Marburg virus RNA synthesis is inhibited by a synthetic anti-VP35 antibody

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Supporting Information:

Supporting Figure Legends

Table S1. Data collection and refinement statistics.

Figure S1. Peptide coverage maps for mVP35 IID, sFab H3 light chain, and sFab H3 heavy chain.

Figure S2. HDX-MS heatmaps of sFab H3.

Figure S3. HDX kinetics curves of mVP35 IID peptides.

Figure S4. HDX kinetics curves of sFab H3 light chain peptides.

Figure S5. HDX kinetics curves of sFab H3 heavy chain peptides.

Figure S6. Fluorescence polarization assay measuring binding of sFab H3 to mVP35 IID with dsRNA.

Figure S7. Pull-down assay of eVP35 IID mutant binding to sFab H5.

Supporting Figure Legends

Table S1. Data collection and refinement statistics.

Figure S1. Coverage maps of all peptides acquired for **A.** mVP35 IID showing 87 peptides with 100% coverage, **B.** sFab H3 light chain showing 200 peptides with 100% coverage, and **C.** sFab H3 heavy chain showing 219 peptides with 99.6% coverage.

Figure S2. HDX-MS heatmaps of unbound **A.** sFab H3 light chain and **B.** sFab H3 heavy chain. The extent of deuteration is depicted following the included gradient bars.

Figure S3. HDX kinetics curves of all mVP35 IID peptides of every observed charge state (89 total) when (black) unbound, (red) bound to sFab H3, and (blue) bound to sFab H5. Some peptides from the original mapping experiments were excluded due to low signal and/or interference. Each page contains the results for 40 peptides ordered from N-terminal to C-terminal.

Figure S4. HDX kinetics curves of all sFab H3 light chain peptides of every observed charge state (151 total) when (black) unbound and (red) bound to mVP35 IID. Each page contains the results for 40 peptides ordered from N-terminal to C-terminal.

Figure S5. HDX kinetics curves of all sFab H3 heavy chain peptides of every observed charge state (170 total) when (black) unbound and (red) bound to mVP35 IID. Each page contains the results for 40 peptides ordered from N-terminal to C-terminal.

Figure S6. mVP35 IID binds to dsRNA with similar affinity in the presence or absence of sFab H3. Fluorescence polarization assay based on the interaction of mVP35 IID to a FITC-labeled 19 bp dsRNA. 1 nM FITC-19 bp dsRNA has low fluorescence polarization across 5 samples (black squares). Addition of 100 nM mVP35 IID results in an increase in fluorescence polarization (red squares), indicating binding. Addition of 50 nM of sFab H3 to 1 nM FITC-19 bp dsRNA containing 100 nM of mVP35 IID results in a further increase in fluorescence polarization (blue squares) due

to formation of mVP35 IID-sFab H3-dsRNA complex. No increase in fluorescence polarization was observed upon addition of 50 nM sFab H3 to 1 nM FITC-dsRNA (compare green circles with cyan circles) or 100 nM maltose binding protein to 1 nM FITC-dsRNA (compare yellow triangles to pink triangles).

Figure S7. In vitro pull-down assay of eVP35 IID mutant binding to sFabs H5. Lane 1, sFab; lane 2, sFab + resin negative control; lane 3, immobilized MBP-fusion protein; lane 4, input; lanes 5-6, washes; lane 7, final bound resin; lane 8, eluate.

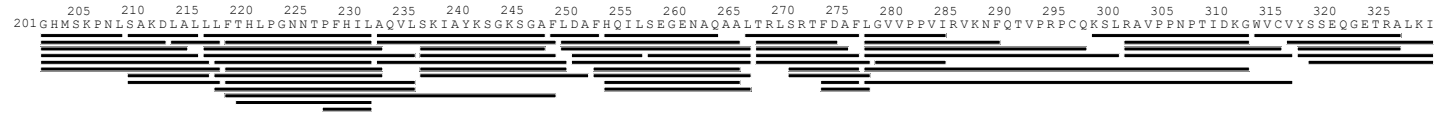
Table S1. Data collection and refinement statistics

Data collection	
Space Group	$P2_12_12$
Unit cell parameters	
a, b, c (Å)	57.83, 97.42, 110.33
α, β, γ (°)	90, 90, 90
Resolution range (Å)[1,2]	50.00-1.70 (1.73 – 1.70)
Limit along a*	~1.85
b*	~1.75
c*	~1.60
Unique reflections	68713 (3348)
Redundancy	6.9 (7.1)
Completeness (%)	99.4 (99.1)
Wilson B factor	
Average B, all atoms (Å^2)	20.16
R_{merge} (%)	12.8 (117)
$\langle I \rangle / \langle \sigma(I) \rangle$	15.8 (1.72)
CC1/2 last shell	(0.721)
Refinement	
Resolution (Å)	36.96 – 1.70 (1.75 – 1.70)
No. of reflections	61776 (3022)
Completeness (%)	93.83 (63.15)
Total No. of non-hydrogen atoms	5064
R_{work}/R_{free} (%)	19.58/23.30 (25.1/30.4)
R.m.s. deviations	
Bond lengths (Å)	0.006
Bond angles (°)	1.307
B-factors (Å^2)	
VP35 IID	27.8 (chain A)
sFab H3 heavy chain	17.1 (chain H)
sFab H3 light chain	21.2 (chain L)
water	27.8 (chain W)
Ramachandran plot outliers (%)	0.18%
Molprobity Clashscore, all atoms	2.6 (99th percentile)
Molprobity Score	1.16 (99th percentile)

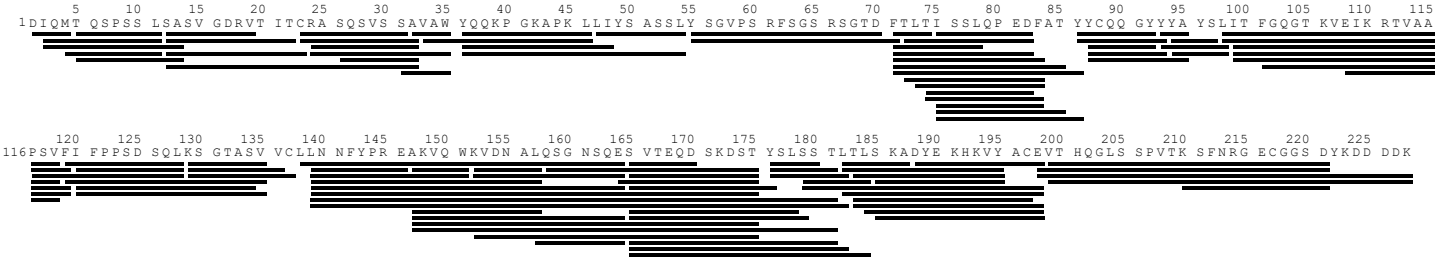
[1]Values in parentheses are for the highest resolution shell.
[2]Anisotropy is presented along three directions of reciprocal lattice. The anisotropy of diffraction is the reason for the ellipsoidal truncation during refinement that results in lower completeness in the highest resolution shell.

Figure S1

A



B



C

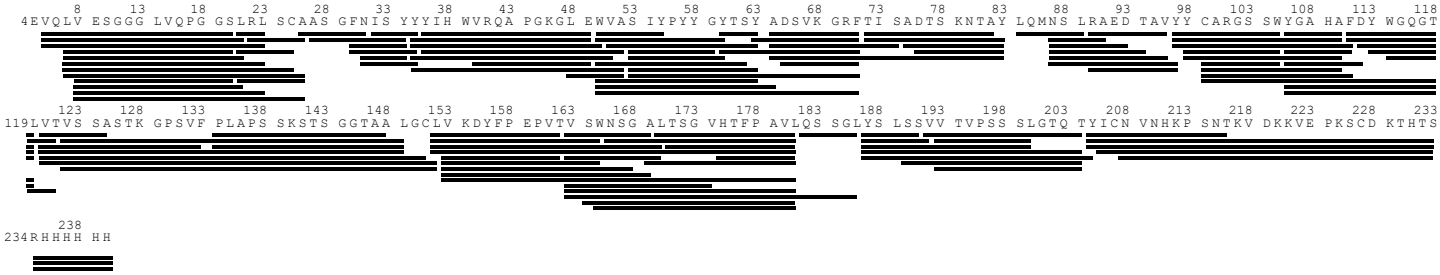
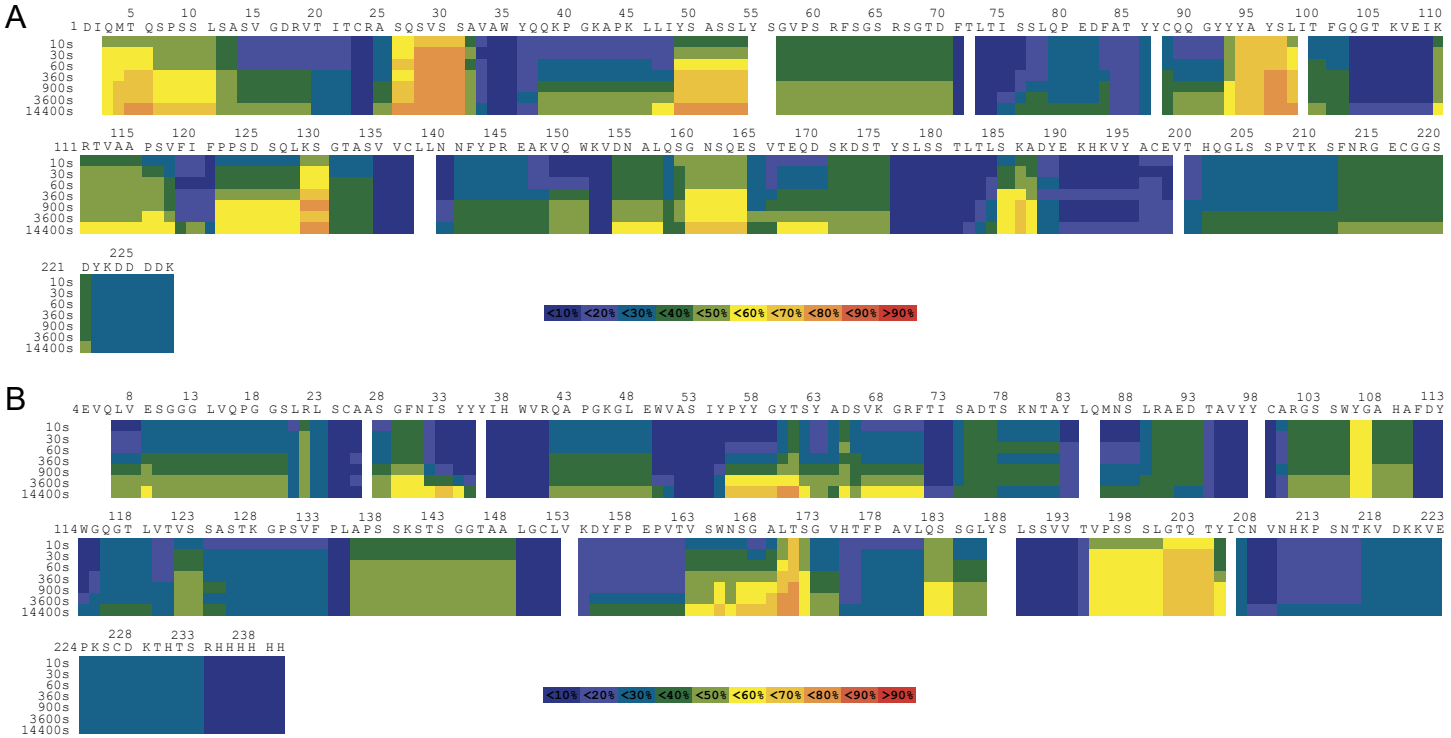


Figure S2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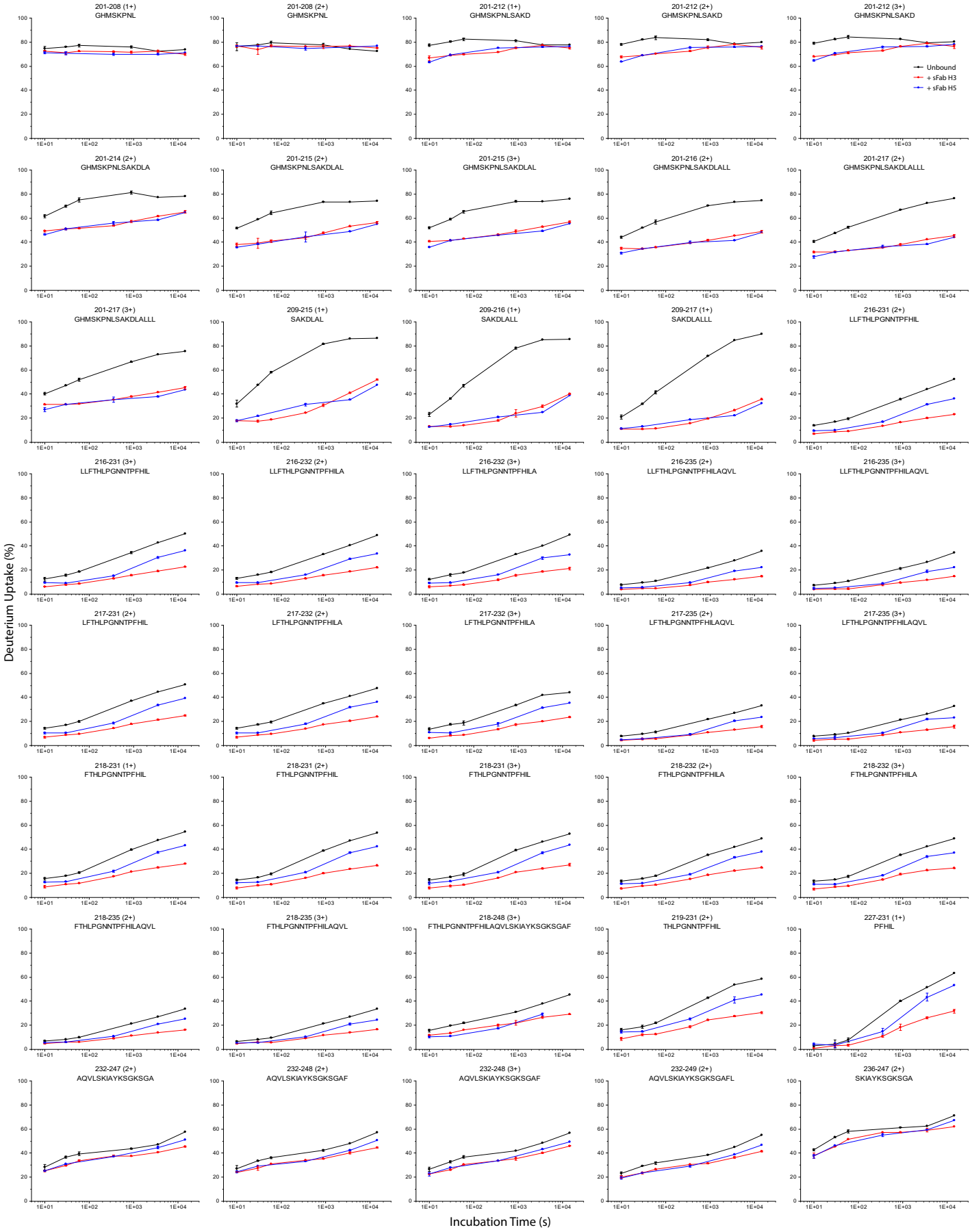
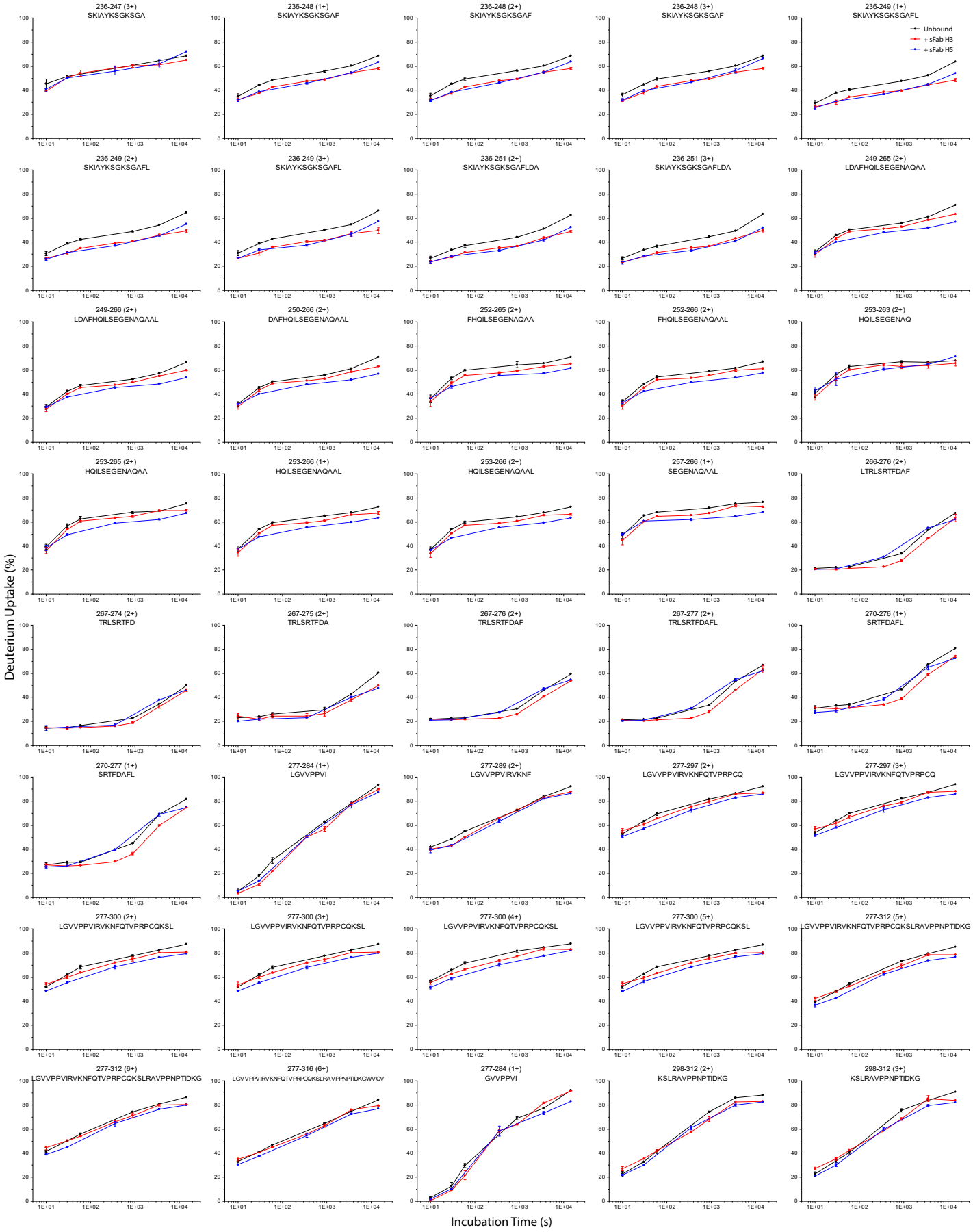
<70%
<80%
<90%
>90%

Figure S3-1. mVP35 IID



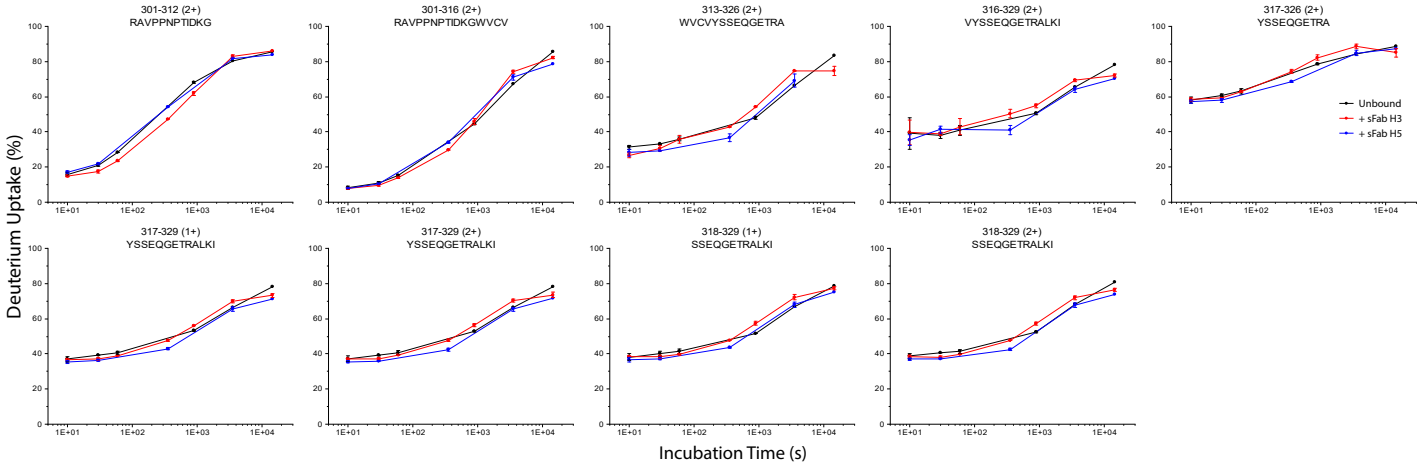
HDX kinetics curves of all mVP35 IID peptides when (black) unbound, (red) bound to Fab H3, and (blue) bound to Fab H5.

Figure S3-2. mVP35 IID



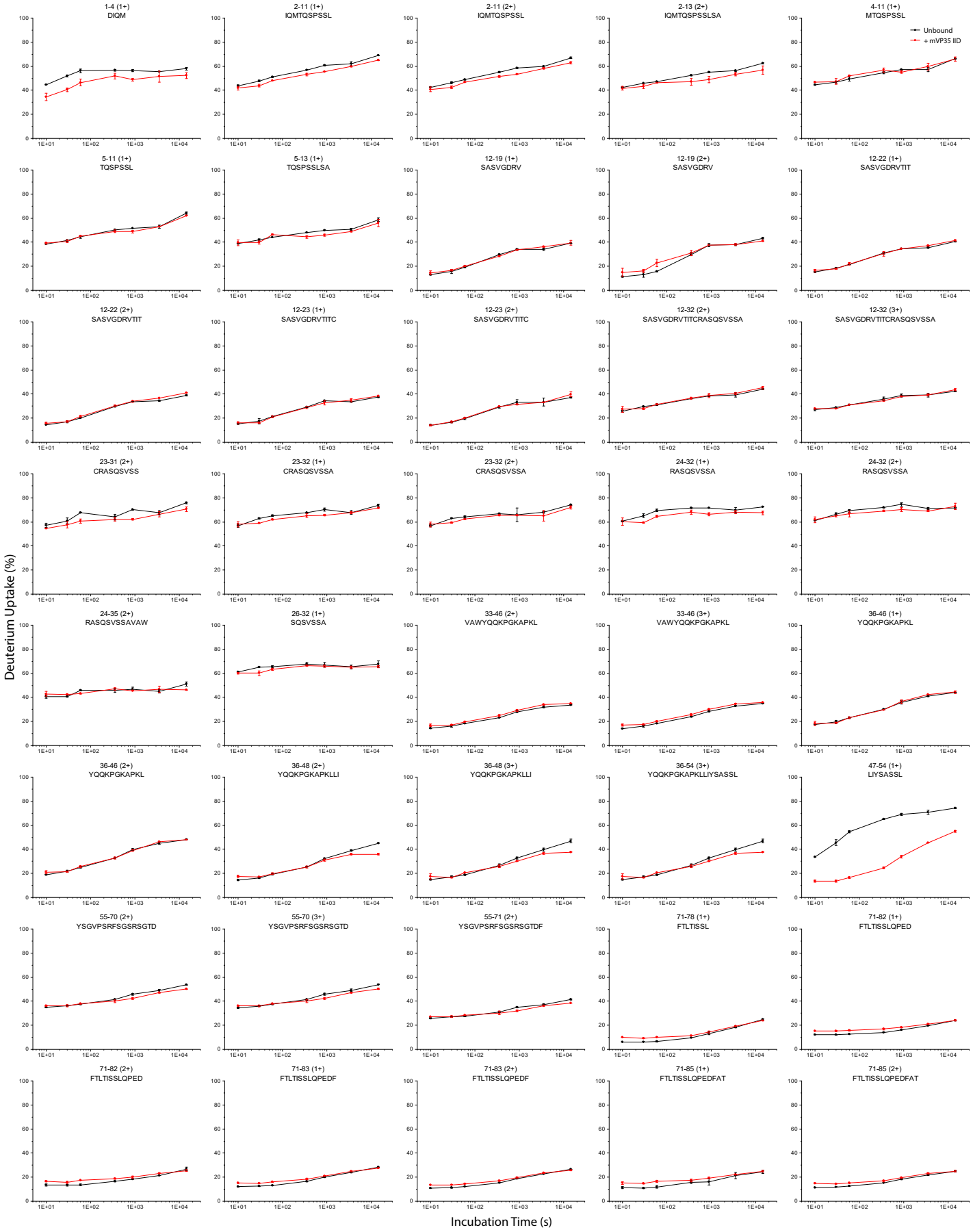
HDX kinetics curves of all mVP35 IID peptides when (black) unbound, (red) bound to Fab H3, and (blue) bound to Fab H5.

Figure S3-3. mVP35 IID



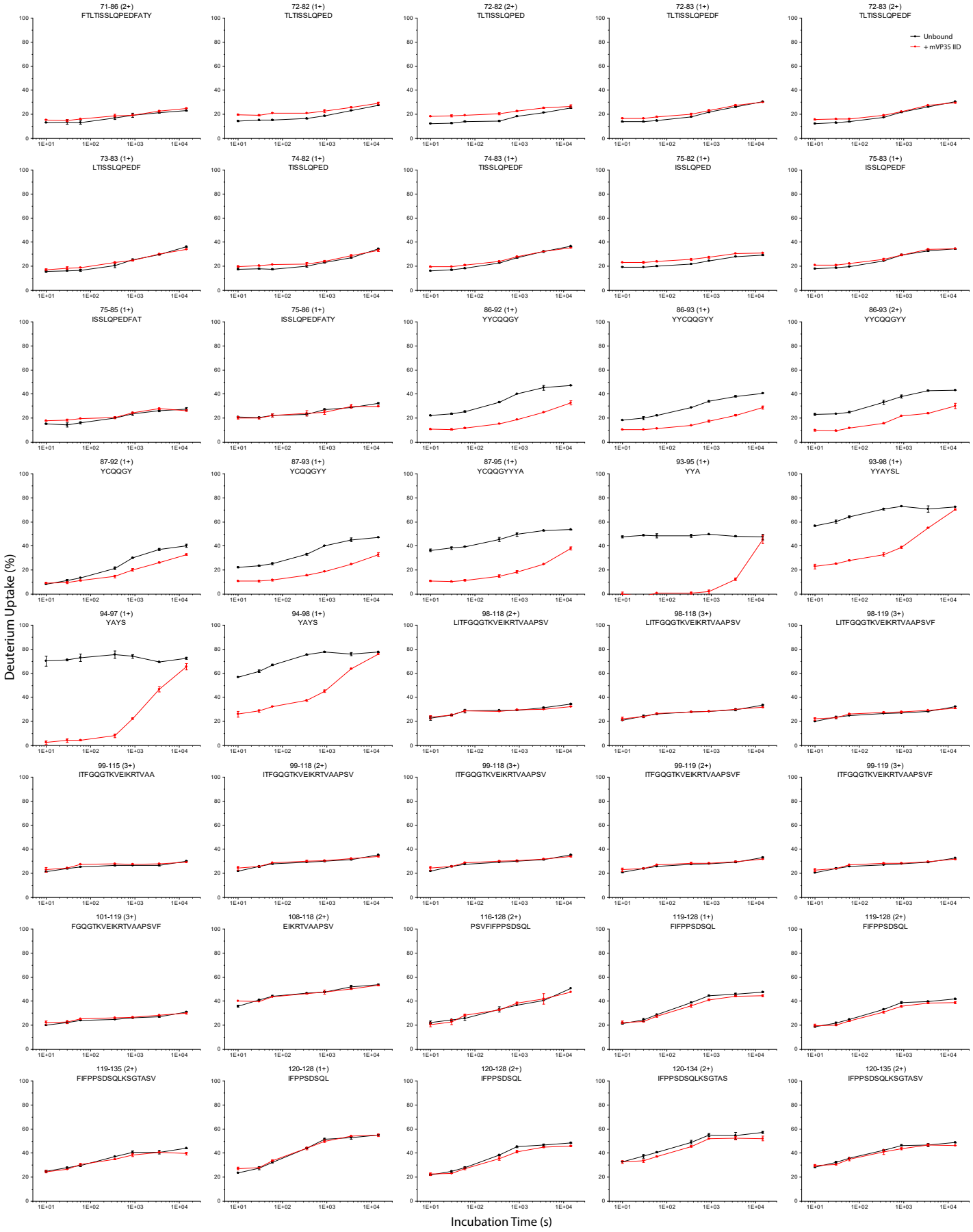
HDX kinetics curves of all mVP35 IID peptides when (black) unbound, (red) bound to Fab H3, and (blue) bound to Fab H5.

Figure S4-1. Fab H3 Light Chain



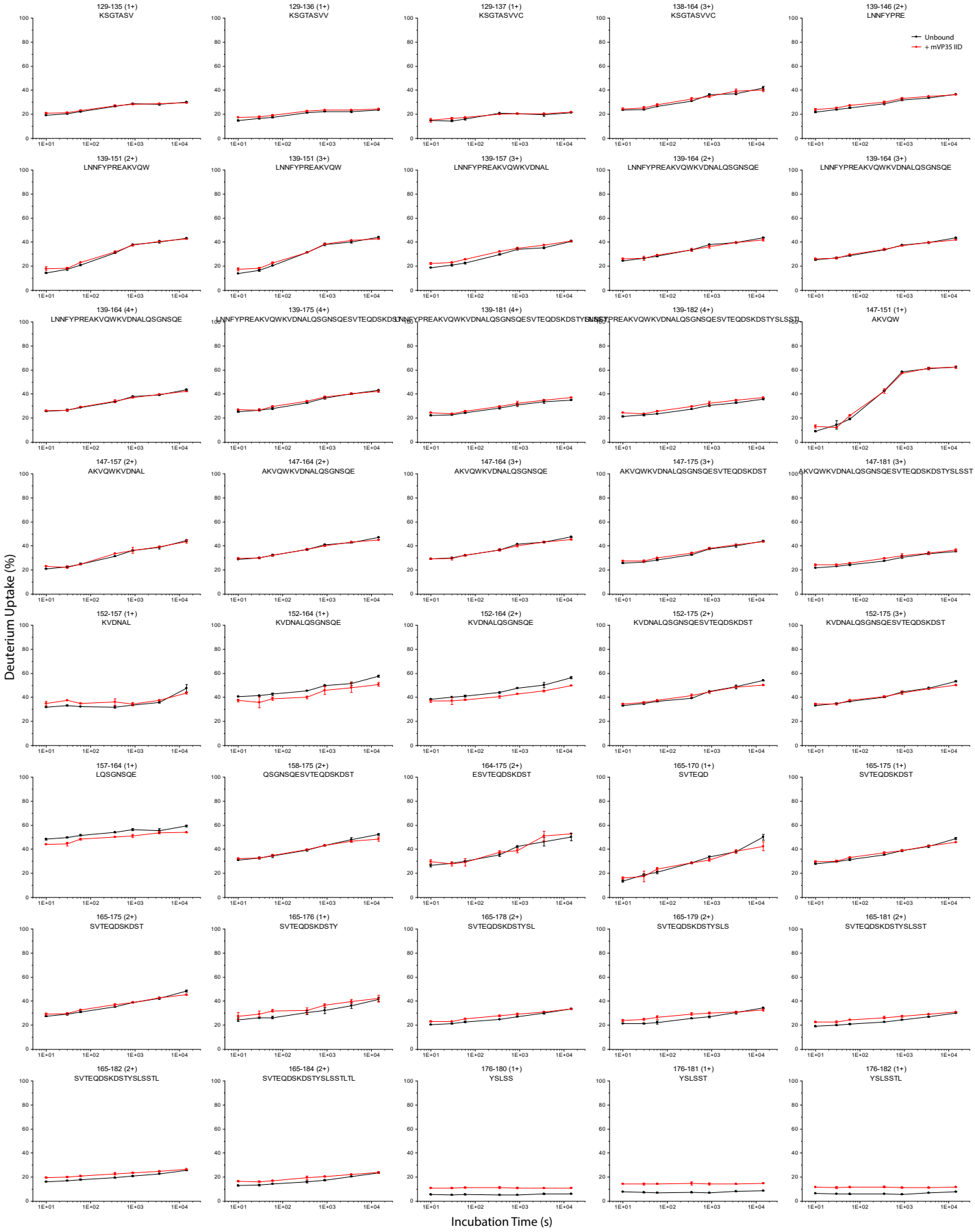
HDX kinetics curves of all Fab H3 light chain peptides when (black) unbound and (red) bound to mVP35 IID.

Figure S4-2. Fab H3 Light Chain



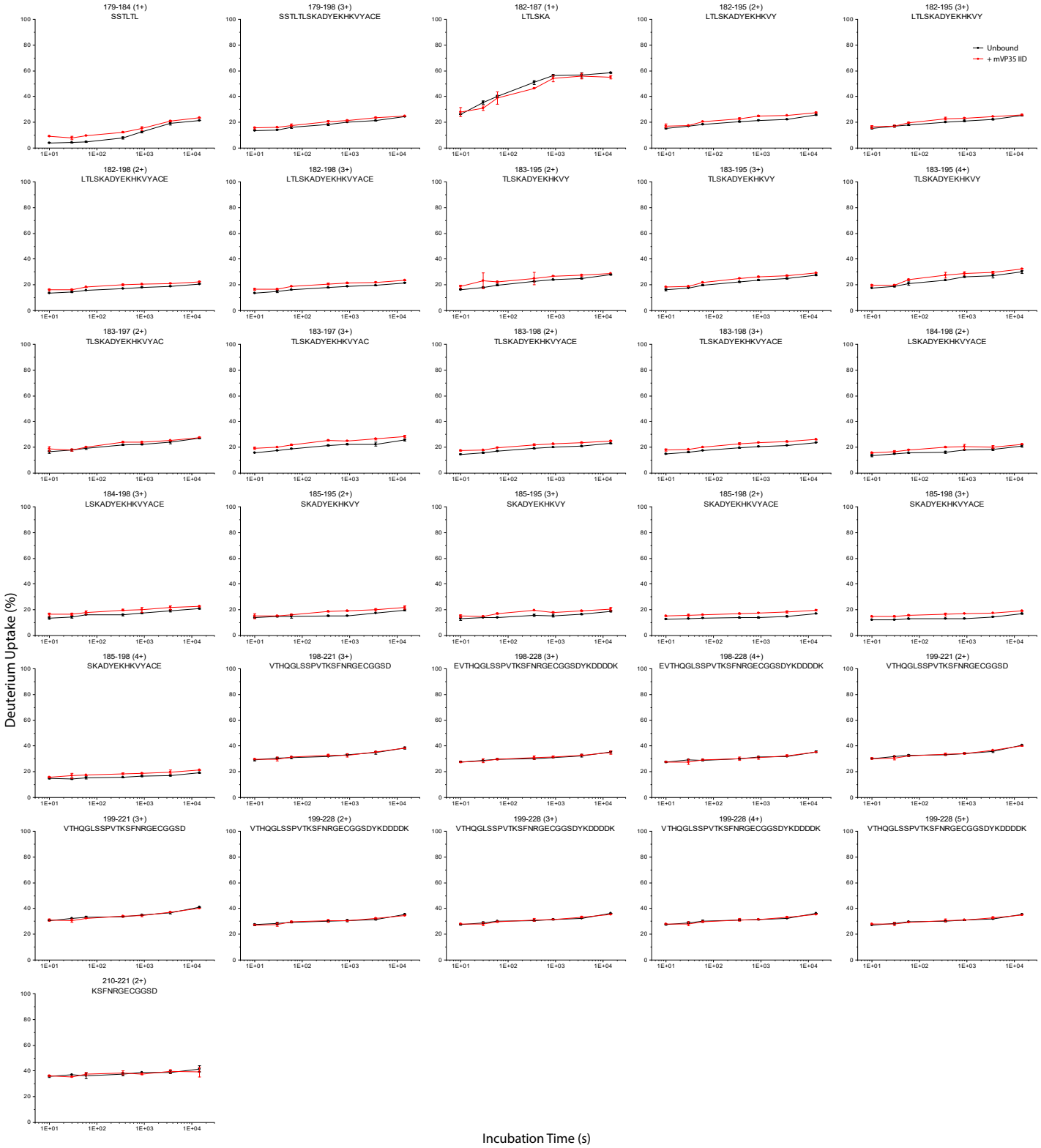
HDX kinetics curves of all Fab H3 light chain peptides when (black) unbound and (red) bound to mVP35 IID.

Figure S4-3. Fab H3 Light Chain



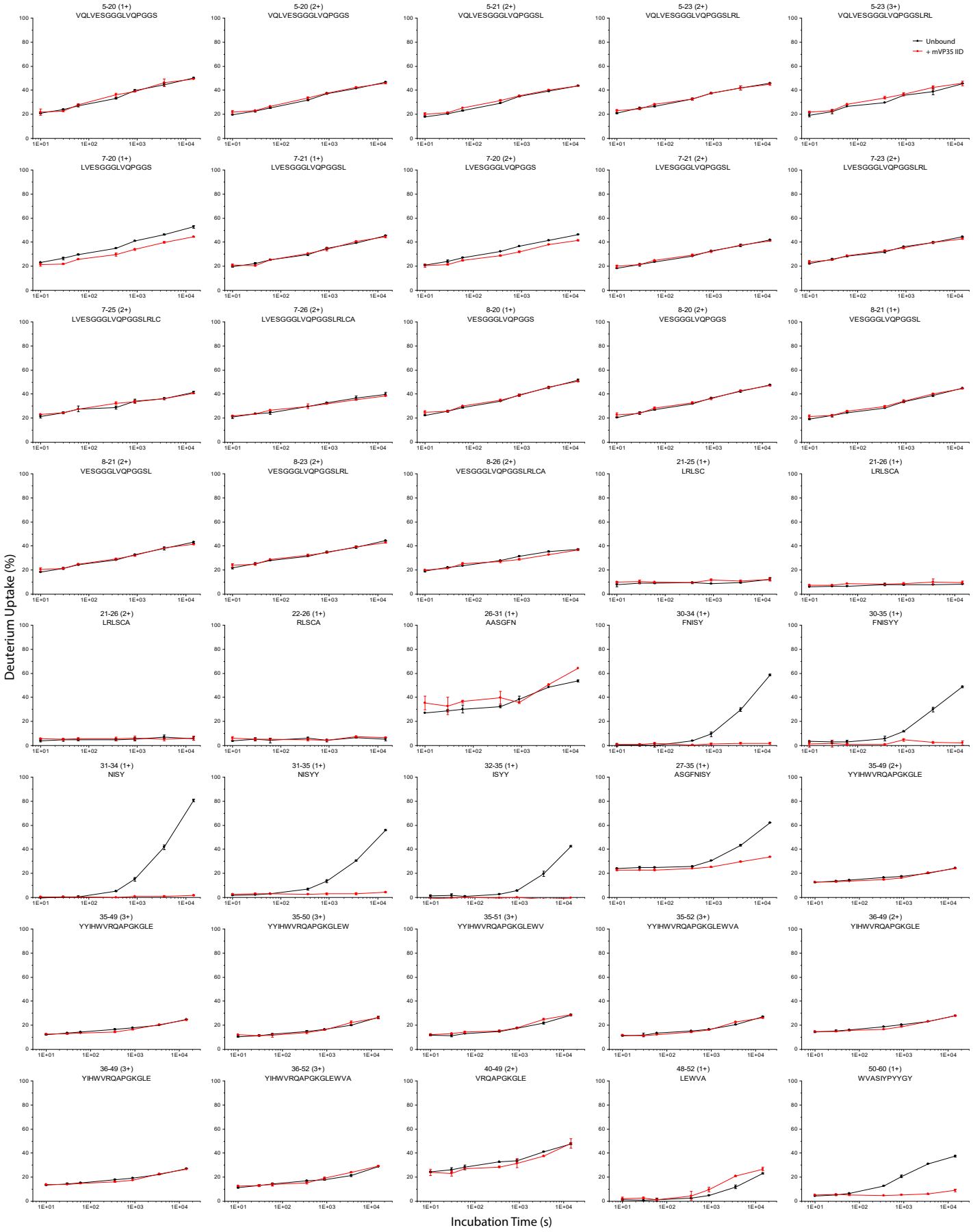
HDX kinetics curves of all Fab H3 light chain peptides when (black) unbound and (red) bound to mVP35 IID.

Figure S4-4. Fab H3 Light Chain



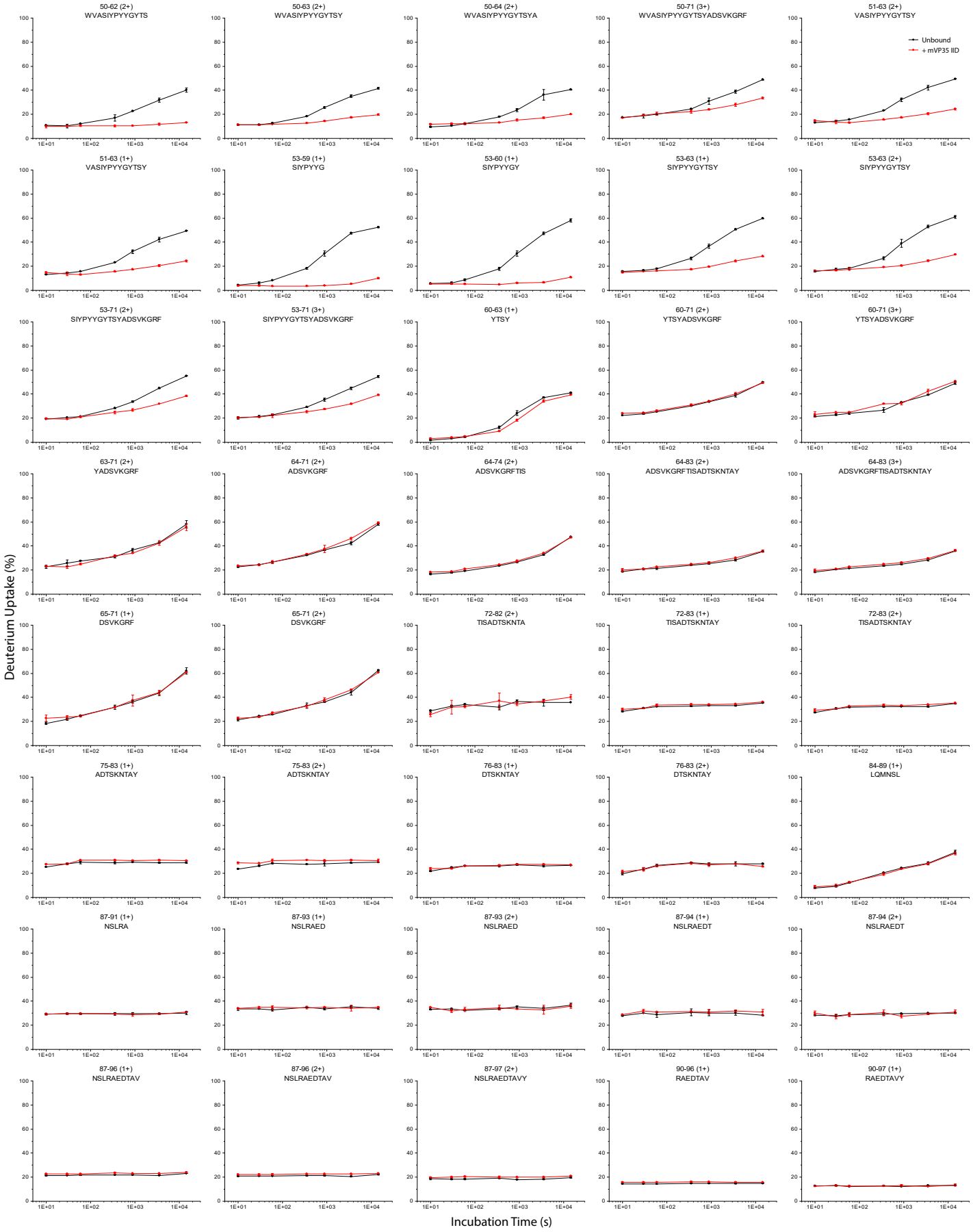
HDX kinetics curves of all Fab H3 light chain peptides when (black) unbound and (red) bound to mVP35 IID.

Figure S5-1. Fab H3 Heavy Chain



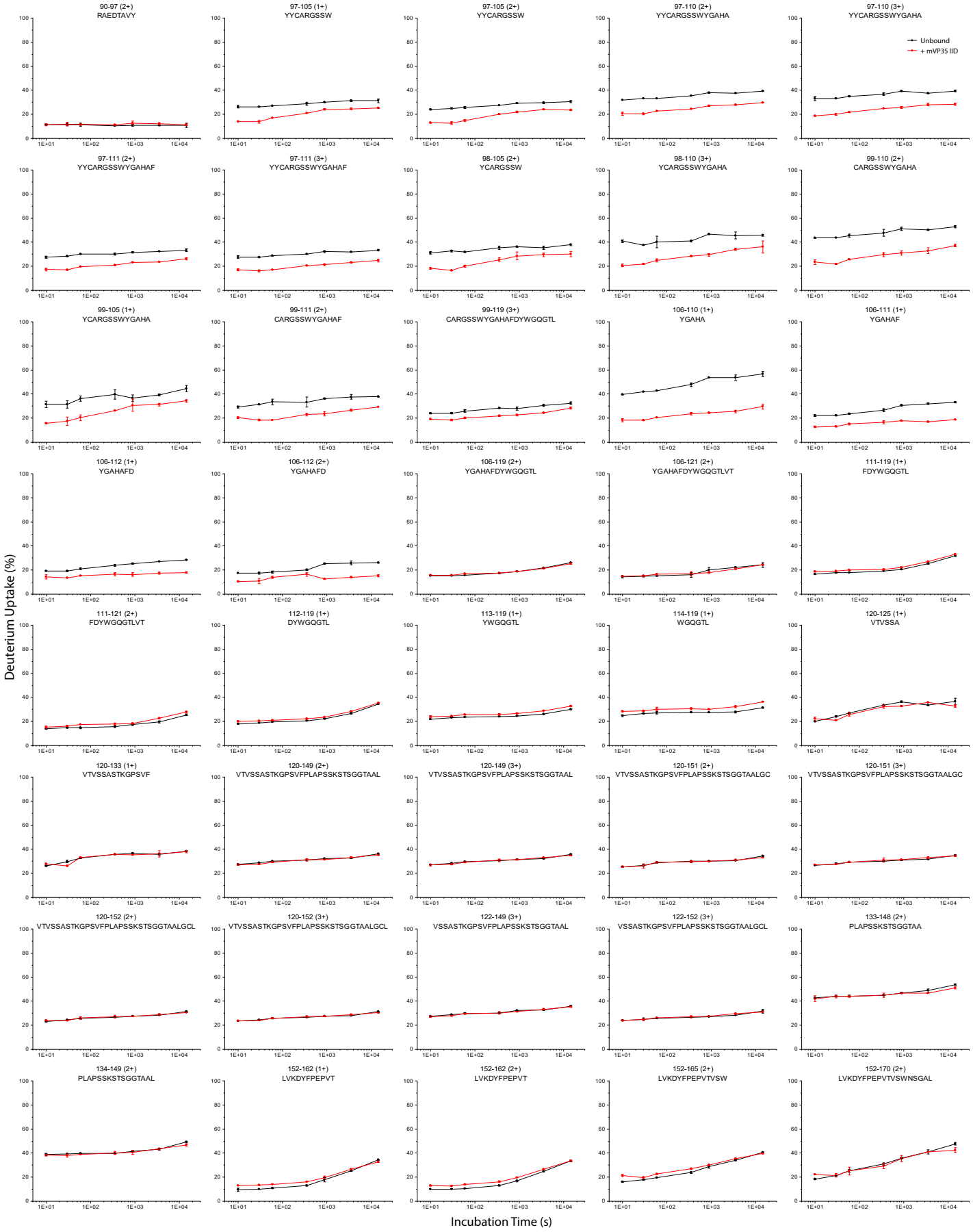
HDX kinetics curves of all Fab H3 heavy chain peptides when (black) unbound and (red) bound to mVP35 IID.

Figure S5-2. Fab H3 Heavy Chain



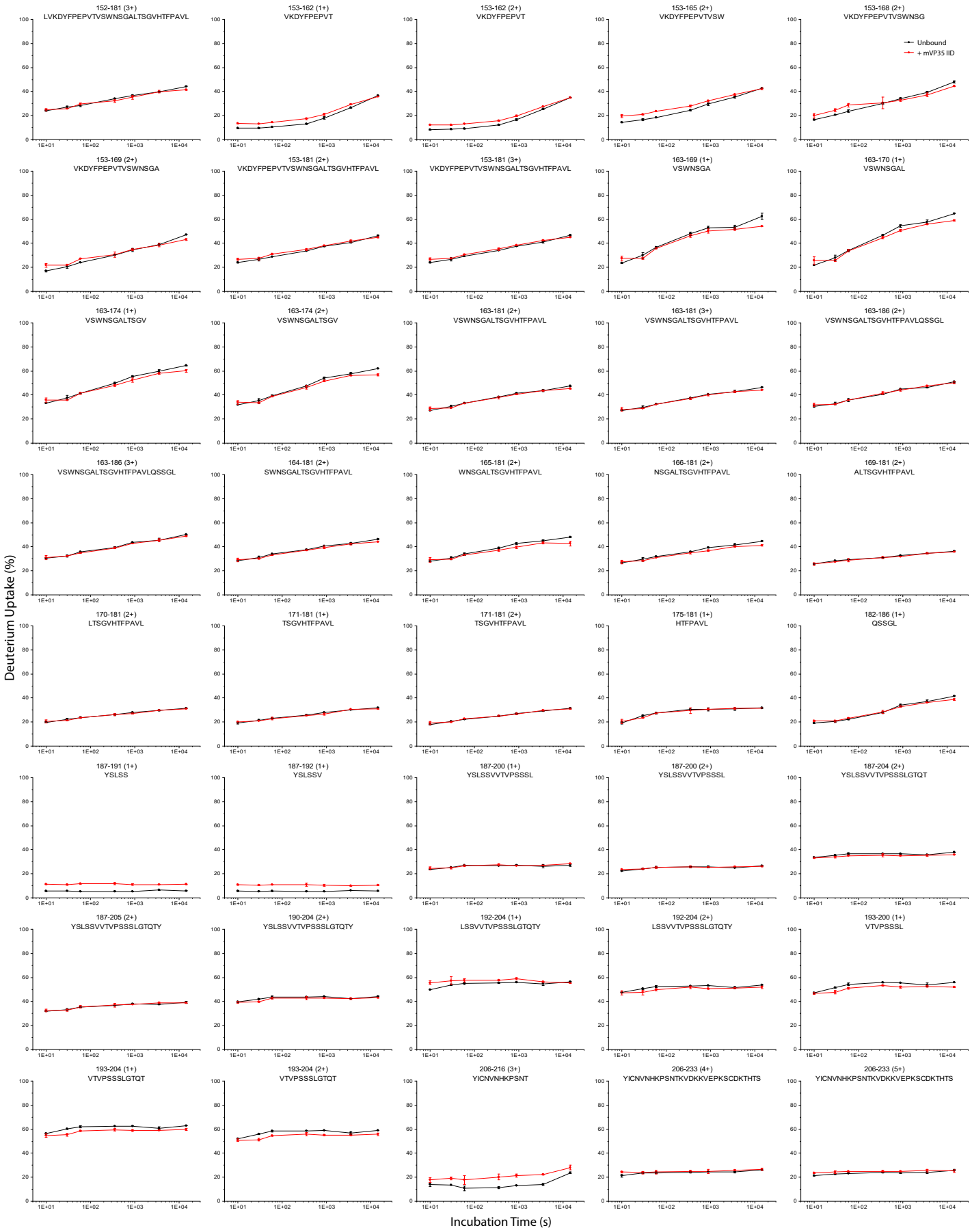
HDX kinetics curves of all Fab H3 heavy chain peptides when (black) unbound and (red) bound to mVP35 IID.

Figure S5-3. Fab H3 Heavy Chain



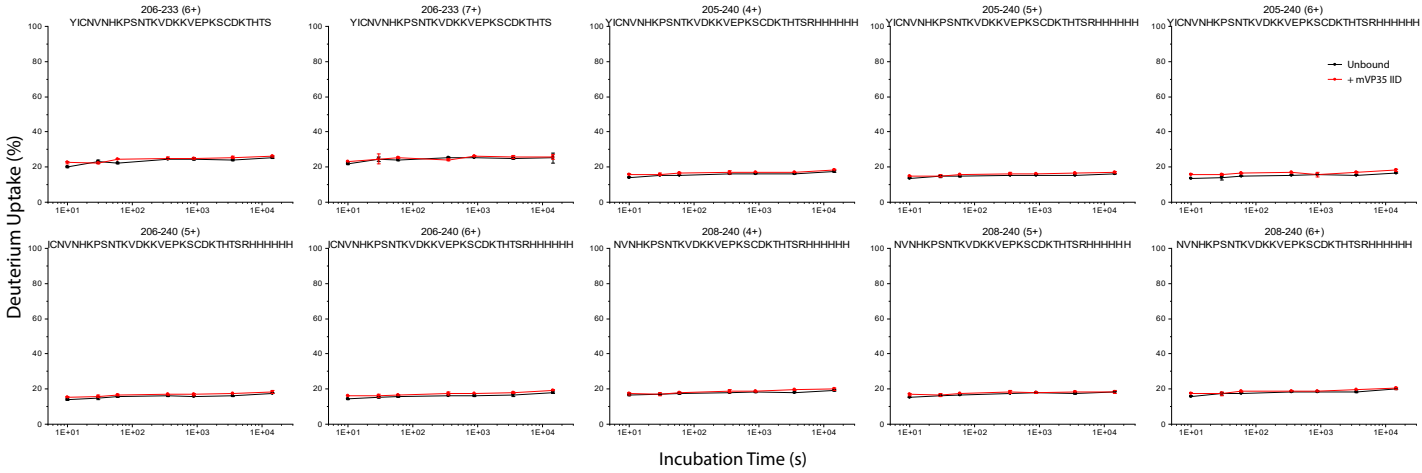
HDX kinetics curves of all Fab H3 heavy chain peptides when (black) unbound and (red) bound to mVP35 IID.

Figure S5-4. Fab H3 Heavy Chain



HDX kinetics curves of all Fab H3 heavy chain peptides when (black) unbound and (red) bound to mVP35 IID.

Figure S5-5. Fab H3 Heavy Chain



HDX kinetics curves of all Fab H3 heavy chain peptides when (black) unbound and (red) bound to mVP35 IID.

Figure S6

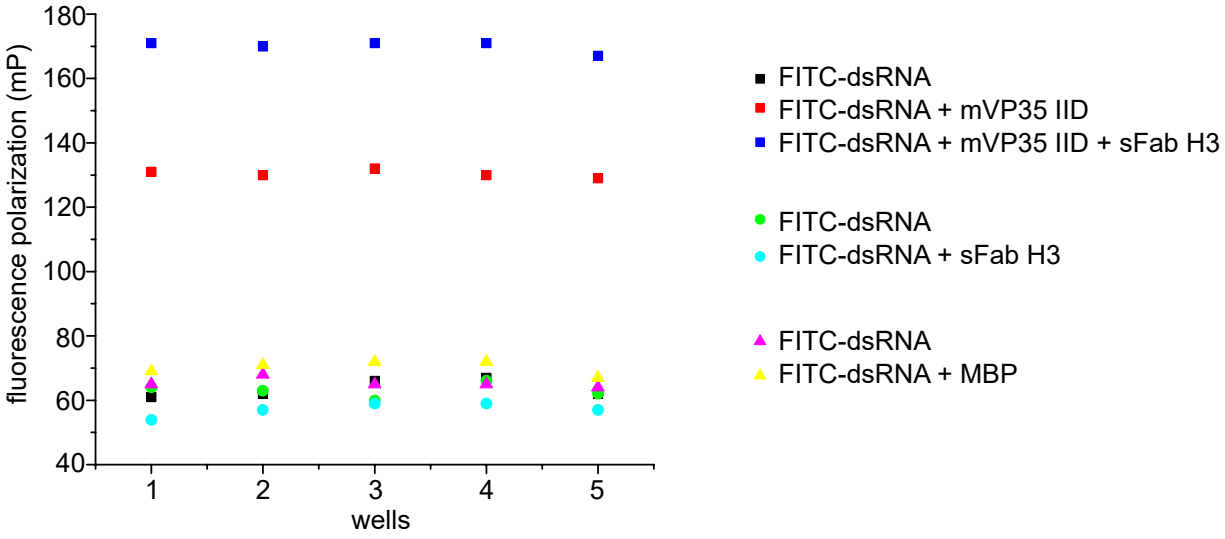
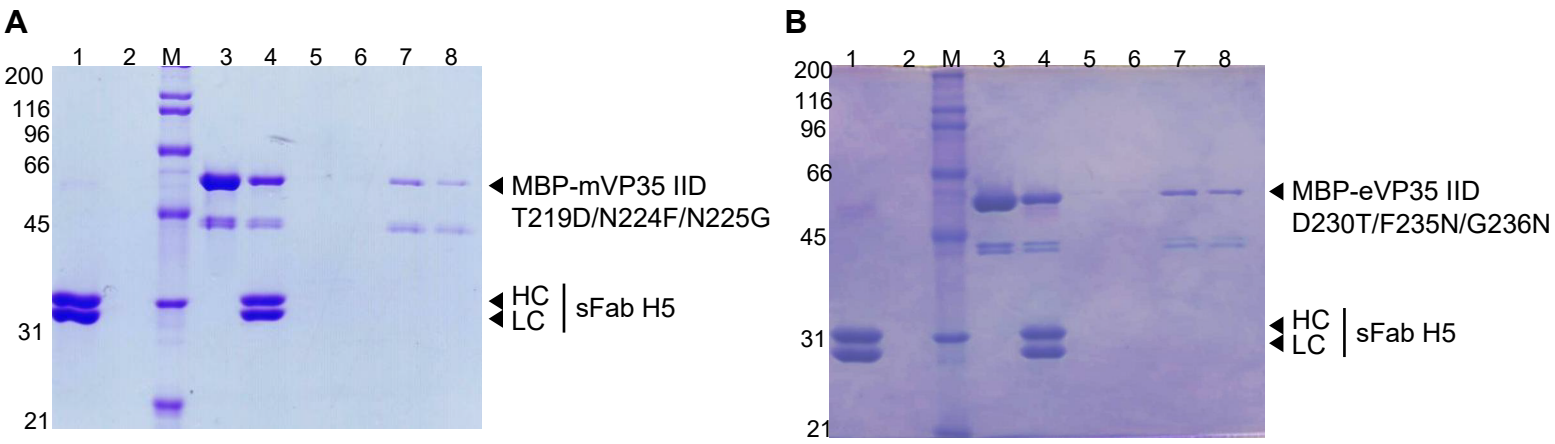

fluorescence polarization (mP)
180
160
140
120
100
80
60
40
1
2
3
4
5
wells
FITC-dsRNA
FITC-dsRNA + mVP35 IID
FITC-dsRNA + mVP35 IID + sFab H3
FITC-dsRNA
FITC-dsRNA + sFab H3
FITC-dsRNA
FITC-dsRNA + MBP

Figure S7


A
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96
66
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31
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MBP-mVP35 IID
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HC
LC
sFab H5
B
1 2 M 3 4 5 6 7 8
200
116
96
66
45
31
21
MBP-eVP35 IID
D230T/F235N/G236N
HC
LC
sFab H5