Expanded View Figures

Figure EV1. Correlated light and electron microscopy (CLEM) shows endothelial uptake of circulating EVs.

CLEM has been performed on Fli1:GFP zebrafish embryos injected with Memglow labeled EVs first imaged live by confocal imaging and the same region where EVs accumulate in endothelial cells imaged by electron microscopy (the sample is identical with that shown in Fig 1A). The top and the left images are consecutive sections (100 nm apart) and have been imaged by serial Transmission Electron Microscopy (sTEM) and stitched using TrakEM (FIJI). The right images are high-resolution images of the highlighted boxes.

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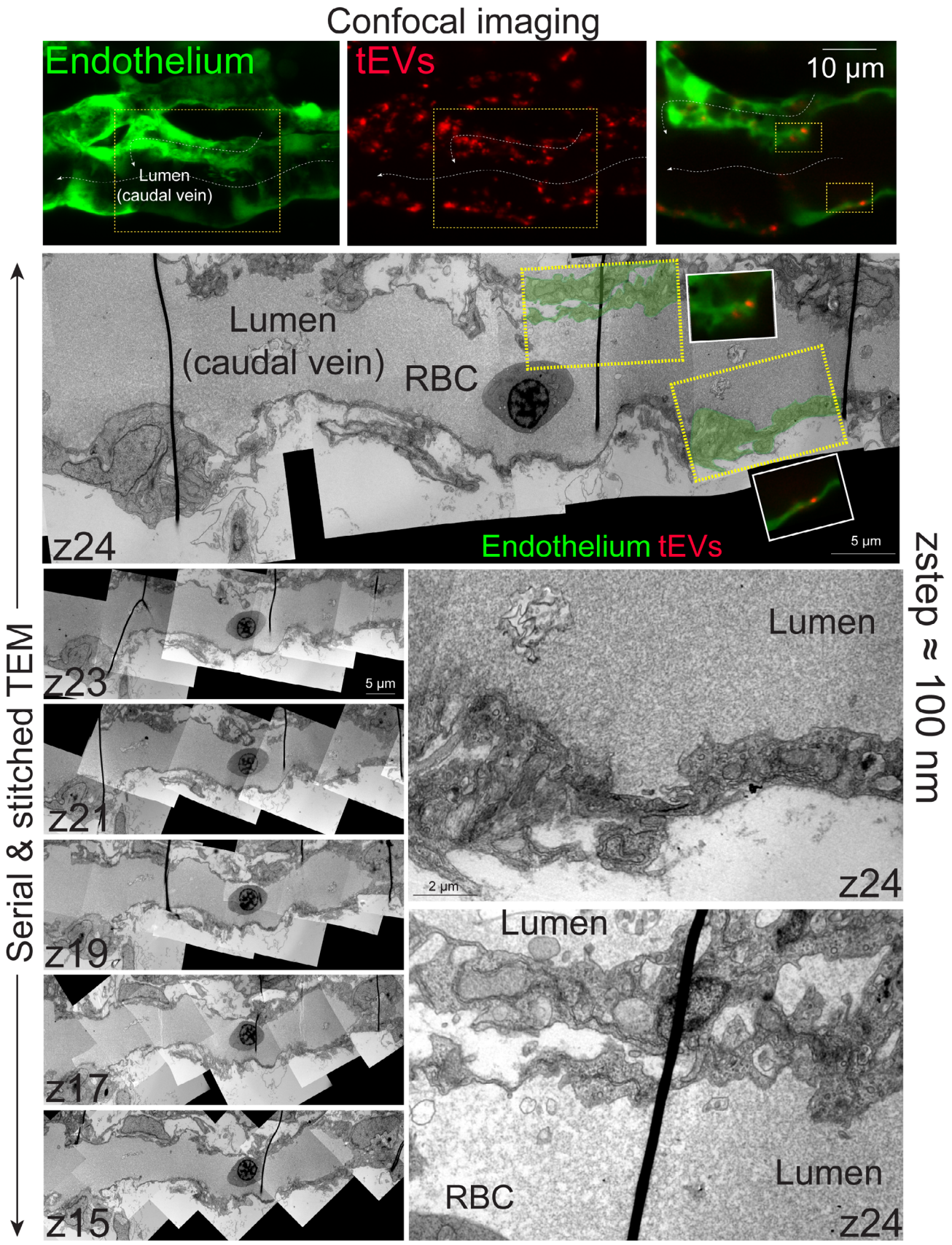

Figure EV1.

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Figure EV2. Impact of flow velocity on tEVs uptake by endothelial cells.

A, B Graph showing the correlation between RBC velocities and internalized 4T1 tEVs isolated by SEC or ultracentrifugation (A) or 4T1 and AT3 EVs (B) in the same regions of zebrafish caudal plexus as in Fig 1A.

C Heartbeat frequency (left; 1 dot: 1 fish, ****$P < 0.0001$ Student *t*-test) and RBC velocity (middle; 1 dot: 1 fish, Kruskal–Wallis, *$P < 0.05$) measured by spinning disk confocal in zebrafish embryos treated with IBMX or lidocaïn. Right: Correlation between blood flow velocity (measured by RBC tracking as in A) and endothelial EV uptake in embryos treated with IBMX, lidocaine or their respective vehicles (vehic.).

D Representative images and quantification of the uptake of 4T1 CD63-GFP EVs by endothelial cells cultured in microfluidics in static conditions or at two flow speeds (400 μm/s and 4,400 μm/s) (data per cell, normalized to static; 1 dot: 1 field of view; experiment performed in triplicate). (a.u. arbitrary units). *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ****$P < 0.0001$ Kruskal–Wallis. n.s., non significative.

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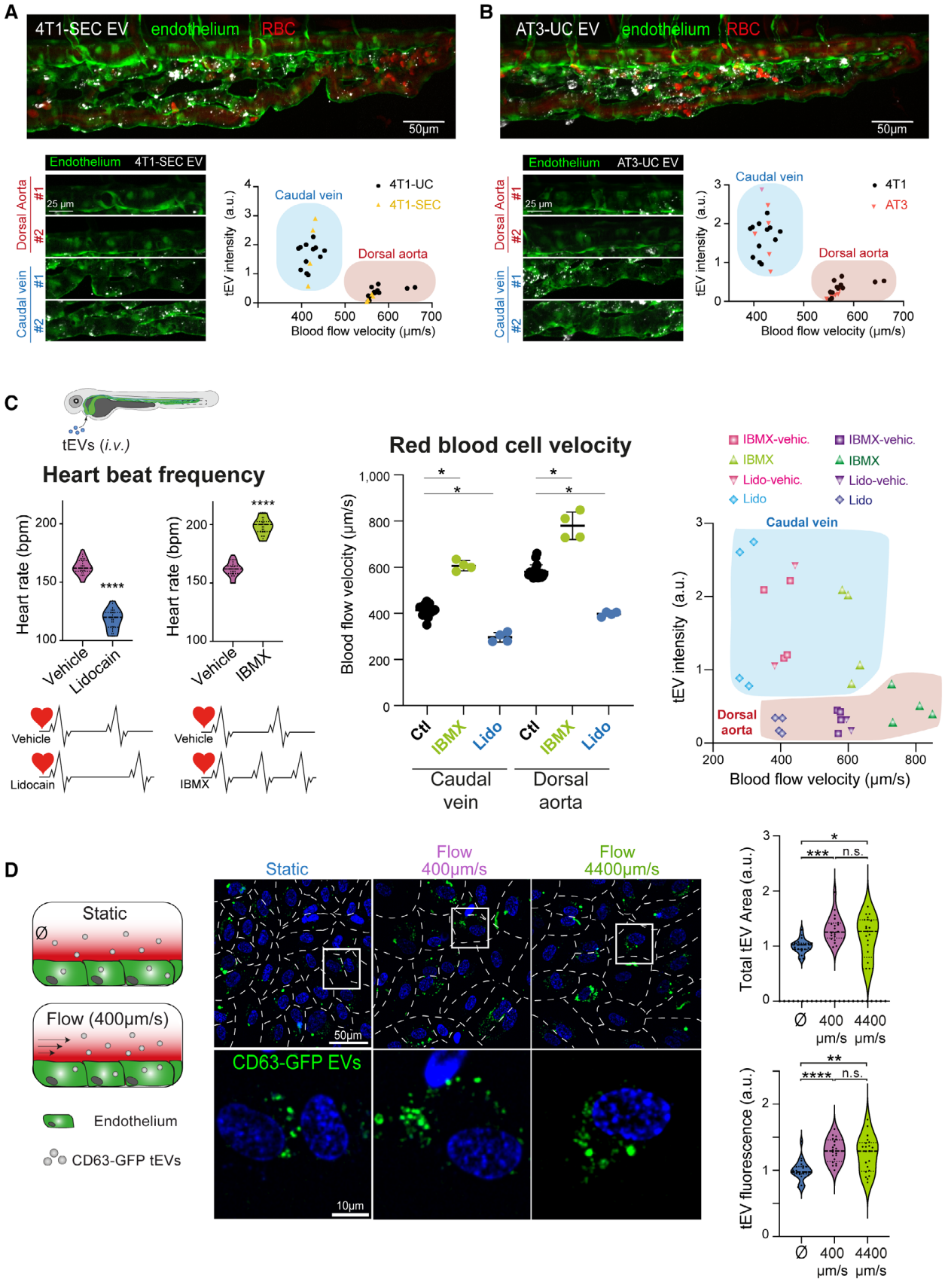

Figure EV2.

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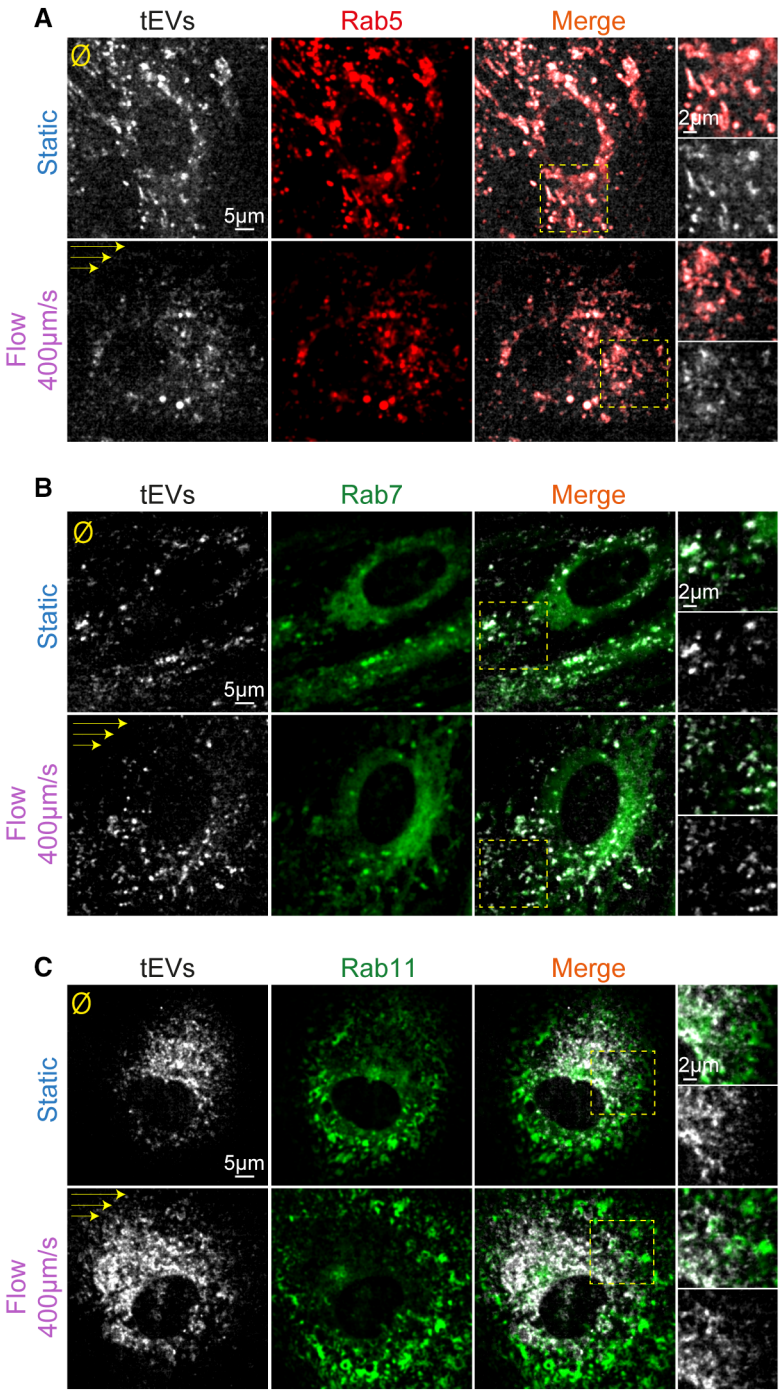

Figure EV3. tEVs trafficking in endothelial cells in flow and static conditions.

A–C Representative confocal images (single plane) of internalized EVs in vHUVECs cells cultured in flow or static conditions and expressing either mCherry-RAB5 (A), mEmerald-RAB7 (B), eGFP-RAB11 (C).

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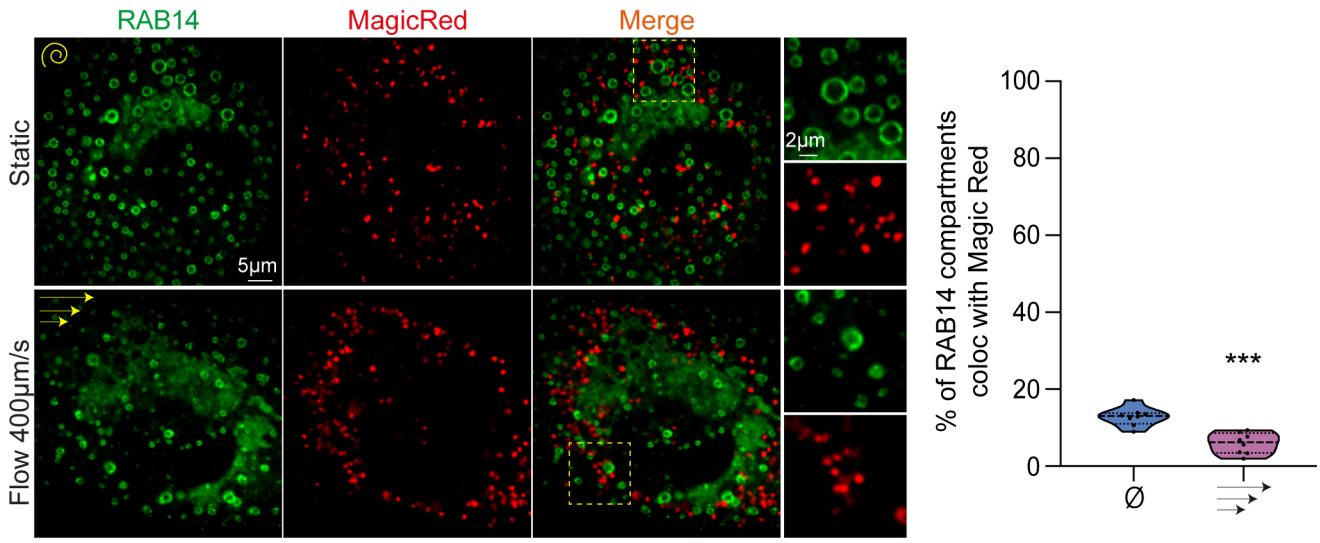

Figure EV4. RAB14 compartments show low colocalization with Magic Red in flow and static conditions.

Representative confocal images (single plane) of vHUVECs cells expressing GFP-RAB14 cultured in flow or static conditions with Magic Red. (1 dot = 1 FOV ***P < 0.001 Mann–Whitney).

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