1. Start ImageJ
2. Using ImageJ, File > Open > select your leaf image and leaf mask files
3. Click on your leaf mask image
4. Image > Overlay > Add Image…
5. Select your main leaf image at 70% opacity
6. Double click eyedropper tool to select white as primary color, black as negative (background)
7. Can right-click paintbrush to set width—40 pixels is precise enough with a few exceptions on pointy regions of leaf
8. Use pencil tool with Alt button to draw black over pixels incorrectly labeled as leaf
   1. Save frequently in case you need to go back a few steps. ImageJ’s Undo function only goes 1 step back.
   2. DO NOT CLICK CONTROL! It will slide your mask off of the image, making it very difficult to correctly re-align them!
9. Use pencil tool to draw white over pixels incorrectly labeled as not-leaf
   1. Lesions ARE included in the white (leaf) region of the image
10. If you set your background color to black as well via the eyedropper, you can also use the square tool to select a large area, and then black it out using Edit > Cut.
11. Can also use paint bucket to roughly fill in white shapes, but you’ll need to go back and remove any white specks with the brush tool
12. For any objects that are touching, draw a ≥1 pixel line between them in black
13. Double-check by looking through the image at high magnification (≥400%)
14. Go to Image > Overlay > Remove Overlay
15. Save the revised leaf mask
16. Repeat with lesion masks but:
    1. You can black out entire area first: If you set your background color to black via the eyedropper, you can use the square tool to select an area, and then black it out using Edit > Cut.
    2. Set pencil diameter to 10 pixels for greater precision in lesion marking

\*\* Note: if this is your first time masking lesions and leaves, show Nicole your first mask before moving forward.

\*\* Note: Since each individual will draw masks a bit differently, it is best to have ONE person draw all leaf masks, and ONE person draw all lesion masks, within a single experiment (or if that is too much work, one person within a single replicate of an experiment).