Supplemental Information

Plasmid constructions. Plasmid manipulations were performed using chemically competent MAX Efficiency® DH5α™ *E. coli* cells.

Plasmid pHWG640:eGFP-TL-REACh1His. The coding sequence of YFP was amplified from pcDNA™6.2/C-YFP-DEST (Invitrogen, Carlsbad, CA) using forward (5′-CGTCATATGGTGAGCAAGGGCGAGGAG-3′) and reverse (5′-CGTAAGCTTTTAATGGTGATGGTGATGGTGCTTGTACAGCTCGTCC-3′) primers. The PCR product was digested with *Nde*I and *Hind*III and cloned into the same sites of pET21a(+) (Novagen). Sequential site-directed mutagenesis of YFP was performed with the QuikChange® II Site-Directed Mutagenesis kit (Stratagene). The coding sequence of eGFP was amplified from pIVEX2.3d-eGFP using forward (5′-CGTCATATGGTGAGCAAGGGCGAGGAG-3′) and reverse (5′-CGTGGATCCGGCCTTCTTGTACAGGCTCTTGTACAGCTCGTC-3′) primers. REACh1 was amplified using forward (5′-GTGGATCCGAAAACCTGTACTTCCAGAGCGGCACCGTGAGCAAGGGCGAA-3′) and reverse (5′-CGTAAGCTTTTAATGGTGATGGTGATGGTGCTTGTACAGCTCGTCC-3′) primers. The PCR products amplified from eGFP and REACh1 were then digested with *Nde*I-*BamH*I and *BamH*I-*Hind*III, respectively, gel purified and three-way ligated into the *Nde*I-*Hind*III sites of *E. coli* expression vector pHWG640 to yield the pHWG640:eGFP-TL-REACh1 plasmid. This method of construction introduces a 17 amino acid linker peptide (SLYKKAGSENLYFQSGT), which joins the C-terminus of eGFP to the N-terminus of REACh1, and also places a 6XHis-tag at the N-terminus of REACh1. The linker contains a TEV protease cleavage site (underlined) and flanking spacer arms to increase TEV protease accessibility.

Plasmid pSAL:TEVHis. TEV protease coding sequence was amplified using forward (5′-CGTGGTACCAGACAACAAGATGGGAGAAAGTCTG-3′) and reverse (5′-CGTAAGCTTTTAATGGTGATGGTGATGGTGTTGCGAGTACACCAATTC-3′) primers. Purified PCR products were digested with *Kpn*I and *Hind*III, gel purified and cloned into *Kpn*I and *Hind*III sites of bacterial expression vector pSAL to yield the pSAL:TEV and pSAL:TEVHis plasmids. Expression of these plasmids in *E. coli* provides the "positive" control.

Plasmid pSAL:RSTEVHis. TEV protease coding sequence was amplified using forward (5′-TGACTGGGTACCGGTGATACCAGC-3′) and the same reverse primer as in previous case. Purified PCR products were digested with *Kpn*I and *Hind*III, gel purified and cloned into *Kpn*I and *Hind*III sites of bacterial expression vector pSAL to yield the pSAL:RSTEV and pSAL:RSTEVHis plasmids.

Plasmid pSAL:RSTEVC151A. (C151A mutation in TEV protease sequence). The mutation C151A that yields inactive TEV protease was prepared using the QuikChange® II Site-Directed Mutagenesis kit (Stratagene) using pSAL:RSTEV as a template and forward (5′-AACCAAGGATGGGCAGGCTGGCAGTCCATTAGTATC-3′) and reverse (5′-GATACTAATGGACTGCCAGCCTGCCCATCCTTGGTT-3′) primers.

TEV protease purification. 6XHis-tagged TEV protease expression plasmid pHWG640:TEVHis was transformed into *E. coli* BL21 cells and a single colony was inoculated into 5 ml of LB medium containing 25 μg/ml chloramphenicol. The cell culture was grown overnight at 37ºC and used to inoculate 250 ml culture in shaking flask. The cell culture was incubated at 37ºC until OD_{600} reached 0.6. Gene expression was induced by addition of L-rhamnose to final concentration 2%, the temperature was lowered to 20ºC and the induction continued for 20 h. The cells were harvested by centrifugation at 5000 x g for 15 min. The cell lysis and 6X His tagged TEV protease purification

was performed according to the manufacturer's instruction of Ni-NTA Fast Start Kit purchased from Qiagen (Valencia, CA). The eluant was dialyzed against 50 mM Tris-HCl pH 7.5 supplemented with 1mM EDTA and 5 mM DTT.

Western blot. His-tagged TEV protease was detected using the SuperSignal West HisProbe Kit from Pierce (Rockford, IL). Images were captured using the Syngene G:Box Gel Documentation System and GeneSnap software, version 6.08 from Synoptics Ltd. (Cambridge, England.)

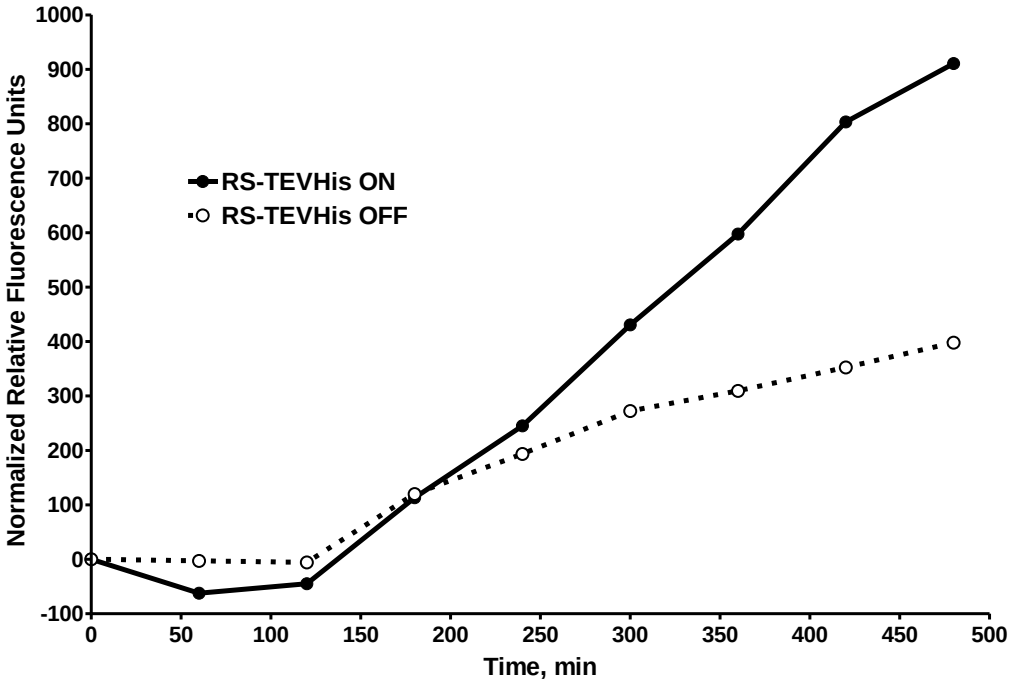

Figure SI.1: Fluorescence signal change in cell culture in the presence of LB media showed a 2-fold difference between the "on" and "off" states of riboswitch. Relative fluorescence units have been normalized to the fluorescent units for culture at 0 min. Time 0 min represents the time the inducer (rhamnose) is added to promote the expression of eGFP-TL-REACh, the FRET-based substrate for the TEV protease.

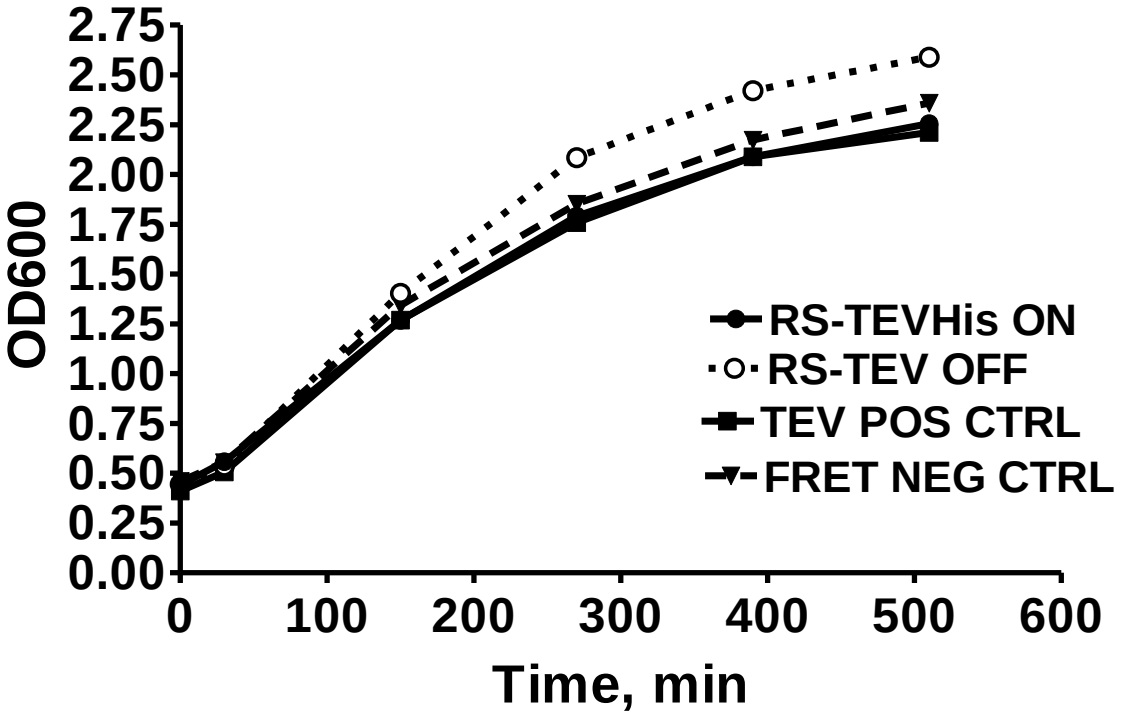

Figure SI.2: TOP10 *E. coli* cells growth in the presence of 2.5 mM theophylline.

Different conditions of *E. coli* TOP10 cells harboring either the positive control (pHWG640:eGFP-TL-REACh1His and pSAL:TEVHis), riboswitch (pHWG640:eGFP-TL-REACh1His and pSAL:RSTEVHis), or negative control (pHWG640:eGFP-TL-REACh1His and pSAL:RSTEVC151A) were grown to mid-log phase at 37˚C and then induced with rhamnose. Theophylline was added after 60 min to the "on" state of riboswitch.

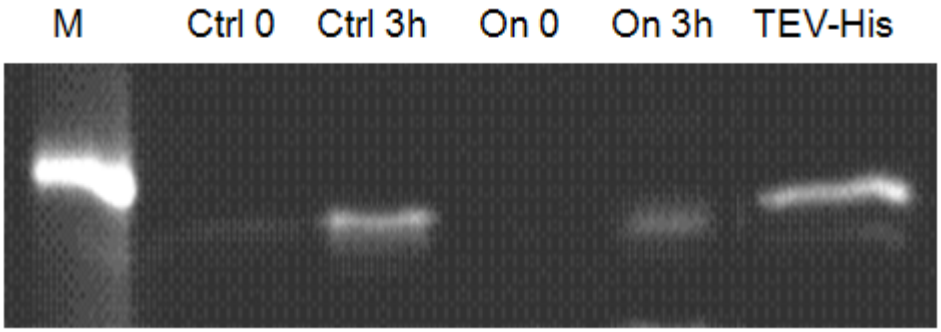

Figure SI.3: Western blot analysis of cell lysates from *E. coli* cells harboring TEVHis and RS-TEVHis constructs. Positive control shows TEV protease expression without riboswitch control (no aptamer) and TEV protease expressions under the riboswitch control are shown at 0h and 3h after the addition of theophylline. The total protein concentration was determined using the BCA assay and all lanes were loaded with 50 μg of total protein. In Lane "M", BenchMark™ His-tagged Protein Standard was loaded at 7 μl according to manufacturer instructions (the band shown corresponds to 30 kDa). Lane "TEV-His" contains 2.5 μg of purified His-tagged TEV protease.

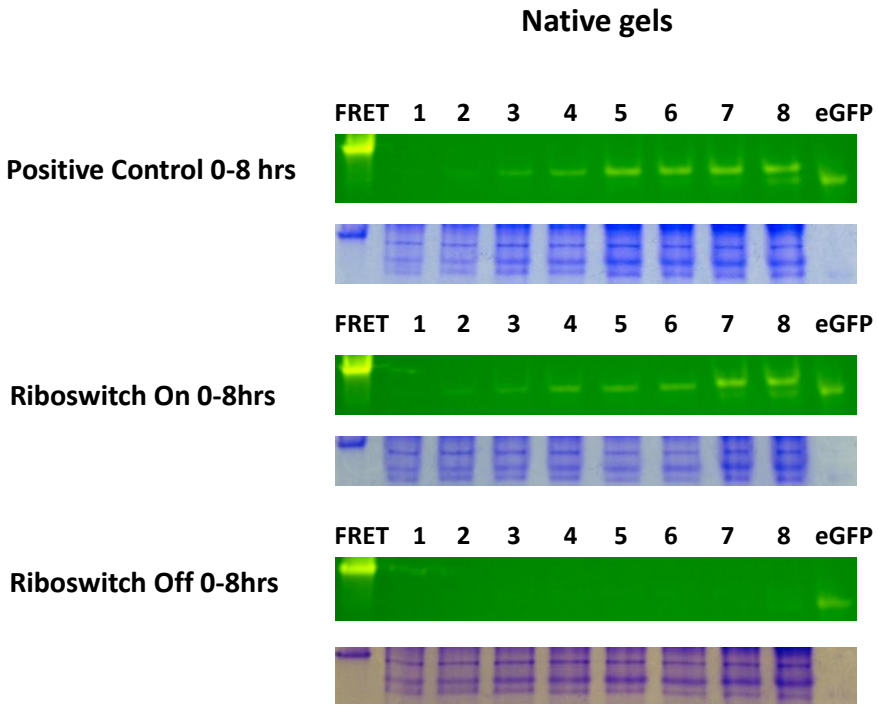

Figure SI.4: Visualization of TEV protease activity as seen by the increase in the fluorescence signal of eGFP on nondenaturing polyacrylamide gel. Aliquots of cultures were harvested hourly pre-and post-theophylline exposure. 12% Tris-HCl Nondenaturing PAGE shows the purifed FRET eGFP-TL-REACh (5.7 µg) in the first lane, purifed eGFP (0.8 µg) in last lane, and aliquot of each lysate (36 µg of total protein) for each time point over 8 hours as indicated above. Each gel was visualized with a transilluminator at 457 nm and a second image is provided of each gel after Coomassie blue staining.

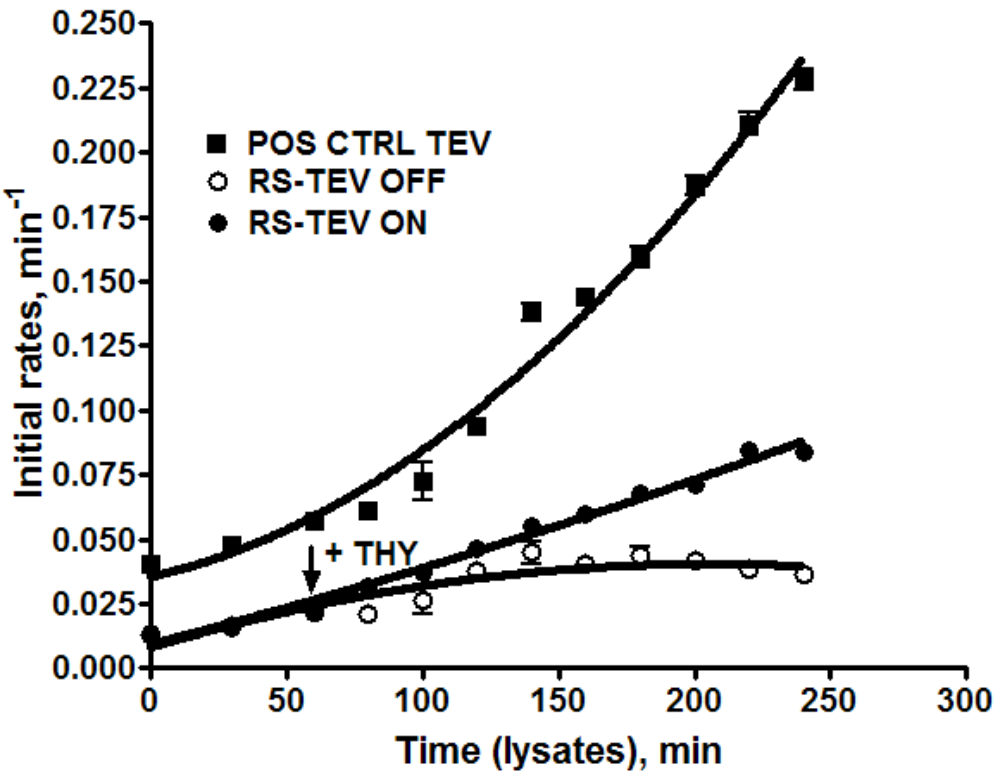

Figure SI.5: **Kinetics of Riboswitch-TEV protease from cellular extracts.**

TEV protease activity examined with 50 nM eGFP-TL-REACh. The riboswitch "off " and "on" states are displayed in terms of TEV protease activity in response to no small molecule (white circles) and 2.5 mM theophylline at 60 min (black circles), respectively. The positive control shows TEV protease activity which is not modulated by the riboswitch at the 5′-UTR (black squares). All curves were fit with a second order polynomial, specifically $Y=A + B*X + C*X^2$.

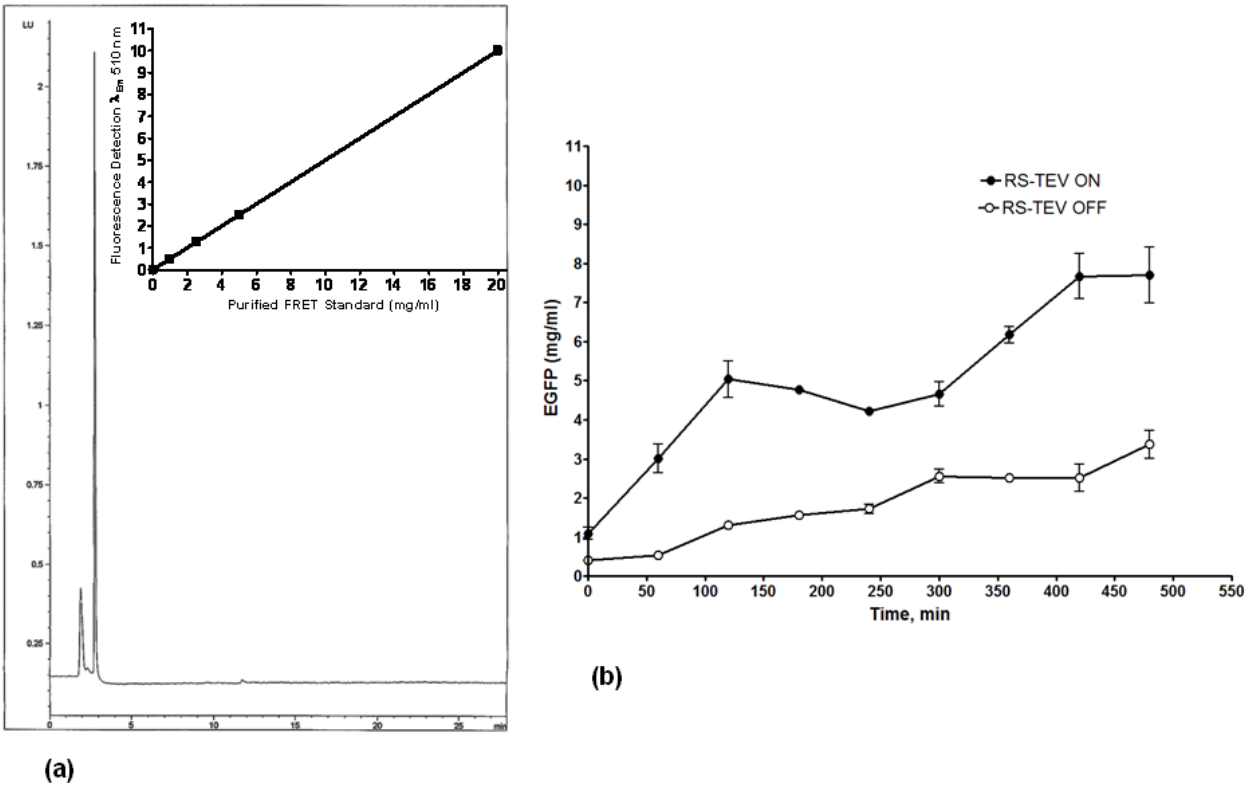

Figure SI.6: HPLC analysis of eGFP concentration in cellular extracts.

(A) A representative chromatogram of the eGFP peak utilizing fluorescence detection at 510 nm is shown with an inset graph depicting a standard curve which is calculated from purified eGFP. (B) Time course profiles of riboswitch activation in terms of eGFP concentration are determined via HPLC analysis.

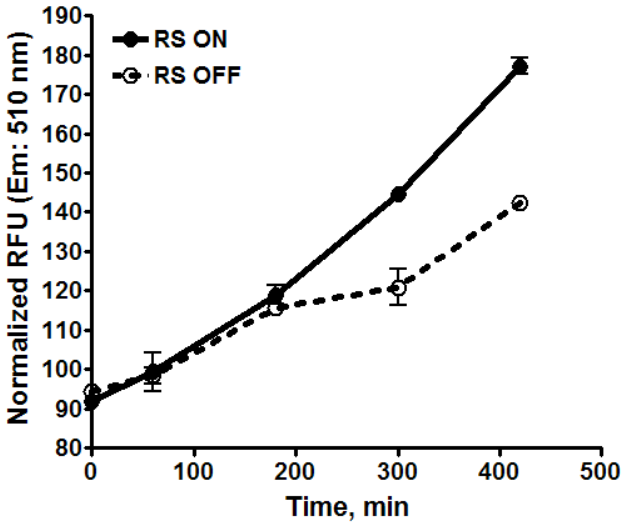

Figure SI.7: Kinetics of Riboswitch-eGFP activation from cellular extracts. The riboswitch "off " and "on" states are displayed in terms of fluorescence increase in response to no analyte (open circles) and 2.5 mM theophylline at 60 min (filled circles), respectively. RFU has been normalized to total protein concentration consistent with the normalization methodology presented in Figure 3.