

# **Chemistry—A European Journal**

## **Supporting Information**

### **Controlling Site-Directed RNA Editing by Chemically Induced Dimerization**

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## Cloning of editase constructs I – IV

Constructs **I – IV** for co-expression of GAI<sub>1-92</sub>-ADAR1(Q) and SNAP<sub>f</sub>-GID1A were cloned in a pcDNA 5 vector via restriction/ligation. ADAR1(Q) and SNAP<sub>f</sub> were amplified from our own plasmids, GAI<sub>1-92</sub> and GID1A coding sequences were kindly provided by Dr. R. Wombacher, Ruprecht Karls University, Heidelberg, Germany.<sup>[1]</sup> GAI<sub>1-92</sub>-ADAR1(Q) and SNAP<sub>f</sub>-GID1A were then successively ligated into one common pcDNA 5 vector. Constructs **I** and **III** were generated by ligation into a vector with two consecutive CMV enhancers and CMV promoters, each followed by two copies of the *tet* operator (TetO<sub>2</sub>) via BamHI/Ascl/Apal and NotI/Nhel/Clal. Constructs **II** and **IV** were generated by ligation into a vector with one CMV enhancer and CMV promoter, followed by two copies of the *tet* operator (TetO<sub>2</sub>) and a central self-cleaving P2A via BamHI/Ascl/NotI and Xhol/Nhel/Pacl. The sequences of the respective constructs are attached as Appendix.

## General cultivation & generation of stable cell lines

In general, cells were cultivated in Dulbecco's Modified Eagle Medium (DMEM, LIFE TECHNOLOGIES) supplemented with 10 % fetal bovine serum (FBS, LIFE TECHNOLOGIES) and 100 U/ml penicillin-streptomycin (LIFE TECHNOLOGIES), short DMEM/FBS/P/S, at 37 °C with 5 % CO<sub>2</sub> in a water saturated steam atmosphere. Stable, inducible cell lines **1 – 4** with integrated constructs **I – IV** respectively were generated with the Flp-In™ T-REx™ system by LIFE TECHNOLOGIES. 4·10<sup>6</sup> 293 Flp-In™ T-REx™ cells were seeded in 10 ml DMEM/10 % FBS/100 µg/ml zeocin/15 µg/ml blasticidin (DMEM/FBS/Z/B) in a 10 cm dish. After 23 h, medium was replaced with DMEM/10 % FBS (DMEM/FBS) and 1 h later 9 µg pOG 44 and 1 µg of the respective construct in a pcDNA 5 vector were forward transfected with 30 µl Lipofectamine 2000 (THERMO FISHER SCIENTIFIC). After 24 h, medium was replaced with 15 ml DMEM/FBS/15 µg/ml blasticidin/100 µg/ml hygromycin (DMEM/FBS/B/H), followed by selection for approximately two weeks. Then, the stable cell lines were transferred to a 75 cm<sup>2</sup> cell culture flask and subsequently cultivated in DMEM/FBS/B/H.

## Western Blotting

### Preparation of lysates from wildtype 293T cells transiently expressing constructs I – IV

2·10<sup>5</sup> wildtype 293T cells were seeded in 500 µl DMEM/FBS/P/S in 3 wells of a 24 well plate per condition. After 24 h, medium was replaced with 450 µl DMEM/FBS and 300 ng of constructs **I – IV** in pcDNA 5 respectively were forward transfected with 1.2 µl Lipofectamine 2000 (LIFE TECHNOLOGIES). 24 h thereafter, medium was removed and cells were first washed with 500 µl PBS and then detached and suspended in 500 µl fresh PBS per well. Cells from one condition were combined and then centrifuged for 5 min at 1.600 rpm, followed by removal of PBS and resuspension of the cell pellets in 75 µl urea lysis buffer (8 M urea, 100 mM NaH<sub>2</sub>PO<sub>4</sub>, 10 mM Tris, pH 8.0). Cells were then lysed via shear force by drawing the solution 15× up and out a 19 gauge syringe. After centrifugation for 15 min at 16.000 rpm and 4 °C, the supernatant lysates were transferred to fresh reaction tubes.

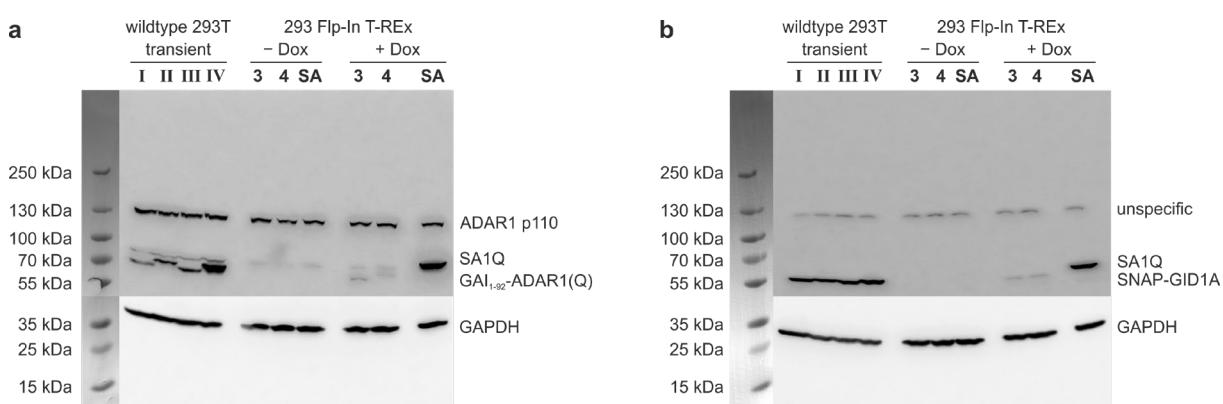
### Preparation of lysates from 293 Flp-In™ T-REx™ cell lines 3 & 4

Lysates from stable, inducible cell lines **3** and **4** expressing the hyperactive ADAR1Q were also analyzed via Western Blot. For comparison, our previously established SNAP-ADAR1Q 293 Flp-In™ T-REx™ cell line (SA1Q)<sup>[2]</sup> was examined in parallel. 1·10<sup>6</sup> 293 Flp-In™ T-REx™ cells from the respective cell line were seeded in 2500 µl medium in one well of a 6 well plate per condition. For the uninduced samples (– Dox) DMEM/FBS/B/H was used as medium, for the samples with doxycycline induction (+ Dox)

DMEM/FBS/B/H/ 10 ng/ml doxycycline (DMEM/FBS/B/H/D) was used. After 24 h, medium was removed and cells were first washed with 1000 µl PBS and then detached and suspended in 1000 µl fresh PBS. Centrifugation for 5 min at 1.600 rpm was followed by removal of PBS and resuspension of the cell pellets in 75 µl urea lysis buffer (8 M urea, 100 mM NaH<sub>2</sub>PO<sub>4</sub>, 10 mM Tris, pH 8.0). Cells were then again lysed via shear force by drawing the solution 15× up and out a 19-gauge syringe. After centrifugation for 15 min at 16.000 rpm and 4 °C, the supernatant lysates were transferred to fresh reaction tubes.

### PAGE & Western Blot

Total protein concentrations of all samples were determined via Bradford assay (SIGMA ALDRICH B6916) and equal amounts of proteins in 16.66 µl urea lysis buffer were heated with 3.33 µl 6× Laemmli buffer (0.4 M SDS, 60 mM Tris pH 6.8, 6.5 M glycerol, 0.6 M dithiothreitol, 0.9 mM bromophenol blue) for 5 min at 95 °C and 700 rpm. Subsequently, samples and PageRuler™ Plus protein ladder (THERMO FISHER 26620) were loaded to a Novex™ WedgeWell™ 8–16 % Tris-Glycine gel (THERMOM FISHER XP08165BOX), which was run at 90 V for 5 min followed by 160 V for 90 min. Proteins were transferred onto a PVDF membrane (BIO-RAD LABORATORIES) at 30 V and 4 °C for 18 h, followed by blocking in 5 % dry milk in TBST for 1 h. For characterization of GAI<sub>1-92</sub>-ADAR1(Q) expression, the respective blot was incubated with rabbit α-ADAR1 (1:1.000, BETHYL LABORATORIES A303-884A) in 5 % DryMilk-TBST at 4 °C overnight as primary antibody. For characterization of SNAP-GID1A expression, the respective blot was incubated with rabbit α-SNAP-tag (1:1.000, NEW ENGLAND BIOLABS P9310S) in 5 % DryMilk-TBST at room temperature for 2 h. In both cases, this was followed by incubation with goat α-rabbit HRP (1:5.000, JACKSON IMMUNORESEARCH 111-035-003) for 2 h at room temperature as secondary antibody. Chemiluminescence was measured with a FUSION FX by VILBER. As loading control, α-GAPDH (1:3.333, THERMO FISHER AM4300) in 5 % DryMilk-TBST was applied at 4 °C overnight, followed by goat α-mouse HRP (1:5.000, JACKSON IMMUNORESEARCH 115-035-003) in 5 % DryMilk-TBST for 2 h at room temperature. Chemiluminescence was again measured with a FUSION FX by VILBER.



**Figure S1. Full Western Blots (corresponding to sections shown in Figure 2b,c) of wildtype 293T cells transiently transfected with constructs **I – IV**, as well as GAI<sub>1-92</sub>-ADAR1Q/SNAP-GID1A 293 Flp-In T-REx cell lines **3** and **4** without (– Dox) and with 24 h (+ Dox) doxycycline induction. SA1Q 293 Flp-In T-REx cell line (**SA**) shown for comparison. **a)** Detection of the different ADAR1 proteins with α-ADAR1. GAI<sub>1-92</sub>-ADAR1(Q) from constructs **II** and **IV** is of slightly larger size due to the residual P2A peptide. An additional band originating from endogenous ADAR1 p110 can be observed equally for all samples. **b)** Detection of different SNAP proteins with α-SNAP-tag. Blots were cut above the GAPDH loading control before detection.**

## Generation of guideRNAs

NH<sub>2</sub>-guideRNAs were purchased from BIOSPRING in ion exchange HPLC-purified quality. guideRNAs were 22 to 25 nt long, containing a 5'-C6-aminolinker, and were chemically stabilized similar as described before.<sup>[3]</sup> Table S1 provides the sequences, modification patterns and extinction coefficients at 260 nm of all guideRNAs. The snap-GAPDH guideRNA was generated from the NH<sub>2</sub>-GAPDH guideRNA by a post-synthesis labeling protocol analogous to a previously published protocol,<sup>[4]</sup> by applying snap (35 eq). (snap)<sub>2</sub>-STAT1 and (snap)<sub>2</sub>-MECP2 were produced analogous to the previously reported improved protocol with DIC activation,<sup>[5]</sup> using (snap)<sub>2</sub> (17.5 eq).

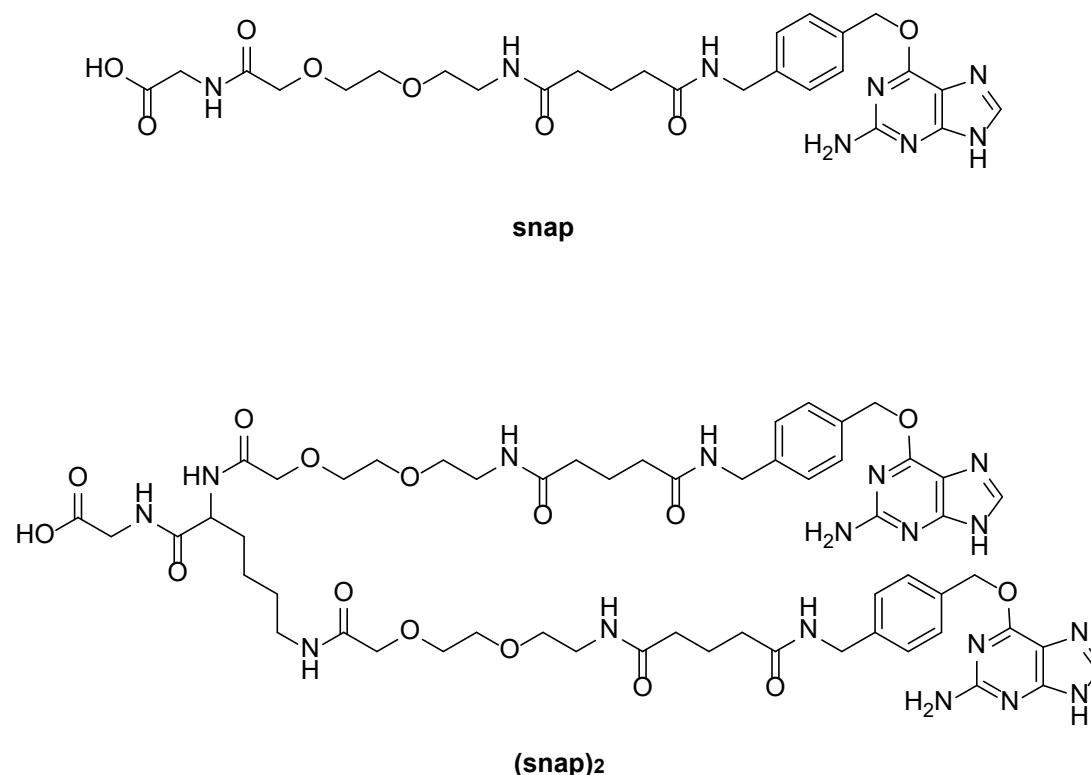


Figure S2. Structures of snap and (snap)<sub>2</sub>, which were pre-activated at their carboxylic acids and coupled to the 5'-C6-aminolinker of NH<sub>2</sub>-guideRNA.

Table S1. Sequences and  $\epsilon_{260\text{ nm}}$  of used guideRNAs. Normal uppercase = ribonucleotide, *italics* = 2'OMe, bold = LNA, *s* = phosphorothioate linkage.

guideRNA	Target	Sequence	$\epsilon_{260\text{ nm}} / \text{mM}^{-1}\text{cm}^{-1}$
NH <sub>2</sub> -GAPDH	GAPDH 3'-UTR	AsAs <i>U</i> AAGGGGU CCA CAUGG <i>s</i> CsAsAs <i>C</i>	232.00
snap-GAPDH	GAPDH 3'-UTR	AsAs <i>U</i> AAGGGGU CCA CAUGG <i>s</i> CsAsAs <i>C</i>	234.50
NH <sub>2</sub> -STAT1	STAT1 Y701C	As <i>G</i> s <i>U</i> GUC <i>U</i> UGAU ACA UCCAGU <i>U</i> sCs <i>C</i> s <i>U</i> <i>T</i>	246.50
(snap) <sub>2</sub> -STAT1	STAT1 Y701C	As <i>G</i> s <i>U</i> GUC <i>U</i> UGAU ACA UCCAGU <i>U</i> sCs <i>C</i> s <i>U</i> <i>T</i>	251.50
NH <sub>2</sub> -MECP2	MECP2 R106Q	As <i>C</i> s <i>A</i> T <i>U</i> AAG <i>C</i> UU UCG UGUCCA <i>A</i> s <i>C</i> s <i>C</i> s <i>U</i> <i>T</i>	245.65
(snap) <sub>2</sub> -MECP2	MECP2 R106Q	As <i>C</i> s <i>A</i> T <i>U</i> AAG <i>C</i> UU UCG UGUCCA <i>A</i> s <i>C</i> s <i>C</i> s <i>U</i> <i>T</i>	250.65

## Editing experiments

All editing experiments depicted in bar graphs were conducted in biological triplicates and standard deviations are shown.

### Editing of endogenous GAPDH under genomic expression of editase constructs

4·10<sup>5</sup> of the respective 293 Flp-In™ T-REx™ cells were seeded in 500 µl DMEM/FBS/B/H/D in a 24 well plate. After 24 h, 8·10<sup>4</sup> cells were reverse transfected in a 96 well plate with 5.0 pmol of the respective guideRNA with 0.5 µl Lipofectamine 2000 and 10 µM GA<sub>3</sub>-AM (from a 10 mM stock in DMSO) were added to the medium of the indicated samples. Doxycycline concentration was kept at 10 ng/ml and after further 24 h cells were harvested. RNA isolation was performed with the Monarch® RNA cleanup kit from NEW ENGLAND BIOLABS, followed by DNase I digestion. Purified RNA was then reverse transcribed and amplified with the One Step RT-PCR Kit from BIOTECHRABBIT and subsequently analyzed with Sanger sequencing (MICROSYNTH). A-to-I editing yields were determined by dividing the peak height for guanosine by the sum of the peak heights for both adenosine and guanosine.

### Determination of dose-response to GA<sub>3</sub>-AM

To determine the dose-response to GA<sub>3</sub>-AM, editing experiments were performed in 293 Flp-In™ T-REx™ cells from cell line 4 as described for the editing of endogenous GAPDH. 0.01, 0.03, 0.1, 0.3, 1, 3, 10, 30, 60 or 100 µM GA<sub>3</sub>-AM were applied from the respective 1000× stocks in DMSO. As negative controls, cells were treated with NH<sub>2</sub>-GAPDH without GA<sub>3</sub>-AM, NH<sub>2</sub>-GAPDH + 100 µM GA<sub>3</sub>-AM and snap-GAPDH without GA<sub>3</sub>-AM, none of which showed substantial editing.

The experiment was conducted in biological triplicates and the mean editing yields after treatment with snap-GAPDH and the respective concentration of GA<sub>3</sub>-AM were plotted on a logarithmic scale against the concentration of GA<sub>3</sub>-AM. The following logistic dose response curve was fitted to the data:

$$\text{editing } e / \% = \frac{e_{\min} - e_{\max}}{1 + \left(\frac{x}{x_0}\right)^p} + e_{\max}$$

with  $e_{\min} = 1.5\%$ : minimal editing,  $e_{\max} = 31\%$ : maximal editing,  $x: c(\text{GA}_3\text{-AM})$ ,  $x_0 = 0.29$ : center,  $p = 0.56$ : power and corrected  $R^2 = 0.9945$ . The resulting half maximal effective concentration of GA<sub>3</sub>-AM is EC<sub>50</sub> ≈ 290 nM.

### Editing of endogenous STAT1 under genomic expression of editase constructs

4·10<sup>5</sup> of the respective 293 Flp-In™ T-REx™ cells were seeded in 500 µl DMEM/FBS/B/H/D in a 24 well plate. After 24 h, 8·10<sup>4</sup> cells were reverse transfected in a 96 well plate with 5.0 pmol of the respective guideRNA with 0.5 µl Lipofectamine 2000 and 100 µM GA<sub>3</sub>-AM (from a 100 mM stock in DMSO) were added to the medium of the indicated samples. Doxycycline concentration was kept at 10 ng/ml and after further 24 h cells were harvested. RNA isolation was performed with the Monarch® RNA cleanup kit from NEW ENGLAND BIOLABS. Purified RNA was then treated with a DNA oligonucleotide of complementary sequence to the STAT1 guideRNA (5'-aaggaaactggatcttatcaagacacc, 1 µM) at 95 °C for 1 min to trap the guideRNA, followed by reverse transcription and amplification with the One Step RT-PCR Kit from BIOTECHRABBIT. A-to-I editing yields were again determined by Sanger sequencing (MICROSYNTH) by dividing the peak height for guanosine by the sum of the peak heights for both adenosine and guanosine.

#### **Editing of endogenous STAT1 under transient expression of editase constructs**

$2 \cdot 10^5$  wildtype 293T cells were seeded in 500 µl DMEM/FBS/P/S in a 24 well plate. After 24 h, medium was replaced with 450 µl DMEM/FBS and 300 ng of either construct **I**, **II**, **III** or **IV** in pcDNA 5 were forward transfected with 1.2 µl Lipofectamine 2000. After further 24 h,  $8 \cdot 10^4$  cells were reverse transfected in a 96 well plate with the respective amount of the indicated guideRNA with 0.5 µl Lipofectamine 2000 and 10 µM or 100 µM GA<sub>3</sub>-AM (from a 10 mM or 100 mM stock in DMSO) were added to the medium as indicated. Doxycycline concentration was kept at 10 ng/ml and after further 24 h cells were harvested and proceeded as for the editing of STAT1 under genomic expression of the editase constructs.

#### **Editing of transfected MECP2 under transient expression of editase constructs**

$2 \cdot 10^5$  wildtype 293T cells were seeded in 500 µl DMEM/FBS/P/S in a 24 well plate. After 24 h, medium was replaced with 450 µl DMEM/FBS and 300 ng mMECP2 R106Q in pEGFP-N3 (kindly provided by Dr. G. Mandel, Oregon Health and Science University, Portland, USA)<sup>[6]</sup> together with 300 ng of either construct **III**, **IV** or SNAP-ADAR1Q in pcDNA 5 were forward transfected with 2.4 µl Lipofectamine 2000. After further 24 h,  $8 \cdot 10^4$  cells were reverse transfected in a 96 well plate with 1.0 pmol of the indicated guideRNA with 0.5 µl Lipofectamine 2000 and 10 µM or 100 µM GA<sub>3</sub>-AM (from a 10 mM or 100 mM stock in DMSO) were added to the medium as indicated. Doxycycline concentration was kept at 10 ng/ml and after further 24 h cells were harvested and proceeded as for the editing of GAPDH.

#### **Supporting literature**

- [1] K. M. Schelkle, T. Griesbaum, D. Ollech, S. Becht, T. Buckup, M. Hamburger, R. Wombacher, *Angew. Chem. Int. Ed.* **2015**, *54*, 2825–2829; *Angew. Chem.* **2015**, *127*, 2867–2871.
- [2] P. Vogel, M. Moschref, Q. Li, T. Merkle, K. D. Selvasaravanan, J. B. Li, T. Stafforst, *Nat. Methods* **2018**, *15*, 535–538.
- [3] P. Vogel, T. Stafforst, *ChemMedChem* **2014**, *9*, 2021–2025.
- [4] A. Hanswillemenke, T. Kuzdere, P. Vogel, G. Jékely, T. Stafforst, *J. Am. Chem. Soc.* **2015**, *137*, 15875–15881.
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- [6] J. R. Sinnamon, S. Y. Kim, G. M. Corson, Z. Song, H. Nakai, J. P. Adelman, G. Mandel, *Proc. Natl. Acad. Sci. U. S. A.* **2017**, *114*, E9395–E9402.

## Appendix

### Constructs I – IV

Construct I: CMV-enhancer – CMV promoter – TetO<sub>2</sub> – GAI<sub>1-92</sub> – ADAR1 – bGH – CMV-enhancer – CMV promoter – TetO<sub>2</sub> – SNAP<sub>f</sub>-tag – GID1A – bGH

GACATTGATTATTGACTAGTTATTAAATAGTAATCAATTACGGGGTATTAGTCATAGCCCCATATATG  
GAGTTCCCGTACATACTTACGGTAAATGGCCGCCTGGCTGACCGCCAAACGACCCCCGCCATT  
GACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTCCATTGACGTCAATGGGTGG  
AGTATTTCAGGTAAACTGCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCTATT  
GACGTCAATGACGGTAAATGGCCGCCTGGCATTATGCCAGTACATGACCTATGGGACTTCCATT  
TTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTATGCCAGTACATCAATG  
GGCGTGGATAGCGGTTGACTCACGGGATTCCAAGTCTCCACCCATTGACGTCAATGGGAGTTG  
TTTGGCACCAAATCAACGGACTTCCAAAATGTCGAACAACCGCCATTGACGCAAATGGG  
CGTAGGCGTGTACGGTGGGAGGTCTATATAAGCAGAGCTCCCTATCAGTGATAGAGATCTCCCTA  
TCAGTGATAGAGATCGCGACGAGCTCGTTAGTGAACCGTCAGATGCCCTGGAGACGCCATCCACGC  
TGTGACCTCCATAGAACGACCGGGACCGATCCAGCCTCCGGACTCTAGCGTTAAACTTAAGCT  
TGGTACCGAGCTCGGATCCCCACCATGAAGAGAGATCATCATCATCATCAAGATAAGAAGAC  
TATGATGATGAATGAAGAACGACGGTAACGGCATGGATGAGCTCTAGCTGTTCTGGTTACAAGG  
TTAGGTATCCGAAATGGCTGATGTTGCTCAGAAACTTGTGAGCAGCTGAAGTTATGATGCTAAATGTT  
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GCTTGATTCTATGCTCACCGACCTTAATCCTGCAGGGGAGGCGCCAGGGTCTGGCGCGAGTA  
AGGCAGAACGATGGGTTCACAGAGGTAAACCCAGTGACAGGGGCCAGTCTCAGAAGAACTATGCTC  
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ACAGGGAAATCGCTGTGAAAAGGAGATTCTCAGCCTAAAGGAGAAACTGTCAATGACTGCCATGC  
AGAAATAATCTCCGGAGAGGCTCATCAGGTTCTACAGTGAGTTAATGAAATACAACCTCCAGA  
CTGCGAAGGATAGTATATTGAACCTGCTAAGGGAGGAGAAAGCTCCAAATAAAAGACTGTGTC  
TTCATCTGTATATCAGCACTGCTCGTGGAGATGGCGCCCTTTGACAAGTCCTGCAGCGACCG  
TGCTATGAAAGCACAGAACCTCCGCCACTACCCTGTCTCGAGAACATCCAAACAAGGAAAGCTCCGCA  
CCAAGGTGGAGAACGGAGAACGGCACAATCCCTGTGGAAATCCAGTGACATTGTGCCTACGTGGGATGGC  
ATTCGGCTGGGGAGAGACTCCGTACCATGTCCTGTAGTGACAAAATCCTACGCTGGAACGTGCTGGG  
CCTGCAAGGGCACTGTTGACCCACTTCTGCAGCCCATTATCTCAAATCTGCACATTGGTTACC  
TTTCAGCCAAGGGCATCTGACCCGTCTATTGCTGTCGTGACAAGAGATGGGAGTGCATTGAG  
GATGGACTACGACATCCCTTATTGTCAACCACCCAAAGGTGGCAGAGTCAGCATATGATTCAA  
AAGGCAATCCGGGAAGACTAAGGAGACAAGCGTAACGGTGTCTGGCTGATGGCTATGACCTGGAGA  
TCCTGGACGGTACCGAGGGACTGTGGATGGGCCACGGAATGAATTGTCGGGTCTCCAAAAGAAC  
ATTTTCTCTATTAAAGAAGCTCTGCTCCTCCGTACCGCAGGGATCTACTGAGACTCTCTATGG  
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CCCATGTACGATTTAATTATGCGGACGTGATGAGCGAAGTACGATCCCACGACCGAGGCCGTTAAA  
CCCGCTGATCAGCCTCGACTGTCCTCTAGTGCCAGCCATCTGTGTTGCCCTCCCCGTGCCT  
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TCTGAGTAGGTGTCATTCTATTCTGGGGGTGGGGCAGGACAGCAAGGGGAGGATTGGGAAG  
ACAATAGCAGGCATGCTGGGATGCGGTGGCTATGGCTCTGAGGCGGAAAGAACCAGCTGGGC  
TCTAGGGGTATCCCCACGCCCTGTAGCGGCCATTAAGCGCGGGGTGTGGTGGTTACGCGCAG  
CGTGACCGCTACACTGCCAGGCCCTAGGCCCGCTCCTTCGCTTCTCCCTTCTCGCCA  
CGTCGCCGGTCGATGTACGGGCCAGATAACGCGTTGACATTGATTGACTAGTTATTAGTA  
ATCAATTACGGGTATTAGTCATAGCCATATGGAGTTCCGCGTTACATAACTACGGTAAATG  
GCCCGCCTGGCTGACCGCCAAACGACCCCCGCCATTGACGTCAATAATGACGTATGTCCTAGTA  
ACGCCAATAGGACTTCCATTGACGTCAATGGGTGGAGTATTACGGTAAACTGCCACTTGGCAGT  
ACATCAAGTGTATCATATGCCAAGTACGCCCTATTGACGTCAATGACGGTAAATGCCCGCCTGGC

ATTATGCCAGTACATGACCTTATGGGACTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCT  
ATTACCATGGTATGCGGTTTGGCAGTACATCAATGGCGTGGATAGCGGTTGACTCACGGGGATT  
TCCAAGTCTCCACCCATTGACGTCAATGGGAGTTGGCACAAAATCAACGGGACTTCAA  
AATGTCGTAACAACCTCCGCCCCATTGACGCAAATGGCGGTAGGCAGTACGGTGGGAGGTCTATATA  
AGCAGAGCTCTCCCTATCAGTGATAGAGATCTCCCTATCAGTGATAGAGAGCGTGCATAGGAAACATC  
CACCACTTAGTGAATTGTAGCACGGCTTCAGAACGGCCGCCACCAGTGGACAAAGACTGCGAAATG  
AAGCGCACCCCTGGATAGCCTCTGGCAAGCTGGAACTGTCTGGGTGCGAACAGGGCTGCACCG  
TATCATCTCCTGGCAAAGGAACATCTGCCGCCAGCCGTGGCTCAACGCTACTTCACCAGCCTGAGGCC  
TGGCGGACCAGAGCCACTGATGCAGGCCACCGCCTGGCTCAACGCTACTTCACCAGCCTGAGGCC  
ATCGAGGAGTTCCCTGTGCCAGCCCTGCCACCAGTGTCCAGCAGGAGAGCTTACCCGCCAGGT  
GCTGTGGAAACTGCTGAAAGTGGTGAAGTTCGGAGAGGTCATCAGCTACAGCCACCTGGCGCCCTGG  
CCGGCAATCCCGCCACCGCCCGCTGAAAACCGCCCTGAGCGGAAATCCCGTGCCTATTCTGATC  
CCCTGCCACCGGGTGGTGCAGGGCAGCTGGACGTGGGGGCTACGAGGGCAGGCTGCCGTGAAAGA  
GTGGCTGCTGGCCCACGAGGGCACAGACTGGCAAGCCTGGCTGGTCCAGGAGCCGGAGCTAGCG  
GCCCAACAGGAATGGCTGCGAGCGATGAAGTTAATCTTATTGAGAGCAGAACAGTGGTCTCTCAAT  
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CCGACACTAGCTGAGTATCTAGACCGTAAAGTCACTGCAAACGCCAATCGGTTGATGGGTTTCT  
CGTCGATGTCTGATTGATCGCAGGATCAATCTCTAAGCAGAGTCTAGACAGCTTGTGAGCTTGT  
CAAGAGCAACCTCCTAGTATTAGCTGGAGAACGCTGTTGATGGCGACATTGTCCTGTTATATT  
GTTCTCCATGGAGGTAGCTTGCTATTCTGCAAACAGTGCATCTACGATACTCTTGTGCA  
GGCTTGTGGTTGTCAAGTGTGTTGTCCTGTGAATTATCGCGTGCACCAGAGAACCATAC  
CCTTGTGTTATGATGGTGGATTGCTCTTAATTGGGTTAACTCCAGATCTGGCTAAATCCAA  
GAAAGACTCAAAGGCCATATTCTGGCTGGTGTAGCTCTGGAGGTAACATCGCATAATGTGG  
CTTAAGAGCGGGTGAATCGGAAATTGATGTTGGGAACATTCTGCTGAATCCTATGTTGGTGG  
AATGAGAGAACGGAGTCTGAGAAAAGTTGGATGGAAATACTTGTGACGGTTAGAGACCGCGATTG  
GTACTGGAAAGCGTTTACCGAGGGAGAACGATAGAGAGCATCCAGCGTGTAACTCGTTAGCCCGA  
GAGGGAAAAGCTAGAAGGAGTGAGTTCCCAAGAGTCTGTGTTGTCGCGGGTTGGATTGATT  
AGAGATTGGCAGTTGGCATACCGGAAAGGGCTCAAGAAAGCGGGTCAAGAGGTTAACGTTATGCATT  
AGAGAAAGCAACTGTTGGTTTACCTCTGCCTAATAACAATCATTCCATAATGTTATGGATGAGA  
TTCCGGCGTTGTAAACCGGAATTGATGTTGAATCGATATTTCAGATATCGTGTAGTAGGGTTGCACCG  
ACCGCGATGTGGATTAGTGCCTGCCTCTAGTGCCTGCCTCTAATAAAATGAGGAAATTGCATCGCATT  
TTCCTTGACCCCTGGAAGGTGCCACTCCACTGTCCTTCTTAATAAAATGAGGAAATTGCATCGCATT  
GTCTGAGTAGGTGTCAATTCTATTCTGGGGGTGGGAGGACAGCAAGGGGGAGGATTGGGAA  
GACAATAGCAGGCATGCTGGGATGCGGTGGCTATGG

**Construct II:** CMV-enhancer – CMV promoter – TetO<sub>2</sub> – GAI<sub>1-92</sub> – ADAR1 – P2A – SNAP<sub>f</sub>-tag – GID1A–bGH

GACATTGATTATTGACTAGTTATTAAATAGTAATCAATTACGGGGTATTAGTCATAGCCCATAATAG  
GAGTTCCCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCCGCCATT  
GACGTCAATAATGACGTATGTCCCATAGTAACGCCAATAGGGACTTCCATTGACGTCAATGGGTGG  
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GAAGACAATAGCAGGCATGCTGGGATGCGGTGGCTATGG

**Construct III:** CMV-enhancer – CMV promoter – TetO<sub>2</sub> – GAI<sub>1-92</sub> – ADAR1Q – bGH – CMV-enhancer – CMV promoter – TetO<sub>2</sub> – SNAP<sub>r</sub>-tag – GID1A – bGH

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GACAATAGCAGGCATGCTGGGATGCGGTGGCTATGG

**Construct IV: CMV-enhancer – CMV promoter – TetO<sub>2</sub> – GAI<sub>1-92</sub> – ADAR1Q – P2A – SNAP<sub>r</sub>-tag – GID1A–bGH**

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