Chronic cypermethrin exposure alters mouse embryonic stem cell growth kinetics, induces Phase II detoxification response and affects pluripotency and differentiation gene expression

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**Supplementary Table 1.** Sequences of the forward and reverse primers used for the gene expression analysis.

| Gene  | Forward                        | Reverse                        | Amplicon length - bp |
|-------|-------------------------------|--------------------------------|----------------------|
| Cat   | 5’TCATCAGGGATGCCCATATTTG 3’   | 5’ACTCCAGAAGTCCCAGACCAT 3’    | 101                  |
| Gpx1  | 5’CTCACCCAGAGTGAACGTCTG 3’    | 5’CTTGGCATTTCTGTTGTC 3’       | 100                  |
| Gpx4  | 5’TGTGGAATGATGGAAATGC 3’      | 5’ACGCAGCGGTCTTATCAAT 3’      | 100                  |
| Sod1  | 5’TGTCCATTGAGATCGTGTA 3’      | 5’TGCACCAGTGCTTGGTCTTC 3’     | 94                   |
| Sod2  | 5’CTACAGATGCTGCTGCTC 3’       | 5’GTAGTAAGCGTGCTCCACAC 3’     | 97                   |
| Cyp1a1| 5’CTGCCCATCTTCCCTGAT 3’       | 5’ATGTTGCCTTCTCTCAAATGTC 3’   | 107                  |
| Cyp1b1| 5’CCAGTGCCAAATGGAGACA 3’      | 5’TGTCTGCACAAAGCTGTTG 3’      | 250                  |
| Nqo1  | 5’TTCCTTGCGGATTCCAGATG 3’     | 5’TCTGTTGTCAGCGTGAAATG 3’     | 306                  |
| Gsta1 | 5’CTCCTGACCCCTTTCCTCCTC 3’    | 5’GCCAGTATCTGTGGCCTCAT 3’     | 183                  |
| Ugt1a6| 5’ATGGCCCATAGACCTTCTCAA 3’    | 5’GAGACCAATGGATCCCCAAAGA 3’   | 236                  |
| Oct-4 | 5’TGTGAGGAAAGCGAGAACAATG 3’   | 5’CACCCTCACAGGGTTCTCAATGC 3’  | 107                  |
| Nanog | 5’CTGCTCTGAGATGCTGCAC 3’      | 5’AGCTTTTGGTGGAACCTGATG 3’    | 106                  |
| Fgf5  | 5’TGTGCTCTGAGGGATGTTAGG 3’    | 5’CATCCGTAATTTGGCGACTTG 3’    | 101                  |
| Brachyury | 5’CTCTAAGGAACCGGCTGTC 3’    | 5’AGCATGGACAGACAAACAGA 3’    | 100                  |
| Foxa2 | 5’AAATGAGGAGGTGAGGTGGAAGA 3’ | 5’GGCCCATCTTATTTAGGGAACAC 3’ | 110                  |
| β2m   | 5’GAATTCACCCCACTGAGACT 3’     | 5’TGTGATCATCATGTCCTGAT 3’     | 103                  |
**Supplementary Table 2.** Distribution of control (CTR) and exposed R1 ESCs and 3T3 cells to cypermethrin (CYP) at their respective LD$_{50}$ dose (0.3 or 0.6 mM) during the cell cycle phases.

| Samples (µM) | Hours | Frequency of cells (mean±SD) |  |
|--------------|-------|-----------------------------|---|
|              |       | G0/G1 | S                | G2/M         |
| **R1**       |       |       |                  |              |
| CTR          | 12    | 26.9±4.4 | 48.9±2.8        | 24.4±1.6     |
| CYP          | 12    | 29.7±1.1 | 47.6±1.0        | 22.7±0.7     |
| CTR          | 24    | 31.6±2.9 | 54.2±3.1        | 14.2±0.3     |
| CYP          | 24    | 31.5±0.5 | 53.3±0.7        | 15.2±0.3     |
| CTR          | 48    | 29.1±2.2 | 52.8±1.6        | 18.1±1.0     |
| CYP          | 48    | 25.7±0.6* | 51.6±0.3     | 22.7±0.6*    |
| CTR          | 72    | 30.9±1.4 | 54.0±1.4        | 15.1±0.8     |
| CYP          | 72    | 24.3±1.3* | 54.6±0.9     | 21.1±1.8*    |
| **3T3**      |       |       |                  |              |
| CTR          | 8     | 65.0±4.0 | 15.9±1.9        | 19.0±2.7     |
| CYP          | 8     | 62.3±1.5 | 17.1±0.6        | 20.6±2.2     |
| CTR          | 24    | 67.1±1.7 | 14.3±1.1        | 18.6±1.4     |
| CYP          | 24    | 67.9±3.2 | 15.1±1.4        | 17.0±1.8     |
| CTR          | 48    | 68.6±1.4 | 14.0±0.6        | 17.4±0.8     |
| CYP          | 48    | 79.2±1.4* | 11.1±0.8*   | 9.7±2.0*     |
| CTR          | 72    | 66.6±0.7 | 15.9±0.4        | 17.5±0.9     |
| CYP          | 72    | 68.1±1.3 | 15.2±0.7        | 16.6±1.4     |

*P<0.001, when compared to CTR.
**Supplementary Table 3.** Fold-change values of redox-related, phase I and phase II gene transcripts, relative to CTR, of R1 cells exposed to 0.3 mM cypermethrin for 12, 24, 48 or 72 h.

| Groups      | Genes   | 12          | 24          | 48          | 72          |
|-------------|---------|-------------|-------------|-------------|-------------|
| Redox-related | Catalase | 1.49 ± 0.64 | 1.05 ± 0.11 | 4.06 ± 1.69* | 2.67 ± 1.71 |
|             | Sod1    | 1.19 ± 0.47 | 0.67 ± 0.13*| 2.24 ± 0.68* | 2.02 ± 0.55*|
|             | Sod2    | 1.42 ± 0.50 | 0.98 ± 0.11 | 3.17 ± 0.70* | 1.93 ± 0.77*|
|             | Gpx1    | 1.29 ± 0.20 | 0.83 ± 0.10 | 1.19 ± 0.38 | 1.52 ± 0.41*|
|             | Gpx4    | 1.15 ± 0.46 | 1.10 ± 0.09 | 2.00 ± 0.01* | 1.72 ± 0.20*|
| Phase I     | Cyp1a1  | 0.95 ± 0.37 | 0.64 ± 0.28*| 0.33 ± 0.32* | 0.77 ± 0.30 |
|             | Cyp1b1  | 1.36 ± 0.51 | 0.64 ± 0.28*| 0.47 ± 0.21* | 3.52 ± 1.42*|
| Phase II    | Nqo1    | 0.90 ± 0.09 | 1.18 ± 0.35 | 1.22 ± 0.36 | 2.36 ± 0.31*|
|             | Gstal   | 0.94 ± 0.46 | 1.20 ± 0.40 | 2.06 ± 1.11* | 6.30 ± 2.61*|
|             | Ugt1a6  | 0.59 ± 0.03*| 2.85 ± 0.81*| 2.19 ± 0.01* | 5.14 ± 2.47*|

*P<0.05; #P<0.001.
**Supplementary Table 4.** Fold-change values of redox-related, phase I and phase II gene transcripts relative to CTR samples (set at 1) of 3T3 cell line exposed to 0.6 mM cypermethrin for 8, 24, 48 or 72 h.

| Groups      | Genes    | 8         | 24         | 48         | 72         |
|-------------|----------|-----------|------------|------------|------------|
| Redox-related | Catalase | 0.78 ± 0.29 | 1.19 ± 0.02* | 1.08 ± 0.12 | 2.03 ± 0.51* |
|             | Sod1     | 0.94 ± 0.08 | 0.92 ± 0.08 | 0.89 ± 0.32 | 0.94 ± 0.44 |
|             | Sod2     | 0.72 ± 0.16 | 0.83 ± 0.04* | 0.72 ± 0.02* | 1.26 ± 0.39 |
|             | Gpx1     | 0.93 ± 0.01 | 0.63 ± 0.03* | 0.76 ± 0.09 | 0.99 ± 0.14 |
|             | Gpx4     | 0.81 ± 0.07 | 1.01 ± 0.05 | 0.86 ± 0.31 | 1.32 ± 0.12* |
| Phase I     | Cyp1a1   | 0.91 ± 0.07 | 1.51 ± 0.25* | 1.00 ± 0.01 | 1.00 ± 0.11 |
|             | Cyp1b1   | 1.00 ± 0.23 | 0.92 ± 0.07 | 0.85 ± 0.43 | 1.15 ± 0.70 |
| Phase II    | Nqo1     | 0.97 ± 0.04 | 0.98 ± 0.04 | 1.17 ± 0.22 | 1.14 ± 0.04 |
|             | Gsta1    | 0.76 ± 0.10 | 1.14 ± 0.11 | 3.80 ± 0.04* | 2.43 ± 0.42* |
|             | Ugt1a6   | 1.22 ± 0.21 | 2.32 ± 0.22* | 4.43 ± 1.23* | 3.56 ± 1.63* |

*P<0.05; **P<0.001.
**Supplementary Table 5.** Fold-change values of pluripotency gene transcripts of R1 ESCs exposed to 0.3 mM cypermethrin for 12, 24, 48 or 72 h relative to control ESCs (set at 1).

| Group       | Genes | Hours |       |       |       |
|-------------|-------|-------|-------|-------|-------|
|             |       | 12    | 24    | 48    | 72    |
| **Pluripotency** |       |       |       |       |       |
|             | Oct-4 | 0.71 ± 0.15* | 0.75 ± 0.11* | 1.29 ± 0.34* | 1.48 ± 0.20* |
|             | Nanog | 0.71 ± 0.11* | 0.85 ± 0.06* | 1.35 ± 0.22* | 1.74 ± 0.55* |

*P<0.001.

**Supplementary Table 6.** Fold-change values of early ectoderm, mesoderm and endoderm gene transcripts of EBs differentiated for 5 days from R1 ESCs exposed to 0.3 mM cypermethrin for 12, 24, 48 or 72 h relative to EBs differentiated from control (not exposed) ESCs (set at 1).

| Group      | Genes | Hours |       |       |       |
|------------|-------|-------|-------|-------|-------|
|            |       | 12    | 24    | 48    | 72    |
| **Early germ layers** |       |       |       |       |       |
|            | Fgf5  | 1.00 ± 0.72 | 0.06 ± 0.01* | 0.39 ± 0.08* | 0.64 ± 0.34 |
|            | Brachyury | 0.16 ± 0.05* | 1.11 ± 0.04* | 1.21 ± 0.26* | 0.64 ± 0.06* |
|            | Foxa2  | 7.28 ± 3.85* | 1.82 ± 1.19 | 7.19 ± 2.69* | 3.42 ± 0.12* |

*P<0.001.
**Supplementary Figure 1.**
Dose/response curve to CYP of R1 and 3T3 after 72 h exposure. Data represent the mean ± SD of 3 independent experiments.