

## Description of Additional Supplementary Files

**File Name:** Supplementary Movie 1

**Description:** Time-lapse imaging of *VE-cad-Venus* ( $Tg(BAC(cdh5:cdh5-ts))$ ) during DLAV formation starting at 29 hpf. Arrows point to edges of the elongating junction. Disappearance of junctions indicated that segment has achieved multicellular architecture. Time-lapse was imaged at 10min/stack (displayed at 5 fps).

**File Name:** Supplementary Movie 2

**Description:** High-resolution time-lapse imaging of *VE-cad-Venus* ( $Tg(BAC(cdh5:cdh5-ts))$ ) in the junction of the DLAV around 32hpf. Some of the JBL are marked with an arrow. Time-lapse was imaged at 60 sec/stack (displayed at 5 fps).

**File Name:** Supplementary Movie 3

**Description:** High-resolution time-lapse imaging of EGFP-UCHD ( $Tg(fli:Gal4ff^{ubs3}; UAS:EGFP-UCHD^{ubs18})$ ) in the junction of DLAV around 32 hpf. Some of the JBL are marked with an arrow. Time-lapse was imaged at 90 sec/stack (displayed at 5 fps).

**File Name:** Supplementary Movie 4

**Description:** High-resolution time-lapse imaging of EGFP-UCHD ( $Tg(fli:Gal4ff^{ubs3}; UAS:EGFP-UCHD^{ubs18})$ ) in the junction of DA around 32hpf. Some of the JBL are marked with an arrow. Time-lapse was imaged at 1min/stack (displayed at 10 fps).

**File Name:** Supplementary Movie 5

**Description:** High-resolution time-lapse imaging of mRuby-UCHD (red) and *VE-cad-Venus* (green) ( $Tg(fli:Gal4ff^{ubs3}; UAS:mRuby2-UCHD^{ubs20}; BAC(cdh5:cdh5-ts))$ ) in the junction of DLAV around 32hpf. The time-lapse was imaged at 30 sec/stack (displayed at 5fps).

**File Name:** Supplementary Movie 6

**Description:** High-resolution time-lapse imaging of mRuby-UCHD (red) and EGFP-ZO1 (green) ( $Tg(fli:Gal4ff^{ubs3}; UAS:mRuby2-UCHD^{ubs20}; UAS:EGFP-hZO1^{ubs5})$ ) in the junction of DLAV around 32 hpf. The double-junctions are marked with arrows. Time-lapse was imaged at 12 sec/stack (displayed at 5 fps). Scale bar 1  $\mu$ m.

**File Name:** Supplementary Movie 7

**Description:** High-resolution time-lapse imaging of mCherry-ZO1 (red) and VE-cad-Venus (green) (*Tg(BAC(cdh5:cdh5-ts);fli:Gal4ff<sup>ubs3</sup>;UAS:mCherry-ZO1)*) in the junction of SeA around 32 hpf. Time-lapse was imaged at 1 min/stack (displayed at 5 fps). Scale bar 1  $\mu$ m.

**File Name:** Supplementary Movie 8

**Description:** High-resolution time-lapse imaging of DMSO-treated embryo expressing *VE-cad-Venus* (*Tg(BAC(cdh5:cdh5-ts))*) in the junction of DLAV around 32hpf. Time-lapse was imaged at 60 sec/stack (displayed at 5 fps).

**File Name:** Supplementary Movie 9

**Description:** High-resolution time-lapse imaging of Latrunculin B-treated embryo expressing *VE-cad-Venus* (*Tg(BAC(cdh5:cdh5-ts))*) in the junction of DLAV around 32hpf. Time-lapse was imaged at 60 sec/stack (displayed at 5 fps).

**File Name:** Supplementary Movie 10

**Description:** High-resolution time-lapse imaging of NSC23766-treated embryo expressing *VE-cad-Venus* (*Tg(BAC(cdh5:cdh5-ts))*) in the junction of DLAV around 32hpf. Time-lapse was imaged at 60 sec/stack (displayed at 5 fps).

**File Name:** Supplementary Movie 11

**Description:** Video of blood flow in the trunk of 2dpf wild-type and *cdh5(ubs25)* mutant embryos (both mild and severe phenotypes are shown).