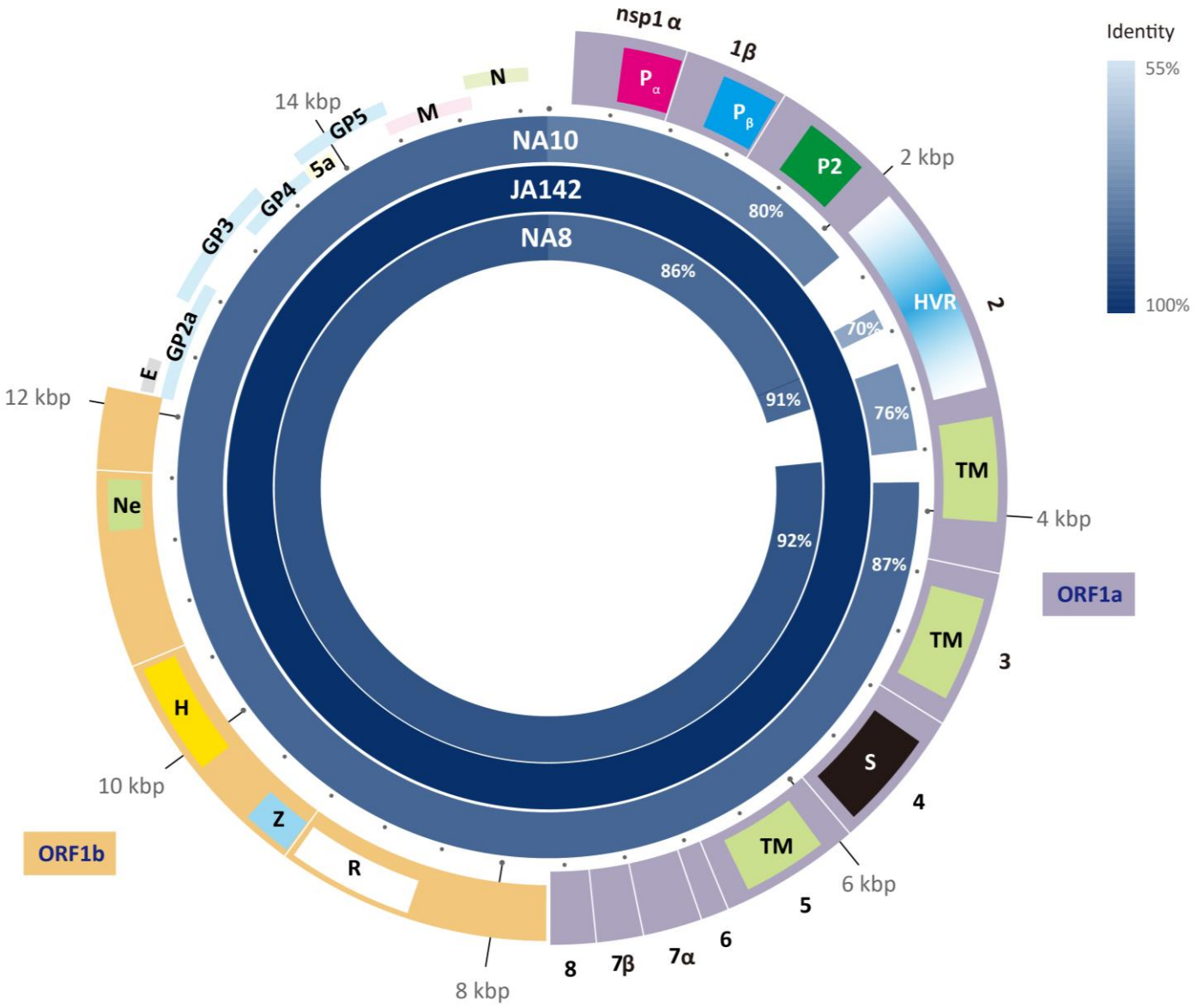
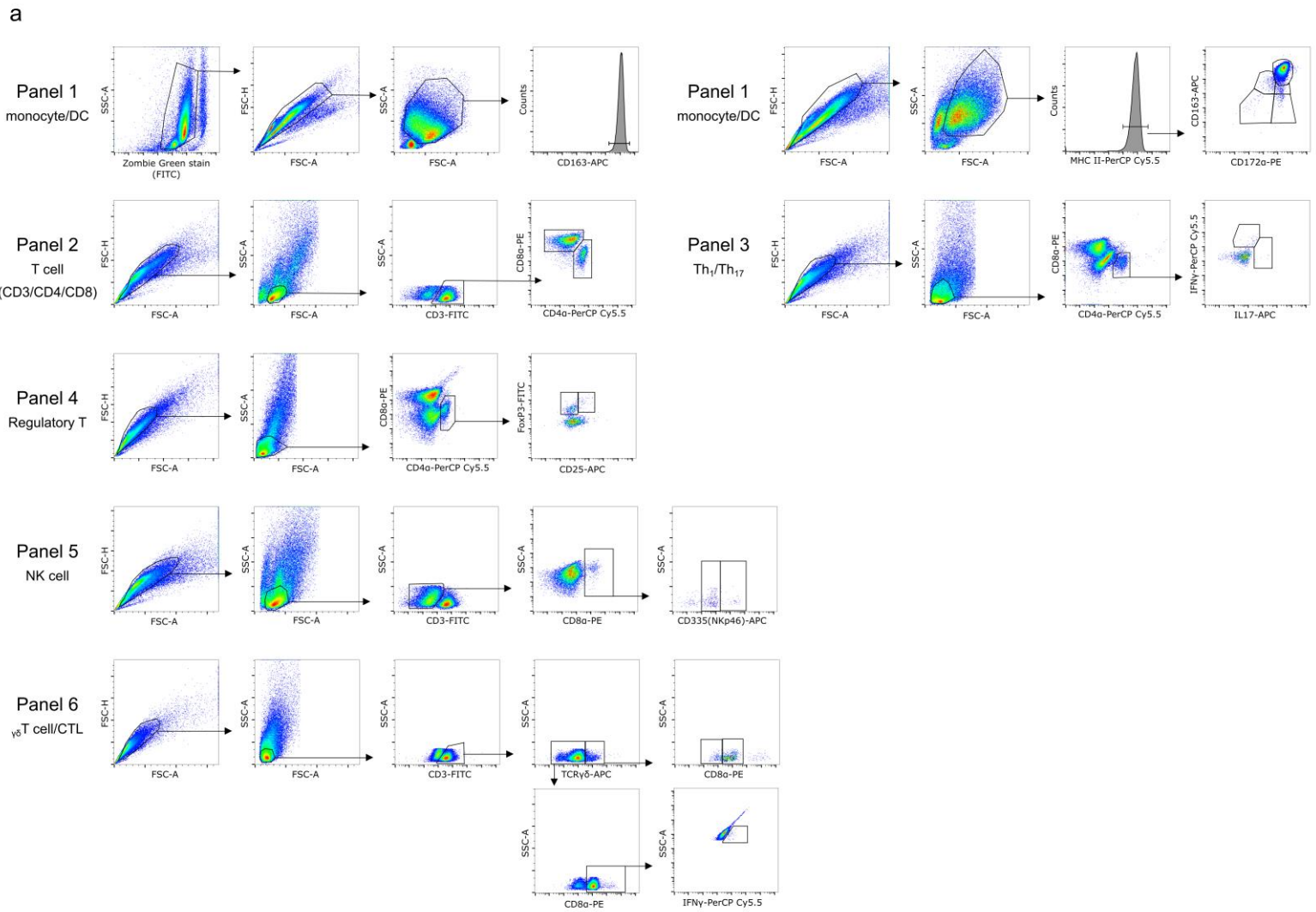
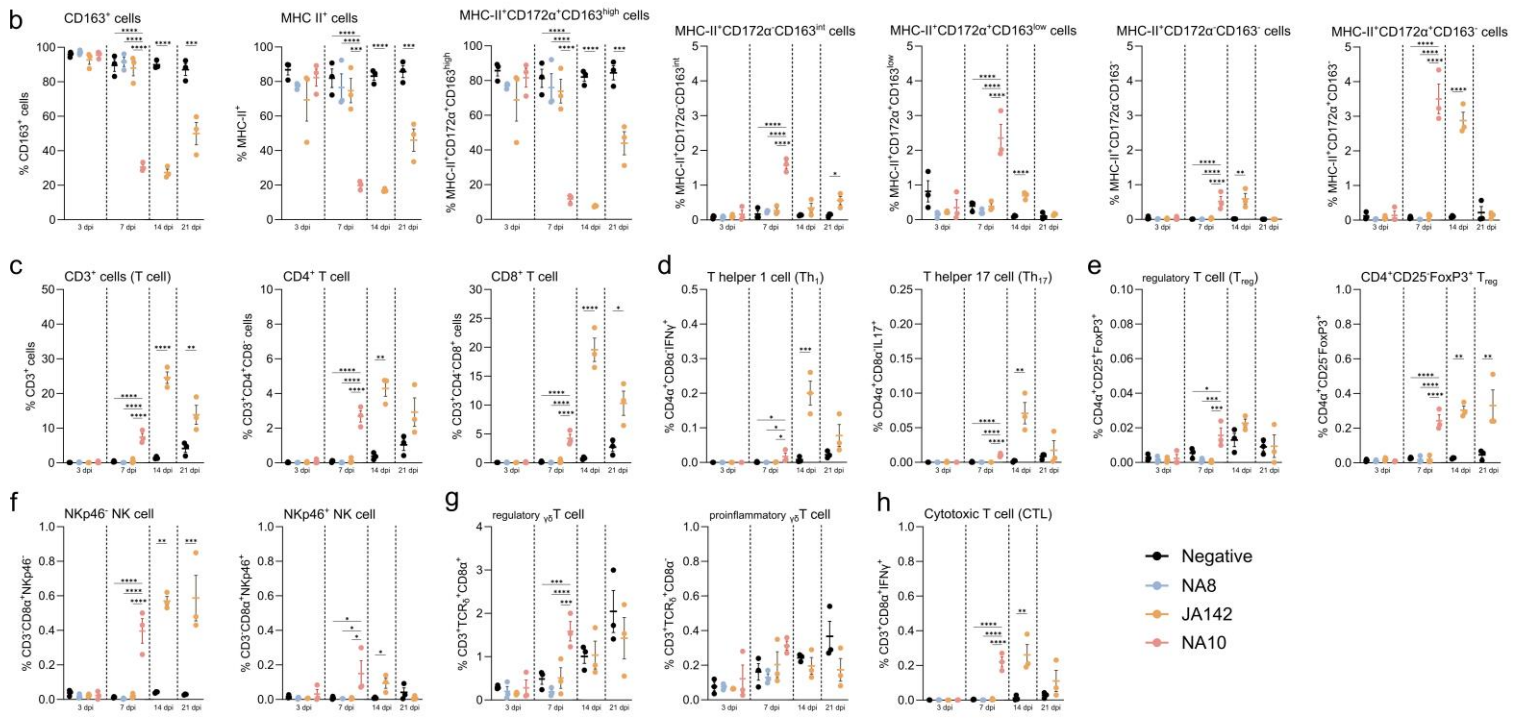
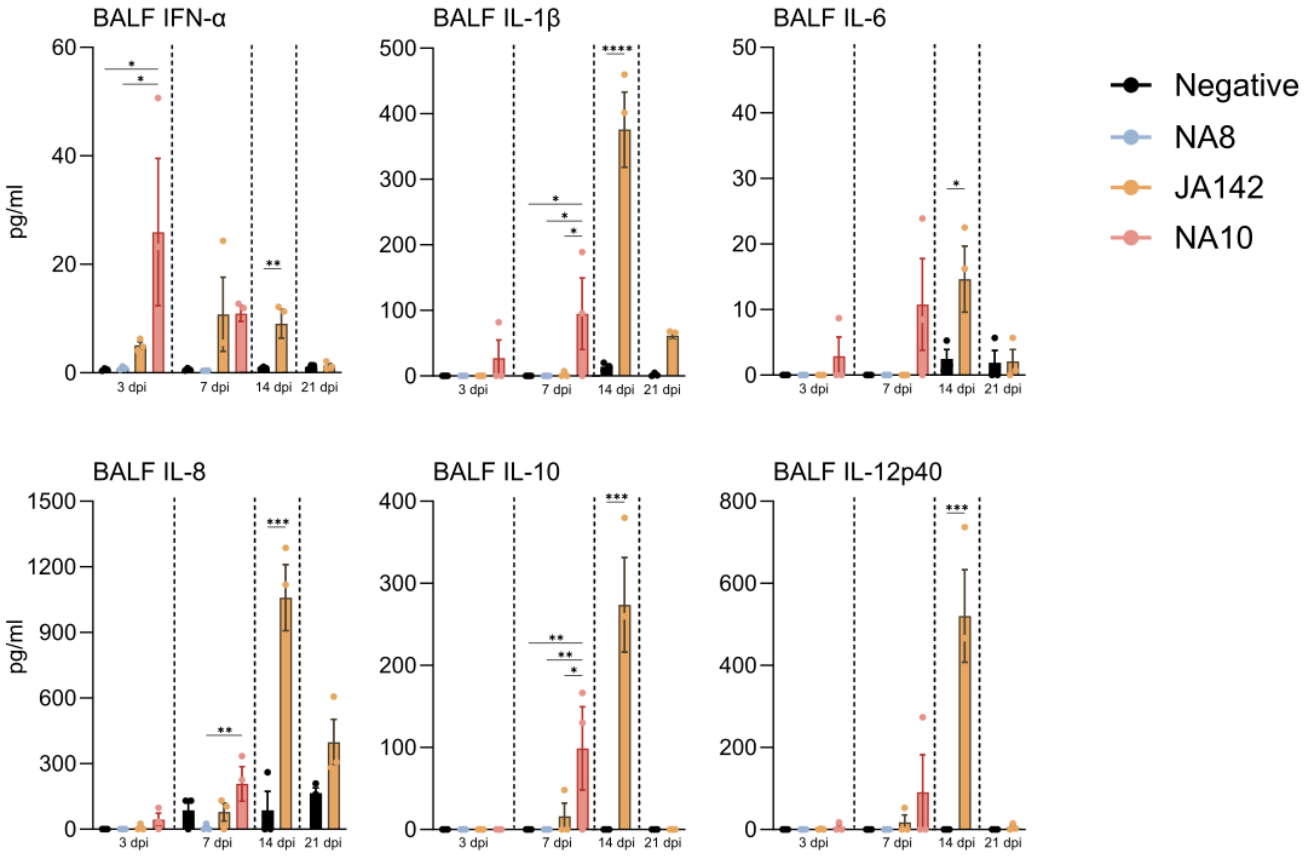


Supplementary Fig. 1 Genome structure of PRRSV. The virus consists of 14 nonstructural proteins (nsps) and 7 structural proteins. Papain-like cysteine protease (PLP)1α (P_α), PLP1β (P_β), and PLP2 (P_2) reside in nsp1α, nsp1β, and nsp2. Transmembrane (TM) domain resides in nsp2, nsp3, and nsp5. Hypervariable region (HVR) resides in nsp2. Serine protease (S) resides in nsp4. RNA-dependent RNA polymerase (R) resides in nsp9. Multinuclear zinc-binding domain (Z) and RNA helicase (H) reside in nsp10. NendoU endoribonuclease domain (Ne) resides in nsp11. Major envelope proteins (GP5 and M), minor envelope proteins (GP2a, GP3, GP4, and E), and nucleocapsid protein (N) are in the ORFs regions 2–7.

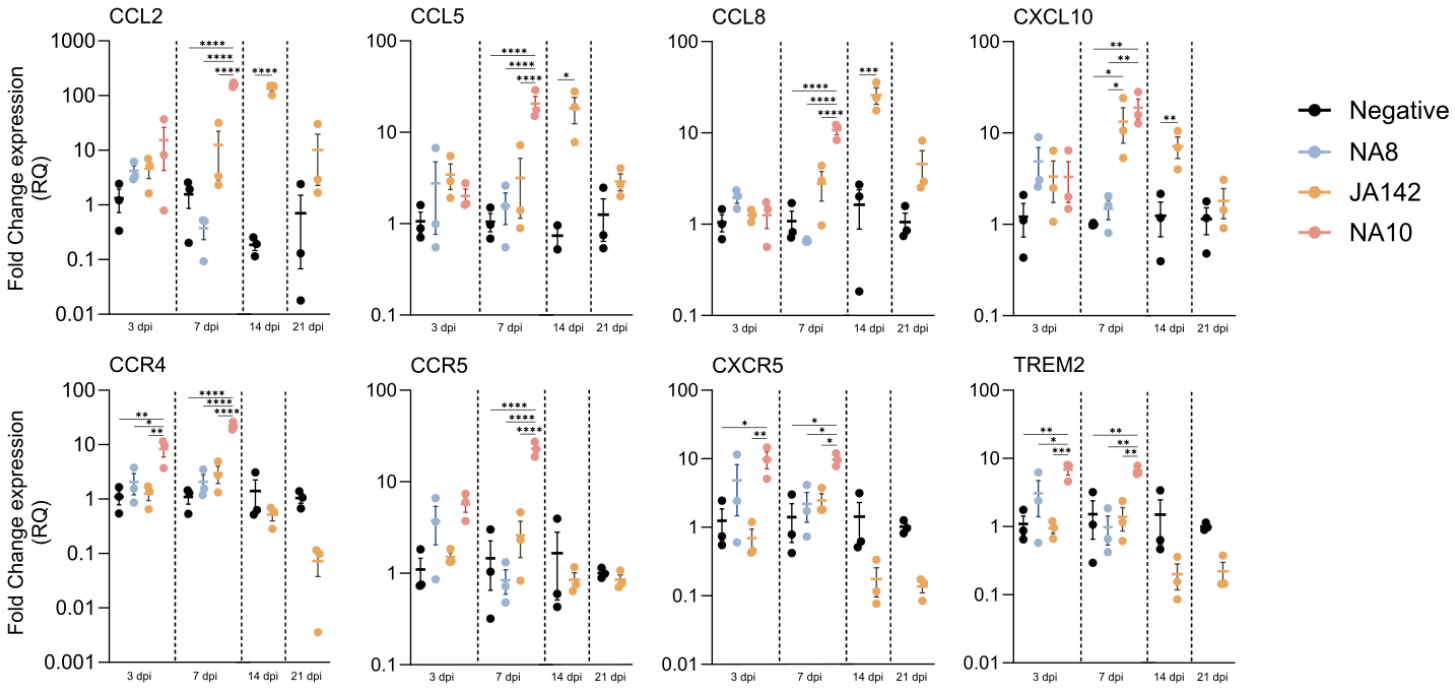

a
Panel 1
monocyte/DC
SSC-A
Zombie Green stain (FITC)
FSC-H
FSC-A
SSC-A
FSC-A
Counts
CD163-APC
Panel 1
monocyte/DC
FSC-H
FSC-A
SSC-A
FSC-A
Counts
MHC II-PerCP Cy5.5
CD163-APC
CD172α-PE
Panel 2
T cell
(CD3/CD4/CD8)
FSC-H
FSC-A
SSC-A
FSC-A
SSC-A
CD3-FITC
CD8α-PE
CD4α-PerCP Cy5.5
Panel 3
Th1/Th17
FSC-H
FSC-A
SSC-A
FSC-A
CD8α-PE
CD4α-PerCP Cy5.5
IFNγ-PerCP Cy5.5
IL17-APC
Panel 4
Regulatory T
FSC-H
FSC-A
SSC-A
FSC-A
CD8α-PE
CD4α-PerCP Cy5.5
FoxP3-FITC
CD25-APC
Panel 5
NK cell
FSC-H
FSC-A
SSC-A
FSC-A
SSC-A
CD3-FITC
SSC-A
CD8α-PE
SSC-A
CD335(NKp46)-APC
Panel 6
γδT cell/CTL
FSC-H
FSC-A
SSC-A
FSC-A
SSC-A
CD3-FITC
SSC-A
TCRγδ-APC
SSC-A
CD8α-PE
SSC-A
CD8α-PE
SSC-A
IFNγ-PerCP Cy5.5


b
CD163+ cells
% CD163+ cells
MHC II+ cells
% MHC-II+
MHC-II+CD172α+CD163high cells
% MHC-II+CD172α+CD163high
MHC-II+CD172α-CD163int cells
% MHC-II+CD172α-CD163int
MHC-II+CD172α+CD163low cells
% MHC-II+CD172α+CD163low
MHC-II+CD172α-CD163- cells
% MHC-II+CD172α-CD163-
MHC-II+CD172α+CD163- cells
% MHC-II+CD172α+CD163-
3 dpi
7 dpi
14 dpi
21 dpi
c
CD3+ cells (T cell)
% CD3+ cells
CD4+ T cell
% CD3+CD4+CD8- cells
CD8+ T cell
% CD3+CD4-CD8+ cells
d
T helper 1 cell (Th1)
% CD4+CD8αlowIFNγ+
T helper 17 cell (Th17)
% CD4+CD8αlowIL17+
e
regulatory T cell (Treg)
% CD4+CD25+FoxP3+
CD4+CD25-FoxP3+ Treg
% CD4+CD25-FoxP3+
f
NKp46- NK cell
% CD3-CD8α+NKp46-
NKp46+ NK cell
% CD3-CD8α+NKp46+
g
regulatory γδT cell
% CD3+TCRδ+CD8α+
proinflammatory γδT cell
% CD3+TCRδ+CD8α-
h
Cytotoxic T cell (CTL)
% CD3+CD8α+IFNγ+
Negative
NA8
JA142
NA10

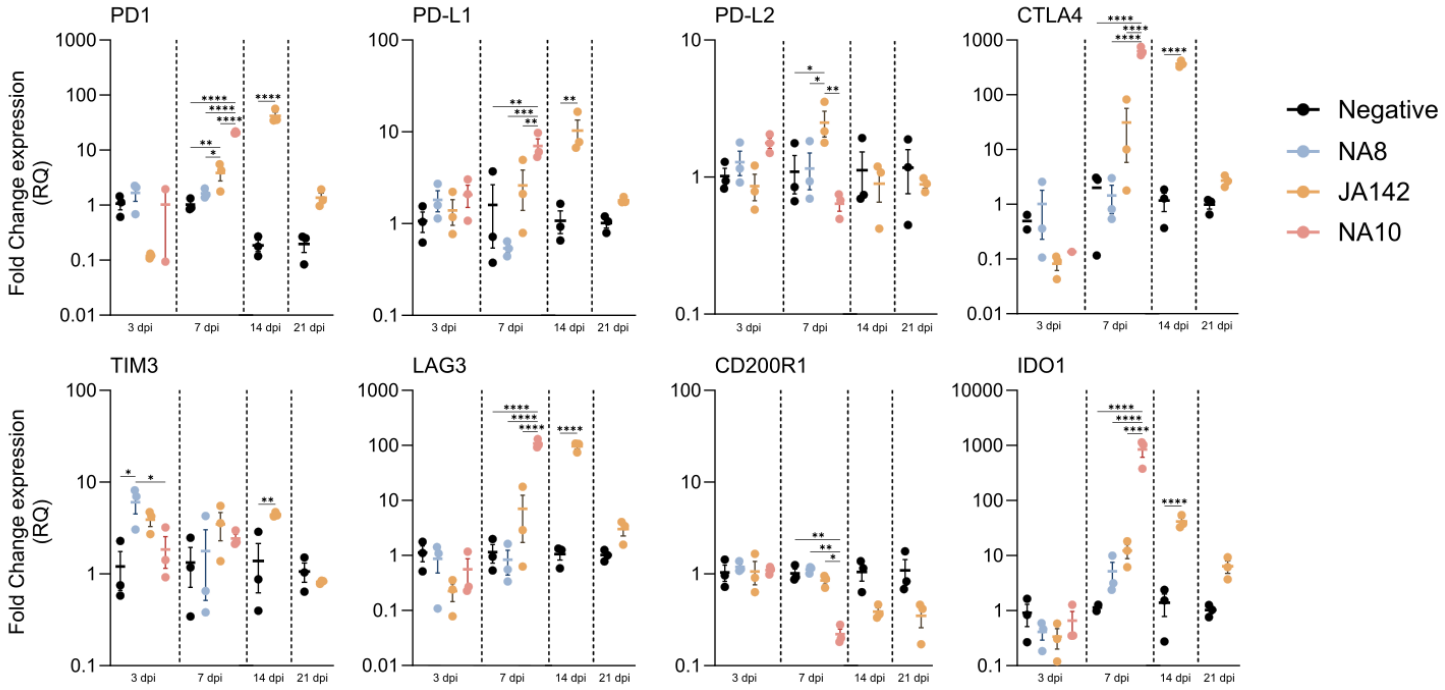
Supplementary Fig. 2 Flow cytometry analysis summary for the BALF cells. a Gating strategy for the flow cytometry of the BALF cells. Proportional cell populations for the **b** monocyte/DC lineage; **c** $CD3^+$, $CD4^+$, and $CD8^+$ T cells; **d** Th1 ($CD4\alpha^+CD8\alpha^-IFN\text{-}\gamma^+$) and Th17 ($CD4\alpha^+CD8\alpha^-IL17^+$) cells; **e** regulatory T cells (T_{reg}) and $CD4\alpha^+CD8\alpha^-CD25^+FoxP3^+$ cells; **f** $NKp46^+$ NK cells ($CD3^-CD8\alpha^+NKp46^+$) and $NKp46^-$ NK cells; **g** regulatory γδT cells and proinflammatory γδT cells; **h** cytotoxic T cell (CTL). The cell populations are presented as a scatter dot plot with error bars (mean ± SEM [standard error mean]). Circles indicate a single biological replication. Statistical analysis was performed using two-way ANOVA analysis with Tukey's multiple comparisons test for four groups (3 and 7 dpi) or Sidak's multiple comparisons test for two groups (14 and 21 dpi). Statistically significant differences between groups are marked with asterisks (*, *P*-value ≤ 0.05. **, *P*-value ≤ 0.01. ***, *P*-value ≤ 0.001. ****, *P*-value ≤ 0.0001).



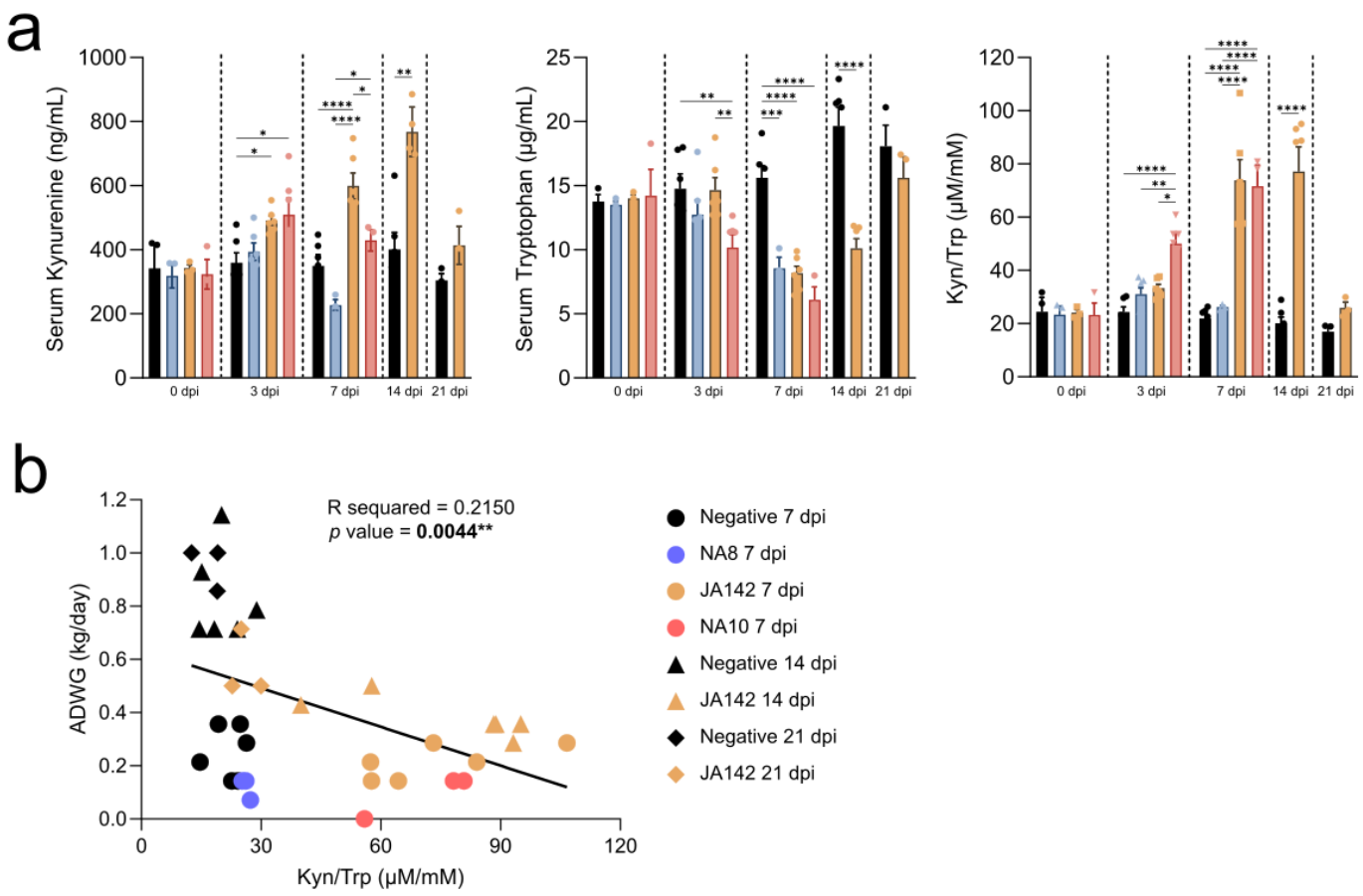
Supplementary Fig. 3 Cytokine immunoassay for BALF. The cytokine levels of BALF IFN-α, IFN-γ, IL-1β, IL-4, IL-6, IL-8, IL-10, IL-12p40, and TNF-α were measured using a Luminex assay. BALF IFN-γ, IL-4, and TNF-α were under the detection limits. The protein levels are presented as bar plot overlaid with scatter dots with error bars (mean ± SEM [standard error mean]). Circles indicate a single biological replication. Statistical analysis was performed using two-way ANOVA analysis with Tukey's multiple comparisons test for four groups (3 and 7 dpi) or Sidak's multiple comparisons test for two groups (14 and 21 dpi). Statistically significant differences between groups are marked with asterisks (*, *P*-value ≤ 0.05. **, *P*-value ≤ 0.01. ***, *P*-value ≤ 0.001. ****, *P*-value ≤ 0.0001).



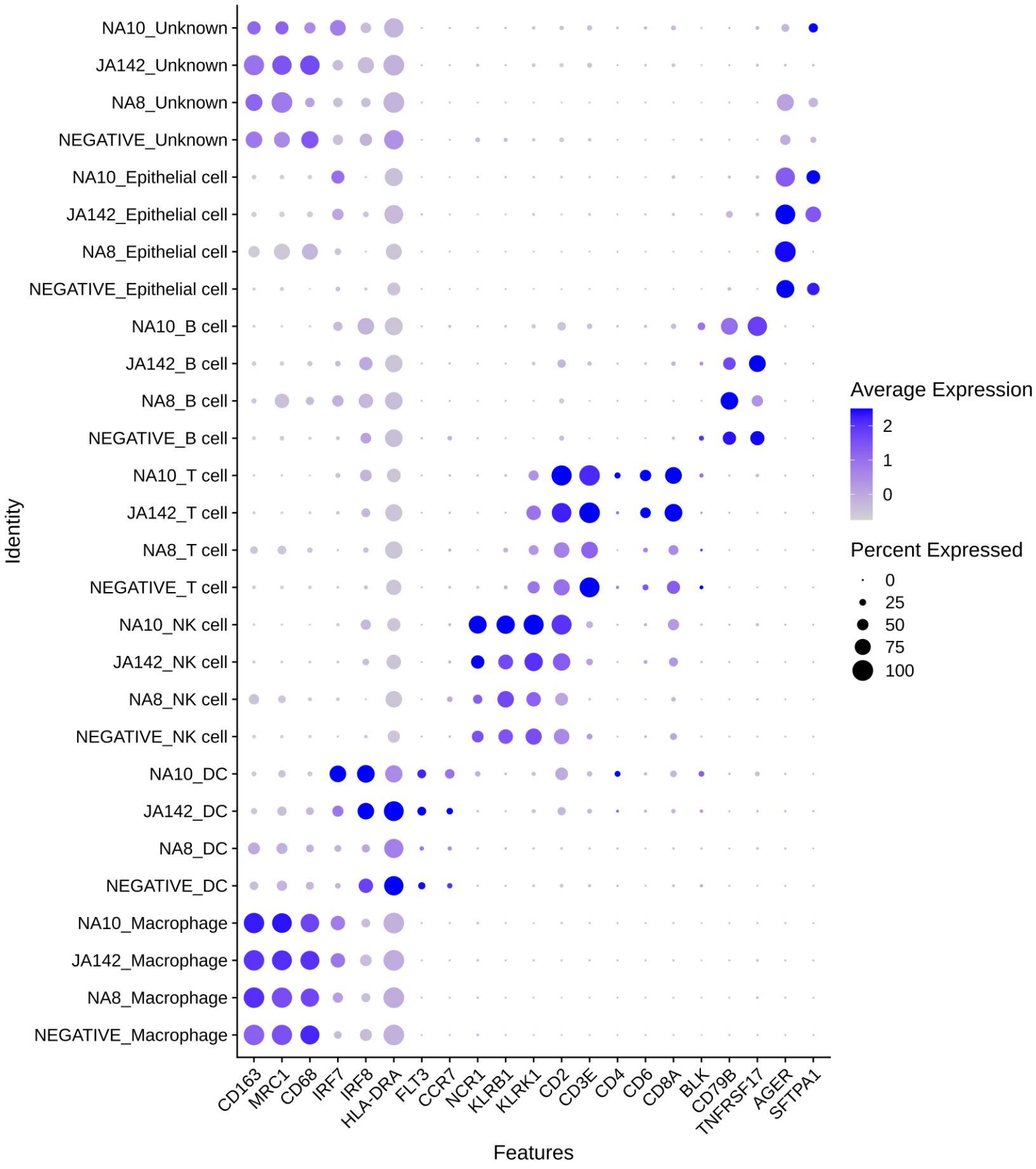
Supplementary Fig. 4 mRNA expression levels for the chemokines and receptors from the BALF cells. The mRNA expression levels are expressed as fold change expression (RQ value) and are presented as scatter dot plots with error bars (mean ± SEM [standard error mean]). Circles indicate a single biological replication. Statistical analysis was performed using two-way ANOVA analysis with Tukey's multiple comparisons test for four groups (3 and 7 dpi) or Sidak's multiple comparisons test for two groups (14 and 21 dpi). Statistically significant differences between groups are marked with asterisks (*, *P*-value ≤ 0.05. **, *P*-value ≤ 0.01. ***, *P*-value ≤ 0.001. ****, *P*-value ≤ 0.0001).



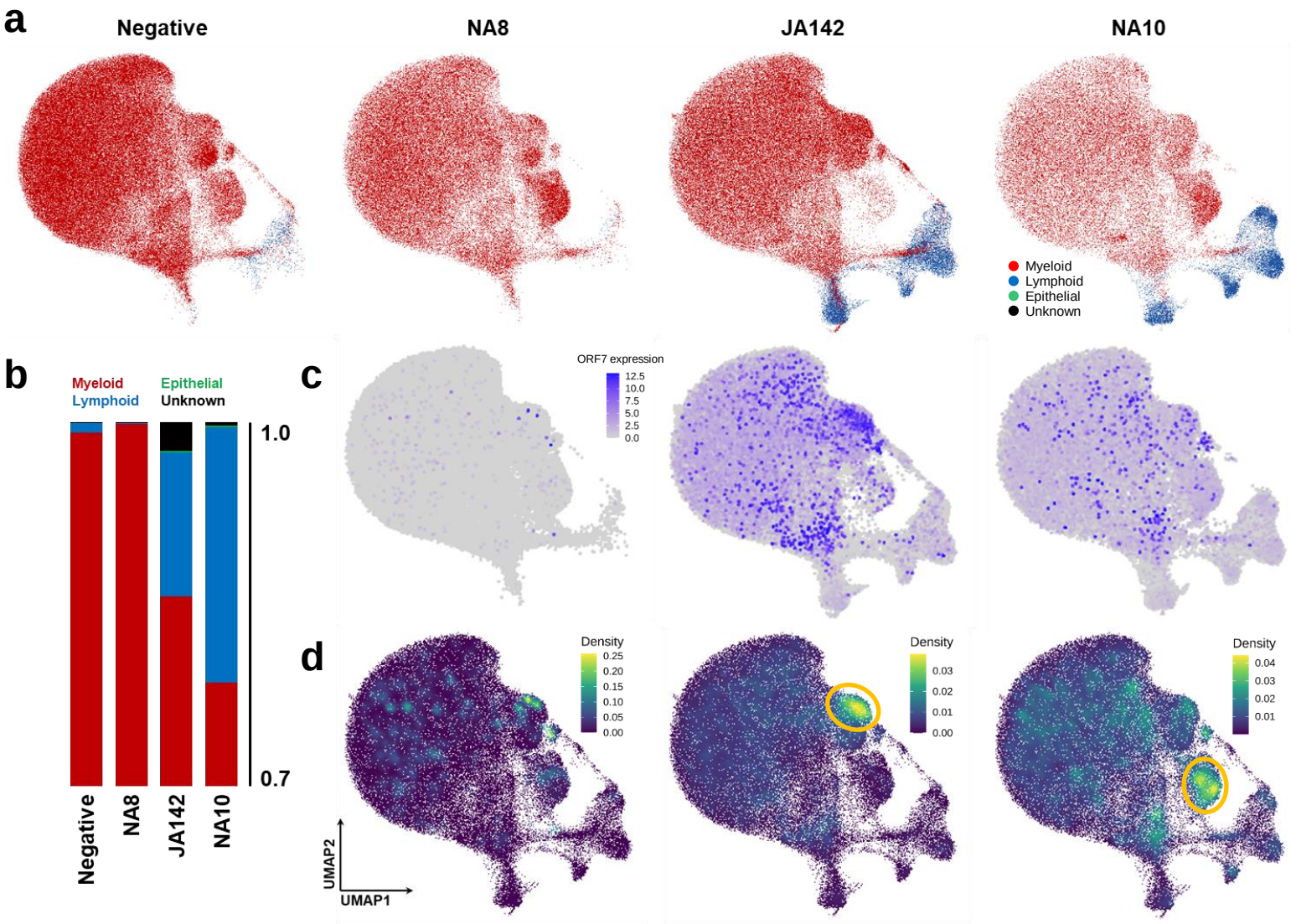
Supplementary Fig. 5 mRNA expression levels for the immune checkpoint molecules from the BALF cells. mRNA expression levels are expressed as fold change expression (RQ value) and are presented as scatter dot plots with error bars (mean ± SEM [standard error mean]). Circles indicate a single biological replication. Statistical analysis was performed using two-way ANOVA analysis with Tukey's multiple comparisons test for four groups (3 and 7 dpi) or Sidak's multiple comparisons test for two groups (14 and 21 dpi). Statistically significant differences between groups are marked with asterisks (*, *P*-value ≤ 0.05. **, *P*-value ≤ 0.01. ***, *P*-value ≤ 0.001. ****, *P*-value ≤ 0.0001).



Supplementary Fig. 6 Serum kynurenine/tryptophan (Kyn/Trp) ratio and correlation with average daily weight gain (ADWG). a The serum kynurenine and tryptophan protein levels measured from samples taken from a PRRSV-infected group, and presented as a bar plot overlaid with scatter dots with error bars (mean ± SEM [standard error mean]). Circles indicate a single biological replication. Statistical analysis was performed using two-way ANOVA analysis with Tukey's multiple comparisons test for four groups (0, 3 and 7 dpi) or Sidak's multiple comparisons test for two groups (14 and 21 dpi). Statistically significant differences between groups are marked with asterisks (*, *P*-value ≤ 0.05. **, *P*-value ≤ 0.01. ***, *P*-value ≤ 0.001. ****, *P*-value ≤ 0.0001). **b** Correlation plot between the serum Kyn/Trp ratio and ADWG with spearman correlation analysis.



Supplementary Fig. 7 Specific marker gene expression levels for seven basic cell types in each group. Average expression levels were calculated using log-normalized values.



Supplementary Fig. 8 Single-cell transcriptomes of the bronchoalveolar lavage fluid (BALF) cells in each group (negative, NA8, JA142, and NA10). a UMAP projection of the negative (*n* = 117,039), NA8 (*n* = 61,915), JA142 (*n* = 83,808), and NA10 (*n* = 43,290) groups with annotation of cell categories (myeloid, lymphoid, and epithelial). **b** Proportion of cell categories. Viral tracking based on the log normalized ORF7 **c** expression levels in each group (NA8, JA142, and NA10) and **d** expression density. The yellow circles indicate the highest density region.

a

P-values		Negative				NA8		JA142				NA10	
		3 dpi	7 dpi	14 dpi	21 dpi	3 dpi	7 dpi	3 dpi	7 dpi	14 dpi	21 dpi	3 dpi	7 dpi
Negative	3 dpi	-	0.9945	0.0120	1.151E-05	1	0.0217	0.0093	0.0054	4.319E-10	1.838E-10	0.8782	1.477E-11
	7 dpi		-	0.1173	0.0001	0.9958	0.1902	0.0942	0.0587	2.655E-09	1.069E-09	1	6.847E-11
	14 dpi			-	0.1927	0.0130	1	1	1	1.480E-06	4.896E-07	0.3317	1.723E-08
	21 dpi				-	1.242E-05	0.119	0.2338	0.3377	1.342E-03	3.867E-04	5.488E-04	7.774E-06
NA8	3 dpi					-	0.0234	0.0100	0.0059	4.567E-10	1.940E-10	0.8912	1.545E-11
	7 dpi						-	1	1	8.488E-07	2.855E-07	0.4756	1.059E-08
JA142	3 dpi							-	1	1.884E-06	6.190E-07	0.2787	2.130E-08
	7 dpi								-	3.113E-06	1.008E-06	0.1887	3.312E-08
	14 dpi									-	1	7.811E-09	0.5766
	21 dpi										-	3.041E-09	0.8668
NA10	3 dpi											-	1.758E-10
	7 dpi												-

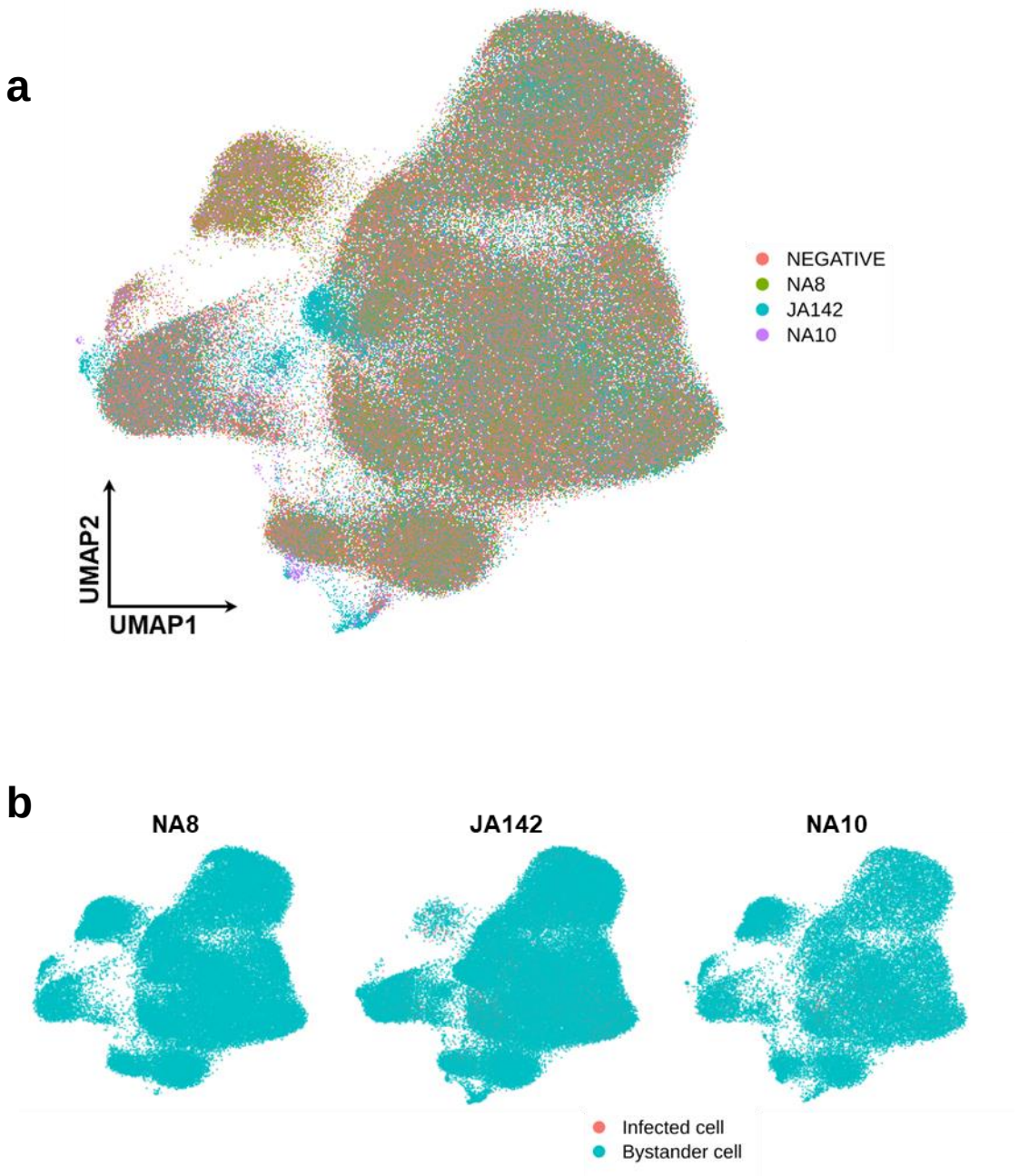
0 $-\log_{10}$ *P*-value 11

b

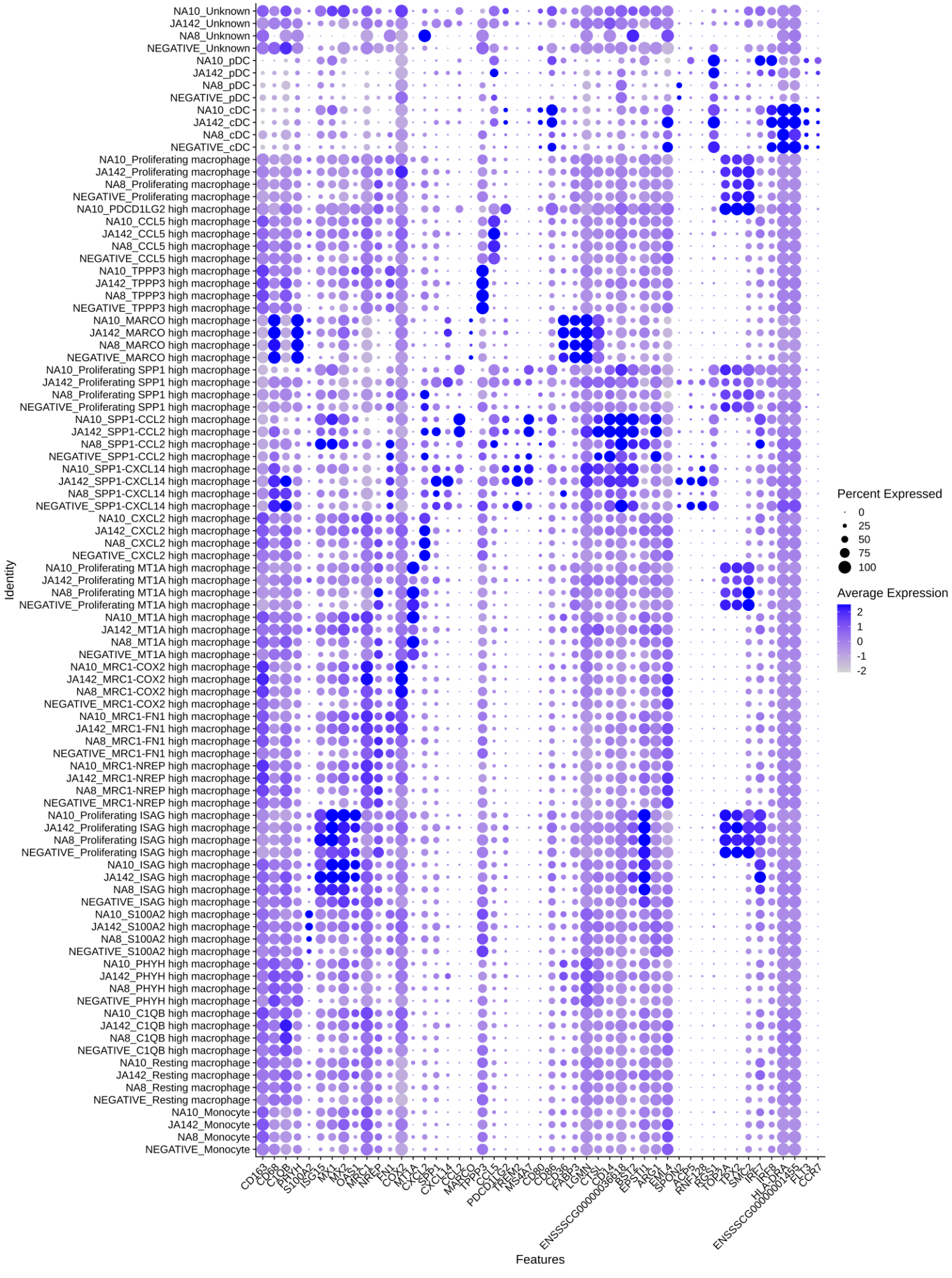
P-values		NA8		JA142				NA10	
		3 dpi	7 dpi	3 dpi	7 dpi	14 dpi	21 dpi	3 dpi	7 dpi
NA8	3 dpi	-	1	1	0.4043	0.9999	1	0.9985	0.9995
	7 dpi		-	1	0.4151	0.9999	1	0.9988	0.9996
JA142	3 dpi			-	0.4043	0.9999	1	0.9985	0.9995
	7 dpi				-	0.6400	0.4186	0.7436	0.6927
	14 dpi					-	0.9999	1	1
	21 dpi						-	0.9989	0.9996
NA10	3 dpi							-	1
	7 dpi								-

0 $-\log_{10}$ *P*-value 0.4

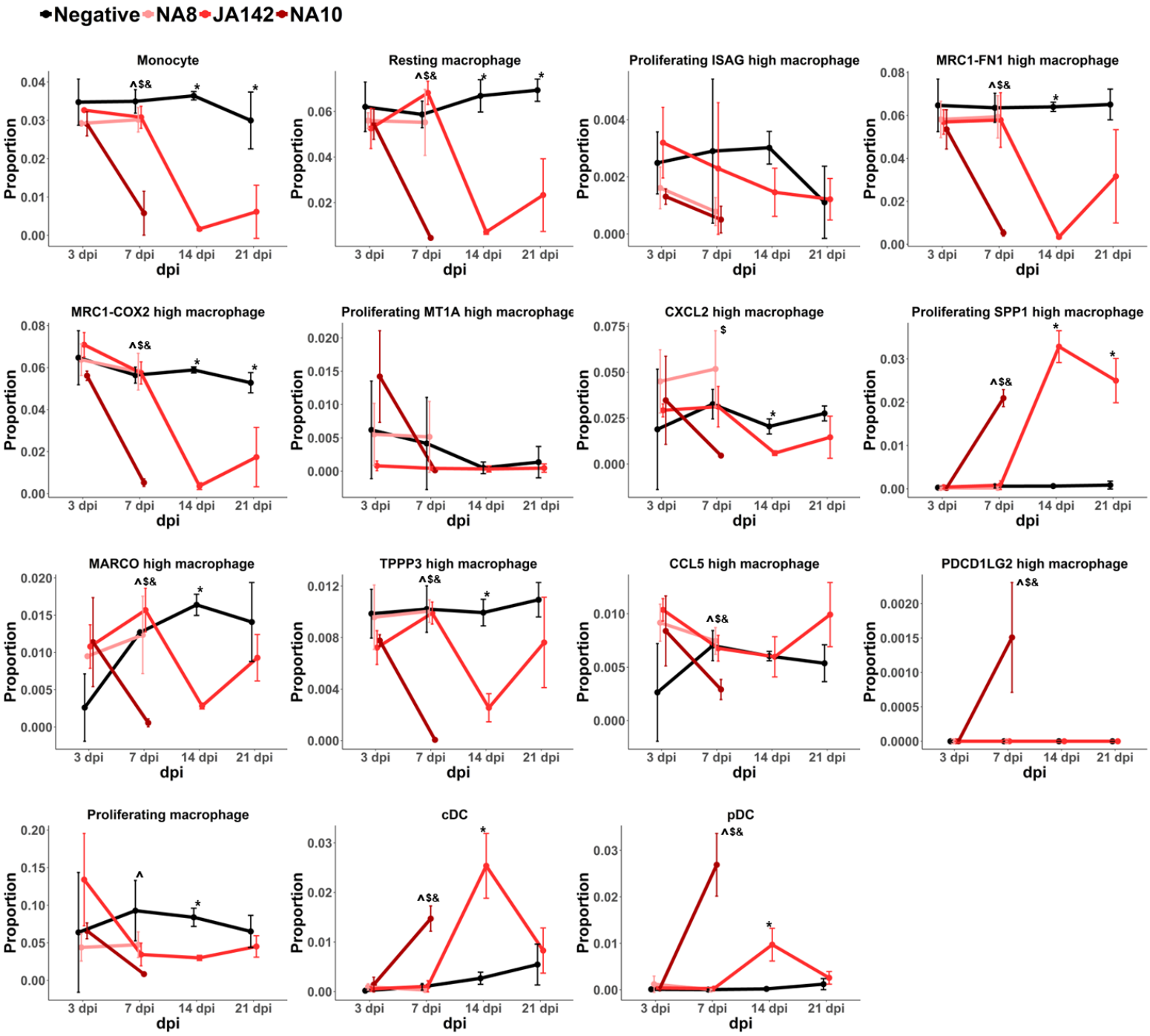
Supplementary Fig. 9 Significance information in cell numbers and proportions of infected cells for the calculated differences. a *P*-values for the average of cell numbers in each time point (3, 7, 14, and 21 dpi) and group (negative, NA8, JA142, and NA10). **b** *P*-values for the average proportions of infected cells in the myeloid at each time point (3, 7, 14, and 21 dpi) and for each group (NA8, JA142, and NA10).

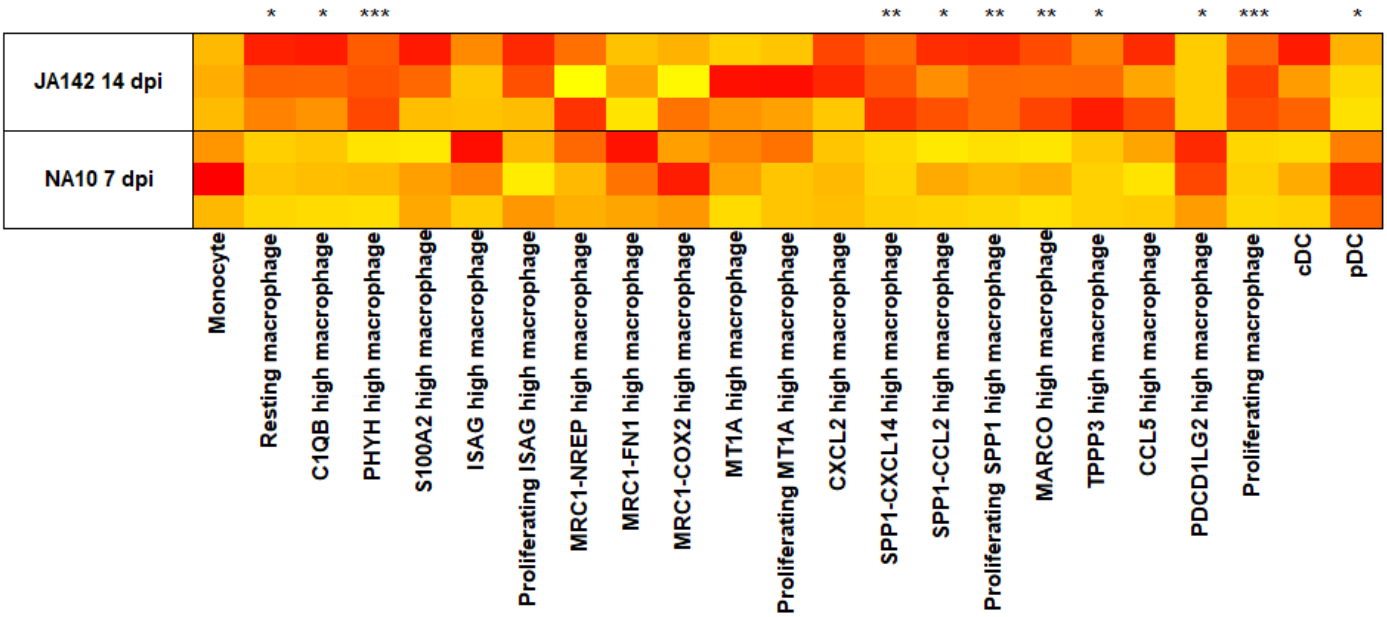


Supplementary Fig. 10 Additional annotation information for the macrophage and dendritic cell (DC) subpopulation. UMAP with **a** group annotation and **b** infection annotation.

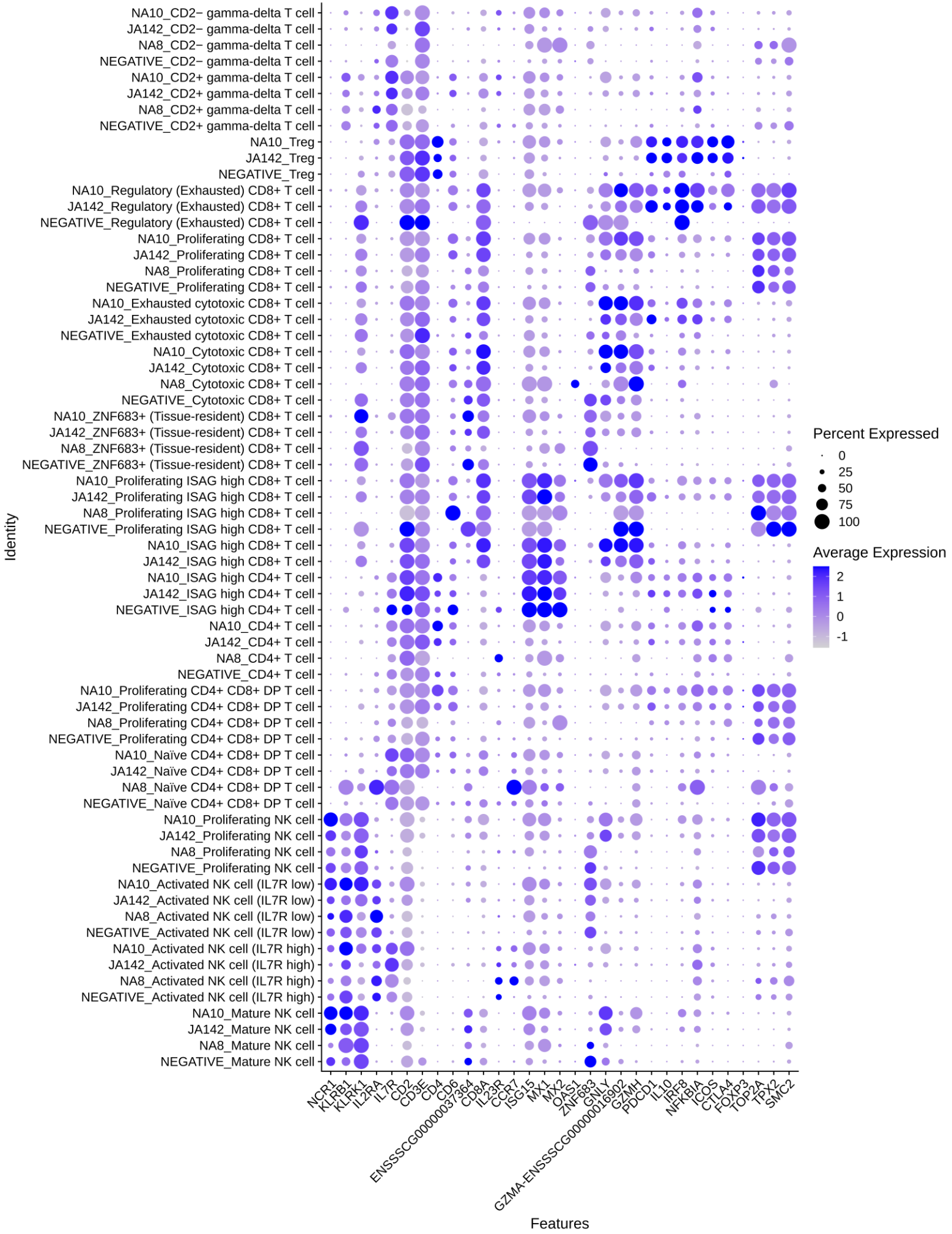


Supplementary Fig. 11 Specific marker gene expression levels for 23 sub-cell types of macrophage and DC subpopulation in each group. Average expression levels were calculated using log-normalized values.

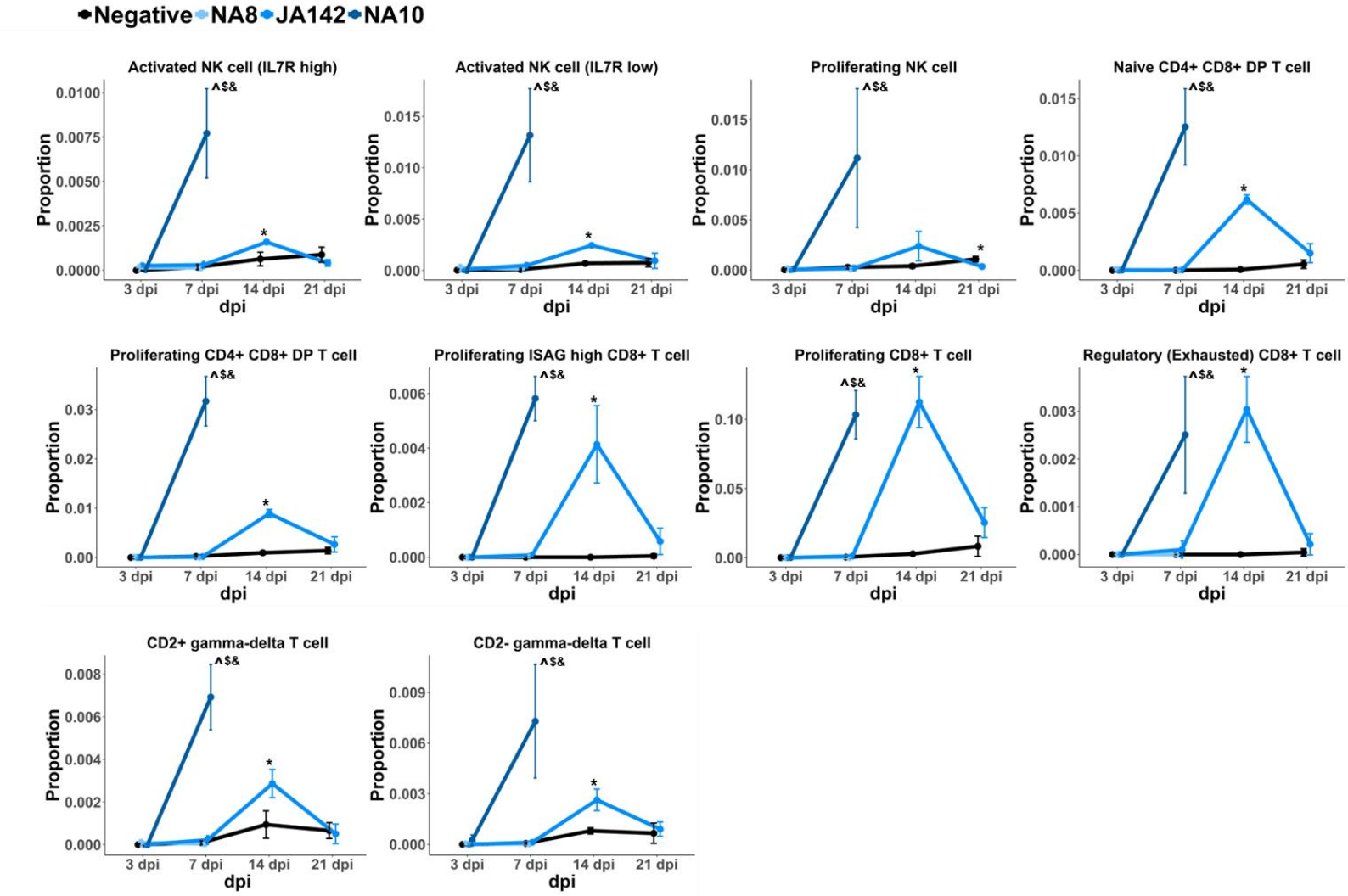

Negative
NA8
JA142
NA10
Monocyte
Resting macrophage
Proliferating ISAG high macrophage
MRC1-FN1 high macrophage
MRC1-COX2 high macrophage
Proliferating MT1A high macrophage
CXCL2 high macrophage
Proliferating SPP1 high macrophage
MARCO high macrophage
TPPP3 high macrophage
CCL5 high macrophage
PDCD1LG2 high macrophage
Proliferating macrophage
cDC
pDC
Proportion
dpi
3 dpi
7 dpi
14 dpi
21 dpi

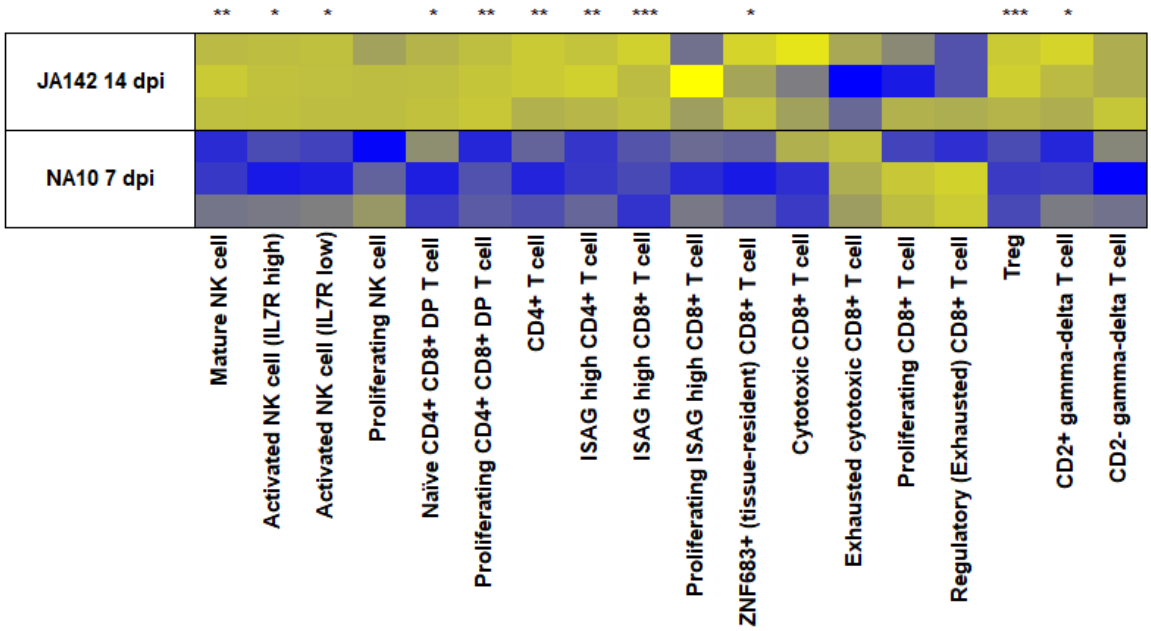

JA142 14 dpi
NA10 7 dpi
Monocyte
Resting macrophage
C1QB high macrophage
PHYH high macrophage
S100A2 high macrophage
ISAG high macrophage
Proliferating ISAG high macrophage
MRC1-NREP high macrophage
MRC1-FN1 high macrophage
MRC1-COX2 high macrophage
MT1A high macrophage
Proliferating MT1A high macrophage
CXCL2 high macrophage
SPP1-CXCL14 high macrophage
SPP1-CCL2 high macrophage
Proliferating SPP1 high macrophage
MARCO high macrophage
TPPP3 high macrophage
CCL5 high macrophage
PDCD1LG2 high macrophage
Proliferating macrophage
cDC
pDC

Supplementary Fig. 12 Time-series proportions for the other sub-cell types for the macrophage and dendritic cell (DC) subpopulation in each group (negative; black, NA8; light red, JA142; red, and NA10; dark red) against PRRSV infection. Dots indicate three biological replications and the data are presented as mean values ± SD. Statistically significant differences between groups are marked with exclamation ($^{!}$, NA8-negative), asterisk (*, JA142-negative), circumflex ($^{\wedge}$, NA10-negative), ampersand ($^{\&}$, NA10-JA142), hash ($^{\#}$, JA142-NA8), and dollar ($^{\$}$, NA10-NA8) signs. Significant differences ($p < 0.05$) determined using one-way ANOVA analysis with Tukey's multiple comparison test. The heatmap shows the differences in the cell proportions between JA142 14 dpi and NA10 7 dpi. Red indicates a higher number. Significance was determined using a two-tailed t-test. *P-value < 0.05, **P-value < 0.01, ***P-value < 0.001. Source data are provided as a Source Data file.



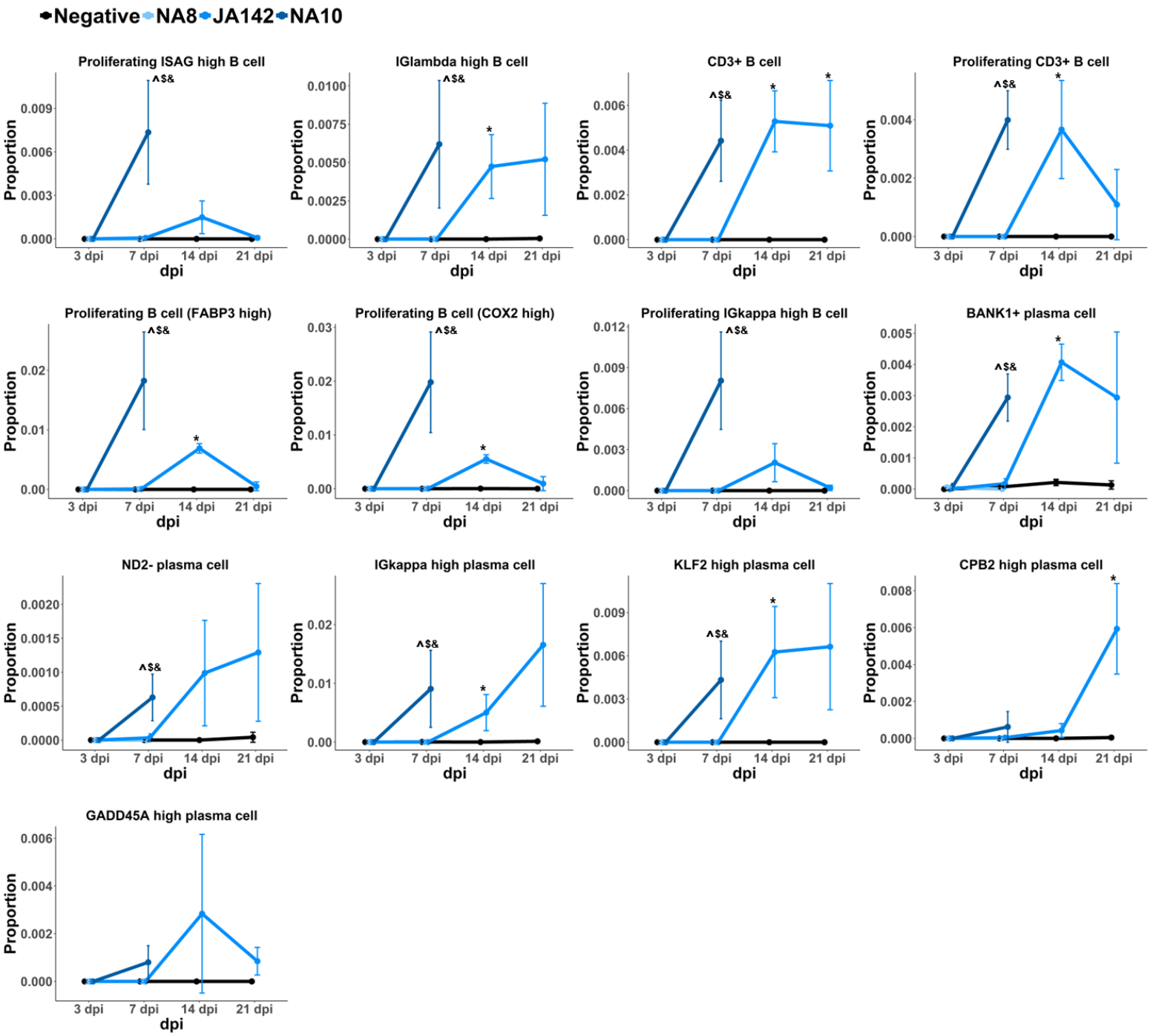
Supplementary Fig. 13 Specific marker gene expression levels for 18 sub-cell types of NK and T cell subpopulation in each group. Average expression levels were calculated using log-normalized values.

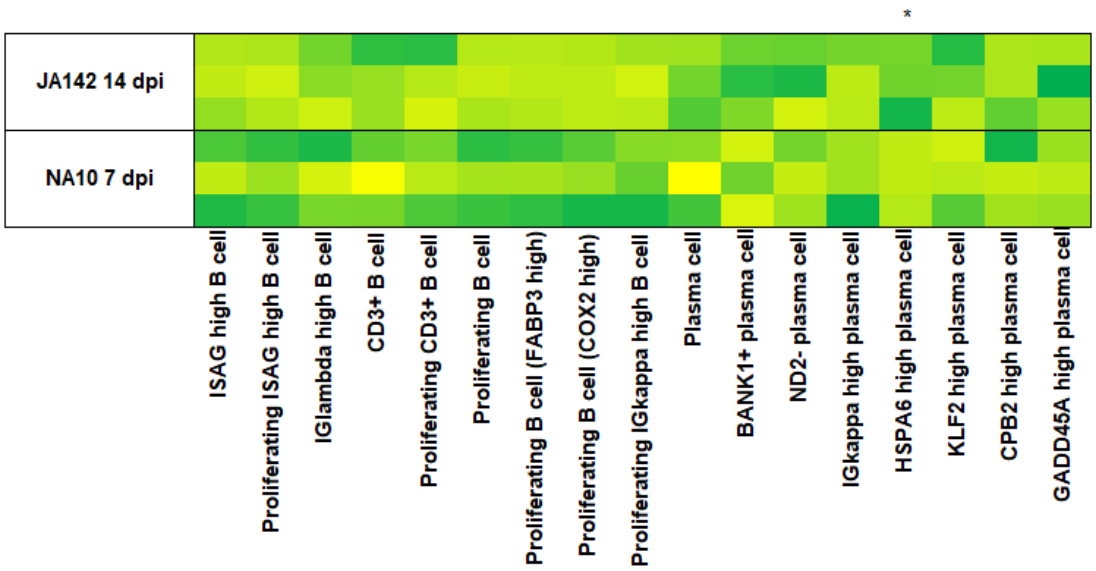

Negative
NA8
JA142
NA10
Activated NK cell (IL7R high)
Activated NK cell (IL7R low)
Proliferating NK cell
Naive CD4+ CD8+ DP T cell
Proliferating CD4+ CD8+ DP T cell
Proliferating ISAG high CD8+ T cell
Proliferating CD8+ T cell
Regulatory (Exhausted) CD8+ T cell
CD2+ gamma-delta T cell
CD2- gamma-delta T cell
Proportion
dpi
3 dpi
7 dpi
14 dpi
21 dpi
^$&
*


JA142 14 dpi
NA10 7 dpi
Mature NK cell
Activated NK cell (IL7R high)
Activated NK cell (IL7R low)
Proliferating NK cell
Naïve CD4+ CD8+ DP T cell
Proliferating CD4+ CD8+ DP T cell
CD4+ T cell
ISAG high CD4+ T cell
ISAG high CD8+ T cell
Proliferating ISAG high CD8+ T cell
ZNF683+ (tissue-resident) CD8+ T cell
Cytotoxic CD8+ T cell
Exhausted cytotoxic CD8+ T cell
Proliferating CD8+ T cell
Regulatory (Exhausted) CD8+ T cell
Treg
CD2+ gamma-delta T cell
CD2- gamma-delta T cell

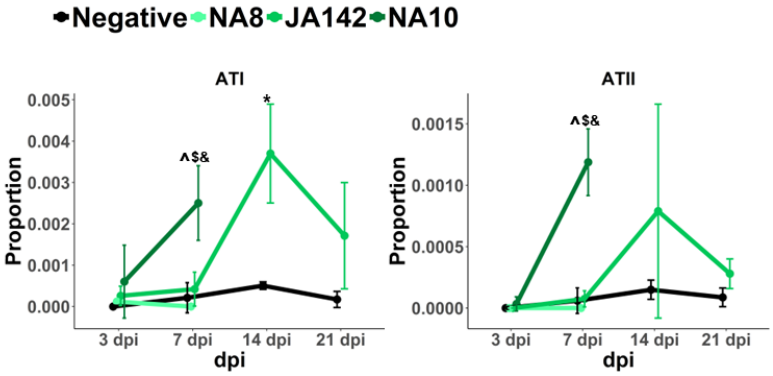
Supplementary Fig. 14 Time-series proportions of the other sub-cell types for the natural killer (NK) and T cell subpopulations in each group (negative; black, NA8; light blue, JA142; blue, and NA10; dark blue) against PRRSV infection. Dots indicate three biological replications and the data are presented as mean values ± SD. Statistically significant differences between groups are marked with exclamation ($^{!}$, NA8-negative), asterisk (*, JA142-negative), circumflex ($^{\wedge}$, NA10-negative), ampersand ($^{\&}$, NA10-JA142), hash ($^{\#}$, JA142-NA8), and dollar ($^{\$}$, NA10-NA8) signs. Significant differences ($p < 0.05$) determined using one-way ANOVA analysis with Tukey's multiple comparison test. The heatmap shows differences in the cell proportions between JA142 14 dpi and NA10 7 dpi. Blue indicates a higher number. Significance was determined using a two-tailed t-test. *P-value < 0.05, **P-value < 0.01, ***P-value < 0.001. Source data are provided as a Source Data file.

Supplementary Fig. 15 Specific marker gene expression levels for 17 sub-cell types of B cell subpopulation in each group. Average expression levels were calculated using log-normalized values.


Negative
NA8
JA142
NA10
Proliferating ISAG high B cell
IGlambda high B cell
CD3+ B cell
Proliferating CD3+ B cell
Proliferating B cell (FABP3 high)
Proliferating B cell (COX2 high)
Proliferating IGkappa high B cell
BANK1+ plasma cell
ND2- plasma cell
IGkappa high plasma cell
KLF2 high plasma cell
CPB2 high plasma cell
GADD45A high plasma cell
Proportion
dpi
3 dpi
7 dpi
14 dpi
21 dpi
^$&
*


*
JA142 14 dpi
NA10 7 dpi
ISAG high B cell
Proliferating ISAG high B cell
IGlambda high B cell
CD3+ B cell
Proliferating CD3+ B cell
Proliferating B cell
Proliferating B cell (FABP3 high)
Proliferating B cell (COX2 high)
Proliferating IGkappa high B cell
Plasma cell
BANK1+ plasma cell
ND2- plasma cell
IGkappa high plasma cell
HSPA6 high plasma cell
KLF2 high plasma cell
CPB2 high plasma cell
GADD45A high plasma cell

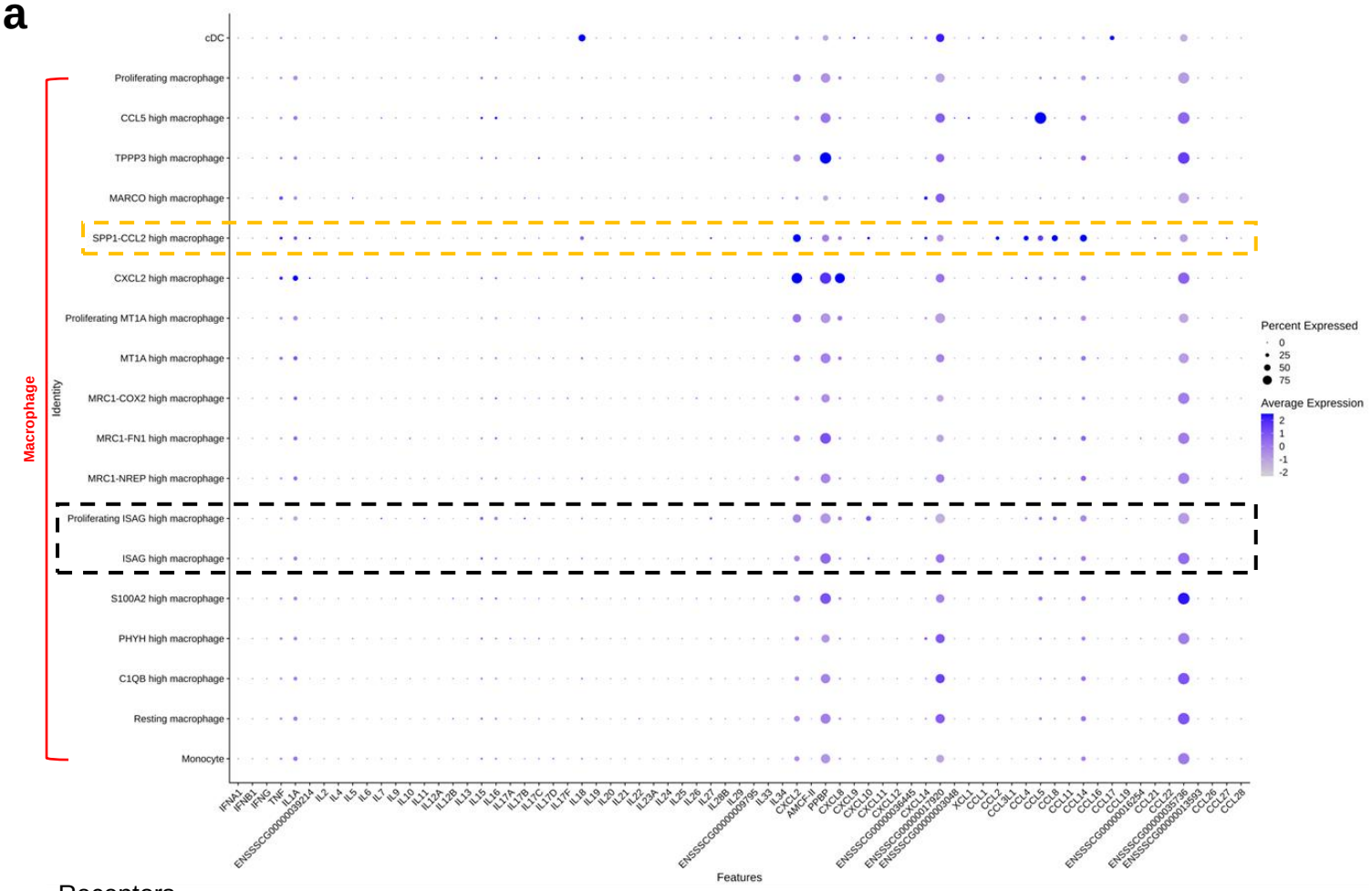
Supplementary Fig. 16 Time-series proportions of the other sub-cell types for the B cell subpopulation in each group (negative; black, NA8; light blue, JA142; blue, and NA10; dark blue) against PRRSV infection. Dots indicate three biological replications and the data are presented as mean values ± SD. Statistically significant differences between groups are marked with exclamation ($^{!}$, NA8-negative), asterisk (*, JA142-negative), circumflex (^, NA10-negative), ampersand ($^{\&}$, NA10-JA142), hash ($^{\#}$, JA142-NA8), and dollar ($^{\$}$, NA10-NA8) signs. Significant differences ($p < 0.05$) determined using one-way ANOVA analysis with Tukey's multiple comparison test. The heatmap shows differences in the cell proportions between JA142 14 dpi and NA10 7 dpi. Green indicates a higher number. Significance was determined using a two-tailed t-test. *P-value < 0.05, **P-value < 0.01, ***P-value < 0.001. Source data are provided as a Source Data file.



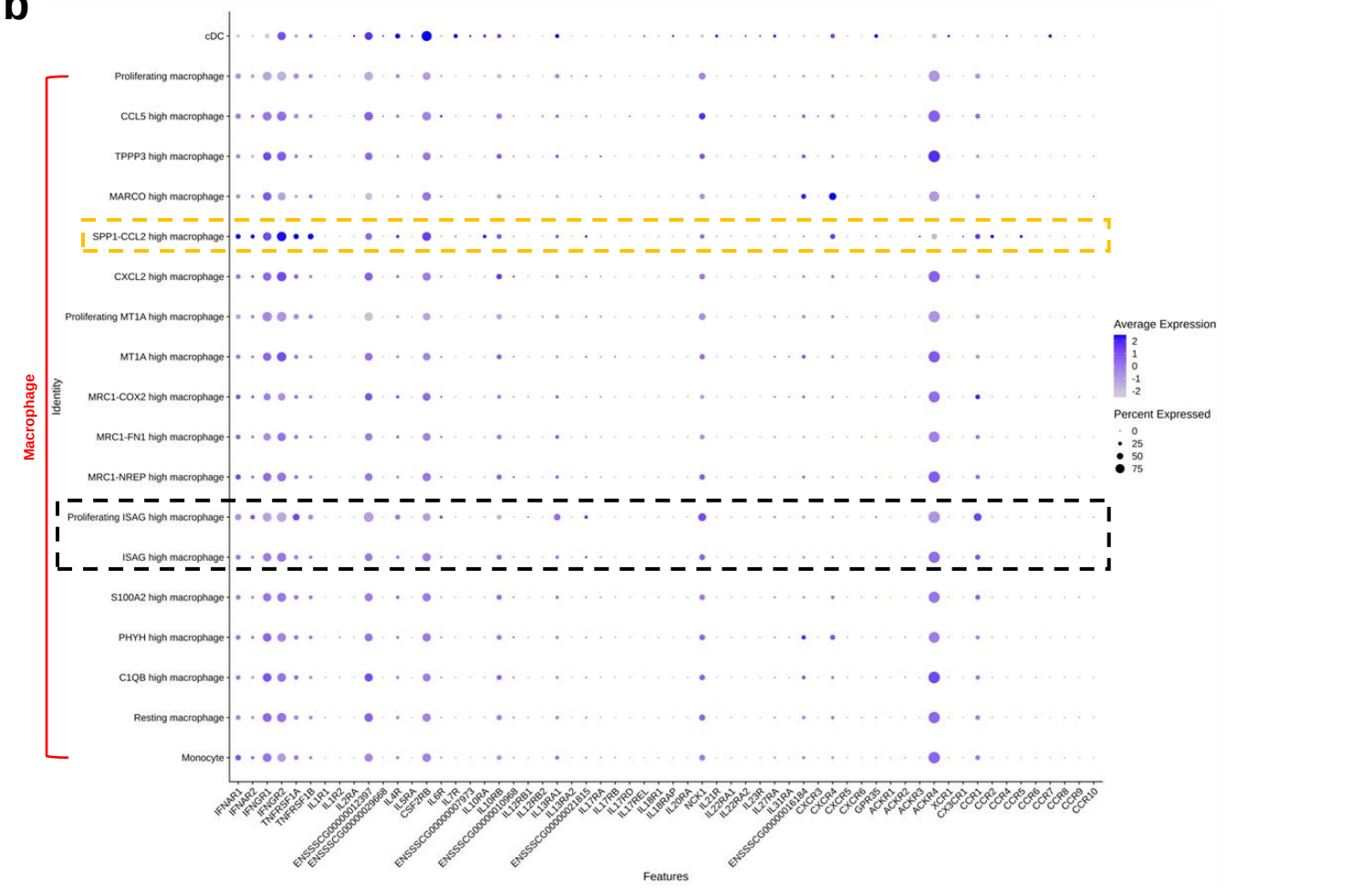
Supplementary Fig. 17 Time-series proportions of the sub-cell types for the epithelial cells in each group (negative; black, NA8; light green, JA142; green, and NA10; dark green) against PRRSV infection. Dots indicate three biological replications and the data are presented as mean values ± SD. Statistically significant differences between groups are marked with exclamation ($^{!}$, NA8-negative), asterisk (*, JA142-negative), circumflex (^, NA10-negative), ampersand ($^{\&}$, NA10-JA142), hash ($^{\#}$, JA142-NA8), and dollar ($^{\$}$, NA10-NA8) signs. Significant differences ($p < 0.05$) determined using one-way ANOVA analysis with Tukey's multiple comparison test. Source data are provided as a Source Data file.

Negative 3 dpi

Cytokines & chemokines (ligands)



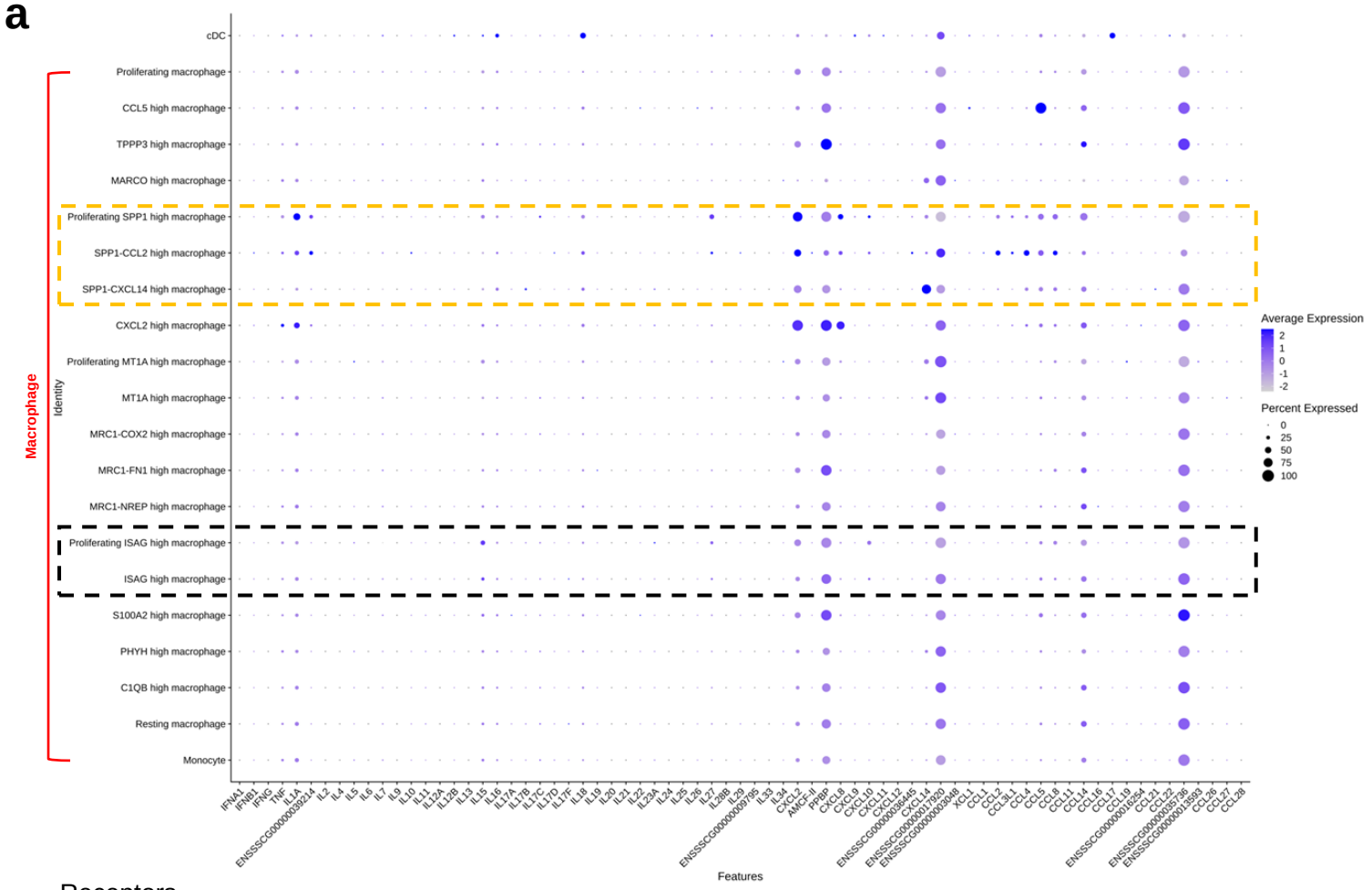
Receptors



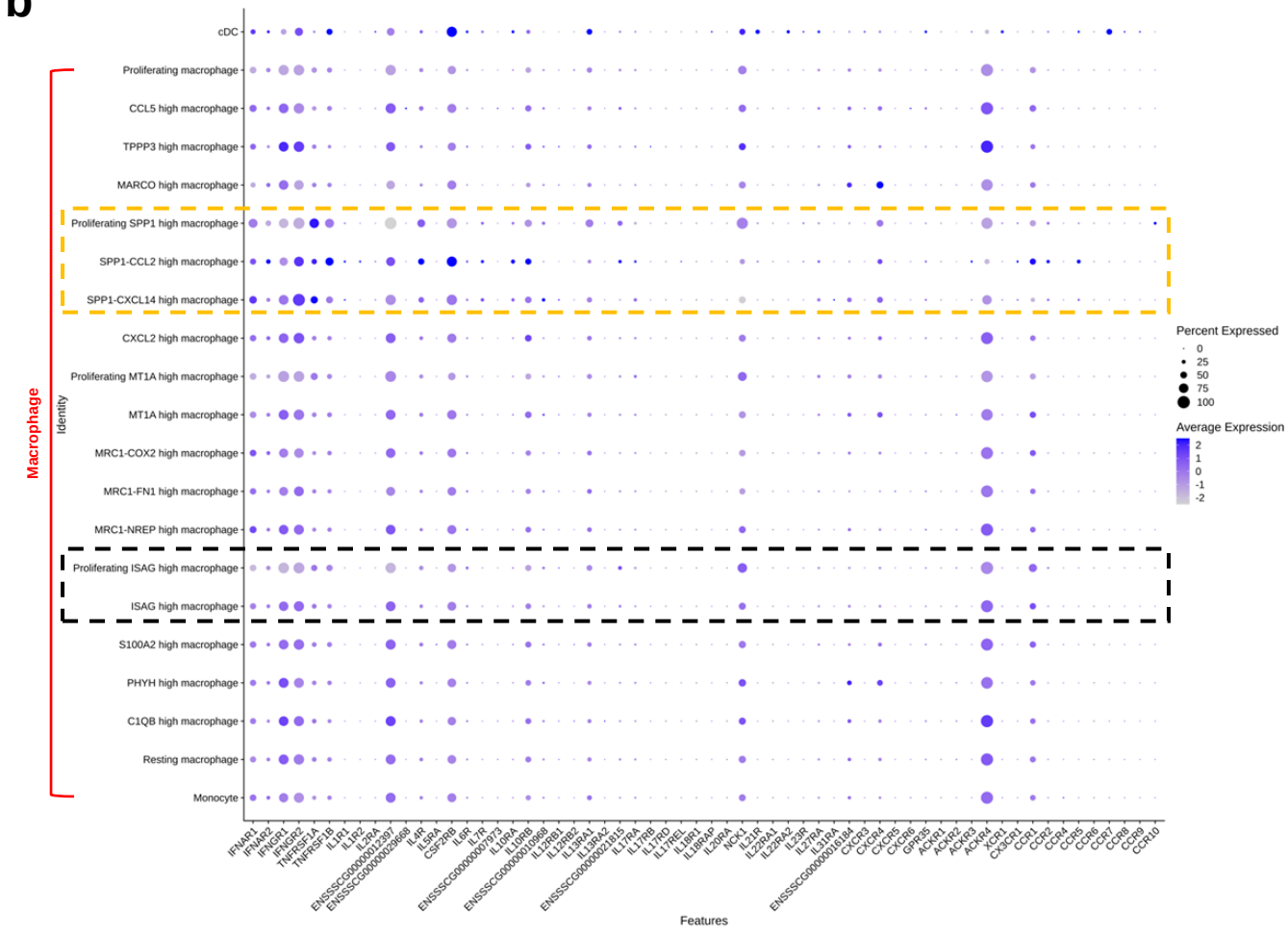
Supplementary Fig. 18 RNA expression levels for ligands (cytokines and chemokines) and their receptors in the negative group 3 dpi. a Cytokines and chemokines, and **b** their receptors. The yellow dotted line indicates SPP1-expressing macrophages and the black dotted line indicates ISAG-expressing macrophages. Average expression levels were calculated using log-normalized values. To eliminate bias from a small number of cells, only sub-cell types with more than 20 cells were included in the dot plot.

Negative 7 dpi

Cytokines & chemokines (ligands)



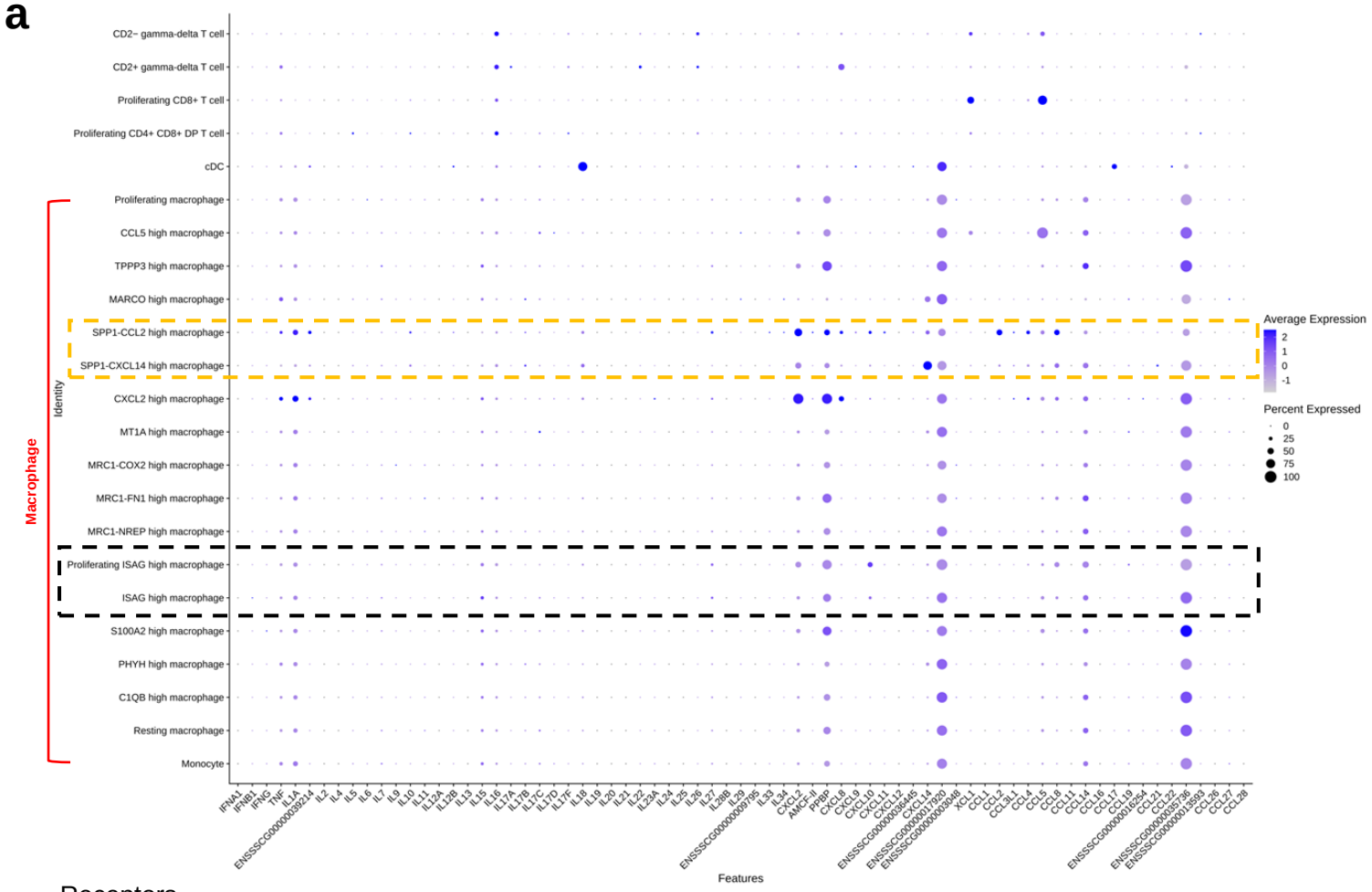
Receptors



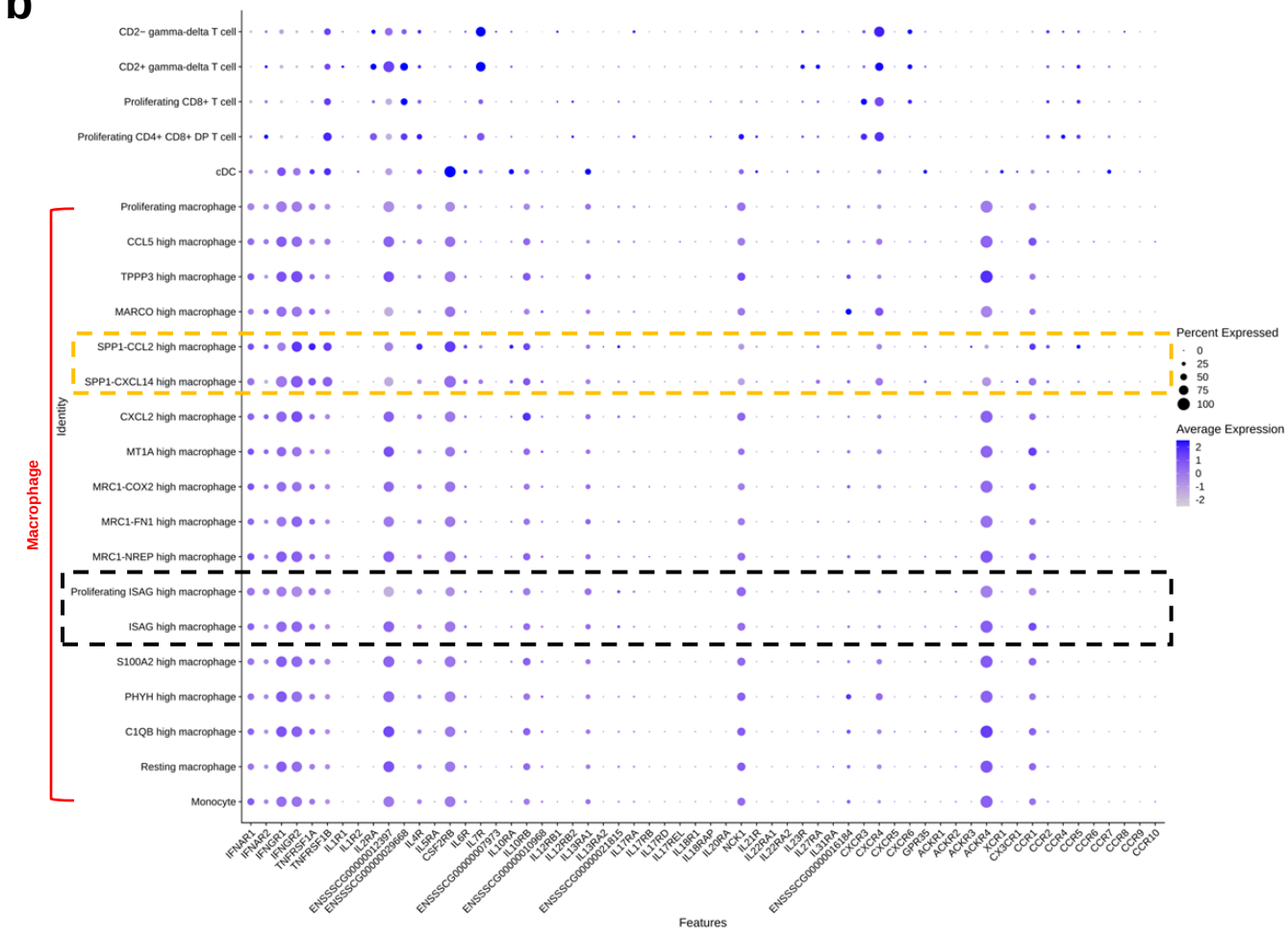
Supplementary Fig. 19 RNA expression levels for ligands (cytokines and chemokines) and their receptors in the negative group 7 dpi. a Cytokines and chemokines, and **b** their receptors. The yellow dotted line indicates SPP1-expressing macrophages and the black dotted line indicates ISAG-expressing macrophages. Average expression levels were calculated using log-normalized values. To eliminate bias from a small number of cells, only sub-cell types with more than 20 cells were included in the dot plot.

Negative 14 dpi

Cytokines & chemokines (ligands)



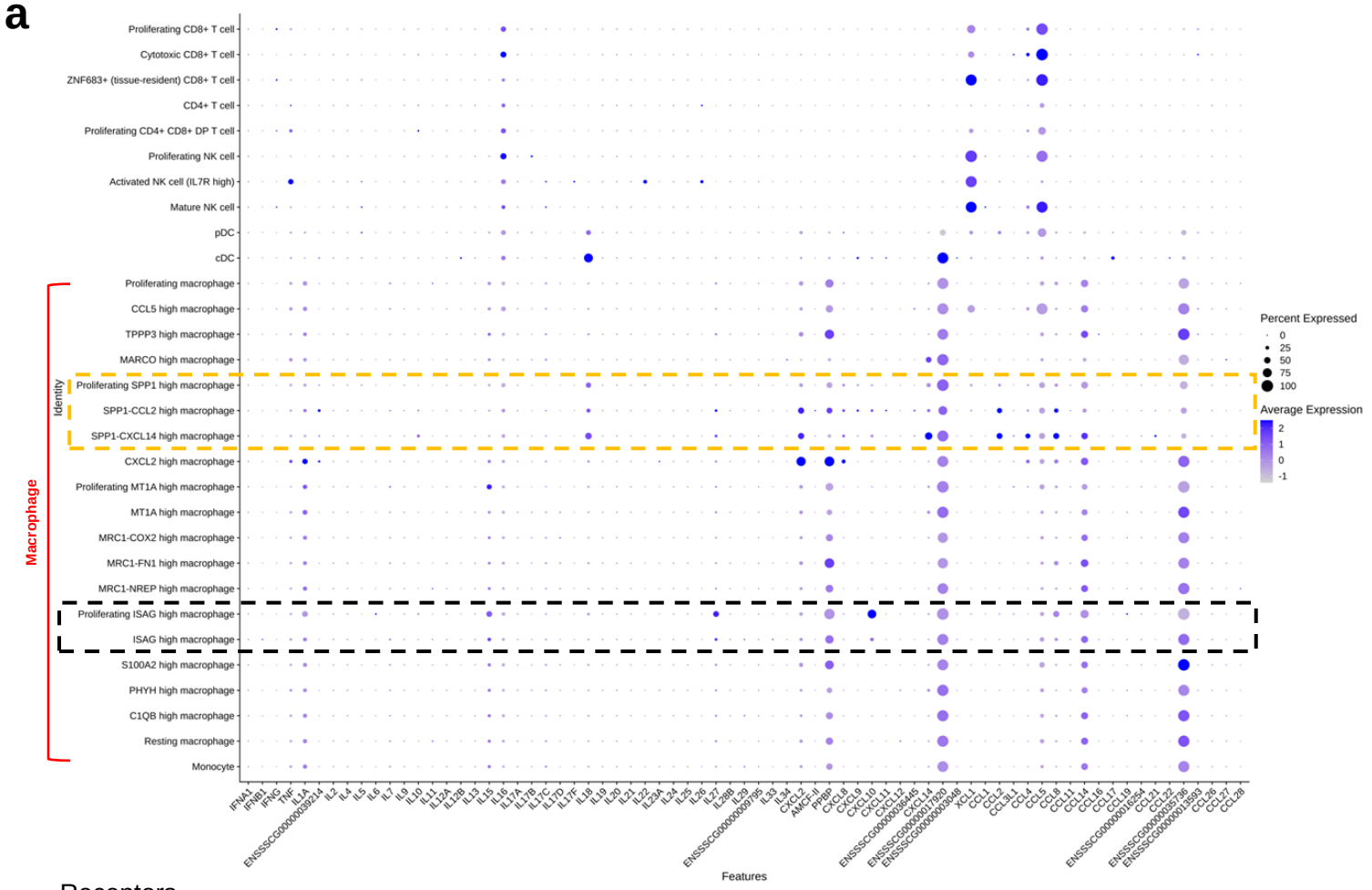
Receptors



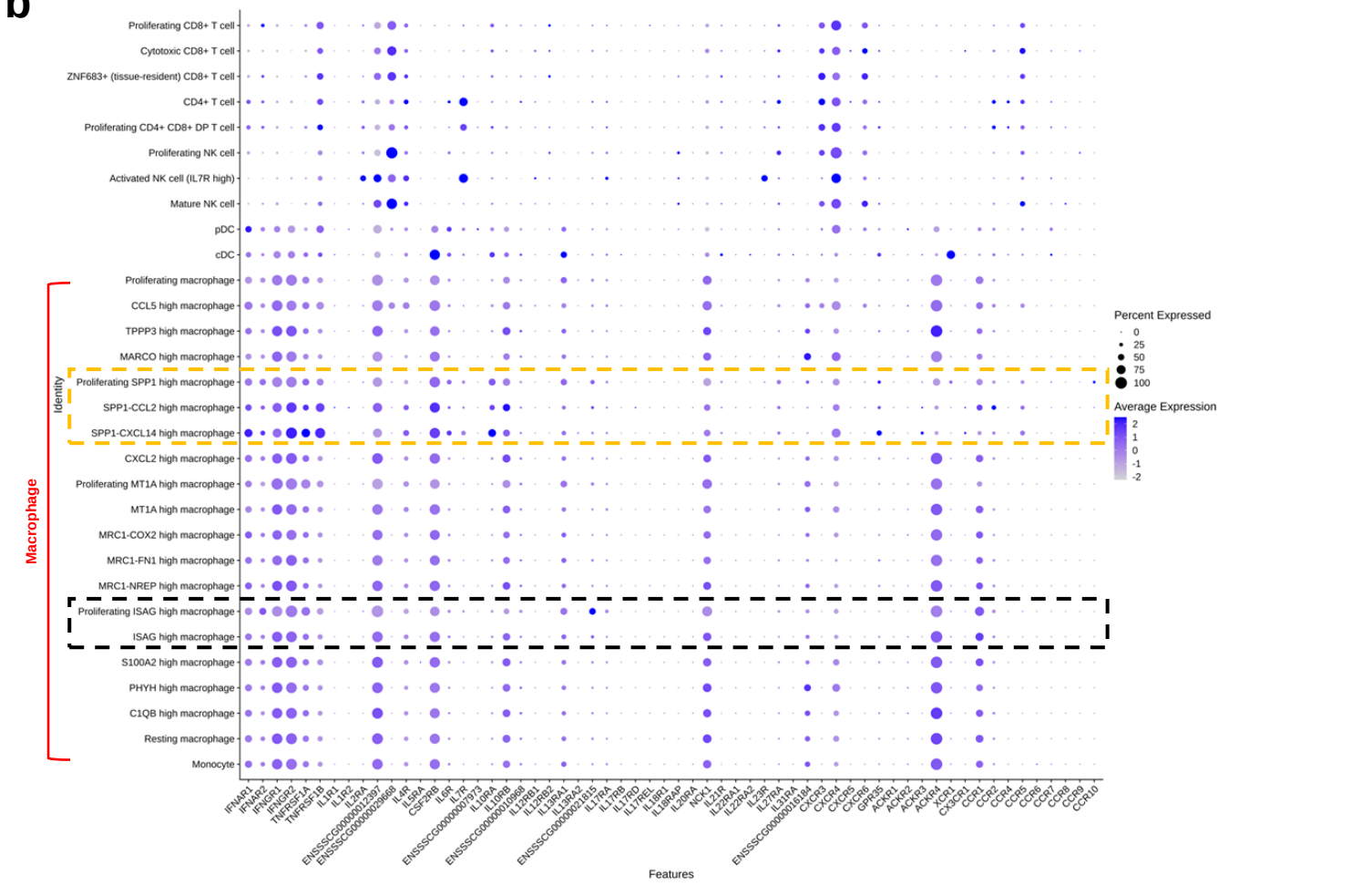
Supplementary Fig. 20 RNA expression levels for ligands (cytokines and chemokines) and their receptors in the negative group 14 dpi. a Cytokines and chemokines, and **b** their receptors. The yellow dotted line indicates SPP1-expressing macrophages and the black dotted line indicates ISAG-expressing macrophages. Average expression levels were calculated using log-normalized values. To eliminate bias from a small number of cells, only sub-cell types with more than 20 cells were included in the dot plot.

Negative 21 dpi

Cytokines & chemokines (ligands)



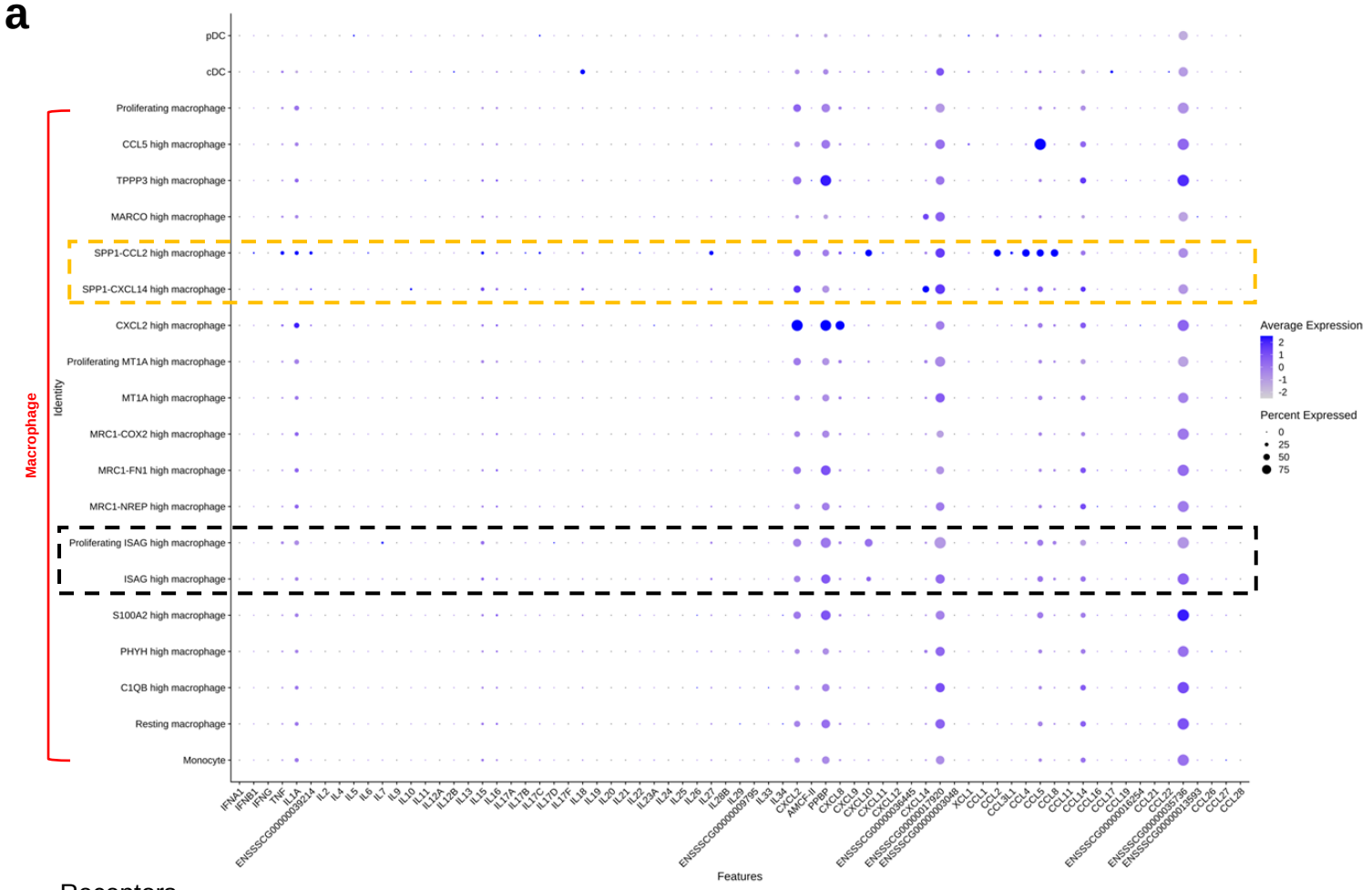
Receptors



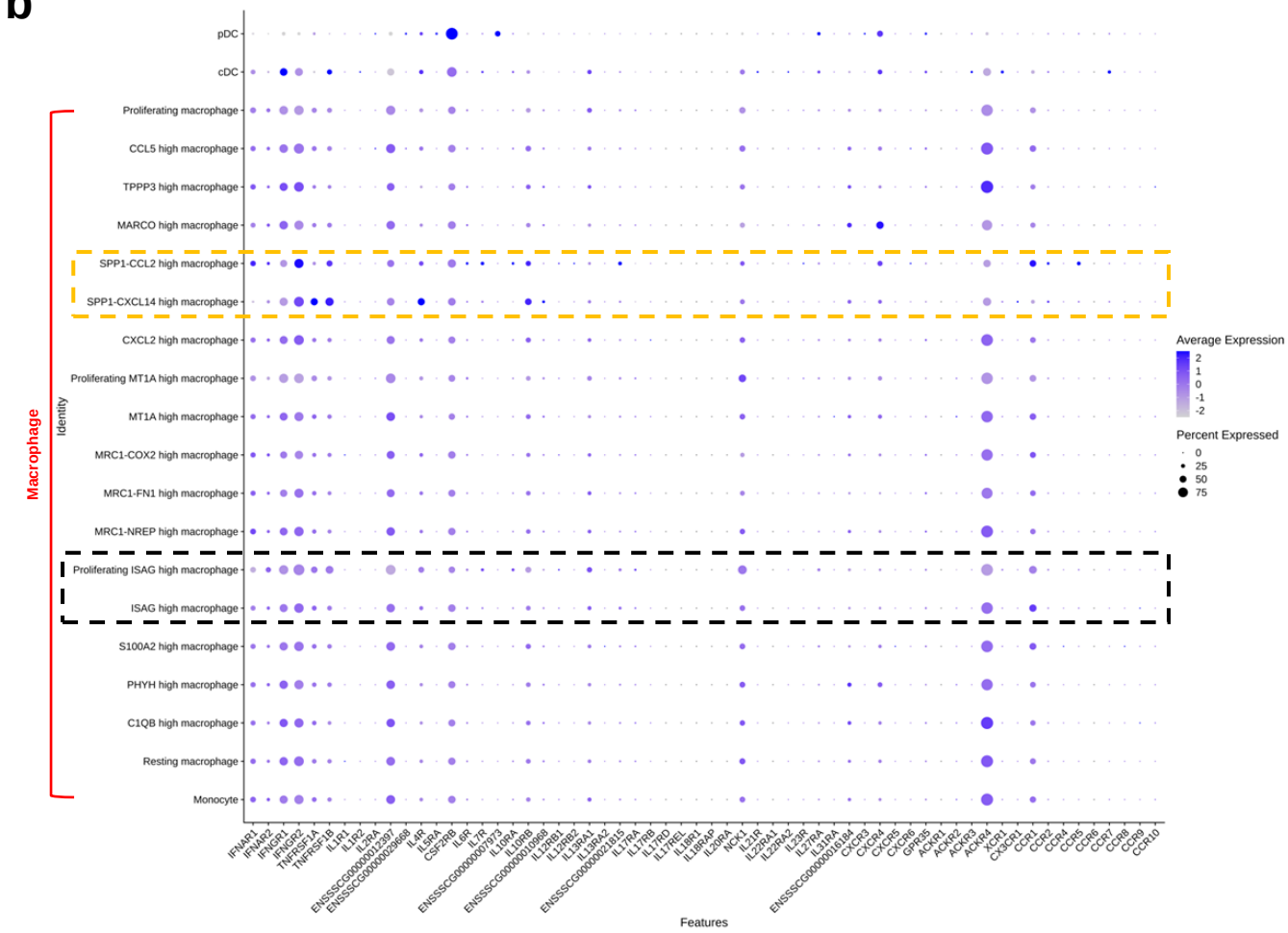
Supplementary Fig. 21 RNA expression levels for ligands (cytokines and chemokines) and their receptors in the negative group 21 dpi. a Cytokines and chemokines, and **b** their receptors. The yellow dotted line indicates SPP1-expressing macrophages and the black dotted line indicates ISAG-expressing macrophages. Average expression levels were calculated using log-normalized values. To eliminate bias from a small number of cells, only sub-cell types with more than 20 cells were included in the dot plot.

NA8 3 dpi

Cytokines & chemokines (ligands)



Receptors

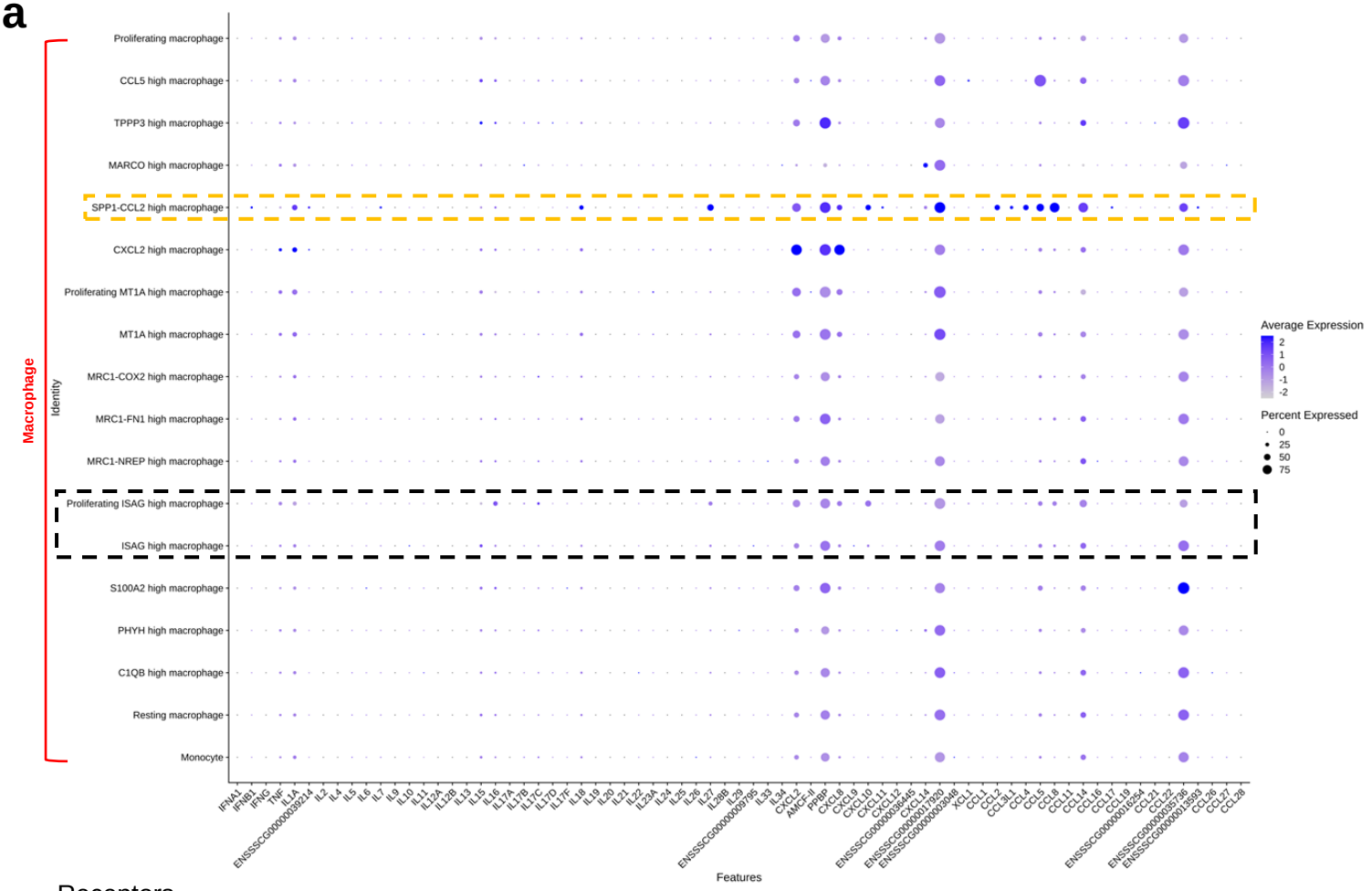


Supplementary Fig. 22 RNA expression levels for ligands (cytokines and chemokines) and their receptors in the NA8 group 3 dpi. a Cytokines and chemokines, and **b** their receptors. The yellow dotted line indicates SPP1-expressing macrophages and the black dotted line indicates ISAG-expressing macrophages. Average expression levels were calculated using log-normalized values. To eliminate bias from a small number of cells, only sub-cell types with more than 20 cells were included in the dot plot.

NA8 7 dpi

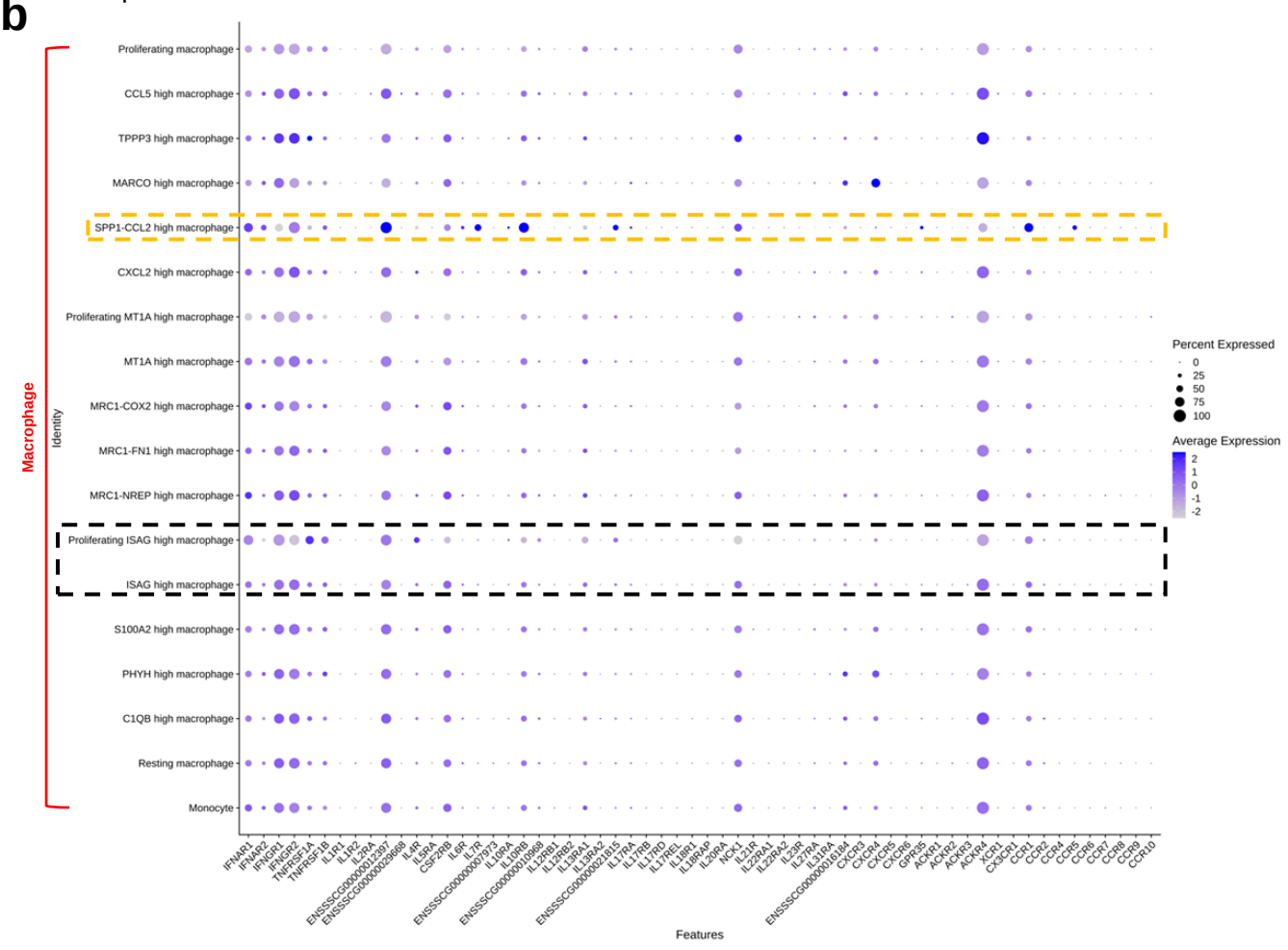
a

Cytokines & chemokines (ligands)



b

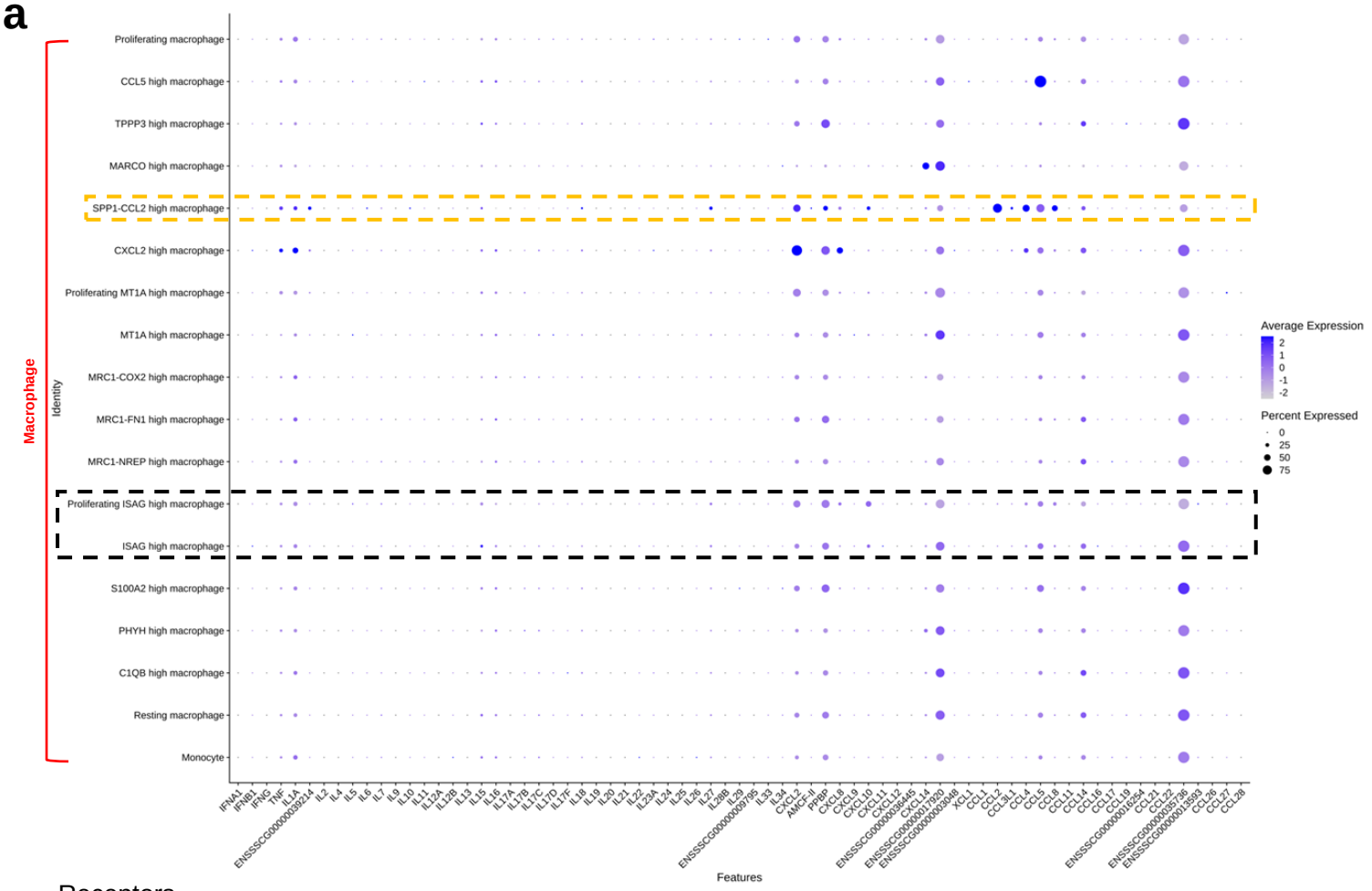
Receptors



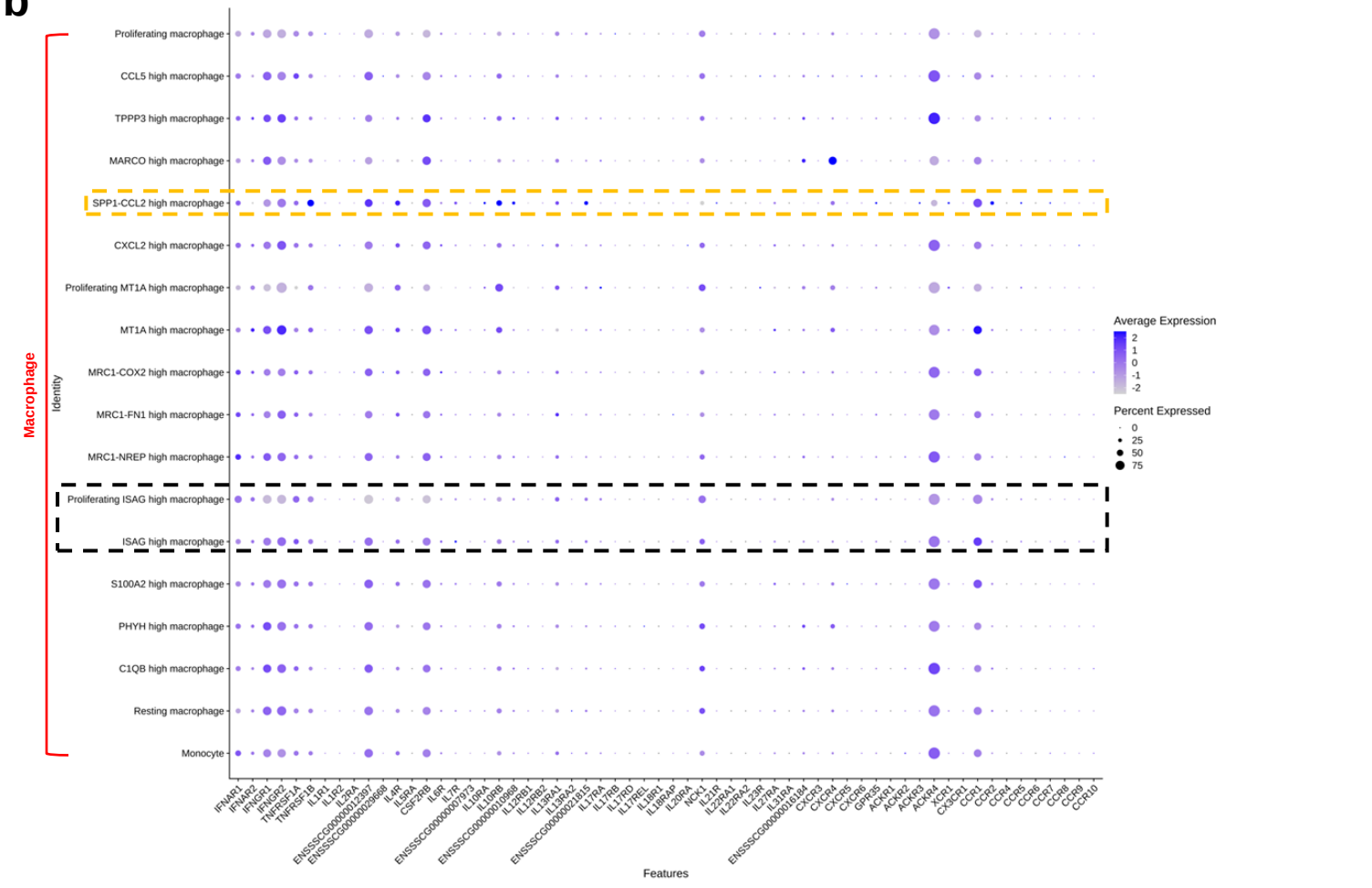
Supplementary Fig. 23 RNA expression levels for ligands (cytokines and chemokines) and their receptors in the NA8 group 7 dpi. a Cytokines and chemokines, and **b** their receptors. The yellow dotted line indicates SPP1-expressing macrophages and the black dotted line indicates ISAG-expressing macrophages. Average expression levels were calculated using log-normalized values. To eliminate bias from a small number of cells, only sub-cell types with more than 20 cells were included in the dot plot.

JA142 3 dpi

Cytokines & chemokines (ligands)



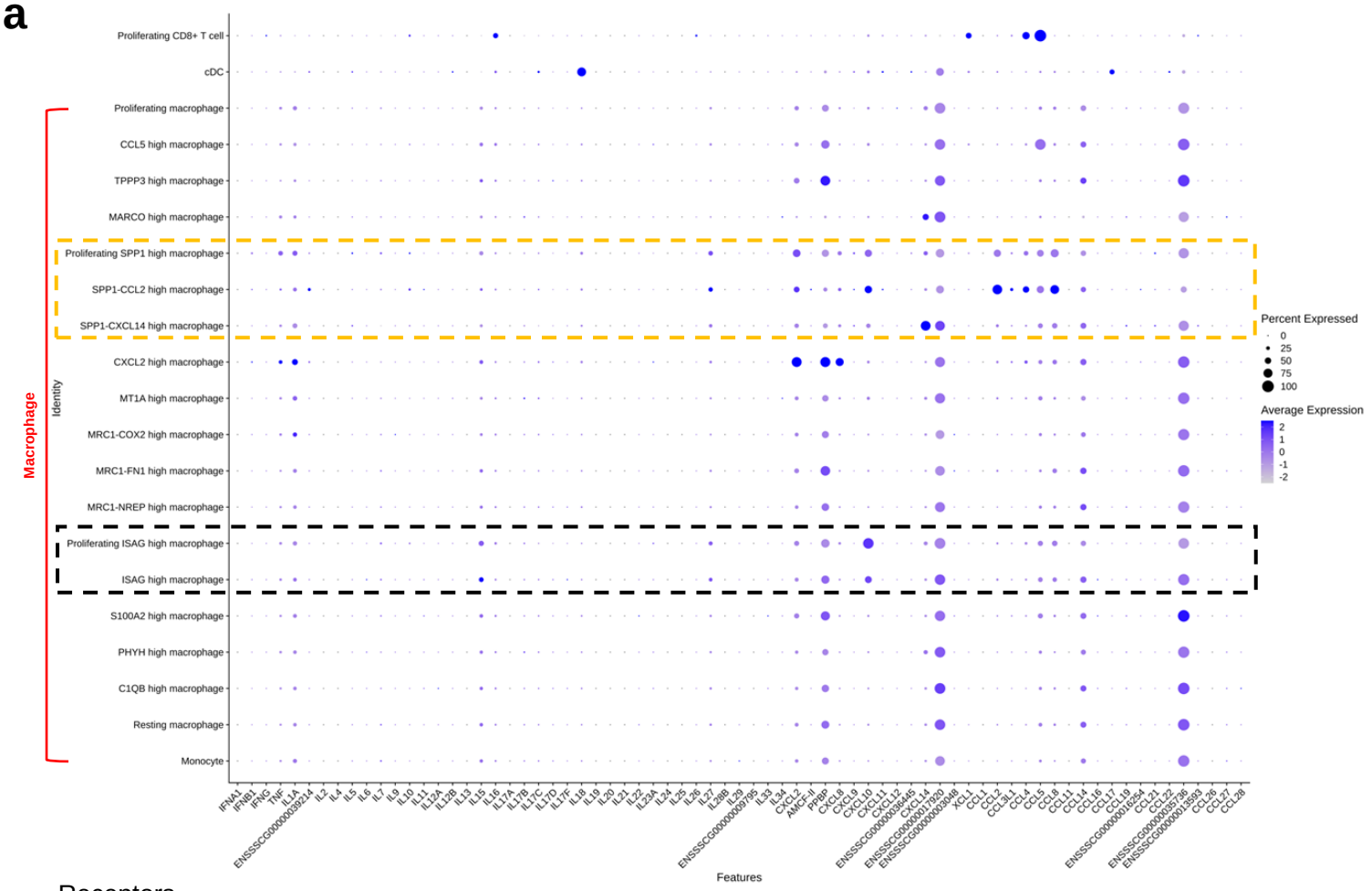
Receptors



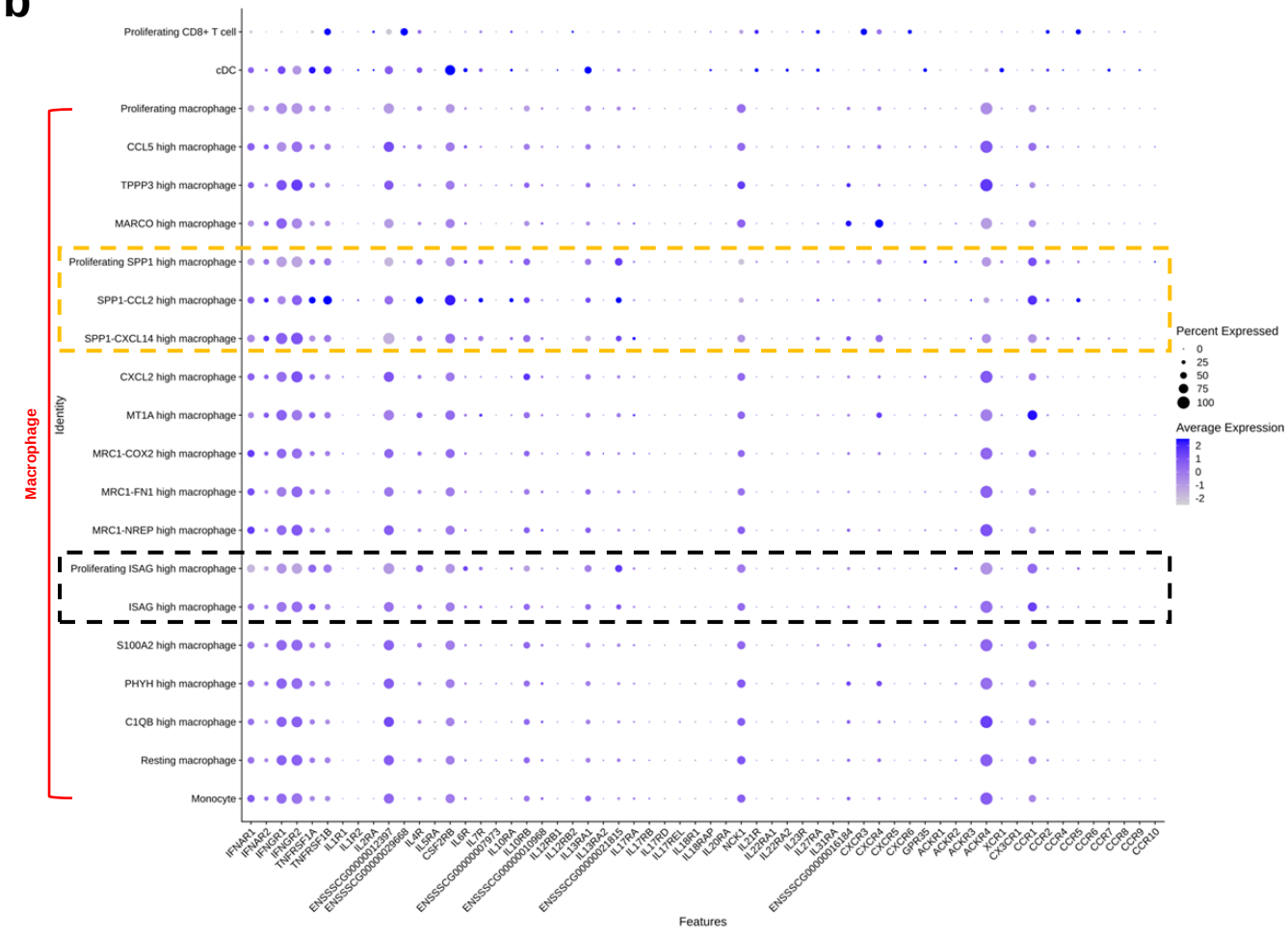
Supplementary Fig. 24 RNA expression levels for ligands (cytokines and chemokines) and their receptors in the JA142 group 3 dpi. a Cytokines and chemokines, and **b** their receptors. The yellow dotted line indicates SPP1-expressing macrophages and the black dotted line indicates ISAG-expressing macrophages. Average expression levels were calculated using log-normalized values. To eliminate bias from a small number of cells, only sub-cell types with more than 20 cells were included in the dot plot.

JA142 7 dpi

Cytokines & chemokines (ligands)



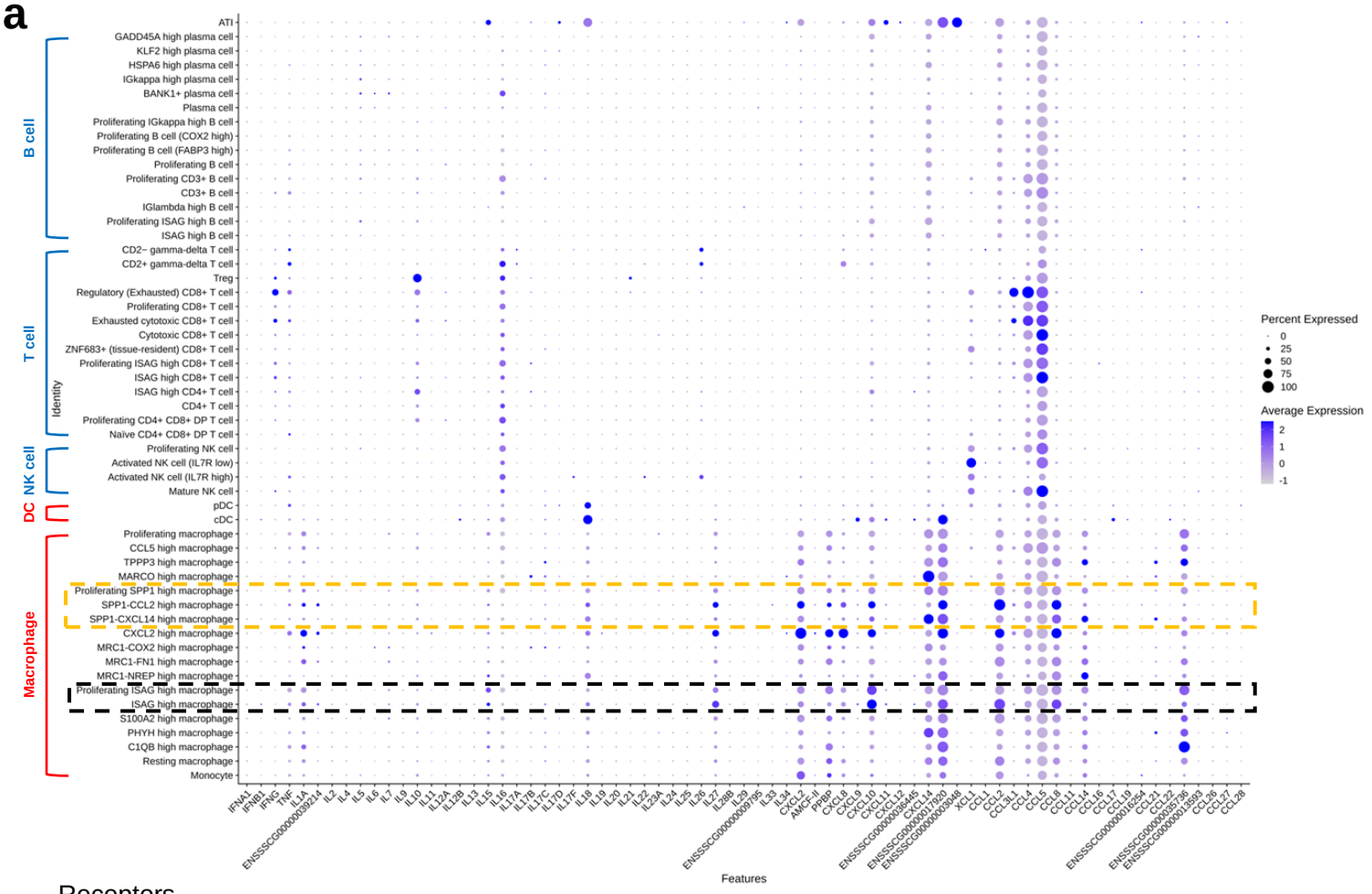
Receptors



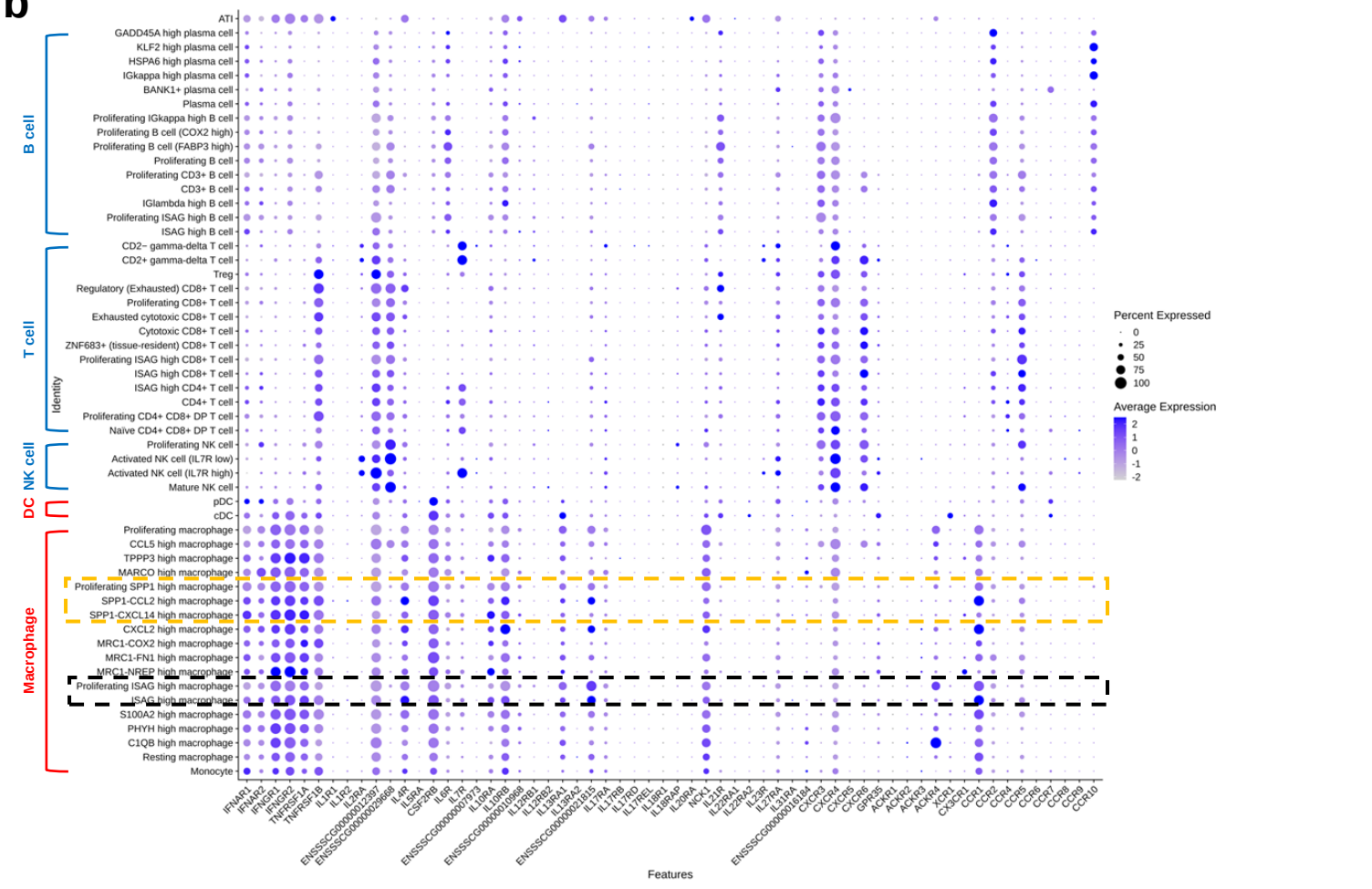
Supplementary Fig. 25 RNA expression levels for ligands (cytokines and chemokines) and their receptors in the JA142 group 7 dpi. a Cytokines and chemokines, and **b** their receptors. The yellow dotted line indicates SPP1-expressing macrophages and the black dotted line indicates ISAG-expressing macrophages. Average expression levels were calculated using log-normalized values. To eliminate bias from a small number of cells, only sub-cell types with more than 20 cells were included in the dot plot.

JA142 14 dpi

Cytokines & chemokines (ligands)



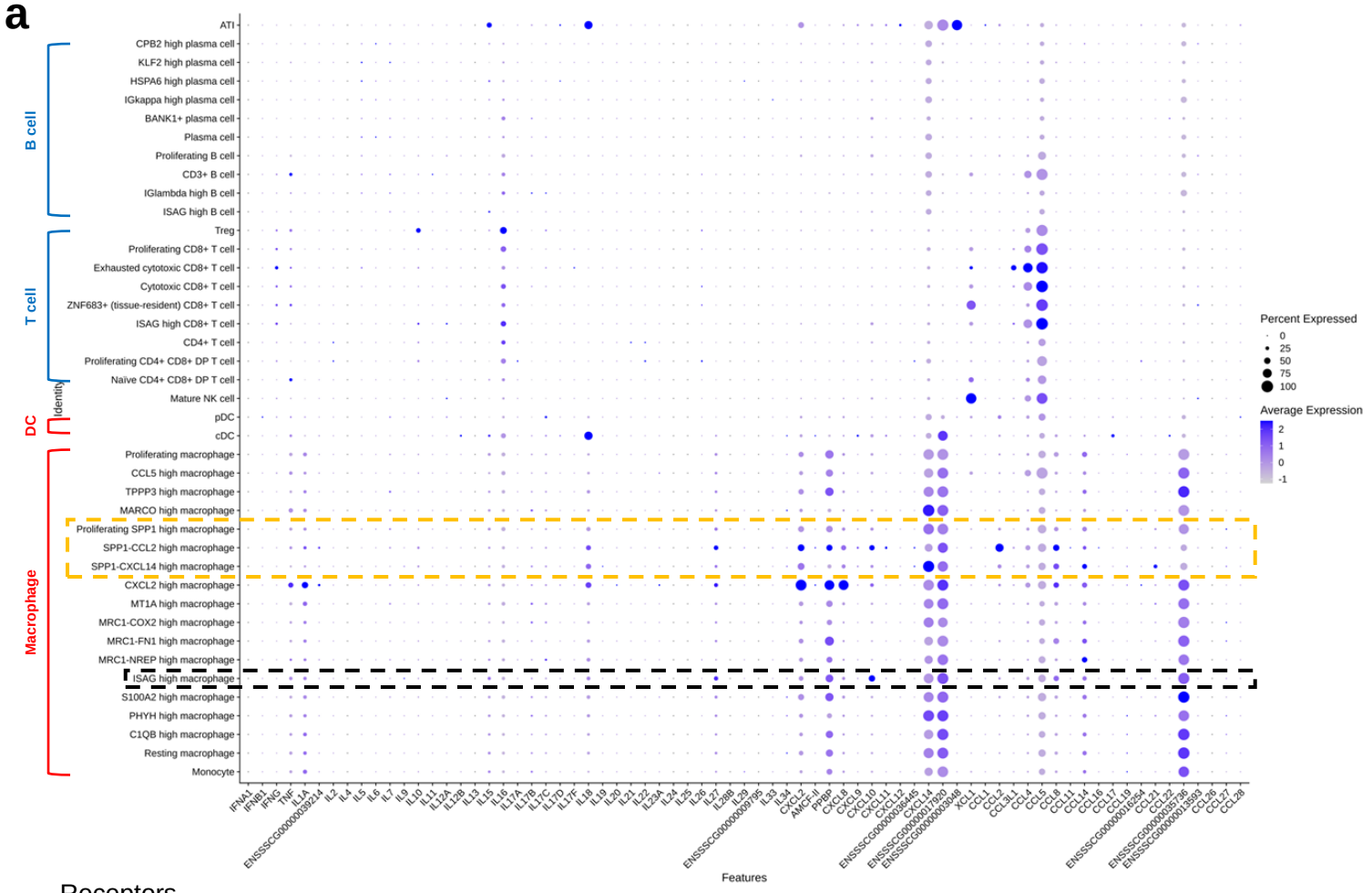
Receptors



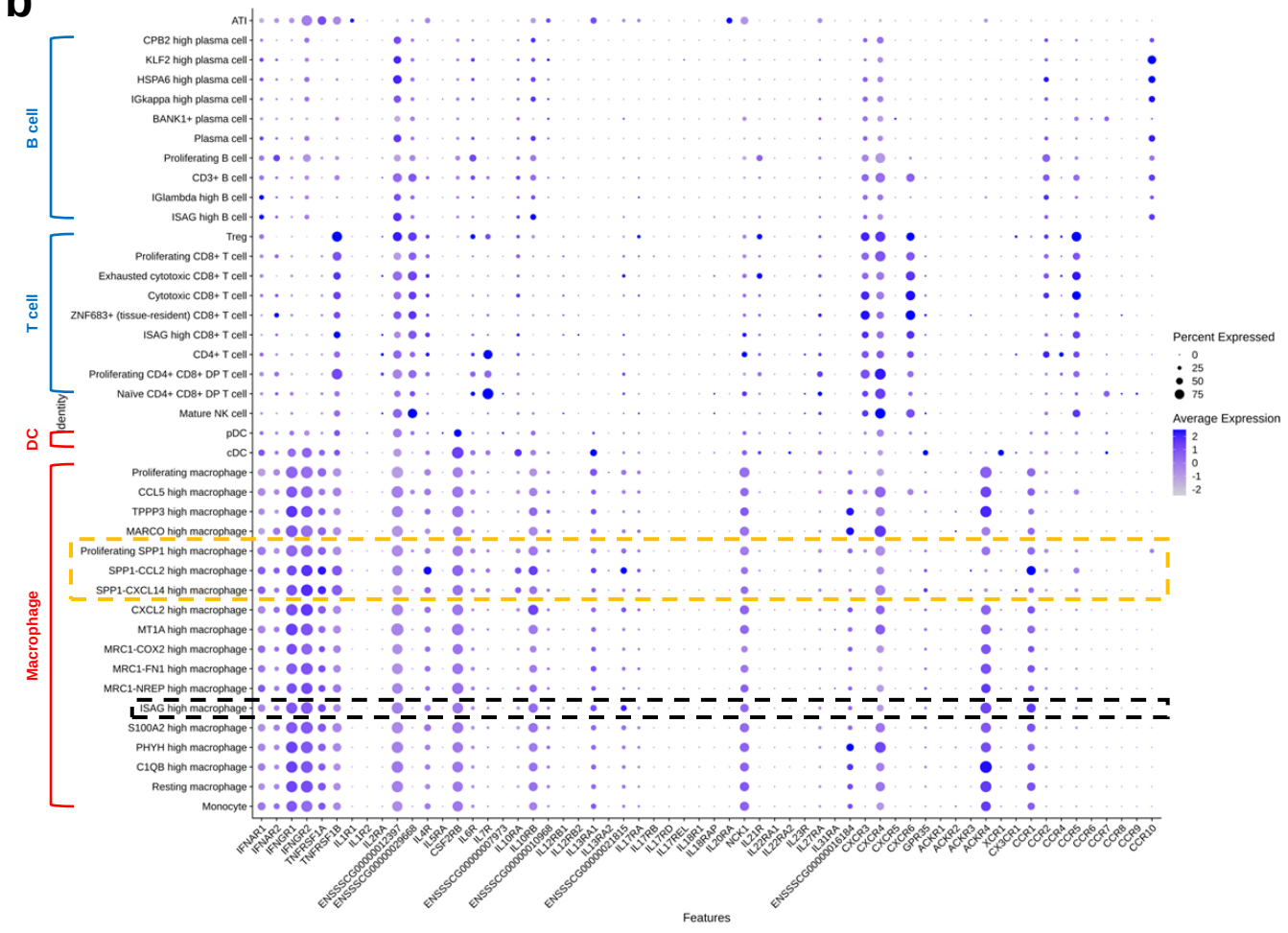
Supplementary Fig. 26 RNA expression levels for ligands (cytokines and chemokines) and their receptors in the JA142 group 14 dpi. a Cytokines and chemokines, and **b** their receptors. The yellow dotted line indicates SPP1-expressing macrophages and the black dotted line indicates ISAG-expressing macrophages. Average expression levels were calculated using log-normalized values. To eliminate bias from a small number of cells, only sub-cell types with more than 20 cells were included in the dot plot.

JA142 21 dpi

Cytokines & chemokines (ligands)



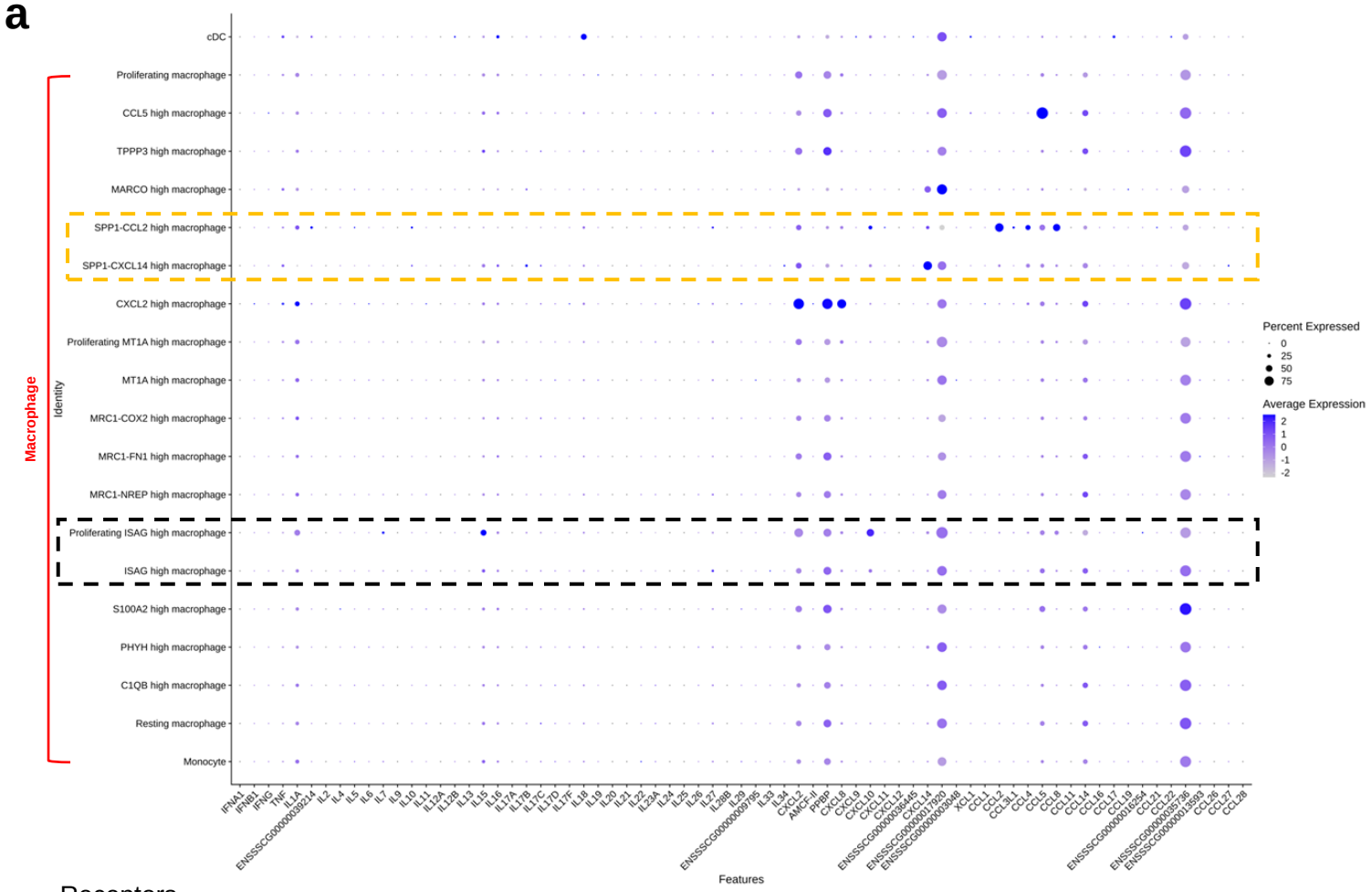
Receptors



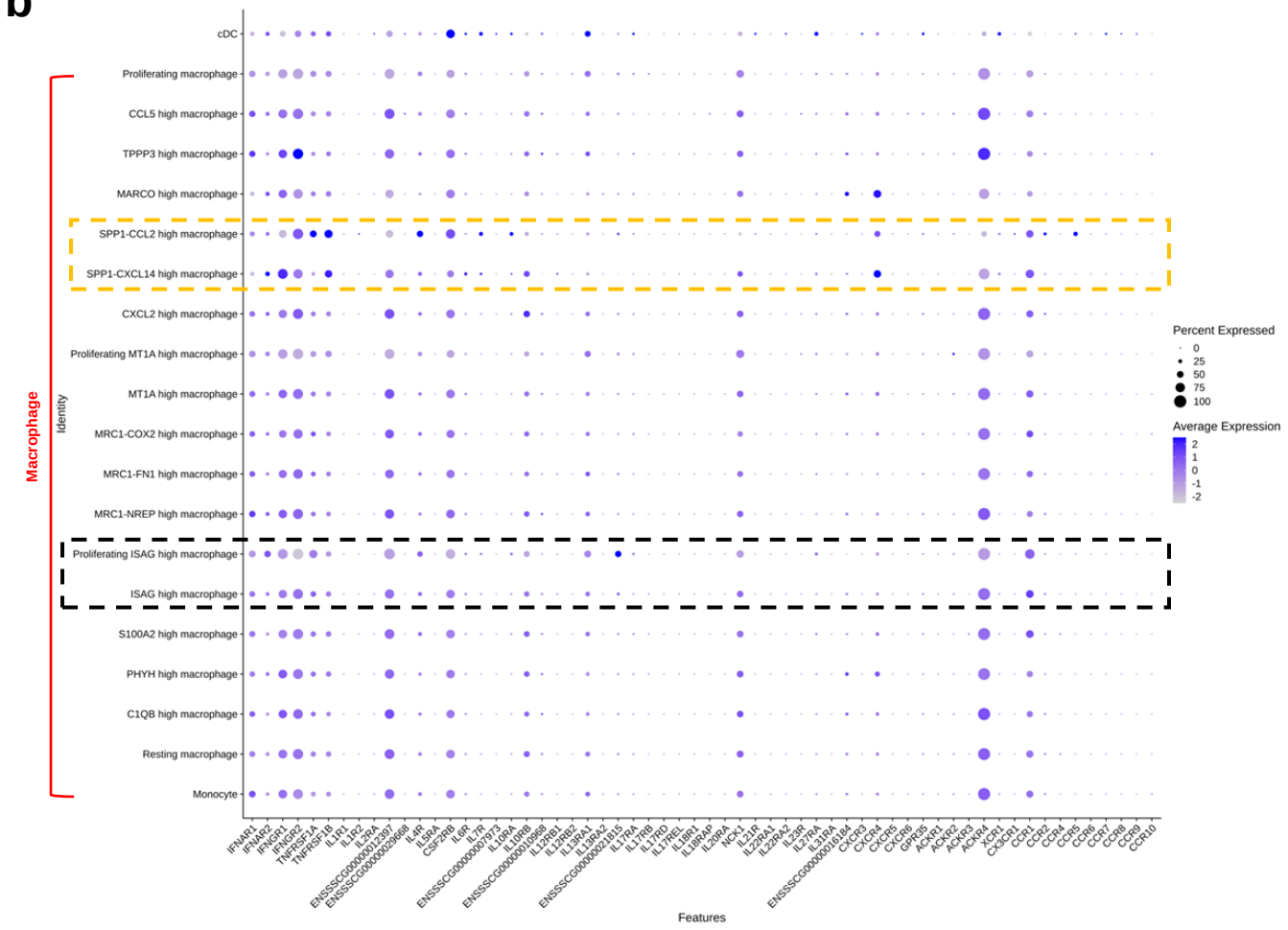
Supplementary Fig. 27 RNA expression levels for ligands (cytokines and chemokines) and their receptors in the JA142 group 21 dpi. a Cytokines and chemokines, and **b** their receptors. The yellow dotted line indicates SPP1-expressing macrophages and the black dotted line indicates ISAG-expressing macrophages. Average expression levels were calculated using log-normalized values. To eliminate bias from a small number of cells, only sub-cell types with more than 20 cells were included in the dot plot.

NA10 3 dpi

Cytokines & chemokines (ligands)



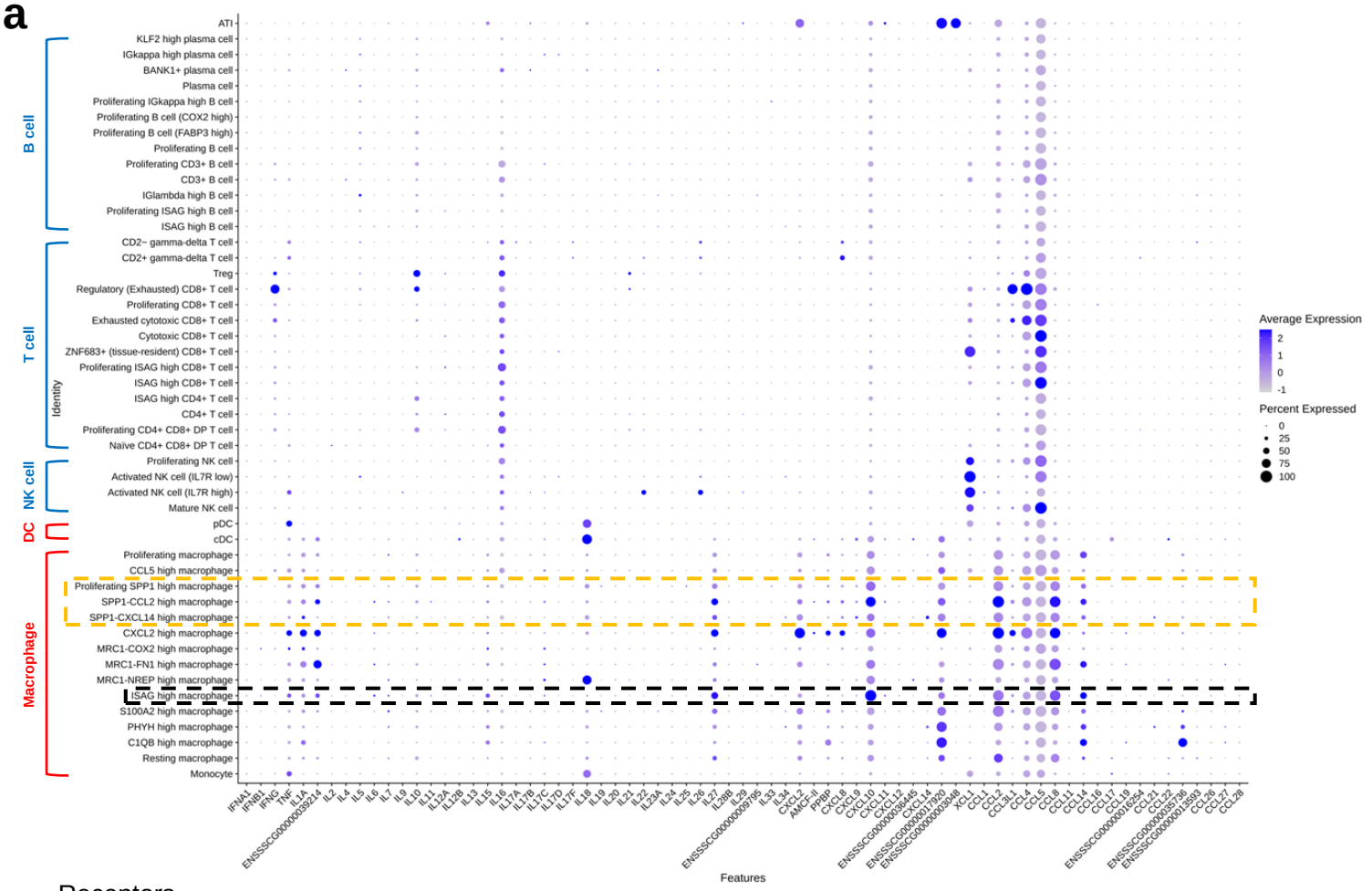
Receptors



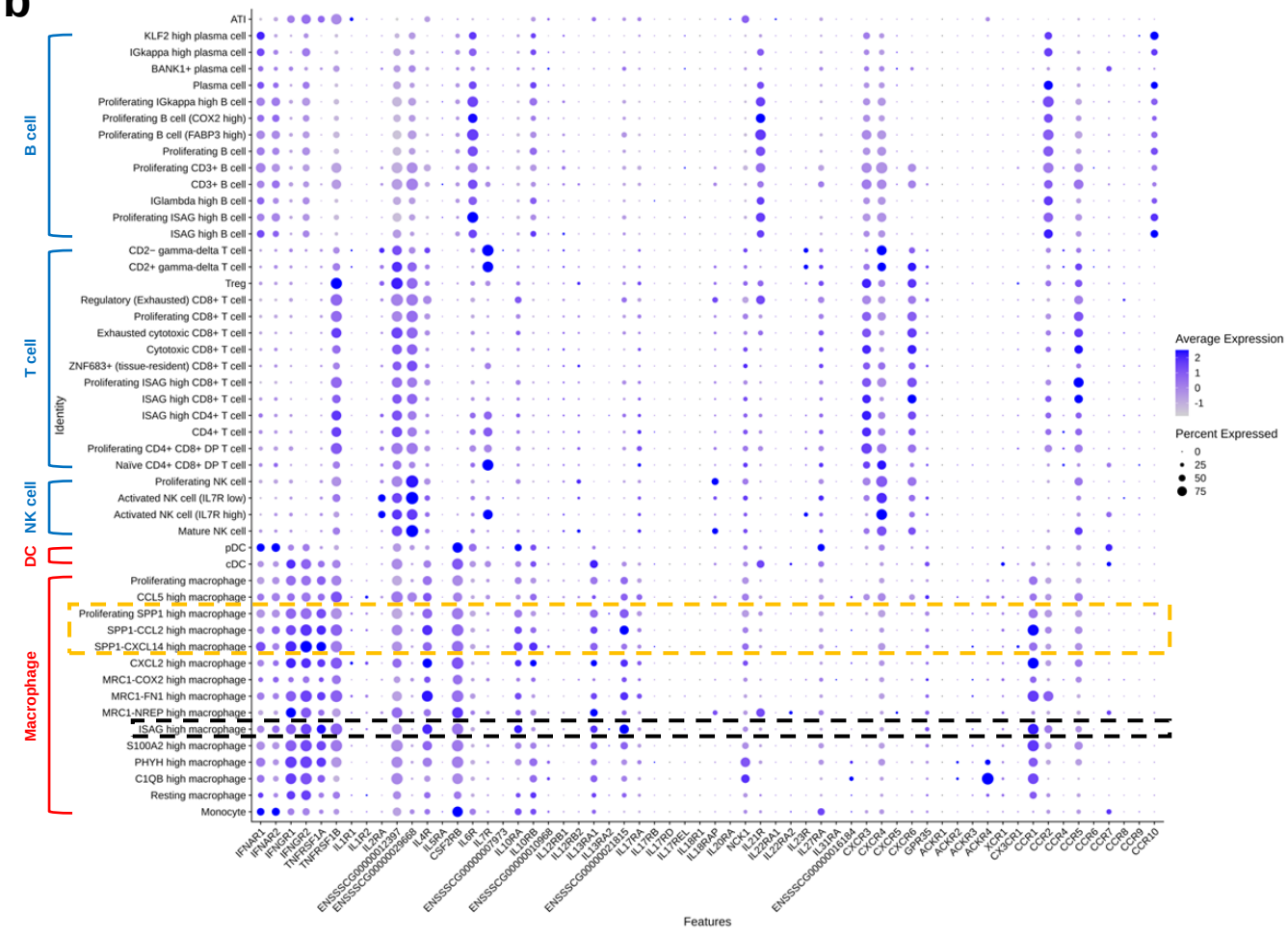
Supplementary Fig. 28 RNA expression levels for ligands (cytokines and chemokines) and their receptors in the NA10 group 3 dpi. a Cytokines and chemokines, and **b** their receptors. The yellow dotted line indicates SPP1-expressing macrophages and the black dotted line indicates ISAG-expressing macrophages. Average expression levels were calculated using log-normalized values. To eliminate bias from a small number of cells, only sub-cell types with more than 20 cells were included in the dot plot.

NA10 7 dpi

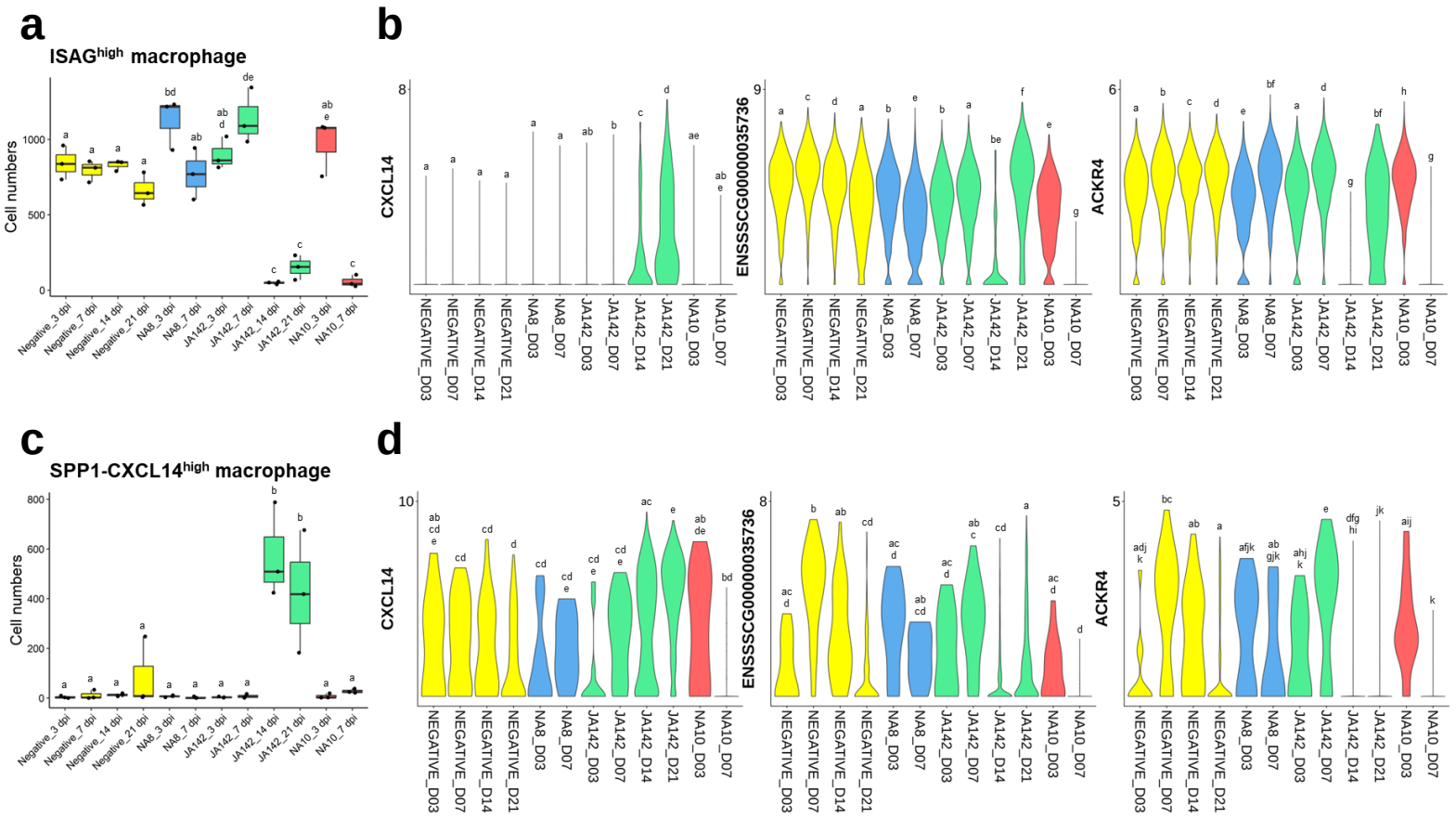
Cytokines & chemokines (ligands)



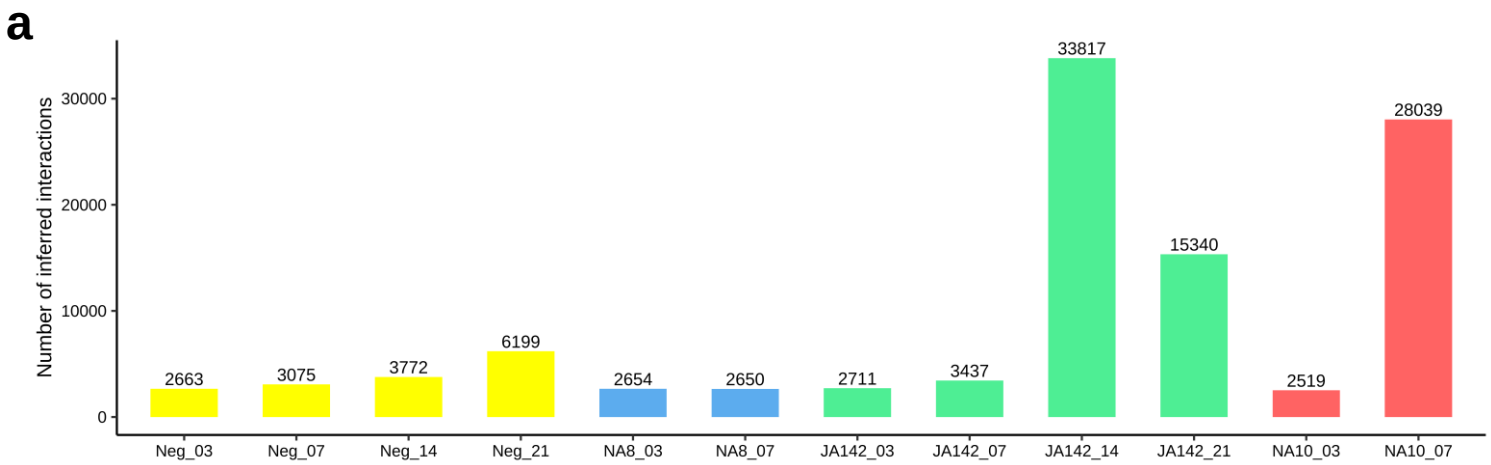
Receptors

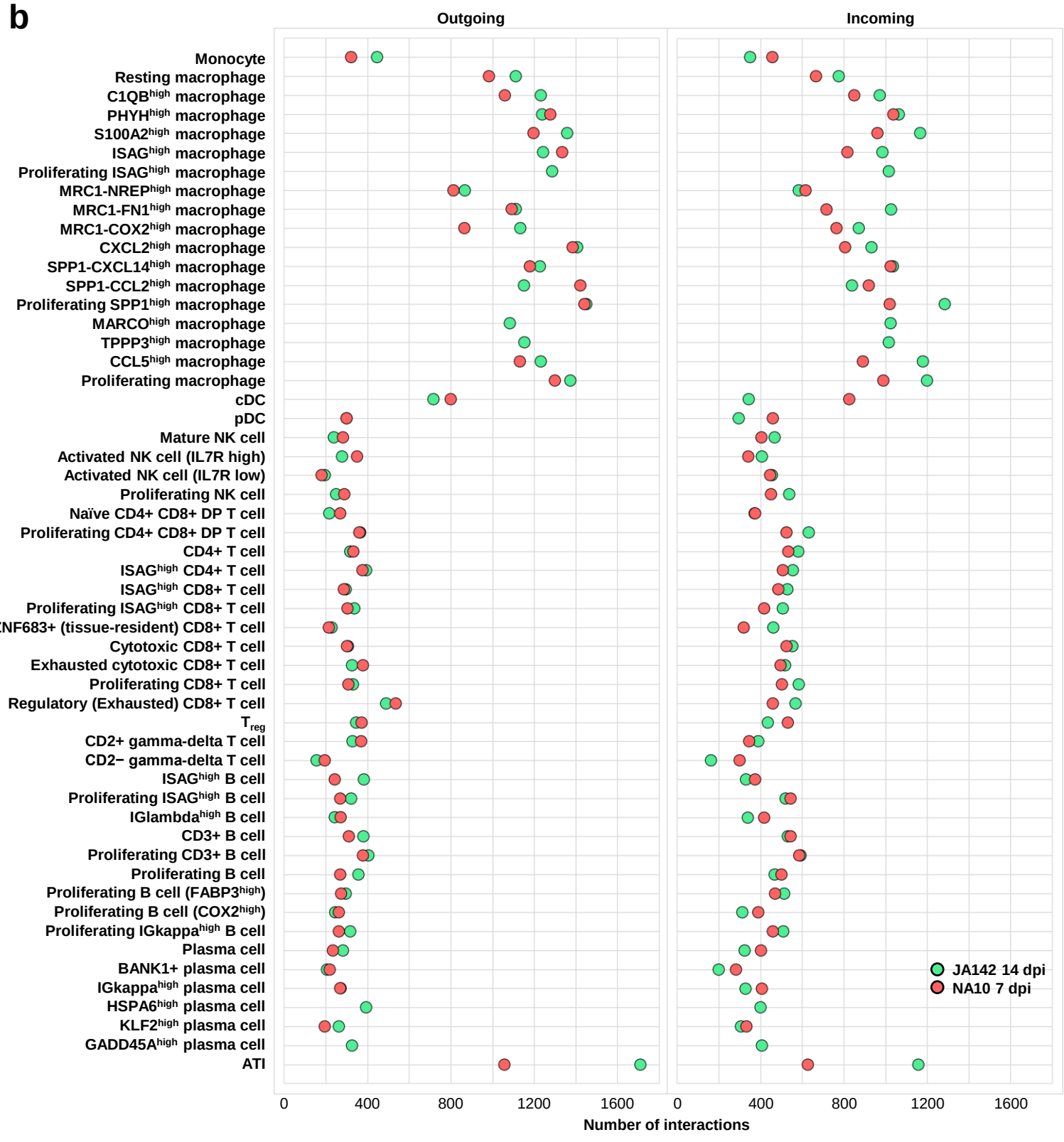


Supplementary Fig. 29 RNA expression levels for ligands (cytokines and chemokines) and their receptors in the NA10 group 7 dpi. a Cytokines and chemokines, and **b** their receptors. The yellow dotted line indicates SPP1-expressing macrophages and the black dotted line indicates ISAG-expressing macrophages. Average expression levels were calculated using log-normalized values. To eliminate bias from a small number of cells, only sub-cell types with more than 20 cells were included in the dot plot.

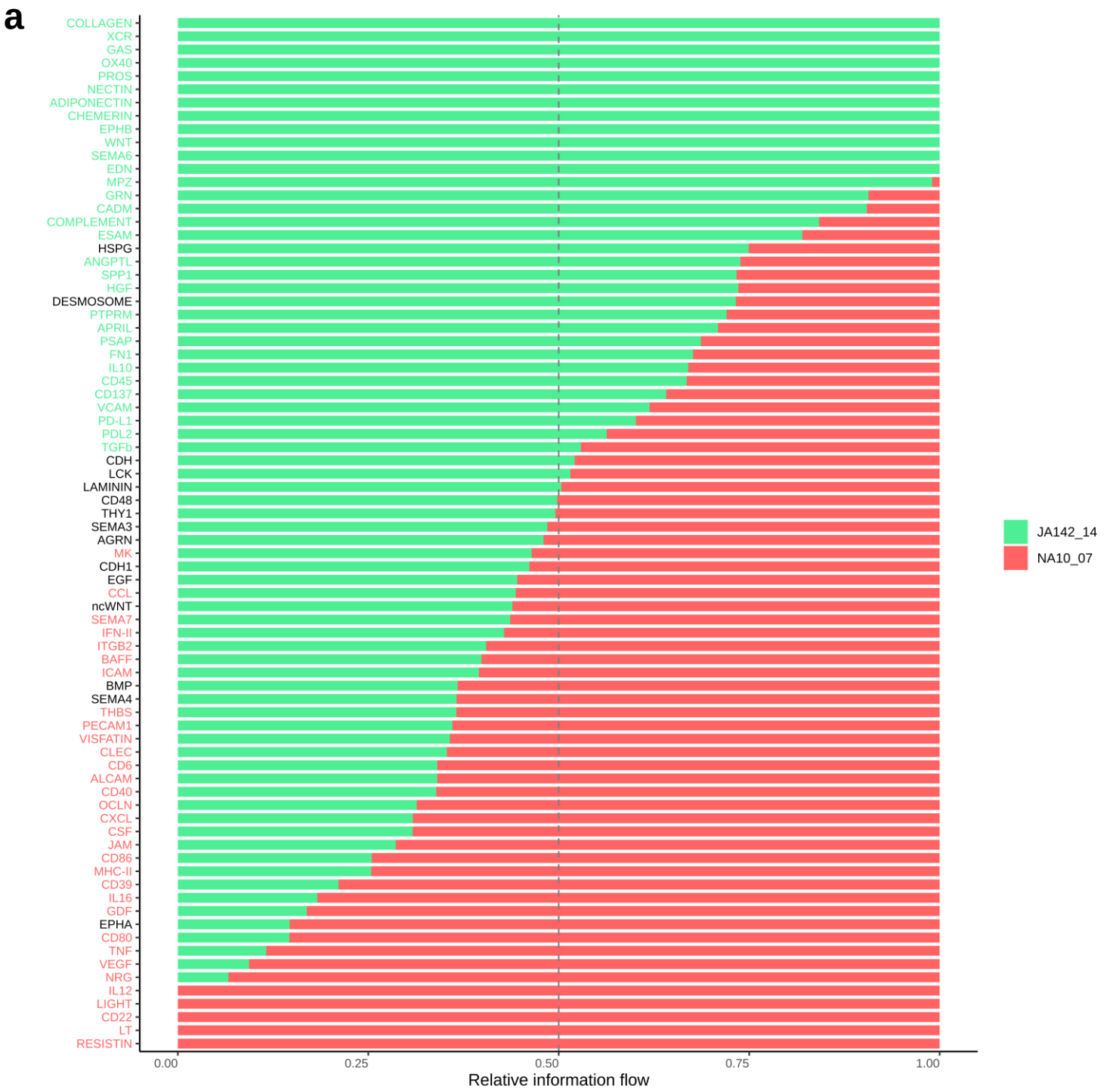


Supplementary Fig. 30 Cell numbers and RNA expression in specific sub-cell types. a Cell numbers of ISAG^{high} macrophages. **b** RNA expression of key genes based on the differential expression gene analyses in each virulent type. **c** Cell numbers of SPP1-CXCL14^{high} macrophages. **d** RNA expression of key genes based on the differential expression gene analyses in each virulent type. **a, c** Each group is depicted using a box-and-whisker plot, illustrating three biological replications. The lower boundary of the box corresponds to the first quartile (Q1), the middle line marks the median, and the upper boundary represents the third quartile (Q3). The whiskers extend to indicate the minimum and maximum values within the dataset, respectively. Different lowercase letters above box indicate significant differences ($p < 0.05$) determined using one-way ANOVA analysis with Tukey's multiple comparison test. **b, d** Different lowercase letters above violin indicate significant differences ($p < 0.05$) determined using one-way ANOVA analysis with Tukey's multiple comparison test. Source data are provided as a Source Data file.

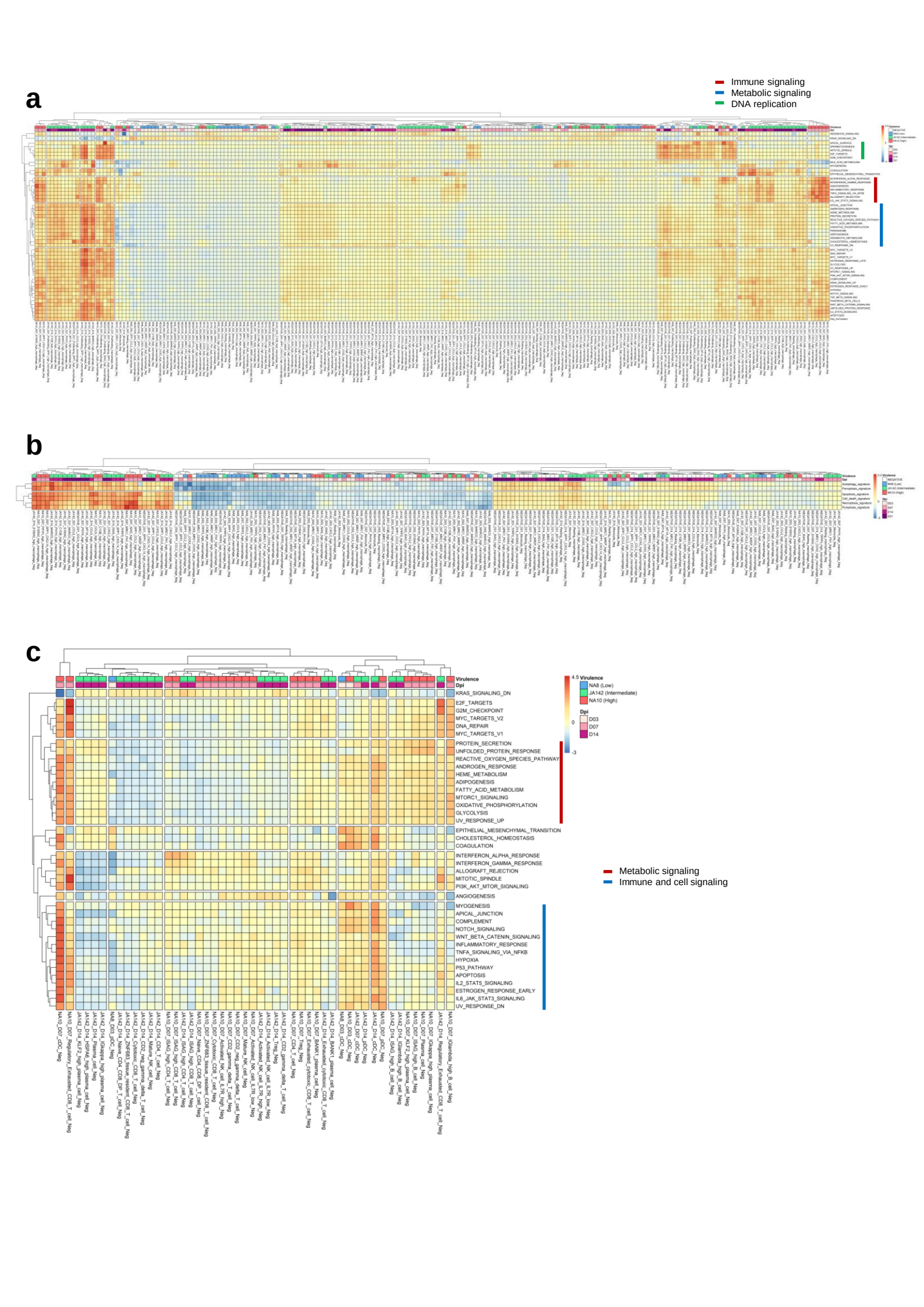

a
Number of inferred interactions
30000
20000
10000
0
2663
3075
3772
6199
2654
2650
2711
3437
33817
15340
2519
28039
Neg_03
Neg_07
Neg_14
Neg_21
NA8_03
NA8_07
JA142_03
JA142_07
JA142_14
JA142_21
NA10_03
NA10_07


b
Outgoing
Incoming
Monocyte
Resting macrophage
C1QB^high macrophage
PHYH^high macrophage
S100A2^high macrophage
ISAG^high macrophage
Proliferating ISAG^high macrophage
MRC1-NREP^high macrophage
MRC1-FN1^high macrophage
MRC1-COX2^high macrophage
CXCL2^high macrophage
SPP1-CXCL14^high macrophage
SPP1-CCL2^high macrophage
Proliferating SPP1^high macrophage
MARCO^high macrophage
TPPP3^high macrophage
CCL5^high macrophage
Proliferating macrophage
cDC
pDC
Mature NK cell
Activated NK cell (IL7R high)
Activated NK cell (IL7R low)
Proliferating NK cell
Naïve CD4+ CD8+ DP T cell
Proliferating CD4+ CD8+ DP T cell
CD4+ T cell
ISAG^high CD4+ T cell
ISAG^high CD8+ T cell
Proliferating ISAG^high CD8+ T cell
ZNF683+ (tissue-resident) CD8+ T cell
Cytotoxic CD8+ T cell
Exhausted cytotoxic CD8+ T cell
Proliferating CD8+ T cell
Regulatory (Exhausted) CD8+ T cell
T_reg
CD2+ gamma-delta T cell
CD2− gamma-delta T cell
ISAG^high B cell
Proliferating ISAG^high B cell
IGlambda^high B cell
CD3+ B cell
Proliferating CD3+ B cell
Proliferating B cell
Proliferating B cell (FABP3^high)
Proliferating B cell (COX2^high)
Proliferating IGkappa^high B cell
Plasma cell
BANK1+ plasma cell
IGkappa^high plasma cell
HSPA6^high plasma cell
KLF2^high plasma cell
GADD45A^high plasma cell
ATI
JA142 14 dpi
NA10 7 dpi
0
400
800
1200
1600
Number of interactions

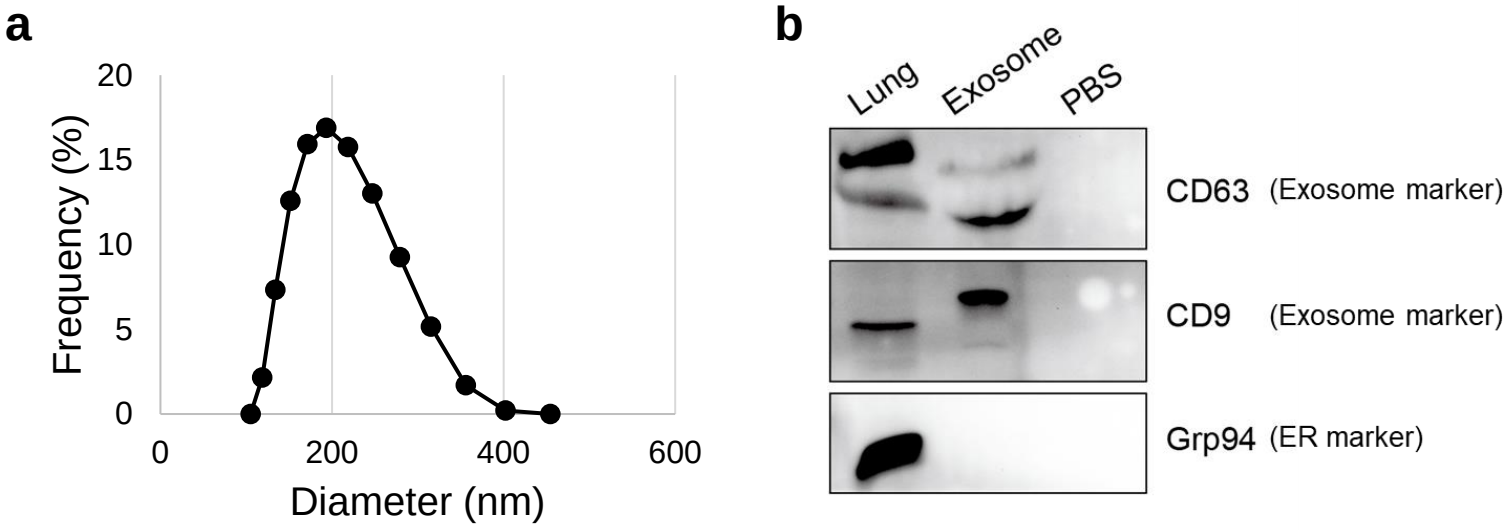
Supplementary Fig. 31 Predicted interactions (ligand-receptor pairs) and signals against PRRSV infection. a The number of interactions for each time point and group. **b** The number of sent (outgoing) and received (incoming) signals for each sub-cell type in the JA142 14 dpi and the NA10 7 dpi.


a
COLLAGEN
XCR
GAS
OX40
PROS
NECTIN
ADIPONECTIN
CHEMERIN
EPHB
WNT
SEMA6
EDN
MPZ
GRN
CADM
COMPLEMENT
ESAM
HSPG
ANGPTL
SPP1
HGF
DESMOSOME
PTPRM
APRIL
PSAP
FN1
IL10
CD45
CD137
VCAM
PD-L1
PDL2
TGFb
CDH
LCK
LAMININ
CD48
THY1
SEMA3
AGRN
MK
CDH1
EGF
CCL
ncWNT
SEMA7
IFN-II
ITGB2
BAFF
ICAM
BMP
SEMA4
THBS
PECAM1
VISFATIN
CLEC
CD6
ALCAM
CD40
OCLN
CXCL
CSF
JAM
CD86
MHC-II
CD39
IL16
GDF
EPHA
CD80
TNF
VEGF
NRG
IL12
LIGHT
CD22
LT
RESISTIN
0.00
0.25
0.50
0.75
1.00
Relative information flow
JA142_14
NA10_07
b
SPP1 - CD44
SPP1 - (ITGAV+ITGB6)
SPP1 - (ITGAV+ITGB5)
SPP1 - (ITGAV+ITGB3)
SPP1 - (ITGAV+ITGB1)
SPP1 - (ITGA9+ITGB1)
SPP1 - (ITGA5+ITGB1)
SPP1 - (ITGA4+ITGB1)
HLA-DRA - CD4
CXCL10 - CXCR3
CCL8 - CCR2
CCL8 - CCR1
CCL8 - ACKR4
CCL5 - CCR5
CCL5 - CCR4
CCL5 - CCR1
CCL4 - CCR5
CCL2 - CCR2
CCL14 - CCR1
Monocyte
Resting macrophage
C1QB^high macrophage
PHYH^high macrophage
S100A2^high macrophage
ISAG^high macrophage
MRC1-NREP^high macrophage
MRC1-FN1^high macrophage
MRC1-COX2^high macrophage
CXCL2^high macrophage
SPP1-CXCL14^high macrophage
SPP1-CCL2^high macrophage
Proliferating SPP1^high macrophage
CCL5^high macrophage
Proliferating macrophage
cDC
pDC
Mature NK cell
Activated NK cell (IL7R^high)
Activated NK cell (IL7R^low)
Proliferating NK cell
Naive CD4+ CD8+ DP T cell
Proliferating CD4+ CD8+ DP T cell
CD4+ T cell
ISAG^high CD4+ T cell
ISAG^high CD8+ T cell
Proliferating ISAG^high CD8+ T cell
ZNF683+ (tissue-resident) CD8+ T cell
Cytotoxic CD8+ T cell
Exhausted cytotoxic CD8+ T cell
Proliferating CD8+ T cell
Regulatory (Exhausted) CD8+ T cell
T_reg
CD2+ gamma-delta T cell
CD2− gamma-delta T cell
ISAG^high B cell
Proliferating ISAG^high B cell
ISlambda^high B cell
CD3+ B cell
Proliferating CD3+ B cell
Proliferating B cell
Proliferating B cell (FABP3^high)
Proliferating B cell (COX2^high)
Proliferating IGkappa^high B cell
Plasma cell
BANK1+ plasma cell
IGkappa^high plasma cell
KLF2^high plasma cell
ATI
Commun. Prob.
max
min
p-value
0.01 < p < 0.05
p < 0.01

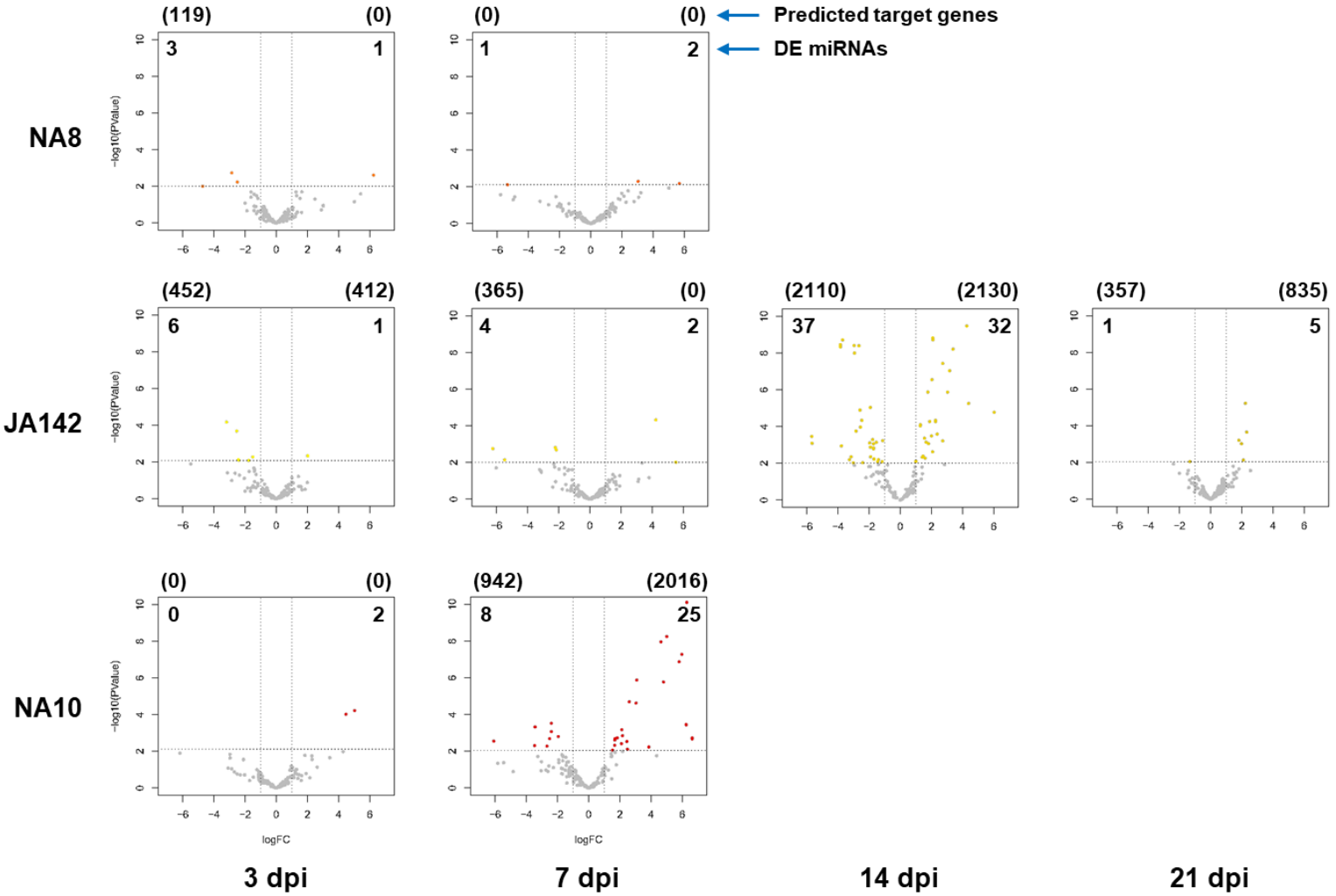
Supplementary Fig. 32 Difference of signaling interactions within BALF after intermediate (JA142 14 dpi) and high (NA10 7 dpi) PRRSV infection. a The sum of the interaction probability differences among whole pairs for each intermediate (JA142 14 dpi) and high (NA10 7 dpi) PRRSV infection group. **b** Dot plot for ligand-receptor pairs between SPP1-CXCL14^{high} macrophage and other sub-cell types. Significant ligands and receptors related SPP1, MHC-II, chemokine, and cytokine signaling are shown.


a
Immune signaling
Metabolic signaling
DNA replication
b
c
Virulence
Dpi
KRAS_SIGNALING_DN
E2F_TARGETS
G2M_CHECKPOINT
MYC_TARGETS_V2
DNA_REPAIR
MYC_TARGETS_V1
PROTEIN_SECRETION
UNFOLDED_PROTEIN_RESPONSE
REACTIVE_OXYGEN_SPECIES_PATHWAY
ANDROGEN_RESPONSE
HEME_METABOLISM
ADIPOGENESIS
FATTY_ACID_METABOLISM
MTORC1_SIGNALING
OXIDATIVE_PHOSPHORYLATION
GLYCOLYSIS
UV_RESPONSE_UP
EPITHELIAL_MESENCHYMAL_TRANSITION
CHOLESTEROL_HOMEOSTASIS
COAGULATION
INTERFERON_ALPHA_RESPONSE
INTERFERON_GAMMA_RESPONSE
ALLOGRAFT_REJECTION
MITOTIC_SPINDLE
PI3K_AKT_MTOR_SIGNALING
ANGIOGENESIS
MYOGENESIS
APICAL_JUNCTION
COMPLEMENT
NOTCH_SIGNALING
WNT_BETA_CATENIN_SIGNALING
INFLAMMATORY_RESPONSE
TNFA_SIGNALING_VIA_NFKB
HYPOXIA
P53_PATHWAY
APOPTOSIS
IL2_STAT5_SIGNALING
ESTROGEN_RESPONSE_EARLY
IL6_JAK_STAT3_SIGNALING
UV_RESPONSE_DN
4.5 Virulence
NA8 (Low)
JA142 (Intermediate)
NA10 (High)
0
-3
Dpi
D03
D07
D14
Metabolic signaling
Immune and cell signaling

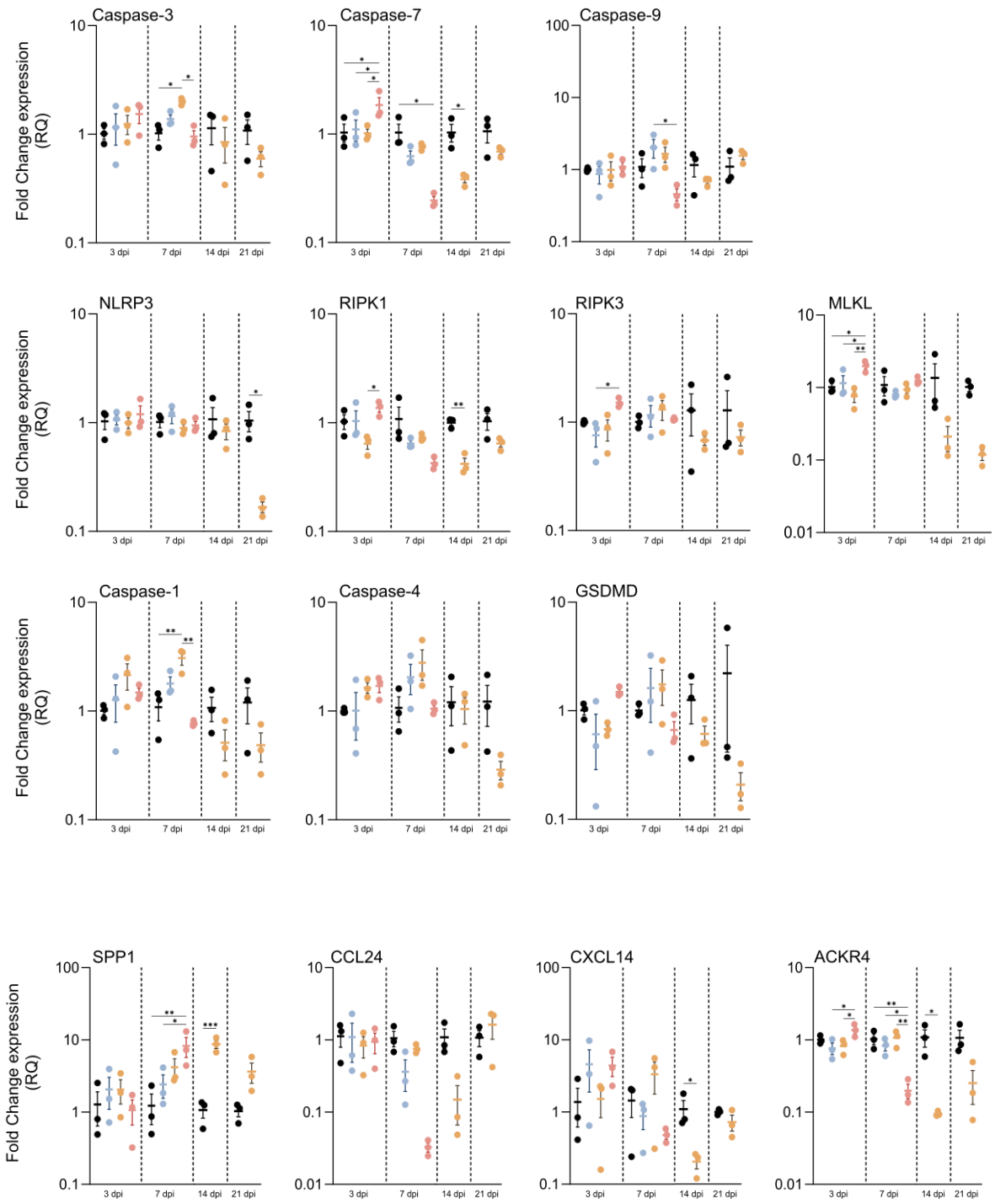
Supplementary Fig. 33 Gene set enrichment analysis of negative and bystander macrophages, and other sub-cell types (DCs, NK, T, and B cells) at each time point and for each virulent group. Functional enrichment based on the **a** Hallmark database in negative and bystander macrophages, **b** cell death signatures in negative and bystander macrophages, and **c** Hallmark databased in sub-cell types of DCs, NK, T, and B cells. The heatmap was generated using z-score standardized enrichment scores. At least one significant term (*P*-value < 0.05) in each sub-cell type (> 20 cells) was used.



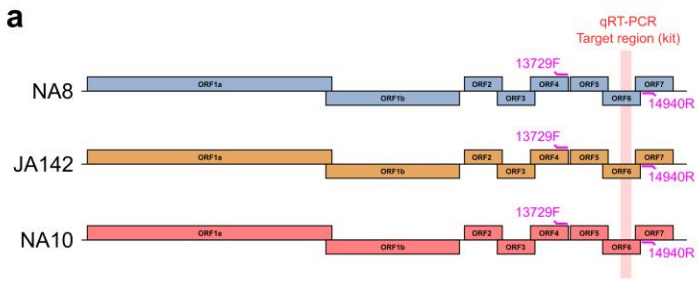
Supplementary Fig. 34 Characteristics of the BALF exosomes. a Size distribution of the exosomes measured using a nanoparticle analyzer. **b** Western blot analysis of the exosome (CD63 and CD9) and endoplasmic reticulum (ER) markers.



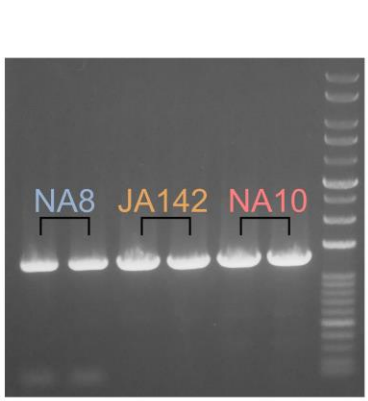
Supplementary Fig. 35 Time-series differentially expressed (DE) exosomal miRNAs in each virulent group (NA8, JA142, and NA10). Volcano plots indicate DE miRNAs (FDR < 0.05 and absolute $\log_2$ FC ≥ 1) in each virulent group based on the negative group. The number of up- and down-regulated miRNAs are identified in the top right and left corners, respectively. Above the number of miRNAs, the number of predicted target genes are written.

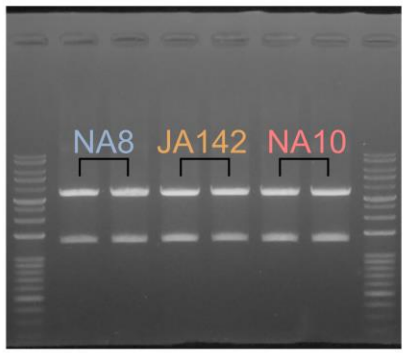


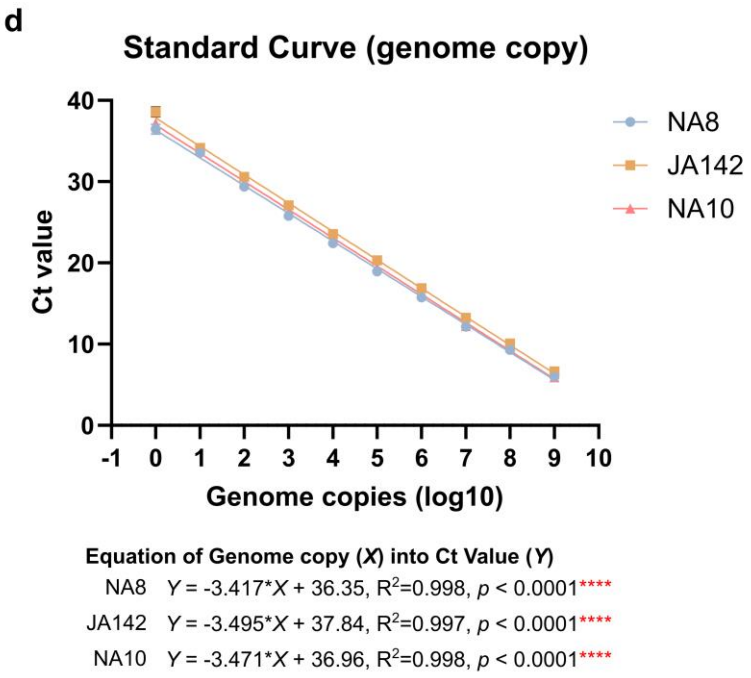
Supplementary Fig. 36 Relative mRNA expression levels for cell death-associated genes and genes for validation. Relative quantification (RQ) values for the cell death-associated genes (Caspase-3, Caspase-7, Caspase-9, NLRP3, RIPK1, RIPK3, MLKL, Caspase-1, Caspase-4, and GSDMD) and the gene set for validation which includes CXCL14, CCL24, SPP1, and ACKR4, are presented as scatter dot plots with error bars (mean ± SEM (standard error mean)). Circles indicate a single biological replication. Statistical analysis was performed using two-way ANOVA analysis with Tukey's multiple comparisons test for four groups (3 and 7 dpi) or Sidak's multiple comparisons test for two groups (14 and 21 dpi). Statistically significant differences between groups are marked with asterisks (*, *P*-value ≤ 0.05. **, *P*-value ≤ 0.01. ***, *P*-value ≤ 0.001.).



Primer	Sequence (5' - 3')	Reference
13729F	GGTGGGCAACTGTTTTAGCCT	This study
14940R	GGCACAGCTGATTGACTGGC	This study





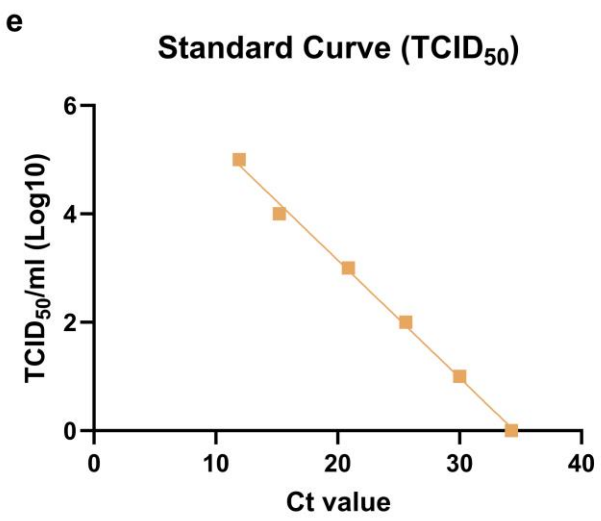


Equation of Genome copy (*X*) into Ct Value (*Y*)

NA8 $Y = -3.417*X + 36.35$, $R^2=0.998$, $p < 0.0001$****

JA142 $Y = -3.495*X + 37.84$, $R^2=0.997$, $p < 0.0001$****

NA10 $Y = -3.471*X + 36.96$, $R^2=0.998$, $p < 0.0001$****



Equation of Ct Value (*X*) into log10 TCID50/ml (*Y*)

JA142 $Y = -0.217*X + 7.4873$, $R^2=0.997$, $p < 0.0001$****

Supplementary Fig. 37 Standard curves for correlating Ct value with TCID. a Primers designed for cloning PRRSV ORF5 and ORF6 including the target of qRT-PCR kit. **b** Gel image of PCR products. All viruses have been tested in duplication. **c** Gel image of constructed TOPO-clones after cut with restriction enzymes (*XbaI* and *SacI*). **d** Standard curve correlating Ct value with genome copies based on constructed plasmids. Equations for the standard curves are as followed: NA8, $Y = -3.417 \times X + 36.35$; JA142, $Y = -3.495 \times X + 37.84$; NA10, $Y = -3.471 \times X + 36.96$. **e** Standard curve correlating Ct value with $TCID_{50}$/ml using JA142. Equation for the standard curve is as followed: $Y = -0.217 \times X + 7.4873$