

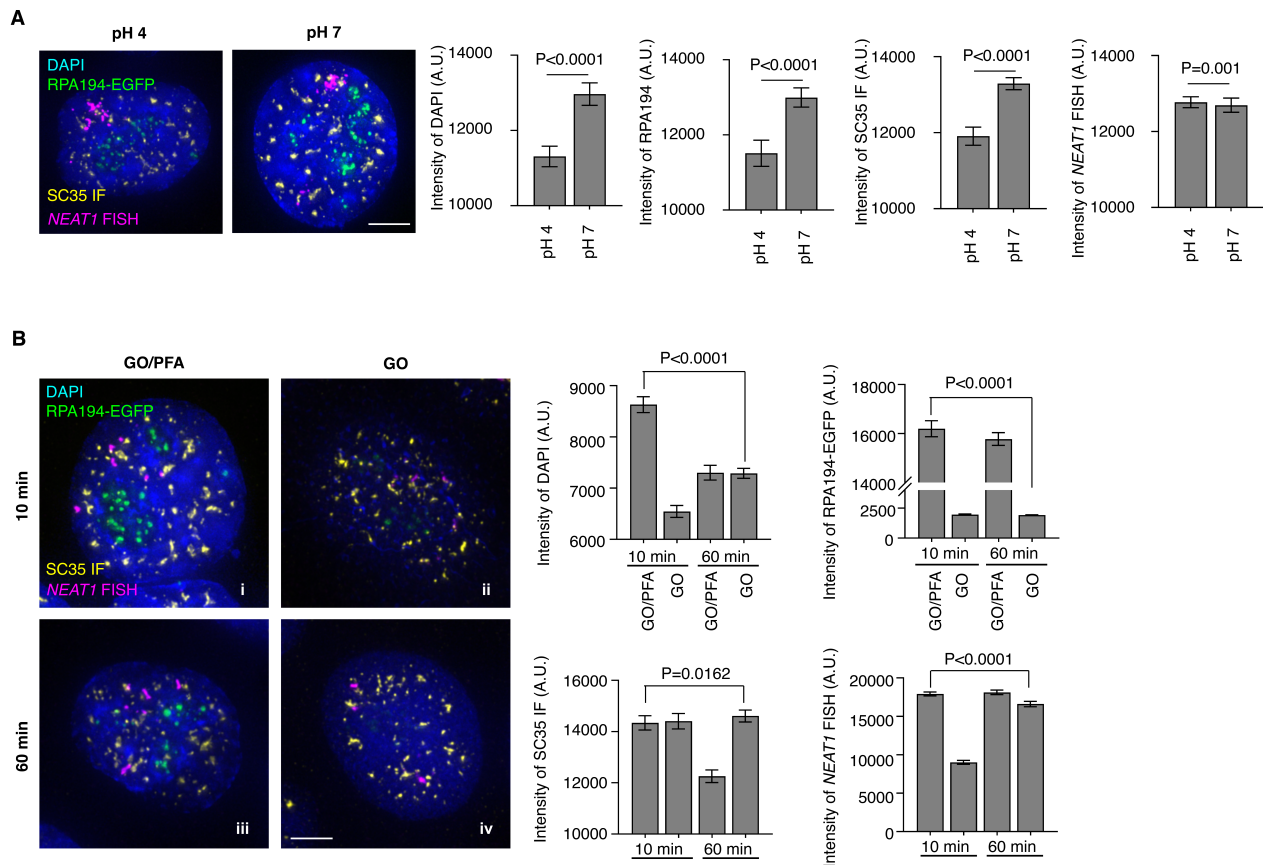


Figure S5. Comparison of pH condition and fixation duration on IF and RNA FISH in NBs.

(A) A neutral pH condition is suitable for GO/PFA fixation. Compared to signals acquired from a neutral pH fixation (pH 7), an acidic pH condition (pH 4) resulted in reduced signals for DAPI (blue), fluorescent protein (green, RPA194-EGFP) and immunofluorescence (yellow, SC35), but with comparable signals for smFISH (magenta, *NEAT1*). Left, representative images, scale bar: 5 μ m; Right, Mean $\pm$ SEM and Mann-Whitney test are shown across three independent experiments.

(B) Prolonged GO/PFA fixation does not further enhance RNA or protein signals in NBs. GO/PFA fixation (i, iii) or GO alone fixation according to the reported protocol (Richter et al., 2018) (ii, iv) were performed for 10 min or 60 min, respectively. Left, representative images, scale bar: 5 μ m; Right, Mean $\pm$ SEM and Mann-Whitney test are shown across three independent experiments.