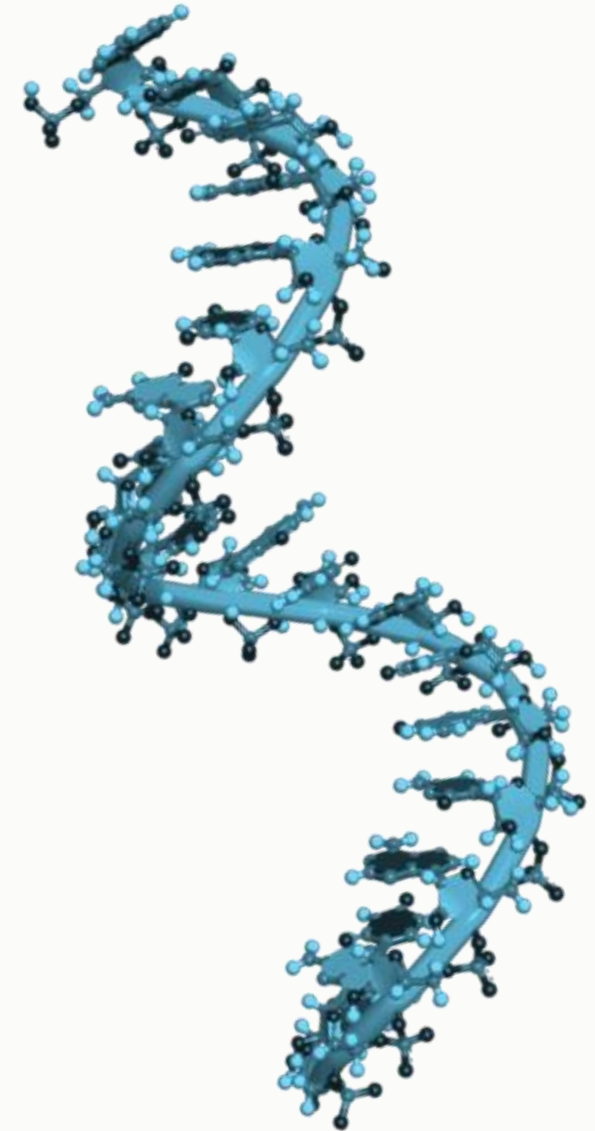


# BULK RNA-SEQ ANALYSIS

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# 1. Quality control

Our tissue and replicates: **Brain** (33, 34, 38), **Colon** (32,33,34), **Muscle** (32,33,34)

	Brain 33	Brain 34	Brain 38	Colon 32	Colon 33	Colon 34	Muscle 32	Muscle 33	Muscle 34
RIN	6.7	6	7.2	6.2	6.3	6.1	7.5	6.2	8.1
% of rRNA reads	6.4	6.7	7.8	0.5	1.4	0.5	1.2	5.0	0.9
% of uniquely mapped reads	91.4	87.5	91.3	89.6	88.9	91.7	89.6	92.2	89

## Thresholds used:

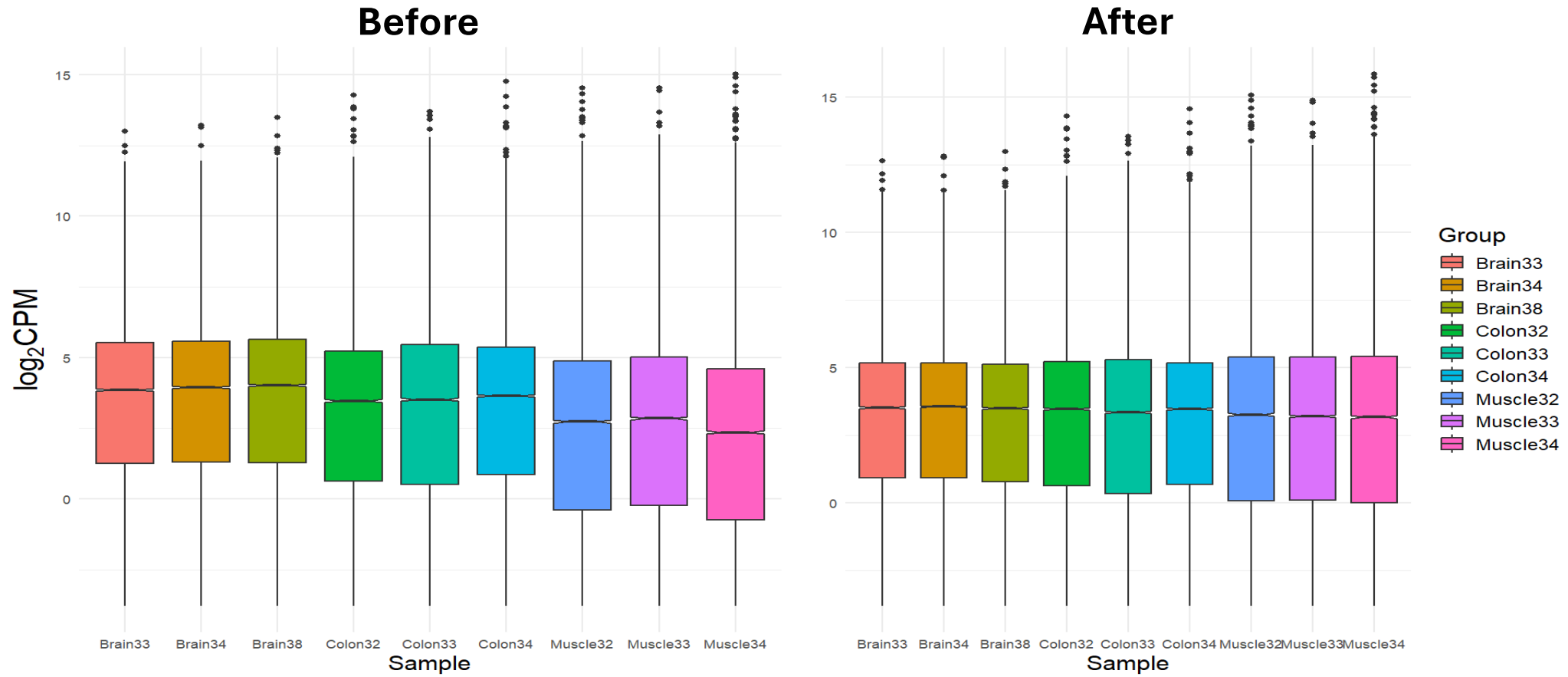
- $RIN \geq 6.0$
- % of rRNA reads  $\leq 10\%$
- % of uniquely mapped reads  $\geq 85\%$



We started with 32, 33 and 34 for all tissues, but then we needed to discard the brain samples 32, 35, 36 and 37 because:

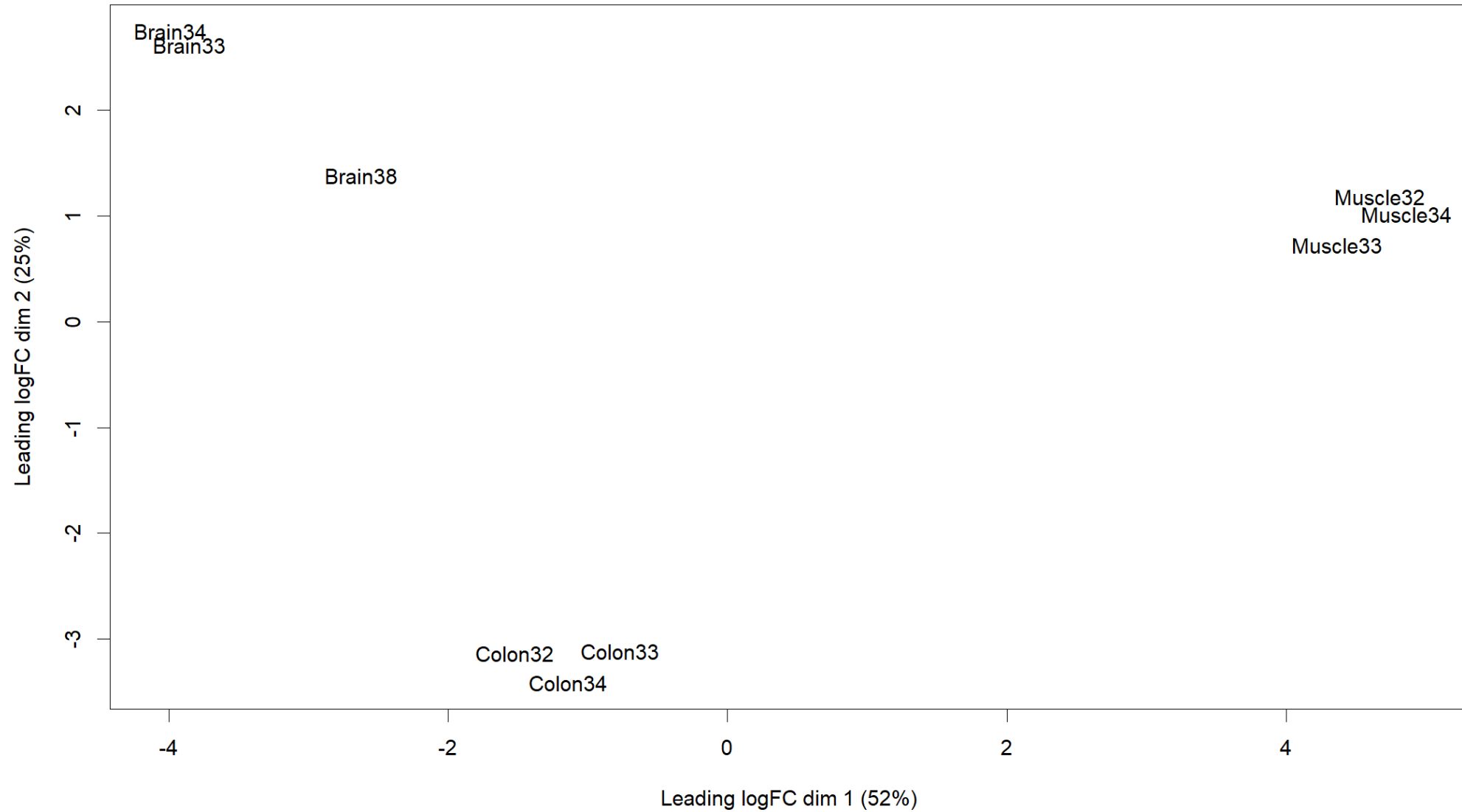
- Brain32 has a % of uniquely mapped reads of 80.4%
- Brain35,36,37 have a RIN equal to 5.7, 5.7 and 5.9, respectively

## 2. Normalization

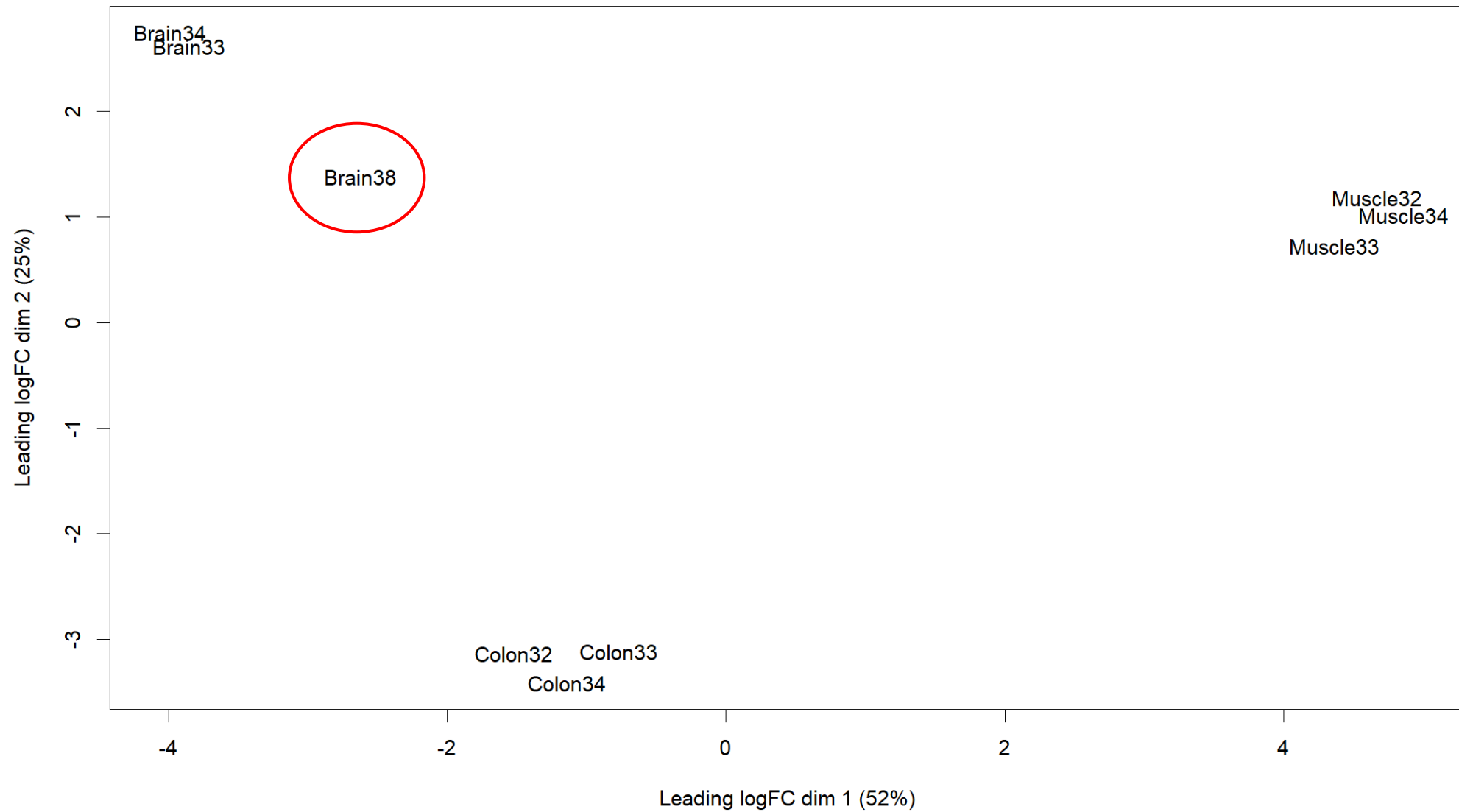


	Brain 33	Brain 34	Brain 38	Colon 32	Colon 33	Colon 34	Muscle 32	Muscle 33	Muscle 34
Normalization factor	1.29	1.32	1.45	1.01	1.13	1.15	0.69	0.79	0.57

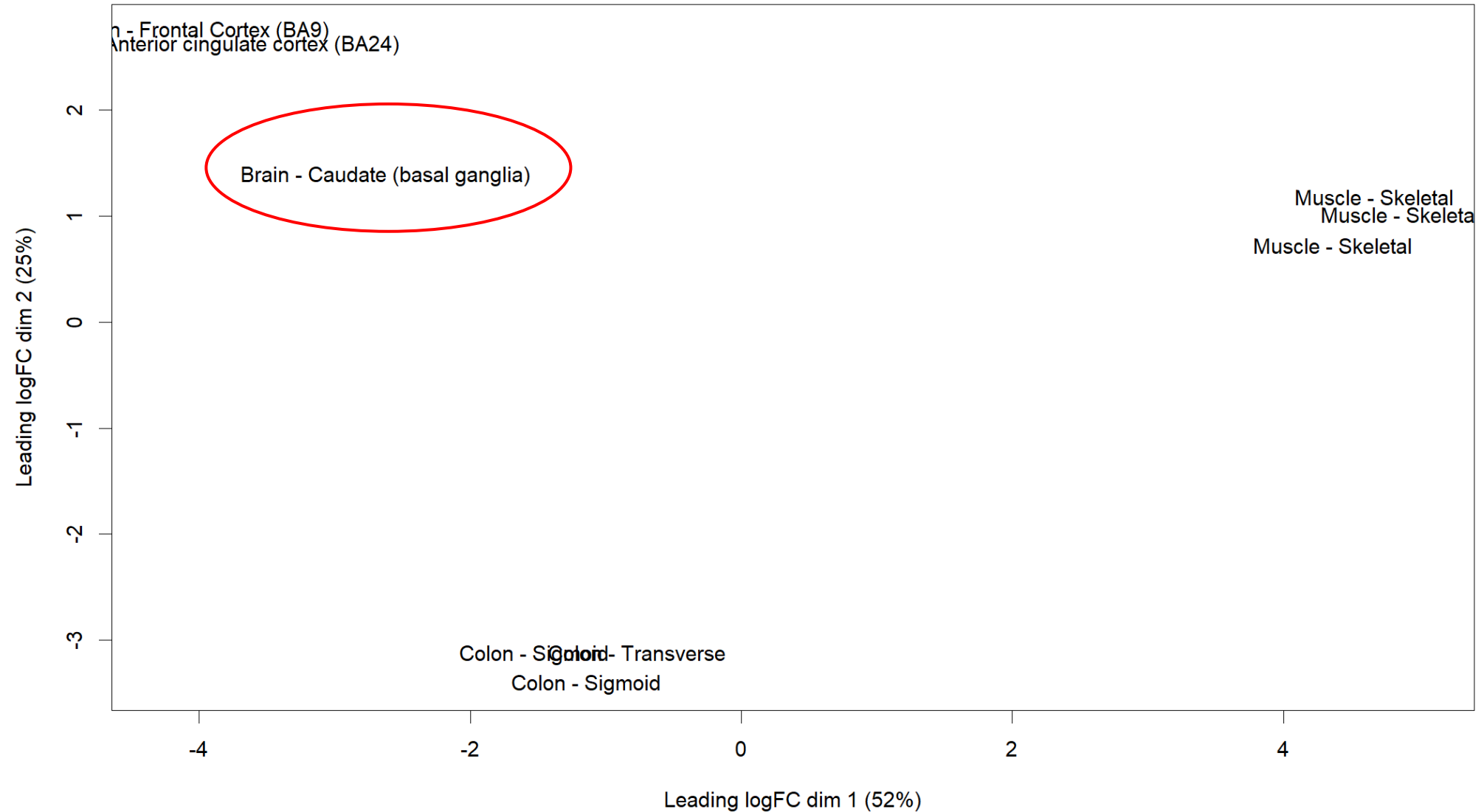
### 3. 2D-projection

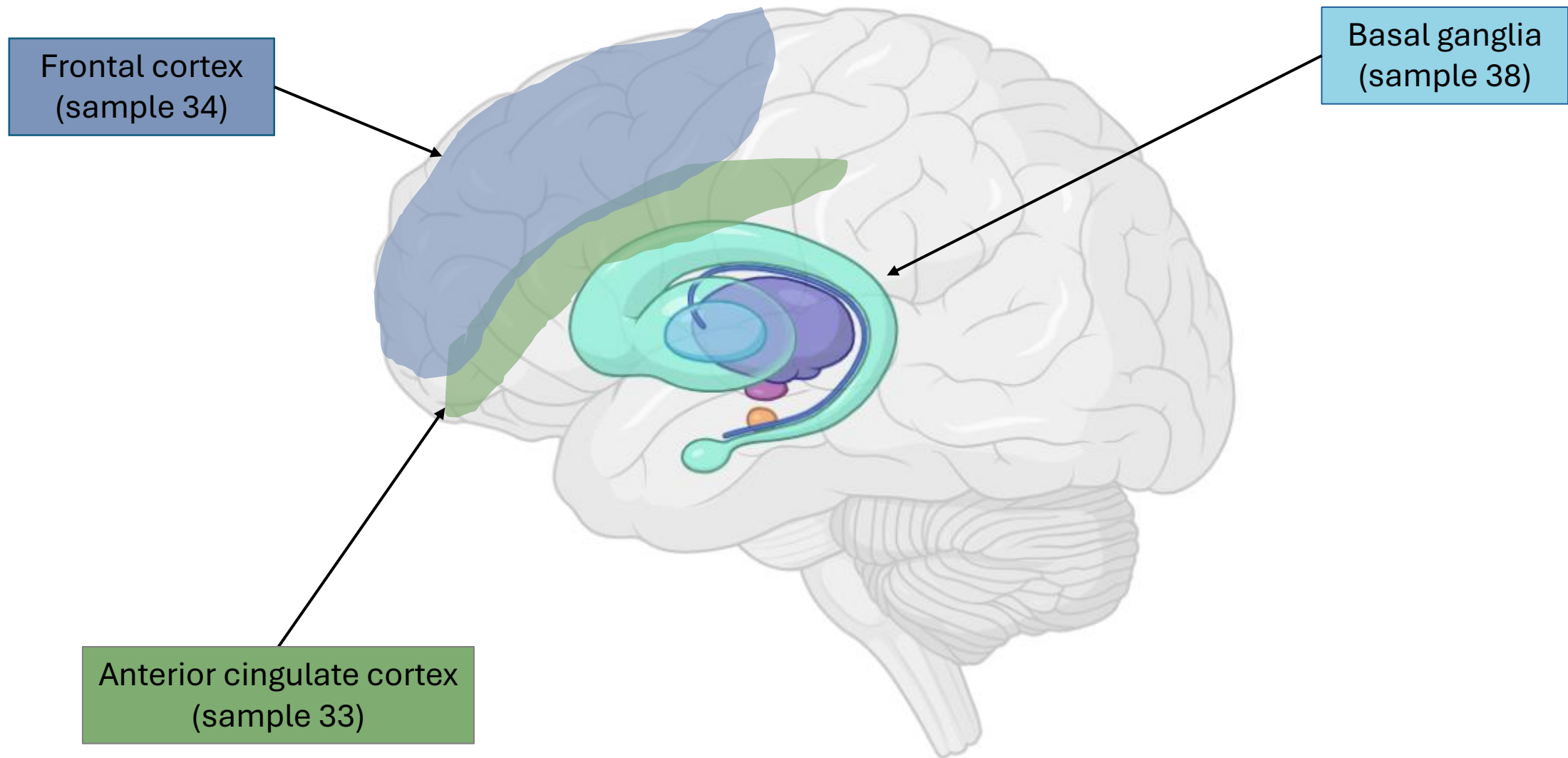


### 3. 2D-projection



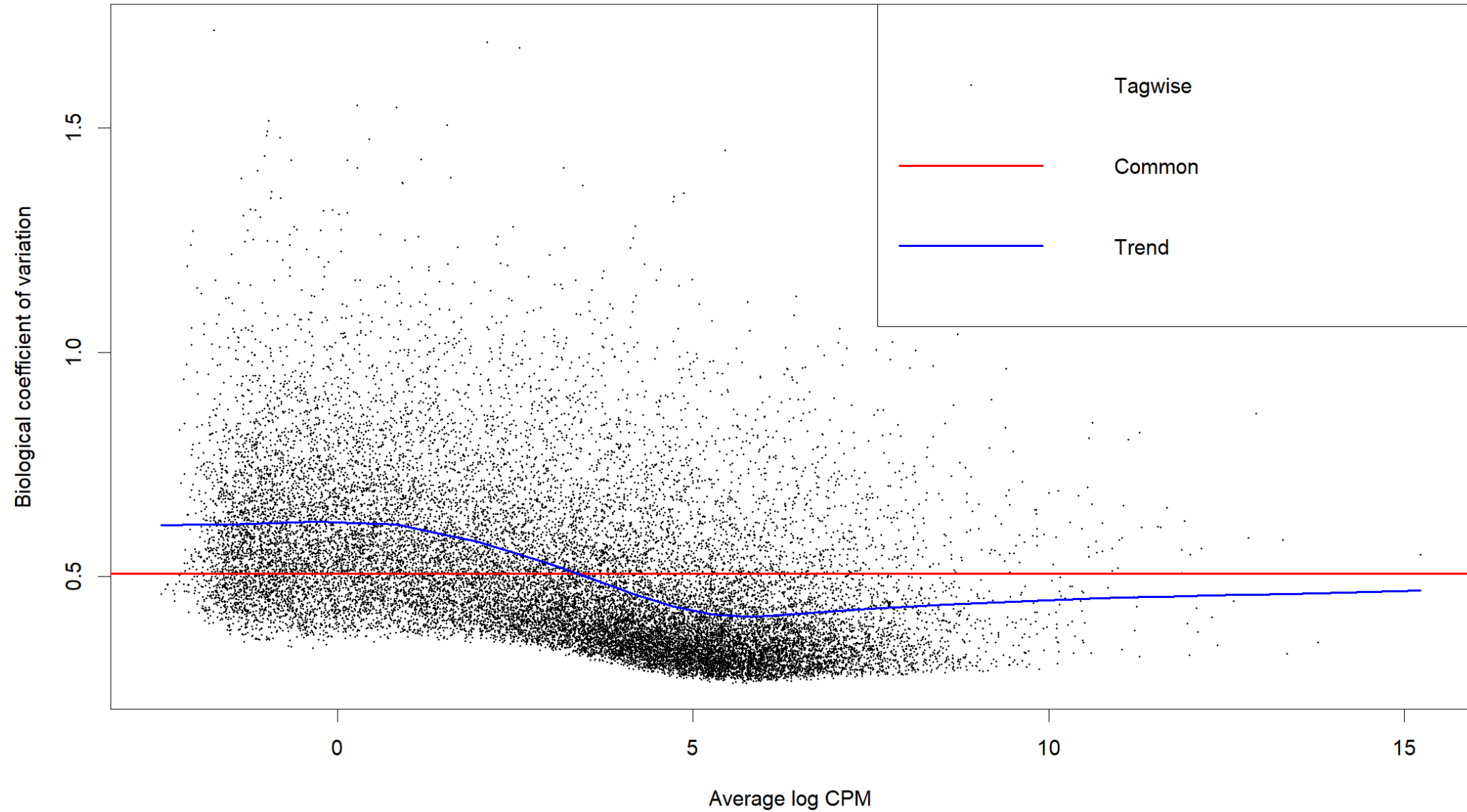
### 3.1. 2D-projection: slice



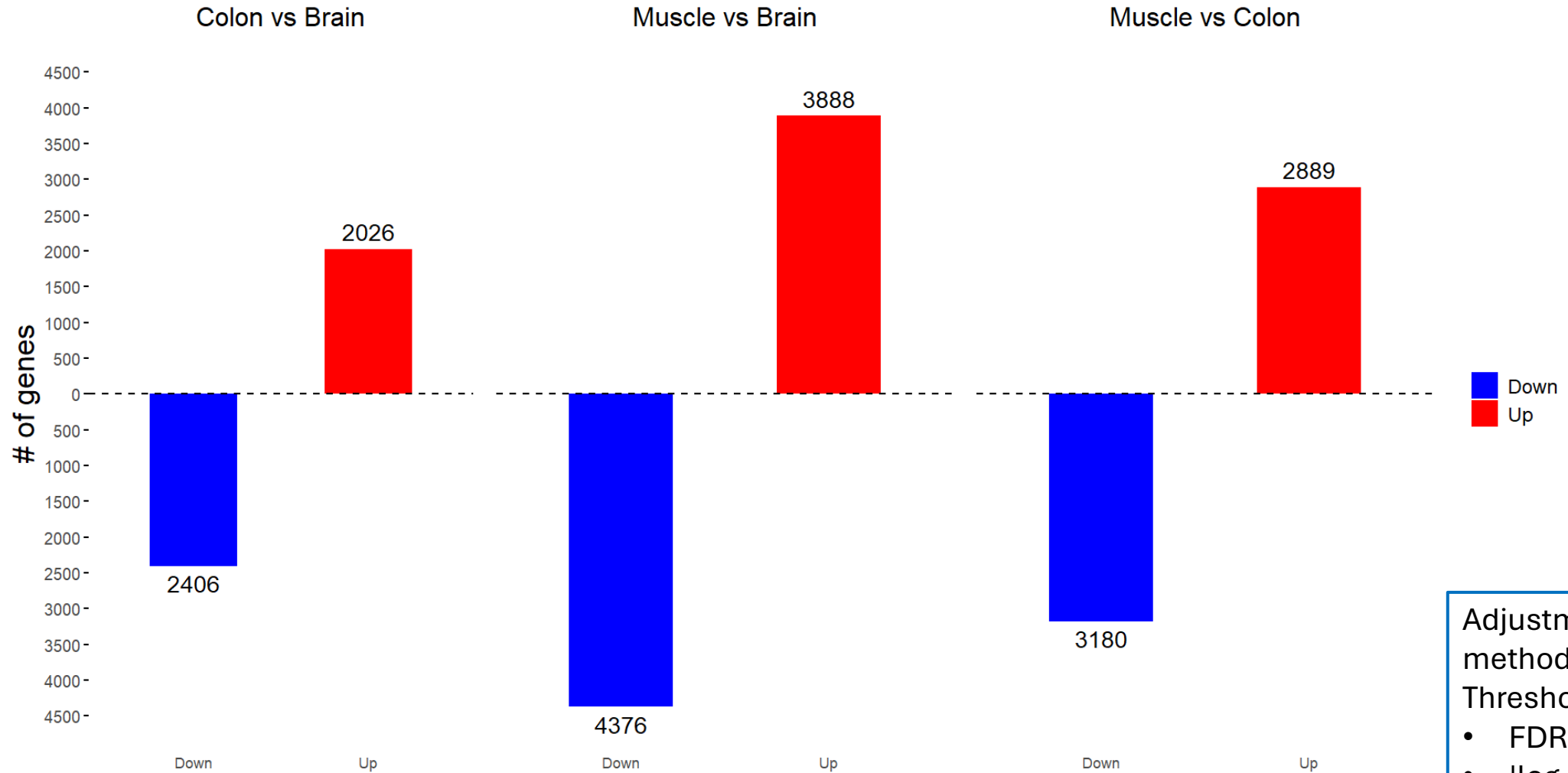




## 4. Relationship mean-variance



## 5. DE genes

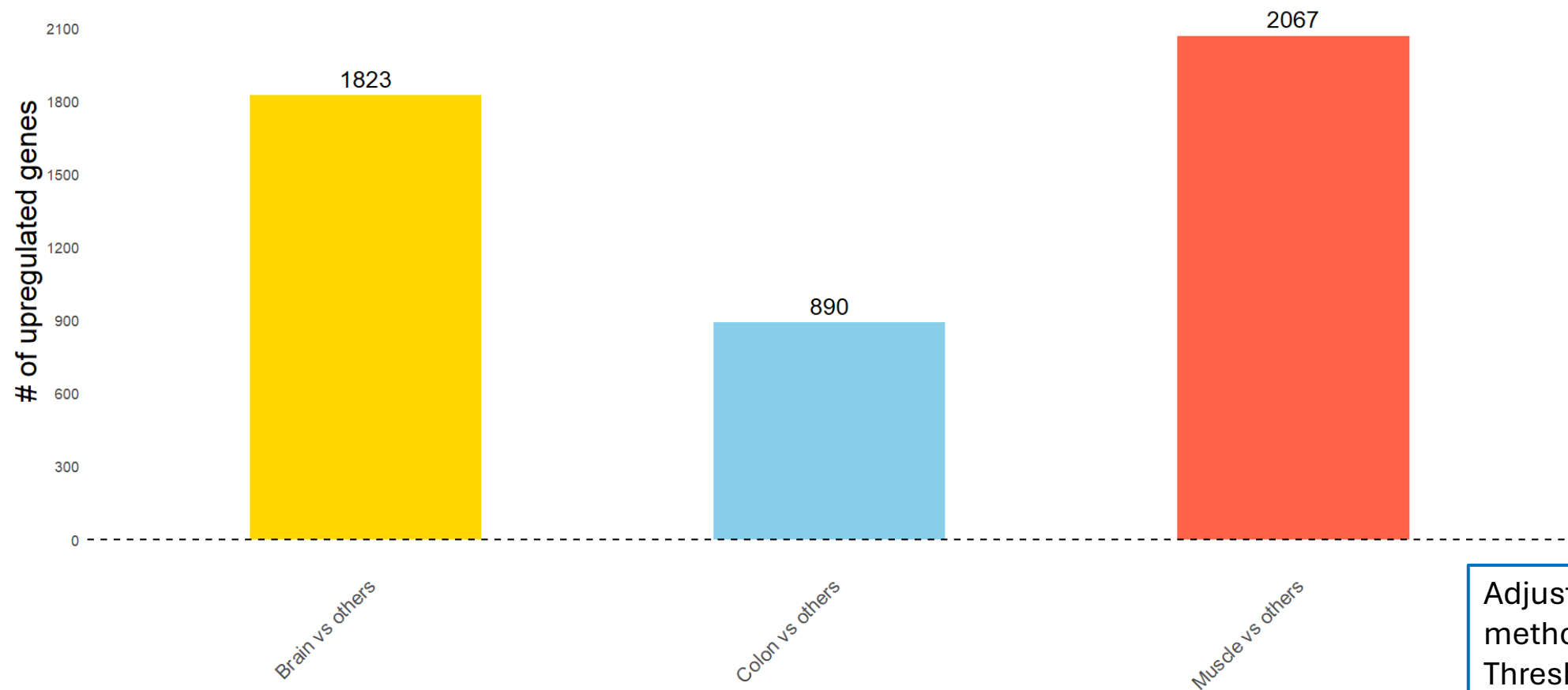


Adjustment  
method: BH  
Thresholds used:

- $FDR < 0.05$
- $|\log_2 FC| > 0$

*Genes are up- and down-regulated in the first condition w.r.t. the second one.*

## 5. DE genes

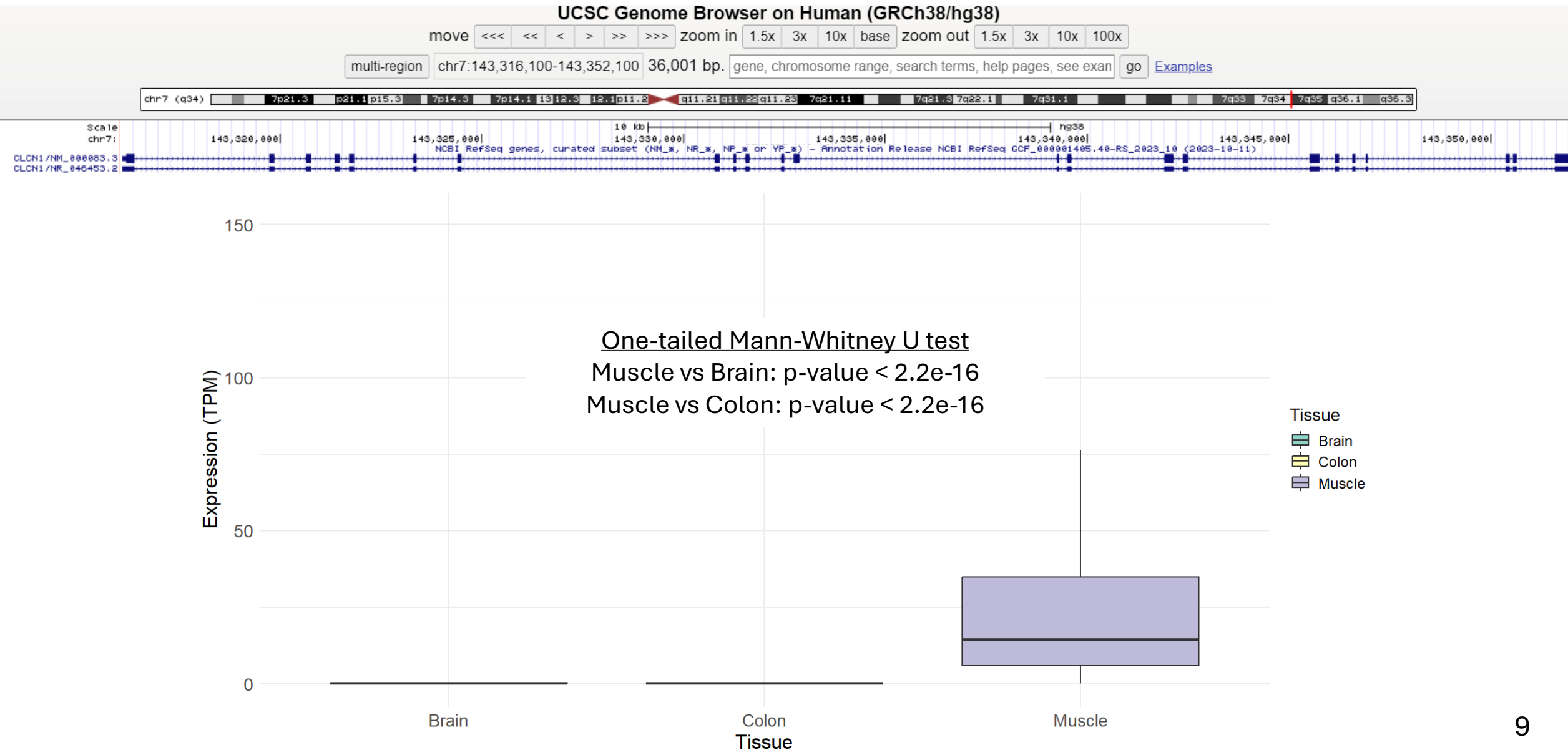


Adjustment  
method: BH  
Thresholds used:

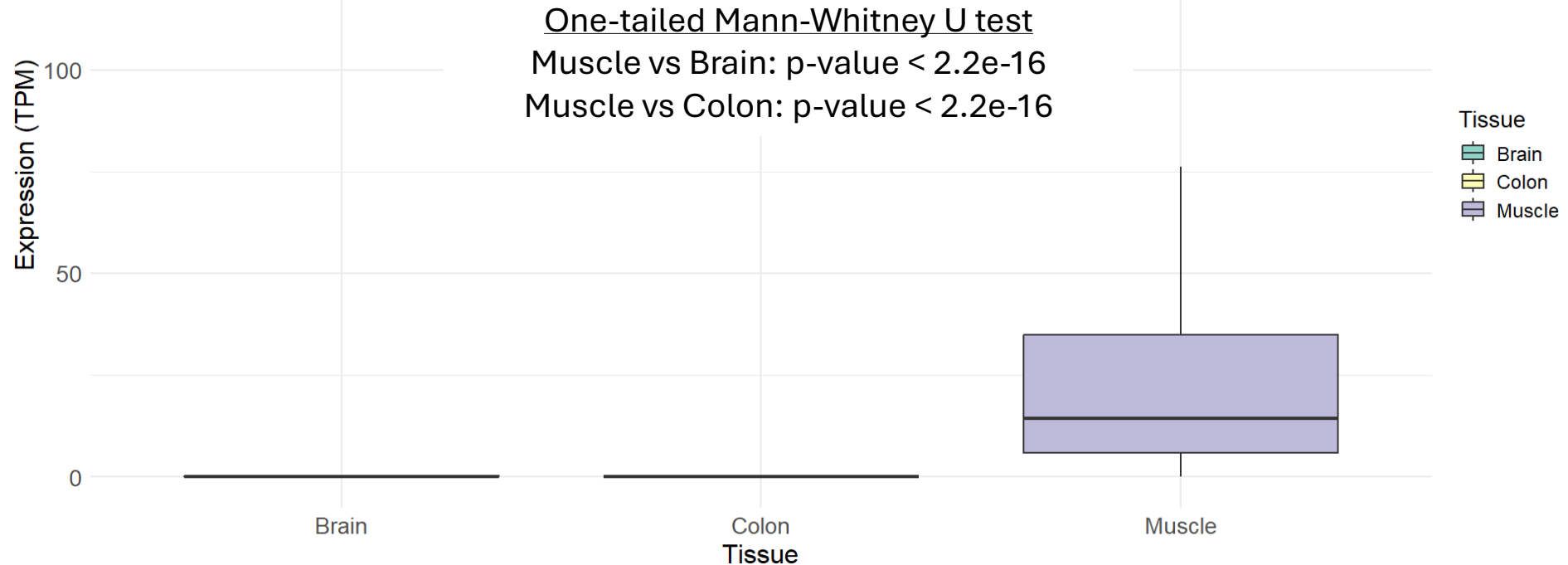
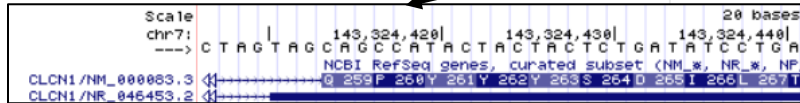
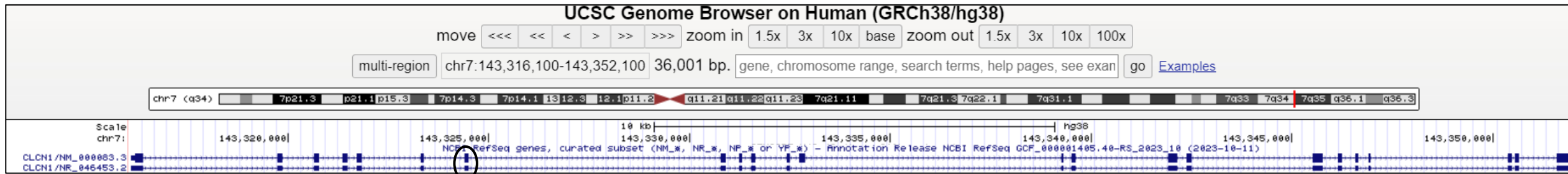
- $\text{FDR} < 0.05$
- $|\log_2\text{FC}| > 0$

*Genes are upregulated in the first condition w.r.t. the others two.*

## 6. One example gene: CLCN1



## 6. One example gene: CLCN1



## 7. Functional enrichment analysis

### Brain

#### GO Cellular Component 2023

Neuron Projection (GO:0043005)  
Postsynaptic Density (GO:0014069)  
Postsynaptic Density Membrane (GO:009888)  
Postsynaptic Specialization Membrane (GO:009889)  
Dendrite (GO:0030425)

#### Reactome 2022

Neuronal System R-HSA-112316  
Transmission Across Chemical Synapses R-HSA-112317  
Neurotransmitter Receptors And Postsynaptic Receptors R-HSA-112318  
Protein-protein Interactions At Synapses R-HSA-112319  
Neurotransmitter Release Cycle R-HSA-112320

### Colon

#### KEGG 2021 Human

Protein digestion and absorption  
Neuroactive ligand-receptor interaction  
Calcium signaling pathway  
ECM-receptor interaction  
Focal adhesion

#### GWAS Catalog 2023

Tryptase Beta-2 Levels  
PR Interval  
Chronic Obstructive Pulmonary Disease Liability  
Joint Mobility (Beighton Score)  
Hernia (Any Subtypes)

### Muscle

#### MGI Mammalian Phenotype Level 4 2021

impaired skeletal muscle contractility MP:0000001  
centrally nucleated skeletal muscle fibers MP:0000002  
abnormal muscle physiology MP:0002106  
abnormal skeletal muscle fiber morphology MP:0000003  
muscle weakness MP:0000747

#### GO Biological Process 2023

Cellular Respiration (GO:0045333)  
Mitochondrial ATP Synthesis Coupled Electron Transport (GO:0006713)  
Oxidative Phosphorylation (GO:0006119)  
Aerobic Electron Transport Chain (GO:0019608)  
Proton Motive Force-Driven Mitochondrial ATP Synthesis (GO:0006714)

**THANKS FOR YOUR ATTENTION!**