Supplemental Data Appendix III

Matlab validation

Branching density data was validated using Matlab version 8.0.2.701 (R2013b; MathWorks, Natick, MA). Glands were sampled by randomly generating 2, 3, and 4 mm lines throughout each gland and the number of intersections/mm were determined according to the following protocol.

1. A box was drawn around the gland such that the gland was completely contained.
2. Matlab created 500 lines of defined distance randomly dispersed throughout the box.
3. Any lines that did not have at least one intersection were excluded.
   a. These included regions outside the gland or void space occupied by the lymph node.
4. The process was conducted three times using 2, 3, and 4 mm lines.
5. Mean $N/mm^2 \pm$ SEM for treated and vehicle groups were determined for each line length and means were assessed for differences by t-test.

Table 1. Mean N/mm for 2 mm sample lines

	Total Lines	N/mm
Vehicle (8)	349 ± 7	3.66 ± 0.15
5 µg EE/kg BW (7)	341 ± 8	4.22 ± 0.06 ***

*(n); N = intersections; ***p<0.001*

Table 2. Mean N/mm for 3 mm sample lines

	Total Lines	N/mm
Vehicle (8)	348 ± 7	5.63 ± 0.20
5 µg EE/kg BW (7)	388 ± 8	6.34 ± 0.13 **

*(n); N = intersections; **p<0.01*

Table 3. Mean N/mm for 4 mm sample lines

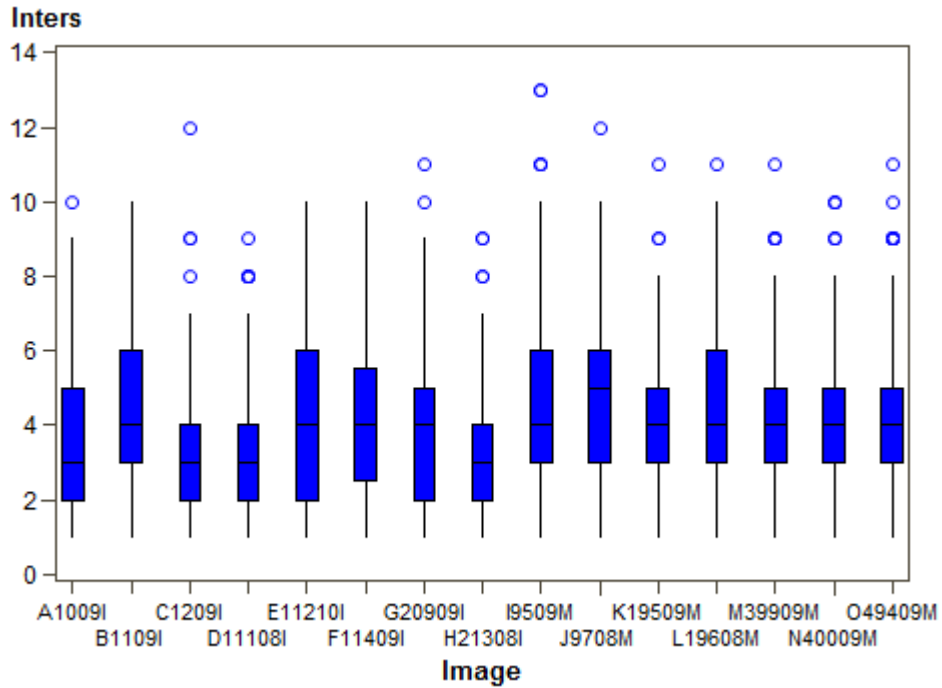|	Total Lines	N/mm
Vehicle (8)	360 ± 11	6.94 ± 0.26
5 µg EE/kg BW (7)	398 ± 5	8.02 ± 0.17 ***

*(n); N = intersections; ***p<0.001*

Figure 1. Box and whisker plot of 2 mm sample lines. Images ending in "I" are from vehicle rats and those ending in "M" are from treated rats.
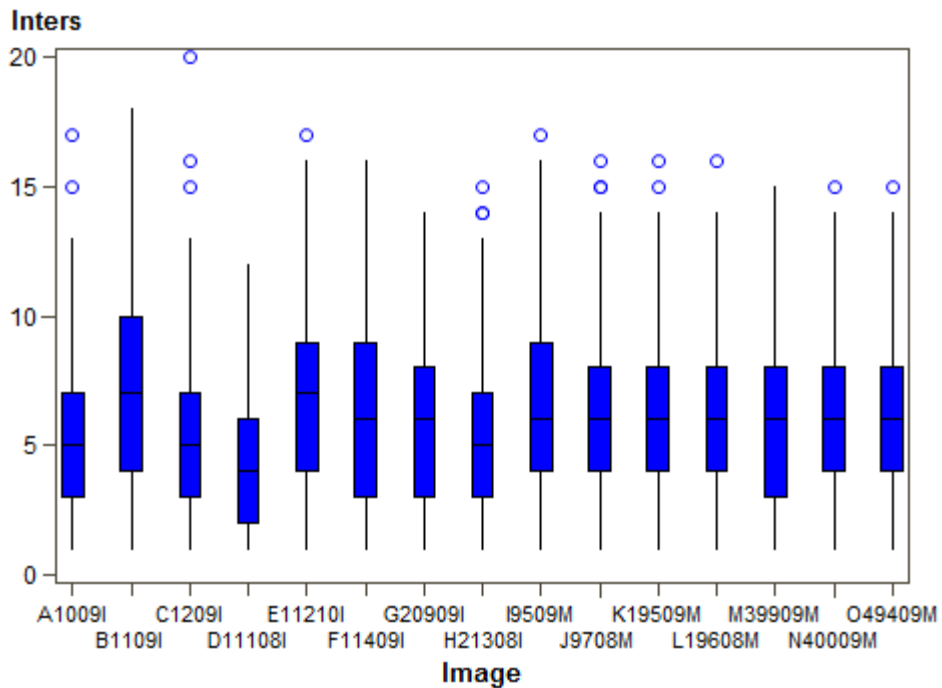

Figure 2. Box and whisker plot of 3 mm sample lines. Images ending in "I" are from vehicle rats and those ending in "M" are from treated rats.
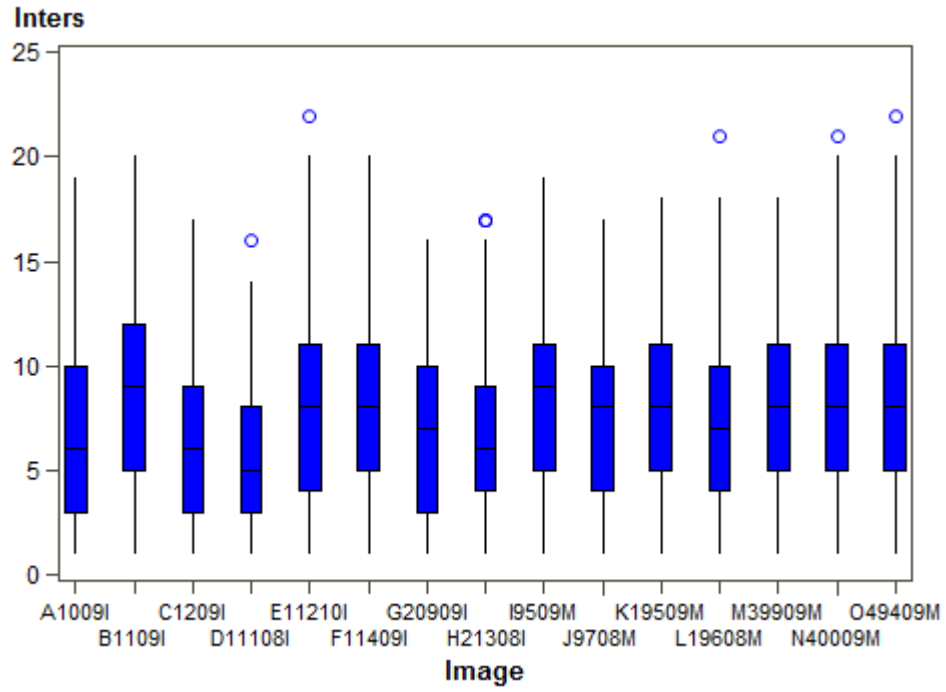
Figure 3. Box and whisker plot of 4 mm sample lines. Images ending in "I" are from vehicle rats and those ending in "M" are from treated rats.