Supplementary data

Cytoskeletal protein 4.1G is essential for the primary ciliogenesis and osteoblast differentiation in bone formation

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Table S1. Primer sequences

Primer name	Sequence
4.1G Wt-Neo Both Left1	GTGGTCTTGAGTTGGCTGTG
4.1G Wt Right2	TTATCCCAAACCAGCCAGTC
4.1G Neo Right1	TCGCCTTCTTGACGAGTTCT
SX-F	GATGATTTGAGTGGAAATGTGAGGTA
SX-R	CTTATGTTTATAGGCATGCACCATGTA
4.1G-F	GGAAGTCCCCATTGTTCAAA
4.1G-R	AGAGCTGCGTCTCCTGTGAT
Gli1-F	GGTGCTGCCTATAGCCAGTGTCCTC
Gli1-R	GTGCCAATCCGGTGGAGTCAGACCC
Ptch 1-F	CTCTGGAGCAGATTTCCAAGG
Ptch 1-R	TGCCGCAGTTCTTTTGAATG
Osterix-F	AGCGACCACTTGAGCAAACAT
Osterix-R	GCGGCTGATTGGCTTCTTCT
Osteocalcin-F	AAGCAGGAGGGCAATAAGGT
Osteocalcin-R	ACTTGCAGGGCAGAGAGAGA
Runx2-F	ACAACCACAGAACCACAAG
Runx2-R	TCTCGGTGGCTGGTAGTGA
GAPDH-F	AGGTCGGTGTGAACGGATTTG
GAPDH-R	TGTAGACCATGTAGTTGAGGTCA

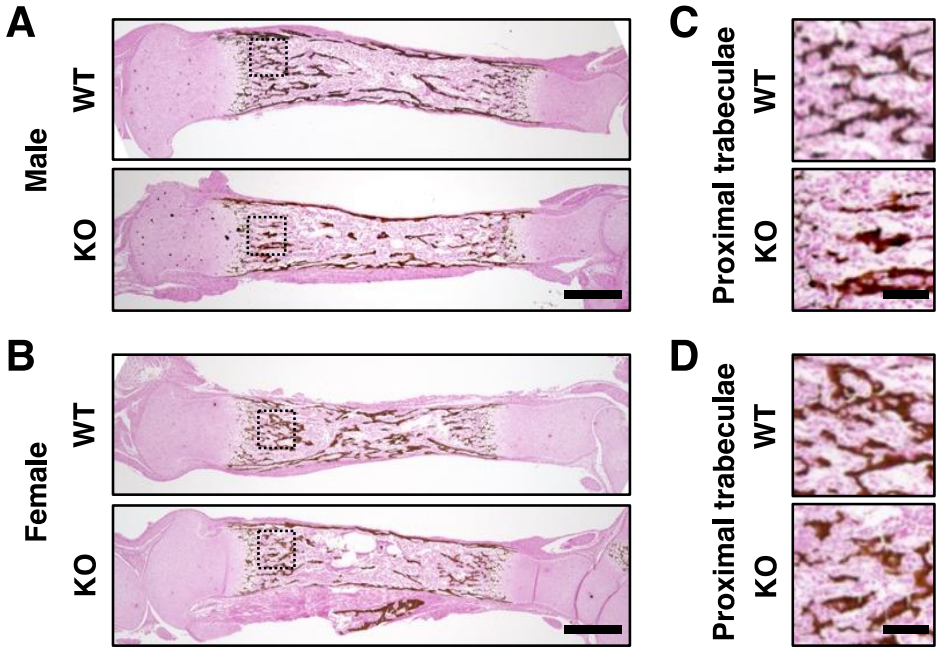

Figure S1. Protein 4.1G promotes bone formation at the trabecular bone in newborn tibia. The newborn tibia sections from the male (**A, C**) and female (**B, D**) mice were stained by modified von Kossa's reaction. (**A**, **B**) Images are the same as the images of figures **1A** and **1B**. (**C**, **D**) Enlarged views of the boxed areas in **A** and **B**, respectively. Scale bars, 500 μm (**A**, **B**) and 100 μm (**C**, **D**).

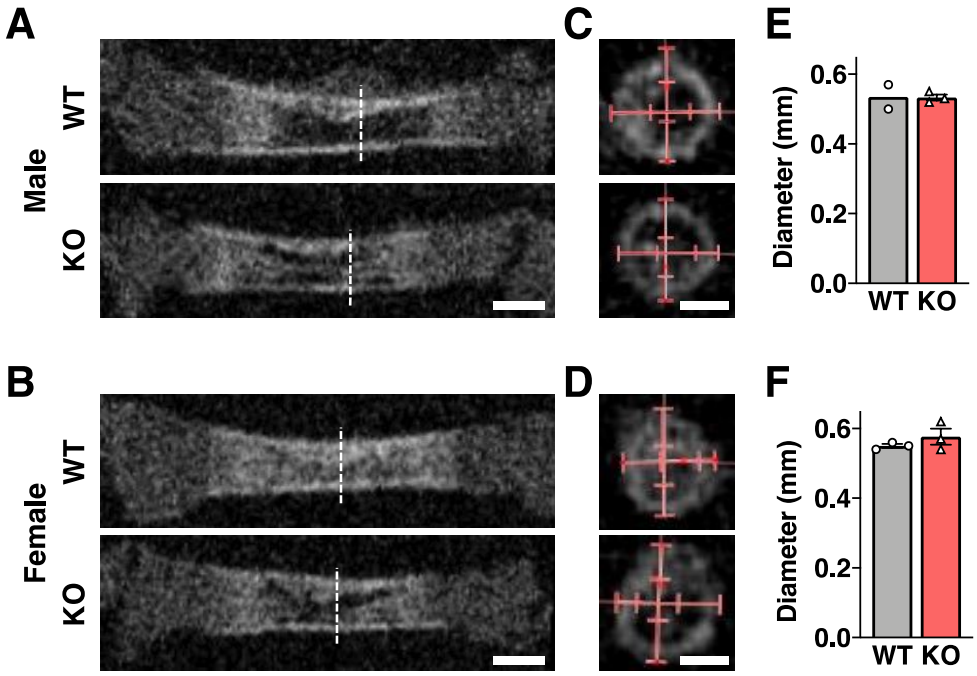

Figure S2. Protein 4.1G has no effect on the tibia diaphyseal diameter. (**A, B**) Micro-CT images of the male (**A**) and female (**B**) mice. (**C, D**) Cross-section images obtained at white dashed bars in (**A**) and (**B**) were shown, respectively. Two transverse diameters that cross at a right angle were measured (illustrated as the red lines), and the longer diameter was applied for the quantification. (**E**, **F**) Quantified diameter. Scale bars, 500 μm (**A**, **B**) and 300 μm (**C**, **D**).

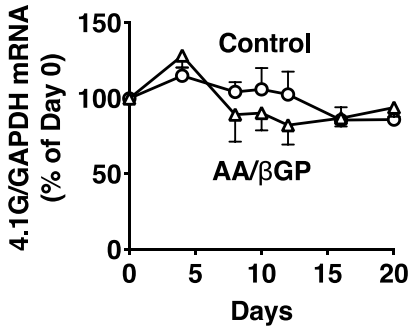

Figure S3. mRNA expression of 4.1G is not changed during the differentiation of MC3T3-E1 cells. The confluent MC3T3-E1 cells were treated with (triangle) or without (circle) 250 μM ascorbic acid and 50 μM β-glycerol phosphate (AA/βGP) for up to 20 days. mRNA expression of 4.1G was analyzed by RT-qPCR. mRNA content of 4.1G was normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Data are presented as the mean ± standard error of the mean (S.E.M.) from three independent experiments.

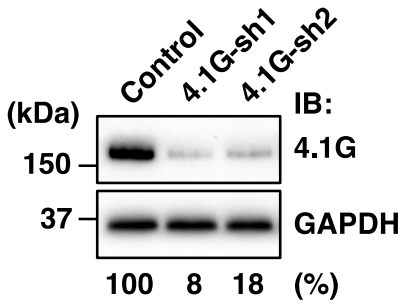

Figure S4. Knockdown efficiency of 4.1G-shRNA1 (4.1G-sh1) and 4.1G-shRNA2 (4.1G-sh2) in MC3T3-E1 cells. The whole cell lysates were immunoblotted (IB) with anti-4.1G or anti-GAPDH antibody. One of two independent representative immunoblots was shown. The relative amount of 4.1G was normalized to GAPDH shown below the blots (%).

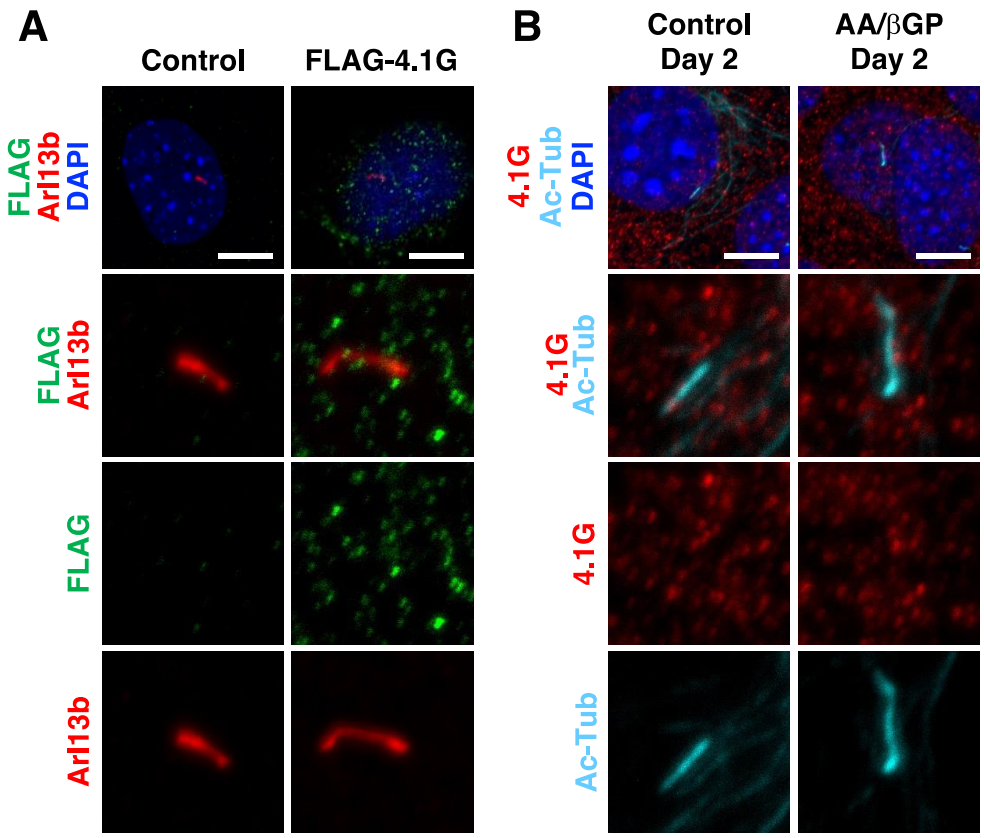

Figure S5. 4.1G is not accumulated at primary cilium or periciliary region. (**A**) Distribution of exogenous 4.1G in MC3T3-E1 cells. MC3T3-E1 cells expressing FLAG-tagged 4.1G were co-immunolabeled with the anti-FLAG (green) and anti-Arl13b (red) antibodies. (**B**) Distribution of endogenous 4.1G in MC3T3-E1 cells. MC3T3-E1 cells treated with AA/bGP for 2 days were co-immunolabeled with the anti-4.1G (red) and anti-Ac-Tub (cyan) antibodies. The cell nuclei were labeled by DAPI. Scale bars, 10 μm.

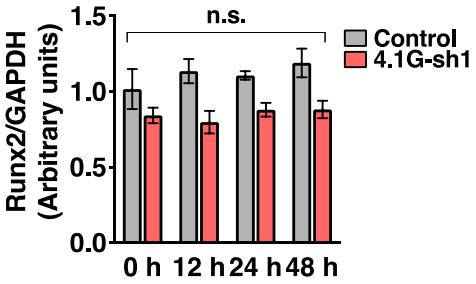

Figure S6. Runx2 mRNA level is not influenced by 4.1G expression. MC3T3-E1 cells transfected with control or 4.1G-sh1 were treated with AA/bGP for 2 days. The cells were further stimulated with 2 μM of purmorphamine at indicated time points. Relative mRNA expression of Runx2 was normalized to GAPDH. Data are presented as the mean ± S.E.M. from three independent experiments. n.s., not significant.