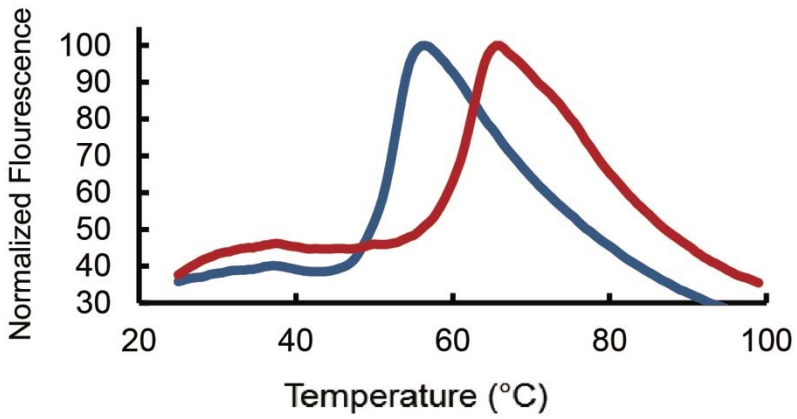
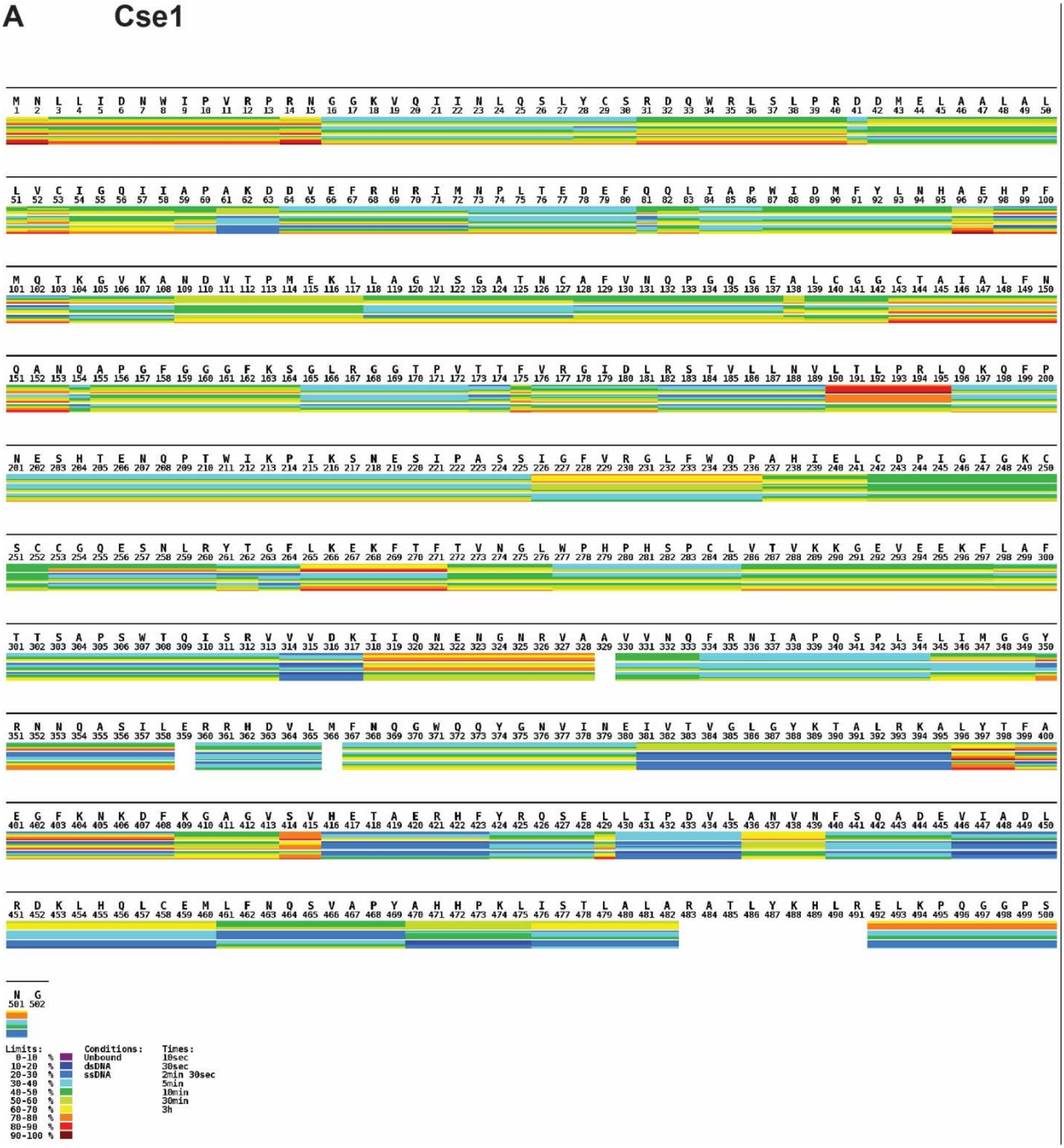
Supplemental Information



Supplemental Figure 1. DNA binding increases the stability of Cascade. Differential scanning fluorimetry of unbound Cascade (blue) and the dsDNA bound form of Cascade (red). The increase in melting temperature after dsDNA binding indicates that the complex is more stable.

1

A Cse1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N G
501 502
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Times:
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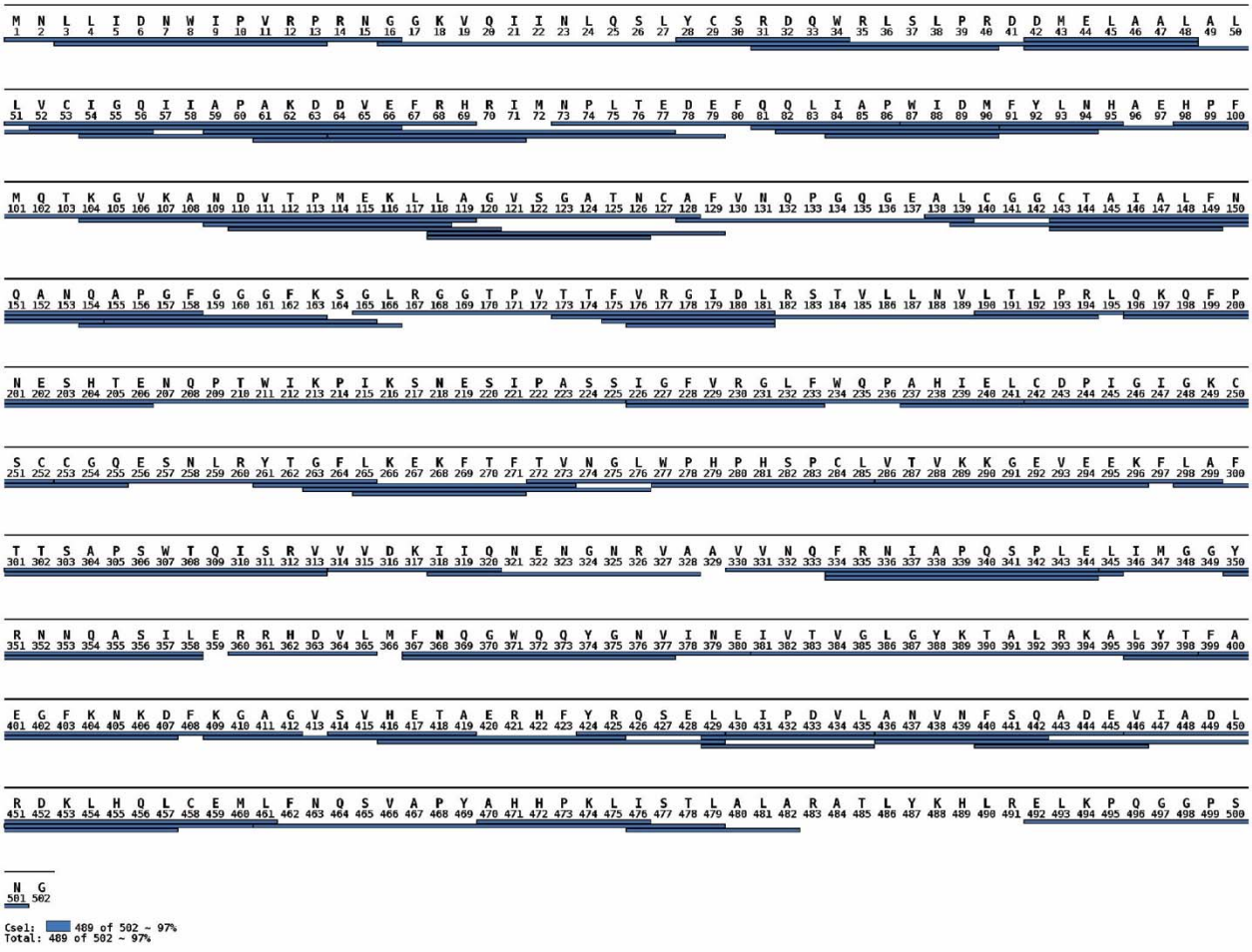
30sec
2min 30sec
5min
10min
30min
3h

2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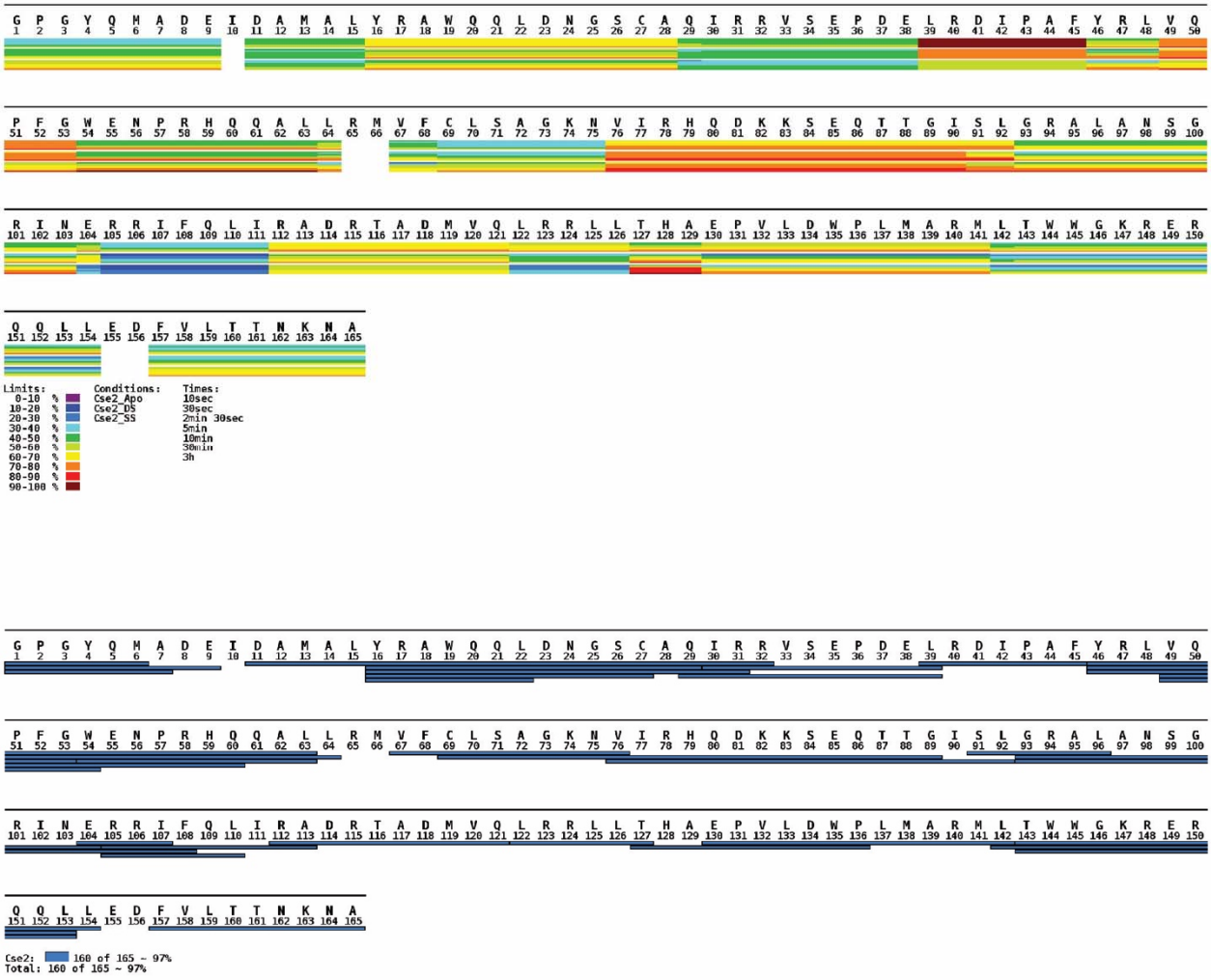
N G
501 502
Csel: 489 of 502 ~ 97%
Total: 489 of 502 ~ 97%

2

B **Cse2**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Cse2_DS
Cse2_SS
Times:
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30sec
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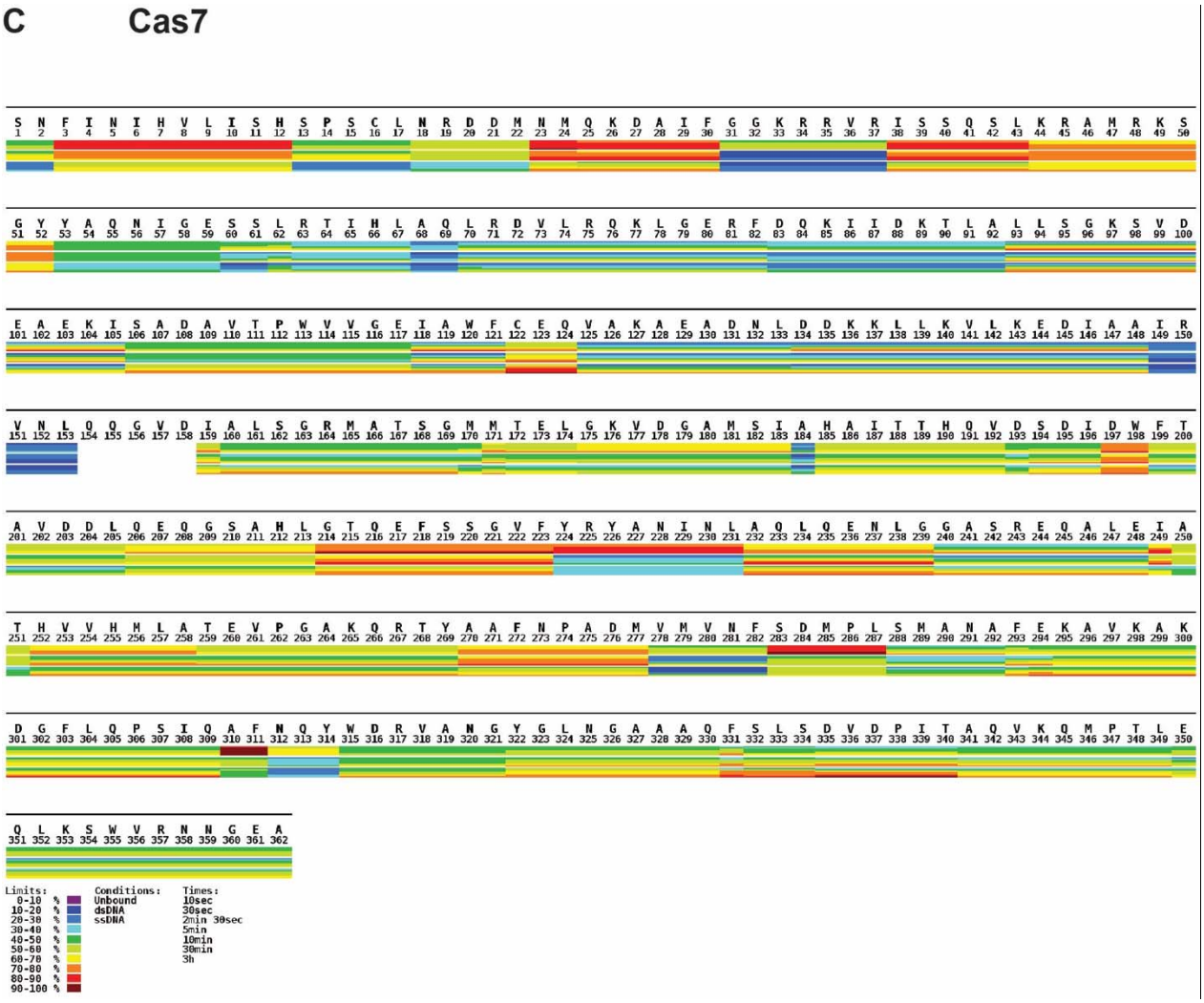
5min
10min
30min
3h
Cse2: 160 of 165 ~ 97%
Total: 160 of 165 ~ 97%

2

C Cas7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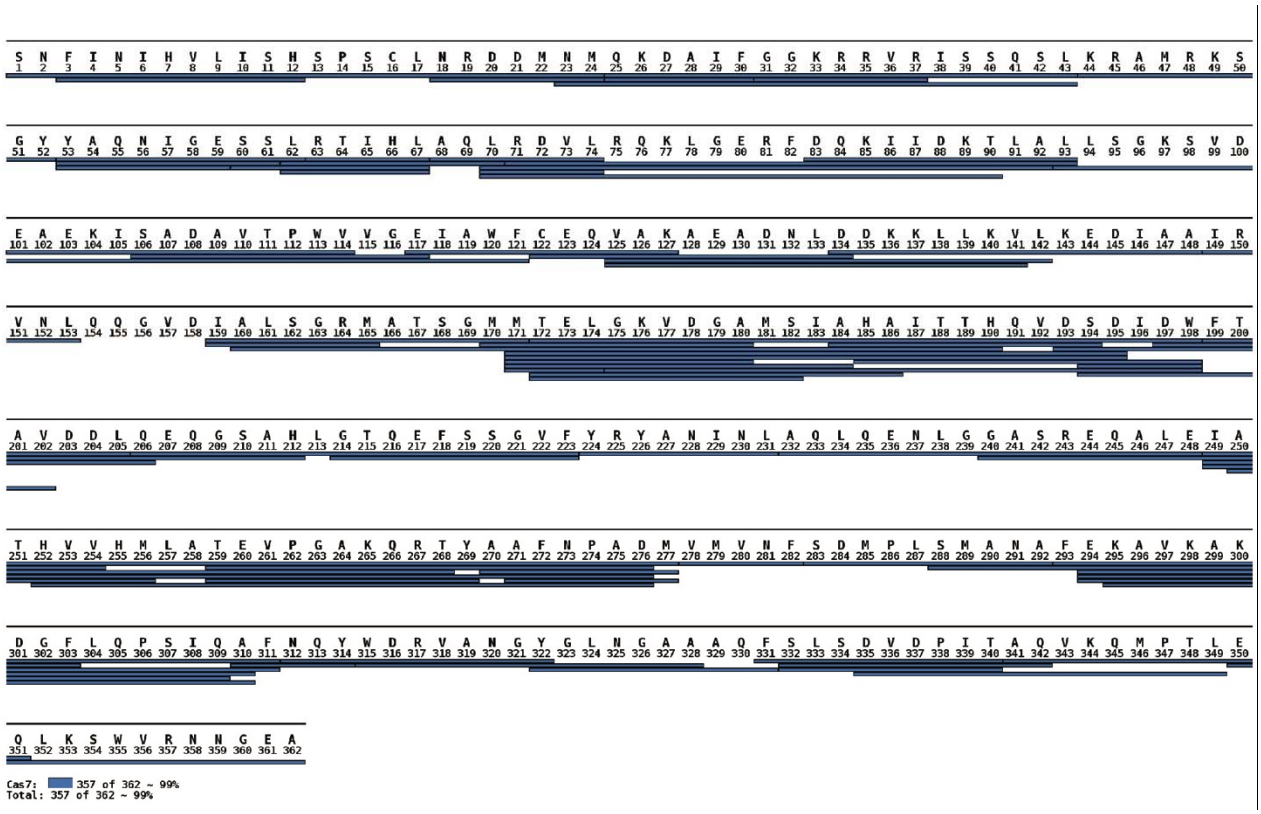
2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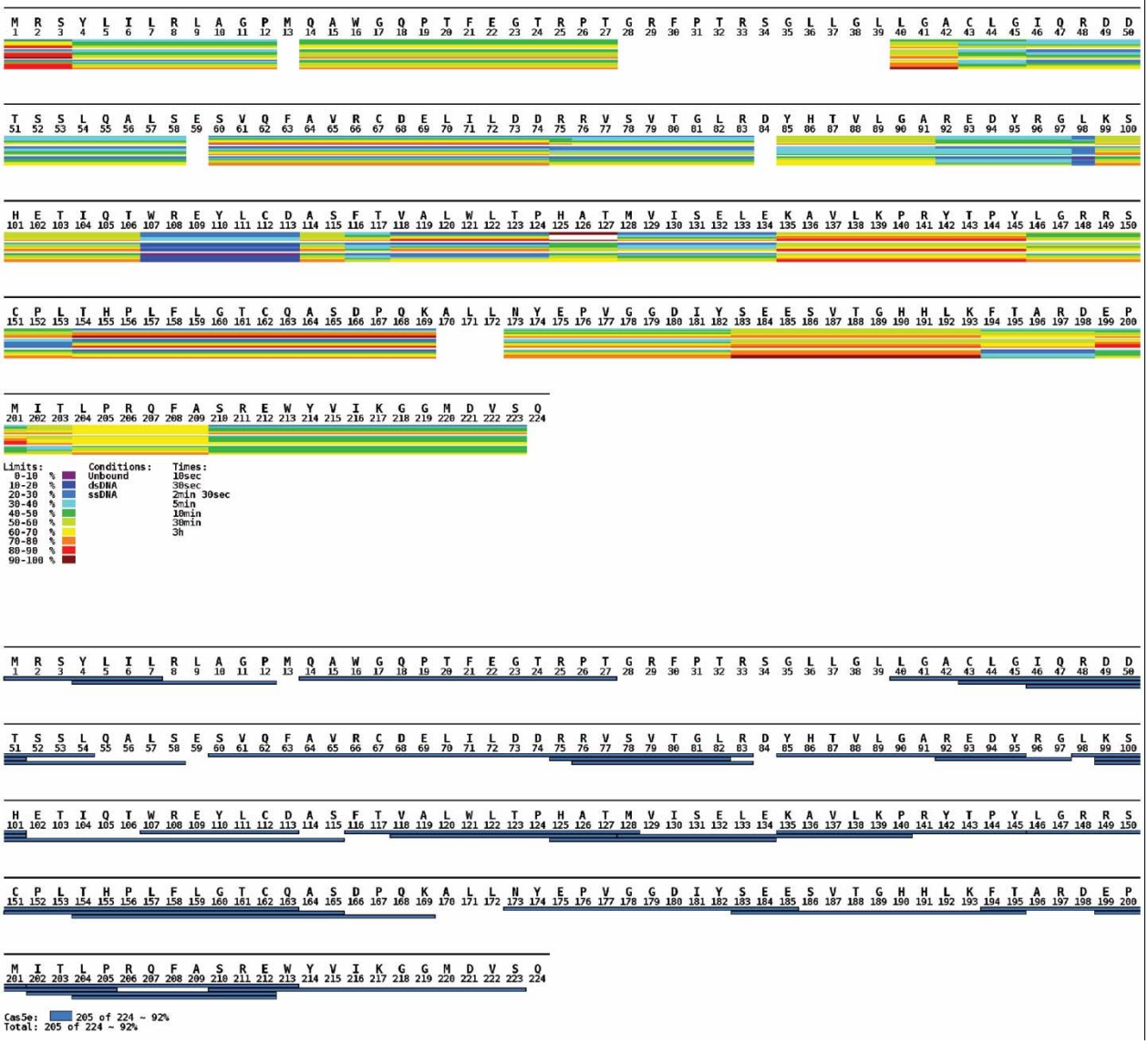
Cas7: 357 of 362 ~ 99%
Total: 357 of 362 ~ 99%

2

D **Cas5**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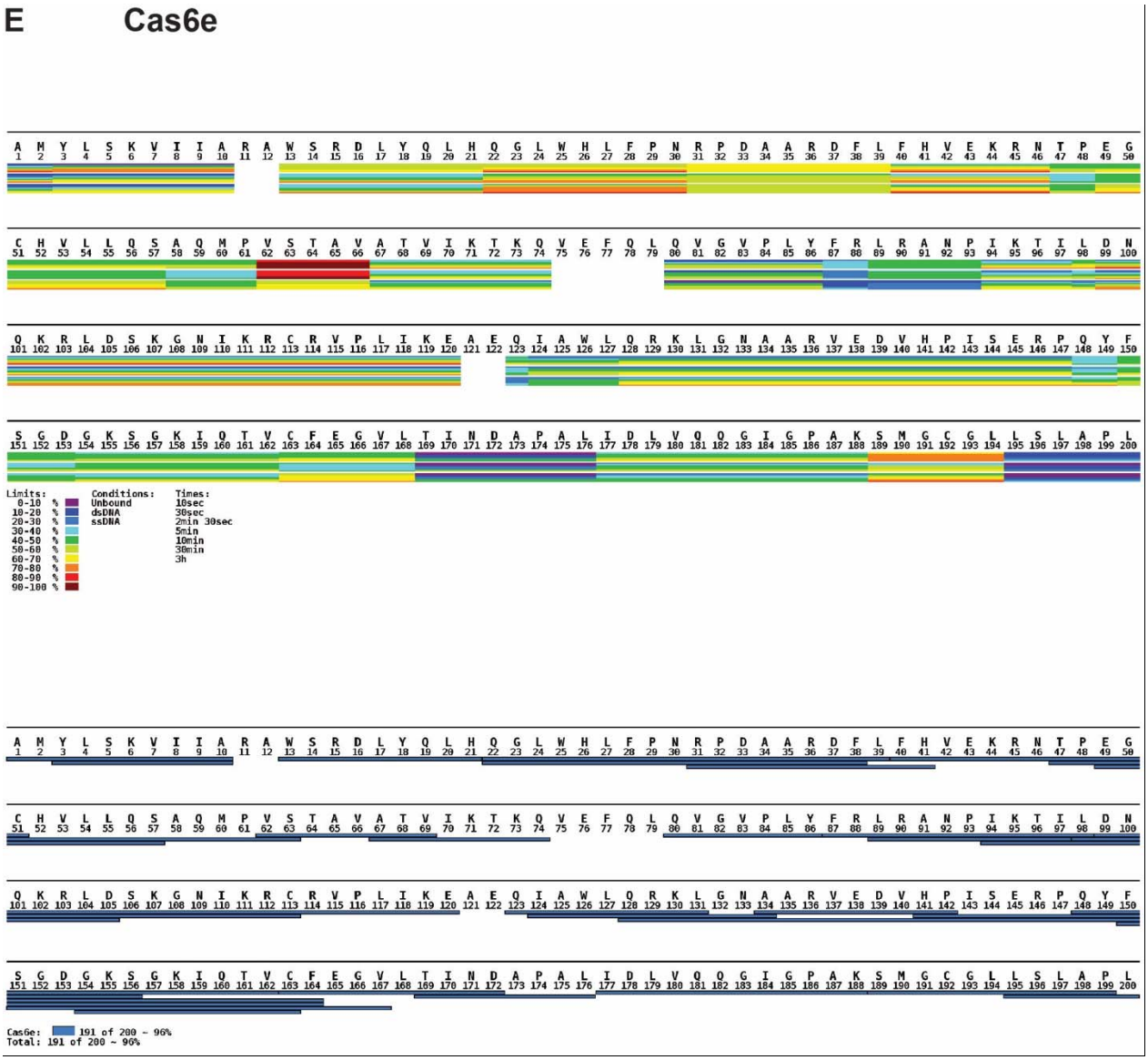
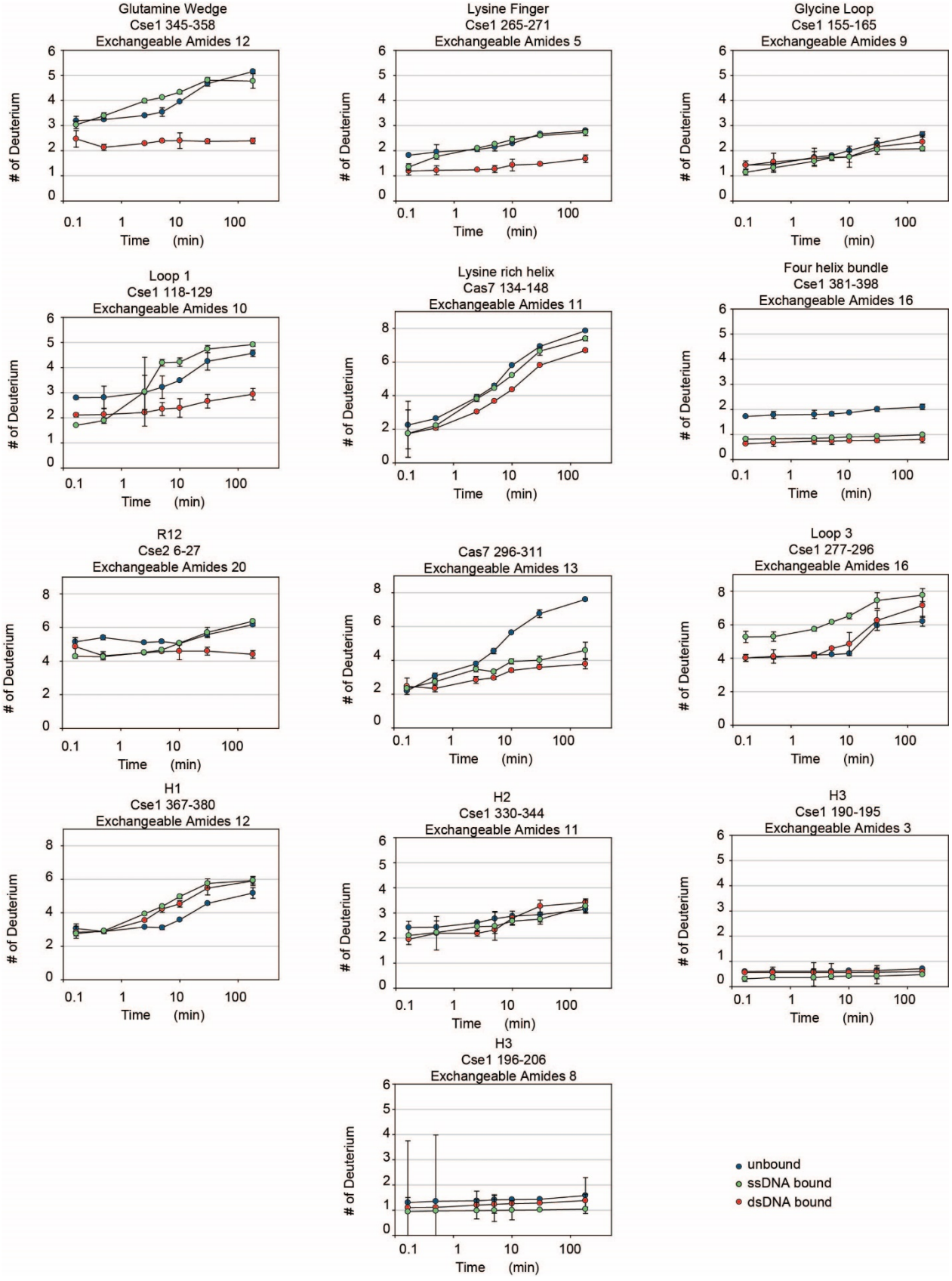
80-90 %
90-100 %
Conditions:
Unbound
dsDNA
ssDNA
Times:
10sec
30sec
2min 30sec
5min
10min
30min
3h
Cas5e: 205 of 224 ~ 92%
Total: 205 of 224 ~ 92%

2

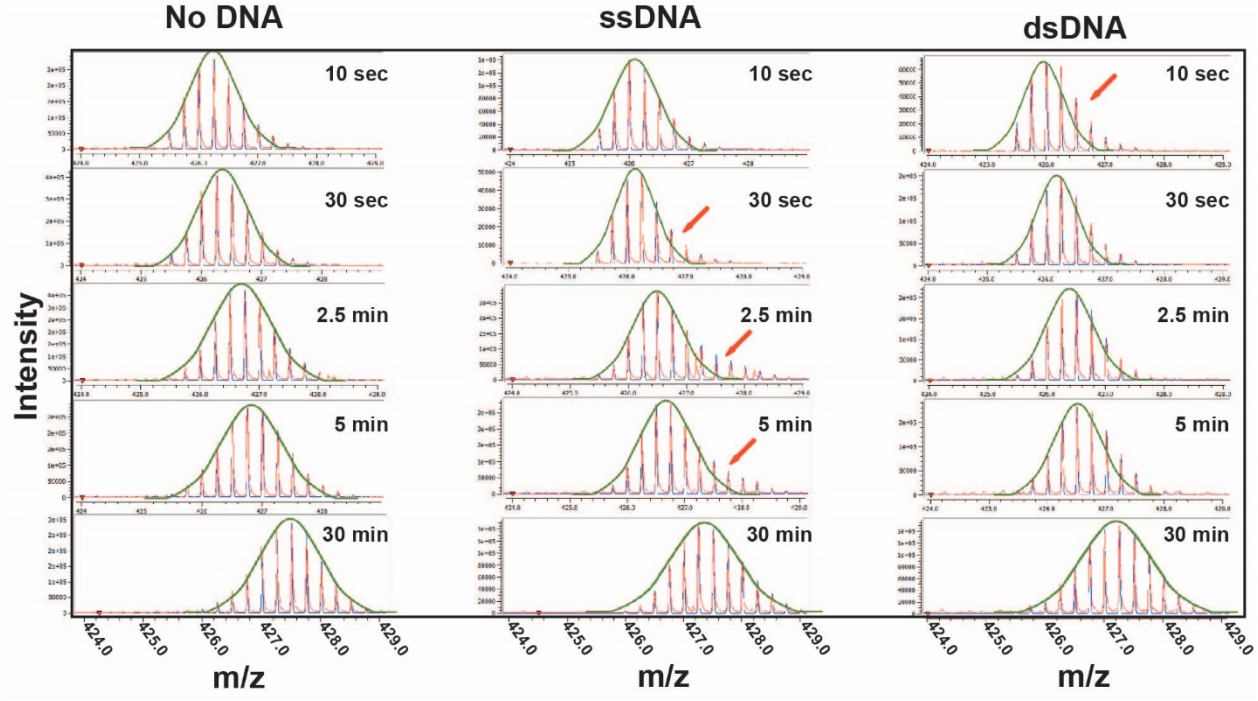


Supplemental Figure 2. Heat and coverage maps for all Cascade subunits. Heat map (colored) and coverage map (blue) for each subunit (Cse1 **A**, Cse2 **B**, Cas7 **C**, Cas5e **D**, and Cas6e **E**). The heat map shows the percentage of deuterium exchanged for each peptide at every time point. Each condition (no DNA, dsDNA bound, and ssDNA bound) are displayed as separate rows respectively, with each block further divided into rows indicating the tested time points. The percent deuterium uptake is indicated by differing colors. Coverage maps for each subunit are shown below each heat map. Each line represents a peptide used to measure the deuterium uptake in all conditions. Over 90% sequence coverage was achieved for all peptides.

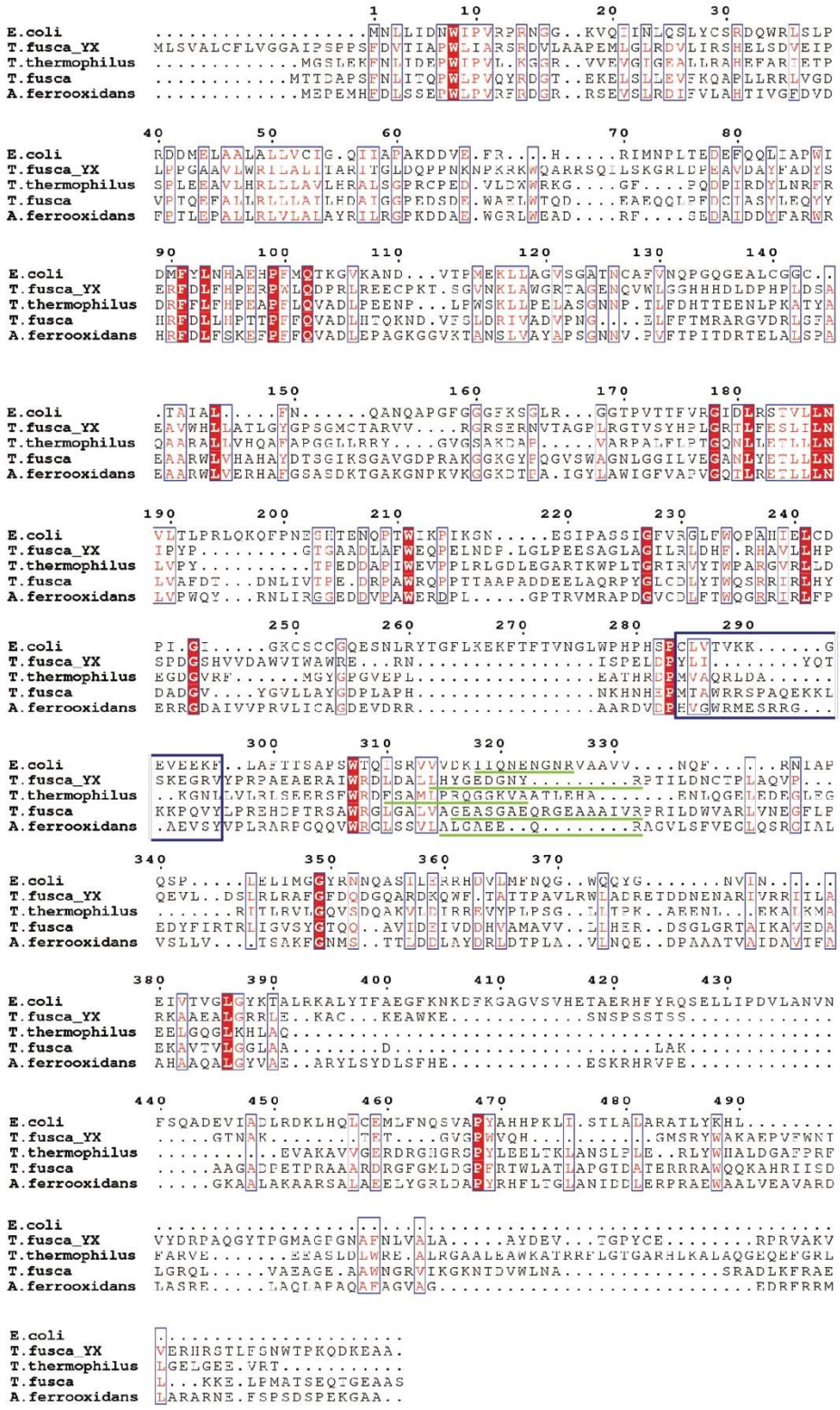

Glutamine Wedge
Cse1 345-358
Exchangeable Amides 12
Lysine Finger
Cse1 265-271
Exchangeable Amides 5
Glycine Loop
Cse1 155-165
Exchangeable Amides 9
Loop 1
Cse1 118-129
Exchangeable Amides 10
Lysine rich helix
Cas7 134-148
Exchangeable Amides 11
Four helix bundle
Cse1 381-398
Exchangeable Amides 16
R12
Cse2 6-27
Exchangeable Amides 20
Cas7 296-311
Exchangeable Amides 13
Loop 3
Cse1 277-296
Exchangeable Amides 16
H1
Cse1 367-380
Exchangeable Amides 12
H2
Cse1 330-344
Exchangeable Amides 11
H3
Cse1 190-195
Exchangeable Amides 3
H3
Cse1 196-206
Exchangeable Amides 8
# of Deuterium
Time (min)
unbound
ssDNA bound
dsDNA bound

1
2

Supplemental Figure 3. HD-exchange over time. The number of deuteriums incorporated by specific peptides are plotted over time. HD-exchange is measured for peptides from Cascade prior to binding DNA (blue), bound to ssDNA (green), and dsDNA (red) at time points 10 and 30 seconds, 2.5, 5, 10, 30 and 180 minutes. With the exception of the 10-minute data point from the unbound form of Cascade which was only performed once, the remaining data points are the average of three replicates with the standard deviation shown as error bars. Error bars are too small to see in some cases. The maximum number of exchangeable amide hydrogens is indicated and calculated by subtracting two possible exchangeable amides hydrogens from the peptide length (back exchange) and one additional for each proline present.

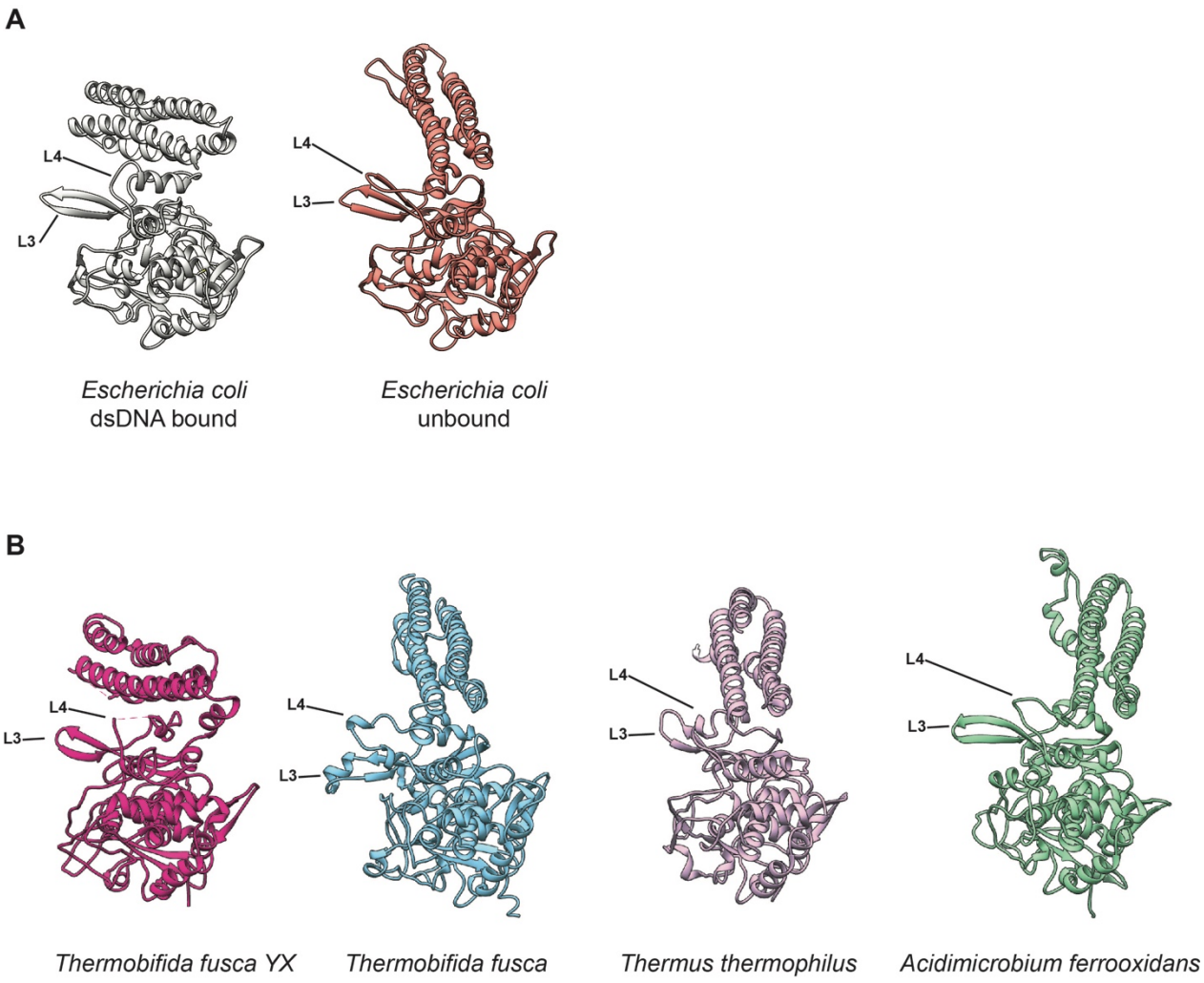


Supplemental Figure 4. Peptide covering the lysine-rich helix (Cas7 134-148) displays a bimodal distribution. Measured isotopic distributions for the Cas7 peptide 134-148 in Cascade prior to binding DNA, bound to ssDNA, and dsDNA bound. The expected isotopic distribution during HDX-MS is shown in green. In the absence of DNA (left column), the peptide shows a normal isotopic distribution, which shifts to the right over time. This shows the incorporation of deuterium. In the ssDNA bound form (middle column), the peptide shows bimodal behavior, which appears at the "leading edge" (red arrows) of the normal Gaussian distribution. This leading edge appears after 30 seconds and becomes continuously more exaggerated until 30 minutes when the isotopic envelope re-adopts a near Gaussian distribution. When dsDNA is bound (right column), the bimodal behavior is observed at the first-time point. Similar to behavior measured for the ssDNA bound complex, the profile returns to a near Gaussian distribution by 30 minutes. However, the centroid of the isotopic distribution for the dsDNA bound form of Cascade lies at a lower m/z value than in the ssDNA bound form. This shows that the dsDNA bound form of this peptide is more protected from deuterium exchange than the ssDNA bound form.

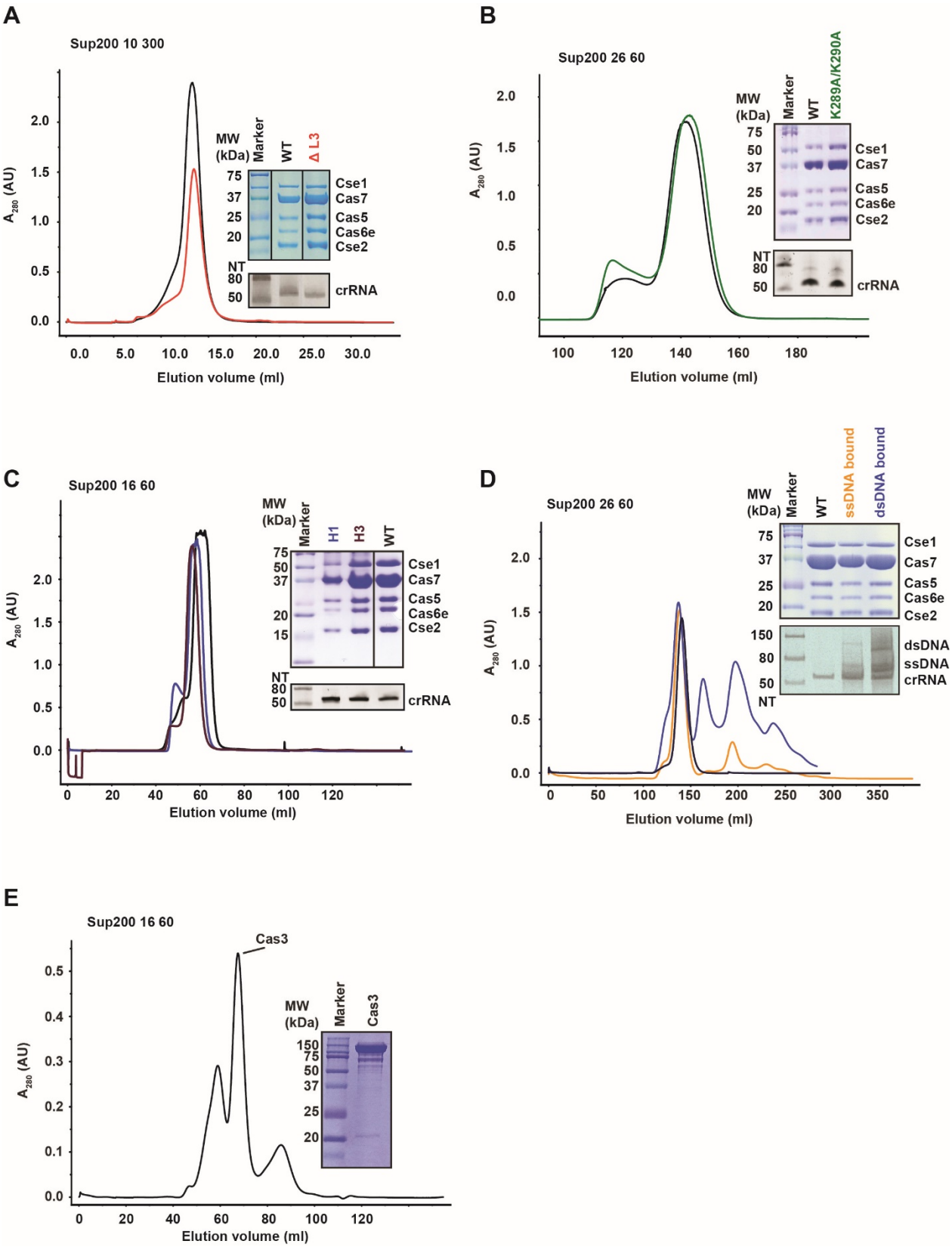


Supplemental Figure 5. Sequence alignment of Cse1 shows little conservation in L3 and L4. Protein sequence alignment of Cse1 subunits of different organisms for which structures are available.

Escherichia coli (5CD4), *Thermobifida fusca YX* (5U0A), *Thermus thermophilus* (4AN8), *Thermobifida fusca* (3WVO) and *Acidimicrobium ferrooxidans* (4H3T). Identical residues are highlighted in red and similar residues are in red text. L3 (blue box) and L4 (green, underlined) are indicated and show little sequence conservation.

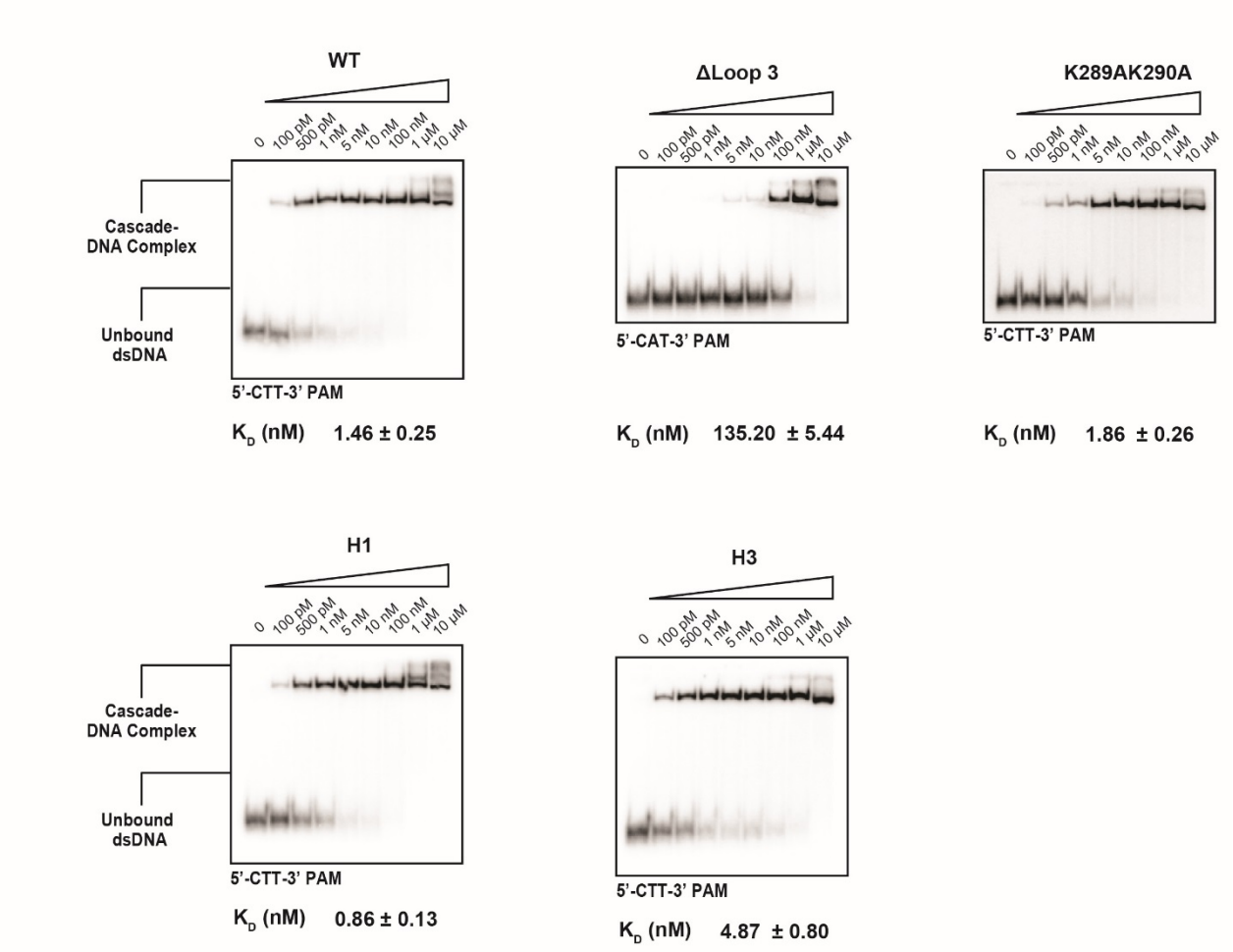


Supplemental Figure 6. Structures of the Cse1 subunit. A) Structures of the *E. coli* Cse1 subunit from the forked dsDNA bound Cascade (left, 5H9F) and from the unbound Cascade (right, 5CD4). L4 in the dsDNA bound structure has moved 11 Å away from L3. **B)** The Cse1 subunit form *Thermobifida fusca YX* (5U0A), *Thermobifida fusca* (3WVO), *Thermus thermophilus* (4AN8) and *Acidimicrobium ferrooxidans* (4H3T) all share the conserved structural features L3 and L4.


A
Sup200 10 300
MW (kDa)
Marker
WT
Δ L3
75
37
25
20
Cse1
Cas7
Cas5
Cas6e
Cse2
NT
80
50
crRNA
A280 (AU)
Elution volume (ml)
B
Sup200 26 60
K289A/K290A
C
Sup200 16 60
H1
H3
D
ssDNA bound
dsDNA bound
150
dsDNA
ssDNA
E
Cas3

1

Supplemental Figure 7. Cascade mutants express and purify as WT Cascade. A) Elution profile of WT Cascade (black) and ΔLoop3 (Cse1 ΔL285-K296, red). The insert shows a SDS-PAGE gel and (top) and denaturing polyacrylamide gel (bottom). **B)** Elution profile of WT Cascade (black), Cascade H1 (Cse1 N379A/E380K, blue) and Cascade H3 (Cse1 R194E/K197E, purple). **C)** Elution profile of WT Cascade (black) and Cascade K289A/K290A (Cse1 K289A/K290A, green). **D)** Elution profile of WT Cascade (black), Cascade bound to 72-nt ssDNA containing P7 protospacer and 3'-TTC-5' PAM (orange) and Cascade bound to 72-bp dsDNA containing P7 protospacer and 3'-TTC-5'PAM. **E)** Elution profile of Cas3. The insert shows a SDS-PAGE gel.



Supplemental Figure 8. Deletion of Loop3 results in a major dsDNA binding defect. Electrophoretic Mobility Shift Assays (EMSA) show equilibrium dissociation constants for Cascade and Cascade mutants binding to 72-bp dsDNA containing P7 protospacer and 3'-TTC-5' or 3'-TAC-5' PAM. Only deletion of Loop 3 resulted in a major binding defect. The H3 mutant resulted in a minor binding defect. Equilibrium dissociation constant (K_D) is calculated using three replicates. Error represents standard deviation.

Description	Target strand (top) and non-target strand (bottom); PAM in black and spacer in blue
72 bp dsDNA target with 3'-TAC-5' PAM	3'-CGCGCCGTTCGGCTTTCGTACTGCCATAACAAGTCTAGGACCGAACGGTTGTCACTAACGAGCCCTCAGCGA-5' 5'-GCGCGGCAAGCCGAAAGCATGACGGTATTGTTCAGATCCTGGCTTGCCAACAGTGATTGCTCGGGAGTCGCT-3'
72 bp dsDNA target with 3'-TTC-5' PAM	3'-CGCGCCGTTCGGCTTTCGTTCTGCCATAACAAGTCTAGGACCGAACGGTTGTCACTAACGAGCCCTCAGCGA-5' 5'-GCGCGGCAAGCCGAAAGCAAGACGGTATTGTTCAGATCCTGGCTTGCCAACAGTGATTGCTCGGGAGTCGCT-3'

Supplemental Table 1. Oligonucleotides used in this study.