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Supplementary Material

A Suite of Therapeutically-Inspired Nucleic Acid Logic Systems for Conditional Generation of Single-Stranded and Double-Stranded Oligonucleotides

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Detailed description of conditional RNA/DNA hybrid constructs:

Adjacent-targeting hybrids

The system was designed to release a 25/27 Dicer substrate siRNA (DsiRNA) product from a sense and antisense RNA/DNA hybrid pair following interaction with a fragment of the CTGF mRNA. The sense hybrid (*sH*$_{DOWN}$) contains a 5′ DNA toehold designed to bind a sequence region of the CTGF trigger downstream of the binding site for the antisense hybrid's (*aH*$_{UP}$) 3′ DNA toehold. The basic *aH*$_{UP}$ and *sH*$_{DOWN}$ hyrbid constructs were designed with 12 nucleotide (nt) toeholds emanating from the RNA/DNA hybrid duplex region. The upstream and downstream regions for toehold binding are separated by only a single nucleotide in the RNA trigger. This is designed to position the RNA/DNA hybrid regions next to one another in 3D space, while the single nucleotide gap between the trigger-bound toeholds provides some steric flexibility. Additional DNA nucleotides were eventually inserted between the RNA/DNA hybrid regions and the toeholds. These DNA nucleotides were complementary between cognate *sH*$_{DOWN}$ and *aH*$_{UP}$ hyrbid pairs, and acted to as a nucleation site for the strand exchange reaction.

CTGF-induced hybrids

The system was designed to release a 25/27 Dicer substrate siRNA (DsiRNA) product from a sense and antisense RNA/DNA hybrid pair following interaction with a fragment of the CTGF mRNA. The sense hybrid (*sH*$^{\wedge CTGF}$) contained a DNA strand that was complementary to the sense RNA,. The DNA strand was extended in the 5′ direction to encode a sequence that formed the diagnostic domain. A structured DNA hairpin was designed immediately 5′ adjacent to the RNA/DNA hybrid region. Initially, this hairpin contained a 12bp stem and 8nt loop (*sH*$^{\wedge CTGF.12/8}$), but multiple variants with differences in the stem length and loop size were ultimately constructed. Flanking the hairpin on the 5′ side is a diagnostic toehold 20nt in length for most *sH*$^{\wedge CTGF}$ constructs. The diagnostic toehold of *sH*$^{\wedge CTGF.20/8}$ was reduced in length to 16nt to keep the total length of the DNA strand from exceeding 90nt. For *sH*$^{\wedge CTGF}$ hyrbids with a 12bp hairpin stem the diagnostic toehold, 5′ side of the hairpin stem, and the first four nucleotides of the hairpin loop were designed to be complementary to a continuous region of the CTGF mRNA. For *sH*$^{\wedge CTGF}$ hybrids with 16bp or 20bp hairpin stems complementarity to the CTGF trigger extended up the entirety of the hairpin stem, but did not include any loop nucleotides. The exchange toehold for *sH*$^{\wedge CTGF}$ hybrids was encoded in the DNA sequence immediately 5′ to the region hybridized to the sense RNA strand, and were ultimately sequestered to serve at the 3′ side of the DNA hairpin stem in the initially folded structure. The cognate antisense hybrids (*aH*$^{\wedge CTGF\text{-}cgnt}$) contained a DNA strand that hybridized to the antisense RNA strand at it 5′ end. From this RNA/DNA hybrid duplex region the 3′ end of the DNA strand was

extended to encode the complementary exchange toehold. Two variants were created. One contained a 12nt toehold, while the other contained a 16 nt toehold.

KRAS-repressed hybrids

The system was designed to release a 25/27 Dicer substrate siRNA (DsiRNA) product from a sense and antisense RNA/DNA hybrid pair in the absence of any interaction with a fragment of the KRAS mRNA. The antisense hybrid (*aH*$_{vKRAS}$) contained a DNA strand that was designed at its 5′ end to be complementary to the antisense RNA, creating the RNA/DNA hybrid region. Immediately adjacent to the hybrid region, the DNA strand encodes the 12nt exchange toehold followed by a DNA hairpin. The DNA hairpin contains a 14 bp stem and 12 nt loop. The 12 nt hairpin loop is designed to be complementary to the 12nt exchange toehold adjacent to the base of the hairpin stem, which can fold to form a less stable alternative hairpin. These complementary loop and toehold sequences that defined the stem of the alternative hairpin were designed to be AU-rich in order to initially favor formation of the primary 14 bp hairpin. This pair of alternative hairpin structures provides the mechanism to repress strand exchange. An 11 nt single-strand diagnostic toehold is incorporated that exits directly from the 3′ side of the 14 bp hairpin. The diagnostic toehold and the adjacent 3′ side of the hairpin are complementary to a continuous region of the KRAS mRNA. Binding of the KRAS trigger is designed to unzip the primary hairpin and induce a conformational change that results in formation of the alternative hairpin, sequestering the exchange toehold within its stem, and ultimately represses dsRNA release. The cognate sense hybrid (*sH*$_{vKRAS\text{-}cgnt}$) contained a DNA strand that contained a sequence at its 3′ to hybridized to the sense RNA strand. From this RNA/DNA hybrid duplex region the 5′ end of the DNA strand was extended to encode the complementary 12 exchange toehold.

Sequences and assemblies used in this study:

**Sequences are indicated as either RNA or DNA*

Beacon-derived switch (KRAS triggered)

DNA diagnostic strand:
TTTGTTCGTTTCATTGCACTGTACTCCTCTTGGCTCGCTGTGA
RNA output strand (anti-miR 375):
UCACGCGAGCCGAACGAACAAA

Adjacent-targeting RNA/DNA hybrids (CTGF triggered):

0bp aH$_{UP}$: aRNA CGGUGGUGCAGAUGAACUUCAGGGUCA
a'DNA tgaccctgaagttcatctgcaccaccgagttgtaatggc
0bp sH$_{DOWN}$: sRNA ACCCUGAAGUUCAUCUGCACCACCG
s'DNA ttgtctccgggacggtggtgcagatgaacttcagggt

+1bp aH$_{UP}$: aRNA CGGUGGUGCAGAUGAACUUCAGGGUCA
a'DNA tgaccctgaagttcatctgcaccaccggagttgtaatggc
+1bp sH$_{DOWN}$: sRNA ACCCUGAAGUUCAUCUGCACCACCG
s'DNA ttgtctccgggaccggtggtgcagatgaacttcagggt

+2bp aH$_{UP}$: aRNA CGGUGGUGCAGAUGAACUUCAGGGUCA
a'DNA tgaccctgaagttcatctgcaccaccgcgagttgtaatggc
+2bp sH$_{DOWN}$: sRNA ACCCUGAAGUUCAUCUGCACCACCG
s'DNA ttgtctccgggacgcggtggtgcagatgaacttcagggt

+3bp aH$_{UP}$: aRNA CGGUGGUGCAGAUGAACUUCAGGGUCA
a'DNA Tgaccctgaagttcatctgcaccaccggcgagttgtaatggc
+3bp sH$_{DOWN}$: sRNA ACCCUGAAGUUCAUCUGCACCACCG

s'DNA Ttgtctccgggacgccggtggtgcagatgaacttcagggt

+4bp aH$_{UP}$: aRNA CGGUGGUGCAGAUGAACUUCAGGGUCA
a'DNA Tgaccctgaagttcatctgcaccaccgggcgagttgtaatggc
+4bp sH$_{DOWN}$: sRNA ACCCUGAAGUUCAUCUGCACCACCG
s'DNA Ttgtctccgggacgcccggtggtgcagatgaacttcagggt

Inducible activation hybrids (CTGF triggered):

aH$^{CTGF\text{-}cgnt.12}$: aRNA CGGUGGUGCAGAUGAACUUCAGGGUCA
a'DNA tgaccctgaagttcatctgcaccaccg aagatgtcattg

aH$^{CTGF\text{-}cgnt.16}$: aRNA CGGUGGUGCAGAUGAACUUCAGGGUCA
a'DNA tgaccctgaagttcatctgcaccaccg aagatgtcattgtctc

sH$^{CTGF.12/8}$: sRNA ACCCUGAAGUUCAUCUGCACCACCG
s'DNA tcctgtagtacagcgattca aagatgtcattg tctcaacc caatgacatctt cggtggtgcagatgaacttcagggtca

sH$^{CTGF.12/12}$: sRNA ACCCUGAAGUUCAUCUGCACCACCG
s'DNA tcctgtagtacagcgattca aagatgtcattg tctcaacaccat caatgacatctt cggtggtgcagatgaacttcagggtca

sH$^{CTGF.16/8}$: sRNA ACCCUGAAGUUCAUCUGCACCACCG
s'DNA tcctgtagtacagcgattca aagatgtcattgtctc aagcggac gagacaatgacatctt cggtggtgcagatgaacttcagggtca

sH$^{CTGF.20/8}$: sRNA ACCCUGAAGUUCAUCUGCACCACCG
s'DNA tagtacagcgattca aagatgtcattgtctccggg aagcggac cccggagacaatgacatctt cggtggtgcagatgaacttcagggtca

3. -piece inducible activation hybrid (CTGF triggered):

sH$^{CTGF.20split}$: sRNA ACCCUGAAGUUCAUCUGCACCACCG
s'DNA1 TAGTACAGCGATTCA AAGATGTCATT-GTCTCCGGG
s'DNA2 CCCGGAGACAATGACATCTT CGGTGGTG-CAGATGAACTTCAGGGTCA

Trigger repressible RNA/DNA hybrids (CTGF or KRAS triggered):

aH$_{vKRAS}$: aRNA CGGUGGUGCAGAUGAACUUCAGGGUCA
a'DNA TGACCCTGAAGTTCATCTGCACCACCG AA-GATGTCATTG GCAATGAGGGACCA CAATGACATCTT TGGTCCCTCATTGC ACTGTACTCCT

aH$_{vCTGF.cgnt}$: aRNA CGGUGGUGCAGAUGAACUUCAGGGUCA
a'DNA tgaccctgaagttcatctgcaccaccg ACTGTAATGCTA

sH$_{vKRAS.cgnt}$: sRNA ACCCUGAAGUUCAUCUGCACCACCG
s'DNA CAATGACATCTT cggtggtgcagatgaacttcagggt

sH_{vCTGF}: sRNA ACCCUGAAGUUCAUCUGCACCACCG
s'DNA AGATGTCATTGTC TCCGGGACAGTTGT ACTG-TAATGCTA
ACAACTGTCCCGGA TAGCATTACAGT CGGTGGTGCAGATGAACTTCAGGGT

3. -piece trigger repressible hybrids (KRAS triggered):

$aH_{vKRAS.nick14}$: aRNA CGGUGGUGCAGAUGAACUUCAGGGUCA
a'DNA1TGACCCTGAAGTTCATCTGCACCACCG AA-GATGTCATTG
GCAATGAGGGACCA CAATGACATCTT
a'DNA2TGGTCCCTCATTGC ACTGTACTCCT

$aH_{vKRAS.nick10}$: aRNA CGGUGGUGCAGAUGAACUUCAGGGUCA
a'DNA1TGACCCTGAAGTTCATCTGCACCACCG AA-GATGTCATTG
GCAATGAGGGACCA CAATGACATCTT TGGT
a'DNA2CCCTCATTGC ACTGTACTCCT

$aH_{vKRAS.nick8}$: aRNA CGGUGGUGCAGAUGAACUUCAGGGUCA
a'DNA1TGACCCTGAAGTTCATCTGCACCACCG AAGATGTCATTG
GCAATGAGGGACCA CAATGACATCTT TGGTCC
a'DNA2CTCATTGC ACTGTACTCCT

$aH_{vKRAS.nick6}$: aRNA CGGUGGUGCAGAUGAACUUCAGGGUCA
a'DNA1TGACCCTGAAGTTCATCTGCACCACCG AAGATGTCATT
GCAATGAGGGACCA CAATGACATCTT TGGTCCCT
a'DNA2CATTGC ACTGTACTCCT

RNA trigger sequences

*underlined bases were added to the 5′ end for the purpose in vitro transcription and are not present in the endogenous sequence from which the truncated mRNA fragments are derived

CTGF: gggaAAGACCUGUGCCUGCCAUUAC*AACUGUCCCGGAGA*CAAU-GACAUCUUUGAAUCGCUG UACUACAGGAAGAUGUACGG

KRAS: gggCUCGACACAGCAGGUCAAGAGGAGUACAGUGCAAU-GAGGGACCAGUACAUGAGGACUG
GG

Random SEQ: GGCAACU-UUGAUCCCUCGGUUUAGCGCCGGCCUUUUCUCCCACACUUUCACG

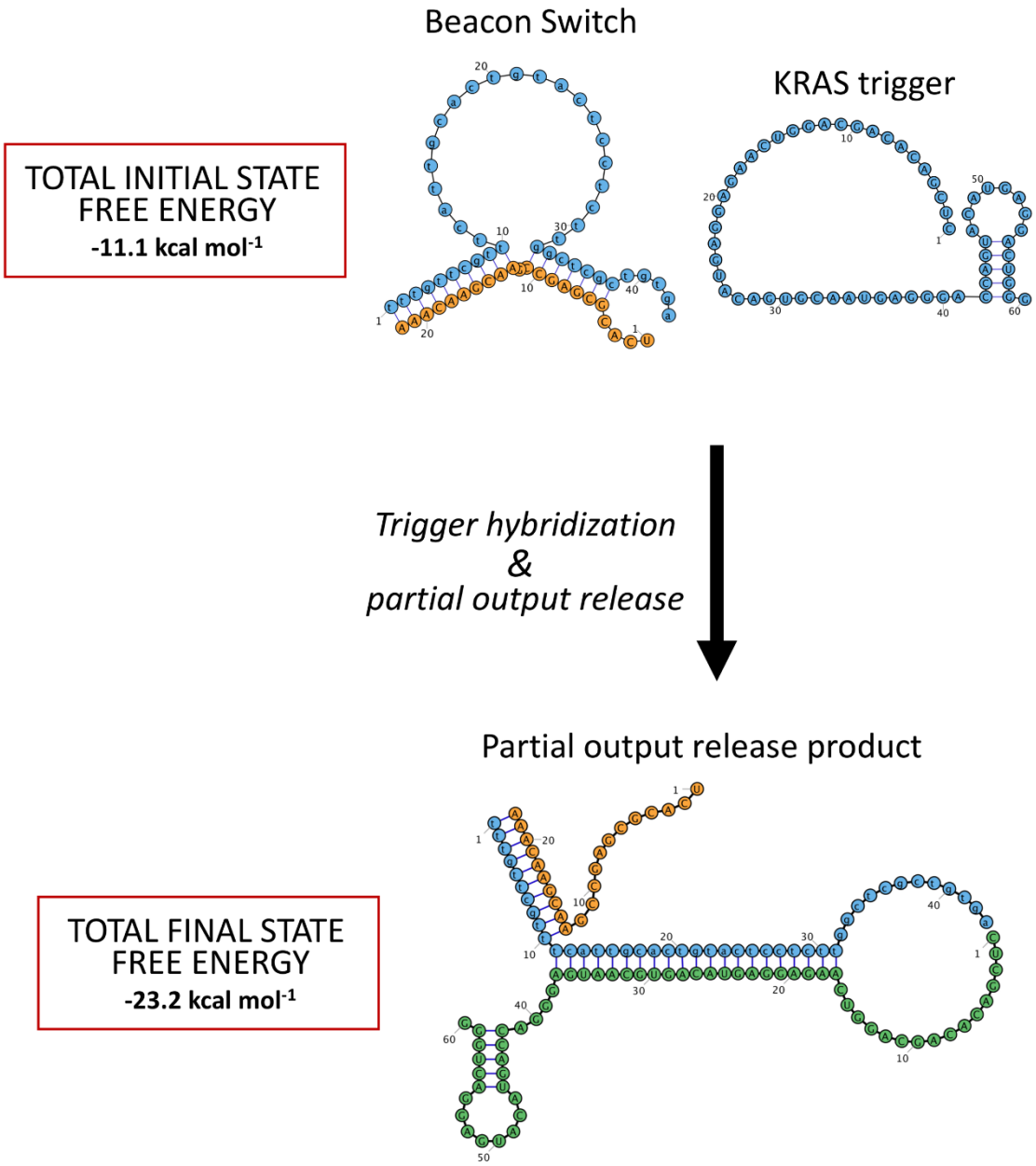

Figure S1. Free energy calculations of the predicted initial and final states for the beacon-derived switch interacting with the KRAS trigger. The final state shows a structure in which only the 5′ end of the output strand is separated from the diagnostic strand. Energy calculations and secondary structure predictions were performed using Hyperfold. Lowercase letters indicate DNA nucleotides. Uppercase letters indicate RNA nucleotides.

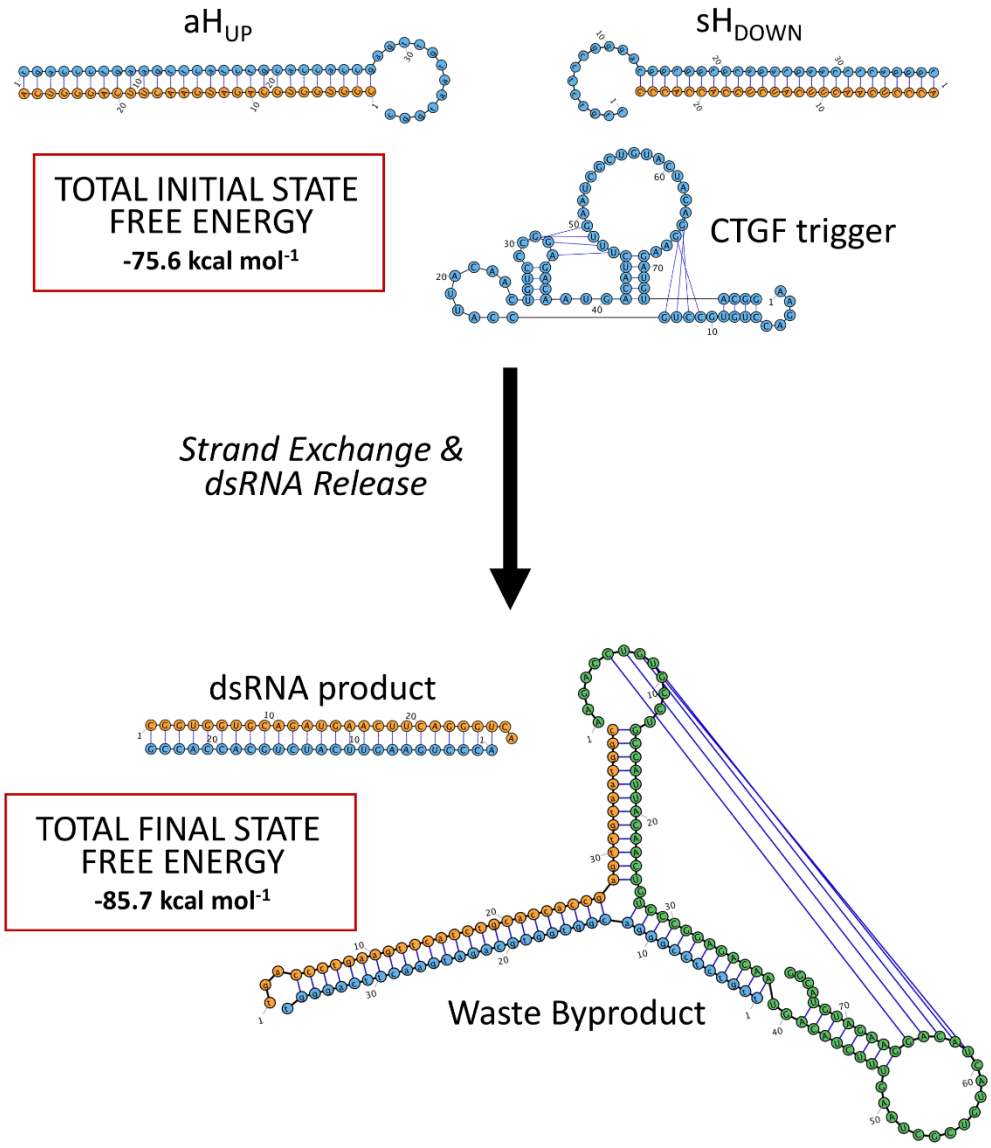

Figure S2. Free energy calculations of the initial and final states for the "+0 bp" adjacent targeting hybrid system. Energy calculations and secondary structure predictions were performed using Hyperfold. Lowercase letters indicate DNA nucleotides. Uppercase letters indicate RNA nucleotides.

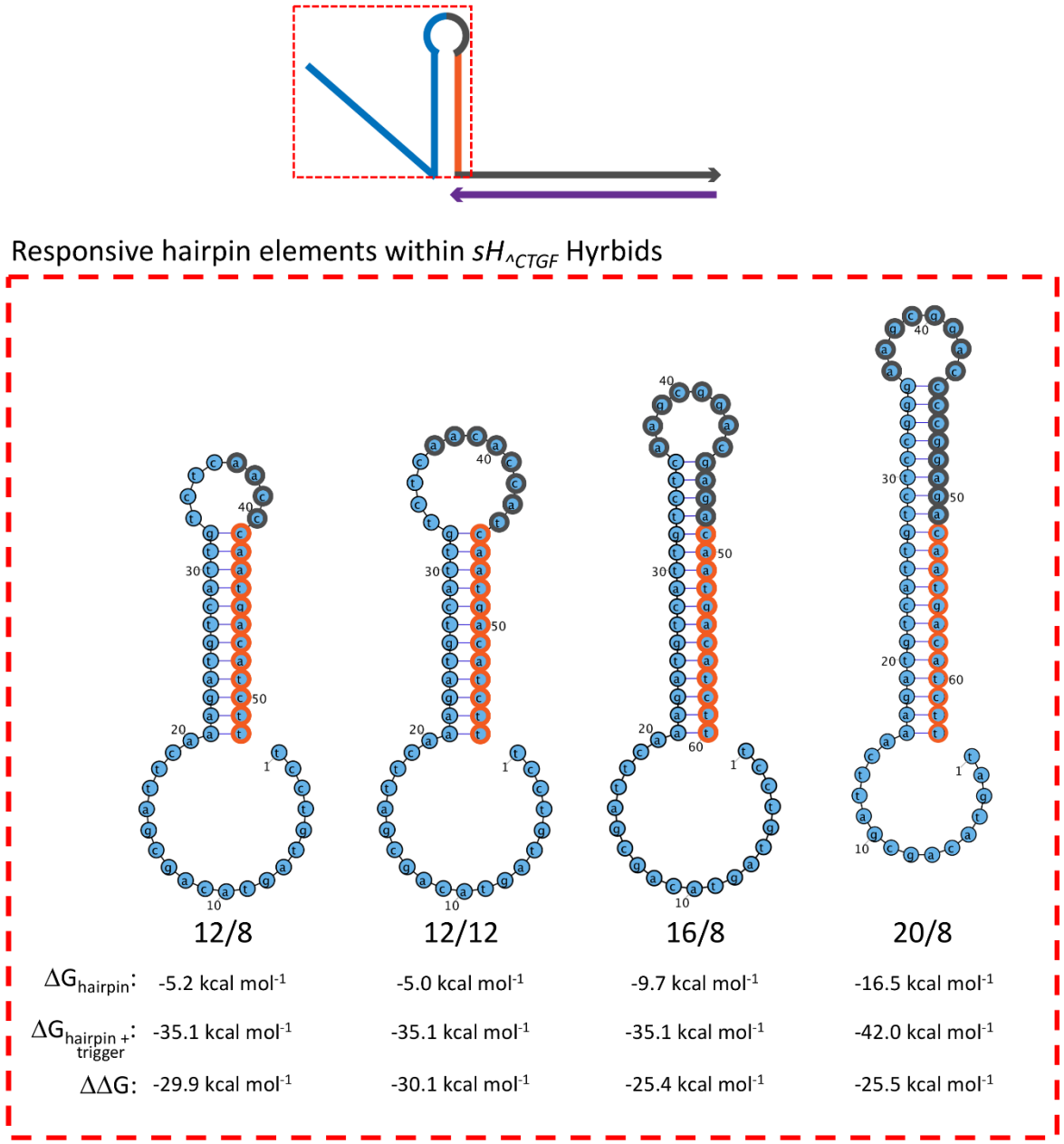

Figure S3. Free energy calculations of the responsive DNA hairpin elements of variant *sH^CTGF* hybrids as predicted by Hyperfold. Free energies are given for the initial hairpin/toehold structure (structures shown, $\Delta G_{hairpin}$), the hairpin/toehold bound to the CTGF trigger ($\Delta G_{hairpin+trigger}$), as well as the difference in free energy between these two states ($\Delta\Delta G$). Nucleotides that define the hybrids' exchange toehold are outlined in orange. Nucleotides that are complementary to the CTGF trigger are outlined in blue. Nucleotides between the region complementary to the trigger and the exchange toehold are outline in black. The distance between these two regions in terms of the primary sequence increases within the structures moving from left to right.

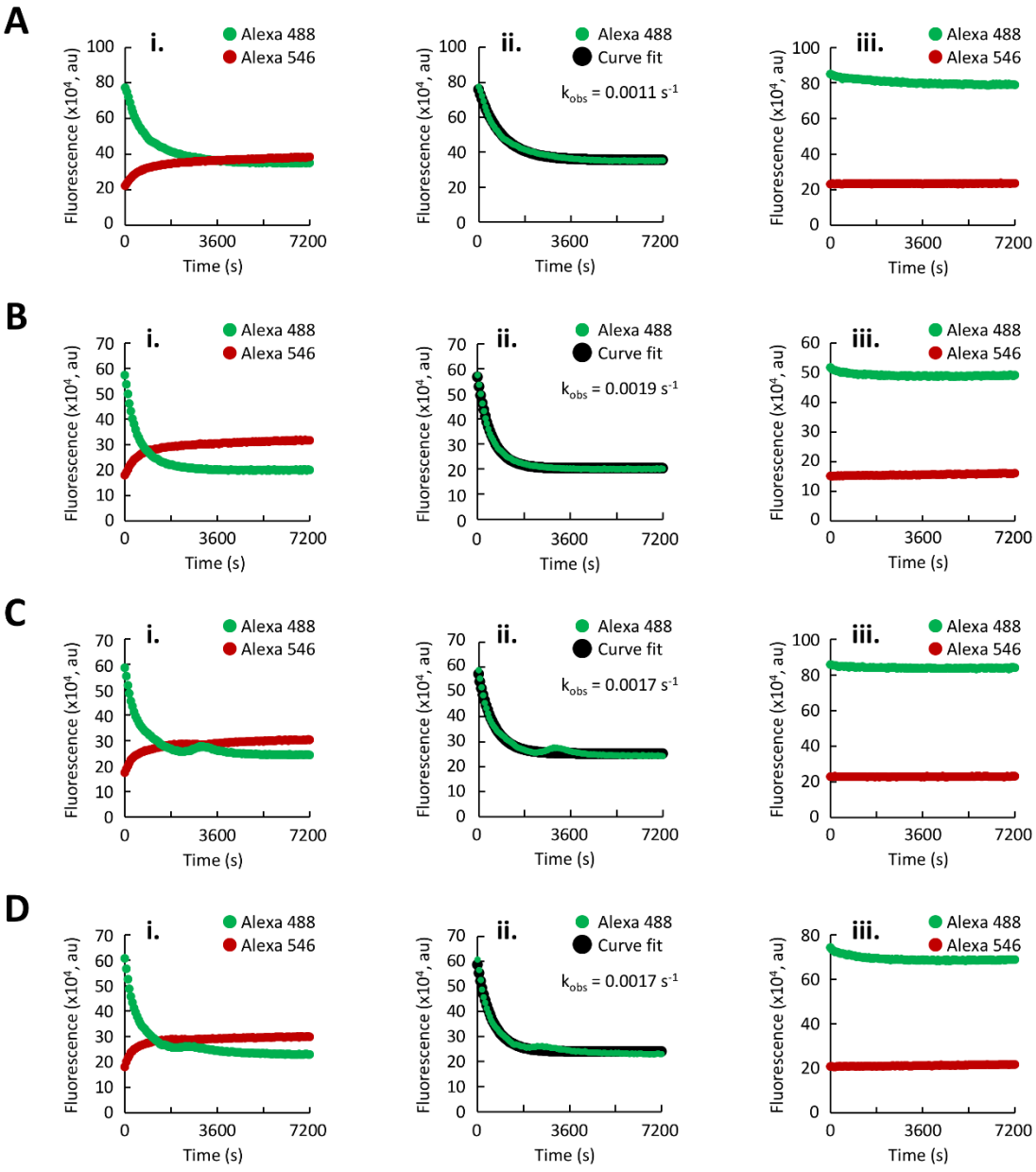

Figure S4. Time course FRET experiments were performed for *aH^CTGF-cgnt.12* paired with (A) *sH^CTGF.12/8*, (B) *sH^CTGF.12/12*, (C) *sH^CTGF.16/8* and (D) *sH^CTGF.20/8*. (i.) CTGF trigger was spiked into the cuvette containing premixed hybrids to monitor triggered DsiRNA generation. (ii) The Alexa488 signal measure in (i.) was used to determined observed rate constants for each hybrid pair in presence of the CTGF trigger. (iii.) Hybrid pairs were observed in the absence of CTGF trigger molecule. All experiments were conducted at 37 °C using 500 nM concentration of each hybrid. CTGF trigger was added to a final concentration of 1 µM in (i.).

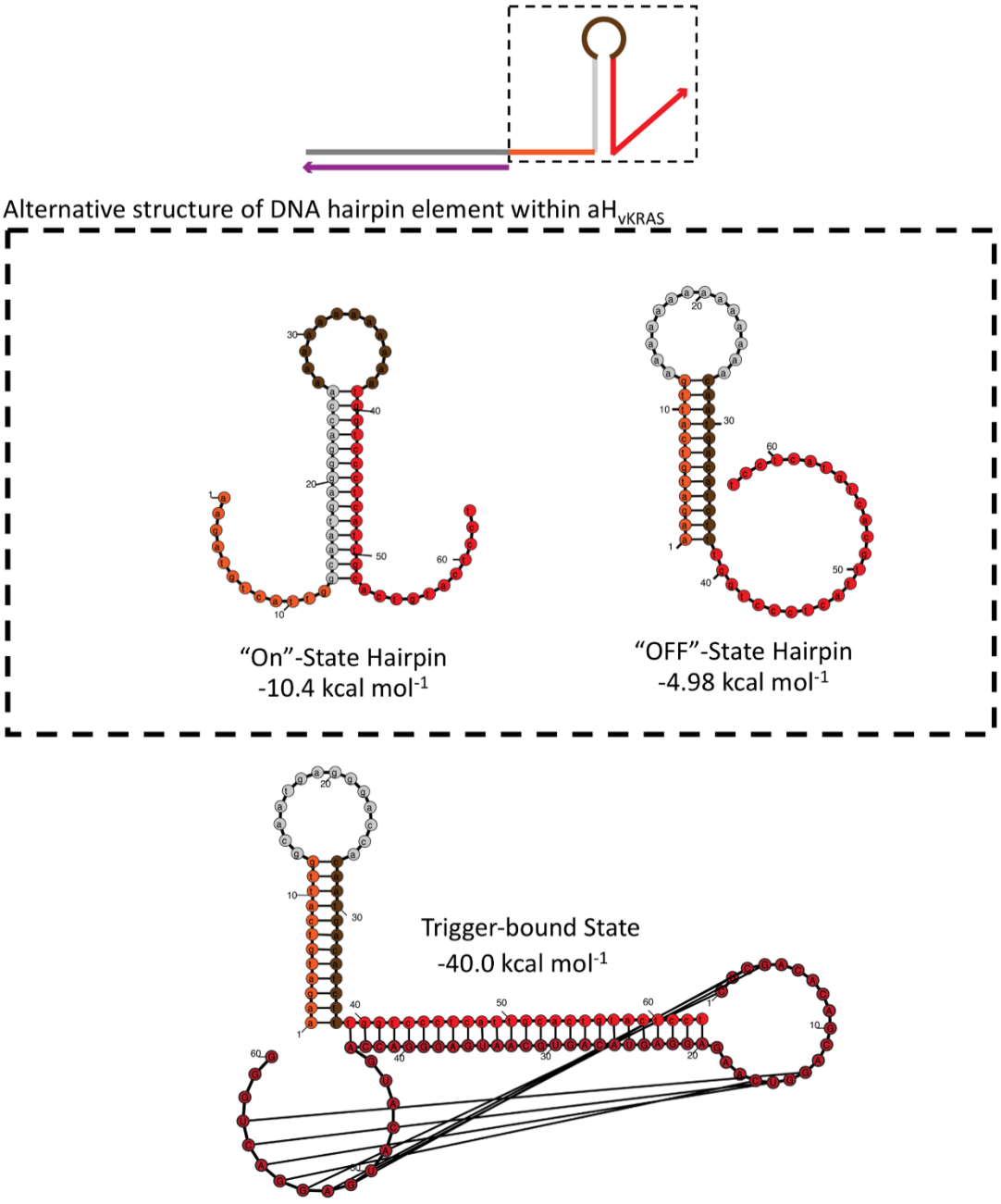

Figure S5. Alternate structures adopted by the responsive hairpin/toehold region of *aH$_{vKRAS}$* as predicted by Hyperfold. Stretches of poly-A were inserted into the loop sequence of each state to avoid pseudoknots and examine the energies of the two distinct hairpins. The "on" state is initially energetically preferred in absence of trigger, but the "off" state structure is stabilized by hybridization of the KRAS trigger. Nucleotide coloring is consistent between all structures throughout the figure. Lowercase letters indicate DNA nucleotides. Uppercase letters indicate RNA nucleotides.

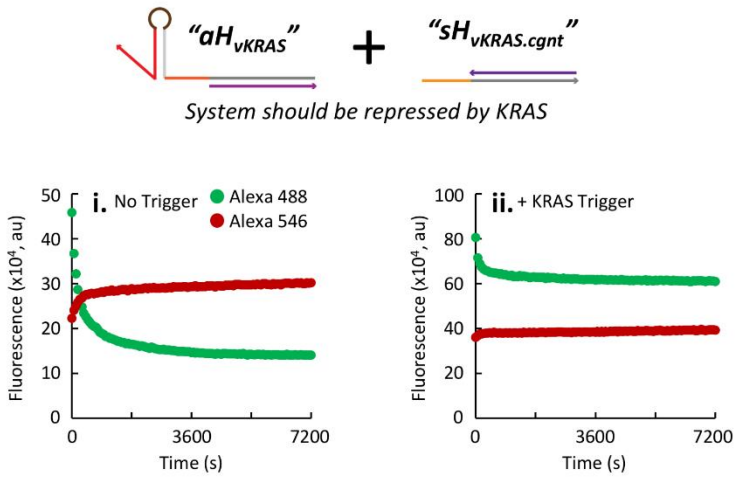

Figure S6. FRET time course experiments were used to monitor dsRNA release the KRAS-responsive repressible hybrid system. $aH_{vKRAS.}$ and $sH_{vKRAS.cgnt}$ hybrids were combined to a final concentration of 500 nM final in (i.) the absence of trigger, or (ii.) the presence of a 3-fold excess of KRAS.

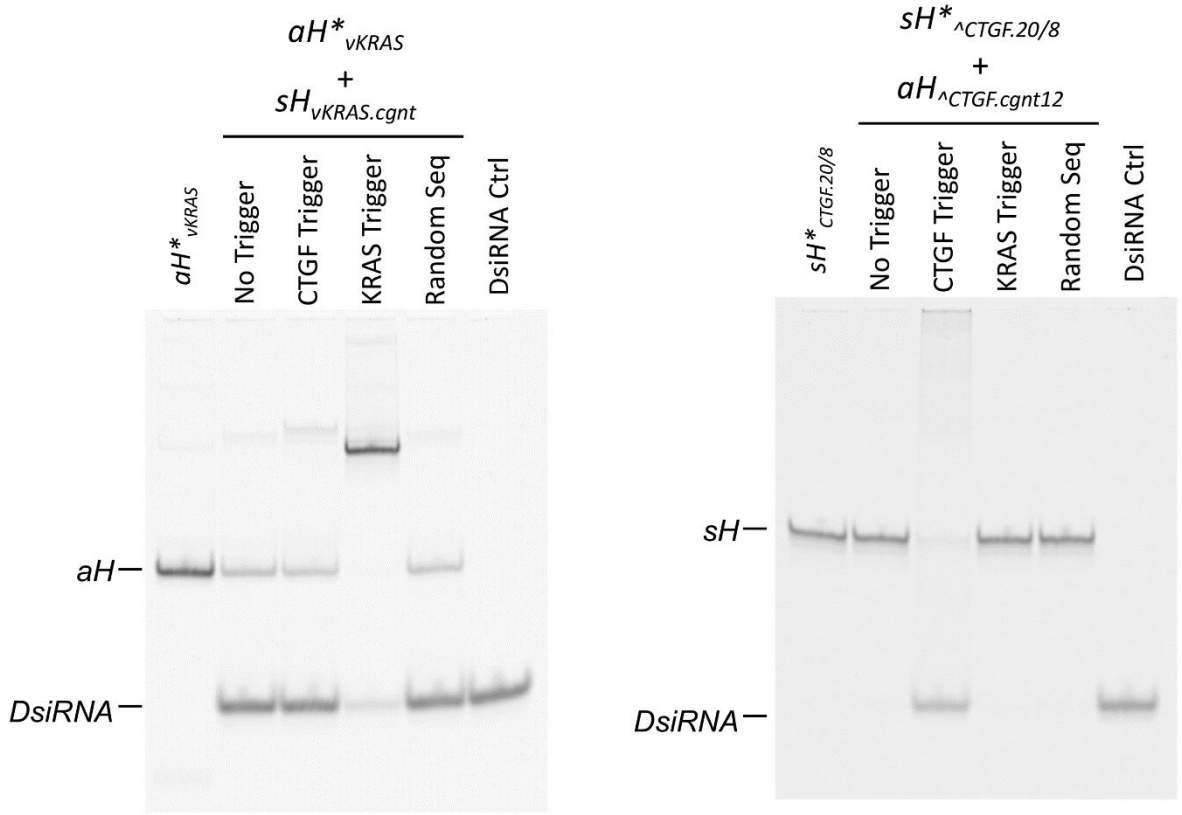

Figure S7. Trigger-responsive RNA/DNA hybrid pairs respond only to their specific cognate triggers. In order to display the specificity and generality of the trigger-responsive systems two different pairs of hybrids were examined for their ability to function in the presence of various trigger

sequences. The $aH_{vKRAS}/sH_{vKRAS.cgnt}$ pair (left) is designed to repress dsRNA release in presence of the KRAS trigger. The $sH^{\wedge CTGF.20/8}/aH^{\wedge CTGF.cgnt12}$ pair (right) is designed to induce dsRNA release in presence of the CTGF trigger. The extent of dsRNA release was examined by non-denaturing PAGE. $aH^{*}{}_{vKRAS}$ and its corresponding DsiRNA control contain a 5′-AlexaFluor546 labeled RNA antisense strand for visualization, while $sH^{*}{}_{CTGF.20/8}$ and the corresponding DsiRNA control contain 3′- 6-FAM labeled sense RNA strands.

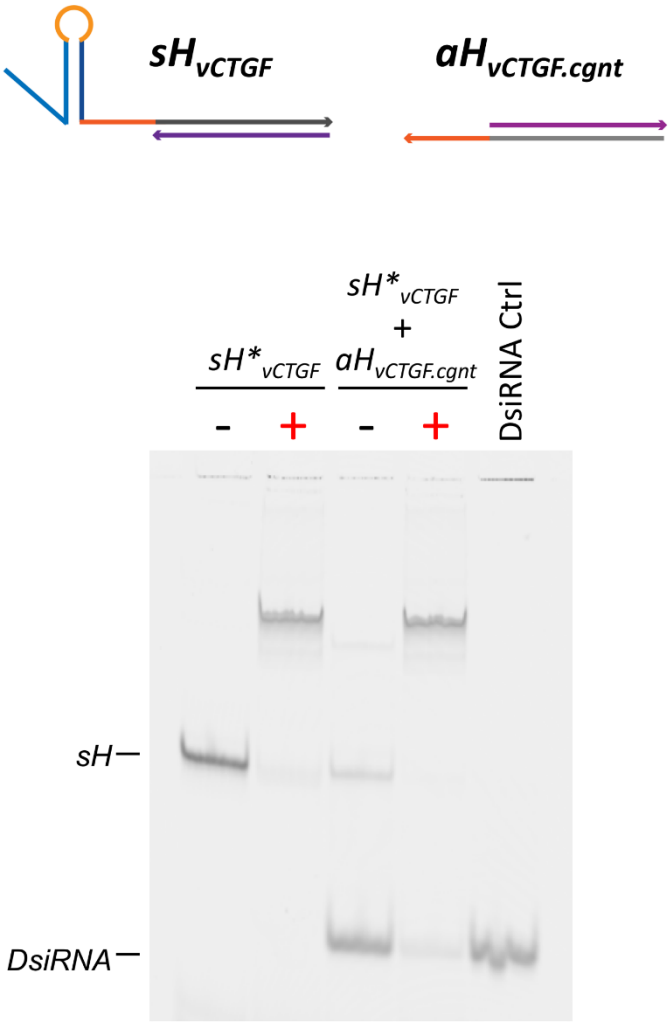

Figure S8. A repressive hybrid system was designed that is responsive to CTGF in order to show that such repressible systems can be generally applied. Not only does the CTGF-repression system respond to a different trigger sequence than the vKRAS system, but the vCTGF system is designed as the mirror opposite of the KRAS-repression system. The vCTGF system contains the responsive DNA structural element on the 5′ end of the sense hybrid, whereas the responsive element of the vKRAS system is on the 3′ end of the antisense hybrid. The function of the vCTGF system was examined by non-denaturing PAGE. The sense hybrid and DsiRNA control contain a 3′- 6-FAM labeled sense RNA for visualization. The presence (+) or absence (-) of the CTGF trigger is indicated above each lane.

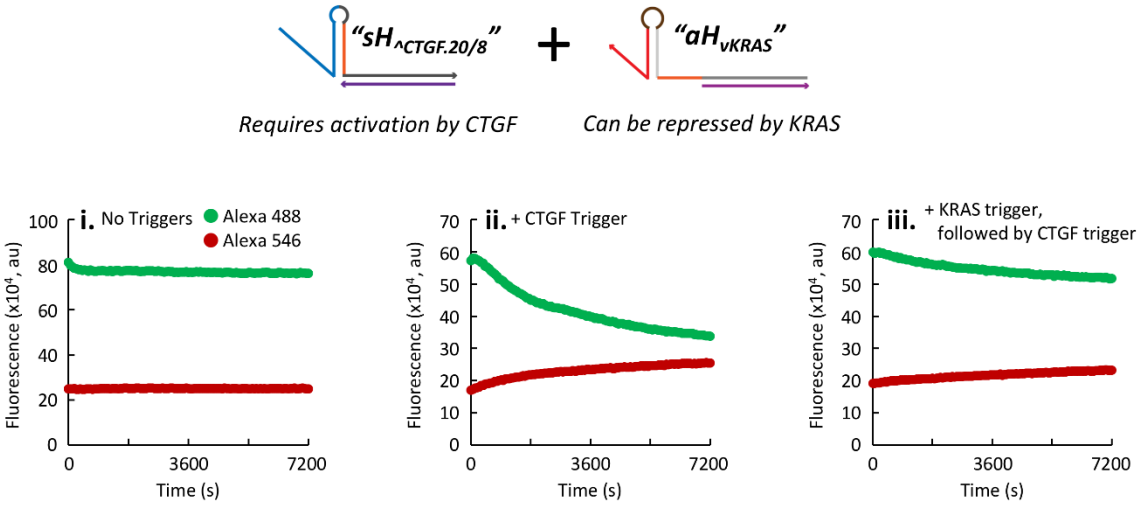

Figure S9. FRET time course experiments were used to monitor dsRNA release for a hybrid system where the sense hybrid requires CTGF to become active, while the function of the antisense hybrid can be repressed by interaction with KRAS. Hybrids *sH^CTGF.20/8* and *aH vKRAS.* were combined to a final concentration of 500 nM final (i.) in the absence of any trigger molecules, (ii.) in the presence of a the 2-fold excess of CTGF, or (iii.) in a context when a 3-fold excess of KRAS followed by a 2-fold excess of CTGF are added to the hybrids in a sequential fashion.

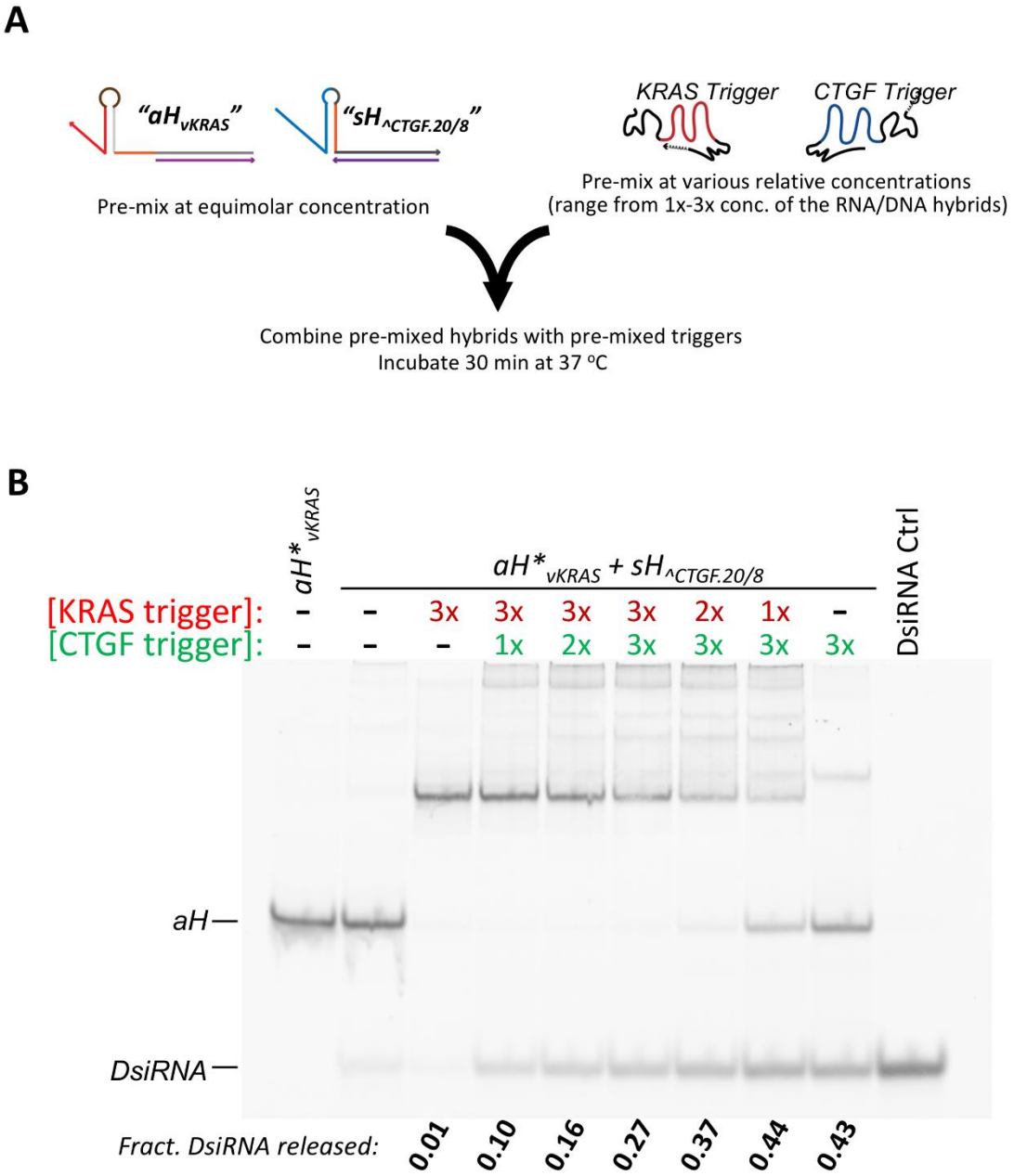

Figure S10. The effect of difference in trigger concentration on dsRNA release from a multi-trigger RNA/DNA hybrid system. (A) The *aH*$_{vKRAS}$ and *sH*$_{\wedge CTGF.20/8}$ hybrids were initially premixed at equimolar concentrations. Multiple tubes of the cognate KRAS and CTGF triggers were also pre-mixed, at various relative concentrations, ranging from 0x-3x the concentration of the hybrid concentration. An aliquot of the hybrid mixture was then added to each tube containing triggers and incubated at 37 °C for 30 minutes. The experiment was designed to reduce any kinetic bias on the system based on the order of construct addition to the reaction. (B) The extent of DsiRNA release was examined by non-denaturing PAGE and is reported for each trigger concentration. *aH**$_{vKRAS}$ and the DsiRNA control were assembled with a 5′-AlexaFluor546 labeled RNA antisense strand for visualization and quantitation.

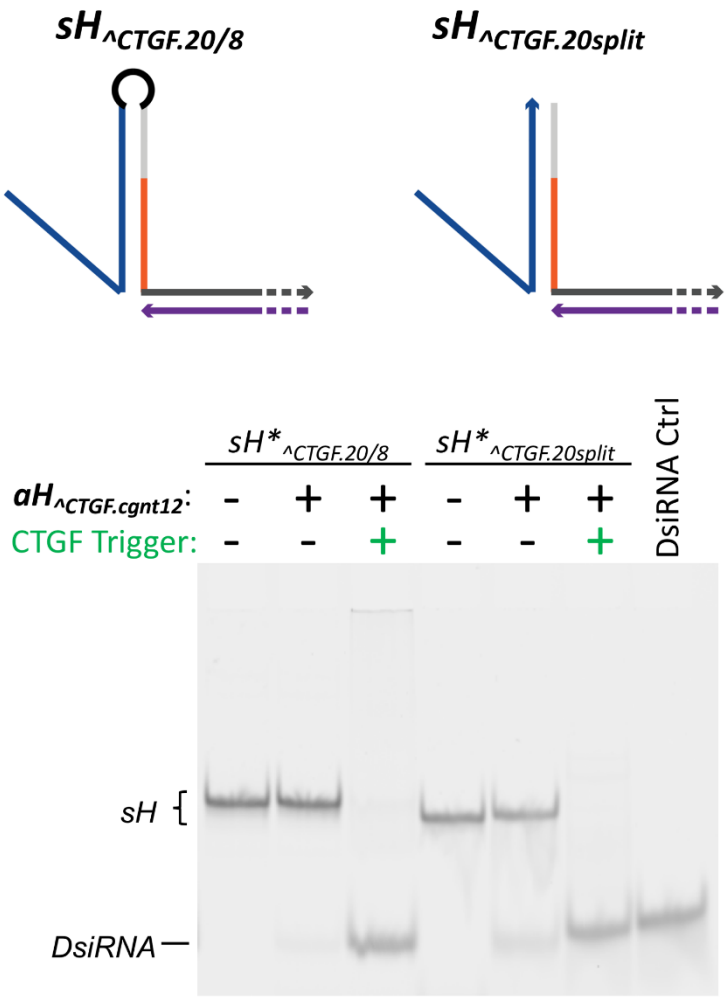

Figure S11. A 3-piece trigger-inducible RNA/DNA hybrid was examined for its ability to release dsRNA in a conditional fashion. Analysis was performed by non-denaturing PAGE. The sense hybrids and DsiRNA control contain a 3'- 6-FAM labeled sense RNA for visualization.

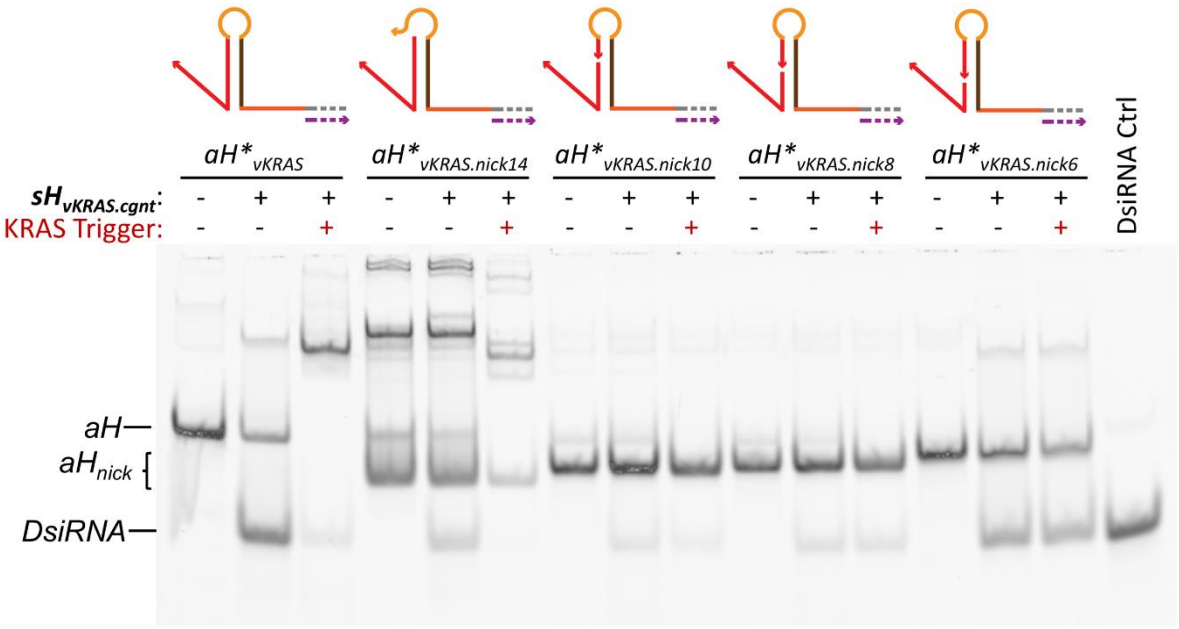

Figure S12. Various 3-piece trigger-repressible RNA/DNA hybrids were examined for their ability to release dsRNA in a conditional fashion. The different 3-piece *aH*$_{vKRAS}$ hybrids were created by inserting a nick in the stem of the responsive DNA element. Each 3-piece *aH*$_{vKRAS}$ hybrid was partnered with *sH*$_{vKRAS.cgnt}$. DsiRNA release was analyzed by non-denaturing PAGE. The ability of the 3-piece hybrids to maintain conditional function decreases as the nick is moved further away from the apical loop of the DNA hairpin.

Table S1. Statistical significance in the difference of dsRNA fraction released from individual adjacent targeting hybrid pairs at various time points, in presence or absence of the CTGF RNA trigger. P-values indicated as follows: not significant (ns) if > 0.05; * if < 0.05; ** if < 0.01; *** if < 0.001.

+0 bp		**Non-Triggered**			**CTGF-Triggered**		
hybrid pair		**30 min**	**90 min**	**180 min**	**30 min**	**90 min**	**180 min**
Non-Triggered	**30 min**	-----					
	90 min	ns	-----				
	180 min	ns	ns	-----			
CTGF-Triggered	**30 min**	*	ns	ns	-----		
	90 min	ns	ns	ns	ns	-----	
	180 min	ns	ns	ns	ns	ns	-----

+1 bp		**Non-Triggered**			**CTGF-Triggered**		
hybrid pair		**30 min**	**90 min**	**180 min**	**30 min**	**90 min**	**180 min**
Non-Triggered	**30 min**	-----					
	90 min	ns	-----				
	180 min	ns	ns	-----			
CTGF-Triggered	**30 min**	ns	ns	ns	-----		
	90 min	ns	ns	ns	ns	-----	
	180 min	ns	ns	ns	ns	ns	-----

+2 bp	**Non-Triggered**	**CTGF-Triggered**

hybrid pair		30 min	90 min	180 min	30 min	90 min	180 min
Non-Triggered	30 min	-----					
	90 min	ns	-----				
	180 min	ns	ns	-----			
CTGF-Triggered	30 min	ns	ns	*	-----		
	90 min	ns	ns	ns	ns	-----	
	180 min	**	**	*	ns	ns	-----

+3 bp		Non-Triggered			CTGF-Triggered		
hybrid pair		30 min	90 min	180 min	30 min	90 min	180 min
Non-Triggered	30 min	-----					
	90 min	ns	-----				
	180 min	ns	ns	-----			
CTGF-Triggered	30 min	ns	*	*	-----		
	90 min	*	**	*	ns	-----	
	180 min	***	***	***	ns	ns	-----

+4 bp		Non-Triggered			CTGF-Triggered		
hybrid pair		30 min	90 min	180 min	30 min	90 min	180 min
Non-Triggered	30 min	-----					
	90 min	ns	-----				
	180 min	ns	ns	-----			
CTGF-Triggered	30 min	**	**	**	-----		
	90 min	**	**	**	ns	-----	
	180 min	***	**	**	ns	*	-----

Table S2. Statistical significance in the difference of dsRNA fraction released from differing adjacent targeting hybrid pairs at a single time point, in presence or absence of the CTGF RNA trigger. P-values indicated as follows: not significant (ns) if > 0.05; * if < 0.05; ** if < 0.01; *** if < 0.001.

No trigger, 30m

	+0 bp	+1 bp	+2 bp	+3 bp	+4 bp
+0 bp	-----				
+1 bp	ns	-----			
+2 bp	ns	ns	-----		
+3 bp	ns	ns	ns	-----	
+4 bp	ns	ns	ns	ns	-----

CTGF trigger, 30m

	+0 bp	+1 bp	+2 bp	+3 bp	+4 bp
+0 bp	-----				
+1 bp	ns	-----			
+2 bp	ns	ns	-----		
+3 bp	*	*	**	-----	
+4 bp	**	**	**	*	-----

No trigger, 90m

	+0 bp	+1 bp	+2 bp	+3 bp	+4 bp
+0 bp	-----				
+1 bp	ns	-----			
+2 bp	ns	ns	-----		
+3 bp	ns	ns	ns	-----	
+4 bp	ns	ns	ns	ns	-----

CTGF trigger, 90m

	+0 bp	+1 bp	+2 bp	+3 bp	+4 bp
+0 bp	-----				
+1 bp	ns	-----			
+2 bp	ns	ns	-----		
+3 bp	*	**	ns	-----	
+4 bp	**	**	*	*	-----

No trigger, 180m

	+0 bp	+1 bp	+2 bp	+3 bp	+4 bp
+0 bp	-----				
+1 bp	ns	-----			
+2 bp	ns	ns	-----		
+3 bp	ns	ns	ns	-----	
+4 bp	ns	ns	ns	*	-----

CTGF trigger, 180m

	+0 bp	+1 bp	+2 bp	+3 bp	+4 bp
+0 bp	-----				
+1 bp	*	-----			
+2 bp	**	*	-----		
+3 bp	***	**	**	-----	
+4 bp	**	**	**	ns	-----

Table S3. Statistical significance in the difference of dsRNA fraction released from differing inducible *sH^CTGF* hybrids paired with either *aH^CTGF-cgnt.12* or *aH^CTGF-cgnt.16* at a single time point, in presence or absence of the CTGF RNA trigger. P-values indicated as follows: not significant (ns) if > 0.05; * if < 0.05; ** if < 0.01.

CTGF Inducible sense hybrids after 30 minutes, no trigger

	Paired with *aH^CTGF-cgnt.12*:			
	sH^CTGF.12/8	**sH^CTGF.12/12**	**sH^CTGF.16/8**	**sH^CTGF.20/8**
sH^CTGF12/8	-----			
sH^CTGF.12/12	ns	-----		
sH^CTGF.16/8	ns	ns	-----	
sH^CTGF.20/8	ns	*	*	-----

	Paired with *aH^CTGF-cgnt.16*:			
	sH^CTGF.12/8	**sH^CTGF.12/12**	**sH^CTGF.16/8**	**sH^CTGF.20/8**
sH^CTGF12/8	-----			
sH^CTGF.12/12	ns	-----		
sH^CTGF.16/8	ns	ns	-----	
sH^CTGF.20/8	ns	ns	ns	-----

CTGF Inducible sense hybrids after 30 minutes, in presence of CTGF trigger

	Paired with *aH^CTGF-cgnt.12*:				Paired with *aH^CTGF-cgnt.16*:				
	sH^CTGF.12/8	sH^CTGF.12/12	sH^CTGF.16/8	sH^CTGF.20/8		sH^CTGF.12/8	sH^CTGF.12/12	sH^CTGF.16/8	sH^CTGF.20/8
sH^CTGF12/8	-----				sH^CTGF12/8	-----			
sH^CTGF.12/12	ns	-----			sH^CTGF.12/12	ns	-----		
sH^CTGF.16/8	ns	ns	-----		sH^CTGF.16/8	ns	*	-----	
sH^CTGF.20/8	ns	ns	ns	-----	sH^CTGF.20/8	ns	*	ns	-----

CTGF Inducible sense hybrids after 90 minutes, no trigger

	Paired with *aH^CTGF-cgnt.12*:				Paired with *aH^CTGF-cgnt.16*:				
	sH^CTGF.12/8	sH^CTGF.12/12	sH^CTGF.16/8	sH^CTGF.20/8		sH^CTGF.12/8	sH^CTGF.12/12	sH^CTGF.16/8	sH^CTGF.20/8
sH^CTGF12/8	-----				sH^CTGF12/8	-----			
sH^CTGF.12/12	ns	-----			sH^CTGF.12/12	ns	-----		
sH^CTGF.16/8	ns	*	-----		sH^CTGF.16/8	ns	ns	-----	
sH^CTGF.20/8	ns	**	ns	-----	sH^CTGF.20/8	ns	ns	**	-----

CTGF Inducible sense hybrids after 90 minutes, in presence of CTGF trigger

	Paired with *aH^CTGF-cgnt.12*:				Paired with *aH^CTGF-cgnt.16*:				
	sH^CTGF.12/8	sH^CTGF.12/12	sH^CTGF.16/8	sH^CTGF.20/8		sH^CTGF.12/8	sH^CTGF.12/12	sH^CTGF.16/8	sH^CTGF.20/8
sH^CTGF12/8	-----				sH^CTGF12/8	-----			
sH^CTGF.12/12	ns	-----			sH^CTGF.12/12	ns	-----		
sH^CTGF.16/8	ns	ns	-----		sH^CTGF.16/8	*	*	-----	
sH^CTGF.20/8	ns	ns	ns	-----	sH^CTGF.20/8	***	ns	ns	-----

CTGF Inducible sense hybrids after 180 minutes, no trigger

	Paired with *aH^CTGF-cgnt.12*:				Paired with *aH^CTGF-cgnt.16*:				
	sH^CTGF.12/8	sH^CTGF.12/12	sH^CTGF.16/8	sH^CTGF.20/8		sH^CTGF.12/8	sH^CTGF.12/12	sH^CTGF.16/8	sH^CTGF.20/8
sH^CTGF12/8	-----				sH^CTGF12/8	-----			
sH^CTGF.12/12	ns	-----			sH^CTGF.12/12	ns	-----		
sH^CTGF.16/8	ns	ns	-----		sH^CTGF.16/8	*	ns	-----	
sH^CTGF.20/8	ns	ns	ns	-----	sH^CTGF.20/8	**	ns	*	-----

CTGF Inducible sense hybrids after 180 minutes, in presence of CTGF trigger

	Paired with *aH^CTGF-cgnt.12*:				Paired with *aH^CTGF-cgnt.16*:				
	sH^CTGF.12/8	sH^CTGF.12/12	sH^CTGF.16/8	sH^CTGF.20/8		sH^CTGF.12/8	sH^CTGF.12/12	sH^CTGF.16/8	sH^CTGF.20/8
sH^CTGF12/8	-----				sH^CTGF12/8	-----			
sH^CTGF.12/12	ns	-----			sH^CTGF.12/12	ns	-----		
sH^CTGF.16/8	ns	*	-----		sH^CTGF.16/8	*	*	-----	
sH^CTGF.20/8	ns	ns	ns	-----	sH^CTGF.20/8	*	*	ns	-----

Table S4. Statistical significance in the difference of dsRNA fraction released for a given *sH^CTGF* hybrid paired with *aH^CTGF-cgnt.16* compared to *aH^CTGF-cgnt.12*, in either the presence or absence of the CTGF RNA trigger. P-values indicated as follows: not significant (ns) if > 0.05; * if < 0.05; ** if < 0.01.

		30 min	90 min	180min
Non-Triggered	sH^CTGF12/8	ns	ns	ns
	sH^CTGF.12/12	ns	ns	ns
	sH^CTGF.16/8	ns	ns	ns
	sH^CTGF.20/8	ns	ns	ns
CTGF-Triggered	sH^CTGF12/8	*	**	*
	sH^CTGF.12/12	*	*	*
	sH^CTGF.16/8	ns	*	*
	sH^CTGF.20/8	*	*	ns

Table S5. Statistical significance in the difference of dsRNA fraction released from individual CTGF-inducible hybrid pairs at various time points, in presence or absence of the CTGF RNA trigger. P-values indicated as follows: not significant (ns) if > 0.05; * if < 0.05; ** if < 0.01; *** if < 0.001.

***sH^CTGF.12/8* /**		**Non-Triggered**			**CTGF-Triggered**		
aH^CTGF-cgnt.12		**30 min**	**90 min**	**180 min**	**30 min**	**90 min**	**180 min**
Non-Triggered	**30 min**	-----					
	90 min	ns	-----				
	180 min	ns	ns	-----			
CTGF-Triggered	**30 min**	**	**	**	-----		
	90 min	**	***	**	ns	-----	
	180 min	***	***	**	*	*	-----
***sH^CTGF.12/12* /**		**Non-Triggered**			**CTGF-Triggered**		
aH^CTGF-cgnt.12		**30 min**	**90 min**	**180 min**	**30 min**	**90 min**	**180 min**
Non-Triggered	**30 min**	-----					
	90 min	ns	-----				
	180 min	ns	ns	-----			
CTGF-Triggered	**30 min**	***	**	*	-----		
	90 min	**	***	**	ns	-----	
	180 min	***	***	**	**	ns	-----
***sH^CTGF.16/8* /**		**Non-Triggered**			**CTGF-Triggered**		
aH^CTGF-cgnt.12		**30 min**	**90 min**	**180 min**	**30 min**	**90 min**	**180 min**
Non-Triggered	**30 min**	-----					
	90 min	*	-----				
	180 min	ns	ns	-----			
CTGF-Triggered	**30 min**	**	**	*	-----		
	90 min	**	**	**	ns	-----	
	180 min	***	***	**	ns	ns	-----
***sH^CTGF.20/8* /**		**Non-Triggered**			**CTGF-Triggered**		
aH^CTGF-cgnt.12		**30 min**	**90 min**	**180 min**	**30 min**	**90 min**	**180 min**

Non-Triggered	30 min	-----					
	90 min	ns	-----				
	180 min	ns	ns	-----			
CTGF-Triggered	30 min	**	**	**	-----		
	90 min	**	**	**	ns	-----	
	180 min	**	**	***	ns	ns	-----

sH^CTGF.12/8* / *aH^CTGF-cgnt.16		Non-Triggered			CTGF-Triggered		
		30 min	**90 min**	**180 min**	**30 min**	**90 min**	**180 min**
Non-Triggered	30 min	-----					
	90 min	ns	-----				
	180 min	*	ns	-----			
CTGF-Triggered	30 min	*	ns	ns	-----		
	90 min	**	**	**	ns	-----	
	180 min	**	*	*	ns	ns	-----

sH^CTGF.12/12* / *aH^CTGF-cgnt.16		Non-Triggered			CTGF-Triggered		
		30 min	**90 min**	**180 min**	**30 min**	**90 min**	**180 min**
Non-Triggered	30 min	-----					
	90 min	ns	-----				
	180 min	ns	ns	-----			
CTGF-Triggered	30 min	*	*	ns	-----		
	90 min	***	***	**	ns	-----	
	180 min	***	**	**	*	*	-----

sH^CTGF.16/8* / *aH^CTGF-cgnt.16		Non-Triggered			CTGF-Triggered		
		30 min	**90 min**	**180 min**	**30 min**	**90 min**	**180 min**
Non-Triggered	30 min	-----					
	90 min	ns	-----				
	180 min	ns	ns	-----			
CTGF-Triggered	30 min	*	*	*	-----		
	90 min	***	***	**	ns	-----	
	180 min	***	***	**	ns	ns	-----

sH^CTGF.20/8* / *aH^CTGF-cgnt.16		Non-Triggered			CTGF-Triggered		
		30 min	**90 min**	**180 min**	**30 min**	**90 min**	**180 min**
Non-Triggered	30 min	-----					
	90 min	ns	-----				
	180 min	*	ns	-----			
CTGF-Triggered	30 min	**	**	**	-----		
	90 min	**	**	**	ns	-----	
	180 min	***	***	***	*	ns	-----

Table S6. Statistical significance in the difference of dsRNA fraction released the KRAS-repressible hybrid pair aH_{vKRAS} and $sH_{vKRAS\text{-}cgnt}$ in various molecular environments, at multiple time points. P-values indicated as follows: not significant (ns) if > 0.05; * if < 0.05; ** if < 0.01; *** if < 0.001.

		30 minutes									90 minutes									180 minutes								
		a	H_v	*KR*	*a*	H_v	*KR*	*a*	H_v	*KR*	*a*	H_v	*KR*	*a*	H_v	*KR*	*a*	H_v	*KR*	*a*	H_v	*KR*	*a*	H_v	*KR*	*a*	H_v	*KR*
30 minutes	aH_{vKRAS} + $sH_{vKRAS\text{-}cgnt}$	-----																										
	aH_{vKRAS} + mixture of KRAS/$sH_{vKRAS\text{-}cgnt}$	**			-----																							
	aH_{vKRAS} + KRAS, followed by $sH_{vKRAS\text{-}cgnt}$	**			**			-----																				
90 minutes	aH_{vKRAS} + $sH_{vKRAS\text{-}cgnt}$	*			***			***			-----																	
	aH_{vKRAS} + mixture of KRAS/$sH_{vKRAS\text{-}cgnt}$	**			*			**			***			-----														
	aH_{vKRAS} + KRAS, followed by $sH_{vKRAS\text{-}cgnt}$	**			**			*			***			**			-----											
180 minutes	aH_{vKRAS} + $sH_{vKRAS\text{-}cgnt}$	ns			***			***			ns			***			***			-----								
	aH_{vKRAS} + mixture of KRAS/$sH_{vKRAS\text{-}cgnt}$	**			ns			**			***			ns			**			***			-----					
	aH_{vKRAS} + KRAS, followed by $sH_{vKRAS\text{-}cgnt}$	***			***			*			***			**			*			***			**			-----		