**Supplementary Table 1a:** *Overview of selected genes for crispant analysis, with reported mutations and/or polymorphisms associated with skeletal and non-skeletal phenotypes in human, mice and zebrafish. The conservation between human and zebrafish is reported in the last column.**The first four genes are associated with the pathogenesis of osteoporosis, while the last six are linked to osteogenesis imperfecta.*

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | Human | | |  | Mouse | | | |  | Zebrafish | | |  |
|  | Variant | Phenotypic features | Skeletal abnormalities |  | Mutation/model | Phenotypic features | Skeletal abnormalities | |  | Mutation/model | Phenotypic features | Skeletal abnormalities | Conservation |
| *ALDH7A1* | c.1279G>C [1] | Pyridoxine-Dependent Epilepsy (PDE) phenotype | n/a |  | *Aldh7a1* KO mice [2] | Early death | | n/a |  | *aldh7a1*-null zebrafish [3] | PDE phenotype with premature death at 14 dpf | n/a | 82% |
|  |  |  |  |  |  |  | |  |  |  |  |  |  |
|  | Polymorphisms [4] [5][6] | n/a | Associated with bone mineral density |  | / | / | | / |  | *aldh7a1* Morpholino knockdown [7] | n/a | Defects in cartilage and pectoral fin development |  |
| *DAAM2* | Missense mutation [8]  Polymorphisms [9][10][11] | Nephrotic syndrome  n/a | n/a  Associated with bone mineral density variation |  | Hypomorphic *Daam2* allele [9]  / | n/a  / | | Reduced bone strength and increased cortical bone porosity  / |  | /  / | /  / | /  / | 72% |
| *ESR1* | Polymorphisms [12][13][14][15][16][17][18] | Associated with different diseases, including breast cancer and cardiovascular risk | Associated with bone mineral density variation |  | *ERα* KO mice [19] | n/a | | Decrease in cortical bone mineral density and increase in trabecular bone mineral density |  | Loss-of-function zebrafish [20] | Viable and overall normal  Role of *esr1* in regulation of heart rate is discovered | n/a | 47% |
| *SOST* | Nonsense mutation [21]  Polymorphisms [12][15][16][17][22] | Sclerosteosis  n/a | Progressive bone thickening and increased BMD  Associated with bone mineral density variation |  | *Sost* KO mice [23]  / | n/a  / | | Increase in bone volume, BMD and osteoblast surface area  / |  | n/a  / | n/a  / | n/a  / | 50% |
| *CREB3L1* | c.934\_936delAAG [24]  c.1284C-A [25]  c.911C-T [26] | n/a | Osteogenesis Imperfecta type XVI |  | *Oasis* KO mice [27] | n/a | | Decreased bone density, delayed osteoblast maturation resulting in severe osteopenia |  | *creb3l1*ΔbZIP/ΔbZIP zebrafish [28] | n/a | Upregulation of *creb3l1* expression | 59% |
| *IFITM5* | c. 14C>T [29]  c.119C>T [30]  c.119C>G [31]  / | n/a  / | Osteogenesis Imperfecta type V  / |  | c. 14C>T [32]  *Ifitm5* KO mice [33] | n/a  n/a | | Severe skeletal defects: fractures and early lethality Abnormalities in mineralization  No skeletal abnormalities |  | n/a  / | n/a  / | n/a  / | 39% |
| *MBTPS2* | Missense mutation [34][35]  / | X-linked Olmsted syndrome and IFAP Syndrome 1, with or without BRESHECK Syndrome  / | X-linked Osteogenesis Imperfecta  / |  | *Mbtps2* Knock-in (N455S) [36]  *Mbtps2* KO mice [36] | n/a  n/a | | Embryonic lethality in hemizygous male mice, early osteoarthritis in heterozygous female mice  Embryonic lethality in hemizygous male mice, early osteoarthritis in heterozygous female mice |  | n/a  / | n/a  / | n/a  / | 60% |
| *SEC24D* | Nonsense variants [37][38]  Missense variants [37][38][39]  Frameshift variants [40][41][39] | n/a | Autosomal recessive Osteogenesis Imperfecta with a Cole-Carpenter syndrome-like phenotype |  | *Sec24d* KO mice [42] | Embryonic lethality | | Embryonic lethality |  | *sec24d* KO zebrafish (*Bulldog*) [43] |  | Abnormalities in pectoral fin and head skeleton | 65% |
| *SERPINF1* | c.696C>G [44]  c.324\_325dupCT [44]  c.1132C>T [44]  c.1118\_1119del [45]  c.1-4796dupT [45]  c.653delT [45] | n/a | Osteogenesis Imperfecta type VI |  | PEDF KO mice [46] | Retinal malformations | | Mild reduction in trabecular bone volume and accumulation of unmineralized bone matrix: increased bone fragility |  | n/a | n/a | n/a | 39% |
| *SPARC* | c.497G>A [47]  c.787G>A [47] | n/a | Osteogenesis Imperfecta type XVII |  | *Sparc* null mice [48] | Abnormal eye phenotype | | Decrease in BMD, bone mineral content and increase in bone fragility |  | n/a | n/a | n/a | 77% |

**Supplementary Table 1b: Assessment of off-target effects in crispants.** *Genes that are possibly targeted by the selected crRNA for each of the crispants are called ‘off-target genes’ in this table (mm=number of mismatches). The top 3 ranked off-target effects, selected based on a high CFD (cutting frequency determination) score, are listed, together with their chromosomal position, forward and reverse primer for amplification, the CFD (cutting frequency determination) score and the off-target percentage, based on NGS analysis of a pool of DNA of 1-day old crispants (n=10).*

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**Supplementary Table 1c: Crispant genotyping.** *Crispant genes with crRNA sequence, forward and reverse primers and assay specifications for Next-generation sequencing (NGS) are listed in this table. The first four genes are associated with the pathogenesis of osteoporosis, while the last six are linked to osteogenesis imperfecta. Primers are designed using Primer3 (*[*https://primer3.ut.ee*](https://primer3.ut.ee)*).*

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Gene** | **crRNA** | **Forward primer** | **Reverse primer** | **Assay** |
| *aldh7a1* | AATTGTTCGACAGATTGGAG | CCTATTTGCTTAGTTAAATTCAGGTGG | CATCCACATACTCCTGCACC | FORD58 |
| *daam2* | GATCTATTGCAGTAAGAAGA | AGTGTGGCTCAGTTGATGTGT | ACATGTCTTTGGATTGCCCCT | FORD60 |
| *esr1* | CAGCTCCTCACACAGGCCCA | TGTTTCCATGGTGATGTCTGG | TGTTAGTAAATACATCAACTCAAAGACC | FORD60 |
| *sost* | GTTTGATCACAATATGCCAG | GTGCCGTCCATCAACAGC | GGTGAACTTGAAATGAACCGC | FORD60 |
| *creb3l1* | ACACAGTTACTCTCTCAGCG | TGTATCCGTTTGCTACCATACC | TGTTTGTGTGGATGGATGGC | FORD60 |
| *ifitm5* | AGCGCAGGAATGCTCAGACA | TCCATCAAGGCTCGAGATCAG | TGAGAAATTCACACAGCCAAGG | FORD60 |
| *mbtps2* | TTCCATATCAAGTGGCACAC | TCTCCTTCTGTCTGTTTCACC | ACTCGGATGGAAAGCAAAGC | FORD60 |
| *sec24d* | GCCTATGGATCTCCAACACA | AAATTGAAACTACAGTGCATAATGG | GGGTCCATTGTTCATCTGAGG | FORD60 |
| *serpinf1* | GTGTTACGAGGAAGAGGGGA | TCTCTCTGTCATTCCGCTGG | GTAAATCTGCTTCTCGGCCC | FORD60 |
| *sparc* | CTGCCAGAGTCTTGCCAGCG | CTACAGCCCTTTCATTCCAGG | TGACCCAAACACATAACTATAAGC | FORD60 |

**Supplementary Table 1d: qPCR primers.***Skeletal marker genes and reference genes with forward and reverse primers are listed in this table. Primers are designed using NCBI PrimerBlast (*[*https://www.ncbi.nlm.nih.gov/tools/primer-blast*](https://www.ncbi.nlm.nih.gov/tools/primer-blast/index.cgi?GROUP_TARGET=on)*).*

|  |  |  |
| --- | --- | --- |
| **Gene** | **FWD primer** | **RVS primer** |
| **Skeletal marker genes** | | |
| *runx2a* | GGAAGAGGAAAGAGCTTCAC | CGTCCACTGTGACCTTTATG |
| *sp7* | CTCTCCTCTCCCGCTTT | GTGTTTCCTCCTCCAGAATC |
| *sox9a* | TGGGAAAACTTTGGAGATTACTGA | AGTCGGGGTGATCTTTCTTGT |
| *sox9b* | GAAGATGGAGAGCAGACGCA | CCTGAGACTGACCGGAGTG |
| *bglap* | TCTTCCTGACTCCTCAGATAC | AGCCCTCTTCTGTCTCAT |
| *col1a1a* | TCTGGTGGCTTTGATGAG | GGGACCAGTAAATCCTGGG |
| *col1a1b* | CTTGCAGTGAGAGGACAA | GCTCGGGTTTCCATACAT |
| *col1a2* | TAACCCTGGTGCTAATGGTA | ACACCAGAATCTCCCTTCA |
| *col2a1a* | GAGAACCAGGCGATATTACA | CACCCTTAGCTCGTCTTTC |
| *col2a1b* | GTTGGTACAGCAGGATCTC | TCCAGGAACACCAGACT |
| **Reference genes** | | |
| *elfa* | GGAGACTGGTGTCCTCAA | GGTGCATCTCAACAGACTT |
| *bactin2* | ACGATGGATGGGAAGACA | AAATTGCCGCACTGGTT |

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