



HAL
open science

Mechanical instabilities of aorta drive blood stem cell production: a live study

Nausicaa Poulet, Ivan Golushko, Vladimir Lorman, Jana Travnickova, Dmitryi Chalin, Sergei Rochal, Andrea Parmeggiani, Karima Kissa

► To cite this version:

Nausicaa Poulet, Ivan Golushko, Vladimir Lorman, Jana Travnickova, Dmitryi Chalin, et al.. Mechanical instabilities of aorta drive blood stem cell production: a live study. 2019. hal-01996796

HAL Id: hal-01996796

<https://hal.umontpellier.fr/hal-01996796>

Preprint submitted on 28 Jan 2019

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 **Mechanical instabilities of aorta drive blood stem cell production:**
2 **a live study.**

3
4 Nausicaa Pouillet¹, Ivan Golushko^{2,3}, Vladimir Lorman², Jana Travnickova¹, Dmitryi Chalin³,
5 Sergei Rochal³, Andrea Parmeggiani^{1,2} and Karima Kissa^{1,*}.

6
7 ¹ DIMNP, CNRS, INSERM, Univ Montpellier, Montpellier, France

8 ² Laboratoire Charles Coulomb, CNRS, Univ Montpellier, Montpellier, France

9 ³ Faculty of Physics, Southern Federal University, 5 Zorge str., Rostov-on-Don, Russia

10

11 *Correspondence: karima.kissa-marin@umontpellier.fr

12

13

14

15

16

17

18

19

20

21

22

23

24

25

26

27

28

29

30

31

During embryogenesis of all vertebrates, haematopoietic stem/progenitor cells (HSPCs) extrude from the aorta by a complex process named Endothelial-to-Haematopoietic Transition (EHT). HSPCs will then colonize haematopoietic organs allowing haematopoiesis throughout adult life. The mechanism underlying EHT including the role of each aortic endothelial cell within the global aorta dynamics remains unknown. In the present study, we show for the first time that EHT involves the remodelling of individual cells within a collective migration of endothelial cells which is tightly orchestrated, resulting in HSPCs extrusion in the sub-aortic space without compromising aorta integrity. By performing a cross-disciplinary study which combines high resolution 4D imaging and theoretical analysis based on the concepts of classical mechanics, we propose that this complex developmental process is dependent on mechanical instabilities of the aorta preparing and facilitating the extrusion of HSPCs.

We dedicate this work to the memory of our friend and colleague, V. Lorman.

32 **Introduction**

33

34 Transplantation of human blood cells is essential to regularly save lives on a large-scale.
35 However, this method requires an exogenous allogenic source to avoid histoincompatibility
36 as well as graft versus host disease associated problems. Currently, haematopoietic
37 stem/progenitor cells (HSPCs) can only be produced *in vitro* by methods involving genetic
38 cellular reprogramming. Different groups succeeded in it, however these approaches remain
39 scientifically and technically challenging¹⁻⁴. Moreover, the presence of transgenes in the
40 genome of reprogrammed human HSPCs represents an important clinical risk⁵.

41 In order to develop new methods to generate HSPCs *in vitro* and control their fate after
42 transplantation, we need to further deepen our knowledge on blood cells ontogenesis at tissue
43 and organism levels, considering also novel features like biomechanical forces experienced by
44 HSPCs in physiological conditions. Indeed, the 3D *in vivo* structure of the tissue from which
45 HSPCs are generated is subjected to mechanical forces that influence cellular properties and
46 processes like cell migration, adhesion and polarity^{6,7}. Moreover, mechanical stress is known
47 to have a major impact on gene expression modulation and consequently on developmental
48 processes⁸, inflammation and cancer⁹.

49 In this article, we address for the first time the question of HSPCs production from the aorta
50 in relationship with the growth of the zebrafish embryo, the most widely used animal model
51 to study developmental processes in real time. We discuss and underline that, together with
52 haemodynamic forces, the growth of the whole embryo generate mechanical stresses on the
53 aorta and play a crucial role in blood production.

54 Previously, we and colleagues have demonstrated that HSPCs emerge in the Aorta Gonad
55 Mesonephros (AGM) region¹⁰⁻¹³, from the ventral wall of the dorsal aorta (DA)¹⁴⁻¹⁶. We
56 named this process Endothelial-to-Haematopoietic Transition or EHT¹⁴. In zebrafish, EHT
57 takes place during a specific time window between 30 and 65 hours post fertilization
58 (h.p.f.)^{12,14,17}. Systematic tracking of aortic endothelial cells (EC) in live embryos showed that
59 HSPCs emerge from the aortic ventral floor through a process that involves a strong shape
60 change followed by the egress of single cells from the aortic ventral wall into the sub-aortic
61 space¹⁴. Moreover, we and colleagues observed that the extrusion of HSPCs was aborted
62 when the *runx1* transcription factor essential for HSPCs emergence was inhibited^{14,18}.
63 Surprisingly, this inhibition affected neither aorta formation nor any events preceding HSPCs
64 extrusion, such as aorta radius dilation and contraction. A more recent study has then put in
65 evidence with wealth of details the cytoskeletal processes occurring in the single cell
66 dynamics during the HSPC egress from the aorta¹⁹.

67 In the present study, with the help of 4D confocal microscopy, we follow and quantify at
68 tissue level, the whole aorta behaviour as well as the one of its cells throughout EHT between
69 24 and 72 h.p.f. We put in evidence important structural changes of the aorta and the
70 collective migration of its lateral cells down to the aorta floor prior to HSPC egress. These
71 phenomena result in a global aorta remodelling in terms of number and localisation of
72 endothelial cells, thus showing an ongoing global cellular reorganisation of the tissue that
73 assures aorta integrity during EHT. We then analyse the role of the actin cytoskeleton both in
74 emerging and neighbouring cells during the HSPCs extrusion process.

75 Based on these observations and applying general principles of mechanics to the novel
76 context of EHT, we relate the overall aorta remodelling and EHT egress to mechanical
77 instabilities. This cross-disciplinary analysis indeed reveals that mechanical instabilities,
78 induced by different stresses arising from the inhomogeneous growth of the aorta and its
79 interaction with surrounding growing tissues, play a key non-specific role in HSPCs
80 extrusion. Thus, not only haemodynamic forces, but also stresses induced by the global
81 zebrafish embryonic growth¹⁷ are essential for haematopoiesis.

82

83 Results

84

85 **EHT is associated with important aorta remodelling.** To investigate the mechanisms
86 underlying EHT, we first carried out a 4D confocal microscopy imaging of this process in a
87 physiological context. For that, we image the trunk of zebrafish embryos (**Fig. 1a**) including
88 diverse structures such as the DA, the cardinal vein (CV), the notochord and the muscles
89 surrounding the DA (**Fig. 1a-c**) between 30 and 65 h.p.f.

90 The imaging revealed strong changes of the morphology of the whole DA with time (**Fig. 1b-**
91 **f, Supplementary Movie S1**). From 24 to 42.5 h.p.f., we observed a drastic increase of the
92 aorta diameter from $24 \pm 0.8 \mu\text{m}$ to $32 \pm 0.9 \mu\text{m}$ (**Fig. 1c, 1g**) followed by the emergence of a
93 pattern with alternating thinner and thicker diameter regions (**Fig. 1c-d, 1g-h**) with a relative
94 amplitude variation ranging from 17% to 33% the average aorta diameter.

95 At 42.5 h.p.f., the average diameter of the DA starts to decrease (**Fig. 1f, 1g**) and at 65 h.p.f.,
96 the aorta original cylindrical shape and diameter are restored which corresponds to the end of
97 EHT (**Fig. 1d-f, 1g**). To determine if there is a causal link between the change in aorta
98 diameter and cell extrusion, we followed the behaviour of individual cells leaving the DA
99 (**Fig. 1e-f, arrowheads correspond to regions where HSPCs emerged**). Our observations
100 showed that interestingly, HSPC extrusion rate peaks between 42.5 and 52 h.p.f. precisely
101 when the aorta diameter starts to decrease.

102

103 **Aorta dilation is not associated with cell mitosis.** To further assess EC behaviour during
104 aorta dilation and HSPC extrusion, we looked at EC division and whether an increase of cell
105 number can explain vessel expansion or compensate for HSPC emerging from the aorta floor.
106 We used a zebrafish transgenic line that expresses a marker of the S/G2/M phase of the cell
107 cycle, mVenus-zGeminin²⁰. This marker was specifically expressed under the *kdrl* promoter,
108 allowing visualization of ECs during mitosis²⁰. We quantified the number of EC divisions in
109 the trunk and in the tail between 27 and 72 h.p.f. We found that many division events occur in
110 the tail region (33.0 ± 2.8 EC divisions at 48 h.p.f), allowing development of the Caudal
111 Haematopoietic Tissue (CHT) (**Fig. 1i**). In contrast, at the peak of EHT (at around 48 h.p.f.),
112 we quantified a mean of 2.2 ± 0.6 ECs dividing in the *whole* AGM area (**Fig. 1i**), far under
113 what would have been needed to compensate for the estimated 25 HSPCs emerging from the
114 aorta during the whole AGM¹⁴. Overall, between 27 and 72 h.p.f., we observed only a few
115 EC divisions in the trunk region: the total number of cells in this area essentially decreases
116 throughout the EHT process. Thus, additional surface area, for the aorta dilation and later for
117 integrity maintenance during HSPC extrusion, should be provided exclusively by cell
118 deformation.

119

120 **HSPCs migrate collectively from the sides towards the ventral part of the aorta.** To
121 quantify the precise contribution of each EC to the EHT process, we followed the fate and
122 behaviour of individual cells between 30 and 60 h.p.f. in the whole trunk region of the DA. To
123 do so, we used the double transgenic line *kdrl:nls-GFP/kdrl:caax-mCherry*, allowing
124 respective visualization of nucleus and membrane of ECs through time. We discovered that
125 ECs undergo substantial rearrangements in the trunk region between 30 and 45 h.p.f. (**Fig. 2a-**
126 **b**). Interestingly, during this time window ECs migrate massively from the lateral side of the
127 DA to the floor (**Fig. 2a-h with arrows**). The number of cells in the DA floor peaks at about
128 40 h.p.f., when cell migration from the sides fully compensate for cell egress, and then
129 steadily declines afterwards (**Fig. 2i**) as lateral cells stop migrating towards the DA floor. The
130 decline is due to the extrusion of cells from the aorta floor into the sub-aortic space. The
131 number of ECs forming the dorsal aorta in the trunk region therefore strongly decreases from
132 the start to the end of the process (from 12.1 ± 0.9 cells/somite at 30 h.p.f. to 7.9 ± 0.3
133 cells/somite at 60 h.p.f., p -value=0.009) (**Fig. 2a-c, 2i-j**). Remarkably, the number of ECs
134 localized in the DA roof (facing the notochord) is essentially constant during the whole
135 process (**Fig. 2i**).

136

137 **HSPCs extrusion requires collective EC morphology changes.** In order to determine the
138 contribution of neighbouring ECs to the extrusion of HSPCs, we compared the morphology of
139 the emerging cells versus the morphology of their neighbours during EHT. Imaging of the
140 double transgenic line *kdrl:utrophin-CH-GFP/kdrl:nls-GFP* allowed to visualize the
141 boundaries of each individual EC together with its nucleus. Quantification of the cell area
142 between 40 and 60 h.p.f. showed that both emerging cells and their neighbours undergo
143 drastic and rapid morphology changes (**Fig. 3**). The area of cells prior to their extrusion
144 significantly reduces and shape changes from flat endothelial to a round plate-like cell (-
145 $69\% \pm 5\%$ decrease in cell area) (**Fig. 3a-b, 3e-g**). In contrast, neighbouring lateral cells
146 increase their area comparably ($+60\% \pm 9\%$ increase in cell area) (**Fig. 3c-d, 3e-f and 3h**).
147 These data confirm that in the absence of divisions in the trunk region, the loss of cells
148 through EHT is mainly compensated by the deformation of the lateral ECs thus assuring
149 global aorta integrity.

150

151 **Extrusion is finalized by actin ring closure around the emerging HSPC.** We then focused
152 on the cytoskeletal activity of ECs to understand the mechanism of this dramatic cell-to-tissue
153 reorganization process. First, we used the *kdrl:utrophin-CH-GFP* line to mark stable F-actin
154 in ECs²¹. 4D confocal imaging during the period of rearrangement (42-60 h.p.f.) showed that
155 EC junctions are highly dynamic and have tight cell-to-cell membrane boundaries
156 (**Supplementary Movie S2**). Because of the significant vascular cell deformation during
157 EHT, we then analysed the interplay between cytoskeleton activity and HSPCs shape during
158 the extrusion process. We imaged a double transgenic line *kdrl:utrophin-CH-GFP/kdrl:caax-*
159 *mCherry* showing respectively stable actin filaments and EC membranes. Live imaging
160 revealed that the aorta diameter decreases while HSPC extrusion in the sub-aortic space are
161 driven by the formation and closure of an actin ring surrounding the emerging cells (**Fig. 4,**
162 **Supplementary Movie S1, Supplementary Movie S3**) as also recently observed by Lancino

163 et al ¹⁹. Transverse view of the aorta shows that the emerging cell membrane and the actin
164 cytoskeleton co-localize to form a perfect circle that closes as the cell exits the DA floor (**Fig.**
165 **4a-c Supplementary Movie S3**). As the *kdrl:utrophin-CH-GFP* line displays a mosaic
166 expression, not all ECs express Utrophin-CH-GFP and we were therefore able to follow EHT
167 in cases where only the neighbouring cells expressed the GFP probe. In this scenario, we still
168 clearly observed the formation (**Fig. 4a, 4d**) and closure of the actin ring (**Fig. 4b-c, e-f**),
169 suggesting that the neighbouring cells also actively participate in the ring formation and actin
170 contractile dynamics.

171 In order to visualize all F-actin in the system, we also used a transgenic line expressing
172 Lifeact (a 17-amino-acid peptide that binds to filamentous actin) fused to green fluorescent
173 protein (GFP) ²². Vascular expression of Lifeact-GFP was driven by the VE-cadherin
174 promoter and this transgenic line was used together with *kdrl:caax-mCherry* to visualize EC
175 membrane as well. Imaging of *VE-cad: Lifeact-GFP* was consistent with the results obtained
176 with *kdrl:utrophin-CH-GFP* and we could follow the formation and closure of the actin ring
177 during EHT (data not shown).

178 To confirm the role of actin polymerization in HSPC extrusion, we treated embryos with
179 drugs blocking actin assembly, Latrunculin B or Rac inhibitor NSC23766. In treated embryos,
180 despite the presence of the aorta diameter modulation, the actin cytoskeleton was highly
181 disrupted and the formation of the actin ring surrounding the emerging cells was not observed
182 (**Supplementary Fig. 2**). Interestingly, the formation of typical plate-like cells in the aorta
183 floor preceding HSPCs extrusion was also not observed (data not shown). We also found that
184 in treated embryos some cells undergoing EHT bent toward the DA lumen: 33%±9% of cells
185 emerged toward DA lumen in Latrunculin B-treated embryos (**Supplementary Fig. 2**). These
186 results show the importance of actin polymerization for the acquisition of cell rigidity and the
187 direction of HSPCs extrusion from the aorta in the sub-aortic space.

188 We further looked at the role of the acto-myosin contraction in HSPC extrusion by subjecting
189 embryos to drugs blocking myosin contraction, Blebbistatin and ROCK inhibitor Y-27632.
190 The action of these drugs was very similar to that of the actin-blocking ones. The actin ring
191 surrounding emerging cells was not observed and HSPC exit took longer with some cells
192 undergoing fragmentation during the process (**Supplementary Fig. 2**).

193 To quantify the effect of actin polymerization and contraction blocking on haematopoietic
194 organs colonization such as the CHT by HSPCs, we treated *cd41:GFP* embryos, which
195 express GFP in HSPCs and thrombocytes ²³, with Latrunculin B, Rho-kinase inhibitor Y-
196 27632, Blebbistatin and Rac inhibitor NSC23766. For all drugs, except with Rho-kinase
197 inhibitor Y-27632, we found that the number of HSPCs colonizing the CHT (cd41+ cells)
198 between 52 and 72 h.p.f. was significantly lower in treated embryos compared to control
199 (**Supplementary Table 1**). Live imaging of EHT reveals that HSPCs bend without achieving
200 complete extrusion. Part of them bents in the wrong direction, i.e. in the aorta lumen, while
201 others cells burst as previously observed after *runx1* gene inactivation ¹⁴. Interestingly, none
202 of the drugs blocking actin polymerisation or contraction was found to affect EC
203 rearrangement occurring during the earlier stages of EHT (data not shown).

204 All together these results show that the acto-myosin cytoskeleton plays an essential role
205 mainly in the final step of EHT, precisely to complete the extrusion of HSPCs from the aorta
206 floor to the sub-aortic space.

207 **Cellular and tissular levels are dynamically coordinated during EHT.** Structuring up our
208 observations, the EHT transition is a collective phenomenon organized on both tissular and
209 cellular spatial scales (**Fig. 5**).

210 At tissue level, EHT is organized in two phases. In the first phase, from 28 to 42.5 h.p.f. the
211 DA diameter increases (**Fig. 1g and 5a**). At 40 h.p.f. aorta's shape of straight cylinder is
212 distorted by an average diameter modulation along the aorta longitudinal direction (**Fig. 1h**
213 **and 5a**).

214 During this first tissular phase, between 30 and 40 h.p.f., and concurrent with the diameter
215 modulation, at cellular level ECs reorganize themselves spatially and cells, localized on the
216 side of the aorta, migrate towards its floor. This cell migration is a quick process, which takes
217 on average $5.9h \pm 0.7h$ (counted for 18 cells in 15 individuals).

218 The second tissular phase corresponds to the diameter reduction and the disappearing of the
219 diameter modulation up to the end of EHT (from about 42.5 to 65 h.p.f.). Here, global
220 morphological reorganization of DA is coupled with the dynamics of cells that, once localized
221 at the aorta floor, undergo a drastic morphological change, shaping from a flat EC to a round
222 plate-like cell. As cells round up, they undergo a strong antero-posterior contraction. In
223 parallel, neighbouring ECs increase their area and compensate for the surface reduction of
224 emerging HPSC cells and eventually for their extrusion. The whole cellular dynamics assures
225 aorta integrity while DA diameter decreases. This important morphology and identity tissular
226 and cellular remodelling take on average $9.2h \pm 1.5h$ (counted for 6 cells in 6 individuals) and
227 occur between 37 and 55 h.p.f. The closure of the actin rings around the emerging cells allows
228 for their individualization from the aorta. The cellular extrusion and release from the aorta
229 take on average $7.5h \pm 1.3h$ (counted for 10 cells in 10 individuals). Remnant ECs finally
230 complete the remodelling of the aorta by fully recovering its initial diameter and cylindrical
231 geometry (**Fig. 1e-f and 5**).

232

233 **Discussion**

234

235 In this article, we study and characterise the EHT process at the cellular and tissular level. We
236 quantify the temporal sequences of cells rearrangements, shape modifications, and their
237 coordination with cellular migration events, whilst the DA tissue takes a peculiar tri-
238 dimensional shape in the trunk region. Thanks to 4D imaging and drug treatment, we have
239 organized the EHT process in two different phases of DA dilation and contraction, and
240 analysed the cellular events occurring.

241 To decipher the mechanisms that control the DA shape at various stages of EHT, we discuss
242 here why the mechanical properties of the DA endothelium play an important role in the
243 global control of the spatiotemporal organisation of the DA.

244 In the last century, theoretical methods of classical elasticity have been successful to describe
245 instabilities in metallic pipes and similar mechanical systems that, once subjected to a critical
246 load, change their shape and buckle. More recently, the generalization and adaptation of linear
247 elasticity theory for the needs of biophysical systems has allowed the mechanical description
248 of living matter like lipid membranes, cells or tissues²⁴⁻²⁶. Moreover, high susceptibility of
249 biological systems, residing near the critical point to the variation of external parameters, is
250 supposed to be often used in nature to control and regulate various processes²⁷.

251 We argue here that mechanical instabilities are involved in the DA shape transition during
252 EHT. More precisely, global variations of aorta shape in the trunk region assisting EHT are
253 the result of instabilities driven by mechanical stresses of various natures acting on the DA.
254 These stresses emerge spontaneously from the DA inhomogeneous growth and its interaction
255 with surrounding tissues rather than from genetically preprogrammed features of
256 embryogenesis. As a matter of fact, the specific diameter modulation in the lower part of the
257 DA appears even when the morphogenetic program of EHT is strongly altered under
258 inhibition of the *runx1* transcription factor¹⁴. Moreover, cytoskeleton-blocking drugs only
259 affect the final step of extrusion of HPSC, but not at all the previous phases involving the
260 cellular tissue remodeling and the global shape change of the DA.

261 The DA is formed by a single monolayer of cells contrary to an adult aorta multilayer
262 complex organization^{14,28}. Therefore, in first approximation, DA can be considered as a thin
263 cylindrical elastic shell. DA is much softer than metallic pipes, and its shape is finally defined
264 by the opposition of different active factors. These factors include: the inner hemodynamic
265 forces, the outer compressions exerted by the surrounding tissues due to embryonic growth
266 and development¹⁷ and the in-plane stresses generated in the aorta tissue by ECs shaping and
267 migration as observed in our experiments. The typical timescales for such developmental
268 events are of several hours, thus much longer than the typical time of aortic elastic response to
269 the heart-beat (fractions of seconds), for instance^{29,30}.

270 Classical mechanics predicts that destabilization of a tubular membrane can be associated
271 with three main modes corresponding to various membrane deformations. These modes are:
272 *Euler buckling* (a tube's longitudinal axis bends while cross-section remains circular, **Fig.**
273 **5b**), *transverse buckling* (axis of a tube remains straight, whereas its cross-section takes an
274 oval shape **Fig. 5c**) and "*corrugation instability*" (a tube preserves its rotational symmetry
275 along the main axis, but its radius is modulated along the main axis, **Fig. 5d**).

276 Euler buckling of the cylindrical tube can be invoked by the compressive stress along the tube
277 main axis. If the surrounding tissues were absent, the DA stability with respect to this mode
278 would have been determined almost exclusively by the bending rigidity of the tube wall.
279 However, as it is proven by the experiment, long-wave buckling instability is irrelevant for
280 the aorta, because notochord and other tissues surrounding the DA prevent its longitudinal
281 axis from bending (**Fig. 1 and 5**).

282 Transverse buckling of the cylindrical shell occurs when transverse isotropic (i.e. possessing
283 the rotational symmetry of the tube) stress in its walls reaches a negative critical value.
284 Stability with respect to transverse buckling is independent on the tension applied along the
285 tube axis and is determined by material constants characterizing the system. This type of
286 mechanical instability also occurs in ordinary rings³¹. Slightly elongated shape of aorta in the
287 dorsal-ventral direction is preserved throughout the whole process and in our opinion this
288 shape is due to an anisotropic compression applied by the muscles located on the sides of the
289 aorta rather than to the transverse buckling instability with spontaneous symmetry breaking.

290 On the contrary, deformation appearing in the DA prior to HSPCs extrusion has a well-
291 pronounced space periodicity and amplitude (**Fig. 1c-e, 1h**, and **Fig. 5**) which makes it similar
292 to the corrugation deformation of an axially compressed thin-walled rigid pipe³². Importantly,
293 space period and amplitude of the DA diameter modulation lay beyond the cell diameter and
294 thickness. As it was mentioned before, the blood pressure creates positive stress in DA walls.
295 Its longitudinal component can be calculated as $\sigma = R \Delta P / 2h$, where ΔP is a pressure difference
296 between the interior and the exterior of DA, R and h are DA's radius and thickness
297 respectively. This positive stress cannot induce corrugation since it makes DA even more

298 stable. However, negative compressive stress (inducing the corrugation in the system) can
299 originate from the difference between the growth rates of the DA and the tissues around it. As
300 it is shown by our data at about 30 h.p.f., this negative stress eventually overpowers the
301 positive one associated with blood pressure and leads to the corrugation of the aorta. This
302 deformation occurs mainly in the ventral part of the DA where the migrating cells converge,
303 and the compressive stress is maximal. Later on, as the zebrafish embryo develops, exit of the
304 cells undergoing EHT decreases the effective stress due to the decrease of the equilibrium
305 surface area of the DA. Consequently, at about 65 h.p.f. the stress drops below the critical
306 value and the tube regains its initial not-deformed shape (**Fig. 1f, Fig. 5**).

307 As we show experimentally, chemical perturbation or inhibition of acto-myosin contractility
308 machinery seriously affects only the final event of EHT, i.e. the extrusion of the EHT cell
309 from the aorta endothelium. Very interestingly, we stress that the initial phases of EHT
310 resulting in DA shape distortion still occurs in presence of chemical perturbations. Moreover,
311 the deformation of HSPCs that are preparing to leave the aorta also occurs. What is different
312 is that, in the drug-treated specimens where the actin rings surrounding the cells are absent,
313 many cells do not bend outside (as usual) but do bend inside of the aorta. Based on this
314 observation, we hypothesize that the EHT process is also associated with additional shape
315 instability of individual cells forming the aorta endothelium. We believe that this second
316 instability is also provoked by the stress exerted on the DA tissue, whereas the polymerization
317 of the actin ring and the actin cytoskeleton activity insures the right direction of future HSPC
318 bending and further facilitates its extrusion in the sub-aortic space toward the cardinal vein.

319 In conclusion, by using 4D fluorescence microscopy, we have characterized qualitatively and
320 quantitatively different dynamical phases of EHT leading to the generation of circulating
321 hematopoietic stem cells from the DA. From the analysis of our observations on wild-type,
322 genetically-modified and chemically-treated zebrafish, we confirmed the important role of the
323 acto-myosin system in EHT single cell final extrusion^{19,33}. Other processes involving the
324 externalisation of a single cell from a cell layer, such as apoptotic extrusion from the
325 epithelial layer of the zebrafish embryo epidermis, confirm that actin/myosin contraction is
326 essential in this process³⁴.

327 In particular, we profiled a general mechanism based on mechanical instabilities that prepare
328 and support the whole EHT prior to a specific genetic control of the process. Importantly, our
329 interpretation suggests a generic and self-organized mechanism that drives unique collective
330 events of tissue reorganization such as EHT in the development and growth of complex
331 organisms.

332 Further on, it will be interesting to develop a more precise mathematical model for the
333 description of aorta dynamics and study EHT transition in other model systems. Since the
334 aorta of the zebrafish embryo consists in a limited number of cells, which decreases even
335 more during the EHT process, it will be important to combine an analytical continuum model
336 with a coarse-grained approach allowing for the description of individual cells, similarly to
337 ones used in^{35-37,38}, but generalized for a curved 2-dimensional surface in 3-dimensional
338 space.

339 We believe that further studies of EHT will shed light on complex HSPC genesis, a
340 fundamental example of developmental process with important applications in tissue
341 engineering and regenerative therapies, but also on mechanical processes resulting in the
342 development of pathologies. Finally, does mechanics prepare the tissue before genetic
343 reprogramming? It is a debate that is developed in this study in an illustrated way with the
344 example of HSPCs ontogenesis.

345 **Methods.**

346 **Zebrafish husbandry**

347 *Tg(kdrl:Has.HRAS-mCherry)* (here cited as *kdrl:caax-mCherry*)³⁹, *Tg(kdrl:utrophin-CH-*
348 *GFP)*, *Tg(Cdh5:Gal4//UAS:lifeact:GFP)*²², *Tg(kdrl:nls-GFP)*⁴⁰, *Tg(flk-1:mV-zGem)*²⁰ and
349 *Tg(cd41:eGFP)*²³ were maintained, crossed, raised and staged as described previously^{41,42}.
350 All animal experiments described in the present study were conducted at the University of
351 Montpellier according to European Union guidelines for handling of laboratory animals
352 (http://ec.europa.eu/environment/chemicals/lab_animals/home_en.htm) and were approved by
353 the Direction Sanitaire et Vétérinaire de l'Hérault and Comité d'Ethique pour
354 l'Expérimentation Animale under reference CEEA-LR-13007.

355

356 **Drug treatments**

357 From 24 h.p.f. onwards, embryos were grown in PTU-containing medium to block
358 pigmentation. Embryos were dechorionated and treated with different drugs after the start of
359 circulation at 26 h.p.f. Drugs were dissolved in DMSO as stock solution and diluted in E3
360 medium up to 1% DMSO final concentration. Control embryos were subjected to the same
361 concentration of DMSO as treated embryos. Embryos were treated with 2µM Latrunculin B,
362 0.8µM Blebbistatin, 50µM Rac inhibitor NSC23766 for or 50µM Rho-kinase (ROCK)
363 inhibitor Y-27632. Embryos were kept in drug solution until image acquisition and
364 appropriate concentrations of drugs were added to the mounting medium for time-lapse
365 acquisitions.

366

367 **Quantification of HSPC colonization using *Tg(cd41:eGFP)***

368 To quantify cd41+ cells in the CHT, embryos were imaged under a fluorescent binocular
369 scope Zeiss V12 at 100X at 52 h.p.f. and 72 h.p.f. Cd41+ cells were then counted using
370 ImageJ.

371

372 **Microscopy**

373 Fluorescence images of transgenic embryos were acquired using Zeiss LSM510 at 20X.
374 Time-lapse imaging was performed using Zeiss LSM510 at 20X or 40X magnification
375 essentially as described⁴³. Embryos were anesthetised with tricaine (0.016%) and mounted on
376 a glass petri dish with 0.7 % low melting agarose and covered with standard E3 medium
377 supplemented with tricaine and 1-phenyl-2-thiourea (PTU) (0.003%) to prevent pigment
378 formation. Temperature was maintained at 28°C by placing the dish in a temperature-control
379 chamber during time-lapse acquisitions. Images were analysed using ImageJ and Imaris
380 (Bitplane).

381

382

383

384 **Aorta diameter measurement**

385 To measure aorta diameter *Tg(kdrl:caax-mCherry)* embryos were imaged using Zeiss
386 LSM510 as described above. Aorta diameter was measured manually using ImageJ at 10
387 different points along the trunk which were then averaged. To show aorta diameter evolution
388 through time, the aorta was measured every 2.5 hours in 5 embryos.

389

390 **Cell surface area measurements**

391 Cell surface area was measured using the Surface Contour tool of the Imaris software
392 (Bitplane). Briefly, the contour of a given cell was outlined on the different stacks where the
393 cell was visible using the Click drawing mode. The software then calculated automatically the
394 total cell surface area (μm^2). A total of 6 ECs starting to emerge from the aorta floor and 5
395 ECs on the side of the aorta were analysed in 5 different embryos.

396

397 **Statistical analysis**

398 Normal distributions were analysed using Shapiro–Wilk test. Non-Gaussian data were
399 analysed using Wilcoxon or Kruskal–Wallis test, Gaussian with Student’s *t*-test or analysis of
400 variance followed by Holm’s multiple comparison. $P < 0.05$ was considered as statistically
401 significant. Statistical analyses were performed using R software.

402

403 **Bibliography.**

- 404 1. Lis, R. *et al.* Conversion of adult endothelium to immunocompetent haematopoietic stem
405 cells. *Nature* **545**, 439–445 (2017).
- 406 2. Batta, K., Florkowska, M., Kouskoff, V. & Lacaud, G. Direct Reprogramming of Murine
407 Fibroblasts to Hematopoietic Progenitor Cells. *Cell Rep.* **9**, 1871–1884 (2014).
- 408 3. Riddell, J. *et al.* Reprogramming Committed Murine Blood Cells to Induced Hematopoietic
409 Stem Cells with Defined Factors. *Cell* **157**, 549–564 (2014).
- 410 4. Lancrin, C. *et al.* The haemangioblast generates haematopoietic cells through a haemogenic
411 endothelium stage. *Nature* **457**, 892–895 (2009).
- 412 5. Ivanovs, A. *et al.* Human haematopoietic stem cell development: from the embryo to the dish.
413 *Development* **144**, 2323–2337 (2017).
- 414 6. Hamill, O. P. & Martinac, B. Molecular Basis of Mechanotransduction in Living Cells. *Physiol.*
415 *Rev.* **81**, 685–740 (2001).
- 416 7. Eyckmans, J., Boudou, T., Yu, X. & Chen, C. S. A Hitchhiker’s Guide to Mechanobiology. *Dev.*
417 *Cell* **21**, 35–47 (2011).
- 418 8. Modesto, K. & Sengupta, P. P. Myocardial mechanics in cardiomyopathies. *Prog. Cardiovasc.*
419 *Dis.* **57**, 111–24 (2014).
- 420 9. Desprat, N., Supatto, W., Pouille, P.-A., Beaurepaire, E. & Farge, E. Tissue Deformation
421 Modulates Twist Expression to Determine Anterior Midgut Differentiation in *Drosophila*
422 Embryos. *Dev. Cell* **15**, 470–477 (2008).

- 423 10. Gering, M. & Patient, R. Hedgehog Signaling Is Required for Adult Blood Stem Cell Formation
424 in Zebrafish Embryos. *Dev. Cell* **8**, 389–400 (2005).
- 425 11. Tavian, M. & Péault, B. Embryonic development of the human hematopoietic system. *Int. J.*
426 *Dev. Biol.* **49**, 243–50 (2005).
- 427 12. Murayama, E. *et al.* Tracing hematopoietic precursor migration to successive hematopoietic
428 organs during zebrafish development. *Immunity* **25**, 963–975 (2006).
- 429 13. Kissa, K. *et al.* Live imaging of emerging hematopoietic stem cells and early thymus
430 colonization. **111**, 1147–1156 (2008).
- 431 14. Kissa, K. & Herbomel, P. Blood stem cells emerge from aortic endothelium by a novel type of
432 cell transition. *Nature* **464**, 112–115 (2010).
- 433 15. Bertrand, J. Y. *et al.* Haematopoietic stem cells derive directly from aortic endothelium during
434 development. *Nature* **464**, 108–11 (2010).
- 435 16. Robin, C. *et al.* Human Placenta Is a Potent Hematopoietic Niche Containing Hematopoietic
436 Stem and Progenitor Cells throughout Development. *Cell Stem Cell* **5**, 385–395 (2009).
- 437 17. Kimmel, C. B., Ballard, W. W., Kimmel, S. R., Ullmann, B. & Schilling, T. F. Stages of embryonic
438 development of the zebrafish. *Dev. Dyn.* **203**, 253–310 (1995).
- 439 18. Chen, M. J., Yokomizo, T., Zeigler, B. M., Dzierzak, E. & Speck, N. A. Runx1 is required for the
440 endothelial to haematopoietic cell transition but not thereafter. *Nature* **457**, 887–891 (2009).
- 441 19. Lancino, M. *et al.* Anisotropic organization of circumferential actomyosin characterizes
442 hematopoietic stem cells emergence in the zebrafish. *Elife* **7**, (2018).
- 443 20. Fukuhara, S. *et al.* Visualizing the cell-cycle progression of endothelial cells in zebrafish. *Dev.*
444 *Biol.* **393**, 10–23 (2014).
- 445 21. Burkel, B. M., von Dassow, G. & Bement, W. M. Versatile fluorescent probes for actin
446 filaments based on the actin-binding domain of utrophin. *Cell Motil. Cytoskeleton* **64**, 822–32
447 (2007).
- 448 22. Helker, C. S. M. *et al.* The zebrafish common cardinal veins develop by a novel mechanism:
449 lumen ensheathment. *Development* **140**, 2776–86 (2013).
- 450 23. Lin, H.-F. *et al.* Analysis of thrombocyte development in CD41-GFP transgenic zebrafish. *Blood*
451 **106**, 3803–10 (2005).
- 452 24. Golushko, I. Y., Rochal, S. B. & Lorman, V. L. Complex instability of axially compressed tubular
453 lipid membrane with controlled spontaneous curvature. *Eur. Phys. J. E* **38**, 112 (2015).
- 454 25. Alstrøm, P., Eguíluz, V. M., Colding-Jørgensen, M., Gustafsson, F. & Holstein-Rathlou, N.-H.
455 Instability and “Sausage-String” Appearance in Blood Vessels during High Blood Pressure.
456 *Phys. Rev. Lett.* **82**, 1995–1998 (1999).
- 457 26. Li, B., Cao, Y.-P., Feng, X.-Q. & Gao, H. Surface wrinkling of mucosa induced by volumetric
458 growth: Theory, simulation and experiment. *J. Mech. Phys. Solids* **59**, 758–774 (2011).
- 459 27. Muñoz, M. A. *Colloquium* : Criticality and dynamical scaling in living systems. *Rev. Mod. Phys.*
460 **90**, 031001 (2018).
- 461 28. Santoro, M. M., Pesce, G. & Stainier, D. Y. Characterization of vascular mural cells during
462 zebrafish development. *Mech. Dev.* **126**, 638–649 (2009).

- 463 29. Campàs, O. A toolbox to explore the mechanics of living embryonic tissues. *Semin. Cell Dev.*
464 *Biol.* **55**, 119–130 (2016).
- 465 30. Wyatt, T., Baum, B. & Charras, G. A question of time: tissue adaptation to mechanical forces.
466 *Curr. Opin. Cell Biol.* **38**, 68–73 (2016).
- 467 31. Landau, L. D., Lifshitz, E. M., Pitaevskii, L. P., Sykes, J. B. & Kearsley, M. J. *Statistical physics.*
468 *Volume 5 of Course of theoretical physics. Part 1.*
- 469 32. Timoshenko, S. & Goodier, J. N. *Theory of Elasticity.* (1951).
- 470 33. Guillot, C. & Lecuit, T. Mechanics of Epithelial Tissue Homeostasis and Morphogenesis. *Science*
471 *(80-.).* **340**, 1185–1189 (2013).
- 472 34. Rosenblatt, J., Raff, M. C. & Cramer, L. P. An epithelial cell destined for apoptosis signals its
473 neighbors to extrude it by an actin- and myosin-dependent mechanism. *Curr. Biol.* **11**, 1847–
474 1857 (2001).
- 475 35. Bi, D., Yang, X., Marchetti, M. C. & Manning, M. L. Motility-Driven Glass and Jamming
476 Transitions in Biological Tissues. *Phys. Rev. X* **6**, 021011 (2016).
- 477 36. Farhadifar, R., Röper, J.-C., Aigouy, B., Eaton, S. & Jülicher, F. The influence of cell mechanics,
478 cell-cell interactions, and proliferation on epithelial packing. *Curr. Biol.* **17**, 2095–104 (2007).
- 479 37. Merkel, M. *et al.* Triangles bridge the scales: Quantifying cellular contributions to tissue
480 deformation. *Phys. Rev. E* **95**, 032401 (2017).
- 481 38. Krajnc, M., Dasgupta, S., Zihler, P. & Prost, J. Fluidization of epithelial sheets by active cell
482 rearrangements. *Phys. Rev. E* (2018). doi:10.1103/PhysRevE.98.022409
- 483 39. Chi, N. C. *et al.* Foxn4 directly regulates tbx2b expression and atrioventricular canal formation.
484 *Genes Dev.* **22**, 734–9 (2008).
- 485 40. Blum, Y. *et al.* Complex cell rearrangements during intersegmental vessel sprouting and vessel
486 fusion in the zebrafish embryo. *Dev. Biol.* **316**, 312–22 (2008).
- 487 41. Kimmel, C. B., Ballard, W. W., Kimmel, S. R., Ullmann, B. & Schilling, T. F. Stages of embryonic
488 development of the zebrafish. *Dev Dyn* **203**, 253–310 (1995).
- 489 42. Westerfield, M. *The Zebrafish Book. A Guide for the Laboratory Use of Zebrafish (Danio rerio),*
490 *4th Edition.* (2000).
- 491 43. Renaud, O., Herbomel, P. & Kissa, K. Studying cell behavior in whole zebrafish embryos by
492 confocal live imaging: application to hematopoietic stem cells. *Nat. Protoc.* **6**, 1897–904
493 (2011).
- 494
- 495
- 496

497 **Acknowledgements**

498 We thank Etienne Lelièvre for his critical reading of the manuscript, A. Sahuquet, C.
499 Chevalier, V. Diakou for their assistance and the MRI facility, N. Abdellaoui for management
500 of zebrafish facility. D. Stainier lab for *Tg(Cdh5:Gal4//UAS:lifeact:GFP)*, S. Shulte-Merker
501 lab for *Tg(kdrl:utrophin-CH-GFP)* and *Tg(kdrl:nls-GFP)* and National Bioresource Project
502 Zebrafish for *Tg(flk-1:mV-zGem)*. This work was supported by the ARC, FRM, ATIP-Avenir
503 fellowships and a fellowship from the Région Languedoc-Roussillon, Chercheur d'Avenir.
504 NP was supported by a fellowship from the ATIP-Avenir, SR and IG are grateful to the RSF
505 grant N 19-12-00032, AP, IG and SR acknowledge NUMEV (AAP-2016-2-025) for financial
506 support. I.G.'s thesis was funded by Campus France (Vernadsky Fellowship) and the France-
507 Russia Cooperation Program, and JT by a fellowship from the MESR and the FRM.

508

509 **Disclosure of Conflicts of Interest**

510 The authors declare no competing financial interests.

511

512

513 **Figure 1. Aorta remodelling during EHT: embryo anatomy and spatiotemporal** 514 **organisation of aorta diameter.**

515 **a.** Drawing showing the AGM localization in the trunk of a 48 h.p.f. zebrafish. Scheme of the
516 AGM in longitudinal or transverse views (grey boxes) consisting of the neural tube, the
517 notochord, the dorsal aorta, the cardinal vein, and the yolk tube. **b-f.** Still frames of time-lapse
518 imaging of *Tg(kdrl:caax-mCherry)* embryo from 30-65 h.p.f. Maximum projection from 40 z-
519 stack spaced by 1µm. Double-headed arrows indicate difference of aorta diameter through
520 time. **b-c.** Between 30 and 42.5 h.p.f., DA diameter expands. Arrowheads indicate
521 localization of cells leaving the aorta and forming a local reduction of the diameter. **d.** Image
522 of *Tg(kdrl:caax-mCherry)* embryo at 50 h.p.f. to illustrate DA diameter measurements
523 throughout the length of 3 somites as represented in **h**. **e.** From 45 to 65 h.p.f., DA diameter
524 decreases, corresponding to the peak and then decline of EHT events. Arrows indicate the
525 contraction of the aorta occurring from the floor. **f.** After EHT, at 65 h.p.f, aorta floor
526 becomes flat. **g.** Graph showing aorta diameter variation in time measured every 2.5h in 5
527 embryos. Letters correspond to the according panels. Error bars represent standard error of the
528 mean. **h.** Graph showing variation of diameter along the antero/posterior axis at 3 time points:
529 50, 55 and 60 h.p.f. (Black box from g) in the embryo imaged in **b-f**. Dotted line indicates
530 intersomitic vessels. Blue arrowheads indicate localization of EHT cells leaving the aorta and
531 forming a local reduction of the diameter. **i.** Graph showing the number of EC divisions
532 (*kdrl:mV-zGmn⁺* cells) occurring between 27 and 72 h.p.f. in the trunk and in the tail region,
533 n=10 cells. Note that a peak of division is observed in the tail region between 36 and 54 h.p.f.
534 corresponding to the formation of the CHT and its colonisation by HSPC. DA: dorsal aorta;
535 isv: intersomitic vessel; CV: cardinal vein. Scale bar: 25 µm. See also **Supplementary Movie**
536 **S1.**

537

538 **Figure 2. Cells undergoing EHT are recruited from the side of the aorta and migrate to**
539 **its floor prior to extrusion.**

540 **a-c.** From 30 to 60 h.p.f., still frames of time-lapse imaging of *Tg(kdrl:ns-GFP)/Tg(kdrl:caax-*
541 *mCherry)* embryo. Maximum projection from 40 z-stack spaced by 1 μ m. Stars indicate the
542 nucleus of the ECs in the dorsal aorta in the 2 central somites of the image. **d-h.** Still frames
543 of time-lapse imaging of *Tg(kdrl:nls-GFP)/Tg(kdrl:caax-mCherry)* embryo. Maximum
544 projection from 40 z-stack spaced by 0.6 μ m. Cells migrating from the side of the aorta toward
545 the floor are numbered 1 and 2 in white. **i.** Graph showing the number of nuclei counted in 3
546 different zones of the dorsal aorta: roof, side and floor, n=5 cells. **j.** Schematic representation
547 of cell rearrangement occurring from 30 to 60 h.p.f. Colour coding of the cells corresponds to
548 the graph in i. DA: dorsal aorta; CV: cardinal vein. Scale bar: 25 μ m (**a-c**), 10 μ m (**d-h**).

549

550 **Figure 3. Endothelial cells adopt collaborative behaviour during EHT: cellular**
551 **contractions and extensions to maintain aorta integrity.**

552 **a-d.** Still frames of time-lapse imaging of *Tg(kdrl:nls-GFP)/Tg(kdrl:utrophin-CH-GFP)*
553 embryo between 40 and 60 h.p.f. Maximum projection from 40 z-stack spaced by 0.6 μ m. Cell
554 A (highlighted in orange) is an endothelial cell located in the floor of the aorta and starting to
555 undergo EHT. Cell A surface reduces importantly between 42 h.p.f. (**a**) and 60 h.p.f. (**b**). Cell
556 B (highlighted in blue) is located in the side of the aorta neighbouring a cell undergoing EHT
557 (star). Cell B surface increases between 42 h.p.f. (**c**) and 54 h.p.f. (**d**). **e-f** Schematic
558 representation of EC surfaces and positions at t1 (**e**) and t2 > t1 (**f**). Colour-code and letters
559 correspond to the panel **a-d**. **g.** Graph showing the temporal evolution of EC starting to
560 emerge from the aorta floor in percentage of difference compared to area at 40 or 42 h.p.f.
561 Each line represents the measurements for one cell. 6 ECs starting to emerge from the aorta
562 floor were analysed in 5 different embryos. For details on cell area (μ m²) calculation, see
563 Material and Methods section. **h.** Graph showing temporal evolution of cell area of lateral EC
564 in percentage of difference compared to area at 40 or 42 h.p.f. Each line represents the
565 measurements for one cell. 5 ECs on the side of the aorta were analysed in 5 different
566 embryos. Scale bar: 10 μ m.

567

568 **Figure 4. Final contraction in EHT is actin-dependent and coordinated with**
569 **surrounding cells.**

570 **a-c.** Horizontal reconstructed view from a maximum projection of a series of z-stack of a
571 *Tg(kdrl:utr-CH-GFP)/Tg(kdrl:caax-mCherry)* embryo during EHT at 42, 44 and 46 h.p.f.
572 Upper panels are *kdrl:utrophin-CH-GFP* alone and lower panels are merged images with
573 *kdrl:caax-mCherry*. Closure of the actin ring by the neighbouring cells is clearly observable
574 in the upper panels. See also **Supplementary Movie S3**. **d-f.** Single z-stacks of time-lapse
575 imaging of the same embryo as in **a-c**. Upper panels are *kdrl:utrophin-CH-GFP* alone and
576 middle panels are merged images with *kdrl:caax-mCherry*. Actin ring (arrows) closes around
577 emerging cell (arrowhead). Note that in this case the emerging cell does not express Utrophin-
578 CH-GFP due to mosaic labelling, allowing us to confirm the role of the neighbouring cells in
579 actin ring closure.

580

581 **Figure 5. Overall schematic representation of Endothelial-to-Haematopoietic Transition**
582 **with mechanical instabilities. a.** The EHT transition starts first by a global aorta dilation (as
583 soon as the heart starts to beat), taking place from 24 to about 42,5 h.p.f., followed by the
584 aorta contraction, occurring from about 42,5 to 65 h.p.f. While the embryo is growing and
585 developing, the first phase of aorta dilation is essentially characterized by cells that start to
586 increase their area, while others, in particular those located on the sides of the aorta, migrate
587 towards the ventral part (aorta floor). During this phase the characteristic modulation of the
588 aorta diameter, the corrugation instability (see main text and below), appears.

589 In the contraction phase, cells localized at the aorta floor undergo a drastic morphological
590 change, shaping from flat ECs to round plate-like cells. As cells round up, they undergo a
591 strong antero-posterior contraction. In parallel, neighbouring ECs compensate for the surface
592 reduction of emerging cells, and eventually for the extrusion of the latter cells from the aorta,
593 by increasing importantly their surface. Finally emerging round cells extrude and
594 individualize from the aorta. This phase coincides with the decrease of the diameter
595 modulation, recovering the initial aorta diameter and its cylindrical geometry.

596 **b-c-d.** The three main mechanical instability modes of a thin cylindrical soft pipe (membrane)
597 under mechanical stresses: **b.** Longitudinal buckling; **c.** Transverse buckling; **d.** Corrugation.

598

Figure 1. Aorta remodelling during EHT: embryo anatomy and spatiotemporal organisation of aorta diameter

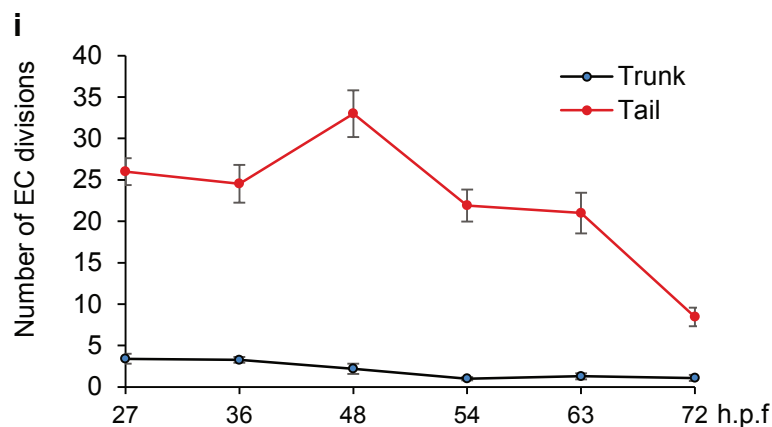
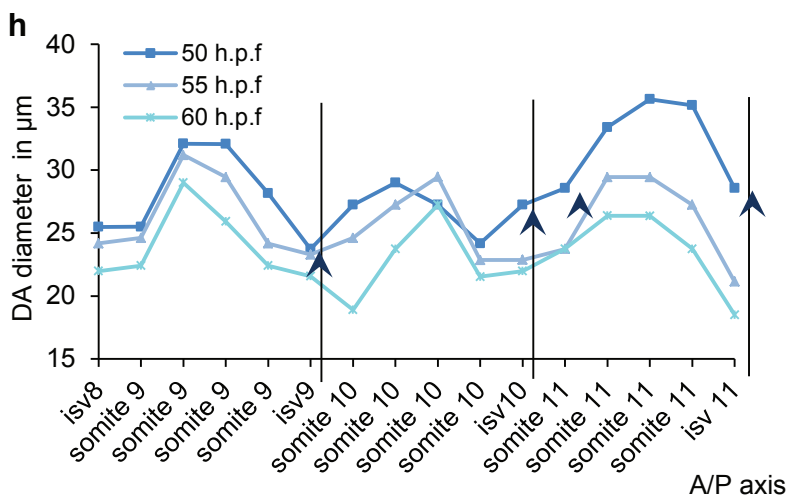
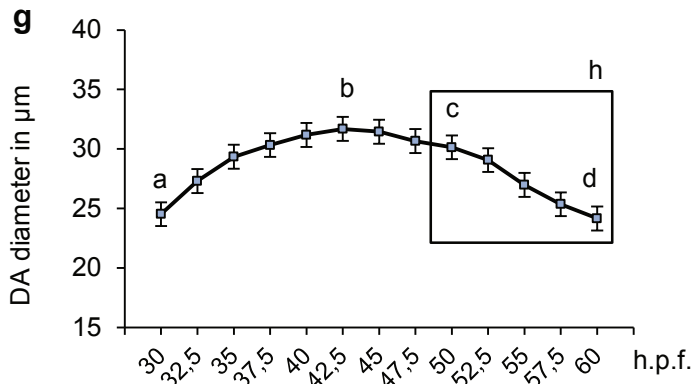
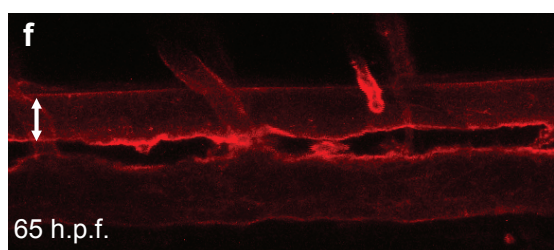
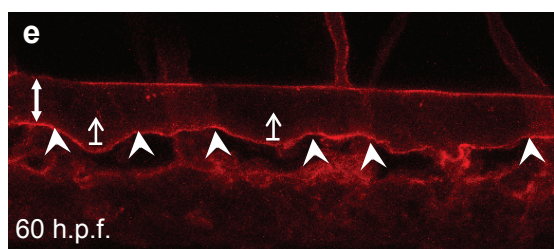
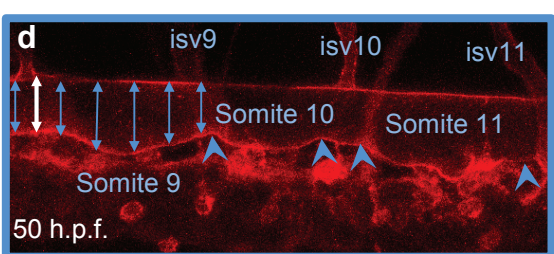
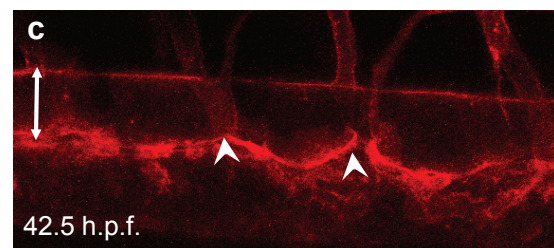
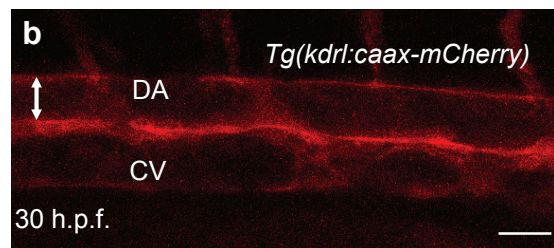
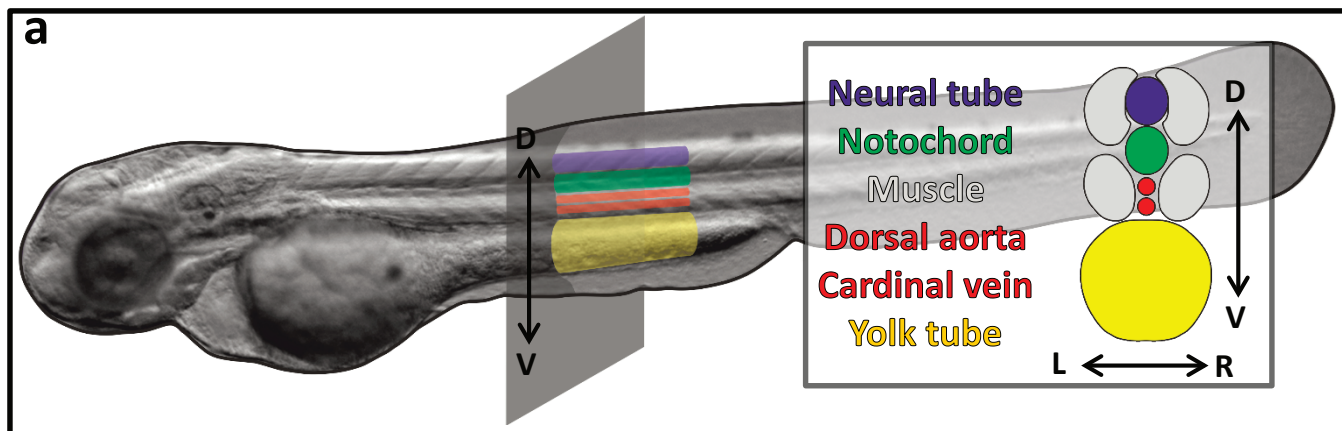


Figure 2. Cells undergoing EHT are recruited from the middle of the aorta and migrate to its floor prior to extrusion.

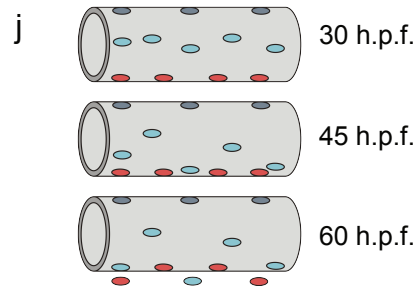
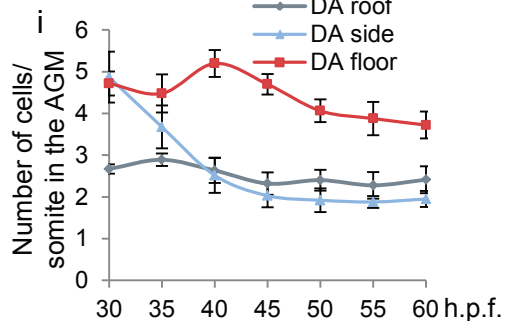
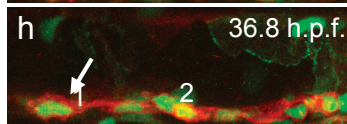
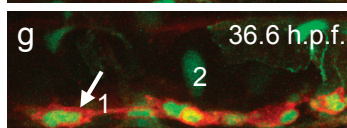
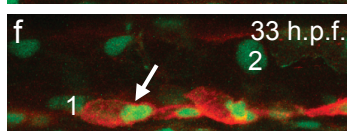
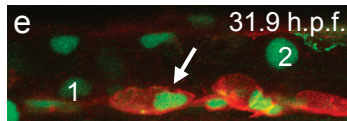
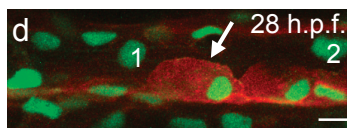
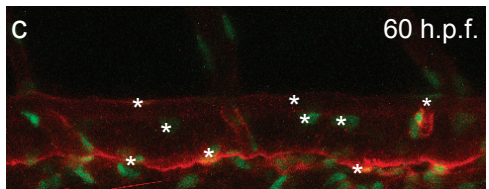
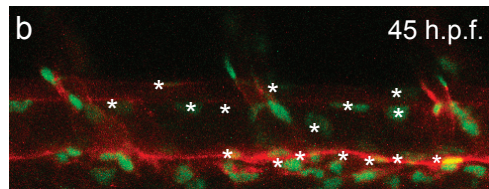
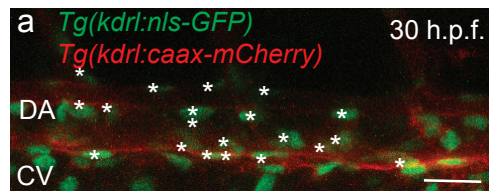


Figure 3. Endothelial cells adopt collaborative behaviour during EHT: cellular contractions and extensions to maintain aorta integrity.

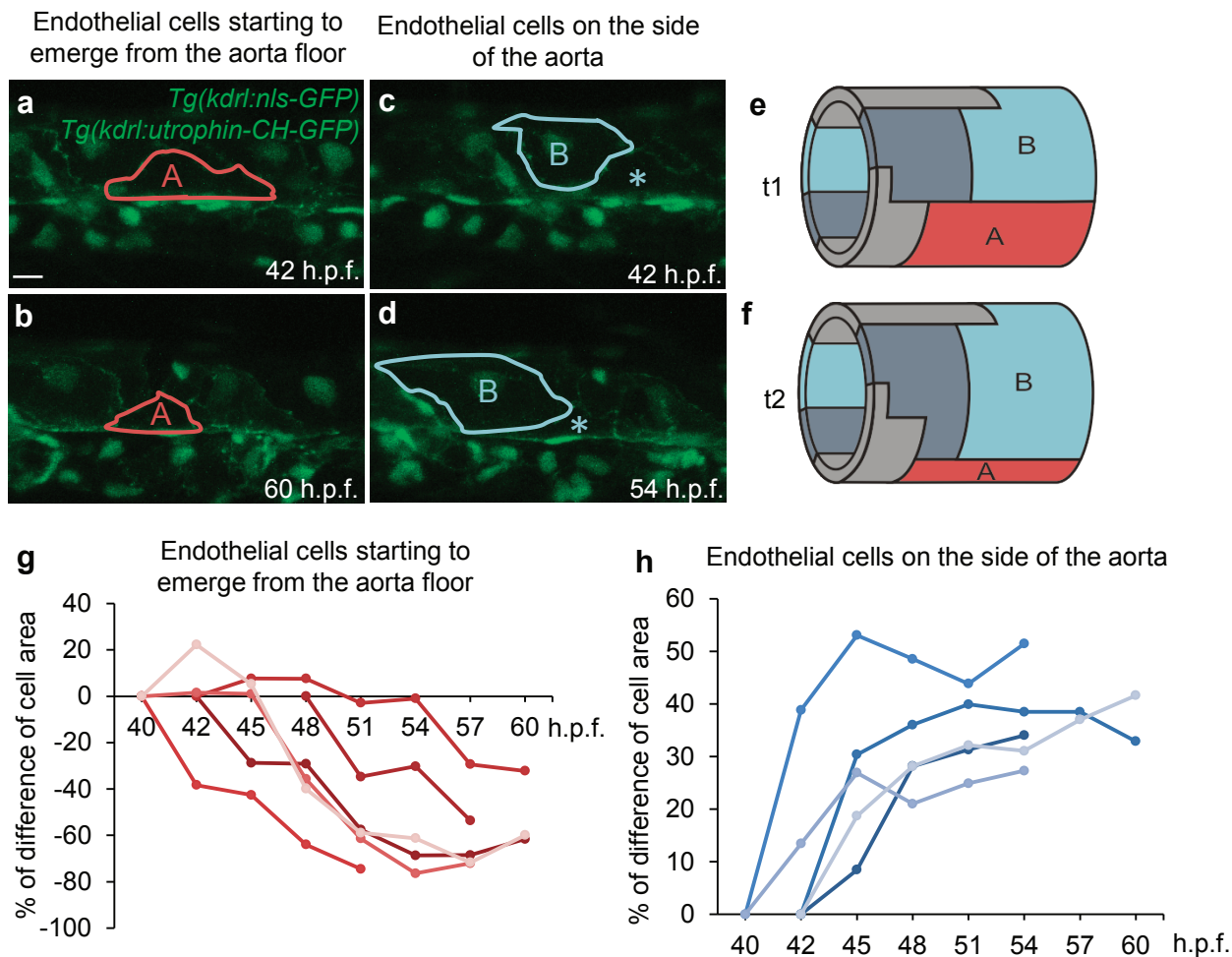


Figure 4. Final contraction in EHT is actin-dependent and coordinated with surrounding cells.

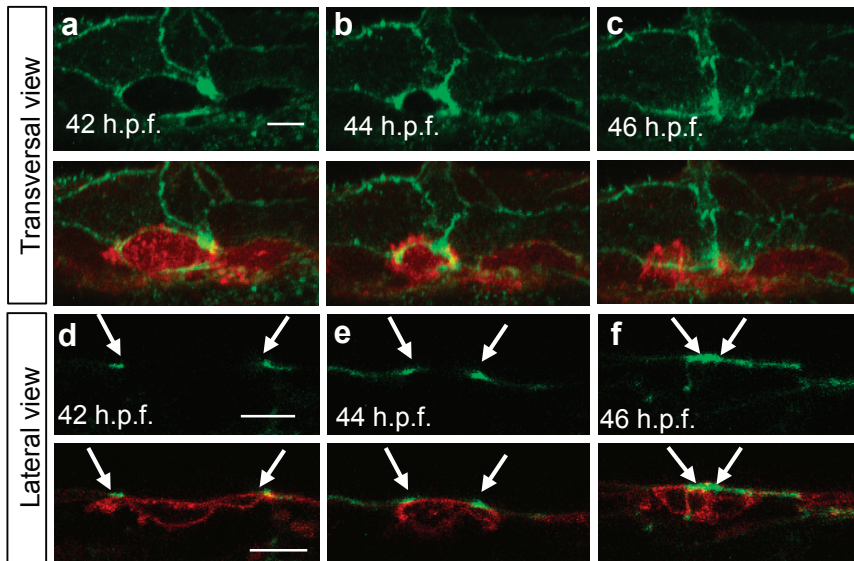
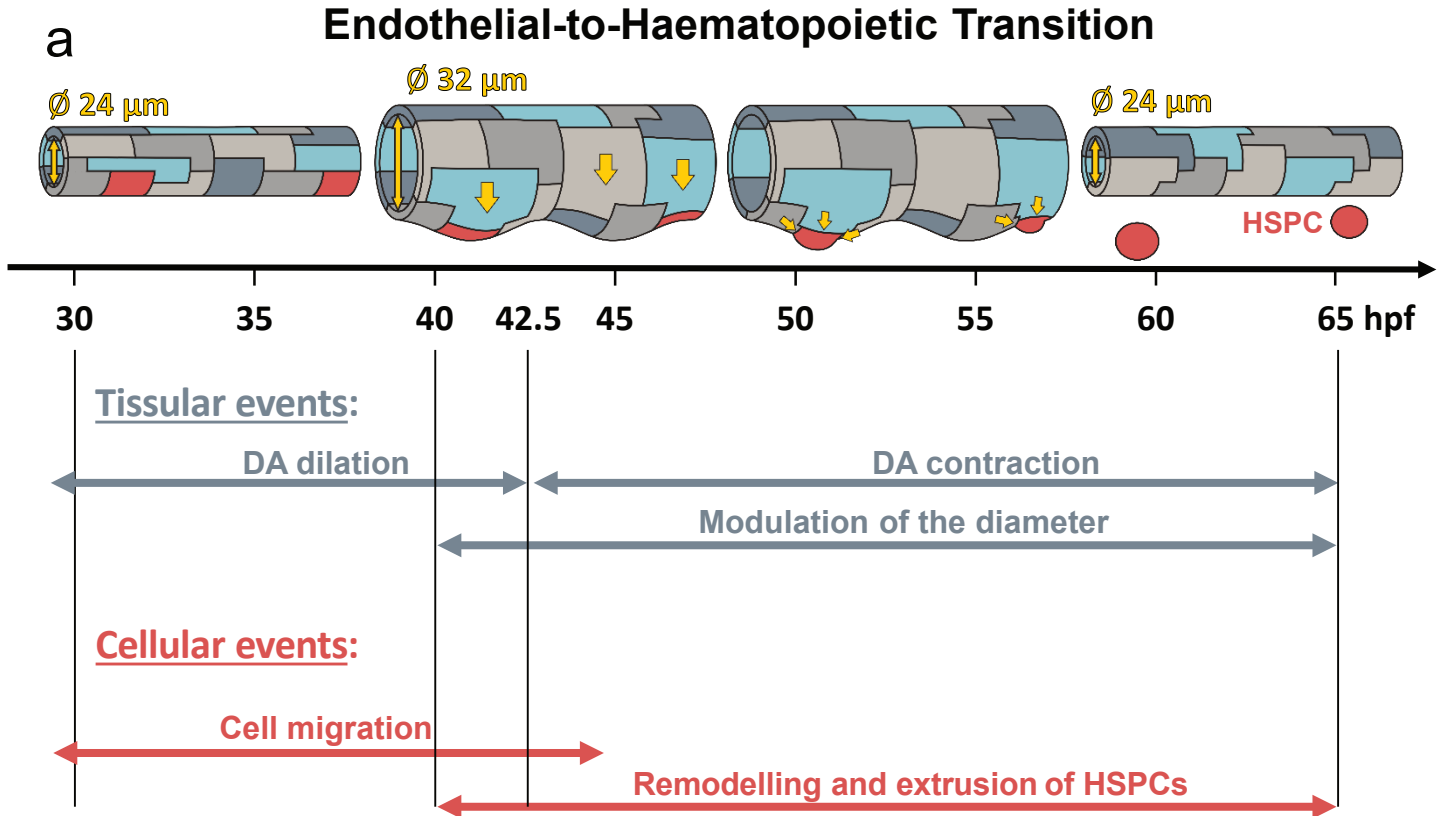


Figure 5. Overall schematic representation of Endothelial-to-Haematopoietic Transition with mechanical instabilities



Mechanical instabilities

