chronic_ACH_exposition_metrics

June 19, 2025

0.1 Control Dataset Overview

This notebook analyzes spontaneous calcium activity in BxPC3 pancreatic cancer cells imaged under identical control conditions across six image sequences.

Experimental context: - Cell line: BxPC3 (pancreatic ductal adenocarcinoma) - Imaging: DIV2, seeded at 1.2 million cells per well (high confluence) - Ca^2 dye: Fluo-4 AM (standard loading protocol) - Nuclei: Hoechst 33342 (10 µg/mL) - Acquisition: FITC, 1 Hz, 30 ms exposure for 30 minutes - CHRONIC exposition to Ach in growth medium after seeding, during incubation & imaging at various concentration.

This is the **first run** of spontaneous activity under control conditions. The goal is to analyze cells' behaviors at different level and compare it to control datasets

0.2 Setup & Utilities

Standard scientific libraries (Pandas, NumPy, Seaborn) and custom plotting utilities are loaded. These functions enable streamlined metric visualization and layout control across multiple datasets.

[1]:
```
# Libraries
import seaborn as sns
import numpy as np
from pathlib import Path
import sys

# Add /Source to sys.path if not already present
project_root = Path.cwd().parent
source_path = project_root / "src"

if source_path.exists() and str(source_path) not in sys.path:
    sys.path.insert(0, str(source_path))

# Utility functions
from calcium_activity_characterization.analysis.visualizers import␣
 ↪plot_metric_by_dataset, plot_category_distribution_by_dataset,␣
 ↪plot_raster_plots_by_dataset
from calcium_activity_characterization.analysis.loaders import␣
 ↪load_and_merge_datasets

sns.set(style="whitegrid")
```

```
# Define paths to control datasets
control_paths = {
    "1 - ACH 10uM - 1": "D:/Mateo/20250618/Output/IS1",
    "1 - ACH 10uM - 2": "D:/Mateo/20250618/Output/IS2",
    "2 - ACH 1uM - 1": "D:/Mateo/20250618/Output/IS3",
    "2 - ACH 1uM - 2": "D:/Mateo/20250618/Output/IS4",
    "3 - ACH 100nM - 1": "D:/Mateo/20250618/Output/IS5",
    "3 - ACH 100nM - 2": "D:/Mateo/20250618/Output/IS6",
    "4 - ACH 10nM - 1": "D:/Mateo/20250618/Output/IS7",
    "4 - ACH 10nM - 2": "D:/Mateo/20250618/Output/IS8",
}

# Load and merge datasets
peaks = load_and_merge_datasets(control_paths=control_paths, file_name="peaks.
 ↪csv")
cells = load_and_merge_datasets(control_paths=control_paths, file_name="cells.
 ↪csv")
events = load_and_merge_datasets(control_paths=control_paths, file_name="events.
 ↪csv")
```

0.3 Preprocessing

Before visual comparison, we normalize event labels (e.g., missing `in_event` entries set to `'individual'`) and compute additional derived fields (`is_active`, `periodicity_score`) to ensure consistent comparisons across all datasets.

These steps are crucial for **ensuring fair metric aggregation** and enabling downstream comparative analysis.

[2]:
```
peaks["in_event"] = peaks["in_event"].fillna("individual").str.lower()

if "is_active" not in cells.columns:
    cells["is_active"] = cells["num_peaks"].apply(lambda x: 1 if x > 0 else 0)

if "periodicity_score" not in cells.columns:
    cells["periodicity_score"] = np.nan
```

0.4 Raster Plot Inspection

Raster plots provide a binary overview of calcium activity over time per cell.

Remarks: TODO

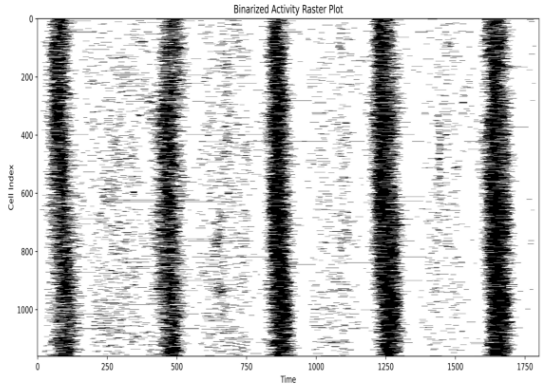
Use these plots to **screen visually for anomalies or batch effects** before statistical analysis.

[3]:
```
plot_raster_plots_by_dataset(
    dataset_paths=control_paths,
    title="Binary Activity Raster Plots by Dataset",
```

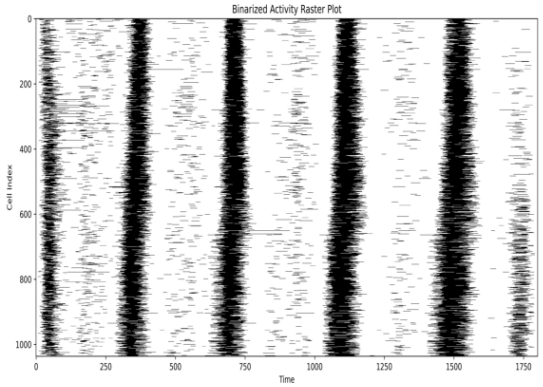
```
    n_cols=2
)
```

Binary Activity Raster Plots by Dataset

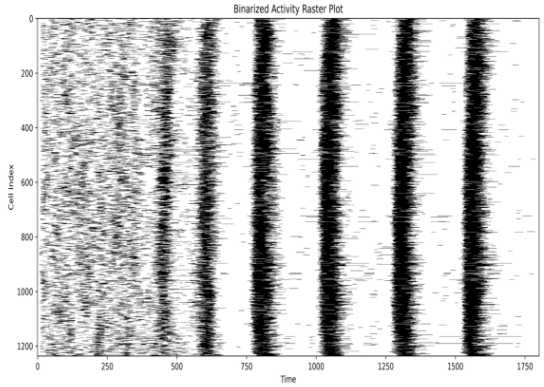
1 - ACH 10uM - 1



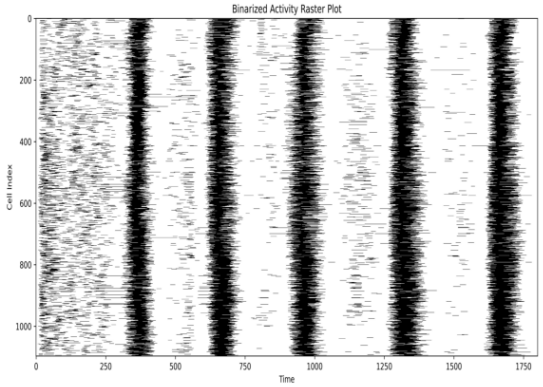
1 - ACH 10uM - 2



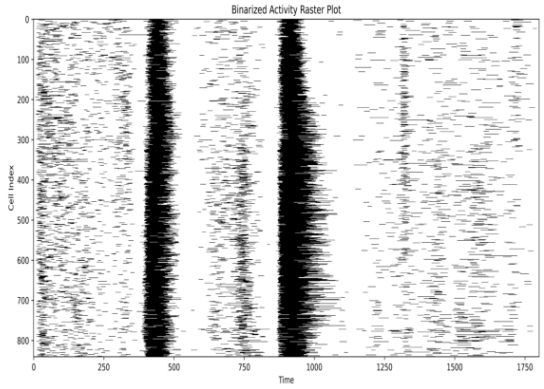
2 - ACH 1uM - 1



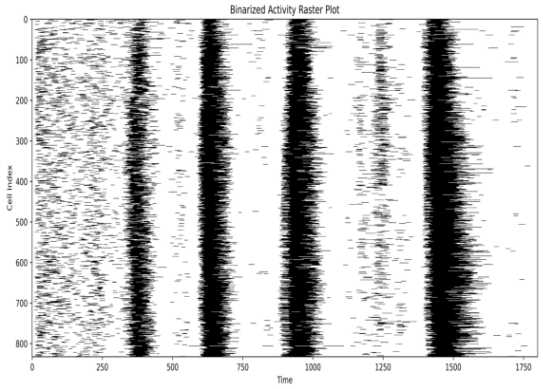
2 - ACH 1uM - 2



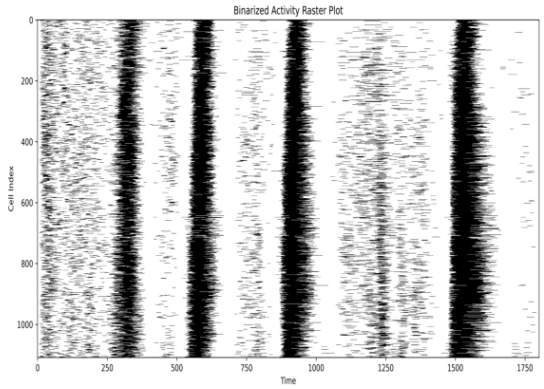
3 - ACH 100nM - 1



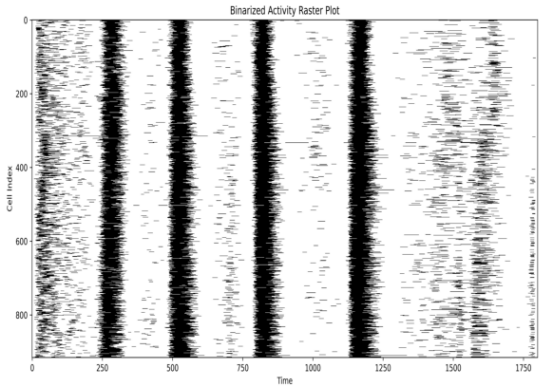
3 - ACH 100nM - 2



4 - ACH 10nM - 1



4 - ACH 10nM - 2



0.5 Peak Type Distribution

Pie charts quantify the proportion of detected calcium peaks classified as: - `individual`: isolated peaks - `sequential`: propagating local events - `global`: large-scale synchronized events

Interpretation:

The distribution of peaks by event type (global, sequential, individual) across First Run control datasets is highly consistent.

- *Approximately **30-40% global events** are consistently observed, except in image sequence `2025-04-04 IS3`, where fewer global events were detected.*
- *Sequential and individual peaks maintain stable proportions across datasets, indicating reproducible spontaneous calcium signaling activity under standard conditions.*

First run image sequences demonstrate reproducible and robust peak-type distribution, validating their reliability as control data.

[4]:
```
peak_type_colors = {
    "global": "#66c2a5",
    "sequential": "#fc8d62",
    "individual": "#8da0cb"
}

plot_category_distribution_by_dataset(
    df=peaks,
    column="in_event",
    category_order=["global", "sequential", "individual"],
    colors=peak_type_colors,
    title="Distribution of Peaks by Event Type",
    n_cols=2
)
```


Distribution of Peaks by Event Type
1 - ACH 10uM - 1
Individual (1303)
15.4%
Sequential (2155)
25.5%
Global (5001)
59.1%
1 - ACH 10uM - 2
Individual (803)
11.8%
Sequential (1190)
17.5%
Global (4794)
70.6%
2 - ACH 1uM - 1
Individual (1174)
9.8%
Sequential (3667)
30.7%
Global (7112)
59.5%
2 - ACH 1uM - 2
Individual (1102)
13.3%
Sequential (1875)
22.6%
Global (5309)
64.1%
3 - ACH 100nM - 1
Individual (1378)
27.3%
Global (1645)
32.6%
Sequential (2029)
40.2%
3 - ACH 100nM - 2
Individual (979)
16.3%
Global (3247)
54.0%
Sequential (1789)
29.7%
4 - ACH 10nM - 1
Individual (1559)
17.6%
Global (4348)
49.2%
Sequential (2932)
33.2%
4 - ACH 10nM - 2
Individual (1023)
15.1%
Sequential (1490)
21.9%
Global (4281)
63.0%

0.6 Peak Metrics per Event Type

Metrics like **duration**, **prominence**, and **symmetry** are visualized per event type.

Interpretation:

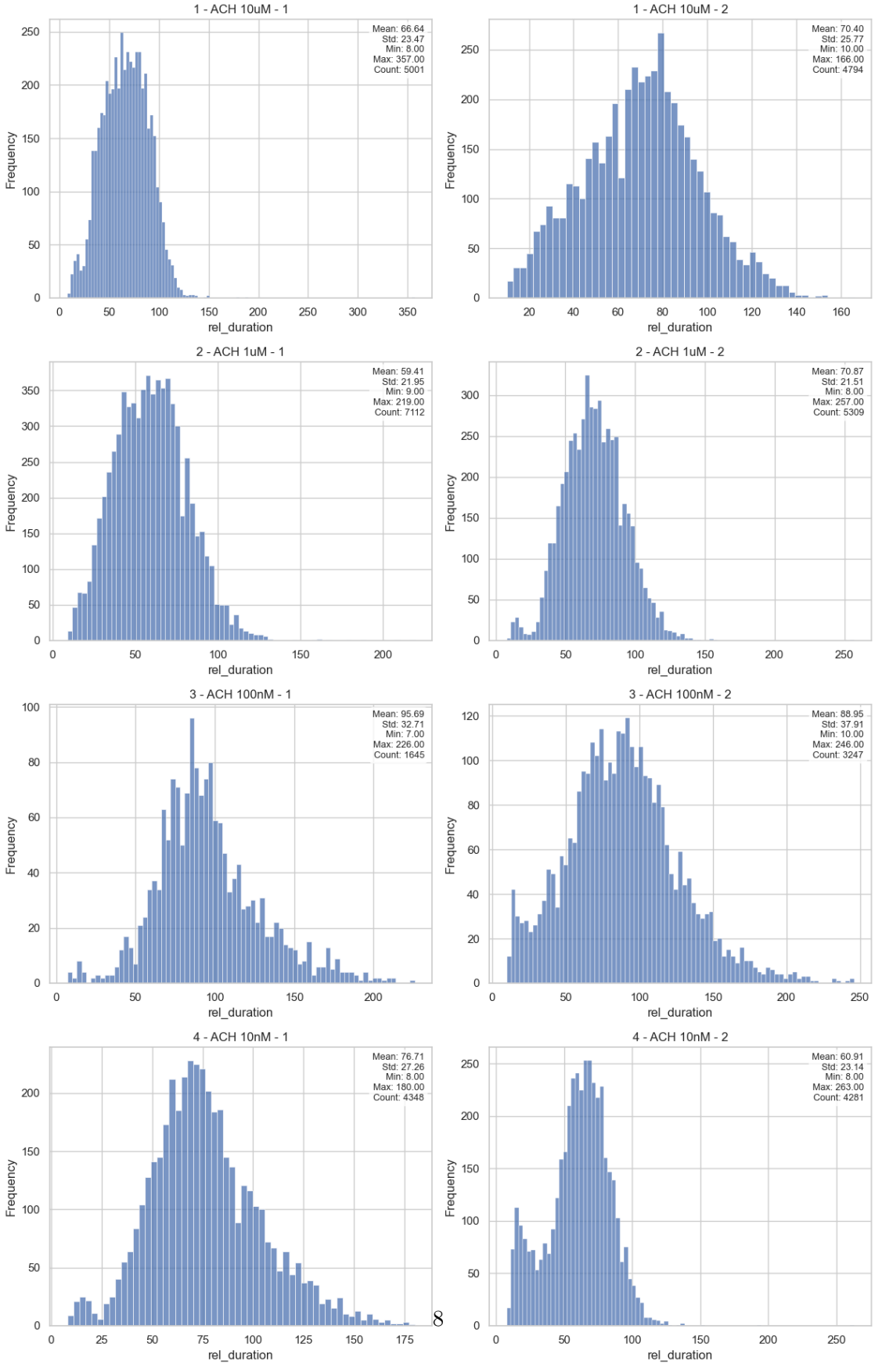
- *Global peaks: Characteristically long durations (~50 frames).*
- *Sequential peaks: Duration means around **26–28 frames**; symmetry values around **0.9 ± 0.12**.*
- *Individual peaks: Duration around **26–27 frames**, also with symmetry ~0.9 ± 0.12.*

Overall, the peak metrics in the First Run are stable, biologically plausible, and reproducible.

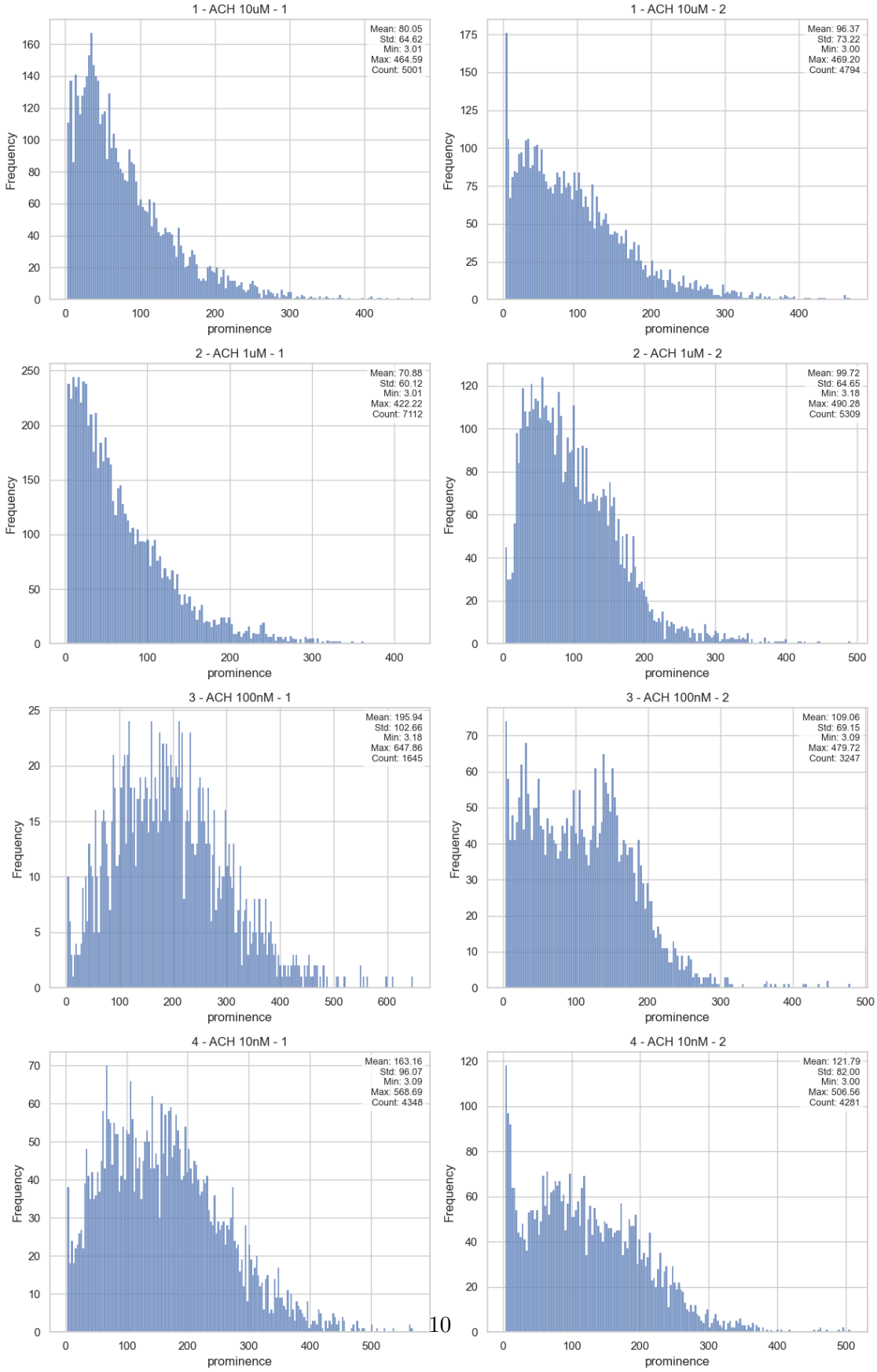
[5]:
```
for event_type in ["global", "sequential", "individual"]:
    subset = peaks[peaks["in_event"] == event_type]
    print(f"\n#  Peak Type: {event_type.title()} - {len(subset)} peaks")
    plot_metric_by_dataset(subset, "rel_duration", f"{event_type.title()} Peaks:
 ↪ Duration", bin_width=3, n_cols=2)
    plot_metric_by_dataset(subset, "prominence", f"{event_type.title()} Peaks:␣
↪Prominence", bin_width=3, n_cols=2)
    plot_metric_by_dataset(subset, "rel_symmetry_score", f"{event_type.title()}␣
↪Peaks: Symmetry", bin_count=30, n_cols=2)
```

```
#  Peak Type: Global - 35737 peaks
```

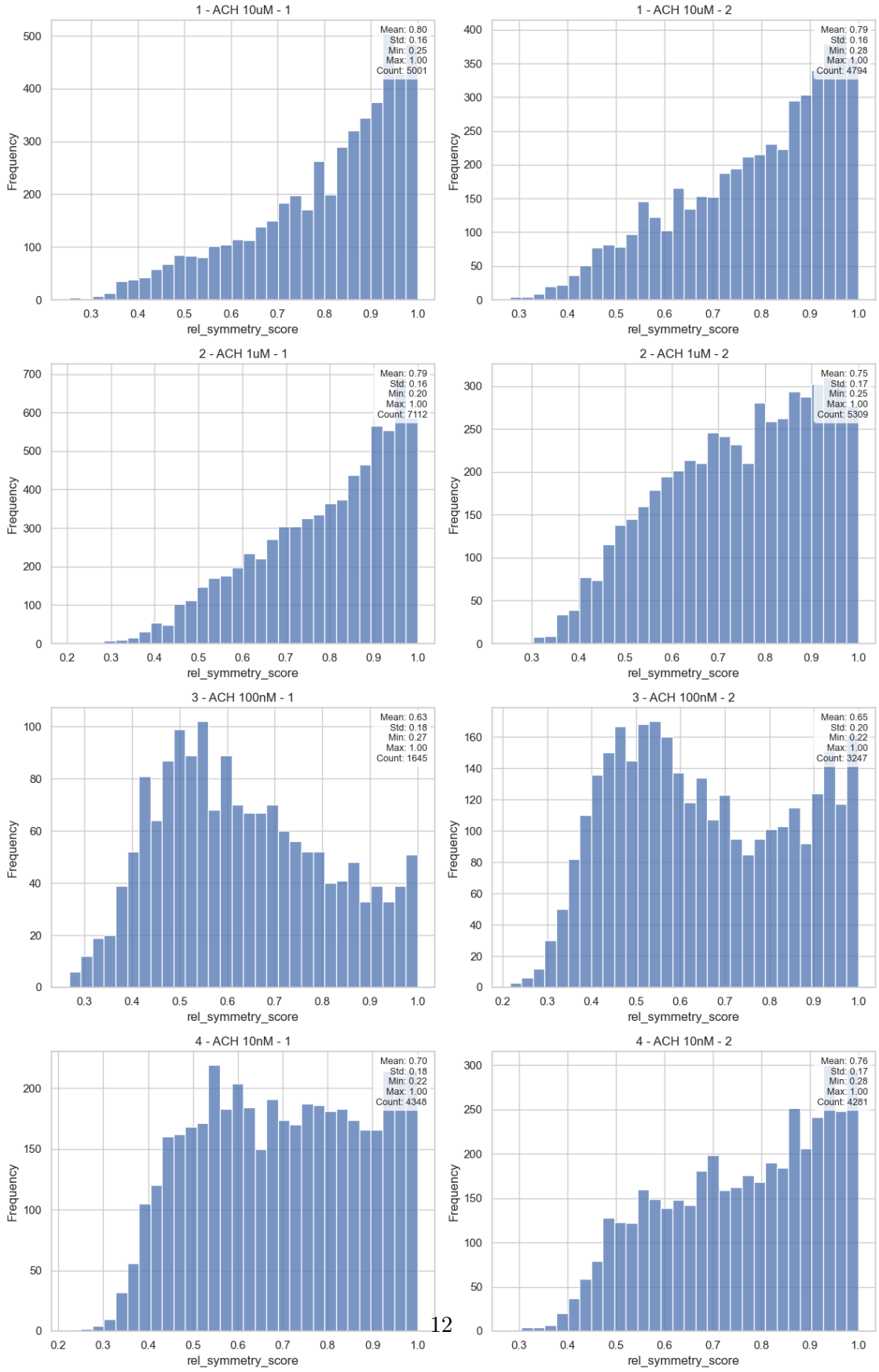
Global Peaks: Duration



Global Peaks: Prominence

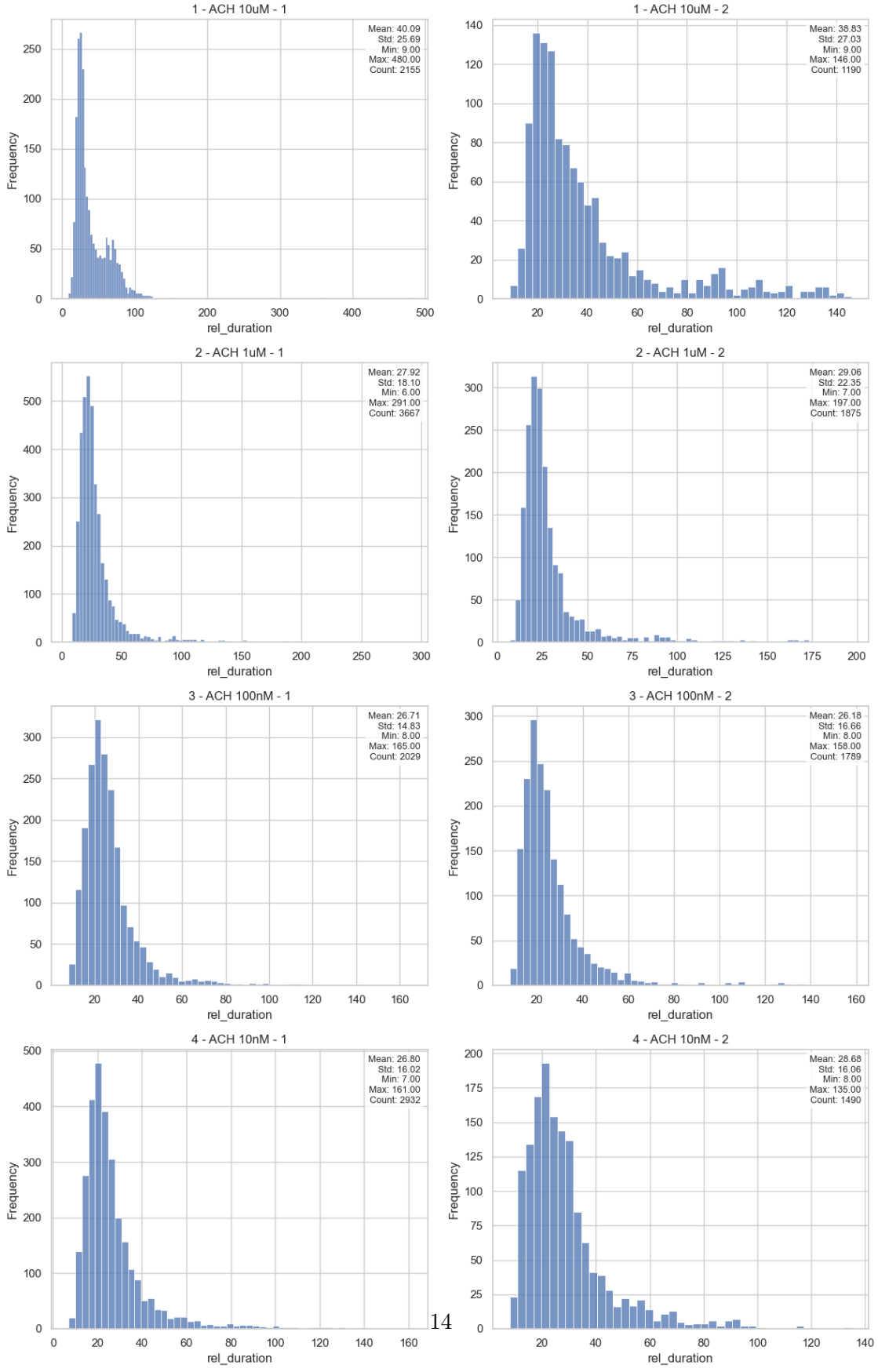


Global Peaks: Symmetry

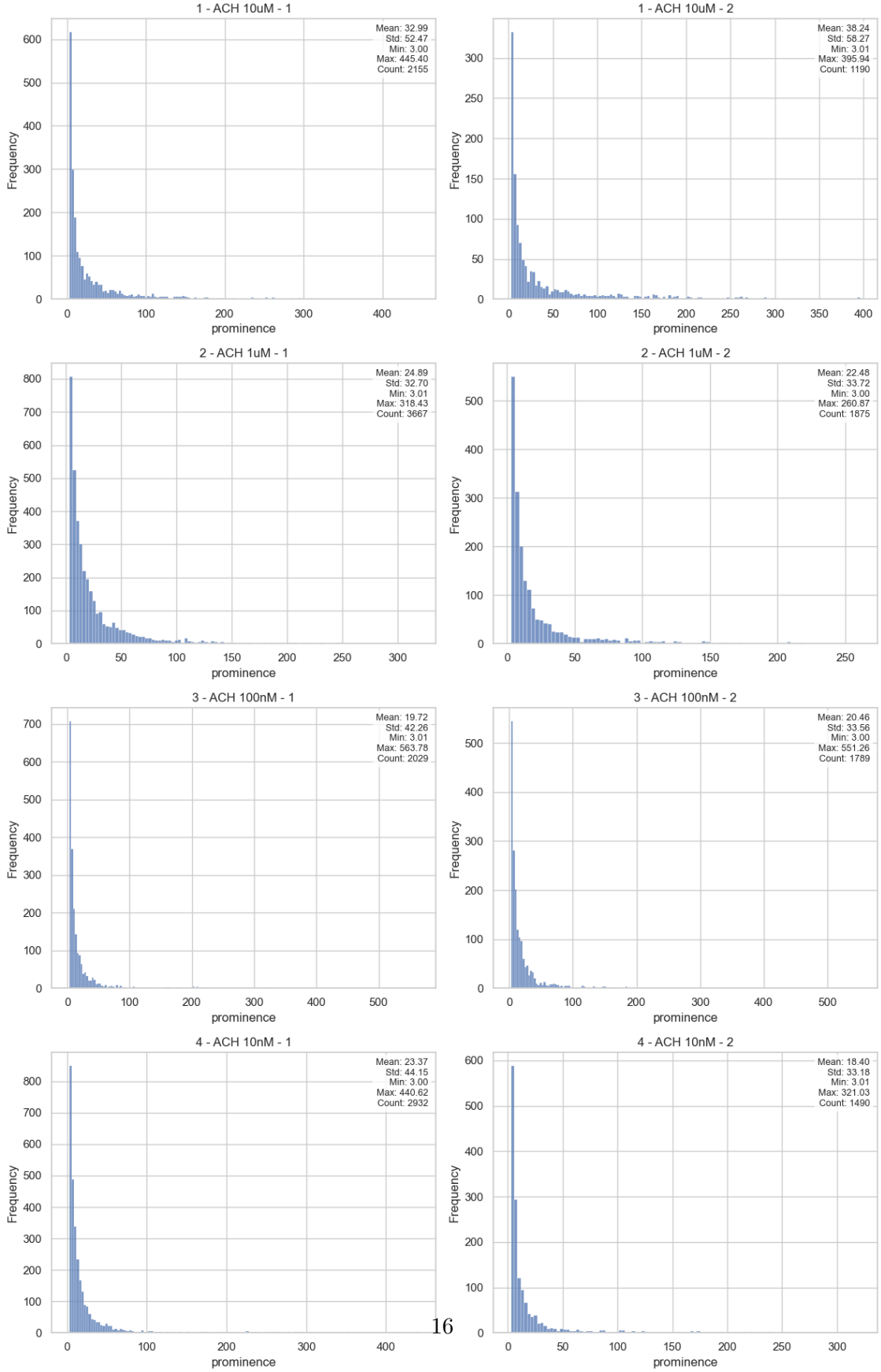


```
#  Peak Type: Sequential - 17127 peaks
```

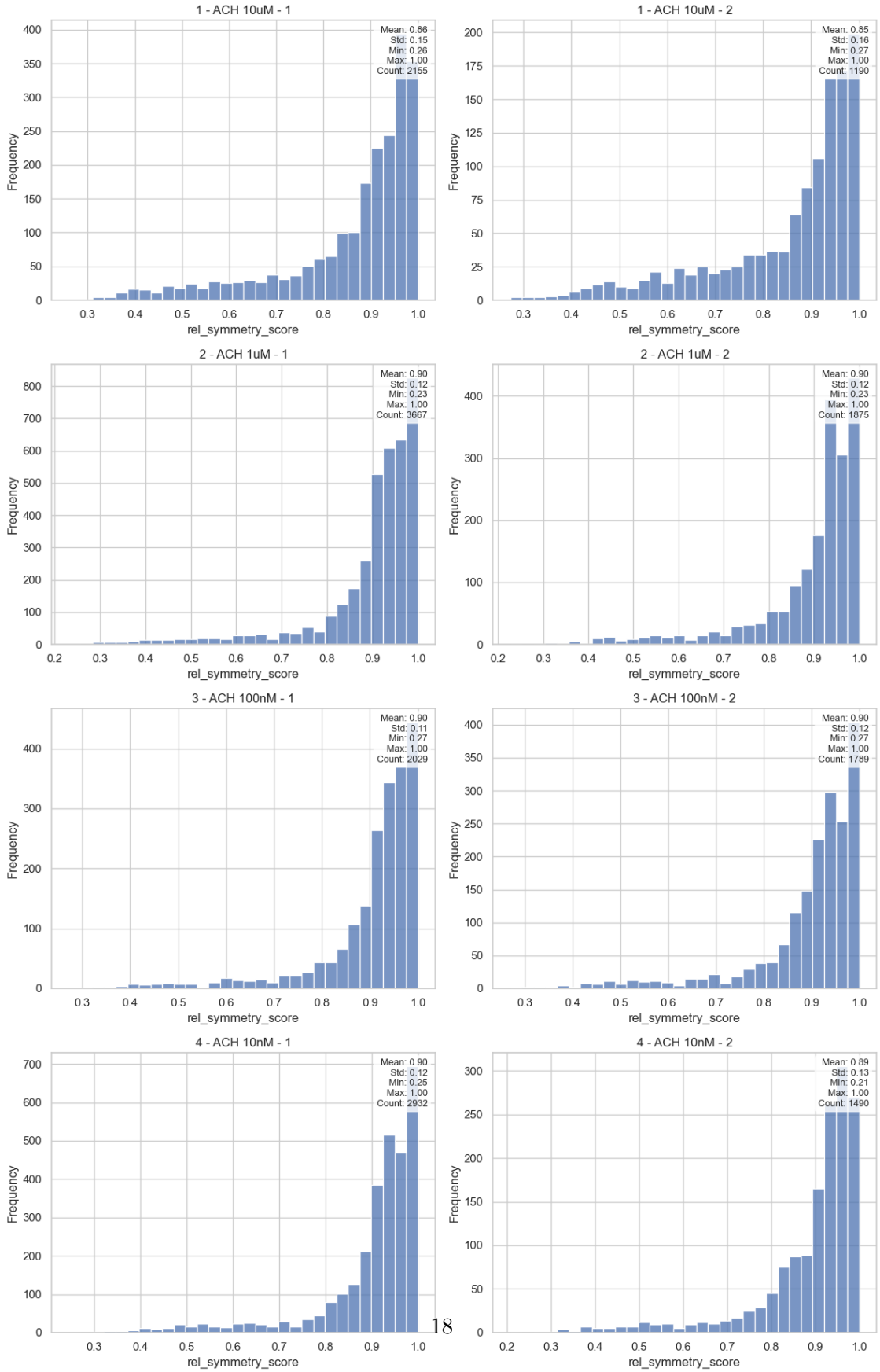
Sequential Peaks: Duration



Sequential Peaks: Prominence

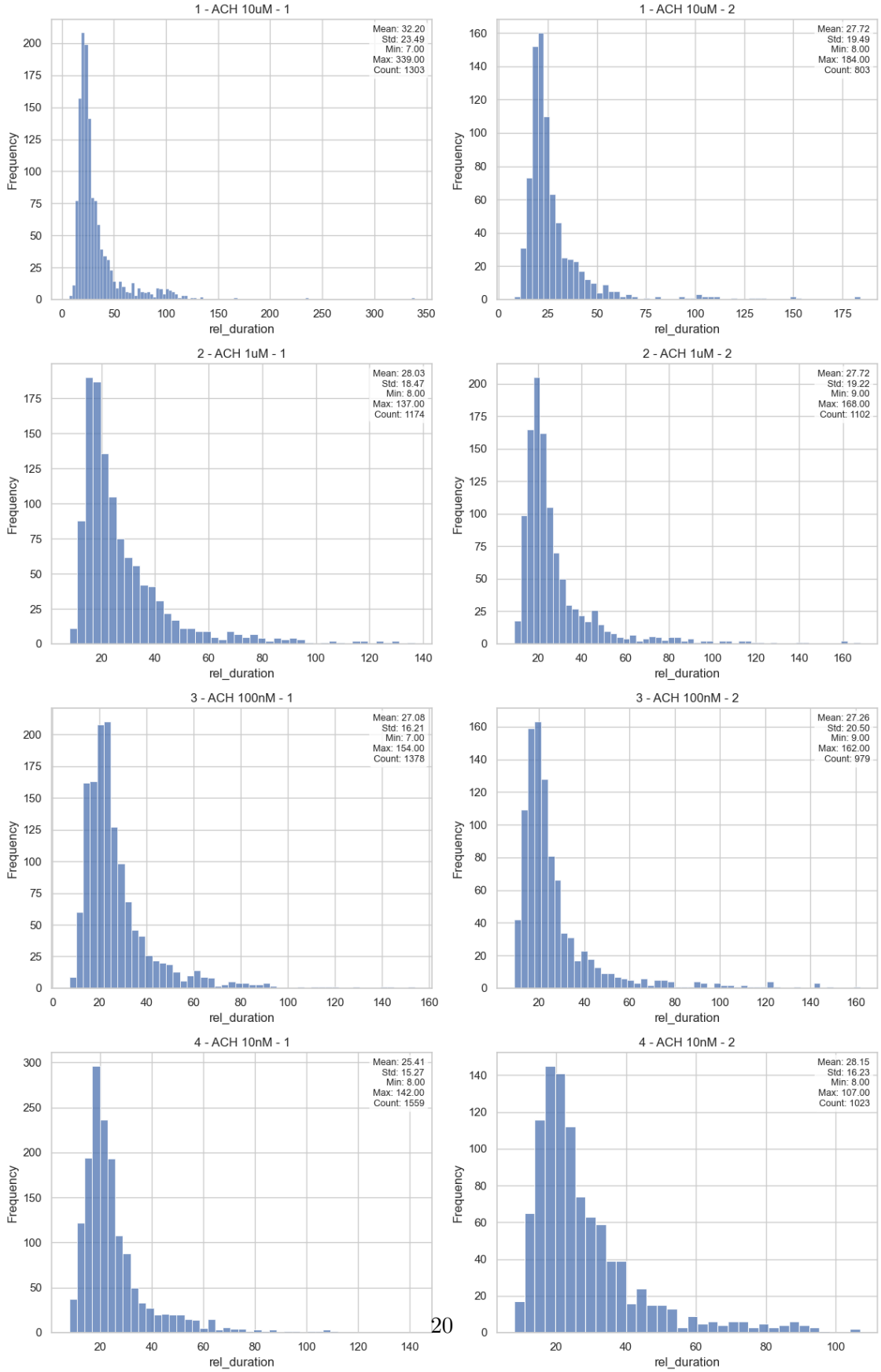


Sequential Peaks: Symmetry

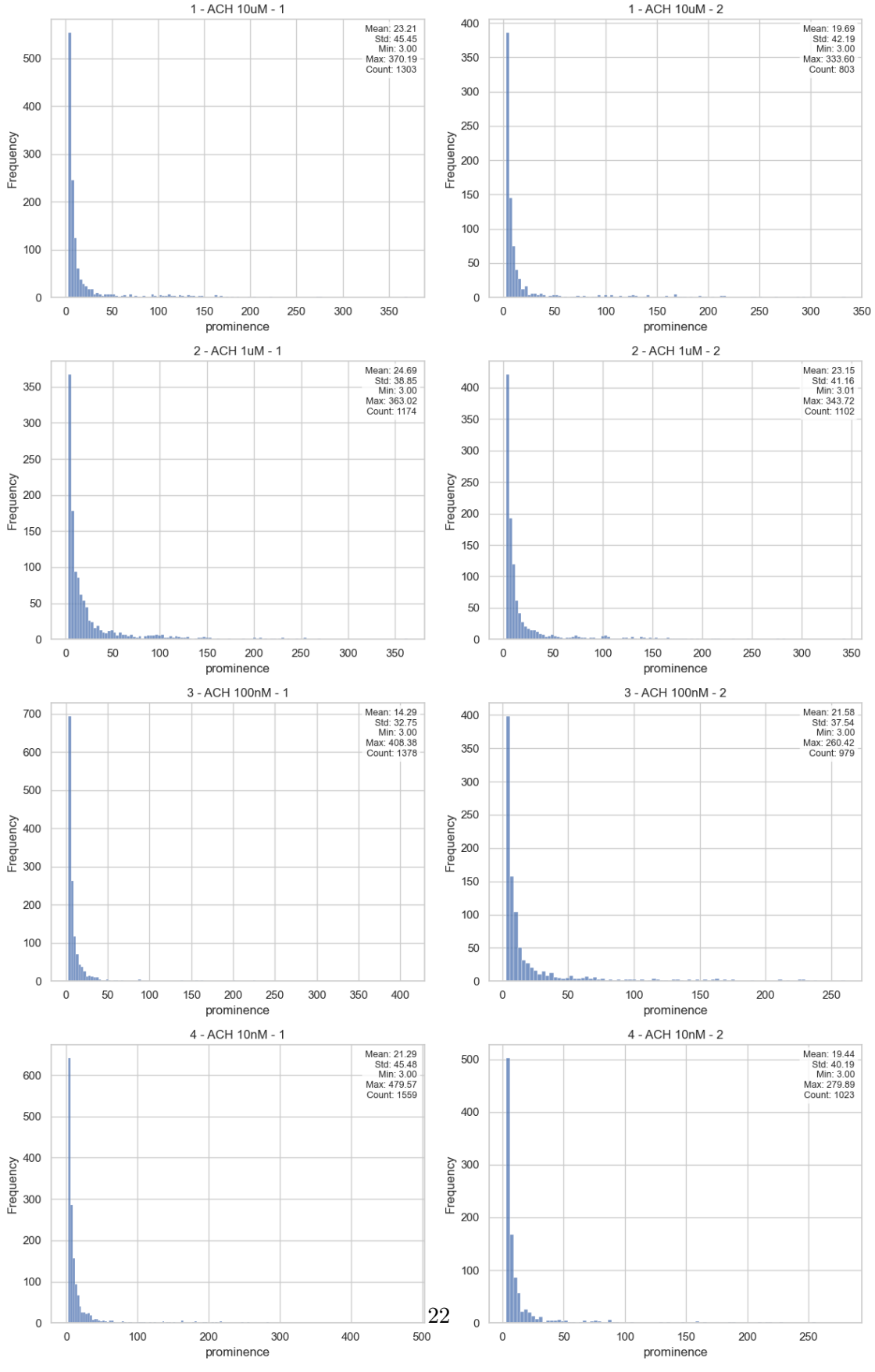


```
# Peak Type: Individual - 9321 peaks
```

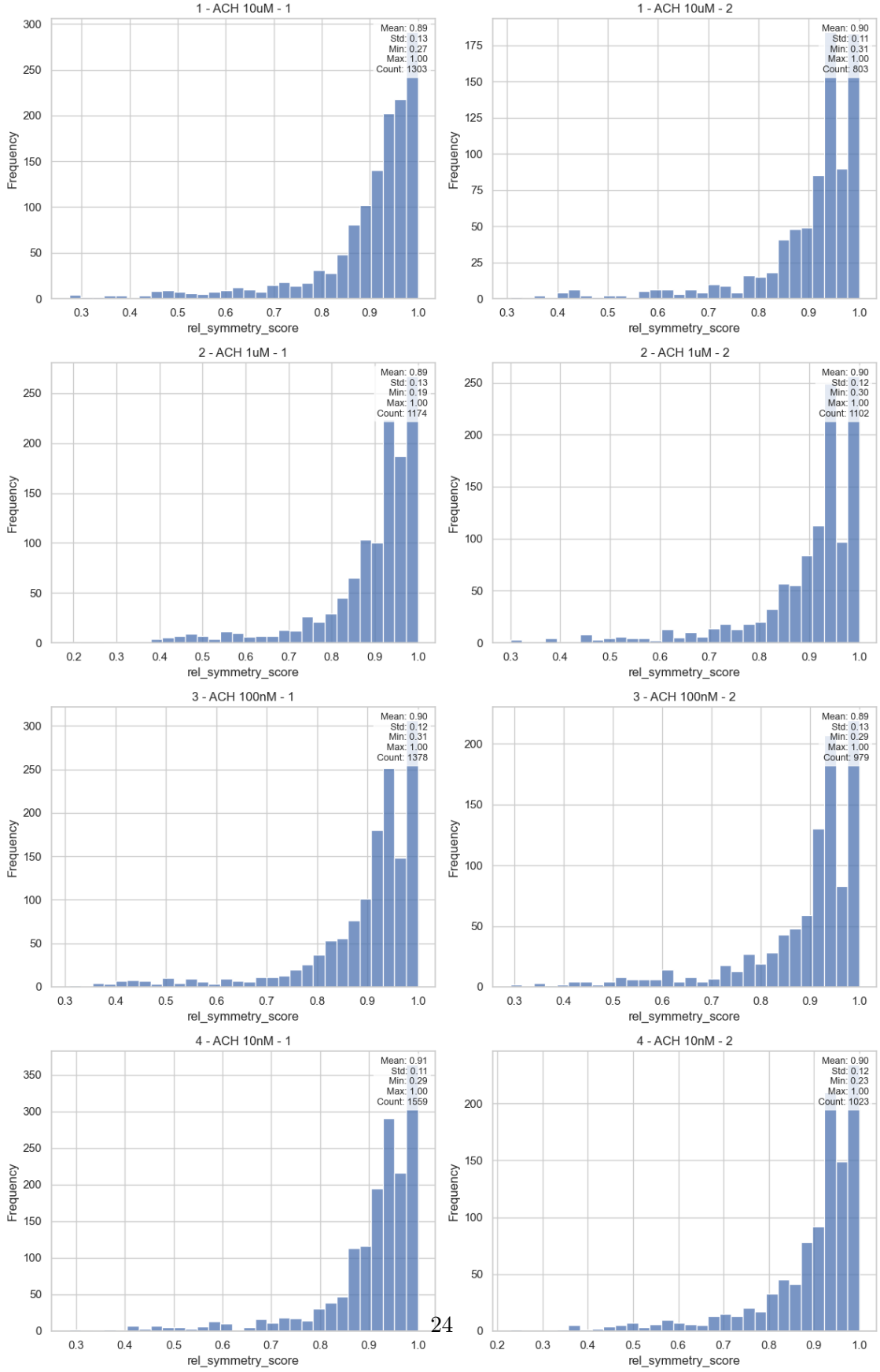
Individual Peaks: Duration



Individual Peaks: Prominence



Individual Peaks: Symmetry



0.7 Cell Metrics

We analyze: - Number of peaks per cell (activity) - Periodicity score (oscillatory behavior) - Proportion of active vs inactive cells

Interpretation:

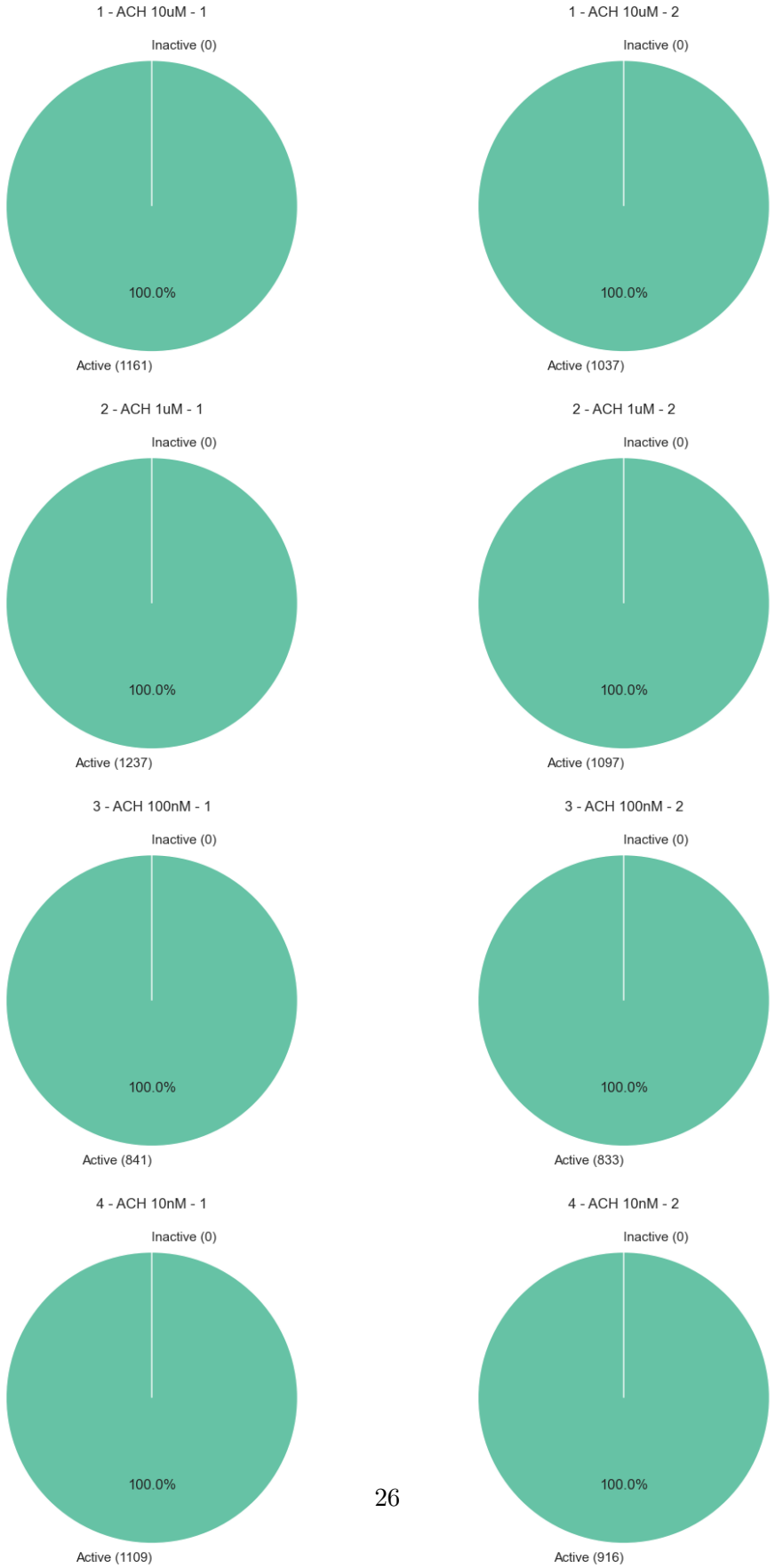
Active cells: Nearly all cells (>99.9%) are active in every dataset. This indicates excellent signal integrity and robust experimental conditions. Activity is highly homogeneous across datasets.

Number of peaks per cell: High total peak count (>10,000 peaks per dataset), with a balanced and near-normal distribution. This reflects widespread and stable activity across the population.

Periodicity: Periodicity scores (~0.7 ± 0.1) remain consistent across both imaging runs. This suggests that rhythmic calcium oscillations are preserved, even when global excitability drops.

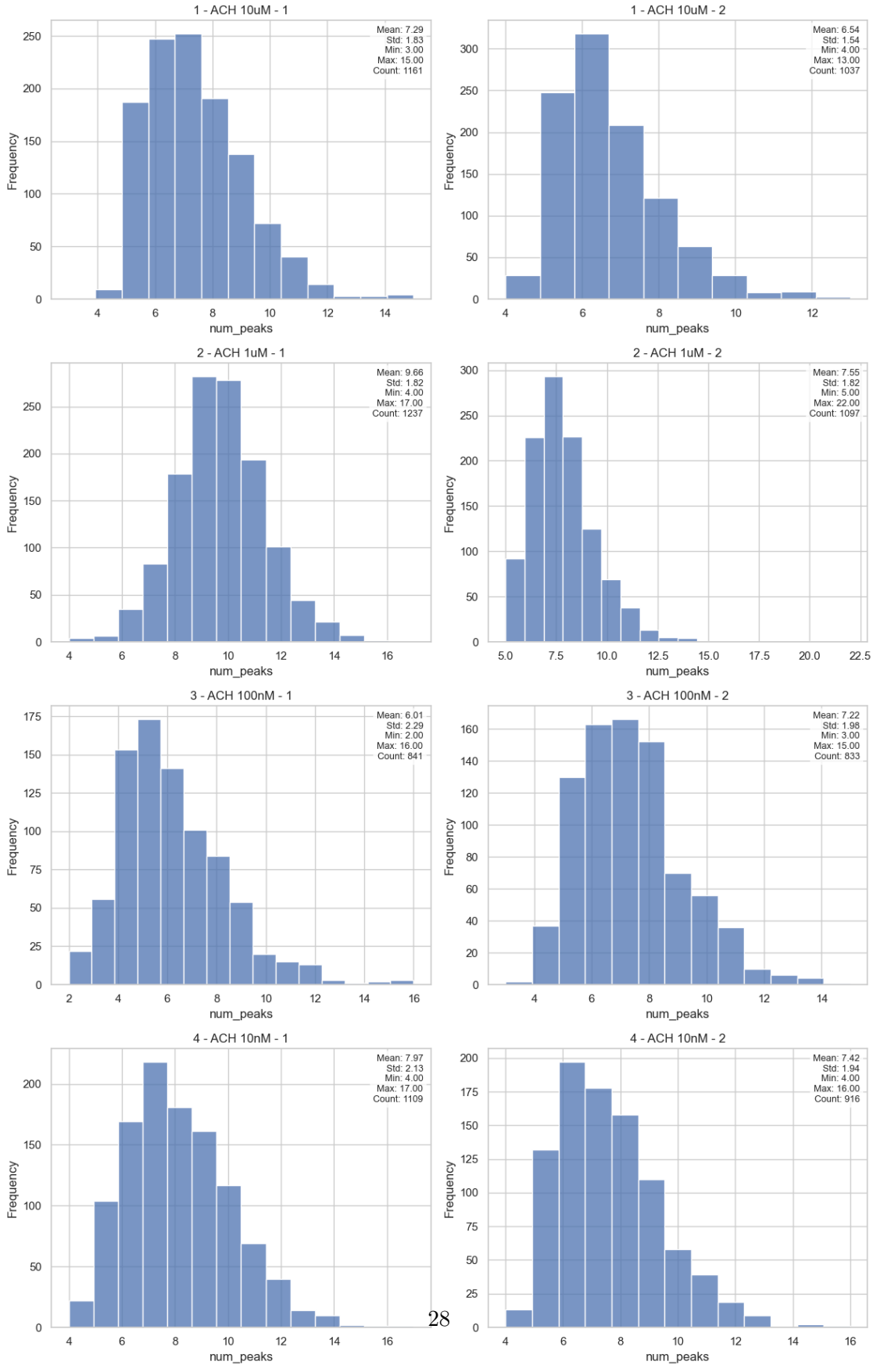
[6]:
```
# Convert is_active to label for display purposes
cells["active_label"] = cells["is_active"].map({1: "Active", 0: "Inactive"})

plot_category_distribution_by_dataset(
    df=cells,
    column="active_label",
    category_order=["Active", "Inactive"],
    colors={"Active": "#66c2a5", "Inactive": "#d3d3d3"},
    title="Active vs Inactive Cells per Dataset",
    n_cols=2
)
```

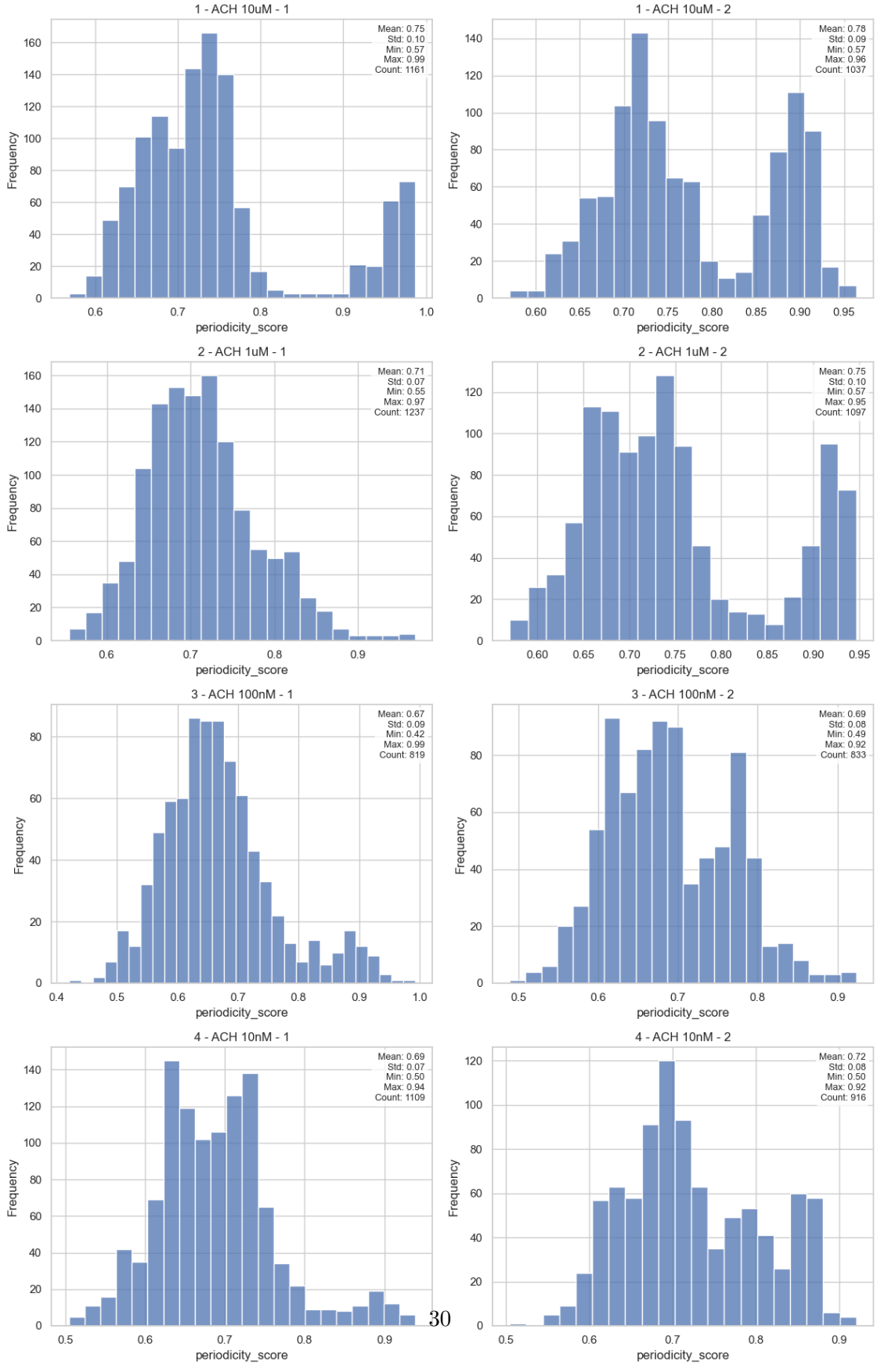

Active vs Inactive Cells per Dataset
1 - ACH 10uM - 1
Inactive (0)
100.0%
Active (1161)
1 - ACH 10uM - 2
Inactive (0)
100.0%
Active (1037)
2 - ACH 1uM - 1
Inactive (0)
100.0%
Active (1237)
2 - ACH 1uM - 2
Inactive (0)
100.0%
Active (1097)
3 - ACH 100nM - 1
Inactive (0)
100.0%
Active (841)
3 - ACH 100nM - 2
Inactive (0)
100.0%
Active (833)
4 - ACH 10nM - 1
Inactive (0)
100.0%
Active (1109)
4 - ACH 10nM - 2
Inactive (0)
100.0%
Active (916)

[7]:
```
plot_metric_by_dataset(cells, "num_peaks", "Cell: Number of Peaks",␣
↪bin_width=1, n_cols=2)
plot_metric_by_dataset(cells, "periodicity_score", "Cell: Periodicity Score",␣
↪bin_width=0.02, n_cols=2)
```

Cell: Number of Peaks



Cell: Periodicity Score



0.8 Sequential Event Metrics

We focus on **spatiotemporal events**, extracting: - Mean communication time and speed - Geometric descriptors (elongation, radiality) - Graph depth, number of involved cells

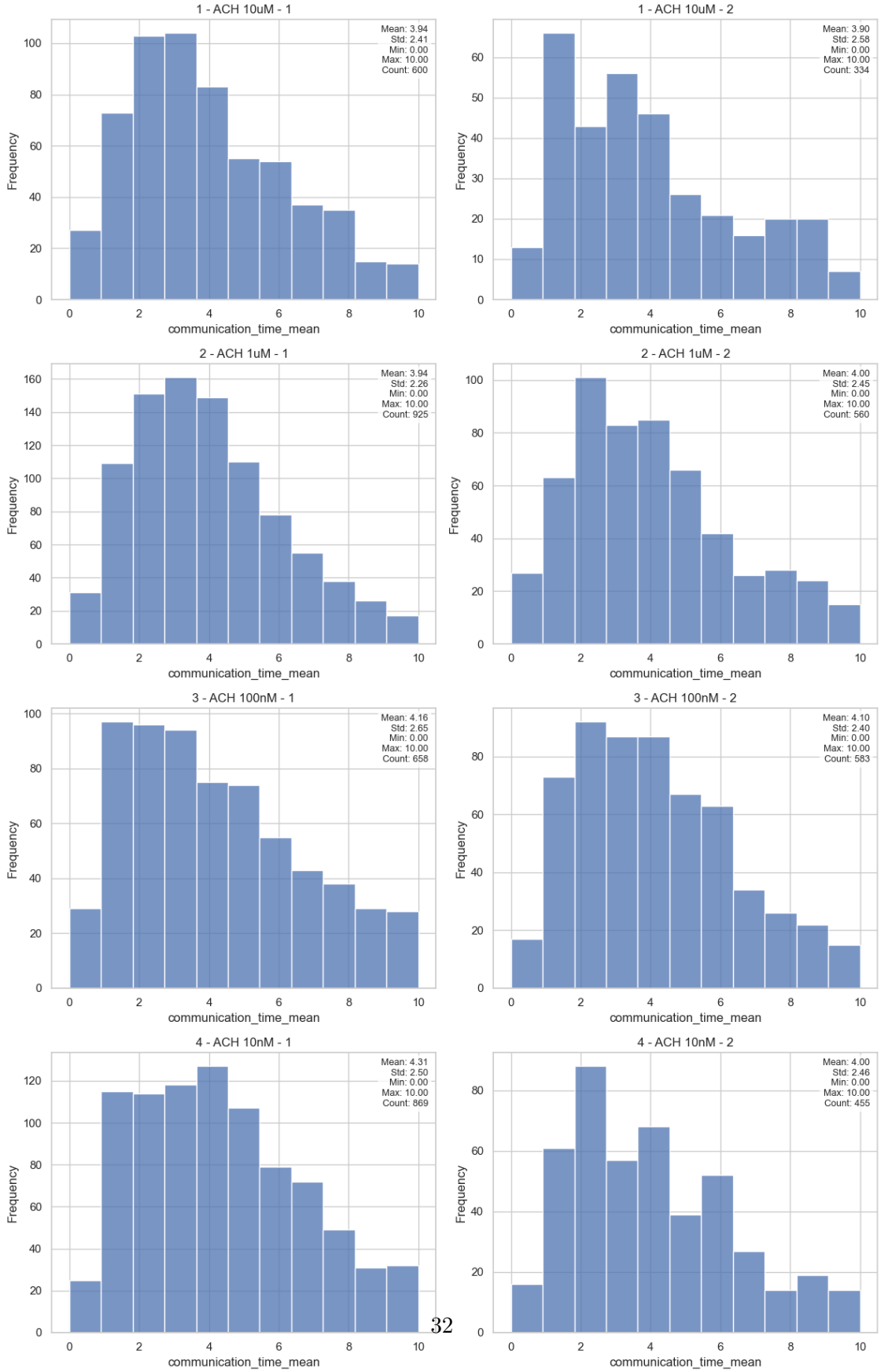
Interpretation:

- *Communication time: ~4.0 frames (STD ~2.5).*
- *Communication speed: ~6.2 units/frame.*
- *Cells involved: ~3.2 ± 2 cells per event.*

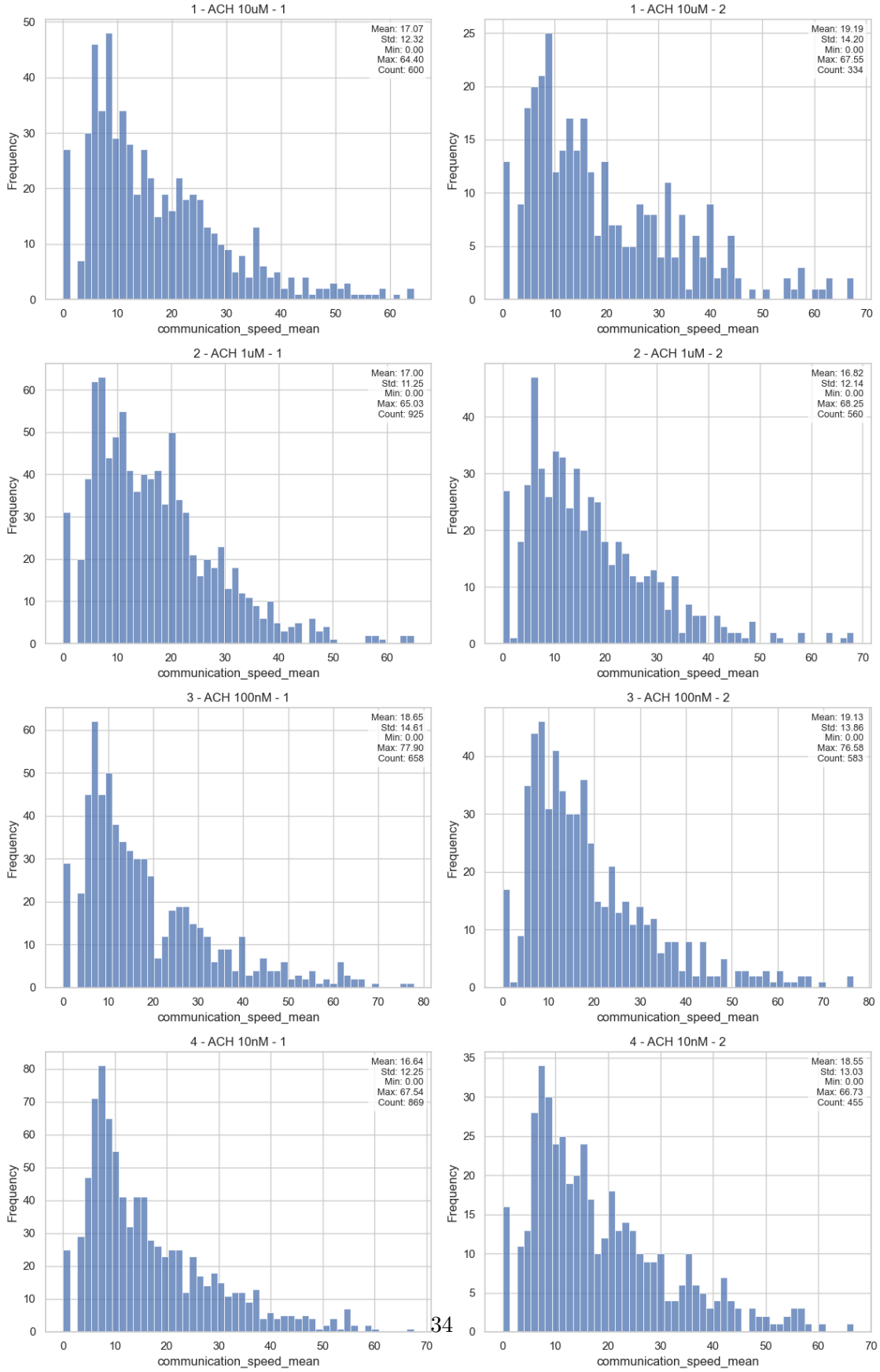
These metrics are consistent across datasets and define the propagation baseline for healthy spontaneous events.

[8]:
```
seq_events = events[events["event_type"] == "SequentialEvent"]
plot_metric_by_dataset(seq_events, "communication_time_mean", "Sequential␣
 ↪Events: Comm Time", bin_width=1, n_cols=2)
plot_metric_by_dataset(seq_events, "communication_speed_mean", "Sequential␣
 ↪Events: Comm Speed", bin_count=50, n_cols=2)
plot_metric_by_dataset(seq_events, "elongation_score", "Sequential Events:␣
 ↪Elongation", bin_count=50, n_cols=2)
plot_metric_by_dataset(seq_events, "radiality_score", "Sequential Events:␣
 ↪Radiality", bin_width=0.05, n_cols=2)
plot_metric_by_dataset(seq_events, "dag_depth", "Sequential Events: DAG Depth",␣
 ↪bin_width=1, n_cols=2)
plot_metric_by_dataset(seq_events, "n_cells_involved", "Sequential Events:␣
 ↪Cells Involved", bin_width=1, n_cols=2)
```

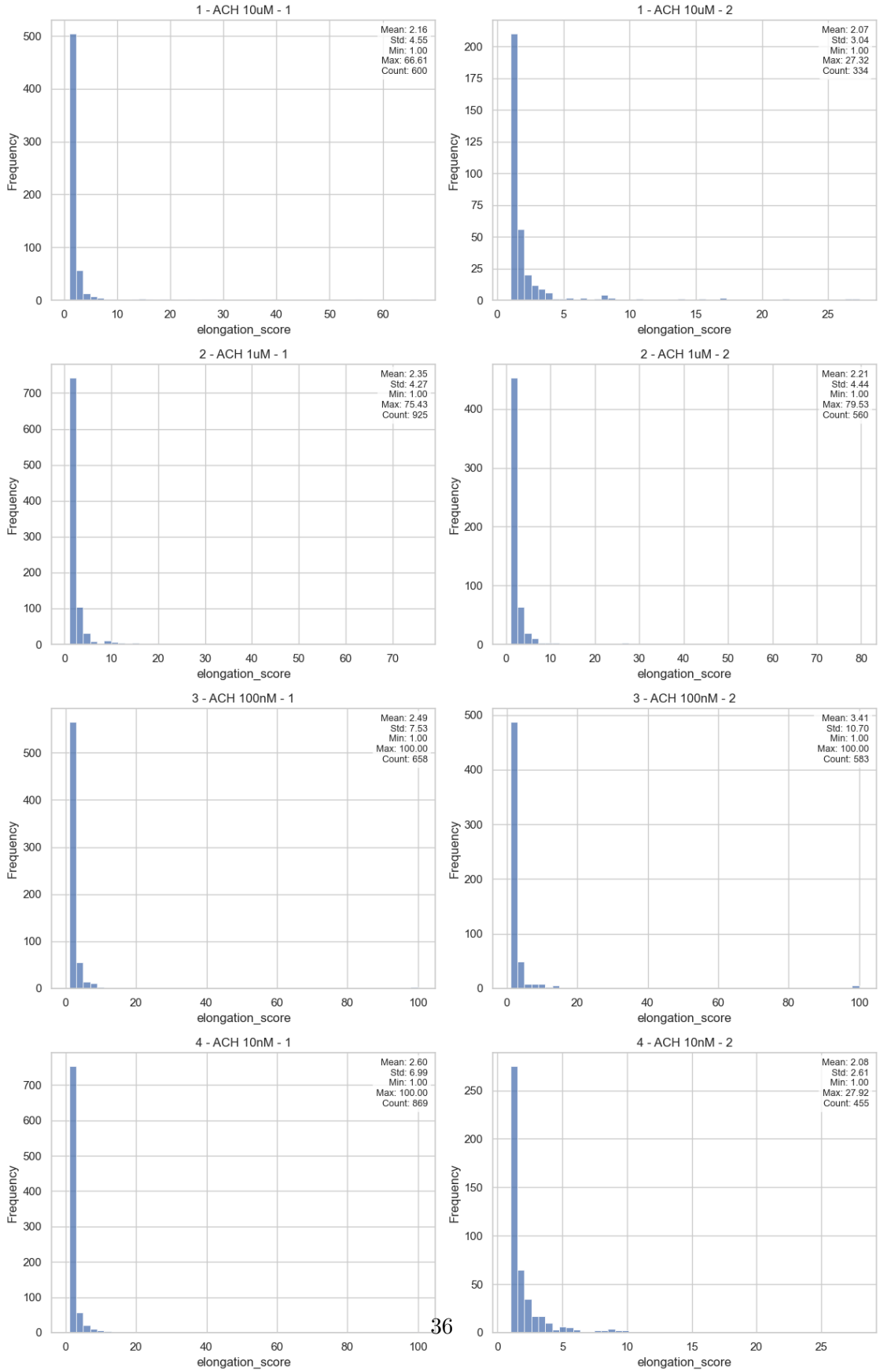
Sequential Events: Comm Time



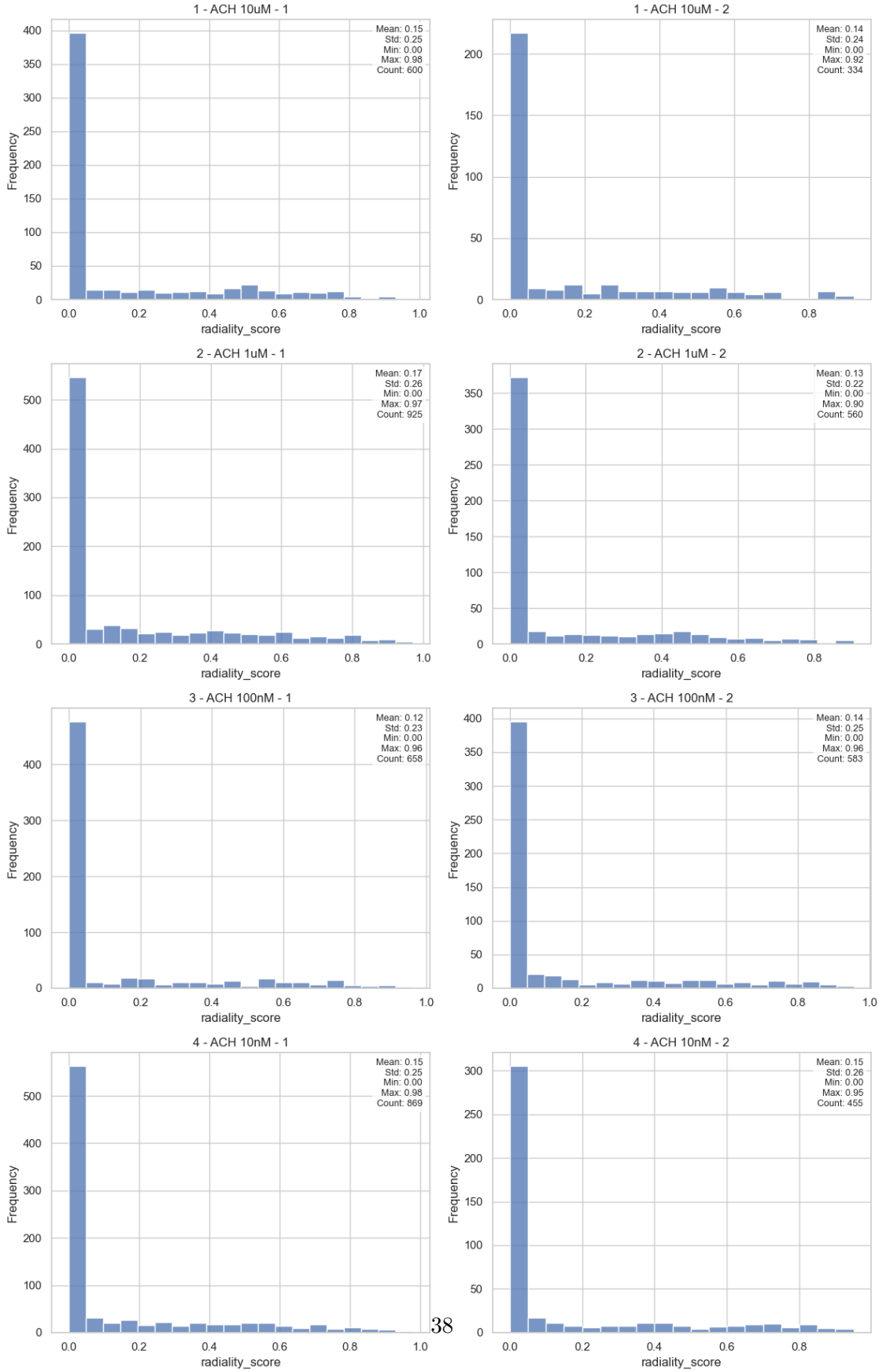
Sequential Events: Comm Speed



Sequential Events: Elongation



Sequential Events: Radiality



Sequential Events: DAG Depth

1 - ACH 10uM - 1 (No Data)

1 - ACH 10uM - 2 (No Data)

2 - ACH 1uM - 1 (No Data)

2 - ACH 1uM - 2 (No Data)

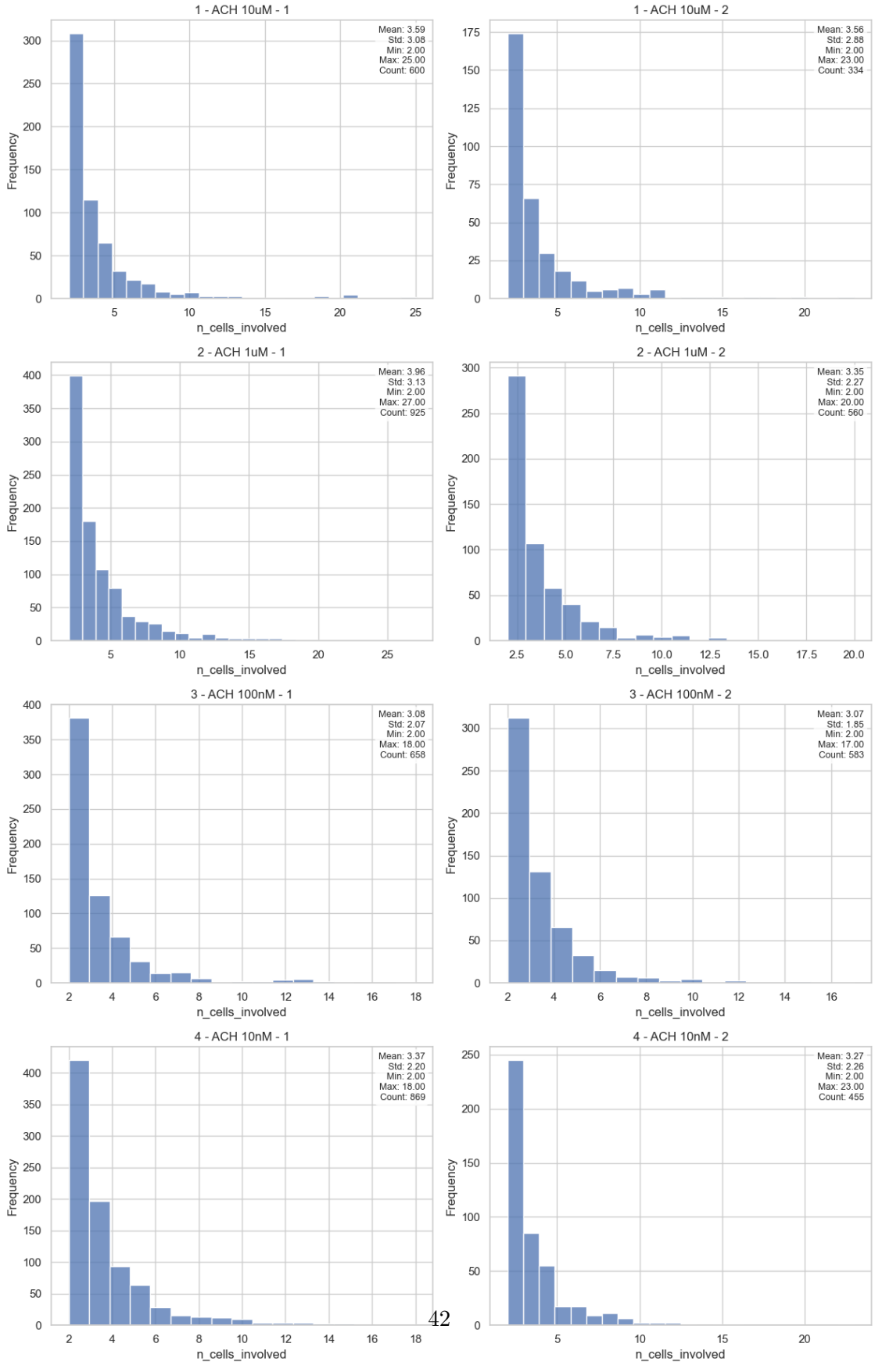
3 - ACH 100nM - 1 (No Data)

3 - ACH 100nM - 2 (No Data)

4 - ACH 10nM - 1 (No Data)

4 - ACH 10nM - 2 (No Data)

Sequential Events: Cells Involved



0.9 Conclusion & Next Steps

0.9.1 Summary of Observations

- **Visual Patterns:** Briefly describe observed visual trends (raster plots, activity levels, etc.).
- **Statistical Results:** Mention key metrics or KS-test outcomes that stood out.

0.9.2 Dataset Reproducibility

- Which datasets appear consistent or divergent?
- Are any replicates potentially faulty or biologically distinct?

0.9.3 Actionable Decisions

☐ Exclude dataset(s) due to technical issues
☐ Flag dataset(s) for further inspection
☐ Select representative samples for downstream analysis

0.9.4 Planned Deep-Dive

- Which direction will the next notebook take? (e.g., spatial clustering, propagation analysis, signal decomposition)

[ ]:
```
from calcium_activity_characterization.analysis.report import␣
↪export_current_notebook_to_pdf
#export_current_notebook_to_pdf("chronic_ACH_exposition_metrics.ipynb")
```

```
Notebook export error: Command '['jupyter', 'nbconvert', '--to', 'pdf', '--no-
input', '--output', 'chronic_ACH_exposition_metrics',
'notebooks\\chronic_ACH_exposition_metrics.ipynb']' returned non-zero exit
status 4294967295.

 Failed to export notebook: Command '['jupyter', 'nbconvert', '--to', 'pdf', '
--no-input', '--output', 'chronic_ACH_exposition_metrics',
'notebooks\\chronic_ACH_exposition_metrics.ipynb']' returned non-zero exit
status 4294967295.
```