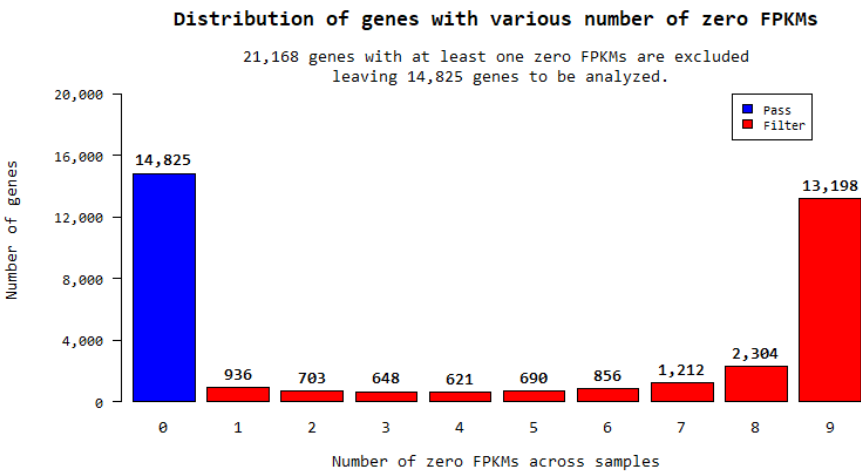
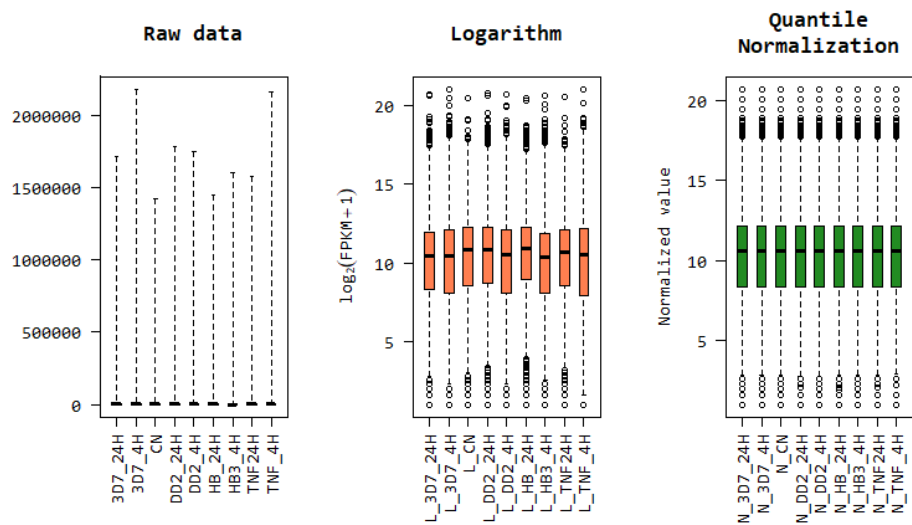


Supplementary Material

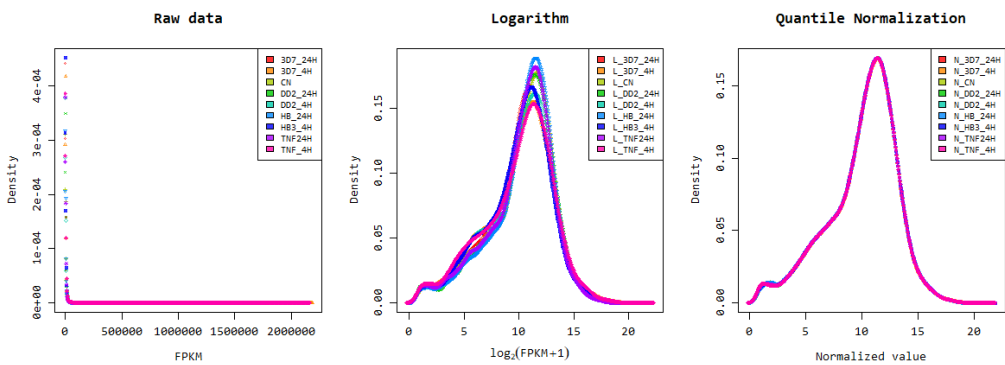
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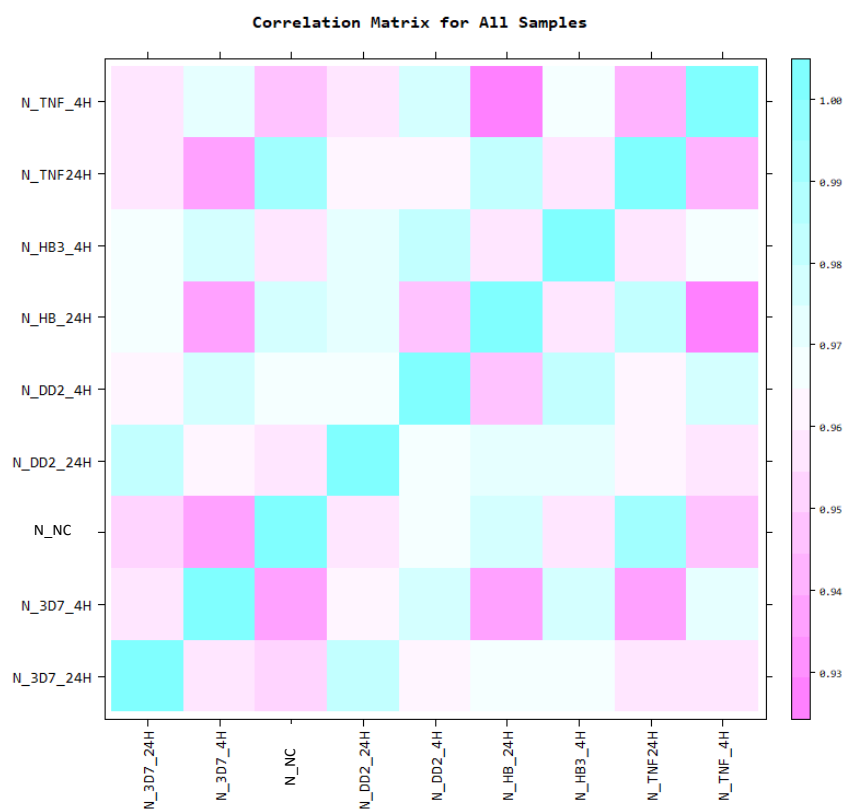
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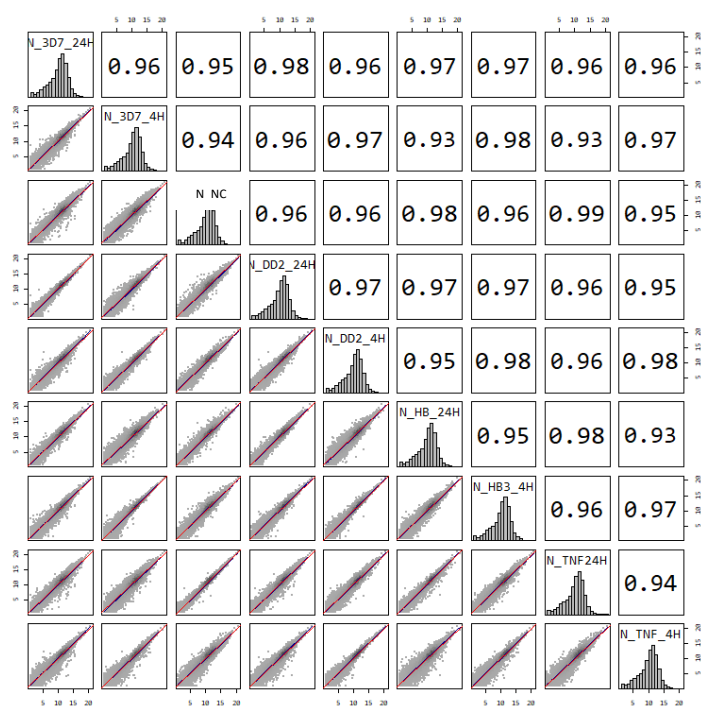
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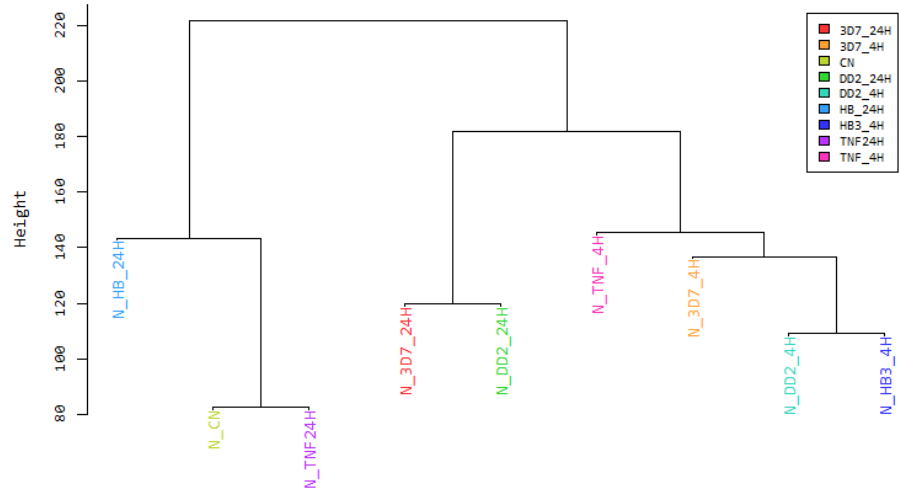
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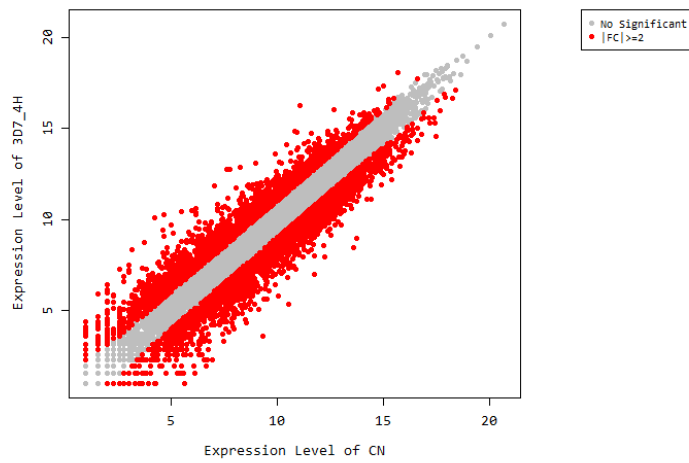
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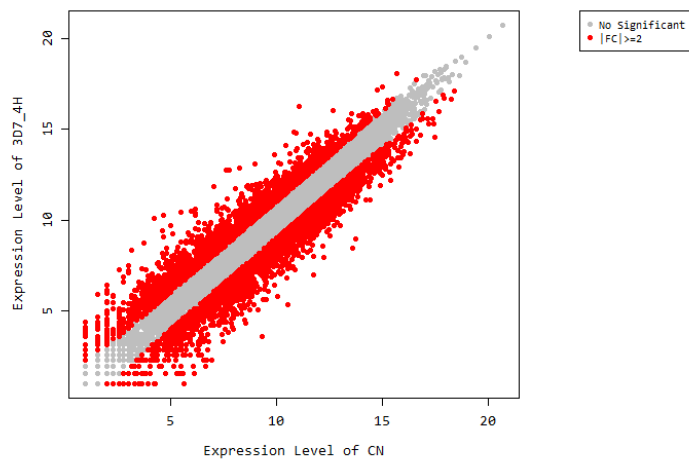
**Figure S1:** HBMEC transcriptome data. (A) The data quality is evaluated through QC for each gene (histogram): For 9 samples, if more than one FPKM value was 0, it was not included in the analysis. Therefore, from the total of 35,993 genes, 21,168 were excluded, and only 14,825 genes were used for statistical analysis. (B) Box plot: Below boxplots show the corresponding sample's expression distribution based on a percentile (median, 50 percentile, 75 percentile, maximum, and minimum) based on raw signal (FPKM), Log2 transformation, and Quantile Normalization. (C) Density plot: Below density plots show the corresponding sample's expression distribution before and after raw signal (FPKM+1), Log2 transformation, and Quantile Normalization. (D) Correlation matrix between Samples: The similarity between samples is obtained through Pearson's coefficient of the sample's normalized value. For range:  $-1 \leq r \leq 1$ , the closer the value is to 1, the more similar the samples were. The correlation matrix of all samples is as follows (X-axis as control and Y-axis as a test is the average normalized value of the group). (E) Reproducibility between samples, the closer the value is to 1, the more similar the samples are. (F) Hierarchical clustering (distance metric = Euclidean distance, linkage method = complete) analysis and graphically represents the similarity of expression patterns between all samples and genes. The samples in the analysis are HBMEC without stimulation, used as negative control (NC), HBMEC with

stimulation, working as a positive control, and TNF- $\alpha$  at 4 h (TNF\_4h) and 24 h (TNF\_24h). HBMEC stimulated with Dd2 (Dd2\_4h Dd2\_24h), HB3 (HB3\_4h, Hb3\_24h), and 3D7 (3D7\_4h, 3D7\_24h) strains of *P. falciparum* parasites at a time point of 4 h and 24 h, respectively.

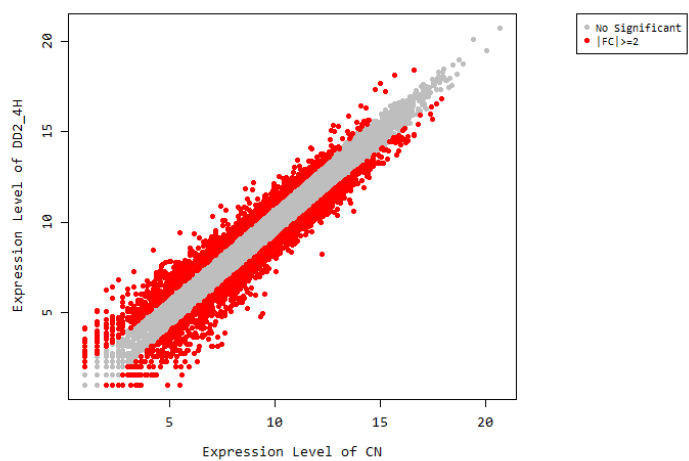
A – Expression level of the genes between 3D7\_4h\_vs\_NC



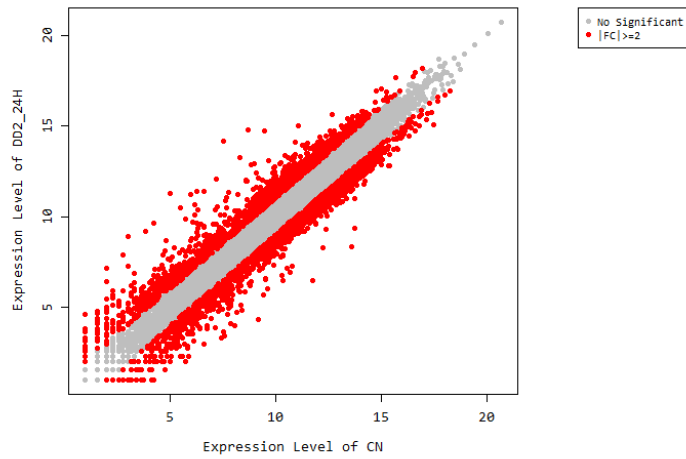
B – Expression level of the genes between 3D7\_24h\_vs\_NC



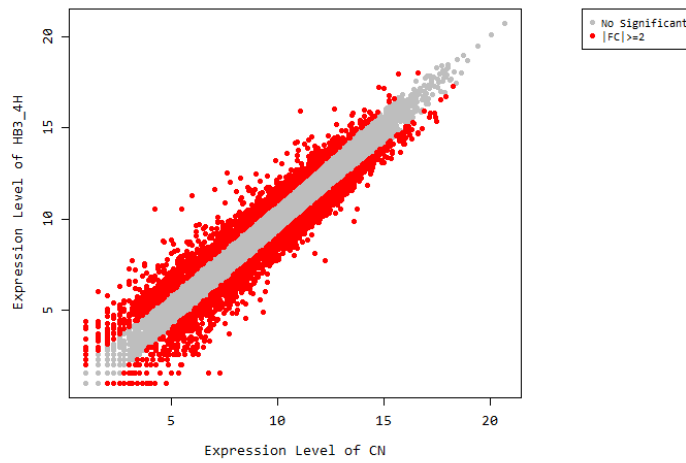
C – Expression level of the genes between Dd2\_4h\_vs\_NC



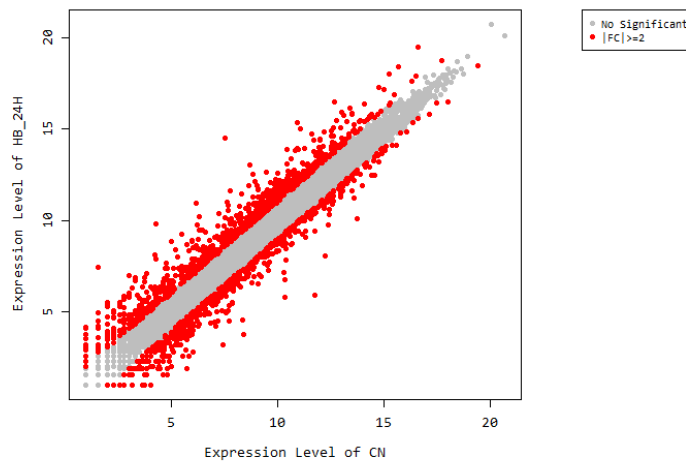
D – Expression level of the genes between Dd2\_24h\_vs\_NC



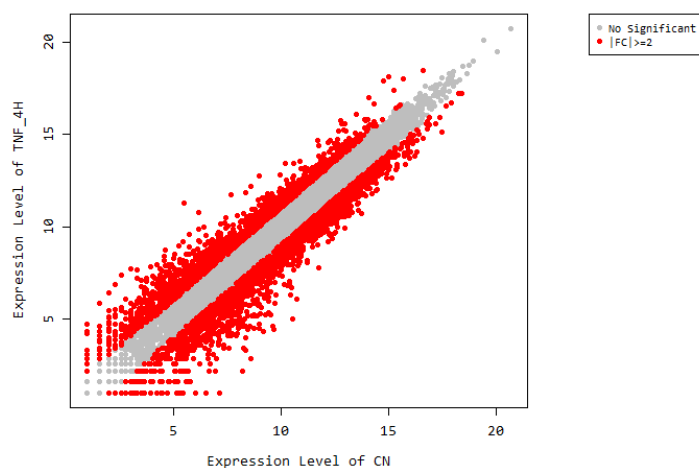
E – Expression level of the genes between HB3\_4h\_vs\_NC



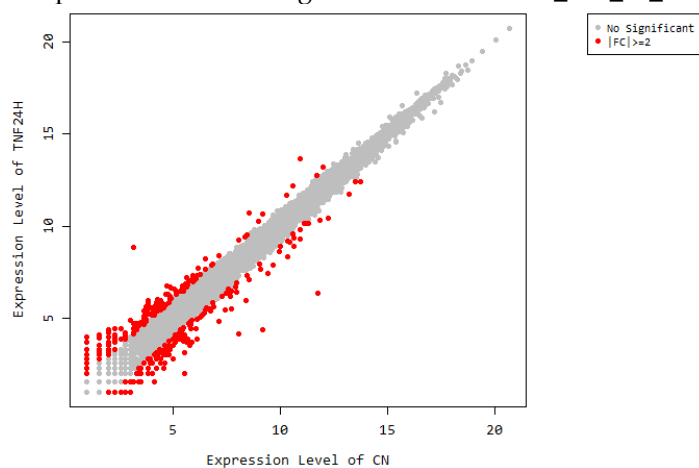
F – Expression level of the genes between HB3\_24h\_vs\_NC



G – Expression level of the genes between TNF- $\alpha$ \_4h\_vs\_NC



H - Expression level of the genes between TNF- $\alpha$ \_24h\_vs\_NC

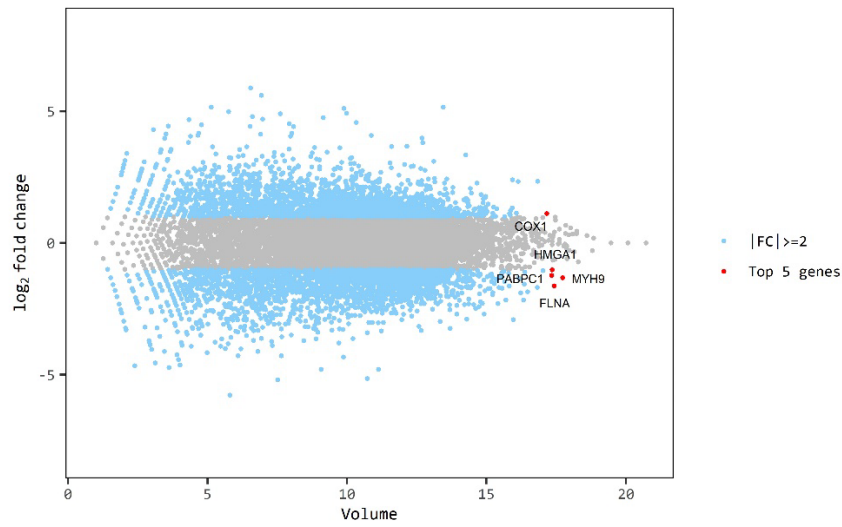


**Figure S2:** Scatter plot of expression level. Shows distribution of normalized expression levels values per group between comparison pairs as a scatter plot by 2-fold-change. The samples in the analysis are HBMEC without stimulation, used as negative control (NC), HBMEC with stimulation, working as a positive control, TNF

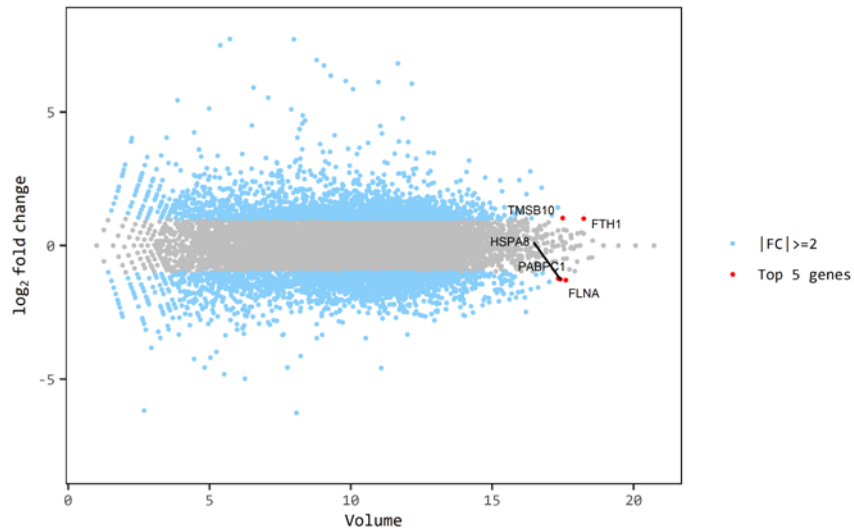
at 4 h (TNF\_4h) and 24 h (TNF\_24h). HBMEC stimulated with Dd2 (Dd2\_4h Dd2\_24h), HB3 (HB3\_4h, Hb3\_24h), and 3D7 (3D7\_4h, 3D7\_24h) strains of *P. falciparum* parasites at a time point of 4 h and 24 h, respectively. The scatter plot of the genes expression level between NC and (A) 3D7 4H, (B) 3D7 24H, (C) Dd2 4H, (D) Dd2 24H, (E) HB3 4H, (F) HB3 24H, (G) TNF 4H, (H) TNF 24H.



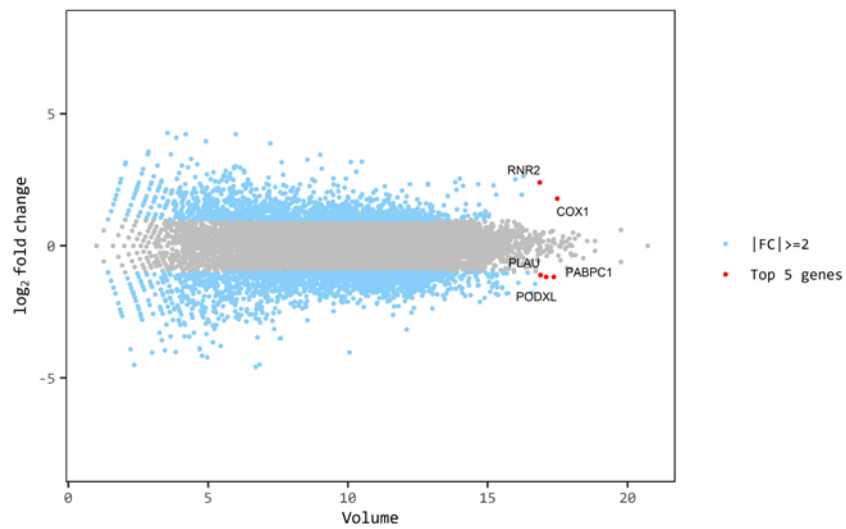
A - Volume Plot Between 3D7\_4h\_vs\_NC



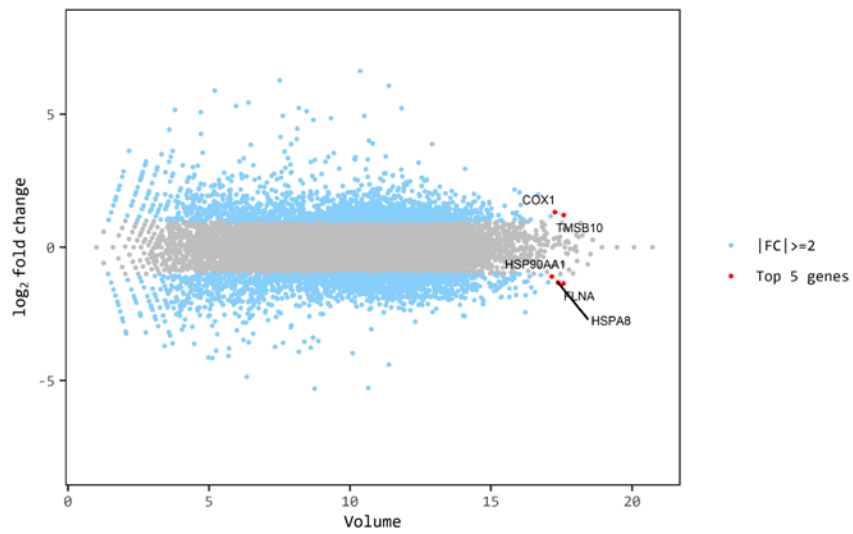
B - Volume Plot Between 3D7\_24h\_vs\_NC



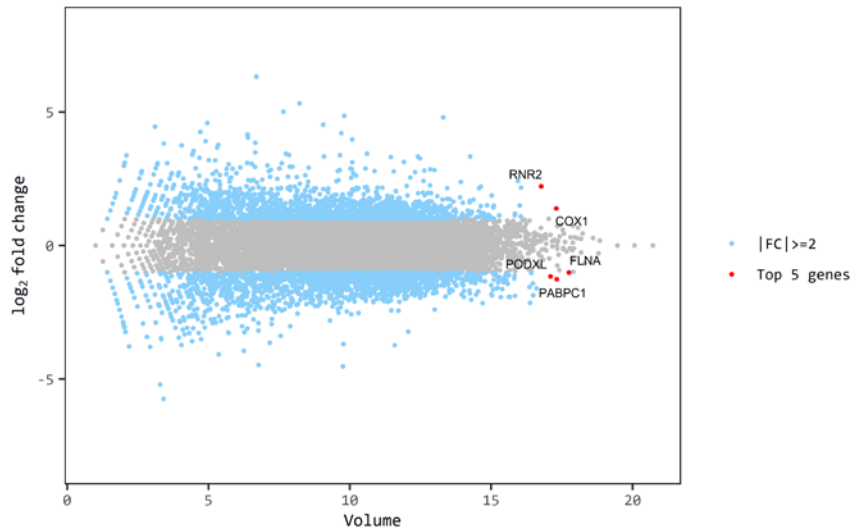
C - Volume Plot Between Dd2\_4h\_vs\_NC



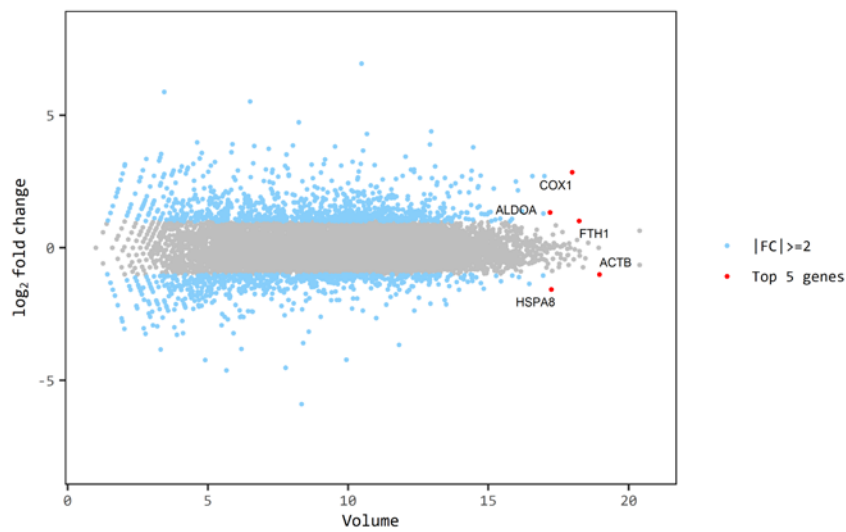
D - Volume Plot Between Dd2\_24h\_vs\_NC



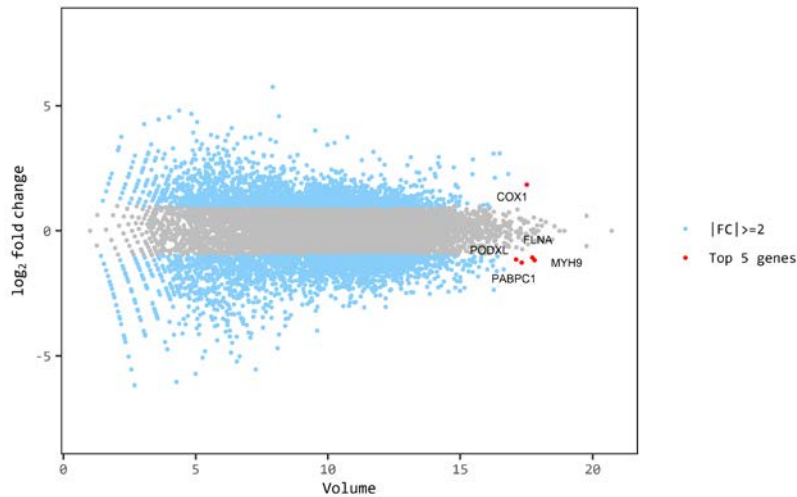
E - Volume Plot Between HB3\_4h\_vs\_NC



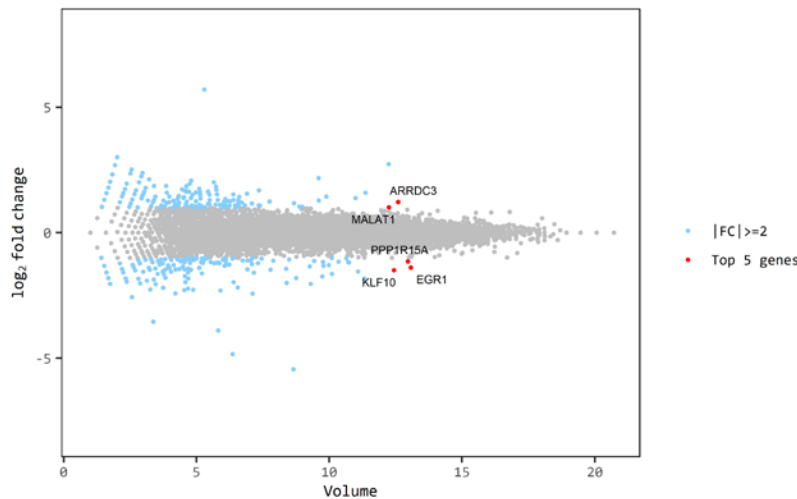
F - Volume Plot Between HB3\_24h\_vs\_NC



G - Volume Plot Between TNF- $\alpha$ \_4h\_vs\_NC



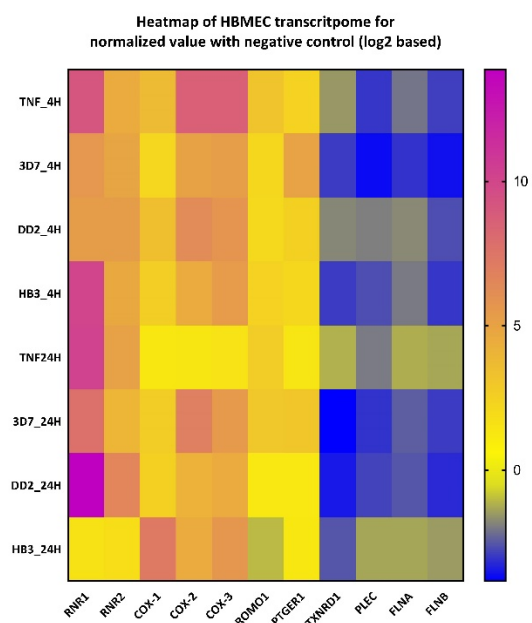
H - Volume Plot Between TNF- $\alpha$ \_24h\_vs\_NC



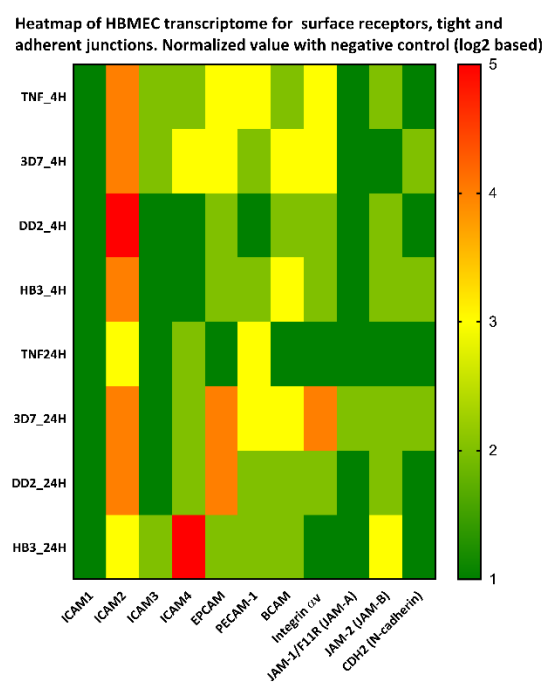
**Figure S3:** Volume plot. Expression volume is the geometric mean of two groups' expression levels. (volume = square-root (Control Normalized value \* Test Normalized value)). To confirm the genes that show higher expression differences compared to the control according to expression volume, a volume plot is drawn. (X-axis: Volume, Y-axis: log<sub>2</sub> Fold Change). For example, even though fold change might differ by 2-fold-change, the genes with higher expression volume may be more credible. Red dot ● means the top five genes by volume, which satisfy  $|fc| \geq 2$ . The samples in the analysis are HBMEC without stimulation, used as negative control (NC), HBMEC with stimulation, working as a positive control, TNF at 4 h (TNF\_4h) and 24 h (TNF\_24h). HBMEC stimulated with Dd2 (Dd2\_4h, Dd2\_24h), HB3 (HB3\_4h, Hb3\_24h),

and 3D7 (3D7\_4h, 3D7\_24h) strains of *P. falciparum* parasites at a time point of 4 h and 24 h, respectively. The volume plot between NC and (A)3D7 4H, (B) 3D7 24H, (C) Dd2 4H, (D) Dd2 24H, (E) HB3 4H, (F) HB3 24H, (G) TNF 4H, (H) TNF 24H.

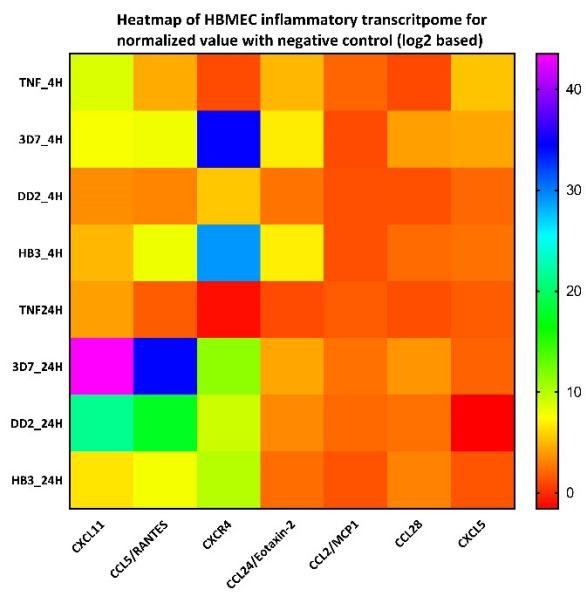
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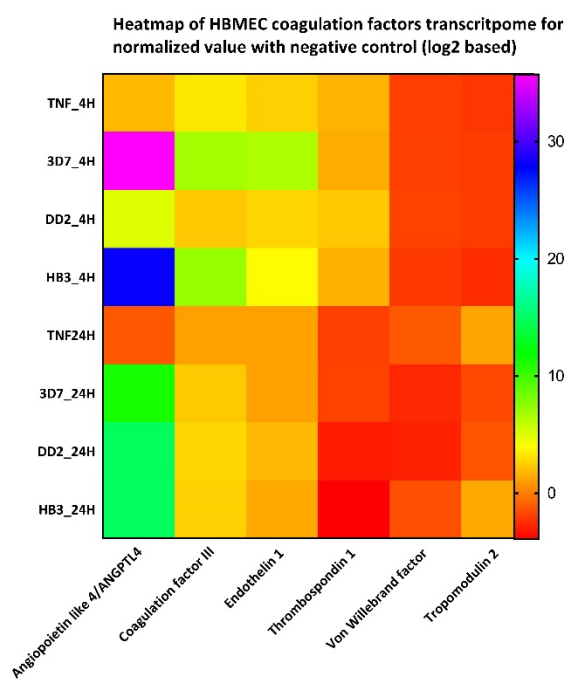
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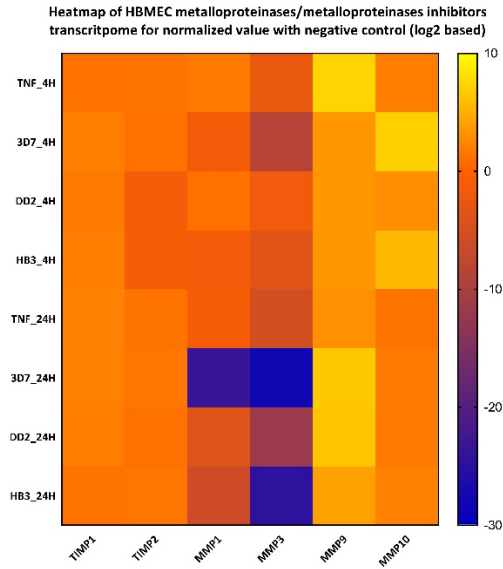
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D



E



**Figure S4:** Heatmaps from HBMEC transcriptomic analysis. The heatmaps from HBMEC transcriptomic analysis using Dd2, HB3, 3D7 strains of *Plasmodium falciparum* parasites and TNF- $\alpha$ , at 4 h and 24 h, Dd2 4H, Dd2 24H, HB3 4H, HB3 24H, 3D7 4H, 3D7 24H, TNF\_4h, and TNF\_24h, respectively. **A)** Heatmap of Ribosomal 45S Cluster 1 (*RNR1*), Ribosomal 45S Cluster 2 (*RNR2*), Cyclooxygenase 1 (*COX-1*), *COX-2* and *COX-3*, Reactive Oxygen Species (ROS) Modulator 1 (*ROMO1*), Prostaglandin E Receptor 1 (*PTGER1*), Thioredoxin Reductase 1 (*TXNRD1*), Plectin (*PLEC*), Filamin A (*FLNA*), Filamin B (*FLNB*) are proteins coding genes. Genes that were downregulated are in blue, and up-regulated are in pink. **B)** Heatmap of transcriptomic expression of gene receptors *ICAM-1*, *ICAM-2*, *ICAM-3*, *ICAM-4*, *EPCAM*, *PECAM-1*, *BCAM*, *Integrin  $\alpha$ V*, *F11R/JAM-A/JAM-1*, *JAM2/JAM-B*, *CDH2/N-cadherin* genes. Genes that were downregulated are in green and the ones up-regulated are in red. **C)** Heatmap of cytokines and chemokines. The data is presented in fold-change of *CXCL11*, *CCL5/RANTES*, *CXCR4*, *CCL24/Eotaxin-2*, *CCL2/MCP-1*, *CCL28*, and *CXCL5* genes. Genes that were downregulated are in blue and the ones up-regulated are in pink. **(D)** Heatmap of coagulation cascade genes. *ANGPTL4* genes that encodes Angiopoietin-like 4 protein, *coagulation factor III*, *endothelin-1*, *thrombospondin-1*, Von Willebrand factor (*vWF*), and *Tmod2* gene that encodes tropomodulin 2. **E)** Heatmap of metalloproteinases (MMP) and tissue

inhibitor metalloproteinase (TIMP). The data is presented in fold-change of *MMP-1*, *MMP-3*, *MMP-10*, *MMP-11*, *MMP-1*, *TIMP1*, and *TIMP-2* genes. The fold-change ratio of gene expression is based on negative control levels. Genes that were downregulated are in blue and the ones up-regulated are in yellow.

	PECAM-1	ICAM-1	CD36	EPCR	N- cadherin	JAM-A	IαVβ3
TNF-α 4H	1.104; ±0.034; <i>p</i> <0.05						



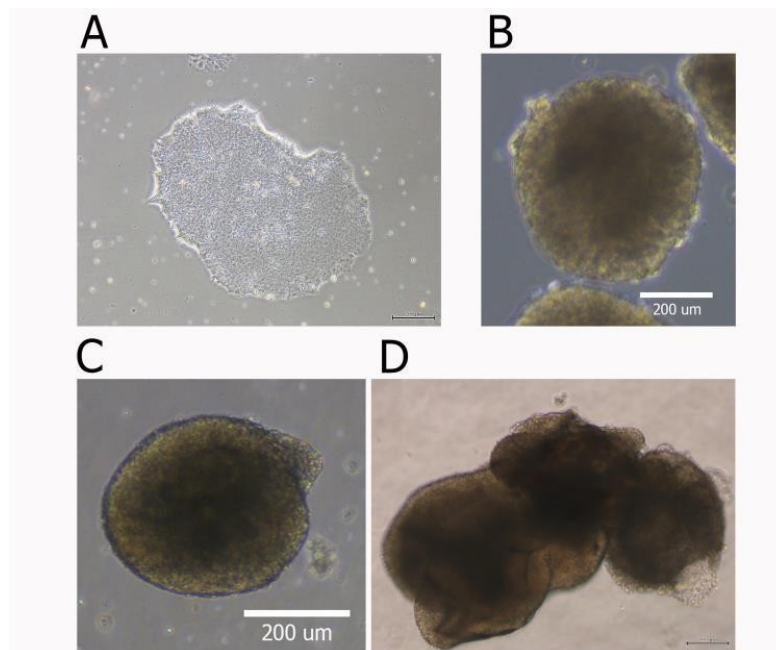
TNF- $\alpha$ 24H	1.189; $\pm 0.063$ ; $p < 0.05$	1.349; $\pm 0.141$ ; $p < 0.05$					
3D7 4H			1,302; $\pm 0,098$ ; $p < 0.05$				0.889; $\pm 0.032$ ; $p < 0.05$
3D7 24H			1.795; $\pm 0.192$ ; $p < 0.0001$	1.461; $\pm 0.142$ ; $p < 0.01$		1.153; $\pm 0.051$ ; $p < 0.05$	1.373; $\pm 0.125$ ; $p < 0.05$
Dd2 4H					(0.933; $\pm 0.026$ ; $p < 0.05$		0.843; $\pm 0.032$ ; $p < 0.001$
Dd2 24H			1.513; $\pm 0,081$ ; $p < 0.01$			1.228; $\pm 0.061$ ; $p < 0.001$	
HB3 4H							0,898; $\pm 0,0371$ ; $p < 0.05$
HB3 24H			1.445; $\pm 0.088$ ; $p < 0.01$			1.153; $\pm 0.052$ ; $p < 0.0001$	

**Table S1:** Significant results from receptor expression of HBMEC. The results express the ratio of receptor expression in comparison to the negative control (NC) levels, the  $\pm$  standard error of the mean and the statistical value ( $p$ ).

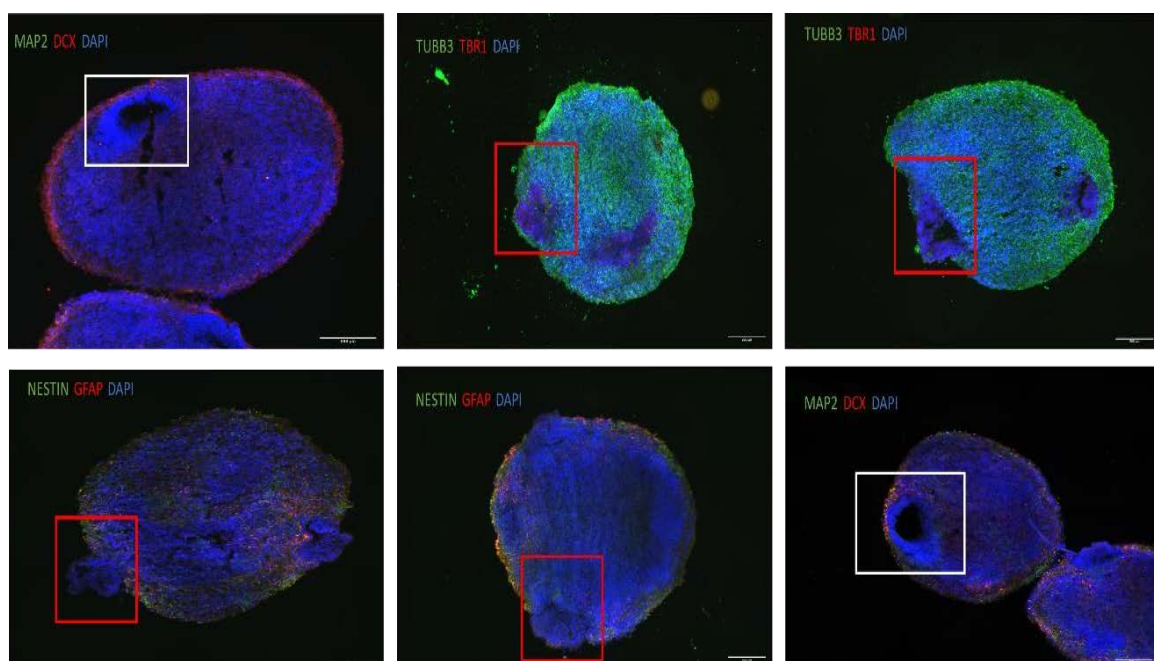
	<i>cxcl11</i>	<i>RANTES</i>	<i>cxcr4</i>	<i>eotaxin-2</i>	<i>MCP-1/ccl2</i>	<i>ccl28</i>	<i>Cxcl5</i>	<i>angptl4</i>	<i>TSP-1</i>	<i>vWF</i>	<i>tropomodulin-2</i>	<i>MMP9</i>
TNF- $\alpha$ 4H	fc $\geq 8$	fc $\geq 3$	-	fc $\geq 2$	fc $\geq 2$	-	fc $\geq 5$	-	-	fc $\leq -2$	fc $\leq -2$	fc $\geq 6$
TNF- $\alpha$ 24H	fc $\geq 4$	-	-	-	-	-	-	-	fc $\leq -2$	-	fc $\leq -2$	fc $\geq 3$
3D7 4H	fc $\geq 7$	fc $\geq 3$	fc $\geq 5$	fc $\geq 6$	fc $\geq 4$	fc $\geq 4$	fc $\geq 4$	fc $\geq 30$	-	fc $\leq -2$	fc $\leq -2$	fc $\geq 3$
3D7 24H	fc $\geq 43$	fc $\geq 7$	fc $\geq 5$	fc $\geq 2$	fc $\geq 2$	fc $\geq 3.5$	-	fc $\geq 10$	-	fc $\leq -2$	-	fc $\geq 6$

Dd2 4H	fc $\geq$ 3	fc $\geq$ 3	fc $\geq$ 5	fc $\geq$ 2	-	fc $\geq$ 2	fc $\geq$ 2		fc $\geq$ 2	fc $\leq$ -2	fc $\leq$ -2	fc $\geq$ 3
Dd2 24H	fc $\geq$ 21	fc $\geq$ 7	fc $\geq$ 5	fc $\geq$ 2	fc $\geq$ 2	fc $\geq$ 2	-	fc $\geq$ 10	fc $\leq$ -3	fc $\leq$ -2	-	fc $\geq$ 3
HB3 4H	fc $\geq$ 4	fc $\geq$ 3	fc $\geq$ 5	fc $\geq$ 6	fc $\geq$ 2	fc $\geq$ 2	fc $\geq$ 2	fc $\geq$ 20	-	fc $\leq$ -2	fc $\leq$ -2	fc $\geq$ 3
HB3 24H	fc $\geq$ 6	fc $\geq$ 7	fc $\geq$ 5	fc $\geq$ 2	fc $\geq$ 3	fc $\geq$ 3	-	fc $\geq$ 10	fc $\leq$ -3	-	-	fc $\geq$ 3

**Table S2: Significant fold-change from HBMEC transcriptome.** The fold-change ratio of gene expression is based on negative control levels.



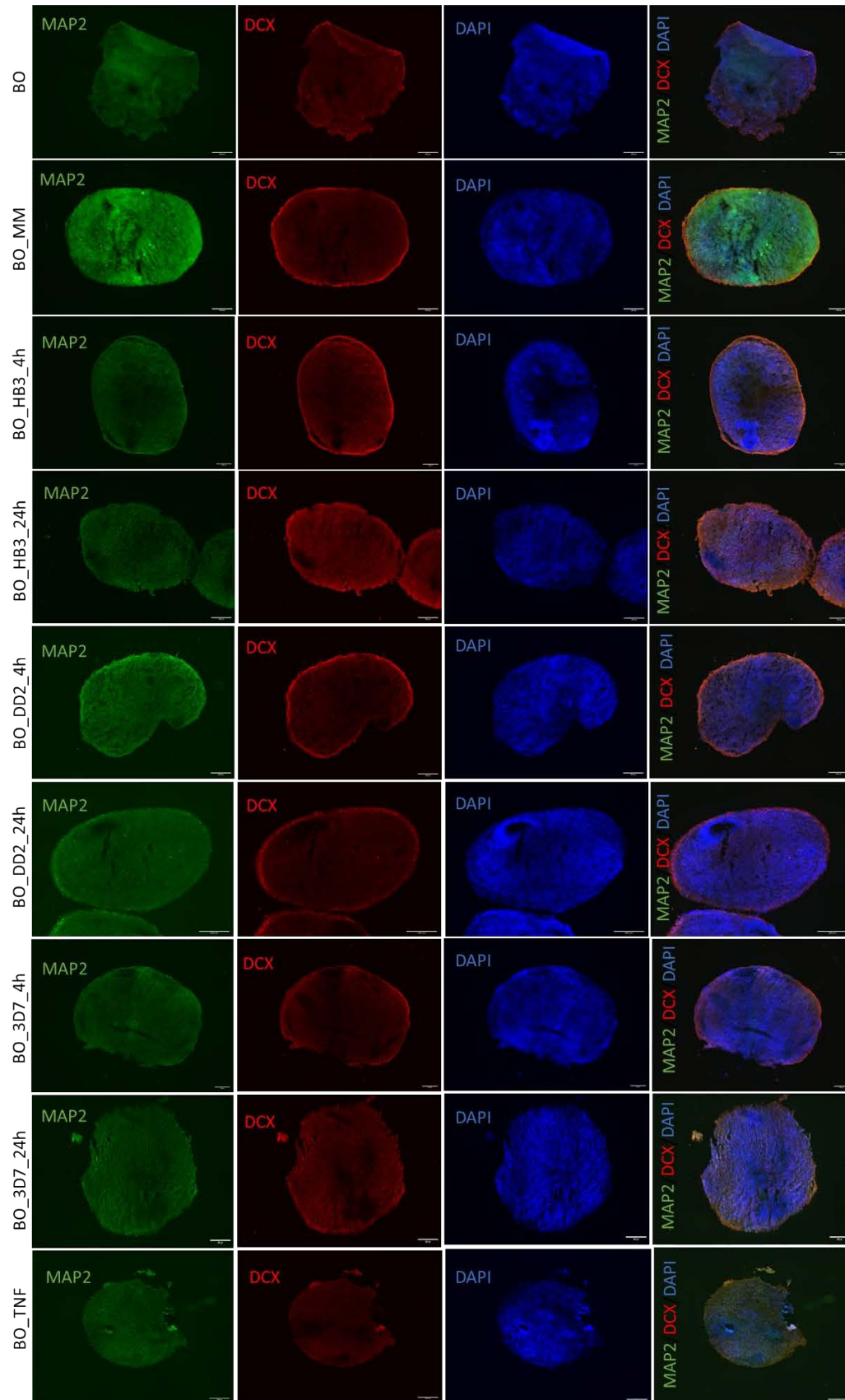
**Figure S5:** Bright field images from human brain organoids culture system. **A)** iPSCs culture used to generate brain organoids. **B)** Embryoid bodies (EB) formation after 6 days of spontaneous differentiation **C)** Neurospheres (NPCs) were formed after 11 days using a neural induction medium. **D)** Brain organoids with 30 days of post-differentiation. Scale bar: 200  $\mu\text{m}$ .

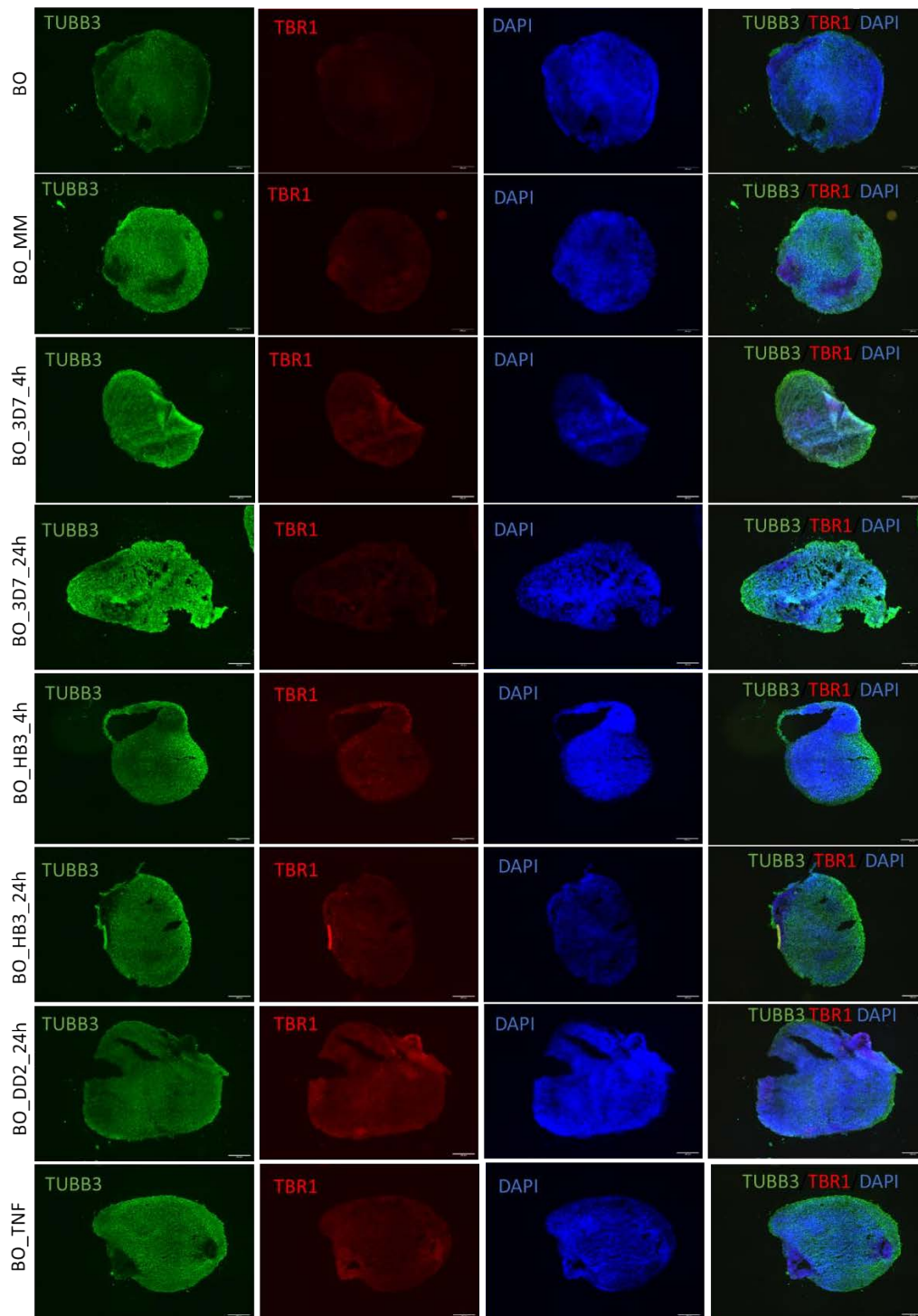


**Figure S6:** The neural tubes and heterogeneous regions of human brain organoids *in vitro*. Representative immunostaining of human brain organoids with 45 days post-differentiation, in cryosections with 10-15  $\mu\text{m}$ . Scale bars: 200  $\mu\text{m}$ . Heterogeneous regions (red square) were similar to the choroid plexus architecture and neural tubes (white square). Nuclear staining with DAPI (Blue), neural stem cell marker (Nestin) (Green), the neuronal marker MAP2 (Green), human astrocytic marker, GFAP (Red), cortical-layer neurons TUBB3 (Green), newborn neurons marker DCX (Red) and pre-plate marker Tbr1 (Red). Brain organoids stimulated with secretome from HBMEC without any stimulation were called modified media (BO\_MM), which means that HBMEC were in stimulated with half HBMEC culture media, and half *P. falciparum* parasites culture media. Brain organoids that grow only

with brain organoid media (BO), which works as a negative control, were also analyzed. The brain organoids were submitted to under several secretome conditions, such as with TFN- $\alpha$  stimulation, which works as a positive control (BO\_TNF), three different wild-type strains, 3D7, Dd2, HB3 of *P. falciparum* parasites induction, at 4 h (BO\_3D7\_4H, BO\_Dd2\_4H, BO\_HB3\_4H) and 24 h (BO\_3D7\_24H, BO\_Dd2\_24H, BO\_HB3\_24H).

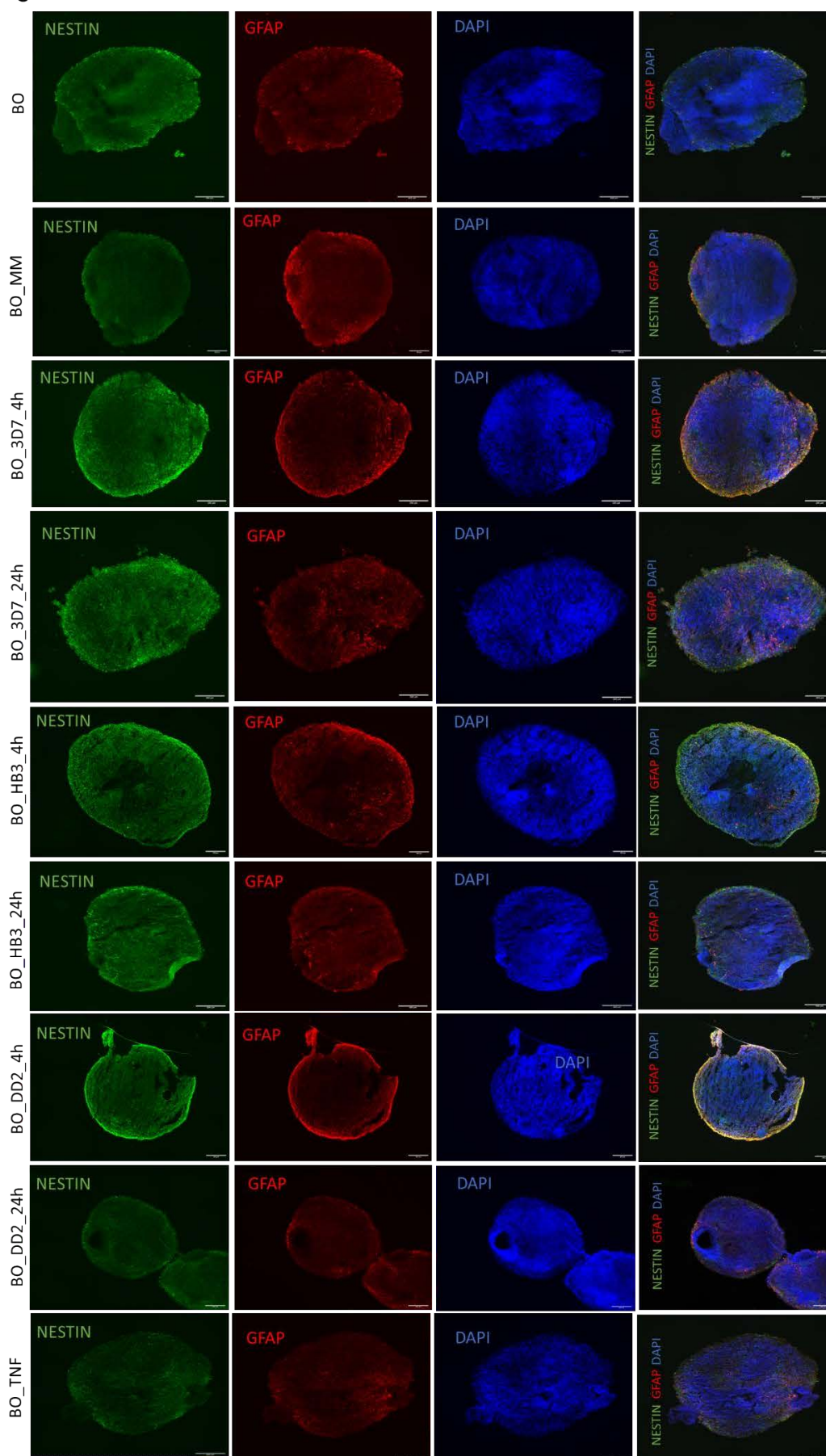
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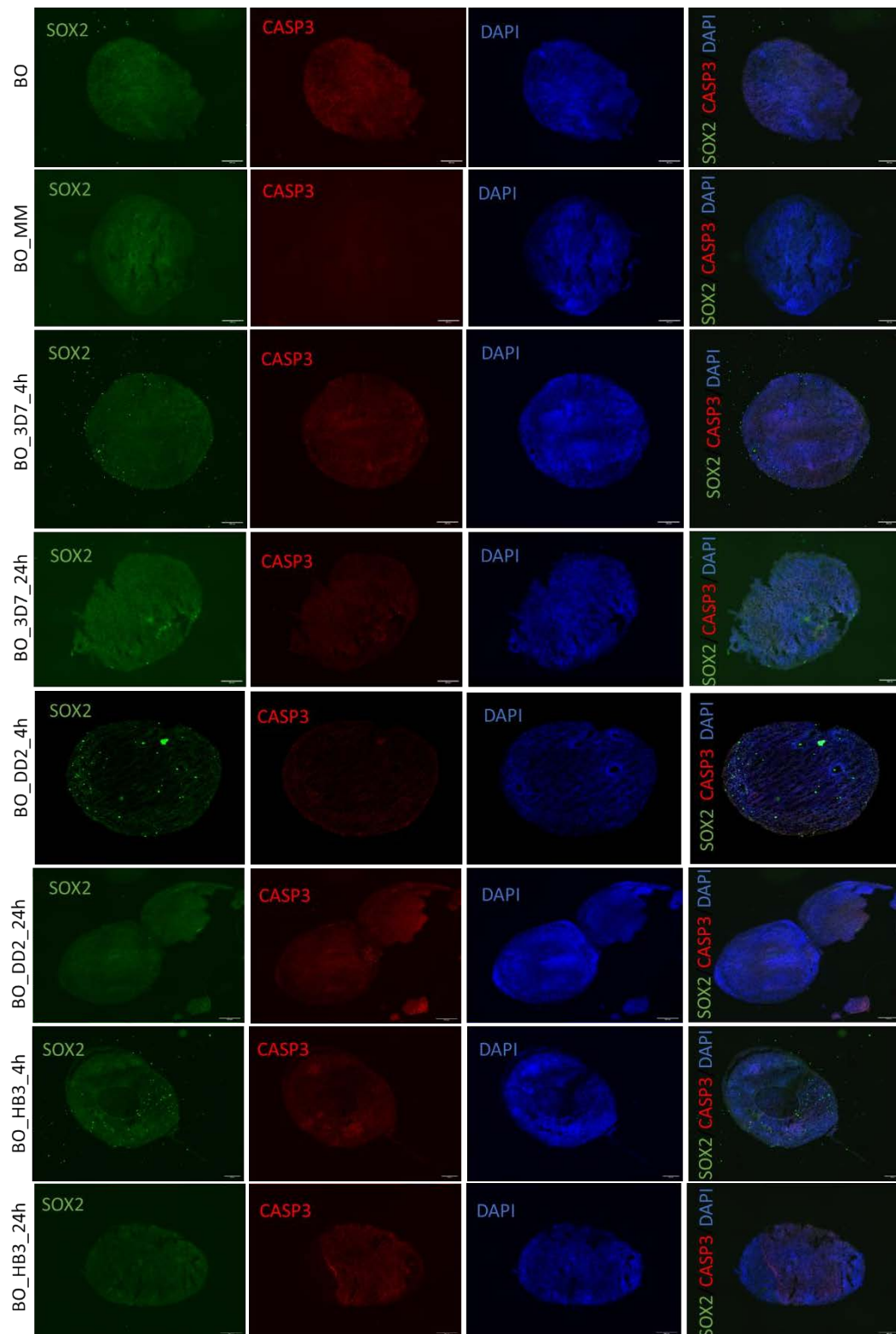
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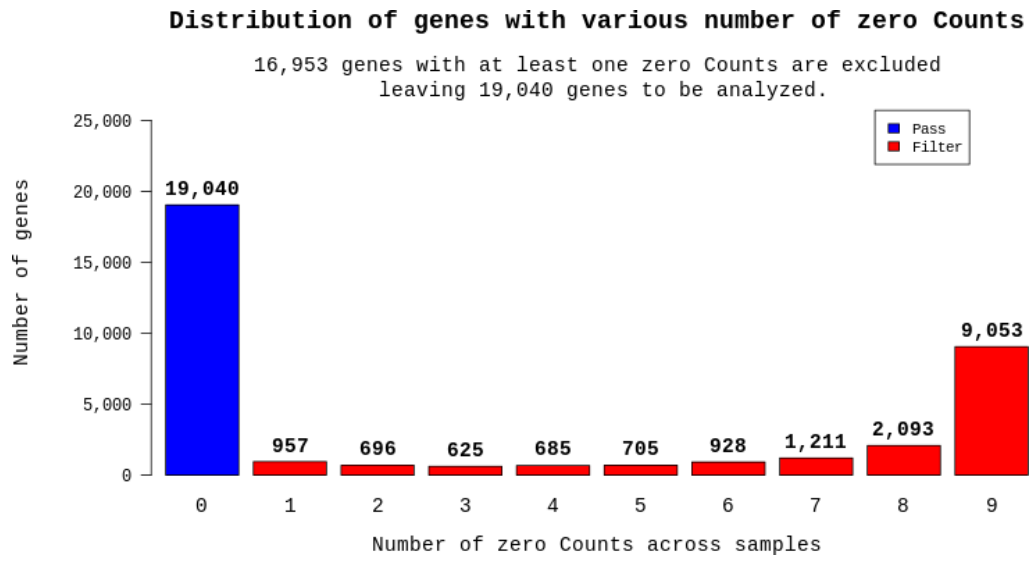


**D**

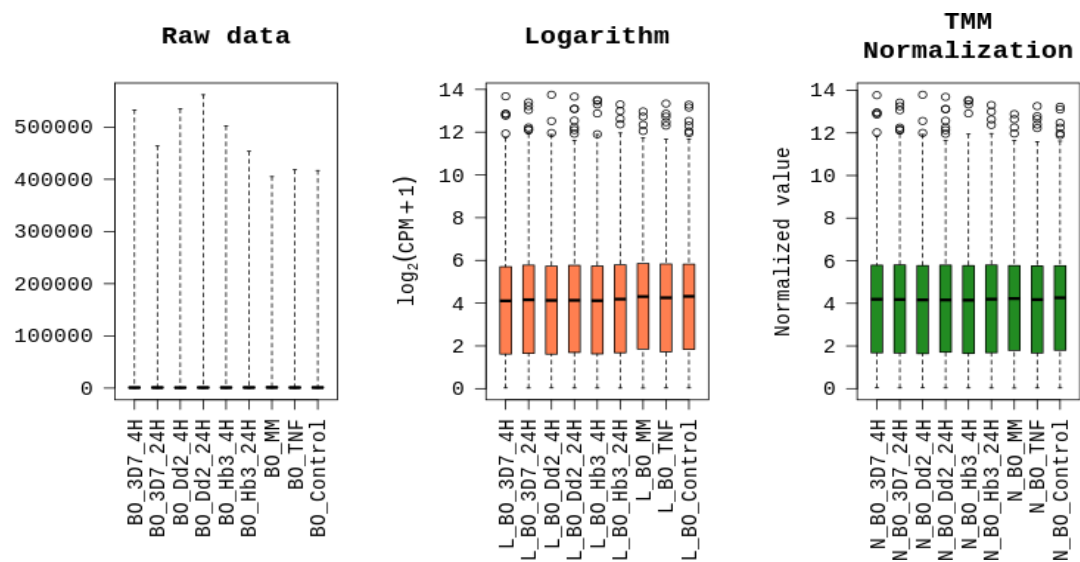
**Figure S7:** The expression of neuronal and glial proteins in human brain organoids. Representative immunostaining of human brain organoids with 45 days post-differentiation, in cryosections with 10-15 μm. Scale bars: 200 μm. Nuclear staining with DAPI (Blue). Brain organoids stimulated with secretome from HBMEC

were called modified media (BO\_MM), which means that HBMEC were cultured with half HBMEC culture media, and half *P. falciparum* parasites culture media. Brain organoids that grow only with brain organoid media (BO) work as a negative control. The brain organoids were submitted to under several secretome conditions in the previous stimulation with HBMEC, such as with TFN- $\alpha$  stimulation, which works as a positive control (BO\_TNF), three different wild-type strains, 3D7, Dd2, HB3 of *P. falciparum* parasites induction, at time points of 4h (BO\_3D7\_4H, BO\_Dd2\_4H, BO\_Hb3\_4H) and 24h (BO\_3D7\_24H, BO\_Dd2\_24H, BO\_Hb3\_24H), respectively. (A) Staining for the neuronal marker MAP2 (Green) revealing neuronal layer and for newborn neurons marker doublecortin (DCX) (Red). (B) Staining for cortical-layer neurons by tubulin beta 3 (TUBB3) (Green) marker, showing a sizeable cortical region. The pre-plate marker TBR1 (Red) for early-born neurons. (C) Immunostaining for a neural stem cell marker (Nestin) (Green) and glial fibrillary acidic protein (GFAP, human astrocytic marker) (Red). (D) Immunostaining with neural progenitor cells (NPC) marker SOX2 for the ventricular zone (VZ) and apoptosis marker Caspase 3 (CASP3). It showed the presence of SOX2<sup>+</sup> cells and some apoptotic cell death (CASP3). It was possible to verify the presence of cavities in the brain organoids.

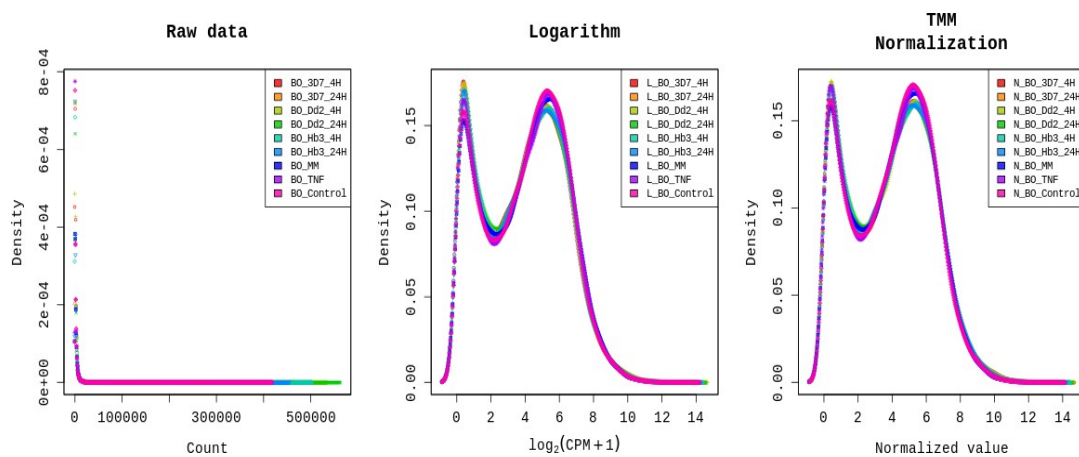
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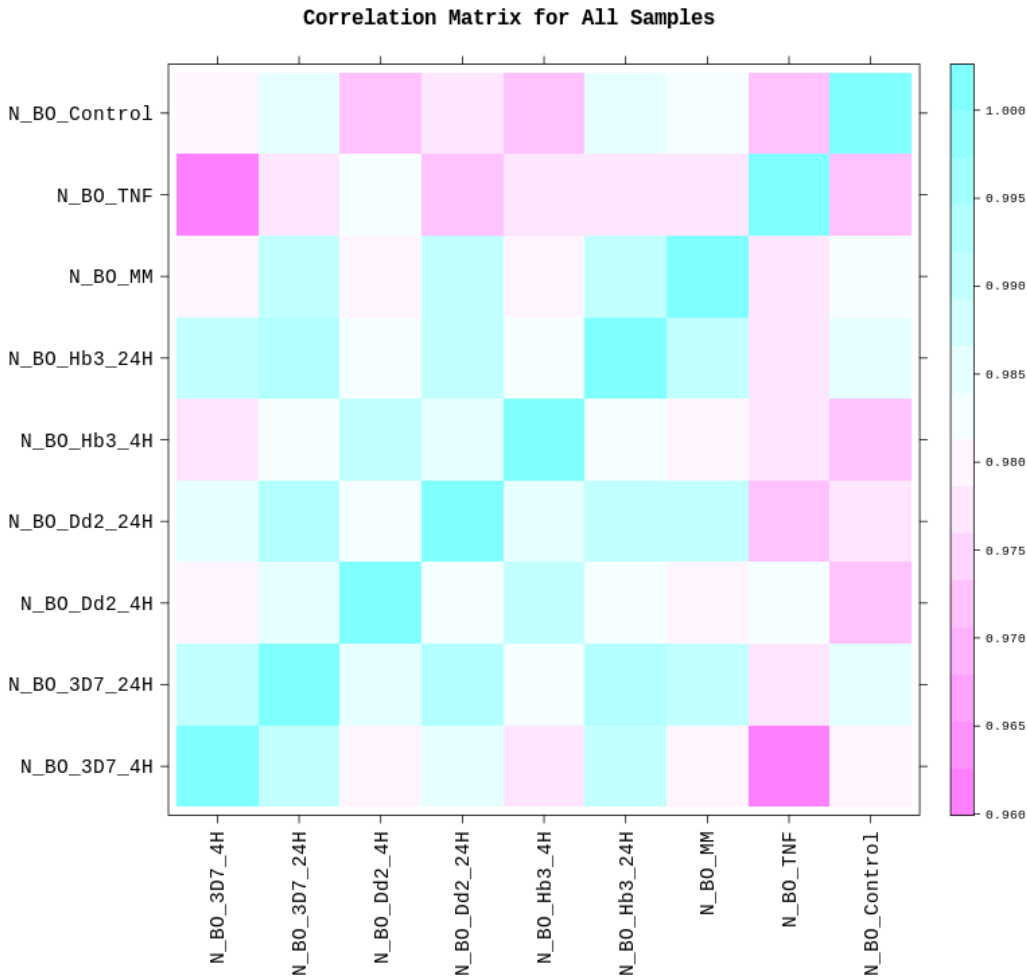
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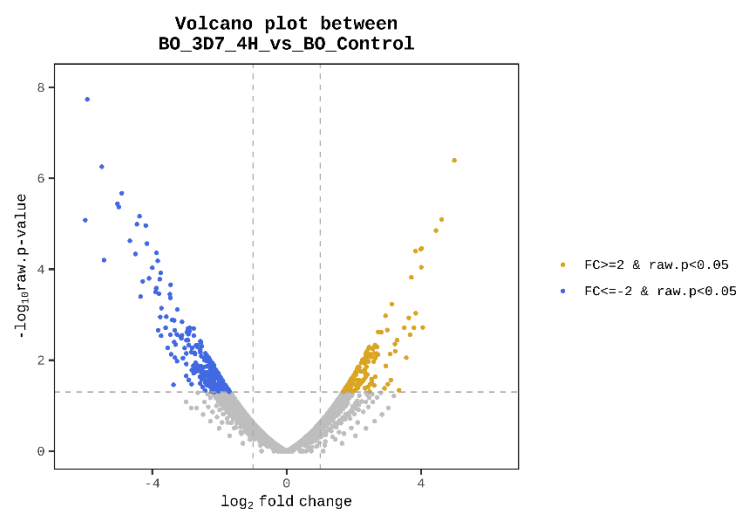
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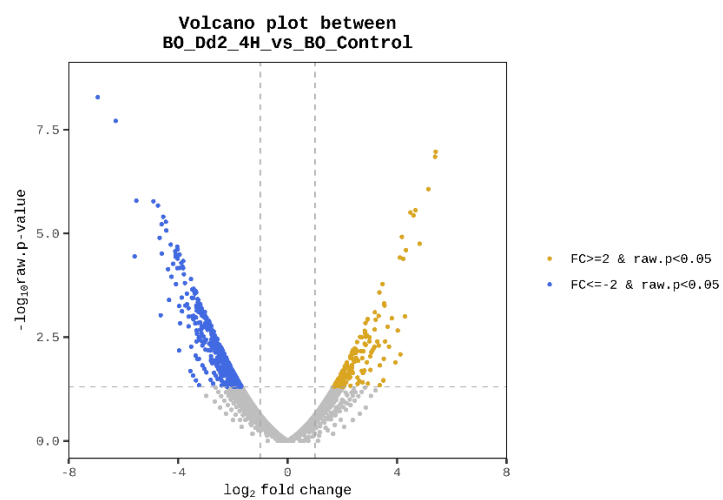
**Figure S8:** Brain organoids transcriptome data. To better understand the influence of secretome in brain organoids, from *P. falciparum* infection in the activation of HBMEC, single-cell RNA sequencing (scRNA-seq) of brain organoids was performed. The brain organoids transcriptomes analyzed were under several secretome conditions, such as with TFN- $\alpha$  stimulation, which works as a positive control (BO\_TNF), three different wild-type strains, 3D7, Dd2, HB3 of *P. falciparum* parasites induction, at time points of 4h (BO\_3D7\_4H, BO\_Dd2\_4H, BO\_HB3\_4H) and 24h (BO\_3D7\_24H, BO\_Dd2\_24H, BO\_HB3\_24H), respectively. Brain organoids stimulation with secretome from HBMEC without any stimulation are called modified media (BO\_MM), which means that HBMEC were in cultured with half HBMEC culture media and half *P. falciparum* parasites culture media. Brain organoids that grow only with brain organoid media (BO\_control), which works as negative control, were also analyzed. (A) The data quality is evaluated through QC for each gene (histogram): For 9 samples, if more than one FPKM value was 0, it was

not included in the analysis. Therefore, from a total of 35,993 genes, 21,168 were excluded, and only 14,825 genes were used for statistical analysis. (B) Box plot: Below boxplots show the corresponding sample's expression distribution based on a percentile (median, 50 percentile, 75 percentile, maximum, and minimum) based on raw signal (FPKM), Log2 transformation, and Quantile Normalization. (C) Density plot: Below density plots show the corresponding sample's expression distribution before and after raw signal (FPKM+1), Log2 transformation, and Quantile Normalization. (D) Correlation matrix between Samples: The similarity between samples is obtained through Pearson's coefficient of the sample's normalized value. For range:  $-1 \leq r \leq 1$ , the closer the value is to 1, the more similar the samples are. The correlation matrix of all samples is as follows (X-axis as control and Y-axis as a test is the average normalized value of the group).

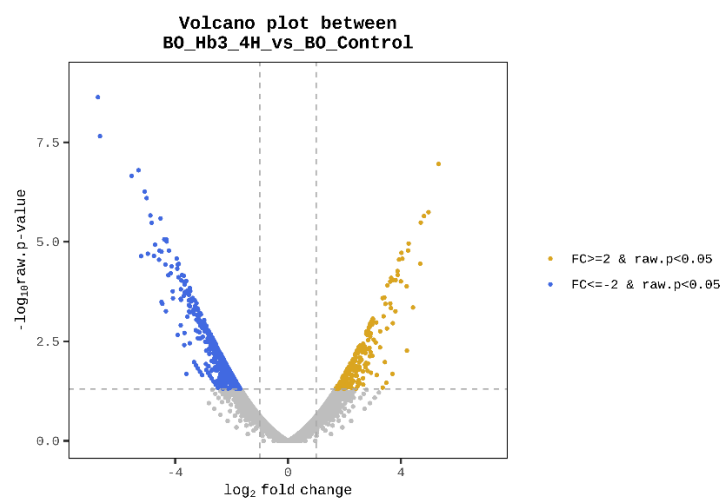
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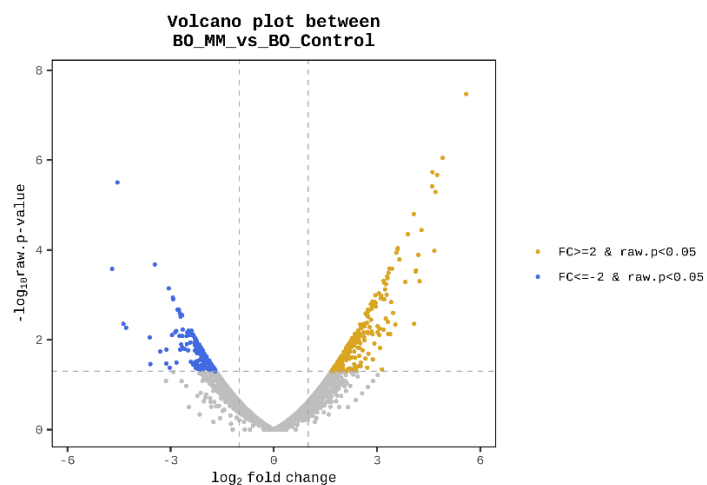
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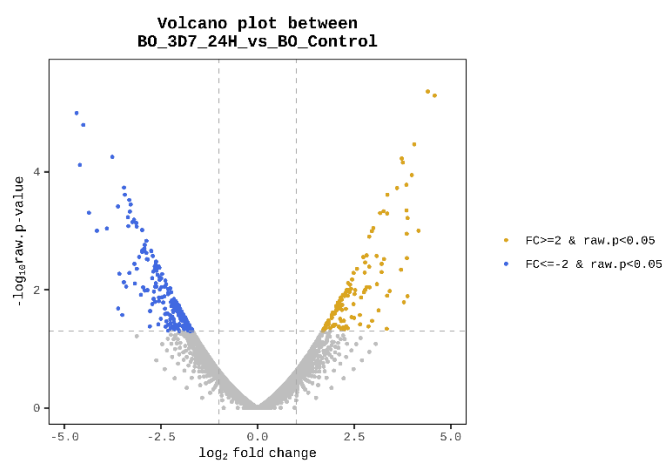
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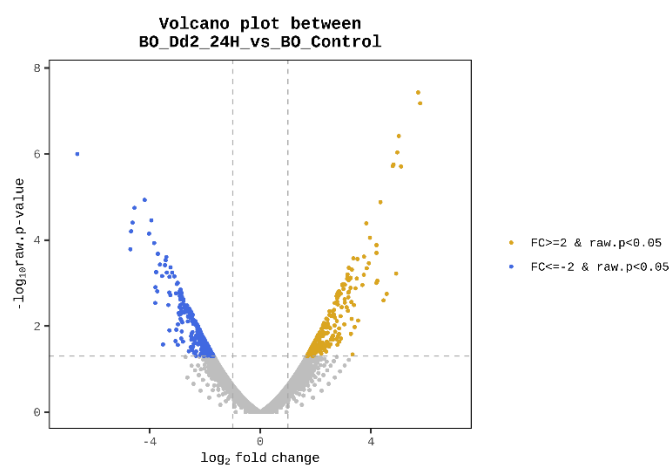
D



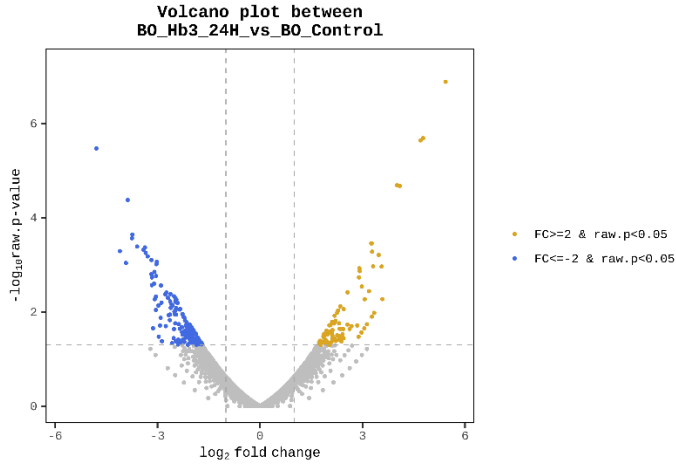
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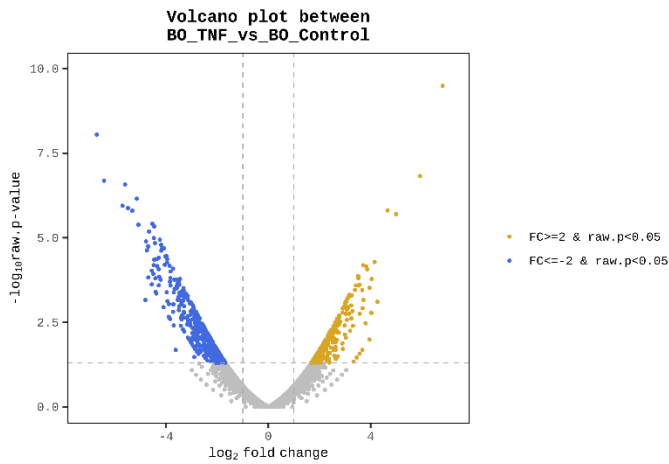
F



G



H

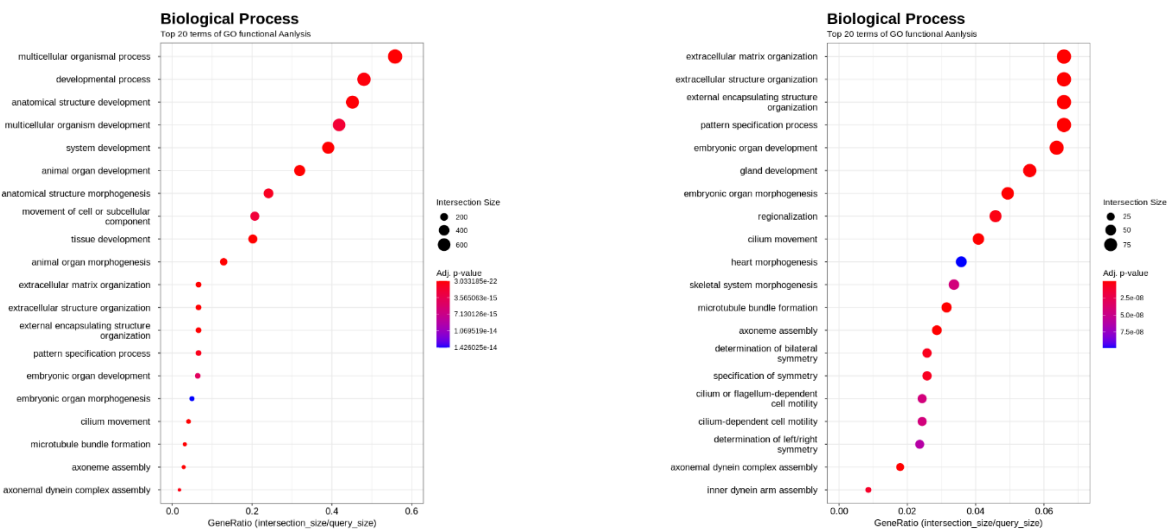


**Figure S9:** Volume plot. Expression volume is the geometric mean of two groups' expression levels. (volume = square-root (Control Normalized value \* Test Normalized value)). To confirm the genes that show higher expression differences compared to the control according to expression volume, a volume plot is drawn. (X-axis: Volume, Y-axis: log2 Fold Change). For example, even though fold change might differ by 2-fold, the genes with higher expression volume may be more credible. ●: Top five genes by volume which satisfy  $|fc| \geq 2$ . The brain organoids transcriptomes analyzed were under several secretome conditions, such as with TFN- $\alpha$  stimulation, which works as a positive control (BO\_TNF), three different wild-type strains, 3D7, Dd2, HB3 of *P. falciparum* parasites induction, at time points of 4h (BO\_3D7\_4H, BO\_Dd2\_4H, BO\_Hb3\_4H) and 24h (BO\_3D7\_24H, BO\_Dd2\_24H,

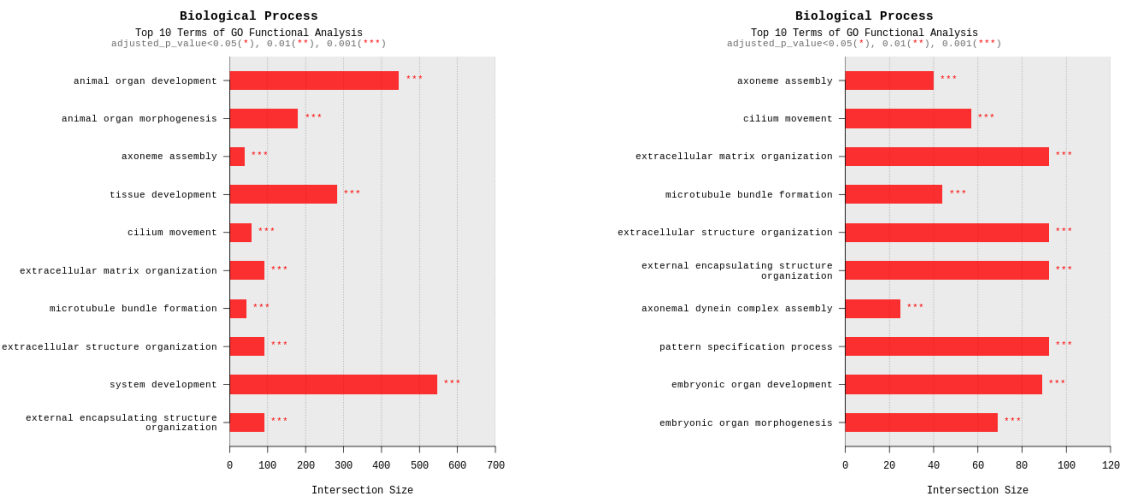


BO\_HB3\_24H), respectively. Brain organoids stimulation with secretome from HBMEC without any stimulation are called modified media (BO\_MM), which means that HBMEC were cultured with half HBMEC culture media and half *P. falciparum* parasites culture media. Brain organoids that grow only with brain organoid media (BO\_control), which works as negative control, were also analyzed. The volume plot between BO and (A) 3D7 4H, (B) Dd2 4H, (C) HB3 4H, (D) MM, (E) 3D7 24H, (F) Dd2 24H, (G) HB3 24H, (H) TNF.

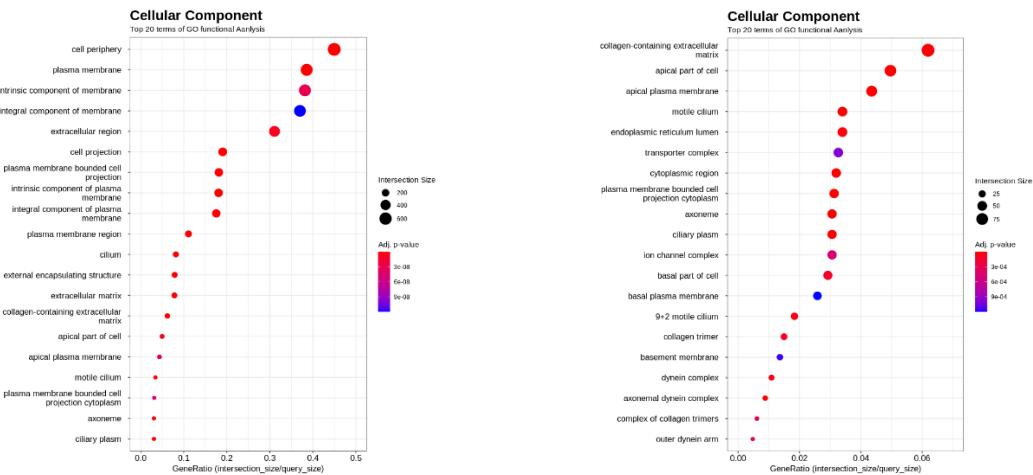
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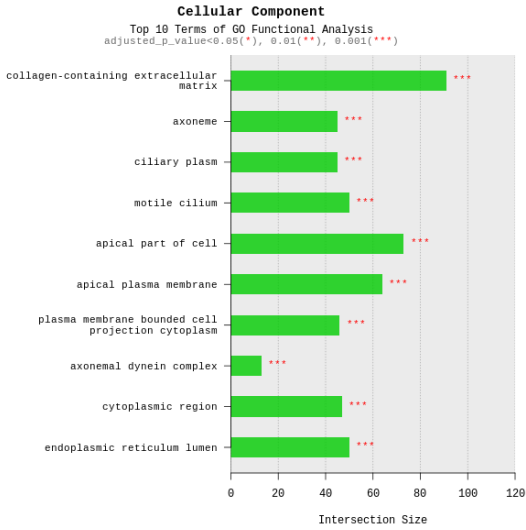
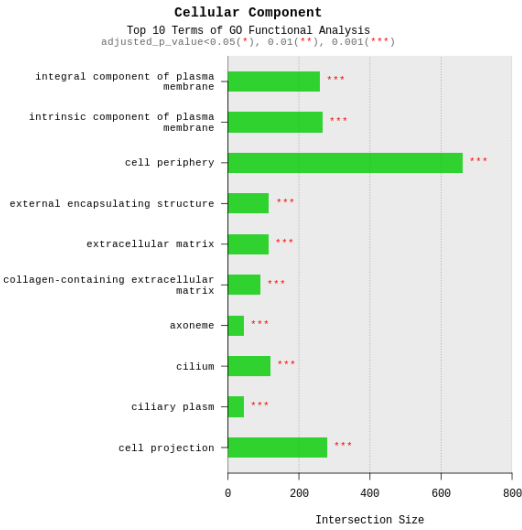
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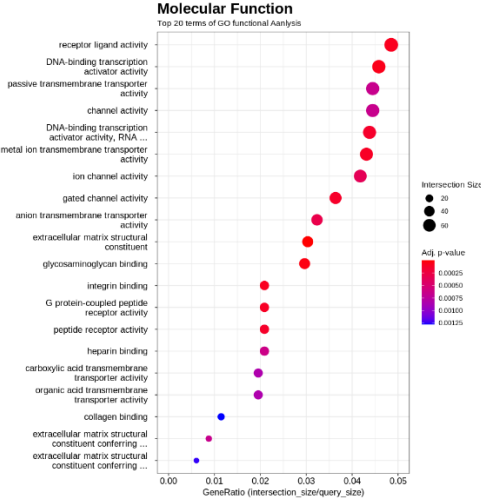
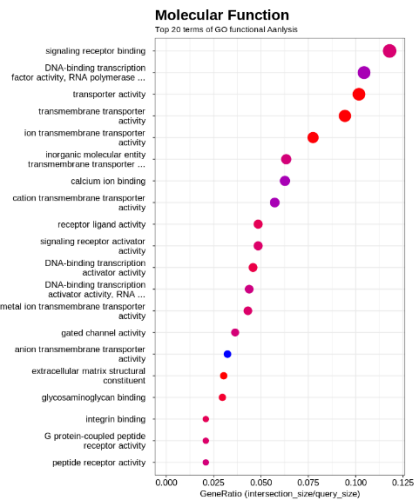
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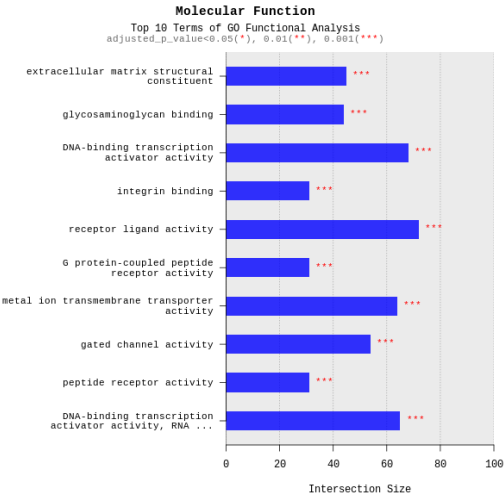
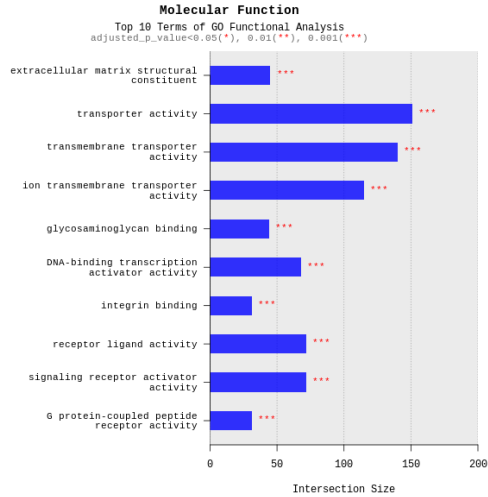
D



E



F



**Figure S10:** Transcriptome data about the biological process (BP), cellular component (CC), and molecular function (MF) of brain organoids within 45 days of post-differentiation. DEG list was further analyzed with gProfiler for gene set enrichment analysis per biological process (BP), cellular component (CC), and molecular function (MF). The Gene Ratio is calculated as the ratio of intersection\_size and query\_size. The presented data is based on a fold-change of 2 and an adjusted *p-value*. (A) Top 20 terms of GO functional analysis of Biological Process. (B) Top 10 terms of GO functional analysis of Biological Process with a *p-value* of <0.05, 0.01, and 0.001. (C) Top 20 terms of GO functional analysis of Cellular component. (D) Top 10 terms of GO functional analysis of Biological Process with a *p-value* of <0.05, 0.01, and 0.001. (E) Top 20 terms of GO functional analysis of Molecular Function (F) Top 10 terms of GO functional analysis of Biological Process with a *p-value* of <0.05, 0.01, and 0.001.