



# ACTIVE MOTIF CHIPSEQ ANALYSIS WT & Q111het

Amol Carl Shetty

October 5, 2021



# Dataset Descriptions

- Dataset (ActiveMotif)

|                            |               |              |
|----------------------------|---------------|--------------|
| Samples                    | 30            |              |
| Conditions                 | EZH2 Wt       | 3 replicates |
|                            | EZH2 Q111     | 3 replicates |
|                            | H3K4me3 Wt    | 3 replicates |
|                            | H3K4me3 Q111  | 3 replicates |
|                            | H3K9me3 Wt    | 3 replicates |
|                            | H3K9me3 Q111  | 3 replicates |
|                            | H3K27me3 Wt   | 3 replicates |
|                            | H3K27me3 Q111 | 3 replicates |
|                            | H3K27Ac Wt    | 3 replicates |
|                            | H3K27Ac Q111  | 3 replicates |
| Matched ChIP-Input samples | No            |              |
| Tissue                     | Striatal      |              |



UNIVERSITY *of* MARYLAND  
SCHOOL OF MEDICINE

---

# REFERENCE-BASED ALIGNMENT



# Alignment Summary Statistics

- Reads assessed for quality and trimmed (if needed)
- Alignment of reads using ??? against mm10
- Alignment Summary for ActiveMotif

| Histone Mark | Genotype | # Total Reads (million) |      |      | # Mapped Reads (million) |      |      | %Mapped Reads |      |      |
|--------------|----------|-------------------------|------|------|--------------------------|------|------|---------------|------|------|
| EZH2         | Wt       | 35.1                    | 32.9 | 27.3 | 30.1                     | 28.2 | 23.3 | 85.7          | 85.9 | 85.4 |
| EZH2         | Q111     | 27.3                    | 33.9 | 50.5 | 22.4                     | 27.6 | 42.8 | 82.0          | 81.2 | 84.7 |
| H3K4me3      | Wt       | 38.6                    | 42.3 | 37.3 | 31.0                     | 35.7 | 30.8 | 80.4          | 84.4 | 82.6 |
| H3K4me3      | Q111     | 38.3                    | 37.4 | 39.9 | 32.7                     | 31.9 | 33.0 | 85.4          | 85.2 | 82.6 |
| H3K9me3      | Wt       | 37.7                    | 34.7 | 29.7 | 33.2                     | 28.7 | 24.9 | 88.1          | 82.8 | 83.5 |
| H3K9me3      | Q111     | 30.6                    | 32.5 | 30.5 | 26.1                     | 27.8 | 26.2 | 85.3          | 85.5 | 85.8 |
| H3K27me3     | Wt       | 26.7                    | 28.8 | 24.5 | 23.5                     | 25.5 | 21.4 | 88.2          | 88.5 | 87.4 |
| H3K27me3     | Q111     | 23.0                    | 24.5 | 25.4 | 20.1                     | 19.6 | 22.1 | 87.2          | 79.8 | 86.6 |
| H3K27Ac      | Wt       | 29.1                    | 26.0 | 27.2 | 26.5                     | 24.4 | 25.6 | 90.8          | 93.8 | 94.1 |
| H3K27Ac      | Q111     | 27.6                    | 26.3 | 21.8 | 26.3                     | 25.2 | 20.3 | 95.5          | 95.7 | 92.9 |
| Input        | NA       | 35.0                    |      |      | 33.3                     |      |      | 95.1          |      |      |



UNIVERSITY *of* MARYLAND  
SCHOOL OF MEDICINE

---

# PEAK DETECTION



# Peak Calling

- Peaks called by ActiveMotif
- Peak calling using ???

| Dataset  | Genotype | # Peaks |        |        | # Merged Peaks<br>(≥3 samples) |
|----------|----------|---------|--------|--------|--------------------------------|
| EZH2     | Wt       | 24,532  | 8,548  | 9,612  | 12,388                         |
| EZH2     | Q111     | 12,019  | 11,247 | 10,285 |                                |
| H3K4me3  | Wt       | 17,956  | 17,492 | 17,997 | 17,672                         |
| H3K4me3  | Q111     | 16,972  | 17,298 | 17,566 |                                |
| H3K9me3  | Wt       | 26,552  | 15,554 | 11,815 | 22,304                         |
| H3K9me3  | Q111     | 26,462  | 23,819 | 23,158 |                                |
| H3K27me3 | Wt       | 23,684  | 31,231 | 26,022 | 25,315                         |
| H3K27me3 | Q111     | 24,059  | 12,356 | 24,192 |                                |
| H3K27Ac  | Wt       | 26,474  | 27,162 | 25,889 | 27,337                         |
| H3K27Ac  | Q111     | 25,353  | 26,781 | 31,162 |                                |



UNIVERSITY *of* MARYLAND  
SCHOOL OF MEDICINE

---

# DIFFERENTIAL HISTONE MARKS

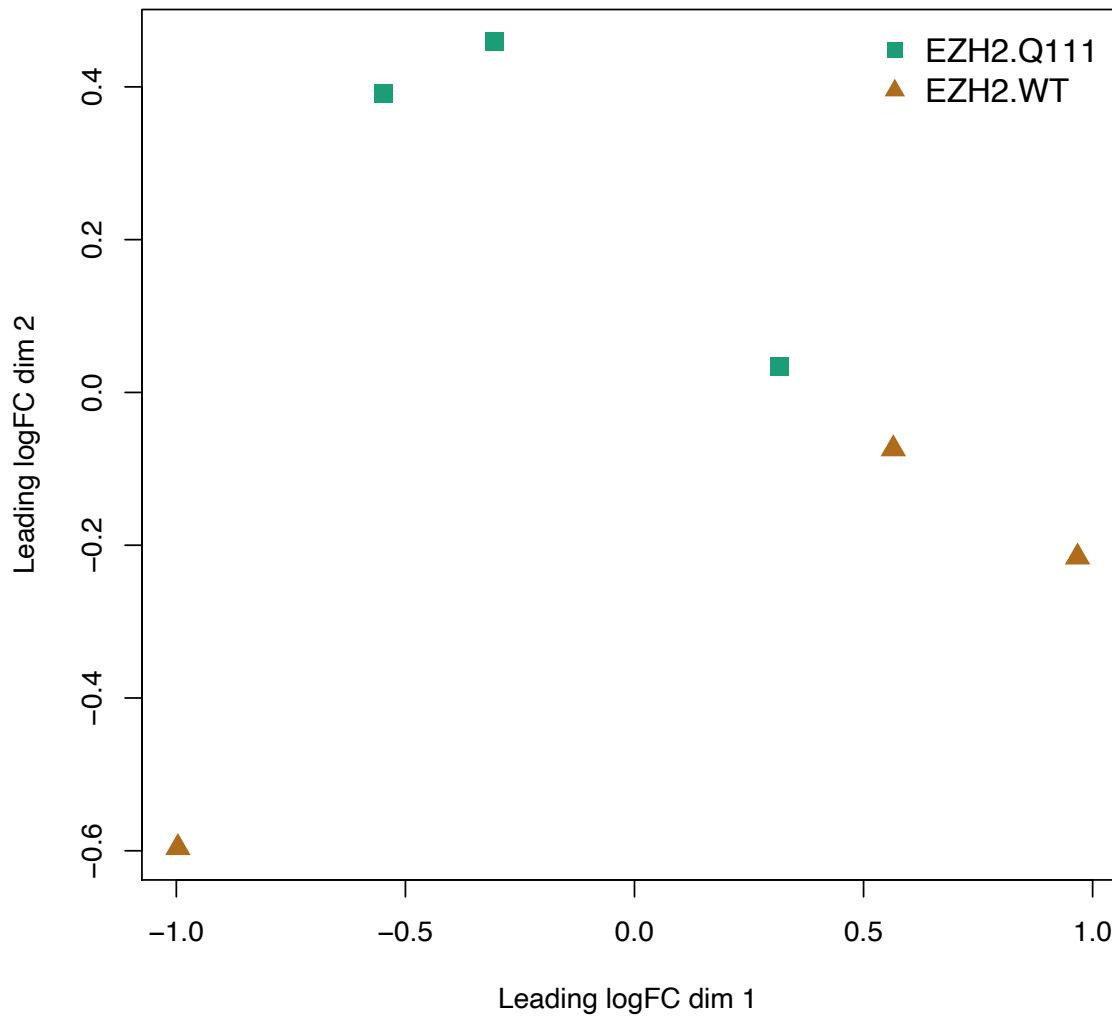


# EZH2 Q111 vs WT



# Differential Binding

- 12,388 total peak regions → peak counts
- Sample clustering





# Differential Binding

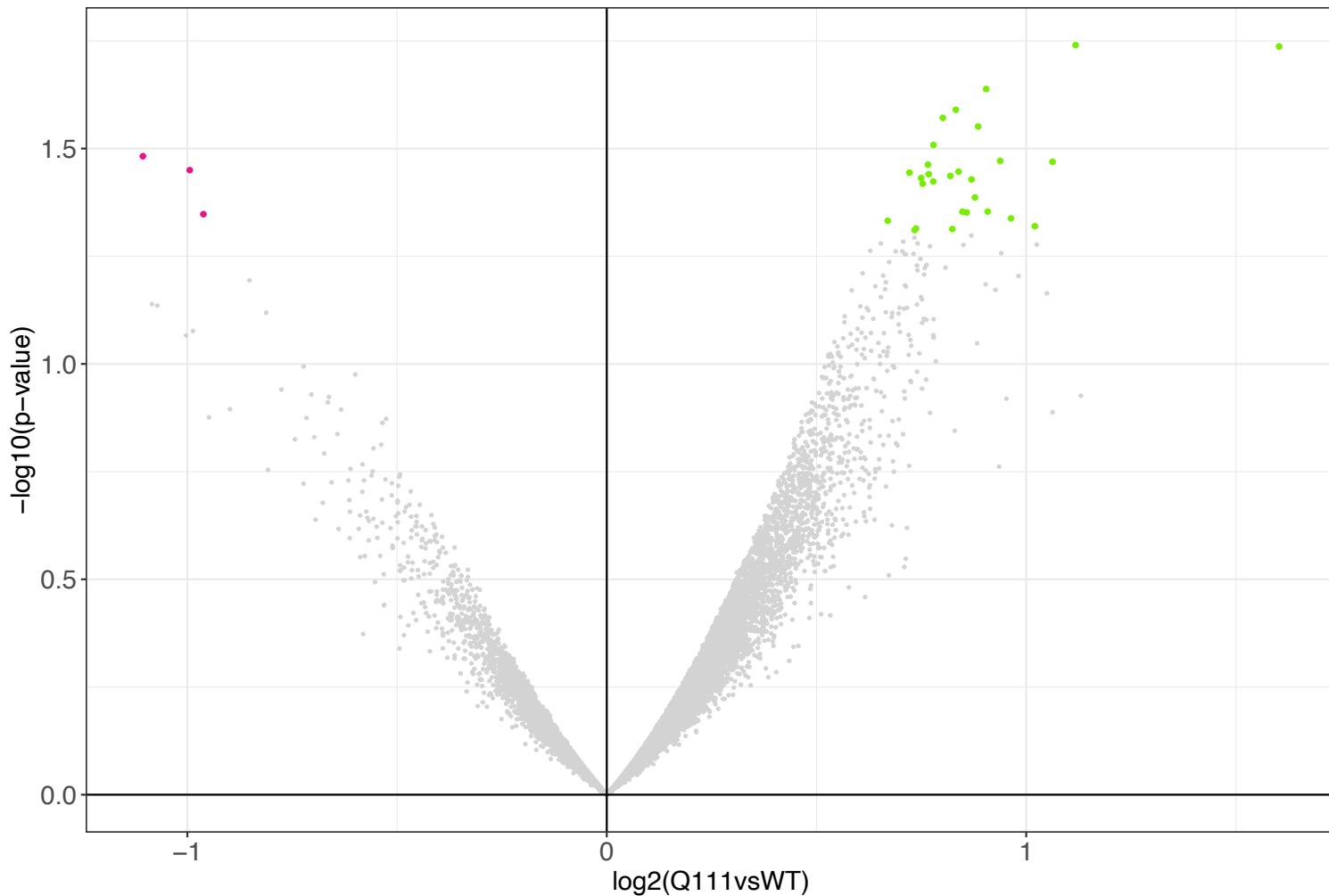
- 12,388 total peak regions → peak counts
- Differential binding between genotypes

| Comparison | Significance Cut-off | # Regions LFC > 0 | # Regions LFC < 0 |
|------------|----------------------|-------------------|-------------------|
| Q111 vs WT | P-value < 0.05       | 28                | 3                 |
| Q111 vs WT | FDR < 0.05           | 0                 | 0                 |



# Differential Binding

- 12,388 total peak regions → peak counts
- Differential binding between genotypes



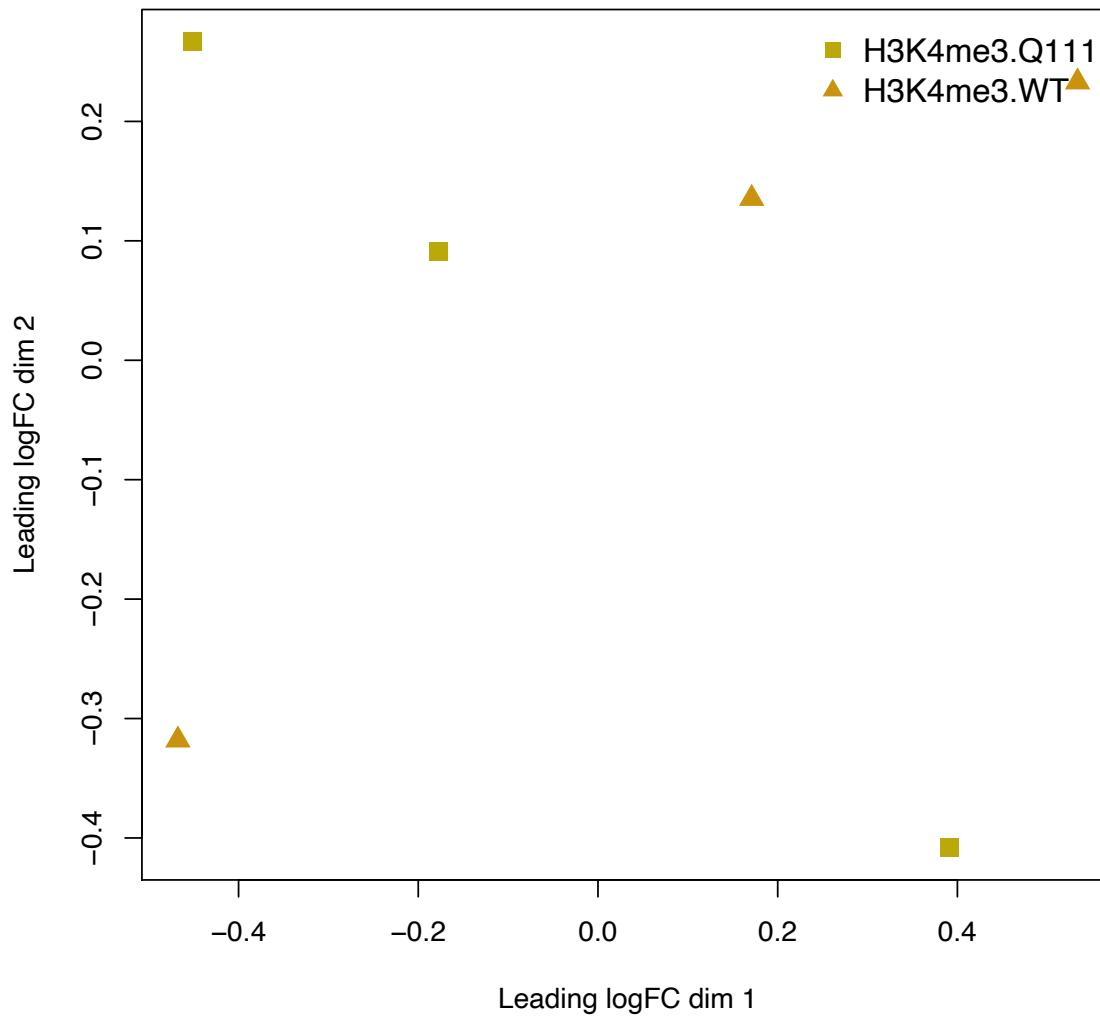


**H3K4ME3 Q111 vs WT**



# Differential Binding

- 17,672 total peak regions → peak counts
- Sample clustering





# Differential Binding

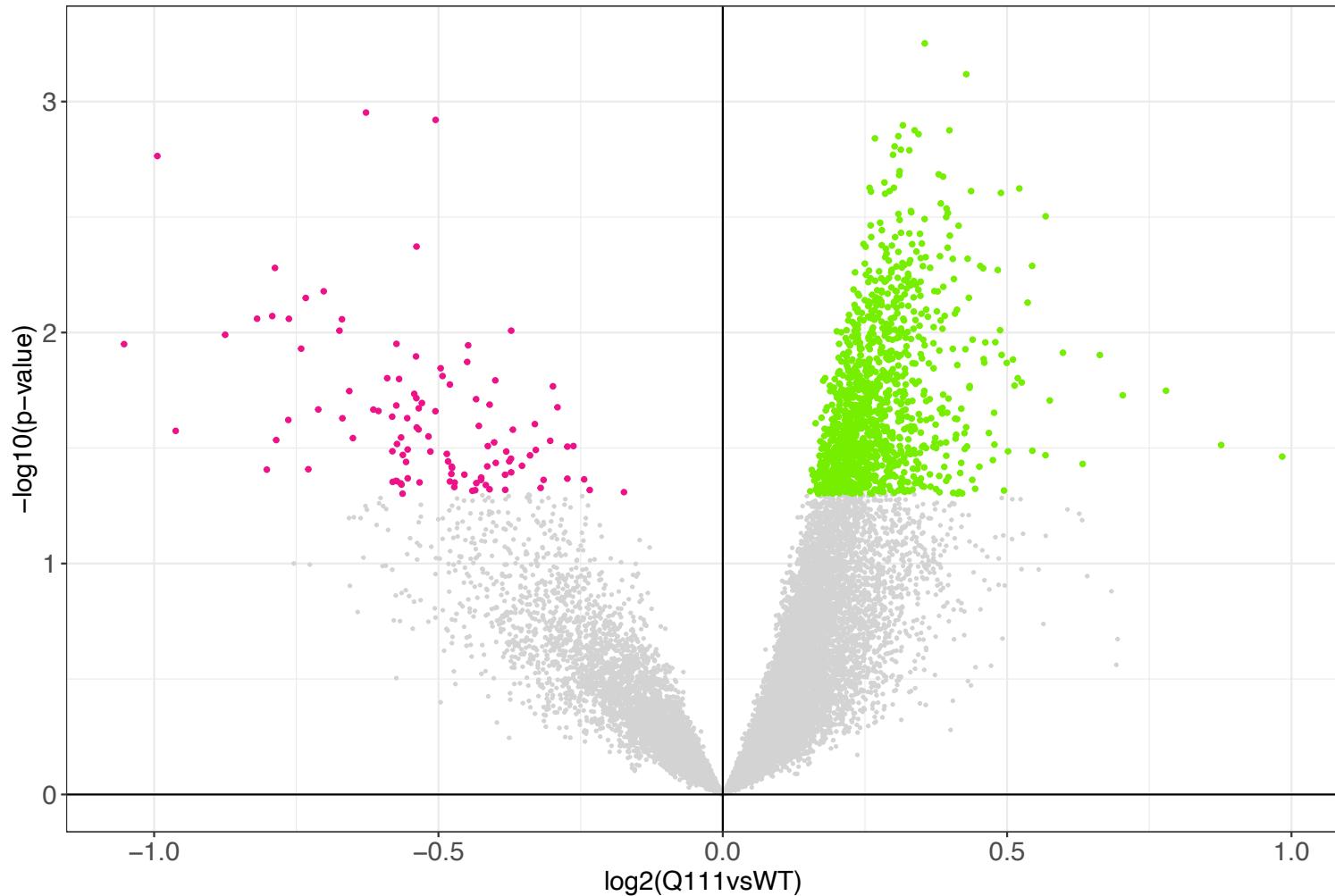
- 17,672 total peak regions → peak counts
- Differential binding between genotypes

| Comparison | Significance Cut-off | # Regions LFC > 0 | # Regions LFC < 0 |
|------------|----------------------|-------------------|-------------------|
| Q111 vs WT | P-value < 0.05       | 1,201             | 109               |
| Q111 vs WT | FDR < 0.05           | 0                 | 0                 |



# Differential Binding

- 17,672 total peak regions → peak counts
- Differential binding between genotypes



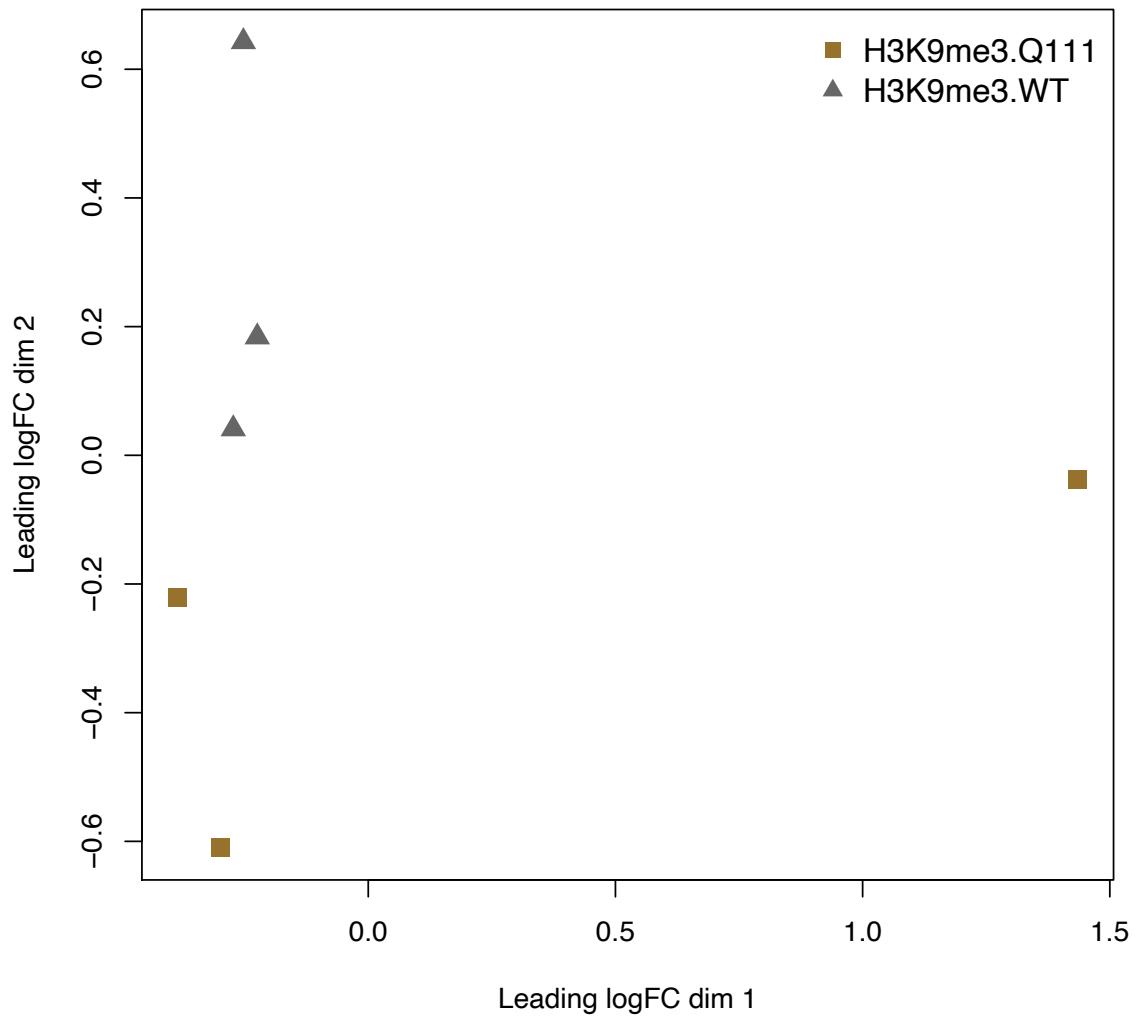


# H3K9ME3 Q111 vs WT



# Differential Binding

- 22,304 total peak regions → peak counts
- Sample clustering





# Differential Binding

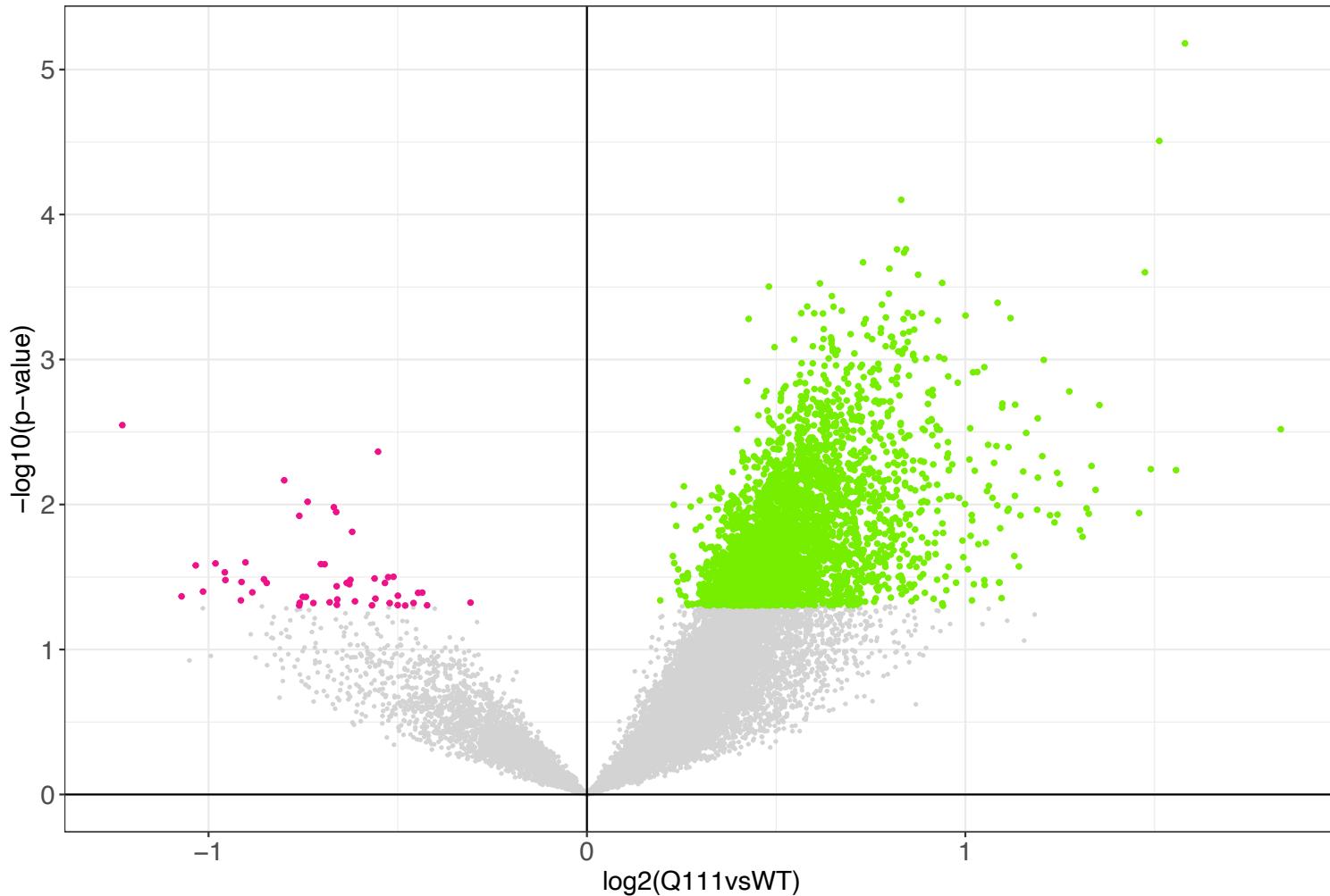
- 22,304 total peak regions → peak counts
- Differential binding between genotypes

| Comparison | Significance Cut-off | # Regions LFC > 0 | # Regions LFC < 0 |
|------------|----------------------|-------------------|-------------------|
| Q111 vs WT | P-value < 0.05       | 3,465             | 50                |
| Q111 vs WT | FDR < 0.05           | 0                 | 0                 |



# Differential Binding

- 22,304 total peak regions → peak counts
- Differential binding between genotypes



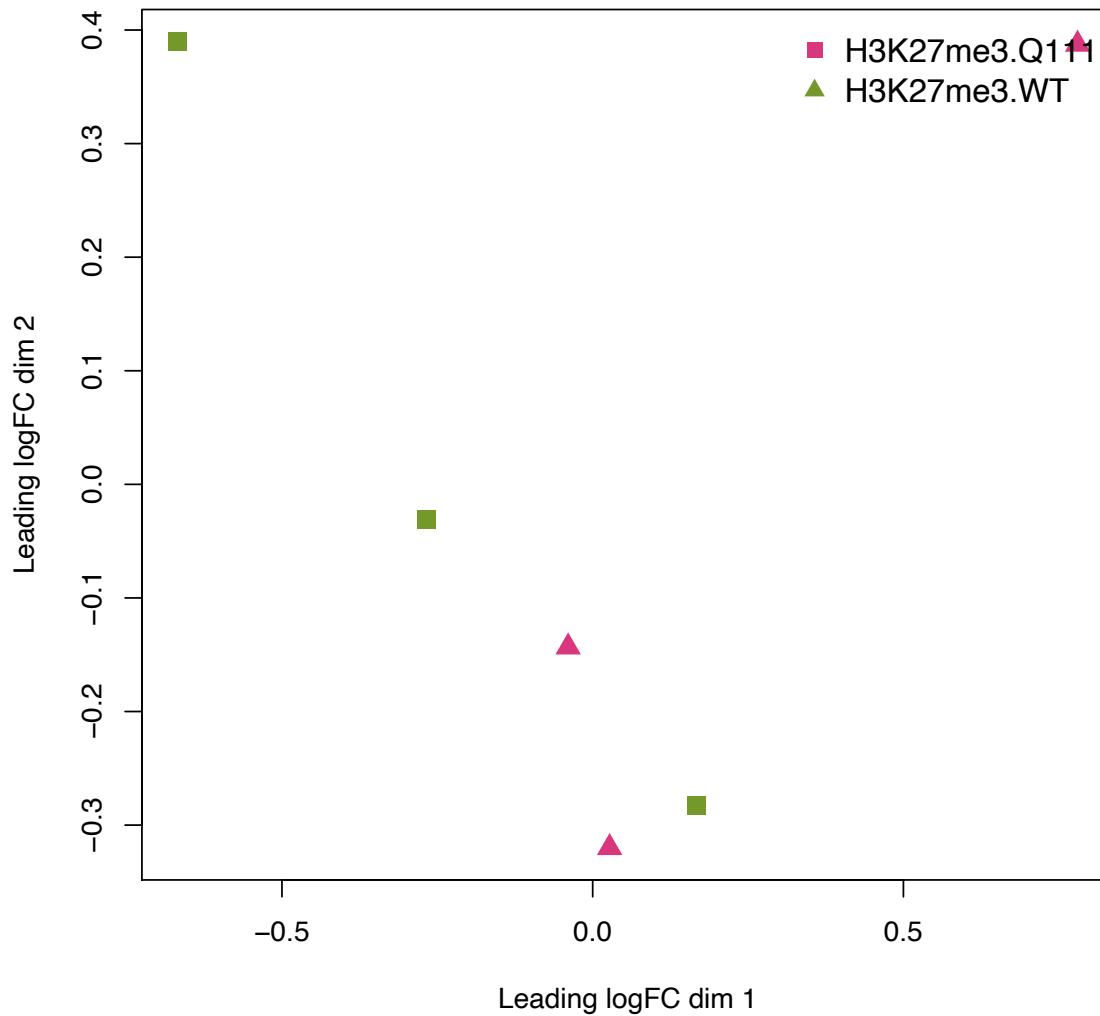


**H3K27ME3 Q111 vs WT**



# Differential Binding

- 25,315 total peak regions → peak counts
- Sample clustering





# Differential Binding

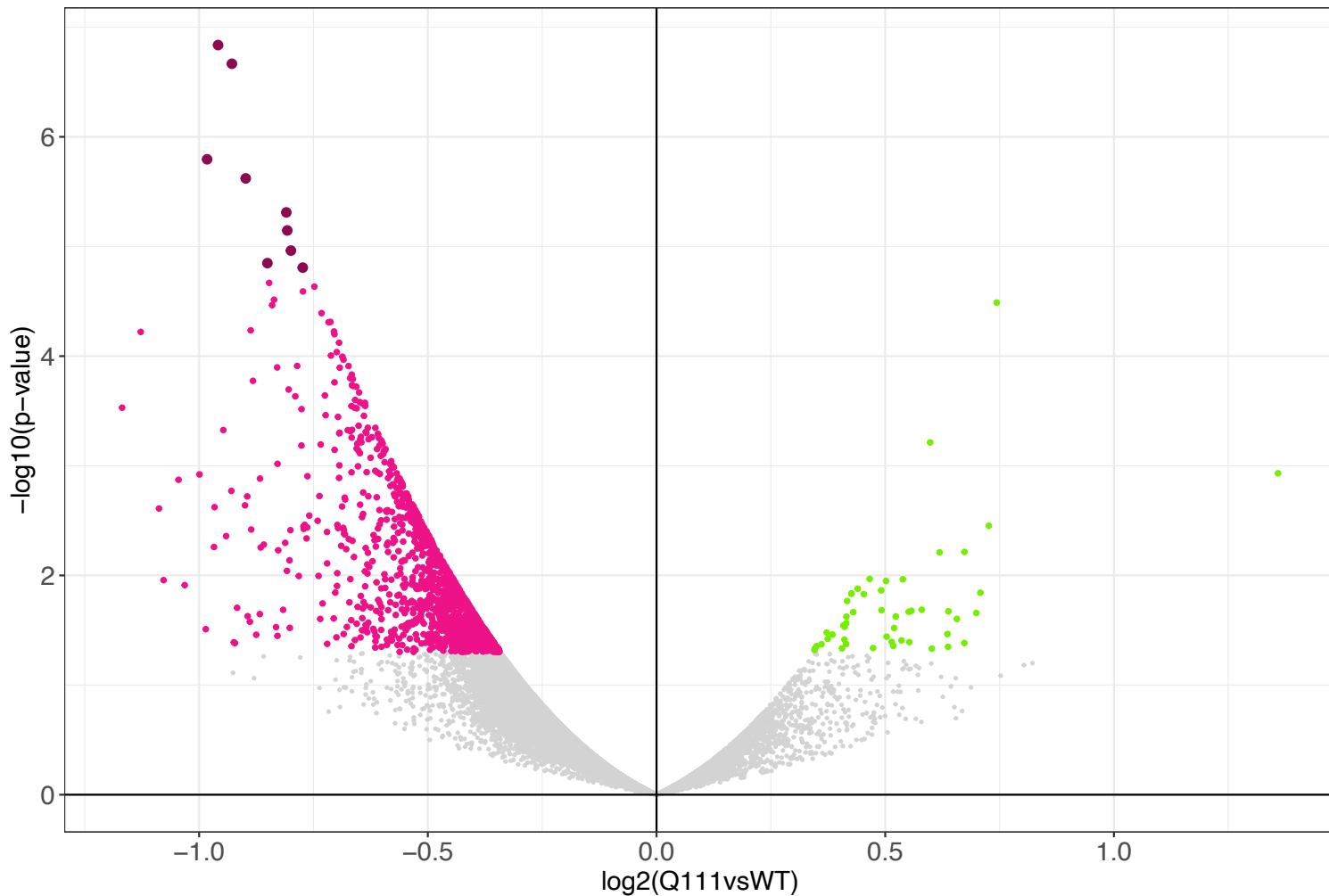
- 25,315 total peak regions → peak counts
- Differential binding between genotypes

| Comparison | Significance Cut-off | # Regions LFC > 0 | # Regions LFC < 0 |
|------------|----------------------|-------------------|-------------------|
| Q111 vs WT | P-value < 0.05       | 50                | 1,481             |
| Q111 vs WT | FDR < 0.05           | 0                 | 9                 |



# Differential Binding

- 25,315 total peak regions → peak counts
- Differential binding between genotypes



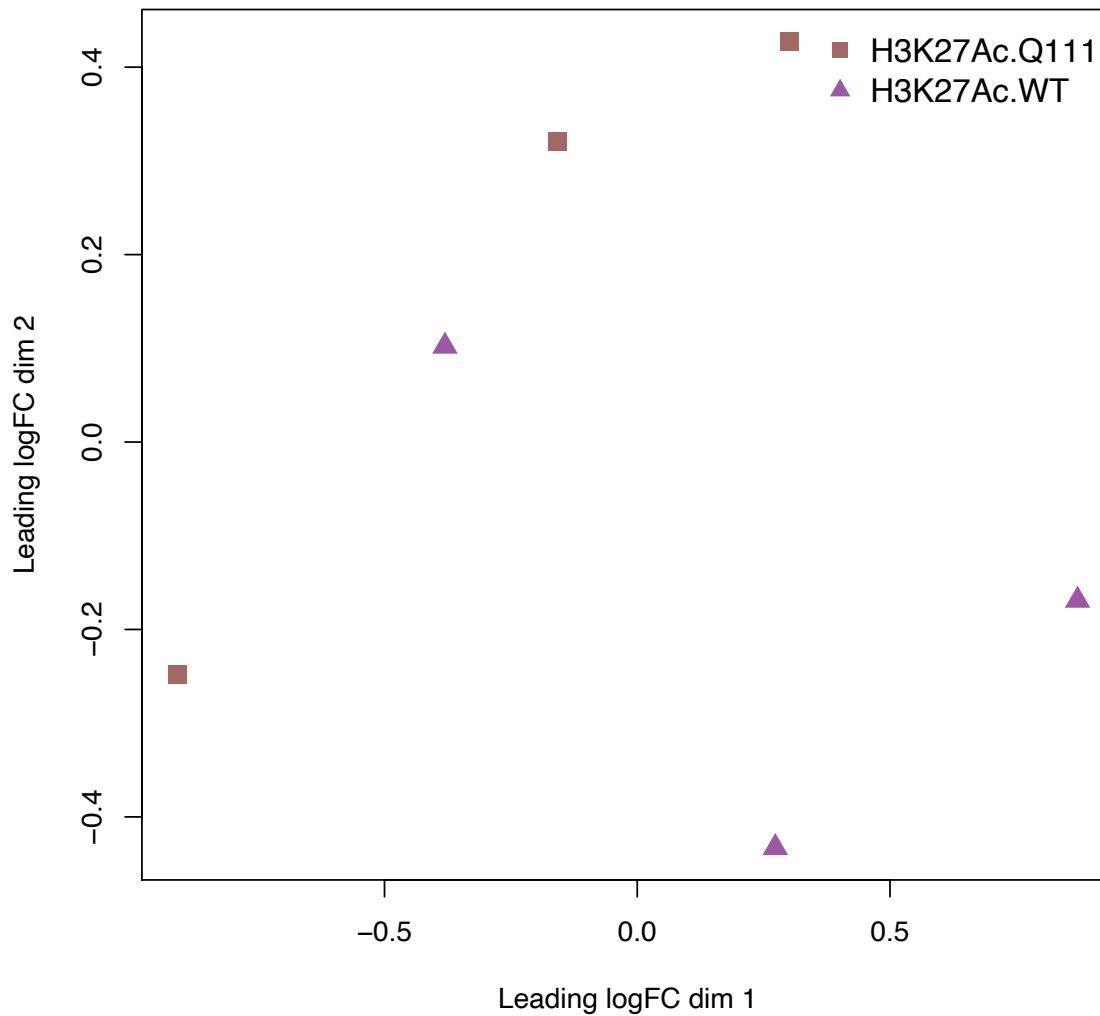


# H3K27AC Q111 vs WT



# Differential Binding

- 27,337 total peak regions → peak counts
- Sample clustering





# Differential Binding

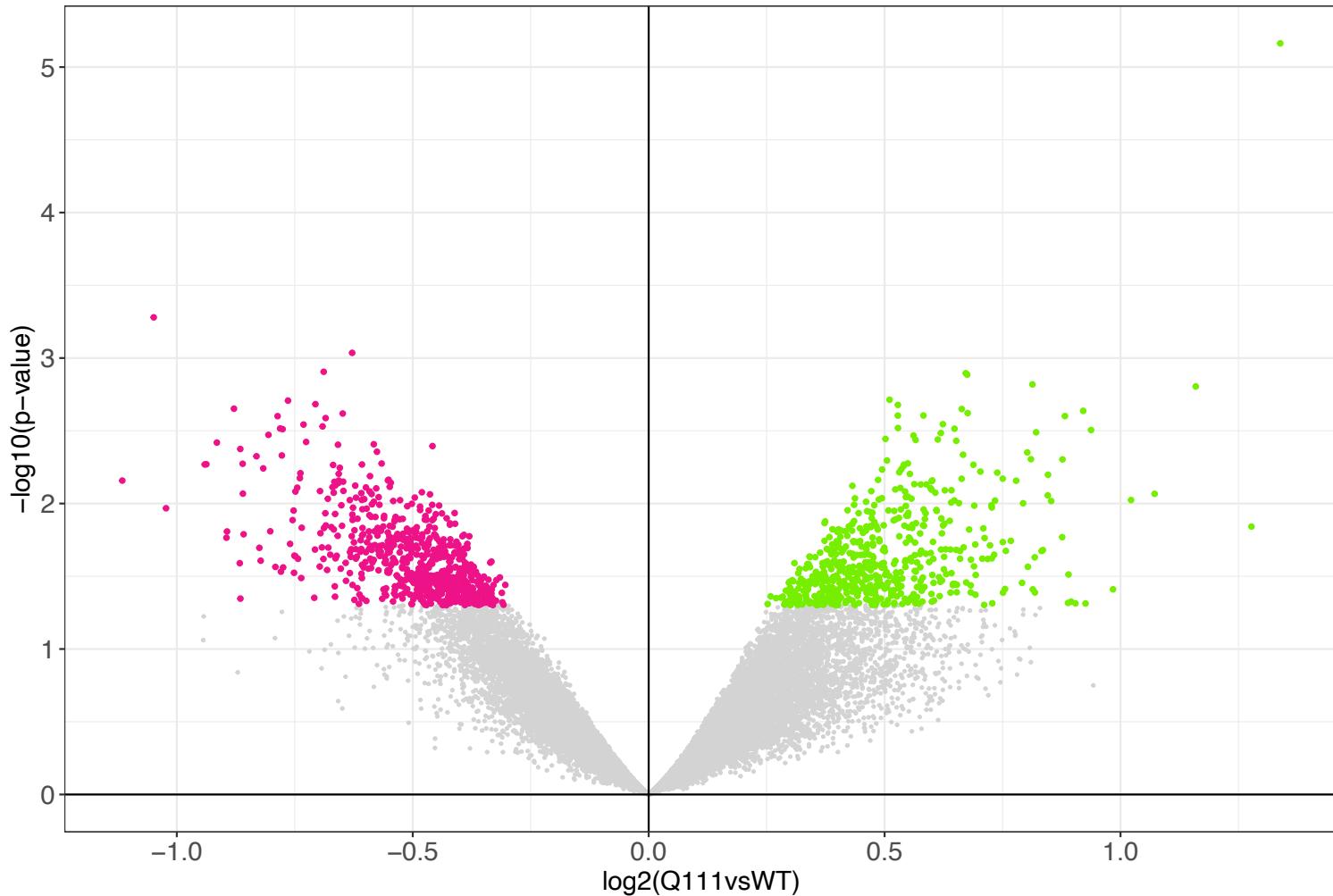
- 27,337 total peak regions → peak counts
- Differential binding between genotypes

| Comparison | Significance Cut-off | # Regions LFC > 0 | # Regions LFC < 0 |
|------------|----------------------|-------------------|-------------------|
| Q111 vs WT | P-value < 0.05       | 595               | 697               |
| Q111 vs WT | FDR < 0.05           | 0                 | 0                 |



# Differential Binding

- 27,337 total peak regions → peak counts
- Differential binding between genotypes





UNIVERSITY *of* MARYLAND  
SCHOOL OF MEDICINE

---

# FUTURE STEPS



---

# Downstream Analysis

- On-going analysis (Questions)
  - What is the expected relationship between genotype and each histone mark
  - Does it corroborate histone mark results from other datasets
- Future analysis (based on relevant set of peak calls)
  - Enrichment against different reference sets (DEGs, HDSigDB, etc)



UNIVERSITY *of* MARYLAND  
SCHOOL OF MEDICINE

---

**QUESTIONS?**