



**Supplementary Figure 1. Vesicle permeability to IPTG.** (A) A shrink-swell<sup>2-4</sup> assay was used to assess whether IPTG was capable of crossing vesicle membranes. POPC:cholesterol vesicles with entrapped calcein were prepared in 10 mM MgCl<sub>2</sub>, 100 mM KCl, 50 mM HEPES, pH 7.6 as described in the methods and subsequently purified by gel filtration chromatography with sepharose-4b. An aliquot of the vesicle sample was diluted two-fold with 1.0 M IPTG (final concentration = 0.5 M) at 37 °C. The reaction was monitored by fluorescence with excitation and emission wavelengths of 495 nm and 515 nm, respectively. The rapid decrease in fluorescence was due to both dilution with the solute solution and calcein self-quenching. If IPTG were capable of crossing the membrane, a recovery of fluorescence would have been observed. (B) The permeability of POPC vesicles to ribose was observable with the shrink-swell assay. The recovery in fluorescence after two-fold dilution with 1.0 M ribose (final concentration = 0.5 M) was due to equilibration of ribose and water across the membrane.

**Supplementary Table 1. DNA sequences used in this study**

NAME	NOTE	SEQUENCE
AS014A	T7 promoter, K30S E31T $\alpha$ HL-sfGFP	<p>ATTTAATACGACTCACTATAG<b>ATG</b>GATTCTGATATCAATATCAAAAACGGCACCACCGATATCGGCTC  CAATACCACCGTTAAAACCGGTGATCTGGTGACCTATGATTCTACCAACGGTATGCATAAAAAAGTGT  TTTACTCGTTTATTGACGATAAAAACCATAACAAAAAACTGCTGGTCATCCGCACCAAAGGCACCATTG  CGGGTCAATACCGTGTGACTCCGAAGAAGGTGCGAACAAAAGCGGTCTGGCTTGCCGTCTGCCTT  TAAAGTGACAGTGCAACTGCCGGATAATGAAGTGCGCAGATTTTCAAGTTATTATCCGCGTAATAGCA  TCGATACCAAAGAATATATGAGTACCCTGACCTATGGTTTTAATGGCAATGTTACCGGTGATGATACG  GGTAAAATTGGCGGTCTGATTGGCGCCAATGTGTCCATTGGTCATACGCTGAAATACGTGCAACCGG  ATTTCAAACCATTTCTGAAAAGTCCGACCGATAAAAAAGTGGGTTGAAAAGTTATCTTCAACAACATG  GTGAATCAGAACTGGGGTCCGTACGATCGCGATTCTGGAATCCGGTTTATGGCAATCAGCTGTTTAT  GAAAACCCGCAACGGTAGTATGAAAGCGGCGGATAATTTTCTGGACCCGAACAAAGCCTCAAGCCTG  CTGTCCAGCGGTTTTAGCCCGATTTTCCACGGTTATTACCATGGATCGAAAGCCAGCAAACAGCA  GACCAACATTGATGTGATCTACGAACGTGTGCGTGATGATTATCAACTGCATTGGACCTCAACCAATT  GGAAAGGCACCAATACCAAAGATAAATGGACGGATCGCAGTTCAGAACGCTACAAAATTGATTGGGA  AAAAGAAGAAATGACCAACGGATCCGGCAGCGGTTCT<b>ATG</b>CGTAAAGGCGAAGAGCTGTTCACTGG  TGTCGTCCCTATTCTGGTGGAACTGGATGGTGTGTCACCGTGCATAAGTTTTCCGTGCGTGGCGAGG  GTGAAGGTGACGCAACTAATGGTAACTGACGCTGAAGTTCATCTGTACTACTGGTAACTGCCGGTA  CCTTGGCCGACTCTGGTAACGACGCTGACTTATGGTGTTCAAGTCTTGTCTGTTATCCGGACCATATG  AAGCAGCATGACTTCTCAAGTCCGCCATGCCGGAAGGCTATGTGCAGGAACGCACGATTTCTTTAA  GGATGACGGCACGTACAAAACGCGTGCGGAAGTGAATTTGAAGGCGATAACCTGGTAAACCGCATT  GAGCTGAAAGGCATTGACTTTAAAGAAGACGGCAATATCCTGGGCCATAAGCTGGAATACAATTTTA  ACAGCCACAATGTTTACATCACCGCCGATAAACAAAAAATGGCATTAAAGCGAATTTTAAATTCGC  CACAACGTGGAGGATGGCAGCGTGCAGCTGGCTGATCACTACCAGCAAACACTCCAATCGGTGATG  GTCCTGTTCTGCTGCCAGACAATCACTATCTGAGCACGCAAAGCGTTCTGTCTAAAGATCCGAACGAG  AAACGCGATCATATGGTTCTGCTGGAGTTCGTAACCGCAGCGGGCATCACGCATGGTATGGATGAAC  TGTACAAA<b>TAA</b>CTCGAGCACCACCACCACCACCCTGAGATCCGGCTGTAACAAAGCCCGAAAGGA  AGCTGAGTTGGCTGCTGCCACCGCTGAGCAATAACTAGCATAACCCCTTGGGGCCTCTAAACGGGTCT  TGAGGGGTTTTTTG</p>
DT101A	T7 promoter, $\alpha$ HL- His tag	<p>TAATACGACTCACTATAGGGGAATTGTGAGCGGATAACAATTCCTCTAGAAATAATTTGTTAACT  TTAAGAAGGAGATATACAT<b>ATG</b>GATTCTGATATCAATATCAAAAACGGCACCACCGATATCGGCTCC  AATACCACCGTTAAAACCGGTGATCTGGTGACCTATGATAAAGAAAACGGTATGCATAAAAAAGTGT  TTTACTCGTTTATTGACGATAAAAACCATAACAAAAAACTGCTGGTCATCCGCACCAAAGGCACCATTG  GGGTCAATACCGTGTGACTCCGAAGAAGGTGCGAACAAAAGCGGTCTGGCTTGCCGTCTGCCTTT  AAAGTGACAGTGCAACTGCCGGATAATGAAGTGCGCAGATTTTCAAGTTATTATCCGCGTAATAGCAT  CGATACCAAAGAATATATGAGTACCCTGACCTATGGTTTTAATGGCAATGTTACCGGTGATGATACGG  GTAAAATTGGCGGTCTGATTGGCGCCAATGTGTCCATTGGTCATACGCTGAAATACGTGCAACCGGAT  TTCAAACCATTTCTGAAAAGTCCGACCGATAAAAAAGTGGGTTGAAAAGTTATCTTCAACAACATGGT  GAATCAGAACTGGGGTCCGTACGATCGCGATTCTGGAATCCGGTTTATGGCAATCAGCTGTTTATGA  AAACCCGCAACGGTAGTATGAAAGCGGCGGATAATTTTCTGGACCCGAACAAAGCCTCAAGCCTGCT  GTCCAGCGGTTTTAGCCCGGATTTTCCACGGTTATTACCATGGATCGCAAAGCCAGCAAACAGCAGA  CCAACATTGATGTGATCTACGAACGTGTGCGTGATGATTATCAACTGCATTGGACCTCAACCAATTGG  AAAGGCACCAATACCAAAGATAAATGGACGGATCGCAGTTCAGAACGCTACAAAATTGATTGGGAAA  AAGAAGAAATGACCAACCTCGAGCACCACCACCACCACC<b>TG</b>AGATCCGGCTGCTAACAAAGCCCG  AAAGGAAGCTGAGTTGGCTGCTGCCACCGCTGAGCAATAACTAGCATAACCCCTTGGGGCCTCTAAAC  GGGTCTTGAGGGGTTTTTTG</p>
JF001A	T7 promoter, <u>theophylline</u> <u>riboswitch</u> $\alpha$ HL	<p>AATTAATACGACTCACTATAGGGTGATACCAGCATCGTCTTGATGCCCTTGGCAGCACCTGCTAAGG  <u>TAACAACAAG<b>ATG</b></u>GATTCTGATATCAATATCAAAAACGGCACCACCGATATCGGCTCCAATACCACCG  TTAAAACCGGTGATCTGGTGACCTATGATAAAGAAAACGGTATGCATAAA AAAGTGTTTTACTCGTTT  ATTGACGATAAAAACCATAACAAAAAACTGCTGGTCATCCGCACCAAAGGCACCATTGCCGGTCAATA  CCGTGTGTACTCCGAAGAAGGTGCGAACAAAAGCGGTCTGGCTTGCCGTCTGCCTTTAAAGTGACG  CTGCAACTGCCGGATAATGAAGTGCGCAGATTTTCAAGTTATTATCCGCGTAATAGCATCGATACCAA</p>

		<p>AGAATATATGAGTACCCTGACCTATGGTTTTAATGGCAATGTTACCGGTGATGATACGGGTAAAATTG  GCGGTCTGATTGGCGCAATGTGTCCATTGGTCATACGCTGAAATACGTGCAACCGGATTTCAAAACC  ATTCTGAAAAGTCCGACCGATAAAAAAGTGGGTTGAAAAGTTATCTTCAACAACATGGTGAATCAGA  ACTGGGGTCCGTACGATCGCGATTCTGGAATCCGGTTTATGGCAATCAGCTGTTTATGAAAACCCGC  AACGGTAGTATGAAAGCGGCGGATAATTTCTGGACCCGAACAAAGCCTCAAGCCTGCTGTCCAGCG  GTTTTAGCCCGATTTTGGCCAGGTTATTACCATGGATCGAAAGCCAGCAAACAGCAGACCAACATT  GATGTGATCTACGAACGTGTGCGTGATGATTATCAACTGCATTGGACCTCAACCAATTGGAAAGGCAC  CAATACCAAAGATAAATGGACGGATCGCAGTTCAGAACGCTACAAAATTGATTGGGAAAAAGAAGAA  ATGACCAAC<b>TAA</b>CTCGAGCACCACCACCACCACCCTGAGATCCGGCTGCTAACAAAGCCCGAAAAGG  AAGCTGAGTTGGCTGCTGCCACCGCTGAGCAATAACTAGCATAACCCCTTGGGGCTCTAAACGGGTC  TTGAGGGGTTTTTTG</p>
RL067A	T7 promoter, αHL	<p>TAATACGACTCACTATAGGGGAATTGTGAGCGGATAACAATCCCCTCTAGAAATAATTTGTTTAACT  TTAAGAAGGAGATATACAT<b>ATG</b>GATTCTGATATCAATATCAAAACCGGCACCACCGATATCGGCTCC  AATACCACCGTTAAAACCGGTGATCTGGTGACCTATGATAAAGAAAACGGTATGCATAAAAAAGTGT  TTACTCGTTTATTGACGATAAAAAACCATAACAAAAAAGTCTGGTTCATCCGACCAAAGGCACCATTGC  GGGTCAATACCGTGTACTCCGAAGAAGGTGCGAACAAAAGCGGTCTGGCTTGGCCGTCTGCCTTT  AAAGTGACGCTGCAACTGCCGGATAATGAAGTGGCGCAGATTTAGATTATTATCCGCGTAATAGCAT  CGATACCAAAGAATATATGAGTACCCTGACCTATGGTTTTAATGGCAATGTTACCGGTGATGATACGG  GTAAAATTGGCGGTCTGATTGGCGCAATGTGTCCATTGGTCATACGCTGAAATACGTGCAACCGGAT  TTCAAAACCACTTCTGAAAAGTCCGACCGATAAAAAAGTGGGTTGAAAAGTTATCTTCAACAACATGGT  GAATCAGAACTGGGGTCCGTACGATCGCGATTCTGGAATCCGGTTTATGGCAATCAGCTGTTTATGA  AAACCCGCAACGGTAGTATGAAAGCGGCGGATAATTTTCTGGACCCGAACAAAGCCTCAAGCCTGCT  GTCCAGCGTTTTAGCCCGATTTTGGCCAGGTTATTACCATGGATCGAAAGCCAGCAAACAGCAGAA  CCAACATTGATGTGATCTACGAACGTGTGCGTGATGATTATCAACTGCATTGGACCTCAACCAATTGG  AAAGGCACCAATACCAAAGATAAATGGACGGATCGCAGTTCAGAACGCTACAAAATTGATTGGGAAA  AAGAAGAAATGACCAAC<b>TAA</b>CTCGAGCACCACCACCACCACCCTGAGATCCGGCTGCTAACAAAGC  CCGAAAGGAAGCTGAGTTGGCTGCTGCCACCGCTGAGCAATAACTAGCATAACCCCTTGGGGCTCT  AAACGGGTCTTGGGGTTTTTTG</p>
RL069A	T7 promoter, <u>theophylline</u> <u>riboswitch</u> , K30S E31T αHL	<p>AATTAATACGACTCACTATAGGGTGATACCAGCATCGTCTTGATGCCCTTGGCAGCACCCCTGCTAAGG  <u>TAACAACAAG</u><b>ATG</b>GATTCTGATATCAATATCAAAACCGGCACCACCGATATCGGCTCCAATACCACC  GTTAAAACCGGTGATCTGGTGACCTATGATTCTACCAACGGTATGCATAAAAAAGTGTTTTACTCGTTT  ATTGACGATAAAAAACCATAACAAAAAAGTCTGGTTCATCCGACCAAAGGCACCATTGCGGGTCAATA  CCGTGTGTACTCCGAAGAAGGTGCGAACAAAAGCGGTCTGGCTTGGCCGTCTGCCTTTAAAGTGACG  CTGCAACTGCCGGATAATGAAGTGGCGCAGATTTAGATTATTATCCGCGTAATAGCATCGATACCAA  AGAATATATGAGTACCCTGACCTATGGTTTTAATGGCAATGTTACCGGTGATGATACGGGTAAAATTG  GCGGTCTGATTGGCGCAATGTGTCCATTGGTCATACGCTGAAATACGTGCAACCGGATTTCAAAACC  ATTCTGAAAAGTCCGACCGATAAAAAAGTGGGTTGAAAAGTTATCTTCAACAACATGGTGAATCAGA  ACTGGGGTCCGTACGATCGCGATTCTGGAATCCGGTTTATGGCAATCAGCTGTTTATGAAAACCCGC  AACGGTAGTATGAAAGCGGCGGATAATTTCTGGACCCGAACAAAGCCTCAAGCCTGCTGTCCAGCG  GTTTTAGCCCGATTTTGGCCAGGTTATTACCATGGATCGAAAGCCAGCAAACAGCAGACCAACATT  GATGTGATCTACGAACGTGTGCGTGATGATTATCAACTGCATTGGACCTCAACCAATTGGAAAGGCAC  CAATACCAAAGATAAATGGACGGATCGCAGTTCAGAACGCTACAAAATTGATTGGGAAAAAGAAGAA  ATGACCAACGGATCCGCGAGCGGTTCT<b>ATG</b>CGTAAAGGCGAAGAGCTGTTCACTGGTGTCTGCCCTA  TTCTGGTGAACCTGGATGGTGTGATGTCAACGGTCATAAGTTTTCCGTGCGTGGCGAGGGTGAAGGTGA  CGCAACTAATGGTAAACTGACGCTGAAGTTCATCTGTACTACTGGTAAACTGCCGTACCTTGGCCGA  CTCTGGTAACGACGCTGACTTATGGTGTTCAGTGCTTTGCTGTTATCCGGACCATATGAAGCAGCAT  GACTTCTTCAAGTCCGCCATGCCGGAAGGCTATGTGCAGGAACGCACGATTTCTTTAAGGATGACGG  CACGTACAAAACGCGTGCGGAAGTGAATTTGAAGGCGATACCTGGTAAACCGCATTGAGCTGAAA  GGCATTGACTTTAAGAAGACGCAATATCCTGGGCCATAAGCTGGAATACAATTTAACAGCCACAA  TGTTTACATCACCGCCGATAAACAAAAAATGGCATTAAAGCGAATTTTAAATTCGCCACAACGTGG  AGGATGGCAGCGTGCAGCTGGCTGATCACTACCAGCAAACACTCCAATCGGTGATGGTCTGTTCTG  CTGCCAGACAATCACTATCTGAGCACGCAAAGCGTTCTGTCTAAAGATCCGAACGAGAAACGCGATCA  TATGGTCTGCTGGAGTTCGTAACCGCAGCGGGCATCACGCATGGTATGGATGAACTGTACAA<b>TAA</b></p>

		CTCGAGCACCACCACCACCACCCTGAGATCCGGCTGCTAACAAAGCCCGAAAGGAAGCTGAGTTGG CTGCTGCCACCGCTGAGCAATAACTAGCATAACCCCTTGGGGCCTCTAACCGGTCTTGAGGGTTTT TTG
SP002A	T7 promoter, αHL-sfGFP	ATTTAATACGACTCACTATAG <b>ATG</b> GATTCTGATATCAATATCAAACCGGCACCACCGATATCGGCTC CAATACCACCGTTAAAACCGGTGATCTGGTGACCTATGATAAAGAAAACGGTATGCATAAAAAAGTGT TTTACTCGTTTTATTGACGATAAAAAACCATAACAAAAAACTGCTGGTCATCCGCACCAAAGGCACCATTG CGGGTCAATACCGTGTGACTCCGAAGAAGGTGCGAACAAAAGCGGTCTGGCTTGGCCGTCTGCCTT TAAAGTGACGCTGCAACTGCCGATAATGAAGTGGCGCAGATTTAGATTATTATCCGCGTAATAGCA TCGATACCAAAGAATATATGAGTACCCTGACCTATGGTTTTAATGGCAATGTTACCGGTGATGATACG GGTAAAATTGGCGGTCTGATTGGCGCAATGTGTCCATTGGTCATACGCTGAAATACGTGCAACCGG ATTTCAAACCACTTCTGGAAAGTCCGACCGATAAAAAAGTGGGTTGGAAAGTTATCTTCAACAACATG GTGAATCAGAAGTGGGGTCCGTACGATCGCGATTCTGGAATCCGGTTTATGGCAATCAGCTGTTTAT GAAAACCGCAACGGTAGTATGAAAGCGGCGGATAATTTTCTGGACCCGAACAAAGCCTCAAGCCTG CTGTCCAGCGTTTTAGCCCGGATTTTGGCACGGTATTACCATGGATCGAAAGCCAGCAAACAGCA GACCAACATTGATGTGATCTACGAACGTGTGCGTGATGATTATCAACTGCATTGGACCTCAACCAATT GGAAAGGCACCAATACCAAAGATAAATGGACGGATCGCAGTTCAGAACGCTACAAAATTGATTGGGA AAAAGAAGAAATGACCAACGGATCCGGCAGCGTTCT <b>ATG</b> CGTAAAGGCGAAGAGCTGTTCACTGG TGTCGTCCCTATTCTGGTGGAACTGGATGGTGTGCAACGGTCATAAGTTTTCCGTGCGTGGCGAGG GTGAAGGTGACGCAACTAATGGTAACTGACGCTGAAGTTTCTGTACTACTGGTAACTGCCGGTA CCTTGGCCGACTCTGGTAACGACGCTGACTTATGGTGTTCAAGTCTTGTCTGTTATCCGGACCATATG AAGCAGCATGACTTCTCAAGTCCGCCATGCCGGAAGGCTATGTGCAGGAACGCACGATTTCTTTAA GGATGACGGCACGTACAAAACGCGTGCAGGAAAGTAAATTTGAAGGCGATACCTGGTAAACCGCATT GAGCTGAAAGGCATTGACTTTAAAGAAGACGGCAATATCCTGGGCCATAAGCTGGAATACAATTTTA ACAGCCACAATGTTTACATCACCGCCGATAAACAAAAAAATGGCATTAAAGCGAATTTTAAATTCGC CACAACGTGGAGGATGGCAGCGTGCAGCTGGCTGATCACTACCAGCAAAACACTCCAATCGGTGATG GTCCTGTTCTGCTGCCAGACAATCACTATCTGAGCACGCAAAGCGTTCTGTCTAAAGATCCGAACGAG AAACGCGATCATATGGTCTGCTGGAGTTCTGTAACCGCAGCGGCATCACGATGGTATGGATGAAC TGTAACA <b>TA</b> ACTCGAGCACCACCACCACCACCCTGAGATCCGGCTGCTAACAAAGCCCGAAAGGA AGCTGAGTTGGCTGCTGCCACCGCTGAGCAATAACTAGCATAACCCCTTGGGGCCTCTAACCGGTCT TGAGGGGTTTTTTG
SP011A	T7 promoter, <u>theophylline</u> <u>riboswitch</u> , αHL-sfGFP	AATTAATACGACTCACTATAGGGTGATACCAGCATCGTCTTGATGCCCTTGGCAGCACCTGCTAAGG <u>TAACAACAAG</u> <b>ATG</b> GATTCTGATATCAATATCAAACCGGCACCACCGATATCGGCTCCAATACCACC GTAAAACCGGTGATCTGGTGACCTATGATAAAGAAAACGGTATGCATAAAAAAGTGTTTTACTCGTT TATTGACGATAAAAAACCATAACAAAAAACTGCTGGTCATCCGCACCAAAGGCACCATTGCGGGTCAAT ACCGTGTGACTCCGAAGAAGGTGCGAACAAAAGCGGTCTGGCTTGGCCGTCTGCCTTTAAAGTGCA GCTGCAACTGCCGATAATGAAGTGGCGCAGATTTAGATTATTATCCGCGTAATAGCATCGATACCA AAGAATATATGAGTACCCTGACCTATGGTTTTAATGGCAATGTTACCGGTGATGATACGGGTAATAAT GGCGGTCTGATTGGCGCAATGTGTCCATTGGTCATACGCTGAAATACGTGCAACCGGATTTCAAAC CATTCTGGAAAGTCCGACCGATAAAAAAGTGGGTTGGAAAGTTATCTTCAACAACATGGTGAATCAG AACTGGGGTCCGTACGATCGCGATTCTGGAATCCGGTTTATGGCAATCAGCTGTTTATGAAAACCGG CAACGGTAGTATGAAAGCGGCGGATAATTTTCTGGACCCGAACAAAGCCTCAAGCCTGCTGTCCAGC GGTTTTAGCCCGGATTTTGGCACGGTATTACCATGGATCGCAAAGCCAGCAAACAGCAGACCAACAT TGATGTGATCTACGAACGTGTGCGTGATGATTATCAACTGCATTGGACCTCAACCAATTGGAAGGCA CCAATACCAAAGATAAATGGACGGATCGCAGTTCAGAACGCTACAAAATTGATTGGGAAAAAGAAGA AATGACCAACGGATCCGGCAGCGTTCT <b>ATG</b> CGTAAAGGCGAAGAGCTGTTCACTGGTGTGTCCTCT ATTCTGGTGGAACTGGATGGTGTGCAACGGTCATAAGTTTTCCGTGCGTGGCGAGGGTGAAGGTG ACGCAACTAATGGTAACTGACGCTGAAGTTTCTGTACTACTGGTAACTGCCGGTACCTTGGCCG ACTCTGGTAACGACGCTGACTTATGGTGTTCAAGTCTTGTCTGTTATCCGGACCATATGAAGCAGCAT GACTTCTCAAGTCCGCCATGCCGGAAGGCTATGTGCAGGAACGCACGATTTCTTTAAGGATGACGG CACGTACAAAACGCGTGCAGGAAAGTAAATTTGAAGGCGATACCTGGTAAACCGCATTGAGCTGAAA GGCATTGACTTTAAAGAAGACGGCAATATCCTGGGCCATAAGCTGGAATACAATTTTAAACGCCACAA TGTTTACATCACCGCCGATAAACAAAAAAATGGCATTAAAGCGAATTTTAAATTCGCCACAACGTGG AGGATGGCAGCGTGCAGCTGGCTGATCACTACCAGCAAAACACTCCAATCGGTGATGGTCTGTTCTG

		CTGCCAGACAATCACTATCTGAGCACGCAAAGCGTTCTGTCTAAAGATCCGAACGAGAAACGCGATCA TATGGTTCTGCTGGAGTTCGTAACCGCAGCGGGCATCACGCATGGTATGGATGAACTGTACAAA <b>TAA</b> CTCGAGCACCACCACCACCACCCTGAGATCCGGCTGCTAACAAAGCCGAAAGGAAGCTGAGTTGG CTGCTGCCACCGCTGAGCAATAACTAGCATAACCCCTTGGGGCCTCTAACGGGTCTTGAGGGTTTTT TTG
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Start and stop codons are in bold and the theophylline riboswitch is underlined. The T7 promoter and T7 terminator sequences are TAATACGACTCACTATA and CTAGCATAACCCCTTGGGGCCTCTAACGGGTCTTGAGGGTTTTTTG, respectively.

**Supplementary Table 2. The activity of cell-free expressed  $\alpha$ HL**

<b>construct name</b>	<b><math>t_{1/2}</math> (min)</b>	<b>Comments</b>
JF001A	> 30	$\alpha$ HL behind theophylline riboswitch in the absence of theophylline
JF001A	16.5	$\alpha$ HL behind theophylline riboswitch in the presence of theophylline
DT101A	> 30	$\alpha$ HL-His tagged
SP002A	4.5	$\alpha$ HL-GFP
RL067A	10.0	$\alpha$ HL
Sigma-Aldrich $\alpha$ HL	9.5	commercial $\alpha$ HL
CD101A <sup>1</sup>	> 30	GFP
AS014A	> 30	K30S E31T $\alpha$ HL-GFP lacking an internal RBS

Each construct was expressed *in vitro* with the PURE system and subsequently added to rabbit red blood cells. Hemolysis was measured by attenuation as described in the methods. When indicated, the theophylline concentration was 1.5 mM. Sigma-Aldrich  $\alpha$ HL indicates purchased purified protein and was not *in vitro* expressed.

## Supplementary References

1. Lentini R., *et al.* Fluorescent proteins and in vitro genetic organization for cell-free synthetic biology. *ACS Synth. Biol.* **2**, 482-489 (2013).
2. Bittman R. & Blau L. Permeability behavior of liposomes prepared from fatty acids and fatty acid methyl esters. *Biochim. Biophys. Acta* **863**, 115-120 (1986).
3. Chen P. Y., Pearce D. & Verkman A. S. Membrane water and solute permeability determined quantitatively by self-quenching of an entrapped fluorophore. *Biochemistry* **27**, 5713-5718 (1988).
4. Sacerdote M. G. & Szostak J.W. Semipermeable lipid bilayers exhibit diastereoselectivity favoring ribose. *Proc. Natl Acad. Sci. USA.* **102**, 6004-6008 (2005).