

Title

In toto light sheet fluorescence microscopy live imaging datasets of *Ceratitis capitata* embryonic development

Authors

Frederic Strobl¹, Marc F. Schetelig² and Ernst H.K. Stelzer¹

Affiliations

¹Physical Biology / Physikalische Biologie (IZN, FB 15)
Buchmann Institute for Molecular Life Sciences (BMLS)
Cluster of Excellence Frankfurt – Macromolecular Complexes (CEF – MC)
Goethe-Universität – Frankfurt am Main (Campus Riedberg)
Max-von-Laue-Straße 15 – D-60438 Frankfurt am Main – Germany

²Justus-Liebig-Universität Gießen
Department of Insect Biotechnology in Plant Protection
Winchesterstraße 2 – D-35394 Gießen – Germany

Corresponding authors:

Frederic Strobl (frederic.strobl@physikalischebiologie.de)

Ernst H.K. Stelzer (ernst.stelzer@physikalischebiologie.de)

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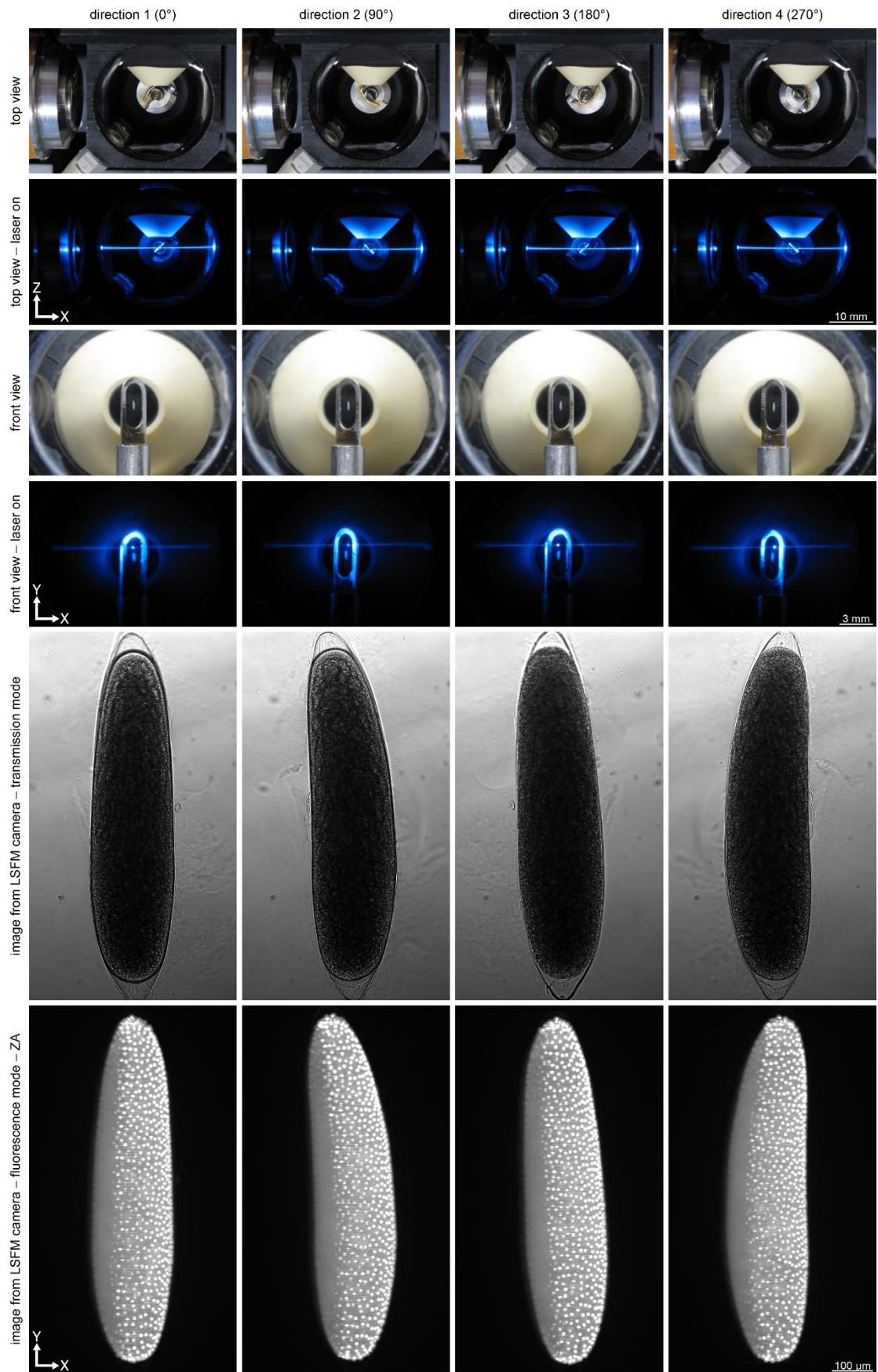
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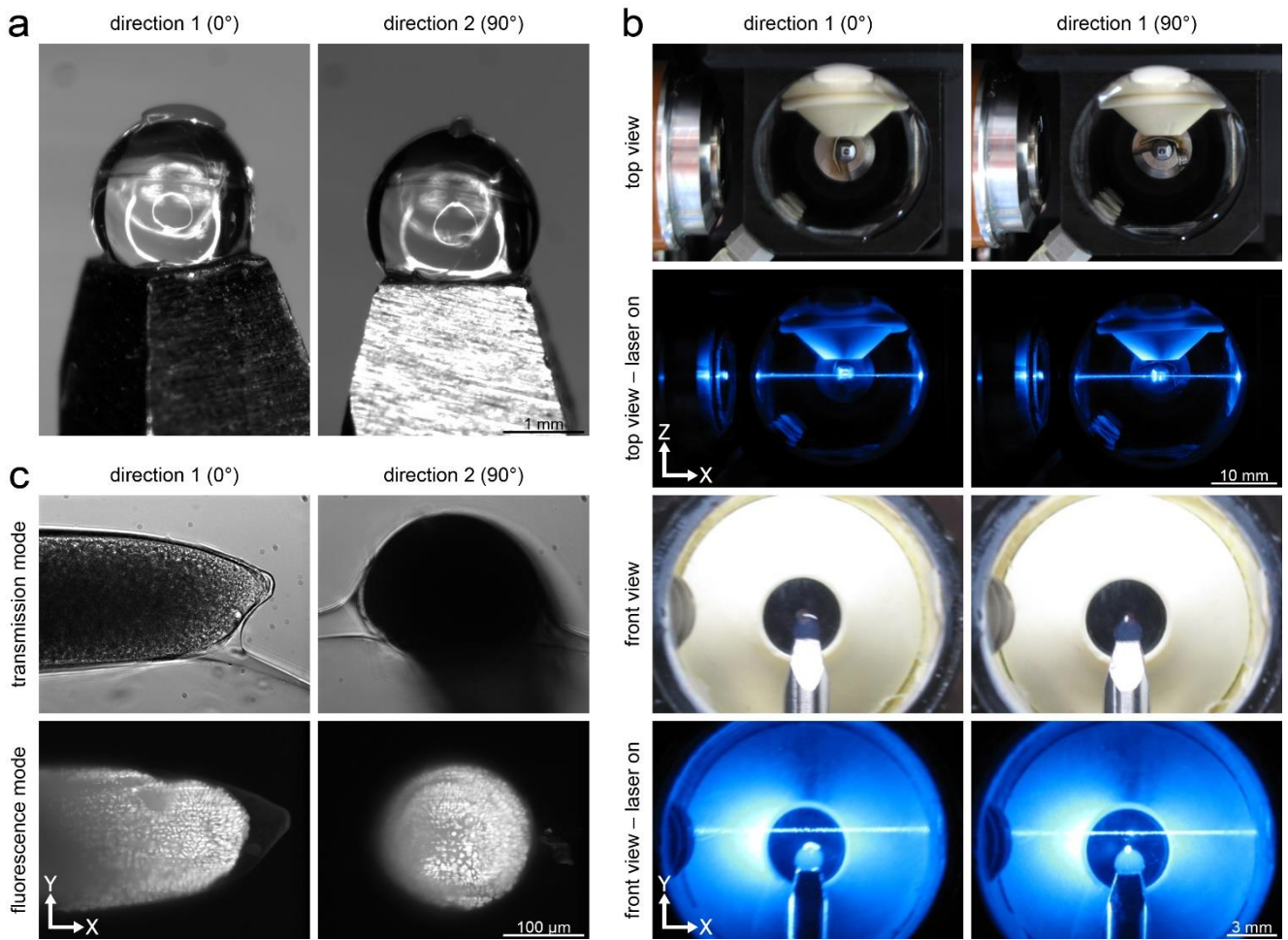
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Supplementary Figure 1 – Illustration of the medfly embryo imaging process with the cobweb holder along four directions. Top view (first and second row) and front view (third and fourth row) of the sample chamber illustrating the illumination process along each direction. Medfly embryo imaged during blastoderm formation in the transmission light (fifth row) and fluorescence (sixth row) channels along four directions (orientations 0°, 90°, 180°, 270°). ZA, z maximum projection with image adjustment.



Supplementary Figure 2 – Illustration of medfly embryo imaging process using the agarose hemisphere mounting method. (a) Front and side view of the sample holder with embryo placed on top of the agarose hemisphere. The shown views correspond to the orientations 0° and 90°. **(b)** Top view (first and second row) and front view (third and fourth row) of the sample chamber using the alternative mounting method. **(c)** Medfly embryo imaged in the transmission light (upper row) and fluorescence channel (lower row) along two directions (orientations 0° and 90°).

a

II – Early Gastrulation

DS0001

DS0002

DS0003

DS0004

DS0005

0° (ventrolateral / ventral) – ZA

90° (dorsolateral / lateral) – ZA

180° (dorsolateral / dorsal) – ZA



100 μ m

b

III – Germband Elongation

DS0001

DS0002

DS0003

DS0004

DS0005

0° (ventrolateral / ventral) – ZA

90° (dorsolateral / lateral) – ZA

180° (dorsolateral / dorsal) – ZA



100 μ m

C

IV – Germband Retraction

DS0001

DS0002

DS0003

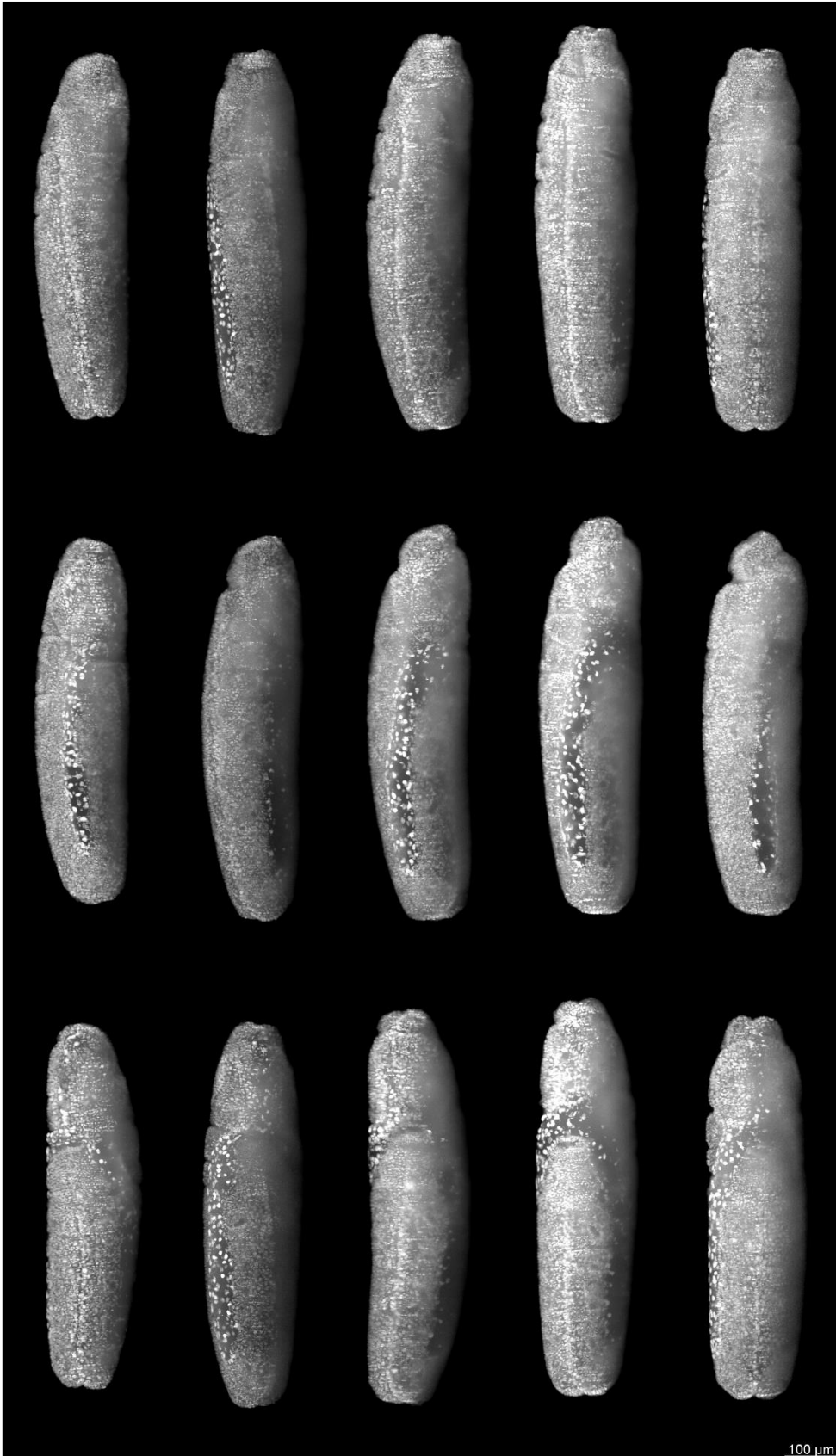
DS0004

DS0005

0° (ventrolateral / ventral) – ZA

90° (dorsolateral / lateral) – ZA

180° (dorsolateral / dorsal) – ZA



d

V – Dorsal Closure

DS0001

DS0002

DS0003

DS0004

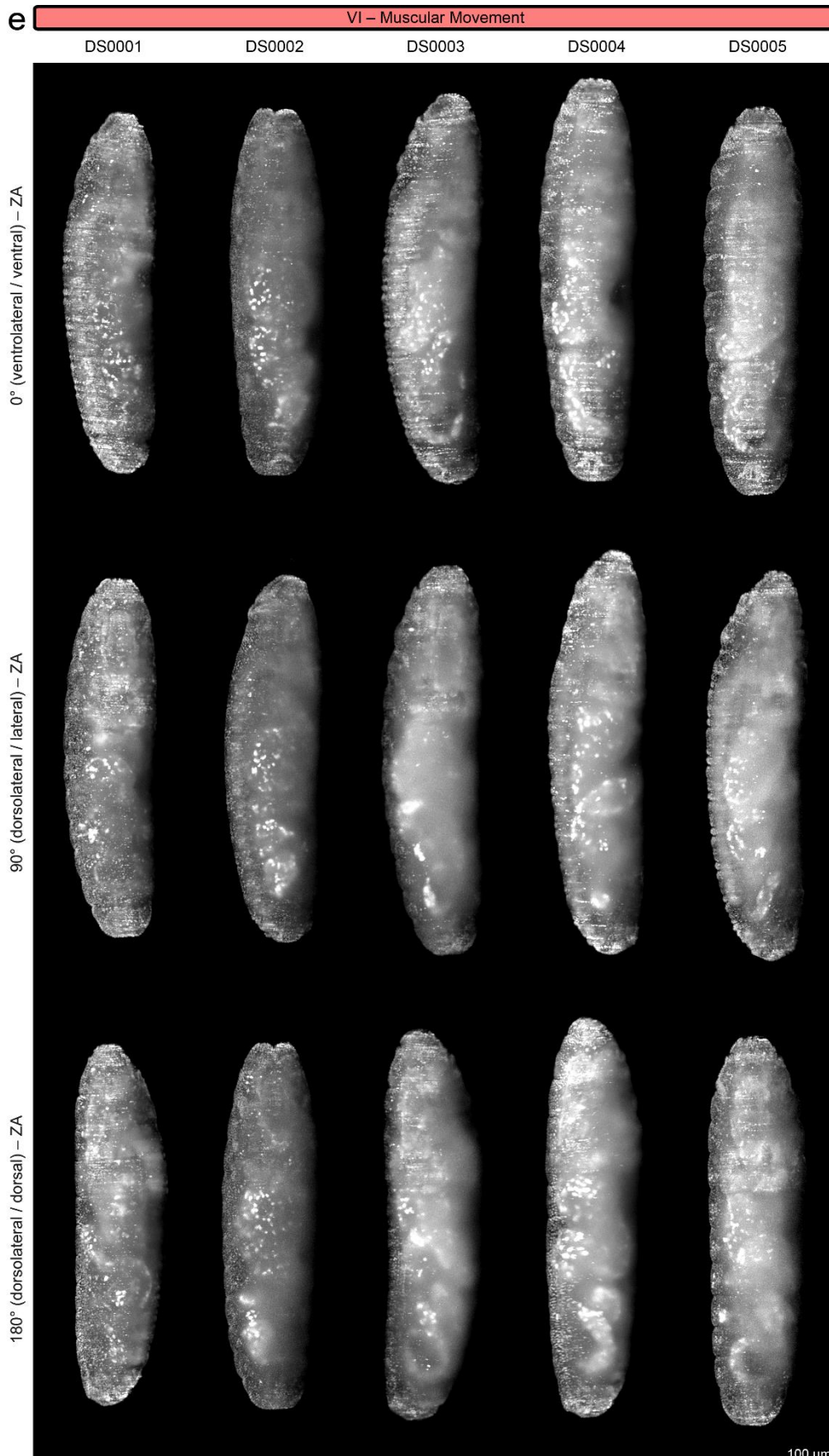
DS0005

0° (ventrolateral / ventral) – ZA

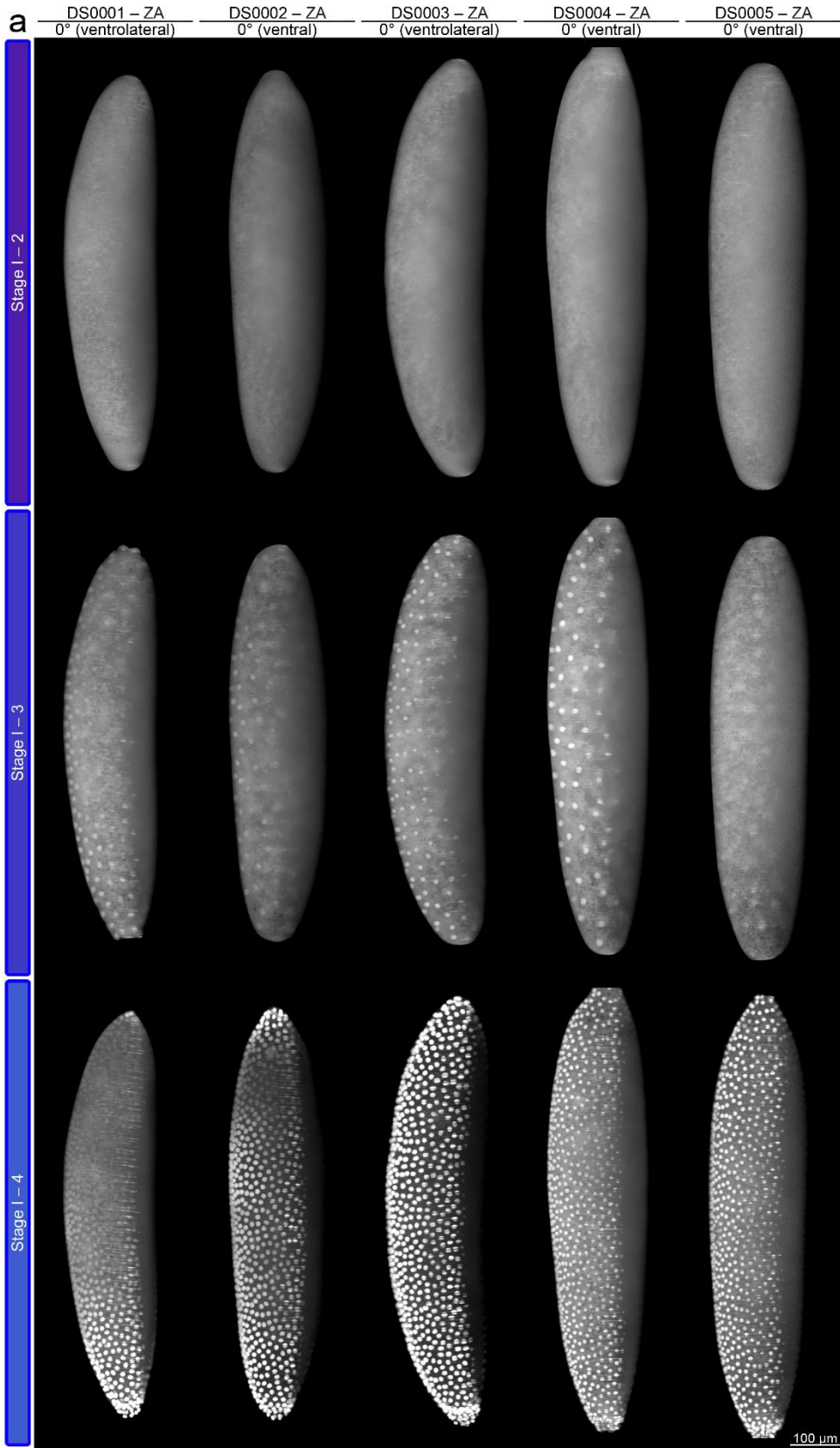
90° (dorsolateral / lateral) – ZA

180° (dorsolateral / dorsal) – ZA

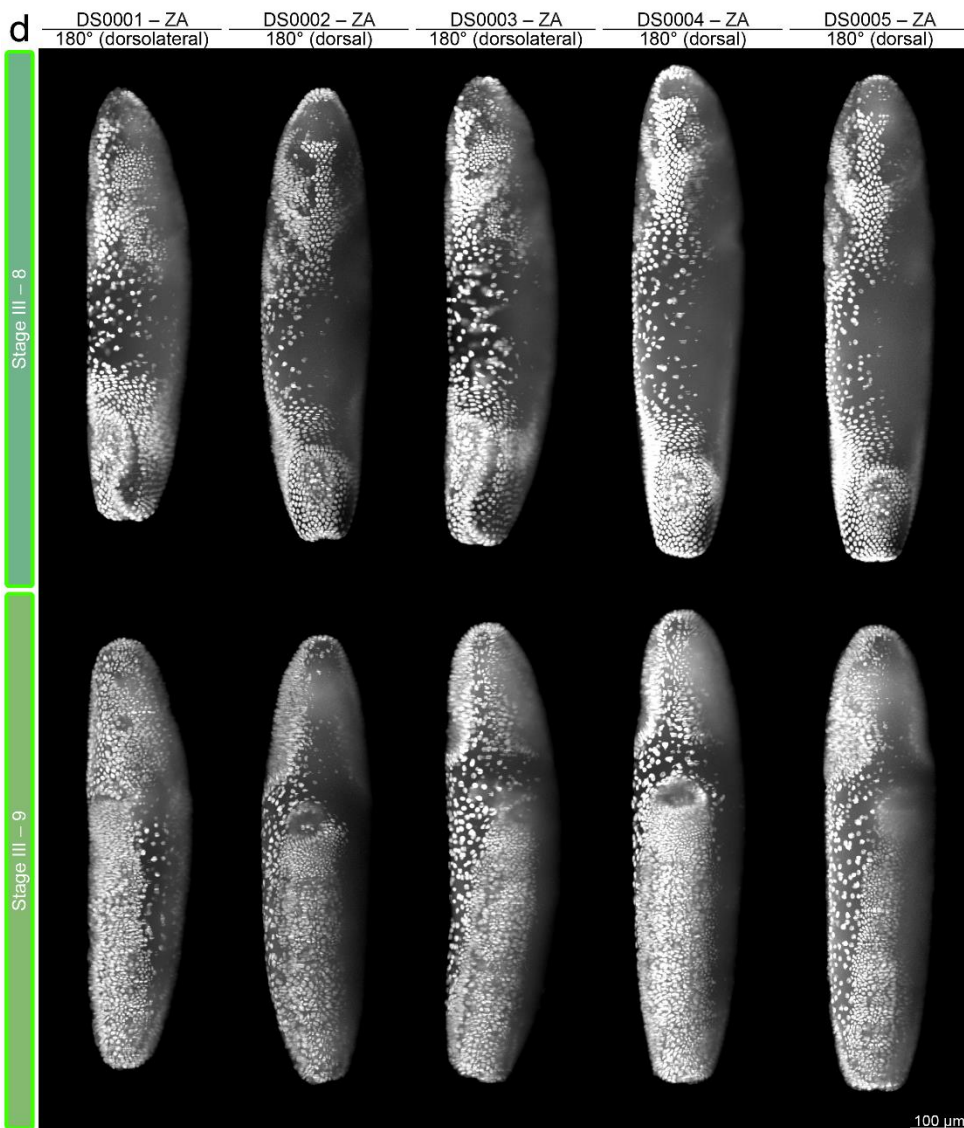
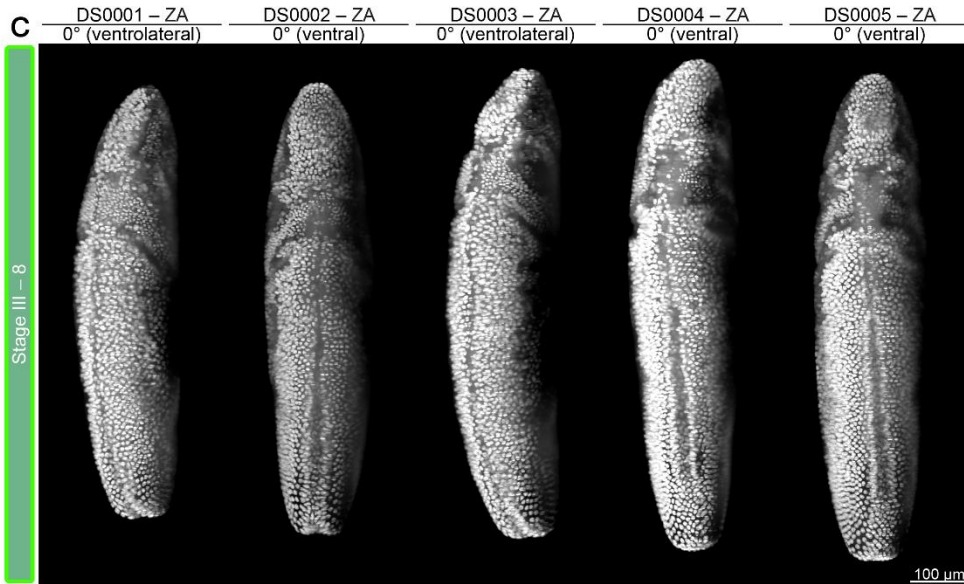




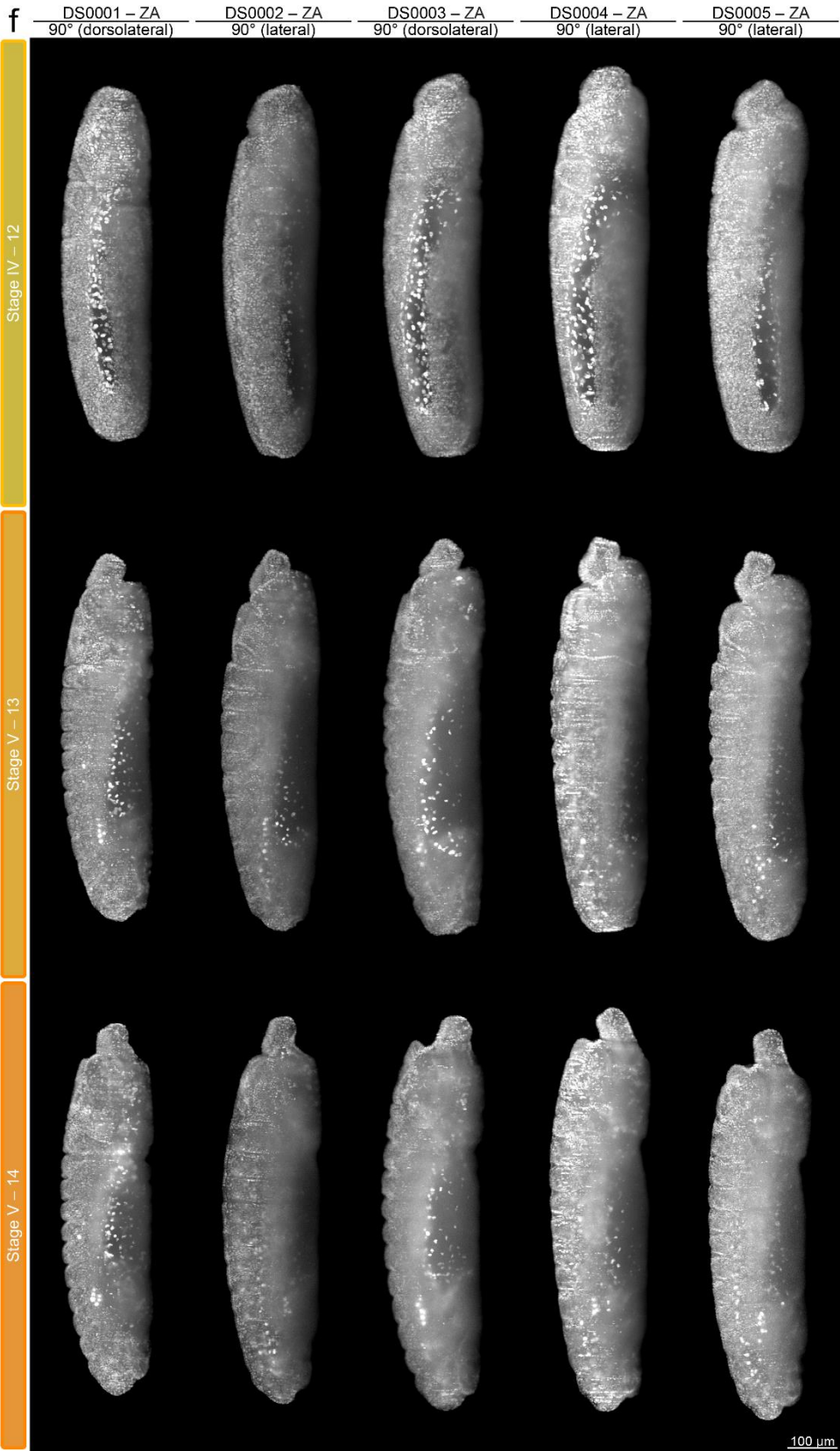
Supplementary Figure 3 – Comparison of datasets DS0001–DS0005 at the onset of the embryogenetic events (a) early gastrulation, (b) germband elongation, (c) germband retraction, (d) dorsal closure and (e) muscular movement along three directions (orientations 0°, 90° and 180°). Since recording started after the onset of blastoderm formation, respective image data is not available. Embryos from datasets DS0001 and DS0003 are depicted ventrolateral, dorsolateral and dorsolateral, whereas embryos from DS0002, DS0004 and DS0005 are depicted ventral, lateral and dorsal. ZA, Z maximum projection with image adjustment.

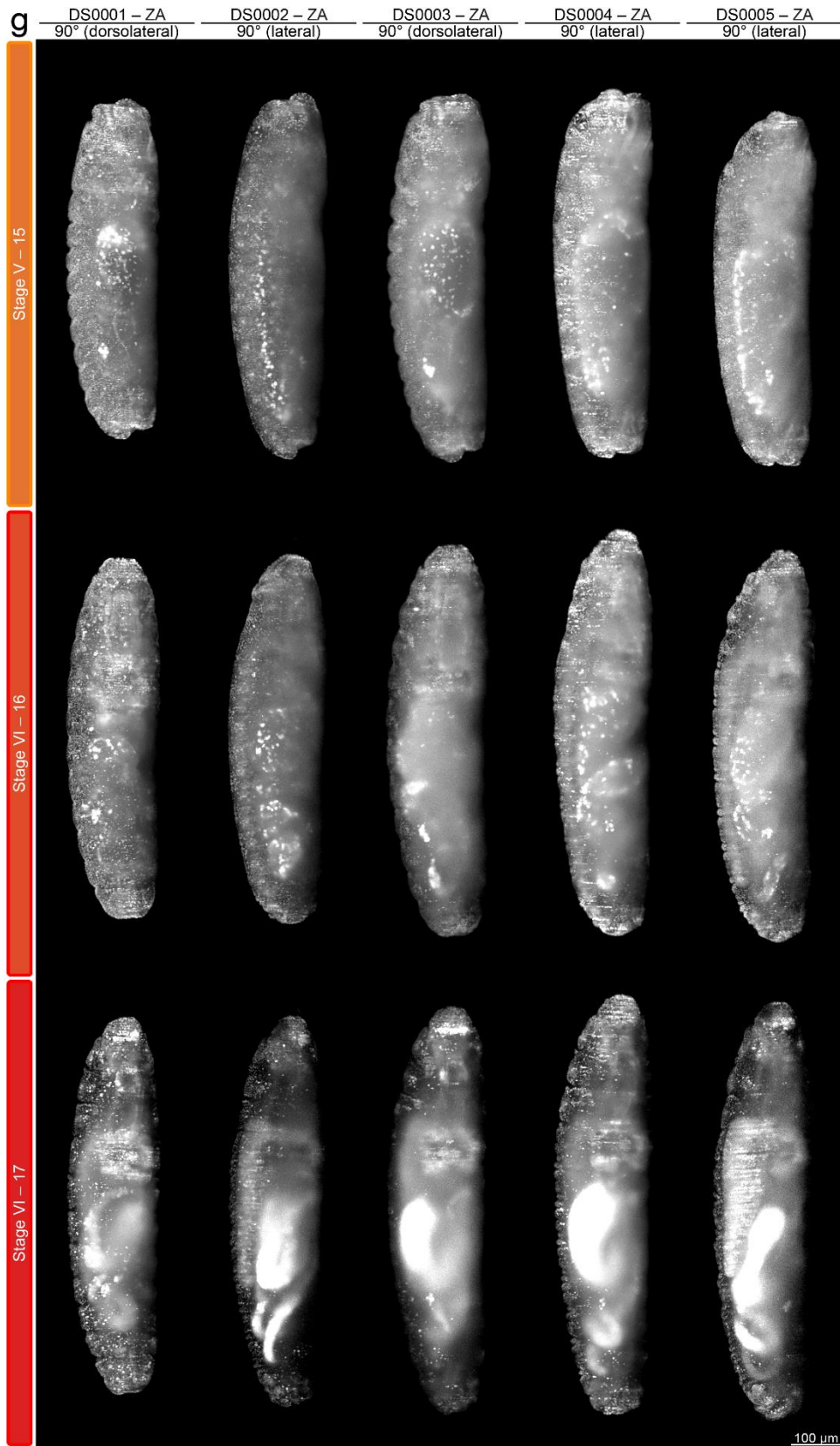












Supplementary Figure 4 – Comparison of datasets DS0001–DS0005 at the onset of the stages (a) I-2 to I-4, (b) I-5 to II-7, (c) III-8, (d) III-8 and III-9, (e) III-10 to IV-12, (f) IV-12 to V-14, (g) V-15 to VI-17. The shown direction changes throughout the figure (at stages III-8 and IV-12), so that characteristic structures for the respective stage can be seen properly. Since the recordings started after the onset of stage I-1, respective image data is not available. Embryos from datasets DS0001 and DS0003 are depicted either ventrolateral or dorsolateral, whereas embryos from DS0002, DS0004 and DS0005 are depicted either ventral, lateral, or dorsal. ZA, z maximum projection with image adjustment.

Supplementary Tables

Supplementary Table 1 – Metadata and access information for datasets DS0001–DS0009.

<i>Dataset (DS)</i>	DS0001	DS0002	DS0003
<i>Species</i>	Mediterranean fruit fly (medfly) <i>Ceratitis capitata</i> (Wiedemann) Arthropoda → Insecta → Diptera → Tephritidae		
<i>Line</i>	<i>TREhs43-hid^{Ala5}_F1m2⁵²</i>		
<i>Line Genotype</i>	single insertion / homozygous		
<i>Stock</i>	~50-80 adults, less than 4 weeks old		
<i>Stock Medium</i>	refined sugar (524973, REWE Markt GmbH, Köln, Germany) supplemented with 33% (wt/wt) inactive dry yeast (62-106, Flystuff, San Diego, CA, USA)		
<i>Stock Conditions</i>	12:00 h light / 12:00 h darkness at 25°C and 70% relative humidity (DR-36VL, Percival Scientific, Perry, IA, USA)		
<i>Embryo Collection</i>	cultures were given 10 min at room temperature (23±1°C) to lay embryos		
<i>Pre-Imaging Incubation</i>	embryo mounting procedure, approximately 00:30 h at room temperature (23±1°C), incubation in the sample chamber, approximately 01:30 h at room temperature (23±1°C) resulting in a total of 02:00 h incubation at room temperature (23±1°C)		
<i>LSFM Implementation</i>	sample chamber-based digital scanned laser light sheet fluorescence microscope (DSL ³)		
<i>Laser Lines</i>	488 nm / 60 mW Diode Laser (PhoxX 488-60, Omicron-Laserage Laserprodukte GmbH, Rodgau-Dudenhofen, Germany)		
<i>Excitation Objective</i>	2.5× NA 0.06 EC Epiplan-Neofluar objective (422320-9900-000, Carl Zeiss, Göttingen, Germany)		
<i>Emission Objective</i>	10× NA 0.3 W N-Achroplan objective (420947-9900-000, Carl Zeiss, Göttingen, Germany)		
<i>Emission Filters</i>	525/50 single-band bandpass filter (FF03-525/50-25, Semrock/AHF Analysentechnik AG, Tübingen, Germany)		
<i>Camera</i>	high-resolution CCD (Clara, Andor, Belfast, United Kingdom), 14 bit, 1040×1392 pixel (pitch 6.45 μm)		
<i>Dataset File Type</i>	TIFF, 16-bit grayscale (planes saved as z stacks in ZIP-compressed container files, indicated as PL(ZS))		
<i>Dechoriation</i>	90 s in a 1:9 mixture of ~10% (vol/vol) sodium hypochlorite (425044-250ML, Sigma Aldrich, Taufkirchen, Germany) and PBS pH 7.4 (10010-023, Gibco Life Technologies GmbH, Darmstadt, Germany)		
<i>Mounting Method</i>	cobweb holder mounting method (embryos are attached to a thin agarose film spanning a slotted hole) ⁵⁰		
<i>Mounting Agarose</i>	1% (wt/vol) low-melt agarose (6351.2, Carl Roth, Karlsruhe, Germany) in PBS pH 7.4 (10010-023, Gibco Life Technologies GmbH, Darmstadt, Germany)		
<i>Imaging Buffer</i>	PBS pH 7.4 (10010-023, Gibco Life Technologies GmbH, Darmstadt, Germany)		
<i>Imaging Temperature</i>	room temperature (23±1°C)	room temperature (23±1°C)	room temperature (23±1°C)
<i>Viewing Perspective</i>	ventrolateral, dorsolateral, dorsolateral, ventrolateral	ventral, lateral, dorsal, lateral	ventrolateral, dorsolateral, dorsolateral, ventrolateral
<i>Developmental Stages</i>	II-2 to VI-17	II-2 to VI-17	II-2 to VI-17
<i>Retrieval</i>	developed to healthy adult	developed to healthy adult	developed to healthy adult

(continued)

<i>Dataset (DS)</i>	DS0001	DS0002	DS0003
<i>Dataset Size</i>	31.1 Gigabyte	31.6 Gigabyte	31.9 Gigabyte
<i>Dataset Access</i>	DOI: 10.5281/zenodo.6448019	DOI: 10.5281/zenodo.6448433	DOI: 10.5281/zenodo.6450798
<i>Figures</i>	2 (first column)	2 (second column)	2 (third column)
<i>Supplementary Figures</i>	3 (first column), 4 (first column)	3 (second column), 4 (second column)	3 (third column), 4 (third column)
<i>Supplementary Videos</i>	1 (first column, first)	1 (first column, second row)	1 (first column third row)
<i>Comment</i>	median development time of datasets DS0001–DS0005	-	-
<i>Time Points (TP)</i>	121 (TP0001-TP0121)	126 (TP0001-TP0126)	122 (TP0001-TP0122)
<i>TP Interval</i>	30 min	30 min	30 min
<i>Total Time</i> ($(TP-1) \times TP$ Interval)	60:00 h	62:30 h	60:30 h
<i>Directions (DR)</i>	4 (DR0001-DR0004)	4 (DR0001-DR0004)	4 (DR0001-DR0004)
<i>DR Orientations</i>	0°, 90°, 180°, 270°	0°, 90°, 180°, 270°	0°, 90°, 180°, 270°
<i>Channels (CH)</i>	1 (CH0001)	1 (CH0001)	1 (CH0001)
<i>CH0001 Excitation</i>	488 nm	488 nm	488 nm
<i>CH0001 Power</i>	135 μ W (close to the embryo)	135 μ W (close to the embryo)	135 μ W (close to the embryo)
<i>CH0001 Exposure Time</i>	50 ms	50 ms	50 ms
<i>CH0001 Emission Filter</i>	525/50 single-band bandpass filter	525/50 single-band bandpass filter	525/50 single-band bandpass filter
<i>CH0001 Theoretical Lateral / Axial Resolution</i>	~850 nm / ~3,200 nm	~850 nm / ~3,200 nm	~850 nm / ~3,200 nm
<i>Planes (PL)</i>	100 (PL0001-PL0100)	100 (PL0001-PL0100)	100 (PL0001-PL0100)
<i>Z Spacing</i>	2.58 μ m	2.58 μ m	2.58 μ m
<i>Z Distance</i> ($PL \times Z$ Spacing)	258.0 μ m	258.0 μ m	258.0 μ m
<i>X-Dimensions (XD)</i>	500 pixels	500 pixels	500 pixels
<i>X Spacing</i>	0.645 μ m	0.645 μ m	0.645 μ m
<i>X Length</i> ($XD \times X$ Spacing)	322.5 μ m	322.5 μ m	322.5 μ m
<i>Y-Dimensions (YD)</i>	1390 pixels	1390 pixels	1390 pixels
<i>Y Spacing</i>	0.645 μ m	0.645 μ m	0.645 μ m
<i>Y Length</i> ($YD \times Y$ Spacing)	896.6 μ m	896.6 μ m	896.6 μ m

(continued)

<i>Dataset (DS)</i>	DS0004	DS0005	DS0006
<i>Species</i>	Mediterranean fruit fly (medfly) <i>Ceratitis capitata</i> (Wiedemann) Arthropoda → Insecta → Diptera → Tephritidae		
<i>Line</i>	<i>TREhs43-hid^{Ala5}_F1m2⁵²</i>		
<i>Line Genotype</i>	single insertion / homozygous		
<i>Stock</i>	~50-80 adults, less than 4 weeks old		
<i>Stock Medium</i>	refined sugar (524973, REWE Markt GmbH, Köln, Germany) supplemented with 33% (wt/wt) inactive dry yeast (62-106, Flystuff, San Diego, CA, USA)		
<i>Stock Conditions</i>	12:00 h light / 12:00 h darkness at 25°C and 70% relative humidity (DR-36VL, Percival Scientific, Perry, IA, USA)		
<i>Embryo Collection</i>	cultures were given 10 min at room temperature (23±1°C) to lay embryos		
<i>Pre-Imaging Incubation</i>	embryo mounting procedure, approximately 00:30 h at room temperature (23±1°C), incubation in the sample chamber, approximately 01:30 h at room temperature (23±1°C) resulting in a total of 02:00 h incubation at room temperature (23±1°C)		
<i>LSFM Implementation</i>	sample chamber-based digital scanned laser light sheet fluorescence microscope (DSLMS) ³		
<i>Laser Lines</i>	488 nm / 60 mW Diode Laser (PhoxX 488-60, Omicron-Laserage Laserprodukte GmbH, Rodgau-Dudenhofen, Germany)		
<i>Excitation Objective</i>	2.5× NA 0.06 EC Epiplan-Neofluar objective (422320-9900-000, Carl Zeiss, Göttingen, Germany)		
<i>Emission Objective</i>	10× NA 0.3 W N-Achroplan objective (420947-9900-000, Carl Zeiss, Göttingen, Germany)		
<i>Emission Filters</i>	525/50 single-band bandpass filter (FF03-525/50-25, Semrock/AHF Analysentechnik AG, Tübingen, Germany)		
<i>Camera</i>	high-resolution CCD (Clara, Andor, Belfast, United Kingdom), 14 bit, 1040×1392 pixel (pitch 6.45 μm)		
<i>Dataset File Type</i>	TIFF, 16-bit grayscale (planes saved as z stacks in ZIP-compressed container files, indicated as PL(ZS))		
<i>Dechoriation</i>	90 s in a 1:9 mixture of ~10% (vol/vol) sodium hypochlorite (425044-250ML, Sigma Aldrich, Taufkirchen, Germany) and PBS pH 7.4 (10010-023, Gibco Life Technologies GmbH, Darmstadt, Germany)		
<i>Mounting Method</i>	cobweb holder mounting method (embryos are attached to a thin agarose film spanning a slotted hole) ⁵⁰		
<i>Mounting Agarose</i>	1% (wt/vol) low-melt agarose (6351.2, Carl Roth, Karlsruhe, Germany) in PBS pH 7.4 (10010-023, Gibco Life Technologies GmbH, Darmstadt, Germany)		
<i>Imaging Buffer</i>	PBS pH 7.4 (10010-023, Gibco Life Technologies GmbH, Darmstadt, Germany)		
<i>Imaging Temperature</i>	room temperature (23±1°C)	room temperature (23±1°C)	room temperature (23±1°C)
<i>Viewing Perspective</i>	ventral, lateral, dorsal, lateral	ventral, lateral, dorsal, lateral	ventrolateral, dorsolateral, dorsolateral, ventrolateral
<i>Developmental Stages</i>	II-2 to VI-17	II-2 to VI-17	II-2 to VI-17
<i>Retrieval</i>	developed to healthy adult	developed to healthy adult	did not eclose from the pupa

(continued)

<i>Dataset (DS)</i>	DS0004	DS0005	DS0006
<i>Dataset Size</i>	31.5 Gigabyte	28.9 Gigabyte	28.1 Gigabyte
<i>Dataset Access</i>	DOI: 10.5281/zenodo.6451099	DOI: 10.5281/zenodo.6453980	DOI: 10.5281/zenodo.6455038
<i>Figures</i>	2 (fourth column)	2 (fifth column)	-
<i>Supplementary Figures</i>	3 (fourth column), 4 (fourth column)	3 (fifth column), 4 (fifth column)	-
<i>Supplementary Videos</i>	1 (second column, first row)	1 (second column, second row)	1 (second column, third row)
<i>Comment</i>	slightly larger than the volume of view along y	slightly larger than the volume of view along y	excluded from analysis
<i>Time Points (TP)</i>	116 (TP0001-TP0116)	115 (TP0001-TP0115)	113 (TP0001-TP0113)
<i>TP Interval</i>	30 min	30 min	30 min
<i>Total Time</i> ($(TP-1) \times TP$ Interval)	57:30 h	57:00 h	56:00 h
<i>Directions (DR)</i>	4 (DR0001-DR0004)	4 (DR0001-DR0004)	4 (DR0001-DR0004)
<i>DR Orientations</i>	0°, 90°, 180°, 270°	0°, 90°, 180°, 270°	0°, 90°, 180°, 270°
<i>Channels (CH)</i>	1 (CH0001)	1 (CH0001)	1 (CH0001)
<i>CH0001 Excitation</i>	488 nm	488 nm	488 nm
<i>CH0001 Power</i>	135 μ W (close to the embryo)	135 μ W (close to the embryo)	135 μ W (close to the embryo)
<i>CH0001 Exposure Time</i>	50 ms	50 ms	50 ms
<i>CH0001 Emission Filter</i>	525/50 single-band bandpass filter	525/50 single-band bandpass filter	525/50 single-band bandpass filter
<i>CH0001 Theoretical Lateral / Axial Resolution</i>	~850 nm / ~3,200 nm	~850 nm / ~3,200 nm	~850 nm / ~3,200 nm
<i>Planes (PL)</i>	100 (PL0001-PL0100)	100 (PL0001-PL0100)	100 (PL0001-PL0100)
<i>Z Spacing</i>	2.58 μ m	2.58 μ m	2.58 μ m
<i>Z Distance</i> ($PL \times Z$ Spacing)	258.0 μ m	258.0 μ m	258.0 μ m
<i>X-Dimensions (XD)</i>	500 pixels	500 pixels	500 pixels
<i>X Spacing</i>	0.645 μ m	0.645 μ m	0.645 μ m
<i>X Length</i> ($XD \times X$ Spacing)	322.5 μ m	322.5 μ m	322.5 μ m
<i>Y-Dimensions (YD)</i>	1390 pixels	1390 pixels	1390 pixels
<i>Y Spacing</i>	0.645 μ m	0.645 μ m	0.645 μ m
<i>Y Length</i> ($YD \times Y$ Spacing)	896.6 μ m	896.6 μ m	896.6 μ m

(continued)

<i>Dataset (DS)</i>	DS0007	DS0008	DS0009
<i>Species</i>	Mediterranean fruit fly (medfly) <i>Ceratitis capitata</i> (Wiedemann) Arthropoda → Insecta → Diptera → Tephritidae		
<i>Line</i>	<i>TREhs43-hid^{Ala5}_F1m2⁵²</i>		
<i>Line Genotype</i>	single insertion / homozygous		
<i>Stock</i>	~50-80 adults, less than 4 weeks old		
<i>Stock Medium</i>	refined sugar (524973, REWE Markt GmbH, Köln, Germany) supplemented with 33% (wt/wt) inactive dry yeast (62-106, Flystuff, San Diego, CA, USA)		
<i>Stock Conditions</i>	12:00 h light / 12:00 h darkness at 25°C and 70% relative humidity (DR-36VL, Percival Scientific, Perry, IA, USA)		
<i>Embryo Collection</i>	cultures were given 10 min at room temperature (23±1°C) to lay embryos		
<i>Pre-Imaging Incubation</i>	embryo mounting procedure, approximately 00:30 h at room temperature (23±1°C), incubation in the sample chamber, approximately 01:30 h at room temperature (23±1°C) resulting in a total of 02:00 h incubation at room temperature (23±1°C)		
<i>LSFM Implementation</i>	sample chamber-based digital scanned laser light sheet fluorescence microscope (DSLMS) ³		
<i>Laser Lines</i>	488 nm / 60 mW Diode Laser (PhoxX 488-60, Omicron-Laserage Laserprodukte GmbH, Rodgau-Dudenhofen, Germany)		
<i>Excitation Objective</i>	2.5× NA 0.06 EC Epiplan-Neofluar objective (422320-9900-000, Carl Zeiss, Göttingen, Germany)		
<i>Emission Objective</i>	20× NA 0.5 W N-Achroplan objective (420957-9900-000, Carl Zeiss, Göttingen, Germany)		
<i>Emission Filters</i>	525/50 single-band bandpass filter (FF03-525/50-25, Semrock/AHF Analysentechnik AG, Tübingen, Germany)		
<i>Camera</i>	high-resolution CCD (Clara, Andor, Belfast, United Kingdom), 14 bit, 1040×1392 pixel (pitch 6.45 μm)		
<i>Dataset File Type</i>	TIFF, 16-bit grayscale (planes saved as z stacks in ZIP-compressed container files, indicated as PL(ZS))		
<i>Dechoriation</i>	90 s in a 1:9 mixture of ~10% (vol/vol) sodium hypochlorite (425044-250ML, Sigma Aldrich, Taufkirchen, Germany) and PBS pH 7.4 (10010-023, Gibco Life Technologies GmbH, Darmstadt, Germany)		
<i>Mounting Method</i>	cobweb holder mounting method (embryos are attached to a thin agarose film spanning a slotted hole) ⁵⁰		
<i>Mounting Agarose</i>	1% (wt/vol) low-melt agarose (6351.2, Carl Roth, Karlsruhe, Germany) in PBS pH 7.4 (10010-023, Gibco Life Technologies GmbH, Darmstadt, Germany)		
<i>Imaging Buffer</i>	PBS pH 7.4 (10010-023, Gibco Life Technologies GmbH, Darmstadt, Germany)		
<i>Imaging Temperature</i>	room temperature (23±1°C)	room temperature (23±1°C)	room temperature (23±1°C)
<i>Viewing Perspective</i>	ventral, lateral, dorsal, lateral	ventral, lateral, dorsal, lateral	anterior, lateral
<i>Developmental Stages</i>	II-2 to IV-12	II-2 to VI-17	II-2 to VI-16
<i>Retrieval</i>	developed to healthy adult	developed to healthy adult	developed to healthy adult

(continued)

Dataset (DS)	DS0007	DS0008	DS0009
<i>Dataset Size</i>	19.4 Gigabyte (TIFF)	41.7 Gigabyte (TIFF)	11.8 Gigabyte (TIFF)
<i>Dataset Access</i>	DOI: 10.5281/zenodo.6456820	DOI: 10.5281/zenodo.6457004	DOI: 10.5281/zenodo.6457894
<i>Figures</i>	-	-	-
<i>Supplementary Figures</i>	-	-	-
<i>Supplementary Videos</i>	-	-	-
<i>Comment</i>	embryo not captured <i>in toto</i> , covers germ cell formation	embryo not captured <i>in toto</i> , covers head involution	embryo not captured <i>in toto</i> , agarose hemisphere mounting
Time Points (TP)	49 (TP0001-TP0049)	123 (TP0001-TP0123)	93 (TP0001-TP0093)
<i>TP Interval</i>	30 min	30 min	30 min
<i>Total Time</i> <i>((TP-1)×TP Interval)</i>	24:00 h	61:00 h	46:00 h
Directions (DR)	4 (DR0001-DR0004)	4 (DR0001-DR0004)	2 (DR0001-DR0002)
<i>DR Orientations</i>	0°, 90°, 180°, 270°	0°, 90°, 180°, 270°	0°, 90°
Channels (CH)	1 (CH0001)	1 (CH0001)	1 (CH0001)
<i>CH0001 Excitation</i>	488 nm	488 nm	488 nm
<i>CH0001 Power</i>	135 μW (close to the embryo)	135 μW (close to the embryo)	135 μW (close to the embryo)
<i>CH0001 Exposure Time</i>	50 ms	50 ms	50 ms
<i>CH0001 Emission Filter</i>	525/50 single-band bandpass filter	525/50 single-band bandpass filter	525/50 single-band bandpass filter
<i>CH0001 Theoretical Lateral / Axial Resolution</i>	~510 nm / ~2,300 nm	~510 nm / ~2,300 nm	~510 nm / ~2,300 nm
Planes (PL)	100 (PL0001-PL0100)	100 (PL0001-PL0100)	DR0001: 115 (PL0001-PL0115), DR0002: 100 (PL0001-PL0100)
<i>Z Spacing</i>	2.58 μm	2.58 μm	2.58 μm
<i>Z Distance</i> <i>(PL×Z Spacing)</i>	258.0 μm	258.0 μm	DR0001: 296.7 μm, DR0002: 258.0 μm
X-Dimensions (XD)	720 pixels	720 pixels	DR0001: 720 pixels, DR0002: 1000 pixels
<i>X Spacing</i>	0.3225 μm	0.3225 μm	0.3225 μm
<i>X Length</i> <i>(XD×X Spacing)</i>	232.2 μm	232.2 μm	DR0001: 232.2 μm, DR0002: 322.5 μm
Y-Dimensions (YD)	1300 pixels	1300 pixels	720 pixels
<i>Y Spacing</i>	0.3225 μm	0.3225 μm	0.3225 μm
<i>Y Length</i> <i>(YD×Y Spacing)</i>	419.25 μm	419.25 μm	232.2 μm

Supplementary Table 2 – Embryogenetic events onset time points for DS0001–DS0005. Embryogenetic events are color-coded (see Methods section). Since imaging started approximately 2 h after embryo collection, the first time point (TP0001) was set to 02:00 h of absolute development. I, blastoderm formation; II, early gastrulation; III, germband elongation; IV, germband retraction; V, dorsal closure; VI, muscular movement. TP, imaging time point; time, absolute time passed from the onset of I until the indicated TP; rel dev, relative progress of embryonic development from the onset of I until the indicated TP.

Event	DS0001 (median)			DS0002 (longer)			DS0003 (longer)		
	TP	time	rel dev	TP	time	rel dev	TP	time	rel dev
I	-	00:00 h	0.0%	-	00:00 h	0.0%	-	00:00 h	0.0%
II	0020	11:30 h	18.4%	0022	12:30 h	19.2%	0021	12:00 h	19.0%
III	0026	14:30 h	23.2%	0027	15:00 h	23.1%	0026	14:30 h	23.0%
IV	0046	24:30 h	39.2%	0048	25:30 h	39.2%	0046	24:30 h	38.9%
V	0061	32:00 h	51.2%	0062	32:30 h	50.0%	0059	31:00 h	49.2%
VI	0087	45:00 h	72.0%	0084	43:30 h	66.9%	0086	44:30 h	70.6%
Hatch	0122	62:30 h	100.0%	0127	65:00 h	100.0%	0123	63:00 h	100.0%

(continued)

Event	DS0004 (shorter)			DS0005 (shorter)			Standard Deviations	
	TP	time	rel dev	TP	time	rel dev	time	rel dev
I	-	00:00 h	0.0%	-	00:00 h	0.0%	±00:00 h	±0.0%
II	0019	11:00 h	18.3%	0019	11:00 h	18.5%	±00:39 h	±0.4%
III	0024	13:30 h	22.5%	0024	13:30 h	22.7%	±00:40 h	±0.3%
IV	0044	23:30 h	39.2%	0044	23:30 h	39.5%	±00:50 h	±0.2%
V	0053	28:00 h	46.7%	0055	29:00 h	48.7%	±01:56 h	±1.7%
VI	0079	41:00 h	68.3%	0082	42:30 h	71.4%	±01:36 h	±2.2%
Hatch	0117	60:00 h	100.0%	0116	59:30 h	100.0%	±02:15 h	±0.0%

Supplementary Table 3 – Stage onset time points for DS0001–DS0005. Onset time points of embryogenetic events are color-coded (see Methods section). Since imaging started approximately 2 h after embryo collection, the first time point (TP0001) was set to 02:00 h of absolute development. TP, imaging time point; time, absolute time passed from the onset of I-1 until the indicated TP; rel dev, relative progress of embryonic development from the onset of I-1 until the indicated TP.

Stage	DS0001 (median)			DS0002 (longer)			DS0003 (longer)		
	TP	time	rel dev	TP	time	rel dev	TP	time	rel dev
I-1	-	00:00 h	0.0%	-	00:00 h	0.0%	-	00:00 h	0.0%
I-2	0001	02:00 h	3.2%	0001	02:00 h	3.1%	0001	02:00 h	3.2%
I-3	0004	03:30 h	5.6%	0004	03:30 h	5.4%	0004	03:30 h	5.6%
I-4	0006	04:30 h	7.2%	0007	05:00 h	7.7%	0006	04:30 h	7.1%
I-5	0010	06:30 h	10.4%	0011	07:00 h	10.8%	0010	06:30 h	10.3%
II-6	0020	11:30 h	18.4%	0022	12:30 h	19.2%	0021	12:00 h	19.0%
II-7	0024	13:30 h	21.6%	0026	14:30 h	22.3%	0025	14:00 h	22.2%
III-8	0026	14:30 h	23.2%	0027	15:00 h	23.1%	0026	14:30 h	23.0%
III-9	0030	16:30 h	26.4%	0031	17:00 h	26.2%	0030	16:30 h	26.2%
III-10	0033	18:00 h	28.8%	0032	17:30 h	26.9%	0032	17:30 h	27.8%
III-11	0041	22:00 h	35.2%	0043	23:00 h	35.4%	0038	20:30 h	32.5%
IV-12	0046	24:30 h	39.2%	0048	25:30 h	39.2%	0046	24:30 h	38.9%
V-13	0061	32:00 h	51.2%	0062	32:30 h	50.0%	0059	31:00 h	49.2%
V-14	0066	34:30 h	55.2%	0068	35:30 h	54.6%	0066	34:30 h	54.8%
V-15	0073	38:00 h	60.8%	0074	38:30 h	59.2%	0071	37:00 h	58.7%
VI-16	0087	45:00 h	72.0%	0084	43:30 h	66.9%	0086	44:30 h	70.6%
VI-17	0106	54:30 h	87.2%	0110	56:30 h	86.9%	0104	53:30 h	84.9%
Hatch	0122	62:30 h	100.0%	0127	65:00 h	100.0%	0123	63:00 h	100.0%

(continued)

Stage	DS0004 (shorter)			DS0005 (shorter)			Standard Deviations	
	TP	time	rel dev	TP	time	rel dev	time	rel dev
I-1	-	00:00 h	0.0%	-	00:00 h	0.0%	±00:00 h	±0.0%
I-2	0001	02:00 h	3.3%	0001	02:00 h	3.4%	±00:00 h	±0.1%
I-3	0004	03:30 h	5.8%	0004	03:30 h	5.9%	±00:00 h	±0.2%
I-4	0006	04:30 h	7.5%	0006	04:30 h	7.6%	±00:13 h	±0.2%
I-5	0010	06:30 h	10.8%	0010	06:30 h	10.9%	±00:13 h	±0.3%
II-6	0019	11:00 h	18.3%	0019	11:00 h	18.5%	±00:39 h	±0.4%
II-7	0023	13:00 h	21.7%	0023	13:00 h	21.8%	±00:39 h	±0.3%
III-8	0024	13:30 h	22.5%	0024	13:30 h	22.7%	±00:40 h	±0.3%
III-9	0029	16:00 h	26.7%	0029	16:00 h	26.9%	±00:25 h	±0.3%
III-10	0031	17:00 h	28.3%	0031	17:00 h	28.6%	±00:25 h	±0.8%
III-11	0036	19:30 h	32.5%	0038	20:30 h	34.5%	±01:23 h	±1.4%
IV-12	0044	23:30 h	39.2%	0044	23:30 h	39.5%	±00:50 h	±0.2%
V-13	0053	28:00 h	46.7%	0055	29:00 h	48.7%	±01:56 h	±1.7%
V-14	0062	32:30 h	54.2%	0062	32:30 h	54.6%	±01:20 h	±0.4%
V-15	0069	36:00 h	60.0%	0071	37:00 h	62.2%	±00:58 h	±1.4%
VI-16	0079	41:00 h	68.3%	0082	42:30 h	71.4%	±01:36 h	±2.2%
VI-17	0097	50:00 h	83.3%	0101	52:00 h	87.4%	±02:27 h	±1.8%
Hatch	0117	60:00 h	100.0%	0116	59:30 h	100.0%	±02:15 h	±0.0%

Supplementary Table 4 – Time point-based alignment of DS0002–DS0005 to DS0001. Onset time points of embryogenetic events are color-coded (see Methods section). Since imaging started approximately 2 h after embryo collection, the first time point (TP0001) was set to 02:00 h of absolute development. Imaging typically begins with stage I-2, the values for I-1 are extrapolated. TP, imaging time point; time, absolute time passed from the onset of I-1 until the indicated TP; rel dev, relative progress of embryonic development from the onset of I-1 until the indicated TP.

Stage	TP	DS0001 (median)		DS0002 (aligned)		DS0003 (aligned)		DS0004 (aligned)		DS0005 (aligned)		Standard Deviation	
		time	rel dev	time	rel dev	time	rel dev	time	rel dev	time	rel dev	time	rel dev
I-1	-	00:00 h	0.0%	00:00 h	0.0%	00:00 h	0.0%	00:00 h	0.0%	00:00 h	0.0%	±0:00 h	±0.0%
	-	00:30 h	0.8%	00:30 h	0.8%	00:30 h	0.8%	00:30 h	0.8%	00:30 h	0.8%	±0:00 h	±0.0%
	-	01:00 h	1.6%	01:00 h	1.5%	01:00 h	1.6%	01:00 h	1.7%	01:00 h	1.7%	±0:00 h	±0.1%
	-	01:30 h	2.4%	01:30 h	2.3%	01:30 h	2.4%	01:30 h	2.5%	01:30 h	2.5%	±0:00 h	±0.1%
I-2	0001	02:00 h	3.2%	02:00 h	3.1%	02:00 h	3.2%	02:00 h	3.3%	02:00 h	3.4%	±0:00 h	±0.1%
	0002	02:30 h	4.0%	02:30 h	3.8%	02:30 h	4.0%	02:30 h	4.2%	02:30 h	4.2%	±0:00 h	±0.1%
	0003	03:00 h	4.8%	03:00 h	4.6%	03:00 h	4.8%	03:00 h	5.0%	03:00 h	5.0%	±0:00 h	±0.2%
I-3	0004	03:30 h	5.6%	03:30 h	5.4%	03:30 h	5.6%	03:30 h	5.8%	03:30 h	5.9%	±0:00 h	±0.2%
	0005	04:00 h	6.4%	04:30 h	6.9%	04:00 h	6.3%	04:00 h	6.7%	04:00 h	6.7%	±0:13 h	±0.2%
I-4	0007	04:30 h	7.2%	05:00 h	7.7%	04:30 h	7.1%	04:30 h	7.5%	04:30 h	7.6%	±0:13 h	±0.2%
	0008	05:00 h	8.0%	05:30 h	8.5%	05:00 h	7.9%	05:00 h	8.3%	05:00 h	8.4%	±0:13 h	±0.2%
	0009	05:30 h	8.8%	06:00 h	9.2%	05:30 h	8.7%	05:30 h	9.2%	05:30 h	9.2%	±0:13 h	±0.2%
	0010	06:00 h	9.6%	06:30 h	10.0%	06:00 h	9.5%	06:00 h	10.0%	06:00 h	10.1%	±0:13 h	±0.3%
I-5	0011	06:30 h	10.4%	07:00 h	10.8%	06:30 h	10.3%	06:30 h	10.8%	06:30 h	10.9%	±0:13 h	±0.3%
	0012	07:00 h	11.2%	07:33 h	11.6%	07:03 h	11.2%	06:56 h	11.6%	06:56 h	11.7%	±0:15 h	±0.2%
	0013	07:30 h	12.0%	08:06 h	12.5%	07:36 h	12.1%	07:23 h	12.3%	07:23 h	12.4%	±0:18 h	±0.2%
	0014	08:00 h	12.8%	08:40 h	13.3%	08:10 h	13.0%	07:50 h	13.1%	07:50 h	13.2%	±0:20 h	±0.2%
	0015	08:30 h	13.6%	09:13 h	14.2%	08:43 h	13.8%	08:16 h	13.8%	08:16 h	13.9%	±0:23 h	±0.2%
	0016	09:00 h	14.4%	09:46 h	15.0%	09:16 h	14.7%	08:43 h	14.5%	08:43 h	14.7%	±0:26 h	±0.2%
	0017	09:30 h	15.2%	10:20 h	15.9%	09:50 h	15.6%	09:10 h	15.3%	09:10 h	15.4%	±0:29 h	±0.3%
	0018	10:00 h	16.0%	10:53 h	16.8%	10:23 h	16.5%	09:36 h	16.0%	09:36 h	16.2%	±0:32 h	±0.3%
	0019	10:30 h	16.8%	11:26 h	17.6%	10:56 h	17.4%	10:03 h	16.8%	10:03 h	16.9%	±0:35 h	±0.4%
	0020	11:00 h	17.6%	12:00 h	18.5%	11:30 h	18.3%	10:30 h	17.5%	10:30 h	17.6%	±0:39 h	±0.4%
II-6	0021	11:30 h	18.4%	12:30 h	19.2%	12:00 h	19.0%	11:00 h	18.3%	11:00 h	18.5%	±0:39 h	±0.4%
	0022	12:00 h	19.2%	13:00 h	20.0%	12:30 h	19.8%	11:30 h	19.2%	11:30 h	19.3%	±0:39 h	±0.4%
	0023	12:30 h	20.0%	13:30 h	20.8%	13:00 h	20.6%	12:00 h	20.0%	12:00 h	20.2%	±0:39 h	±0.4%
	0024	13:00 h	20.8%	14:00 h	21.5%	13:30 h	21.4%	12:30 h	20.8%	12:30 h	21.0%	±0:39 h	±0.3%
II-7	0024	13:30 h	21.6%	14:30 h	22.3%	14:00 h	22.2%	13:00 h	21.7%	13:00 h	21.8%	±0:39 h	±0.3%
	0025	14:00 h	22.4%	14:30 h	22.3%	14:00 h	22.2%	13:00 h	21.7%	13:00 h	21.8%	±0:40 h	±0.3%
III-8	0026	14:30 h	23.2%	15:00 h	23.1%	14:30 h	23.0%	13:30 h	22.5%	13:30 h	22.7%	±0:40 h	±0.3%
	0027	15:00 h	24.0%	15:30 h	23.8%	15:00 h	23.8%	14:10 h	23.6%	14:10 h	23.8%	±0:35 h	±0.1%
	0028	15:30 h	24.8%	16:00 h	24.6%	15:30 h	24.6%	14:50 h	24.7%	14:50 h	24.9%	±0:30 h	±0.1%
	0029	16:00 h	25.6%	16:30 h	25.4%	16:00 h	25.4%	15:30 h	25.8%	15:30 h	26.1%	±0:25 h	±0.3%
III-9	0030	16:30 h	26.4%	17:00 h	26.2%	16:30 h	26.2%	16:00 h	26.7%	16:00 h	26.9%	±0:25 h	±0.3%
	0031	17:00 h	27.2%	17:00 h	26.2%	16:45 h	26.6%	16:15 h	27.1%	16:15 h	27.3%	±0:22 h	±0.5%
	0032	17:30 h	28.0%	17:00 h	26.2%	17:00 h	27.0%	16:30 h	27.5%	16:30 h	27.7%	±0:25 h	±0.7%
III-10	0033	18:00 h	28.8%	17:30 h	26.9%	17:30 h	27.8%	17:00 h	28.3%	17:00 h	28.6%	±0:25 h	±0.8%
	0034	18:30 h	29.6%	18:12 h	28.0%	17:51 h	28.3%	17:17 h	28.8%	17:25 h	29.3%	±0:30 h	±0.7%
	0035	19:00 h	30.4%	18:55 h	29.1%	18:12 h	28.9%	17:34 h	29.3%	17:51 h	30.0%	±0:38 h	±0.6%
	0036	19:30 h	31.2%	19:38 h	30.2%	18:34 h	29.5%	17:51 h	29.8%	18:17 h	30.7%	±0:46 h	±0.7%
	0037	20:00 h	32.0%	20:21 h	31.3%	18:55 h	30.0%	18:08 h	30.2%	18:42 h	31.5%	±0:55 h	±0.8%
	0038	20:30 h	32.8%	21:04 h	32.4%	19:17 h	30.6%	18:25 h	30.7%	19:08 h	32.2%	±0:04 h	±1.0%
	0039	21:00 h	33.6%	21:47 h	33.5%	19:38 h	31.2%	18:42 h	31.2%	19:34 h	32.9%	±0:13 h	±1.2%
	0040	21:30 h	34.4%	22:30 h	34.6%	20:00 h	31.7%	19:00 h	31.7%	20:00 h	33.6%	±0:12 h	±1.4%
III-11	0041	22:00 h	35.2%	23:00 h	35.4%	20:30 h	32.5%	19:30 h	32.5%	20:30 h	34.5%	±0:12 h	±1.4%
	0042	22:30 h	36.0%	23:30 h	36.2%	21:22 h	33.9%	20:22 h	34.0%	21:07 h	35.5%	±0:13 h	±1.1%
	0043	23:00 h	36.8%	24:00 h	36.9%	22:15 h	35.3%	21:15 h	35.4%	21:45 h	36.6%	±0:04 h	±0.8%
	0044	23:30 h	37.6%	24:30 h	37.7%	23:07 h	36.7%	22:07 h	36.9%	22:22 h	37.6%	±0:56 h	±0.5%
	0045	24:00 h	38.4%	25:00 h	38.5%	24:00 h	38.1%	23:00 h	38.3%	23:00 h	38.7%	±0:50 h	±0.2%
IV-12	0046	24:30 h	39.2%	25:30 h	39.2%	24:30 h	38.9%	23:30 h	39.2%	23:30 h	39.5%	±0:50 h	±0.2%
	0047	25:00 h	40.0%	25:57 h	39.9%	24:55 h	39.6%	23:47 h	39.6%	23:51 h	40.1%	±0:54 h	±0.2%
	0048	25:30 h	40.8%	26:25 h	40.7%	25:21 h	40.2%	24:04 h	40.1%	24:12 h	40.7%	±0:58 h	±0.3%
	0049	26:00 h	41.6%	26:53 h	41.4%	25:47 h	40.9%	24:21 h	40.6%	24:34 h	41.3%	±0:03 h	±0.4%
	0050	26:30 h	42.4%	27:21 h	42.1%	26:12 h	41.6%	24:38 h	41.1%	24:55 h	41.9%	±0:07 h	±0.5%
	0051	27:00 h	43.2%	27:49 h	42.8%	26:38 h	42.3%	24:55 h	41.5%	25:17 h	42.5%	±0:12 h	±0.6%
	0052	27:30 h	44.0%	28:17 h	43.5%	27:04 h	43.0%	25:12 h	42.0%	25:38 h	43.1%	±0:17 h	±0.7%
	0053	28:00 h	44.8%	28:45 h	44.2%	27:30 h	43.7%	25:30 h	42.5%	26:00 h	43.7%	±0:21 h	±0.9%
	0054	28:30 h	45.6%	29:12 h	44.9%	27:55 h	44.3%	25:47 h	43.0%	26:21 h	44.3%	±0:26 h	±1.0%
	0055	29:00 h	46.4%	29:40 h	45.7%	28:21 h	45.0%	26:04 h	43.5%	26:42 h	44.9%	±0:31 h	±1.1%
	0056	29:30 h	47.2%	30:08 h	46.4%	28:47 h	45.7%	26:21 h	43.9%	27:04 h	45.5%	±0:36 h	±1.2%
	0057	30:00 h	48.0%	30:36 h	47.1%	29:12 h	46.4%	26:38 h	44.4%	27:25 h	46.1%	±0:41 h	±1.3%
V-13	0058	30:30 h	48.8%	31:04 h	47.8%	29:38 h	47.1%	26:55 h	44.9%	27:47 h	46.7%	±0:46 h	±1.5%
	0059	31:00 h	49.6%	31:32 h	48.5%	30:04 h	47.7%	27:12 h	45.4%	28:08 h	47.3%	±0:51 h	±1.6%
	0060	31:30 h	50.4%	32:00 h	49.2%	30:30 h	48.4%	27:30 h	45.8%	28:30 h	47.9%	±0:56 h	±1.7%
	0061	32:00 h	51.2%	32:30 h	50.0%	31:00 h	49.2%	28:00 h	46.7%	29:00 h	48.7%	±0:56 h	±1.7%
	0062	32:30 h	52.0%	33:07 h	51.0%	31:45 h	50.4%	29:00 h	48.3%	29:45 h	50.0%	±0:46 h	±1.3%
	0063	33:00 h	52.8%	33:45 h	51.9%	32:30 h	51.6%	30:00 h	50.0%	30:30 h	51.3%	±0:37 h	±1.0%
	0064	33:30 h	53.6%	34:22 h	52.9%	33:15 h	52.8%	31:00 h	51.7%	31:15 h	52.5%	±0:28 h	±0.7%
	0065	34:00 h	54.4%	35:00 h	53.8%	34:00 h	54.0%	32:00 h	53.3%	32:00 h	53.8%	±0:20 h	±0.4%
	0066	34:30 h	55.2%	35:30 h	54.6%	34:30 h	54.8%	32:30 h	54.2%	32:30 h	54.6%	±0:20 h	±0.4%
V-14	0067	35:00 h	56.0%	35:55 h	55.3%	34:50 h	55.3%	33:00 h	55.0%	33:10 h	55.7%	±0:15 h	±0.4%
	0068	35:30 h	56.8%	36:20 h	55.9%	35:10 h	55.8%	33:30 h	55.8%	33:50 h	56.9%	±0:10 h	±0.5%
	0069	36:00 h	57.6%	36:45 h	56.5%	35:30 h	56.3%	34:00 h	56.7%	34:30 h	58.0%	±0:06 h	±0.7%
	0070	36:30 h	58.4%	37:10 h	57.2%	35:50 h	56.9%	34:30 h	57.5%	35:10 h	59.1%	±0:03 h	±0.9%
	0071	37:00 h	59.2%	37:35 h	57.8%	36:10 h	57.4%	35:00 h	58.3%	35:50 h	60.2%	±0:00 h	±1.1%
	0072	37:30 h	60.0%	38:00 h	58.5%	36:30 h	57.9%	35:30 h	59.2%	36:30 h	61.3%	±0:58 h	±1.3%

(continued)

V-15	0073	38:00 h	60.8%	38:30 h	59.2%	37:00 h	58.7%	36:00 h	60.0%	37:00 h	62.2%	±00:58 h	±1.4%
	0074	38:30 h	61.6%	38:50 h	59.8%	37:32 h	59.6%	36:20 h	60.6%	37:23 h	62.8%	±00:59 h	±1.4%
	0075	39:00 h	62.4%	39:11 h	60.3%	38:04 h	60.4%	36:41 h	61.2%	37:46 h	63.5%	±01:00 h	±1.4%
	0076	39:30 h	63.2%	39:32 h	60.8%	38:36 h	61.3%	37:02 h	61.7%	38:09 h	64.1%	±01:02 h	±1.4%
	0077	40:00 h	64.0%	39:53 h	61.4%	39:09 h	62.1%	37:23 h	62.3%	38:32 h	64.8%	±01:04 h	±1.4%
	0078	40:30 h	64.8%	40:13 h	61.9%	39:41 h	63.0%	37:43 h	62.9%	38:55 h	65.4%	±01:07 h	±1.5%
	0079	41:00 h	65.6%	40:34 h	62.4%	40:13 h	63.9%	38:04 h	63.5%	39:18 h	66.1%	±01:09 h	±1.5%
	0080	41:30 h	66.4%	40:55 h	63.0%	40:46 h	64.7%	38:25 h	64.0%	39:41 h	66.7%	±01:13 h	±1.6%
	0081	42:00 h	67.2%	41:16 h	63.5%	41:18 h	65.6%	38:46 h	64.6%	40:04 h	67.4%	±01:16 h	±1.7%
	0082	42:30 h	68.0%	41:36 h	64.0%	41:50 h	66.4%	39:06 h	65.2%	40:27 h	68.0%	±01:20 h	±1.7%
	0083	43:00 h	68.8%	41:57 h	64.6%	42:23 h	67.3%	39:27 h	65.8%	40:50 h	68.6%	±01:23 h	±1.8%
	0084	43:30 h	69.6%	42:18 h	65.1%	42:55 h	68.1%	39:48 h	66.3%	41:13 h	69.3%	±01:27 h	±1.9%
	0085	44:00 h	70.4%	42:39 h	65.6%	43:27 h	69.0%	40:09 h	66.9%	41:36 h	69.9%	±01:32 h	±2.0%
	0086	44:30 h	71.2%	43:00 h	66.2%	44:00 h	69.8%	40:30 h	67.5%	42:00 h	70.6%	±01:36 h	±2.1%
VI-16	0087	45:00 h	72.0%	43:30 h	66.9%	44:30 h	70.6%	41:00 h	68.3%	42:30 h	71.4%	±01:36 h	±2.2%
	0088	45:30 h	72.8%	44:11 h	68.0%	44:58 h	71.4%	41:28 h	69.1%	43:00 h	72.3%	±01:37 h	±2.1%
	0089	46:00 h	73.6%	44:53 h	69.1%	45:26 h	72.1%	41:56 h	69.9%	43:30 h	73.1%	±01:38 h	±2.0%
	0090	46:30 h	74.4%	45:35 h	70.1%	45:55 h	72.9%	42:25 h	70.7%	44:00 h	73.9%	±01:39 h	±1.9%
	0091	47:00 h	75.2%	46:16 h	71.2%	46:23 h	73.6%	42:53 h	71.5%	44:30 h	74.8%	±01:41 h	±1.8%
	0092	47:30 h	76.0%	46:58 h	72.3%	46:51 h	74.4%	43:21 h	72.3%	45:00 h	75.6%	±01:43 h	±1.8%
	0093	48:00 h	76.8%	47:40 h	73.3%	47:20 h	75.1%	43:50 h	73.1%	45:30 h	76.5%	±01:45 h	±1.7%
	0094	48:30 h	77.6%	48:21 h	74.4%	47:48 h	75.9%	44:18 h	73.8%	46:00 h	77.3%	±01:48 h	±1.7%
	0095	49:00 h	78.4%	49:03 h	75.5%	48:16 h	76.6%	44:46 h	74.6%	46:30 h	78.2%	±01:50 h	±1.6%
	0096	49:30 h	79.2%	49:45 h	76.5%	48:45 h	77.4%	45:15 h	75.4%	47:00 h	79.0%	±01:53 h	±1.6%
	0097	50:00 h	80.0%	50:26 h	77.6%	49:13 h	78.1%	45:43 h	76.2%	47:30 h	79.8%	±01:57 h	±1.6%
	0098	50:30 h	80.8%	51:08 h	78.7%	49:41 h	78.9%	46:11 h	77.0%	48:00 h	80.7%	±02:00 h	±1.6%
	0099	51:00 h	81.6%	51:50 h	79.7%	50:10 h	79.6%	46:40 h	77.8%	48:30 h	81.5%	±02:03 h	±1.6%
	0100	51:30 h	82.4%	52:31 h	80.8%	50:38 h	80.4%	47:08 h	78.6%	49:00 h	82.4%	±02:07 h	±1.6%
	0101	52:00 h	83.2%	53:13 h	81.9%	51:06 h	81.1%	47:36 h	79.4%	49:30 h	83.2%	±02:11 h	±1.6%
	0102	52:30 h	84.0%	53:55 h	82.9%	51:35 h	81.9%	48:05 h	80.1%	50:00 h	84.0%	±02:15 h	±1.6%
	0103	53:00 h	84.8%	54:36 h	84.0%	52:03 h	82.6%	48:33 h	80.9%	50:30 h	84.9%	±02:19 h	±1.7%
	0104	53:30 h	85.6%	55:18 h	85.1%	52:31 h	83.4%	49:01 h	81.7%	51:00 h	85.7%	±02:23 h	±1.7%
	0105	54:00 h	86.4%	56:00 h	86.2%	53:00 h	84.1%	49:30 h	82.5%	51:30 h	86.6%	±02:27 h	±1.8%
VI-17	0106	54:30 h	87.2%	56:30 h	86.9%	53:30 h	84.9%	50:00 h	83.3%	52:00 h	87.4%	±02:27 h	±1.8%
	0107	55:00 h	88.0%	57:02 h	87.7%	54:06 h	85.9%	50:38 h	84.4%	52:28 h	88.2%	±02:26 h	±1.7%
	0108	55:30 h	88.8%	57:34 h	88.6%	54:42 h	86.8%	51:16 h	85.4%	52:56 h	89.0%	±02:24 h	±1.5%
	0109	56:00 h	89.6%	58:06 h	89.4%	55:18 h	87.8%	51:54 h	86.5%	53:24 h	89.7%	±02:23 h	±1.4%
	0110	56:30 h	90.4%	58:38 h	90.2%	55:54 h	88.7%	52:32 h	87.6%	53:52 h	90.5%	±02:22 h	±1.3%
	0111	57:00 h	91.2%	59:10 h	91.0%	56:30 h	89.7%	53:10 h	88.6%	54:20 h	91.3%	±02:20 h	±1.2%
	0112	57:30 h	92.0%	59:42 h	91.8%	57:06 h	90.6%	53:48 h	89.7%	54:48 h	92.1%	±02:19 h	±1.1%
	0113	58:00 h	92.8%	60:14 h	92.7%	57:42 h	91.6%	54:26 h	90.7%	55:16 h	92.9%	±02:18 h	±0.9%
	0114	58:30 h	93.6%	60:46 h	93.5%	58:18 h	92.5%	55:04 h	91.8%	55:44 h	93.7%	±02:18 h	±0.8%
	0115	59:00 h	94.4%	61:18 h	94.3%	58:54 h	93.5%	55:42 h	92.8%	56:12 h	94.5%	±02:17 h	±0.7%
	0116	59:30 h	95.2%	61:50 h	95.1%	59:30 h	94.4%	56:20 h	93.9%	56:40 h	95.2%	±02:16 h	±0.6%
	0117	60:00 h	96.0%	62:22 h	95.9%	60:06 h	95.4%	56:58 h	94.9%	57:08 h	96.0%	±02:16 h	±0.5%
	0118	60:30 h	96.8%	62:54 h	96.8%	60:42 h	96.3%	57:36 h	96.0%	57:36 h	96.8%	±02:16 h	±0.4%
	0119	61:00 h	97.6%	63:26 h	97.6%	61:18 h	97.3%	58:14 h	97.1%	58:04 h	97.6%	±02:15 h	±0.2%
	0120	61:30 h	98.4%	63:58 h	98.4%	61:54 h	98.3%	58:52 h	98.1%	58:32 h	98.4%	±02:15 h	±0.1%
	0121	62:00 h	99.2%	64:30 h	99.2%	62:30 h	99.2%	59:30 h	99.2%	59:00 h	99.2%	±02:23 h	±0.0%
Hatch	0122	62:30 h	100.0%	65:00 h	100.0%	63:00 h	100.0%	60:00 h	100.0%	59:30 h	100.0%	±02:15 h	±0.0%

Supplementary Videos

Supplementary Video 1 – Medfly embryonic development for the six *in toto* datasets (DS0001–DS0006) along four orientations from 02:00 h to 64:30 h of absolute development time with an interval of 30 min between the time points. The video starts at the onset of stage I-2, when the nuclei move to the surface of the egg and stops at the end of stage VI-17, when larvae hatch. It shows six embryogenetic events: (I) blastoderm formation, (II) early gastrulation, (III) germband elongation, (IV) germband retraction, (V) dorsal closure and (VI) muscular movement. Frame rate is five frames per second. Embryos from DS0001 and DS0003 are shown along the ventrolateral, dorsolateral, dorsolateral, and ventrolateral orientations, embryos from DS0002 and DS0004–DS0006 are shown along the ventral, lateral, dorsal, and lateral orientations. ZA, z maximum projection with image adjustment.