Supplementary material

Title: The integrated signature of renal preconditioning against ischemia reperfusion injury

Running title: Gene expression signature of renal organoprotection

Authors: Marc Johnsen, Torsten Kubacki, Assa Yeroslaviz, Martin Späth, Jannis Mörsdorf, Heike Göbel, Katrin Bohl, Michael Ignarski, Bianca Habermann, Janine Altmüller, Andreas Beyer, Thomas Benzing, Bernhard Schermer, Volker Burst and Roman-Ulrich Müller

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Supplementary methods

Total RNA and miRNA mapping, trimming and adapter removal

Raw mRNA data quality was analyzed using the fastqc software (0.10.1). mRNA trimming and adapter removal were done with the fastx-Toolkit (0.0.14) and cutadapt (1.5) [1] before mapping to the mouse genome from Ensembl (GRCm38) using STAR aligner (2.4.2a) [2]. Mapped reads were run through featureCount (1.4.6-p4) [3].

miRNA adapter removal was done using cutadapt [1], mapping and quantification using the mirDeep2 software package [4]. Products were evaluated for suitability on a thermodynamic scale with bowtie aligner [5] and their structure was using Randfold [6] to classify as possible miRNAs.

Outcome correlation score

Mean of serum urea on day 1 and 3 after IRI and the weight from day 2 post IRI divided by the weight at baseline before IRI were rated from 1 to 8 and summed up to a maximum score of 16.

mg/dl	score
<50	1
50-99	2
100-149	3
150-199	4
200-249	5
250-299	6
300-349	7
349 / deceased	8

% baseline weight	score
105-110	1
104-100	2
95-99	3
90-94	4
85-89	5
80-84	6
75-79	7
70-74	8

Primer IDs for qPCR assays

For qPCR Applied Biosystems Customized TaqMan Arrays were utilized.

Acsm3-Mm00489774_m1	Target
Ubc-Mm01201237_m1	Control
Slc22a7-Mm00460672_m1	Target
Tmprss9-Mm01177636_m1	Target
Tspan13-Mm00481226_m1	Target
Reln-Mm00465200_m1	Target
Cyp7b1-Mm00484157_m1	Target
Ace-Mm00802048_m1	Target
Folh1-Mm00489655_m1	Target
Ctxn3-Mm01718023_m1	Target
18S-Hs99999901_s1	Control
Cndp2-Mm01261959_mH	Target
Gusb-Mm01197698_m1	Control
Pde6a-Mm00476664_m1	Target
Mpv17l-Mm00473833_m1	Target
Hsd17b11-Mm00504410_m1	Target
Als2cr11-Mm00613608_m1	Target
Slc16a13-Mm00655437_m1	Target
Nudt19-Mm00473613_m1	Target
Hmox1-Mm00516005_m1	Target
Aqp4-Mm00802131_m1	Target
Ldhd-Mm00459138_g1	Target
Pdk3-Mm00455220_m1	Target
Ces2c-Mm01250994_m1	Target
9030619P08Rik-Mm01205874_m1	Target
Mogat1-Mm00503358_m1	Target
Apoe-Mm01307193_g1	Target
Amacr-Mm00507717_m1	Target
Slc18a1-Mm00461868_m1	Target
Acadm-Mm01323360_g1	Target
Entpd4-Mm00491888_m1	Target
Acy3-Mm00503587_g1	Target
Hao2-Mm00469507_m1	Target

Cyp4a12a-Mm00514494_m1	Target
Kif20b-Mm01205010_m1	Target
Kynu-Mm00551012_m1	Target
Slc7a12-Mm00499866_m1	Target
Prlr-Mm00599957_m1	Target
Alas1-Mm01235914_m1	Target
Gapdh-Mm99999915_g1	Control
Tnfrsf21-Mm00446361_m1	Target
Wnt5b-Mm01183986_m1	Target
Hbb-b1;Beta-s;Hbb-b2-Mm03646870_gH	Target
Alas2-Mm00802083_m1	Target
Grem2-Mm00501909_m1	Target
Hdc-Mm00456104_m1	Target
Polr2a-Mm00839493_m1	Control
Il34-Mm01243248_m1	Target

Recovery score after IRI

The Score was put together based on the “Belastungskataloge zur Bewertung von Tierversuchen” of the „Forum Tierversuche in der Forschung“.

http://www.dfg.de/download/pdf/dfg_magazin/forschungspolitik/tierschutz2015/dialogforum_tierversuche/belastungskataloge_zur_bewertung_von_tierversuchen.pdf

Weight loss	compared to baseline (day 0) before surgery
0 points	No weight loss
1 point	< 10%
2 points	10-20%
3 points	> 20%
Activity	nest building, flight reflex, fur cleansing, movement in cage
0 points	normal
1 point	slightly reduced
2 points	severly reduced
3 points	no activity

Appearance	
0 points	normal
1 point	shaggy fur
2 points	+ nose bump, elevated respiratory rate, signs of slight dehydration
3 points	+ discharge from eyes and / or nose, standing skin folds
Total Score	
≥ 7 points	sacrifice of animal in anaesthesia
5-7 points	close observation and consultation with experimental supervisor
0-4 points	continue experiment according to protocol

Supplementary Figure / Table Legends

Supplementary Figure 1 Raw count of mapped reads for total RNA samples

Raw count of mapped reads with gene rank on x axis and raw counts on y axis for non-preconditioned animals (CTRL) at baseline – 0 hours (A), 4 (B) and 24 hours (C) after IRI, caloric restricted animals (CR) at baseline (D) and 24 hours after IRI (E), as well as hypoxic preconditioned animals (HP) at baseline (F) and 24 hours after IRI (G).

Supplementary Figure 2 Experimental setup and baseline characteristics of animals

Baseline creatinine (A) and urea (B) values for non-preconditioned animals and animals preconditioned with calorie restriction (CR). Weight curves for animals from A+B (C). Recovery score 24h and 72h after IR (n=9 in each group) D). Lower scores correspond to better recovery. ($p=0.0023$ at 24 h after IRI, $p<0.0001$ at 72 h after IRI) Caloric restriction, CR; hypoxic preconditioning, HP; ischemic reperfusion injury, IRI. N.S. not significant, ** $p<0.01$, **** $p<0.0001$

Supplementary Figure 3 Damage after IRI is strongly ameliorated in preconditioned animals

Representative PAS stainings from post ischemic kidneys 4h and 72h after IRI from non-preconditioned controls, HP and CR animals. * tubular flattening, ➤ nuclear loss, ➚ tubular casts, # denuded tubuli with luminar debris. TUNEL assay from controls, HP and CR group 24h after IRI showing cell death in green (D/H/L). Caloric restriction, CR; hypoxic preconditioning, HP; ischemic reperfusion injury, IRI.

Supplementary Figure 4 Pseudotimeanalysis

Pseudotime analysis including all samples (CR, HP, non-PC) before and after IRI.
Sample ordering ("cell ordering" in the original Monocle workflow) in a reduced dimensional space as determined by the Monocle algorithm
CR; hypoxic preconditioning, HP; non-preconditioned animals, non-PC; ischemic reperfusion injury, IRI

Supplementary Figure 5: Cell specific changes in precondtioned and non-preconditioned animals before and after IRI

Shown are the cell specific changes of different kidney and immune cells according to the expression of their marker genes calculated as Z-score. Z-score is showing the number of standard deviations from the mean expression of each cell specific marker set across all conditions. CR; hypoxic preconditioning, HP; non-preconditioned animals, non-PC; ischemic reperfusion injury, IRI

Supplementary Figure 6 qPCR validation for RNAseq results of selected genes that were commonly regulated in response to CR as well as HP

Validation of RNAseq expression changes with qPCR TaqMan arrays of 44 genes that were commonly regulated in RNAseq in response to preconditioning with CR as well as HP. Log2FC expression changes from RNAseq (dark blue CR / red HP) and qPCR (light blue CR / orange HP). Genes are sorted according to Log2FC in RNAseq for CR animals starting with the most downregulated gene in the upper panel to the most upregulated gene in the lower panel to the right.

Supplementary Figure 7 51 genes from overlapping KEGG Pathways

KEGG pathway analysis was performed separately in HP and CR animals before IRI. Shown are the genes contributing to each of the 3 overlapping KEGG pathways for CR and HP before IRI (padj <0.05, FDR<0.05).

Supplementary Figure 8 Genes in Peroxisome pathway regulated in response to CR and HP

Depiction of Peroxisome KEGG pathway with significantly regulated genes in response to CR (A) and HP (B).

Supplementary Figure 9 Genes in Glutathione pathway regulated in response to CR and HP

Depiction of Glutathione KEGG pathway with significantly regulated genes in response to CR (A) and HP (B).

Supplementary Figure 10 Differential regulation of miRNAs and biological significance of regulation in response to CR and 24 hours after IRI in non-preconditioned

Regulation of miRNAs in CR animals compared to non-preconditioned animals at baseline (A) and 24 hours after IRI (B). Upregulated miRNAs depicted as red bars, downregulated miRNAs as green bars. Percentage of downregulated target mRNAs for significantly up- and downregulated miRNAs at baseline (C) and 24 hours after IRI (D) compared to the whole gene set (black bar). Targets that show a significantly different percentage of downregulation compared to the whole gene set (black bar chart) are marked with an asterix (*). Mean log2foldchange for the whole gene set (black bar) and for targets of significantly regulated miRNAs at baseline (E) and 24 hours after IRI (F).

Significantly regulated miRNAs in response to CR or IRI

Since posttranscriptional regulators are expected to add another layer of complexity to the regulation of gene networks, we performed small RNA sequencing in addition to standard RNAseq. Four microRNAs were significantly regulated in response to CR. No microRNAs were significantly regulated in response to HP. 24 hours after IRI, 91 microRNAs were differentially regulated in non-preconditioned animals. For a

complete list of regulated microRNAs see Table 5. All microRNAs regulated in response to CR were also regulated 24 hours after IRI (A+B). mir-802-5p was upregulated and mir-22-3p downregulated in both situations. To evaluate their functional relevance, we compared the differences between all significantly regulated mRNAs with the significantly regulated predicted miRNA targets in the same condition. If functionally relevant, inverse regulation of a miRNA compared to its targets would be expected as miRNAs are thought to silence their targets. From the 4 miRNAs regulated by CR, such an association was revealed only for miR-22-3p (miR-22-3p downregulated, targets upregulated, mean log2FC (.007) higher than for the regulated mRNAs in the whole sample (p=.007)). Consistently, only 2 downregulated miRNAs showed a pattern of inversely regulated targets 24 hours after IRI: miR-29-3p and miR-182-5p, which both had a higher percentage of upregulated targets and also a higher mean log2FC than regulated genes in the whole sample (C-F).

Supplementary Figure 11 IRI-induced gene expression patterns and pathways at 4h and 24h

Overlap of differentially regulated genes in non-preconditioned animals 4h and 24h after IRI (A) ($p<0.001$). Top ten up- and downregulated genes by IRI after 4h and 24h (B). SPIA analysis showing the Top 20 signalling pathways after 4h and 24h after IRI in non-PC animals (C) (pGfdr <0.05), y-axis: pathway terms, x-axis: observed total preturbation accumulation in the pathway (tA). Heatmap of SPIA pathways of preconditioned animals before damage and non-preconditioned animals 4h and 24 hours after IRI (D). CR; hypoxic preconditioning, HP; non-preconditioned animals, non-PC; ischemic reperfusion injury, IRI

Supplementary Figure 12 Interactome overview of mitochondrial processes (by mitoXplorer)

Interactome view is the visualisation method for all mitochondrial processes of a single dataset. Shown are CR 0h vs non-PC 0h (A), HP 0h vs non-PC 0h (B), non-PC-IR4h vs non-PC 0h (C) and non-PC-IR24h vs non-PC 0h (D). Red - down-regulated, Blue - up-regulated, size of the ball represents higher/lower log2FC (p-value is not considered at this visualisation).

Supplementary Figure 13 Heatmap for outcome correlated genes

Heatmap of 34 genes whose regulation at baseline was highly correlated with individual animal's outcome after IRI (r Spearman >0.95 and <-0.95). Expression of 30 genes is positively correlated and of 4 genes is negatively regulated with outcome.

Supplementary Figure 14 Univariate linear regression analysis of outcome correlated genes

Univariate linear regression analyses for the 16 genes, that were highly correlated with the damage score and that were also identified in the overlap between CR and HP. Regression coefficient B is provided as the change of damage score per change of transcripts by 100 copies.

Supplementary Figure 15A-P Spearman blots for outcome regulated genes that were enriched after CR as well as HP

Raw read count for genes with $r_S > 0.95$ and $r_s < -0.95$ blotted against outcome score.

Supplementary Table Legends

Supplementary Table 1 Differentially regulated genes

Shown are the differentially regulated genes of CR, HP and non-preconditionend animals at different timepoints (see legend in the table)

Supplementary Table 2 GO term analysis

Shown are GO BP, CC and MF terms of CR, HP and non-preconditioned animals at different timepoints (see legend in the table).

Supplementary Table 3 KEGG pathway analysis

Shown are the KEGG pathway terms of CR, HP and non-preconditioned animals at different timepoints (see legend in the table).

Supplementary Table 4 Gene lists of SPIA from CR0h

Shown are the genes contributing to the 7 significant pathways from the Signaling pathway impact analysis (SPIA) in CR0h animals (see legend in the table).

Supplementary Table 5 SPIA

Signalling pathway impact analysis of CR0h, HP0h and non-preconditioned animals 4h and 24h after ischemia-reperfusion injury (see legend in the table).

Supplementary Table 6 Regulation of AUMD RNA degrading proteins after damage

Selected RNA stabilizing and destabilizing adenine/uridine-rich element binding proteins (AUBPs) and their differential expression (Log2FC) in response to IRI induced damage (adapted from von Roretz et al. 2011) (see legend in the table).

Supplementary Table 7 Analysis from PCA with loadings

Shown are the genes from the 3 gene sets (upper, right and lower outliers) from the PCA analysis (->Fig.5) and the GO term analysis (BP, CC, MF) as well as KEGG pathway analysis (see legend in the table).

References for Supplementary Material

1. Martin M: Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet.journal* 17: pp. 10–12, 2011

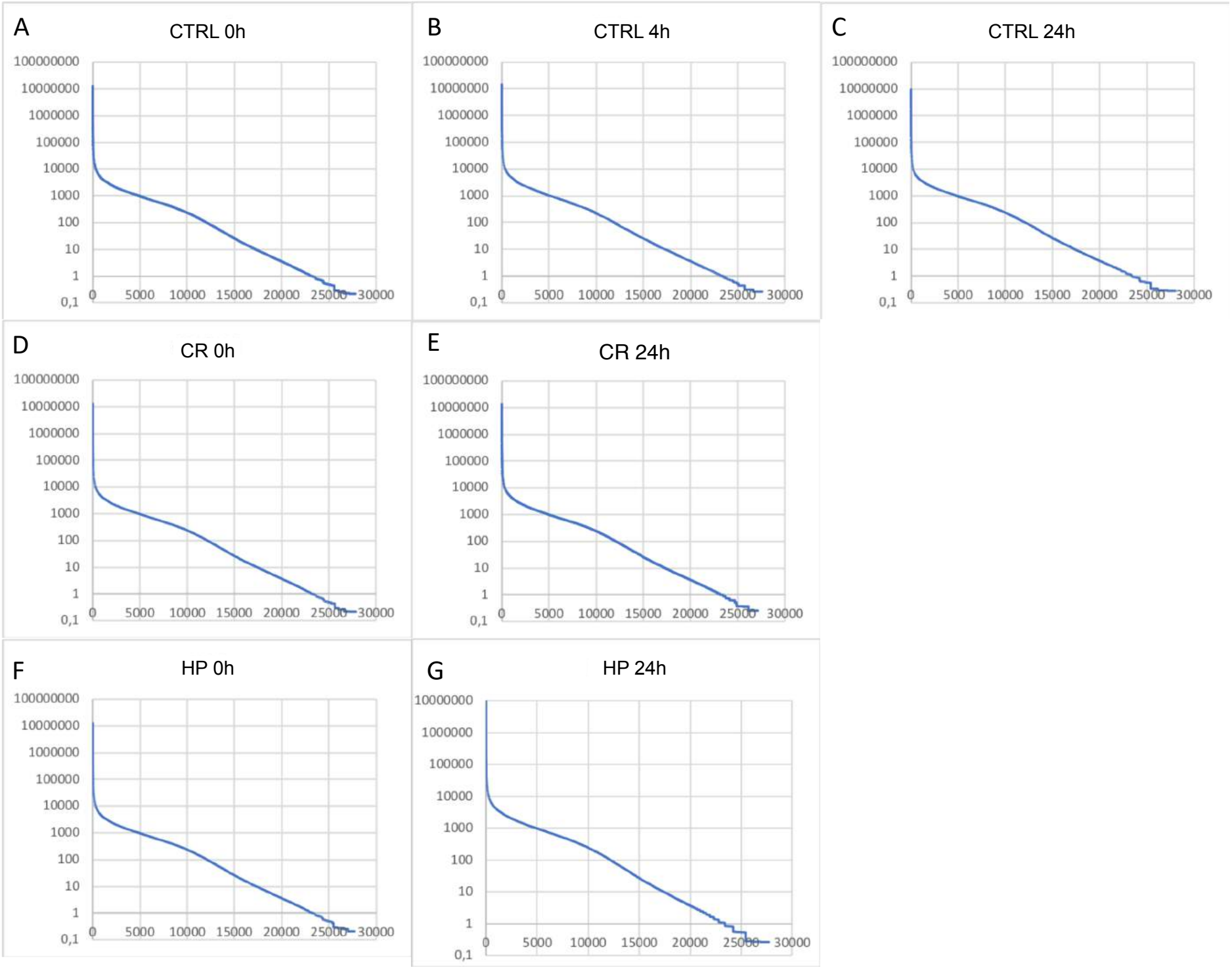
2. Dobin A, Davis CA, Schlesinger F, Drenkow J, Zaleski C, Jha S, Batut P, Chaisson M, Gingeras TR: STAR: ultrafast universal RNA-seq aligner. *Bioinformatics* 29: 15–21, 2013

3. Liao Y, Smyth GK, Shi W: featureCounts: an efficient general purpose program for assigning sequence reads to genomic features. *Bioinformatics* 30: 923–930, 2014

4. Friedländer MR, Mackowiak SD, Li N, Chen W, Rajewsky N: miRDeep2 accurately identifies known and hundreds of novel microRNA genes in seven animal clades. *Nucleic Acids Research* 40: 37–52, 2012

5. Langmead B, Trapnell C, Pop M, Salzberg SL: Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. *Genome Biology* 10: R25, 2009

6. Bonnet E, Wuyts J, Rouzé P, Van de Peer Y: Evidence that microRNA precursors, unlike other non-coding RNAs, have lower folding free energies than random sequences. *Bioinformatics* 20: 2911–2917, 2004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B
CTRL 4h
C
CTRL 24h
D
CR 0h
E
CR 24h
F
HP 0h
G
HP 24h
100000000
10000000
1000000
100000
10000
1000
100
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0
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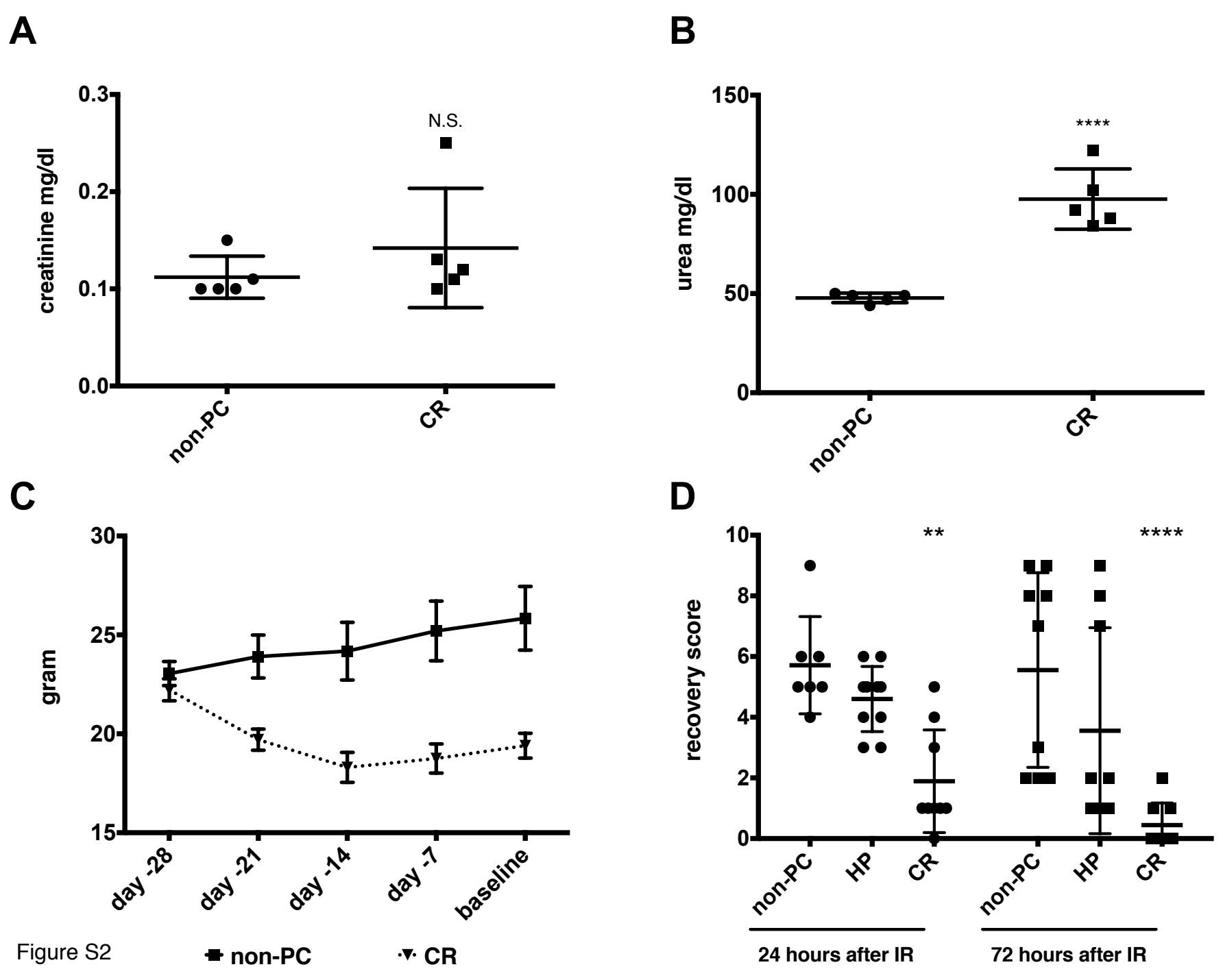
10000
15000
20000
25000
30000

Figure S1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N.S.
non-PC
CR
B
urea mg/dl
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100
50
0
****
non-PC
CR
C
gram
30
25
20
15
day -28
day -21
day -14
day -7
baseline
non-PC
CR
D
recovery score
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6
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**
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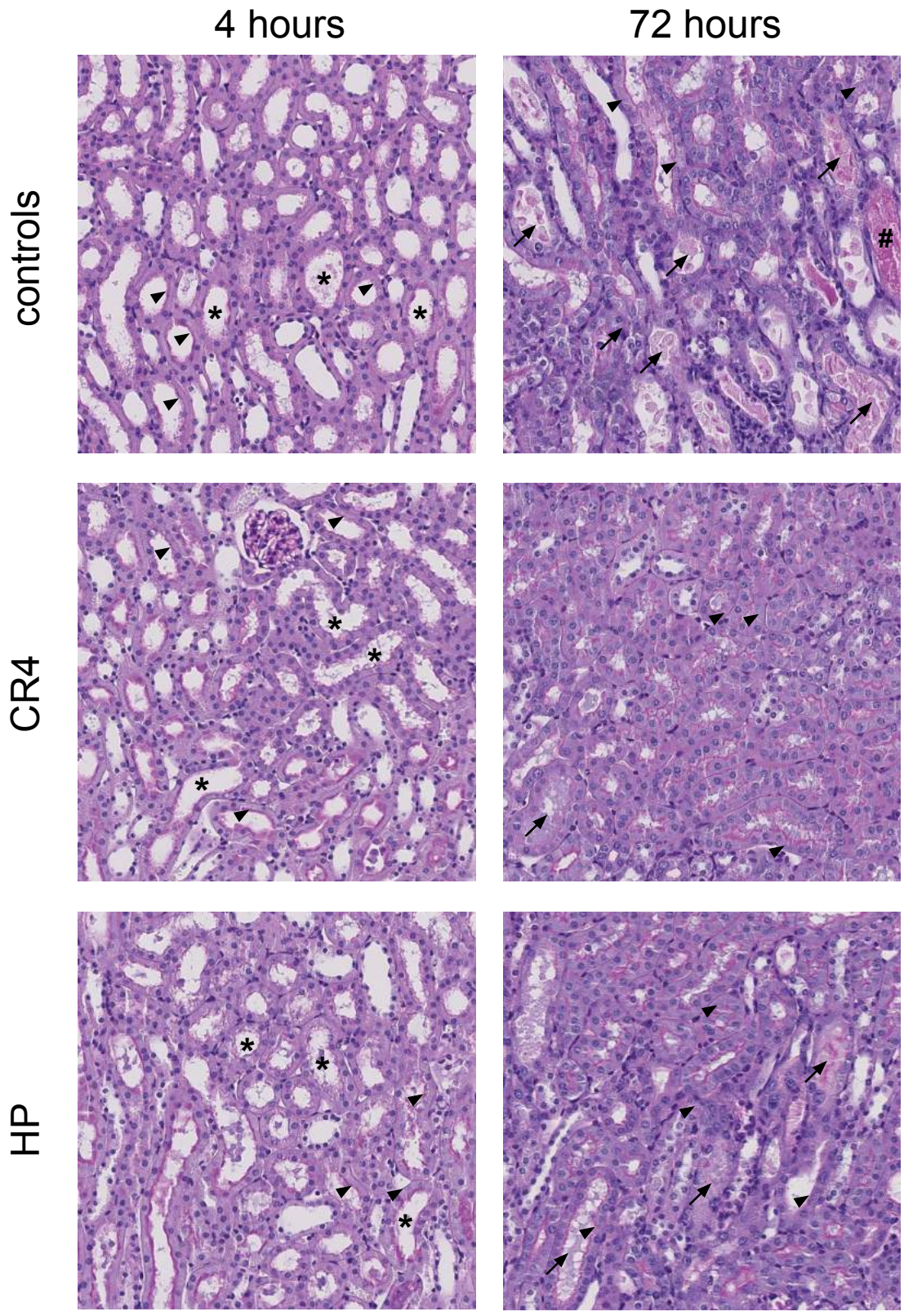
HP
CR
non-PC
HP
CR
24 hours after IR
72 hours after IR

Figure S2



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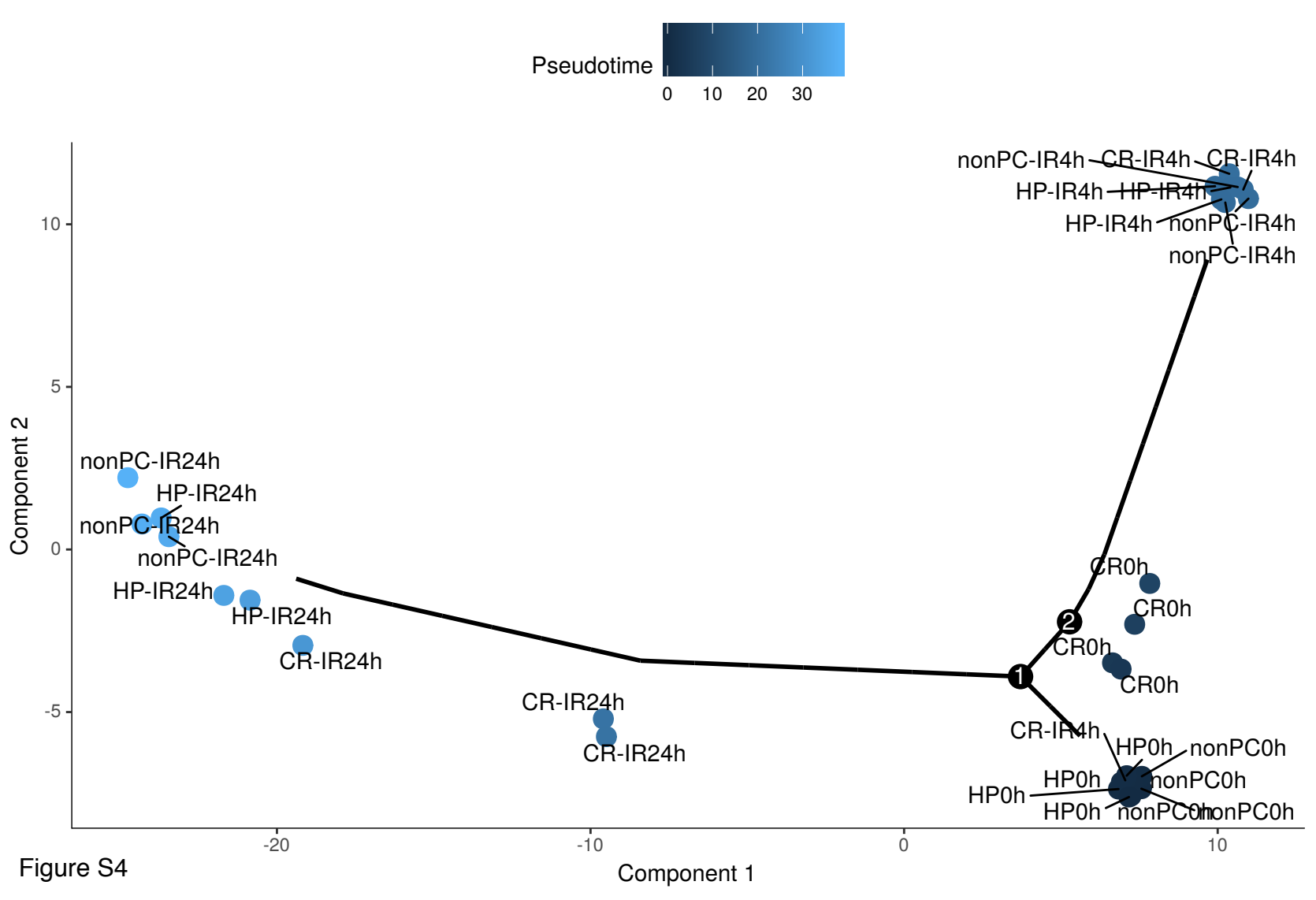
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20
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HP-IR4h
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nonPC0h
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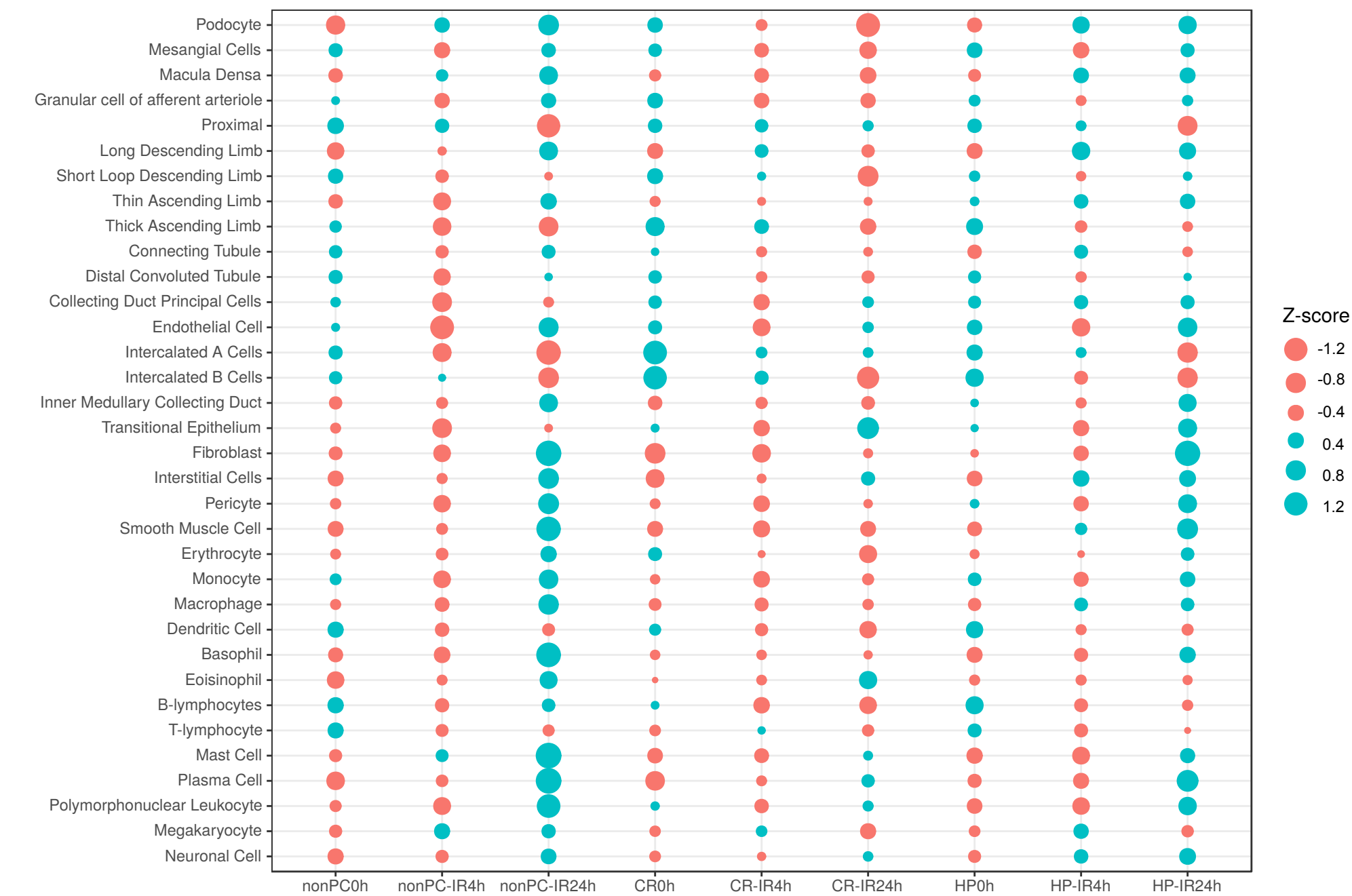
Component 2
Component 1

Figure S4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Macula Densa
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Proximal
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Short Loop Descending Limb
Thin Ascending Limb
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Connecting Tubule
Distal Convoluted Tubule
Collecting Duct Principal Cells
Endothelial Cell
Intercalated A Cells
Intercalated B Cells
Inner Medullary Collecting Duct
Transitional Epithelium
Fibroblast
Interstitial Cells
Pericyte
Smooth Muscle Cell
Erythrocyte
Monocyte
Macrophage
Dendritic Cell
Basophil
Eoisinophil
B-lymphocytes
T-lymphocyte
Mast Cell
Plasma Cell
Polymorphonuclear Leukocyte
Megakaryocyte
Neuronal Cell
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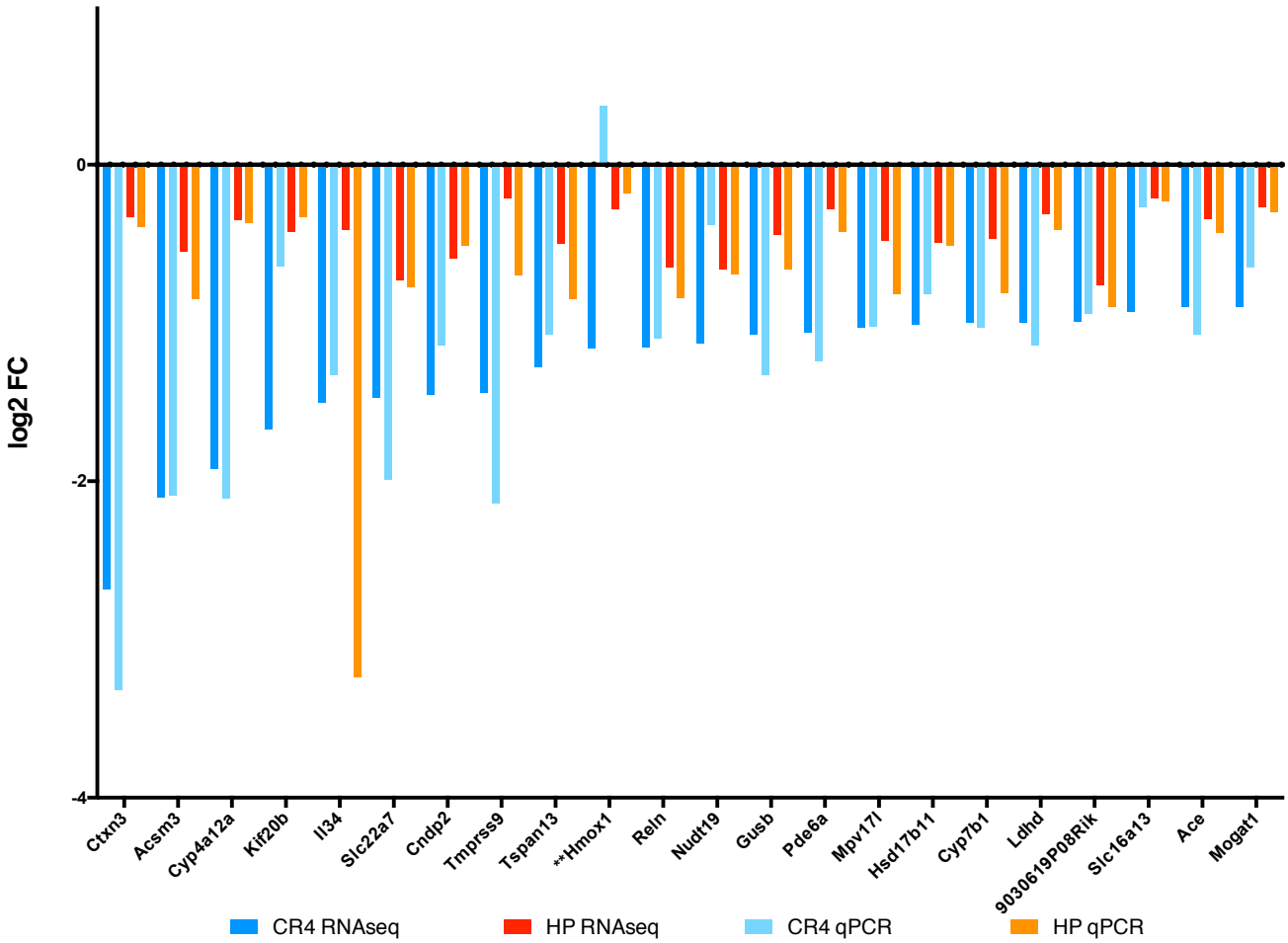
Figure S5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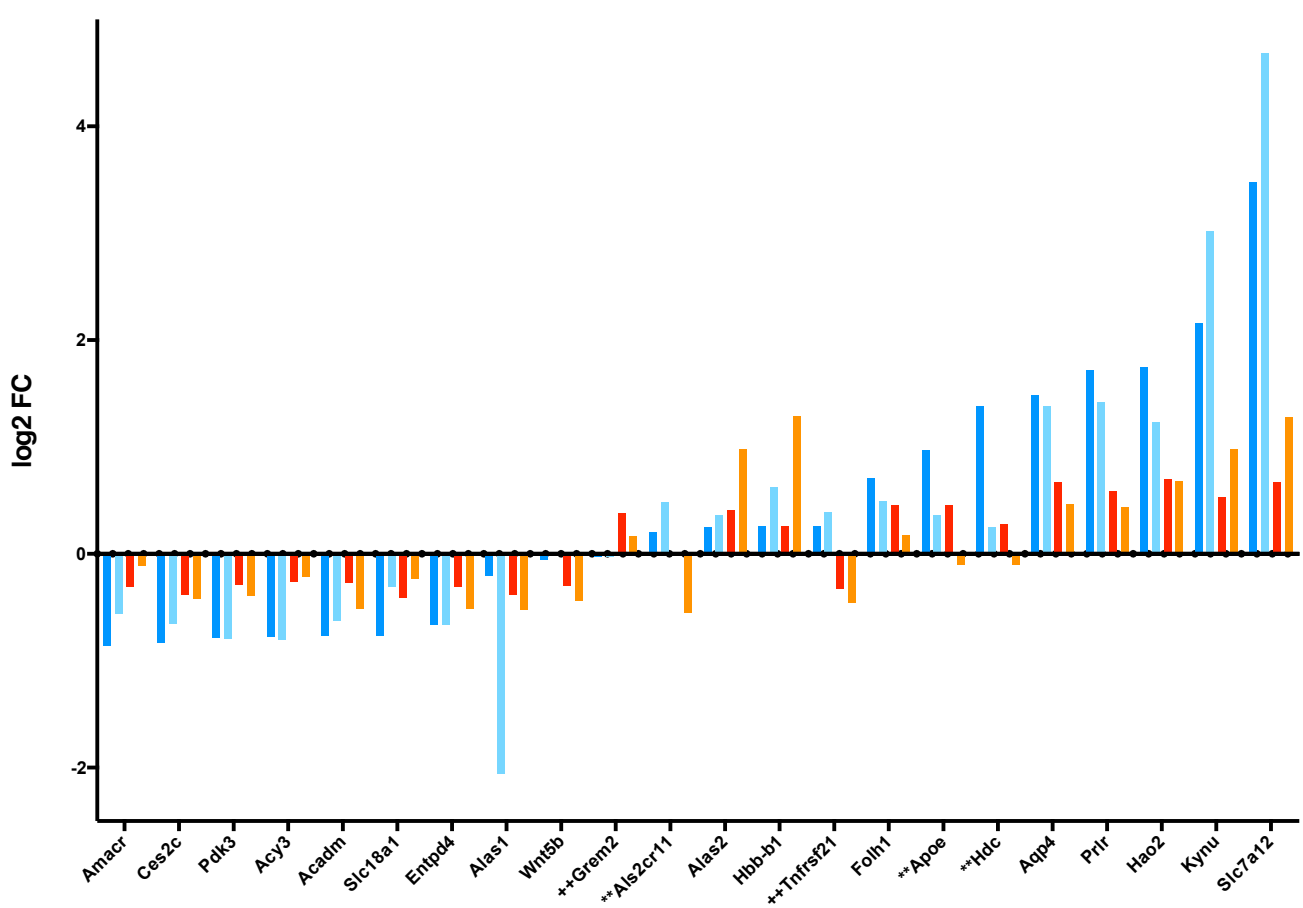
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Alas2
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++Tnfrsf21
Folh1
**Apoe
**Hdc
Aqp4
Prlr
Hao2
Kynu
Slc7a12
CR 0h RNAseq
HP 0h RNAseq
CR qPCR
HP 0h qPCR

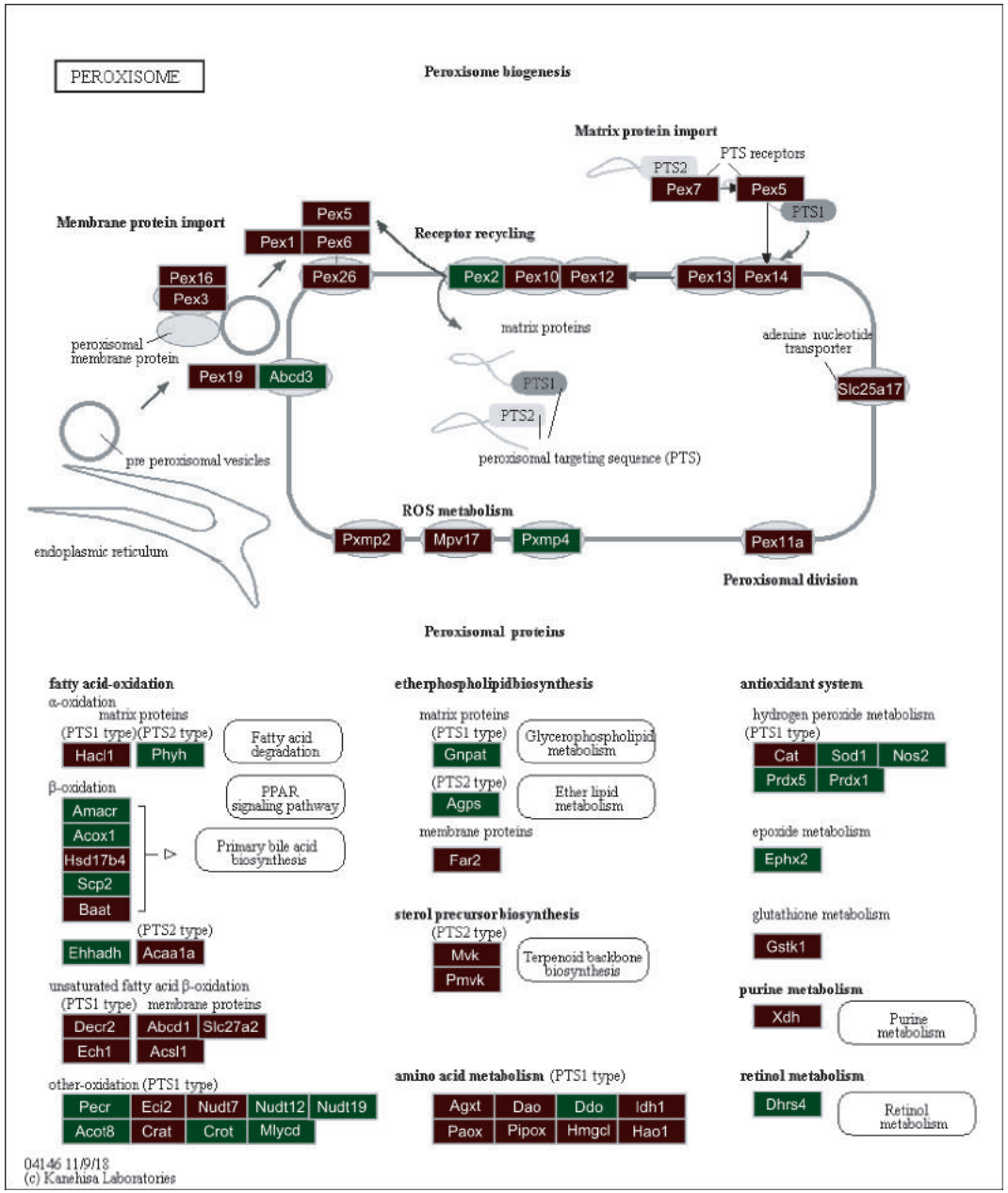
Figure S6

Metabolic Pathways	Acadm, Acat1, Acox1, Acsm3, Agps, Aldh3a2,Amacr, Anpep, Bdh1, Bhmt, Chpt1, Cndp2, Cox15, Cox5a, Cyp2c23,Cyp2e1,Degs2, Dhrs4, Folh1, Galns, Galnt1, Gclc, Gda, Ggt1, Glb1, Gusb, Hao2, Hsd11b1, Kynu, Me1, Ndufa1, Ndufb8, Odc1, Pank3, Papss1, Ppt2, Tpk1, Treh
Glutathione Metabolism	Abcd3, Acox1, Agps, Amacr, Ddo, Dhrs4, Hao2, Mpv17l Nudt19, Phyh, Sod1, Sod2
Peroxisome	Anpep, Gclc, Ggt1, Gpx3, Gsta1, Gsta2, Mgst1, Odc1

Figure S7

A Caloric restriction

B Hypoxic preconditioning



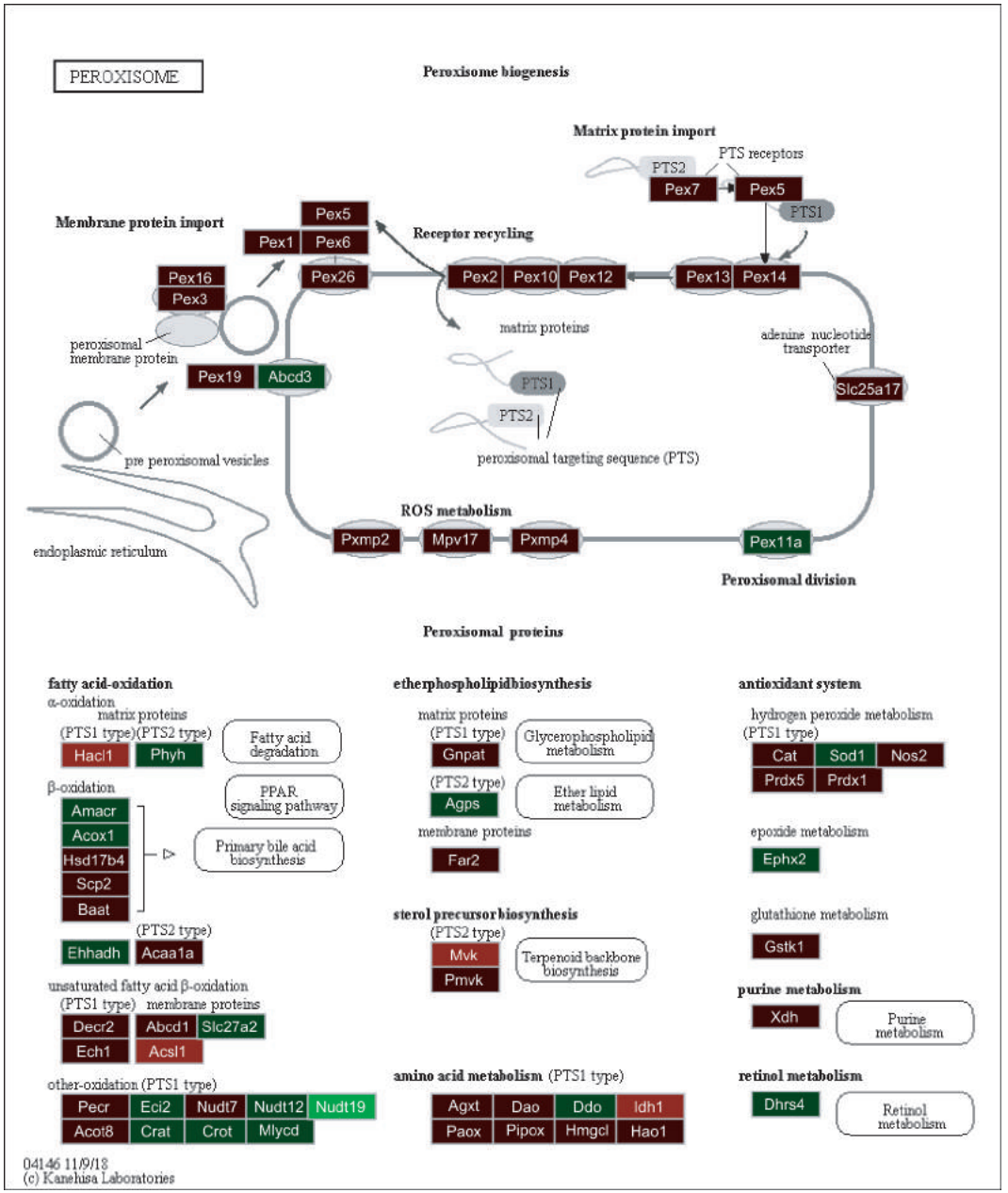
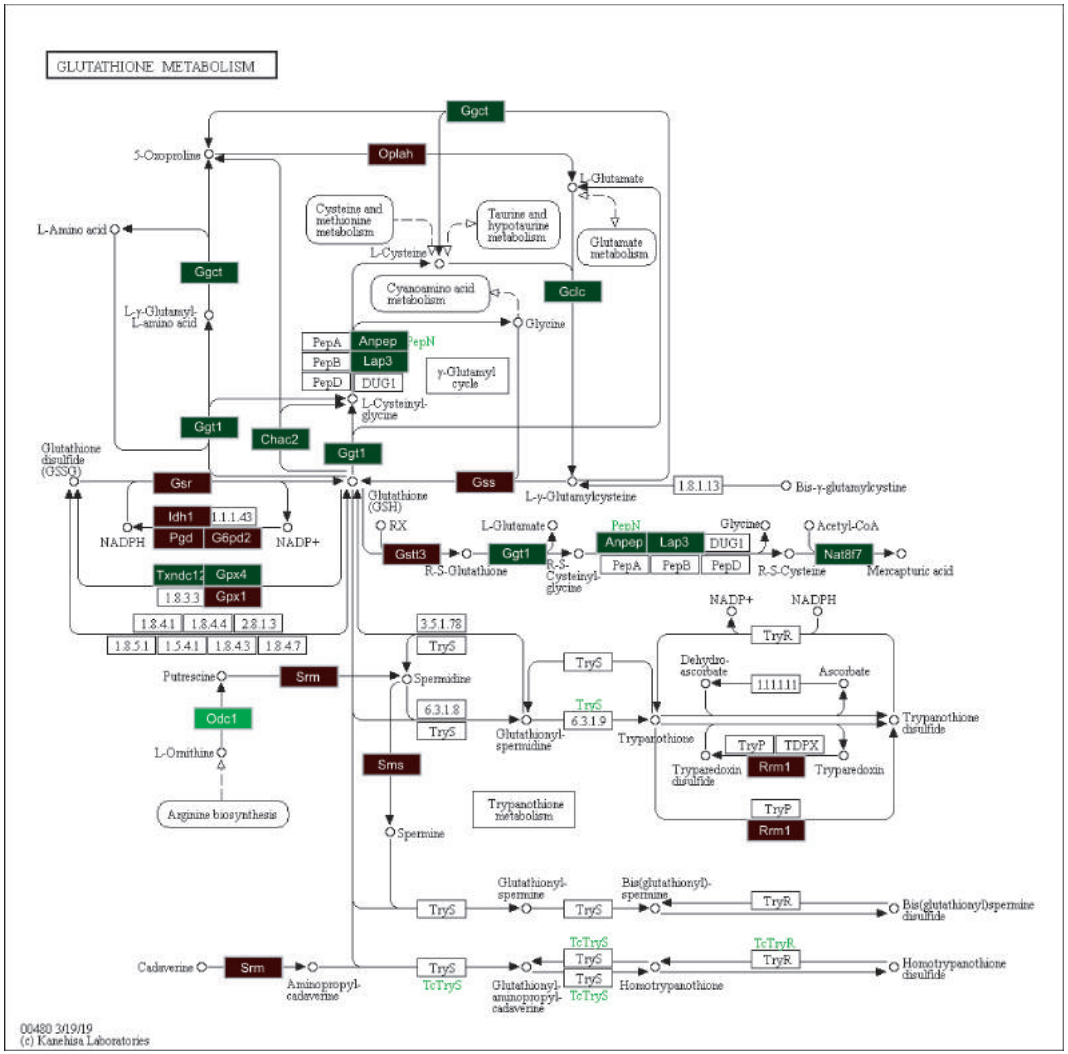


Figure S8

A Caloric restriction



B Hypoxic preconditioning

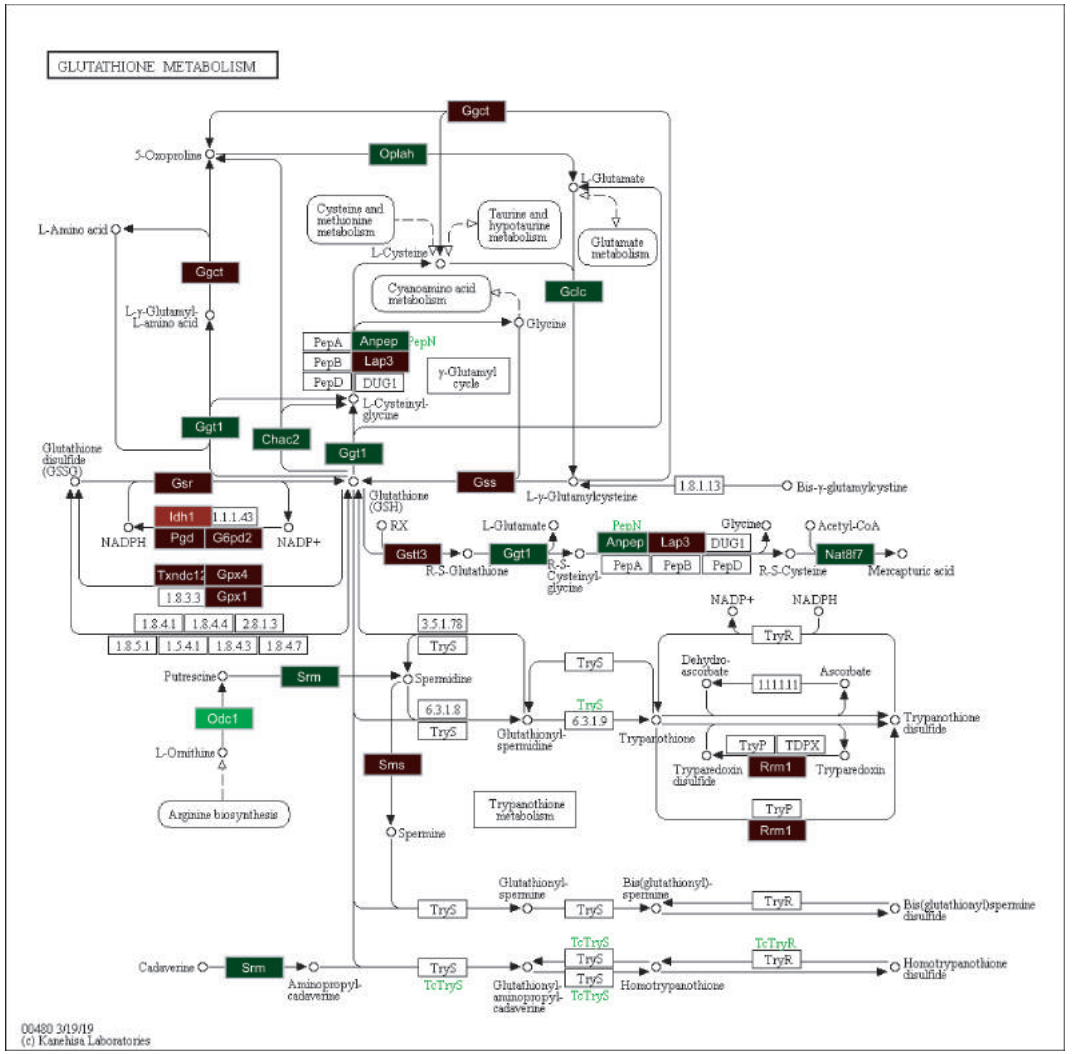
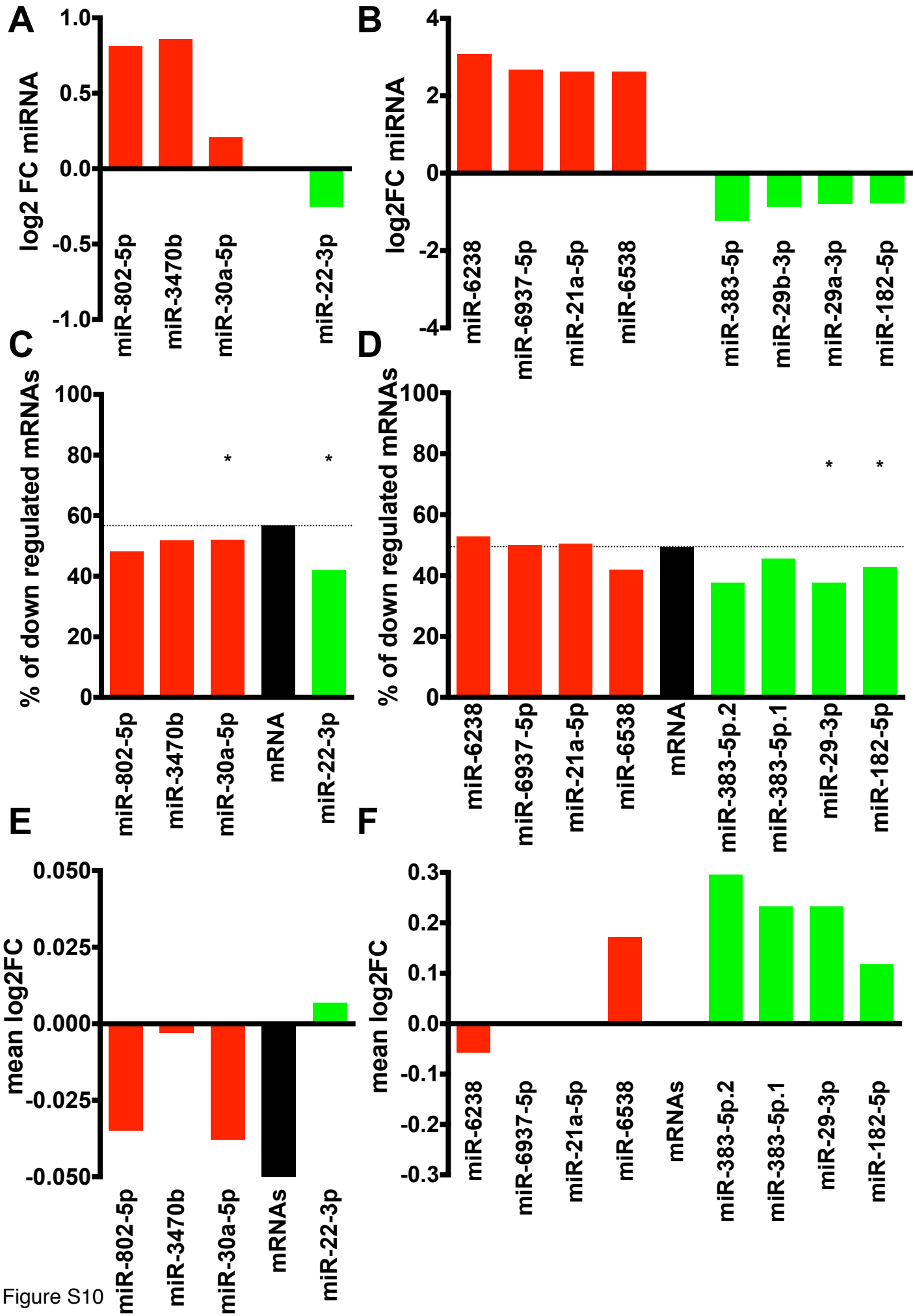


Figure S9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log2FC miRNA
4
2
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miR-6538
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miR-29b-3p
miR-29a-3p
miR-182-5p
C
% of down regulated mRNAs
100
80
60
40
20
0
*
*
miR-802-5p
miR-3470b
miR-30a-5p
mRNA
miR-22-3p
D
% of down regulated mRNAs
100
80
60
40
20
0
*
*
miR-6238
miR-6937-5p
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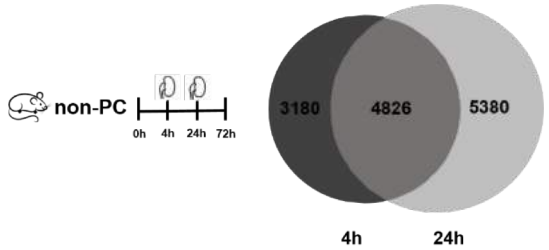
miR-29-3p
miR-182-5p
E
mean log2FC
0.050
0.025
0.000
-0.025
-0.050
miR-802-5p
miR-3470b
miR-30a-5p
mRNAs
miR-22-3p
F
mean log2FC
0.3
0.2
0.1
0.0
-0.1
-0.2
-0.3
miR-6238
miR-6937-5p
miR-21a-5p
miR-6538
mRNAs
miR-383-5p.2
miR-383-5p.1
miR-29-3p
miR-182-5p

Figure S10

A



B

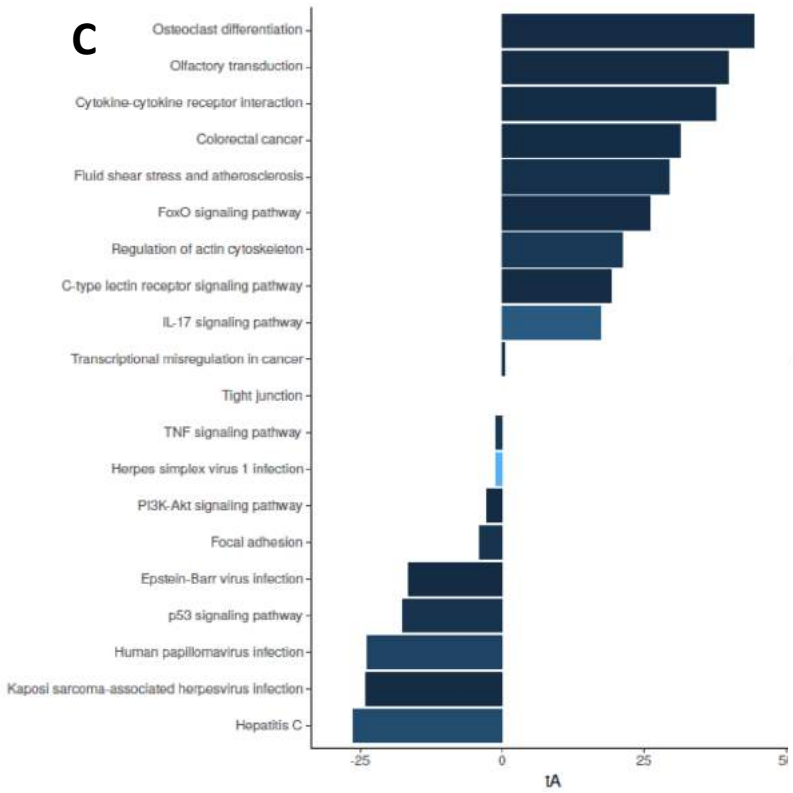
Top 10 upregulated genes 4h after IRI in non-PC animals	
Gene name	Log2FC
Krt20	6,7521275
Atf3	6,57529829
Fosb	6,28389646
Apol8	5,93870774
Dusp5	5,42681188
Cdkn1a	5,36027551
Nr4a3	5,34508134
Maff	5,04920449
Cxcl2	5,00063763
Olfr1443	4,93570337

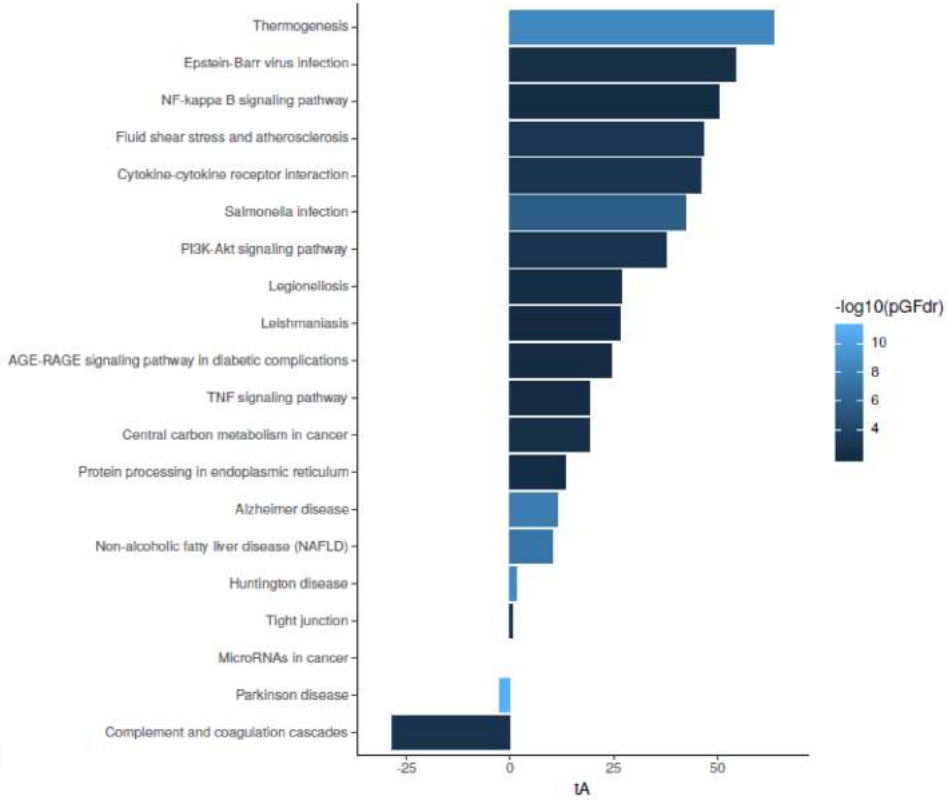
Top 10 upregulated genes 24h after IRI in non-PC animals	
Gene name	Log2FC
Krt20	11.10476278
Havcr1	11.06122650
Lcn2	9.306682026
Sprr2g	7.991778284
Fgb	7.851349821
Sprr2f	7.839730746
Flnc	7.483171049
Timp1	7.436120335
Fgg	7.070138272
Lgi2	6.915045336

Top 10 downregulated genes 4h after IRI in non-PC animals	
Gene name	Log2FC
Gm15354	- 4,31092457
Aplnr	-3,81676949
Hcar1	-3,7924212
Gm24515	-3,63146977
D230017M19Rik	-3,61997396
Kcnh4	-3,55770823
Sox18	-3,38999549
Tnn	-3,37535084
Alx1	-3,32597337
Cck	-3,26778997

Top 10 downregulated genes 24h after IRI in non-PC animals	
Gene name	Log2FC
Vmn1r19	-7.307865845
Vmn1r18	-6.931475388
Vmn1r20	-6.843851905
4930533I22Rik	-6.309045508
Slitrk6	-6.041828417
Tmem207	-5.994182757
Vmn1r-ps11	-5.84681139
Akr1c14	-5.585071372
Tnn	-5.512866539
Prok1	-5.486649894

C





D

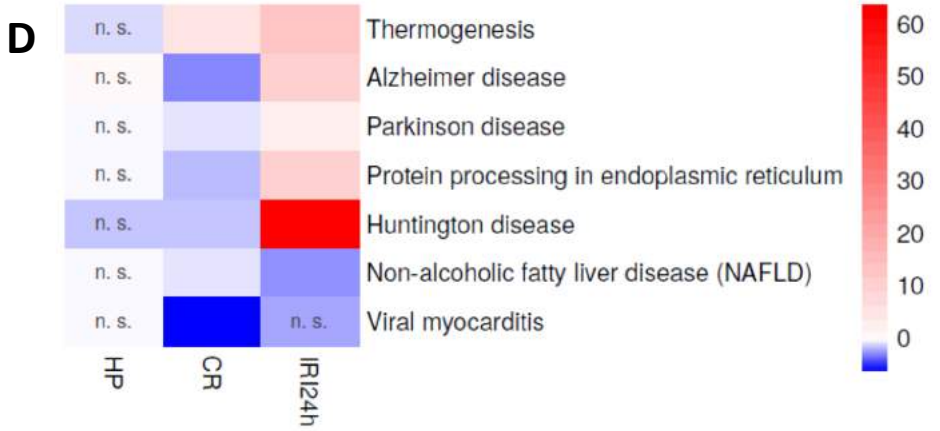


Figure S11

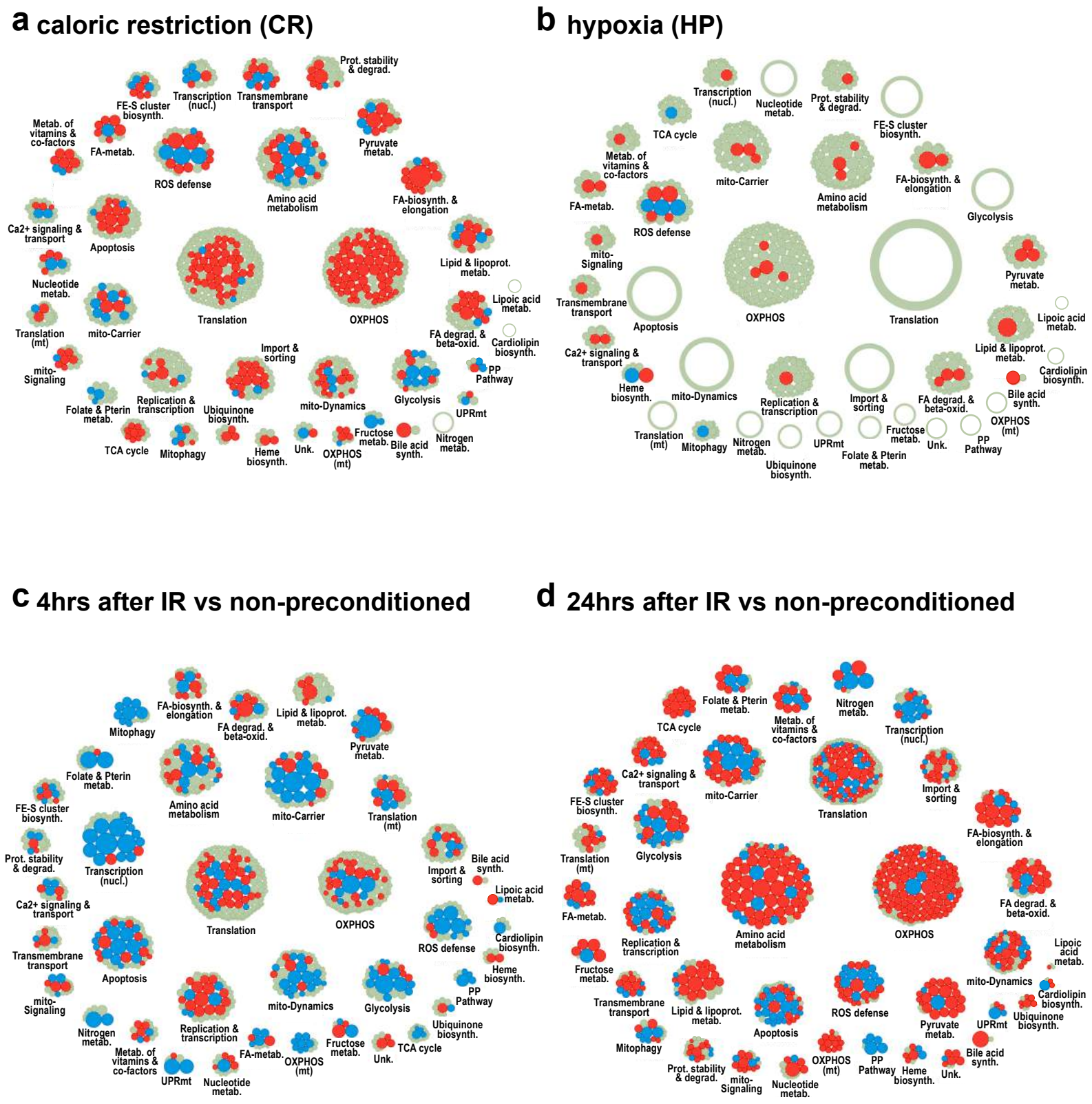

a caloric restriction (CR)
Prot. stability & degrad.
Transcription (nucl.)
Transmembrane transport
FE-S cluster biosynth.
Metab. of vitamins & co-factors
FA-metab.
Pyruvate metab.
ROS defense
Amino acid metabolism
FA-biosynth. & elongation
Ca2+ signaling & transport
Apoptosis
Lipid & lipoprot. metab.
Nucleotide metab.
Translation
OXPHOS
Lipoic acid metab.
Translation (mt)
mito-Carrier
FA degrad. & beta-oxid.
Cardiolipin biosynth.
Import & sorting
mito-Signaling
PP Pathway
Replication & transcription
Ubiquinone biosynth.
mito-Dynamics
Glycolysis
Folate & Pterin metab.
UPRmt
Fructose metab.
Bile acid synth.
Nitrogen metab.
TCA cycle
Mitophagy
Heme biosynth.
Unk.
OXPHOS (mt)
b hypoxia (HP)
Transcription (nucl.)
Nucleotide metab.
Prot. stability & degrad.
FE-S cluster biosynth.
TCA cycle
Metab. of vitamins & co-factors
mito-Carrier
FA-biosynth. & elongation
FA-metab.
Amino acid metabolism
Glycolysis
ROS defense
mito-Signaling
Pyruvate metab.
Transmembrane transport
OXPHOS
Translation
Lipoic acid metab.
Apoptosis
Lipid & lipoprot. metab.
Ca2+ signaling & transport
Cardiolipin biosynth.
Heme biosynth.
mito-Dynamics
Replication & transcription
Import & sorting
FA degrad. & beta-oxid.
Bile acid synth.
OXPHOS (mt)
Translation (mt)
Mitophagy
Nitrogen metab.
UPRmt
Fructose metab.
Unk.
PP Pathway
Folate & Pterin metab.
Ubiquinone biosynth.
c 4hrs after IR vs non-preconditioned
FA-biosynth. & elongation
Lipid & lipoprot. metab.
Mitophagy
FA degrad. & beta-oxid.
Pyruvate metab.
Folate & Pterin metab.
Amino acid metabolism
mito-Carrier
Translation (mt)
FE-S cluster biosynth.
Prot. stability & degrad.
Transcription (nucl.)
Bile acid synth.
Import & sorting
Lipoic acid metab.
Ca2+ signaling & transport
Translation
OXPHOS
ROS defense
Cardiolipin biosynth.
Transmembrane transport
Apoptosis
Heme biosynth.
mito-Signaling
mito-Dynamics
Glycolysis
PP Pathway
Replication & transcription
Ubiquinone biosynth.
Nitrogen metab.
Fructose metab.
TCA cycle
Metab. of vitamins & co-factors
FA-metab.
OXPHOS (mt)
Unk.
UPRmt
Nucleotide metab.
d 24hrs after IR vs non-preconditioned
Folate & Pterin metab.
Nitrogen metab.
TCA cycle
Metab. of vitamins & co-factors
Transcription (nucl.)
Ca2+ signaling & transport
mito-Carrier
Import & sorting
FE-S cluster biosynth.
Translation
FA-biosynth. & elongation
Translation (mt)
Glycolysis
FA-metab.
FA degrad. & beta-oxid.
Amino acid metabolism
OXPHOS
Replication & transcription
Lipoic acid metab.
Fructose metab.
mito-Dynamics
Cardiolipin biosynth.
Transmembrane transport
Lipid & lipoprot. metab.
ROS defense
Ubiquinone biosynth.
Apoptosis
Pyruvate metab.
UPRmt
Mitophagy
Bile acid synth.
Prot. stability & degrad.
mito-Signaling
Nucleotide metab.
OXPHOS (mt)
PP Pathway
Heme biosynth.
Unk.

Figure S12

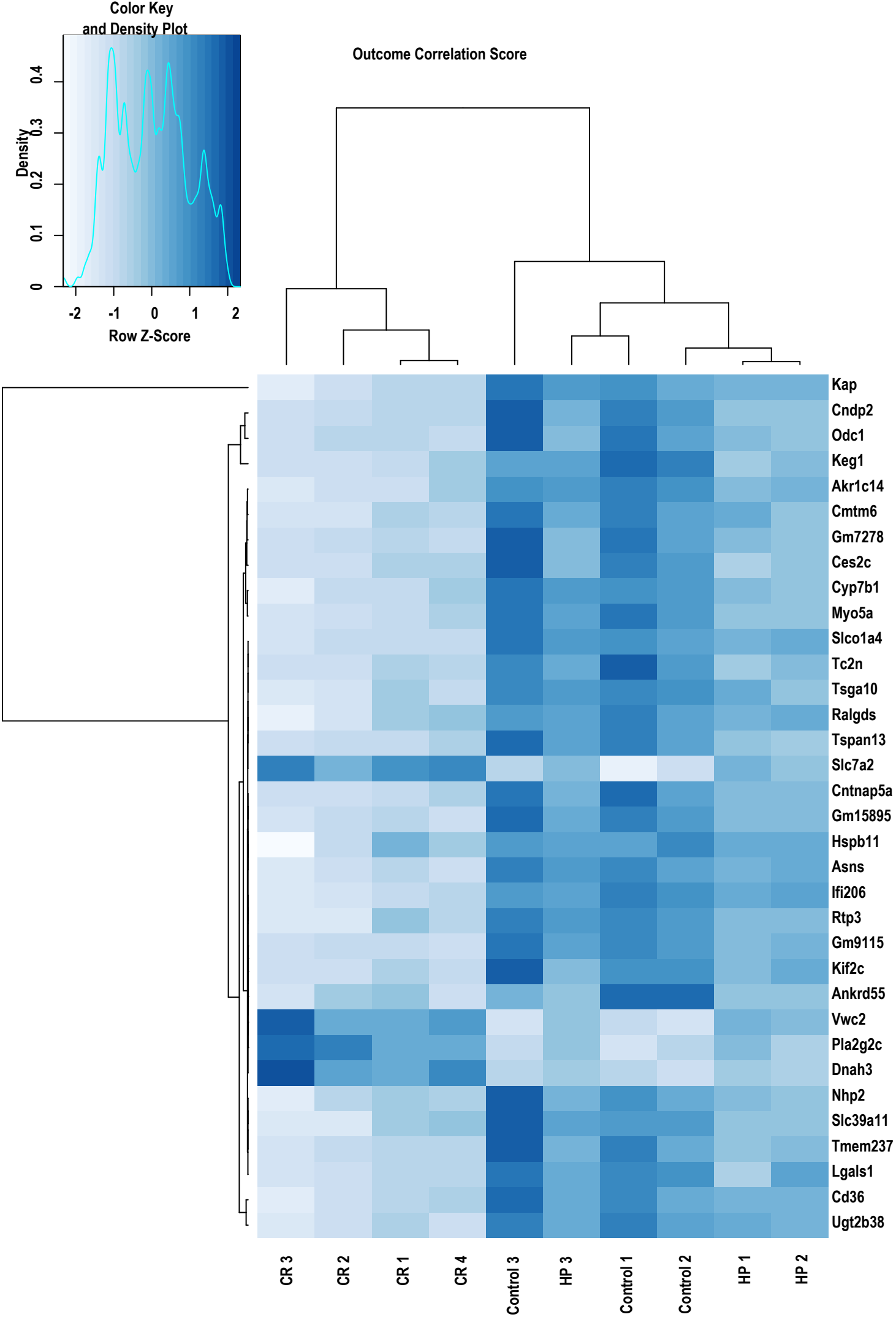

Color Key
and Density Plot
Density
0
0.1
0.2
0.3
0.4
-2
-1
0
1
2
Row Z-Score
Outcome Correlation Score
Kap
Cndp2
Odc1
Keg1
Akr1c14
Cmtm6
Gm7278
Ces2c
Cyp7b1
Myo5a
Slco1a4
Tc2n
Tsga10
Ralgds
Tspan13
Slc7a2
Cntnap5a
Gm15895
Hspb11
Asns
Ifi206
Rtp3
Gm9115
Kif2c
Ankrd55
Vwc2
Pla2g2c
Dnah3
Nhp2
Slc39a11
Tmem237
Lgals1
Cd36
Ugt2b38
CR 3
CR 2
CR 1
CR 4
Control 3
HP 3
Control 1
Control 2
HP 1
HP 2

Figure S13

	Intercept	p	B per 100 copies	β	p	adjusted R^2
Kap	-5,212	0,065	0,004	0,910	<0,001	0,807
Cndp2	-1,760	0,181	0,046	0,962	<0,001	0,917
Odc1	0,062	0,965	0,047	0,936	<0,001	0,861
Cd36	-6,084	0,048	0,114	0,907	<0,001	0,801
Akr1c14	-4,216	0,101	0,215	0,911	<0,001	0,808
mtm6	-7,809	0,024	0,296	0,911	<0,001	0,809
Gm7278	-0,212	0,881	0,168	0,939	<0,001	0,866
Ces2c	-7,646	0,021	0,229	0,919	<0,001	0,824
Cyp7b1	-4,094	0,029	0,288	0,956	<0,001	0,903
Myo5a	-2,599	0,050	0,329	0,970	<0,001	0,934
Nhp2	-8,466	0,031	2,354	0,894	<0,001	0,775
Slc39a11	-13,998	0,003	3,325	0,927	<0,001	0,842
Tmem237	-4,408	0,056	2,100	0,933	<0,001	0,854
Slco1a42	-4,959	0,068	3,461	0,913	<0,001	0,813
Tc2n	-3,106	0,155	3,079	0,920	<0,001	0,828
Tspan13	-3,189	0,034	2,596	0,967	<0,001	0,926

Figure S14

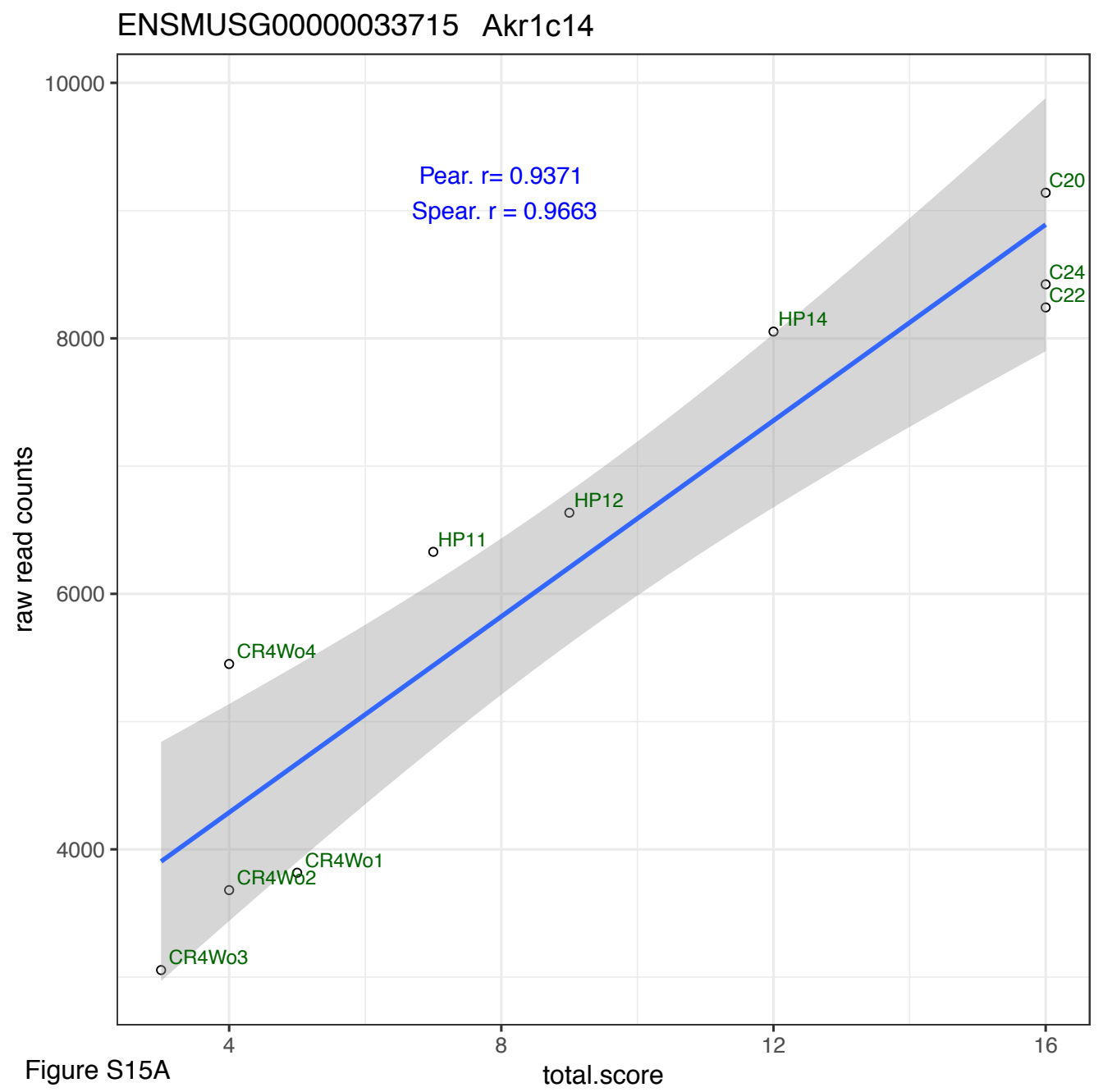

ENSMUSG00000033715 Akr1c14
Pear. r= 0.9371
Spear. r = 0.9663
C20
C24
C22
HP14
HP12
HP11
CR4Wo4
CR4Wo1
CR4Wo2
CR4Wo3
10000
8000
6000
4000
raw read counts
4
8
12
16
total.score

Figure S15A

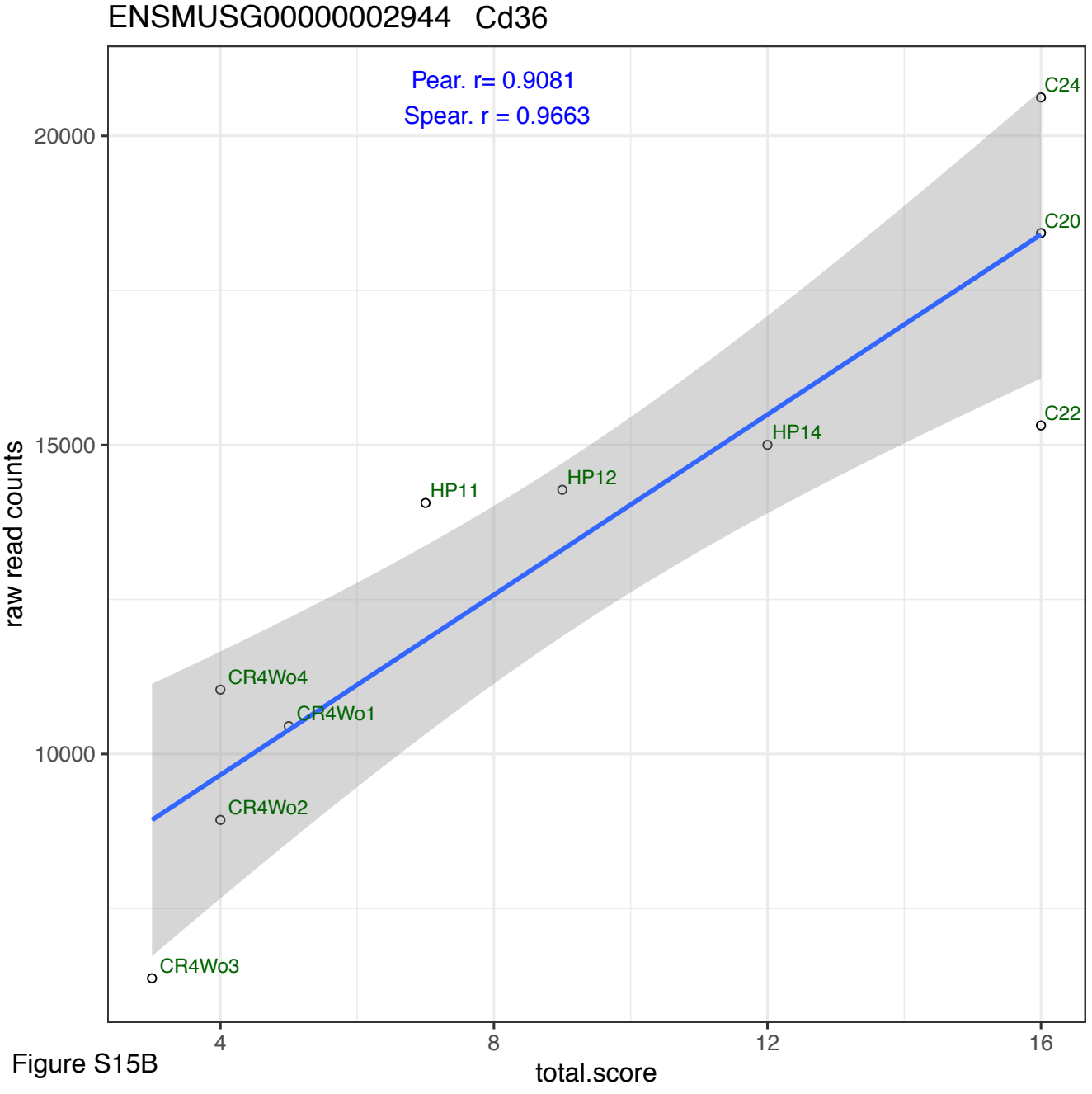

ENSMUSG00000002944 Cd36
Pear. r= 0.9081
Spear. r = 0.9663
C24
C20
C22
HP14
HP12
HP11
CR4Wo4
CR4Wo1
CR4Wo2
CR4Wo3
20000
15000
10000
raw read counts
4
8
12
16
total.score

Figure S15B

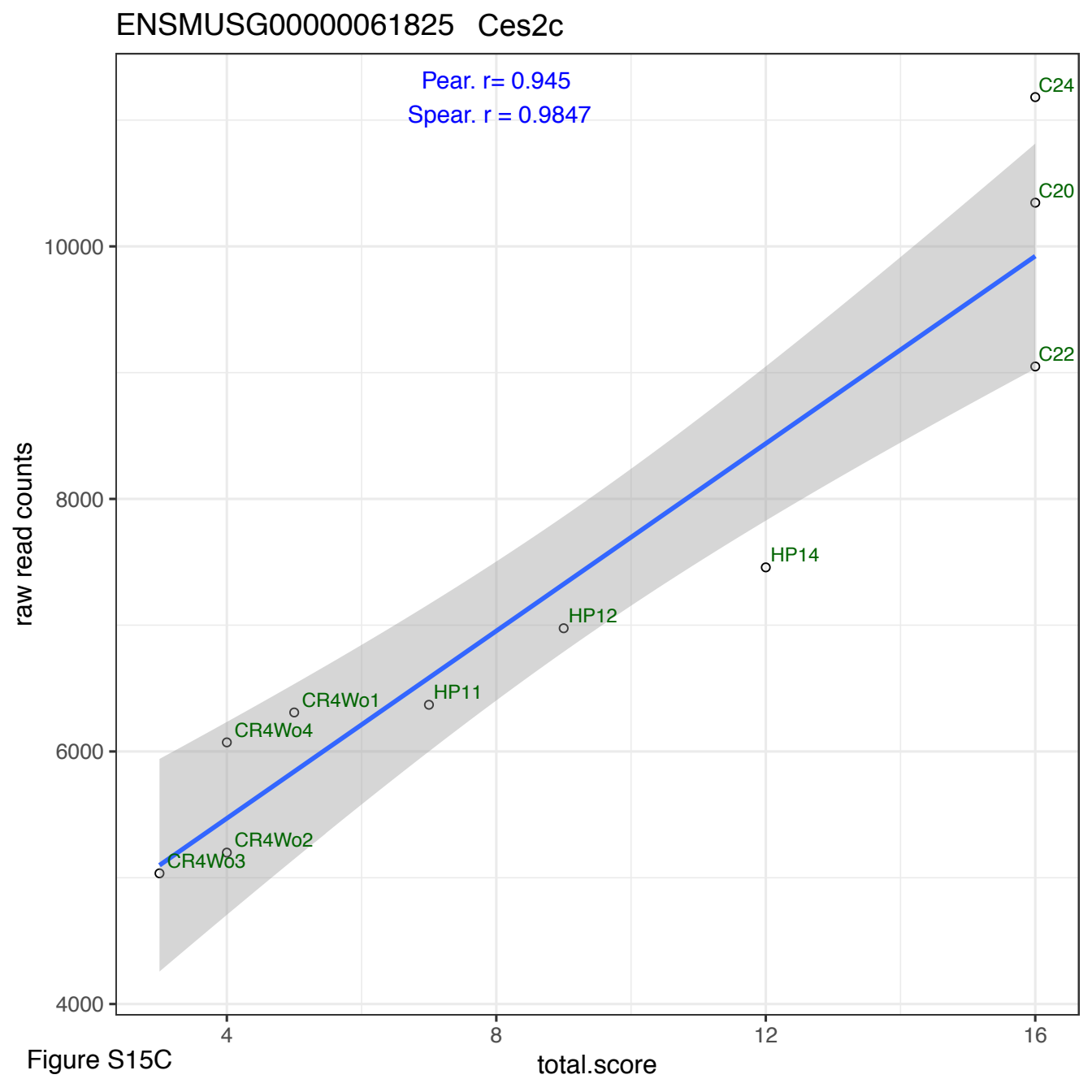

ENSMUSG00000061825 Ces2c
Pear. r= 0.945
Spear. r = 0.9847
C24
C20
C22
HP14
HP12
HP11
CR4Wo1
CR4Wo4
CR4Wo2
CR4Wo3
raw read counts
10000
8000
6000
4000
4
8
12
16
total.score

Figure S15C

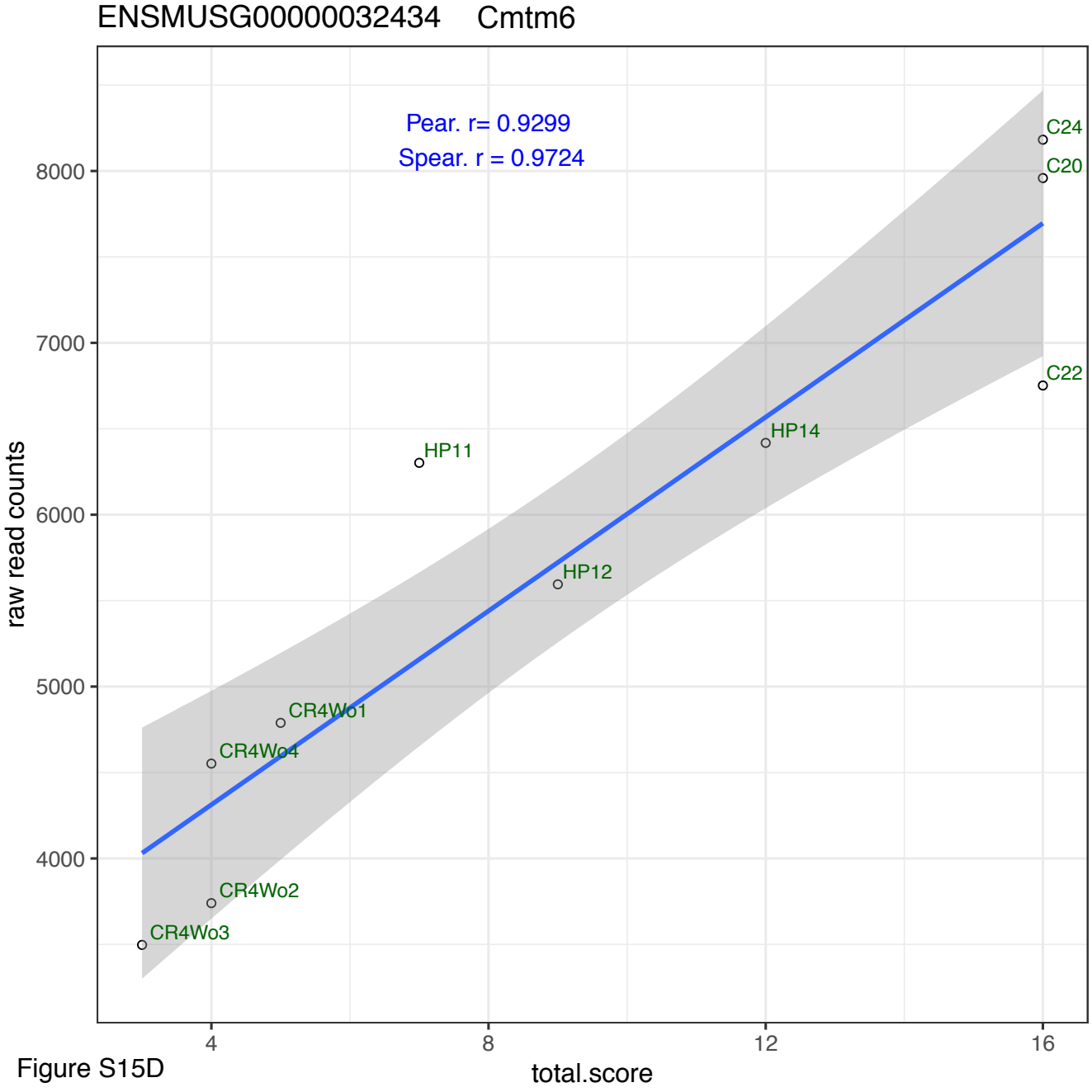

ENSMUSG00000032434 Cmtm6
Pear. r= 0.9299
Spear. r = 0.9724
C24
C20
C22
HP14
HP11
HP12
CR4Wo1
CR4Wo4
CR4Wo2
CR4Wo3
8000
7000
6000
5000
4000
raw read counts
4
8
12
16
total.score

Figure S15D

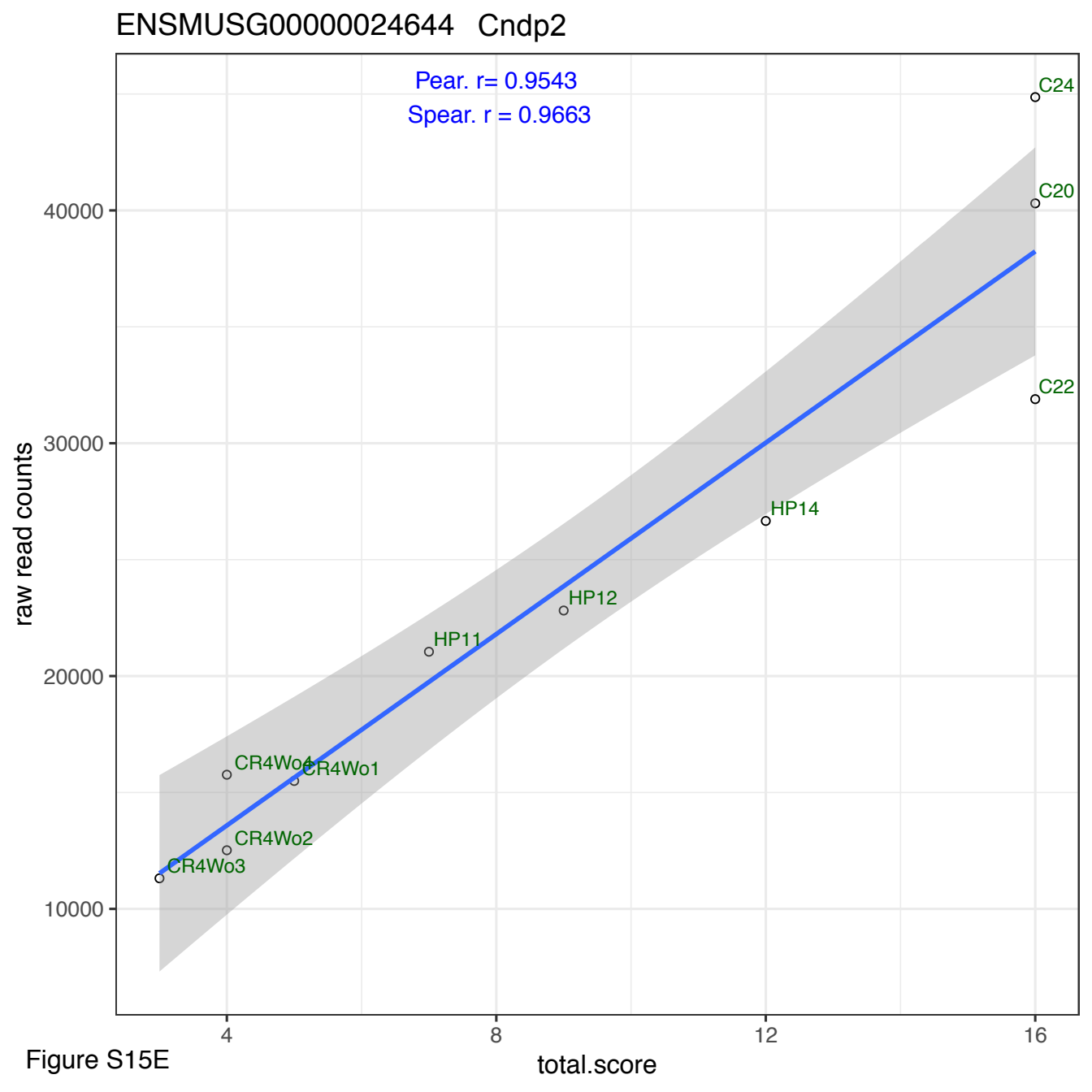

ENSMUSG00000024644 Cndp2
Pear. r= 0.9543
Spear. r = 0.9663
C24
C20
C22
HP14
HP12
HP11
CR4Wo4
CR4Wo1
CR4Wo2
CR4Wo3
raw read counts
40000
30000
20000
10000
4
8
12
16
total.score

Figure S15E

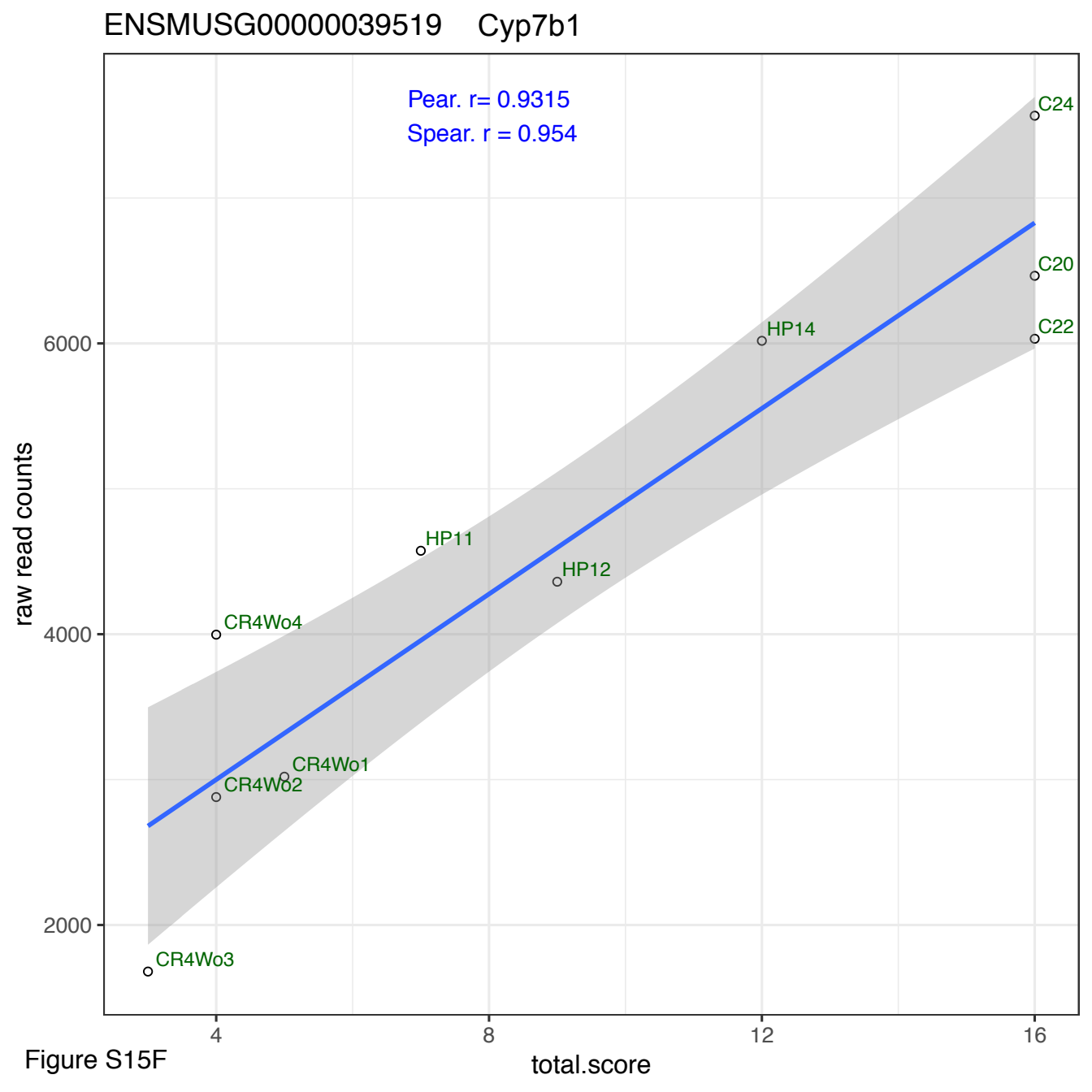

ENSMUSG00000039519 Cyp7b1
Pear. r= 0.9315
Spear. r = 0.954
C24
C20
C22
HP14
HP11
HP12
CR4Wo4
CR4Wo1
CR4Wo2
CR4Wo3
6000
4000
2000
raw read counts
4
8
12
16
total.score

Figure S15F

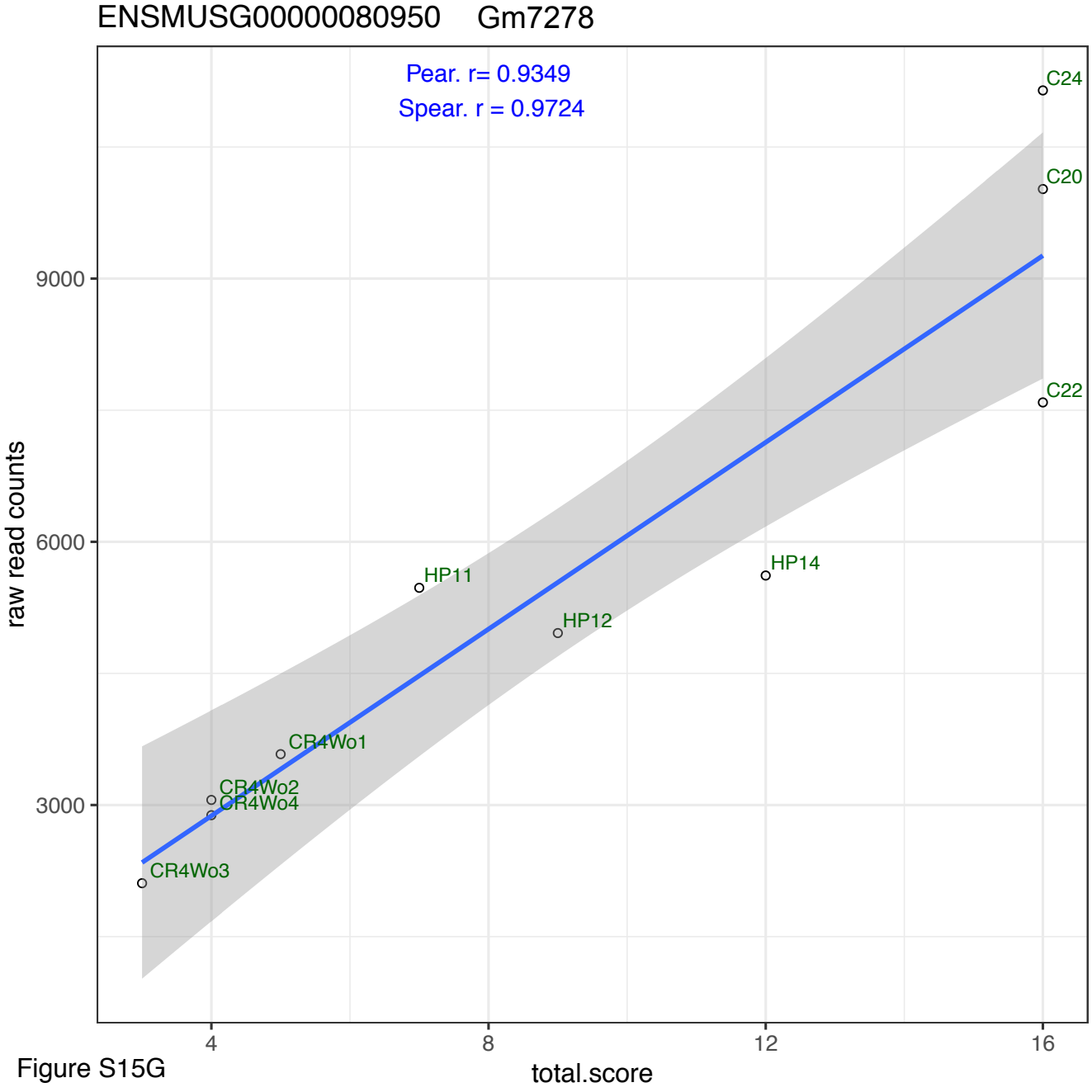

ENSMUSG00000080950 Gm7278
Pear. r= 0.9349
Spear. r = 0.9724
C24
C20
C22
HP11
HP14
HP12
CR4Wo1
CR4Wo2
CR4Wo4
CR4Wo3
9000
6000
3000
raw read counts
4
8
12
16
total.score

Figure S15G

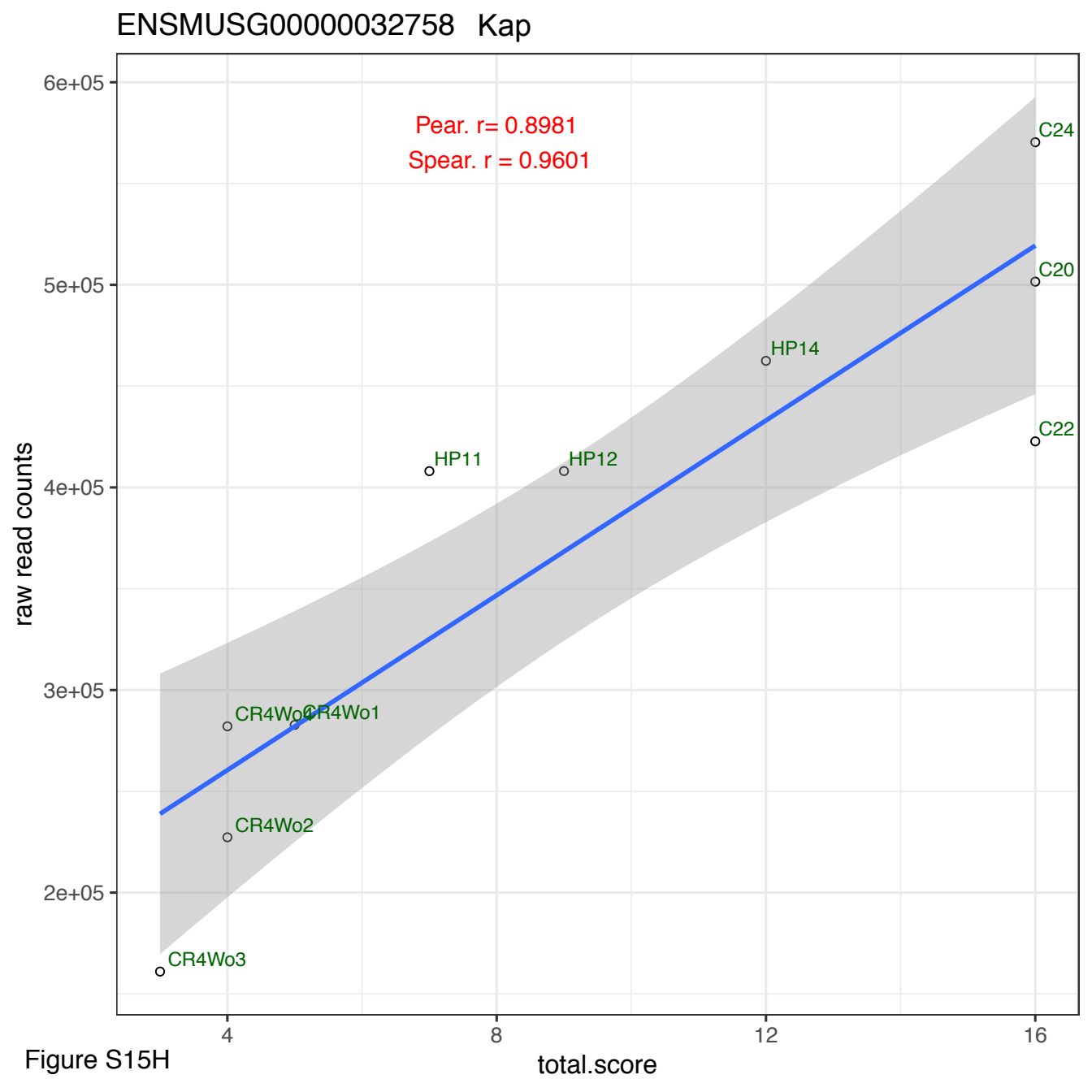

ENSMUSG00000032758 Kap
Pear. r= 0.8981
Spear. r = 0.9601
raw read counts
total.score
6e+05
5e+05
4e+05
3e+05
2e+05
4
8
12
16
C24
C20
C22
HP14
HP11
HP12
CR4Wo4
CR4Wo1
CR4Wo2
CR4Wo3

Figure S15H

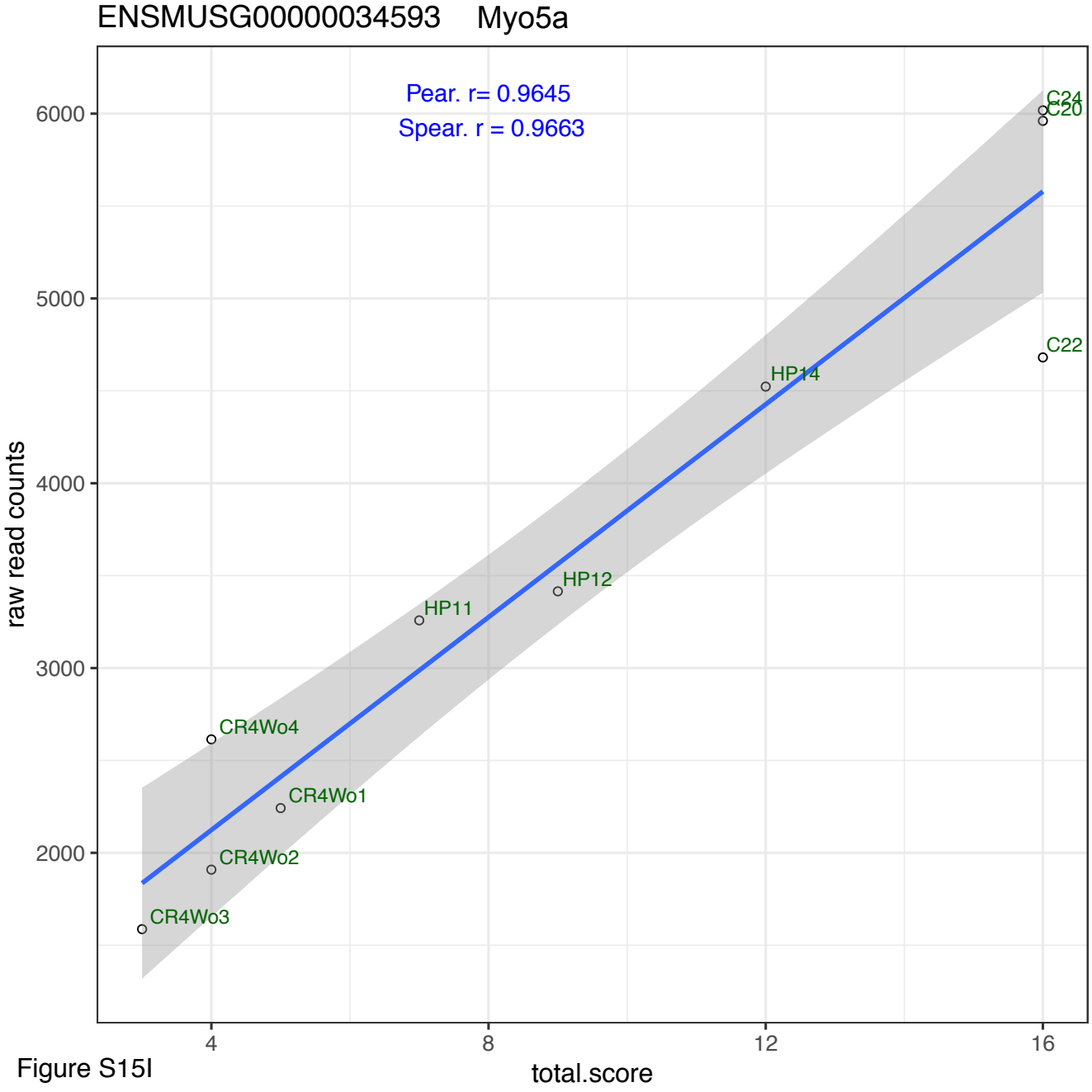

ENSMUSG00000034593 Myo5a
Pear. r= 0.9645
Spear. r = 0.9663
raw read counts
total.score
6000
5000
4000
3000
2000
4
8
12
16
C24
C20
C22
HP14
HP12
HP11
CR4Wo4
CR4Wo1
CR4Wo2
CR4Wo3

Figure S15I

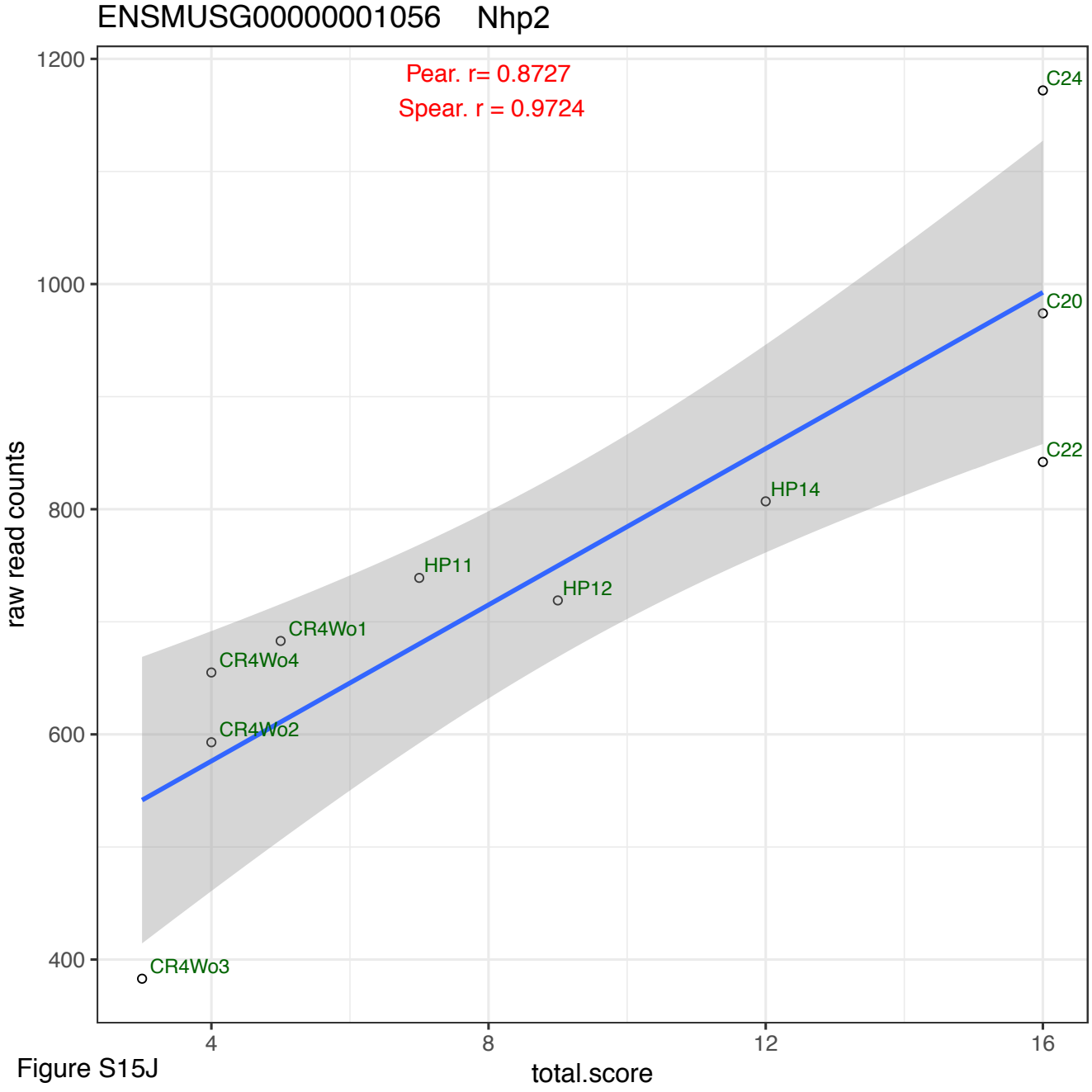

ENSMUSG00000001056 Nhp2
Pear. r= 0.8727
Spear. r = 0.9724
raw read counts
total.score
1200
1000
800
600
400
4
8
12
16
C24
C20
C22
HP14
HP11
HP12
CR4Wo1
CR4Wo4
CR4Wo2
CR4Wo3

Figure S15J

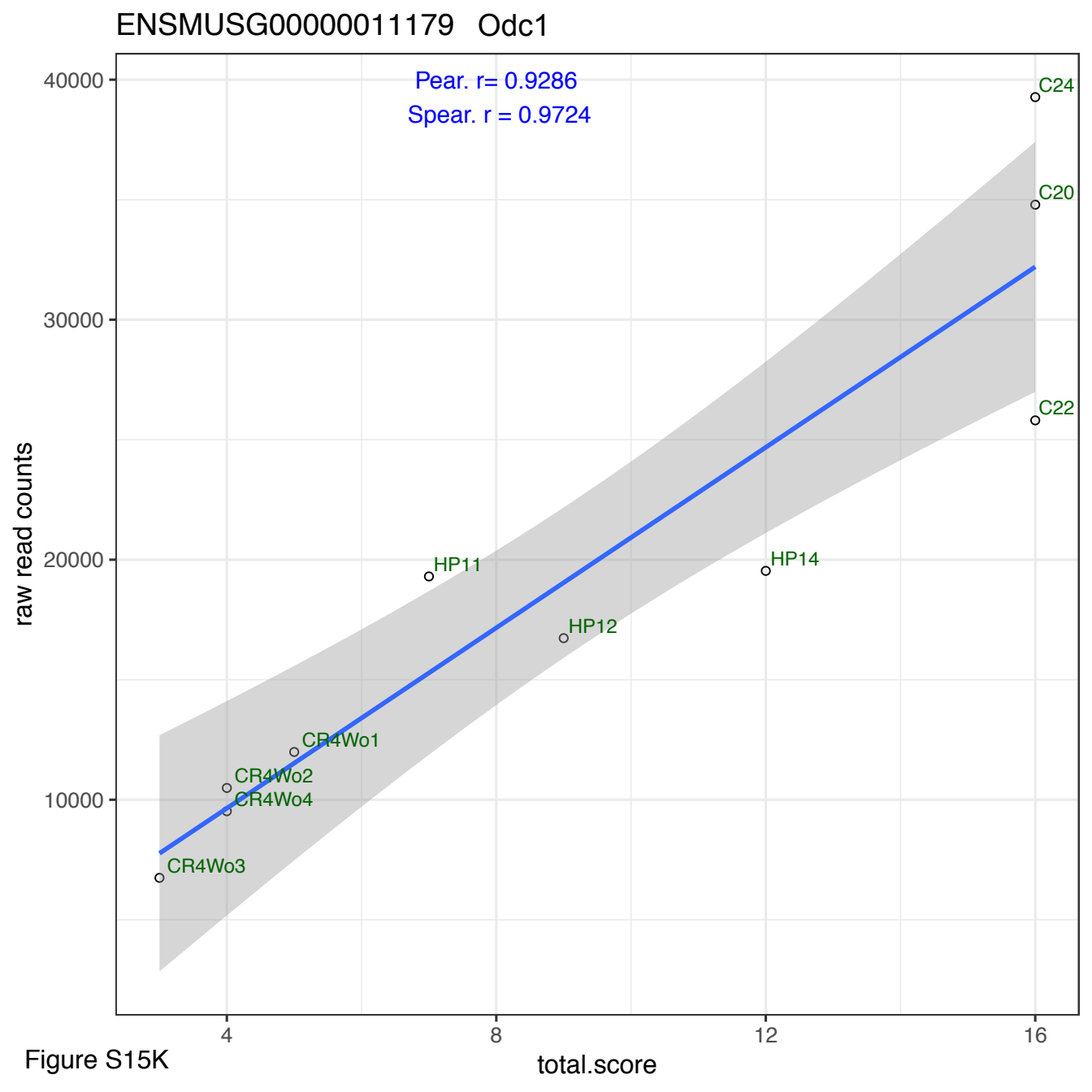

ENSMUSG00000011179 Odc1
Pear. r= 0.9286
Spear. r = 0.9724
raw read counts
total.score
40000
30000
20000
10000
4
8
12
16
C24
C20
C22
HP11
HP14
HP12
CR4Wo1
CR4Wo2
CR4Wo4
CR4Wo3

Figure S15K

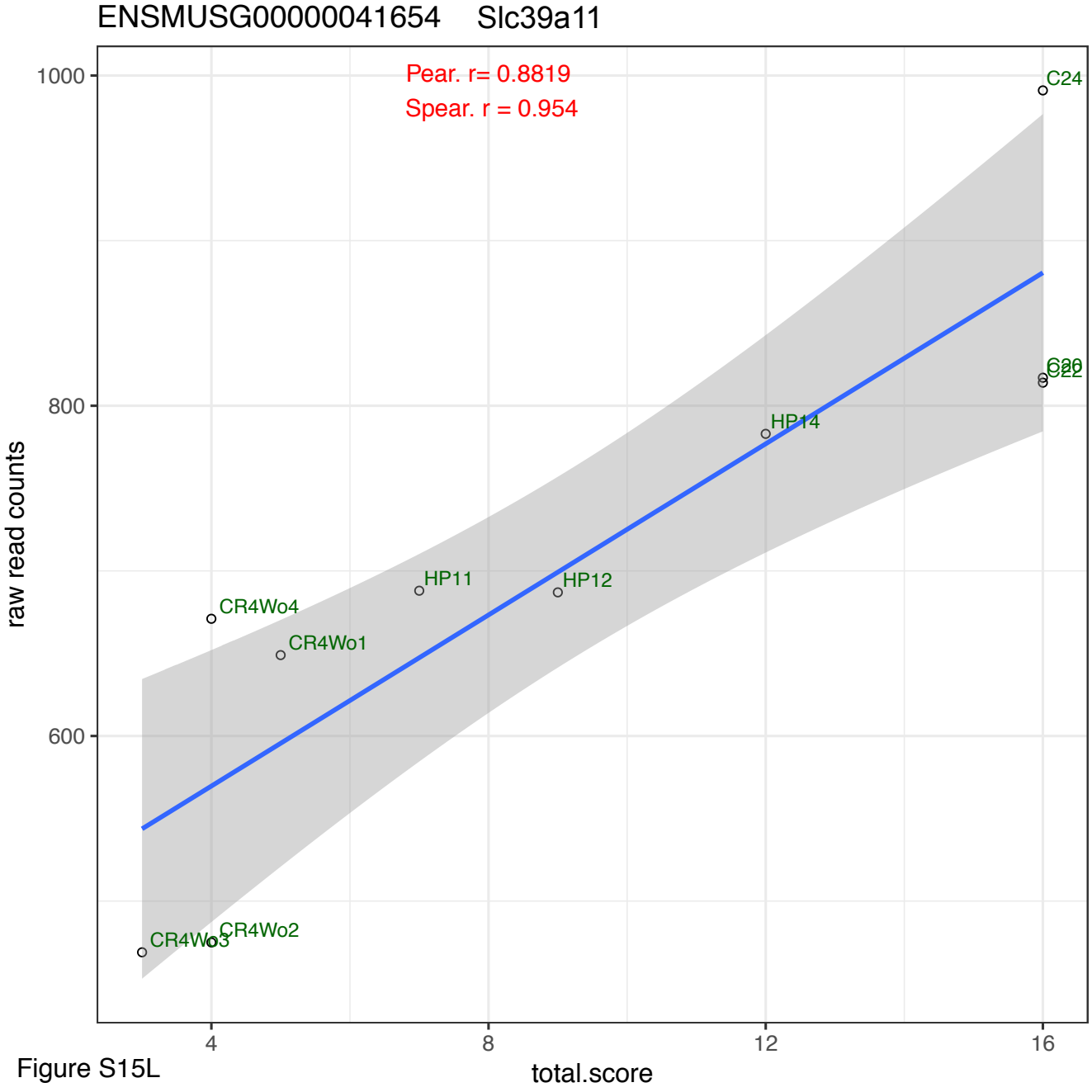

ENSMUSG00000041654 Slc39a11
Pear. r= 0.8819
Spear. r = 0.954
raw read counts
total.score
1000
800
600
4
8
12
16
C24
C20
C22
HP14
HP11
HP12
CR4Wo4
CR4Wo1
CR4Wo3
CR4Wo2

Figure S15L

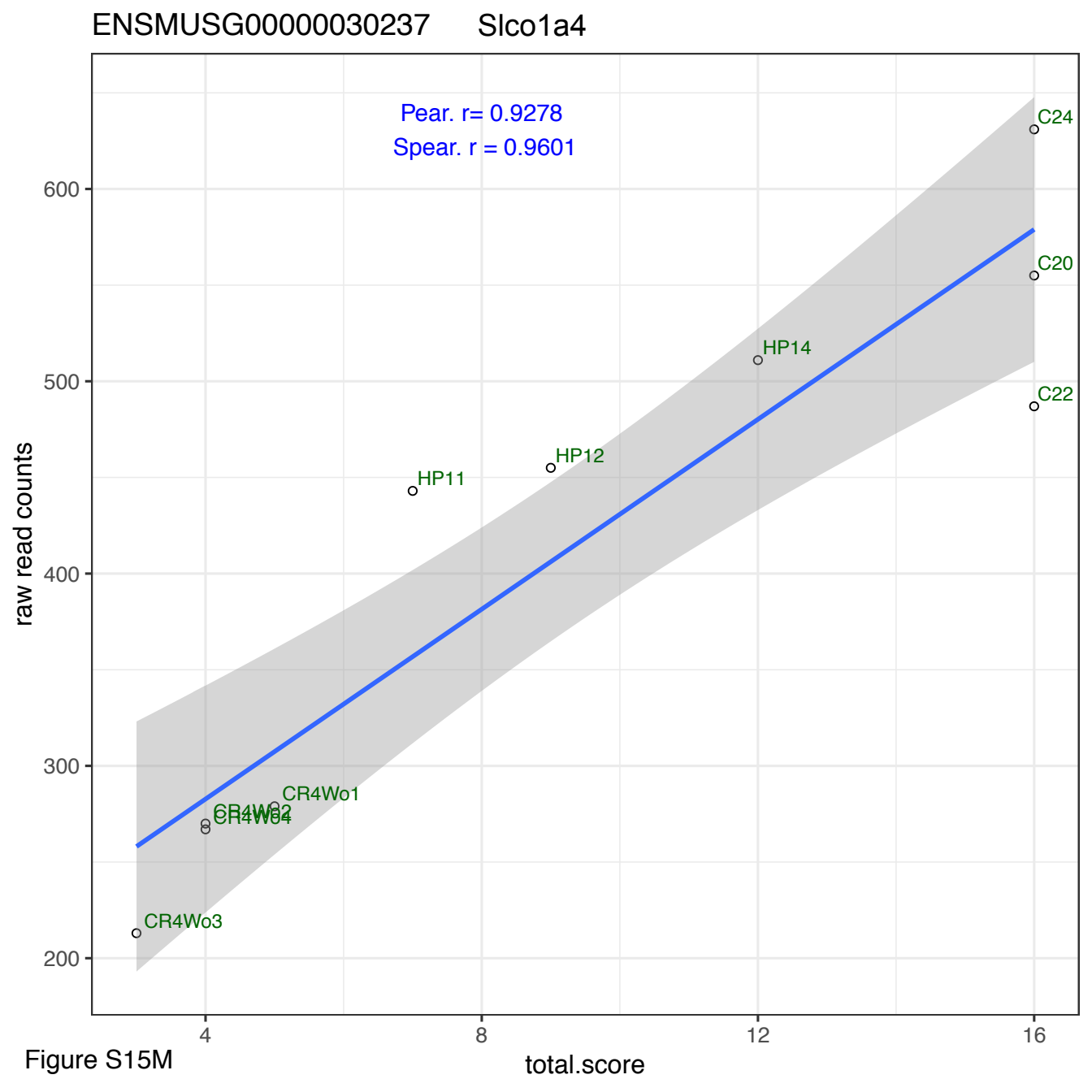

ENSMUSG00000030237 Slco1a4
Pear. r= 0.9278
Spear. r = 0.9601
C24
C20
HP14
C22
HP12
HP11
CR4Wo1
CR4Wo2
CR4Wo4
CR4Wo3
raw read counts
600
500
400
300
200
4
8
12
16
total.score

Figure S15M

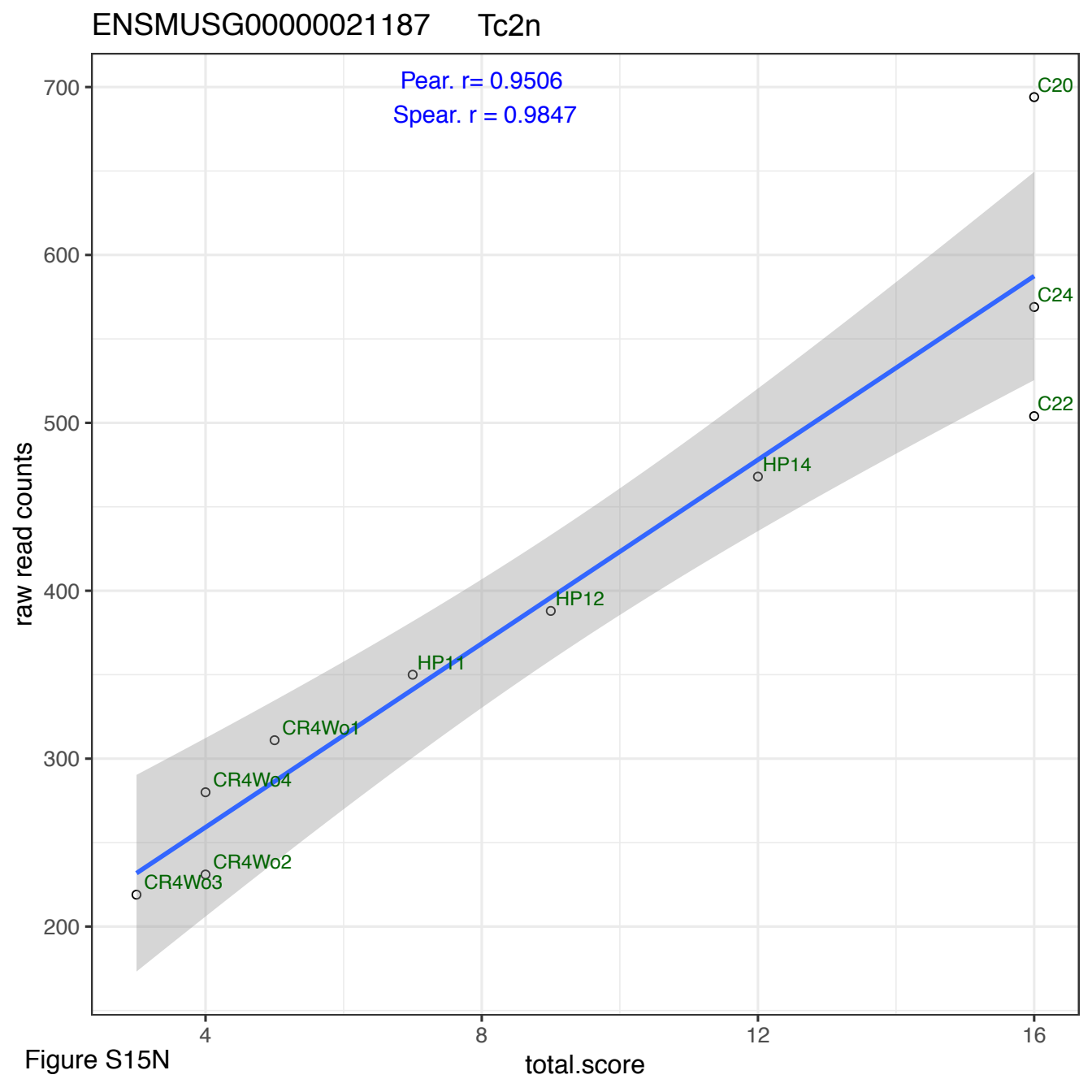

ENSMUSG00000021187 Tc2n
Pear. r= 0.9506
Spear. r = 0.9847
C20
C24
C22
HP14
HP12
HP11
CR4Wo1
CR4Wo4
CR4Wo2
CR4Wo3
raw read counts
total.score
700
600
500
400
300
200
4
8
12
16

Figure S15N

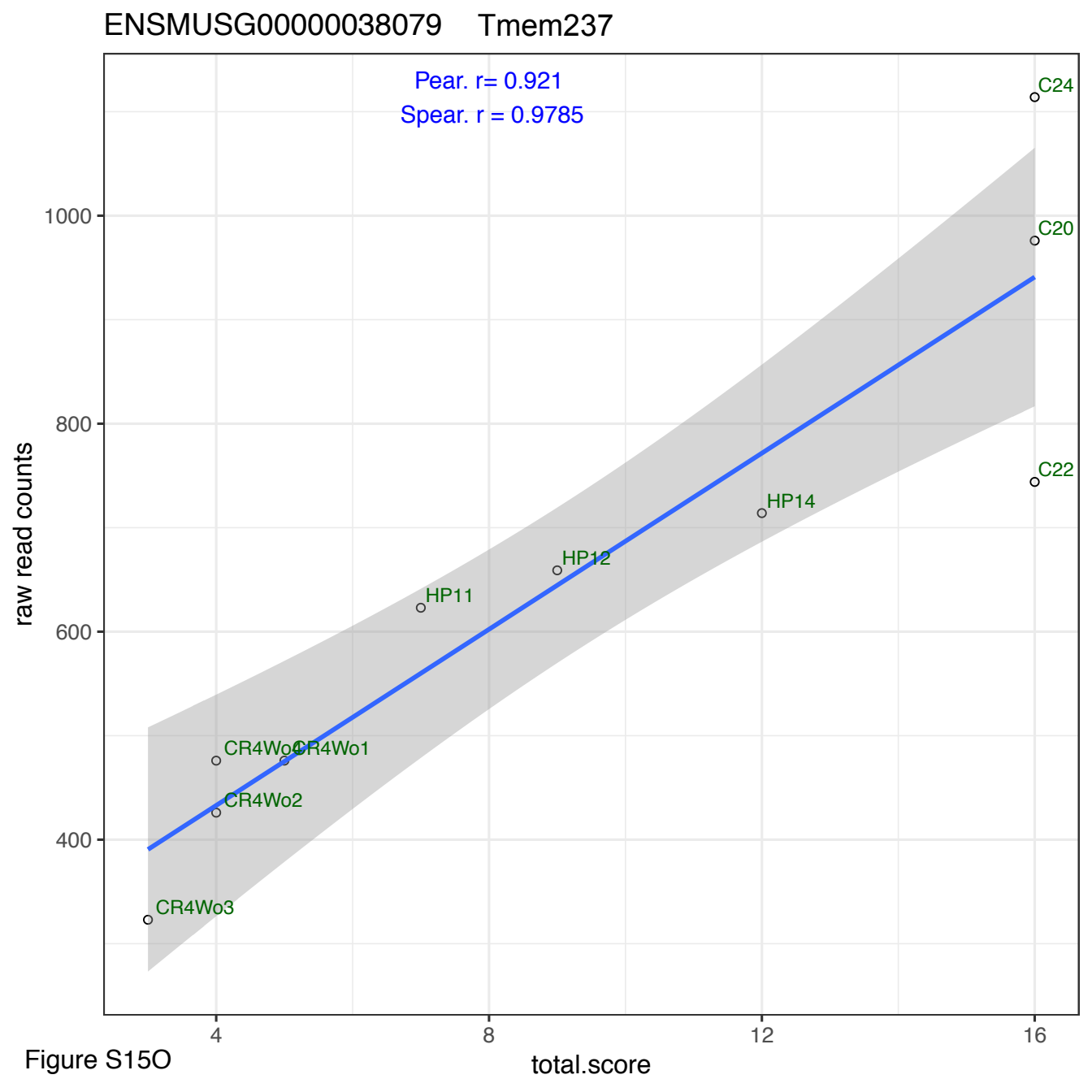

ENSMUSG00000038079 Tmem237
Pear. r= 0.921
Spear. r = 0.9785
C24
C20
C22
HP14
HP12
HP11
CR4Wo4
CR4Wo1
CR4Wo2
CR4Wo3
raw read counts
1000
800
600
400
4
8
12
16
total.score

Figure S15O

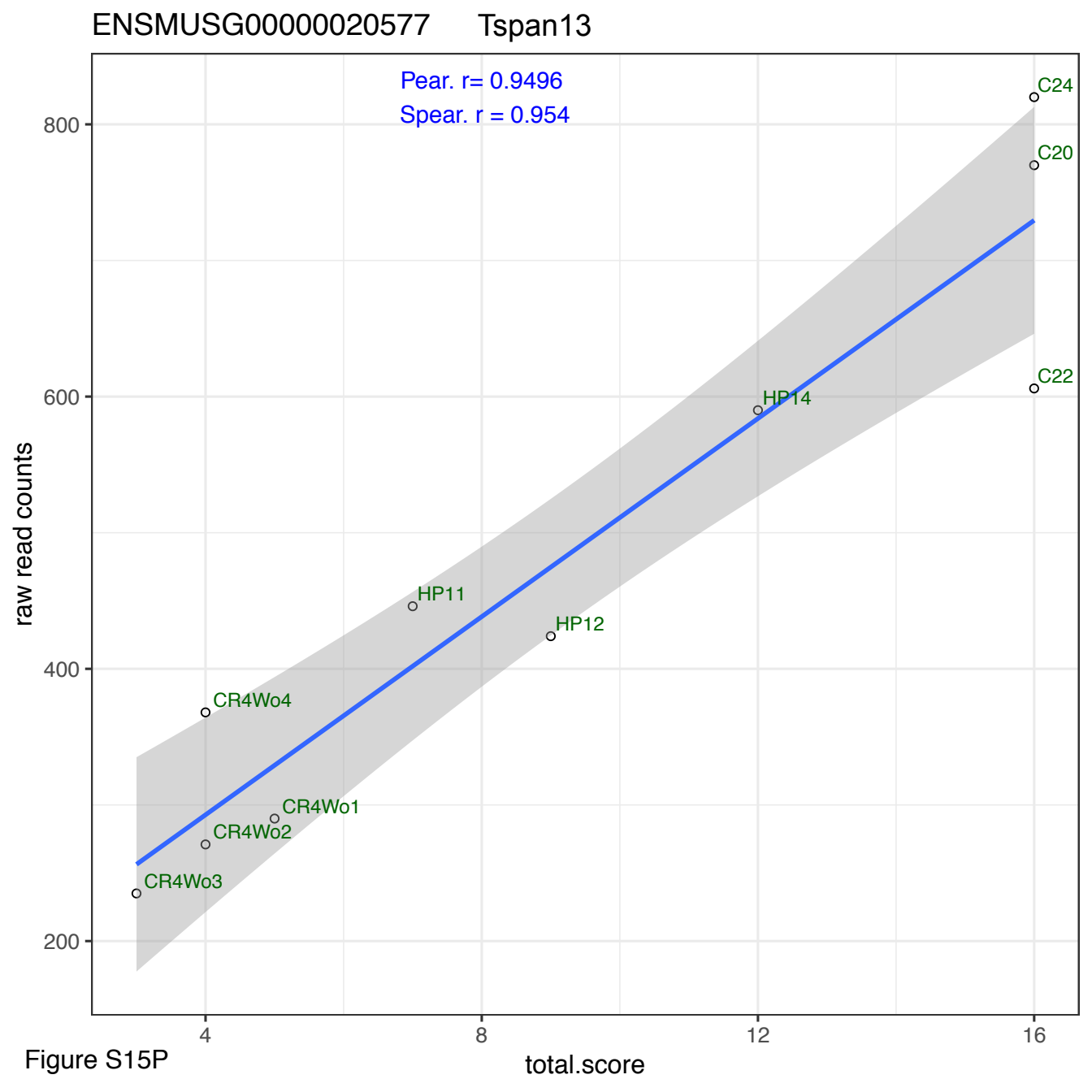

ENSMUSG00000020577 Tspan13
Pear. r= 0.9496
Spear. r = 0.954
C24
C20
C22
HP14
HP11
HP12
CR4Wo4
CR4Wo1
CR4Wo2
CR4Wo3
raw read counts
total.score
800
600
400
200
4
8
12
16

Figure S15P