### Supplemental Data 1

## DNA damage induced G1-S arrest: pathways overview



**Figure S1**: Overview of the p53-p21 pathway and the p16-Rb-E2F pathway for G1-S arrest.

At the beginning of the cell cycle growth factors lead to the formation of an active complex between cyclin D and the cyclin-dependent kinase (CDK) Cdk4/6 [[1](#_ENREF_1)]. Active Cdk4/6 phosphorylates the retinoblastoma protein Rb1, which in turn releases the Rb1-bound transcription factor family E2F1-3 [[2-4](#_ENREF_2)]. Active E2F transcription factors have been shown to trigger the transcription of important cyclins necessary for the G1-S transition and S phase, like Cyclin E and Cyclin A [[4-9](#_ENREF_4)]. Both Cyclin E and Cyclin A build a complex with the CDK Cdk2. The activated Cyclin E-Cdk2 complex is necessary to pass through the G1-S checkpoint by, e.g., further enhancing Rb1 phosphorylation and activating other E2F targets, which eventually leads to formation of active Cyclin A-Cdk2 complex and up-regulation of S phase genes and DNA synthesis [[1](#_ENREF_1)]. Like most other CDKs, the Cyclin E-Cdk2 complex requires post-translational modifications to be activated. One such modification is phosphorylation on Thr160, a highly conserved site among all CDKs, which is carried out by the CDK-activating kinase (CAK) complex [[10](#_ENREF_10)]. However, there are also counteracting modifications. Notably, the Wee1 and Myt1 kinases inhibit the kinase activity of Cdk–cyclin complexes by phosphorylating adjacent threonine and tyrosine residues in the Cdk subunit (Thr14 and Tyr15). Thus, for full Cdk2 activity these inhibitory phosphorylation have to be removed, which is accomplished by the phosphatase Cdc25A [[1](#_ENREF_1), [11-13](#_ENREF_11)]. Notably, both active Cdk2 complexes further activate their own activator Cdc25A and inhibit their own inhibitor Wee1, leading to a positive feedback [[14-18](#_ENREF_14)] and a double-negative feedback, respectively [[19](#_ENREF_19), [20](#_ENREF_20)]. These positive feedbacks involved in Cdk2 activation have been implicated in the robustness and irreversibility of the G1-S transition and S phase entry [[21-24](#_ENREF_21)]. Upon DNA damage the CDK inhibitors (CDKIs) p16 and p21 are up-regulated. The mechanism of p16 up-regulation is still unclear and is probably not directly related to DNA damage but rather a secondary effect [[25](#_ENREF_25), [26](#_ENREF_26)] (indicated by the dashed line in Figure S1). The CDKI p16 binds and inactivates the Cyclin D-Cdk4/6 complex thereby inactivating E2F transcription factors [[3](#_ENREF_3)] leading eventually to down-regulation of important G1 and S phase cyclins, especially Cyclin A [[27](#_ENREF_27)]. This way the cell cycle halts at the G1-S transition. However, the p16-Rb route to senescence seem to be important mainly in replicative and oncogene-induced senescence [[28](#_ENREF_28)]. The up-regulation of the other important CDKI p21 is directly related to DNA damage and is regulated by the transcription factor p53. DNA damage activates the DNA damage sensor ATM leading to phosphorylation and activation of p53, which in turn up-regulates p21 [[29-31](#_ENREF_29)]. Interestingly, activated ATM has also been reported to control down-regulation of the phosphatase Cdc25A through Chk1 kinase, which also de-activates Cdk2 leading to cell cycle arrest in G1 phase [[11](#_ENREF_11), [32](#_ENREF_32), [33](#_ENREF_33)]. Additionally, p21, similar to p16, binds and de-activates the Cyclin E/A-Cdk2 complexes, which is considered to be sufficient to, at least temporarily, engage the G1-S checkpoint halting the cell cycle before DNA synthesis starts [[34-36](#_ENREF_34)] (Figure S1).

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