General discussion
Chapter VIII

The CCAAT/enhancer binding protein β (C/EBPβ) transcription factor is widely expressed and regulates amongst others tissue homeostasis, adipocyte and hematopoietic stem cell differentiation, cell growth, senescence and apoptosis. From the single-exon CEBPB-mRNA three protein isoforms (LAP1, LAP2 and LIP) are translated through the usage of alternative translation initiation sites. Translation of LIP strictly depends on translation of a uORF element in the CEBPB-mRNA 5'-leader sequence and subsequent translation re-initiation. LIP lacks the N-terminal transactivation domains of LAP1/2 but has an otherwise identical amino acid sequence, including the C-terminal DNA-binding/dimerization domain. Thereby, LIP competes for the same DNA binding sites as LAP but inhibits target gene transcription as it cannot recruit transcription co-activators. Therefore, the LIP/LAP ratio determines the transcriptional activity of C/EBPβ. Physiologically, the LIP/LAP ratio can be translationally regulated by for example increased nutrient availability through mTORC1 activation, which stimulates LIP translation.

Deregulated expression of C/EBPβ isoforms affects amongst others breast cancer progression and ageing. For instance, C/EBPβ-LIP is highly expressed in the aggressive triple negative breast cancer (TNBC) subtype. In this thesis we aimed to uncover upstream regulators of C/EBPβ translation and individual isoform function in TNBC. We find the N6-methyladenosine demethylase FTO promotes C/EBPβ-LIP expression and thereby regulates TNBC growth and migration. We further describe a role for the mTORC1 substrate S6K in inhibiting LIP translation. Additional regulators may be identified by unbiased genome-wide CRISPR/Cas9 screening for which we developed reporter systems. We find LIP, but not LAP, promotes aerobic glycolysis and stimulates activity of the malate-aspartate shuttle (MAS), rendering cells with high LIP expression addicted to glucose. Finally, we describe the generation of a C/EBPα-uORF-deficient (C/EBPαΔuORF) mouse model with reduced p30 expression to study the role of individual C/EBPα isoforms in organismal metabolism, differentiation and oncogenesis. In this chapter, I explain how our findings contribute to the understanding of C/EBPβ and TNBC biology and highlight open ends to be investigated in future research.

Identifying upstream regulators of CEBPB-mRNA translation in TNBC

Previous work has shown C/EBPβ is involved in mammary gland development (1) while C/EBPβ-LIP expression is increased in TNBC and promotes disease progression and therapy resistance (2-9). Several factors are known to regulate CEBPB-mRNA translation re-initiation resulting in LIP synthesis. These include mTORC1, elf4E, elf2α, CUG-BP1, DENR/MCT-1 and SBDS, of which mTORC1 has been most well described (10-15). In Chapter II we found inhibition of mTORC1 failed to reduce the LIP/LAP ratio in TNBC in contrast to for example lesser aggressive luminal A type breast cancer cells. This was surprising to us and to our knowledge constitutes the first identified cell type where CEBPB-mRNA
translation is insensitive to mTORC1 inhibition. Under favorable conditions, mTORC1 stimulates translation initiation through phosphorylation of 4E-BPs which causes their release from eIF4E. This allows eIF4E to interact with other translation initiation factors such as eIF4G and eventually the small ribosomal subunit to promote scanning and cap-dependent translation (described in Chapter I and reviewed in (16-19)). The importance of free eIF4E for translation re-initiation following C/EBPβ-uORF translation (i.e. LIP translation) has been previously described (11). In the absence of 4E-BP1/2, LIP levels are increased and become refractory to mTORC1 inhibition (11). However, 4E-BPs are present in TNBC cells and are dephosphorylated normally in response to mTORC1 inhibition. We confirmed protein stability of LIP was not affected and therefore it is likely TNBC cells have activated a distinct, currently unknown, pathway to sustain LIP translation during mTORC1 inhibition.

For unbiassed assessment of the molecular mechanism underlying the high expression levels of C/EBPβ-LIP and its translational insensitivity to mTORC1 inhibition in TNBC, in Chapter II we developed several C/EBPβ reporter systems for use in a genome-wide CRISPR/Cas9 screening approach. CRISPR screens have widely applied since their first application in 2014 (20, 21) and make use of Cas9 nuclease in combination with guide RNAs (gRNAs) to disrupt specific genomic loci and thereby make targeted gene knock-outs. In a pooled screen, a library of gRNAs is transduced into cells expressing Cas9 at such a viral titer that on average a single gRNA (and thereby gene knock-out) is obtained per cell. The readout can be for example cell survival, resistance to chemotherapy, expression of a fluorescent reporter protein (and subsequent sorting by FACS), single-cell transcriptomics or expression/phosphorylation of a specific protein (and subsequent antibody staining and sorting by FACS) (reviewed in: (22)). Our initial efforts were focused on creating a GFP reporter driven by C/EBPβ, a strategy similar to a previously successful screen where a fluorescent NF-κB reporter was used (23). We constructed several C/EBP binding sites in tandem driving luciferase expression, which allowed successful down- and upregulation respectively by LIP or LAP in transient co-transfection experiments (Chapter II, Figure 2). To our disappointment we did not obtain clones of BT-20 TNBC cells stably expressing a GFP variant of the reporter, either through lentiviral transduction or transfection followed by continued antibiotic selection. Silencing of transgene expression in mammalian cells is a known phenomenon often caused by DNA methylation or histone modifications (epigenetic silencing) (24). Treatment of reporter clones with DNA methyltransferase inhibitors marginally increased GFP expression and introduced a minimal responsiveness to LIP or LAP co-transfection which was completely absent in untreated cells. This implies DNA methylation minimally contributes to transgene silencing, which is more likely caused by histone modifications and chromatin condensation. The introduced construct cannot have been completely silenced as cells were cultured under persistent geneticin selection for which the resistance gene lies on the introduced transgene. There was however no selective pressure to sustain GFP expression which might allow reduced GFP expression over time.
by local chromatin condensation, concomitantly causing a lack of regulation by LIP or LAP transfection due to inaccessibility of the C/EBP binding sites. To circumvent the problems posed by chromatin accessibility, we designed a translational reporter modelled closely after the CEBPB-mRNA. A straightforward replacement of the LAP coding sequence with one fluorophore and the LIP coding sequence with another (using the ratio of the two for an eventual FACS readout of LIP/LAP ratio) was no option. This is because no AUG codons can be introduced between the LAP and LIP start codons, as these would likely interfere with re-initiation at the LIP AUG after uORF translation, and all known fluorescent protein contain internal AUGs (already in the main reading frame). Splitting the LAP and LIP translation reporters over two constructs would solve this issue, but would inadvertently introduce additional variability due to potential differences in expression level, as the reporters would not be translated from a single mRNA. For these reasons we chose a single mRNA reporter where initiation at the LAP start codon will produce a truncated sfCherry protein (25), while initiation at the LIP start codon will yield GFP expression. This truncated sfCherry2(11) contains no internal AUGs but needs to complement with the remainder of the sfCherry3V protein (sfCherry3V(1-10)) expressed from a different construct to be fluorescent (25). This setup worked well in transient transfection tests and also upon stable integration of both constructs we were able to sort GFP/sfCherry double positive cells (Chapter II, Figure 4). sfCherry expression was however lost upon culturing of double positive cells and could not be restored by DNA methyltransferase inhibitor treatment. As the reporter construct was still expressed (evidenced by sustained GFP expression), silencing of sfCherry3V(1-10) is the likely cause despite cells being constantly grown under antibiotic selection coded for on the sfCherry3V(1-10) construct. We had anticipated the random integration approach used here would yield clones with sustained expression. This does not seem to be the case, yet the reporters work as intended shortly after transduction and during transient transfection experiments. We therefore propose future integration of the constructs into a targeted locus in order to maintain stable expression over time. Such an approach is widely used to generate genetically engineered mouse models for overexpression of various factors from for example the Col1a1, Rosa26, or Hprt locus (26). In vivo these models are often created using the bacteriophage P1 derived Cre-loxP-system. Cre recombinase can stably integrate novel DNA segments if they are flanked by loxP sites through recombination with loxP sites integrated into the genome (27). A similar system is derived from Saccharomyces cerevisiae and uses Flp recombinase in combination with Flp-Recombination Target (FRT) sites (28). This system also allows recombination mediated integration of an expression construct in a single localized genomic site in mammalian cells (29). For this type of approach first a cell line with integrated recombination sites (loxP or FTR) needs to be obtained as currently there are no TNBC cell lines with integrated recombination sites. Considering the relevance of C/EBPB for TNBC biology, we believe it would be worth the effort to create BT-20 cells with integrated recombination sites in an active chromatin
region. Not only could these be used for the intended stable expression of the translation reporter constructs, but they would also provide a useful tool for future validation and follow-up studies.

To further study the molecular mechanisms downstream of mTORC1, in **Chapter III** we investigated the role of S6K activity in LIP translation. Chemical inhibition of S6K1/2 using DG2 was previously observed to increase LIP expression in MEF cells (11). Although S6K was long thought to specifically regulate translation of 5’ terminal oligopyrimidine (TOP) mRNAs, a mouse model lacking both S6K isoforms displayed normal TOP mRNA translation (30). S6K activity has however been shown to increase the helicase activity of eIF4A through phosphorylation of eIF4B (31) and PDCD4 (32). Our experiments in **Chapter III** indicate DG2 treatment increases expression of LIP in multiple cell lines by increasing LIP translation without affecting protein isoform stability. Interestingly, co-treatment with catalytic mTORC1 inhibitor pp242 abolishes the increase in LIP expression. As pp242 also inhibits mTORC2, it will be interesting to see whether a more specific mTORC1 inhibitor (such as rapamycin) produces similar results in co-treatment with DG2. As mentioned, mTORC1 inhibition in MEFs lacking 4E-BP1/2 does not affect the LIP/LAP ratio (11) and we know from our experiments that DG2 increases LIP in these cells. It will be interesting to see whether mTORC1 inhibition can still prevent an increase in LIP translation by DG2 in cells lacking 4E-BPs. If the increase in LIP translation by DG2 is mediated purely through canonical cap-dependent translation, no effect of co-treatment is to be expected since mTORC1 regulates initiation via (the now absent) 4E-BPs. In addition to determining cap-dependency, future work should aim to identify the downstream substrate(s) responsible for the increase in LIP upon DG2 treatment. Several S6K substrates including rpS6 are not fully dephosphorylated in mouse models lacking both S6K isoforms (30). Indeed, other AGC family kinases such as RSK (p90S6K) and AKT are known to phosphorylate for example rpS6 and eIF4B (31, 33-35), off-setting the effect of S6K deficiency. Furthermore, contrary to previous results in MEFs with a different genetic background (11), knock-down of S6K1 did not affect LIP/LAP ratio and barely affected phosphorylated rpS6 levels. Although DG2 is relatively specific for S6Ks for a kinase inhibitor, it also significantly inhibits related RSKs and MSks (36). It is therefore reasonable to hypothesize that the effect of DG2 on LIP translation is not mediated solely through SK6 inhibition, but rather through inhibition of several structurally related kinases with common downstream substrates. Given the strong effect DG2 has on LIP translation, it will be interesting to identify this/these substrate(s) to improve our understanding of translation re-initiation regulation and to determine if the effect is C/EBPβ-specific or generally applicable to re-initiation following uORF translation.

### The role of FTO-C/EBPβ in regulating breast cancer growth and migration

FTO and C/EBPβ are two factors whose roles in organismal metabolism have been well established and that have become implicated in disease progression of various cancer types in recent years (reviewed
in Chapters I & IV and (37-39)). Single nucleotide polymorphisms (SNPs) in FTO are significantly associated with human obesity (40-43). Although over 100 genomic loci have been found to associate with body mass index (BMI) to date, the strongest genetic link is found for FTO in multiple ancestries (40). Mice deficient in Fto display a healthy metabolic phenotype that includes reduced bodyweight, fat content and weight gain upon high fat diet (HFD) feeding (44), while ubiquitous overexpression of Fto leads to opposite phenotypes and induces glucose intolerance upon HFD feeding (45). Downstream targets of Fto crucial for regulation of metabolism are however poorly explored. In more recent years, additional mouse models have started examining the function of Fto in specific organs. Liver-specific knock-out of Fto for example led to reduced body weight and fat mass similar to whole body Fto deficiency, but did not affect insulin sensitivity or glucose tolerance (46). Also for C/EBPβ organismal metabolism has been studied. The metabolic phenotypes of mice deficient in LIP through a mutation in the C/EBPβ-uORF (C/EBPβ ΔuORF mice) mimics those observed under caloric restriction (47, 48). C/EBPβ ΔuORF mice display reduced body weight, body fat content and steatosis with better insulin sensitivity, glucose tolerance and preservation of motor coordination and naïve T cell population (11, 49).

Molecularly, FTO functions as an N6-methyadenosine (m6A) and N6,2′-O-dimethyadenosine (m6Am) demethylase in mRNA, snRNA and ssDNA (50-52). The function of m6Am, predominantly present at the first transcribed nucleotide of mRNA and snRNA, is still poorly studied but has recently become more accessible by the discovery of the mRNA m6Am methyltransferase phosphorylated C-terminal domain (CTD)-interacting factor 1 (PCIF1) (53, 54) and the snRNA m6Am methyltransferase METTL4 (55, 56). The role of m6A has mainly been studied in mRNA and appears to affect amongst others polyadenylation, splicing, nuclear export, RNA structure, liquid-liquid phase separation, translation and mRNA decay (reviewed in Chapter IV) although it should be noted the field is rapidly evolving and certain aspects of m6A biology, including the role of m6A-binding YTHDF proteins in mRNA translation, are disputed (57-59). Because of the involvement of C/EBPβ in TNBC biology, the similarity of metabolic phenotypes of Fto and C/EBPβ-LIP deficient mouse models and the described function of m6A in regulating translation of specific transcripts, in Chapter V, we assessed whether FTO-mediated m6A demethylation influences C/EBPβ-isoform expression. Indeed, we find knockdown of the m6A demethylase FTO reduced C/EBPβ-LIP expression, while knockdown of the m6A-methyltransferasae complex component WTAP promotes it. The CEBPB-mRNA contains several bioinformatically predicted (60) and experimentally verified (61, 62) m6A-sites. We observe a clear trend towards increased CEBPB-mRNA m6A levels in MeRIP-qPCR experiments upon FTO knockdown in MDA-MB-231 TNBC cells. Cellular growth and migration of breast cancer cells lacking FTO are impaired and gene set enrichment analysis (GSEA) and gene ontology term (GO-term) analysis point towards downregulation of gene expression related to the extracellular matrix (ECM) and epithelial to
mesenchymal transition (EMT). We confirmed the reduced expression of fibronectin, tenacin C, collagens and matrix metalloproteases in qPCR experiments. In a recent study from our lab, depletion of C/EBPβ in TNBC cells reduced migration and invasion with a similar downregulation of these factors (9). In line with a transcriptional role for C/EBPβ in regulating gene expression downstream of FTO, we did not find any changes in m6A levels or mRNA stability for any of the regulated ECM/EMT transcripts. We propose future experiments determine if the FTO-mediated increase in C/EBPβ-LIP expression is necessary and sufficient for FTO to promote TNBC migration; does expression of LIP in FTO-deficient cells rescue their migration phenotype and does FTO-knockdown still negatively affect TNBC cell migration in the absence of C/EBPβ expression? On a molecular level it will be interesting to identify the exact nucleotides in the CEBPB-mRNA that are m6A modified, for example by mutating potentially m6A-modified adenosines and determine if/how this affects C/EBPβ translation regulation by FTO. Further, although several factors including YTHDF1, YTHDF3 and elf3 (63-65), YTHDF2 (indirectly) (66), YTHDC2 (67) and ABCF1 (68) have been suggested to promote translation of specific m6A modified mRNAs, their role in CEBPB-mRNA translation remains unstudied and is worthy of investigation.

We did not expect an increase in E2F target gene expression and reduction in S-phase DNA replication activity upon knockdown of FTO in TNBC cells. E2F family members have long been known to regulate processes such as S-phase entry, DNA replication, mitosis, DNA repair and apoptosis (69). There are studies suggesting a link between C/EBPβ transcriptional activity and activation of E2F target genes (70, 71), but whether any such regulation exists in TNBC remains to be determined. E2F1 can be induced by DNA damage (72) and DNA damage response genes were upregulated in FTO-deficient cells. However, we did not find an increase in basal or hydrogen peroxide-induced γH2AX levels in FTO knockdown cells. Regardless of the mechanism of their upregulation, we reasoned the increase in E2F target expression might induce vulnerabilities in FTO deficient cells exposed to E2F inhibition. Indeed, treatment of breast cancer cells with FTO inhibitor entacapone significantly increased the sensitivity to E2F inhibition with HLM006474. In future work, we aim to establish the mechanism of E2F regulation by FTO and determine if combined therapy targeting FTO and E2F (or other cell cycle regulators) is a valid therapeutic strategy in TNBC.

**LIP-induced MAS activity induces metabolic vulnerabilities in TNBC**

The function of C/EBPβ isoforms in the regulation of cellular metabolism has in part been previously described. LIP is a more potent activator of mitochondrial respiration than LAP and, in contrast to LAP, stimulates glycolysis, thereby inducing a cancer-type metabolic reprogramming of aerobic glycolysis (73). In Chapter VI, we describe inhibition of glycolysis by 2-deoxyglucose (2-DG) treatment inhibits proliferation and induces apoptosis in cells with high LIP/LAP expression ratios. In TNBC cells with endogenously high LIP expression, knockout of C/EBPβ reduces 2-DG sensitivity while LIP
overexpression in T-47D cells (with low endogenous LIP expression) increases it. Using oxygen consumption measurements (Seahorse XF96) and targeted inhibition of relevant metabolic pathways, we show that LIP induces the malate-aspartate-shuttle (MAS) to promote mitochondrial respiration via NADH entering the electron transport chain at complex I. In addition to stimulation of ATP production, this also provides LIP expressing cells with a way to regenerate cytosolic NAD+, which is an essential co-factor used by GAPDH and PHGDH in glycolysis and serine biosynthesis respectively (74). As glycolysis requires NAD+ and cancer cells display high glycolytic activity, cancer cells often have high cellular NADH/NAD+ ratios since NAD+ is depleted from the cytosol (75). Low levels of cytosolic NAD+ can become problematic as cancer cells rely on high glycolytic flux for uncoupling glycolysis from the tricarboxylic acid (TCA) cycle, thereby allowing diversion of glucose carbons to biosynthetic pathways such as the pentose phosphate pathway, serine biosynthesis pathway and hexosamine pathway (76). Therefore, cytosolic NAD+ needs to be regenerated for example by the MAS. Indeed, knockdown of the MAS-component SLC25A11 (malate/α-ketoglutarate carrier) inhibits lung cancer cell growth in xenograft models (77). 2-DG treatment of cells with high LIP levels results in low NADH/NAD+ ratios, as the LIP-driven MAS continues to generate cytosolic NAD+. Our preliminary experiment did not reveal the mechanism by which LIP induced MAS activity, as MDH1 methylation status, basal Ca2+-levels and gene expression of MAS components was hardly affected by LIP. We did observe an increase in SLC25A12 (aralar, Glu/Asp antiporter) in LIP expressing cells which reduced cytosolic NADH/NAD+ ratios in melanoma and lung cancer models (78, 79). The involvement of SLC25A12 in LIP-mediated stimulation of the MAS await further testing. In our experiments low NADH/NAD+ ratios are associated with apoptosis. Restoring their balance through inhibition of NADH-consuming processes such as the MAS or lactate dehydrogenase reduces 2-DG induced cell death. We do not know what causes the induction of apoptosis in high LIP expressing cells with a low NADH/NAD+ ratio. Increased NAD+ levels rather protect from apoptosis through activation of sirtuins (80). NAD+ is however also a precursor for cyclic-APD ribose (cADPR) which increases cellular Ca2+ levels (81). This in turn activates calcium-dependent proteases such as calpains which have been shown, at least in vitro, to cleave apoptosis inducing factor mitochondria associated 1 (AIFM1) (82). Mis localization of Nde1 (a yeast homolog of AIFM1) leads to its proteolytic cleavage and was recently shown to induce apoptosis (83). Whether similar mechanisms exist in mammalian cells and if they are inducible by increased Ca2+ or NAD+ levels caused by high LIP expression under conditions of glycolysis inhibition remains unclear.

**C/EBPαΔuORF mice: a novel mouse model with reduced C/EBPα-p30 expression**

Previously we described mice with a genetic mutation in the C/EBPβ-uORF (C/EBPβΔuORF mice) express reduced levels of LIP resulting in increased health- and lifespan and reduced tumor incidence, reminiscent of metabolic phenotypes of mice under caloric restriction or mTORC1 inhibition (11, 47-
Both C/EBPβ and C/EBPα are expressed as multiple isoforms of which the translation is regulated by mTORC1 signaling through 4E-BPs (10) (reviewed in Chapter I). C/EBPα is a well-known regulator of organismal metabolism (84), highly expressed in metabolic tissues (liver, white and brown adipose tissue (WAT/BAT respectively)), and Cebpa-knockout mice are inviable due to a lack of hepatic glycogen buildup prior to birth and impaired gluconeogenesis (85). However, the importance of uORF-mediated translational control and thereby isoform-specific functions of C/EBPα in metabolism regulation in vivo are not studied yet. In Chapter VII we successfully generated mice with a mutation in the CEBPA-uORF (C/EBPαΔuORF mice) that express reduced C/EBPα-p30/p42 ratios. We find homozygous C/EBPαΔuORF/ΔuORF mice are viable, born to mendelian ratios from heterozygous parents and display no differences in body weight compared to wild type mice in either gender up to 5 months after birth. These results indicate p30 is dispensable for organismal development and no translational compensation of C/EBPs is taking place since C/EBPβ-LIP/LAP ratios were unaffected by the mutation. Initial examination of their glucose metabolism indicates mutant male mice have improved glucose tolerance and mutant female mice display impaired gluconeogenesis while insulin sensitivity is unaltered in either gender. Ablation of C/EBPα in the mouse liver after birth has been shown to reduce glucokinase expression which is associated with decreased glucose tolerance (86). The increased p42/p30 ratios in C/EBPαΔuORF mice might similarly promote glucokinase expression to increase glucose tolerance in male mice. To evaluate this, gene expression of glucose metabolism genes in relevant tissues (liver, adipose tissue and muscle) will be analysed in future work. We were surprised to find reduced gluconeogenesis by female C/EBPαΔuORF mice in i.p. pyruvate tolerance tests. C/EBPα is a known activator of gluconeogenesis through induction of for example Pepck and G6pc expression in the mouse liver (85). This might be an indirect effect however, as hepatic Pepck and G6pc expression are not reduced in mice with liver-specific C/EBPα deficiency (86). Insulin is a known repressor of gluconeogenesis and although insulin sensitivity was unaltered in mutant female mice, they did display a reduced fasted blood glucose level. It will be interesting to determine fed and fasted insulin levels in female mice to see if they might contribute to the observed metabolic phenotypes. Furthermore, in the aforementioned C/EBPαΔuORF mice differences in body weight between mutant and wild type mice increased with age, highlighting the study of C/EBPαΔuORF mice should not be limited to mice of younger age.

In establishing this model, we have also allowed for investigation of isoform-specific functions of C/EBPα in the hematopoietic system and malignancies thereof. C/EBPα promotes differentiation of hematopoietic cells and is often mutated in acute myeloid leukaemia (AML) leading to a malignant block in differentiation, particularly of myeloid lineages (87, 88). Indeed, overexpression of the transcriptional inhibitory p30 isoform promotes AML development with complete penetrance (89). It will be interesting to see whether C/EBPαΔuORF mice display normal hematopoietic differentiation and
cross-breeding with leukaemia models may further reveal the role of C/EBPα-p30 in hematopoietic malignancies. As translation of the CEBPB-mRNA has been shown to be druggable (12), identification of a potentially essential role for p30 in hematopoietic malignancy may promote future efforts targeting CEBPA-mRNA translation for therapeutic purposes.

Concluding remarks
In this thesis we aimed to identify molecular mechanisms that lie upstream of CEBPB-mRNA translation and the effect of individual C/EBPβ isoforms on breast cancer cells. We propose regulation of uORF-mediated translation re-initiation of the CEBPB-mRNA becomes insensitive to mTORC1 activity in TNBC cells, and devised a screening strategy to unbiasedly assess regulators of C/EBPβ translation (Chapter II). Additionally, we show inhibition of mTORC1 target S6K rather increases LIP expression, through as of yet to be identified mechanisms (Chapter III). Expression of C/EBPβ isoforms is further controlled by mRNA m6A modification and demethylation through FTO, which thereby promotes breast cancer growth and migration (Chapter V). In addition to cell migration, LIP promotes aerobic glycolysis with concomitant upregulation of MAS activity which renders cells sensitive to glycolysis inhibition (Chapter VI). Finally, we describe the establishment of C/EBPαΔuORF mice with reduced p30 expression to study C/EBPα isoform-specific functions in organismal metabolism and (hematopoietic) malignancy (Chapter VII). Our studies indicate proper regulation of C/EBPβ and -α isoform expression is key for cellular homeostasis and their deregulation contributes to disease.
General discussion

References


Chapter VIII
