Supporting Information

Site-directed RNA editing in vivo can be triggered by the light-driven assembly of an artificial riboprotein

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Supplementary Information on chemical synthesis, compound characterization and additional editing data is available in the online version of the paper. Correspondence and requests for materials should be addressed to TS (thorsten.stafforst@uni-tuebingen.de).

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Chemical Synthesis

Chemicals
If not stated otherwise, all substrates and reagents required for synthesis and biochemical studies were purchased from commercial providers and used without further purification.

General Methods
All column chromatographic purifications were carried out on self-packed columns of silica gel (0.04-0.063 mm/230-240 mesh). Thin-layer chromatography (TLC) was performed on silica gel sheets (60 F254, 0.2 mm, 5 x 10 cm, Merck) and visualized under UV light (254 nm). All analytical and preparative HPLC runs were performed on a Shimadzu system (SCL-10A VP, SPD-20AV, LC-20AT) running with 0.1% TFA in water (Eluent A) and 0.1% TFA in acetonitrile/water (9:1, Eluent B). Analytical HPLC was performed using an EC 125/4 Nucleodur C18 column by Machery + Nagel and preparative HPLC was performed using a VP 250/10 Nucleodur C18 column by Machery + Nagel. $^1$H and $^{13}$C spectra were recorded on a Bruker ARX 250 or a Bruker Avance 400. $^1$H, $^{13}$C, HCCH-Cosy, HSQC and HMBC experiments for the discrimination of Npom-BG isomers (1a/1b) were performed using a Bruker AMX-600. High resolution mass spectrometry was performed on a maXis4G ESI-TOF-MS by Bruker Daltonics.
Figure S1. Synthesis of Npom-caged BG- derivatives. a) DBU, DMF, r.t, 2.5 h b) K₂CO₃, MeOH, 50°C, 4 h c) OSu-FAM, TEA, DMF, r.t., 1 h d) Solid-phase peptide synthesis (see page S12).

Synthesis of \( ^{N7/N9}\text{Npom-BG-TFA (1a/1b)} \)

BG-TFA (120 mg, 0.33 mmol, 1 eq; synthesized according to Keppler et al.\[^{S1}\]) was solved in DMF (1.2 ml) in a dry flask under argon atmosphere. Diazabicycloundecen (DBU, 150 μl, 153 mg, 1 mmol, 3 eq) was added at r.t. and the solution was stirred for 30 min. Npom-Cl (0.5 mmol, ~1.5 eq, \textit{in situ}; synthesized according to Lusic et al.\[^{S2}\]) was solved in DMF (1.6 ml) and added drop wise. After 2.5 h stirring at r.t., the reaction mixture was diluted with EtOAc, washed three times with 1% citric acid and Brine and dried over Na₂SO₄. Evaporation of the organic phase resulted in 180 mg crude product. Silica gel column chromatography (2-4% MeOH in DCM) yielded 120 mg (63%) total product. The isomers were cleanly separable and were obtained in a 1:2 ratio (21% yield of \( ^{N7}\text{Npom-BG-TFA (1a)} \) and 42% yield of \( ^{N9}\text{Npom-BG-TFA (1b)} \)).

Rf(DCM/MeOH, 98:2): \( N7 = 0.08, N9 = 0.32 \)
Rf(DCM/MeOH, 95:5): \( N7 = 0.50, N9 = 0.55 \)
**N7** Npom-BG-TFA, characterization:

1H NMR (600 MHz, DMSO-d6): δ = 9.99 (t, J = 5.9 Hz, 1H), 8.07 (s, 1H), 7.48 (d, J = 8.1 Hz, 2H), 7.35 (s, 1H), 7.28 (d, J = 8.1 Hz, 2H), 6.84 (s, 1H), 6.22 (s, 2H), 6.16 (s, 1H), 6.04 (s, 1H), 5.54 (m, 2H), 5.46 (m, 2H), 5.13 (q, J = 6.2 Hz, 1H), 4.39 (d, J = 5.9 Hz, 2H), 1.33 (d, J = 6.2 Hz, 3H).

13C NMR (151 MHz, DMSO-d6): 164.1, 159.8, 156.4 (q, 2\(^{13}J(C,F)\) = 36.2 Hz), 156.3, 151.8, 146.5, 145.9, 140.5, 137.2, 136.2, 135.6, 127.9, 127.5, 116.0 (q, 1\(^{13}J(C,F)\) = 288.4 Hz), 105.6, 105.2, 104.3, 103.2, 74.9, 72.0, 66.6, 42.4, 23.3.

HR-ESI-MS: [M+H]+ (theoretical) = 590.16056 for C\(_{25}\)H\(_{23}\)F\(_3\)N\(_7\)O\(_7\); [M+H]+ (found) = 590.16118

**N9** Npom-BG-TFA, characterization:

1H NMR (600 MHz, DMSO-d6): 10.00 (t, J = 6.0 Hz, 1H), 7.80 (s, 1H), 7.49 (d, J = 8.1 Hz, 2H), 7.46 (s, 1H), 7.31 (d, J = 8.1 Hz, 2H), 6.97 (s, 1H), 6.34 (s, 2H), 6.15 (s, 1H), 6.03 (s, 1H), 5.43-5.49 (m, 2H), 5.40 (d, 2\(^{13}J\) = 11.4 Hz), 5.32 (d, 2\(^{13}J\) = 11.4 Hz), 5.21 (q, J = 6.3 Hz, 1H), 4.41 (d, J = 6.0 Hz, 2H), 1.38 (d, J = 6.3 Hz, 3H).

13C NMR (151 MHz, DMSO-d6): 160.8, 157.2 (q, 2\(^{13}J(C,F)\) = 36.2 Hz), 155.3, 152.7, 147.5, 142.1, 140.5, 138.2, 137.0, 136.6, 129.6, 128.3, 116.9 (q, 1\(^{13}J(C,F)\) = 288.4 Hz), 114.3, 106.7, 105.2, 104.1, 73.1, 71.7, 67.5, 43.3, 24.1.

HR-ESI-MS: [M+Na]+ (theoretical) = 612.14250 for C\(_{25}\)H\(_{22}\)F\(_3\)N\(_7\)O\(_7\)Na; [M+Na]+ (found) = 612.14262

The N7- and N9- isomer of Npom-BG-TFA were characterized by 1D- and 2D-NMR spectroscopy (see Figure S2-5). All peaks in the 1H and 13C spectra were assigned supported by the data from heteronuclear multiple-bond correlation spectroscopy (HMBC) experiments. The carbon atoms of the TFA-protection group show characteristic coupling to the 19F atoms.

In the HMBC spectrum of N7Npom-BG-TFA a crosspeak between the protons of the oxymethyl bridge of the Npom-group and C5 of guanosine was observed, while a cross-peak between the oxymethyl bridge and C4 was observed for N9Npom-BG-TFA. Figure S5 shows the HMBC spectra of both isomers and signals relevant for the assignment of the regioisomers are highlighted. The Npom substitution has a strong effect on chemical shifts of C4 (N7: 165.14 ppm, N9: 155.25 ppm) and C5 (N7: 105.23 ppm, N9: 114.28 ppm). Furthermore, the oxymethyl bridge of the Npom group and the oxygen and O6 methylene group of BG are in close proximity in the case of N7Npom-BG-TFA, shifting the 1H signal of the oxymethyl bridge from 5.36 ppm (N9) to 5.53 ppm (N7).
Figure S2. 1H- & 13C-NMR spectra of N7Npom-BG-TFA (1a).
Figure S3. HSQC- and H,H-COSY of $^{N_7}$Pom-BG-TFA (1a).
Figure S4. 1H- & 13C-NMR spectra of \textsuperscript{N\textsubscript{8}}Npom-BG-TFA (1b).
Figure S4 continued H,H-COSY of $^{N6}$Npom-BG-TFA (1b).
Figure S5. Assignment of the regio-isomers via HMBC NMR experiments of compounds 1a (top, N7) versus 1b (bottom, N9). Signals significant for the assignment are marked.
For deprotection of the trifluoroacetamide group, 50 mg of the respective isomer of Npom-BG-TFA (1a/1b, 90 μmol, 1 eq) was solved in 1 ml MeOH and 150 μl water. Potassium carbonate (K₂CO₃, 100 mg, 720 μmol, 8 eq) was added and the reaction mixture was heated to 50°C for 4 h. HPLC analysis showed full conversion of the starting material. No hydrolysis of the Npom-group was observed. 1 M HCl was added to the solution until a neutral pH was reached and all solvent was evaporated. When Npom-BG-NH₂ was used for solid phase synthesis of the Npom-BG-Linker for the later incorporation into guideRNAs, then Npom-BG-NH₂ was purified via preparative HPLC. For this, the crude reaction mixture was neutralized to pH 7 with 1 M HCl, all solvents were evaporated, the crude product was solved in water/ACN/TFA (50:50:0.1), filtered and applied to preparative HPLC. 38 mg (90%) HPLC-pure product was recovered (see Figure S6).

For workup of larger scale reactions MeOH was removed by evaporation and the resulting precipitate was resolved in EtOAc by ultrasonification. The organic phase was washed three times with 1M NaOH/Brine (1:9) and dried over Na₂SO₄. Evaporation yielded ~80% of the respective Npom-BG-NH₂ isomer that was used without further purification for the synthesis of N₇/N₉ Npom-BG-FAM (3a/3b).

N₇ Isomer: HR-ESI-MS: 
[M+H]⁺(theoretical) = 494.1783 for C₂₃H₂₄N₇O₆
[M+H]⁺(found) = 494.1781

N₉ Isomer: HR-ESI-MS: 
[M+H]⁺(theoretical) = 494.1783 for C₂₃H₂₄N₇O₆
[M+H]⁺(found) = 494.1784

Figure S6. Analytical HPLC-analyses of N7 and N9 Npom-BG-NH₂ (2a/2b), top. unpurified compounds for NpomBG-FAM synthesis; bottom: HPLC-purified compounds for incorporation into guideRNAs.
Npom-BG-FAM (3a/3b)

3-4 mg of the respective isomer of Npom-BG-NH₂ (2a/2b, ~7 μmol, 1 eq) were solved in DMF (300 μl) in an amber eppendorf cup (1.5 ml) and trimethylamine (TEA, 5 μl) was added. Then, 5(6)-carboxyfluorescein succinimidyl ester (~3.5 mg, 7.4 μmol, 1.05 eq) was solved in DMF (50 μl) and added to the Npom-BG-NH₂. The reaction cup was incubated at r.t. for 1 h. DMF and TEA were removed under high vacuum and the crude product was solved in water/ACN/TFA (60:40:0.1), filtered and applied to preparative HPLC. For both isomers, the product fractions contained two products referring to the C5 and C6 isomers of carboxyfluorescein (see Figure S7). The main fractions were lyophiliized and resolved in dimethylsulfoxide (DMSO, 50 μl). The concentration was adjusted to 1 mM by spectroscopic measurement of the carboxyfluorescein absorbance at a wavelength of 500 nm (assuming an extinction coefficient of FAM at 500 nm to equal 93.000 M⁻¹cm⁻¹). The extinction was determined in a buffer containing 20 mM Tris-HCl (pH 8), 100 mM NaCl and 5% glycerol.

HR-ESI-MS (N⁷Npom-BG-FAM): [M+H]⁺(theoretical) = 852.22600; [M+H]⁺(found) = 852.22538

HR-ESI-MS (N⁹Npom-BG-FAM): [M+H]⁺(theoretical) = 852.22600; [M+H]⁺(found) = 852.22499

Figure S7. Analytical HPLC of N7- and N9-Npom-BG-FAM (3a/3b)
Synthesis of \( {^N^7}\text{Npom-BG-Linker-COOH} \) (4) via solid phase peptide synthesis

Synthesis of \( {^N^7}\text{Npom-BG-Linker-COOH} \) was performed in a syringe and if not indicated otherwise the resin was washed with N-Methyl-2-pyrrolidon (NMP)/DCM (4 x 1:1, 4 x DCM and 4 x NMP) after every coupling or deprotection step. Fmoc-EEG-COOH (Fmoc-aminoethoxy-ethoxy-acetic acid, synthesized according to Visintin et al. \([S^3]\), 179 mg, 460 \( \mu \)mol, 4.14 eq) was pre-activated with HBTU (162 mg, 427 \( \mu \)mol, 3.85 eq) and HOBt (54 mg, 400 \( \mu \)mol, 3.6 eq) for 10 min and coupled to the H-Gly-2-CITrt resin (EMD Millipore, 178 mg, 0.63 mmol/g, 111 \( \mu \)mol, 1 eq) in DIPEA/NMP (1:8). After 50 min of coupling, the resin was washed and capped with 5 ml NMP/DIPEA/acetic anhydride (10:1:1, 3x). Fmoc-deprotection was performed using 20% piperidine in NMP (3x5 ml, 10 min each). Glutaric anhydride (125 mg ,1.1 mmol, 10 eq) was coupled in NMP (1.2 ml) with DIPEA (200 \( \mu \)l) for 25 min. An additional washing step with 1% NaOH in Dioxan/H\textsubscript{2}O (1:1, 1 min) was performed. Afterwards, the resin was washed with NMP/DCM thoroughly to ensure that all water was removed. The glutaric acid was activated on the solid phase two times with pentafluorophenyl trifluoroacetate (Tokyo chemical industries,155 mg, 95 \( \mu \)l, 555 \( \mu \)mol, 5 eq) in 2 ml pyrimidine/DCM (1:1) and the resin was washed with dry NMP (4 x). \( {^N^7}\text{Npom-BG-NH}_2 \) (2a, 15 mg, 30 \( \mu \)mol, 0.27 eq) was coupled to the resin in NMP/DMSO/DIPEA (100:15:8, 1.2 ml) at r.t. overnight. The syringe was protected from light. Cleavage from the trityl-resin was performed with 0.5% TFA in DCM/hexafluoro-2-propanol (9:1, 50 ml). Evaporation of the cleavage solution yielded 80 mg of crude product which was solved in ACN/water, filtered and applied to preparative HPLC. Product fractions were identified by ESI-MS and analyzed by analytical HPLC (Figure S8). A stock solution of \( {^N^7}\text{Npom-BG-Linker-COOH} \) in DMSO was prepared and the concentration was determined photometrically by observing Npom absorption (\( \varepsilon_{360\text{nm}} = 4300 \text{ M}^{-1}\text{cm}^{-1} \), as determined for (R,S)-1-(6-Nitro-1,3-benzodioxol-5-yl)ethan-1-ol (Npom-OH)). Overall, 3.05 \( \mu \)mol (10% with respect to 2a) of clean product were obtained.

HR-ESI-MS: \([\text{M+H}]^+\text{(theoretical)} = 810.30531 \) for \( \text{C}_{36}\text{H}_{44}\text{N}_{9}\text{O}_{13} \);  
\([\text{M+H}]^+\text{(found)} = 810.30417 \)

Figure S8. Analytical HPLC of N7-Npom-BG-Linker-COOH (4).
Synthesis of Npom-guideRNAs

guideRNAs that carry only the 5′-C6-aminolinker but no further chemical modifications were obtained from Eurofins Germany in HPLC-purified, MALDI-TOF-confirmed quality. The phosphothioate / 2′-OMe modified oligomers were obtained from Biospring GmbH (Frankfurt) in HPLC-clean quality with sodium as counter ion. Npom-guideRNAs were synthesized on a 100 µg scale (~12-15 nmol depending on sequence, length and modification of the guideRNAs). Antogamir-like modified guideRNAs were solved in RNase free water to a concentration of 6 µg/µl. Stop66 NH2-guideRNAs were precipitated with 0.1 volumes of 3 M NaCl and 3 volumes of 100% EtOH, washed with 70% EtOH and dissolved in RNase free water (6 µg/µl) prior to coupling.

A 35 mM stock solution of N7-Npom-BG-Linker-COOH (4) in DMSO was prepared. For activation N7-Npom-BG-Linker-COOH (10 µl, 0.350 µmol, ~25 eq) was incubated with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDCI*HCl, 0.72 µmol, ~50 eq), N-hydroxysuccinimide (NHS, 1.23 µmol, ~90 eq) in 24 µl DMSO. After 15 min. incubation at 30°C, half of the pre-activated linker was added to 17 µl of the NH2-guideRNA solution (6 µg/µl, 100 µg, ~14 µmol, 1 eq) together with DIPEA (2.35 µmol, 170 eq, in 8 µl DMSO) and incubated for another 30 min at 37°C. Then, the remaining pre-activation mix was added and the reaction was continued for 60-90 min.

The crude BG-guideRNA was mixed with 0.1 volumes of 10 x TBE-7M urea containing two dyes (bromophenol blue and xylene cyanol) and purified on a 20% urea-PAGE (four lanes per reaction, 1 x TBE-7 M urea, 120-140 V, 300 min). The PAGE was performed in the dark to prevent Npom-deprotection. While the main part of the gel was stored in the dark, one lane containing ca. 10% of the crude Npom-BG-guideRNA was cut from the gel and analyzed on a TLC plate under low intensity 254 nm UV light. The migration of NH2-guideRNA and Npom-BG-Linker were noted and the gel slice was discarded. The region corresponding to Npom-BG-guideRNA migration was cut out from the remaining lanes and was transferred to a 1.5 ml reaction tube. In the 20% urea PAGE the xylene cyanol band migrated between the NH2- and the Npom-BG-guideRNA and served as additional orientation.

600 µl of RNAase free water were added to the gel slices and the tube was shaken at 4°C overnight to allow the NpomBG-guideRNA to diffuse out of the gel. To remove urea and buffer salts, the NpomBG-guideRNA was precipitated with 0.1 volumes sodium acetate (NaOAc, 3 M) and 3 volumes of EtOH 100% (incubation at -80°C for >4 h). The precipitated RNA was centrifuged (45 min, 14,000 rpm, -4°C), washed with 70% EtOH, dried and dissolved in 50 µl RNase free water. Concentrations were determined photometrically by UV-absorbance of RNA at 260nm and typically around 40% of clean Npom-BG-guideRNA was recovered (compare Table S9).
**Table S9.** Sequences and extinction coefficients of guideRNAs synthesized in this study. The 5’-BG-modified and 5’-(N7Npom-BG)-guideRNAs were prepared from the commercially obtained 5´-NH2-guideRNAs as described above. The nucleotides opposite of the targeted adenosine are unlined and bold, they are normal ribonucleotides. 2′-Methoxylation is indicated by italic characters. Phosphothioate linkages are indicated by “s” subscripts. The 5’-terminal three nucleotides 5’ of each guideRNA are not binding to the mRNA substrate but link the guideRNA to the SNAP-tag. BG-modification adds 2.5 mM-1 cm-1, Npom-BG modification adds 6.5 mM-1 cm-1 to the extinction coefficient of the NH2-guideRNAs.

| Short name   | Sequence                        | $\varepsilon_{260\text{nm}}$ (mM$^{-1}$cm$^{-1}$) |
|--------------|---------------------------------|-----------------------------------------------|
| NH2-Stop66   | UCG GAA CAC CCC AGC ACA GA      | 230                                           |
| NH2-W58X 19nt| UsAsU GUG UCG GCC ACG GAAs CsAsGs G | 226                                           |
| NH2-W58X 21nt| UsAsU GUG UCG GCC ACG GAA CAAsGs GsCsA | 236                                           |
| BG-Stop66    | UCG GAA CAC CCC AGC ACA GA      | 232.5                                         |
| BG-W58X 19nt | UsAsU GUG UCG GCC ACG GAAs CsAsGs G | 228.5                                         |
| BG-W58X 21nt | UsAsU GUG UCG GCC ACG GAA CAAsGs GsCsA | 238.5                                         |
| BG-Stop66 PTO| UsCsG GAA CAC CCC AGC AsCsAs GsA | 232.5                                         |
| Npom-Stop66  | UCG GAA CAC CCC AGC ACA GA      | 236.5                                         |
| Npom-W58X 19nt| UsAsU GUG UCG GCC ACG GAAs CsAsGs G | 232.5                                         |
| Npom-W58X 21nt| UsAsU GUG UCG GCC ACG GAA CAAsGs GsCsA | 242.5                                         |

**MALDI-TOF characterization of guideRNAs**

150-300 pmol of each guideRNA were precipitated by adding 0.5 volumes ammonium acetate (7.5 M) and 2.5 volumes EtOH 100% and subsequent incubation at -80°C for >4 h. The precipitated RNA was centrifuged (45 min, 14 000 rpm, -4°C), washed with 70% EtOH, dried and dissolved in 5 μl RNase free water. The samples were mixed with a matrix of [2,4,6]-trihydroxyacetophenone monohydrate (0.3 M in EtOH) / diammonium citrate (0.1 M in water) (2:1) and the mixture applied on a ground steel target and analyzed using a Bruker Reflex MALDI-TOF mass spectrometer (linear mode, negative mode). All spectra were processed using mMass (Martin Strohalm, Germany, see Figure S10).
Fig S10. MALDI-TOF spectra of NH-guideRNAs
cont. Fig S10. MALDI-TOF spectra of BG-guideRNAs
cont. Fig S10 MALDI-TOF spectra of Npom-guideRNAs
Experiments with Npom-caged O6-Benzylguanine

Photo-deprotection kinetics of N7/N9 Npom-BG-TFA
To determine the decaging efficiency $\varepsilon\Phi$, the decaging of our compounds 1a and 1b was compared to the decay of a commercially available compound DMBN-cAMP (4,5-dimethoxy-2-nitrobenzyl adenosine 3',5'-cyclic monophosphate; Life Technologies). The latter was chosen because of the very similar absorbance properties with our compounds. First, the absorption coefficient $\varepsilon$ of the Npom-BG-TFA was determined. Therefore, the absorption spectra of N7Npom-BG-TFA, BG-NH$_2$ and Npom-OH were recorded (Fig S11). The extinction coefficient of Npom-OH and the caged product N7Npom-BG-TFA are very similar around 360 nm. $\varepsilon_{365\text{nm}}$ of Npom-OH was determined to be 4300 M$^{-1}$cm$^{-1}$. In the range of the methoxy-nitrobenzyl group the presence of the BG moiety didn’t change the UV spectra significantly. Stock solutions of N7Npom-BG-TFA and DMNB-cAMP in DMSO were prepared and diluted in sodium phosphate buffer (NaCl 100 mM, KH$_2$PO$_4$/K$_2$HPO$_4$ 10mM, pH 7.4) to final concentrations of 10 $\mu$M. Decaging was performed in PCR tubes (60 $\mu$L scale) by irradiation with 365 nm light on a UV transilluminator (UVP TFL-40V, 25 W, intensity high) for the indicated amount of time at room temperature. Decaging was performed at low concentrations of the compounds making inner-filter effects negligible. Taking the extinction coefficient and the maximal diameter of the PCR tube (diameter of 5 mm) into consideration a transmission of $>95\%$ is expected. Photodecomposition of the caged substances was monitored in analytical HPLC with UV detection at 280nm and 365 nm. For both substances an exemplary chromatogram is shown in Figure S12. Decomposition of N7Npom-BG-TFA results in two clean products, BG-TFA which shows high absorption at 280nm but now absorption at 365nm and the released photocaging group that shows high absorbance at 365nm. DMNB-cAMP shows two starting materials that refer to the two isomers (axial versus equatorial). The peak areas of the emerging products, BG-TFA and cAMP, were determined and plotted against irradiation time (see Figure S13). By 1st-order fitting, the half-life was determined to be 5.53±0.63 min for DMNB-cAMP decay, 0.57±0.04 min for N7Npom-BG-TFA and 0.78±0.06 min for N9Npom-BG-TFA.

Literature reports a $\varepsilon\Phi$ value of 250 M$^{-1}$cm$^{-1}$ for of cAMP formation at 350 nm with $\varepsilon = 5.0$ mM$^{-1}$cm$^{-1}$ and $\Phi = 5\%$.[S6] $\varepsilon_{365\text{nm}}$ of DMNB-cAMP was determined to be 4.0 mM$^{-1}$cm$^{-1}$ and from this $\varepsilon\Phi$ at 365nm was calculated to be 200 M$^{-1}$cm$^{-1}$. By comparing the half-lives, an $\varepsilon\Phi$ 365nm for N7-Npom-BG-TFA of 2000 M$^{-1}$cm$^{-1}$ results. Taking a $\varepsilon$ of ca. 4 mM$^{-1}$cm$^{-1}$ into account, the quantum yields can be estimated to be $\approx$50$\%$ for the N7- and $\approx$36$\%$ for the N9-isomer.

To test the stability of the Npom-protected BG in the dark, compounds 1a and 1b were dissolved at 10 $\mu$M in phosphate buffer (NaCl 100 mM, KH$_2$PO$_4$/K$_2$HPO$_4$ 10mM, pH 7.4) and kept at r.t. for 3 day. Per HPLC (Figure S14), no release of BG is detectable.
Figure S11. Determination of the extinction coefficients of Npom-OH, N7-Npom-BG-TFA and BG-NH₂.

Figure S12. Analytical HPLC traces of the photo-decaging of reference DMNB-cAMP versus compound N7-Npom-BG-TFA. Shown are the HPLC trace after 5 min (left) and 0.5 min (right) irradiation with 365 nm.

Figure S13. Photodecaging kinetics at 365 nm irradiation for N7-Npom-BG-TFA and N9-Npom-BG-TFA in comparison to commercial reference DMNB-cAMP for the determination of the quantum yield.
Figure S14. Analytical HPLC for N7-Npom-BG-TFA before and after 3 days at r.t. in the dark (NaCl 100 mM, KH2PO4/K2HPO4 10mM, pH 7.4). No release of BG-TFA ($t_R = 10$ min) was observed. The little impurity at $t_R = 10$ min after 3 days is not BG-TFA as it shows absorption at 365 nm (compare Figure S12).

BG-FITC/FAM assay
The BG-FITC/FAM assay (Figure 1c in the manuscript) was performed in 20mM Tris-HCl (pH 8), 100mM NaCl and 5% glycerol. The reaction was performed on a 8 μl scale in PCR reaction tubes. The concentration of SNAP-ADAR1 was adjusted to 1 μM and the respective BG derivatives (BG-FITC, N7Npom-BG-FAM and N9Npom-BG-FAM) were used in a concentration of 7.5 μM. Irradiation was performed on a UV-table (365 nm, power=high). The reaction tubes were incubated at 30°C for 20 min in the dark. The reaction was stopped by adding 0.5 volumes of 4 x SDS-PAGE loading buffer and heating to 95°C for 4 min. 10 μl of each sample were applied to SDS-PAGE (4% stacking gel, 12% separation gel, 100 V, 2 h). Furthermore, GE Healthcare LMW protein marker was applied to one lane. To determine FITC/FAM fluorescence, the gel was scanned in a Fujifilm FLA-5100 fluorescence scanner using an excitation wavelength of 473nm (Intensity = 500V) and recording the emission at 557nm (Cy3 filter set). To verify consistent loading, coomassie staining was performed. The staining solution was composed of Coomassie Brilliant Blue G-250 (0.02% w/v), Al2(SO4)3 (5% w/v), EtOH (10% v/v) and phosphoric acid (2% v/v).
SNAP-ADAR1 band-shift assay
The SNAP-ADAR1 band-shift assay (Figure 2b in the manuscript) was performed in 10 ml Tris-HCl (pH 8), 50 mM NaCl and 2.5% glycerol, 2 mM DTT. The reaction was performed on an 8 μl scale in PCR reaction tubes. The concentration of SNAP-ADAR1 was adjusted to 1 μM and the respective guideRNAs (NH2-Stop66, BG-Stop66, Npom-Stop66) were used in a concentration of 7.5 μM. Irradiation was performed on a UV-table (365 nm, power=high). The reaction tubes were incubated at 30°C in the dark for 40 min. The reaction was stopped by addition of 0.5 volumes of 4 x SDS-PAGE loading buffer and heating to 95°C for 4 min. 10 μl of each sample were applied to SDS-PAGE (4% stacking gel, 12% separation gel, 100 V, 3.5 h). Furthermore, GE Healthcare LMW protein marker was applied to one lane. The proteins were visualized by coomassie staining. The staining solution was composed of Coomassie Brilliant Blue G-250 (0.02% w/v), Al₂(SO₄)₃ (5% w/v), EtOH (10% v/v) and phosphoric acid (2% v/v).
**Primer**

All primers used in this study were purchased from Sigma-Aldrich GmbH (Muenchen) or MWG Eurofins (Ebersberg).

Stop66 fw: GCGGATAACA ATTCGCCCTCT AG
Stop66 rv: CAGCGGCTGC AGCAGCCCAAC

W58X RT: CTAGAAGGCA CAGTCGAGGC
W58X fw: GCGGATCCAC CATGGCTAGC AAAGGAGAAG AACTC
W58X rv: CCTCTAGAGC CGGATTGTA TAGTTCATCC ATGCC
W58Xpos327 fw: GACACGTGCT GAAGTCAAGT TTGAAGGTG

W58X PD fw: ATGGCGCGCC TAGCTAGCAA AGGAGAAGAA CTC
W58X PD bw: TAACCGGGTT TGATATAGTT ATCCATGCCA TG

**Gene sequences**

**PCR-template for the generation of W66amber eCFP mRNA**

The premature stop codon (TAG) at position 66 is underlined and highlighted.
W58XeGFP/wt eGFP in the context of the pcDNA3.1 vector (Invitrogen):
The gene was cloned using the BamH1 and Xba1 (underlined) restriction sites. The
codon 58 (TAG) that is edited is highlighted in yellow. In the wildtype sequence =
positive control plasmid this codon is TGG.
### SNAPf-ADAR1 in the context of the pcDNA3.1 vector

| 1   | GGATCCACCATGGACAAAGACTGCGAAATGAAGCGCACCACCCCTGGATAGCCCTCTGGGC  |
|-----|---------------------------------------------------------------|
| 161 | D K Q  K N G  I  K  V  N  F  K  T  R  H  N  I  E  D  G        |
| 310 | GAAAGGCCACCATCTTCTTCTTCCAAGATGACGGCAACTACAAGACACGTGCTGAAGTCAAGTTT |
| 361 | GAAGTGATACCTCCTTTGTTAATAGAATCCGAGTTAATAAGGTATTTGACCTTCAAGGAGATG  |
| 411 | AGCGTTCACTAGCAGACCATTACTATCAACAAAATACTCAACATCGGATCCGCTTTCCTTC    |
| 511 | TTACCAGACACACCATTACCTGTCCACACAATCTGCCCCCTTCTGAAAGACATCCACGAAAAG |
| 611 | AGAGACCACATGTTGCTGCTGCCCACCAATGTAACAGCTGTGGGATTACACATGGCATGGAT  |
| 711 | GAACATCAAAATACCGGTCTCTAGAGGGCCCTATTCTATAGTGTCACCTAAATGCTAGAGC  |

**BamHI**

| 1   | GGATCCACCATGGACAAAGACTGCGAAATGAAGCGCACCACCCCTGGATAGCCCTCTGGGC  |
|-----|---------------------------------------------------------------|
| 161 | D K Q  K N G  I  K  V  N  F  K  T  R  H  N  I  E  D  G        |
| 310 | GAAAGGCCACCATCTTCTTCTTCCAAGATGACGGCAACTACAAGACACGTGCTGAAGTCAAGTTT |
| 361 | GAAGTGATACCTCCTTTGTTAATAGAATCCGAGTTAATAAGGTATTTGACCTTCAAGGAGATG  |
| 411 | AGCGTTCACTAGCAGACCATTACTATCAACAAAATACTCAACATCGGATCCGCTTTCCTTC    |
| 511 | TTACCAGACACACCATTACCTGTCCACACAATCTGCCCCCTTCTGAAAGACATCCACGAAAAG |
| 611 | AGAGACCACATGTTGCTGCTGCCCACCAATGTAACAGCTGTGGGATTACACATGGCATGGAT  |
| 711 | GAACATCAAAATACCGGTCTCTAGAGGGCCCTATTCTATAGTGTCACCTAAATGCTAGAGC  |

**KpnI**

| 1   | GGATCCACCATGGACAAAGACTGCGAAATGAAGCGCACCACCCCTGGATAGCCCTCTGGGC  |
|-----|---------------------------------------------------------------|
| 161 | D K Q  K N G  I  K  V  N  F  K  T  R  H  N  I  E  D  G        |
| 310 | GAAAGGCCACCATCTTCTTCTTCCAAGATGACGGCAACTACAAGACACGTGCTGAAGTCAAGTTT |
| 361 | GAAGTGATACCTCCTTTGTTAATAGAATCCGAGTTAATAAGGTATTTGACCTTCAAGGAGATG  |
| 411 | AGCGTTCACTAGCAGACCATTACTATCAACAAAATACTCAACATCGGATCCGCTTTCCTTC    |
| 511 | TTACCAGACACACCATTACCTGTCCACACAATCTGCCCCCTTCTGAAAGACATCCACGAAAAG |
| 611 | AGAGACCACATGTTGCTGCTGCCCACCAATGTAACAGCTGTGGGATTACACATGGCATGGAT  |
| 711 | GAACATCAAAATACCGGTCTCTAGAGGGCCCTATTCTATAGTGTCACCTAAATGCTAGAGC  |

**NotI**

| 1   | GGATCCACCATGGACAAAGACTGCGAAATGAAGCGCACCACCCCTGGATAGCCCTCTGGGC  |
|-----|---------------------------------------------------------------|
| 161 | D K Q  K N G  I  K  V  N  F  K  T  R  H  N  I  E  D  G        |
| 310 | GAAAGGCCACCATCTTCTTCTTCCAAGATGACGGCAACTACAAGACACGTGCTGAAGTCAAGTTT |
| 361 | GAAGTGATACCTCCTTTGTTAATAGAATCCGAGTTAATAAGGTATTTGACCTTCAAGGAGATG  |
| 411 | AGCGTTCACTAGCAGACCATTACTATCAACAAAATACTCAACATCGGATCCGCTTTCCTTC    |
| 511 | TTACCAGACACACCATTACCTGTCCACACAATCTGCCCCCTTCTGAAAGACATCCACGAAAAG |
| 611 | AGAGACCACATGTTGCTGCTGCCCACCAATGTAACAGCTGTGGGATTACACATGGCATGGAT  |
| 711 | GAACATCAAAATACCGGTCTCTAGAGGGCCCTATTCTATAGTGTCACCTAAATGCTAGAGC  |

**XhoI**

| 1   | GGATCCACCATGGACAAAGACTGCGAAATGAAGCGCACCACCCCTGGATAGCCCTCTGGGC  |
|-----|---------------------------------------------------------------|
| 161 | D K Q  K N G  I  K  V  N  F  K  T  R  H  N  I  E  D  G        |
| 310 | GAAAGGCCACCATCTTCTTCTTCCAAGATGACGGCAACTACAAGACACGTGCTGAAGTCAAGTTT |
| 361 | GAAGTGATACCTCCTTTGTTAATAGAATCCGAGTTAATAAGGTATTTGACCTTCAAGGAGATG  |
| 411 | AGCGTTCACTAGCAGACCATTACTATCAACAAAATACTCAACATCGGATCCGCTTTCCTTC    |
| 511 | TTACCAGACACACCATTACCTGTCCACACAATCTGCCCCCTTCTGAAAGACATCCACGAAAAG |
| 611 | AGAGACCACATGTTGCTGCTGCCCACCAATGTAACAGCTGTGGGATTACACATGGCATGGAT  |
| 711 | GAACATCAAAATACCGGTCTCTAGAGGGCCCTATTCTATAGTGTCACCTAAATGCTAGAGC  |

**HindIII**

| 1   | GGATCCACCATGGACAAAGACTGCGAAATGAAGCGCACCACCCCTGGATAGCCCTCTGGGC  |
|-----|---------------------------------------------------------------|
| 161 | D K Q  K N G  I  K  V  N  F  K  T  R  H  N  I  E  D  G        |
| 310 | GAAAGGCCACCATCTTCTTCTTCCAAGATGACGGCAACTACAAGACACGTGCTGAAGTCAAGTTT |
| 361 | GAAGTGATACCTCCTTTGTTAATAGAATCCGAGTTAATAAGGTATTTGACCTTCAAGGAGATG  |
| 411 | AGCGTTCACTAGCAGACCATTACTATCAACAAAATACTCAACATCGGATCCGCTTTCCTTC    |
| 511 | TTACCAGACACACCATTACCTGTCCACACAATCTGCCCCCTTCTGAAAGACATCCACGAAAAG |
| 611 | AGAGACCACATGTTGCTGCTGCCCACCAATGTAACAGCTGTGGGATTACACATGGCATGGAT  |
| 711 | GAACATCAAAATACCGGTCTCTAGAGGGCCCTATTCTATAGTGTCACCTAAATGCTAGAGC  |

**SalI**

| 1   | GGATCCACCATGGACAAAGACTGCGAAATGAAGCGCACCACCCCTGGATAGCCCTCTGGGC  |
|-----|---------------------------------------------------------------|
| 161 | D K Q  K N G  I  K  V  N  F  K  T  R  H  N  I  E  D  G        |
| 310 | GAAAGGCCACCATCTTCTTCTTCCAAGATGACGGCAACTACAAGACACGTGCTGAAGTCAAGTTT |
| 361 | GAAGTGATACCTCCTTTGTTAATAGAATCCGAGTTAATAAGGTATTTGACCTTCAAGGAGATG  |
| 411 | AGCGTTCACTAGCAGACCATTACTATCAACAAAATACTCAACATCGGATCCGCTTTCCTTC    |
| 511 | TTACCAGACACACCATTACCTGTCCACACAATCTGCCCCCTTCTGAAAGACATCCACGAAAAG |
| 611 | AGAGACCACATGTTGCTGCTGCCCACCAATGTAACAGCTGTGGGATTACACATGGCATGGAT  |
| 711 | GAACATCAAAATACCGGTCTCTAGAGGGCCCTATTCTATAGTGTCACCTAAATGCTAGAGC  |
SNAPf-ADAR2 in the context of the pcDNA3.1 vector

1  GGATCCACCATGGACAAAAAGACTGCGAAATGAAGCGCACCACCCTGGATAGCCCTCTGGGC
   BamHI  M D K D C E M K R T T L D S P L G

70  80  90  100  110  120

61  AAGCTGGAACTGTCTGGGTGCGAACAGGGCCTGCACCGTATCATCTTCCTGGGCAAAGGA

1921  GATCTGAAATATGCAATCCGGGCTGCTACATCACCACATTGAGTCTAAACCCGCTGTAGCA
   D L N M H T G H H H H H *
341  RSIFQKSERGGFRKLKENVQF
1081  CATCTGTACATCAGAACCTCTTCTCGTGAGATTGCCAGAAATCTTCACCCCATATGAGCCA
361  HLYISTSPCGDARIIFSPHEP
1141  ATCTGGAAAGAGCAGTAGATGACACACAAAACTGTAAGCGAAGGAGACACGCTACGGGAC
381  ILEEPADRPNKRAGQLRT
1201  AAAATAGAGTCTGTTGAGGGGACAGTTCCAGTGCGCTCCAAATGCGAGCATCACAACATTGCGACG
401  KIESGEGTKIPVRSNASIQTW
1261  GCGGGGTGCTGCAAGGGGAGCGGCTGCTCACCATGTCCTGCAGTGACAAGATTGCACGC
421  DGVQLQGERLTTMSCSDOKAR
1321  TGGAAAGCTGGCAGGATCCTGGAGGATCCTGAACCAATTCATGGAGACCCTTACCTTC
441  WNVVGIGQGSLLSIFVEPIYF
1381  TCAGACATCATCCTGGCAGCCTTTACCACGGGGACCACCTTTCCAGGGCATGTACAG
461  SSILGSLHYGDHLSRAMYQ
1441  CGGATCTCCACATAGAGGACCTGCCACCTCTCTACACCCTCAACAAGCCTTTGCATCTAGT
481  RISNIDELPPLYTLNKPLLS
1501  GGCAATCCAAATGAGCAGGAGGGCCACACCCACCTACACCAACAAAGCCTTGGCTACTG
501  GISNAEARQPGKAPNFVS
1561  ACCGATCGAGACATGCCGCTATTGAGGGATCAACGCTACCGAGGAGTGGATGCAGTGAGCC
521  TVGDSAIEVINATTGKDLEG
1621  CGGGGTGCTCCGCTCTGTTAAGGGACGCGGCTGATCCTCATGGATGCGTCTGCGGCTGCAAGCAAG
541  RASRLCKHALYCRWMRVGHK
1681  GTTCCCTCCCAATCTACACGCTCCAAGATTACCACAGGGGCAACTTCAACGCTACAGG
561  VPSHLRSKITYKPNVYHESK
1741  CTGGGAGCAAAAGAGGATACAGCCGCCGCCAGGGCCTGCTGGTCAAGGCTACCTCAGGCGG
581  LAAKEYQAAKARLFTAFKA
1801  GGGCTGGGGGCTCTGTTGGAGAAGCCACCGAAGGGGACTCCTCTACCTCAGCTCAGAGGCG
601  GLGAWEKVPEQDQSFLTSP
1861  AGAGGCCCCCTGAGAACAAAAACTCATCTACGAGAAAGATCTCAATATGCTAAACCCGCTC
621  RGRPFEQKLISEEEDLNMHTGH
1921  CATCACCATCACCATCGATGTTTAAACCCGCTGATCAGCCTCGACTGTGCCTTCTAGTTGC
641  HHHHHH*
**In vitro editing**

The general procedure of *in vitro* editing comprises the actual editing of the mRNA, reverse transcription and amplification of the cDNA by PCR. Subsequent sequencing of the PCR product served as a read-out for the editing yield.\[^{[S4]}\]

**CFP Stop66 mRNA synthesis**

The mRNA substrate for the *in vitro* editing experiments was generated by *in vitro* transcription of a PCR fragment containing the Stop66 gene under the control of a T7 promotor. Using a PCR fragment as template instead of a plasmid decreases the risk of RNase contamination. The *in vitro* transcription reaction mixture contained 100 ng template, transcription buffer (7.5 μl), DTT (100 mM, 7.5 μl), BSA (1 mg/ml, 3 μl), rNTPs (mix 25 mM, 12 μl) and T7 RNA polymerase (4.5 μl). The volume was adjusted to 50 μl with RNase free water. The mRNA was purified using the RNeasy mini kit (Qiagen). To remove traces of the template DNaseI (Qiagen) digest was performed according to the manufactures protocol. Afterwards, the mRNA was again purified using the RNeasy mini kit (Qiagen). Absence of DNA was controlled by PCR using 2 μl of diluted mRNA (OD = 0.2) as template and the primers Stop66 fw and Stop66 rv. If necessary the DNaseI digest was repeated until the PCR control reaction was negative.

**Editing**

All *in vitro* editings were performed on a 25 μl scale in PCR-tubes as previously described.\[^{[S4]}\] The editing buffer was composed of 25 mM Tris·HCl, 75 mM KCl, 10 mM DTT (pH 8.3) and 0.75 mM MgCl\(_2\). The substrate mRNA (eCFP Stop66) was used in a concentration of 10 nM and NH\(_2\)-, BG- or Npom-guideRNA was added in 5-fold excess. The reaction was started by addition of 3.4 equivalents of editing enzyme (SNAP-ADAR1 or SNAP-ADAR2) relative to guideRNA resulting in a final enzyme concentration of 170 nM. Expression and purification of the SNAP-ADAR enzymes were previously described.\[^{[S4,S5]}\] Furthermore, the reaction mixture always contained heparin (2 μM, assuming an average molar mass of 20 kDa) and murine RNase inhibitor (0.5 u/μl, NEB) to prevent degradation of the guideRNAs and the mRNA substrate. Irradiation with 365 nm light was performed on a UV transilluminator (UVP TFL-40V, 25 W, intensity high) for the indicated amount of time at room temperature. The reaction was carried out by cycling between 30°C and 37°C [3x(30 min at 30°C and 30 min at 37°C) for SNAP-ADAR2, 3x(20 min at 30°C and 20 min at 37°C) for SNAP-ADAR1].
Reverse Transcription
Editing was stopped by adding an excess of an antisense DNA oligo (5’-TGACGGCTGGCTGCACCATT, final concentration 20 μM), MgCl₂ (2.6 mM), dNTPs (0.27 mM each), a primer for reverse transcription (Stop66 rv, 0.5 μM), DTT (5.3 mM), and M-MuLV RT buffer (1.25 μl, NEB), and heating to 70°C for 3 min. After heating M-MuLV-reverse transcriptase (50 units, 0.5 μl NEB) was added and the reaction mixture was incubated at 42°C for 2 h. Afterwards the cDNA was purified and concentrated using the NucleoSpin gel and PCR clean-up (Machery-Nagel).

Taq-PCR and agarose gel electrophoresis
The cDNA was amplified by PCR on a 50 μl scale (denaturation: 15 s at 95°C, annealing: 30 s at 56°C, elongation: 60 s at 68°C, 27 cycles). The PCR reaction mix contained 5 μl ThermoPol buffer (10 x, NEB), 2.5 μl of Stop66 fw primer (10 µM), 2.5 μl Stop66 rv primer (10 µM), 1.25 μl dNTPs (10 mM each, NEB) and 0.5 μl Taq DNA polymerase (5 units/μl). 5 μl of cDNA (typical concentration ca. 10 ng/μl) were used as template. Elution buffer from the NucleoSpin kit was used as negative control to preclude contamination of the elution buffer or PCR components. PCR products of previous editings were used as positive control. All PCR reactions were applied to agarose gel electrophoresis (1 x TAE, 1.4%, staining with Rotisafe by Carl Roth GmbH). The eCFP band was cut out under UV light and the PCR product was recovered using the NucleoSpin gel and PCR clean-up kit by Machery-Nagel and sent for sequencing.

Sequencing and processing of sequencing traces
The PCR products (120 ng) were sequenced with Stop66 fw primer () at Eurofins MWG, Ebersberg. The editing yield was determined by comparison of the peak height of adenosine and guanosine in the sequencing traces according to the following formula:

\[ \text{Editing yield} = \frac{h(\text{guanosine})}{h(\text{guanosine}) + h(\text{adenosine})} \times 100 \]  
\[ h = \text{peak height [cm]} \]

Sequencing traces were processed with DNAMAN 7. The Figure S15 on the next page shows the complete sequencing trace of the CFP s top66 mRNA edited in vitro.
Figure S15 RNA Sequencing of the entire ORF of CFP. The RNA was obtained after light-induced transcript repair with \(^{N^7}\)Npom-BG-Stop66 guideRNA and SNAP-ADAR1 (corresponding to experiments shown in Figure 2c, manuscript).
Additional experimental data
The light-dependent RNA editing experiment shown in the manuscript in Figure 2c for SNAP-ADRA1 was also carried out with SNAP-ADAR2 as shown below (Figure S16). Again, the full dynamic range of editing is obtained from no editing prior to irradiation to the editing yield of the positive editing control after >60 sec irradiation. The maximum yield obtained for SNAP-ADAR2 is a little bit reduced compared to SNAP-ADAR1 which is caused by the stronger sensitivity of the first towards heparin.

Figure S16 Light-dependent in-vitro site-directed RNA editing of the amber Stop codon at position 66 in the CFP gene by SNAP-ADAR2.
Editing in cell culture

Cell culture techniques
HEK293T cells (DSMZ Braunschweig, Germany, ACC-635) were grown in Dulbecco’s Modified Eagle Medium (DMEM, Life Technologies) supplemented with 10% fetal bovine serum (FBS, Life Technologies) and 1% penicillin/streptomycin (P/S, Life Technologies) under standard conditions (37°C and 5% CO² in a water saturated steam atmosphere).

Plasmid transfection
HEK 293T cells (DSMZ code ACC-635, passage number < 15) were grown in a 25 cm² cell culture flask to a confluency of 70-90%. The medium was removed; the cells were washed with 5 ml PBS and trypsinized with 500 μl trypsin/EDTA for 3 min at 37°C. Then the protease was blocked by adding 4.5 ml DMEM+FBS+P/S. Cells were incubated with trypan blue for 5 min and the cell number was determined in a hemacytometer. Cells were seeded onto a 24 well plate (200 000 cells/well) in 500 μl DMEM+FBS+P/S grown for 24 h. 60-120 min before plasmid transfection the medium was replaced with 450 μl DMEM+FBS without antibiotics. Lipofectamine 2000 (Life Technologies, 4 μl per 1 μg DNA) was used as transfection reagent. For transfection, plasmid DNA and Lipofectamine were diluted in 50 μl OptiMem (Life Technologies) independently and incubated for 5 min at r.t. allowing micelle formation of the transfection agent. Then both solutions were mixed, incubated for another 20 min at r.t. for complex formation and applied to the cells. The transfected cells were incubated for 24 h.

In a typical experiment 500 ng of W58X coding plasmid (pcDNA3.1, Invitrogen) was co-transfected with 25 to 200 ng SNAPf-ADAR1 coding plasmid (pcDNA3.1, Invitrogen). Furthermore, W58X was co-transfected with empty pcDNA3.1 as negative control. The plasmid coding for functional EGFP was transfected together with SNAPf-ADAR1 as positive control.

guideRNA transfection
After 24 h incubation, the medium was removed; cells were washed with 500 μl PBS, trypsinated (60μl trypsin/EDTA) and taken up in 500 μl DMEM+FBS+P/S. The cell suspension was centrifuged (1600 rpm, 5 min, r.t.), the supernatant was removed, the cell pellet re-suspended in DMEM+FBS without antibiotics and the cell number was determined in a hemacytometer. The cells were reverse transfected onto a 96 well plate (60 000 cells/well, in 100 μl DMEM+FBS) containing the respective guideRNAs (typically 10 pmol/well) pre-treated with Lipofectamine 2000 (0.5 μL/well). guideRNA and Lipofectamine were diluted in 25 μl OptiMem independently, incubated for 5 min, mixed and incubated another 20 min before applying the mixture to the 96 well plate.
All experiments involving Npom-caged guideRNAs were performed in the absence of direct light or under red light (590-660 nm, LED Spot Luexon Red, Conrad Electronic). 96 well plates were wrapped in aluminum foil to protect Npom-guideRNAs from light.

**Npom-decaging**
After 4 h of guideRNA transfection, the medium was replaced by 100 μl DMEM+HEPES (25 mM) without phenol red. Irradiation (365 nm) was performed in a fluorescence microscope (Zeiss CellObserverZ.1, equipped with a 365 nm Colibri.2 LED) at 100% LED power for the indicated amount of time.

**Imaging & data processing**
All cells were imaged 24 h after irradiation using a Zeiss AXIO Observer.Z1 with a Colibri.2 light source. For each sample, a phase contrast image acquired and EGFP fluorescence signal was recorded (Excitation: 465 nm, 50% LED power, 460-480 nm band pass filter, Emission: 500-557 nm band pass filter). All samples of one experiment were recorded with the same settings (exposure time, etc.). Images were processed in ImageJ 1.47h and the adjustment of contrast and brightness was carried out for all fluorescence images identically.

**Cell harvesting and RNA isolation**
After imaging, the cells were harvested and the RNA was isolated and reverse transcribed. The obtained cDNA was amplified by PCR, worked-up and sequenced.

The medium was removed from the 96 well-plates and the cells were trypsinated with 20 μl Trypsin/EDTA for 1 min. Then, DMEM+FBS+P/S (80 μl) was added, the cell suspension was transferred to a 1.5 ml reaction tube and centrifuged (1600 rpm, 5 min, r.t.). The supernatant was removed and the cell pellets were washed with PBS. After another centrifugation step (1600 rpm, 5 min, r.t.) and removal of the PBS, the pellets were frozen in liquid nitrogen and stored for up to 3 days at -80°C. For RNA isolation, the pellets were thawed on ice and suspended in lysis buffer (RLT buffer from Qiagen RNeasy mini kit + 1% mercaptoethanol (v/v)). To enhance cell disruption, the cells were exposed to shearing stress by pipetting the suspension through a needle (Ø = 0.25 mm) several times. After lysis, RNA purification was performed according to the RNeasy mini kit manual (Qiagen). In a typical experiment 600-1800 ng RNA were recovered per well.

**DNase digest and reverse transcription**
For DNase digest 2.9 μl 10 x RDD buffer and 1 μl DNase I (Qiagen) were added to 25 μl RNA sample and incubated for 30 min at 37°C. The DNase was inactivated by adding 1 μl EDTA (25 mM) and heating to 65°C for 10 min. 14.75 μl of the DNase digest were mixed with 1 μl W58X RT (10 μM) primer and 1 μl dNTPs (10mM each) and incubated at 70°C for 3 min. Then, 2 μl 10 x M-MuLV-RT buffer, 0.25 μl murine RNase inhibitor (40 units/μl, NEB) and 1 μl M-MuLV reverse transcriptase (200
units/μl, NEB) were added and the solution was incubated at 42°C for 1 h. M-MuLV reverse transcriptase was inactivated by heating to 90°C for 10 min. The cDNA was purified using the NucleoSpin gel and PCR clean-up kit (Machery-Nagel). In a typical experiment, reverse transcription yielded 150-600 ng cDNA.

**Taq-PCR and agarose gel electrophoresis**
The cDNA was amplified by PCR on a 50 μl scale. Furthermore, a control PCR was performed using the DNase digest as template to verify the absence of interfering plasmid DNA. The PCR reaction mix contained 5 μl ThermoPol buffer (10 x), 2.5 μl of W58X fw primer (10 μM), 2.5 μl W58X rv primer (10 μM), 1.25 μl dNTPs (10 mM each) and 0.5 μl Taq DNA polymerase (5 U/μl, NEB). 5 μl of cDNA sample or 2.5 μl RNA after DNase digest were used as template. Furthermore, RNase free water and elution buffer from the NucleoSpin kit were used as negative control to preclude contamination of the PCR components. W58X plasmid DNA was used as positive control. 10 μl of 6 x blue loading dye (NEB) were added and all PCR reactions were applied to agarose gel electrophoresis (1 x TAE, 1.4%, staining with Rotisafe by Carl Roth GmbH). The W58X/EGFP band was cut out and the PCR product was recovered using the NucleoSpin gel and PCR clean-up kit by Machery-Nagel. If any bands showed up in the DNase control PCR, the samples were processed again starting from DNase digest.

**Sequencing**
For sequencing 120 ng of the PCR products were send to Eurofins MWG. W58X fw (1.5 μl, 10 μM) was used as primer for all samples. The editing yield was determined as described for Stop66 editing *in vitro*. Figure S18 shows the complete sequencing trace of the W58X editing in cell culture. The amplified fragment corresponds to base pairs 44-668 of the EGFP ORF.
**Figure S17.** Light-controlled RNA editing in living 293T cells (corresponding to experiments shown in Figure 3 manuscript). For all samples phase contrast imaging (left), eGFP fluorescence imaging (middle) and Sanger sequencing traces (right) 24 h post transfection is shown.
Figure S18. RNA Sequencing of the isolated eGFP mRNA (amplified fragment corresponds to base pairs 44-668 of the eGFP ORF). The RNA was obtained after light-induced transcript repair with "N7NpomBG-Stop66 guideRNA and SNAPf-ADAR1 (corresponding to experiments shown in Figure 3, manuscript)
Additional experimental data

Figure S19 and S20 demonstrate the dependence of the editing yield and Npom-guideRNA residual activity on the amount of transfected guideRNA and plasmid. Remarkably, the system yields reasonable editing (20-50%) even when the amount of plasmid or BG-guideRNA is varied by about 1 order of magnitude.

With a constant amount of BG-guideRNA (10 pmol/well) the editing yield decreases from ~50% with 200 ng SNAPf-ADAR1 plasmid to ~20% with 25 ng SNAPf-ADAR1. At the same time the residual activity of Npom-guideRNA in the dark is decreases from ~15% to <5%.

With a constant amount of SNAPf-ADAR1 enzyme (100 ng) the editing yield decreases from ~50% with 50 pmol BG-guideRNA to ~20% with 2 pmol BG-guideRNA. Residual activity of Npom-guideRNA can be efficiently suppressed by lowering the amount of guideRNA.

The presented results indicate that SNAPf-ADAR1 expression level and the amount guideRNA can be used to adjust both editing yield and residual activity for future applications.

Figure S19. RNA editing in living 293T cells with different amounts of SNAPf-ADAR1 plasmid transfected. All cells were transfected with a constant amount of guideRNA (10 pmol/well).
Figure S20. RNA editing in living 293T cells with different amounts of guideRNA transfected. All cells were transfected with a constant amount of SNAPf-ADAR1 plasmid (100ng).
Editing in Platynereis Dumerillii

Materials and Methods

Gene (transcript) sequences of eGFP and SNAP-ADAR1

W58X eGFP in the pUC57-T7-RPP2 context

10 20 30 40 50 60
1 TAATACGACTCACTATAGGGAGATTTGATGTTTACAGGGCTATTTATAAACAATTTGTA
T7 promoter
70 80 90 100 110 120
61 ATAATTTAGGGAATAATTGTTGCTCACAACCACATGTTCTAGGAAGCTGTGAGCC
130 140 150 160 170 180
121 CCAAATCTTCTTTCTGTGAGAATATTCTTGTGTCACACGGTTTCTGCTCTTGG
190 200 210 220 230 240
181 AAGACTTAAAAATGCCGCCTAGCTAGCAAAGGAAGAAGACTCTTCACTGGAGTTG
M A R L A S K G E E L F T G V
250 260 270 280 290 300
241 CCAAATCTTGTGAATAGATGTTGATTTACGCCCAAAGTTCTTGCTCAGTGGAG
81 P I L V E L D G D V N G H K F S V S G E
310 320 330 340 350 360
301 GTGGAAGGGTGATGCAACATACGCAAAACTTTACCTGAAGTTCTCAGTCTACTGGCAA
101 G E G D A T Y G K L T L K F I C T T G K
370 380 390 400 410 420
361 CTGCCCTTGTCGCTTAGCACACTAGTGACGCTCTGCTATGCGTCCAGTCTTTTCA
121 L P V P * P T L V T T L C Y G V Q C F S
430 440 450 460 470 480
421 AGATGCCCGGATCACTAGATGCCCCAGGACATTTCTGCTAAGTGGTTCATGCGAA
141 R Y P D H M K R H D F F K S A M P E G Y
490 500 510 520 530 540
481 GTACAGGAAGGGACCCTTTCTTCAAAGATGACGGCAACTCAAGACGCTGTGAAGTC
161 V Q E R T I F F K D D G N Y K T R A E V
550 560 570 580 590 600
541 AAGTTTGAGATGATCCACCCCATTTGTGTTAATAGATCGAGTTAAAAGGTTTGACTTCAAGGAA
181 K F E G D T L V N R I E L K G I D F K E
610 620 630 640 650 660
601 ATGGCAACATTTCTGGGACACAAATTGGGAGATACAAACTTAAACTCACCAAATGTAACTAC
201 D G N I L G H K L E Y N Y N S H N Y I
670 680 690 700 710 720
661 ATGGCAAGCAAAAACAAAAAGAATGGAACAGTTGCAACTTCAAGAGCCCACAACATGGA
221 M A D K Q K N G I K V N F K T R H N I E
730 740 750 760 770 780
721 ATGGGAAGCCGTTCAACACTGACGACATTTACAAAGAAATTACTCAATGCGATGCGCCCT
241 D G S V Q L A D H Y Q Q N T P I G D G P
In yellow the transcription start site and the premature W58X site are highlighted. The ORF is marked by the single letter amino acid code.

SNAP-ADAR1 in the pUC57-T7-RPP2 context

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10  20  30  40  50  60
1   TAATACGACTCACTATAGGGAGATTTGATGTTTACAGGGCTATTTATAAACAAATTGTTA
    T7 promotor

250 260 270 280 290 300
241   AGCCCTCTGGGCAAGCTGGAACTGTCTGGGTGCGAACAGGGCCTGCACCGTATCATCTTC
70  80  90 100 110 120
61   ATAATTTAGGTGAAATTATTTTGGTGTCTCAAACCACATGTTCAGGAAGCTGTGGCCC

310 320 330 340 350 360
181   AAGACTTAAAAATTATGGCCGCTTCTAGACAAAGACTGCGAAATGAAGCGCACCACCACCTGGAT

370 380 390 400 410 420
101   LGKTSAADADVPEVAPAPAAL

430 440 450 460 470 480
361   GGCAGCACCAGGCCACTTGATGCGGCCACCACCCCTGGTCAACGCCTACTCTGAGAGGC

490 500 510 520 530 540
161   FTRQVLWKLLKVVKFGEVISM

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Polyadenylation Eco53kI
550 560 570 580 590 600
TACAGCCACCTGGCCGCCCTGGCCGGCAATCCCGCCGCCACCGCCGCCGTGAAAACCGCC
181
Y S H L A L A G N P A A T A A V K T A

610 620 630 640 650 660
CTGAGCGGAAATCCCGTCCACCCGATCCGGCCACCGGCTGGGTGCGAAGGGCCGGACCC TG
201
L S G N P V P I L I P C H R V V Q G D L

670 680 690 700 710 720
GACGTGGGGGGCTACGAGGGCGGGCTCGCCGTGAAAGAGTGGCTGCTGGCCCACGAGGCC
221
D V G Y E G G L A V K E W L L A H E G

730 740 750 760 770 780
CACAGACTGGCAGGGCTGGGCTGGGTCCTGCAGGCGGAGGCGCGCCAGGGTCTGGCGGC
241
H R L G K P G L G P A G G G A G S G

790 800 810 820 830 840
GGCAGTAAAGCCAGACGCTAGGGGTTCCTACAGAGGTAACCCCAGTGACAGGGGCCAGTCTC
261
G S K A E R M G F T E V T P V T G A S L

850 860 870 880 890 900
AGAAAGACTATATGCTCTCCTCCTCCTCAAGGGTCGCCAAGGGCCAGCCAGCCAAAGCAGTCTCTC
281
R R T M L L S R S P E A Q P K T L P L

910 920 930 940 950 960
ACTGCGACGACCTCTCCTGCAGCAGAAAGGCGACCCGGTCGTTGACATGCGCCAGCGACCTCTCCTG
301
T G S T F H D Q I A M L S H R C F N T L

970 980 990
ACTAACAGCTTCCAGCCCTCCTTGCTCGGCCGCAAGATTCTGGCCGCCATCATTATGAAA
321
T N S F Q P S L L G R K I L A A I I M K

1010
AAAGACTCTGAGGACATGGGTGTCGTCGTCAGCTTGGGAACAGGGAATCGCTGTGTAAAA
341
K D S E D M G V V V S L G T G N R C V K

1090 1100 1110 1120 1130 1140
GGGAAATTCTCTCAGCCTAAAGAGGAAACTGTCAGGCTATTGCTCCAGGCCCTGGGAAAGATCGATGGTAA
361
G D S L S L K G E T V N D C H A E I S

1150 1160 1170 1180 1190 1200
CGGAGAGGCTTCATCAGGTTTCTCTCAGGATGTTAATGAAATACAATCTCCAGACTTCC
381
R R G F I R F L Y S E L M K Y N S Q T A

1210 1220 1230 1240 1250 1260
AAGGATATATATATTTGAACTCTGCTAAGGGAAGAGAAAGCTCATAATAAATAAAGCTCAGTG
401
K D S I F E P A K G G E K L Q I K K T V

1270 1280 1290 1300 1310 1320
TCATTTCCCATGTATACAGCTACATCTGGCTCCCTGGTGAGATGGGCGCCCTTCTTGCAGAATGCC
421
S P H L Y I S A T C P C G D G A L F D K S

1330 1340 1350 1360 1370 1380
TGACGACCGGCTCGACTGTTGGAAGACGAAATCCTCCGACATCACCTCCTGTGCGAGAATCCC
441
C S D R A M E S T E S R H Y P V F E N P

1390 1400 1410 1420 1430 1440
AACAAGACAGCGCTCCGACACCAAGGCGAGAGAGACAGCGCATTCTCTGGGAATCCC
461
K Q G K L R T K V E N E G T I P V E S

1450 1460 1470 1480 1490 1500

S42
In yellow the transcription start site is highlighted. The ORF is marked by the single letter amino acid code.
SNAP-ADAR1 and reporter mRNA synthesis

For the in-vitro transcription of correctly m\textsuperscript{7}G capped and poly(A)-tailed mRNAs of SNAP-ADAR1 and eGFP variants (wt, W58X) the mMESSAGE mMACHINE T7 Ultra Kit (Life Technologies) was used. For additional stabilization, the respective genes were subcloned into the pUC57-T7-RPP2 vector containing a 169 bp 5'-UTR from the Platynereis 60S acidic ribosomal protein P2 downstream of the T7 promoter. To avoid the formation of long heterogeneous RNA transcripts and to facilitate “run-off” transcription, the expression plasmids were linearized prior to in-vitro transcription. The linearization reaction mix contained 5 µL CutSmart buffer (10 x), 15 µg plasmid DNA and 2.5 µL Eco53kl (10 units/µL). The total reaction volume was adjusted to 50 µL with nuclease-free water. To terminate the linearization reaction 2.5 µL 0.5 M EDTA, 0.5 µL 3 M NaOAc and 100 µl ethanol (RNase free) were added. The mixture was cooled for 1h at -20°C, pelleted (15 min, 15000 rpm) and re-suspended in nuclease-free water at a concentration of 1 µg/µL. 2 µg of the respective linearized plasmid template was in-vitro-transcribed with the mMESSAGE mMACHINE T7 Ultra Kit (Life Technologies). All steps were performed according to the manufacturer’s protocol. To verify the correct polyadenylation state, the synthesized transcripts were mixed with 2 µL of 5 x RNA loading buffer (95% formamide, 0.025 % bromophenol blue) denatured (70°C, 5 min) and applied to agarose gel electrophoresis (1 x TBE, 1.4%, staining with Rotisafe by Carl Roth GmbH, see Fig S21).

![Figure S21. Analytical TBE agarose gel (1.4 %) of iv-transcribed mRNAs for microinjection prior to and after polyadenylation.](image_url)
**Ethanol precipitation of guideRNAs**

Prior to the in vivo application of the editing system in Platynereis Dumerilii the synthesized guideRNAs had to be purified. Ethanol precipitation was used to get rid of impurities and detergents and to exchange the counter ion of the guideRNAs to potassium. Therefore, the volume of the guideRNA samples was adjusted to 300 µL with nuclease-free water. Subsequently 33 µL 3 M KOAc and 1 ml 100 % ethanol were added. The mixture was stored at -80°C overnight and centrifuged again (30 min, -4°C, 15000 rpm). The supernatant was removed carefully and the pellet was washed with 500 µl 70% ethanol (10 min, 4 °C, 15000 rpm) and re-suspended in 15 µl nuclease-free water.

**Platynereis dumerilii zygotes**

Full-grown and mature male and female Platynereis were obtained from the in-house breeding culture of the laboratory of Dr. Gáspár Jékely (Max Planck Institute for Developmental Biology, 72076 Tübingen, Germany). Reproduction of the worm was initiated by collecting two males and females in one beaker containing 75 mL natural seawater (NSW). The release of sperm and oocytes was monitored by eye and eventually the animals were removed carefully with a plastic pasteur pipette. The fertilized eggs were washed one time by pouring off half of the NSW and refilling the beaker. To facilitate microinjection, the zygotes were incubated for 55 min at 14.8 °C and the egg jelly was removed by rinsing the zygotes with 500 mL NSW in a 100 µm sieve. The vitelline envelope was softened by a minute-long proteinase K treatment (final concentration: 70 µg/mL) and an additional washing step with 500 µL NSW. Approximately 100 zygotes were embedded into the channel of the injection stage (2% agarose in NSW). During the whole procedure of washing and the following microinjection, care has been taken to always keep the eggs covered with NSW to avoid draining.

**Microinjection of Platynereis zygotes**

Injection samples contained 1.5 µg/µL Rhodamine-dextran (10 kDa MW, Sigma) for injection control, 250 ng/µL of the respective reporter mRNA, 450 ng/µL SNAP-ADAR1 mRNA, and 25 µM of the respective guideRNA. Prior to microinjection the samples were centrifuged and 3.5 µL of the supernatant were loaded into a Femtotips II microcapillarie (Eppendorf). Microinjection was performed with a Femtojet express microinjector (Eppendorf) on a Zeiss Axiovert 40 CL inverted microscope combined with a Luigs and Neumann micromanipulator. The temperature was controlled using a Luigs and Neumann Badcontroller V cooling system and a Cyclo 2 water pump (Roth). The injection session was carried out at 14.8 °C. The start parameter for microinjection (injection pressure: 700 hPa; injection time: 0.1 s; compensation pressure: 35 hPa) were adjusted accordingly to the condition of the microcapillarie. Injection was started 1 hour post fertilization (hpf) and stopped when the first cleavage of the Platynereis zygotes could be detected.
(ca. 2 hpf). Microinjected zygotes were bred at 19°C in Nunclon six-well plates containing 6 mL NSW until the desired stage of development was reached. For decaging of Npom-protected guideRNAs the injected zygotes were collected in a Nunclon petri dish containing 6 ml NSW, put on a UV-table (UVP high performance UV transilluminator, 25 W, 365 nm, power=high) and irradiated for the indicated amount of time.

**Immobilization and imaging of Platynereis larvae**

Platynereis larvae were imaged 24 hpf. Healthy animals had to be immobilized to allow proper imaging. Therefore, glass slides with 3 layers of adhesive tape on both sides were prepared. 10 µl of NSW containing 3-5 larvae were mounted in the middle of the slide and trapped with a coverslip, forming a small chamber for the animals.

Imaging was performed with an AxioCamHRc microscope camera connected to an Axio Imager Z1 widefield fluorescence microscope (Zeiss). The eGFP and the rhodamine-dextran signal were recorded with an exposure time of 750 ms and 350 ms respectively for 40x magnification. For 10x magnification an exposure time of 2000 ms (eGFP) and 1063 ms (rhodamine-dextran) were chosen.

**Platynereis harvesting and RNA isolation**

Larvae were harvested 25 hpf. Healthy ones were separated from unhealthy ones and collected in a 1.5 ml reaction tube. As much NSW as possible was removed from the tube and the larvae were frozen in liquid nitrogen and stored at -80°C till further use. Larvae were thawed on ice and 80–100 animals were used for RNA isolation. If needed, larvae of 2 injection sessions with the same injection sample were pooled. Lysis and RNA purification was performed with the RNeasy MinElute Cleanup Kit (Qiagen). All steps were performed according to the manufacturers protocol. To facilitate lysis of Platynereis, the RLT lysis buffer + 1% mercaptoethanol (v/v), shear forces (passing through 0.6 mm needle) and vortexing (10 s) was used. The purified RNA was eluted from the spin columns in 30 µl RNase free water and the RNA concentration was determined.

**Reverse transcription**

500 ng of extracted total RNA were reverse transcribed into cDNA. The sample was filled up to 14.75 µl with RNase free water, mixed with 1 µl W58X PD bw primer (10 µM) and 1 µl dNTPs (10 mM each) and incubated at 70 °C for 3 min. The mixture was immediately spun down and put on ice. Subsequently 2 µl 10 x M-MuLV-RT buffer, 0.25 µl murine RNase inhibitor (40 units/µl) and 1 µl M-MuLV reverse transcriptase (200 units/µl) were added and the solution was incubated at 42°C for 2 h. M-MuLV reverse transcriptase was inactivated by heating to 90°C for 10 min.
**Taq-PCR and agarose gel electrophoresis**

The cDNA was amplified by PCR on a 50 μl scale. The PCR reaction mix contained 5 μl ThermoPol buffer (10 x), 2.5 μl of W58X PD fw primer (10 μM), 2.5 μl W58X PD bw primer (10 μM), 1.25 μl dNTPs (10 mM each), 1 μl Taq DNA polymerase (5 units/μl) and 7 μl of the unpurified cDNA reaction mixture. 10 μl of 6 x blue loading dye (NEB) were added and all PCR reactions were applied to agarose gel electrophoresis (1 x TAE, 1.4%, staining with Rotisafe by Carl Roth GmbH). The W58X/eGFP band was cut out and the PCR product was recovered using the NucleoSpin gel and PCR clean-up kit by Macherey-Nagel.

**Sequencing**

For sequencing 120 ng of the PCR products were send to Eurofins MWG (Germany). W58X fw (1.5 μl, 10 μM) and W58Xpos327 fw (1.5 μl, 10μM) were used as primers for all samples respectively. The editing yield was determined as described for Stop66 editing *in vitro*. Figure S24-S26 show complete sequencing traces of W58X edited in Platynereis Dumerilii.
Additional experimental data

**Figure S22.** Editing in Platynereis Dumerilii. Showing the full set of controls and additional fluorescence and DIC images at 10x magnification corresponding to the experiment shown in Figure 4, manuscript.
**Table S23.** Statistical overview of detected GFP signal after microinjection. To score the phenotypes, the injected larvae (24hpf) were analyzed by an Imager Z1 widefield fluorescence microscope.

| Injection Sample         | No GFP signal [%] | Low GFP signal [%] | High GFP signal [%] | total number healthy larvae analyzed |
|--------------------------|-------------------|--------------------|---------------------|-------------------------------------|
| eGFP SNAP-ADAR1 BG-guideRNA Figure 4a | 0                 | 4.6                | 95.4                | 195                                 |
| W58X Figure 4b           | 100               | 0                  | 0                   | 200                                 |
| W58X BG-guideRNA Figure 4c | 100               | 0                  | 0                   | 110                                 |
| W58X SNAP-ADAR1 Figure 4d | 100               | 0                  | 0                   | 150                                 |
| W58X SNAP-ADAR1 NH2-guideRNA Figure 4e | 100           | 0                  | 0                   | 120                                 |
| W58X SNAP-ADAR1 BG-guideRNA Figure 4f | 29.1           | 8.2                | 62.7                | 110                                 |
| W58X SNAP-ADAR1 NpomBG-guideRNA No light Figure 5a | 81.2           | 15.2               | 3.6                 | 250                                 |
| W58X SNAP-ADAR1 NpomBG-guideRNA 5 min 365 nm Figure 5b | 48               | 10                 | 42                  | 150                                 |
**Figure S24.** Sequencing obtained from the edited RNA from the experiment shown in Figure 4f in the manuscript (editing with BG-guideRNA & SNAP-ADAR1).
Figure S25. Sequencing obtained from the unedited RNA from the control experiment shown in Figure 4b in the manuscript (W58X eGFP & no SNAP-ADAR1).
Figure S26. Sequencing obtained from the edited RNA from the experiment shown in Figure 5b in the manuscript (editing with NpomBG-guideRNA & SNAP-ADAR1 & 5 min UV light).
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