Comparative transcriptomics between species attributes reactogenicity pathways induced by the capsular group B meningococcal vaccine, 4CMenB, to the membrane-bound endotoxin of its outer membrane vesicle component

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- **Cytokine signal transducer and prostaglandin synthesising enzyme genes are significantly upregulated on brain tissue after 4CMenB and OMV immunisation**

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(n = 6 per group)

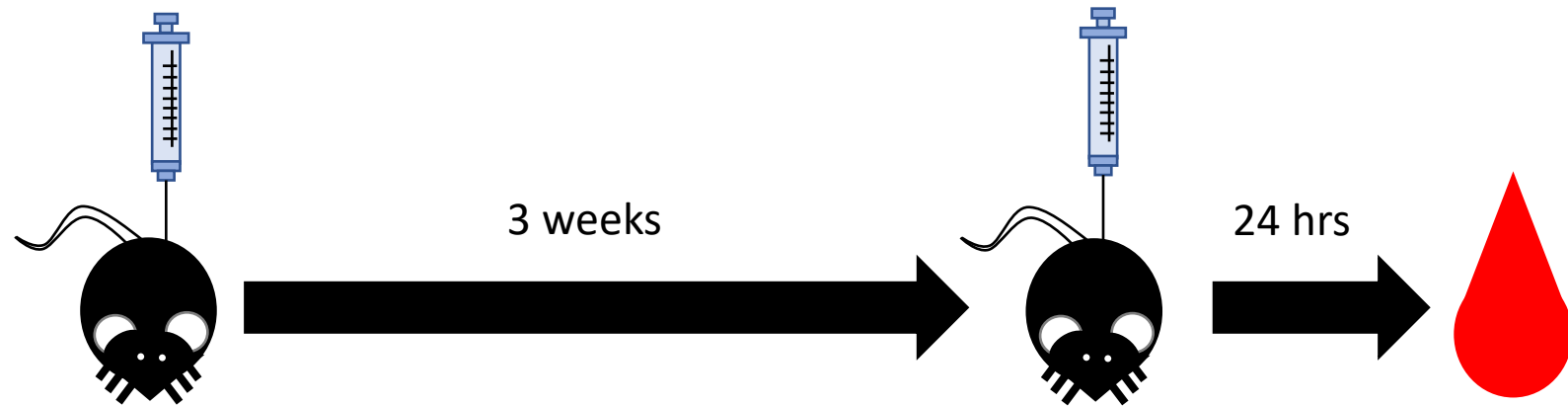4CMenB + routine

4CMenB + DTaP-
IPV-Hib + PCV13
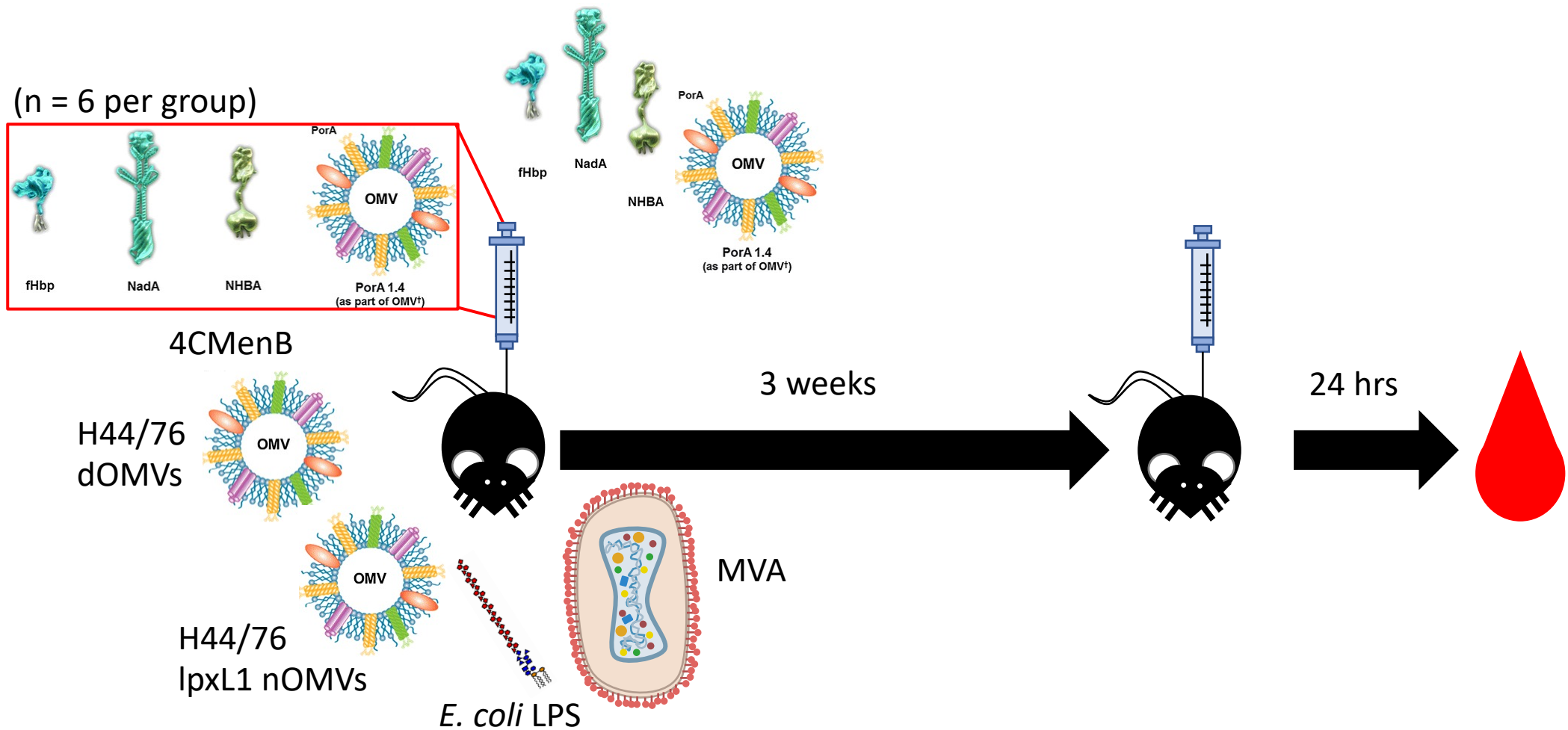
Routine only

DTaP-IPV-Hib +
PCV13 + PBS

4CMenB only

4CMenB + PBS +
PBS

PBS control

PBS + PBS + PBS

3 weeks

24 hrs

Supplementary Fig. 1. Routine versus concomitant immunisations. Schematic of mouse immunisation strategy for concomitant 4CMenB vs. routinely-administered vaccines experiment.

Supplementary Fig. 2. 4CMenB individual components immunisations. Schematic of mouse immunisation strategy 4CMenB, individual component, and comparator vaccine experiment. 4CMenB component figures taken from O'Ryan *et al.*, 2014.
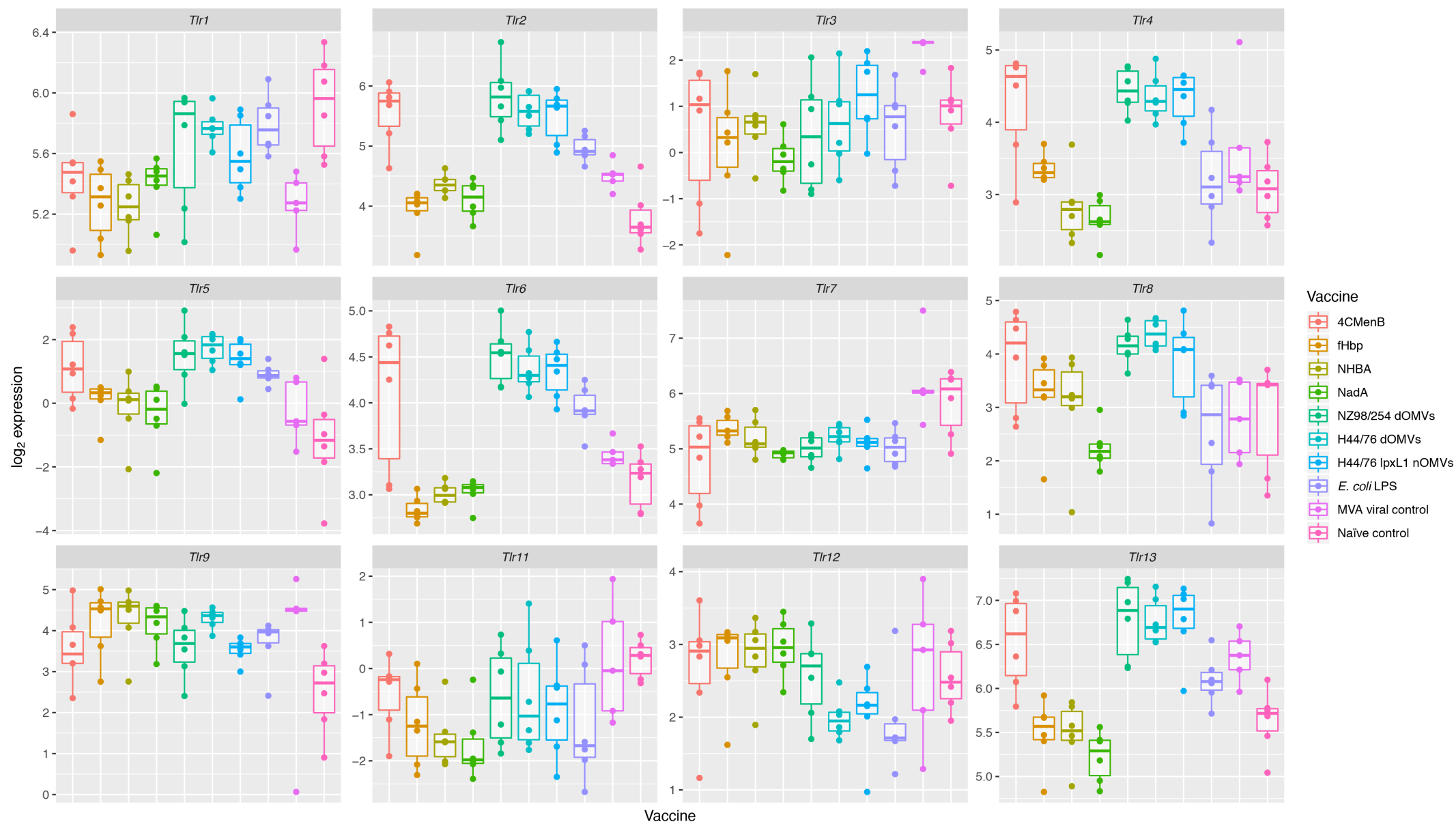
Supplementary Fig. 3. Toll like receptor genes. Log$_2$ normalised expression values for the mouse toll like receptor genes.
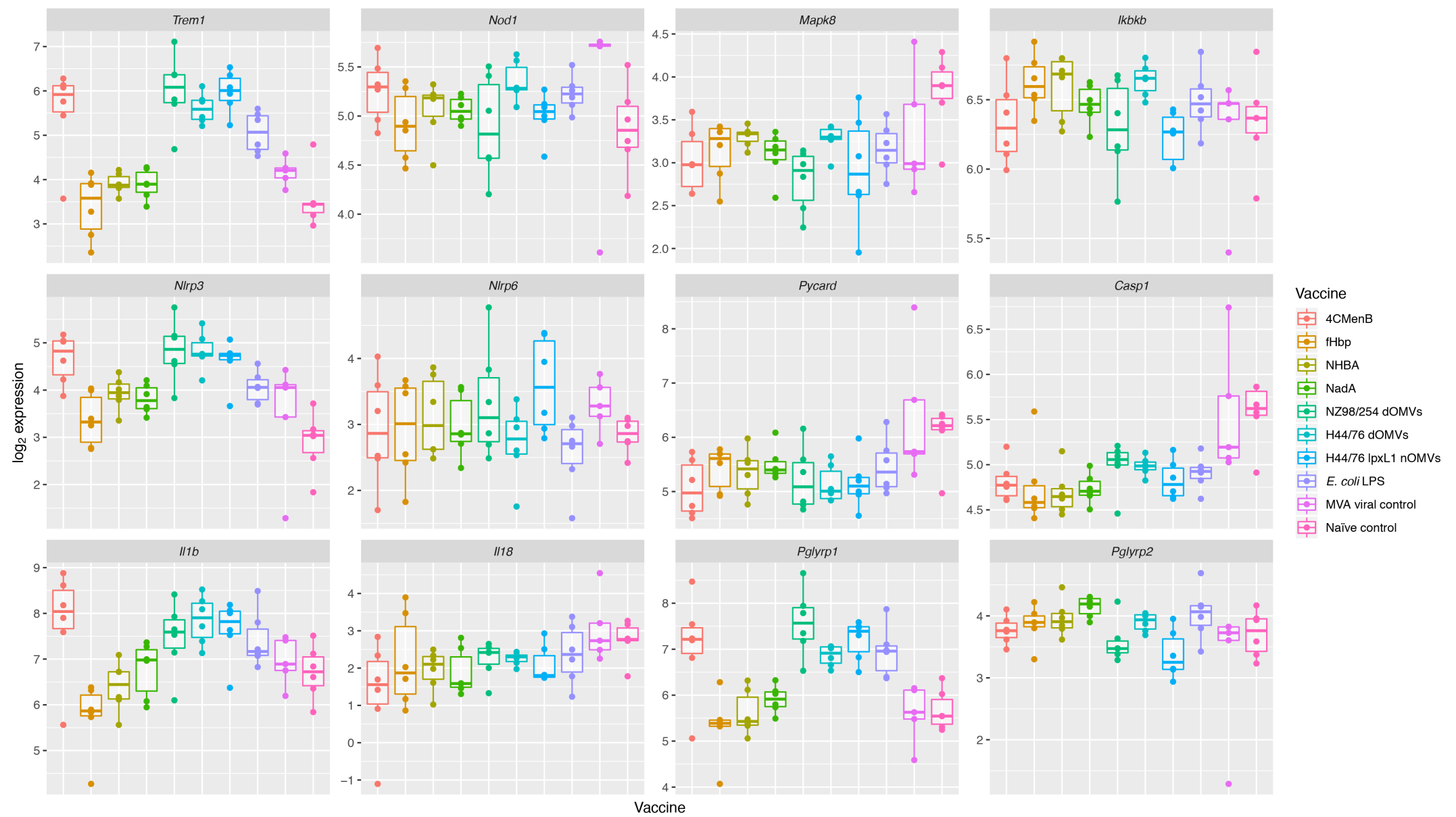
Supplementary Fig. 4. Signalling molecule genes. Log$_2$ normalised expression values for accessory and downstream signalling molecule genes.
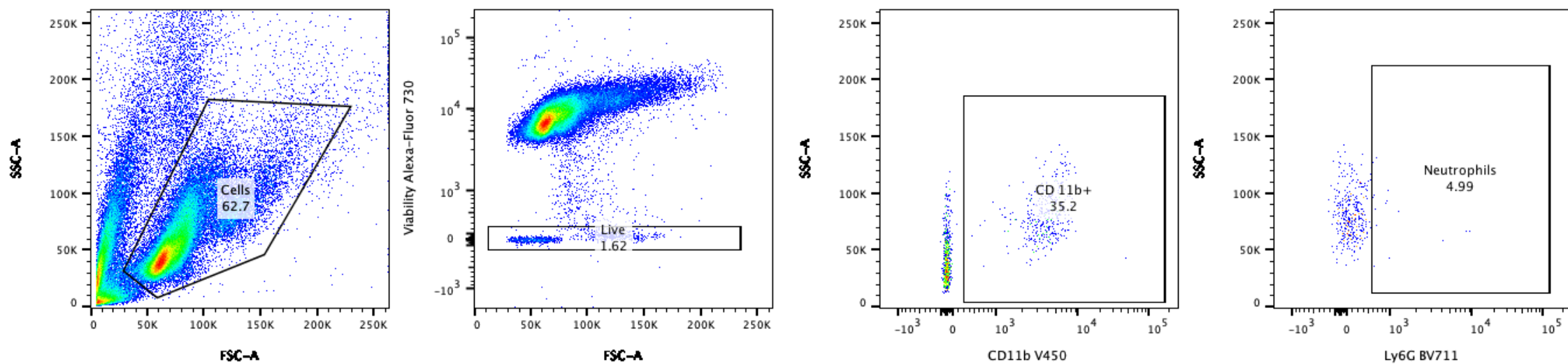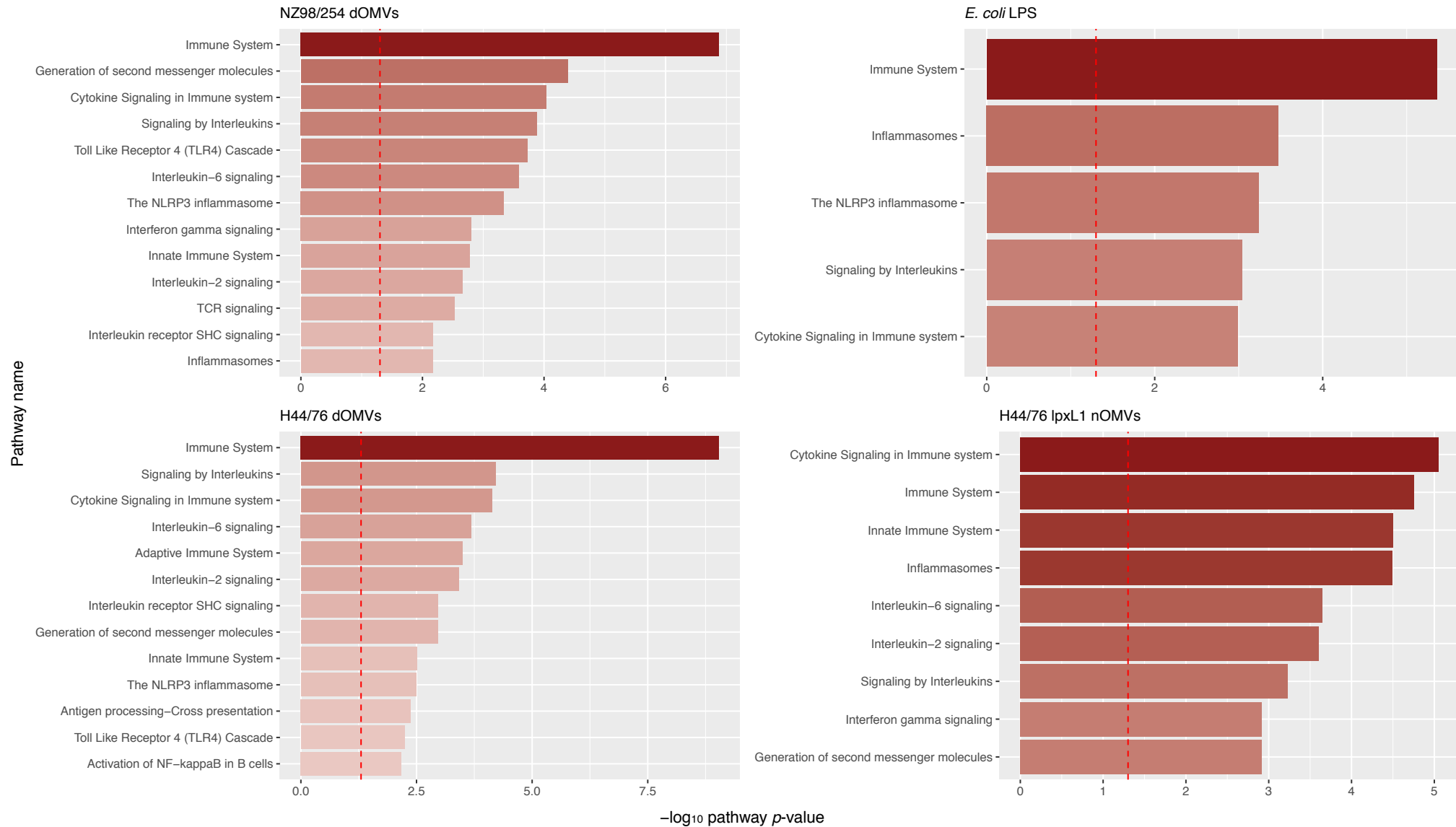
Supplementary Fig. 5. Gating strategy for neutrophil isolation. Neutrophils were identified as Ly6C⁻Cd11b⁺Ly6G⁺ live cells in whole blood.
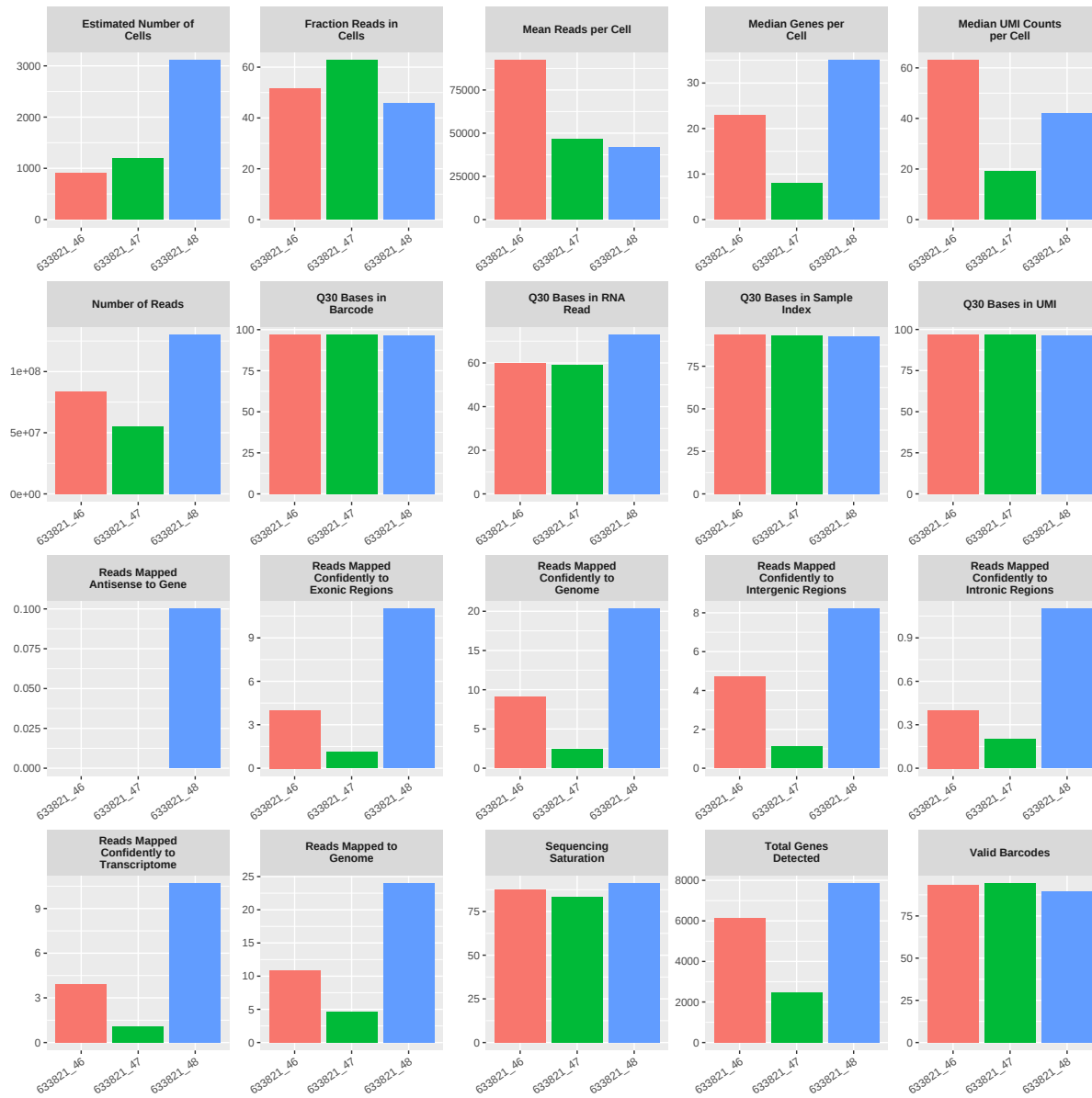
Supplementary Fig. 6. Quality control metrics of neutrophil 10x data. 633821_46 corresponds to the routine only group, 633821_47 to the PBS control group, and 633821_48 to the 4CMenB + routine group.

Supplementary Fig. 7. Reactome pathway over-representation analysis. Significantly enriched immunological pathway categories from the publicly available Reactome database. The NZ98/254 detergent-extracted (d)outer membrane vesicle (OMV, top left), *Escherichia coli* (*E.* coli) lipopolysaccharide (LPS, top right), H44/76 dOMV (bottom right), H44/76 lpxL1 native (n)OMVs (bottom right) groups are presented here. The dashed red line corresponds to a negative $\log_{10}$ pathway adjusted *p*-value of 0.05.
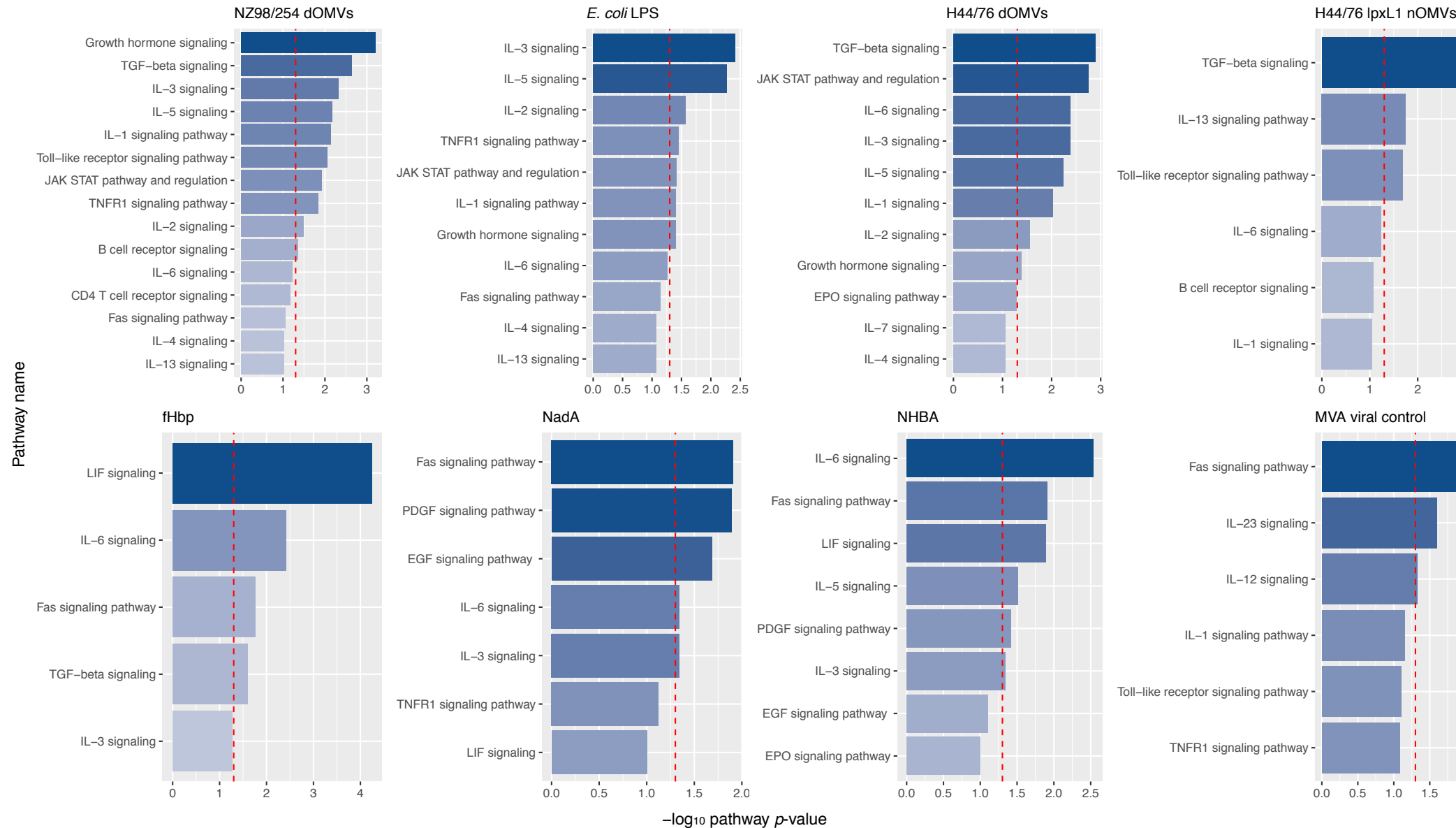
**Supplementary Fig. 8. INOH pathway over-representation analysis.** Significantly differentially expressed genes (FDR < 0.05) determined for each group comparison were assessed for enrichment of genes pertaining to cell signalling pathway categories from the publicly available integrating network objects with hierarchies (INOH) database. The dashed red line corresponds to a negative $\log_{10}$ pathway adjusted *p*-value of 0.05.
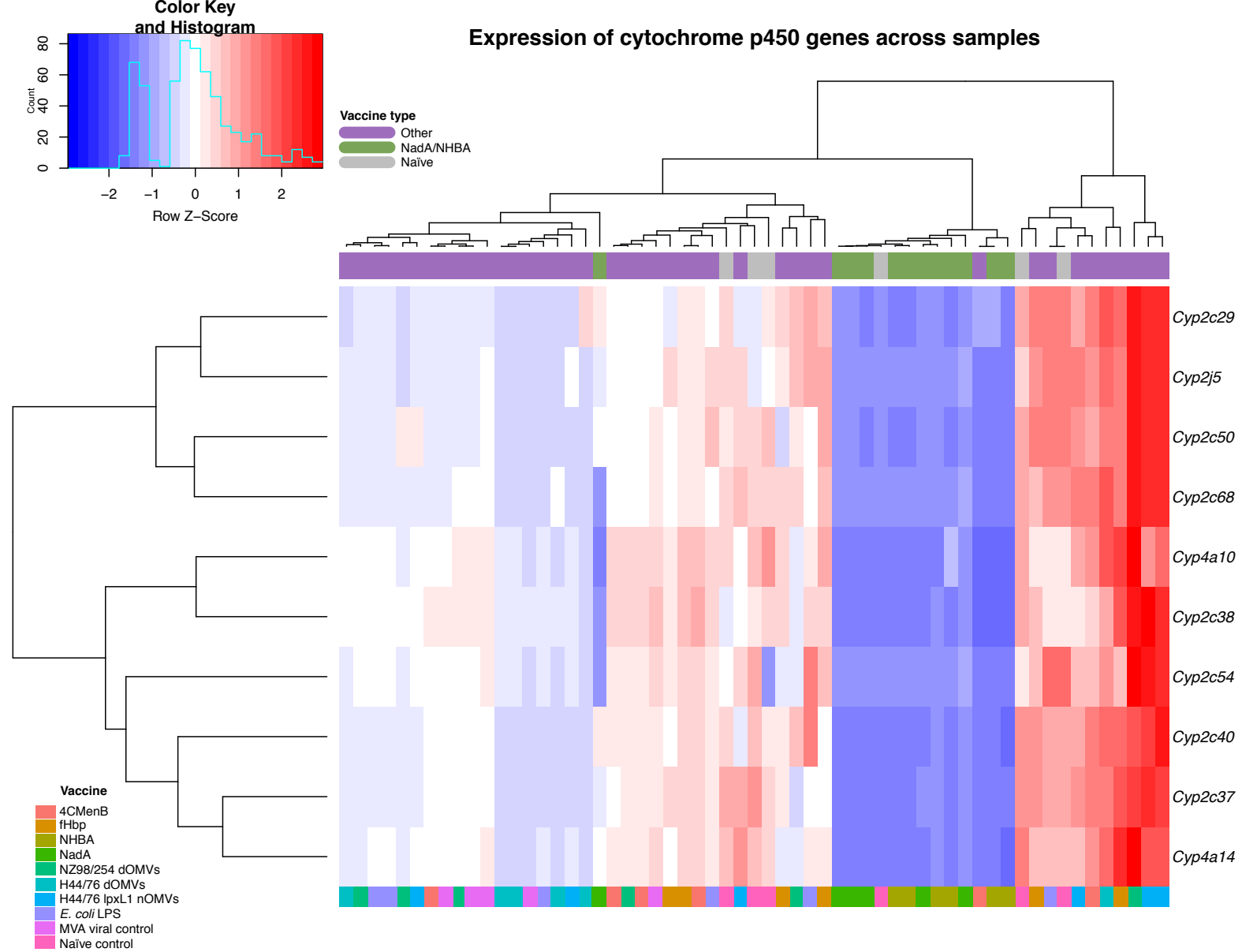
Supplementary Fig. 9. Hierarchical clustering of cytochrome P450 genes. Clustering across all samples of cytochrome P450 family of genes, a pathway identified as significantly downregulated in the NHBA and NadA groups.
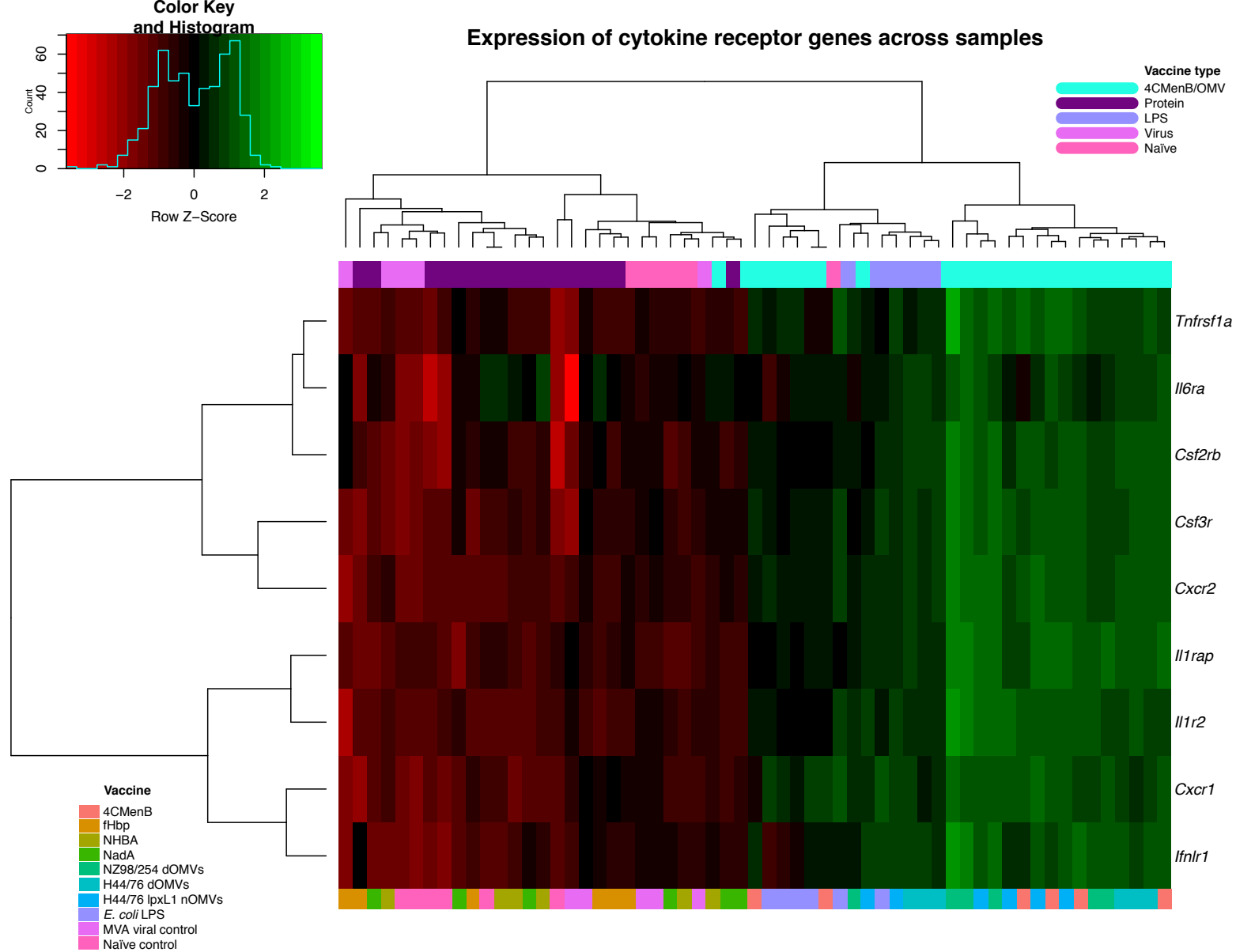
Supplementary Fig. 10. Hierarchical clustering of cytokine/chemokine receptors. Clustering across all samples of cytokine/chemokine receptor genes identified among the top significantly differentially expressed genes in the 4CMenB, OMV, and LPS groups.
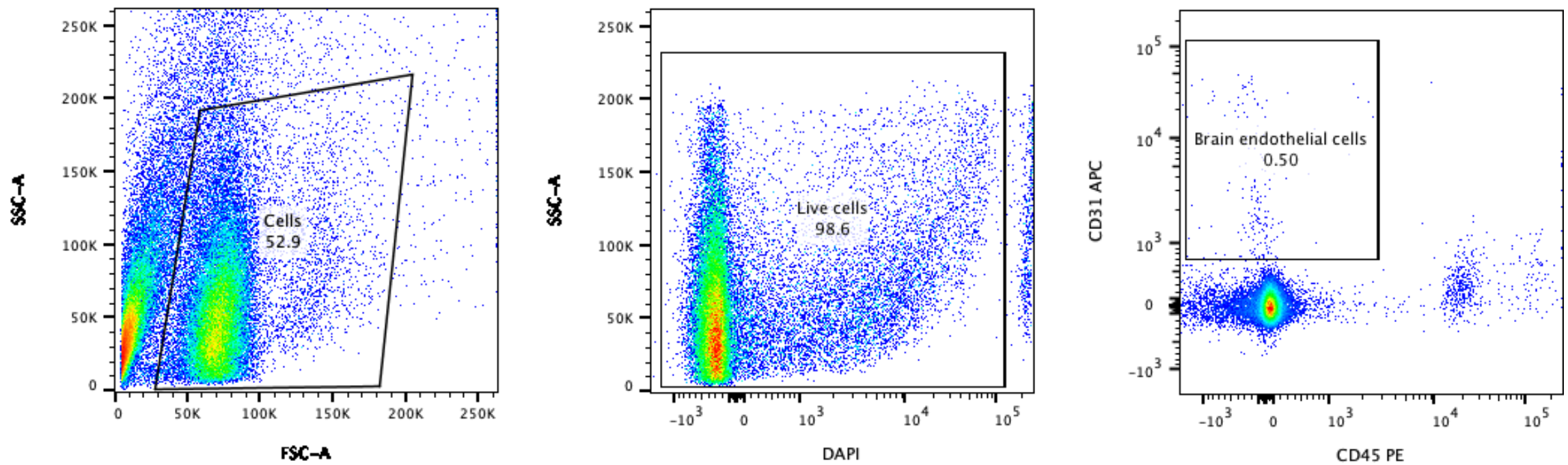
Supplementary Fig. 11. Gating strategy for brain endothelial cell isolation. Brain endothelial cells were identified as CD31+CD45- live cells.

Target gene	Primer
Il1r1	Mm00434237_m1
Il1rap	Mm00492638_m1
Il6ra	Mm00439653_m1
Tnfrsf1a	Mm00441883_g1
Tnfrsf1b	Mm00441889_m1
Ptges	Mm00452105_m1
Ptgs2	Mm00478374_m1
Pgk1	Mm01225301_m1

Supplementary Table 1. Primers used for real-time quantitative PCR of brain endothelial cells.

A   Preamplification parameters

Step	Temperature (°C)	Time	Repeat
Enzyme activation	95	10 m	-
Denaturation	95	15 s	14 times
Anneal/extend	60	4 m	
Enzyme inactivation	99	10 m	-
	4		Hold

B   RT-qPCR parameters

Step	Temperature (°C)	Time	Repeat
UNG incubation	50	2 m	-
Enzyme activation	95	10 m	-
Denaturation	95	1 s	40 times
Anneal/extend	60	20 s	

Supplementary Table 2. Thermocycling parameters. A. Parameters for preamplification of cDNA. **B.** Parameters for StepOnePlus™ real-time quantitative PCR.