

In vitro angiogenesis · HUVEC

Scientific report · MAPSNY · Cell Biology

Tube formation: *junctions, segments and meshes*

Experiment	Prueba Angiogénesis HUVEC (Control vs Inhibidor)
Date	2026-10-08
Cells	HUVEC (tube formation assay)
Control condition	Control
Units	µm and µm² (image scale)
2 conditions · 6 wells · 12 images	

Condition	Wells	Images	Meshes	Network pieces
Control	3	6	192	1588
Inhibidor	3	6	65	1162

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1. Summary of findings

26 per-image metrics were compared across 2 conditions (6 wells, 12 images; replicate = well). After FDR correction (Benjamini-Hochberg), no metric reaches FDR $p < 0.05$.

With 3 replicates per condition the exact Mann-Whitney test cannot go below $p = 0.10$: even when differences are consistent (Cliff's $\delta = \pm 1$ means every replicate of one condition exceeds every replicate of the other), they cannot reach significance. At least 4–5 wells per condition are recommended for formal inference.

Main assay metrics (plus any other significant one). Mean $\pm$ SEM across replicates; in parentheses, percentage change of the mean relative to control.

Metric	Control	Inhibitor	p	p FDR	Effect (Cliff's δ)
Total tube length (μm)	27,835 $\pm$ 81	19,659 $\pm$ 1,013 (-29%)	0.100	0.118	-1.00
Junctions	137.0 $\pm$ 1.3	80.50 $\pm$ 8.84 (-41%)	0.100	0.118	-1.00
Master segments	108.3 $\pm$ 4.2	36.83 $\pm$ 8.82 (-66%)	0.100	0.118	-1.00
Mesh index (μm)	253.4 $\pm$ 4.9	309.5 $\pm$ 23.7 (+22%)	0.100	0.118	1.00
Meshes	32.00 $\pm$ 0.50	10.83 $\pm$ 2.05 (-66%)	0.077	0.118	-1.00
Total mesh area (μm^2)	956,465 $\pm$ 5,202	184,967 $\pm$ 19,082 (-81%)	0.100	0.118	-1.00

Table 1. Main findings: mean $\pm$ SEM per condition, test, p, FDR p and effect size.

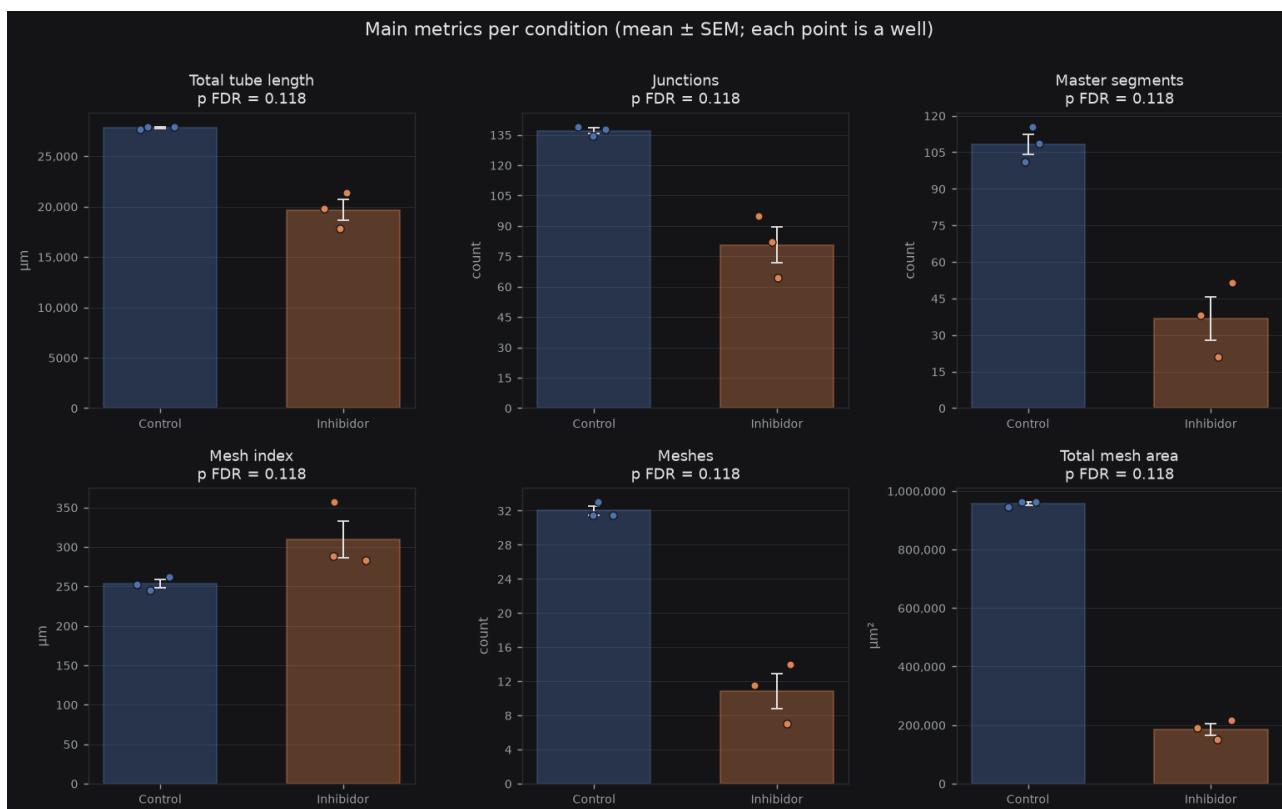


Figure 1. Main metrics per condition: mean $\pm$ SEM across replicates, each point is a replicate; FDR-corrected p above each panel.

2. Methods

Tubular network analysis

Tubular networks were analysed with the MAPSNY in vitro Angiogenesis module. In each image the system automatically reconstructs the network formed by the endothelial cells, completes short interruptions of the tubes by following the cells and discards fragments without structure. The standard tube-formation parameters are measured on the network with the Angiogenesis Analyzer definitions (junctions, segments, branches, master network, meshes, mesh index and branching interval). Detection settings were tuned once on a representative image of the experiment and applied identically to every image.

Analysis settings

Settings chosen in the portal preview step and applied identically to every image of the experiment (values on the scale of the portal controls).

Setting	Value
Tube thickness	6
Sensitivity (lower = fainter tubes)	0.9
Gap closing	1 · conservative
Minimum branch	25
Minimum mesh	600
Image type	Automatic (detected per image)

Table 2. Analysis settings used in this experiment.

Acquisition and scale

12 images from 6 wells were analysed. Lateral scale was 1.6 $\mu\text{m}/\text{px}$ in all images; the analysed field measures 2.83 mm^2 per image. Lengths are given in μm , areas in μm^2 and densities per mm^2 of field.

Condition	Well	Image	Size (px)	$\mu\text{m}/\text{px}$	Image type
Control	1	pocillo_campo_1	904 × 1224	1.6	Bright field
Control	1	pocillo_campo_2	1224 × 904	1.6	Bright field
Control	2	pocillo_campo_1	1224 × 904	1.6	Bright field
Control	2	pocillo_campo_2	904 × 1224	1.6	Bright field
Control	3	pocillo_campo_1	904 × 1224	1.6	Bright field
Control	3	pocillo_campo_2	1224 × 904	1.6	Bright field
Inhibidor	1	pocillo_campo_1	904 × 1224	1.6	Bright field
Inhibidor	1	pocillo_campo_2	904 × 1224	1.6	Bright field
Inhibidor	2	pocillo_campo_1	1224 × 904	1.6	Bright field
Inhibidor	2	pocillo_campo_2	1224 × 904	1.6	Bright field
Inhibidor	3	pocillo_campo_1	904 × 1224	1.6	Bright field
Inhibidor	3	pocillo_campo_2	904 × 1224	1.6	Bright field

Table 3. Size, scale and detected image type per image.

Statistical analysis

Replicate unit. Each well (folder {rank}_{Condition}_{n}) is a replicate: the median of its images is used for every metric. Individual images are shown as distributions but never as independent replicates (no pseudoreplication). If a condition has a single well, the image becomes the replicate and this is stated.

Tests. Two conditions: Mann-Whitney U with Cliff's δ . Three or more: Kruskal-Wallis with ϵ^2_H and Dunn post hoc comparisons against control. Benjamini-Hochberg (FDR) correction across metrics.

Units. Lengths and areas in μm and μm^2 when the image carries a scale or the resolution was declared; otherwise in pixels, as in the Angiogenesis Analyzer. Densities per mm^2 exist only with a scale.

3. Representative images

For each condition the image whose total tube length is closest to the condition median is shown. A: original image. B: reconstructed network with the Angiogenesis Analyzer colours.

Network colour legend

 Segment	 Branch	 Isolated	 Master segment
 Mesh	 Junction	 Master junction	 Extremity

Control

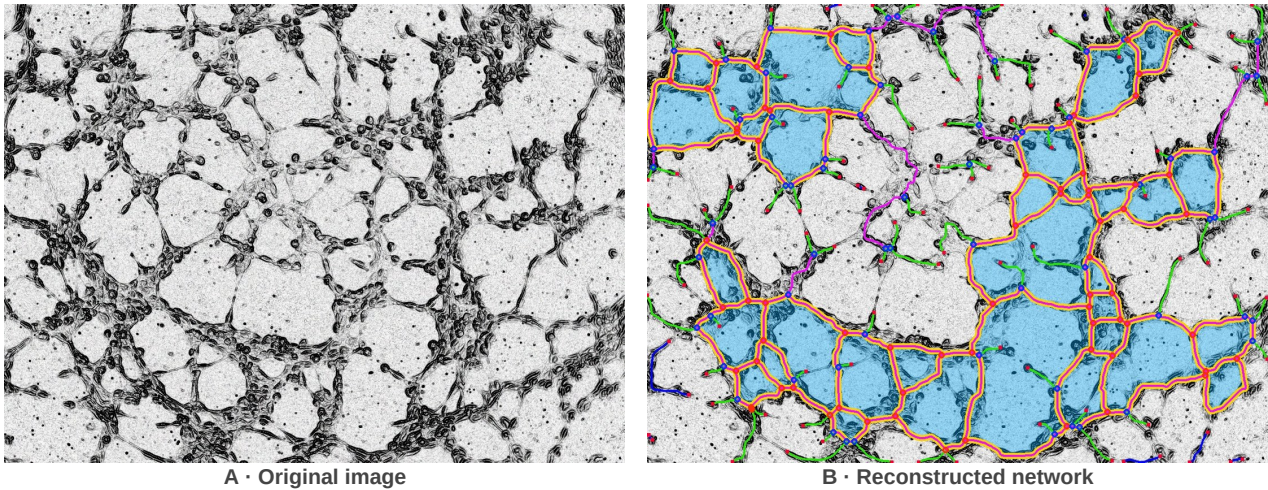


Figure 2. Control — well 2, pocillo_campo_1: 135 junctions, 33 meshes, total length 27,783 μm (condition median 27,831 μm).

Inhibidor

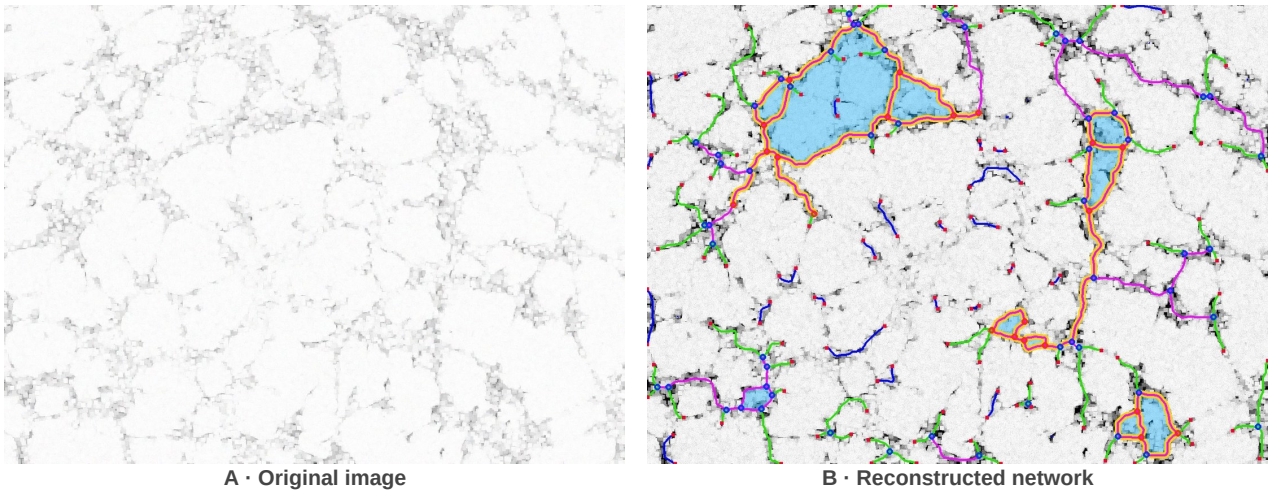


Figure 3. Inhibidor — well 2, pocillo_campo_2: 77 junctions, 10 meshes, total length 19,083 μm (condition median 19,592 μm).

4. Results per condition

Mean $\pm$ standard error of the mean (SEM) across replicates (wells; each replicate contributes the median of its images). The last column is the p-value of the comparison between conditions, FDR-corrected across all metrics; bold = FDR $p < 0.05$.

Metric	Unit	Control	Inhibitor	p FDR
Wells		3	3	
Images		6	6	
Network extent				
Total tube length	μm	27,835 $\pm$ 81	19,659 $\pm$ 1,013	0.118
Total branching length	μm	27,387 $\pm$ 97	17,662 $\pm$ 1,011	0.118
Total segment length	μm	19,349 $\pm$ 116	9,568 $\pm$ 1,020	0.118
Total branch length	μm	8,038 $\pm$ 21	8,094 $\pm$ 148	0.728
Network coverage	fraction 0–1	0.406 $\pm$ 0.000	0.279 $\pm$ 0.011	0.118
Topology				
Junctions	count	137.0 $\pm$ 1.3	80.50 $\pm$ 8.84	0.118
Extremities	count	93.17 $\pm$ 0.44	121.2 $\pm$ 2.3	0.118
Segments	count	177.2 $\pm$ 3.1	90.83 $\pm$ 12.32	0.118
Branches	count	80.50 $\pm$ 0.29	77.17 $\pm$ 3.68	0.728
Isolated elements	count	7.00 $\pm$ 0.29	25.67 $\pm$ 2.32	0.118
Mean segment length	μm	109.3 $\pm$ 1.3	106.3 $\pm$ 3.6	1.000
Branching interval	μm	240.4 $\pm$ 2.2	123.7 $\pm$ 9.8	0.118
Master network				
Master segments	count	108.3 $\pm$ 4.2	36.83 $\pm$ 8.82	0.118
Master segment length	μm	17,079 $\pm$ 249	6,340 $\pm$ 1,370	0.118
Master junctions	count	67.50 $\pm$ 2.31	21.33 $\pm$ 5.55	0.118
Mesh index	μm	253.4 $\pm$ 4.9	309.5 $\pm$ 23.7	0.118
Meshes (closed polygons)				
Meshes	count	32.00 $\pm$ 0.50	10.83 $\pm$ 2.05	0.118
Total mesh area	μm^2	956,465 $\pm$ 5,202	184,967 $\pm$ 19,082	0.118
Mean mesh size	μm^2	29,908 $\pm$ 620	18,276 $\pm$ 2,257	0.118
Mesh coverage	fraction 0–1	0.338 $\pm$ 0.002	0.065 $\pm$ 0.007	0.118
Mesh circularity	fraction 0–1	0.639 $\pm$ 0.004	0.667 $\pm$ 0.008	0.118
Density per mm^2				
Junction density	/ mm^2	48.36 $\pm$ 0.47	28.42 $\pm$ 3.12	0.118
Mesh density	/ mm^2	11.30 $\pm$ 0.18	3.82 $\pm$ 0.72	0.118
Segment density	/ mm^2	62.54 $\pm$ 1.08	32.07 $\pm$ 4.35	0.118
Branch density	/ mm^2	28.42 $\pm$ 0.10	27.24 $\pm$ 1.30	0.728
Tube length density	mm/mm^2	9.83 $\pm$ 0.03	6.94 $\pm$ 0.36	0.118

Table 4. All metrics per condition (mean $\pm$ SEM; replicate = well).

5. Statistical analysis

Comparison between conditions

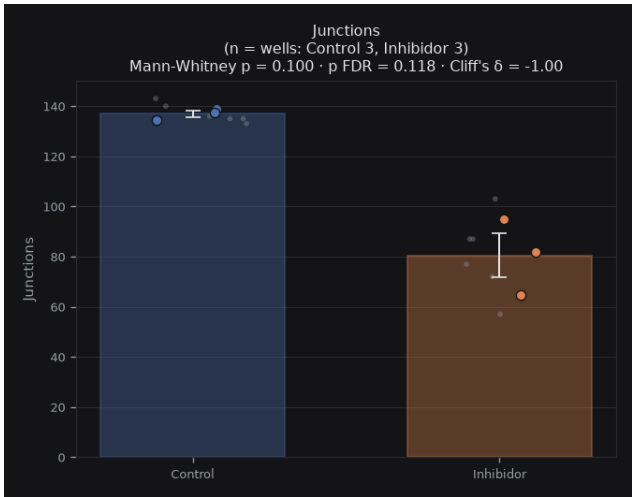
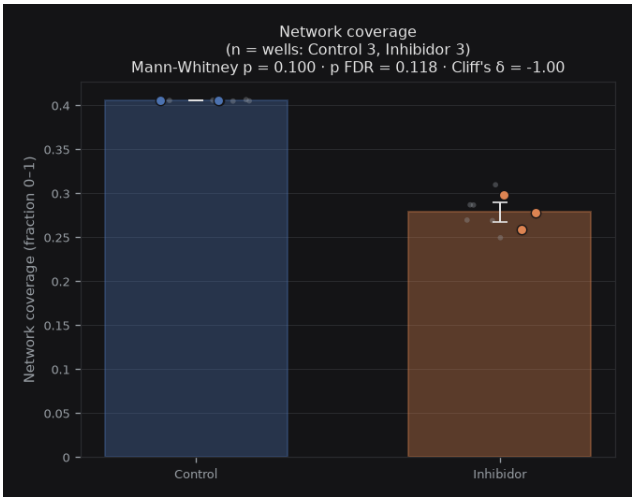
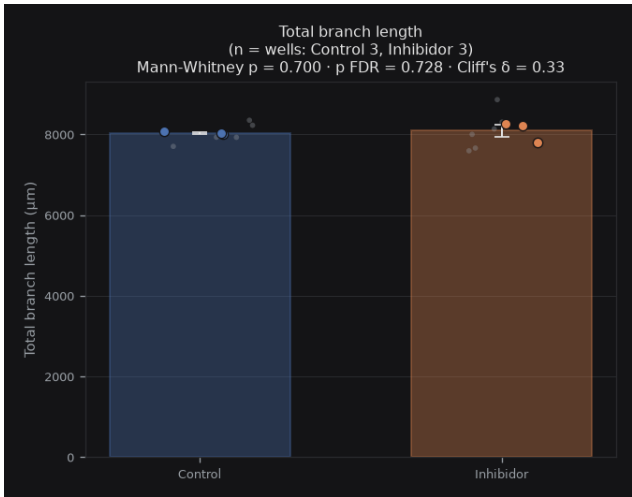
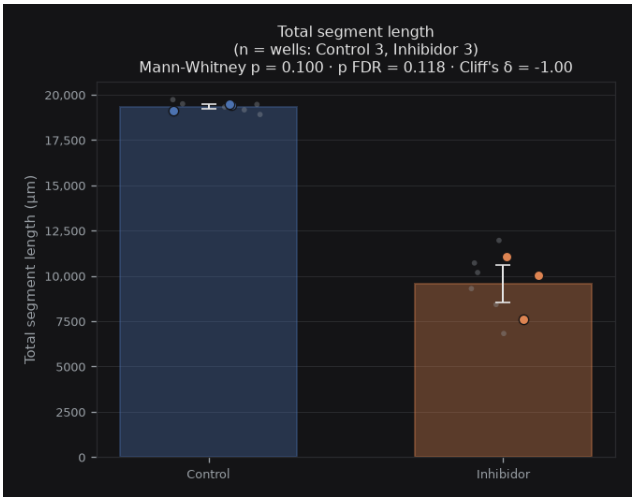
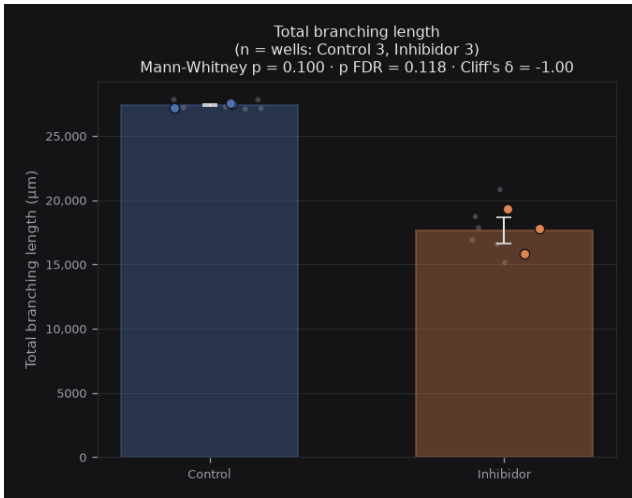
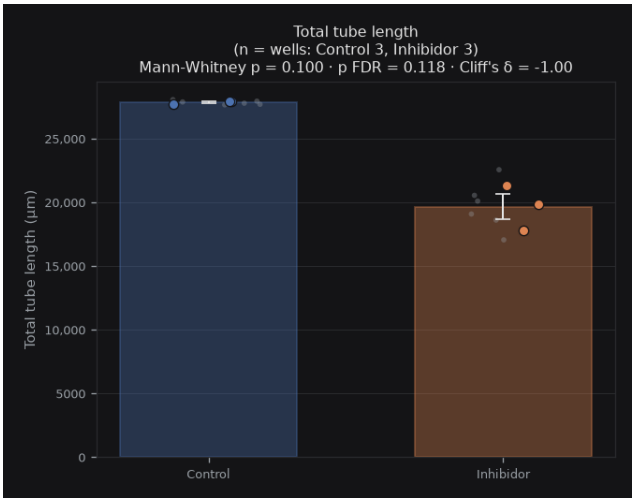
One row per metric: test applied, uncorrected p, FDR-corrected p, effect size (Cliff's δ) and percentage change of the mean relative to control.

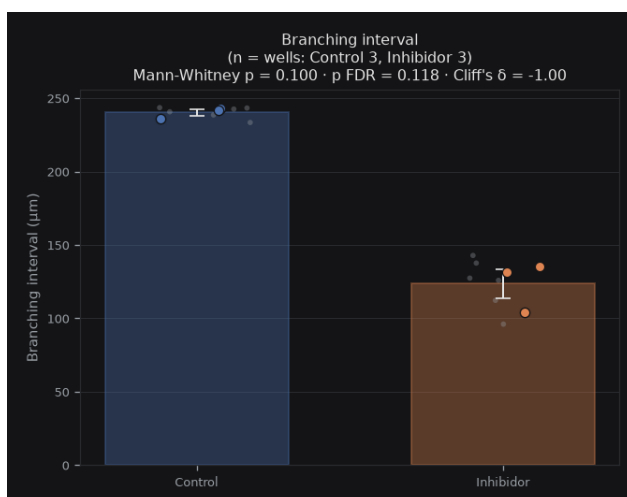
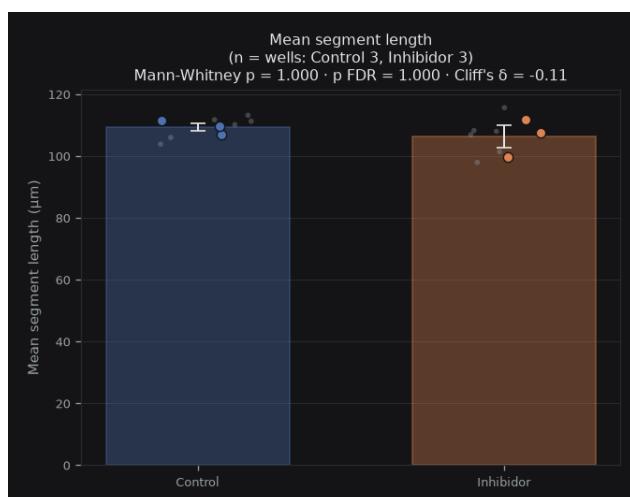
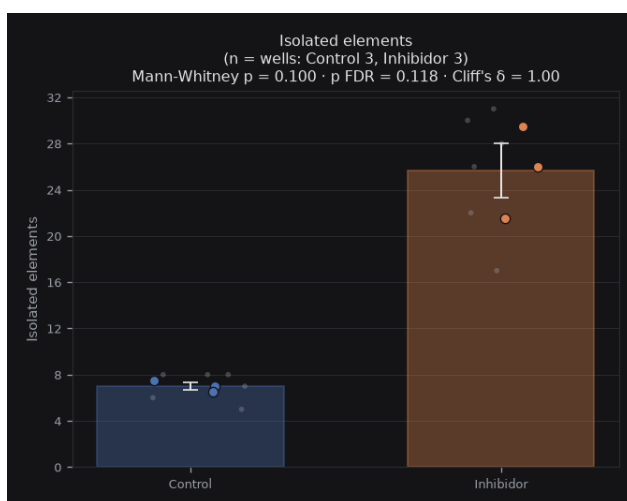
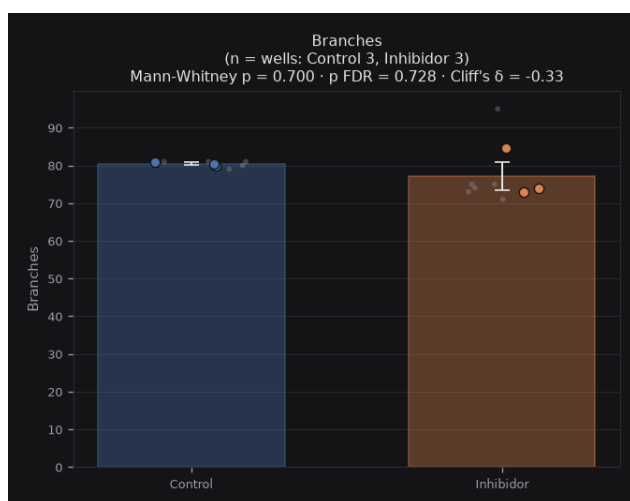
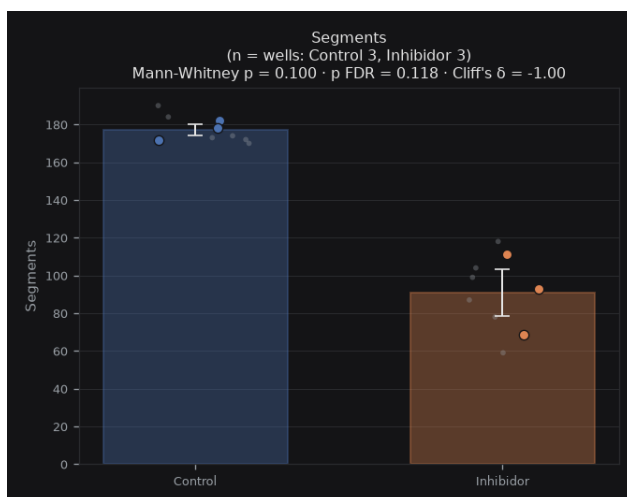
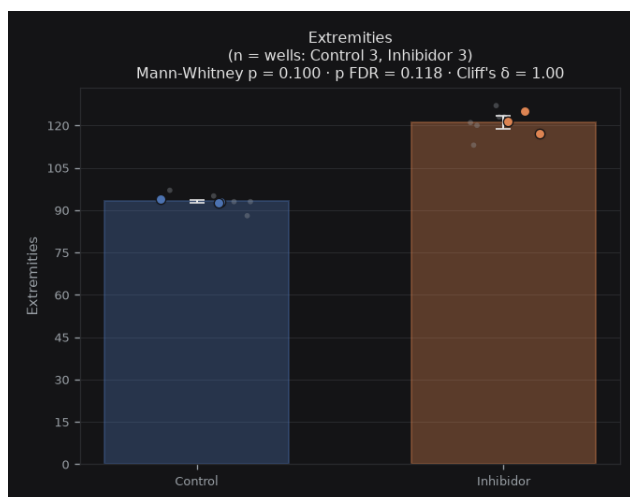
Metric	Test	p	p FDR	Effect (Cliff's δ)	Δ Inhibidor
Network extent					
Total tube length (μm)	Mann-Whitney	0.100	0.118	-1.00	-29%
Total branching length (μm)	Mann-Whitney	0.100	0.118	-1.00	-36%
Total segment length (μm)	Mann-Whitney	0.100	0.118	-1.00	-51%
Total branch length (μm)	Mann-Whitney	0.700	0.728	0.33	+1%
Network coverage (fraction 0–1)	Mann-Whitney	0.100	0.118	-1.00	-31%
Topology					
Junctions	Mann-Whitney	0.100	0.118	-1.00	-41%
Extremities	Mann-Whitney	0.100	0.118	1.00	+30%
Segments	Mann-Whitney	0.100	0.118	-1.00	-49%
Branches	Mann-Whitney	0.700	0.728	-0.33	-4%
Isolated elements	Mann-Whitney	0.100	0.118	1.00	+267%
Mean segment length (μm)	Mann-Whitney	1.000	1.000	-0.11	-3%
Branching interval (μm)	Mann-Whitney	0.100	0.118	-1.00	-49%
Master network					
Master segments	Mann-Whitney	0.100	0.118	-1.00	-66%
Master segment length (μm)	Mann-Whitney	0.100	0.118	-1.00	-63%
Master junctions	Mann-Whitney	0.100	0.118	-1.00	-68%
Mesh index (μm)	Mann-Whitney	0.100	0.118	1.00	+22%
Meshes (closed polygons)					
Meshes	Mann-Whitney	0.077	0.118	-1.00	-66%
Total mesh area (μm^2)	Mann-Whitney	0.100	0.118	-1.00	-81%
Mean mesh size (μm^2)	Mann-Whitney	0.100	0.118	-1.00	-39%
Mesh coverage (fraction 0–1)	Mann-Whitney	0.077	0.118	-1.00	-81%
Mesh circularity (fraction 0–1)	Mann-Whitney	0.100	0.118	1.00	+4%
Density per mm^2					
Junction density (/mm ^{2})	Mann-Whitney	0.100	0.118	-1.00	-41%
Mesh density (/mm ^{2})	Mann-Whitney	0.077	0.118	-1.00	-66%
Segment density (/mm ^{2})	Mann-Whitney	0.100	0.118	-1.00	-49%
Branch density (/mm ^{2})	Mann-Whitney	0.700	0.728	-0.33	-4%
Tube length density (mm/mm ^{2})	Mann-Whitney	0.100	0.118	-1.00	-29%

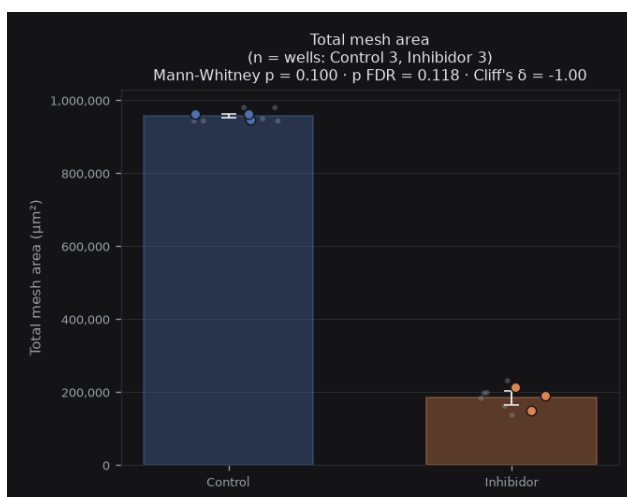
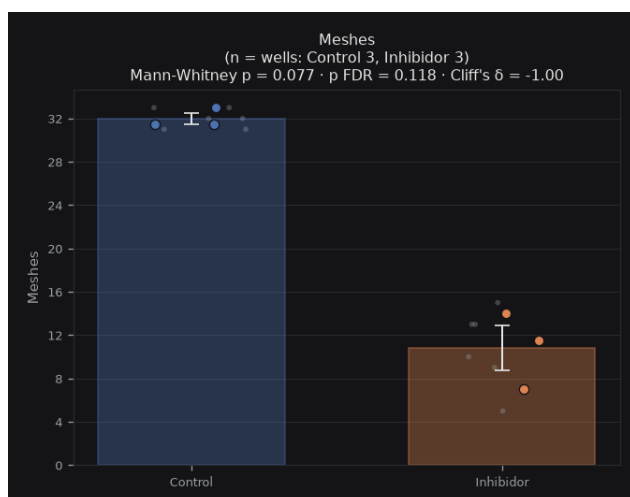
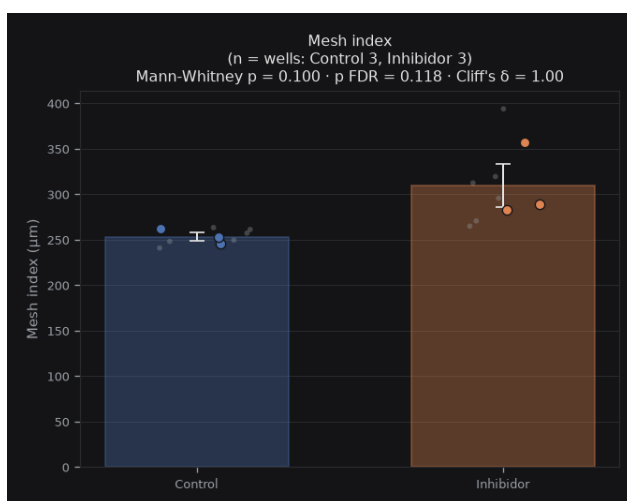
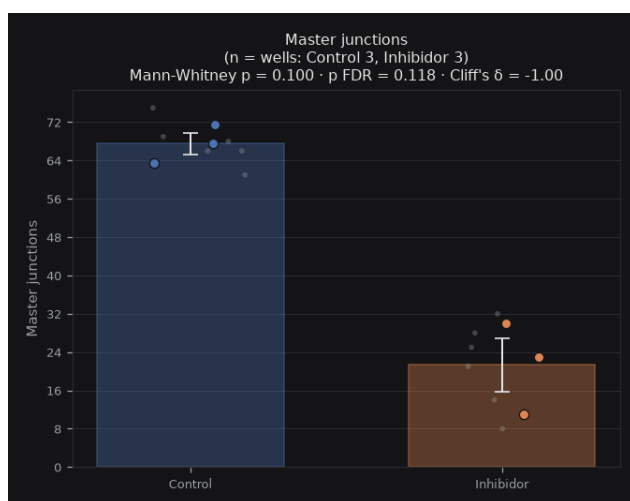
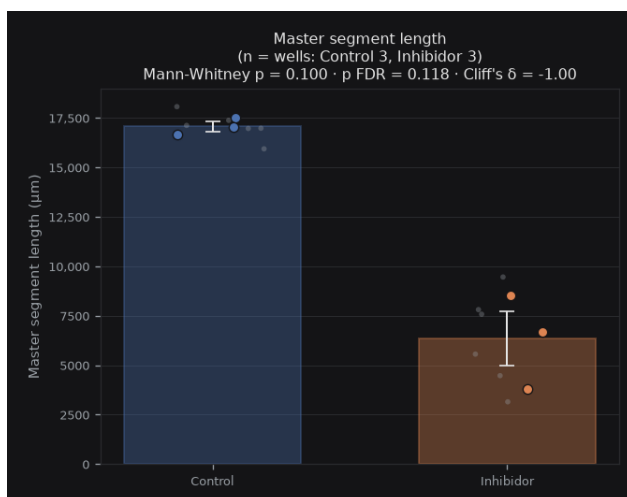
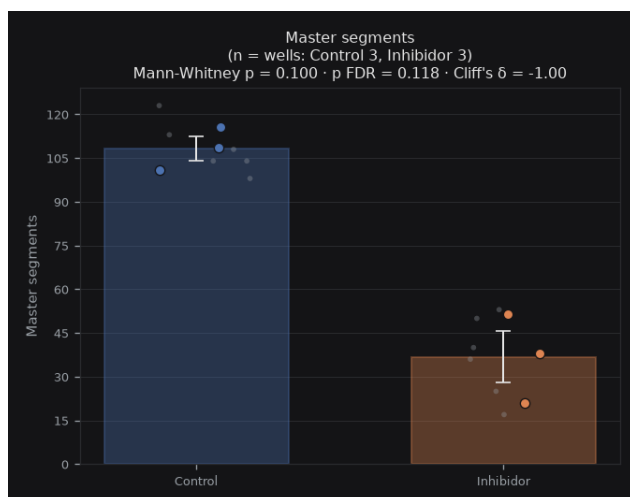
Table 5. Comparison between conditions (all metrics).

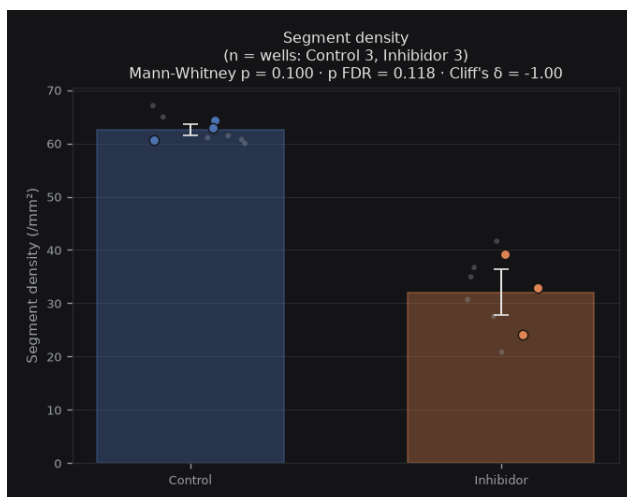
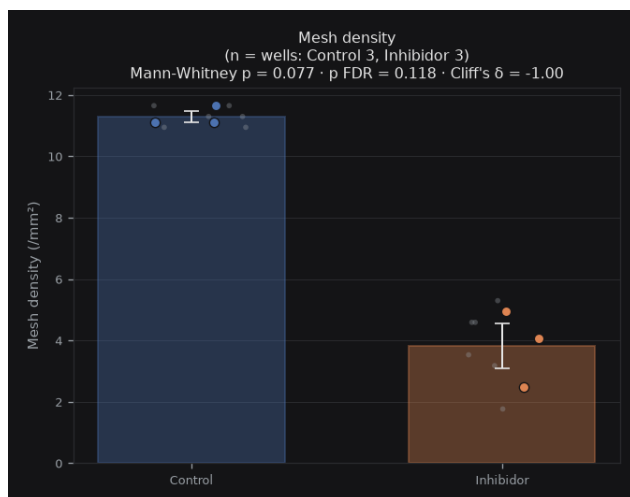
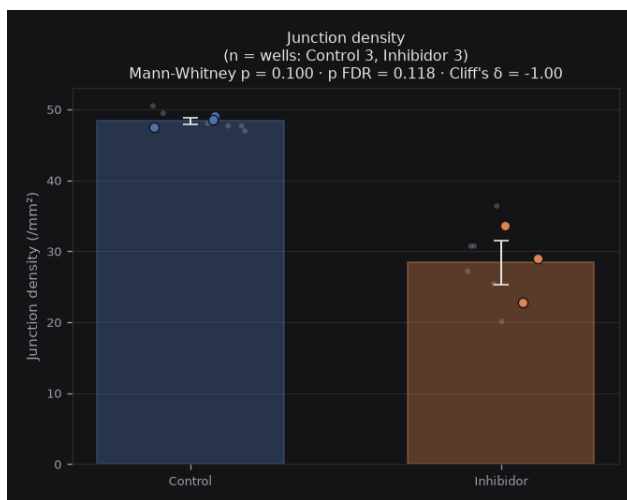
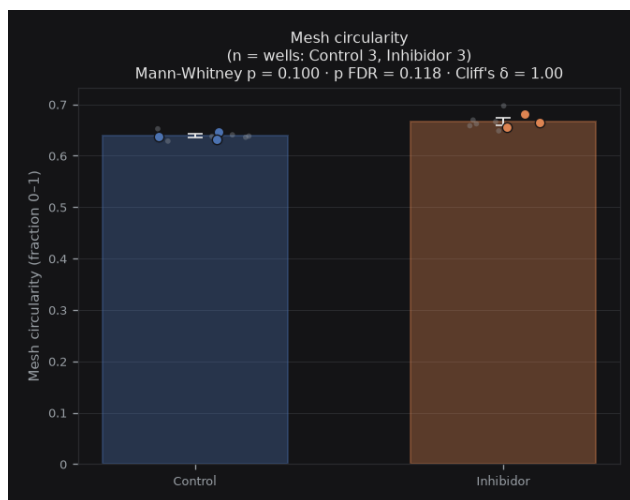
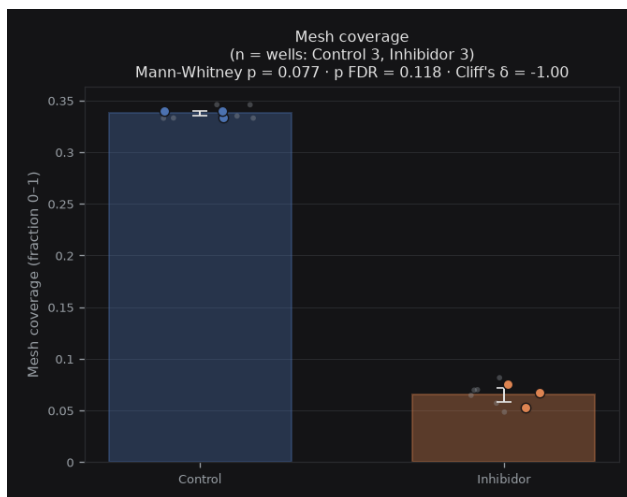
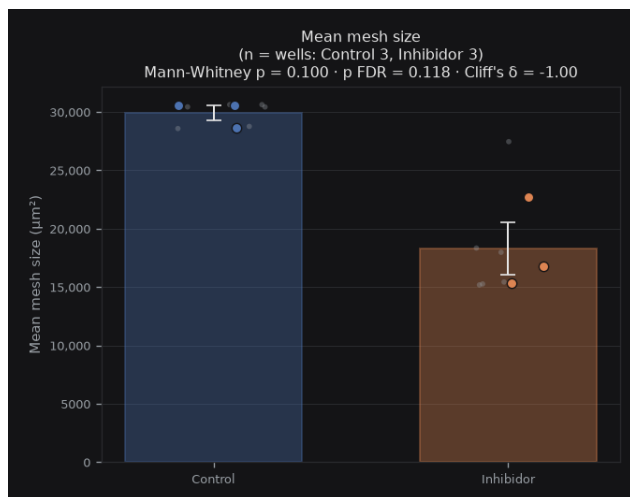
Per-metric figures

Bars: mean ± SEM across replicates; coloured points: replicates; grey points: individual images (shown as distribution only, not used in the test).









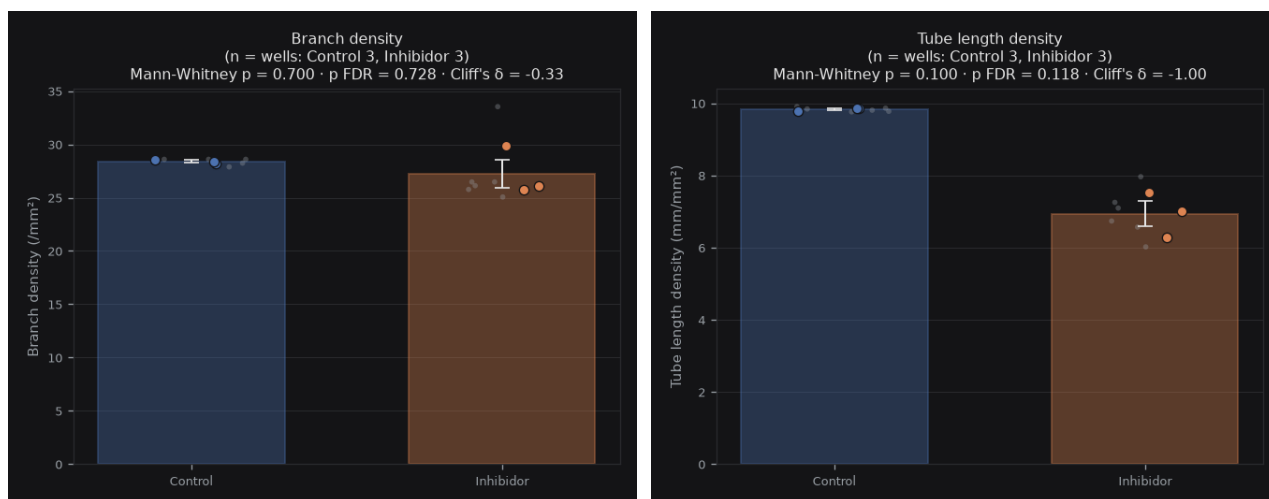


Figure 4. Per-metric comparison between conditions (26 figures; each title gives the test, p, FDR p and effect).

Statistical engine figures

Per-condition violins with the points of each replicate. In these figures the test is chosen from normality and variance homogeneity (Welch parametric or non-parametric) with automatic post hoc, so their p can differ from the comparison table.

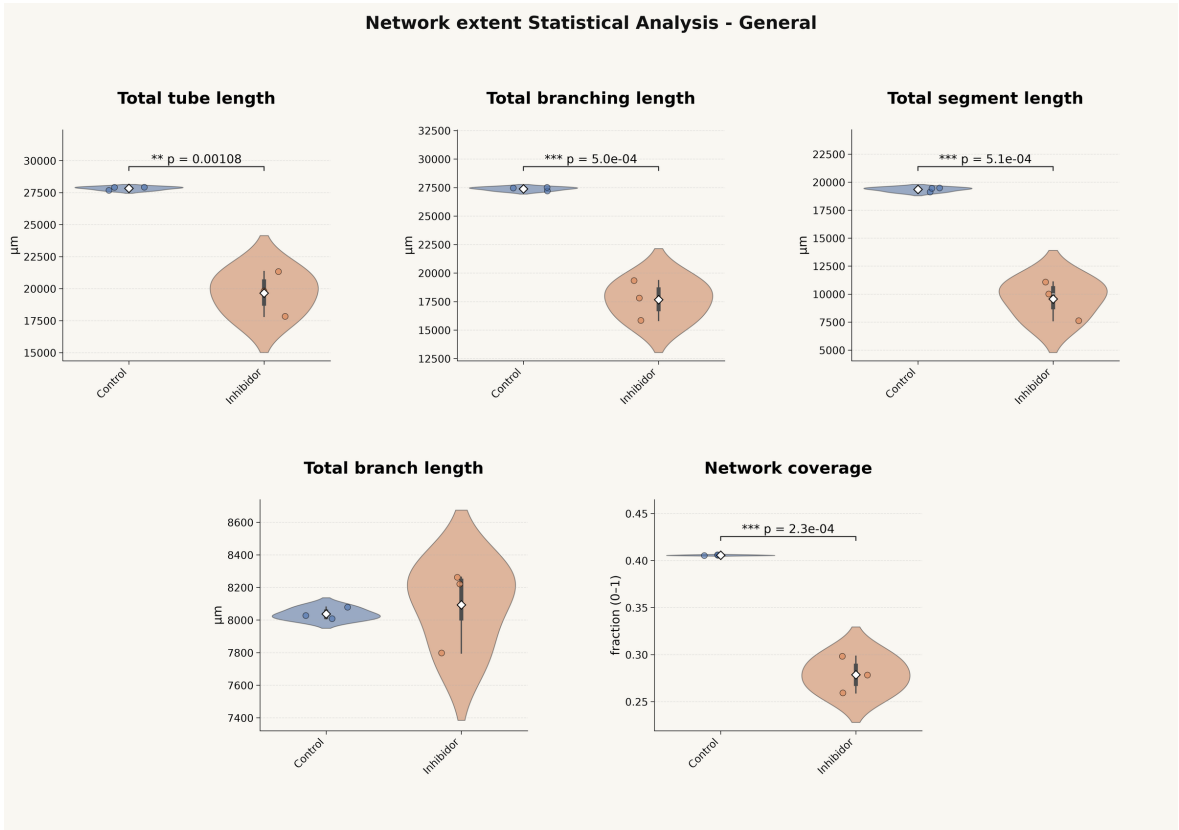


Figure 5. Per-condition violins with omnibus test, p and effect size — Network extent group. Metrics: Total tube length (μm); Total branching length (μm); Total segment length (μm); Total branch length (μm); Network coverage (fraction 0–1).

Topology Statistical Analysis - General

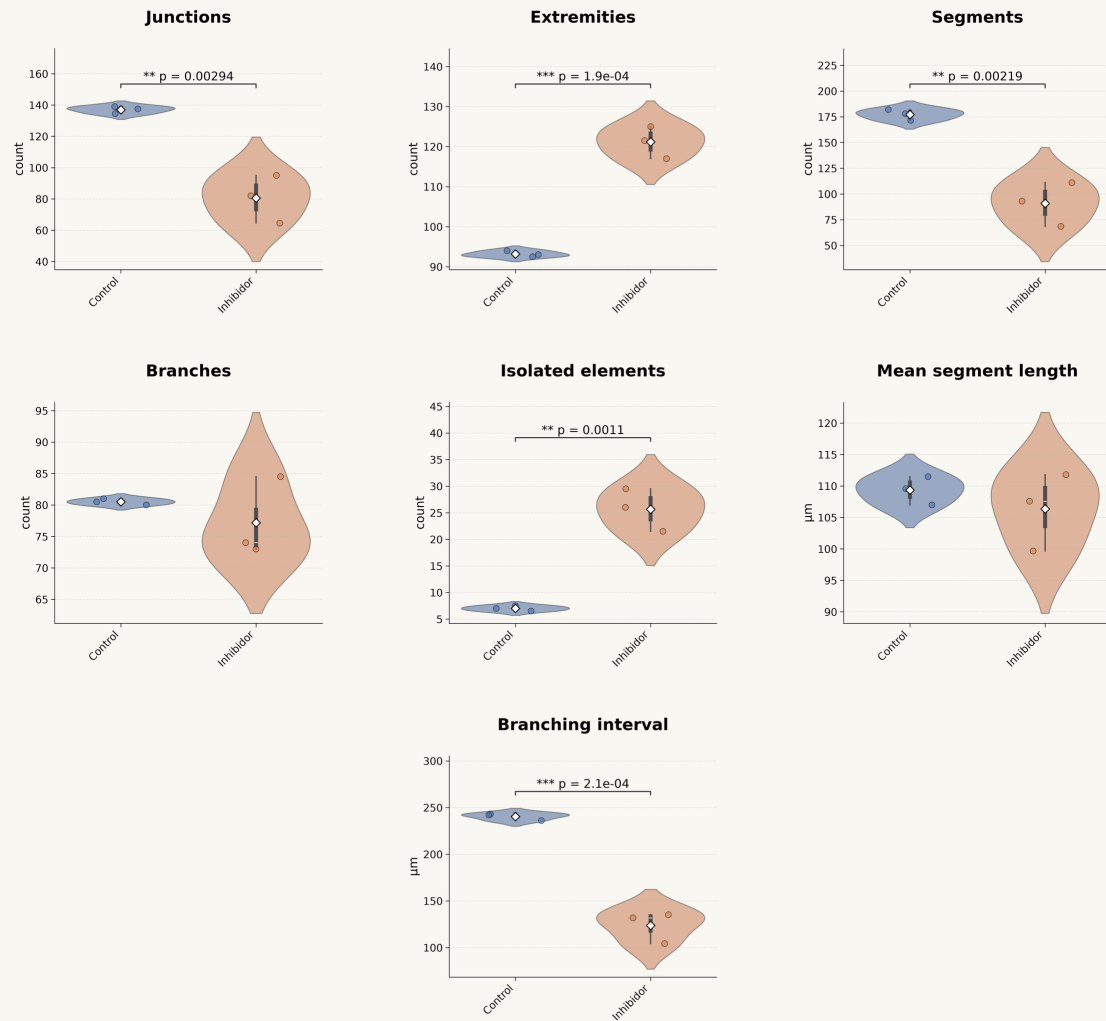


Figure 6. Per-condition violins with omnibus test, p and effect size — Topology group. Metrics: Junctions; Extremities; Segments; Branches; Isolated elements; Mean segment length (μm); Branching interval (μm).

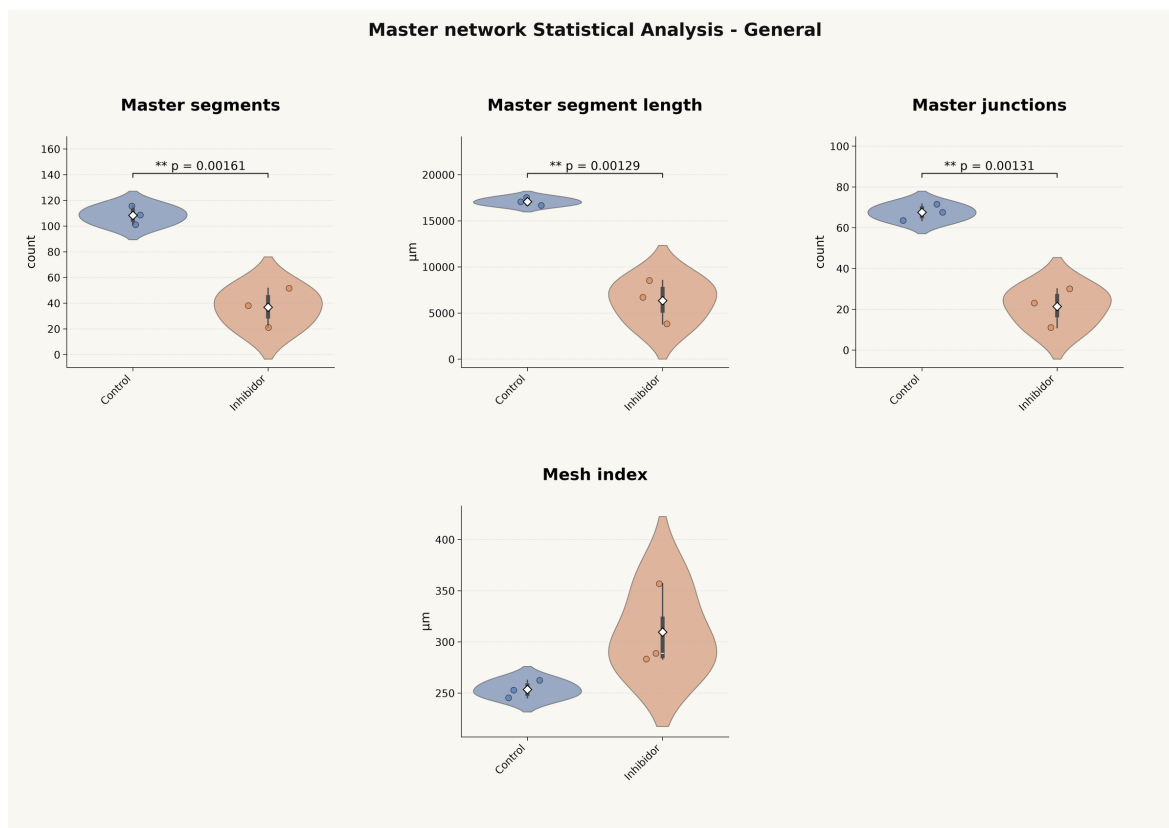


Figure 7. Per-condition violins with omnibus test, p and effect size — Master network group. Metrics: Master segments; Master segment length (μm); Master junctions; Mesh index (μm).

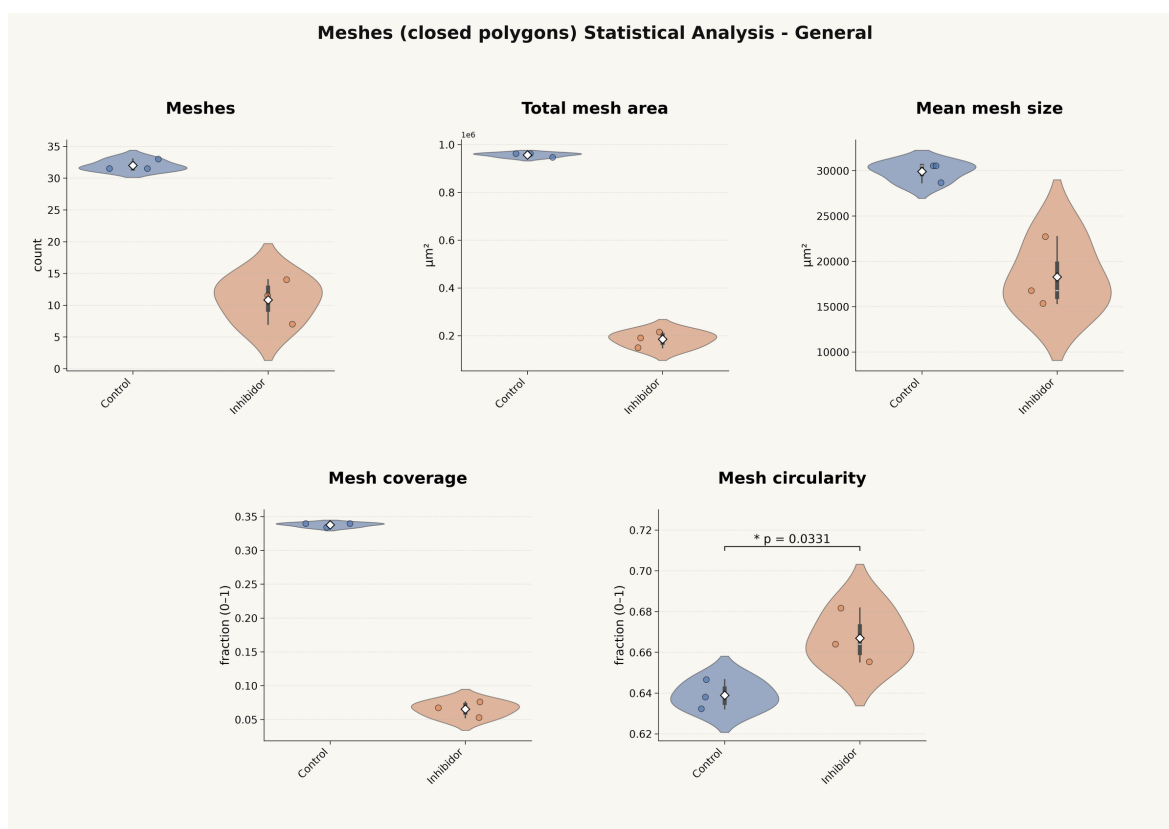


Figure 8. Per-condition violins with omnibus test, p and effect size — Mesher (closed polygons) group. Metrics: Mesher; Total mesh area (μm^2); Mean mesh size (μm^2); Mesh coverage (fraction 0–1); Mesh circularity (fraction 0–1).

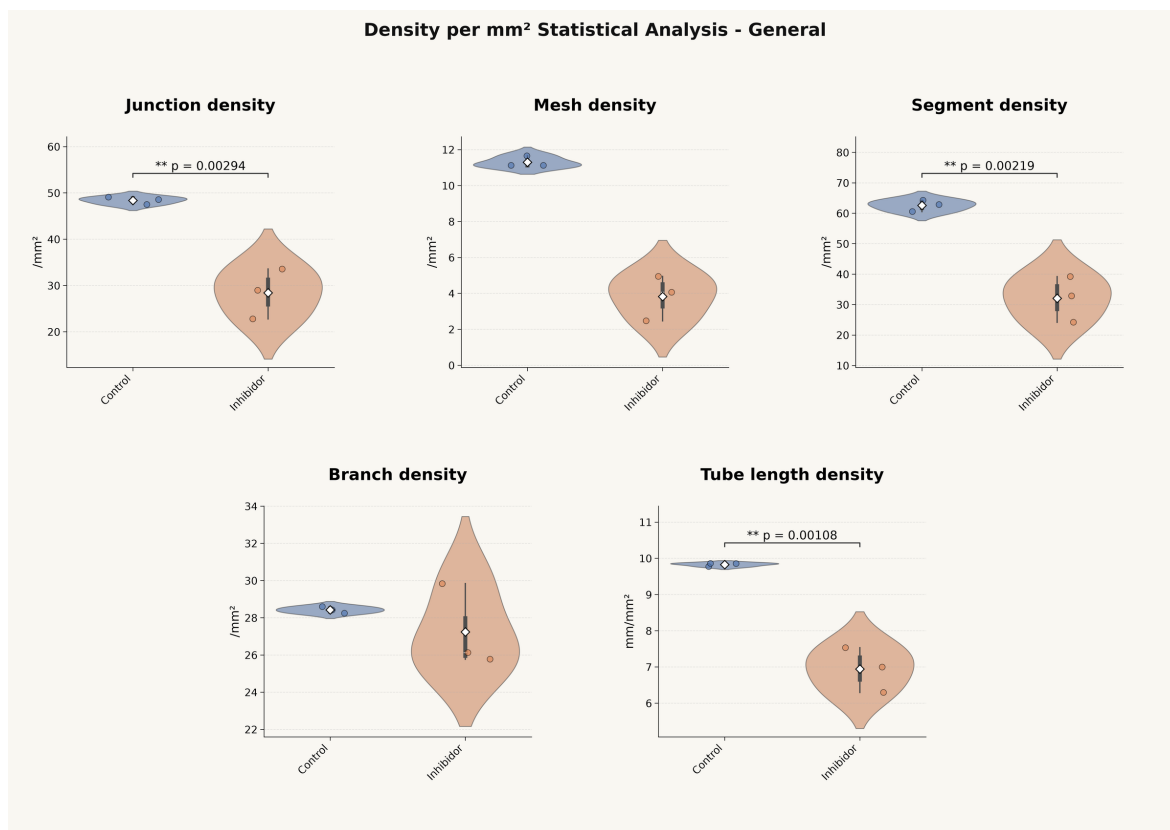


Figure 9. Per-condition violins with omnibus test, *p* and effect size — Density per mm² group. Metrics: Junction density (/mm²); Mesh density (/mm²); Segment density (/mm²); Branch density (/mm²); Tube length density (mm/mm²).

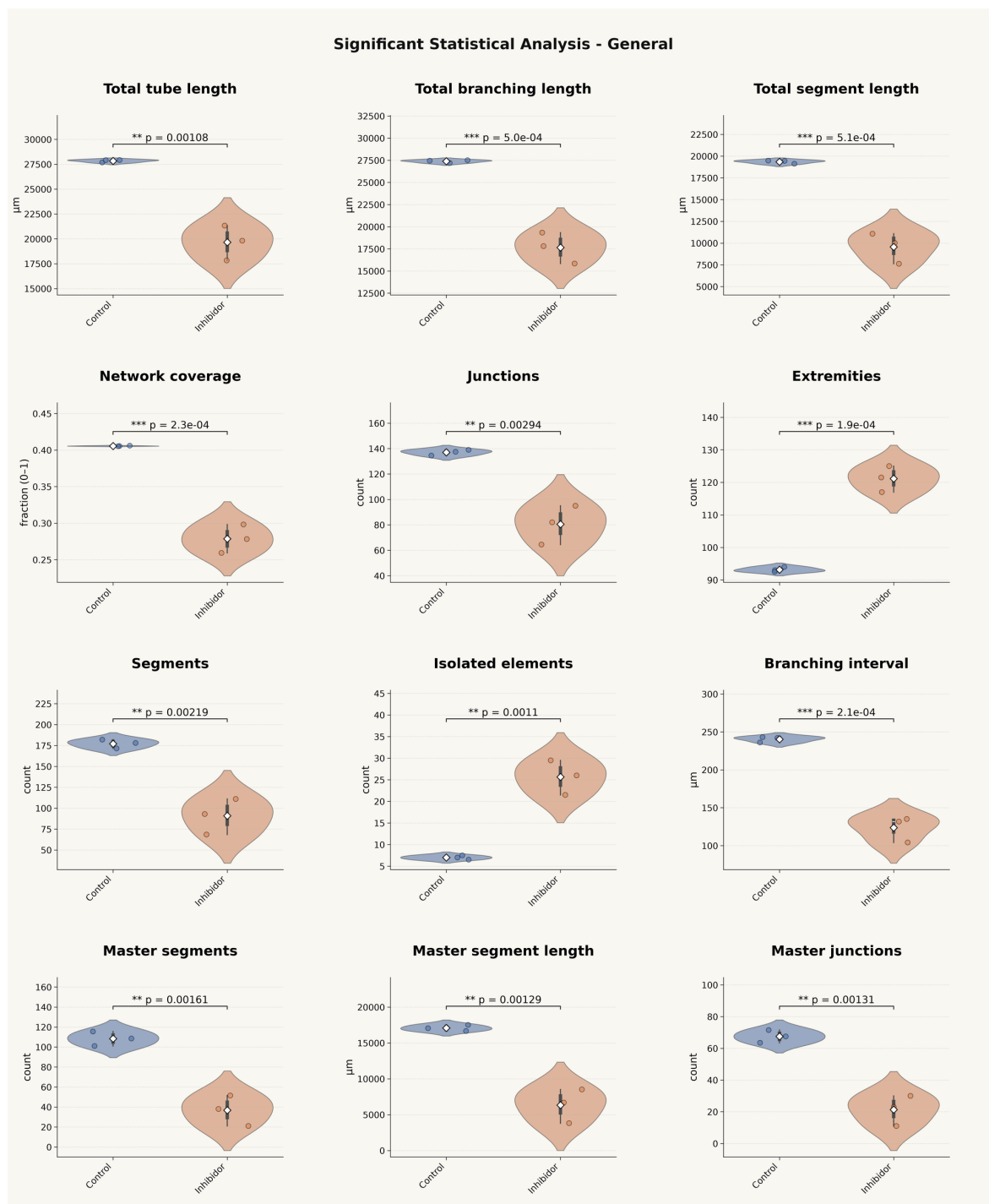


Figure 10. Synthesis of the significant metrics in the statistical engine (part 1 of 2).

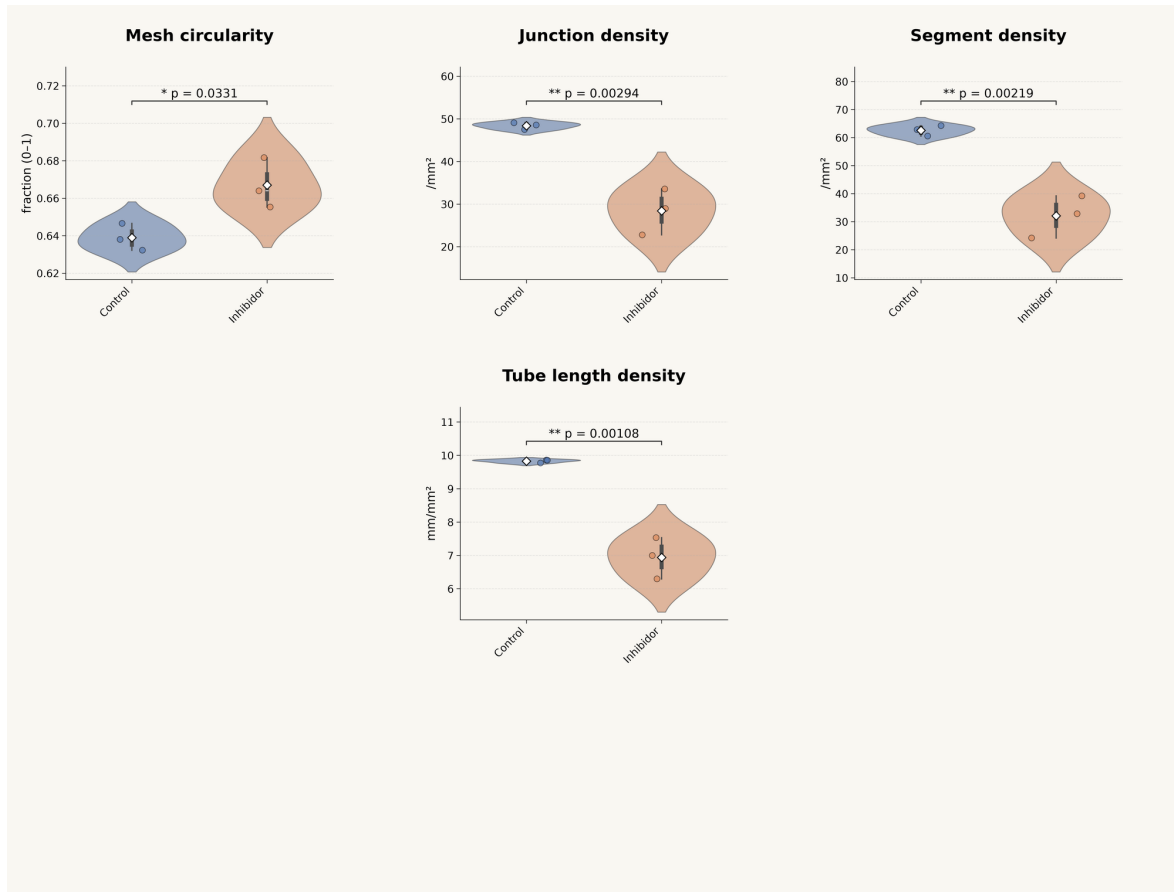


Figure 11. Synthesis of the significant metrics in the statistical engine (part 2 of 2).

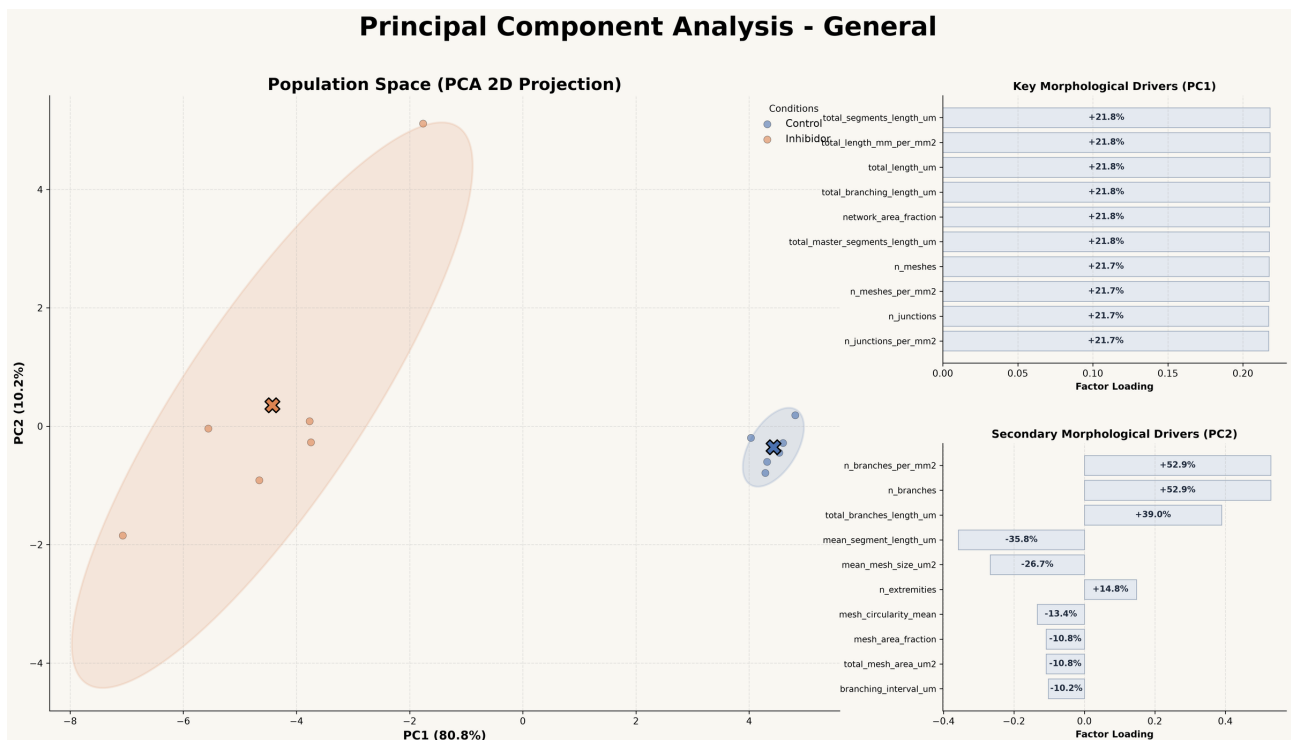


Figure 12. Principal component analysis of the standardised per-image metrics; each point is an image coloured by condition.

6. Network morphometry

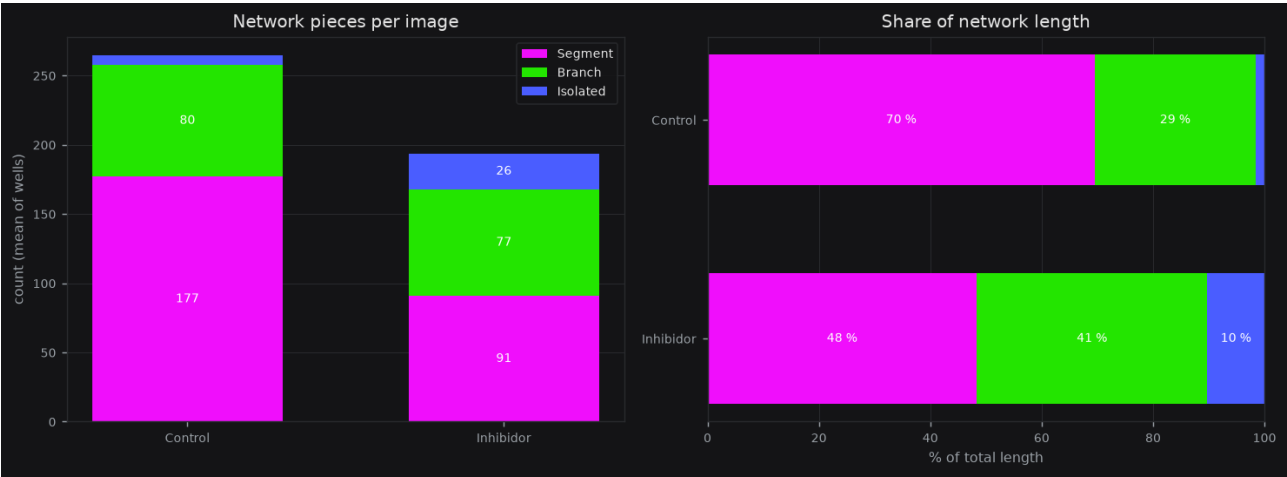


Figure 13. Network composition. Left: number of segments (magenta), branches (green) and isolated elements (blue) per image, mean of replicates. Right: share of total network length among the three piece types. A mature network concentrates its length in segments; a disassembling network gains branches and isolated elements.

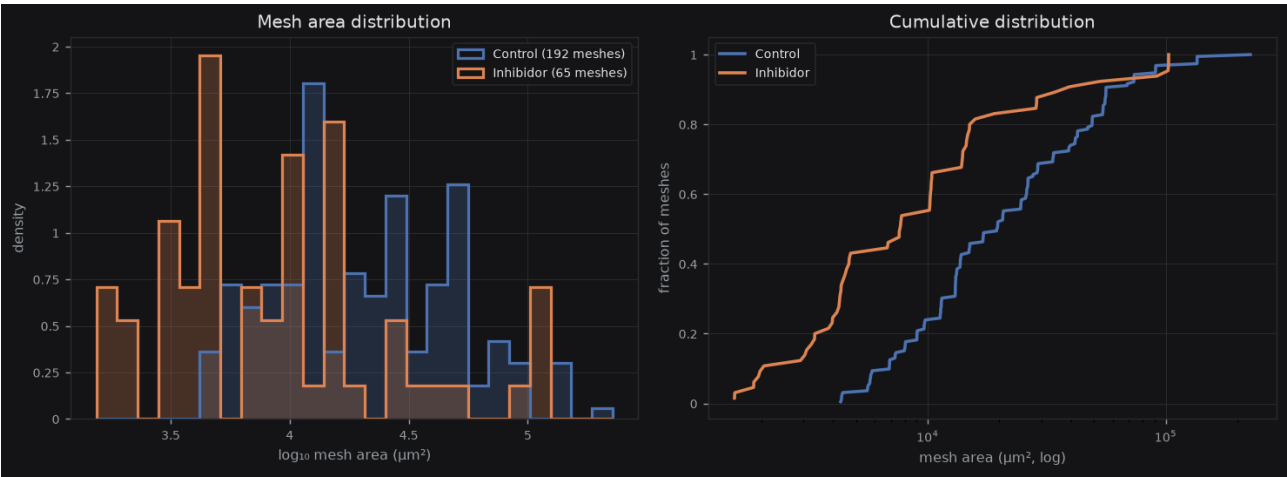


Figure 14. Distribution of mesh (closed polygon) area over all images of each condition. Left: histogram of log area. Right: cumulative distribution.

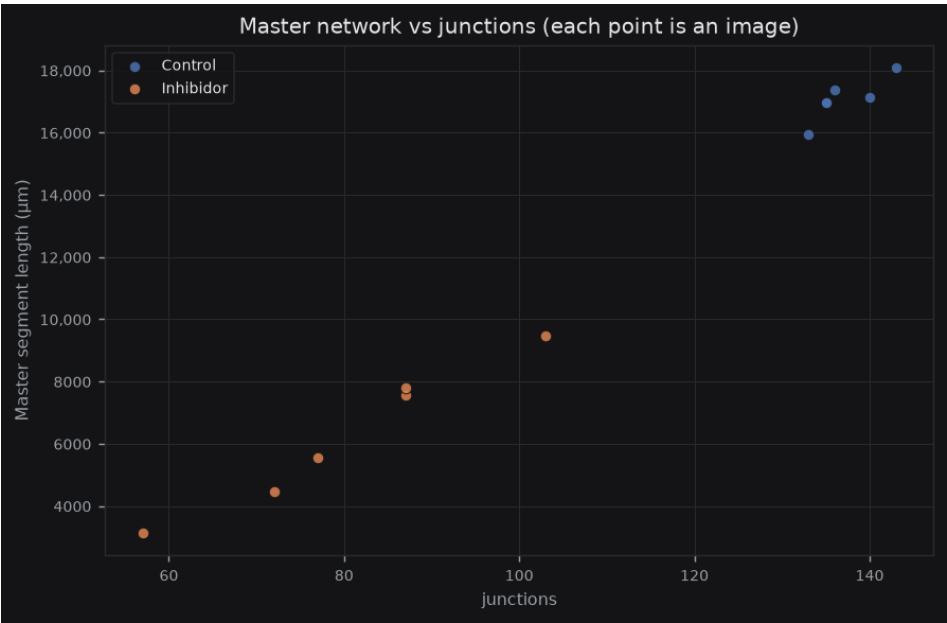


Figure 15. Master network length versus number of junctions; each point is an image.

7. Per-image quality control

Contrast: spread of the normalised intensity (very low values mean a washed-out image). Signal coverage: fraction of the field occupied by signal before cleaning. Gaps closed: short interruptions of a tube that the system completed by following the cells. In red, images with contrast below half the experiment median or with no junctions detected: they should be checked in the dashboard.

Condition	Well	Image	Type	Contrast	Coverage	Gaps closed	Junctions	Meshes
Control	1	pocillo_campo_1	Bright field	0.290	37 %	32	136	32
Control	1	pocillo_campo_2	Bright field	0.290	37 %	31	133	31
Control	2	pocillo_campo_1	Bright field	0.290	37 %	30	135	33
Control	2	pocillo_campo_2	Bright field	0.290	37 %	35	143	33
Control	3	pocillo_campo_1	Bright field	0.290	37 %	31	140	31
Control	3	pocillo_campo_2	Bright field	0.290	37 %	35	135	32
Inhibidor	1	pocillo_campo_1	Bright field	0.165	29 %	28	103	15
Inhibidor	1	pocillo_campo_2	Bright field	0.164	26 %	16	87	13
Inhibidor	2	pocillo_campo_1	Bright field	0.164	26 %	21	87	13
Inhibidor	2	pocillo_campo_2	Bright field	0.164	25 %	21	77	10
Inhibidor	3	pocillo_campo_1	Bright field	0.164	25 %	16	72	9
Inhibidor	3	pocillo_campo_2	Bright field	0.162	23 %	17	57	5

Table 6. Quality indicators per image.

All images have comparable contrast and a network with detected junctions.

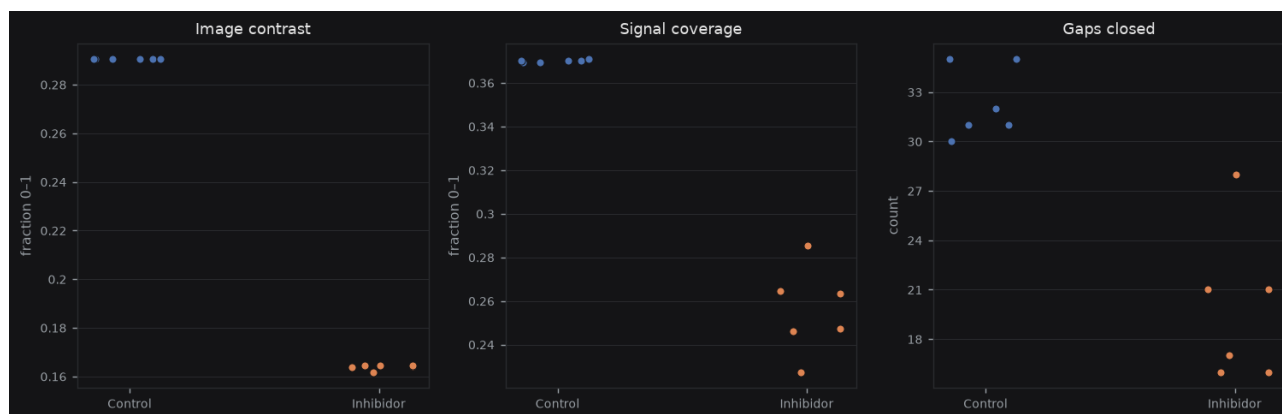


Figure 16. Contrast, signal coverage and gaps closed per image and condition.

8. Metric glossary

Metric	Definition	Unit
Network extent		
Total tube length	Summed length of the whole tubular network (segments, branches and isolated elements).	μm
Total branching length	Length of the tree-like structures (segments + branches), excluding isolated elements.	μm
Total segment length	Summed length of segments (tubes delimited by two junctions).	μm
Total branch length	Summed length of branches (tubes between a junction and a free end).	μm
Network coverage	Fraction of the field occupied by the cellular network.	fraction 0–1
Topology		
Junctions	Branch points of the network (nodes where three or more tubes meet, merged when they lie close together).	count
Extremities	Free ends of the network.	count
Segments	Tubes delimited by two junctions.	count
Branches	Tubes delimited by a junction and a free end.	count
Isolated elements	Tubes with two free ends, not connected to the network.	count
Mean segment length	Total segment length divided by the number of segments.	μm
Branching interval	Mean distance separating two branches along the network. <i>segment length / branches</i>	μm
Master network		
Master segments	Segments of the master network: what remains after iteratively removing the branches, i.e. the closed, load-bearing part of the network.	count
Master segment length	Summed length of the master segments.	μm
Master junctions	Junctions that connect at least three master segments.	count
Mesh index	Mean distance between two master junctions of the meshed network. <i>master length / master junctions</i>	μm
Meshes (closed polygons)		
Meshes	Areas fully enclosed by the network (closed polygons).	count
Total mesh area	Summed area of all meshes.	μm ²
Mean mesh size	Total mesh area divided by the number of meshes.	μm ²
Mesh coverage	Fraction of the field enclosed by meshes.	fraction 0–1
Mesh circularity	Mean roundness of the meshes (1 = circle); immature networks form irregular polygons. $C = 4\pi A/P^2$	fraction 0–1
Density per mm²		
Junction density	Count per mm ² of imaged field.	/mm ²
Mesh density	Count per mm ² of imaged field.	/mm ²
Segment density	Count per mm ² of imaged field.	/mm ²
Branch density	Count per mm ² of imaged field.	/mm ²
Tube length density	Total tube length (mm) per mm ² of field.	mm/mm ²
Image quality control		
Image contrast	Spread of the normalised intensity; very low values mean a washed-out image.	fraction 0–1
Signal coverage	Fraction of the field occupied by signal before cleaning.	fraction 0–1
Gaps closed	Short interruptions of a tube that the system completed following the cells.	count

9. References

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The network reconstruction method is proprietary to MAPSNY and is described only in general terms. Visual review of the reconstructed networks (dashboard, images tab) is recommended before publication. Confidential document automatically generated by MAPSNY (mapsny.com).