

## Supplemental Information

### Optogenetic Inhibition of Synaptic Release

#### with Chromophore-Assisted Light Inactivation (CALI)

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#### Inventory of Supplemental Materials

**Figure S1. Representative images of SYP1-eGFP fluorescence and FM4-64 dye uptake in cultured cortical neurons.**

This supplemental figure shows the representative images of the data used in Figure 1E.

**Figure S2. Additional examples and summaries of whole-cell patch-clamp recording of electrically-evoked EPSC and miniature EPSC in organotypic hippocampal slices and primary cultured neurons.**

This supplemental figure provides additional representative electrophysiological recordings and summary graphs not shown Figure 2.

**Figure S3. Expression of miniSOG-Citrine and SNT-1-miniSOG-Citrine in *C. elegans*.**

This supplemental figure provides the representative fluorescent images of miniSOG-Citrine and SNT1-miniSOG-Citrine worms that are acquired similarly to Figure 4A.

**Figure S4. Analysis of spontaneous EPSCs recorded from miniSOG-VAMP2-Citrine (miniSOG-VAMP2) expressing worms and non-expressing (Wt) N2 worms.**

This supplemental figure provides the properties of the spontaneous EPSC recorded from non-expressing (Wt) worms and miniSOG-VAMP2-Citrine expressing worms prior to light illumination to demonstrate the over-expression of miniSOG-VAMP2-Citrine in Wt

worms does not significantly changed the vesicular release. This supplemental figure complements the data shown in Figure 4B.

**Table S1. Analysis of spontaneous EPSCs recorded from miniSOG-VAMP2-Citrine (miniSOG-VAMP2) expressing worms and non-expressing (Wt) N2 worms.**

This supplemental table provides values shown in **Figure S4**.

**Movie S1. An example of the inhibition of *C. elegans* movements with light illumination.**

This movie shows the typical movies used for the quantification shown in **Figure 3A-C**.

**Additional Supplemental Material**

Protein and DNA sequences of InSynC

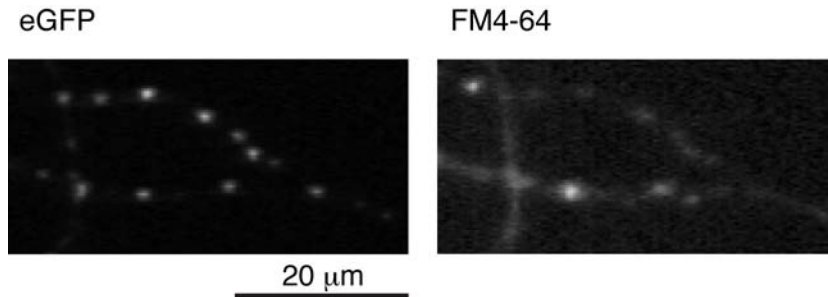
- miniSOG-VAMP2-T2A-mCherry
- miniSOG-VAMP2-Citrine
- SYP1-miniSOG-T2A-mCherry
- SYP1-miniSOG-Citrine
- SNT-1-miniSOG-Citrine

## Supplemental Material

### Optogenetic inhibition of synaptic release with chromophore-assisted light inactivation (CALI)

John Y. Lin *et al.*

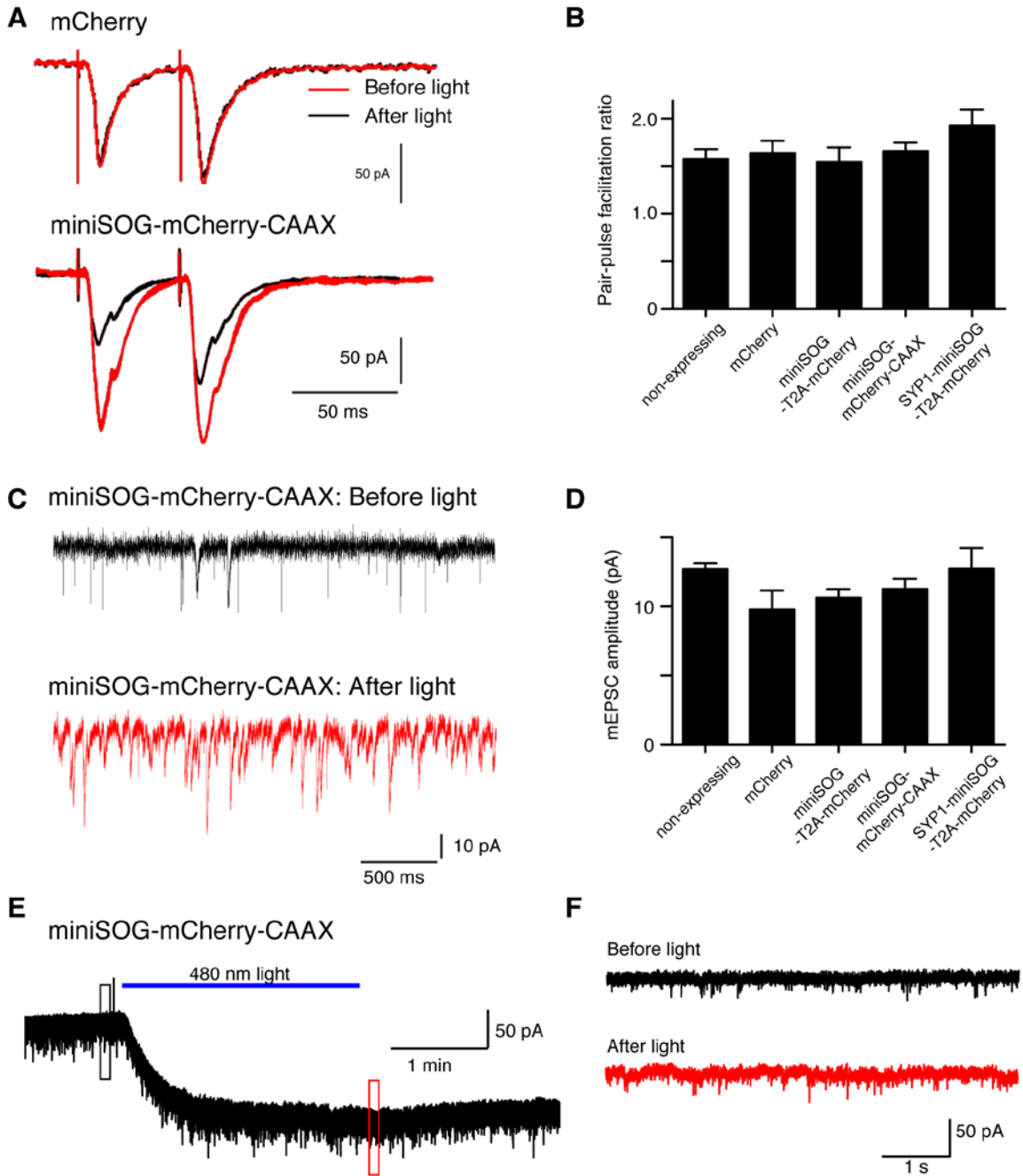
#### Figure S1



**Figure S1. Representative images of SYP1-eGFP fluorescence and FM4-64 dye uptake in cultured cortical neurons.**

Fluorescence images of eGFP puncta (left) and the corresponding FM4-64 dye uptake (right) after stimulation with 40 mM KCl. Not all eGFP positive puncta had detectable dye uptake, and FM4-64 on the plasma membrane was not removed completely after washout. Fluorescence was quantified to produce the graph shown in Figure 1E.

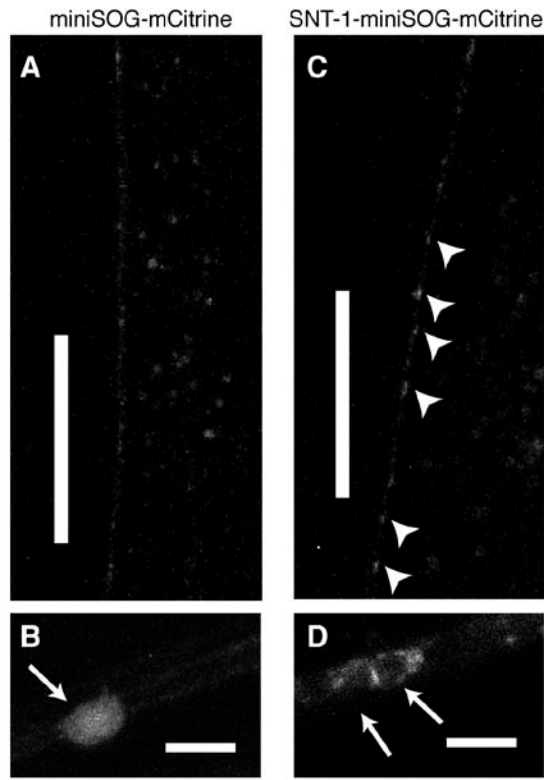
**Figure S2**



**Figure S2. Additional examples and summaries of whole-cell patch-clamp recording of electrically-evoked EPSC and miniature EPSC in organotypic hippocampal slices and primary cultured neurons.**

(A) In the organotypic hippocampal slices infected with rAAV expressing mCherry alone, the electrically-evoked EPSC was not inhibited after 480 nm light illumination. In the organotypic hippocampal slices infected with rAAV expressing membrane tethered miniSOG and mCherry (miniSOG-mCherry-CAAX), the electrically-evoked EPSC was sometimes increased after 480 nm light illumination. (B) Expression of mCherry, miniSOG-T2A-mCherry, miniSOG-mCherry-CAAX and SYP1-miniSOG-T2A-mCherry did not significantly change the pair-pulse facilitation ratio. (C) In the organotypic hippocampal slices infected with rAAV expressing miniSOG-mCherry-CAAX, light illumination increased the frequency mEPSC. (D) The mean mEPSC amplitudes in the slices expressing the different constructs prior to light illumination were not significantly different. (E) Voltage-clamp recording of miniSOG-mCherry-CAAX-expressing cortical neurons before and after light illumination (illumination indicated by blue horizontal bar). A long-lasting inward current is observed after light illumination. (F) Higher magnification of the boxed area in (E). Both graphs are shown as mean  $\pm$  S.E.M.. This supplemental figure provides additional representative electrophysiological recordings and summary graphs not shown Figure 2.

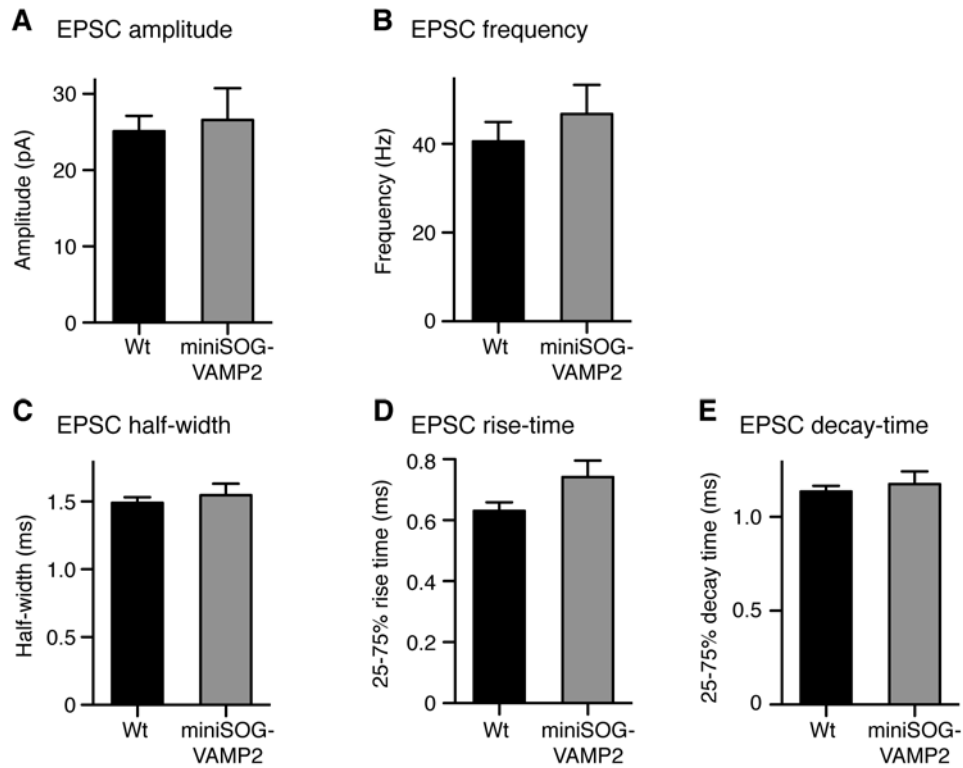
**Figure S3**



**Figure S3. Expression of miniSOG-Citrine and SNT-1-miniSOG-Citrine in *C. elegans*.**

Confocal images of Citrine fluorescence in miniSOG-mCitrine- (A and B) and SNT-1-miniSOG-Citrine- (C and D)-expressing worms. (A) In miniSOG-Citrine-expressing animals, punctate fluorescence signals corresponding to presynaptic terminals were not observed at the dorsal cord (A) although fluorescence is observed in the motor neuron somas located ventrally (B; arrowed). (C) In SNT-1-miniSOG-Citrine-expressing worms, punctate fluorescence signals at the dorsal cord (indicated by arrowheads) were consistent with synaptic localization along axonal projections (C). Somatic fluorescence signals (arrowed) were also observed in SNT-1-miniSOG-Citrine-expressing worms, presumably from newly synthesized protein prior to axonal trafficking (D). The animals expressing miniSOG-VAMP2-Citrine show similar expression pattern to SNT-1-miniSOG-Citrine-expressing animals. Scale bar: 25  $\mu\text{m}$  for (A) and (C) and 5  $\mu\text{m}$  for (B) and (D). These images were acquired similarly to Figure 4A

**Figure S4**



**Figure S4. Analysis of spontaneous EPSCs recorded from miniSOG-VAMP2-Citrine (miniSOG-VAMP2) expressing worms and non-expressing (Wt) N2 worms prior to light illumination.**

Analysis of the mean spontaneous EPSC amplitudes (A), frequency (B), half-width (C), rise time (D) and decay time (E) of whole-cell patch-clamp recordings from the muscle of miniSOG-VAMP2 (n = 11) and wild-type (n = 15) worms. None of the parameter compared were significantly different between the two groups. All graphs are shown as mean  $\pm$  S.E.M..

**Table S1. Analysis of spontaneous EPSCs recorded from miniSOG-VAMP2-Citrine (miniSOG-VAMP2) expressing worms and non-expressing (Wt) N2 worms.**

	Amplitude (pA)	Frequency (Hz)	Half-width (ms)	Rise-time (ms)	Decay-time (ms)
Wt (N2)	$25.10 \pm 2.01$	$40.57 \pm 4.39$	$1.492 \pm 0.041$	$0.063 \pm 0.028$	$1.137 \pm 0.029$
miniSOG-VAMP2	$26.57 \pm 4.18$	$46.73 \pm 6.55$	$1.548 \pm 0.083$	$0.742 \pm 0.054$	$1.175 \pm 0.068$



**Movie S1. An example of the inhibition of *C. elegans* movements with light illumination.**

This composite movie shows the inhibition and the recovery of the movements of a miniSOG-VAMP2-mCitrine expressing worm (N2 wild-type background) on a bacteria-free agar surface after the illumination of light. The movie is shown in real time.

## Protein and DNA sequences of InSynC

### miniSOG-VAMP2-T2A-mCherry

miniSOG  
Linker  
VAMP2  
T2A sequence  
mCherry

gctagc NheI restriction site  
cgtacg BsiWI restriction site

```
1  M E K S F V I T D P R L P D N P I I F A 20
1068 ATgGAGAAAAGTTTCGTGATAAcTGATCCACGGCTGCCAGACAATCCCATCATCTTCGCA 1127

21  S D G F L E L T E Y S R E E I L G R N G 40
1128 TCCGATggCTTCCTGAGCTGACCGAGTATTCCAGAGAGGAGATCCTGGGCCGCAATGgC 1187

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1248 AGAGATCAGCGGAGATTACCGTGCAGCTGATAAACTACACAAAAAGCGGAAGAAATTC 1307

81  W N L L H L Q P M R D Q K G E L Q Y F I 100
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121 T V P P A A P A G E G G P P A P P P N I 140
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141 T S N R R L Q Q T Q A Q V D E V V D I M 160
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161 R V N V D K V L E R D Q K L S E L D D R 180
1548 AGGGTGAATGTGGACAAGGTCTGGAGCGGGACCAGAAGTTGTGGAGCTGGATGACCGT 1607

181 A D A L Q A G A S Q F E T S A A K L K R 200
1608 GCAGATGCCCTCCAGGCAGGGGCCCTCCAGTTTGAACAAGTGCAGCCAAGCTCAAGCGC 1667

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241 G R G S L L T C G D V E E N P G F R T M 260
1788 ggcagaggaagtcttctaacatgcggtgacgtggaggagaatcccggcctcgtacgatg 1847

261 V S K G E E D N M A I I K E F M R F K V 280
1848 gtgagcaagggcgaggaggataaacatggccatcatcaaggagttcatgcgcttcaaggtg 1907

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1968 ccctacgagggcaccagaccgccaagctgaaggtgaccaaggggtgccccctgccttc 2027

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## miniSOG-VAMP2-Citrine

miniSOG  
Linker  
VAMP2  
Citrine

ACCGGT AgeI restriction site  
ATCGAT ClaI restriction site

1 **M E K S F V I T D P R L P D N P I I F A** 20  
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21 **S D G F L E L T E Y S R E E I L G R N G** 40  
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## SYP1-miniSOG-T2A-mCherry

Synaptophysin (SYP1)

Linker

miniSOG

T2A sequence

mCherry

accggt AgeI restriction site

gctagc NheI restriction site

cgtacg BsiWI restriction site

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2163 ctgcaggaccagagacagaccaggccacagtgcagaagattcgcgatgccattagagat 2222

381 Q R E I T V Q L I N Y T K S G K K F W N 400  
2223 cagcgcgagattaccgtgcagctgataaactacacaaaaagcgggaagaaattctggaac 2282

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## SYPI-miniSOG-Citrine

SYPI  
miniSOG  
Linker

Citrine

ACCGGT AgeI restriction site

ATCGAT ClaI restriction site

1 M D V V N Q L V A G G Q F R V V K E P I 20  
1065 ATGGACGTGGTGAATCAGCTGGTGGCTGGGGGTCAAGGAGCCCTT 1124

21 G F V K V L Q W V F A I F A F A T C G S 40  
1125 GGCTTCGTGAAGGTGCTGCAGTGGGTCTTTGCCATCTTCGCCTTTGCTACGTGTGGCAGC 1184

41 Y T G E L R L S V E C A N K T E S A L N 60  
1185 TACACCGGGGAGCTTCGCTGAGCGTGGAGTGTGCCACAAGACGGAGAGTGCCTCAAC 1244

61 I E V E F E Y P F R L H Q V Y F D A P S 80  
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1305 TGCGTCAAAGGGGGCACTACCAAGATCTTCTGGTTGGGGACTACTCCTCGTCGGCTGAA 1364

101 F F V T V A V F A F L Y S M G A L A T Y 120  
1365 TTCTTTGTCACCGTGGCTGTGTTTGCCTTCTCTACTCCATGGGGCCCTGGCCACTAC 1424

121 I F L Q N K Y R E N N K G P M M D F L A 140  
1425 ATCTTCTGCAGAACAAGTACCGAGAGAACAACAAGGGCCTATGATGGACTTTCTGGCT 1484

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161 D V K M A T D P E N I I K E M P M C R Q 180  
1545 GATGTGAAGATGGCCACGGACCCAGAGAACATTATCAAGGAGATGCCATGTGCCGCCAG 1604

181 T G N T C K E L R D P V T S G L N T S V 200  
1605 ACAGGGAACACATGCAAGGAAGTGGGACCCCTGTGACTTCAGGACTCAACACCTCAGTG 1664

201 V F G F L N L V L W V G N L W F V F K E 220  
1665 GTGTTTGGCTTCTGAACCTGGTGTCTTGGGTTGGCAACTTATGGTTCGTGTTCAAGGAG 1724

221 T G W A A P F M R A P P G A P E K Q P A 240  
1725 ACAGGCTGGGCAGCCCCATTCATGCGCGACCTCCAGGCGCCCCGAAAAGCAACCAGCA 1784

241 P G D A Y G D A G Y G Q G P G G Y G P Q 260  
1785 CCTGGCGATGCCTACGGCGATGCGGGCTACGGGCAGGGCCCCGAGGCTATGGGCCCAg 1844

261 D S Y G P Q G G Y Q P D Y G Q P A S G G 280  
1845 GACTCCTACGGGCTCAGGGTGGTTATCAACCCGATTACGGGCAGCCAGCCAGCGGTGGC 1904

281 G G Y G P Q G D Y G Q Q G Y G Q Q G A P 300  
1905 GGTGGCTACGGGCTCAGGGCGACTATGGGCAGCAAGGCTATGGCCAACAGGGTGCGCC 1964

301 T S F S N Q M K T G G G G S G G S M E 320  
1965 ACCTCCTTCTCCAATCAGATGaaaaccggTGGTGGCGCAGTGGTGGCGGCAGCATgGAG 2024

321 K S F V I T D P R L P D N P I I F A S D 340  
2025 AAAAGTTTCGTGATAAcTGATCCACGGCTGCCAGACAATCCCATCATCTTCGCATCCGAT 2084

341 G F L E L T E Y S R E E I L G R N G R F 360  
2085 ggCTTCTGAGCTGACCGAGTATTCCAGAGAGGAGATCCTGGGCCGAATGgCCGCTTT 2144

361 L Q G P E T D Q A T V Q K I R D A I R D 380  
2145 CTGCAGGACCCAGAGACAGACCAGGCCACAGTGCAGAAGATTTCGCGATGCCATTAGAGAT 2204

381 Q R E I T V Q L I N Y T K S G K K F W N 400  
 2205 CAGCGCGAGATTACCGTGCAGCTGATAAACTACACAAAAGCGGGAAGAAATTCGGAAC 2264  
  
 401 L L H L Q P M R D Q K G E L Q Y F I G V 420  
 2265 CTCcTgCACCTCCAGCCCATGAGGGACCAGAAGGGTGAGCTCCAGTATTTTCATCGGAGTG 2324  
  
 421 Q L D G S G G G S G G G G A G I D M V S 440  
 2325 CAGCTGGATGGATCAGGAGGAGGCTCAGGAGGAGGAGGCGCGggAATCGATatggtgagc 2384  
  
 441 K G E E L F T G V V P I L V E L D G D V 460  
 2385 aagggcgaggagctgttcaccgggggtggtgcccatcctggtcgagctggacggcgacgta 2444  
  
 461 N G H K F S V S G E G E G D A T Y G K L 480  
 2445 aacggccacaagttcagcgtgtccggcgagggcgagggcgatgccacctacggcaagctg 2504  
  
 481 T L K F I C T T G K L P V P W P T L V T 500  
 2505 accctgaagttcatctgcaccaccggcaagctgcccgtgccctggcccaccctcgtgacc 2564  
  
 501 T F G Y G L M C F A R Y P D H M K Q H D 520  
 2565 accttcggctacggcctgatgtgcttcgcccgtaccccgaccacatgaagcagcagcagc 2624  
  
 521 F F K S A M P E G Y V Q E R T I F F K D 540  
 2625 ttcttcaagtccgccatgcccaaggtacgtccaggagcgcaccatcttcttcaaggac 2684  
  
 541 D G N Y K T R A E V K F E G D T L V N R 560  
 2685 gacggcaactacaagaccgcgcccaggtgaagttcgagggcgacaccctggtgaaccgc 2744  
  
 561 I E L K G I D F K E D G N I L G H K L E 580  
 2745 atcgagctgaagggcatcgacttcaaggaggacggcaacatcctggggcacaagctggag 2804  
  
 581 Y N Y N S H N V Y I M A D K Q K N G I K 600  
 2805 tacaactacaacagccacaacgtctatatcatggccgacaagcagaagaacggcatcaag 2864  
  
 601 V N F K I R H N I E D G S V Q L A D H Y 620  
 2865 gtgaacttcaagatccgccacaacatcgaggacggcagcgtgcagctcgccgaccactac 2924  
  
 621 Q Q N T P I G D G P V L L P D N H Y L S 640  
 2925 cagcagaacacccccatcggcgacggccccgtgctgctgcccgacaaccactacctgagc 2984  
  
 641 Y Q S A L S K D P N E K R D H M V L L E 660  
 2985 taccagtccgccctgagcaaagacccaacgagaagcgcgatcacatggtcctgctggag 3044  
  
 661 F V T A A G I T L G M D E L Y K \* 677  
 3045 ttcgtgaccgcccgggatcactctcggcatggacgagctgtacaagTAG 3095

## SNT-1-miniSOG-Citrine

miniSOG  
 Linker  
 SNT-1  
 Citrine

accggt AgeI restriction site  
 ATCGAT ClaI restriction site

1 M V K L D F S S Q D E E N D E D L T K E 20  
 692 ATGGTGAAATTAGACTTTTCGTGCGCAAGACGAAGAGAACGACGAAGACTTGACAAAAGAG 751  
  
 21 F V R D E A P M E E T T S E A V K Q I A 40



752 TTTGTAAGGGATGAAGCACCAATGGAAGAAACAACATCGGAAGCAGTAAAGCAAATAGCA 811

41 T T T K E T L K D V V V N K V I D V K D 60  
812 ACAACGACAAAGGAGACGCTGAAAGATGTGGTTGTAATAAAGTGATTGATGTGAAAGAC 871

61 V V K E K V M Q Q T G M P E W A F V F L 80  
872 GTTGTGAAAAGAAAGTTATGCAACAACTGGGATGCCTGAATGGGCGTTCGTATTTCTT 931

81 G F V F I L L V L A C A F C L I R K L F 100  
932 GGATTCGTATTTATTTCTGCTGGTTCCTCGCGTGTGCATTCTGTCTCATTTCGGAAGTTATTT 991

101 G K K R H G E K N K K G G L K G F F G K 120  
992 GGAAAAAAGCGGCATGGTGAGAAGAACAAAAAGGTTGATTGAAAGGATTCTTTGGTAAA 1051

121 G Q D V V D G K N I Q G M A Q D L E E L 140  
1052 GGACAGGATGTCGTTGATGGAAAAATATTCAAGGGATGGCTCAAGACTTGAAGAAGT 1111

141 G D A M E Q N E K E Q A E E K E E V K L 160  
1112 GGTGATGCGATGGAACAAAATGAAAAAGAACAAGCTGAAGAAAAAGAAGAAGTGAAGT 1171

161 G R I Q Y K L D Y D F Q Q G Q L T V T V 180  
1172 GGAAGGATACAATATAAACTTGATTATGATTTCCAACAAGGTCAACTAACTGTAAGT 1231

181 I Q A E D L P G M D M S G T S D P Y V K 200  
1232 ATTCAAGCAGAAGATTTACCAGGAATGGACATGTTCAGGAACATCAGATCCATATGTAAA 1291

201 L Y L L P E K K K K V E T K V H R K T L 220  
1292 TTGTATTTGTTACCGGAGAAAAAGAAGAGTTGAGACGAAAGTACATCGAAAAACTCTT 1351

221 N P V F N E T F I F K V A F N E I T A K 240  
1352 AATCCAGTATTCAATGAGACATTCATTTTAAAGTCGCTTTCAACGAAATTACGGCAAAA 1411

241 T L V F A I Y D F D R F S K H D Q I G Q 260  
1412 ACTCTTGTCTTTGCAATTTATGATTTTCGATCGGTTTCAGTAAGCACGATCAAATCGGACAA 1471

261 V L I P L G K I D L G A V I E E W K D I 280  
1472 GTTCTCATTCCGCTTGAAAAATTGATTTGGGAGCTGTATCGAAGAATGGAAGGATATT 1531

281 A P P P D D K E A E K S L G D I C F S I 300  
1532 GCACCACCACAGATGACAAAGAAGCTGAGAAGAGTCTTGGTGACATTTGCTTCTCACTT 1591

301 R Y V P T A G K L T V V I L E A K N L K 320  
1592 CGGTACGTCCCAACTGCTGGTAAATTGACAGTGGTCATTCTGGAAGCAAAAAATCTTAAG 1651

321 K M D V G G L S D P Y V K I V L M Q G G 340  
1652 AAAATGGACGTCGGTGGTTTATCAGATCCTTATGTGAAGATTGTGTTGATGCAAGGTGGA 1711

341 K R L K K K K T S I K K C T L N P Y Y N 360  
1712 AAACGACTGAAAAAGAAGAAGACATCAATCAAAAAGTGTACACTTAACCCATATTATAAC 1771

361 E S F S F E V P F E Q I Q K V S L M I T 380  
1772 GAATCGTTCAGCTTTGAAGTGCCTTTGAAACAAATTCAGAAAGTTTCCCTTATGATCACT 1831

381 V M D Y D K L G S N D A I G R C L L G C 400  
1832 GTGATGGATTATGATAAACTTGGATCCAATGACGCTATTGGAAGGTGTCTATTGGGATGT 1891

401 N G T G A E L R H W M D M L A S P R R P 420  
1892 AATGGAACCGGTGCCGAGCTGAGGCATTGGATGGATATGTTGGCTTACCACGTCGTCCA 1951

421 I A Q W H T L G P V E E E G D K K D D K 440  
1952 ATTGCTCAATGGCATACTTGGACCAGTTGAAGAAGAAGGTGATAAGAAAGATGATAAG 2011

441 K T G G G G S G G G S M E K S F V I T D 460

2012 AAAaccggtGGTGGCGGCAGTGGTGGCGGCAGCATgGAGAAAAGTTTCGTGATAAcTGAT 2071

461 P R L P D N P I I F A S D G F L E L T E 480  
2072 CCACGGCTGCCAGACAATCCCATCATCTTCGCATCCGATggCTTCCTGGAGCTGACCGAG 2131

481 Y S R E E I L G R N G R F L Q G P E T D 500  
2132 TATTCCAGAGAGGAGATCCTGGGCCGAATGgCCGCTTCTGCAGGGACCAGAGACAGAC 2191

501 Q A T V Q K I R D A I R D Q R E I T V Q 520  
2192 CAGGCCACAGTGCAGAAGATTTCGCATGCCATTAGAGATCAGCGCGAGATTACCGTGCAG 2251

521 L I N Y T K S G K K F W N L L H L Q P M 540  
2252 CTGATAAACTACACAAAAAGCGGGAAGAAATTCTGGAACCTCtGcACCTCCAGCCCATG 2311

541 R D Q K G E L Q Y F I G V Q L D G S G G 560  
2312 AGGGACCAGAAGGGTGAGCTCCAGTATTTTCATCGGAGTGCAGCTGGATGGATCAGGAGGA 2371

561 G S G G G G A G I D M V S K G E E L F T 580  
2372 GGCTCAGGAGGAGGAGGCGGGGAATCGATatggtgagcaagggcgaggagctgttcacc 2431

581 G V V P I L V E L D G D V N G H K F S V 600  
2432 ggggtggtgcccatcctggtcgagctggacggcgacgtaaacggccacaagttcagcgtg 2491

601 S G E G E G D A T Y G K L T L K F I C T 620  
2492 tccggcgagggcgagggcgatgccacctacggcaagctgaccctgaagttcatctgcacc 2551

621 T G K L P V P W P T L V T T F G Y G L M 640  
2552 accggcaagctgcccgtgccctggcccaccctcgtgaccaccttcggctacggcctgatg 2611

641 C F A R Y P D H M K Q H D F F K S A M P 660  
2612 tgcttcgcccgtacccccgaccacatgaagcagcagcacttcttcaagtccgccatgccc 2671

661 E G Y V Q E R T I F F K D D G N Y K T R 680  
2672 gaaggctacgtccaggagcgcaccatcttcttcaaggacgacggcaactacaagaccgcg 2731

681 A E V K F E G D T L V N R I E L K G I D 700  
2732 gccgaggtgaagttcgagggcgacaccctggtgaaccgcatcgagctgaagggcatcgac 2791

701 F K E D G N I L G H K L E Y N Y N S H N 720  
2792 ttcaaggaggacggcaacatcctggggcacaagctggagtacaactacaacagccacaac 2851

721 V Y I M A D K Q K N G I K V N F K I R H 740  
2852 gtctatatcatggccgacaagcagaagaacggcatcaaggtgaacttcaagatccgccac 2911

741 N I E D G S V Q L A D H Y Q Q N T P I G 760  
2912 aacatcgaggacggcagcgtgcagctcggcaccactaccagcagaacacccccatcggc 2971

761 D G P V L L P D N H Y L S Y Q S A L S K 780  
2972 gacggccccgtgctgctgcccgacaaccactacctgagctaccagtcgcgcctgagcaaa 3031

781 D P N E K R D H M V L L E F V T A A G I 800  
3032 gaccccaacgagaagcgcgatcacatggtcctgctggagttcgtgaccgcccgggatc 3091

801 T L G M D E L Y K \* 810  
3092 actctcggcatggacgagctgtacaagTAG 3121