Supplemental Material
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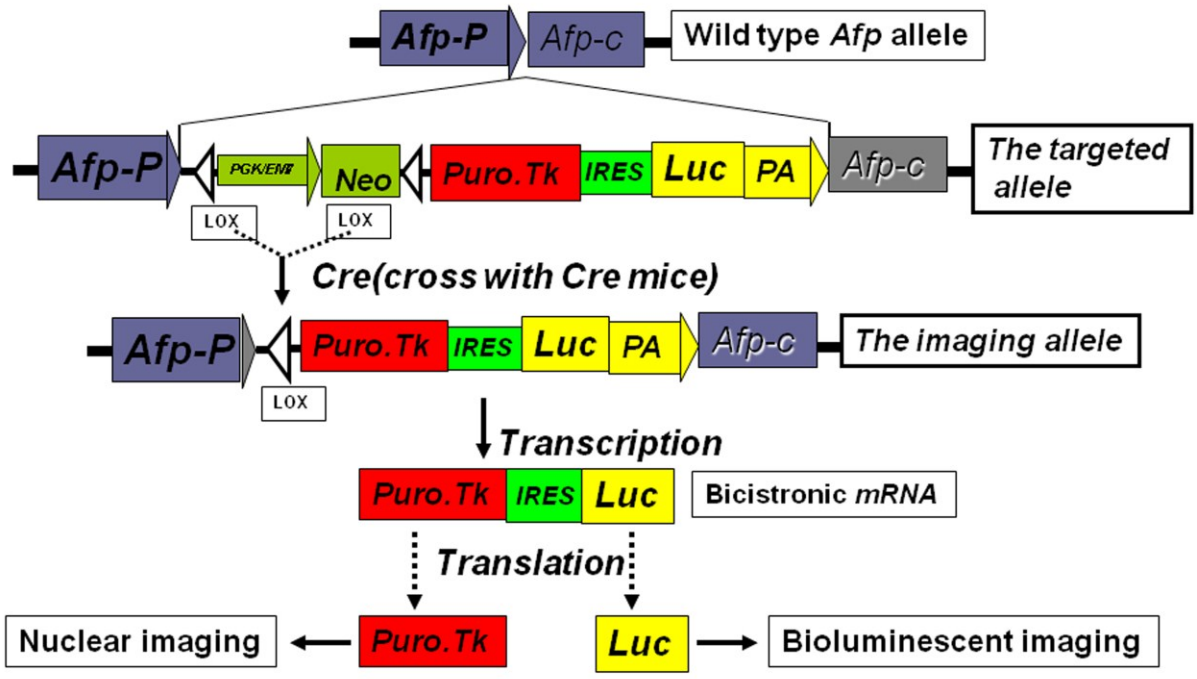

Figure S1. Gene targeting strategy for a mouse model with dual-reporter system for imaging HCC. In the DNA cassette, the coding sequence of *tk* was linked with that of luc via an intra-ribosomal entry sequence (IRES). Thus, when expressed in mouse cells, it is expected to generate a bi-cistronic mRNA that could produce both thymidine kinase and luciferase, two enzymes that are commonly used for facilitating PET and BLI imaging, respectively. The cassette was introduced into Afp locus of the mouse genome in mouse embryonic stem cell by standard knock-in gene targeted approach so that its transcription is under the control of an endogenous Afp promotor and to create a new mouse strain. AFP(+/-) knock-in don't affect the size and weight of the targeted mouse.

Figure S2. Mice were generated on C57 BL/6j-129 background originally and backcrossed to C3H and FVB/N mice for 5 generations by standard genetic crosses, which were then bred with each other to generate cohorts of Afp-*tk*-IRES-luc positive or negative mice that were used in subsequent studies. Lower-left is the BLI on one-day-old mouse litters. After crossing, about half of the litters were positive for the reporter allele. Injection of the imaging substrate D-luciferin proved to be difficult. The litters were imaged without any anesthesia.

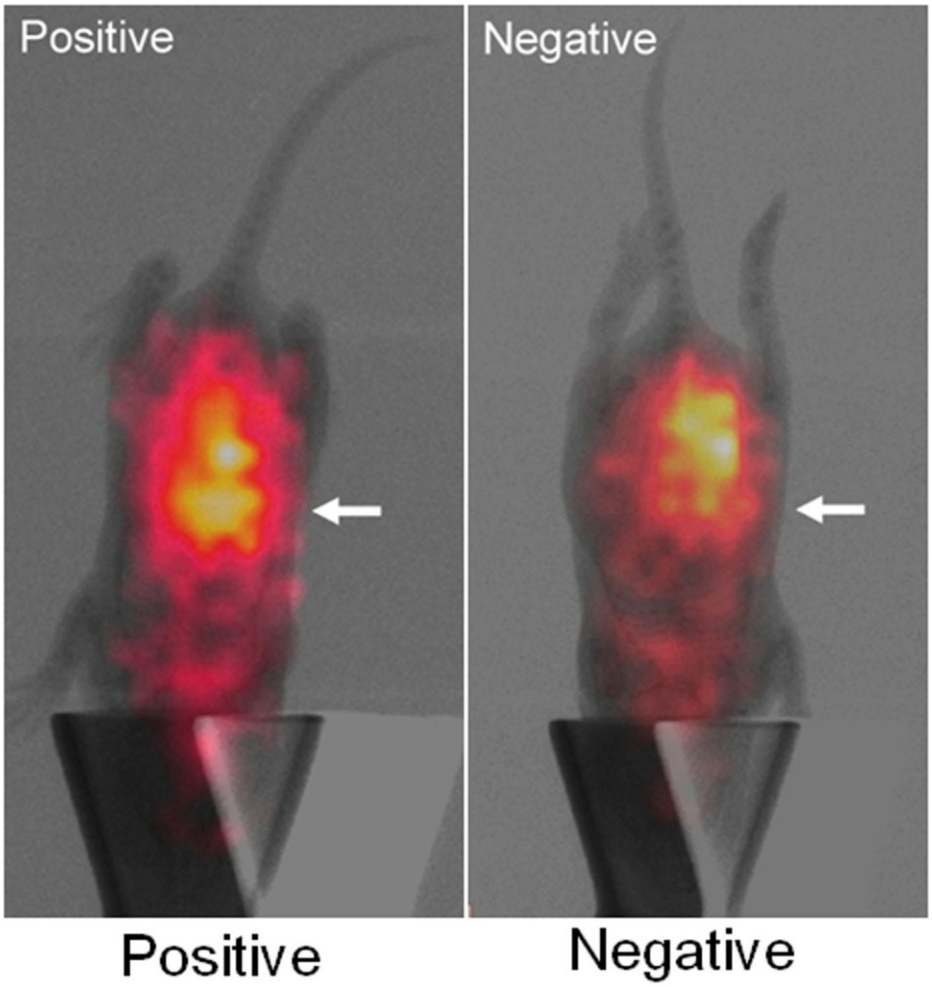

Figure S3. Planar gamma scintigraphic imaging two days after FIAU injection (five days after birth). It is challenging to inject radio-tracer i.v. into the newborns. Three day after birth, pups were successfully injected i.v. with a small amount of [^{125}I]-FIAU. During the two days after injection for tracer washout, the pups grew rapidly. 5 days after birth, planar scintigraphy was performed while the young pups were under much lower anesthesia (<0.5% of isoflurane mixed with oxygen) for fear of loosing them. Despite of some movement that cause image blurring during longer (2 minutes comparing to seconds during x-ray) scintigraphic scan, positive mice (left) showed liver uptake vs. lower abdominal non-specific uptake in the negative controls (right).