Supplemental Tables and Figures

Table S1 Primary antibodies used for immunohistochemistry or Western Blotting

Primary antibody	Cat. number	Source	Dilution
Nf2/merlin	12888S	Abcam, Cambridge, United Kingdom	1:1000(WB)
Nf2/merlin	AF7563	Beyotime Biotechnology, Shanghai, China	1:100(IF)
GAPDH	60004-1-Ig	Proteintech, Wuhan, China	1:5000(WB)
Runx2	AF2593	Beyotime Biotechnology, Shanghai, China	1:1000(WB) 1:500(IF)
Sp7	ab227820	Abcam, Cambridge, United Kingdom	1:1000(WB) 1:500(IF)
GM130	11308-1-Ig	Proteintech, Wuhan, China	1:500(IF)
COL1	ab21286	Abcam, Cambridge, United Kingdom	1:500(IF)
COL1	AF1840	Beyotime Biotechnology, Shanghai, China	1:1000(WB, IHC)
COL1	SP1.D8	Developmental Studies Hybridoma Bank, DHSB	1:100(IF)
AKT	9272S	Cell Signaling Technology, Danvers, MA	1:1000(WB) 1:500(IF)
P-AKT(S473)	4060S	Cell Signaling Technology, Danvers, MA	1:1000(WB) 1:100(IF)
FGFR1	60325-1-Ig	Proteintech, Wuhan, China	1:1000(WB) 1:100(IF)
PLCγ	2822S	Cell Signaling Technology, Danvers, MA	1:1000(WB)
P-PLCγ	3285S	Cell Signaling Technology, Danvers, MA	1:1000(WB)
GRP78 BiP	200310-4F11	Zen-bio, Chengdu, China	1:1000(WB)
ATF4	R381426	Zen-bio, Chengdu, China	1:1000(WB)
FLAG	1479S	Cell Signaling Technology, Danvers, MA	1:1000(WB)
HA	3724S	Cell Signaling Technology, Danvers, MA	1:1000(WB)
FLAG	66008-4-Ig	Proteintech, Wuhan, China	1:1000(WB)
HA	66006-2-Ig	Proteintech, Wuhan, China	1:1000(WB)
Normal Rabbit IgG	2729	Cell Signaling Technology, Danvers, MA	1:1000(WB)
Alexa Fluor 488-labeled Goat Anti-Rabbit IgG(H+L)	111-545-144	Jackson ImmunoResearch, West Grove, PA	1:500
Alexa Fluor 594-labeled Goat Anti-Rabbit IgG(H+L)	705-585-003	Jackson ImmunoResearch, West Grove, PA	1:500
Alexa Fluor 488-labeled Goat Anti-Mouse IgG(H+L)	715-545-150	Jackson ImmunoResearch, West Grove, PA	1:500
Alexa Fluor 594-labeled Goat Anti-Mouse IgG(H+L)	715-585-150	Jackson ImmunoResearch, West Grove, PA	1:500
Peroxidase AffiniPure Donkey Anti-Rabbit IgG (H+L)	711-035-152	Jackson ImmunoResearch, West Grove, PA	1:500
Peroxidase AffiniPure	715-035-150	Jackson ImmunoResearch, West	1:500

Donkey Anti-Mouse IgG (H+L)		Grove, PA	
Goat Anti-Rabbit IgG (H+L)-Biotinylated	BA-1000-1.5	Vector Laboratories, Newark, CA	1:500

Table S2 Primers used in Nf2 site mutation construction.

Gene	Forward (5'-3')	Reverse (5'-3')
overlap	*tagagctagcgaaTTATGGCCGGAGCCATCG*	*tcgcggccgcggatcTCAAATGCAGATAGGTCTTCTGCCTTG*
Ser10A	*TCTCGCATGAGATTCAGCTCA*	*TGAGCTGAATCTCATGCGAGA*
Thr230A	*TAAAAAGGGCGCGGAGTTGCTGC*	*GCAGCAACTCCGCGCCCTTTTTA*
Ser315A	*AAAGCTGACGCTTTAGAAGTT*	*AACTTCTAAAGCGTCAGCTTT*
Ser518A	*AGCGACTTTCTATGGAGAT*	*TATCTCCATAGAAAGTCGCT*
Ser10D	*TTCTCGCATGGACTTCAGCTCA*	*TGAGCTGAAGTCCATGCGAGAA*
Thr230D	*TAAAAAGGGCGACGAGTTGCTGC*	*GCAGCAACTCGTCGCCCTTTTTA*

Table S3 Primers used in qRT-PCR analysis.

Gene	Forward (5'-3')	Reverse (5'-3')
Nf2	*CATGAGCTTCAGCTCACTCAAGAGGAAG*	*ATCCCCGCTTGTGCACAGAGGGGTCATAG*
Alp	*GGACAGGACACACACACACA*	*CAAACAGGAGAGCCACTTCA*
OCN	*TGAGCTTAACCCTGCTTGTG*	*TAGGGCAGCACAGGTCCTA*
Col1	*ATAGCTCGTCACAAGCAGGG*	*TGACAAAGCCTTCATGTCCA*
Sp7	*CCTACTTACCCATCTGACTTTGCT*	*CTTATAGACATCTTGGGGTAGGACA*

Supplemental Figures

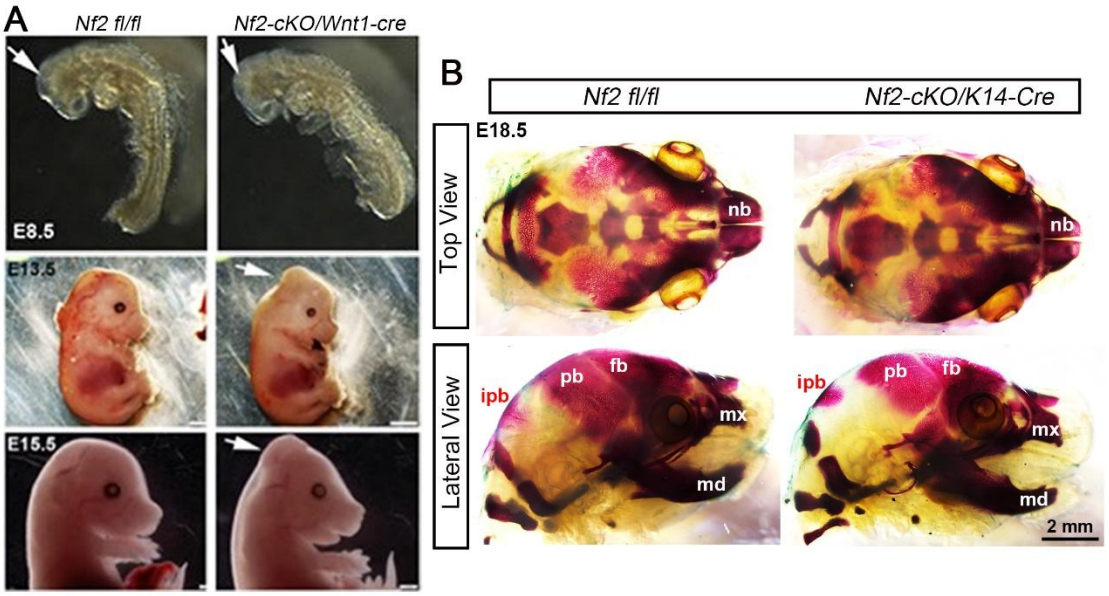

Fig 1S ***Epithelial-specific Nf2 deletion reveals normal craniofacial bone development.***

A: The appearance of the phenotype of the cranial neural crest-specific *Nf2* deletion at E8.5, E13.5 and E15.5, with visible midfacial hypoplasia. Scale bars: 2 mm.

B: Alizarin red-stained skulls of the *Nf2 fl/fl* and *Nf2-cKO/K14-Cre* mice at E18.5 show normal craniofacial bone development. fb, frontal bone; pb, parietal bone; ib, interparietal bone; oc, occipital bone; na, nasal bone; ma, mandible; mx, maxilla. Scale bars: 2 mm.

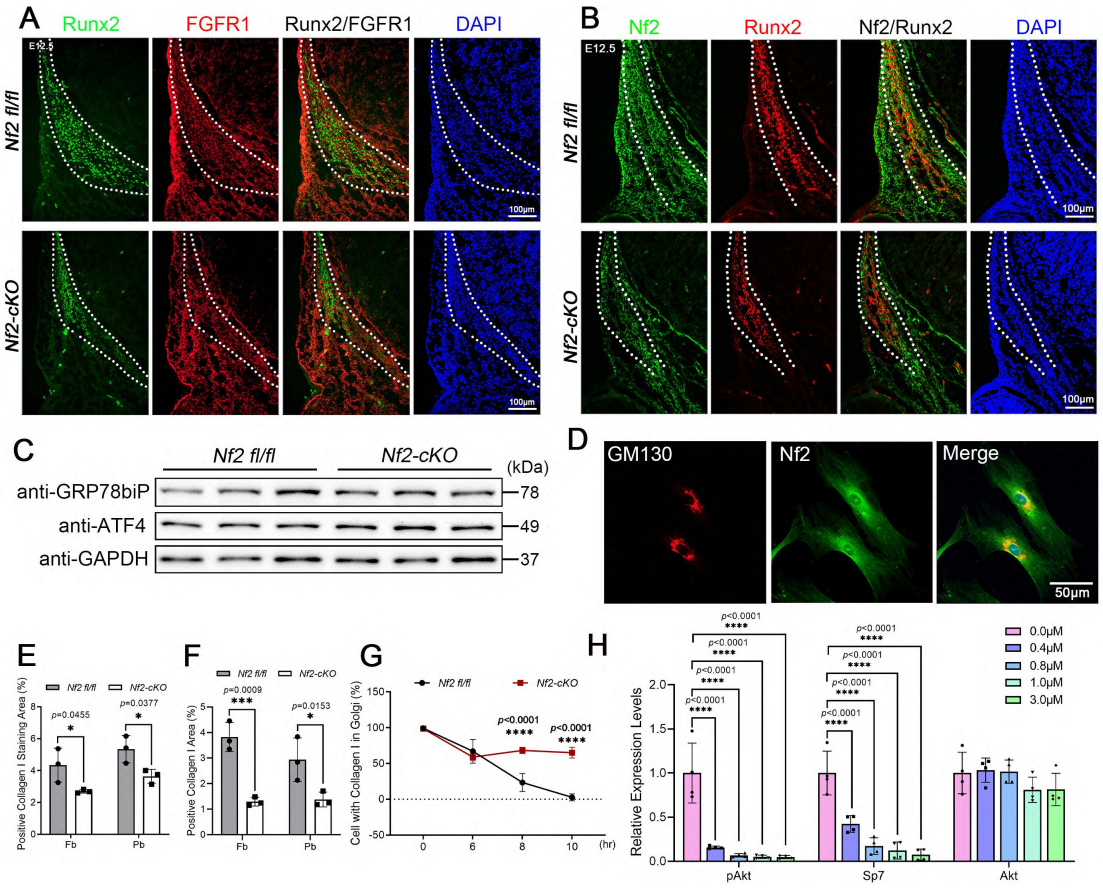

Fig 2S Localization of FGFR1 and Nf2 in early osteoprogenitors and analysis of ER stress in *Nf2* mutants.

A: Co-localization of Runx2^{+} osteoprogenitors and FGFR1 in E12.5 coronal sections (dotted lines demarcate osteogenic zone). *Nf2* mutant mice maintains normal FGFR1 expression in Runx2^{+} osteoprogenitors. Scale bars: 100 μm.

B: Co-localization of Runx2^{+} osteoprogenitors and Nf2 in E12.5 coronal sections (dotted lines demarcate osteogenic zone). *Nf2* mutant mice maintains normal FGFR1 expression in Runx2^{+} osteoprogenitors. Scale bars: 100 μm.

C: Western blot analysis of ER stress sensor molecules in *Nf2* mutant CNC-derived osteoblasts.

D: Colocalization of Nf2 and GM130 was analyzed by immunofluorescence staining in *Nf2* mutant CNC-derived osteoblasts. Scale bars: 50 μm.

E: Quantification of positive Collagen I staining area in *Nf2* mutant.

F: Quantification of positive Collagen I area by polarized Sirius Red staining in polarized light.

G: Quantification of the percentage of the cells with collagen I in the Golgi.

H: Quantification of expression level of pAkt, Sp7 and Akt (normalized to GAPDH).

Data were expressed as means ± SD and each dot represents an individual biological replicate. *P* values were calculated by unpaired Student's *t*-test with two-tailed

analysis without adjustments (E, F and G) and 2-way ANOVA with Tukey's multiple-comparison test (H). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$.

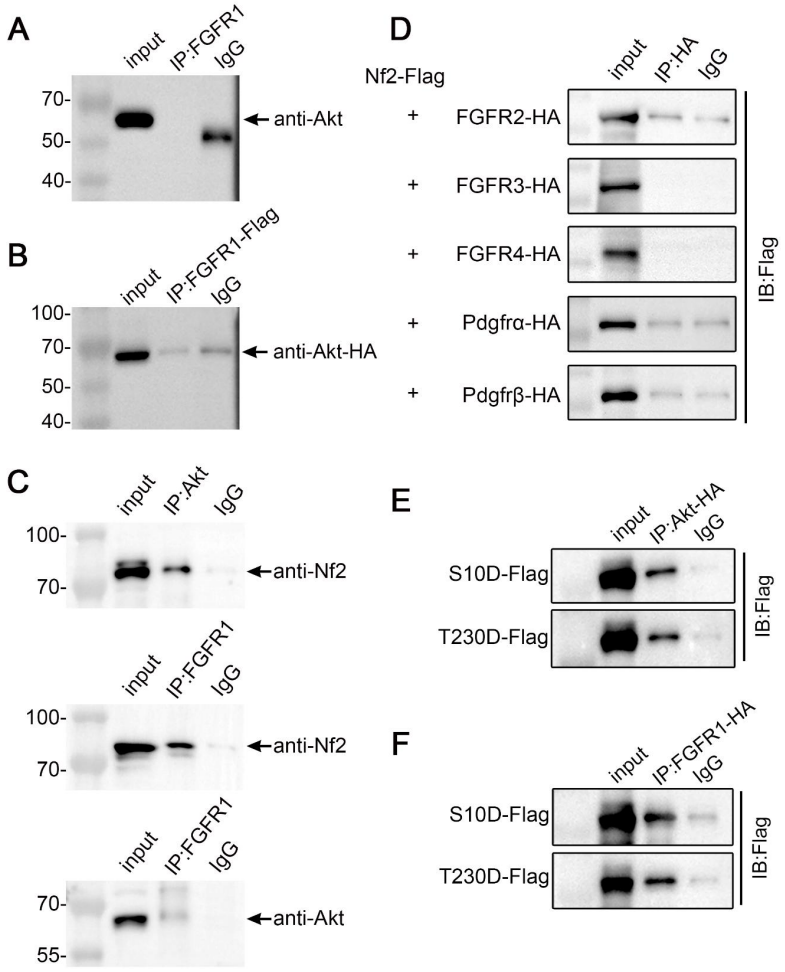

Fig 3S Different receptors were evaluated the interactions with Nf2.

A-B: No direct FGFR1-Akt complex in primary osteoblasts (**B**) and HEK293T cells using co-IP assay.

C: co-IP assay was performed with HEK293T cells transfected with the indicated plasmids, *FGFR1-Flag*, *Akt-HA*, and co-expression of Nf2. co-IP was performed using FGFR1, Akt and Nf2 antibody. Western blot analysis showed that Nf2 interacts with both FGFR1 and Akt individually. However, no interaction was detected between *FGFR1-Flag* and *Akt-HA*, suggesting that Nf2 does not bridge FGFR1 and Akt into a stable ternary complex under the tested conditions.

D: co-IP assay showed that *Nf2-Flag* and *FGFR2-HA*, *FGFR3-HA*, *FGFR4-HA*, *Pdgfrα-Flag*, *Pdgfrβ-Flag* could not form an interaction in HEK293T cells.

E: co-IP assay was performed with HEK293T cells transfected with the indicated plasmids. The same amount of tagged Nf2 single site continuous phosphorylation plasmids (*S10D-Flag, T230D-Flag*) and *Akt-HA* plasmids were co-transfected. Immunoprecipitation was performed using a FLAG, HA antibody.

F: co-IP assay was performed with HEK293T cells transfected with the indicated

plasmids. The same amount of tagged Nf2 single site continuous phosphorylation plasmids (*S10D-Flag, T230D-Flag* and *FGFR1-HA* plasmids were co-transfected. Immunoprecipitation was performed using a FLAG, HA antibody.