Oxidation of protein disulfide bonds by singlet oxygen gives rise to glutathionylated proteins

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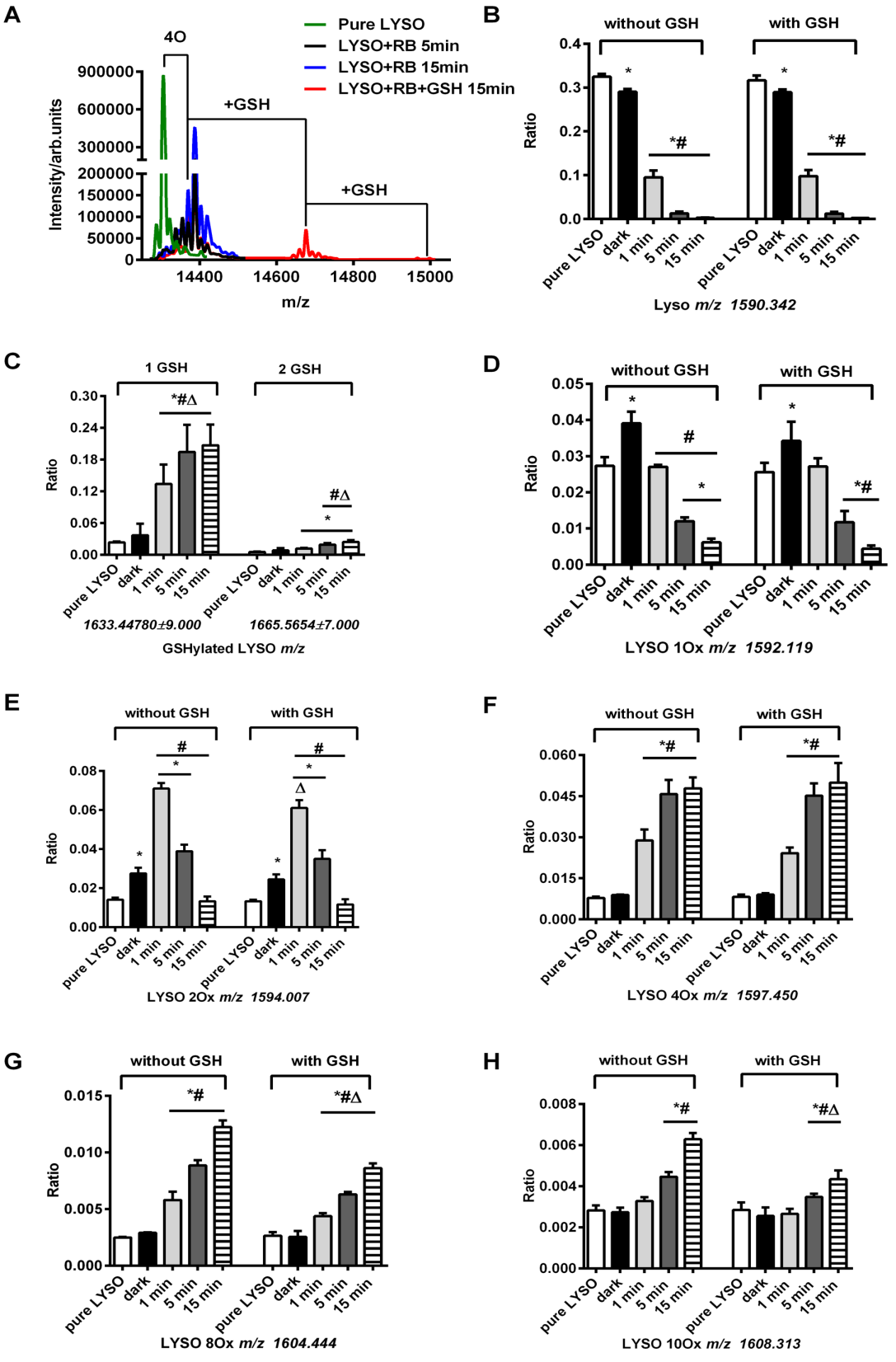
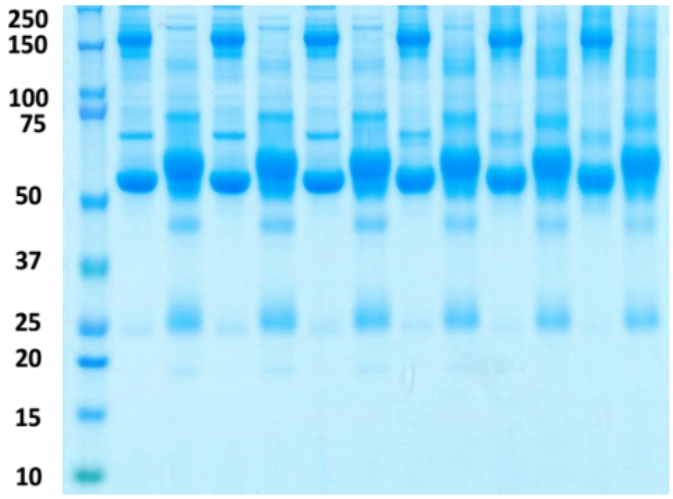
Supplementary Data


A
Pure LYSO
LYSO+RB 5min
LYSO+RB 15min
LYSO+RB+GSH 15min
4O
+GSH
+GSH
Intensity/arb.units
900000
600000
300000
200000
150000
100000
50000
0
14400
14600
14800
15000
m/z
B
without GSH
with GSH
Ratio
0.4
0.3
0.2
0.1
0.0
*
*#
*#
pure LYSO
dark
1 min
5 min
15 min
Lyso m/z 1590.342
C
1 GSH
2 GSH
Ratio
0.30
0.24
0.18
0.12
0.06
0.00
*#Δ
#Δ
*
1633.44780±9.000
1665.5654±7.000
GSHylated LYSO m/z
D
without GSH
with GSH
Ratio
0.05
0.04
0.03
0.02
0.01
0.00
*
#
*
*
*#
LYSO 1Ox m/z 1592.119
E
without GSH
with GSH
Ratio
0.08
0.06
0.04
0.02
0.00
#
*
#
*
Δ
LYSO 2Ox m/z 1594.007
F
without GSH
with GSH
Ratio
0.060
0.045
0.030
0.015
0.000
*#
*#
LYSO 4Ox m/z 1597.450
G
without GSH
with GSH
Ratio
0.015
0.010
0.005
0.000
*#
*#Δ
LYSO 8Ox m/z 1604.444
H
without GSH
with GSH
Ratio
0.008
0.006
0.004
0.002
0.000
*#
*#Δ
LYSO 10Ox m/z 1608.313

Supplementary Figure 1. LC-MS demonstrates incorporation of oxygen into Lyso after photo-oxidation and subsequent glutathionylation. Panel A: Deconvoluted spectrum of Lyso after photolysis and addition of GSH (green line: pure Lyso; black line: photolysis for 5 min; blue line: photolysis for 15 min; red line: glutathionylation of oxidized Lyso after 15 min illumination); Panel B: Ratio of parent Lyso; Panel C: Ratio of glutathionylated Lyso; Panel D: Ratio of oxidized Lyso with 1O incorporation; Panel E: Ratio of oxidized Lyso with 2O incorporation; Panel F: Ratio of oxidized Lyso with 4O incorporation; Panel G: Ratio of oxidized Lyso with 8O incorporation; Panel H: Ratio of oxidized Lyso with 10O incorporation. The *m/z* ion ratio is calculated by taking the sum of the signal of detected species and dividing by the total signal of protein envelope in the mass spectrum. Statistical differences are indicated as follows: $^{*}P < 0.05$ vs. native Lyso (CON); $^{\#}P < 0.05$ vs. dark samples (0 min); $^{\Delta}P < 0.05$ vs. samples without addition of GSH. Quantitative data are presented as mean $\pm$ SD from three independent experiments.

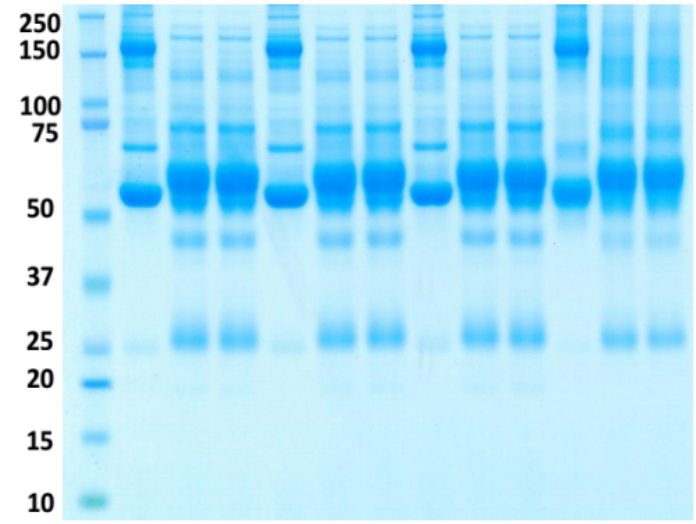
A

Time(min)	-	-	15	15	0	0	1	1	5	5	15	15
RB	-	-	-	-	+	+	+	+	+	+	+	+
GSH	-	+	-	+	-	+	-	+	-	+	-	+
Lane No.	1	2	3	4	5	6	7	8	9	10	11	12



B

RB	-	-	-	+	+	+	-	-	-	+	+	+
GSH	-	+	+	-	+	+	-	+	+	-	+	+
NEM	-	-	+	-	-	+	-	-	+	-	-	+
Lane No.	1	2	3	4	5	6	7	8	9	10	11	12



Supplementary Figure 2. Plasma proteins exposed to $^1O_2$ undergo structural changes. Panel A: Representative SDS-PAGE of plasma (2 mg protein mL^{-1}) after photo-oxidation and then reaction with GSH. Panel B: Plasma (2 mg protein mL^{-1}) was pretreated with NEM (1 mM, as indicated) before exposure to $^1O_2$ for 15 min and reaction with GSH for 1 h.