

**CRISPR-mediated multiplexed live cell imaging of nonrepetitive  
genomic loci with one guide RNA per locus**

Supplementary Information

Clow *et al.*

**Supplementary Table 1. Comparison of Casilio with published CRISPR-based imaging systems for visualizing endogenous unmodified genomic loci.**

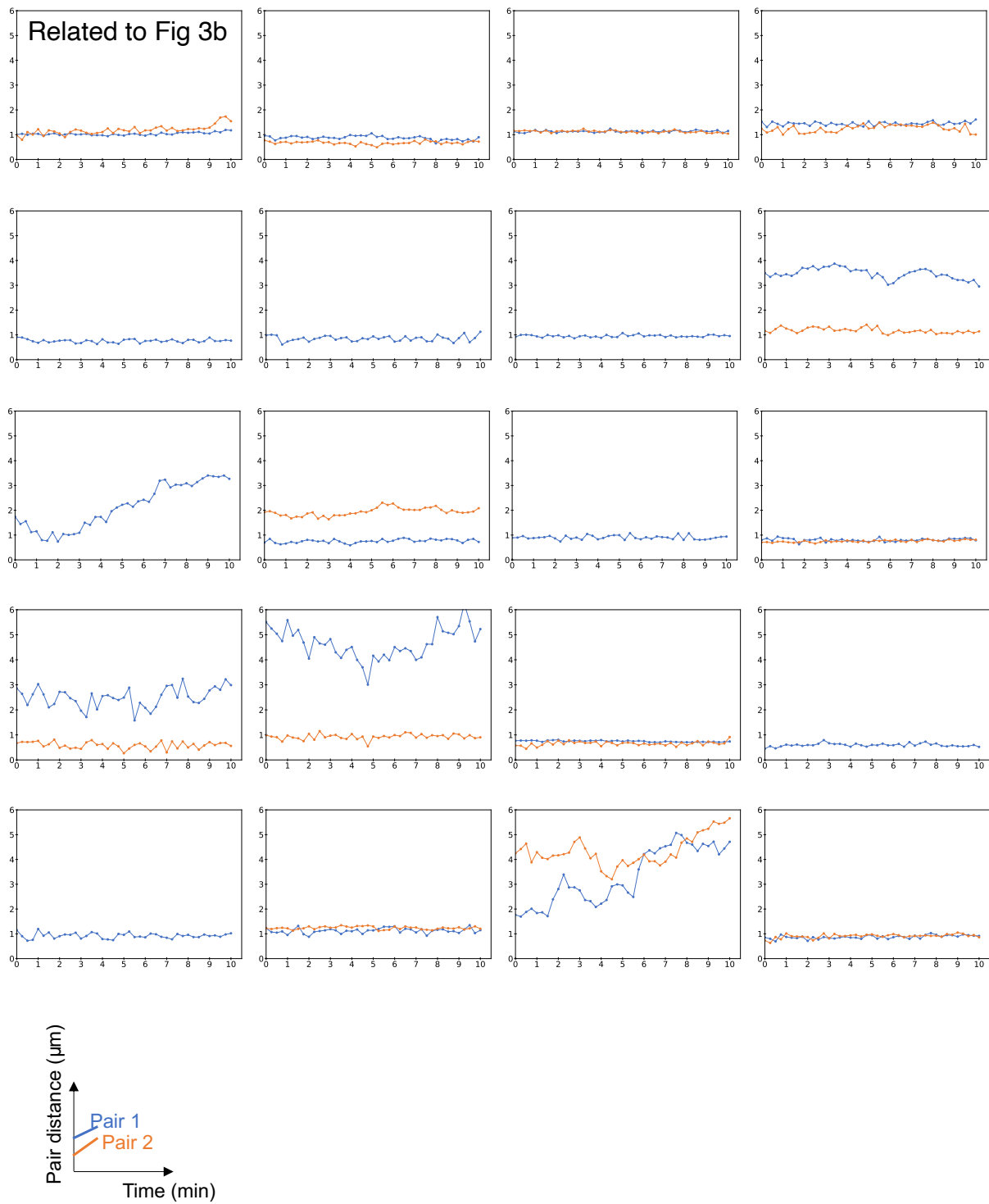
<b>Reference in main text</b>	<b>Composition</b>	<b>Minimal number of gRNAs for non-repeat imaging</b>	<b>Number of colors</b>
Chen 2013 <sup>14</sup>	dCas9-EGFP	36	1
Tanenbaum 2014 <sup>15</sup>	dCas9:SunTag <sub>24x</sub> :GFP	N.A. (only repeats)	1
Ma 2016 <sup>16</sup>	dCas9:MS2/PP7/boxB:FP	N.A. (only repeats)	3 <sup>a</sup>
Qin 2017 <sup>17</sup>	dCas9:16xMS2:mCherry	4	1
Ma 2018 <sup>18</sup>	dCas9:8xMS2/PP7:FP	N.A. (only repeats)	2
Maas 2018 <sup>19</sup>	dCas9:MS2/PP7:FP	2 <sup>b</sup>	2
Gu 2018 <sup>20</sup>	dCas9-EGFP	12	1
Wang 2019 <sup>21</sup>	dCas9:gRNA-Cy3	N.A. (only repeats)	2
Mao 2019 <sup>22</sup>	dCas9:gRNA:MB(FRET)	3	1
Casilio (this study)	dCas9:PUF:FP	1	3

<sup>a</sup>Using combination of 3 colors, co-imaging of a maximum of 6 loci was demonstrated. However, this relies on color combination. When labeling very close-by loci, deconvolution of color combinations may become a challenge.

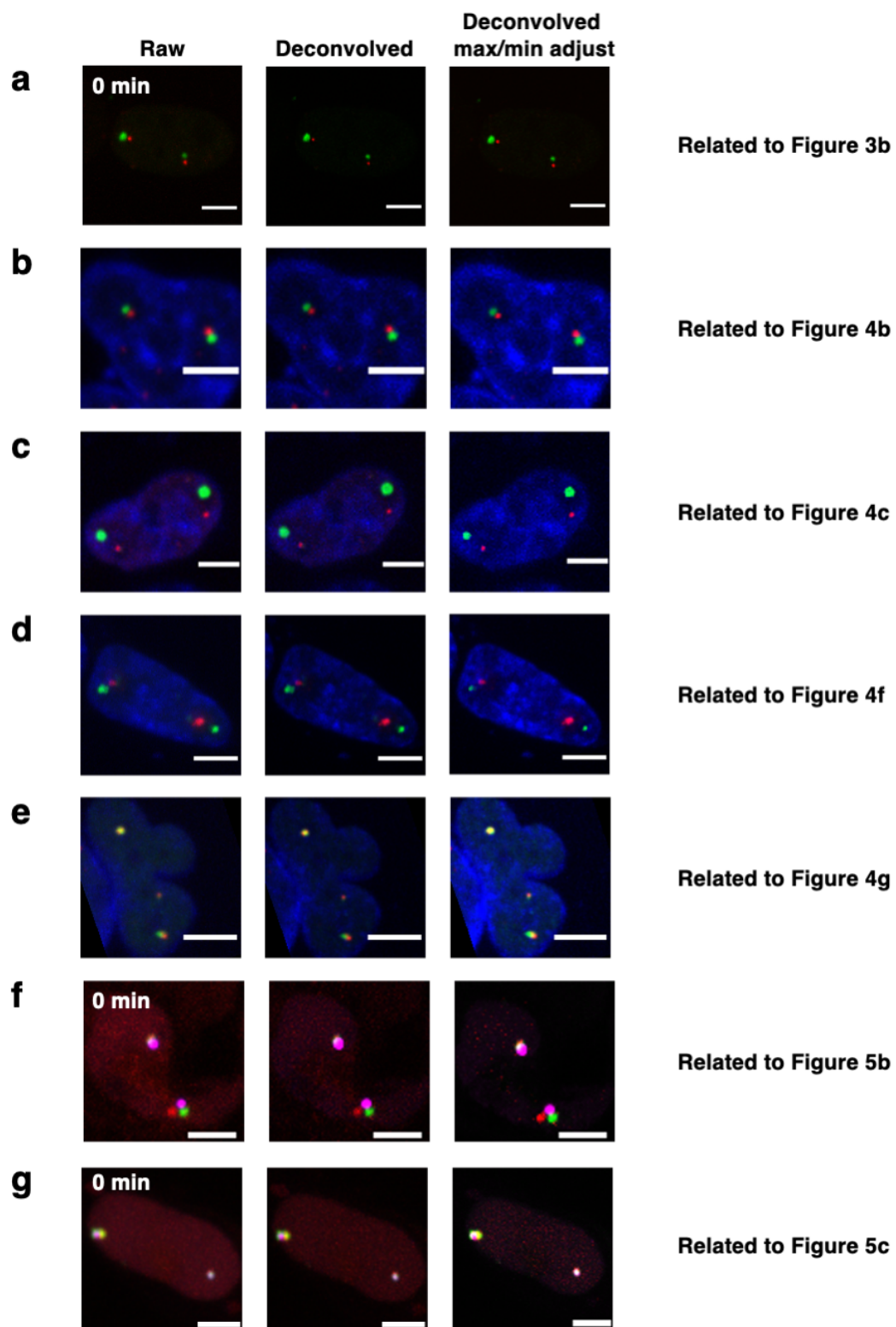
<sup>b</sup>More than expected number of spots observed, requiring elaborate filtering algorithm keeping two brightest spots (see supplementary figure 3 of that reference).

**Supplementary Table 2. Listing of Plasmids with links to plasmid maps and Addgene.**

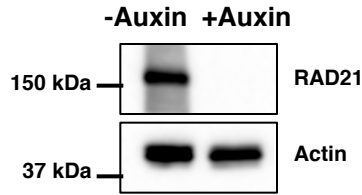
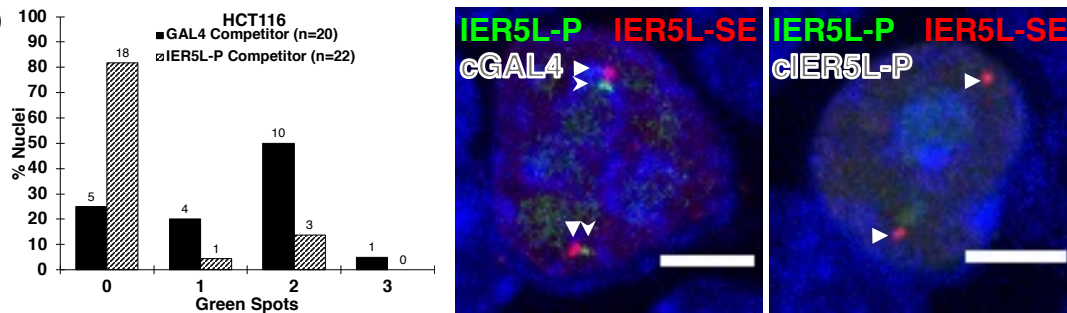
<b>Code</b>	<b>Plasmid Name</b>	<b>Plasmid map on Benchling</b>	<b>Addgene Link</b>
pAC11001	lenti_dCas9	<a href="https://benchling.com/s/seq-nwhb6COmj4HyvRDaltcT?m=slm-0GaEau5AEgsDsPVvBi9D">https://benchling.com/s/seq-nwhb6COmj4HyvRDaltcT?m=slm-0GaEau5AEgsDsPVvBi9D</a>	<a href="https://addgene.org/183208">https://addgene.org/183208</a>
pAC1447	pmax-Clover_PUFc	<a href="https://benchling.com/s/seq-DjznMYu2SHLIVqh8JjQ3?m=slm-XhF9XSW3mbTfMEgZzO4W">https://benchling.com/s/seq-DjznMYu2SHLIVqh8JjQ3?m=slm-XhF9XSW3mbTfMEgZzO4W</a>	<a href="https://addgene.org/73689">https://addgene.org/73689</a>
pAC11002	pmax-PUF9R_iRFP670	<a href="https://benchling.com/s/seq-ymk2raAqiMP1wZEIDtmX?m=slm-XLBZX3AQozvu2RDxWiUi">https://benchling.com/s/seq-ymk2raAqiMP1wZEIDtmX?m=slm-XLBZX3AQozvu2RDxWiUi</a>	<a href="https://addgene.org/183209">https://addgene.org/183209</a>
pAC11003	pmax-PUF9R_mRuby2	<a href="https://benchling.com/s/seq-05CQGYUdjnefm6nwJrkl?m=slm-meYSbUjvCBrc744J7tSZ">https://benchling.com/s/seq-05CQGYUdjnefm6nwJrkl?m=slm-meYSbUjvCBrc744J7tSZ</a>	<a href="https://addgene.org/183210">https://addgene.org/183210</a>
pAC11004	pmax-Clover_PUF48107	<a href="https://benchling.com/s/seq-fRaBdNkydw9dDeyowhdd?m=slm-rDks2uTpsak4mVz0w3MI">https://benchling.com/s/seq-fRaBdNkydw9dDeyowhdd?m=slm-rDks2uTpsak4mVz0w3MI</a>	<a href="https://addgene.org/183211">https://addgene.org/183211</a>
pAC11005	pmax-iRFP670_PUFc	<a href="https://benchling.com/s/seq-6tmSjU4WcZpzy03YcpEv?m=slm-eDyvA9z21iWDM1HU8IH1">https://benchling.com/s/seq-6tmSjU4WcZpzy03YcpEv?m=slm-eDyvA9z21iWDM1HU8IH1</a>	<a href="https://addgene.org/183212">https://addgene.org/183212</a>
pAC11006	pCR8-sgRNA-15xPBSc	<a href="https://benchling.com/s/seq-XFhAw0mDVUoNViBilYlH?m=slm-BkR0AYIqz45RygiksM1X">https://benchling.com/s/seq-XFhAw0mDVUoNViBilYlH?m=slm-BkR0AYIqz45RygiksM1X</a>	<a href="https://addgene.org/183213">https://addgene.org/183213</a>
pAC11007	pCR8-sgRNA-15xPBS9R	<a href="https://benchling.com/s/seq-mj21Noq9avBms6Ooa31D?m=slm-nQbglQHY0PjDh1r1EVTT">https://benchling.com/s/seq-mj21Noq9avBms6Ooa31D?m=slm-nQbglQHY0PjDh1r1EVTT</a>	<a href="https://addgene.org/183214">https://addgene.org/183214</a>
pAC11008	pCR8-sgRNA-20xPBS48107	<a href="https://benchling.com/s/seq-BMHoDIBHYEfLM9Meyp4p?m=slm-L00jM5Knz4LpNvSFSG6Y">https://benchling.com/s/seq-BMHoDIBHYEfLM9Meyp4p?m=slm-L00jM5Knz4LpNvSFSG6Y</a>	<a href="https://addgene.org/183215">https://addgene.org/183215</a>
pAC11009	pCR8-sgRNA-20xPBSc	<a href="https://benchling.com/s/seq-MHL6lsmXICnDbdldVjFD?m=slm-1gbixcNOTO25ngnYOhYo">https://benchling.com/s/seq-MHL6lsmXICnDbdldVjFD?m=slm-1gbixcNOTO25ngnYOhYo</a>	<a href="https://addgene.org/183216">https://addgene.org/183216</a>
pAC11010	pCR8-sgRNA-25xPBS9R	<a href="https://benchling.com/s/seq-fOBZj7IIuZjAoxDEy44I?m=slm-17OYnvymuAzct16VuTes">https://benchling.com/s/seq-fOBZj7IIuZjAoxDEy44I?m=slm-17OYnvymuAzct16VuTes</a>	<a href="https://addgene.org/183217">https://addgene.org/183217</a>



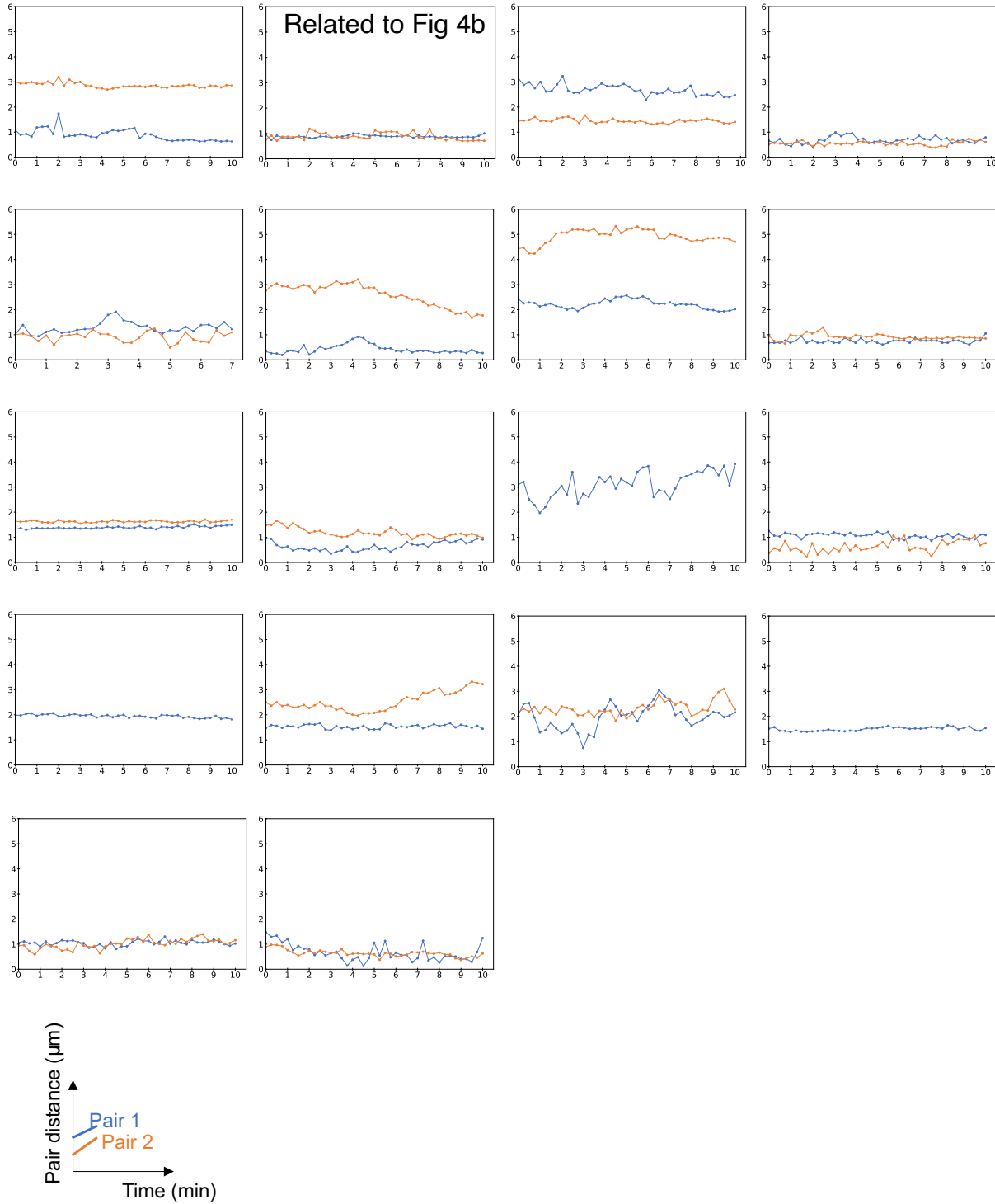
**Supplementary Fig. 1.** Pairwise 3D distances of Clover and iRFP670 foci labeling the *MASP1-BCL6* loop in 20 ARPE-19 nuclei (33 pairs) over time. Source data are provided as a Source Data file.



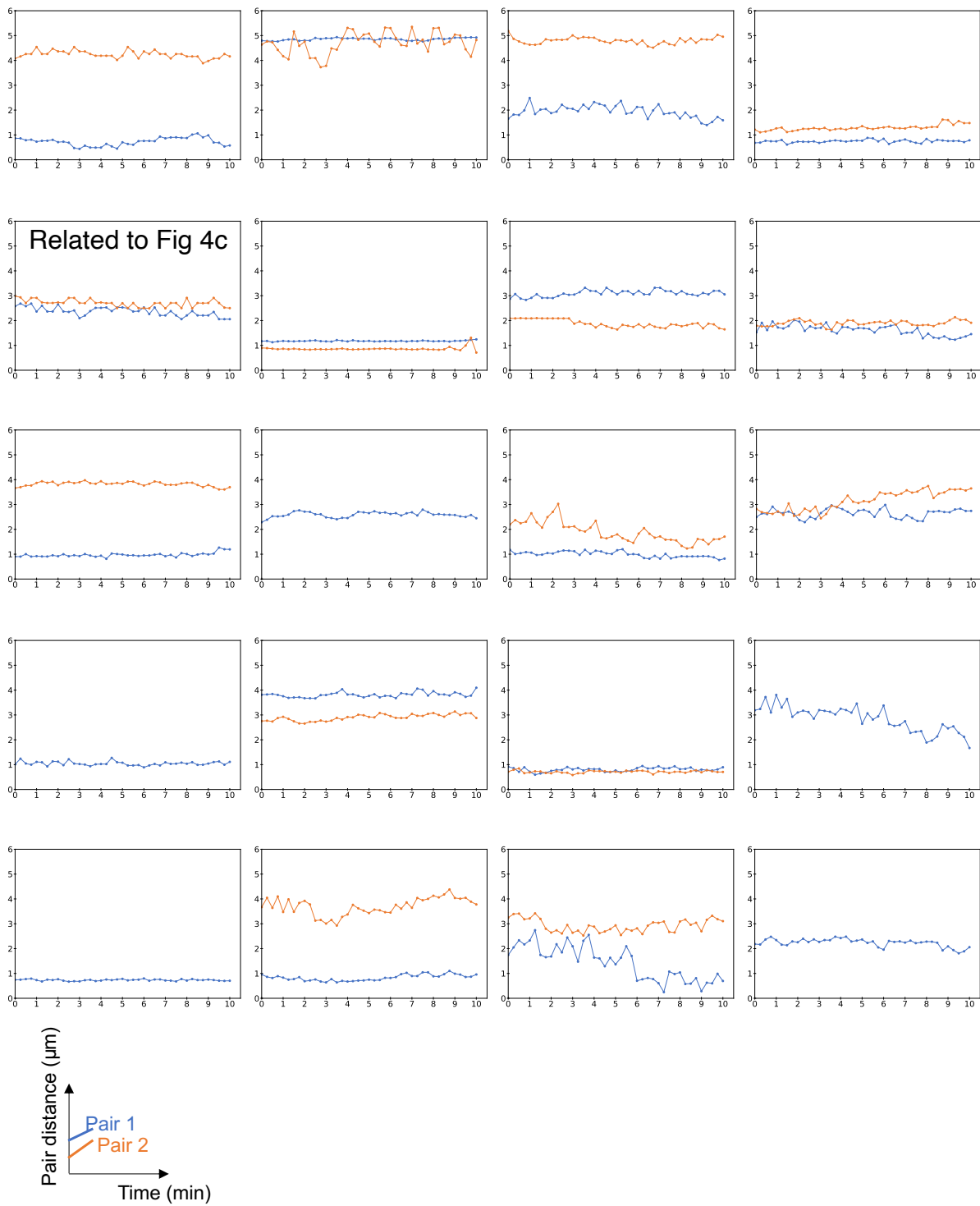
Supplementary Fig. 2. Example images through each step of image processing.

**a****b**

**Supplementary Fig 3. (a)** RAD21 and actin immunoblot of HCT116/RAD21-mAID cell extracts without and with auxin treatment. Experiment repeated 3 times. **(b)** Competitor experiments for validating *IER5L* P-SE labeling. Column plots show percentages of nuclei with the indicated numbers of Clover (green) spots (counts shown on top of columns) in HCT116 cells co-transfected with Clover-PUFc/gIER5L-P-15xPBSc, PUF9R-iRFP670/gIER5L-SE-15xBPS9R and either a non-competing GAL4 gRNA (black columns) or a gRNA competing with the IER5L-P (patterned columns). Representative images in the non-competed or competed samples (Clover, green spots and stealth arrowheads; iRFP670, red spots and triangle arrowheads) are shown to the right. Scale bars, 5  $\mu\text{m}$ . Experiment repeated 3 times. Source data are provided as a Source Data file.

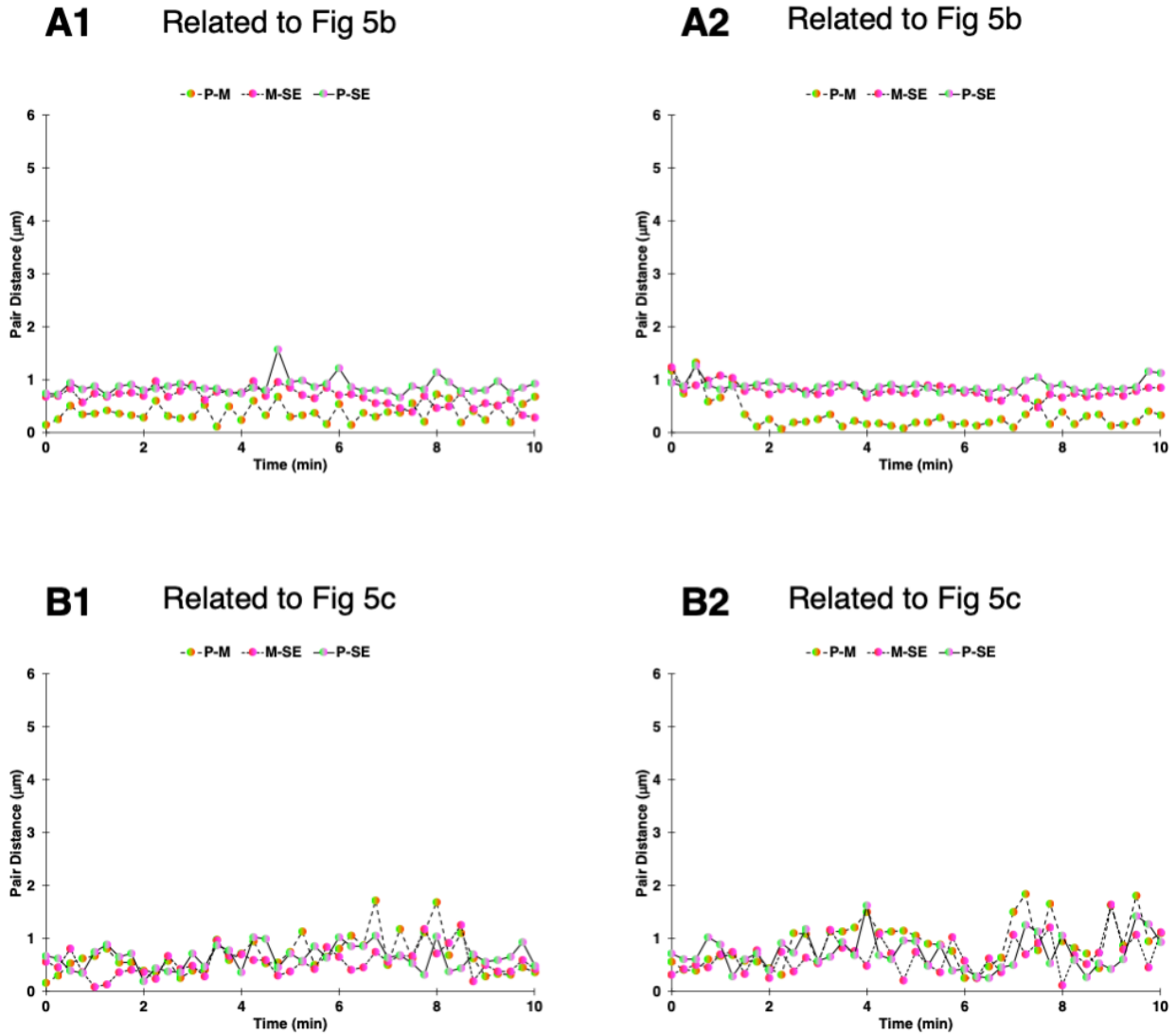


**Supplementary Fig. 4.** Pairwise 3D distances of Clover and iRFP670 foci labeling the *IER5L*-P locus and *IER5L*-SE locus in 18 nuclei (33 pairs) of untreated HCT116/RAD21-mAID cells over time. Source data are provided as a Source Data file.

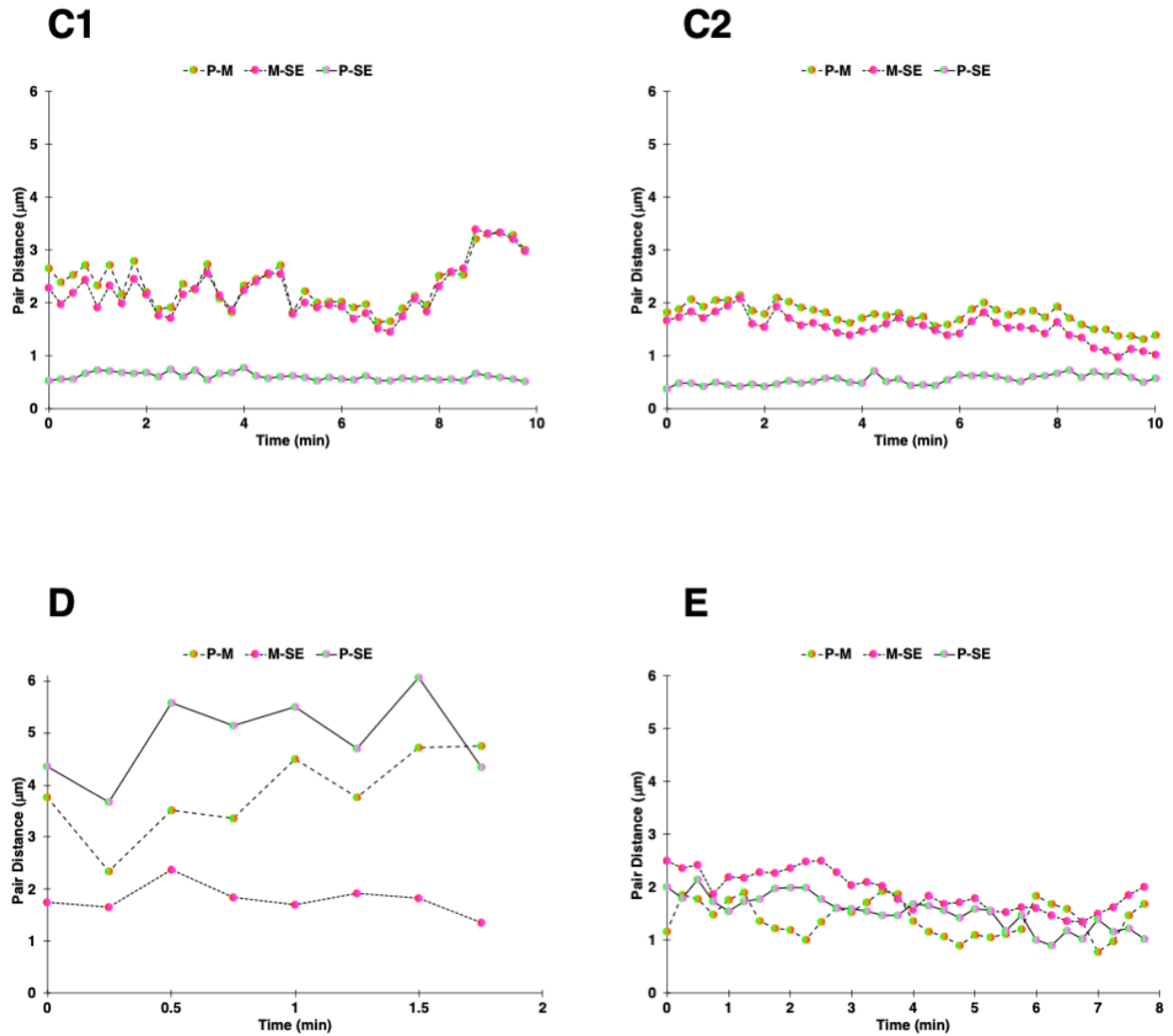


**Supplementary Fig. 5.** Pairwise 3D distances of Clover and iRFP670 foci labeling the *IER5L*-P locus and *IER5L*-SE locus in 20 nuclei (35 pairs) of auxin-treated HCT116/RAD21-mAID cells over time. Source data are provided as a Source Data file.

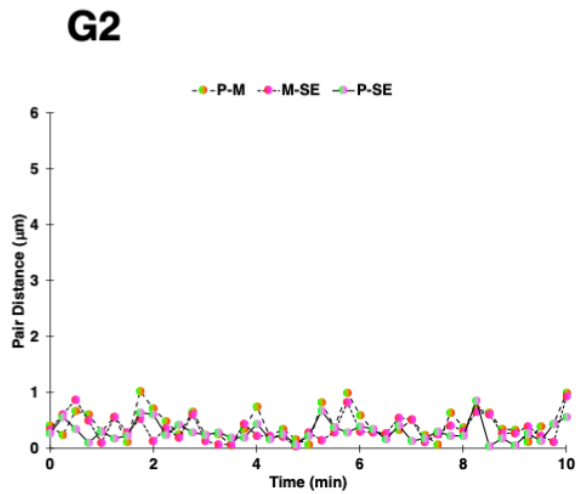
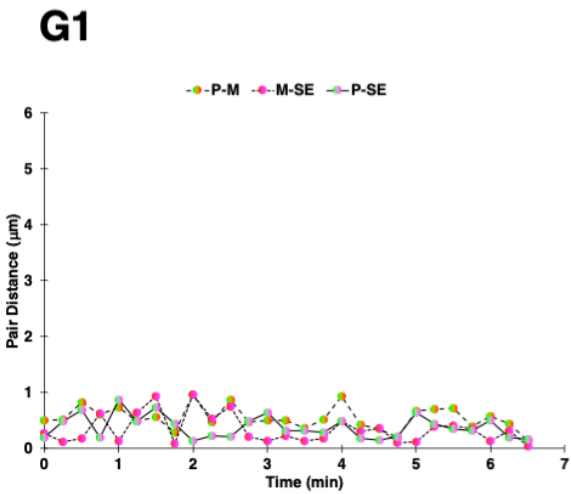
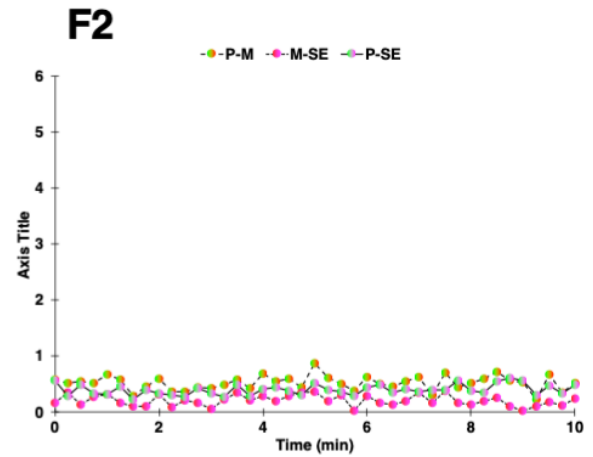
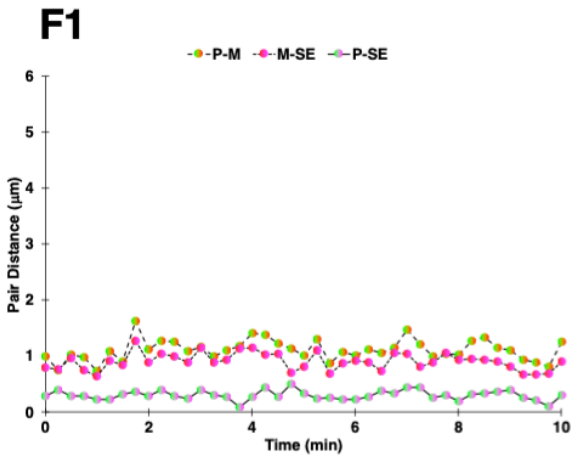




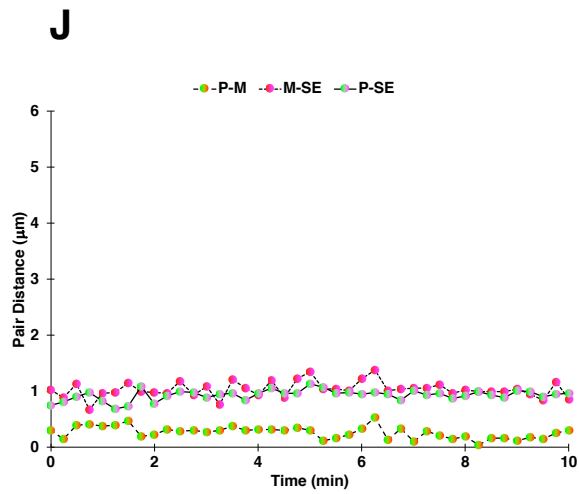
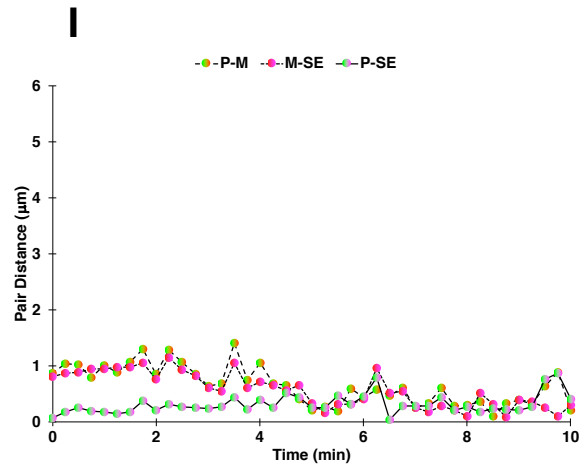
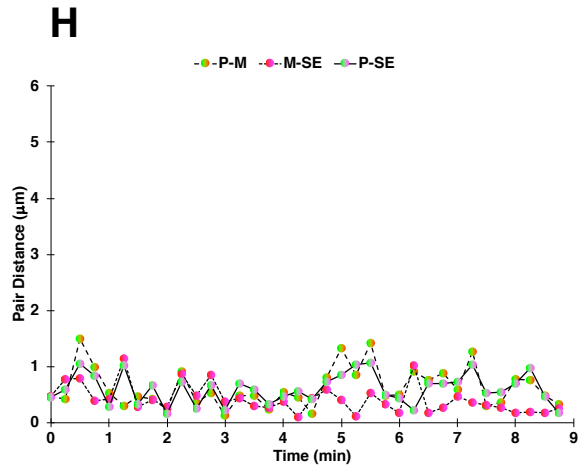
**Supplementary Fig. 6.** All combinations of pairwise 3D distances of Clover (locus P), iRFP670 (locus M) and mRuby2 (locus SE) spots on the *IER5L* P-SE loop in 10 HCT116/RAD21-mAID nuclei (15 alleles) over time. Source data are provided as a Source Data file.



**Supplementary Fig. 6 (continued).** All combinations of pairwise 3D distances of Clover (locus P), iRFP670 (locus M) and mRuby2 (locus SE) spots on the *IER5L* P-SE loop in 10 HCT116/RAD21-mAID nuclei (15 alleles) over time. Source data are provided as a Source Data file.



**Supplementary Fig. 6 (continued).** All combinations of pairwise 3D distances of Clover (locus P), iRFP670 (locus M) and mRuby2 (locus SE) spots on the *IER5L* P-SE loop in 10 HCT116/RAD21-mAID nuclei (15 alleles) over time. Source data are provided as a Source Data file.



**Supplementary Fig. 6 (continued).** All combinations of pairwise 3D distances of Clover (locus P), iRFP670 (locus M) and mRuby2 (locus SE) spots on the *IER5L* P-SE loop in 10 HCT116/RAD21-mAID nuclei (15 alleles) over time. Source data are provided as a Source Data file.