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### Supplemental information

## Alterations induced by the PML-RAR $\alpha$ oncogene revealed by image

### cross correlation spectroscopy

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## SUPPORTING INFORMATION FOR:

# Alterations induced by the PML-RAR $\alpha$ oncogene revealed by Image Cross-Correlation Spectroscopy

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Content:

Supporting Figure S1-S4



### Fig. S1. Generation of the 1D spatial correlation functions.

Representative 2D spatial correlation functions (left) and corresponding 1D spatial correlation functions (right) obtained by angular averaging. Shown are (from top to bottom) the autocorrelation function (ACF) of the green and red channel and the cross-correlation function (CCF). The shown correlation functions are calculated from the image shown in Fig.2B.



### Fig. S2. Confocal vs STED imaging of PML-RARa microspeckles.

A,B) (left) Representative confocal (A) and STED (B) images of PML-RAR $\alpha$  microspeckles in U937-PR9 cells at 8h after PML-RAR $\alpha$  expression induction. Scale bar 3  $\mu$ m. (right) QuICS analysis showing the value R representing the average apparent size of the PML-RAR $\alpha$  microspeckles in the selected images.



### Fig. S3. Comparison between different STED imaging modalities.

A) Representative STED image of Pol2S2 in U937-PR9 cells (left) and evaluation of image quality by QuICS (right). B) Representative gated-STED (gating = 1 ns) image of Pol2S2 in U937-PR9 cells (left) and evaluation of image quality by QuICS (right). C) Representative Tau-STED image of Pol2S2 in U937-PR9 cells (left) and evaluation of image quality by QuICS (right). Scale bar 3  $\mu$ m.



### Fig. S4. Confocal ICCS of PML/PML-RAR $\alpha$ and elongating Pol2

A) Representative 2-color confocal images of PML/PML-RAR $\alpha$  (green) and Pol2S2 (red) in U937-PR9 cells before (ctrl) and after PML-RAR $\alpha$  expression induction, at the specified time point. Scale bar 3 um. B) Histograms show the distribution of the colocalization fraction f<sub>Pol2S2</sub>, representing the fraction of Pol2S2 molecules colocalized with PML/PML-RAR $\alpha$  molecules. Red lines are Gaussian fits. Numbers represent the peak values.