SUMMARY

Osteoporosis is a common disorder affecting more than 200 million people worldwide. With rising treatment costs and increasing socioeconomic issues, disease prevention and effective therapy options become more important by the day. As of today, almost three-quarter of elderly people worldwide are affected by low bone mass and increased fracture risk. Interestingly, genetic predisposition was shown to be relevant in 60 – 80 % of osteoporosis patients.

Mutations in plastin 3 (*PLS3*) cause X-linked primary osteoporosis with fractures in men and a variable phenotype, ranging from unaffected to mild fractures, in women. Additionally, a single nucleotide polymorphism (SNP) in *PLS3* shows the strongest association for the increased risk of developing postmenopausal osteoporosis in women up to date. PLS3 is an F-actin bundling and binding protein which is overexpressed in around 5% of the general population, with yet unknown consequences. Furthermore, PLS3 is a protective modifier of motor neuron disorder spinal muscular atrophy (SMA) when overexpressed. Hitherto, the role of PLS3 in bone homeostasis and its implications in osteoporosis remain elusive. However, *PLS3* is expressed in all essential cell types in bone, namely osteoblasts, osteocytes and osteoclasts. Remarkably, morpholino-mediated *pls3* knock-down in zebrafish causes a malformed craniofacial morphology, especially present in the jaw. Additional overexpression of human *PLS3* mRNA in zebrafish was able to rescue the phenotype, emphasizing the role of PLS3 in bone development.

This thesis aims to unravel the function of PLS3 in skeletal health by studying genetically engineered mice in which *Pls3* has been ubiquitously knocked-out (KO) or a human *PLS3* transgene is overexpressed (OE). Hence, the following research questions were applied: 1) What is the pathophysiological effect on bone morphology and strength in *Pls3* KO and *PLS3* OE animals? 2) Which bone cell type is functionally or morphologically affected by *Pls3* KO and *PLS3* OE? 3) What is the underlying molecular mechanism causing the observed phenotype? 4) Which other bone related pathways might be affected by the loss or overexpression of *PLS3*?

Here, we examined bone morphology in ubiquitous *Pls3* KO mice by micro-CT and 3-point-bending-test. *Pls3* KO causes an osteoporotic phenotype, which was more pronounced in males than females, resembling the human phenotype. Contrarily, ubiquitous *PLS3* OE mice exhibit an increased cortical thickness in males and females as well as augmented bone strength in females. Immunohistological stainings of femoral sections revealed that osteoclast numbers were significantly reduced in 5-day-old *PLS3* OE animals but restored in 3-month-old mice. Osteoclast number was unaffected in *Pls3* KO mice and early mineralization as well as chondrocyte differentiation was unaffected in both mouse models. Thus, osteoclast function was further examined in primary osteoclast cultures, which were differentiated from femoral

bone marrow of 3-month-old mice, and displayed an increase in resorptive activity in *Pls3* KO and a decrease in *PLS3* OE mice. Furthermore, immunofluorescence stainings of osteoclast cultures revealed that podosome structures in osteoclasts of both *Pls3* KO and *PLS3* OE was affected, presenting rather podosome clusters than rings. Interestingly, *PLS3* OE osteoclasts were almost devoid of any podosome structures at all. To unravel the molecular mechanism underlying osteoclast dysfunctions, essential protein players of the NFκB pathway, essential in osteoclast differentiation, were investigated by western blotting of extracted proteins from primary osteoclast cultures. Remarkably, RELA (NFκB subunit p65) was highly increased in *PLS3* OE osteoclasts, but unchanged in *Pls3* KO. Concomitantly, NFκB repressing factor (NKRF) was found to be a novel interaction partner of PLS3 in mass-spectrometry as well as co-immunoprecipitation experiments. Interestingly, we demonstrated by quantitative real-time PCR that mRNA expression of master regulator of osteoclastogenesis nuclear factor of activated T cells cytosplasmic 1 (*Nfatc1*) was downregulated in *PLS3* OE and upregulated in *Pls3* KO osteoclasts. Immunofluorescence stainings in cell cultures revealed that this change was correlating with the nuclear translocation of NKRF. Precisely, NKRF nuclear localization was significantly diminished in *Pls3* KO osteoclasts thereby causing increased *Nfatc1* expression. Contrarily, decreased *Nfatc1* mRNA levels were caused upon increased NKRF nuclear translocation in *PLS3* OE osteoclasts. Thus, we hypothesized that transcriptional repression of *Nfatc1* by PLS3-mediated NKRF translocation modifies osteoclast function, thereby causing either osteoporosis in *Pls3* KO or an augmented bone phenotype in *PLS3* OE. Taken together, PLS3 is a novel and crucial regulator of osteoclast function through alterations in the NFκB-NKRF-NFATC1 pathway.

Finally, transcriptome analysis of primary osteoclasts from male and female 3-month-old *Pls3* KO and *PLS3* OE mice was studied to unravel differentially expressed genes. We discovered changes in several candidate genes involved in the development of osteoporosis as well as osteoarthritis. Furthermore, differentially expressed genes were found pointing at a potential dysregulation of intracellular trafficking in *Pls3* KO and *PLS3* OE osteoclasts possibly contributing to the bone phenotype. While these are preliminary data, an in depth analysis is required in future to better understand the entire interactome of PLS3 and its impact on bone homeostasis.