Supplementary Information

Supplementary Figure Legends

Supplementary Figure 1. 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, p*m*GENIE2-luc and p*m*GENIE3-luc.

Supplementary Figure 2. 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 (kb) window surrounding the sites of *piggyBac* integration events on chromosomes 6, 13, 14, and X from all liver samples transfected with p*m*GENIE3-luc were analyzed and averaged. **(b)** Analysis of the average number of TTAA dinucleotide sequences in the same 2.0 kb window.

Supplementary Tables

***In vitro* transposition site sequences in MEF 3T3 cells**	***m*Ch. No.**
agtggcacagcagttaggAGAGGGAACTTGTAGAGTCCATCTTCAGTAGAAAGACAGGGCATCAA GTGGAGGGATGGGGTTGCCATCCAACAGTCAAAAACTCTGACCCAGAATTGTTCCTGTCT AAAAGAACTCCAGCAACAAAAATGAAGAGGAGACTGAGGGAAAGAAAGGCAGTCCAGTGA CTGGCCCAACTTGAGATCCATCTCAAGGAGAGTCTCTAAGACCTGACACTAACTGATGCTA TGGTGTGCTTATAGATATGAGCCTAGCATGGCTGTCCTCCAAGAGGCCCAACAAGCAGCT GGCTGAGACATTTGCAGATACTTAAACCCAACCAATTCACTGCAGTAAAGCACTAAAAGGA AAACTTTGGTTGAATTAGGGAATGGCTGGAAGAAGCTGAGGAGGAGGGCAACCCCATAG GAAAACCAGCAGTCTTAATTAACCTGGAACCAGAGCTCTCTCAGACACAGAGCCACCAAC CAGGAAGCATACATGAGCTGGTCTGAGGCCCA	15
agtggcacagcagttaggAGAGTGGACTTGGAACCAGATCTCTTGGGTCTGAGTGGCAGCTAAGC TATTTAATAGTTGTATAGCTTTGAAAAATTTAGCTAGCCACTCTGTGCCTTCATTTTTAATCT TTGAACGAGCATATTTTCAACCCATACAATTGCTGTGAGGATCAAACACATAAACAATTAGT ACAGTTGTTGGTGTA	9
agtggcacagcagttaggGATGCAGTCAGCCTGCTGGATTCAAACTCCAGCCTGGCCACTCCCCA GCTGTACTTCAGCAACTGCTTGACCTCTCTTTGTGACTTGATTTTCTCATCTACAAAATAAA AACAATAAGGCTATCAGTCACCTCAGGACTGGAGAGGCGGCTCAGTGGTTAGAGCACTCA AATAGCTTCGGTGCATCTGCCGCCACCGACAGAGCTGCCCCACCCTTCCTTCAAAGTCGT GATGGATTAAAGACCCTCTGAAACCTGAAGCTGAAATCAACCCCCCTCTCTTAATCCGAGT CAGTTATTCTGTCGCAATAATACAAAAGGAATACAGAGCTCGCTTATCTTCAGCTTCCGTAT ATGGTGTGGGGCTTCTCAAACGGTTTGATTTAAATTTTA	9
agtggcacagcagttaggAAGTAGCAACAAAATAGTTTTATGGCTGGGGCTCACCACAGTATGTGA AACTCTATTAAAAGGTTGAATCATTAGGAGGTTTGAGAAGCACTGGTTTAAAGTAAGTGAA TTGAAAAACACAACATGAGAGTATAAAGATGAGAAGCTGGACTTTTATGACATACTTTGGA TTTTTTTTTCTTTTCTATGAAAAAGAAAGCAGAACAGTGCATTGTCCTTTAAAACTTGAAGTC TCTAGTCCTAAGCTAACGAATTCATTTCCACATTTGACTTAATGCCCCAGAGATAACAGTCC TTTGGAATTTTTTCATTTCAGTTACATTCTAGTGTCTAATTGAATAGCGATTACAATACTAAA TGAGCATTAGTGAACACTTTCTGTGTAACAAAAGCGGAGTACCTTCATTGTATTTAATGTAG CTATGATTCATCCACCACCAGTGTGGGCTTATCATTACAAATATTGTTTTGGAAATTCTTGA GATATTAGATTATAAATAGTTCTCC	X
agtggcacagcagttaggTAGGGACTAGGTGGGAACTTGTCAGGATGTGGTAGGGGCTGTGTGAT TTAACCAGCGAGAGGCATAGAGAACCTCAGGCTGGGCCATCTGGGTAGCGAAAACTTTAT ACCTTGAGAATACCTACAAAGCTGGGTGGCACAGCACTTGCCTGGCATCAAGGAGGCCCT GGGTTTTAGCCCCAGCACCAGGTAGAGGAAGGAGAGGGAGGGAAAATGGGGTGGGGAA TGAATATCCATAAAATATGCTAATTGGATTGGAGGTTCAATTGTTCGAAAACTTAAAAGATT TTTATGCTACTCTTCTGCATATGGCTGTCCCTATTACTCAAGGTTCTGTTTGAGCAATGACT GTGTTTTACCCTTATAACCTAGTGAGGTAAAAGTGCTCAGGACGTCCTCATAGCTCTGTCC CAACCCCATAGCCTTTTCCTAGTTCTGCATGAACTAGTTCCTTCTAGAGAAGGAACCTGGA TGTTCTCGCCCATACCTAGCTTTCTACACTACTCCCAGGTTACCCCCTCCAACCCTGTTTC CTCTGCTTAACCTTGCCAGCCCTTGCTTGCACCTGGCCTAGCTAGGAGAAAGGGCCCCTT GGGGTCCTCATACGTGGGTCTGCAGGGGTAGCACTGTGCCCTGTCAGCATGTCTGATGG GCATCGAGAATTTGGTTCTCTCTAAGAGAGAAGAATGAGGCTCGGTCTAGACTCACCCCA CTGGCCAACCCAATGCCCCAGGACCCATTCCCAGCACCACAAACACTTTTAGACTAAGAT GATTAAG	6

agtggcacagcagttaggGTGTTCTTCAGAGTCACATCTCTTCTCTAAAGCTTTGAAGAACCACCT GTCTGAGGGCCAGAAGCTCTTGTGAGTATCTGCTGATGCAGAGTTAGAAACAGCTCTACT AAGTCCTCAGCC	14
agtggcacagcagttaggGACACTTAGGGGATGCAATTCTTTGTCTTTCACTGCATATGCACCAGT GAGTTACTCGGGATCCAGTGGAGAGCTTCGAATCCATGCTCATGGTAGTGGTCTTGACTA AACTTAGTGGGTCACAACACAAAAACCAAAGCCAAACAAAAAAGCATCAAAGTGGGGTTG GGCCTTGAAGATAGAGGGAGCCTGGGAATTAATTATTAATTAATTGAAAAACCAGAGAGCT TGAAAATATCAACTCTATCAACCCTGCCATACAGAAGCACAACATGAGCAACTAATTATGAT AATTCGGGGTTCACCCGGTCCACATATCACCTAGAATCTACATCTCAACAAGTTCTGGCAA AATTC	1
agtggcacagcagttaggAACGAAGAAGCACTTTTTAAAACAATGGCATTTGGAAGAAGTCACACC ATCACATCTGGGACTACTCCAGTGGGCTTTATTGGTTACCTATGGCCTGGCTAAAGCACTG AA	13
agtggcacagcagttaggGTGCCTTGGGCACAAGCTGTCCAGCGTCTCTTTCCACCGCATTTCTT CCCCTTCCTCAGAAGCTGCTAACATCTGCAGAATGATCCATTTTCCTTCGCACGCATGCTC GTTTTCAACATCAAGTGCTTCCTACTCAGCTTGTTCTCTCCTTCCGATGCAGTGACGCCTC TGGGGACCTGCCATTAATCATTCTTAATTTGATCCCCTTCCTTGGTTCCCACTGGACTTGT GTTCAGGCCTCTCCTGCTCAACATAATCACTCCAACCTGTGATTTCTCCTTCTCTGTGAGC TGCTTCTCCCATTTAAATAGCTCGCTCATAAACAGCATCATTGAGACTGAAGATAAGTAGAT GTACAAGTGCCGGTGCACTTGAAGCCTCCCATCGGGCAAACCCTCATCCTCCCAC	3
agtggcacagcagttaggGAATCTGAATTCTTCCTTTATATAACCTCTTCTAACCATGAGTTCTAGA CCTGCTTCTGACCCATGAATAAACAGTTAGGGAATTATTGTTAAAGGAAGGAATCACACCC AGTGCAAGTTTGACCTACCCCAGACAATCCTAAAAGCTTCTTTAGGTGGCTCAGAGTAGCT ACATTTTTGTTTTCCTTAAAGATTAACTATCTATCCTTTGTCTCTAAAACTCAACAATCAGCC CGTGAATTGTCAGGAATAAATATGATCGTCAAATATTTACTTGACATAGTAAGGGCTTGGA GAATCCAGTGATATTATTACTGCCATGAAAATTGGTATACCCAGCATGCATGGGAAAGAAT AAGAGTTATCACATTTAGAAAGAAAGAATTCCTCTGTATCTCTCATAAGACAATATTTTAGTT CTTCTTTTTTTGTTTGTTTGTCTTTTTGTTTGTTTTCTCCGAAATATAGGGATCTTTGCTTGA CTAAAACACCAAGGTGGCTTTGTATATACACAGTCTGGAATAAGCCTTTAAGGGGAGCTCA TAAACTGTTGCCCGTACATAATTACTGTATTTAGCCAGCTACAGTGAATGCTAAAGGTAAC CTAGAATACTGCATTTTTAGGTGGGGAACCACCATTGAAAAGAAGAAATTATTCAGCTCTTT TAACATAATGCTATTTCATTTCTCTAGCTATTGATGAGATGAATACTCATCAATGGATGATC GAGAATTATGGGCTCTTCTATATATGGGATTTTTCCAGTCAGTTAAACTTTATATCTTCTTAT ATTGGTTGTACTACCTGAAATATTTTTGTCACAATGGTTGTAGGAAAAAGAATACAAAACAG GAACTCCCACCAAATAGCGTTGGCTCTTGTTCTCCCTAAGTTTAAGACATAGAATGGGCAA AGGAGGGATAGTTCCTCAAAAACATTAGCCAATTTCTCTCTCTAATCA	6
agtggcacagcagttaggAGGCAGAGGCAGGCAGATTCCTGAGTTCAAGGCCAGCGTGGTCAAC AGAGTGAGTTCCAAGACAGTCCTGTTACAATAAGAAATCTTGTCTCAAAAAACAAAAACAA AAACAAAAATTACAGCAGTGGAAACAGCCTAAGAAATTTTAGGAAACAGTGTCACCTAGTG GTCACACACGGCACTGCAGAAATGTTAACTTGATTTCGTGTCCCATTTCACTTACAAAGAC CACGCTGACCCTGAAATTAGAGAACCAGTTGCCTGTGCTTCCCAAATGTTGTGATTAAAGG AGAGAGACACCATGCCAAGATTCCTGCCCTACTTTTGAAACAGCCTCTCAGGGCTGTTTTT ACTCTCAGGTAAAATTTCTGAATTCTATTCTGCCTCTTAGTGGCTGGCATAAATCAAATTAT AAAGACTCTTTTGGTCTGGAGAGTTCACTGAACTATCCAGAAATGAAAACAGCCAAGTGGG TGCTGCAGCTCACCTCCAGCTCTATTAGTTCCCAGGGAGACTCCAGTAGCCAGAGCATTA TCTCCGCTGCAATTCGGCTACCTAAAGGAGGAAGGCTTTCTTCCCAGAATATCTCCCTTTA ACTCTTTTTTAATGTGTTATTATGTGTGGCCATGATGTTGGAGAATTGCCTTGGTACACACG	1

TGGAGGCCATGTGGAGTTGGCCTCTGCTTCCACCTTCGCCATGGGTTCCAGGAAGTGAAC TCAGATCAATAGGCTTGCCTGGAAAAGAGCTTTAGGCACTGAGCCATGCCATCGACCCTG TATAGTTCAAGGATGAATGTCAACCAGGCTTAG	

Supplementary Table 1. Transposition site sequences are shown for MEF 3T3 cells as determined by nrLAM PCR, TOPO TA cloning, and sequencing. Each sequence begins with an 18bp sequence (in lower case letters) that indicates the priming site specific for the DNA linker sequence that was ligated to the unknown genomic DNA ends of captured biotinylated sequences from nrLAM PCR. The remaining sequence for each sample contains the mouse genomic sequence connected to the linker, (listed in the last column of the tables). Sequences were localized by BLAST analysis.

***In vivo* transposition site sequences in C57Bl/6 mice**	***m*Ch. No.**
agtggcacagcagttaGGGTGTTCTTCAGAGTCACATCTCTTCTCTAAAGCTTTGAAGAACCACC TGTCTGAGGGCCATAAGCTCTTGTGAGTATCTGCTGATGCAGAGTTAGAAACAGCTCTAC TAAGTCCTCAGCC	14
agtggcacagcagttaggAAGTAGCAACAAAATAGTTTTATGGCTGGGGCTCACCACAGTATGTG AAACTCTATTAAAAGGTTGAATCATTAGGAGGTTTGAGCAGCACTGGTTTAAAGTAAGTGA ATTGAAAAACACAACATGAGAGTATAAAGATGAGAAGCTGGACTTTTATGACATACTTTGG ATTTTTTTTTCTTTTCTACGAAAAAGAAAGCAGAACAGTGCATTGTCCTTTAAAACTTGAAG TCTCTAGTCCTAAGCTAACGAATTCATTTCCACATTTGACTTAATGCCCCAGAGATAACAG TCCTTTGGAATTTTTTCATTTCAGTTACATTCTAGTGTCTAATTGAATAGCGATTACAATAC TAAATGAGCATTAGTGAACACTTTCTGTGTAACAAAAGCGGAGTACCTTCATTGTATTTAA TGTAGCTATGATTCATCCACCACCAGTGTGGGCTTATCATTACAAATATTGTTTTGGAAAT TCTTGAGATATTAGATTATAAATAGTTCTCCTTTTTCCATTCTTTTTAAAAACAATTATCTTC AATTTTTATTAACATTTATTTATT	X
agtggcacagcagttaggAACGAAGAAGCACTTTTTAAAACAATGGCATTTGGAAGAAGTCGCAC CATCACATCTGGGACTACTCCAGTGGGCTTTGTTGGTTACCTATGGCCTGGCTAAAGCAC TGAA	13
agtggcacagcagttaggAAGTAGCAACAAAATAGATTATGGCTGGGGCTCACCACAGTATGTGA AACTCTATTAACACTTTGAATCATTAGGAGGT	X
ccgataaaacacatgcgtcaTGATTAGAGAGAGAAATTGGCTAATGTTTTTGAGGAACTATCCCTC CTTTGCCCATTCTATTGTCTTAAACTTAGGGAGAACAAGAGCCAACGCTATTTGGTGGGA GTTCCTGTTTTGTATTCTTTTTCCTACAACCATTGTGACAAAAATATTTCAGGTAGTACAAC CAATATAAGAAGATATAAAGTTTAACTGACTGGAAAAATCCCATATATAGAAGAGCCCATA ATTCTCGATCATCCATTGATGAGTATTCATCTCATCAATAGCTAGAGAAATGAAATAGCAT TATGTTAAAAGAGCTGAATAATTTCTTCTTTTCAATGGTGGTTCCCCACCTAAAAATGCAG TATTCTAGGTTACCTTTAGCATTCACTGTAGCTGGCTAAATACAGTAATTATGTACGGGCA ACAGTTTATGAGCTCCCTTTAAAGGCTTATTCCAGACTGTGTATATACAAAGCCACCTTGG TGTTTTAGTCAAGCAAAGATCCCTATATTTCGGAGAAAACAAACAAAAAGACAAACAAACA AAAAAAGAAGAACTAAAATATTGTCTTATGAGAGACACAGAGGAATTCTTTCTTTCTAAAT GTGATAACTCTTATTCCTTTCCCATGCATGCTGGGTATACCAATTTTCATGGCATTATAATA TCACTGGATTCTCCAAGCCCTTACTATGTCAAGAAAATATTTGACGATTCATATTTATTTCT GATATTCACGTGCTGATTCGTTGAGTTTTAGAGACCAAGGATAGATAGCTTAAT	6

Supplementary Table 2. Transposition site sequences are shown for the mouse liver as determined by nrLAM PCR, TOPO TA cloning, and sequencing. Each sequence begins with an 18bp sequence (in lower case letters) that indicates the priming site specific for the DNA linker sequence that was ligated to the unknown genomic DNA ends of captured biotinylated sequences from nrLAM PCR. The remaining sequence for each sample contains the mouse genomic sequence connected to the linker, (listed in the last column of the tables). Sequences were localized by BLAST analysis.