

## **SUPPLEMENTARY FIGURE LEGENDS**

**FIG S1.** Amino acid conservation amongst different DENV serotypes and related flaviviruses. The amino acid sequences of representative flavivirus isolates for DENV serotypes 1-4, WNV, YFV and JEV were aligned and compared using JalView software and the *ClustalW* algorithm, using default settings (see Materials and Methods for accession numbers). Residues are color-coded according to the *ClustalW* algorithm. As generated by JalView, ‘Conservation’ provides a graphical and numerical indication of the number of conserved physico-chemical properties of residues at each position. ‘Quality’ provides a measure of the likelihood of observing mutations at a given position. ‘Consensus’ depicts the percentage of the modal residue for each position. The location of the 25 top-ranked tolerated insertion sites (P2 / P0) are highlighted in yellow.

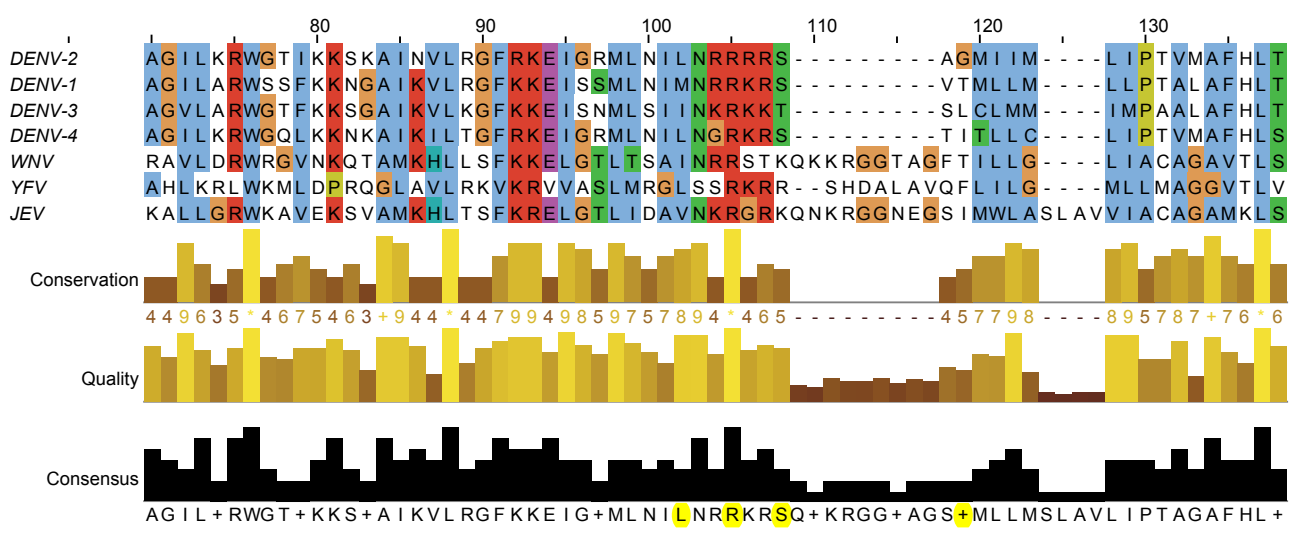
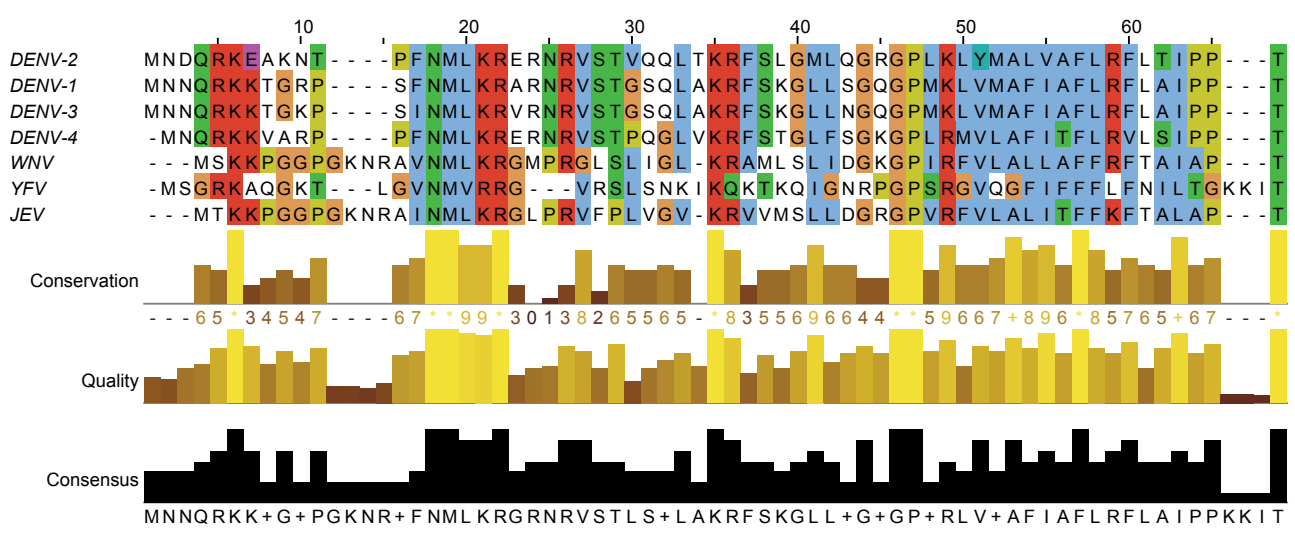
## **SUPPLEMENTARY MOVIE LEGENDS**

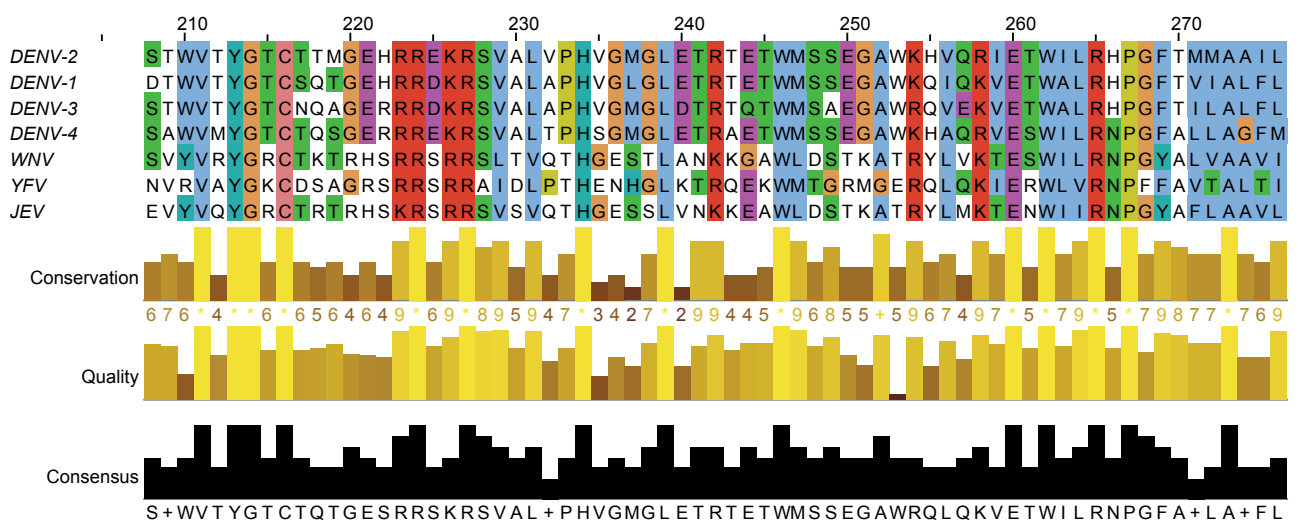
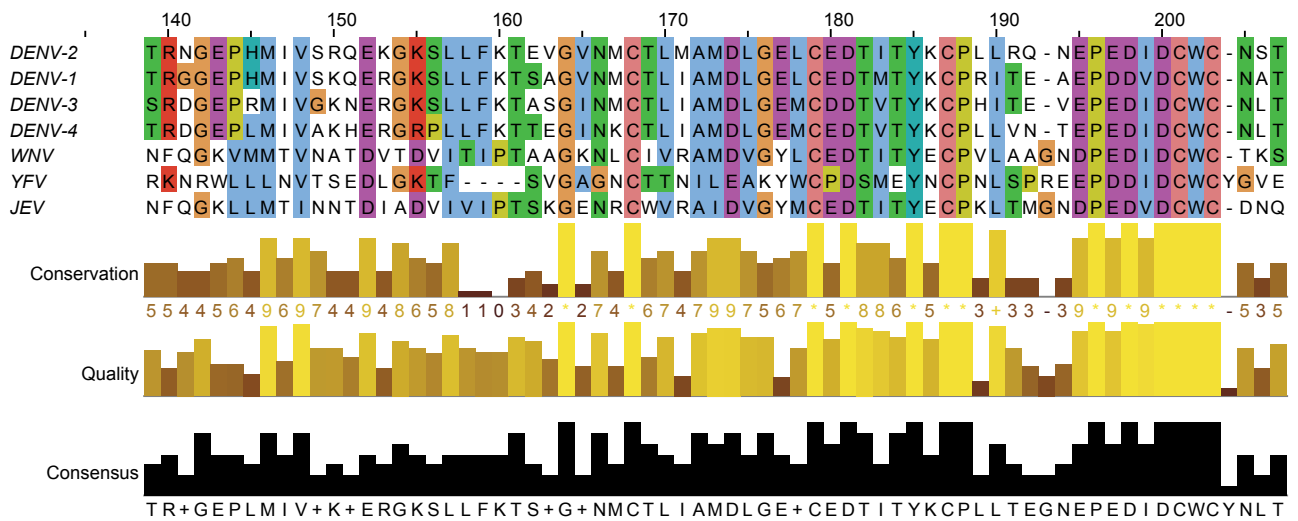
**Movie S1.** Live cell imaging of NS1-mScarlet localization and traffic. Huh-7.5 cells were transfected with DENV2-NS1-mScarlet RNA transcripts and cultured for 6 days prior to live cell imaging. Images were acquired every 1.5 s for 5 minutes. To enhance the visibility of weakly fluorescent structures the ‘Yellow Fire’ lookup table and scaling was applied according to the lookup tables displayed in the upper-right corner of each panel using NIS Elements v.3.22 software (Nikon). The ‘time stamp’ is displayed in the upper-left corner of each panel. Playback speed is 5 frames/s (~8× original speed). Scale bars are 10 μm for the main image (top panel) and 5 μm for ‘Insets’ (bottom panels).

**Movie S2.** Intense juxta-nuclear NS1-mScarlet foci are relatively static over extended periods (~50 mins). Huh-7.5 cells were infected with DENV2-NS1-mScarlet (M.O.I. ~0.01) for 3 d before live cell imaging. Images were acquired every 10 s for 50 minutes. To enhance the

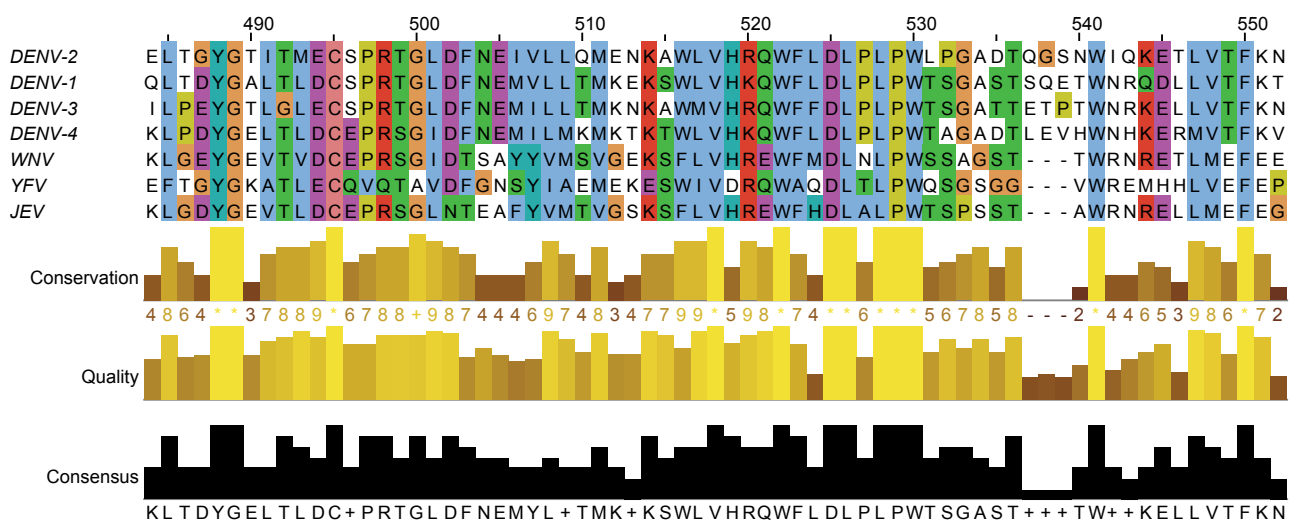
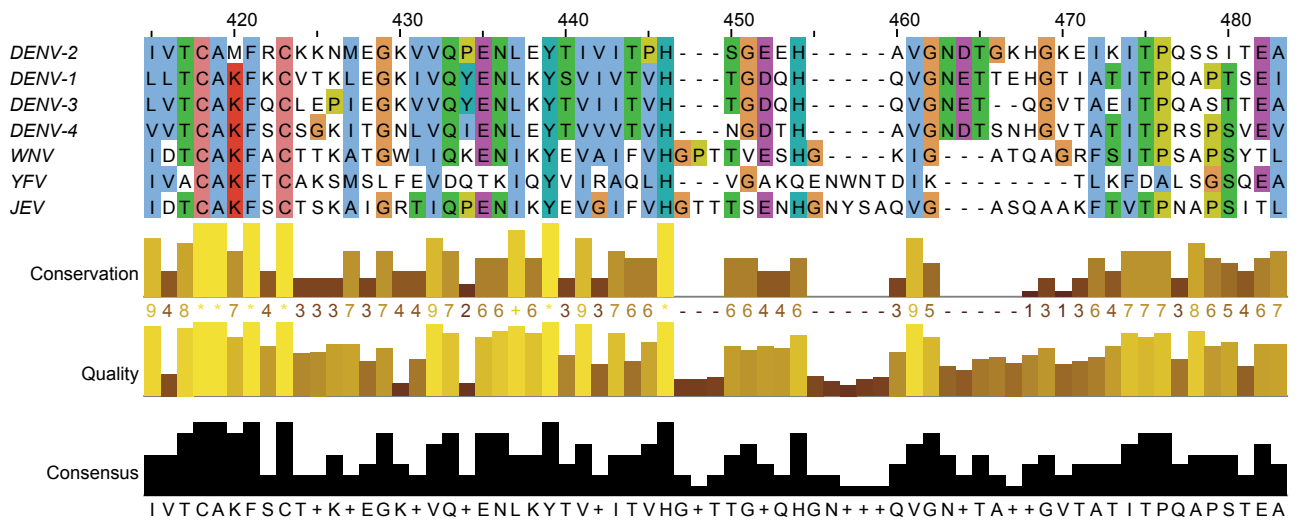
visibility of weakly fluorescent structures the 'Yellow Fire' lookup table and scaling was applied according to the lookup table displayed in the upper-right corner using NIS Elements v.3.22 software (Nikon). The 'time stamp' is displayed in the upper-left corner. Playback speed is 5 frames/s (~50× original speed). The scale bars represents 10  $\mu\text{m}$ .

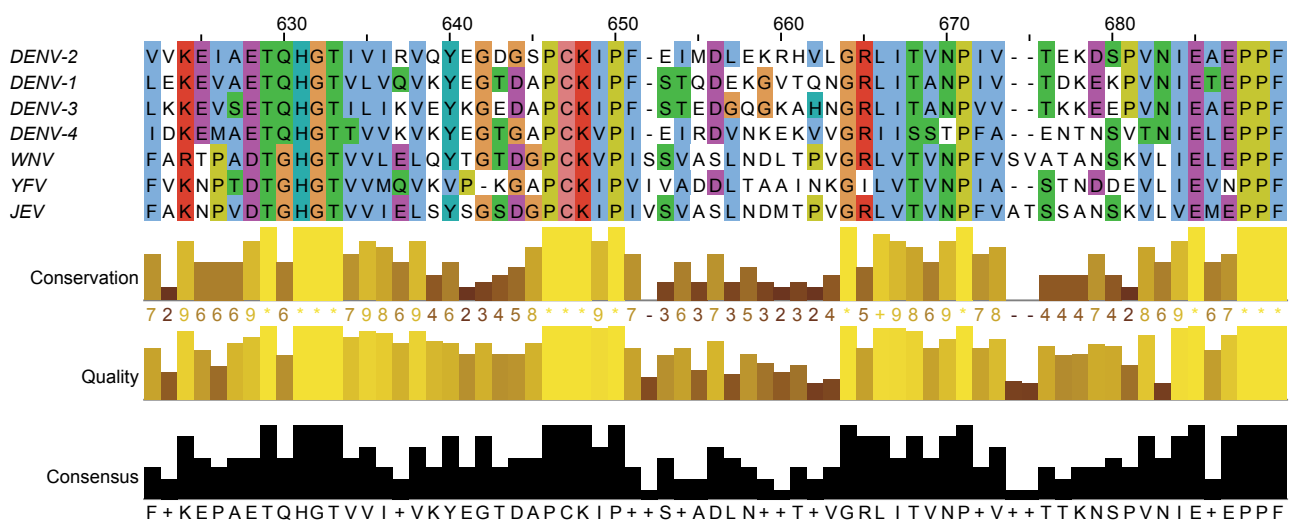
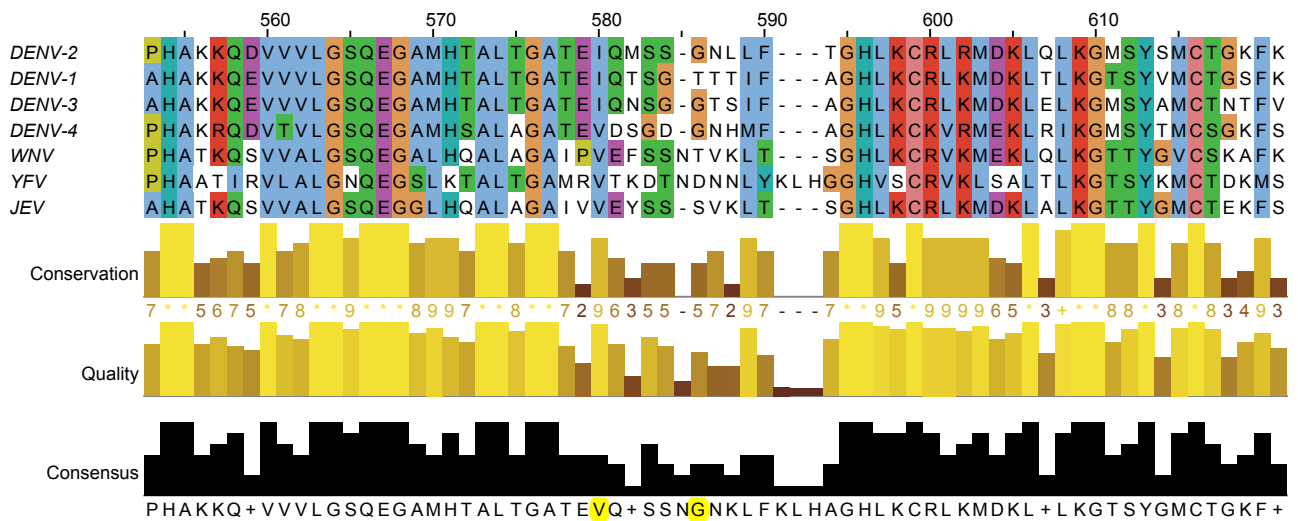
**Fig. S1**

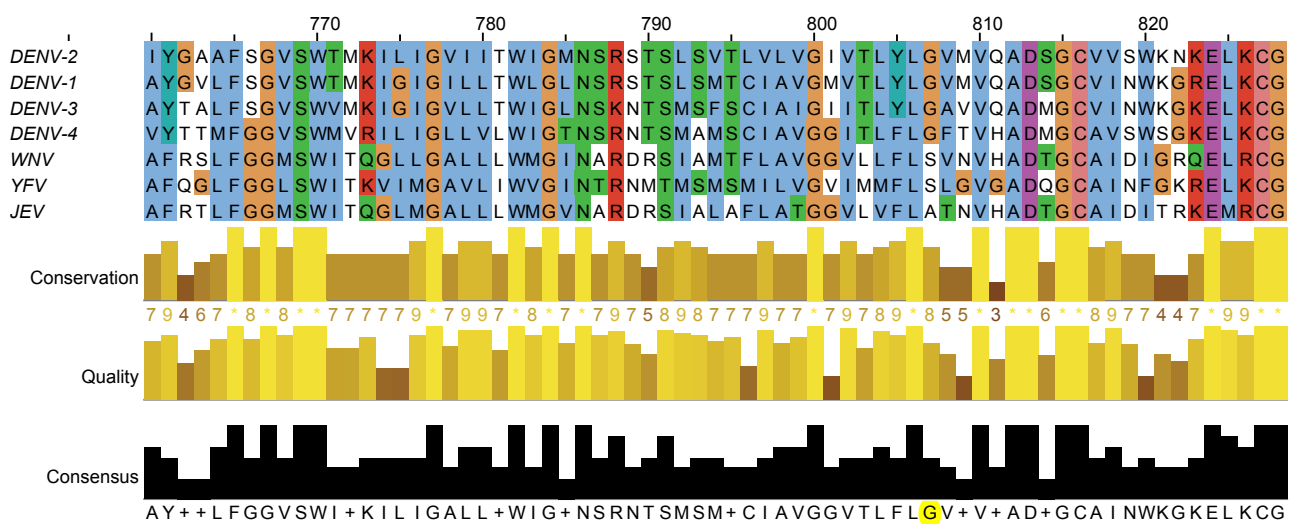
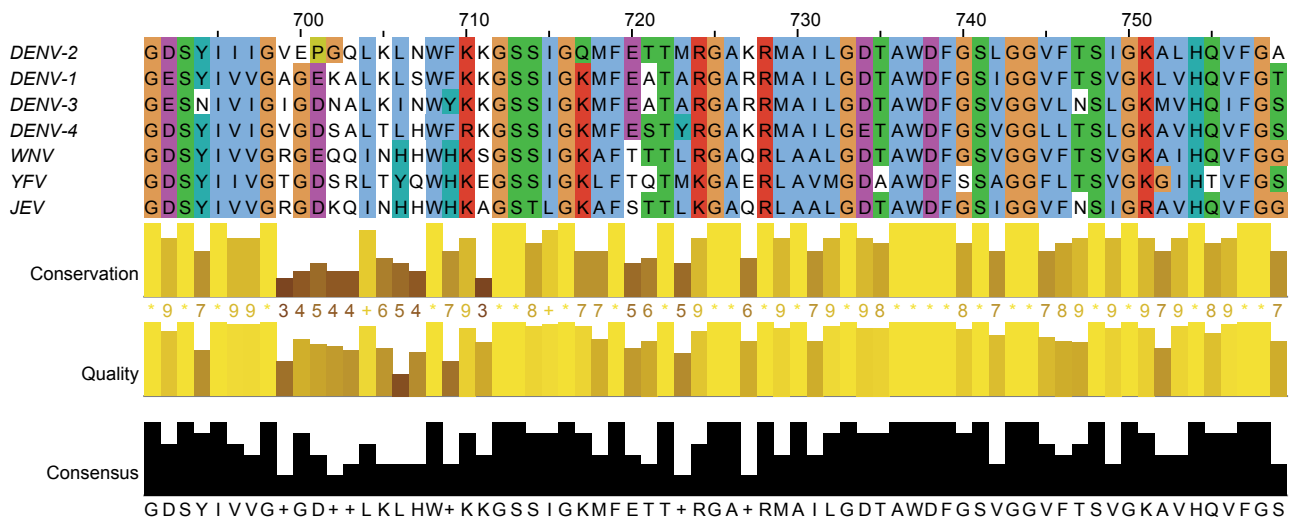




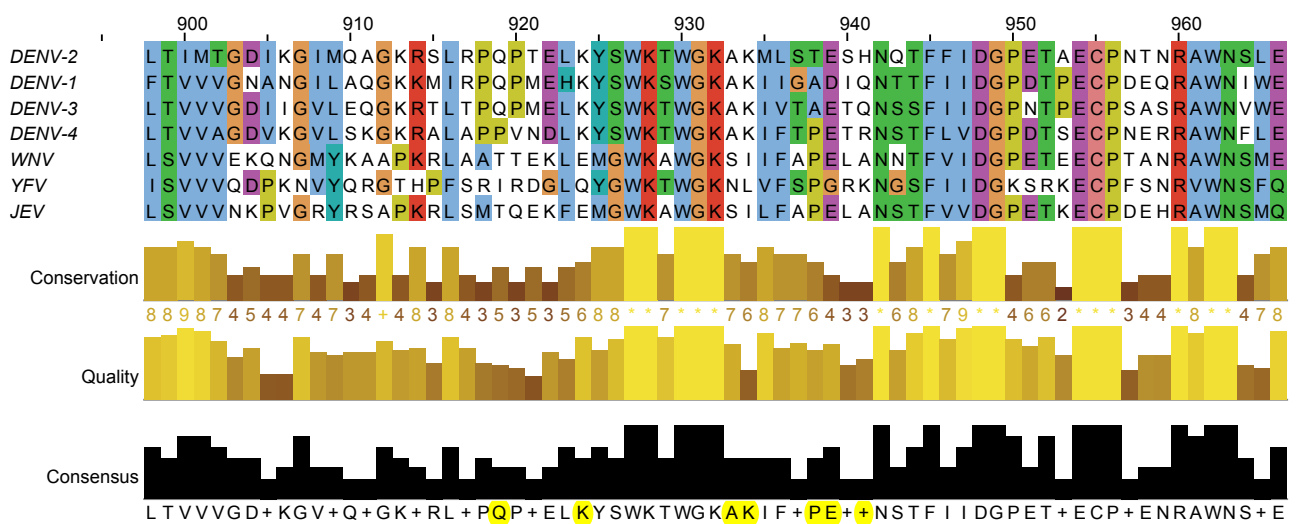
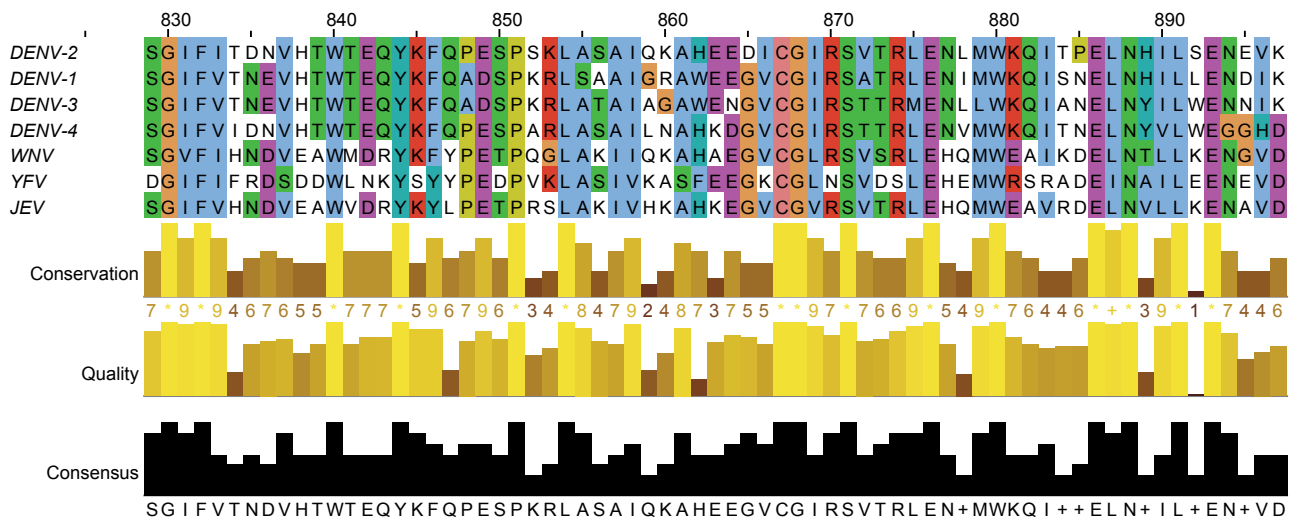


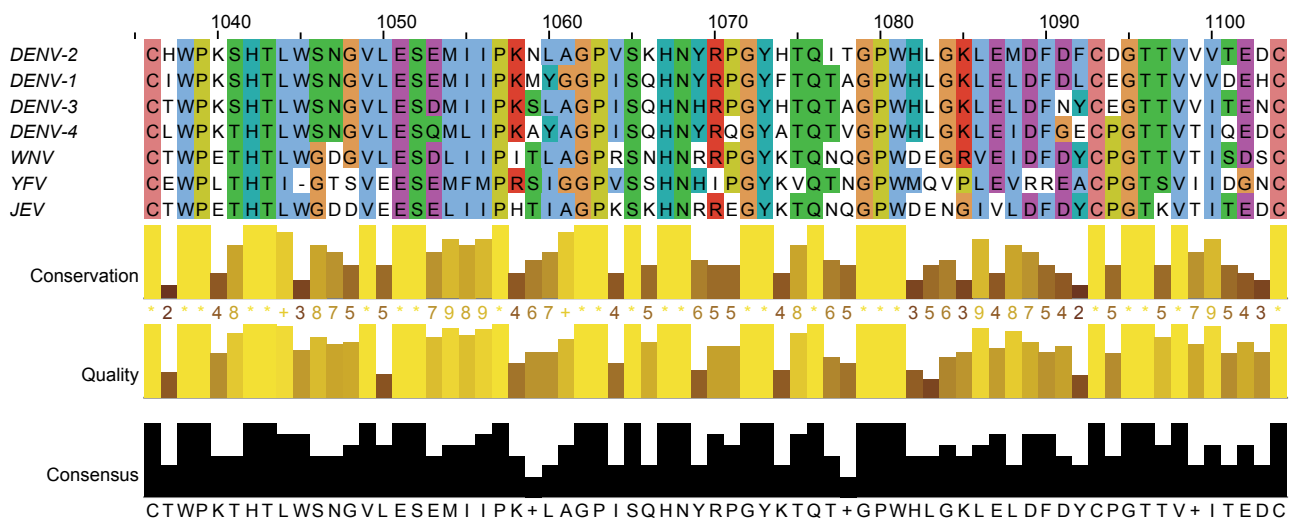
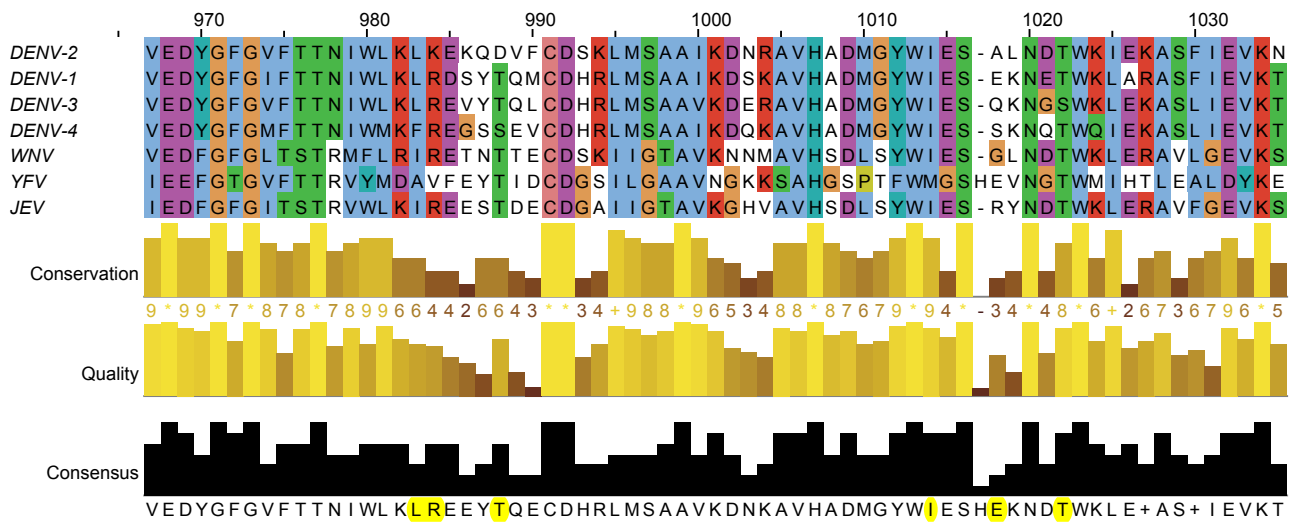


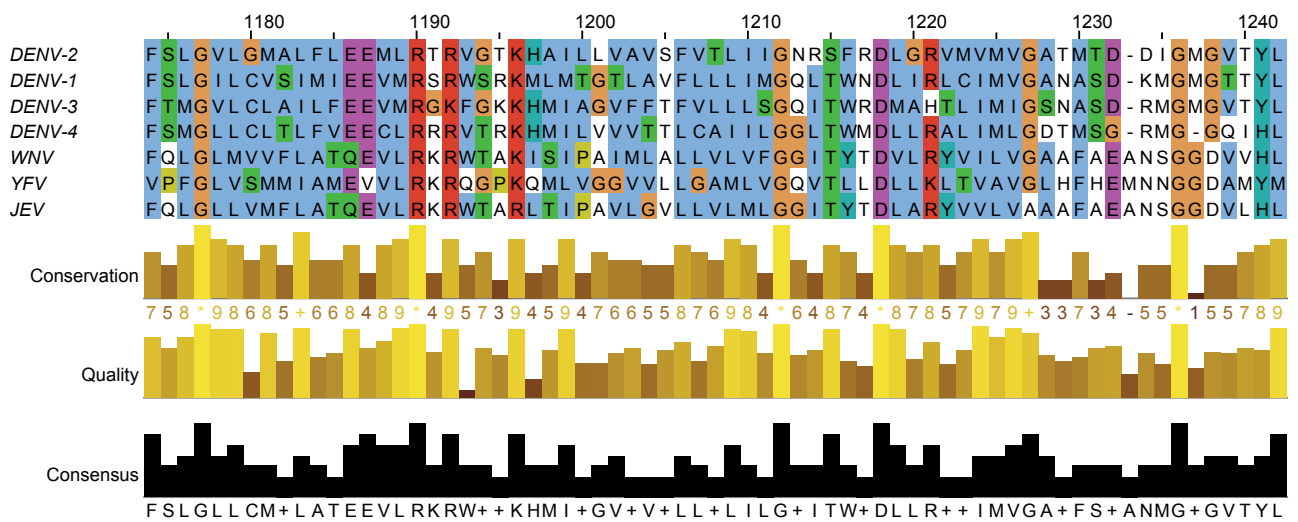
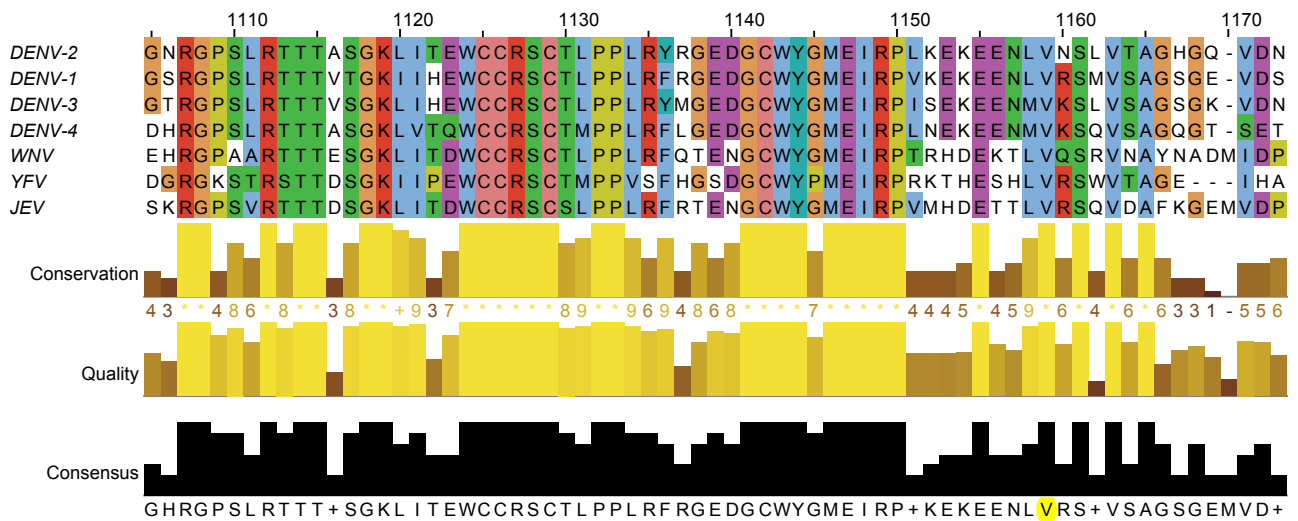


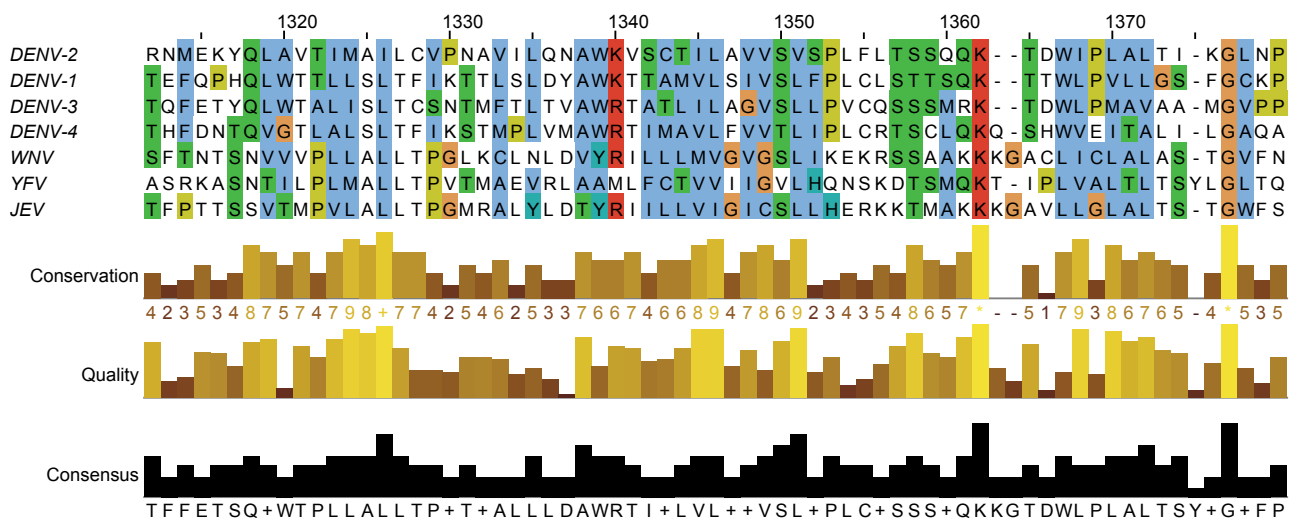
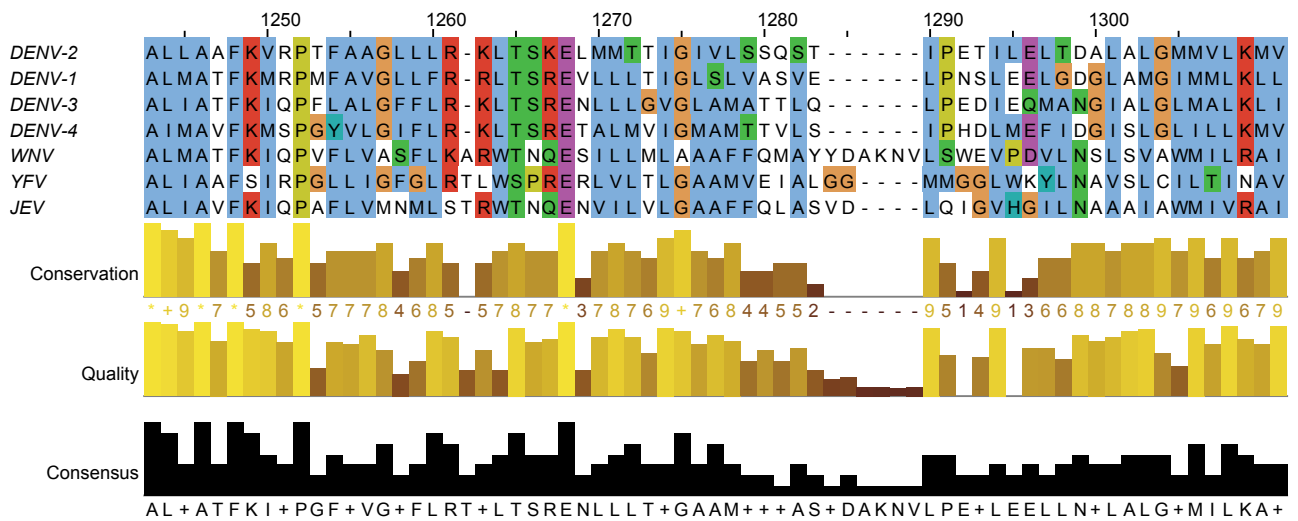


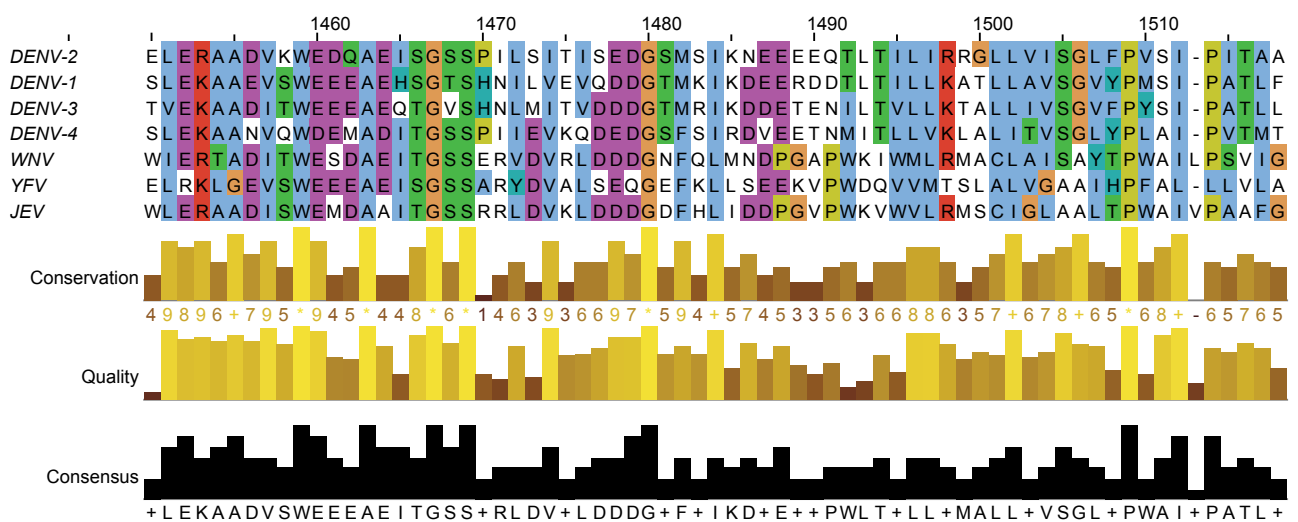
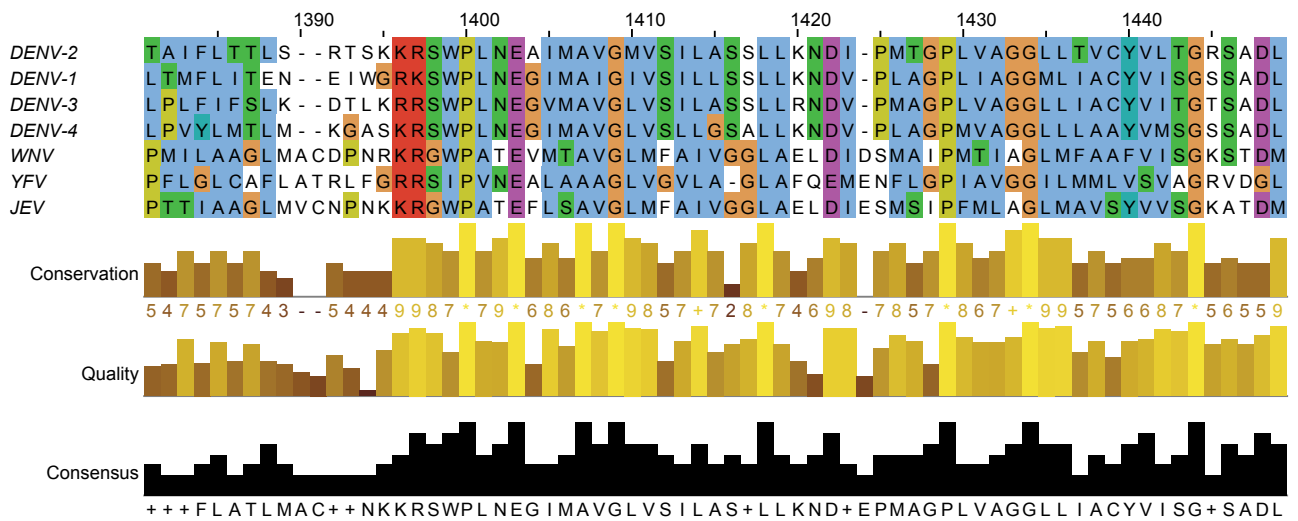


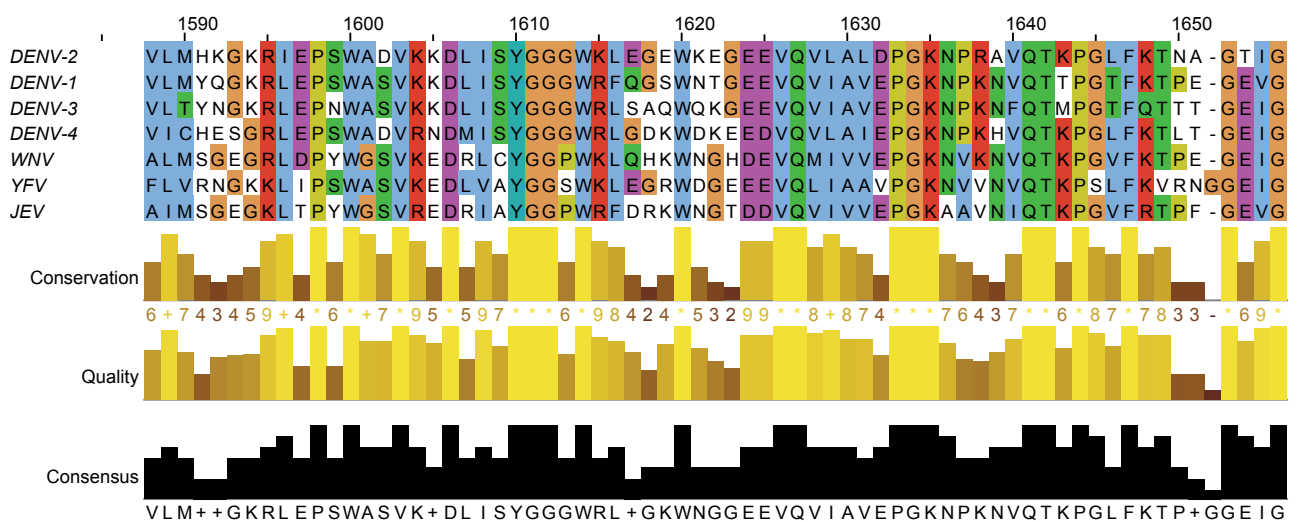
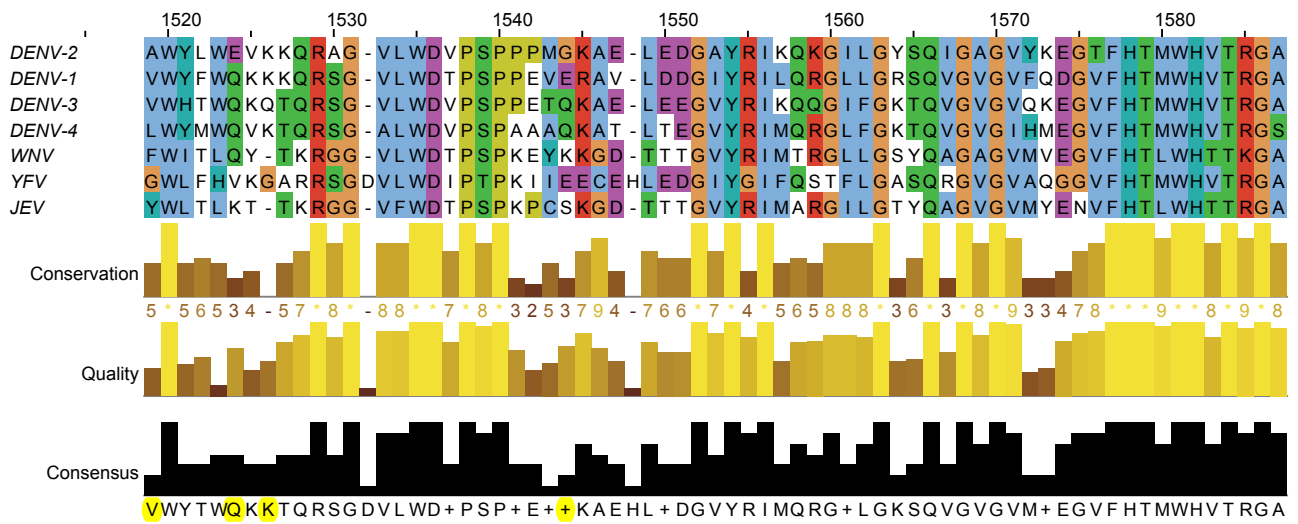


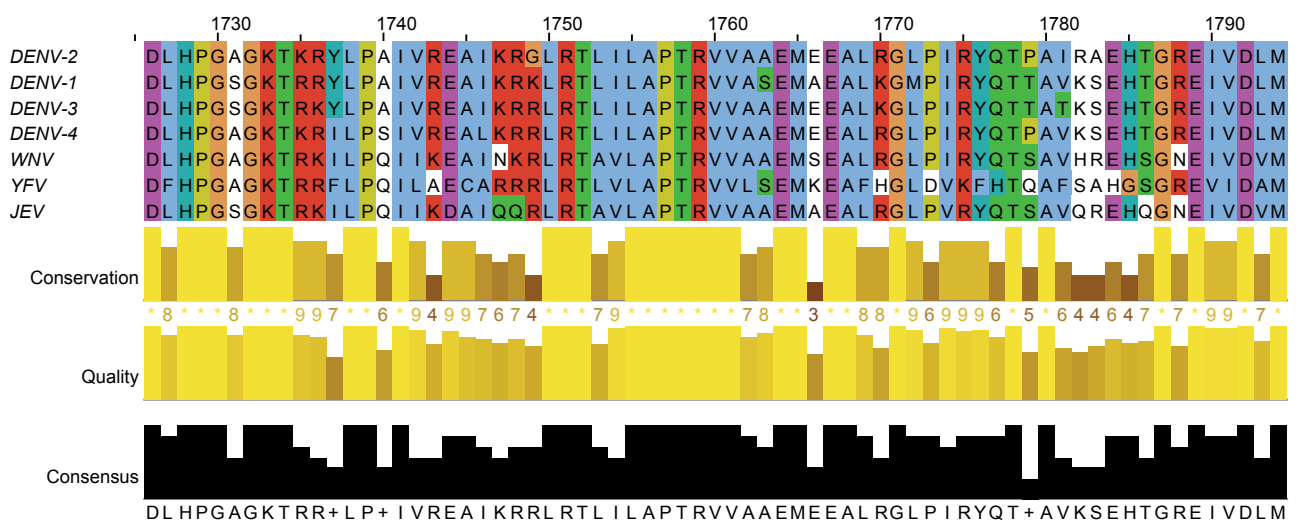
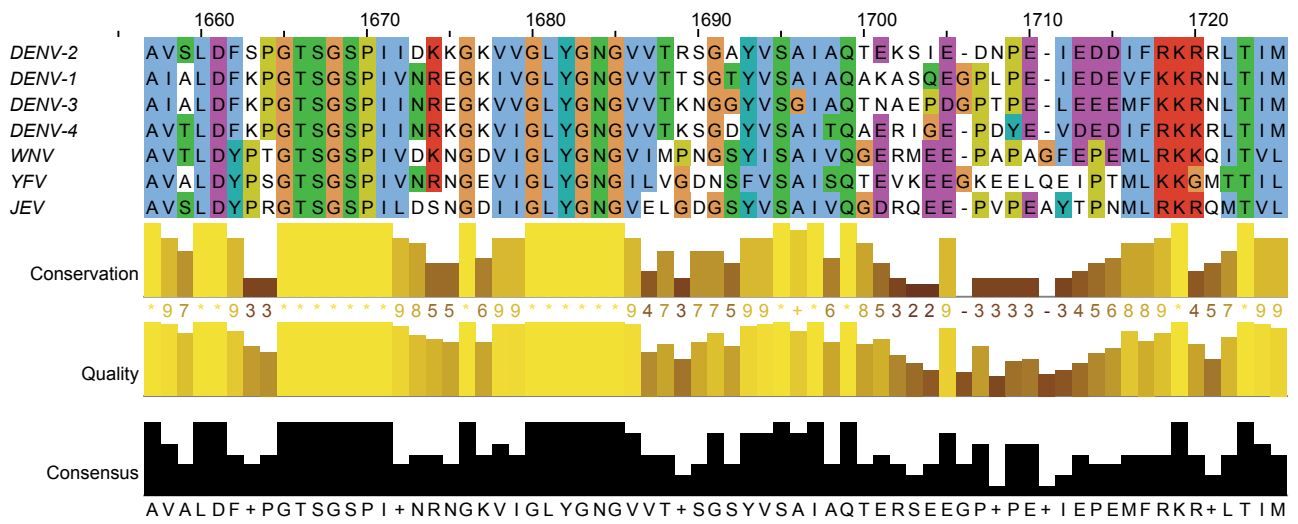


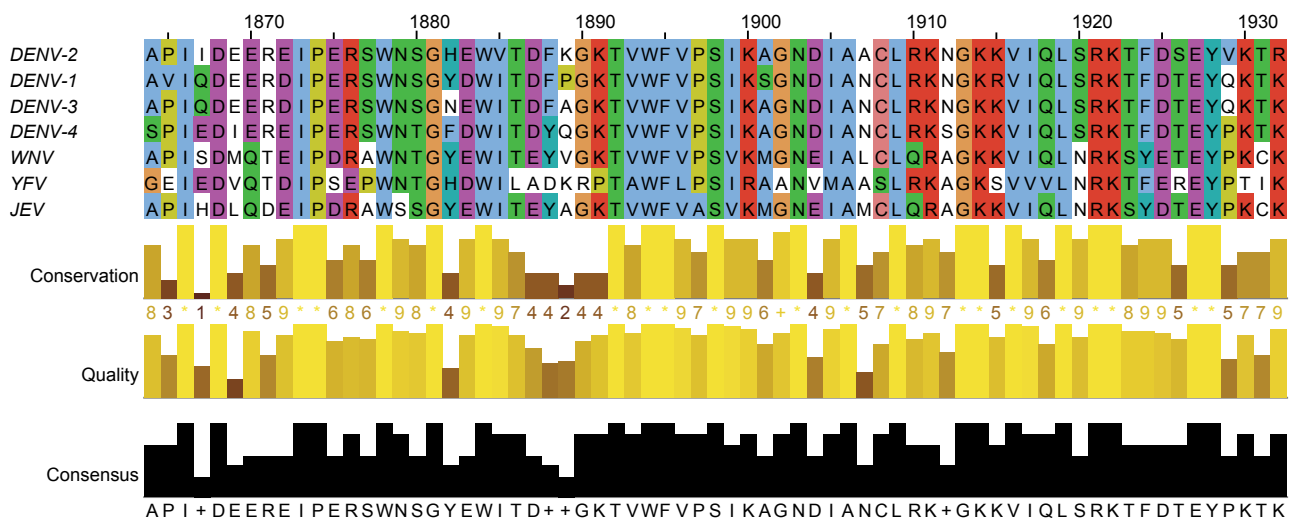
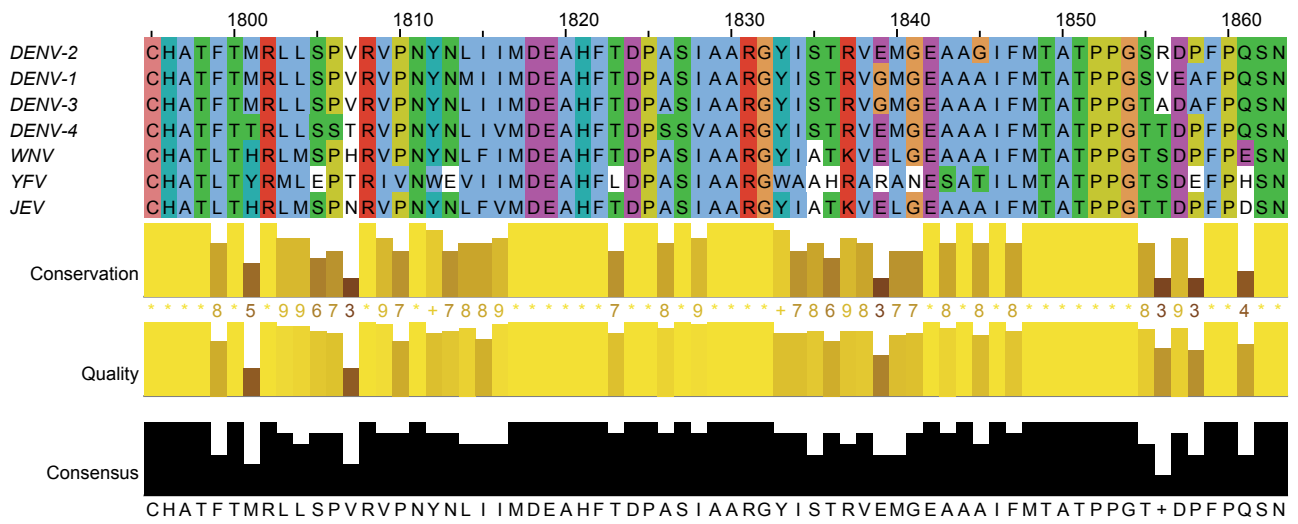




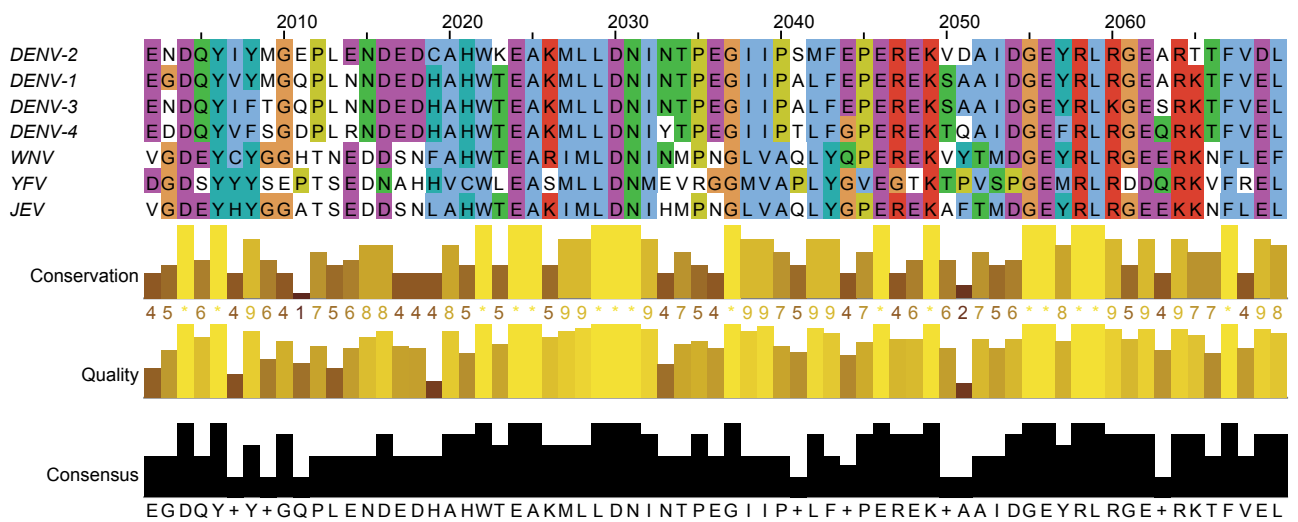
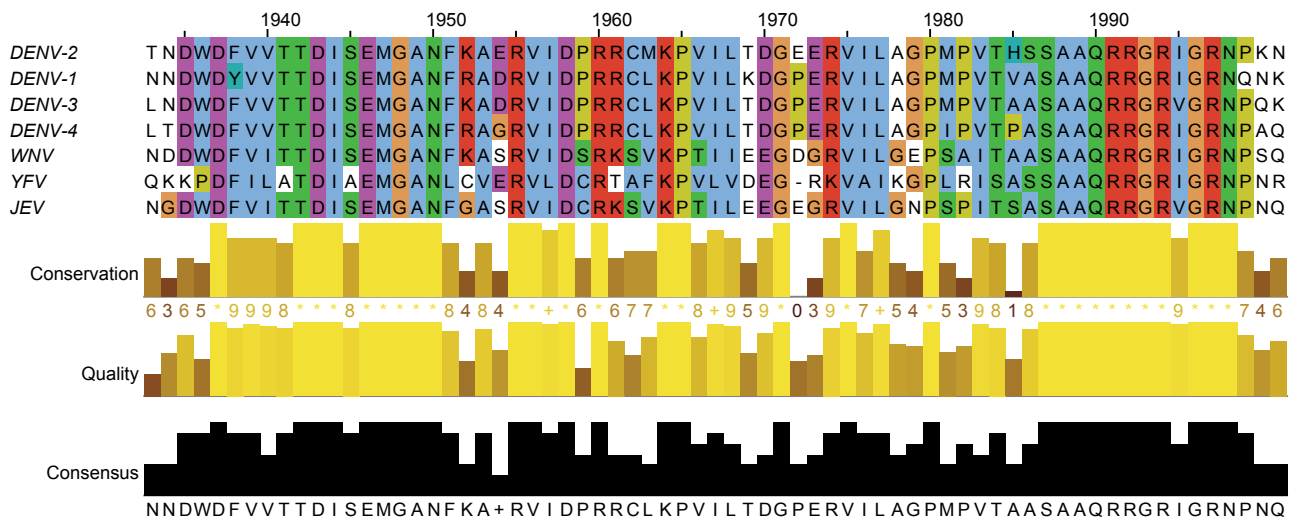


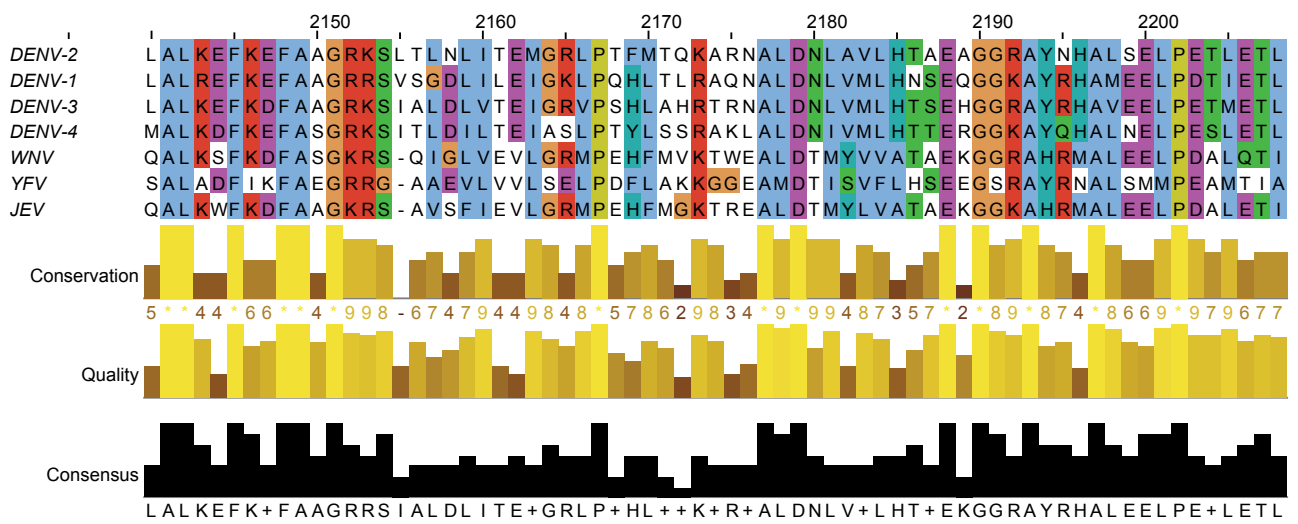
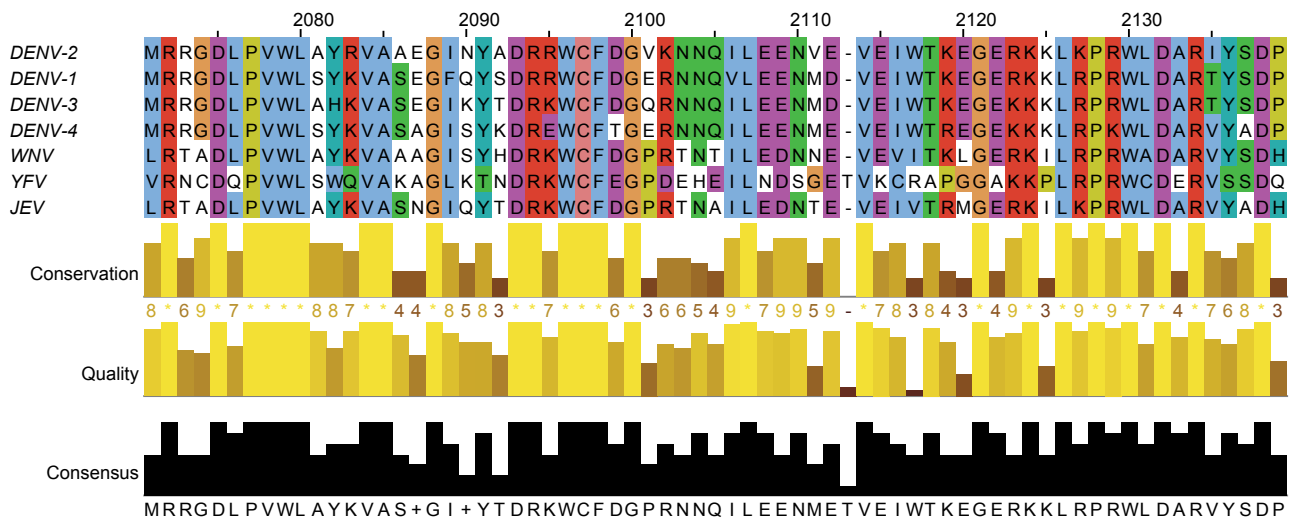


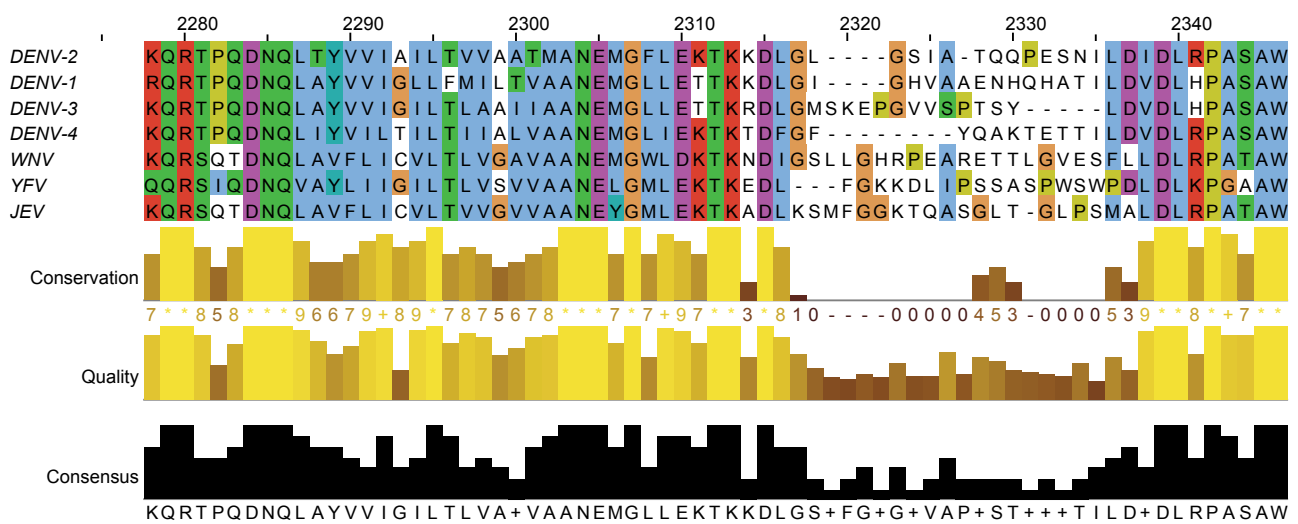
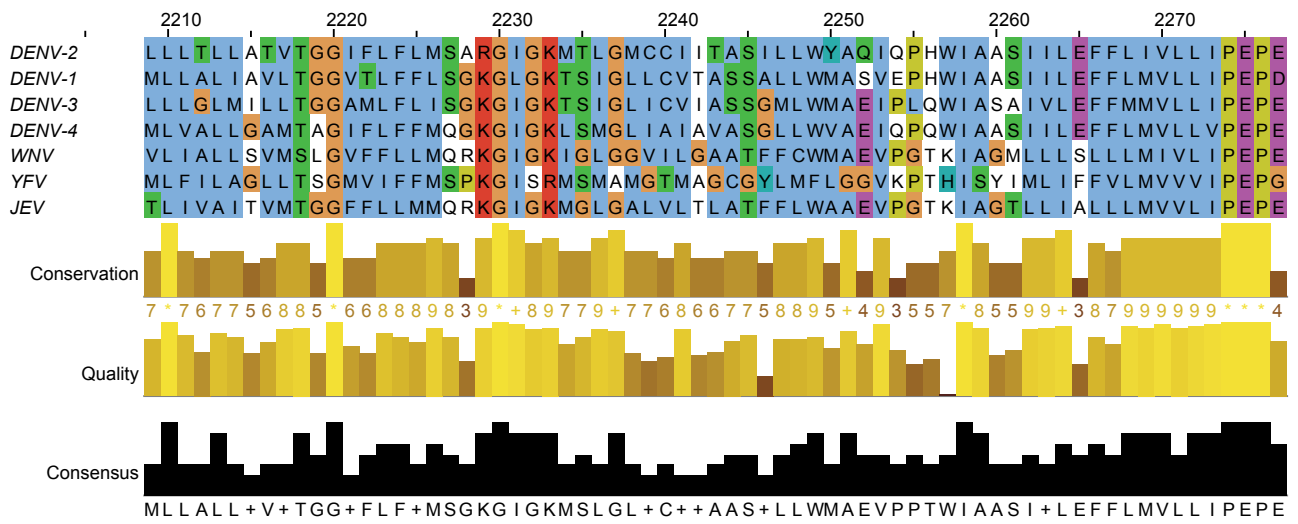


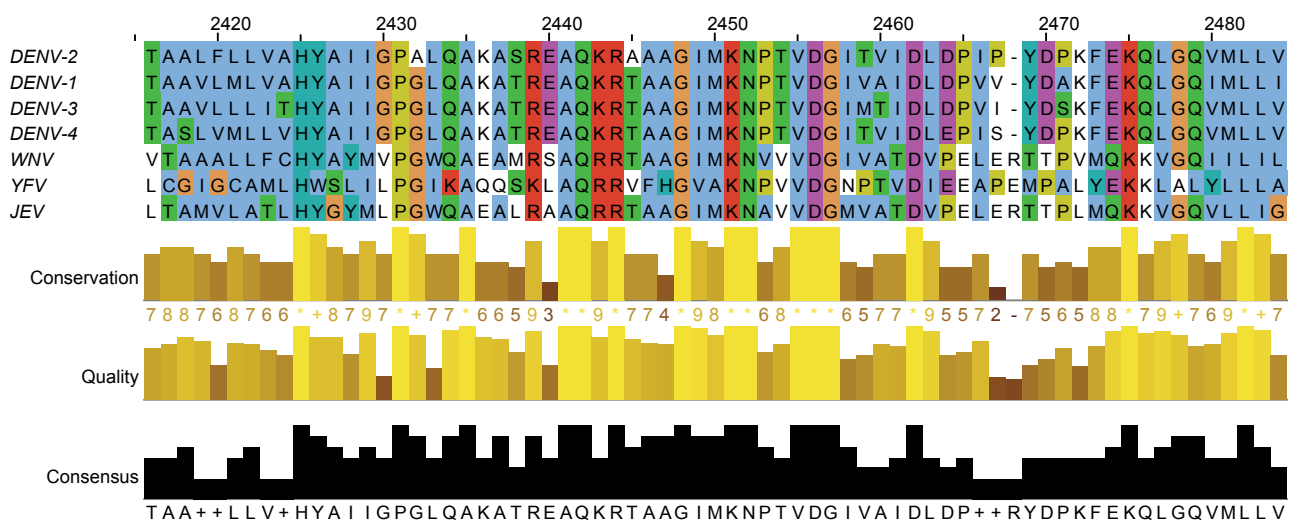
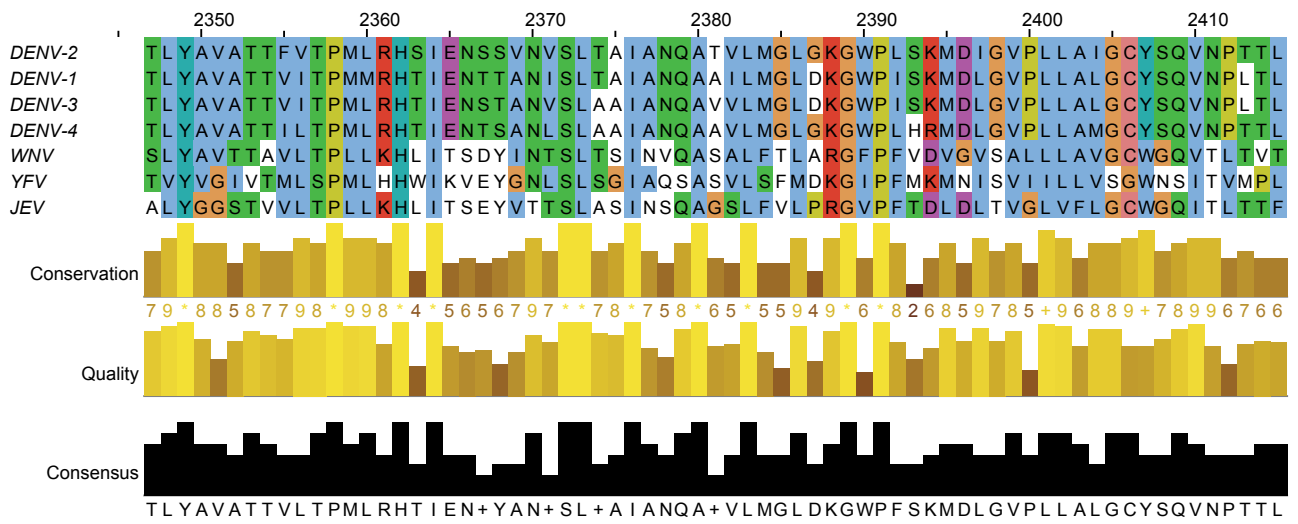


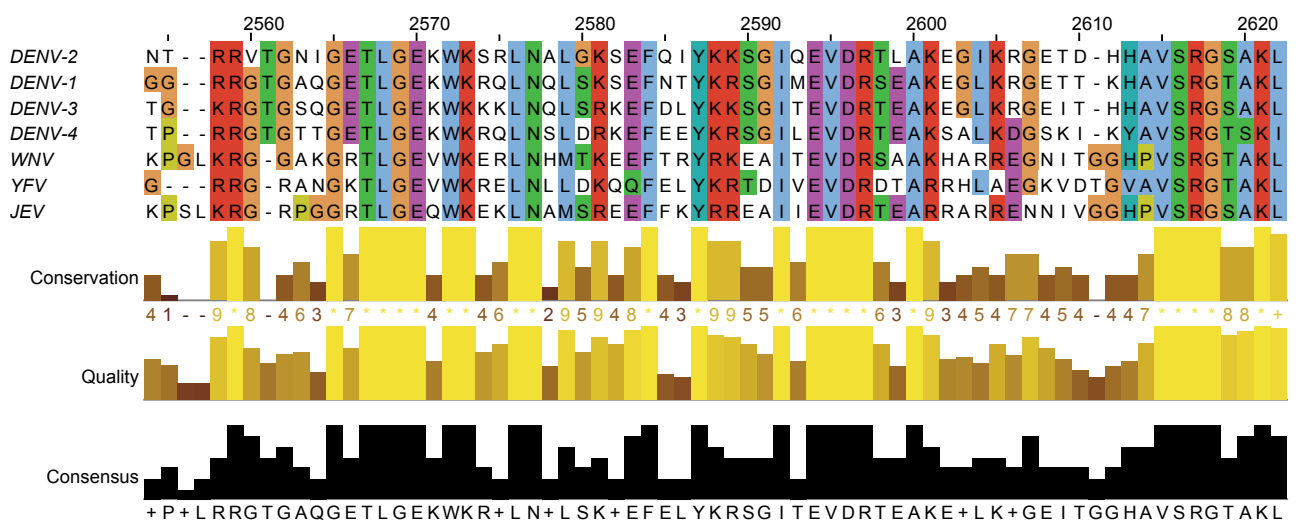
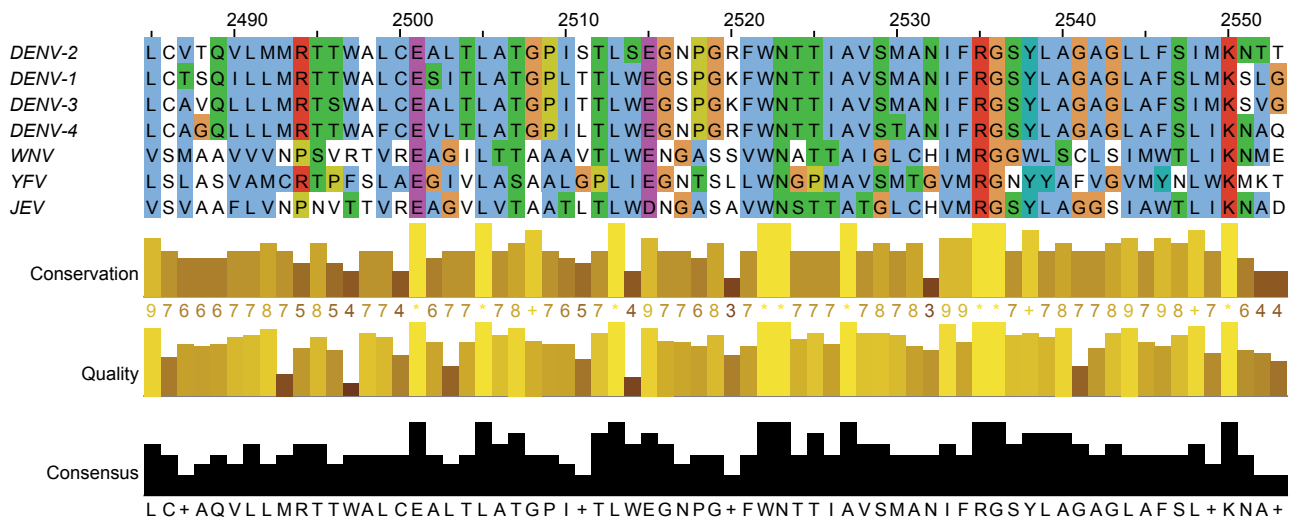


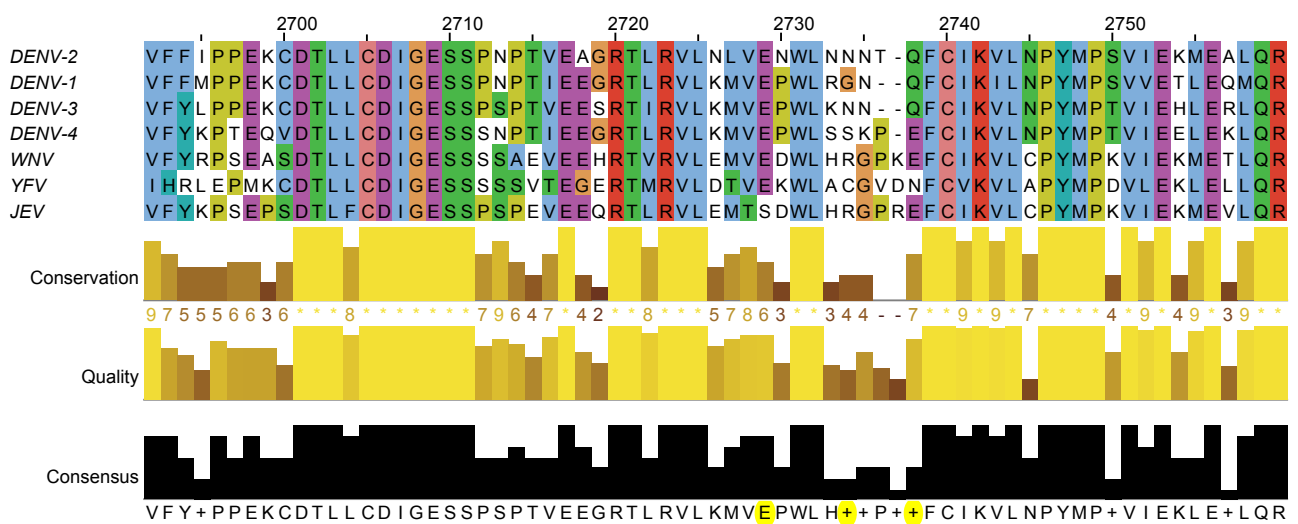
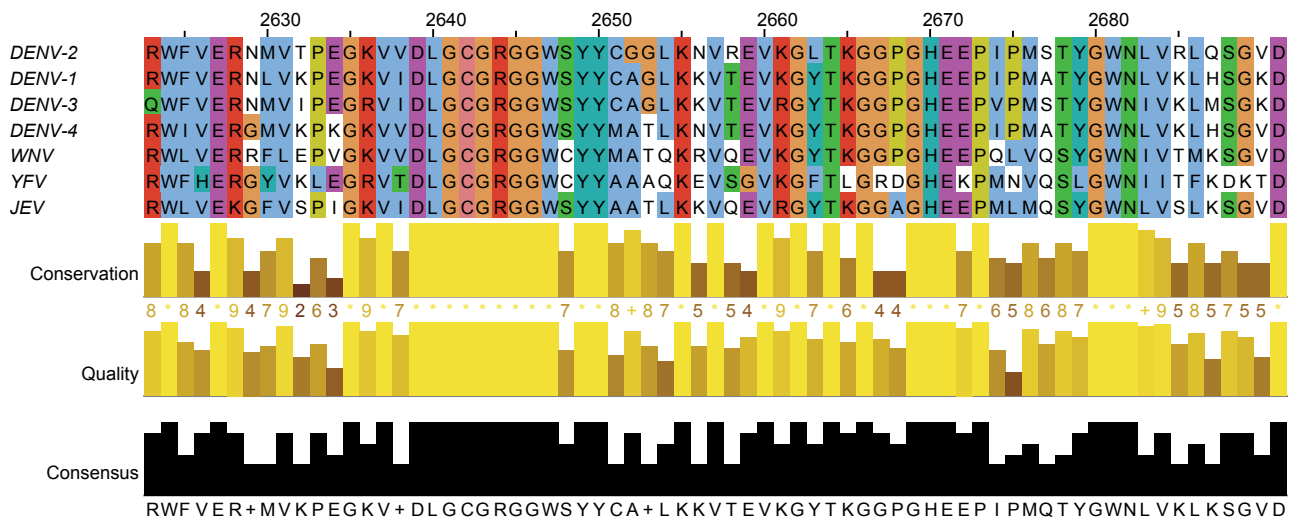


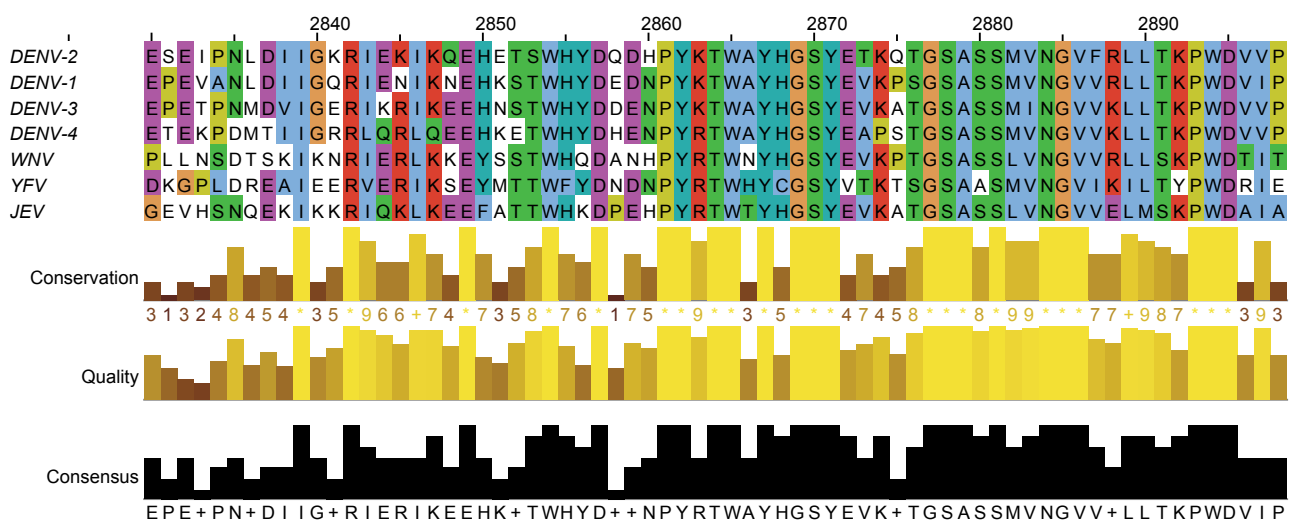
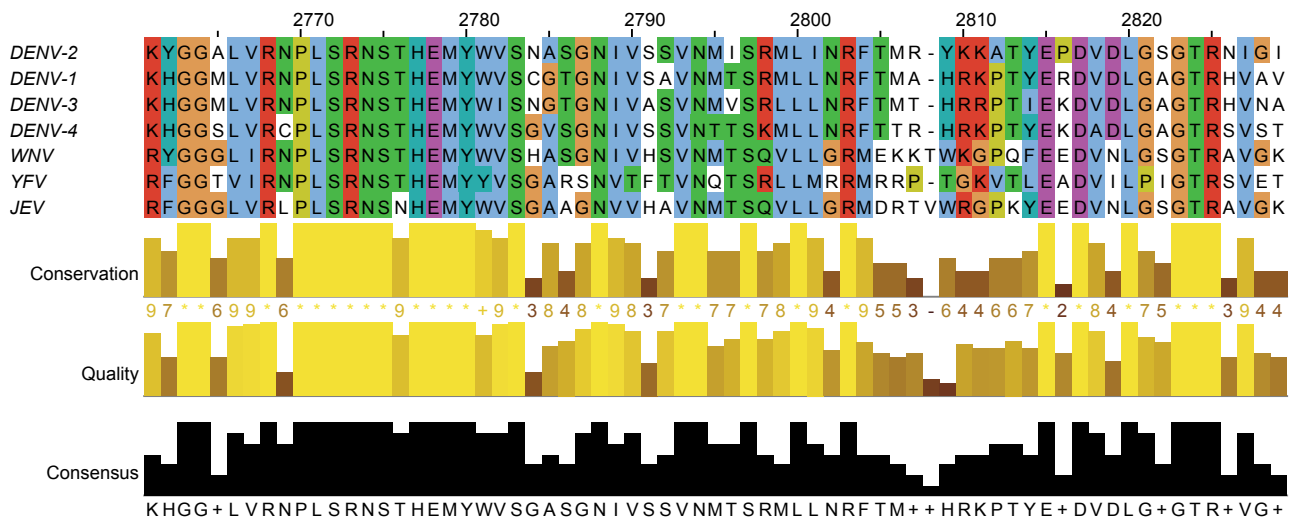


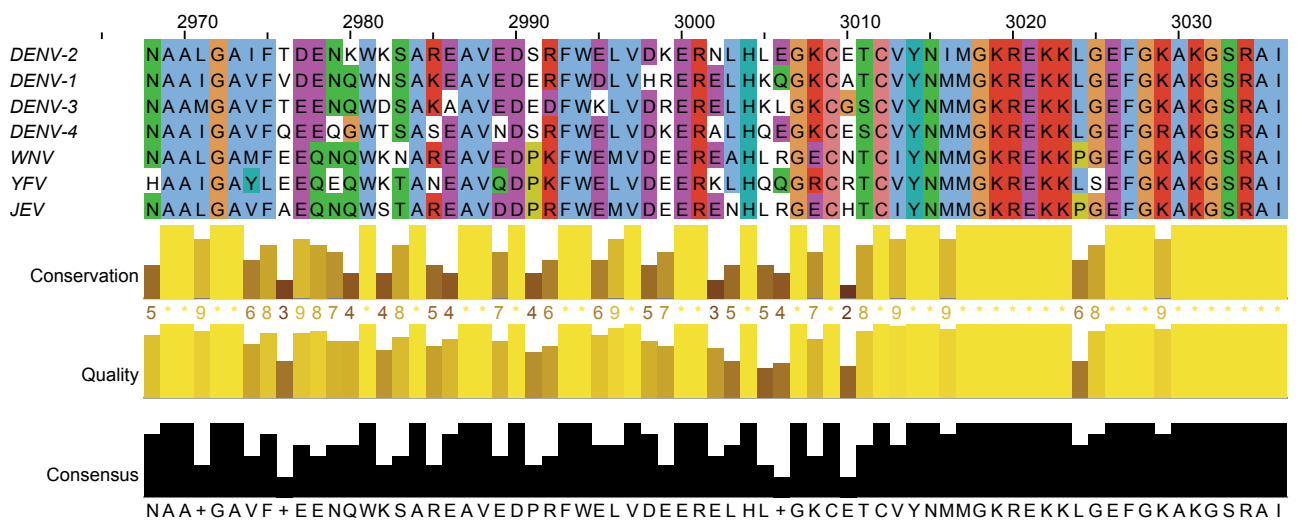
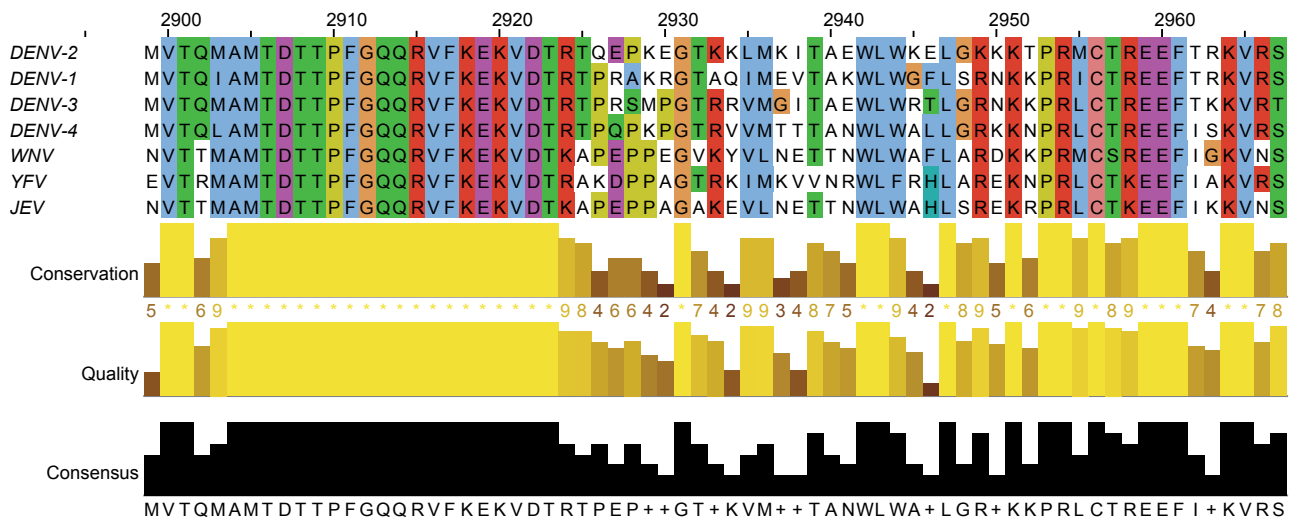




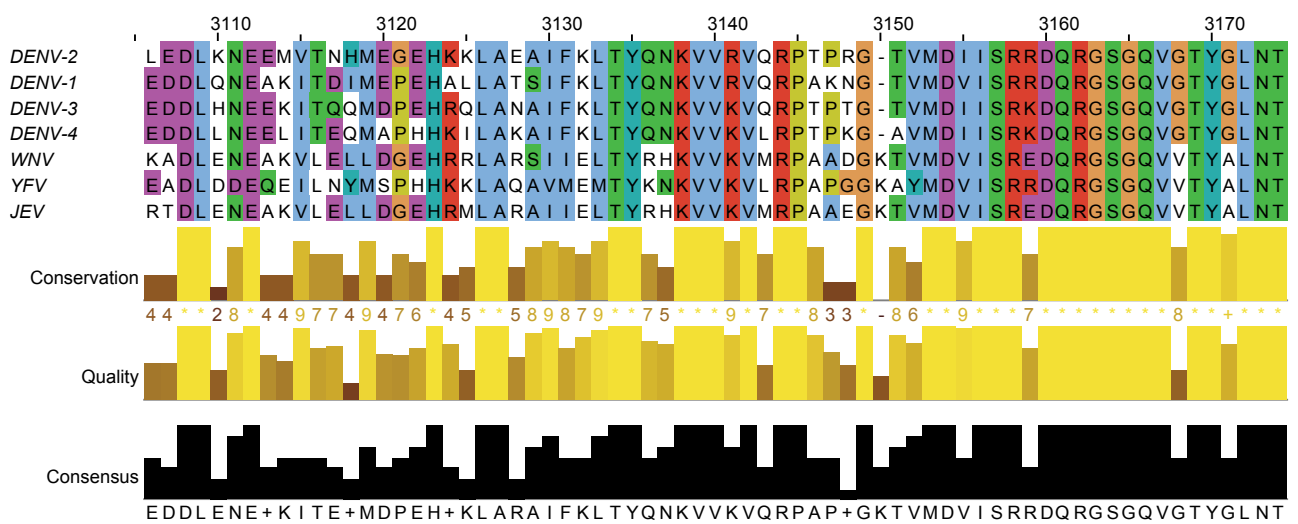
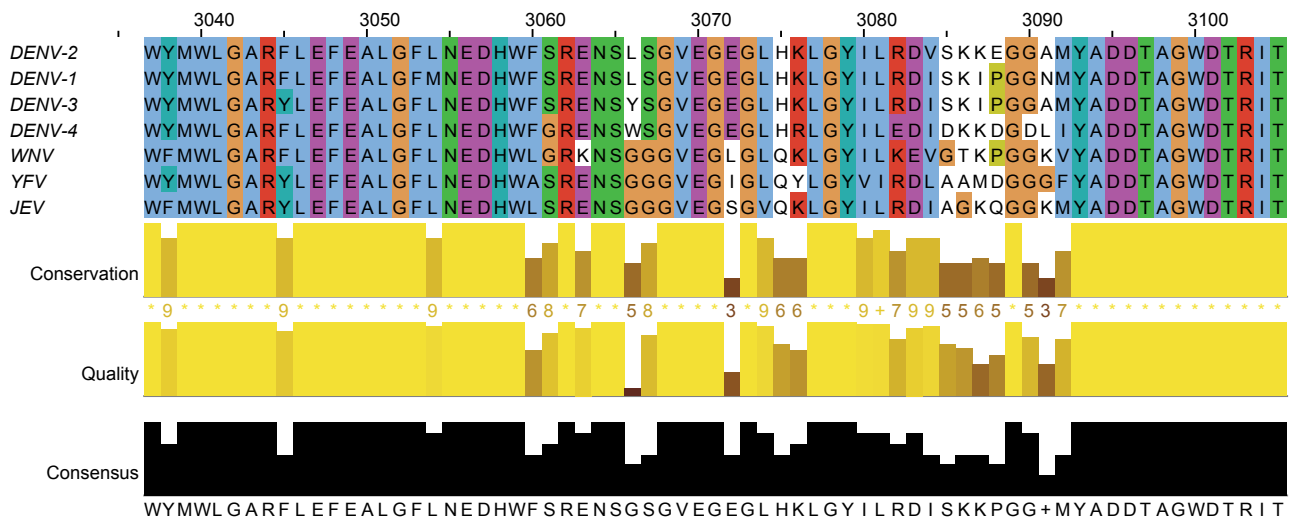


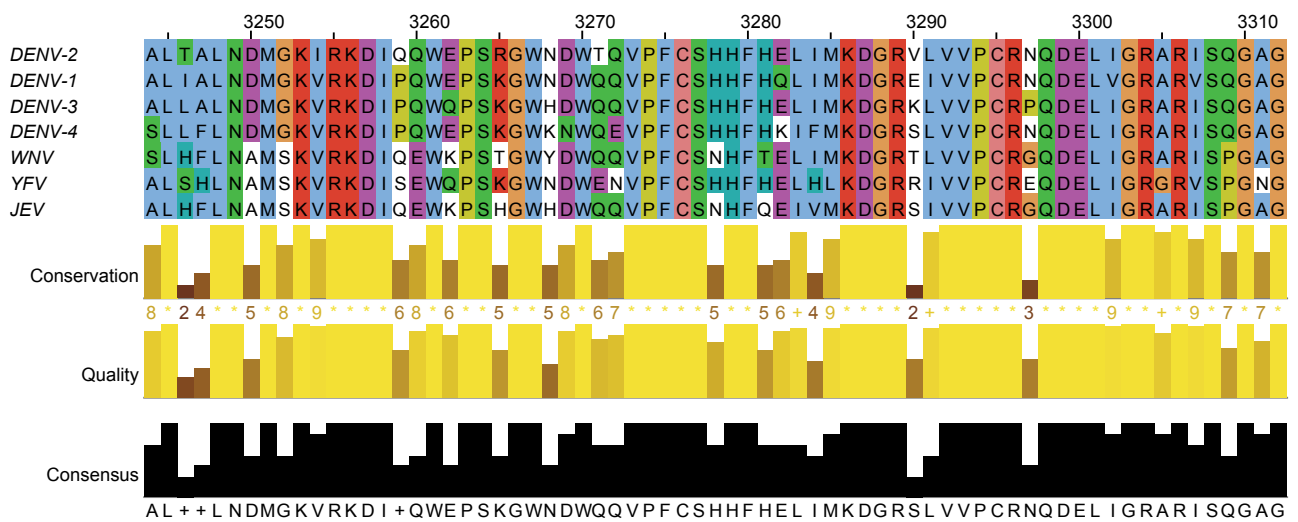
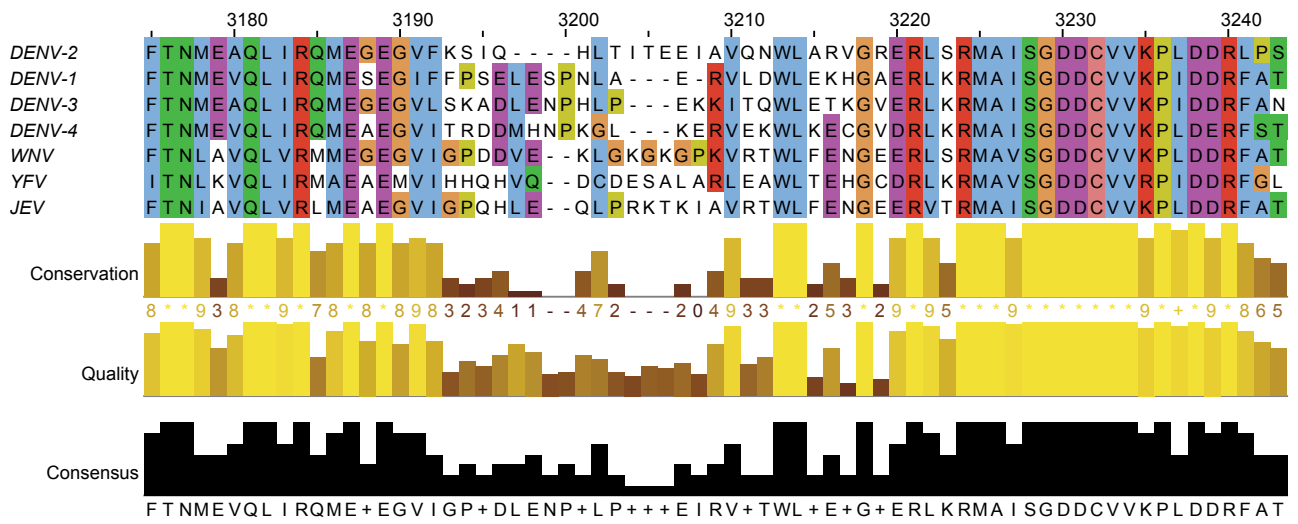


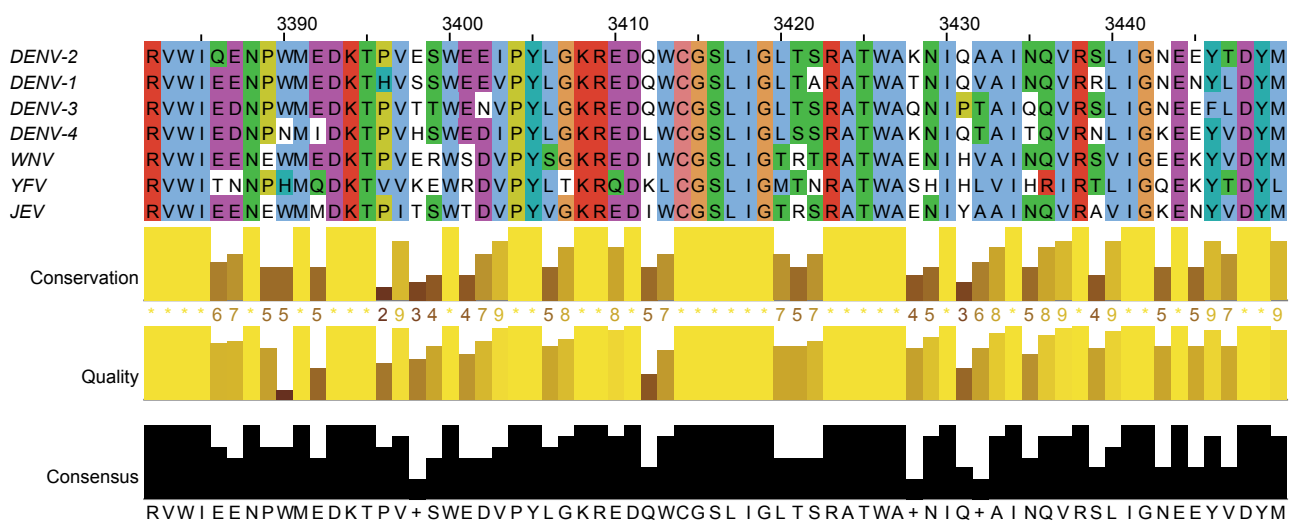
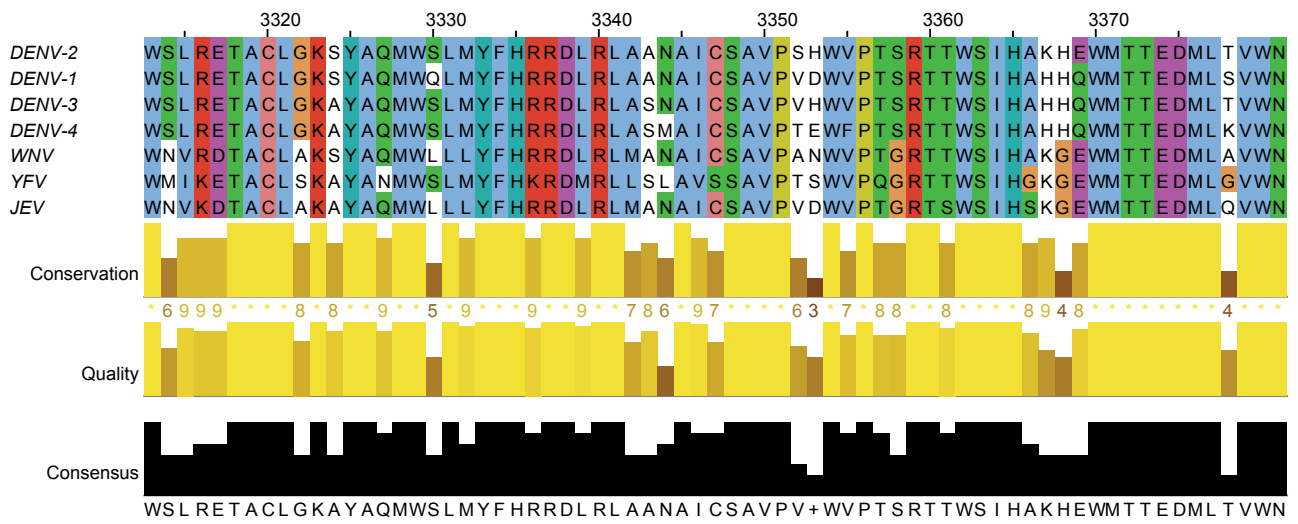












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<i>DENV-2</i>	P	S	M	K	R	F	R	-	R	E	E	E	E	A	G	V	L	W
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<i>DENV-3</i>	P	S	M	K	R	F	R	-	K	E	E	E	S	E	G	A	I	W
<i>DENV-4</i>	P	V	M	K	R	Y	S	-	A	H	F	E	S	E	G	V	L	-
<i>WNV</i>	S	S	L	R	R	Y	E	-	D	T	I	V	V	E	D	T	V	L
<i>YFV</i>	T	V	M	D	R	Y	S	V	D	A	D	L	Q	P	G	E	L	I
<i>JEV</i>	T	S	L	R	R	Y	E	-	D	V	L	I	Q	E	D	R	V	I

