

Supplementary data

Supplementary table S1: Antibodies and markers used for the visualization of blood vessels and cells respectively.

<i>Primary antibodies</i>	<i>Target</i>	<i>Dilution</i>	<i>Species</i>	<i>Suppliers</i>	<i>Secondary antibodies</i>	<i>Dilution</i>
Podocalyxin	Capillaries	1/200	goat	RetD Systems (#AF1556)	DAG-Alexafluor 594	1/400
PECAM-1	Endothelial cells	1/200	rat	BD Pharmingen (#550274)	DAR-Alexafluor 594	1/400
α SMA-Cy3	Arteries	1/500	mouse	Sigma Aldrich (#C16198)	NA	NA
TO-PRO 3 iodide	DNA intercalator	1/3 000	NA	Thermo Fisher (#T3605)	NA	NA

α SMA: alpha smooth muscle actin; DAG: donkey anti-goat; DAR: donkey anti-rat; NA: not applicable; PECAM-1: platelet endothelial cell adhesion molecule 1. The secondary antibodies were purchased from Molecular Probes.

Supplementary table S2: Adaptation of the iDISCO protocol depending on the age of the embryos/pups.

<i>Steps</i>	<i>Solvents</i>	<i>Samples</i>				
		E11 (whole embryo)	E13 (whole embryo)	E15 (whole embryo)	E17 (head)	P0 (dissected brain)
1) Dehydration	20%MeOH	NA	1h	1h	1h	1h
	40%MeOH	NA	1h	1h	1h	1h
	60%MeOH	NA	1h	1h	1h	1h
	80%MeOH	NA	1h	1h	1h	1h
	100%MeOH	NA	1h	1h	1h	1h
	100%MeOH	NA	1h	1h	1h	1h
2) Depigmentation	95%MeOH + 5% H2O2	NA	24h	48h	48h	48h
3) Rehydration	80%MeOH	NA	1h	1h	1h	1h
	60%MeOH	NA	1h	1h	1h	1h
	40%MeOH	NA	1h	1h	1h	1h
	20%MeOH PBS	NA	1h	1h	1h	1h
	Rinsing PTx.2 (2x30 min)	1h	1h	1h	1h	1h
		30 min	30 min	30 min	30 min	30 min
4-9) IHC	4) Permeabilization	24h	48h	48h	48h	48h
	5) Blocking	24h	24h	24h	24h	24h
	6) Primary antibodies	5 days	6 days	6 days	6 days	6 days
	7) Rinsing (6 times)	1h	1h	1h	1h	1h
	8) Secondary antibodies	5 days	6 days	6 days	6 days	6 days
	9) Rinsing	1h	1h	1h	1h	1h
Embedding	Agarose	30 min	NA	NA	NA	NA
10) Dehydration	20%MeOH	1h	1h	1h	1h	1h
	40%MeOH	1h	1h	1h	1h	1h
	60%MeOH	1h	1h	1h	1h	1h
	80%MeOH	1h	1h	1h	1h	1h
	100%MeOH	1h	1h	1h	1h	1h
	100%MeOH	1h	1h	1h	1h	1h
	Méthanol /DCM	1 night	1 night	1 night	1 night	1 night
11) Delipidation	100% DCM (2 times)	15 min	15 min	15 min	15 min	15 min
12) Clearing	DBE	storage solvent	storage solvent	storage solvent	storage solvent	storage solvent

DBE: dibenzylether; DCM: dichloromethane; MeOH: methanol; PBS: phosphate-buffered saline.

Supplementary table S3: Summary of the statistical methods used for the analysis of the different variable types.

<i>Variable analyzed</i>	<i>Statistical Test performed</i>	<i>Analysis type</i>
Total artery volume/length per age	Kruskal-Wallis test	Global difference during development
	Dunn's test	Differences between two specific ages
Individual artery volume/length per age	Two-way ANOVA	Global difference during development
	Tukey post-test	Differences between two specific ages
Artery volume per individual	Wilcoxon Friedman	Global difference in paired arteries per age
	Dunn's test	Differences between two specific arteries
	Mixed-effects analysis	Interindividual/inter-artery dispersion
Analysis of the topographic variability of the three cerebellar arterial pairs	Fisher's exact test	Sex and age differences of arterial morphology or branching
Internal vasculature analysis per age	Principal component analysis	Overview of the differences of multiple measured parameters during development
Internal vascular parameters per age	Kruskal-Wallis test	Global difference during development
	Dunn's test	Differences between two specific ages

Supplementary table S4: Sex effect analyses assessed using the Wilcoxon test. Variables with a significant sex effect (Wilcoxon rank-sum test, p-value < 0.05) are shown in bold.

<i>Analysis</i>	<i>Categories</i>	<i>Age</i>	<i>p-value</i>
Figure 6B Total artery volume per age		E11	0.0199* ♂ > ♀
		E13	0.1437
		E15	0.1207
		E17	0.5309
Figure 6B' Artery volume per age	Left SCA	E11	0.1780
		E13	0.4034
		E15	0.7841
		E17	0.8345
	Right SCA	E11	0.0661
		E13	0.7841
		E15	0.2353
		E17	0.6761
	Left AICA	E13	0.5308
		E15	0.7841
		E17	1.0000
	Right AICA	E13	0.2962
		E15	0.2353
		E17	0.8345
	Left PICA	E13	0.2100
		E15	0.7841
		E17	0.5308
	Right PICA	E13	0.2962
		E15	0.0828
		E17	0.2962
Figure 6C Total artery length per age		E11	0.5402
		E13	0.6761
		E15	0.0357* ♂ < ♀
		E17	0.2100
Figure 6C' Artery length per age	Left SCA	E11	0.5402
		E13	0.4033
		E15	0.3153
		E17	0.6761
	Right SCA	E11	0.3912
		E13	0.6761
		E15	0.0081** ♂ < ♀
		E17	0.6761
	Left AICA	E13	0.4033
		E15	0.3153
		E17	0.6761

<i>Analysis</i>	<i>Categories</i>	<i>Age</i>	<i>p-value</i>
	Right AICA	E13	0.6761
		E15	0.01371* ♂ < ♀
		E17	0.0601
	Left PICA	E13	0.5308
		E15	0.2353
		E17	0.0601
	Right PICA	E13	0.2962
		E15	0.0137* ♂ < ♀
		E17	0.8345
Figure 10C Network volume		E11	1.000
		E13	0.7841
		E15	0.4033
		E17	0.0367* ♂ > ♀
		P0	0.2100
Figure 10C Network length		E11	1.000
		E13	0.3153
		E15	0.5308
		E17	0.0121* ♂ > ♀
		P0	0.1436
Figure 10D Number of segments		E11	1.000
		E13	0.3153
		E15	0.2962
		E17	0.0121* ♂ > ♀
		P0	0.2100
Figure10D Branchpoints		E11	1.000
		E13	0.6480
		E15	0.2962
		E17	0.0121* ♂ > ♀
		P0	0.1436
Figure 10D Segment partitioning		E11	0.3912
		E13	0.6480
		E15	0.6761
		E17	0.0601
		P0	1.000
Figure 10E Mean segment volume		E11	0.5402
		E13	0.9272
		E15	0.5308
		E17	0.0946
		P0	0.5308
Figure 10E		E11	0.0661
		E13	0.9272

<i>Analysis</i>	<i>Categories</i>	<i>Age</i>	<i>p-value</i>
Mean segment length		E15	0.5308
		E17	0.0946
		P0	0.5308
Figure 10E Mean segment radius		E11	0.2703
		E13	0.6480
		E15	0.4033
		E17	0.0367* ♂ > ♀
		P0	0.6761

Supplementary table S5: Summary of the statistical analyses used for each quantitative parameter.

<i>Analysis</i>	<i>Test</i>	<i>n</i>	<i>p-value</i>
Figure 6B Total artery volume per age	Kruskal-Wallis Dunn's test	E11 n=9 (5♂/4♀) E13 n=10 (5♂/5♀) E15 n=12 (7♂/5♀) E17 n=10 (5♂/5♀)	Kruskal-Wallis Stat.: 15.60 p= 0.0014 E11 vs E13 – p= 0.0248* E11 vs E15 – p= 0.0008*
Figure 6B' Artery volume per age	Two way ANOVA Tukey post-test	E11 n=9 (5♂/4♀) E13 n=10 (5♂/5♀) E15 n=12 (7♂/5♀) E17 n=10 (5♂/5♀) Left and right arteries	ANOVA Interaction F 4.614 – p<0.0001**** Row factor F 31.40 – p<0.0001**** Column factor F 3.621 – p= 0.0036** Tukey's <i>Left AICA</i> E13 vs E17 – p=0.0006*** <i>Right AICA</i> E13 vs E17 – p<0.0001**** E15 vs E17 – p<0.0001**** <i>Right PICA</i> E13 vs E17 – p=0.0175*
Figure 6C Total artery length per age	Kruskal-Wallis Dunn's test	E11 n=9 (5♂/4♀) E13 n=10 (5♂/5♀) E15 n=12 (7♂/5♀) E17 n=10 (5♂/5♀)	Kruskal-Wallis Stat.: 15.60 p<0,0001**** E11 vs E13 – p=0.0007*** E11 vs E15 – p=0.0037** E13 vs E17 – p=0.0185 *
Figure 6C' Artery length per age	Two way ANOVA Tukey post-test	E11 n=9 (5♂/4♀) E13 n=10 (5♂/5♀) E15 n=12 (7♂/5♀) E17 n=10 (5♂/5♀) Left and right arteries	ANOVA Interaction F 9.407 – p= 0.0002*** Row factor F 18.53 – p<0.0001**** Column factor F 28.92 – p<0.0001**** Tukey's <i>Left SCA</i> E15 vs E17 – p=0.0265* <i>Right SCA</i> E11 vs E17 – p=0.0239* E13 vs E17 – p=0.0215* E15 vs E17 – p=0.0141* <i>Left AICA</i> E15 vs E17 – p=0.0265* <i>Right AICA</i> E13 vs E17 – p= 0.0408*
Figure 6D E11	Wilcoxon	E11 n=9 (5♂/4♀) Left and right arteries	p=0.6523 – ns

<i>Analysis</i>	<i>Test</i>	<i>n</i>	<i>p-value</i>
Artery volume per individual			
Figure 6D E13 Artery volume per individual	Friedman Dunn's test	E13 n=10 (5♂/5♀) Left and right arteries	Friedman Friedman stat.: 13.76 p=0.0172* Mixed-effects analysis Treatment: p=0.0275* Matching effect: p=0.0056** $V_{\text{indiv}} = 2,186 \times 10^{-6}$ $V_{\text{residual}} = 5.171 \times 10^{-6}$ $V_{\text{indiv}} < V_{\text{resid}}$
Figure 6D E15 Artery volume per individual	Friedman Dunn's test	E15 n=12 (7♂/5♀) Left and right arteries	Friedman Friedman stat.: 22.63 p=0.0004*** Mixed-effects analysis Treatment: p=0.021** Matching effect: p=0.0069** $V_{\text{indiv}} = 3.981 \times 10^{-6}$ $V_{\text{residual}} = 1.064 \times 10^{-5}$ $V_{\text{indiv}} < V_{\text{resid}}$
Figure 6D E17 Artery volume per individual	Friedman Dunn's test	E17 n=10 (5♂/5♀) Left and right arteries	Friedman Friedman stat.: 33.54 p<0.0001**** Mixed-effects analysis Treatment : p=0.0006*** Matching effect : p=0.0671 ns $V_{\text{indiv}} = 2.682 \times 10^{-6}$ $V_{\text{residual}} = 1.172 \times 10^{-5}$ $V_{\text{indiv}} < V_{\text{resid}}$
Figure 6D' E11 Artery length per individual	Wilcoxon	E11 n=9 (5♂/4♀) Left and right arteries	p=0.5703 – ns
Figure 6D' E13 Artery length per individual	Friedman Dunn's test	E13 n=10 (5♂/5♀) Left and right arteries	Friedman Friedman stat.: 39.31 p<0.0001**** Dunn <i>Left AICA vs Right AICA</i> – p=0.0824 – ns Mixed-effects analysis Treatment: p=0.0002***

<i>Analysis</i>	<i>Test</i>	<i>n</i>	<i>p-value</i>
			Matching effect: $p < 0.0001$ **** $V_{\text{indiv}} = 3.130 \times 10^{-12}$ $V_{\text{residual}} = 1.307 \times 10^{-12}$ $V_{\text{indiv}} > V_{\text{resid}}$
Figure 6D' E15 Artery length per individual	Friedman Dunn's test	E15 n=12 (7♂/5♀) Left and right arteries	Friedman Friedman stat.: 47.30 $p < 0.0001$ *** Dunn <i>Left AICA vs Right AICA</i> – $p = 0.0157^*$ Mixed-effects analysis Treatment: $p < 0.0001$ **** Matching effect: $p < 0.0001$ **** $V_{\text{indiv}} = 6.154 \times 10^{-12}$ $V_{\text{residual}} = 1.256 \times 10^{-12}$ $V_{\text{indiv}} > V_{\text{resid}}$
Figure 6D' E17 Artery length per individual	Friedman Dunn's test	E17 n=10 (5♂/5♀) Left and right arteries	Friedman Friedman stat.: 40.16 $p < 0.0001$ **** Dunn <i>Left AICA vs Right AICA</i> – $p = 0.0824$ – ns Mixed-effects analysis Treatment: $p = 0.0002$ *** Matching effect: $p = 0.0044$ ** $V_{\text{indiv}} = 1.063 \times 10^{-13}$ $V_{\text{residual}} = 2.187 \times 10^{-13}$ $V_{\text{indiv}} > V_{\text{resid}}$
Figure 7C Analysis of the topographic variability of the three cerebellar arterial pairs	Fisher's exact test	E13 n=12 (7♂/5♀) E15 n=12 (6♂/6♀) E17 n=10 (5♂/5♀) Left and right arteries	Fisher exact's test Sex-based morphology AICA $p = 0.7249$ – ns Sex-based morphology PICA $p = 0.9214$ ns Age-based morphology AICA $p = 0.3576$ – ns Age-based morphology PICA $p = 0.1902$ – ns Sex-based branching $p = 0.4348$ – ns Age-based branching $p = 0.1902$ E13 vs E17 – $p = 0.05341$ – ns

<i>Analysis</i>	<i>Test</i>	<i>n</i>	<i>p-value</i>
Figure 10AB	Principal component analysis	E11 n=9 (5♂/4♀) E13 n=11 (6♂/5♀) E15 n=10 (5♂/5♀) E17 n=10 (5♂/5♀) 14 components analyzed	Principal component analysis Percentage of cumulative variance Dim1: 47.43% Dim2: 73.92% Dim3: 89.48% Dim4: 95.09%
Figure 10C Network volume	Kruskal-Wallis Dunn's test	E11 n=9 (5♂/4♀) E13 n=11 (6♂/5♀) E15 n=10 (5♂/5♀) E17 n=10 (5♂/5♀)	Kruskal-Wallis Stat.: 29.80 p<0,0001**** E11 vs E15 – p=0.0006*** E11 vs E17 – p=0.0007*** E13 vs E15 – p=0,0350* E13 vs E17 – p=0.0427* E15 vs P0 – p=0.0044** E17 vs P0 – p=0.0056**
Figure 10C Network length	Kruskal-Wallis Dunn's test	E11 n=9 (5♂/4♀) E13 n=11 (6♂/5♀) E15 n=10 (5♂/5♀) E17 n=10 (5♂/5♀)	Kruskal-Wallis Stat.: 20.54 p=0.0004*** E11 vs E17 – p=0.0244* E15 vs P0 – p=0.0216* E17 vs P0 – p=0.0033**
Figure 10D Number of segments	Kruskal-Wallis Dunn's test	E11 n=9 (5♂/4♀) E13 n=11 (6♂/5♀) E15 n=10 (5♂/5♀) E17 n=10 (5♂/5♀)	Kruskal-Wallis Stat.: 18.21 P=0,0011** E11 vs E17 – p=0.0105* E13 vs E17 – p=0.0421* E17 vs P0 – p=0.0194*
Figure 10D Branchpoints	Kruskal-Wallis Dunn's test	E11 n=9 (5♂/4♀) E13 n=11 (6♂/5♀) E15 n=10 (5♂/5♀) E17 n=10 (5♂/5♀)	Kruskal-Wallis Stat.: 22.87 p=0,0001*** E11 vs. E15 – p=0.0446* E11 vs E17 – p=0.0026** E13 vs. E17 – p=0.0120* E17 vs P0 – p=0.0059**
Figure 10D Segment partitioning	Kruskal-Wallis Dunn's test	E11 n=9 (5♂/4♀) E13 n=11 (6♂/5♀) E15 n=10 (5♂/5♀) E17 n=10 (5♂/5♀)	Kruskal-Wallis Stat.: 42.98 p<0,0001**** E11 vs E17 – p=0.0001*** E11 vs P0 – p<0.0001**** E13 vs E17 – p=0.0396* E13 vs P0 – p<0.0001**** E15 vs P0 – p=0.0109*
Figure 10E Mean segment volume	Kruskal-Wallis Dunn's test	E11 n=9 (5♂/4♀) E13 n=11 (6♂/5♀) E15 n=10 (5♂/5♀) E17 n=10 (5♂/5♀)	Kruskal-Wallis Stat.: 37.21 p<0,0001**** E11 vs E15 – p=0.0039** E11 vs E17 – p<0.0001**** E11 vs P0 – p<0.0001****

<i>Analysis</i>	<i>Test</i>	<i>n</i>	<i>p-value</i>
			E13 vs E17 – p=0.0059** E13 vs P0 – p=0.0052**
Figure 10E Mean segment length	Kruskal-Wallis Dunn's test	E11 n=9 (5♂/4♀) E13 n=11 (6♂/5♀) E15 n=10 (5♂/5♀) E17 n=10 (5♂/5♀)	Kruskal-Wallis Stat.: 43.13 p<0,0001**** E11 vs E15 – p=0.0398* E11 vs E17 – p=0.0002*** E11 vs P0 – p<0.0001**** E13 vs P0 – p=0.0001*** E15 vs P0 – p=0.0128*
Figure 10E Mean segment radius	Kruskal-Wallis Dunn's test	E11 n=9 (5♂/4♀) E13 n=11 (6♂/5♀) E15 n=10 (5♂/5♀) E17 n=10 (5♂/5♀)	Kruskal-Wallis Stat.: 41.20 p<0,0001**** E11 vs E15 – p=0.0164* E11 vs E17 – p<0.0001**** E11 vs P0 – p<0.0001**** E13 vs E17 – p=0.0135* E13 vs P0 – p=0.0004***