Table S1. Antibodies and dyes used in this study.

	Antibody and dye	Marking target	Company	Cat No.	Ratio
1	PRDM10 rabbit antibody	Prdm10	Biorbyt	orb312613	1:100
2	PRDM13 rabbit antibody	Prdm13	Biorbyt	orb312614	1:100
3	Goat anti-rabbit IgG H&L (Alexa Fluor® 488)		Abcam	ab150077	1:100
4	DyLight 594 labeled Lycopersicon Esculentum (Tomato) Lectin (LEL, TL)	Blood vessel	Vector laboratories	DL-1177	-
5	DAPI Hoechst 33342	Nuclear	Invitrogen	R37605	-

Table S2. An outline of PRDM10 and PRDM13 mouse embryonic expression.

		PRDM10	PRDM13
Mouse embryonic date	**E9.5**	Craniofacial structures Somites Notochord Telencephalon Tegmentum Cerebellum Midbrain Dorsal root ganglia Hindbrain	Craniofacial structures Somites Notochord Telencephalon Tegmentum Cerebellum Midbrain Dorsal root ganglia Hindbrain
	E10.5	Craniofacial structures Brain Somites Heart Tegmentum Spinal cord Spinal ganglia	Craniofacial structures Brain Somites Heart Tegmentum Spinal cord Spinal ganglia
	E13.5	Brain Spinal cord Skeletal cartilage Ventricle Tongue Olfactory epithelium Umbilical cord	Brain Spinal cord Skeletal cartilage Lung Olfactory epithelium Eye

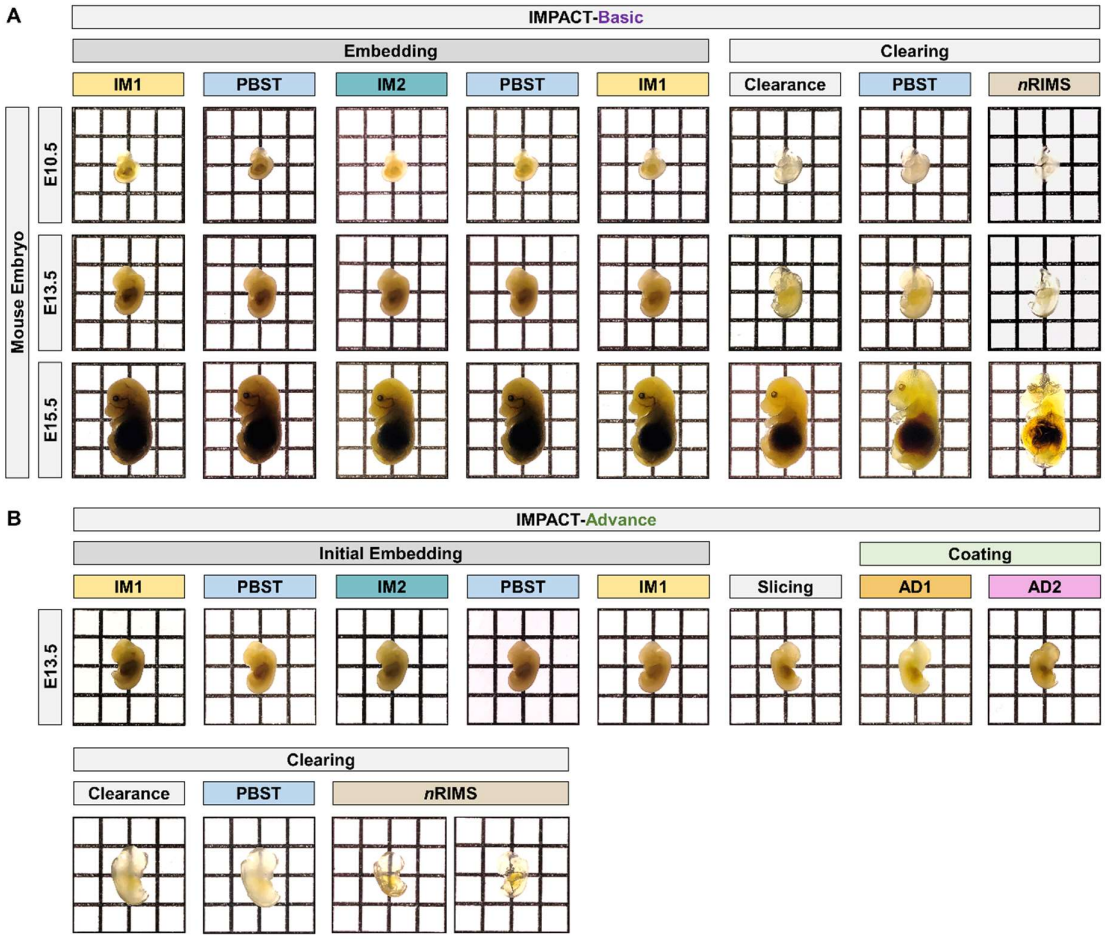

Figure S1. Comparison of optical transparency achieved in mouse embryos processed via IMPACT-Basic and IMPACT-Advance.

Comparison of optical transparency achieved in E9.5, E10.5, E13.5 and E15.5 mouse processed via IMPACT-Basic (**A**) and IMPACT-Advance (**B**). The transparency of all cleared samples was assessed against a patterned background (length:width=5-mm:5-mm).

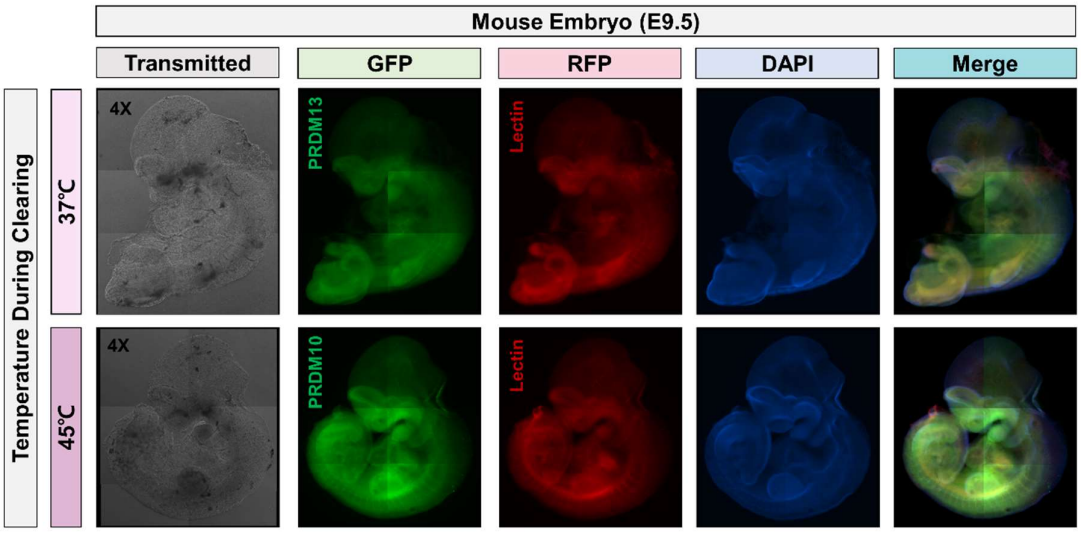

Figure S2. Fluorescence images in E9.5 mouse embryos via IMPACT-Basic.
Comparison of additional fluorescence images in E9.5 mouse embryos achieved by clearing at 37°C and 45°C using IMPACT-Basic. The whole image of each sample was created using fluorescent microscopy, and the microscope was focused on 2 × 3 panels (horizontal × vertical). Merged images with PRDM10 and PRDM13 in green, lectin in red, and DAPI in blue.

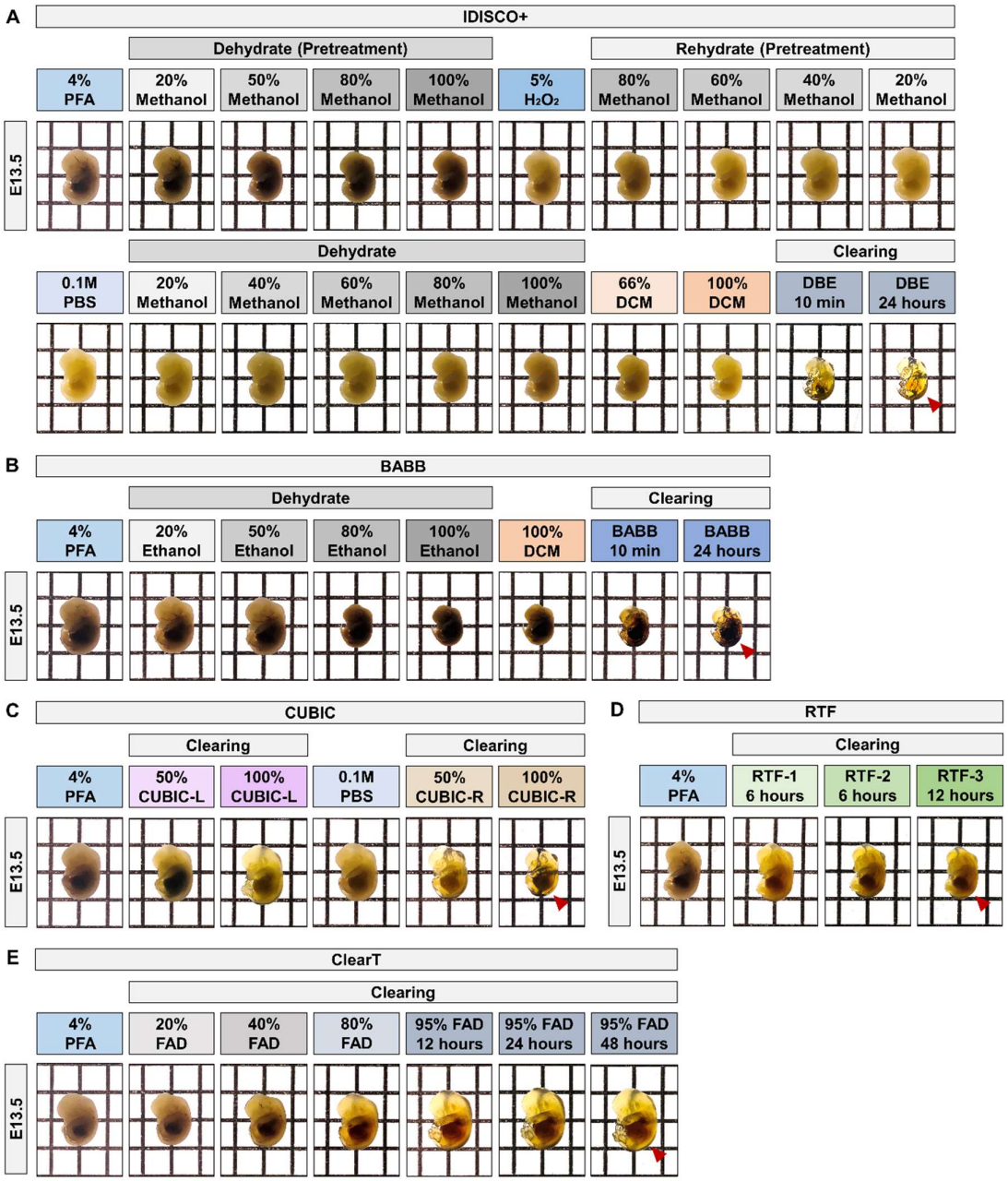

Figure S3. Comparison of optical transparency in E13.5 mouse embryos achieved by iDISCO+, BABB, CUBIC, RTF and Clear^{T}.

Comparison of optical transparency achieved in E13.5 processed via iDISCO+ (**A**), BABB (**B**), CUBIC (**C**), RTF (**D**) and Clear^{T} (**E**). The transparency of all cleared samples was assessed against a patterned background (length:width=5-mm:5-mm). Red arrows point to areas of opaque heart and liver.

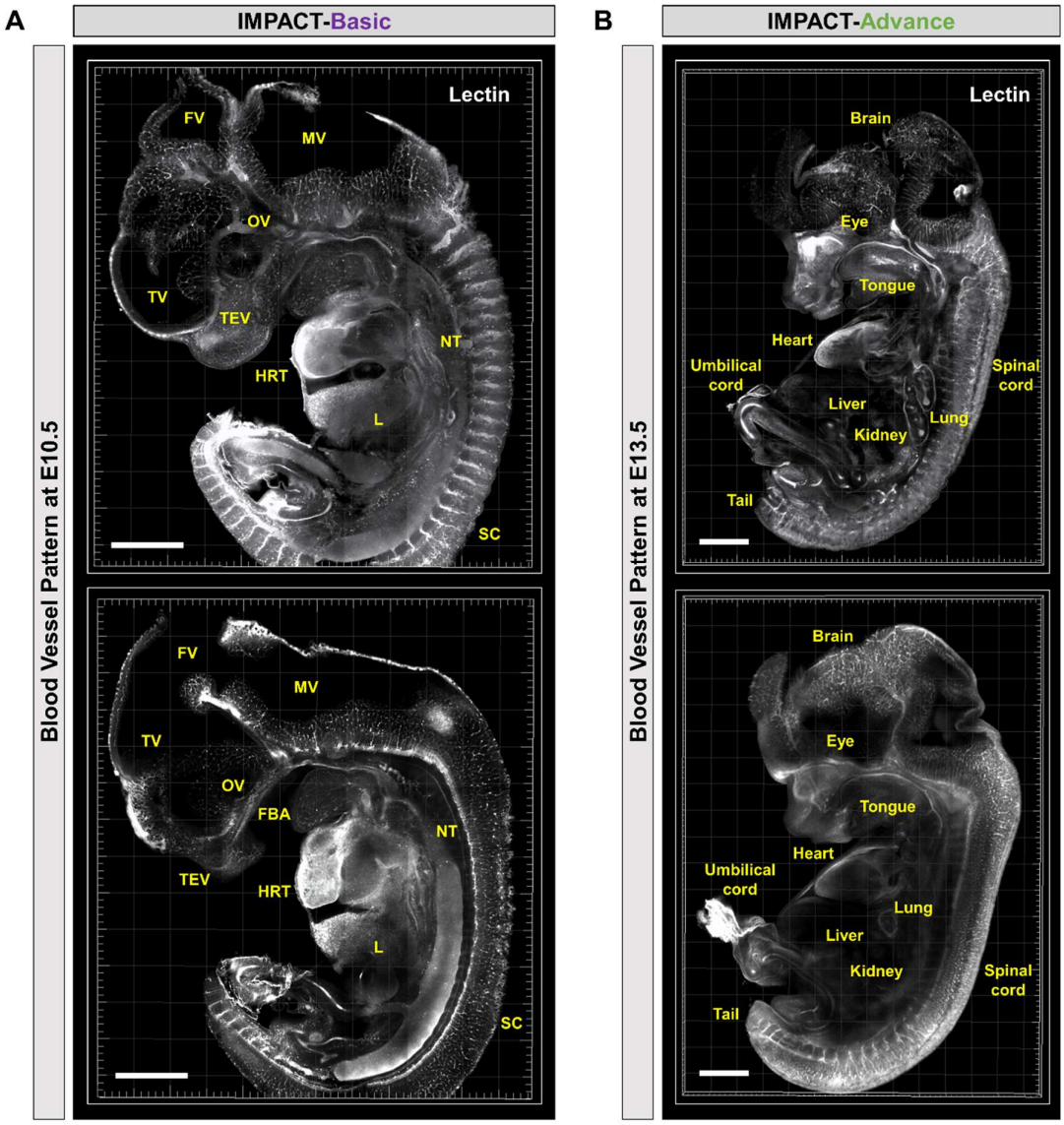

Figure S4. Blood vessel imaging in mouse embryos at E10.5 and E13.5.
Lectin immunostaining in E10.5 (**A**) and E13.5 (**B**) mouse embryos processed via IMPACT-Basic and IMPACT-Advance. FBA = first branchial arch; FV = fourth ventricle; HRT = heart; MV = mesencephalic vesicle; SC = spinal cord; NT = neural tube; TEV = telencephalic vesicle; TV = third ventricle; T = tegmentum; FL = fore limb; L = liver. Scale bar (white: 1000-μm).

Video S1. Three-dimensional images of PRDM10 immunostaining in a cleared E9.5 mouse embryo.

Video S2. Three-dimensional images of PRDM13 immunostaining in a cleared E9.5 mouse embryo.

Video S3. Three-dimensional images of PRDM10 immunostaining in focused midbrain in a cleared E13.5 mouse embryo.