

## SUPPLEMENTARY MATERIAL

### *Movie S1\_Young embryo 240nM SiR-actin*

Darkfield and widefield fluorescent time lapse movie of chicken embryo cultured *in vitro* in a submerged filter paper sandwich in the presence of 240nM SiR-actin in the culture medium. Development progressed from the 3-somite stage embryo (HH7 up to 10-somite stage (HH10) during 12 hours of incubation.

[Movie S1\\_Young embryo 240nM SiR-actin](#)

Video URL: <https://youtu.be/DtfjRQWlqvg>

### *Movie S2\_240 nM SiR-actin high magnification*

Formation of somites in chicken embryo, pre-incubated with 240nM SiR-actin overnight prior to imaging.

[Movie S2\\_240 nM SiR-actin high magnification](#)

Video URL: <https://youtu.be/6le4w6mynZI>

### *Movie S3\_older embryo 50nM SiR-actin*

Darkfield and widefield fluorescent time lapse movie of chicken embryo cultured *in vitro* in a submerged filter paper sandwich in the presence of 50nM SiR-actin in the culture medium. Developed progressed from the 8-somite stage embryo (HH9 to 9+) up to 21-somite stage (HH13-14) during 28 hours of incubation.

[Movie S3\\_older embryo 50nM SiR-actin](#)

Video URL: <https://youtu.be/YcHdMUbtQg>

### *Movie S4\_SiR-actin stretched embryo*

DF and WF microscopic time lapse images of the segmenting paraxial mesoderm in a stretched chicken embryo cultured *in vitro* in a submerged filter paper sandwich in presence of 50 nM SiR-actin. The movie starts after the end of stretching protocol.

[Movie S4 SiR-actin stretched embryo](#)

Video URL: <https://youtu.be/2b-gCWH-Gf0>

**Table S1. Microscope settings Leica SP5.** Overview of settings used to acquire micrograph presented in Figure 3 B (left table) and Figure 3 C (right table).

Dimension	Logical Size	Physical Length	Physical Design	Dimension	Logical Size	Physical Length	Physical Design
X	1024	492.05 $\mu\text{m}$	0 $\mu\text{m}$	X	1024	580.48 $\mu\text{m}$	0 $\mu\text{m}$
Y	1024	492.05 $\mu\text{m}$	0 $\mu\text{m}$	Y	1024	580.48 $\mu\text{m}$	0 $\mu\text{m}$
T	5	8m0.000 s	0 s	T	24	40m0.000 s	0 s
<b>Scanner Settings</b>				<b>Scanner Settings</b>			
UV Shutter		0		UV Shutter		0	
Visible Shutter		1		Visible Shutter		1	
Scan Mode		xyt		Scan Mode		xyt	
Pinhole (m)		50 $\mu\text{m}$		Pinhole (m)		110 $\mu\text{m}$	
Pinhole (airy)		896.83		Pinhole (airy)		1.97	
Size-Width		492.06 $\mu\text{m}$		Size-Width		590.48 $\mu\text{m}$	
Size-Height		492.06 $\mu\text{m}$		Size-Height		590.48 $\mu\text{m}$	
Voxel-Width		481 nm		Voxel-Width		577.2 nm	
Voxel-Height		481 nm		Voxel-Height		577.2 nm	
Zoom		3		Zoom		2.5	
Scan-Direction		2		Scan-Direction		2	
Sequential Mode		0		Sequential Mode		0	
Frame Accumulation		1		Frame Accumulation		1	
Frame Average		1		Frame Average		1	
Line Average		3		Line Average		5	
Resolution		8 bits		Resolution		8 bits	
Channels		2		Channels		2	
Format-Width		1024 pixels		Format-Width		1024 pixels	
Format-Height		1024 pixels		Format-Height		1024 pixels	
Line-Accumulation		1		Line-Accumulation		1	
Iterations		1000		Iterations		1000	
Size-Depth		480 s		Size-Depth		2.8 ks	
AOTF(405)		0.00 %		AOTF(405)		0.00 %	
AOTF(458)		0.00 %		AOTF(458)		0.00 %	
AOTF(476)		0.00 %		AOTF(476)		0.00 %	
AOTF(488)		0.00 %		AOTF(488)		0.00 %	
AOTF(496)		0.00 %		AOTF(496)		0.00 %	
AOTF(514)		0.00 %		AOTF(514)		0.00 %	
AOTF(561)		0.00 %		AOTF(561)		0.00 %	
AOTF(633)		20.00%		AOTF(633)		13.00%	
HyD 1		Active		HyD 1		Active	
HyD 1 (Gain)		61.0		HyD 1 (Gain)		61.0	
HyD 1 (AcquisitionMode)		Standard		HyD 1 (AcquisitionMode)		Standard	
HyD 1 (OverloadState)		OK		HyD 1 (OverloadState)		OK	
PMT 2		Active		PMT 2		Active	
PMT 2 (Offs)		0.0 %		PMT 2 (Offs)		0.0 %	
PMT 2 (HV)		0.0		PMT 2 (HV)		0.0	
PMT 2 (HV_Unit)		V		PMT 2 (HV_Unit)		V	
PMT 2 (Preamp)		Direct		PMT 2 (Preamp)		Direct	
PMT 3		Inactive		PMT 3		Inactive	
System Number		5100001274		System Number		5100001274	
Laser (405 Diode, UV)		Off		Laser (405 Diode, UV)		Off	
Laser (Argon, visible)		Off		Laser (Argon, visible)		Off	
Laser (Argon, visible) (Power)		-11 %		Laser (Argon, visible) (Power)		-11 %	
Laser (DPSS 561, visible)		On		Laser (DPSS 561, visible)		On	

Laser (HeNe 633, visible)	On	Laser (HeNe 633, visible)	On
Scan Field Rotation	8 degrees	Scan Field Rotation	8 degrees
Scan Speed	200 Hz	Scan Speed	200 Hz
Objective	10.0x0.40 DRY UV	Objective	10.0x0.40 DRY UV
Numerical aperture (Obj.)	400	Numerical aperture (Obj.)	400
Refraction index	1	Refraction index	1
DM6000 Stage Pos x	0.01279385575423	DM6000 Stage Pos x	0.01284464186101
DM6000 Stage Pos 7	0.01040080522166	DM6000 Stage Pos 7	0.01033960896393
Emission bandwidth PMT 2: begin - end	782nm – 790nm	Emission bandwidth PMT 2: begin - end	782nm – 790nm

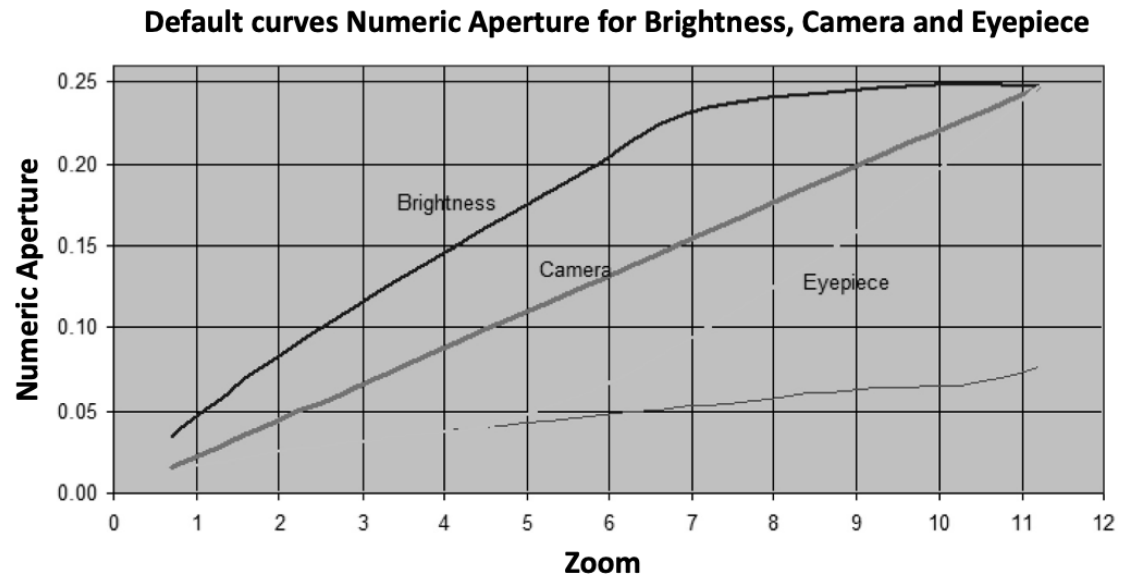
**Table S2. Microscope settings Leica SP5.** Overview of settings used to acquire micrograph presented in Figure 3 D.

Dimension	Logical Size	Physical Length	Physical Design
X	512	234.32 $\mu$ m	0 $\mu$ m
Y	512	234.32 $\mu$ m	0 $\mu$ m
<b>Scanner Settings</b>			
UV Shutter		0	
Visible Shutter		1	
Scan Mode		xyt	
Pinhole (m)		600 $\mu$ m	
Pinhole (airy)		3.85	
Size-Width		234.32 $\mu$ m	
Size-Height		234.32 $\mu$ m	
Size-Depth		0 $\mu$ m	
StepSize		0.05 $\mu$ m	
Voxel-Width		458.5 nm	
Voxel-Height		458.5 nm	
Zoom		1	
Scan-Direction		2	
Sequential Mode		0	
Frame Accumulation		1	
Frame Average		1	
Line Average		1	
Resolution		8 bits	
Channels		2	
Format-Width		512 pixels	
Format-Height		512 pixels	
Line-Accumulation		1	
Section		1	
AOTF(405)		0.00 %	
AOTF(458)		0.00 %	
AOTF(476)		0.00 %	
AOTF(488)		0.00 %	
AOTF(496)		0.00 %	
AOTF(514)		0.00 %	
AOTF(561)		0.00 %	
AOTF(633)		25.00%	
HyD 1		Active	
HyD 1 (Gain)		50.0	

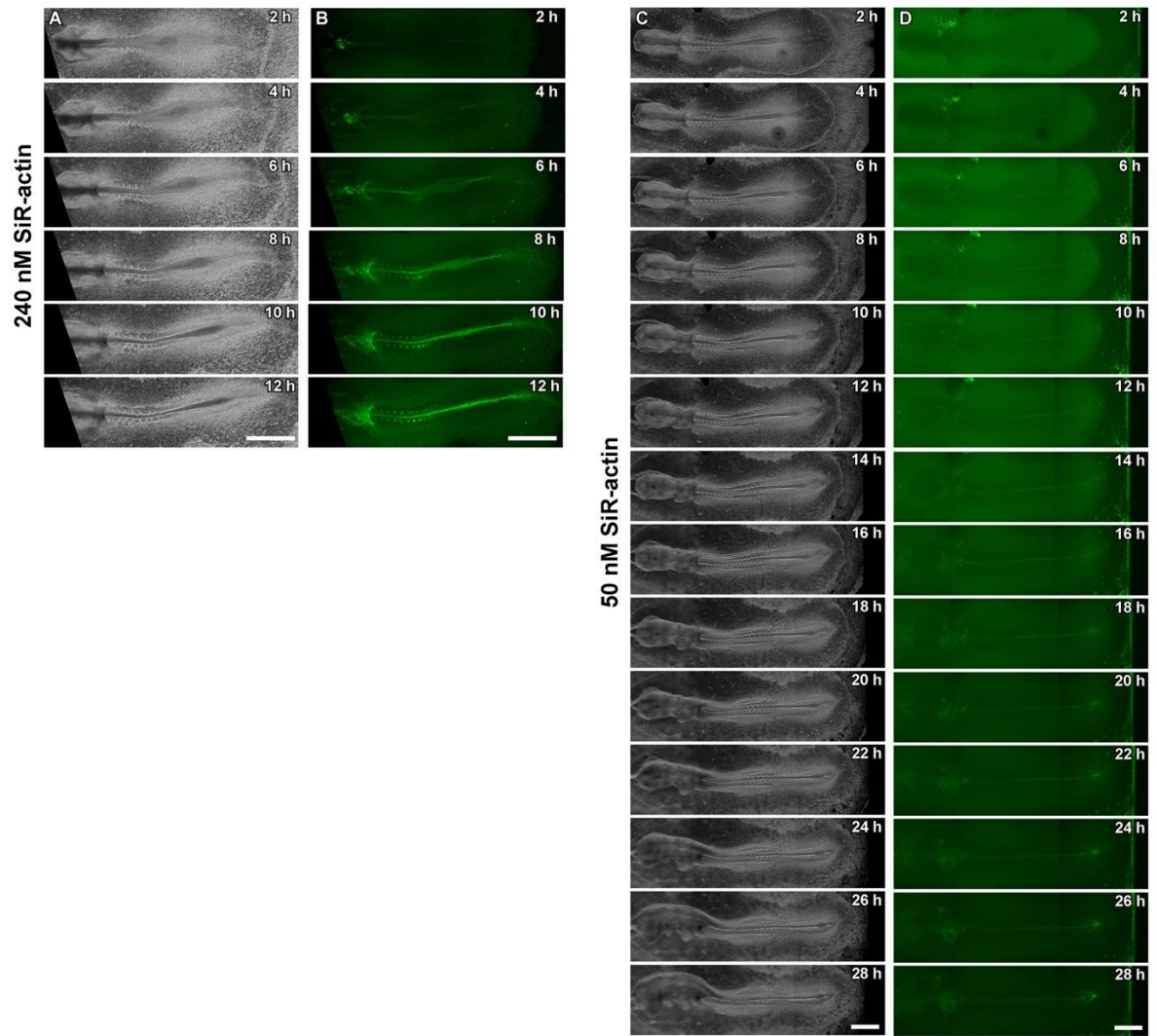
HyD 1 (AcquisitionMode)	Standard
HyD 1 (OverloadState)	OK
PMT 2	Active
PMT 2 (Offs)	0.0 %
PMT 2 (HV)	0.0
PMT 2 (HV_Unit)	V
PMT 2 (Preamp)	Direct
PMT 3	Inactive
System Number	5100001274
Laser (405 Diode, UV)	Off
Laser (Argon, visible)	Off
Laser (Argon, visible) (Power)	-11 %
Laser (DPSS 561, visible)	On
Laser (HeNe 633, visible)	On
Scan Field Rotation	0 degrees
Scan Speed	100 Hz
Objective	HCX APO L U-V-I 63.0x0.90 Water UV
Numerical aperture (Obj.)	900
Refraction index	1.33
DM6000 Stage Pos x	0.01261429041648
DM6000 Stage Pos 7	0.01029187949618
Emission bandwidth PMT 2: begin - end	782nm – 790nm

**Table S3. Average somite formation time.** Number of somites that were completely separated from the anterior tip of the PSM were counted at selected time-points during imaging. Darkfield microscopic time lapse images were used.

	Sample	SiR-actin concentration [nM]	No. somites formed	Elapsed time [min]	Average time/somite [min]
Control	1	0	11	770	77
	2	0	16	1300	86.7
	3	0	19	1329	73.8
SiR	1	50	14	1166	89.7
	2	50	15	1130	80.7
	3	240	9	711	88.9



**Figure S1. Numerical aperture Zeiss PlanNeofluar 1.0x objective.** NA of objective in dependence of zoom value as received as information from Zeiss (black line).



**Figure S2. Embryonic development and gradual increase of SiR-actin fluorescence in chick embryos cultured ex ovo with 240 nM SiR-actin (left) and 50 nM SiR-actin (right) at intervals of two hours. .**