Supporting Information for

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

Supporting Materials and Methods:

Obtaining Absorption coefficients for wildtype *As*LOV2, Q513N, and Q513L

To obtain absorption coefficients we employed trichloroacetic acid (TCA) precipitation of our proteins to isolate FMN (*1*). 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^{o}C for 10 min. to clarify supernatant. An FMN standard curve was prepared using the A_{446} measurements of FMN at concentrations of 1 μM, 10 μM, 50 μM, 100 μM, and 250 μM 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:

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

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

To convert the $\varepsilon_{446,TCA}$ 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 $\varepsilon_{446,TCA}$ by this ratio. The resulting absorption coefficient (ε_{446}) for wildtype *As*LOV2, Q513N, and Q513L are reported in Table 1.

Supporting Figure Legends

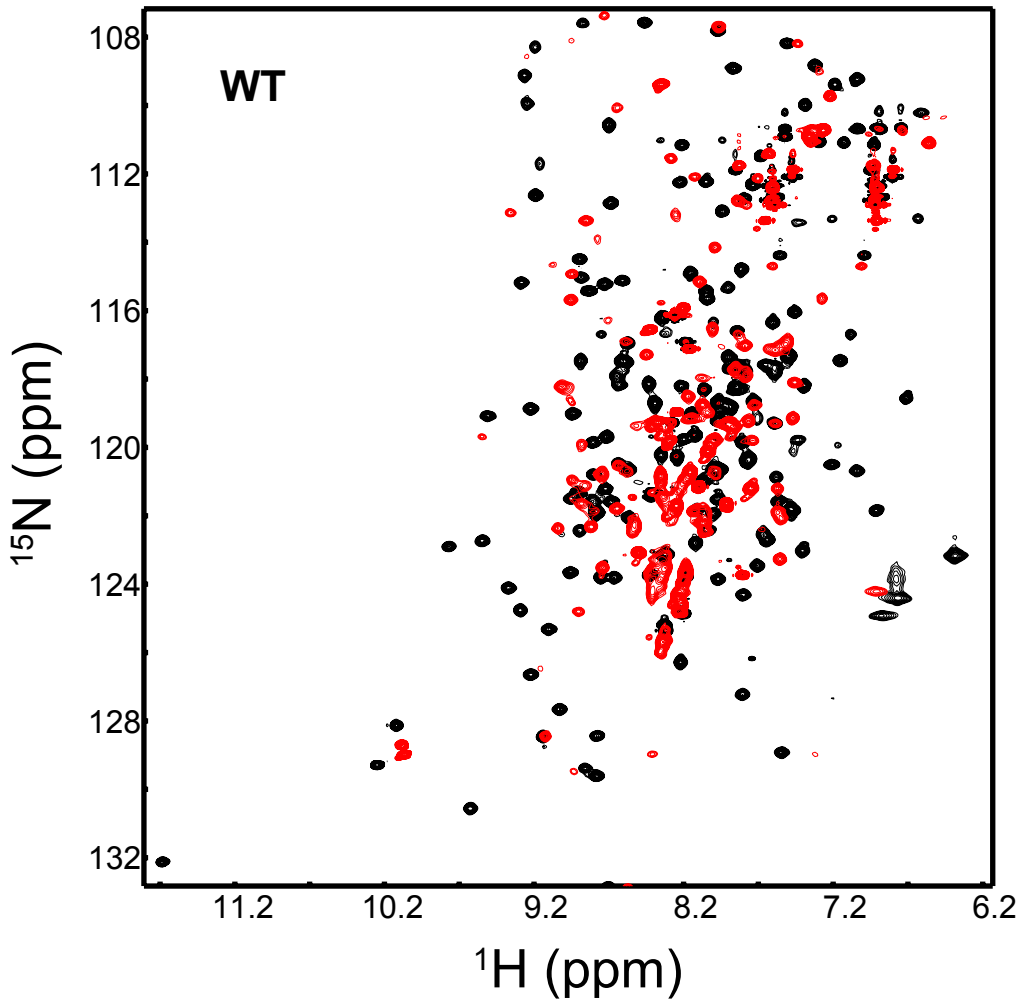
Supporting Figure 1. Overlay of dark (black) and lit (red) state $^{15}N/^{1}H$ HSQC correlation spectra for (a) wildtype *As*LOV2 domain, (b) Q513L, and (c) Q513N. Spectra were recorded at 25°C at 500 MHz. Black spectra represent the dark state and red spectra represent the lit state. See materials 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:

1. Christie, J. M., Salomon, M., Nozue, K., Wada, M., and Briggs, W. R. (1999) 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
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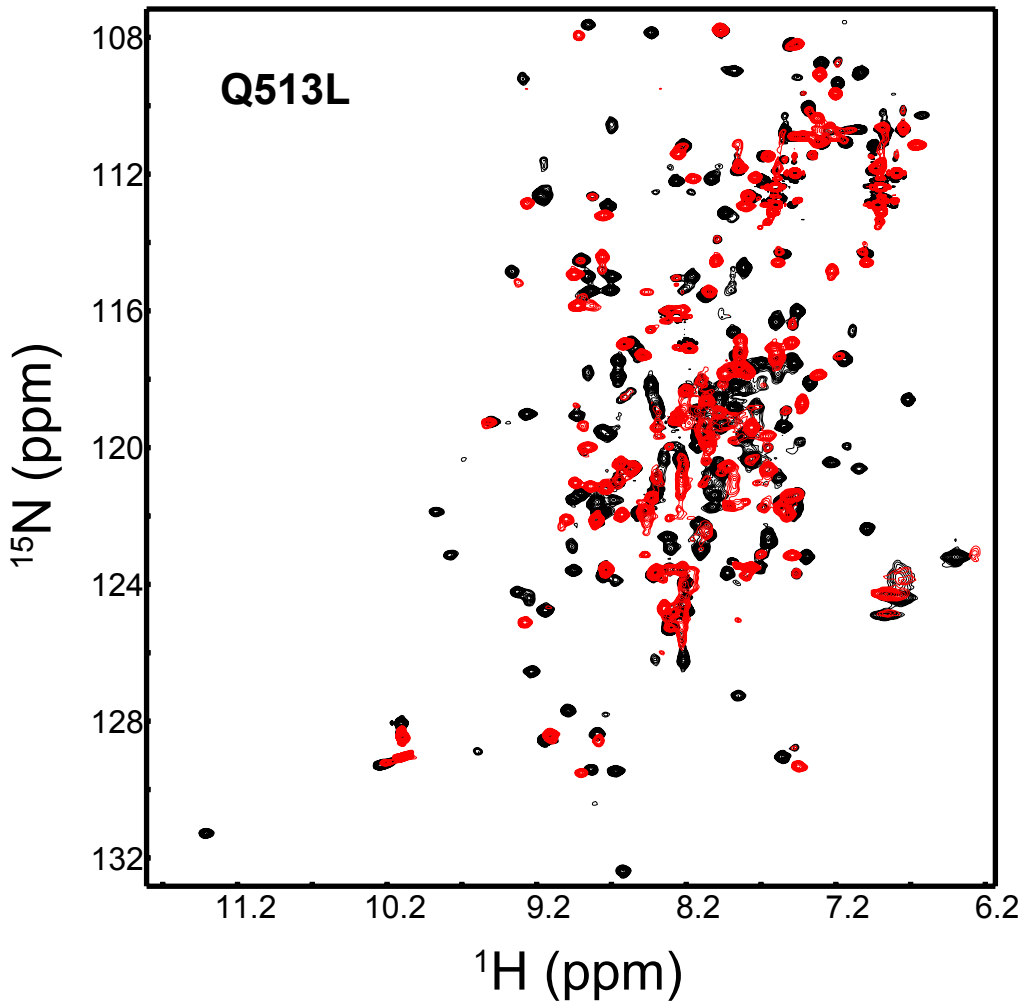

Supporting Fig. 1b
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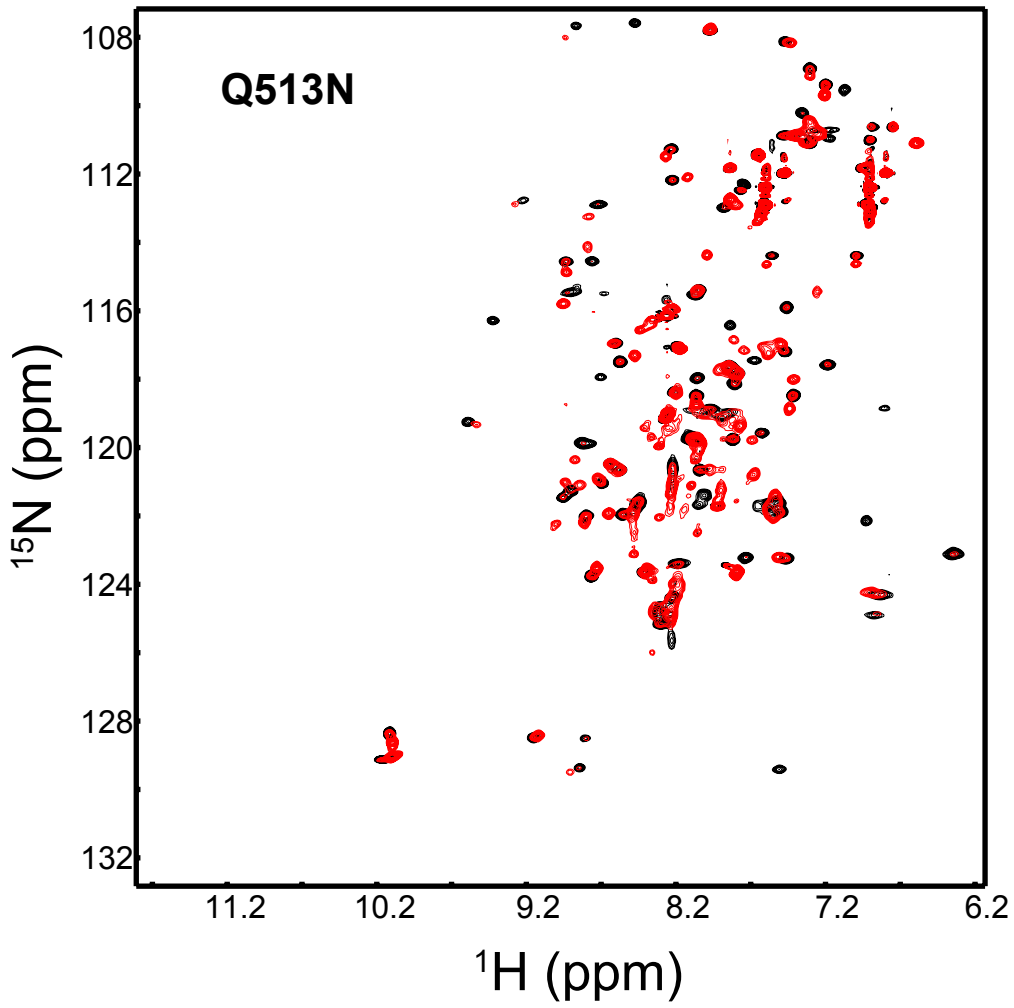

Supporting Fig. 1c
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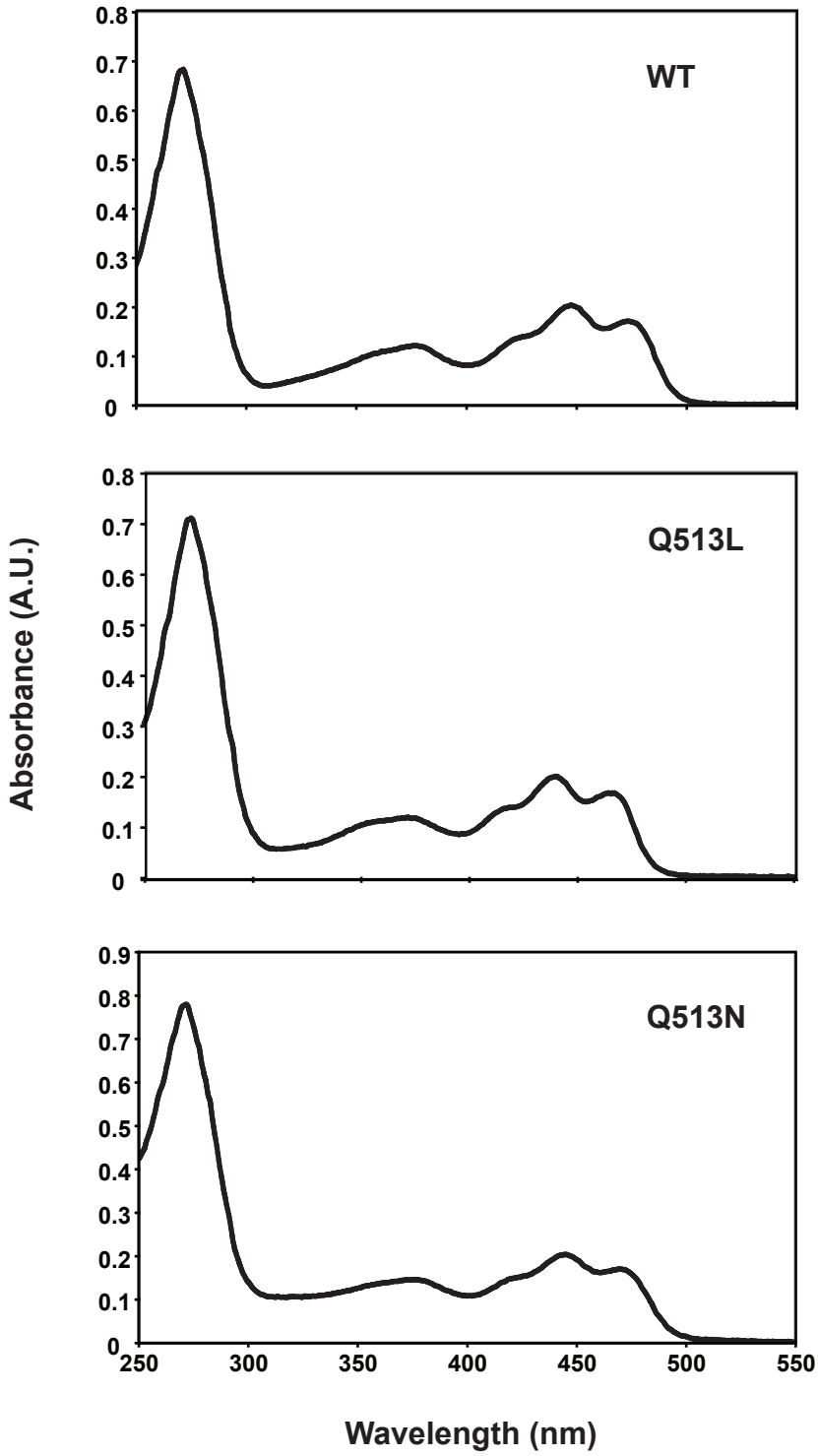

Supporting Fig. 2
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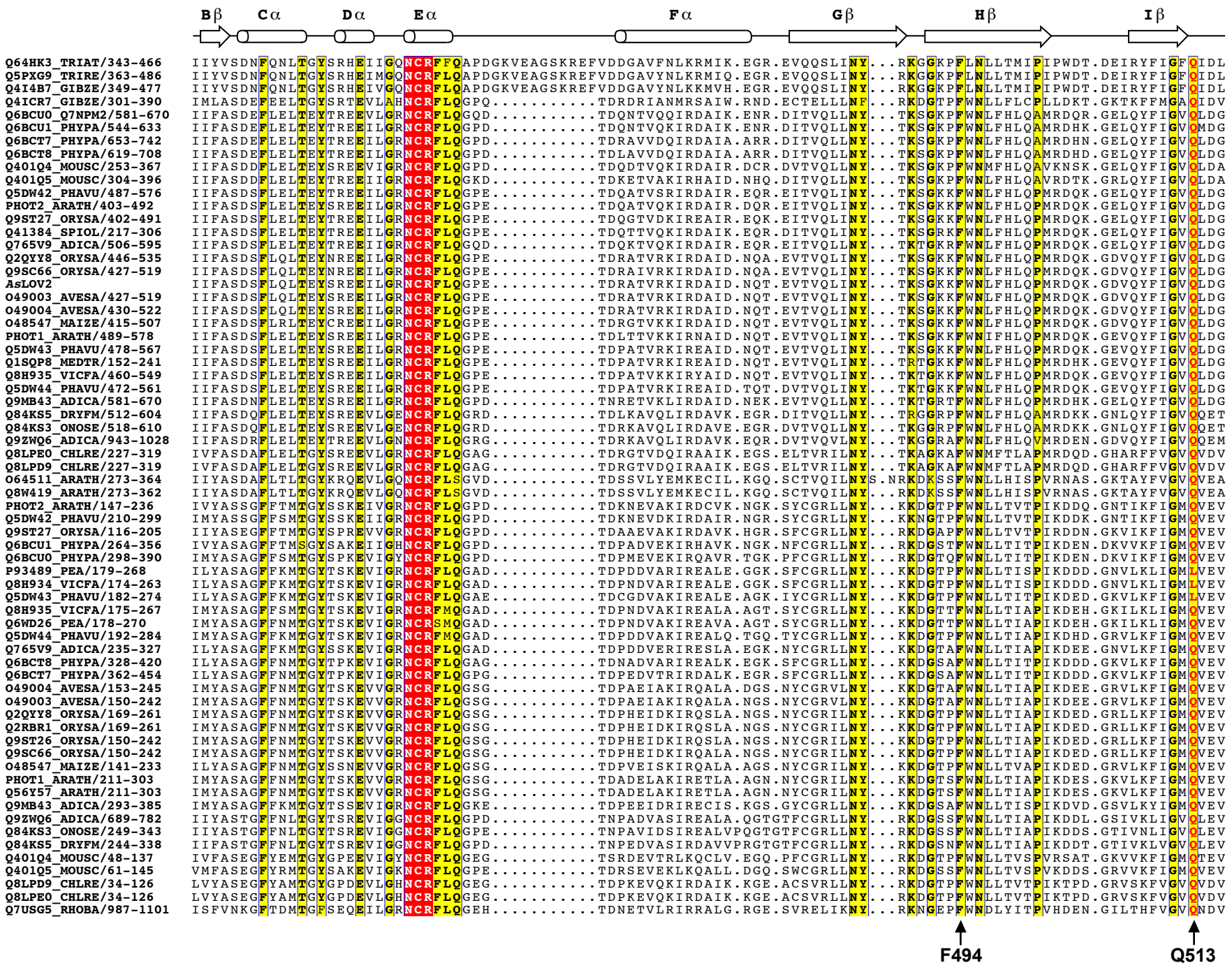

Supporting Fig. 3
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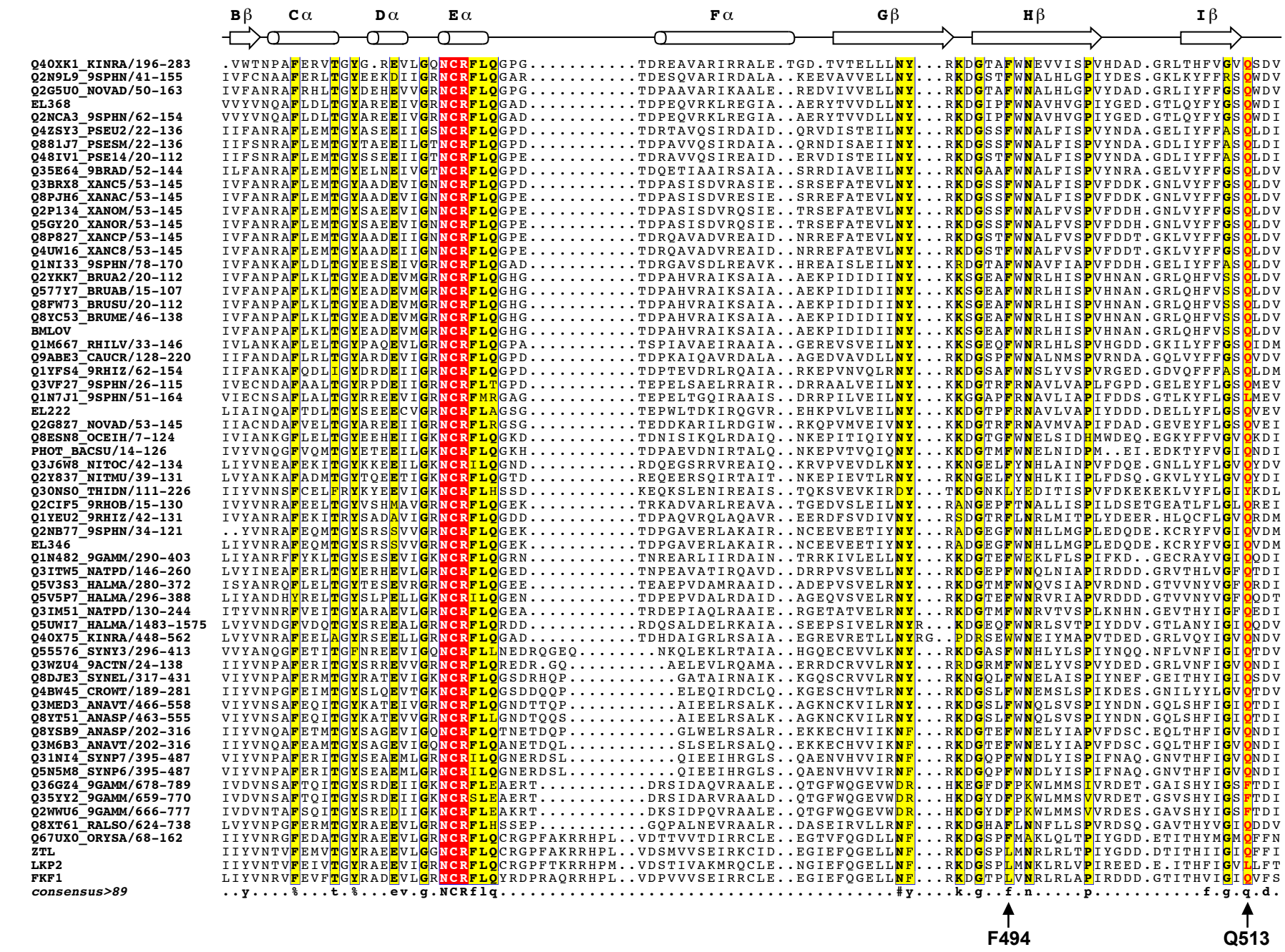

Supporting Fig. 3, cont.
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