## **Supplemental Methods**

#### Animals and reagents

The *Apc<sup>lox/lox</sup>* mice, which received intra-peritoneally one injection of 1.5 mg tamoxifen (MP Biomedicals), constitute the so-called "pretumoral" model[1]. This dose results in a deletion of *Apc* in all hepatocytes. A unique injection of 0,75mg results in the deletion of *Apc* in 10 to 50% hepatocytes, which was sufficient to obtain few isolated hepatocytes activated for  $\beta$ -catenin 90 days after tamoxifen injection. Tumors were obtained from these isolated hepatocytes after 6 to 8 months[2]. The tumor development was followed twice monthly by 2D-ultrasound (Vevo 770). *Apc<sup>lox/lox/</sup>*HNF-4a<sup>lox/lox/</sup>TTR-Cre<sup>Tam</sup> mice were generated using *hnf-4a* floxed mice created by the Gonzalez laboratory[3].The mice received a unique injection of 1,5mg tamoxifen, and diet for five days (TD135859, 400 mg/kg, Harlan). Mice with Apc<sup>KO</sup> tumors received intraperitoneal injections of 10 mg/kg LNA-scramble or LNA against miR-34a (LNA-34a, Exiqon) in saline. The LNA injections were realized once a week since tumors were detectable and lasted until 1 to 2 months according to tumor size and mouse care.

#### **Transfection assays**

We plated 8 x  $10^5$  isolated hepatocytes per well, in six-well plates. The cells were incubated for 4 h and then transfected, in the presence of Lipofectamine 2000 (Life Technologies), with 100 nmol LNA-scramble or LNA-34a, a control mimic or miR-34a mimic and siRNA directed against  $\beta$ -catenin (Qiagen). For FOP experiments, cells were transfected with 3 µg of a luciferase reporter plasmid under the control of a HNF-4 $\alpha$  response element (SuperFOPflash M51, reference 12457, Addgene), and 1 µg of a *Renilla* luciferase expression plasmid (Promega). FOP plasmid is a mutant

form of the TOP plasmid containing 8 Wnt responsive elements. In fact, the mutations create a HNF-4 $\alpha$  binding motif (9).

We assessed miR-34a activity by transfection with 1  $\mu$ g of a luciferase reporter plasmid under the control of a miR-34a-binding site (pmiR-34a-LUC, Signosis). Luciferase activity was measured 24 h after transfection, with the DUAL-LUC kit (Promega), after lysis of the cells by incubation in passive cell lysis buffer for 20 minutes.

For caspase-2L detection in western-blot, the cells had to be transfected with 100nmol LNA-34a once a day during three days successively.

#### xCELLigence assay

Hepatocyte adhesion and cell cycle progression were measured with the xCELLigence system (Roche). This system monitors the viability of cultured cells in real time, by measuring electrical impedance in microtiter plates with interdigitated gold microelectrodes. For this experiment, we plated 8 x  $10^5$  isolated hepatocytes per well in 96-well plates. Cells were transfected with a LNA or a mimic 4 h after plating, as described above. The impedance was then measured for 48 h.

### Measurement of lactate deshydrogenase activity

Isolated hepatocytes were transfected with the LNA-Scramble or the LNA-34a. Cell supernatants were collected 48h after and lactate dehydrogenase activity was measured on 0,2 mL supernatant during 3 min following the addition of 50  $\mu$ L NADH 2,4mM in a spectrophotometer at 340nm.

## Western-Blotting

We lysed the hepatocytes, 48 h after their transfection with LNA, by incubation in RIPA buffer (Sigma) plus protease inhibitors (Roche) for 30 minutes on ice. The cells were then centrifuged at 13000 x g for 10 minutes, and the supernatants were boiled for 10 minutes in Laemmli sample buffer (Sigma). We then ran the extracts obtained (equivalent of 50 µg of protein per lane) on a 10% polyacrylamide gel. The resulting protein bands were electrotransferred onto a nitrocellulose membrane. The membrane was then blocked by incubation with 5% blocking agent (Biorad) in TBS/Tween 0.1% for 1 h at room temperature, probed overnight with a primary antibody against the protein of interest, and then incubated with a HRP-conjugated secondary antibody (Cell Signaling) for detection with the ECL system (Biorad). Primary antibodies against the following proteins were used: HNF-4 $\alpha$  (1/200, Santa-Cruz, SC-6556), cyclin D1 (1/500, Thermo Scientific, RM9104), LEF-1 (1/100, Cell Signaling, 2230). *β*-actin was used as a reference (Sigma, A5441), anti-cleaved caspase 3 (1/50, Cell Signaling, 9661), anti-cleaved caspase 2 (1/100, ABCAM, AB2251), anti-caspase 2 (1/100, Santa-Cruz, SC-623), anti-PARP-1 (1/500, BD pharmingen).

## Immunostaining

Paraffin-embedded liver sections were treated as previously described[2]. The following antibodies were used: anti-HNF-4 $\alpha$  (1/250, Santa-Cruz, SC-8987), anti-cyclin D1 (1/100, Epitomics, 1476-1), anti-cleaved caspase 3 (1/600, Cell Signaling, 9661), anti-cleaved caspase 2 (1/60, ABCAM, AB2251), anti-glutamine synthetase (1/400, BD Transduction Laboratories, 61517), and Ki-67 (1/300, Leica ncl-Ki67p).

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### Samples processing for miRNA-seq:

Total RNA was extracted from purified hepatocytes with Trizol® reagent (Life Technologies). RNA integrity was assessed with a RNA nano kit and an Agilent 2100 bioanalyzer. The efficiency of  $\beta$ -catenin activation or inactivation was determined by RT-qPCR through the measurement of glutamine synthase (Mm00725701, Life Technologies) and axin2 (Mm00443610, Life Technologies) mRNAs as compared to 18S mRNA (4308329, Life Technologies).

For each set of conditions, we analyzed four distinct hepatocyte preparations by deep sequencing on a Genome Analyzer II Illumina platform (Fasteris. Switzerland). Small RNAs were selected by electrophoresis in an acrylamide gel. Illumina adapters were then ligated to the 3' and 5' ends of the RNAs before reverse transcription and PCR amplification. Sequencing was performed by multiplexing in the same channel with a 1x50 bp run. The reads generated by the HiSeq were then analyzed with miRDeep2 and miRAnalyzer software.

#### Chromatin sonication and immunoprecipitation

The ChIP protocol was adapted from that of Nelson[4], as previously described[1].  $\beta$ -catenin and TCF-4 binding to the miR-34a promoter, relative to that of the immunoglobulin isotype control, was assessed by SYBR green with the following oligonucleotides: F: CTG-AGT-AGC-GTG-TTG-CGT-GT, R: AGC-GAG-AGC-TCA-GAG-TTT-GC (Eurogentec). HNF-4 $\alpha$  binding to the cyclin D1 promoter was measured as follows: for peak 1: (F) CAC-CGC-GGG-AAA-GAC-CAG, (R)AAG-CCA-ATG-TAA-AGT-TAA-AAT-TGC-AA, for peak 4: (F) GCTTTCATCATTGGGTGGCA; (R) TGGCAAGACTCCTGTGATGT

## **RNA extraction and RT-qPCR**

Total RNA was extracted from purified hepatocytes with Trizol® reagent (Life Technologies). Levels of miR-34a and miR-127 level were determined, for 2 ng total mRNA, with a specific Taqman miRNA assay provided by Applied Biosystems (assays 000426 and 000452. respectively), and were normalized with respect to the endogenous snoRNA snoRNA135 (001230) for mouse and RNU49 (001005) and RNU24 (001001). HNF-4 $\alpha$  (assay Mm01247715) and cyclin D1 (assay Mm00432359)levels were assessed with 100 ng total mRNA, in an assay provided by Applied Biosystems, with normalization against ribosomal RNA. We used the following primers to determine p53 mRNA levels:p53 (F) ccgggtggaaggaaatttgt and p53 (R) aggataggtcggcggttcat.

## **Supplemental Results**



#### Figure S1: The discriminating miRNAs interfere with the $\beta$ -catenin pathway.

The KEGG pathway was obtained from Dianalab software (http://diana.cslab.ece.ntua.gr/). The proteins in yellow are targets of miRNAs; green squares were not targeted.

# Discarding the miRNAs from the imprinted Dlk1/Gtl2 locus, as relevant targets of $\beta$ -catenin in human HCCs.

We investigated the levels of miR-127, one of the miRNAs generated from this locus, in tumor samples from patients with HCC. Unfortunately, miR-127, overexpressed in Apc<sup>KO</sup> hepatocytes, as revealed by the deep-sequencing of miRNAs (Table S1), was not significantly induced in human HCCs with or without activating mutations of *CTNNB1* (Figure S2 below and Table S3). This suggests that a control of  $\beta$ -catenin on miR-127 and on the Dlk1/Gtl2 locus occurs only in mouse hepatocytes.



## Figure S2: miR-127 expression is not altered in HCC samples

We determined miR-127 levels, relative to RNU24 and RNU49 levels, for 24 samples of HCCs with  $\beta$ -catenin mutations (mut- $\beta$ -cat) and 20 samples of non-mutated (non-mut- $\beta$ -cat) HCCs, and compared them with those in normal tissues. ns : non-significant.



**Figure S3:** TCF-4 and  $\beta$ -catenin binding to a proximal site upstream from the miR-34a promoter in wt (CTRL) and Apc<sup>KO</sup>hepatocytes, as compared with the isotype control (IgG). Results are expressed as the binding value ± SEM normalized with respect to three control sites in the *myc* and *Alb* genes. The means of at least three independent experiments are shown.



Figure S4: Inverse correlation between miR-34a levels and HNF-4 $\alpha$  expression in HCCs with  $\beta$ -catenin mutations.



**Figure S4: A : miR-34a levels in mouse tumours are independent on p53 mRNA level.** Results are shown as the mean RQ values for p53 relative to ribosomal RNA (n=42). n.s : non significant.





**Figure S6:** A : Apc<sup>KO</sup> hepatocytes have a doubling time halfthat of wt hepatocytes. Cell proliferation was measured for wt and Apc<sup>KO</sup>(Apc-/-) hepatocytes, via microelectrodes under the plate, over a period of 48h. Results are expressed as impedance normalized against the time to complete hepatocyte adhesion (vertical line).**B** : thelactate dehydrogenase activity is measured in the cell supernatant of wt or APC<sup>KO</sup> cells transfected with 100nmol LNA-scramble or 34a for 48h. The results are represented as the mean of three experiments +/-SEM. **C** : PARP protein level in wt or APC<sup>KO</sup> cells transfected with 100nmol LNA-scramble or 34a for 48h.**D** : cleaved caspase 2 level in liver 7 days after injection of LNA-scramble (LNA-Scr) or LNA-34a to wt or Apc<sup>KO</sup> mice.



**Figure S7 : Hnf4 bindings within human and murine Cyclin D1 genomic environment.** Cyclin D1 has been described as a Hnf4 negative target but there was no evidence for a direct binding of Hnf4. We extracted ChIP-Seq data: in vivo Hnf4 bindings (trplicates) in the murine liver [5], and Hnf4 bindings in HepG2 human liver cancer cells (ENCODE data). We focused on four putative Hnf4 binding sites: one is located at -1750 base pairs from the transcription start site (TSS) and had been found experimentally in Apcko chromatin. Its DNA sequence is conserved in mouse and human genomes. The second one surrounds the TSS: it was found in HepG2 cells but not in Apcko chromatin. The third one is in one intron, but is specific for the mouse gene. The fourth one is in the 3' region of CCND1: its sequence and its binding to Hnf4 is conserved between mouse liver and human HepG2. Due to their relevance to human HCCs, we studied the effect of LNA-miR34a on the first and the fourth binding sites.



## Figure S8: Ultrasound follow-up

Images of tumours treated with LNA-scramble or LNA-34a and followed by ultrasound for 15 days (D0 to D15). The lines in blue indicate the size of the tumours.



**Figure S9:** A : Cyclin D1 and HNF-4 $\alpha$ expression in mouse tumors with $\beta$ -catenin activation treated with LNA-scramble or LNA-34a on parallel sections.**B** : Ki-67 is not altered in the tumors treated with LNA-34a.

| miRNA             | Mean read numbers ApcKO | Mean read numbers wt | ApcKO/wt   | Pvalue ApcKO/wt    |
|-------------------|-------------------------|----------------------|------------|--------------------|
| mmu-miR-136       | 22956                   | 218                  | 105.4      | 3.7E-72            |
| mmu-miR-127       | 14388                   | 244                  | 58.8       | 1.2E-53            |
| mmu-miR-134       | 3161.3                  | 87.4                 | 36.2       | 2.1E-34            |
| mmu-miR-379       | 9005                    | 386                  | 23.4       | 7.7E-34            |
| mmu-miR-341       | 497.1                   | 4.6                  | 107.3      | 5.0E-33            |
| mmu-miR-434-3p    | 2094.9                  | 50                   | 41.9       | 5.2E-33            |
| mmu-miR-541       | 9861                    | 483                  | 20.4       | 2.8E-32            |
| mmu-miR-434-5p    | 1678.3                  | 41.6                 | 40.3       | 2.1E-31            |
| mmu-miR-381       | 860                     | 20.7                 | 41.6       | 2.1E-28            |
| mmu-miR-409-3p    | 1368.4                  | 42.4                 | 32.3       | 2.8E-28            |
| mmu-miR-665       | 271.5                   | 2.6                  | 105.9      | 1.0E-27            |
| mmu-miR-369-3p    | 1185                    | 40                   | 29.6       | 8.3E-27            |
| mmu-miR-431       | 257.7                   | 4.6                  | 56.6       | 9.5E-24            |
| mmu-miR-376b      | 345.0                   | 9.4                  | 36.6       | 8.1E-23            |
| mmu-miR-299       | 669.6                   | 26.5                 | 25.3       | 1.2E-22            |
| mmu-miR-300       | 708.6                   | 29.2                 | 24.2       | 3.2E-22            |
| mmu-miR-411       | 1490.8                  | 79.0                 | 18.9       | 3.4E-22            |
| mmu-miR-494       | 601                     | 28                   | 21.7       | 2.2E-20            |
| mmu-miR-540-3p    | 276.6                   | 8.8                  | 31.6       | 3.2E-20            |
| mmu-miR-370       | 135.6                   | 2.7                  | 51.1       | 1.8E-19            |
| mmu-miR-376a      | 184.7                   | 5.0                  | 36.8       | 5.5E-19            |
| mmu-miR-433       | 96                      | 1.1                  | 83.7       | 7.4E-19            |
| mmu-miR-376c      | 304                     | 13                   | 24.0       | 8.0E-19            |
| mmu-miR-485       | 181.5                   | 6.4                  | 28.5       | 1.2E-17            |
| mmu-miR-543       | 66.0                    | 1.8                  | 37.3       | 3.4E-14            |
| mmu-miR-667       | 184.1                   | 10.8                 | 17.1       | 4.1E-14            |
| mmu-miR-409-5p    | //.1                    | 2.8                  | 27.8       | 1.2E-13            |
| mmu-miR-337-5p    | 157.5                   | 9.3                  | 16.9       | 3.9E-13            |
| mmu-miR-495       | 52.3                    | 1.5                  | 34.7       | 7.0E-13            |
| mmu-miR-382       | 189.3                   | 13.7                 | 13.8       | 7.3E-13            |
| mmu-miR-487b      | 37.7                    | 0.9                  | 43.6       | 5.3E-12            |
| mmu-miR-337-3p    | 62.6                    | 2.9                  | 21.7       | 1.6E-11            |
| mmu-miR-673-5p    | 67.4                    | 3.5                  | 19.4       | 2.6E-11            |
| mmu-miR-1193-3p   | 93.8                    | 6.9                  | 13.5       | 6.2E-11            |
| mmu-miR-410       | 19.6                    | 0                    |            | 2.4E-10            |
| mmu-miR-496       | 22.3                    | 0.5                  | 41.9       | 4.8E-09            |
| mmu-miR-412-5p    | 29.6                    | 1.8                  | 16.0       | 1.3E-08            |
| mmu-miR-770-3p    | 31.1                    | 1./                  | 18.3       | 1.6E-08            |
| mmu-miR-329       | 16.7                    | 0.3                  | 59.4       | 2.3E-08            |
| mmu-miR-369-5p    | 27.5                    | 1.8                  | 15         | 3.2E-08            |
| mmu-miR-377       | 16                      | 0.3                  | 60.6       | 5.8E-08            |
| mmu-miR-154       | 26.8                    | 2.5                  | 10.6       | 3.1E-07            |
| mmu-miR-673-3p    | 12.7                    | 0.8                  | 15.6       | 8.0E-06            |
| mmu-miR-380-5p    | 13.1                    | 1.1                  | 11.6       | 2.0E-05            |
| mmu-miR-666-3p    | 13.3                    | 1.1                  | 12.5       | 2.7E-05            |
| mmu-miR-380-3p    | 5.5                     | 0                    |            | 5.2E-05            |
| mmu-mik-34a       | 4927                    | 1935                 | 2.5        | 1.1E-04            |
| mmu-miR-323-3p    | 1.6                     | 0.6                  | 13.4       | 3.6E-04            |
| mmu-mik-758       | b.1                     | 0.3                  | 24.1       | 5.1E-04            |
| mmu miP 540 5n    | 2 4                     | 0.2                  | 3<br>17    | 3.0E-04            |
| mmu miP 670       | 3.4                     | 0.2                  | 17         | 1.22-03            |
| minu-miR-670      | 3.1                     | 0                    | 17.0       | 1.7E-03            |
| mmu miP 1046h     | 4.0                     | 0.3                  | 12.0       | 1.0E-U3            |
| mmu-miR-1946b     | 3.1                     | 0.3                  | 12.2       | 4.4E-03            |
| mmu miP 1107      | 22                      | 1.3                  | 3.0        | 0.9E-03            |
| mmu miP 1102 5r   | 2                       | 0                    |            | 9.4E-03            |
| mmu miD 1100      | 22                      | 0                    |            | 9.5E-03            |
| mmu miP 10270     | 3.3                     | U<br>67 F            | 25         | 9.9E-03            |
| mmu miD 2072      | 00.9                    | C.10                 | 2.0        | 1.1E-02<br>2.1E-02 |
| mmu-miD 2127      | 1650                    | U<br>800             | 20         | 2.1E-02<br>2.1E-02 |
| mmu-miP 120 5n    | 30.2                    | 11 0                 | 2.0        | 2.1E-02<br>2.3E-02 |
| mmu-miD 660       | 27                      | 0.6                  | 2.0        | 2.5E-02            |
|                   | 2.7                     | 1100                 | 10.0       | 2.5E-02            |
| mmu-miD 192       | 12/0                    | 653                  | 1.0        | 3 1E 02            |
| mmu-miP-197       | 1243<br><u>/</u> 52     | 1/0                  | 1.0        | 3.12-02            |
| mmu_miD_2/72      | 10.3                    | 5                    | 1.8<br>0.0 | 4 0E-02            |
| 11111u-1111K-3473 | 10.3                    | 3                    | 2.2        | 4.02-02            |

## **Table S1: microRNAs upregulated in the Apc<sup>KO</sup> model, in miRNA-seq analysis** The miRNAs more strongly expressed in the Apc<sup>KO</sup> model than in wt mice were identified as follows: p<0.05, at least 1.5-fold induction. The miRNAs in red are those produced from the Dlk1/Gtl2 locus.

| miRNA           | Mean read numbers wt | Mean read numbers wt | ApcKO/wt | Pvalue ApcKO/wt |
|-----------------|----------------------|----------------------|----------|-----------------|
| mmu-miR-200b    | 131                  | 1814                 | 0.1      | 1.9E-12         |
| mmu-miR-200a    | 126                  | 1395                 | 0.1      | 5.5E-11         |
| mmu-miR-429     | 15.2                 | 164.0                | 0.1      | 4.8E-09         |
| mmu-miR-1       | 6.3                  | 56.5                 | 0.1      | 4.7E-07         |
| mmu-miR-224     | 2.1                  | 30.0                 | 0.1      | 6.7E-07         |
| mmu-miR-1948    | 224                  | 884                  | 0.3      | 1.3E-06         |
| mmu-miR-451     | 17                   | 135                  | 0.1      | 1.7E-06         |
| mmu-miR-141     | 17.4                 | 92.7                 | 0.2      | 1.3E-05         |
| mmu-miR-143     | 16240                | 44516                | 0.4      | 1.8E-05         |
| mmu-miR-150     | 22.4                 | 127.9                | 0.2      | 9.5E-05         |
| mmu-miR-10b     | 25.1                 | 121.6                | 0.2      | 1.7E-04         |
| mmu-miR-206     | 0.2                  | 4.9                  | 0.0      | 1.8E-04         |
| mmu-miR-200c    | 40                   | 167                  | 0.2      | 1.8E-04         |
| mmu-miR-126-3p  | 27886                | 60454                | 0.5      | 2.5E-04         |
| mmu-miR-195     | 2588                 | 6898                 | 0.4      | 3.0E-04         |
| mmu-miR-144     | 2.7                  | 14.1                 | 0.2      | 3.2E-04         |
| mmu-miR-146a    | 7238                 | 18190                | 0.4      | 7.7E-04         |
| mmu-miR-10a     | 8872                 | 19395                | 0.5      | 1.0E-03         |
| mmu-miR-135b    | 0                    | 2.1                  |          | 1.0E-03         |
| mmu-miR-142-5p  | 227                  | 751                  | 0.3      | 1.0E-03         |
| mmu-miR-708     | 0                    | 2.2                  |          | 1.1E-03         |
| mmu-miR-181c    | 52.4                 | 171.2                | 0.3      | 1.4E-03         |
| mmu-miR-223     | 2358                 | 6136                 | 0.4      | 1.5E-03         |
| mmu-miR-34c     | 7.1                  | 25.6                 | 0.3      | 2.2E-03         |
| mmu-miR-497     | 1093                 | 2619                 | 0.4      | 2.9E-03         |
| mmu-miR-335-5p  | 314                  | 807                  | 0.4      | 3.7E-03         |
| mmu-miR-125b-1- |                      |                      |          |                 |
| Зр              | 2.2                  | 9.4                  | 0.2      | 5.4E-03         |
| mmu-miR-181d    | 28.6                 | 80.2                 | 0.4      | 6.1E-03         |
| mmu-miR-135a    | 12.1                 | 37.5                 | 0.3      | 7.3E-03         |
| mmu-miR-126-5p  | 9036                 | 17243                | 0.5      | 7.7E-03         |
| mmu-miR-3/5     | 11.7                 | 28.1                 | 0.4      | 7.7E-03         |
| mmu-miR-199a-5p | 180                  | 444                  | 0.4      | 7.9E-03         |
| mmu-miR-145     | 708                  | 1634                 | 0.4      | 8.6E-03         |
| mmu miD 977     | 0.0                  | 7.9                  | 0.3      | 9.2E-03         |
| mmu miB 700     | 2.1                  | 40.0                 | 0.4      | 1.1E-02         |
| mmu miD 214     | 3.1                  | 5.7                  | 0.3      | 1.1E-02         |
| mmu miP 142 2n  | 20.9                 | 07.3<br>10115        | 0.4      | 1.2E-02         |
| mmu miP 222     | 1059                 | 007                  | 0.5      | 1.2E-02         |
| mmu-miR-335-3n  | 21.5                 | 34.6                 | 0.3      | 1.3E-02         |
| mmu-miR-542-3p  | 172                  | 228                  | 0.4      | 1.3E-02         |
| mmu-miR-23a     | 430                  | 618                  | 0.4      | 1.6E 02         |
| mmu-miR-205     | 60                   | 34                   | 0.4      | 1.4E 02         |
| mmu-miR-130b    | 5.0                  | 93                   | 0.0      | 1.0E 02         |
| mmu-miR-1895    | 25.5                 | 30.3                 | 0.5      | 2.2E-02         |
| mmu-miR-450b-3p | 2.3                  | 3.7                  | 0.3      | 2.4E-02         |
| mmu-miR-547     | 8.2                  | 19.4                 | 0.4      | 2.5E-02         |
| mmu-miR-342-3p  | 1690                 | 2490                 | 0.5      | 2.7E-02         |
| mmu-miR-450b-5p | 10.6                 | 9.2                  | 0.4      | 2.8F-02         |
| mmu-miR-467e    | 1.1                  | 2.8                  | 0.2      | 3.5E-02         |
| mmu-miR-714     | 0.2                  | 0.7                  | 0.1      | 3.9E-02         |
| mmu-miR-511-3p  | 228                  | 257                  | 0.5      | 4.1E-02         |
| mmu-miR-100     | 2287                 | 2463                 | 0.6      | 4.4E-02         |
| mmu-miR-700     | 16.2                 | 18.3                 | 0.4      | 4.5E-02         |
| mmu-miR-3082-5p | 1.3                  | 1.7                  | 0.4      | 4.7E-02         |

# Table S2: microRNAs downregulated in the Apc<sup>KO</sup> model, in miRNA-seq

analysis The miRNAs less strongly expressed in the Apc<sup>KO</sup> model than in wt mice were identified as follows: p<0.05, at least 1.5-fold inhibition.

| samples  | etiology | qPCR GS | IHC GS | bcat mutation | IHC p53 | sex | Age |
|----------|----------|---------|--------|---------------|---------|-----|-----|
| K103T    | HBV      | 1       | 0      | wt            | n.d     | М   | 65  |
| G172     | ОН       | 0.43    | 0      | wt            | 0       | М   | 77  |
| G6855bis | HBV      | 1.6     | 0      | wt            | 1       | М   | 43  |
| G8341    | HBV      | 3.5     | 1      | wt            | 2       | М   | 25  |
| G29372   | HCV      | 1.1     | 0      | wt            | 0/1     | F   | 35  |
| F46220   | HCV      | 1       | 0      | wt            | 1       | F   | 55  |
| F58680   | HCV      | 1.2     | 0      | wt            | n.d     | М   | 57  |
| F59770   | HCV      | 1.3     | 0      | wt            | n.d     | М   | 54  |
| F79918T  | CBP      | 2       | 0      | wt            | 0       | F   | n.d |
| F85548   | HCV      | 0.7     | 0      | wt            | 1       | М   | 55  |
| F86725   | HBV      | 2.4     | 1      | wt            | 0       | М   | 58  |
| F92656T  | OH       | 2.2     | 0      | wt            | 0       | М   | 55  |
| F93869T  | ОН       | 2.2     | 1      | wt            | n.d     | М   | 68  |
| F96609   | HBV      | 6       | 0      | wt            | 0       | F   | 42  |
| F96869t1 | HCV      | 1.1     | 0      | wt            | 0       | М   | 55  |
| G2911    | HCV      | 2.5     | 1      | wt            | 0       | М   | 47  |
| G5595    | OH       | 1.2     | 1      | wt            | 1       | М   | 71  |
| G33171   | OH       | 2.5     | 1      | wt            | 0       | М   | 72  |
| F99980   | 0        | 3       | 0      | wt            | n.d     | М   | 57  |
| G31473   | NASH     | 5       | 1      | wt            | 0       | М   | 61  |
| K22T     | 0        | 59      | 2      | S33F          | n.d     | М   | 67  |
| K30T     | 0        | 29      | 2      | delG34-H36    | n.d     | М   | 24  |
| K90T     | 0        | 49      | 2      | delD32-H36    | n.d     | М   | 44  |
| K107T    | OH       | 45      | 2      | D32G          | n.d     | М   | 60  |
| K111T    | HBV HCV  | 18      | 1      | delP44-G50    | n.d     | М   | 67  |
| F50472   | HVC      | 29      | 2      | T41A          | 0       | F   | 78  |
| F50582   | OH       | 70      | 2      | D32G          | 0       | М   | 53  |
| F59541   | OH       | 61      | 2      | 135S          | 0       | М   | 61  |
| F64236   | HBV      | 41      | 2      | T41A          | 0       | М   | 53  |
| F92010   | HCV      | 25      | n.d    | S45F          | n.d     | М   | 48  |
| F93123T  | HVC      | 35      | 2      | D32Y          | 0       | F   | 51  |
| 226692T  | HCV      | 52      | 2      | T41A          | n.d     | М   | 73  |
| K51T     | HBV      | 56      | 2      | delF1-R2      | n.d     | М   | 75  |
| K108T    | HCV      | 46      | 2      | T41A          | n.d     | М   | 65  |
| K21T     | 0        | 21      | 2      | S33F          | n.d     | М   | 60  |
| K92T     | OH       | 35      | 2      | T41A          | n.d     | М   | 73  |
| F49311   | HBV      | 26      | 2      | D32G          | 0       | М   | 79  |
| F81378   | 0        | 9       | 2      | T41I          | 0       | М   | 54  |
| 232556   | OH       | 141     | 1      | S33A+V22G     | 0       | М   | 75  |
| K23T     | HEM      | 39      | 2      | S33C          | n.d     | М   | 67  |
| K97T     | HCV      | 64      | 2      | delA39-S47    | n.d     | М   | 60  |
| K101T    | HCV      | 23      | 2      | D32Y          | n.d     | М   | 71  |
| K102T    | HCV      | 22      | 2      | S37A          | n.d     | М   | 68  |
| K105T    | HCV      | 45      | 2      | S33C          | n.d     | М   | 64  |

## Table S3: Etiological characteristics of HCC samples

The mutational activation status of the *CTNNB1* gene (samples in yellow) was assessed both by characterization of the mutation and by the induction of glutamine synthetase (GS), by both RT-qPCR analysis (RQ value) and the immunostaining of paraffin sections (score 0-2), as previously described [6]. OH: alcohol, HBV: hepatitis B virus, HCV: hepatitis C virus, NASH: non-alcoholic steatohepatitis, PBC: primary biliary cirrhosis, 0: no particular etiology, HEM: hepatic hemangioma, n.d: not determined.

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