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Supporting Information

Protein-Functionalized DNA Nanostructures as Tools to Control Transcription in Zebrafish Embryos

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Supporting Information

Experimental Procedures / Materials

Design of origami structures: The three-dimensional rigid-rod tubular DNA origami structure (DON) used in this work was assembled from the single stranded scaffold 109Z5 (5438 bases) and 130 staple strand oligonucleotides. The scaffold 109Z5 was prepared as previously described.[1] A series of staple strands was selected to selectively position the Cy5 fluorophore, benzylguanine (BG) and chlorohexan (CH). All oligonucleotides were purchased from Sigma Aldrich. The sequences of the unmodified staple strands are listed in Table S2.

Assembly and purification of DNA origami: DNA origami nanostructures were assembled according to Rothemund´s procedure. The assembly of DON-1 was achieved by temperature-dependent annealing, using a 1:20 molar ratio between the single-stranded plasmid DNA (20 nM) and each of the staple strands in 1X TEMg buffer (20 mM Tris base, 2 mM EDTA, 12.5 mM $MgCl_2$, pH = 7.6) and a total volume of 500 µl. After the denaturing step at 95 °C, the annealing in solution was performed by decreasing the temperature from 85°C to 65°C at -1°C/cycle with each step holding the temperature for 10 minutes and subsequently from 65°C to 25°C at -1°C/cycle with each step holding the temperature for 15 minutes. After annealing, excess staple strands were removed by PEG precipitation according to Dietz´s procedure.[2] The origami structures were precipitated by adding a 1:1 volume ratio of precipitating buffer (5 mM Tris base, 1 mM EDTA, 505 mM NaCl, 15% PEG-8000, pH 8), followed by centrifugation at 16,100 g for 30 minutes. The resulting pellet was resuspended in 40 µl TM buffer (20 mM Tris base, 6 mM $MgCl_2$ pH 7.6).

Agarose gel electrophoresis: DON samples were analyzed by gel electrophoresis on 0.5-2% agarose gels in 0.5x TBE buffer supplemented with 10 mM $MgCl_2$ (44.5 mM Tris, 44.5 mM boric acid, 1 mM EDTA, 10 mM $MgCl_2$, pH 8.3). Electrophoresis was performed at 70 V for 2-3 h at 4 °C. Subsequently, the gels were stained with ethidium bromide. The standard markers used for agarose gels were GeneRuler DNA Ladder Mix (Thermo Scientific , SM0333) or MassRuler™ DNA Ladder Mix (Thermo Scientific, SM0403).

SDS-gel electrophoresis: Protein sample were incubated with 25% of SDS loading dye (500 mM Tris pH 6.8, 20% glycerol, 4 % SDS) at 95°C for 10 minutes and then analyzed by 16 % denaturing SDS-PAGE, run in 1x Maniatis buffer (0.1 M Tris, 1.25 M Glycin, 0.1% SDS) at 20 V/cm for 1 h. Bands were visualized by Coomasie staining or Silver staining (Biorad). The standard marker used for these gels was Page Ruler™ prestained protein ladder (Thermo scientific).

AFM analysis: The sample was diluted in TEMg (20 mM Tris base, 1 mM EDTA, 12.5 mM $MgCl_2$, pH 7.6) up to 20 times to a final concentration of 10 nM. A drop of 5 µl DON sample was deposited on freshly cleaved mica surface (Plano GmbH) and allowed to adsorb for 3 min at room temperature. Subsequently the drop was removed and the mica carefully dried under nitrogen stream. The sample was scanned with a MultiModeTM 8 microscope (Bruker, Billerica, MA) equipped with a Nanoscope V controller. The analyses were performed in Air-ScanAsyst mode with silicon tip on nitride lever cantilevers with sharpened pyramidal tips (ScanAsyst-Air+ tips 0.4 N/m, Bruker). The obtained images were analyzed using the NanoScope 8.15 software.

Origami quantification via qPCR: The concentration of the purified origami structures was determined by quantitative PCR (qPCR). For the quantitative analysis of the unknown purified origami samples, a calibration curve was generated from serial 1:2 dilutions of ss109Z5 plasmid in the range of 10 nM-15 pM. To selectively amplify a fragment of ss109Z5 origami scaffold, a PCR-mix was prepared by mixing 1 mL 10x PCR-buffer (160 mM Ammoniumsulfat 670 mM Tris-HCl, 0.1% Tween 20, pH 8.8, autoclaved H_2O), 500 µl KCl [50 mM], 600 µl $MgCl_2$ [50 mM], 200 µl dNTP Mix [10 mM each, Thermo Scientific], 100 µl primer FW_109Z5 [100 µM, CACAATCTTCTCGCGCAACG], 100 µl primer RV_109Z5 [100 µM, AATGCGACCAGATGCTCCAC], 20 µl TaqMan3_109Z5 probe [100 µM, FAM-ACCAGGATGCCATTGCTGTG-TAMRA] 100 µM in H_2O] and 20 µl TaqMoltaq Polymerase 16S (5U/µL) (Molzym) in 7.4 mL H_20. 20 µL PCR-mix were pipetted in each well of a PCR microplate and 1.5 µL of ss109Z5 calibration standards or origami samples were added. qPCR was performed using the real-time thermocycler RotorGene 6000 (Corbett research). The threshold cycle (Ct) was manually adjusted. ΔCt values were calculated by subtraction of the Ct signal from the maximal number of cycles (C_{Max}). To calculate the concentration of the origami samples, the ΔCt values were plotted against the log concentration of the ss109Z5 calibration samples and a linear regression was used for quantification.

Synthesis of CH- and BG-modified DNA oligonucleotides: Amino groups coupled to DNA oligonucleotides were used for conjugation with CH and BG ligands (see Figure S1 for details). Lyophilized amino-conjugated DNA oligonucleotides were resuspended in ddH_2O to a final concentration of 100 µM and purified with the commercially available kit oligo Clean & Concentrator® (Zymo Reasearch, D4060). The resulting pellet at the end of the purification procedure was resuspended in 50 µl of 0.5x PBS (3.5 mM NaH_2PO_4, 8.0 mM Na_2HPO_4, 50 mM NaCl, pH 8.6) to a final concentration of 250-300 µM. Subsequently, this solution was mixed with 50 µl of a 10 mM solution of BG-NHS (NEB, S9151S) or CH-NHS (Promega, P6751) and incubated

overnight at 25 °C under shaking at 1000 rpm. Upon completion of the reaction, the DNA oligonucleotides were purified again with the Clean&Concentrator kit and resuspended in 30-40 µl of 1x TE.

Characterization of CH- and BG-modified DNA oligonucleotides: Samples of amino- and ligand-modified oligonucleotides were analyzed by 20 % denaturing urea-PAGE in 1 x TBE (89 mM Tris, 89 mM boric acid, 2 mM EDTA, pH 8.0) at 28 V/cm for 1 h. Bands were visualized by SybrGold staining.

Cloning of genetic constructs: The genetic constructs were generated following either the protocol for isothermal assembly[3] or Gateway® reactions, carried out according to manufacturer's instructions (Invitrogen). All DNA constructs were purchased from the supplier ThermoFisher Scientific as Geneart® DNA fragments. The sequences were codon optimized for expression in *E. coli* using the algorithm provided in the online ordering system from Geneart®.[4] All constructs were verified by sequencing at LGC Genomics. All PCR reactions were carried out using the Phusion polymerase (NEB). The sequences of all primers are listed in Table S3.

To enable the genetic fusion and expression of N-terminal Halo-tag fused proteins, the destination vector pDESTn25 was created as follows. The plasmid pEXPn9-GFP[5] was linearized using the primers P49 and P51. The HaloTag® sequence flanked by 30 bp overlaps with the terminal regions of the linearized target vector was purchased. These DNA constructs were assembled and the resulting vector pEXPn25-GFP was subsequently recombined with pDONR221 in a BP reaction, leading to pDESTn25. The DNA sequence coding for the VP16 trans-activator domains (residues 410-490)[6, 7] was purchased and subsequently amplified by PCR using the primers P43 and P55. Owing to the att-sites B1 and B2 added by the primers at the two ends of the coding region, the DNA fragment could be assembled with the vector pDONR221 to obtain the pENTR-N-VP16. The expression vector pEXPn25-VP16 was finally obtained by LR reaction between the pENTR-N-VP16 and the pDESTn25.

The sequence of the DNA binding domain of GAL4 (residues 7-40) followed by the dimerization element (residues 50-94) was purchased as "attB1-RBS-GAL4-linker-attB2". It contains the att-sites for the Gateway-based recombination, the ribosome binding site (RBS) for prokaryotic expression and a flexible linker $(GGGGS)_3$.[8] The DNA fragment was assembled with the vector pDONR221 to obtain the pENTR-C-GAL4, which was further recombined with the pDESTn8[5] to yield the expression vector pEXPn8-GAL4.

For the generation of the luciferase reporter plasmid pT2-n2, the vector pT2-luci was linearized with the primers P16 and P24, designed to exclude the originally present minimal promoter (TATA box). The linearized plasmid will be referred

as fragment A in the following. In separate PCRs, two different DNA fragments (fragment B and C) were further obtained. The fragment B was amplified with the primers P26 and P34 from the pENTR221-C-mKate.[5] It contains the TE, flanked by the att-sites B1 and B2. The fragment C contains the minimal promoter Citomegalovirus (CMV) and was amplified from the vector pCSegfpcherry (Addgene, #22465) with the primers P31 and P32. All the used primers were designed to insert a 40 bp overlap region between the fragments in order to allow their assembly in configuration A-B-C and thereby obtaining the vector pT2-luci-mCMV-TE (Figure S1C). The resulting plasmid was subjected to BP reaction with pDONR221 to obtain the destination vector pT2-n2.

The sequence containing five repetitions of the binding sequence for GAL4 (5xUAS, Upstream Activating Site) followed by a spacer was purchased. The sequence of the spacer was designed to give no significant similarities in a blastn analysis in order to exclude the possibility of non-specific binding. The purchased UAS-containing sequence was amplified by PCR using the primers attB1-UAS fwd and UAS 550bp-attB2 rev, which amplify the 5xUAS followed by 550 bp of the spacer, flanked by the att-sites B1 and B2. The resulting DNA fragment was assembled with the vector PDONR211 to obtain the vector pEntry-UAS-550bp. This entry vector was further recombined in a LR reaction with the destination vector pT2-n2 to achieve the plasmid pT2-n2-UAS-550, named also Test gene reporter A.

Protein expression and purification: The expression plasmids were transformed in chemically competent *E. coli* BL21 DE3. Individual clones were cultivated in 2 L LB medium with 100 µg/ml Ampicillin at 37 °C. Once the OD_{600} had reached a value of 0.6–0.9, protein expression was induced by adding IPTG up to a concentration of 0.5 mM and the temperature was decreased to 25 °C. For GAL4-SNAP cultures, $ZnSO_4$ was added to the growth medium concurrently to the IPTG addition at a final concentration of 100 µM to allow its incorporation in the GAL4 DNA binding domains and thereby to ensure the correct protein folding and activity.[9] After 18–20 hours, the cells were harvested by centrifugation. The cell pellet was resuspended in resuspension buffer (10 mM Tris base, 500 mM NaCl, 0.1 % Tween-20, pH 8.0) supplemented with 1mM Dithiothreitol (DTT), 700 µg/ml lysozyme (Applichem) and 25 µg/ml DNAse (Applichem). After sonication, the His-tagged proteins were isolated from the cell lysate by Ni-NTA affinity chromatography. Prior to protein binding, the His Trap FF 1 ml or 5 ml column (GE Healthcare) was pre-equilibrated with NPI10 buffer (10 mM NaH_2PO_4, 100 mM NaCl, 10 mM imidazole, pH 8.0). The protein elution from the column was conducted following optimized gradients of NPI500 buffer (10 mM NaH_2PO_4, 100 mM NaCl, 500 mM imidazole, pH 8.0) with a flow rate of 1 ml/min or 5 ml/min, respectively. Details of the specific gradients are shown in Table S1a.

The further purification of the protein Halo-VP16 and Halo-GFP was achieved by anion exchange chromatography using HiTrap HP 5 ml columns (GE Healthcare). Prior to protein binding, the column was pre-equilibrated with the buffer Tris A (20 mM Tris, 0.1 % Tween, pH 8.0). The protein elution from the column was conducted following an optimized gradient of Tris B (20 mM Tris, 1 M NaCl, 0.1 % Tween, pH 8.0) with a flow rate of 3 ml/min. Details of the specific gradients are shown in Table S1b.
The further purification of the protein GAL4-SNAP was achieved by Heparin-affinity chromatography using HiTrap Heparin 5 ml columns (GE Healthcare). Prior to protein binding, the column was pre-equilibrated with a mix of the Heparin binding buffer (3.5 mM NaH_2PO_4, 8 mM Na_2HPO_4, 50 mM NaCl, 1mM DTT (added directly before use), pH 8.0) and 20 % of Heparin elution buffer (3.5 mM NaH_2PO_4, 8 mM Na_2HPO_4, 1050 mM NaCl, 1mM DTT (added directly before use), pH 8.0). The protein elution from the column was conducted following an optimized gradient of Heparin elution buffer with a flow rate of 3 ml/min. Details of the specific gradients are shown in Table S1c.
The proteins were analyzed on 16% SDS polyacrylamide gels, run at 20 V/cm for 1 h and using the Page Ruler® prestained protein ladder (Thermo scientific). The bands were visualized by Coomassie staining. The concentrations were determined by UV-Vis spectroscopy, using the theoretical molar extinction coefficients at 280 nm estimated by the program Geneious® (Biomatters Ltd).

Table S1a: Purification protocol for Ni-NTA affinity chromatography.

Phase	Elution Buffer (%)	Column volume
Binding	0	-
Washing	2	30
Elution	2-50 (gradient)	15
	100 (isocratic)	6

Table S1b: Purification protocol for Anion exchange chromatography.

Phase	Elution Buffer (%)	Column volume
Binding	0	-
Washing	20	10
Elution	20-40	65

Table S1c: Purification protocol for Heparin-affinity chromatography.

Phase	Elution Buffer (%)	Column volume
Binding	20	-
Washing	25	10
Elution	25-45	25

Western blot analysis: The proteins to be investigated were first separated by 16% SDS-PAGE run at 20 V/cm for 1 h, then blotted onto a PVDF membrane at 300 mA for at least 3 hours in WTB buffer (2.9 g Tris base, 14.5 g Glycine, 100 ml MeOH in 1 L ddH_2O). Subsequently, the membrane was stained for the visualization of the total protein content and to assess the successful protein transfer. The staining was performed by incubation in Coomassie PVDF staining solution (0.1% (w/v) Coomassie R-250, 50% (v/v) MeOH in ddH_2O) for 30-60 seconds, followed by destaining in destaining solution (15% (v/v) Acetic acid, 50% (v/v) MeOH in ddH_2O) and imaging was performed using a flatbed scanner. Prior to all further antibodies incubation steps, the staining reagents were completely removed from the membrane with incubation in destaining solution for 1 hour and subsequent washing for three times in TBS (2.42 g Tris base, 8.0 g NaCl in 1 L ddH_2O, pH 7.6) for 15 minutes. The membrane was subsequently blocked in 5% milk in tween-TBS (TBS with 0.2 % Tween-20) for 1 hour. The membrane was then incubated with 5 ml of a 1:10000 dilution of an anti-VP16 primary antibody (Rabbit anti-VP16 polyclonal, Abcam, ab4808) in 5% milk-tween-TBS for 2 hours. The membrane was washed three times in tween-TBS and incubated with 10 ml of a 1:5000 dilution of a secondary antibody (Goat anti-Rabbit IgG, AP-Conjugated, polyclonal, Rockland, 611-1524) in 5% milk-tween-TBS for 1 hour. The membrane was washed three times in tween-TBS and finally developed by Alkaline Phosphatase Conjugate substrate Kit (Biorad) by immersion in AP-staining solution. The development was blocked by immersion in ddH_2O for 15 minutes.

GAL4 DNA binding activity analysis (Figure S7): The 254 bp and 217 bp DNA fragments containing or not containing 5 copies of the consensus binding site for GAL4 (UAS), respectively, were PCR amplified using biotin-modified forward primers and Cy5-modified reverse primers, using as template the plasmids pEntry-UAS-550bp. The sequences of all primers are listed in Table S2. The obtained DNA fragments were immobilized onto streptavidin-coupled Dynabeads® M-280 (NP-STV, ThermoFisher, 11206D). All washing steps were performed by repetition of magnetic precipitation of the beads and buffer exchange, whereas all incubation steps were performed at room temperature under shaking conditions at 600 rpm. For each assay, four samples containing 15 µl aliquots of NP-STV (10 mg/ml) were prepared and washed three times with 75 µl TPBS (3.5 mM NaH_2PO_4, 8.0 mM Na_2HPO_4, 50 mM NaCl, 0.01% Tween-20, pH 8). After the last washing step, the NP-STV pellets were resuspended in 15 µl 2x Dynabeads binding/wash buffer (10 mM Tris-HCl, 2 M NaCl, 1 mM EDTA, pH 7.5). The samples were further mixed with 15 µl of the desired biotinylated DNA (130 nM) and incubated for 15 minutes. Generally, two samples were incubated with the UAS construct, whereas the other two samples were mixed with the DNA fragment without UAS. The

obtained NP-STV-DNA were collected by magnetic precipitation, the excess of DNA was removed and the NP-STV-DNA were washed three times with 75 µl TPBS (3.5 mM NaH_2PO_4, 8.0 mM Na_2HPO_4, 50 mM NaCl, 0.01% Tween-20, pH 8). After the last washing step, the NP-STV-DNA pellet was resuspended in Biotin-binding/wash buffer (5 mM Tris-HCl, 1 M NaCl, 0.5 mM EDTA, 800 µM biotin, pH 7.5) and incubated for 15 minutes to quench the remaining streptavidin activity. Subsequently, the NP-STV-DNA were washed twice with 75 µl Wash buffer (20 mM Tris base, 6mM $MgCl_2$, 100 mM NaCl, 1 mM ß-mercaptoethanol, 0.01% Tween-20, pH 8) and three times with 75 µl Binding buffer (20 mM Tris base, 6mM $MgCl_2$, 100 mM NaCl, 1 mM ß-mercaptoethanol, pH 8). Finally, each "test" sample was mixed with 5 pmol of GAL4-SNAP in a total volume of 30 µl Binding buffer and incubated for 20 minutes to allow protein-DNA interaction. In each "negative" sample, the incubation step with the protein GAL4-SNAP was substituted by incubation with binding buffer only. After incubation, the samples were washed four times with 100 µl Binding buffer and twice with 100 µl Wash buffer. After the last washing step, the obtained NP pellets were resuspended in 20 µl 4x SDS-LD (500 mM Tris base, 600 mM Glycine, 4% SDS, 50 µl/ml β-mercaptoethanol, 20% Glycerol and traces of bromophenol blue) and denatured by incubation at 95 °C for 10 minutes. Directly after this denaturing step, the NP were immediately pelleted and the supernatant was analyzed by SDS-PAGE.

Protocols for zebrafish experiments: All zebrafish husbandry and experimental procedures were performed in accordance to the German law on Animal Protection and were approved by Local Animal-Protection Committee (Regierungspräsidium Karlsruhe, Az.35-9185.64) and the Karlsruhe Institute of Technology (KIT). Breeding zebrafish (Danio rerio) were maintained at 28 °C on a 14 h light/10 h dark cycle. Embryos were raised in 0.2 mM 1-phenyl-2-thiourea (PTU, Sigma) after 24 hour-post-fertilization to prevent skin pigment formation. For the experiments shown in Figure 4, main text, the transgenic Tg(h2afva:h2afva-GFP) line was used.[10] In order to obtain embryos for the injection, zebrafish male and female were placed in tanks separated by a network overnight. The next morning, the females and males were transferred to fresh-water-containing tanks and after approx. 15 minutes the eggs were collected. Embryos were decorionated before injection to allow confocal microscopy analysis. For decorionation, the eggs were treated with 10 mg/ml pronase (from Streptomyces griseus, Sigma-Aldrich) for 5 minutes to partially digest the chorion and immediately washed 3 times with fishwater in a beaker. Subsequently, the embryos were collected with glass pipette onto 1 % agarose plates and aligned along pre-casted grooves present in the agarose. DON samples supplemented with 0.01 % phenol red (Sigma-Aldrich) were injected into the 1- or 2-cell stage eggs yolk using micro-needles. After injection the embryos were incubated at 28 °C in E3 medium (0.1% NaCl,

0.003% KCl, 0.004% $CaCl_2$ x $2H_2O$, 0.016% $MgSO_4$ x $7H_2O$) until further analysis. Before imaging, the injected zebrafish embryos were embedded in 0.7% low melting agarose immersed in E3 medium. Images were acquired using a Leica TCS SP5 X confocal laser-scanning microscope with a 63x dip-in objective in 3 different channels for DON signal (Cy5, red channel), eGFP-H2 histones signal (eGFP, green channel) and images in bright field. The images were recorded along the z-axis with a stacking size of 0.3 µm and further processed using the Imaris 7.1 software.

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Supporting Figures

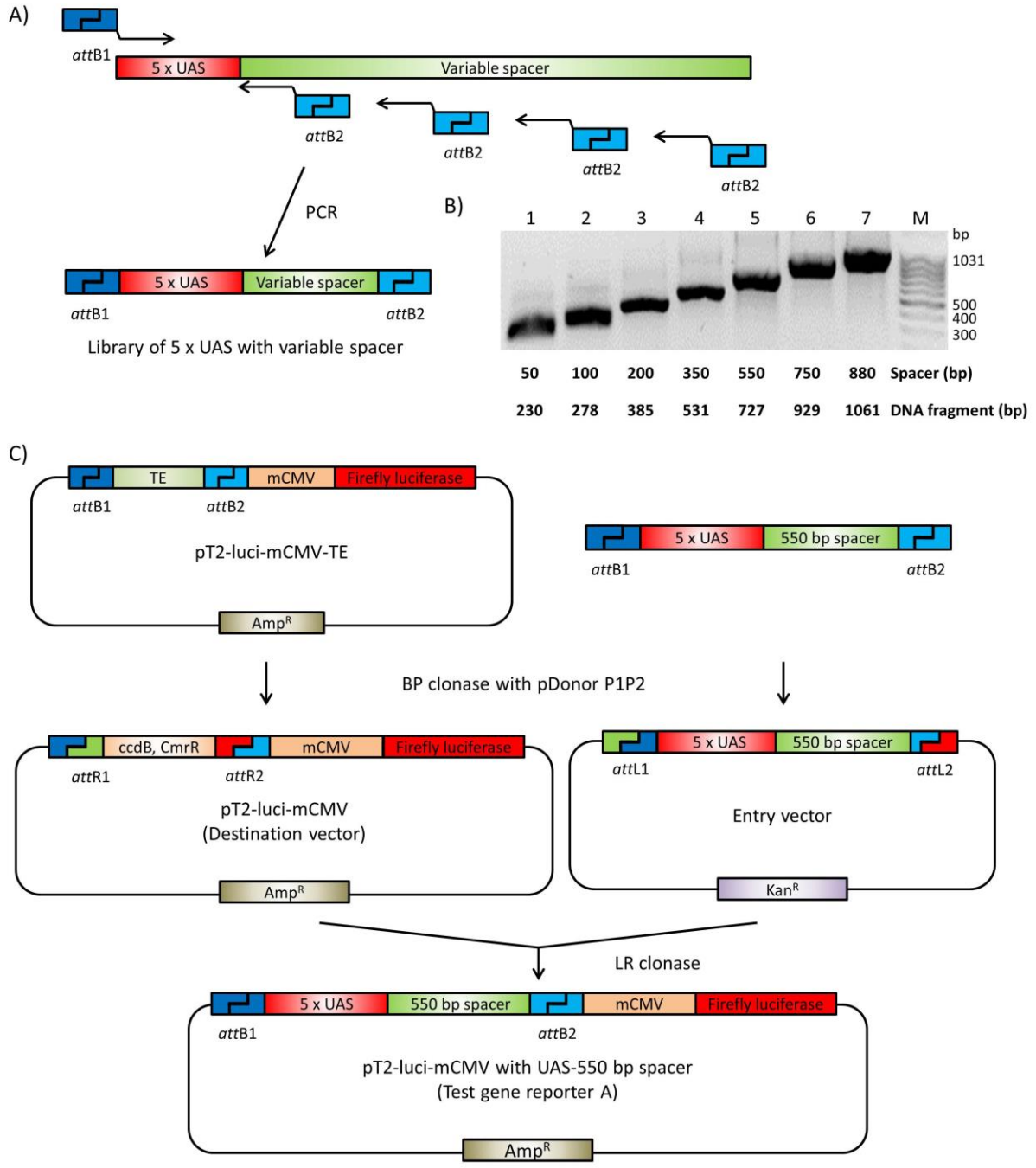

Figure S1: Creation of a plasmid library for the Test reporter gene A. DNA fragments containing the 5xUAS cassette and different spacers were amplified by PCR (**A**) and analyzed by gel electrophoretic analysis (**B**). The gel analysis was carried out with a 1 % agarose gel, run at 90 V for 1 h. M: MassRuler DNA Ladder Mix (Thermo scientific). The procedure illustrated in **C**) allows the creation of a small library with various spacers between the UAS binding sites and the minimal CMV promoter (mCMV) driving Firefly luciferase as a reporter. To this end, the plasmid pT2-luci-mCMV-TE was cloned by Gibson cloning and the illustrated method was used to create a full library by combination of the plasmid pT2-luci-CMV-TE and the different DNA fragments illustrated in panel A.

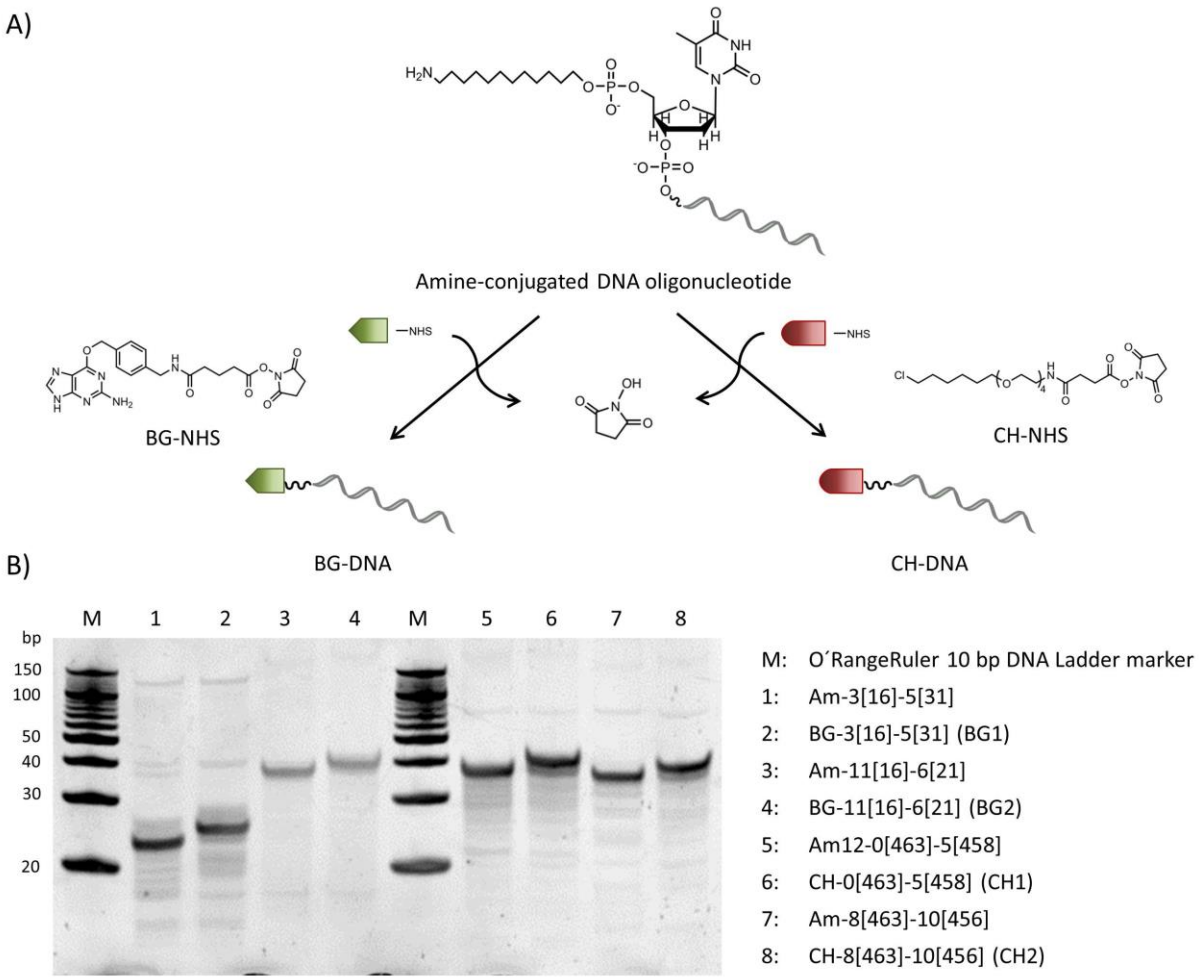

Figure S2: Chemical coupling of olionucleotides. **A**) Schematic representation of the chemical coupling of amino-modified DNA oligonucleotides with benzylguanine (BG, left) or clorohexane (CH, right) groups using commercially available NHS-derivates of the two tags.[5, 11] **B**) Gel electrophoretic analysis (20 % denaturing urea-PAGE, TBE buffer, 17 V/cm, 1 h) of aminoalkyl-derivatized oligonucleotides with CH- and BG-ligands for the decoration of DON-1 (Figure 2, main manuscript). For details on the oligonucleotide names and sequences, see Table S3. Note that the ligand-conjugated oligonucleotides have a lower electrophoretic mobility than their respective amino-modified oligonucleotide educts, thereby indicating the successful conjugation.

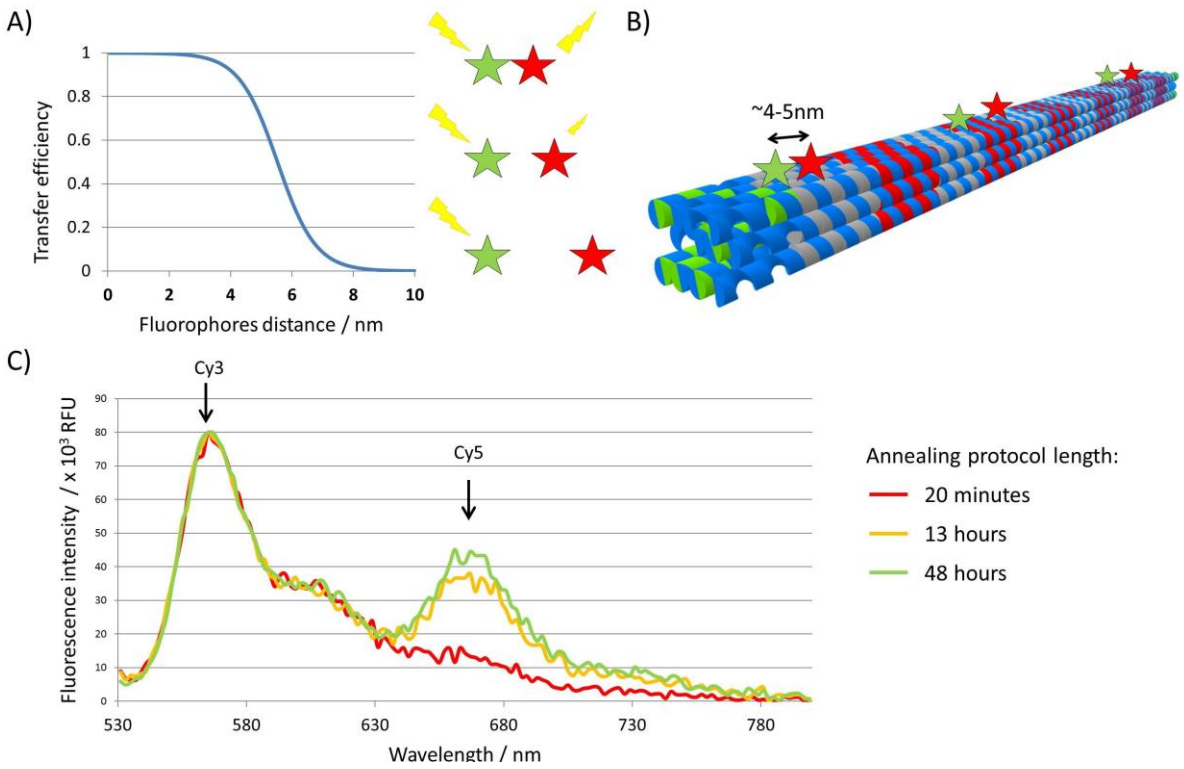

Figure S3: FRET-dependent annealing studies to analyze the folding of DON-1. Since FRET relies on the partial transfer of the excitation energy absorbed by the Cy3 donor to the Cy5 acceptor, which strongly depends on the distance between the two fluorophores (**A**), it can be exploited to spectroscopically monitor the DON assembly. To this end, DON constructs were decorated with six FRET pairs with an inter-fluorophore distance of 4-5 nm (**B**) and the assembly upon three different annealing protocols (annealing time of 20 minutes, 13 hours and 48 hours) was followed. Subsequent to DON purification, the Cy3 emission spectra were recorded (**C**). Each spectrum shows two peaks centered at 570 nm and 670 nm, which correspond to the respective Cy3 and Cy5 emission wavelengths. The presence of the Cy5 peaks upon Cy3 excitation clearly indicates the occurrence of FRET between the two fluorophores, thereby indicating a distance of <6 nm. This confirms the successful assembly. Note that the Cy5 peak intensities significantly increase upon elongation of the annealing process. This indicates that the DON assembly for 13 and 48 hours led to higher yields of the target nanostructures as compared to short annealing for only 20 minutes. Since the DON assembly for 13 h shows almost equally good results as the 48 h assembly, this protocol was chosen as a good compromise between synthesis efficiency and yields.

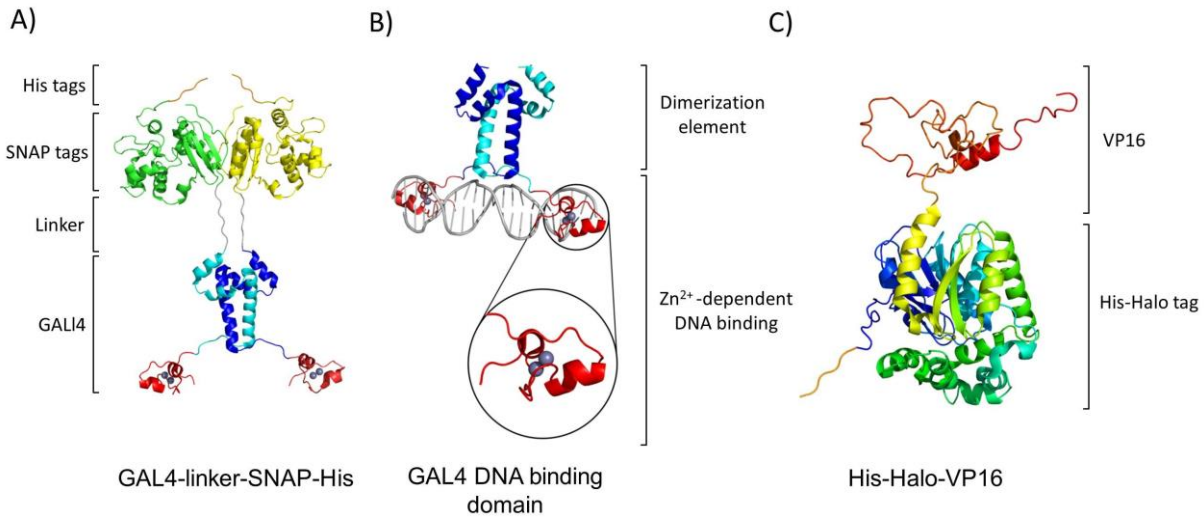

Figure S4: Schematic representation of the GAL4-SNAP and Halo-VP16 proteins. The protein GAL4-SNAP (**A**) contains the DNA-binding domain of GAL4 (GAL4-DBD, **B**) which was genetically fused with the SNAP-tag and a His-tag appended to the GAL4's C-terminus through a flexible $(GGGGS)_3$ linker. Protein Halo-VP16 (**C**) contains the transcription activator domain of VP16, which was genetically fused with the Halo-tag and the His-tag appended to the VP16's N-terminus. The schematic representations of the protein structures are based on the pdb entries 3fbw (Halo tag), 3coq (GAL4) and 1eh6 (SNAP tag). The structure of the VP16 transcription activator domain was calculated by the software I-TASSER based on the amino acid sequence (C-score=-2.51 Estimated TM-score = 0.42±0.14, Estimated RMSD = 9.0±4.6Å).[12] The flexible linker and the His-tag were manually added in the images.

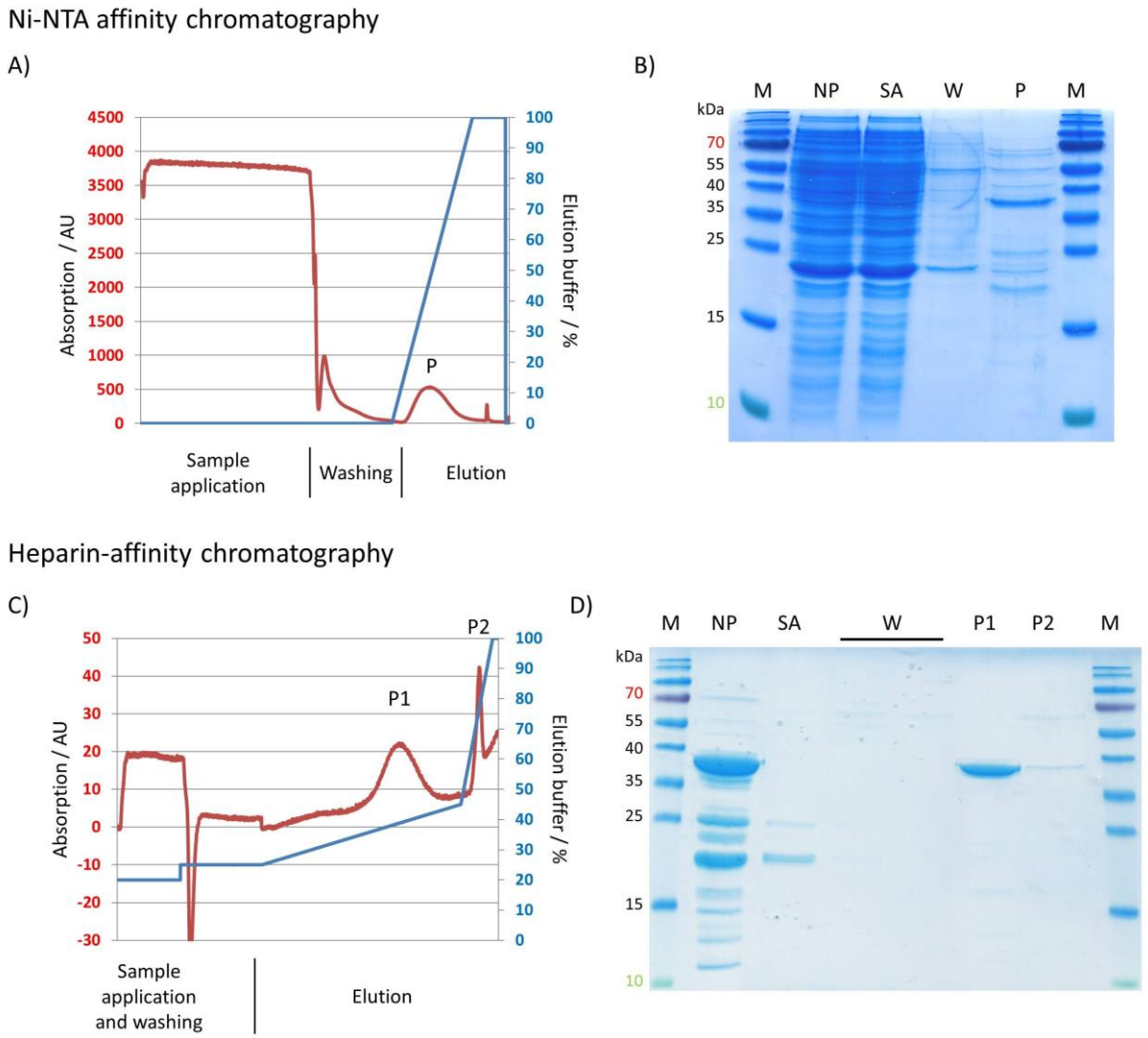

Figure S5: Purification of GAL4-SNAP. The protein was overexpressed in *E. coli* cells and first purified by Ni-NTA affinity chromatography. The representative chromatogram (**A**) and the electrophoretic characterization of the obtained fractions (**B**) show the successful isolation of the fusion protein (P, ~39 kDa) from the cell lysate. The protein was further purified by Heparin-affinity chromatography (**C**). The obtained fractions were analyzed by electrophoresis (**D**), indicating the successful preparation of highly pure protein (P1). NP: Not purified *E.coli* cell lysate; SA: Sample application; W: washing steps; P, P1 and P2: peaks of purified protein. The gels are 16% SDS gels, run at 20 V/cm for 1 h and stained with Coomassie blue. M: Page Ruler® prestained protein ladder (Thermo scientific).

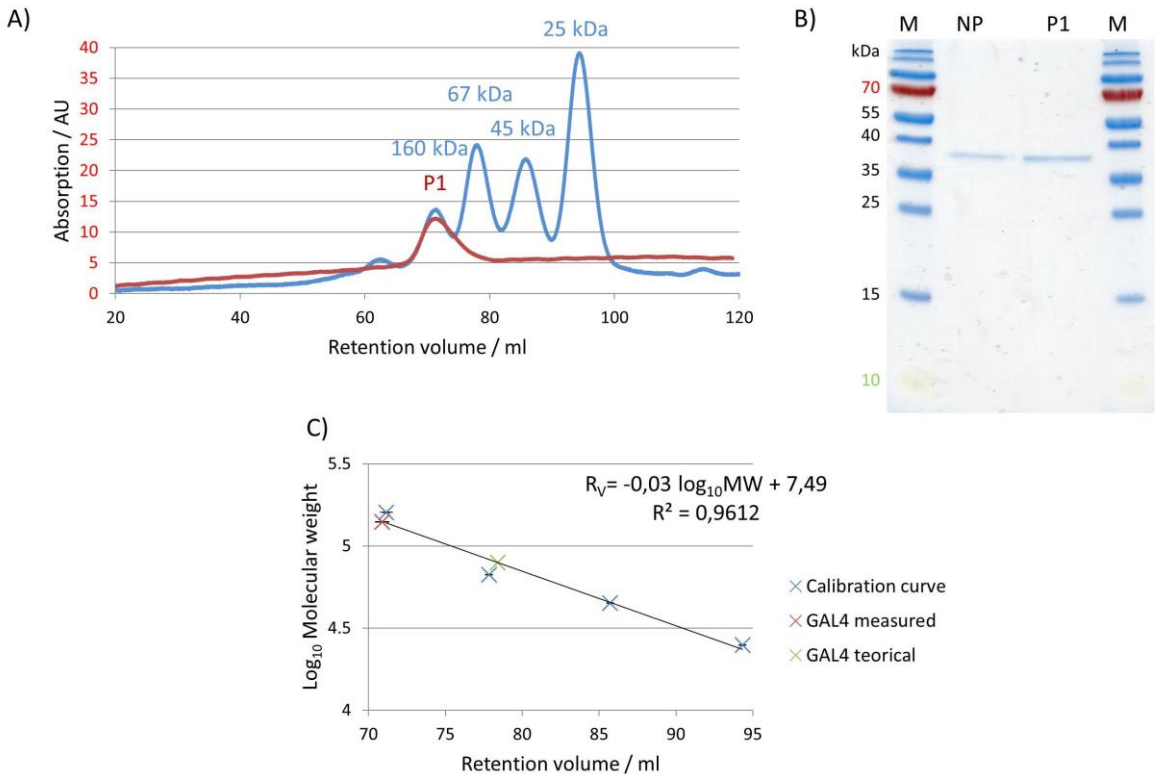

Figure S6: Analysis of the native homodimer conformation of the purified GAL4-SNAP by size-exclusion chromatography (SEC). **A**) Representative chromatogram obtained by SEC analysis under native conditions of the pure GAL4-SNAP (red curve) and of a set of calibration proteins with known molecular weight (blue curve). **B**) Analysis of the peak P1 by gel electrophoresis confirmed the presence of GAL4-SNAP (native protein, NP). This is a 16% SDS gel, 20 V/cm, 1 h, Coomassie staining, M: Page Ruler® prestained protein ladder). Note that the occurrence of only a single peak in SEC indicates that GAL4-SNAP only adopts a single quaternary structure. The graph in **C**) shows the calibration curve obtained from the calibration marker proteins (blue curve in A). The linear regression of the data points was used to estimate the molecular weight GAL4-SNAP as 140 kDa which is higher than the expected value of 79.2 kDa, presumably due to the non-globular nature of the GAL4-SNAP construct, which is a pre-requisite for an accurate determination of the protein´s molecular weight by SEC analysis.[13].

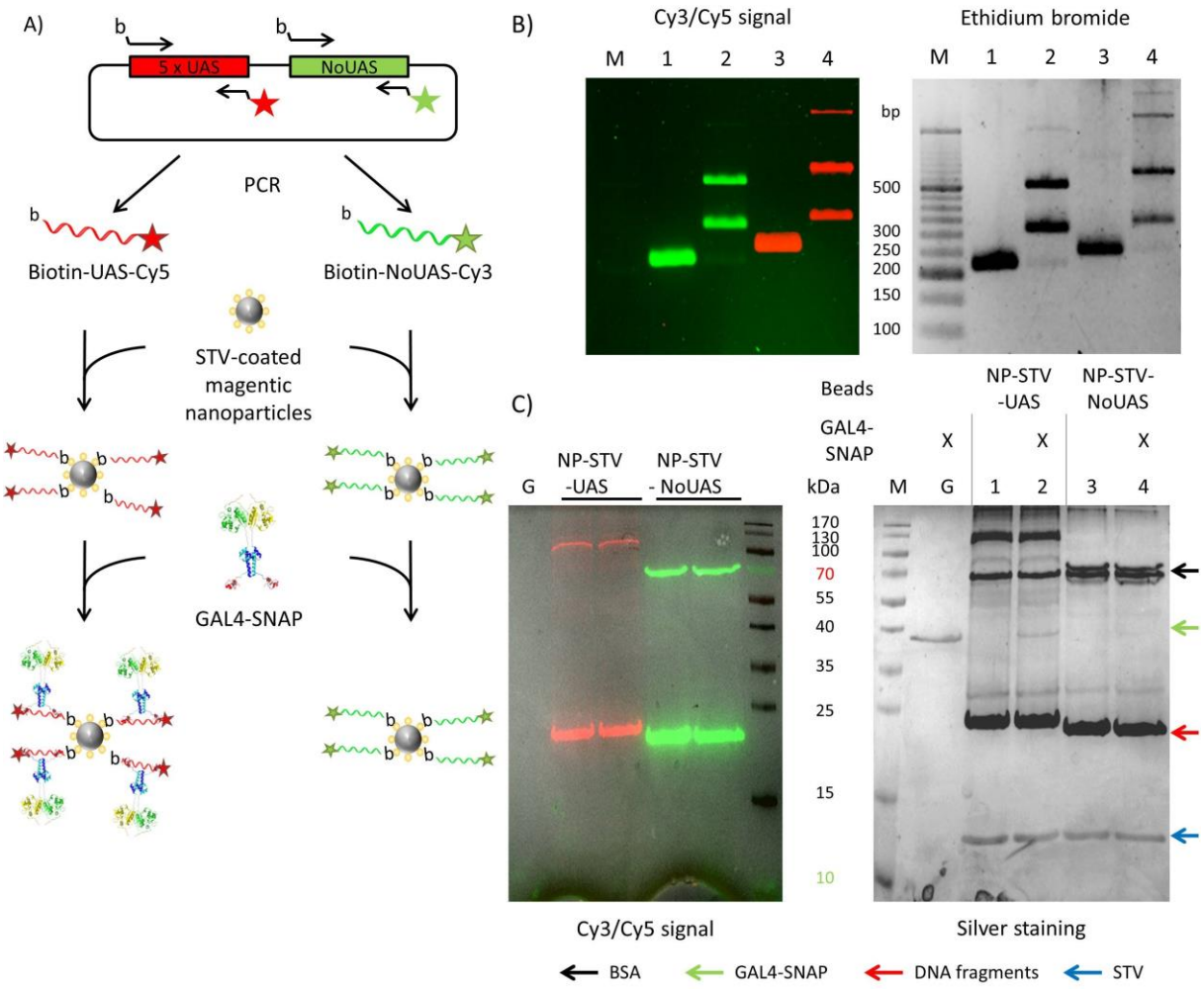

Figure S7: DNA binding activity of GAL4-SNAP. **A**) Schematic illustration of the bead-based pull-down assay developed to assess the activity of recombinant GAL4-SNAP. Two DNA stretches containing either the 5xUAS binding site cassette (UAS) or no UAS binding sites (NoUAS) were synthesized by PCR amplification using a biotinylated and a fluorophore-labeld primer. 5xUAS and NoUAS were labeled with Cy3 and Cy5, respectively (for details on the primer names and sequences, see Table S3). **B**) The PCR fragments were characterized with a 3% agarose gel, 70 V, 2 h, M: O´RangeRuler 50 bp DNA Ladder; lanes 1-2: No-UAS DNA fragment without and with STV; lanes 3-4: UAS DNA fragment without and with STV. The two PCR fragments were then immobilized onto STV-coated magnetic beads and allowed to bind the GAL4-SNAP. Subsequent to washing, the samples were analyzed by SDS-gel electrophoresis (16% SDS gel, 20 V/cm, 1 h) (**C**). The fluorescence image of the gel (left) confirms the presence of the DNA fragments. Silver staining (right) revealed the presence of GAL4-SNAP only in the sample which contained the UAS DNA fragment (lane 2, green arrow). Lane G: pure GAL4-SNAP, MW ~39.4 kDa.

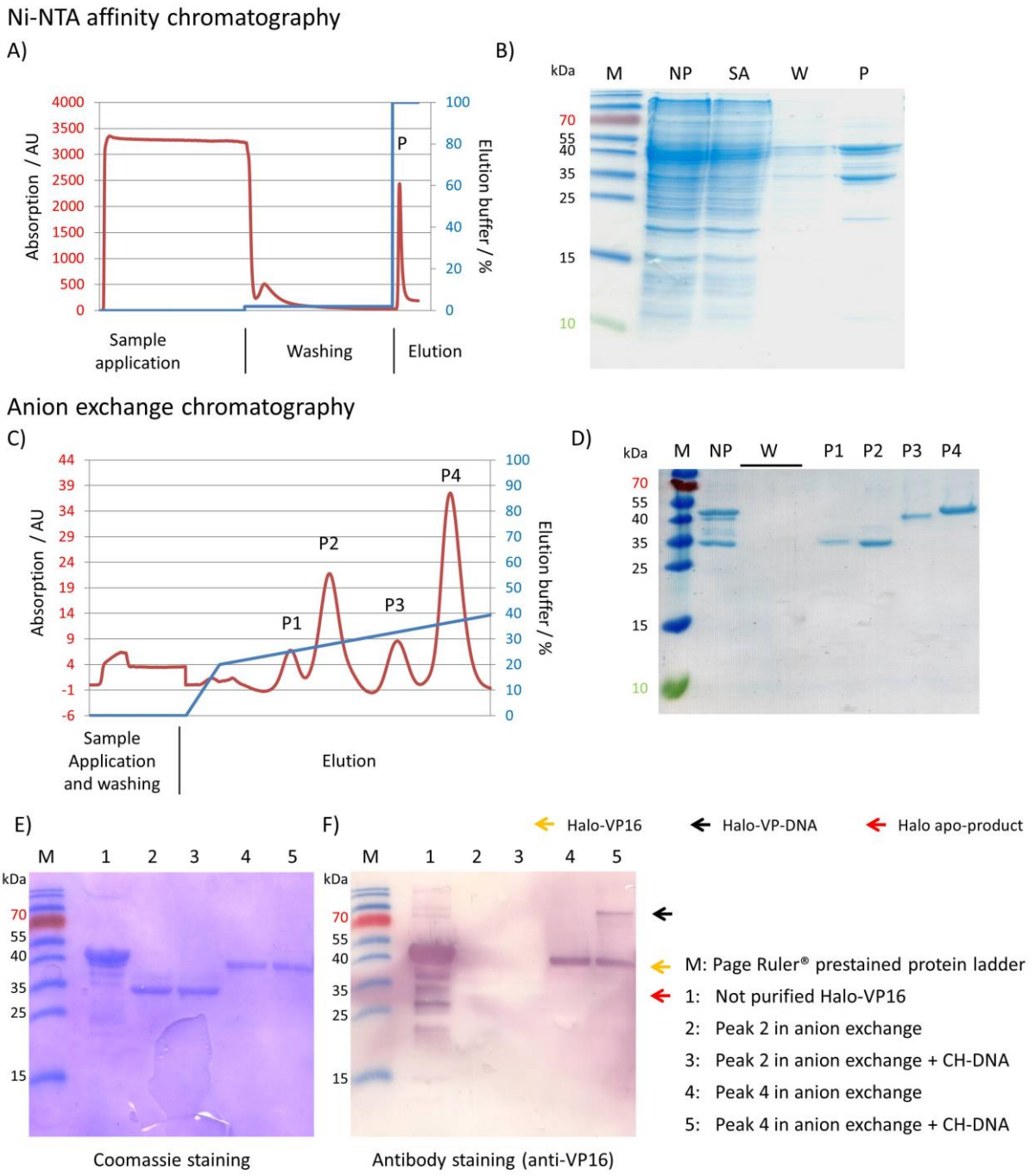

Figure S8: Purification of Halo-VP16. The protein was overexpressed in *E. coli* cells and first purified by Ni-NTA affinity chromatography. The representative chromatogram (**A**) and the gel electrophoretic analysis of the obtained fractions (**B**) show the successful isolation from the cell lysate of the desired protein (~44.9 kDa) along with other three protein species. The eluted proteins were separated by anion exchange chromatography. The representative chromatogram (**C**) along with the electrophoretic characterization of the obtained peak fractions (**D**), shows the successful purification of Halo-VP16 (P4). The proteins obtained from peaks P2 and P4 were further analyzed by western blot to confirm their identity. To this end, both P2 and P4 were incubated with a CH-DNA

oligonucleotide and subjected to SDS PAGE with Coomassie staining (**E**). Subsequent Western blotting of the gel using an anti-VP16 antibody (**F**) revealed that only P4 reacted with the oligonucleotide. The nature of P2 is currently unknown.

Table S2: Unmodified staple strands used for the assembly of the DNA origami structure. The staple strands chosen for modification of the DNA origami structure are highlighted in grey.

Name	Sequence (5´-3´)	Modification
0[103]-11[103]	GTATAGGGGGCCTGCAGGGTGTCTCATTCAGCTTAATACGCATGAAGC GCACCGCGAAGCGAGACCGTCTTCGACATGGCGCGTAGAGACC	
0[135]-11[135]	ACCAC	
0[167]-11[167]	CTTCCATTCAGCCTCGATGCGACCTATCGGCTTGGCGCCGGGCAAGCC	
0[199]-11[199]	GAGTGGTGGCCCAGCGGCCCGCTATATGCCAGGTCGGCGAAATCAAGG	
0[231]-11[231]	TCTCCGCAATGCCCGGCCCAGCTTACCTACACAAGTGGCGAATAATAT	
0[263]-11[263]	TTCTGCCACAACTGGCGGTCGGGCGCTATGAGATCGGAGGCAACATTT	
0[295]-11[295]	GGCTGCTGAAAAATCACTCAAGACGAAAGGCGATAACACTTGCGGCAT	
0[327]-11[327]	GGACCCAATTAATACAAGTGGCGAGTCGGAACAGAATTATCAGAAACG	
0[359]-11[359]	GACAGGAGCAGCAGCACTCTGCTAAGGGGGAAGAAAAGCAGATCAGTT	
0[39]-11[39]	GCCGTGACTAAGAGCCATGGCATCGCGCGCATTTAACTTTCAGGCTCT	
0[391]-11[391]	GGCAACCCAATGGTGCCAAGAACTTCGGGTTTAGAATGACACTGGATC	
0[423]-11[423]	GCGTATCGGACTTTACCTTCTAGTTTTTTGTGAGAGCAACGTTTTCGC	
0[463]-5[458]	TCGCAGACGTTTTGCACATGTTGTGCTTCAGCATGGAAAAACG	5´ CH (CH1)
0[71]-11[71]	CGTTCCATTGATGGTCCCGGAACAGGCAACCAGGATAACAAAGGGCGA	
1[120]-7[127]	GCCGCCGGACGCAACGCCACGCTG	5´ Cy5
1[152]-7[159]	AGGCCATCCAGGTCGATTAATCGC	5´ Cy5
1[184]-7[191]	AAGACGTAAATCCGTTCAGGGCCA	
1[216]-7[223]	GGCGATAAAGAATTGAGCAACGAC	5´ Cy5
1[248]-7[255]	AGGGTAAAAGGGTTGGGTGAAGAT	5´ Cy5
1[280]-7[287]	GACCAGAGGAGATGGCAATCCCTT	
1[312]-7[319]	GCGCTTCGCGCTGCCCCGTCAGAC	
1[344]-7[351]	AGGGTAGCCACGATCATCTTGAGA	
1[376]-7[383]	CGGAACATCGCCAGCCCTGCTTGC	5´ Cy5
1[408]-7[415]	CGTTTCCAGTGATTCACGGTGGTT	
1[56]-7[63]	GCTGTCCCGTGCTCGCGCGAGAAG	5´ Cy5
1[88]-7[95]	GACAGCATCGGCGCCTTTCGACGC	5´ Cy5
2[103]-4[96]	GGTCCTCGGAGTGAGCGGTTAACG	
2[135]-4[128]	CAGGCCGATCACTGCCTGCCCCAG	
2[167]-4[160]	AGGGCGTGGTGCCAGCGAGAGAGT	
2[199]-4[192]	TGGTGGCGCGGGGAGACAACAGCT	
2[231]-4[224]	TGATGAACGGTTGGTGCGCAAGGT	
2[263]-4[256]	CGAGAGAGTGCCGCATTTCTCCTT	
2[295]-4[288]	TTCTGTTCATCGCTACCGAGGAAG	
2[327]-4[320]	TTTGGTCAGACACCCGAGCCGAAC	
2[359]-4[352]	AAGCGGGCCGGGCTTGCCGCCTTT	
2[391]-4[384]	TCTCCAGAACAAGCTGGTTATCCC	
2[423]-4[416]	TGCCTGTTGTCAGAGGTGCTGGCC	

2[71]-4[64]	GGCACCTGCAAGGGCACGGTATCG	
3[120]-8[120]	CGTTGCGCTCATCGTCGAAAACCGATGGGAGAGAGGCCGTTGGTTCAC	5´ Cy3
3[152]-8[152]	AACCTGTCCAAGATTCCGTTCCGCAGATGCTCGTGCATGCTTTCCCGC	5´ Cy3
3[16]-5[31]	AGTCATGCCCCGCGCCTAAGTGCGCGGTAATG	5´ BG (BG1)
3[184]-8[184]	CCAACGCGGGACCAGTGAGATATTACAGCGCGCCGGCCACGTAATTCA	
3[216]-8[216]	GGCGCCAGTGGCCTGCGCGGAACGGGAGCCCGGAAACAAGCCCGCCAG	5´ Cy5
3[248]-8[248]	TGCTCTGAGATGCTCACAGCGTGATGAACGGGGGGGATCAATATACTT	5´ Cy5
3[280]-8[280]	ACTCCGCTATGGGGGTCGAAGGGAGATAGTTAACCGGAGCGCA TTGGT	
3[312]-8[312]	TGCGCCCCCTGATGCCGCGGCAGGTAAGTCGTAGCGTGACCAG ATCGC	
3[344]-8[344]	CGCCCTGACATGTTAAGAGCTTCCATCCTGTTACAACGTTGGAGTCAG	
3[376]-8[376]	GCTTACAGAGCGTTAATAGTCCTGCTGTAGCAACTTACTCGCCCTCCC	5´ Cy5
3[408]-8[408]	CTGCATGTCATCCGCGAGCGTCGAGTAGCCGTACTGGATGGGTATCAT	
3[440]-8[440]	CGAAACGCGTCGTGAACGGAGCCTAGAGCGCACTTCTGCGATAAATCT	
3[56]-8[56]	AAGGCTCTTCCTACGATGATCGTTTTAGTGCAGTCCGGGATTACTGGT	5´ Cy5
3[88]-8[88]	TGCCTAATCCGAAAATGATGCCCTGGTCAGAGTCCTGCATGACACCGG	5´ Cy3
4[127]-10[120]	CAGGCGAACGTGAACAGATCGAGA	
4[159]-10[152]	TGCAGCAAAGGGCGAAGTGATGCC	
4[191]-10[184]	GATTGCCCACCATGAGTATAGGCG	
4[223]-10[216]	TTTTCTTTAATGCTTCAGCCCGAT	
4[255]-10[248]	ACGCATCTTGAGTATTACCGAAGG	
4[287]-10[280]	CGGAAGAGCCCTTTTTGCGGCCAA	
4[319]-10[312]	GACCGAGCTGCTCACCGCAGTGCT	
4[351]-10[344]	GAGTGAGCATGCTGAATCTTACGG	
4[383]-10[376]	CTGATTCTTACATCGATTGGTTGA	
4[415]-10[408]	TTTTGCTCCCTTGAGATCGGTCGC	
4[63]-10[56]	TCGTATCCTGGTGTCTATTCCCCT	
4[95]-10[88]	GCGGGATAAACATGCAACTCACTA	
7[128]-9[135]	GCACCCAGGGCTGGCCTGAGCACC	
7[160]-9[167]	CGCGACAATTCCACTTAAGGAGAT	
7[192]-9[199]	GACTGGAGTTGGGAATGGGGCCTG	
7[224]-9[231]	TGTTTTTATTCATTTGCGCTCATG	
7[256]-9[263]	CCTTTTTGTTACTCATTGTAACTC	
7[288]-9[295]	AACGTGAGCTGATTAATGAATGAA	
7[320]-9[327]	CCCGTAGAGAAATAGAACCACGAT	
7[352]-9[359]	TCCTTTTTCACGACGGGCGCAAAC	
7[384]-9[391]	AAACAAAAGATGGTAATAGCTTCC	
7[416]-9[423]	TGTTTGCCGGTCTCGCGAGGCGGA	
7[64]-9[71]	ATTGTGCAGTATAACGTCTCGACG	
7[96]-9[103]	CGCTTCGTTGATAAGATAGGAAGC	
8[119]-2[104]	CACGCGGGAAACGGTCTCTACCATCTGTTGATACGCGGGCGCGAAAGC	
8[151]-2[136]	GTTTTCGCAGAAACGTTTGATCGGGTCGCGTAAGAATCATGCAAGCGA	
8[183]-2[168]	GCTCCGCCATCGCCGCTTTGCGACGTGACCCACGTCGCGACTTGAGCG	5´ Cy5
8[215]-2[200]	TTGTTGTGCCACGCGGGTGGCAACCTTAATGGCGTCGGCCGAAACGTT	5´ Cy5
8[247]-2[232]	TAGATTGATTTAAAACATTTAAAAGCACACAGTTACTGGAGTTACTGA	
8[279]-2[264]	AACTGTCAGACCAAGTATAATCTCGGCGCAGCGGTATGGACGATGAAA	5´ Cy5
8[311]-2[296]	TGAGATAGGTGCCTCATTTTCGTTGGGTTGGACTCAGGGTAGGGGGAT	5´ Cy5

8[343]-2[328]	GCAACTATGGATGAACAAAGATCACTGCTGCCGATGTAGGTTTTCCTG	5´ Cy5
8[375]-2[360]	GTATCGTAGTTATCTATTCTGCGCATACCTCGTCCTGCGATTCTGATA	5´ Cy5
8[407]-2[392]	TGCAGCACTGGGGCCAAAACCACCCACCACTTAGGGCGCTGTTGAGTT	
8[439]-2[424]	GGAGCCGGTGAGCGTGGGATCAAGATACTGTCGAAACACGCAGATGTC	
8[463]-10[456]	GGCTGGTTTATTGCTGCTCGGCCCTTCCGGCTTGCTATGT	5´ CH (CH2)
8[55]-2[40]	TTCACATTCACCACCCCCCACTGACCACAGCAGCGAGCGAATAAAGAA	
8[87]-2[72]	CATACTCTGCGACATCCCGCCGCTAAATAACGGTCATCTAGCGCTGCC	
9[104]-3[119]	AGCCCAGTCCGCGAAAATTTGCATTTGATGGTTAACTTACATTAATTG	
9[136]-3[151]	GCCGCCGCTGCGTCCGACTCCAGTCGCTGGTTCGCTTTCCAGTCGGGA	
9[168]-3[183]	GGCGCCCACGCACCTGGAATTTGACTGGCCCTTGCATTAATGAATCGG	
9[200]-3[215]	CCACCATATCGGTGATCCAGCCAGGAGACGGGGGCGGTTTGCGTATTG	
9[232]-3[247]	AGCTTTGCGCTTTCCGCGAACTGATTTCACACCACTCTCAGTACAATC	
9[264]-3[279]	GCCTTGATTGACAACGAAAGCGCCGCGGTATTAGTTAAGCCAGTATAC	
9[296]-3[311]	GCCATACCCATGAGTGGACAGGTATCAGTGAGGTGACTGGGTCATGGC	
9[328]-3[343]	GCCTGCAGACAGTAAGAGGAGAGCCTCGCCGCCCAACACCCGCTGACG	
9[360]-3[375]	TATTAACTCAGTCACAACGCCTGGCCGTATTATCTGCTCCCGGCATCC	
9[392]-3[407]	CGGCAACACTATTCTCCGCCACCTTTTCCTGCTGACCGTCTCCGGGAG	
9[40]-3[55]	TGCGCCATAATTTTGTTGCGCCCAAGATATCCGGAGCTGACTGGGTTG	
9[424]-3[439]	TAAAGTTGGCCGGGCAATGCTCGTTGGCCTTTTTTTCACCGTCATCAC	
9[72]-3[87]	CTCTCCCTTTGTGAGCGCATCGCAGCTGTCTTTCGGTCGAGATCCCGG	
10[119]-0[104]	TCTCGATCAGTAGGTTAAATAATACGACACCACGGGGAGGCAGACAAG	
10[151]-0[136]	GGCCACGAAAGGAATGCACGCCCACGCGAGATGGTGGCCCGGCTCCAT	
10[183]-0[168]	CCAGCAACACAGTCCCATTTGCTGGGCGCGTGAGCGAGGTGCCGCCGG	5´ Cy3
10[215]-0[200]	CTTCCCCACCCACGCCAGACAGAAGCCAATCATTGGCTCCAATTCTTG	5´ Cy5
10[247]-0[232]	AGCTAACCACAACATGGGGTTCGTGGATCTAGTTTGCGCATTCACAGT	
10[279]-0[264]	CTTACTTCCGTTGGGACCGGATAAATGACCAAGGACGCGATGGATATG	5´ Cy5
10[311]-0[296]	GCCATAACAAACGACGGTCTTACCCCACTGAGGAGATGCGCCGCGTGC	5´ Cy5
10[343]-0[328]	ATGGCATGCAATGGCAACCAGTGGAAGGATCTTGCGCACCCGTGGCCA	5´ Cy3
10[375]-0[360]	GTACTCACGGCGAACTCCGCCTACGTAATCTGTAGCCGGGTCCTCAAC	5´ Cy3
10[407]-0[392]	CGCATACAATTAATAGAGTTAGGCGCTACCAGTTCTGCTAACCAGTAA	
10[439]-0[424]	GTGTTGACCAGGACCAGATACCAAAGCTACCACGCTTCACGTTCGCTC	
10[55]-0[40]	CTAGAAATTCGATGGTGGCAGCTTCGCGTTGCCGAGGCGGCATAAATC	
10[87]-0[72]	TAGGGGAATATGCGACACATCAAGTTACAGGCACAATCCATGCCAACC	
11[104]-1[119]	TGTACATGGAGGGCACAATCCTGTGGTTTGTTGCGCTCCAATCCCGAT	
11[136]-1[151]	TTCAAGTGCACATCCGGCGGTCCACGCCTTCCCGAATACCAATGGGGA	
11[16]-6[21]	AGTTTGTACAAAAAAGAAGAAGGAATATCACAATCATGCCCCA	5´ BG (BG2)
11[168]-1[183]	CTACGAGGGCACCCAGTTCACCGCTTGCGAGTGACGAAGGACGCCAGC	
11[200]-1[215]	TGGTCTAACCCTGATATCACCAGTACGCAGACTTCTCGCCGCCATGCC	
11[232]-1[247]	TGAAAAAGGAAGAGTAGTGCGGTAGATACCTACGATACGGACGTTGTG	
11[264]-1[279]	CCGTGTCGCCCTTATTCGCCTGATACGCTTCCAATGATACTGCGGCGG	
11[296]-1[311]	TTTGCCTTCCTGTTTTGCAGCGAGTCCGGTAATCCGTGTACAATGCCA	
11[328]-1[343]	CTGGTGAAAGTAAAAGTGATACCGGCACGAGGGGGCGGTTTGTTCCAC	
11[360]-1[375]	GGGTGCACGAGTGGGTGTGGATAATATCTTTATGTCTGGCTGCAGATC	
11[392]-1[407]	TCAACAGCGGTAAGATACATGTTCCTGACTTGTCCAGCTCGACTTCCG	
11[40]-1[55]	AAGGAGGATAGAACCACACTACCGGCGCCATCGTTGCATGTCCTTGAA	
11[424]-1[439]	CCCGAAGAACGTTTTCACGGTTCCCAGGGGGGGCGATTCAGAAACCGA	
11[72]-1[87]	AGAGCTGATTAAGGAGTAACATGAGTGGGAACGACCCAGACCTGCCTG	

Table S3: Sequences of DNA fragments and primers used in this study.

Name	Sequence (5´-3´)
P15	GGGTATATAATGGGAGCTCGAATTCCAGCT
P16	TCTAGAGTCTCCGCTGTCGACCTCGAGGGC
P24	ATGGGAGCTCGAATTCCAGCTTGGCATTCC
P26	GGCCAGATGGGCCCTCGAGGTCGACAGCGGAGACTCTAGACAAGTTTGTACAAAAAAGCAGGCTTAAAAGAAGGAGATATAC ATATGGTGTCTAAGGGCGAAGAG
P27	CGGAATGCCAAGCTGGAATTCGAGCTCCCATTATATACCCCCACTTTGTACAAGAAAGCTGGGTACTACTAATTAAGTTTGT GCCCCAGTTTGCT
P31	CAACAGTACCGGAATGCCAAGCTGGAATTCGAGCTCCCATGGTGGCCCAGCCTGCTCTTCTGTACAATCTCATTGATCCTTG AATTCGAATCGATGGGATCCT
P32	TAATTAGTAGTACCCAGCTTTCTTGTACAAAGTGGGGGTACGTAAATGGGCGGTAGGCGTG
P33	TAATTAGTAGTACCCAGCTTTCTTGTACAAAGTGGGGGTAATATCTATTAATAGTAACTTGGCAAGTACA
P34	TACCCCCACTTTGTACAAGAAAGCTGGGTA
P43	GGGGACAAGTTTGTACAAAAAAGCAGGCTTAGGTAGTCCGGGTATTAGCACCGC
P49	AGATATACATATGTCGTACTACCATCACCATCACCATCACGGTAGGAAATTGGCACCGGTTTTCCG
P51	TGCTTTTTTGTACAAACTTGTTGATTCGAGACCGCTAATTTCCAGGGTGCTCAGCCAACG
P55	GGGGACCACTTTGTACAAGAAAGCTGGGCTACTAACCACCATATTCATCAATACCCAGGGC
F1	CCTGCGTCGTTTAAGGAAGTAC
cF1	GTACTTCCTTAAACGACGCAGG
UAS-Fwd	Cy5-CTTTGTACAAAAAAGCAGGCTTATA
UAS-Rev	Biotin-ATGATGGTAAATGAGCTATGAAGAT
NoUAS-Fwd	Cy3-AAGCTGCGTGCCGTTGTATGCT
NoUAS-Rev	Biotin-TTGATAAATGGTTGAAAAAGGCACA