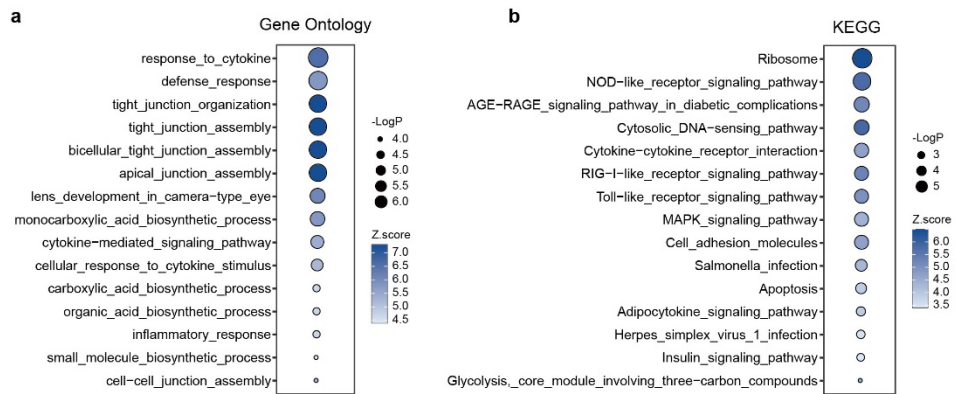
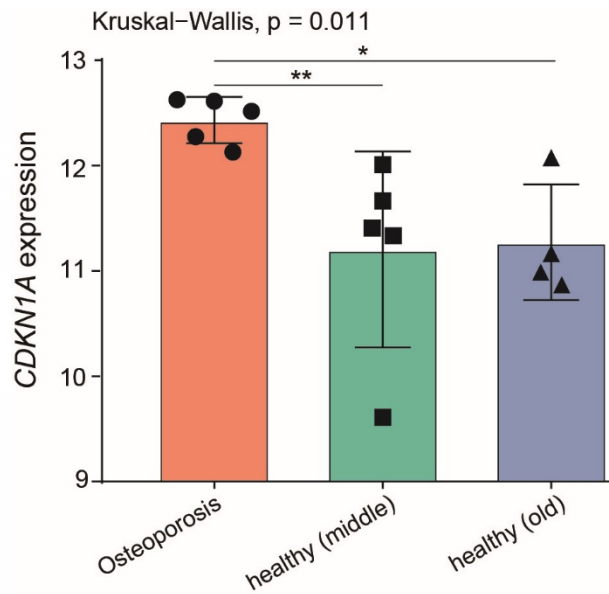


# 1 Supplementary information



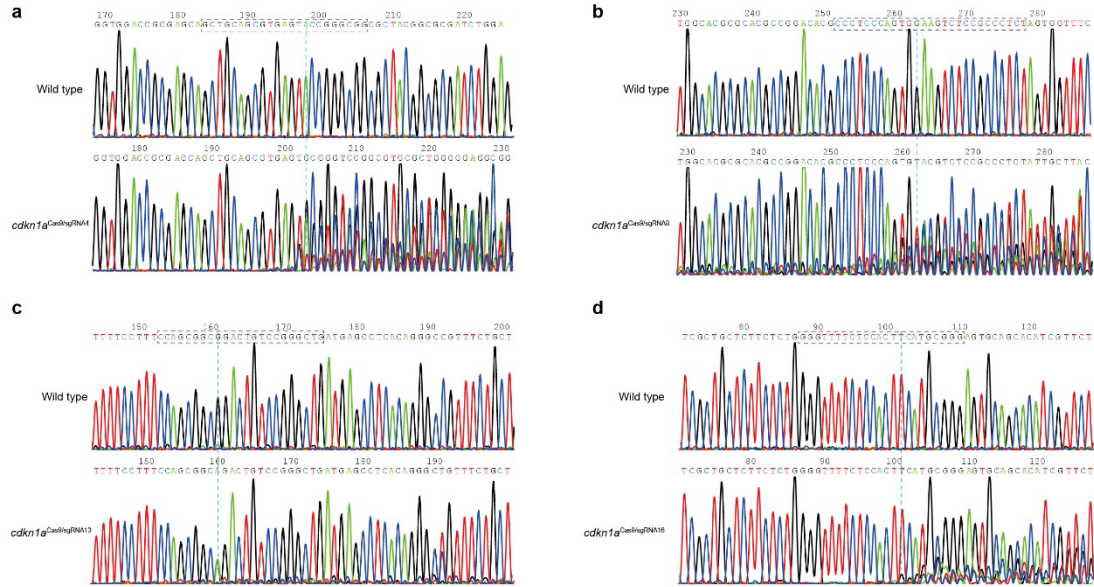
2

3 **Supplementary Fig. 1. Gene ontology (GO) and pathway enrichment analysis of**  
 4 **DNB genes. (a) GO terms enriched in the differentially expressed genes (DEGs). (b)**  
 5 **Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways enriched in the DEGs.**



6

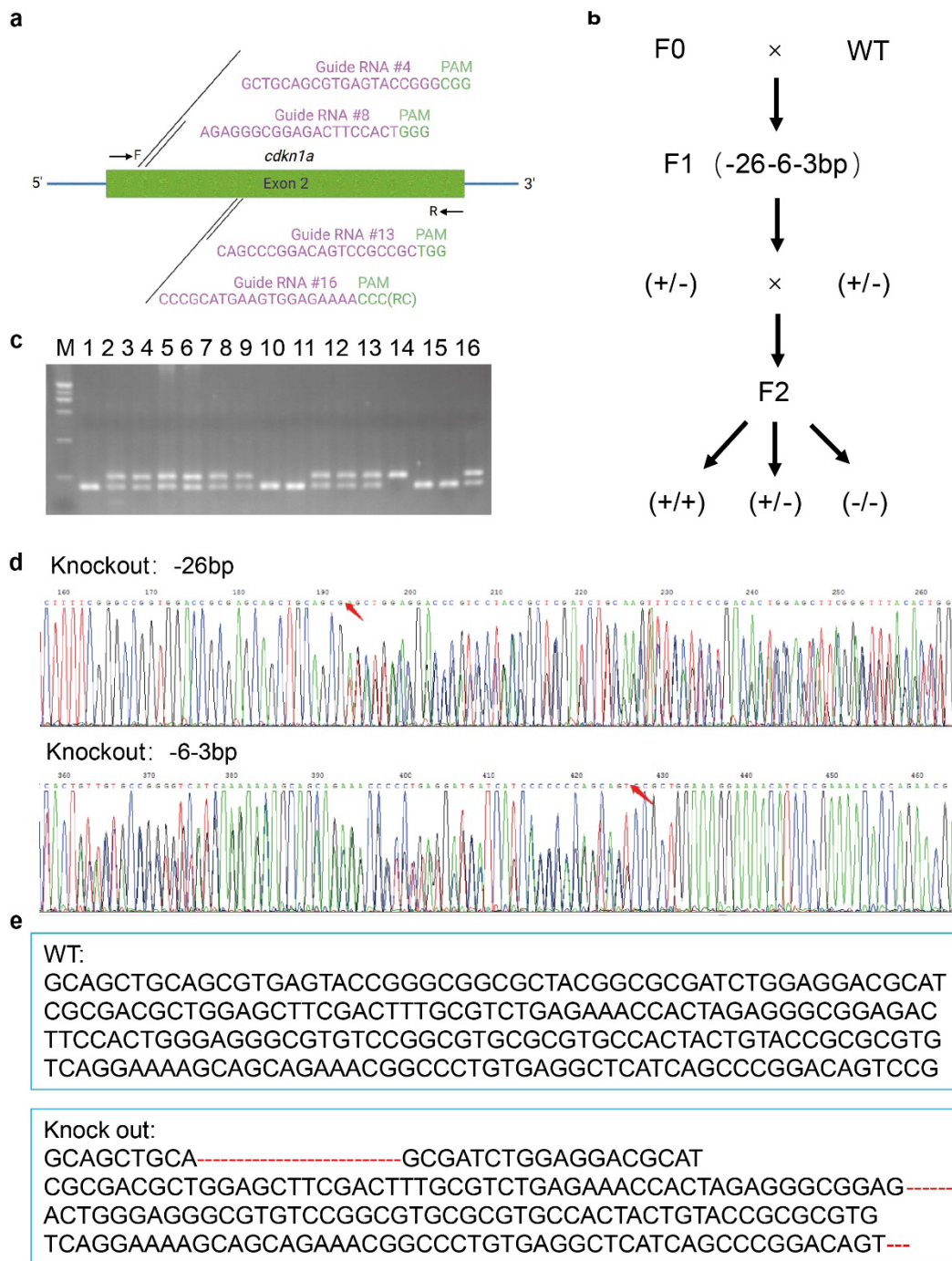
7 **Supplementary Fig. 2. Differentially expression analysis indicated that *CDKN1A***  
 8 **was remarkably upregulated in primary osteoporosis patients in contrast with**  
 9 **healthy donors with both middle and old ages.**



10

11 **Supplementary Fig. 3. Mutation of the target sites with the gRNAs was**  
 12 **confirmed by Sanger sequencing.** (a) Sequencing peaks for wild type (WT) and  
 13 *cdkn1a*<sup>Cas9/sgRNA4</sup> crisprants. (b) Sequencing peaks for WT and *cdkn1a*<sup>Cas9/sgRNA8</sup>  
 14 crisprants. (c) Sequencing peaks for WT and *cdkn1a*<sup>Cas9/sgRNA13</sup> crisprants. (d) Sequencing  
 15 peaks for WT and *cdkn1a*<sup>Cas9/sgRNA16</sup> crisprants. The black dotted box marks the target  
 16 sequence. The blue dotted line marks the start position of mutation.

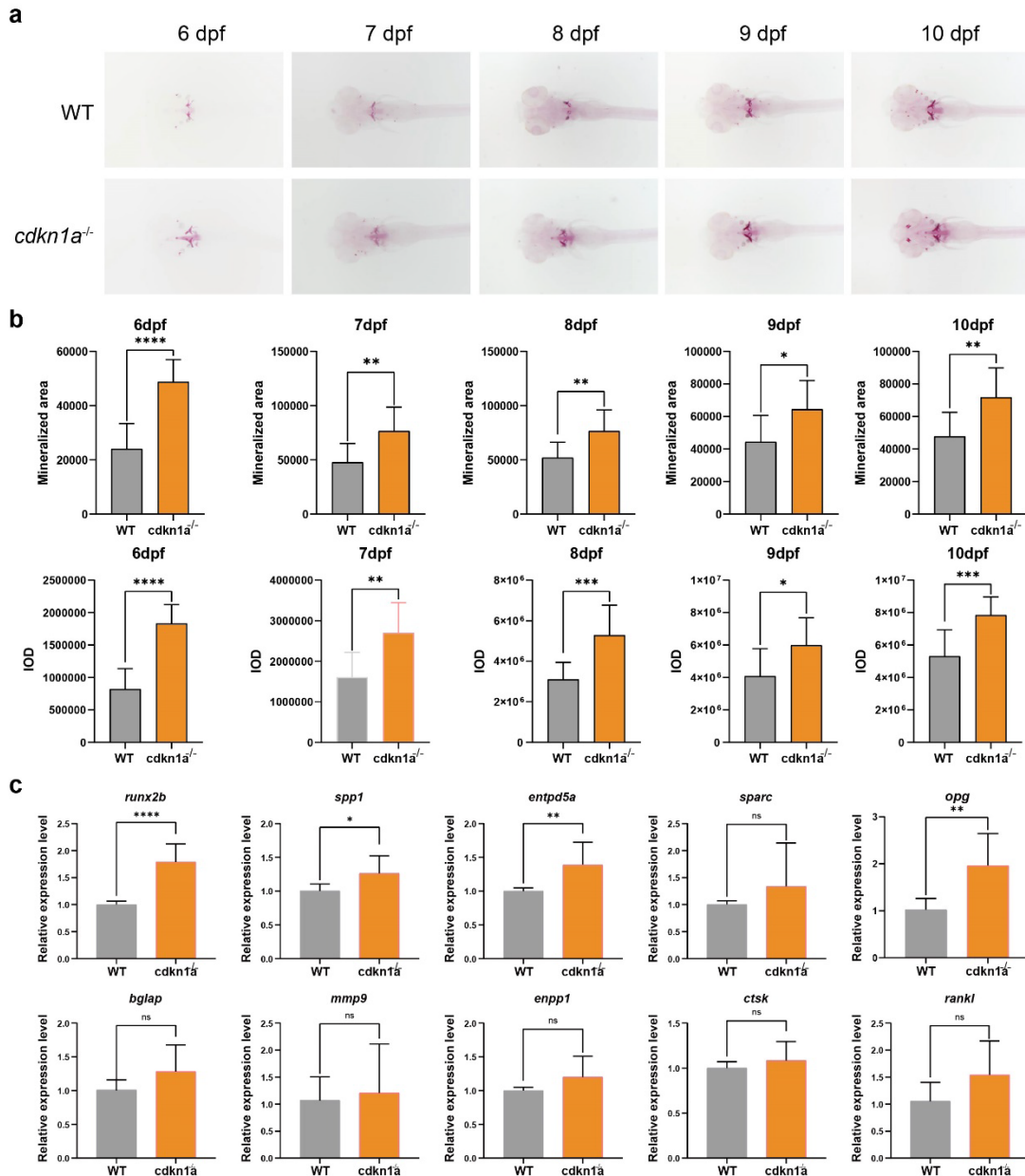
17



18

19 **Supplementary Fig. 4. Establishment of a homozygous knockout model of the**  
 20 **cdkn1a gene in zebrafish.** (a) The *cdkn1a* sgRNAs site in exon-2. (b) Diagram  
 21 detailing crossing scheme used to obtain a homozygous strain. (c) Gel electrophoresis  
 22 of PCR products of F1 mutant. (d) The F1 mutant sequencing result. The red arrow  
 23 indicates the starting point of the frameshift mutation. (e) Sequence alignment result.  
 24 The red strikethroughs indicate frameshift mutations.

25



26

27 **Supplementary Fig. 5. The *cdkn1a*-mutant zebrafish larvae show increased bone**  
 28 **mineralization.** (a) Alizarin red staining evaluated the *cdkn1a*-mutant group on the  
 29 bone mineralization at 6, 7, 8, 9, and 10 days post-fertilization (dpf) compared to the  
 30 WT group. Scale bars: 1 mm. (b) Semiquantitative analysis of mineralization area and  
 31 IOD (n = 10). (c) mRNA expression levels of genes related to osteoblasts (*runx2b*, *spp1*,  
 32 *entpd5a*, *sparc*, *bglap*) and osteoclasts (*mmp9*, *enpp1*, *ctsk*, *rankl*, *opg*) in WT and  
 33 *cdkn1a*-mutant group (n = 20). \*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001, \*\*\*\*P < 0.0001,  
 34 Student's *t*-test.

35

36 **Supplementary Table 1. Exon 2 sequence of *cdkn1a*.**

```
ATGGCGGCGCACAAGCGGATCCTACGTTCACTCGGTAATGGGCCGACTAGGCGGA  
GTCTTTTCGGGCCGGTGGACCGCGAGCAGCTGCAGCGTGAGTACCGGGCGGCCTA  
CGGCGCGATCTGGAGGACGCATCGCGACGCTGGAGCTTCGACTTTGCGTCTGAGAA  
ACCACTAGAGGGCGGAGACTTCCACTGGGAGGGCGTGTCCGGCGTGCGCGTGCCA  
CTACTGTACCGCGCGTGTTCAGGAAAAGCAGCAGAAACGGCCCTGTGAGGCTCATC  
AGCCCGGACAGTCCGCCGCTGGAAAAGGAAAACATCCCGAAAACACCAGAACGATG  
TGCTGCACTCCCGCATGAAGTGGAGAAAACCCAGAGAAGAGCAGCGAGCTGAAG  
CGCAAACAGACCAACATCACAG
```

37

38 **Supplementary Table 2. *cdkn1a* RNA guides for CRISPR.**

Guides	Target sequence	Reverse complement sequence
Guide 1	ATCCTACGTTCACTCGGTAATGG	CCATTACCGAGTGAACGTAGGAT
Guide 2	CGACTAGGCGGAGTCTTTTCGGG	CCCGAAAAGACTCCGCCTAGTCG
Guide 3	TGCAGCTGCTCGCGGTCCACCGG	CCGGTGGACCGCGAGCAGCTGCA
Guide 4	GCTGCAGCGTGAGTACCGGGCGG	CCGCCCGGTACTCACGCTGCAGC
Guide 5	GGCGGCGCTACGGCGCGATCTGG	CCGCCGCGATGCCGCGCTAGACC
Guide 6	TGGAGGACGCATCGCGACGCTGG	CCAGCGTCGCGATGCGTCCTCCA
Guide 7	TGCGTCTGAGAAACCACTAGAGG	CCTCTAGTGGTTTCTCAGACGCA
Guide 8	AGAGGGCGGAGACTTCCACTGGG	CCCAGTGGAAGTCTCCGCCCTCT
Guide 9	CAGTAGTGGCACGCGCACGCCGG	CCGGCGTGC GCGTGCCACTACTG
Guide 10	ACTACTGTACCGCGCGTGT CAGG	CCTGACACGCGCGGTACAGTAGT
Guide 11	GCAGCAGAAACGGCCCTGTGAGG	CCTCACAGGGCCGTTTCTGCTGC
Guide 12	TCCGGGCTGATGAGCCTCACAGG	CCTGTGAGGCTCATCAGCCCGGA
Guide 13	CAGCCCGGACAGTCCGCCGCTGG	CCAGCGGCGGACTGTCCGGGCTG
Guide 14	GATGTTTTCTTTCCAGCGGCGG	CCGCCGCTGGAAAGGAAAACATC
Guide 15	CACATCGTTCTGGTGT TTTTCGGG	CCCGAAAACACCAGAACGATGTG
Guide 16	GGGTTTTCTCCACTTCATGCGGG	CCCGCATGAAGTGGAGAAAACCC

39

40 **Supplementary Table 3. Primer sequences for the genes observed in qRT-PCR.**

Gene	Forward Primer (5'-3')	Reverse Primer (5'-3')
<i>β-actin</i>	ACGAACGACCAACCTAAACTCT	TTAGACAACCTACCTCCCTTTGC
<i>cdkn1a</i>	CGCAAACAGACCAACATCAC	CGGAATAAACGGTGTCTGTCT
<i>runx2b</i>	GAAGCGGAAGAGGGAAGAGC	AATGGCTCTGTGGTAAGTGCC
<i>sp7</i>	TCCTCTCCCGCTTTTGGATT	TCCCGAATTTGTTGCAGGTC
<i>col2a1a</i>	GATGTGGAGATCAGAGCAGAGGG	CATGATGGGCAGTCTGGATGTCT
<i>sparc</i>	CCCTGCAAATACATCGCTCC	TCTCGTAAATCTTCTTCACCCTG
<i>mmp9</i>	CAGAGAGGAAAAGGCAAGGTG	AGGAATACATCATGTGAATCAATG
<i>mmp13</i>	AGACCAGGACACACTCGCAGAG	CTGCTGCATCTCCTTGAGCCT
<i>acp5a</i>	CCCCATAGAGACCGCTACAG	ACATCAGTGACGCCCTTGTA
<i>enpp1</i>	TTGCAACCAACTCAGCTCTG	CCCCTTTTACACAGTCTGC
<i>sost</i>	TACCAGAATACGCGGAGGAC	AAGTCCGTGTGTTTGCTGAC
<i>entpd5a</i>	CGCTACCGTGCAATCAATCA	AAGACGCGACTGAAGTTGAC
<i>bglap</i>	TGAAGAGCCTGACAGTCCTG	CACGCTTCACAAACACACCT
<i>ctsk</i>	CTATAAAGAGATTCTCAGGG	ACACGGGTCCCACATTGG
<i>rankl</i>	CTCACCTTCCAATCAAGACGCC	CTTTCATGCCATCCCAGGCTATCT
<i>opg</i>	GTCAAACCGCTGGAACGCC	CAGCAGATGCTCTTCCCCCTG

41



- 42 **Supplementary Table 4. (separate file)**
- 43 **7344 differentially expressed genes (DEGs).**

44 **Supplementary Table 5. (separate file)**

45 **The total genes of “osteoblast differentiation” and “osteoclast differentiation”.**