

Figure S1: Atypical E2Fs and RB cooperate to prevent liver cancer. **(A)** Transcript levels of Rb, E2f7 and E2f8 from *Alb-7-/-8-/-* and *Alb-Rb-7-/-8-/-* mice in non-tumor areas. Fold changes were adjusted to average of controls and *Actb*, *Gapdh* and *Rsp18* were used to normalize the expression. Data represent average \pm SEM ($n=4-5$ mice). **(B)** Kaplan-Meier overall survival curves of males and females from *control*, *Alb-Rb-/-*, *Alb-7-/-8-/-* and *Alb-Rb-7-/-8-/-* mice. **(C)** Table indicating tumor incidence (%) of males and females of the indicated genotypes. Tumors collected at the end of life span. **(D)** Tumor latency distribution. Bar charts represent number of animals with tumors at the indicated age period. Dash lines indicate distribution of the data. **(E)** Body weights of *control* and *Alb-7-/-8-/-* and *Alb-Rb-7-/-8-/-* knock-out mice. Data represents average \pm SEM ($n=15-20$ controls; $n=20$ *Alb-Rb-7-/-8-/-* ko; $n=10$ *Alb-7-/-8-/-* ko). **(F)** Liver weights of *control* and *Alb-7-/-8-/-* and *Alb-Rb-7-/-8-/-* ko mice. Data represents average \pm SEM ($n=15-20$ controls; $n=20$ *Alb-Rb-7-/-8-/-* ko; $n=10$ *Alb-7-/-8-/-* ko).

Data information: in (B: survival and C: latency) Log Rang (Mantel-Cox) test and in (E and F) Mann-Whitney Rank Sum test; n.s not significant, * $p<0.05$, ** $p<0.01$, *** $p<0.001$

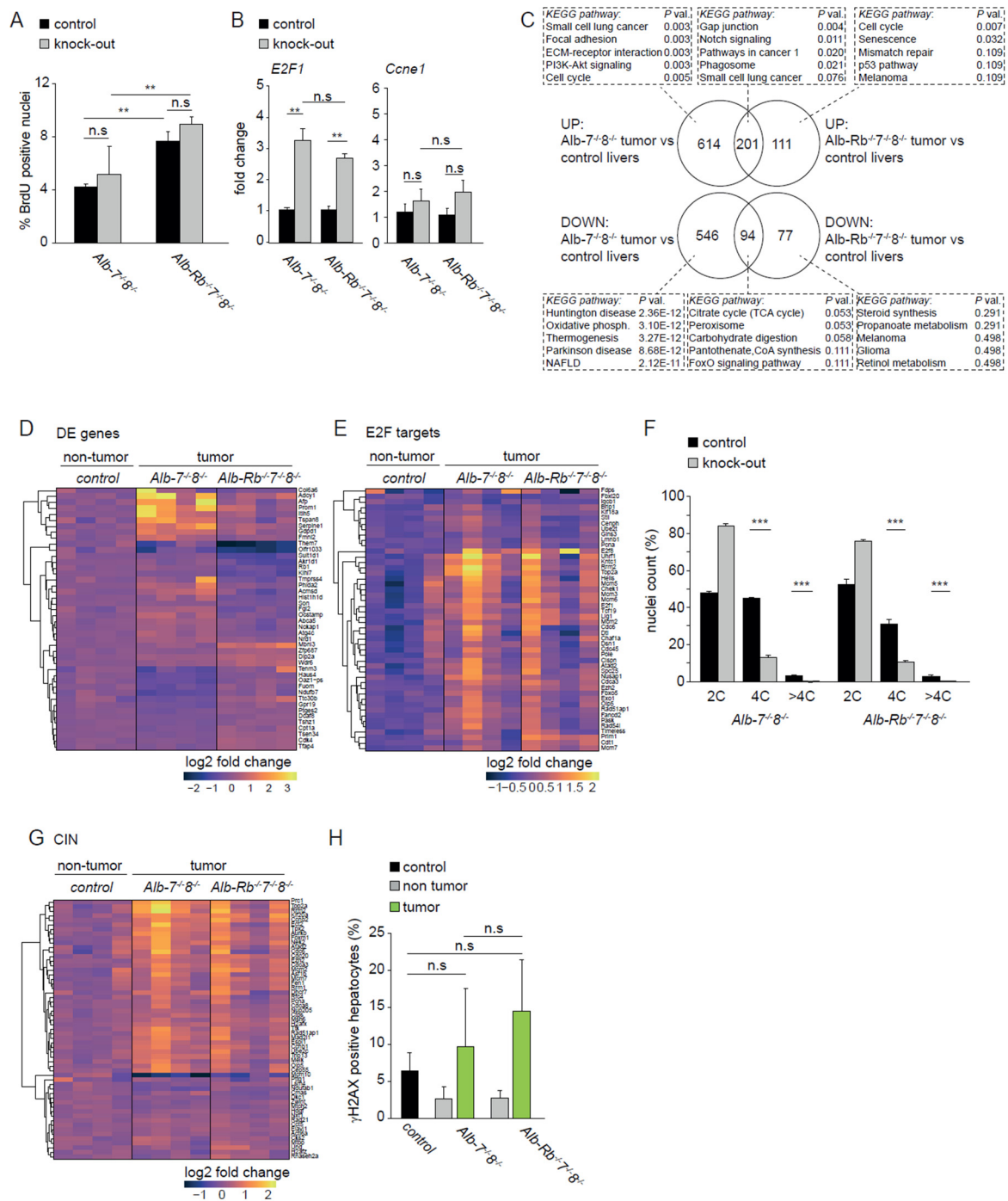


Figure S2: Loss of RB results in deregulation of cell cycle control in atypical E2F-deficient liver tumors. (A) Quantification of the percentage of BrdU-positive nuclei in control (Cre-negative) and knockout (Cre-positive) livers from *Alb-7-8-/-* and *Alb-Rb-7-8-/-* mice, 4 weeks after birth, measured by FACS. Data represent average \pm SEM ($n=7-14$ mice). (B) Transcript levels of E2F targets from *Alb-7-8-/-* and *Alb-Rb-7-8-/-* 4 weeks old mice. Fold changes were adjusted to average of controls and *Actb* and *Gapdh* were used to normalize the expression. Data represent average \pm SEM ($n=4-5$ mice). (C) Venn diagram showing KEGG pathway enrichment analysis from the genes significantly up- or down-regulated in liver tumor samples from *Alb-7-8-/-* and *Alb-Rb-7-8-/-* mice compared to non-tumor control livers in RNA sequencing ($n=4$ mice/ condition). P val.= Benjamini-Hochberg-corrected P value. (D) RNA-sequencing analysis of transcripts that were significantly differentially expressed in *Alb-Rb-7-8-/-* versus *Alb-7-8-/-* tumor samples. Color scale represents log₂-fold changes relative to the normal control livers in RNA sequencing ($n=4$). (E) RNA-sequencing data showing expression of E2F targets in samples from the indicated genotypes ($n=4$ livers/genotype). Color scale represents log₂-fold changes relative to age-matched wild-type livers ($n=4$). (F) Quantification of 2C, 4C and >4C nuclei in 4 weeks old livers

from control and knock-out *Alb-7^{-/-}8^{-/-}* and *Alb-Rb^{-/-}7^{-/-}8^{-/-}* mice. Data represent average \pm SEM ($n=7-14$ mice). (G) RNA sequencing data showing expression of CIN (chromosomal instability)-associated genes (signature CIN70) from the indicated genotypes ($n=4$ livers/ genotype). Color scale represents log2-fold changes relative to age-matched wild-type livers ($n=4$). (H) Quantification of γ -H2AX immunohistochemistry in 5 fields (40x objective) in tumor and adjacent non tumor areas of the livers from the indicated genotypes. Controls represent Alb-Cre negative littermates analyzed at the same time point as Alb-Cre positive ones. The data are presented as average \pm SEM (1 tumor per mice was analyzed/mouse; $n=6$ mice).

Data information: in (A, B, F and H) n.s not significant, * $p<0.05$, ** $p<0.01$, *** $p<0.001$ (Mann-Whitney Rank Sum test).

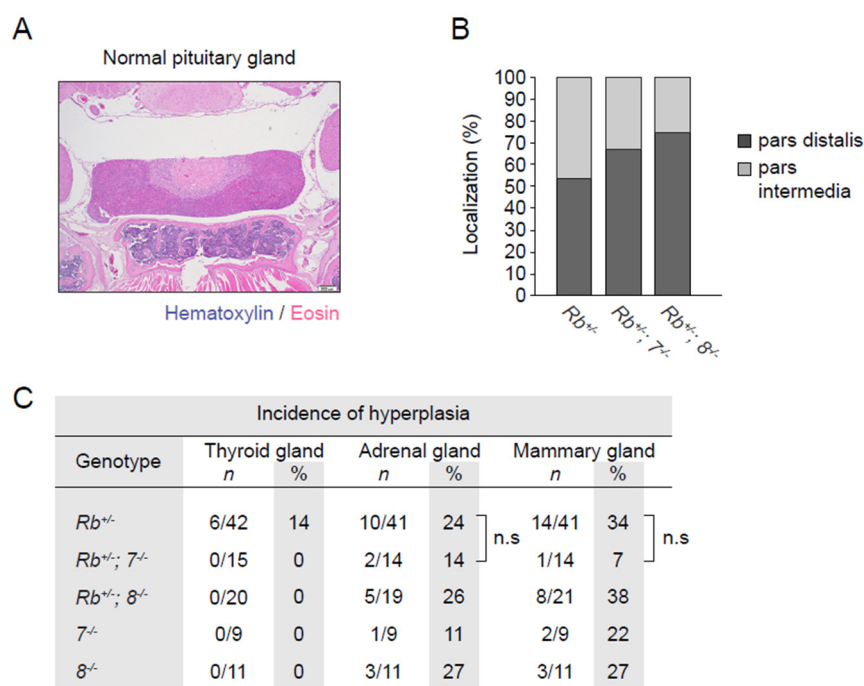


Figure S3: Effect of E2F7 and -8 deletion in pituitary glands of RB heterozygous mice. (A) Representative HE-stained image of a normal mouse pituitary gland. (B) Percentage of the location of the pituitary tumors in the indicated genotypes. (C) Incidence of hyperplasia in the additional neuroendocrine glands analyzed by a board-certified veterinary pathologist. n.s non significant (Chi-square)

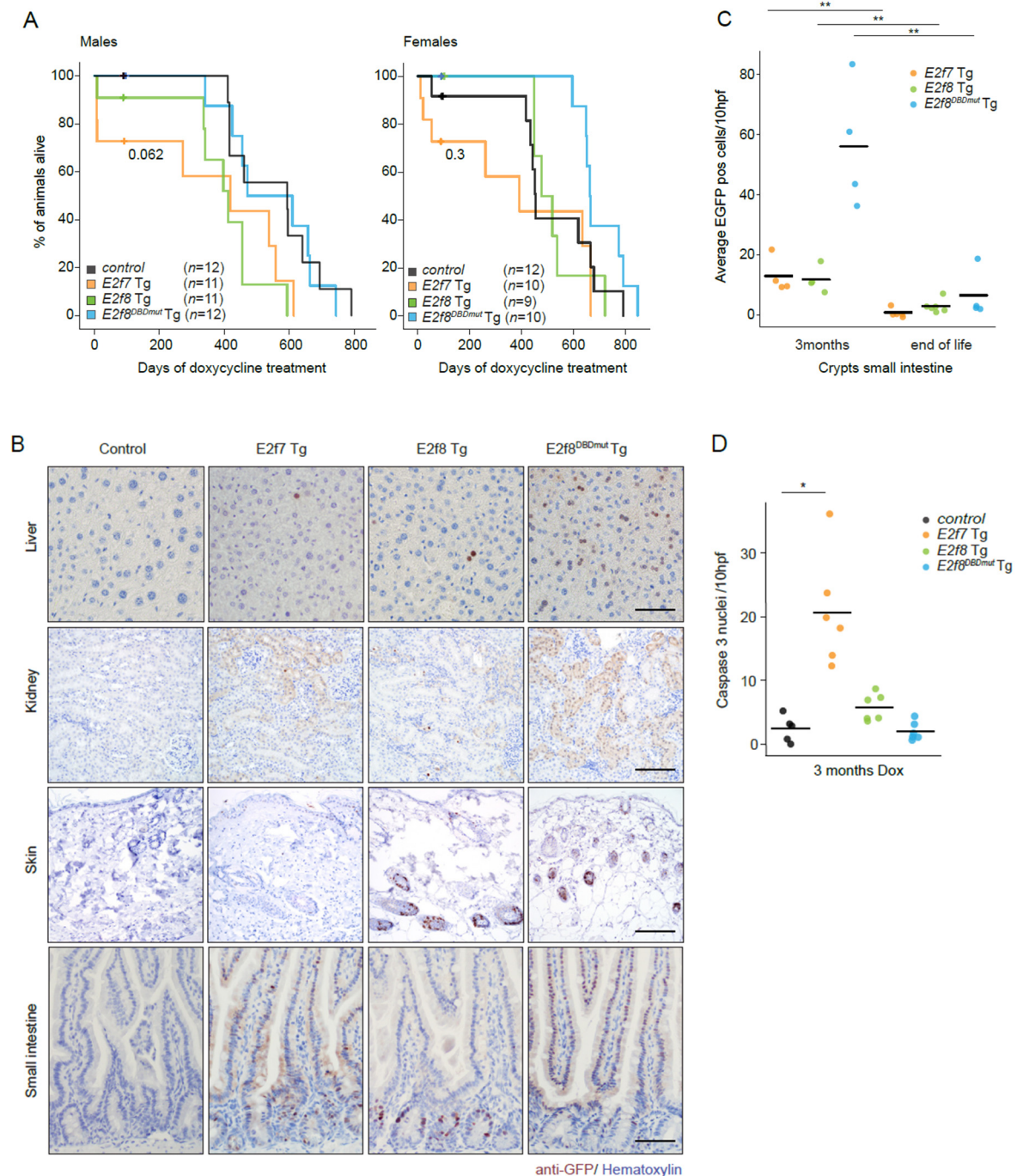


Figure S4: Overexpression of atypical E2Fs affects rapidly proliferating tissues in mice. **(A)** Kaplan-Meier overall survival of transgenic mice, split by gender. **(B)** Representative anti-EGFP immunohistochemistry pictures in the indicated tissues showing transgene expression in the indicated genotypes after 3 months of doxycycline treatment. Scale bars: 100µm. **(C)** Dot plot representing the counts of EGFP-positive cells in ten fields (40x objective) of the small intestinal crypts of 3 months-old and mice harvested at the end of life. Cross black line represents average. ** $p < 0.01$ (Mann-Whitney Rank Sum Test). **(D)** Dot plot representing the counts of Caspase 3-positive nuclei in ten fields (40x objective) of the small intestinal crypts of mice treated for 3 months with doxycycline. Cross black line represents average. * $p < 0.05$ (Kruskal Wallis One Way Analysis of Variance on Ranks and Dunnett's Method for Multiple Comparisons vs. control).

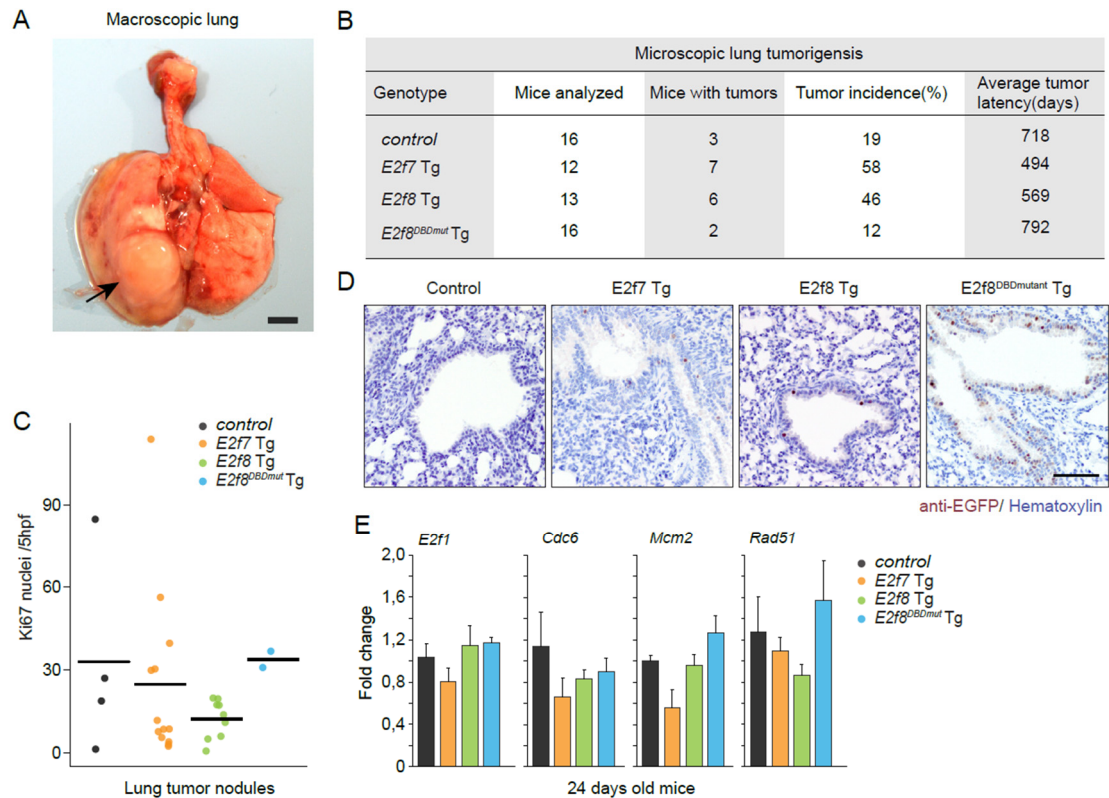


Figure S5: E2F7 or -8 overexpression promotes spontaneous lung tumorigenesis. **(A)** Representative image of a macroscopic lung lesion. Arrow indicates a lung lesion. Scale bar: 50mm. **(B)** Microscopic lung tumor incidence (%) and latency (days) of the indicated genotypes. Tumor incidence was determined on mice at the end of their life span. **(C)** Dot plot representing the counts of Ki67-positive nuclei in five fields (40x objective) of lung tumor nodules from the different genotypes. Cross black line represents average/ tumor nodule (control ($n=4$); *E2f7* Tg ($n=13$); *E2f8* Tg ($n=9$); *E2f8*^{DBDmut} Tg ($n=2$) mice. **(D)** Representative anti-EGFP immunohistochemistry pictures to detect transgene expression in lungs of 24 days old mice treated 3d with doxycycline. Scale bar: 50µm. **(E)** Transcript levels of E2F targets in lungs of juvenile mice from the indicated genotypes treated for 3 days with doxycycline. Fold changes were adjusted to average of controls and *Gapdh* was used to normalize the expression. Data represent average \pm SEM ($n=5$ mice).

Table S1: The table list the 44 differentially expressed genes that were significantly up- or down- regulated in Alb-Rb-/-7-/-8-/- versus Alb-7-/-8-/- tumors.

Gene symbol	Gene name	Log2 fold change
<i>Upregulated</i>		
<i>Mbnl3</i>	Muscle blind-Like Splicing Regulator 3	2,37
<i>Tenm3</i>	Teneurin Transmembrane Protein 3	1,83
<i>Ttc30b</i>	Tetratricopeptide Repeat Domain 30B	1,60
<i>Ndufb7</i>	NADH: Ubiquinone Oxidoreductase Subunit B7	1,10
<i>Gpr19</i>	G protein-coupled receptor 19	1,08
<i>Tfap4</i>	Transcription factor AP-4	1,03
<i>Oaz1-ps</i>	Ornithine decarboxylase antizyme 1, pseudogene	0,93
<i>Fuom</i>	Fucose mutarotase	0,90
<i>Haus4</i>	HAUS augmin-like complex, subunit 4	0,75
<i>Tsen34</i>	tRNA splicing endonuclease subunit 34	0,74
<i>Cdk4</i>	Cyclin-dependent kinase 4	0,72
<i>Wdr6</i>	WD repeat domain 6	0,71
<i>Ptges2</i>	Prostaglandin E synthase 2	0,65
<i>Dip2a</i>	Disco interacting protein 2 homolog A	0,64
<i>Cpt1a</i>	Carnitine palmitoyltransferase 1A	0,63
<i>Zfp687</i>	Zinc finger protein 687	0,60
<i>Tshz1</i>	Teashirt zinc finger family member 1	0,55
<i>Dcaf8</i>	DDB1 and CUL4 associated factor 8	0,45
<i>Downregulated</i>		
<i>Son</i>	Son DNA binding protein	-0,38
<i>Nrd1</i>	Nardilysin, N-arginine dibasic convertase, NRD convertase 1	-0,48
<i>Nckap1</i>	NCK-associated protein 1	-0,75
<i>Abca5</i>	ATP-binding cassette, subfamily A (ABC1) member 5	-0,76
<i>Klhl7</i>	Kelch-like 7	-0,85
<i>Atg4c</i>	Autophagy related 4C, cysteine peptidase	-0,86
<i>Rb1</i>	RB transcriptional corepressor 1	-1,02
<i>Akr1d1</i>	Aldo-keto reductase family 1 member D1	-1,04
<i>Sult1d1</i>	Sulfotransferase family 1D member 1	-1,09
<i>Fmn12</i>	Formin-like 2	-1,14
<i>Gdpd1</i>	Glycerophosphodiester phosphodiesterase domain containing 1	-1,31
<i>Fgl2</i>	Fibrinogen-like protein 2	-1,40
<i>Acmsd</i>	Amino carboxymuconate semialdehyde decarboxylase	-1,64
<i>Serpine1</i>	Serine (or cysteine) peptidase inhibitor, clade E, member 1	-1,80
<i>Tspan8</i>	Tetraspanin 8	-2,39
<i>Olfr1033</i>	Olfactory receptor 1033	-2,53
<i>Ocstamp</i>	Osteoclast stimulatory transmembrane protein	-2,66
<i>Itih5</i>	Inter-alpha (globulin) inhibitor H5	-2,70

<i>Prom1</i>	Prominin 1	-2,77
<i>Phlda2</i>	Pleckstrin homology like domain, family A, member 2	-2,86
<i>Them7</i>	Thioesterase superfamily member 7	-2,94
<i>Adcy1</i>	adenylate cyclase 1	-3,20
<i>Hist1h1d</i>	H1.3 linker histone, cluster member	-3,24
<i>Tmprss4</i>	Transmembrane protease, serine 4	-3,29
<i>Afp</i>	Alpha fetoprotein	-3,43
<i>Col6a6</i>	Collagen, type VI, alpha 6	-3,72

Table S2: mouse qPCR primers

	<i>Forward primer (5'-3')</i>	<i>Reverse primer (3'-5')</i>
<i>Actb</i>	AGCTCCTTCGTTGCCGGTCCA	TTTGACATGCCGGAGCCGTTG
<i>Cdc6</i>	AGTTCTGTGCCCCGAAAGTG	AGCAGCAAAGAGCAAACCAGG
<i>Cdt1</i>	ACAGCCGGGCAAGATCCCCT	GGTCCCAACTTCCGTGCCC
<i>Ccnb1</i>	AAAGGGAAGCAAAAACGCTAGG	TGTTCAAGTTCAGGTTCAAGGCTC
<i>Ccne1</i>	AGCGAGGATAGCAGTCAGCC	GGTGGTCTGATTTTCCGAGG
<i>Ccna2</i>	CTTCTTCCTTTTCCCTTGGC	TTTCAGAGTCCCAGTGACCC
<i>E2f1</i>	ACATCACCAATGTCCTGGAGGG	AGCCGCTTACCAATCCCCAC
<i>E2f7</i>	GATGCGTTCGTGAACTCCCTG	AGAAACTTCTGGCACAGCAGCC
<i>E2f8</i>	GAGAAATCCCAGCCGAGTC	CATAAATCCGCCGACGTT
<i>Gapdh</i>	GAAGGTCGGTGTGAACGG	TGAAGGGGTCGTTGATGG
<i>Mcm2</i>	TCTCTCTCAGCATCTAGCCCTG	AGACTCATCTTCAAATGGGGG
<i>Rad51</i>	CTCATGCGTCAACCACCAG	GCTTCAGGAAGACAGGGAGAG
<i>Rb1</i>	TCTCCAGGGTAACCATACTGC	CAAGGGAGGTAGATTTCAATGG