haematologica
Journal of The Ferrata Storti Foundation

The multifaceted role of protein kinase CK2 in high-risk acute lymphoblastic leukemia

by Yun Zhou, Haiwei Lian, Ning Shen, Sovannarith Korm, Andrew Kwok Ping Lam, Olivia Layton, Leah N. Huiting, Dun Li, Kelly Miao, Aozhuo Zeng, Esther Landesman-Bollag, David C. Seldin, Hui Fu, Li Hong, and Hui Feng

Haematologica 2020 [Epub ahead of print]

Citation: Yun Zhou, Haiwei Lian, Ning Shen, Sovannarith Korm, Andrew Kwok Ping Lam, Olivia Layton, Leah N. Huiting, Dun Li, Kelly Miao, Aozhuo Zeng, Esther Landesman-Bollag, David C. Seldin, Hui Fu, Li Hong, and Hui Feng. The multifaceted role of protein kinase CK2 in high-risk acute lymphoblastic leukemia.
Haematologica. 2020; 105:xxx
doi:10.3324/haematol.2020.246918

The multifaceted role of protein kinase CK2 in high-risk acute lymphoblastic leukemia

Yun Zhou[†1,2], Haiwei Lian[†2,3], Ning Shen[2,4], Sovannarith Korm[2], Andrew Kwok Ping Lam[2], Olivia Layton[2], Leah N. Huiting[2], Dun Li[2], Kelly Miao[2], Aozhuo Zeng[2], Esther Landesman-Bollag[4], David C. Seldin[4], Hui Fu[3], Li Hong[1*], and Hui Feng[2,4*]

[1]Department of Gynecology, Wuhan University Renmin Hospital, Wuhan, Hubei, P. R. China

[2]Department of Pharmacology & Experimental Therapeutics, Boston University School of Medicine, Boston, MA, USA

[3]Department of Anatomy and Embryology, Wuhan University School of Basic Medical Sciences, Wuhan, Hubei, P. R. China

[4]Department of Medicine, Section of Hematology and Medical Oncology, Boston University School of Medicine, Boston, MA, USA

[†]equal contribution

***Correspondence:** *HUI FENG. huifeng@bu.edu and HONG LI. drhongli1011@yeah.net*

Letters To The Editor

Acute lymphoblastic leukemia (ALL) is an aggressive malignancy of developing lymphocytes. Despite outstanding overall cure rates, patients with the refractory or relapsed disease have a poor prognosis (1). To improve treatments for these high-risk (HR) ALL patients, it is critical to gain an in-depth understanding of the disease pathogenesis. The enhanced expression of the protein kinase *CK2* gene and proto-oncogene *MYC* are common in HR T and B-ALL (2-6). CK2 is a constitutively active serine/threonine kinase composed of two catalytic (α or α') and two regulatory (β) subunits that are overexpressed in a broad spectrum of human cancers (7). Despite the demonstrated anti-leukemic efficacy of CK2 inhibitors (8), how CK2 contributes to HR-ALL development remains incompletely understood. Here we utilized transgenic zebrafish models to elaborate the multifaceted role of CK2 in HR-ALL pathogenesis, providing therapeutic implications for this stubborn disease.

Overexpression of the *CK2α* subunit under the immunoglobulin gene promoter induces low penetrance of T-cell lymphomas in a murine model (9). To further understand the oncogenic potential of CK2 in T and B lineages, we generated transgenic zebrafish that overexpress the wild-type or the kinase-dead version (*CK2αK68M*) of human *CK2α* gene in T and B cells through the tyrosine kinase gene (*lck*) promoter (2, 10). Western blotting analysis revealed elevated expression of CK2α in transgenic *CK2* fish, compared to age-matched wild-type fish (*Supplementary Figure S1A*). Despite relatively normal thymus development and no difference in fish survival observed (*Supplementary Figure S1B*), lymphocytes in *Tg(lck:CK2αwt;rag2:mCherry)* fish survived much longer than the control *Tg(lck:EGFP)* or *Tg(rag2:mCherry)* transgenic fish (*Supplementary Figure S1C*). By 8 months *CK2* transgenic fish still had clearly defined red-fluorescent thymi, while control transgenic fish began to lose

their thymic fluorescence as early as 5 months of age (*Supplementary Figure S1C* and data not shown). Strikingly, *CK2* transgenic fish can retain their thymic fluorescence till one and a half years. To determine the effect of CK2α in inducing lymphoid malignancies in zebrafish, starting at 21 days post-fertilization (dpf), we monitored both wild-type and mutant *CK2* transgenic fish at least once a month until two years of age and found no tumors developed in these fish (*Supplementary Figure S1C* and data not shown). Additionally, we also overexpressed *CK2α* under the zebrafish *rag2* promoter and also failed to observe tumor development in this fish line. These results indicate that *CK2* overexpression alone has very limited oncogenic potentials.

Despite the early knowledge that CK2 accelerates MYC-induced T-ALL (9), several questions remain: a) can CK2 and MYC synergize to promote B-ALL? b) does CK2's tumor-promoting effect solely depend on its enzymatic activity? and c) how does CK2 contribute to different stages of ALL development? To this end, we bred our *lck*-promoter-driven wild-type or kinase-dead *CK2* transgenic fish to conditional *Tg(rag2:MYC-ER;lck:EGFP)* fish, in which aberrant MYC activity is regulated by tamoxifen and induces leukemia in both T and B lineages (2, 11). We raised their offspring in fish water containing 4-hydroxytamoxifen (4HT) beginning at 5 dpf when thymic fluorescence was first visible (Figure 1A), and monitored the fish for tumor onset using previously defined criteria (12, 13). At 4 weeks of age, all groups showed normal-sized thymi. However, by six weeks, all three fish lines exhibited evidence of tumor initiation compared to *Tg*(*lck:EGFP*) and *Tg*(*rag2:mCherry*) controls (Figure 1C and *Supplementary Figure S1B*). By 12 weeks of life, tumors developed in more than 90% of *Tg(rag2:MYC-ER;lck:EGFP;lck:CK2αwt;rag2:mCherry)* fish, referred to as *MYC-ER;CK2αwt* (Figure 1B-C). However, tumors developed in less than 60% of *Tg(rag2:MYC-ER;lck:EGFP)* fish, referred to as *MYC-ER* (Figure 1B-C). Interestingly, overexpressing the enzyme-dead version of *CK2α* in

Tg(rag2:MYC-ER;lck:EGFP;lck:CK2αK68M;rag2:mCherry) fish, referred to as *MYC-ER;CK2αK68M*, failed to accelerate the disease, with ~ 50% of fish developing tumors at 12 weeks of life (Figure 1B-C). These results demonstrate that the HR-ALL development depends on the enzymatic activity of *CK2* since wild-type, but not kinase-dead CK2α, significantly accelerated the onset of MYC-induced ALL.

Next, we questioned whether CK2α could hasten the progression of MYC-induced ALL by quantifying the tumor burden in the above three groups of fish. We found that ALL developed in *MYC-ER;CK2αwt* fish much more aggressively, as demonstrated by a significantly heavier tumor burden in these fish compared to those in *MYC-ER* sibling fish (*Supplementary Figure S2A,B*). However, overexpression of *CK2αK68M* failed to enhance disease aggression as the tumor burden in *MYC-ER;CK2αK68M* fish was similar to those in *MYC-ER* fish (data not shown). Since *MYC-ER* fish develop both T and B-ALL (2, 11), we then asked which types of leukemia *MYC-ER;CK2αwt* fish developed by performing semi-quantitative RT-PCR using zebrafish T and B-cell specific primers (14). Our results show that *MYC-ER;CK2αwt* fish also developed ALL of T and B lineages (*Supplementary Figure S3*).

To determine whether MYC-induced transformation is restricted to the particular stages of lymphocyte development, we treated *MYC-ER* fish with 4HT at 30 dpf instead of 5 dpf, and monitored fish for tumor development with weekly fluorescent imaging (Figure 2A). Surprisingly, none of these *MYC-ER* fish developed tumors after 8 weeks of 4HT treatment (Figure 2B-C). However, if these fish were treated with 4HT at 5 dpf, more than 30% of *MYC-ER* fish had already developed tumors at this time (Figure 1C). Next, we determined if the enhanced *CK2α* expression could overcome this temporal restriction of lymphocyte transformation. To do so, we bred *CK2α* transgenic fish to *MYC-ER* fish and treated the fish with

4HT at 30 dpf. Strikingly, tumors start to arise in the *MYC-ER;CK2αwt* fish within one week of 4HT treatment (Figure 2C). With less than two weeks of 4HT treatment, ~80% of *MYC-ER;CK2αwt* fish developed aggressive ALL (Figure 2B-C). These results demonstrate that CK2 can overcome the temporal restriction of *MYC*-mediated lymphocyte transformation and induce ALL at a later developmental stage.

Since the aggressive nature of leukemia in *MYC-ER;CK2αwt* fish depends on the kinase activity of CK2 (Figure 1), we next performed phos-tag Western blotting to determine whether enforced CK2 expression increases MYC phosphorylation *in vivo*. Compared to tumors in *MYC-ER* fish, we detected increased CK2α and relatively more phosphorylated MYC (upper bands) protein levels in tumors from *MYC-ER;CK2αwt* fish (*Supplementary Figure S4A*). To determine whether increased phosphorylation of MYC led to the stabilization of MYC protein *in vivo*, we analyzed the half-life of MYC-ER protein in the presence or absence of *CK2α* overexpression in zebrafish developing lymphocytes. We isolated premalignant thymocytes from 5-week-old *MYC-ER* and *MYC-ER;CK2αwt* fish, dissociated the thymocytes, and treated them with cycloheximide (CHX) to inhibit protein synthesis. Western blotting analysis was then performed to measure MYC-ER protein levels at different time points. We found that MYC-ER was stabilized in lymphocytes with *CK2α* overexpression, compared to those without *CK2α* overexpression (*Supplementary Figure S4B*). To understand whether CK2 can promote MYC-mediated leukemogenesis through other mechanisms, we performed qRT-PCR analysis of zebrafish homologs of human anti-apoptotic genes, *BCL2, BCL-XL,* and *MCL1*. No significant difference was found in leukemic cells from *MYC-ER* versus *MYC-ER;CK2αwt* fish (*Supplementary Figure S5*). Together, these data indicate that CK2's ability in phosphorylating and stabilizing MYC *in vivo* serves as one mechanism to promote leukemia initiation and aggressiveness.

To determine whether overexpression of *CK2α* alleviates the necessity of MYC in established tumors, we treated fish with 4HT starting at 5 dpf for 11 weeks to induce tumor development. We then removed 4HT from *MYC-ER* and *MYC-ER;CK2αwt* tumor fish to inactivate MYC and monitored disease regression for 8 weeks by fluorescent imaging (Figure 3A). We categorized tumor phenotypes based on the extent of change in tumor size as previously described: complete regression, partial regression, stable disease, and progression (11). By 4 weeks post-withdrawal of 4HT, ~35% of *MYC-ER* fish and ~50% of *MYC-ER;CK2αwt* fish had already exhibited complete tumor regression (Figure 3B). We found that there were no statistically significant differences between *MYC-ER* versus *MYC-ER;CK2αwt* fish for the changes of tumor status at both 4 and 8 weeks post 4HT removal (Figure 3C and data not shown). These results demonstrate that *CK2* overexpression alone cannot substitute for aberrant MYC activity in maintaining the established disease.

In this study, we elaborated on the contribution of CK2 to different stages of HR-ALL development using the tamoxifen-regulated zebrafish model of MYC-induced ALL. Our data show that the kinase activity of CK2 promotes both the onset and progression of T and B-ALL in the presence of aberrant MYC activation, but cannot maintain the disease upon MYC inactivation through the removal of 4HT. When *MYC-ER* fish are treated with 4HT to activate MYC at a later stage of development, these fish can no longer develop leukemia, indicating a temporal restriction of MYC-induced lymphocyte transformation. Strikingly, however, this temporal restriction can be overcome by enforced CK2 expression, leading to high penetrance of leukemia development. Although *CK2α* overexpression alone cannot induce leukemia, it promotes the survival of lymphocytes. Hence, it is likely that MYC activation at a later stage of

development induces apoptosis in lymphocytes that is overcome by *CK2* overexpression, enabling the rapid induction of leukemia in these fish.

Because CK2 inhibition with the selective and potent inhibitor, CX-4945, exhibits anti-tumor activities (7), CX-4945 has been included in clinical testing to treat hematological malignancies (NCT01199718) and solid cancers (NCT03897036, NCT03904862, NCT00891280, and NCT0357143). Based on our findings that MYC, but not CK2, is the key factor for HR-ALL maintenance, it is important to simultaneously target MYC and CK2. Although directly targeting MYC remains challenging, combination treatment of CX4945 with inhibitors targeting MYC-regulated oncogenic pathways, such as metabolism and stress response pathways, may be highly effective and beneficial to patients with HR-ALL, and possibly other cancers with high expression of MYC and CK2.

Funding

H. Feng acknowledges the grant support from the National Institutes of Health (NIH) (CA134743 and CA215059), Boston University (Ralph Edwards Career Development Professorship and 1UL1TR001430 grant from the Clinical & Translational Science Institute), the Leukemia Research Foundation (Young Investigator Award), the American Cancer Society (RSG-17-204-01-TBG), and the St. Baldrick Foundation (Career Development Scholar Award); Y.Z. and S.K. are grateful for the International Scholar grant from the Dahod family; H.W.L acknowledges an International Scholar Grant from the St. Baldrick Foundation; N.S. and D.L. received training support through NHLB1 T32 HL7501 from the NIH, H. Fu acknowledges NSFC 81371338 grant from the National Science Foundation of China and Wuhan University

Intramural Funding. The content of this research is solely the responsibility of the authors and does not necessarily represent the official views of the NIH.

Acknowledgments

We thank Dr. Alejandro Gutierrez for providing us the *MYC-ER* fish and Dr. David M. Langenau for sharing primer sequences for genes specifically expressed in zebrafish T and B cells.

Conflict of interest

The authors declare no conflict of interest.

References

1. Ko RH, Ji L, Barnette P, et al. Outcome of patients treated for relapsed or refractory acute lymphoblastic leukemia: a Therapeutic Advances in Childhood Leukemia Consortium study. J Clin Oncol. 2010;28(4):648-654.

2. Borga C, Foster CA, Iyer S, et al. Molecularly distinct models of zebrafish Myc-induced B cell leukemia. Leukemia. 2019;33(2):559-562.

3. Bonaccorso P, La Rosa M, Andriano N, et al. Clinical Significance of Ck2 (CSNK2) and C-Myc Expression in Childhood Acute Lymphoblastic Leukemia. Blood. 2016;128(22):5269.

4. Borgo C, Ruzzene M. Role of protein kinase CK2 in antitumor drug resistance. J Exp Clin Cancer Res. 2019;38(1):287.

5. Piazza F, Manni S, Ruzzene M, et al. Protein kinase CK2 in hematologic malignancies: reliance on a pivotal cell survival regulator by oncogenic signaling pathways. Leukemia. 2012;26(6):1174-1179.

6. Piazza F. Protein kinase CK2 in normal and malignant hematopoiesis. In: Pinna LA, editor. Protein Kinase CK2. 2013. Chapter 13:344-362.

7. Chua MM, Ortega CE, Sheikh A, et al. CK2 in Cancer: Cellular and Biochemical Mechan isms and Potential Therapeutic Target. Pharmaceuticals (Basel). 2017;10(1):18.

8. Gowda C, Sachdev M, Muthusami S, et al. Casein Kinase II (CK2) as a Therapeutic Target for Hematological Malignancies. Curr Pharm Des. 2017;23(1):95-107.

9. Seldin DC, Leder P. Casein kinase II alpha transgene-induced murine lymphoma: relation to theileriosis in cattle. Science. 1995;267(5199):894-897.

10. Penner CG, Wang Z, Litchfield DW. Expression and localization of epitope-tagged protein kinase CK2. J Cell Biochem. 1997;64(4):525-537.

11. Gutierrez A, Grebliunaite R, Feng H, et al. Pten mediates Myc oncogene dependence in a conditional zebrafish model of T cell acute lymphoblastic leukemia. J Exp Med. 2011;208(8):1595-1603.

12. Feng H, Langenau DM, Madge JA, et al. Heat-shock induction of T-cell lymphoma/leukaemia in conditional Cre/lox-regulated transgenic zebrafish. Br J Haematol. 2007;138(2):169-175.

13. Borga C, Park G, Foster C, et al. Simultaneous B and T cell acute lymphoblastic leukemias in zebrafish driven by transgenic MYC: implications for oncogenesis and lymphopoiesis. Leukemia. 2019;33(2):333-347.

14. Garcia EG, Iyer S, Garcia SP, et al. Cell of origin dictates aggression and stem cell number in acute lymphoblastic leukemia. Leukemia. 2018;32(8):1860-1865.

FIGURE LEGENDS

Figure 1. Overexpression of wild-type but not enzyme dead *CK2α* promotes the onset of MYC-induced ALL in zebrafish. (A) Diagram of the experimental design. (B) Thymic fluorescence in the *Tg(rag2:MYC-ER;lck:EGFP)* (left), *Tg(rag2:MYC-ER;lck:EGFP;lck:CK2αwt; rag2:mCherry)* (middle), and *Tg(rag2:MYC-ER;lck:EGFP;lck:CK2αK68M;rag2:mCherry)* (right) fish raised in 50 μg/L (129 nM) 4HT at the indicated age. One representative fish is shown for each group. (C) Kaplan-Meier analysis of tumor-free fish revealed that overexpression of *CK2αwt* but not *CK2αK68M* significantly accelerated the onset of MYC-induced ALL (*P=0.0013* for *MYC-ER* [green line] *vs. MYC-ER;CK2αwt* [red line]*;* n=19 and 22, respectively; and *P=0.0008* for *MYC-ER;CK2αwt* [red line] *vs. MYC-ER;CK2αK68M* [black line], n=22 and 13, respectively). There was no statistical significance between *MYC-ER* and *MYC-ER;CK2αK68M* fish. Statistical analysis was performed using the log-rank test. The scale bar in the left and middle panel of Figure 1B = 1 mm and in right panel = 200 μm.

Figure 2. *CK2α* overexpression overcomes temporal restriction of MYC-induced lymphocyte transformation and induces leukemia at the later stage of development. (A) Diagram of the experimental design. (B) Thymic fluorescence in the *Tg(rag2:MYC-ER;lck:EGFP),* referred as *MYC-ER* and *Tg(rag2:MYC-ER;lck:EGFP;lck:CK2αwt;rag2:mCherry),* referred as *MYC-ER;CK2αwt* zebrafish that were raised in 50 μg/L (129 nM) 4HT beginning at 30 dpf. (C) Kaplan-Meier analysis of tumor-free fish based on genotype (*P<0.0001;* n=18 for *MYC-ER* and n=34 for *MYC-ER;CK2αwt* fish). Statistical analysis was performed using the log-rank test and scale bars = 1 mm.

Figure 3. ***CK2α*** **overexpression alone cannot maintain ALL in the absence of aberrant MYC activation.** (A) Diagram of the experimental design. (B, top panel) Thymic fluorescence in *MYC-ER* (left) and *MYC-ER;CK2αwt* (right) zebrafish raised in 50 μg/L (129 nM) 4HT for 5 weeks showing tumor initiation in *MYC-ER;CK2αwt* fish. (B, middle panel) show both *MYC-ER* (left) and *MYC-ER;CK2αwt* (right) with aggressive disease at 11 weeks although *MYC-ER;CK2αwt* fish has more aggressive ALL than *MYC-ER* fish. (B, bottom panel) show thymic fluorescence 4 weeks after 4HT withdrawal. One representative fish is shown for each group. (C) Zebrafish were classified by the indicated tumor phenotype at 8 weeks post 4HT removal (*P=0.13; MYC-ER vs. MYC-ER;CK2αwt*; n=11 per group). The difference in observed tumor phenotypes between each group as a whole was statistically insignificant, as calculated by a two-way ANOVA test. Scale bars = 1 mm.

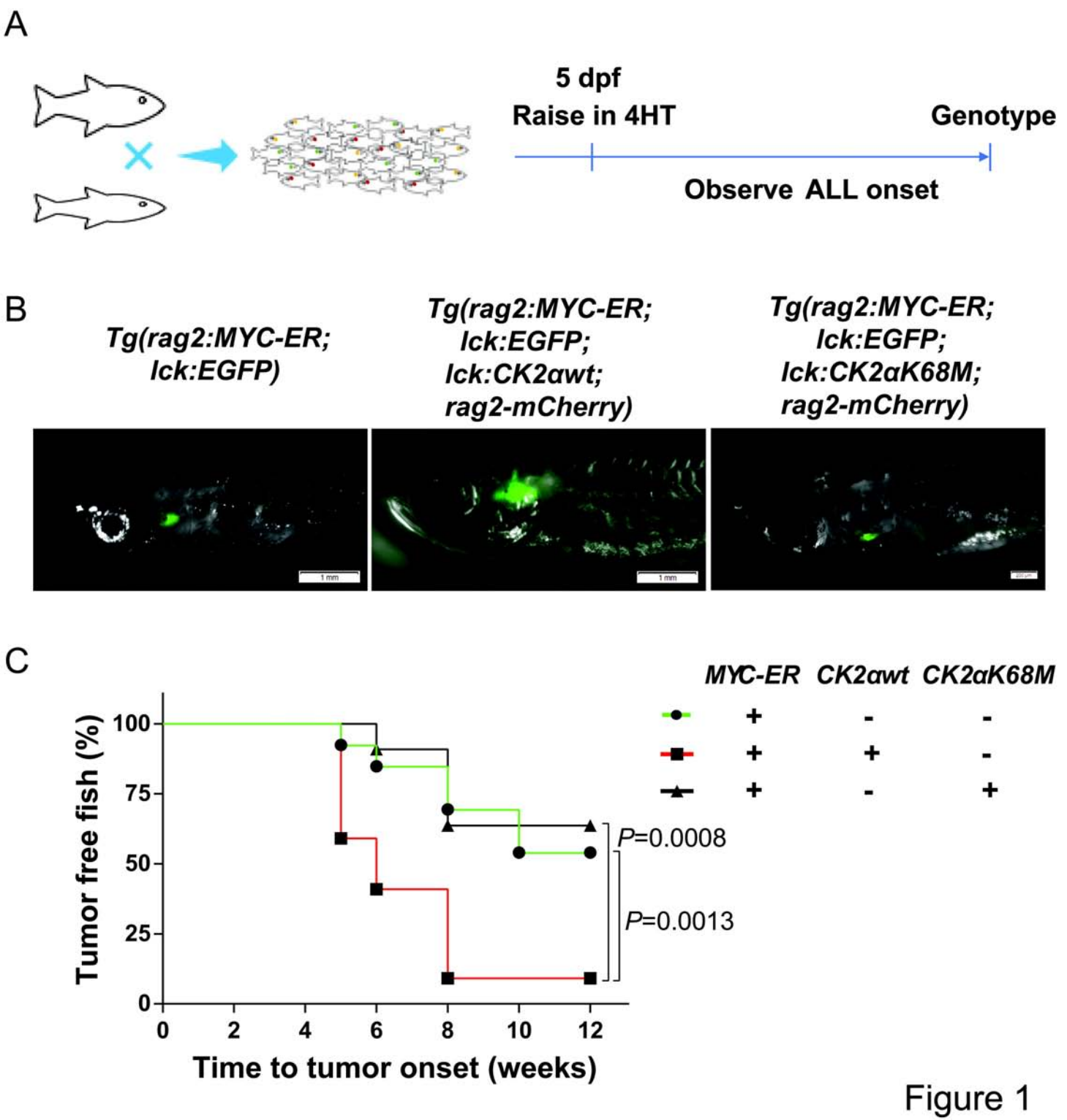

A
5 dpf
Raise in 4HT
Genotype
Observe ALL onset
B
Tg(rag2:MYC-ER; lck:EGFP)
Tg(rag2:MYC-ER; lck:EGFP; lck:CK2αwt; rag2-mCherry)
Tg(rag2:MYC-ER; lck:EGFP; lck:CK2αK68M; rag2-mCherry)
C
MYC-ER
CK2αwt
CK2αK68M
P=0.0008
P=0.0013
Tumor free fish (%)
Time to tumor onset (weeks)

Figure 1

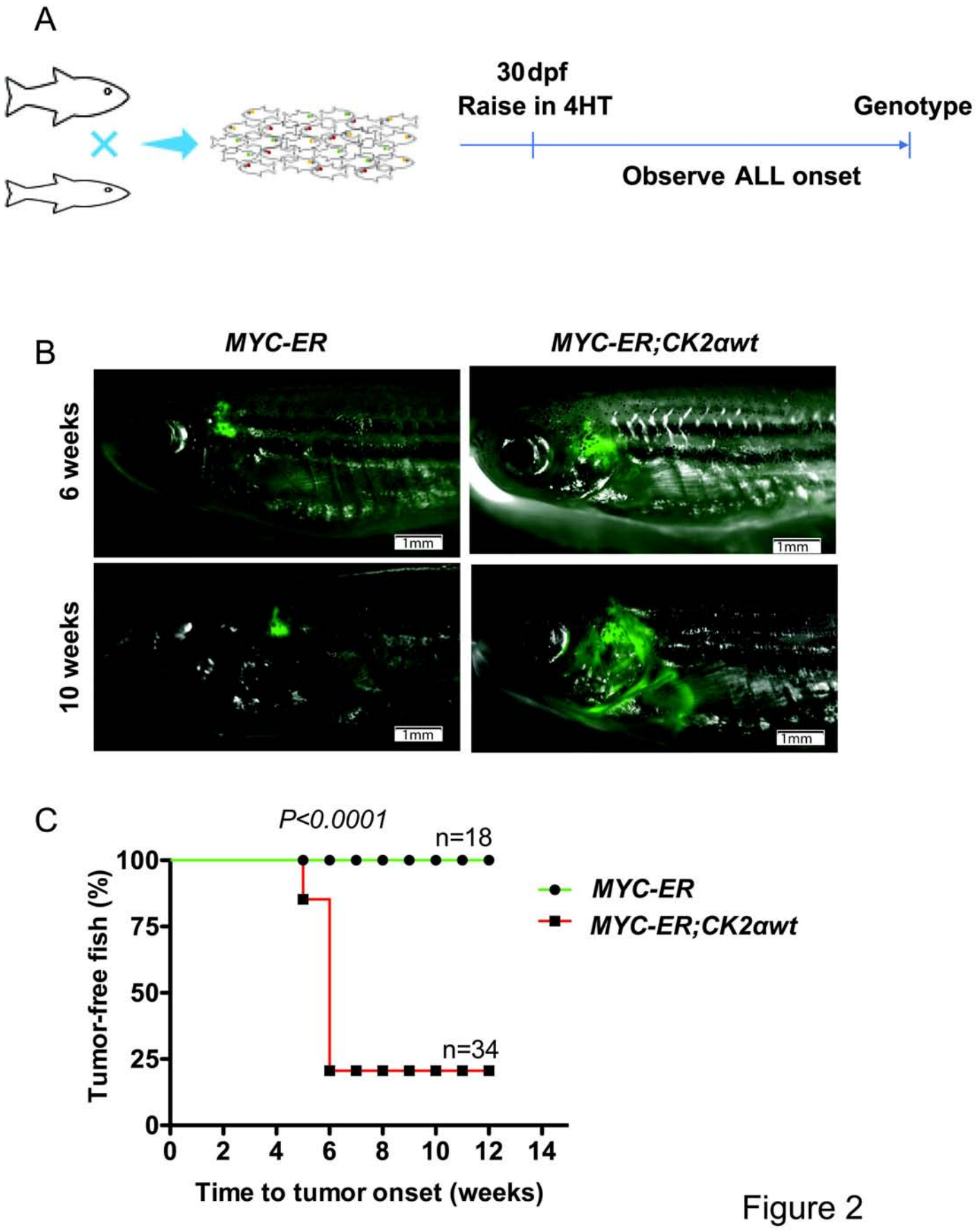
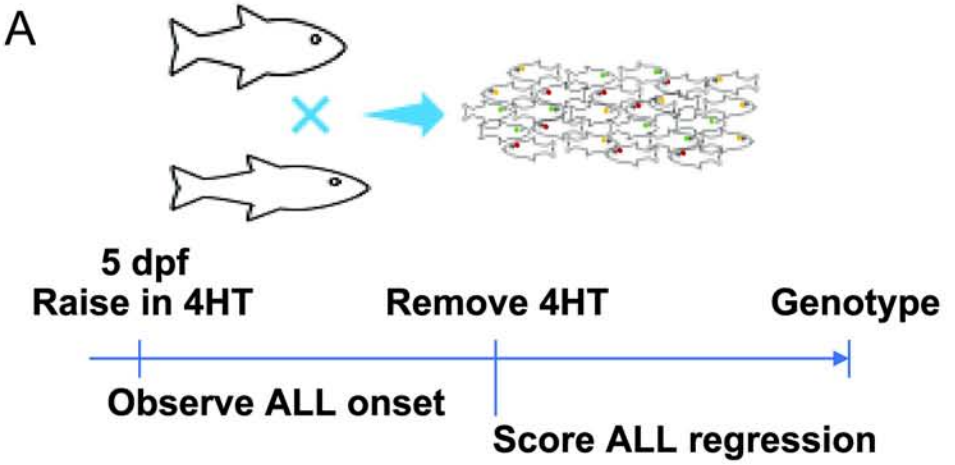
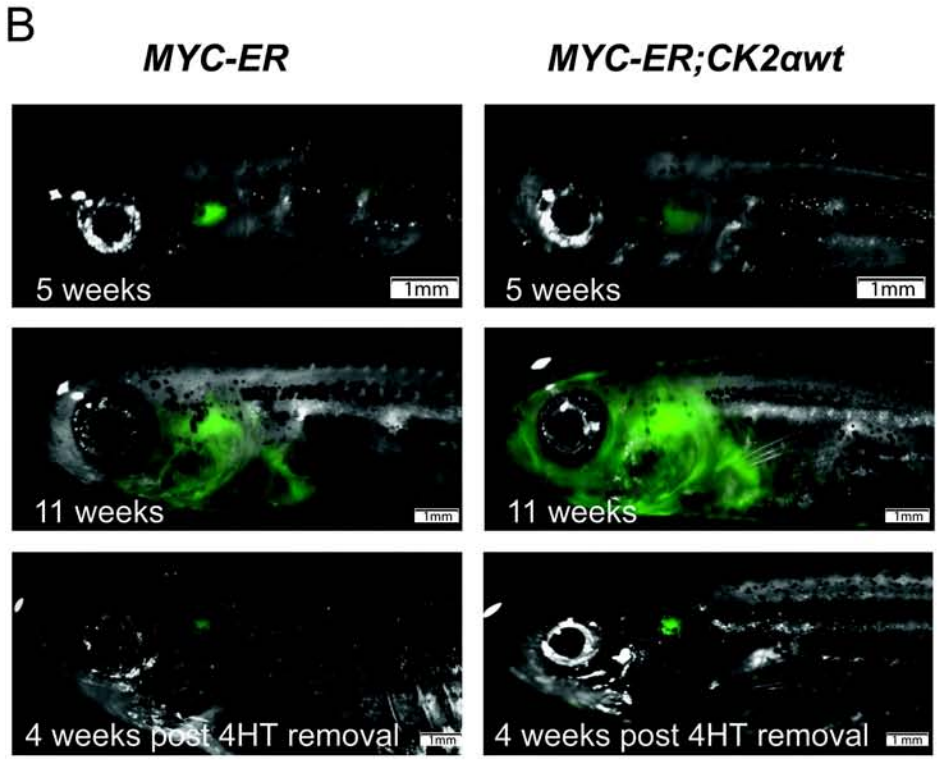

A
30dpf
Raise in 4HT
Genotype
Observe ALL onset
B
MYC-ER
MYC-ER;CK2αwt
6 weeks
10 weeks
1mm
C
P<0.0001
n=18
n=34
MYC-ER
MYC-ER;CK2αwt
Tumor-free fish (%)
100
75
50
25
0
0
2
4
6
8
10
12
14
Time to tumor onset (weeks)

Figure 2


A
5 dpf
Raise in 4HT
Remove 4HT
Genotype
Observe ALL onset
Score ALL regression


B
MYC-ER
MYC-ER;CK2αwt
5 weeks
1mm
5 weeks
1mm
11 weeks
11 weeks
4 weeks post 4HT removal
4 weeks post 4HT removal

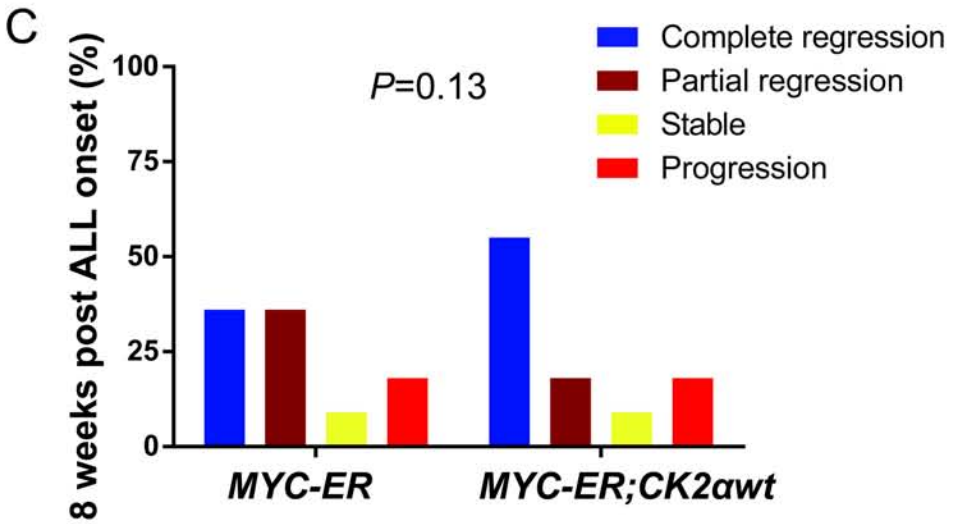

C
P=0.13
Complete regression
Partial regression
Stable
Progression
8 weeks post ALL onset (%)
100
75
50
25
0
MYC-ER
MYC-ER;CK2αwt

Figure 3

Supplementary Information

Materials and Methods

Fish husbandry

Zebrafish (*Danio rerio*) husbandry was performed as described (1), in zebrafish facilities at the Boston University (BU) Medical Campus following protocols approved by the Institutional Animal Care and Use Committee at BU.

Subcloning

The human cDNA of wild-type *CK2α* (*CK2αwt*) and mutant *CK2α* (*CK2αK68M*) was amplified by PCR from pZW6 and pGV15 vector, respectively (2). Mutant *CK2αK68M* was previously generated by mutating the lysine (K) codon at amino acid position 68 to methionine (M), resulting in the loss of kinase activity (3). PCR products were subsequently cloned into the *I-SceI-lck/pKS* or *I-SceI-rag2/pKS* vector, containing a zebrafish *lck* or *rag2* promoter sequence and flanked by the I-SceI endonuclease recognition sites. The forward primer contained an Age-I enzyme site and *CK2α* translation start sequence. The reverse primer contained the *CK2α* translation termination codon and a ClaI enzyme site. PCR-purified fragments were digested with AgeI-HF and ClaI restriction enzymes and then cloned into the respective *I-SceI/pKS* vector through AgeI-HF and ClaI sites. Clones containing inserts were fully sequenced. PCR primer pairs used for amplification of *CK2α* cDNA are shown in Supplementary Table 1.

Generation of stable *CK2αwt* and *CK2αK68M* transgenic fish

The *I-SceI-lck:CK2αwt-I-SceI*, *I-SceI-rag2:CK2αwt-I-SceI*, and *I-SceI-lck:CK2αK68M-I-SceI* constructs were microinjected with meganuclease (New England Biolabs, Ipswich, MA, USA)

into *nacre* (*mitfa*$^{-/-}$) zebrafish embryos at the one-cell stage of development, thereby generating mosaic F0 founder fish. The *I-SceI-lck:CK2αwt-I-SceI* construct was co-injected with the *I-SceI-rag2:mCherry-I-SceI* construct as previously described (4). The founder fish were outcrossed and their progeny were screened for the presence of human *CK2α* gene by gene-specific PCR.

4-hydroxytamoxifen treatment and ALL monitoring

The conditional zebrafish ALL model, *Tg(rag2:MYC-ER)*, was previously generated (5). In this transgenic fish line, the zebrafish *rag2* promoter drives the expression of human *MYC* fused to a modified estrogen receptor that binds to 4-hydroxytamoxifen (4HT, Sigma-Aldrich, St. Louis, MO, USA) but not endogenous estrogens (6). For all experiments described in this study, treatment with 4HT began at 5 dpf, and each clutch of larvae was placed in 1 L of water containing 50 μg/L 4HT with a weekly water change. Zebrafish raised in 4HT were imaged weekly by fluorescent microscopy beginning at 5 weeks of age to monitor the onset of ALL as previously described (5). Fish were imaged by brightfield and GFP/mCherry fluorescent microscopy using an Olympus fluorescent dissecting microscope (MVX10; Olympus, Center Valley, PA, USA). Fluorescent and brightfield images were merged using Adobe Photoshop (version 7.0). The onset of ALL was defined as the development of a fluorescent mass that was more than twice the size of a normal thymus or kidney together with fluorescent-labeled cell infiltration into local tissues. All fish were genotyped for *MYC-ER*, *CK2αwt,* or *CK2αK68M* transgenes using genomic DNA extracted from the individual fish fin upon the completion of tumor surveillance. Genotyping primers are listed in Supplementary Table 2.

Tumor regression was monitored after the removal of 4HT from the fish water. Tumor fish were placed in individual tanks and imaged weekly for a total of 8 weeks after the removal of 4HT.

Based on the diameter of the largest contiguous tumor mass at the end of the 8-week monitoring period, tumor phenotypes were classified into 4 groups as described in the results section. Fish that became moribund with leukemia <8 weeks after 4HT removal were placed in the progression category. Genotyping for fish was performed as described above.

Protein extraction and Western blotting

Whole-cell lysates were prepared from tumors in RIPA buffer (1% NP-40, 0.1% SDS, 50 mM Tris-HCl pH 7.4, 150 mM NaCl, 0.5% sodium deoxycholate, and 1 mM EDTA) supplemented with 1x or 2x Halt proteinase and phosphatase inhibitor cocktail (Thermo Scientific, Cambridge, MA, USA). To visualize the levels of phosphorylated MYC, a phosphate-binding molecule (Phos-Tag, AAL-107; FUJIFILM Wako Pure Chemical Co., Richmond, VA, USA) was used. Specifically, the 8% resolving gel for the SDS-PAGE was freshly prepared according to the manufacture's instruction with 0.04 mM $MnCl_2$ (Sigma, St. Louis, MO, USA) and 0.02 mM Phos-tag (TM) Acrylamide AAL-107 (FUJIFILM Wako Pure Chemical Co., Richmond, VA, USA). Primary antibodies included anti-CK2 (SC-9030, Santa Cruz Biotechnology, Dallas, TX, USA), anti-MYC (5605S, Cell Signaling, Danvers, MA, USA), and anti-ACTIN (SC-47778, Santa Cruz Biotechnology, Dallas, TX, USA). Secondary antibodies included horseradish-peroxidase-conjugated anti-mouse (31430, Thermo Scientific, Waltham, MA, USA) or anti-rabbit (65-6120, Thermo Scientific, Waltham, MA, USA) antibody. Autoradiographs were obtained with a G:BOX Chemi XT4 (Syngene, Frederick, MD, USA) and a CCD camera, and quantification analysis were performed using Syngene GeneTools software (Syngene, Frederick, MD, USA).

Pulse-chase analysis

Premalignant thymi were isolated from *MYC-ER* and *MYC-ER;CK2αwt* zebrafish. Specifically, adult zebrafish were euthanized in ice-cold fish water for approximately 10 minutes. The thymus of each fish was immediately dissected with sterilized forceps and kept in ice-cold 1x RPMI-1640 medium (Corning, NY, USA) supplemented with 10% FBS (Life Technology, Carlsbad, CA, USA). Cell suspensions were made by pipetting several times in medium, then aliquoted into 12-well plates (Thermo Scientific, Waltham, MA, USA), treated with 50 μg/mL cycloheximide (Sigma, St. Louis, MO, USA), and collected at 0, 15, 30, 60, and 180 minutes post-treatment. Protein was then extracted for Western blotting analysis to detect MYC and Actin levels.

RT-PCR

The tumor was dissected from each fish under a fluorescent microscope and subjected to RNA extraction using Trizol reagent (Thermo Scientific, Waltham, MA, USA) following the manufacturer's instruction. cDNA was synthesized with QuantiTect Reverse Transcription Kit (QIAGEN, Germantown, MA, USA). 100 ng of cDNA was used for semi-quantitative RT-PCR with gene-specific RT primers as previously described (7). Alternatively, 200 ng of cDNA was applied for qRT-PCR for anti-apoptotic genes with the SYBR green PCR master mix (Genecopoeia, Rockville, MD, USA) and a Step-One PCR instrument (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's manual. The primer sequences for qRT-PCR are included in Supplementary Table 2.

Statistical analyses

GraphPad Prism software (GraphPad Software, Inc., La Jolla, CA, USA) was used to calculate *p*-values using a log-rank test for Kaplan-Meier curves and two-tailed t-test for tumor burden and

anti-apoptotic gene expression. A two-way ANOVA test was used to calculate significance for tumor changes among fish groups after 4HT removal. *P*-values less than or equal to 0.05 were considered statistically significant.

Supplementary Figure Legends

Supplementary Figure 1. Overexpression of *CK2α* promotes the survival of lymphocytes but does not induce leukemia in zebrafish. (A) CK2α protein expression in lymphocytes of wild-type (wt) and *Tg(lck:CK2αwt;rag2:mCherry)* zebrafish. (B-C) GFP and/or mCherry fluorescence in the transgenic *Tg(lck:EGFP)* (left panels), *Tg(rag2:mCherry)* (middle panels), and *Tg(lck:CK2αwt;rag2:mCherry)* (right panels) fish at 6 weeks (B) or 8 months (C) old. Scale bars in (B) = 1 mm and in (C) = 2 mm.

Supplementary Figure 2. Overexpression of human *CK2αwt* promotes leukemia aggressiveness in zebrafish. (A) Representative images of *MYC-ER* (left, overlay of brightfield and GFP channel) and *MYC-ER;CK2αwt* (middle and right panel, overlay of brightfield and RFP or GFP channel) fish at 8-month old. (B) Fluorescent intensity quantification of GFP channel of tumor from *MYC-ER* and *MYC-ER;CK2αwt* fish (*P=0.02*; n=6 and 8, respectively). Scale bars = 2 mm.

Supplementary Figure 3. Similar to *MYC-ER* fish, *MYC-ER;CK2αwt* fish develop both T and B-ALL. Semi-quantitative RT-PCR analysis show that tumors from both *MYC-ER* and *MYC-ER;CK2αwt* fish express T and B cell markers.

Supplementary Figure 4. CK2 phosphorylates and stabilizes MYC in leukemic cells from *MYC-ER;CK2αwt* zebrafish. (A) Western blotting analysis of CK2 and MYC (phosphorylated [upper bands] and unphosphorylated [the lowest band]) in tumors dissected from individual *MYC-ER* and *MYC-ER;CK2αwt* fish. The number above the upper bands of MYC are relative values of

the phosphorylated MYC versus the total MYC. (B) Cycloheximide (CHX) chase analysis of MYC stability in the premalignant thymus of *MYC-ER* and *MYC-ER;CK2αwt* fish. Levels of Actin were analyzed as a loading control. MYC band intensities were normalized to the corresponding Actin levels, and the value for cells isolated immediately before CHX treatment was set to 100 for ease of comparison.

Supplementary Figure 5. *CK2* overexpression does not increase the expression of anti-apoptotic genes. qRT-PCR analysis of the transcript levels of zebrafish anti-apoptotic genes in leukemic cells from *MYC-ER* or *MYC-ER;CK2αwt* fish (n=15 per group). Statistical analysis was performed using the two-tailed t-test.

Supplementary Tables

Supplementary Table 1. **PCR primer pairs used for amplification of *CK2α* cDNA for subcloning**

	Primer sequences (5′-3′)
Forward	GGGGACAAGTTTGTACAAAAAAGCAGGCTCACCACCGGTATGTCGGGAC CCGTGCCAAGCAG
Reverse	GGGGACCACTTTGTACAAGAAAGCTGGGTATCGATTTACTGCTGAGCGCC AGCGGCAG

Supplementary Table 2. **Genotyping or qRT-PCR primers**

Target gene	Primer sequences (5'-3')
MYC-ER	Forward: AGTCCTGAGACAGATCAGCA
	Reverse: TCATCATGCGGAACCGACTT
CK2αwt	Forward: ATGTCGGGACCCGTGCCAAGCAG
	Reverse: TTACTGGCTTGAGAATTTTAAC
CK2αK68M	Forward: ATGTCGGGACCCGTGCCAAGCAG
	Reverse: TTACTGGCTTGAGAATCATAAC
bcl2a	Forward: AGATGGCGTCCCAGGTAGAT
	Reverse: GAAGGCATCCCAACCTCCAT
bcl2l1	Forward: AGGGCTTGTTTGCTTGGTTG
	Reverse: GCAATGGCTCATACCCATAACAC
mcl1a	Forward: GTCACTAACTGGGGCCGAAT
	Reverse: AACCCATGCCAGCTCTTGTT

Supplementary References

1. Westerfield M. The Zebra fish Book: A Guide for the Laboratory Use of Zebra fish (Brachydanio rerio) Eugene, OR, USA: University of Oregon Press. 1994.

2. Vilk G, Saulnier RB, St Pierre R, Litchfield DW. Inducible expression of protein kinase CK2 in mammalian cells. Evidence for functional specialization of CK2 isoforms. J Biol Chem. 1999;274(20):14406-14414.

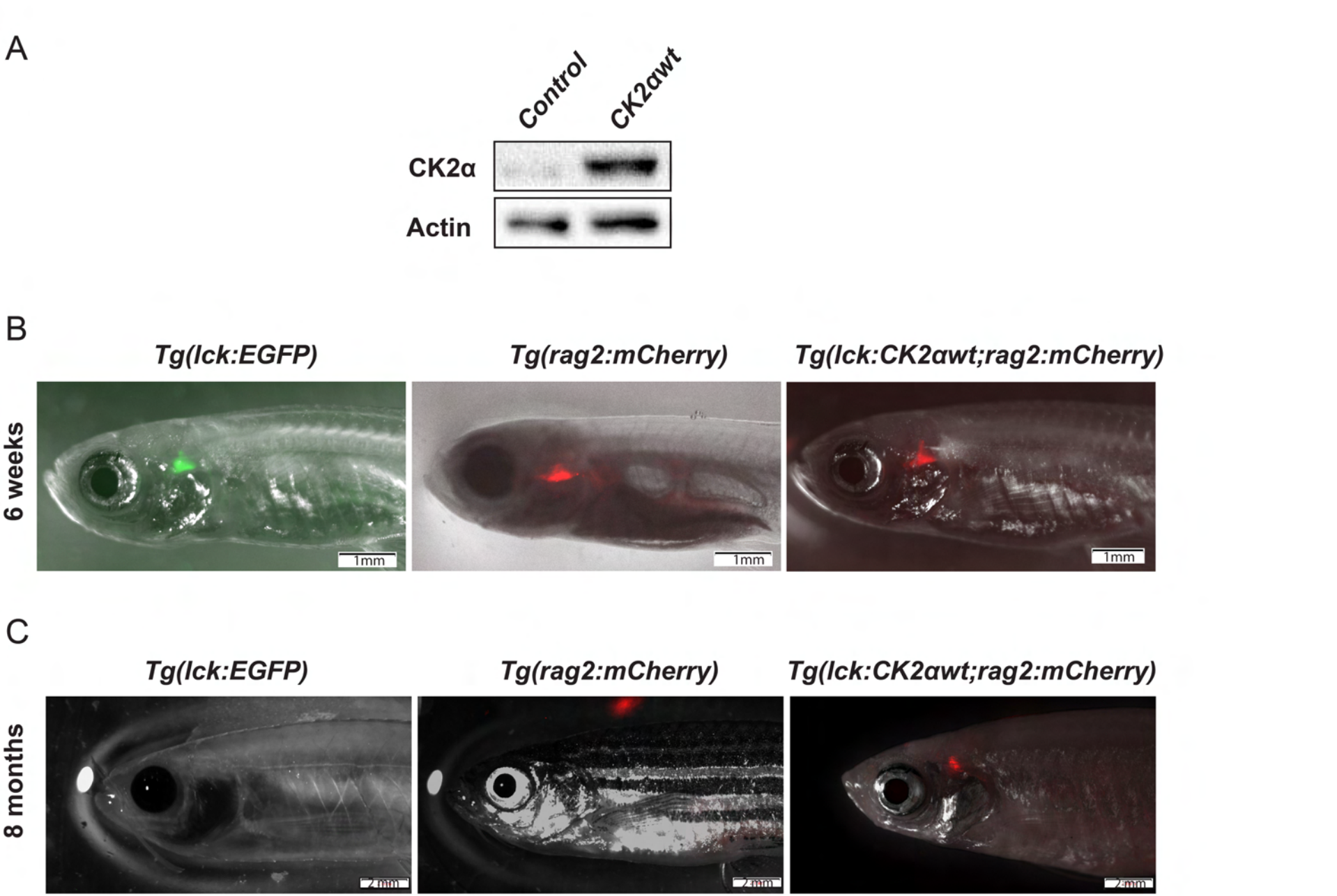
3. Penner CG, Wang Z, Litchfield DW. Expression and localization of epitope-tagged protein kinase CK2. J Cell Biochem. 1997;64(4):525-537.

4. Rembold M, Lahiri K, Foulkes NS, Wittbrodt J. Transgenesis in fish: efficient selection of transgenic fish by co-injection with a fluorescent reporter construct. Nat Protoc. 2006;1(3):1133-1139.

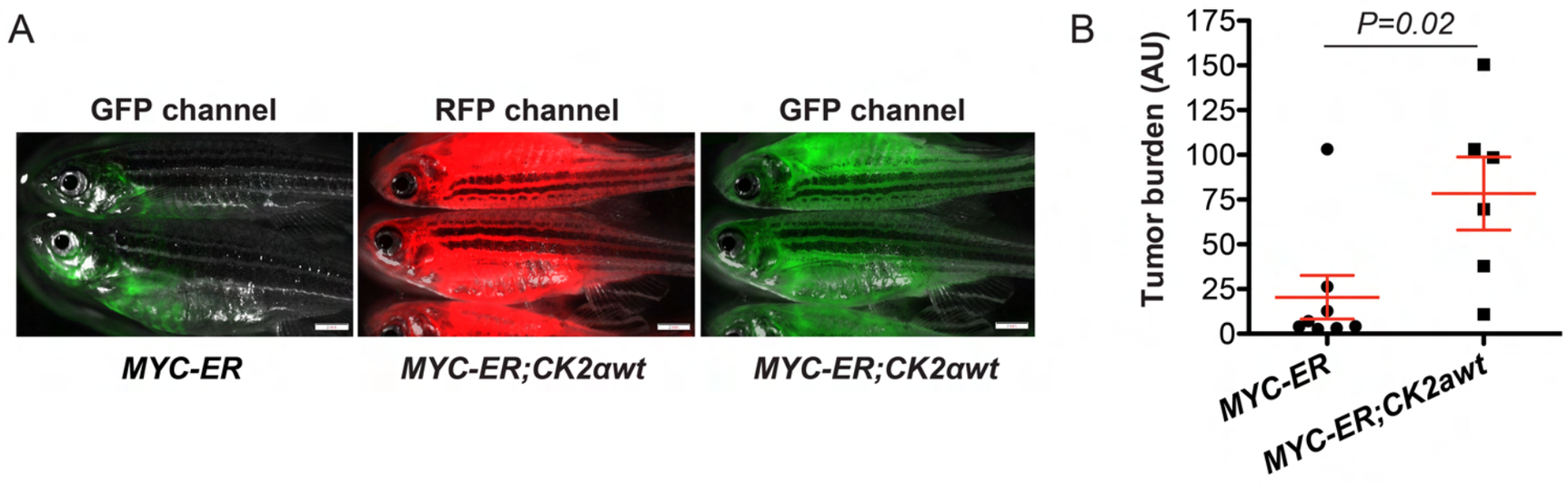
5. Gutierrez A, Grebliunaite R, Feng H, et al. Pten mediates Myc oncogene dependence in a conditional zebrafish model of T cell acute lymphoblastic leukemia. J Exp Med. 2011;208(8):1595-1603.

6. Littlewood TD, Hancock DC, Danielian PS, Parker MG, Evan GI. A modified oestrogen receptor ligand-binding domain as an improved switch for the regulation of heterologous proteins. Nucleic Acids Res. 1995;23(10):1686-1690.

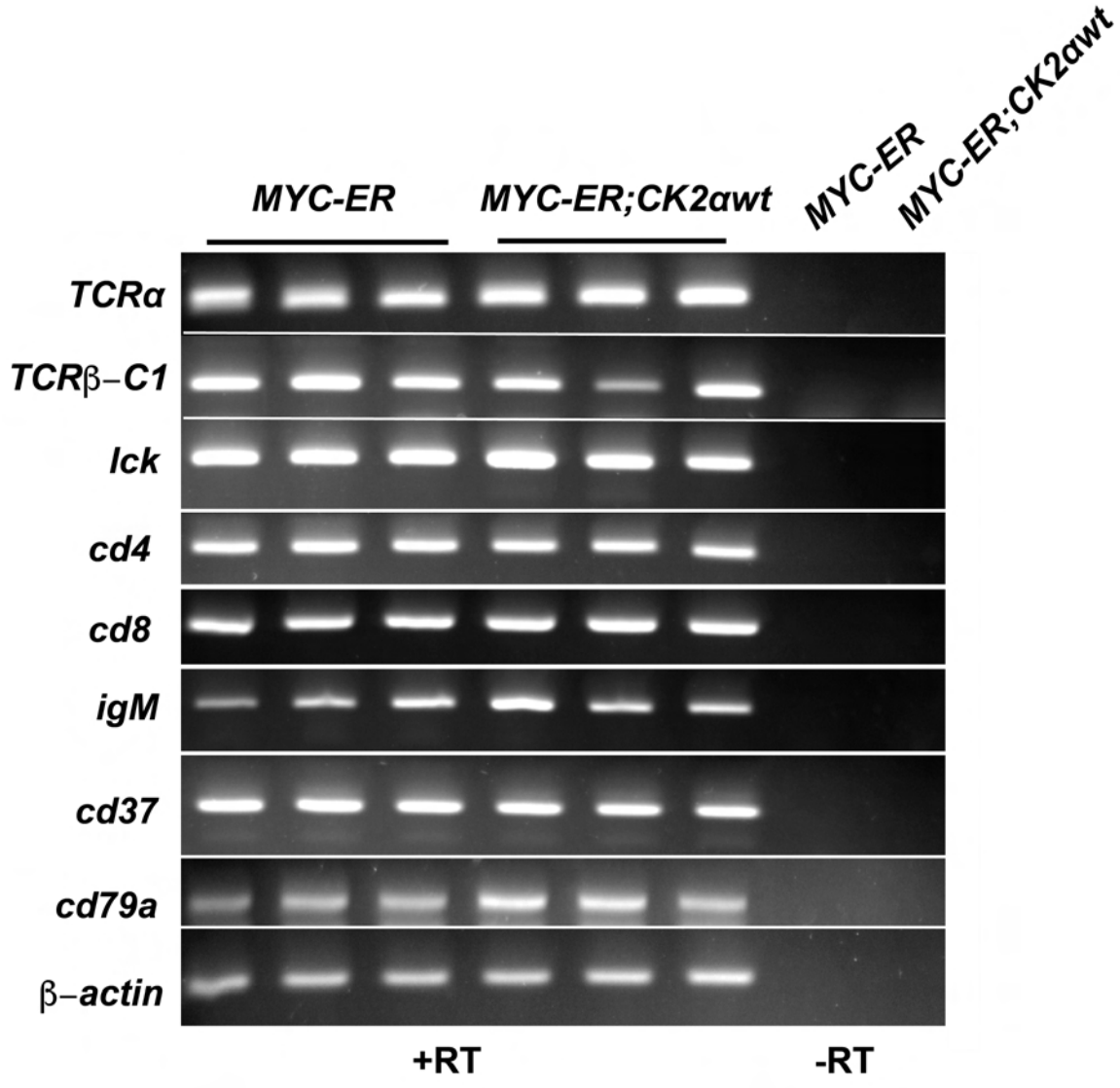
7. Garcia EG, Iyer S, Garcia SP, et al. Cell of origin dictates aggression and stem cell number in acute lymphoblastic leukemia. Leukemia. 2018;32(8):1860-1865.



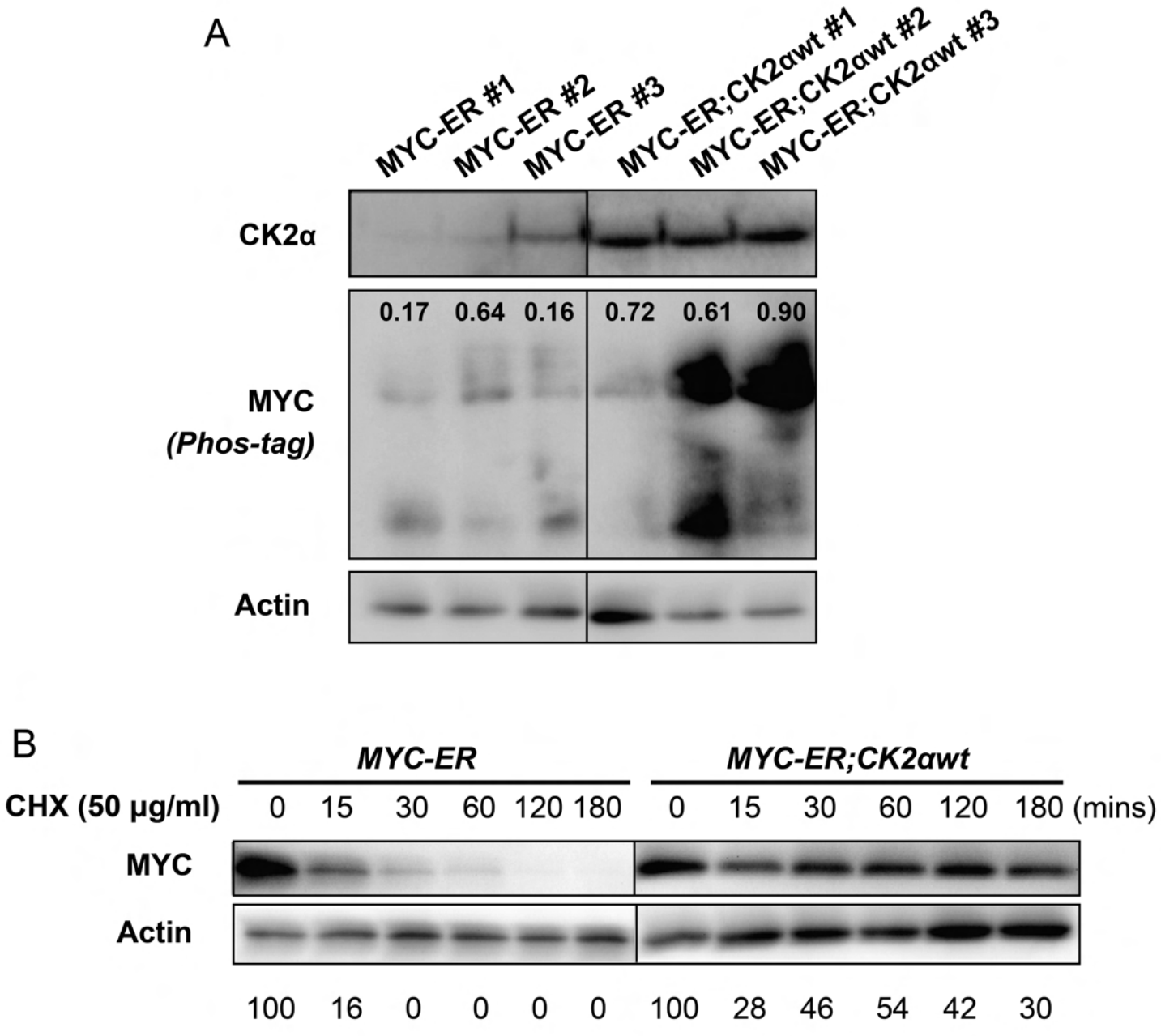
Supplementary Figure 1



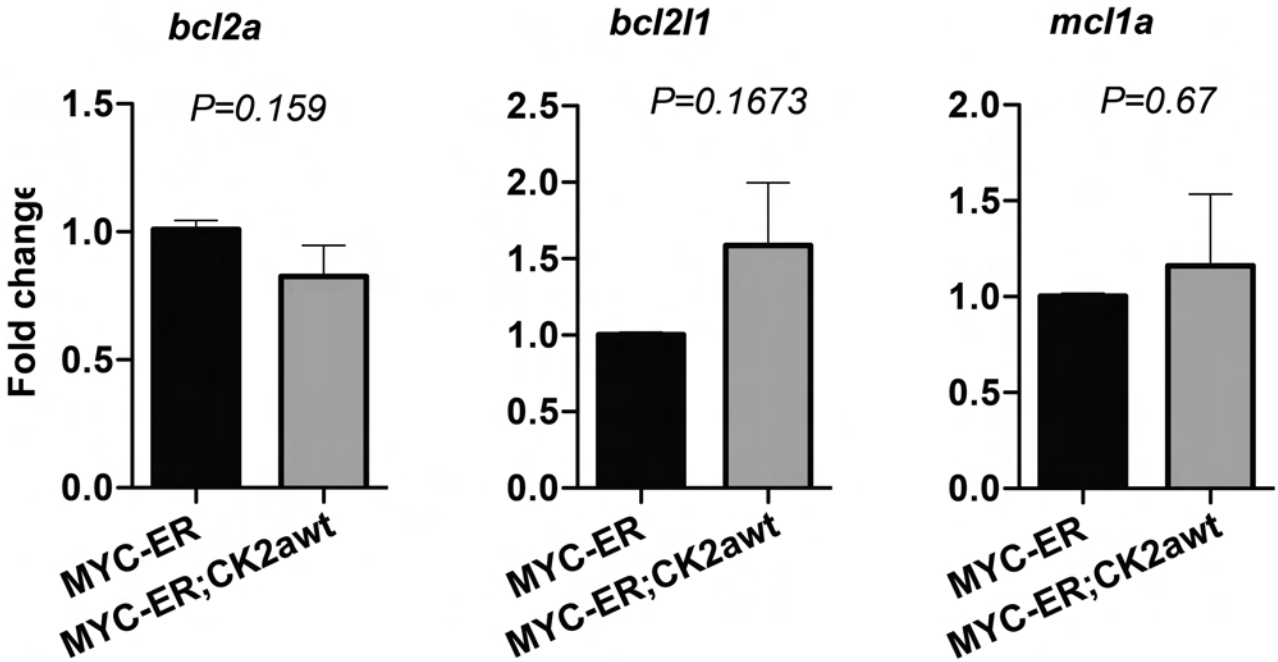
Supplementary Figure 2



Supplementary Figure 3



Supplementary Figure 4



Supplementary Figure 5