Supplementary Note 1: Identification and characterization of -1 PRF motifs from NGS data.

Raw sequencing data can be downloaded from the following web address: https://rabadan.c2b2.columbia.edu/public/PRF/frameshift_selection.fastq.gz. The software and resources for the analysis pipeline can be accessed at: https://github.com/szairis/frameshift.

A pipeline was established for processing and analyzing NGS data for motif identification. Step 1 of the pipeline beings by processing the raw sequencing fastq file and generates a file containing every unique sequence and its associated read count. The HiSeq run yielded roughly 56 million sequencing reads after processing, from which >3 million unique sequences were recovered. Abundances (or read counts) of these sequences ranged from 1 read to >800,000 reads. 50% of the total sequencing reads are accounted for by the top 6,752 most abundant unique sequences. We set out to identify -1 PRF motifs from this dataset with a specific focus on RNA pseudoknots (PKs), which are known to promote -1 PRF. To assign a PK structure to each sequence, we chose to assess *compatibility* with PK configurations without accounting for energetic contributions. Because this approach is tailored to our restricted scaffold, it is computationally more efficient than absolute prediction. Notably, this approach often leads to PK assignments that are in agreement with pseudoknot prediction algorithms (such as pKiss). We created a hairpin-type (H-type) PK feature space using the starting library's sequence constraints and our imposed structural constraints. Every H-type PK can be defined by the lengths of its segments: (a) stem 1; (b) loop 1; (c) stem 2; (d) loop 2; and (e) loop 3 (**Supplementary Fig. 3**). To establish the PK position within the library scaffold, we also define the length of the segment of unpaired nucleotides preceding (5' to) the PK, the length of the segment downstream of (3' to) the PK, and fix the total length to 44 nucleotides.

Using the library scaffold's constraints and our imposed constraints, a total of 2,068 PK features were generated. The top 20,000 most abundant sequences from the NGS, as well as every theoretical starting library sequence, were evaluated for their compatibility with the PK feature space (step 2 of the pipeline). A sequence was deemed compatible with a given PK feature if it supported the base pairs present within the PK feature (allowable base pairs include the standard Watson-Crick A-U, U-A, G-C, and C-G, along with the wobble G·U and U·G pairs). Of the PK features supported by a given sequence, the feature with the most base pairs is chosen as the assigned PK feature for that sequence. In the case of a tie, both PK features are

assigned to the sequence. From the theoretical starting library, 1,507 PK features were selected by at least 1 sequence.

In step 3 of the pipeline, we determined the enrichment of each PK feature by calculating its pre-selection probability and comparing it to its post-selection probability (**Supplementary Fig. 4**). Explicitly, pre-selection probability was defined as the total number of sequences from the theoretical starting library that were assigned to the PK feature divided by the total starting sequences (2.68×10^8). Post-selection probability was defined as the total number of sequencing reads that fit to the PK feature (number of unique sequences in that feature multiplied by the mean read-count of sequences within that PK feature) divided by the total sequencing reads (approximately 5.6×10^7). The enrichment factor (EF) is then defined as the ratio of the post-selection probability to the pre-selection probability.

$$EF = \frac{\left(post\_selection\ probability\right)}{\left(pre\_selection\ probability\right)} = \frac{(\#\,PK\ reads\,/\,total\ reads)}{(\#\,PK\ initial\,/\,total\ initial)}$$

Each PK feature was ranked according to its EF, and PK features that showed low occupancy (low total read mass) were removed. Then, PK features ranked within the top 10% of EFs were nominated for primary sequencing clustering analysis. Primary sequence clustering was performed using a greedy algorithm, resulting in families of sequences that comprise the motifs. Clusters containing less than 5 sequences were discarded or ignored. From this analysis, 115 clusters were nominated as final -1 PRF motifs (**Supplementary Data 1**).

The analysis pipeline generates a summary report for all nominated motifs with the following fields: 1) an ID number; 2) the number of sequences supporting the motif; 3) the mean and 4) standard deviation of abundance level for sequences in the motif; 5) the assigned PK secondary structure in dot-bracket notation; 6) agreement with pKiss; 7) the sequence mode; 8) the neighborhood occupancy about the mode; and 9) the normalized entropy of the mode neighborhood. Additionally, for each motif, the analysis pipeline generates a fasta file containing the motif's supporting sequences and a logo[1] displaying information content (see **Supplementary Fig. 5** for an example). An additional variant analysis can be performed as step 4 in the pipeline to analyze the sequence neighborhood of a particular sequence of interest. This output provides a plot of single nucleotide variants for any sequence of interest, as well as a heat map describing abundance for variants with two nucleotide (pairwise) differences. Notably, the pairwise output may highlight positions where mutual information is present.

Supplementary Note 2: Thermodynamic calculations for -1 PRF ON-switches.

Calculations were performed using NUPACK[2] with Turner parameters[3] and the temperature set to 30 ˚C. By default, NUPACK does not predict pseudoknots and therefore will predict the lowest energy non-pseudoknotted secondary structure for the RNA. pKiss[4] was used to predict FS-2 pseudoknot energy at 30 ˚C. **Supplementary Table 1** summarizes the results of the calculations, which are outlined in more detail in **Supplementary Data 2**. RNA segments used for calculations spanned from the start of the FS-2 pseudoknot to the unstructured mid-insulator region. Ligand binding energy contributions were estimated based on the K_d of the ligand aptamer interaction and the concentration of the ligand that was used. We estimated thermodynamic contributions of RNA folding and ligand binding for 4 hypothetical scenarios: (1) ΔG of the system in the OFF state not bound to ligand; (2) ΔG of the system in the ON state not bound to ligand; (3) ΔG of the system in the OFF state when bound to ligand; and (4) ΔG of the system in the ON state when bound to ligand. The ON state is defined by a folded FS-2 pseudoknot structure. The following energies were calculated as contributors to each of the scenarios:

(1) ΔG of the system in the OFF state not bound to ligand, Σ of:
- Ensemble ΔG of the unfolded pseudoknot
- Ensemble ΔG of the switching hairpin
- Ensemble ΔG of the aptamer region

(Note: Σ of ΔG's can be estimated by the ensemble ΔG of the entire sequence)

(2) ΔG of the system in the ON state not bound to ligand, Σ of:
- ΔG of the folded pseudoknot
- Ensemble ΔG of the sequence 3' to the pseudoknot

(3) ΔG of the system in the OFF state when bound to ligand, Σ of:
- Ensemble ΔG of sequence 5' to the aptamer
- ΔG of the folded aptamer
- ΔG of ligand binding

(4) ΔG of the system in the ON state when bound to ligand, Σ of:
- ΔG of the folded pseudoknot
- ΔG of the folded aptamer
- ΔG of ligand binding

Unbound		*Ligand Bound*	
ΔG OFF (kcal/mol)	**ΔG ON** (kcal/mol)	**ΔG OFF** (kcal/mol)	**ΔG ON** (kcal/mol)
Unfolded FS-2 (ensemble ΔG)	Folded FS-2 (MFE ΔG)	5' of aptamer (ensemble ΔG)	Folded FS-2 (MFE ΔG)
Hairpin (ensemble ΔG)	3' of FS-2 (ensemble ΔG)	Folder Aptamer (MFE ΔG)	Folder Aptamer (MFE ΔG)
3' of Hairpin (ensemble ΔG)		Ligand Binding (ΔG)	Ligand Binding (ΔG)
Σ ΔG Unbound OFF	Σ ΔG Unbound ON	Σ ΔG Ligand OFF	Σ ΔG Ligand ON

The relative calculated energies of the ON and OFF states were used to guide decision for constructing switches, with the desirable property that the OFF state is preferred in the absence of ligand and the ON state is preferred in the presence of ligand (i.e. minimize ΔΔG ON – OFF in the absence of ligand, and maximize ΔΔG ON – OFF in the presence of ligand). When ligand is absent from the system, only scenarios (1) and (2) are considered. However, in the presence of ligand, energies of all 4 scenarios must be considered (ON unbound and ligand bound, OFF unbound and ligand bound). Therefore, for calculating ΔΔG between the ON and OFF states in the presence of ligand, the Helmholtz free energy was used (-kT ln Q) for the ON and OFF states using the two contributing scenarios (unbound and ligand bound).

Supplementary Table 1. Summary of thermodynamic calculations for -1 PRF ON-switches.

Sequence	Σ ΔG (kcal/mol) Unbound OFF	Σ ΔG (kcal/mol) Unbound ON	Σ ΔG (kcal/mol) Ligand OFF	Σ ΔG (kcal/mol) Ligand ON	ΔΔG (kcal/mol) OFF-ON Unbound	ΔΔG (kcal/mol) OFF-ON Ligand
Theo-ON-1	-32.44	-33.58	-33.16	-36.75	+1.14	+3.43
Theo-ON-2	-34.14	-33.75	-34.86	-36.75	-0.39	+1.73
Theo-ON-3	-35.87	-33.65	-36.59	-36.75	-2.22	+0.00
Theo-ON-4	-35.69	-35.04	-37.35	-38.58	-0.65	+1.19
Theo-ON-5	-36.69	-35.95	-36.77	-39.99	-0.74	+2.84
Theo-ON-6	-36.45	-39.00	-38.18	-43.27	+2.55	+5.05
Neo-ON-1	-25.70	-28.65	-25.71	-32.64	+2.95	+6.51
Neo-ON-2	-27.46	-28.62	-27.55	-32.64	+1.19	+4.73
Neo-ON-3	-31.15	-29.82	-31.02	-32.64	-1.33	+1.14
Neo-ON-4	-29.11	-29.16	-29.02	-32.64	+0.05	+3.16

Supplementary Table 2. Sequences of -1 PRF OFF-switches.

Switch Name	**Sequence**
Theo-OFF-1	TTTAAACTAGTTGACGCGGTTCTATCTAGTTACGCGTTAAA CCAAC**TAGAGATACCAGCATCGTCTGATGCCCTTGGCAGC TCTA**AAAAAAATAAATAATAAAATTAAA
Theo-OFF-2	TTTAAACTAGTTGACGCGGTTCTATCTAGTTACGCGTTAAA CCAA**CTAGAGATACCAGCATCGTCTGATGCCCTTGGCAGC TCTAG**AAAAATAAATAATAAAATTAAA
Theo-OFF-3	TTTAAACTAGTTGACGCGGTTCTATCTAGTTACGCGTTAAA CCAA**CTAGAGATACCAGCATCGTCTGATGCCCTTGGCAGC TCTAGT**AAAATAAATAATAAAATTAAA
Neo-OFF-1	TTTAAACTAGTTGACGCGGTTCTATCTAGTTACGCGTTAAA CCAAC**TAGAGGCTTGTCCTTTAATGGTCCCTCTA**AAAAACA TAAATAATAAAATTAAA
Neo-OFF-2	TTTAAACTAGTTGACGCGGTTCTATCTAGTTACGCGTTAAA CCAA**CTAGAGGCTTGTCCTTTAATGGTCCCTCTAG**AAAACA TAAATAATAAAATTAAA
Neo-OFF-3	TTTAAACTAGTTGACGCGGTTCTATCTAGTTACGCGTTAAA CCAA**CTAGAGGCTTGTCCTTTAATGGTCCCTCTAGT**AAACA TAAATAATAAAATTAAA
Neo-OFF-4	TTTAAACTAGTTGACGCGGTTCTATCTAGTTACGCGTTAAA CCAA**CTAGAAGCTTGTCCTTTAATGGTCCTTCTAGT**AAACA TAAATAATAAAATTAAA
Neo-OFF-DE	TTTAAACTAGTTGACGCGGTTCTATCTAGTTACGCGTTAAA CCAA**CTAGTTGCTTGTCCTTTAATGGTCCAACTAG**AAAACA TAAATAATAAAATTAAA

Sequences begin with the slippery site and end with the insulating sequence. The pseudoknot sequence is underlined and the aptamer sequence bolded.

Supplementary Table 3. Sequences of -1 PRF ON-switches.

Switch Name	Sequence (5' to 3')
Theo-ON-1	TTTAAACTAGTTGACGCGGTTCTATCTAGTTACGCGTTAA*A* *CCAACTAGACATACTA****GTTGGT*****GATACCAGCATCGTCTTGA** **TGCCCTTGGCAGCACC**CAAAATAAATAATAAAATTAAA
Theo-ON-2	TTTAAACTAGTTGACGCGGTTCTATCTAGTTACGCGTTAA*A* *CCAACTAGACATTCTAGTT****GGT*****GATACCAGCATCGTCTTGA** **TGCCCTTGGCAGCACC**CAAAATAAATAATAAAATTAAA
Theo-ON-3	TTTAAACTAGTTGACGCGGTTCTATCTAGTTACGCGTTAA*A* *CCAACTAGAGCATCCTCTA****GTTGGT*****GATACCAGCATCGTCT** **TGATGCCCTTGGCAGCACC**CAAAATAAATAATAAAATTAAA
Theo-ON-4	TTTAAACTAGTTGACGCGGTTCTATCTAGTTACGCGTTAA*A* *CCAACTAGAGCATCCTCTAGT****TGGT*****GATACCAGCATCGTCT** **TGATGCCCTTGGCAGCACCA**CAAATAAATAATAAAATTAAA
Theo-ON-5	TTTAAACTAGTTGACGCGGTTCTATCTAGTTACGCGTTAA*A* *CCAACTAGAGCATCCTCTAG****TTGGT*****GATACCAGCATCGTCT** **TGATGCCCTTGGCAGCACCAA**AAATAAATAATAAAATTAAA
Theo-ON-6	TTTAAACTAGTTGACGCGGTTCTATCTAGTTACGCGTTAA*A* *CCAACTAGAGCATCCTCTA****GTTGGT*****GATACCAGCATCGTCT** **TGATGCCCTTGGCAGCACCAAC**AATAAATAATAAAATTAAA
Neo-ON-1	TTTAAACTAGTTGACGCGGTTCTATCTAGTTACGCGTTAA*A* *CCAACTAGACATTCTA****GTTGGT*****GCTTGTCCTTTAATGGTCC** **ACCAAC**AAATAAATAATAAAATTAAA
Neo-ON-2	TTTAAACTAGTTGACGCGGTTCTATCTAGTTACGCGTTAA*A* *CCAACTAGAGCATCCTCTA****GTTGGT*****GCTTGTCCTTTAATGG** **TCCACCAAC**AAATAAATAATAAAATTAAA
Neo-ON-3	TTTAAACTAGTTGACGCGGTTCTATCTAGTTACGCGTTAA*A* *CCAACTAGAGGAAACTCTA****GTTGGT*****GCTTGTCCTTTAATGG** **TCCACCAAC**AAATAAATAATAAAATTAAA
Neo-ON-4	TTTAAACTAGTTGACGCGGTTCTATCTAGTTACGCGTTAA*A* *CCAACTAGAGGATACTCTA****GTTGGT*****GCTTGTCCTTTAATGG** **TCCACCAAC**AAATAAATAATAAAATTAAA

Sequences begin with the slippery site and end with the insulating sequence. The pseudoknot sequence is underlined, the switching hairpin is italicized, and the aptamer sequence bolded.

Supplementary Table 4. Sequences of logic gates and the apoptosis module.

Construct	Sequence (5' to 3')
NOR gate	**TTTAAACTAGTTGACGCGGTTCTATCTAGTTACGCGTTAAACCAACT AGAGATACCAGCATCGTCTGATGCCCTTGGCAGCTCTAG**AAAAATA AATAATAAAATTAAAATGGGTTCAGGTGAACAATCAAAGAC**TTTAAAC TAGTTGACGCGGTTCTATCTAGTTACGCGTTAAACCAACTAGTTGC TTGTCCTTTAATGGTCCAACTAG**AAAACATAAATAATAAAATTAAA
AND gate	**TTTAAACTAGTTGACGCGGTTCTATCTAGTTACGCGTTAAACCAACT AGAGCATCCTCTAGTTGGTGATACCAGCATCGTCTTGATGCCCTTG GCAGCACCAA**AAATAAATAATAAAATTAAAATGGGTTCAGGTGAACA ATCAAAGAC**TTTAAACTAGTTGACGCGGTTCTATCTAGTTACGCGTT AAACCAACTAGAGGATACTCTAGTTGGTGCTTGTCCTTTAATGGTC CACCAAC**AAATAAATAATAAAATTAAA
Apoptosis module open reading frame	ATGCAGATTTTCGTCAAGACTTTGACCGGTAAAACCATAACATTGGAAG TTGAATCTTCCGATACCATCGACAACGTTAAGTCGAAAATTCAAGACAA GGAAGGTATCCCTCCAGATCAACAAAGATTGATCTTTGCCGGTAAGCAG CTAGAAGACGGTAGAACGCTGTCTGATTACAACATTCAGAAGGAGTCC ACCTTACATCTTGTGCTAAGGCTAAGAGGTGGTAGGCATGGATCCGGAG CTTGGCTGTTGCCCGTCTCACTGGTGAAAAGAAAAACCACCCTGGCGCC CAATACGCAAACCGCCTCTCCCCGCGCGTTGGCCGATTCATTAATGCAG GGCAGCCTGCAGGGCGACTACAAGGACGACGACGACAAGAC**TTTAAA CTGGTTGACGCGGTTCGTATCTTGTTACGCGTTAAACCAACAAGAG AATCTCTTGTTGGTGATACCAGCATCGAAAGATGCCCTTGGCAGCA CCAA**AATAATAATAAAATTAAAAATGTCGACGGTTCTGGTGGTTCTGGT GAAGGTAGAGGTTCTTTGCTTACTTGTGGTGATGTCGAAGAGAATCCTG GT*CCGAAGTTTGGTATGGGTTCTGCTCAAGCTTGTCCATGTCAAGTCCC AAGAGCTGCTTCCACTACCTGGGTCCCATGTCAAATTTGTGGTCCTCAA CCATCTTTATCCTTGGCCGAACAACACTTGGAATCCCCAGTTCCATCCG CTCCTGGTGCCTTGGCCGGTGGTCCTACCCAAGCTGCTCCAGGTGTTAG AGGTGAAGAAGAACAATGGGCTAGAGAAATCGGTGCCCAATTGCGTAG AATGGCCGATGATCTTAACGCTCAATACGAAAGAAGAAGACAAGAAGA ACAACAAAGACATAGACCATCCCCTTGGAGAGTTTTATATAACCTTATT ATGGGTTTGTTGCCATTACCTAGAGGTCACCGTGCTCCAGAAATGGAAC CAAATTAAGCTAGCAGGTTCAGGTGAACAATCAAAGAC**TTTAAACTAG TTGACGCGGTTCTATCTAGTTACGCGTTAAACCAACTAGTTGCTTGT CCTTTAATGGTCCAACTAG**AAAACATAAATAATAAAATTAAAGACGT CGGTTCTGGTGGTTCTGGTGAAGGTAGAGGTTCATTGTTAACATGTGGA GATGTTGAGGAAAATCCTGGT*CCGATGTCCCAATCTAACAGAGAATTA GTCGTTGACTTCTTGTCTTACAAATTGTCTCAAAAGGGTTACTCTTGGTC CCAATTTTCCGATGTCGAAGAAAATAGAACTGAAGCTCCAGAAGGTAC CGAGTCTGAAATGGAAACTCCATCCGCTATCAACGGTAACCCATCCTGG CACTTGGCTGATTCTCCAGCTGTCAACGGTGCCACTGGTCACTCCTCCTC CTTAGATGCTCGTGAGGTTATTCCAATGGCCGCCGTCAAGCAAGCTTTG AGAGAAGCTGGTGATGAATTCGAATTGAGATACAGAAGAGCCTTCTCT

	GACTTGACCTCTCAATTGCATATCACTCCAGGTACTGCTTACCAATCCTT CGAACAAGTTGTTAACGAATTGTTCAGAGACGGTGTTAACTGGGGTAG AATTGTCGCTTTTTTCTCTTTCGGTGGTGCCTTATGTGTTGAATCTGTTG ACAAGGAAATGCAAGTCTTGGTTTCCAGAATTGCTGCTTGGATGGCTAC CTACTTGAATGACCACTTGGAACCATGGATTCAAGAAAACGGTGGTTGG GATACTTTCGTCGAGTTGTACGGTAATAACGCTGCCGCTGAATCTAGAA AGGGTCAAGAAAGATTCAATCGTTGGTTCTTGACTGGTATGACTGTCGC TGGTGTTGTCTTGTTGGGTTCCTTATTCTCCAGAAAGTGA

-1 PRF switches are bolded and underlined. * indicates position of 2A peptide cleavage.

Supplementary Table 5. Sequences of oligonucleotides used in this work.

Name	Sequence (5' to 3')
AVA-95	TTT TTT TTT TTT ATA GCC GCT GCC
AVA105	TCT AAT ACG ACT CAC TAT AGG GAC AAT TAC TAT TTA CAA TTA CAA TGG ACT ACA AGG ACG
AVA106	CGC GTC AAC TAG TTT AAA GTC TTG TCG TCG TCG TCC TTG TAG TCC ATT GTA ATT GTA AAT
AVA107	GAC AAG ACT TTA AAC TAG TTG ACG CG**N NN**C TA**N NNN NNN N**CG CGT TAA AC**N NN**C TAG AAG GCG GTT CTA TGG GAA TGT C
AVA108	ATA GCC GCT GCC GTG GTG ATG GTG ATG ATG GCT TAA TCC AGA CAT TCC CAT AGA ACC GCC
AVA109	TCT AAT ACG ACT CAC TAT AGG GAC AAT TAC TAT TTA CAA TTA CAA TGG ACT ACA AGG ACG ACG ACG ACA AGA CTT TAA ACT AG
AVA111	ATA AAC ACA CAT AAA CAA ACA AAG AAT TCA TGT CTA AAG GTG AAG AAT TAT TCA CTG GTG
AVA112	GCT AGC TTT GTA CAA TTC ATC CAT ACC ATG GGT AAT ACC
AVA117	AGC TAG CGG CAG CGG CGA CTA CAA GGA CGA CGA CGA CAA GAC
AVA118	CAT ATT ATC TTC TTC ACC TTT TGA AAC CAT GAC GTC TCC AGA CAT TCC CAT AGA ACC GCC
AVA119	ATT ACC CAT GGT ATG GAT GAA TTG TAC AAA GCT AGC GGC AGC GGC GAC TAC
AVA155	GTA GTG CGC CAG AAC CAC TGC CGG ATC CCG ATA CAG TCA ACT GTC TTT GAC
AVA156	CGG CAG TGG TTC TGG CGC ACT ACA AGG ACG ACG ACA CAA GAC TTT AAA CTA
AVA158	TAT GGG AAT GTC TGG AAA GCT TGC CAA TTT TAA TCA AAG TGG GAA TAT TGC
AVA161	TAA CAA AGG TCA AAG ACA GTT GAC TGT ATC GGG ATC CGG CAG TGG TTC TGG CGC ACT AC
AVA162	AGC AAT ATT CCC ACT TTG ATT AAA ATT GGC AAG CTT TCC AGA CAT TCC CAT AGA ACC GCC
AVA208	CAC ACA GGA AAC AGC TAT GAC CAT G
AVA245	AGT CTT TGA TTG TTC ACC TGA ACC CAT TTT AAT TTT ATT ATT TAT TTT TGG TGC TGC C
AVA246	ATG GGT TCA GGT GAA CAA TCA AAG ACT TTA AAC TAG TTG ACG CGG TTC
AVA247	ATA GCC ATA TTA TCT TCT TCA CCT TTT GAA ACC
AVA248	ATG GTT TCA AAA GGT GAA GAA GAT AAT ATG GC
AVA276	AGC AAT ATT CCC ACT TTG ATT AAA ATT GGC AAG CTT AGC CAT ATT ATC TTC TTC AC
AVA317	TTC CCT ACA CGA CGC TCT TCC GAT CTN NNN NGA CAA GAC TTT AAA CTA GTT GAC GCG
AVA318	TCC CTA CAC GAC GCT CTT CCG ATC TNN NNN NNN NGA CAA GAC TTT AAA CTA GTT GAC GCG

AVA319	AAT GAT ACG GCG ACC ACC GAG ATC TAC ACT CTT TCC CTA CAC GAC GCT CTT CCG ATC
AVA321	CAA GCA GAA GAC GGC ATA CGA GAT CGT GAT GTG ACT GGA GTT CAG ACG TGT GCT C
AVA322	GAC TGG AGT TCA GAC GTG TGC TCT TCC GAT CAC ATT CCC ATA GAA CCG CCT TCT AG
AVA345	GAC ACA AGA CTT TAA ACT AGT TGA CGC GGT TCT ATC TAG TTA CGC GTT AAA CCA ACT AG
AVA346	CAA GCT TAG CCA TAT TAT CTT CTT CAC CTT TTG AAA CCA TTT TAA TTT TAT TAT TTA TGT
AVA347	CCT TTT GAA ACC ATT TTA ATT TTA TTA TTT ATG TTT NCT AGN NGG ACC ATT AAA GGA CAA GCN NCT AGT TGG TTT AAC GCG TAA CTA GAT
AVA348	CAC CTT TTG AAA CCA TTT TAA TTT TAT TAT TTA TGT NNN CTA GNG GAC CAT TAA AGG ACA TCN CTA GTT GGT TTA ACG CGT AAC TAG AT
AVA357	CTT AGC CAT ATT ATC TTC TTC ACC TTT TGA AAC C
AVA358	GGT TTC AAA AGG TGA AGA AGA TAA TAT GGC
AVA364	CCT TGT AGT CGC CCT GCA GGC TGC CCT GCA TTA ATG AAT C
AVA367	TTA AAA ATG TCG ACG GTT CTG GTC AAT TGC TTA ACT TCG ATT TAC TTA AAT TGG
AVA406	GTC TTT GAT TGT TCA CCT GAA CCC ATT TTA ATT TTA TTA TTT ATT TTT CTA GAG CTG CC
AVA462	CAA AAT GCA GAT TTT CGT CAA GAC TTT GAC CGG
MS049	GAT ATC GAC GTC ATG AAG CTA CTG TCT TCT ATC GAA C
MS050	GTA TGC GCT AGC TTA CTC TTT TTT TGG GTT TGG TGG GG

Supplementary Table 6. Colony counts[a] for viability assay of apoptosis module.

Conditions[b]	Plate 1	Plate 2	Plate 3	Average	Stand. Err.
T-0 N-0	64	70	62	65.3	2.4
T-1 N-0	77	52	68	65.7	7.3
T-2 N-0	90	78	67	78.3	6.6
T-3 N-0	102	114	100	105.3	4.4
T-0 N-1	82	78	72	77.3	2.9
T-1 N-1	63	51	55	56.3	4.3
T-2 N-1	24	34	31	29.7	3.6
T-3 N-1	10	7	7	8.0	1.0
T-0 N-2[c]	62.7	65	61.5	63.1	1.0
T-1 N-2[c]	24.5	26.2	29.5	26.7	1.8
T-2 N-2[c]	2.5	4.1	3.8	3.5	0.5
T-3 N-2[c]	1.3	1.2	0.6	1.0	0.2
T-0 N-3[c]	21.8	18.3	16.8	19.0	1.5
T-1 N-3[c]	3.3	3.5	4.4	3.7	0.3
T-2 N-3[c]	0.3	0.8	0.3	0.5	0.2
T-3 N-3[c]	0.3	0.1	0.1	0.2	0.1
T-0 N-0[d]	75	72	80	75.7	2.3
T-3 N-0[d]	99	101	94	98	2.1
T-0 N-3[d]	90	103	110	101	5.9
T-3 N-3[d]	127	129	139	131.7	3.7

[a]Cultures were diluted to a standard cell density based on OD_{600} and so that approximately 10^3 cells were plated on SC(gluc) L- selection. Colony counts are reported as number of colonies per 10^3 cells plated.

[b]Ligand concentrations. T-0: No theophylline; T-1: 1 mM theophylline; T-2: 5 mM theophylline; T-3: 20 mM theophylline. N-0: No neomycin; N-1: 40 μg/mL neomycin; N-2: 150 μg/mL neomycin; N-3: 650 μg/mL neomycin.

[c]Cultures were diluted 10-fold less in this case so that 10^4 cells were plated.

[d]Cultures in which Bax expression was not induced with galactose, grown with raffinose as the sole carbon source.

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