Supplemental Figures

FIGURE S1. Catalytic activities of MTSL-labeled ExoU variants. (A) Relative activity of each spin label derivative compared to unlabeled recombinant wild type ExoU. Black bars indicate spin-labeled variants with PED6 hydrolysis rates less than 0.25 wild type. Bars at the top of the Figure indicate locations of helices, lines represent the interhelical loops, and arrows the positions of variants with reduced activity. (B) Structural model of the C-terminal four-helix bundle indicating the position of derivatives with less than 25% activity as compared to wild type (blue spheres).

FIGURE S2. Far-UV circular dichroism spectra of ExoU and spin-labeled derivatives. Molar ellipticity (θ) in the region from 250-195 nm for wild type (WT) ExoU and several MTSL spin-labeled derivatives that exhibit reduced catalytic activity.

FIGURE S3A. EPR spectra of MTSL-labeled helical sites in the presence of liposomes. (A) Spectral overlays of spin labeled mutants in the first helix of the C-terminal four-helix bundle. Green spectra are in the presence of 100-fold excess liposome substrate and black spectra correspond to the apoenzyme state. (B-D): Spectral overlays of spin labeled sites in the second through fourth helices of the C-terminal bundle, respectively. Spectra were normalized to integrated intensity. Scaling factors for presentation are shown at the right of each spectrum. All scan widths are 100 G.

FIGURE S3B. EPR spectra MTSL-labeled loop sites in the presence of liposomes. (A) Spectral overlays of spin label mutants in the first loop of the helical bundle. Green spectra correspond to ExoU in the presence of 100-fold excess liposome substrate and black spectra correspond to the apoenzyme state. (B-D): Spectral overlays of spin label sites in the second and third interhelical loops of the C-Terminal bundle, respectively. Spectra were normalized to integrated intensity. Scaling factors for presentation are shown at the right of each spectrum. Scan widths 100 G.

FIGURE S4A. EPR spectral overlays of helical sites in the Apo and Holo states. (A) Spectral overlays of R1-labeled mutants in the first helix of the C-terminal four-helix bundle. Red spectra are in the presence of liposomes and diUb (Holo state) and black spectra correspond to the Apo state. (B-D): Spectral overlays of R1-labeled sites in the second through fourth helices of the C-terminal bundle, respectively. Spectra were normalized to integrated intensity. Scaling factors are shown at the right of each spectrum. Scan widths are 100 G.

FIGURE S4B. EPR spectral overlays of loop sites in the Apo and Holo states. (A) Spectral overlays of R1-labeled mutants in the first helix of the C-terminal four-helix bundle. Red spectra are in the presence of liposomes and diUb (Holo state) and black spectra correspond to the Apo state. (B-D): Spectral overlays of R1-labeled sites in the second through fourth helices of the C-terminal bundle, respectively. Spectra were normalized to integrated intensity. Scaling factors are shown at the right of each spectrum. Scan widths are 100 G.

FIGURE S5. Simulation of V602R1 EPR spectra in the apo (Left) and holo (Right) states. (Top) Overlay of the experimental (black) and fitted (red) spectra. (Bottom and middle) Individual components used to achieve the fit. A small (~2%) contribution from rapidly-tumbling was included to improve the overall fit in the apo state. The holo state could not be adequately fit with a single component. Component fractions, rotational correlation times, and order parameters are indicated in the *insets*. The order parameter was 0 unless otherwise noted.

FIGURE S6. Simulation of R611R1 EPR spectra in the apo (Left) and holo (Right) states. (Top) Overlay of the experimental (black) and fitted (red) spectra. (Bottom and middle) Individual components used to achieve the fit. Component fractions, rotational correlation times, and order parameters are indicated in the *insets*. The order parameter was 0 unless otherwise noted.

FIGURE S7. Experimentally determined Φ values and calculated distances from the lipid phosphates based on equation 4. (Open circles, apo), (green triangles, liposomes only), (orange squares, holo), (black squares, PCSL standards). Negative distances indicate sites exposed to the aqueous phase.

FIGURE S1.

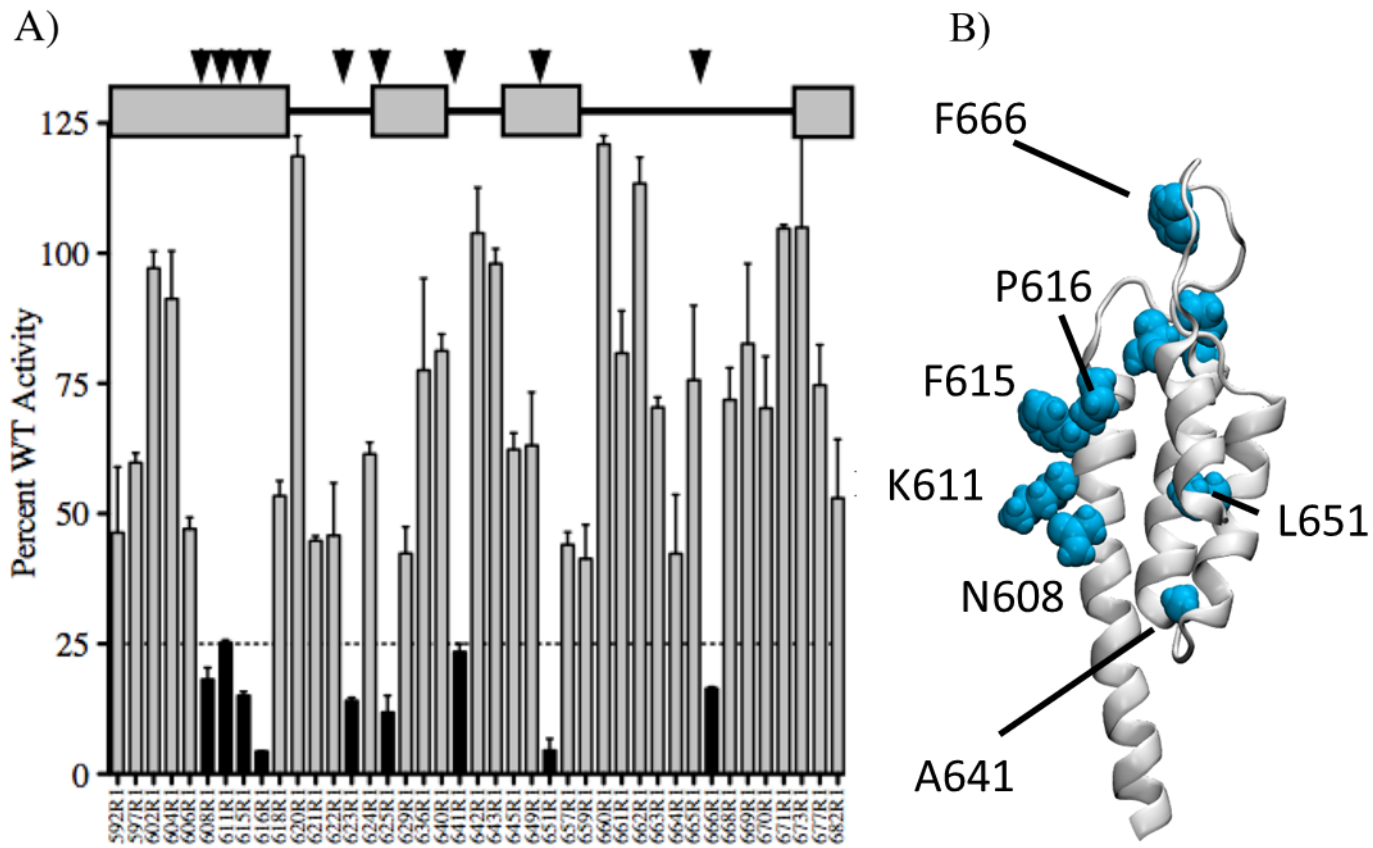

A)
B)
Percent WT Activity
125
100
75
50
25
0
592R1
597R1
602R1
604R1
606R1
608R1
611R1
615R1
616R1
618R1
620R1
621R1
622R1
623R1
624R1
625R1
629R1
636R1
640R1
641R1
642R1
643R1
645R1
649R1
651R1
657R1
659R1
660R1
661R1
662R1
663R1
664R1
665R1
666R1
668R1
669R1
670R1
671R1
673R1
677R1
682R1
F666
P616
F615
K611
L651
N608
A641

FIGURE S2.

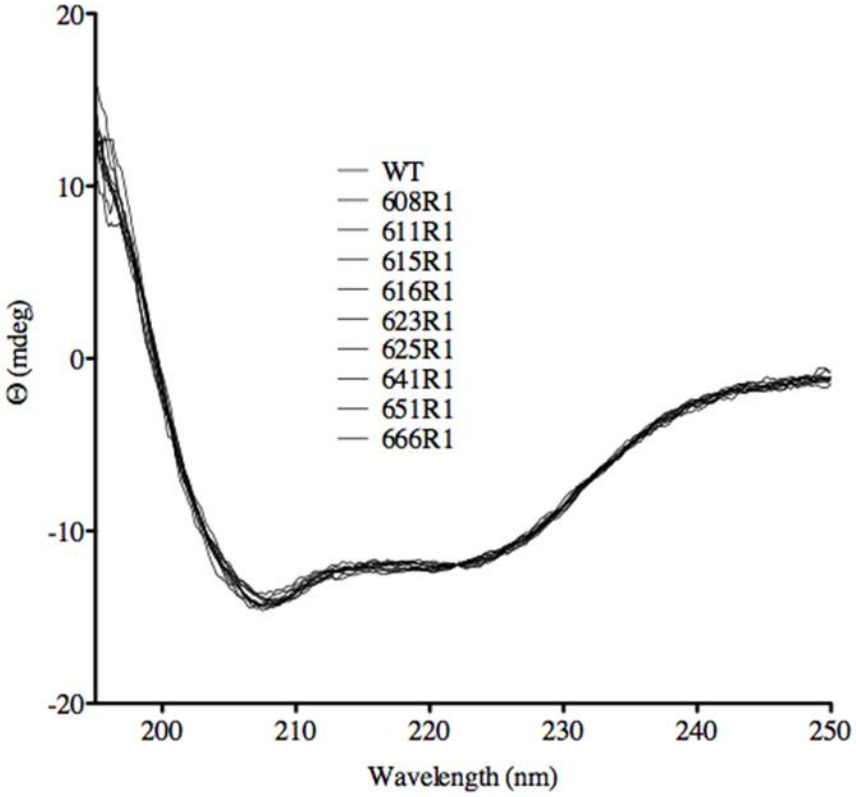

20
10
0
-10
-20
Θ (mdeg)
200
210
220
230
240
250
Wavelength (nm)
WT
608R1
611R1
615R1
616R1
623R1
625R1
641R1
651R1
666R1

FIGURE S3A.

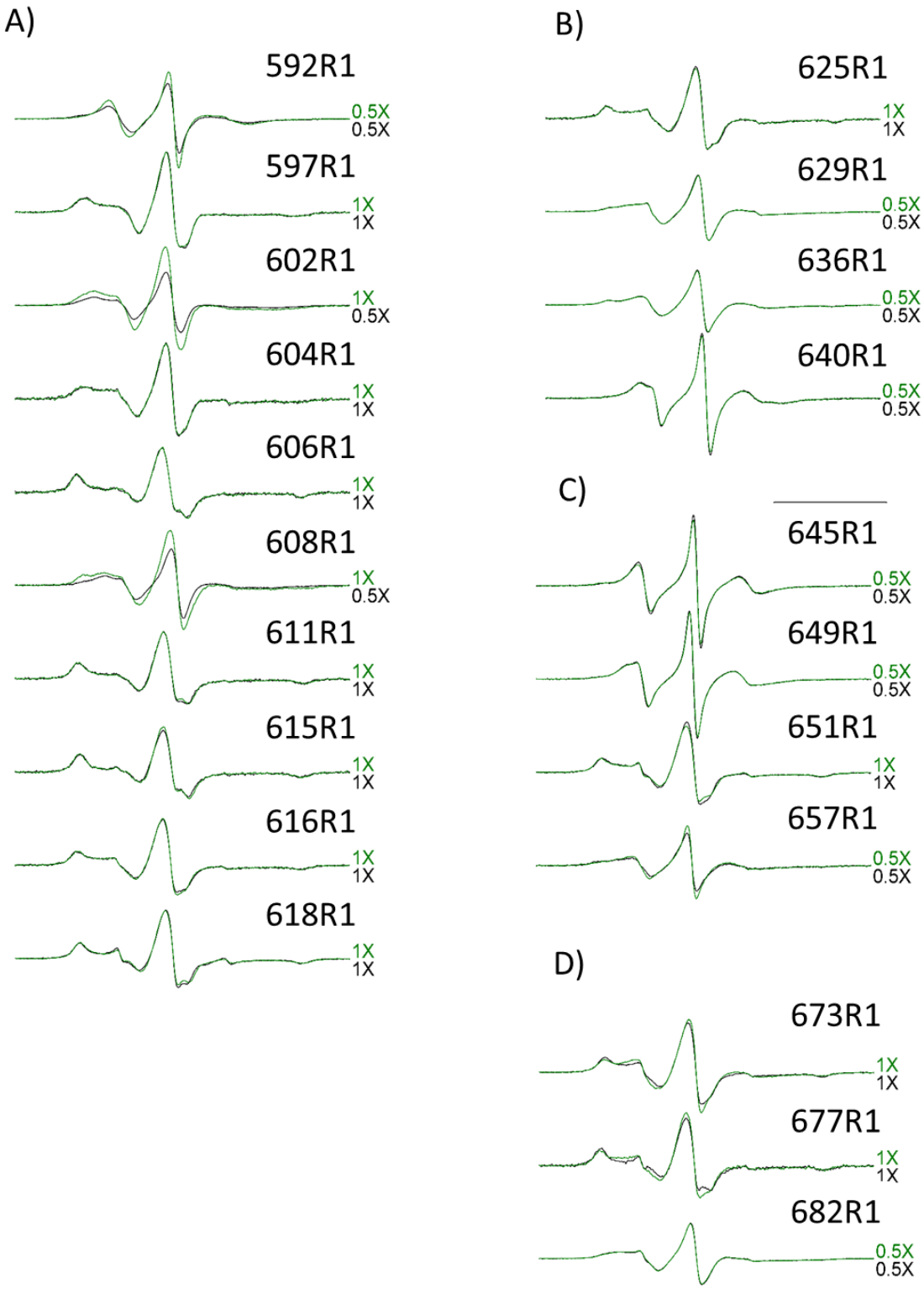

A)
592R1
0.5X
0.5X
597R1
1X
1X
602R1
1X
0.5X
604R1
1X
1X
606R1
1X
1X
608R1
1X
0.5X
611R1
1X
1X
615R1
1X
1X
616R1
1X
1X
618R1
1X
1X
B)
625R1
1X
1X
629R1
0.5X
0.5X
636R1
0.5X
0.5X
640R1
0.5X
0.5X
C)
645R1
0.5X
0.5X
649R1
0.5X
0.5X
651R1
1X
1X
657R1
0.5X
0.5X
D)
673R1
1X
1X
677R1
1X
1X
682R1
0.5X
0.5X

FIGURE S3B.

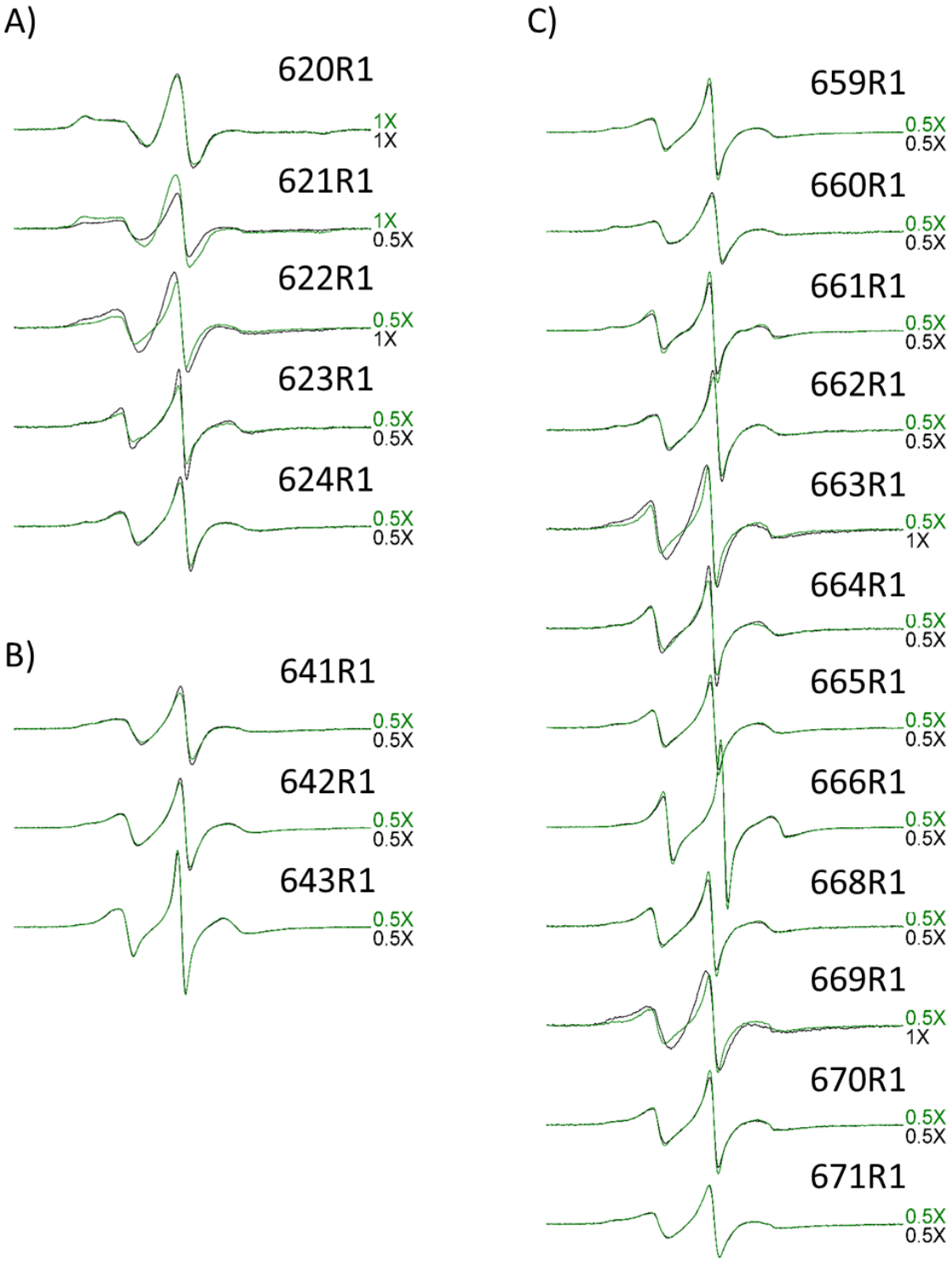

A)
620R1
1X
1X
621R1
1X
0.5X
622R1
0.5X
1X
623R1
0.5X
0.5X
624R1
0.5X
0.5X
B)
641R1
0.5X
0.5X
642R1
0.5X
0.5X
643R1
0.5X
0.5X
C)
659R1
0.5X
0.5X
660R1
0.5X
0.5X
661R1
0.5X
0.5X
662R1
0.5X
0.5X
663R1
0.5X
1X
664R1
0.5X
0.5X
665R1
0.5X
0.5X
666R1
0.5X
0.5X
668R1
0.5X
0.5X
669R1
0.5X
1X
670R1
0.5X
0.5X
671R1
0.5X
0.5X

FIGURE S4A.

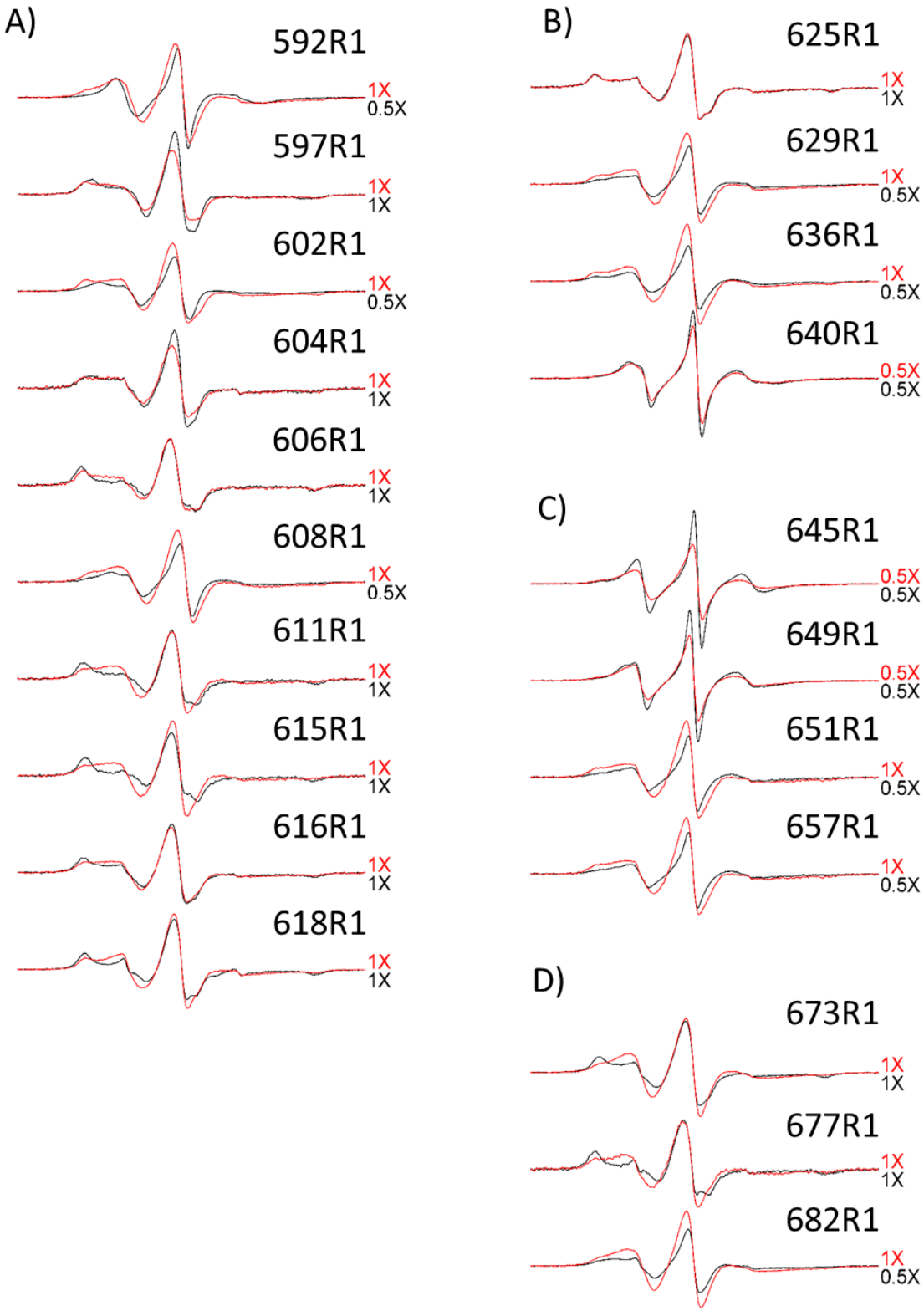

A)
592R1
1X
0.5X
597R1
1X
1X
602R1
1X
0.5X
604R1
1X
1X
606R1
1X
1X
608R1
1X
0.5X
611R1
1X
1X
615R1
1X
1X
616R1
1X
1X
618R1
1X
1X
B)
625R1
1X
1X
629R1
1X
0.5X
636R1
1X
0.5X
640R1
0.5X
0.5X
C)
645R1
0.5X
0.5X
649R1
0.5X
0.5X
651R1
1X
0.5X
657R1
1X
0.5X
D)
673R1
1X
1X
677R1
1X
1X
682R1
1X
0.5X

FIGURE S4B.

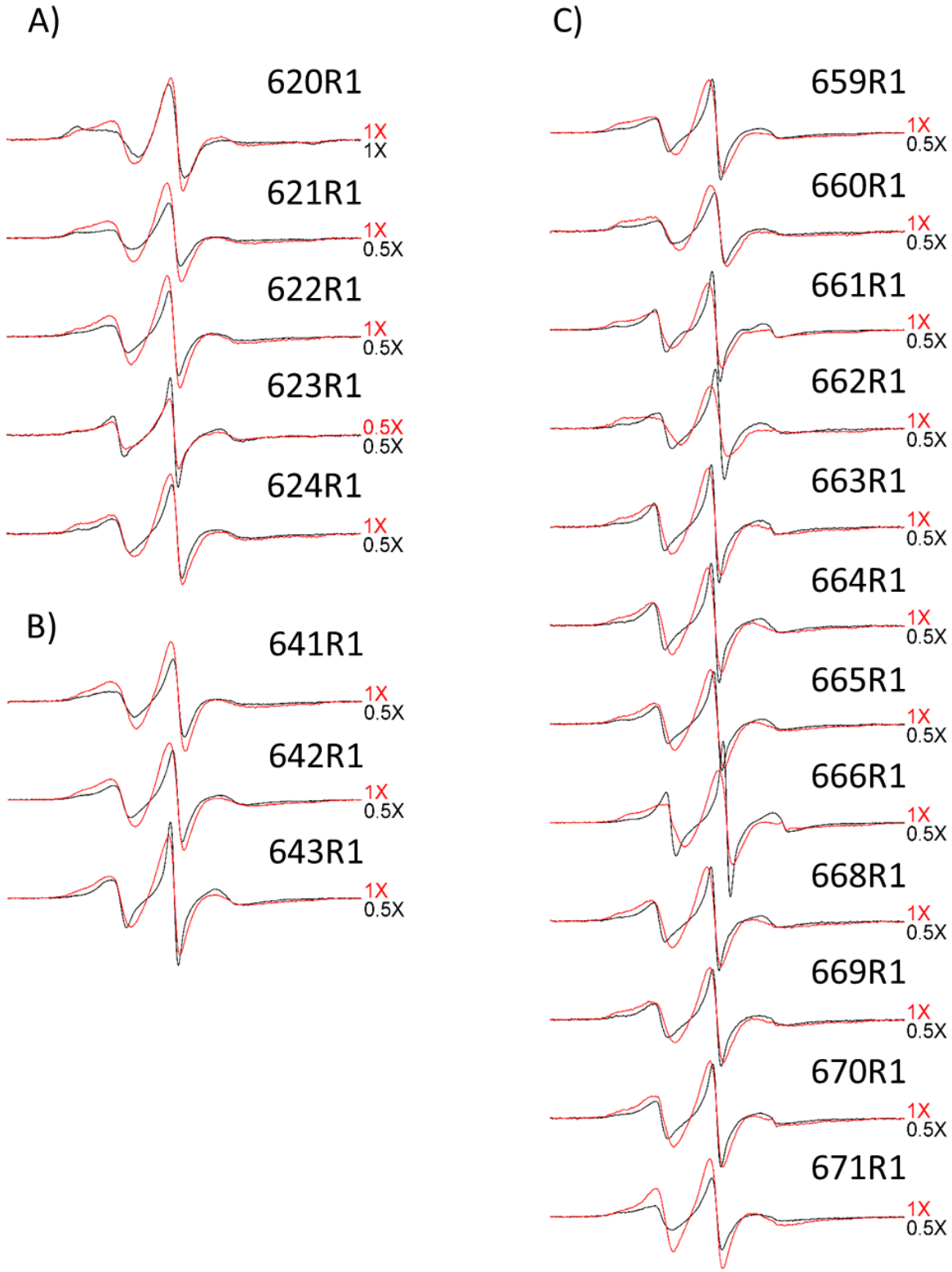

A)
620R1
1X
1X
621R1
1X
0.5X
622R1
1X
0.5X
623R1
0.5X
0.5X
624R1
1X
0.5X
B)
641R1
1X
0.5X
642R1
1X
0.5X
643R1
1X
0.5X
C)
659R1
1X
0.5X
660R1
1X
0.5X
661R1
1X
0.5X
662R1
1X
0.5X
663R1
1X
0.5X
664R1
1X
0.5X
665R1
1X
0.5X
666R1
1X
0.5X
668R1
1X
0.5X
669R1
1X
0.5X
670R1
1X
0.5X
671R1
1X
0.5X

FIGURE S5.

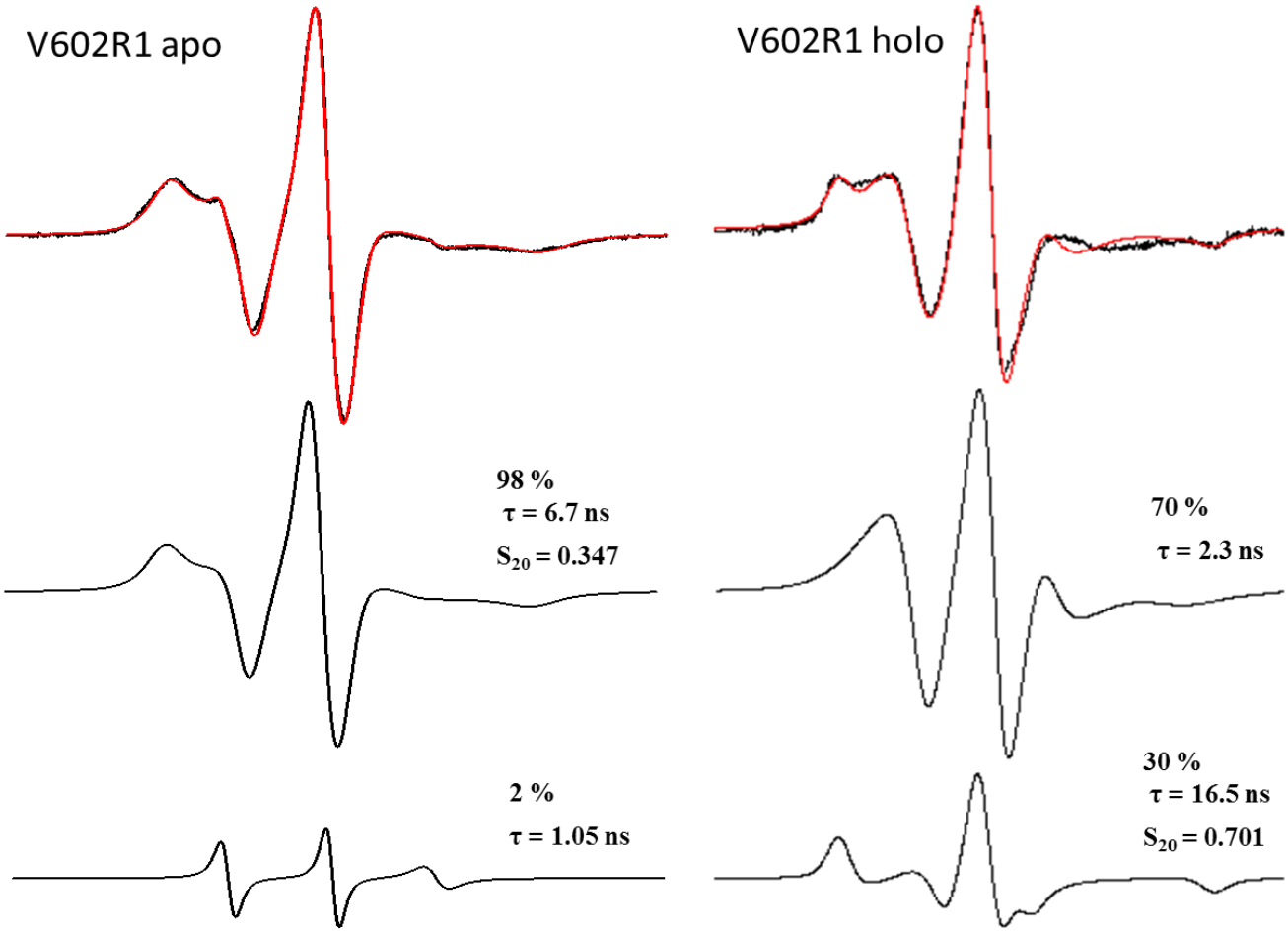

V602R1 apo
98 %
τ = 6.7 ns
S20 = 0.347
2 %
τ = 1.05 ns
V602R1 holo
70 %
τ = 2.3 ns
30 %
τ = 16.5 ns
S20 = 0.701

FIGURE S6.

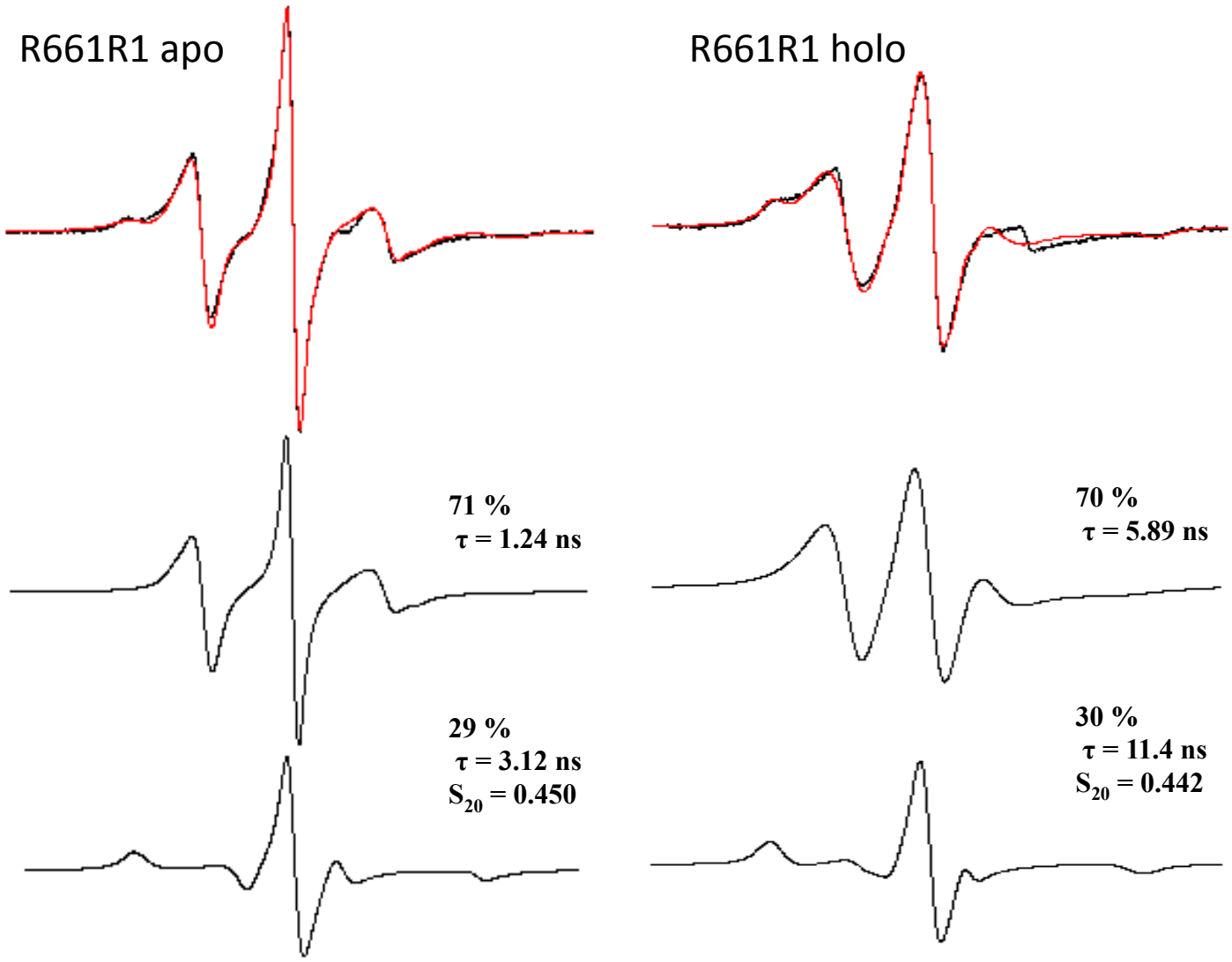
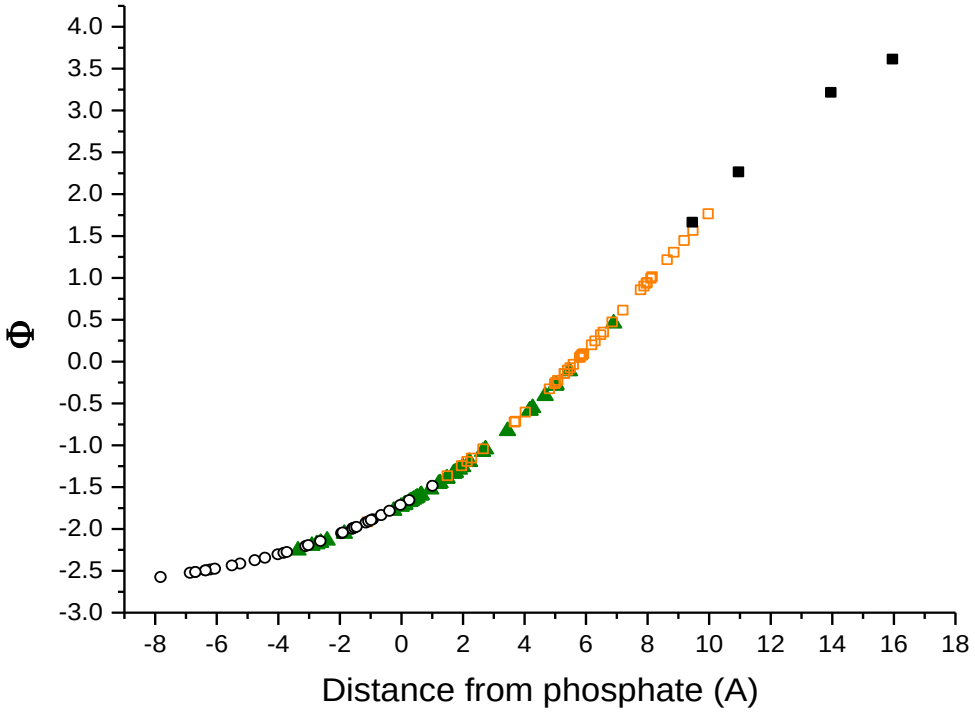

R661R1 apo
R661R1 holo
71 %
τ = 1.24 ns
29 %
τ = 3.12 ns
S20 = 0.450
70 %
τ = 5.89 ns
30 %
τ = 11.4 ns
S20 = 0.442

FIGURE S7.


Φ
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0
-1.5
-2.0
-2.5
-3.0
-8
-6
-4
-2
0
2
4
6
8
10
12
14
16
18
Distance from phosphate (A)