

### **Supplementary material and methods**

Total DNA was isolated from liver samples by mechanical procedures using a buffer containing 10mM Tris-HCl, 20mM EDTA, 2%SDS and 0.1 µg/µl proteinase K. DNA was purified using phenol-chloroform. U1 snRNA copy number was quantified by real-time PCR (BioRad) with primers designed to detect exogenous U1 snRNA (U1Fw: GATCTCATAGTTCCATGGCAGGGGAGATAACCAT and U1Rev: CGAGTTTGGCACATTTGGCC) and normalized to mouse β-actin (mAct Fw GGGATGTTTGCTCCAACCAA and mAct Rv GCGCTTTTGACTCAAGGATTTAA). PreS1 mRNA expression was quantified from pA<sup>+</sup> liver RNA by real-time PCR (BioRad) with primers S1Fw: TCTACAGCATGGGGCAGAAT and S1Rev: TGCTCCAGCTCCTACCTTGT. Oligo hybridization was performed at 60 °C.

**Supplementary Figure 1. Quantification of luciferase expression from pCH-Fluc in mice.** pCH-Fluc plasmid was introduced in C57BL/6 mice by hydrodynamic injection and luciferase activity was quantified in living mice with a CCD camera at the indicated times post-injection. Data are mean ± SD from a minimum of 2 independent experiments with n=5.

**Supplementary Figure 2. Analysis of the inhibition of HBV with different doses of shRNAs or U1in in mice.** C57BL/6 mice were injected with pCH-Fluc plasmid (HBVLuc) combined or not with decreasing amounts of plasmids expressing sh1 (**a**), sh2 (**b**), UA (**c**) or the combination of UA and sh1 (**d**) or UA and sh2 (**e**). Luciferase activity was measured in living mice with a CCD camera at the indicated times post-injection (**a to e**) or in liver extracts obtained 8 days post-injection (**f**). Luciferase

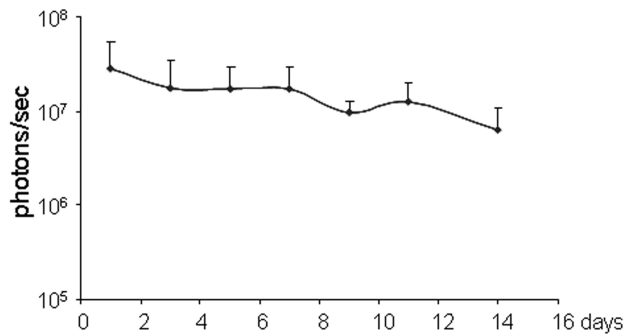
activity measured in vivo was quantified and plotted (**a to e**). RLU indicates relative light units (**f**). Data are mean  $\pm$  SD from at least 5 mice.

**Supplementary Figure 3. Quantification of PreS1 Luc mRNA in vivo.** PreS1 Luc mRNA was quantified by RT-PCR from pA+ RNA isolated from liver extracts obtained as described in Fig. 5. The relative number of copies of PreS1 Luc mRNA obtained from 67 ng of pA+ mRNA compared to a standard plasmid is shown. Data are mean  $\pm$  SD from at least 2 independent experiments.

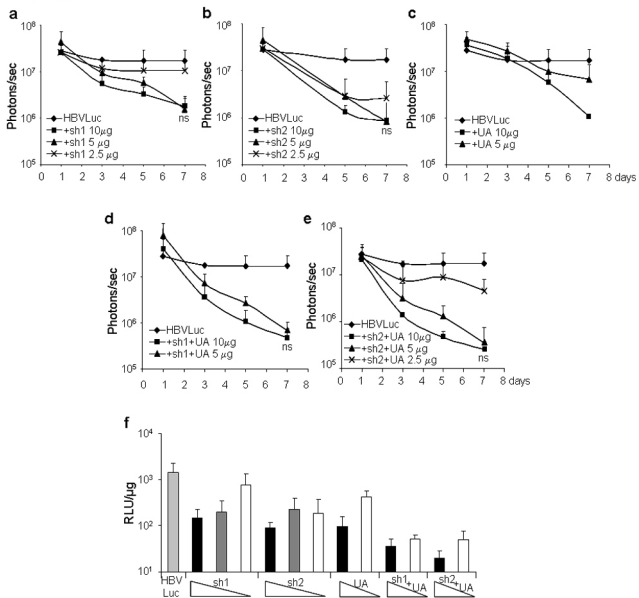
**Supplementary Figure 4. Quantification of U1in and mutated U1in in vivo.** Exogenous U1in expression was quantified by RT-PCR from liver extracts obtained as described in Fig. 6 (**b**). Actin mRNA was also quantified to allow comparison among different samples. Data are mean  $\pm$  SD from at least 2 independent experiments.

**Supplementary Figure 5. Quantification of U1in DNA in vivo.** Plasmid expressing U1in was quantified by PCR from liver extracts obtained as described in Fig. 4 and 5. Actin DNA was also quantified to allow comparison among different samples. Data are mean  $\pm$  SD from 2 independent experiments.

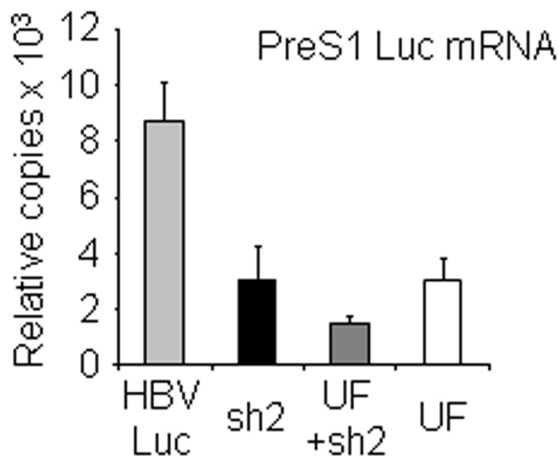
**Sup Fig. 1**



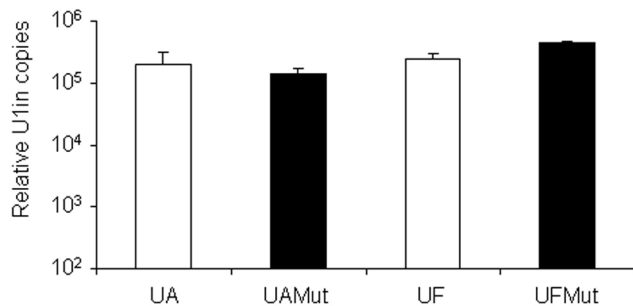
Sup Fig. 2



### Sup Fig. 3



## Sup Fig. 4



**Sup Fig. 5**

