

Fig. S1. Illustration of mapping accuracy using WlzWarp. Examples of mapped data (bright-field image on left) compared to equivalent virtual sections through 3D reconstructions of the original gene expression data (on right) for genes as indicated. All data are from E10.5 embryos through the forebrain and midbrain (Wnt3a, Wnt5a, Wnt4 (upper)), the hindbrain (Wnt4 lower), the forelimb (Wnt6 left) and hindbrain and forebrain (Wnt6 right). Note the mapping accuracy overall but the mis-mapping of ectodermal Wnt6 on one side of the embryo (arrows). Please note that mapped data are used for integrated analysis and any interpretation is supported by viewing the original scanned expression pattern. Relative scale as indicated on Figure 1.

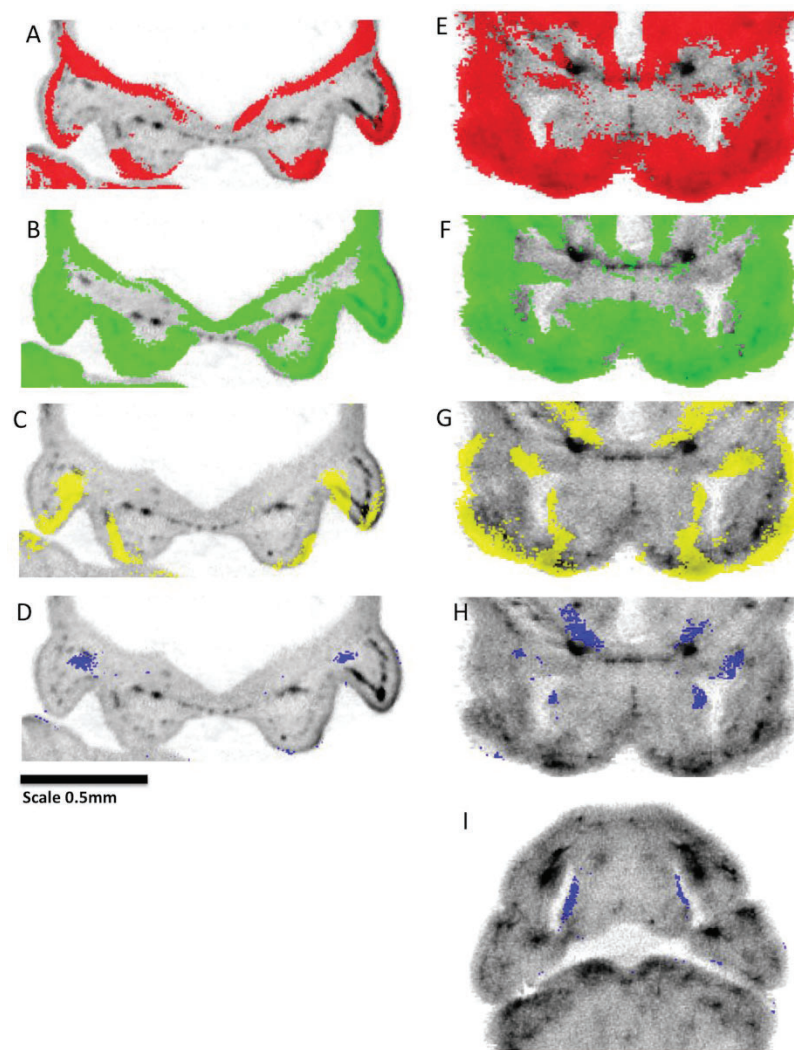


Fig S2. Canonical Wnt pathway activity in the context of detectable Wnt and Fzd expression in the nasal region. A-H: Frontal sections in the nasal region in OPT reference models of E10.5 (A-D) and E11.5 (E-H) embryos; I is a transverse section at E11.5. A and E, show combined expression domain for all Wnt genes; B and F, combined expression domain for all Fzd genes; C and G, Tcf/Lef-GFP; D, H and I shows the domain of Tcf/Lef-GFP activity where neither Wnt nor Fzd expression was detected.

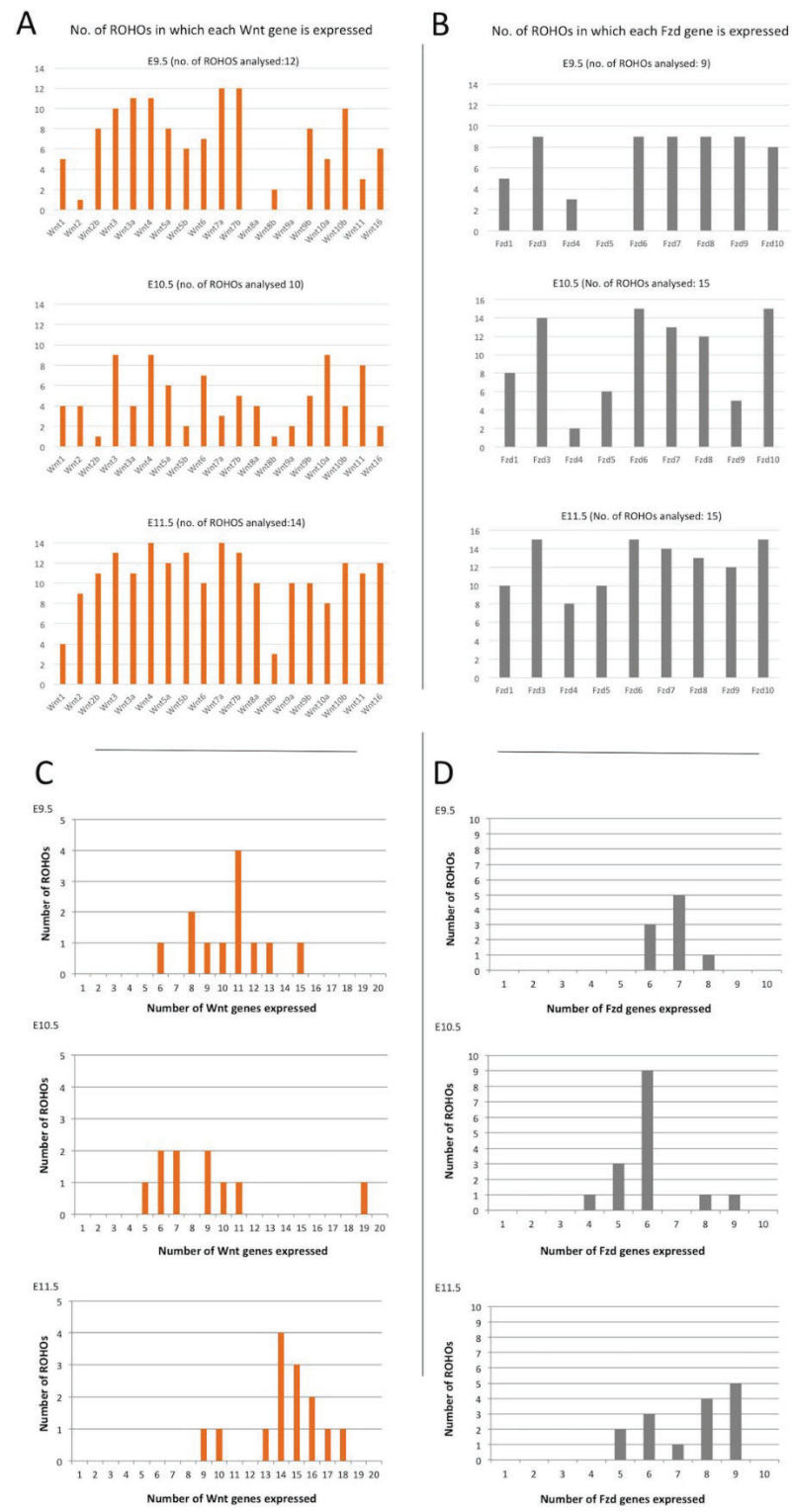


Fig. S3. Analysis of Wnt and Fzd ROHOs. A and B show the number of ROHOs in which each Wnt (A) and Fzd (B) gene are expressed, across stages. C and D graphically represent the number of Wnt (C) and Fzd (D) genes expressed per ROHO. Note the shift to more genes expressed per ROHO at E11.5

Table S2.

- (i) Proportion of the single-Wnt gene expression domain occupied by each Wnt across stages

Wnt gene	9.5 dpc	10.5 dpc	11.5 dpc
Wnt1	0.00684	0.00861	0.00454
Wnt2b	0.00466	0.00073	0.08383
Wnt2	0.07945	0.10506	0.16993
Wnt3a	0.02398	0.01722	0.07222
Wnt3	0.01189	0.06084	0.00597
Wnt4	0.0538	0.02939	0.03607
Wnt5a	0.07124	0.02666	0.11043
Wnt5b	0.00835	0.00074	0.16437
Wnt6	0.03493	0.09375	0.00921
Wnt7a	0.39379	0.05526	0.07819
Wnt7b	0.10618	0.09457	0.03283
Wnt8a	0	0.0059	0.02262
Wnt8b	0.00159	0.0053	0.003
Wnt9a	0	0.01962	0.06795
Wnt9b	0.01084	0.00259	0.02925
Wnt10a	0.00186	0.02698	0.00243
Wnt10b	0.1139	0.00136	0.01015
Wnt11	0.01592	0.39775	0.02279
Wnt16	0.06071	0.0476	0.07415

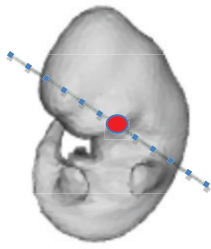
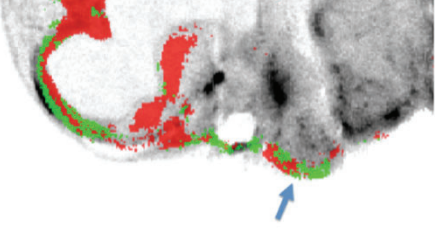
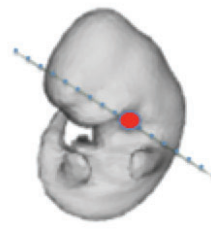
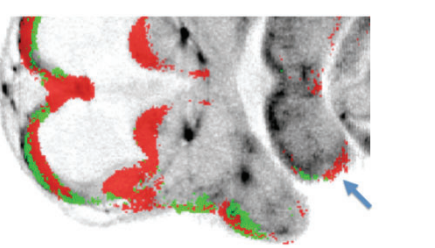
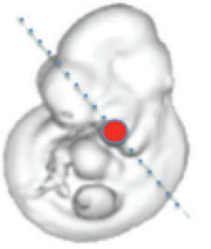
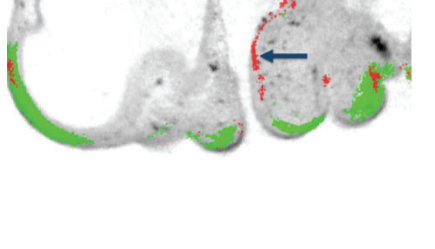
- (ii) Proportions of each Wnt gene expression domain (and canonical read-out) within the single Wnt expression domain (normalised by the individual Wnt expression domain volume).

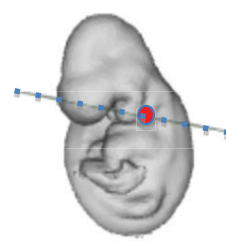
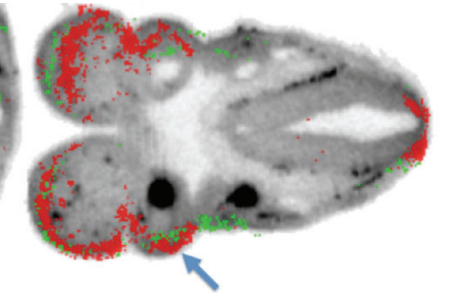
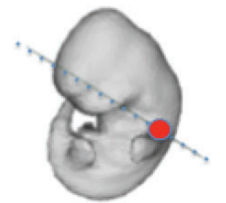
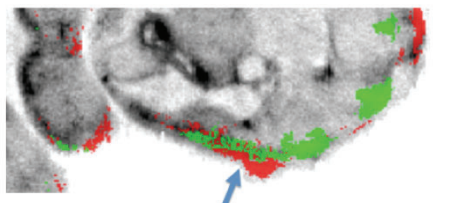
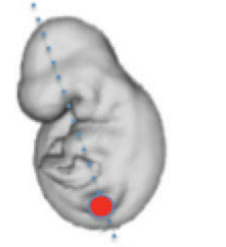
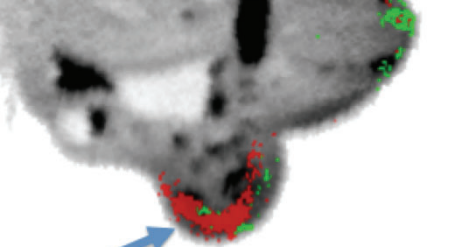
Wnt	9.5 dpc	10.5 dpc	11.5 dpc
Wnt1	0.11466	0.28179	0.06599
Wnt2	0.48927	0.54252	0.37266


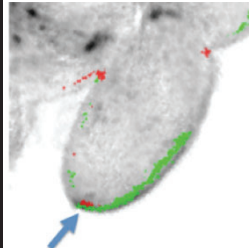
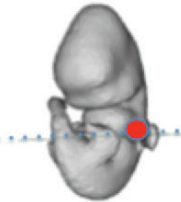
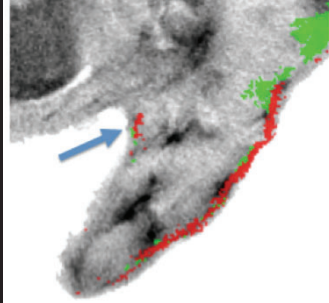
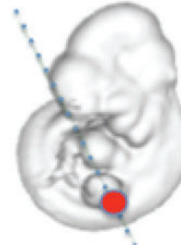
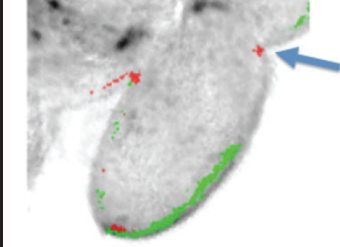
Wnt2b	0.1242	0.07291	0.16949
Wnt3	0.11151	0.29977	0.02701
Wnt3a	0.10426	0.23143	0.12668
Wnt4	0.1944	0.25136	0.05721
Wnt5a	0.17406	0.31449	0.13857
Wnt5b	0.11239	0.21475	0.36803
Wnt6	0.1731	0.3844	0.0875
Wnt7a	0.3396	0.31664	0.13011
Wnt7b	0.17472	0.40856	0.05277
Wnt8a	0	0.20901	0.04197
Wnt8b	0.09059	0.24741	0.03749
Wnt9a	0	0.3669	0.18468
Wnt9b	0.13795	0.15841	0.12216
Wnt10a	0.11904	0.3306	0.04382
Wnt10b	0.25689	0.13489	0.10676
Wnt11	0.27627	0.63064	0.09769
Wnt16	0.30858	0.52106	0.11702
Canonical readout	0.31377	0.35058	0.18978

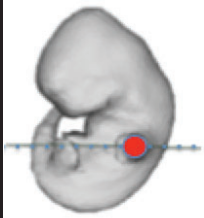
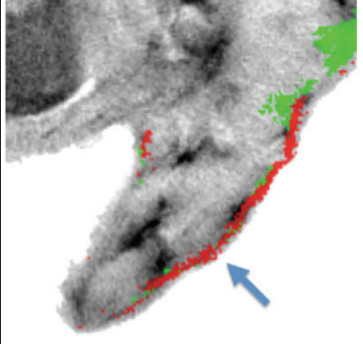
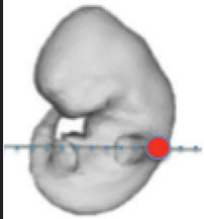
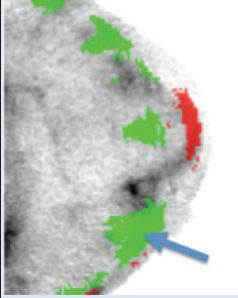
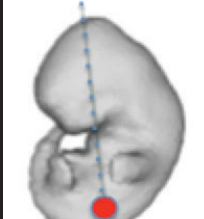
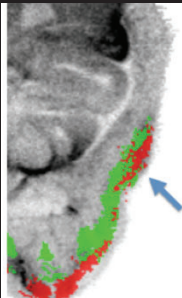
Note that the domains represented in Supplementary Table 2 were obtained by subtraction of multiple mapped expression domains and, as such, are sensitive to cumulative effects of noise in the data for each gene, in particular small differences in thresholding the original, continuously variable signals into binary (expressed versus not detected) values. The volumes should therefore be considered as approximate.

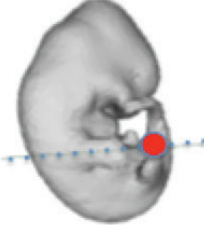
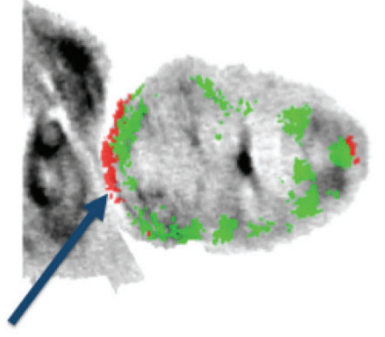
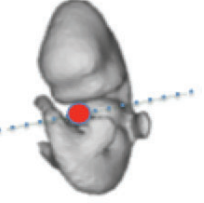
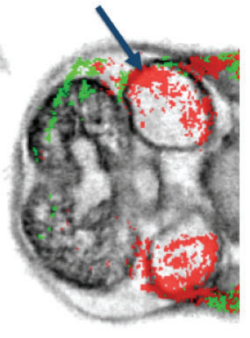
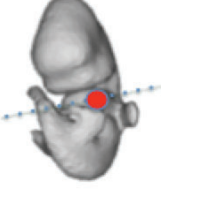
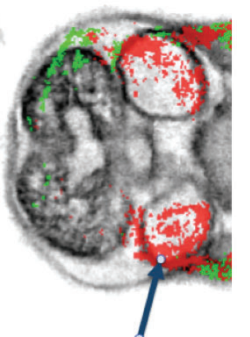
Table S3. Genes expressed in each of the Wnt and Fzd ROHOs examined. Right hand columns show location with Wnt ROHO in red and Fzd ROHO in green

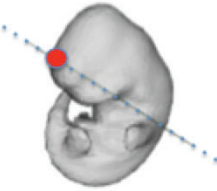
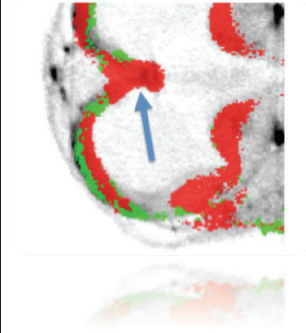
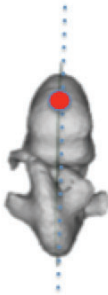
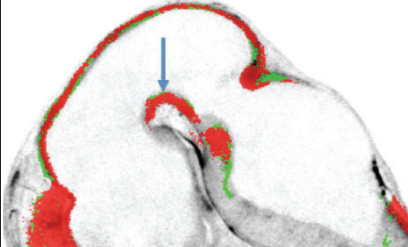
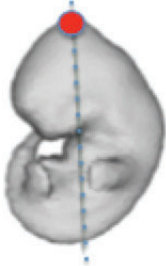
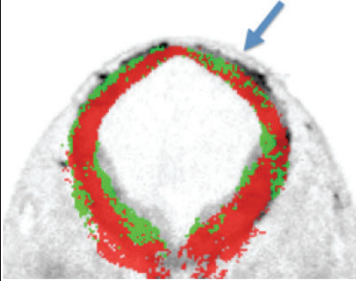
STRUCTURE	ROHO	E9.5 set	E10.5 set	E11.5set	Location	Wnt ROHO and Fzd ROHO
Maxilla	W1	2b, 3, 3a, 4, 6, 7a, 7b, 9b, 10b	2, 3, 3a, 4, 5a, 6, 7b, 9b, 10a, 11	2, 2b, 3, 3a, 4, 5a, 5b, 6, (7a), 7b, 8a, 9a, 9b, 10b, 11, (16)		
Maxilla	F1	1, 3, 4, 6, 7, 8, (9), (10)	(1), 3, 6, 7, 8, 10	1, 3, 4, 6, 7, 8, (9), 10		Location indicated above (in green)
Mandible	W2 Postero-lateral	(2b), 3, 3a, 4, 5a, 6, 7a, 7b, 9b, 10b, (16)	NO ROHO	2, 2b, 3, 3a, 4, 5a, 5b, 6, 7a, 7b, 8a, 9b, 10a, 10b, 11, 16		
Mandible	F2 Postero-lateral	(1), 3, 6, 7, 8, 9, 10	(1), 3, 6, 7, 8, 10	3, 4, 6, 7, (9), 10		Location indicated above (in green)
Mandible	W3 Antero-medial	2b, (3), 3a, 4, 5a, 6, 7a, 7b, 9b, 10b	3, 4, 5a, 6, 7b, 8a, 9b, 10a, 11	NO ROHO		

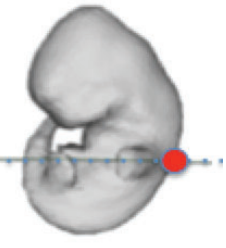
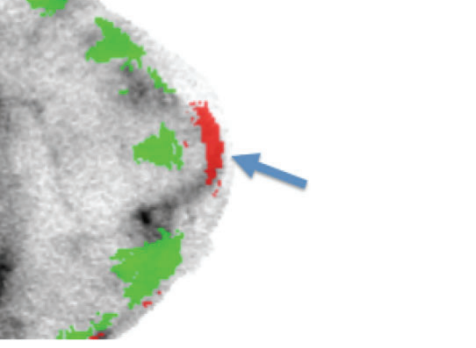
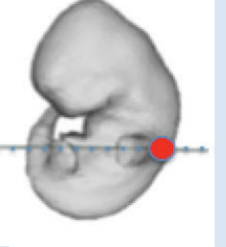
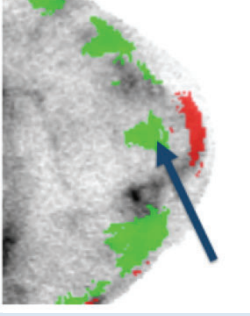
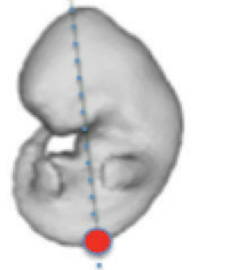
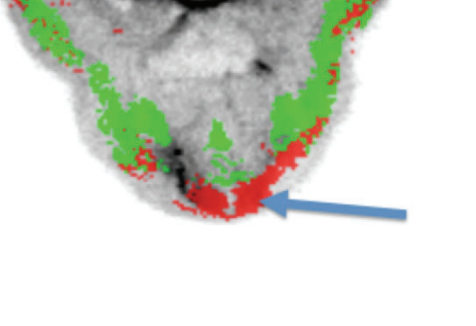
Mandible	F3 Antero-medial	3, 4, 6, 7, 8, 9, 10	1, 3, 6, 10	NO ROHO		Location indicated above (in green)
Arch 2	W4	(2b), 3, 3a, 4, 5a, (5b), 7a, 7b, 9b, 10b, (16)	NO ROHO	NO ROHO		
Arch 2	F4	(1), 3, 6, 7, 8, 9, 10	3, 4, 6, 7, 8, 10	1, 3, 4, (5), 6, 7, (8), (9), 10		Location indicated above (in green)
Neck	W5	No ROHO	2, 3, (4), 5a, 6, 7a, 9b, 10a, 11	2, 2b, 3, 3a, 4, 5a, 5b, 6, 7a, 7b, 8a, 9a, 9b, 10a, 10b, 11, 16		
Neck	F5	(1), 3, 4, (6), 7, 8, 9,	1, 3, (5), 6, 7, 8, (9), (10)	1, 3, 4, 5, 6, 7, 8, 9, 10		Location indicated above (in green)
Fore Limb	W6 Early distal E9.5 only	(2b), 3, 3a, 4, 5a, (5b), 6, 7a, 7b, (8b), 9b, 10a, 10b, 11, (16)				
Fore Limb	F6 Early distal E9.5 only	1, 3, 6, 7, 8, (9), 10				Location indicated above (in green)

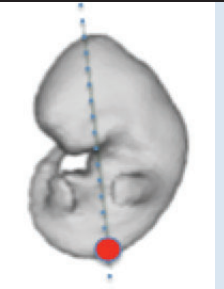
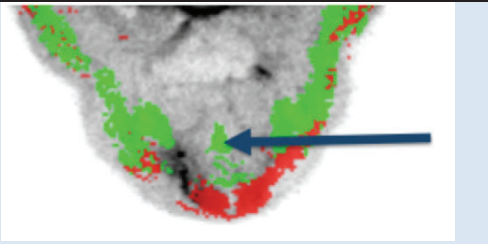
Fore Limb	W7 Distal	N/A	5a, 6, 7a, 10a, 10b, 11	NO ROHO		
Forelimb	F7 Distal	N/A	continuous with ROHO F10 (5), 6, 7, 8, 9, 10	NO ROHO		Location indicated above (in green)
Forelimb	W8 Proximo-ventral	No ROHO	2, 3, 4, 6, 7b, 8a, 9a, 9b, 10a, 10b, 11	2, 3, 3a, 4, 5a, 5b, 6, 7a, 7b, 8a, 9a, 9b, 10b, 11, (16)		
Forelimb	F8 Proximo-ventral	NO ROHO	1, 3, 6, 7, 10	1, 3, 5, 6, 7, 8, 10		Location indicated above (in green)
Forelimb	W9 Proximo-dorsal	NO ROHO	3, 4, 6, 10a, 10b, 11, 16	NO ROHO		
Forelimb	F9 Proximo-dorsal	NO ROHO	1, 3, 6, 7, 8, 10	1, (3), 4, (5), 6, 7, 8, 10		Location indicated above (in green)

Forelimb	W10 Dorsal surface	NO ROHO	NO ROHO	2, 2b, 3a, 4, 5a, 5b, 6, 7a, (7b), 8a, (9b), 10a, 10b, 11		
Forelimb	F10 Dorsal surface	NO ROHO	3, 6, 7, 8, (9), 10	1, (3), 4, 5, 6, 7, 8, 9, 10		Location indicated above (in green)
Flank	F11 Flank dorsal to fore limb	NO ROHO	1, 3, 6, 7, 8, 10	1, (3), (5), 6, 7, 8, (9), 10		
Flank	W12 Mid Flank	2, (2b), 3, (3a), 4, 5a, 6, 7a, 7b, 9b, (10a), 10b, 11 Small but distinct ROHO	NO ROHO	2, 2b, (3), 3a, 4, 5a, 5b, 6, 7a, (7b), 8a, (8b), 9a, 9b, (10a), 10b, 11, 16		
Flank	F12 Mid Flank	NO ROHO	3, 6, 7, 8, 10	1, 3, (4), 5, 6, 7, 8, 9, 10		Location indicated above (in green)

Ventral tail	W13	2b, 3, 3a, 4, 5a, 5b, (6), 7a, 7b, 11, 16	NO ROHO	2, (2b), 3, 3a, 4, 5a, 5b, 6, 7a, 7b, 9a, 9b, (10b), 16		
Ventral tail	F13	NO ROHO	NO ROHO	3, 6, 7, 9, 10		Location indicated above (in green)
Heart	W14 Right atrium	NO ROHO	NO ROHO	1, 2 2b, 3, 4, 5a, 5b, 6, 7a, 7b, 9a, 9b, 10a, 10b, 11, 16.		
Heart	W15 Left atrium	NO ROHO	NO ROHO	2, 3, 4, 5a, 5b, 6, 7a, 7b, 8a, 9a, 10a, 10b, 11, 16		

Cortical hem	W16	(1), 3a, 4, 5b, 7a, 7b, 8b, 9b, 10a 10b, (16)	NO ROHO	3,3a, 4,5a,5b,7a,7b,8a,8b,9a, 9b, 11,16		
Cortical hem	F16	NO ROHO	NO ROHO	3, 5, 6, 8, 9, 10		Location indicated above (in green)
Isthmus	W17	1, 5a, 5b, 7a, 7b, 10b	1, 3, (3a), 4, 5a, 5b, 7b	1, 2b, 3, 4, 5a, 5b, 7a, 7b, 8a, 8b, 9a, 9b, (10b), 11, 16		
Isthmus	F17	NO ROHO	3, (5), 6, 7, 9, 10,	1, 3, 5, 6, 7, 8, 9, (10)		Location indicated above (in green)
Midbrian	W18	1, 2b, 3, 3a, 4, (5a), 5b, 6, 7a, 7b, 10a, 10b	1, (2), 2b, 3, 3a, 4, 5a, 5b, 6, 7a, 7b, 8a, 8b, 9a, 9b, 10a, 10b, 11, 16,	1, 2b, 3, 3a, 4, 5a, 5b 6, 7a, 9a, 10a, 10b (11), 16		
Midbrian	F18	3, 6, 7, 8, 9, 10	1, 3, 4, 5, 6, 7, 8, 9, 10	1, 3, 4, 5, 6, 7, 8, 9, 10		Location indicated above (in green)

Dorsal anterior NT	W19	1, 3, 3a, 4, 7a, 7b, 10b, 16	1, 3, 4, (8a), 10a	1, 2b, 3, 3a, 4, 7a, 7b, 8a, 10a, 16,		
Dorsal anterior NT	F19	3, 6, 7, 8, (9), 10	3, (5), 6, 8, 10	NO ROHO		Location indicated above (in green)
Mid-d-v anterior NT	F20	NO ROHO	NO ROHO	3, 6, 7, 8, 10		
Dorsal trunk NT	W21	v. small ROHO 1, (3), 3a, 4, 7a, 7b, 9b, (10a)	1, 3, 3a, 4, 10a, 11	1, 2b, 3, 3a, 4, (5b), 7a, 7b, 10b		
Dorsal trunk NT	F21	3, (6), 7, 8, (9), 10	3, (5), 6, 7, 8, 10	NO ROHO		Location indicated above (in green)

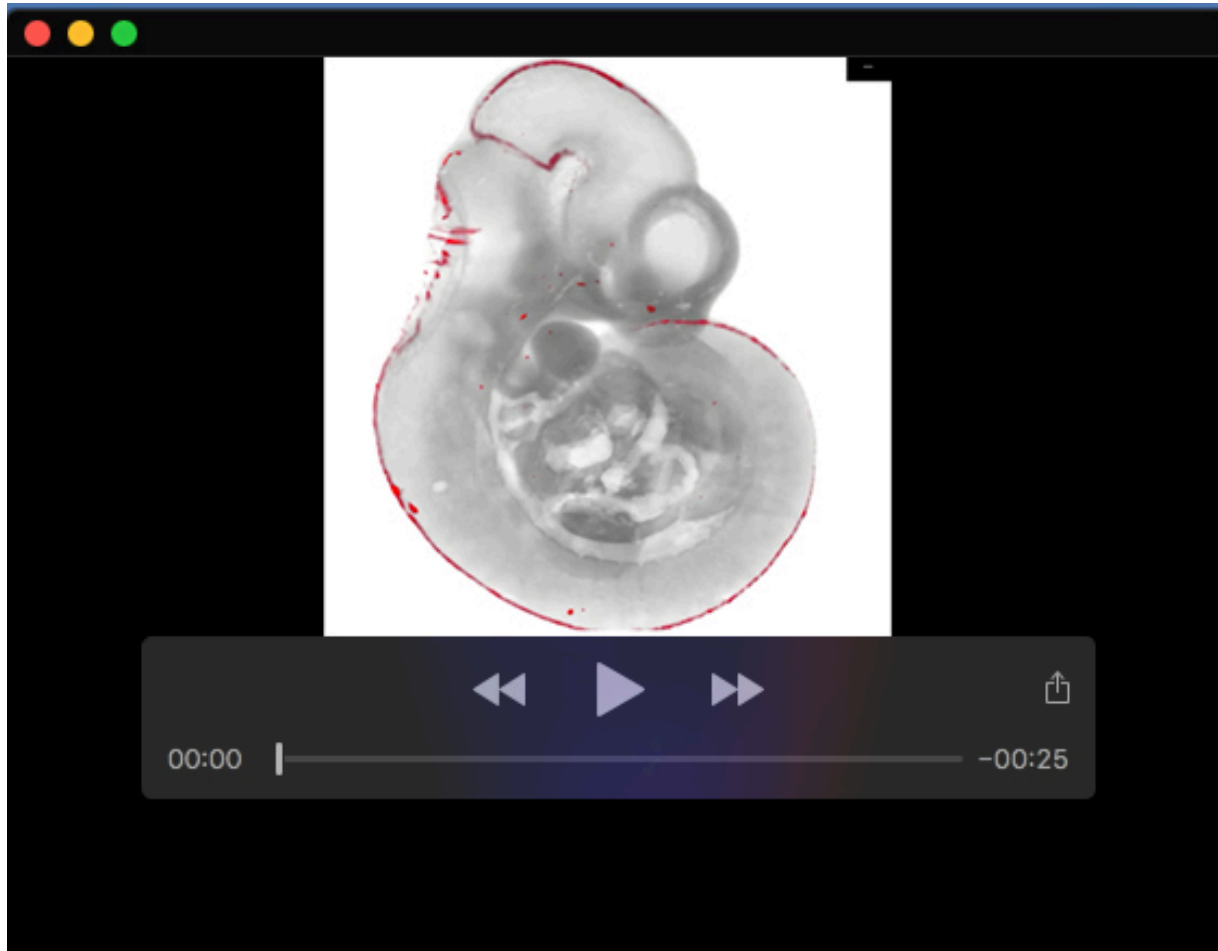
Mid-ventral alar plate trunk NT	F22	NO ROHO	NO ROHO	3, 6, 7, 8, 9, 10		
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In the Table above, the Wnt and Fzd ROHOs are numbered W1, W2, etc., F1, F2, etc. respectively. The location of each ROHO is illustrated at the latest stage the ROHO is present. **Bold** gene numbers indicate genes expressed in the ROHO at all stages when this ROHO is present. Gene numbers in parenthesis indicate that the expression domain or intersection with the ROHO is small, comprising only a few voxels. Note the number of genes listed can exceed the maximum occupancy for that ROHO because some genes intersect only part of the ROHO.

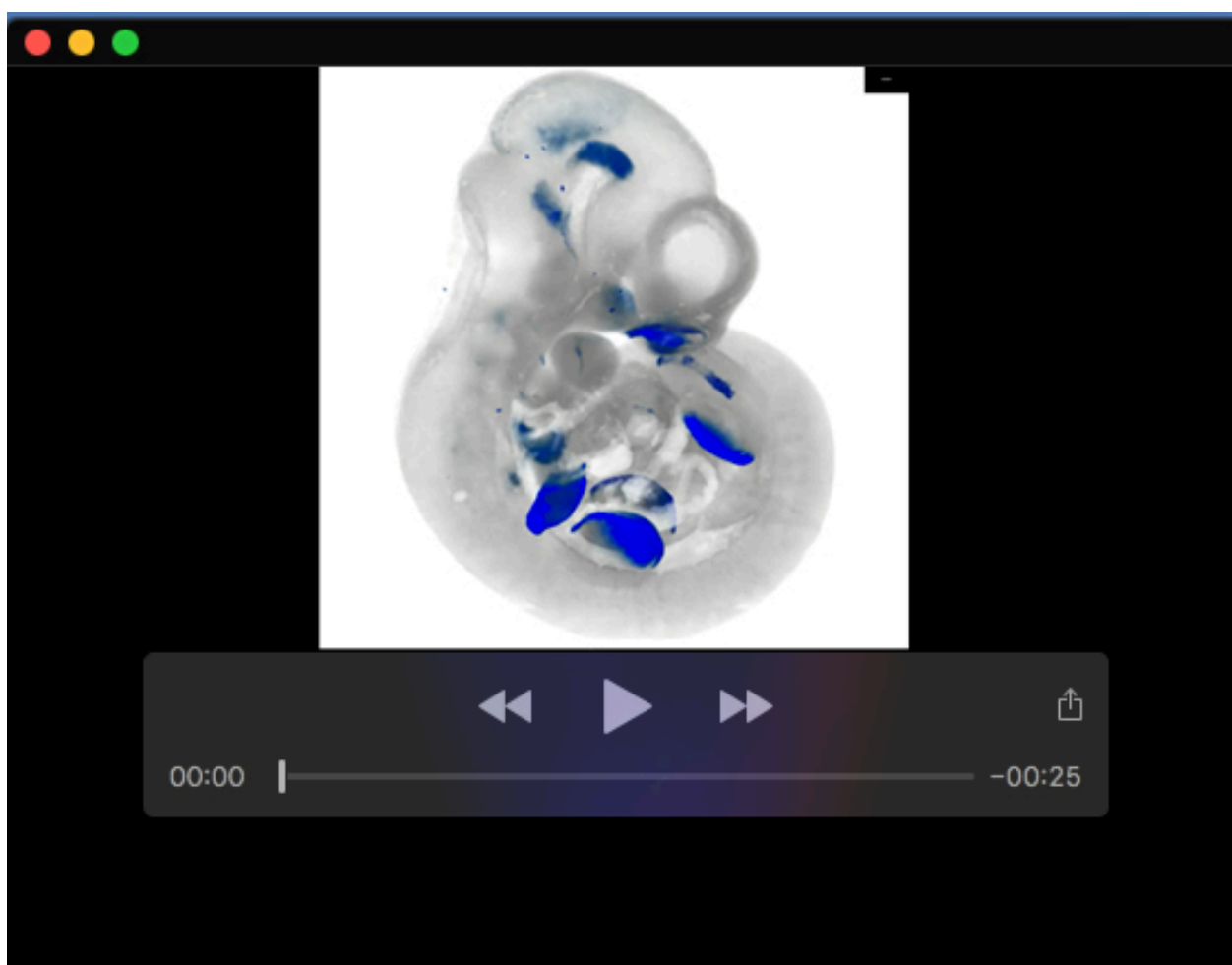
Table S5. details of gene expression probes and EMAGE entry IDs

*Note no specific expression detected for Fzd2 and Sfrp5 at these stages

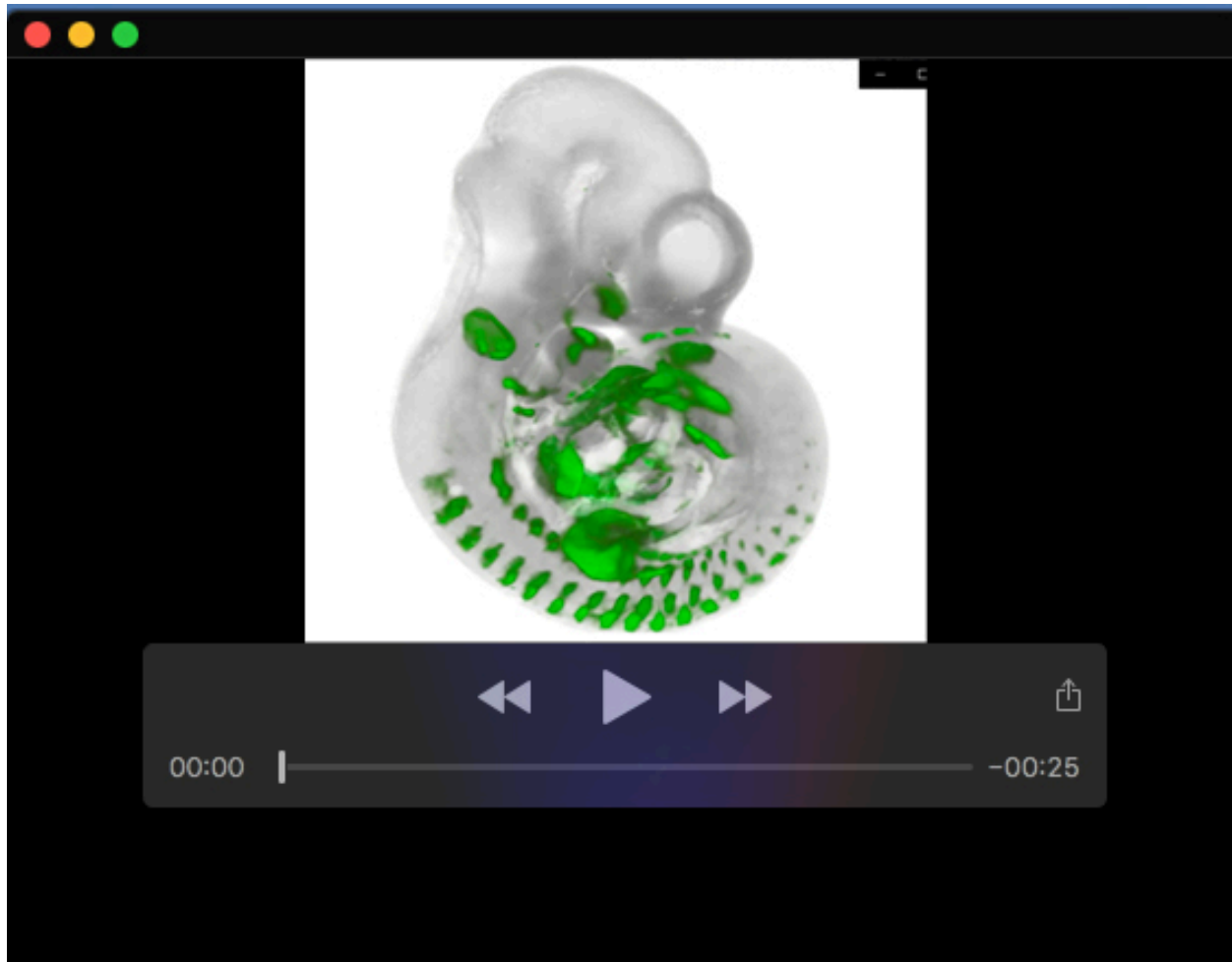
Gene	Extent of Probe on Genbank Sequence	Source	EMAGE IDs
Wnt1	Nucleotide 138 to 2345 on genbank sequence BC005449.1	A. McMahon	EMAGE:6131 EMAGE:6132 EMAGE:3949
Wnt2	Nucleotide 19 to 1493 on genbank sequence BC026373	A. McMahon	EMAGE:6133 EMAGE:6134 EMAGE:3950
Wnt2b	Nucleotide 882 to 1103 on genbank sequence AF070988	L. Zakin	EMAGE:6135 EMAGE:6136 EMAGE:3951
Wnt3	Nucleotide 1610 to 2973 on genbank sequence NM_009521.1	P. Salinas	EMAGE:6137 EMAGE:6138 EMAGE:3952
Wnt3a	Nucleotide 2310 to 2676 on genbank sequence NM_009522.1	RT-PCR generated	EMAGE:6139 EMAGE:6141 EMAGE:3953
Wnt4	Nucleotide 853 to 1315 on genbank sequence AK012727	A. McMahon	EMAGE:6140 EMAGE:6142 EMAGE:3954
Wnt5a	Nucleotide 193 to 2324 on genbank sequence BC018425	A. McMahon	EMAGE:6143 EMAGE:6144 EMAGE:3955
Wnt5b	Nucleotide 111 to 1429 on genbank sequence BC010775	A. McMahon	EMAGE:6145 EMAGE:6146 EMAGE:3956
Wnt6	Nucleotide 27 to 2066 on genbank sequence NM_009526.3	A. McMahon	EMAGE:6147 EMAGE:6148 EMAGE:3957
Wnt7a	Nucleotide 24 to 3164 on genbank sequence BC049093	A. McMahon	EMAGE:6149 EMAGE:6150 EMAGE:3958
Wnt7b	Nucleotide 93 to 1581 on genbank sequence NM_009528.2	A. McMahon	EMAGE:6151 EMAGE:6152 EMAGE:3959
Wnt8a	Nucleotide 87 to 1746 on genbank sequence NM_009290.1	P. Chambon	EMAGE:6153 EMAGE:3960
Wnt8b	Nucleotide 942 to 1631 on genbank sequence NM_011720.2	J. Mason	EMAGE:6166 EMAGE:6154 EMAGE:3961
Wnt9a	Nucleotide 1698 to 2247 on genbank sequence NM_139298.2	RT-PCR generated	EMAGE:6155 EMAGE:6156 EMAGE:3962
Wnt9b	Nucleotide 2834 to 3436 on genbank sequence NM_011719.3	RT-PCR generated	EMAGE:6158 EMAGE:6157 EMAGE:3963
Wnt10a	Nucleotide 289 to 2469 on genbank sequence NM_009518.1	IMAGE clone	EMAGE:6159 EMAGE:6160 EMAGE:3964
Wnt10b	Nucleotide 27 to 473 on genbank sequence U61970	A. McMahon	EMAGE:6167 EMAGE:6161 EMAGE:3965
Wnt11	Nucleotide 169 to 1789 on genbank sequence NM_009519.1 + 419bp of 3' UTR	A. McMahon	EMAGE:6162 EMAGE:6163 EMAGE:3966
Wnt16	Nucleotide 538 to 1532 on genbank sequence NM_053116	PCR cloned	EMAGE:6164 EMAGE:6165 EMAGE:3967
Fzd1	Nucleotide 2774 to 3663 on genbank sequence NM_021457.2	PCR cloned	EMAGE:6129 EMAGE:6130 EMAGE:3939
Fzd2*	Nucleotide 3106 to 3441 on genbank sequence BC049774	IMAGE clone	Not detected
Fzd3	Nucleotide 127 to 1485 on genbank sequence BC050965	U. Borello/C. Cossu	EMAGE:6101 EMAGE:6115 EMAGE:3941
Fzd4	Nucleotide 1419 to 1862 on genbank sequence BC015256	U. Borello/C. Cossu	EMAGE:6116 EMAGE:6117 EMAGE:3942
Fzd5	Nucleotide 640 to 868 on genbank sequence AB052910	U. Borello/C. Cossu	EMAGE:6118 EMAGE:6119 EMAGE:3943
Fzd6	Nucleotide 1794 to 2642 on genbank sequence NM_008056.2	U. Borello/C. Cossu	EMAGE:6120 EMAGE:6121 EMAGE:3944
Fzd7	Nucleotide 191 to 1013 on genbank sequence BC049781	U. Borello/C. Cossu	EMAGE:6122 EMAGE:6123 EMAGE:3945
Fzd8	Nucleotide 968 to 1965 on genbank sequence NM_008058.1	U. Borello/C. Cossu	EMAGE:6124 EMAGE:6125 EMAGE:3946
Fzd9	Nucleotide 678 to 1178 on genbank sequence AF033585	U. Borello/C. Cossu	EMAGE:6126 EMAGE:3947
Fzd10	Nucleotide 1892 to 2988 on genbank sequence NM_175284.3	IMAGE library	EMAGE:6127 EMAGE:6128 EMAGE:3948
Lef1	Nucleotide 371 on NM_010703.3	J. Meeldijk	EMAGE:5753 (TS16) EMAGE:5757 EMAGE:5758
Tcf7	Nucleotide 18 to 1572 on NM_009331.3	R.Grosschedl	EMAGE:5765 EMAGE:5759 EMAGE:5760
Tcf7I1	Nucleotide 86 to 1582 on NM_009332.2	Cloned: library screen	EMAGE:5766 EMAGE:5761 EMAGE:5762
Tcf7I2	Nucleotide 946 to 1361 on NM_009333.3	J.Rubenstein	EMAGE:5754 (TS16) EMAGE:5763 EMAGE:5764
β -catenin	Nucleotide 2194 to 2621 on NM_007614.2	U.Borello/ G. Cossu	EMAGE 5752 EMAGE 5755 EMAGE 5756
Sfrp1	Nucleotide 12 to 784 on NM_013834	A Rattner	Not on EMAGE
Sfrp2	Nucleotide 82 to 852 on U88569	A Rattner	"
Sfrp3	Nucleotide 2059-2522 on NM_011356.4	PCR cloned	"
Sfrp4	Nucleotide 9 to 1756 on NM_016687.3	A Rattner	"
Sfrp5*	Nucleotide 1 to 1081 on NM_018780.3	PCR cloned	Not detected
Wif1	Nucleotide 876 to 2237 on NM_011915.2	IMAGE library	Not on EMAGE
Wise	Nucleotide 81 to 1038 on NM_025312.3	IMAGE library	"
Ror2	Nucleotide 1 to 3968 on NM_013846.3	IMAGE library	"



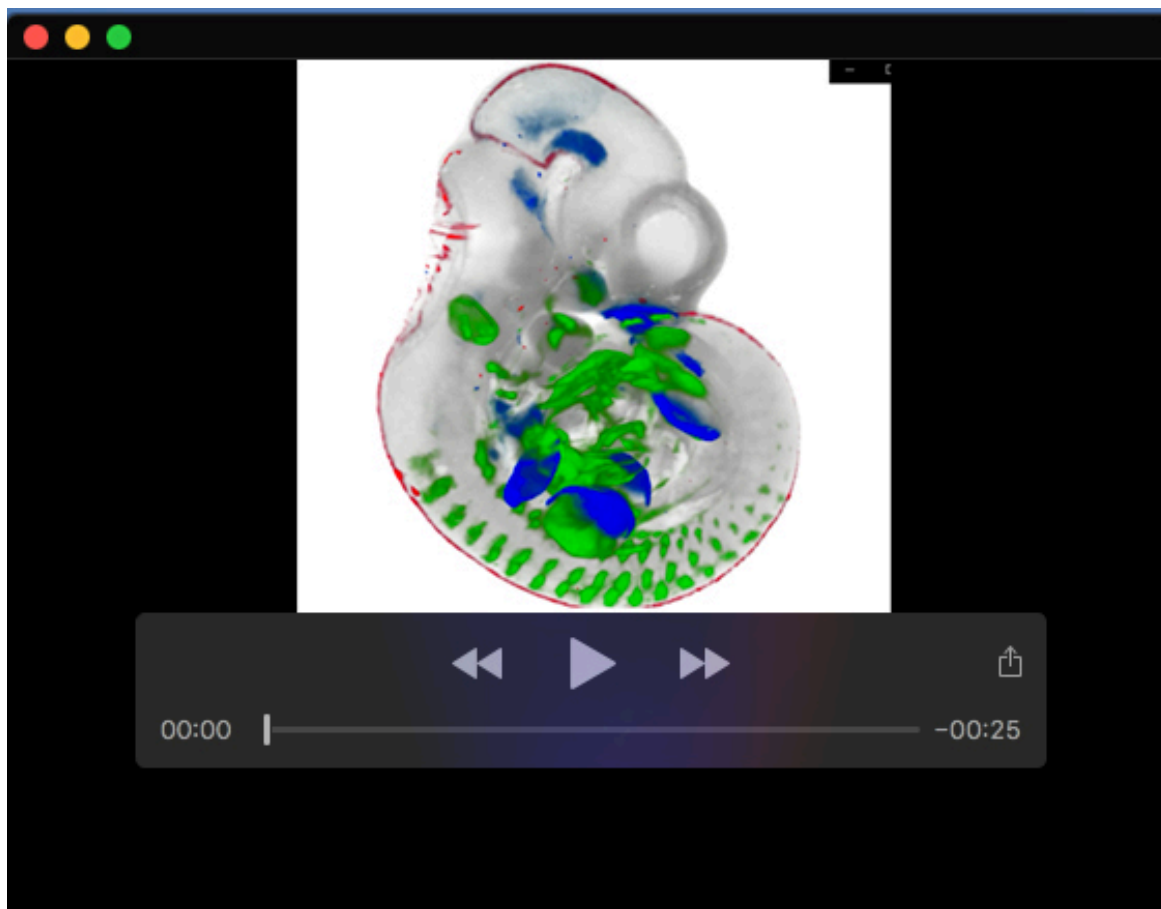
Movie 1. 3D movie of Wnt1 expression pattern at E10.5 mapped onto reference embryo



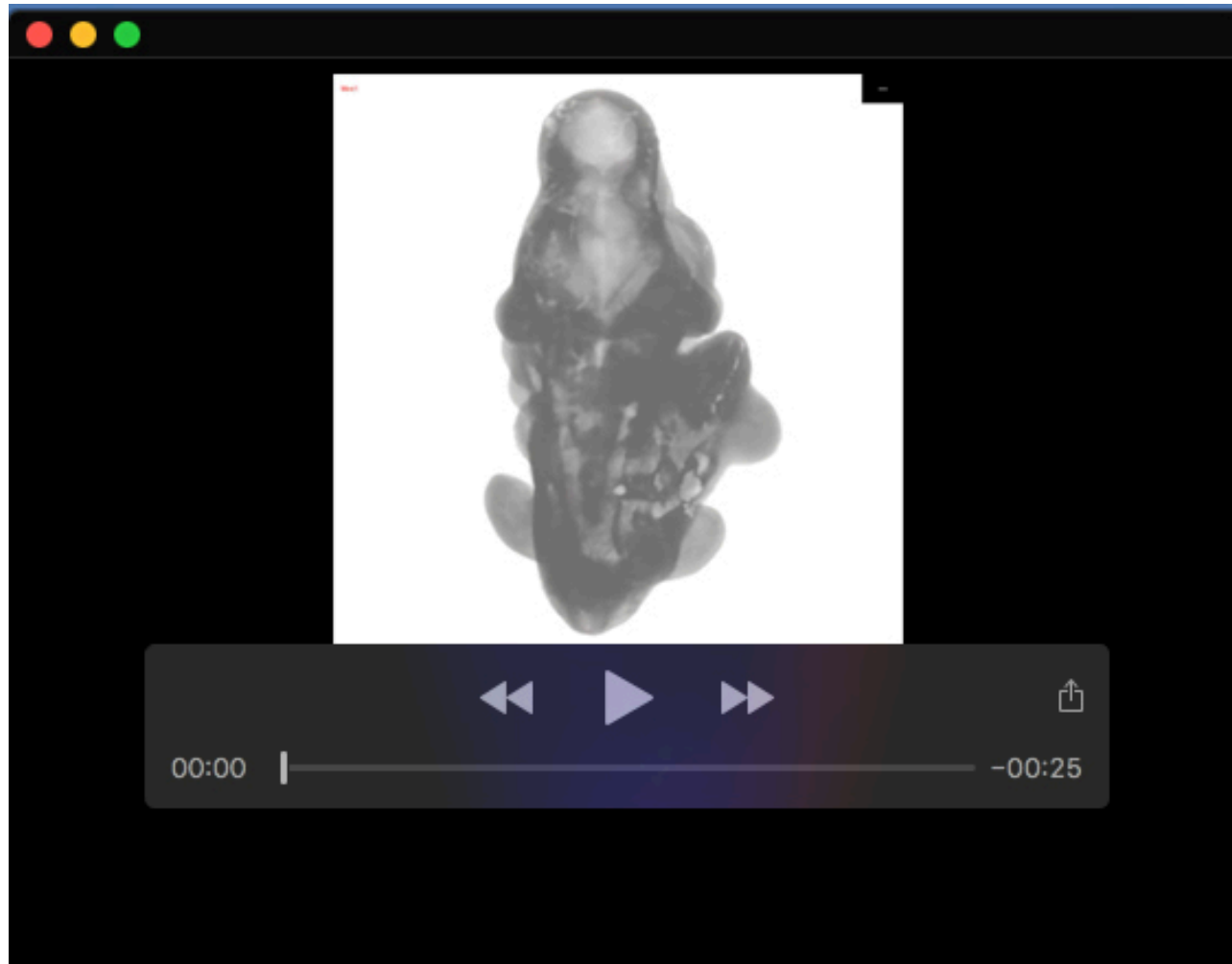
Movie 2. 3D movie of Wnt5a expression pattern at E10.5 mapped onto reference embryo



Movie 3. 3D movie of Wnt11 expression pattern at E10.5 mapped onto reference embryo



Movie 4. 3D movie of integrated Wnt1 (red), Wnt5a (blue) and Wnt11 (green) expression patterns at E10.5 mapped onto reference embryo



Movie 5. 3D movie of integrated expression patterns of all 19 Wnt genes (colour codes indicated on movie) at E10.5 mapped onto reference embryo.