Myeloperoxidase-catalyzed oxidation of cyanide to cyanate: A potential carbamylation route involved in the formation of atherosclerotic plaques?

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Quantification of cyanate formation from cyanide

Cyanate production was carried out at 37 °C in a final volume of 1.0 mL. The reaction mixture contained the following reagents at the final concentrations indicated between brackets: ammonium acetate buffer, pH 7.4 (10 mM), H_2O_2 (1 mM), cyanide (1 mM). The reaction was started by addition of MPO (300 nM). Alternatively, cyanate was obtained by mixing isotopomers of cyanide (1 mM) and HOCl (1 mM) in the same buffer. After incubation for 1 h, the solution was analyzed by infusion of the samples at 2mL/hour during 3 min into an Agilent 6520 Electrospray ionization (ESI) source Quadrupole Time of flight (QTOF) mass spectrometer (Palo Alto, CA, USA) in negative mode with a source temperature at 325 °C, a VCap at 4500 V, a drying gas at 5 L/min, a nebulizer gas at 10 psig, a fragmentor at 150 V and a skimmer at 80 V. Spectra were accumulated during 2 min.Cyanate concentration was calculated thanks to a calibration curve from 50 to 1000 µM of KOCN.

The results are as follows:

KOCN conc. (µM)	Average Abundance (n=3) (counts)	SD (counts)
50	15	7
100	46	10
500	402	11
1000	904	36

Conditions	Average Abundance (n=3) (counts)	Calculated concentration in ^-OCN (µM)	SD (µM)
CN- + MPO + H_2O_2	77	131	5
CN^- + HOCl	145	202	5

Legends of supporting materials

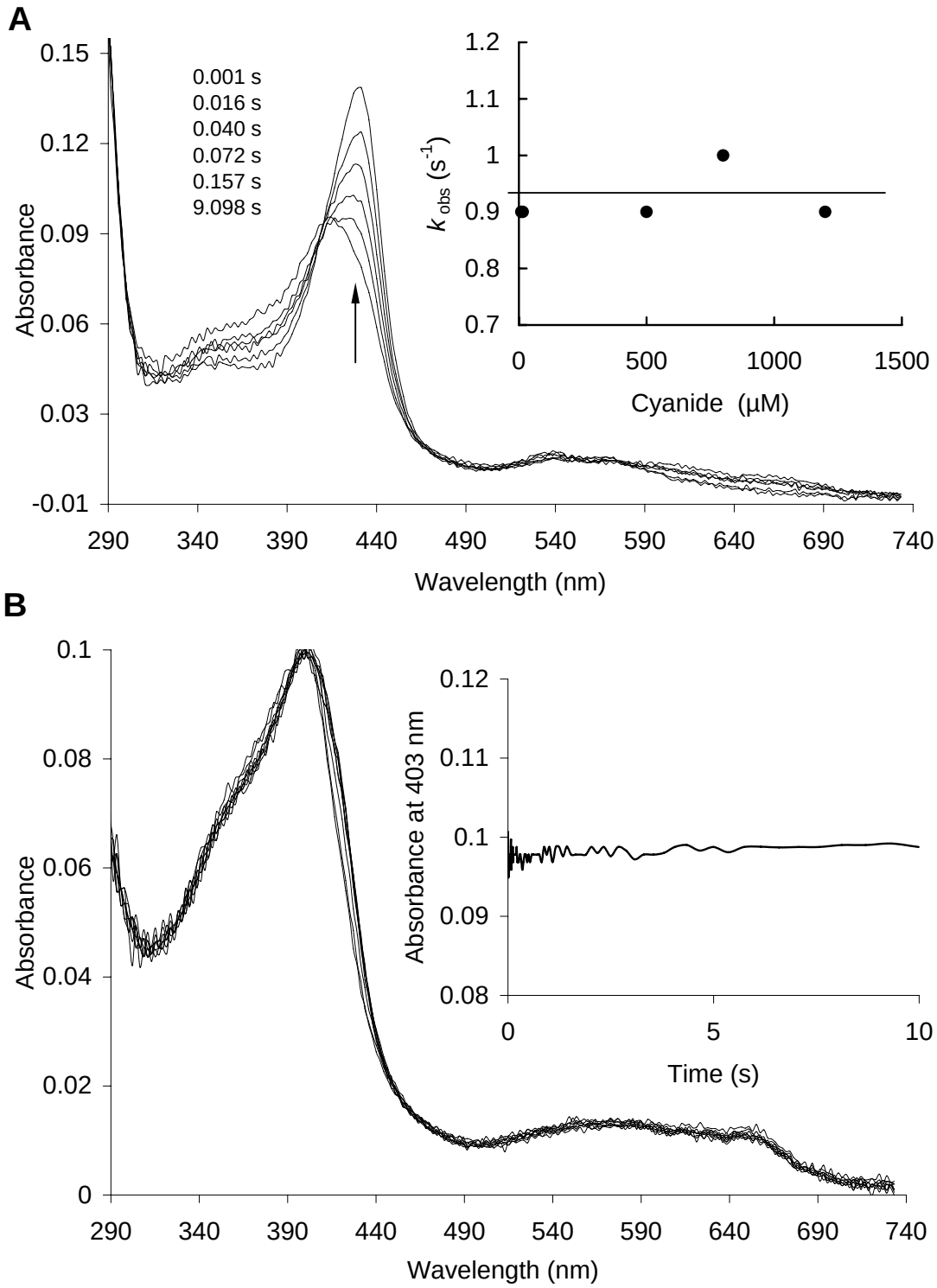
Suppl. Figure 1. Decay of lactoperoxidase compound I in the presence of cyanide. (**A**) Spectral changes upon addition of 1 mM cyanide to 2 µM compound I in the sequential-mixing stopped-flow mode (see Methods). Compound I was formed by mixing 8 µM ferric LPO with 8 µM H_2O_2 and waiting for 100 ms. First spectrum was recorded at 1.3 ms, subsequent spectra at 6 ms, 16 ms, 40 ms, 73 ms 157 ms and 9.9 s. Reaction conditions: 100 mM phosphate buffer, pH 7.0, and 25°C. Inset to Figure 1A shows a plot of k_{obs} *versus* cyanide concentration. (**B**) Spectral changes of HRP compound I (2µM) upon addition of 10 mM cyanide. Inset to Suppl Figure 1B shows absorbance change at 403 nm.

Suppl. Figure 2. MS spectra illustrating the carbamyllysine production. The production of carbamyllysine was demonstrated with peaks (positive mode) at respectively 190.1177 and 192.1197 *m/z* corresponding to ^{12}C- and $^{13}C^{15}N$-carbamyllysine. Peak at 191.077 did not allow isolation of the ^{13}C-isotope. Performed in negative mode, peaks at 188.1079, 189.1120 and 190.1078 *m/z* were observed corresponding to the isotopomers of carbamyllysine.

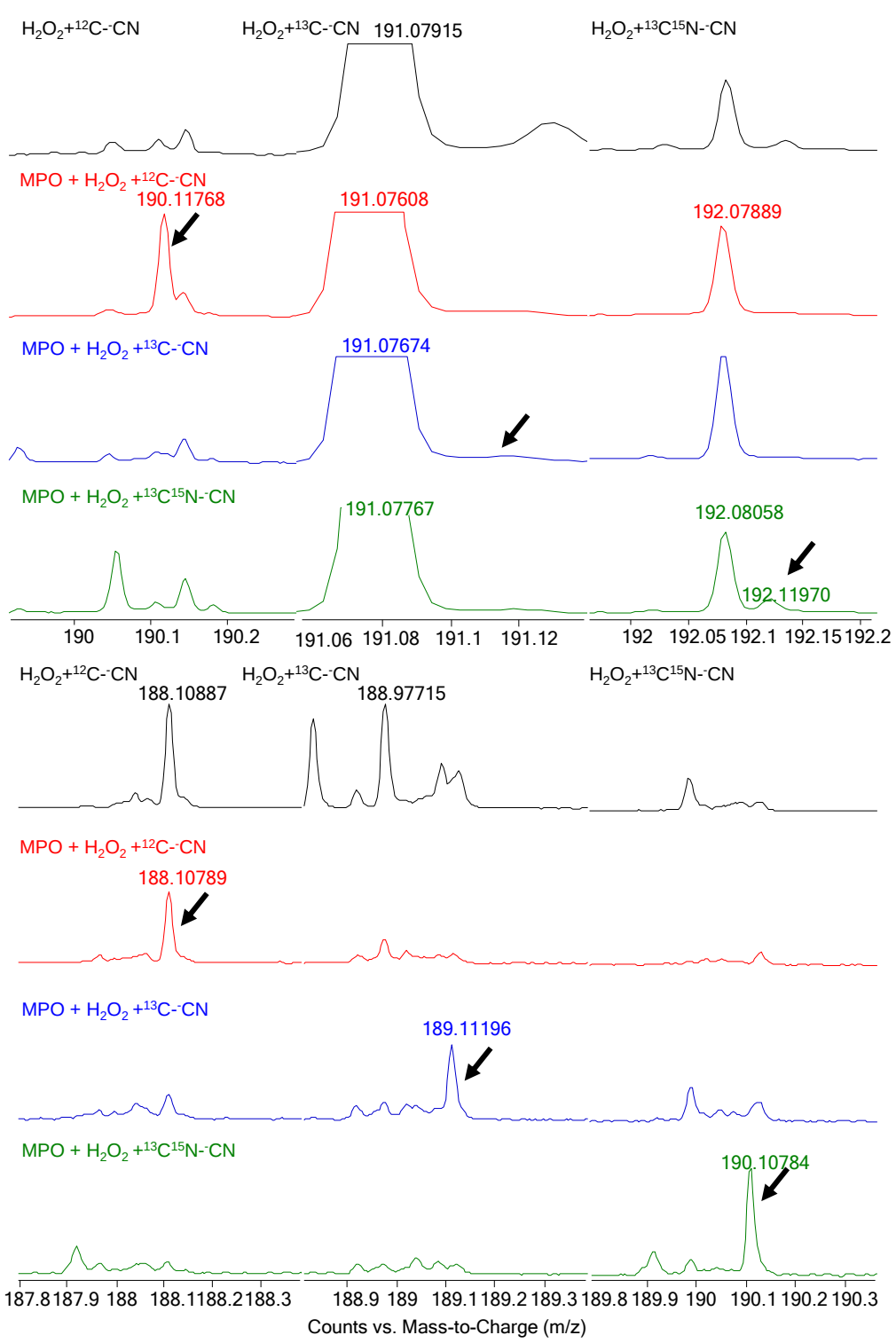
Suppl. Figure 3. HOCl mediates cyanate and carbamyltaurine production. Addition of hypochlorous acid (HOCl) promotes oxidation of cyanide into cyanate such as indicated by the peaks at 43.0028 and 43.9999 *m/z* corresponding to ^{13}C- and $^{13}C^{15}N$-cyanate. In the presence of taurine, addition of HOCl has also allowed the production of carbamyltaurine (right panel).

Suppl. Figure 4. Reaction of recombinant myeloperoxidase compound I with cyanide. Typical time traces with single exponential fit showing the transition of 1.6 µM compound I to the ferric MPO cyanide complex. Reactions were followed at 456 nm, the maximum absorbance of MPO cyanide complex. Pseudo-first-order rate constants for compound I reduction by cyanide. The second-order rate constants were calculated from the slope ($k_{app} = 2.5 \times 10^5\ M^{-1}\ s^{-1}$).

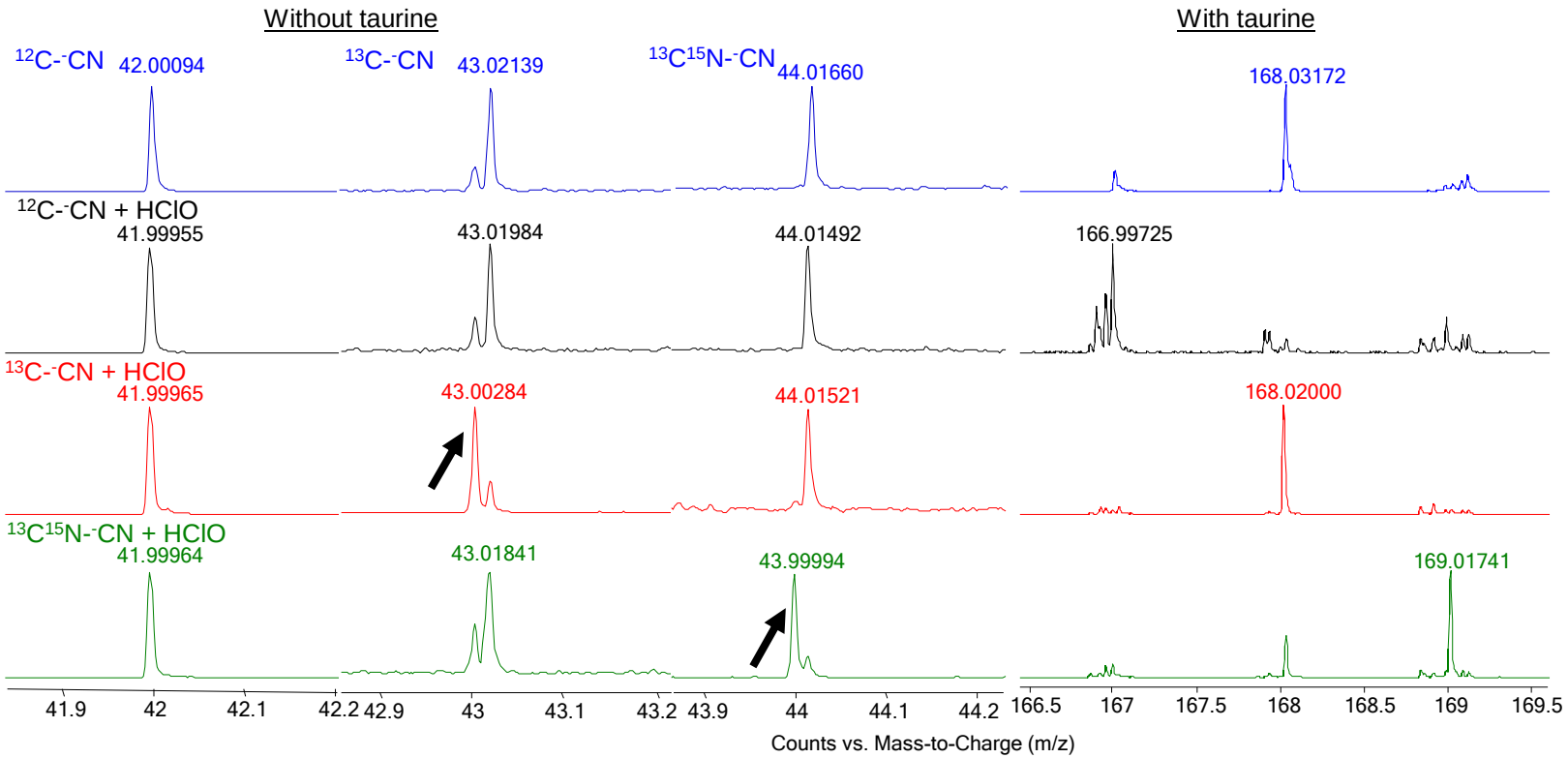
Suppl. Figure 5. Absorbance spectra of myeloperoxidase compound II and myeloperoxidase-cyanide complex. Absorbance spectra were acquired between 290 and 740 nm for the myeloperoxidase compound II (MPO-Compound II) and myeloperoxidase cyanide complex (MPO-CN^-). For both spectrum, a Soret maximum at 456 nm is observed.



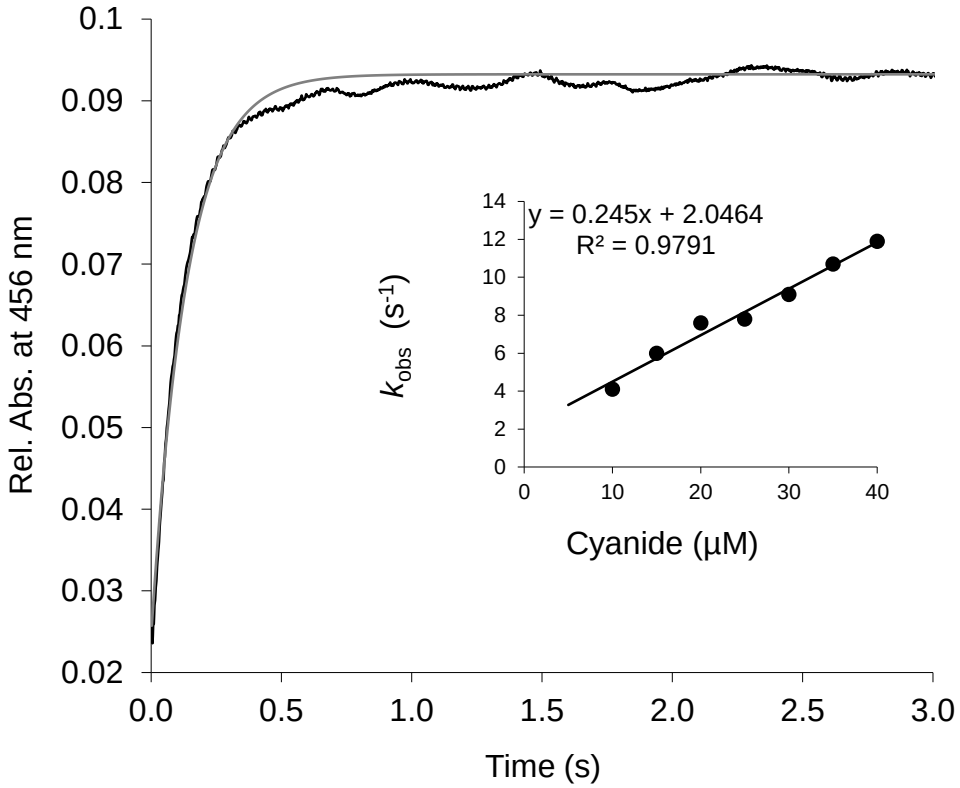
Suppl. Figure 1, Delporte, C., et al



Suppl. Figure 2, Delporte, C., et al.

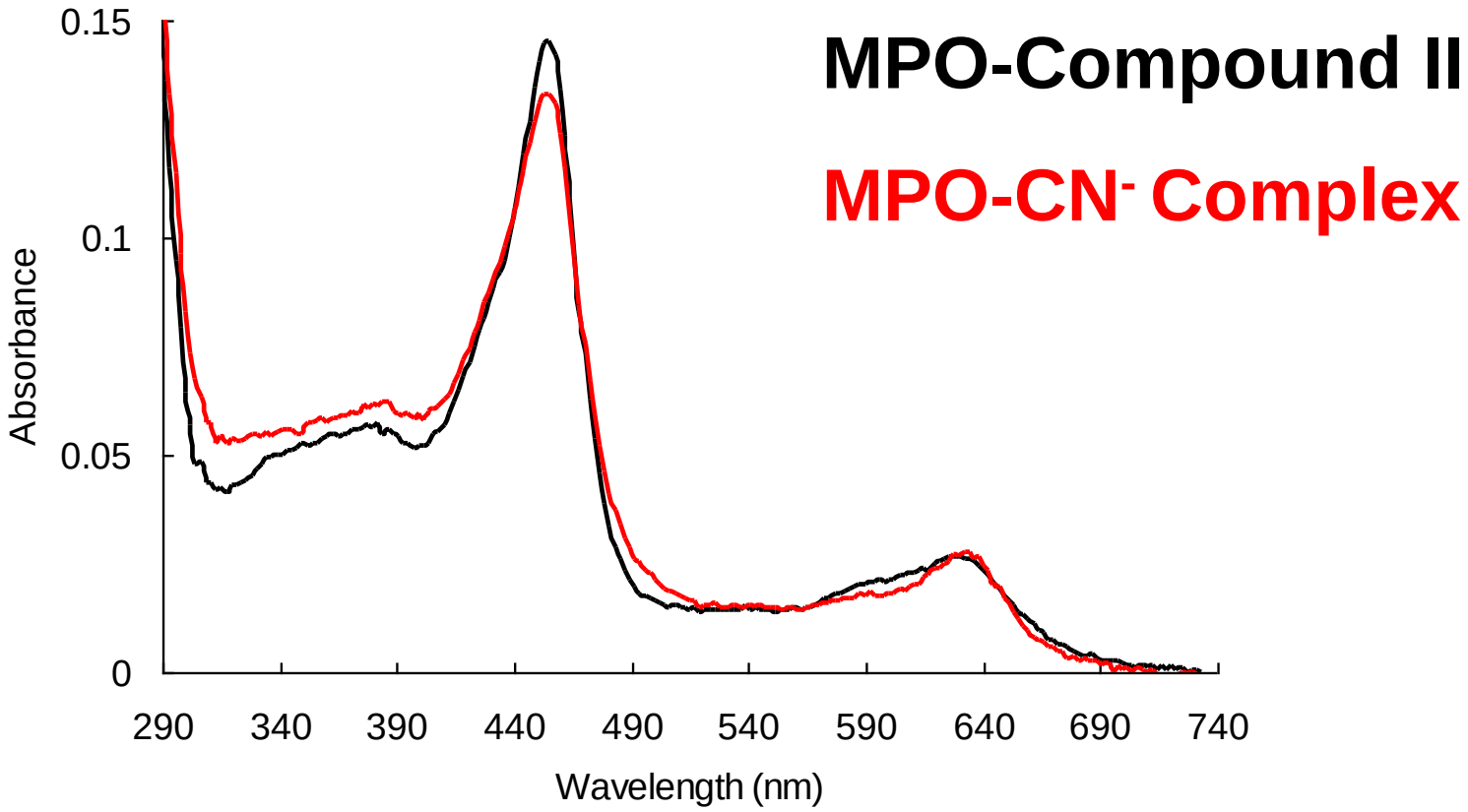


Suppl. Figure 3, Delporte, C., et al.



k_{app} = 2.5 x 10^5 M^{-1} s^{-1}

Suppl Figure 4, Delporte, C., et al.



Delporte, C., et al. Suppl. Figure 5