



Mouse Cre Models for the Study of Bone Diseases

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Published online: 22 June 2018

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Abstract

Purpose Transgenic Cre lines are a valuable tool for conditionally inactivating or activating genes to understand their function. Here, we provide an overview of Cre transgenic models used for studying gene function in bone cells and discuss their advantages and limitations, with particular emphasis on Cre lines used for studying osteocyte and osteoclast function.

Recent Findings Recent studies have shown that many bone cell-targeted Cre models are not as specific as originally thought. To ensure accurate data interpretation, it is important for investigators to test for unexpected recombination events due to transient expression of Cre recombinase during development or in precursor cells and to be aware of the potential for germ line recombination of targeted genes as well as the potential for unexpected phenotypes due to the Cre transgene.

Summary Although many of the bone-targeted Cre-deleter strains are imperfect and each model has its own limitations, their careful use will continue to provide key advances in our understanding of bone cell function in health and disease.

Keywords Osteocyte · Osteoclast · Cre recombinase · Conditional knockout · Cre-loxP · Bone

Introduction

Transgenic Cre lines have been an extremely valuable tool for conditionally inactivating or activating genes in bone cell lineages in order to understand the function of these genes in skeletal development, growth, remodeling, and disease. Conditional gene deletion can be especially informative in situations where global deletion of the gene is embryonic lethal (which is particularly problematical for bone research if lethality occurs prior to formation of the skeletal elements) or where postnatal lethality prevents researchers from examining the function of the gene in the postnatal or adult skeleton. Bone-specific Cre mouse models have also provided a powerful tool for dissecting out the precise function of genes in specific bone cell lineages [1–5]. Additionally, Cre models have been used with great success for lineage tracing studies in mineralized tissues [6–8]. This review provides an overview of the different types of Cre transgenic models that have

been used for the study of gene function in bone and their advantages and limitations. We have chosen to focus the review on Cre lines used for studying osteocytes and osteoclasts, their limitations, and important considerations in using them. Many of the guiding principles and limitations of these Cre models are also applicable to working with the numerous osteoblast-specific Cre mouse models that are also available.

The Cre-loxP System

The conditional gene inactivation or activation approach exploits the Cre-loxP system in which Cre recombinase (Cre) is used to excise or invert “target” DNA sequences that have been engineered to be flanked by two 34-bp DNA sequences, termed loxP sites (reviewed in [9, 10]). Cre recombinase is a 38-kDa protein originally discovered in bacteriophage P1 [11] that mediates site-specific intermolecular DNA recombination between two loxP sites. Cre recombinase recognizes the loxP sequences flanking a targeted DNA sequence and creates a DNA loop. The Cre then excises or inverts the looped DNA, depending on the orientation of the loxP sites (see Fig. 1). To take advantage of this technology, a Cre-driver mouse strain is first generated in which Cre expression is driven by a promoter that specifically targets the cell type of interest (e.g., osteoblast, osteocyte, or osteoclast). Conditional knockout or knock-in (KI) animals are then generated by crossing the

This article is part of the Topical Collection on *Genetics*

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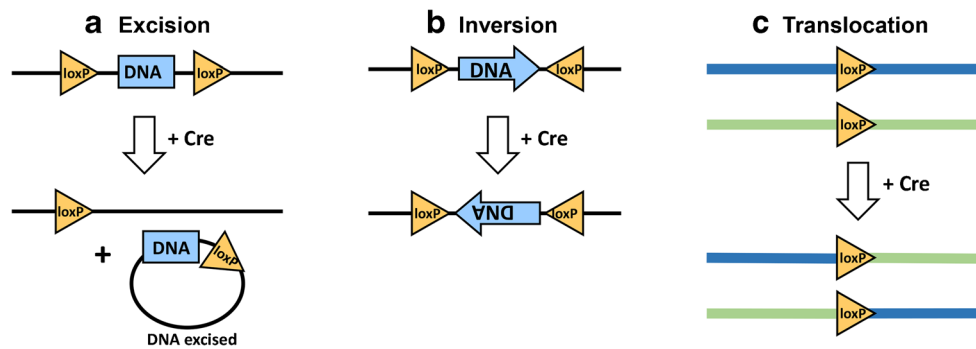


Fig. 1 The orientation of loxP sites determines whether Cre-mediated recombination results in DNA excision, inversion, or translocation. The paired loxP sites (orange triangles) have directionality and can be engineered either on the same DNA strand (cis) or on different DNA strands (trans). **a** Cre recombinase creates a DNA loop and excises the intervening DNA between two LoxP sites placed in a cis arrangement and

oriented in the same direction. **b** Cre inverts the DNA sequence between two LoxP sites placed in a cis arrangement and oriented in opposite directions. **c** Cre-mediated recombination between loxP sites on two different DNA strands (trans) results in translocation of the DNA segments flanking the loxP sites (modified from Kuhn and Torres [82] and Bouabe et al. [10])

Cre-driver strain with a second “floxed” mouse strain in which a region of DNA is engineered to be flanked by “loxP” sequences. Depending on how the floxed gene locus is designed, Cre-mediated DNA recombination will inactivate (knockout) or activate a gene of interest or knock-in a desired disease mutation. Cre-driver strains in which the Cre is driven by a ubiquitous promoter can also be used to generate a global gene knockout for comparison to tissue-specific knockouts. In addition, Cre-driver lines can be used for lineage tracing when crossed with reporter lines in which Cre-mediated recombination induces expression of a reporter such as lacZ [8] or a fluorescent reporter [6]. When using such Cre-loxP reporter models, it is important to keep in mind that, because the Cre-mediated DNA recombination event is irreversible, positive expression of the reporter does not necessarily mean that the cell is currently or has been recently expressing Cre. Rather, it means that the Cre transgene was expressed at some time point in the cell’s life history, including in an earlier precursor cell, for example during embryonic development. This explains why Cre-induced reporter models can often show a wider tissue expression pattern than reporters that are directly driven by the promoter and therefore only reflect current or recent expression of the promoter. As will be demonstrated in this review, it also explains why unexpected off-target gene deletion or activation can sometimes occur in Cre-loxP models.

Many of the earlier Cre-driver mouse lines used relatively short promoter regions fused to Cre recombinase to drive its expression. Larger bacterial artificial chromosomes (BAC) of ~200 kb that include additional promoter regulatory regions for the gene of interest have also been used for Cre insertion to provide more faithful Cre expression. In both of these approaches, the transgene is integrated randomly into the genome. While this approach has been valuable, there is the potential for mis-expression or disruption of other important genes, depending on the insertion site. An alternative strategy

is to use gene targeting by homologous recombination in embryonic stem cells. This has recently been superseded by using clustered regularly interspaced short palindromic repeats/CRISPR-associated protein 9 (CRISPR/Cas9) technology. With both these approaches, the Cre recombinase is expressed using the endogenous gene promoter, which gives more faithful Cre expression, but insertion of the Cre recombinase is likely to disrupt expression of the gene, creating a null allele. Therefore, this strategy is less useful where haploinsufficiency of the gene results in a baseline phenotype in the absence of any floxed transgene. The bone field has readily embraced Cre-loxP approaches, resulting in the generation of numerous Cre-driver strains specific for osteoblast, osteocyte or osteoclast cell lineages that fall into the above categories. In addition, many floxed mouse lines have been developed in which DNA sequences for genes important in bone cell function have been flanked by loxP sites. The following sections will review Cre-transgenic mice used for the study of osteocytes and osteoclasts.

Cre Transgenic Mice Used for Studies in Osteocytes

Osteocytes were previously viewed as inactive cells. However, in the past 15 years they have come to the forefront of bone research and are now known to have diverse functions in the skeleton, including regulation of osteoblast and osteoclast activity, mechanosensation, and regulation of phosphate homeostasis (reviewed in [12, 13]). Several marker genes are highly expressed in osteocytes, all of which are candidate promoters for generating transgenic Cre lines. These include among others, dentin matrix protein-1 (*Dmp1*) and *E11/gp38* (a.k.a. podoplanin), which are expressed in the early osteocyte, and sclerostin (encoded by the *Sost* gene), which is expressed in mature osteocytes (reviewed in [12, 13]). The

most widely used Cre transgenic lines for conditional in vivo gene deletion in osteocytes have used various versions of the *Dmp1* promoter to drive Cre recombinase expression. These include a 10-kb Dmp1-Cre mouse [14], an 8-kb Dmp1-Cre mouse [1], and more recently, a tamoxifen-inducible 10kbDmp1-Cre-ERT2 transgenic line for deletion of genes in osteocytes in an inducible (time-dependent) manner [15].

The 10-kb Dmp1-Cre model has been used for osteocyte-specific deletion of numerous important regulatory molecules in the skeleton. It is not possible to describe all here, but some interesting examples include β -catenin [16, 17], *Rankl* [18, 19], and *Fgfr1* [2]. This work has revealed a key role for osteocyte β -catenin signaling in maintaining bone mass [17] and in mechanical responses to loading [16] and has also shown that osteocytes are a key source of RANKL for osteoclast formation in postnatal bone remodeling [18] as well as in bone loss in secondary hyperparathyroidism induced by dietary calcium deficiency [19]. Deletion of *Fgfr1* in osteocytes using Dmp1-Cre showed a dramatic reduction in FGF23 expression and serum FGF23 concentrations, suggesting an important role for osteocyte FGFR1 signaling in phosphate regulation [2]. An example of the 10-kb Dmp1-Cre being used for gene activation is from the study of Chen et al. [20], in which β -catenin was constitutively activated in osteocytes by crossing *Catnb+lox(exon 3)* mice with the 10-kb Dmp1-Cre mice. These mice showed increased cancellous bone mass, but surprisingly showed reduced bone strength due to cortical thinning and increased cortical porosity. The 8-kb Dmp1-Cre model has been used for osteocyte-specific deletion of connexin 43 (*Cx43*) [1, 21]. This work revealed a key role for this gap junction protein in osteocyte survival and in controlling osteoblast and osteoclast activity via regulation of sclerostin and osteoprotegerin levels [1], as well as a negative regulatory role in bone mechanoresponsiveness.

Based on earlier studies, *Dmp1* expression was thought to be mainly restricted to preosteocytes, osteocytes, odontoblasts, and some pulp cells. These reports included studies examining expression of *Dmp1* mRNA and protein [22, 23] and expression studies using lacZ knocked in to the *Dmp1* locus or lacZ or GFP driven by various *Dmp1* promoter fragments [14, 22, 24]. In their original paper describing the 10-kb-Dmp1-Cre mouse using a ROSA26R-LacZ reporter model, Lu and colleagues reported Cre expression in osteocytes and odontoblasts postnatally, but saw little or no Cre expression during embryonic development and did not report notable off-target expression in other tissues [14]. However, recent studies using more sensitive TdTomato reporter lines crossed with Dmp1-Cre models have shown that Cre-mediated recombination may also occur in other cell types, leading to concerns about off-target effects [25••, 26]. Kalajzic and colleagues used a reporter line, termed Ai9, which carries the Rosa-CAG-LSL-tdTomato-WPRE conditional allele (Jackson labs, #007905) in which Cre-mediated recombination

causes activation of TdTomato expression. Using this reporter, they showed expression of Dmp1-Cre in osteoblasts, skeletal muscle, a few cells within bone marrow, and in subsets of cells in brain and kidney [25••]. The 8-kb Dmp1-Cre model showed a similar expression pattern, but with slightly less expression in osteoblasts. Lim and colleagues, using the same TdTomato reporter line [26], showed similar off-target Dmp1-Cre expression and reported expression in a subset of gastric and intestinal mesenchymal cells. They further showed that deletion of the type IA BMP receptor using Dmp1-Cre resulted in the formation of polyps in the gastrointestinal tract, showing that Dmp1-Cre-mediated recombination caused an off-target phenotype. Studies in our own laboratory using the Ai9 TdTomato reporter line have shown Dmp1-Cre expression in some osteoblasts, skeletal muscle, a subset of cells in the marrow, stomach, small intestine, skin, brain, eye, and an even more restricted subset of cells in the lung and kidney (see Fig. 2). Unlike skeletal muscle, there was negligible expression in heart muscle and lung was also negative.

Off-target Cre-mediated DNA recombination may be construct-dependent, since some floxed loci seem to be less efficiently recombined by Cre than others [27]. For example, Gorski and colleagues used the 10-kb Dmp1-Cre to delete *Mtbp1* in osteocytes and showed effects on muscle size and contractility in the absence of any detectable reduction of MTBPS1 protein in muscle [28]. Detection of off-target Cre activity may also be dependent on the sensitivity of the reporter system. For example, lacZ-based reporters appear generally less sensitive than fluorescence-based reporters. Some of the highly sensitive Cre reporter lines, such as the Ai9 TdTomato reporter strain described above, may be exquisitely sensitive to trace amounts of Cre expression that are not sufficient to delete other floxed transgenes. Therefore, using these reporter models likely indicates a “worst case scenario” of where the off-target expression could occur, which may end up being less extreme with other floxed constructs.

As an approach to overcome off-target Cre expression, inducible Cre models can be very useful. This allows the investigator to induce Cre expression at a specific time, in a tissue-specific manner. Powell and colleagues developed a tamoxifen-inducible Dmp1-Cre transgenic line (10kbDmp1-Cre-ERT2) [15]. In this model, the Cre is theoretically induced only in cells expressing *Dmp1* at the time of the tamoxifen treatment. This model was shown to have a much more restricted expression pattern compared to the 8- and 10-kb Dmp1-Cre mice, with robust expression in osteocytes and minimal expression in osteoblasts, especially at lower doses of tamoxifen. There was no detectable Cre activity in muscle [15, 25••]. Although advantageous for some applications, inducible models are not without their own limitations. Doses of inducer agents, such as tamoxifen, must be carefully optimized to avoid effects on bone formation and resorption. Also, some amount of “leaky” expression often occurs in the

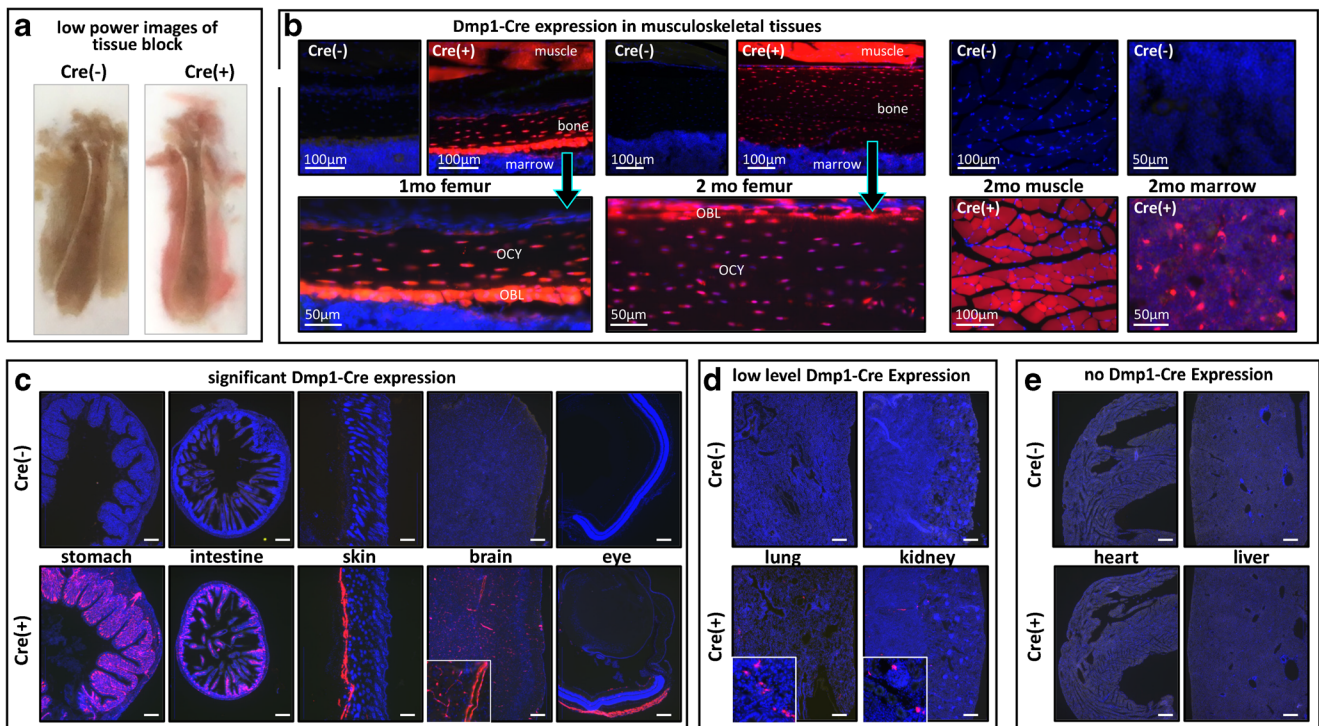


Fig. 2 Tissue distribution of 10-kb-Dmp1-Cre Expression Revealed by the Ai9 TdTomato Reporter Mouse [red = TdTomato, blue = DAPI nuclear stain]. **a** Low-power image of the cut face of the tissue block showing the femur from a 2-week-old 10-kb Dmp1-Cre(+) mouse compared to a Cre(-) littermate. Both mice carry the Ai9 TdTomato reporter. Note that even without fluorescence illumination, the muscle tissue in the Cre(+) mouse appears red, suggesting muscle expression of 10-kb Dmp1-Cre. **b** Fluorescence images showing TdTomato reporter expression in 10-kb Dmp1-Cre(+) mice and Cre(-) littermates which both carry the Ai9 TdTomato reporter. Note the lack of baseline TdTomato expression in Cre(-) mice. In bone tissues in 1mo and 2mo femur, expression is seen in osteocytes (OCY) and in the osteoblast layer (OBL). Strong expression is also seen in muscle fibers and in a subset of cells in the marrow. **c** Soft tissue panel, including stomach, intestine, skin,

brain, and eye showed significant TdTomato reporter expression in 10-kb-Dmp1-Cre(+) mice compared to Cre(-) littermates. All mice carry the Ai9 TdTomato reporter. Inset shows a higher power view of TdTomato positive areas in brain. Bars = 200 μ m. **d** Lung and kidney showed a low level of TdTomato reporter expression in 10-kb Dmp1-Cre(+) mice compared to Cre(-) littermates. The expression was seen in only a small subset of cells within the tissue. All mice carry the TdTomato reporter. Insets show higher power views of the TdTomato positive cells. Bars = 200 μ m. **e** Heart and liver showed no detectable TdTomato reporter expression in either the 10-kb-Dmp1-Cre(+) mice or Cre(-) littermates. Bars = 200 μ m. Similar results as those depicted (a–e) were seen in tissues from mice at all ages examined (2 weeks and 1, 2, and 6 months)

absence of the inducer. This was shown to be the case in the 10kbDmp1-Cre-ERT2 model using the Ai9 TdTomato reporter, where a small proportion of osteocytes (10–20%) showed Cre activity in 3–4-week-old mice without tamoxifen treatment [25•]. Also, the inducible Dmp1-Cre model may not hit mature osteocytes that have already progressed past expressing *Dmp1* at the time of the tamoxifen injection. Therefore, the osteocyte population as a whole may only be “deficient” in the gene rather than achieving a complete knockout. The degree of leaky expression and Cre-mediated recombination appears to be both age- and construct-dependent. In a recent study, Kang and co-workers used the 10kbDmp1-Cre-ERT2 to delete β -catenin in osteocytes in skeletally mature mice [29]. An approximately 40% reduction in β -catenin expression was seen in cortical bone in the 10kbDmp1-Cre-ERT2/ β -catenin^{fl/fl} mice at 18 weeks even without tamoxifen treatment, suggesting significant leaky

expression of the Cre transgene. Tamoxifen injection induced further gene deletion, resulting in an 80% reduction in β -catenin expression. This adult-onset reduction in osteocyte β -catenin expression by about 80% was associated with reduced skeletal mass but no impairment in the periosteal bone anabolic response to applied mechanical loading of the ulna.

For targeting the mature osteocyte, the most obvious osteocyte-specific marker gene is *Sost*, which encodes the protein sclerostin and is expressed in mature osteocytes but not osteoblasts and lining cells [30]. Xiong and colleagues generated a *Sost*-Cre transgenic line by inserting Cre recombinase into the second exon of the murine *Sost* gene using a BAC clone [3]. Using this line crossed with the Ai9 TdTomato reporter, they observed recombination in osteocytes but not osteoblasts or lining cells. Although this promoter showed greatly improved osteocyte specificity among cells in the osteoblastic lineage, surprisingly, some recombination

was observed in osteoclasts on the bone surface and about 90% of the bone marrow cells expressed TdTomato. Their data further suggested that Sost-Cre was expressed in an early hemopoietic progenitor. This provides an explanation for recombination occurring in osteoclasts, which are of hemopoietic origin. These investigators used the Sost-Cre strain to delete *Tnfrsf11* (the gene encoding RANKL) in osteocytes, while avoiding deletion in osteoblasts or lining cells. Their data showed that Sost-Cre-mediated deletion of *Tnfrsf11* resulted in increased cancellous bone mass and mimicked the phenotype obtained by deletion of *Tnfrsf11* using Dmp1-Cre, providing further evidence that osteocytes rather than osteoblasts or lining cells provide a critical source of RANKL to support osteoclast formation for cancellous bone remodeling. Overall, while osteocyte-targeted Cre models have provided exciting insights into the biology of these unique cells, the advantages and disadvantages of each model must be considered when using them and when interpreting the data. A summary of osteocyte-targeted Cre models and their advantages and disadvantages is provided in Table 1. In addition to these models, an inducible SOST-Cre-ERT2 strain has recently been developed and may soon be available to mineralized tissue researchers [31].

Although not the main focus of this review, a number of Cre-transgenic lines have also been developed for gene deletion in osteoblasts. These include, but are not limited to, osterix-Cre (*Osx*-Cre) [32] osteocalcin-Cre [4], *Runx2*-Cre [33], and transgenic lines in which Cre is driven by various fragments of the type I collagen promoter with or without tamoxifen induction [7, 34, 35]. These Cre lines have their own advantages and limitations similar to those described above for osteocyte-Cre models. Particularly noteworthy, is the *Osx*-Cre model, which was shown to have off-target expression in stromal cells, adipocytes, perivascular cells in bone marrow, olfactory glomerular cells, and a subset of gastric and intestinal epithelial cells [36]. The *Osx*-Cre model was also shown to exhibit unexpected skeletal phenotypes of delayed calvarial ossification and multiple fracture calluses, which could complicate bone studies performed using these mice [37•, 38]. This phenotype occurred with the *Osx*-Cre transgene alone (i.e., without crossing to any floxed mouse strain). It is also important to keep in mind that Cre models that are targeted to osteoblasts will actually delete genes in both osteoblasts and osteocytes, since osteocytes are terminally differentiated osteoblasts. Therefore, phenotypes obtained using osteoblast-specific Cre-driver lines can be due to deletion of the gene in osteoblasts, osteocytes or both.

Cre Transgenic Mice Used for Studies in Osteoclasts

Targeting gene deletion to the osteoclast lineage can provide key insights into the molecular mechanisms of osteoclast

formation and function and their role in regulating bone mass in vivo under physiological and pathological conditions. A number of Cre transgenic lines have been developed as tools to investigate gene functions in osteoclasts. In general, these can be divided into Cre drivers that target the mature osteoclasts (cathepsinK, TRAP) and those that target earlier steps in the osteoclast lineage (e.g., myeloid lineage).

The most widely used Cre transgenic mouse line for the in vivo study of osteoclasts is the CathepsinK (CtsK)-Cre knock-in (KI) mouse [39]. In this line, selectivity of Cre expression in osteoclasts is thought to be reliable because the Cre gene is knocked into the CtsK gene locus, a well-accepted mature osteoclast marker gene. In spite of its many advantages, researchers using this transgenic line need to be aware that a study by Winkeler and colleagues has shown Cre activity in the testis and ovary in these mice [40•]. Our laboratory has also observed germ line deletion on the C57BL/6 and 129Sv mixed background (YU, personal communication). Thus, it is possible that the targeted (floxed) genes may be deleted in the germ line, which can potentially cause systemic deletion of target genes. However, some studies suggest that this germ line deletion of target genes occurs in a gene- or gender-dependent manner or only under certain genetic or environmental conditions. For example, when genes essential for development are deleted using this *Ctsk*-Cre KI line, embryonic lethality is avoided in the cases of PI3Ks and *Rac1/2* [5, 41, 42]. Routine genotyping by PCR with genomic DNA isolated from non-bone tissues with a primer set that distinguishes the deleted allele of the target gene from the wild-type and floxed alleles is therefore essential to identify any mice that underwent unexpected germ line deletion. It is also important to keep in mind that because the *CtsK*-Cre mice are KI mice, endogenous cathepsin K expression is disrupted. Since complete deficiency of *Ctsk* leads to an osteopetrotic phenotype in these mice [43–47], generation of conditional knockout mice under a homozygous *CtsK*-Cre/*CtsK*-Cre KI background must be avoided. Also, mice on a heterozygous KI (*Ctsk*-Cre/+) background should be used as controls. Homozygous and heterozygous *Ctsk*-Cre KI alleles can be distinguished by PCR [40••].

Ctsk-Cre-KI mice have been used to delete numerous genes in mature osteoclasts. Although it is not possible to review all here, some notable examples include estrogen receptor- α (ER α) [39], *BclxL* [48], and *dicer* [49]. This work has revealed a key role for osteoclast ER α signaling in females in maintaining bone mass through promoting osteoclast apoptosis [39]. *BclxL*, which is normally known as an anti-apoptotic factor, was shown to have an unexpected role in osteoclasts to promote osteoclastic bone resorption through upregulation of c-Src and decreased ECM production [48]. Osteoclast-targeted deletion of *dicer* resulted in increased bone mass due to suppression of bone resorption. This revealed a previously unknown role for this protein, important

Table 1 Comparison of osteocyte-targeted Cre models

Cre Model	Targeted cells	Advantages	Limitations	Ref
10-kb Dmp1-Cre (overexpression)	Early and mature osteocytes, preosteocytes, odontoblasts, some pulp cells	<ul style="list-style-type: none"> • Can be used to determine gene function in early and late osteocytes • Robust expression and efficient recombination • Available from JAX 	<ul style="list-style-type: none"> • 10-kb fragment of Dmp1 promoter • Potential for off-target Cre-mediated recombination in osteoblasts, muscle and several soft tissues 	[13]
8-kb Dmp1-Cre (overexpression)	Early and mature osteocytes, preosteocytes, odontoblasts, some pulp cells	<ul style="list-style-type: none"> • Can be used to determine gene function in early and late osteocytes • Robust expression and efficient recombination 	<ul style="list-style-type: none"> • 8-kb fragment of Dmp1 promoter • Potential for off-target Cre-mediated recombination in osteoblasts, muscle, and some soft tissues 	[1]
10kbDmp1-Cre-ERT2 (inducible overexpression)	Early and mature osteocytes, preosteocytes, odontoblasts, some pulp cells	<ul style="list-style-type: none"> • Can be used to determine gene function in early and some late osteocytes • Inducible to avoid effects of gene deletion during development/growth • No off-target expression in muscle and much less off target Cre expression in other tissues compared to 8- and 10-kb Dmp1-Cre models • Fairly robust expression and efficient recombination • Available from JAX 	<ul style="list-style-type: none"> • 10-kb fragment of Dmp1 promoter • Some degree of “leaky” expression without tamoxifen induction • Tamoxifen dose must be optimized to avoid effects on bone • Mature osteocytes that have progressed past expressing Dmp1 at the time of tamoxifen treatment may escape gene deletion 	[15]
SOST-Cre (overexpression/BAC construct)	Mature osteocytes	<ul style="list-style-type: none"> • Construct made using Sost gene within a BAC clone for more faithful expression • Can be used to determine gene function in mature osteocytes • No off-target expression in osteoblasts or muscle 	<ul style="list-style-type: none"> • Potential for off-target Cre-mediated recombination in hemopoietic cells/osteoclasts and possibly other soft tissues 	[3]

JAX the Jackson laboratory

in generation of siRNAs and miRNAs, in regulating osteoclast differentiation and function. Other CtsK-Cre lines have also been reported that are not knock-in lines, but based on CtsK-Cre transgenic overexpression [50, 51]. These have been used for deletion of *Fcgr4* [52], *Vdr* [53], and *Pstpip1* [54] and have shown roles for these genes in osteoclasts in physiological and pathological conditions.

Tartrate-resistant acid phosphatase (TRAP) is another mature osteoclast marker gene that is strongly induced during osteoclastogenesis. Two TRAP-Cre overexpression transgenic lines have been created as models for osteoclast-specific Cre recombination [50, 55]. One of these lines has been used for deletion of *Ilk* [55], *Nfl* [56], and *Pdgfb* [57] genes in osteoclasts. These studies revealed a role for *Ilk* in regulating osteoclast resorptive activity but not differentiation and showed a mild bone phenotype of reduced tibial bone perimeter and reduced bone marrow cavity diameter in mice with

osteoclast-targeted *Nfl* deletion. Osteoclast-specific deletion of *Pdgfb* revealed a previously unknown role for osteoclast-derived PDGF-BB in regulating blood vessel formation during bone formation. However, off-target Cre activity was detected in chondrocytes in one of these TRAP-Cre-deleter strains and some expression of Cre mRNA was detected in colon, liver, kidney and testes, depending on the founder line [50]. Therefore, a more thorough evaluation of off-target TRAP-Cre expression in non-bone tissues is needed to confirm the selectivity of the TRAP promoter in osteoclasts in vivo in these models.

One of the most widely used mouse lines for targeting earlier stages in the osteoclastic lineage is the LysM-Cre knock-in mouse in which Cre recombinase is targeted into the endogenous lysozyme M (*LysM*) locus [58]. Thus, similar to the CtsK-Cre KI mouse described above, endogenous LysM expression is disrupted. The popularity of this Cre line

is partly due to the fact that LysM-positive macrophages are precursors of osteoclasts and that the mice are available from The Jackson Laboratory (#004781). The Cre activity in LysM-Cre KI mice is restricted to the myeloid cell lineage [58], but is also observed in neurons [59]. Deletion of various genes such as *Syk* in bone marrow-derived macrophages using LysM-Cre has been reported [60, 61]. For in vivo studies, careful attention should be given to the fact that the Cre is expressed in cells other than osteoclast precursor cells, including mature macrophages, monocytes, and neutrophils. While LysM is highly expressed in myeloid lineage cells, it was reported that deletion of the *Nfatc1* gene did not occur in bone marrow-derived macrophages in *Nfatc1* floxed mice crossed with LysM-Cre mice and that these mice do not show abnormal skeletal phenotypes [62]. These results suggest that the deletion efficiency may be dependent on the type of floxed allele; therefore, target gene deletion always needs to be confirmed when using LysM-Cre mice in osteoclast studies. Unsuccessful or inefficient deletion of the target gene with heterozygous LysM-Cre KI mice can be overcome on a homozygous LysM-Cre KI background (YU, personal experience), which can be distinguished from wild-type and heterozygous LysM-Cre alleles by PCR [63]. In these cases, mice that are wild-type for the target gene on a homozygous LysM-Cre KI background should be used as controls. There have been no reports showing a skeletal phenotype in hetero- or homozygous LysM-Cre KI mice in the absence of a floxed target gene. The CtsK-Cre KI and LysM-Cre KI lines were used for the etiological study of rare bone disorders such as Hajdu-Cheney syndrome and cherubism [60, 64]. These studies have shown that accumulation of NOTCH2 protein in osteoclasts and increased activation of SYK by accumulation of SH3BP2 protein in myeloid lineage cells are responsible for the bone phenotypes, respectively.

Receptor activator of nuclear factor- κ B (RANK) is expressed in a premature macrophage population [65], and RANK-Cre KI mice have been used as another deleter strain for osteoclasts that is more specific to the osteoclast lineage [66]. Endogenous RANK expression is likely not abrogated in the mice because the GFP-Cre cassette is inserted into the 3' UTR of the *Rank* gene. Analysis using a reporter mouse crossed with the RANK-Cre mice revealed that Cre activity is found in osteoclast lineage cells [66], and that genes such as *Dnmt3a* [67], *Ahr* [68], and *Ror2* [66] were successfully deleted in osteoclasts. These studies showed important roles for these genes in osteoclast precursors in regulating bone mass. However, a drawback is that RANK is also expressed in various cell types other than hematopoietic cells. Therefore, attention should be given to potential off-target effects in other cells [41].

Several other myeloid-targeted Cre expressing strains have been developed [69]. Previous studies using CD11b-Cre mice showed that genes were successfully deleted in the osteoclast lineage [70–73], although Cre activity can also be detected in T cells, B cells, dendritic cells, neutrophils, mast cells, NK

cells, basophils, and eosinophils in CD11b-Cre overexpression transgenic mice [69, 74]. Therefore, this mouse line does not specifically target the osteoclasts. Other myeloid-selective Cre transgenic lines on an FVB background have been developed, and could potentially be useful for gene deletion in osteoclasts, although there are not yet any reports of osteoclast-related studies using these lines. Cre is expressed under control of a 7.2-kb fragment of the colony stimulating factor 1 receptor (*Csf1r*) promoter in *Csf1r*-iCRE mice for constitutive Cre expression [75] and *Csf1r*-Mer-iCre-Mer mice for tamoxifen-inducible Cre expression [76]. These strains are available from the Jackson laboratory (#021024 and 019098, respectively). A summary of osteoclast-targeted Cre models and their advantages and disadvantages is provided in Table 2.

Designing Appropriate Controls when Using Cre-Mediated Recombination

When using Cre-loxP models for gene activation or inactivation, careful consideration of the appropriate control genotypes is important in order to accurately attribute phenotypic differences to the gene activation or inactivation rather than other factors. In general, controls carrying the floxed gene but negative for Cre are often used. However, in many cases, it is important to also include controls expressing Cre but without the floxed gene. This is particularly important for models where the Cre is knocked in to the endogenous gene locus and therefore inactivates one or more alleles of the gene (e.g., the CtsK-Cre KI model described above [39]). For inducible Cre expression models, it is critical to include controls with an identical genotype to the experimental animals (e.g., carrying the floxed allele and positive for the inducible Cre transgene) but treated with vehicle instead of the inducing agent. This controls for any leaky expression of the Cre recombinase that may occur even in the absence of the inducer. Ideally, littermates should always be used to make accurate comparisons and offspring from more than one set of parents should be evaluated. In some cases, investigators have combined conditional knockout mouse models with global knockouts, such that one allele of the gene of interest is globally deleted and the other allele is floxed. Therefore, Cre recombinase in the target tissues only needs to delete one allele of the gene to achieve a complete gene knockout in the target cells. In this type of model, the appropriate controls would be Cre-negative mice with one globally deleted and one floxed allele of the target gene.

Summary and Conclusions

It is clear from the above discussion that caution must be used when employing any tissue-specific Cre model. As more studies are performed using these Cre models, it is becoming

Table 2 Comparison of Cre Mouse lines used in osteoclast studies

Cre model	Targeted cells	Advantages	Limitations	Ref
CtsK-Cre KI (knock-in)	Mature osteoclasts	<ul style="list-style-type: none"> Knocked in to CtsK locus, so Cre expression under control of authentic expression machinery 	<ul style="list-style-type: none"> CtsK gene is knocked out by Cre insertion Potential for deletion in germ line cells 	[39]
CtsK-Cre (overexpression and inducible overexpression)	Mature osteoclasts	<ul style="list-style-type: none"> Tamoxifen-inducible Cre expression [51] 	<ul style="list-style-type: none"> 5.0-kb fragment of the CtsK promoter [50] 3.48-kb fragment of the CtsK promoter [51] Tamoxifen dose must be optimized to avoid effects on bone [51] 	[50, 51]
TRAP-Cre (overexpression)	Mature osteoclasts	<ul style="list-style-type: none"> Promoter is well-characterized [77] 	<ul style="list-style-type: none"> 0.62-kb fragment of the TRAP promoter [50, 78] 1.8-kb fragment of the TRAP promoter [55, 77]. Potential off-target expression in chondrocytes [50]. No successful report of gene deletion using one of the lines [41] 	[50, 55]
LysM-Cre KI (knock-in)	Myeloid lineage cells	<ul style="list-style-type: none"> Knocked in to LysM locus, so Cre expression under control of authentic expression machinery Available from JAX 	<ul style="list-style-type: none"> LysM gene is knocked out by Cre insertion Off-target expression in neutrophils [58] and neurons [59] 	[58]
RANK-Cre KI (knock-in)	Osteoclast precursors	<ul style="list-style-type: none"> Knocked in to RANK locus, so Cre expression under control of authentic expression machinery RANK gene expression is not disrupted by Cre insertion 	<ul style="list-style-type: none"> Off-target expression in a subset of intestinal epithelial immune cells [79, 80] and neurons [81] 	[66]
CD11b-Cre (overexpression)	Macrophages and granulocytes	<ul style="list-style-type: none"> Cell types with off target expression are characterized [69] 	<ul style="list-style-type: none"> 1.7-kb fragment of the CD11b promoter [74] Off-target expression in other types of immune cells [69] 	[74]
Csf1r-Cre (overexpression and inducible overexpression)	Monocyte/macrophage lineage cells	<ul style="list-style-type: none"> Tamoxifen-inducible Cre expression [76] Available from JAX 	<ul style="list-style-type: none"> 7.2-kb fragment of the Csf1r promoter [75] Tamoxifen dose must be optimized to avoid effects on bone [76] No report of using the lines in osteoclast studies 	[75, 76]

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increasingly apparent that many tissue-specific Cre models are not as specific as originally thought and that different loxP-flanked alleles may vary in their recombination efficiency when crossed with the same deleter strain [9]. Accurate data interpretation with these models is dependent on a complete understanding of the tissue specificity for Cre expression. Investigators should always confirm that the desired DNA recombination has occurred in the target cells and that the targeted gene has been activated or inactivated. In addition, other tissues should be examined to determine whether off-target recombination has occurred in other cell types or

whether there was any germ line recombination. This should be performed for each floxed strain and for different genetic backgrounds. It should be done by a combination of PCR on the genomic DNA to detect the recombined DNA sequence and qPCR or western blotting to confirm whether mRNA or protein expression levels of the target gene are altered in targeted and non-targeted tissues. Additional information on how to evaluate Cre transgenic lines and avoid problems when using them is provided in an excellent review by Song and Palmiter [9]. While off-target Cre expression does not necessarily negate the results of conditional knockout experiments,

it must factor into a more nuanced interpretation of the data. Off-target Cre expression may also be less important when the floxed gene being deleted has a restricted expression pattern as compared to genes that are widely expressed in other tissues (for example, if the targeted gene is not normally expressed in a particular tissue, Cre-mediated recombination will likely have negligible effect in that tissue). It is increasingly apparent that few to none of the above osteocyte, osteoblast or osteoclast-targeted Cre-deleter strains are perfect. Each model has its own limitations, but these models still provide a powerful tool for interrogating bone cell biology and function.

Funding Information SLD was supported by NIH grants P01-AG039355 and R21-AR071563. YU was supported by NIH grants R01-DE025870 and R21-AR070953. YX and LAS were supported by NIH grant P01-AG039355, which also supported the data in Fig. 2.

Compliance with Ethical Standards

Conflict of Interest Lora Shiflett, Sarah Dallas, Yixia Xie and Yasuyoshi Ueki declare no conflict of interest.

Human and Animal Rights and Informed Consent All reported studies/experiments with animal subjects performed by the authors have complied with all applicable ethical standards (including the Helsinki declaration and its amendments, institutional/national research committee standards, and international/national/institutional guidelines).

References

Papers of particular interest, published recently, have been highlighted as:

•• Of major Importance

- Bivi N, Condon KW, Allen MR, Farlow N, Passeri G, Brun LR, et al. Cell autonomous requirement of connexin 43 for osteocyte survival: consequences for endocortical resorption and periosteal bone formation. *J Bone Miner Res.* 2012;27(2):374–89. <https://doi.org/10.1002/jbmr.548>.
- Xiao Z, Huang J, Cao L, Liang Y, Han X, Quarles LD. Osteocyte-specific deletion of Fgfr1 suppresses FGF23. *PLoS One.* 2014;9(8):e104154. <https://doi.org/10.1371/journal.pone.0104154>.
- Xiong J, Piemontese M, Onal M, Campbell J, Goellner JJ, Dusevich V, et al. Osteocytes, not osteoblasts or lining cells, are the main source of the RANKL required for osteoclast formation in remodeling bone. *PLoS One.* 2015;10(9):e0138189. <https://doi.org/10.1371/journal.pone.0138189>.
- Zhang M, Xuan S, Bouxsein ML, von Stechow D, Akeno N, Faugere MC, et al. Osteoblast-specific knockout of the insulin-like growth factor (IGF) receptor gene reveals an essential role of IGF signaling in bone matrix mineralization. *J Biol Chem.* 2002;277(46):44005–12. <https://doi.org/10.1074/jbc.M208265200>.
- Zhu M, Sun BH, Saar K, Simpson C, Troiano N, Dallas SL, et al. Deletion of Rac in mature osteoclasts causes Osteopetrosis, an age-dependent change in osteoclast number, and a reduced number of osteoblasts in vivo. *J Bone Miner Res.* 2016;31(4):864–73. <https://doi.org/10.1002/jbmr.2733>.
- Balani DH, Ono N, Kronenberg HM. Parathyroid hormone regulates fates of murine osteoblast precursors in vivo. *J Clin Invest.* 2017;127(9):3327–38. <https://doi.org/10.1172/JCI91699>.
- Liu F, Woitge HW, Braut A, Kronenberg MS, Lichtler AC, Mina M, et al. Expression and activity of osteoblast-targeted Cre recombinase transgenes in murine skeletal tissues. *Int J Dev Biol.* 2004;48(7):645–53. <https://doi.org/10.1387/jdb.041816fl>.
- Maes C, Kobayashi T, Selig MK, Torrekens S, Roth SI, Mackem S, et al. Osteoblast precursors, but not mature osteoblasts, move into developing and fractured bones along with invading blood vessels. *Dev Cell.* 2010;19(2):329–44. <https://doi.org/10.1016/j.devcel.2010.07.010>.
- Song AJ, Palmiter RD. Detecting and avoiding problems when using the Cre-lox system. *Trends Genet.* 2018;34:333–40. <https://doi.org/10.1016/j.tig.2017.12.008>.
- Bouabe H, Okkenhaug K. Gene targeting in mice: a review. *Methods Mol Biol.* 2013;1064:315–36. https://doi.org/10.1007/978-1-62703-601-6_23.
- Sternberg N, Hamilton D. Bacteriophage P1 site-specific recombination. I Recombination between loxP sites. *J Mol Biol.* 1981;150(4):467–86.
- Dallas SL, Prideaux M, Bonewald LF. The osteocyte: an endocrine cell ... and more. *Endocr Rev.* 2013;34(5):658–90. <https://doi.org/10.1210/er.2012-1026>.
- Prideaux M, Findlay DM, Atkins GJ. Osteocytes: the master cells in bone remodelling. *Curr Opin Pharmacol.* 2016;28:24–30. <https://doi.org/10.1016/j.coph.2016.02.003>.
- Lu Y, Xie Y, Zhang S, Dusevich V, Bonewald LF, Feng JQ. DMP1-targeted Cre expression in odontoblasts and osteocytes. *J Dent Res.* 2007;86(4):320–5. <https://doi.org/10.1177/154405910708600404>.
- Powell WF Jr, Barry KJ, Tulum I, Kobayashi T, Harris SE, Bringhurst FR, et al. Targeted ablation of the PTH/PTHrP receptor in osteocytes impairs bone structure and homeostatic calcemic responses. *J Endocrinol.* 2011;209(1):21–32. <https://doi.org/10.1530/JOE-10-0308>.
- Javaheri B, Stern AR, Lara N, Dallas M, Zhao H, Liu Y, et al. Deletion of a single beta-catenin allele in osteocytes abolishes the bone anabolic response to loading. *J Bone Miner Res.* 2014;29(3):705–15. <https://doi.org/10.1002/jbmr.2064>.
- Kramer I, Halleux C, Keller H, Pegurri M, Gooi JH, Weber PB, et al. Osteocyte Wnt/beta-catenin signaling is required for normal bone homeostasis. *Mol Cell Biol.* 2010;30(12):3071–85. <https://doi.org/10.1128/MCB.01428-09>.
- Xiong J, Onal M, Jilka RL, Weinstein RS, Manolagas SC, O'Brien CA. Matrix-embedded cells control osteoclast formation. *Nat Med.* 2011;17(10):1235–41. <https://doi.org/10.1038/nm.2448>.
- Xiong J, Piemontese M, Thostenson JD, Weinstein RS, Manolagas SC, O'Brien CA. Osteocyte-derived RANKL is a critical mediator of the increased bone resorption caused by dietary calcium deficiency. *Bone.* 2014;66:146–54. <https://doi.org/10.1016/j.bone.2014.06.006>.
- Chen S, Feng J, Bao Q, Li A, Zhang B, Shen Y, et al. Adverse effects of osteocytic constitutive activation of ss-catenin on bone strength and bone growth. *J Bone Miner Res.* 2015;30(7):1184–94. <https://doi.org/10.1002/jbmr.2453>.
- Bivi N, Pacheco-Costa R, Brun LR, Murphy TR, Farlow NR, Robling AG, et al. Absence of Cx43 selectively from osteocytes enhances responsiveness to mechanical force in mice. *J Orthop Res.* 2013;31(7):1075–81. <https://doi.org/10.1002/jor.22341>.
- Kaljajic I, Braut A, Guo D, Jiang X, Kronenberg MS, Mina M, et al. Dentin matrix protein 1 expression during osteoblastic differentiation, generation of an osteocyte GFP-transgene. *Bone.* 2004;35(1):74–82. <https://doi.org/10.1016/j.bone.2004.03.006>.
- Toyosawa S, Shintani S, Fujiwara T, Ooshima T, Sato A, Ijuhin N, et al. Dentin matrix protein 1 is predominantly expressed in chicken and rat osteocytes but not in osteoblasts. *J Bone Miner Res.*

- 2001;16(11):2017–26. <https://doi.org/10.1359/jbmr.2001.16.11.2017>.
24. Feng JQ, Huang H, Lu Y, Ye L, Xie Y, Tsutsui TW, et al. The dentin matrix protein 1 (Dmp1) is specifically expressed in mineralized, but not soft, tissues during development. *J Dent Res*. 2003;82(10):776–80. <https://doi.org/10.1177/154405910308201003>.
 25. Kalajzic I, Matthews BG, Torreggiani E, Harris MA, Divieti Pajevic P, Harris SE. In vitro and in vivo approaches to study osteocyte biology. *Bone*. 2013;54(2):296–306. <https://doi.org/10.1016/j.bone.2012.09.040>. **This article provides an excellent overview of the Dmp1-Cre tissue expression profiles for the 8- and 10-kb Dmp1-Cre and 10kbDmp1-CreERT2 mouse models and was the first to report off target Cre expression in muscle and some marrow cells.**
 26. Lim J, Burclaff J, He G, Mills JC, Long F. Unintended targeting of Dmp1-Cre reveals a critical role for Bmpr1a signaling in the gastrointestinal mesenchyme of adult mice. *Bone Res*. 2017;5:16049. <https://doi.org/10.1038/boneres.2016.49>.
 27. Schmidt-Supprian M, Rajewsky K. Vagaries of conditional gene targeting. *Nat Immunol*. 2007;8(7):665–8. <https://doi.org/10.1038/ni0707-665>.
 28. Gorski JP, Huffman NT, Vallejo J, Brotto L, Chittur SV, Breggia A, et al. Deletion of Mbtps1 (Pcsk8, S1p, Ski-1) gene in osteocytes stimulates soleus muscle regeneration and increased size and contractile force with age. *J Biol Chem*. 2016;291(9):4308–22. <https://doi.org/10.1074/jbc.M115.686626>.
 29. Kang KS, Hong JM, Robling AG. Postnatal beta-catenin deletion from Dmp1-expressing osteocytes/osteoblasts reduces structural adaptation to loading, but not periosteal load-induced bone formation. *Bone*. 2016;88:138–45. <https://doi.org/10.1016/j.bone.2016.04.028>.
 30. van Bezooijen RL, Roelen BA, Visser A, Van der Wee-Pals L, de Wilt E, Karperien M, et al. Sclerostin is an osteocyte-expressed negative regulator of bone formation, but not a classical BMP antagonist. *J Exp Med*. 2004;199(6):805–14. <https://doi.org/10.1084/jem.20031454>.
 31. Maurel D, Johnson ML, SE H, Harris MA, Bonewald LF. Characterization of a new Cre model targeting osteocytes. *J Bone Miner Res*. 2015;30(Suppl 1):S100.
 32. Rodda SJ, McMahon AP. Distinct roles for hedgehog and canonical Wnt signaling in specification, differentiation and maintenance of osteoblast progenitors. *Development*. 2006;133(16):3231–44. <https://doi.org/10.1242/dev.02480>.
 33. Rauch A, Seitz S, Baschant U, Schilling AF, Illing A, Stride B, et al. Glucocorticoids suppress bone formation by attenuating osteoblast differentiation via the monomeric glucocorticoid receptor. *Cell Metab*. 2010;11(6):517–31. <https://doi.org/10.1016/j.cmet.2010.05.005>.
 34. Dacquin R, Starbuck M, Schinke T, Karsenty G. Mouse alpha1(I)-collagen promoter is the best known promoter to drive efficient Cre recombinase expression in osteoblast. *Dev Dyn*. 2002;224(2):245–51. <https://doi.org/10.1002/dvdy.10100>.
 35. Kim JE, Nakashima K, de Crombrughe B. Transgenic mice expressing a ligand-inducible cre recombinase in osteoblasts and odontoblasts: a new tool to examine physiology and disease of postnatal bone and tooth. *Am J Pathol*. 2004;165(6):1875–82. [https://doi.org/10.1016/S0002-9440\(10\)63240-3](https://doi.org/10.1016/S0002-9440(10)63240-3).
 36. Chen J, Shi Y, Regan J, Karuppaiah K, Ornitz DM, Long F. Osx-Cre targets multiple cell types besides osteoblast lineage in postnatal mice. *PLoS One*. 2014;9(1):e85161. <https://doi.org/10.1371/journal.pone.0085161>.
 37. Huang W, Olsen BR. Skeletal defects in Osterix-Cre transgenic mice. *Transgenic Res*. 2015;24(1):167–72. <https://doi.org/10.1007/s11248-014-9828-6>. **This paper showed that there is a skeletal phenotype in the osterix-Cre transgenic mouse without crossing it to a mouse carrying a floxed transgene. This illustrates the need for using controls expressing Osx-Cre or expressing Osx-Cre and heterozygous floxed targeting gene. It also shows that investigators need to be alert for unexpected phenotypes in Cre expressing transgenic mouse lines.**
 38. Wang L, Mishina Y, Liu F. Osterix-Cre transgene causes craniofacial bone development defect. *Calcif Tissue Int*. 2015;96(2):129–37. <https://doi.org/10.1007/s00223-014-9945-5>.
 39. Nakamura T, Imai Y, Matsumoto T, Sato S, Takeuchi K, Igarashi K, et al. Estrogen prevents bone loss via estrogen receptor alpha and induction of Fas ligand in osteoclasts. *Cell*. 2007;130(5):811–23. <https://doi.org/10.1016/j.cell.2007.07.025>.
 40. Winkler CL, Kladney RD, Maggi LB Jr, Weber JD. Cathepsin K-Cre causes unexpected germline deletion of genes in mice. *PLoS One*. 2012;7(7):e42005. <https://doi.org/10.1371/journal.pone.0042005>. **This study crossed CatK-Cre mice with Arf floxed mice to delete Arf1 in osteoclasts and unexpectedly observed germline loss of Arf. This was found to be due to expression of Cre in both ovary and testes. This illustrates that investigators need to be alert to potential off-target and/or germline recombination when using Cre transgenic lines.**
 41. Okamoto K, Nakashima T, Shinohara M, Negishi-Koga T, Komatsu N, Terashima A, et al. Osteoimmunology: the conceptual framework unifying the immune and skeletal systems. *Physiol Rev*. 2017;97(4):1295–349. <https://doi.org/10.1152/physrev.00036.2016>.
 42. Shinohara M, Nakamura M, Masuda H, Hirose J, Kadono Y, Iwasawa M, et al. Class IA phosphatidylinositol 3-kinase regulates osteoclastic bone resorption through protein kinase B-mediated vesicle transport. *J Bone Miner Res*. 2012;27(12):2464–75. <https://doi.org/10.1002/jbmr.1703>.
 43. Saftig P, Hunziker E, Wehmeyer O, Jones S, Boyde A, Rommerskirch W, et al. Impaired osteoclastic bone resorption leads to osteopetrosis in cathepsin-K-deficient mice. *Proc Natl Acad Sci U S A*. 1998;95(23):13453–8.
 44. Gowen M, Lazner F, Dodds R, Kapadia R, Feild J, Tavarina M, et al. Cathepsin K knockout mice develop osteopetrosis due to a deficit in matrix degradation but not demineralization. *J Bone Miner Res*. 1999;14(10):1654–63. <https://doi.org/10.1359/jbmr.1999.14.10.1654>.
 45. Kiviranta R, Morko J, Alatalo SL, NicAmhlaibh R, Risteli J, Laitala-Leinonen T, et al. Impaired bone resorption in cathepsin K-deficient mice is partially compensated for by enhanced osteoclastogenesis and increased expression of other proteases via an increased RANKL/OPG ratio. *Bone*. 2005;36(1):159–72. <https://doi.org/10.1016/j.bone.2004.09.020>.
 46. Li CY, Jepsen KJ, Majeska RJ, Zhang J, Ni R, Gelb BD, et al. Mice lacking cathepsin K maintain bone remodeling but develop bone fragility despite high bone mass. *J Bone Miner Res*. 2006;21(6):865–75. <https://doi.org/10.1359/jbmr.060313>.
 47. Chen W, Yang S, Abe Y, Li M, Wang Y, Shao J, et al. Novel pycnodysostosis mouse model uncovers cathepsin K function as a potential regulator of osteoclast apoptosis and senescence. *Hum Mol Genet*. 2007;16(4):410–23. <https://doi.org/10.1093/hmg/ddl474>.
 48. Iwasawa M, Miyazaki T, Nagase Y, Akiyama T, Kadono Y, Nakamura M, et al. The antiapoptotic protein Bcl-xL negatively regulates the bone-resorbing activity of osteoclasts in mice. *J Clin Invest*. 2009;119(10):3149–59. <https://doi.org/10.1172/JCI39819>.
 49. Mizoguchi F, Izu Y, Hayata T, Hemmi H, Nakashima K, Nakamura T, et al. Osteoclast-specific Dicer gene deficiency suppresses osteoclastic bone resorption. *J Cell Biochem*. 2010;109(5):866–75. <https://doi.org/10.1002/jcb.22228>.
 50. Chiu WS, McManus JF, Notini AJ, Cassady AI, Zajac JD, Davey RA. Transgenic mice that express Cre recombinase in osteoclasts. *Genesis*. 2004;39(3):178–85. <https://doi.org/10.1002/gene.20041>.

51. Sanchez-Fernandez MA, Sbacchi S, Correa-Tapia M, Naumann R, Klemm J, Chambon P, et al. Transgenic mice for a tamoxifen-induced, conditional expression of the Cre recombinase in osteoclasts. *PLoS One*. 2012;7(5):e37592. <https://doi.org/10.1371/journal.pone.0037592>.
52. Seeling M, Hillenhiuff U, David JP, Schett G, Tuckermann J, Lux A, et al. Inflammatory monocytes and Fcγ receptor IV on osteoclasts are critical for bone destruction during inflammatory arthritis in mice. *Proc Natl Acad Sci U S A*. 2013;110(26):10729–34. <https://doi.org/10.1073/pnas.1301001110>.
53. Starczak Y, Reinke DC, Barratt KR, Ryan JW, Russell PK, Clarke MV, et al. Absence of vitamin D receptor in mature osteoclasts results in altered osteoclastic activity and bone loss. *J Steroid Biochem Mol Biol*. 2018;177:77–82. <https://doi.org/10.1016/j.jsbmb.2017.10.022>.
54. Sztacho M, Segeletz S, Sanchez-Fernandez MA, Czupalla C, Niehage C, Hoflack B. BAR proteins PSTPIP1/2 regulate podosome dynamics and the resorption activity of osteoclasts. *PLoS One*. 2016;11(10):e0164829. <https://doi.org/10.1371/journal.pone.0164829>.
55. Dossa T, Arabian A, Windle JJ, Dedhar S, Teitelbaum SL, Ross FP, et al. Osteoclast-specific inactivation of the integrin-linked kinase (ILK) inhibits bone resorption. *J Cell Biochem*. 2010;110(4):960–7. <https://doi.org/10.1002/jcb.22609>.
56. Alanne MH, Siljamaki E, Peltonen S, Vaananen K, Windle JJ, Parada LF, et al. Phenotypic characterization of transgenic mice harboring Nf1+/- or Nf1-/- osteoclasts in otherwise Nf1+/+ background. *J Cell Biochem*. 2012;113(6):2136–46. <https://doi.org/10.1002/jcb.24088>.
57. Xie H, Cui Z, Wang L, Xia Z, Hu Y, Xian L, et al. PDGF-BB secreted by preosteoclasts induces angiogenesis during coupling with osteogenesis. *Nat Med*. 2014;20(11):1270–8. <https://doi.org/10.1038/nm.3668>.
58. Clausen BE, Burkhardt C, Reith W, Renkawitz R, Forster I. Conditional gene targeting in macrophages and granulocytes using LysMcre mice. *Transgenic Res*. 1999;8(4):265–77.
59. Orthgiess J, Gericke M, Immig K, Schulz A, Hirrlinger J, Bechmann I, et al. Neurons exhibit Lyz2 promoter activity in vivo: implications for using LysM-Cre mice in myeloid cell research. *Eur J Immunol*. 2016;46(6):1529–32. <https://doi.org/10.1002/eji.201546108>.
60. Yoshitaka T, Mukai T, Kittaka M, Alford LM, Masrani S, Ishida S, et al. Enhanced TLR-MYD88 signaling stimulates autoinflammation in SH3BP2 cherubism mice and defines the etiology of cherubism. *Cell Rep*. 2014;8(6):1752–66. <https://doi.org/10.1016/j.celrep.2014.08.023>.
61. Hume DA. Applications of myeloid-specific promoters in transgenic mice support in vivo imaging and functional genomics but do not support the concept of distinct macrophage and dendritic cell lineages or roles in immunity. *J Leukoc Biol*. 2011;89(4):525–38. <https://doi.org/10.1189/jlb.0810472>.
62. Aliprantis AO, Ueki Y, Sulyanto R, Park A, Sigrist KS, Sharma SM, et al. NFATc1 in mice represses osteoprotegerin during osteoclastogenesis and dissociates systemic osteopenia from inflammation in cherubism. *J Clin Invest*. 2008;118(11):3775–89. <https://doi.org/10.1172/JCI35711>.
63. Goren I, Allmann N, Yogev N, Schurmann C, Linke A, Holdener M, et al. A transgenic mouse model of inducible macrophage depletion: effects of diphtheria toxin-driven lysozyme M-specific cell lineage ablation on wound inflammatory, angiogenic, and contractile processes. *Am J Pathol*. 2009;175(1):132–47. <https://doi.org/10.2353/ajpath.2009.081002>.
64. Fukushima H, Shimizu K, Watahiki A, Hoshikawa S, Kosho T, Oba D, et al. NOTCH2 Hajdu-Cheney mutations escape SCF(FBW7)-dependent proteolysis to promote osteoporosis. *Mol Cell*. 2017;68(4):645–58.e5. <https://doi.org/10.1016/j.molcel.2017.10.018>.
65. Mass E, Ballesteros I, Farlik M, Halbritter F, Gunther P, Crozet L, et al. Specification of tissue-resident macrophages during organogenesis. *Science*. 2016;353(6304):aaf4238. <https://doi.org/10.1126/science.aaf4238>.
66. Maeda K, Kobayashi Y, Udagawa N, Uehara S, Ishihara A, Mizoguchi T, et al. Wnt5a-Ror2 signaling between osteoblast-lineage cells and osteoclast precursors enhances osteoclastogenesis. *Nat Med*. 2012;18(3):405–12. <https://doi.org/10.1038/nm.2653>.
67. Nishikawa K, Iwamoto Y, Kobayashi Y, Katsuoka F, Kawaguchi S, Tsujita T, et al. DNA methyltransferase 3a regulates osteoclast differentiation by coupling to an S-adenosylmethionine-producing metabolic pathway. *Nat Med*. 2015;21(3):281–7. <https://doi.org/10.1038/nm.3774>.
68. Yu TY, Pang WJ, Yang GS. Aryl hydrocarbon receptors in osteoclast lineage cells are a negative regulator of bone mass. *PLoS One*. 2015;10(1):e0117112. <https://doi.org/10.1371/journal.pone.0117112>.
69. Abram CL, Roberge GL, Hu Y, Lowell CA. Comparative analysis of the efficiency and specificity of myeloid-Cre deleting strains using ROSA-EYFP reporter mice. *J Immunol Methods*. 2014;408:89–100. <https://doi.org/10.1016/j.jim.2014.05.009>.
70. Ishii M, Egen JG, Klauschen F, Meier-Schellersheim M, Saeki Y, Vacher J, et al. Sphingosine-1-phosphate mobilizes osteoclast precursors and regulates bone homeostasis. *Nature*. 2009;458(7237):524–8. <https://doi.org/10.1038/nature07713>.
71. Sugatani T, Hruska KA. Impaired micro-RNA pathways diminish osteoclast differentiation and function. *J Biol Chem*. 2009;284(7):4667–78. <https://doi.org/10.1074/jbc.M805777200>.
72. Soung do Y, Kalinowski J, Baniwal SK, Jacome-Galarza CE, Frenkel B, Lorenzo J, et al. Runx1-mediated regulation of osteoclast differentiation and function. *Mol Endocrinol*. 2014;28(4):546–53. <https://doi.org/10.1210/me.2013-1305>.
73. Yuan X, Cao J, Liu T, Li YP, Scannapieco F, He X, et al. Regulators of G protein signaling 12 promotes osteoclastogenesis in bone remodeling and pathological bone loss. *Cell Death Differ*. 2015;22(12):2046–57. <https://doi.org/10.1038/cdd.2015.45>.
74. Ferron M, Vacher J. Targeted expression of Cre recombinase in macrophages and osteoclasts in transgenic mice. *Genesis*. 2005;41(3):138–45. <https://doi.org/10.1002/gene.20108>.
75. Deng L, Zhou JF, Sellers RS, Li JF, Nguyen AV, Wang Y, et al. A novel mouse model of inflammatory bowel disease links mammalian target of rapamycin-dependent hyperproliferation of colonic epithelium to inflammation-associated tumorigenesis. *Am J Pathol*. 2010;176(2):952–67. <https://doi.org/10.2353/ajpath.2010.090622>.
76. Qian BZ, Li J, Zhang H, Kitamura T, Zhang J, Campion LR, et al. CCL2 recruits inflammatory monocytes to facilitate breast-tumour metastasis. *Nature*. 2011;475(7355):222–5. <https://doi.org/10.1038/nature10138>.
77. Reddy SV, Hundley JE, Windle JJ, Alcantara O, Linn R, Leach RJ, et al. Characterization of the mouse tartrate-resistant acid phosphatase (TRAP) gene promoter. *J Bone Miner Res*. 1995;10(4):601–6. <https://doi.org/10.1002/jbmr.5650100413>.
78. Luchin A, Suchting S, Merson T, Rosol TJ, Hume DA, Cassady AI, et al. Genetic and physical interactions between Microphthalmia transcription factor and PU.1 are necessary for osteoclast gene expression and differentiation. *J Biol Chem*. 2001;276(39):36703–10. <https://doi.org/10.1074/jbc.M106418200>.
79. Nagashima K, Sawa S, Nitta T, Tsutsumi M, Okamura T, Penninger JM, et al. Identification of subepithelial mesenchymal cells that induce IgA and diversify gut microbiota. *Nat Immunol*. 2017;18(6):675–82. <https://doi.org/10.1038/ni.3732>.
80. Powell JJ, Thomas-McKay E, Thoree V, Robertson J, Hewitt RE, Skepper JN, et al. An endogenous nanomineral chaperones luminal

- antigen and peptidoglycan to intestinal immune cells. *Nat Nanotechnol.* 2015;10(4):361–9. <https://doi.org/10.1038/nnano.2015.19>.
81. Hanada R, Leibbrandt A, Hanada T, Kitaoka S, Furuyashiki T, Fujihara H, et al. Central control of fever and female body temperature by RANKL/RANK. *Nature.* 2009;462(7272):505–9. <https://doi.org/10.1038/nature08596>.
82. Kuhn R, Torres RM. Cre/loxP recombination system and gene targeting. *Methods Mol Biol.* 2002;180:175–204. <https://doi.org/10.1385/1-59259-178-7:175>.