Supporting Information

Structural basis of the atypical activation mechanism for KRAS V14I

Asim K Bera[1], Jia Lu[1], Thomas E. Wales[2], Sudershan Gondi[1], Deepak Gurbani[1], Andrew Nelson[1], John R. Engen[2], and Kenneth D. Westover[1]*

[1]Departments of Biochemistry and Radiation Oncology, The University of Texas Southwestern Medical Center at Dallas, Dallas, Texas 75390, United States.

[2]Department of Chemistry and Chemical Biology, Northeastern University, Boston, MA 02115, United States.

Running title: Structural basis of KRAS V14I activation

*Correspondence should be addressed to K.D.W. (Kenneth.Westover@UTSouthwestern.edu).

Table S1. HDX Data Summary and list of experimental parameters

Data Set	wild-type KRAS	V14I KRAS	A146T KRAS
HDX reaction details[a]	Final D_2O concentration=94.6%, pH_{read}=7.6, 21 °C.		
HDX time course	0.083, 0.167, 1, 5, 10, 60, and 240 minutes		
HDX controls	6 undeuterated controls, three for each state		
Back-exchange	30-35%		
Number of peptides [b]	64 followed	67 followed	58 followed
	56 coincident peptides followed		
Sequence coverage	92.3%	92.9%	92.3%
	77.5% coincident coverage		
Average peptide length; Redundancy	11.4;4.6		
Replicates	4 technical	2 technical	2 technical
Repeatability	+/- 0.30 relative Da		
Significant differences	> 0.8 Da		

[a] 18-fold dilution with labeling buffer [10mM Hepes, 150mM NaCl, 5mM $MgCl_2$ and 5 □M GDP, 99.9% D_2O (pD 8.0)]. 1-fold dilution with quench buffer [300 mM Sodium phosphate pH 2.47, H_2O].

[b] Parameters to filter peptides for identification from the 6 undeuterated controls were: 2 consecutive products, 0.25 products per amino acid.

Figure S1: Side chain residues involved in interaction with GDP in (A) $KRAS^{WT}$ and (B) $KARS^{V14I}$. Figures are generated from crystal structures by using Maestro.

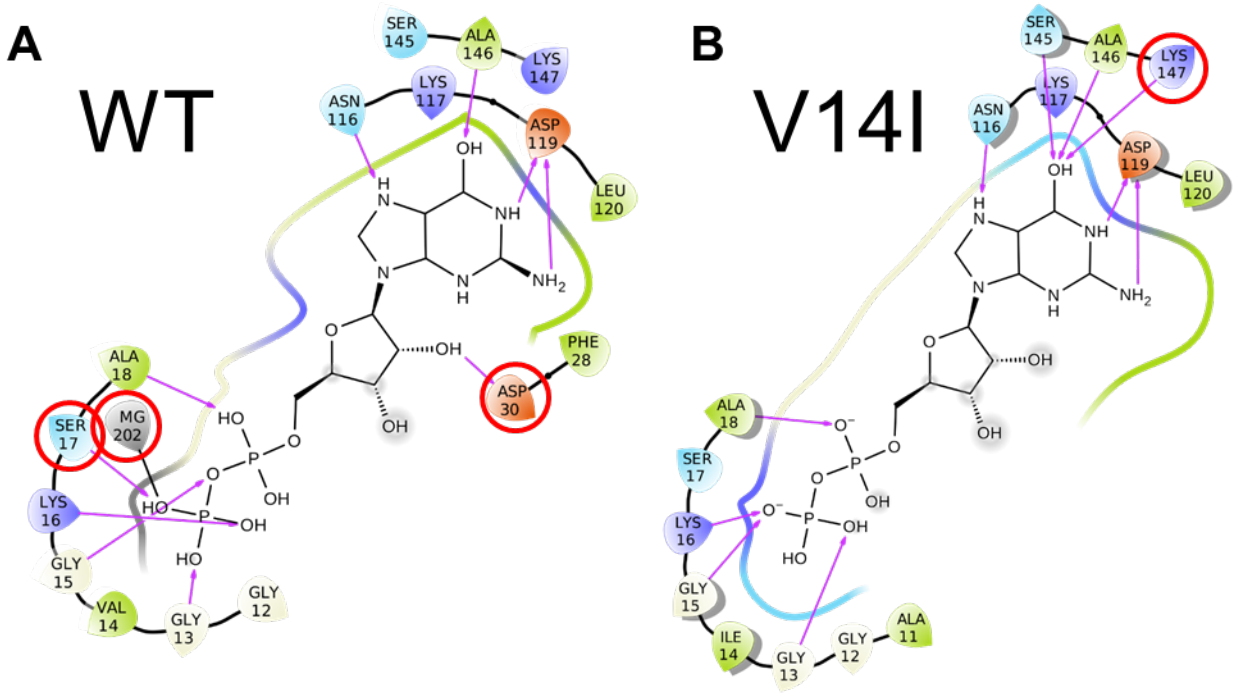
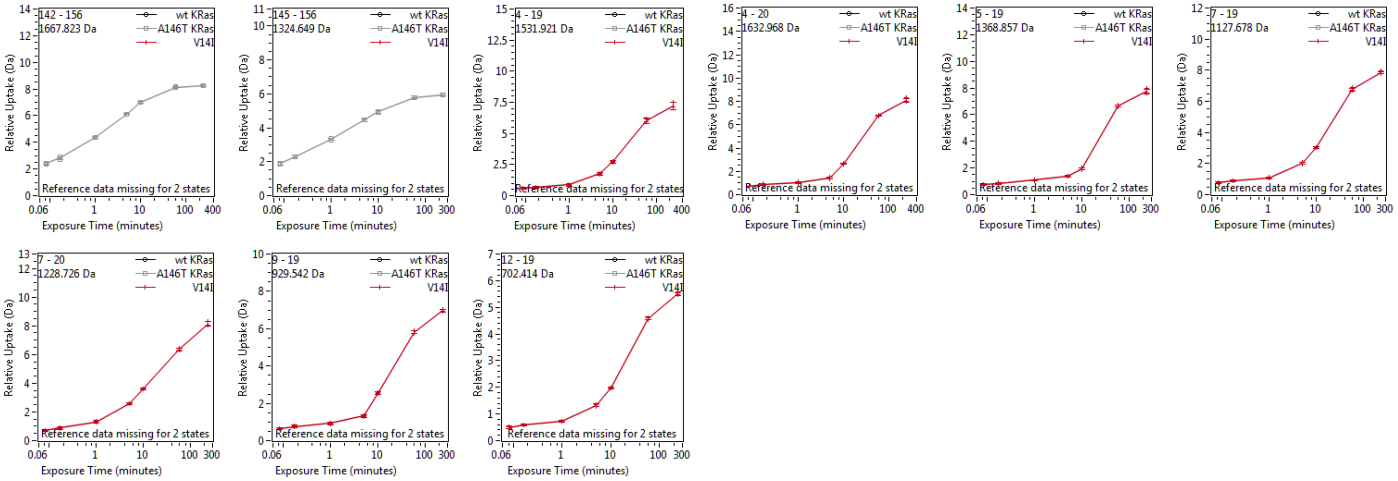
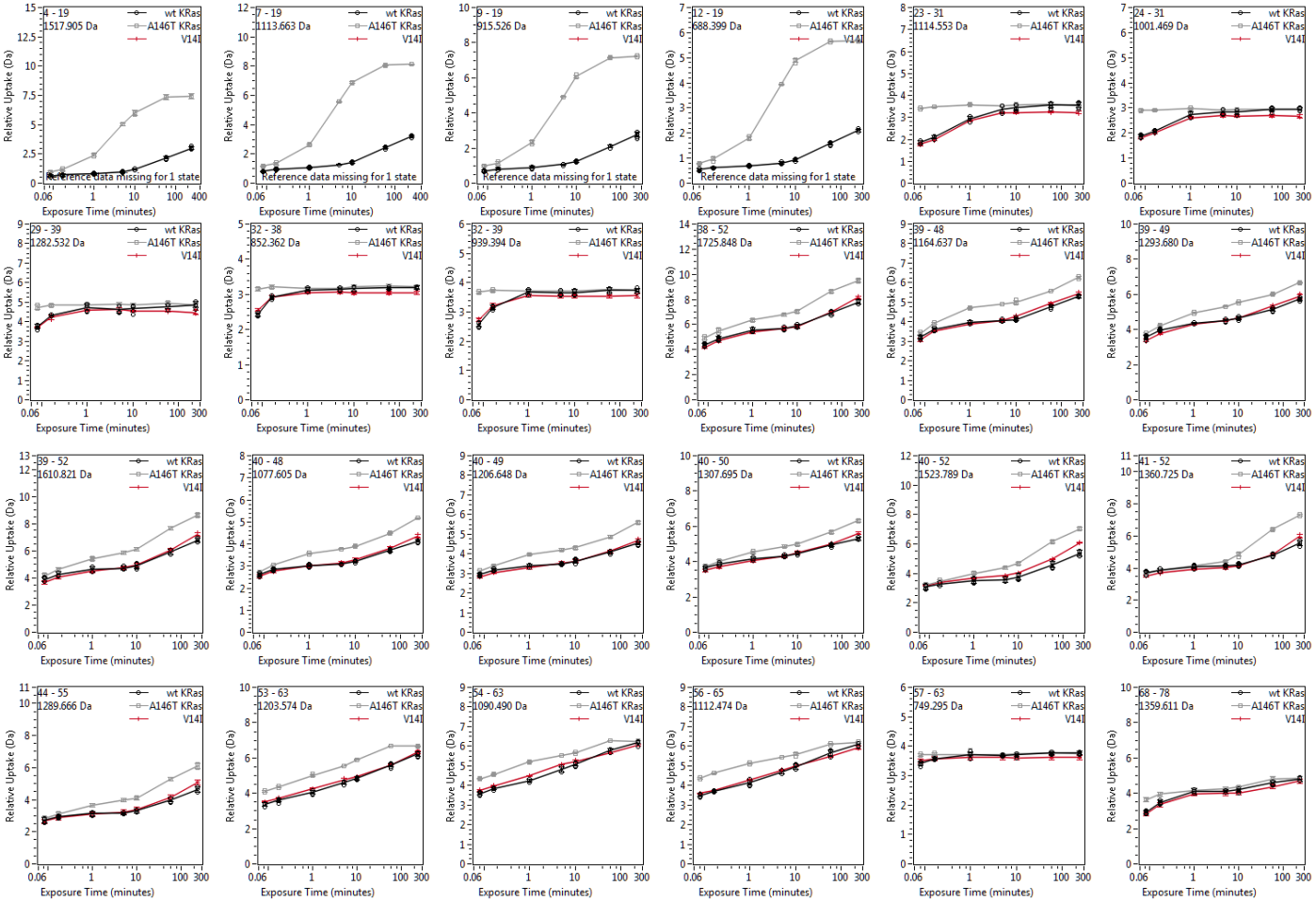


Figure S2: Deuterium incorporation plots for all peptides followed in the HDX experiments

Mutation containing peptides



Coincident peptides



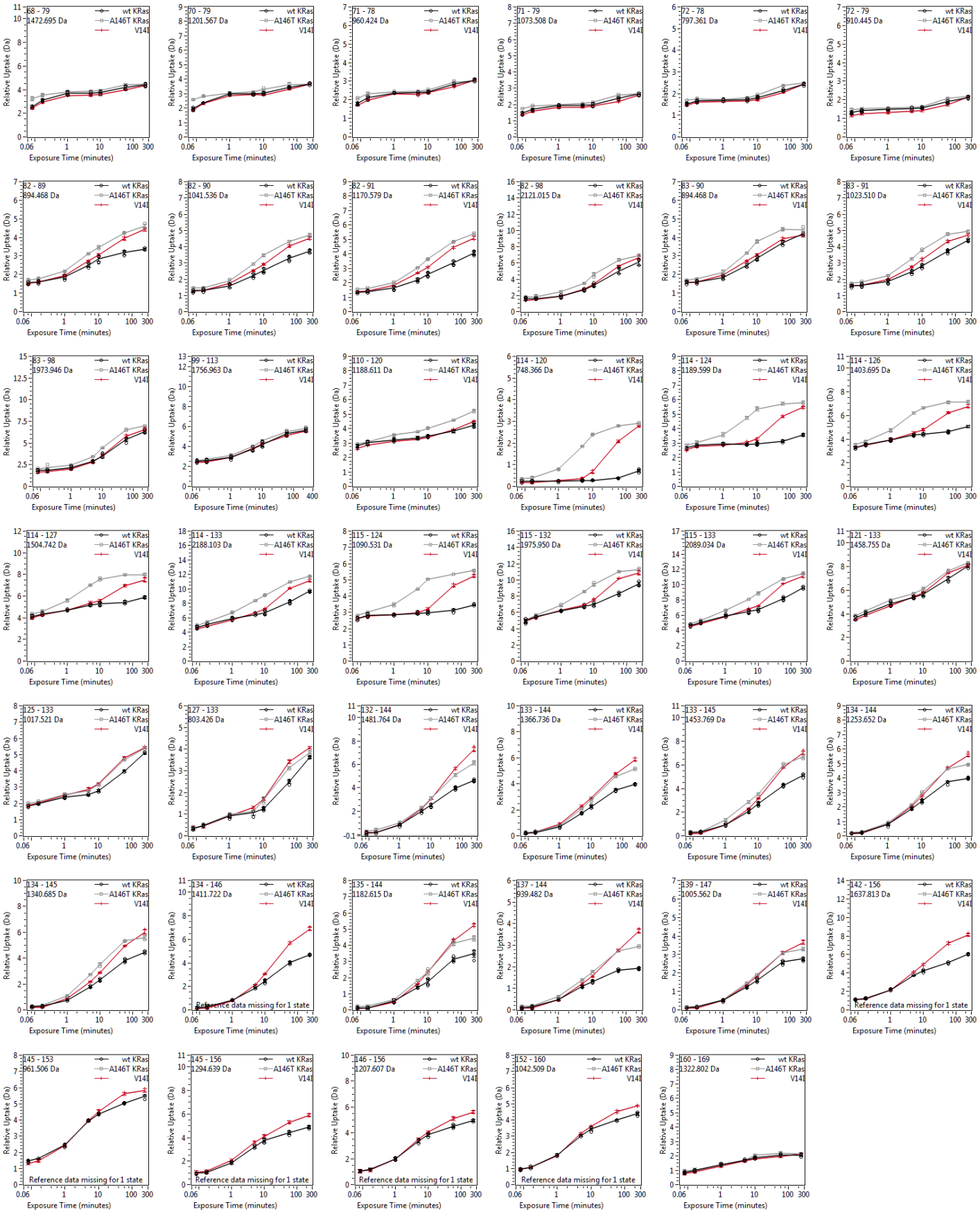
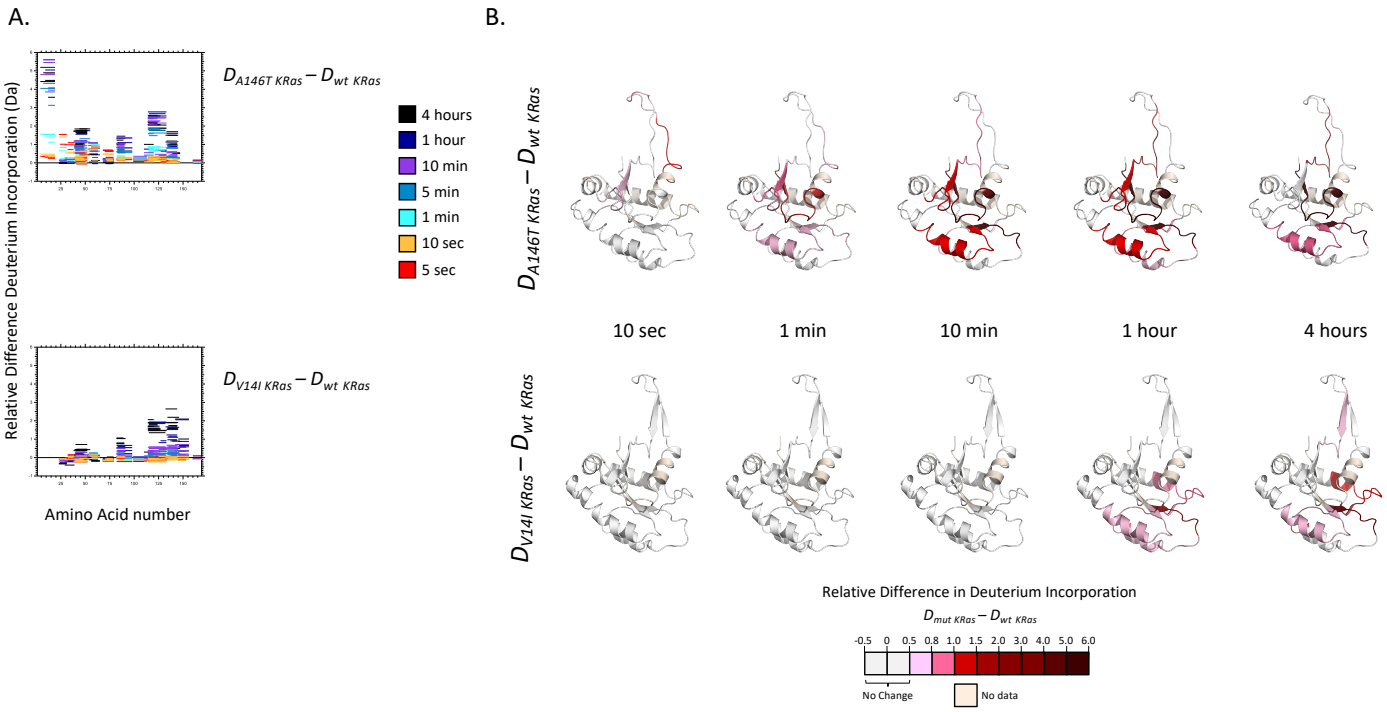

68 - 79
1472.695 Da
70 - 79
1201.567 Da
71 - 78
960.424 Da
71 - 79
1073.508 Da
72 - 78
797.361 Da
72 - 79
910.445 Da
82 - 89
894.468 Da
82 - 90
1041.536 Da
82 - 91
1170.579 Da
82 - 98
2121.015 Da
83 - 90
894.468 Da
83 - 91
1023.510 Da
83 - 98
1973.946 Da
99 - 113
1756.963 Da
110 - 120
1188.611 Da
114 - 120
748.366 Da
114 - 124
1189.599 Da
114 - 126
1403.695 Da
114 - 127
1504.742 Da
114 - 133
2188.103 Da
115 - 124
1090.531 Da
115 - 132
1975.950 Da
115 - 133
2089.034 Da
121 - 133
1458.755 Da
125 - 133
1017.521 Da
127 - 133
803.426 Da
132 - 144
1481.764 Da
133 - 144
1366.736 Da
133 - 145
1453.769 Da
134 - 144
1253.652 Da
134 - 145
1340.685 Da
134 - 146
1411.722 Da
135 - 144
1182.615 Da
137 - 144
939.482 Da
139 - 147
1005.562 Da
142 - 156
1637.813 Da
145 - 153
961.506 Da
145 - 156
1294.639 Da
146 - 156
1207.607 Da
152 - 160
1042.509 Da
160 - 169
1322.802 Da
wt KRas
A146T KRas
V14I
Relative Uptake (Da)
Exposure Time (minutes)
Reference data missing for 1 state

Figure S3: Illustrating the relative differences in hydrogen-deuterium exchange mass spectrometry of KRAS^{V14I} and KRAS^{A146T}.



Illustrating the relative differences in hydrogen-deuterium exchange mass spectrometry of KRAS4B^{V14I} and KRAS^{A146T}. **(A)** Relative differences in deuterium incorporation shown in all peptides coincident between KRAS4B^{A146T} vs KRAS^{WT} (top panel) and KRAS4B^{V14I} vs KRAS^{WT} (bottom panel). The relative difference (y-axis) for each peptide is shown for each time point (time points are colored according to scale at the right). The peptides in the regions of mutation are not shown as a subtraction cannot be made against the wildtype (see uptake graphs in Figure S4 for comparison of deuterium levels in the peptides). **(B)** The differences in deuterium incorporation between the mutant and wild-type KRAS constructs at 5 time points are identified on the cartoon structures of KRAS^{A146T} (top panel) and KRAS4B^{V14I} (bottom panel). Relative differences are calculated as mutant minus wt KRAS and are colored according to the color scale at the bottom.