SUPPLEMENTARY MATERIAL

Myc stimulates cell cycle progression through the activation of Cdk1 and phosphorylation of p27

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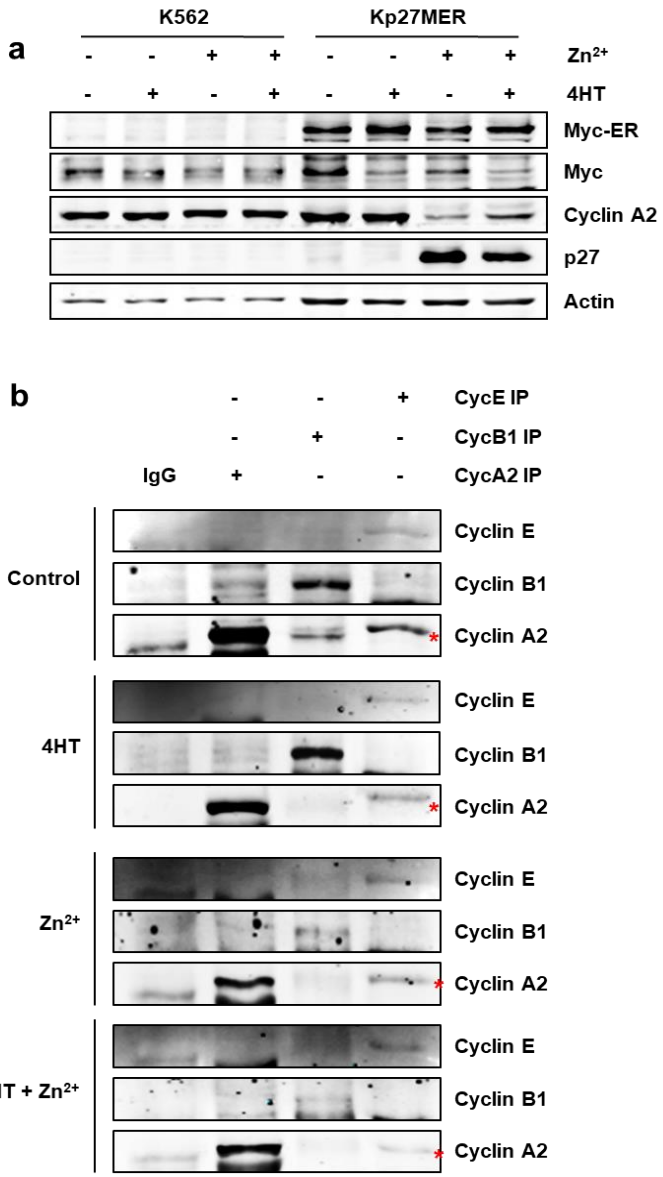
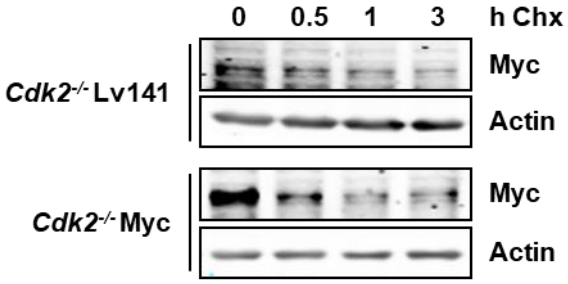
Running Title: Myc activates Cdk1 to phosphorylate p27

Supplementary Table S1. List of plasmids used in this work.

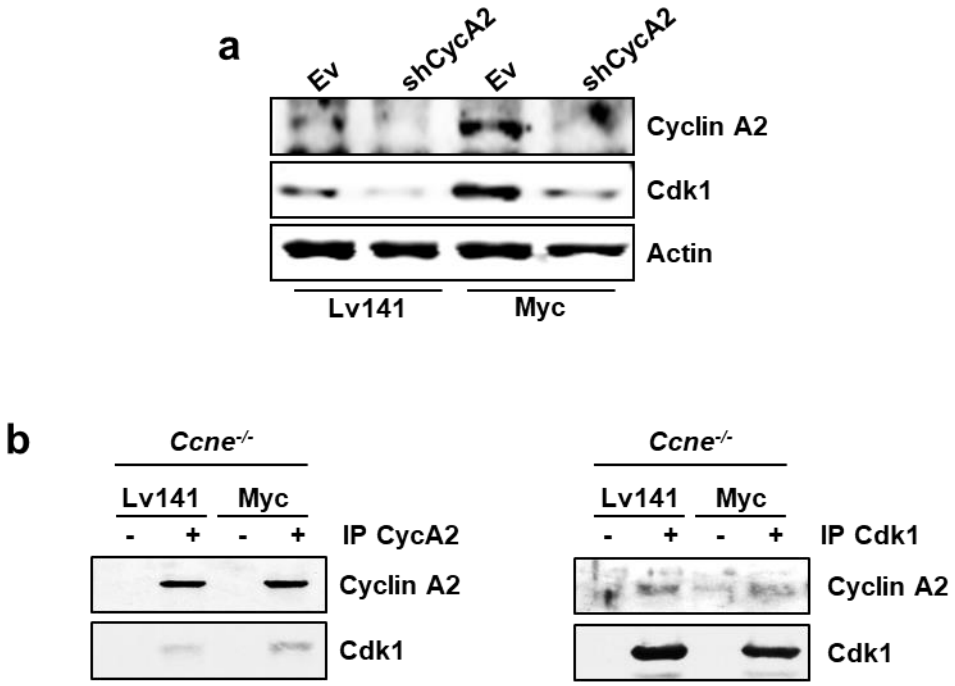
Plasmid	Construct	Origin
pEYFP	Empty vector containing YFP protein	Clontech
pEYFP-p27	Human p27 fused with YFP	Our lab
pCMV-Bam-HA-EV	Empty vector	Addgene
pCMV-cdc2-HA WT	Cdk1 wild type gene tagged with HA	Addgene
pCMV-cdc2-HA DN	CDK1 D146N gene tagged with HA	Addgene
EX-NEG-Lv141	Lentiviral empty vector	Genecopoeia
EX-Z2845-Lv141-MYC Flag	Human Myc tagged with Flag; IRES GFP	Genecopoeia
pLKO.1 control	Lentiviral empty vector	SigmaMission
pLKO.1 shMYC TRCN0000039640	Lentiviral shRNA for human MYC gene	SigmaMission
pLKO.1 shMYC TRCN0000039642	Lentiviral shRNA for human MYC gene	SigmaMission
pLKO.1 shMYC TRCN0000039655	Lentiviral shRNA for human MYC gene	SigmaMission
pLKO.1 shCyclin A2 TRCN00000	Lentiviral shRNA for mousecyclin A2 gene	SigmaMission
pCMV-VSV-G	VSV-G gene encoding envelope lentiviral protein	Addgene
psPAX2	GAG and POL genes encoding packaging lentiviral proteins	Addgene



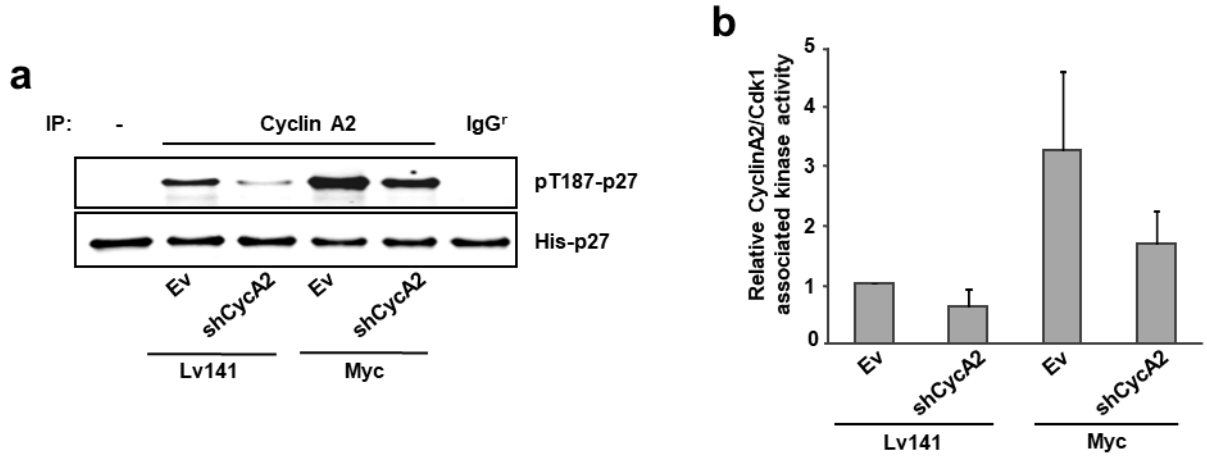
Supplementary Figure S1. Induction of p27 and activation of Myc-ER in Kp27MER cells. **(a)** Protein levels of Myc, Myc-ER, cyclin A2 and p27 of Kp27MER cells upon the indicated treatments for 24h hours compared with K562 parental cells. Actin levels were measured as loading control. **(b)** Levels of cyclins E, B1 and A2 after immunoprecipitation from Kp27MER treated as indicated for 24 hours. The asterisk marks a previous incubation with anti-cyclin E antibody.



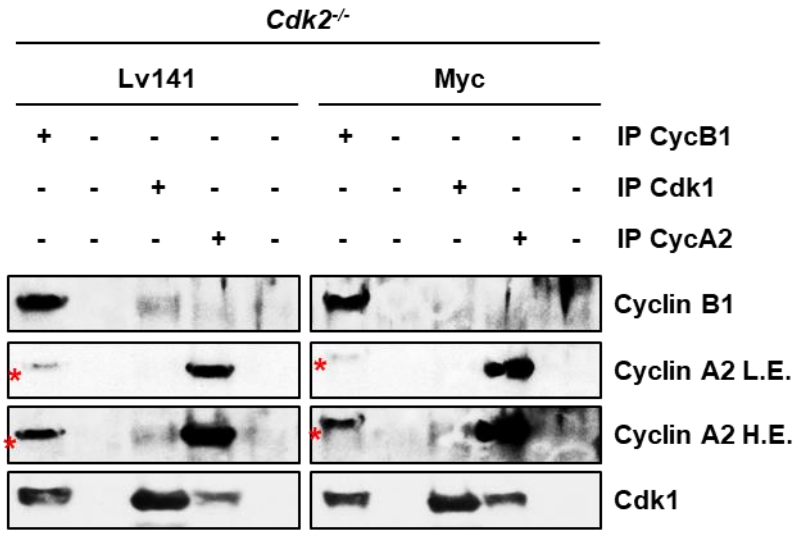
Supplementary Figure S2. Cycloheximide treatment of *Cdk2*$^{-/-}$ Lv141 or *Cdk2*$^{-/-}$ Myc MEFs leads to Myc rapid degradation. Protein stability of Myc as control of cycloheximide effectivity in *Cdk2*$^{-/-}$ Lv141 and *Cdk2*$^{-/-}$ Myc MEFs transfected with a p27-YFP construct measured by western blot. Levels of Myc were detected after 0, 0.5, 1 and 3 hours of cycloheximide treatment (30 µg/mL). Actin levels were used as loading control.



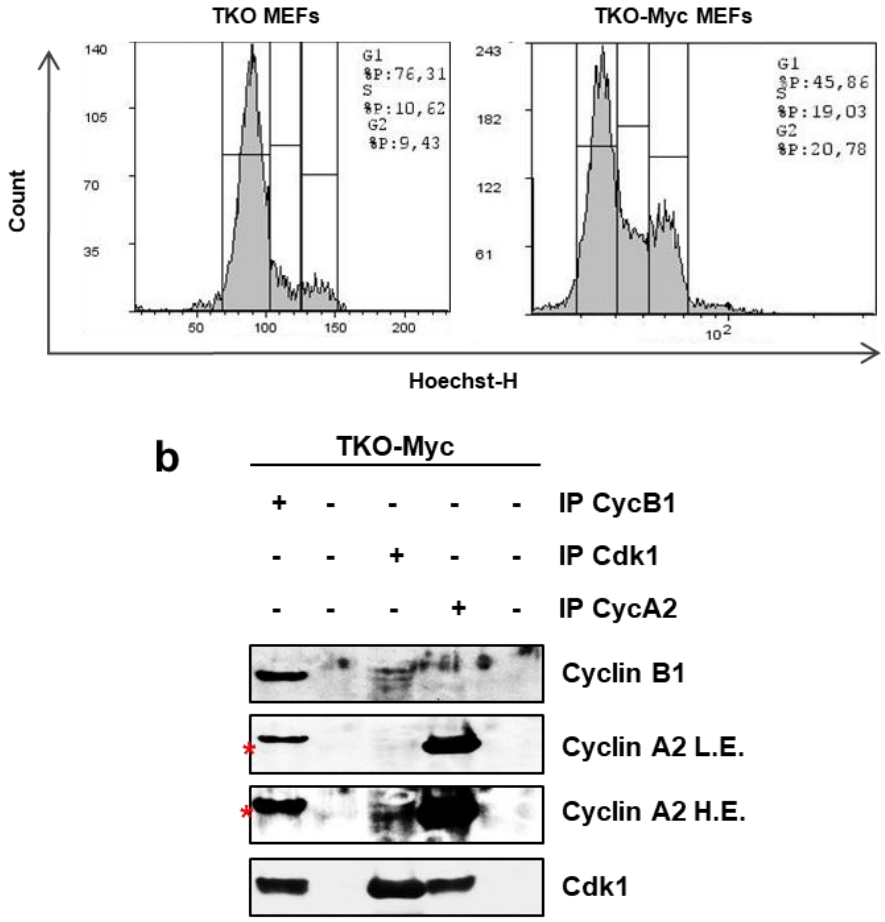
Supplementary Figure S3. Total lysates and immunoprecipitations from *Ccne*$^{-/-}$ Lv141 and *Ccne*$^{-/-}$ Myc MEFs. (a) Protein levels of cyclin A2 and Cdk1 from *Ccne*$^{-/-}$ Lv141 and *Ccne*$^{-/-}$ Myc MEFs transduced with shCyclin A2 lentiviral particles or the corresponding control (Ev). Actin levels were used as loading control. **(b)** Co-immunoprecipitation of cyclin A2 and Cdk1 in *Ccne*$^{-/-}$ Lv141 and *Ccne*$^{-/-}$ Myc MEFs. Unspecific IgG was used as negative control for the specificity of the antibody used for immunoprecipitation.



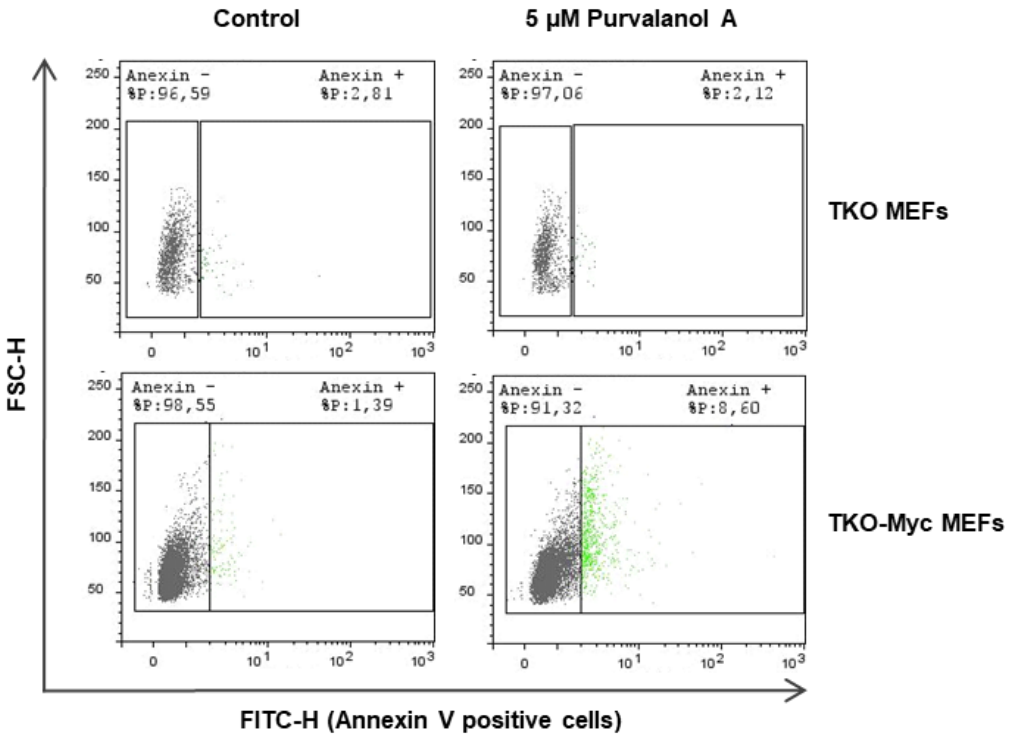
Supplementary Figure S4. Efficiency of cyclin A2 knock down in *Cdk2*$^{-/-}$ MEFs measured by its kinase activity over 27. (a) Kinase assay of cyclin A2 immunocomplexes obtained from *Cdk2*$^{-/-}$ Lv141 and *Cdk2*$^{-/-}$ Myc MEFs transduced with shCyclin A2 lentiviral particles or the corresponding control (Ev). **(b)** Densitometric quantification of the relative cyclin A2 kinase activity. Error bars represent ±SD of the quantification of three independent experiments. Kinase buffer with His-p27 was used as negative control (No IP) and unspecific IgG was used as negative control for the specificity of the antibody used for immunoprecipitation.



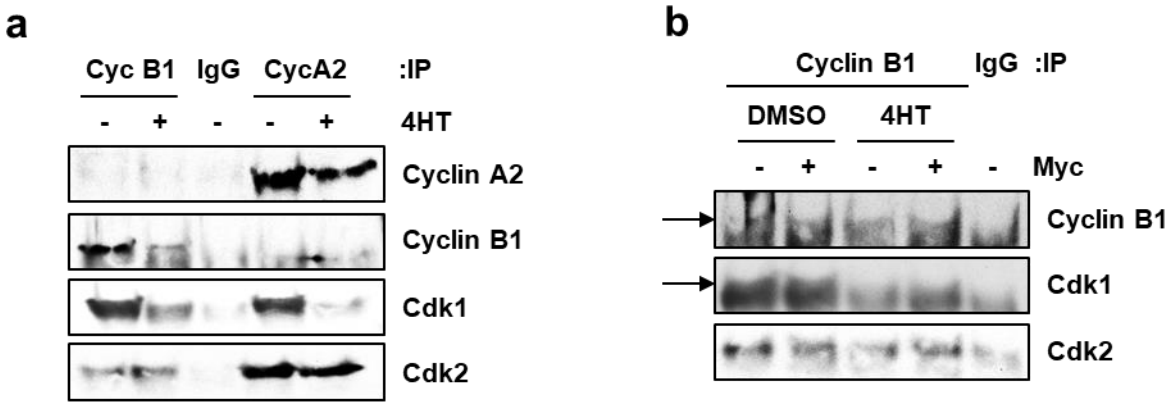
Supplementary Figure S5. Co-immunoprecipitation of cyclins A2 and B1 with Cdk1 in *Cdk2*$^{-/-}$ MEFs. Immunoprecipitated levels of cyclins A2 and B1 and Cdk1 from *Cdk2*$^{-/-}$ Lv141 and *Cdk2*$^{-/-}$ Myc MEFs. Unspecific IgG was used as negative control for the specificity of the antibody used for immunoprecipitation. The asterisk marks cyclin B1 from a previous incubation with anti-cyclin B1 antibody.



Supplementary Figure S6. Cell cycle profile of TKO and TKO-Myc MEFs and Cdk1 interaction with cyclins A2 and B1. (a) Distribution of cells among G_1, S and G_2 phases of the cycle of TKO and TKO-Myc MEFs. DNA content was measured using Hoechst staining by flow cytometry. **(b)** Immunoprecipitated levels of cyclins A2 and B1 and Cdk1 from TKO-Myc MEFs. Unspecific IgG was used as negative control for the specificity of the antibody used for immunoprecipitation. L.E, low exposure; H.L., high exposure. The asterisk marks cyclin B1 from a previous incubation with anti-cyclin B1 antibody.



Supplementary Figure S7. Apoptotic assay measured by Annexin V positive cells. Original plots of Annexin V positive cells of TKO vs TKO-Myc cells treated with Purvalanol A for 24 h measured by flow cytometry.



Supplementary Figure S8. Co-immunoprecipitation of cyclins A2 and B1 with Cdk1 and Cdk2 in *Cdk1*$^{lox/lox}$ MEFs. (a) Cyclin B1 and A2 immunoprecipitates from *Cdk1*$^{lox/lox}$ MEFs after treatment with 0.6 µM of 4HT or DMSO for 3 days. Levels of co-immunoprecipitated Cdk1 and Cdk2 are shown. **(b)** Myc overexpressing *Cdk1*$^{lox/lox}$ MEFs or their corresponding control cells treated for 3 days with 4HT 0.6 µM. Levels of cyclin B1, Cdk1 and Cdk2 after cyclin B1 immunoprecipitation are shown.