

SUPPLEMENTARY FIGURES

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          Sac1          Not1          BstAP
          Ecl2 |      EcoRV |      Eag1 |      Nco1 |      AlwN1 |
          | |      | |      | |      | |      Pvu2 |      |
1  CGAGCTCGGATCGATATCTGCGGCCGCCACCATGGACCTGTGGCAGCTGCTGACCCCT
-----+-----+-----+-----+-----+-----+-----+-----+
GCTCGAGCCTAGCTATAGACGCCGCGGTGGTACCTGGACACCGTCGACGACGACTGGGA
          M D L W Q L L L T L

          Nae1
          NgoM4 |
          BseR1 | |
          | | |
61 GGCCCTGGCCGGCTCCTCTGACGCCACCACCACCATCACCACGACTACGACATCCCTAC
-----+-----+-----+-----+-----+-----+-----+
CCGGGACCGCCGAGGAGACTGCGGGTGGTGGTGGTAGTGGTGCTGATGCTGTAGGGATG
          A L A G S S D A H H H H H H D Y D I P T

          BssS1
          |
121 CACCGAGAACCTGTACTTTTCAGGGCGACAAGCGGCTGCGGGACAACCACGAGTGGAAAAA
-----+-----+-----+-----+-----+-----+-----+
GTGGCTCTTGGACATGAAAGTCCCGCTGTTCGCGGACGCCCTGTTGGTGCTCACCTTTTT
          T E N L Y F Q G D K R L R D N H E W K K

181 ACTGATCATGGTCCAGCACTGGCCTGAGACAGTGTGCGAGAAGATCCAGAACGACTGCCG
-----+-----+-----+-----+-----+-----+-----+
TGACTAGTACCAGGTTCGTGACCGGACTCTGTACACGCTCTTCTAGGTCTTGCTGACGGC
          L I M V Q H W P E T V C E K I Q N D C R

          SanD1
          |
241 GGACCCTCCTGACTACTGGACCATCCACGGCCTGTGGCCTGACAAGTCCGAGGGCTGCAA
-----+-----+-----+-----+-----+-----+-----+
CCTGGGAGGACTGATGACCTGGTAGGTGCCGGACACCGGACTGTTTCAGGCTCCCGACGTT
          D P P D Y W T I H G L W P D K S E G C N

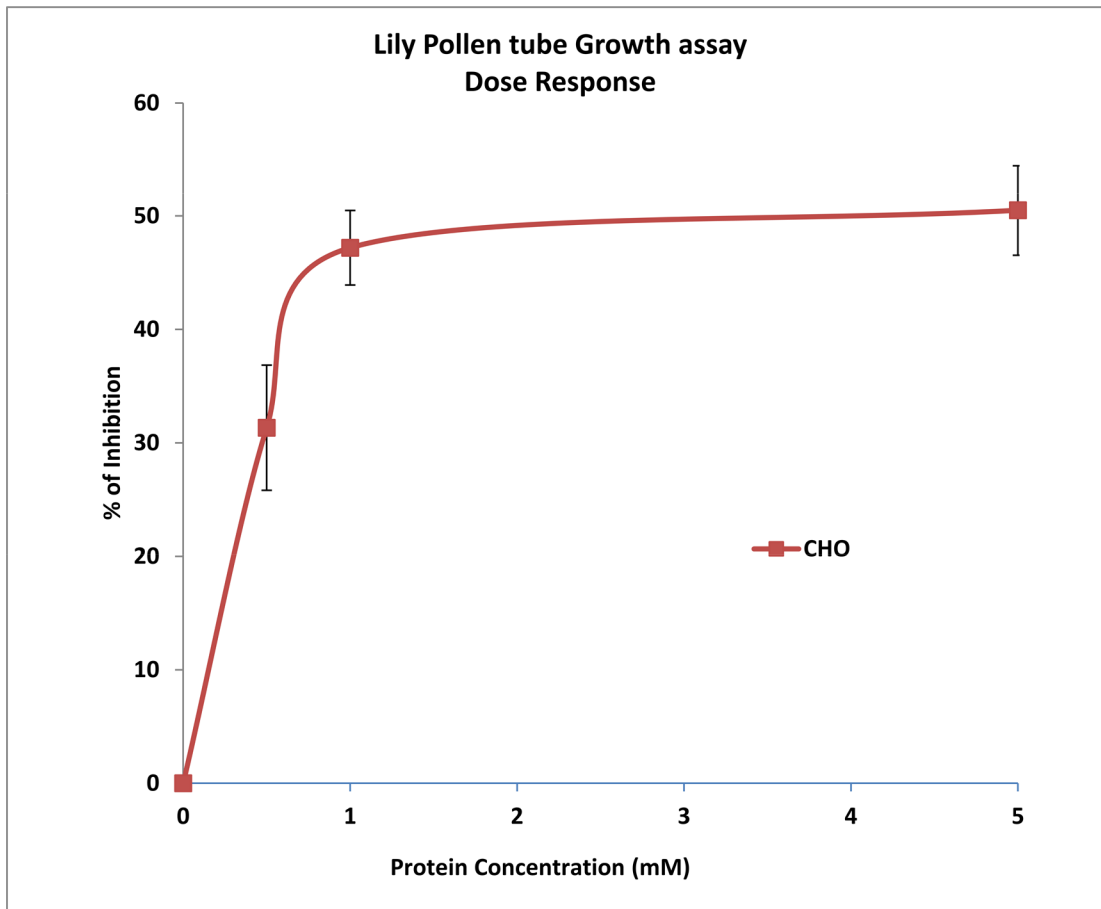
          Age1          SpAcc
          |          |
301 CCGGTCCTGGCCTTTCAACCTGGAAGAAATCAAGGATCTGCTGCCTGAGATGCGGGCCTA
-----+-----+-----+-----+-----+-----+-----+
GGCCAGGACCGGAAAGTTGGACCTTCTTTAGTTCCTAGACGACGGACTCTACGCCCGGAT
          R S W P F N L E E I K D L L P E M R A Y

          BmgB1          Age1
          |          |
361 CTGGCCTGACGTGATCCACTCCTTCCTAACCAGTCCCGGTTCTGGAAGCATGAGTGGGA
-----+-----+-----+-----+-----+-----+-----+
GACCGGACTGCACTAGGTGAGGAAGGGATTGGCCAGGGCCAAGACCTTCGTACTCACCT
          W P D V I H S F P N R S R F W K H E W E
    
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(Continued)

	<u>Sall</u> 		<u>XmnI</u> <u>ScaI</u> <u>EagI</u> 		
421	GAAGCACGGCACTTGTGCTGCCAGGTCGACGCCCTGAACTCCCAGAAGAAGTACTTCGG				
	-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+				
	CTTCGTGCCGTGAACACGACGGGTCCAGCTGCGGGACTTGAGGGTCTTCTTCATGAAGCC				
	K H G T C A A Q V D A L N S Q K K Y F G				
	<u>BsaXb</u> 		<u>BseY1</u> <u>BsaXa</u> 		
481	CCGGTCCCCTGGAAGTGTACCGGGAGCTGGACCTGAACTCCGTGCTGCTGAAGCTGGGCAT				
	-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+				
	GGCCAGGGACCTTGACATGGCCCTCGACCTGGACTTGAGGCACGACACTTCGACCCGTA				
	R S L E E L Y R E L D L N S V L L K L G I				
	<u>Eco57</u> 		<u>SmaI</u> 		
541	CAAGCCTTCCATCAACTACTACCAGGTGGCCGACTTCAAGGACGCCCTGGCCC GGGTGTA				
	-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+				
	GTTCCGGAAGGTAGTTGATGATGGTCCACCGGCTGAAGTTCCCTGCGGGACCGGCCACAT				
	K P S I N Y Y Q V A D F K D A L A R V Y				
	<u>AlwN1</u> 		<u>SpAcc</u> 		
601	CGGCGTGATCCCTAAGATCCAGTGCCTGCCTCCTTCCCAGGACGAAGAAGTGCAGACCAT				
	-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+				
	GCCGCACTAGGGATTCTAGGTCACGGACGGAGGAAGGGTCTGCTTCTTCACGTCTGGTA				
	G V I P K I Q C L P P S Q D E E V Q T I				
	<u>BsgI</u> 	<u>Pvu2</u> 	<u>BsgI</u> <u>AlwN1</u> <u>PstI</u> 		
661	CGGCCAGATCGAGCTGTGCTGACCAAGCAGGACCAGCAGCTGCAGAAGTGCACCCGAGCC				
	-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+				
	GCCGGTCTAGCTCGACACGGACTGGTTCGTCTGCTGGTCGACGCTTTCGACGTGGCTCGG				
	G Q I E L C L T K Q D Q Q L Q N C T E P				
	<u>Bpu10</u> 	<u>MscI</u> 			
721	TGCGAGCAGCCTTCCCCTAAGCAGGAAGTCTGGCTGGCCAACGGCGCTGCCGAGTCCAG				
	-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+				
	ACCGCTCGTCGGAAGGGGATTCGTCCCTCAGACCGACCCGGTTGCCGCGACGGCTCAGGTC				
	G E Q P S P K Q E V W L A N G A A E S R				
	<u>StuI</u> 	<u>BseR1</u> 			
781	AGGCCTGCGCGTGTGCGAGGACGGCCCTGTGTTCTACCCTCCTCCCAAGAAAACCAAGCA				
	-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+				
	TCCGGACGCGCACACGCTCCTGCCGGACACAAGATGGGAGGAGGGTCTTTTGGTTTCGT				
	G L R V C E D G P V F Y P P P K K T K H				
	<u>EcoRI</u> 	<u>BamH1</u> 	<u>SpeI</u> 	<u>EagI</u> 	<u>BstX1</u>
841	CTAGGAATTCAGTGGATCCACTAGTAACGGCCGCCAGTGTGCTGGAA				
	-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+				
841	GATCCTTAAGTCACCTAGGTGATCATTGCCGGCGGTACACGACCTT				
	* 887				

Supplementary Figure S1 (Continued): Restriction map. The human recombinant RNASET2 construct. The amino acid sequence is in bold. The human growth hormone receptor signal peptide (magenta), 6 x His tag (green), spacer region (blue) and TEV protease cleavage site (grey) were situated upstream to the mature RNASET2 sequence. The c-DNA sequence was optimized for expression in CHO. The open reading frame ends with a stop codon (asterisk).



Supplementary Figure S2: Lily pollen tube results.

Lilly pollen tube growth (PTG) assay

Pollen tube growth was assessed as previously described (4), with slight modifications. *Lilium longiflorum* var. white heaven flowers were allowed to open in the lab. Anthers were excised and brought to complete dehiscence and pollen release for 24-48 h at room temperature. The pollen was suspended at a concentration of about 40 mg per ml culture medium (7% sucrose (w/v), 1.27 mM $\text{Ca}(\text{NO}_3)_2$, 0.16 mM H_2BO_3 , 1 mM KNO_3 and 3 mM KH_2PO_4 in water) (5), vigorously stirred by vortex and centrifuged for 2 min at 13,400 rpm at room temperature, to release the lipid phase from pollen grains. The cell pellet was suspended in 1 ml culture medium, in a fresh 1.5 ml Eppendorff tube. The same procedure was repeated three more times. Cells were counted and suspended in culture medium at a density of 25,000 cells/ml. Pollen was germinated in 100 μl culture medium containing 1 mM hrRNASET2 or PBS (as control) and incubated for 1-2 h at 25°C in the dark, to maximum 500 μm . The pollen tubes were fixed and stained by adding 100 μl Alexander stain (20 mg malachite green, 100 mg acid fuchsin, 5 gr phenol, 2 ml lactic acid, 20 ml ethanol, 40 ml glycerol, 50 ml water) (6) to each tube and gently mixing. Pollen tube length were imaged under a microscope (Leica DMi 3000M, Germany) equipped with a Digital Sight DS-Fi1 camera (Nikon, Japan) and analyzed using NIS-Elements Br software (Nikon, Japan). N=3 and at least 100 pollen tubes were measured in each determination.