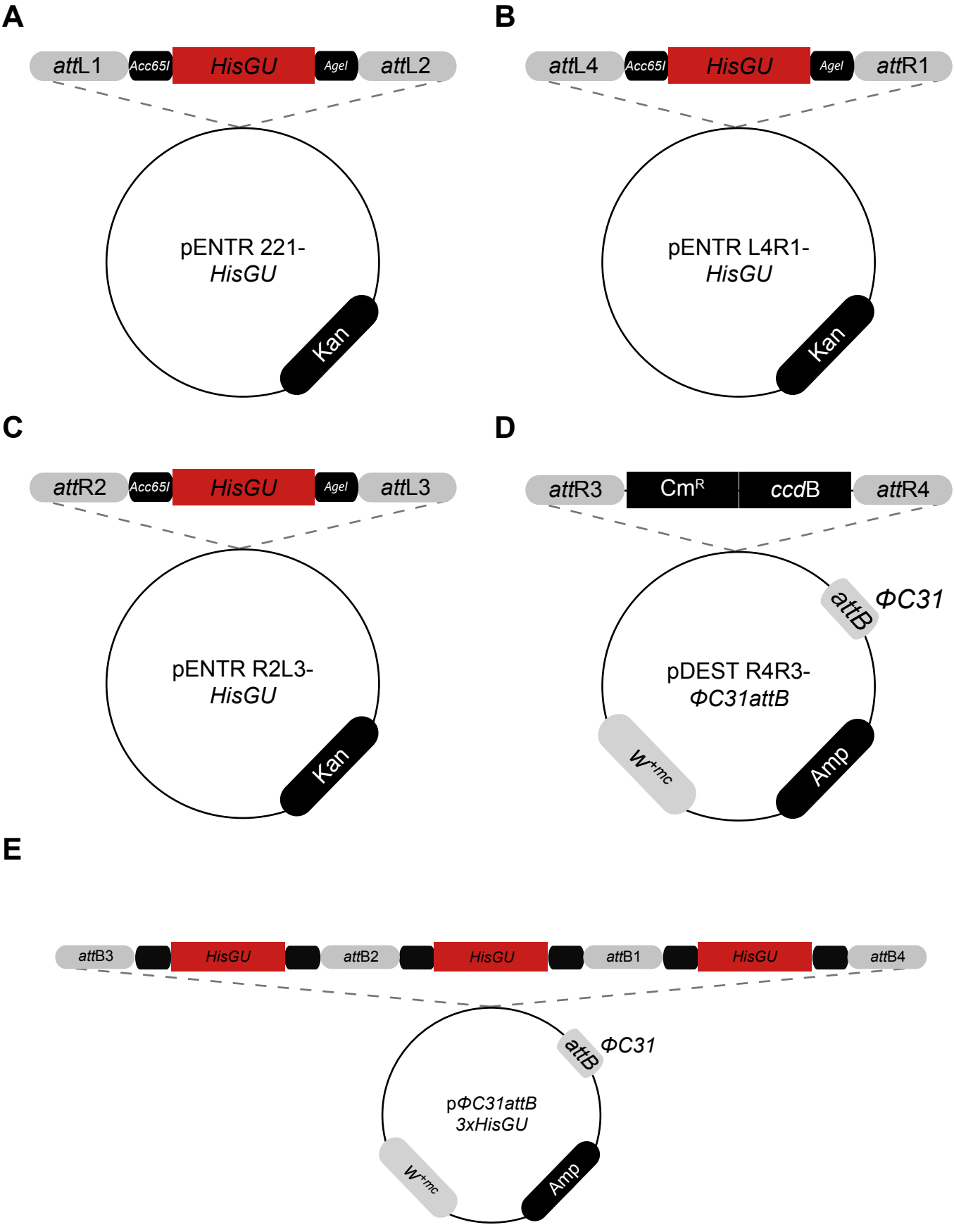
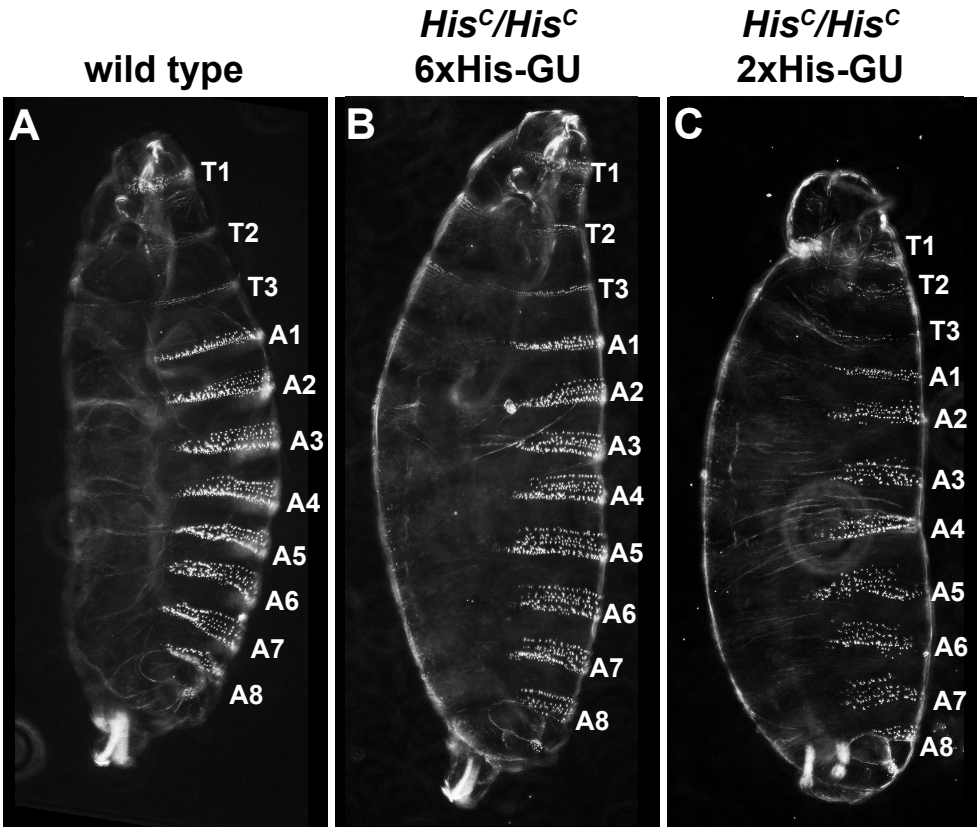
Figure S1


A
attL1
Acc65I
HisGU
AgeI
attL2
pENTR 221-
HisGU
Kan
B
attL4
Acc65I
HisGU
AgeI
attR1
pENTR L4R1-
HisGU
Kan
C
attR2
Acc65I
HisGU
AgeI
attL3
pENTR R2L3-
HisGU
Kan
D
attR3
CmR
ccdB
attR4
ΦC31
attB
pDEST R4R3-
ΦC31attB
W+mc
Amp
E
attB3
HisGU
attB2
HisGU
attB1
HisGU
attB4
ΦC31
attB
pΦC31attB
3xHisGU
W+mc
Amp

Supplementary Fig S1: Transgenes and their construction.

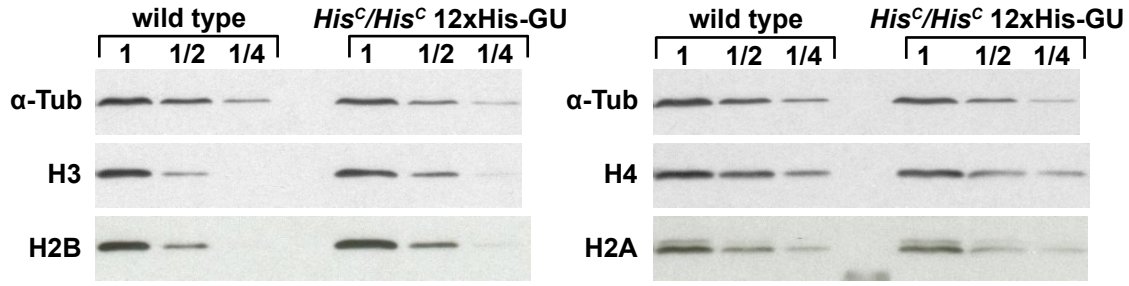
(A-E) Schematic representation of vectors and transgenes. The MultiSite-Gateway recombination of entry vectors pENTR 221-*HisGU* (A), pENTR L4R1-*HisGU* (*B*) and pENTR R2L3-*HisGU* (C) with the destination vector pDEST R3R4-*φC31att*B (D) resulted in the transgene integration construct p*ΦC31att*B *3xHisGU* (E). For details see methods section.

Figure S2


wild type
His^{C}/His^{C} 6xHis-GU
His^{C}/His^{C} 2xHis-GU
A
T1
T2
T3
A1
A2
A3
A4
A5
A6
A7
A8
B
T1
T2
T3
A1
A2
A3
A4
A5
A6
A7
A8
C
T1
T2
T3
A1
A2
A3
A4
A5
A6
A7
A8

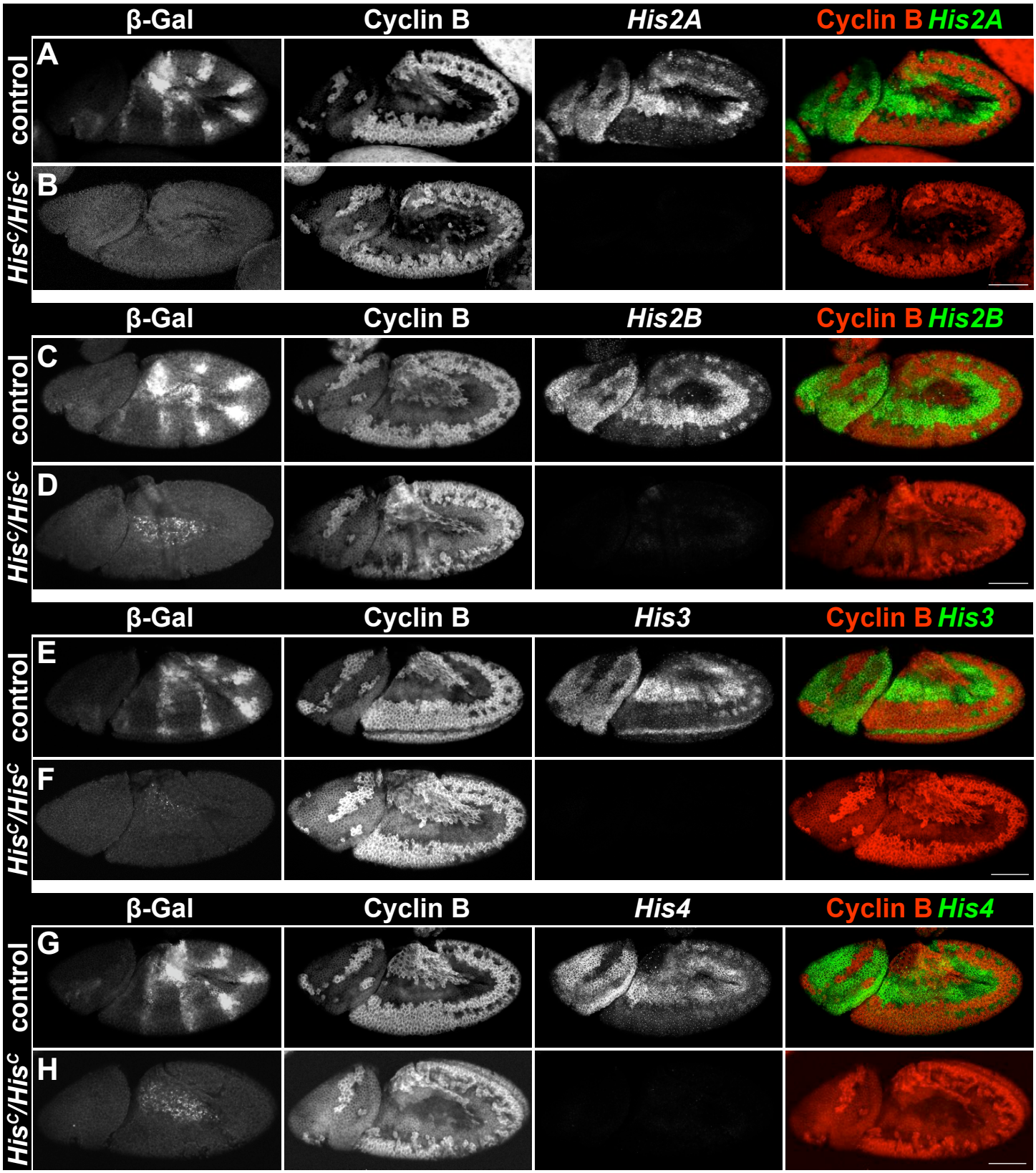
Supplementary Fig S2: Cuticle phenotypes of *His^{C}* mutant flies with 6 or 2 His-GUs. (A-C) Lateral views on cuticle preparations of unhatched first instar larvae from wild type (A), *His^{C}* mutants with 6 His-GUs (B; *His^{C}*/*His^{C}* 6xHis-GU) and *His^{C}* mutants with 2 His-GUs (C; *His^{C}*/*His^{C}* 2xHis-GU), respectively. (A) Wild type cuticles show a characteristic pattern of denticle belts in each of the thoracic (T1-T3) and abdominal segments (A1-A8). (B) The cuticle of *His^{C}* mutants with 6 His-GUs does not show any abnormalities compared to wild type (A). (C) *His^{C}* mutants with 2 His-GUs exhibit a cuticle with all segments but abnormal morphology.

Figure S3

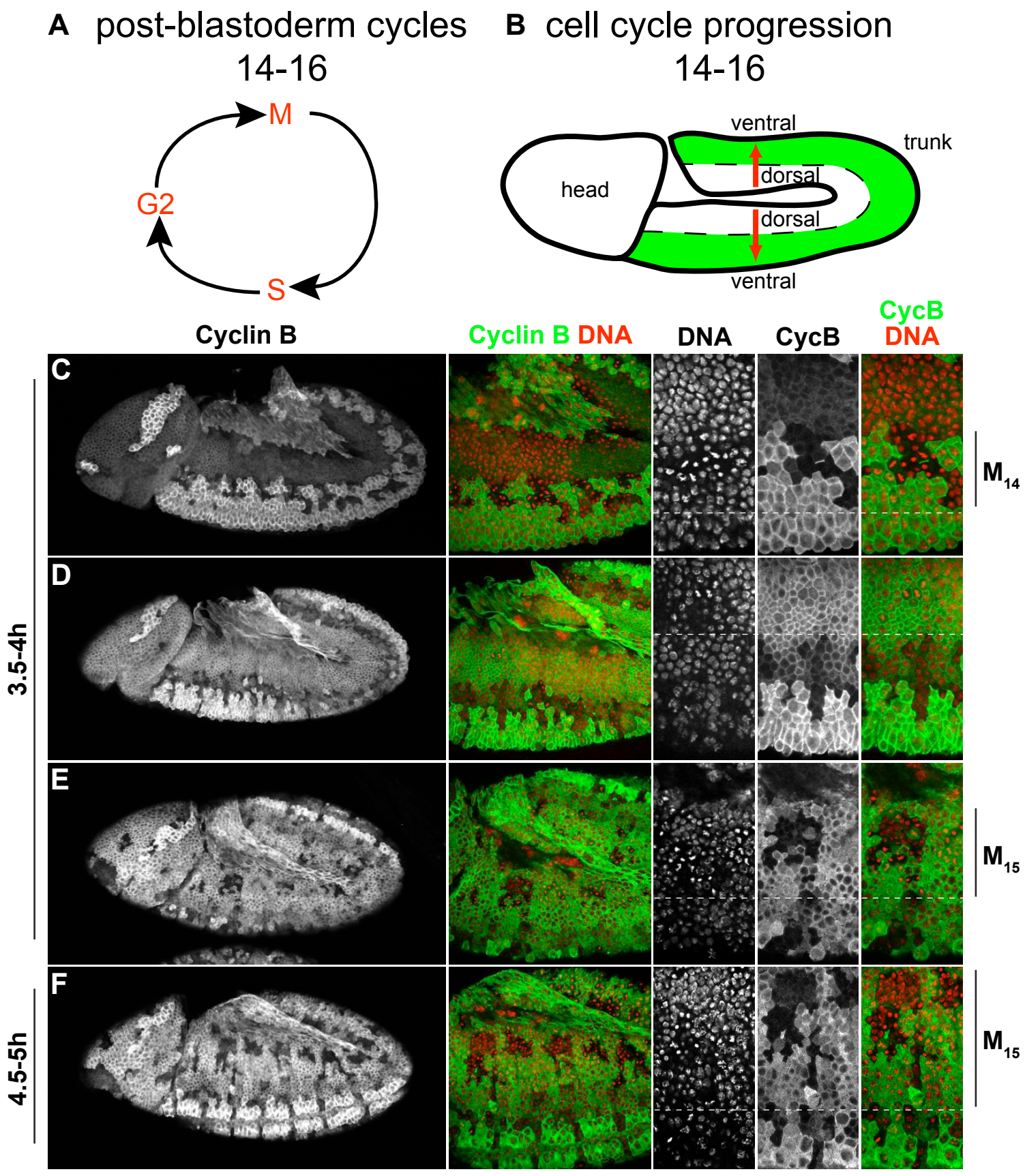

wild type
His^{C}/*His*^{C} 12xHis-GU
1 1/2 1/4
1 1/2 1/4
α-Tub
H3
H2B
wild type
His^{C}/*His*^{C} 12xHis-GU
1 1/2 1/4
1 1/2 1/4
α-Tub
H4
H2A

Supplementary Fig S3: Histone protein levels in rescued *His*^{C} mutant flies with 12 His-GUs. Quantitative western blotting analyses with protein extracts from wild type flies and *His*^{C} mutant flies rescued by 12 transgene coded His-GUs (*His*^{C}/*His*^{C} 12xHis-GU) using antibodies against histones H3, H2B, H4 and H2A, respectively. The total amount of loaded protein extract was bisected from lane to lane (1; 1/2; 1/4). α-Tubulin (α-Tub) detection served as loading control. *His*^{C} mutant flies with 12 His-GUs do not have reduced protein levels of histones compared to wild type.

Figure S4


β-Gal
Cyclin B
His2A
Cyclin B His2A
control
A
His^C/His^C
B
β-Gal
Cyclin B
His2B
Cyclin B His2B
control
C
His^C/His^C
D
β-Gal
Cyclin B
His3
Cyclin B His3
control
E
His^C/His^C
F
β-Gal
Cyclin B
His4
Cyclin B His4
control
G
His^C/His^C
H

Supplementary Fig S4: *His^{C}* mutant embryos lack zygotic histone expression. (A-H) Embryos were labeled with antibodies against β-Galactosidase (β-Gal), Cyclin B (Cyclin B, red in merge) and with probes detecting the mRNA of *His2A* (A, B), *His2B* (C, D), *His3* (E, F), or *His4* (G, H) (green in merge). Internal control embryos are positive for β-Gal labeling (control). Homozygous mutant *His^{C}* embryos are negative for β-Gal labeling (*His^{C}/His^{C}*). In control embryos, histone expression is high in S_{15} cells, which show low Cyclin B levels. In *His^{C}* mutant embryos, no histone expression is detected. Scale bar: 100 μm.


A post-blastoderm cycles 14-16
M
G2
S
B cell cycle progression 14-16
ventral
trunk
dorsal
head
dorsal
ventral
Cyclin B
Cyclin B DNA
DNA
CycB
CycB DNA
C
D
E
F
3.5-4h
4.5-5h
M14
M15
M15

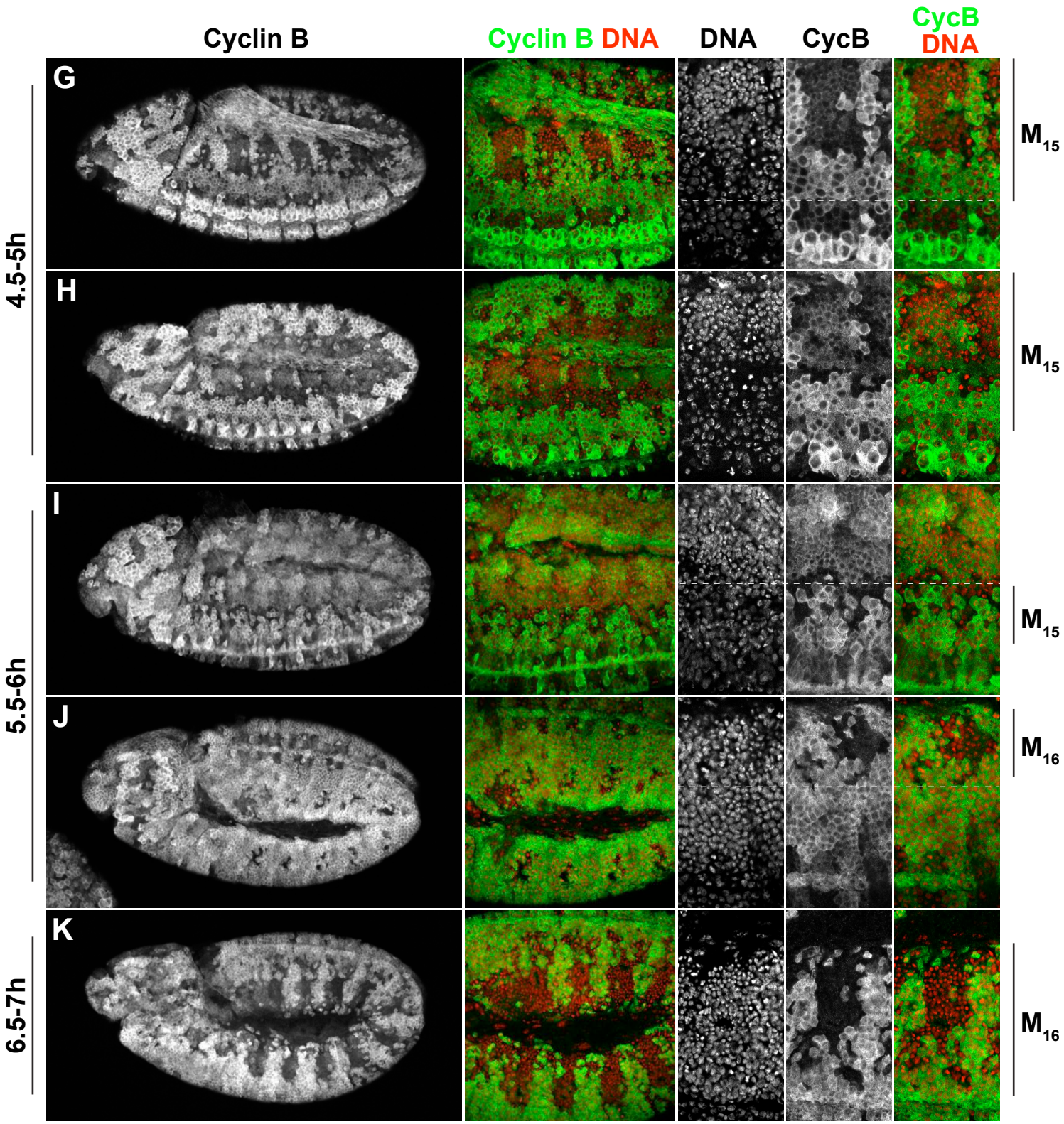
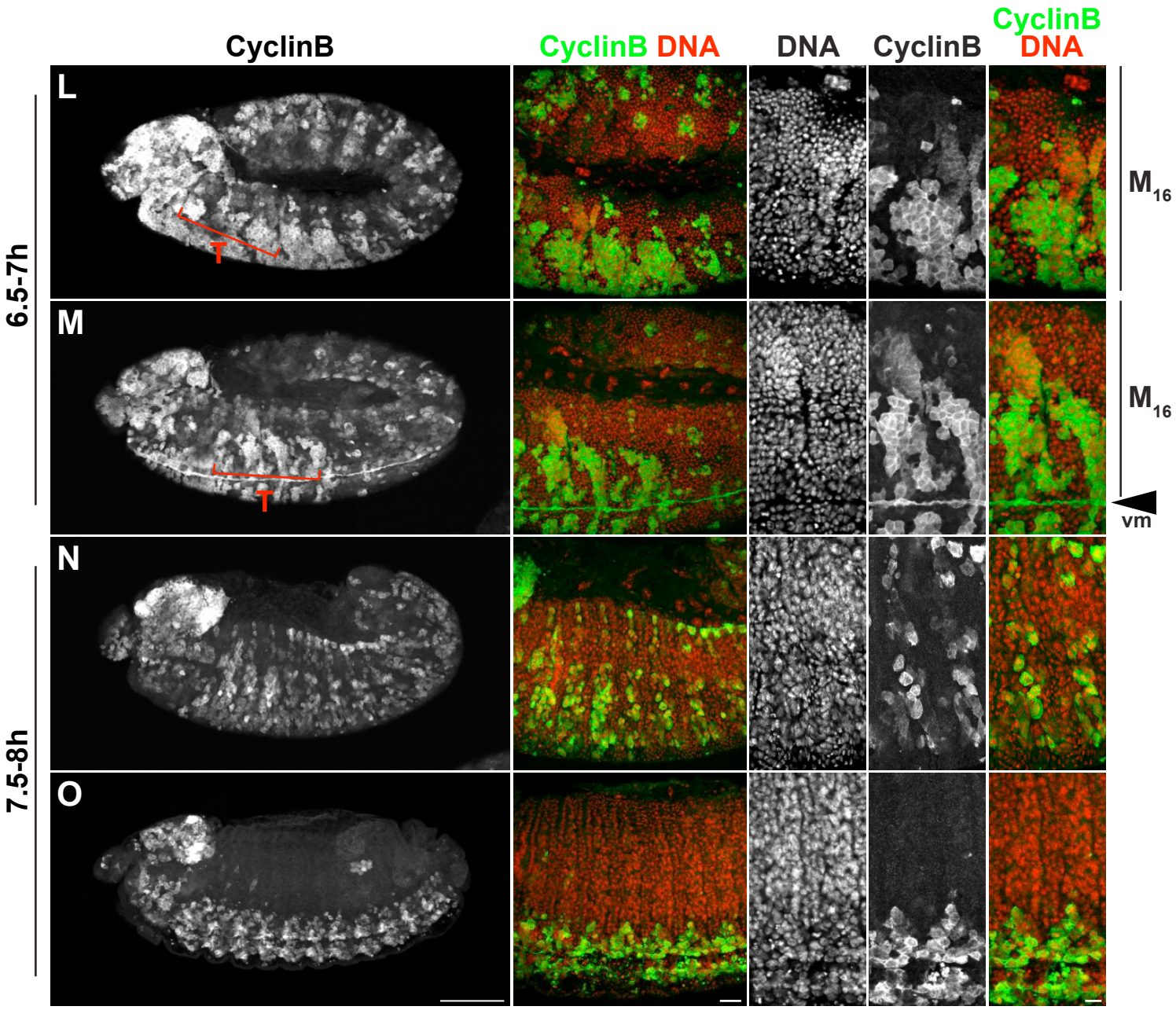

Cyclin B
Cyclin B DNA
DNA
CycB
CycB DNA
G
H
I
J
K
4.5-5h
5.5-6h
6.5-7h
M15
M15
M15
M16
M16

Figure S5 part I



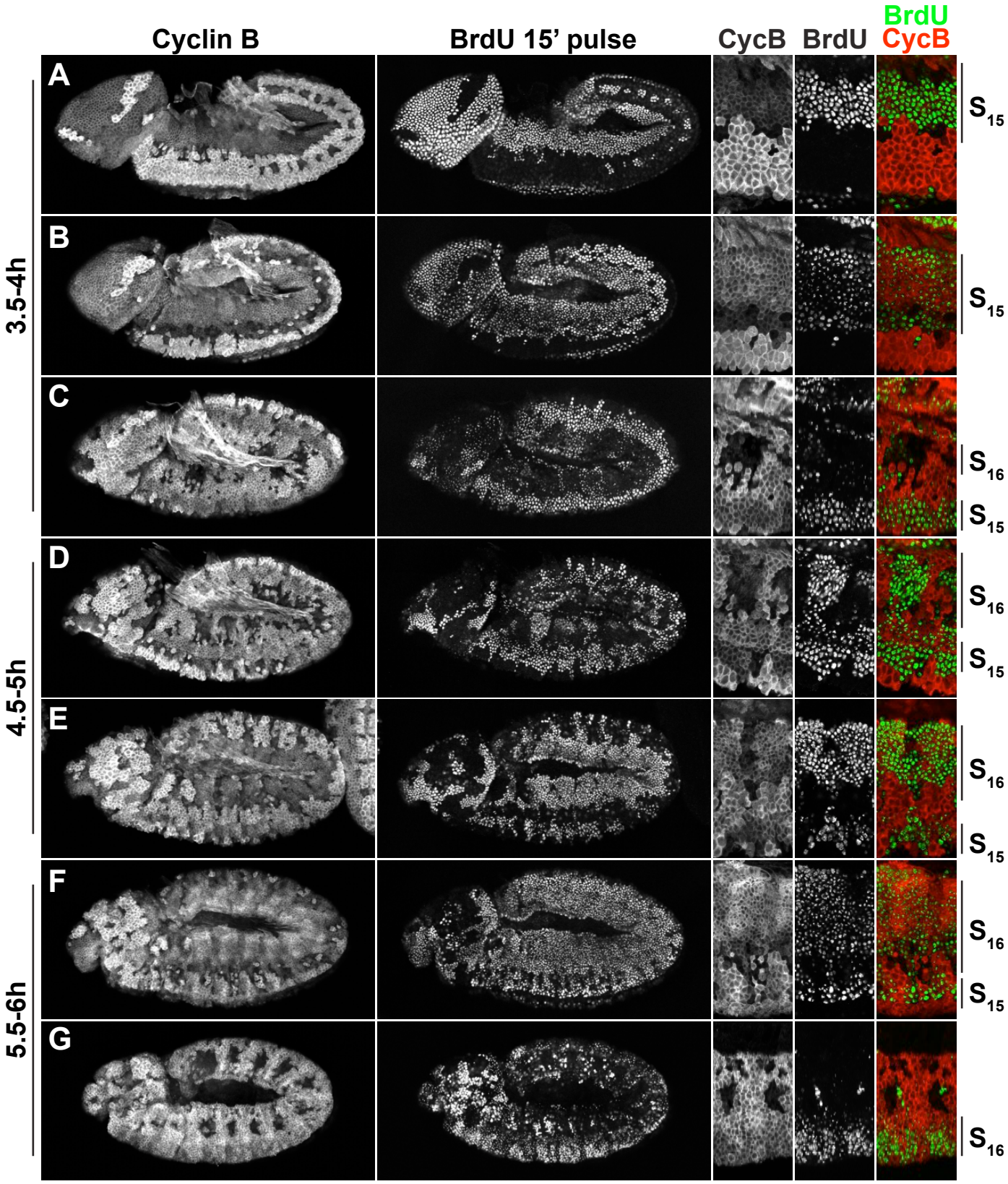
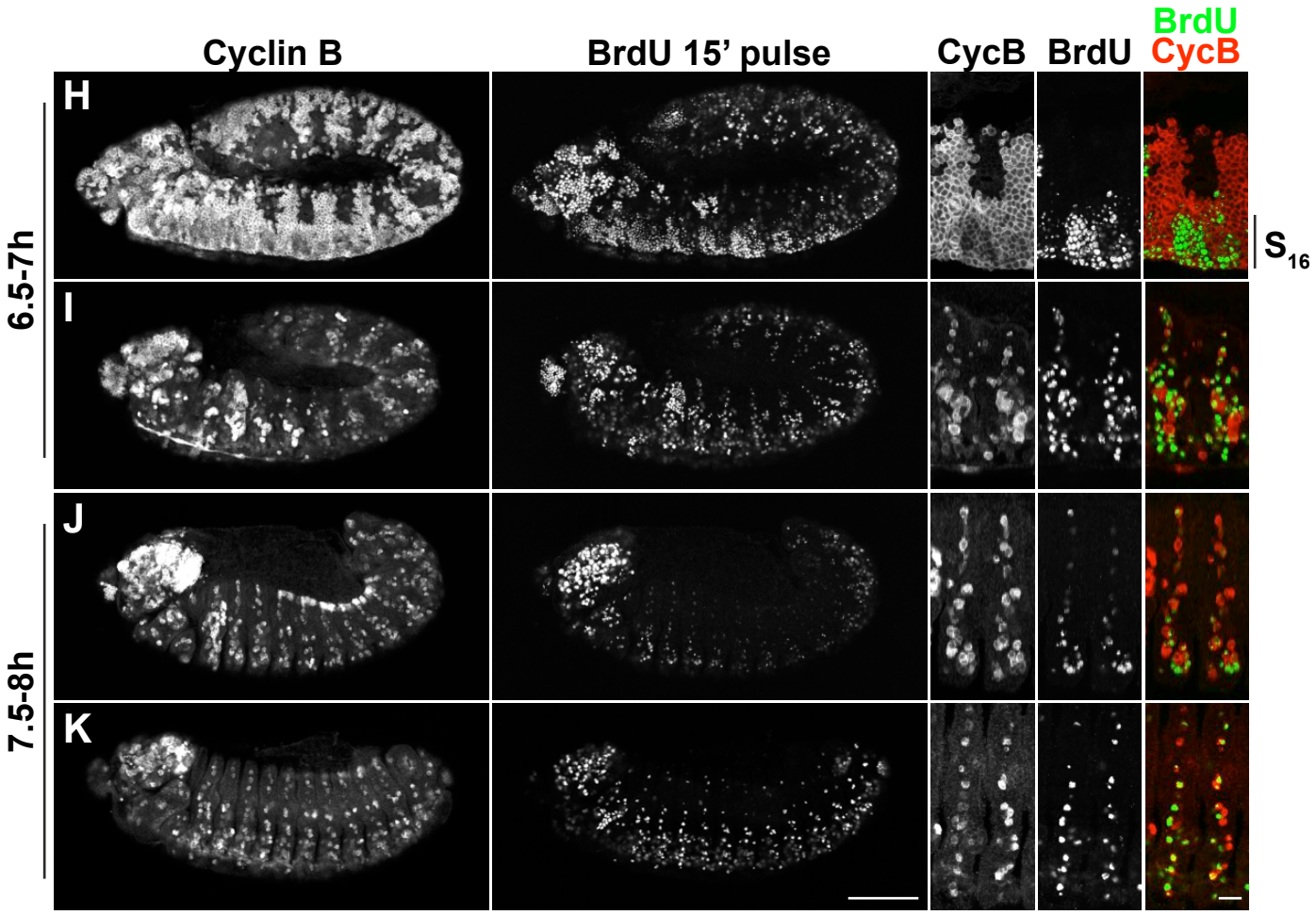

Cyclin B
BrdU 15' pulse
CycB
BrdU
BrdU
CycB
A
B
C
D
E
F
G
3.5-4h
4.5-5h
5.5-6h
S15
S15
S16
S15
S16
S15
S16
S15
S16

Figure S5 part II



Supplementary Fig S5: Cell division pattern of wild type epidermal cells.

Part I: Cell division pattern of wild type epidermal cells - mitosis.

(A) The embryonic post-blastoderm cell division cycles 14-16 lack G_1 gap phases. Cells directly enter S phase after they exit from mitosis. Only after completion of cell cycle 16 most epidermal cells enter a quiescent G_1/G_0 phase. (B) Embryonic cell division cycles 14-16 are accompanied by morphogenetic movements, which lead to the extension and subsequent retraction of the trunk germ band. The scheme shows an embryo with fully extended germ band. At the anterior, the head is clearly separated from the trunk. The trunk appears 'folded' with the dorsal epidermis pointing to the 'interior' of the egg and the ventral epidermis to the 'outside'. In the epidermis of the trunk, the spatial pattern of each post-blastoderm cycle is propagated from the dorsal to the ventral part, which leads to a 'mirrored' appearance in germ band extended embryos.

(C-O) Individual embryos were labeled for Cyclin B and DNA. The embryos represent typical developmental stages, which were found in time-matched collections as indicated to the left of the panels. The first row in the panels shows a whole mount Cyclin B staining. The second row shows a magnification of the trunk region (Cyclin B in green, DNA in red). Rows 3-5 show magnifications of an epidermal region with single channel detections of DNA or Cyclin B (CycB) and the merge of both channels (Cyclin B in green, DNA in red). These magnifications are oriented with dorsal up. Arrowheads to the right of the panels point towards to ventral midline (vm).

(C-E) In the dorsal epidermis cells already re-accumulate Cyclin B during S_{15}; in cells of the lateral epidermis Cyclin B is degraded in M_{14} (C). M_{14} is further propagated ventrally, but some cells remain in $G2_{14}$ with high Cyclin B levels. Cell size decreases with every cell division during the post-blastoderm cycles. Therefore $G2_{14}$ cells can be easily distinguished from S_{15} cells by size (D). M_{15} is then initiated in patches of cells in the dorsal epidermis (E).

(F-H) Patches of cells that undergo M_{15} are clearly visible in the dorsal epidermis as segmentally repeated domains with low Cyclin B (F). These patches extend laterally and to the ventral side, as more cells transit M_{15} (G). A hallmark of M_{15} is, that this mitosis is first completed in the dorsal epidermis, before M_{15} will take place in the ventral epidermis (H). Finally most cells of the dorsal epidermis have completed M_{15} and re-accumulate Cyclin B as (smaller) S_{16} cells (H). At this stage of development

the epidermis contains cells in three different cycles, S_{16} dorsally, $S_{15}/G2_{15}$ more ventrally and $G2_{14}$ most ventral (H).

(I-J) M_{15} in the dorsal epidermis is completed, and is further propagated ventrally (I). The progression through M_{15} in the dorsal epidermis is accompanied by completion of M_{14} in the ventral epidermis. By the time M_{15} is completed about half way, M_{14} cells appear as non-interrupted belt (G). When M_{15} is almost completed, M_{14} cells appear as interrupted belt (H). When all cells of the dorsal epidermis are in S_{16}, M_{14} is eventually completed (I). Interphase 15 is short in the cells of the ventral most epidermis. Therefore most of these cells already transited M_{15}, when M_{16} is initiated in the dorsal epidermis (J).

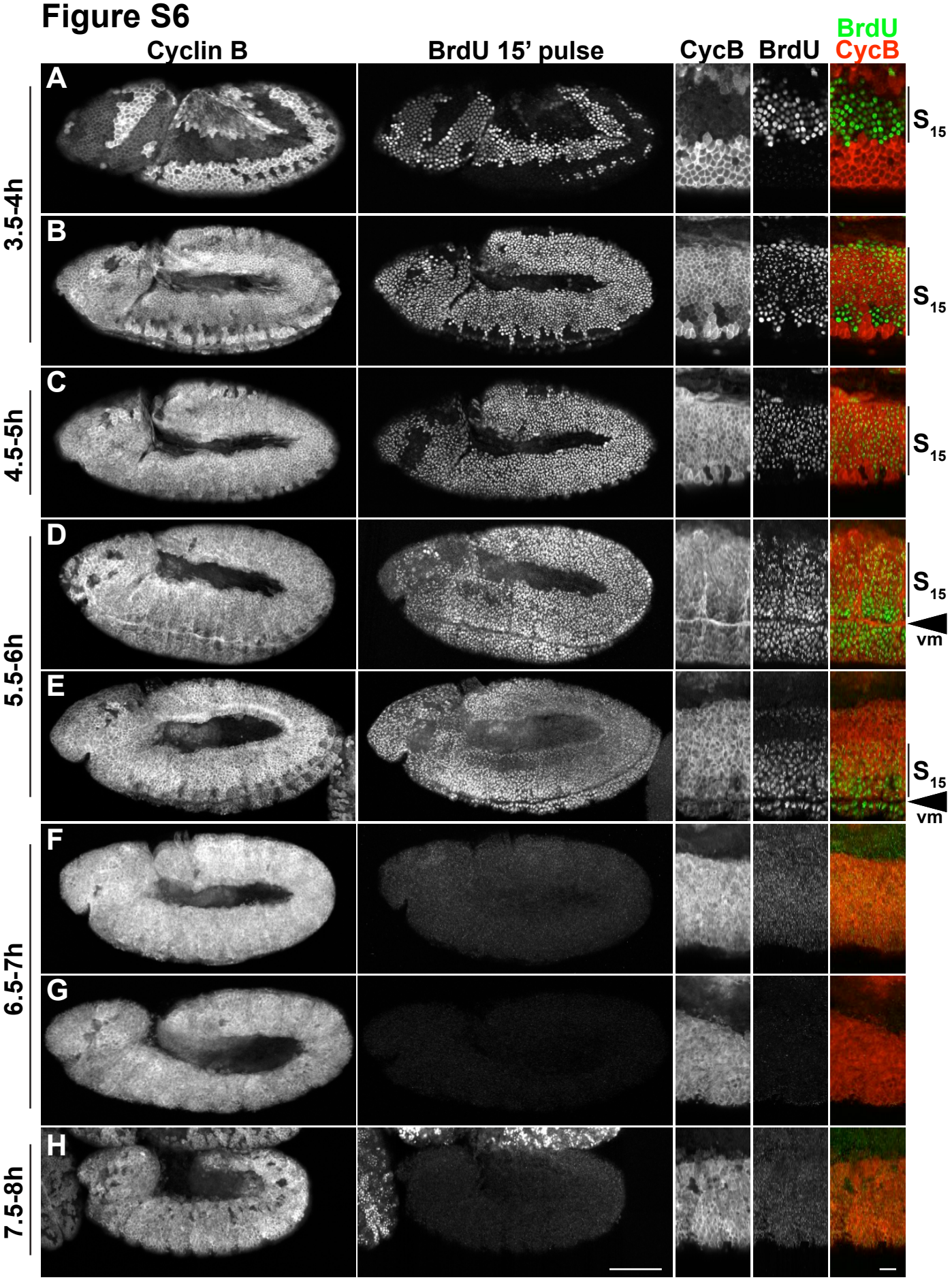
(K-O) M_{16} starts around the tracheal pits (J), which are segmentally repeated structures in the posterior of each parasegment. From there, M_{16} proceeds to cover the posterior half of each parasegment in the dorsal epidermis (K). M_{16} then proceeds ventrally and laterally, with the ventral part of the three thoracic segments (T, red bracket) lagging behind (L, M). There is a small patch of cells in thoracic segment 1 that re-accumulates Cyclin B and will undergo M_{17}, whereas most other epidermal cells enter a quiescent G_1/G_0 state.

(N-O), during germband retraction segmentally repeated cells in the epidermis accumulate Cyclin B (N). These cells presumably contribute to the peripheral nervous system and undergo additional division(s). With a fully retracted germband, Cyclin B expression gets confined to proliferating cells of the central nervous system and to the primordial gonads (O). Scale bars: 100 μm in whole mounts, and 10 μm in magnifications, anterior: left.

Part II: Cell division pattern of wild type epidermal cells – S phase.
(A-K) panels show individual embryos labeled for Cyclin B and for DNA-synthesis by a 15' BrdU incorporation pulse immediately before fixation. The embryos represent typical developmental stages, which were found in time-matched collections as indicated to the left of the panels. The first row in the panels shows a whole mount Cyclin B labeling; the second row the respective pattern of BrdU incorporation. Rows 3-5 show magnifications of an epidermal region with single channel detections of Cyclin B (CycB), BrdU incorporation and the merge of both channels (BrdU in green, Cyclin B in red). These magnifications are oriented with dorsal up. Scale bars:
100 µm in row 1-2, 10 µm in row 3-5, anterior: left.
The pattern of mitosis is discussed based on Cyclin B stainings in **part I.**

Determination of 'S_{15}-exit' and 'M_{15}-entry' (Fig 2): The most anterior domain in the dorsal epidermis first exits S_{15} (BrdU negative) and initiates M_{15} (C). Subsequently, patches of cells in the whole dorsal epidermis exit S_{15} and initiate M_{15} (C, D). Embryos that exited S_{15} in more then the most anterior domain were counted as "S_{15}-exit" (C). Embryos that initiated M_{15} in more then the most anterior domain were counted as "M_{15}-entry" (C).

Figure S6


Cyclin B
BrdU 15' pulse
CycB
BrdU
BrdU
CycB
A
B
C
D
E
F
G
H
3.5-4h
4.5-5h
5.5-6h
6.5-7h
7.5-8h
S15
S15
S15
S15
vm
vm

Supplementary Fig S6: Cell division pattern of *His^{C}* mutant epidermal cells.
(A-H) Panels show individual embryos labeled for Cyclin B and for DNA-synthesis by a 15' BrdU incorporation pulse immediately before fixation. The embryos represent typical *His^{C}* mutant embryos, which were found in time-matched collections as indicated to the left of the panels. The first row in the panels shows a whole mount labeling of Cyclin B, the second row the respective pattern of BrdU incorporation. Rows 3-5 show magnifications of an epidermal region with single channel detections of Cyclin B (CycB), BrdU incorporation and the merge of both channels (BrdU in green, Cyclin B in red). These magnifications are oriented with dorsal up. Arrowheads point towards the ventral midline (vm). Scale bars: 100 µm in row 1-2, 10 µm in row 3-5, anterior: left.
The pattern of wild type mitosis is discussed based on Cyclin B staining and BrdU incorporation in **supplementary Fig S5 - part I**. Therefore, aside from giving an overview on the temporal succession of individual stages, we emphasize specific aspects here.

M_{14} is completed with normal kinetics:
Similar to wild type, M_{14} in the dorsal epidermis was already completed by 3.5h AEL (A). In the ventral epidermis M_{14} is completed between 5.5-6h AEL, like in wild type.

M_{15} is permanently blocked in mutant embryos:
During the entire time-course, no Cyclin B degradation was observed that resembled M_{15} in wild type.

Figure S7

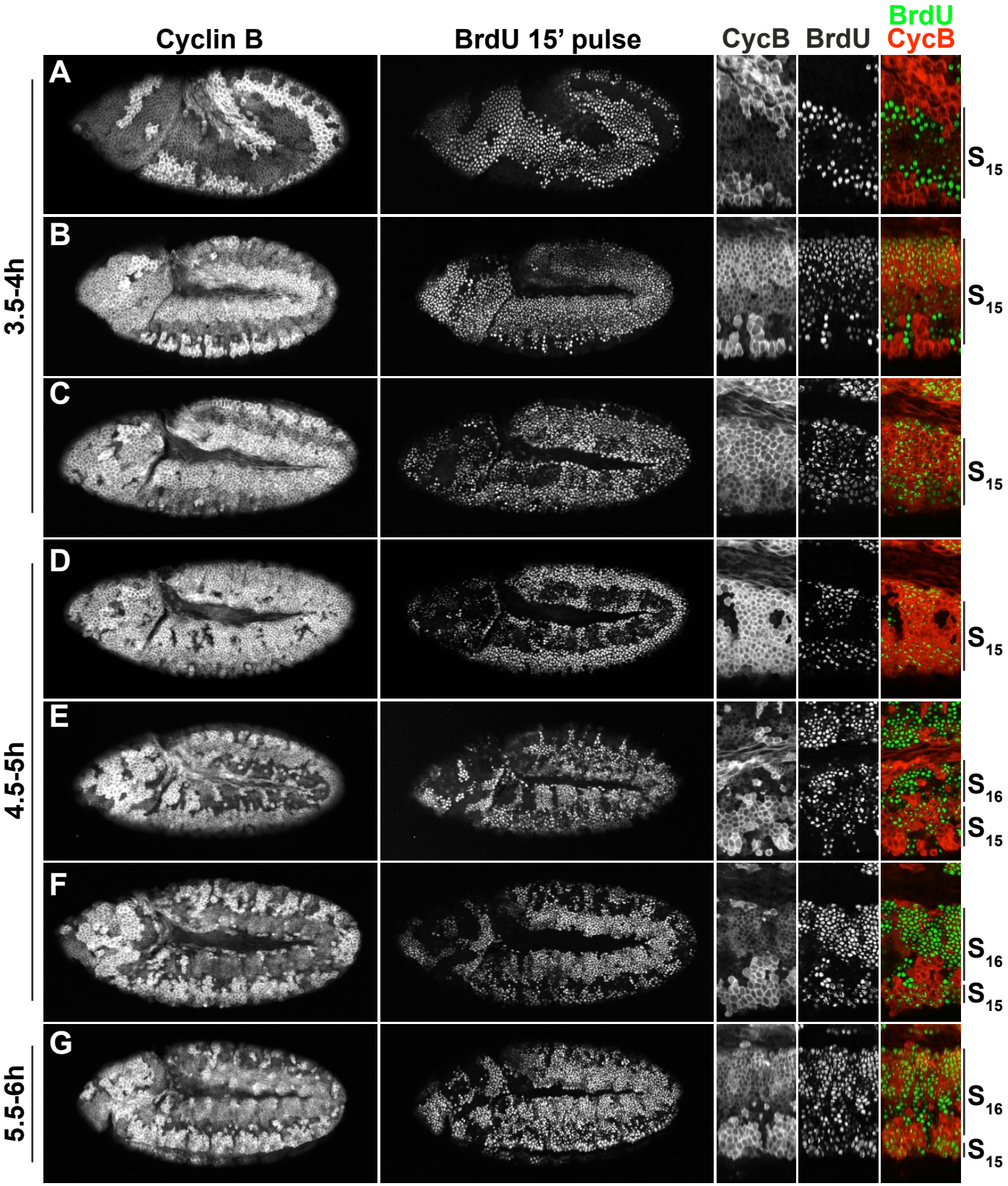
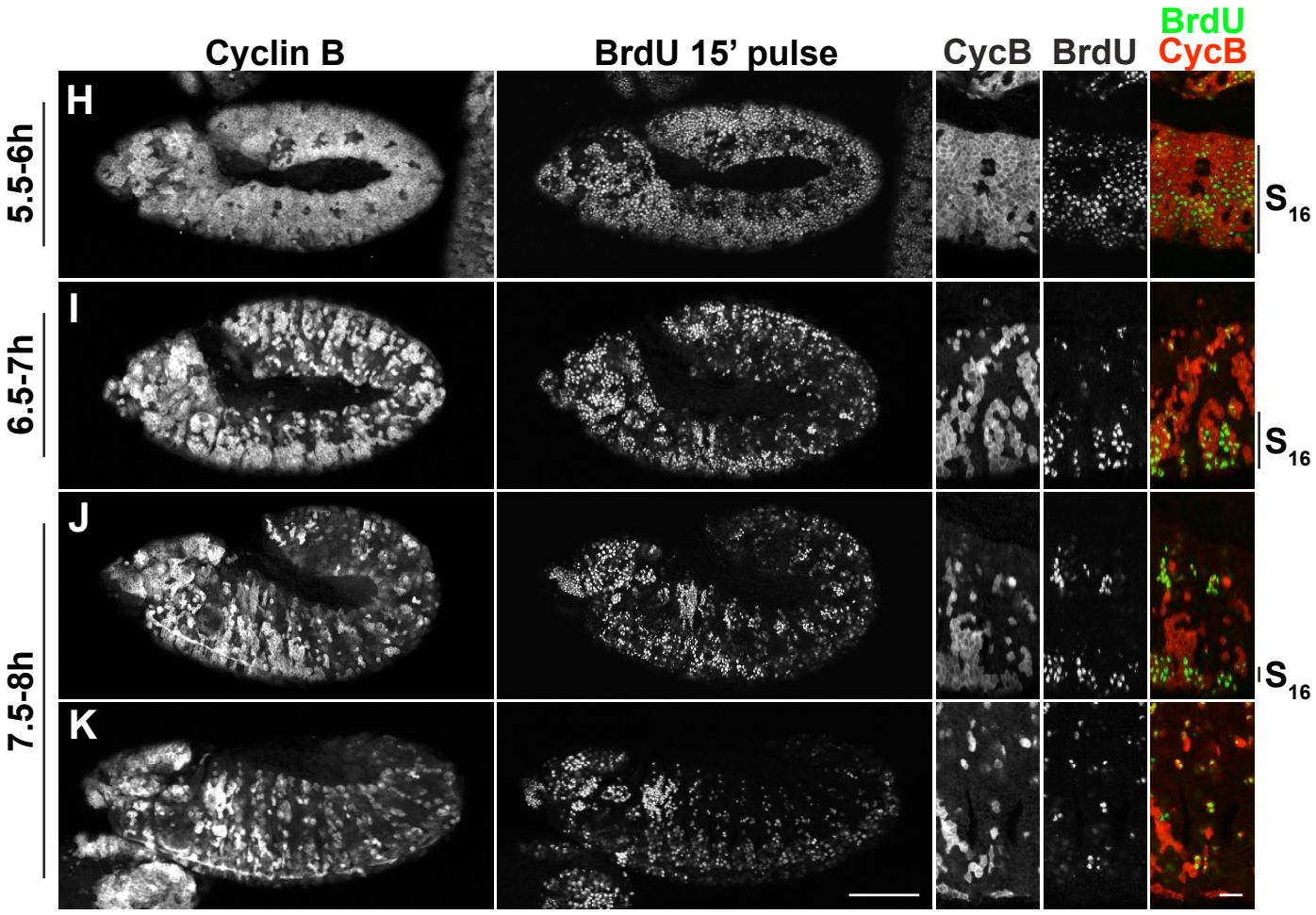

Cyclin B
BrdU 15' pulse
CycB
BrdU
BrdU
CycB
A
B
C
D
E
F
G
3.5-4h
4.5-5h
5.5-6h
S15
S16
S15
S15
S15
S16
S15
S16
S15

Figure S7


Cyclin B
BrdU 15' pulse
CycB
BrdU
BrdU
CycB
H
I
J
K
5.5-6h
6.5-7h
7.5-8h
S16
S16
S16

Supplementary Fig S7: Cell division pattern of *His*^{C} mutant epidermal cells rescued by histone transgenes.

(A-K) Panels show representative time-matched (indicated to the left) embryos that were labeled for Cyclin B and for DNA-synthesis by a 15' BrdU incorporation pulse immediately before fixation. The first row in the panels shows a whole mount Cyclin B labeling; the second row the respective pattern of BrdU incorporation. Rows 3-5 are magnifications of an epidermal region with single channel detections of Cyclin B (CycB), BrdU incorporation and the merge of both channels (BrdU in green, Cyclin B in red). These magnifications are oriented with dorsal up. Arrowheads to the right of the panels point towards to ventral midline (vm). The pattern of wild type mitosis is discussed based on Cyclin B staining and BrdU incorporation in **supplementary Fig S5**. This figure illustrates that 12 transgene based His-GUs restore the wild type pattern of epidermal cell divisions. Scale bars: 100 μm in row 1-2, 10 μm in row 3-5, anterior: left.