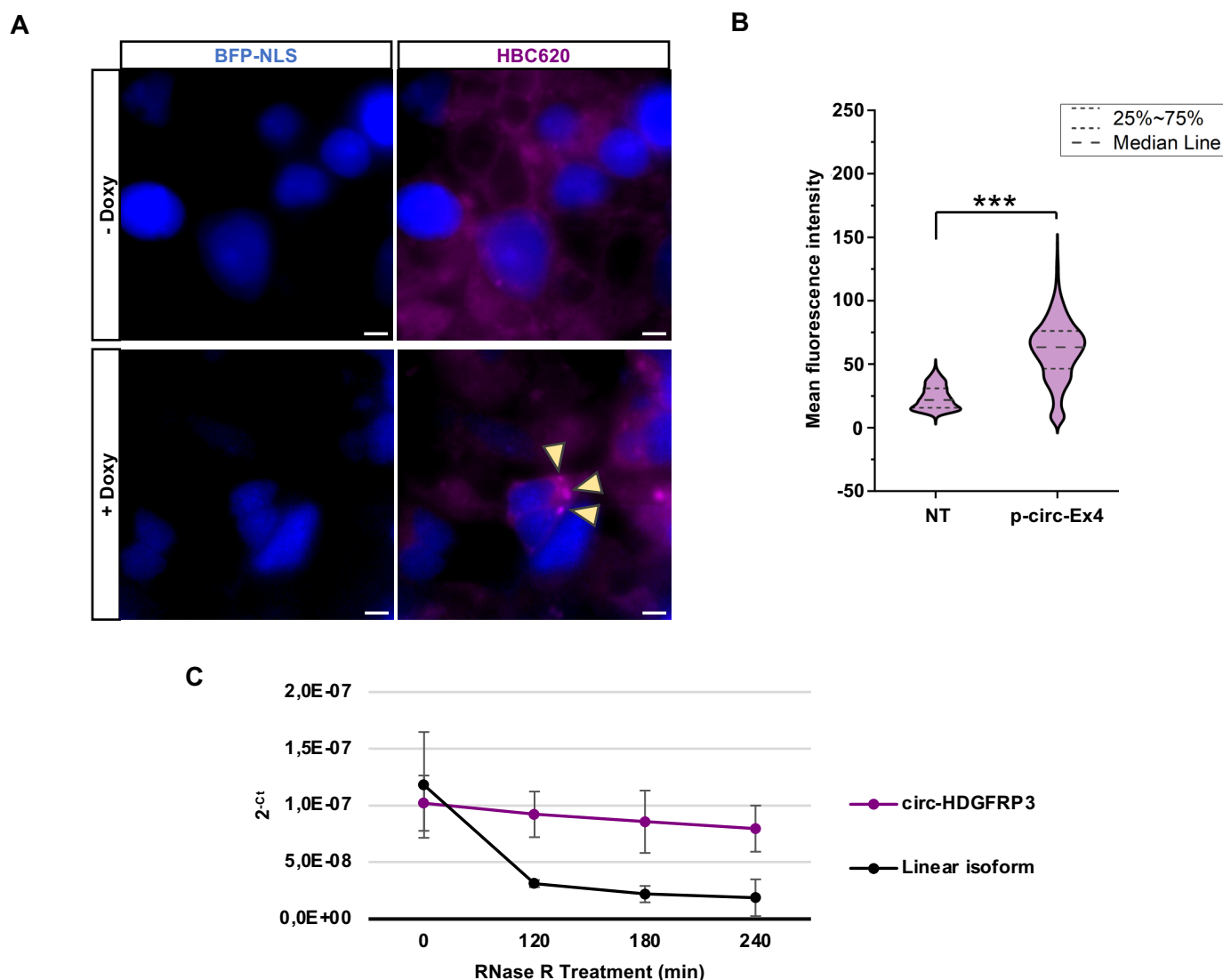




Supplementary Fig 1 - Pepper provides a detectable and robust signal for circ-HDGFRP3 visualization in living mammalian cells.

A Representative image of HEK 293T cells transfected with p-circ-Ex4 overexpression construct. Signal of BFP-NLS reporter protein (blue) allows the identification of successfully transfected cells. HBC620 (magenta) provides background signal in cells not treated with Doxycycline (- Doxy, upper panel), while binds to p-circ-Ex4 transcript in cells treated with Doxycycline (+ Doxy, lower panel). Yellow arrows highlight focal foci of p-circ-Ex4 signal. All scale bars correspond to 5 μ m.

B Violin plot comparing mean fluorescence intensity of non-transfected HEK 293T cells treated only with HBC with cells transfected with p-circ-Ex4 and treated with HBC dye. *** $p \leq 0,001$ corresponds to two tails unpaired Student's t-test (N=3) calculated on fluorescent intensity measurements of >5000 cells imaged from 3 independent biological replicates.

C Chart representing unnormalized RNA levels of circ-HDGFRP3 (magenta) and of the linear isoform (black) exposed to 120, 180 and 240 minutes of RNase R treatment. Error bars represent $\pm$ SEM (N=3).