Supporting Information

Section 1. Methods

cgRNA preparation. gBlocks containing the cgRNA sequence following a T7 promoter sequence were ordered from IDT. The gBlocks were amplified using NEB Q5 High-Fidelity DNA Polymerase 2X Master Mix and purified using QIAquick PCR Purification Kit. Purified PCR products were used for *in vitro* transcription.

Trigger DNA/RNA preparation. Trigger DNA and RNA strands were ordered directly from Integrated DNA Technologies, except for trigger RNAs aT1 and aT1_2OR_B. For preparation of these two trigger RNAs, complementary DNA strands were ordered from IDT and resuspended in IDT Duplex Buffer to 100 μM. The forward strand contained the trigger sequence following a T7 promoter sequence. Forward and reverse strands were combined in equal volumes to 50 μM duplex concentration, and duplexed strands were used for *in vitro* transcription.

***In vitro* RNA transcription.** RNA for cgRNA, standard gRNA, and some trigger RNAs were transcribed from their corresponding DNA templates using the Invitrogen MEGAScript T7 transcription kit and cleaned using Zymo RNA Clean and Concentrator Kit. Concentrations were determined using a NanoDrop2000 spectrophotometer.

Cas9 cleavage/dCas9 binding assay. Target DNA was ordered dry as two 60-nt complementary strands from IDT, with one having a 5' Cy3 label, and resuspended in IDT Duplex Buffer. The two complementary strands were annealed at 50 μM at 95°C for 4 minutes and cooled down to room temperature at 1°C/min.

In vitro cleavage experiments were conducted using 10 μL reactions containing 1X NEBuffer 3.1 (New England Biolabs), 10 nM 5'-Cy3 labeled double-stranded target DNA, 125 nM Cas9 (PNA Bio), 250 nM standard gRNA or cgRNA, and 1 μM trigger RNA. Reactions were incubated for 1 hour at 37°C, followed by addition of 5 μL formamide-TE-SDS (30 mM EDTA, 10 mM Tris-HCl pH 8.0, and 0.24% SDS in formamide) and incubation for 10 minutes at 65°C.

For de-repression experiments, reactions were similarly assembled to a total volume of 9.5 μL. Reactions were incubated at 37°C for 30 minutes, after which either 0.5 μL of 10 μM de-repressing trigger or 0.5 μL 0.1x TE (IDTE, Integrated DNA Technologies) as a control was added to each reaction to produce a final reaction volume of 10 μL. All final concentrations for target DNA, Cas9, and gRNA/cgRNA were as listed above. Final trigger concentrations were 500 nM for repressing trigger and 100-1000 nM for de-repressing trigger. Samples were then incubated for an additional 30 minutes, followed by addition of formamide-TE-SDS and 10 minutes of incubation at 65°C as described above.

dCas9 binding experiments were conducted using 10 μL reactions containing 1X NEBuffer 3.1 (New England Biolabs), 10 nM 5'-Cy3 labeled double-stranded target DNA, 1 μM Cas9 (PNA Bio), 1 μM standard gRNA or cgRNA, and 125-2000 nM trigger RNA. Reactions were incubated for 1 hour at 37°C, followed by addition of 2.5 uL 50% glycerol.

Gel electrophoresis. For Cas9 cleavage experiments, products were separated and analyzed by 6% denaturing PAGE (TBE-urea) at 65°C in 1X TBE (200V for 20 minutes), and immediately scanned on Cy3 channel, followed by staining and visualization with 1X SYBR Gold for verification. For dCas9 binding experiments, products were separated and analyzed by 8% native PAGE (TBE) at 4°C in 1X TBE + 5 mM $MgCl_2$ (200V for 20 minutes), and immediately scanned on Cy3 channel, followed by staining and visualization with 1X SYBR Gold for verification.

Image acquisition and quantification. Gels were scanned on a Typhoon FLA 9000 and analyzed using ImageJ. Cleaved and uncleaved bands on the Cy3 channel were quantified in each lane by capturing a rectangular region containing the two bands such that the midpoint of the rectangle separates the two bands, and saving the profile data containing the average pixel intensity of each pixel row. The profile data for each lane was smoothed using a moving average filter with a span of 5 pixels, and then background

subtracted by using the minimum value in the lane's *smoothed* profile data as the background value. Each half of the smoothed, background-subtracted profile data was then integrated using Simpson's method to yield two integrated intensities U and C for the halves containing the uncleaved DNA and cleaved DNA, respectively. The fraction of DNA template that was cleaved was then calculated by C/(U+C).

NUPACK sequence design. NUPACK software was used to design agRNA using multiple structural constraints. After receiving up to 10 candidate output sequences from NUPACK, sequences were vetted starting with the one with the lowest normalized ensemble defect (a percentage measure of how well the sequence folds into the desired structure, weighted by the abundance of multiple possible conformations) by folding the agRNA sequence individually, the trigger individually, and then together, to manually check for similarity between the minimum free energy structures and the desired conformations. RNA energy parameters used were those of Serra and Turner[1], and the following sequences were excluded in order to avoid long strings of nucleotide repeats: AAAA, CCCC, GGGG, UUUU, KKKKKK, MMMMMM, RRRRRR, SSSSSS, WWWWWW, YYYYYY.

All agRNA-trigger pairs for a given guide sequence were designed using three structural constraints: 1) the desired structure of the agRNA, 2) the desired structure of the trigger (always 100% single-stranded), and 3) the desired structure of the agRNA-trigger complex. All agRNA structures were specified without the additional 3' ssRNA tail attached to the toehold domain (Figure S3). The treatment of the constant gRNA scaffold region for constraints 1 and 3 differed between designs. agG1T1 and agG1T4 were designed with constraints 1 and 3 having an unstructured constant scaffold region. agG1T2 and agG1T3 were designed with constraints 1 and 3 having a structure-specified scaffold region based on the expected secondary structure. agG1T5 was designed with constraint 1 having an unstructured scaffold region, but with constraint 3 having a structure-specified scaffold region. agRNAs with guide sequences other than G1 were obtained by merely swapping out only the guide sequence on the corresponding G1 switch (i.e., agG2T3 is the same as agG1T3, but with only the 20 nt guide sequence changed from G1 to G2).

The next section ("Example NUPACK script") shows an example script for generating sequences for an agRNA with guide sequence G1 specified but unspecified trigger sequence, and constraints 1 and 3 (see paragraph above) having an unstructured and structured constant scaffold region, respectively.

Generation of agRNA for optimization of various domain lengths for agG1T1 was done similarly, with the appropriate modification to structural constraints: the stem (see Figure S5a) was specified as double stranded, and the linker **y** and spacer **s** (Figure S5b and S5c, respectively) specified as single-stranded. Testing of 3' tail extensions of various lengths on agRNA (Figure S3) was done by designing a 20 nt tail in NUPACK specified to be single-stranded when appended to the toehold domain of agRNA with guide 1 and trigger 1. The extensions tested were 5, 10, and 15nt truncations of this 20nt sequence.

rgRNA was prepared as a truncation of the corresponding agRNA by using an appropriate reverse PCR primer which truncated it at the end of the gRNA scaffold and appended 4 U's. rgG1T1re was prepared as a truncation of agG1T1_sgsp10.

Guide sequences G1 and G2 were obtained from working sequences in the literature.[2] Guides G3 and G4 were obtained by direct permutation of G1, and G5 was randomly generated with approximate mirroring of the A, U, C, and G fractions within guide sequence G1.

Self-assembling trigger sequences for NAND logic implementation were designed using NUPACK where individual triggers were specified to have no secondary structure. Hybridization domains between triggers were specified to be 21 nt, and for the triggers with an rgRNA-binding domain, a 3 nt spacer was included between its rgRNA-binding domain and its 21 nt trigger hybridization domain in order to compensate, at least in part, for the extra distance between rgRNA-binding domains created when forming a multi-trigger complex. The hybridization and spacer domains were allowed to take on any sequence. 21 nt was chosen as the hybridization domain length because it is optimal according to previous work in fine tuning of self-assembling trigger complex design for AND logic activation of RNA ribocomputing switch devices.[3]

cgRNA and trigger designs produced a range of ON/OFF ratios as seen in the data. While cgRNA design is straightforward and quite simple, not all sequence combinations produce switching behavior with the same degree of robustness, and it is to be expected that there exist parts of the guide/trigger sequence space with less robust switching due to unanticipated secondary structure or other factors.

Example NUPACK script. The example script below designs an agRNA with guide sequence G1 (GAUUUCUUCUUGCGCUUUUU) and unspecified trigger sequence. In the structural constraints specified in the script, the constant scaffold region is given as unstructured for the agRNA alone, but structured according to the expected gRNA secondary structure for the agRNA-trigger duplex (see previous section “NUPACK sequence design”).

```
#
# design material, temperature, and trials
# see NUPACK User Guide for valid options for
# material, sodium, magnesium, and dangles
#
material = rna
temperature[C] = 37.0    # optional units: C (default) or K
trials = 10
sodium[M] = 1.0    # optional units: M (default), mM, uM, nM, pM
dangles = some

#
# target structure using DU+ notation
#
structure switch = D16 U106 U14
structure trigger = U30
structure duplex = U37 D5 (U2 D4 U4 U4) U3 D2 U5 U7 D4 U4 U1 D6 U3 U10 D30 +

# sequence domains
#
domain a = GG N14 #stem seq
domain b = GAUUUCUUCUUGCGCUUUUU #guide
domain c =
GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCAC
CGAGUCGGUGC #scaffold
domain d = N10 #linker
domain e = N14 #toehold

#
# strands (optional, used for threading sequence information
# and for displaying results)
#
strand J = a b c d a* e
strand M = e* a

#
# thread sequence domains onto target structures
#
switch.seq = J
trigger.seq = M
duplex.seq = J M

#
# specify stop conditions for normalized ensemble defect
```

```
# default: 1.0 (percent) for each target structure
#
switch.stop = 5.0
trigger.stop = 5.0
duplex.stop = 5.0

#
# prevent sequence patterns
#
prevent = AAAA, CCCC, GGGG, UUUU, KKKKKK, MMMMMM, RRRRRR, SSSSSS, WWWWWW,
YYYYYY
```

Section 2. Sequences

A. Target DNA sequences

Target DNA (Guide G1)	/5Cy3/GTACCTCTGAGACCAAGT**GATTTCTTCTTGCGCTTTTT**TGGTAACTATCTCGATCGTAGC
Target DNA (Guide G1, complement)	GCTACGATCGAGATAGTTACCAAAAAAGCGCAAGAAGAAATCACTTGGTCTCAGAGGTAC
Target DNA (Guide G2)	/5Cy3/TAACCCATCAACCTGTAC**GGTTCACAGTCGGTCACATT**GGGCTACTCCTTGGCTTTTCCG
Target DNA (Guide G2, complement)	CGGAAAAGCCAAGGAGTAGCCCAATGTGACCGACTGTGAACCGTACAGGTTGATGGGTTA
Target DNA (Guide G3)	/5Cy3/GTACCTCTGAGACCAAGT**TGATTCTTCTCTCTGTGTTT**TGGTAACTATCTCGATCGTAGC
Target DNA (Guide G3, complement)	GCTACGATCGAGATAGTTACCAAAACACAGAGAGAAGAATCAACTTGGTCTCAGAGGTAC
Target DNA (Guide G4)	/5Cy3/GTACCTCTGAGACCAAGT**GTCTCTCTATTCTTTGTTTG**TGGTAACTATCTCGATCGTAGC
Target DNA (Guide G4, complement)	GCTACGATCGAGATAGTTACCACAAACAAAGAATAGAGAGACACTTGGTCTCAGAGGTAC
Target DNA (Guide G5)	/5Cy3/GTACCTCTGAGACCAAGT**TTGCTATGATTCCGTTTTAT**TGGTAACTATCTCGATCGTAGC
Target DNA (Guide G5, complement)	GCTACGATCGAGATAGTTACCAATAAAACGGAATCATAGCAAACTTGGTCTCAGAGGTAC

Table S1. Common sequences used in Cas9 cleavage assays. /5Cy3/ denotes Cy3 fluorophore conjugated on 5' end of DNA oligo. Guide sequences are in bold.

B. cgRNA and gRNA sequences

cgRNA nomenclature: prefix “ag” denotes activatable cgRNA design, “rg” denotes repressible cgRNA design. G1, G2, etc. denote the particular guide sequence used in that design. T1, T2, etc. denote the particular activating or repressing trigger sensed by that design. I.e., agG1T1 and rgG1T1 have the same guide sequence G1 but different trigger sequences aT1 and rT1, whereas rgG1T1 and rgG2T1 have the same trigger sequence rT1 (see Table S3). Numbers appearing after an underscore in the cgRNA name denote nucleotide length, e.g. agG1T1_stm8 has a stem length of 8 nt.

agG1T1	GGCACAAUUACAACUC**GAUUUCUUCUUGCGCUUUUU**GUUUUAGA GCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUG AAAAAGUGGCACCGAGUCGGUGCGCCUACUCAUGAGUUGUAAUU GUGCCUUGUAUAUGUGUCCCGUCCUAAUCCCAAU
rgG1T1	GGCACAAUUACAACUC**GAUUUCUUCUUGCGCUUUUU**GUUUUAGA GCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUG AAAAAGUGGCACCGAGUCGGUGCUUUU
rgG1T1e (extended variant used in NAND and NOR logic gate implementations: Figures 3d-f, S7-S9)	GGCACAAUUACAACUCAAUUCGGAAC**GAUUUCUUCUUGCGCUUU UU**GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGU UAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUUUU
agG1T2	GGCCAUUAACACUACA**GAUUUCUUCUUGCGCUUUUU**GUUUUAGA GCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUG AAAAAGUGGCACCGAGUCGGUGCGCCUACUCAUUGUAGUGUUAA UGGCCGUUCUGUUUCUGUGCGUCCUAAUCCCAAU
agG1T3	GGCCUACAUACACUAC**GAUUUCUUCUUGCGCUUUUU**GUUUUAGA GCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUG AAAAAGUGGCACCGAGUCGGUGCGCCUACUCAUGUAGUGUAUGU AGGCCGAAUUCUUGUAUGUCGUCCUAAUCCCAAU
rgG1T3	GGCCUACAUACACUAC**GAUUUCUUCUUGCGCUUUUU**GUUUUAGA GCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUG AAAAAGUGGCACCGAGUCGGUGCUUUU
agG1T4	GGCACCAUACUACUAC**GAUUUCUUCUUGCGCUUUUU**GUUUUAGA GCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUG AAAAAGUGGCACCGAGUCGGUGCAUCCUAUGCUGUAGUAGUAUG GUGCCGAAGUUGUAUGUGACGUCCUAAUCCCAAU
agG1T5	GGCCCUAACAUCACAC**GAUUUCUUCUUGCGCUUUUU**GUUUUAGA GCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUG AAAAAGUGGCACCGAGUCGGUGCAAAUUCUACCGUGUGAUGUUA GGGCCUGUUGAUUGAUUGUCGUCCUAAUCCCAAU
agG2T1	GGCACAAUUACAACUC**GGUUCACAGUCGGUCACAUU**GUUUUAGA GCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUG AAAAAGUGGCACCGAGUCGGUGCGCCUACUCAUGAGUUGUAAUU GUGCCUUGUAUAUGUGUCCCGUCCUAAUCCCAAU
rgG2T1	GGCACAAUUACAACUC**GGUUCACAGUCGGUCACAUU**GUUUUAGA GCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUG AAAAAGUGGCACCGAGUCGGUGCUUUU
rgG2T3	GGCCUACAUACACUAC**GGUUCACAGUCGGUCACAUU**GUUUUAGA GCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUG AAAAAGUGGCACCGAGUCGGUGCUUUU
agG3T1	GGGCACAAUUACAACUC**UGAUUCUUCUCUCUGUGUUU**GUUUUAG AGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUU GAAAAAGUGGCACCGAGUCGGUGCGCCUACUCAUGAGUUGUAAU UGUGCCUUGUAUAUGUGUCCCGUCCUAAUCCCAAU

agG4T1	GGGCACAAUUACAACUC**GUCUCUCUAUUCUUUGUUUG**GUUUUAG AGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUU GAAAAAGUGGCACCGAGUCGGUGCGCCUACUCAUGAGUUGUAAU UGUGCCUUGUAUAUGUGUCCCGUCCUAAUCCCAAU
agG5T1	GGGCACAAUUACAACUC**UUGCUAUGAUUCCGUUUUAU**GUUUUAG AGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUU GAAAAAGUGGCACCGAGUCGGUGCGCCUACUCAUGAGUUGUAAU UGUGCCUUGUAUAUGUGUCCCGUCCUAAUCCCAAU
agG5T4	GGCACCAUACUACUAC**UUGCUAUGAUUCCGUUUUAU**GUUUUAGA GCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUG AAAAAGUGGCACCGAGUCGGUGCGCCUACUCAUGUAGUAGUAUG GUGCCGAAGUUGUAUGUGACGUCCUAAUCCCAAU
agG1T1D (variant used for dCas9 experiment in Figure S12)	GGCACAAUUACAACUC**GAUUUCUUCUUGCGCUUUUU**GUUUUAGA GCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUG AAAAAGUGGCACCGAGUCGGUGCGCCUACUCAUGAGUUGUAAUU GUGCCUUGUAUAUGUGUCCAAAGACCAUCUCGCUCGAGA
agG1T1LS (variant used for tail truncation experiments in Figure S3)	GGCACAAUUACAACUC**GAUUUCUUCUUGCGCUUUUU**GUUUUAGA GCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUG AAAAAGUGGCACCGAGUCGGUGCGCCUACUCAUGAGUUGUAAUU GUGCCUUGUAUAUGUGUCCCGUCCUAAUCCCAAUUCCUC
agDep	**GAUUUCUUCUUGCGCUUUUU**GUUUUAGAGCUAGAAAUAGCAAGU UAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAG UCGGUGCAAUACAUCCCAGCGCAAGAAGAAAUCCUUUGGAGUAU GACAAAGACCAUCUCGCUCGAGA

Table S2.1. List of cgRNA sequences used with different combinations of guide and trigger sequences. Guide sequences are in bold, trigger toehold/binding regions are underlined. All agRNA sequences include 15 nt ssRNA 3' extension (see main text and Figure S3 for details), except agG1T1D, agG1T1LS, and agDep, all of which have a 20 nt ssRNA 3' extension.

agG1T1_stm8	GG**UACAACUC**GAUUUCUUCUUGCGCUUUUUGUUUUAGAGCU AGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUG AAAAAGUGGCACCGAGUCGGUGCGCCUACUCAUGAGUUGUAA UUGUGCCUUGUAUAUGUGUCCCGUCCUAAUCCCAAU
agG1T1_stm12	GG**CAAUUACAACUC**GAUUUCUUCUUGCGCUUUUUGUUUUAG AGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAA CUUGAAAAAGUGGCACCGAGUCGGUGCGCCUACUCAUGAGU UGUAAUUGUGCCUUGUAUAUGUGUCCCGUCCUAAUCCCAAU
agG1T1_stm20	GG**ACAAGGCACAAUUACAACUC**GAUUUCUUCUUGCGCUUUU UGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCC GUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCGCCUACU CAUGAGUUGUAAUUGUGCCUUGUAUAUGUGUCCCGUCCUAAU CCCAAU
agG1T1_sp0	GGCACAAUUACAACUCGAUUUCUUCUUGCGCUUUUUGUUUUA GAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCA ACUUGAAAAAGUGGCACCGAGUCGGUGCGAGUUGUAAUUGU GCCUUGUAUAUGUGUCCCGUCCUAAUCCCAAU
agG1T1_sp5	GGCACAAUUACAACUCGAUUUCUUCUUGCGCUUUUUGUUUUA GAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCA ACUUGAAAAAGUGGCACCGAGUCGGUGC**GCCUA**GAGUUGUA AUUGUGCCUUGUAUAUGUGUCCCGUCCUAAUCCCAAU
agG1T1_sp15	GGCACAAUUACAACUCGAUUUCUUCUUGCGCUUUUUGUUUUA GAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCA ACUUGAAAAAGUGGCACCGAGUCGGUGC**GCCUACUCAUAUC CC**GAGUUGUAAUUGUGCCUUGUAUAUGUGUCCCGUCCUAAU CCCAAU
agG1T1_sgsp5	GGCACAAUUACAACUC**AAUUC**GAUUUCUUCUUGCGCUUUUUG UUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGU UAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCGCCUACUCA UGAGUUGUAAUUGUGCCUUGUAUAUGUGUCCCGUCCUAAUC CCAAU
agG1T1_sgsp10	GGCACAAUUACAACUC**AAUUCGGAAC**GAUUUCUUCUUGCGCU UUUUGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAG UCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCGCCU ACUCAUGAGUUGUAAUUGUGCCUUGUAUAUGUGUCCCGUCC UAAUCCCAAU
agG1T1_sgsp20	GGCACAAUUACAACUC**AAUUCGGAACCUACAUACCU**GAUUUC UUCUUGCGCUUUUUGUUUUAGAGCUAGAAAUAGCAAGUUAAA AUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGU CGGUGCGCCUACUCAUGAGUUGUAAUUGUGCCUUGUAUAUG UGUCCCGUCCUAAUCCCAAU
agG1T1_sgsp30	GGCACAAUUACAACUC**AAUUCGGAACCUACAUACCUCACUAC UACA**GAUUUCUUCUUGCGCUUUUUGUUUUAGAGCUAGAAAUA GCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGU GGCACCGAGUCGGUGCGCCUACUCAUGAGUUGUAAUUGUGC CUUGUAUAUGUGUCCCGUCCUAAUCCCAAU

Table S2.2. List of agRNA sequences used in parameter space exploration experiments (Figure S5). Stm* variants vary the length of the double-stranded stem region (Figure S5a), sp* variants vary the length of the linker region between the gRNA scaffold and the double-stranded stem (Figure S5b), and sgsp* variants vary the distance between the guide sequence and the double-stranded stem (Figure S5c). Varied sequences are in bold, trigger toehold/binding regions are underlined. All sequences include 15 nt ssRNA 3' extension (Figure S3).

gRNA_G1	G**GAUUUCUUCUUGCGCUUUUU**GUUUUAGAGCUAGAAAUAGCA AGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGC ACCGAGUCGGUGCUUUU
gRNA_G2	**GGUUCACAGUCGGUCACAUU**GUUUUAGAGCUAGAAAUAGCAA GUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCA CCGAGUCGGUGCUUUU
gRNA_G5	GG**UUGCUAUGAUUCCGUUUUAU**GUUUUAGAGCUAGAAAUAG CAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUG GCACCGAGUCGGUGCUUUU

Table S2.3. List of canonical gRNAs used as positive controls. Guide sequences are in bold.

C. Trigger sequences

aT1	GGACACATATACAAGGCACAATTACAACTC
rT1	GAGTTGTAATTGTGCC
aT2	CACAGAAACAGAACGGCCATTAACACTACA
aT3	ACATACAAGAATTCGGCCTACATACACTAC
rT3	GTAGTGTATGTAGGCC
aT4	TCACATACAACTTCGGCACCATACTACTAC
aT5	ACAATCAATCAACAGGCCCTAACATCACAC
aTDep	GTCATACTCCAAAGGATTTCTTCTTGCGCT

Table S3. List of trigger DNA sequences.

aT1_1SNP	GGACACATATACAA**C**GCACAATTACAACTC
aT1_2SNP	GGACACATA**A**ACAAGGCAC**T**ATTACAACTC
aT1_3SNP	GGACAC**T**TATACA**T**GGCACAA**A**TACAACTC
aT1_4SNP	GGACA**G**ATATA**G**AAGGC**T**CAATT**T**CAACTC

Table S4. List of trigger DNA sequences used for SNP sensitivity testing. SNPs are in bold.

aT1_2AND_A	GGACACATATACAAGGCACA
aT1_2AND_B	CAACTCATGAGTAGGC
aT1_2OR_A (=aT1)	GGACACATATACAAGGCACAATTACAACTC
aT1_2OR_B	GGCACAATTACAACTCATGAGTAGGC
rT1_2NAND_A	GTTCCGAACCCATGGGTAAAGATAGGATAAGA
rT1_2NAND_B (=3NAND_C)	TCTTATCCTATCTTTACCCATCACTTGAGTTG
rT1_3NAND_A	GTTCCGAACCCTTACTCTATCTTACTTTACTC
rT1_3NAND_B	GAGTAAAGTAAGATAGAGTAAATGGGTAAAGATAGGATAAGA
rT1_3NAND_C (=2NAND_B)	TCTTATCCTATCTTTACCCATCACTTGAGTTG
rT1_2NOR_A	GTTCCGAATT
rT1_2NOR_B	GAGTTGTAATTGTGCC

Table S5. List of trigger DNA sequences used in logic gate implementation.

T1Rp	GAGTTGTAATTGTGCCTGGGATGTGA
T1Rp*	TCACATCCCAGGCACAATTACAACTC

Table S6. List of DNA oligos used for de-repression experiments.

aT1_IVT	GAAATTAATACGACTCACTATAGGACACATATACAAGGCACAATTACAACTC
aT1_IVT_comp	GAGTTGTAATTGTGCCTTGTATATGTGTCCTATAGTGAGTCGTATTAATTTC
aT1_2OR_B_IVT	GAAATTAATACGACTCACTATAGGCACAATTACAACTCATGAGTAGGC
aT1_2OR_B_IVT_comp	GCCTACTCATGAGTTGTAATTGTGCCTATAGTGAGTCGTATTAATTTC

Table S7. List of DNA oligos used for in vitro transcription of RNA triggers. T7 promoter is underlined.

aT1_2AND_A	GGACACAUAUACAAGGCA
aT1_2AND_B	ACUCAUGAGUAGGC
aT1_2OR_A (=aT1)	GGACACAUAUACAAGGCACAAUUACAACUC
aT1_2OR_B	GGCACAAUUACAACUCAUGAGUAGGC
rT1_2NAND_A	GUUCCGAACCCAUGGGUAAAGAUAGGAUAAGA
rT1_2NAND_B	UCUUAUCCUAUCUUUACCCAUCACUUGAGUUG
rT1_2NOR_A	GUUCCGAAUU
rT1_2NOR_B	GAGUUGUAAUUGUGCC

Table S8. List of trigger RNA sequences used in logic gate implementation. Sequences with italicized names were generated using *in vitro* transcription (see Methods), using DNA oligos listed in Table S7.

Section 3. Supporting Figures

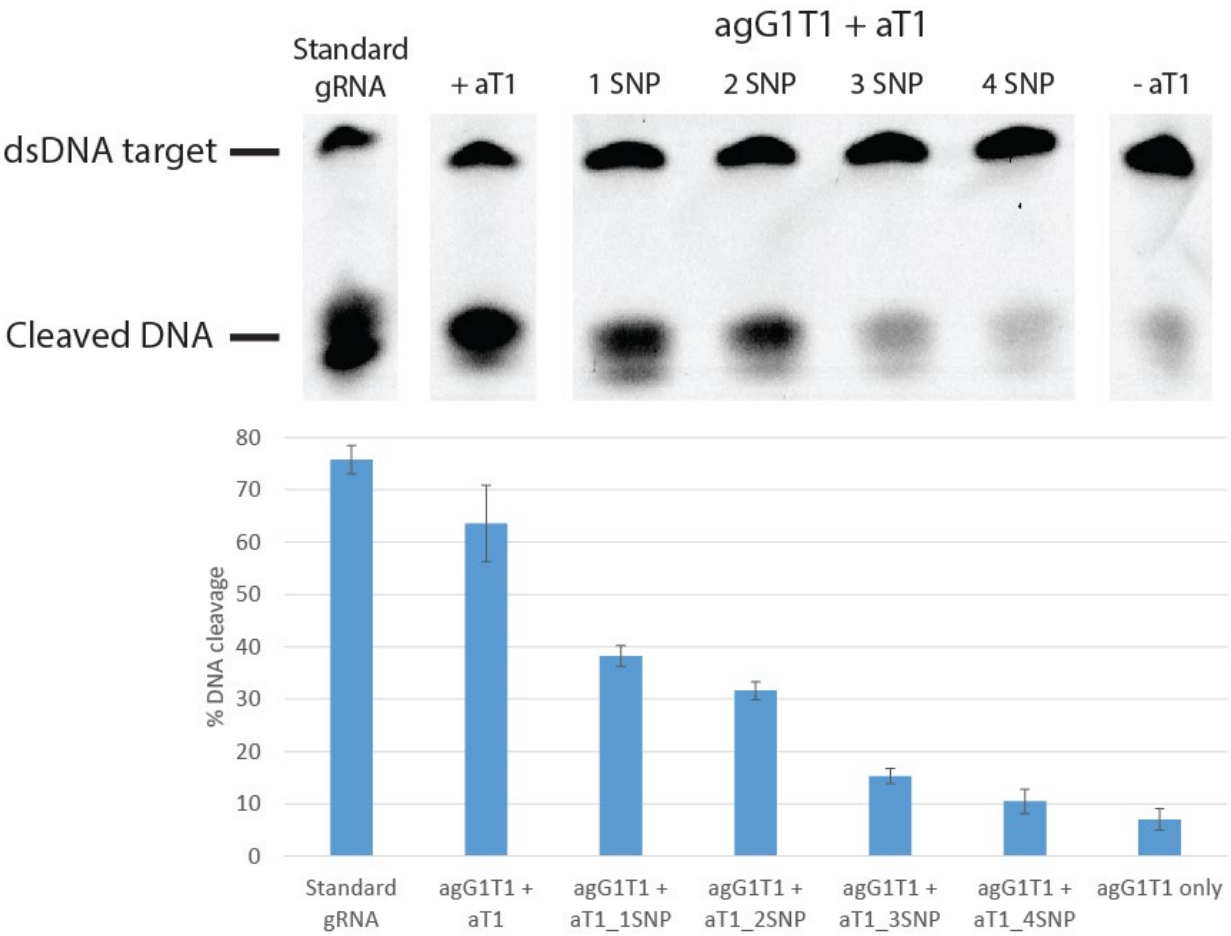

Figure S1. Sensitivity of agG1T1 to SNPs in activating trigger aT1. SNPs were introduced evenly spaced throughout trigger and maintained purine/pyrimidine status (Table S4). Denaturing polyacrylamide gel images show cleavage of 10 nM dsDNA target by 125 nM Cas9 and 250 nM agRNA in the presence of 1 μM activating trigger with varying numbers of SNPs after 1 hour incubation at 37°C.

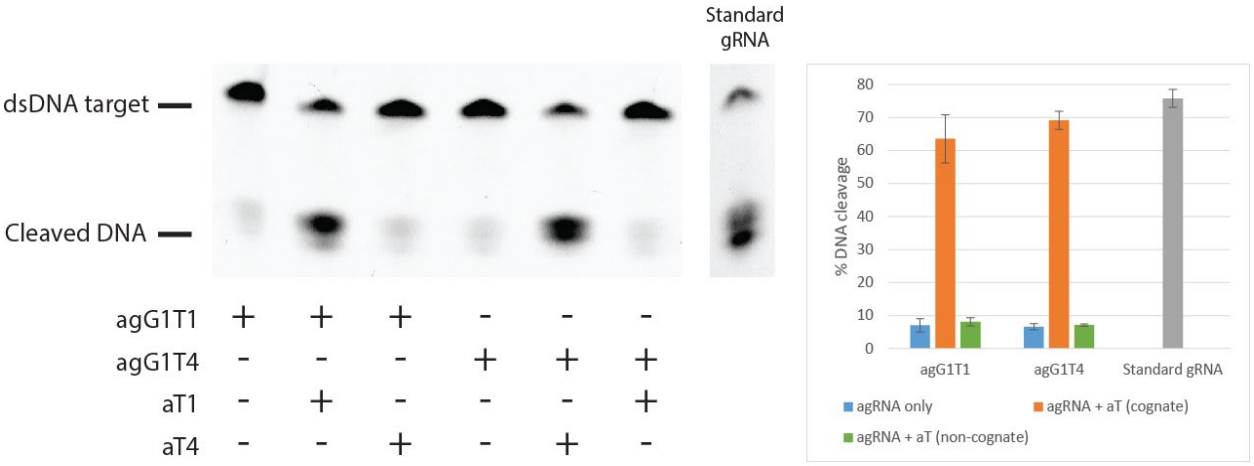

Figure S2. Cross-reactivity testing between agG1T1 and agG1T4 using DNA triggers aT1 and aT4. Denaturing polyacrylamide gel images show cleavage of 10 nM dsDNA target by 125 nM Cas9 and 250 nM agRNA in the presence of 1 μM cognate or non-cognate activating trigger after 1 hour incubation at 37°C.

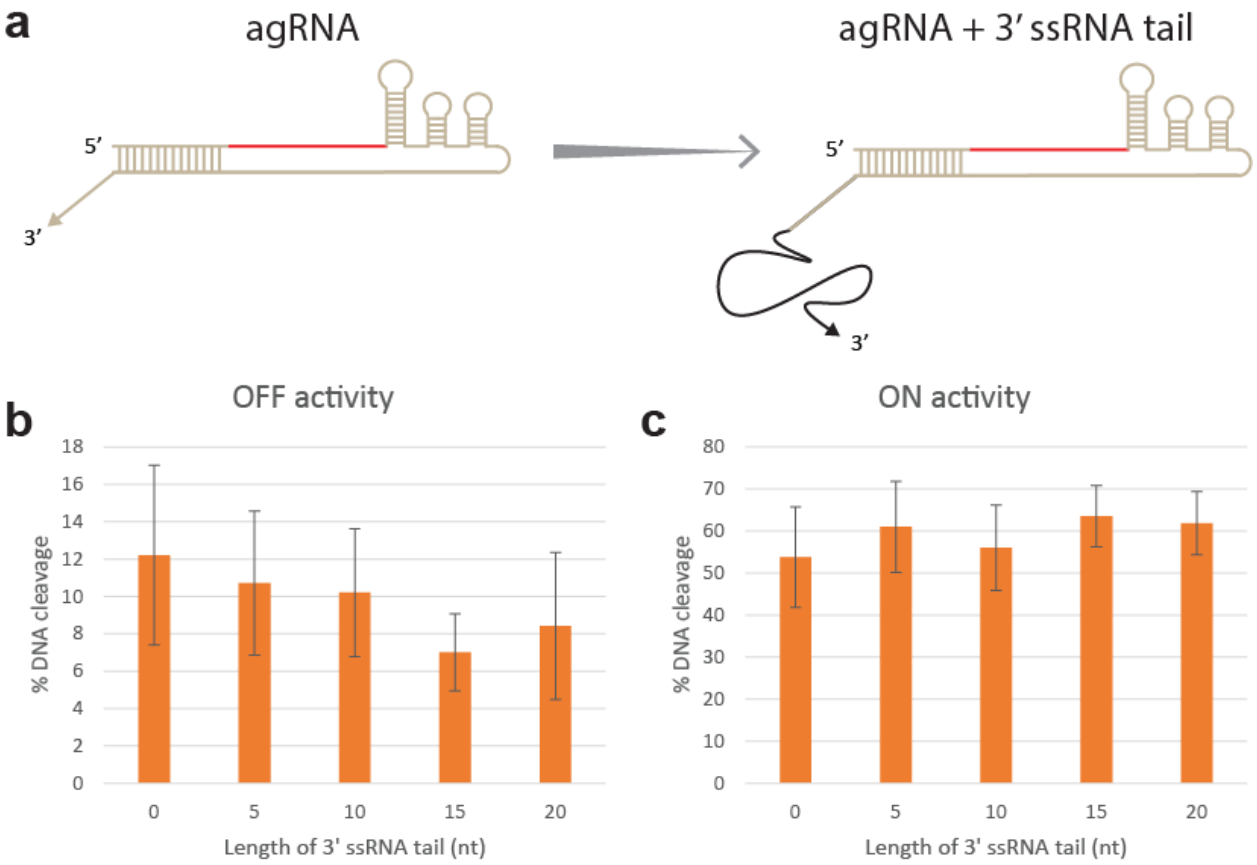

Figure S3. (a) Schematic showing the location of the addition of a 3' ssRNA tail extension to the 3' end of the agRNA toehold to enhance performance. (b) Cas9 activity using agRNA in the absence of activating trigger, with varying lengths of ssRNA tail appended to the 3' end of the agRNA toehold domain. Leakage activity reaches a minimum around 15 nt tail. (c) Cas9 activity using agRNA with activating DNA trigger. Sequence for agRNA with 20 nt tail is given in Table S2.1. Shorter tail sequences are truncations of the 20 nt tail from its 3' end.

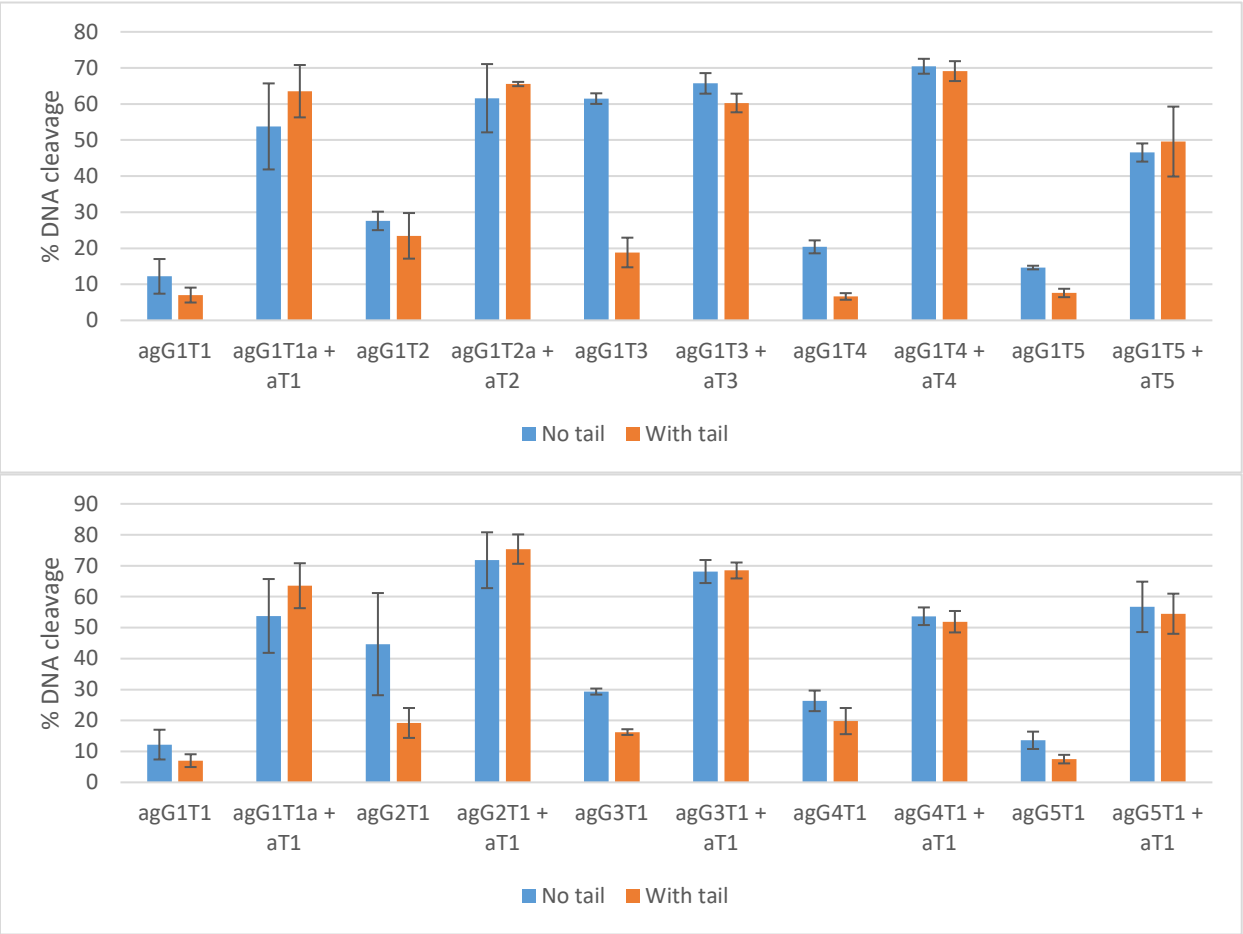

Figure S4. Cas9 ON/OFF state activity for agRNAs with and without 15 nt 3' ssRNA extension on agRNA toehold domain. (a) agRNA with guide G1 combined with triggers aT1-aT5. (b) agRNA with guides G1-G5 combined with trigger aT1. Sequences are given in Table S2.1.

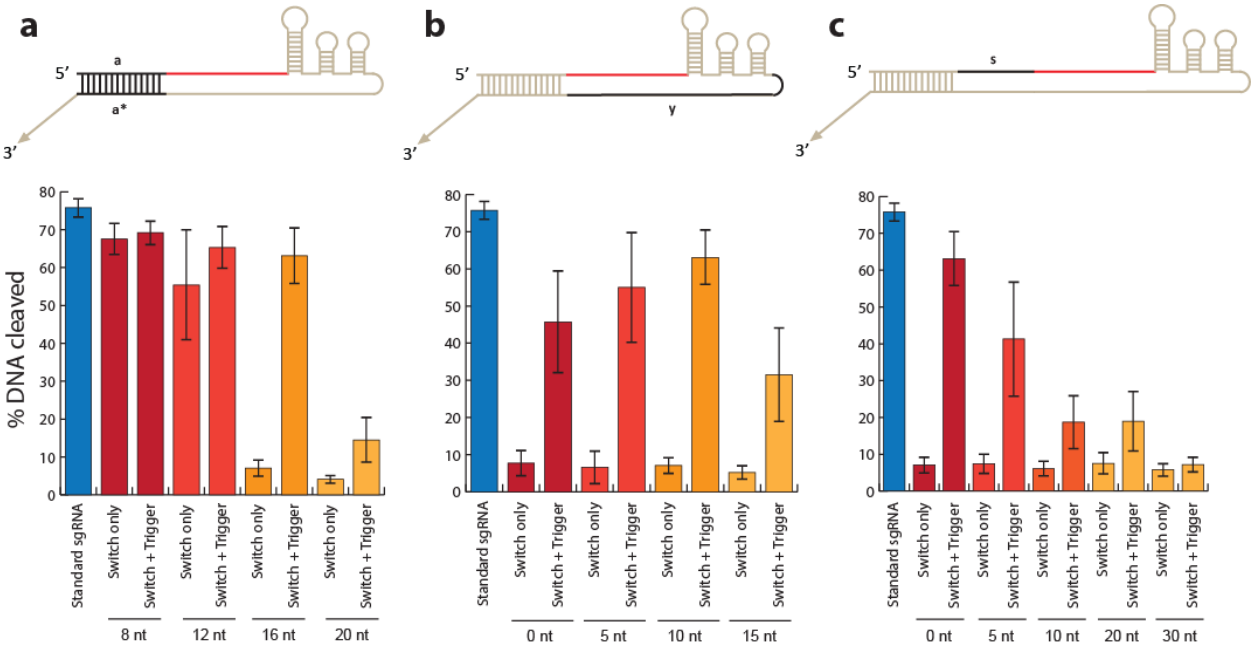

Figure S5. Parameter space exploration for agG1T1. (a) Varying the length of the double-stranded stem region **a** from 8 nt to 20 nt. The decreased ON activity with a 20 nt stem may have been due to unanticipated secondary structure formation. (b) Varying the length of the linker region **y** between the gRNA scaffold and the double-stranded stem from 0 nt to 15 nt. (c) Varying the distance **s** between the guide sequence and the double-stranded stem from 0 nt to 30 nt. agRNA sequences are given in Table S2.2.

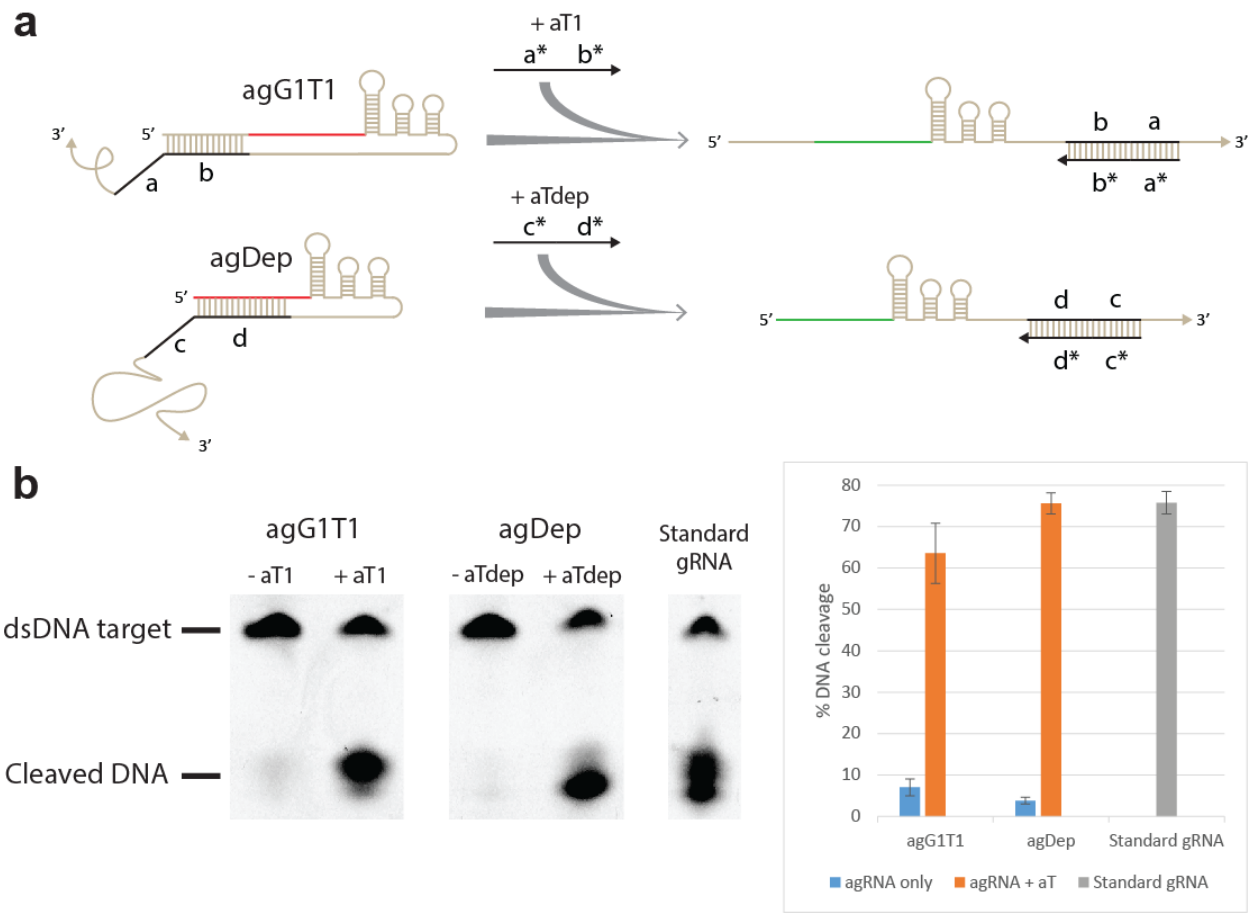

Figure S6. ON/OFF activity for sequence independent design agG1T1 and sequence dependent variant agDep, wherein the double-stranded stem is moved to directly and partially overlap the guide sequence for 16 bp. Domain lengths **a** = 14 nt, **b** = 16 nt, **c** = 14 nt, **d** = 16 nt. The 3' tail extensions are 15 nt for agG1T1 and 20 nt for agDep. Denaturing polyacrylamide gel images show cleavage of 10 nM dsDNA target by 125 nM Cas9 and 250 nM agRNA in the presence of 1 μM activating trigger after 1 hour incubation at 37°C. agRNA sequences are given in Table S2.1.

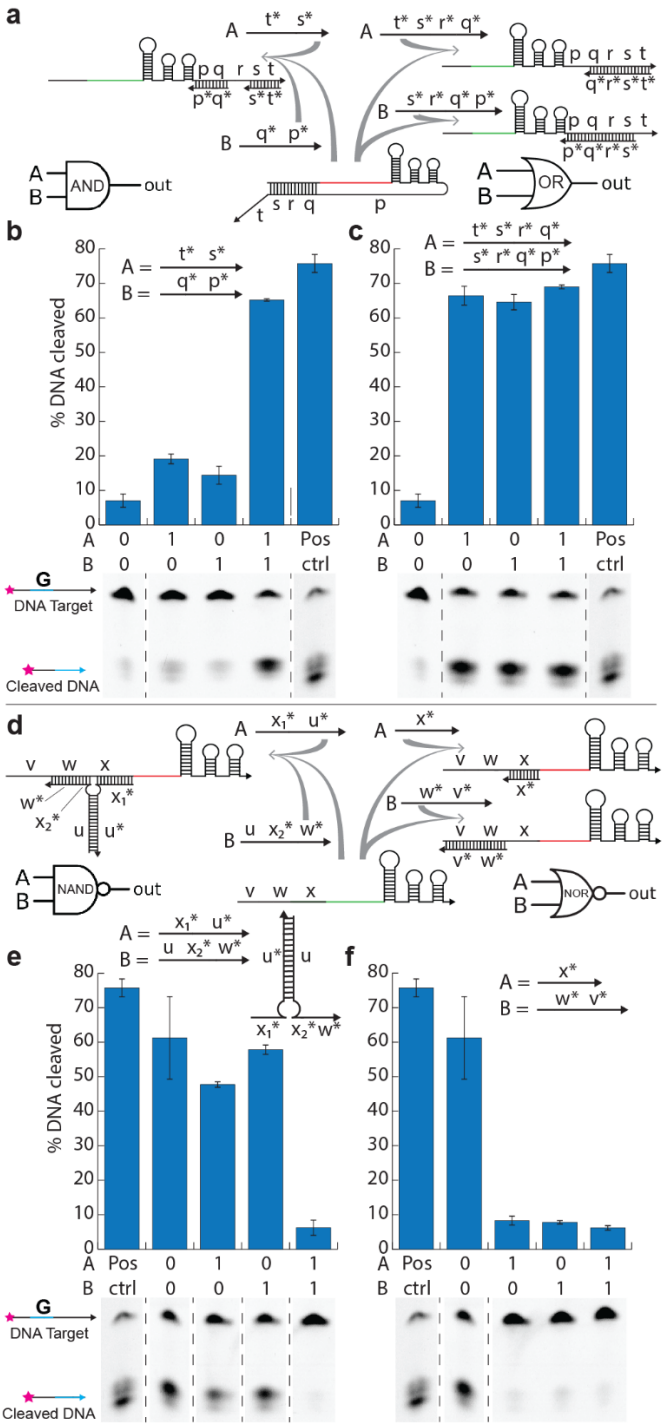

Figure S7. Logic gate implementation using DNA triggers on cgRNA designs. (a-c) Implementation of 2-input AND (both inputs must be present for activation) and 2-input OR (either input present results in activation) with agG1T1. Domain lengths **p** = 10 nt, **q** = 6 nt, **r** = 4 nt, **s** = 6 nt, **t** = 14 nt. (d-f) Implementation of 2-input NAND (both inputs must be present for repression) and 2-input NOR (either input present results in repression) with rgG1T1e. Domain lengths **u** = 21 nt, **v** = 10 nt, **w** = 6 nt, **x** = 10 nt, $\mathbf{x_1}$ = 8 nt, $\mathbf{x_2}$ = 2 nt. Denaturing polyacrylamide gel images below each graph show cleavage of 10 nM dsDNA target by 125 nM Cas9 and 250 nM cgRNA (or standard gRNA as positive control) in the presence of 1 μM of each trigger after 1 hour incubation at 37°C. See Figure S8 for uncropped gel image for (e). See Methods and Table S2.1, Table S5 in Supplementary Information for additional details.

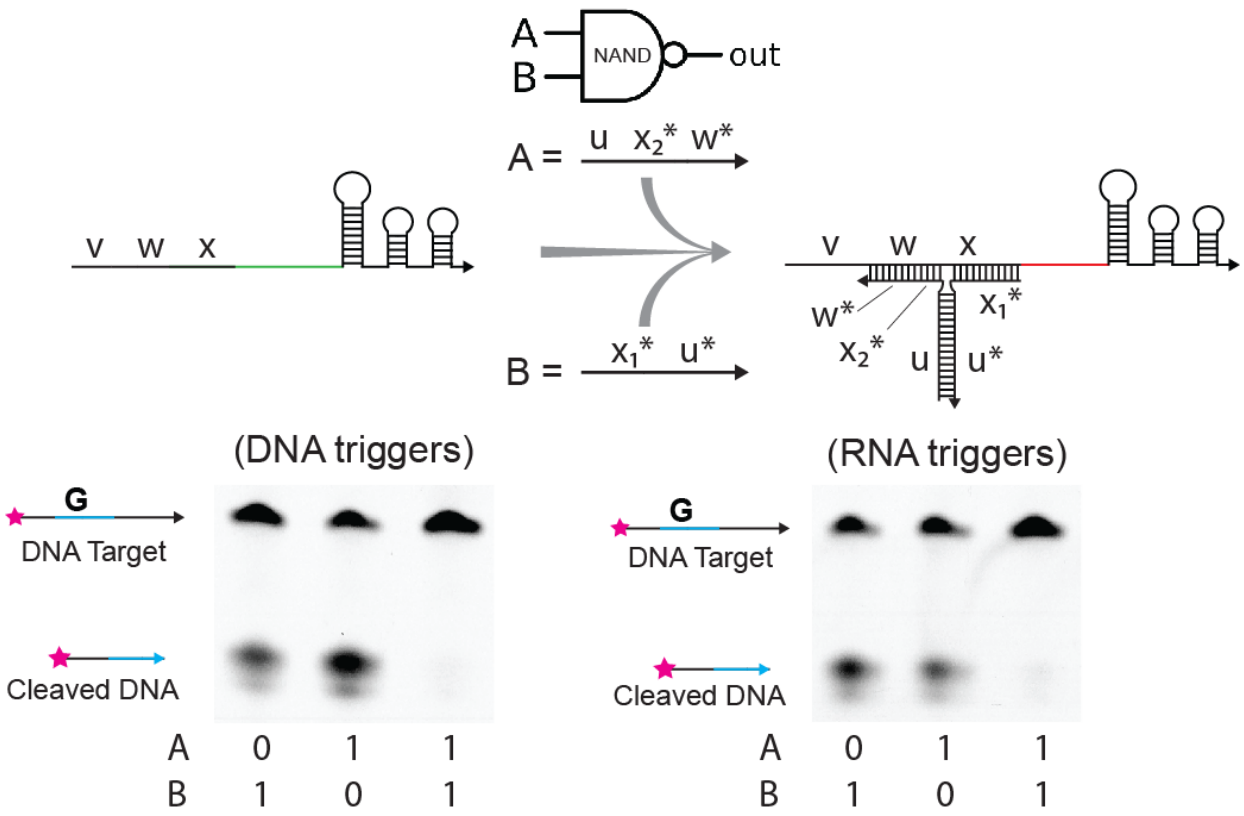

Figure S8. Denaturing polyacrylamide gel images for 2-input NAND implementation using RNA and DNA triggers. In Figures 3e and S7e, these lanes were re-ordered for presentation purposes. Gel images show cleavage of 10 nM dsDNA target by 125 nM Cas9 and 250 nM rgRNA (or standard gRNA as positive control) in the presence of 1 μM of each trigger after 1 hour incubation at 37°C. Sequences are given in Tables S5 and S8.

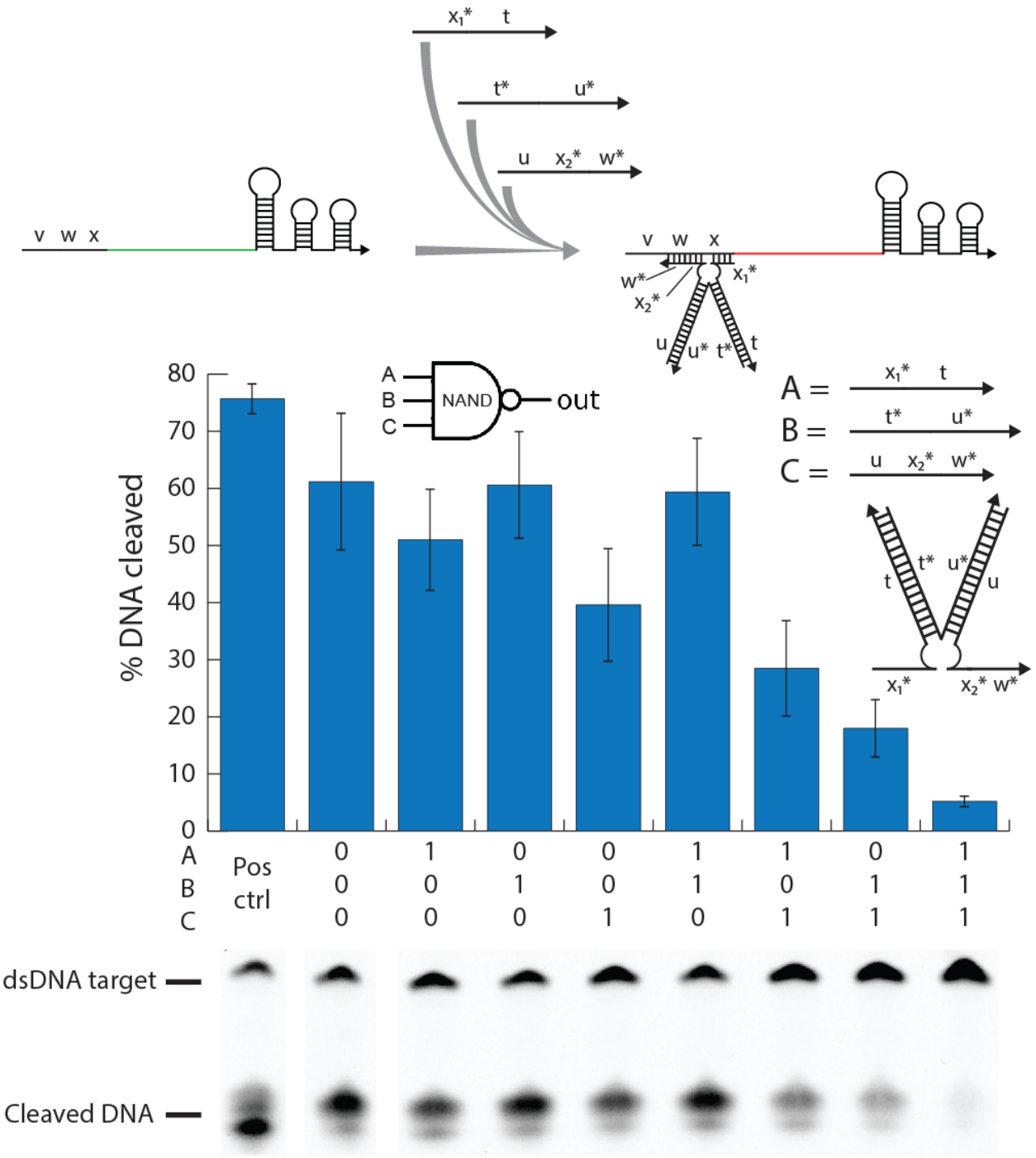

Figure S9. Implementation of 3-input NAND gate using rgG1T1e (sequence in Table S2.1) and DNA triggers. All three triggers must be present for full repression. Denaturing polyacrylamide gel images show cleavage of 10 nM dsDNA target by 125 nM Cas9 and 250 nM rgRNA in the presence of 1 μM of each trigger after 1 hour incubation at 37°C. Domain lengths **t** = 21 nt, **u** = 21 nt, **v** = 10 nt, **w** = 6 nt, **x** = 10 nt, $\mathbf{x_1}$ = 8 nt, $\mathbf{x_2}$ = 2 nt. Sequences for DNA triggers are given in Table S5.

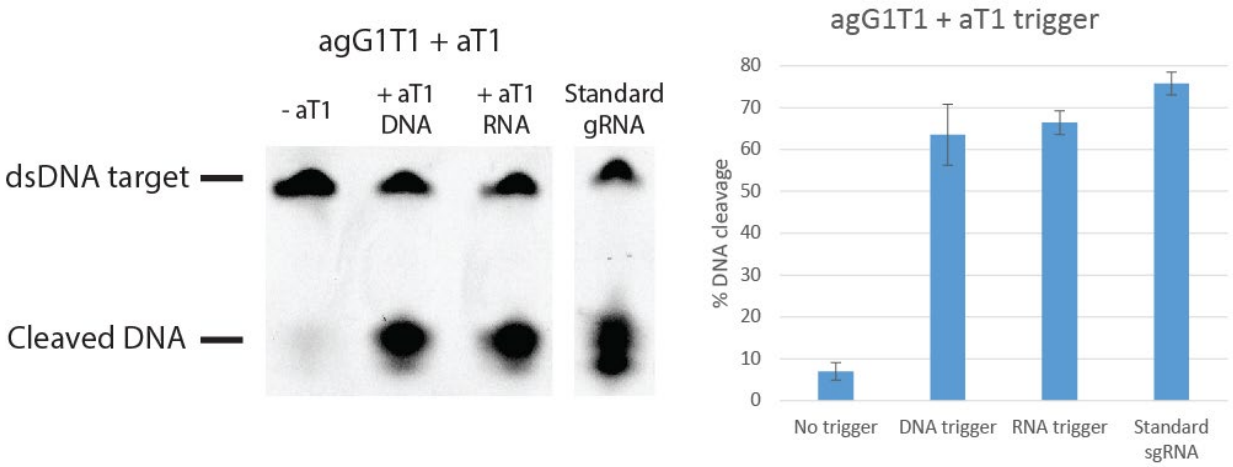

Figure S10. Efficacy of both ssDNA and ssRNA triggers for agG1T1. aT1 RNA was produced by *in vitro* transcription (see Methods); DNA oligo sequences used in transcription are given in Table S7. Denaturing polyacrylamide gel images show cleavage of 10 nM dsDNA target by 125 nM Cas9 and 250 nM agRNA in the presence of 1 μM activating DNA or RNA trigger aT1 after 1 hour incubation at 37°C.

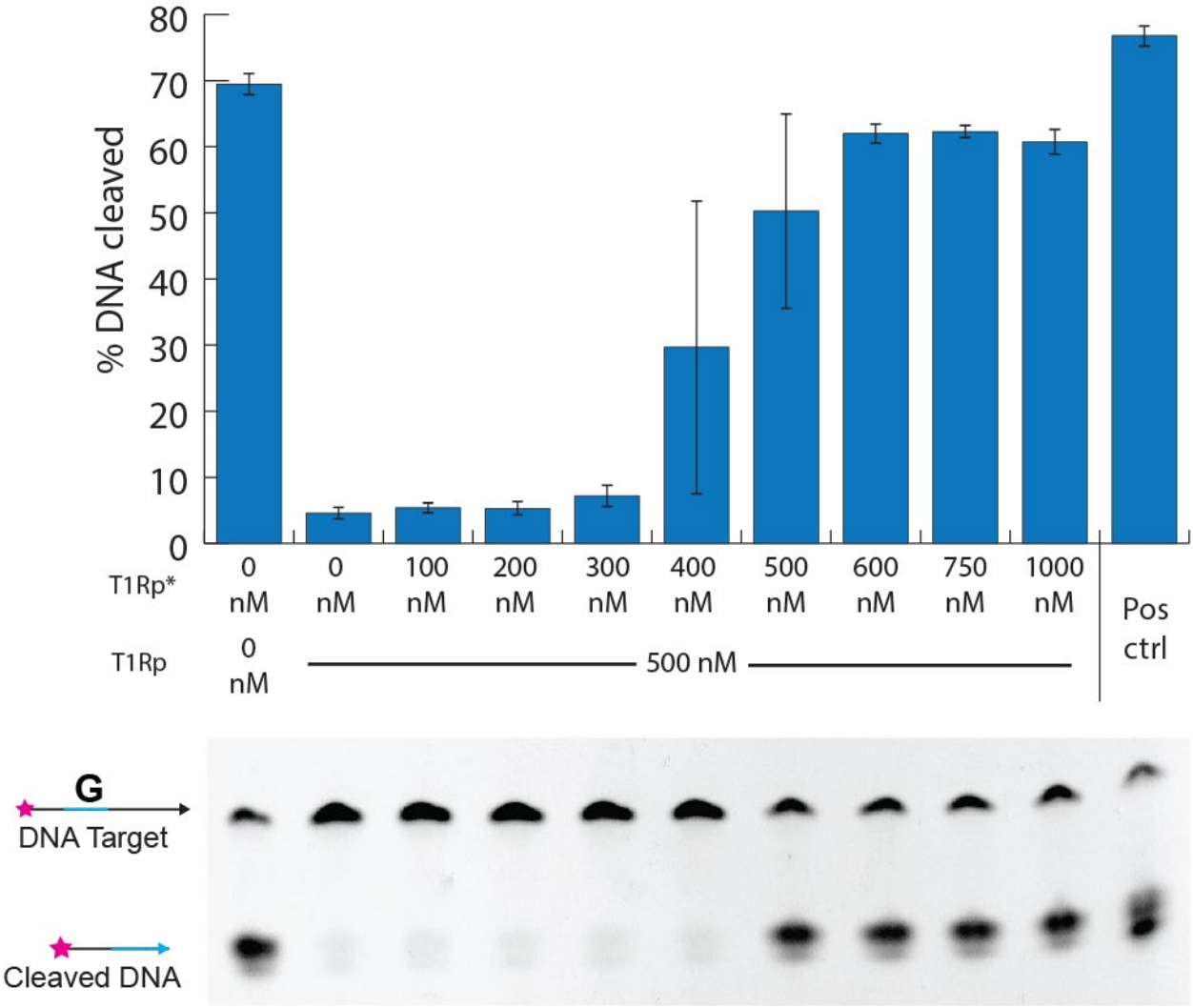

Figure S11. Denaturing polyacrylamide gel showing cleavage of 10 nM dsDNA target by 125 nM Cas9 and 250 nM rgRNA or standard gRNA as positive control in the presence of varying concentrations of derepressing trigger T1Rp* to remove repressor strand T1Rp at threshold concentration 500 nM. T1Rp* was added halfway (30 minutes) through 1 hour incubation at 37°C. Trigger sequences are given in Table S6. In addition to enabling concentration thresholds for cgRNA activation at de-repressor concentrations around the rgRNA concentration or higher (see main text; Figure 4), this scheme can in principle also be used to set a threshold for sharp, programmable activation of Cas9 activity.[4]

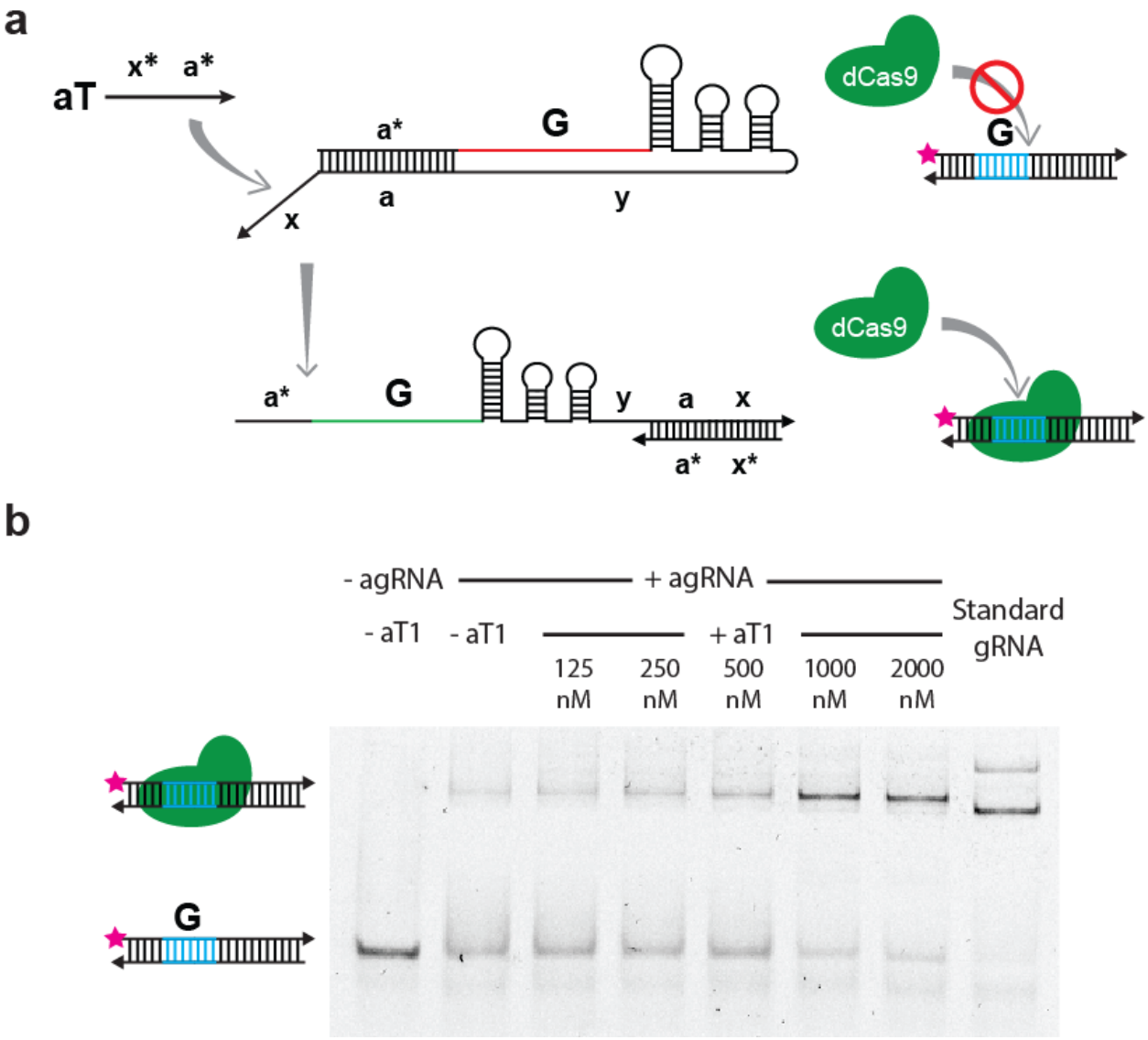

Figure S12. (a) Activation of dCas9 activity using an agRNA with 20 nt 3' extension (sequence agG1T1D given in Table S2.1). Sequence-specific binding of dCas9 to target sequence **G** is greatly increased upon introduction of trigger **aT**. (b) Native polyacrylamide gel shows increased binding of 1 μM dCas9 and 1 μM agRNA to 10 nM dsDNA target with increasing trigger concentration after 1 hour incubation at 37°C.

References

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4. Buchler, N. E.; Cross, F. R., Protein sequestration generates a flexible ultrasensitive response in a genetic network. Mol Syst Biol 2009, 5, 272.