

Supplementary Figure Legends:

Fig S-1. Ablation of *E2f1-3* does not affect erythroid or lymphoid lineages in BM.

Cells isolated from femur of mice of indicated genotypes stained with lineage markers Ter119⁺, CD3⁺, B220⁺ and CD11b⁺ representing the erythroid, lymphoid and myeloid lineages respectively. (A) E2F single knockout mice. (B) E2F double knockout mice.

Fig S-2. Ablation of *E2f1-3* after BM transplant leads to reduction in myeloid population.

(A) Schematic diagram of experimental design. Bone marrow from *E2f1*^{-/-}*2*^{-/-}*3*^{ff} and *Mx-Cre;E2f1*^{-/-}*2*^{-/-}*3*^{ff} mice was transplanted into wild type mice and 5 weeks post-transplant mice were injected with pIpC. (B) FACS profile of bone marrow cells isolated from *E2f1*^{-/-}*2*^{-/-}*3*^{ff} (control) and *Mx-Cre;E2f1*^{-/-}*2*^{-/-}*3*^{ff} mice. (C) Bone marrow cellularity from the femur of the mice. Values are mean ± SD (p<0.004). (D) Total number of Cd11b⁺ and Gr-1⁺ cells isolated from BM. Values are mean ± SD (p<0.0006). (E) The percentage of apoptotic cells in BM co-stained with myeloid marker Cd11b. (F) Flow diagram depicting the hematopoietic lineages in BM. Lineages affected by the ablation of *E2f1-3* are shown in red.

Fig S-3. ChIP assay to confirm the genes involved in G₁/S and G₂/M regulation are direct targets of E2F3.

CD11b⁺ myeloid cells isolated from BM of mice were subjected to ChIP assays using E2F3 antibody. (A&B) E2F3 recruitment on promoters of G₁/S and G₂/M targets in wild type myeloid cells. (C&D) ChIP assay performed in *E2f3a*^{-/-} and *E2f3b*^{-/-} myeloid cells to show E2F3 loading on promoters of G₁/S and G₂/M genes. (E) RT-PCR to measure the expression of *E2f4-8* in *E2f1*^{-/-} (open bars) and *Mx-cre;E2f1*^{-/-}*E2f2*^{-/-}*E2f3*^{ff} (closed bars) in myeloid cells.

Figure S-4. c-Myc regulates the expression of *E2f1-3*.

(A) *c-Myc* PCR genotyping on genomic DNA isolated from the control and *cre*-infected *c-Myc*^{ff}-BMM. (B) BrdU incorporation in serum (red bars), and CSF-1 (blue bars) stimulated *c-Myc*^{ff}-CSF1R cells. *c-Myc*^{ff}-CSF1R cells infected with either the control- or *cre*- retroviruses were serum starved for 60 hours. Quiescent MEFs were then re-stimulated with media containing serum or CSF-1 and assessed for BrdU incorporation at the indicated time points as described in "Materials and Methods". (C) *c-Myc*^{ff}-CSF1R MEFs were transfected with the *E2f3a*-luciferase plasmid, along with thymidine kinase renilla luciferase construct as an internal control. Transfected cells were incubated in low-serum and then stimulated with either serum or CSF-1 and luciferase activity was measured at the indicated time points.

Figure S-5. *E2f3a* can rescue the proliferation defect in *c-Myc* deleted cells.

The *c-Myc*^{ff}-CSF-1R cell were infected with vector alone or *E2f3a*-expressing retroviruses and then with either vector alone (red) or *cre*-expressing retroviruses (blue), selected for hygromycin and puromycin resistance, and used in the following assays. (A) BrdU incorporation of *c-Myc*^{ff}-CSF-1R cell line. Cells were synchronized by serum starvation, stimulated by addition of CSF-1. Cells were incubated with BrdU for 2 hours and then fixed and stained at the indicated time points. A total of 500 DAPI-stained nuclei from each cell line were counted, and the percent positive for BrdU incorporation is shown. (B) *c-Myc* PCR genotyping of the population of cells from the experiment described above showing efficient deletion of *c-Myc*. The floxed allele (*c-Myc*^{ff}) produces a 450-bp PCR fragment, and the knockout *c-Myc*^{-/-} allele produces a 300-bp fragment. (C) Cells from the experiment described above were also tested for their long-term growth potential by colony formation assay. Values shown have been corrected for deletion of *c-Myc* by colony PCR. (D) *c-Myc* PCR genotyping on genomic DNA from the *cre*-infected *c-Myc*^{ff}-CSF-1R colonies.

Figure S-6. List of primers used for RT- PCR, ChIP assay and genotyping.

Supplementary Table- I. List of E2F targets upregulated and downregulated in *Mx-cre;E2f1^{-/-}E2f2^{-/-}E2f3^{fl/fl}* myeloid cells.

Supplementary Table- II. List of genes upregulated and downregulated in *Mx-cre; E2f1^{-/-}E2f2^{-/-}E2f3^{fl/fl}* myeloid cells (p<0.001).

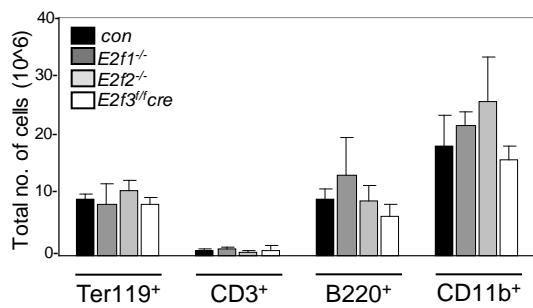
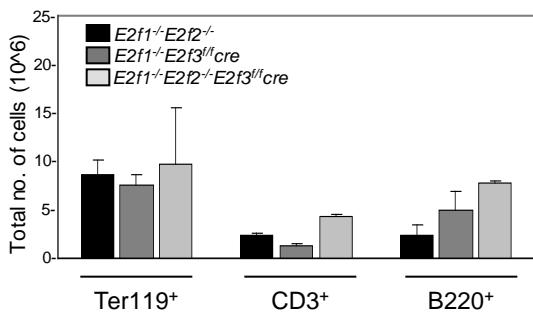
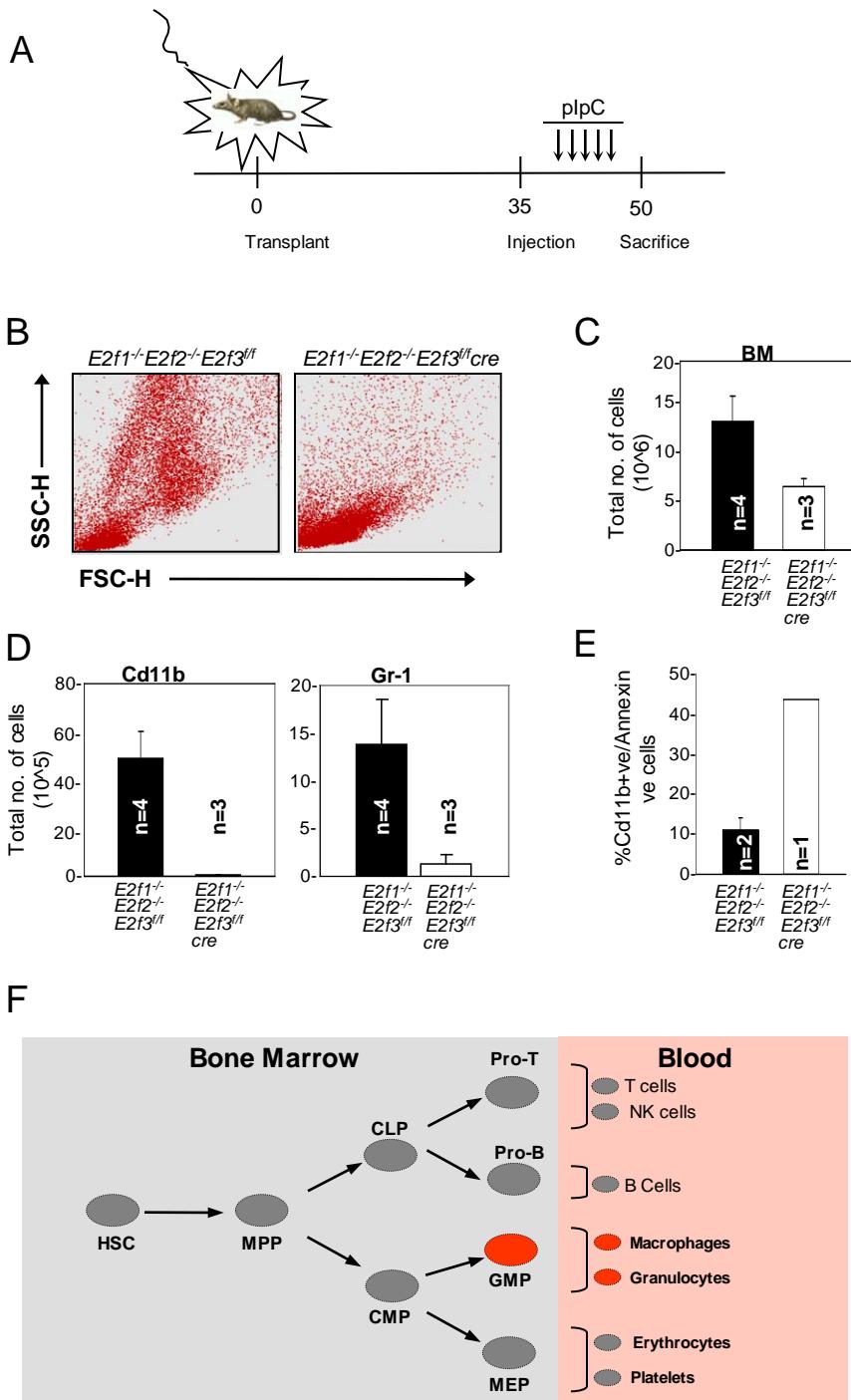
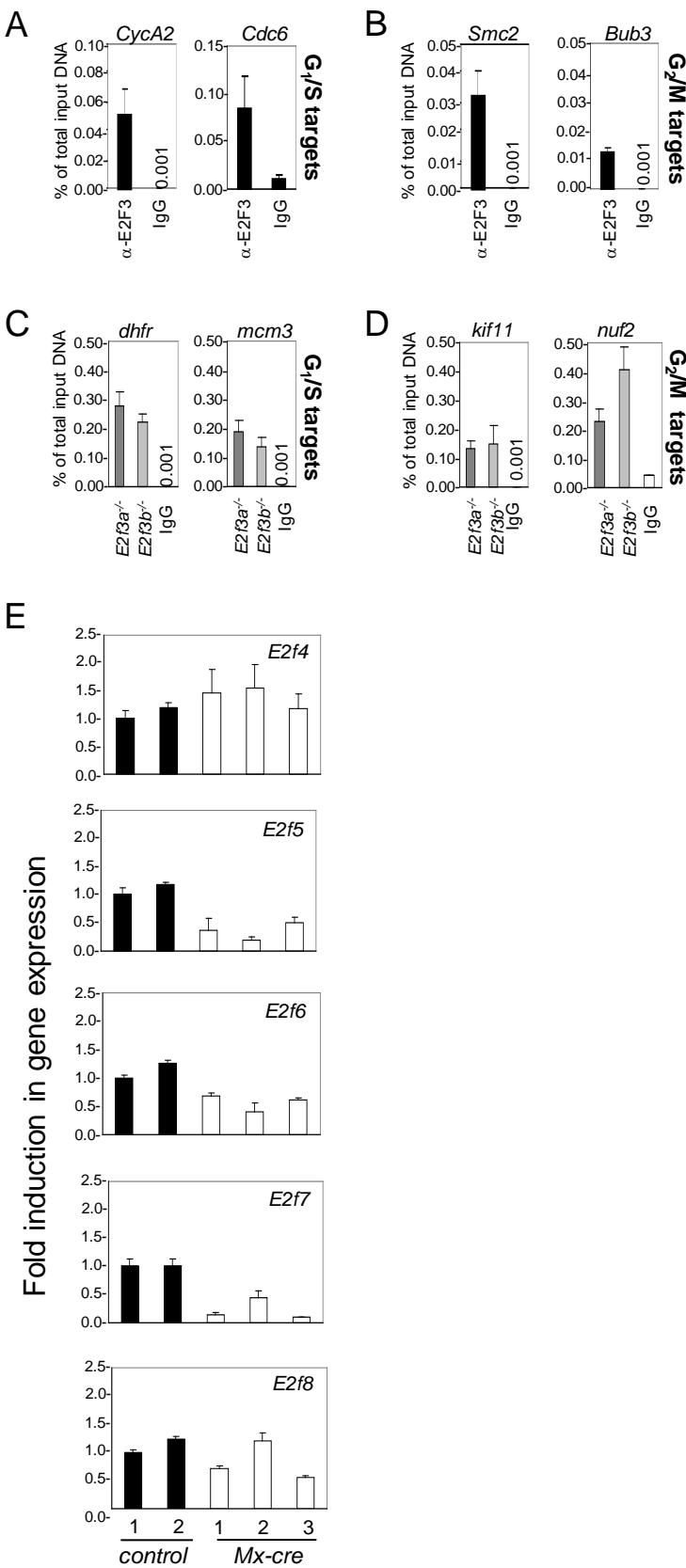
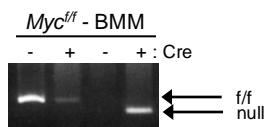
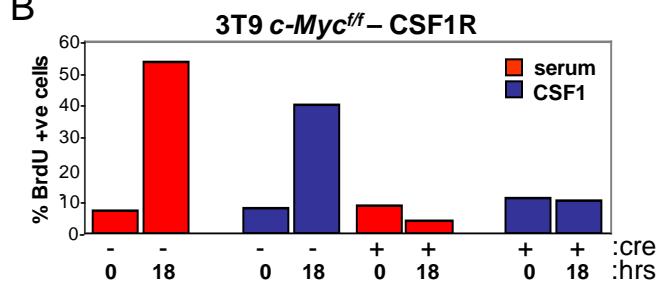
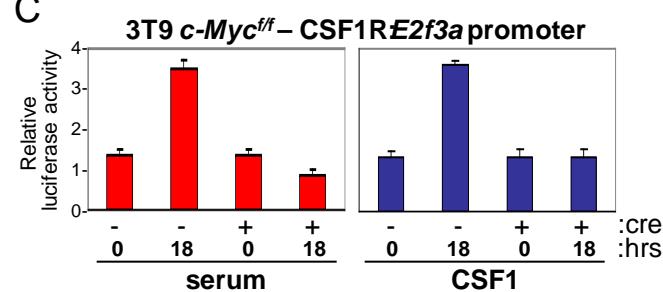
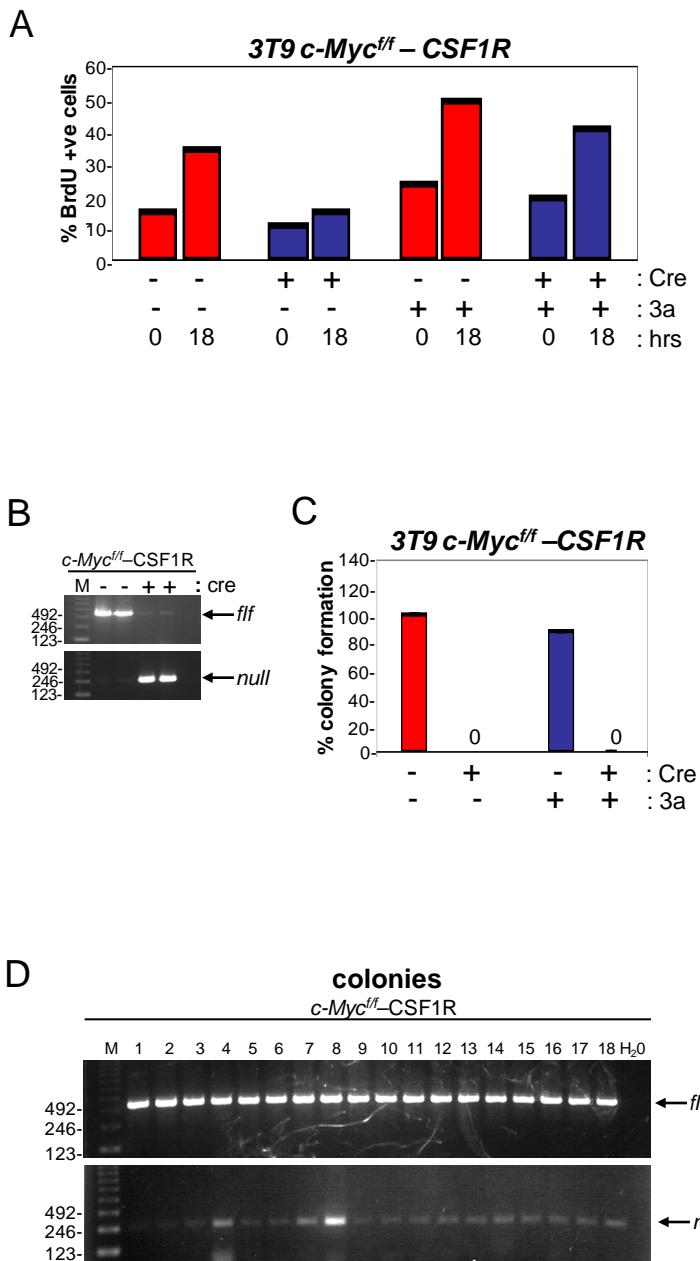
A**B**

Figure S2





A**B****C**



Gene	Sense primer(s)	Antisense primer(s)
RT-PCR		
<i>Mcm3</i>	CGCAGGAAGAATGAAAAGAGGG	CTGAGGAAGCAGGAAGTGAGAGTC
<i>Cdc6</i>	AGTTCTGTGCCGCAAAGTG	AGCAGCAAAGAGCAAACCAGG
<i>Cdt1</i>	TATGGAGGTGGTCTGTGCAA	GGCAAGGAGCTCAAAGTCAC
<i>Nuf2</i>	TTGGACGCTCGGTTTTAAC	TCGGATTGGAAGAAAATCG
<i>Nusap1</i>	GAGCAAGCTGAGACGGAATC	CTGTATCCCTGGGTCTTGA
<i>CcnF</i>	TGAAGCAGAGGTTCGAGGAT	TCTGTCCTCCTGAAGGCTGT
ChIP :		
<i>Dtl</i>	CAGCACCGCCATCTTCTC	AACTCCCGCCACTAAGAGC
<i>Mcm3</i>	ATAACCGGTCTCTGCTCCAT	CACCTCGTCATCCAGGAAGT
<i>Cdc6</i>	TGATGAGTGACAACATAATCAG	GAGCTTGCACCTTCAGG
<i>Cyc A2</i>	TGTAAGATTCCCGTCGGGCCTTC	AGGCAGGGAGGAGCGTAGAGCC
<i>Dhfr</i>	CGGCAATCCTAGGGTGAAGGCTGGT	GGCTCCATTAGCGACGAAAGGTGC
<i>Bub3</i>	AGGAGAAGGATGTCACAAGAGC	ATTTCCGCAGTCATCCTGT
<i>Kif11</i>	GGAATCGTTGCTGATTCG	TTACCTGCATCTCACCACCA
<i>Smc2</i>	TCCTAGGGCAAAAGAGGAA	GGCCAAGAGCAGCACTAC
<i>Nuf2</i>	AGGGGCTAGAAACCGTCTGT	CCGAGAAGGCTCAGAAGACC
Genotype :		
<i>E2f1</i>	AGAACGTCACGCTATGAAACCTCAC AGTGCCAGCAGGGCTGCTAAAG	AGCCACTGGATATGATTCTGGAC
<i>E2f2</i>	CCTGAGCGAGTCGGAGGATGG ACCAAAGAACGGAGCCGGTGGCG	GCCCCTAACACATGCACCCATTGG
<i>E2f3</i>	TGTGAATAATTTTGGCATGTTT	AAGGGAAGGGAAAATTAAATCTGA CTTATTCTGAGTGTGGACATACCG
<i>c-Myc</i>	CACCGCCTACATCCTGTCCATT	TACAGTCCCAAAGCCCCAGCCAAG AATTAAAGCCTGACCCCCGGCGCA
<i>Mx-Cre</i>	CTGCATTACCGGTCGATGCAAC	GCATTGCTGTCACTTGGTCGTG

Supplementary Table-1**Upregulated Genes :**

Gene symbol	Description	Function	Ref
Mcm5	minichromosome maintenance deficient 5, cell division cycle 46 (<i>S. cerevisiae</i>)	S	(1,2)
Mcm7	minichromosome maintenance deficient 7 (<i>S. cerevisiae</i>)	S	(2)
Mcm4	minichromosome maintenance deficient 4 homolog (<i>S. cerevisiae</i>)	S	(2)
Mcm6	minichromosome maintenance deficient 6 (MIS5 homolog, <i>S. pombe</i>) (<i>S. cerevisiae</i>)	S	(3)
Rpa2	replication protein A2	S	(4)
Lig1	ligase I, DNA, ATP-dependent	S	(5)
Cdc6	cell division cycle 6 homolog (<i>S. cerevisiae</i>)	S	(6)
Rfc2	replication factor C (activator 1) 2	S	(7)
Fen1	flap structure specific endonuclease 1	S	(8)
Rad51	RAD51 homolog (<i>S. cerevisiae</i>)	S	(9)
Orc11	origin recognition complex, subunit 1-like (<i>S. cerevisiae</i>)	S	(10)
Chaf1b	chromatin assembly factor 1, subunit B (p60)	S	(11)
Cdt1	chromatin licensing and DNA replication factor 1	S	(12)
Mcm3	minichromosome maintenance deficient 3 (<i>S. cerevisiae</i>)	S	(13)
Dtl	denticleless homolog (<i>Drosophila</i>)	S	(14)
Mcm2	minichromosome maintenance deficient 2 mitotin (<i>S. cerevisiae</i>)	S	(15)
Pola2	polymerase (DNA directed), alpha 2	S	(16)
Rad54l	RAD54 like (<i>S. cerevisiae</i>)	S	(17)
Gins1	GINS complex subunit 1 (Psf1 homolog)	S	(18)
Rpa1	replication protein A1	S	(19)
E2f1	E2F transcription factor 1	G ₁ /S	(20)
Cdkn1a	cyclin-dependent kinase inhibitor 1A (P21)	G ₁ /S	(21)
Ddb2	damage specific DNA binding protein 2	G ₁ /S	(22)
Chek1	checkpoint kinase 1 homolog (<i>S. pombe</i>)	G ₁ /S	(23)
Shmt1	serine hydroxymethyltransferase 1 (soluble)	G ₁ /S	(24)
Dhfr	dihydrofolate reductase	G ₁ /S	(25)
Cdca7	cell division cycle associated 7	Others	(26)
Prkcbp1	protein kinase C binding protein 1	Others	(27)
Cenph	centromere protein H	Others	(28)
Pnpo	pyridoxine 5'-phosphate oxidase	Others	(29)
Mthfd1	methylenetetrahydrofolate dehydrogenase (NADP ⁺ dependent), methenyltetrahydrofolate	Others	(30)

	cyclohydrolase, formyltetrahydrofolate synthase		
Elov13	elongation of very long chain fatty acids (FEN1/Elo2, SUR4/Elo3, yeast)-like 3	Others	(31)
Mybbp1a	MYB binding protein (P160) 1a	Others	(32)
Isg20l1	interferon stimulated exonuclease gene 20-like 1	Others	(33)
Usp37	ubiquitin specific peptidase 37	Others	(34)
Gls2	glutaminase 2 (liver, mitochondrial)	Others	(35)
Trim37	tripartite motif protein 37	Others	(36)
Ssbp4	single stranded DNA binding protein 4	Others	(37)
Dcp2	DCP2 decapping enzyme homolog (S. cerevisiae)	Others	(38)
Gtf2f2	general transcription factor IIF, polypeptide 2	Others	(39)
Ung	uracil DNA glycosylase	Others	(40)
Pold1	polymerase (DNA directed), delta 1, catalytic subunit	Others	(41)
Syce2	synaptonemal complex central element protein 2	Others	(42)
Fancg	Fanconi anemia, complementation group G	Others	(43)
Rad51l1	RAD51-like 1 (S. cerevisiae)	Others	(44)

Downregulated Genes :

Gene symbol	Description	Function	Ref
Nusap1	nucleolar and spindle associated protein 1	G ₂ /M	(45)
H2afx	H2A histone family, member X	G ₂ /M	(46)
Spc25	SPC25, NDC80 kinetochore complex component, homolog (<i>S. cerevisiae</i>)	G ₂ /M	(47)
Sgol1	shugoshin-like 1 (<i>S. pombe</i>)	G ₂ /M	(48)
Ncaph	non-SMC condensin I complex, subunit H	G ₂ /M	(49)
Ccnf	cyclin F	G ₂ /M	(50)
Aspm	Asp (abnormal spindle)-like, microcephaly associated (<i>Drosophila</i>)	G ₂ /M	(51)
Prc1	protein regulator of cytokinesis 1	G ₂ /M	(52)
Mki67	antigen identified by monoclonal antibody Ki 67	G ₂ /M	(53)
Cenpf	centromere protein F	G ₂ /M	(54)
Cenpl	centromere protein L	G ₂ /M	(55)
Fbxo5	F-box protein 5	G ₂ /M	(56)
Smc2	structural maintenance of chromosomes 2	G ₂ /M	(57)
Nuf2	NUF2, NDC80 kinetochore complex component, homolog (<i>S. cerevisiae</i>)	G ₂ /M	(58)
Kif 11	kinesin family member 11	G ₂ /M	(59)
Skp2	S-phase kinase-associated protein 2 (p45)	G ₂ /M	(60)
Kif2c	kinesin family member 2C	G ₂ /M	(61)
Ndc80	NDC80 homolog, kinetochore complex component (<i>S. cerevisiae</i>)	G ₂ /M	(62)
Cenpe	centromere protein E	G ₂ /M	(63)
Cenpc1	centromere protein C1	G ₂ /M	(64)
Pbk	PDZ binding kinase	G ₂ /M	(65)
Kif20a	kinesin family member 20A	G ₂ /M	(66)
Cks2	CDC28 protein kinase regulatory subunit 2	G ₂ /M	(67)
Bub3	budding uninhibited by benzimidazoles 3 homolog (<i>S. cerevisiae</i>)	G ₂ /M	(68)
Stag3	stromal antigen 3	G ₂ /M	(69)
Lmnb1	lamin B1	G ₂ /M	(70)
Esco2	establishment of cohesion 1 homolog 2 (<i>S. cerevisiae</i>)	G ₂ /M	(71)
Cdkn2c	cyclin-dependent kinase inhibitor 2C (p18, inhibits CDK4)	G ₂ /M	(72)
Ccng2	cyclin G2	G ₂ /M	(73)
Tyms	Thymidylate synthase	G ₁ /S	(74)
Ccne2	cyclin E2	G ₁ /S	(75)
Ccna2	cyclin A2	G ₁ /S	(76)
Tk2	thymidine kinase 2, mitochondrial	G ₁ /S	(77)
Gspt2	G1 to S phase transition 2	G ₁ /S	(78)

Sp1	trans-acting transcription factor 1	Others	(79)
Ier5	immediate early response 5	Others	(80)
E2f2	E2F transcription factor 2	Others	(81)
Phc1	polyhomeotic-like 1 (<i>Drosophila</i>)	Others	(82)
Junb	Jun-B oncogene	Others	(83)
Dbp	D site albumin promoter binding protein	Others	(84)
Syk	spleen tyrosine kinase	Others	(85)
Serbp1	Serpine1 mRNA binding protein 1	Others	(86)
Calm2	calmodulin 2	Others	(87)
Ube2t	ubiquitin-conjugating enzyme E2T (putative)	Others	(88)
Eps8	epidermal growth factor receptor pathway substrate 8	Others	(89)
Tcf19	transcription factor 19	Others	(90)
Sfrs7	splicing factor, arginine/serine-rich 7	Others	(91)
Depdc1a	DEP domain containing 1a	Others	(92)
Cklf	chemokine-like factor	Others	(93)
Brd8	bromodomain containing 8	Others	(94)
Dleu2	deleted in lymphocytic leukemia, 2	Others	(95)
H2afv	H2A histone family, member V	Others	(96)
Dhx40	DEAH (Asp-Glu-Ala-His) box polypeptide 40	Others	(97)
Dck	deoxycytidine kinase	Others	(23)
Capns2	calpain, small subunit 2	Others	(98)
Prr11	proline rich 11	Others	(99)
Top2a	topoisomerase (DNA) II alpha	Others	(100)
Letm2	leucine zipper-EF-hand containing transmembrane protein 2	Others	(101)
Dctn4	dynactin 4	Others	(102)
Mrp63	mitochondrial ribosomal protein 63	Others	(103)
Rnf167	ring finger protein 167	Others	(99)
Id3	inhibitor of DNA binding 3	Others	(104)
Mycn	v-myc myelocytomatosis viral related oncogene, neuroblastoma derived (avian)	Others	(105)
Apoe	apolipoprotein E	Others	(106)
Sft2d3	SFT2 domain containing 3	Others	(107)
Dbp	D site albumin promoter binding protein	Others	(108)
Hist2h2aa2	histone cluster 2, H2aa2	Others	(109)
Hist3h2a	histone cluster 3, H2a	Others	(110)
Hist1h1e	histone cluster 1, H1e	Others	(111)
Asf1b	ASF1 anti-silencing function 1 homolog B (<i>S. cerevisiae</i>)	Others	(112)

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