

## Supplemental Video Legends

**Video S1: Third instar larval neuroblast mitotic division with I-Crel-induced acentrics and GFP-tagged X chromosomes.** Chromosomes are labeled with H2Av histone variant tagged with RFP (magenta) and X chromosomes are labeled with MSL3 tagged with GFP (green). Time lapse: 5 seconds. 9 frames per second. Scale bar: 2  $\mu$ m. This video corresponds to Figure 1b.

**Video S2: Third instar larval neuroblast wild-type mitotic division with GFP-tagged X chromosomes.** Chromosomes are labeled with H2Av histone variant tagged with RFP (magenta) and X chromosomes are labeled with GFP-tagged MSL3 (green). Time lapse: 5 seconds. 9 frames per second. Scale bar: 2  $\mu$ m. This video corresponds to Figure 1a.

**Video S3: 3D rendering of third instar larval neuroblast at metaphase of mitosis with GFP-tagged X chromosomes.** Chromosomes are labeled with H2Av histone variant tagged with RFP (magenta) and X chromosomes are labeled with MSL3 tagged with GFP (green). Image rotates 180°. Scale bar: 1  $\mu$ m. This video corresponds to Figure 3a.

**Video S4: 3D rendering of third instar larval neuroblast at metaphase of mitosis with I-Crel-induced acentrics and GFP-tagged X chromosomes.** Chromosomes are labeled with H2Av histone variant tagged with RFP (magenta) and X chromosomes are labeled with MSL3 tagged with GFP (green). Image rotates 180°. Scale bar: 3  $\mu$ m. This video corresponds to Figure 3b.

**Video S5: Third instar larval neuroblast mitotic division with I-Crel-induced acentrics and GFP-tagged X chromosomes.** Chromosomes are labeled with H2Av histone variant tagged with RFP (magenta) and X chromosomes are labeled with MSL3 tagged with GFP (green). Time lapse: 5 seconds. 9 frames per second. Scale bar: 2  $\mu$ m. This video corresponds to Figure 4b.

**Video S6: Third instar larval neuroblast mitotic division with I-Crel-induced acentrics and GFP-tagged telomeres.** Chromosomes are labeled with H2Av histone variant tagged with RFP (magenta) and telomeres are labeled with HOAP tagged with GFP (green). Time lapse: 5 seconds. 9 frames per second. Scale bar: 2  $\mu$ m. This video corresponds to Figure 5a.

**Video S7: 3D rendering of third instar larval neuroblast at metaphase of mitosis with I-Crel-induced acentrics and GFP-tagged telomeres.** Chromosomes are labeled with H2Av histone variant tagged with RFP (magenta) and telomeres are labeled with HOAP tagged with GFP (green). Image rotates 180°. Scale bar: 0.5  $\mu$ m. This video corresponds to Figure 5d.

**Video S8: Third instar larval neuroblast mitotic division expressing I-Crel to generate acentrics and RNAi against EB1.** Chromosomes are labeled with H2Av histone variant tagged with RFP (white). Time lapse: 20 seconds. 9 frames per second. Scale bar: 2  $\mu$ m. This video corresponds to Figure 6b.

**Video S9: Third instar larval neuroblast mitotic division expressing I-Crel to generate acentrics and a *k/p3a*<sup>2</sup> hypomorphic allele.** Chromosomes are labeled with H2Av histone variant tagged with RFP (white). Time lapse: 5 seconds. 9 frames per second. Scale bar: 2  $\mu$ m. This video corresponds to Figure 7b.

**Video S10: Third instar larval neuroblast mitotic division expressing I-Crel to generate acentrics and RNAi against Klp31E.** Chromosomes are labeled with H2Av histone variant tagged with RFP (magenta) and microtubule-associated protein Jupiter tagged with GFP (green). Time lapse: 5 seconds. 9 frames per second. Scale bar: 2  $\mu$ m. This video corresponds to Figure 8b.