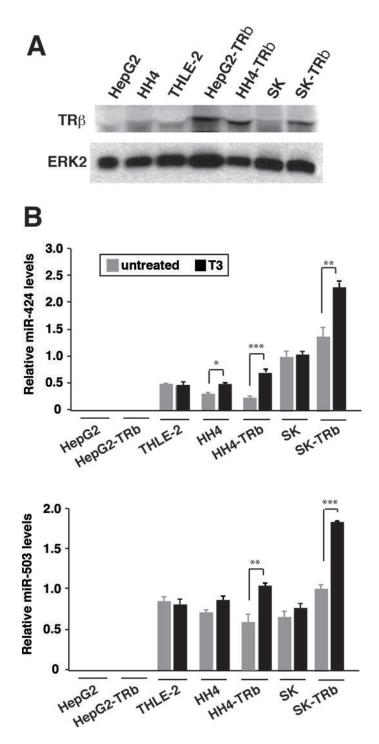


Supplementary Figure S1: (A) The levels of TRb were determined by western blot in parental SK-hep1 hepatocarcinoma cells (SK) and in cells stably transfected with TRb (SK-TRb). ERK2 was used as a loading control. (B) miR-424 and miR-503 levels were measured after 36 h of incubation in the presence and absence of 5 nM T3 in SK and SK-TRb cells. Results are expressed as fold induction with respect to the untreated parental cells. Statistically significant differences between untreated and T3-treated cells are indicated with asterisks.



Supplementary Figure S2: (A) The levels of TRb were determined by western blot in the parental cell lines and in cells stably transfected with TR β ERK2 was used as a loading control. (B) miR-424 and miR-503 levels were determined after 36 h of incubation in the presence and absence of 5 nM T3 in the cells indicated. Results are expressed as the percentage to the values obtained in untreated SK-TRb cells. Statistically significant differences between untreated and T3-treated cells are shown with asterisks.

Cdc25 3'-UTR

UGUGGUACUGGGGCUGCUA 660/679

IIIIII CGACGA

seed sequence

GAAGUUACACAGAAAUGCUGCUG 352/370

CGACGA

c-Myb 3'-UTR

AAAACGUUUUUUGCUGCUA 643/661

IIIIIII CGACGA

UUAGCCUGUAGACAUGCUGCUA 667/688

IIIIIII CGACGA

Chk1 3'-UTR

UGGUGAAUAUAGUGCUGCUA 26/45

IIIIIII CGACGA

Wee1 3'-UTR

CCACUGUGGUGGUGCUGCUA 251/272

CGACGA

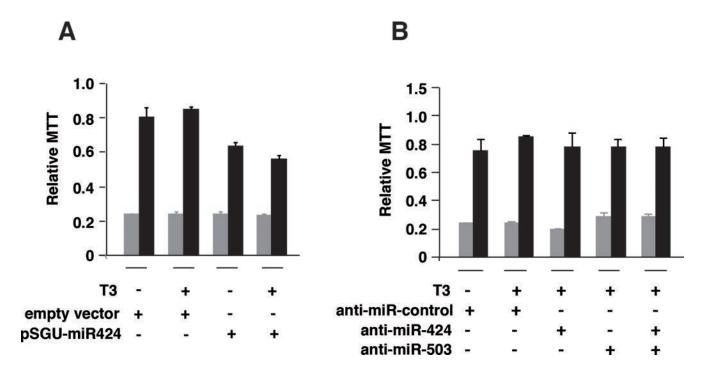
AUUGCCUUGUGAAUUUGCUGCUA 529/551

CGACGA

UAUGUGAAAUGUAUAGCUGCUU 628/649

CGACGA

Supplementary Figure S3: Sequences of the 3'-UTRs of Cdc25, c-Myb, Chk1 and Wee1 mRNAs containing putative binding sites for miR-424 and miR-503. The position of the nucleotides is shown at the right and the seed sequence of the miRNA family annealing with the 3'-UTRs is shown in red.



Supplementary Figure S4: (A) HepG2-TRb cells were transfected with the expression vector for miR424 or with an empty vector. After 24h were detached from the plates and grown in suspension under rocking conditions during an additional 24 h period in the presence and absence of T3. Cells were then plated and MTT was assayed. Results were expressed relative to MTT values obtained in cells inoculated in parallel without agitation. (B) HepG2-TRb cells were transfected with the indicated antimiRNAs and after 24 h grown in suspension with and without T3 as indicated.

PRIMERS AND ANTIBODIES

PRIMERS

Chk1 3'-UTR (+28953/+29193)

Forward: 5'-CCA GAA GAT TTG GCT TCC TG-3' Reverse: 5'-CCT TTC CCC AAA GTT TTG CT-3'

Wee1 3'-UTR (+13948/+14644)

Forward: 5'-TGA CAA GAG GAA GCT AGG TTG A-3' Reverse: 5'-GAA AGG GTG CAT TTT TCC AA-3'

APO

Forward: 5'-AAG TGC TGT AGG CCA TTT AGA TTA G-3' Reverse: 5'-AGT CTT CGA TGA CAG CGT ATA CAG A-3'

miR424/503 promoter

+59/-382

Forward: 5'-GGC TTC CTT CAG TCA TCC AG-3' Reverse: 5'-GGA AAC AAT ACC CCA GAG CA-3'

-34/-923

Forward: 5'-AGA GGG CAT GCA TGT GAC TT-3' Reverse: 5'-GCG GGT GAA TAA AGA CTG GA-3'

-910/-1793

Forward: 5'-GAC AAG ACG CCC CTT TGT AA-3' Reverse: 5'-ATG CAT GCC CTC TTT TTG TT-3'

-1773/-2574

Forward: 5'-AAC CTT TGC AAA AAG CTT GC-3' Reverse: 5'-TTA CAA AGG GGC GTC TTG TC-3'

miR424/503 promoter (ChIP assays)

-244/-84

Forward: 5'- TCA CCC ACT ACG TTG TTC CA Reverse: 5'- GGC AAG GAA TAG GGC AAA AC

-330/-181

Forward: 5'- TTG CCT CTA CTT AAC CTT ATT CGT C

Reverse: 5'- AGC CGA AAG TGT GGC TCT C

-3723/-3911

Forward: 5'-GAA AGA ACG GTA CAA CGA ACG Reverse: 5'- TTG AAG ATG CAT GCT GAA GTG

ANTIBODIES

| Antibody | Dilution | Use | Provider |
|----------|----------|-----|---------------------|
| CDK-6 | 1:500 | WB | Sigma-Aldrich C8343 |
| WEE1 | 1:1000 | WB | Santa Cruz sc-9037 |
| CDC25A | 1:500 | WB | Santa Cruz sc-7389 |

| Antibody | Dilution | Use | Provider |
|----------|----------|------|-------------------------|
| CyclinD2 | 1:500 | WB | Santa Cruz sc-56305 |
| MYB | 1:500 | WB | Pierce MA5-12339 |
| CHK1 | 1:1000 | WB | Novus Biologicals 19210 |
| E2F3 | 1:1000 | WB | Santa Cruz sc-878 |
| ERK2 | 1:1000 | WB | Santa Cruz sc-154 |
| TRβ | 5 μg | ChIP | Santa Cruz sc-737 |
| NCoR | 5 μg | ChIP | Santa Cruz sc-8994 |
| CBP | 5 μg | ChIP | Santa Cruz sc-369 |
| p/CIP | 5 μg | ChIP | Santa Cruz sc-9119 |
| SRC-1 | 5 μg | ChIP | Santa Cruz sc-6096X |
| Ac-H4 | 5 μg | ChIP | Millipore 06-866 |