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Supplemental Information

**FGFR3 in Periosteal Cells Drives Cartilage-to-Bone Transformation in
Bone Repair**

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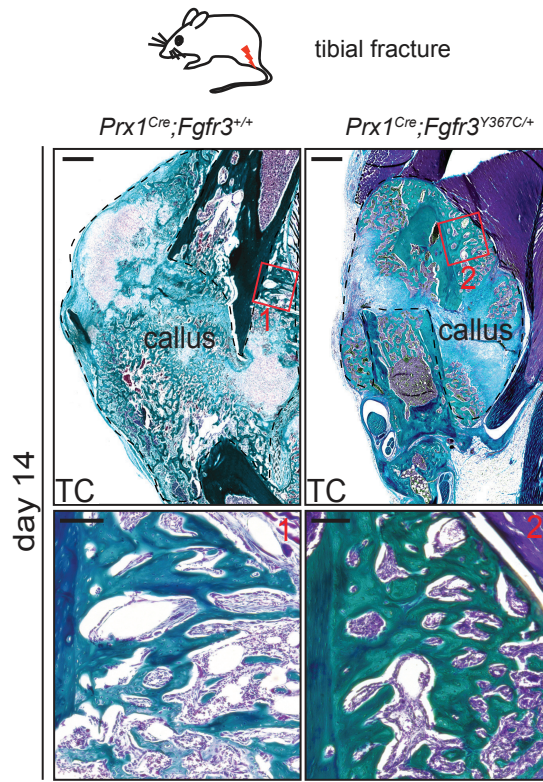


Figure S1. Direct bone formation at the periphery of the callus is not affected in *Prx1^{Cre};Fgfr3^{Y367C/+}* mice.

(A) Representative Masson's Trichrome (TC) staining of tibial fracture calluses from *Prx1^{Cre};Fgfr3^{+/+}* (control) and *Prx1^{Cre};Fgfr3^{Y367C/+}* (mutant) mice at day 14 post-fracture and high magnification of new bone area at the periphery of the callus showing normal bone deposition in mutant compared to control. Scale bar: 1mm, high magnification: 100 μ m.

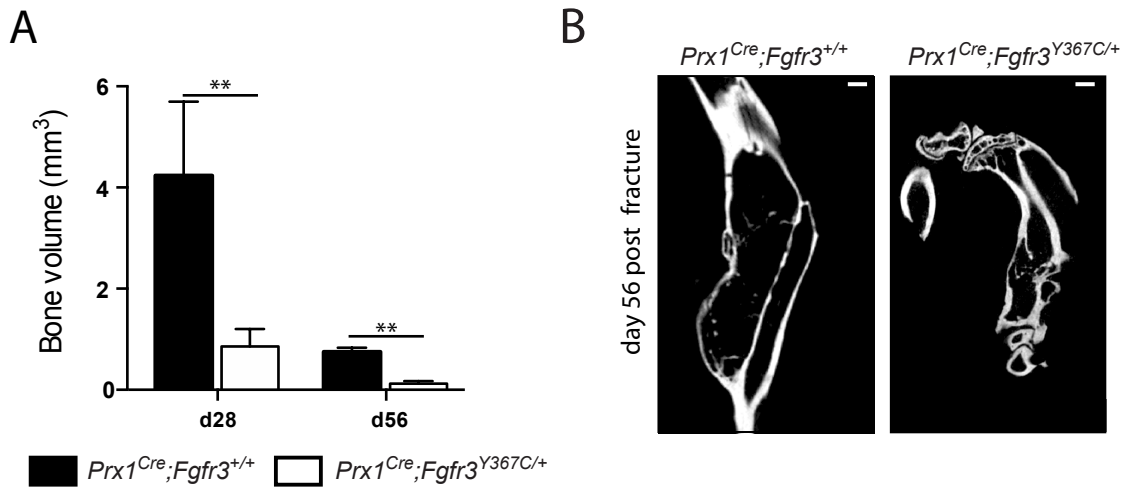


Figure S2. Reduced bone formation in *Prx1^{Cre};Fgfr3^{Y367C/+}* mutant calluses.

(A) Quantification of bone volume via μ CT in the callus of *Prx1^{Cre};Fgfr3^{+/+}* control and *Prx1^{Cre};Fgfr3^{Y367C/+}* mutant mice at day 28 and 56 post-fracture (n=4-5 per group). (B) Representative μ CT images of *Prx1^{Cre};Fgfr3^{Y367C/+}* and *Prx1^{Cre};Fgfr3^{+/+}* calluses at 56 days post-fracture. Values represent the mean \pm SD. **p<0.01 using Mann-Whitney test. Scale bar: 1mm.

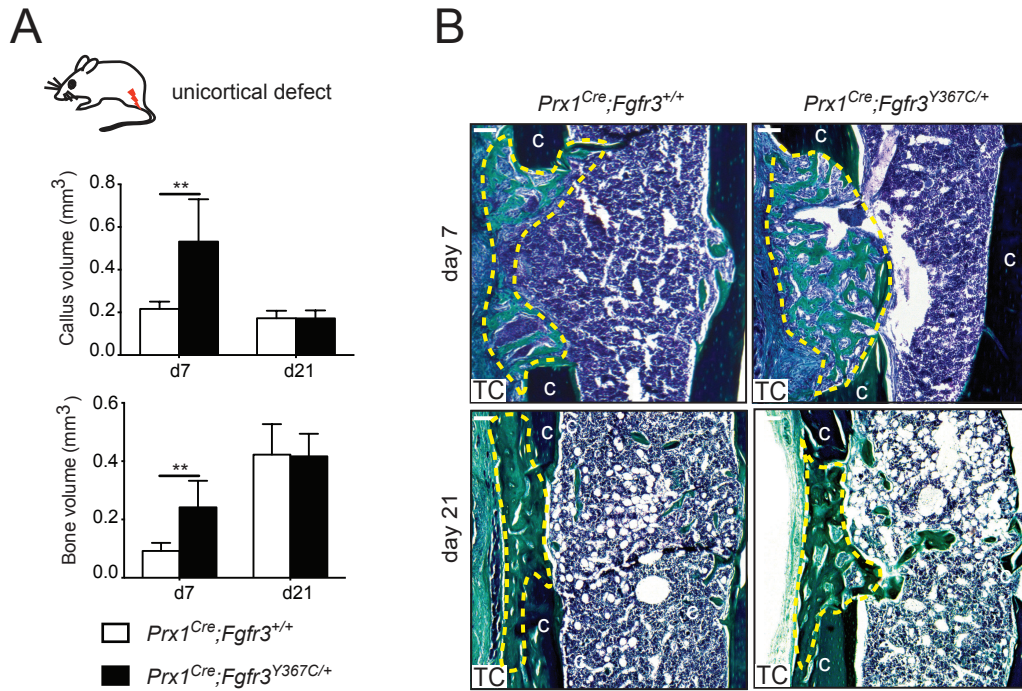


Figure S3. Healing via intramembranous ossification is unaltered in *Prx1^{Cre};Fgfr3^{Y367C/+}* mice.

(A) Histomorphometric analyses of callus and bone volumes at day 7 and 21 post-unicortical defect in the tibia of *Prx1^{Cre};Fgfr3^{+/+}* and *Prx1^{Cre};Fgfr3^{Y367C/+}* mice. n=5 per group. (B) Representative TC staining of cortical defect calluses from *Prx1^{Cre};Fgfr3^{+/+}* and *Prx1^{Cre};Fgfr3^{Y367C/+}* mice at day 7 (upper panels) and day 21 (lower panels). Scale bar: 0.1mm. Values represent the mean \pm SD. **p<0.01 using Mann-Whitney test.

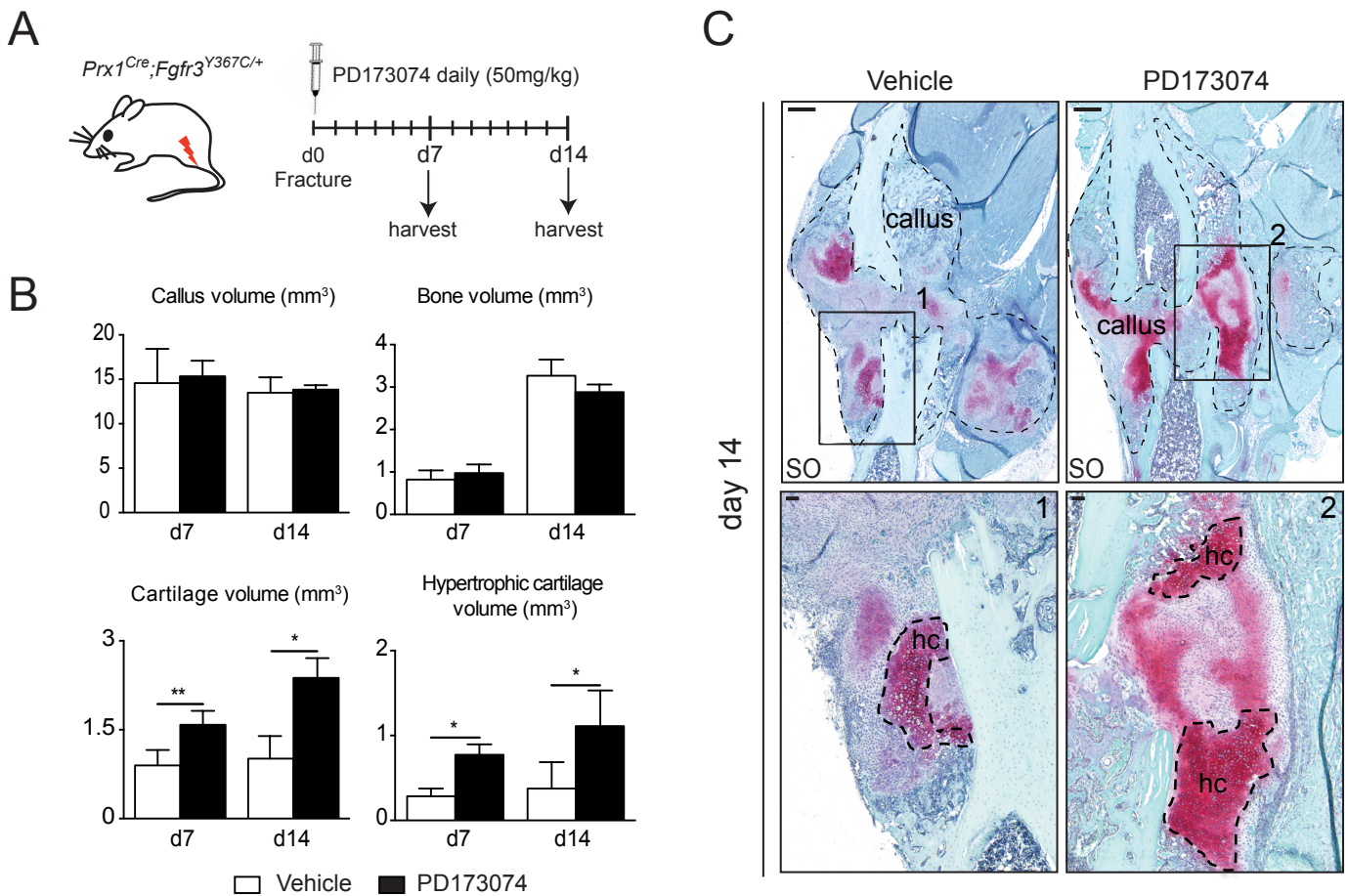


Figure S4. PD173074 treatment increases cartilage and hypertrophic cartilage in the callus of *Prx1^{Cre};Fgfr3^{Y367C/+}* mice.

(A) Experimental design of daily injection of PD173074 (50mg/kg) or vehicle (control) in *Prx1^{Cre};Fgfr3^{Y367C/+}* mice with tibial fracture. (B) Histomorphometric analysis of total callus, bone, cartilage and hypertrophic cartilage volumes of PD173074 treated and control mice at days 7 and 14 post-fracture. (n=4 or 5 per group). (C) Representative callus sections of *Prx1^{Cre};Fgfr3^{Y367C/+}* treated and control mice stained with Safranin'O (SO) at day 14 post-fracture and high magnification of hypertrophic cartilage area (Box 1-2). Scale bar: 1mm, high magnification: 150 μ m.

Values represent the mean \pm SD. **p<0.01 using Mann-Whitney test.

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Goat polyclonal anti-CD31	BioTechne	AF3628
Rat polyclonal anti-EMCN	Santa Cruz	sc-65495
Rabbit polyclonal anti-COLX	Abcam	ab58362
Rabbit polyclonal anti-OSX	Abcam	ab22552
Rabbit polyclonal anti-SOX2	Abcam	ab97959
Rabbit monoclonal anti-SOX9	Abcam	ab182530
Rabbit polyclonal anti-KI67	Abcam	ab15580
Rabbit polyclonal anti-VEGFA	Abcam	ab46154
Goat polyclonal anti-POSTN	BioTechne	AF2955
Mouse monoclonal anti-COLX	BIOCYC	N.2031501005
Alexa Fluor 488 goat anti-rabbit	Invitrogen	A11034
Alexa Fluor 488 donkey anti-goat	Invitrogen	A11055
Alexa Fluor 546 goat anti-rabbit	Invitrogen	A11056,
Alexa Fluor 647 goat anti-rabbit	Invitrogen	A-21245
Rhodamine donkey anti-rat	Jackson immunoresearch	712-025-150
streptavidin-HRP	BD Biosciences	554066
CD45-BV650	BD Biosciences	563410
TER119-BV650	BD Biosciences	747739
CD51-BV711	BD Biosciences	740755
THY1-BV786	BD Biosciences	564365
TIE2-APC	BioLegend	124009
6C3-PeCy7	BioLegend	BLE108313
Chemicals		
PD173074	Sigma	P2499
Experimental Models		
Mouse : C57BL/6ScNj, Prx1 ^{Cre}	Jackson Laboratory	005584
Mouse : Rosa-tdTomato-EGFP	Jackson Laboratory	007676
Mouse : Fgfr3 ^{Y367C}	Laurence Legeai Mallet Laboratory	
Softwares		
FlowJo, LLC software	FlowJo, LLC	
ZEN software	Carl Zeiss	
Ctan	Bruker	
GraphPad Prism	GraphPad	