Cell proliferation is tightly controlled by inhibitors that block cell cycle progression until growth signals relieve this inhibition, allowing cells to divide. In several tissues, including the liver, cell proliferation is inhibited at mitosis by the transcriptional repressors E2F7 and E2F8, leading to formation of polyploid cells. Whether growth factors promote mitosis and cell cycle progression by relieving the E2F7/E2F8-mediated inhibition is unknown. We report here on a mechanism of cell division control in the postnatal liver, in which Wnt/β-catenin signaling maintains active hepatocyte cell division through Tbx3, a Wnt target gene. The Tbx3 protein directly represses transcription of E2F7 and E2F8, thereby promoting mitosis. This cascade of transcriptional repressors, initiated by Wnt signals, provides a paradigm for exploring how commonly active developmental signals impact cell cycle completion.

Significance
As a general model for cell cycle control, repressors keep cells quiescent until growth signals remove the inhibition. For S phase, this is exemplified by the Retinoblastoma (RB) protein and its inactivation. It was unknown whether similar mechanisms operate in the M phase. The Wnt signaling pathway is an important regulator of cell proliferation. Here, we find that Wnt induces expression of the transcription factor Tbx3, which in turn represses mitotic inhibitors E2F7 and E2F8 to permit mitotic progression. Such a cascade of transcriptional repressors may be a general mechanism for cell division control. These findings have implications for tissue homeostasis and disease, as the function for Wnt signaling in mitosis is relevant to its widespread role in stem cells and cancer.
and used Glutamine synthetase (GS), a Wnt target (26), to assess pathway activity. Using CRISPR-Cas9 gene editing, we deleted Apc, a negative regulator of Wnt signal transduction in hepatocytes, and observed ectopic expression of Tbx3 (Fig. 1A). Conversely, mice carrying mutations for the Wnt secretion machinery gene Wntless (Wls) exhibited a partial loss of Tbx3 expression (Fig. 1A). These findings agree with Tbx3 acting as a target of Wnts in other contexts (25, 27).

To examine the function of Tbx3 in hepatocytes under defined conditions, we employed a recently developed method for long-term culture and genetic manipulation of primary hepatocytes, where cells multiply rapidly in a Wnt-dependent manner (31). We knocked down Tbx3 in cultured hepatocytes using two different shRNA constructs (SI Appendix, Fig. S1B). This resulted in significant slowing down of hepatocyte proliferation (Fig. 1B) and increased numbers of polyploid cells (Fig. 1C and SI Appendix, Fig. S1C and Table S1), suggesting that hepatocytes failed to complete M phase. Conversely, overexpression of Tbx3 in hepatocytes was sufficient to increase proliferation (Fig. 1D and SI Appendix, Fig. S1D and E), even in the absence of CHIR99021, a GSK3 inhibitor that activates Wnt signaling (Fig. 1D). Additionally, overexpression of Tbx3 increased the percentage of diploid cells (Fig. 1E and SI Appendix, Table S1). As expected, CHIR99021 alone enhanced proliferation of hepatocytes albeit not to the same degree as Tbx3 overexpression (Fig. 1D).

**Deletion of Tbx3 and Changes in Ploidy.** We examined Tbx3 function in the growing postnatal liver by generating inducible Tbx3 loss-of-function mutant mice (Tbx3 knockout [KO]) carrying the Axin2-rTA; TetO-H2B-GFP; TetO-Cre; Tbx3fl/fl genotype. Axin2-rTA is a doxycycline-inducible transgene, which leaves the

**Fig. 1.** Tbx3 acts downstream of Wnt signaling and promotes proliferation of cultured primary hepatocytes. (A) Tbx3 expression is downstream of Wnt signaling. Immunofluorescence for GS and TBX3 is shown in control livers, CRISPR-Cas9–generated Apc KO cells and Wls KO (Ve-CadCreERT2; Wlsfl/fl) livers. Arrows: Apc KO cells that express Tbx3. Arrowheads: Wls KO cells that do not express Tbx3. Dashed lines delineate central veins. (B) Tbx3 knockdown (Tbx3 KD) slows down hepatocyte proliferation. Growth curves show the number of cells in control (GFP) and two different Tbx3 KD (shRNA #348092 and #334009) mouse primary hepatocyte cultures at days 0, 2, 4, and 6 after seeding. Statistical comparisons were made within control groups and Tbx3 KD groups. (C) Tbx3 knockdown leads to increased numbers of polyplid hepatocytes in culture. Representative flow cytometry plots show ploidy distribution of control and Tbx3 KD cells, stained with PI. Percentages of ploidy classes are reported as averages from three independent experiments. Statistical significance was determined by Student’s t test. Error bars represent SDs from three independent replicates. (Scale bars, 100 μm.) CV, central vein; ns, not significant.
endogenous Axin2 gene intact and is expressed in a pattern similar to Tbx3 (Fig. 2A) (17). To obtain maximal elimination of Tbx3, doxycycline was administered from postnatal week 2 to week 4, at which point tissues were harvested for analysis (Fig. 2B). Nuclear size measurement (Fig. 2C) and nuclear DNA content analysis (Fig. 2D) of GFP-labeled hepatocytes revealed a significant increase in the proportion of polypliod nuclei in Tbx3 KO livers (>2N: 59.8% in control and 92% in Tbx3 KO) (SI Appendix, Table S1). The decrease in diploidy and the concomitant increase in polypliody indicated that in the absence of Tbx3, hepatocytes were able to complete S phase but not M phase. In parallel, we used the inducible and hepatocyte-specific Albumin-CreERT2 (Alb-CreERT2) driver to eliminate Tbx3 in all hepatocytes. We administered a single dose of tamoxifen at postnatal day 3 (P3) and analyzed the livers at different timepoints into adulthood (Fig. 2E and SI Appendix, Fig. S2A and B). Loss of Tbx3 did not affect liver shape or relative mass, at any timepoint (SI Appendix, Fig. S2C and D). Consistent with the observations above, Tbx3 KO livers were comprised of larger nuclei at all analyzed timepoints compared to control (Fig. 2F and G) and DNA content analysis confirmed increased polypliody in mutant tissues (Fig. 2H and SI Appendix, Table S1). Taken together, we conclude that
TBX3 Represses Transcription of E2f7 and E2f8. To identify mechanisms of Tbx3 function, a known repressor of transcription, we investigated potential target genes of TBX3 using chromatin immunoprecipitation-sequencing (ChIP-Seq) in Tbx3-overexpressing mouse primary hepatocytes. We found that the TBX3 protein binds to promoter and enhancer regions of E2f7 and E2f8, respectively, and verified this interaction by ChIP-qPCR. (Fig. 3A and B). This suggested that TBX3 represses E2f7 and E2f8, and indeed E2f7 and E2f8 transcripts were both ectopically increased in Tbx3 KO livers (Fig. 3C and D). Knockdown and overexpression of Tbx3 in cultured primary hepatocytes further corroborated the interactions with E2f7 and E2f8 (Fig. 3E and F).

To verify that repression of E2f7 and E2f8 by TBX3 occurs on the regulatory sequences of these genes, we employed a luciferase reporter assay in human hepatoblastoma HepG2 cells, which express TBX3 at high levels (32). Addition of the mouse E2f7 or E2f8 enhancer regions containing the TBX3 binding sites to an HSV-TK reporter construct led to significant repression of the luciferase reporter activity compared to control vector with HSV-TK promoter only (Fig. 3G). Moreover, reporter activity was increased when Tbx3 was knocked down or T-box binding motifs were mutated or deleted from the enhancer regions, confirming that TBX3 is a specific transcriptional repressor of E2f7 and E2f8 (Fig. 3G and SI Appendix, Fig. 3A). In the adult human liver, TBX3 is specifically expressed in pericentral hepatocytes, similar to the mouse liver (SI Appendix, Fig. 3B). Analysis of ChIP-Seq data from the ENCODE database (GSE105374)
(33, 34) showed that human TBX3 protein also binds to E2F7 and E2F8 enhancers (SI Appendix, Fig. S3C), suggesting that TBX3 has conserved functions in both human and mouse livers. Hence, a variety of different experiments provided evidence that TBX3 directly regulates E2F7 and E2F8 expression.

Epistatic Relationships between Tbx3 and E2f7/E2f8. The negative interactions between Tbx3 and E2f7/E2f8 and the up-regulation of E2f7 and E2f8 in the absence of Tbx3 would imply that loss of all three genes would suppress the Tbx3 knock-out phenotype. To test possible epistatic interactions by mouse genetics, we generated triple conditional mutants of Tbx3, E2f7, and E2f8 (Tbx3-E2f7-E2f8 TKO). First, we used the Axin2-rtTA; TetO-GFP; TetO-Cre system to delete all three genes by continuously administering doxycycline from postnatal week 2 to week 4 (Fig. 4A). GFP-labeled nuclei in Tbx3-E2f7-E2f8 TKO livers were indistinguishable from controls in ploidy and size (Fig. 4B–D and SI Appendix, Table S1). Similar results were obtained in the pan-hepatocyte deletion model of Tbx3-E2f7-E2f8 TKO livers (SI Appendix, Fig. S4A–C and Table S1). These findings show that simultaneous removal of E2f7, E2f8, and Tbx3 resolves the polyploid phenotype caused by removal of Tbx3 alone. These genetic interactions imply a linear pathway whereby Wnt activates Tbx3, which represses the mitotic inhibitors E2f7 and E2f8, this therefore relieves cell cycle arrest and maintains hepatocytes in a diploid, dividing state.

Loss of Tbx3 Leads to Fibrosis. Despite the functions attributed here to Tbx3 in postnatal liver growth, we did not observe major changes in liver weight or morphology (SI Appendix, Fig. S2C and D). To identify physiological impact of long-term loss of Tbx3 on the liver, we ablated the gene in neonates with the Alb-CreERT2 driver and aged the animals to adulthood (Fig. 5A). Upon histological analysis, we observed cytoplasmic vacuoles and anomalies in the pericentral zone (Fig. 5B). In addition, collagen staining revealed fibrotic areas in five of eight adult Tbx3 KO livers, while none of the five control animals exhibited fibrosis (Fig. 5C).

Discussion

In contrast to the extensive insight on the role of growth factors that initiate S phase of the cell cycle, little is known about extracellular signals that regulate M phase. The Wnt/β-catenin signaling pathway is one of the mechanisms known to control G1/S transition (35–38). A role for Wnt signaling during M phase has also been suggested based on high Wnt/β-catenin signaling activity during cell division, through an unknown mechanism (39, 40). In this work we have shown that in the liver, M phase is regulated by Wnt signals through the Wnt target transcription factor Tbx3, which in turn represses the mitotic inhibitors E2f7 and E2f8. Interestingly, activating mutations in Wnt signaling components are common in liver cancer (41, 42), while E2f7 and E2f8, as well as polyploidy, are known to suppress tumorigenesis (43–46). These mitotic inhibitors are expressed in several other tissues, such as the placenta and pancreas, where they are also implicated in arresting the cell cycle (8, 47–49). Whether Wnt signaling likewise regulates mitosis by suppressing E2f7 and E2f8 in other tissues and contexts is relevant to identifying the widespread functions of Wnts in development and disease (41).

Our data indicated that Tbx3, acting in the Wnt signaling network, fine tunes the degree and onset of zonated polyploidy in the liver, by promoting mitosis and cell division. Tbx3, which has been shown to act in a dosage-sensitive manner in other contexts (50), seems to be expressed in a gradient in the pericentral zone (SI Appendix, Fig. S1A). Whether there are differences in Tbx3 function or rate of proliferation in Tbx3-high cells adjacent to the central vein and Tbx3-low cells further into the lobule is unclear. Tbx3-expressing cells may proliferate at different rates or capacities in the context of injury when additional cell cycle inducers promote tissue repair (19). Moreover, Tbx3-negative hepatocytes in the periperipheral zone seem to maintain low ploidy levels through the course of the animal’s lifetime (51). Whether mitosis is regulated by a periporal signal in these cells remains to be studied.

While we did not observe major changes in liver weight or morphology due to loss of Tbx3 or increased polyploidy, the fibrotic phenotype of Tbx3 KO mice indicates an important role for cell cycle regulation in the tissue. In the absence of a capacity for cell division, fibrosis becomes a mode of tissue repair and leads to tissue scarring (52, 53). Appearance of fibrotic areas in Tbx3 KO livers suggests that loss of Tbx3 is not compatible with long-term homeostatic tissue renewal, likely due to failure in cell division, which leads to compensatory fibrotic repair.

Regarding an absence of growth abnormalities during postnatal development, we hypothesize that the increased polyploidy itself contributes to growth of the tissue and compensates for the lack of cell division, as it has been observed with partial hepatectomy (54). In addition, in the growing and adult liver, Tbx3 is expressed primarily in the pericentral zone, and it is likely that cells from other zones are able to compensate and fill the growth gap in the absence of Tbx3.

Our explorations of the mammalian liver highlight it as a useful and unique model for cell cycle studies. Hepatocytes have distinct modes of cell cycle activity. They first undergo complete cycles and proliferate, then face roadblocks and become polyploid. The temporal regulation of these events provides distinct windows to query the extracellular cues that drive the cell cycle and to understand how these signals are linked to the intrinsic cell cycle machinery.

Materials and Methods

Animals. The Institutional Animal Care and Use Committee at Stanford University approved all animal methods and experiments. Wild-type C57BL/6J mice, Axin2-rtTA [B6.Cg-Tg(Axin2-rtTA2S*M2)7Cos/J] (55), TetO-H2B-GFP [Tg[tetO-H1St123B2/GFP]47Efu/J] (56), and TetO-Cre [B6.Cg-Tg(tetO-cre)1Jaw/J], Wlsfl/fl (129S5/SvImJ-0.1ani/J) (57) strains were obtained from The Jackson Laboratory (JAX). Tbx3f/f mice were a gift from Anne Moon (Geisinger Clinic, Danville, PA) (58). Alb-CreERT2 mice were a gift from Julien Sage (Stanford University, Stanford, CA) (59). E2f7fl/fl; E2f8fl/fl mice were gifted by Alain de Bruin (Utrecht University, Utrecht, The Netherlands) (60) and were rederived at the Stanford Transgenic Facility. Cdhs5-CreERT2 mice were used as previously described (61). For knocking out Tbx3 alone or Tbx3; E2f7; E2f8 together using the Axin2-rtTA driver, animals were given 1 mg/mL doxycycline (Sigma D9891) to drinking water from P14 until P28. In experiments involving the Alb-CreERT2 driver, neonatal P3 mice received a single intragastric injection of 0.08 mg tamoxifen (Sigma T5648), dissolved in corn oil with 10% ethanol. For knocking out Wls, Cdhs5-CreERT2; Wlsfl/fl mice 8 to 10 wk of age received intraperitoneal injections of tamoxifen on 4 consecutive days and tissues were harvested at 7 d after the last dose of tamoxifen. All mice were housed with a 12-h light/dark cycle with ad libitum access to water and normal chow.

CRISPR-Cas9-Mediated Apc Deletion. The adeno-associated virus with a single guide RNA targeting the Apc gene (AAV-sgApc) was produced from the pAAV-Guide-It-Down construct (Clontech Laboratories Inc., 041315) using assembly primers: (forward) 5′-CCGAGGCTGATGAGAGCACCTGAGAAACATGGTGCTCGATGAGAGCACTTG3 and (reverse) 5′-AACGAGCAGAGAGCAGGGTGCAGAGGCTGCATGAGAGCACTTG3. AAV-sgApc contains a U6 promoter and an sgRNA targeting the sequence 5′-AGCTGATGAGAGCACCTGAGAAACATGGTGCTCGATGAGAGCACTTG3 in exon 13 of Apc. Adult CRISPR-Cas9 knockin mice were obtained from JAX and a single intraperitoneal
injection of AAV-sgApc was administered at a dose of 1 × 10^13 genome copies/kg. Livers were collected for analysis 4 wk after induction.

Tissue Collection and Processing. Livers were collected, fixed overnight at room temperature in 10% neutral buffered formalin, dehydrated, cleared in Histoclear (Natural Diagnostics), and embedded in paraffin. Sections were cut at 5-μm thickness, deparaffinized, rehydrated, and processed for further staining via immunofluorescence or in situ hybridization assays as described below.

Immunofluorescence and Immunocytochemistry. Tissue slides were subjected to antigen retrieval with Tris buffer pH = 8.0 (Vector Labs H-3301) in a pressure cooker. They were then blocked in 5% normal donkey serum in phosphate buffered saline (PBS) containing 0.1% Triton X, in combination with the avidin/biotin blocking reagent (Vector Labs SP-2001). Sections were incubated with primary and secondary antibodies and mounted in Prolong Gold with DAPI medium (Invitrogen). Biotinylated goat antibody (Jackson Immunoresearch 705-065-147) was applied to sections stained with TBX3, before detection with Streptavidin-647. GS and β-ACTIN staining was performed with the Mouse-on-Mouse detection kit (Vector Labs) according to the manufacturer’s protocol. The following antibodies were used: GFP (chicken, 1:500; Abcam ab13970), TBX3 (goat, 1:50; Santa Cruz sc-17871), GS (mouse, 1:500; Millipore MAB302), and HNF4α (rabbit 1:50; Santa Cruz sc8987). Samples were imaged at 20× magnification using a Zeiss Imager Z.2 and processed and analyzed with ImageJ software. For immunocytochemistry,

![Fig. 4. Genetic epistasis test showing Tbx3 controls hepatocyte ploidy by repressing E2f7 and E2f8. (A) Visualization of Axin2-rtTA-driven GFP expression in control, Tbx3 KO, or Tbx3-E2f7-E2f8 TKO livers. Representative images of GFP and TBX3 immunofluorescence in the pericentral zone in control (Axin2-rtTA; TetO-H2B-GFP; Tbx3f/f; E2f7f/f; E2f8f/f), Tbx3 KO (Axin2-rtTA; TetO-H2B-GFP; TetO-Cre; Tbx3f/f), and Tbx3; E2f7; E2f8 TKO (Axin2-rtTA; TetO-H2B-GFP; TetO-Cre; Tbx3f/f; E2f7f/f; E2f8f/f) livers postnatal week 4 are shown. (B–D) Deletion of E2f7 and E2f8 along with Tbx3 restores the balance of ploidy. (B) Nuclear ploidy plots of GFP+ hepatocytes in control and Tbx3-E2f7-E2f8 TKO mice, stained with PI and measured by flow cytometry. Plots shown are chosen as representatives of each genotype and noted percentages are averages from n = 3 control and n = 4 Tbx3-E2f7-E2f8 TKO mice. (C) Bar graph summarizes nuclear ploidy distribution of GFP+ nuclei from each genotype at postnatal week 4. (D) Measurements of nuclear area from GFP+ pericentral hepatocytes in control (n = 3 mice) and Tbx3-E2f7-E2f8 TKO (n = 4 mice). Bars indicate mean and SD. P = 0.2249, t test with Welch’s correction comparison of mean nuclear area from biological replicates. (Scale bars, 100 μm). ns, not significant.](https://doi.org/10.1073/pnas.2203849119)
plated cells were fixed with 4% paraformaldehyde, blocked in 5% normal donkey serum in PBS containing 0.1% Triton-X, and stained with primary and secondary antibodies as indicated above. Cells were imaged using a Zeiss Spinning Disk Confocal Microscope.

mRNA In Situ Hybridization. In situ hybridizations were performed using the manual RNAscope 2.5 HD assay code (Advanced Cell Diagnostics) according to the manufacturer’s instructions. Images were taken at 20× magnification on a Zeiss Imager Z2 and processed using ImageJ software. Probes used in this study were E2F7 (target region: 612 to 1526) and E2F8 (Mm00618098_m1), and E2F7 (Mm99999915_g1).

RNA Isolation and qRT-PCR. Liver samples were homogenized in Tissue-Tek (Agros Technologies A0001) or bead homogenization (Qiagen) and reverse transcribed (High-Capacity cDNA Reverse Transcription Kit; Applied Biosystems) on a StepOne. Analysis and two out of five Tbx3 KO shRNA were verified to be effectively knocking down Tbx3. A total of 50,000 cells from these two plates were used for growth curve analysis. Cell counts were performed using the Cellometer (Nexcelom).

Generation of Tbx3-HA Ectopic Expression Vector. Tbx3 was amplified from the Mammalian Gene Collection (MGC) fully sequenced mouse Tbx3 cDNA (Ge Dharmacon M00113-20207797681) with forward primer: 5′-TAAGCTTCTGAGGGTCAGTATAGGCCTCTGCCTICAAGATGCC-3′ and reverse primer: 5′-GAACATCGTAGTGTCATTCTGGACCGCCCTGCTACACG-3′ and inserted into pK3H (Addgene 12255) to add HA tag at C-terminal of Tbx3 by Nucleofector HiFi DNA Assembly Cloning Kit (E95205). Then Tbx3-HA was amplified with forward primer: 5′-TGAGAGGAGACCTGCCATATGAGGCTTCCTAGAGAG-3′ and reverse primer: TGAATGCTGGGTATCAAATCTGTTAACCAGCGACTG-3′ and inserted into p2SwNeo-\_EFl-eGFP_P2A_EcoRI (modified from p2SwNeo-EFl-eGFP-P2A_EcoRI) from Eric Rullfson (Stanford University, Stanford, CA).

Applying Sleeping Beauty Transposon System in Primary Hepatocytes. Primary hepatocytes were co-cultured with expansion media till 60% confluence. The Sleeping Beauty System was applied as described in the plasmid DNA transfection protocol from TransIT-X2 Dynamic Delivery System (Mirus Biotech) with modifications. A total of 2.25 μg of p2SwNeo-\_EFl-eGFP_P2A or p2SwNeo-\_EFl-eGFP-P2A-Tbx3-HA along with the transposase in a ratio of 10:1 were mixed with TransIT-X2 and incubated for 48 h. Then cells were put on G418 selection with expansion media for 48 h. Cells expressing GFP only or GFP together with Tbx3 were expanded for growth curve or flow cytometry analysis.

Hepatocyte Nuclei Isolation and Ploidy Analysis. Hepatocyte nuclei preparation was developed by modifying the chromatin preparation protocols described previously (63, 64). Liver lobes were fixed for 10 min at room temperature. Samples were incubated for 5 min with glycine at a final concentration 0.125 M and centrifuged at 300 x g for 10 min, at 4°C. Pellets were washed in PBS and resuspended with 10 μl cell lysis buffer (10 mM Tris-HCl, 10 mM NaCl, 0.5% IGEPA) and filtered through 100μm cell strainers. After incubation at 4°C for 30 min with ploidy analysis. Nuclei were resuspended in 5 μl of PBS and approximately 1 million nuclei were stained with 0.5 μl of FACSCycle PI/RNAse (Thermo Fisher, FITC, 107977) staining solution for 30 min to 30 min at room temperature. Primary hepatocytes were fixed and stained the same way. Both nuclei and cells were analyzed on FACS ARIA II (BD). Data were processed with FACS Diva 8.0 software (BD) and FlowJo v10 (FlowJo). Doublets were excluded by forward-scatter width (FSC-W)/forward-scatter height (FSC-H) and
side-scatter width (SSC-W) / side-scatter height (SSC-H) analysis. Single-stained cells were used for compensation and fluorochrome minus one control was used for gating.

**ChIP-qPCR and Sequencing.** Three to five million Tbx3 overexpressing primary hepatocytes were used for each ChIP reaction as recommended (65). Chromatin was prepared with a truChIP Chromatin Shearing Reagent Kit (Covaris 520154) and shared with Covaris S220 according to the kit manual. A total of 4 μg anti-α-HA antibody (Roche Sigma 11867423001) was used for IgG control. ChIP washing steps, input, and ChIP DNA preparation were modified as previously described (64) and sent for sequencing through the NextSeq 500 system (Illumina). Sequencing data were mapped as previously described with modifications (65). Raw reads were called using MASC2 as previously described (68-70) with modifications from subcommands. Purified input, IgG, and ChIP DNA following the chromatin immunoprecipitation were also used in ChIP-qPCR and calculated as described previously (63). Forward: 5′-AACGTCATCCCATCAGTGAA-3′ and reverse: 5′-CTGGTTCACCCACCTTGCA-3′ primers detecting Gapdh promoter area were used as internal controls. E2f7 forward: 5′-CAGGAGGAGGGATGTTAG-3′ and reverse: 5′-CCGAGCGTGTTTAGTAT3′ or E2f8 forward: 5′-GAAACCTTGGGCACCTGGA-3′ and reverse: 5′-CAAGGGAATGCACTTGCC-3′ were used to detect the TBX3-bound E2f7 or E2f8 promoters, respectively.

**Luciferase Assay for Promoter Function.** The HSV-TK promoter was cloned upstream of luciferase in the pGL4.10[luc2] plasmid (Promega). A 122-bp region promoter area were used as inter- interfere. E2f7 or upstream of luciferase in the pGL4.10[luc2] plasmid (Promega). A 122-bp region promoter area were used as inter- interfere. E2f7 or upstream of luciferase in the pGL4.10[luc2] plasmid (Promega). A 122-bp region promoter area were used as inter- interfere. E2f7 or upstream of luciferase in the pGL4.10[luc2] plasmid (Promega). A 122-bp region promoter area were used as inter- interfere. E2f7 or upstream of luciferase in the pGL4.10[luc2] plasmid (Promega). A 122-bp region promoter area were used as inter- interfere. E2f7 or upstream of luciferase in the pGL4.10[luc2] plasmid (Promega). A 122-bp region promoter area were used as inter- interfere. E2f7 or upstream of luciferase in the pGL4.10[luc2] plasmid (Promega). A 122-bp region promoter area were used as inter- interfere. E2f7 or upstream of luciferase in the pGL4.10[luc2] plasmid (Promega). A 122-bp region promoter area were used as inter-
