

1 **IFT20 governs mesenchymal stem cell fate through positively regulating TGF- β -**
2 **Smad2/3-Glut1 signaling mediated glucose metabolism**

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31 **Supplemental Figure Legends**

32 Figure S1. IFT20 deficiency in Prx1-expressing cells causes a severe limb shortening
33 in mice. (A) IFT20 expression. qRT-PCR analysis using RNA from MSCs from Prx1-
34 Cre;IFT20^{f/f} mice and controls. (B) Representative whole-mount skeletal-stained image
35 of Prx1-Cre;IFT20^{f/f} mice and age-mated controls at different timepoints of embryo
36 (E16.5 and E18.5) as indicated. (C) Quantitative analysis of the length of femurs and
37 tibiae from Prx1-Cre;IFT20^{f/f} mice and age-mated controls as indicated. (D, E)
38 Representative images of Prx1-Cre;IFT20^{f/f} mice and controls at age of 1 month. (F)
39 Quantitative analysis of the length of femurs and tibiae from 1-month-old Prx1-
40 Cre;IFT20^{f/f} mice and controls. Error bars were the means ± SEM from three
41 independent experiments. *P < 0.05, **P < 0.01, ***P < 0.001.

42 Figure S2. IFT20 deficiency in Prx1-expressing cells impaired bone formation. (A)
43 Representative micro-CT image of femurs of Prx1-Cre;IFT20^{f/f} mice and age-matched
44 controls at P7. Scale bars, 1 mm. (B) Representative micro-CT image of femurs of Prx1-
45 Cre;IFT20^{f/f} mice and age-matched controls at 1 month. Scale bars, 1 mm.

46 Figure S3. IFT20 deficiency in Lerp-expressing cells causes bone-fat imbalance. (A)
47 Representative micro-CT image of femurs of Lepr-Cre;IFT20^{f/f} mice and controls at 1
48 month. Scale bars, 1 mm. (B-E) Histomorphometric analysis of bone parameters in the
49 femurs of 1-month-old Lepr-Cre;IFT20^{f/f} mice and controls. Bone volume fraction
50 (BV/TV); trabecular thickness (Tb.Th); trabecular number (Tb.N); trabecular spacing
51 (Tb.Sp). N=5 mice/group. (F) Quantitative measurements of BMD of femurs from
52 Lepr-Cre;IFT20^{f/f} mice and controls at 1 month. (G) The serum level of OCN from
53 Lepr-Cre;IFT20^{f/f} mice and controls at 1 month. (H-J) Calcein double labeling in tibia
54 of 1-month-old Lepr-Cre;IFT20^{f/f} mice and controls. Scale bar, 50 μm. (K)
55 Representative H&E-stained image of femur sections from Lepr-Cre;IFT20^{f/f} mice and
56 controls at 1 month. Scale bars, 200 μm. (L) Representative TRAP-stained image of
57 femur sections from 1-month-old Lepr-Cre;IFT20^{f/f} mice and controls. The
58 corresponding quantitative analysis was performed at lower panel. (M) OsO₄ micro-CT
59 staining of decalcified tibiae by micro-CT analysis as indicated. Error bars were the

60 means \pm SEM from three independent experiments. * $P < 0.05$, ** $P < 0.01$.
61 Figure S4. The effect of IFT20 on mature osteoblasts is dispensable. (A) Representative
62 micro-CT image of femurs of DMP1-Cre;IFT20^{f/f} mice and controls at 1 month. Scale
63 bars, 1 mm. (B) Histomorphometric analysis of bone parameters in the femurs of 1-
64 month-old DMP1-Cre;IFT20^{f/f} mice and controls.

65 Figure S5. IFT20 expression. (A) IFT20 expression was identified by qRT-PCR after
66 transfection for 48 hr with Ad-Cre or Ad-GFP in the MSCs from IFT20^{f/f} mice. (B)
67 MSCs were transfected with Myc-IFT20 plasmid or empty vector, respectively. After
68 transfection of 48 hr, the IFT20 expression was identified by qRT-PCR. Error bars were
69 the means \pm SEM from three independent experiments. *** $P < 0.001$.

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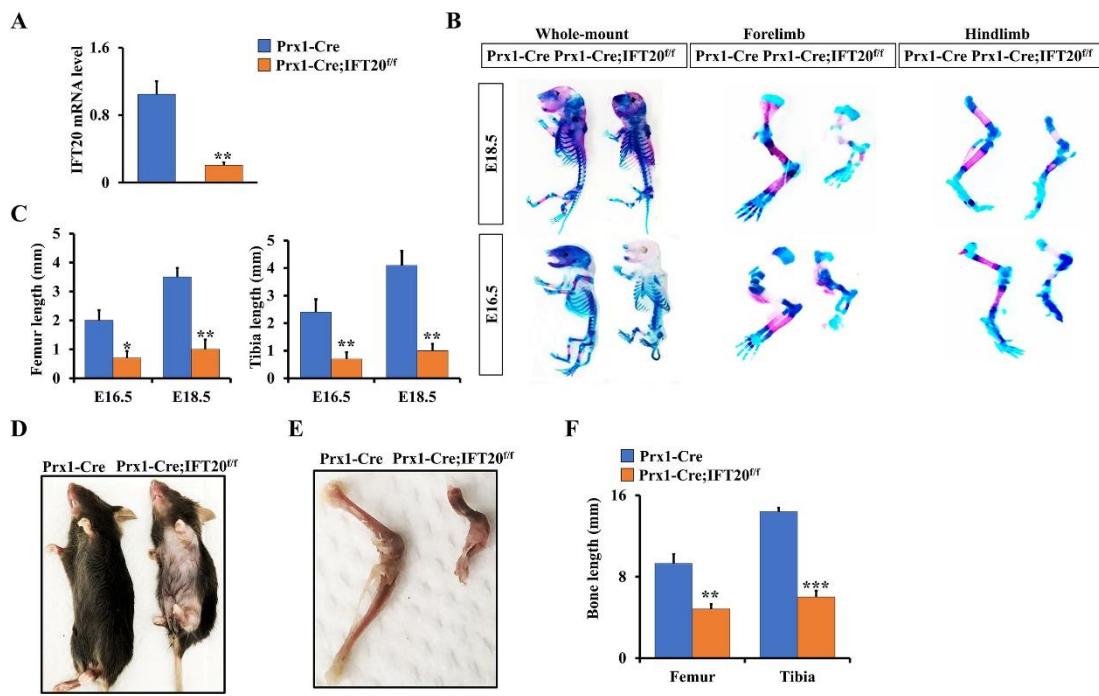
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85 **Figure. S1**



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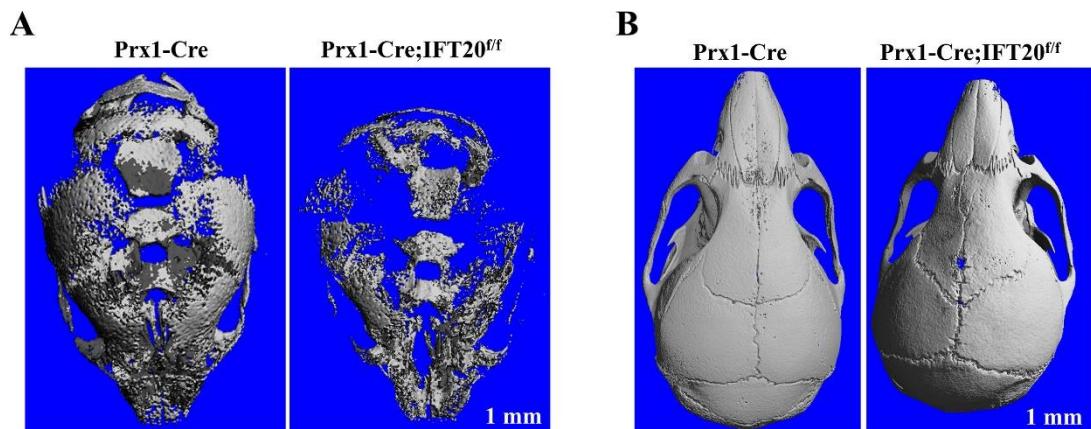
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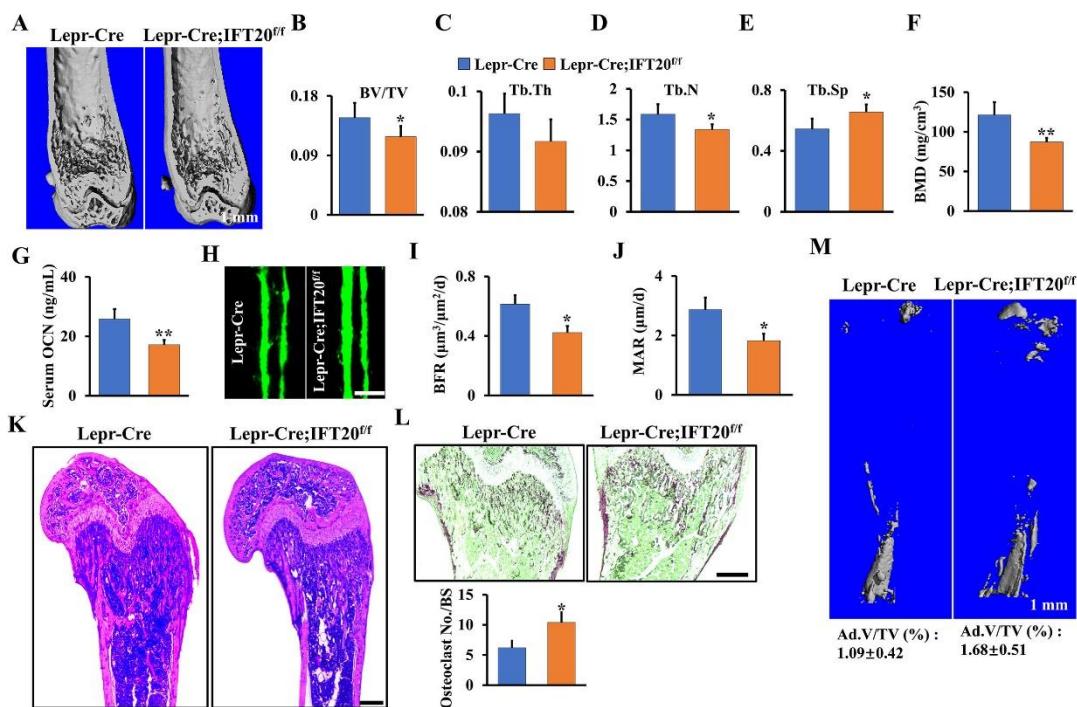
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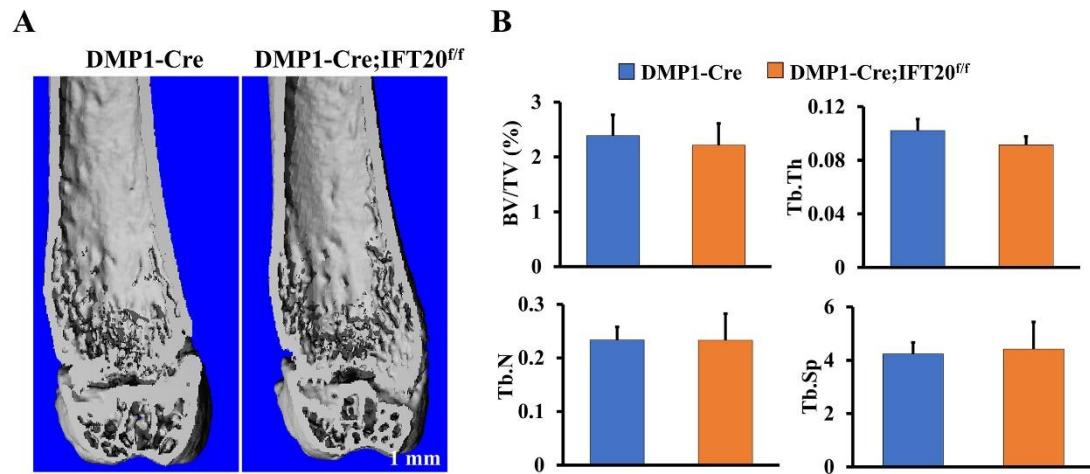
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133 **Figure. S4**

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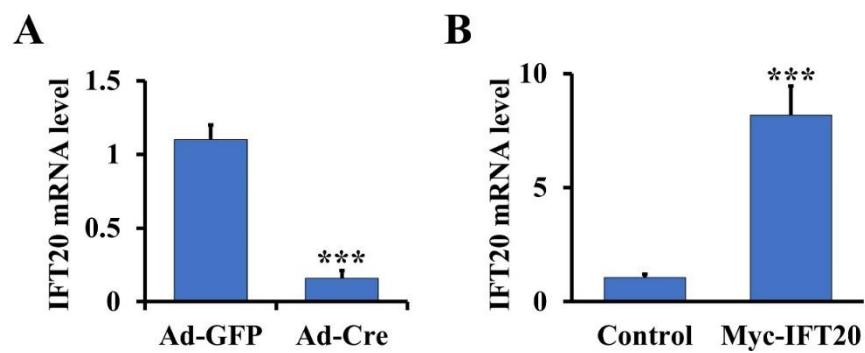
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150 **Figure. S5**

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169 **Supplementary information, Table S1**

Gene	sequence (5'-3')	Gene	sequence (5'-3')
GAPDH-F	CCTGGTCACCAGGGCTGCCATT	Runx2	GCCGGGAATGATGAGAACTA
GAPDH-R	CGTTGAATTGCCGTGAGTGGAG	Runx2	GGACCGTCCACTGTCACTTT
Glut2-F	ATCCCTGGTTCATGGTGCTG	ALP-F	AAGGCTTCTTCTGCTGGTG
Glut2-R	TCCGCAATGTACTGGAAGCAG	ALP-R	GCCTTACCCCATGATGTCC
Glut3-F	TGGTAGCTCAGATCTTGGTTGG	OSX-F	GGAGGCACAAAGAACCCATACGC
Glut3-R	GATCTCTGTAGCTTGGCTTCCTC	OSX-R	TGCAGGAGAGAGGAGTCCATTG
Glut4-F	CCAGCCACGTTGCATTGTA	OCN-F	CTTGGTGCACACCTAGCAGA
Glut4-R	ACACTGGCCTAGCTGTATTCT	OCN-R	ACCTTATTGCCCTCCTGCTT
IFT20-F	GCGAGGCAGGGCTGCATTTGAT	Glut1-F	GGGCATGTGCTTCCAGTATGT
IFT20-R	CCTTGCACTCCTCCTTGAGCTCC	Glut1-R	ACGAGGAGCACCGTGAAGAT
HK2-F	TGATGCCCTGCTTATTACCGG	Pfkfb3-F	CTCCCAGCCCCGGTAAGACTTACA
HK2-R	AACCGCCTAGAAATCTCCAGA	Pfkfb3-R	GCTTCACAGCCTCACGCCATA
Pfkfb4-F	CCGACACTCATTGTCATGGTGG	Ldha-F	TGTCTCCAGCAAAGACTACTGT
Pfkfb4-R	CACGCCAATCCAGTTGAGGTAC	Ldha-R	GAUTGTACTTGACAATGTTGGGA
PPARr-F	ACAGAGATGCCATTCTGGCCCACCAAC	Fabp4-F	ATGTGTGATGCCCTTGTGGGAACC
PPARr-R	GCTGGAGAAATCAACTGTGGTAAAGGGC	Fabp4-R	CCATGCCCTGCCACTTCCTGTG
C/EPBa-F	CCGGTGGGGCAAAGCCAAGAAG	Adiponectin-F	ATGCTACTGTTGCAAGCTCTCCTG
C/EPBa-R	TCTTGCGCACCGCGATGTTGTTG	Adiponectin-R	AGGGACCAAAGCAGGAGCTAGCT
ChIP1-F	CCTGAAGCTAGCAACAGACT	ChIP3-F	CTTGGGCACAGGAACACGGGA
ChIP1-R	TCACCAATCAGCCATTTT	ChIP3-R	GGTGTGTTACAACCGCGTGTG
ChIP2-F	GGCAAAGTGGTGATCAGGAG	ChIP4-F	CTGGGACTGCAGGTTCTAGC
ChIP2-R	CTAATTGAGCATGGACCCCT	ChIP4-R	TCTGAGAGGCGTGGTTCTGT

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