

## ***Supplementary Material***

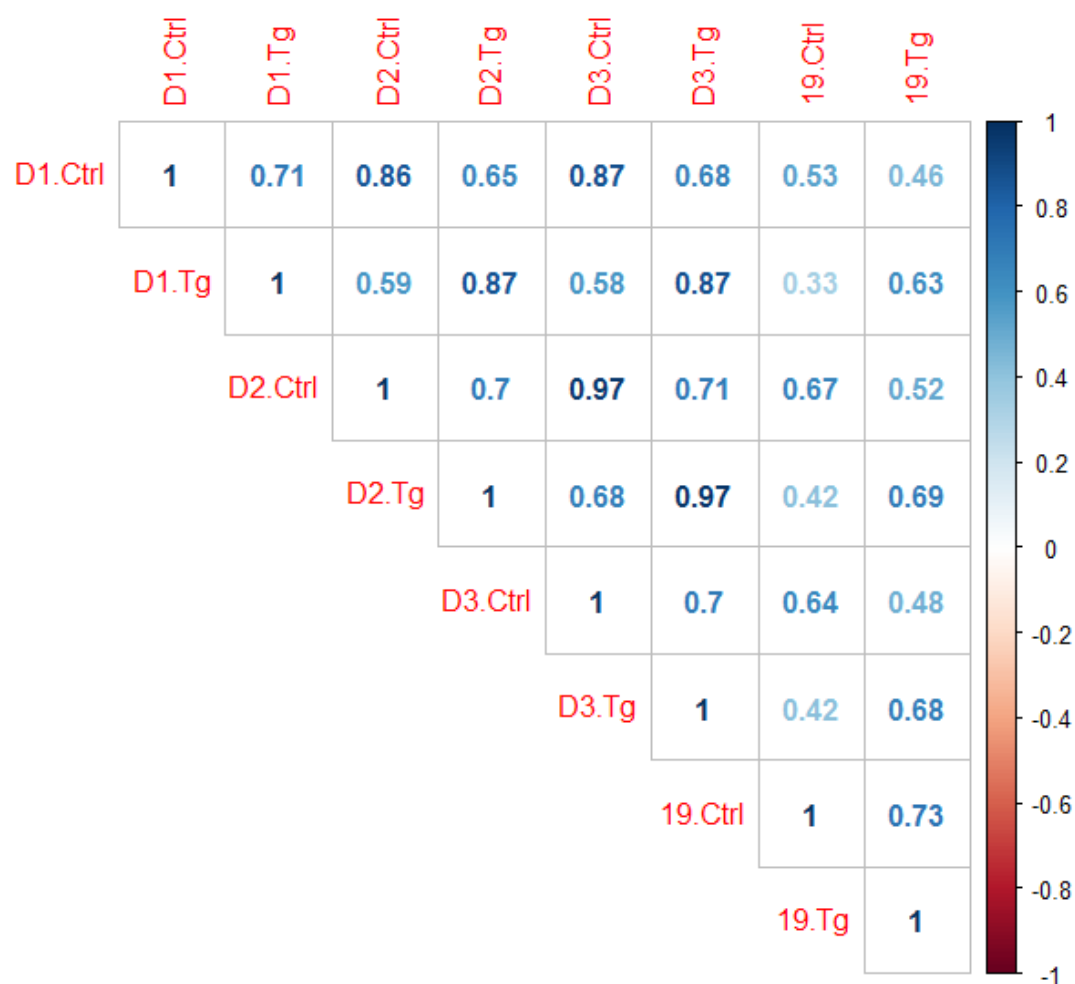
### **Immunological Molecular Responses of Human Retinal Pigment Epithelial Cells to Infection with *Toxoplasma gondii***

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Supplementary Figure 1, and Tables S1-S3, S10 and S11 are included in this document. Supplementary Tables S4-S9 and S12-S15 are presented as separate .xlsx files.

**Supplementary Figure S1.** Correlogram comparing gene expression in uninfected and *T. gondii* infected samples for the top 2000 most variable genes. D = donor; 19 = ARPE19; Ctrl = control; Tg = *T. gondii*.



**Supplementary Table S1.** Primer pairs and product sizes for protein-coding and long non-coding transcripts.

| Transcript*         | Primer pair                                                                         | Product size (bp) |
|---------------------|-------------------------------------------------------------------------------------|-------------------|
| ANGTPL7             | Forward 5'- TGGAAGCAGTACAAGCAGGG -3'<br>Reverse 5'- GCTATACTCAGCGTAGCGCA -3'        | 147               |
| BIRC3               | Forward 5'- AGCAAAGCCATGCACAAAAC -3'<br>Reverse 5'- ACGTGTTGGCGCTTTTCATC -3'        | 127               |
| CCL2 <sup>1</sup>   | Forward 5'- AATCAATGCCCCAGTCACCTGC -3'<br>Reverse 5'- CGGAGTTTGGGTTTGCTTGTCC -3'    | 211               |
| CXCL3 <sup>2</sup>  | Forward 5'- TCATCAAACATAGCTCAGTCCTG -3'<br>Reverse 5'- GGCTGACACATTATG GTCTCC -3'   | 100               |
| CXCL8 <sup>2</sup>  | Forward 5'- GTGTGAAGGTGCAGTTTTC -3'<br>Reverse 5'- TCTGCACCCAGTTTTCCTTG -3'         | 203               |
| FGF2                | Forward 5'- CAAGCAGAAGAGAGAGGAGTTG -3'<br>Reverse 5'- GCTCTTAGCAGACATTGGAAGA -3'    | 273               |
| ICAM-1 <sup>4</sup> | Forward 5'- TAAGCCAAGAGGAAGGAGCA -3'<br>Reverse 5'- CATATCATCAAGGGTTGGGG -3'        | 289               |
| IL1RN <sup>2</sup>  | Forward 5'- TCATGCTCTGTTCTTGGAAT -3'<br>Reverse 5'- GCTTGTCCTGCTTCTGTTC -3'         | 131               |
| IL-6 <sup>3</sup>   | Forward 5'- ATGAACTCC TTC TCCACAAGCGC -3'<br>Reverse 5'- GAAGAGCCCTCAGGCTGGACTG -3' | 628               |
| IL-11               | Forward 5'- GTCCCGGATTCTTGGGTCTC -3'<br>Reverse 5'- AAACCCAGGCTTCCCTTTCC -3'        | 151               |
| IL-12A              | Forward 5'- GTGGAGGCCTGTTTACCATT -3'<br>Reverse 5'- CGGTTCTTCAAGGGAGGATTT -3'       | 315               |
| IL17RB              | Forward 5'- GGGACCTCCGAGTAGAACCT -3'<br>Reverse 5'- CTTGGTGGCCTTCAACAAGC -3'        | 119               |
| LINC00968           | Forward 5'- AATTGTGTCCCCTGTCCACC -3'<br>Reverse 5'- CTGTGCTGAGCTGTCTGGAA -3'        | 126               |
| LINC01105           | Forward 5'- AGGAGAAGGAATTGTGGCGG -3'<br>Reverse 5'- CAGTCCCTGCTGTAGCG AA -3'        | 137               |
| LRP8                | Forward 5'- AAGTGTGTACCTGCCTCGTG -3'<br>Reverse 5'- CCGTCACCACAGTCGTCG -3'          | 167               |
| MALAT1              | Forward 5'- ATTCCGGTGATGCGAGTTGT -3'<br>Reverse 5'- ATTCGGGGCTCTGTAGTCCT -3'        | 396               |
| MIR155HG            | Forward 5'- CTTGGCTCTCCCACCCAATG -3'<br>Reverse 5'- CCTACAGCAAGCCTTCAGCA -3'        | 115               |
| NEAT1               | Forward 5'- CTTGGCACTGGTACTGGGAG -3'<br>Reverse 5'- ACCCACGCACTAAATTCCCC -3'        | 137               |
| NFκB1 <sup>5</sup>  | Forward 5'- CCCAGTGAAGACCACCTCTC -3'<br>Reverse 5'- CTGAGTTTGCGGAAGGATGT -3'        | 132               |
| PPIA                | Forward 5'- GAGCACTGGAGAGAAAGGATT T -3'<br>Reverse 5'- GGTGATCTTCTTGCTGGTCTT -3'    | 355               |

|                     |                                                                                   |     |
|---------------------|-----------------------------------------------------------------------------------|-----|
| PTGS2               | Forward 5'- CCAGAGCAGGCAGATGAAATA -3'<br>Reverse 5'- CCAGTAGGCAGGAGAACATATAAG -3' | 277 |
| RP11-572C15.6       | Forward 5'- ATCAGACATCTCCCAGGGCT -3'<br>Reverse 5'- ATTCACCCTACTCTCGGGCT -3'      | 202 |
| RPLP0               | Forward 5'- GCAGCATCTACAACCCTGAA -3'<br>Reverse 5'- GCAGATGGATCAGCCAAGAA -3'      | 235 |
| SNAP25              | Forward 5'- GTCACATGGCCCTGGATATGG -3'<br>Reverse 5'- CACTTAACCACTTCCCAGCAT -3'    | 143 |
| TGF- $\beta$ 2      | Forward 5'- GTACTACGCCAAGGAGGTTTAC -3'<br>Reverse 5'- TGTGGAGGTGCCATCAATAC -3'    | 568 |
| TSLP                | Forward 5'- AAGGCAACAGCATGGGTGAA -3'<br>Reverse 5'- TACGTGGACACCCAATTCCA -3'      | 160 |
| TSP1                | Forward 5'- AAAGGATAATTGCCCCAACC -3'<br>Reverse 5'-CGGTCTCCCACATCATCTCT -3'       | 177 |
| VCAM-1 <sup>6</sup> | Forward 5'- CGTCTCCATTGACTTGCAGCACC -3'<br>Reverse 5'- GTGATCGGCTTCCCAGCCTC -3'   | 276 |

\*References for primers sequences sourced from the literature are provided below. For primer pairs designed in-house, products were confirmed by sequencing.

ANGTPL7= angiopoietin-like 7; BIRC3= baculoviral IAP repeat containing 3; FGF2= fibroblast growth factor 2; ICAM1= intercellular adhesion molecule 1; Linc= long intergenic non-protein coding RNA; IL = interleukin; LRP8= low-density lipoprotein receptor-related protein 8; MALAT1= metastasis associated lung adenocarcinoma transcript 1; MIR155HG= MIR155 host gene; NEAT1= nuclear paraspeckle assembly transcript 1; NF $\kappa$ B1= nuclear factor kappa B subunit 1; PPIA= peptidylprolyl isomerase A; PTGS2= prostaglandin-endoperoxide synthase 2; RPLP0= ribosomal protein lateral stalk subunit P0; SNAP25= synaptosomal-associated protein 25; TGF- $\beta$ 2 = transforming growth factor  $\beta$ 2; TSLP= thymic stromal lymphopoietin; TSP1 = thrombospondin 1; VCAM-1: vascular cell adhesion molecule 1.

## References

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5. Rao NA, McCalman MT, Moulos P, et al. Coactivation of GR and NFkB alters the repertoire of their binding sites and target genes. *Genome Res*. 2011;21:1404-1416.
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**Supplementary Table S2.** Primer kits for microRNA (MystiCq MicroRNA Quantification System, Sigma-Aldrich Merck, St. Louis, MO).

| MicroRNA    | Catalogue number | Mature sequence                                                |
|-------------|------------------|----------------------------------------------------------------|
| miR-10b-5p  | MIRAP00023       | UACCCUGUAGAACCGAAUUUGUG                                        |
| miR-449c-3p | MIRAP00404       | UUGCUAGUUGCACUCCUCUCUGU                                        |
| miR-449b-3p | MIRAP00402       | CAGCCACAACUACCCUGCCACU                                         |
| miR-3130-5p | MIRAP00863       | UACCCAGUCUCCGGUGCAGCC                                          |
| miR-670-5p  | MIRAP00662       | GUCCCUGAGUGUAUGUGGUG                                           |
| miR-212-3p  | MIRAP00264       | UACAGUCUCCAGUCACGGCC                                           |
| miR-146b-5p | MIRAP00185       | UGAGAACUGAAUCCAUAAGGCU                                         |
| miR-191-5p  | MIRAP00226       | CAACGGAAUCCCAAAGCAGCUG                                         |
| SNORD44     | MIRCP00005       | CCUGGAUGAUGAUAAGCAAAUGCUGACUGAACAUGAAGGUCUAAUUAAGCUCUAAACUGACU |

**Supplementary Table S3.** Alignment statistics for RNA-Sequencing on total and small RNA extracted from *T. gondii*-infected or uninfected human retinal pigment epithelial cells.

|           | Status              | Donor 1 |                  | Donor 2 |                  | Donor 3 |                  | ARPE19-1 |                  |
|-----------|---------------------|---------|------------------|---------|------------------|---------|------------------|----------|------------------|
|           |                     | Ctrl    | <i>T. gondii</i> | Ctrl    | <i>T. gondii</i> | Ctrl    | <i>T. gondii</i> | Ctrl     | <i>T. gondii</i> |
| Total RNA | %mapped             | 35.71   | 17.62            | 73.07   | 35.73            | 52.64   | 46.94            | 79.95    | 34.76            |
|           | % Assigned to genes | 71.46   | 58.36            | 71.81   | 66.32            | 71.81   | 72.95            | 69.96    | 65.54            |
| Small RNA | %mapped             | 0.26    | 0.11             | 0.16    | 0.17             | 0.24    | 0.15             | 0.26     | 0.15             |
|           | % Assigned to genes | 0.74    | 0.57             | 0.61    | 0.58             | 0.66    | 0.57             | 0.64     | 0.63             |

**Supplementary Table S10.** Relative normalized expression by RT-qPCR of selected protein-coding and long non-coding transcripts expressed by *T. gondii*-infected or uninfected human retinal pigment epithelial cells (presented with corresponding fold-change by RNA-sequencing).

| Molecule  | RNA-seq | RT-qPCR |                      |         |                      |         |                      |
|-----------|---------|---------|----------------------|---------|----------------------|---------|----------------------|
|           |         | Donor 4 |                      | Donor 5 |                      | Donor 6 |                      |
|           |         | FC      | p-value              | FC      | p-value              | FC      | p-value              |
| ANGPTL7   | -9.2    | -14.9   | 1.3x10 <sup>-2</sup> | -29.3   | 1.3x10 <sup>-5</sup> | -4.2    | 1.1x10 <sup>-3</sup> |
| BIRC3     | 62.2    | 312.7   | 6.6x10 <sup>-3</sup> | 56      | 4.1x10 <sup>-5</sup> | 295.2   | 9.2x10 <sup>-3</sup> |
| CCL2      | 4.21    | 10      | 2x10 <sup>-6</sup>   | 18.5    | 1.3x10 <sup>-3</sup> | 8.9     | 1.4x10 <sup>-4</sup> |
| CXCL3     | 7.6     | 8.4     | 1.7x10 <sup>-2</sup> | 45.7    | 2x10 <sup>-3</sup>   | 19.1    | 5.5x10 <sup>-2</sup> |
| CXCL8     | 5.6     | 7.7     | 4.1x10 <sup>-3</sup> | 8.6     | 2.5x10 <sup>-5</sup> | [1.4]   | NS                   |
| FGF2      | -2.8    | [1.0]   | NS                   | -3.1    | 2.6x10 <sup>-2</sup> | -1.5    | 7.1x10 <sup>-2</sup> |
| ICAM-1    | 5.2     | 4.6     | 4.2x10 <sup>-4</sup> | 3.1     | 1.9x10 <sup>-3</sup> | 25.5    | 3.3x10 <sup>-4</sup> |
| IL1RN     | 149.5   | 882.1   | 1.3x10 <sup>-5</sup> | 243.2   | 5.2x10 <sup>-4</sup> | 5282.4  | 2.8x10 <sup>-2</sup> |
| IL-6      | 6.3     | 60.3    | 4.5x10 <sup>-3</sup> | 82.4    | 7.3x10 <sup>-4</sup> | 77.8    | 1.6x10 <sup>-3</sup> |
| IL-11     | 5.8     | 12.9    | 4.7x10 <sup>-4</sup> | 15.9    | 4.5x10 <sup>-3</sup> | 7       | 1.2x10 <sup>-4</sup> |
| IL-12A    | -5.2    | -4.1    | 8.9x10 <sup>-3</sup> | -10.4   | 1.4x10 <sup>-3</sup> | -7      | 8.8x10 <sup>-4</sup> |
| IL17RB    | 49.9    | 45.9    | 1.3x10 <sup>-4</sup> | 165.9   | 7.8x10 <sup>-4</sup> | 267.4   | 1.3x10 <sup>-2</sup> |
| LINC00968 | -8.8    | -5.7    | 3.4x10 <sup>-2</sup> | -5      | 7.8x10 <sup>-4</sup> | -5.3    | 7.3x10 <sup>-3</sup> |
| LINC01105 | -5.3    | -8      | 3.5x10 <sup>-3</sup> | -8.9    | 7x10 <sup>-6</sup>   | -8.9    | 2.3x10 <sup>-2</sup> |
| LRP8      | 6.9     | 4.9     | 6.6x10 <sup>-3</sup> | 11.2    | 3.6x10 <sup>-3</sup> | 11.3    | 1.9x10 <sup>-3</sup> |
| MALAT1    | -2.3    | -2.5    | 2x10 <sup>-2</sup>   | -2.3    | 3.8x10 <sup>-2</sup> | -2.6    | 3.5x10 <sup>-2</sup> |
| MIR155HG  | 14.2    | 14.2    | 8x10 <sup>-6</sup>   | 5.3     | 5.3x10 <sup>-4</sup> | 12.6    | 6.2x10 <sup>-5</sup> |
| NEAT1     | -3.1    | -3.4    | 9.4x10 <sup>-3</sup> | -3      | 4.2x10 <sup>-2</sup> | [-1.2]  | NS                   |
| NFκB1     | 3.7     | 4.1     | 4.1x10 <sup>-2</sup> | 2.4     | 7.1x10 <sup>-2</sup> | 5.24971 | 7.4x10 <sup>-3</sup> |
| PTGS2     | 37.4    | 13.7    | 5.1x10 <sup>-3</sup> | 20.8    | 1.4x10 <sup>-3</sup> | 6.9     | 3.9x10 <sup>-3</sup> |

|                                |      |       |                      |        |                      |        |                      |
|--------------------------------|------|-------|----------------------|--------|----------------------|--------|----------------------|
| <b>RP11-572C15.6</b>           | -7.3 | -4.3  | $4.6 \times 10^{-2}$ | -6.1   | $6 \times 10^{-6}$   | [-1.1] | NS                   |
| <b>SNAP25</b>                  | 49.1 | 114.1 | $9.3 \times 10^{-3}$ | 34.3   | $2.3 \times 10^{-4}$ | 40.5   | $2.4 \times 10^{-4}$ |
| <b>TGF-<math>\beta</math>2</b> | -3.1 | -2.7  | $6.5 \times 10^{-2}$ | -3.8   | $1.3 \times 10^{-5}$ | -2.2   | $5.0 \times 10^{-2}$ |
| <b>TSLP</b>                    | 17.3 | 69.9  | $5.6 \times 10^{-2}$ | 45.7   | $1.7 \times 10^{-4}$ | 47.5   | $9 \times 10^{-5}$   |
| <b>TSP1</b>                    | -3.7 | -2.6  | $4.4 \times 10^{-2}$ | -2.5   | $2.4 \times 10^{-4}$ | -2.5   | $3.1 \times 10^{-2}$ |
| <b>VCAM-1</b>                  | 15.7 | 14.3  | $6.8 \times 10^{-4}$ | [-1.7] | $1.3 \times 10^{-2}$ | [1.2]  | NS                   |

Abbreviations: FC= fold-change; NS= not significant; ANGPTL7= angiopoietin-like 7; BIRC3= baculoviral IAP repeat containing 3; FGF2= fibroblast growth factor 2; ICAM1= intercellular adhesion molecule 1; Linc= long intergenic non-protein coding RNA; IL = interleukin; LRP8= low-density lipoprotein receptor-related protein 8; MALAT1= metastasis associated lung adenocarcinoma transcript 1; MIR155HG= MIR155 host gene; NEAT1= nuclear paraspeckle assembly transcript 1; NF $\kappa$ B1= nuclear factor kappa B subunit 1; PTGS2= prostaglandin-endoperoxide synthase 2; SNAP25= synaptosomal-associated protein 25; TGF- $\beta$ 2 = transforming growth factor  $\beta$ 2; TSLP= thymic stromal lymphopoietin; TSP1 = thrombospondin 1; VCAM-1: vascular cell adhesion molecule 1.

**Supplementary Table S11.** Relative normalized expression by RT-qPCR of selected microRNA expressed by *T. gondii*-infected or uninfected human retinal pigment epithelial cells (presented with corresponding fold-change by RNA-sequencing).

| Molecule          | Strand | RNA-seq | RT-qPCR |                      |         |                      |         |                      |
|-------------------|--------|---------|---------|----------------------|---------|----------------------|---------|----------------------|
|                   |        |         | Donor 4 |                      | Donor 5 |                      | Donor 6 |                      |
|                   |        |         | FC      | p-value              | FC      | p-value              | FC      | p-value              |
| <b>miR-670</b>    | +      | 169.9   | [2.0]   | NS                   | [1.5]   | NS                   | 3.7     | 6.5x10 <sup>-2</sup> |
| <b>miR-146b</b>   | +      | 13.2    | 4.1     | 8.3x10 <sup>-3</sup> | 3.7     | 6.7x10 <sup>-4</sup> | [1.2]   | NS                   |
| <b>miR-10b</b>    | +      | 12.2    | [-1.0]  | NS                   | [-1.3]  | NS                   | [-1.3]  | NS                   |
| <b>miR-543</b>    | +      | 19.4    |         |                      |         |                      |         |                      |
| <b>miR-449c</b>   | -      | 7.5     | -1.7    | 3.8x10 <sup>-2</sup> | [1.1]   | NS                   | [2.1]   | NS                   |
| <b>miR-449b</b>   | -      | 13.4    | [-1.6]  | NS                   | [-1.4]  | NS                   | [1.0]   | NS                   |
| <b>miR-212</b>    | -      | 6.6     | 8.2     | 7.2x10 <sup>-4</sup> | 1.8     | 2.2x10 <sup>-2</sup> | 6.7     | 3.9x10 <sup>-4</sup> |
| <b>miR-3130-1</b> | +      | -45.0   | [-1.4]  | NS                   | [-1.3]  | NS                   | 2.1     | 6.5x10 <sup>-4</sup> |

Abbreviations: FC= fold-change; NS= not significant