

Supplementary information S1 | Genetic mouse models that revealed the role of cell cycle proteins in tumorigenesis

Gene	Gain-of-function by overexpression and/or point mutation	Constitutive heterozygous deletion	Constitutive homozygous loss-of-function by deletion or point mutation	Acutely induced homozygous deletion
<i>Ccnd1</i>	↑ Mammary cancer (spontaneous) ¹ ↑ Hepatocellular carcinomas (spontaneous) ² ↑ Lymphomas (spontaneous, <i>Myc</i> , <i>MycL</i> , <i>Mycln</i> or <i>Nras</i> ^{G12D}) ³⁻⁵ ↑ Skin papillomas (DMBA) ⁶	↓ Intestinal adenomas (<i>Apc</i> ^{Min/+}) ⁷	↓ Intestinal adenomas (<i>Apc</i> ^{Min/+}) ⁷ ↓ Skin carcinomas (<i>vHras</i> +TPA or DMBA+TPA) ⁸ ↓ Rhabdoid tumours (<i>Ini1</i> ^{-/-}) ⁹ ↓ Mammary cancer (<i>ErbB2</i> ^{V664D} or <i>vHras</i>) ¹⁰⁻¹²	↓ Mammary cancer (<i>ErbB2</i> ^{V664D}) ¹³
<i>Ccnd2</i>	↑ Skin carcinomas (DMBA+TPA) ¹⁴		↓ Intestinal adenomas (<i>Apc</i> ^{Min/+}) ¹⁵ ↓ Ovarian, testicular and adrenal tumours (<i>Inha</i> ^{-/-}) ¹⁶ ↓ Skin carcinomas (DMBA+TPA) ¹⁴	
<i>Ccnd3</i>	↑ Mammary cancer (spontaneous) ¹⁷ ↓ Skin carcinomas (DMBA+TPA) ¹⁴		↓ T-ALL (<i>Lck</i> or <i>Notch1</i> ^{ICD}) ¹⁸	↓ T-ALL (<i>Notch1</i> ^{ICD}) ¹³
<i>Cdk4</i>	↑ Endocrine tumours and haemangiomas (spontaneous) ¹⁹ ↑ Skin tumours (DMBA+TPA) ^{20, 21} ↑ Pituitary and pancreatic tumours (<i>Men1</i> ^{-/-}) ²² ↑ Pituitary carcinomas (<i>Cdkn1b</i> ^{-/-}) ²³		↓ Odontogenic tumours (<i>Myc</i>) ²⁴ ↓ Skin tumours (DMBA+TPA) ²⁵ ↓ Mammary cancer (<i>ErbB2</i> ^{V664D} or <i>vHras</i>) ²⁶⁻²⁸ ↓ Oligodendrogiomas (<i>PDGF</i>) ²⁹ ↑ B-cell lymphomas (<i>Myc</i>) ³⁰	↓ Lung cancer (<i>Kras</i> ^{G12V/+}) ³¹
<i>Cdk6</i>	↓ Skin papillomas (DMBA+TPA) ³²		↓ Lymphomas (<i>vAkt1</i>) ³³	
<i>Ccne1</i>	↑ Mammary cancer (spontaneous or <i>Trp53</i> ^{-/-}) ^{34, 35} ↑ Lung cancer (spontaneous or <i>Kras</i> ^{G12D/+}) ^{36, 37} ↑ Pituitary adenomas (spontaneous) ³⁸ ↑ T-cell lymphomas (<i>Cdkn1b</i> ^{-/-}) ³⁹			
<i>Cdk2</i>			↓ Skin tumours (<i>CDK4</i> +DMBA+TPA) ⁴⁰ ↓ Mammary cancer (<i>CCNE1</i> ^{LMMW} or <i>ErbB2</i> ^{V664D}) ^{41, 42}	
<i>Ccna2</i>			↓ Liver tumours (<i>NRAS</i> ^{G12V} + <i>p53</i> shRNA) ⁴³	
<i>Ccnb1</i>	↑ Lung cancer (spontaneous) ⁴⁴ ↑ Skin tumours (DMBA) ⁴⁴ ↑ Intestinal adenocarcinomas (<i>Apc</i> ^{Min/+}) ⁴⁴			
<i>Ccnb2</i>	↑ Lung cancer (spontaneous) ⁴⁴ ↑ Skin and lung tumours (DMBA) ⁴⁴ ↑ Intestinal adenocarcinomas (<i>Apc</i> ^{Min/+}) ⁴⁴			
<i>Cdk1</i>			↓ Liver tumours (<i>NRAS</i> ^{G12V}) ⁴⁵	
<i>Chek1</i>		↑ Mammary cancer (<i>Wnt1</i> or <i>Trp53</i> ^{+/+}) ^{46, 47} ↑ Skin carcinomas (DMBA+TPA) ⁴⁸ ↑ Lymphomas (<i>Chek2</i> ^{+/+}) ⁴⁹	↓ Skin papillomas (DMBA+TPA) ⁴⁸ ↓ Mammary cancer (<i>Trp53</i> ^{+/+}) ⁴⁷	
<i>Chek2</i>		↑ Lymphomas (<i>Chek1</i> ^{+/+}) ⁴⁹	↑ Lung cancer (spontaneous) ⁵⁰ ↑ Mammary cancer (spont., DMBA or <i>Brca1</i> ^{-/-}) ⁵⁰⁻⁵² ↑ Skin tumours (DMBA) ⁵³ ↑ T-cell lymphomas (<i>Brca1</i> ^{-/-}) ⁵¹ ↓ B-cell lymphomas (<i>Mus81</i> ^{-/-}) ⁵⁴	
<i>Wee1</i>		↑ Mammary cancer (spontaneous) ⁵⁵		
<i>Plik1</i>		↑ Lymphomas (spontaneous) ⁵⁶		
<i>Plik3</i>			↑ Lung adenocarcinomas (spontaneous) ⁵⁷	
<i>Plik4</i>		↑ Hepatocellular carcinomas and lung adenocarcinomas (spontaneous) ⁵⁸		
<i>Aurka</i>	↑ Mammary cancer (spontaneous) ⁵⁹ ↑ Skin carcinomas (DMBA+TPA) ⁶⁰	↑ Lymphomas (spontaneous) ⁶¹		
<i>Aurkb</i>	↑ Lymphomas (spontaneous) ⁶²	↑ Hepatocellular carcinomas, pituitary adenomas and skin papillomas (spontaneous) ⁶³		

Genetically engineered mouse models with gain-of-function or loss-of-function alterations of cell cycle genes showing enhanced (↑) or reduced (↓) formation of spontaneous tumours or tumours induced by genetic alterations or carcinogen treatment (the trigger indicated in parentheses). Only observations with an impact on tumorigenesis are detailed for each mouse model. DMBA, 7,12-Dimethylbenz[a]anthracene; T-ALL, T cell acute lymphoblastic leukaemia; TPA, 12-O-Tetradecanoylphorbol-13-acetate.

REFERENCES

1. Wang, T.C. et al. Mammary hyperplasia and carcinoma in MMTV-cyclin D1 transgenic mice. *Nature* **369**, 669-71 (1994).
2. Deane, N.G. et al. Hepatocellular carcinoma results from chronic cyclin D1 overexpression in transgenic mice. *Cancer Res* **61**, 5389-95 (2001).
3. Bodrug, S.E. et al. Cyclin D1 transgene impedes lymphocyte maturation and collaborates in lymphomagenesis with the myc gene. *EMBO J* **13**, 2124-30 (1994).
4. Lovec, H., Grzeschiczek, A., Kowalski, M.B. & Moroy, T. Cyclin D1/bcl-1 cooperates with myc genes in the generation of B-cell lymphoma in transgenic mice. *EMBO J* **13**, 3487-95 (1994).
5. Gladden, A.B., Woolery, R., Aggarwal, P., Wasik, M.A. & Diehl, J.A. Expression of constitutively nuclear cyclin D1 in murine lymphocytes induces B-cell lymphoma. *Oncogene* **25**, 998-1007 (2006).
6. Yamamoto, H. et al. Enhanced skin carcinogenesis in cyclin D1-conditional transgenic mice: cyclin D1 alters keratinocyte response to calcium-induced terminal differentiation. *Cancer Res* **62**, 1641-7 (2002).
7. Hulit, J. et al. Cyclin D1 genetic heterozygosity regulates colonic epithelial cell differentiation and tumor number in ApcMin mice. *Mol.Cell Biol.* **24**, 7598-7611 (2004).
8. Robles, A.I. et al. Reduced skin tumor development in cyclin D1-deficient mice highlights the oncogenic ras pathway in vivo. *Genes Dev* **12**, 2469-74 (1998).
9. Tsikitis, M., Zhang, Z., Edelman, W., Zagzag, D. & Kalpana, G.V. Genetic ablation of Cyclin D1 abrogates genesis of rhabdoid tumors resulting from *Ini1* loss. *Proc Natl Acad Sci U S A* **102**, 12129-34 (2005).
10. Yu, Q., Geng, Y. & Sicinski, P. Specific protection against breast cancers by cyclin D1 ablation. *Nature* **411**, 1017-21 (2001).
11. Bowe, D.B., Kenney, N.J., Adereth, Y. & Maroulakou, I.G. Suppression of Neu-induced mammary tumor growth in cyclin D1 deficient mice is compensated for by cyclin E. *Oncogene* **21**, 291-8 (2002).
12. Landis, M.W., Pawlyk, B.S., Li, T., Sicinski, P. & Hinds, P.W. Cyclin D1-dependent kinase activity in murine development and mammary tumorigenesis. *Cancer Cell* **9**, 13-22 (2006).
13. Choi, Y.J. et al. The requirement for cyclin D function in tumor maintenance. *Cancer Cell* **22**, 438-451 (2012).
14. Rojas, P. et al. Cyclin D2 and cyclin D3 play opposite roles in mouse skin carcinogenesis. *Oncogene* **26**, 1723-30 (2007).
15. Cole, A.M. et al. Cyclin D2-cyclin-dependent kinase 4/6 is required for efficient proliferation and tumorigenesis following *Apc* loss. *Cancer Res* **70**, 8149-58 (2010).
16. Burns, K.H., Agno, J.E., Sicinski, P. & Matzuk, M.M. Cyclin D2 and p27 are tissue-specific regulators of tumorigenesis in inhibin alpha knockout mice. *Mol Endocrinol* **17**, 2053-69 (2003).
17. Pirkmaier, A. et al. Alternative mammary oncogenic pathways are induced by D-type cyclins; MMTV-cyclin D3 transgenic mice develop squamous cell carcinoma. *Oncogene* **22**, 4425-33 (2003).
18. Sicinska, E. et al. Requirement for cyclin D3 in lymphocyte development and T cell leukemias. *Cancer Cell* **4**, 451-461 (2003).
19. Sotillo, R. et al. Wide spectrum of tumors in knock-in mice carrying a Cdk4 protein insensitive to INK4 inhibitors. *EMBO J* **20**, 6637-47 (2001).
20. Sotillo, R. et al. Invasive melanoma in Cdk4-targeted mice. *Proc.Natl.Acad.Sci.U.S.A* **98**, 13312-13317 (2001).
21. Miliani de Marval, P.L., Macias, E., Conti, C.J. & Rodriguez-Puebla, M.L. Enhanced malignant tumorigenesis in Cdk4 transgenic mice. *Oncogene* **23**, 1863-73 (2004).
22. Gillam, M.P. et al. MEN1 tumorigenesis in the pituitary and pancreatic islet requires Cdk4 but not Cdk2. *Oncogene* **34**, 932-8 (2015).
23. Macias, E., Miliani de Marval, P.L., Senderowicz, A., Cullen, J. & Rodriguez-Puebla, M.L. Expression of CDK4 or CDK2 in mouse oral cavity is retained in adult pituitary with distinct effects on tumorigenesis. *Cancer Res* **68**, 162-71 (2008).
24. Miliani de Marval, P.L. et al. Lack of cyclin-dependent kinase 4 inhibits c-myc tumorigenic activities in epithelial tissues. *Mol.Cell Biol.* **24**, 7538-7547 (2004).

25. Rodriguez-Puebla, M.L. et al. Cdk4 deficiency inhibits skin tumor development but does not affect normal keratinocyte proliferation. *Am J Pathol* **161**, 405-11 (2002).
26. Yu, Q. et al. Requirement for CDK4 kinase function in breast cancer. *Cancer Cell* **9**, 23-32 (2006).
27. Reddy, H.K. et al. Cyclin-dependent kinase 4 expression is essential for neu-induced breast tumorigenesis. *Cancer Res* **65**, 10174-8 (2005).
28. Reddy, H.K., Grana, X., Dhanasekaran, D.N., Litvin, J. & Reddy, E.P. Requirement of Cdk4 for v-Ha-ras-Induced Breast Tumorigenesis and Activation of the v-ras-Induced Senescence Program by the R24C Mutation. *Genes Cancer* **1**, 69-80 (2010).
29. Ciznadija, D., Liu, Y., Pyontek, S.M., Holland, E.C. & Koff, A. Cyclin D1 and cdk4 mediate development of neurologically destructive oligodendrogloma. *Cancer Res* **71**, 6174-83 (2011).
30. Lu, Y. et al. CDK4 deficiency promotes genomic instability and enhances Myc-driven lymphomagenesis. *J Clin Invest* **124**, 1672-84 (2014).
31. Puyol, M. et al. A synthetic lethal interaction between K-Ras oncogenes and Cdk4 unveils a therapeutic strategy for non-small cell lung carcinoma. *Cancer Cell* **18**, 63-73 (2010).
32. Wang, X., Sistrunk, C. & Rodriguez-Puebla, M.L. Unexpected reduction of skin tumorigenesis on expression of cyclin-dependent kinase 6 in mouse epidermis. *Am J Pathol* **178**, 345-54 (2011).
33. Hu, M.G. et al. A requirement for cyclin-dependent kinase 6 in thymocyte development and tumorigenesis. *Cancer Res* **69**, 810-818 (2009).
34. Smith, A.P. et al. Deregulated cyclin E promotes p53 loss of heterozygosity and tumorigenesis in the mouse mammary gland. *Oncogene* **25**, 7245-59 (2006).
35. Bortner, D.M. & Rosenberg, M.P. Induction of mammary gland hyperplasia and carcinomas in transgenic mice expressing human cyclin E. *Mol Cell Biol* **17**, 453-9 (1997).
36. Ma, Y. et al. Transgenic cyclin E triggers dysplasia and multiple pulmonary adenocarcinomas. *Proc Natl Acad Sci U S A* **104**, 4089-94 (2007).
37. Loeb, K.R. et al. A mouse model for cyclin E-dependent genetic instability and tumorigenesis. *Cancer Cell* **8**, 35-47 (2005).
38. Roussel-Gervais, A. et al. Cooperation between cyclin E and p27(Kip1) in pituitary tumorigenesis. *Mol Endocrinol* **24**, 1835-45 (2010).
39. Geisen, C., Karsunky, H., Yucel, R. & Moroy, T. Loss of p27(Kip1) cooperates with cyclin E in T-cell lymphomagenesis. *Oncogene* **22**, 1724-9 (2003).
40. Macias, E., Kim, Y., Miliani de Marval, P.L., Klein-Szanto, A. & Rodriguez-Puebla, M.L. Cdk2 deficiency decreases ras/CDK4-dependent malignant progression, but not myc-induced tumorigenesis. *Cancer Res* **67**, 9713-20 (2007).
41. Akli, S., Van Pelt, C.S., Bui, T., Meijer, L. & Keyomarsi, K. Cdk2 is required for breast cancer mediated by the low-molecular-weight isoform of cyclin E. *Cancer Res* **71**, 3377-86 (2011).
42. Ray, D., Terao, Y., Christov, K., Kaldis, P. & Kiyokawa, H. Cdk2-null mice are resistant to ErbB-2-induced mammary tumorigenesis. *Neoplasia* **13**, 439-44 (2011).
43. Gopinathan, L. et al. Loss of Cdk2 and cyclin A2 impairs cell proliferation and tumorigenesis. *Cancer Res* **74**, 3870-9 (2014).
44. Nam, H.J. & van Deursen, J.M. Cyclin B2 and p53 control proper timing of centrosome separation. *Nat Cell Biol* **16**, 538-49 (2014).
45. Diril, M.K. et al. Cyclin-dependent kinase 1 (Cdk1) is essential for cell division and suppression of DNA re-replication but not for liver regeneration. *Proc Natl Acad Sci U S A* **109**, 3826-31 (2012).
46. Liu, Q. et al. Chk1 is an essential kinase that is regulated by Atr and required for the G(2)/M DNA damage checkpoint. *Genes Dev* **14**, 1448-59 (2000).
47. Fishler, T. et al. Genetic instability and mammary tumor formation in mice carrying mammary-specific disruption of Chk1 and p53. *Oncogene* **29**, 4007-17 (2010).
48. Tho, L.M., Libertini, S., Rampling, R., Sansom, O. & Gillespie, D.A. Chk1 is essential for chemical carcinogen-induced mouse skin tumorigenesis. *Oncogene* **31**, 1366-75 (2012).
49. Niida, H. et al. Cooperative functions of Chk1 and Chk2 reduce tumour susceptibility in vivo. *EMBO J* **29**, 3558-70 (2010).
50. Bahassi el, M. et al. Mice with the CHEK2*1100delC SNP are predisposed to cancer with a strong gender bias. *Proc Natl Acad Sci U S A* **106**, 17111-6 (2009).

51. McPherson, J.P. et al. Collaboration of Brca1 and Chk2 in tumorigenesis. *Genes Dev* **18**, 1144-53 (2004).
52. Kwak, E.L. et al. Mammary tumorigenesis following transgenic expression of a dominant negative CHK2 mutant. *Cancer Res* **66**, 1923-8 (2006).
53. Hirao, A. et al. Chk2 is a tumor suppressor that regulates apoptosis in both an ataxia telangiectasia mutated (ATM)-dependent and an ATM-independent manner. *Mol Cell Biol* **22**, 6521-32 (2002).
54. El Ghamrasni, S. et al. Inactivation of chk2 and mus81 leads to impaired lymphocytes development, reduced genomic instability, and suppression of cancer. *PLoS Genet* **7**, e1001385 (2011).
55. Vassilopoulos, A. et al. WEE1 murine deficiency induces hyper-activation of APC/C and results in genomic instability and carcinogenesis. *Oncogene* **34**, 3023-35 (2015).
56. Lu, L.Y. et al. Polo-like kinase 1 is essential for early embryonic development and tumor suppression. *Mol Cell Biol* **28**, 6870-6 (2008).
57. Yang, Y. et al. Polo-like kinase 3 functions as a tumor suppressor and is a negative regulator of hypoxia-inducible factor-1 alpha under hypoxic conditions. *Cancer Res* **68**, 4077-85 (2008).
58. Ko, M.A. et al. Plk4 haploinsufficiency causes mitotic infidelity and carcinogenesis. *Nat Genet* **37**, 883-8 (2005).
59. Wang, X. et al. Overexpression of aurora kinase A in mouse mammary epithelium induces genetic instability preceding mammary tumor formation. *Oncogene* **25**, 7148-7158 (2006).
60. Torchia, E.C. et al. A genetic variant of Aurora kinase A promotes genomic instability leading to highly malignant skin tumors. *Cancer Res* **69**, 7207-15 (2009).
61. Lu, L.Y. et al. Aurora a is essential for early embryonic development and tumor suppression. *J Biol Chem.* **283**, 31785-31790 (2008).
62. Gonzalez-Loyola, A. et al. Aurora B Overexpression Causes Aneuploidy and p21Cip1 Repression during Tumor Development. *Mol Cell Biol* **35**, 3566-78 (2015).
63. Fernandez-Miranda, G. et al. Genetic disruption of aurora B uncovers an essential role for aurora C during early mammalian development. *Development* **138**, 2661-72 (2011).