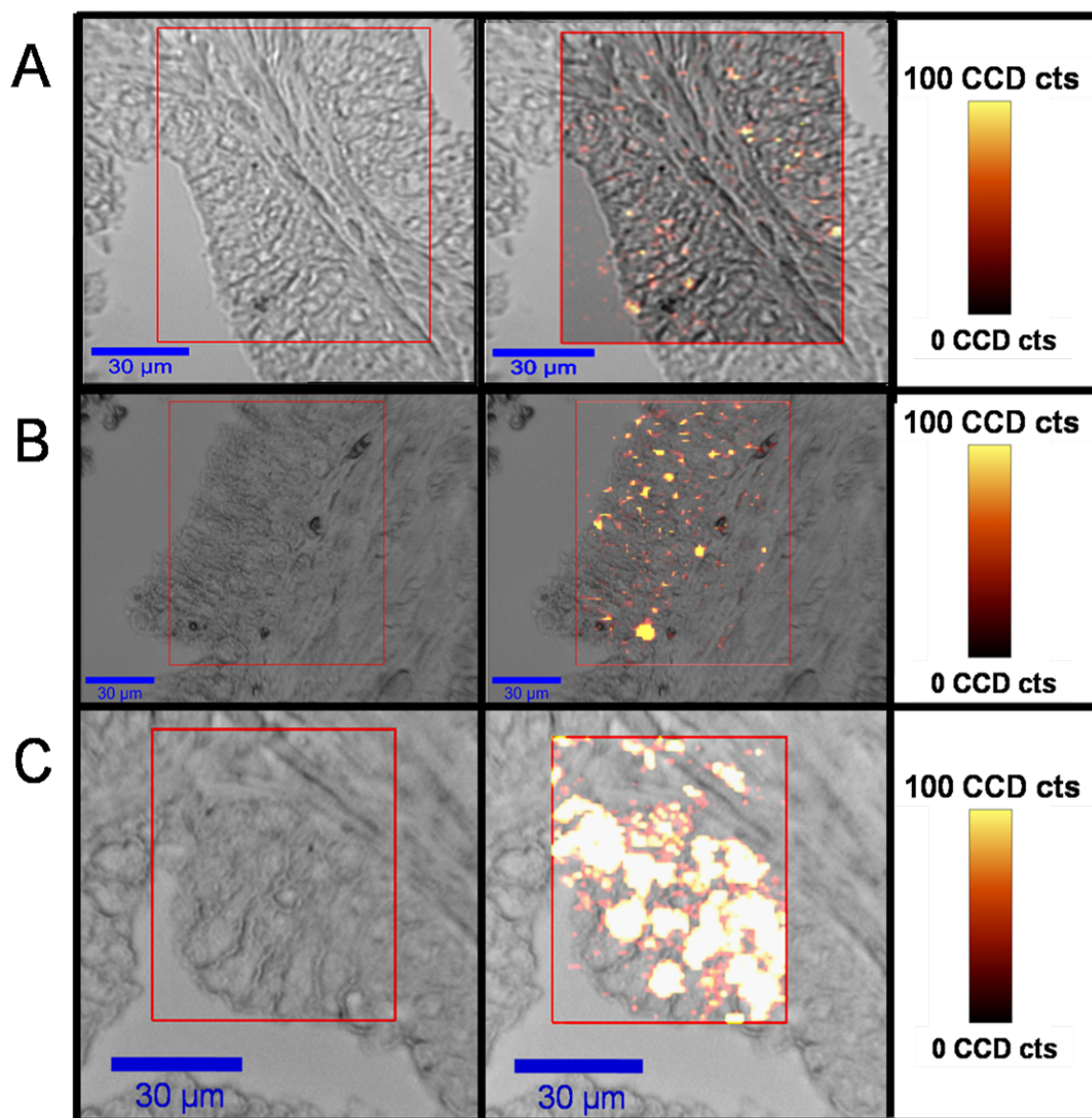


## Supporting Information

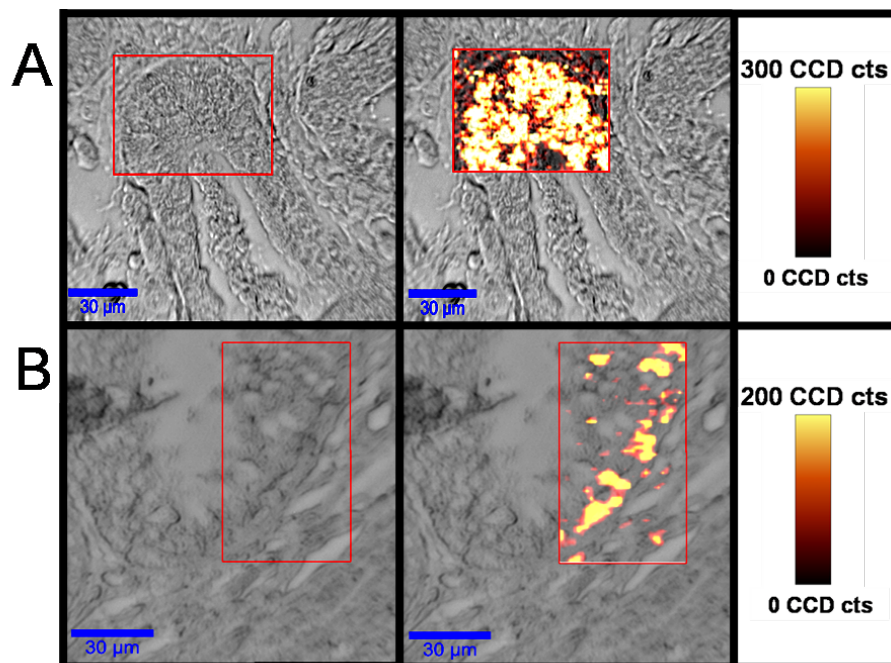
For these systematic SERS microscopic experiments, gold/silver nanoshells as SERS labels were employed.<sup>[1,3,6,24]</sup>

*Influence of blocking solutions on protein detection by SERS microscopy:*



**Figure S1:** Three tissue sections were pre-treated with home-made citrate buffer solution and blocked with different blocking buffers before incubation with  $\alpha$ -PSA/SERS Au/Ag nanoshell-conjugates. Tissue sample A was blocked for 5 minutes with commercial blocking solution. Tissue sample B was blocked for 20 min with 0.2% skim milk. The third sample was blocked with 2% BSA for 20 min (C). The white light images are shown on the left, while on the right their overlay with the corresponding false color SERS images is shown. The color coding (right) is based on the SERS intensity of the 4-TNB Raman band at  $1340\text{ cm}^{-1}$ . The same laser power and exposure time was used for samples A and B (5 mW and 0.2 sec). Sample C was scanned with the same laser power as samples A and B. The exposure time for sample C was 0.1 sec.

*Influence of antigen unmasking methods on protein localization by SERS-microscopy:*



**Figure S2:** Tissue unmasking with home-made citrate buffer solution. Results for  $\alpha$ -PSA/SERS Au/Ag nanoshell-conjugates (A) and  $\alpha$ -p63/SERS-Au/Ag nanoshell-conjugates (B). The incubation time was 20 min. The white light images are shown on the left, while on the right their overlay with the corresponding false color SERS images is shown. The color coding (right) is based on the SERS intensity of the 4-TNB Raman band at  $1340\text{ cm}^{-1}$ . The laser power for both samples was 5 mW. The exposure time for sample A (PSA) was 0.1 sec and 0.2 sec for sample B (p63). The same number of antibody/SERS NP-conjugates was used. For both samples 200  $\mu\text{L}$  of a colloidal suspension with OD = 0.25 was employed.

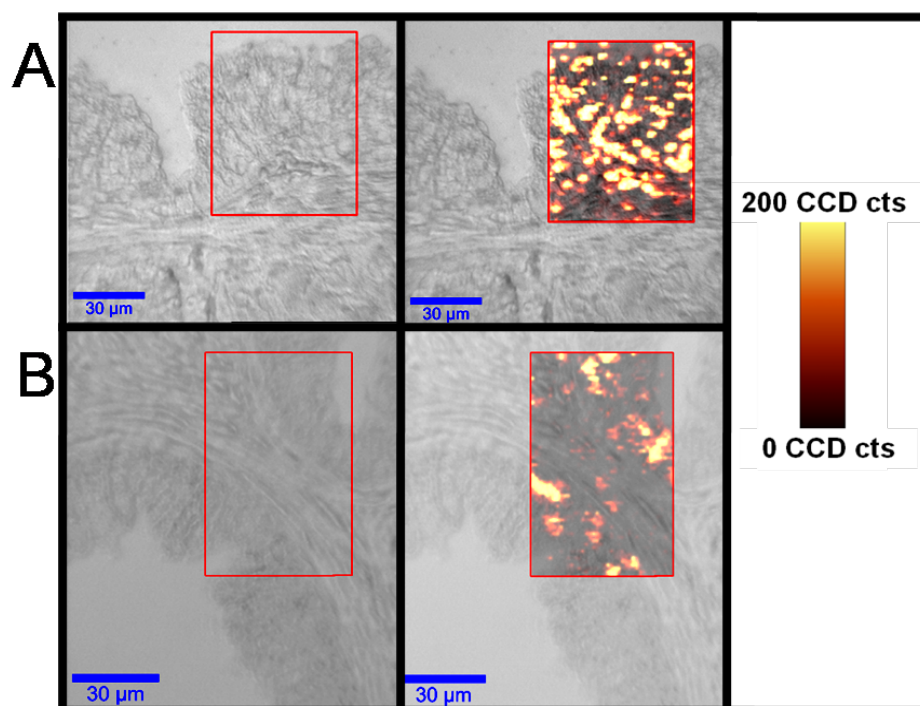


Figure S3: Tissue unmasking with commercial citrate buffer solution. Results from the antigen detection with  $\alpha$ -PSA/SERS Au/Ag nanoshell-conjugates (A) and  $\alpha$ -p63/SERS Au/Ag nanoshell-conjugates (B) are shown. The incubation time was 20 min. The white light images are shown on the left, while on the right their overlay with the corresponding false color SERS images is shown. The color coding (right) is based on the SERS intensity of the 4-TNB Raman band at  $1340\text{ cm}^{-1}$ . In both cases the same exposure time (0.2 sec), laser power (5 mW) and the same number of antibody/SERS NP-conjugates (200  $\mu\text{L}$  colloid with OD = 0.25) was employed.

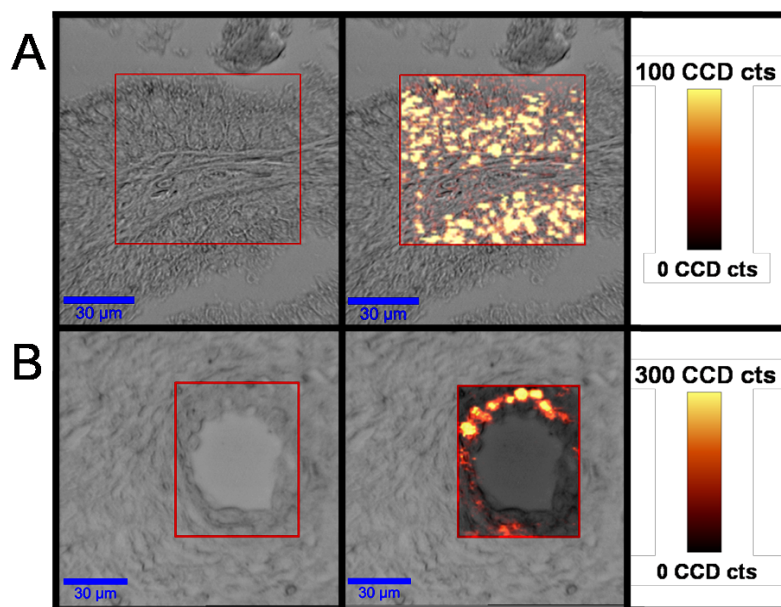


Figure S4: Tissue unmasking with home-made EDTA-Tris-buffer solution. Results from the antigen detection with  $\alpha$ -PSA/SERS Au/Ag nanoshell-conjugates (A) and  $\alpha$ -p63/SERS Au/Ag nanoshell-conjugates (B) are shown. The white light images are shown on the left, while on the right their overlay with the corresponding false color SERS images is shown. The color coding (right) is based on the SERS intensity of the 4-TNB Raman band at  $1340\text{ cm}^{-1}$ . In both cases the same exposure time (0.2 sec), laser power (5 mW) and the same number of antibody/SERS NP-conjugates (200  $\mu\text{L}$  colloid with OD = 0.25) was employed.

*The role of incubation time with SERS NPs on the tissue and formation of non-specific binding:*

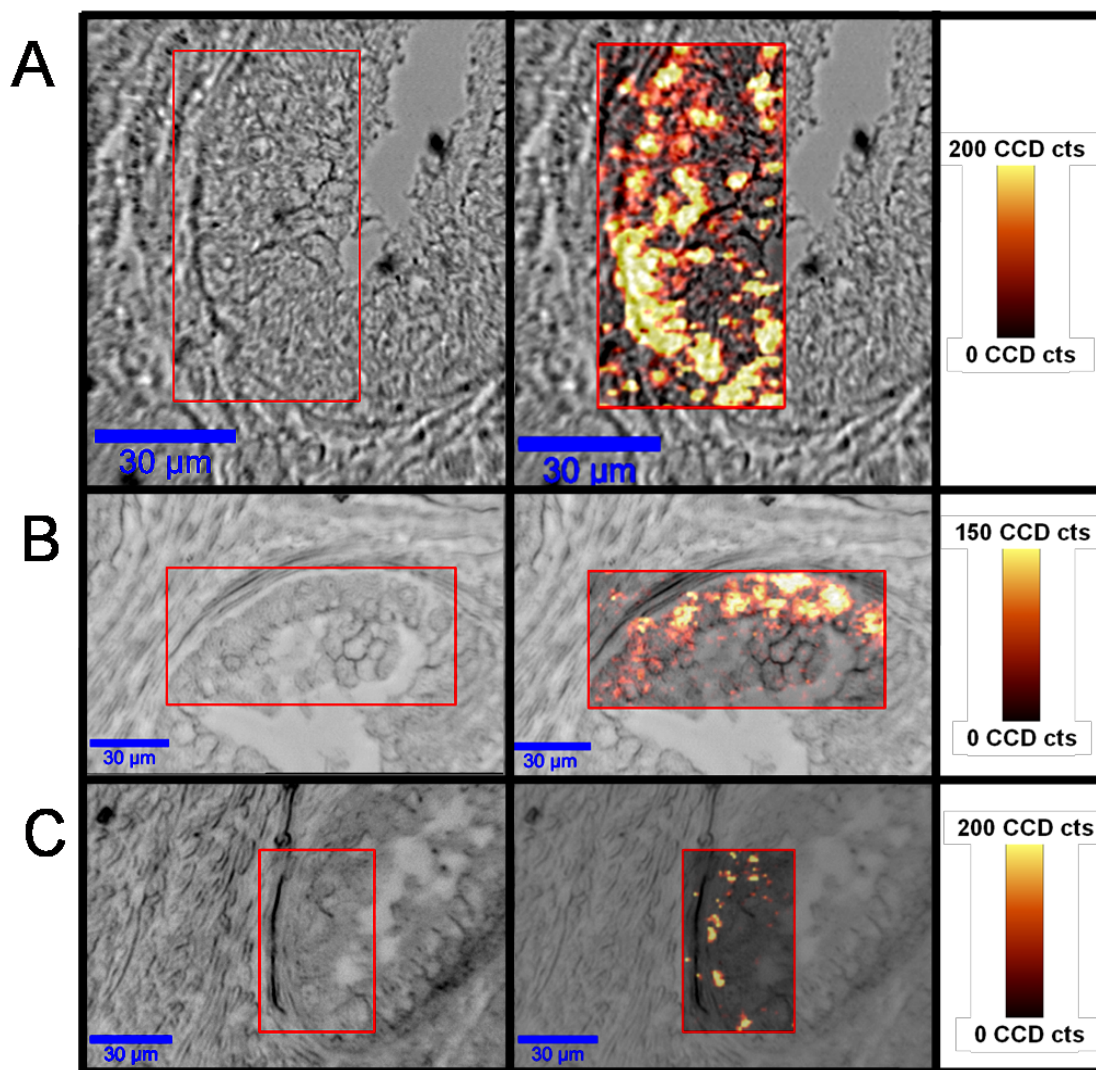
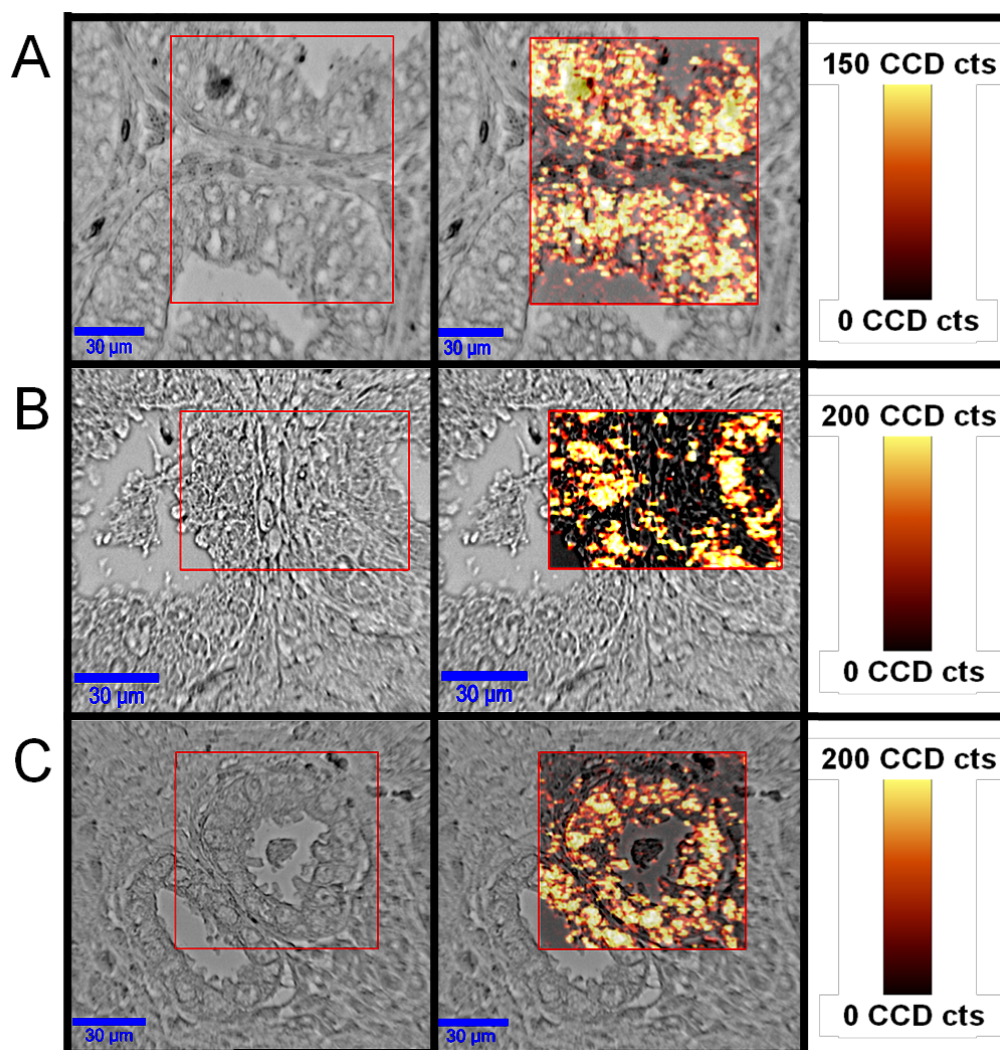


Figure S5: Unmasking with commercial citrate buffer solution for three tissue sections. All samples were blocked for 20 minutes with 2% BSA solution (in PBST) before incubation with SERS-labeled p63 antibodies. The samples were incubated with  $\alpha$ -p63/SERS Au/Ag nanoshell-conjugates for 40 min (A), 30 min. (B) and 20 min. (C), respectively. The white light images are shown on the left, while on the right their overlay with the corresponding false color SERS images is shown. The color coding (right) is based on the SERS intensity of the 4-TNB Raman band at 1340 cm<sup>-1</sup>. In both cases the same exposure time (0.2 sec), laser power (5 mW) and the same number of antibody/SERS NP-conjugates (200  $\mu$ L colloid with OD = 0.25) was employed.

*Influence of NP concentration on protein localization by SERS microscopy:*



**Figure S6:** Tissue unmasking with homemade citrate buffer solution and protein detection with different concentrations of  $\alpha$ -PSA/SERS Au/Ag nanoshell-conjugates. All samples were blocked for 20 minutes with 2% BSA solution (in PBST) before incubation with the SERS-labeled antibodies. A volume of 250  $\mu$ l for all samples was used, but the optical density of the samples differed: OD= 1 in **A**, OD=0.5 in **B** and OD=0.25 in **C**.