

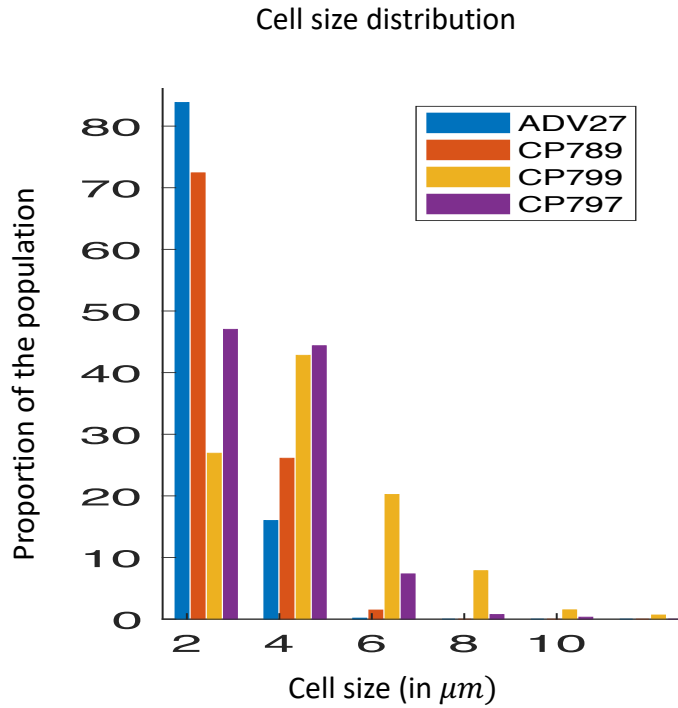
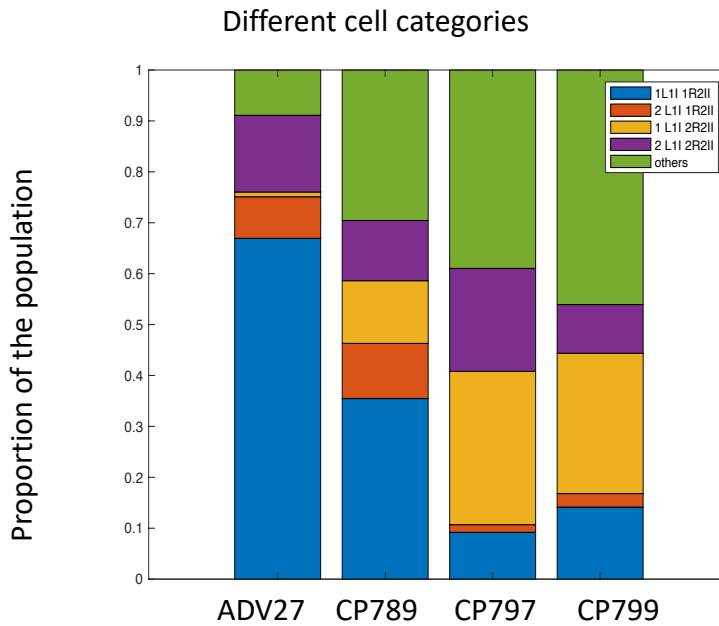
A**B**

Figure S1: Characteristics of strains ADV27 (WT), CP789 ($\Delta\text{parAB2} + \text{ori2}::2\text{parS1}$), CP797 ($\Delta\text{hubPA} \Delta \text{parAB2} + \text{ori2}::2\text{parS1}$) and CP799 ($\Delta\text{parS1} \Delta \text{parAB2} + \text{ori2}::2\text{parS1}$). **(A)** Histogram representing the proportion of cells with a length ranging within the different 2 μm size intervals, from 2 to 8 μm . **(B)** Proportion of cells ranging in different categories defined from the number of « red » and « green » foci as indicated in the figure inset.

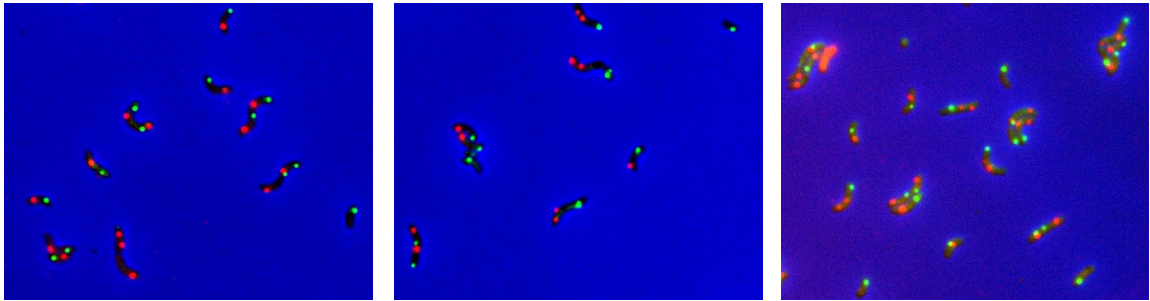
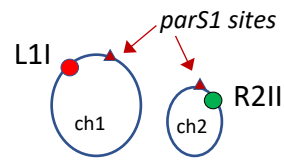


Figure. S2A: Representative Snapshots of CP789 ($\Delta parAB2 + parS1^{ectopic} :: ori2$) containing dual labelling system (red-L1I (300kb from oriC1) and green R2II (180kb from oriC2))

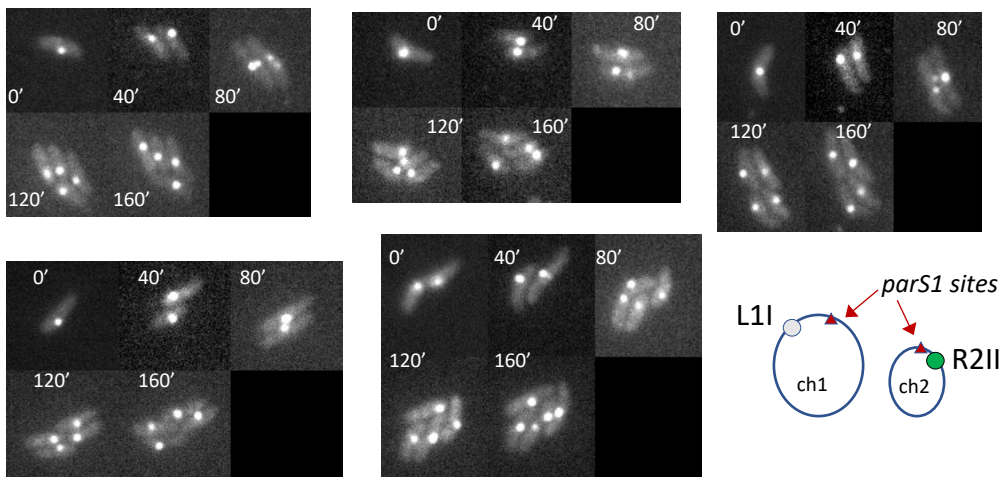


Figure. S2B: Representative timelapses of CP789 ($\Delta parAB2 + parS1^{ectopic} :: ori2$) presenting only the GFP signal of R2II (180kb from oriC2), as the mCherry signal had photobleached.

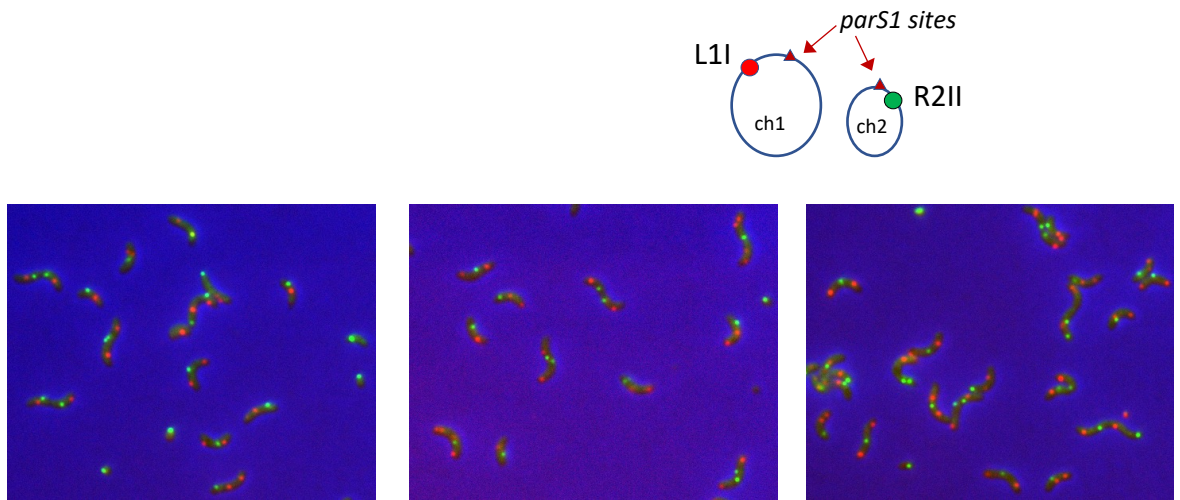


Figure. S3A: Representative Snapshots of CP797 ($\Delta hubP \Delta parAB2 + parS1^{ectopic}::ori2$) containing dual labelling system (red-L1I (300kb from oriC1) and green R2II (180kb from oriC2))

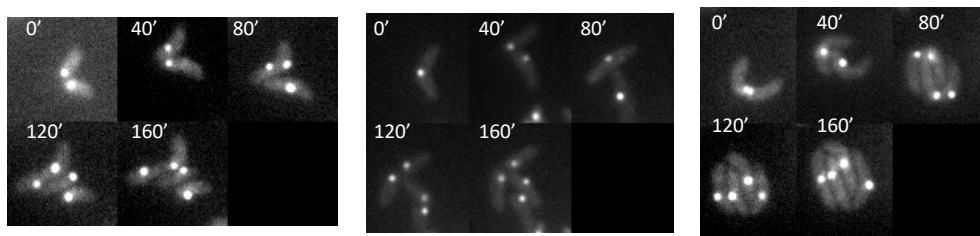


Figure. S3B: Representative timelapses of CP797 ($\Delta hubP \Delta parAB2 + parS1^{ectopic}::ori2$) presenting only the GFP signal of R2II (180kb from oriC2), as the mCherry signal had photobleached.

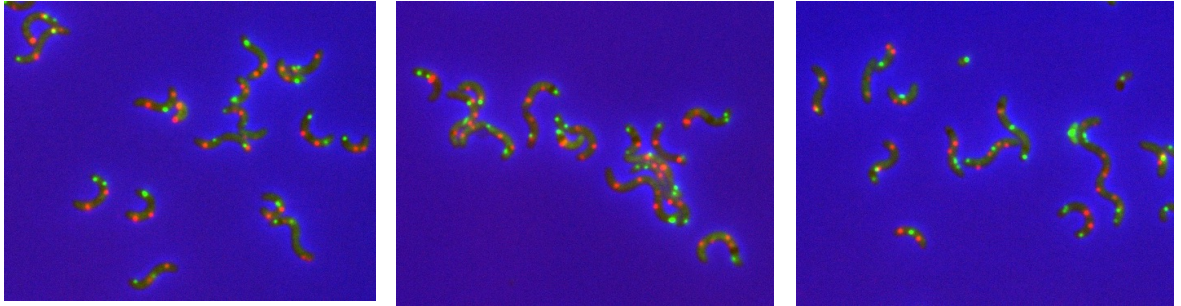
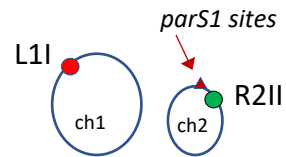


Figure. S4A: Representative Snapshots of CP799 ($\Delta parS1 \Delta parAB2 + parS1^{ectopic::ori2}$) containing dual labelling system (red-L1I (300kb from oriC1) and green R2II (180kb from oriC2))

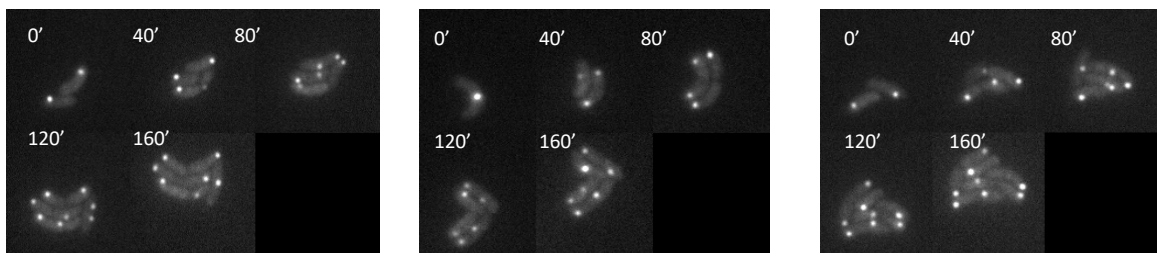


Figure. S4B: Representative timelapses of CP799 ($\Delta parS1 \Delta parAB2 + parS1^{ectopic::ori2}$) presenting only the GFP signal of R2II (180kb from oriC2), as the mCherry signal had photobleached.

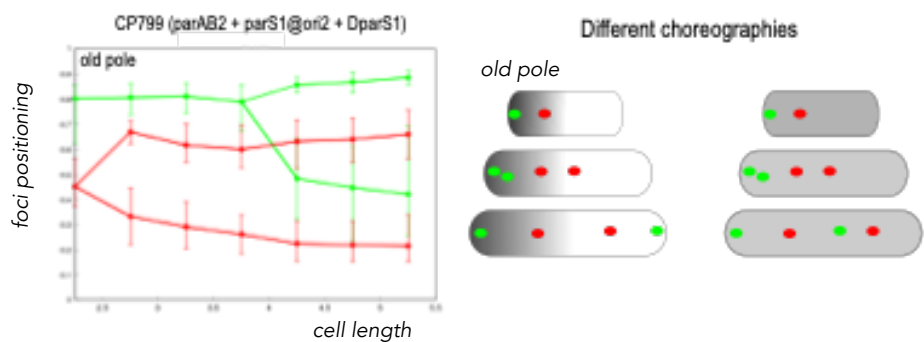
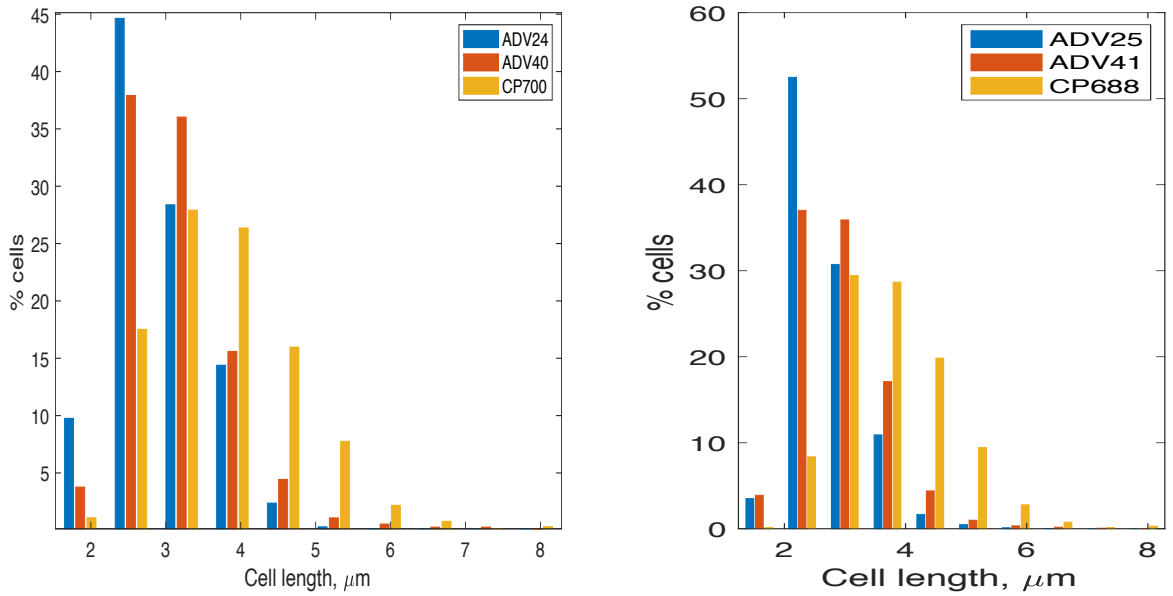


Figure S4C: Graph of reconstituted choreographies of L1I (red) and R2II (green) loci in CP799 ($\Delta parS1 \Delta parAB2 + parS1^{ectopic::ori2}$). The old pole is indicated by a gray gradient; while if no orientation biased is observed the entire cell in uniformly gray.

A



B

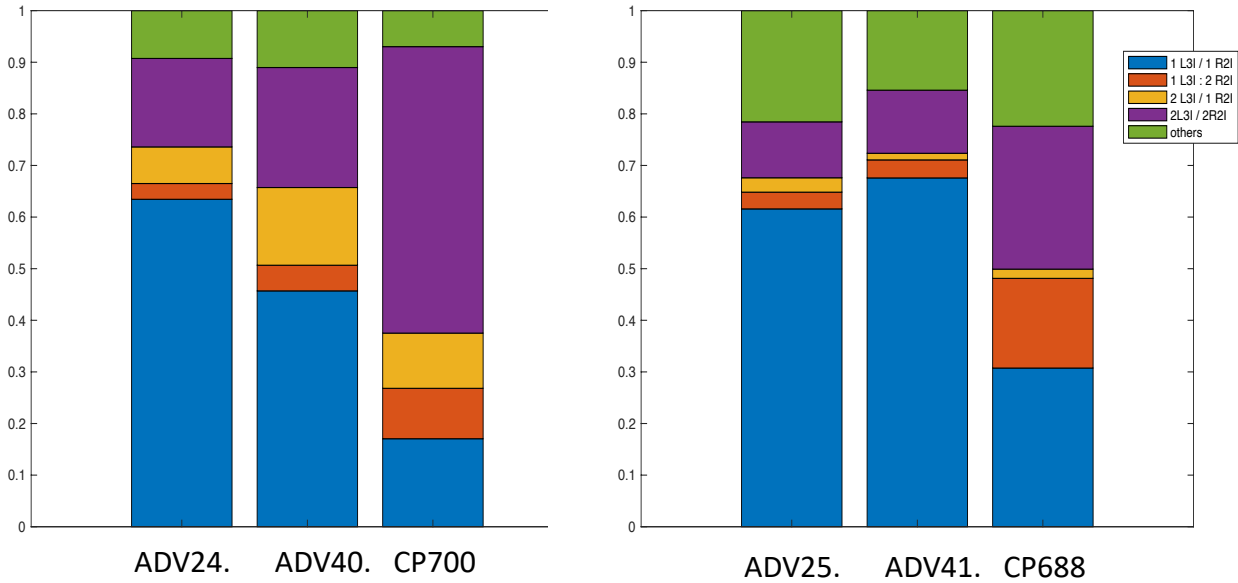
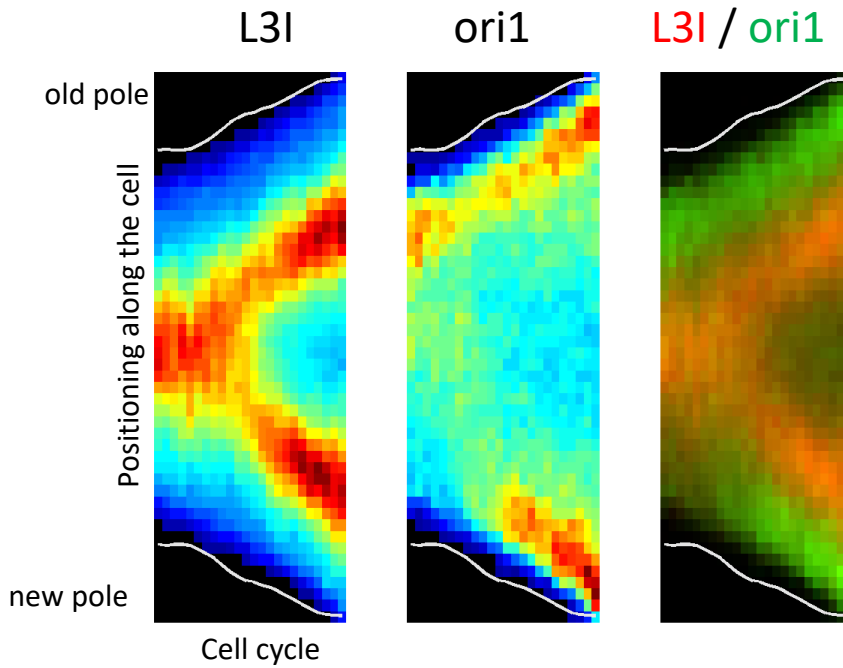


Figure S5: Characteristics of different strains: ADV24 (WT), ADV40 (*parS1*-deleted), CP700 (*hubP*-deleted) tagged at *ori1* and *L3I* and ADV25 (WT), ADV41 (*parS1*-deleted), CP688 (*hubP*-deleted) tagged at *L3I* and *R2I*. **(A)** Histogram representing the proportion of cells with a length ranging within the different 2 μm size intervals, from 2 to 8 μm . **(B)** Proportion of cells ranging in different categories defined from the number of « red » and « green » foci as indicated in the legend inset.

A



B

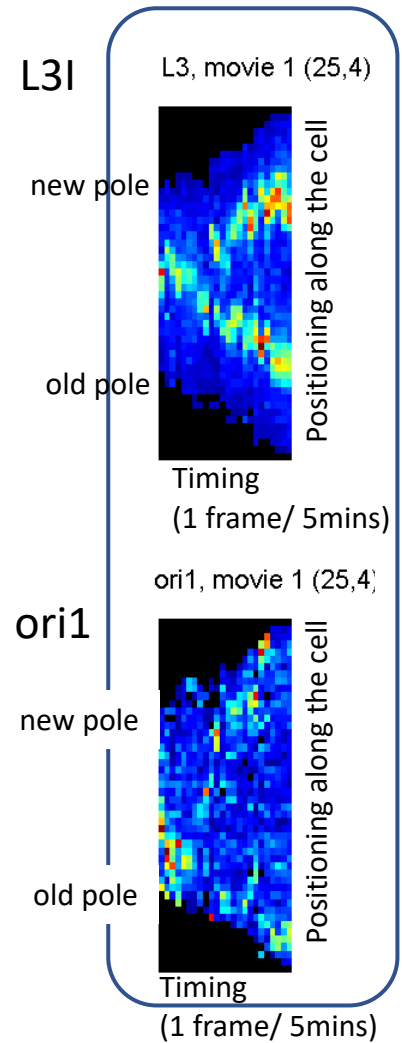
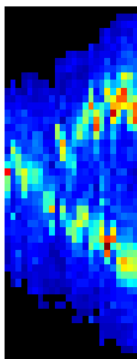


Figure. S6: Reconstituted choreographies of L3I and ori1 foci in CP700 from kymographs. **(A)** Compilation of the 32 lineages. Heatmap of L3I (left), ori1 (middle) positionings and an overlay of L3I and ori1 positionings in green and red, respectively. The positionings along the cell (y axis, old pole top and new pole bottom and heatmap color corresponds to the fluorescence intensity) are in function of the progression of the cell cycle (x axis, birth to division). **(B and B1-B4)** 32 individual lineages: each composed of the two kymographs of L3I (top) and ori1 (bottom). For each kymograph, X axis corresponds to the time (1 frame/ 5mins) and Y axis corresponds to the fluorescence data projected on the long axis of the cell for each images (here, old pole bottom and new pole bottom). The intensity of the fluorescence is indicated by the heatmap color. The number of frames depends of the duration of each independent cell cycle (birth to division). The numbers in brackets allowed to identify the lineage in the timelapse.

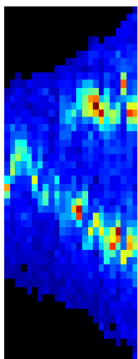
Figure.S6B1

L3I

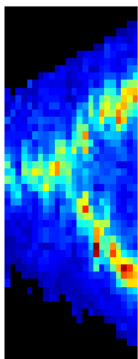
L3, movie 1 (25,4)



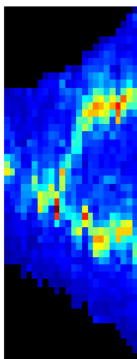
L3, movie 1 (25,3)



L3, movie 1 (32,2)

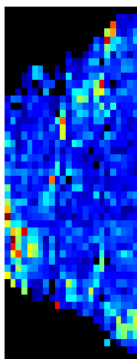


L3, movie 1 (32,1)

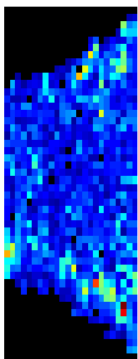


ori1

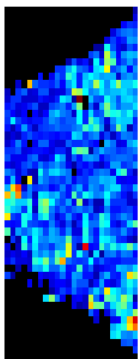
ori1, movie 1 (25,4)



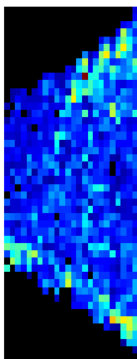
ori1, movie 1 (25,3)



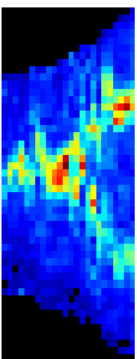
ori1, movie 1 (32,2)



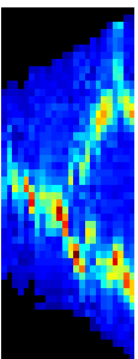
ori1, movie 1 (32,1)



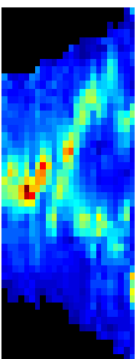
L3, movie 1 (36,9)



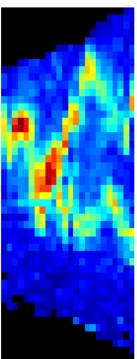
L3, movie 1 (36,10)



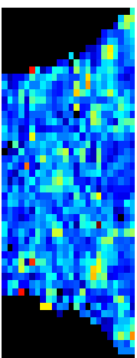
L3, movie 1 (47,13)



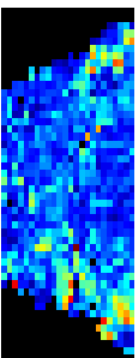
L3, movie 1 (45,15)



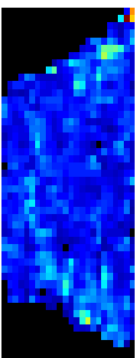
ori1, movie 1 (36,9)



ori1, movie 1 (36,10)



ori1, movie 1 (47,13)



ori1, movie 1 (45,15)

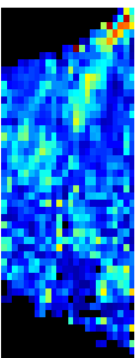


Figure.S6B3

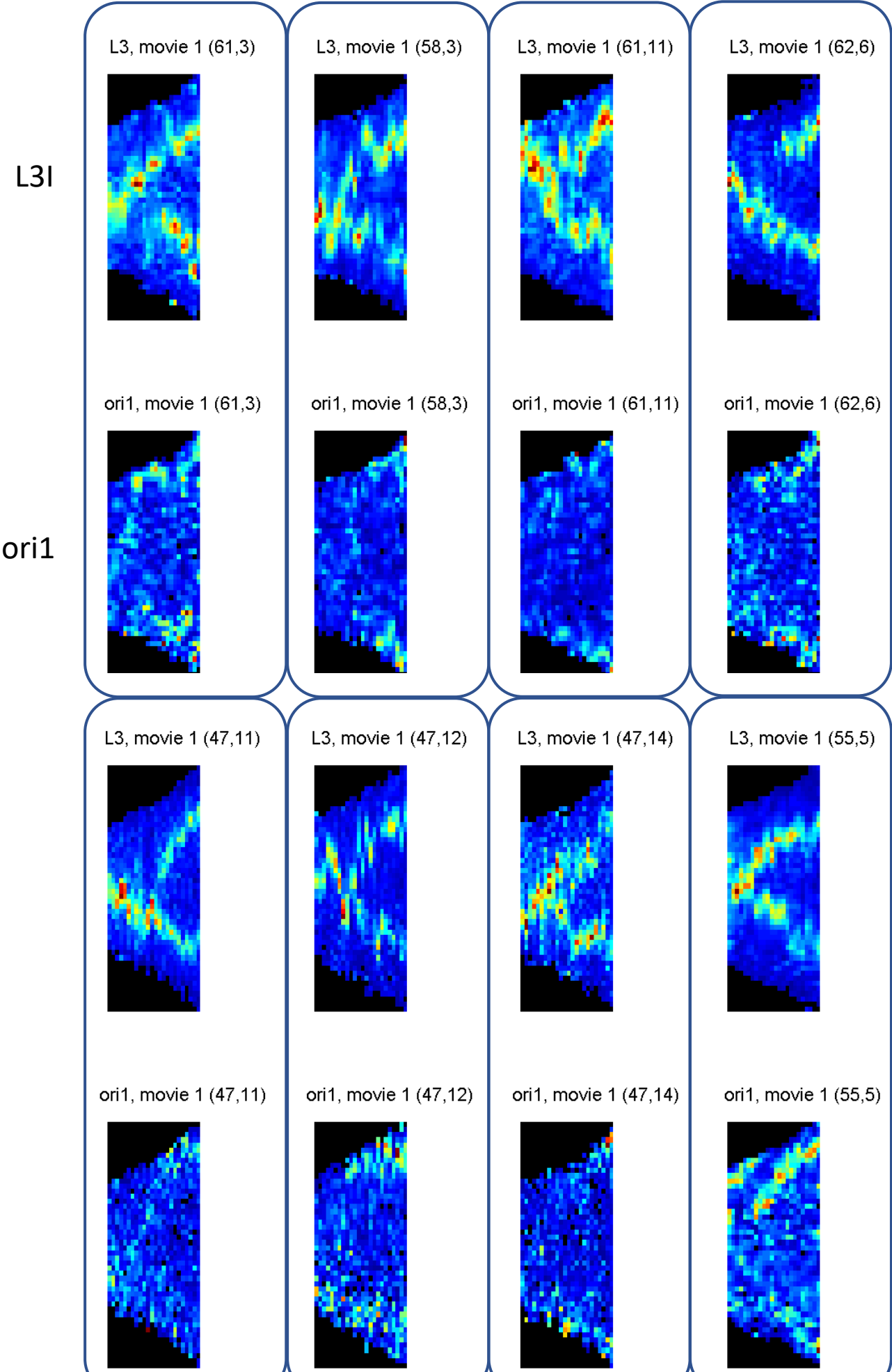
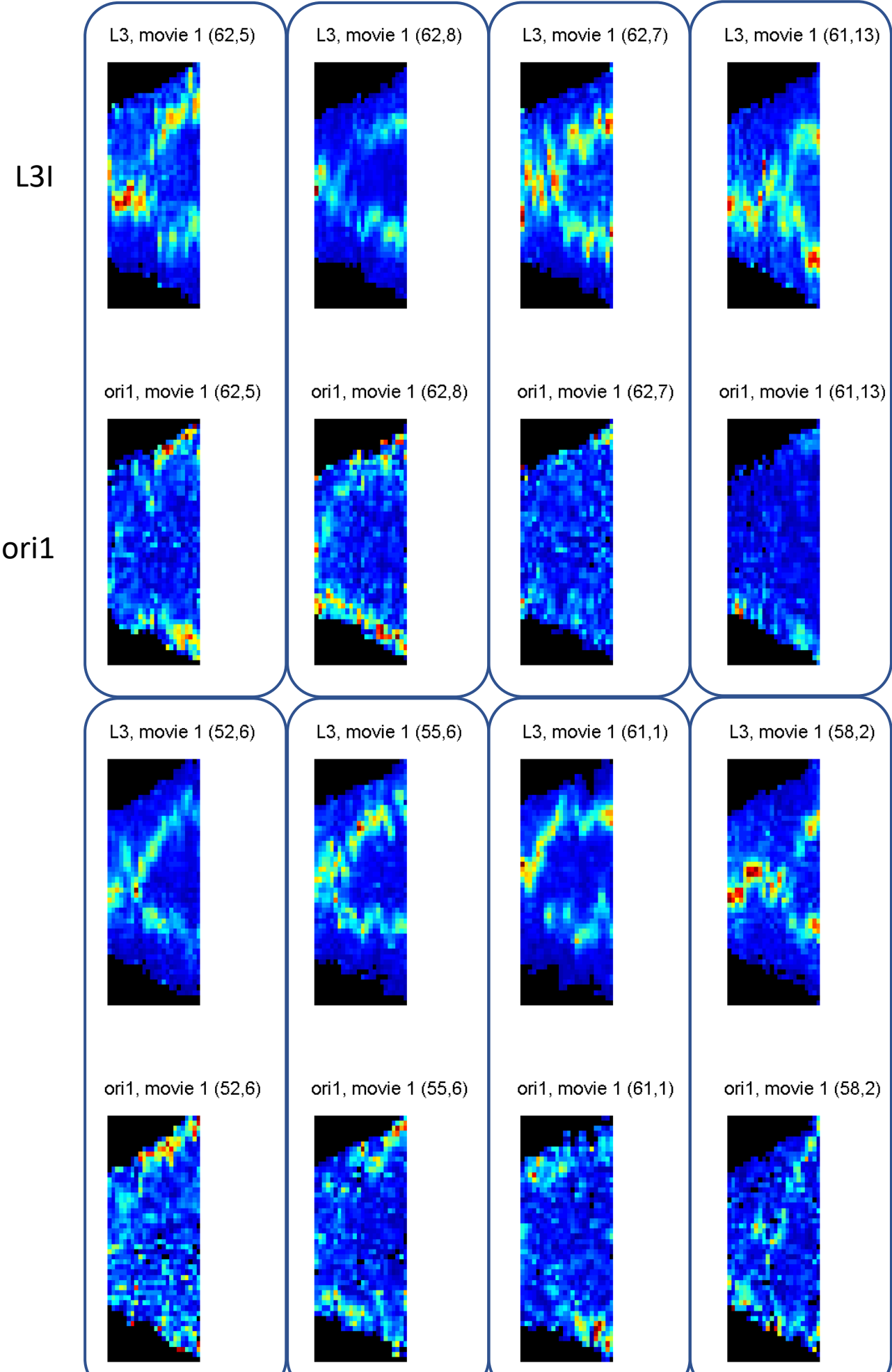
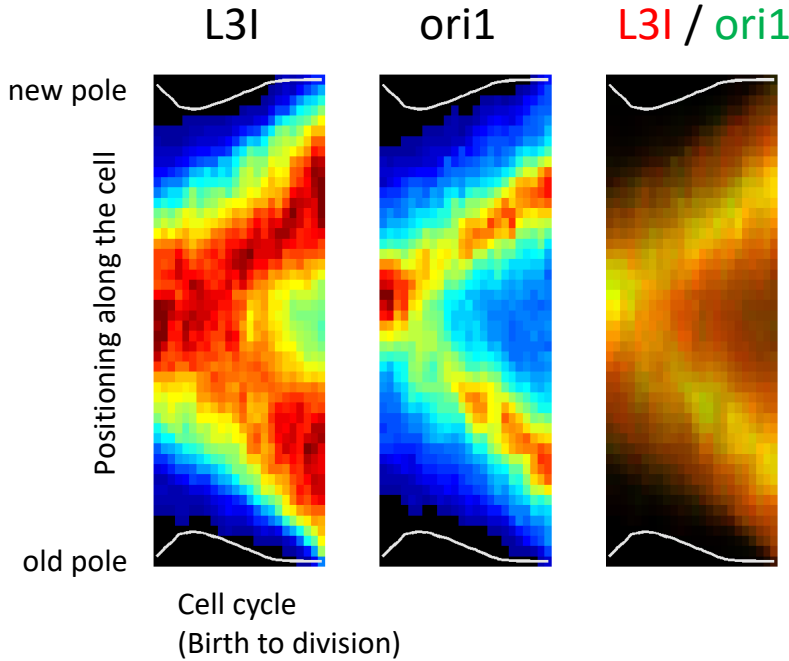


Figure.S6 B4



A



B

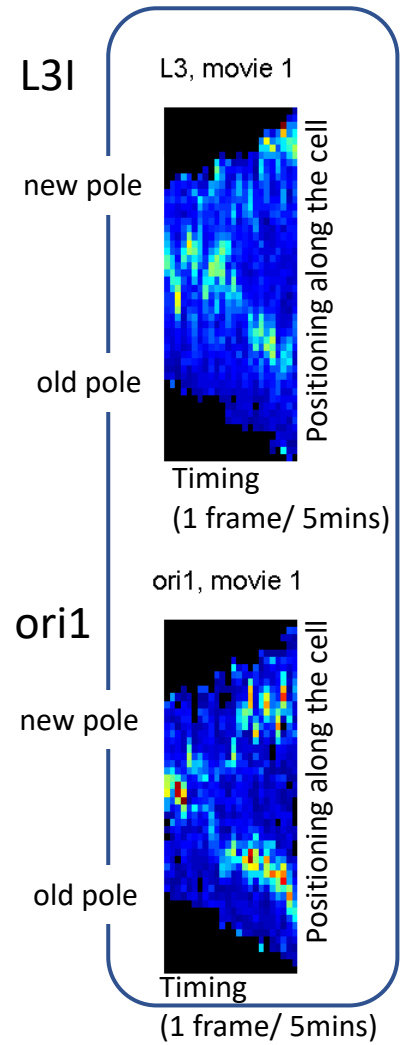


Figure. S7: Reconstituted choreographies of L3I and ori1 foci in CP700 from kymographs. **(A)** Compilation of the 56 lineages. Heatmap of L3I (left), ori1 (middle) positionings and an overlay of L3I and ori1 positionings in green and red, respectively. The positionings along the cell (y axis, old pole top and new pole bottom) are in function of the progression of the cell cycle (x axis, birth to division and heatmap color corresponds to the fluorescence intensity). **(B and B1-B7)** 56 individual lineages: each composed of the two kymographs of L3I (top) and ori1 (bottom). For each kymograph, X axis corresponds to the time (1 frame/ 5mins) and Y axis corresponds to the fluorescence data projected on the long axis of the cell for each images (here, old pole bottom and new pole bottom). The intensity of the fluorescence is indicated by the heatmap color. The number of frames depends of the duration of each independent cell cycle (birth to division). The numbers in brackets allowed to identify the lineage in the timelapse.

Figure.S7B1

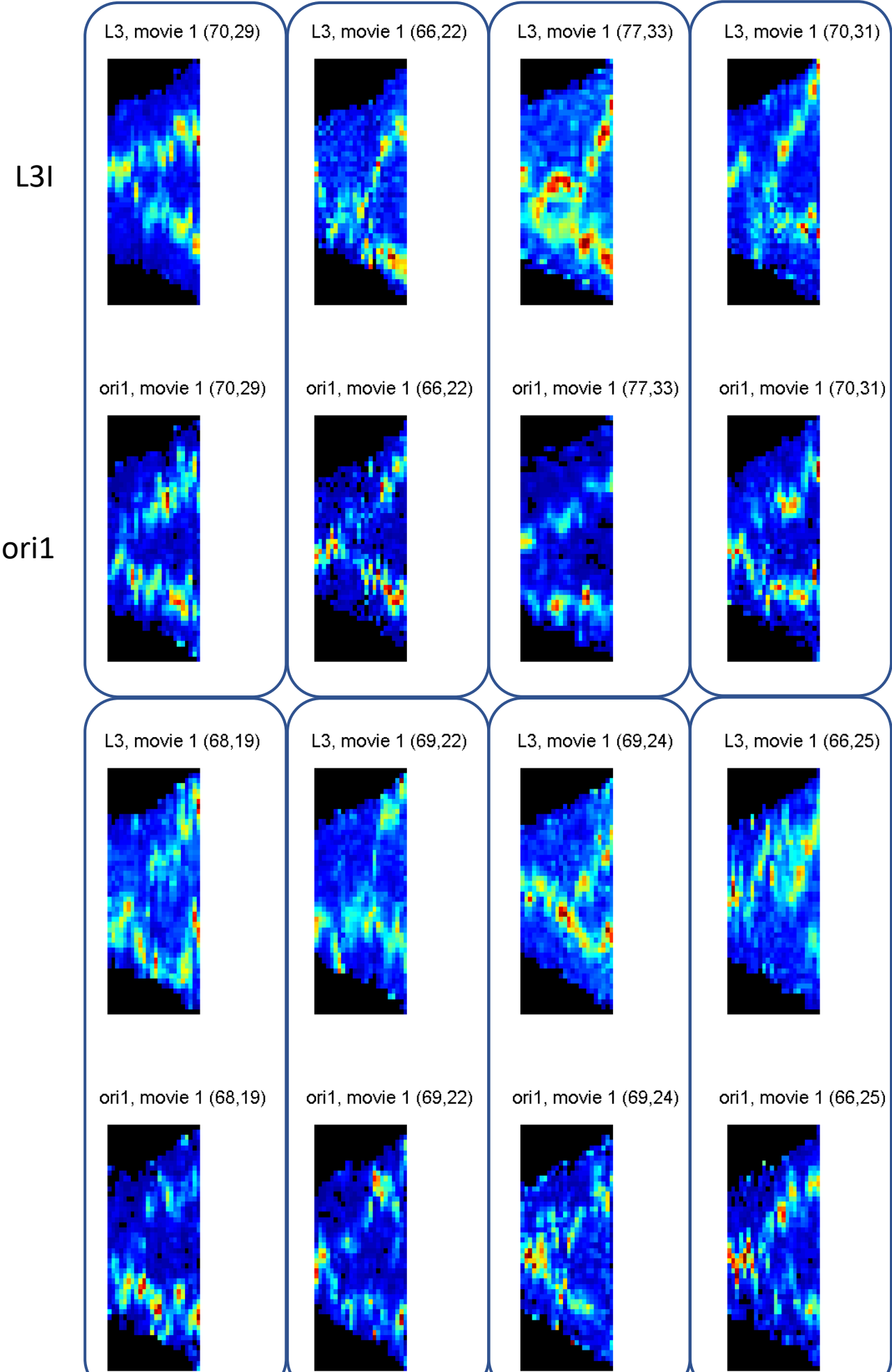


Figure.S7B2

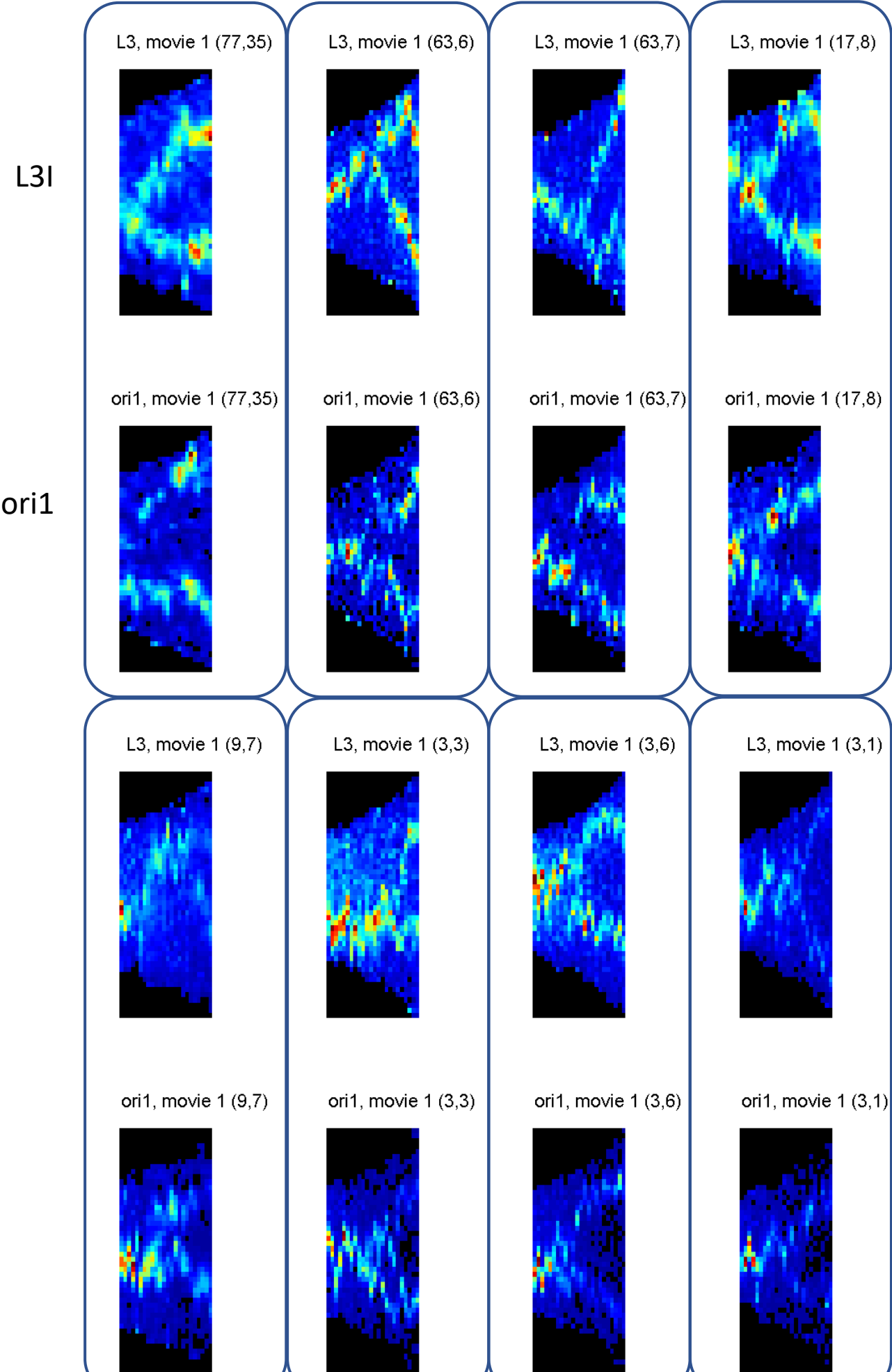


Figure.S7B3

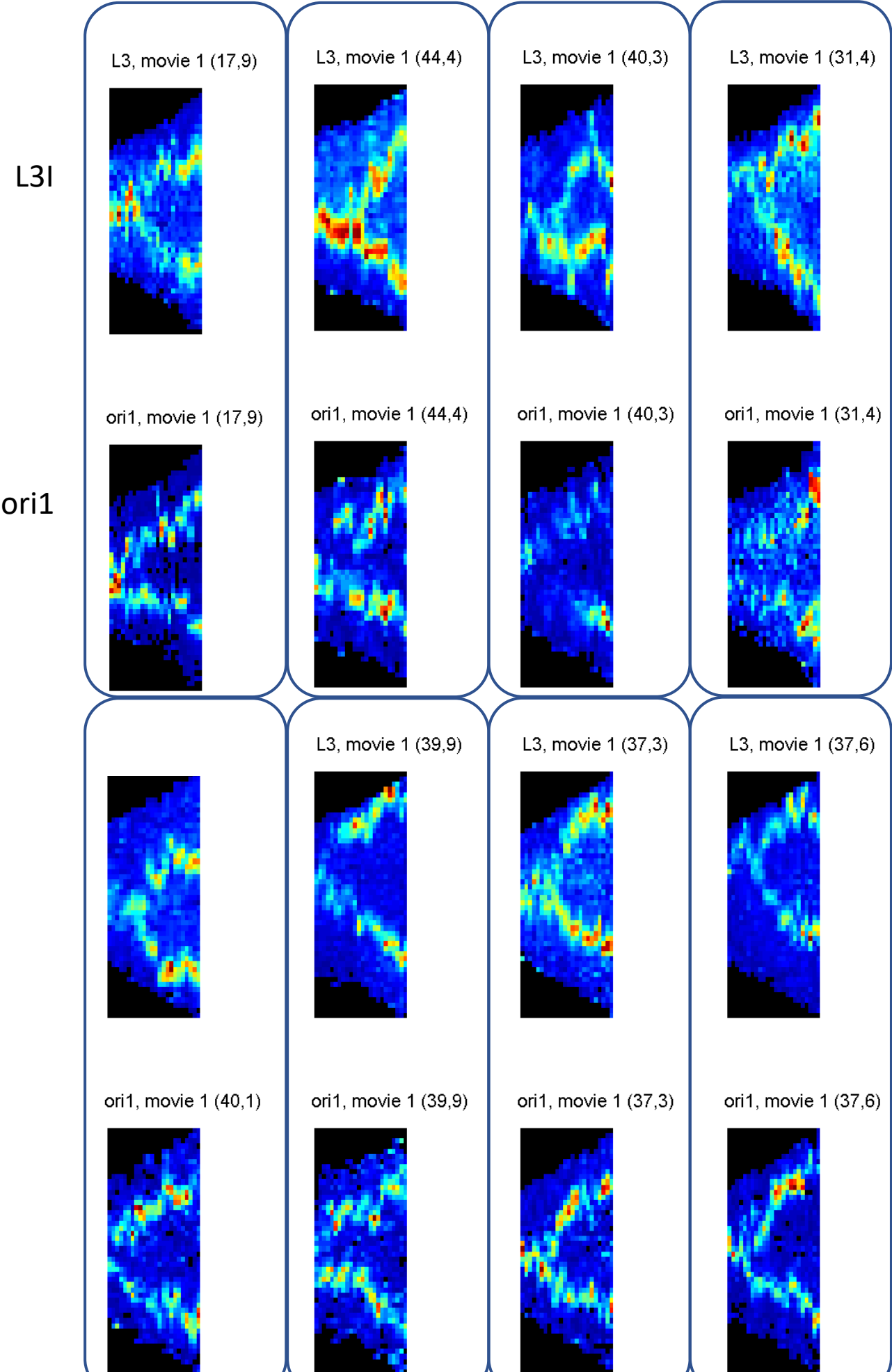


Figure.S7B4

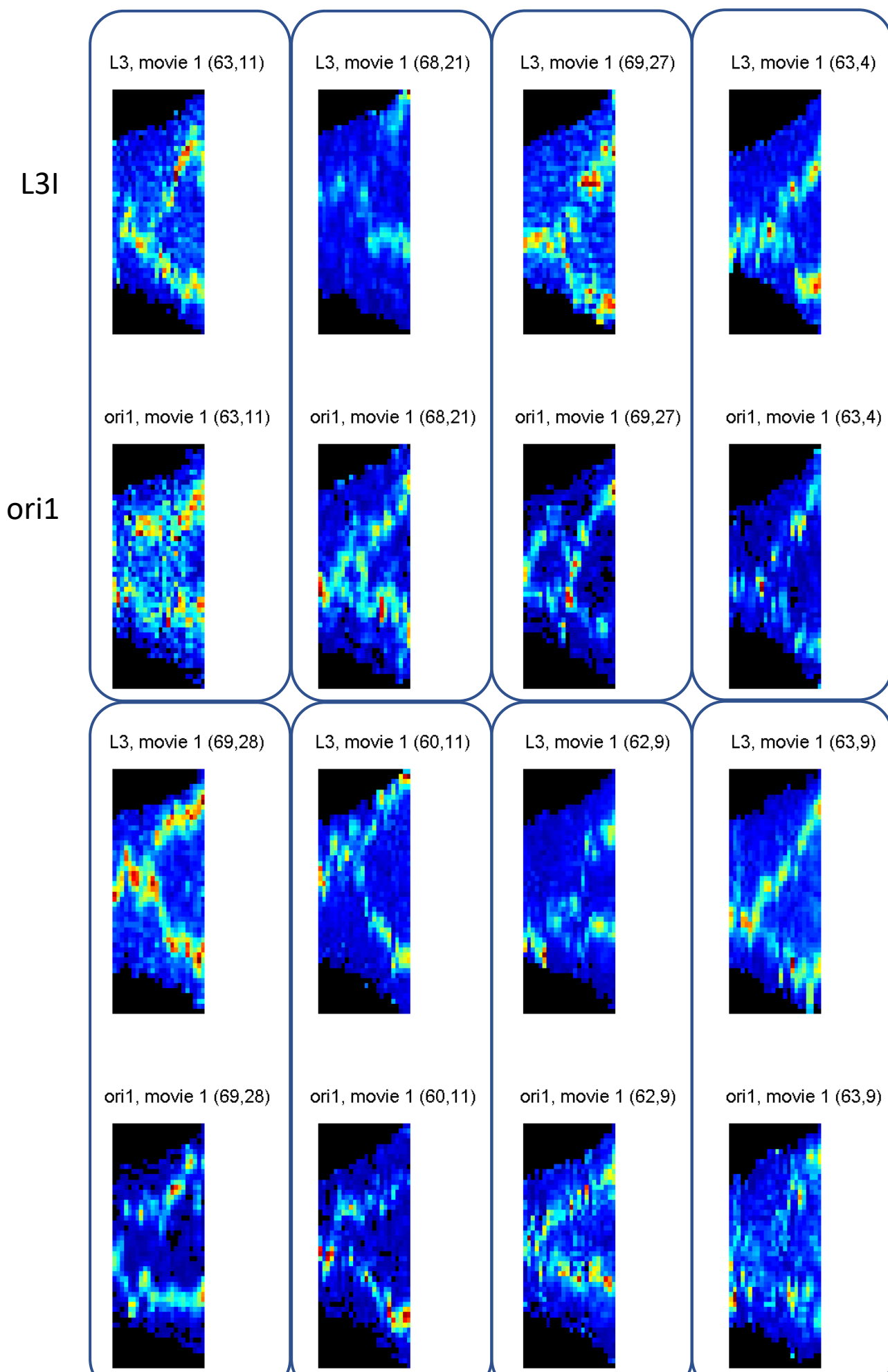
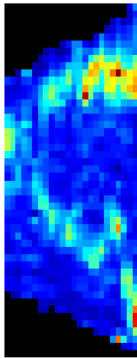


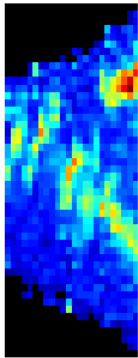
Figure.S7B5

L3I

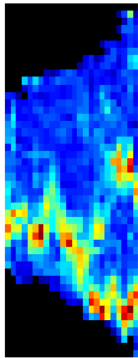
L3, movie 1 (70,34)



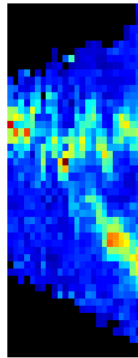
L3, movie 1 (66,30)



L3, movie 1 (64,4)

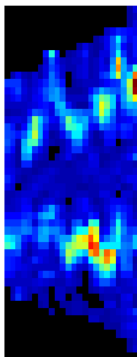


L3, movie 1 (62,10)

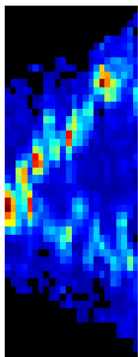


ori1

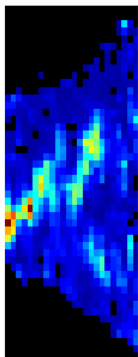
ori1, movie 1 (70,34)



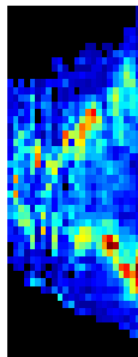
ori1, movie 1 (66,30)



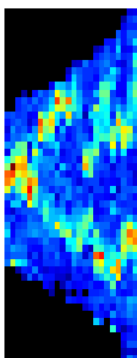
ori1, movie 1 (64,4)



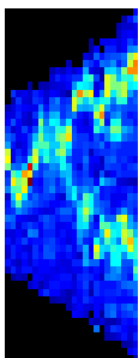
ori1, movie 1 (62,10)



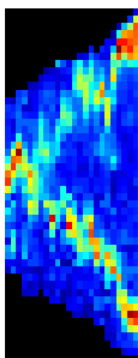
L3, movie 1 (56,9)



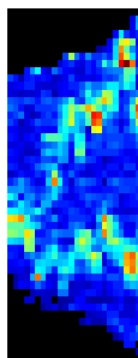
L3, movie 1 (60,6)



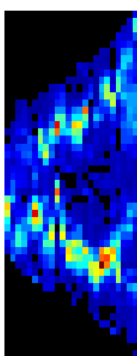
L3, movie 1 (59,13)



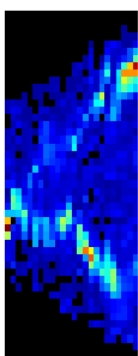
L3, movie 1 (70,30)



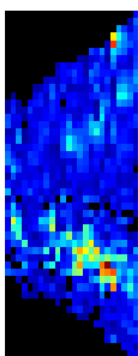
ori1, movie 1 (56,9)



ori1, movie 1 (60,6)



ori1, movie 1 (59,13)



ori1, movie 1 (70,30)

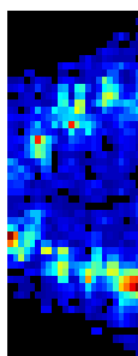


Figure.S7B6

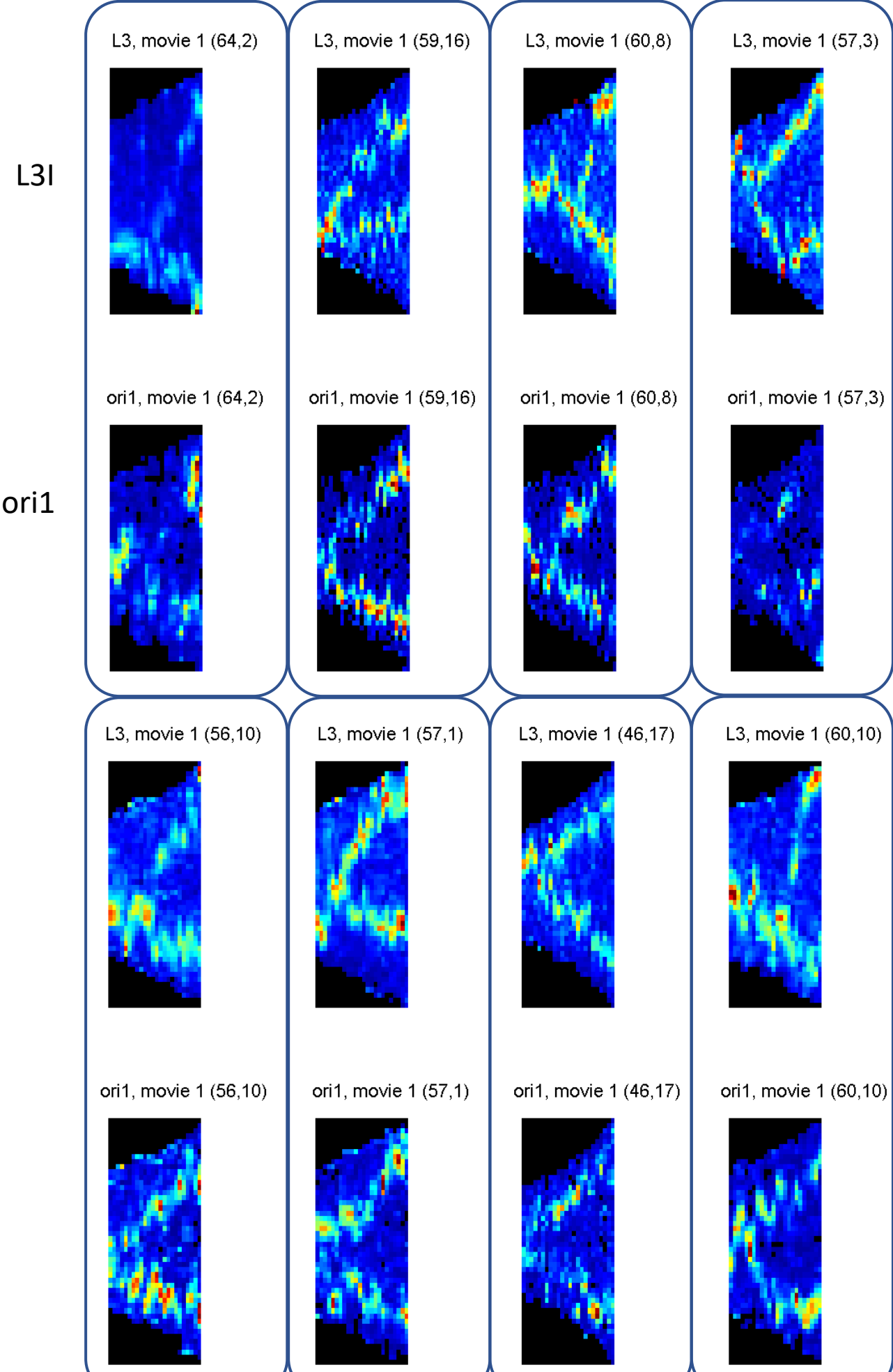
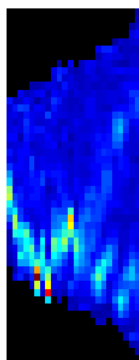


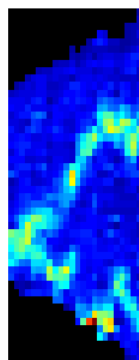
Figure.S7B7

L3I

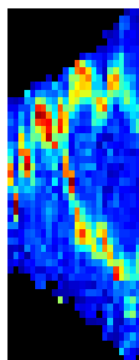
L3, movie 1 (44,10)



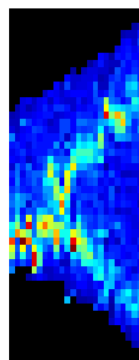
L3, movie 1 (51,14)



L3, movie 1 (43,12)

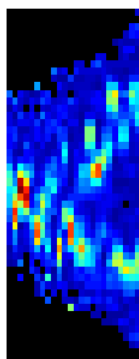


L3, movie 1 (43,13)

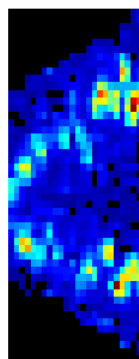


ori1

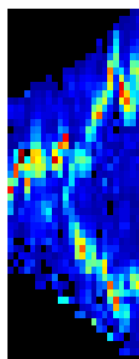
ori1, movie 1 (44,10)



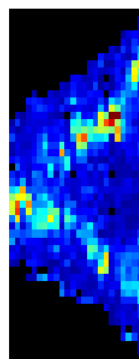
ori1, movie 1 (51,14)



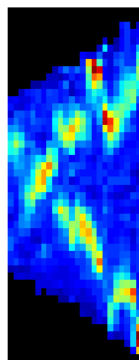
ori1, movie 1 (43,12)



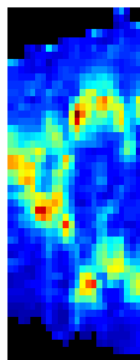
ori1, movie 1 (43,13)



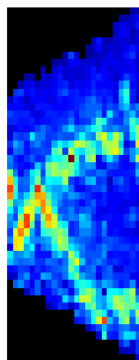
L3, movie 1 (44,2)



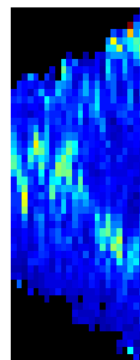
L3, movie 1 (51,12)



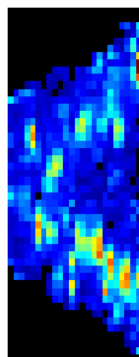
L3, movie 1 (46,16)



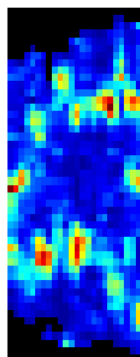
L3, movie 1 (44,11)



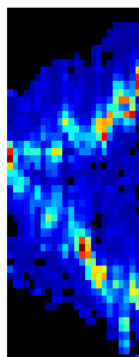
ori1, movie 1 (44,2)



ori1, movie 1 (51,12)



ori1, movie 1 (46,16)



ori1, movie 1 (44,11)

