Artemis Regulates the Mammalian Cell Cycle Via Interactions with Cullin E3 Ligases

Randy J. Legerski, Ph.D.
Department of Genetics
MD Anderson Cancer Center
Houston, TX

The SNM1 gene of S. cerevisiae

S. cerevisiae mutant: snm1/pso2

- sensitivity to nitrogen mustard

- **Sensitive to bifunctional alkylating agents**
  e.g., nitrogen mustard, cis-platin, psoralen plus UVA
- **Not sensitive to IR, UV, or monofunctional alkylating agents**
- **Proficient in initiation of ICL repair**
- **Appears to be defective in repair of DSBs created by ICLs**
- **Has 5' exonuclease activity on single-stranded DNA**
- **Mammalian orthologues: SNM1A, SNM1B/Apollo, Artemis**

Brief History of Artemis

Moushous *et al*. Cell 105, 177 (2001)

- Radiosensitive severe combined immunodeficiency syndrome (RS-SCID)
- Defect in V(D)J recombination - lack of T and B cell maturation
- Sensitivity to IR (double-strand breaks)

Ma et al. Cell 108, 781 (2002)

- Artemis with DNA-PKcs acquires hairpin opening activity during V(D)J recombination
- Phosphorylated by DNA-PKcs in vitro

Riballo *et al*. Mol. Cell 16, 715 (2004)

- Artemis is required in approx. 10% of NHEJ reactions
- Artemis is phosphorylated by ATM

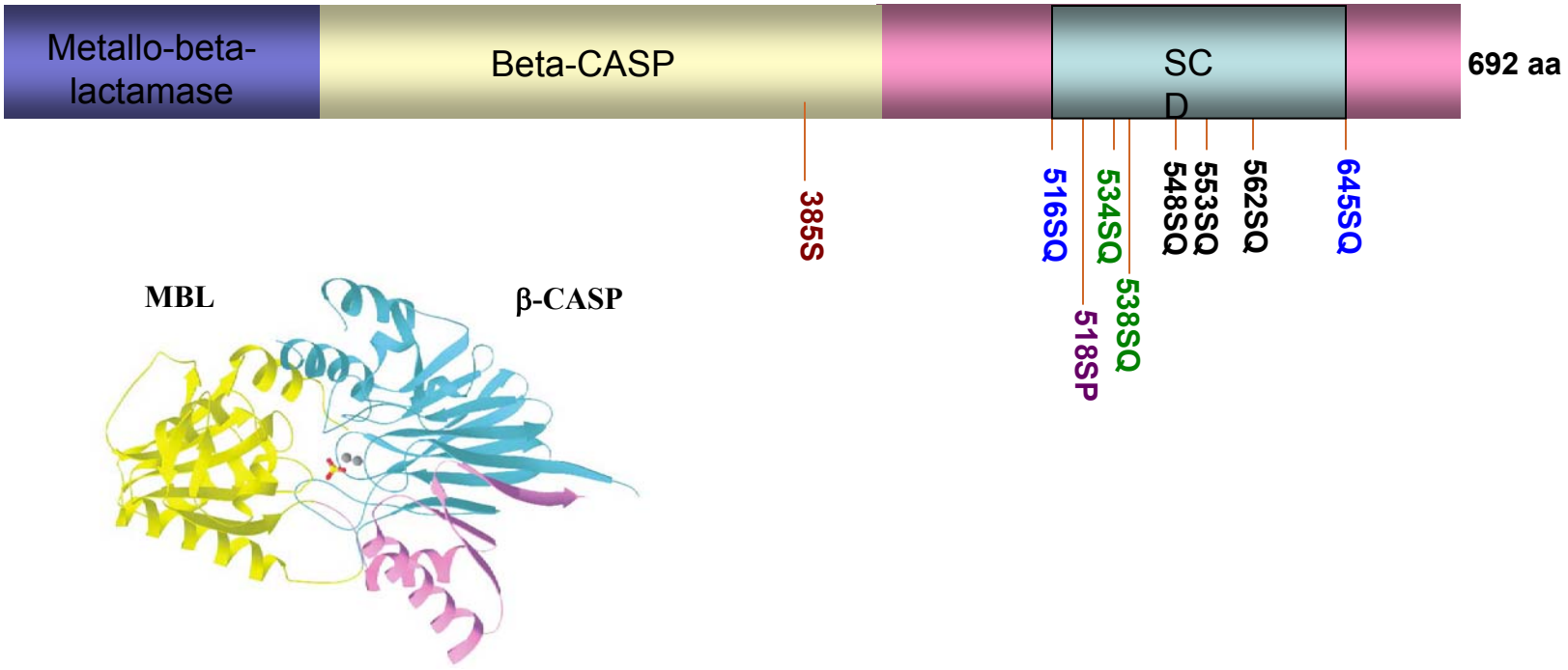
Beucher *et al*. EMBO J (2009)

- ATM and Artemis promote HR repair of DSBs

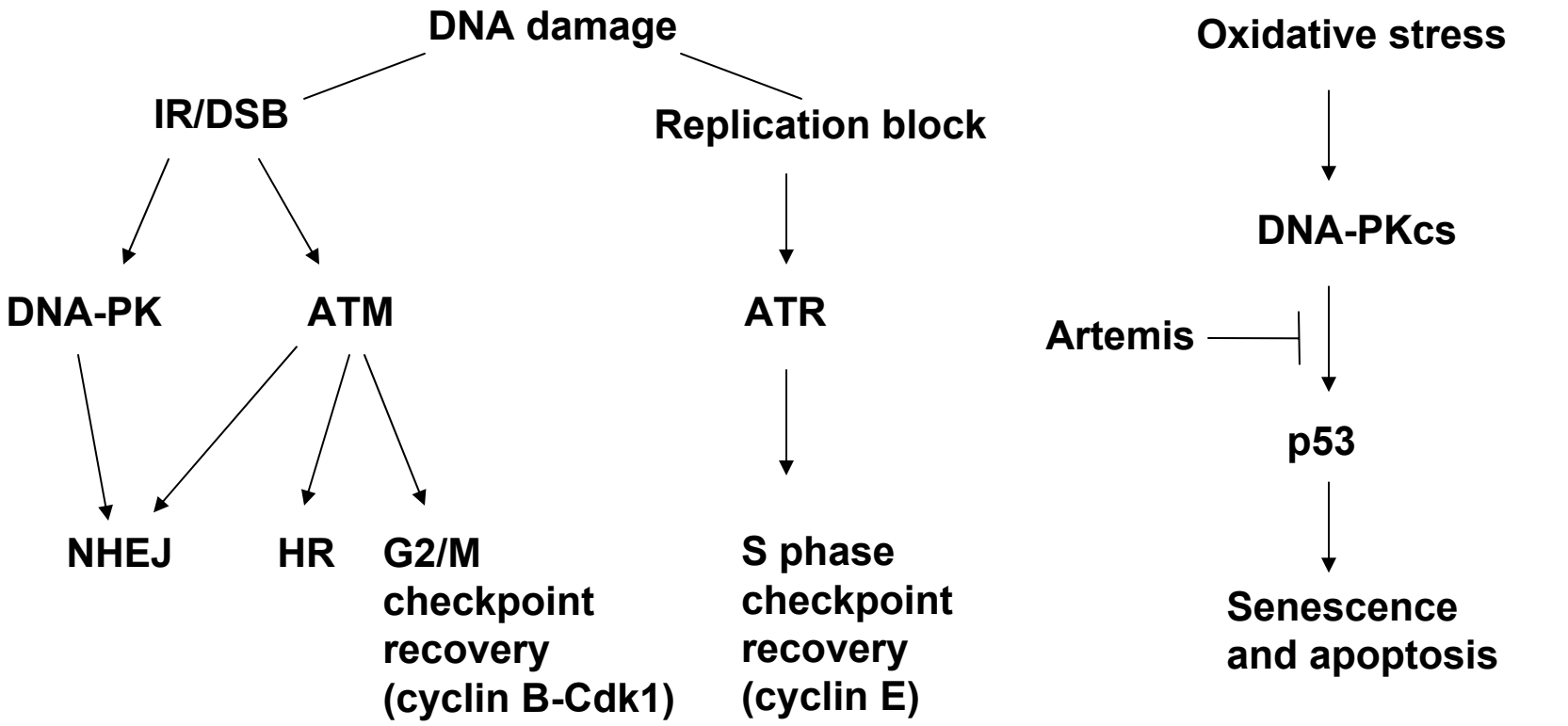
All these studies are consistent with the concept of Artemis as a nuclease

Structure of Artemis

- Artemis contains metallo-β-lactamase and β-CASP domains
- Artemis is a phospho-protein



Artemis and Cellular Stress Responses

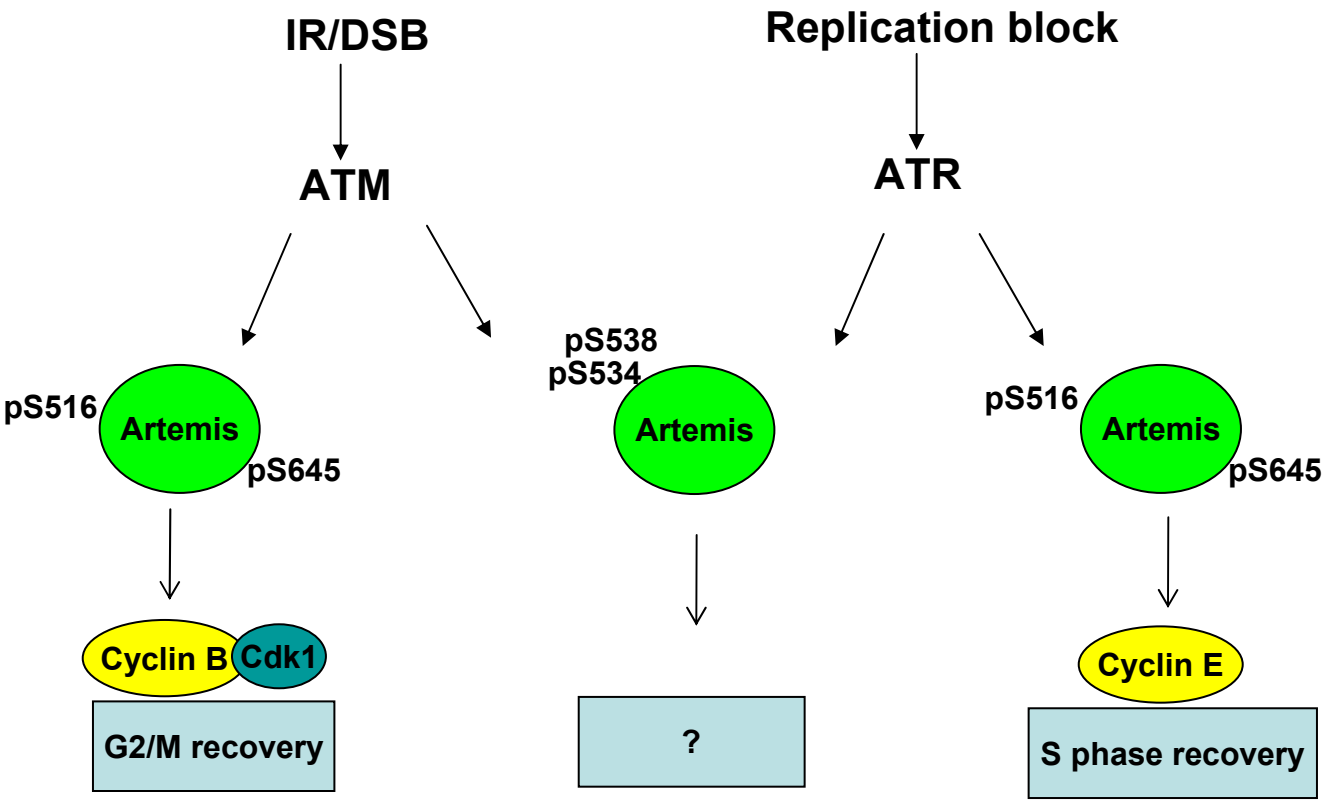


Zhang et al. Mol. Cell. Biol. (2004)
Geng et al. Mol. Cell. Biol. (2007)

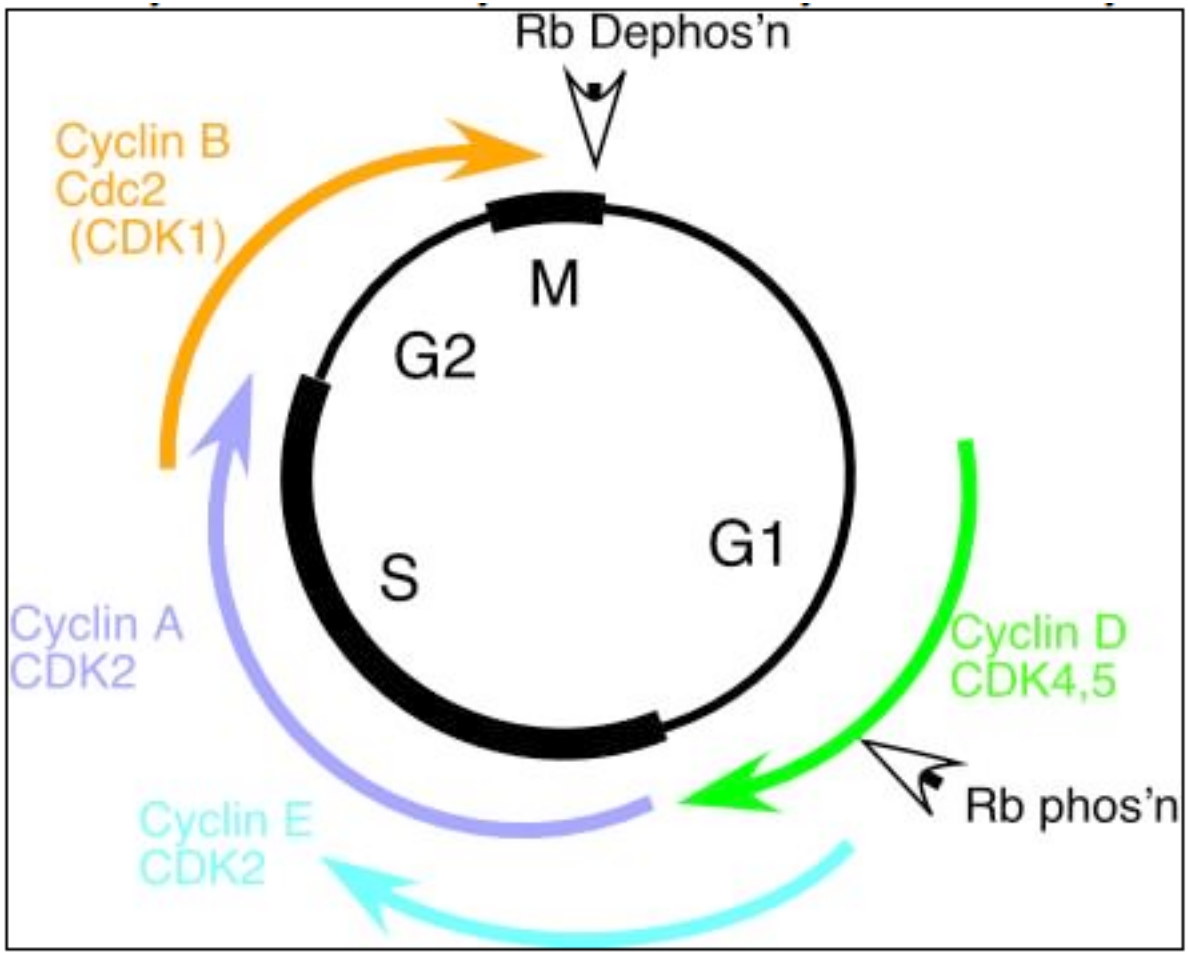
Wang et al. JBC (2009)

Zhang et al. Oncogene (2009)

Function of Artemis phosphorylation

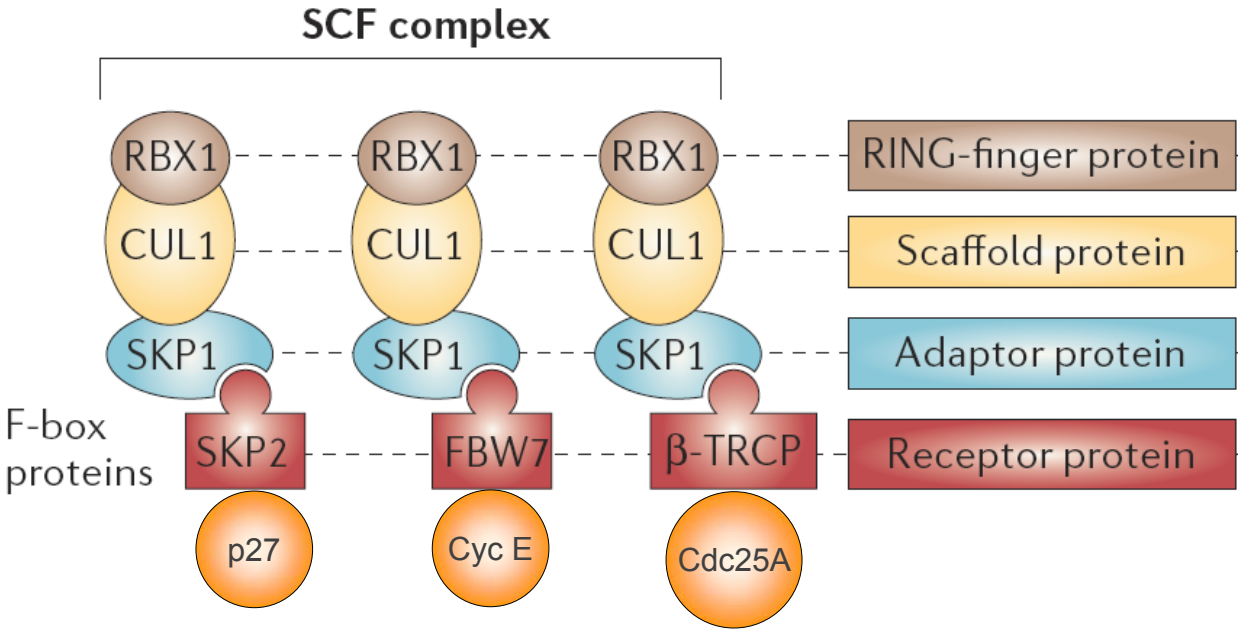


Cyclin E is stabilized in response to replication stress

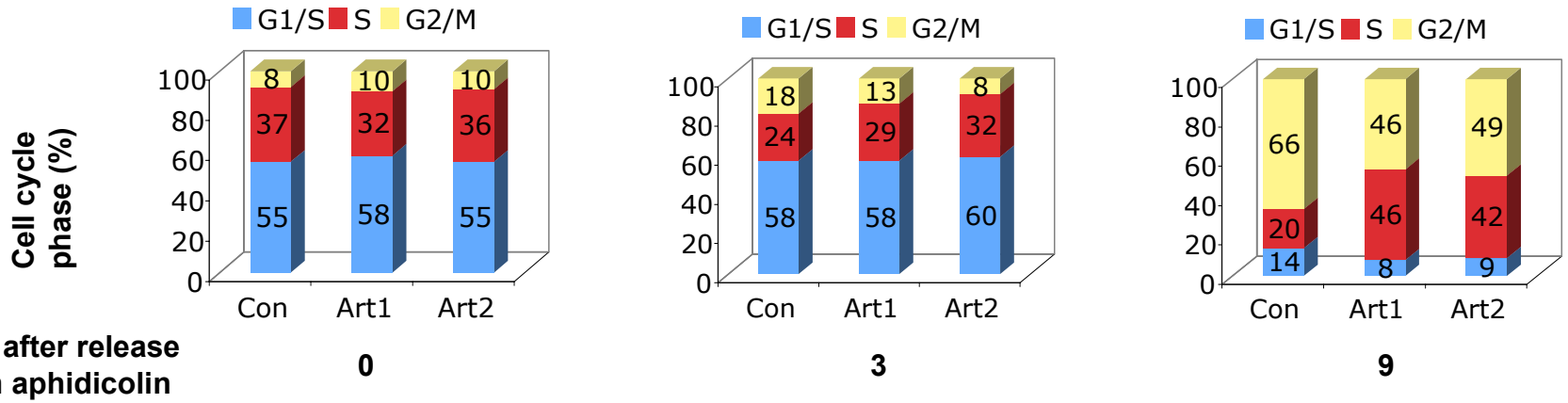


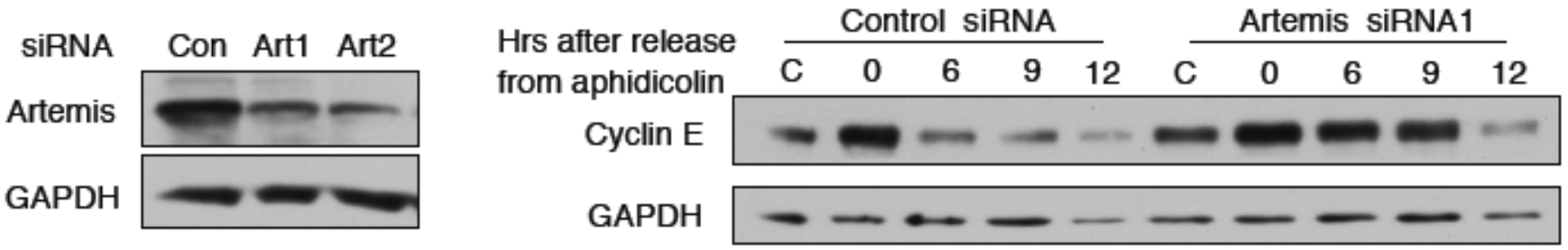
Lu et al. JBC, in press (2009)

Cyclin E degradation is mediated by the SCF-FBW7 E3 ligase

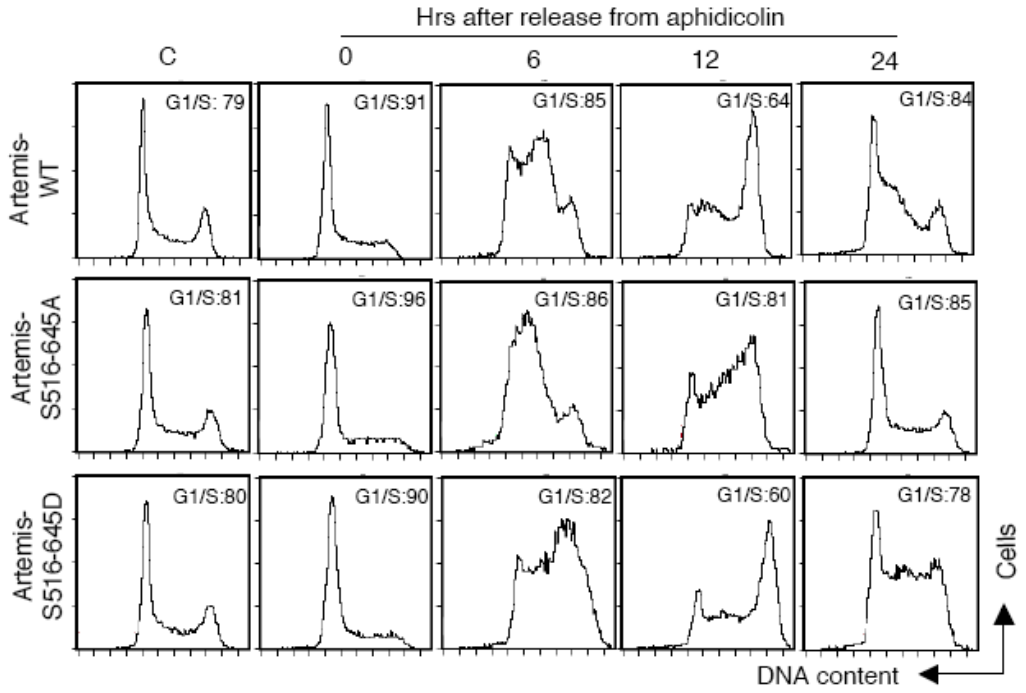


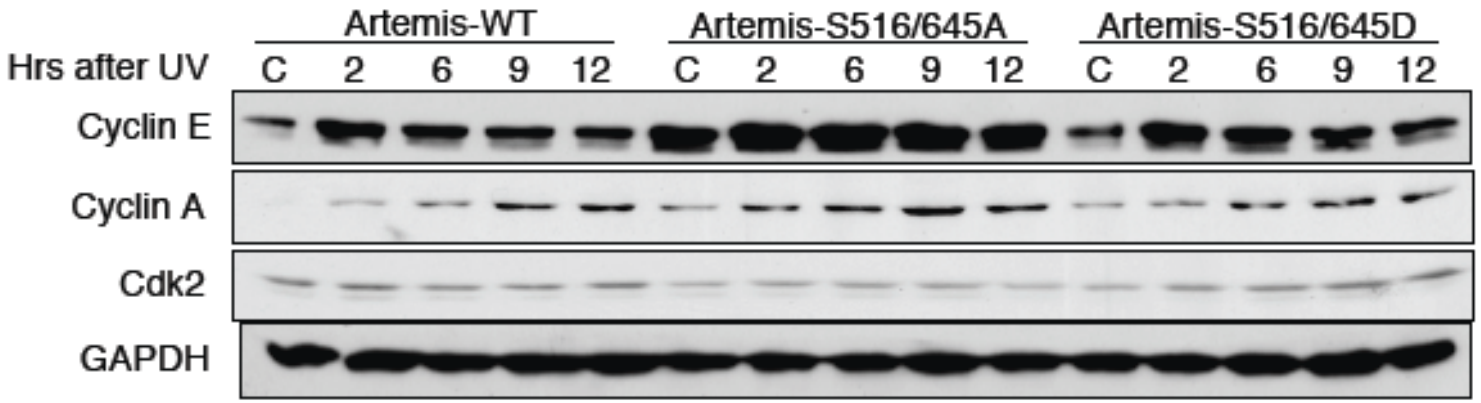
Depletion of Artemis stabilizes cyclin E and delays recovery from the S phase checkpoint



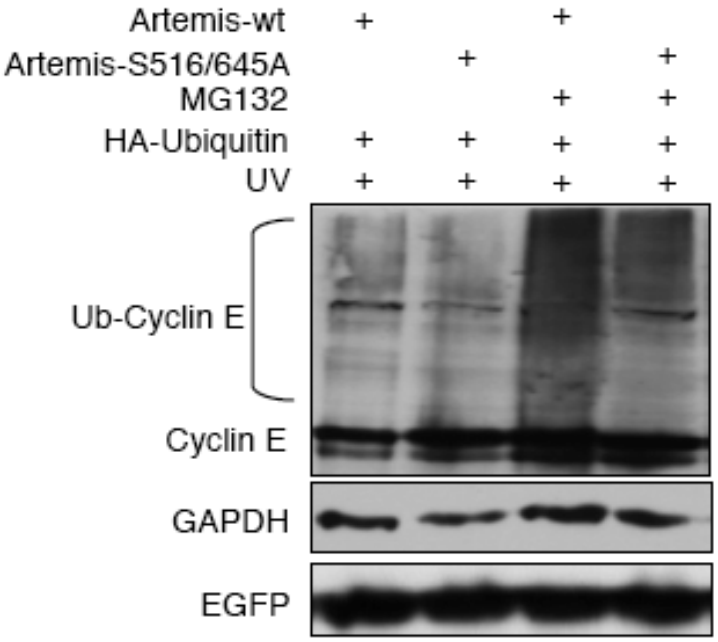


Mutation of S516 and S645 results in prolonged S phase arrest in response to replication stress

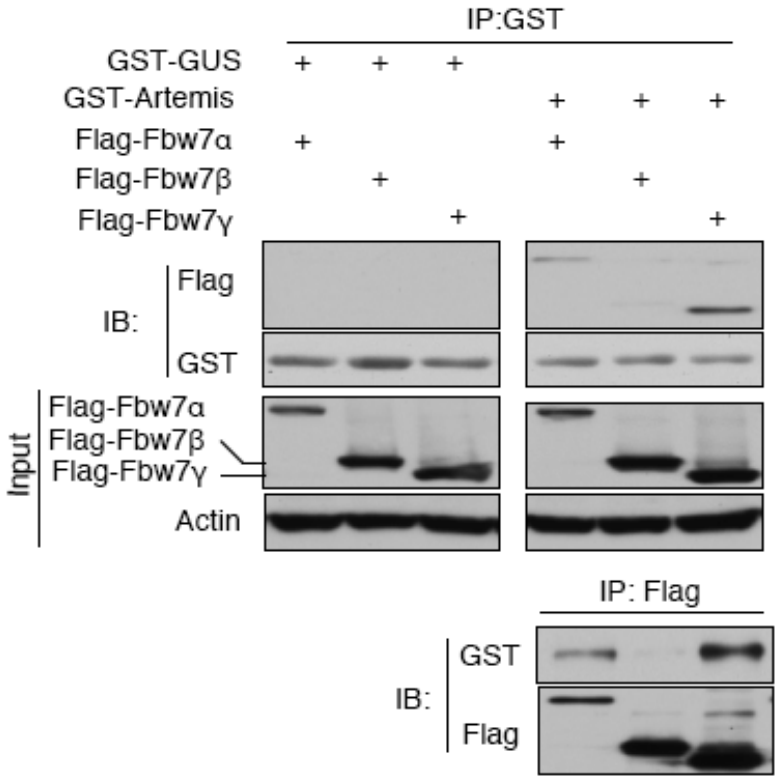


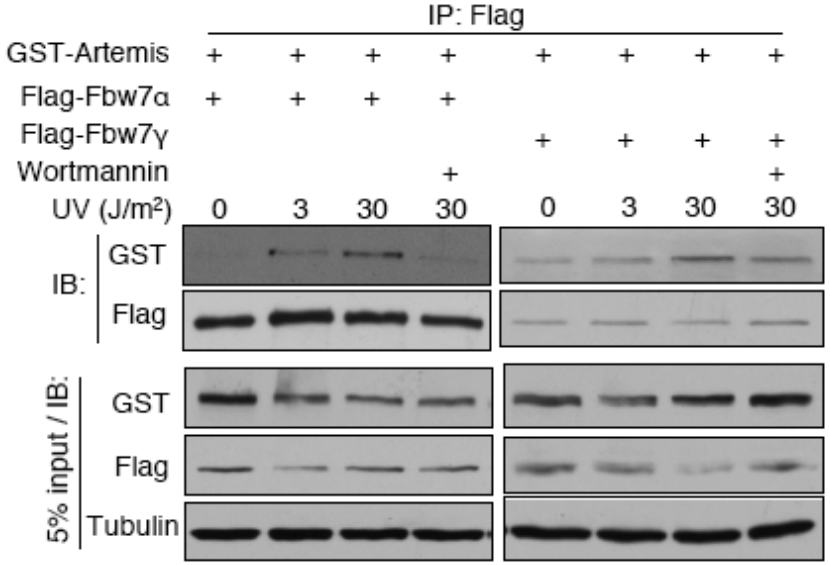


Artemis regulates cyclin E stability via ubiquitylation

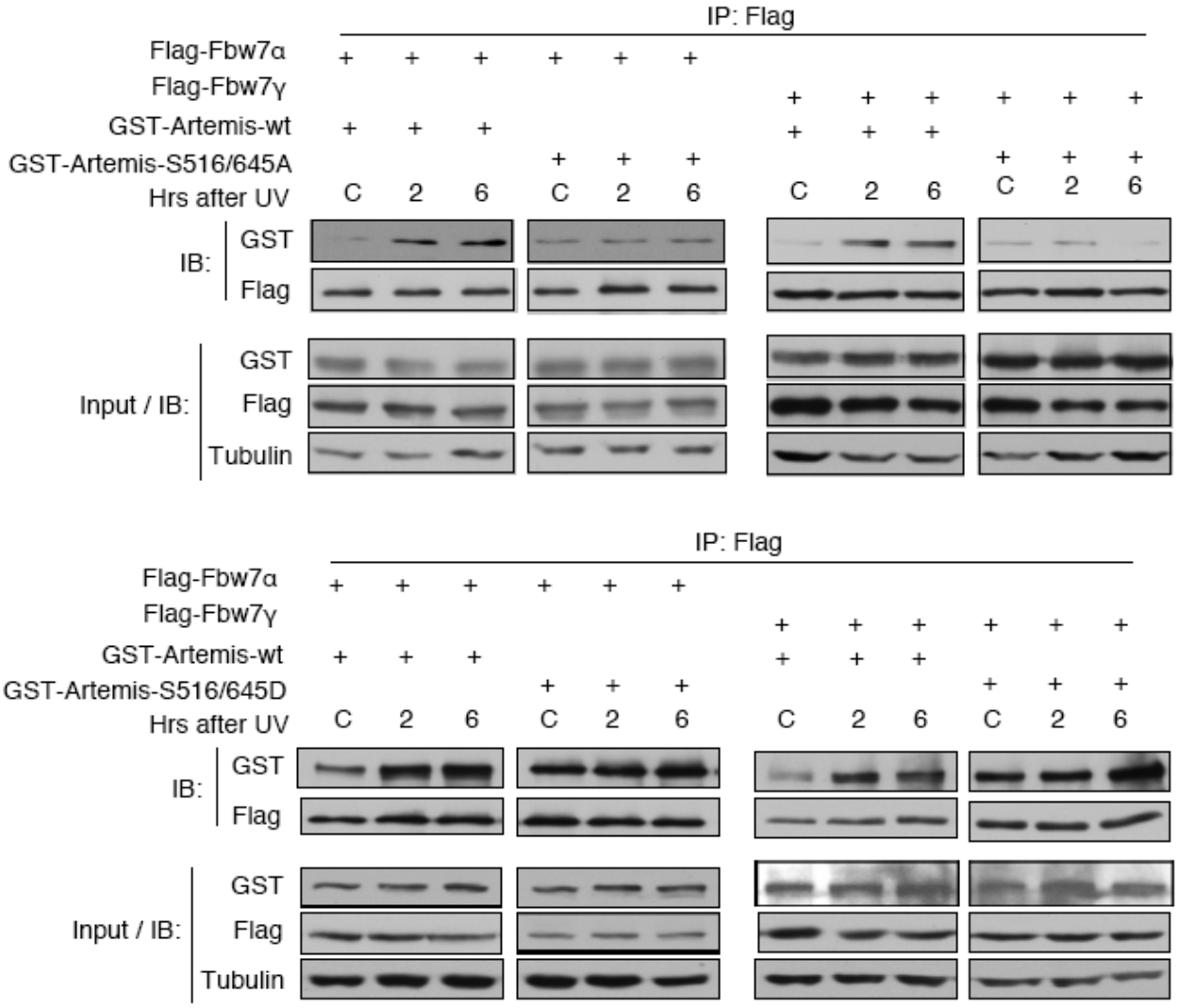


Artemis interacts with the α and γ isoforms of the F box protein Fbw7

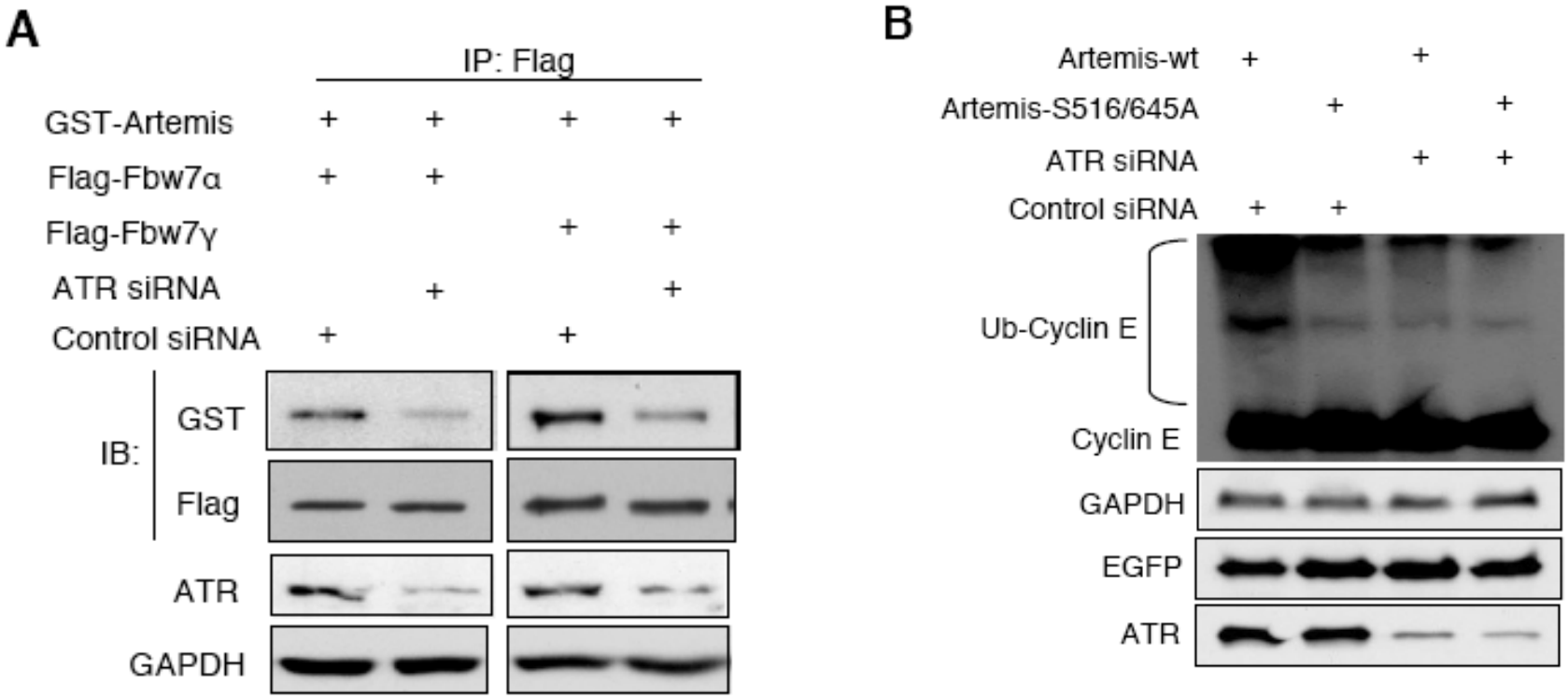




The interaction of Artemis with Fbw7 is regulated by phosphorylation



ATR is required for Artemis-mediated degradation of cyclin E



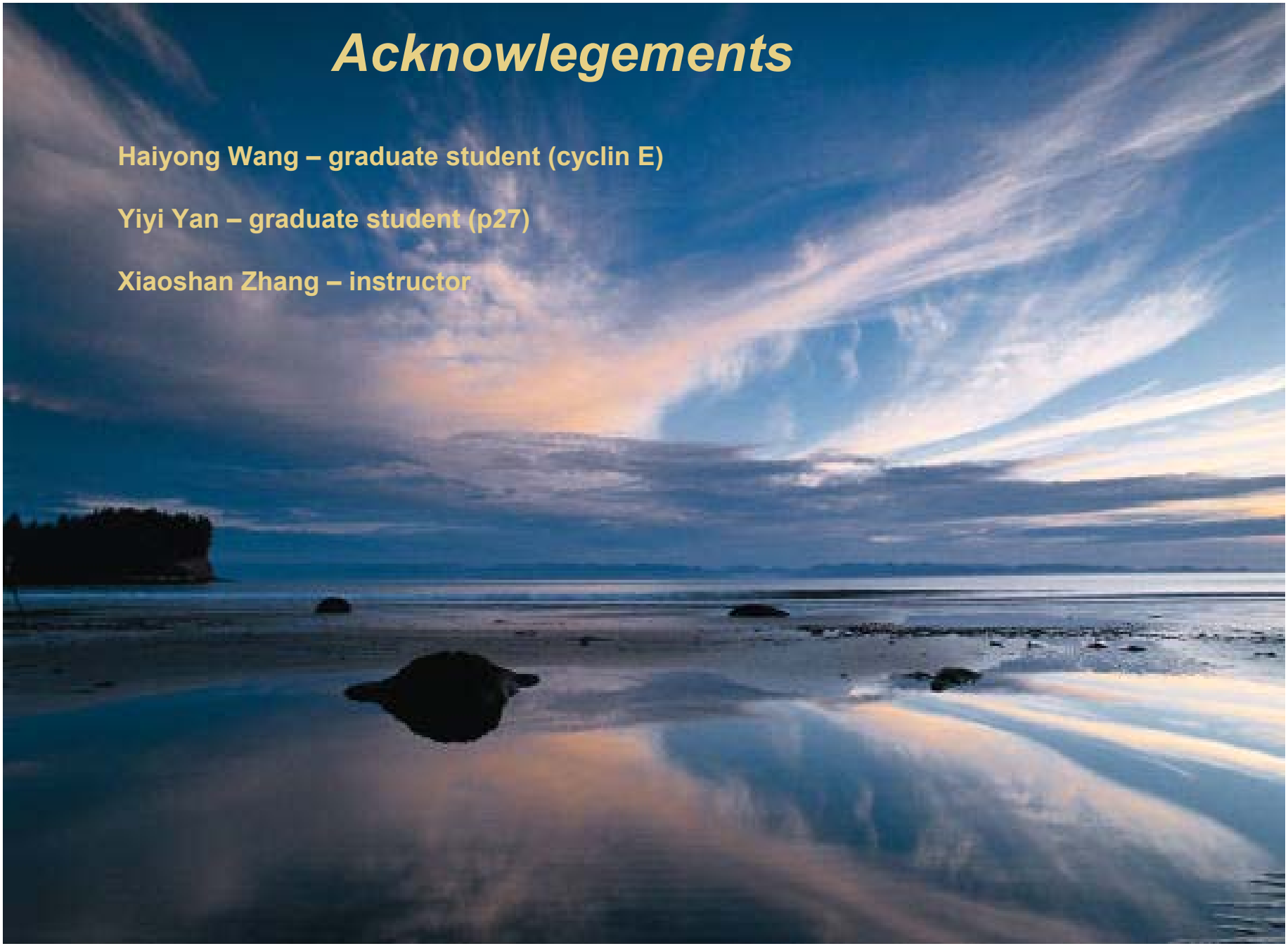
Conclusions

1. *Artemis regulates recovery from the S phase replication checkpoint*

2. *Artemis interacts with SCF^{Fbw7} to promote degradation of cyclin E*

3. *Phosphorylation of Artemis at S516 and S645 by ATR in response to replication stress enhances its association with Fbw7*

4. *ATR is required for Artemis-mediated degradation of cyclin E*



Acknowlegements

Haiyong Wang – graduate student (cyclin E)

Yiyi Yan – graduate student (p27)

Xiaoshan Zhang – instructor