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Michael Lardelli

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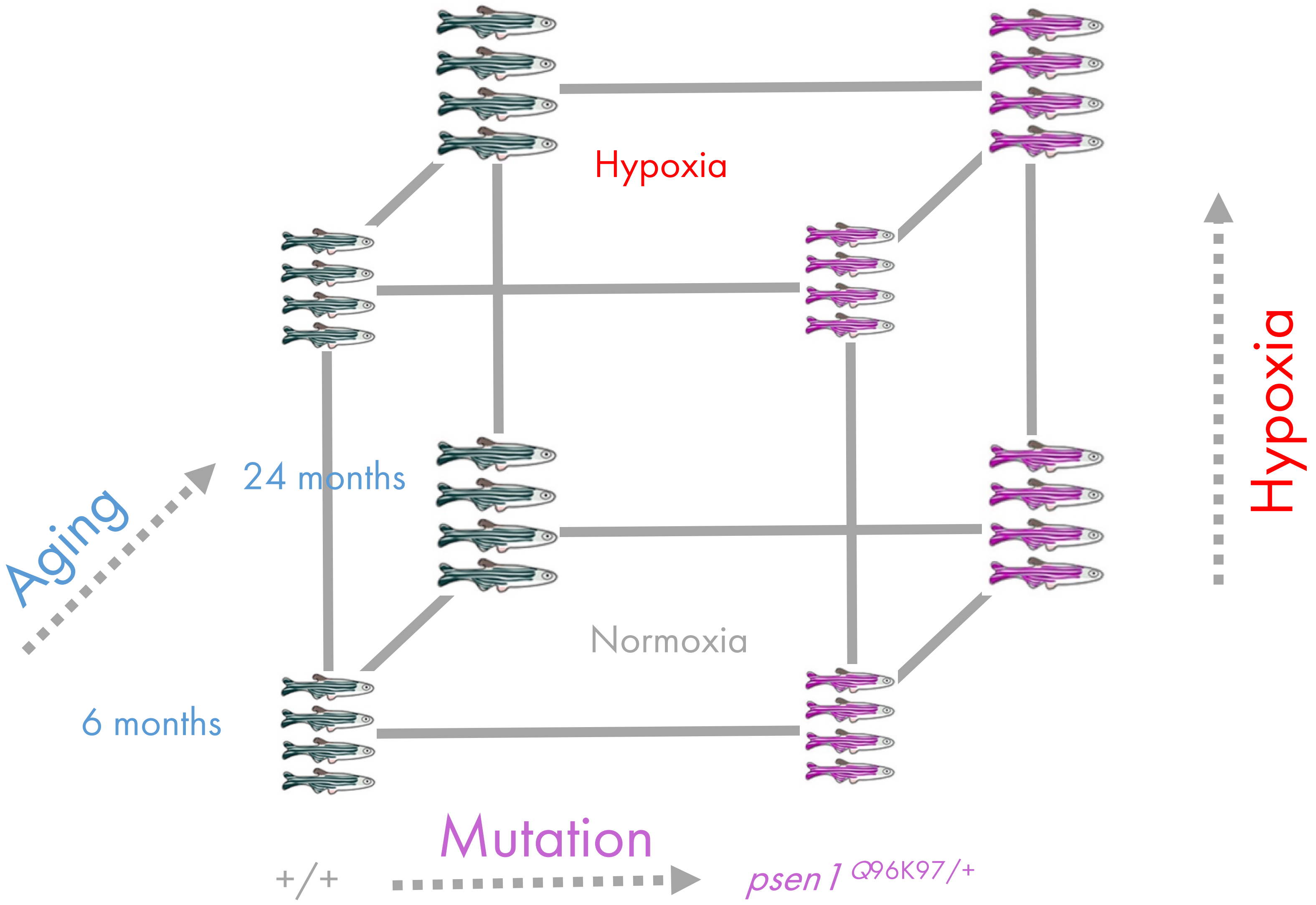
Summary

- Evidence in a mutation model of familial Alzheimer's disease to support iron deficiency as an early step in disease progression.
- Our approach is an extension to standard RNA-seq analysis that assesses iron homeostasis and its disruption in different conditions (e.g. aging, hypoxia).

RNA-seq Samples & Study Design

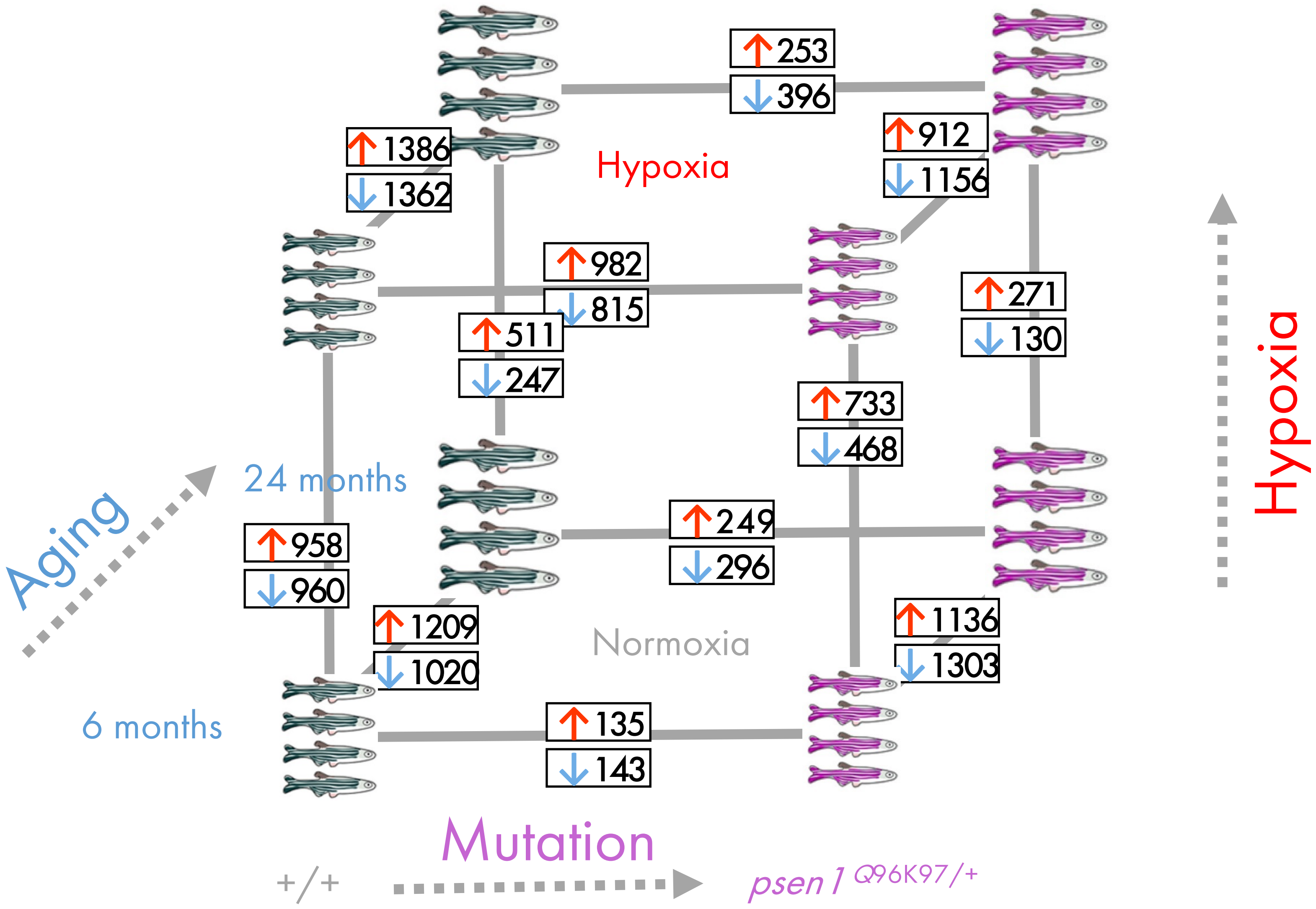
Library prep

- cDNA from whole brains extracted and prepared for RNA-seq by Morgan.

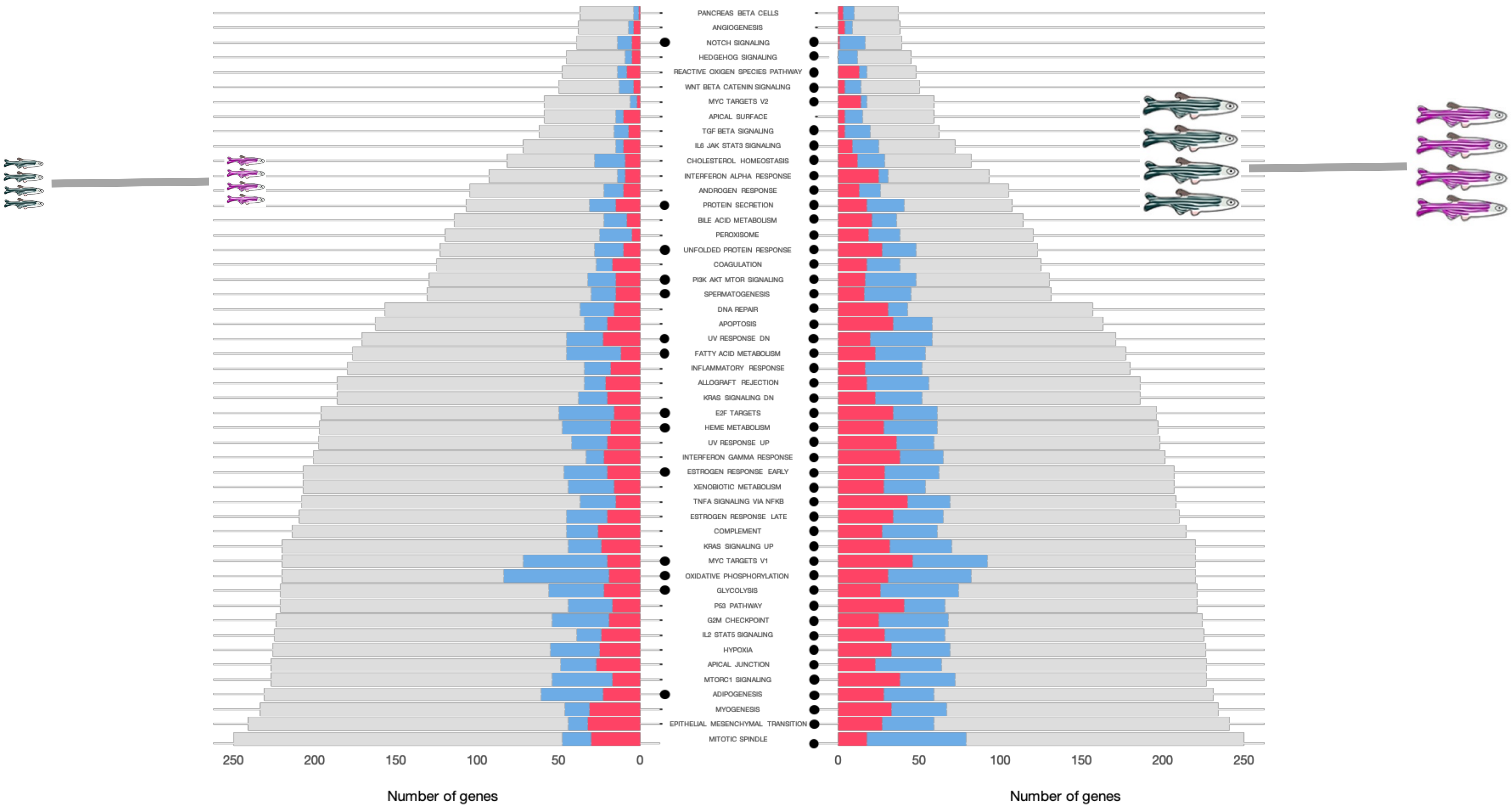


RNA-seq + data analysis

- Expression of ~18,000 genes quantified.
- Determined differentially expressed genes with linear modelling + moderated *t*-test (FDR $p < 0.05$).

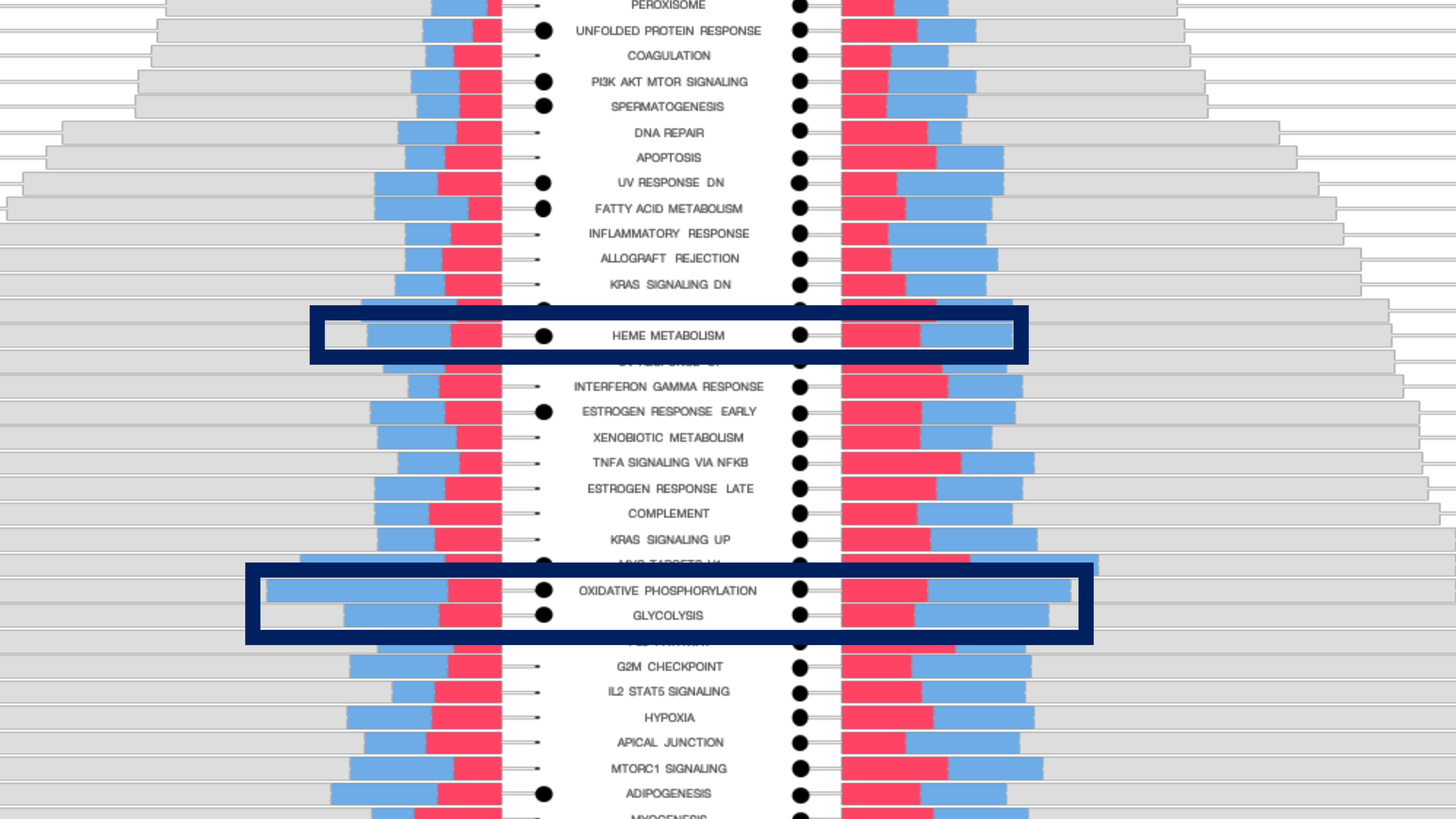


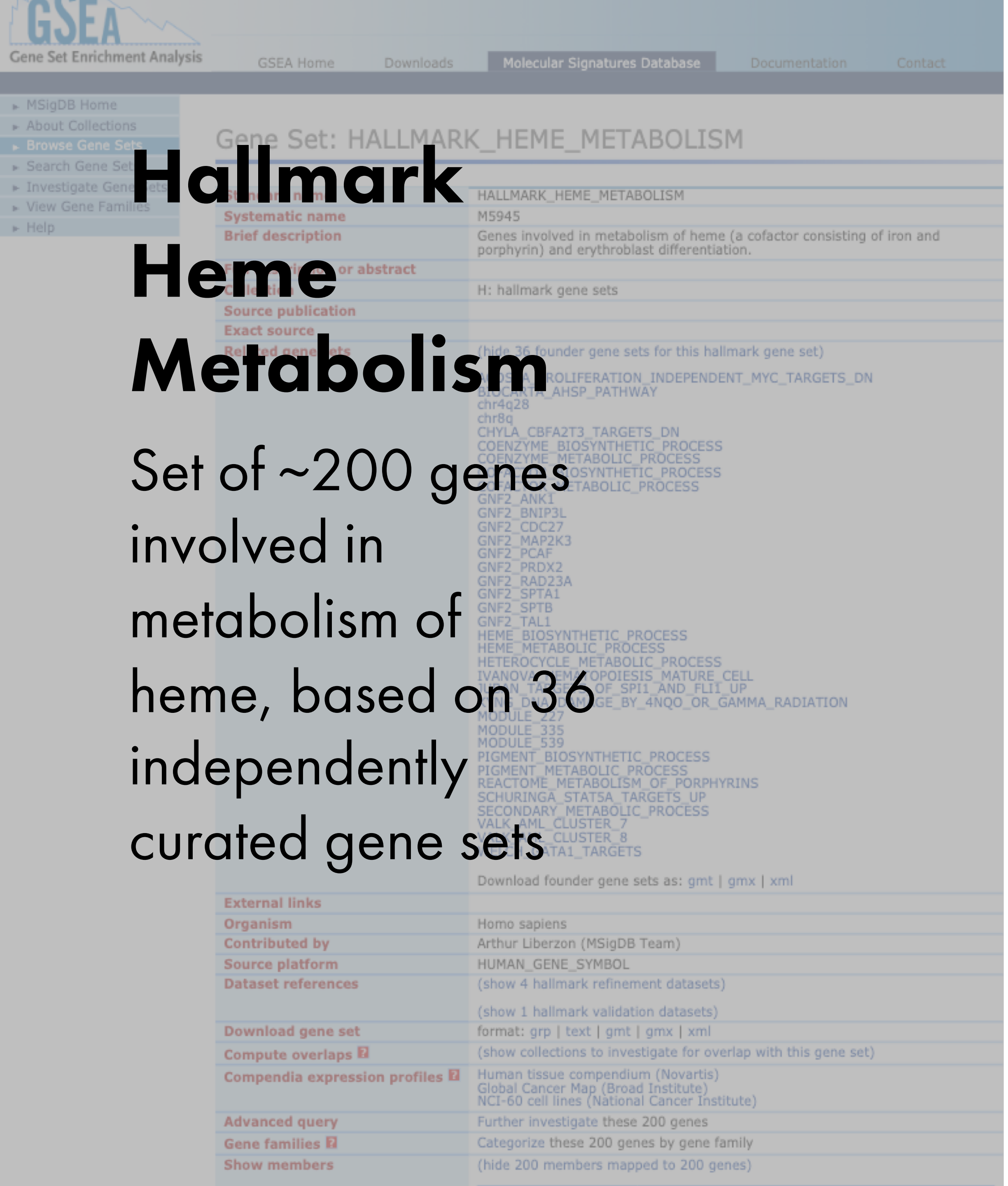
Law et al. 2014; Ritchie et al. 2015



Down 🟦🟥 Up

● FDR-adjusted *p*-value from Gene Set Enrichment Analysis with *fry*, *camera*, and *fgsea* < 0.05


PEROXISOME
UNFOLDED PROTEIN RESPONSE
COAGULATION
PI3K AKT MTOR SIGNALING
SPERMATOGENESIS
DNA REPAIR
APOPTOSIS
UV RESPONSE DN
FATTY ACID METABOLISM
INFLAMMATORY RESPONSE
ALLOGRAFT REJECTION
KRAS SIGNALING DN
HEME METABOLISM
INTERFERON GAMMA RESPONSE
ESTROGEN RESPONSE EARLY
XENOBIOTIC METABOLISM
TNFA SIGNALING VIA NFKB
ESTROGEN RESPONSE LATE
COMPLEMENT
KRAS SIGNALING UP
OXIDATIVE PHOSPHORYLATION
GLYCOLYSIS
G2M CHECKPOINT
IL2 STAT5 SIGNALING
HYPOXIA
APICAL JUNCTION
MTORC1 SIGNALING
ADIPOGENESIS



Gene Set: HALLMARK_HEME_METABOLISM

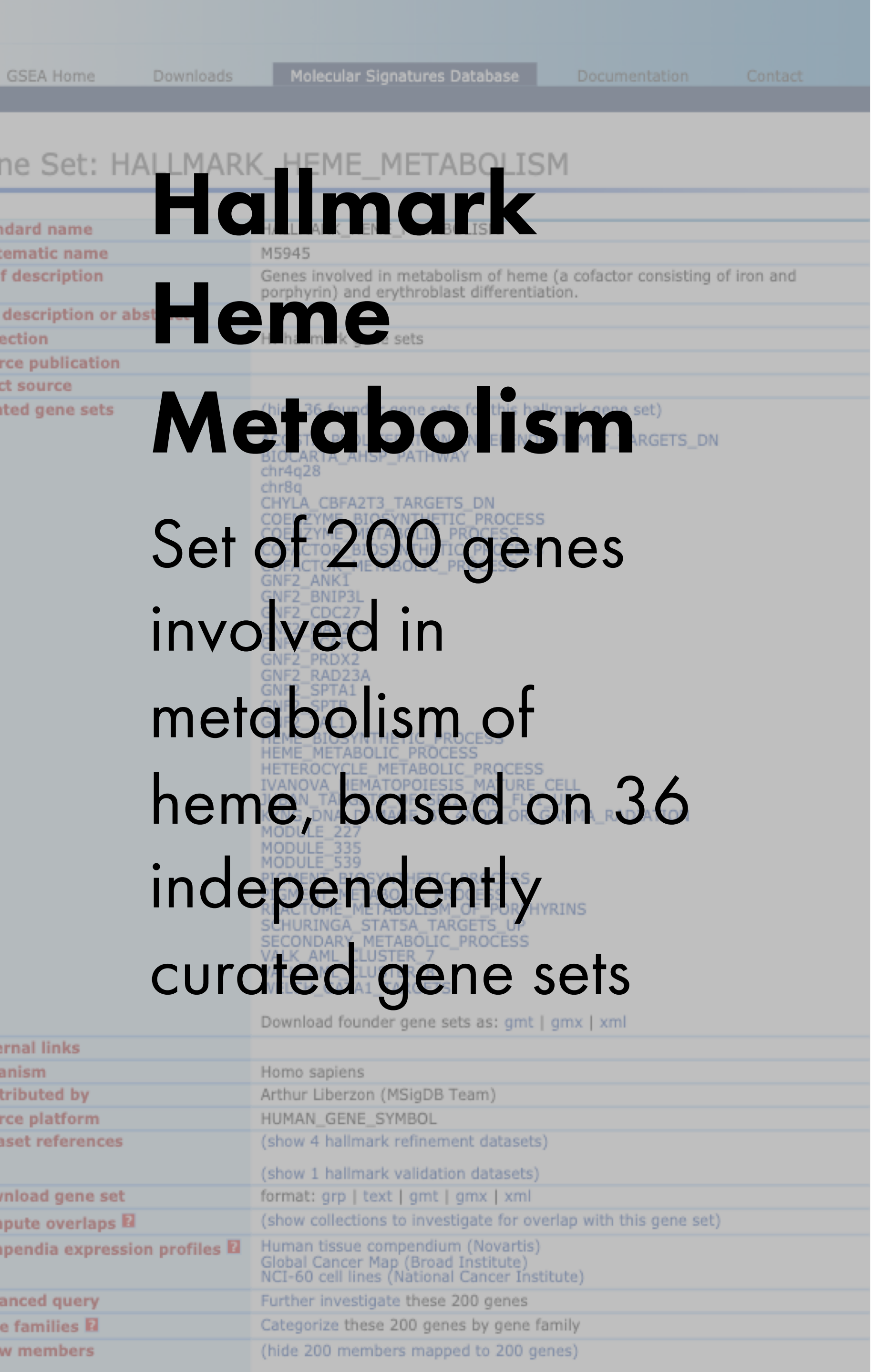
Standard name	HALLMARK_HEME_METABOLISM
Systematic name	M5945
Brief description	Genes involved in metabolism of heme (a cofactor consisting of iron and porphyrin) and erythroblast differentiation.
Full description or abstract	
Collection	H: hallmark gene sets
Source publication	
Exact source	
Related gene sets	(hide 36 founder gene sets for this hallmark gene set) ACOSTA_PROLIFERATION_INDEPENDENT_MYC_TARGETS_DN BIOCARTA_AHSP_PATHWAY chr4q28 chr8q CHYLA_CBFA2T3_TARGETS_DN COENZYME_BIOSYNTHETIC_PROCESS COENZYME_METABOLIC_PROCESS COFACTOR_BIOSYNTHETIC_PROCESS COFACTOR_METABOLIC_PROCESS GNF2_ANK1 GNF2_BNIP3L GNF2_CDC27 GNF2_MAP2K3 GNF2_PCAF GNF2_PRDX2 GNF2_RAD23A GNF2_SPTA1 GNF2_SPTB GNF2_TAL1 HEME_BIOSYNTHETIC_PROCESS HEME_METABOLIC_PROCESS HETEROCYCLE_METABOLIC_PROCESS IVANOVA_HEMATOPOIESIS_MATURE_CELL JUBAN_TARGETS_OF_SPI1_AND_FLI1_UP KYNG_DNA_DAMAGE_BY_4NQO_OR_GAMMA_RADIATION MODULE_227 MODULE_335 MODULE_539 PIGMENT_BIOSYNTHETIC_PROCESS PIGMENT_METABOLIC_PROCESS REACTOME_METABOLISM_OF_PORPHYRINS SCHURINGA_STAT5A_TARGETS_UP SECONDARY_METABOLIC_PROCESS VALK_AML_CLUSTER_7 VALK_AML_CLUSTER_8 WELCH_GATA1_TARGETS Download founder gene sets as: gmt | gmx | xml
External links	
Organism	Homo sapiens
Contributed by	Arthur Liberzon (MSigDB Team)
Source platform	HUMAN_GENE_SYMBOL
Dataset references	(show 4 hallmark refinement datasets) (show 1 hallmark validation datasets)
Download gene set	format: grp | text | gmt | gmx | xml
Compute overlaps ?	(show collections to investigate for overlap with this gene set)
Compendia expression profiles ?	Human tissue compendium (Novartis) Global Cancer Map (Broad Institute) NCI-60 cell lines (National Cancer Institute)
Advanced query	Further investigate these 200 genes
Gene families ?	Categorize these 200 genes by gene family
Show members	(hide 200 members mapped to 200 genes)

Hallmark Heme Metabolism

Set of ~200 genes involved in metabolism of heme, based on 36 independently curated gene sets

How can we define a comprehensive and sensitive set of genes that we can use to assess **iron homeostasis** responses and how they differ between conditions?

Iron Responsive Elements (IREs)



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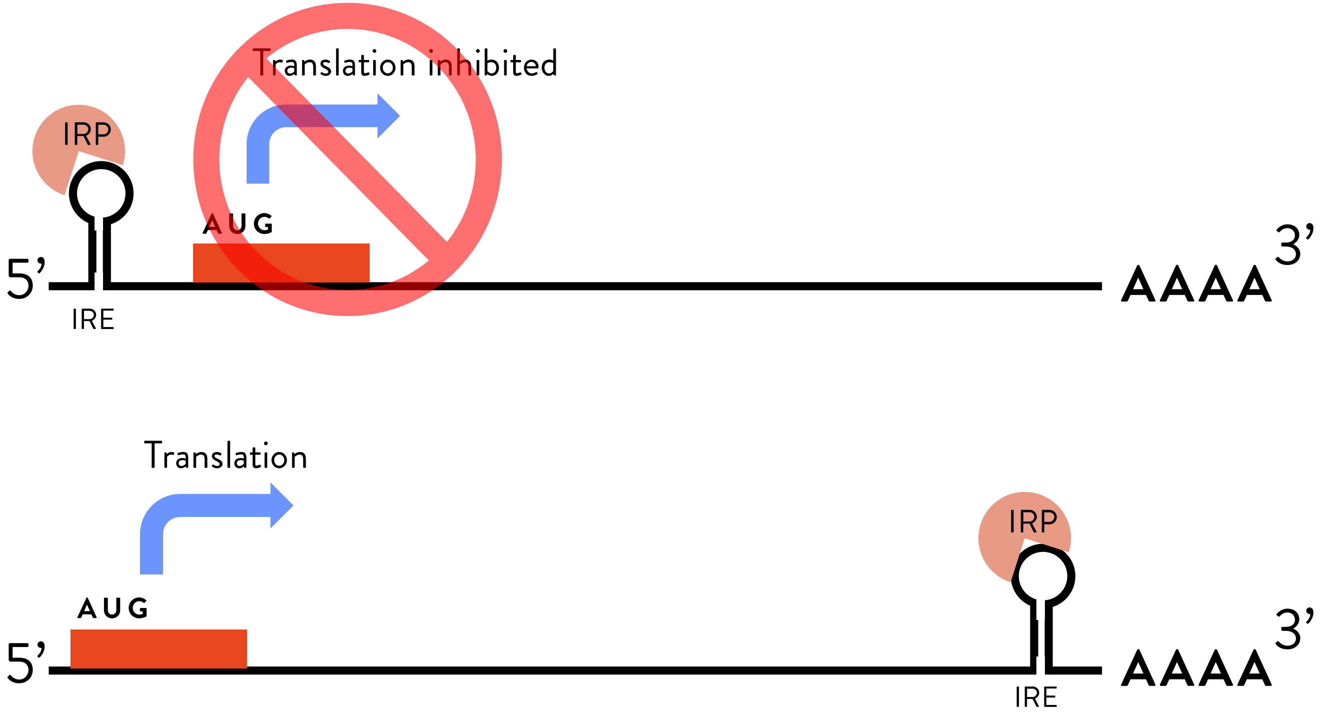
Iron Responsive Elements (IREs)

Under iron deficient conditions:



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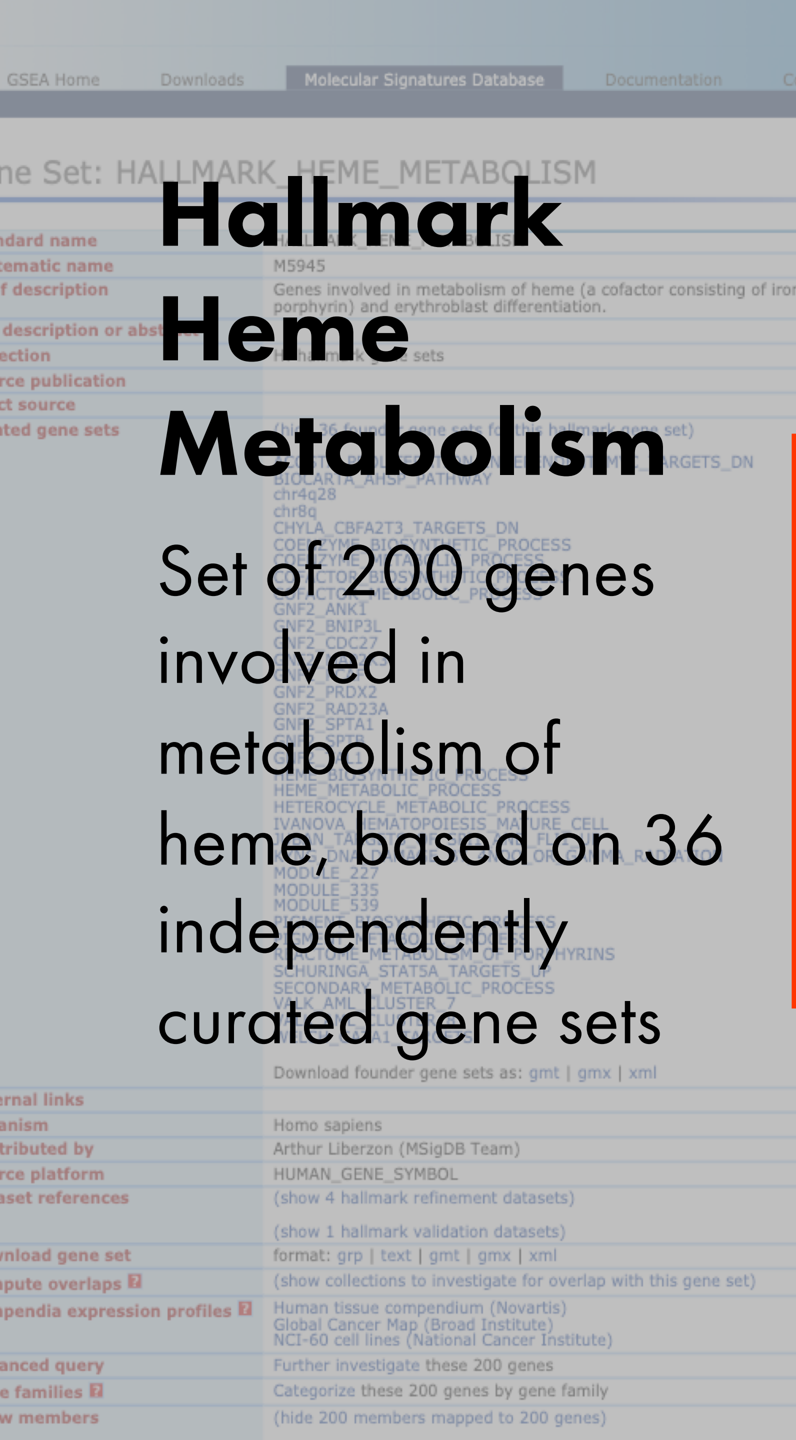

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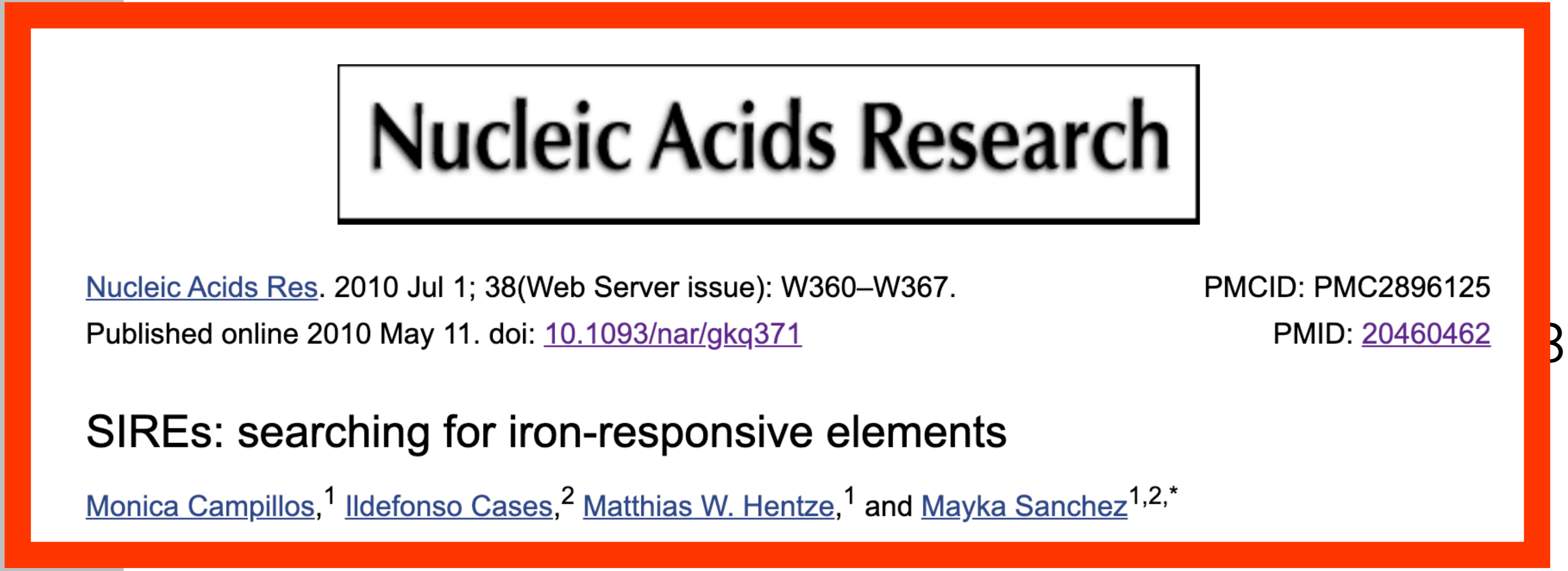


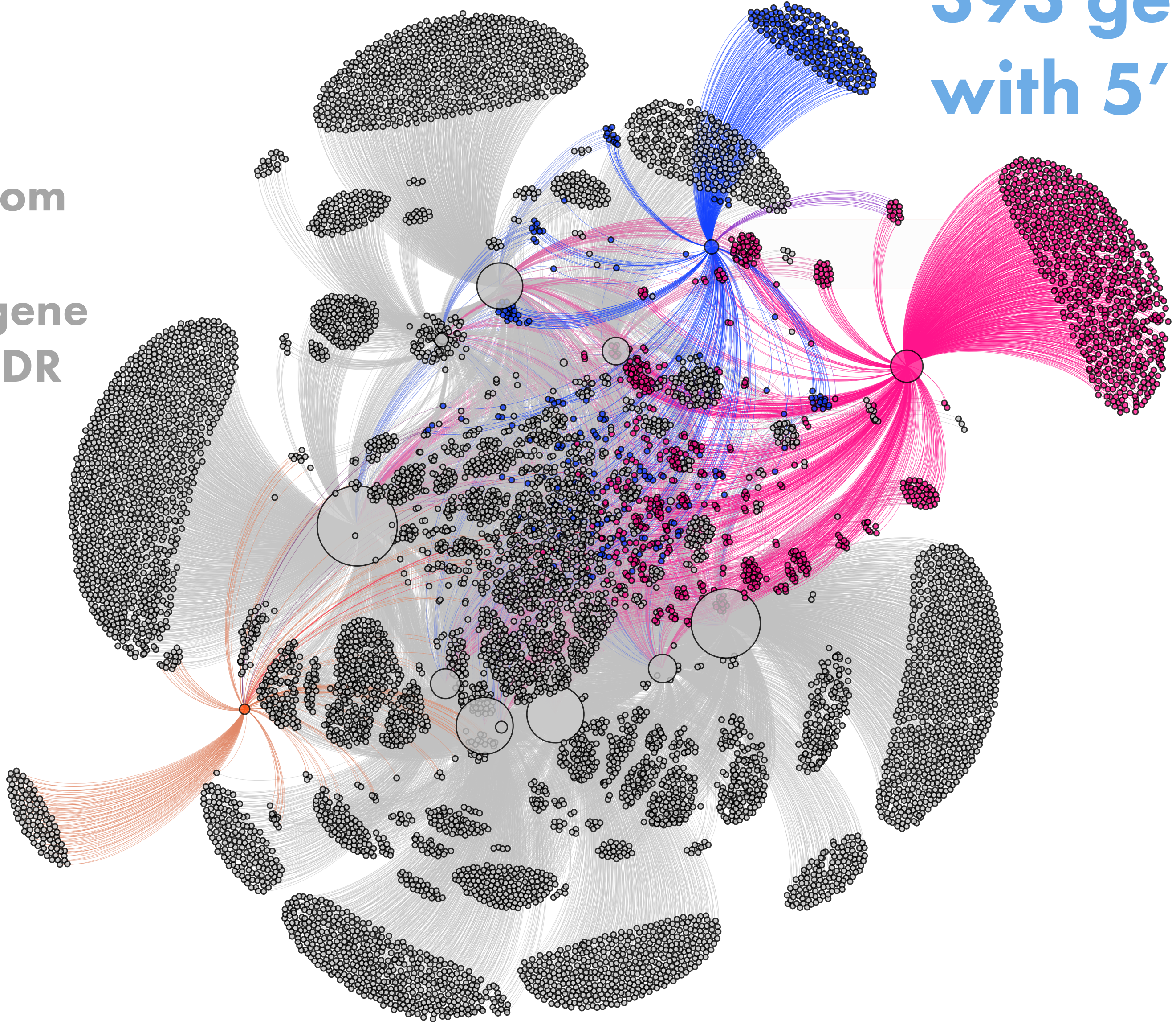
Nucleic Acids Research

Nucleic Acids Res. 2010 Jul 1; 38(Web Server issue): W360–W367.
Published online 2010 May 11. doi: 10.1093/nar/gkq371

PMCID: PMC2896125
PMID: 20460462

SIREs: searching for iron-responsive elements

Monica Campillos,[1] Ildefonso Cases,[2] Matthias W. Hentze,[1] and Mayka Sanchez[1,2,*]



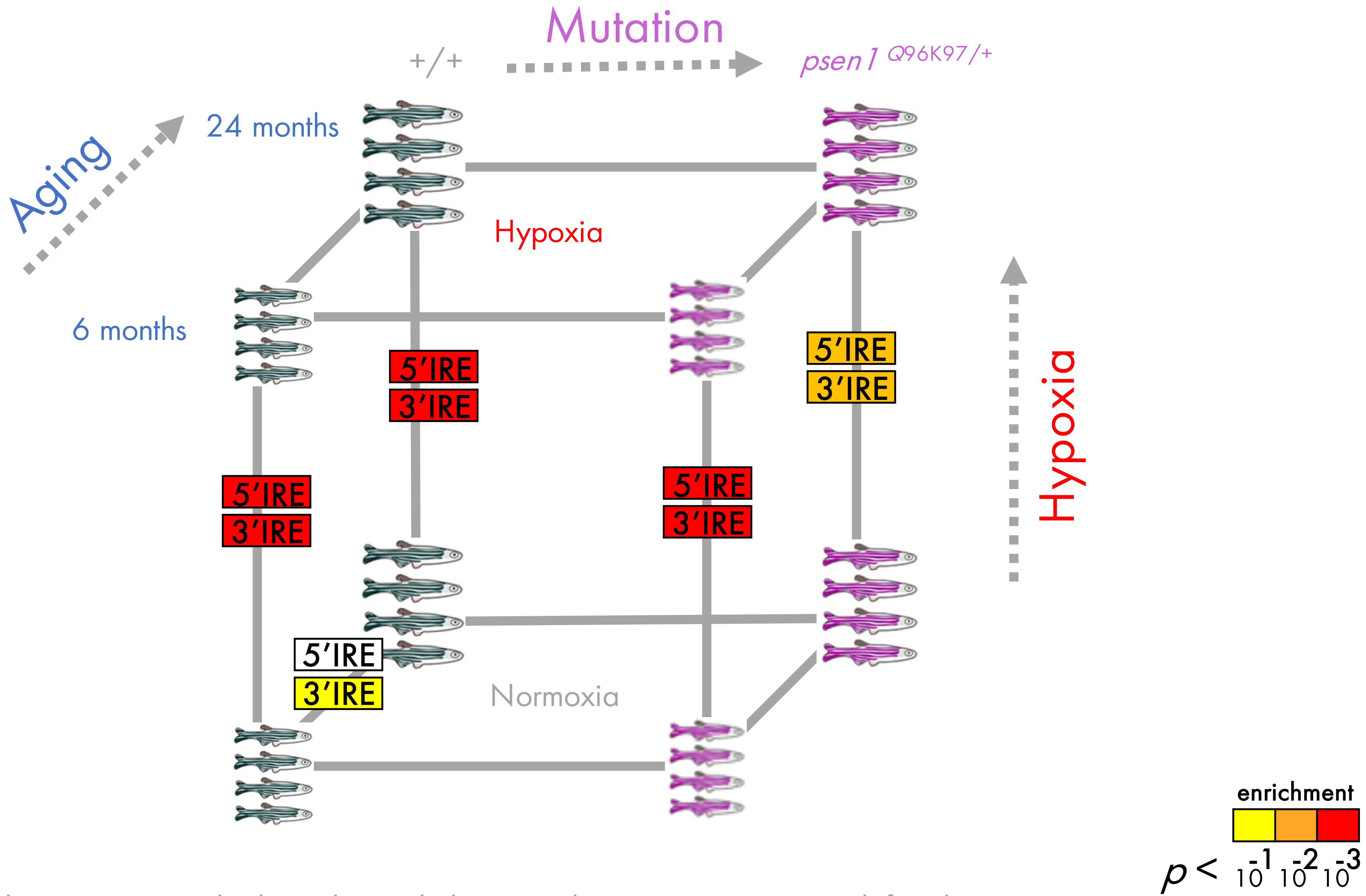
393 genes with 5′ IREs

1,207 genes with 3′ IREs

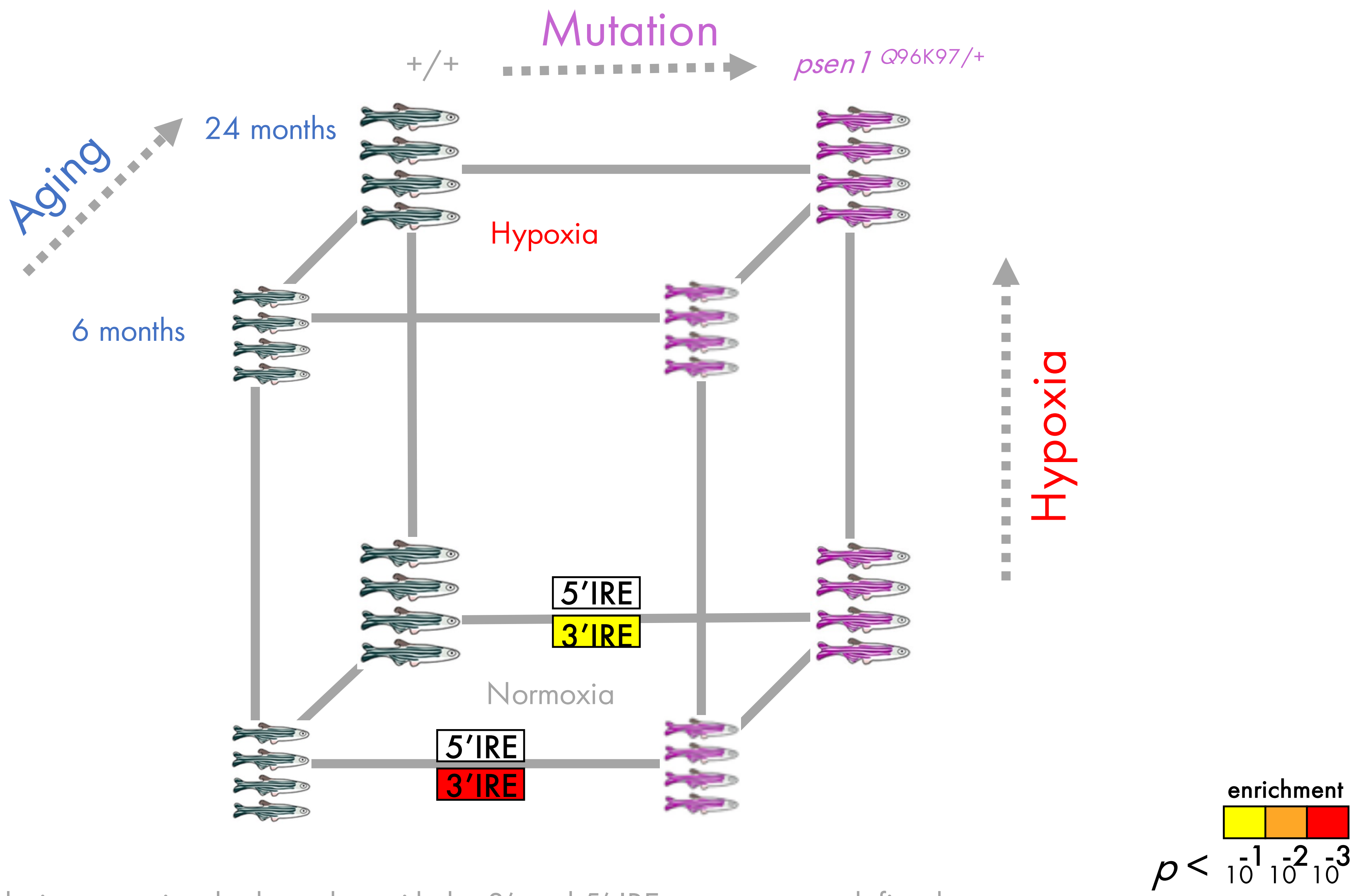
Other gene sets from MSigDB showing overlap with IRE gene sets (enrichment FDR *p*-value < 0.05)

Hallmark Heme Metabolism

Genes with IREs were identified by searching all reference zebrafish transcripts for IRE stem-loop motifs with SIREs.



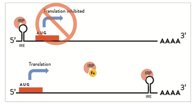
Gene Set Enrichment Analysis as previously done, but with the 3′ and 5′ IRE gene sets we defined



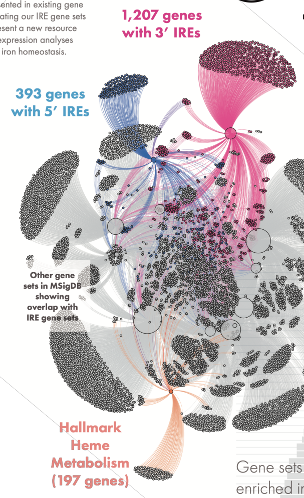
Gene Set Enrichment Analysis as previously done, but with the 3′ and 5′ IRE gene sets we defined

1 Defining IRE gene sets for monitoring iron homeostasis

Under iron-deficient conditions, Iron Responsive Proteins bind to Iron Responsive Elements, which are located in UTRs of genes involved in iron homeostasis. Generally, this results in increased 3′ IRE gene expression and decreased 5′ IRE gene expression[1].



We searched[2] all zebrafish transcript UTRs for IRE stem-loop motifs to form comprehensive sets of genes regulated by 3′ and 5′ IREs. Many genes in these lists are not represented in existing gene sets, indicating our IRE gene sets may represent a new resource for gene expression analyses exploring iron homeostasis.



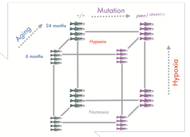
We observed iron deficiency in the brain with aging, hypoxia, & a familial Alzheimer's disease-like mutation.

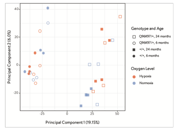
A straightforward approach using RNA-seq data to explore iron homeostasis.

2 Characterising brain gene expression changes in mutants

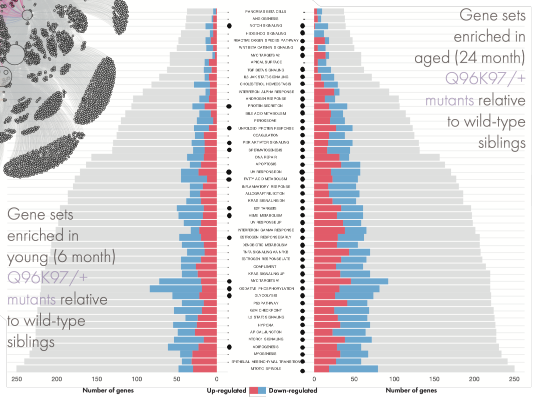
Whole zebrafish brains were subjected to poly-A-enriched RNA-sequencing. Pairwise comparisons between conditions were used to explore effects of the fAD-like mutation, aging, and hypoxia on gene expression.

Principal Component Analysis (PCA) was used to visualise overall similarity between samples. In the PCA plot of ~20,000 expressed genes, the effects of aging appeared to obscure gene expression changes due to genotype or hypoxia.



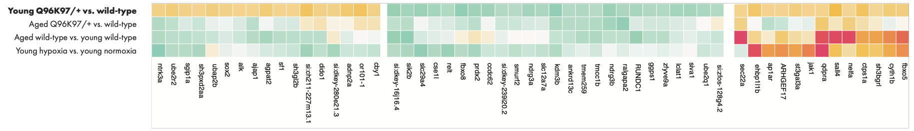


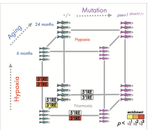
To explore coordinated gene expression changes in Q96K97/+ mutants relative to wild-type siblings, we performed Gene Set Enrichment Analysis on MSigDB Hallmark gene sets[3]. Significant gene sets at 6 months are useful clues for pathways altered earlier in disease pathogenesis (● indicates GSEA enrichment FDR-adj. $p < 0.05$). Up/Down-regulated indicate genes in gene sets that were significantly differentially expressed via *limma*[4] analysis (FDR-adj. $p < 0.05$).

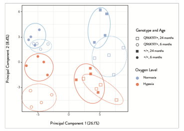


3 Exploring iron deficiency through 3′ IRE gene expression

We focused on iron homeostasis through the IRE genes defined earlier to see whether evidence of an iron deficiency response was present in certain conditions. Together, the Gene Set Enrichment Analysis, PCA, and heatmap of DE genes containing IREs in the young mutant vs. wild-type siblings below all suggest that an 3′ IRE response occurs in aging, hypoxia, and Q96K97/+ mutants, and shared gene expression changes are present. Our findings represent the first evidence in a genetic model of familial Alzheimer's disease supporting a recently-proposed hypothesis that positions disrupted iron homeostasis as a key effect of familial Alzheimer's disease mutations[5].







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1. Alzheimer's Disease Genetics Laboratory, School of Biological Sciences, The University of Adelaide
2. Bioinformatics Hub, School of Biological Sciences, The University of Adelaide

1. Zhou, ZD & Tan, EK 2017, 'Iron regulatory protein (IRP)-iron responsive element (IRE) signaling pathway in human neurodegenerative diseases', *Molecular Neurodegeneration*, vol. 12, no. 1, p. 75.
2. Campillos, M et al. 2010, 'SIREs: searching for iron-responsive elements', *Nucleic Acids Research*, vol. 38, pp. 360-367.
3. Liberzon, A et al. 2015, 'The Molecular Signatures Database (MSigDB) hallmark gene set collection', *Cell Systems*, vol. 1, no. 6, pp. 417-425.
4. Law C, et al. 2014, 'voom: precision weights unlock linear model analysis tools for RNA-seq read counts', *Genome Biology*, vol. 15, no. 29, pp. 1-17.
5. Lumsden A, et al. 2018, 'Dysregulation of Neuronal Iron Homeostasis as an Alternative Unifying Effect of Mutaations Causing Familial Alzheimer's Disease', *Frontiers in Neuroscience*, vol. 12, no. 533.

Acknowledgements

Michael Lardelli

Alzheimer's Disease Genetics Laboratory

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+ Dave Adelson

Stephen Pederson

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