# SUPPLEMENTARY FIGURES

# Structured illumination microscopy improves spot detection performance in spatial transcriptomics

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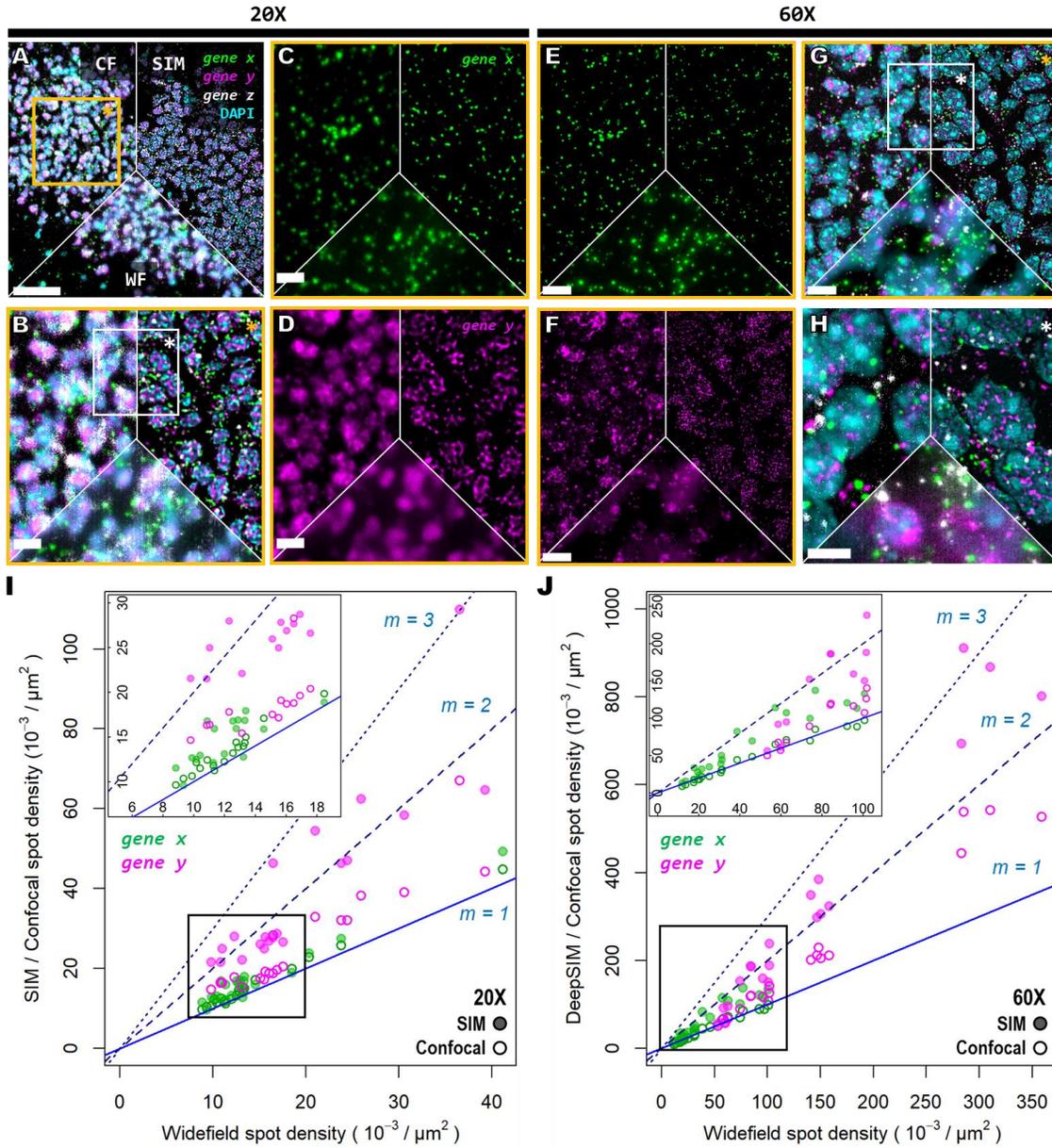
**Figure S1.** Structured illumination enhances spot contrast and resolution and allows resolving individual transcripts across various spot densities, magnification levels and imaging modalities. (**A**) Three different groups of 50 genes are visualized in each channel (Cy3, Cy5 and Cy7) referred to as *gene x*, *gene y* and *gene z* for simplicity. A single FOV of a mouse brain coronal section stained with DAPI is shown, imaged with a 20X objective. For panels (**A**) through (**H**), a comparison of the same FOV imaged in widefield (lower sector), spinning disk confocal (upper-right) and SIM (upper-right) modalities is shown. (**B**) Zoomed image of the yellow box in (**A**). Panels (**C**) through (**F**) correspond to the same zoomed FOV as in (**B**), displaying only the channels for either *gene x* (upper row) or *gene y* (lower row) alone, both in 20X (**C,D**) and 60X (**E,F**) magnification. (**G**) FOV corresponding to the region inside the yellow box in (**A**), but imaged with a 60X objective. (**H**) Zoomed image of the white box in (**G**) and (**B**). In all panels, color-wise asterisks indicate micrographs corresponding to the same physical region of the sample. Plots show the ratio of spot detection density when comparing imaging of *gene x* (green dots) and *gene y* (magenta dots) in widefield against spinning disk confocal (hollow dots) and SIM (colored dots) modes, both for the 20X (**I**) and 60X (**J**) objectives. Blue and dotted blue lines correspond to an increasing spot density ratio from 1 to 3. The inner plot shows a zoomed version of the regions in the black boxes. Scale bars: (**A**) = 50 μm; (**B,C,D,E,F**) = 10 μm; (**H**) = 5 μm.