Supplementary Material

An essential role for Ink4 and Cip/Kip Cell Cycle Inhibitors in preventing replicative Stress

Victor Quereda,[1] Eva Porlan,[1] Marta Cañamero,[2] Pierre Dubus,[3] and Marcos Malumbres[1]

[1] Cell Division and Cancer Group, Spanish National Cancer Research Centre (CNIO), Madrid, Spain; [2] Histopathology Unit, CNIO; [3] EA2406 University of Bordeaux 2, France

Supplementary Figures

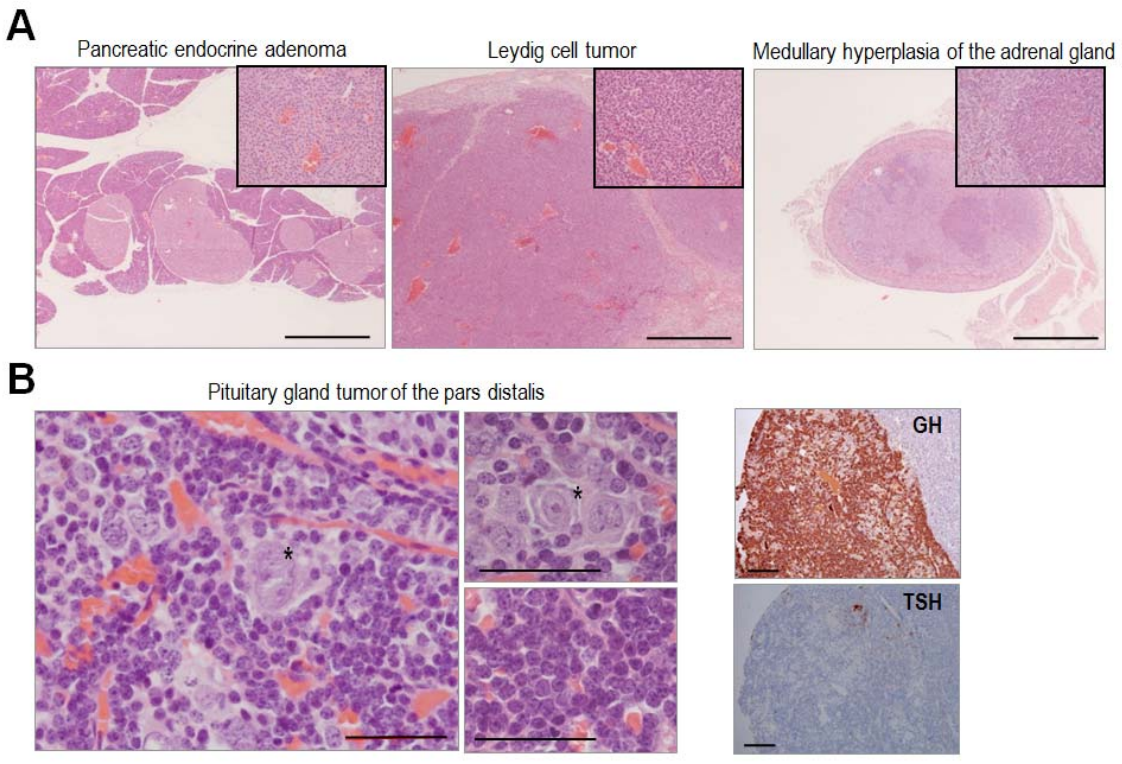

Figure S1. Pathologies observed in mice with combined Cdk4 R24C, p21-null, p27-null alleles. (A) Microscopic images of hematoxylin-eosin stained sections showing the indicated pathologies. Scale bars, 1 mm, magnifications are 10X. (B) Pituitary gland tumor of the pars distalis with giant cells (asterisks). Scale bars, 100 μm. Representative stainings for growth hormone (GH) and thyroid stimulating hormone (TSH) are shown (right panels; Scale bar, 100 μm).

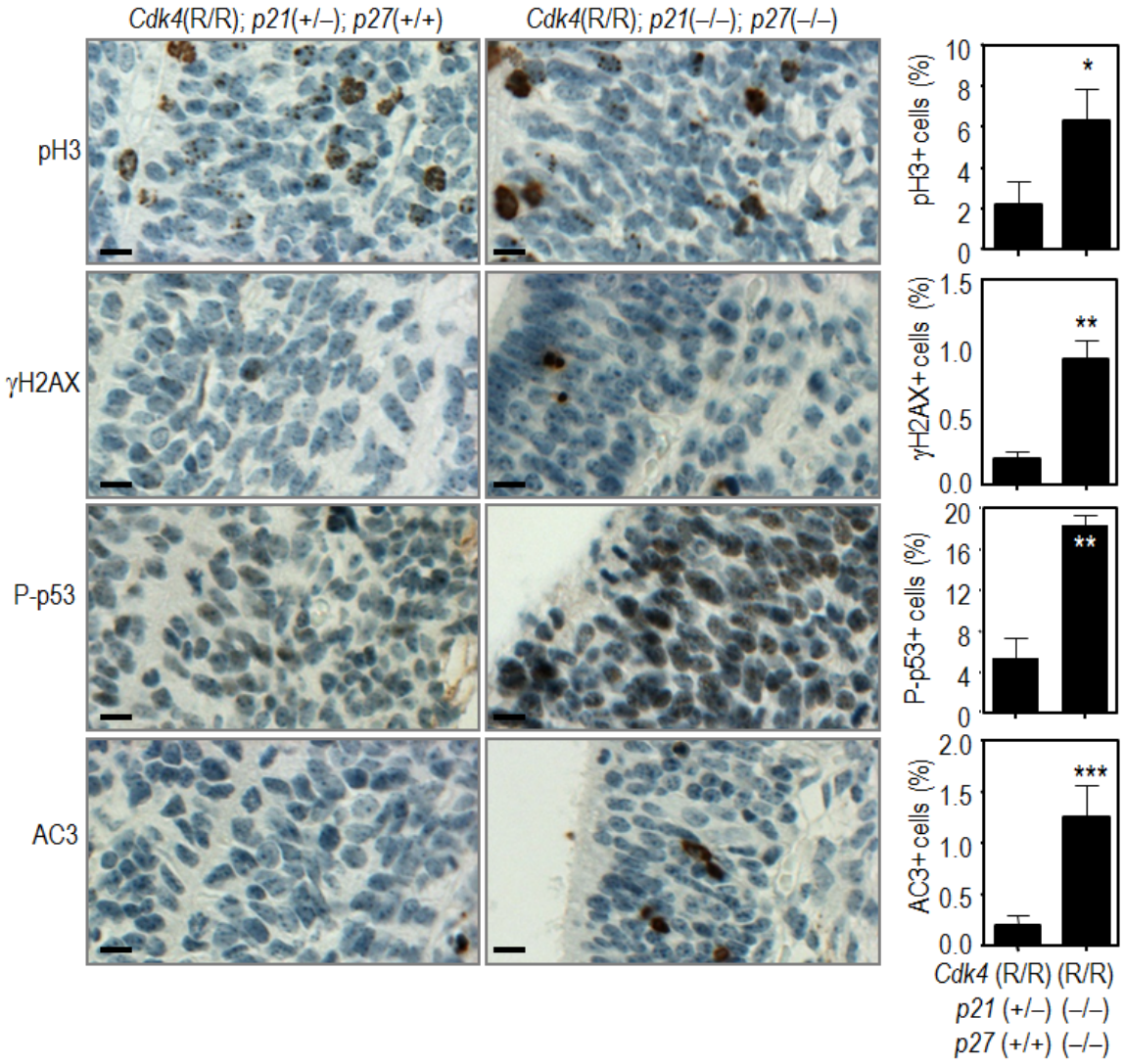

Figure S2. Increased DNA damage and apotosis in brains from triple mutant embryos. Microscopic images of subventricular zone sections from embryonic (E) day 17.5 brains from triple mutants and control littermates immunostained with antibodies against phospho-histone H3 (pH3), γH2AX, phosphorylated p53 (Ser15; P-p53), and active Caspase 3 (AC3). Scale bars, 10 mm. Respective quantifications are shown on the right.

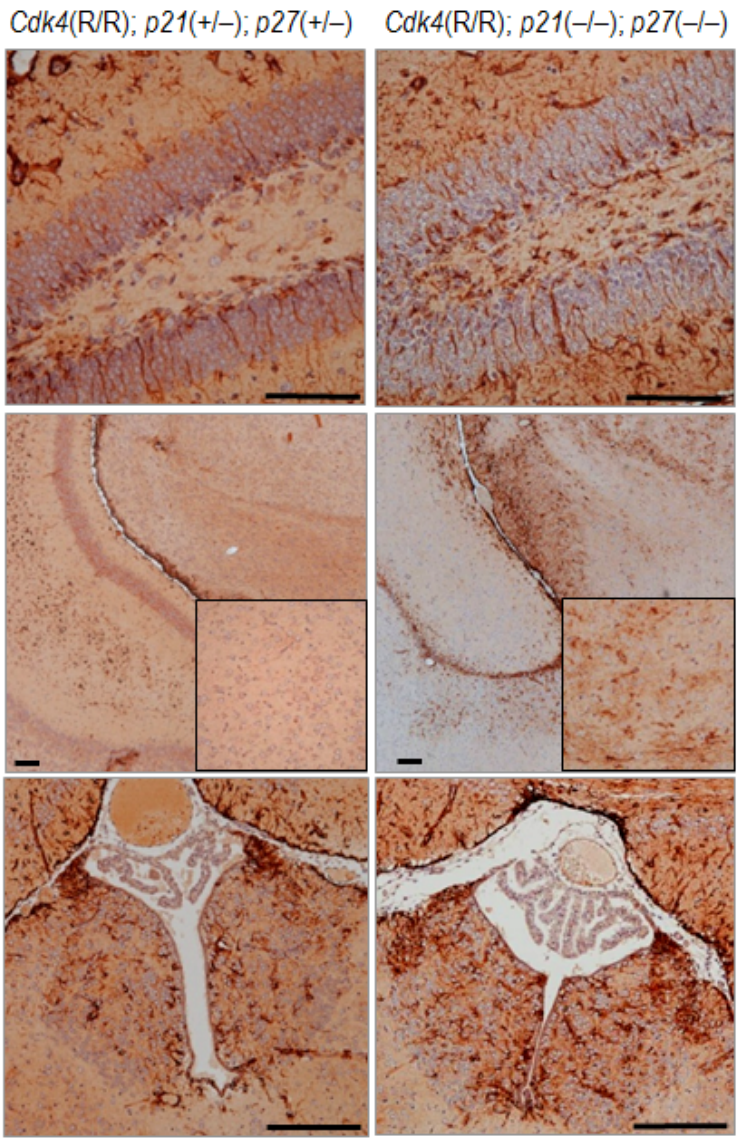

Figure S3. GFAP immunostaining in the dentate gyrus of the hippocampus (top), amygdala (middle), and nucleus medialis habenulae (bottom) areas of the brain in triple mutant mice and control littermates. Scale bar, 100 μm. Insets are 4X magnification.

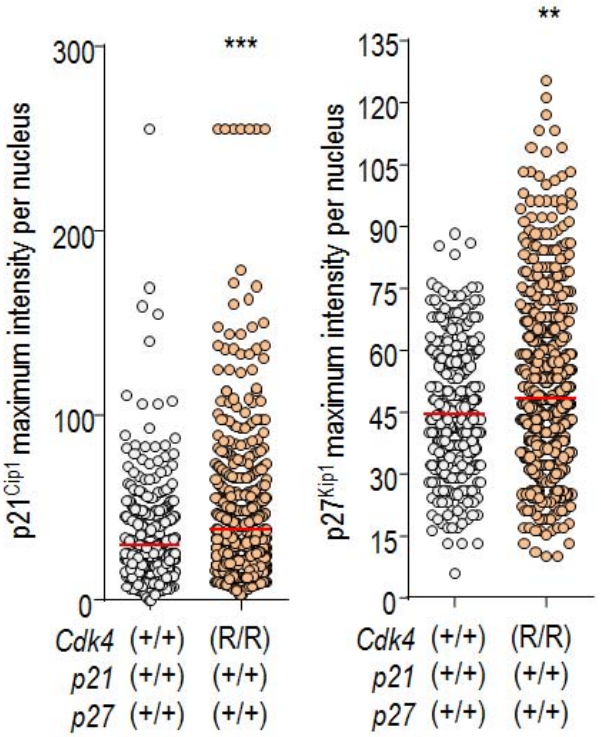

Figure S4. Expression of Cdk4 R24C results in an increase in the levels of $p21^{Cip1}$ and $p27^{Kip1}$ in neurospheres. Each dot represents the maximum fluorescence intensity per nucleus of $p21^{Cip1}$ or $p27^{Kip1}$ in neurospheres of the indicated genotypes after Immunodetection with specific antibodies. Student's t-test; **, <0.01; ***, p<0.001.

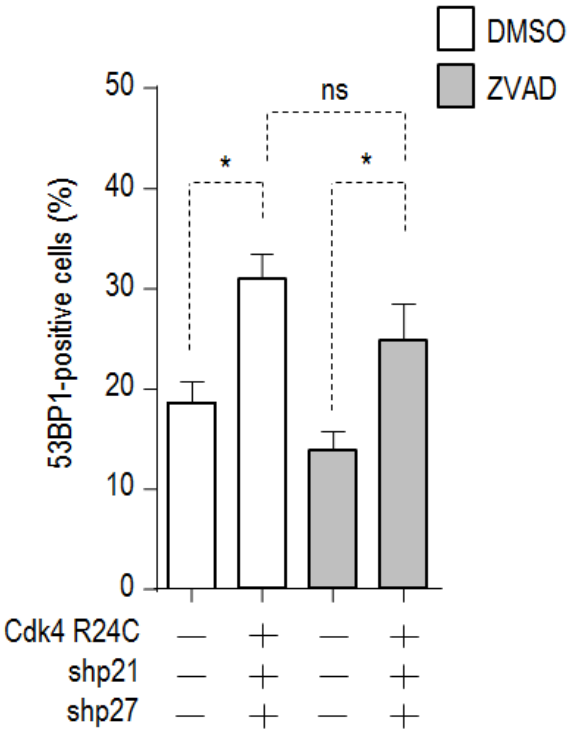

Figure S5. 53BP1 foci are not a consequence of caspase activity. The percentage of C17.2 neural progenitor cells positive for 53BP1 foci was scored per microscopy field and data from n=3 independent experiments for ZVAD and n=5 for DMSO were pooled. Two-tailed, unpaired t test. SEM Student's t-test; **, <0.01; ***, p<0.001.

Supplementary Videos

Reduced mobility and abnormal behaviour in a *Cdk4*(R/R); *p21*(–/–); *p27*(–/–) newborn, (Video S1) compared to a wild-type littermate (Video S2).

Supplementary Tables

Table S1. Percentage of non-tumoral pathologies in Cdk4 R24C; $p21^{Cip1}$; $p27^{Kip1}$ mutant mice

	Cdk4(+/+) *p21*(+/+) *p27*(+/+)	(+/+) (–/–) (–/–)	(+/R) (+/–) (+/–)	(+/R) (–/–) (+/–)	(+/R) (+/–) (–/–)	(+/R) (–/–) (–/–)	(R/R) (+/+) (+/+)	(R/R) (–/–) (+/+)	(R/R) (+/–) (+/–)	(R/R) (–/–) (+/–)	(R/R) (+/+) (–/–)	(R/R) (+/–) (–/–)
	N=23	N=22	N=6	N=17	N=6	N=10	N=21	N=19	N=25	N=20	N=7	N=10
Leydig cell hyperplasia	0	0	0	0	0	0	14	21	16	0	29	0
Pancreatic islet hyperplasia	4	0	17	29	0	10	38	37	72	50	57	40
Adrenal medullary hyperplasia	0	0	67	6	1	40	5	0	28	5	43	30
Myocardiopathy	9	0	17	6	17	0	0	0	0	0	0	0
Hydrocephaly	0	0	0	0	0	0	0	0	16	0	0	0
Follicular lymphoid hyperplasia	20	0	0	0	84	40	19	0	20	30	43	20
Atrophy spleen/thymus	9	0	0	0	0	20	0	0	0	0	0	30
Nephropathy	20	0	0	0	0	0	25	10	0	0	0	50

Table S2. Incidence (%) of pituitary pathologies (*) in Cdk4 R24C; $p21^{Cip1}$; $p27^{Kip1}$ mutant mice

	Cdk4(+/+) *p21*(+/+) *p27*(+/+)	(+/+) (–/–) (–/–)	(+/R) (+/-) (+/-)	(+/R) (-/-) (+/-)	(+/R) (+/-) (-/-)	(+/R) (-/-) (-/-)	(R/R) (+/+) (+/+)	(R/R) (-/-) (+/+)	(R/R) (+/-) (+/-)	(R/R) (-/-) (+/-)	(R/R) (+/+) (-/-)	(R/R) (+/-) (-/-)
	N=23	N=22	N=6	N=17	N=6	N=10	N=12	N=5	N=25	N=20	N=7	N=10
PCPI or PTPI	0	46	40	0	0	11	0	0	21	6	0	0
PAPI	0	18	20	11	33	11	8	40	42	13	0	10
PHPI	9	36	0	33	17	22	0	0	17	38	29	20
PCPD or PTPD	0	0	40	11	0	22	0	20	25	25	29	40
PAPD	0	0	20	33	50	0	50	20	21	13	0	0
PHPD	9	0	0	22	17	11	50	20	8	19	0	10
PIT	0	0	0	22	17	33	0	0	25	25	71	70
PD&PI	0	0	20	22	17	11	17	0	29	44	29	30

* PCPI: Pituitary carcinoma pars intermedia; PTPI: Pituitary tumour pars intermedia; PAPI: Pituitary adenoma pars intermedia; PHPI: Pituitary hyperplasia pars intermedia; PCPD: Pituitary carcinoma pars distalis; PTPD: Pituitary tumour pars distalis; PAPD: Pituitary adenoma pars distalis; PHPD: Pituitary hyperplasia pars distalis; PIT: pituitary neoplasias whose cell-of-origin is difficult to assess characterized by the presence of giant-neuron-like cells and small endocrine-like cells. PD&PI indicate pathologies that affect both the pars distalis and pars intermedia.