Supplement 2. QuPath procedures and settings

For immunohistochemical quantification, using QuPath Bioimage analysis v0.2.0-m8 (University of Edinburgh, Scotland, UK) the following protocol was used:

1. A new "Project" was created within QuPath for each immunohistochemical marker analyzed.
2. Then, WSIs in ScanScope Virtual Slide (.svs) format were imported into each project file setting as "Heme/DAB brightfield" images for further analysis.
3. For the automated cell counting the followed steps were used:
   a- At 10× viewing magnification, using the polygon annotation tool, the region of interest (ROI) was outlined in selected tumor areas;
   b- RGB pixel depth stain vectors recalibration setting the "Estimate Stain Vectors function" with the default "auto" detection;
   c- “Positive Cell Detection” function used for automated cell counting;
   d- After computation, the tumor cells within the fixed-shaped annotations (ROIs) were automatically counted and visualized as red (positive) or blue (negative).

4- The procedures and settings in QuPath software using the “Positive Cell Detection” function for the digital counting method is detailed below:
   - **Image file type**: ScanScope Virtual Slide (.svs)
   - **Image set (upon import to QuPath)**: Heme/DAB brightfield
   - **Representative tumor areas (n=5):** Selection at 10x magnification
   - **Pixel depth separation vectors:** “Estimate Stain Vectors” function with “Auto” calibration.
   - **Heme threshold for counterstain (default: 0.1):** 0.20 in areas with low cellularity and increased stroma/ 0.10 or 0.01 in areas with high cellularity and scarce stroma
   - **“Threshold Compartment” (depends on the antibody staining patterns):** Nucleus: DAB OD mean or Cytoplasm: DAB OD mean
   - **“Threshold Positive 1”:** 0.2 (default)