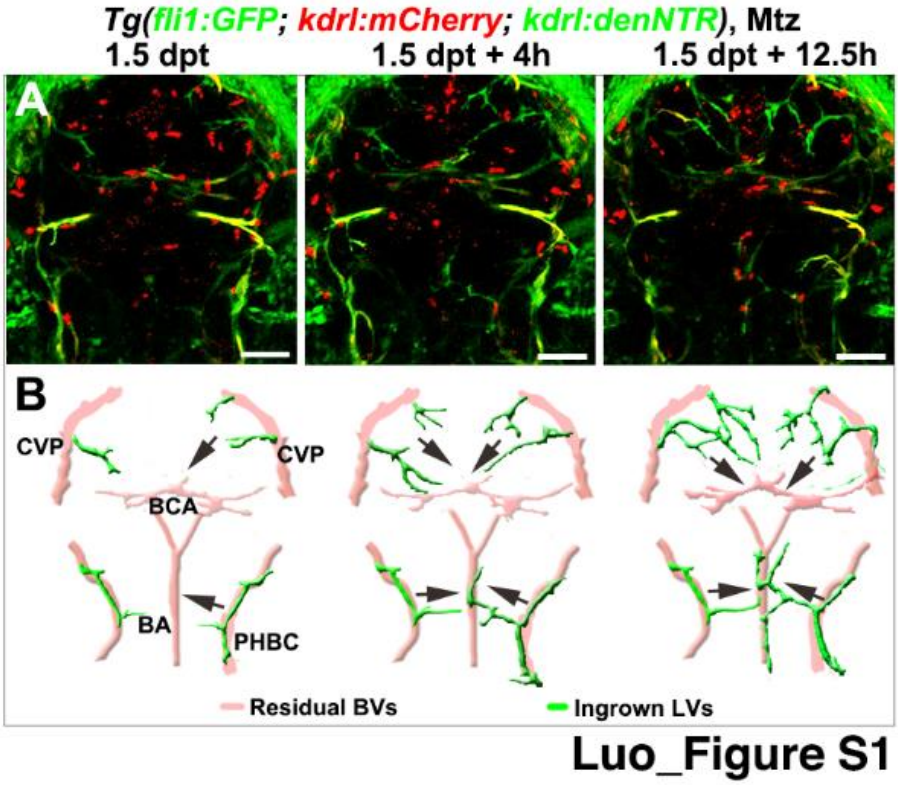


Fig. S1. The *fli1+kdrl*- iLVs ingrow into the injured brain parenchyma with certain directionality. (A) Rapid and active ingrowth of *fli1+kdrl*- iLVs from peri- to central-injured area from 1.5 dpt/4.5 dpf (days post fertilization) (n=11/11). **(B)** The explanatory schematic diagrams of the ingrowth pattern of iLVs. Arrows indicate they directionally ingrow from CVP to BCA, and from PHBC to BA. The elapsed time is indicated in hours:minutes after 1.5 dpt. iLV, ingrown lymphatic vessels. Dorsal view. Scale bar, 50 μm.

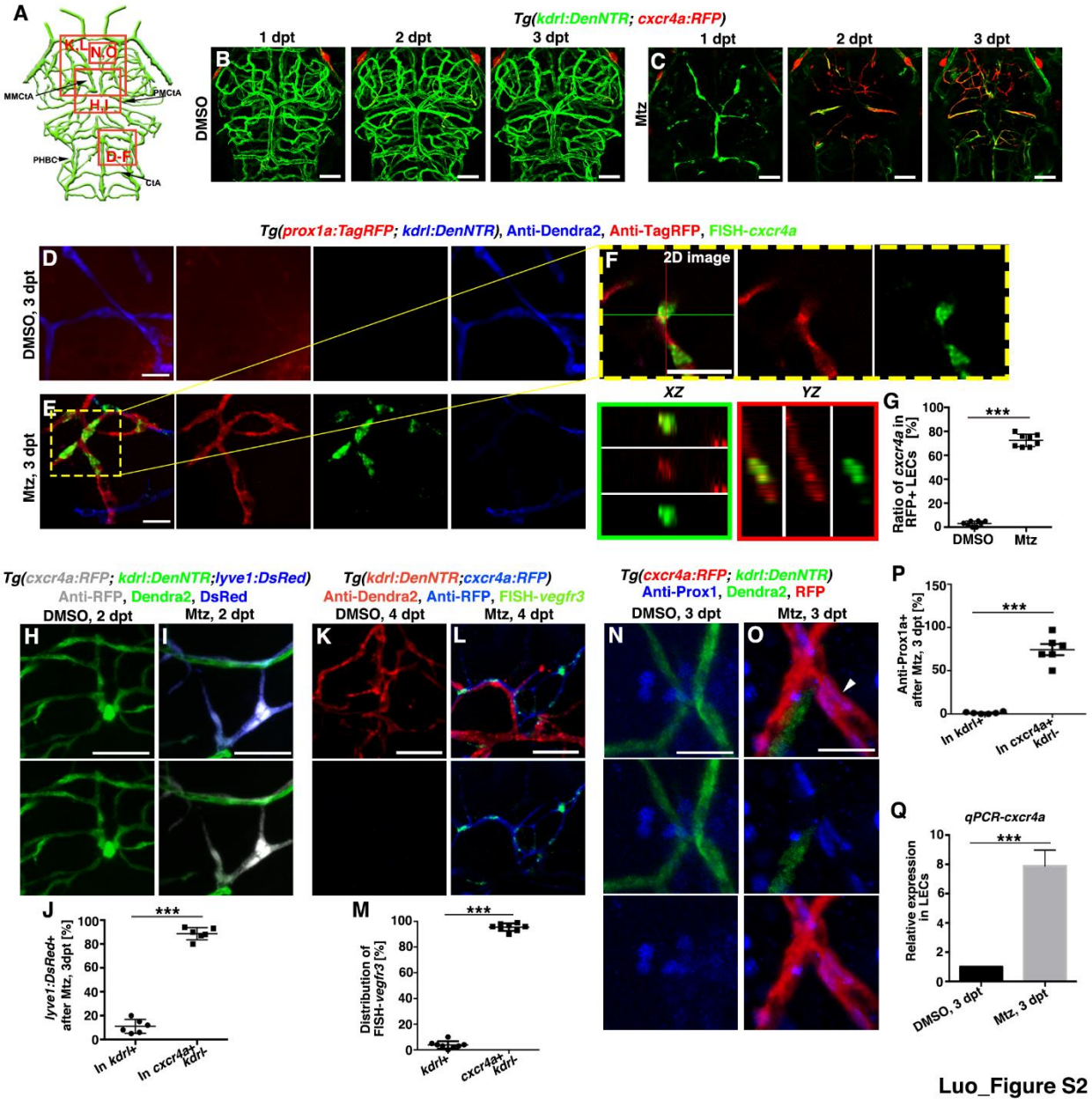


Fig. S2. Transcription of *cxcr4a* is active in the iLVs after cerebrovascular injury.

(A) Red boxes indicate the image areas in the corresponding panels. (**B** and **C**) The expression of *cxcr4a:RFP* transgene from 1 dpt to 3 dpt after DMSO (**B**, n=24/24) and Mtz treatment (**C**, n=17/20). **(D-G)** Triple labeling of FISH-*cxcr4a*, anti-TagRFP and Dendra2 at 3 dpt after DMSO (**D**, n=19/20) or Mtz treatment (**E**, n=18/23). Enlarged 2D images with higher resolution to show the co-localization of FISH-*cxcr4a* and *prox1a:TagRFP*, including XZ, YZ, and separated channels (**F**). Statistics show the ratio

of *cxcr4a* expressed in RFP+ iLECs after DMSO and Mtz treatment (**G**, n=8, $p<0.0001$, Two-tailed unpaired t-test, Mean±SD). (**H**-**J**) In contrast to the uninjured control (**H**, n=22/22), the *lyve1b:DsRed*+ vessels are shown to be positive for the *cxcr4a:RFP* transgene at 2 dpt after Mtz treatment (**I**, n=21/22). Statistics show *lyve1b:DsRed*+ in *kdrl*+ vessels and in *cxcr4a*+*kdrl*- vessels at 3 dpt after Mtz (**J**, n=6, $p<0.0001$, Two-tailed unpaired t-test, Mean±SD). **(K**-**M)** In contrast to the uninjured control (**K**, n=15/17), the *cxcr4a:RFP*+ vessels are shown to be positive for FISH-*vegfr3* signals at 4 dpt after Mtz treatment (**L**, n=14/17). Statistics show the distribution of FISH-*vegfr3* in *kdrl*+ vessels and in *cxcr4a*+*kdrl*- vessels at 4 dpt after Mtz (**M**, n=8, $p<0.0001$, Two-tailed unpaired t-test, Mean±SD). **(N**-**P)** In contrast to the uninjured control (**N**, n=19/20), the *cxcr4a:RFP*+ vessels are also positive for anti-Prox1 signals at 3 dpt after Mtz treatment (**O**, n=18/23). Arrowhead indicates an example. Statistics show Anti-Prox1 in *kdrl*+ vessels and in *cxcr4a*+*kdrl*- vessels at 3 dpt after Mtz (**P**, n=6, $p<0.0001$, Two-tailed unpaired t-test, Mean±SD). **(Q)** RT-PCR of *cxcr4a* expression in FACS sorted LECs from uninjured and injured brain (n=3 technical replicates. $p<0.0001$, Two-tailed unpaired t-test, Mean±SD). Dorsal view in A-C. Ventral view in D-O. B, C, K, and L, Scale bar, 50 μm. D-F, H, I, N, and O, Scale bar, 20 μm.

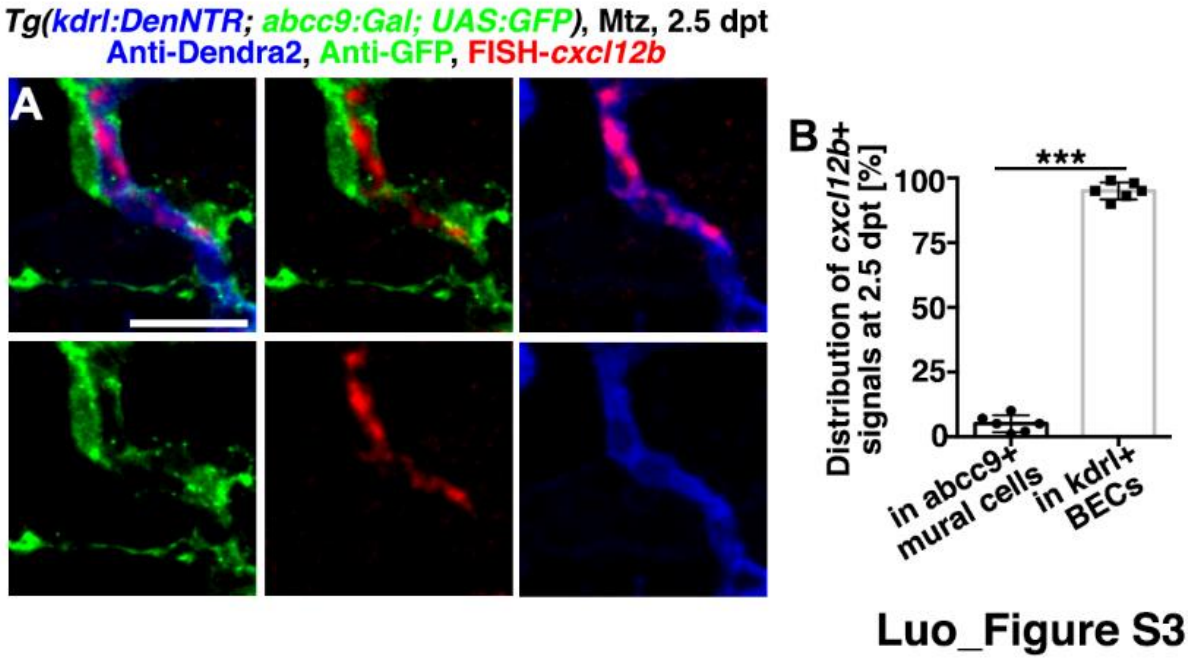
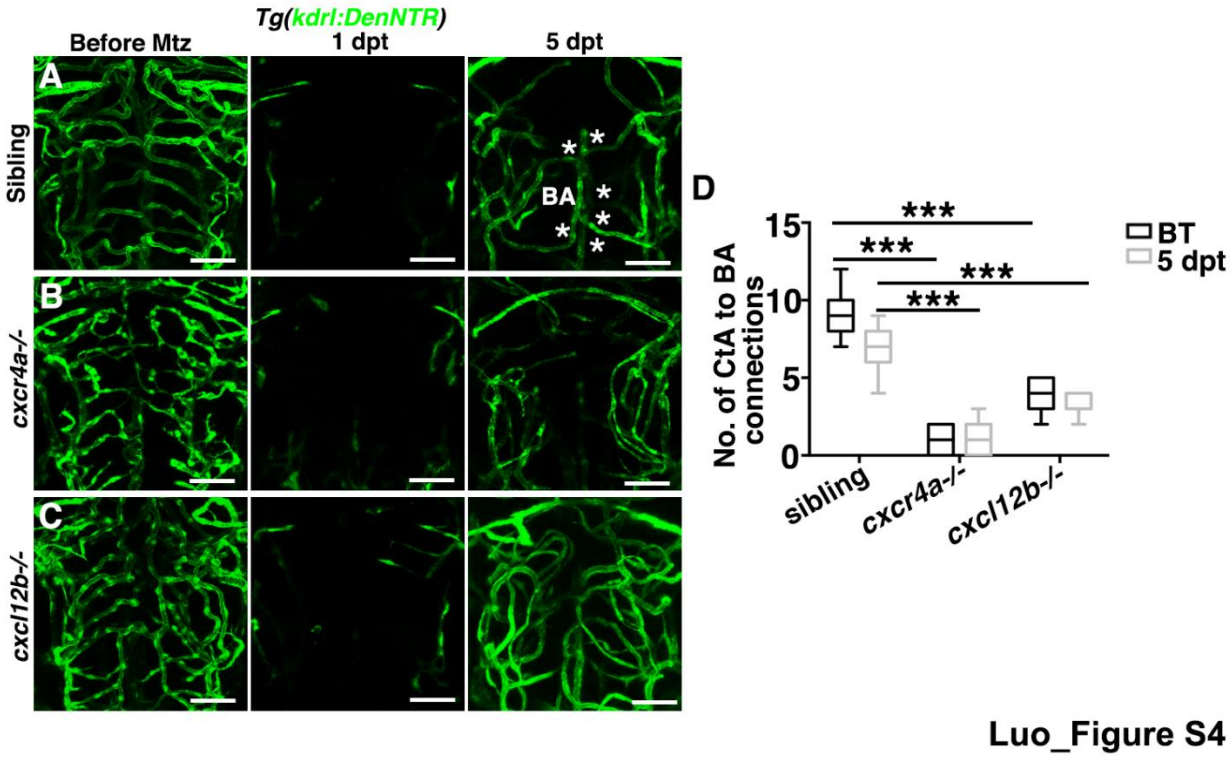


Fig. S3. Transcription of *cxcr12b* is not active in the mural cells. (**A** and **B**) Under *Tg(kdrl:DenNTR; abcc9^{BAC}:Gal4; UAS:GFP)* double transgenic line, triple labeling of FISH-*cxcl12b*, anti-GFP and Dendra2 at 2.5 dpt after Mtz treatment shows that *cxcl12b* are accumulated in the *kdrl*+ BECs, but not the *abcc9*+ mural cells (**A**, n=15/15). Statistics show the distribution of *cxcl12b*+ signals in the mural cells and BECs (**B**, n=6, p<0.0001, Two-tailed unpaired t-test, Mean±SD). Ventral view. Scale bar, 20 μm



Luo_Figure S4

Fig. S4. Cxcl12b/Cxcr4a is required for determining the ingrowing direction of nascent BVs. (**A**–**D**) In contrast to the siblings (**A**, asterisks, n=24/24), lateral connections of CtAs with BA rarely regenerate in the *cxcr4a* mutant (**B**, n=21/24), randomize but less regenerate in the *cxcl12b* mutant (**C**, n=19/20) at 5 dpt. Statistics show the number of CtA to BA connections at BT and 5 dpt (**D**, n=15, all ***, p<0.0001. Two-way ANOVA by Sidak's multiple comparisons test, Min to Max). Dorsal view. Scale bar, 50 μm.

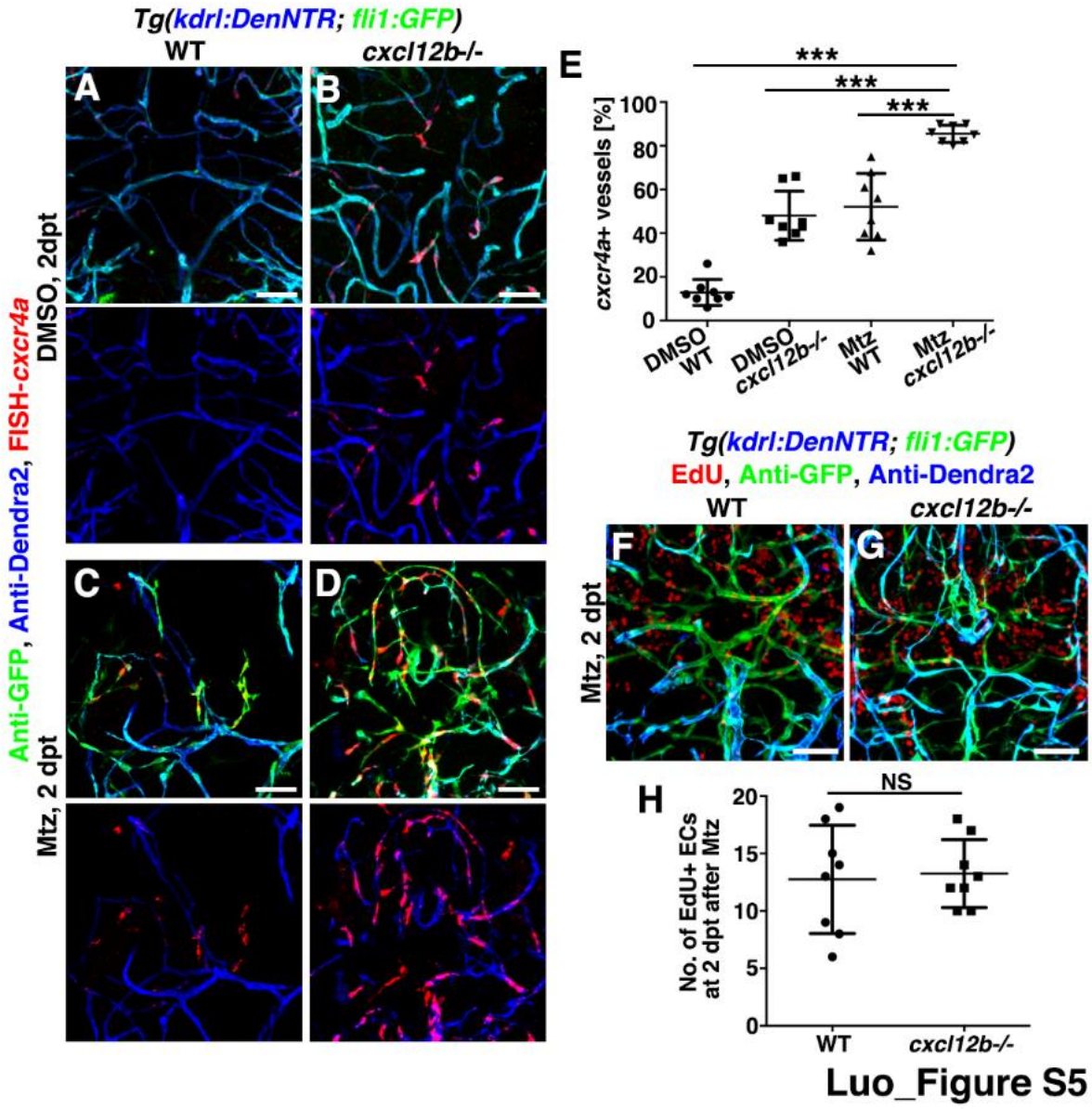


Fig. S5. Compensatory upregulation of *cxcr4a* in the *cxcl12b* mutant. (A–E) Triple labeling of FISH-*cxcr4a*, anti-GFP and anti-Dendra2 antibodies. Regardless of DMSO or Mtz treatment at 2 dpt, the *cxcl12b* mutant (**B**, n=15/18; **D**, n=16/19) exhibited compensatory upregulation of *cxcr4a* in both iLECs and brain BECs comparing to the wild-type (**A**, n=24/24; **C**, n=19/21). The statistics show the ratios of the vessels positive for *cxcr4a* (**E**, n=8, Two-tailed unpaired t-test, ***, p<0.0001). **(F–H)** Triple labeling of EdU, anti-GFP and anti-Dendra2 antibodies in the wild-type (**F**, n=17/17) and *cxcl12b*

mutant (**G**, n=16/18). The statistics show the ratios of the endothelial cells positive for EdU labeling are unaffected in the *cxcl12b* mutant (**H**, n=8, Two-tailed unpaired t-test, NS, p=0.8032). Data are represented as Mean±SD. Ventral view. Scale bar, 50 μm.

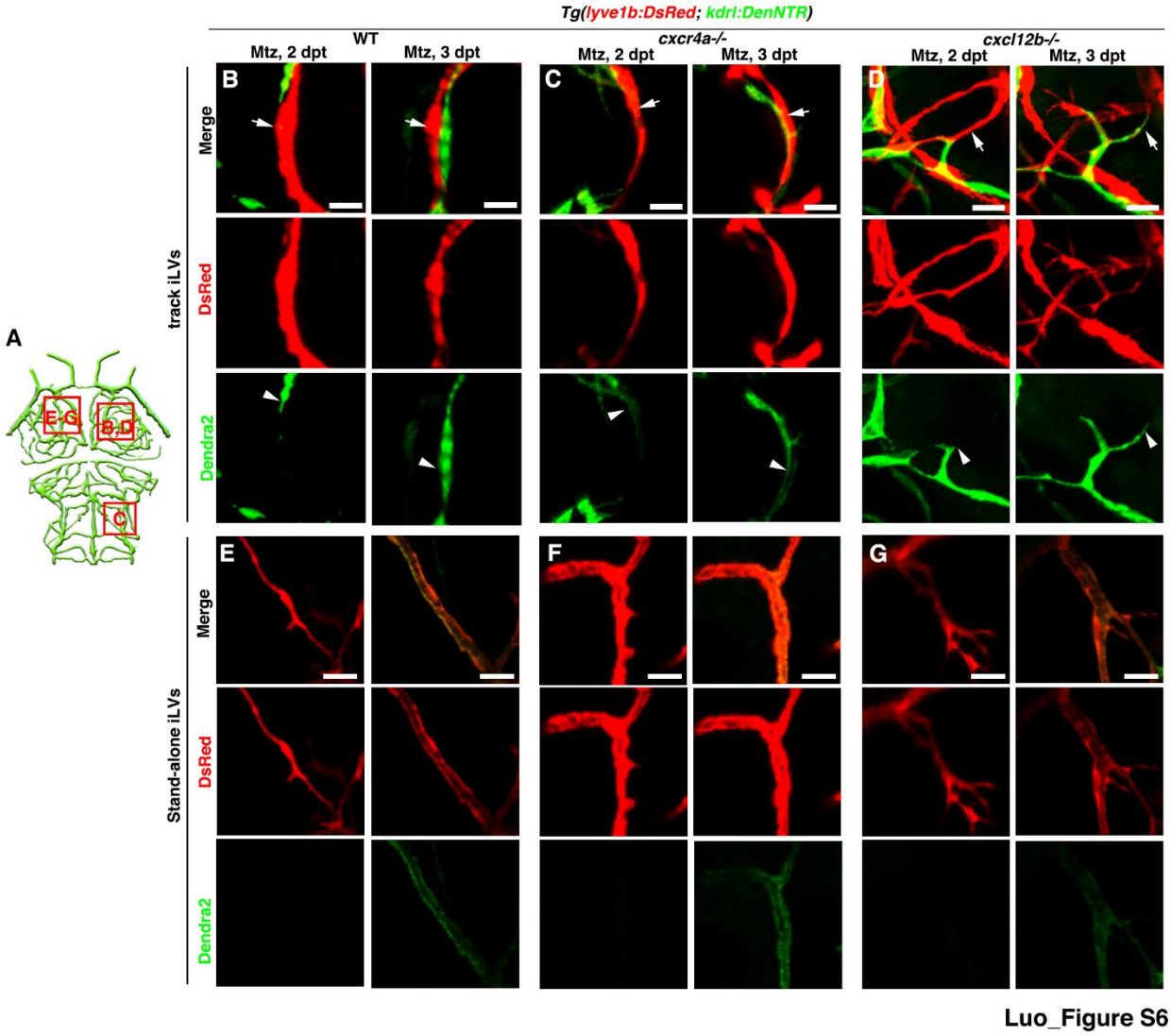
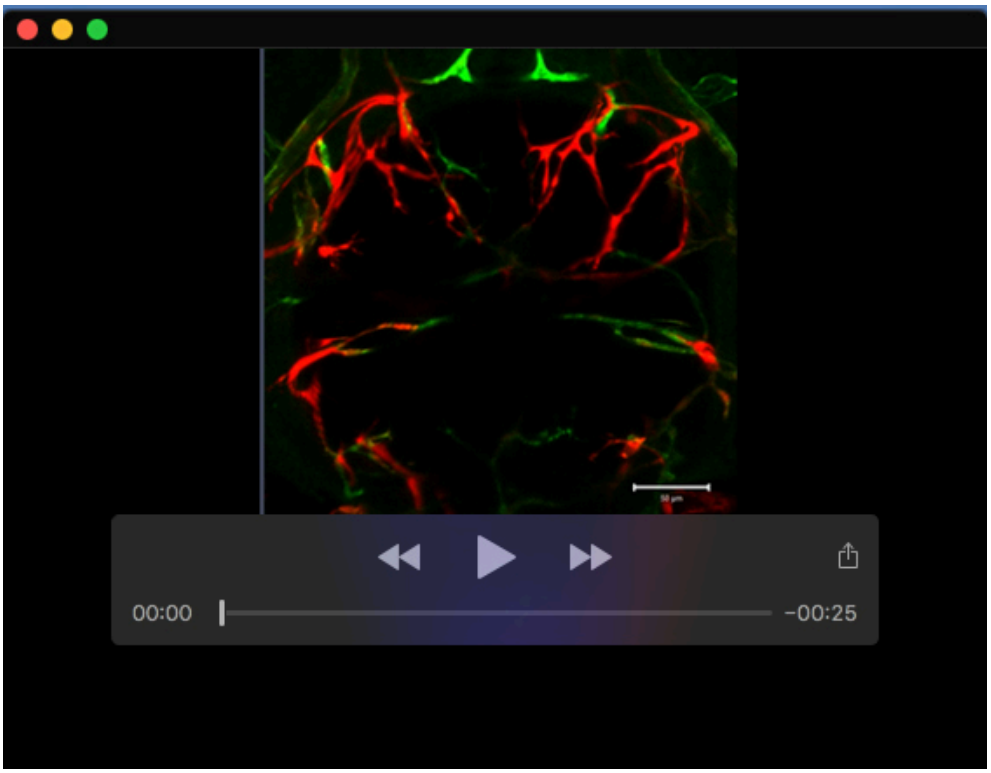
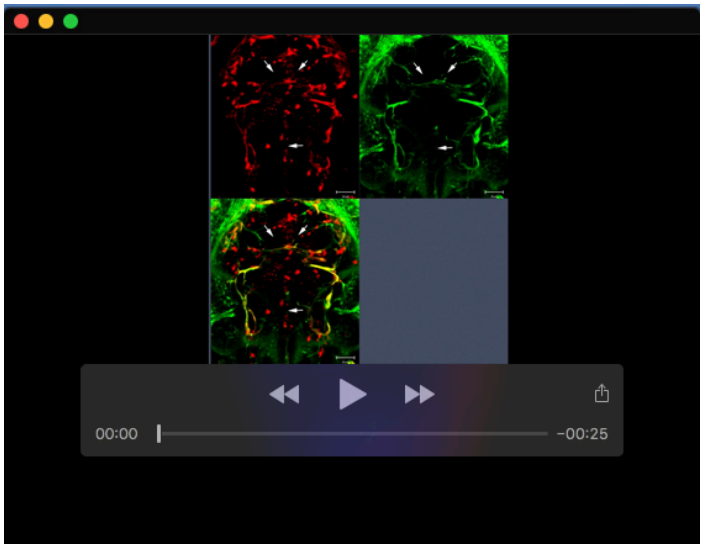


Fig. S6. Cxcl12b/Cxcr4a are dispensable for the iLVs acting as "growing tracks" or transdifferentiation. (A) Red box indicates the image area in the corresponding panels. **(B–G)** Compared to wild-type (**B**, **E**, n=20/20, n=18/18), in both *cxcr4a* (**C**, **F**, n=11/13, n=14/15) and *cxcl12b* (**D**, **G**, n=19/23, n=14/16) mutants, the Dendra2^{+} nascent BVs (**B-D**, arrowheads) keep growing along the DsRed^{+} track iLVs (**B-D**, arrows), while the stand-alone iLVs keep transdifferentiating (**E-G**). Dorsal view. Scale bar, 20 μm.



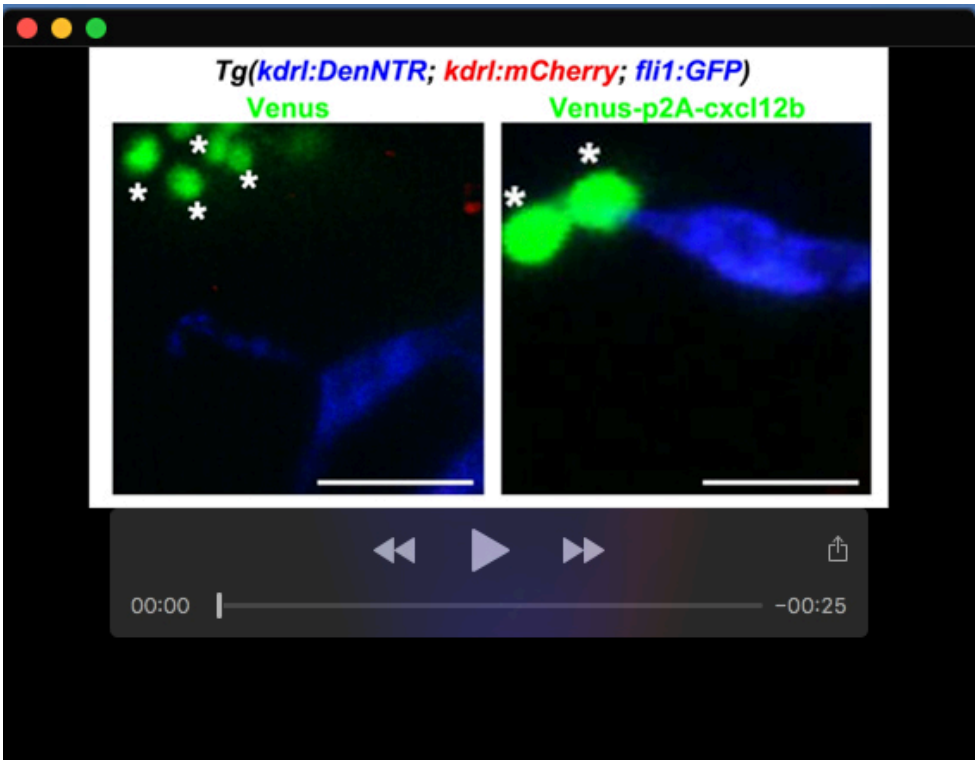
Movie 1. Rapid and active ingrowth of the *lyve1b+kdrl-* iLVs from peri-injured area into the central area from 1.5 dpt.

The *Tg(kdrl:DenNTR; lyve1b:DsRed)* double transgenic line was used for the time-lapse image (n=13/13). The *lyve1b*+ lymphatic vessels quickly and directionally ingrow into the injured parenchyma. Duration of imaging was 850 minutes. Dorsal view. Scale bar, 50 μm.



Movie 2. Rapid and active ingrowth of the *fli1+kdrl*- iLVs from peri-injured area into the central area from 1.5 dpt.

The *Tg(kdrl:DenNTR; kdrl:mCherry; fli1:GFP)* triple transgenic line was used for the time-lapse image (n=15/19). Arrows indicate the growth direction (arrows). Duration of imaging was 750 minutes. Dorsal view. Scale bar, 50 μm.



Movie 3. Ectopically expressed Cxcl12b is sufficient to determine the directionality of ingrown lymphangiogenesis in the *cxcl12b* mutant.

The *Tg(kdrl:DenNTR; kdrl:mCherry; fli1:GFP)* triple transgenic line was used for the time-lapse image. Note that the blue fluorescent (GFP artificial fluorescence) iLVs ingrow towards the ectopically expressed Venus-p2A-Cxcl12b (right side, green fluorescence, asterisks), but not the Venus control (left side, green fluorescence, asterisks). Durations of left and right imaging were 410 and 1000 minutes, respectively. Dorsal view. Scale bar, 20 μm.