**Additional file 1**

**Assessment of HCMV-encoded microRNAs in plasma as potential biomarkers in pregnant women with adverse pregnancy outcomes**

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**Table S1** TaqMan Advanced miRNA assays (Applied Biosystems)

|  |  |  |
| --- | --- | --- |
| **Assay Name** | **Mature miRNA Sequence** | **Assay ID** |
| hcmv-miR-UL22A-5p | UAACUAGCCUUCCCGUGAGA | 007677 |
| hcmv-miR-UL22A-3p | UCACCAGAAUGCUAGUUUGUAG | 006040 |
| hcmv-miR-UL36-5p | UCGUUGAAGACACCUGGAAAGA | 197212-mat |
| hcmv-miR-UL36-3p | UUUCCAGGUGUUUUCAACGUGC | 006481 |
| hcmv-miR-UL112-5p | CCUCCGGAUCACAUGGUUACUCA | 469687 |
| hcmv-miR-UL112-3p | AAGUGACGGUGAGAUCCAGGCU | 006621 |
| hcmv-miR-UL148D | UCGUCCUCCCCUUCUUCACCG | 197215-mat |
| hcmv-miR-US33-5p | GAUUGUGCCCGGACCGUGGGCG | 197227-mat |
| hcmv-miR-US33-3p | UCACGGUCCGAGCACAUCCAA | 467895-mat |
| hcmv-miR-US5-1 | UGACAAGCCUGACGAGAGCGU | 004641-mat |
| hcmv-miR-US25-1-5p | AACCGCUCAGUGGCUCGGACC | 197211-mat |
| hcmv-miR-US25-1-3p | UCCGAACGCUAGGUCGGUUCU | 467895-mat |
| hcmv-miR-US25-2-5p | AGCGGUCUGUUCAGGUGGAUGA | 197201-mat |
| hcmv-miR-US25-2-3p | AUCCACUUGGAGAGCUCCCGCGGU | 468261-mat |
| hcmv-miR-US4-5p | UGGACGUGCAGGGGGAUGUCUG | 469977-mat |
| hcmv-miR-US4-3p | UGACAGCCCGCUACACCUCU | 469699-mat |
| hcmv-miR-UL70-5p | UGCGUCUCGGCCUCGUCCAGA | 003183-mat |
| hcmv-miR-UL59 | GUUCUCUCGCUCGUCAUGCCGU | 469253-mat |
| hcmv-miR-UL69 | CCAGAGGCUAAGCCGAAACCG | 468703-mat |
| hcmv-miR-US5-2-5p | CUUUCGCCACACCUAUCCUGAAAG | 469274-mat |
| hcmv-miR-US5-2-3p | UAUGAUAGGUGUGACGAUGUCU | 469255-mat |
| hcmv-miR-US29-5p | UGGAUGUGCUCGGACCGUGACG | CS1RUMR |
| hcmv-miR-US29-3p | CCCACGGUCCGGGCACAAUCA | 468621-mat |
| hcmv-miR-US22-5p | UGUUUCAGCGUGUGUCCGCGGG | 468736-mat |
| hcmv-miR-US22-3p | UCGCCGGCCGCGCUGUAACCAGG | 468548-mat |
| peu-MIR2911 | GGCCGGGGGACGGGCUGGGA | 242025-mat |
| hsa-miR-16 | UAGCAGCACGUAAAUAUUGGCG | 00391 |

**Table S2** The concentration of HCMV-encoded miRNAs in pregnant women with APO in the validation seta

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **HCMV encoded miRNAs** | **APO (n=20)**  **Mean ± SEM** | **Controls (n=27)**  **Mean ± SEM** | **Fold change** | ***P* valuea** |
| hcmv-miR-UL148D | 3.91±0.25 | 3.08±0.27 | 1.27 | 0.035 |
| hcmv-miR-US25-1-5p | 0.11±0.01 | 0.08±0.01 | 1.38 | 0.006 |
| hcmv-miR-US5-1 | 3.18±0.43 | 1.90±0.14 | 1.67 | 0.003 |
| hcmv-miR-US33-3p | 0.06±0.01 | 0.06±0.01 | 1.05 | 0.875 |
| hcmv-miR-UL59 | 0.78±0.14 | 1.27±0.23 | 0.62 | 0.096 |

a Data are presented as the Mean ± SEM

b Student-t test.

**Table S3** The concentration of HCMV-encoded miRNAs in pregnant women with APO and normal controlsa

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **HCMV encoded miRNAs** | **APO (n=40)**  **Mean ± SEM** | **Controls (n=55)**  **Mean ± SEM** | **Fold change** | ***P* valuea** |
| hcmv-miR-UL148D | 5.01±0.49 | 3.32±0.20 | 1.51 | <0.001 |
| hcmv-miR-US25-1-5p | 0.11±0.01 | 0.08±0.01 | 1.58 | <0.001 |
| hcmv-miR-US5-1 | 3.44±0.35 | 2.11±0.16 | 1.63 | <0.001 |
| hcmv-miR-US33-3p | 0.09±0.01 | 0.06±0.01 | 1.43 | 0.041 |
| hcmv-miR-UL59 | 1.02±0.10 | 1.11±0.12 | 0.92 | 0.603 |

a Data are presented as the Mean ± SEM

b Student-t test.

**Table S4** The concentration of HCMV-encoded miRNAs in two types of pregnant women with APO and controlsa

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **HCMV encoded miRNAs** | **FSA (n=25)** | **SA (n=15)** | **Controls (n=55)** | **Fold change** | | |  | ***P* valueb** | | |
| **Mean ± SEM** | **Mean ± SEM** | **Mean ± SEM** | **FSA vs Controls** | **SA vs Controls** | **FSA vs SA** |  | **FSA vs Controls** | **SA vs Controls** | **FSA vs SA** |
| hcmv-miR-UL148d | 5.41±0.53 | 4.35±0.96 | 3.32±0.20 | 1.63 | 1.31 | 1.24 |  | <0.001 | 0.105 | 0.298 |
| hcmv-miR-US25-1-5p | 0.16±0.02 | 0.10±0.01 | 0.09±0.01 | 1.83 | 1.16 | 1.57 |  | <0.001 | 0.356 | 0.018 |
| hcmv-miR-US5-1 | 3.42±0.38 | 3.46±0.70 | 2.11±0.16 | 1.62 | 1.64 | 0.99 |  | <0.001 | 0.005 | 0.954 |

a Data are presented as the Mean ± SEM

b Student-t test.

**Table S5** The respective AUCs of candidate miRNAs in ROC curve analyses for control and APO cohorts

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Test Result Variable(s)** | **APO vs Controls** | | | | |
| **Area** | **Std. Error** | **Asymptotic Sig.** | **Asymptotic 95% Confidence Interval** | |
| **Lower Bound** | **Upper Bound** |
| **hcmv-miR-UL148D** | 0.689 | 0.055 | 0.002 | 0.581 | 0.797 |
| **hcmv-miR-US25-1-5p** | 0.735 | 0.051 | <0.0001 | 0.635 | 0.836 |
| **hcmv-miR-US5-1** | 0.688 | 0.058 | 0.002 | 0.574 | 0.801 |
| **The panela** | 0.685 | 0.058 | 0.002 | 0.573 | 0.798 |

a Combination of 3 miRNAs.

**Table S6** Univariate logistic regression analyses of plasma HCMV miRNAs for pregnant women with APO

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Variables** | | **B** | **Std. Error** | | **OR** | **95% CI for OR** | | ***P* value** |
| **Lower** | **Upper** |
| hcmv-miR-US25-1-5p | | 10.185 | 4.662 | | 26500.657 | 2.848 | 246567602.202 | 0.029 |
| hcmv-miR-US5-1 | | 0.341 | 0.162 | | 1.406 | 1.024 | 1.931 | 0.035 |
|  |  | |  |  | |  |  |  |