Supplemental Information

1   **Differential but complementary roles of HIF-1α and HIF-2α in the regulation of**

2   **bone homeostasis**

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4   Sun Young Lee[1†], Su-Jin Kim[1,2†], Ka Hyon Park[1,2], Gyuseok Lee[1], Youngsoo Oh[1], Je-Hwang
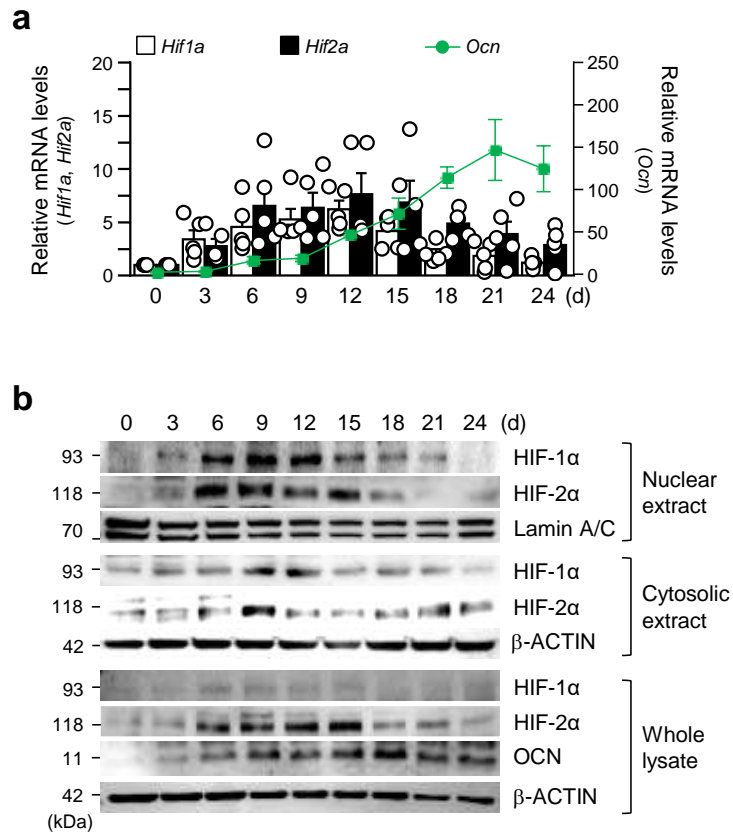
5   Ryu[1,2*], and Yun Hyun Huh[3*]

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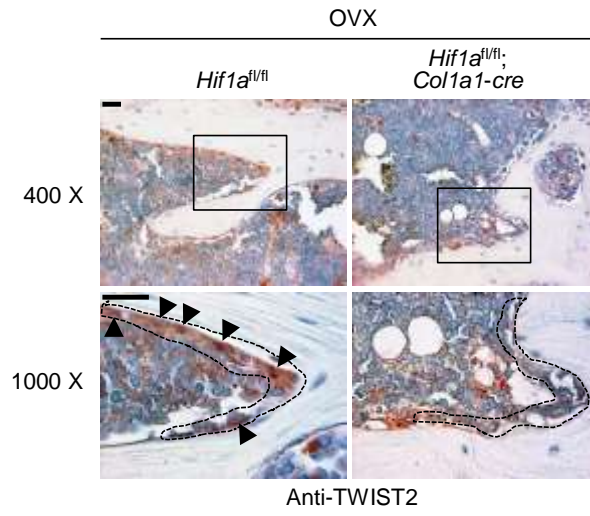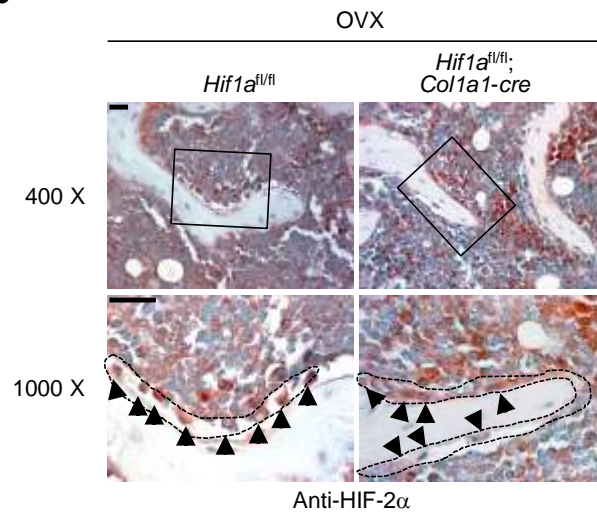Supplementary Figure 1, 2, 3 and 4

Supplementary Table 1

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4 **Supplementary Fig. 1 *Hif1a* is up-regulated during osteoblast differentiation. a, b** Primary

5 calvarial pre-steoblasts isolated from WT mice were cultured in the DM. Relative transcript

6 levels of *Hif1a, Hif2a, and Ocn* at indicated culture day (0-24 days) were determined by qRT-

7 PCR ($n = 5$) **(a)**. The protein levels and nuclear translocation were determined by western

8 blotting ($n = 3$) **(b)**.

a

OVX

$Hif1a^{fl/fl}$ $Hif1a^{fl/fl}$; $Col1a1$-cre

400 X

1000 X

Anti-TWIST2

b

OVX

$Hif1a^{fl/fl}$ $Hif1a^{fl/fl}$; $Col1a1$-cre

400 X

1000 X

Anti-HIF-2α

c

OVX

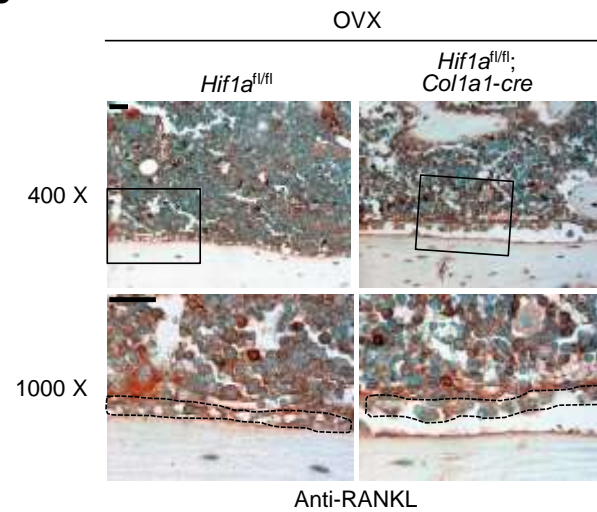$Hif1a^{fl/fl}$ $Hif1a^{fl/fl}$; $Col1a1$-cre

400 X

1000 X

Anti-RANKL

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1 **Supplementary Fig. 2 Representative images of immunohistochemical staining in bone**

2 **tissue from OVX model in *Hif1a*$^{fl/fl}$ and *Hif1a*$^{fl/fl}$;*Col1a1-Cre* mice. a-c** Representative

3 mouse femur sections stained TWIST2 **(a)**, HIF-2a **(b)**, and RANKL **(c)**. The lower panel

4 images are the enlarged frames of the upper panel. The dotted lines indicate osteoblasts, and

5 the nuclear positively stained osteoblasts in brown are indicated by arrows.

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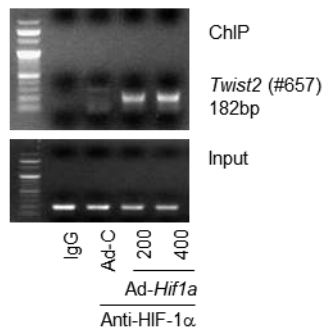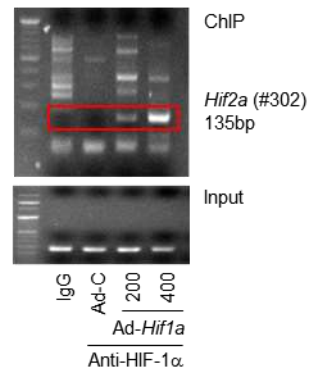11

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Figure 2h

ChIP

Twist2 (#657)
182bp

Input

IgG Ad-C 200 400

Ad-*Hif1a*

Anti-HIF-1α

Figure 5a

421bp — *Hif1a*

347bp — *Hif2a*

353bp — *Rankl*

431bp — *Gapdh*

Figure 5b

ChIP

Hif2a (#302)
135bp
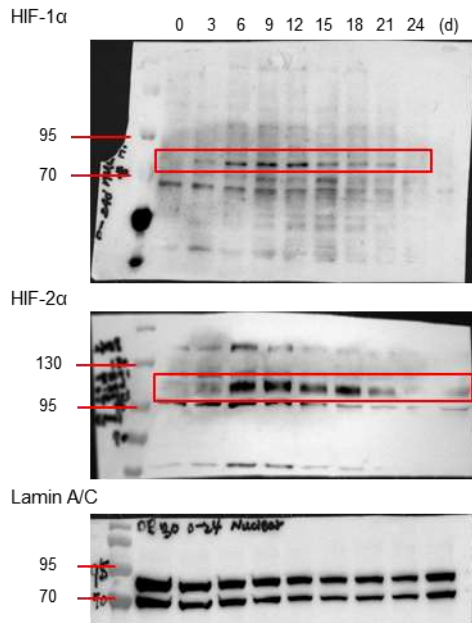
Input

IgG Ad-C 200 400

Ad-*Hif1a*

Anti-HIF-1α

2

3 **Supplementary Fig. 3 Unedited/uncropped gel for main figures.**

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Supplementary Figure 1b

Nuclear extract

HIF-1α

Cytosolic extract

HIF-1α

HIF-2α

HIF-2α

Lamin A/C

β-ACTIN

Whole lysate

HIF-1α

HIF-2α

OCN

β-ACTIN
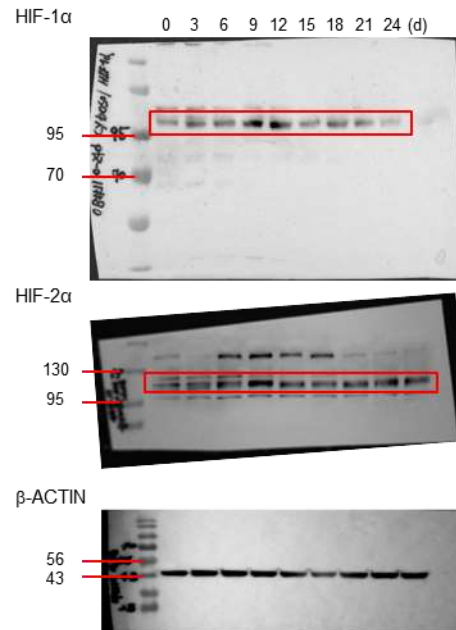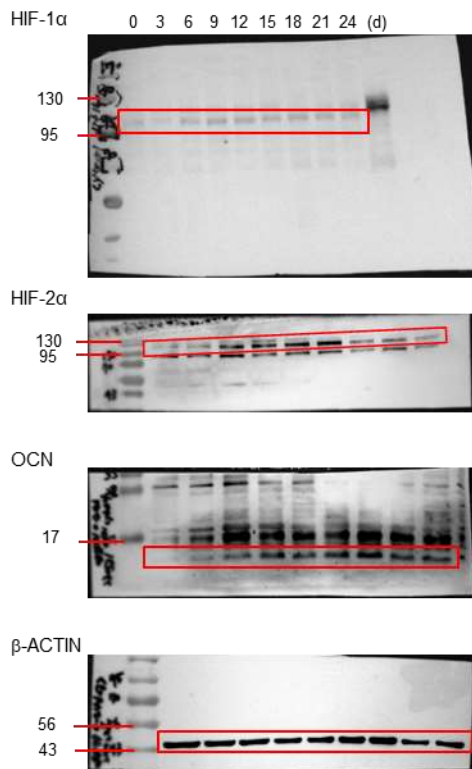
Supplementary Fig. 4 Unedited/uncropped western blots for supplementary figures.

1 **Supplementary Table 1** PCR primers and conditions

Gene	Strand	Sequences (5'-3')	Size (bp)	[a]AT (°C)
Alp	[b]S	TACACCACAACACGGGCGAGGAGAC	356	60
	[c]As	CCTCTGGTGGCATCTCGTTATCCG		
Hif1a	S	AGGCTCACCATCAGTTATTTACGTGTG	421	60
	As	TAGACATGAATATGGCCCGTGCAGTG		
Hif2a	S	AGAAGAGCAAAGACGTGTCCACCGAG	347	63
	As	GTAGAACTCATAGGCAGAGCGTCCAAG		
Ocn	S	CTCCTGAGAGTCTGACAAAGCCTT	320	55
	As	GCTGTGACATCCATTACTTGC		
Opg	S	CTTGCCCTGACCACTCTTATACGG	359	60
	As	CGTTGTCATGTGTTGCATTTCCTTTC		
Rankl	S	GACTCGACTCTGGAGAGTGAAGAC	353	60
	As	AATGTTGGCGTACAGGTAATAGAAG		
Runx2	S	GCCACCTTTACCTACACCCC	363	55
	As	GACTCATCCATTCTGCCGCT		
Twist1	S	TGAGCAACAGCGAGGAGGAG	417	60
	As	GCCAGTTTGAGGGTCTGAATC		
Twist2	S	GGCCGCCAGGTACATAGAC	103	60
	As	GTAGCTGAGACGCTCGAGA		
Gapdh	S	TCACTGCCACCCAGAAGA	431	60
	As	TGTAGGCCATGAGGTCCA		
Twist2 ChIP	S	CCAACACTCAGGGCAAGGTC	182	60
	As	CTCGGGCCAGTATGCAAGTTG		

| Hif2a | S | AATCGCCCCCACCCCAAGAC | 135 | 60 |
| ChIP | As | CAATTGCGGAGAAAGCACGG | | |

1 [a]AT, annealing temperature; [b]S, sense primer; [c]As, antisense primer

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