

Supporting Information for

A. I. Nash, W-H. Ko, S. M. Harper, K. M. Gardner

A Conserved Glutamine Plays a Central Role in LOV Domain Signal Transmission and Duration

Supporting Materials and Methods:

Obtaining Absorption coefficients for wildtype *AsLOV2*, Q513N, and Q513L

To obtain absorption coefficients we employed trichloroacetic acid (TCA) precipitation of our proteins to isolate FMN (*I*). Following addition of 10% TCA, each protein sample was incubated in the dark at room temperature 5 min., then centrifuged at 20,000 x g, 4°C for 10 min. to clarify supernatant. An FMN standard curve was prepared using the A_{446} measurements of FMN at concentrations of 1 μ M, 10 μ M, 50 μ M, 100 μ M, and 250 μ M in 50 mM sodium phosphate, 100 mM NaCl, pH 6.0 with 10% TCA. The UV-visible absorbance spectrum of each sample's supernatant was recorded and the concentrations of FMN determined using the standard curve. Assuming a 1:1 protein/FMN stoichiometry, we assume the concentration of FMN is equal to total concentration of protein and calculate an absorption coefficient for each protein using the following formula:

$$\epsilon_{446,TCA} = A_{446,TCA} / [C_{TCA} * 1 \text{ nm}]$$

Where ϵ is the absorption coefficient, A is the absorption, and C is the concentration of total protein.

To convert the $\epsilon_{446,TCA}$ to the an ϵ_{446} under non-acidic buffer conditions, we measured the ratio between the A_{446} measured under non-acidic versus acidic conditions ($A_{446,non-$

acidic/ $A_{446,TCA}$) and multiplied the $\epsilon_{446,TCA}$ by this ratio. The resulting absorption coefficient (ϵ_{446}) for wildtype *AsLOV2*, Q513N, and Q513L are reported in Table 1.

Supporting Figure Legends

Supporting Figure 1. Overlay of dark (black) and lit (red) state $^{15}\text{N}/^1\text{H}$ HSQC correlation spectra for (a) wildtype *AsLOV2* domain, (b) Q513L, and (c) Q513N.

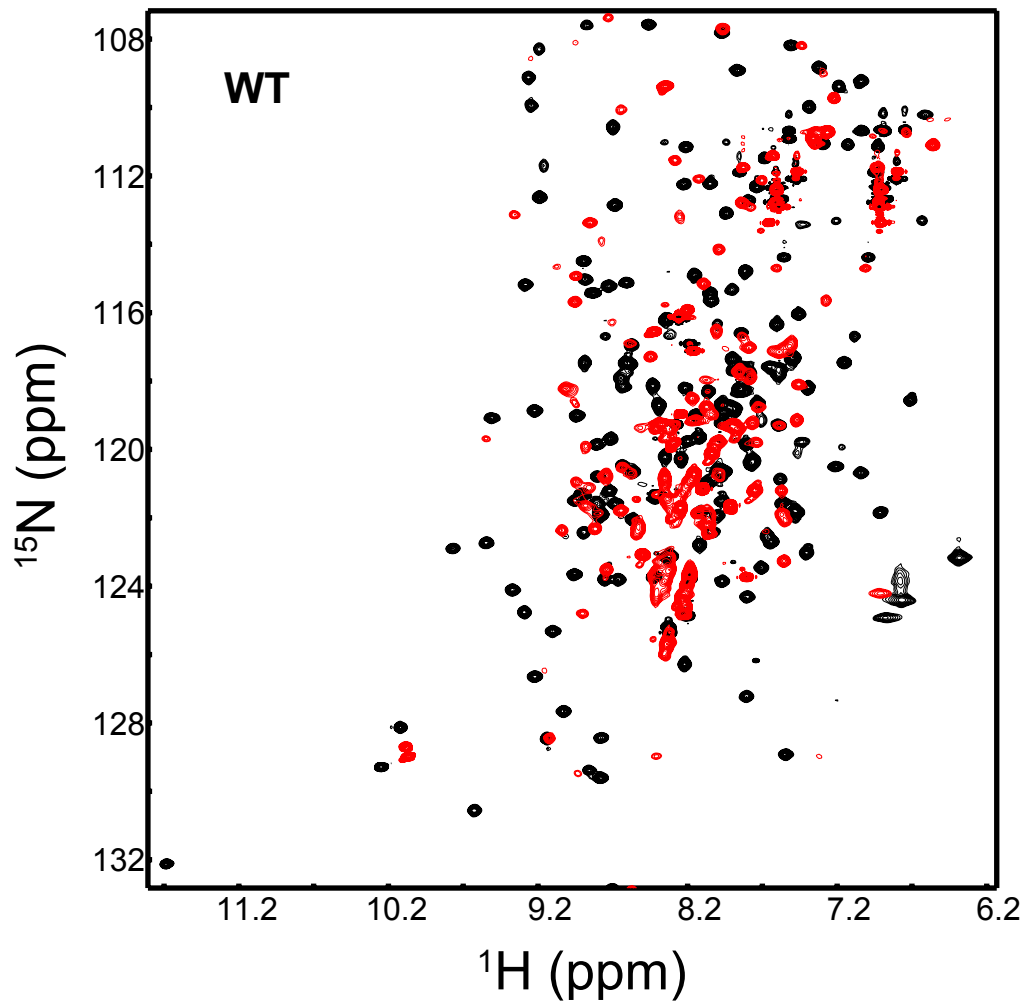
Spectra were recorded at 25°C at 500 MHz. Black spectra represent the dark state and red spectra represent the lit state. See materials and methods for more details. Mutations lead to chemical shift changes as well as differential broadening across each spectrum.

Supporting Figure 2. UV-visible absorbance spectra of wildtype, Q513L and Q513N mutants. UV-visible absorbance spectra shown here were recorded from 250nm to 550 nm for each protein at concentrations between 50 μM and 70 μM in buffer containing 50 mM sodium phosphate (pH 6.0) and 100 mM NaCl. Calculated A_{280}/A_{446} ratios are 2.60 for wildtype, 2.63 for Q513N and 2.76 for Q513L (Q513L ratio taken at A_{438} due to blue shift).

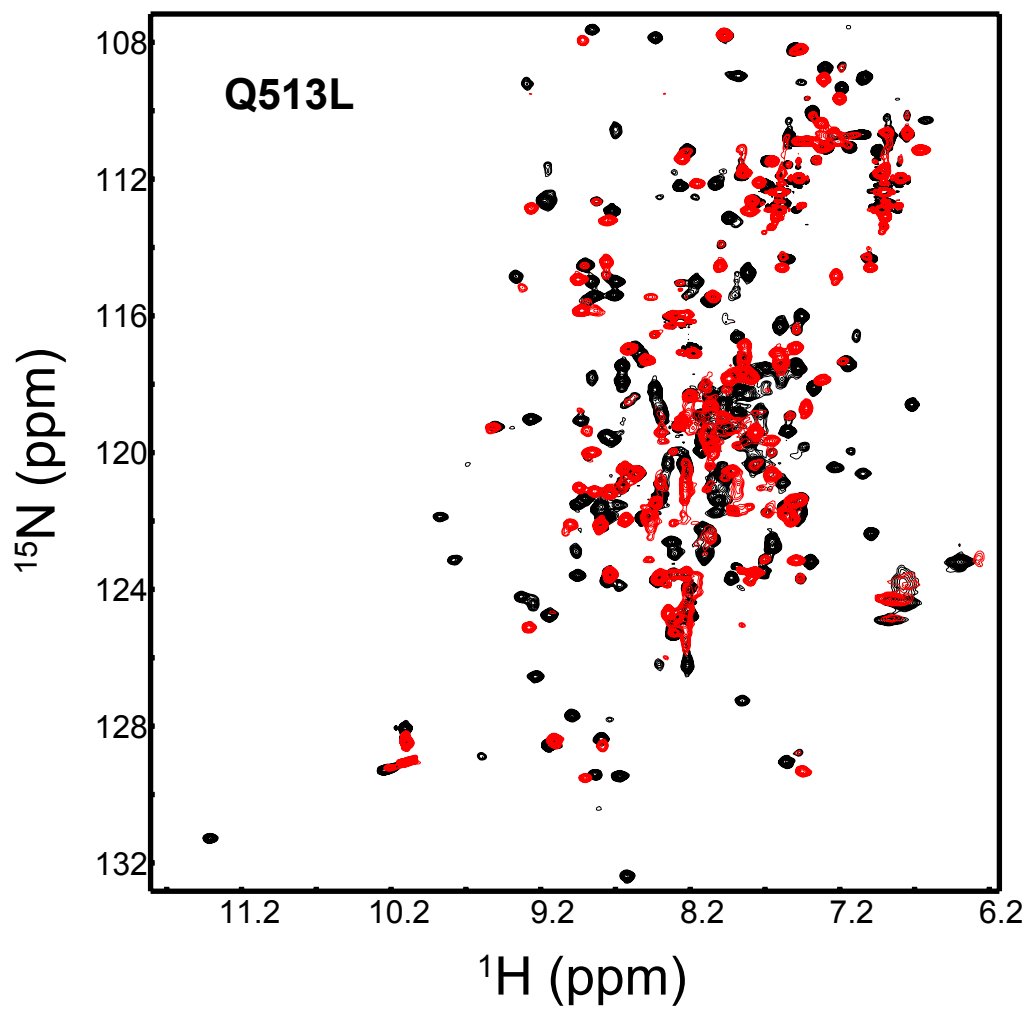
Supporting Figure 3. Sequence alignment of known LOV domains. Sequence alignments of 128 known LOV domains are shown with the predicted secondary structure indicated on the top of the sequences. Residues Q513 and F494 are indicated on the bottom. Red boxes with white characters indicate strict identity. Red characters or bold black characters indicate similarity within a group, while yellow boxes indicate similarity across groups.

Reference:

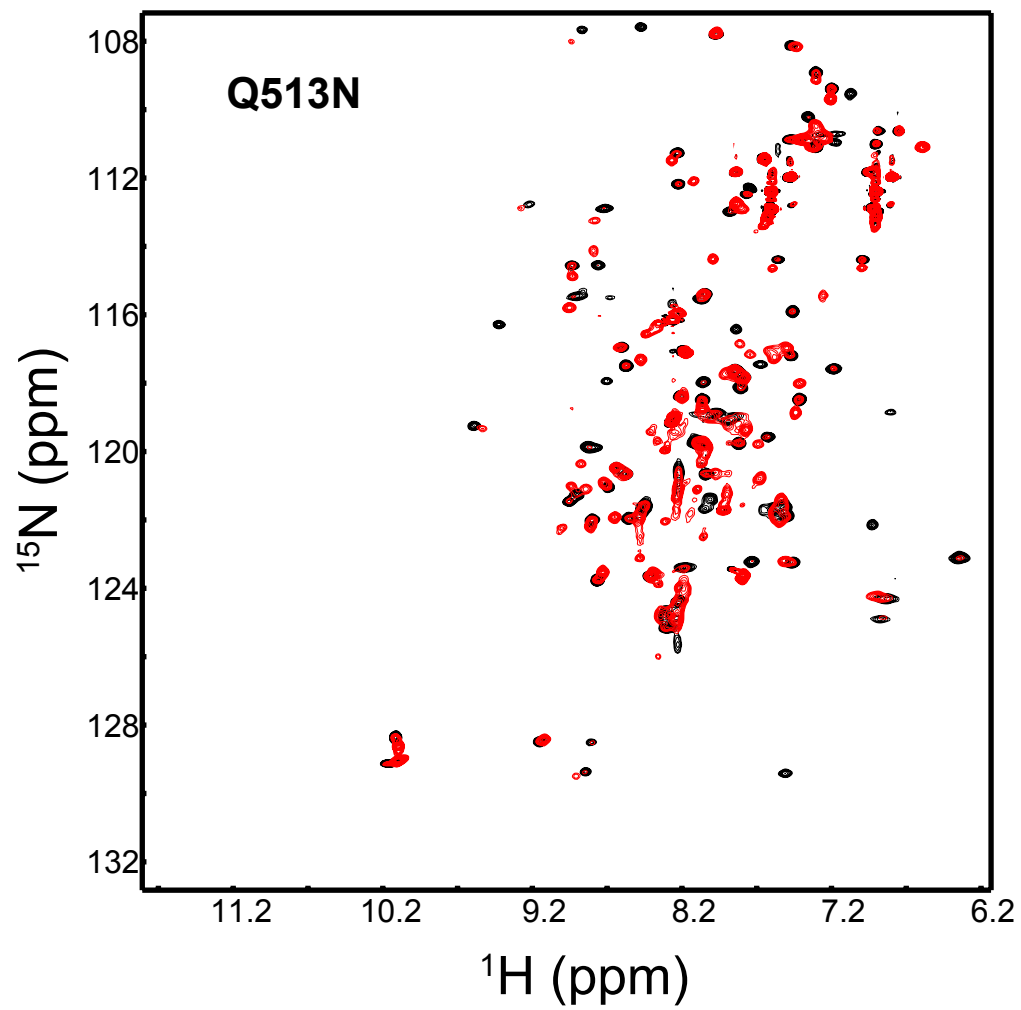
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LOV (light, oxygen, or voltage) domains of the blue-light photoreceptor
phototropin (nph1): binding sites for the chromophore flavin mononucleotide,
Proc Natl Acad Sci U S A 96, 8779-8783.



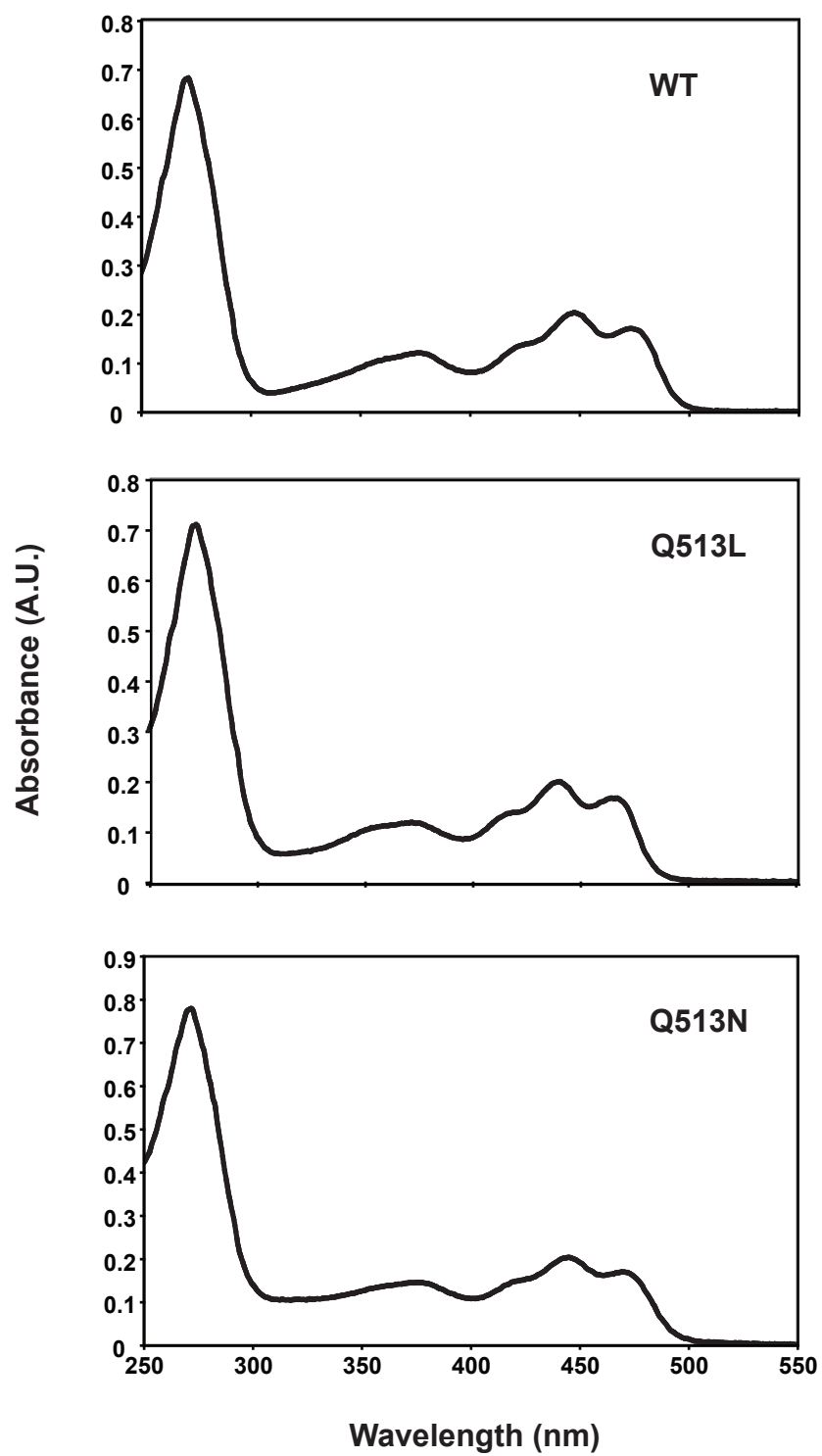
Supporting Fig. 1a
Nash *et al.*



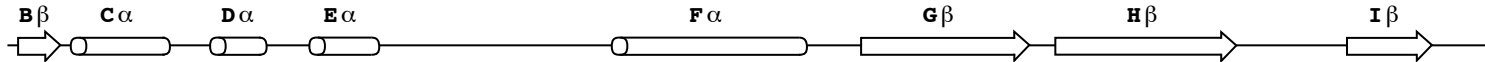
Supporting Fig. 1b
Nash *et al.*



Supporting Fig. 1c
Nash *et al.*



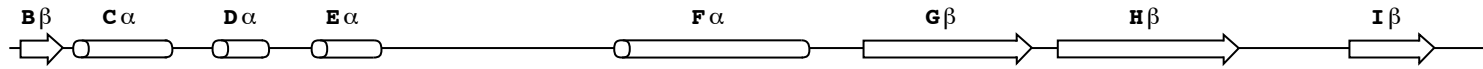
Supporting Fig. 2
Nash *et al.*



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Q84K55_DRYFM/244-338	I I F A S T G F F N L T G Y T S R E V I G G N C R F L O G P D T N P E D V A S I R D A V P P R G T G T F C G R L L N Y . . . R K D G S N F W N L L T I A P I K D D T . G T I V K L V G V L E V
Q401Q4_MOUSE/48-137	I V F A S E G F Y E M T G Y G P E E V I G N C R F L O G E G T S R D E V T R L K Q C L L V . E G Q . P F C G R L L N Y . . . R K D G T P F W N L L T V S P V R S A T . G R V V K F I G M O V E V
Q401Q5_MOUSE/61-145	V M F A S E G F Y R M T G Y S A K E V I G N C R F L O G P E T D R S E V E K L Q A L L . D G Q . S W C G R L L N Y . . . R K D G S S F W N L L T V S P V K D D S . G R V V K F I G M O V E V
Q8LPP9_CHLRE/34-126	L V Y A S E G F Y A M T G Y G P D E V L G H N C R F L O G E G T D P K E V Q K I R D A I K . K G E . A C S V R L L N Y . . . R K D G T P F W N L L T V T P I K T P D . G R V S K F V G V D V D
Q8LPE0_CHLRE/34-126	L V Y A S E G F Y A M T G Y G P D E V L G H N C R F L O G E G T D P K E V Q K I R D A I K . K G E . A C S V R L L N Y . . . R K D G T P F W N L L T V T P I K T P D . G R V S K F V G V D V D
Q7USG5_RHOBA/987-1101	I S F V N K G F T D M T G F S E Q E I L G R N C R F L O G E H T D N E T V L R I R R A L G . R G E . S V R E L I K N Y . . . R K N G E P F W N D L Y I T P V H D E N . G I L T H F V G V D N D V

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Supporting Fig. 3
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Q40XK1_KINRA/196-283
 Q2N9L9_9SPHN/41-155
 Q2G5UO_NOVAD/50-163
 EL368
 Q2NCA3_9SPHN/62-154
 Q4ZSY3_PSEU2/22-136
 Q881J7_PSEIM/22-136
 Q48IV1_PSEL4/20-112
 Q35E64_9BRAD/52-144
 Q3BRX8_XANC5/53-145
 Q8PJH6_XANAC/53-145
 Q2P134_XANOM/53-145
 Q5GY20_XANOR/53-145
 Q8P827_XANCP/53-145
 Q4UW16_XANC8/53-145
 Q1NI33_9SPHN/78-170
 Q2YKK7_BRUA2/20-112
 Q57Y7_BRHIZ/15-107
 Q8FW73_BRUSU/20-112
 Q8YC53_BRUME/46-138
 BML0V
 Q1M667_RHILV/33-146
 Q9ABE3_CAUCR/128-220
 Q1YFS4_9RHIZ/62-154
 Q3VF27_9SPHN/26-115
 Q1N7J1_9SPHN/51-164
 EL222
 Q2G8Z7_NOVAD/53-145
 Q8ESN8_OCRH/7-124
 PHOT_BACSU/14-126
 Q3J6W8_NITOC/42-134
 Q2Y837_NITUM/39-131
 Q3ONSO_THIDN/111-226
 Q2CIF5_9RHOB/15-130
 Q1YEU2_9RHIZ/42-131
 Q2NB77_9SPHN/34-121
 EL346
 Q1N482_9GAMM/290-403
 Q3ITW5_NATPD/146-260
 Q5V3S3_HALMA/280-372
 Q5V5P7_HALMA/296-388
 Q3IM51_NATPD/130-244
 Q5UW17_HALMA/1483-1575
 Q40X75_KINRA/448-562
 Q55576_SYNY3/296-413
 Q3WZU4_9ACTN/24-138
 Q8DJE3_SYNEL/317-431
 Q4BW45_CROWT/189-281
 Q3MED3_ANAVT/466-558
 Q8YT51_ANASP/463-555
 Q8YSB9_ANASP/202-316
 Q3M6B3_ANAVT/202-316
 Q3INI4_SYNP7/395-487
 Q5N5M8_SYNP6/395-487
 Q3GGZ4_9GAMM/678-789
 Q35YY2_9GAMM/659-770
 Q2WNU6_9GAMM/666-777
 Q8XT61_RALSO/624-738
 Q67UX0_ORYSA/68-162
 ZTL
 LKP2
 FKF1
 consensus>89

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.VWTNPAFERVTVYGR.EVVLGQNCRFLOGPG.....TDREAVARIRRALE.TGD.TVTELLLNY...RKDGTAFFWNEVVISFVHADAD.GRLTHFVGVQSDV
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IVFANRAFRLHLYGYDEHEVVRNCRFLQGP.....TDPAAVARIKAALE..REDVIVVELLNY...RKDGTAFFWNNALHLGFIYDAD.GRLIYFFRQOWDV
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IIFSNRAFLMTGYTAEELGTNCRFLQGPD.....TDPAVQVSIRDAIA..QRNDISAEIINNY...RKDGSSFFWNALFISPVYNDAGDLIYFFASOLDI
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IVFANPAFLKLTGYEADVVMGRNCRFLQGHG.....TDPAHVRAIKSAIA..AEKPIDIDIIINNY...KKSGEAFWNNALFISPVHNNAGRLQHFVSSOLDV
IVLANKAFLLELTGYPAQEVLRNCRFLQGPA.....TSPVAVAEIRAAIA..GEREVSVIELNY...KKSGEQFVNNALFISPVHGGD.GKLYYFFRQOLDM
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VIECNDAFLALTYGYRREIVGNCRFLMRGAG.....TEPELTGQIRAAIS..DRRPILVEILNY...KKGGAFFRNVAVLIAPVIFDSD.GTLKYFLVSSLMEV
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IVVYNQGFVQMTGYETEELIGKNCRFLQGGK.....TDPAEVDNIRTLAQ..NKEPTVQIQINNY...KKGDTGFVNNELSDHMMWDEG.EGKYFFVGVQKDI
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IIYVNNSECELTFRYKYEIVGKNCRFLHSSD.....KEQKSLENIREAIS..TKQSVVKIRNY...TKDGNKLYEDIETISPVFDEKEKLVYFLVSSYKDL
IVYVNRFAEELTYGVSHMAVGNCRFLQGGK.....TRKADVARLREAVA..TGEDVSLIENNY...RANGEPFNALLIPVLDSETGEATFLVGLGOREI
IVYANRAFEKITGYRSADVIVGNCRFLQGGK.....TDPQAVRQLQAQAVR..NCEEVVIVDNY...RSDGTRFLNRLHISPVLYDER.HLQCFVGVQRDM
..YVNRFAEQMTGYRSRSVIVGNCRFLQGGK.....TDPGAVERLAKAIR..NCEEVVETIYNY...RADGEGFWNNHLLMGLPQEDQE.KCRYVFGIQRDM
LIYVNRFAEQMTGYRSRSVIVGNCRFLQGGK.....TDPGAVERLAKAIR..NCEEVVETIYNY...RADGEGFWNNHLLMGLPQEDQE.KCRYVFGIQRDM
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LVYINEAFERLTYGERHEVLRNCRFLQGED.....TNPEAVATIRQAVD..DRRPVSVELLNY...RKDGEPFVNNALFISPVIRDD.GRVTHLVGFTDI
ISYANRQFLELTGYTESVVRGNCRFLQGE.....TEAEPVDAMRAID..ADEPVSVELRNY...RKDGTFMFWNNALFISPVIRDD.GTVVNVGFRDI
LIYANDHYRELTYGYSLELLGKNCRFLQGEN.....TDPEPVDALRDAID..AGEQVSVELRNY...RKDGTEFFWNNALFISPVIRDD.GTVVNVGFRDI
ITYVNNRFFVEITGYARAIVLGNCRFLQGEA.....TRDEPIAQLRAALE..RGETATVELRNY...RKDGTFMFWNNALFISPVIRDD.GTVVNVGFRDI
LVYVNDGFVDQTYGYSREELGNCRFLQRDD.....RDQSAIGLRLKAIA..SEEPSVIVELRNY...KKGTEFFWNNALFISPVIRDD.GTLNLIYGIQRDV
LVYVNRFAEELTYGRSEELGNCRFLQGGAD.....TDHDAIGLRLKAIA..SEEPSVIVELRNY...KKGTEFFWNNALFISPVIRDD.GTLNLIYGIQRDV
VVYANQGFETITGFNREIVGQNCRFLLNEDRQGEQ.....NKQLEKLRTAIA..HGQCEVVLKNY...RKDGASFFWNNALFISPVYNDAGELVYFFRQOLDV
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VIYVNSAFEQITGYKATEIVGNCRFLQGNDDTQP.....ELEQIRDCLO..KGESCHVTLRNY...RKDGSLFWNNALFISPVYNDAGELVYFFRQOLDV
VIYVNSAFEQITGYKATEIVGNCRFLQGNDDTQP.....ELEQIRDCLO..KGESCHVTLRNY...RKDGSLFWNNALFISPVYNDAGELVYFFRQOLDV
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VIYVNPFAFERITGYSEAMLGNCRFLQGNERSL.....QEBEIHRLGLS..QAENVHVIRNF...RKDGQPFVNDLYSIPVFNAG.GNVTHFVGIQRDI
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IIYVNTVFEMVTVGYRAEIVLGNCRFLQCRGPFQAKRRHPL..VDSMVVSEIRKCID..EGIEFQGEVWDR...HKGDFDFPKWLMMSVVRDET.GAISHYVGIQRDI
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Supporting Fig. 3, cont.
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