

Supplementary Information

Single-cell analysis highlights differences in druggable pathways underlying adaptive or maladaptive kidney regeneration

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Supplementary Figures

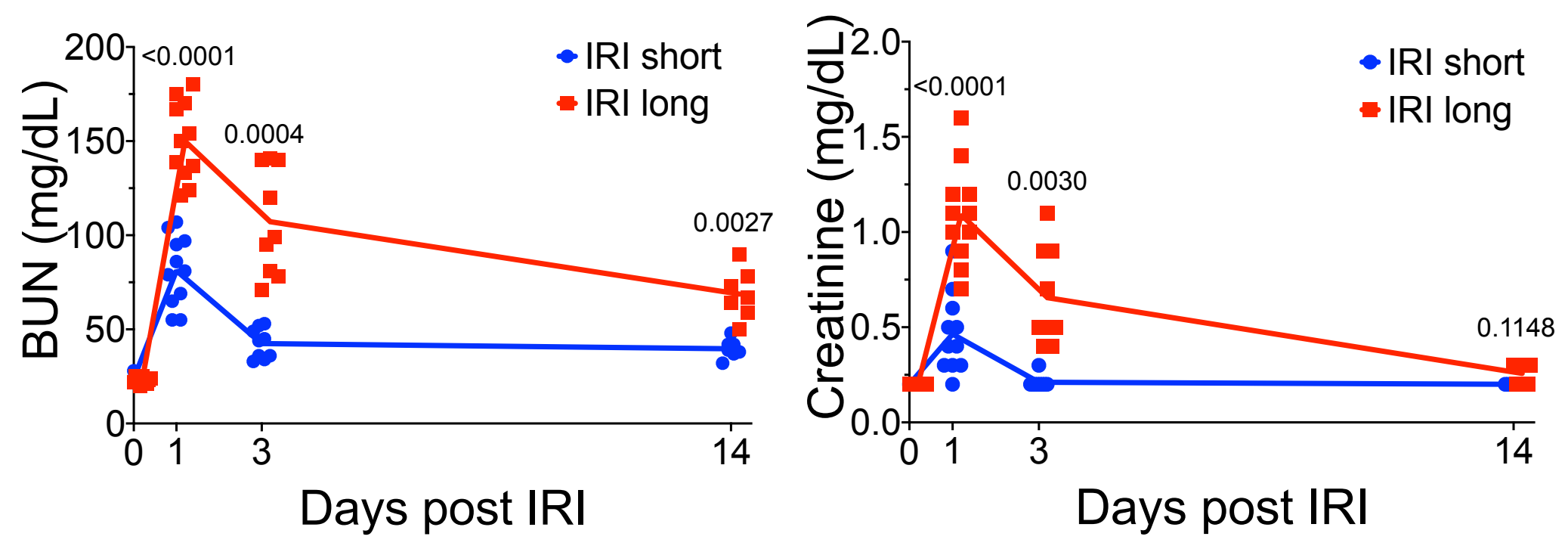
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Supplementary Tables

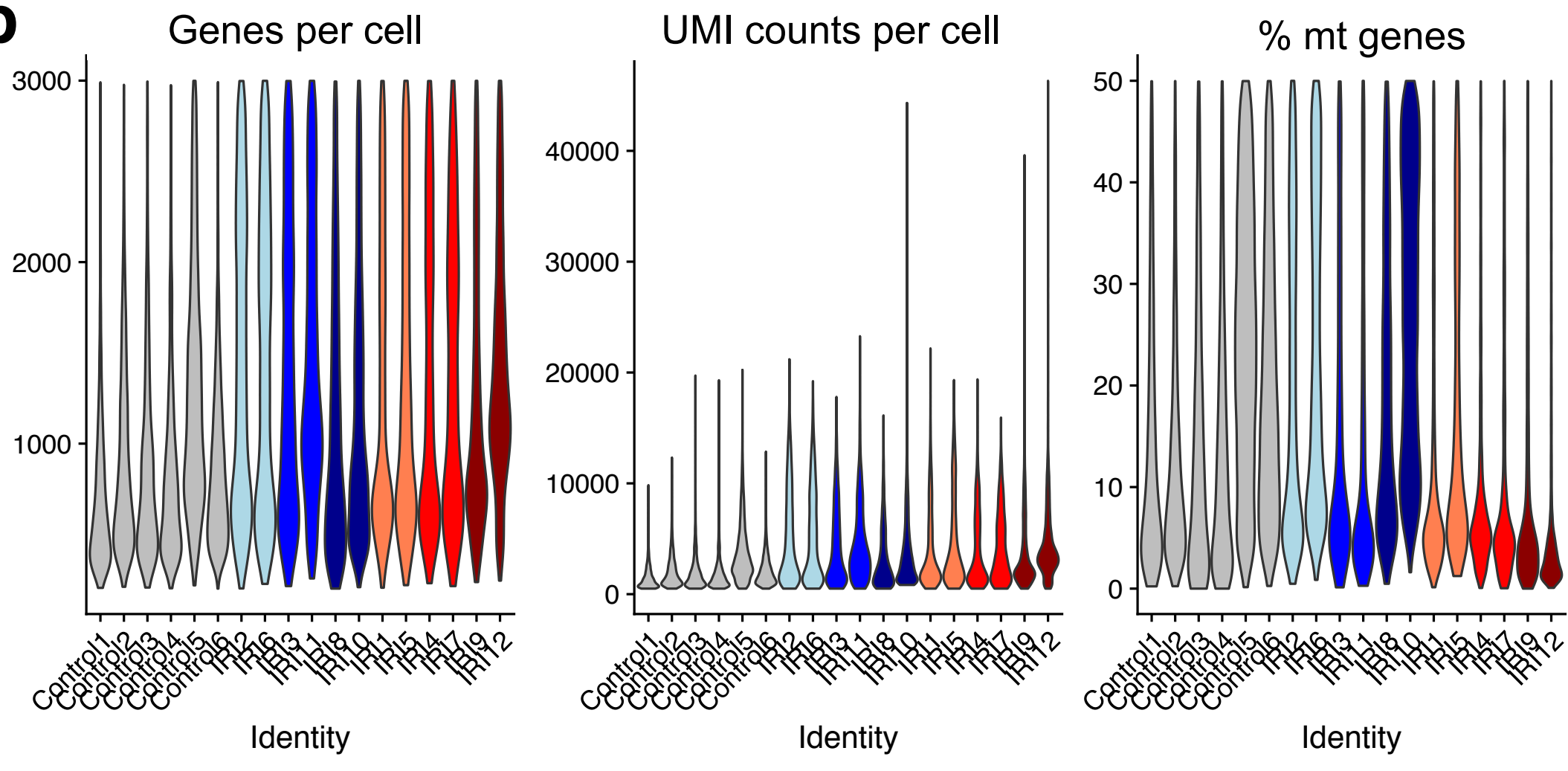
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Fig. S1

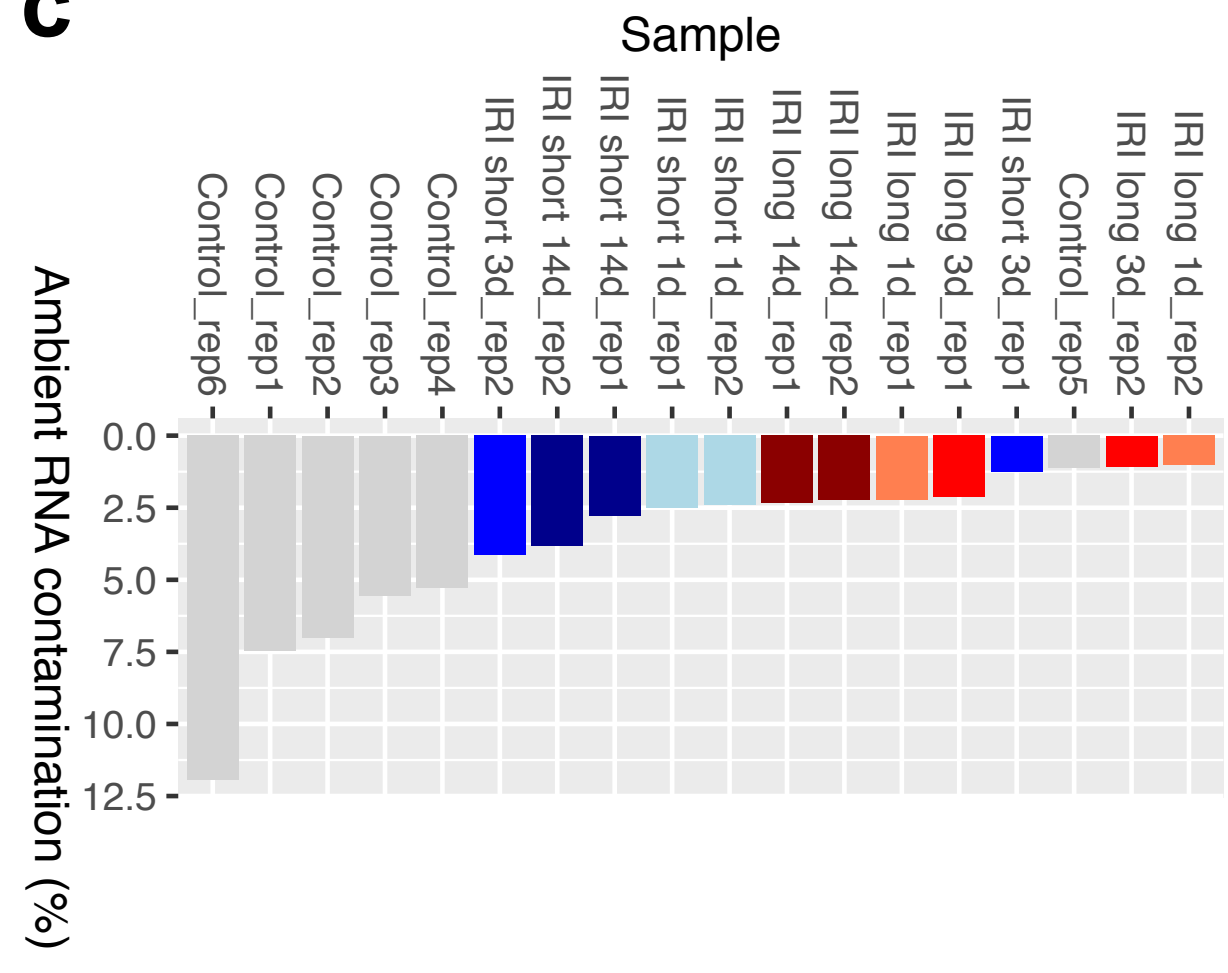
a



b



c



d

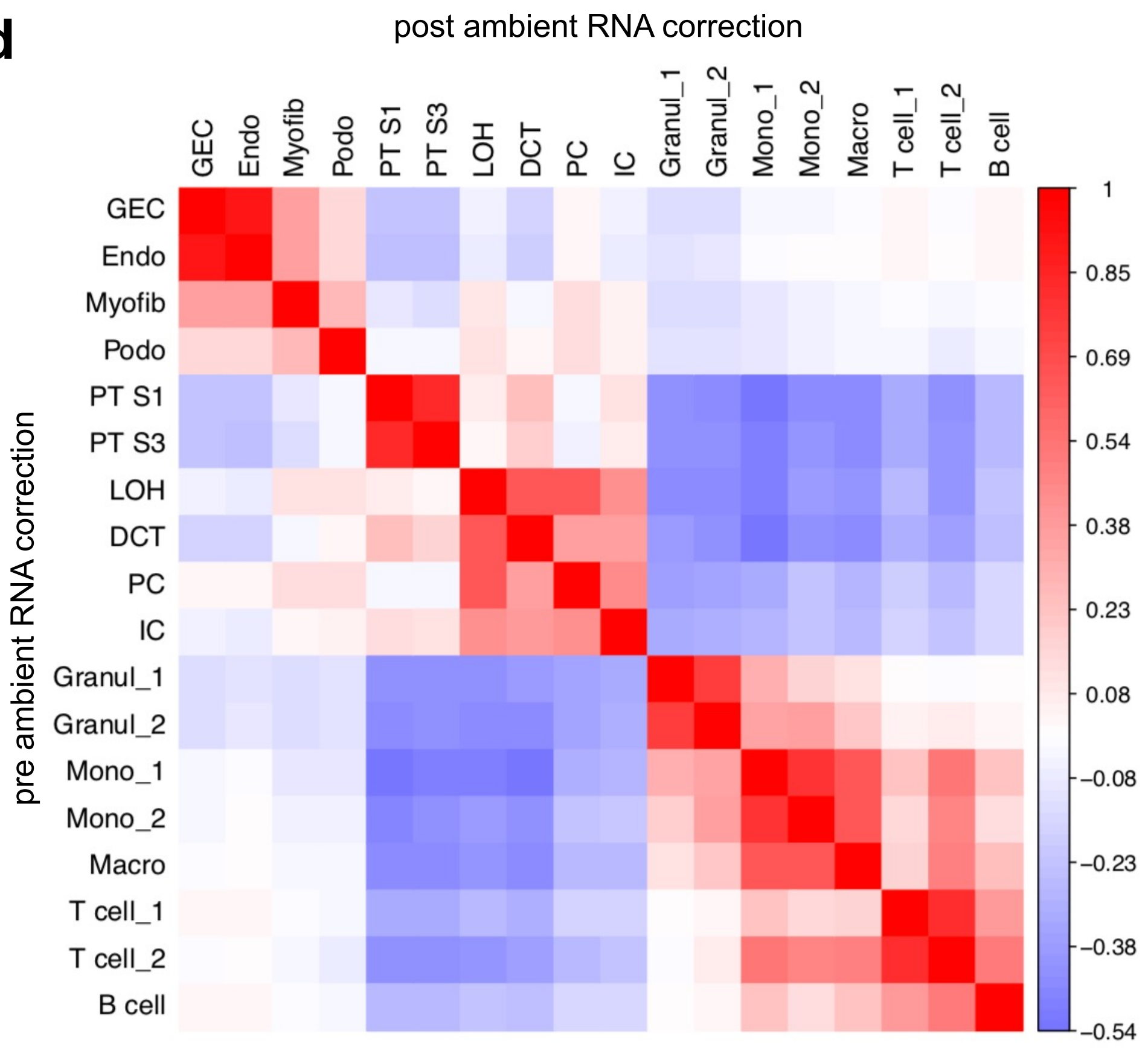


Figure S1. Ischemia reperfusion injury model titration & scRNA-seq quality control.

- (a) BUN and blood creatinine 1, 3, and 14d post-IRI. The figure includes additional animals not used for scRNA-seq and bulk RNA-seq analysis, whereas **Fig. 1b** shows only animals used for scRNA-seq. p values are given for mixed-effects model (Sidak corrected) across n=5 independent experiments.
- (b) Violin plots showing number of informative genes per single cell, unique molecular identifiers (UMIs) per single cell and percentage of mitochondrial genes per single cell, all split by batches. Dotted red lines indicate quality control limits and thresholds, respectively.
- (c) Bar graph demonstrating the percentage of ambient RNA contamination per individual sample replicates, as estimated by SoupX. Gray bars denote Control samples, blue bars IRI short, red bars IRI long.
- (d) Correlation matrix visualizing Pearson correlation coefficients (PCC) between average cell type gene expressions prior to (rows) and post (columns) correction for ambient RNA using SoupX.

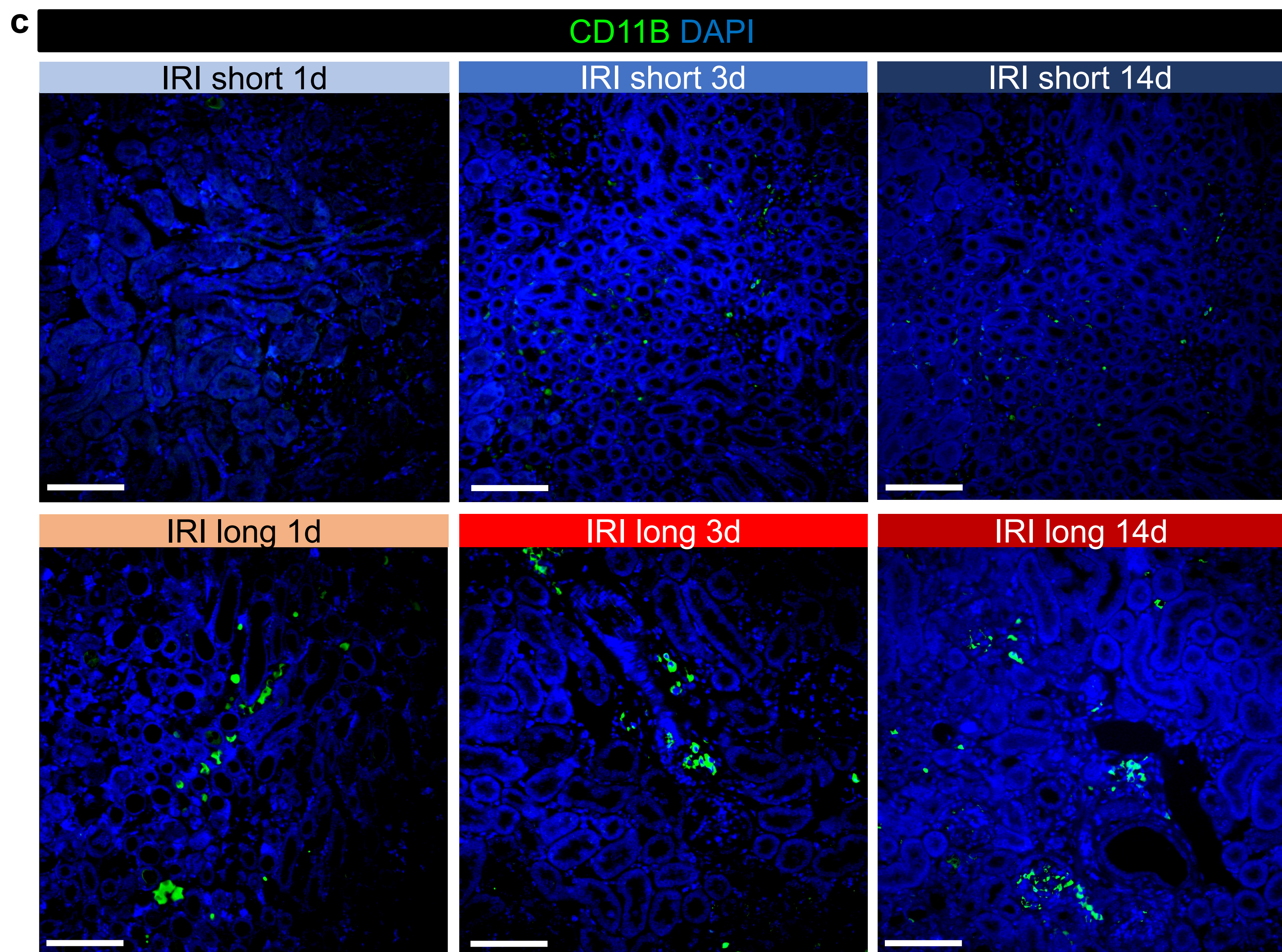
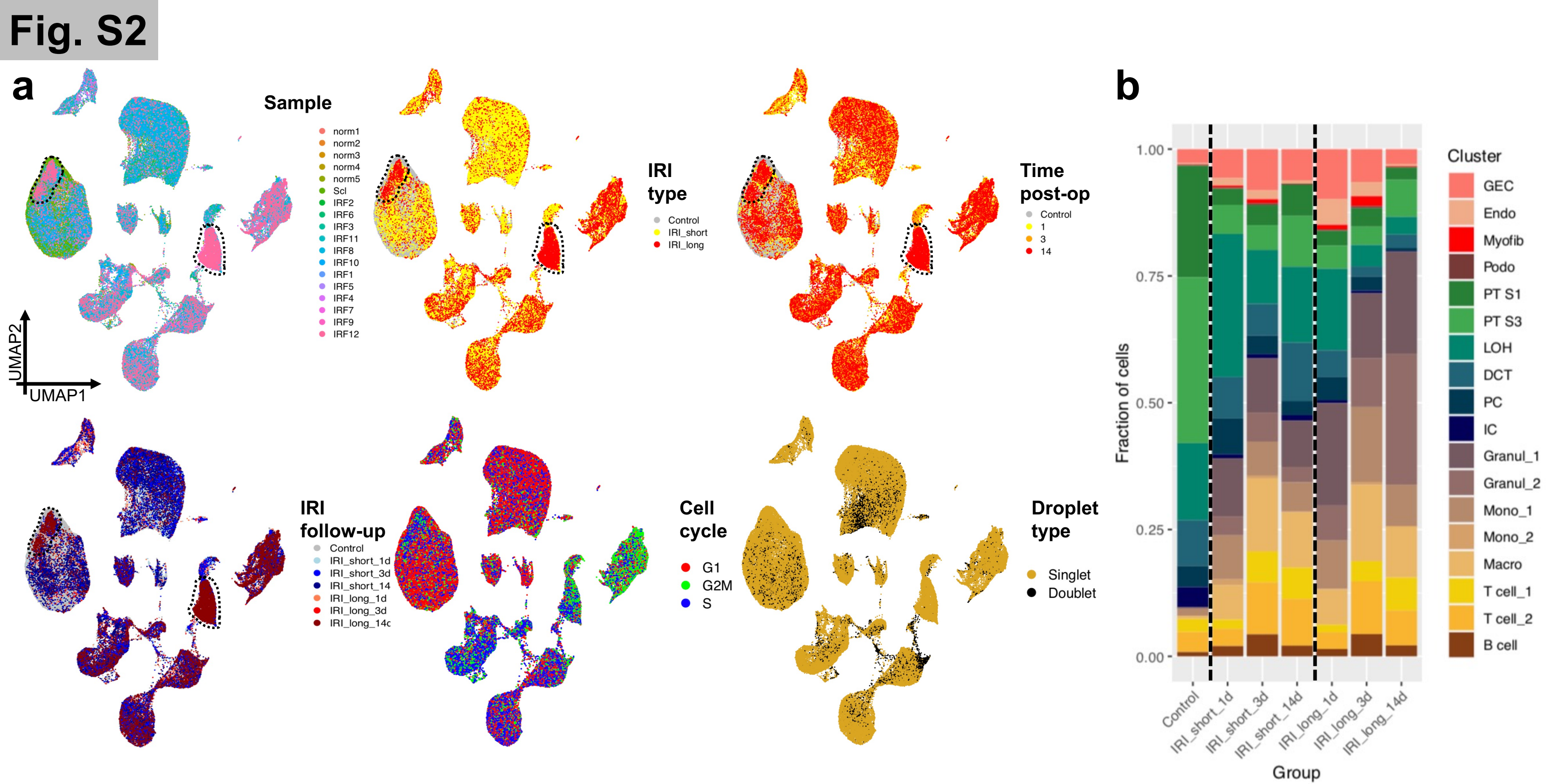


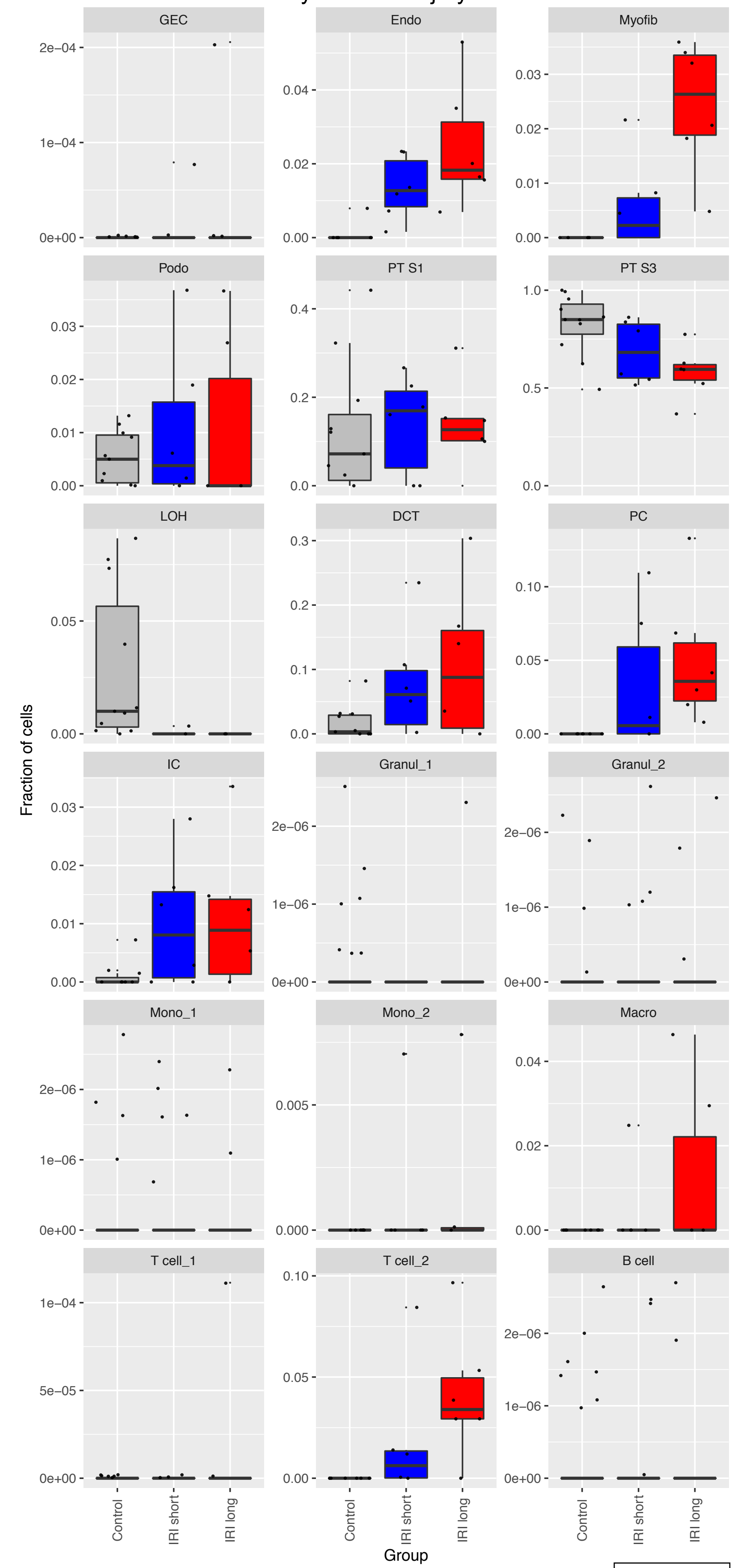
Figure S2. scRNA-seq cell type distribution, principal component heterogeneity, and myeloid cell infiltration.

- (a) UMAP projection of 113,579 cells of n=6 controls, n=6 short IRI and n=6 long IRI samples colored by sample, IRI type (Control, short IRI, long IRI), time post-op (Control, 1d, 3d, 14d), IRI follow-up (Control, IRI short 1d, IRI short 3d, IRI short 14d, IRI long 1d, IRI long 3d, IRI long 14d), cell cycle phase (G1, G2M, S) as well as projection of 122,973 cells colored by droplet type (9,394 doublets were excluded in all other plots).
- (b) Stacked bar graph showing the fraction of different cell types across different treatment groups and time points, respectively (Control, IRI short 1d, IRI short 3d, IRI short 14d, IRI long 1d, IRI long 3d, IRI long 14d): GEC, glomerular endothelial cell; Endo, endothelial cell; Myofib, myofibroblast; Podo, podocyte; PT S1, proximal convoluted tubule; PT S3, proximal straight tubule; LOH, loop of Henle; DCT, distal convoluted tubule; PC, principal cell; IC, intercalated cell; Granul, granulocyte; Mono, monocyte; Macro, macrophage; T cell; B cell.
- (c) Immunofluorescence representative of n=3 independent experiments staining for myeloid cell marker CD11B; scale bars=50 μm .

Fig. S3

a

Bulk RNA-seq deconvolution
by ischemic injury dose



b

Bulk RNA-seq deconvolution
by ischemic injury dose & time post-ischemia

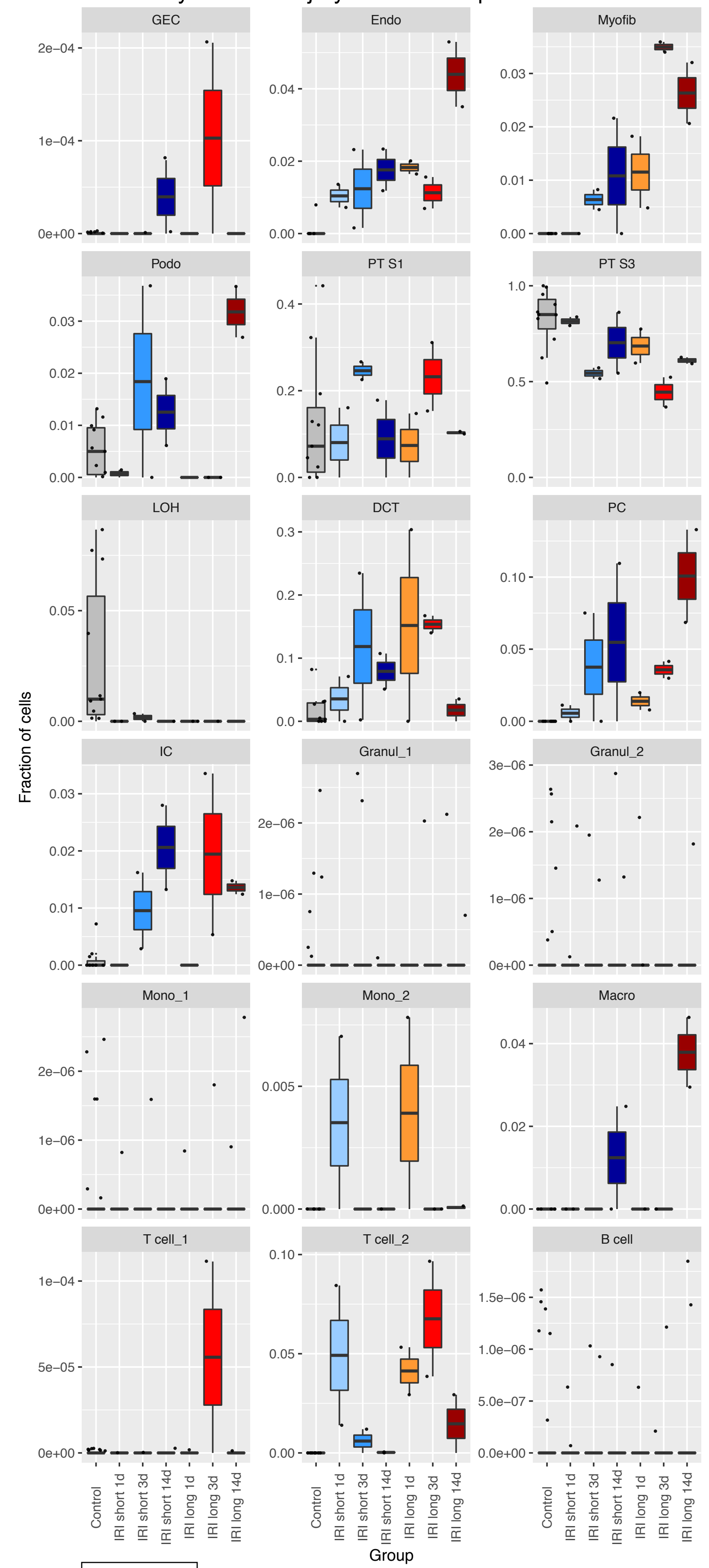


Figure S3. Bulk kidney RNA-seq deconvolution by IRI dose and time post-IRI.

(a-b) Cell fractions displayed as Tukey box plots after deconvolution of bulk RNA-seq data, stratified by injury dose only **(a)** and both injury dose and time post-ischemia ischemia **(b)**, respectively.

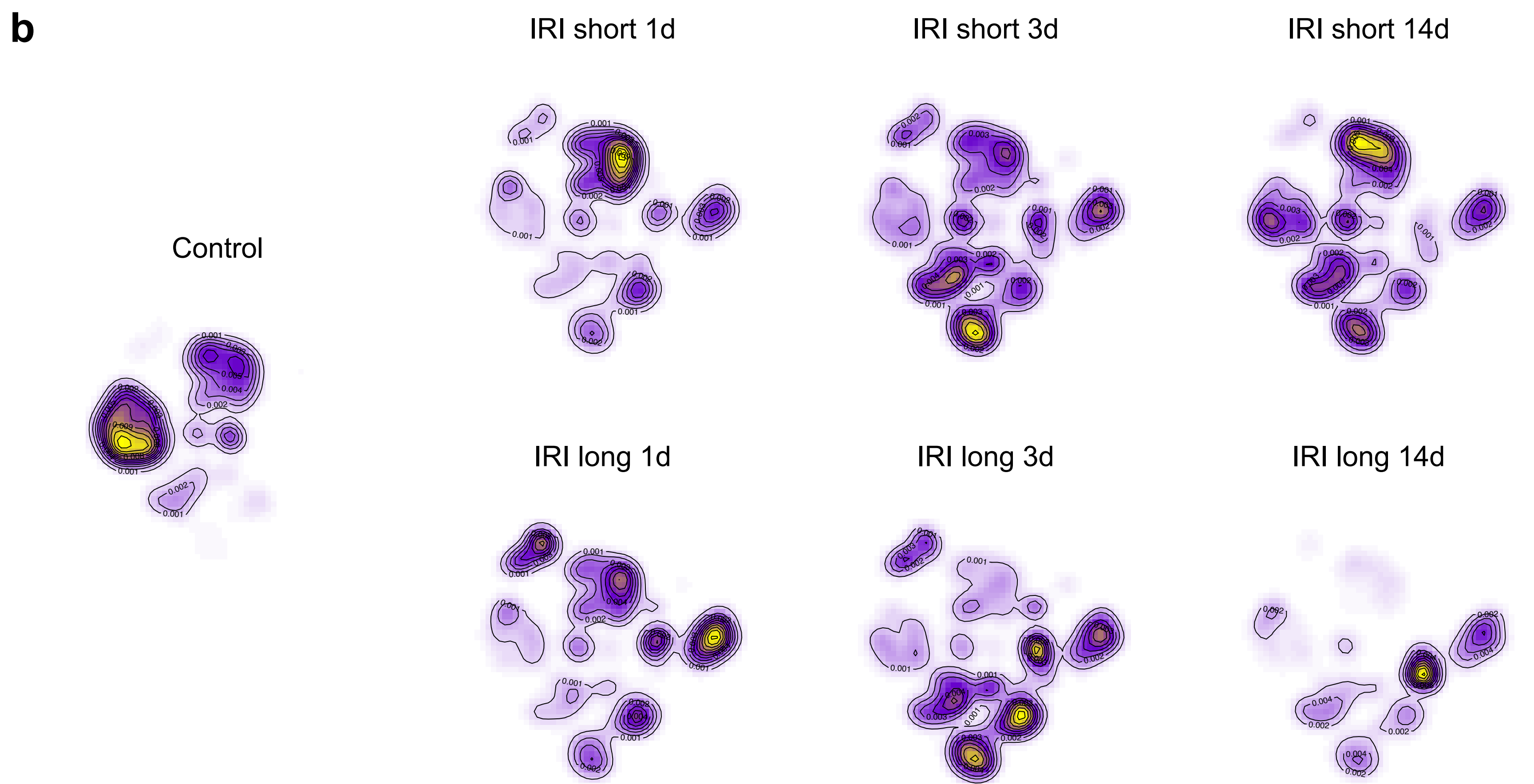
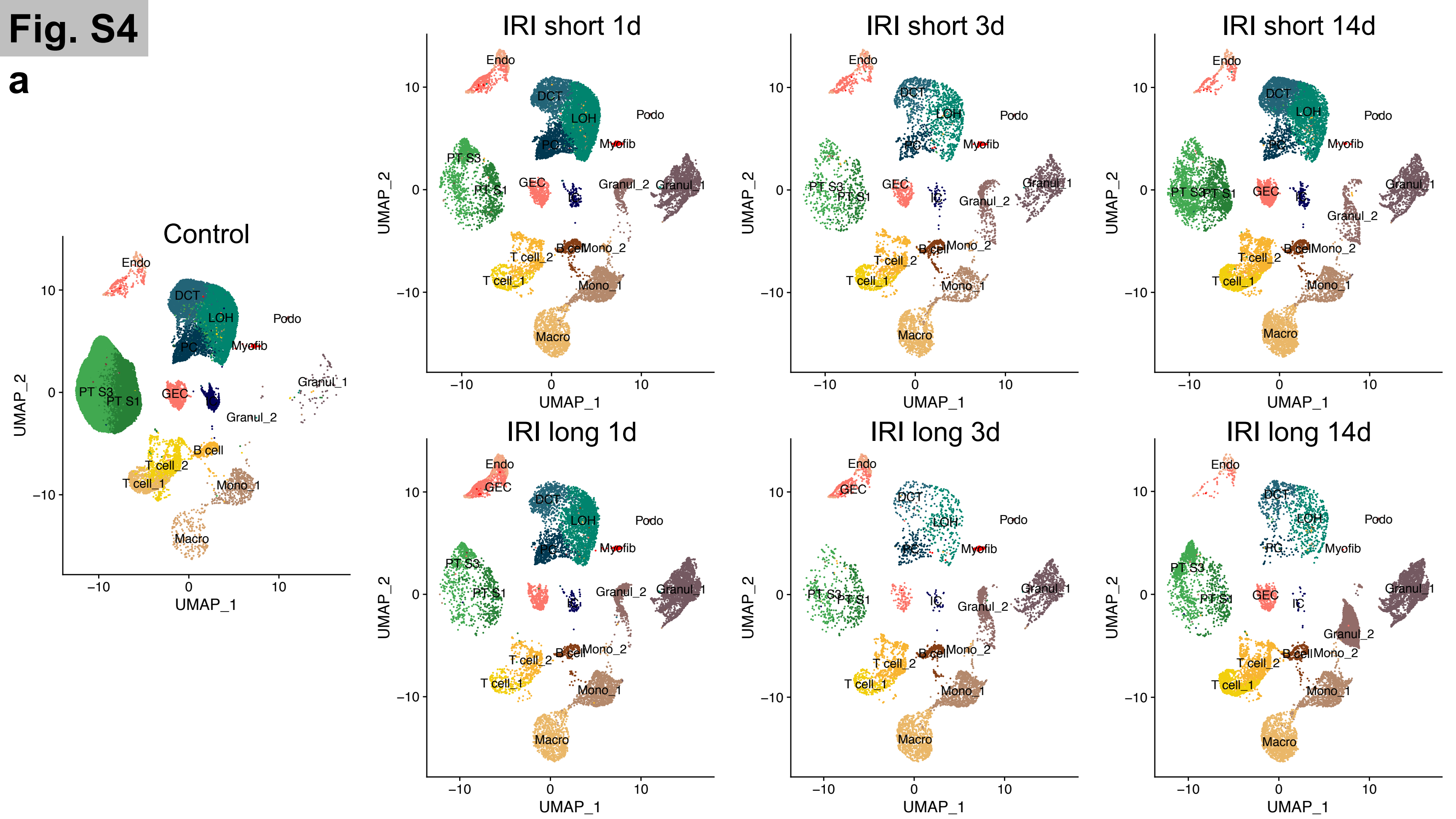


Figure S4. Cell clusters and cell density stratified by injury dose and time post-ischemia.

(a-b) UMAP projections **(a)** and density plots **(b)** corresponding to **Figs. 1g** and **1k**, respectively, stratified by injury dose and time post-ischemia.

Fig. S5

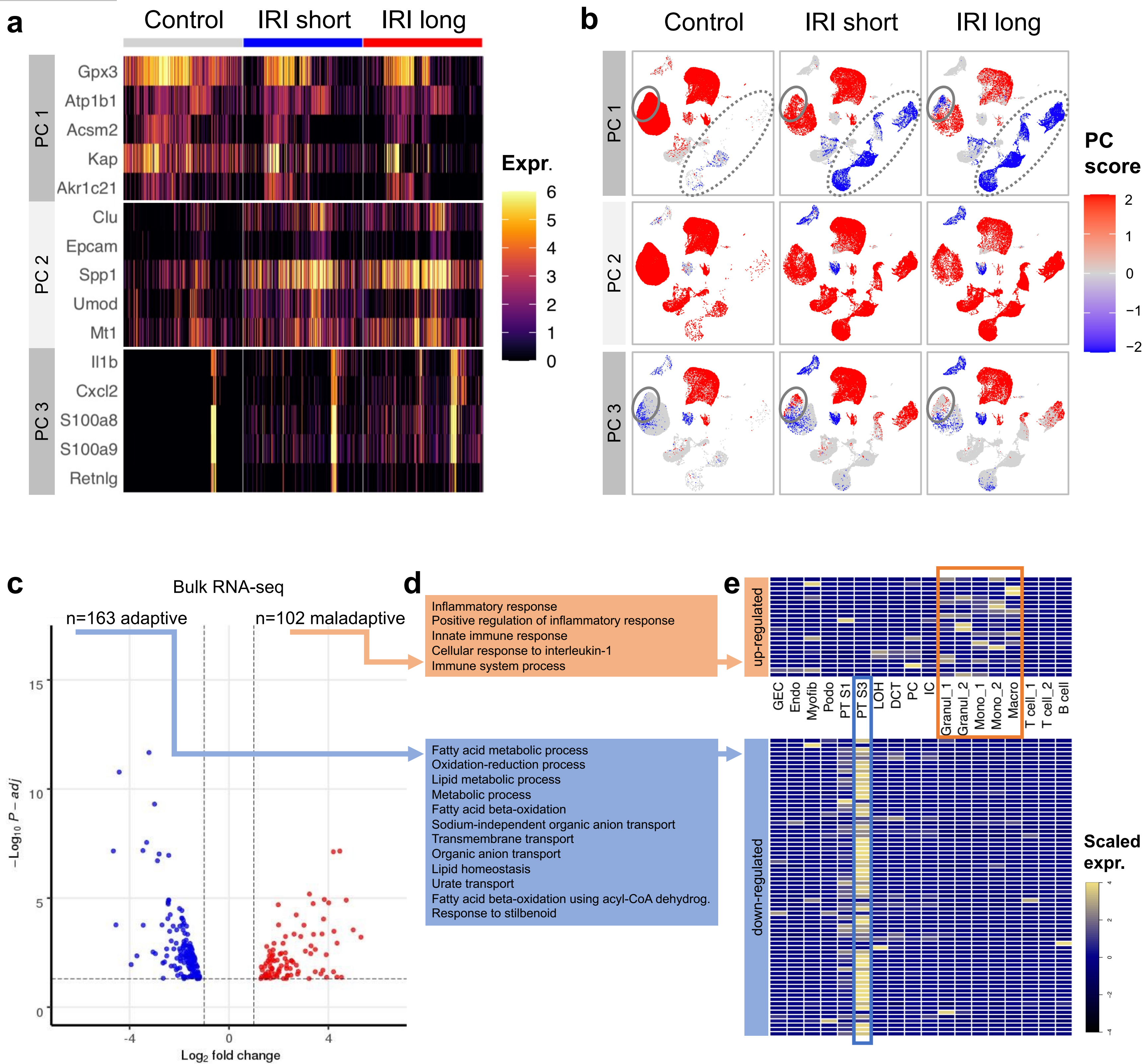


Figure S5. scRNA-seq principal component heterogeneity, and bulk RNA-seq deconvolution.

- (a) Heatmap visualizing the expression of top high-loading genes for the first three principal components (PC) based on PC analysis of the integrated dataset, illustrating recurrent aspects of transcriptional heterogeneity between treatment conditions (Control; IRI short; IRI long).
- (b) Corresponding projections of scaled and normalized PC scores across different treatment conditions. The first 3 PCs are visualized. Maladapted PT cells (circle) and myeloid cells (dotted circle) contributed the most to PC heterogeneity.
- (c) Volcano plot of differentially expressed genes (DEGs) comparing bulk RNA-seq data from samples 14d after short and long IRI, respectively. Genes upregulated in maladaptive samples are colored red, those in adaptive samples are colored blue. X axis indicates $\log_2$ -fold change for two-tailed Wald test and y axis indicates statistical significance-adjusted $p = -\log_{10}$.
- (d) Corresponding Gene Ontology (GO) analysis showing statistically significant GO terms corresponding to up- and downregulated DEGs from **(h)**.
- (e) Heatmap showing scaled cell type-specific expression of top DEGs identified in bulk RNA-seq analysis from **(h)** in the scRNA-seq dataset. Mean expression values of the genes were calculated for each cluster.

Fig. S6

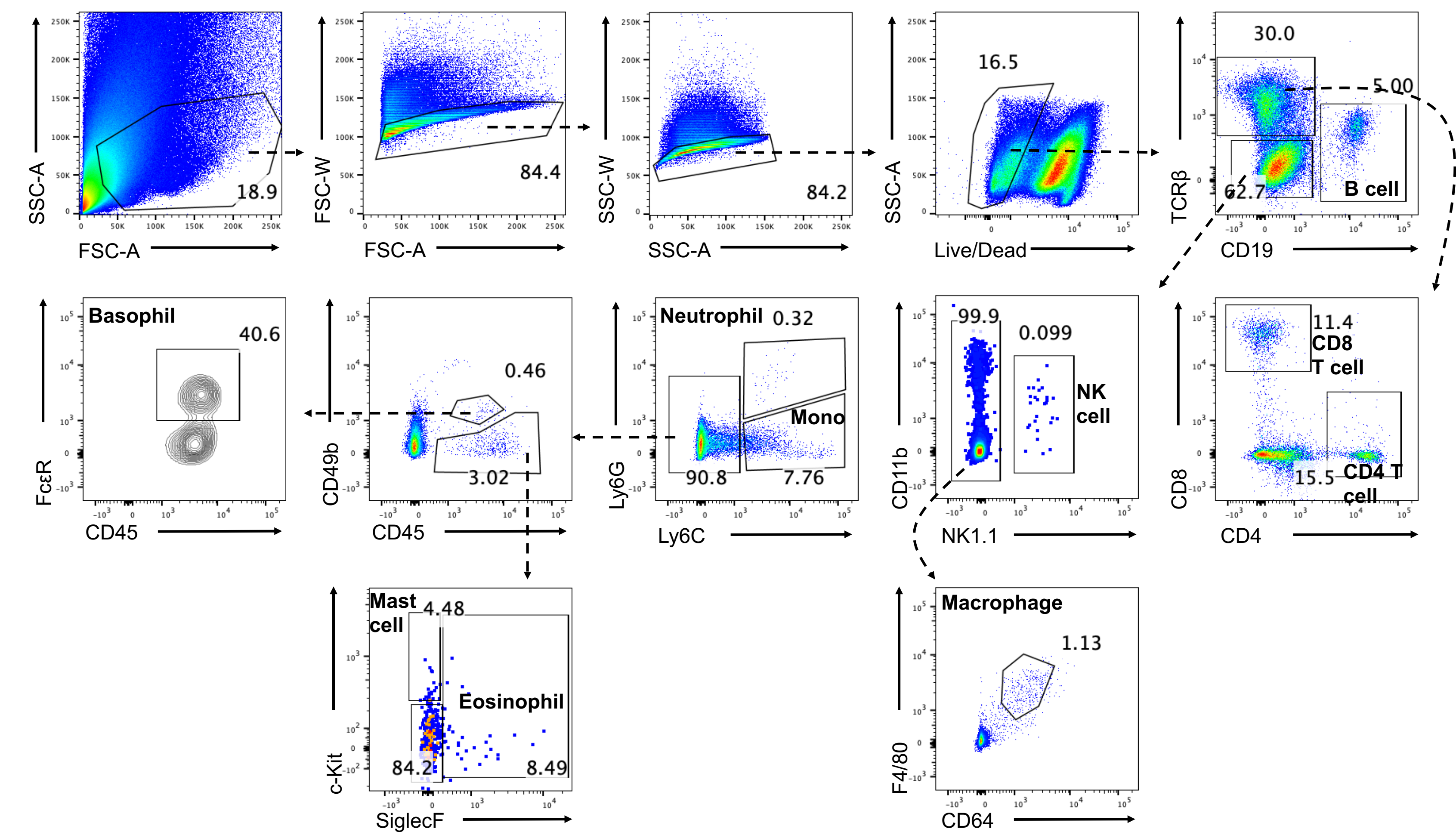


Figure S6. Gating strategy for flow cytometry.

Cell (surface) markers used to determine the cell identity of B, CD4 T, CD8 T, natural killer (NK) cells, macrophages, monocytes, neutrophils, basophils, eosinophils, mast cells, and dendritic cells, respectively; SSC, side scatter; FSC, forward scatter.

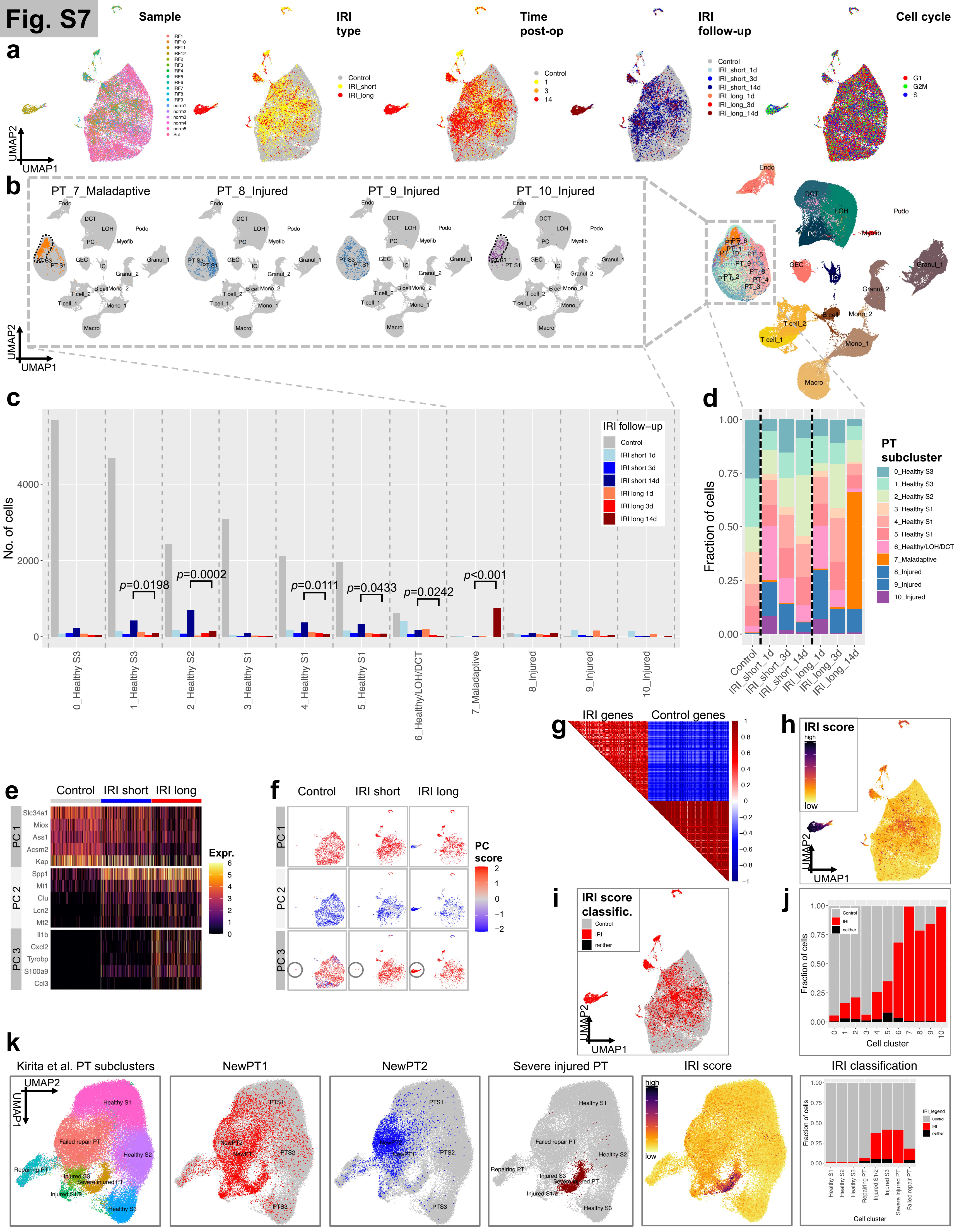


Figure S7. PT cell quality control, cell fractions, heterogeneity, and IRI score.

- (a) UMAP projection of 28,385 PT cells of n=6 controls, n=6 short IRI and n=6 long IRI samples colored by sample, IRI type (Control, short IRI, long IRI), time post-op (Control, 1d, 3d, 14d), IRI follow-up (Control, IRI short 1d, IRI short 3d, IRI short 14d, IRI long 1d, IRI long 3d, IRI long 14d), cell cycle phase (G1, G2M, S), and GMM cluster (1-5) as inferred by Slingshot analysis of sub-sampled cells.
- (b) Right panel shows a UMAP projection of the whole dataset with PT cells colored by PT subcluster as in **Fig. 1f**, as inferred by separate subclustering analysis. Left panels show projections of Maladaptive and Injured PT subclusters within the whole dataset.
- (c) Number of PT subcluster cells across ischemia dose and time post-IRI (Control, IRI short 1d, IRI short 3d, IRI short 14d, IRI long 1d, IRI long 3d, IRI long 14d). Statistical significance for comparisons between short and long IRI was derived using differential proportion analysis, with a mean error of 0.1 over 100,000 iterations; p values are given for time point comparisons between IRI short and long.
- (d) Stacked bar graph showing the fraction of PT subclusters across ischemia dose and time post-IRI (Control, IRI short 1d, IRI short 3d, IRI short 14d, IRI long 1d, IRI long 3d, IRI long 14d).
- (e) Heatmap visualizing the expression of top high-loading genes for the first three principal components (PC) based on PC analysis of all PT cells, illustrating recurrent aspects of transcriptional heterogeneity between treatment conditions (Control; IRI short; IRI long).
- (f) Corresponding projections of scaled and normalized PC scores across different treatment conditions. The first 3 PCs are visualized. Maladapted PT cells (circled) contributed the most to PC heterogeneity.
- (g) Top 100 IRI genes and 100 Control genes used to create an IRI score show very high intra-group correlation and inter-group anticorrelation; scale represents Pearson correlation coefficients.
- (h) Calculated IRI scores projected on every single PT cell in UMAP embedded space highlights stark phenotype differences between Healthy and Maladaptive PT subclusters.
- (i) IRI score-derived corresponding cell classification into either Control, IRI or neither of the two.
- (j) Fraction of cells classified as in (i), stratified by PT subcluster.
- (k) Validation of IRI scoring method in PT cells from an external renal IRI dataset (Kirita et al.). Original PT cluster annotations from the external dataset, NewPT1, NewPT2, and Severe injured PT cells are visualized in UMAP space. Note that PT cells with the highest IRI score are located in cluster “Severe injured PT”. Right subpanel shows cell classification according to IRI score into either Control, IRI or neither of the two and confirms the presence of IRI-classified cells exclusively in non-healthy cell clusters.

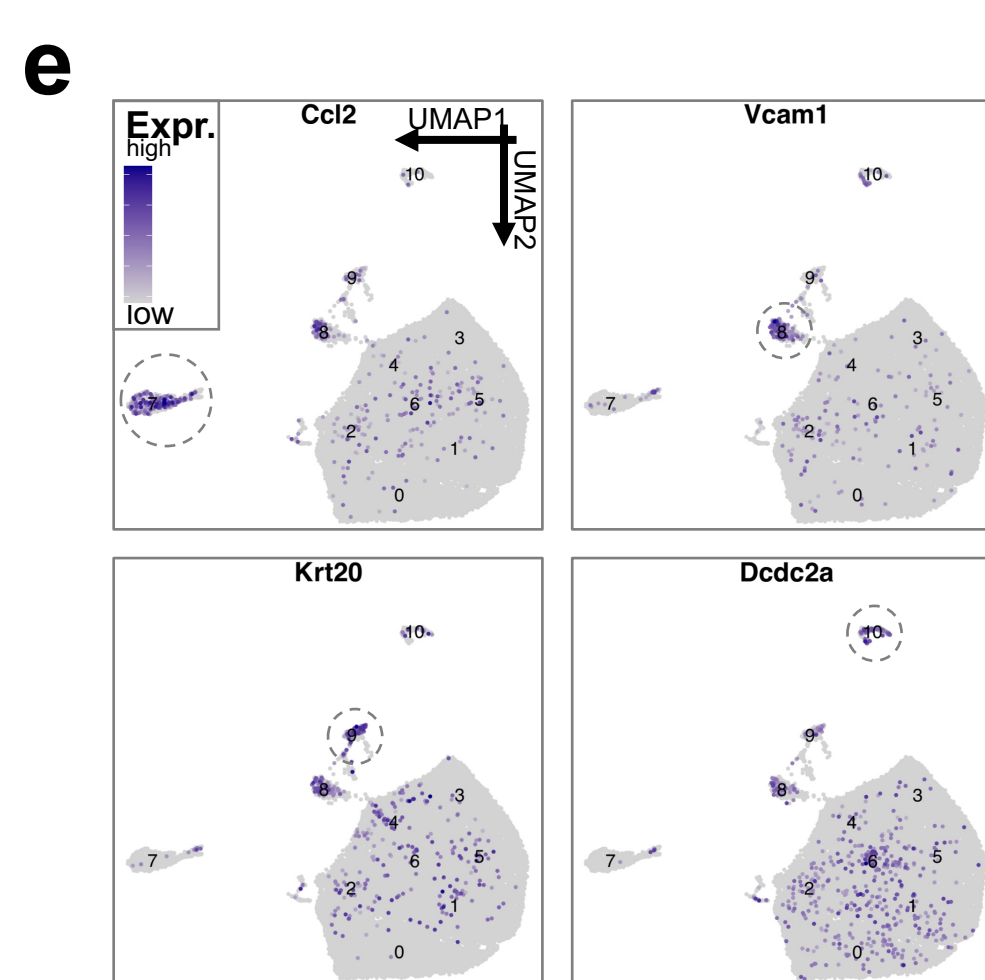
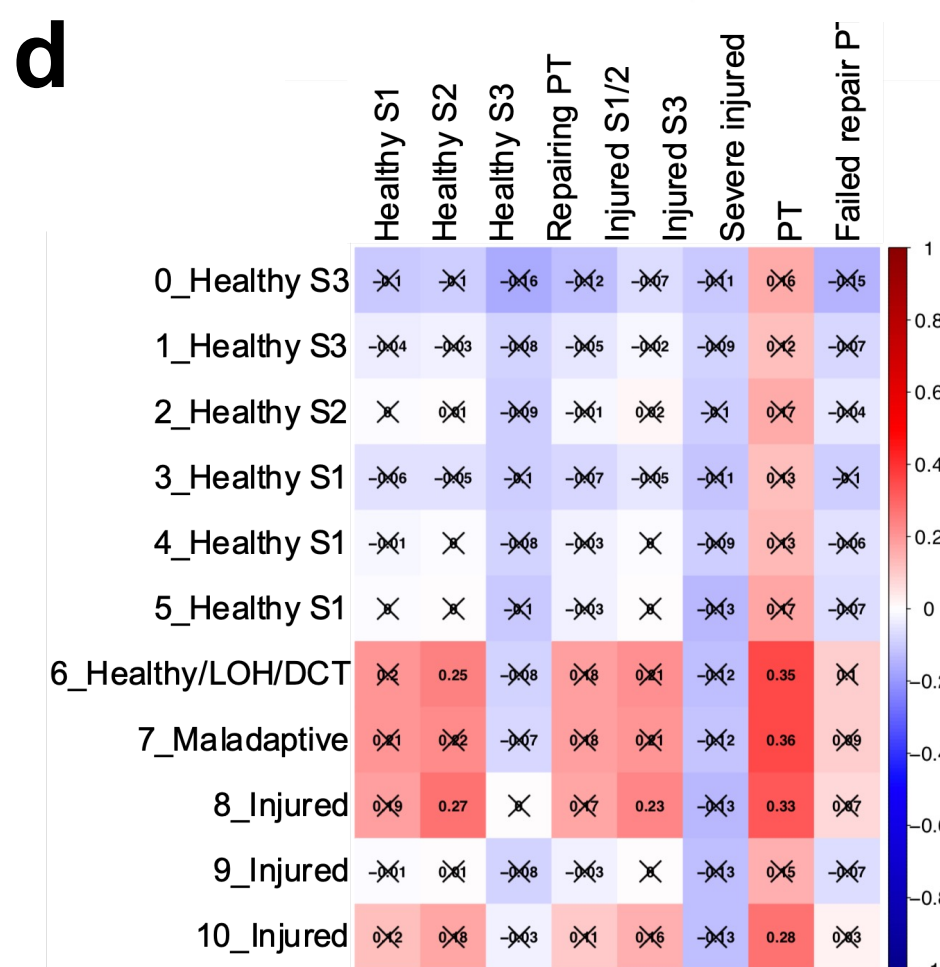
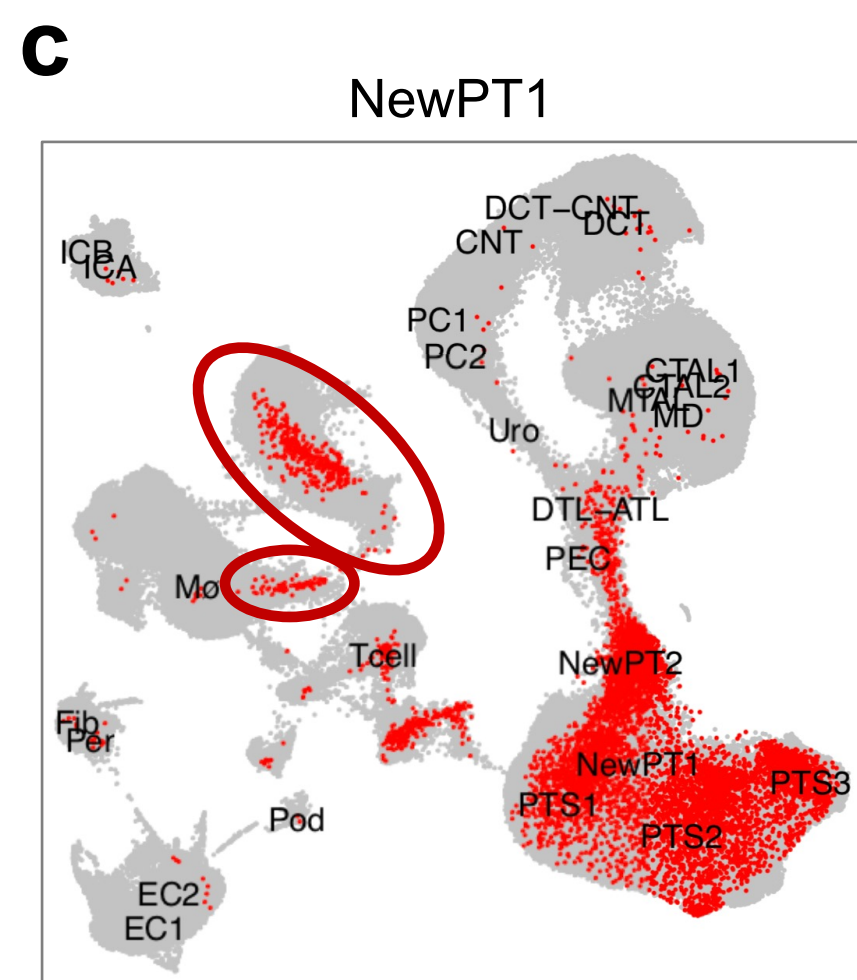
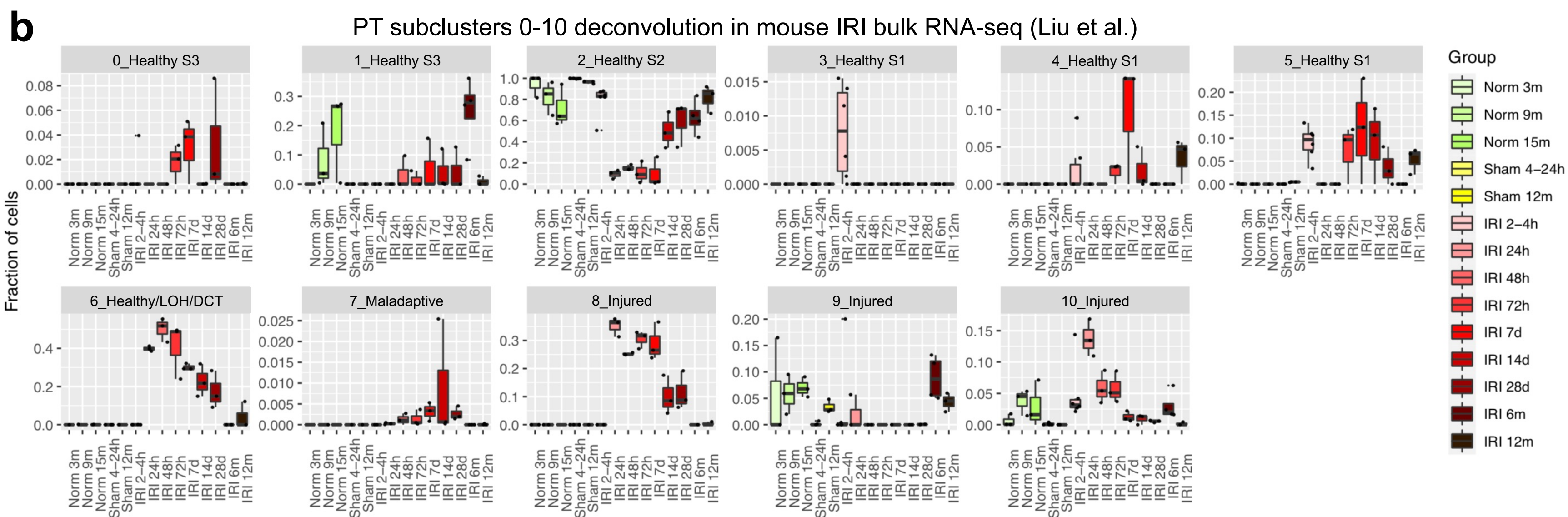
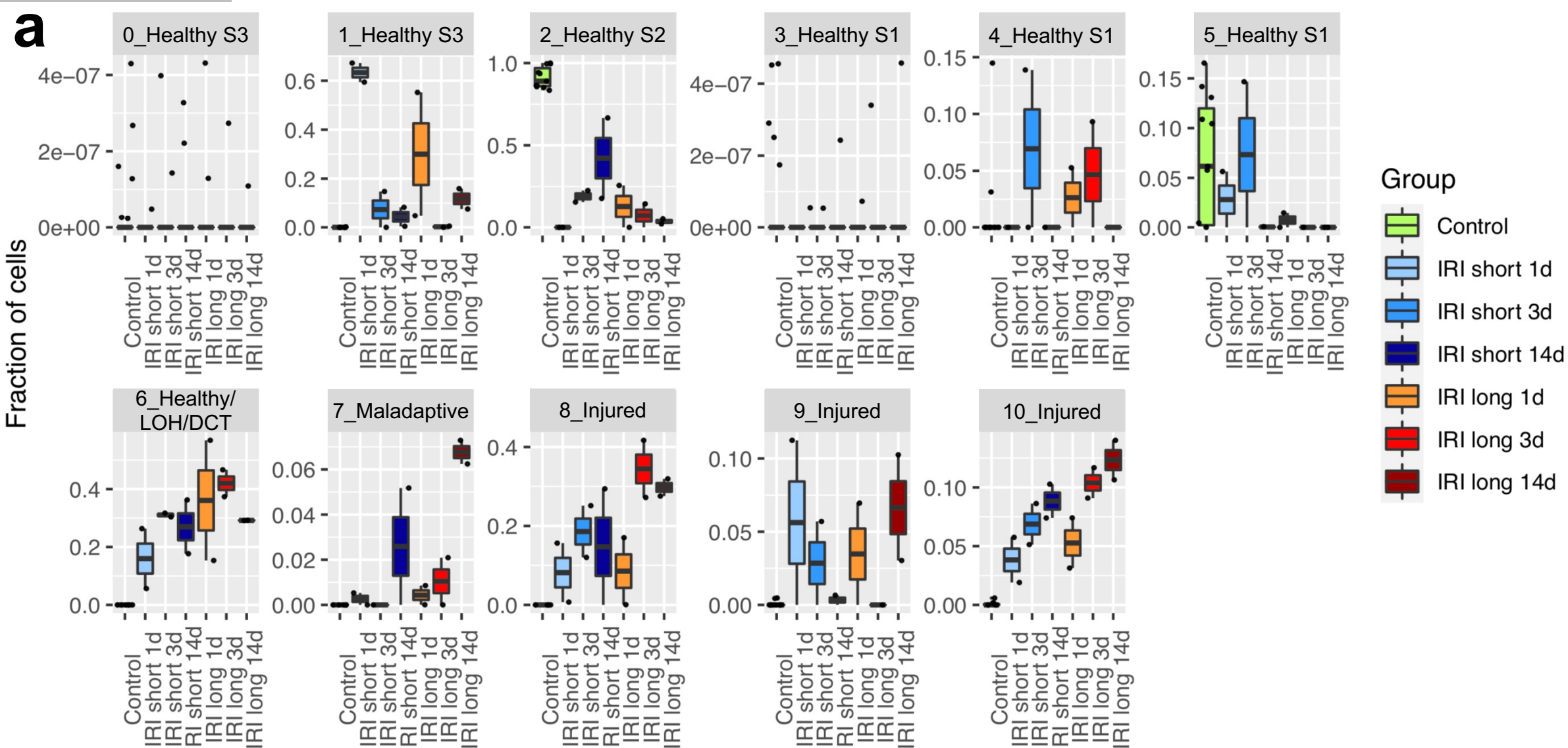
Fig. S8**PT subclusters 0-10 deconvolution**

Figure S8. PT subclusters bulk RNA-seq deconvolution.

- (a) Fraction of Healthy, Injured, and Maladaptive PT subclusters displayed as Tukey box plots after bulk kidney RNA-seq data convolution using the MuSiC pipeline. X axis denotes the different treatment groups (Control, IRI short 1d, IRI short 3d, IRI short 14d, IRI long 1d, IRI long 3d, IRI long 14d).
- (b) Same as (a) for a mouse kidney IRI dataset from Liu et al. X axis denotes the different treatment groups for Controls (Norm), sham, and IRI kidneys after several different time points.
- (c) Cells annotated as “NewPT1” from Kirita et al. are colored red in the joint UMAP embedding. Note significant overlap with Granul_1, Granul_2, and Macro clusters. Cluster labels as in Kirita et al.
- (d) Correlation matrix visualizing Pearson correlation coefficients (PCC) of cluster-averaged expression of top 50 DEGs from PT subclusters 0-10 (rows) and PT subclusters from Kirita et al., columns. Non-significant PCC values are crossed out.
- (e) Feature plots of selected marker genes from Kirita et al. PT subclusters projected onto UMAP space as in **Fig. 2a**.

Fig. S9

a

UMAP2

UMAP1

GMM cluster

notsubset

5

4

3

2

1

pseudotemporal ordering

b

Lineage 1

Lineage 2

GMM cluster

Slingshot pseudotime

c

dim2

dim1

PT subcluster

0_Healthy S3

1_Healthy S3

2_Healthy S2

3_Healthy S1

4_Healthy S1

5_Healthy S1

6_Healthy/LOH/DCT

7_Maladaptive

8_Injured

9_Injured

10_Injured

d

GMM cluster

Scaled regulon activity

3

2

1

0

-1

-2

-3

e

n=1,650 PT cells

Comp 1

Comp 2

0_Healthy S3

1_Healthy S3

2_Healthy S2

3_Healthy S1

4_Healthy S1

5_Healthy S1

6_Healthy/LOH/DCT

7_Maladaptive

8_Injured

9_Injured

10_Injured

f

Lineage 1

Lineage 2

Cell Type

Pre-branch

Health

IRI

Cluster

1

2

Norm. Expr.

3

2

1

0

-1

-2

-3

g

Cebpb

Cxcl2

Il1b

Junb

S100a8

Tyropb

Control

IRI_short_1d

IRI_short_3d

IRI_short_14d

IRI_long_1d

IRI_long_3d

IRI_long_14d

h

Cebpb

Junb

Il1b

Cxcl2

S100a8

Slc34a1

Slc27a2

Acsm2

Slc22a30

Timp3

i

Lineage 1

Lineage 2

Control

IRI_short_1d

IRI_short_3d

IRI_short_14d

IRI_long_1d

IRI_long_3d

IRI_long_14d

j

Slc34a1

Slc27a2

Acsm2

Timp3

Cebpb

Junb

Il1b

S100a8

Expr.

low

high

Figure S9. PT injury trajectories and differential driver patterns of adaptive and maladaptive PT cell repair.

- (a-b) 1,650 randomly sampled PT cells **(a)** are ordered by Slingshot pseudotime along lineages 1 (dark blue label) and 2 (yellow label) **(b)**. Clusters correspond to Gaussian mixed modeling (GMM) clustering, as inferred by Slingshot.
- (c) Dimensional reduction representation of Slingshot-derived cell trajectories of PT cells, coloring corresponds to PT subclusters as in **Figs. 2a-c**.
- (d) Heatmap of GMM cluster-specific SCENIC-inferred scaled regulon activity. Rows represent GMM clusters, columns represent regulons, as inferred by Slingshot in **Fig. 3a**.
- (e) Monocle2-derived trajectory of the same PT cell selection, confirming lineages 1 and 2. R denotes the root node where branching of lineages 1 and 2 occurs.
- (f) Heatmap visualizing pseudotime-dependent gene expression along lineages 1 and 2, respectively. R denotes the root node where branching of lineages 1 and 2 occurs, as shown in **(e)**.
- (g) Gene expression of DEGs from **(f)** colored by experimental sample group stratified by IRI degree and time post-ischemia.
- (h) Corresponding Monocle2 visualization of gene expression in reduced dimension space.
- (i) Monocle3-derived trajectories of lineages 1 and 2 of the same PT cell selection, coloring as per experimental group.
- (j) Corresponding Monocle3 visualization of gene expression in reduced dimension space.

a

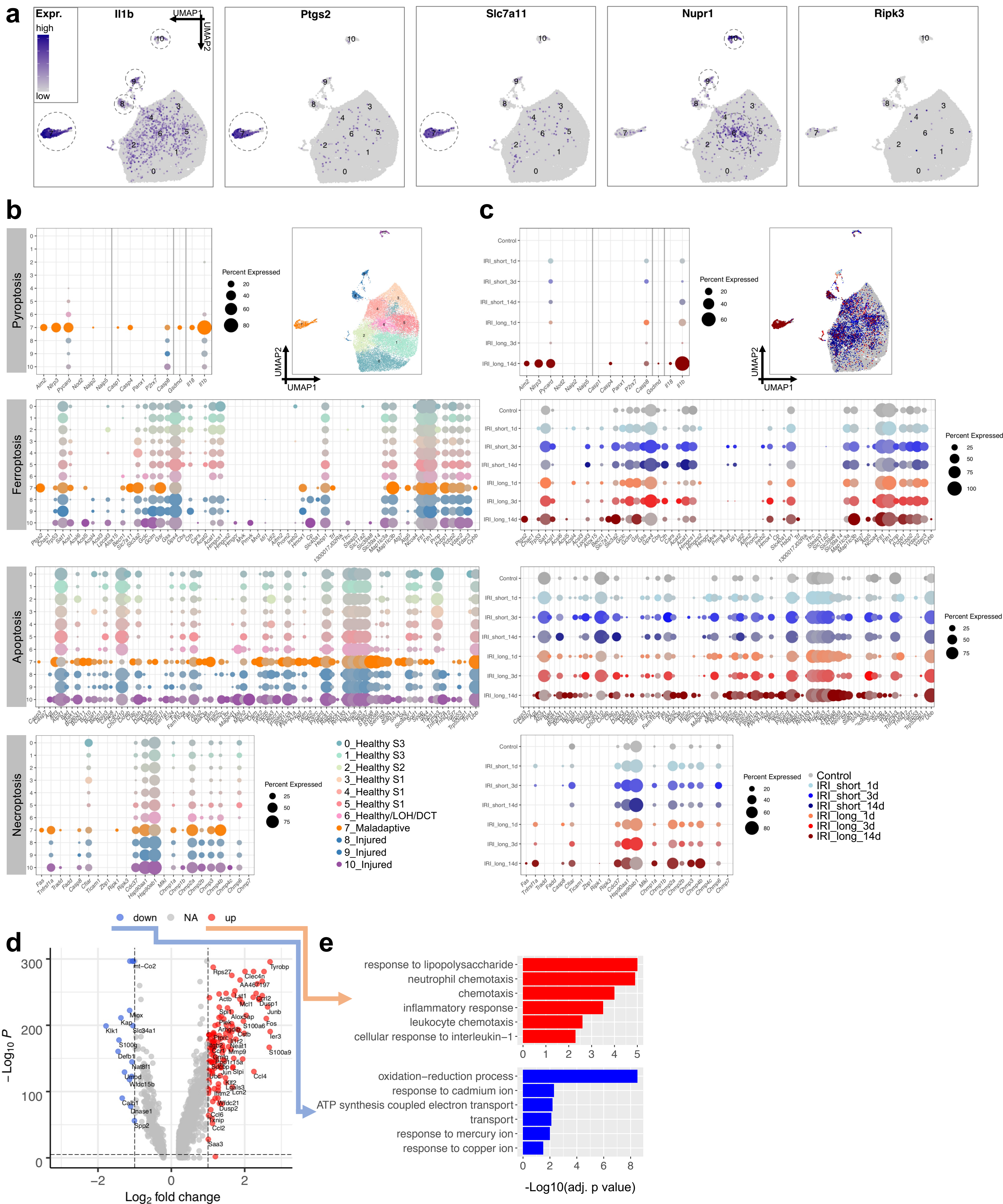


Figure S10. Sustained regulated cell death signatures in PT cells after long IRI.

- (a) Feature plots of selected genes from **Fig. 4b** projected onto UMAP space as in **Fig. 2a**.
- (b-c) Dot plots showing comprehensive marker gene expression in PT cells for regulated cell death necrosis pathways pyroptosis, ferroptosis, apoptosis, and necroptosis. Cells are stratified by PT subclusters **(b)** or ischemia dose and time post-IRI **(c)**. Dot size denotes percentage of cells expressing the marker.
- (d) Volcano plot of differentially expressed genes (DEGs) comparing PT cells from samples 14d after short and long IRI, respectively. Genes upregulated in IRI long compared to IRI short samples are colored red, downregulated genes are colored blue. X axis indicates $\log_2$ -fold change (Model-based Analysis of Single-cell Transcriptomics; MAST) and y axis indicates statistical significance-adjusted $p = -\log_{10}$.
- (e) Corresponding Gene Ontology (GO) analysis showing statistically significantly enriched GO terms (over-representation analysis with Benjamini-Hochberg FDR method) corresponding to up- and downregulated DEGs from **(d)**.

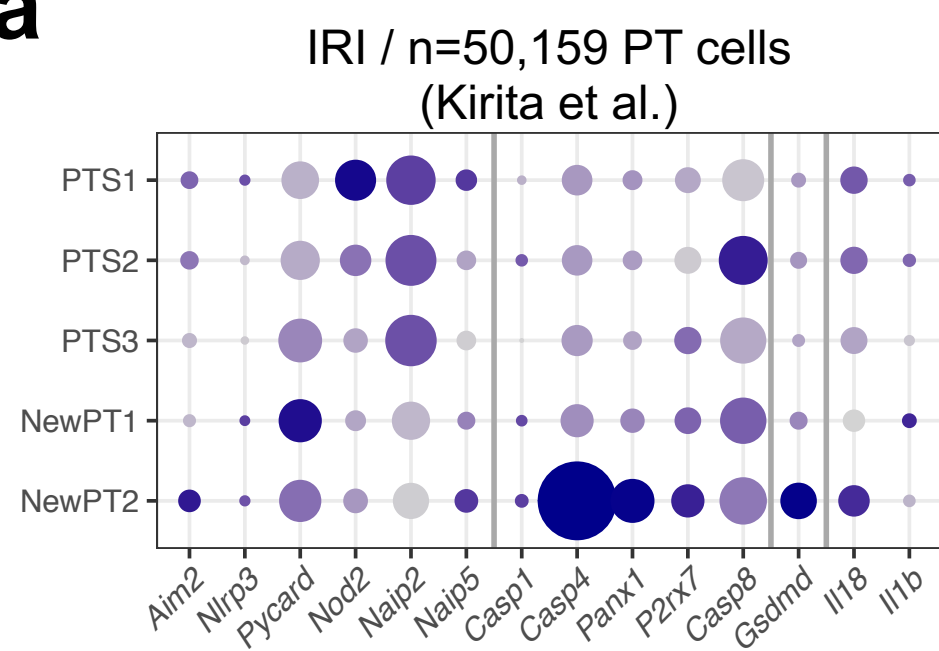
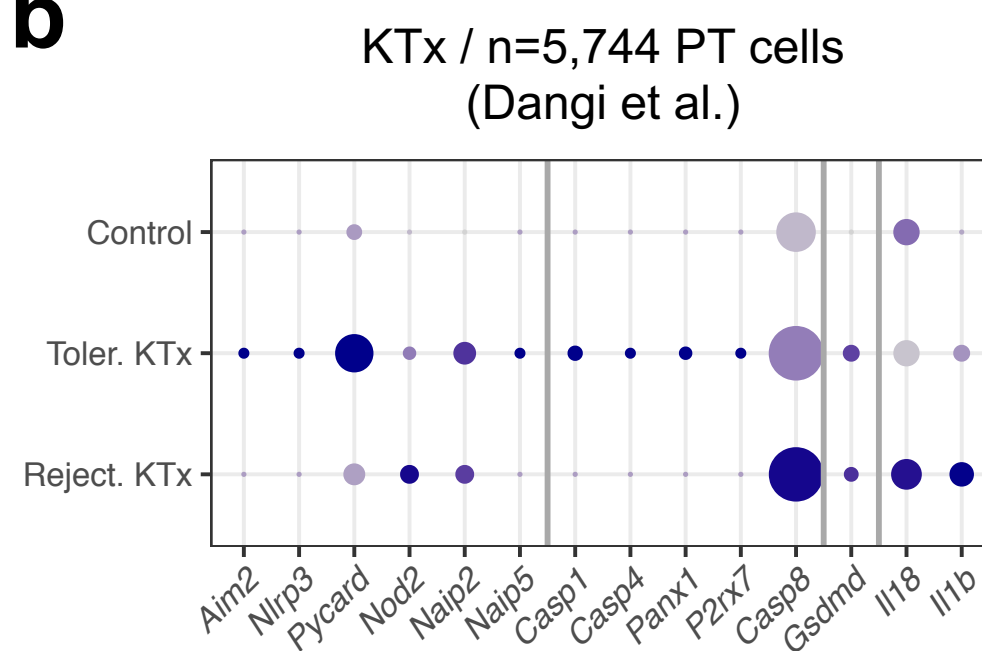
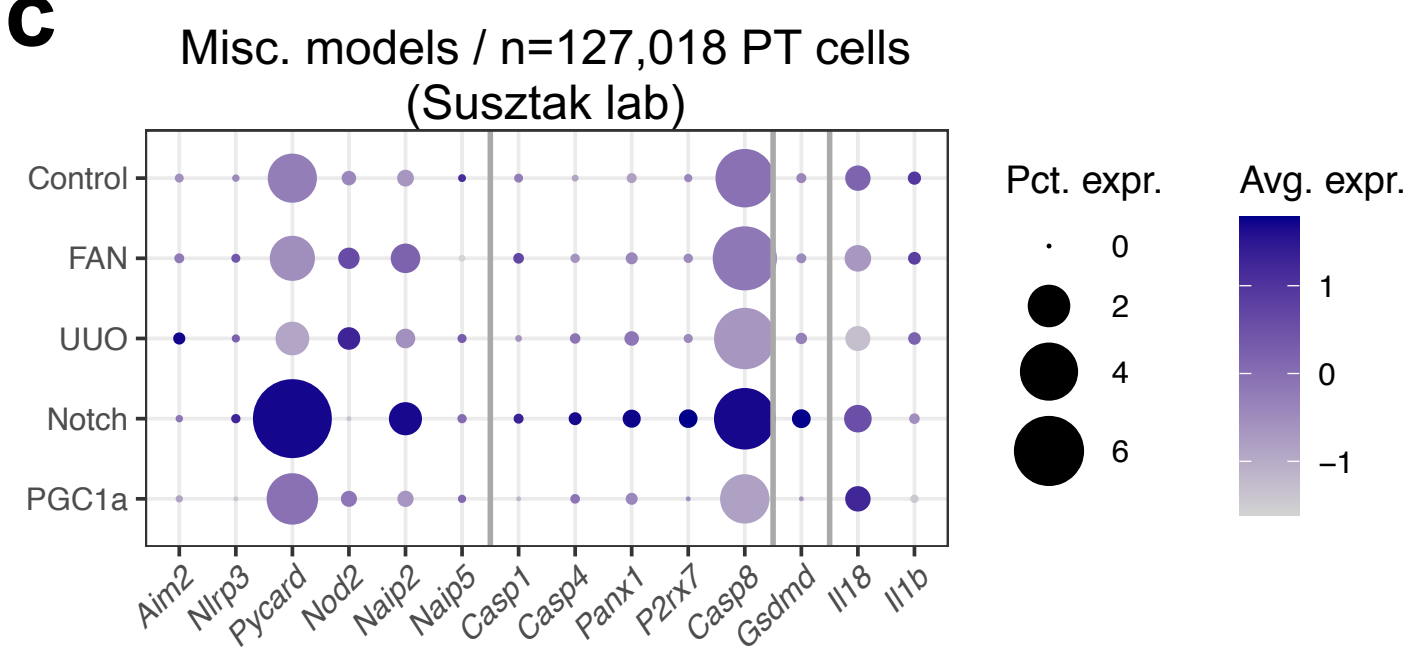
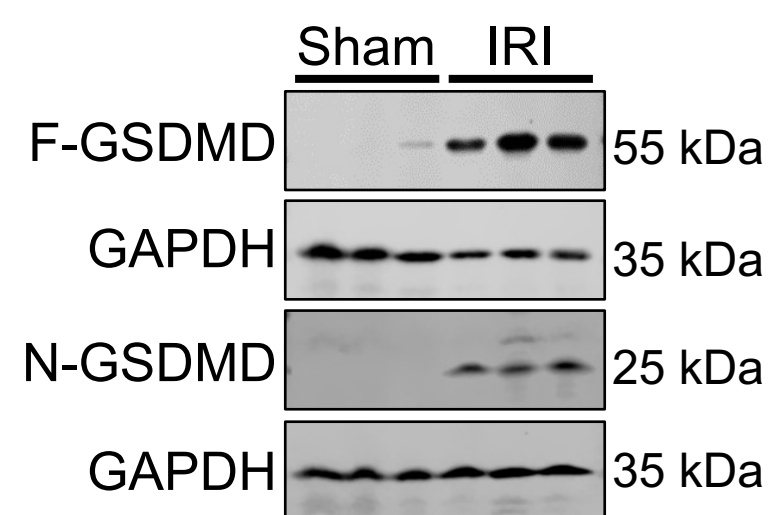
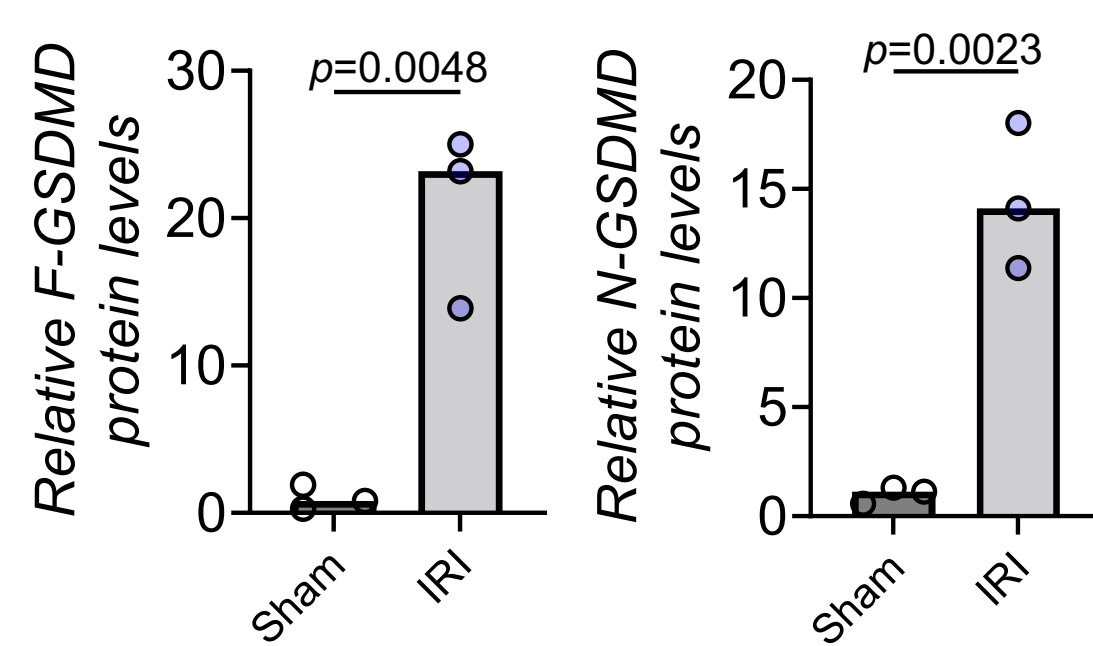
Fig. S11**a****b****c****d****e**

Figure S11. Pyroptosis gene signature in PT cells of other kidney scRNA-seq datasets.

- (a-c) Dot plots showing pyroptosis marker gene expression in PT cells of 2 external datasets (Kirita et al. and Dangi et al.) and several mouse models of our own lab, respectively. Dot size denotes percentage of cells expressing the marker, color scale represents average gene expression values. FAN, folic acid nephropathy; Notch; conditional tubular overexpression of Notch mouse model; UUO, unilateral ureteric obstruction; KTx, kidney transplant; reject., rejecting; toler., tolerated.
- (d) Western blot for full length (GSDMD-F) and cleaved GSDMD (GSDMD-N) in full kidney lysates. GAPDH was used as loading control. IRI, long ischemia reperfusion injury.
- (e) Corresponding quantification of Western blot in **(d)**; n=3 independent experiments, data shown are from n=1 experiment with n=3 biological replicates per group; p values are given for Wilcoxon-Mann test.

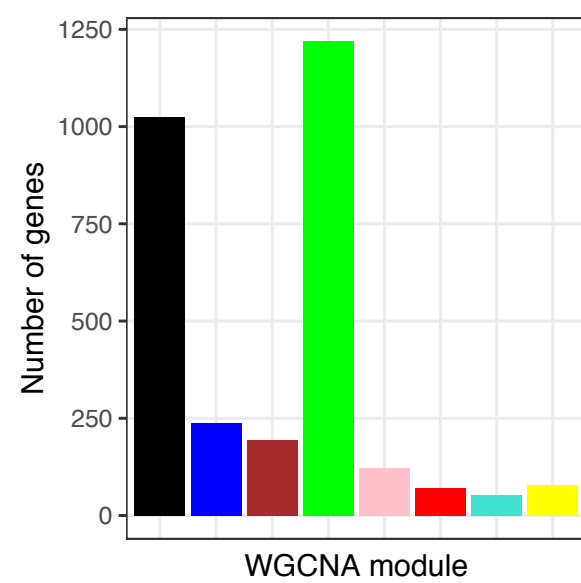
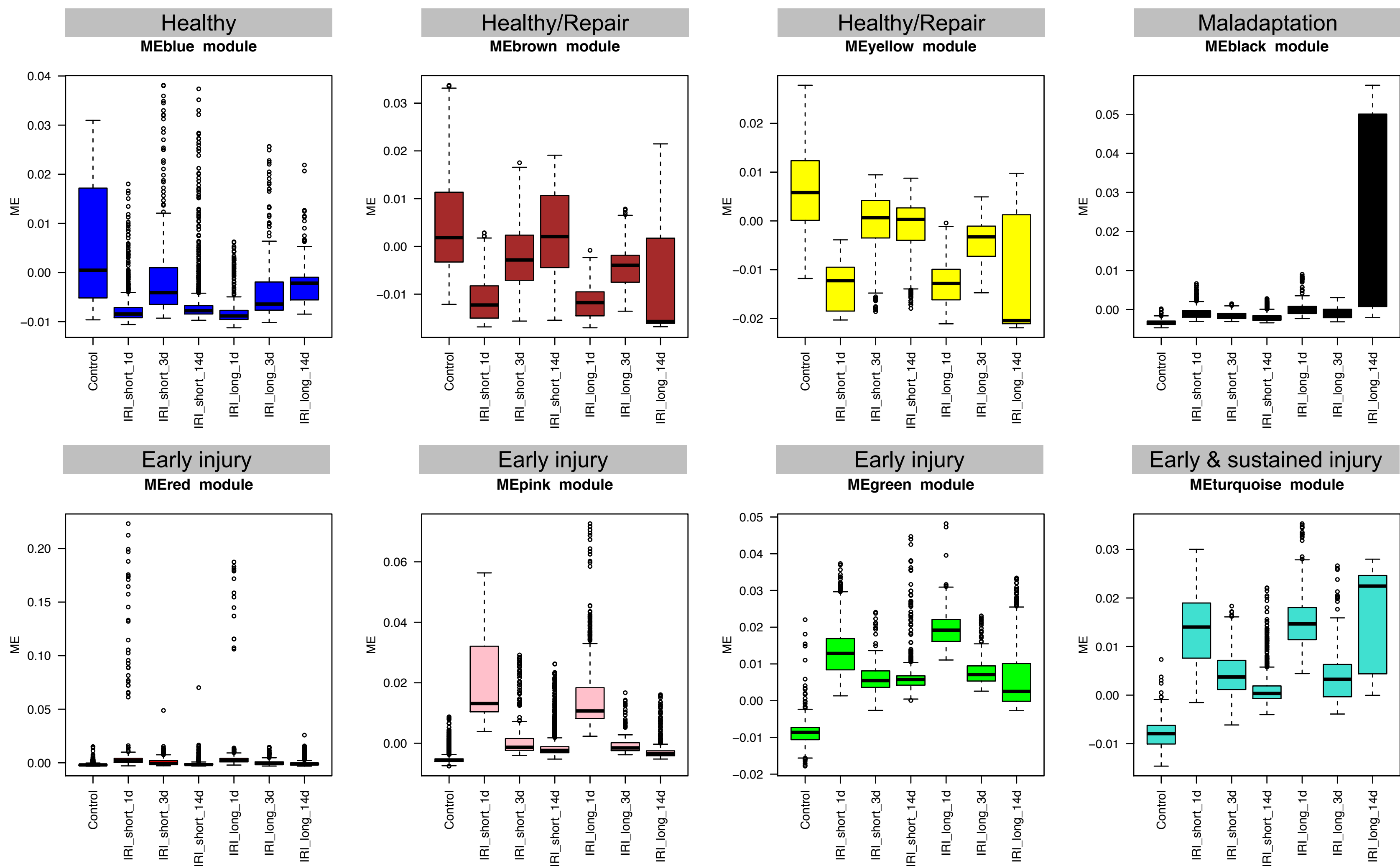
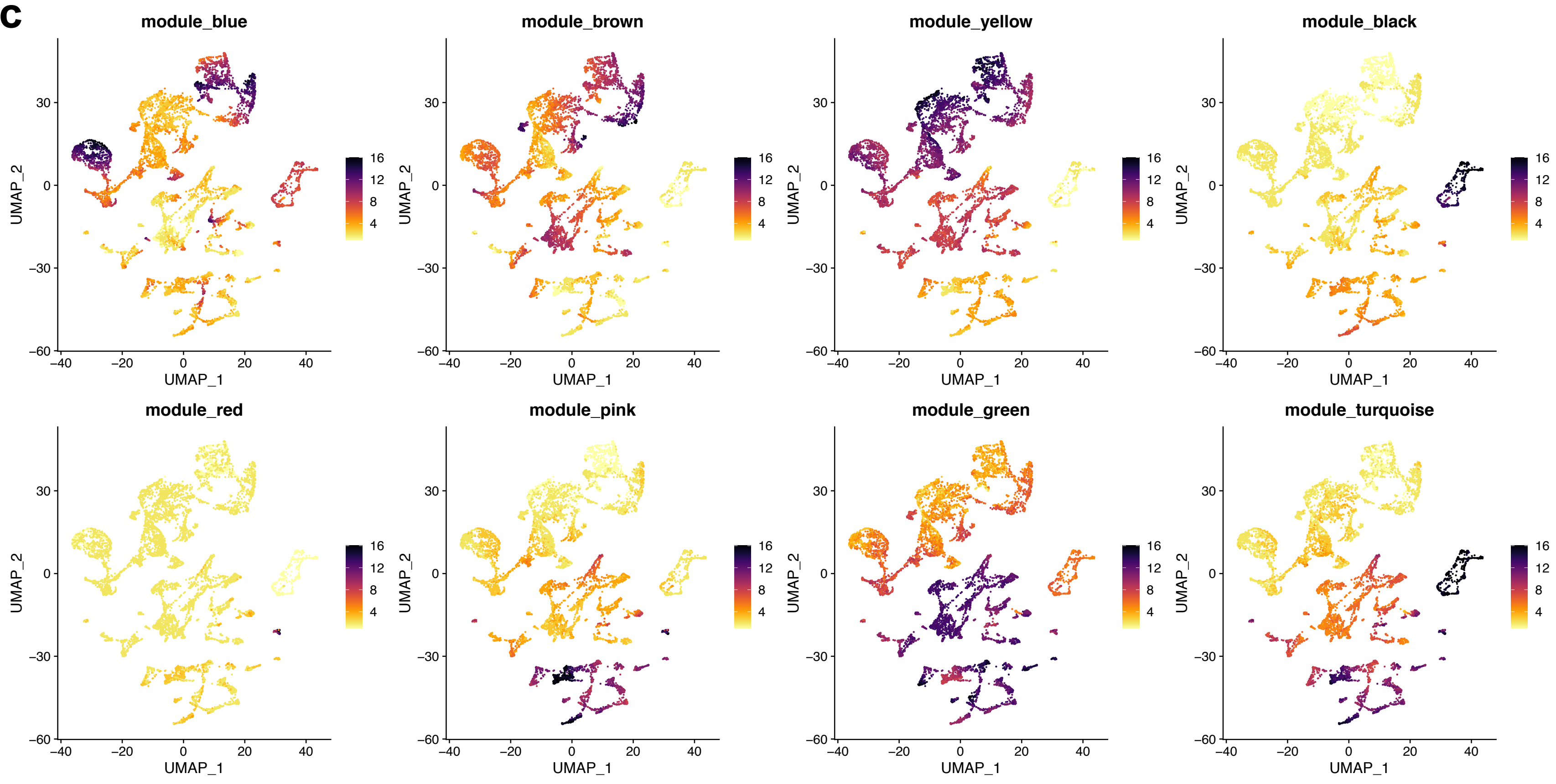
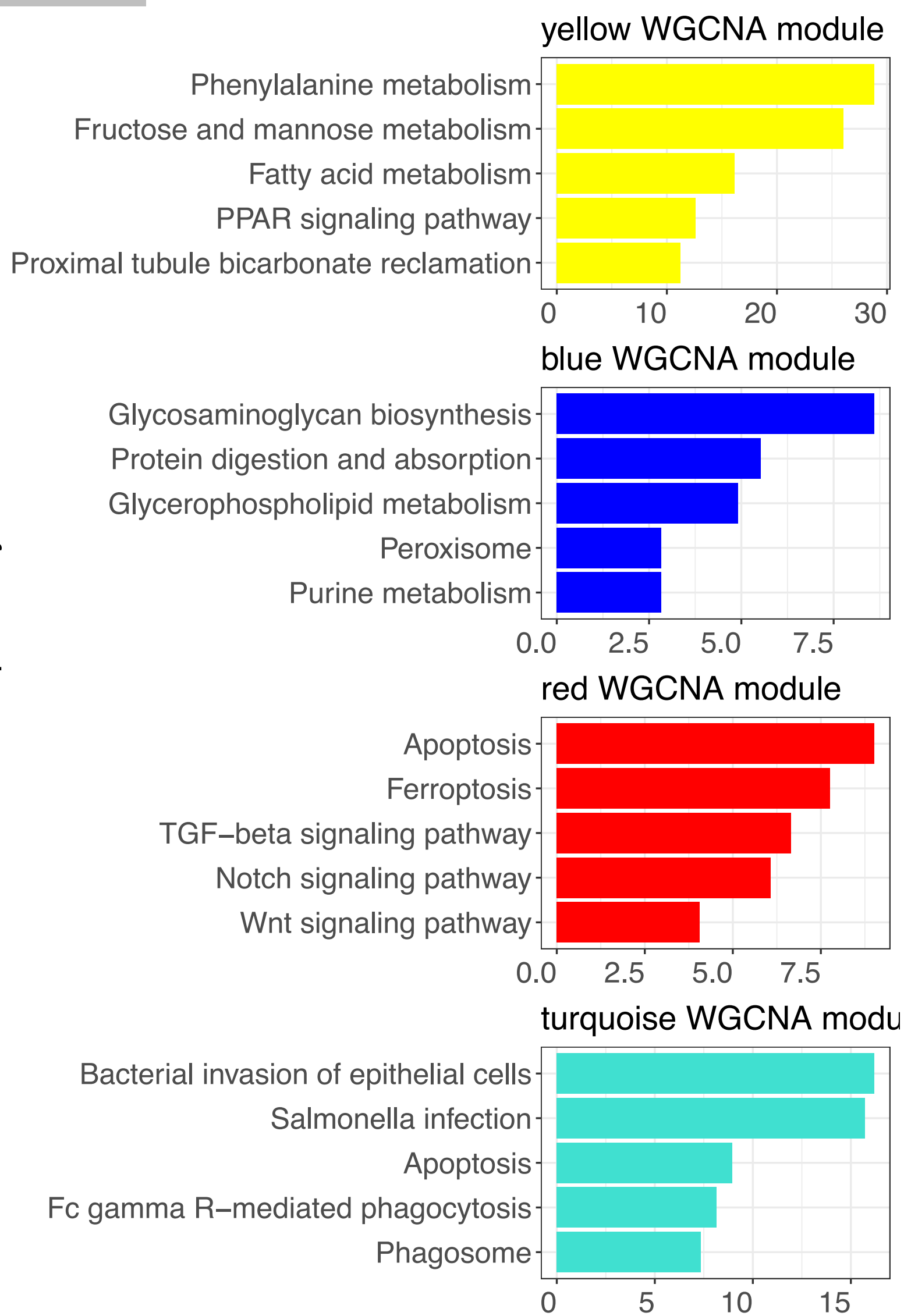
Fig. S12**a****b****c**

Figure S12. WGCNA analysis in PT metacells.

- (a) Bar graph depicting the number of genes in WGCNA-derived modules.
- (b) Module eigengene (ME) scores of WGCNA modules displayed as Tukey box plots (outliers denoted as dots outside box plot whiskers) by IRI degree and time post-ischemia (n=8,777 metacells).
- (c) kME values of individual WGCNA modules visualized in UMAP space.

Fig. S13

KEGG pathway



Enrichment ratio

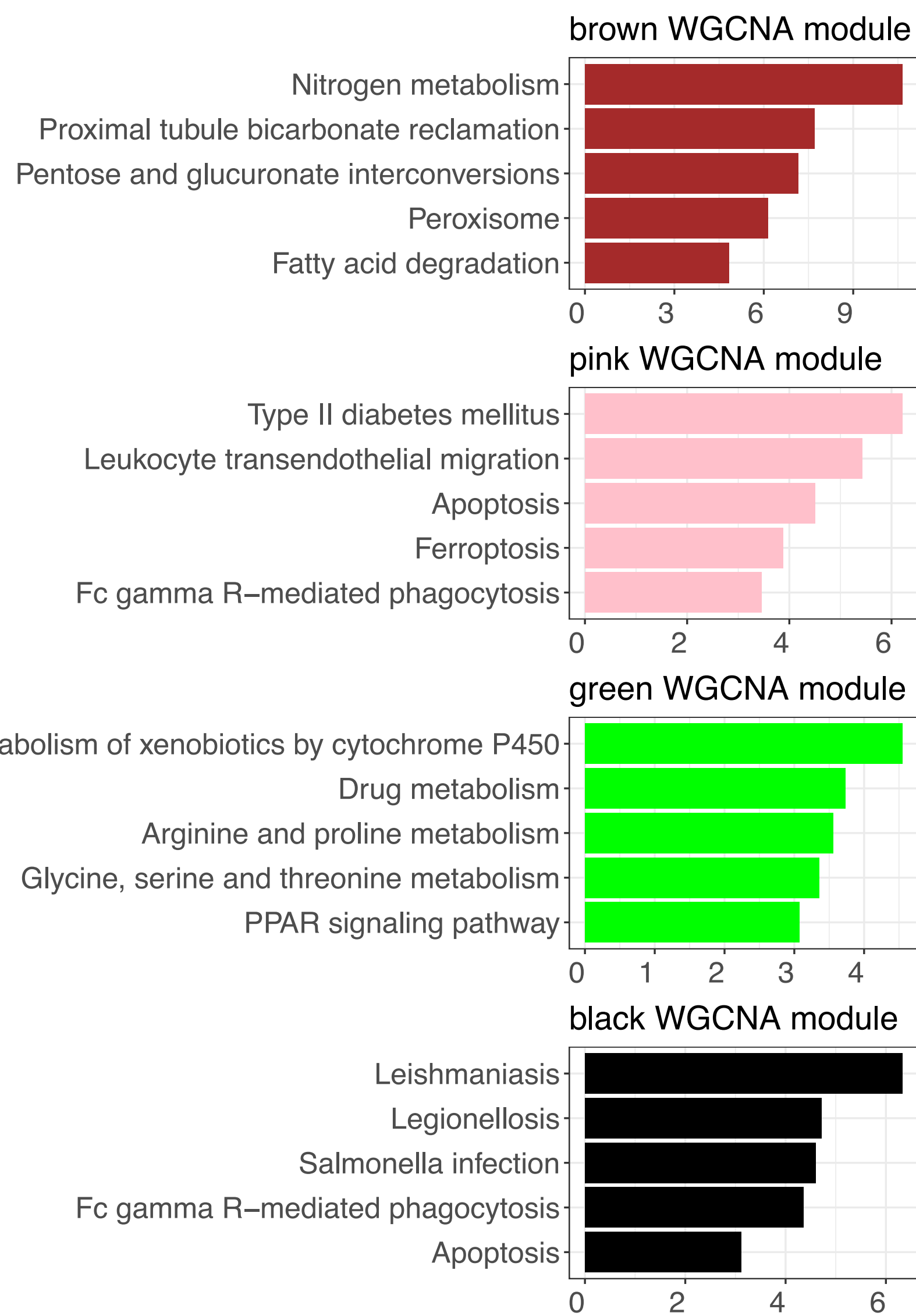


Figure S13. KEGG pathway analysis for WGCNA modules.

Enrichment ratios of top enriched KEGG pathway terms, derived by overrepresentation analysis of WGCNA module genes in proximal tubule metacells. The graph is split into subpanels by WGCNA module.

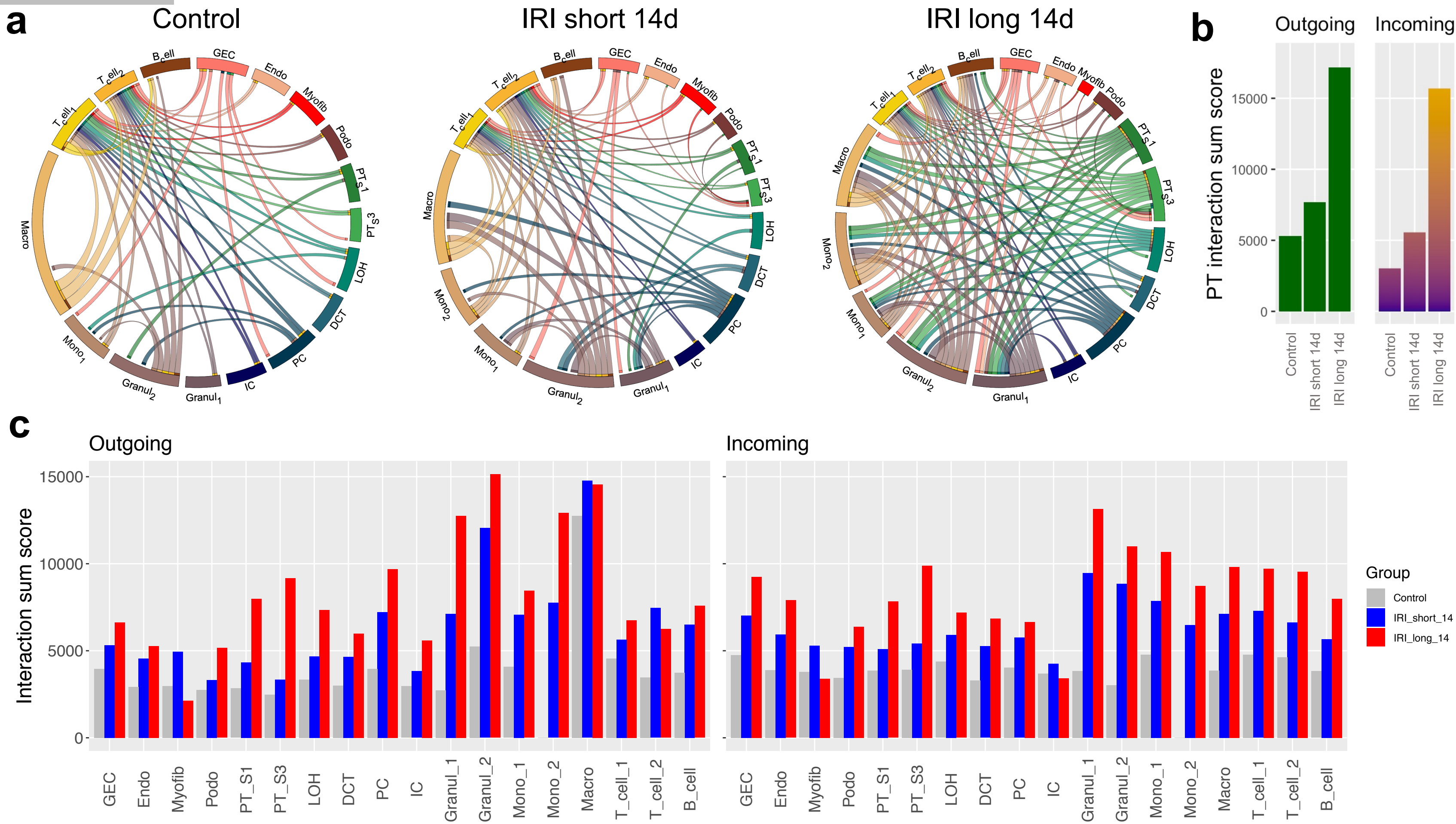
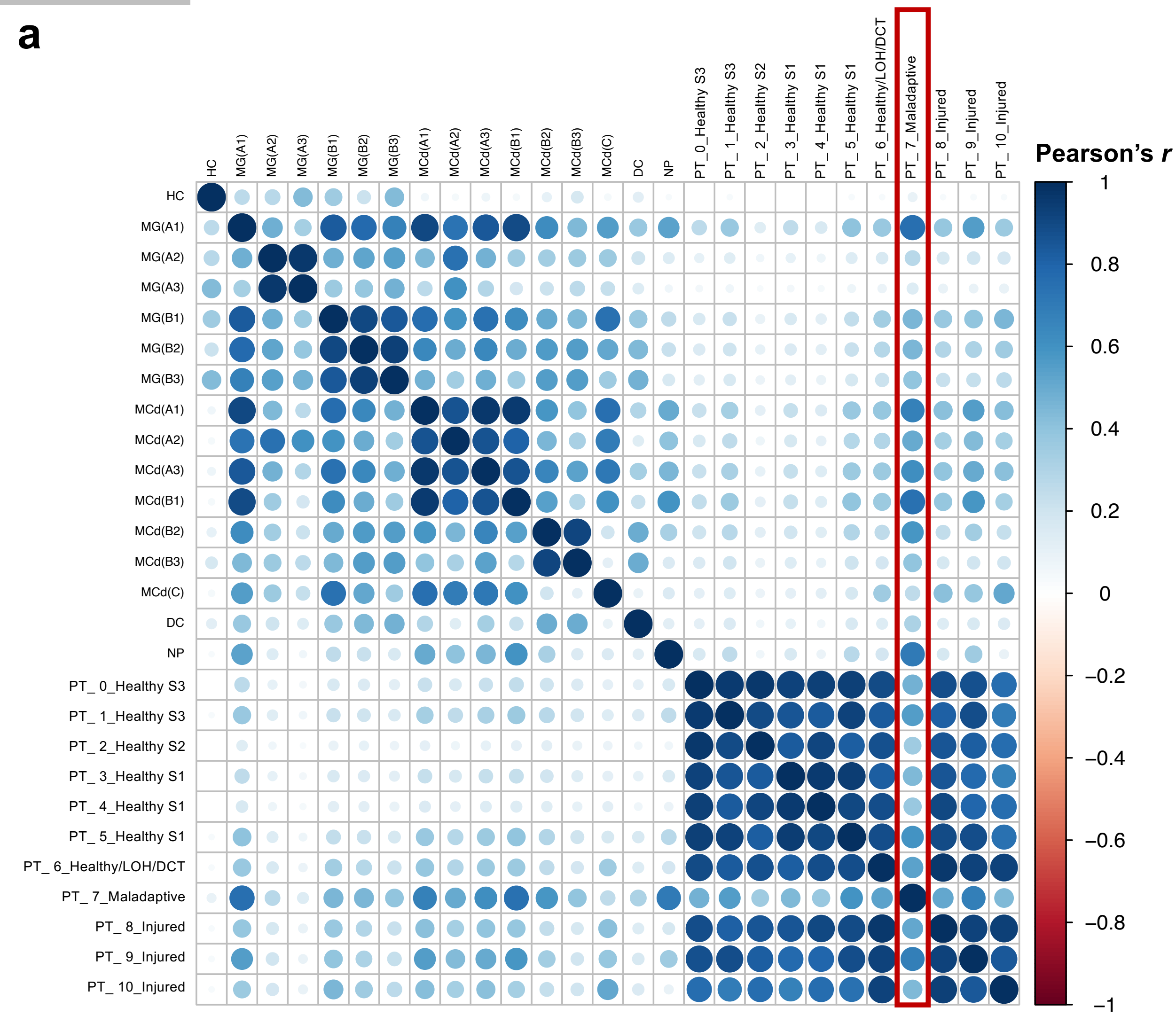
Fig. S14

Figure S14. Cell-cell interaction of kidney cells in health, adaptive repair, and maladaptive repair.

- (a) Circos plots visualizing the degree of cell-cell interactions as inferred by CellPhoneDB between healthy (Control), adapted (IRI short 14d), and maladapted (IRI long 14d) kidney cells. Width of connecting lines is proportional to the strength of interaction, as measured by combined means of ligands and respective receptors by CellPhoneDB. For clarity only the top 50% of interactions are visualized.
- (b) Bar graphs showing the sum score of outgoing and incoming connections of proximal tubule (PT) cells across treatment conditions (Control; IRI short 14d; IRI long 14d).
- (c) Bar graphs showing the sum score of outgoing and incoming connections of all cell types across treatment conditions (Control; IRI short 14d; IRI long 14d).

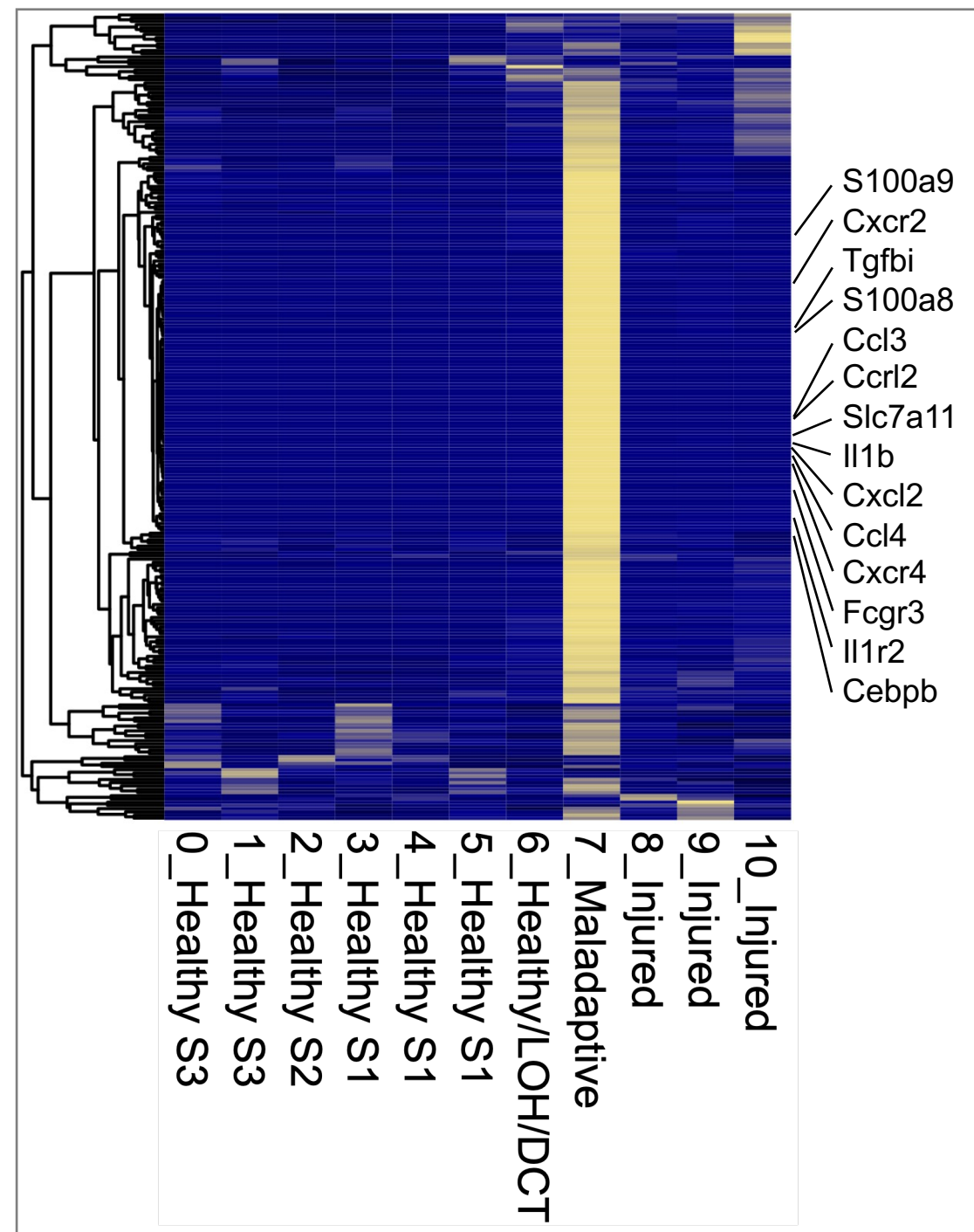
Fig. S15

a



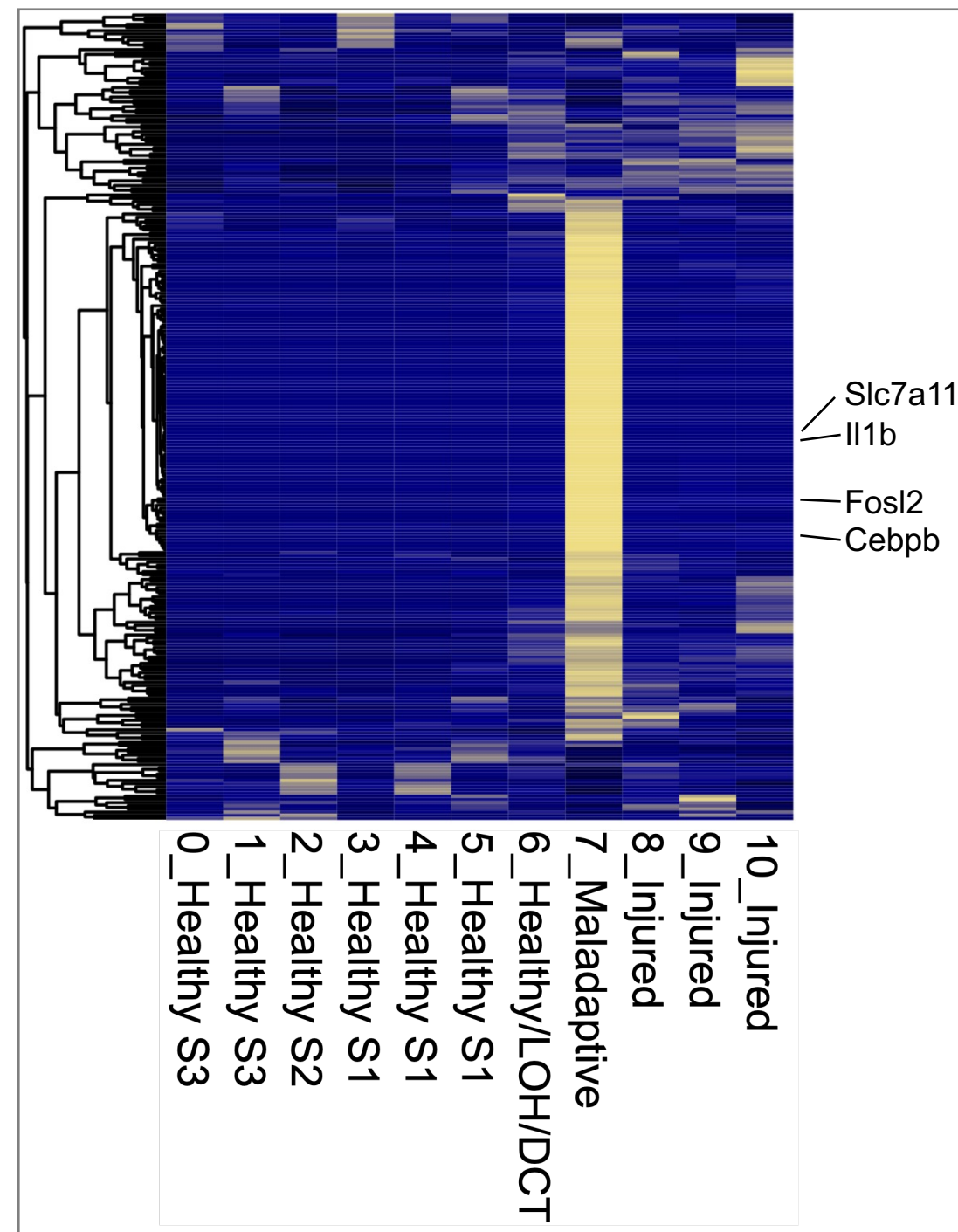
b

n=28,385 PT cells
top250 Neutro DEGs



c

n=28,385 PT cells
top250 Monocyte-derived Macro (B2) DEGs



d

n=28,385 PT cells
top250 Monocyte-derived Macro (B1) DEGs

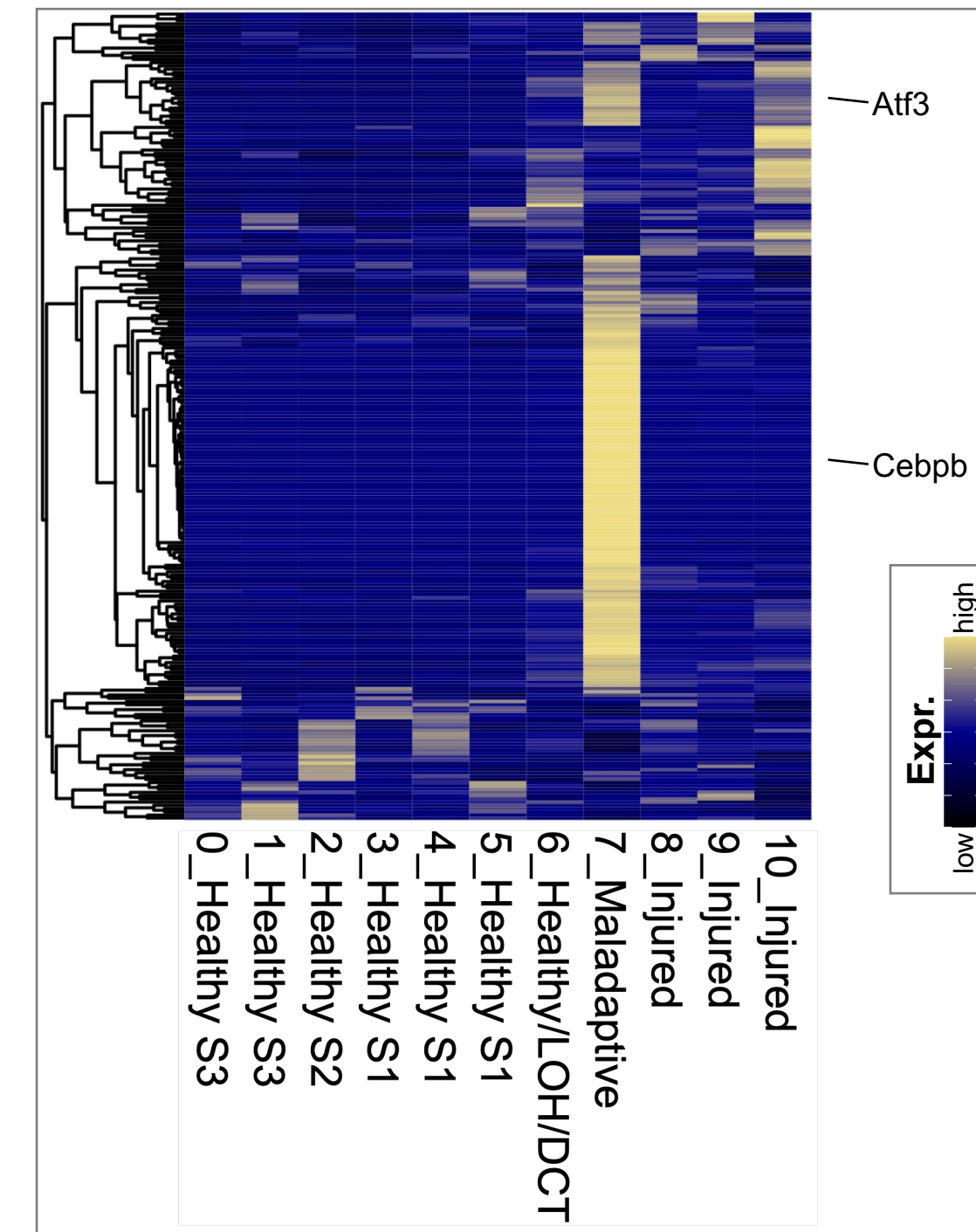


Figure S15. Validation of myeloid phenotype of maladaptive PT cells.

- (a) Correlation matrix visualizing Pearson correlation coefficients between average expressions of clusters from an external dataset of immune cells in spinal cord injury (Hamel et al.) and our PT subclusters. PT_7_Maladaptive gene signature correlates best with neutrophils (NP), monocyte-derived macrophages (MCd) B1 and B2, corresponding to different pseudotime points on a trajectory characterized by cell death.
- (b-d) Heatmaps visualizing gene expression in 11 subclusters of 28,385 PT cells of marker genes from an external dataset of immune cells in spinal cord injury (Hamel et al.). **(b)** depicts top 250 marker genes for neutrophils, **(c)** and **(d)** for monocyte-derived macrophages at different pseudotime points on a trajectory characterized by cell death.

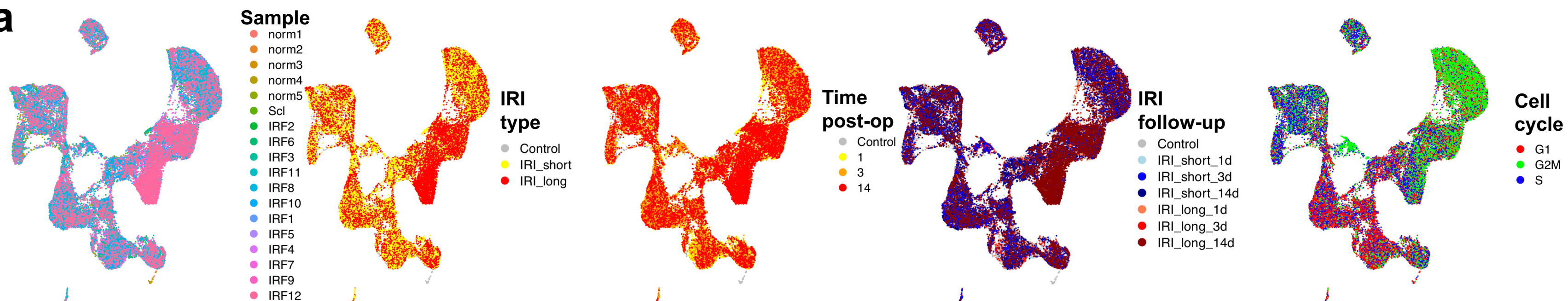
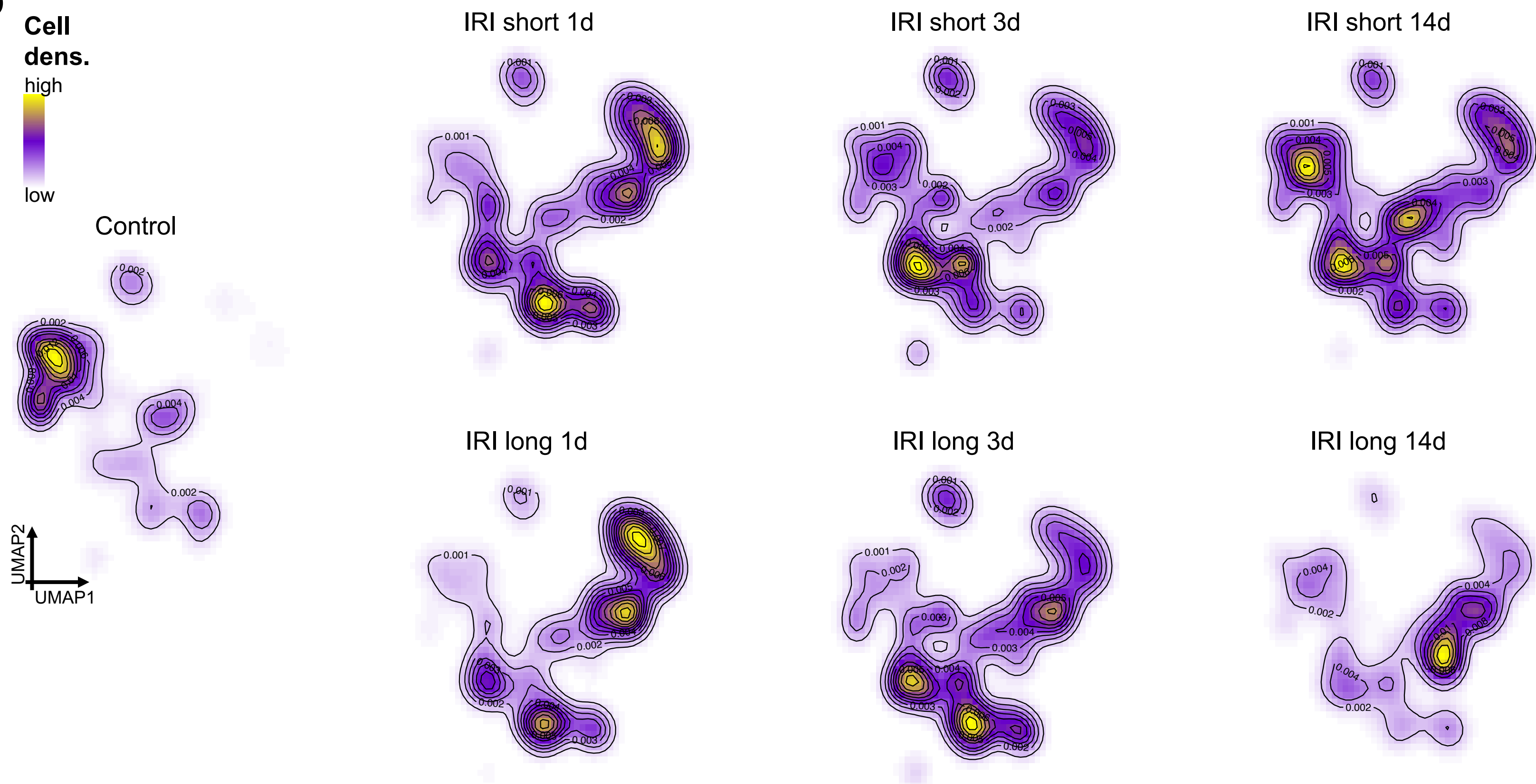
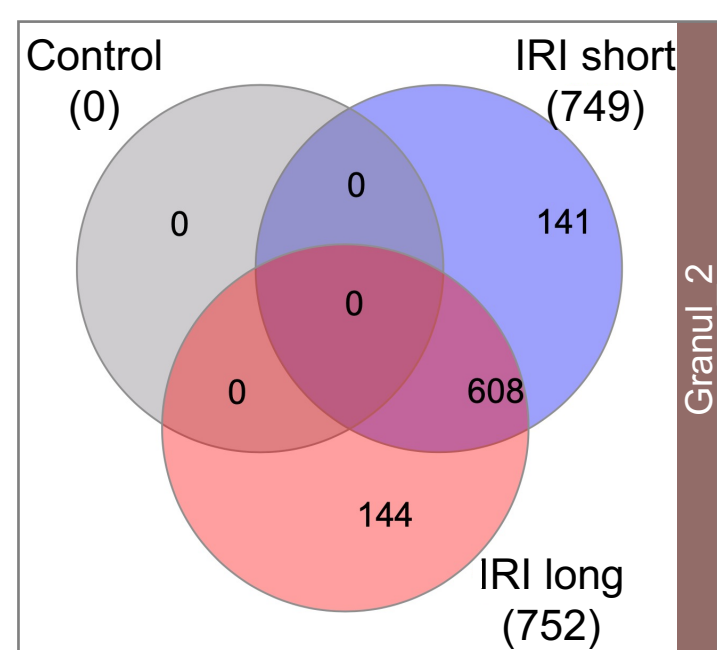
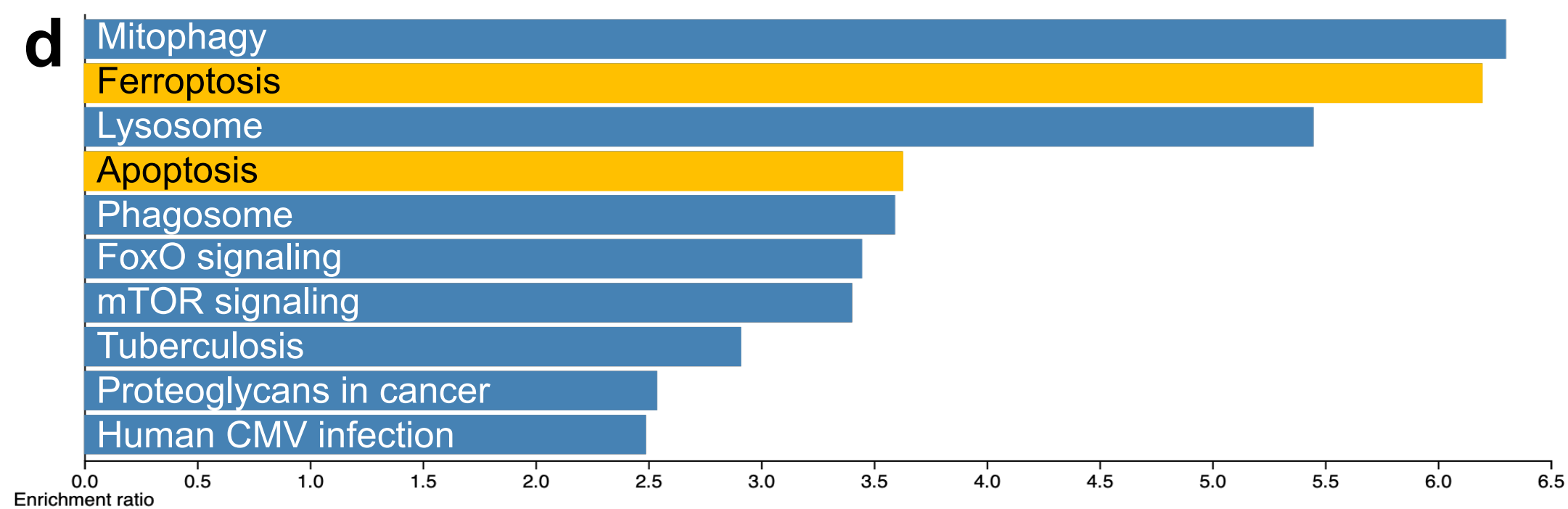
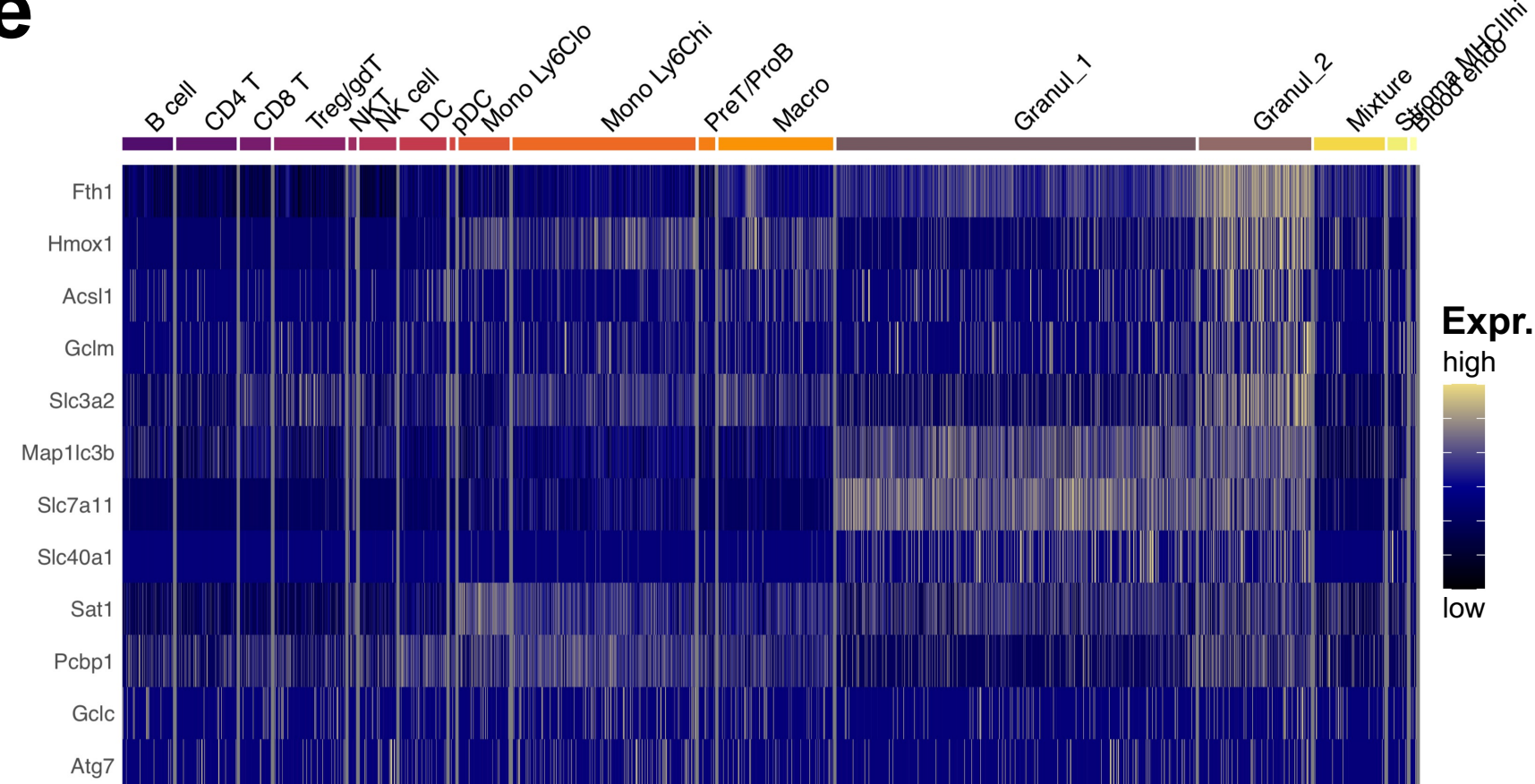
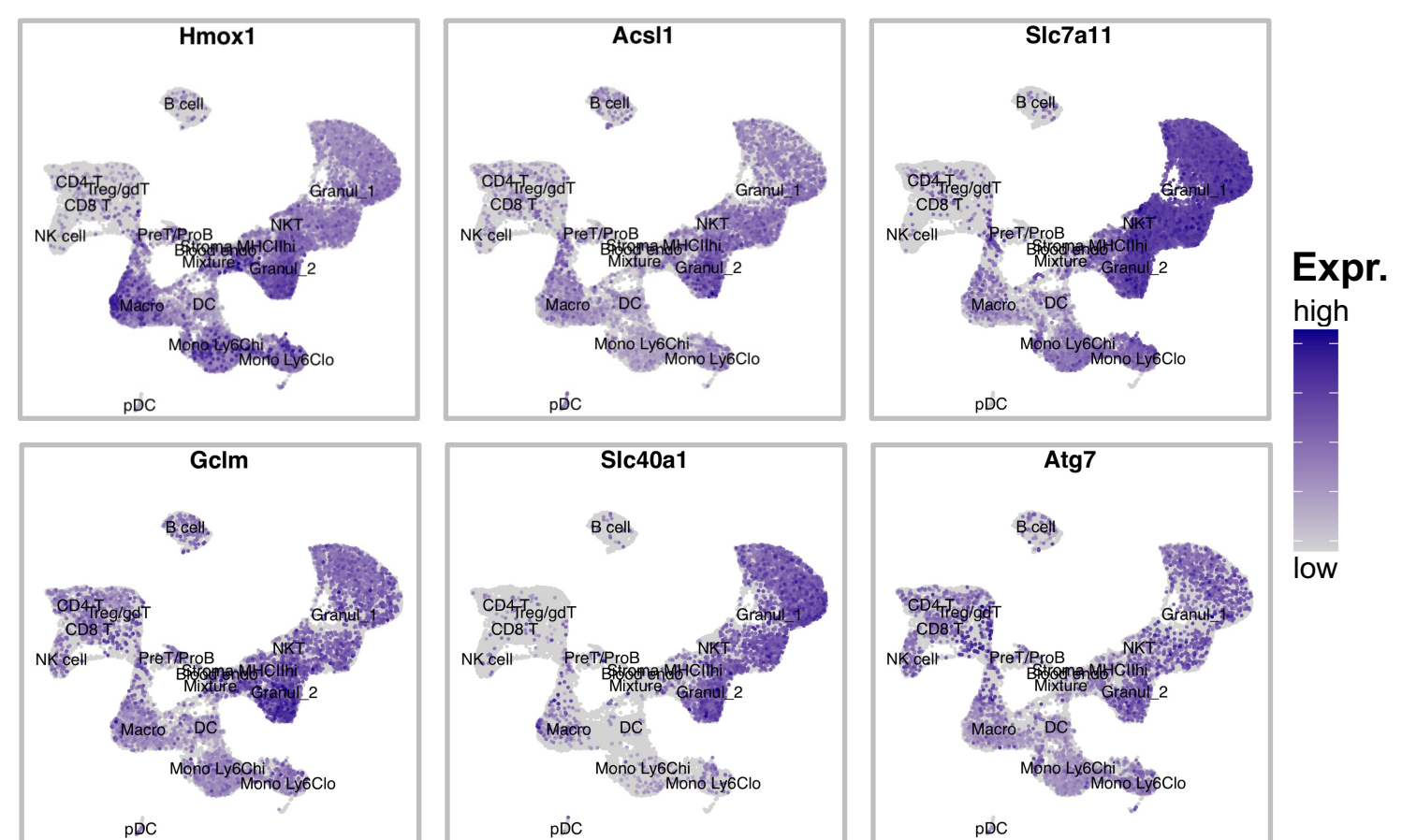
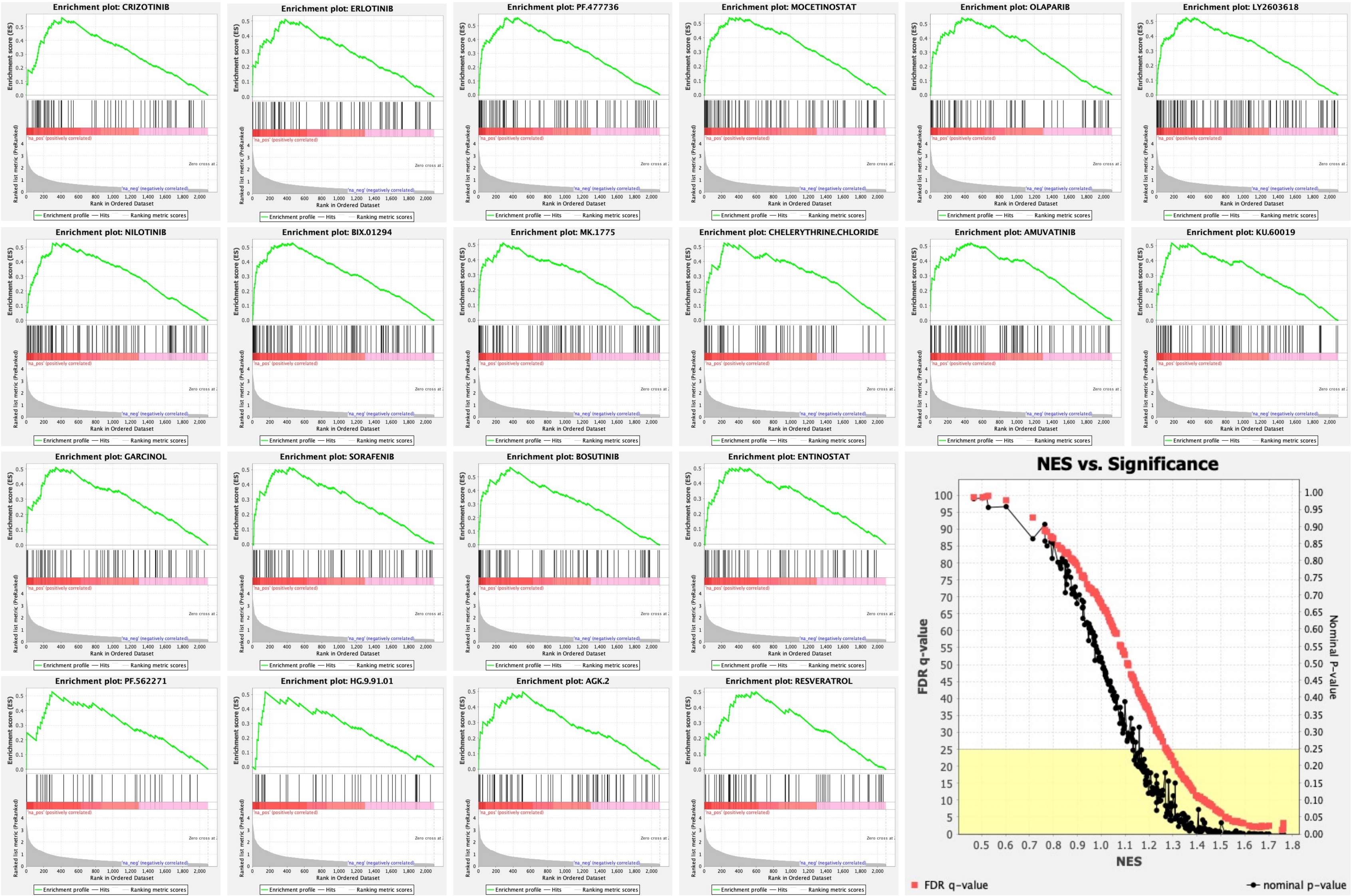
Fig. S16**a****b****c****d****e****f**

Figure S16. Transcriptional atlas of immune cells in acutely injured kidneys.

- (a) UMAP projection of 45,327 immune cells of n=6 controls, n=6 short IRI and n=6 long IRI samples colored by sample, IRI type (Control, short IRI, long IRI), time post-op (Control, 1d, 3d, 14d), IRI follow-up (Control, IRI short 1d, IRI short 3d, IRI short 14d, IRI long 1d, IRI long 3d, IRI long 14d), and cell cycle phase (G1, G2M, S).
- (b) Density plots visualizing changes in cell type proportions between different treatment groups.
- (c) Venn diagram visualizing the number of DEGs from Granul_2 cluster, respectively, and their overlap according to treatment group (Control; IRI short; IRI long). Healthy Control samples did not contribute a single cell to Granul_2 cluster.
- (d) Gene ontology (GO) terms analysis for DEGs from Granul_2 cluster in **(b)** yielded high enrichment ratios for cell death pathways such as ferroptosis and apoptosis, respectively.
- (e) Heatmap showing the expression of genes known to be involved in ferroptosis by immune cell type. Note the enrichment of myeloid clusters, especially Granul_2, for high expressing cells.
- (f) Corresponding feature plots visualizing the expression of select examples from **(d)** in the joint UMAP embedding space.

Fig. S17

a



b

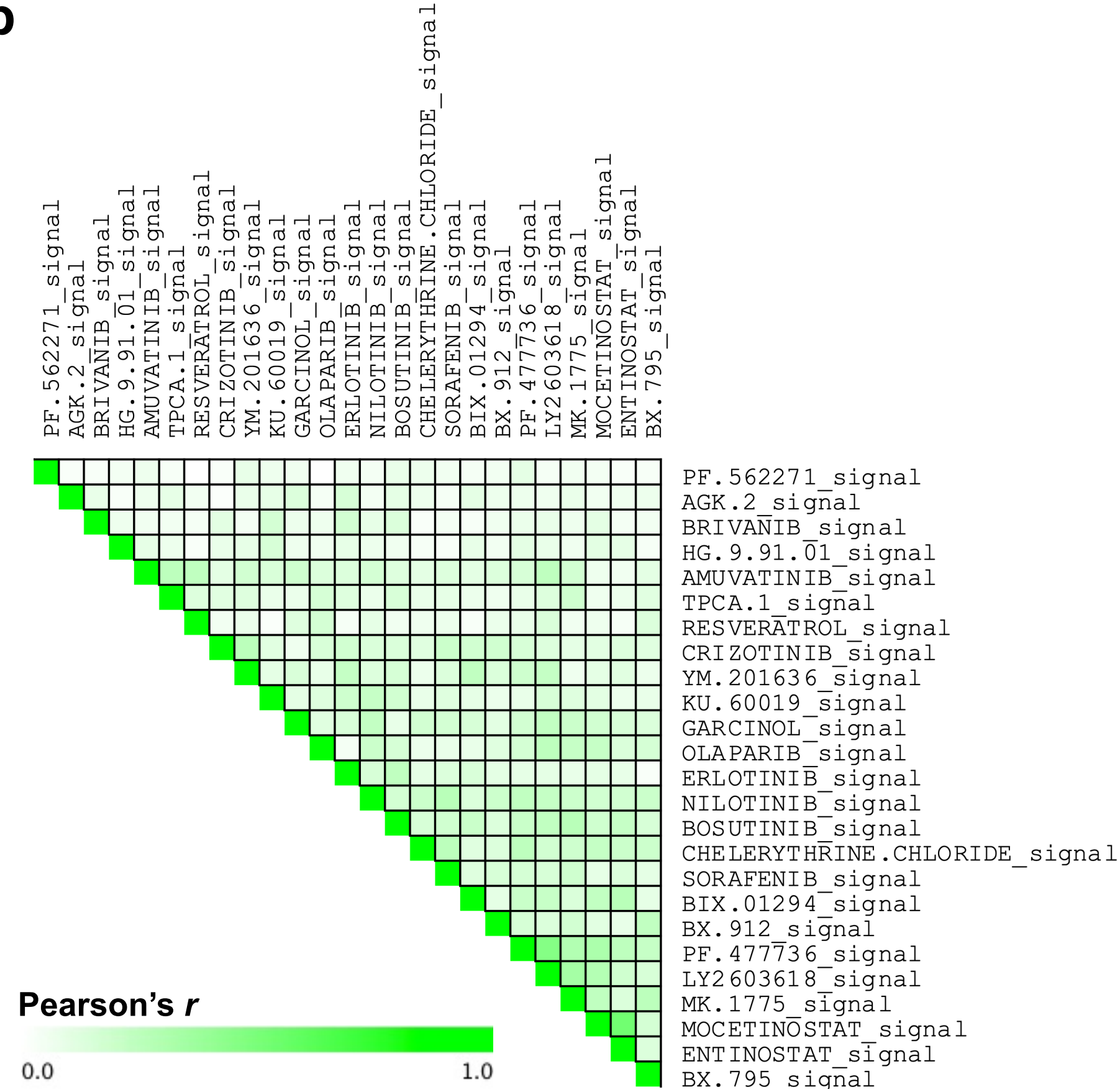


Figure S17. Gene set enrichment analysis (GSEA) of maladaptive DEGs within drug response lists.

- (a) GSEA plots of top 20 drug response patterns overlapping with pre-ranked maladaptive DEGs (Methods). Large subpanel x axis denotes normalized enrichment score (NES), y axis denotes FDR q-value of all tested drug response patterns.
- (b) Correlation matrix of top 25 positively enriched drug response patterns.

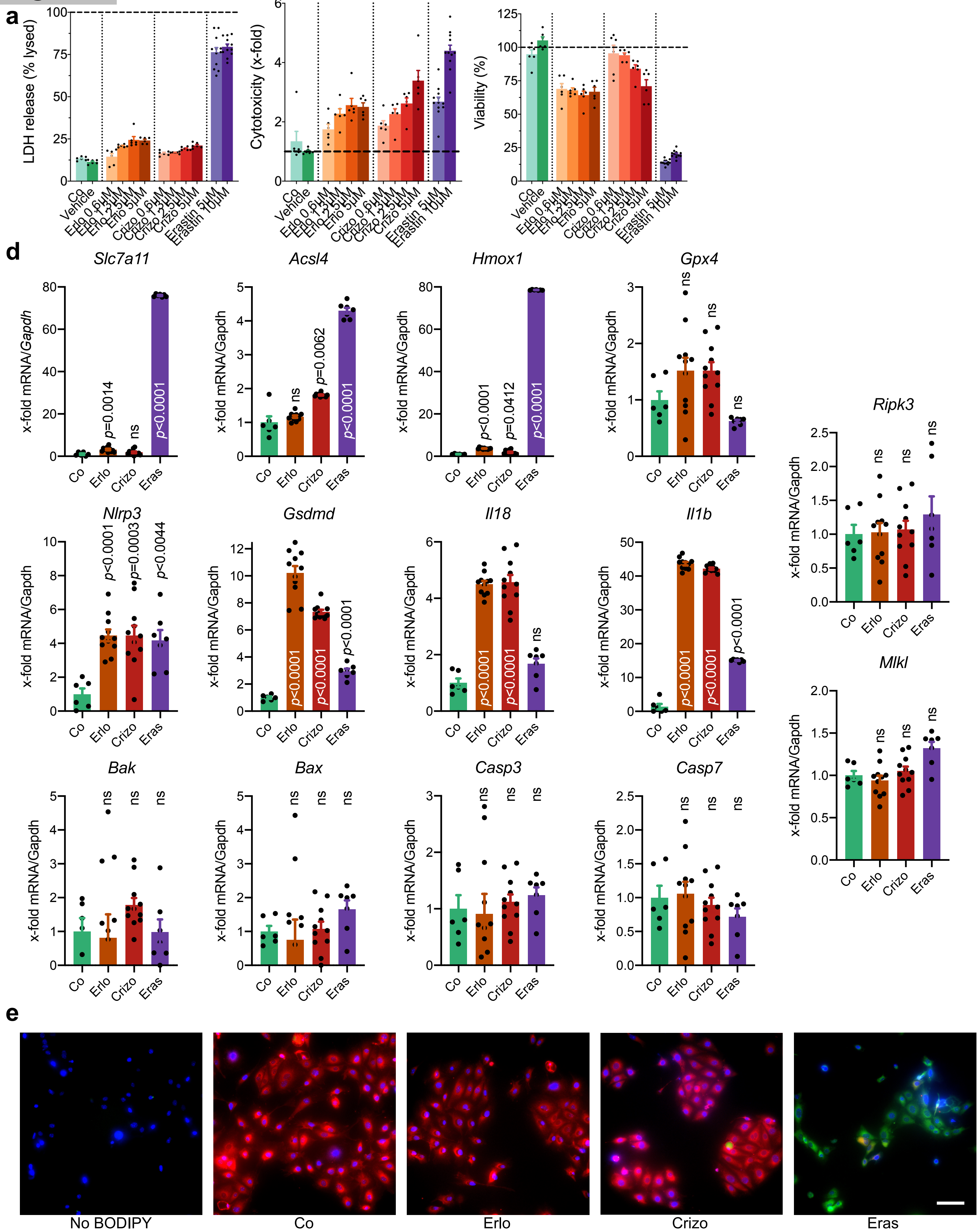
Fig. S18

Figure S18. Erlotinib and crizotinib induced pyroptosis, not ferroptosis, apoptosis, or necroptosis in primary mouse tubular epithelial cell culture *in vitro*.

- (a-c) Lactate dehydrogenase (LDH) release, cytotoxicity, and viability of primary mouse tubular epithelial cells treated for 24 h with culture media only (control, Co), vehicle, erlotinib, crizotinib, and ferroptosis inducer erastin, respectively. LDH release is given as percentage of maximum LDH release in fully lysed cells, cytotoxicity and viability are normalized to Co and vehicle groups; n=2 independent experiments; means $\pm$ SEM. 5 μ M concentrations were hence used for further experiments.
- (d) Relative mRNA levels of ferroptosis (*Slc7a11*, *Acs14*, *Hmox1*, *Gpx4*), pyroptosis (*Nlrp3*, *Gsdmd*, *Il18*, *Il1b*), apoptosis (*Bak*, *Bax*, *Casp3*, *Casp7*), and necroptosis markers (*Ripk3*, *Mlkl*), respectively, after treatment of cells for 24 h with 5 μ M concentrations of DMSO as vehicle (Co), erlotinib (Erlo), crizotinib (crizo), and erastin (Eras), respectively; *Gapdh* was used as reference gene; n=3 independent experiments; means $\pm$ SEM; p values are given for comparisons to control (Co) using Browne-Forsythe and Welch ANOVA (Dunnett corrected).
- (e) Representative live cell images representative of n=3 independent experiments after staining with BODIPY 581/591 C11. Color change from red to green indicates lipid peroxidation, a hallmark of ferroptosis; blue color denotes Hoechst; scale bar = 50 μ m.

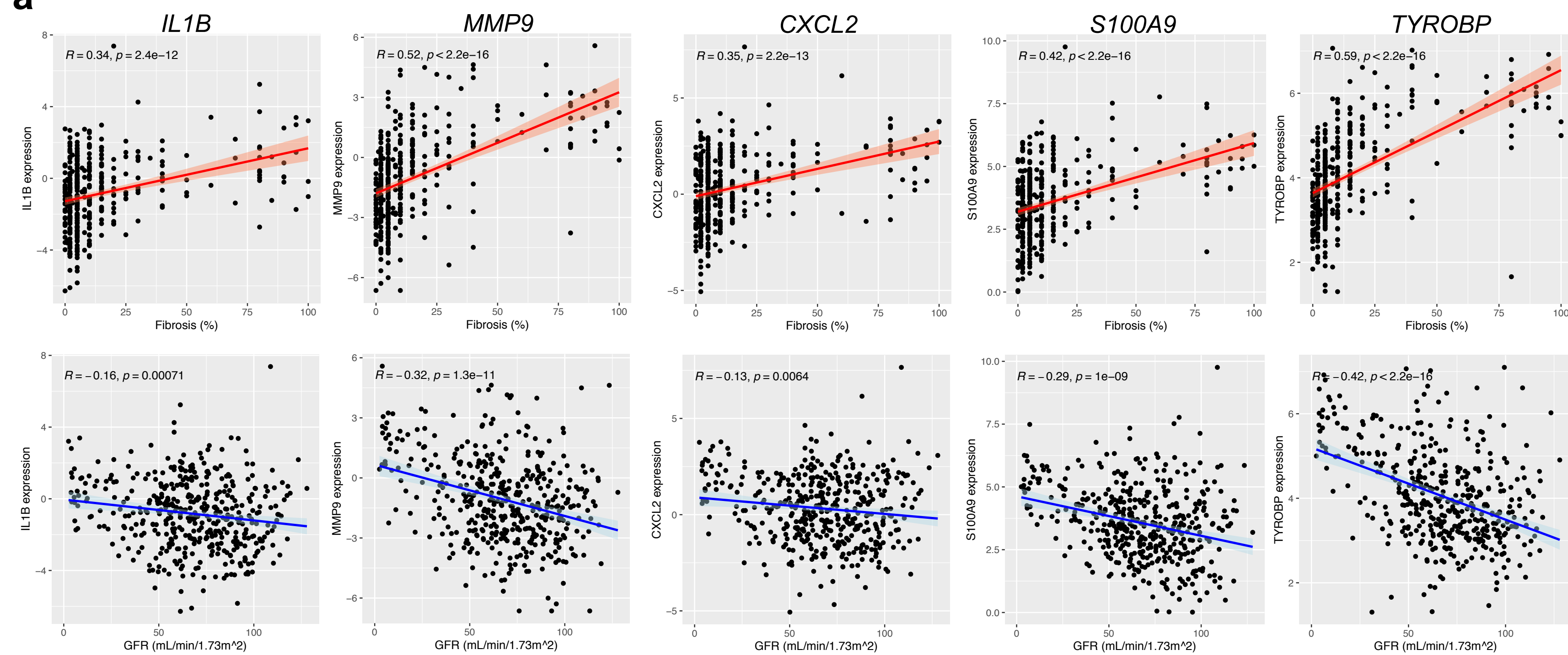
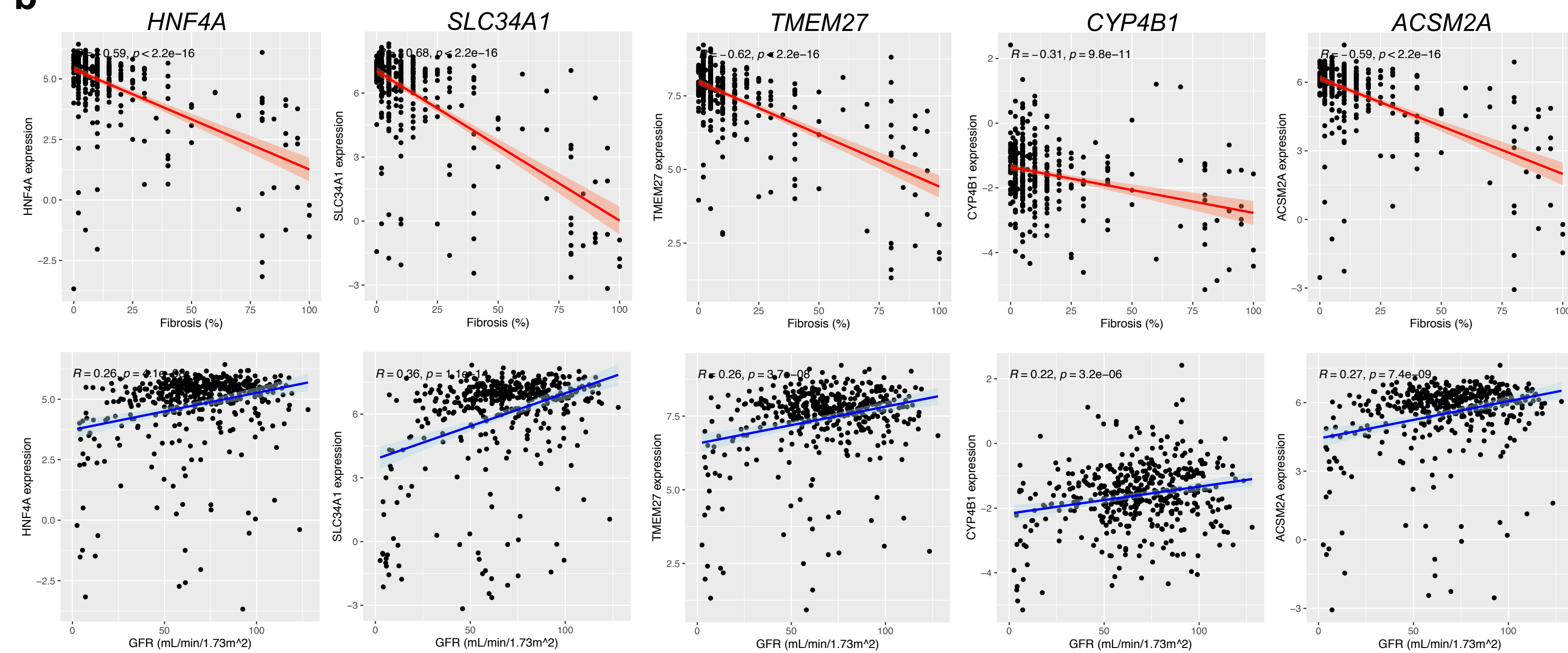
Fig. S19**a****b**

Figure S19. Gene set enrichment analysis (GSEA) of maladaptive DEGs within drug response lists.

(a-b) Correlation of maladaptive repair (*IL1B*, *MMP9*, *CXCL2*, *S100A9*, *TYROBP*) (**a**) and successful repair gene (*HNF4A*, *SLC34A1*, *TMEM27*, *CYP4B1*, *ACSM2A*) (**b**) expression in microdissected human kidney tubules with fibrosis (top row) and eGFR (bottom row); error bands represent 95% confidence interval.

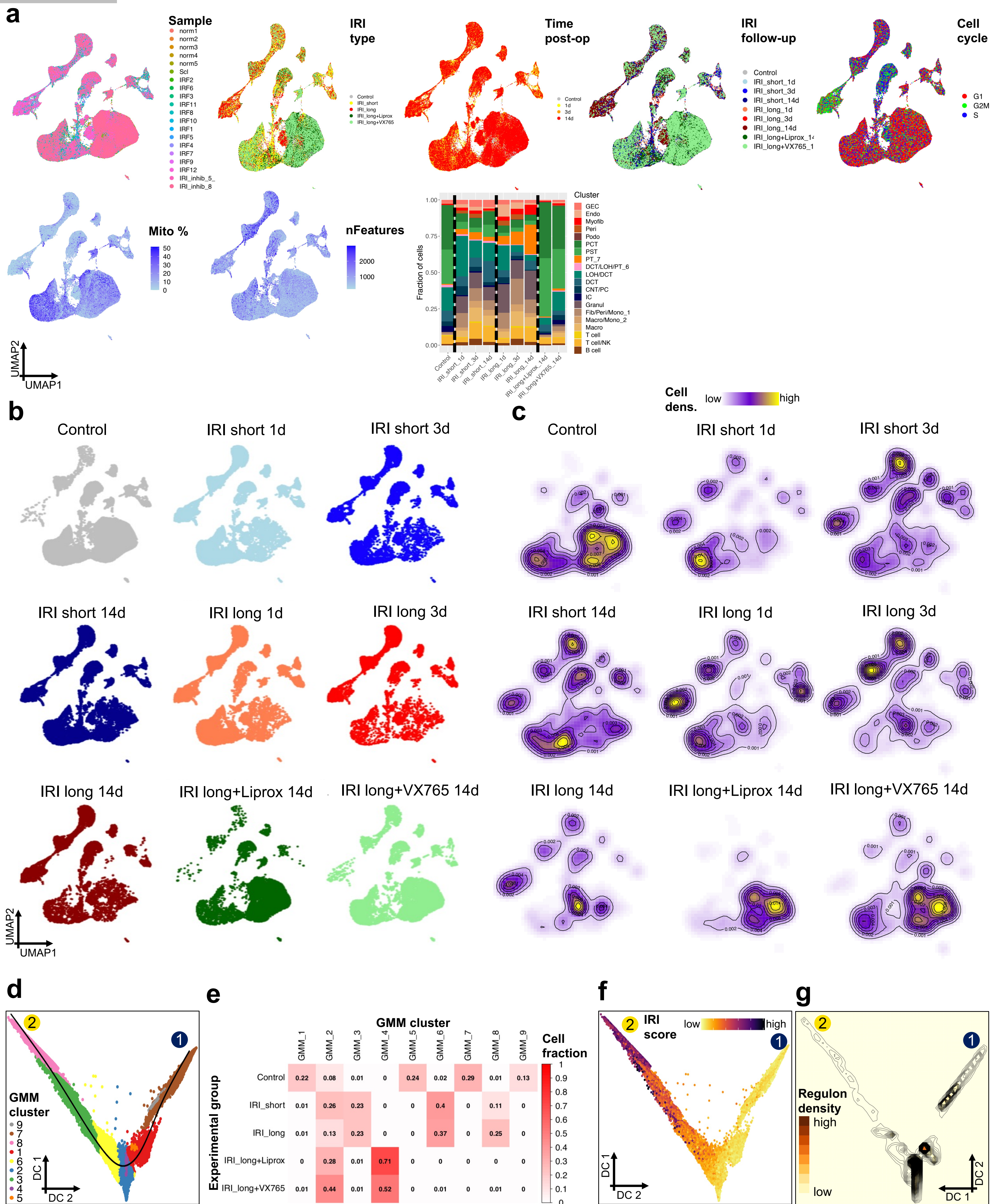
Fig. S20

Figure S20. Pharmacological inhibition of pyroptosis and ferroptosis ameliorates maladaptive kidney response and fibrosis after severe IRI.

- (a) UMAP projection of 133,433 kidney cells of Control, IRI short, IRI long and IRI long+VX765, and IRI long+Liproxstatin-treated samples. Coloring by sample, IRI type (Control, short IRI, long IRI, long IRI+VX765, long IRI+Liprox), time post-op (Control, 1d, 3d, 14d), IRI follow-up (Control, IRI short 1d, IRI short 3d, IRI short 14d, IRI long 1d, IRI long 3d, IRI long 14d, IRI long+VX765 14d, IRI long+Liprox 14d), cell cycle phase (G1, G2M, S), mitochondrial %, and number of features per cell. Last subpanel shows cell fraction across ischemia dose, time post-IRI, and treatment (Control, IRI short 1d, IRI short 3d, IRI short 14d, IRI long 1d, IRI long 3d, IRI long 14d, IRI long+VX765 14d, IRI long+Liprox 14d). Note reduction of PT_7_Maladaptive cells and general preservation of convoluted (PCT) and straight (PST) PT segments in inhibitor-treated samples.
- (b) UMAP projections are split by treatment group for better viewability.
- (c) Changes in cell type proportions of cell types between different treatment groups. Inhibitor-treated kidneys display cell densities similar to Control samples. Highest density in IRI long 14d kidneys corresponds to PT_7_Maladaptive.
- (d) Diffusion map dimensional reduction representation of Slingshot-derived cell trajectories of 47,791 PT cells using a Gaussian mixture modeling (GMM) approach yielded 9 clusters in 2 trajectories. GMM clusters demonstrated major overlap with treatment groups (**Fig. 9h**). Inhibitor-treated samples overlapped with GMM clusters 2 and 4 at the junction right between lineages 1 and 2.
- (e) Correlation matrix of cell fractions of GMM clusters across experimental groups, row sums equal 100%, identifying GMM clusters 2 and 4 as “drug response”-specific.
- (f) Corresponding IRI scores projected onto diffusion map embedding space demonstrated a steady IRI score increase along trajectory 2, whereas IRI score was lowest in clusters from inhibitor-treated and Control samples.
- (g) Corresponding SCENIC-derived regulon density is highest in clusters of inhibitor-treated samples at the junction of lineages 1 and 2. Corresponding tSNE visualization in **Fig. S21a**.

Fig. S21

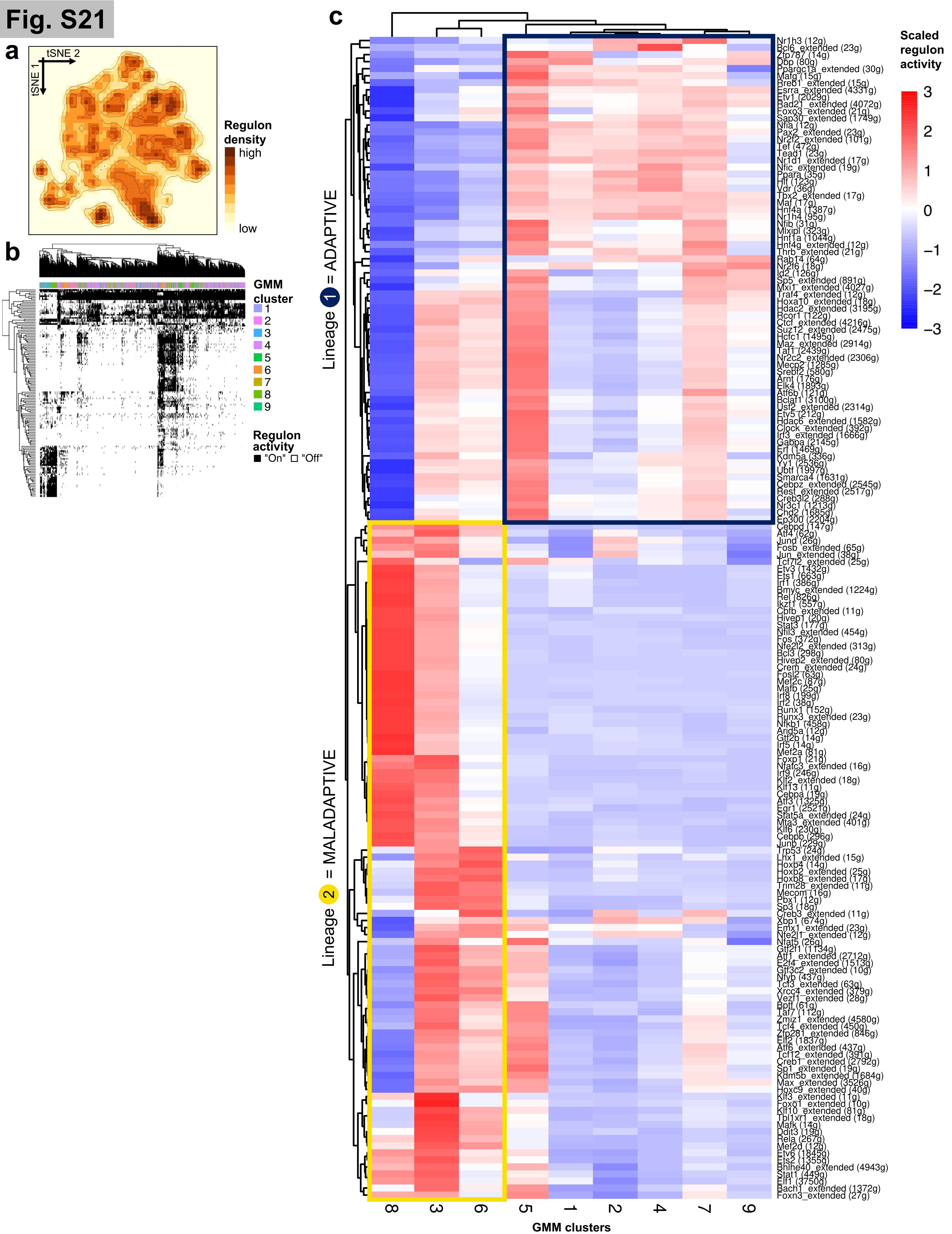
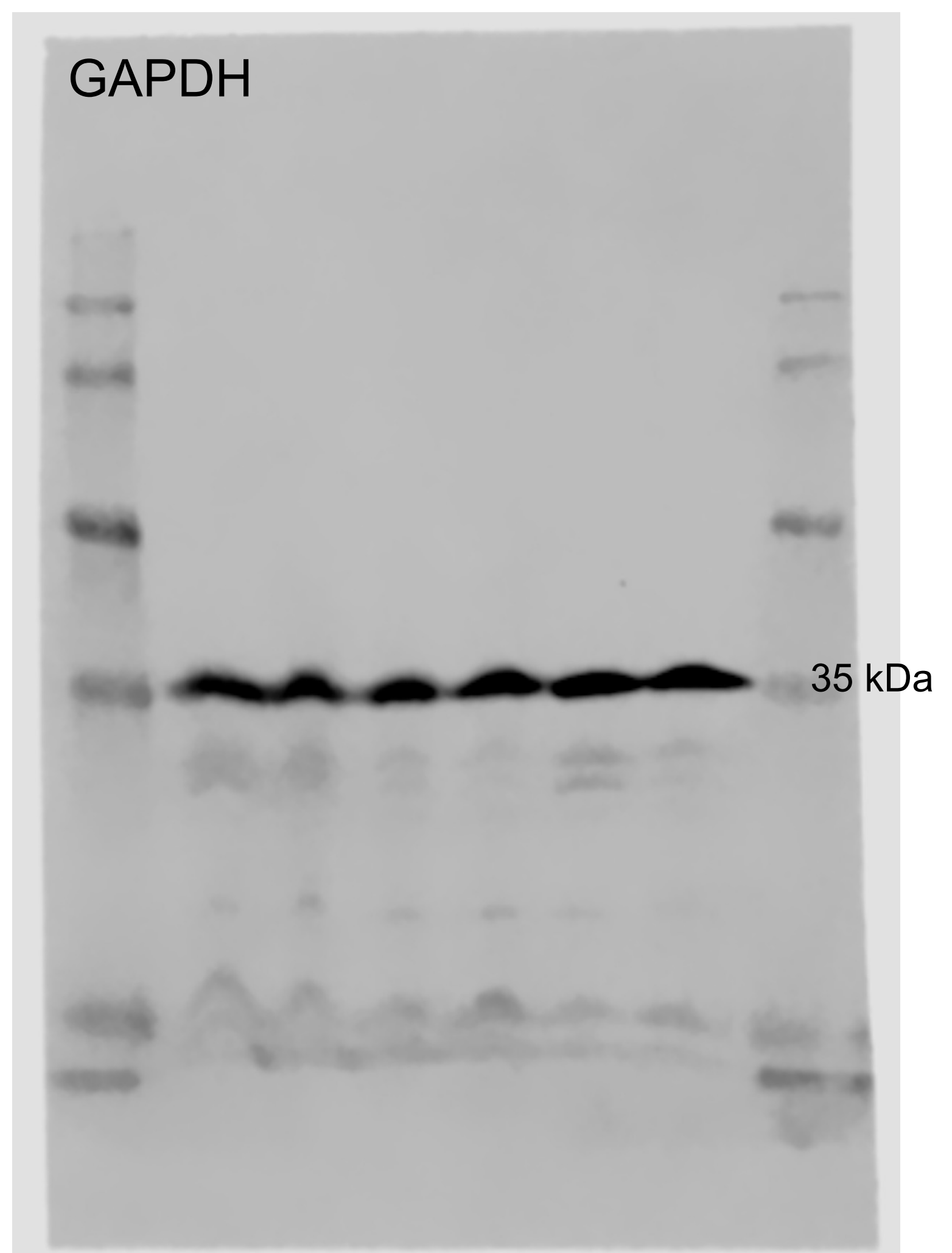
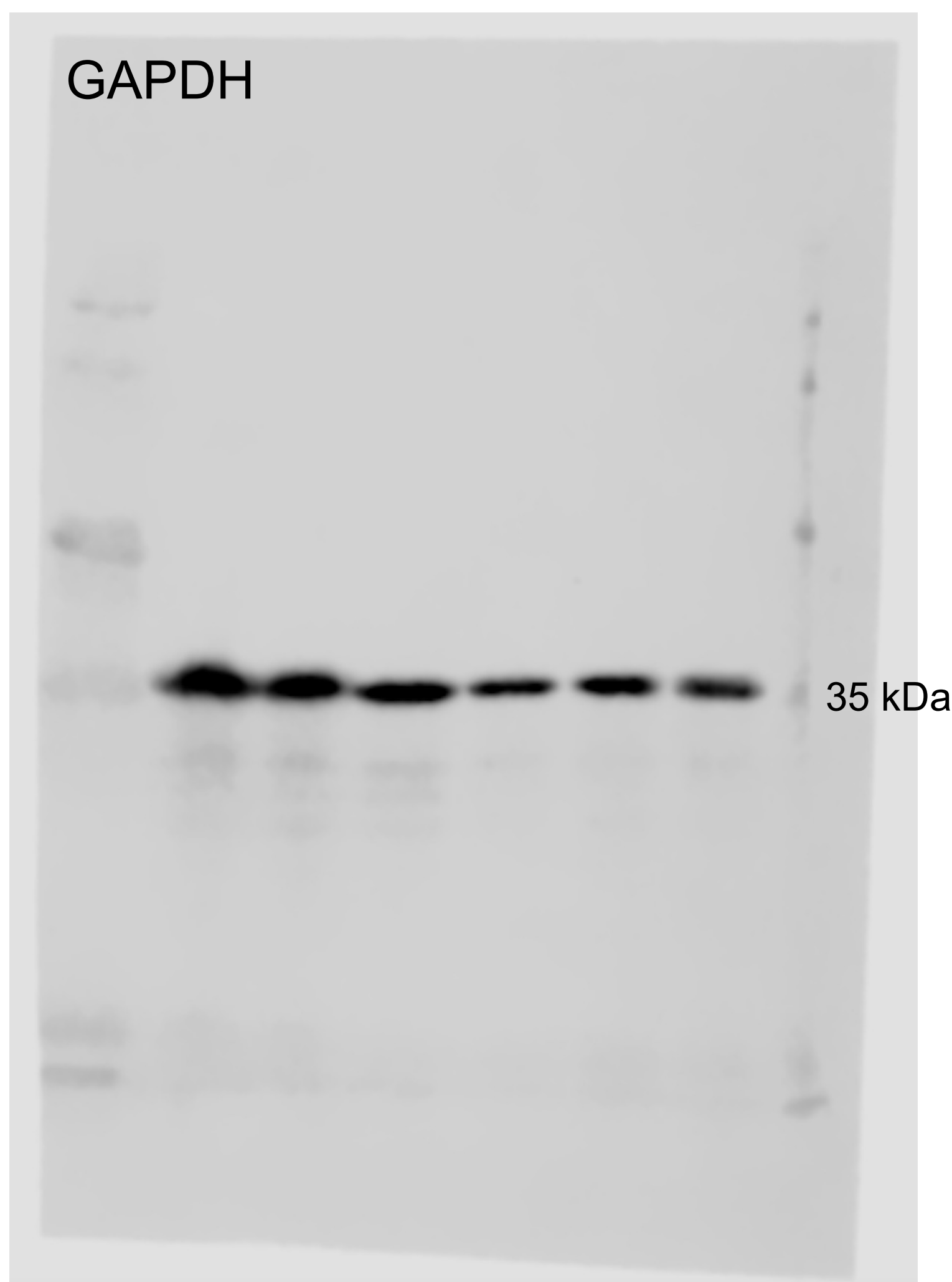
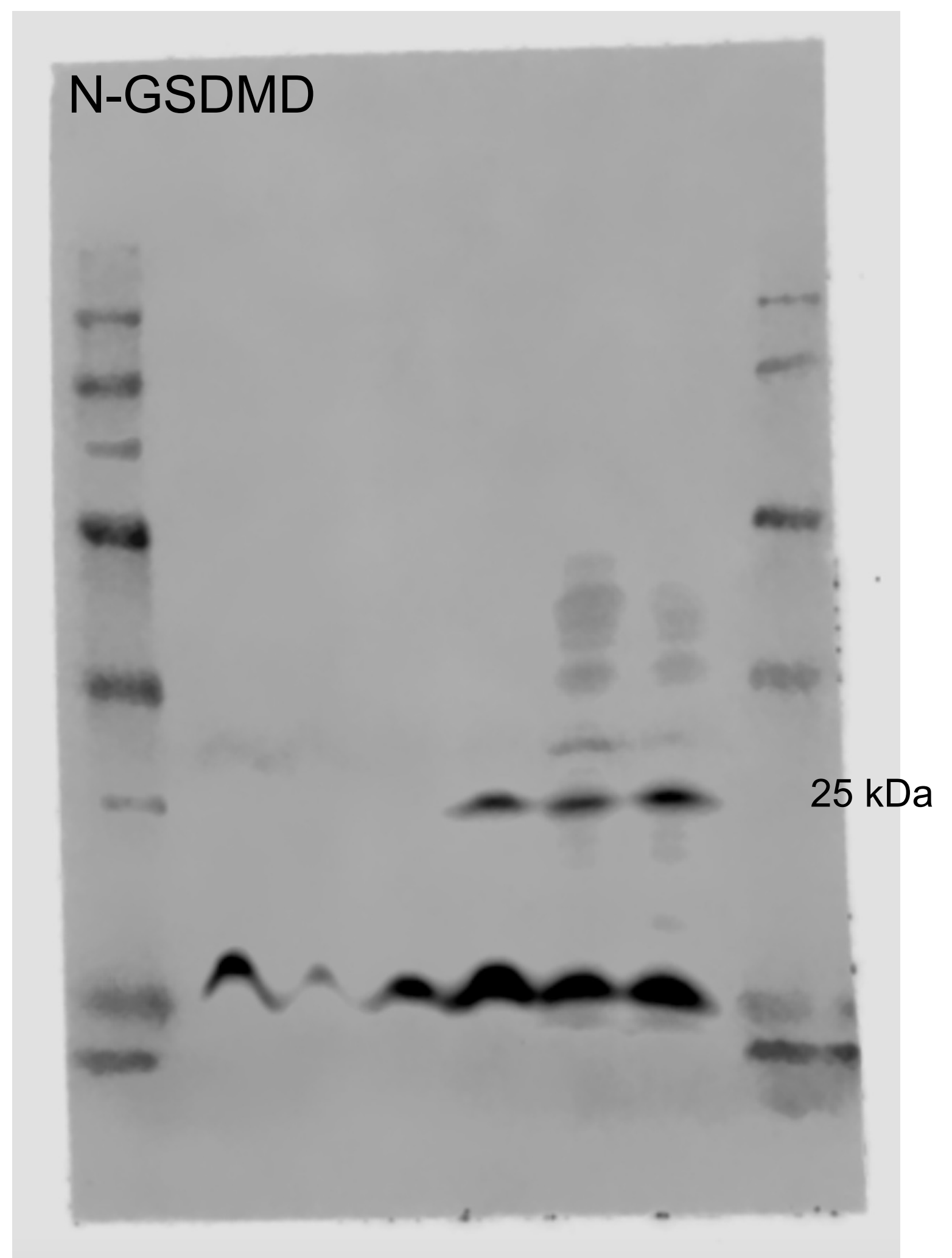
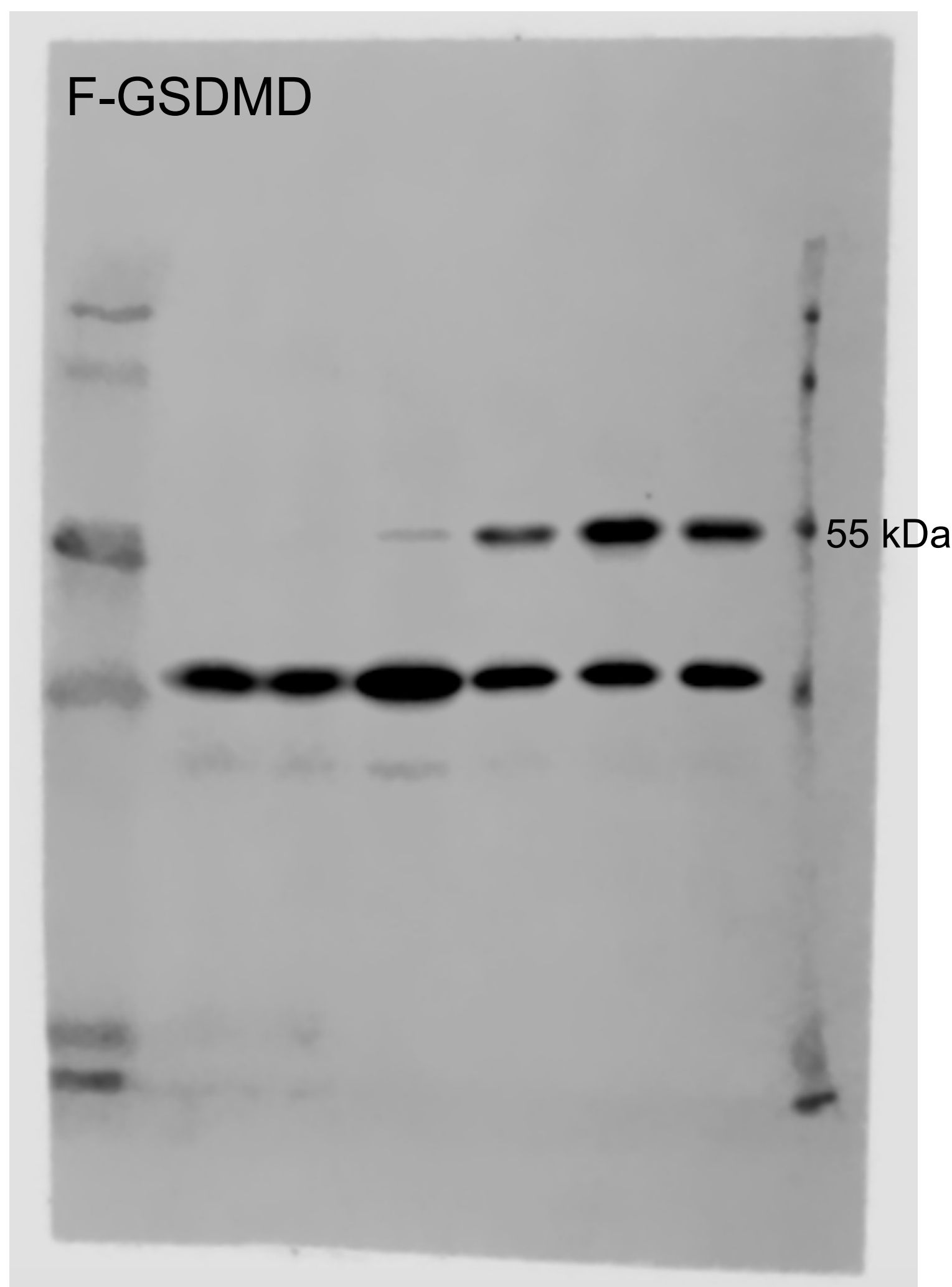


Figure S21. Gene regulatory network of pharmacological inhibition of pyroptosis and ferroptosis.

- (a) tSNE representation of regulon density as a surrogate for stability of regulon states, as inferred by SCENIC algorithm, corresponding to diffusion map representation in **Fig. S20g**.
- (b) Heatmap of cell type-specific binarized regulon activity, as inferred by SCENIC. Regulon activity was binarized to “on” (black) or “off” (white). Rows represent regulons, columns represent individual PT cells. Color legend at the top displays GMM clusters as derived from previous trajectory analysis in **Fig. S20d**.
- (c) Heatmap of GMM cluster-specific scaled regulon activity. Rows represent regulons, columns represent GMM clusters as in **(b)**. GMM clusters 1, 2, 4, 5, 7, and 9 represented lineage 1, clusters 3, 6, and 8 lineage 2, respectively.

Full scan blots for Fig. S11d



Supplementary Table 1. Flow cytometry antibodies.

Target	Dilution	Clone	Company	Fluorophore	Cat.#
CD3	1:200	145-2C11	BD bioscience	BUV395	100308
Ly6G	1:300	1A8	BD bioscience	BUV563	127602
CD11b	1:600	M1/70	BD bioscience	BUV661	612977
CD45	1:200	30-F11	BD bioscience	BUV805	103102
F4/80	1:200	BM8	Invitrogen	ef450	MF48000
CD19	1:300	6D5	Biolegend	BV605	115508
CD4	1:300	RM4-5	Biolegend	BV650	100506
NK1.1	1:200	PK136	Biolegend	BV711	108702
Ly6C	1:400	HK1.4	Biolegend	BV785	128016
CD11c	1:200	N418	eBioscience	FITC	117302
CD49b	1:100	DX5	eBioscience	PerCP-ef710	108908
SiglecF	1:200	E50-2440	BD bioscience	PE	562068
CD8a	1:300	53-6.7	BD bioscience	PE-cf594	562283
CD64	1:200	X54-5/7.1	Biolegend	PE-Cy7	139304
FcεR	1:200	MAR-1	Invitrogen	APC	17-5898-82
MHCII (IA/IE)	1:300	M5/114.15.2	Biolegend	AF700	107622
cKit (CD117)	1:200	ACK2	Invitrogen	APC-e780	47-1172-82

Supplementary Table 2. Materials table.

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Chemicals, Antibodies, Peptides, and Recombinant Proteins		
Buprenorphine SR	midwest Veterinary Supply	191.26505.3
Liproxstatin	Cayman Chemical	17730
VX-765	InvivoGen	inh-vx765i-5
VetScan iSTAT CHEM8+	Abaxis	016.30270.2
DPBS	Corning	21-031-CV
RPMI 1640	Gibco	21875-034
Epithelial growth factor	Peprotech	AF-100-15
ITS	Gibco	51500--56
Penicillin-Streptomycin	Corning	30-002-CI
Dimethyl sulfoxide	Merck	D2650
Erlotinib	Cayman Chemical	10483
Crizotinib	Cayman Chemical	12087
Erastin	Cayman Chemical	17754
BODIPY™ 581/591 C11	Thermo Fisher	D3861
Hoechst 33342	Invitrogen	H1399
Tet System Approved FBS	Clontech	631106
Nonidet™ P40 Substitute	Sigma	74385
Magnesium Chloride Solution	Sigma	M1028
Ultrapure BSA (50 mg/ml)	Thermo Fisher	AM2616
Trizol	Invitrogen	15596018
RNase inhibitor	Applied Biosystems	100021540
SYBR Green PCR Master Mix	Applied Biosystems	4367659
cDNA Reverse Transcription Kit	Applied Biosystems	4368813
RNAscope® 2.5 HD Duplex Detection Kit	ACD	322436
Mm-Gsdmd probe	ACD	537601
SDS lysis buffer	CST	7722
anti-GSDMD antibody	Santa Cruz	393656
anti-SLC34A1 antibody	Novus	NBP2-13328
anti-CD11B antibody	BD	555386
AF555-conj. donkey anti-rabbit	LifeSciences	A31572
AF488-conj. goat anti-mouse	LifeSciences	A11029
anti-GSDMD antibody	Abcam	ab209845
anti-N-GSDMD antibody	CST	36425S
HRP-conjugated secondary antibody	CST	7074
ECL SuperSignal West Femto	Thermo Fisher	34096
Critical Commercial Assays		
RNeasy mini kit	Qiagen	74106
Bioanalyzer High Sensitivity DNA kit	Agilent Technologies	5067-4626
Chromium Cell B Chip	10X Genomics	PN-120262
Chromium Controller	10X Genomics	PN-120223
Chromium i7 Multiplex Kit	10X Genomics	PN-120262
Chromium Single Cell 3' Reagent Kits v3	10X Genomics	PN-1000092
Countess AutoCounter	Invitrogen	C10227
Illumina Library Quantification Kit	KAPA Biosystems	KK4824

Multi Tissue dissociation kit	Miltenyi	130-110-201
CytoTox 96 Non-Radioactive cytotoxicity assay	Promega	G1780
MultiTox-Fluor Multiplex Cytotoxicity assay	Promega	G9200
Deposited Data		
Mouse folic acid injury scRNA-seq data	(Dhillon et al.)	GSE156686 [https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE156686]
Mouse acute kidney injury snRNA-seq data	(Kirita et al.)	GSE139107 [https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE139107]
Mouse acute kidney injury bulk Affymetrix microarray data	(Liu et al.)	GSE52004 [https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi]
Mouse kidney transplant scRNA-seq data	(Dangi et al.)	GSE157292 [https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi]
Mouse acute spinal cord injury scRNA-seq data	(Hamel et al.)	GSE15638 [https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi]
Software and Algorithms		
a4Base v1.34.1	open source	https://bioconductor.org/packages/release/bioc/html/a4Base.html
Biobase v2.40.0	open source	https://bioconductor.org/packages/release/bioc/html/Biobase.html
BisqueRNA v1.0.3	open source	https://cran.r-project.org/web/packages/BisqueRNA/index.html
Cell Ranger v3.1.0	10X Genomics	https://support.10xgenomics.com/single-cell-gene-expression/software/downloads/latest
CellPhoneDB v2.1.2	open source	https://www.cellphonedb.org
circize v0.4.11	open source	https://github.com/jokergoo/circize
Circos Table Viewer v0.63-9	open source	http://mkweb.bcgsc.ca/tableviewer/
clusterProfiler v3.10.1	open source	https://guangchuangyu.github.io/software/clusterProfiler/
DESeq2 v1.10.1	open source	https://bioconductor.org/packages/release/bioc/html/DESeq2.html
destiny v3.1.1	open source	https://github.com/theislab/destiny
DoubletFinder v2.0	open source	https://github.com/chris-mcginnis-ucsf/DoubletFinder
EnhancedVolcano v1.0.1	open source	https://bioconductor.org/packages/release/bioc/html/EnhancedVolcano.html
FlowJo v10.8	open source	https://www.flowjo.com
gam v1.20	open source	https://cran.r-project.org/web/packages/gam/index.html
GSEA v4.0.3	open source	http://www.gsea-msigdb.org/gsea/index.jsp
htmlwidgets v1.5.1	open source	https://github.com/ramnathv/htmlwidgets/releases
LIGER v0.5.0	open source	https://github.com/MacoskoLab/liger
mclust v5.4.6	open source	https://mclust-org.github.io/mclust/
monocle2 v2.14.0	open source	http://cole-trapnell-lab.github.io/monocle-release/
monocle3 v0.1.3	open source	https://cole-trapnell-lab.github.io/monocle3/
MRI fibrosis tool (unversioned)	open source	https://github.com/MontpellierRessourcesImagerie/imagej_macros_and_scripts/wiki/MRI_Fibrosis_Tool#myfootnote1
MuSiC v0.1.1	open source	https://github.com/xuranw/MuSiC
OneR v2.2	open source	https://cran.r-project.org/web/packages/OneR/index.html
pagoda2 v0.1.1	open source	https://github.com/kharchenkolab/pagoda2/releases
RSEM v1.3.0	open source	https://github.com/deweylab/RSEM
SCENIC v1.1.2.2	open source	https://aertslab.org/#scenic
Seurat v3.1.2	open source	https://satijalab.org/seurat/
SeuratWrappers v0.1.0	open source	https://github.com/satijalab/seurat-wrappers
Slingshot v1.6.1	open source	https://bioconductor.org/packages/release/bioc/html/slingshot.html
SoupX v1.4.5	open source	https://github.com/constantAmateur/SoupX
STAR v2.4.1d	open source	https://github.com/alexdobin/STAR
TradeSeq v1.2.01	open source	https://statomics.github.io/tradeSeq/index.html
Velocity v0.6	open source	https://github.com/velocity-team/velocity.R
WGCNA v1.70-3	open source	https://github.com/cran/WGCNA
xbioc v0.1.18	open source	https://rdrr.io/github/renozao/xbioc/