Revising the ortholog conjecture in cross-species comparison of single-cell transcriptomics

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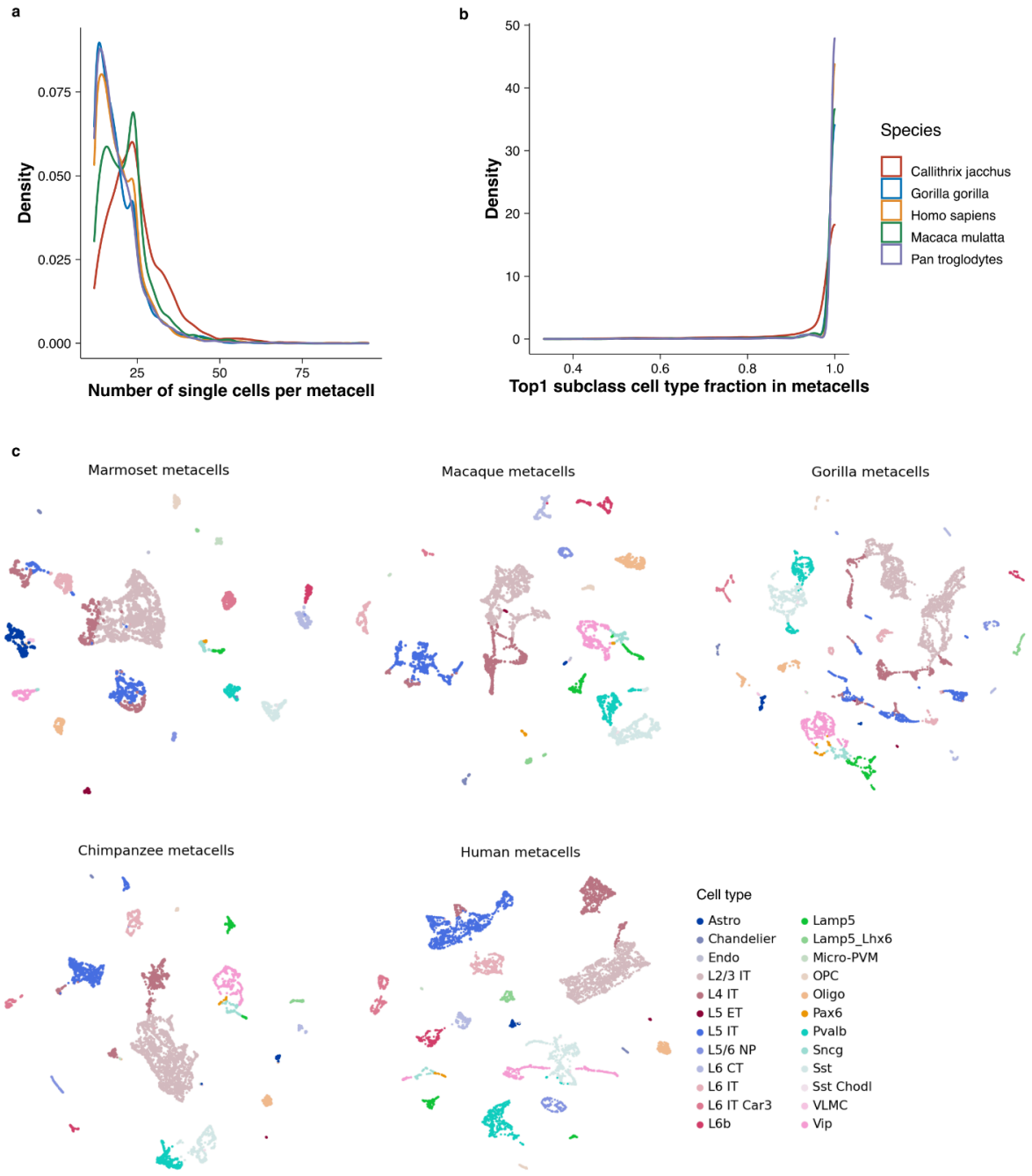
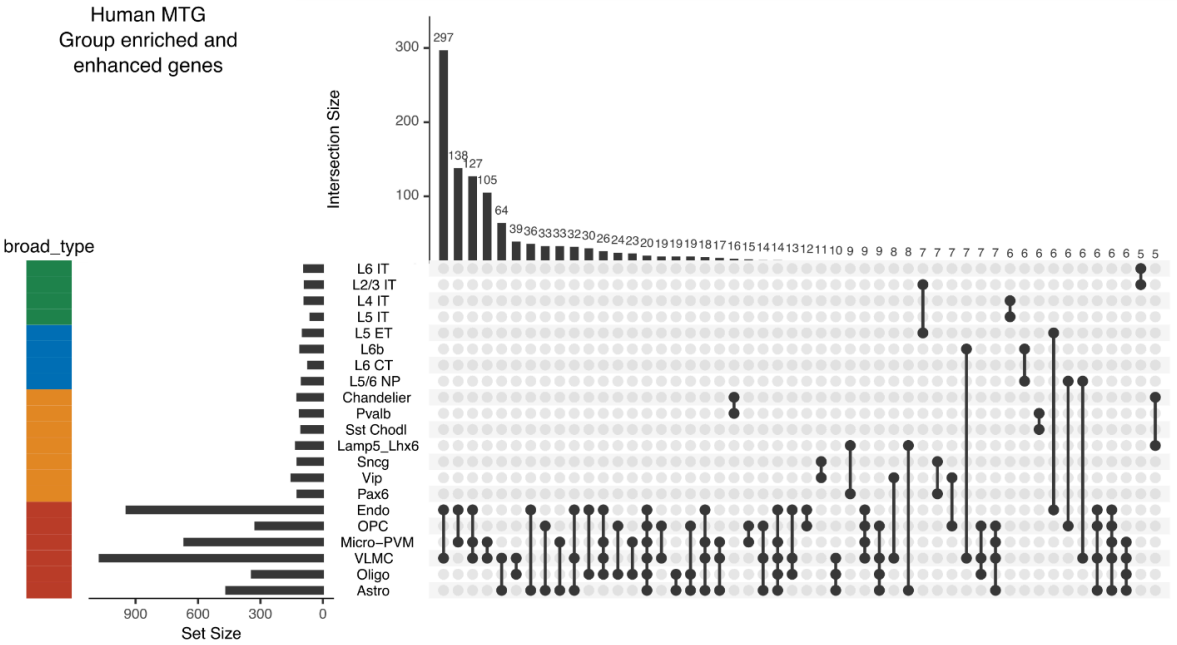
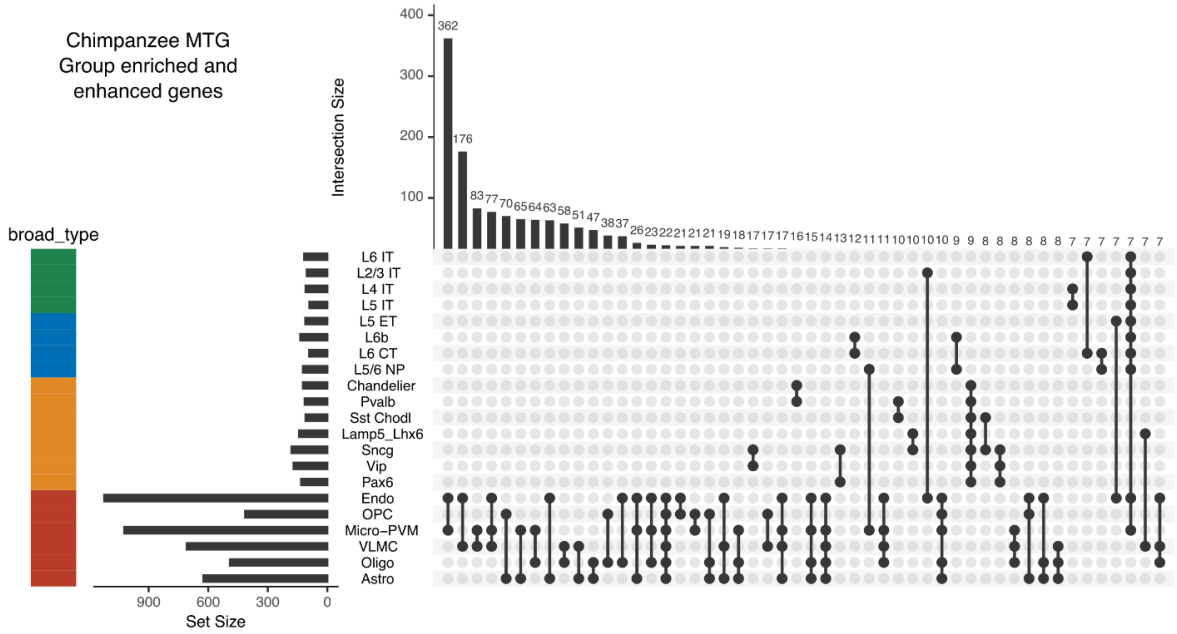
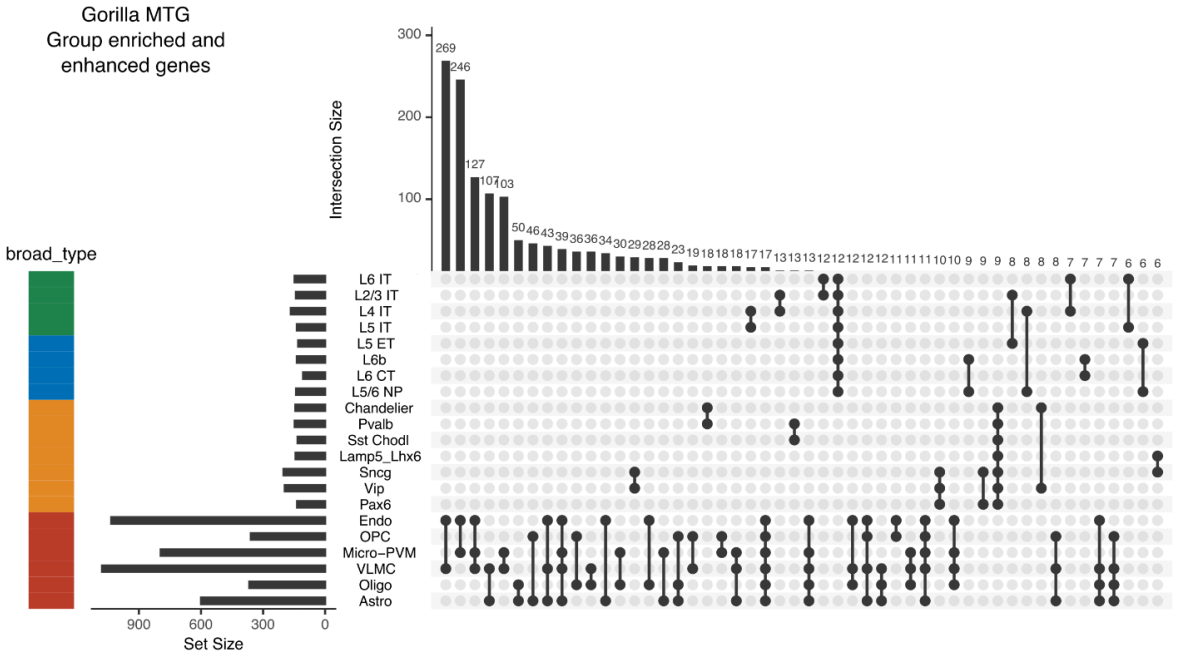
Supplementary Figures 1-16

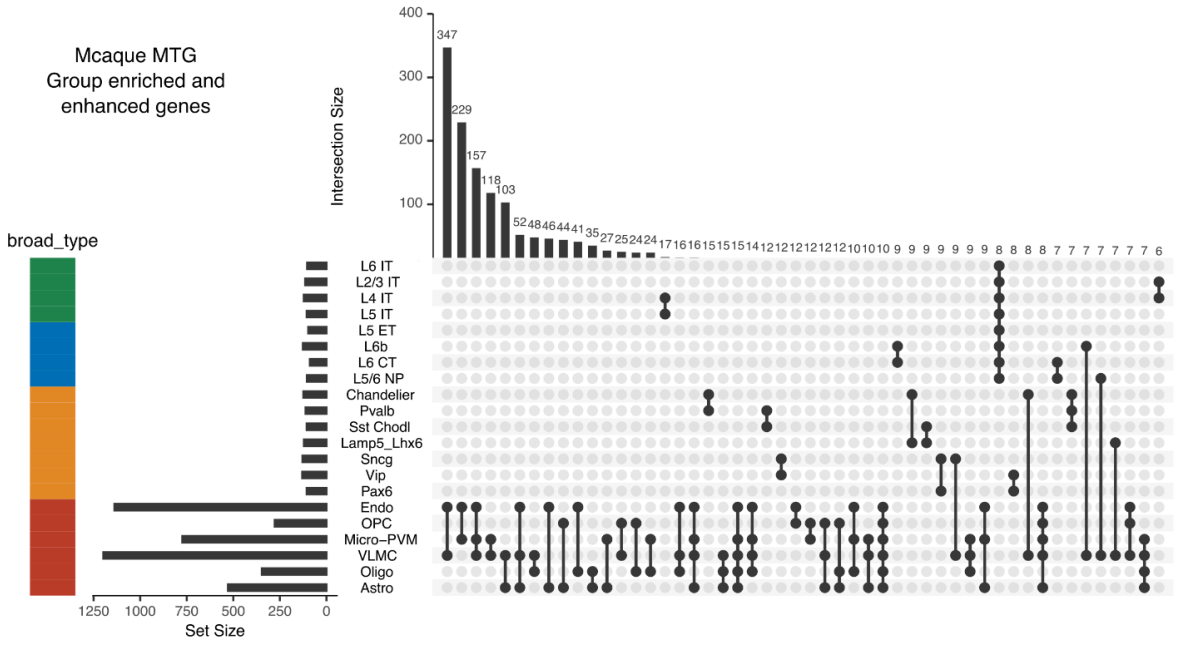


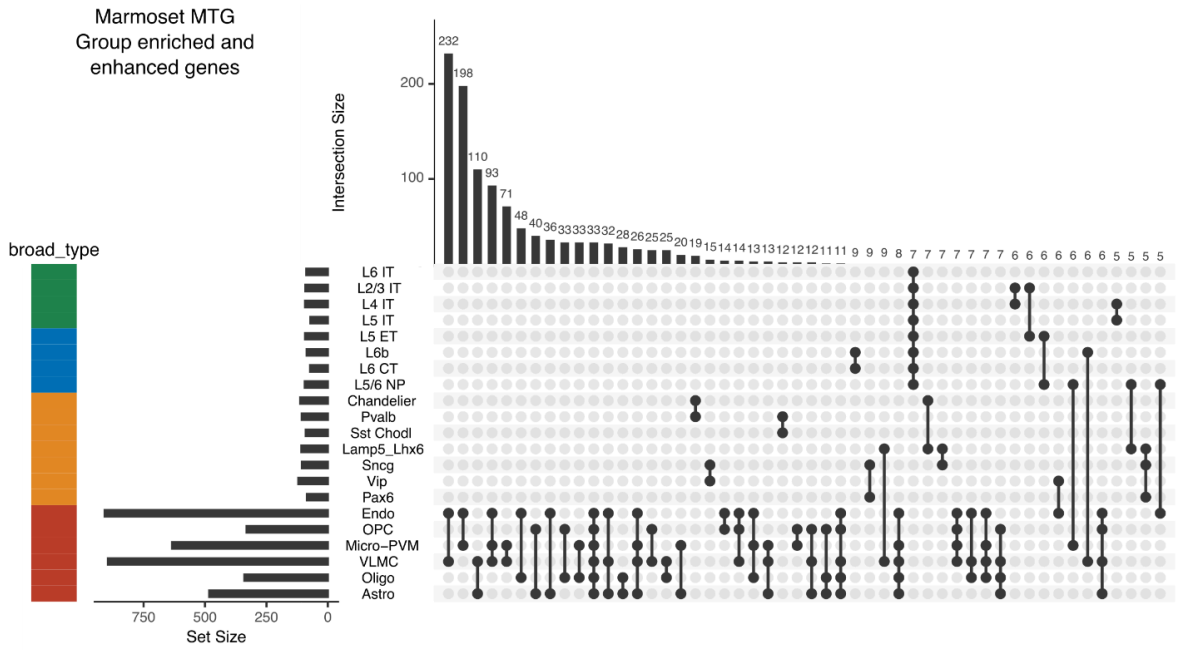
Supplementary Figure 1 Metacells in the primate MTG data. (a) Number of single cells per metacell in each species. (2) Fraction of the top 1 cell type in each metacell in each species. (c) UMAP visualisation of metacells in each species with cell type annotation (“Subclass" in original study). MTG, middle temporal gyrus.


Human MTG
Group enriched and
enhanced genes
Intersection Size
broad_type
L6 IT
L2/3 IT
L4 IT
L5 IT
L5 ET
L6b
L6 CT
L5/6 NP
Chandelier
Pvalb
Sst Chodl
Lamp5_Lhx6
Sncg
Vip
Pax6
Endo
OPC
Micro−PVM
VLMC
Oligo
Astro
Set Size

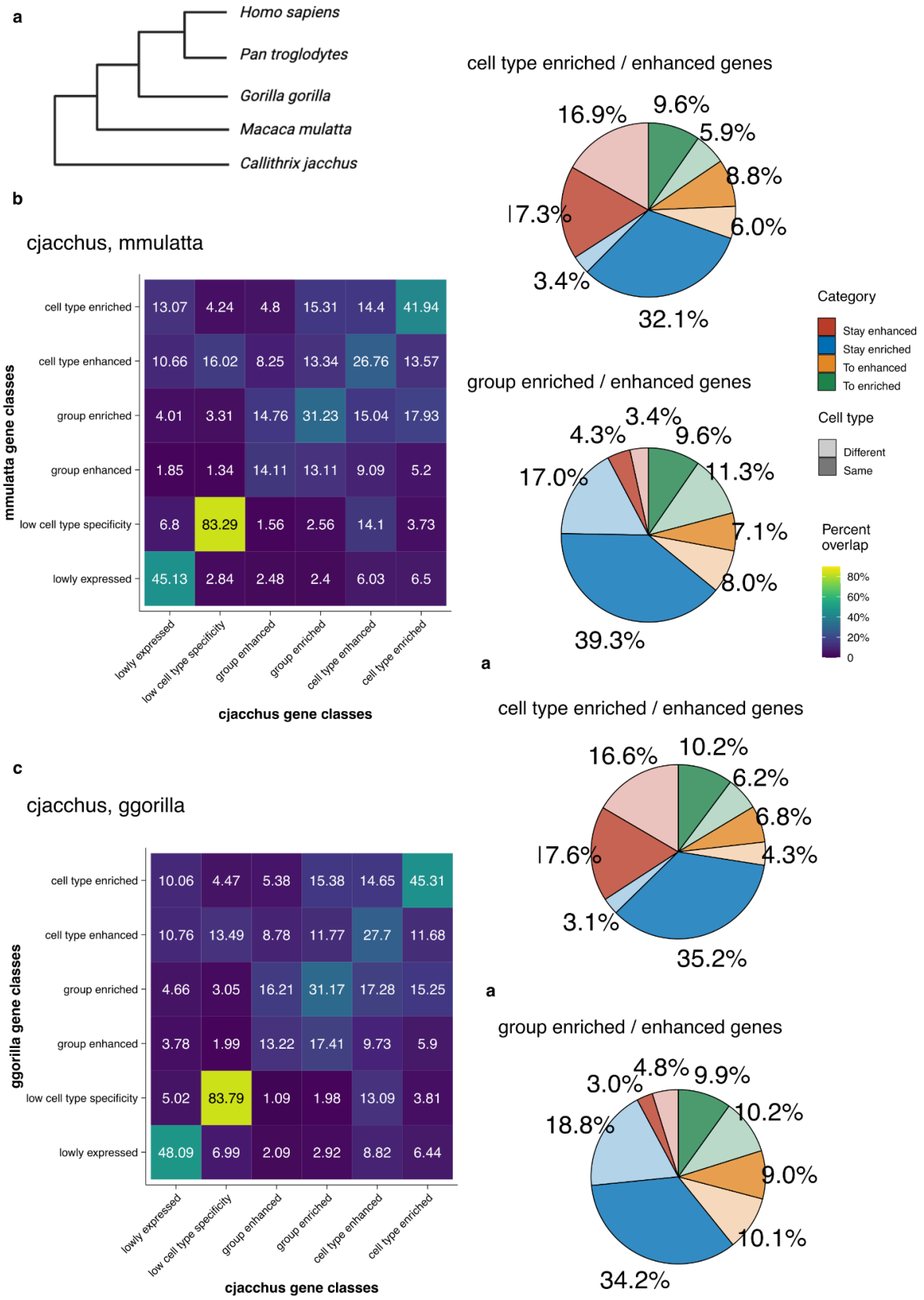

Chimpanzee MTG
Group enriched and
enhanced genes
Intersection Size
broad_type
L6 IT
L2/3 IT
L4 IT
L5 IT
L5 ET
L6b
L6 CT
L5/6 NP
Chandelier
Pvalb
Sst Chodl
Lamp5_Lhx6
Sncg
Vip
Pax6
Endo
OPC
Micro−PVM
VLMC
Oligo
Astro
Set Size

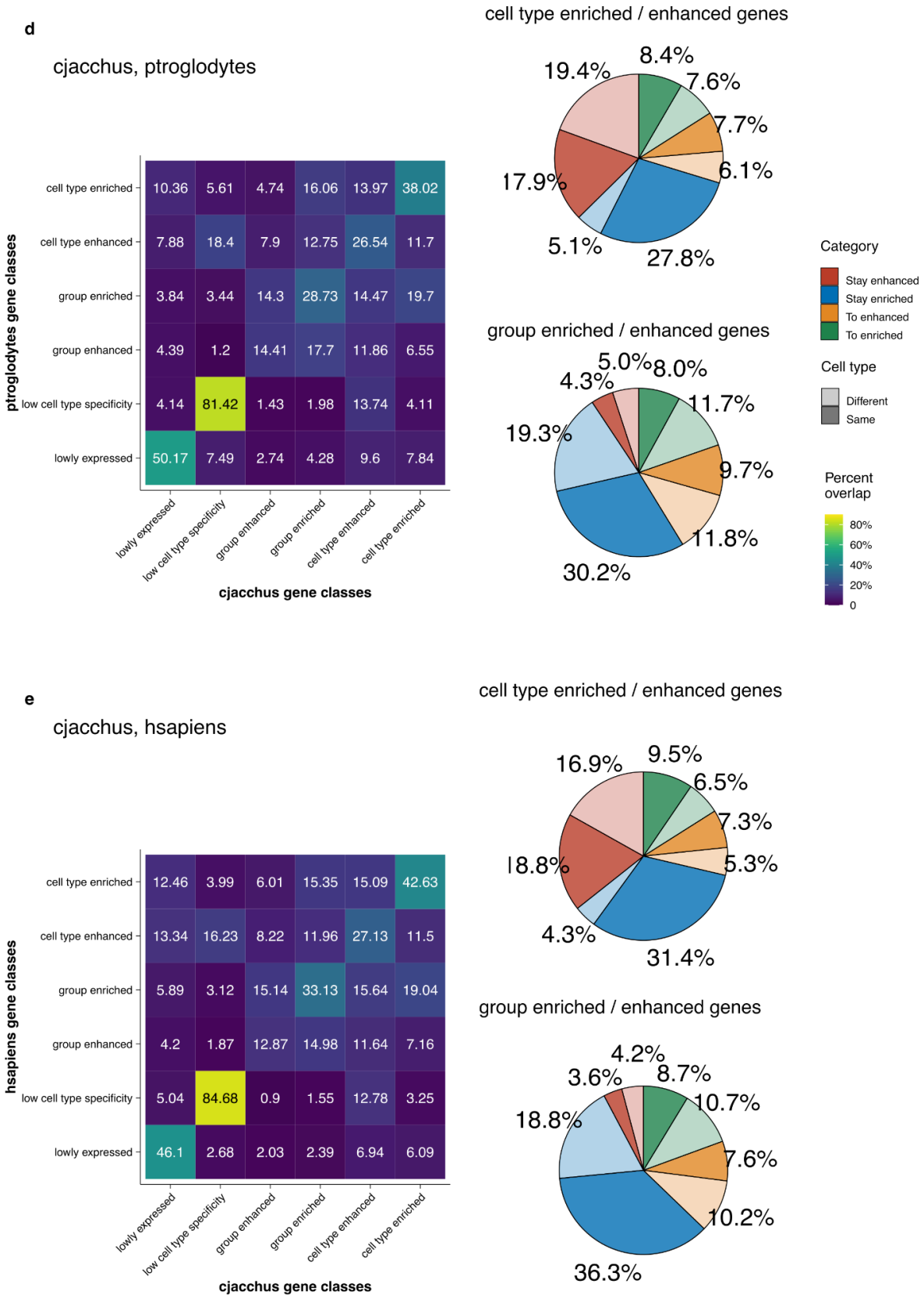

Gorilla MTG
Group enriched and
enhanced genes
Intersection Size
300
200
100
269
246
127
107
103
50 46 43 39 36 36 34 30 29 28 28 23 19 18 18 18 17 17 13 13 13 12 12 12 12 12 12 11 11 11 11 10 10 9 9 9 8 8 8 8 7 7 7 7 6 6 6
broad_type
L6 IT
L2/3 IT
L4 IT
L5 IT
L5 ET
L6b
L6 CT
L5/6 NP
Chandelier
Pvalb
Sst Chodl
Lamp5_Lhx6
Sncg
Vip
Pax6
Endo
OPC
Micro−PVM
VLMC
Oligo
Astro
900
600
300
0
Set Size


Mcaque MTG
Group enriched and
enhanced genes
Intersection Size
400
300
200
100
347
229
157
118
103
52 48 46 44 41 35 27 25 24 24 17 16 16 15 15 15 14 12 12 12 12 12 12 12 10 10 10 9 9 9 9 9 9 9 8 8 8 8 7 7 7 7 7 7 6
broad_type
L6 IT
L2/3 IT
L4 IT
L5 IT
L5 ET
L6b
L6 CT
L5/6 NP
Chandelier
Pvalb
Sst Chodl
Lamp5_Lhx6
Sncg
Vip
Pax6
Endo
OPC
Micro−PVM
VLMC
Oligo
Astro
1250
1000
750
500
250
0
Set Size



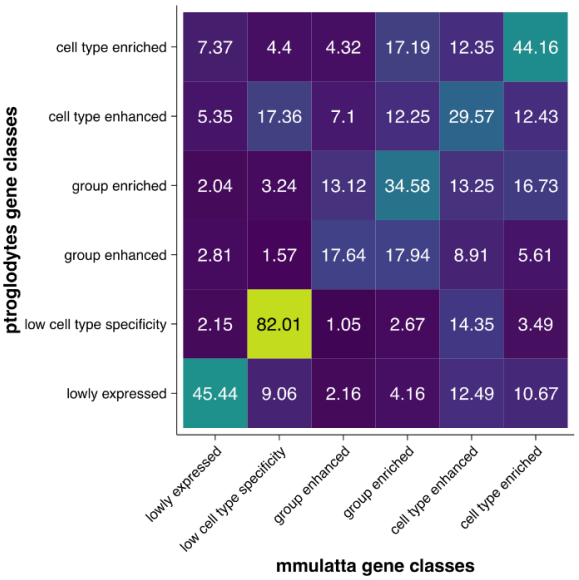
Supplementary Figure 2 Group specific genes shared among cell types. Upset plots showing which cell types share the most group specific (enriched and enhanced) genes in each species. Intersections are ordered by intersection size, while set size is ordered by broad type.


a
Homo sapiens
Pan troglodytes
Gorilla gorilla
Macaca mulatta
Callithrix jacchus
b
cjacchus, mmulatta
mmulatta gene classes
cell type enriched
cell type enhanced
group enriched
group enhanced
low cell type specificity
lowly expressed
13.07 4.24 4.8 15.31 14.4 41.94
10.66 16.02 8.25 13.34 26.76 13.57
4.01 3.31 14.76 31.23 15.04 17.93
1.85 1.34 14.11 13.11 9.09 5.2
6.8 83.29 1.56 2.56 14.1 3.73
45.13 2.84 2.48 2.4 6.03 6.5
lowly expressed
low cell type specificity
group enhanced
group enriched
cell type enhanced
cell type enriched
cjacchus gene classes
cell type enriched / enhanced genes
16.9%
9.6%
5.9%
8.8%
17.3%
6.0%
3.4%
32.1%
group enriched / enhanced genes
3.4%
4.3%
9.6%
17.0%
11.3%
7.1%
8.0%
39.3%
Category
Stay enhanced
Stay enriched
To enhanced
To enriched
Cell type
Different
Same
Percent overlap
80%
60%
40%
20%
0
c
cjacchus, ggorilla
ggorilla gene classes
cell type enriched
cell type enhanced
group enriched
group enhanced
low cell type specificity
lowly expressed
10.06 4.47 5.38 15.38 14.65 45.31
10.76 13.49 8.78 11.77 27.7 11.68
4.66 3.05 16.21 31.17 17.28 15.25
3.78 1.99 13.22 17.41 9.73 5.9
5.02 83.79 1.09 1.98 13.09 3.81
48.09 6.99 2.09 2.92 8.82 6.44
lowly expressed
low cell type specificity
group enhanced
group enriched
cell type enhanced
cell type enriched
cjacchus gene classes
a
cell type enriched / enhanced genes
16.6%
10.2%
6.2%
6.8%
17.6%
4.3%
3.1%
35.2%
a
group enriched / enhanced genes
3.0%
4.8%
9.9%
18.8%
10.2%
9.0%
10.1%
34.2%

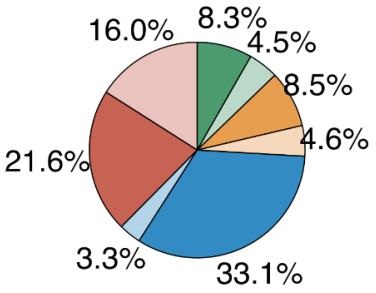

d
cjacchus, ptroglodytes
ptroglodytes gene classes
cell type enriched
cell type enhanced
group enriched
group enhanced
low cell type specificity
lowly expressed
10.36 5.61 4.74 16.06 13.97 38.02
7.88 18.4 7.9 12.75 26.54 11.7
3.84 3.44 14.3 28.73 14.47 19.7
4.39 1.2 14.41 17.7 11.86 6.55
4.14 81.42 1.43 1.98 13.74 4.11
50.17 7.49 2.74 4.28 9.6 7.84
lowly expressed
low cell type specificity
group enhanced
group enriched
cell type enhanced
cell type enriched
cjacchus gene classes
cell type enriched / enhanced genes
19.4%
8.4%
7.6%
7.7%
6.1%
17.9%
5.1%
27.8%
group enriched / enhanced genes
5.0%
4.3%
8.0%
11.7%
19.3%
9.7%
11.8%
30.2%
Category
Stay enhanced
Stay enriched
To enhanced
To enriched
Cell type
Different
Same
Percent overlap
80%
60%
40%
20%
0
e
cjacchus, hsapiens
hsapiens gene classes
cell type enriched
cell type enhanced
group enriched
group enhanced
low cell type specificity
lowly expressed
12.46 3.99 6.01 15.35 15.09 42.63
13.34 16.23 8.22 11.96 27.13 11.5
5.89 3.12 15.14 33.13 15.64 19.04
4.2 1.87 12.87 14.98 11.64 7.16
5.04 84.68 0.9 1.55 12.78 3.25
46.1 2.68 2.03 2.39 6.94 6.09
lowly expressed
low cell type specificity
group enhanced
group enriched
cell type enhanced
cell type enriched
cjacchus gene classes
cell type enriched / enhanced genes
16.9%
9.5%
6.5%
7.3%
18.8%
5.3%
4.3%
31.4%
group enriched / enhanced genes
4.2%
3.6%
8.7%
10.7%
18.8%
7.6%
10.2%
36.3%

f

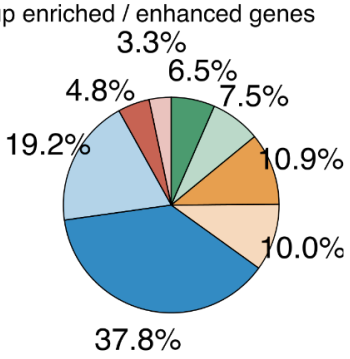
mmulatta_ptroglodytes

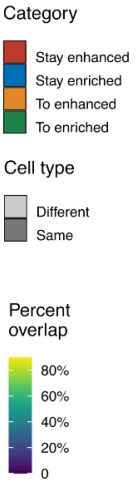


cell type enriched / enhanced genes



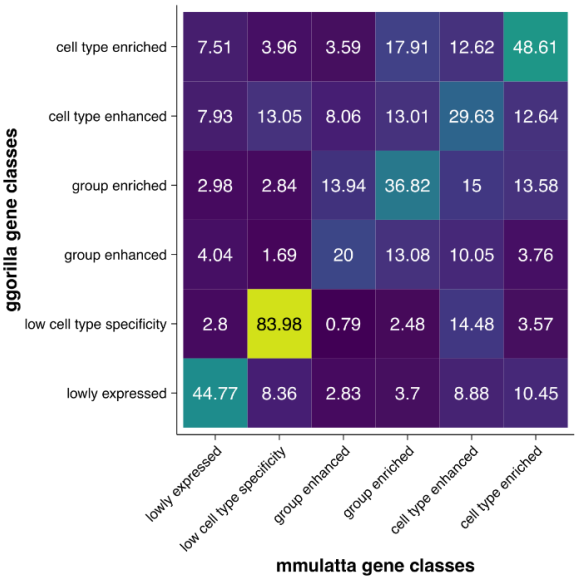
group enriched / enhanced genes



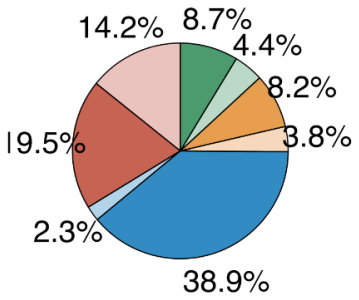


g

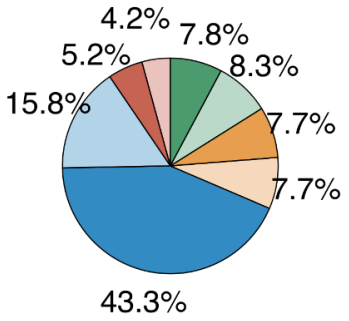
mmulatta_ggorilla



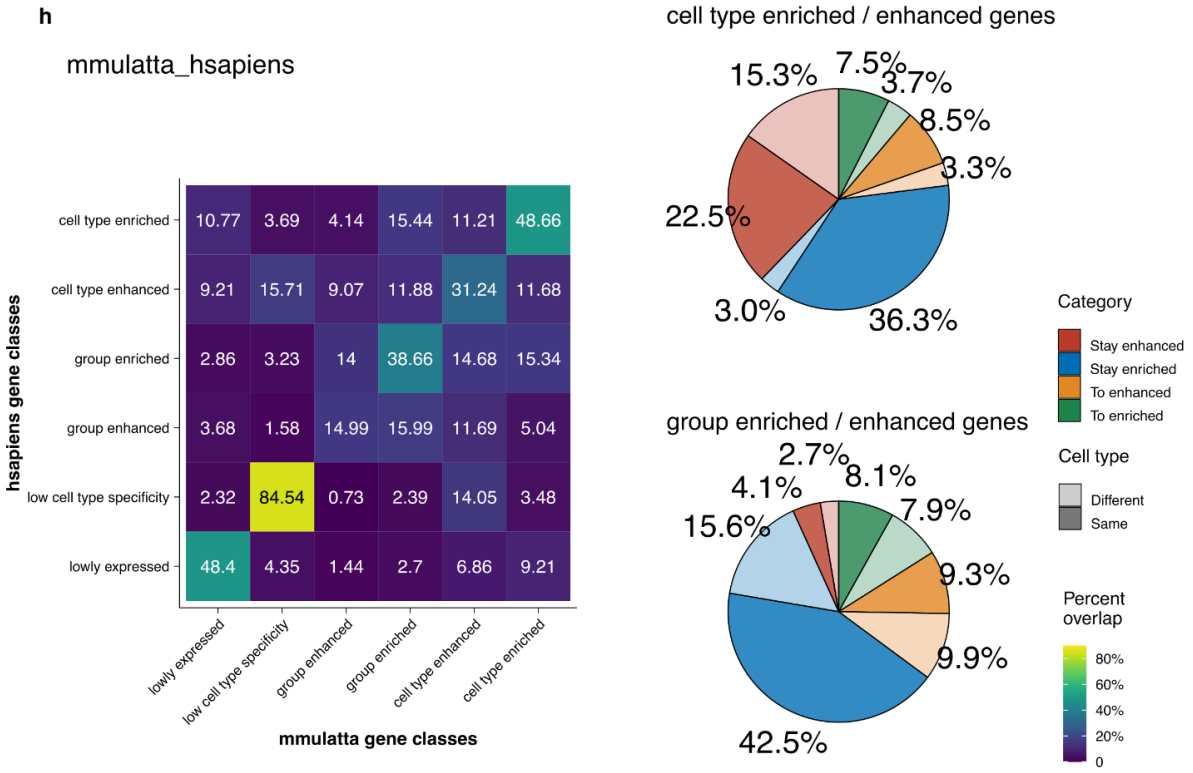
cell type enriched / enhanced genes



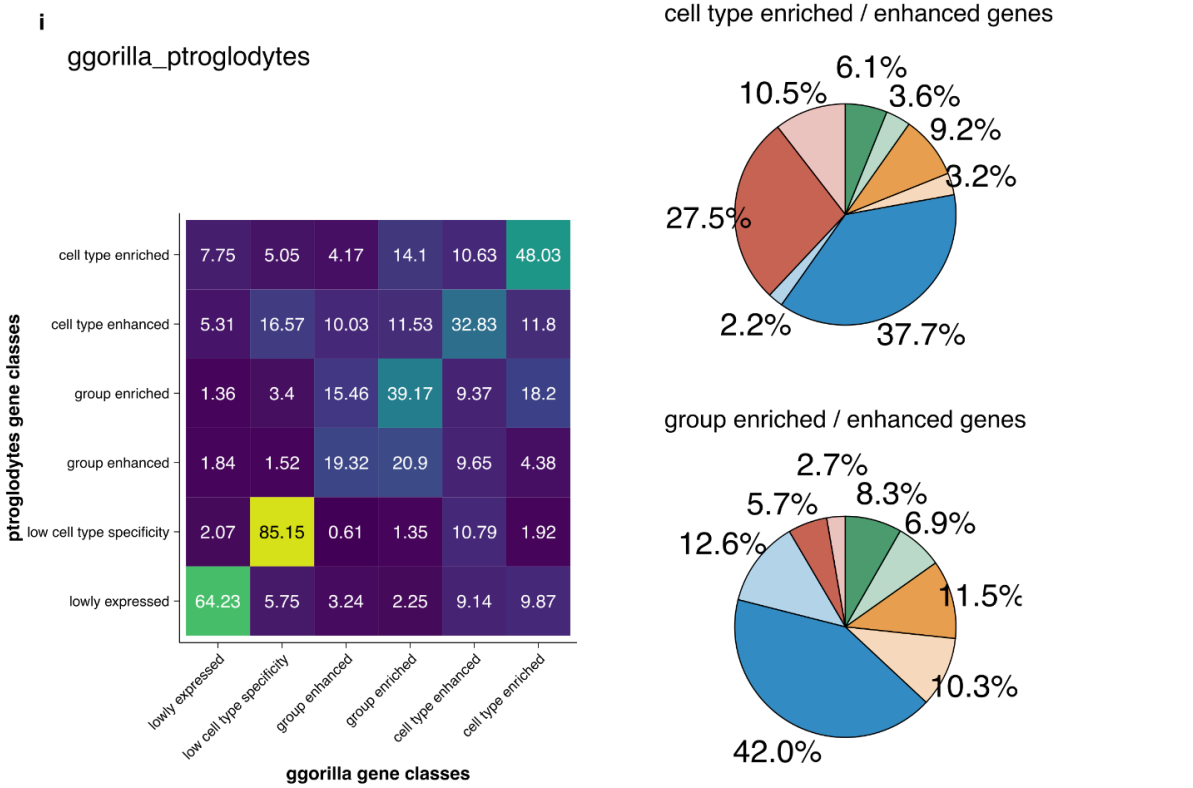
group enriched / enhanced genes

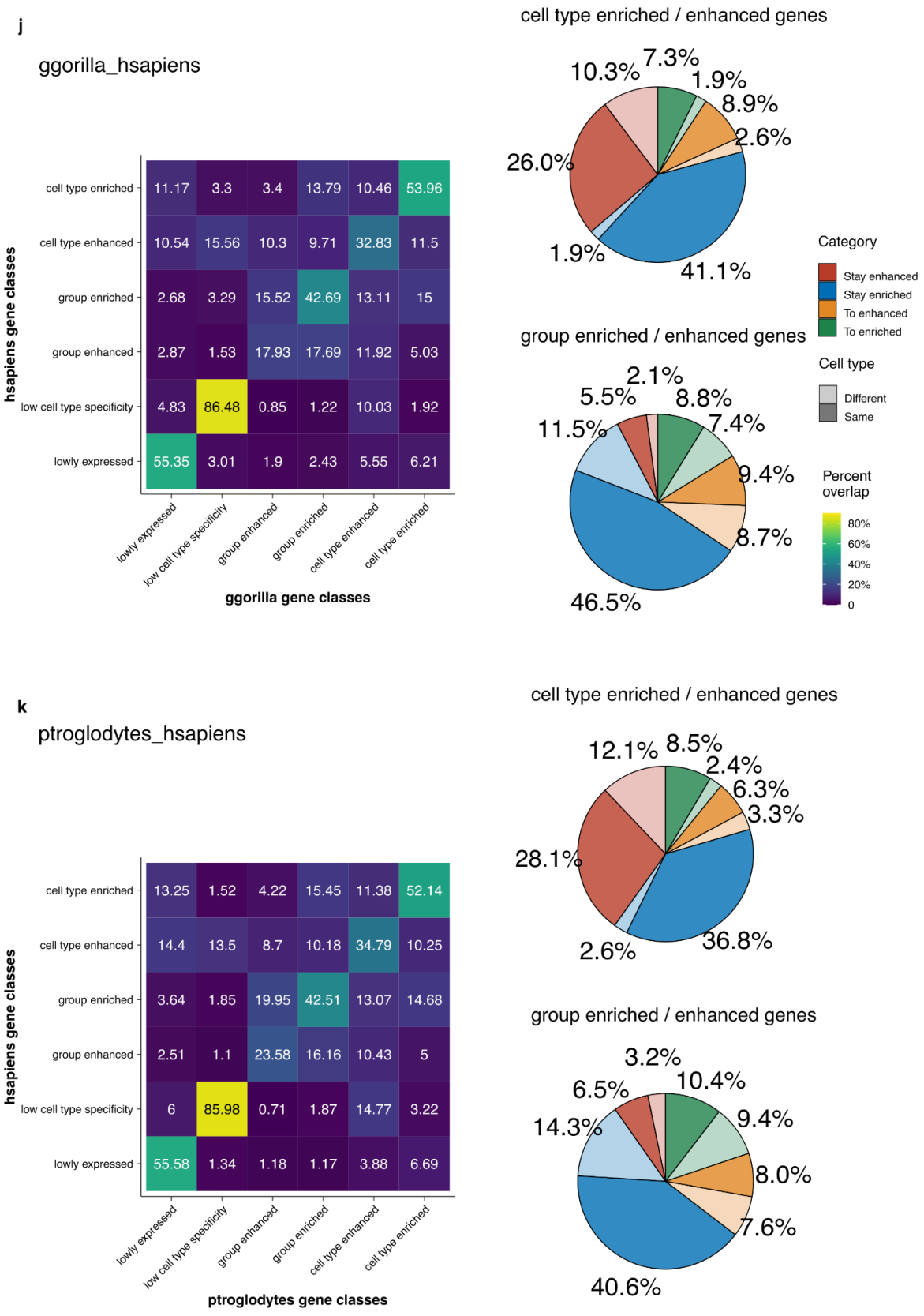


h


mmulatta_hsapiens
cell type enriched
cell type enhanced
group enriched
group enhanced
low cell type specificity
lowly expressed
hsapiens gene classes
10.77 3.69 4.14 15.44 11.21 48.66
9.21 15.71 9.07 11.88 31.24 11.68
2.86 3.23 14 38.66 14.68 15.34
3.68 1.58 14.99 15.99 11.69 5.04
2.32 84.54 0.73 2.39 14.05 3.48
48.4 4.35 1.44 2.7 6.86 9.21
lowly expressed
low cell type specificity
group enhanced
group enriched
cell type enhanced
cell type enriched
mmulatta gene classes
cell type enriched / enhanced genes
15.3%
7.5%
3.7%
8.5%
3.3%
22.5%
3.0%
36.3%
group enriched / enhanced genes
2.7%
4.1%
8.1%
15.6%
7.9%
9.3%
9.9%
42.5%
Category
Stay enhanced
Stay enriched
To enhanced
To enriched
Cell type
Different
Same
Percent overlap
80%
60%
40%
20%
0

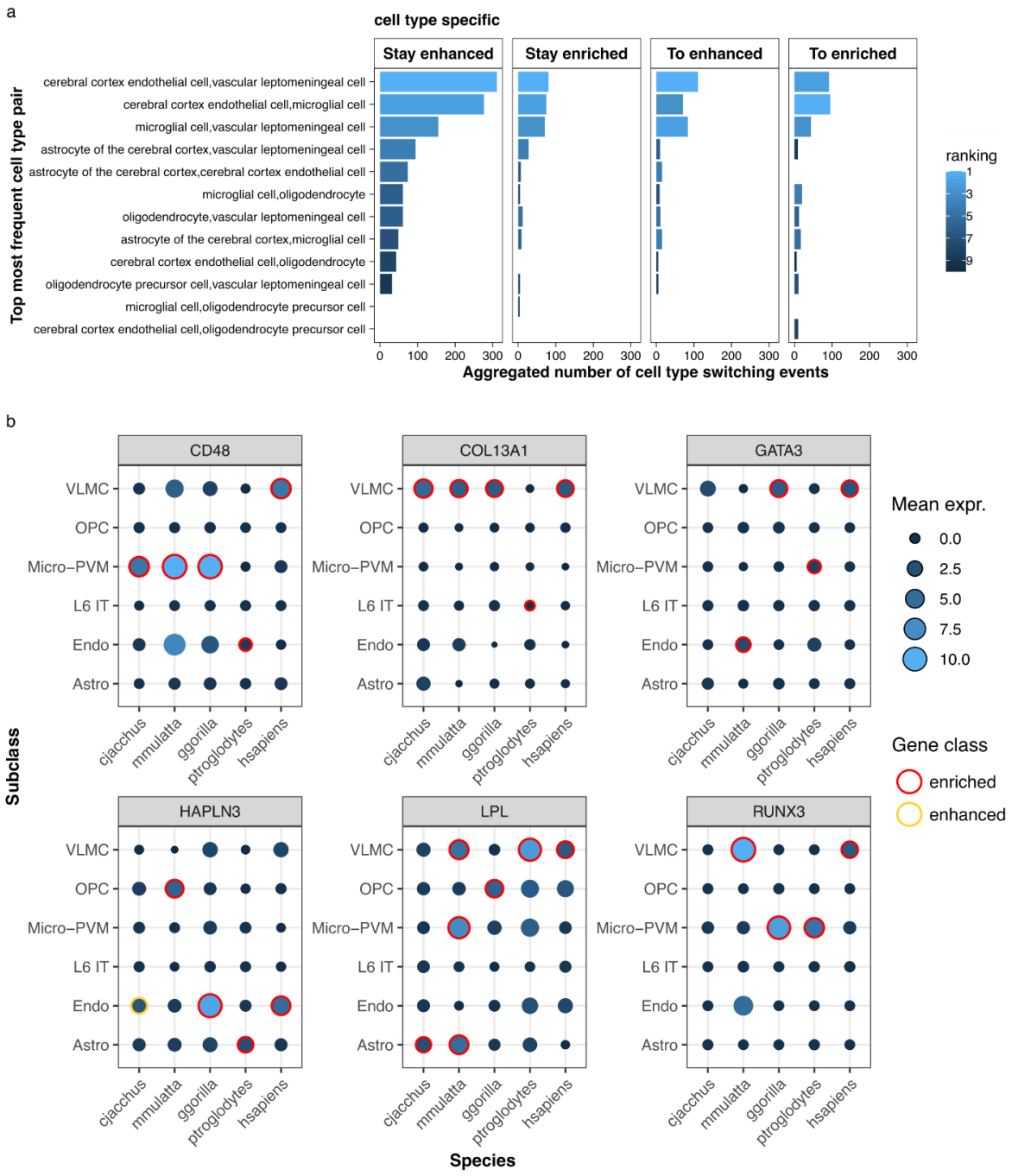
i


ggorilla_ptroglodytes
cell type enriched
cell type enhanced
group enriched
group enhanced
low cell type specificity
lowly expressed
ptroglodytes gene classes
7.75 5.05 4.17 14.1 10.63 48.03
5.31 16.57 10.03 11.53 32.83 11.8
1.36 3.4 15.46 39.17 9.37 18.2
1.84 1.52 19.32 20.9 9.65 4.38
2.07 85.15 0.61 1.35 10.79 1.92
64.23 5.75 3.24 2.25 9.14 9.87
lowly expressed
low cell type specificity
group enhanced
group enriched
cell type enhanced
cell type enriched
ggorilla gene classes
cell type enriched / enhanced genes
6.1%
10.5%
3.6%
9.2%
3.2%
27.5%
2.2%
37.7%
group enriched / enhanced genes
2.7%
5.7%
8.3%
12.6%
6.9%
11.5%
10.3%
42.0%



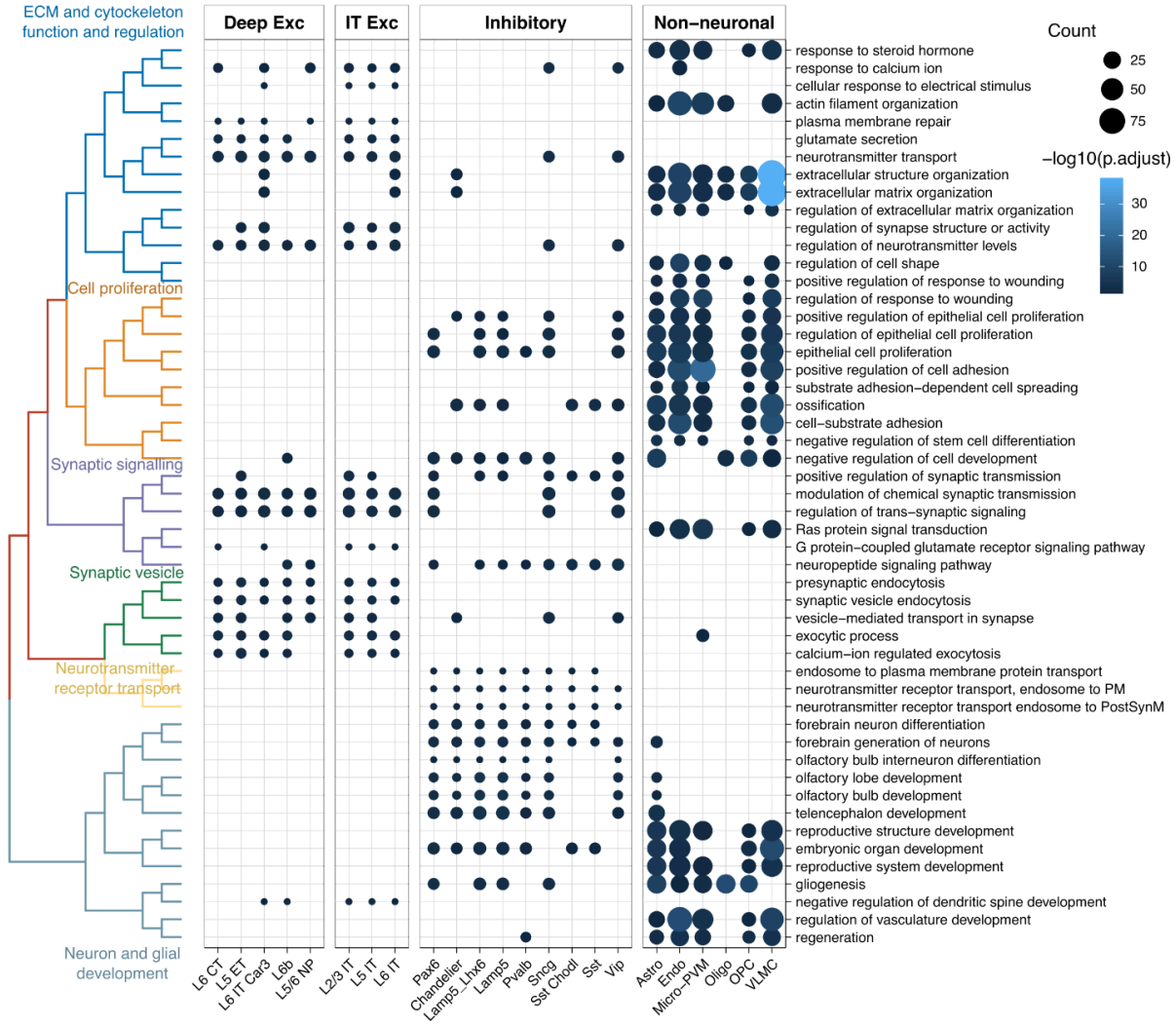
Supplementary Figure 3 Gene specificity class conservation among O2O orthologs in the primate MTG dataset, for each species pair. Average specificity class conservation heatmap showing the percent overlap among one-to-one orthologs in various specificity class

combinations (calculation process see methods). Pie chart showing the aggregated percentage of cell type conservation and class conservation for genes with cell type level specificity or group level specificity.

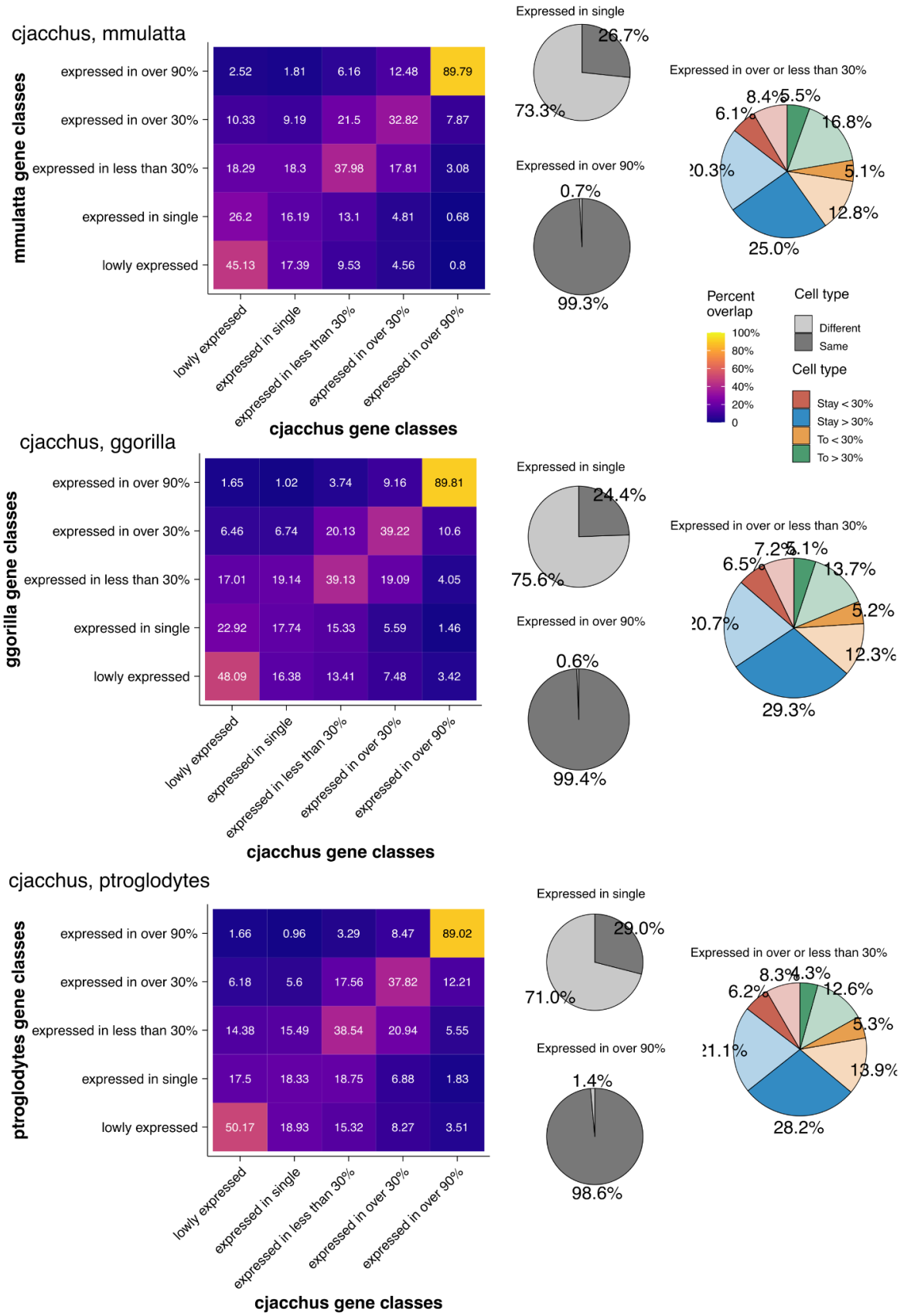


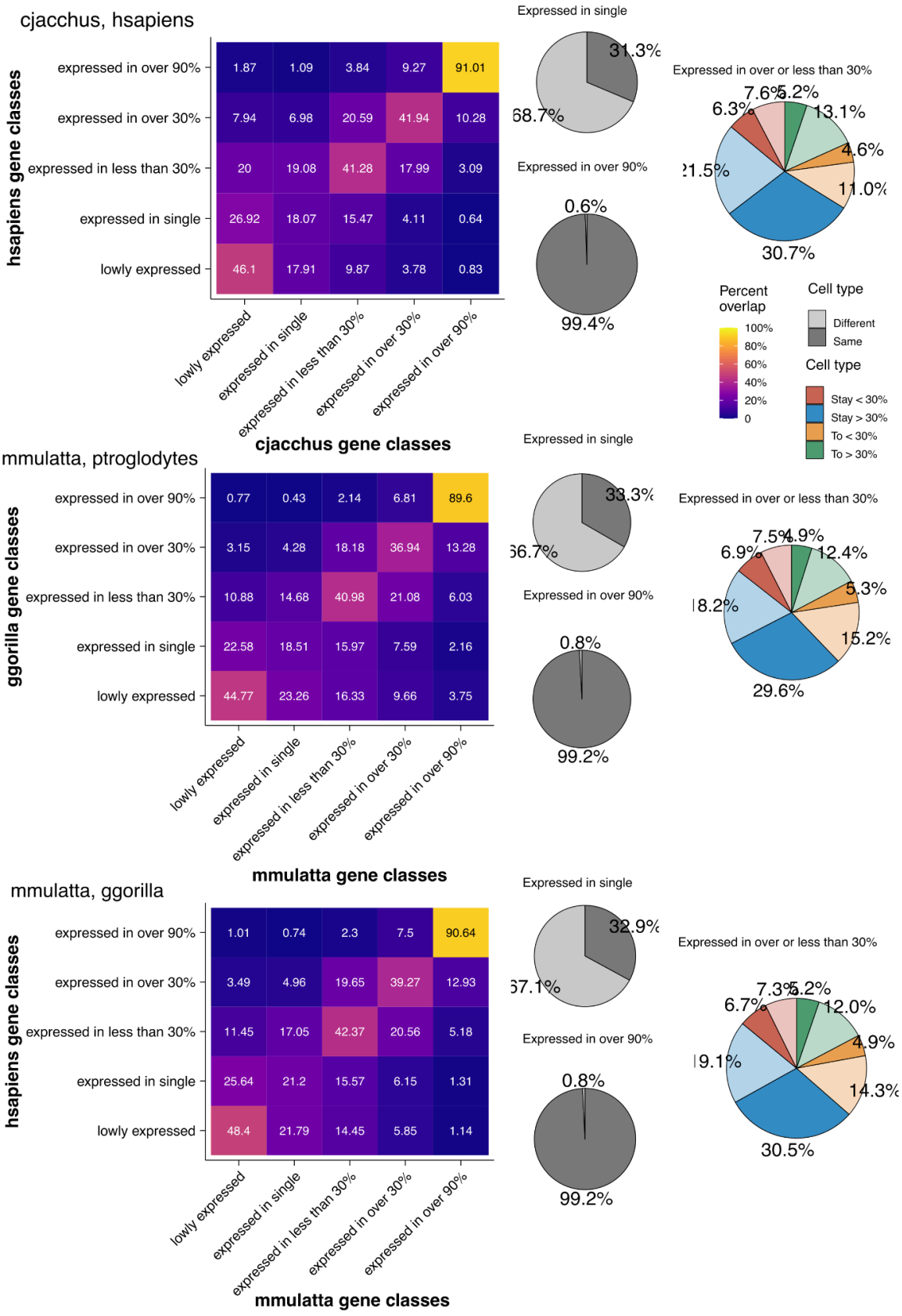
Supplementary figure 4 Gene specificity class switching mostly happens between transcriptomically similar cell types. (a) Showing the top cell type pairs in which cell type-specific genes switched between them. (b) Showing examples of cell type enriched genes that switched cell type between species with the top frequency. Mean expr., mean scaled expression. VLMC, vascular leptomeningeal cell; OPC, oligodendrocyte precursor cell;

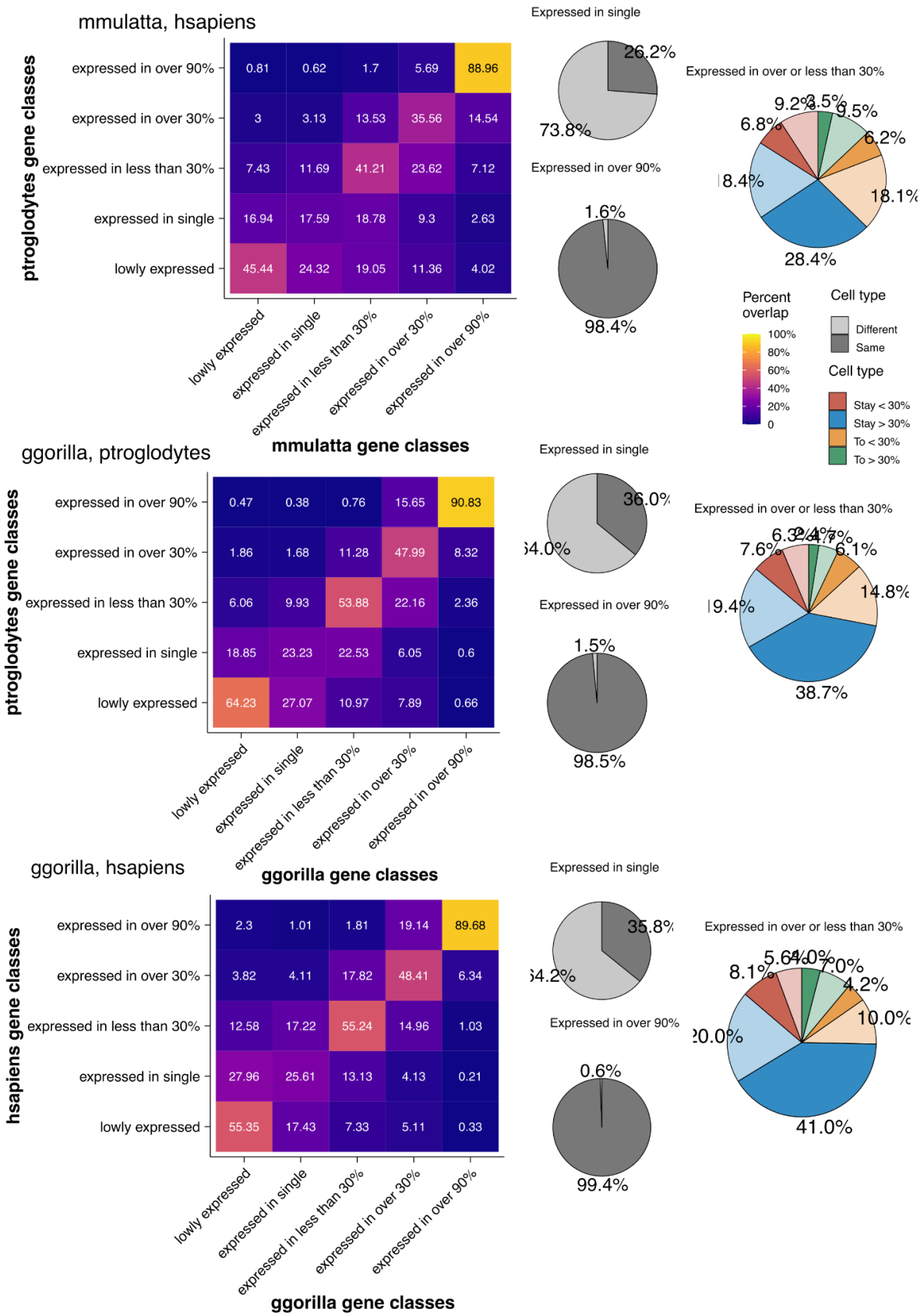
Micro-PVM, microglial cell; L6 IT, L6 intratelencephalic neurons; Endo, cerebral cortex endothelial cell; Astro, astrocyte of the cerebral cortex.

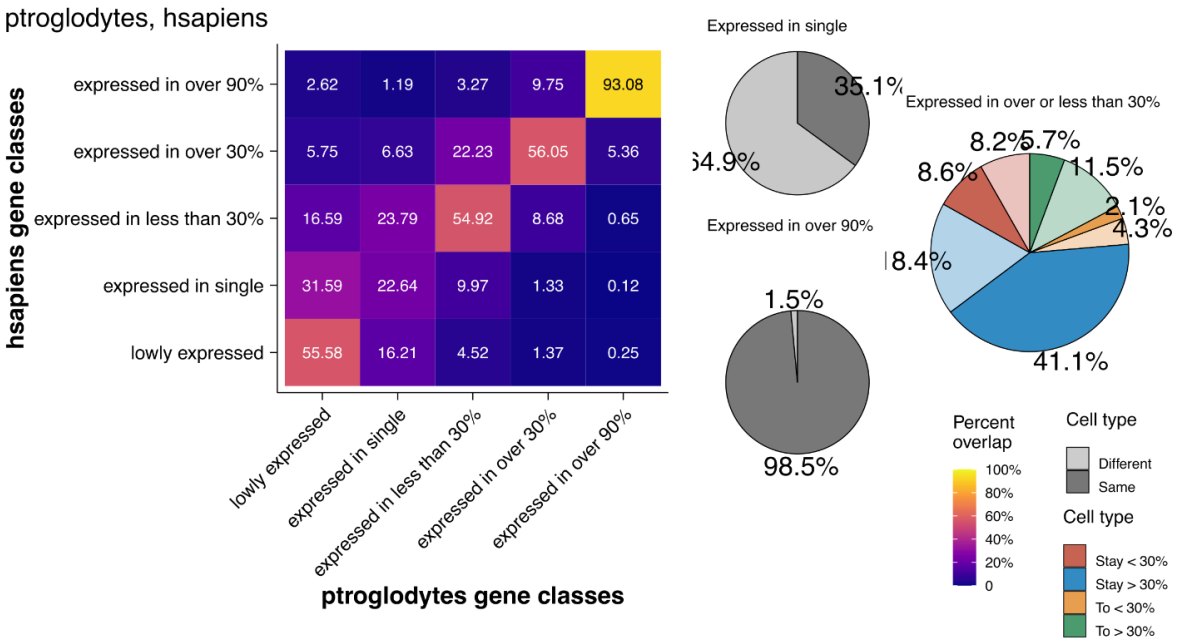


Supplementary figure 5 GO BP enrichment of genes that stayed in the same specificity class. Here shows the GO BP enrichment of genes that stayed enriched in the same cell type in any pair of species. Top most frequent enriched terms across each broad type were selected and plotted in the figure. Semantic similarity between the selected GO BP terms were calculated and used for hierarchical clustering, showing on the left and summarised by keywords.


cjacchus, mmulatta
mmulatta gene classes
expressed in over 90%
expressed in over 30%
expressed in less than 30%
expressed in single
lowly expressed
2.52 1.81 6.16 12.48 89.79
10.33 9.19 21.5 32.82 7.87
18.29 18.3 37.98 17.81 3.08
26.2 16.19 13.1 4.81 0.68
45.13 17.39 9.53 4.56 0.8
lowly expressed
expressed in single
expressed in less than 30%
expressed in over 30%
expressed in over 90%
cjacchus gene classes
Expressed in single
26.7%
73.3%
Expressed in over 90%
0.7%
99.3%
Expressed in over or less than 30%
8.4%
5.5%
6.1%
16.8%
5.1%
12.8%
25.0%
Percent overlap
100%
80%
60%
40%
20%
0
Cell type
Different
Same
Cell type
Stay < 30%
Stay > 30%
To < 30%
To > 30%
cjacchus, ggorilla
ggorilla gene classes
expressed in over 90%
expressed in over 30%
expressed in less than 30%
expressed in single
lowly expressed
1.65 1.02 3.74 9.16 89.81
6.46 6.74 20.13 39.22 10.6
17.01 19.14 39.13 19.09 4.05
22.92 17.74 15.33 5.59 1.46
48.09 16.38 13.41 7.48 3.42
lowly expressed
expressed in single
expressed in less than 30%
expressed in over 30%
expressed in over 90%
cjacchus gene classes
Expressed in single
24.4%
75.6%
Expressed in over 90%
0.6%
99.4%
Expressed in over or less than 30%
7.2%
6.5%
13.7%
5.2%
12.3%
29.3%
cjacchus, ptroglodytes
ptroglodytes gene classes
expressed in over 90%
expressed in over 30%
expressed in less than 30%
expressed in single
lowly expressed
1.66 0.96 3.29 8.47 89.02
6.18 5.6 17.56 37.82 12.21
14.38 15.49 38.54 20.94 5.55
17.5 18.33 18.75 6.88 1.83
50.17 18.93 15.32 8.27 3.51
lowly expressed
expressed in single
expressed in less than 30%
expressed in over 30%
expressed in over 90%
cjacchus gene classes
Expressed in single
29.0%
71.0%
Expressed in over 90%
1.4%
98.6%
Expressed in over or less than 30%
8.3%
4.3%
6.2%
12.6%
5.3%
13.9%
28.2%

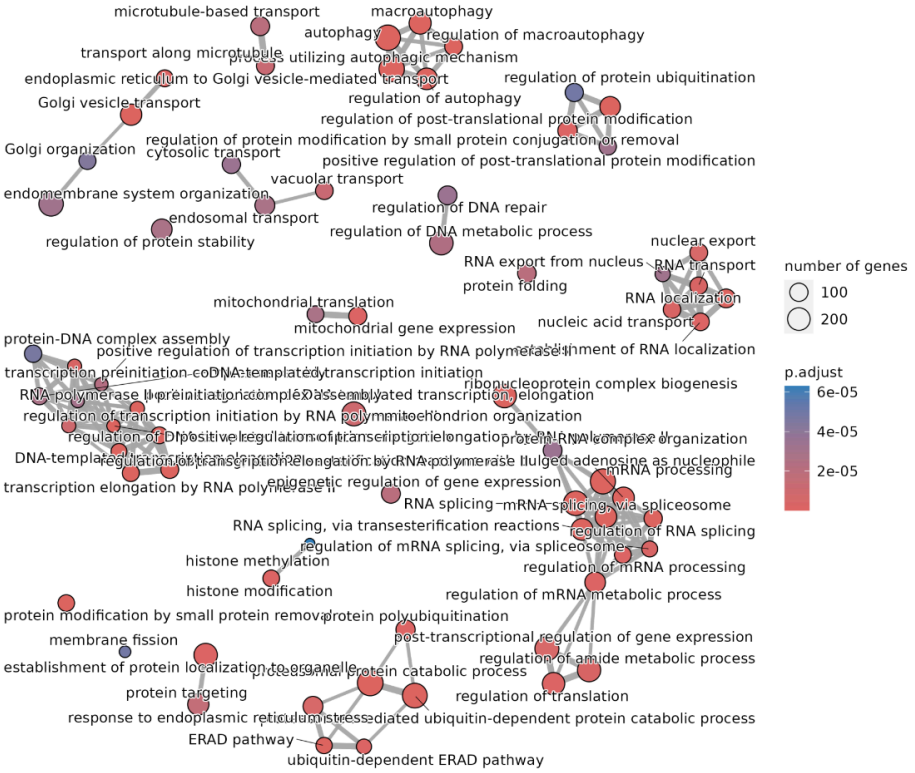

cjacchus, hsapiens
hsapiens gene classes
expressed in over 90%
expressed in over 30%
expressed in less than 30%
expressed in single
lowly expressed
1.87 1.09 3.84 9.27 91.01
7.94 6.98 20.59 41.94 10.28
20 19.08 41.28 17.99 3.09
26.92 18.07 15.47 4.11 0.64
46.1 17.91 9.87 3.78 0.83
lowly expressed
expressed in single
expressed in less than 30%
expressed in over 30%
expressed in over 90%
cjacchus gene classes
Expressed in single
31.3%
68.7%
Expressed in over 90%
0.6%
99.4%
Expressed in over or less than 30%
7.6%
5.2%
6.3%
13.1%
4.6%
21.5%
11.0%
30.7%
Percent overlap
100%
80%
60%
40%
20%
0
Cell type
Different
Same
Cell type
Stay < 30%
Stay > 30%
To < 30%
To > 30%
mmulatta, ptroglodytes
ggorilla gene classes
expressed in over 90%
expressed in over 30%
expressed in less than 30%
expressed in single
lowly expressed
0.77 0.43 2.14 6.81 89.6
3.15 4.28 18.18 36.94 13.28
10.88 14.68 40.98 21.08 6.03
22.58 18.51 15.97 7.59 2.16
44.77 23.26 16.33 9.66 3.75
lowly expressed
expressed in single
expressed in less than 30%
expressed in over 30%
expressed in over 90%
mmulatta gene classes
Expressed in single
33.3%
66.7%
Expressed in over 90%
0.8%
99.2%
Expressed in over or less than 30%
7.5%
4.9%
6.9%
12.4%
5.3%
18.2%
15.2%
29.6%
mmulatta, ggorilla
hsapiens gene classes
expressed in over 90%
expressed in over 30%
expressed in less than 30%
expressed in single
lowly expressed
1.01 0.74 2.3 7.5 90.64
3.49 4.96 19.65 39.27 12.93
11.45 17.05 42.37 20.56 5.18
25.64 21.2 15.57 6.15 1.31
48.4 21.79 14.45 5.85 1.14
lowly expressed
expressed in single
expressed in less than 30%
expressed in over 30%
expressed in over 90%
mmulatta gene classes
Expressed in single
32.9%
67.1%
Expressed in over 90%
0.8%
99.2%
Expressed in over or less than 30%
7.3%
5.2%
6.7%
12.0%
4.9%
19.1%
14.3%
30.5%


mmulatta, hsapiens
ptroglodytes gene classes
expressed in over 90%
expressed in over 30%
expressed in less than 30%
expressed in single
lowly expressed
0.81 0.62 1.7 5.69 88.96
3 3.13 13.53 35.56 14.54
7.43 11.69 41.21 23.62 7.12
16.94 17.59 18.78 9.3 2.63
45.44 24.32 19.05 11.36 4.02
lowly expressed
expressed in single
expressed in less than 30%
expressed in over 30%
expressed in over 90%
mmulatta gene classes
Expressed in single
26.2%
73.8%
Expressed in over 90%
1.6%
98.4%
Expressed in over or less than 30%
9.2%
3.5%
9.5%
6.8%
6.2%
8.4%
18.1%
28.4%
Percent overlap
100%
80%
60%
40%
20%
0
Cell type
Different
Same
Cell type
Stay < 30%
Stay > 30%
To < 30%
To > 30%
ggorilla, ptroglodytes
ptroglodytes gene classes
expressed in over 90%
expressed in over 30%
expressed in less than 30%
expressed in single
lowly expressed
0.47 0.38 0.76 15.65 90.83
1.86 1.68 11.28 47.99 8.32
6.06 9.93 53.88 22.16 2.36
18.85 23.23 22.53 6.05 0.6
64.23 27.07 10.97 7.89 0.66
lowly expressed
expressed in single
expressed in less than 30%
expressed in over 30%
expressed in over 90%
ggorilla gene classes
Expressed in single
36.0%
64.0%
Expressed in over 90%
1.5%
98.5%
Expressed in over or less than 30%
7.6%
6.1%
14.8%
9.4%
38.7%
ggorilla, hsapiens
hsapiens gene classes
expressed in over 90%
expressed in over 30%
expressed in less than 30%
expressed in single
lowly expressed
2.3 1.01 1.81 19.14 89.68
3.82 4.11 17.82 48.41 6.34
12.58 17.22 55.24 14.96 1.03
27.96 25.61 13.13 4.13 0.21
55.35 17.43 7.33 5.11 0.33
lowly expressed
expressed in single
expressed in less than 30%
expressed in over 30%
expressed in over 90%
ggorilla gene classes
Expressed in single
35.8%
64.2%
Expressed in over 90%
0.6%
99.4%
Expressed in over or less than 30%
8.1%
4.2%
10.0%
20.0%
41.0%

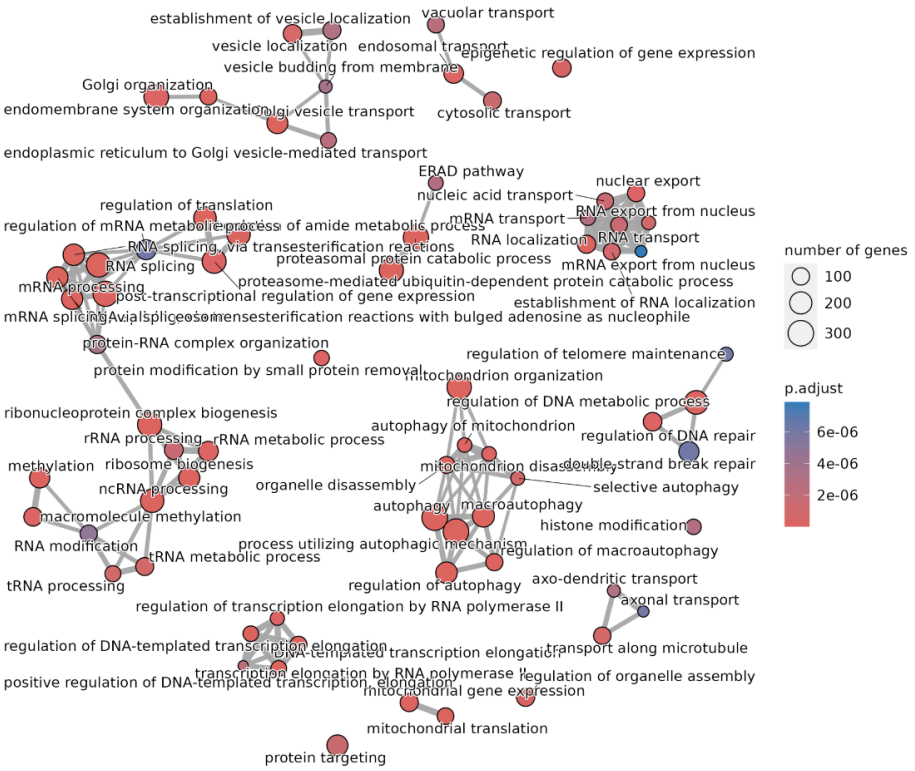


Supplementary figure 6 Gene distribution class conservation matrix for each species pair. Showing the average distribution class conservation heatmap (calculation process see methods). Pie chart showing the aggregated percentage of cell type conservation of genes that stayed expressed in over 90% of cell types; genes stayed expressed in a single cell type, and genes expressed in some cell types.

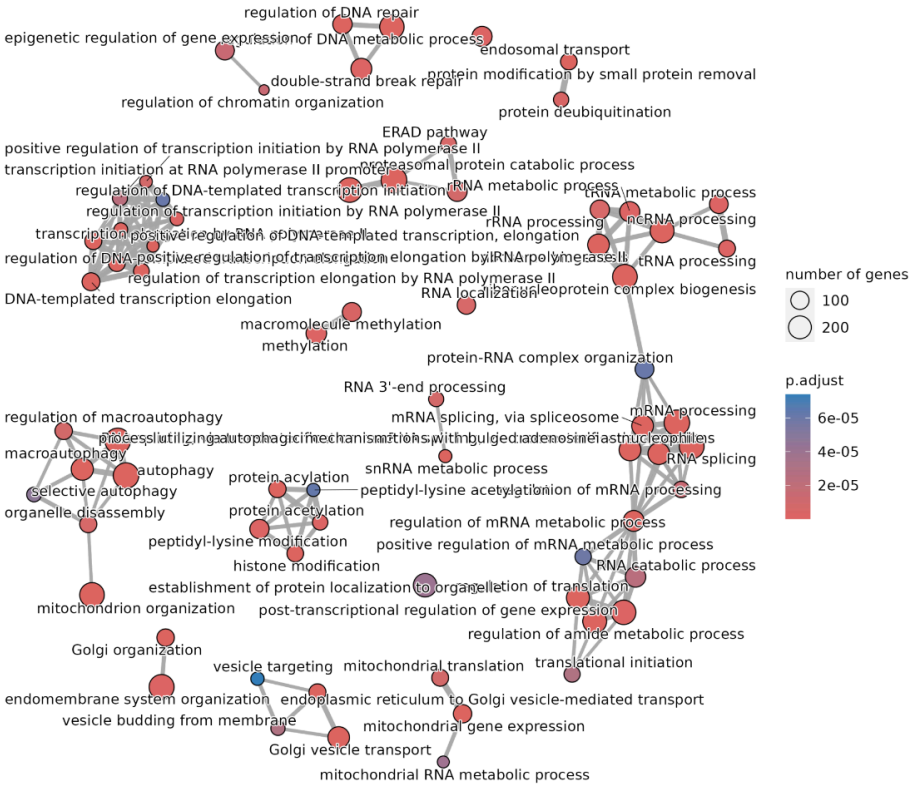
a *H.sapiens* and *P.troglodytes* conserved lowly specific and broadly expressed genes



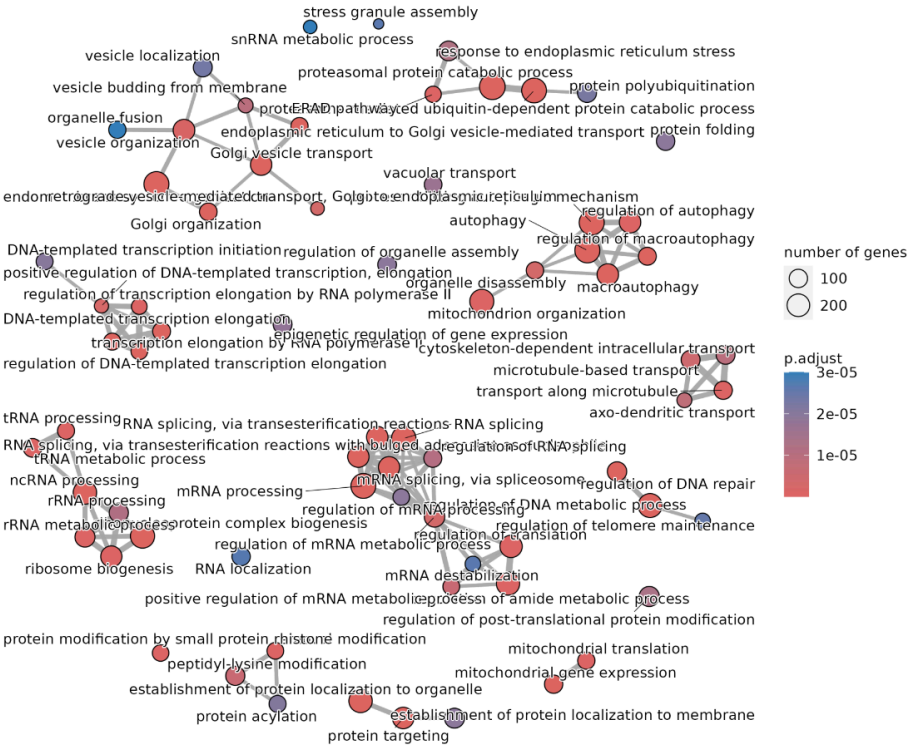
b *H.sapiens* and *G.gorilla* conserved lowly specific and broadly expressed genes



c *H.sapiens* and *M.mulatta* conserved lowly specific and broadly expressed genes

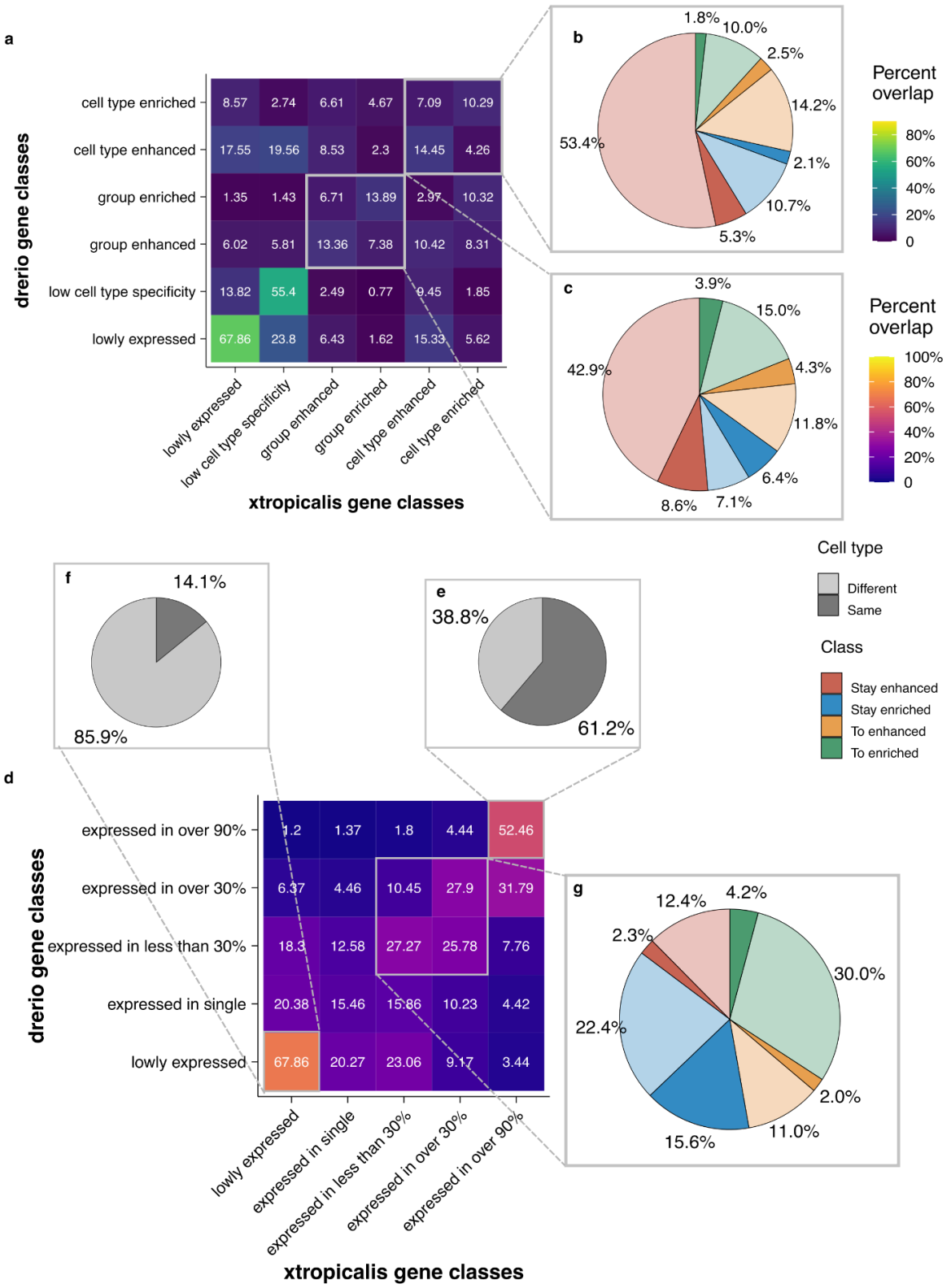


d *H.sapiens* and *C.jacchus* conserved lowly specific and broadly expressed genes

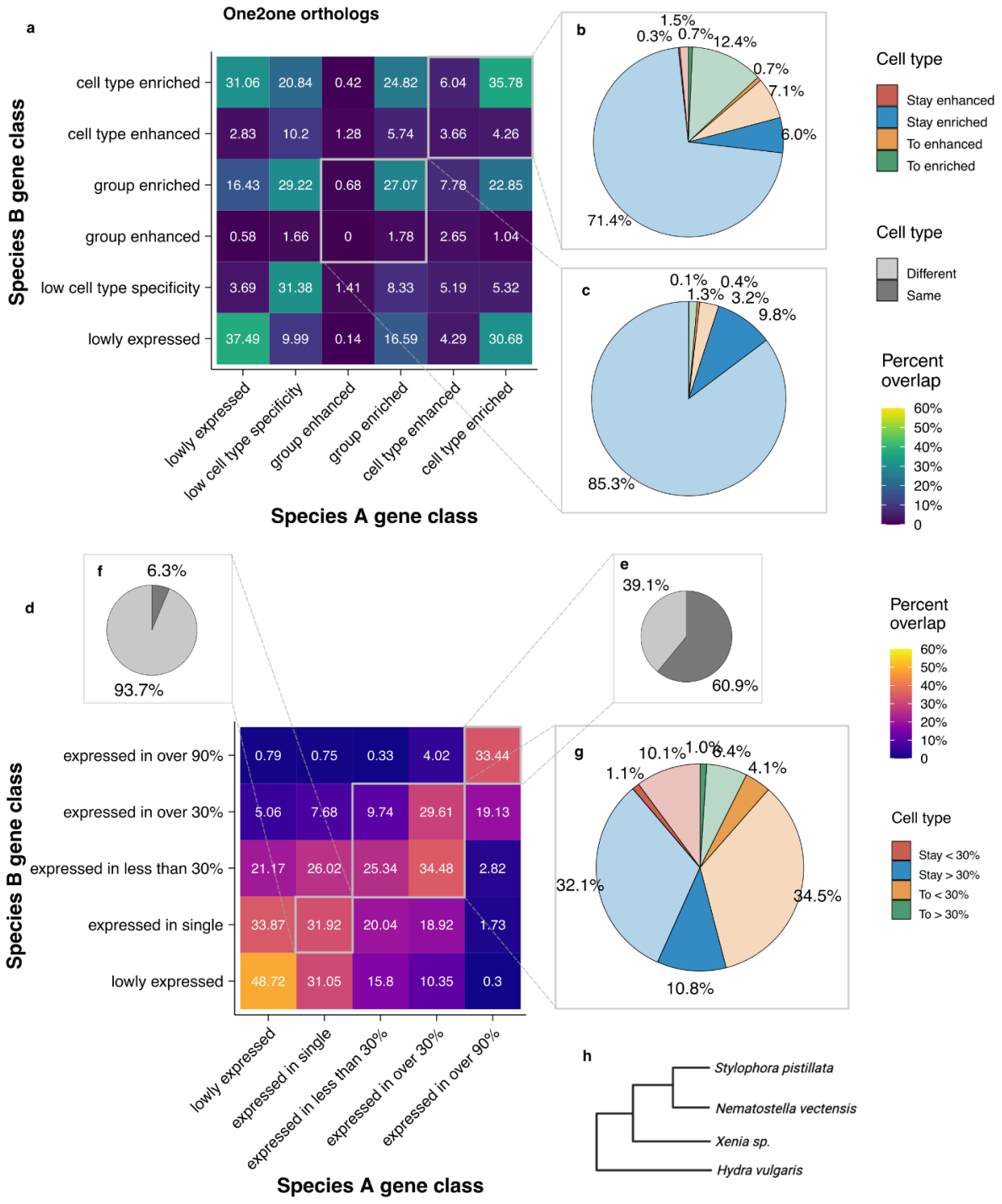


Supplementary figure 7 Gene ontology enrichment results for genes stayed lowly specific and broadly expressed between human and other species in the primate MTG data. Figure made with emapplot from the R package enrichplot. Showing 70 categories. P-values were

corrected for multiple comparisons using the Benjamini-Hochberg method, and results were considered significant if P < 0.01.

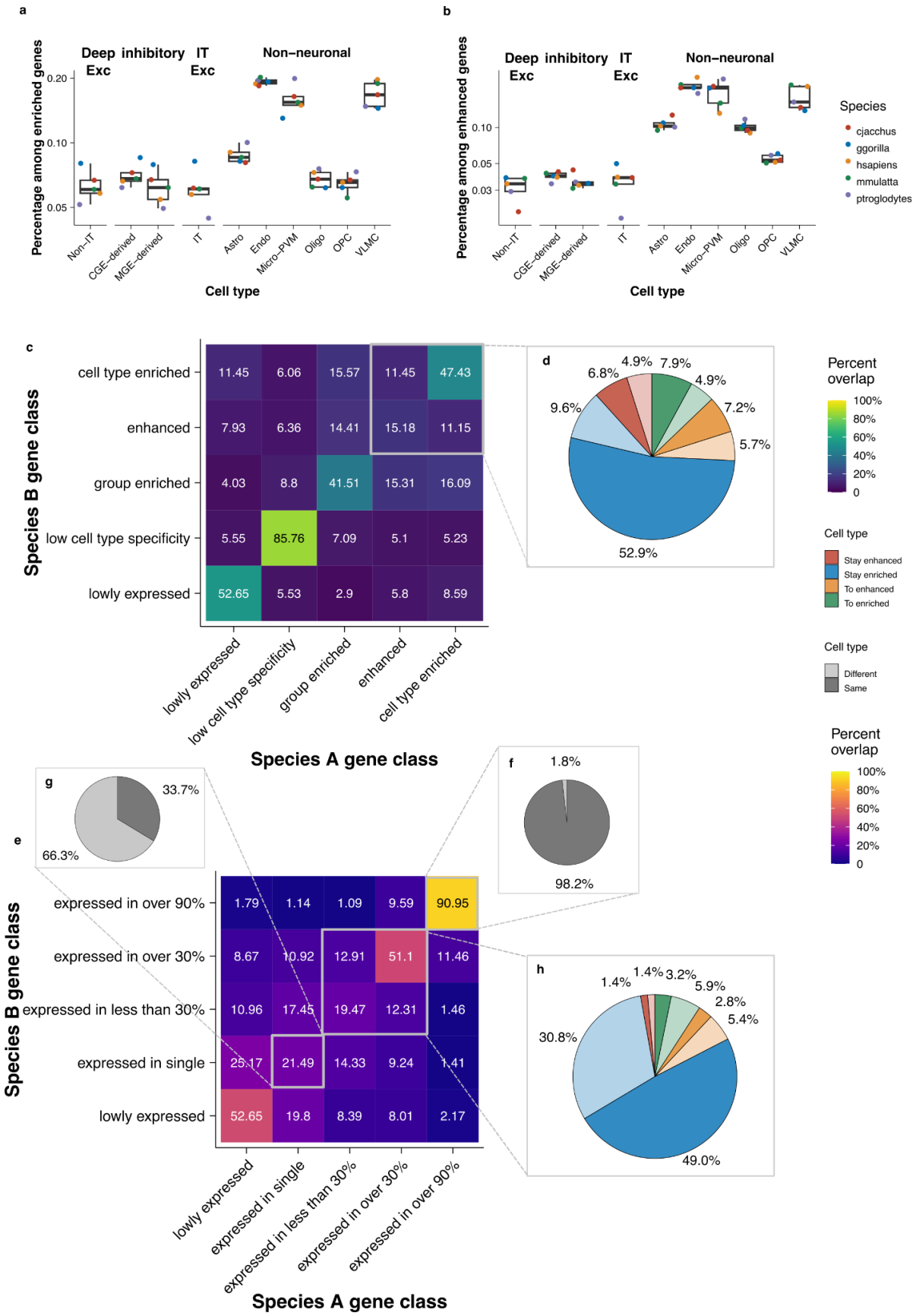


Supplementary figure 8 Gene class conservation among O2O orthologs in the embryogenesis dataset. (a) Average specificity class conservation heatmap showing the percent overlap among 1-2-1 orthologs in various specificity class combinations (calculation process see methods). (b)-(c) Pie chart showing the aggregated percentage of cell type conservation and class conservation for genes with (b) cell type level specificity or (c) group level specificity. (d) Average distribution class conservation heatmap (calculation process see methods). (e)-(g) Pie chart showing the aggregated percentage of cell type conservation of genes stayed expressed in (e) over 90% of cell types; genes stayed expressed in (f) a single cell type, and genes expressed in (g) some cell types.



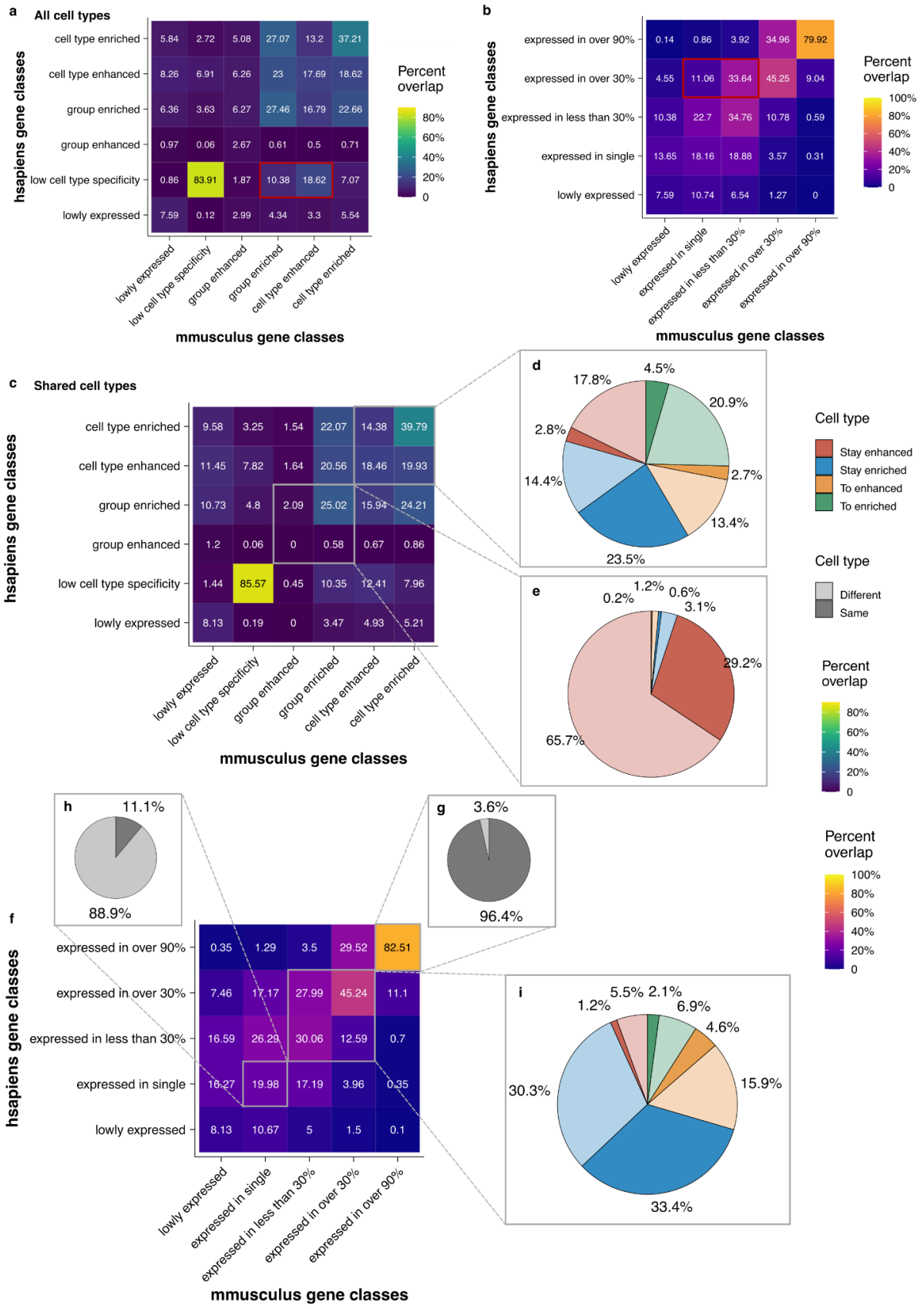
Supplementary figure 9 Gene class conservation among O2O orthologs in the Cnidarian dataset. (a) Average specificity class conservation heatmap showing the percent overlap among 1-2-1 orthologs in various specificity class combinations (calculation process see

methods). (b)-(c) Pie chart showing the aggregated percentage of cell type conservation and class conservation for genes with (b) cell type level specificity or (c) group level specificity. (d) Average distribution class conservation heatmap (calculation process see methods). (e)-(g) Pie chart showing the aggregated percentage of cell type conservation of genes stayed expressed in (e) over 90% of cell types; genes stayed expressed in (f) a single cell type, and genes expressed in (g) some cell types. (h) species tree of this dataset.



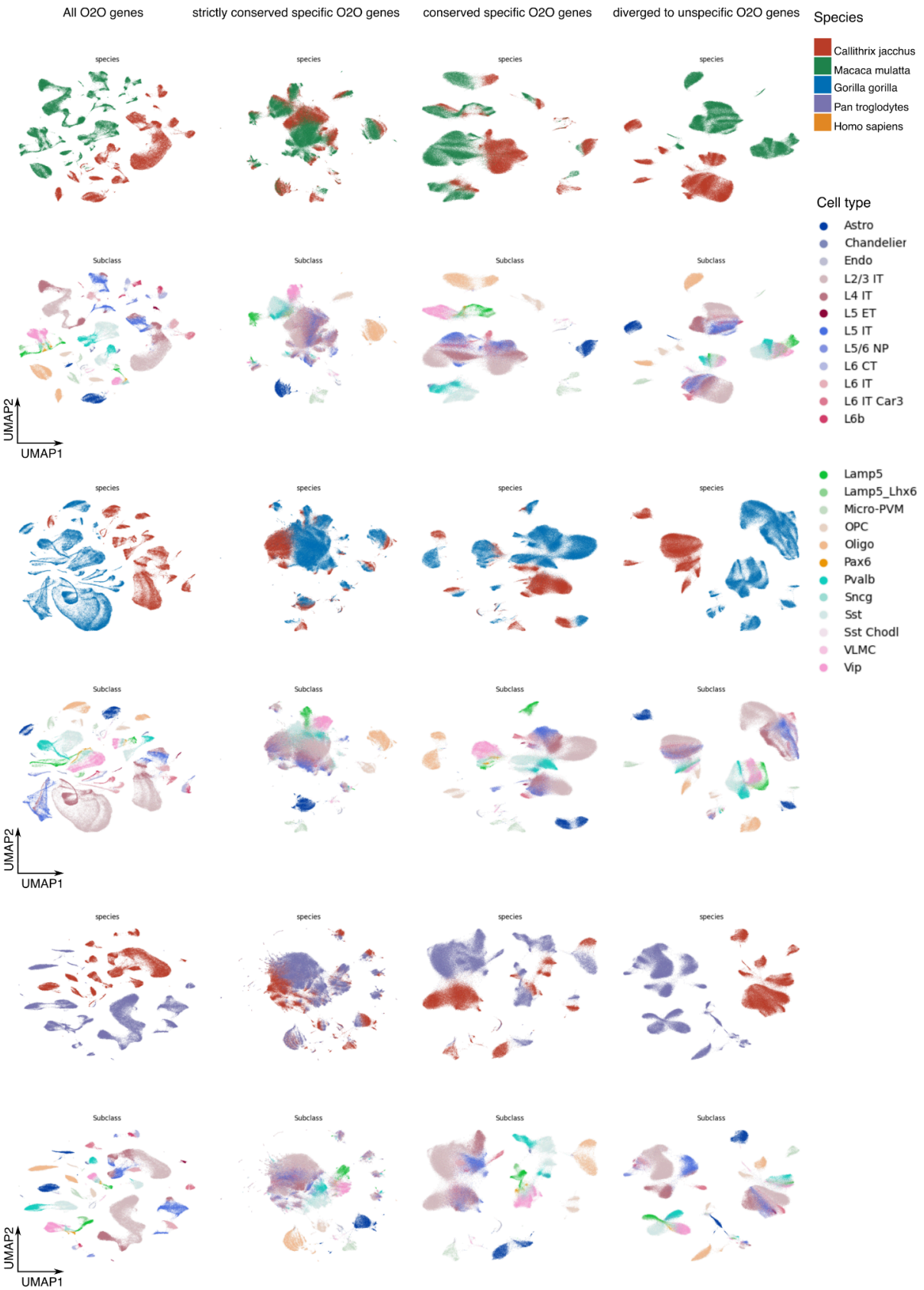
Supplementary figure 10 Gene class conservation among O2O orthologs in primate MTG dataset using reduced cell type granularity. Neurons are grouped by reduced granularity but

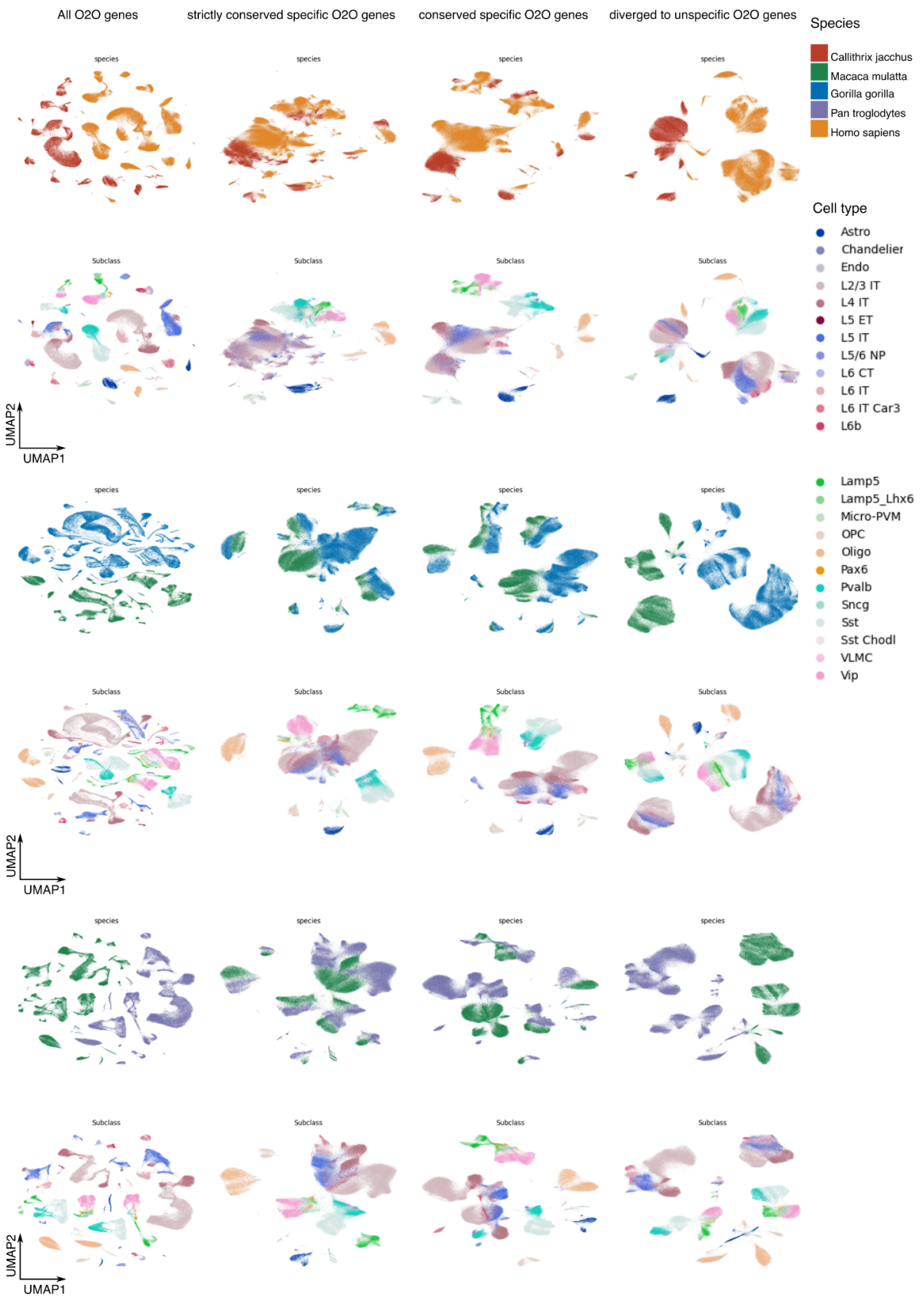
not non-neuronal cells. (a)-(b) Number of cell type-specific genes across species for each cell type. (c) Average specificity class conservation heatmap showing the percent overlap among one-to-one orthologs in various specificity class combinations (calculation process see methods). (d) Pie chart showing the aggregated percentage of cell type conservation and class conservation for genes with cell type level specificity. (e) Average distribution class conservation heatmap (calculation process see methods). (f)-(h) Pie chart showing the aggregated percentage of cell type conservation of genes stayed expressed in (f) over 90\% of cell types; genes stayed expressed in (g) a single cell type and genes expressed in (h) some cell types.

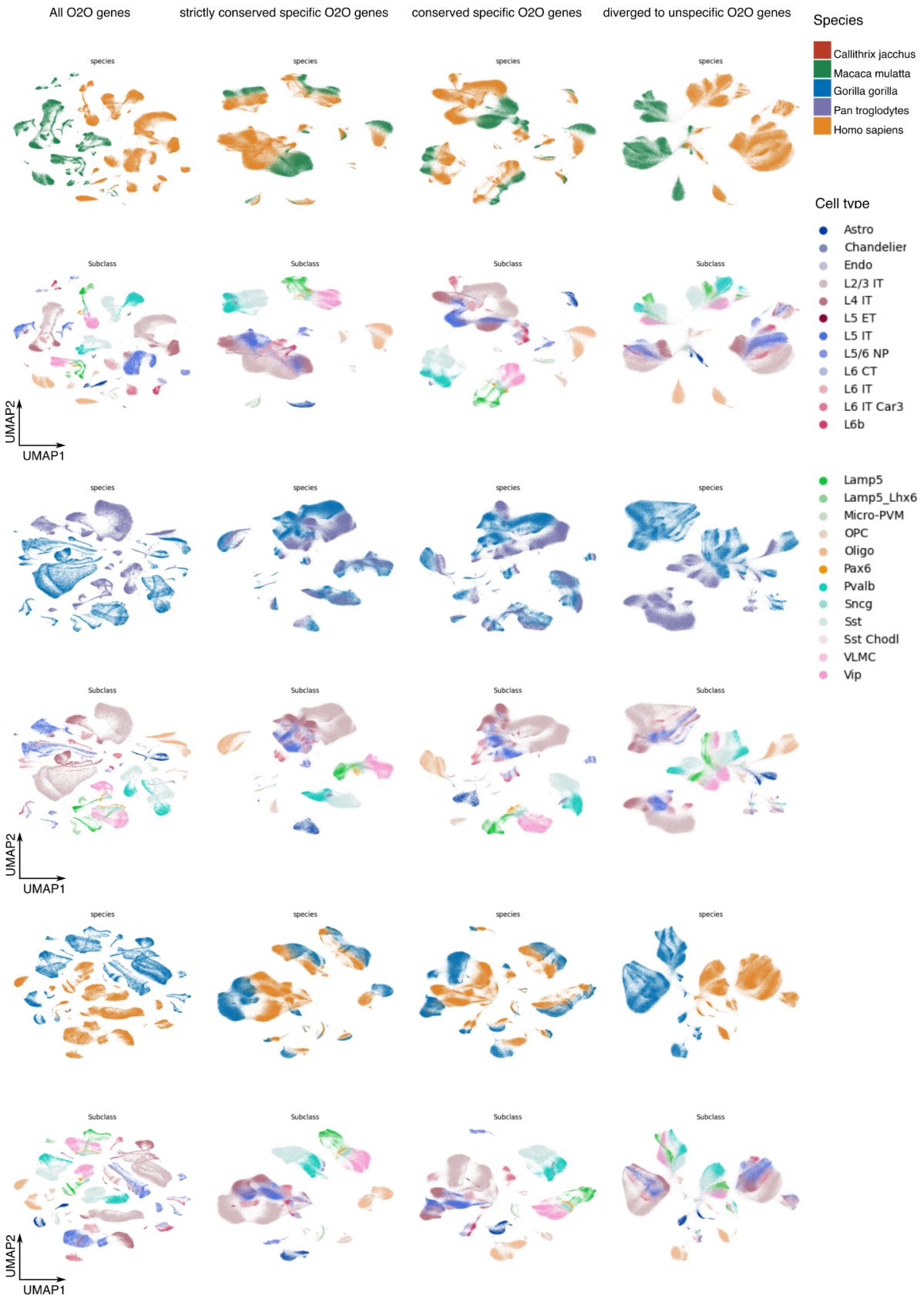


Supplementary figure 11 Gene class conservation among O2O orthologs in the human-mouse bone marrow dataset using all cell types, compared with using only shared

cell types. (a) Specificity class conservation heatmap showing the percent overlap among 1-2-1 orthologs in various specificity class combinations using all cell types (b) distribution class conservation heatmap using all cell types. Red boxes indicate the stripe pattern, indicating genes with specificity in mouse but not human, attributed to mouse data-specific cell types. (c) Specificity class conservation heatmap showing the percent overlap among 1-2-1 orthologs in various specificity class combinations using shared cell types. (d)-(e) Pie chart showing the aggregated percentage of cell type conservation and class conservation for genes with cell type level specificity (d) or group level specificity (e). (f) Distribution class conservation heatmap using shared cell types. (g)-(i) Pie chart showing the aggregated percentage of cell type conservation of genes stayed expressed in over 90% of cell types (g); genes stayed expressed in a single cell type (h,) and genes expressed in some cell types (i).

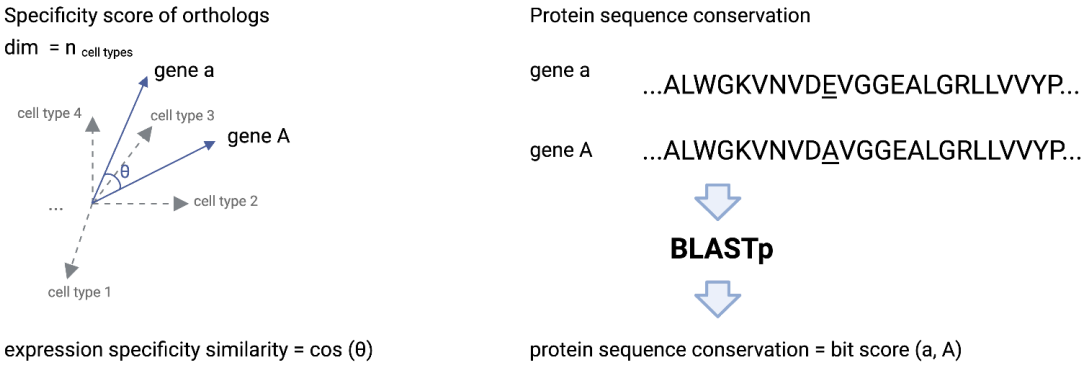

All O2O genes
strictly conserved specific O2O genes
conserved specific O2O genes
diverged to unspecific O2O genes
species
Subclass
UMAP2
UMAP1
Species
Callithrix jacchus
Macaca mulatta
Gorilla gorilla
Pan troglodytes
Homo sapiens
Cell type
Astro
Chandelier
Endo
L2/3 IT
L4 IT
L5 ET
L5 IT
L5/6 NP
L6 CT
L6 IT
L6 IT Car3
L6b
Lamp5
Lamp5_Lhx6
Micro-PVM
OPC
Oligo
Pax6
Pvalb
Sncg
Sst
Sst Chodl
VLMC
Vip


All O2O genes
strictly conserved specific O2O genes
conserved specific O2O genes
diverged to unspecific O2O genes
species
Subclass
UMAP2
UMAP1
Species
Callithrix jacchus
Macaca mulatta
Gorilla gorilla
Pan troglodytes
Homo sapiens
Cell type
Astro
Chandelier
Endo
L2/3 IT
L4 IT
L5 ET
L5 IT
L5/6 NP
L6 CT
L6 IT
L6 IT Car3
L6b
Lamp5
Lamp5_Lhx6
Micro-PVM
OPC
Oligo
Pax6
Pvalb
Sncg
Sst
Sst Chodl
VLMC
Vip

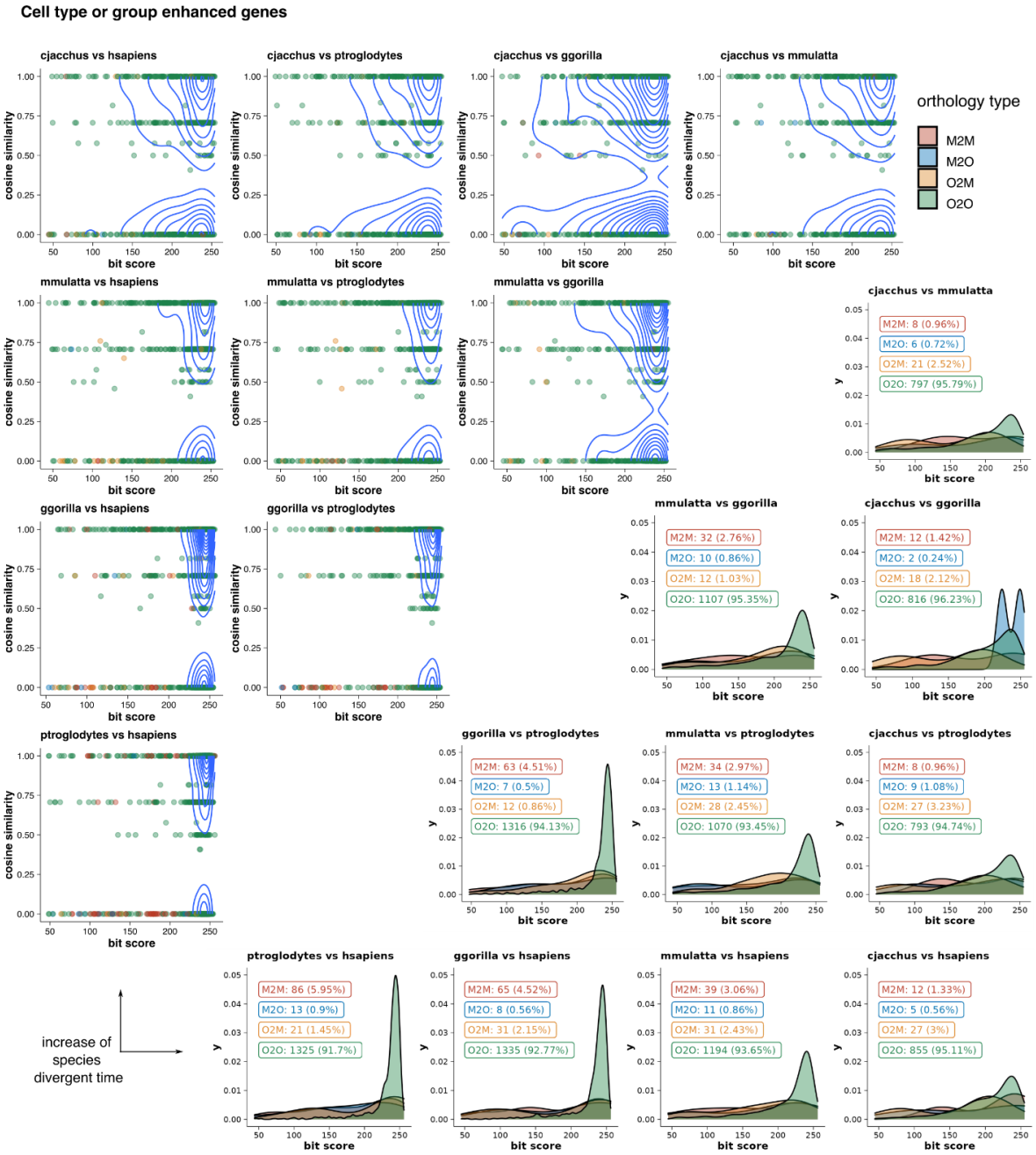


Supplementary figure 12 Gene class switching explains species effect on transcriptomic space for each species pair. (a) UMAP visualisation using different sets of O2O orthologs

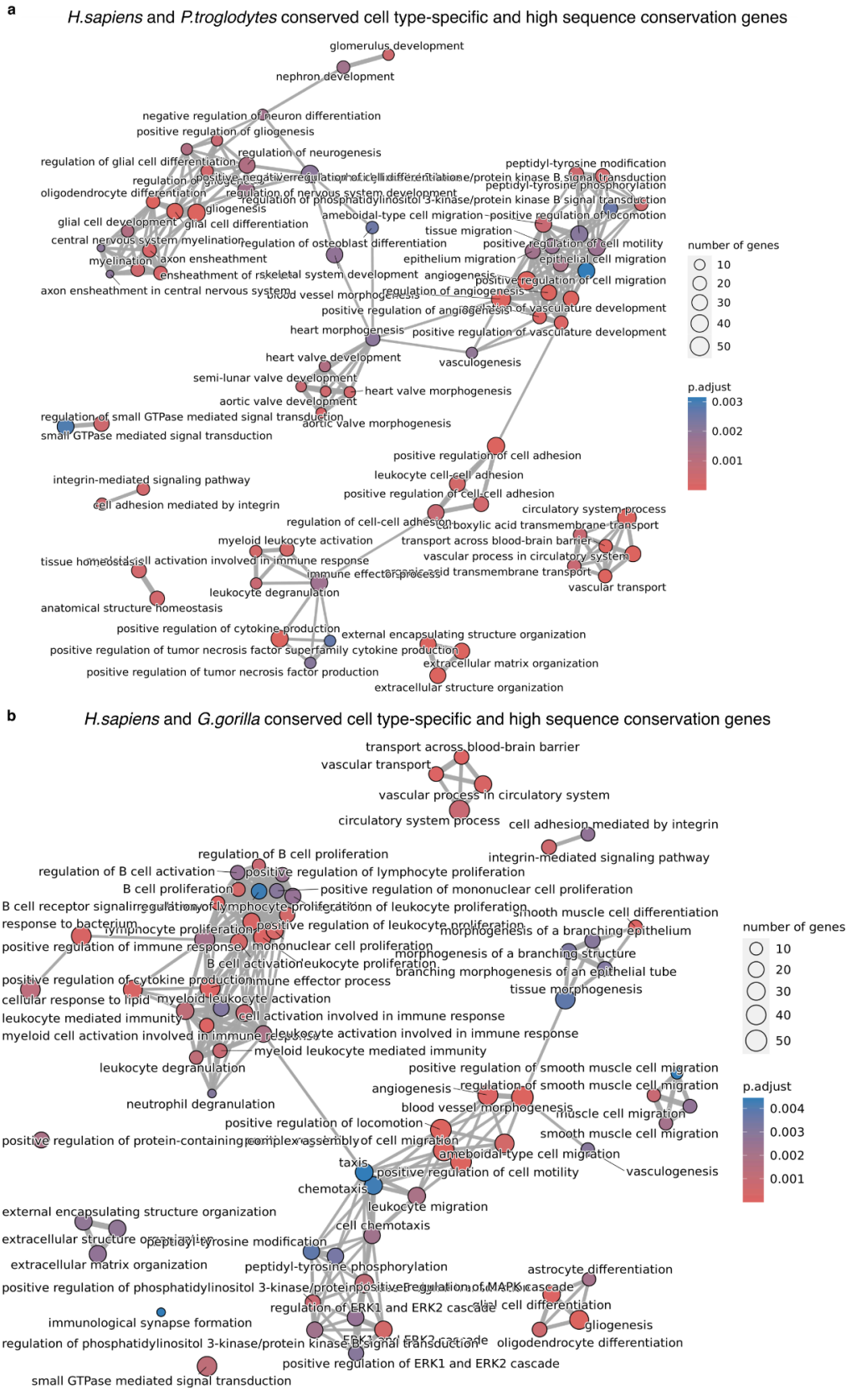
for all species data. Showing the strong species effect using all genes, the reduced species effect using (strictly) conserved specific genes, as well as a purified species effect observed in data using diverged to unspecific genes. O2O, one-to-one; UMAP, Uniform Manifold Approximation and Projection; PC, principal component.

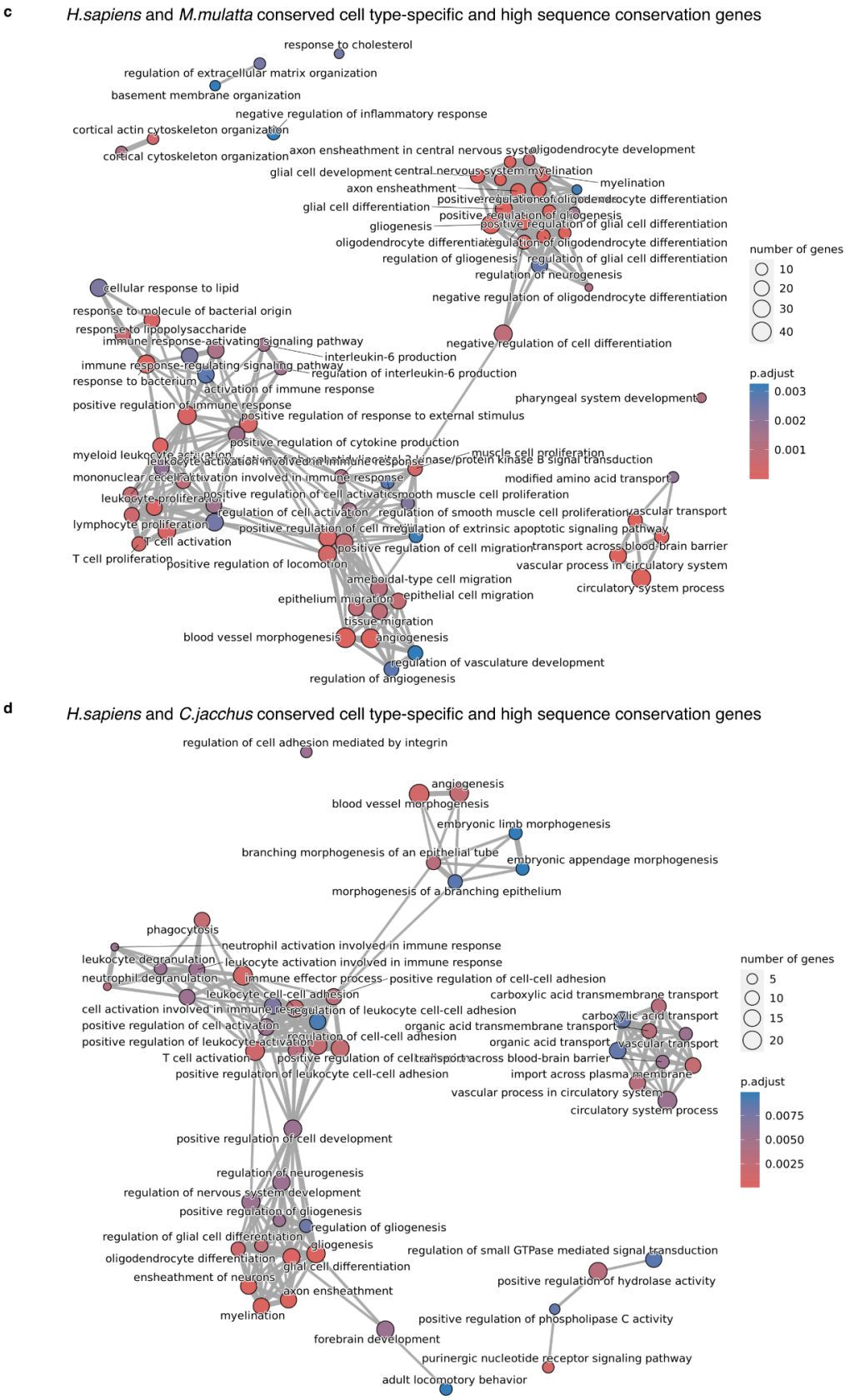


Supplementary figure 13 Schematic of the comparison of expression specificity similarity and sequence conservation of orthologs. Gene A and gene a represent orthologs from different species. Expression specificity similarity is calculated with the cosine similarity of gene specificity scores across cell types. Protein sequence conservation is the bit score of BLASTp.



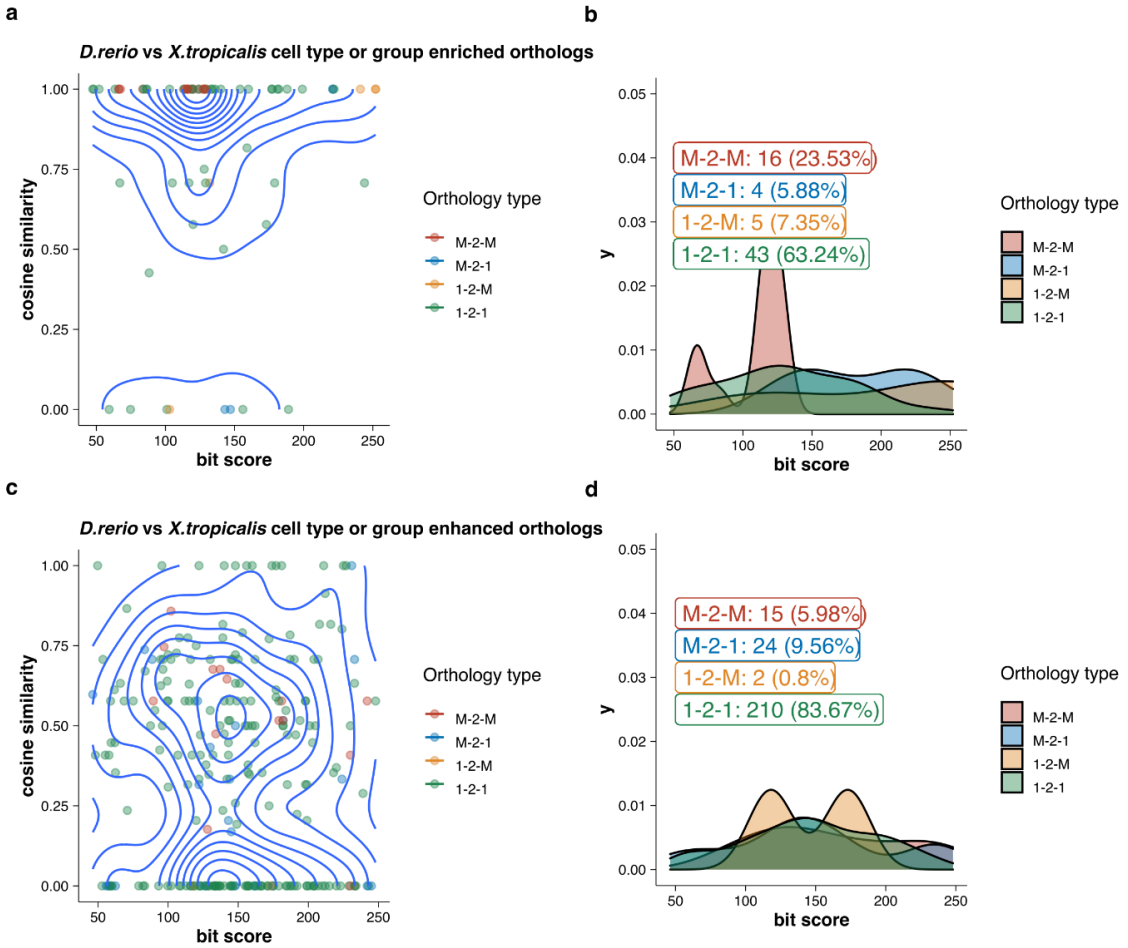
Supplementary figure 14 Comparing sequence conservation with cell type expression specificity similarity across species distance for enhanced genes. The top left triangle plots the sequence conservation (bit score) against the expression similarity (cosine similarity) for each pair of cell type or group enhanced orthologs in each pair of species. The bottom right triangle shows the distribution of bit scores for different types of orthologs for the same set of genes. O2O: one-to-one, O2M: one-to-many, M2O: many-to-one, M2M: many-to-many.


a
H.sapiens and P.troglodytes conserved cell type-specific and high sequence conservation genes
glomerulus development
nephron development
negative regulation of neuron differentiation
positive regulation of gliogenesis
regulation of neurogenesis
regulation of glial cell differentiation
peptidyl-tyrosine modification
peptidyl-tyrosine phosphorylation
regulation of nervous system development
oligodendrocyte differentiation
gliogenesis
glial cell development
glial cell differentiation
central nervous system myelination
myelination
axon ensheathment
ensheathment of rskeletal system development
axon ensheathment in central nervous system
ameboidal-type cell migration
positive regulation of locomotion
tissue migration
regulation of osteoblast differentiation
positive regulation of cell motility
epithelium migration
epithelial cell migration
angiogenesis
positive regulation of cell migration
blood vessel morphogenesis
regulation of angiogenesis
positive regulation of angiogenesis
regulation of vasculature development
heart morphogenesis
positive regulation of vasculature development
heart valve development
vasculogenesis
semi-lunar valve development
heart valve morphogenesis
aortic valve development
aortic valve morphogenesis
regulation of small GTPase mediated signal transduction
small GTPase mediated signal transduction
positive regulation of cell adhesion
leukocyte cell-cell adhesion
integrin-mediated signaling pathway
positive regulation of cell-cell adhesion
cell adhesion mediated by integrin
circulatory system process
regulation of cell-cell adhesion
carboxylic acid transmembrane transport
myeloid leukocyte activation
transport across blood-brain barrier
tissue homeostasis
cell activation involved in immune response
vascular process in circulatory system
immune effector process
amino acid transmembrane transport
vascular transport
leukocyte degranulation
anatomical structure homeostasis
positive regulation of cytokine production
external encapsulating structure organization
positive regulation of tumor necrosis factor superfamily cytokine production
extracellular matrix organization
positive regulation of tumor necrosis factor production
extracellular structure organization
number of genes
10
20
30
40
50
p.adjust
0.003
0.002
0.001
b
H.sapiens and G.gorilla conserved cell type-specific and high sequence conservation genes
transport across blood-brain barrier
vascular transport
vascular process in circulatory system
circulatory system process
cell adhesion mediated by integrin
integrin-mediated signaling pathway
regulation of B cell proliferation
regulation of B cell activation
positive regulation of lymphocyte proliferation
B cell proliferation
positive regulation of mononuclear cell proliferation
B cell receptor signaling pathway
regulation of leukocyte proliferation
smooth muscle cell differentiation
response to bacterium
lymphocyte proliferation
morphogenesis of a branching epithelium
positive regulation of immune response
morphogenesis of a branching structure
B cell activation
leukocyte proliferation
branching morphogenesis of an epithelial tube
positive regulation of cytokine production
immune effector process
tissue morphogenesis
cellular response to lipid
myeloid leukocyte activation
leukocyte mediated immunity
cell activation involved in immune response
myeloid cell activation involved in immune response
leukocyte activation involved in immune response
myeloid leukocyte mediated immunity
leukocyte degranulation
positive regulation of smooth muscle cell migration
angiogenesis
regulation of smooth muscle cell migration
neutrophil degranulation
blood vessel morphogenesis
muscle cell migration
positive regulation of locomotion
smooth muscle cell migration
positive regulation of protein-containing complex assembly
regulation of cell migration
ameboidal-type cell migration
taxis
vasculogenesis
positive regulation of cell motility
chemotaxis
leukocyte migration
external encapsulating structure organization
cell chemotaxis
extracellular structure organization
peptidyl-tyrosine modification
extracellular matrix organization
peptidyl-tyrosine phosphorylation
astrocyte differentiation
positive regulation of phosphatidylinositol 3-kinase/protein
positive regulation of MAPK cascade
regulation of ERK1 and ERK2 cascade
glial cell differentiation
immunological synapse formation
gliogenesis
regulation of phosphatidylinositol 3-kinase/protein kinase B signal transduction
oligodendrocyte differentiation
positive regulation of ERK1 and ERK2 cascade
small GTPase mediated signal transduction
number of genes
10
20
30
40
50
p.adjust
0.004
0.003
0.002
0.001



Supplementary figure 15 Gene ontology enrichment results for genes that have complete specificity conservation and high sequence similarity between human and other species.

Figure made with emapplot from the R package enrichplot. Showing 70 categories. P-values were corrected for multiple comparisons using the Benjamini-Hochberg method, and results were considered significant if P < 0.01.



Supplementary figure 16 Comparing sequence conservation with cell type expression specificity similarity in embryogenesis data. (a) and (c) plots the sequence conservation (bit score) against the expression similarity (cosine similarity) for each pair of cell type enriched or enhanced orthologs, respectively. (d) and(e) shows the distribution of bit scores for different types of orthologs for the same set of genes. 1-2-1: one-to-one, 1-2-M: one-to-many, M-2-1: many-to-one, M-2-M: many-to-many.