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Supplementary material: 1.4 nm gold nanoparticle-antibody conjugates for in situ gold immunolabelling after transduction into living human cells

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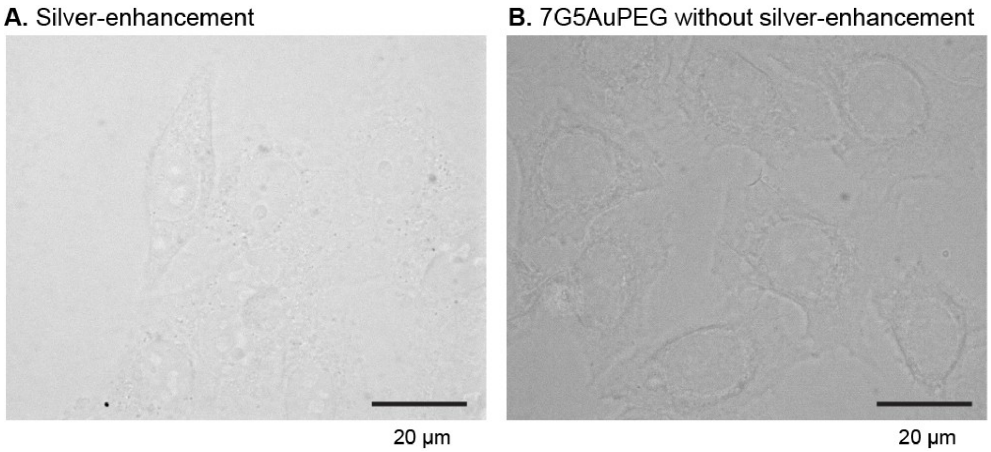
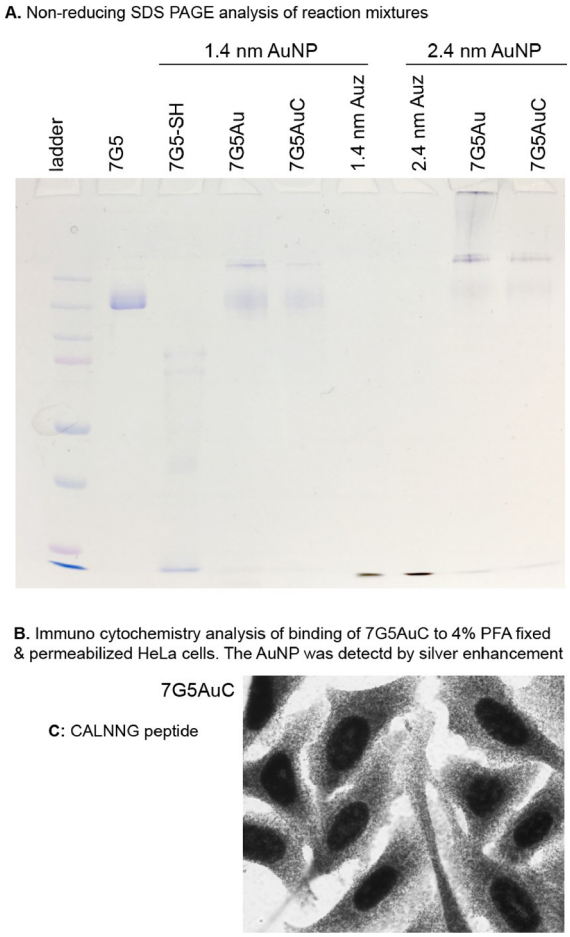
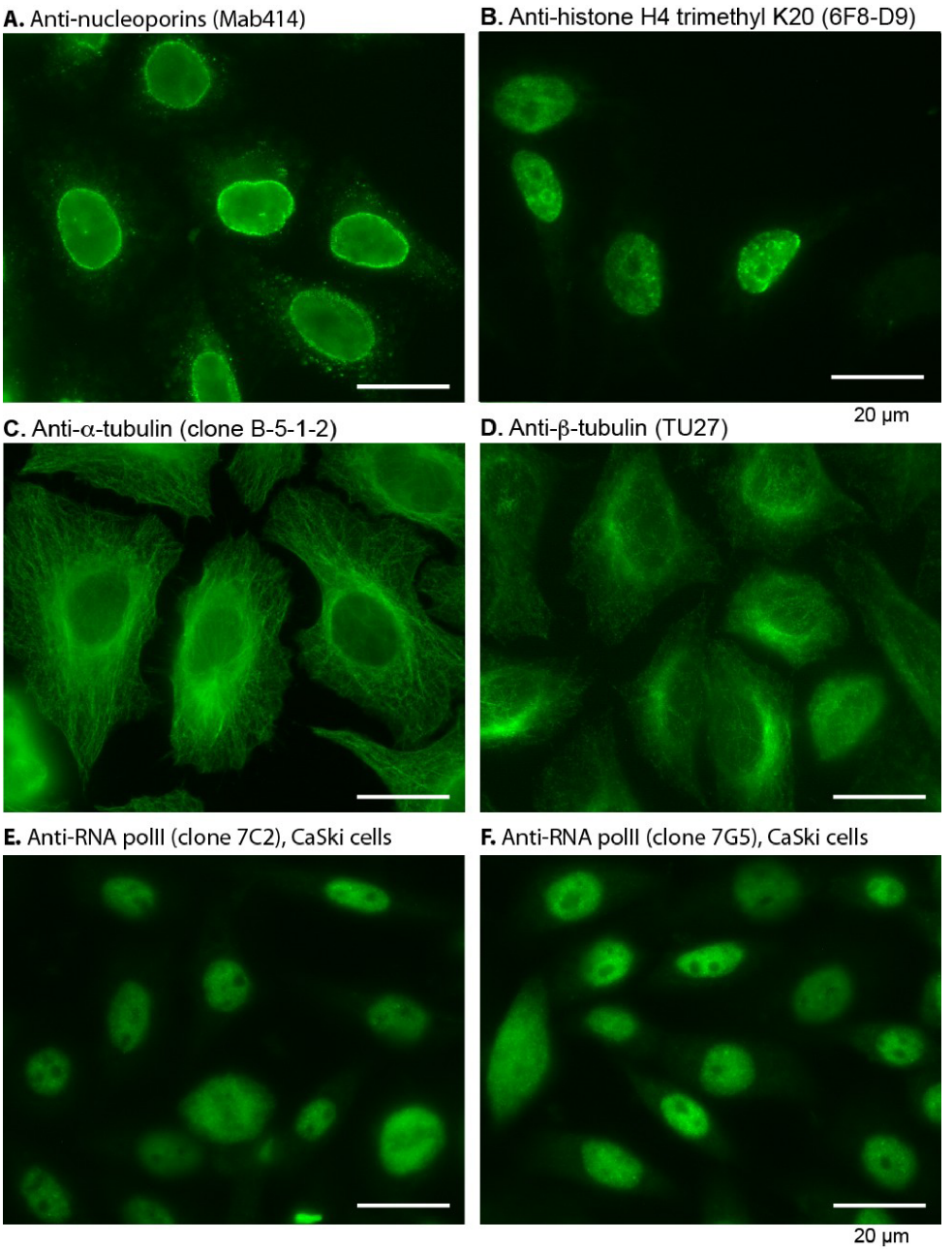


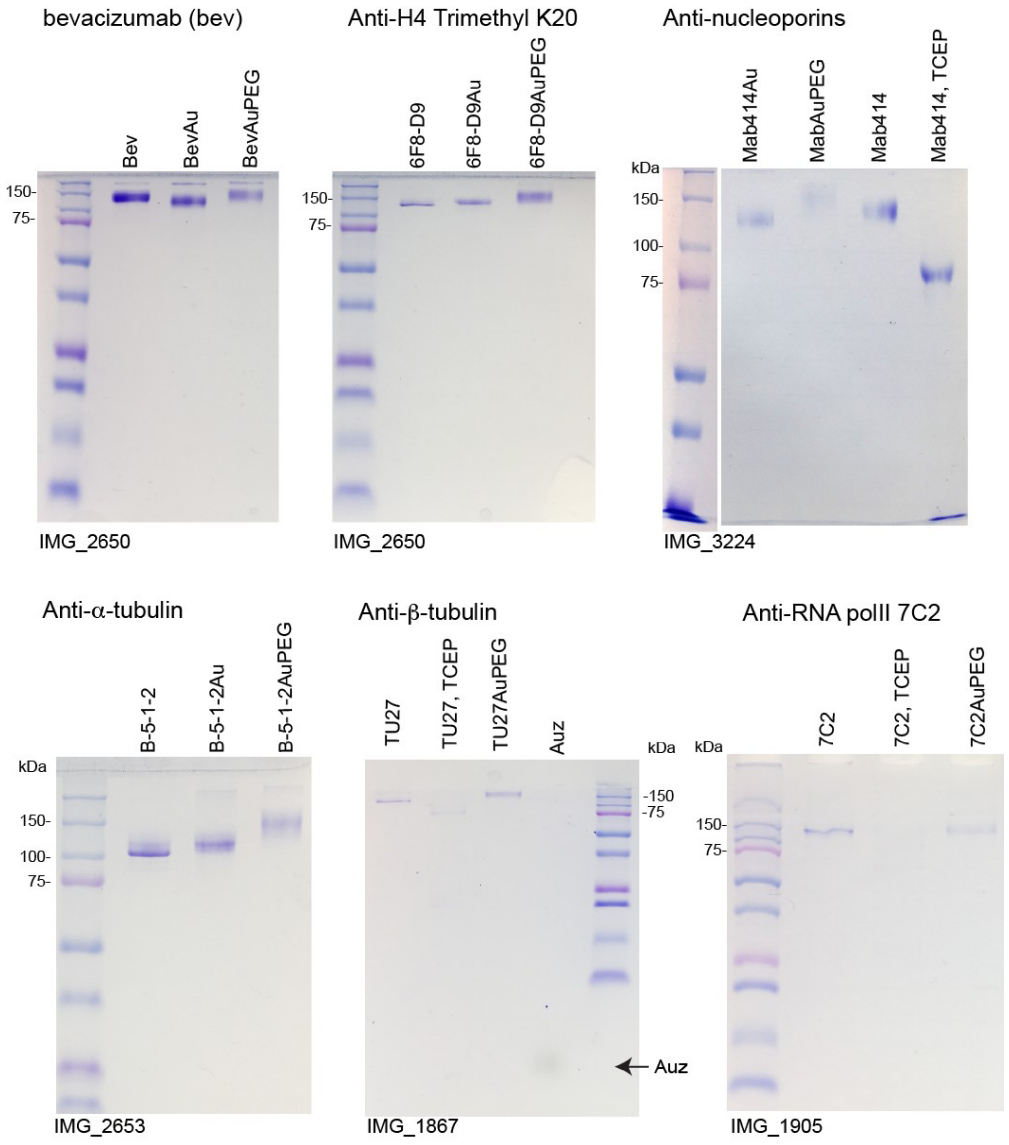
Supplementary Figure S1. Controls experiments. Image of 4% PFA-fixed and 0.1% Triton X-100 permeabilized HeLa cells. (A) After permeabilization, the cells were treated with silver-enhancement solution. (B) Gold immunolabelling of HeLa with the 7G5AuPEG anti-RPB1 without silver-enhancement solution.



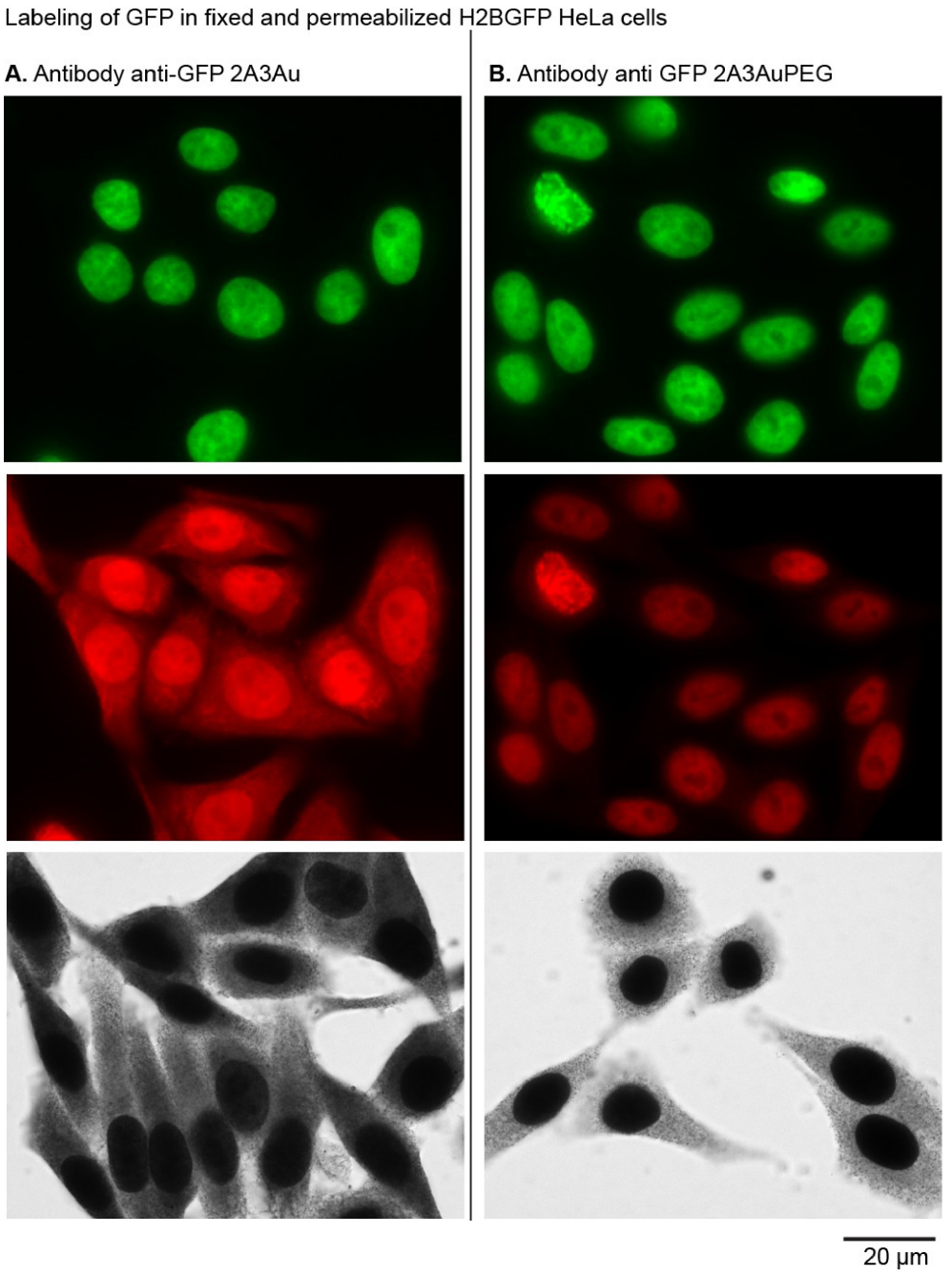
Supplementary Figure S2. Analysis of the production of 7G5AuC and binding specificity using fixed HeLa cells. (A) The 7G5 was firstly reduced with TCEP, reacted with Auz and purified by size-exclusion chromatography. The purified conjugate was then reacted for passivation with the peptide CALNNG (C). (B) The CALNNG appeared to promote unspecific binding of the probe the fixed cell ultrastructure.



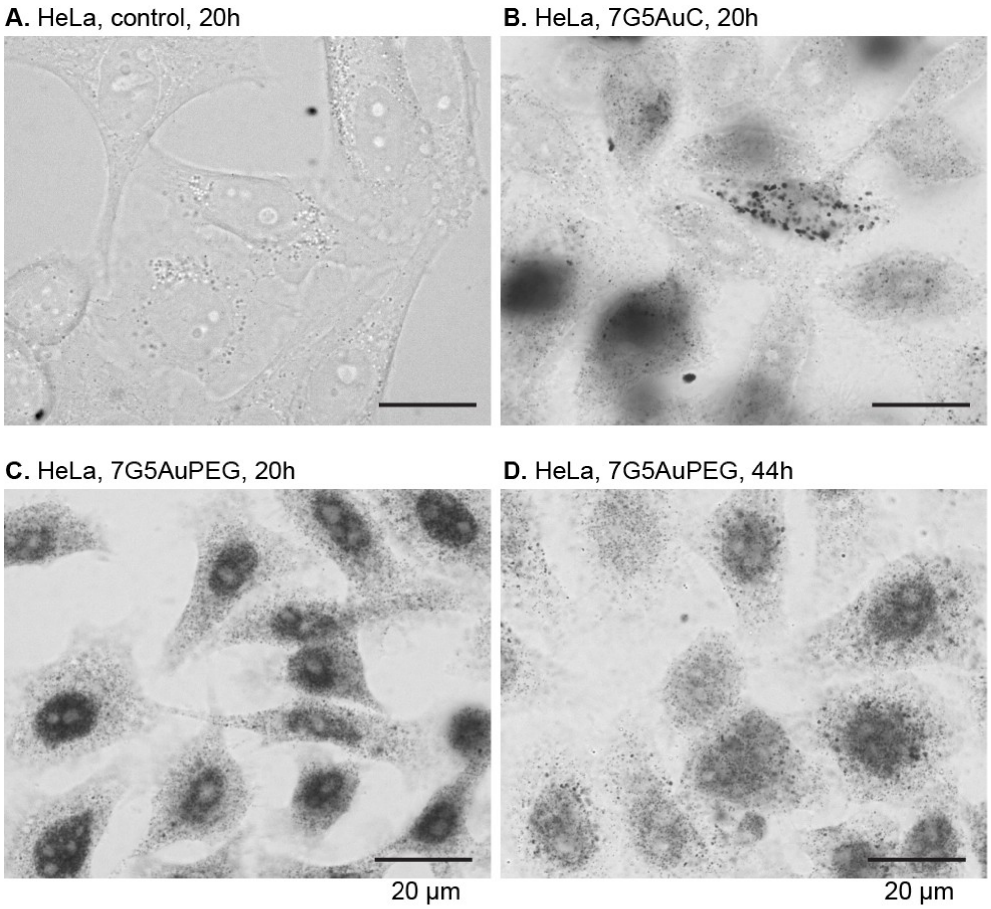
Supplementary Figure S3. Immunofluorescence imaging. Otherwise indicated, HeLa cells adhered onto glass coverslips were fixed with 4% PFA for 20 min. The plasma membrane was permeabilized with 0.1% Triton X-100 before incubation with the indicated primary mouse mAbs in PBS containing 10% FBS. The mouse antibody was detected with a goat anti-mouse AlexaFluor488-antibody conjugate.



Supplementary Figure S4. Non-reducing SDS-PAGE analysis of the indicated AuNP-mAb conjugates. The proteins were stained with Coomassie blue.



Supplementary Figure S5. HeLa cells stably expressing H2B-GFP were fixed with 4% PFA for 20 min, permeabilized with 0.1% Triton X-100. The H2BGFP fusion protein was then detected by binding of 2A3Au and 2A3AuPEG conjugates to the GFP. Detection was done either directly using the GFP green fluorescence (top images), an anti-mouse AlexaFluor-594-Ab conjugate (middle images) or the AuNP via a silver enhancement procedure. Results showed that coating of the AuNP with PEG limit unspecific binding.



Supplementary Figure S6. HeLa cells were incubated with the indicated AuNP-7G5 conjugates and treated with three pulses of 10 ms at 517 V·cm^{-1}. After 20 h or 44 h of cell culture, the cells were fixed with 4% PFA for 20 min, permeabilized with Triton X-100 and the gold particles were enhanced with silver.