

Inter- and transgenerational impacts of in ovo nutriepigenetic intervention on chicken male gonadal transcriptome

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1. Introduction

In ovo technology

In ovo stimulation

Prebiotics
Synbiotics
Methyl donors



ED 12

In ovo feeding

Carbohydrates
Hormones



ED 14

Probiotics
Vitamins
Amino acids



ED 17.5-ED 19

In ovo chick model for epigenetic research

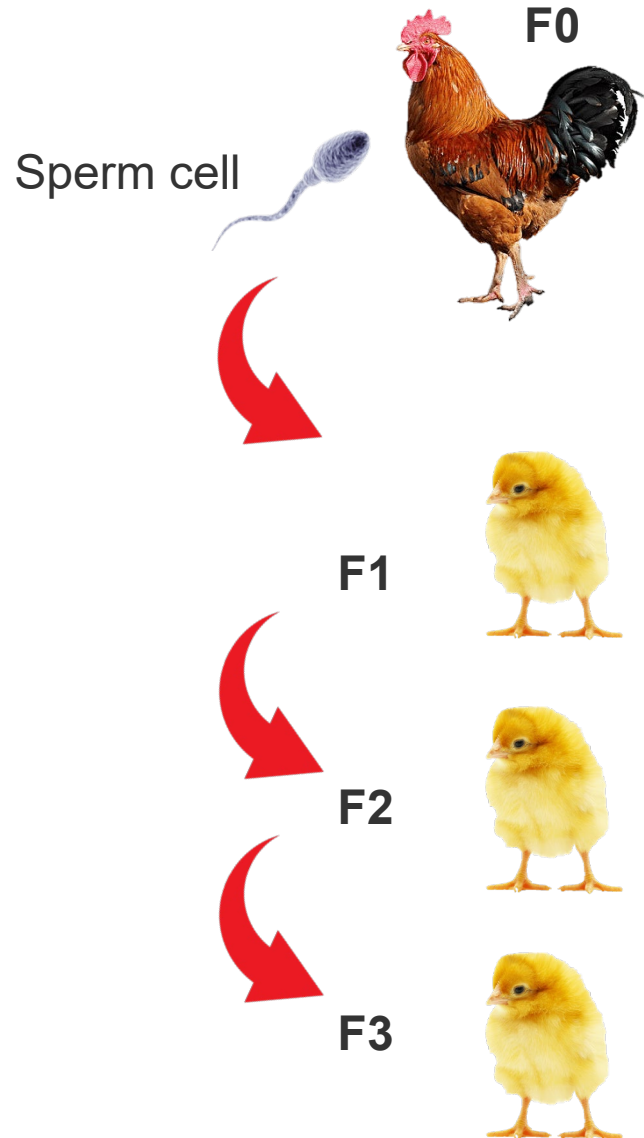
- Rapid embryo development
- Easy access
- External growth
- Manipulability at any developmental stage
- Short generation interval
- Well-characterized genome
- Economic and ethical advantages

Variability in Results

Influenced by injection route, dose, method, timing, and host factors

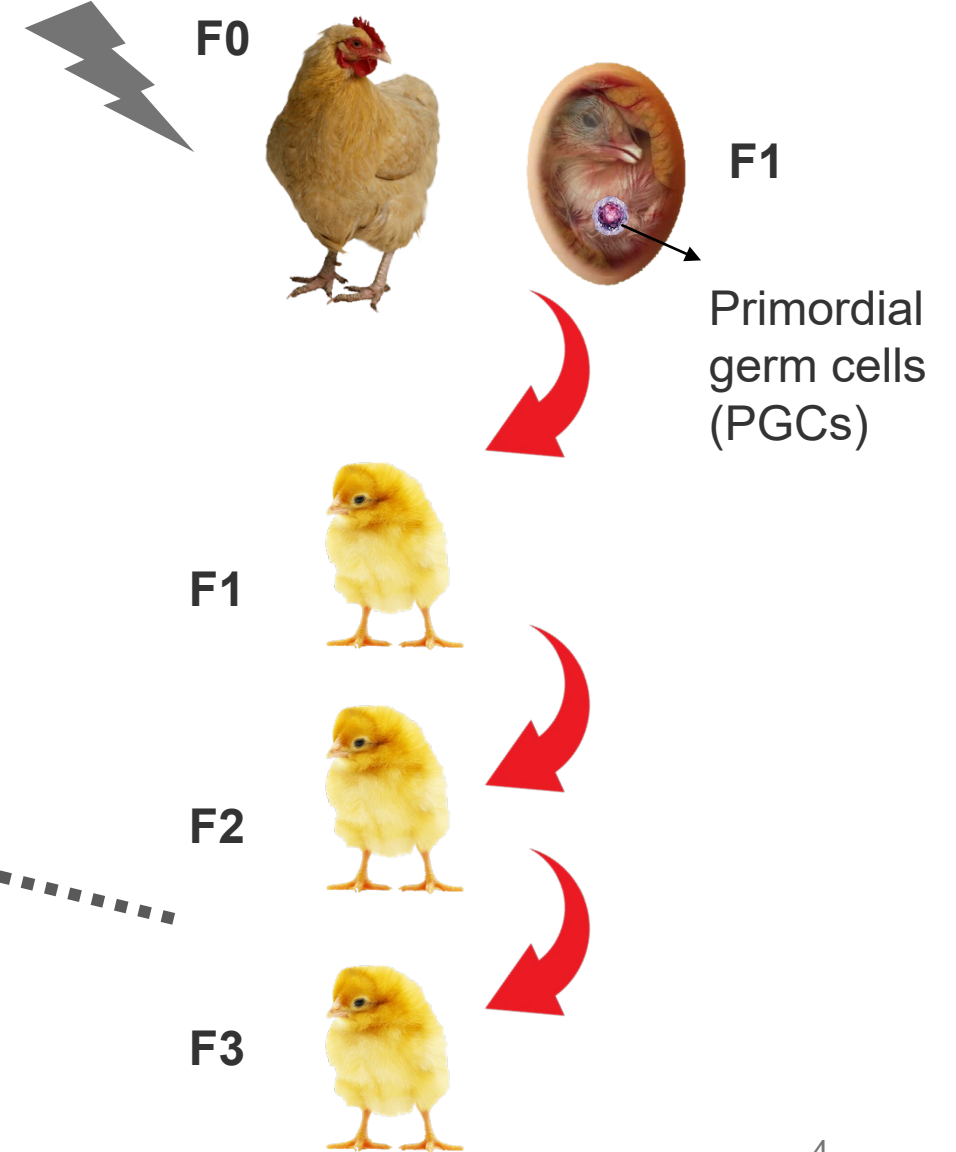
Epigenetic effect inheritance

Paternal epigenetic transmission



Environmental
change

Maternal epigenetic transmission



Intergenerational
Inheritance

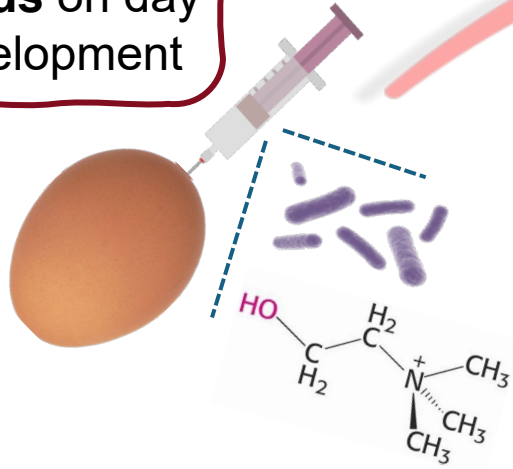
Transgenerational
Inheritance



2. Hypothesis and Aim

Hypothesis

In ovo injection of **bioactive compounds** on day 12 of development



Changes in the epigenome

Long-term effects in adult chicken

Inheritance of epigenetic marks via the germline components

Aim

Investigate the effect of in ovo stimulation with synbiotic and choline on the gonadal transcriptome of Green-Legged Partridgeline adult chicken in two consecutive generations

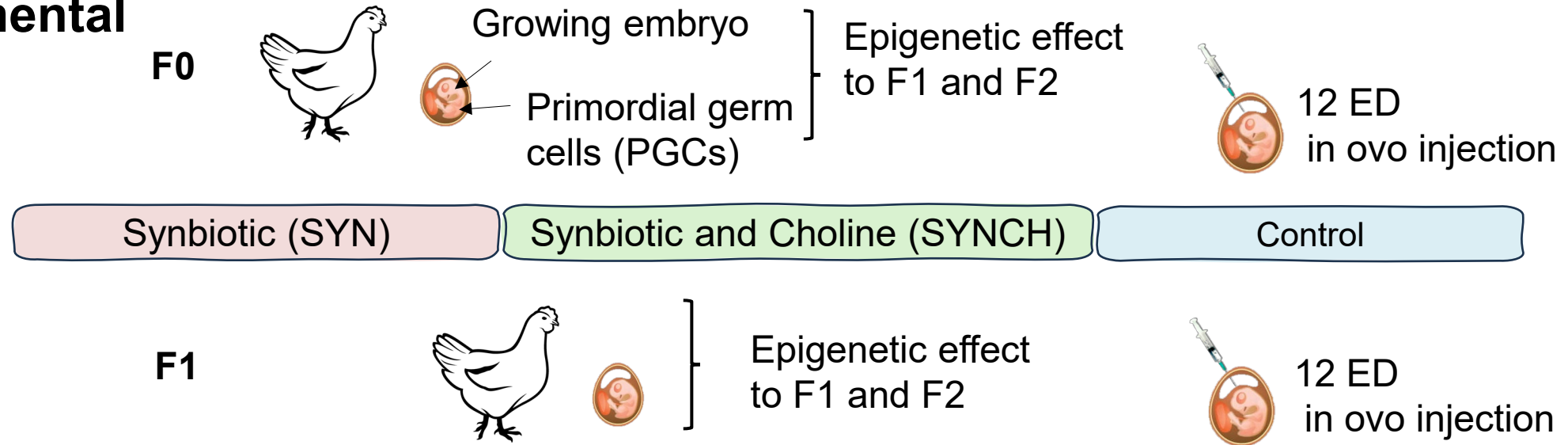


Green-legged Partridgeline chicken



3. **Materials and Methods**

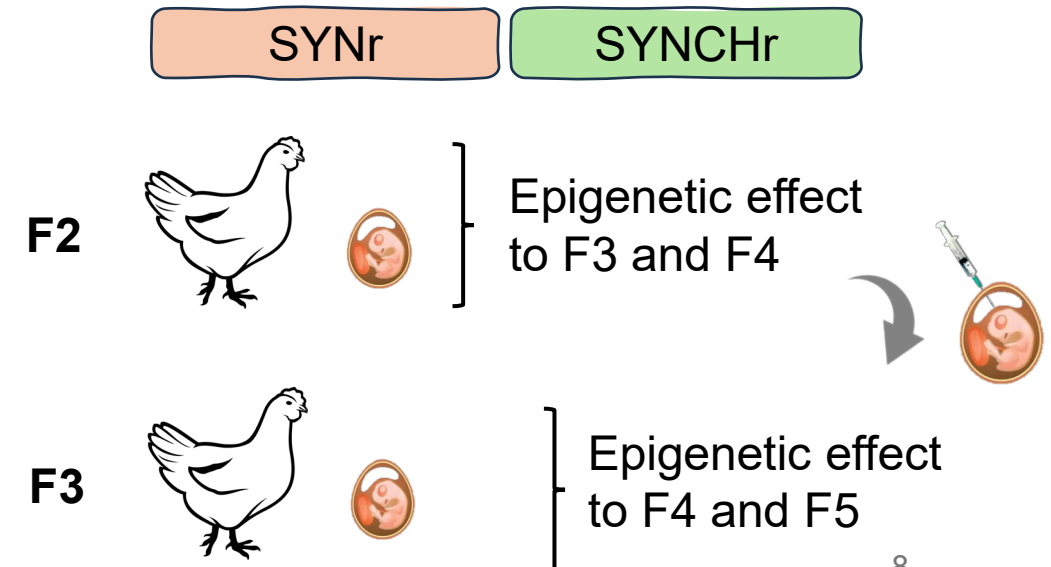
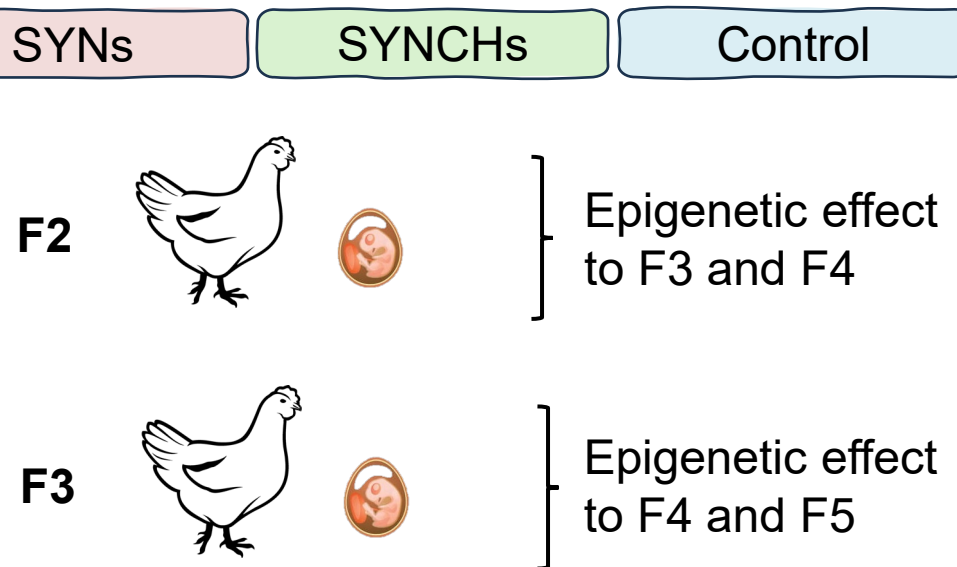
Experimental design

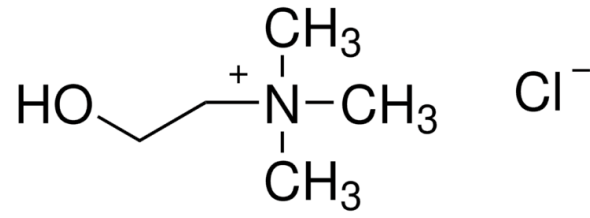


Splitting the group treatments for F2-F3

Single in ovo injection at F1 ↙

↘ Repeated in ovo injection





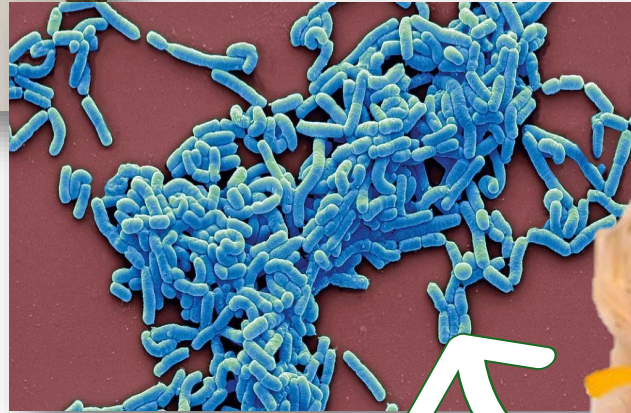
Choline



Choline:
0.25mg/embryo



Prebiotic
Fructooligosaccharides



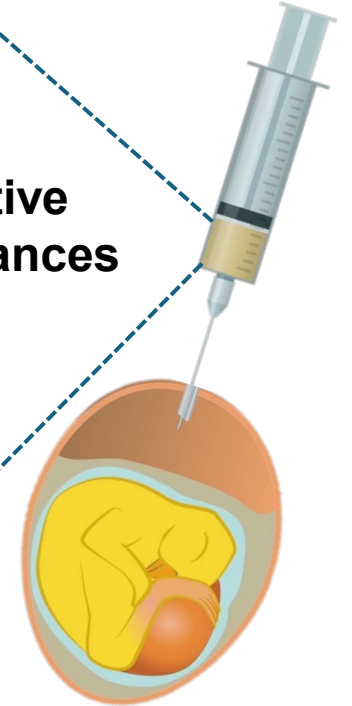
Probiotic strains belonging
to the genera *Enterococcus*,
Pediococcus, *Lactobacillus*,
Bifidobacterium

BIOMIN
PoultryStar®
Synbiotic



**Bioactive
substances**

Synbiotic:
2mg/embryo



Total injected volume:
200µl



**Males
21 weeks old**

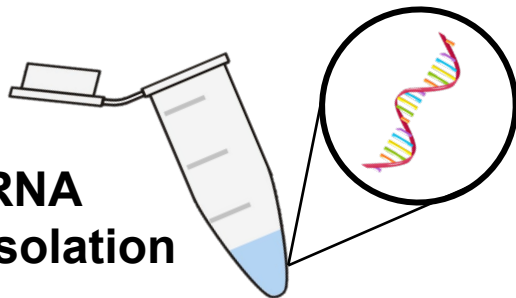
1

**Tissue sampling
Gonadal tissues**



2

**RNA
isolation**



3

**Transcriptomic
sequencing
(RNA-Seq)**

Platform: Illumina
Novaseq6000
Strategy: PE150



4

Bioinformatic analysis

Quality control of raw data
FastQC software

Trimming
Fastp software

Quality control
FastQC software

Mapping
(Gallus gallus
bGalGal1.mat.broiler.GRCg7b),
STAR v2.7.10a software

Differential expression analysis
DESeq2 v.1.34.0, an R/Bioconductor
package

5

Enrichment



GO:
Gene
Ontology

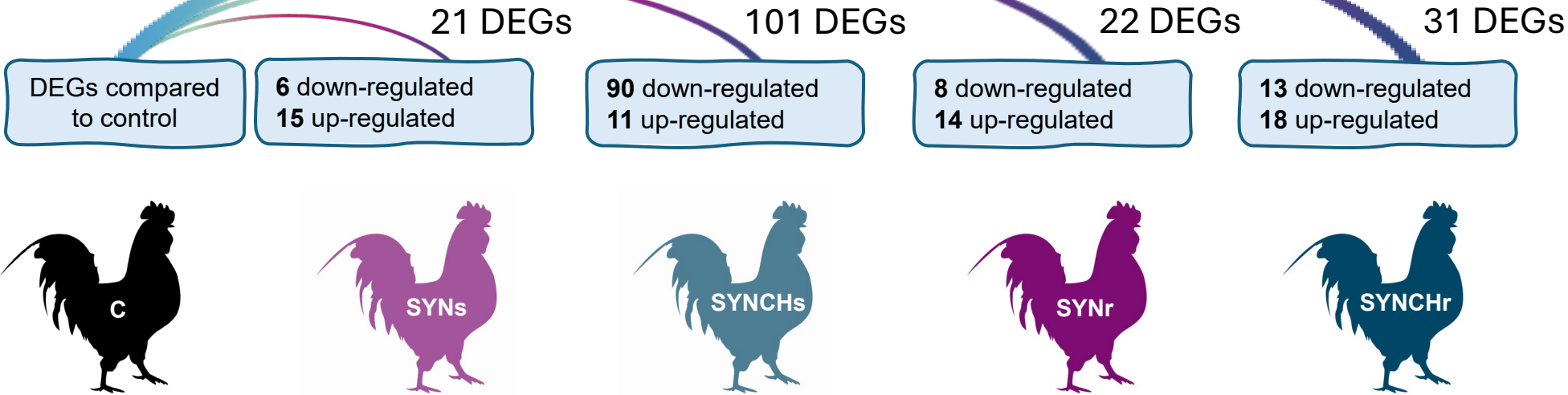
KEGG:
Kyoto
Encyclopedia of
Genes and
Genomes



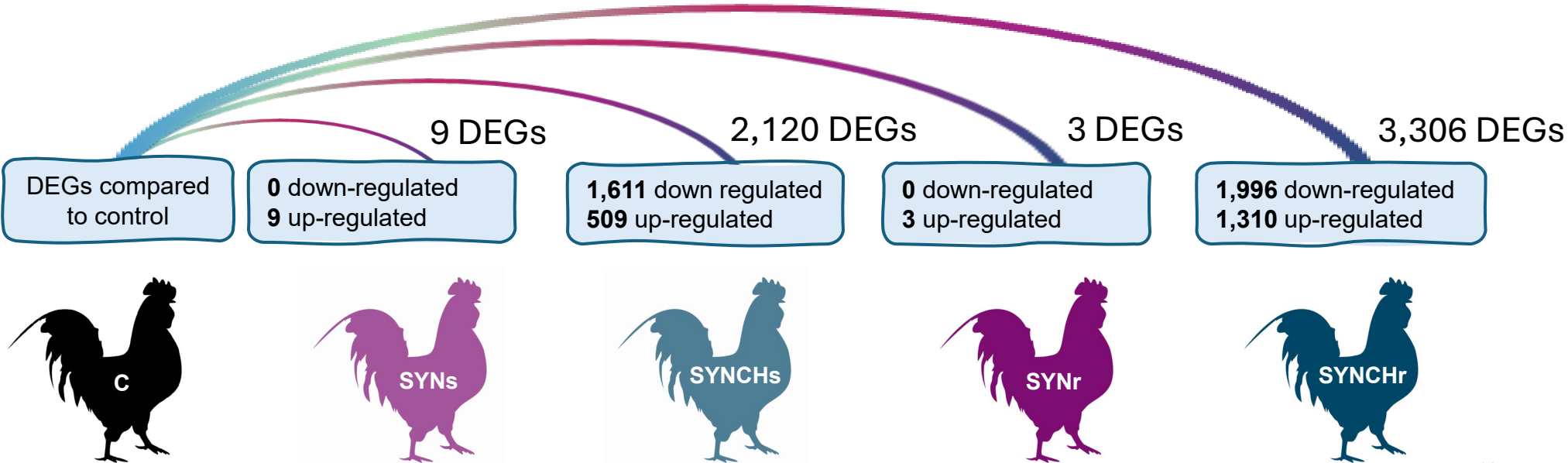
4. Results

Differentially expressed genes (DEGs)

F2 generation



F3 generation

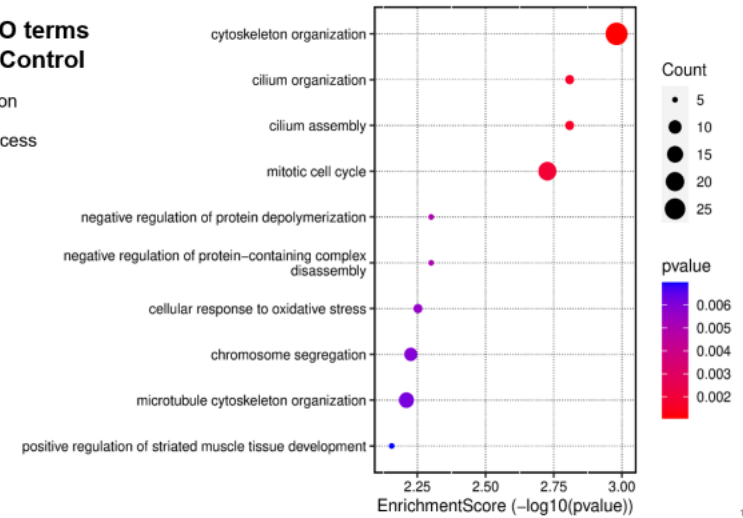


Significant GO terms SYNCHs vs. Control

F3 generation

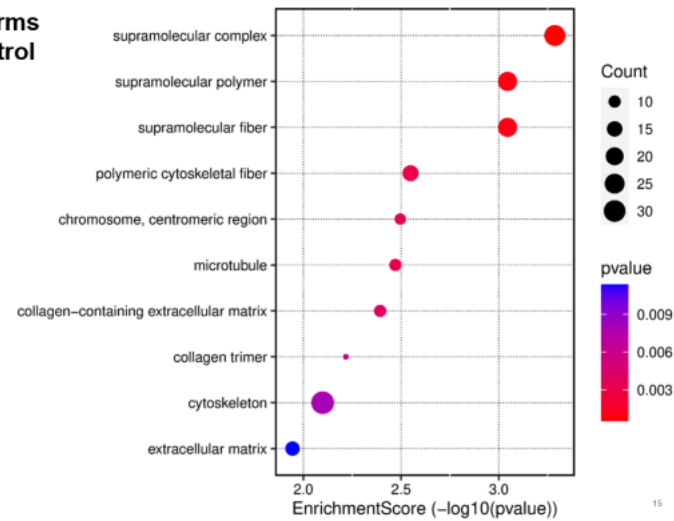
Significant GO terms SYNCHs vs. Control

F3 generation
Biological Process



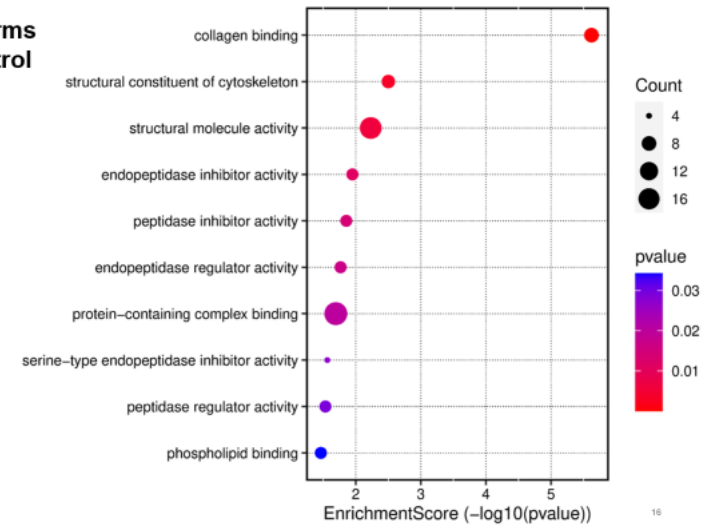
Significant GO terms SYNCHs vs. Control

F3 generation
Cellular Component



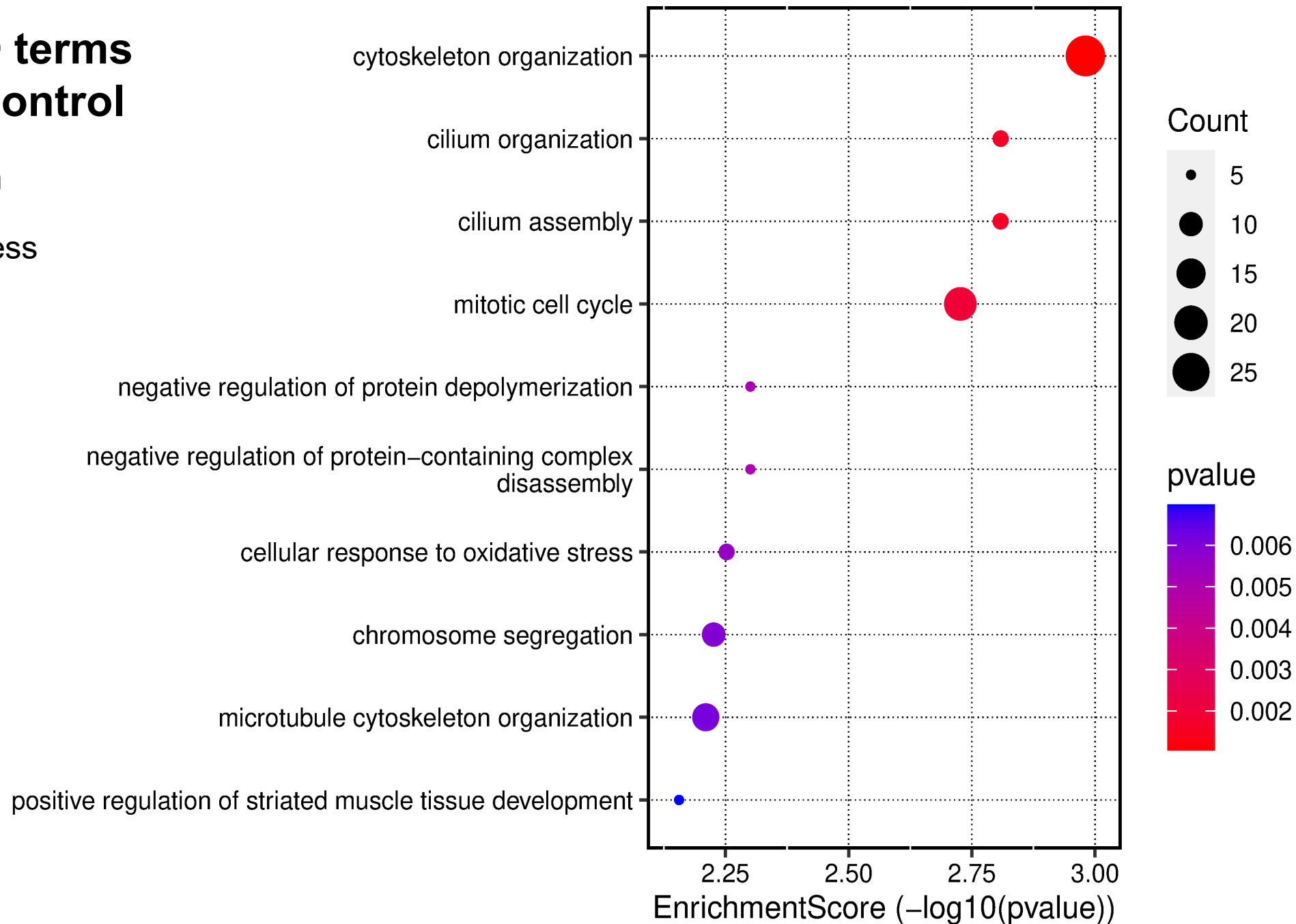
Significant GO terms SYNCHs vs. Control

F3 generation
Molecular Function



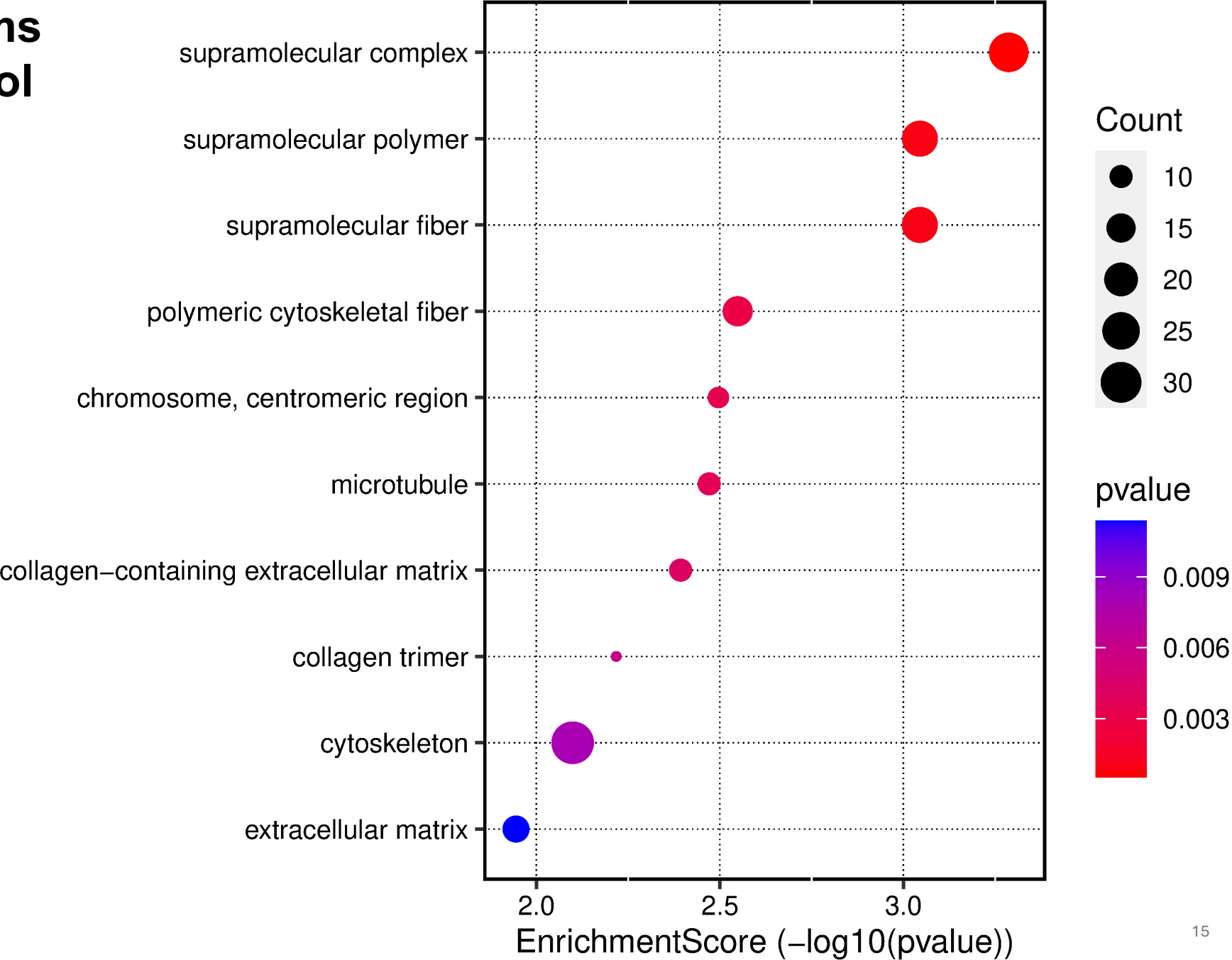
Significant GO terms SYNCHs vs. Control

F3 generation
Biological Process



Significant GO terms SYNCHs vs. Control

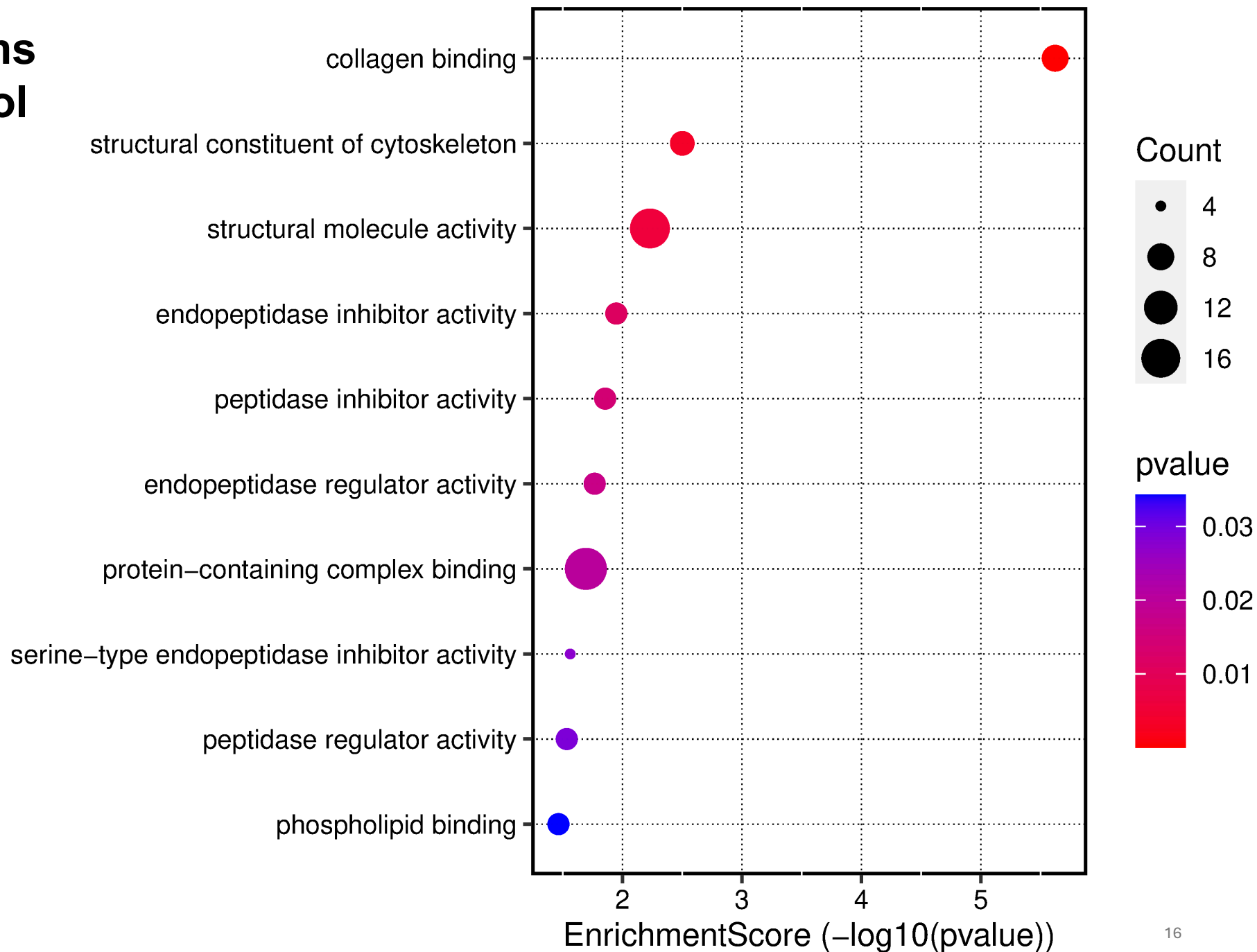
F3 generation
Cellular Component



Significant GO terms SYNCHs vs. Control

F3 generation

Molecular Function

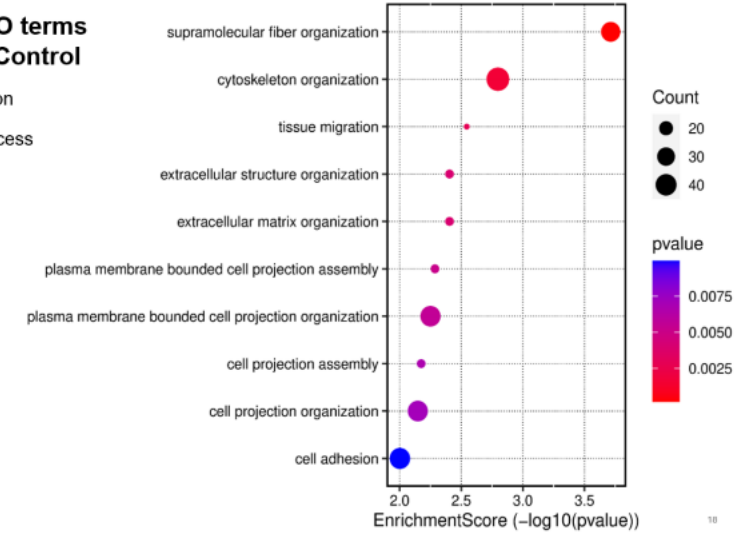


Significant GO terms SYNCHr vs. Control

F3 generation

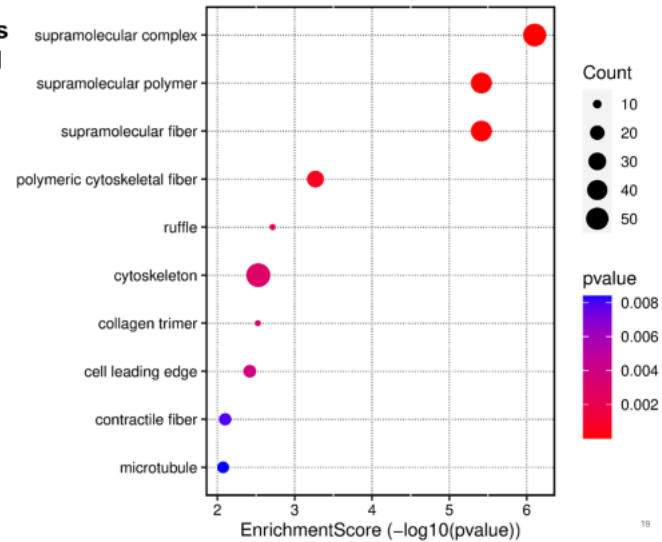
Significant GO terms SYNCHr vs. Control

F3 generation
Biological Process



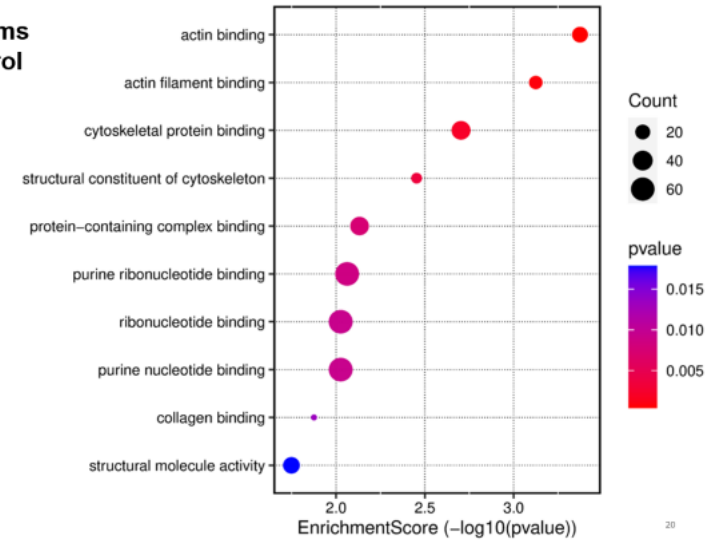
Significant GO terms SYNCHr vs. Control

F3 generation
Cellular Component



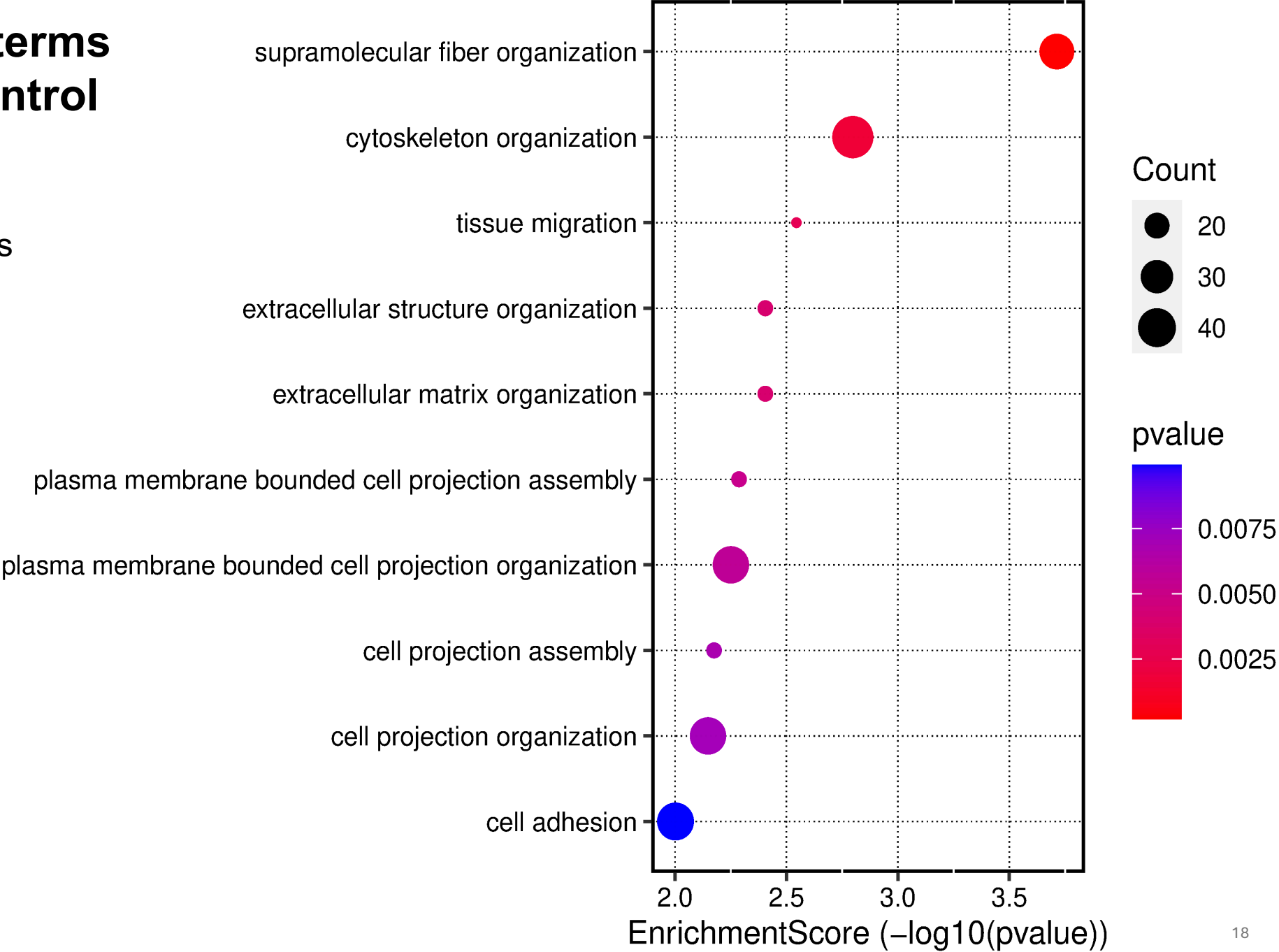
Significant GO terms SYNCHr vs. Control

F3 generation
Molecular Function



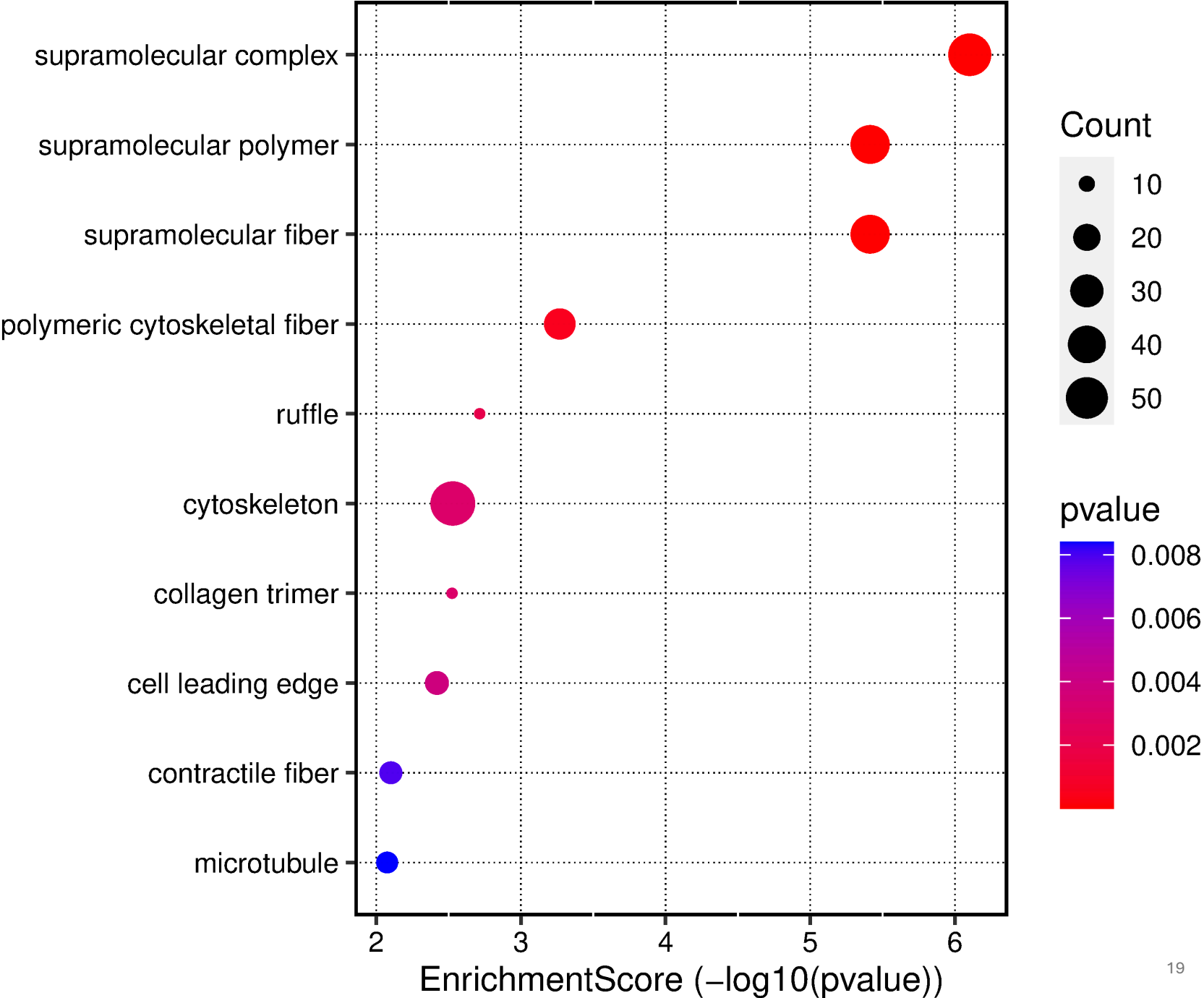
Significant GO terms SYNCHr vs. Control

F3 generation
Biological Process



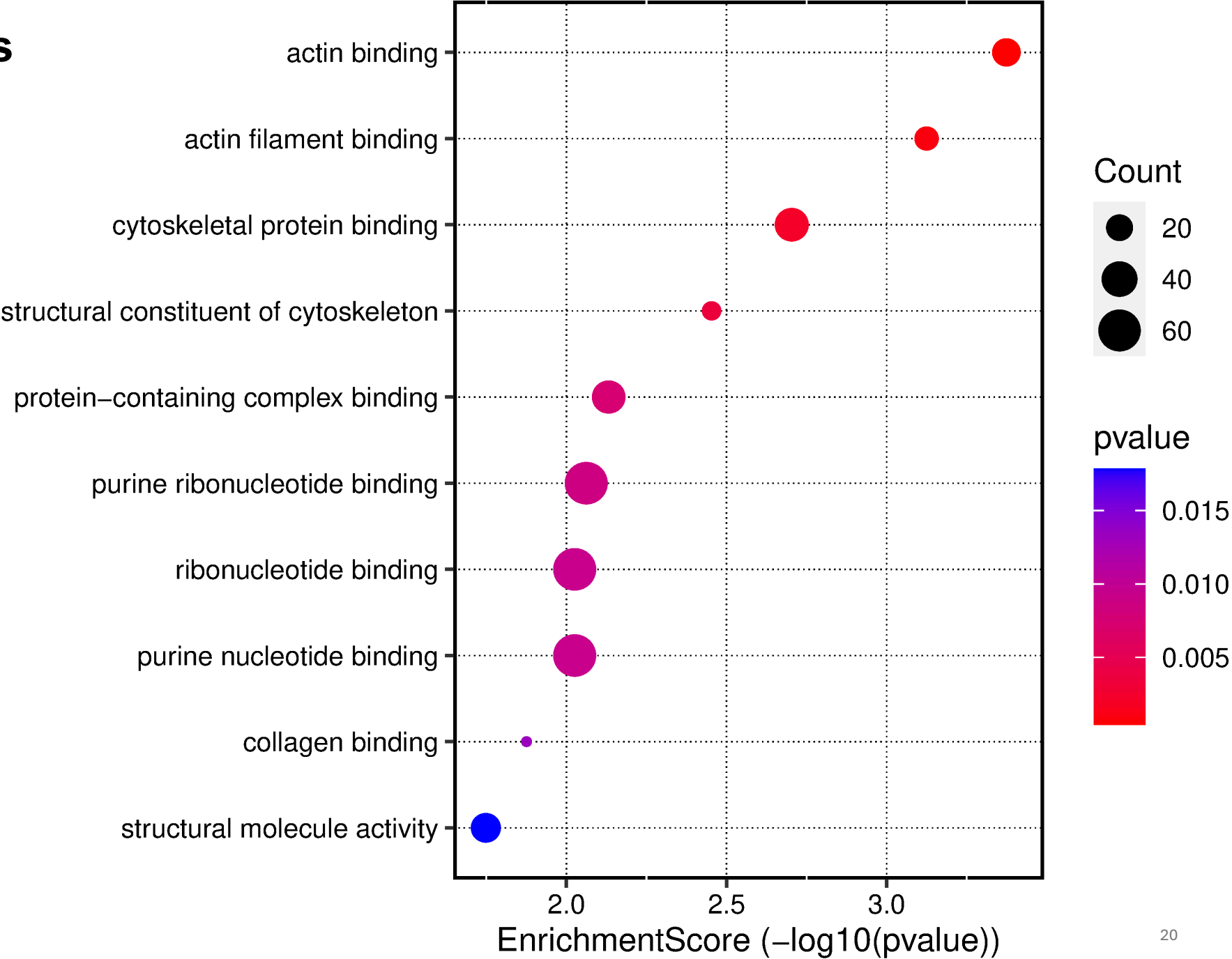
Significant GO terms SYNCHr vs. Control

F3 generation
Cellular Component



Significant GO terms SYNCHr vs. Control

F3 generation
Molecular Function



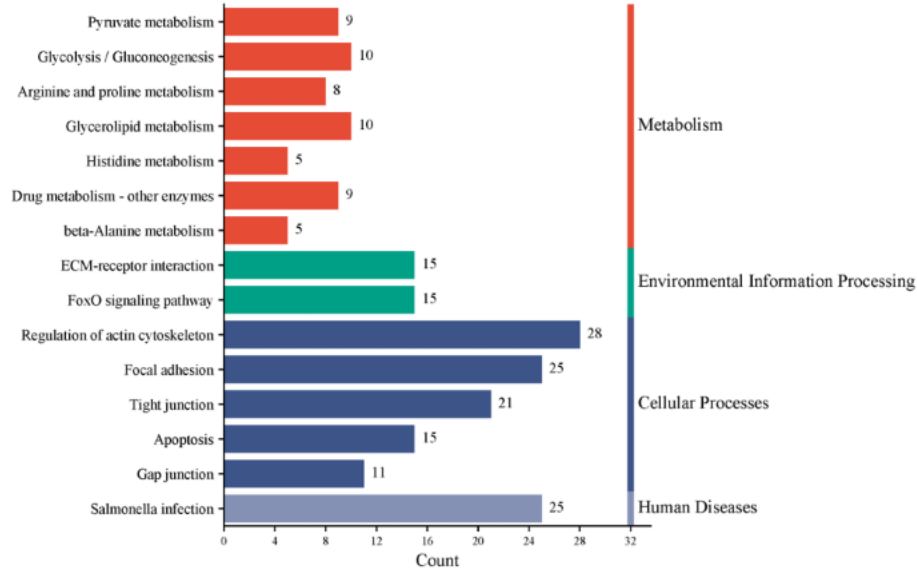
KEGG pathway enrichment category plot

F3 generation

KEGG pathways

F3 generation

SYNCHs vs. Control

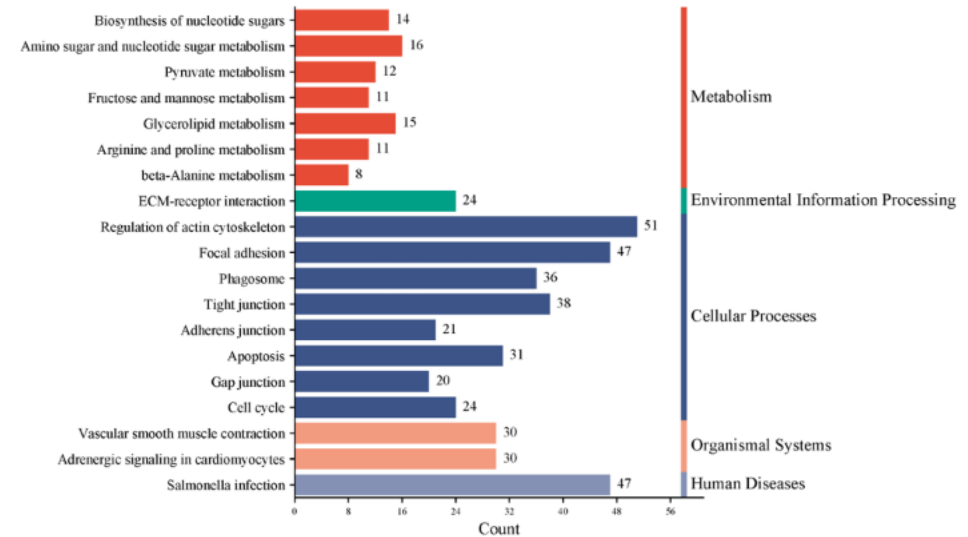


22

KEGG pathways

F3 generation

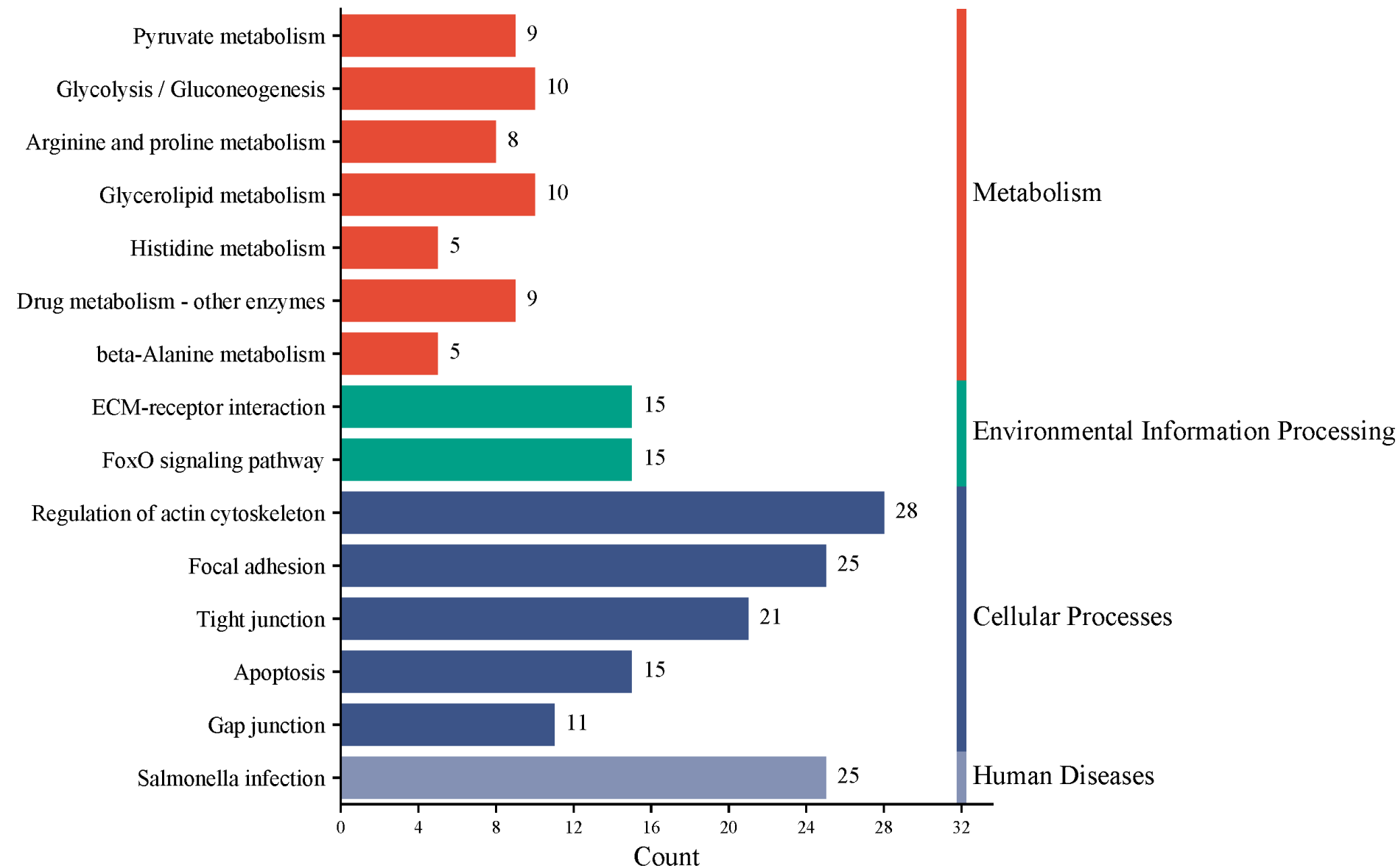
SYNCHr vs. Control



23

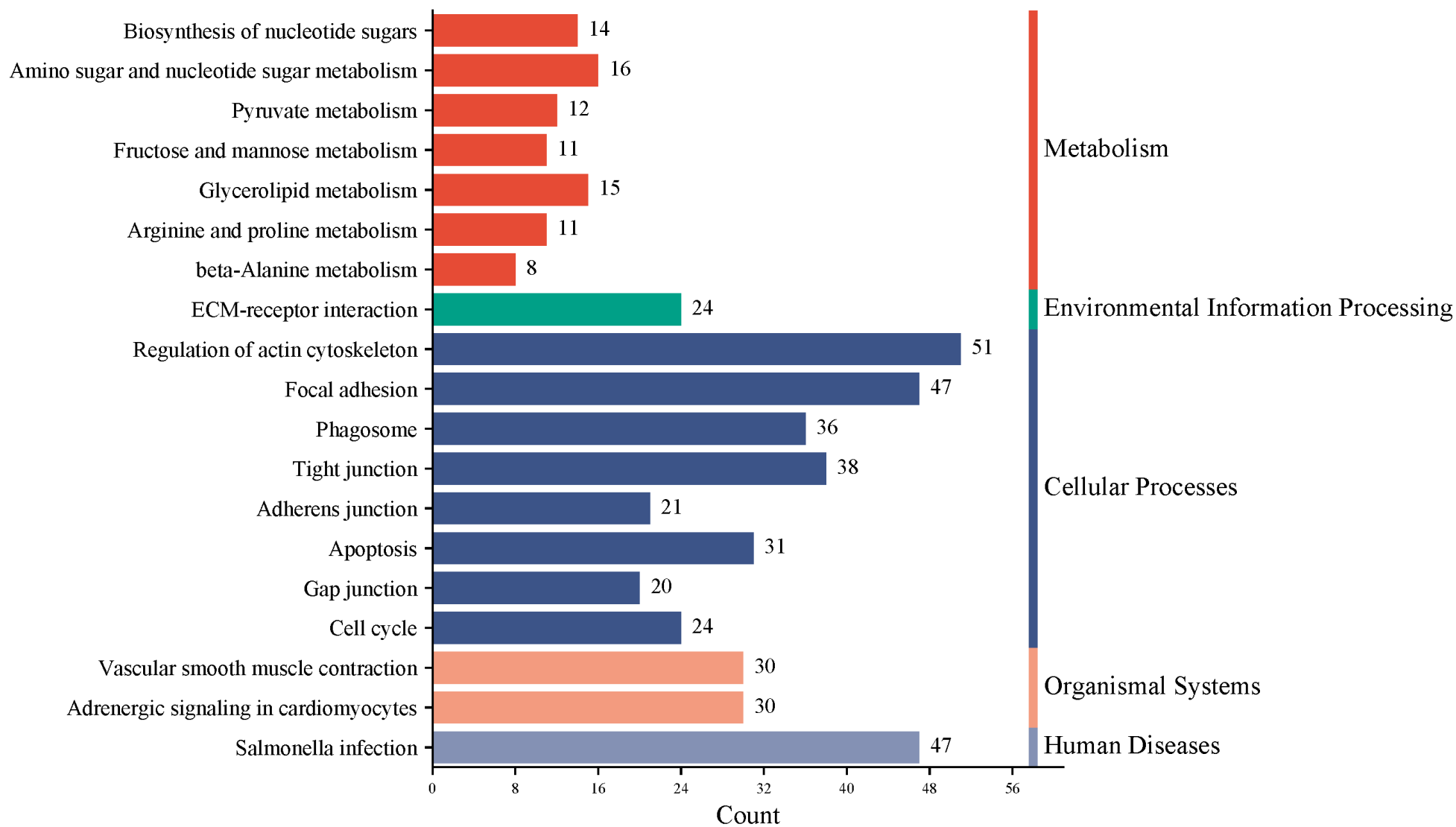
KEGG pathways

F3 generation
SYNCHs vs. Control



KEGG pathways

F3 generation
SYNCHr vs. Control





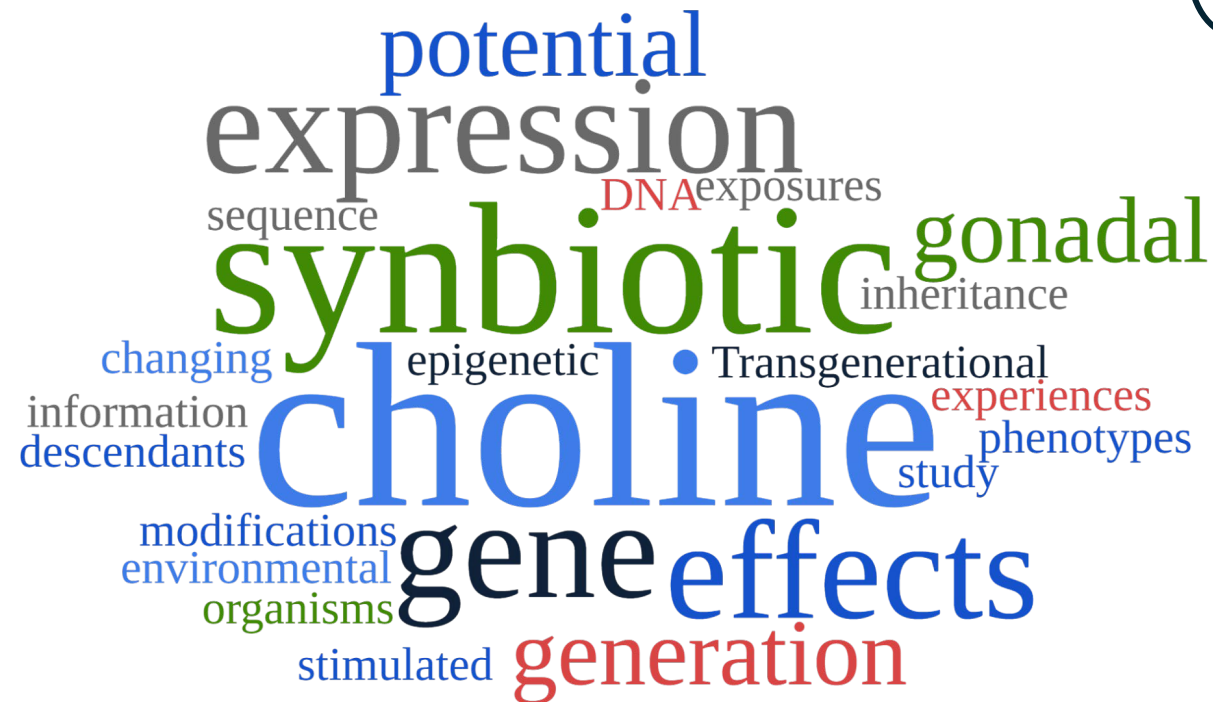
5. Conclusion

Minimal Impact of Synbiotic Alone

Synbiotic administration alone resulted in minimal changes to gene expression in adult male gonads, even with repeated administration.

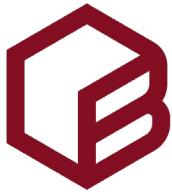
Enhanced Impact with Choline

Adding choline to the synbiotic resulted in a significant effect on gene expression. This effect was amplified in the F3 generation, even with a single injection in F1.



Transgenerational Effect

The combination of choline and synbiotic suggests a potential transgenerational effect, particularly evident in the F3 generation



**POLITECHNIKA
BYDGOSKA**
im. Jana i Jędrzeja Śniadeckich



**NICOLAUS COPERNICUS
UNIVERSITY
IN TORUŃ**



**Thanks for your
attention!**

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