

Transcriptome and proteome analysis of innate immune responses to inactivated *Leptospira* and bivalent *Leptospira* vaccines in canine 030-D cells

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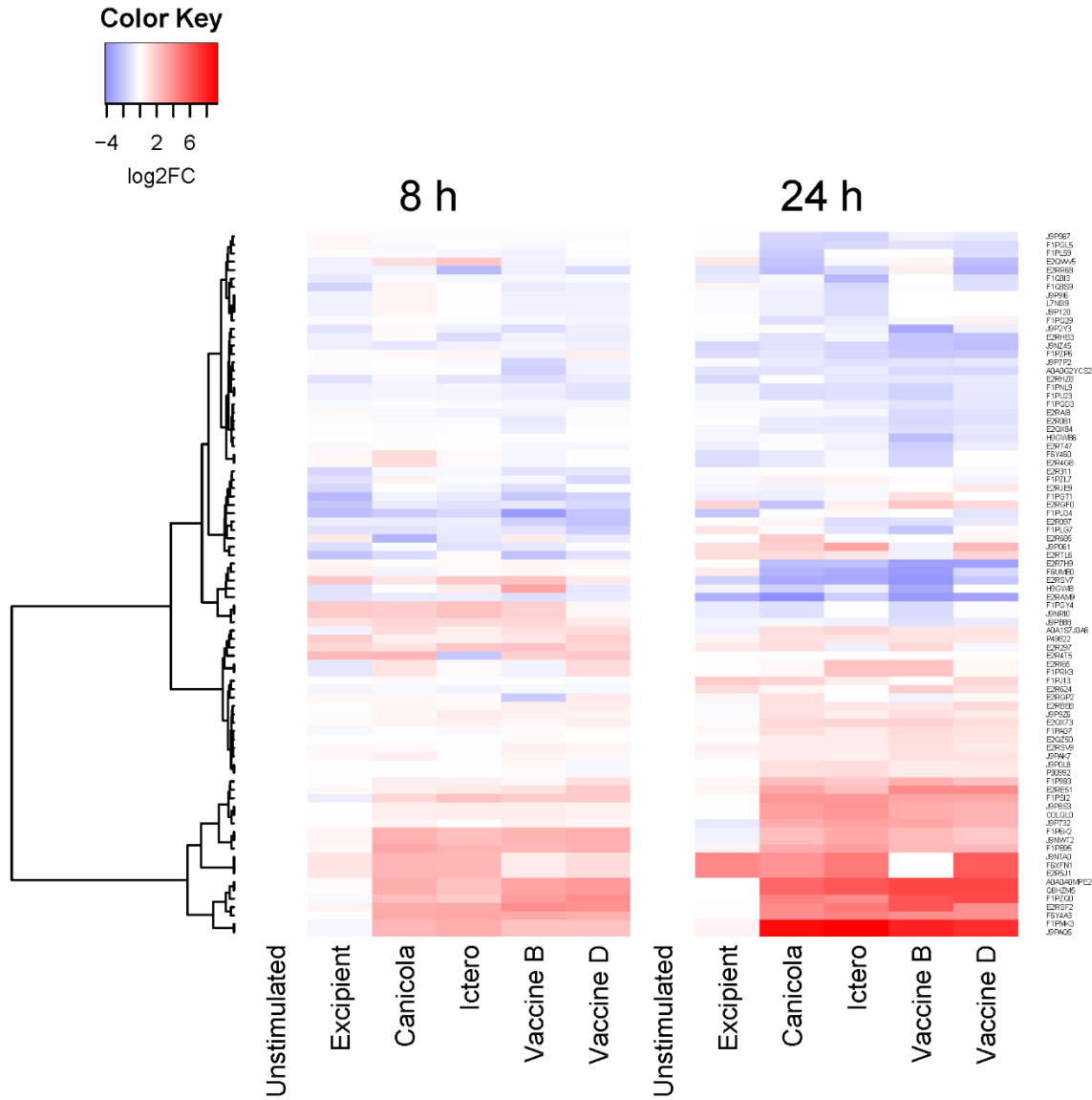


Figure S1. Heatmap of quantitative DEPs identified in 030-D cells. Shown are 36 quantitative DEPs found at 8 h together with 55 quantitative DEPs that were found at 24 h stimulation with the inactivated *L. interrogans* serogroup Canicola or Icterohaemorrhagiae, a bivalent *Leptospira* vaccine from manufacturer B (vaccine B), a bivalent *Leptospira* vaccine from manufacturer D (vaccine D), or vaccine excipient (84 quantitative DEPs in total). Proteins were considered quantitative DEPs if found in at least two of the triplicates, showed at least 2-fold change in expression ($FC \geq 2$) and reached statistical significance ($P < 0.01$). The color key represents the changes (log₂ scale), from most downregulated (dark blue) to most upregulated genes (red). Each row is a UniProt ID, and each column is a stimulus (average of n=3 replicates).

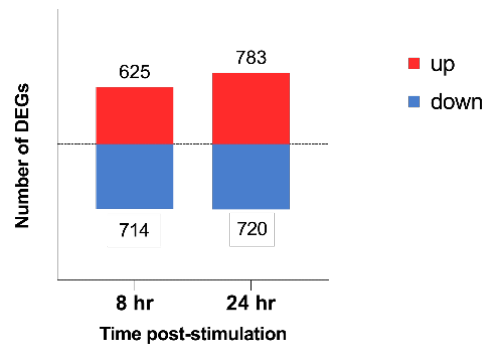
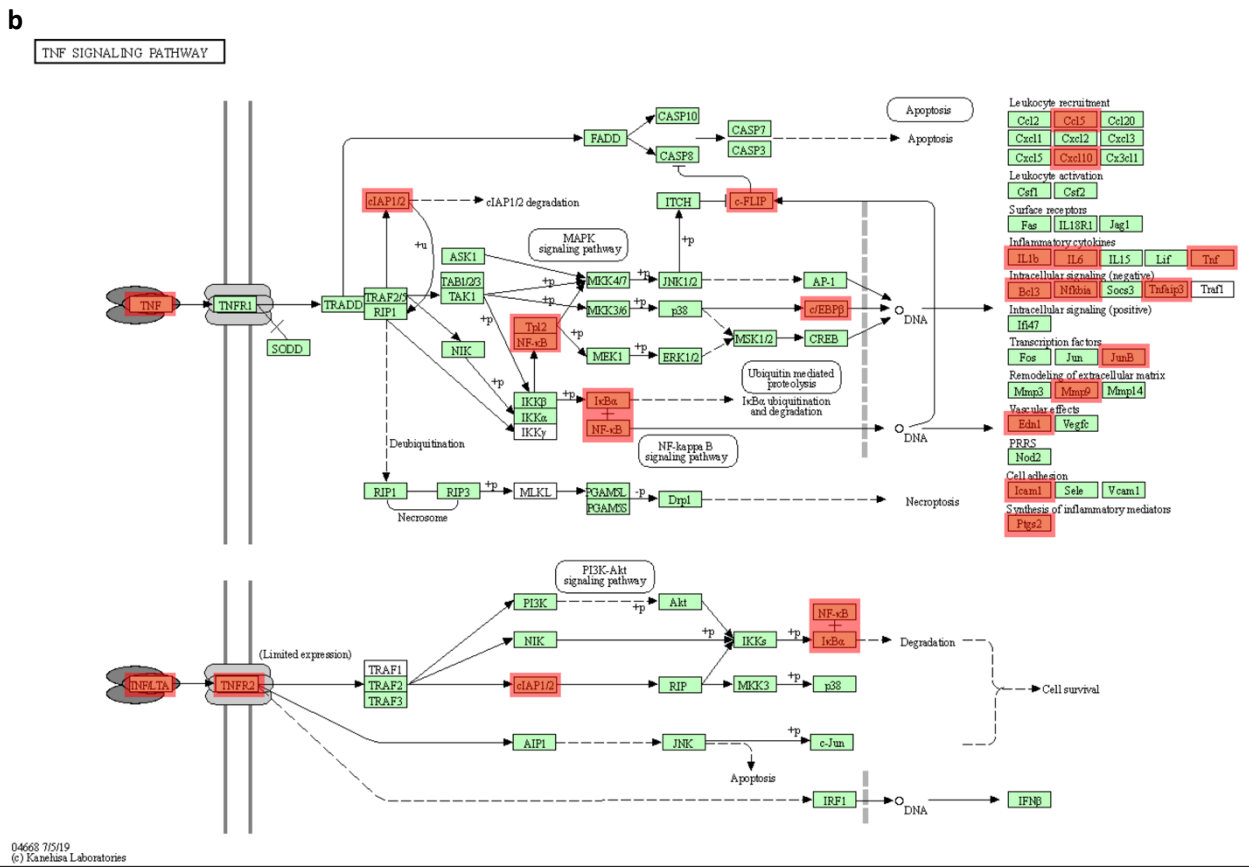
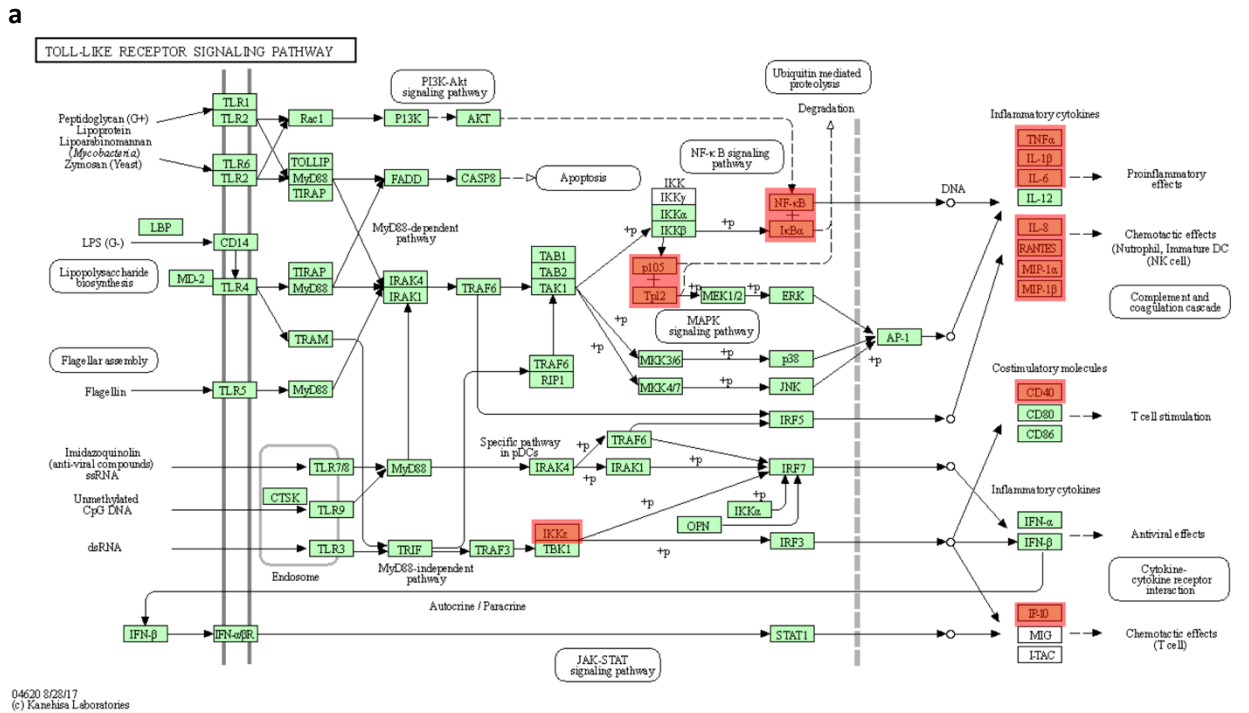
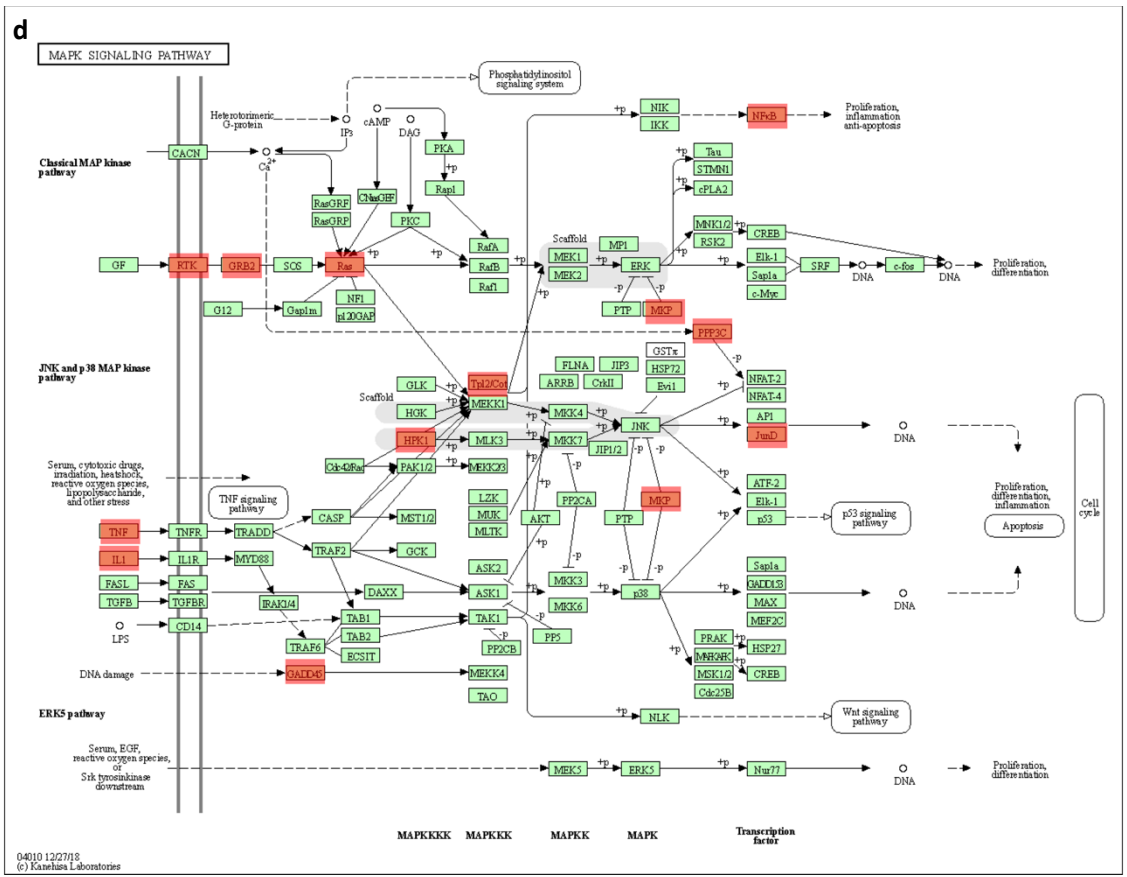
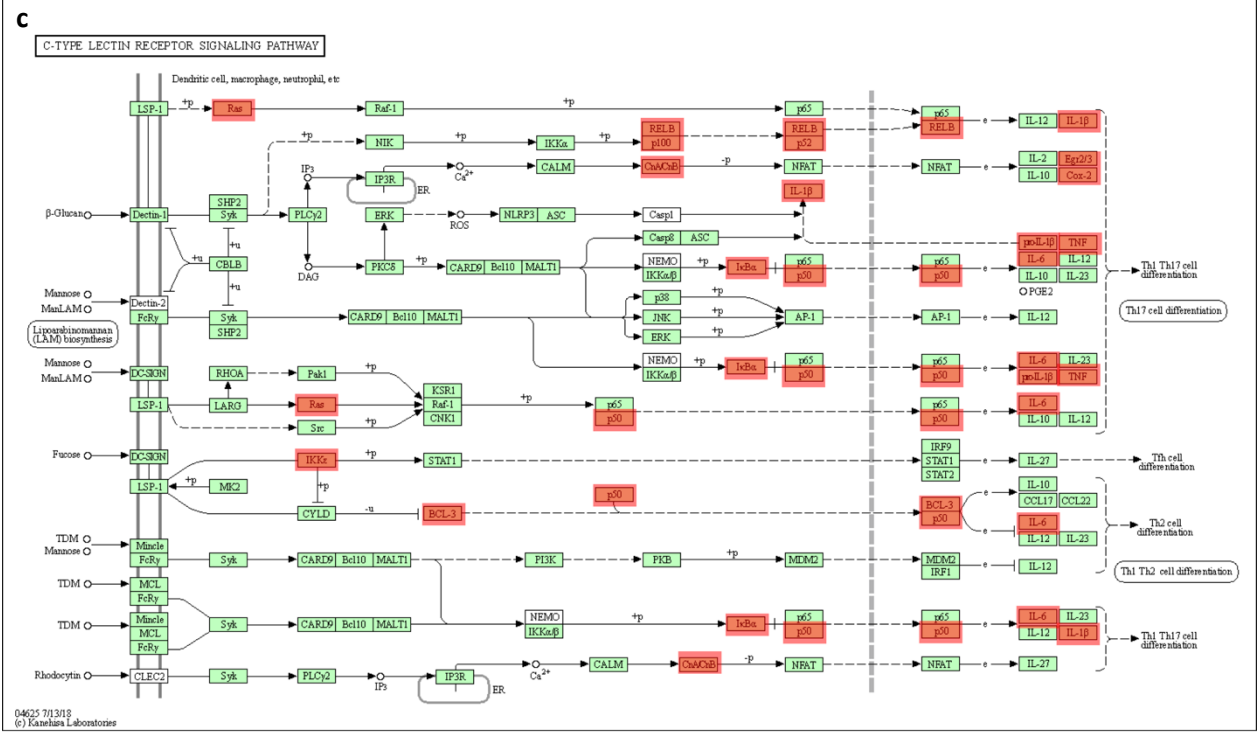


Figure S2. DEGs ($FC \geq 2$; $P < 0.001$, FDR 5%) in canine 030-D cells stimulated with bivalent vaccine B and D. The number (y-axis) and directionality (upregulated=positive y-space, downregulated=negative y-space) of DEGs after 8 and 24 hr stimulation with bivalent *Leptospira* vaccines from different manufacturers (coded B and D).





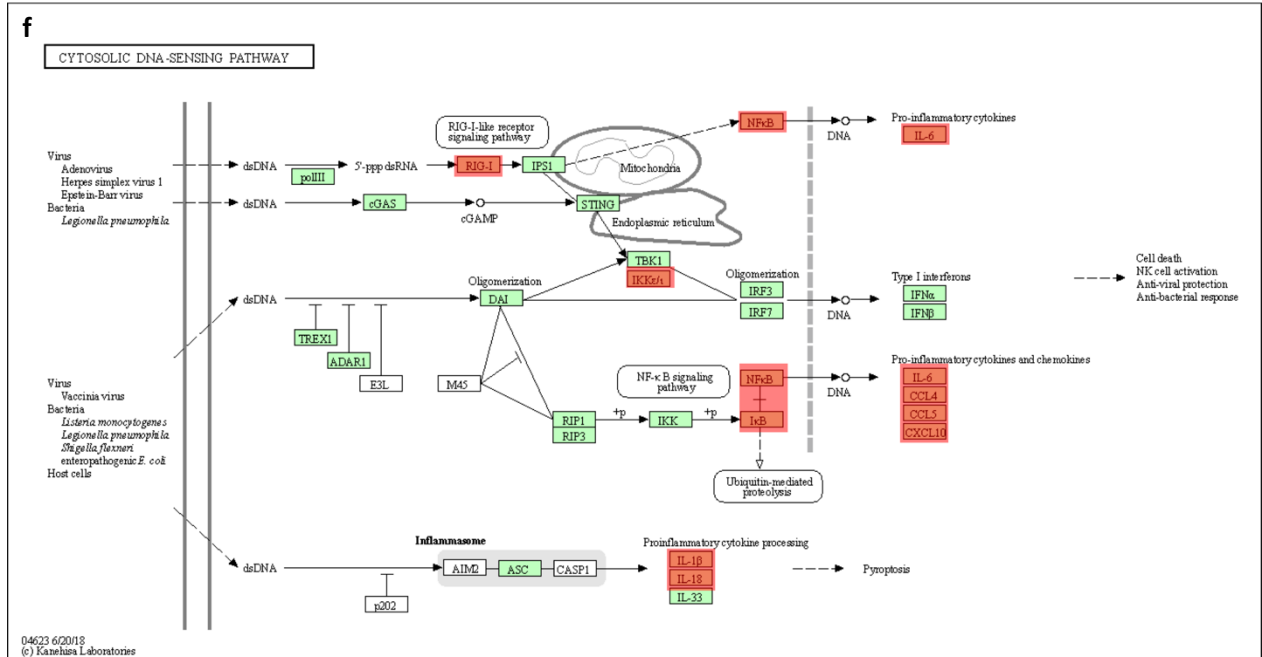
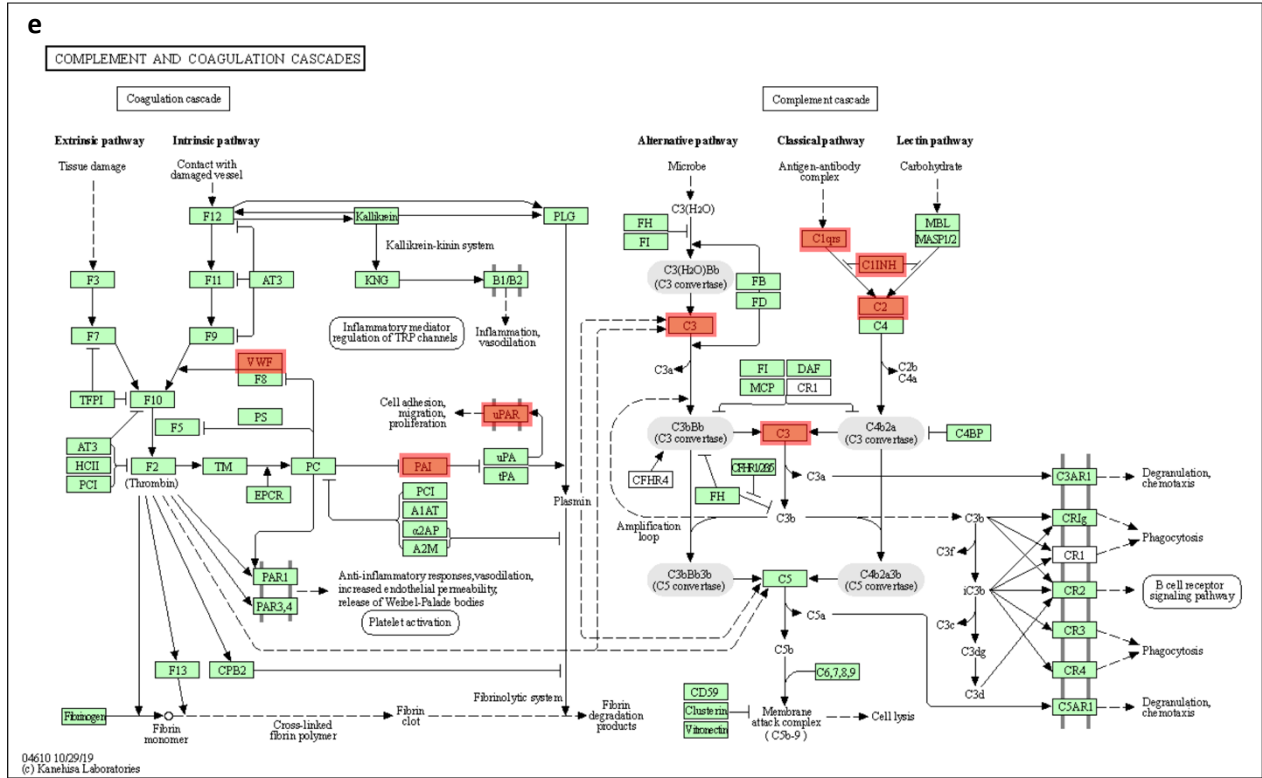


Figure S3. Upregulated pathways in canine O30-D cells stimulated with the inactivated *L. interrogans* serogroup Canicola or Icterohaemorrhagiae, or bivalent *Leptospira* vaccine B. Shown are a) Toll-like receptor b) TNF c) C-type lectin receptor and d) MAPK signaling pathways, e) complement and coagulation cascades, and f) cytosolic DNA sensing pathway. Upregulated DEGs (FC ≥ 2; P < 0.001, FDR 5%) found in stimulated cells at 8 h are highlighted in red. cfa04620 (a), cfa04668 (b), cfa04625 (c), cfa04010 (d), cfa04610 (e), cfa04623 (f) pathway maps were obtained with permission from <https://www.kegg.jp/kegg/kegg1.html>.

Table S1. Transcriptomic immune profile of 030-D cells

Cell type	Average%	SD%
Monocytes NC+I	6.74	2.06
Monocytes C	0.26	0.56
pDCs	0.25	0.03
mDCs	-0.01	0.03
Neutrophils LD	0.67	0.48
Basophils LD	0.07	0.01
NK	-0.30	0.13
T CD4 Naive	3.82	0.59
T CD4 Memory	-1.63	0.36
T CD8 Naive	-1.81	0.42
T CD8 Memory	0.78	0.20
MAIT	-0.15	0.06
T gd Vd2	0.52	0.18
T gd non-Vd2	0.04	0.01
B Naive	-0.02	0.05
B Memory	0.00	0.05
Plasmablasts	0.15	0.02

Gene expression profiles of the 030-D gene expression dataset were deconvoluted to those of 17 human cell types found in PBMC using the ABIS RNA-seq tool described by Monaco *et al.*³⁵. The values represent the average gene expression score and standard deviation (SD) for the 39 samples analyzed in this study. NC, non-classical; I, intermediate; C, classical, pDC, plasmacytoid dendritic cells; mDC, myeloid dendritic cells; LD, low-density.

Table S4. The concentration of S100A12 protein in the supernatant of 030-D cells stimulated for 24 h with whole inactivated *L. interrogans* serogroup Canicola, Icterohaemorrhagiae, bivalent *Leptospira* vaccine B, its excipient, or bivalent *Leptospira* vaccine D.

Condition	Absorbance
Unstimulated 0 h	<0.115
Unstimulated 24 h	>2.19
Excipient	>2.19
Canicola	>2.19
Ictero	>2.19
Vaccine B	>2.19
Vaccine D	>2.19

The supernatants were assayed for the presence of S100A12. Stimulations were performed in triplicate. Three independent experiments were performed with similar outcome. A representative experiment is shown. Values of 0.115 and 2.19 were the means of the lowest and the highest absorbance measured in the S100A12 standard curve, respectively. The S100A12 values in all samples were above the highest concentration of the standard curve except the unstimulated 0 h.