS 930 EVHEDGSPNLAPIPVTVKREPEELDQLYLDDVNEIIRNDLSSTSTHS 943 716



Supplementary Figure 5. Bioinformatics analysis of NFATc1.

I

(a-j) Human and mouse NFATc1.

(a) Examples of known mTOR phosphorylated motifs. (b) Phosphopeptide with serine-proline motif of NFATc1 detected in HEK-293E by mass spectrometry.

(c-h) Bioinformatics analysis of human NFATc1 (c-e) and mouse NFATc1 (f-h). (c,f) potential mTOR phosphorylation motifs colors coded as consensus sites; (d,g) number of serine and threonine residues; (e,h) quantification of consensus sites in NFATc1.

(i) Protein alignment of human and mouse NFATc1. SRR, serine rich region, SP, serine/proline repeats; NLS, nuclear localization signal; green underlined, phosphopeptide detected by mass spectrometry.

(j) quantification of consensus sites overlapping between human and mouse NFATc1.

(k-I) Vertebrate NFATc1 orthologs.

(k) Protein alignment of various NFATc1 orthologs. Domestic pig (Sus scrofa); Mouse (Mus musculus); Rat (Rattus norvegicus); Rhesus monkey (Macaca mulatta); Human (Homo sapiens); Chimpanzee (Pan troglodytes).

(I) quantification of consensus sites overlapping between vertebrate NFATc1 orthologs.

k







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^{fl/fl};Vav1-iCre Mice (n=2)

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

Supplementary Table 2. Bone Marrow Cellularity of Raptor^{fl/fl}-Lyz-Cre Mice (n=5)

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

Supplementary Table 3. Bone Marrow Cellularity of Tsc1^{fl/fl}-Vav1-iCre Mice (n=3)

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

Supplementary Table 4. Bone Marrow Cellularity of Tsc1^{fl/fl}-Lyz-Cre Mice (n=5)

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