Supplemental data

Cholesterol Hydroxylase CYP46A1: Mapping of the Allosteric Site for Efavirenz, a Drug that Stimulates Enzyme Activity

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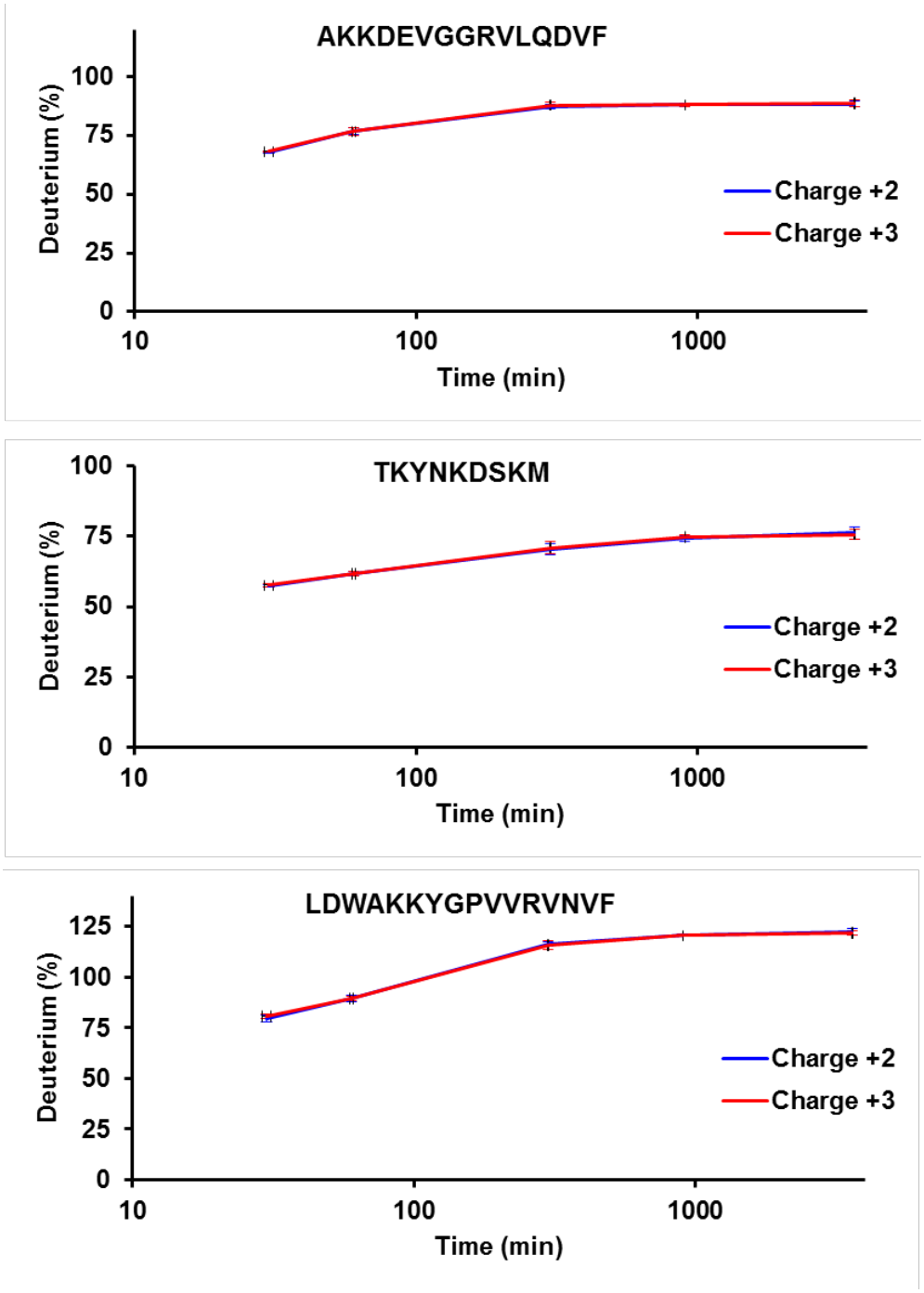


FIGURE S1. **Peptide charge consensus and precision in MS measurements.** More than one charge state was detected for many CYP46A1 peptides as indicated by deuterium uptake plots for three representative CYP46A1 peptides. Despite different charge states, the plots are nearly identical, both in averages and standard deviations. Accordingly, either charge state could be selected for analysis to reduce redundancy in measurements. The high degree of consensus between charge states and negligible standard deviations also demonstrates the precision in individual measurements.

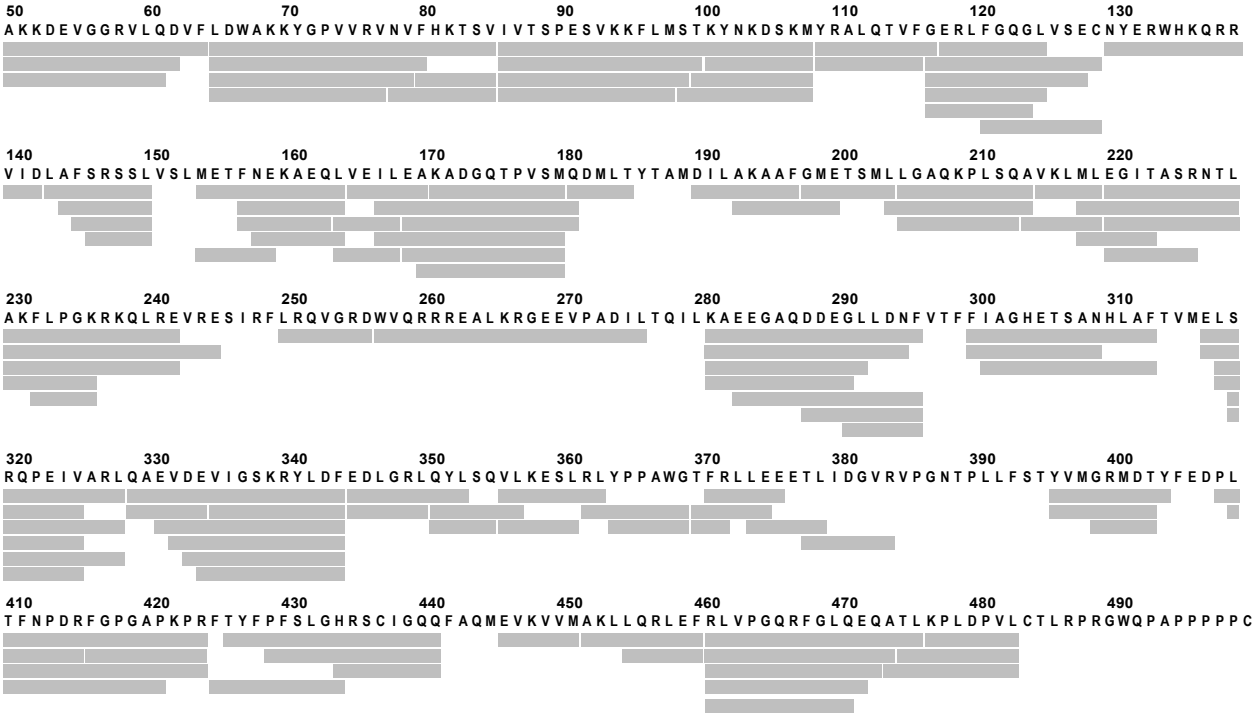
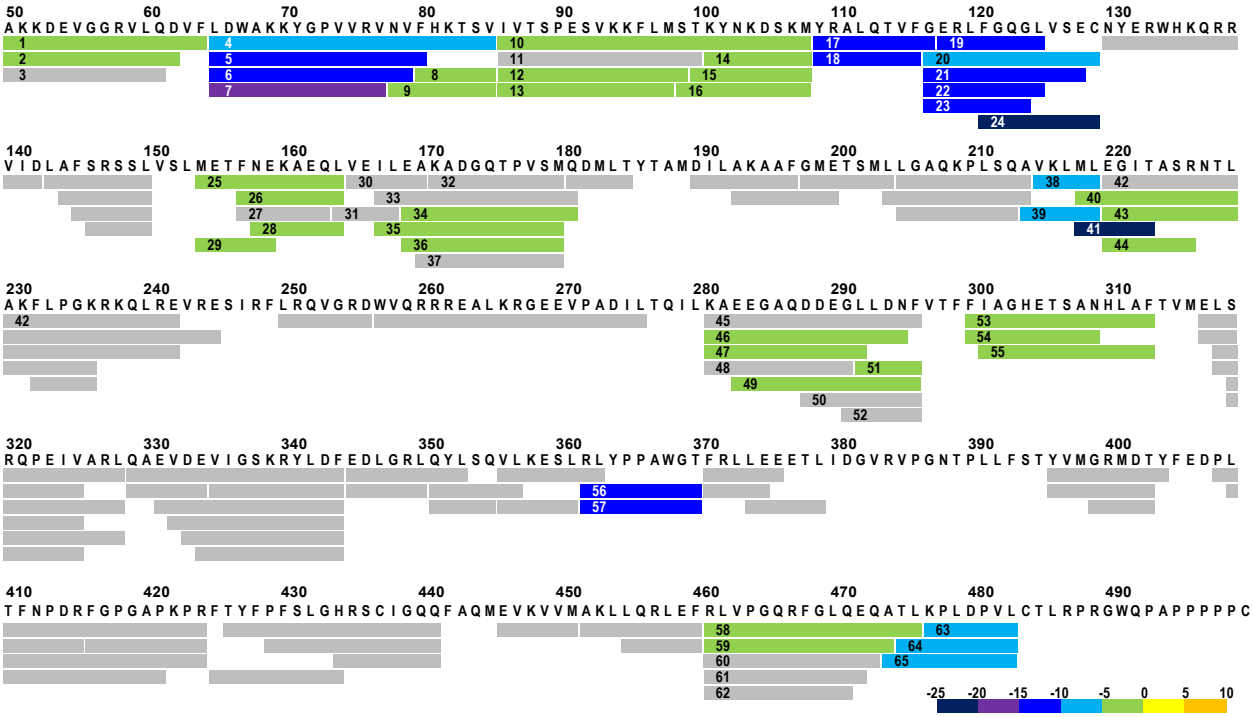


FIGURE S2. **Differential HDX analysis of EFV-bound CYP46A1 *vs* substrate-free CYP46A1.** Each bar below CYP46A1 primary sequence indicates a peptide identified by MS. All identified peptides are colored *gray* because there were no significant changes in ΔD%.

A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140 150 160 170 180 190 200 210 220
V I D L A F S R S S L V S L M E T F N E K A E Q L V E I L E A K A D G Q T P V S M Q D M L T Y T A M D I L A K A A F G M E T S M L L G A Q K P L S Q A V K L M L E G I T A S R N T L
230 240 250 260 270 280 290 300 310
A K F L P G K R K Q L R E V R E S I R F L R Q V G R D W V Q R R R E A L K R G E E V P A D I L T Q I L K A E E G A Q D D E G L L D N F V T F F I A G H E T S A N H L A F T V M E L S
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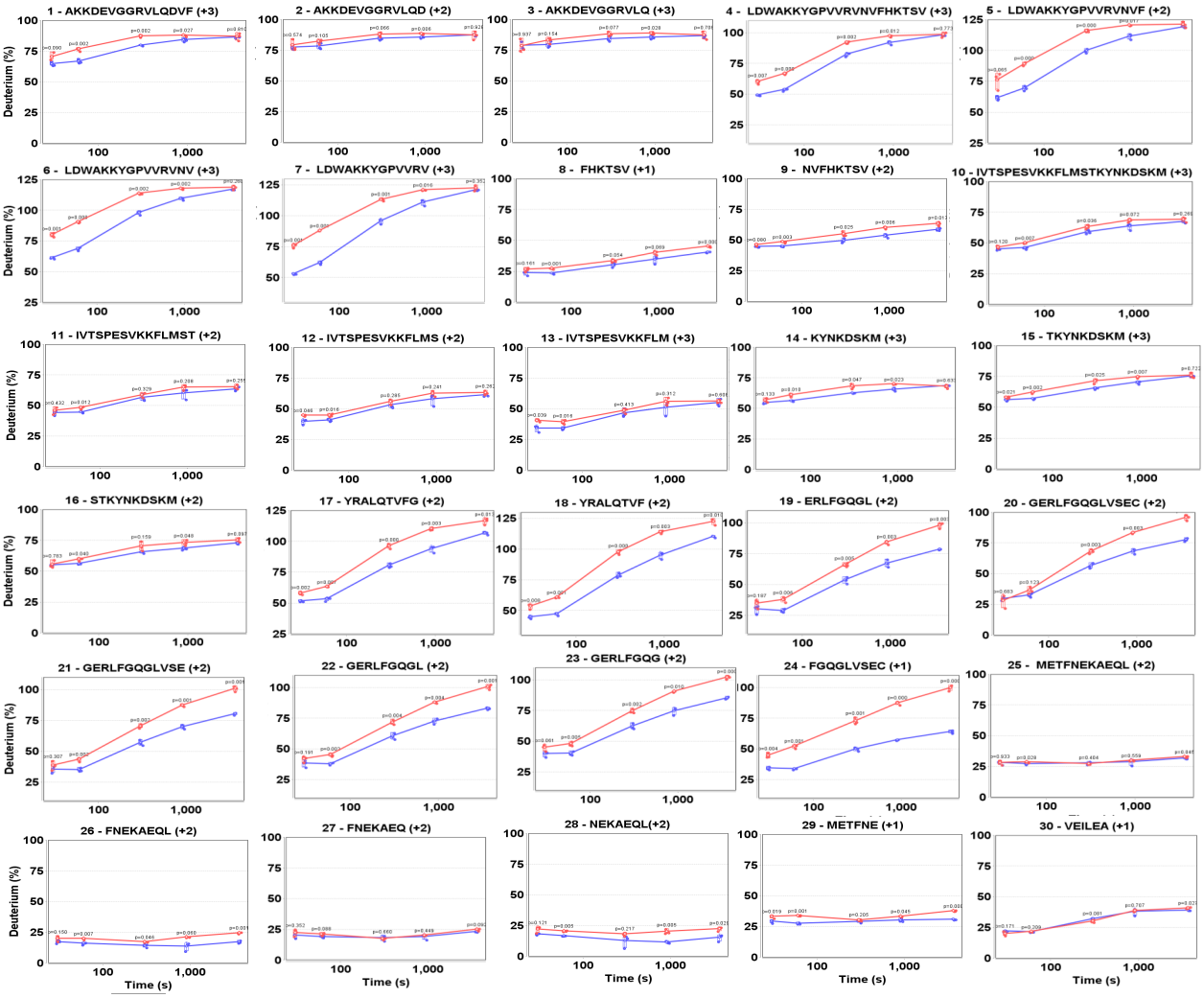
R Q P E I V A R L Q A E V D E V I G S K R Y L D F E D L G R L Q Y L S Q V L K E S L R L Y P P A W G T F R L L E E E T L I D G V R V P G N T P L L F S T Y V M G R M D T Y F E D P L
410 420 430 440 450 460 470 480 490
T F N P D R F G P G A P K P R F T Y F P F S L G H R S C I G Q Q F A Q M E V K V V M A K L L Q R L E F R L V P G Q R F G L Q E Q A T L K P L D P V L C T L R P R G W Q P A P P P P P C
-25 -20 -15 -10 -5 0 5 10

B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2 - AKKDEVGGRVLQD (+2)
3 - AKKDEVGGRVLQ (+3)
4 - LDWAKKYGPVVRVNVFHKTSV (+3)
5 - LDWAKKYGPVVRVNVF (+2)
6 - LDWAKKYGPVVRVNV (+3)
7 - LDWAKKYGPVVRV (+3)
8 - FHKTSV (+1)
9 - NVFHKTSV (+2)
10 - IVTSPESVKKFLMSTKYNKDSKM (+3)
11 - IVTSPESVKKFLMST (+2)
12 - IVTSPESVKKFLMS (+2)
13 - IVTSPESVKKFLM (+3)
14 - KYNKDSKM (+3)
15 - TKYNKDSKM (+3)
16 - STKYNKDSKM (+2)
17 - YRALQTVFG (+2)
18 - YRALQTVF (+2)
19 - ERLFGQGL (+2)
20 - GERLFGQGLVSEC (+2)
21 - GERLFGQGLVSE (+2)
22 - GERLFGQGL (+2)
23 - GERLFGQG (+2)
24 - FGQGLVSEC (+1)
25 - METFNEKAEQL (+2)
26 - FNEKAEQL (+2)
27 - FNEKAEQ (+2)
28 - NEKAEQL(+2)
29 - METFNE (+1)
30 - VEILEA (+1)
Deuterium (%)
Time (s)

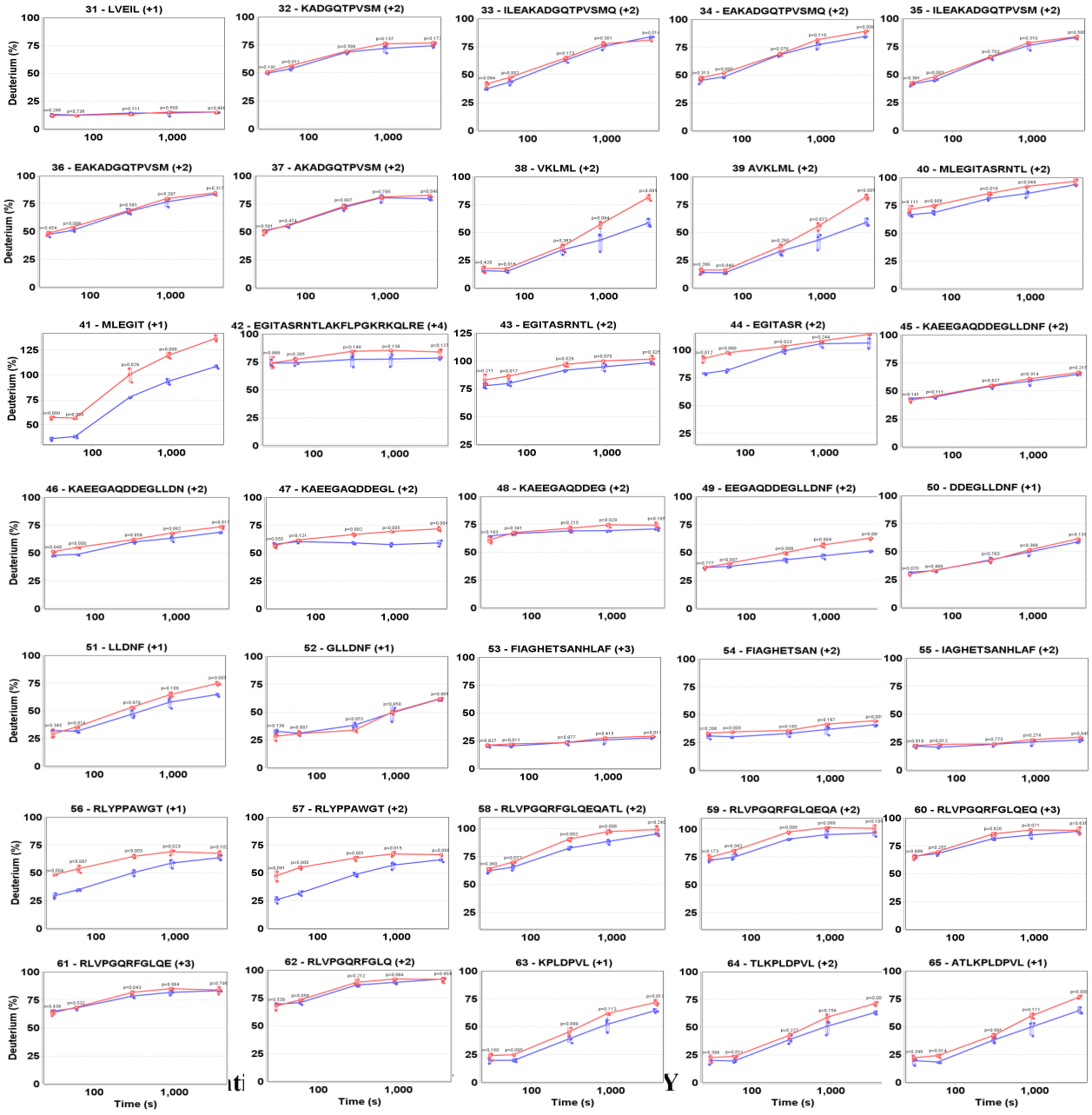

FIGURE S3. **Differential HDX analysis of cholesterol-bound CYP46A1 *vs* substrate-free CYP46A1.** *A*, Sequence coverage map of CYP46A1 with each bar indicating a peptide identified by MS. Inside numbers represent peptide numeration. Colored bars show negative ΔD% values according to the color code at the panel bottom. Gray bars indicate CYP46A1 regions with no changes in ΔD%. *B*, Kinetics of deuterium incorporation in 65 CYP46A1 peptides for substrate-bound CYP46A1 (blue) and substrate-free CYP46A1 (red). Most of these 65 peptides were used for the consolidation of the CYP46A1 regions with statistically significant changes in ΔD%. The results are means ± SD of triplicate measurements.

A


50 60 70 80 90 100 110 120 130
AKKDEVGGRVLQDVFLDWAKKYGPVVRVNVFHKTSVIVTSPESVKKFLMSTKYNKDSKMYRALQTVFGERLFGQGLVSECNYERWHKQRR
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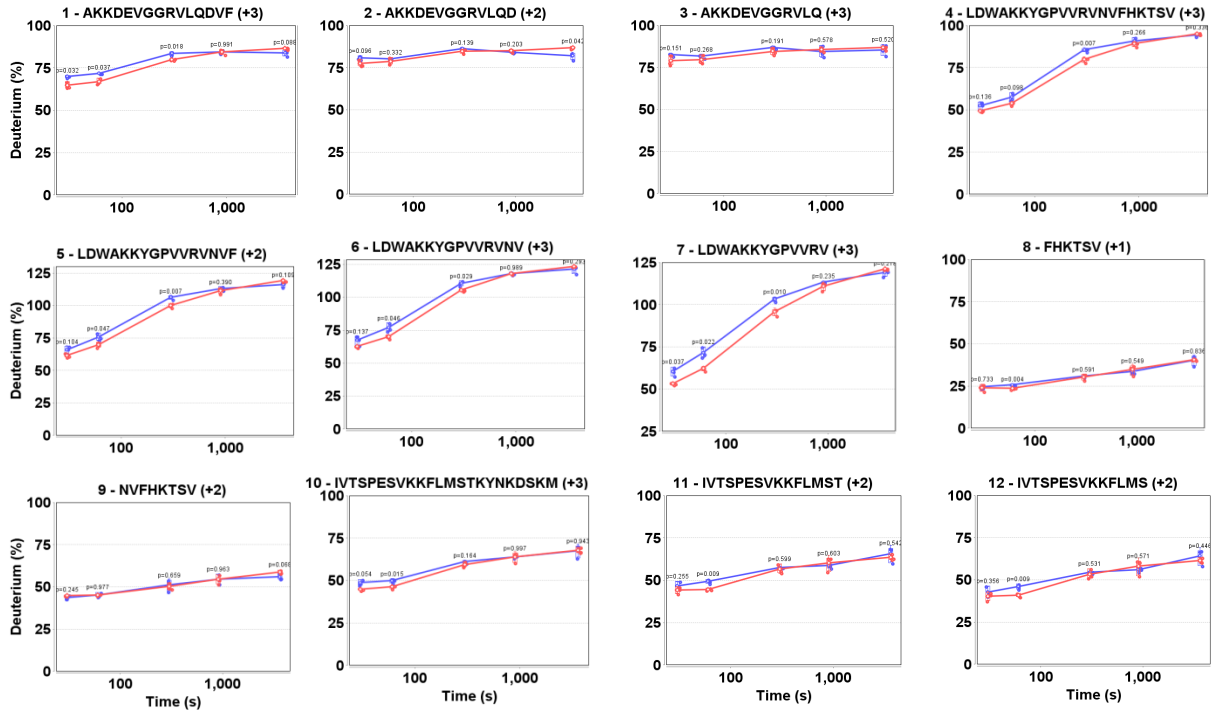
230 240 250 260 270 280 290 300 310
AKFLPGKRKQLREVRESIRFLRQVGRDWVQRRREALKRGEEVPADILTQILKAEEGAQDDEGLLDNFVTFFIAGHETSANHLAFTVMELS
320 330 340 350 360 370 380 390 400
RQPEIVARLQAEVDEVIGSKRYLDFEDLGRLQYLSQVLKESLRLYPPAWGTFRLLEEETLIDGVRVPGNTPLLFSTYVMGRMDTYFEDPL
410 420 430 440 450 460 470 480 490
TFNPDRFGPGAPKPRFTYFPFSLGHRSCIGQQFAQMEVKVVMAKLLQRLEFRLVPGQRFGLQEQATLKPLDPVLCTLRPRGWQPAPPPPPC
-25 -20 -15 -10 -5 0 5 10

B


1 - AKKDEVGGRVLQDVF (+3)
2 - AKKDEVGGRVLQD (+2)
3 - AKKDEVGGRVLQ (+3)
4 - LDWAKKYGPVVRVNVFHKTSV (+3)
5 - LDWAKKYGPVVRVNVF (+2)
6 - LDWAKKYGPVVRVNV (+3)
7 - LDWAKKYGPVVRV (+3)
8 - FHKTSV (+1)
9 - NVFHKTSV (+2)
10 - IVTSPESVKKFLMSTKYNKDSKM (+3)
11 - IVTSPESVKKFLMST (+2)
12 - IVTSPESVKKFLMS (+2)
Deuterium (%)
Time (s)

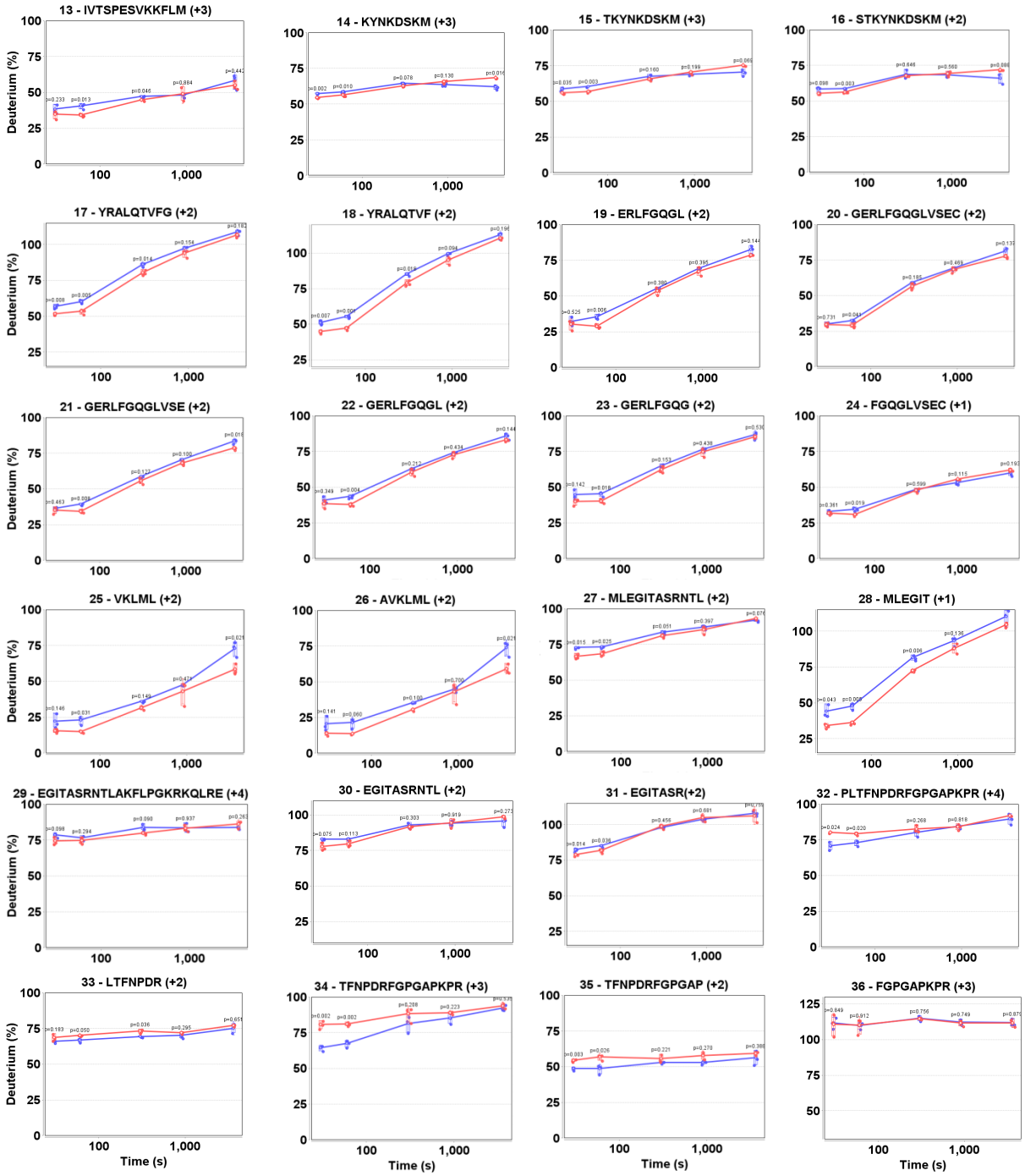


FIGURE S4. **Differential HDX analysis of the CYP46A1 double complex with EFV and cholesterol *vs* CYP46A1 complex with cholesterol.** *A*, Sequence coverage map of CYP46A1 with each bar indicating a peptide identified by MS. Inside numbers represent peptide numeration. Colored bars show negative and positive ΔD% values according to the color code at the panel bottom. Gray bars indicate CYP46A1 regions with no changes in ΔD%. *B*, Kinetics of deuterium incorporation in 36 CYP46A1 peptides for the P450 double complex with cholesterol and EFV (blue) and single complex with cholesterol (red). Most of these 36 peptides were used for the consolidation of the CYP46A1 regions with statistically significant changes in ΔD%. The results are means ± SD of triplicate measurements.