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Bone

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A B S T R A C T

H *Lrp5* *Lrp5* *β-catenin.* *Wntless* (*Wls*) *Wls* (B C) /L 5 *Lrp5* *β-catenin* fi fi B C B C

2013 E I . A

Introduction

(B) 1 . D fi F GF-β (B C) B 2 . A KL 3–8 . B2–E B4 9,10 4D 1 3A– 1 11,12 . I B C B 13–19 . L 5 β . L 20 , 21–23 . *Lrp5* *Lrp5* β A

13,14,24–26 . β-C 3–5 . , β G 27 . D L 5 . B *Lrp5*, fi *Lrp5* *Lrp6* 16 . L 5 . H *Lrp5* *Lrp5* 28 . L 5 . G 29–32 . 33 . I 5 34 , 10 B C 17 . H

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Wntless fi [35,36]. ( ), fi [37,38]. *Wls* [39]. C *Wls* [37], [40] [41]. I *Wls* fi B C fi B C

Materials and methods

Mice

Col1-cre [42], *Osteocalcin-Cre* [43,44], *Wls*$^{c/c}$ [38], β-cat$^{e\ 3/+}$ [45]. E *Col1-Cre* *Osc-Cre* *Wls*$^{c/c}$ β-cat$^{e\ 3/+}$ 129 1 I J C57B /6J. A B I J

Cal arial cell culture and ALP staining

fi 0.05% -ED A (G) 0.5 / (G) α- E (G) 37 C 15 . I 24- , 5 10^4 10 β ( ), 50 μ / -2 ( ) 10^{-7} ( ) fl AL ( )

Immunohistological and histomorphometric anal ses

B fi , 7 14 fi 4% FA 4 C fi 15% ED A 7 H&E (B), ( ) E17.5 K fi . F 10- 5 (&D), 5 (A), 10 ( ), (A), (A) -B (A). A F 488 A F 488 (I). (B) ( ) 100 μ / 2 fi . F 24- , 5 10^4 24 . C 10 μ B ( ) 2 . F B

Microcomputed tomograph (μCT)

70% E H 4 C. ( ), 50 200 μA, 280- 18.26 μ . D C [46] (I) : 44 (0.8) 22 (0.4) fi : (1) (B /); (2) fi (B /B); (3) (.); (4) (B D); (5) ((6) ( ); (7) (C).

RNA e traction and quantitati e real-time PCR

A (I) A II (I) 800 A. C ABI 7500 D (A B) B G K ( ). A A 1.

Flo c tometric assa and CFU-F assa s

B fl B C fi L ( ), CD31( ), CD45( ), CD29( ) 1( ). fi CD11 ( ), B220(BD), CD3( ), -K ( ) CD115(B). F CF -F fl , 1.0 10^6 6- C).

Serolog

B 2- *Wls*- C- (C), G A KL L EL A K (1 L4000, B , H D)

BMMs isolation and coculture e periment

B fl 8 . A α- E 5 / -C F. A 24 fl α- E 10 / -C F. A 24 fl α- E (B) F , 1 10^5 8 24- . 1 10^5 B α- E 10 / -C F 50 / A KL, 5 A

Statistical anal sis

t . F F .2–7 2 5, <0.05, ** <0.01 *** <0.001

Results

Multiple Wnt proteins are d namicall e pressed in differentiating osteoblasts

fi *Wnts* C C) *Wnt4, Wnt5a, Wnt5b, Wnt7b* *Wnt16* *Wnt10b,* (F . 1A). *Wnt4, Wnt7b* *Wnt16* (F . 1A). I (IHC)

10 5 E16.5 (F . 1C–D). A 7 14 5 10 (F . 1E, G, I), 5 (F . 1D, H). 5 + B C (F . 1E).

Depletion of osteoblastic Wls results in osteopenia

Wntless (*Wls*) *Wls*$^{c/c}$ *Col1a1-3.6kb-Cre* (*Col1-Cre*) *Osteocalcin-Cre* (*Osc-Cre*) 42,44 . I *Col1-Cre; R26R* C *Col1-Cre*

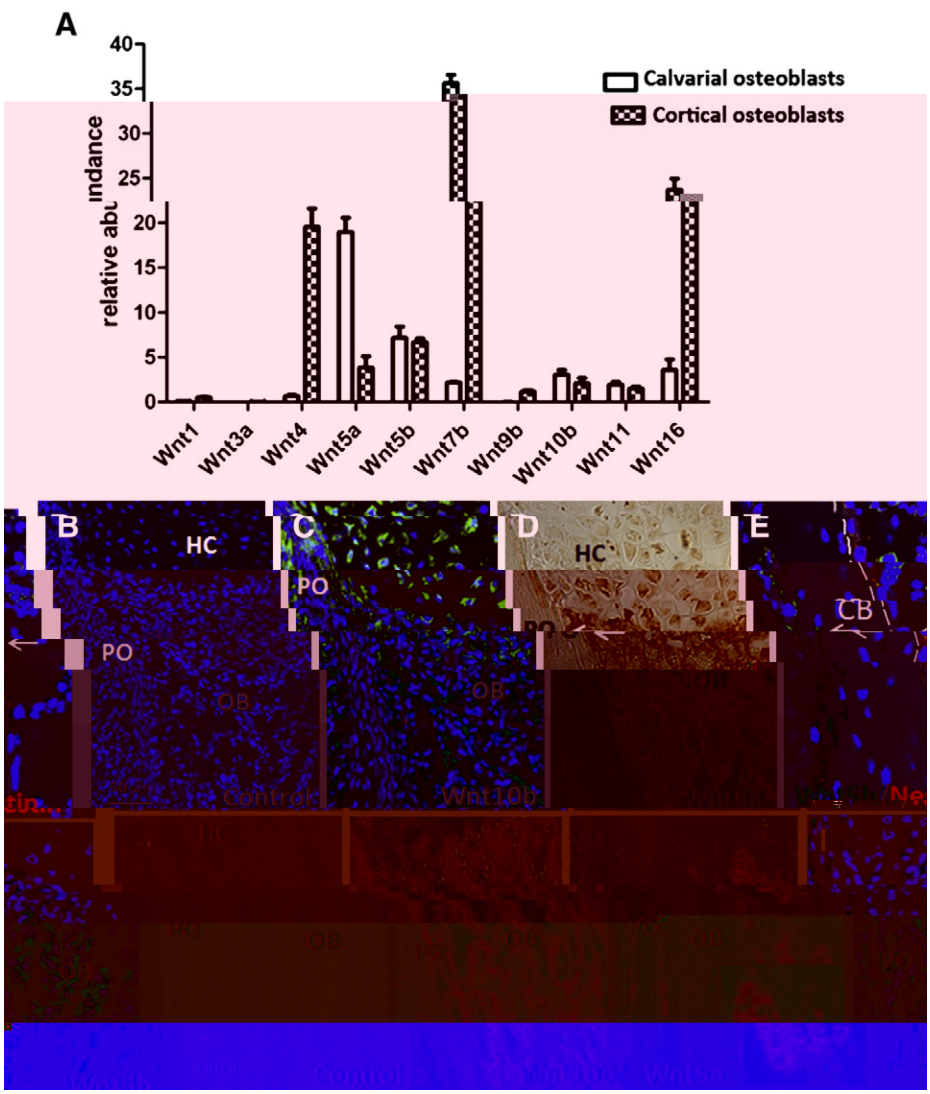

Fig. 1. C (=3). B–I. . A. *Wnt* 10 (C, G), 5 (D, H) 5 (E, I) fi E16.5 (B–D) 7 (E) 14 (F–I) (IHC) 5 E). + B C 5 + 7 (E). 8. (D, F–J) . HC, ; B, ; B,

(Fig. 1). *Col1-Cre; Wls$^{c/c}$* ... H&E ... K ... E17.5 (Fig. 2). H ... fi ... (Fig. 2A, B). ... *Col1-Cre; Wls$^{c/c}$* ... (Fig. 2C, D). fl ... *Col1-Cre; Wls$^{c/c}$* ... (Fig. 2D, E). C ... *Col1-Cre; Wls$^{c/c}$* ... μCT ... 14 ... B, D, ... B/B (Fig. 3). I ... *Wls* ... *Osc-Cre; Wls$^{c/c}$* ... (Fig. 4). *Osc-Cre; Wls$^{c/c}$* ... fi

Osteoblastic Wnts promotes osteoblastogenesis in postnatal bone turnover

fi ... H ... H&E ... *Col1-Cre; Wls$^{c/c}$*

(Fig. 4A–C). I ... B ... *Col1-Cre; Wls$^{c/c}$* ... B ... (Fig. 4D–I). ... *Col1-Cre; Wls$^{c/c}$* ... *Runx2* ... *Osterix* ... C ... fi ... (Fig. 4J), ... H ... *Col1a1* ... *Osteocalcin*, ... (Fig. 4J). I ... (ALP) ... (Fig. 4K). A ... *Lef1* ... β ... *Wls* ... (Fig. 4L–), ... fi ... fi ... *Wls*- fi

Ablation of osteoblastic Wls enhances osteoclast differentiation

Wls- fi ... FACS ... A ... fi ... CD11^{-}B220^{-}CD3^{-}CD117^{+}CD115^{+} 47 . FACS

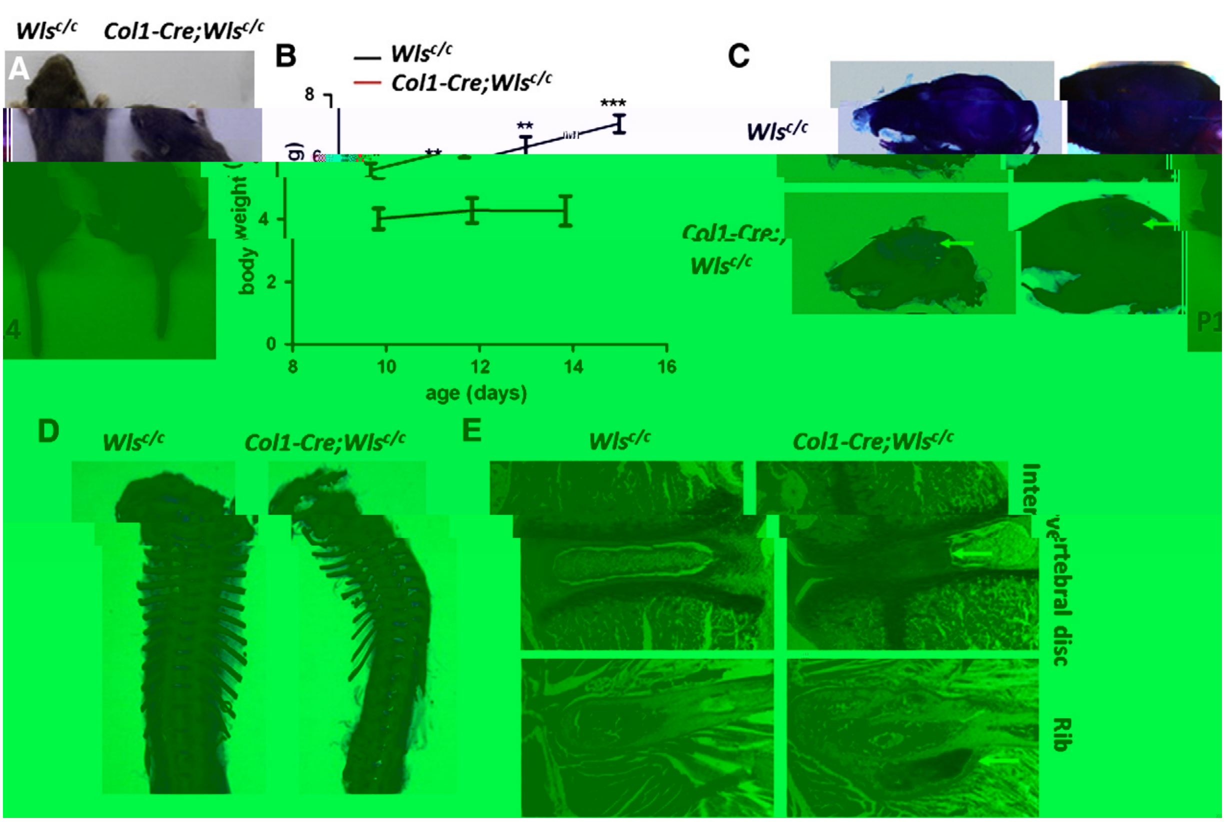

Fig. 2. A ... *Wls* ... A. *Col1-Cre; Wls$^{c/c}$* ... 14. B. ... *Col1-Cre; Wls$^{c/c}$* ... D. ... 10. C. ... fi ... fl ... E. E ... ( ). (F ... fi ... .)

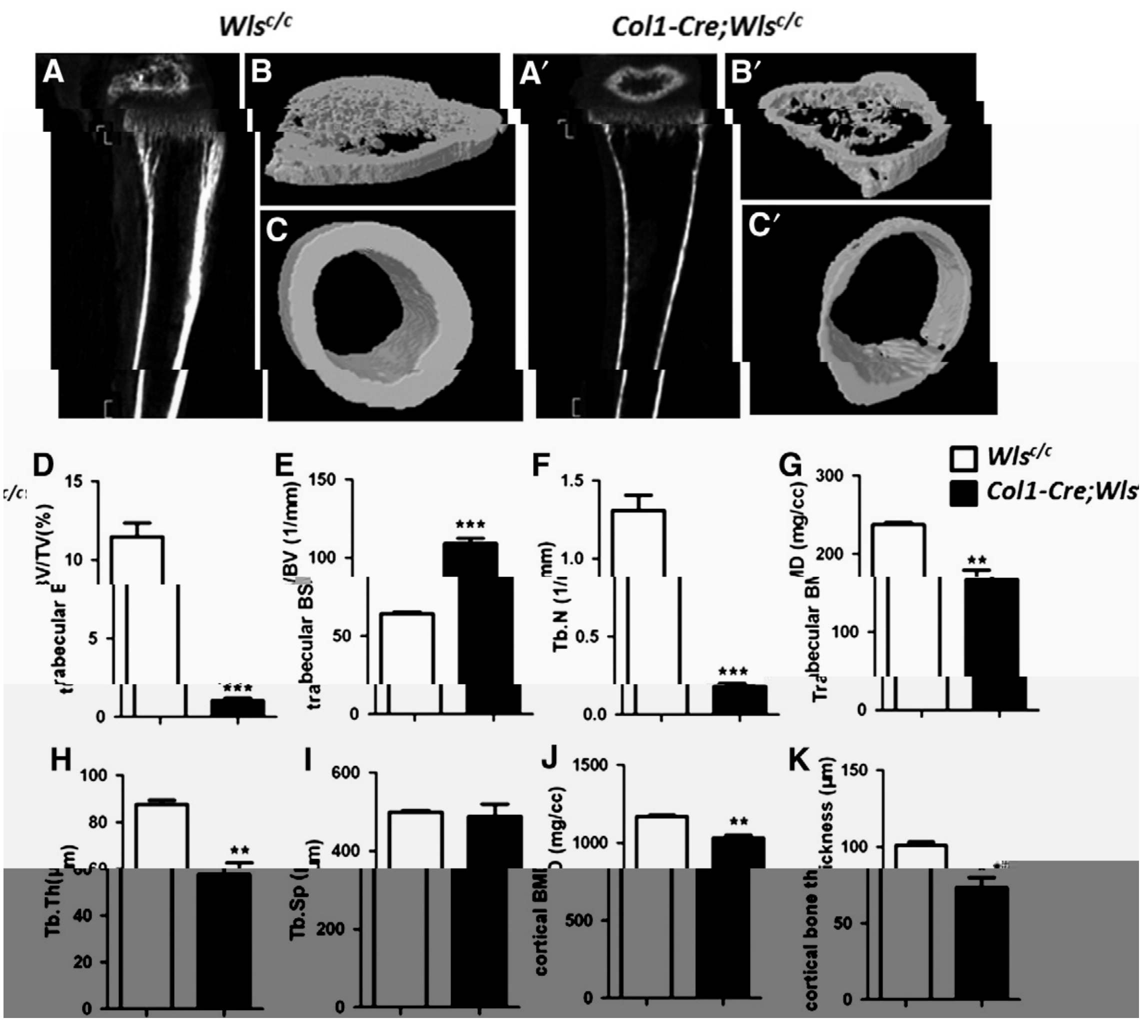

Fig. 3. μC ... (B, B′ ... *Col1-Cre; Wls^c/c^* ... A–C′. ... μC ... 14 ... (A, A′) ... 3-D ...

... *Col1-Cre; Wls^c/c^* ... (Fig. 5A, B). I ... A ... (Fig. 5C, D). ... fi ... *Wls*- fi ... G, A KL ... -C F, ... 48 ... ELI A. ... *Col1-Cre; Wls^c/c^* ... 14 ... G, A KL ... G/ A KL ... (Fig. 5E, F ... H). ... (C), ... (Fig. 5G). ... *OPG*, *RANKL* ... *M-CSF* ... C ... (Fig. 5I). ... G, A KL ... C F ...

The paracrine effect of osteoblastic Wnts on osteoclast differentiation

... *Col1-Cre; Wls^c/c^; β-cat^e 3/+^* ...

... *Wls*- fi ... β ... /β ... I ... *Col1-Cre; β-cat^e 3/+^* ... *Col1-Cre; Wls^c/c^; β-cat^e 3/+^* ... μC ... H&E ... A ... *Col1-Cre; Wls^c/c^; β-cat^e 3/+^* ... *Col1-Cre; Wls^c/c^* ... 7 (Fig. 6A). ... *Col1-Cre; β-cat^e 3/+^* ... (Fig. 6F). ... *Col1-Cre; β-cat^e 3/+^* ... (Fig. 6C, D). H ... *Col1-Cre; Wls^c/c^; β-cat^e 3/+^* ... *Col1-Cre; β-cat^e 3/+^* ... (Fig. 6F, G ... H). ... *OPG* ... *RANKL* ... *M-CSF* ... (Fig. 6I). ... (B) ... A KL/ -C F. ... A ... 5 ... B ... A KL/ -C F- ... *Wls^c/c^* ...

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Fig. 4. I Wls . A–H. H&E (A, B), B (D, E) (G, H) 14 . (C) / . A) (A, B) (=3). . H (F) (I) (=3). J. 8 (=3). Col1 Osc . K. A . L– . Wls (L) Lef1 ( ) (=3). . β . .) . (F fi

Col1-Cre; Wls c/c (F . 7A, B). Wls c/c B Wls c/c Col1-Cre; Wls c/c (F . 7C, D) fl A KL/ -C F . I A KL/ -C F Wls c/c B Col1-Cre; Wls c/c Wls c/c (F . 7C′, D′). OPG/RANKL OPG, RANKL M-CSF fi- (F . 7E).

5 2 34 .

Osteoblastic Wnts sustain BMSC maintenance

I 5 + B C ((F . 1F). B C 49 . I B C CF -F Col1-Cre; Wls c/c (F . 8D, E). A B C L −CD31−CD45−CD29 1+ 2 . FAC B C (F . 8A–C).

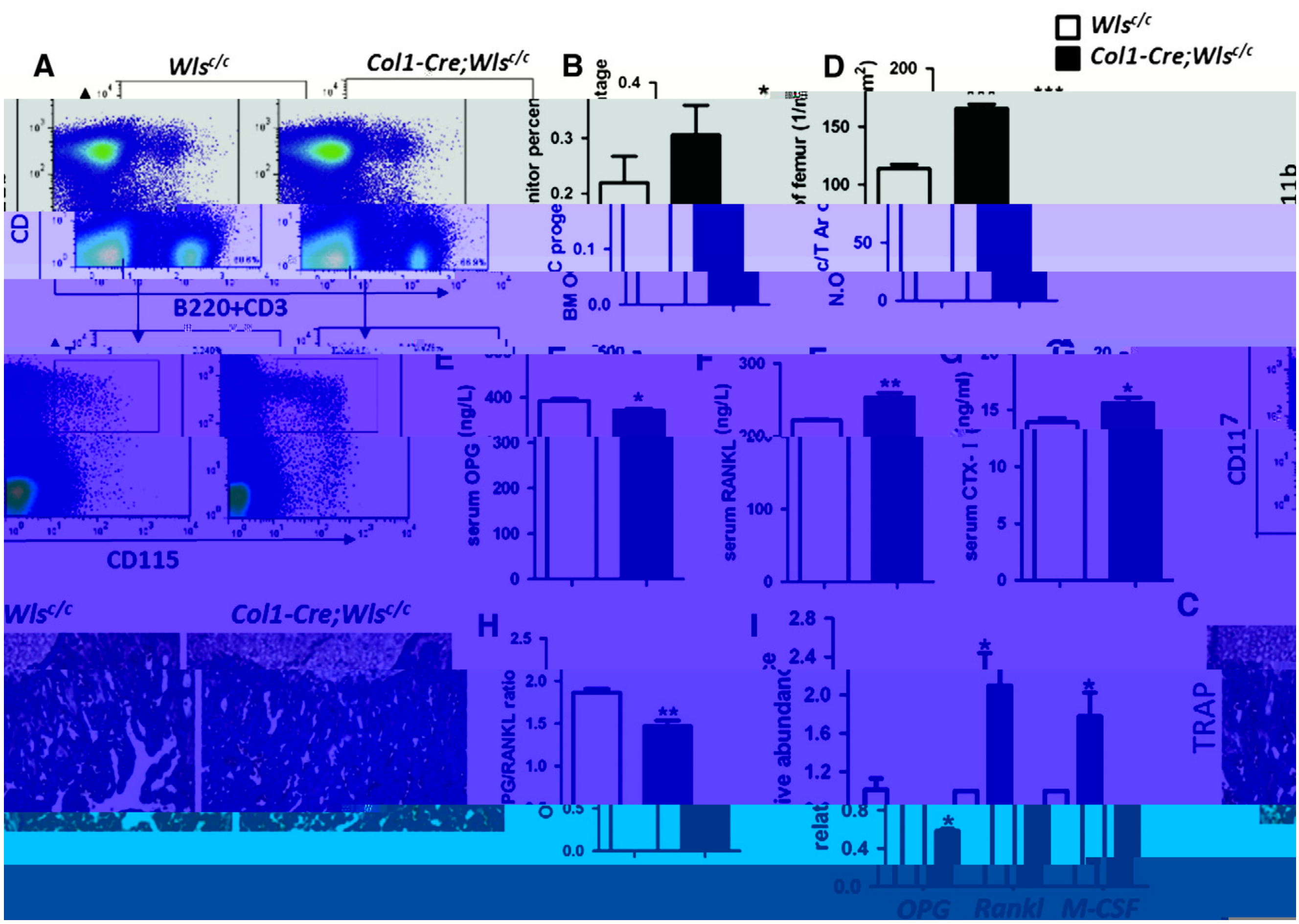

Fig. 5. I Wls . A. A FAC Col1-Cre; Wls$^{c/c}$ CD11 B220$^-$CD3$^-$CD117$^+$CD115$^+$. B. G (A) (=4). C. A (C) (=3). E–G. ELI A G (E), A KL (F), C (G) 14 . D. H 14 (=4). H G/ A KL (E, F) (=4). I. OPG, Rankl M-CSF 8.

A fi CF -F B C .

Discussion

I Wls fi Cre, Wls- fi B C fi L 5 β 15,24–26,31 . B C A Wls Ocn-Cre Col1-Cre; Wls$^{c/c}$ 41 . C Osteocalcin 10 Ocn-Cre 50 , 1.3 Osc-Cre 44 , Col1-Cre 42 . Wls (F . 4),

Wls Ocn-Cre Osc-Cre 41 . I β 24–26,31 . fi I Wnts Wnt5a, Wnt5b and Wnt16 Wnt4, Wnt5b, Wnt7, Wnt10b Wnt16 (F . 1). 10 17,32 . 7 (F . 1), fi 30 . 5 10 29 . 10 5/ 7 , OPG 5 /β 34 . I Wls G A KL

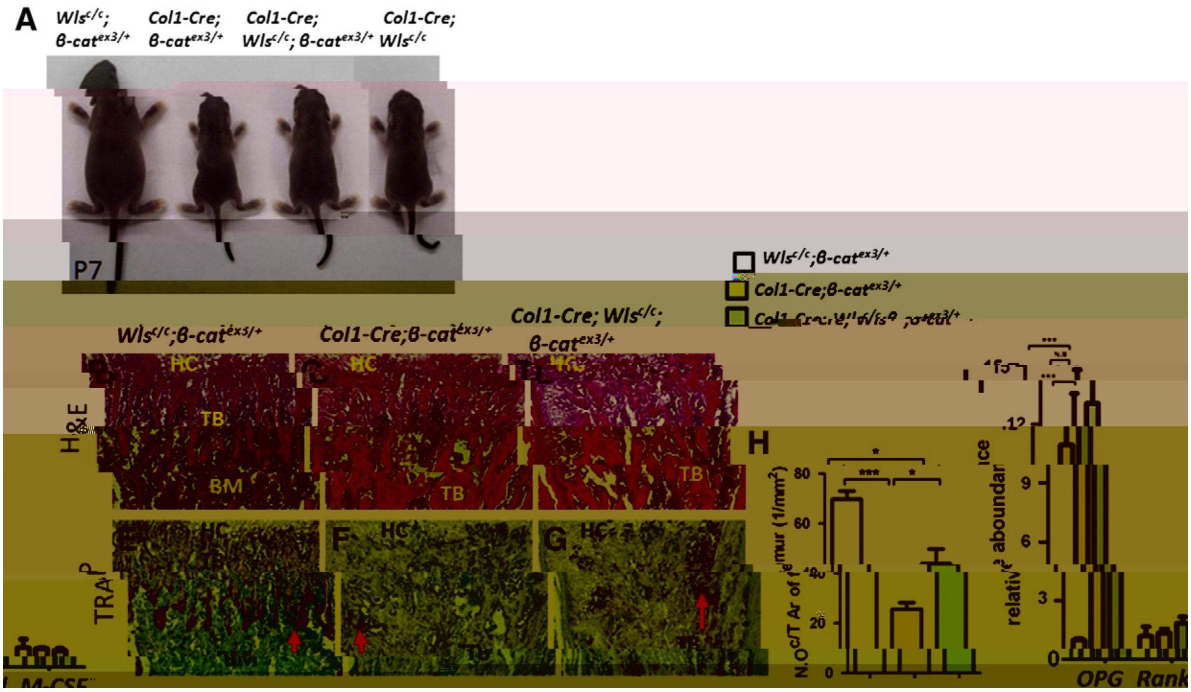

Fig. 6. Col1-Cre; β-cat e 3/+; Wls c/c Col1-Cre; β-cat e 3/+ . A. , Col1-Cre; Wls c/c, Col1-Cre; β-cat e 3/+ Col1-Cre; β-cat e 3/+; Wls c/c 7. . B–G. H&E (B–D) (E–G) 7. HC, ; B, ; B H. H (.A) (E–G) (= 3). C OPG, RANKL, M-CSF (= 3).

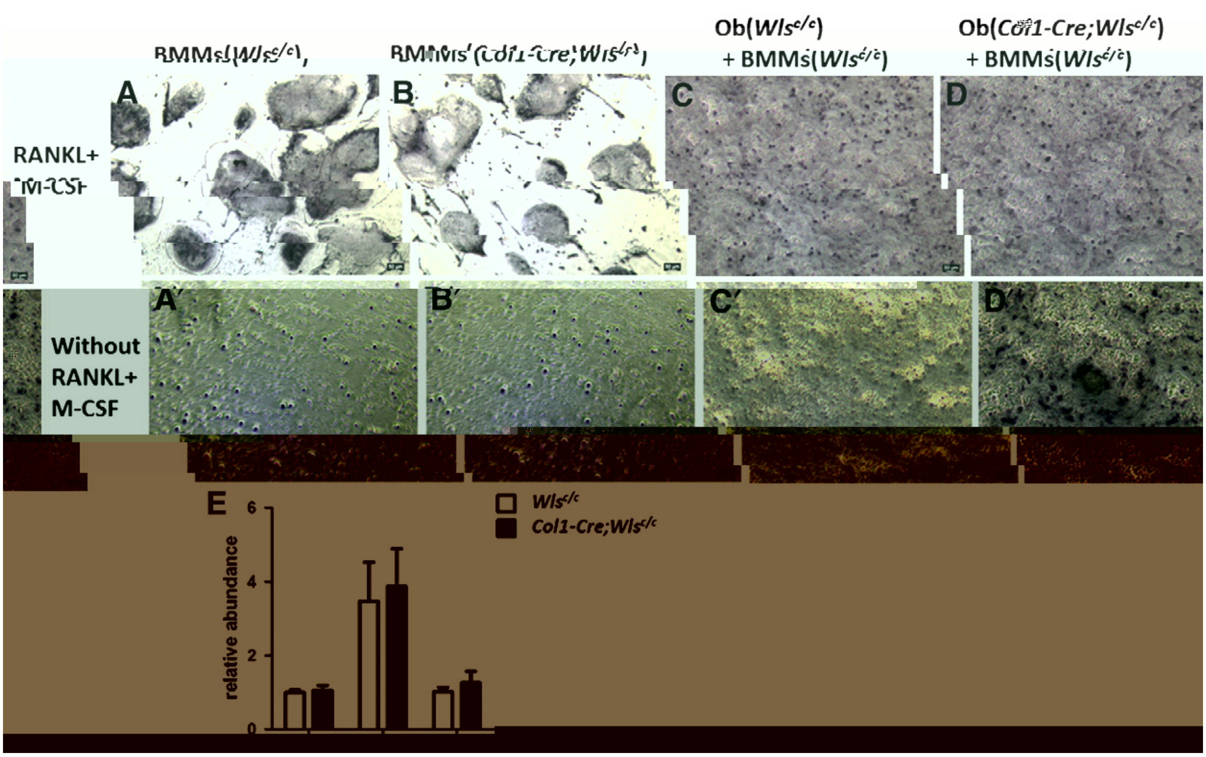

Fig. 7. (B) A KL/ -C F. A B Wls c/c 8. B B′. B Col1-Cre; Wls c/c 8. C–C′. . A–A′. B Wls c/c ( ) Wls c/c 8. D–D′. B Wls c/c 8. Wls c/c Col1-Cre; Wls c/c 8. (A-D) -C F A KL (A′–D′). E. C OPG, RANKL, M-CSF ( ), Wls c/c Col1-Cre; Wls c/c 8 (= 3).

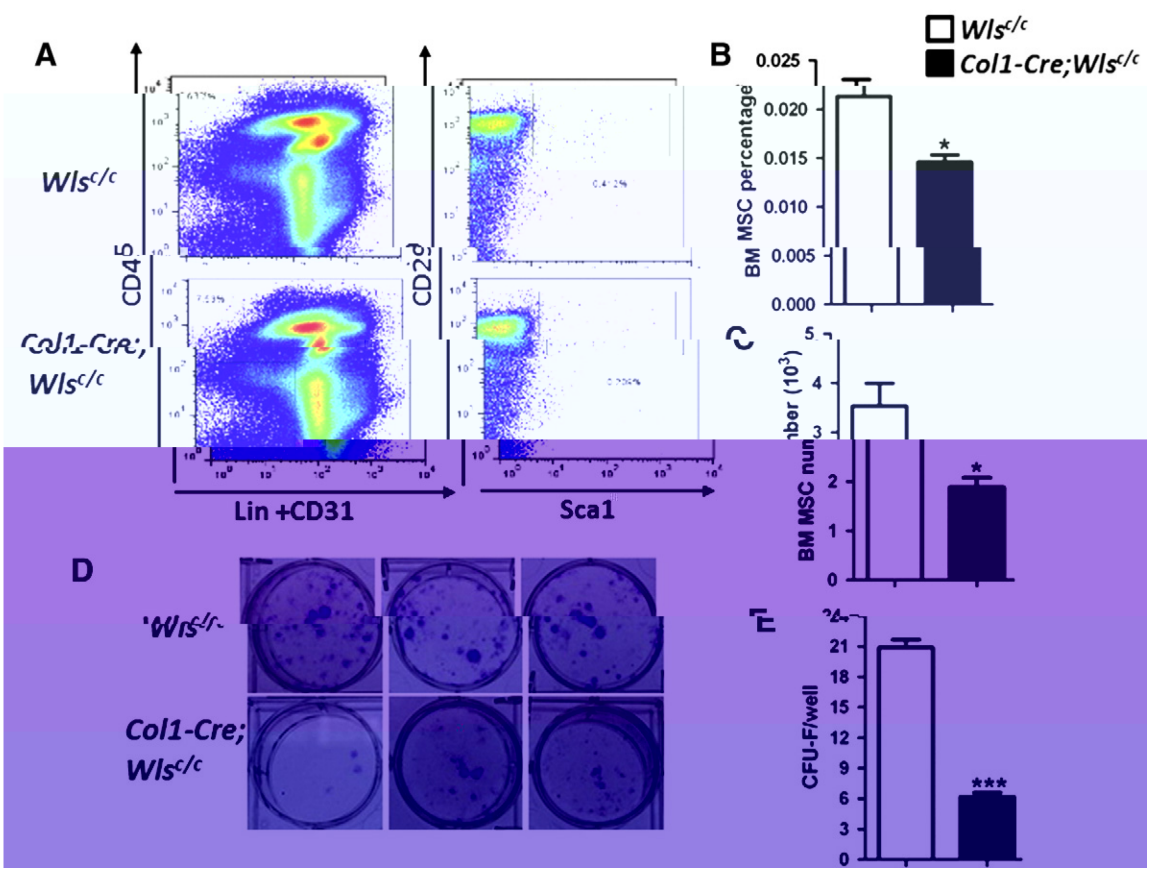

Fig. 8. B C. A. A FACS B C L $^-$CD31$^-$CD45$^-$CD29$^+$ 1$^+$. B–C. G B C (B) (C) (= 4). D. CF -F B C E CF -F (D) (= 3). B fl *Col1-Cre; Wls$^{c/c}$* 8-10 (A–D).

G/ A KL B *Col1-Cre; Wls$^{c/c}$* *Wls$^{c/c}$* (F . 7E, F). *OPG*, *RANKL* *M-CSF* . A *Wls-* fi β *Col1-Cre;* *β-catenin$^{e\ 3/+}$* *OPG* (F . 6) *Col1-Cre (3.6kb)* C 51 *Wls* *Wls* fi . I *Wls* *Ocn-Cre* G/ AKL 41 fi G/ A KL β 27 . (H C) fi 52 . H B C *Wls-* fi CF -F B C (F . 7).

fi B C 10 17 . H B C *Wnt10b-* B C *Wls-* fi 10 B C I fi B C G/ A KL B C fi B C ://. . /10.1016/ . .2012.12.052.

Ackno ledgments

973 C F 2007CB947301 2012CB966903 F C 31171396, 31271553, 81121001 31100624 .

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