Photomicrograph showing spiral artery (SpA) selected for quantification of vascular smooth muscle. The vessel has been cropped to eliminate most of the background. The histogram tab (blue arrow) shows ‘expanded view’ to show the pixel count. The magnetic lasso tool (black arrow) is selected to outline the SpA wall.

The SpA wall has been carefully drawn around with the magnetic lasso tool, then copied and pasted into a new layer (layer 1, black arrow). The luminal part of the SpA is then selected using the magnetic lasso tool and deleted from the image. The background is then deselected by clicking on the eye icon (blue arrow).

The image now shows the SpA wall without background and lumen. The source in the histogram box (black arrow) is changed to selected layer. This gives the total pixel count for the selected SpA wall; the total pixel count is noted. The magic wand tool (blue arrow) is then used to highlight the positive pixels.

The positive pixels have been selected using the magic wand tool ensuring that ‘similar’ is highlighted in the ‘Select’ menu (black arrow).

The immunostained selection has been copied into another layer (black arrow). The pixel count of this layer is now displayed. The percentage positive pixels in the SpA wall is calculated from positive pixels + total pixels x 100.

**Supplementary figure 1**: Screenshots with explanatory text to illustrate the stages of quantification of vascular smooth muscle in a cross section of a spiral artery.