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SUPPLEMENTARY INFORMATION

Supplementary Figure S1 Plasmid maps of pCS321-based vectors.

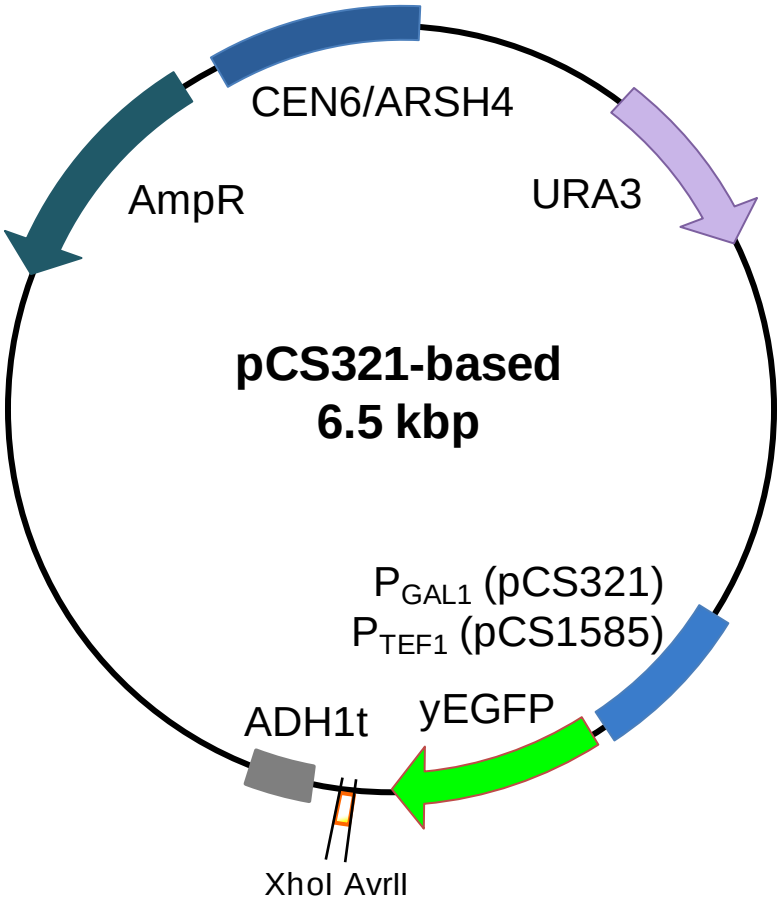
Supplementary Figure S2 FACS analysis and gating procedure for pCS1585 system on FACSAria.

Supplementary Figure S3 FACS analysis and gating procedure for pCS1748 system on FACSAria II.

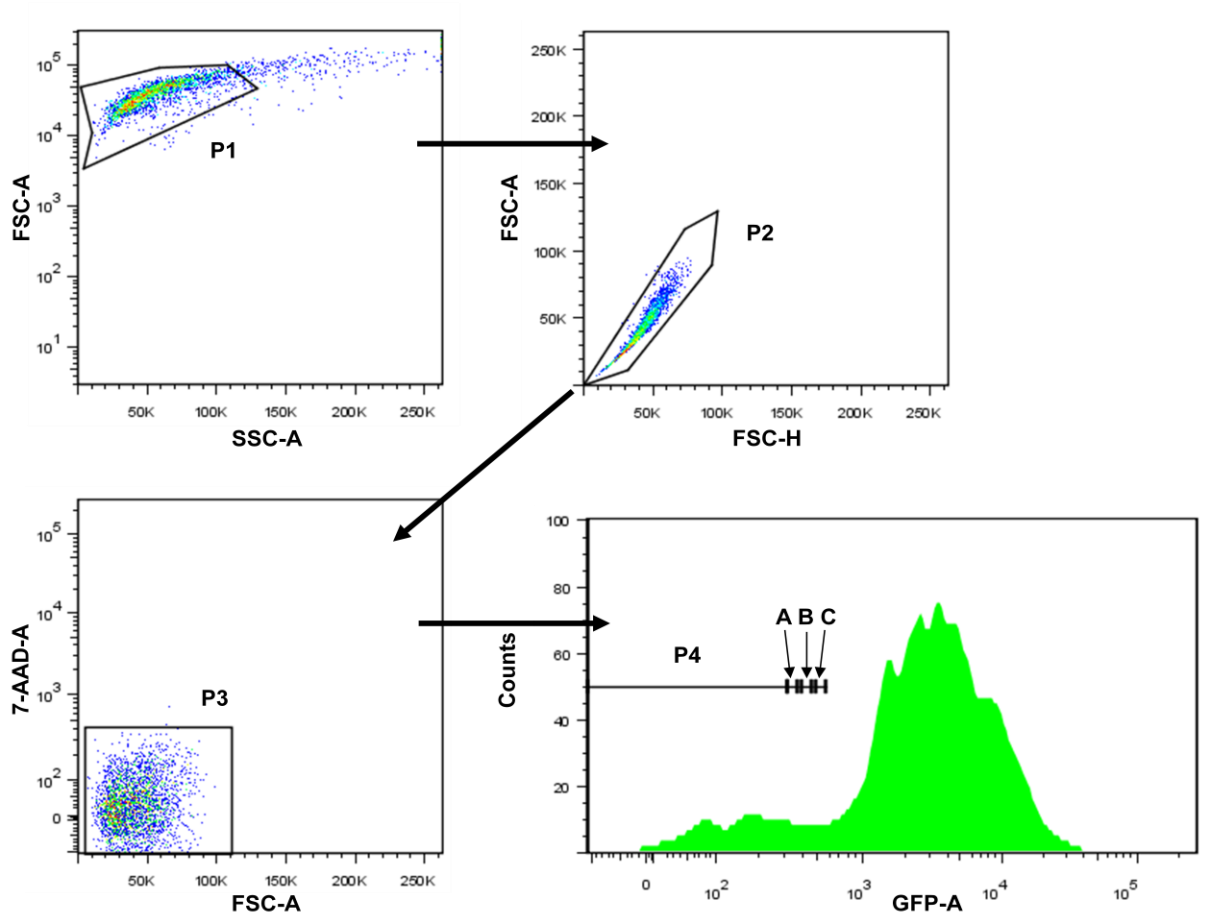
Supplementary Figure S4 Sequences and structures of the selected Rnt1p binding library and control hairpins containing the 'parent' BSB.

Supplementary Figure S5 The integration of different synthetic binding stability box (BSB) modules tune baseline levels of the Rnt1p switch (RS).

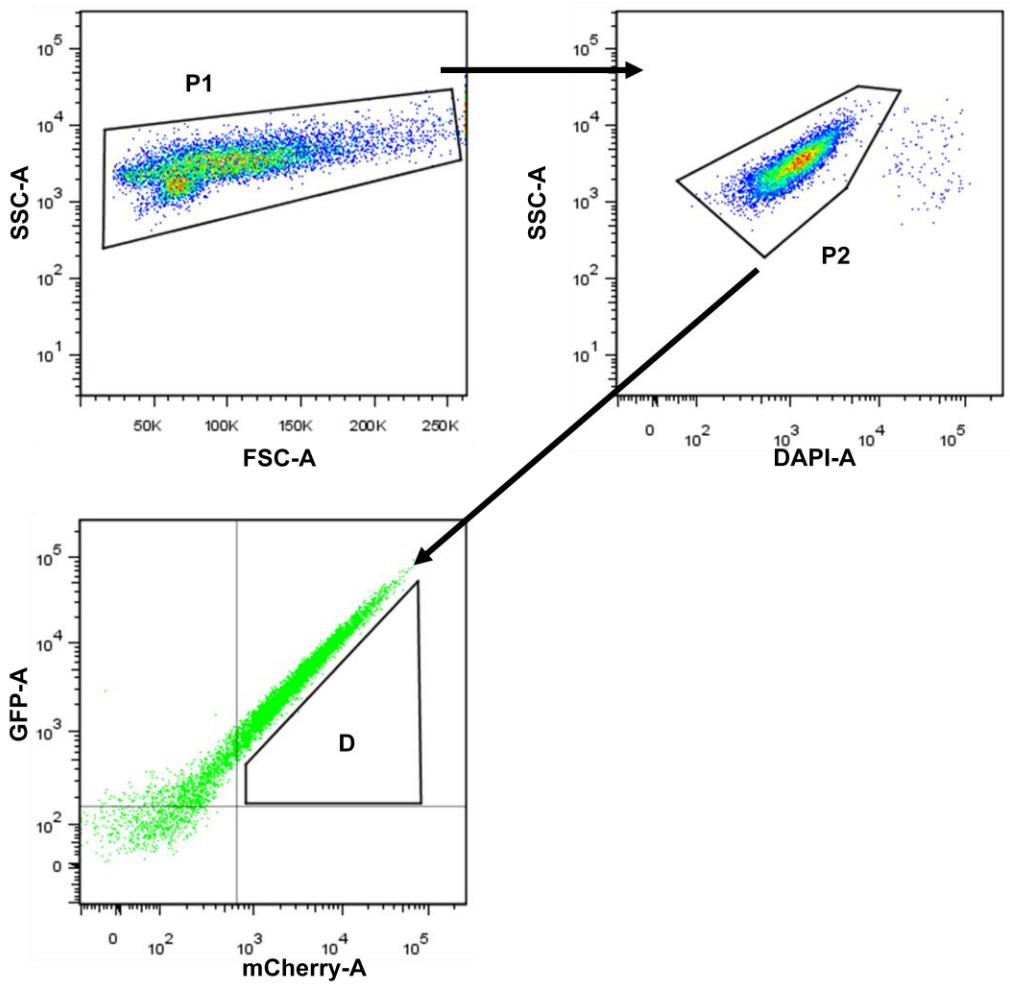
Supplementary Table S1 Sequence and *in vivo* characterization data of all tested Rnt1p hairpins.



Supplementary Figure S1. Plasmid maps of pCS321-based vectors. pCS321 is the characterization plasmid and GFP expression is driven by the GAL1 promoter. pCS1585 is a screening plasmid used with FACS and GFP expression is driven by the TEF1 promoter.



Supplementary Figure S2. FACS analysis and gating procedure for pCS1585 system on FACSAria. As an example, data for the construct baring no Rnt1p hairpin is presented. Dot plots show initial gating of stable cells (P1), followed by gating for cell uniformity (P2), and finally gating for live cells with the 7-AAD stain (P3). GFP-negative cells (P4) were gated initially with a construct lacking a fluorescent gene (empty vector). Cells outside of P4 represent GFP-positive cells. Fractions A, B, and C are represented on this graph, but collections were only performed with the binding library sample. The fractions cover the range of expression seen with the C13-B00 hairpin. With the binding library sample, ~120,000 cells were analyzed with 719, 841, and 943 cells collected in fractions A, B, and C respectively.



Supplementary Figure S3. FACS analysis and gating procedure for pCS1748 system on FACSAria II. As an example, data for the GFP positive construct (no Rnt1p hairpin) is presented. Dot plots show initial gating of stable cells (P1) and subsequent gating of uniform, live cells (P2) with DAPI used for the viability stain. A construct lacking fluorescent genes (empty vector) was used to set the gates for mCherry- and GFP-positive cells. A single gate (D) was set to collect all GFP-positive cells that exhibited lower GFP fluorescence than cells containing the positive construct. With the binding library sample, 1,000,000 cells were analyzed with 18,416 cells collected in fraction D.

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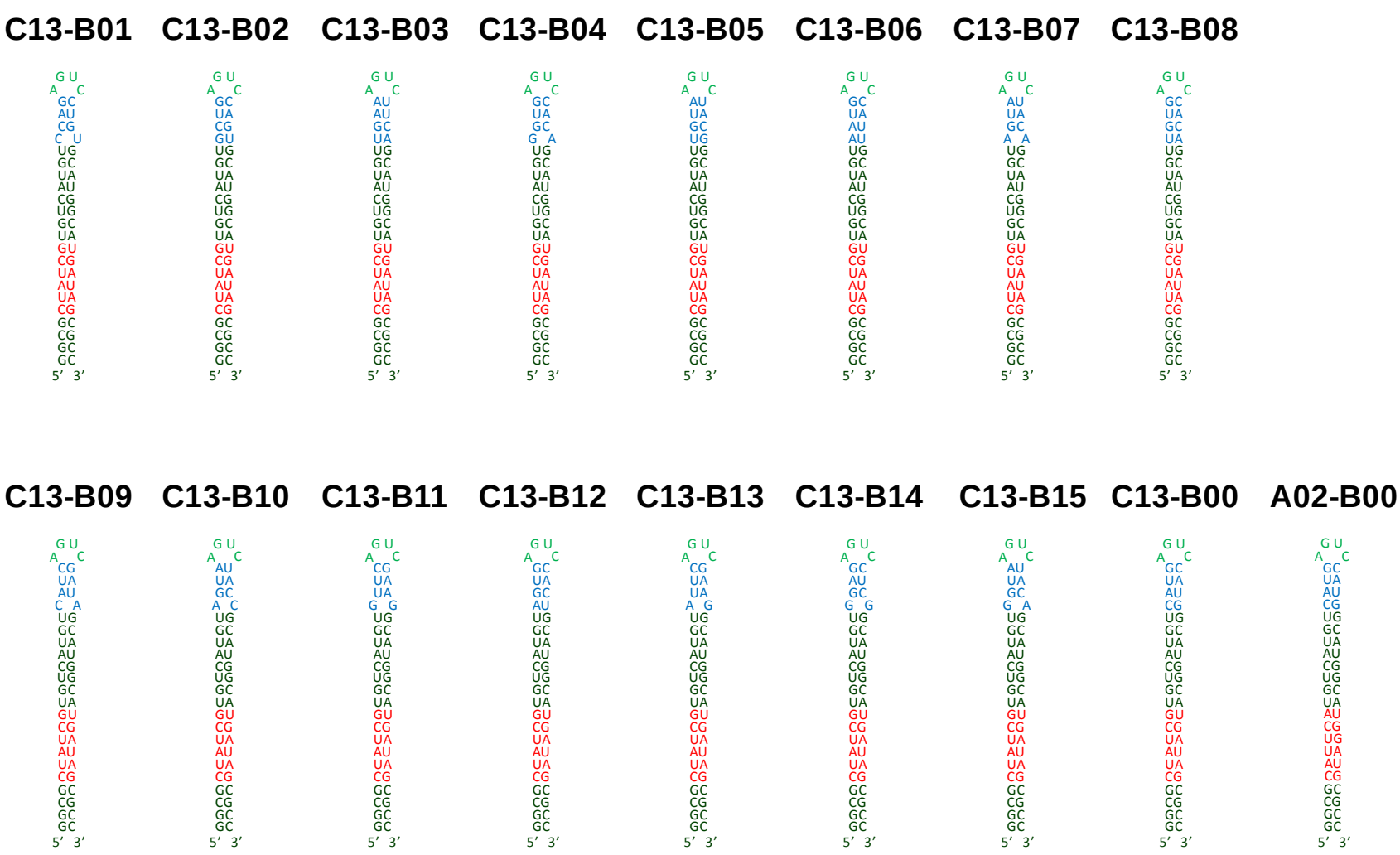
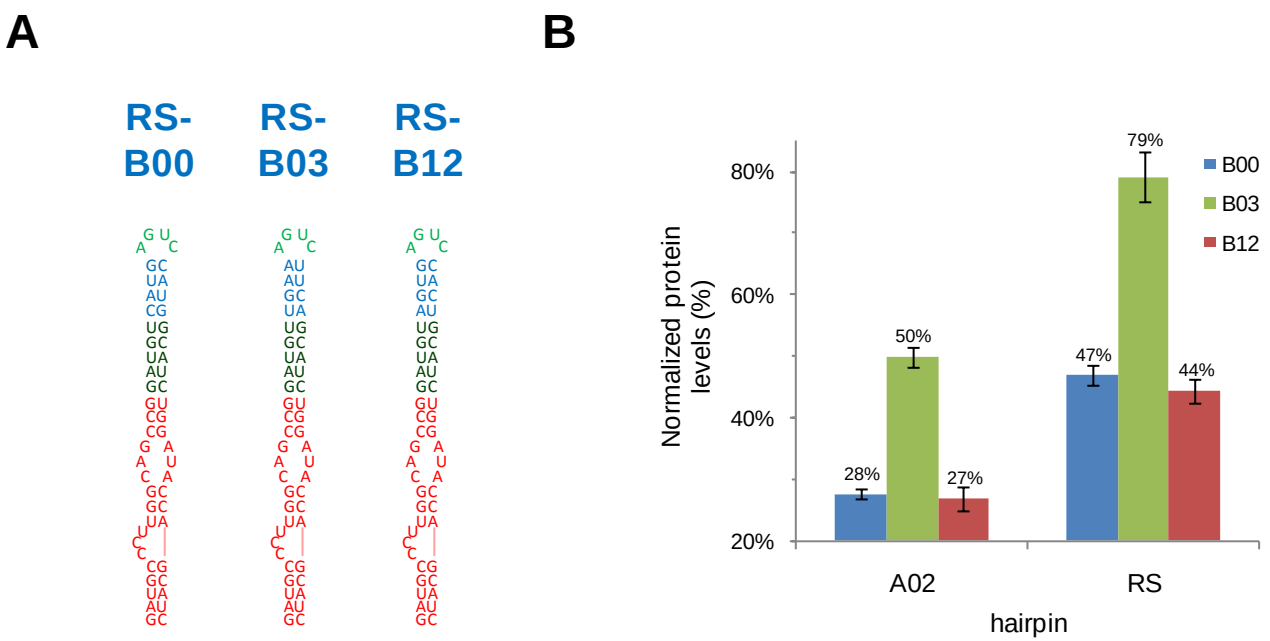


Supplementary Figure S4. Sequences and structures of the selected Rnt1p binding library and control hairpins containing the 'parent' BSB. The binding library was initially sequenced when in the context of the C13 CEB.



Supplementary Figure S5. The integration of different synthetic binding stability box (BSB) modules tune baseline levels of the Rnt1p switch (RS). (A) Switch sequences indicating the different BSB modules utilized (B00, B03, and B12). Color scheme is as follows: cleavage efficiency box (CEB) and aptamer region, red; BSB, blue; initial binding and positioning box (IBPB), green. (B) The incorporation of the B03 and B12 BSBs modulate baseline levels from the parent switch (RS-B00). For comparison, the gene regulatory of the same BSB modules are displayed in the context of the A02 stem. Normalized protein levels were determined as indicated in Figure 3A.

Supplementary Table S1. Sequence and *in vivo* characterization data of all tested Rnt1p hairpins. The nucleotides of the BSB are indicated in blue. The CEB sequences in the 'parent' hairpins (xx-B00) are indicated in red. All normalized protein and transcript levels were determined as described in Figure 3A and Figure 3B, respectively.

Substrate	Sequence	Normalized protein levels (%)	Normalized transcript levels (%)
A02-B00	GGCGCAUUCAUGUCAUGUCAUGAGUCCAUGGCAUGGCAUGGAUGCGCC	28% ± 1%	43% ± 8%
A02-B01	GGCGCAUUCAUGUCAUGUCCAGAGUCCUGUGCAUGGCAUGGAUGCGCC	75% ± 3%	78% ± 11%
A02-B02	GGCGCAUUCAUGUCAUGUGCUGAGUCCAGUGCAUGGCAUGGAUGCGCC	62% ± 2%	64% ± 6%
A02-B03	GGCGCAUUCAUGUCAUGUUGAAAGUCUUCAGCAUGGCAUGGAUGCGCC	50% ± 2%	53% ± 5%
A02-B04	GGCGCAUUCAUGUCAUGUGGUGAGUCCACAGCAUGGCAUGGAUGCGCC	32% ± 1%	57% ± 7%
A02-B05	GGCGCAUUCAUGUCAUGUUGUAAGUCUACGGCAUGGCAUGGAUGCGCC	25% ± 0%	36% ± 5%
A02-B06	GGCGCAUUCAUGUCAUGUAAUGAGUCCAUUGCAUGGCAUGGAUGCGCC	27% ± 2%	60% ± 6%
A02-B07	GGCGCAUUCAUGUCAUGUAGUAAGUCUACAGCAUGGCAUGGAUGCGCC	37% ± 3%	51% ± 3%
A02-B08	GGCGCAUUCAUGUCAUGUUGUGAGUCCACAGCAUGGCAUGGAUGCGCC	30% ± 2%	53% ± 4%
A02-B09	GGCGCAUUCAUGUCAUGUCAUCAGUCGAUAGCAUGGCAUGGAUGCGCC	36% ± 3%	60% ± 9%
A02-B10	GGCGCAUUCAUGUCAUGUAGUAAGUCUACCGCAUGGCAUGGAUGCGCC	42% ± 3%	55% ± 4%
A02-B11	GGCGCAUUCAUGUCAUGUGUUCAGUCGAAGGCAUGGCAUGGAUGCGCC	32% ± 2%	51% ± 4%
A02-B12	GGCGCAUUCAUGUCAUGUAGUGAGUCCACUGCAUGGCAUGGAUGCGCC	27% ± 2%	47% ± 5%
A02-B13	GGCGCAUUCAUGUCAUGUAUUCAGUCGAAGGCAUGGCAUGGAUGCGCC	39% ± 4%	53% ± 5%
A02-B14	GGCGCAUUCAUGUCAUGUGGAGAGUCCUCGGCAUGGCAUGGAUGCGCC	48% ± 4%	74% ± 8%
A02-B15	GGCGCAUUCAUGUCAUGUGGUAAGUCUACAGCAUGGCAUGGAUGCGCC	48% ± 4%	58% ± 7%
C13-B00	GGCGCUAUCGUGUCAUGUCAUGAGUCCAUGGCAUGGCAUGAUAGCGCC	8% ± 0%	12% ± 1%
C13-B01	GGCGCUAUCGUGUCAUGUCCAGAGUCCUGUGCAUGGCAUGAUAGCGCC	48% ± 2%	
C13-B02	GGCGCUAUCGUGUCAUGUGCUGAGUCCAGUGCAUGGCAUGAUAGCGCC	37% ± 2%	
C13-B03	GGCGCUAUCGUGUCAUGUUGAAAGUCUUCAGCAUGGCAUGAUAGCGCC	20% ± 1%	
C13-B04	GGCGCUAUCGUGUCAUGUGGUGAGUCCACAGCAUGGCAUGAUAGCGCC	9% ± 0%	
C13-B05	GGCGCUAUCGUGUCAUGUUGUAAGUCUACGGCAUGGCAUGAUAGCGCC	8% ± 0%	
C13-B06	GGCGCUAUCGUGUCAUGUAAUGAGUCCAUUGCAUGGCAUGAUAGCGCC	8% ± 0%	
C13-B07	GGCGCUAUCGUGUCAUGUAGUAAGUCUACAGCAUGGCAUGAUAGCGCC	11% ± 1%	
C13-B08	GGCGCUAUCGUGUCAUGUUGUGAGUCCACAGCAUGGCAUGAUAGCGCC	10% ± 0%	
C13-B09	GGCGCUAUCGUGUCAUGUCAUCAGUCGAUAGCAUGGCAUGAUAGCGCC	15% ± 1%	
C13-B10	GGCGCUAUCGUGUCAUGUAGUAAGUCUACCGCAUGGCAUGAUAGCGCC	14% ± 1%	

Supplementary Table S1 cont'd.

Substrate	Sequence	Normalized protein levels (%)
C13-B11	GGCGCUAUCGUGUCAUGUGUUCAGUCGAAGGCAUGGCAUGAUAGCGCC	11% ± 1%
C13-B12	GGCGCUAUCGUGUCAUGUAGUGAGUCCACUGCAUGGCAUGAUAGCGCC	10% ± 0%
C13-B13	GGCGCUAUCGUGUCAUGUAUUCAGUCGAAGGCAUGGCAUGAUAGCGCC	14% ± 0%
C13-B14	GGCGCUAUCGUGUCAUGUGGAGAGUCCUCGGCAUGGCAUGAUAGCGCC	17% ± 0%
C13-B15	GGCGCUAUCGUGUCAUGUGGUAAGUCUACAGCAUGGCAUGAUAGCGCC	14% ± 1%