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Supplemental information

**Development of a pan-neuronal genetic driver
in *Aedes aegypti* mosquitoes**

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Supplemental Information

Table S1. sgRNA cutting efficiency. Related to STAR Methods.

sgRNA name	Target gene	sgRNA cut location	Target sequence (PAM underlined)	Evaluation method	Evaluation criteria	Cutting efficiency	Evaluation primer forward	Evaluation primer reverse	Note
sgRNA-syt1	<i>Syt1</i> (AAEL000704)	Chr2: 459,055,055	GCACACTCTTAAAGA CCCGG <u>AGG</u>	Fragment analysis	Variability of fragment length	High	TGAAAACGACGG CCAGTTGTGGTGG ATTACGATCGCA	GTGTCTTTCAGT GTCTCTTGTCTC TCG	Used for HDR knock-in
sgRNA-syt2	<i>Syt1</i> (AAEL000704)	Chr2: 459,054,995	ATGCCAGCATGTCTG ACCAGT <u>G</u> G	Fragment analysis	Variability of fragment length	Low	TGAAAACGACGG CCAGTTGTGGTGG ATTACGATCGCA	GTGTCTTTCAGT GTCTCTTGTCTC TCG	
sgRNA-syt3	<i>Syt1</i> (AAEL000704)	Chr2: 459,055,074	GAGGACGACAAGAA GGACTA <u>AGG</u>	Fragment analysis	Variability of fragment length	Medium	TGAAAACGACGG CCAGTTGTGGTGG ATTACGATCGCA	GTGTCTTTCAGT GTCTCTTGTCTC TCG	
sgRNA-syt4	<i>Syt1</i> (AAEL000704)	Chr2: 459,039,097	CTAAAGAAGATGGAC GTCGG <u>AGG</u>	Fragment analysis	Variability of fragment length	Low	TGAAAACGACGG CCAGTATAACAAG CTGGGAGACATC	GTGTCTTTCAGT ATCTCATAGTAG GC	
sgRNA-syt5	<i>Syt1</i> (AAEL000704)	Chr2: 459,016,400	CAAAGCGTCGAAGG CGAAGG <u>CGG</u>	Fragment analysis	Variability of fragment length	Low	TGAAAACGACGG CCAGTGTATGCCG ACGCGATGAAC	GTGTCTTCATAA ACCTGACTATGG ATCGGAAC	
sgRNA-syt6	<i>Syt1</i> (AAEL000704)	Chr2: 459,055,052	ATGGCACACTCTTAA AGACCC <u>G</u> G	Fragment analysis	Variability of fragment length	Low	TGAAAACGACGG CCAGTTGTGGTGG ATTACGATCGCA	GTGTCTTTCAGT GTCTCTTGTCTC TCG	
sgRNA-syt7	<i>Syt1</i> (AAEL000704)	Chr2: 459,039,336	GAGAACGACTCATTG TAGTAG <u>G</u> G	Fragment analysis	Variability of fragment length	Low	TGAAAACGACGG CCAGTCATACGTA AAGATTGCACTG	GTGTCTTACAGT ACGGCATGTATA G	
sgRNA-brp1	<i>brp</i> (AAEL018153)	Chr2: 234,771,924	AAGCTCTTCAGGAAA CCGGCT <u>TGG</u>	MiSeq	Fraction of reads that have indels	0.052	TCGTCGGCAGCGT CAGATGTGTATAA GAGACAGCTGGT GCTCTGCAGTTTG AAG	GTCTCGTGGGC TCGGAGATGTG TATAAGAGACAG ACTGCAACTGGT ACAGATGACA	
sgRNA-brp2	<i>brp</i> (AAEL018153)	Chr2: 234,771,909	CCGGCTGGTCCAGC ATCTT <u>GCGG</u>	MiSeq	Fraction of reads that have indels	0.447	TCGTCGGCAGCGT CAGATGTGTATAA GAGACAGCTGGT GCTCTGCAGTTTG AAG	GTCTCGTGGGC TCGGAGATGTG TATAAGAGACAG ACTGCAACTGGT ACAGATGACA	
sgRNA-brp3	<i>brp</i> (AAEL018153)	Chr2: 234,771,739	GCAACTGGTACAGAT GACAC <u>AGG</u>	MiSeq	Fraction of reads that have indels	0.585	TCGTCGGCAGCGT CAGATGTGTATAA GAGACAGACTGTG CCAATACTAATGC CAG	GTCTCGTGGGC TCGGAGATGTG TATAAGAGACAG TGGAGGTACAG CAGCAGC	Used for HDR knock-in

sgRNA-elav5	<i>elav</i> (AAEL008164)	Chr3: 9,232,508	GCTGACTTGTAACAC CCGCTGG	Fragment analysis	Variability of fragment length	Low	TGTA AACGACGG CCAGTAGATTCGG CGACAAACCACT	GTGTCTTTGGAA AGGAGATAGAA ATCTGTT
sgRNA-elav6	<i>elav</i> (AAEL008164)	Chr3: 9,232,875	TTCTGATTTTCAGCGC CGAAATGG	Fragment analysis	Variability of fragment length	Low	TGTA AACGACGG CCAGTCCGCGATT GTGGAATTCCTTG	GTGTCTTAGTAC TCGCGTCTTGAT GCG
sgRNA-elav7	<i>elav</i> (AAEL008164)	Chr3: 9,232,504	ACGGATACACCCTTG GCCAGCGG	Fragment analysis	Variability of fragment length	Low	TGTA AACGACGG CCAGTAGATTCGG CGACAAACCACT	GTGTCTTTGGAA AGGAGATAGAA ATCTGTT
sgRNA-elav8	<i>elav</i> (AAEL008164)	Chr3: 9,232,454	CGTCACGATGACCA ACTACGAGG	MiSeq	Fraction of reads that have indels	0.121	TCGTCGGCAGCGT CAGATGTGTATAA GAGACAGCAAAGA TTCGGCGACAAAC CA	GTCTCGTGGGC TCGGAGATGTG TATAAGAGACAG GGATTGAGAATC GTTTCAGGCG
sgRNA-elav9	<i>elav</i> (AAEL008164)	Chr3: 9,232,501	TTGTAACACCCGCTG GCCAAGGG	MiSeq	Fraction of reads that have indels	0.011	TCGTCGGCAGCGT CAGATGTGTATAA GAGACAGAGGGA TACGGCTTCGTCA C	GTCTCGTGGGC TCGGAGATGTG TATAAGAGACAG GGATTGAGAATC GTTTCAGGCG
sgRNA-elav10	<i>elav</i> (AAEL008164)	Chr3: 9,232,485	CTGGCGATTTCGATCT CTCAACGG	MiSeq	Fraction of reads that have indels	0.085	TCGTCGGCAGCGT CAGATGTGTATAA GAGACAGCAAAGA TTCGGCGACAAAC CA	GTCTCGTGGGC TCGGAGATGTG TATAAGAGACAG GGATTGAGAATC GTTTCAGGCG
sgRNA-nsyb1	<i>nSyb</i> (AAEL024921)	Chr2: 99,332,681	AGATCCTGTACGAGT GCCGCCGG	MiSeq	Fraction of reads that have indels	0.352	TCGTCGGCAGCGT CAGATGTGTATAA GAGACAGACCACA ACAGCCGATGATG A	GTCTCGTGGGC TCGGAGATGTG TATAAGAGACAG AGAGTTGTGTTT GTCGTCCTAT
sgRNA-nsyb2	<i>nSyb</i> (AAEL024921)	Chr2: 99,332,676	GCAAGTCCCAGCTG CTCCGGCGG	MiSeq	Fraction of reads that have indels	0.232	TCGTCGGCAGCGT CAGATGTGTATAA GAGACAGACCACA ACAGCCGATGATG A	GTCTCGTGGGC TCGGAGATGTG TATAAGAGACAG AGAGTTGTGTTT GTCGTCCTAT
sgRNA-nsyb3	<i>nSyb</i> (AAEL024921)	Chr2: 99,332,646	GATGCAGCAGATGC CAATGCAGG	MiSeq	Fraction of reads that have indels	0.176	TCGTCGGCAGCGT CAGATGTGTATAA GAGACAGATGCTG ATTAGAACTAGT GACGAG	GTCTCGTGGGC TCGGAGATGTG TATAAGAGACAG CCTCACTATCT CGGTAGAAGTT G

Table S2. HDR efficiency. Related to STAR Methods.

Construct	Target gene	Donor construct	Insert length (bp)	Homology arm length: left (bp)	Homology arm length: right (bp)	No. injected embryos	No. dsRed+ G1 families	No. dsRed+ G2 families	No. PCR verified families	Note
<i>Syt1-T2A-3XGCaMP6s</i>	<i>Syt1</i> (AAEL000704)	Syt1LeftArm-T2A-GCaMP6s-T2A-GCaMP6s-T2A-GCaMP6s-SV40-3XP3-dsRed-SV40-Syt1RightArm	5723	1374	1822	1098	>=2	>=2	1	Bred and screened in bulk, so the exact number of families is unknown
<i>Syt1-T2A-QF2-QUAS-GCaMP6s</i>	<i>Syt1</i> (AAEL000704)	Syt1LeftArm-T2A-QF2-Hsp70-5XQUAS-GCaMP6s-SV40-3XP3-dsRed-SV40-Syt1RightArm	4689	1374	1822	1200	5	NA	NA	Two G1 families had strong green fluorescence in the central and peripheral nervous system, but died at the late larval stage. The other three families were not positive at G2.
<i>Syt1-T2A-GAL4d-UAS-GCaMP6s</i>	<i>Syt1</i> (AAEL000704)	Syt1LeftArm-T2A-GAL4d-Hsp70-3XUAS-GCaMP6s-SV40-3XP3-dsRed-SV40-Syt1RightArm	4480	1374	1822	3500	8	4	1	
<i>Syt1:GCaMP6s</i>	<i>Syt1</i> (AAEL000704)	Syt1LeftArm-3XGS-GCaMP6s-SV40-3XP3-dsRed-SV40-Syt1RightArm	2923	1374	1822	1200	2	2	1	
<i>brp-T2A-QF2w</i>	<i>brp</i> (AAEL018153)	brpLeftArm-T2A-QF2w-Hsp70-3XP3-dsRed-SV40-brpRightArm	2799	1011	1043	1533	6	2	1	

Table S3. Verify insertion. Related to STAR Methods.

Construct	Verification primer forward	Verification primer reverse	Expected product length (bp)	Observed band length	Sanger sequencing alignment file
<i>Syt1-T2A-3XGCaMP6s</i>	GCTCTCAATTCGCTC ATGTGAT	CTTCAGCTTCAGGG CCTT	6861	~7kb	Syt13XGCaMP6s_Verification_Alignment_PCR_product
<i>Syt1-T2A-QF2-QUAS-GCaMP6s</i>	GCACAAGTCTCCCAA CTGGT	CGTAGTTGTGGGTC CCAGAC	1669	1.5kb - 2kb	Syt1QF2QUASGCaMP6s_Verification_Alignment_PCR_product
<i>Syt1-T2A-GAL4d-UAS-GCaMP6s</i>	GCTCTCAATTCGCTC ATGTGAT	CTTCAGCTTCAGGG CCTT	5618	5kb - 6kb	Syt1T2AGal4dUASGCaMP6s_Verification_Alignment_PCR_P roduct
<i>Syt1:GCaMP6s</i>	GCTCTCAATTCGCTC ATGTGAT	GCATGAACTCCTTG ATGACGT	3594	3kb - 4kb	Syt1GCaMP6s_Verification_Alignment_PCR_product
<i>brp-T2A-QF2w</i>	TCCGGAAAGCATGGT CAGTC	CGTAGTTGTGGGTC CCAGAC	1642	1.5kb - 2kb	BrpT2AQF2w_Verification_Alignment_PCR_product