Supplemental Material to:

Mir-33 regulates cell proliferation, cell cycle progression and liver regeneration in mice

Daniel Cirera-Salinas, Montse Pauta, Ryan M. Allen, Alessandro G. Salerno, Cristina M. Ramírez, Aranzazu Chamorro-Jorganes, Amarylis C. Wanschel, Miguel A. Lasunción, Manuel Morales-Ruiz, Yajaira Suárez, Ángel Baldán, Enric Esplugues and Carlos Fernández-Hernando.

<u>Supplemental Figure legends</u>

Supplemental Figure 1. CDK6 and CCDN1 miR-33 binding site conservation between species. Mmu=mouse; Hsa=human; Ptr=chimpanzee; Rno=rat; Cfa=cat).

Supplemental Figure 2. HeLa (A and C) and MCF-7 (B and D) cells stably transduced with empty or miR-33 over-expression lentiviral vectors were serum starved for forty-eight hours in DMEM supplemented with 0.2% BSA. In two independent experiments, growth following the return serum was assayed at 24, 48 and 72h qualitatively by crystal violet staining (A and B) and quantitatively by MTT assay (Cayman Chemical Inc; C and D). Data are represented as mean +/- standard deviation. ** $P < 0.01$.

Supplemental Figure 3. HEK-293 cells stably transduced with empty or miR-33 over-expression lentiviral vectors were serum starved for forty-eight hours in DMEM supplemented with 0.2% BSA. mRNA levels of CDK6, CDK4, CCND1, CCND2, and CCND3 were determined at the end of the 48h starvation, as well as at eight and twenty-four hours following the return of serum (DMEM supplemented with 10%FBS). Data are represented as mean +/- standard deviation. ** $P < 0.05$.

Supplemental Figure 4. A model of the miR-33a/b in regulating cell proliferation and cell cycle progression. miR-33 induces a G1 arrest through negative effects on cell cycle related proteins including CDK6 and CCND1. Molecules highlighted in red show predicted targets for miR-33a/b.

Cdk6 3'UTR (10.208 kb)

Site 1 (175-181)

```
Hsa  5' –AAAUCAAUGCAAGAG…
Ptr  5' –AAAUCAAUGCAAGAG…
Mmu  5' –AGAUCAAUGCAAGGG…
Rno  5' –AGAUCAAUGCAAGAG…
Ocu  5' –AAAUCAAUGCAAGAG…
```

Site 2 (4525-4531)

```
Hsa  5' AUCAAUGCAAUU…
Ptr  5' AUCAAUGCAAUU…
Mmu  5' CUCAGUCCAGUU…
Rno  5' UUCAGUCCAGUU…
Ocu  5'---------AGUU…
```

Site 3 (7111-7117)

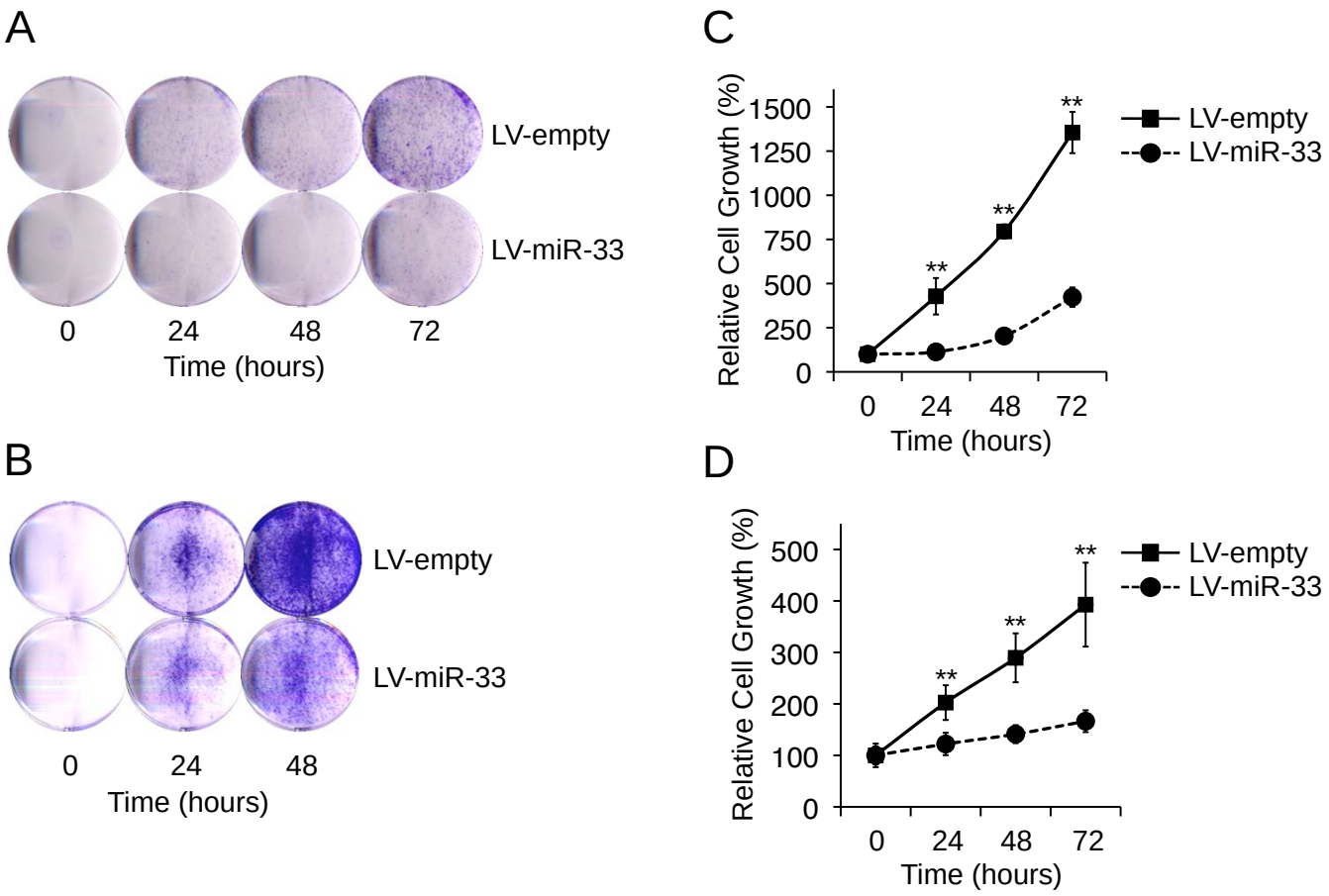
```
Hsa  5' AUGAAAUGCAAUAAU…
Ptr  5' AUGAAAUGCAAUAAU…
Mmu  5' AUGAAAUGCAAUAAC…
Rno  5' AUGGAAUGCAAUAAC…
Ocu  5' ACAAAAUGCAAUAAC…
```

Ccnd1 3'UTR (3.191 kb)

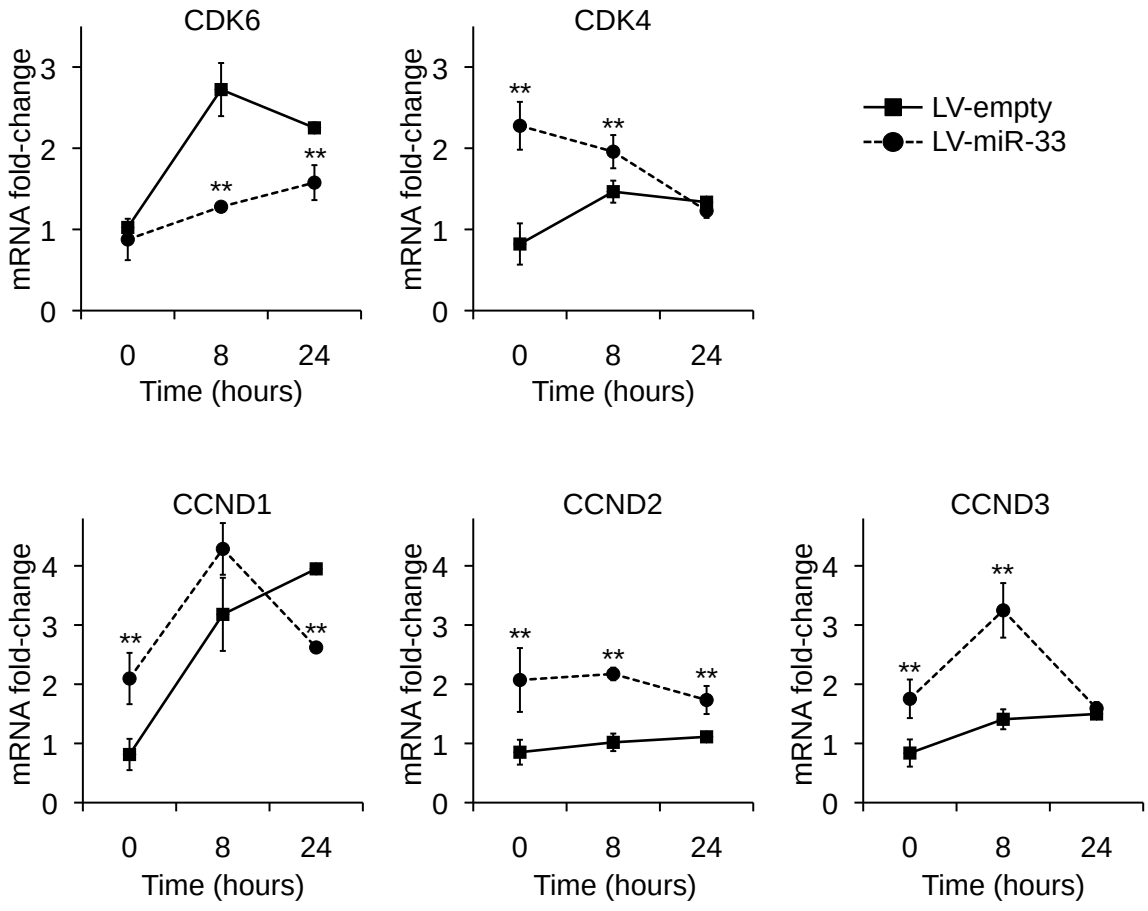
Site 1 (1465-1471)

```
Hsa  5' UAUAAAUGCAAUCUC…
Ptr  5' UAUAAAUGCAAUCUC…
Mmu  5' AAGCAAUGUGAUCUC…
Rno  5' AAGCAAUGUGAUCUU…
Ocu  5' GAGCGAUGCCGUCCC
```

Supplemental Figure 1



Supplemental Figure 2



Supplemental Figure 3

Supplemental Figure 4