

**A three-dimensional vessel-on-chip model to study Puumala orthohantavirus pathogenesis**

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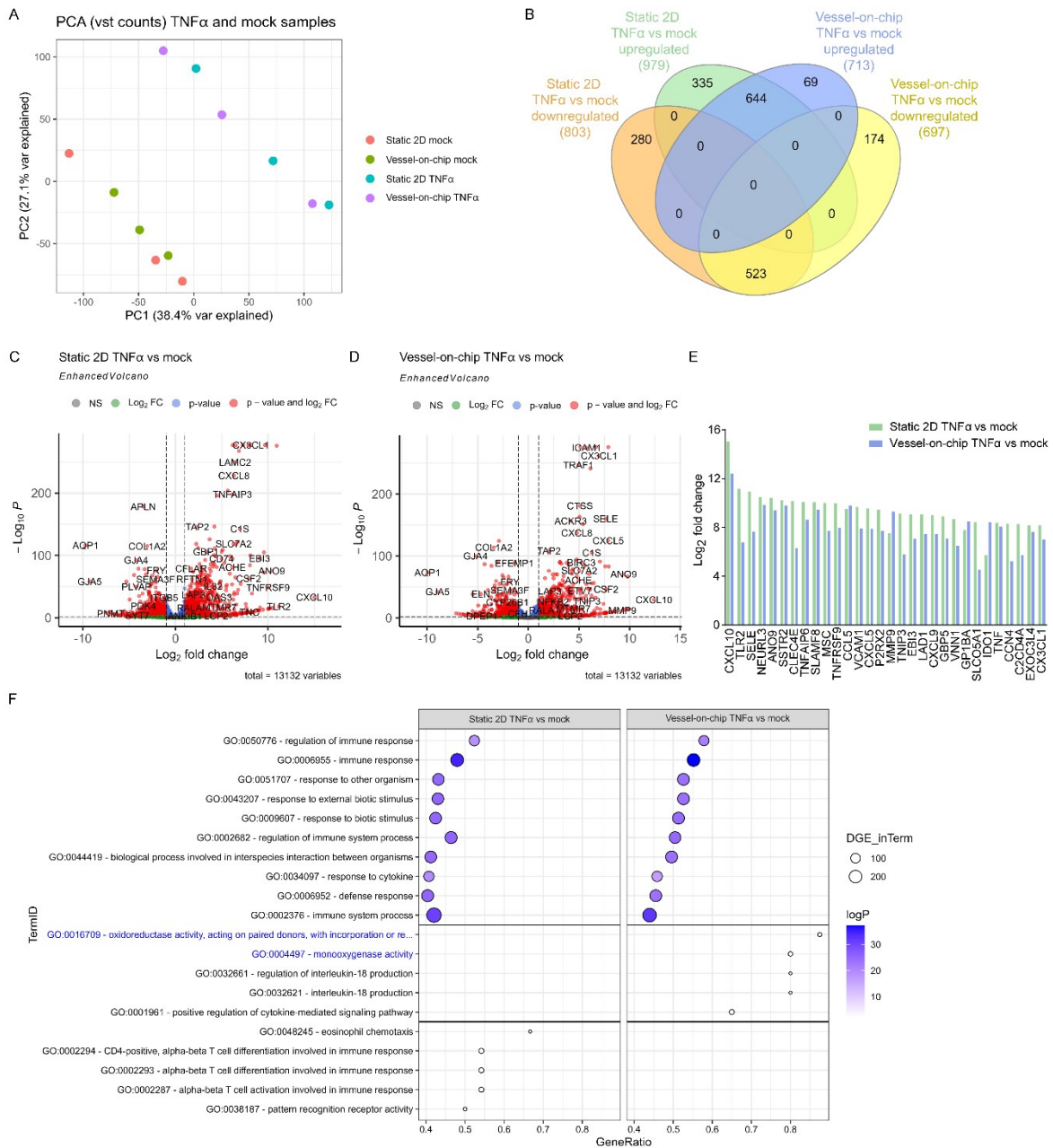
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**The supplementary information includes:**

Video S1  
Fig. S1

**Video S1 Three-dimensional representation of a perfused vessel-on-chip.** Representative three-dimensional (3D) render of a vessel-on-chip, demonstrating the 3D architecture tube-like structures of an EC monolayer. Nuclei are expressed in grey and VE-cadherin in red. Z-stack images were obtained from confocal microscopy and rendered with Dragonfly software.



**Fig. S1 RNA-Seq analyses of ECs cultured in static 2D and vessel-on-chip models upon TNF $\alpha$  treatment. (A)** Principal component analysis (PCA) plot based on vst normalized counts of mock- and 24 hours 100 ng/ml tumor necrosis factor alpha (TNF $\alpha$ )-treated samples. **(B)** Venn diagram comparing significantly upregulated and downregulated genes in response to TNF $\alpha$  treatment in static 2D and vessel-on-chip EC cultures. For Volcano plots, grey dots represent genes which expressions neither significantly altered nor exceeded the fold change cut-off. Blue dots represent significantly altered genes that did not exceed the fold change cut-off. Green dots represent genes which expressions were not significantly altered, but exceeded the fold change cut-off. Red dots represent genes with significantly altered expressions that also exceeded the fold change cut-off. **(C)** Volcano plot demonstrating significantly upregulated and downregulated gene expressions following TNF $\alpha$ -treatment compared to mock-treated cells in static 2D-cultured ECs. **(D)** Volcano plot demonstrating significantly upregulated and downregulated gene expressions following TNF $\alpha$ -treatment compared to mock-treated cells in vessel-on-chip-cultured ECs. **(E)** Bar charts demonstrating top 25 highest fold changes of common significantly upregulated genes due to TNF $\alpha$  treatment, ranked on the fold change in static 2D cultures. **(F)** TNF $\alpha$ -induced transcriptomic gene expression profile overview for ECs cultured in static 2D or vessels-on-chip. Balloon plot displaying significantly (Benjamini-Hochberg adjusted P-value < 0.05) enriched GO-terms upon TNF $\alpha$  treatment. Size of the balloon represents the number of differentially expressed genes within a GO-term (DGE\_inTerm) while the color of the balloon represents the negative base-10 logarithm of the P-value. GeneRatio on the x-axis is the number of differentially expressed genes divided by the total number of genes involved in the GO-term. Significant GO-terms were ranked on highest GeneRatio, displaying the top 10 common pathways, and top 5 for unique pathways for vessel-on-chip and static 2D cultures. Blue font indicates that the enrichment of the GO-term was mainly based on downregulated genes. For illustrative purposes, GO-terms were cut-off to 90 characters, complete term: GO:0016709 – oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen, NAD(P)H as one donor, and incorporation of one atom of oxygen.