

Cell Cycle News & Views

Similar, not the same: Diverse roles and regulation of cyclin Es

Comment on: Caldon CE, et al. *Cell Cycle* 2013; 12:606–17
PMID:23324395; <http://dx.doi.org/10.4161/cc.23512>

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Cell cycle deregulation and genomic instability play a major role in the aberrant cell proliferation that characterizes tumorigenesis. A novel role of the cyclin E isoform cyclin E2 in these processes is reported in the manuscript “Cyclin E2 induces genomic instability by mechanisms distinct from cyclin E1” by Caldon et al.¹ In another issue of *Cell Cycle*, evidence that regulation of cyclin E2 stability is uncoupled from cyclin E1 regulation in cancer cells is described in the manuscript “Differences in degradation lead to asynchronous expression of cyclin E1 and cyclin E2 in cancer cells” by the same research group.²

The major role of cyclin E is promotion of G₁- to S-phase transition through Cdk2 activation. Involvement in other activities such as pre-replication complexes formation³ and centrosome duplication has also been identified for cyclin E1.⁴ The cyclin E/Cdk2 complex is in part regulated by the increased expression of cyclin E in late G₁ phase⁵ and its destruction by ubiquitin-mediated proteasomal degradation in S phase (reviewed in ref. 6). Due to their high sequence similarity, cyclin E1 and E2 have been regarded as functionally redundant, and where the isoforms are even considered, cyclin E1 is generally studied as the prototypic cyclin E. However, knockout mouse models have revealed tissue-specific functions in male fertility⁷ and liver regeneration.⁸ In cancer, there is also evidence for cyclin E isoform-specific functions (reviewed in ref. 9). Cyclin E1 and cyclin E2 are independent prognostic indicators in different breast cancer cohorts, and unlinked co-expression of cyclin E1 and E2 has been observed in several other types of cancers, with cyclin E2 commonly associated with the relapsing

forms of the disease. However, while there is strong evidence for cyclin E1 overexpression promoting tumorigenesis, there is much less evidence for cyclin E2 oncogenicity.

In the first manuscript, Caldon et al. investigated the effect of breast cancer cells' overexpression of cyclin E1 and cyclin E2 on cell cycle and genomic instability.¹ Similarly to E1, overexpression of cyclin E2 resulted in chromosome aberrations, but it did not prolong the duration of mitosis and was not associated with cdh1 or increased association with p107, which are observed with cyclin E1 overexpression. These differences suggest cyclin E1 and cyclin E2 overexpression trigger genomic instability in distinct manners, cyclin E1 possibly dependent on its ability to form complexes with cdh1 and sequester p107. It will be important to analyze cyclin E2 complexes and identify its specific interacting partners to determine how cyclin E2 overexpression promotes the genomic instability observed.

In the second paper, the authors analyzed the cell cycle-dependent abundance of cyclin E1 and cyclin E2 in cancer cells and in their normal/immortalized counterparts.² In normal cells, cyclin E1 and E2 levels were coordinately regulated, peaking at G₁/S-phase transition. However, in cancer cells cyclin E2, but not E1, levels were maintained through S-phase. This increased stability of cyclin E2 was found to be linked to failed targeting by Fbw7, a component of the Skp1-Cul1-Rbx1-Fbw7 ubiquitin ligase complex. This data may provide the molecular basis for the higher levels of cyclin E2 not correlated with cyclin E1 in cancer cells. Why Fbw7 does not recognize cyclin E2 in cancer cells, whereas it is responsible for its destruction in normal cells and is

normally functional toward cyclin E1 in the same cancer cells, is still an open question. It is possible that cyclin E2 recognition by Fbw7 is impaired, possibly due a lack of appropriate phosphorylation to generate a phosphodegron or due to proteins specifically associated with overexpressed cyclin E2 that in some way regulate Fbw7 binding. This study is further evidence that the unlinked expression of these two closely related cyclins might translate to different disease outcomes.

In summary, the two reports from Caldon et al. provide novel evidence of cyclin E2 oncogenicity, underline important differences in their mechanisms of action and, finally, demonstrate that the term “cyclin E” denotes a functionally diverse family of important cell cycle regulators that contributes to the transformed phenotype.

References

1. Caldon CE, et al. *Cell Cycle* 2013; 12:606-17; PMID:23324395; <http://dx.doi.org/10.4161/cc.23512>
2. Caldon CE, et al. *Cell Cycle* 2013; 12:596-605; PMID:23324394; <http://dx.doi.org/10.4161/cc.23409>
3. Geng Y, et al. *Mol Cell* 2007; 25:127-39; PMID:17218276; <http://dx.doi.org/10.1016/j.molcel.2006.11.029>
4. Matsumoto Y, et al. *Science* 2004; 306:885-8; PMID:15514162; <http://dx.doi.org/10.1126/science.1103544>
5. Lauper N, et al. *Oncogene* 1998; 17:2637-43; PMID:9840927; <http://dx.doi.org/10.1038/sj.onc.1202477>
6. Hwang HC, et al. *Oncogene* 2005; 24:2776-86; PMID:15838514; <http://dx.doi.org/10.1038/sj.onc.1208613>
7. Geng Y, et al. *Cell* 2003; 114:431-43; PMID:12941272; [http://dx.doi.org/10.1016/S0092-8674\(03\)00645-7](http://dx.doi.org/10.1016/S0092-8674(03)00645-7)
8. Nevzorova YA, et al. *Gastroenterology* 2009; 137:691-703, 703, e1-6; PMID:19445941; <http://dx.doi.org/10.1053/j.gastro.2009.05.003>
9. Caldon CE, et al. *Cell Div* 2010; 5:2; PMID:20180967; <http://dx.doi.org/10.1186/1747-1028-5-2>

Phosphorylation: A key regulator of meiosis

Comment on: Kovacikova I, et al. *Cell Cycle* 2013; 12:618–24;
PMID:23370392; <http://dx.doi.org/10.4161/cc.23513>

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A characteristic feature of meiosis is that chromosome replication in S-phase is followed by two consecutive cell divisions to produce haploid cells. Orchestrated regulation of meiotic divisions is critical for proper segregation of chromosomes. Identification and characterization of proteins involved in this process is essential for our understanding of how chromosome number is reduced during meiosis.

One of the main cell division control mechanisms operating during both mitosis and meiosis is the spindle assembly checkpoint (SAC), which monitors the proper attachment of chromosomes to spindle fibers and prevents anaphase until all kinetochores are properly attached.^{1,2} SAC proteins play a particularly important role in preventing degradation of cyclin B and securin until completion of the process of attachment of chromosomes to spindle microtubules in metaphase. The SAC inhibits anaphase-promoting complex/cyclosome, and the anaphase onset is delayed until all chromosomes are properly attached to microtubules.² Protein kinases are known to play important roles in SAC regulation and other processes required for proper segregation of chromosomes during both mitosis and meiosis.^{3–6}

A recent paper by Kovacikova et al.⁷ systematically analyzes the role of non-essential *S. pombe* protein kinases in meiotic chromosome segregation. Interestingly, the new role for protein kinases Mph1 and Spo4 was discovered. First, they found that Mph1 protein kinase, member of Mps1 family of SAC kinases, is required for proper segregation of recombined homologous chromosomes during meiosis I. This chromosome segregation defect caused by *mph1Δ* is probably due to precocious start of anaphase I, as is the case in other SAC-defective mutant cells. Second, a new role for Spo4 protein kinase, the fission yeast ortholog of Dbf4-dependent Cdc7 kinase,⁸ was discovered. In *S. cerevisiae* Cdc7 kinase plays role in setting up mono-orientation of

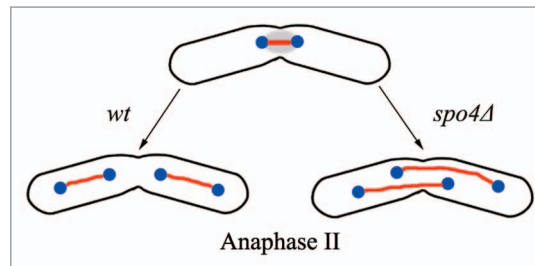


Figure 1. A comparison of anaphase II in wild-type (wt) and in *spo4Δ* mutant (*spo4Δ*) shows that anaphase II spindles are abnormally expanded in *spo4Δ* mutant cells.⁷ Tubulin is in red, DNA in blue.

sister kinetochores during the first meiotic division.⁹ Is this function conserved, and are Spo4 kinase and its regulatory subunit Spo6 required for proper segregation of sister centromeres during meiosis in *S. pombe*? The advantage of *S. pombe* as a model object is in production of linear asci in which the order of spores reflects the descent of nuclei from the two meiotic divisions. Kovacikova et al. scored the segregation of sister centromeres in a strain with only one copy of chromosome I marked with GFP (*lys1-GFP*). They observed that in 40% of *spo4Δ* and *spo6Δ* asci with four nuclei, *lys1-GFP* dots occupied both halves of the ascus, which indicated possible missegregation of sister centromeres during meiosis I. However, using more direct method based on staining with antibodies against tubulin and GFP, in *spo4Δ* and *spo6Δ* mutants, surprisingly no missegregation in anaphase I and anaphase II was detected. Unexpectedly, formation of extremely elongated anaphase II spindles was observed. These elongated spindles overlapped, and, as a result, corresponding nuclei separated during meiosis II were no longer adjacent (**Fig. 1**). This observation explained the abnormal pattern of *lys1-GFP* dots in spores of *spo4Δ* and *spo6Δ* mutants and suggested that Spo4 and Spo6 are important for keeping proper length of anaphase II spindles. Although Kovacikova et al. suggested that dysregulation of the

activity of the cyclin-dependent kinase may cause abnormal elongation of anaphase II spindles in *spo4Δ* mutant cells, more work will be needed to understand the mechanism how Spo4 regulates timely anaphase II completion.

Kovacikova et al. provide another piece of evidence that reversible phosphorylation and protein kinases play an important role in ensuring complete and proper chromosome segregation during meiosis.

References

- Sebestova J, et al. *Cell Cycle* 2012; 11:3011-8; PMID:22871737; <http://dx.doi.org/10.4161/cc.21398>
- Musacchio A. *Philos Trans R Soc Lond B Biol Sci* 2011; 366:3595-604; PMID:22084386; <http://dx.doi.org/10.1098/rstb.2011.0072>
- Domingo-Sananes MR, et al. *Philos Trans R Soc Lond B Biol Sci* 2011; 366:3584-94; PMID:22084385; <http://dx.doi.org/10.1098/rstb.2011.0087>
- Cipak L, et al. *Cell Cycle* 2012; 11:1626-33; PMID:22487684; <http://dx.doi.org/10.4161/cc.20052>
- Rumpf C, et al. *Cell Cycle* 2010; 9:2657-62; PMID:20581463; <http://dx.doi.org/10.4161/cc.9.13.12146>
- Ricke RM, et al. *Cell Cycle* 2011; 10:3645-51; PMID:22033440; <http://dx.doi.org/10.4161/cc.10.21.18156>
- Kovacikova I, et al. *Cell Cycle* 2013; 12: (Epub Ahead of Print.); PMID:23370392
- Nakamura T, et al. *Mol Cell Biol* 2002; 22:309-20; PMID:11739743; <http://dx.doi.org/10.1128/MCB.22.1.309-320.2002>
- Matos J, et al. *Cell* 2008; 135:662-78; PMID:19013276; <http://dx.doi.org/10.1016/j.cell.2008.10.026>

Protein kinases required for proper segregation of chromosomes during meiosis

Comment on: Kovacikova I, et al. *Cell Cycle* 2013; 12:618–24;
PMID:23370392; <http://dx.doi.org/10.4161/cc.23513>

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Cellular division involves complex processes in which protein phosphorylation plays crucial role. As chromosome segregation is a key process in both mitosis and meiosis, its proper progression is essential for cell growth and viability. Although numerous studies investigating the role of protein kinases on chromosome segregation have been performed,^{1–3} no systematic analysis of their involvement in meiosis has been performed so far. The fission yeast *Schizosaccharomyces pombe*, a widely used model organism for studying eukaryotic biology, possesses more than one hundred protein kinases. From these, 96 are non-essential and can be deleted from the yeast genome without affecting cell viability. Kovacikova et al. analyzed chromosome segregation during meiosis in yeast strains carrying the *S. pombe* non-essential kinase knockout alleles and found seven mutants with apparent defect in meiosis.⁴ Strong defect in meiotic chromosome segregation was observed in *bub1Δ* and *mph1Δ* strains, while the remaining strains, *hph2Δ*, *ppk24Δ*, *mug27Δ*, *spo4Δ* and *atg1Δ*, showed various alterations, such as weak missegregation phenotype, asci with more than four DNA masses or lagging chromosomes.

In their study, Kovacikova et al. focused on the analysis of *mph1Δ* and *spo4Δ/spo6Δ* mutations, as phenotypes of remaining strains, except for *ppk24Δ*, have previously been described.^{5,6}

First, authors analyzed cells lacking the Mph1 protein kinase. They marked chromosome II in *mph1Δ* knockout cells with GFP dots and tested chromosome segregation. Analysis of *mph1Δ* mutant cells carrying homozygous *cen2-GFP* dots revealed 10% homolog non-disjunction during first meiotic division. When

GFP dots were present only on one copy of the chromosome II (heterozygous *cen2-GFP*), no major defect in sister chromatid segregation was observed when compared with wild-type cells. This indicates that the fission yeast Mph1, similar to other components of the spindle assembly checkpoint, plays a crucial role in homolog disjunction during meiosis I.

The other question was if Spo4/Spo6, a meiosis-specific kinase complex that was shown to be important for progression of the second meiotic division,⁷ and its ortholog Dbf4-dependent Cdc7 kinase, required for DNA replication in most eukaryotes,⁸ is also required for proper sister chromatid segregation during meiotic divisions. Kovacikova and co-authors analyzed *spo4Δ* and *spo6Δ* mutant cells, where one copy of the first chromosome was marked with GFP (*lys1-GFP*). Although, most of cells arrested at the binucleate stage, a small fraction went through both meiotic divisions. These cells were able to produce asci with four nuclei. Scoring GFP dots in such asci showed that more than 40% contained GFP dots in both halves, suggesting missegregation of sister centromeres during meiosis I. Unexpectedly, missegregation of sister chromatids was not observed when the authors analyzed anaphase I cells. Importantly, Kovacikova et al. noticed that anaphase II spindles in *spo4Δ* and *spo6Δ* cells were significantly longer as compared with wild-type spindles. Live-cell imaging showed that these spindles pushed sister nuclei apart, leading to abnormal position of spore in mutant asci. This defect could be rescued only with the wild-type allele and not the “kinase dead” allele of Spo4 (Spo4K95A), which indicates that altered Spo4 kinase activity is responsible for abnormal meiosis II spindle elongation.

Moreover, duration of anaphase II in *spo4Δ* cells was more than twice as long as compared with wild-type cells. This implies that Spo4 is required for proper timing of anaphase II. Interestingly, similar results were observed with another sporulation-defective strain, *spo5Δ*, which points out the possible link between sporulation and proceeding of anaphase II. Concomitant inhibition of the *S. pombe* CDK kinase, Cdc2, together with *spo4* deletion caused reduction of the *spo4Δ* mutant phenotype to approximately one-half, suggesting that altered Cdc2 activity is responsible for abnormal spindle elongation in cells lacking Spo4. Taken together, Kovacikova et al. showed that protein kinases such as Mph1 or Spo4 play important roles during meiosis, and detailed studies of protein kinases and reversible protein phosphorylation are essential for our understanding of processes ensuring proper chromosome segregation.

References

1. Bimbó A, et al. *Eukaryot Cell* 2005; 4:799–813; PMID:15821139; <http://dx.doi.org/10.1128/EC.4.4.799-813.2005>
2. Guerra-Moreno A, et al. *Cell Cycle* 2012; 11:1621–5; PMID:22456336; <http://dx.doi.org/10.4161/cc.20051>
3. Cipak L, et al. *Cell Cycle* 2012; 11:1626–33; PMID:22487684; <http://dx.doi.org/10.4161/cc.20052>
4. Kovacikova I, et al. *Cell Cycle* 2013; 12: (Epub Ahead of Print); PMID:23370392
5. Bernard P, et al. *Nat Cell Biol* 2001; 3:522–6; PMID:11331883; <http://dx.doi.org/10.1038/35074598>
6. Rumpf C, et al. *Cell Cycle* 2010; 9:2657–62; PMID:20581463; <http://dx.doi.org/10.4161/cc.9.13.12146>
7. Nakamura T, et al. *Genes Cells* 2000; 5:463–79; PMID:10886372; <http://dx.doi.org/10.1046/j.1365-2443.2000.00343.x>
8. Nakamura T, et al. *Mol Cell Biol* 2002; 22:309–20; PMID:11739743; <http://dx.doi.org/10.1128/MCB.22.1.309-320.2002>

A low-carb diet kills tumor cells with a mutant p53 tumor suppressor gene: The Atkins diet suppresses tumor growth

Comment on: Rodriguez OC, et al. *Cell Cycle* 2012; 11:4436–46;
PMID:23151455; <http://dx.doi.org/10.4161/cc.22778>

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The p53 tumor suppressor gene plays a critical role in maintaining tissue homeostasis through both transcriptionally dependent and independent mechanisms. The response of p53 is dictated by the type of stress (genotoxic, oncogenic, hypoxia, metabolic, etc.) through a complex and incompletely understood number of post-translational modifications. Wild type p53 promotes apoptosis, cell cycle arrest and senescence in response to growth-restrictive conditions, including glucose deprivation.^{1,2} Under such conditions, nuclear p53 has also been reported to stimulate autophagy through the transcriptional activation of genes that comprise the autophagic network, such as ULK1 and DRAM1, and modulators of autophagy, such as Sestrin2.^{3–6} Unlike nuclear p53,

cytoplasmic p53 appears to inhibit autophagy through mechanisms that are not well understood, but appear to involve localization of p53 to the endoplasmic reticulum (ER) and binding to FIP200, the human ortholog of yeast Atg17.⁷ In fact, some cancer-associated forms of mutant p53 found predominantly in the cytoplasm can also inhibit autophagy.⁸ This negative regulation of autophagy by p53 has been proposed to act as rheostat to prevent an excessive amount of autophagy from occurring. Thus, the role of p53 in autophagy is complex and may be dependent on the autophagic stimulus and the mutational status of p53 in the cell.

Studies describing the role p53 in the response to metabolic stress have focused

primarily on the wild-type version of the protein. However, the vast majority of human tumors express high levels of mutant p53 protein that have acquired novel tumor-promoting functions distinct from those of wild type p53.⁹ To date, little is known about how mutant versions of p53 respond to growth-restrictive conditions, including the absence of glucose. In an elegant paper by Rodriguez et al.,¹⁰ the authors explored the effects of glucose restriction on the stability of a number of common cancer-associated p53 mutants and found that glucose deprivation resulted in degradation of mutant p53 protein levels. Interestingly, the negative regulation of p53 by glucose restriction was specific to mutant p53, since glucose restriction had a stabilizing effect on wild type p53. Surprisingly, the decreased levels of mutant p53 proteins were associated with rapid deacetylation and degradation through an autophagy-dependent but proteasome-independent process. Through the use of a constitutive acetylation-mimetic mutant p53, the authors demonstrated that autophagic degradation of p53 was dependent on the acetylation status of the protein. A major consequence of mutant p53 degradation in tumor cells after glucose deprivation is the loss of a critical check on the autophagic process that results in increased autophagy and leads to cell death (Fig. 1). Importantly, wild type p53 has been previously demonstrated to protect cells from glucose deprivation through induction of a reversible G₁/S phase cell cycle arrest, suggesting that normal tissues will respond to glucose shortage differently than tumors harboring mutant p53.¹

The authors also provided evidence that mutant p53A135V knock-in mice fed a low-carbohydrate diet expressed reduced levels of the mutated transgene compared with mice fed on a normal or high-carbohydrate diet. Importantly the low-carbohydrate diet had no effect on wild-type p53 levels in multiple tissues studied. Critically, the authors also demonstrated that a low-glucose diet inhibited the tumor-forming ability of cells

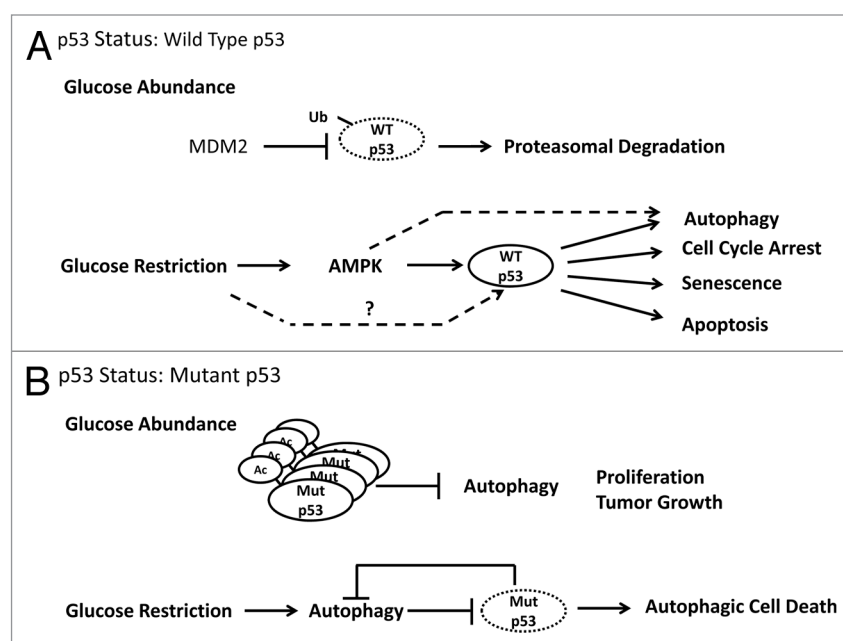


Figure 1. Glucose deprivation leads to autophagic degradation of mutant p53. (A) Wild type p53 is degraded by the proteasome under basal conditions but can be activated in an AMPK-dependent manner after glucose deprivation. Activated wild type p53 can induce a variety of cellular responses to glucose deprivation including autophagy, cell cycle arrest, senescence and apoptosis. (B) Cancer-associated p53 mutants are constitutively expressed at high levels and inhibit basal autophagy. Glucose deprivation leads to rapid de-acetylation of mutant p53 and subsequent degradation through autophagy. Because mutant p53 suppresses autophagy, its degradation leads to a feedforward autophagic loop that results in cell death.

that possess mutant forms of p53, and that this was dependent on acetylation status of the mutant p53 protein. Taken together, these findings strongly indicate that some tumor-promoting forms of mutant p53 can be targeted for autophagic degradation through glucose restriction. These exciting results could be tested in the clinic by randomizing patients with tumors that harbor similar p53 mutations to a glucose-restrictive, low-carbohydrate diet compared with a normal diet. The expectation of such studies would be that the tumors of patients on a glucose-restrictive diet would see their tumors regress or grow more slowly than those on an unrestrictive glucose diet. However, the next step in this saga will be to see what combinations of chemotherapy or targeted therapy will be more effective against mutant p53 tumors that are glucose-restrictive.

References

1. Jones RG, et al. *Mol Cell* 2005; 18:283-93; PMID:15866171; <http://dx.doi.org/10.1016/j.molcel.2005.03.027>
2. Zhao Y, et al. *J Biol Chem* 2008; 283:36344-53; PMID:18990690; <http://dx.doi.org/10.1074/jbc.M803580200>
3. Gao W, et al. *Cell Death Differ* 2011; 18:1598-607; PMID:21475306; <http://dx.doi.org/10.1038/cdd.2011.33>
4. Mah LY, et al. *Autophagy* 2012; 8:18-28; PMID:22082963; <http://dx.doi.org/10.4161/auto.8.1.18077>
5. Maiuri MC, et al. *Cell Cycle* 2009; 8:1571-6; PMID:19377293; <http://dx.doi.org/10.4161/cc.8.10.8498>
6. Feng Z, et al. *Proc Natl Acad Sci USA* 2005; 102:8204-9; PMID:15928081; <http://dx.doi.org/10.1073/pnas.0502857102>
7. Morselli E, et al. *Cell Cycle* 2011; 10:2763-9; PMID:21775823; <http://dx.doi.org/10.4161/cc.10.16.16868>
8. Morselli E, et al. *Cell Cycle* 2008; 7:3056-61; PMID:18818522; <http://dx.doi.org/10.4161/cc.7.19.6751>
9. Brosh R, et al. *Nat Rev Cancer* 2009; 9:701-13; PMID:19693097
10. Rodriguez OC, et al. *Cell Cycle* 2012; 11:4436-46. PMID:23151455; <http://dx.doi.org/10.4161/cc.22778>