

## **Appendix A**

## Appendix A

Table A1

LOX-1 expression in DFO, EDTA, and BHT pretreatment groups

| Group <sup>a</sup>                             | Density<br>(mean $\pm$ SEM) | P-value |
|------------------------------------------------|-----------------------------|---------|
| Control                                        | 53.494 $\pm$ 3.600          |         |
| 0.3 mM EDTA                                    | 53.259 $\pm$ 8.500          | 1.000   |
| 0.3 mM EDTA+ 40 $\mu$ g/ml mil-oxLDL           | 72.570 $\pm$ 3.690          | 0.105   |
| 0.3 mM EDTA + 40 $\mu$ g/ml mo-oxLDL           | 75.760 $\pm$ 1.670          | 0.019*  |
| 0.3 mM EDTA + 40 $\mu$ g/ml fully-oxLDL        | 84.200 $\pm$ 2.550          | 0.002*  |
| 0.3 mM EDTA+ 80 $\mu$ g/ml mil-oxLDL           | 70.000 $\pm$ 2.740          | 0.095   |
| 0.3 mM EDTA+ 80 $\mu$ g/ml mo-oxLDL            | 80.289 $\pm$ 4.279          | 0.005*  |
| 0.3 mM EDTA + 80 $\mu$ g/ml fully-oxLDL        | 86.280 $\pm$ 2.387          | 0.033*  |
| 10 $\mu$ M DFO                                 | 51.323 $\pm$ 5.856          | 0.938   |
| 10 $\mu$ M DFO+ 40 $\mu$ g/ml mil-oxLDL        | 103.420 $\pm$ 8.853         | 0.144   |
| 10 $\mu$ M DFO+ 40 $\mu$ g/ml mo-oxLDL         | 97.330 $\pm$ 7.250          | 0.000*  |
| 10 $\mu$ M DFO+ 40 $\mu$ g/ml fully-oxLDL      | 86.490 $\pm$ 8.250          | 0.000*  |
| 10 $\mu$ M DFO+ 80 $\mu$ g/ml mo-oxLDL         | 97.620 $\pm$ 4.188          | 0.953   |
| 10 $\mu$ M DFO+ 80 $\mu$ g/ml fully-oxLDL      | 114.850 $\pm$ 5.060         | 1.000   |
| 100 $\mu$ g/ml BHT                             | 76.683 $\pm$ 1.717          | 0.012*  |
| 100 $\mu$ g/ml BHT + 40 $\mu$ g/ml mil-oxLDL   | 84.736 $\pm$ 1.590          | 0.002*  |
| 100 $\mu$ g/ml BHT + 40 $\mu$ g/ml mo-oxLDL    | 97.474 $\pm$ 2.294          | 0.000*  |
| 100 $\mu$ g/ml BHT + 40 $\mu$ g/ml fully-oxLDL | 110.202 $\pm$ 1.830         | 0.000*  |
| 100 $\mu$ g/ml BHT + 80 $\mu$ g/ml mil-oxLDL   | 83.467 $\pm$ 2.680          | 0.003*  |
| 100 $\mu$ g/ml BHT + 80 $\mu$ g/ml mo-oxLDL    | 95.680 $\pm$ 3.275          | 0.001*  |
| 100 $\mu$ g/ml BHT + 80 $\mu$ g/ml fully-oxLDL | 118.814 $\pm$ 4.596         | 0.000*  |

\* Significant difference is P value < 0.05 when compared with control.

<sup>a</sup>EDTA = ethylenediaminetetraacetic acid; DFO = Desferoxamine;

BHT = butylated hydroxytoluene; mil-oxLDL = mildly oxidized LDL; mo-oxLDL = moderately oxidized LDL; fully = fully oxidized LDL

Table A2

Mean density/mm<sup>2</sup> of p38 MAPK activity activated by various doses of oxLDL.

(a) Mean density/mm<sup>2</sup> of p38 MAPK activity compared with control

| Group <sup>a</sup>        | Density/mm <sup>2</sup><br>(mean $\pm$ SEM) | Percent (%) mean | P- value |
|---------------------------|---------------------------------------------|------------------|----------|
| control                   | 87.031 $\pm$ 5.844                          | 100              |          |
| 10 $\mu$ g/ml mo-oxLDL    | 117.426 $\pm$ 9.021                         | 134.924          | 0.210    |
| 20 $\mu$ g/ml mo-oxLDL    | 155.321 $\pm$ 9.442                         | 178.466          | 0.000*   |
| 40 $\mu$ g/ml mo-oxLDL    | 177.758 $\pm$ 14.296                        | 204.246          | 0.000*   |
| 80 $\mu$ g/ml mo-oxLDL    | 218.557 $\pm$ 15.134                        | 251.125          | 0.000*   |
| 10 $\mu$ g/ml fully-oxLDL | 132.062 $\pm$ 8.782                         | 151.741          | 0.004*   |
| 20 $\mu$ g/ml fully-oxLDL | 191.164 $\pm$ 21.453                        | 219.650          | 0.003*   |
| 40 $\mu$ g/ml fully-oxLDL | 201.012 $\pm$ 16.063                        | 230.966          | 0.000*   |
| 80 $\mu$ g/ml fully-oxLDL | 269.158 $\pm$ 24.442                        | 309.266          | 0.000*   |

\* Significant difference is P value < 0.05.

<sup>a</sup> mo-oxLDL= moderately oxidized LDL; fully-oxLDL = fully oxidized LDL

(b) Mean density/mm<sup>2</sup> of p38 MAPK activity compared between mo-oxLDL and fully-oxLDL

| Between group <sup>a</sup>                           | p-value |
|------------------------------------------------------|---------|
| 10 $\mu$ g/ml mo-oxLDL and 10 $\mu$ g/ml fully-oxLDL | 1.000   |
| 20 $\mu$ g/ml mo-oxLDL and 20 $\mu$ g/ml fully-oxLDL | 0.980   |
| 40 $\mu$ g/ml mo-oxLDL and 40 $\mu$ g/ml fully-oxLDL | 1.000   |
| 80 $\mu$ g/ml mo-oxLDL and 80 $\mu$ g/ml fully-oxLDL | 0.922   |

<sup>a</sup> mo-oxLDL= moderately oxidized LDL; fully-oxLDL = fully oxidized LDL

Table A3

Mean density/mm<sup>2</sup> of p38 MAPK activity activated by various doses and degrees moderately and fully oxLDL in 0.3 mM EDTA pretreatment.

(a) Mean density/mm<sup>2</sup> of p38 MAPK activity compared with control

| Group <sup>a</sup>                     | Density/mm <sup>2</sup><br>(mean $\pm$ SEM) | Percent (%) | p-value |
|----------------------------------------|---------------------------------------------|-------------|---------|
| control                                | 87.031 $\pm$ 5.844                          | 100         |         |
| 0.3 mM EDTA                            | 79.778 $\pm$ 5.135                          | 91.666      | 1.000   |
| 0.3 mM EDTA+ 10 $\mu$ g/ml mo-oxLDL    | 98.259 $\pm$ 5.715                          | 112.900     | 1.000   |
| 0.3 mM EDTA+ 20 $\mu$ g/ml mo-oxLDL    | 131.861 $\pm$ 8.407                         | 151.510     | 0.012*  |
| 0.3 mM EDTA+ 40 $\mu$ g/ml mo-oxLDL    | 165.675 $\pm$ 8.227                         | 190.363     | 0.000*  |
| 0.3 mM EDTA+ 80 $\mu$ g/ml mo-oxLDL    | 205.265 $\pm$ 12.647                        | 235.853     | 0.000*  |
| 0.3 mM EDTA+ 10 $\mu$ g/ml fully-oxLDL | 121.877 $\pm$ 8.140                         | 140.039     | 0.142   |
| 0.3 mM EDTA+ 20 $\mu$ g/ml fully-oxLDL | 148.742 $\pm$ 10.164                        | 170.906     | 0.001*  |
| 0.3 mM EDTA+ 40 $\mu$ g/ml fully-oxLDL | 165.730 $\pm$ 8.859                         | 190.426     | 0.000*  |
| 0.3 mM EDTA+ 80 $\mu$ g/ml fully-oxLDL | 170.819 $\pm$ 10.956                        | 196.273     | 0.000*  |

\*Significant difference is P value < 0.05.

<sup>a</sup> mo-oxLDL= moderately oxidized LDL; fully-oxLDL = fully oxidized LDL

(b) Mean density/mm<sup>2</sup> of p38 MAPK activity compared between 0.3 mM EDTA + mo-oxLDL and 0.3 mM EDTA + fully-oxLDL

| Between group <sup>a</sup>                                                 | P-value |
|----------------------------------------------------------------------------|---------|
| 0.3mM EDTA+10 $\mu$ g/ml mo-oxLDL and 0.3mM EDTA+10 $\mu$ g/ml fully-oxLDL | 0.879   |
| 0.3mM EDTA+20 $\mu$ g/ml mo-oxLDL and 0.3mM EDTA+20 $\mu$ g/ml fully-oxLDL | 1.000   |
| 0.3mM EDTA+40 $\mu$ g/ml mo-oxLDL and 0.3mM EDTA+40 $\mu$ g/ml fully-oxLDL | 1.000   |
| 0.3mM EDTA+80 $\mu$ g/ml mo-oxLDL and 0.3mM EDTA+80 $\mu$ g/ml fully-oxLDL | 0.984   |

<sup>a</sup> mo-oxLDL= moderately oxidized LDL; fully-oxLDL = fully oxidized LDL

(c) Mean density/mm<sup>2</sup> of p38 MAPK activity compared with dose-degree of oxLDL and 0.3 mM EDTA pretreatment group

| Between group <sup>a</sup>                               | P-value |
|----------------------------------------------------------|---------|
| 10 µg/ml mo-oxLDL and 0.3mM EDTA+10 µg/ml mo-oxLDL       | 0.999   |
| 20 µg/ml mo-oxLDL and 0.3mM EDTA+20 µg/ml mo-oxLDL       | 0.998   |
| 40 µg/ml mo-oxLDL and 0.3mM EDTA+40 µg/ml mo-oxLDL       | 1.000   |
| 80 µg/ml mo-oxLDL and 0.3mM EDTA+80 µg/ml mo oxLDL       | 1.000   |
| 10 µg/ml fully-oxLDL and 0.3mM EDTA+10 µg/ml fully-oxLDL | 1.000   |
| 20 µg/ml fully-oxLDL and 0.3mM EDTA+20 µg/ml fully-oxLDL | 0.998   |
| 40 µg/ml fully-oxLDL and 0.3mM EDTA+40 µg/ml fully-oxLDL | 0.994   |
| 80 µg/ml fully-oxLDL and 0.3mM EDTA+80 µg/ml fully-oxLDL | 0.103   |

<sup>a</sup> mo-oxLDL= moderately oxidized LDL; fully-oxLDL = fully oxidized LDL

Table A4

Mean density/mm<sup>2</sup> of p38 MAPK activity activated by various doses and degrees moderately and fully oxLDL in 10 µM DFO pretreatment.

(a) Mean density/mm<sup>2</sup> of p38 MAPK activity compared with control

| Group <sup>a</sup>               | Density/mm <sup>2</sup><br>(mean ± SEM) | Percent (%) | p-value |
|----------------------------------|-----------------------------------------|-------------|---------|
| control                          | 87.031 ± 5.844                          | 100         |         |
| 10 µM DFO                        | 148.772 ± 13.536                        | 170.941     | 0.038*  |
| 10 µM DFO + 10 µg/ml mo-oxLDL    | 152.125 ± 8.121                         | 174.794     | 0.000*  |
| 10 µM DFO + 20 µg/ml mo-oxLDL    | 190.480 ± 10.185                        | 218.864     | 0.000*  |
| 10 µM DFO + 40 µg/ml mo-oxLDL    | 198.076 ± 10.903                        | 227.592     | 0.000*  |
| 10 µM DFO + 80 µg/ml mo-oxLDL    | 211.956 ± 7.450                         | 243.540     | 0.000*  |
| 10 µM DFO + 10 µg/ml fully-oxLDL | 145.424 ± 8.449                         | 167.094     | 0.000*  |
| 10 µM DFO + 20 µg/ml fully-oxLDL | 158.080 ± 7.144                         | 181.636     | 0.000*  |
| 10 µM DFO + 40 µg/ml fully-oxLDL | 163.192 ± 8.846                         | 187.510     | 0.000*  |
| 10 µM DFO + 80 µg/ml fully-oxLDL | 180.907 ± 4.046                         | 207.864     | 0.000*  |

\*Significant difference is P value < 0.05.

(b) Mean density/mm<sup>2</sup> of p38 MAPK activity compared between 10  $\mu$ M DFO + mo-oxLDL and 10  $\mu$ M DFO + fully-oxLDL

| Between group <sup>a</sup>                                                       | p-value |
|----------------------------------------------------------------------------------|---------|
| 10 $\mu$ M DFO +10 $\mu$ g/ml mo- and 10 $\mu$ M DFO +10 $\mu$ g/ml fully ox-LDL | 1.000   |
| 10 $\mu$ M DFO +20 $\mu$ g/ml mo- and 10 $\mu$ M DFO +20 $\mu$ g/ml fully ox-LDL | 0.811   |
| 10 $\mu$ M DFO +40 $\mu$ g/ml mo- and 10 $\mu$ M DFO +40 $\mu$ g/ml fully ox-LDL | 0.742   |
| 10 $\mu$ M DFO +80 $\mu$ g/ml mo- and 10 $\mu$ M DFO +80 $\mu$ g/ml fully ox-LDL | 0.098   |

<sup>a</sup> mo-oxLDL= moderately oxidized LDL; fully-oxLDL = fully oxidized LDL

(c) Mean density/mm<sup>2</sup> of p38 MAPK activity compared with dose-degree of oxLDL and 10  $\mu$ M DFO pretreatment group

| Between group <sup>a</sup>                                              | P-value |
|-------------------------------------------------------------------------|---------|
| 10 $\mu$ g/ml mo-oxLDL and 10 $\mu$ M DFO +10 $\mu$ g/ml mo-oxLDL       | 0.514   |
| 20 $\mu$ g/ml mo-oxLDL and 10 $\mu$ M DFO +20 $\mu$ g/ml mo-oxLDL       | 0.846   |
| 40 $\mu$ g/ml mo-oxLDL and 10 $\mu$ M DFO +40 $\mu$ g/ml mo-oxLDL       | 1.000   |
| 80 $\mu$ g/ml mo-oxLDL and 10 $\mu$ M DFO +80 $\mu$ g/ml mo oxLDL       | 1.000   |
| 10 $\mu$ g/ml fully-oxLDL and 10 $\mu$ M DFO +10 $\mu$ g/ml fully-oxLDL | 1.000   |
| 20 $\mu$ g/ml fully-oxLDL and 10 $\mu$ M DFO +20 $\mu$ g/ml fully-oxLDL | 1.000   |
| 40 $\mu$ g/ml fully-oxLDL and 10 $\mu$ M DFO +40 $\mu$ g/ml fully-oxLDL | 0.979   |
| 80 $\mu$ g/ml fully-oxLDL and 10 $\mu$ M DFO +80 $\mu$ g/ml fully-oxLDL | 0.155   |

<sup>a</sup> mo-oxLDL= moderately oxidized LDL; fully-oxLDL = fully oxidized LDL

Table A5

Mean density/mm<sup>2</sup> of p38 MAPK activity activated by various doses and degrees moderately and fully oxLDL in 100 µg/ml BHT pretreatment.

(a) Mean density/mm<sup>2</sup> of p38 MAPK activity compared with control

| Group <sup>a</sup>                   | Density/mm <sup>2</sup><br>(mean ± SEM) | Percent (%) | p-value |
|--------------------------------------|-----------------------------------------|-------------|---------|
| control                              | 87.031 ± 5.844                          | 100         |         |
| 100 µg/ml BHT                        | 111.507 ± 6.793                         | 128.123     | 0.622   |
| 100 µg/ml BHT + 10 µg/ml mo-oxLDL    | 137.417 ± 6.426                         | 157.894     | 0.000*  |
| 100 µg/ml BHT +20 µg/ml mo-oxLDL     | 152.498 ± 4.742                         | 175.222     | 0.000*  |
| 100 µg/ml BHT + 40 µg/ml mo-oxLDL    | 175.903 ± 13.639                        | 202.114     | 0.000*  |
| 100 µg/ml BHT + 80 µg/ml mo-oxLDL    | 217.441 ± 13.770                        | 299.842     | 0.000*  |
| 100 µg/ml BHT + 10 µg/ml fully-oxLDL | 119.643 ± 7.814                         | 137.417     | 0.196   |
| 100 µg/ml BHT + 20 µg/ml fully-oxLDL | 155.915 ± 8.113                         | 179.149     | 0.000*  |
| 100 µg/ml BHT + 40 µg/ml fully-oxLDL | 192.875 ± 12.080                        | 221.616     | 0.000*  |
| 100 µg/ml BHT + 80 µg/ml fully-oxLDL | 236.527 ± 14.707                        | 271.773     | 0.000*  |

\*Significant difference is P value < 0.05.

<sup>a</sup> mo-oxLDL= moderately oxidized LDL; fully-oxLDL = fully oxidized LDL

(b) Mean density/mm<sup>2</sup> of p38 MAPK activity compared with dose-degree of oxLDL and 100 µg/ml BHT pretreatment group

| Between Group <sup>a</sup>                                                 | P-value |
|----------------------------------------------------------------------------|---------|
| 100 µg/ml BHT +10 µg/ml mo-oxLDL- and 100 µg/ml BHT +10 µg/ml fully ox-LDL | 0.999   |
| 100 µg/ml BHT +20 µg/ml mo-oxLDL and 100 µg/ml BHT +20 µg/ml fully ox-LDL  | 1.000   |
| 100 µg/ml BHT +40 µg/ml mo-oxLDL and 100 µg/ml BHT +40 µg/ml fully ox-LDL  | 1.000   |
| 100 µg/ml BHT +80 µg/ml mo-oxLDL and 100 µg/ml BHT +80 µg/ml fully ox-LDL  | 1.000   |

<sup>a</sup> mo-oxLDL= moderately oxidized LDL; fully-oxLDL = fully oxidized LDL

(c) Mean density/mm<sup>2</sup> of p38 MAPK activity compared with dose-degree of oxLDL and 100 µg/ml BHT pretreatment group

| Between group <sup>a</sup>                                   | P-value |
|--------------------------------------------------------------|---------|
| 10 µg/ml mo-oxLDL and 100 µg/ml BHT +10 µg/ml mo-oxLDL       | 0.999   |
| 20 µg/ml mo-oxLDL and 100 µg/ml BHT +20 µg/ml mo-oxLDL       | 1.000   |
| 40 µg/ml mo-oxLDL and 100 µg/ml BHT +40 µg/ml mo-oxLDL       | 1.000   |
| 80 µg/ml mo-oxLDL and 100 µg/ml BHT +80 µg/ml mo oxLDL       | 1.000   |
| 10 µg/ml fully-oxLDL and 100 µg/ml BHT +10 µg/ml fully-oxLDL | 1.000   |
| 20 µg/ml fully-oxLDL and 100 µg/ml BHT +20 µg/ml fully-oxLDL | 1.000   |
| 40 µg/ml fully-oxLDL and 100 µg/ml BHT +40 µg/ml fully-oxLDL | 1.000   |
| 80 µg/ml fully-oxLDL and 100 µg/ml BHT +80 µg/ml fully-oxLDL | 1.000   |

<sup>a</sup> mo-oxLDL= moderately oxidized LDL; fully-oxLDL = fully oxidized LDL