# Supporting Information

## S1 Text: Autoradiography in electron microscopy

Autoradiography is a classic technique that allows the EM visualization of a radioactive marker, usually targeting a certain process, and thus reveals the subcellular localization of that process [1-2]. Tritiated uridine, for example, can be used to locate active RNA synthesis [3-5], as also shown in this study. A clear advantage over the use of alternatives for metabolic labelling of newly-synthesized RNA (e.g. Br-uridine, Br-UTP, 5-ethynil uridine) is that the radioactive precursor is chemically identical to the natural substrate.

After labelling, the samples are immediately fixed and processed for EM. The location of the radioactive marker can then be made apparent by applying a highly-sensitive photographic emulsion (a nuclear emulsion) on top of the cell sections and exposing it for several weeks to months. The beta particles that are emitted as a result of tritium disintegrations generate electrons that get trapped in the silver halide emulsion and create a “latent image”. When the emulsion is developed, these negative charges promote the reduction to metallic silver, generating electron-dense grains that are visible by EM. In principle, given enough time to accumulate enough radioactive disintegrations, even low levels of the radioactive marker could be detected. In practice, other factors (e.g. background radiation, emulsion aging) set some limits to autoradiography, which is nonetheless a very sensitive technique.

The resolution of EM autoradiography is limited by the fact that radioactive disintegrations generate beta particles that are emitted in random directions. Importantly, the probability of giving rise to signal decreases with the distance from the radioactive source; however, some beta particles may travel up to a few hundred nanometers before striking the photographic emulsion [2]. Therefore, it is important to keep in mind that the silver grains may not directly overlay the structure containing the radioactive source. Quantitative analyses of the signal that take this factor into account, like those presented in this study, become indispensable to maximize the information that autoradiography can provide.

## References

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