

## Supplementary Information

### Supplementary Figure Legends

**Supplementary Figure 1.** Acoustic peak negative pressure beam profiles for the Olympus A303 unfocused transducer. (a) The beam profile of the acoustic peak negative pressure in megapascals (MPa) across the face of the transducer (measured at a depth of ~0.2 mm from the face) with a frequency set to 1.0 MHz using the UTMD ultrasound experiment parameters described in the methods. (b) The axial beam profile of the acoustic peak negative pressure (MPa) readout from the transducer relative to the distance from the face of the transducer.

**Supplementary Figure 2.** Preliminary evaluation of *piggyBac* and *Sleeping Beauty* reporter expression in HEK 293 cells. Average luciferase expression three days and three weeks after transfection, measured in relative light units/mg protein.

Transfections were performed in triplicate using equimolar amounts of DNA for the conventional plasmid, pcDNA3-luc; *Sleeping Beauty* transposase, SB100x with luciferase transposon, pT2/c-luciferase; and helper-independent *piggyBac* plasmids, pmGENIE2-luc and pmGENIE3-luc. HEK: human embryonic kidney, SB: *Sleeping Beauty*; luc: luciferase; RLU: relative light units.

**Supplementary Figure 3.** Evaluation of the AT and TTAA content of the chromosomal DNA sequences surrounding the *in vitro* and *in vivo* *piggyBac* integration sites. (a) The AT base percentages in an evenly distributed 2.0 kilobase window surrounding the sites of *piggyBac* integration events on chromosomes 6, 13, 14, 17, and X from all liver samples transfected with pmGENIE3-luc were analyzed and averaged.

**(b)** Analysis of the average number of TTAA dinucleotide sequences in the same 2.0kb window. luc: luciferase; kb: kilobase.

**Supplementary Figure 4.** Immunofluorescence evaluation of luciferase expression in additional organs (heart, kidney, and spleen) collected from mice transfected with pcDNA3-luc and pmGENIE-luc plasmids in liver-directed UTMD studies. Primary mouse monoclonal antibody to luciferase with a secondary AlexaFluor 568 mouse antibody (red), and DAPI stain for nuclei (blue) is displayed in panels A-F. Panels A-C display images of mouse organs collected from a pcDNA3-luc UTMD treatment, **(a)** heart, **(b)** kidney, **(c)** spleen. Panels D-F display images of mouse organs collected from a pmGENIE-luc UTMD treatment, **(d)** heart, **(e)** kidney, **(f)** spleen. Scale bars = 50  $\mu$ m for all tissues.