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## **Supplemental Information**

### **Molecular Diversity of Midbrain Development in Mouse, Human, and Stem Cells**

**Gioele La Manno, Daniel Gyllborg, Simone Codeluppi, Kaneyasu Nishimura, Carmen Salto, Amit Zeisel, Lars E. Borm, Simon R.W. Stott, Enrique M. Toledo, J. Carlos Villaescusa, Peter Lönnerberg, Jesper Ryge, Roger A. Barker, Ernest Arenas, and Sten Linnarsson**

<b>Table S1. Related to Figure 1. Tissue used per experiment</b>						
Mouse (embryonic) CD-1						
Embryonic Age (E)	Embryos used per experiment (each cell is a separate day; number represents litter mates collected from one mother)					Total (n=)
11.5	12	15	12	11	13	5
12.5	11	15	13	14		4
13.5	15	11	14			3
14.5	7	16	12	13	12	5
15.5	15	14	10			3
18.5	6	10				2
Total embryos collected					271	
Total embryonic experiments						n=22
Mouse (postnatal) CD-1						
Age (days)	Brains used per experiment (each cell is a separate day)					Total (n=)
P19	2					1
P20	2	3				2
P21	2	3	3			3
P23	2	3				2
P24	3	3	3			3
P25	3					1
P26	3	3				2
Mouse (postnatal) Dat1-Cre/tdTomato						
Age (days)	Brains used per experiment (each cell is a separate day)					Total (n=)
P26	1					1
P28	1					1
P56	1	1	1			3
Total postnatal experiments						n=19
Human (fetal)						
Age (weeks)	Each cell is a separate day. 1+1 means that a second experiment is completed 24h after first with the other half of same tissue					Total fetuses
6	1					1
7	1	1				2
7.5	1					1
8	1+1					1
9	1+1	1+1				2
10	1	1+1				2
11	1+1					1
Total						n=10

hPSC cultures						
	Each cell is a separate day. Number indicates number of separate biological replicates (different cultures) run on that day. samples not pooled.					
H9						
Day 0	2					
Day 12	4					
Day 18	2					
Day 35	4					
HS401						
Day 0	2					
Day 12	4					
Day 17	2					
Day 35	4					
iPS (iCell DopaNeurons)						
Day 5 (47)	4					
Day 21 (63)	4	4				

**Table S3. Related to Figure 3. Probe sequences (5' -> 3') for single molecule RNA FISH**

Probe	Ednrb	Cd36	Foxj1
1	agtaagttaagttgcgcg	actctgaaaggatcagcact	ccagaggaaggttctggaag
2	tcgaaaaacacactagccc	acagcaatgatgagctgtt	ctccgaacacgaatgtgagg
3	acaggacacttaagtcagct	ccggttctactaattcatgc	gaacactcactccattctg
4	tttcagctcgagaccaac	ccaggtaaatcacagtgtt	cagactccagttacacagtc
5	cgattgcatgttacagcttg	gcagaatcaagggagagcac	gtcaacatccacaggctgag
6	ttagtcccgagaagtgcacag	taacagctccagcaatgagc	cttggcgttgagaatggaga
7	aggaacgcatcagactggag	tcicaataagcatgtctccg	gagatgtgcacgacgatgtg
8	caataattcgttggcacgga	acaacttccctttgattgt	ataggagtgggtggcttca
9	acacagggcacgcacaatc	ttgaaagcagtggttcctt	catggccatgcagatgagag
10	agatgatcttagcagcgtg	aactgtctgtacacagtgtt	cgacagagtgtatcttgggg
11	gcgatcaagatattgggacc	atctgggtttgcacatcaa	ccgtgatccactttagatg
12	tgatgatgtgcagtaggtct	gattttgctgctgttctttg	catggcgggaagtagcagaag
13	cgagcaactgtagggttta	atgtgtaaggacctctttgt	aggcactttgatgaagcact
14	gagcacaagactcagcact	gagttatatttcttggct	aaaggcaggggtgatgtgga
15	ccaagaagcaacagctcgat	aaagacacagtggttcctc	tcaaactctgaagcagctg
16	gaacccaattccttaaft	gtgaaggctcaaagatggct	cagcagtaaggtgctgggag
17	aatttctactgctgcatt	cacaccaactgtgtactta	ttacctttgagtggtccag
18	cacagagaccacccaaatta	ccatctacagtgctattgta	aaagatggcctcccagtcaa
19	ctttgtagtccgacgtaac	cttatgtatcctttccatt	ggactgtaagatccacatct
20	aggctgttttctgaaagggga	tgccattaatcatgtcgcaa	gtagcagggcagttgatgtg
21	ggctgtcttctgaaactgca	ccgaacacagcgtatagata	atcaaagtccaggctgtcag
22	aagactgcagtgatggctag	ggattccttaaggtcgatt	gaaggatgtggccaagaagg
23	caaagcaatctgcataccgc	ggcattggctggaagaacaa	ttcaaagatgggttctgggg
24	tcgtctctgcttaagtgat	aatggtgtctggattctgg	ttttacaagaaggcaccac
25	aaagctcagaagctcacacc	tctggactgcatgtaggaa	ttgagcactgtccggagatg
26	gttgataccaatgtagtcca	gtaagccttcaataggttct	ggcttgatagaggacatc
27	tgacaggagtcaagaagcc	cctatgctcatcttcattg	aaacactgtgtgatggggg
28	tgctcaccaaatacagagcg	gttatgggtccacatctaa	tgaggcgaagcttgggtgag
29	accagcagcacaacatgac	accaatatgtgacctgcag	ccagagaagcagcagaagtg
30	aggactgcttttctcaaac	cttctattttctagctggc	ggaactttctctgggtagag
31	tggaaccggaagttgtcatat	catttagccacagtataggt	taatgagcgtttgtgtacc
32	ccttcaagacgagctgtatt	atgccaaggagcttgatttt	cctcctgggaattaattgt
33	agaatgggaatatttcccca	caacaaacatcaccactcca	cgggcttagagaccatttcg
34	atgacgtggcagtgacgaac	ttctggatttgcaagcaca	aggtgatggaccagggaaag
35	acaaggttaagctctcggg	gctcatccactactatttt	aagtcaggctggaaggttg
36	cattttcaacatgtggcctg	gatgtagccagtgatatgt	catcttctgatgctcttag
37	ccggttgggtttattgtatt	ttttgtagatcggtttacc	cccagttaagcttagctat
38	tggtctggtttttacctc	agtccactcagttgcaaatg	tagtatagacagggcctag
39	catgtgtcacgtggattcag	cctccagtgagggaaaacaa	aggggtcaaggtggagcaag
40	actgtgggtgactcctaag	agttgcaagccaaattgtct	cacgtggaggttagattcag
41	aattgaagatctggggcgtc	gttatacttatgccatgtgc	
42	actactggggagccaataac	cagtggtcacatgtgaatt	
43	attattgccatgaagaggg	cttctgttagccaagaactc	
44	tgccatttcataatttgca	tgacagcatggaacatgacga	
45	gaccttaacatccttgta	ttgctttgtaggattccat	
46	ttggctgttgacattatc	aaaccagtcctttttggg	
47	atgttaggtacaacacacc	acaacctatcactgtgtctt	
48	itagcaacgacacaagctgc	taatgacatcgtttcccaca	

Probe	Msx2	Rfx4	Slc6a11
1	ctcaagcgaagactccaact	agtaatccagtcgggcaag	cagctacgctcaacacgaac
2	ggagaagccatgactttctg	gagacatcttcaatccagc	ttttagcacaggtagggaa
3	gaaacaagtaccgccttt	ccagagatgtgtgactggaa	caccacgtaaggaatcagga
4	cttctgtcggacatgagcg	gcaatctcatagtttctc	cgttccaggaagaagacgg
5	ctcgaagggctgacgagag	cttctcacagaaatccaggt	gccaggatgatgatgtagta
6	tcttcggaatttccgactt	aaagctggcagcattgacag	ggggaagcaattgcttaggt
7	ttggtctgtgttctctcag	aaactgtcccttatgatct	tggaactccacacatttctc
8	gggatgtggtgaaggggtg	aatgtactttgactgtcct	tgtagttgctgaagttcagc
9	ttgcgctccaaggctagaag	ctggcagatttaggtctttg	tcaatgccatcgatatagc
10	atggacaggctactgtttctg	tctgacagtggttctgtac	caggagacataatgccagtt
11	tctggaaccagatttgacc	gaacctcatcaaagttggct	tgcagaagtagcagatggtc
12	ttgcagcttttcgcttag	ccttgccaaaagtcgagaag	ctttccagtcgacttagtac
13	attttcagctttccagttc	gtagaggatggagtcacaca	aggttcagtgacatacacg
14	aagggcagactgaagcctga	tccatggtatacagagtctg	gaggtcaggttacagataga
15	ttgcaggggtgagttgatag	aactctggtacaactggat	ctgtttagacagcagagcat
16	taggatgcgccgtatatgga	atccagccattctatgtaag	ctgagaagatggcaaaccca
17	gagcacaggtctatggaagg	ctttacaacgcacatcggtcta	cttgggtaggcgatgaaag
18	cgtggcatagagccaacag	aagcaagaccacatcagcag	acacaaactgactgtccagg
19	tggtacatgccatatccaac	aggtgaaaagaccggaagct	ggaagaccttagggtagatg
20	atctggtcttcttaggata	ttctagaacagtagagcacgt	tggcggcgtatgagtcacaaa
21	cgattcaggagcagagttg	gcctctgtcaagataatctc	acaaagagcaagcacatgcc
22	tgtcgttagggtgacaatg	cacagatgtggcagactttg	ccgatgcagacacactcaaa
23	gtgcctctcacctaaataac	tagcgaccacgtatagact	attgtcatagaaccgggtgc
24	tgagaggaagggggcattt	tagttgtgggagttctcag	cggtaaccaatcatatcctc
25	gaagtgacatcctgatgga	tcctgtggaagggggaatag	ccacttgatgagtgacagtg
26	ccttcaactgcaagaatctg	tggggctattgaaagggtag	tgtacttgagcgggtttgac
27	attctcatctcttttcatc	tcaacaaggttccatcctg	ccaagcaggatagtgtgaca
28	ttaccctgagtaactctcag	gggtgacatgtcactagagt	ttctgtaatttctcggggag
29	ctgacatggagtttctatt	ctcatactcagaattcctgc	ctcacagtcattaacggta
30	aatatttcaccttcaaccc	aagccaggaaagtgttgcac	tctctgtgatggcagagatg
31	ggactgatgtagatagcatt	taaagtcttctggaggggtg	gtacgaggttcaggattcat
32	caatcacctctgtaccgtgt	cccgaagttatgatgtatt	atctgctagtcaactgtacag
33	gaagagatttaccatgtcct	acatctctgttctcattagg	gactctagaaaacaccggct
34	ggccacagttataagacact	tttagtgactcctgtttct	gaacatcagaggcagatggg
35	cctcattctatggactgttg	cttggctacagaacccaat	ttactcaggagtcttaacga
36	ttccctaagaagtttagcac	ccaagatagtgctctatag	tgaaggacagtcagcaact
37	gccaacacgtagagtata	aaggtcatggaacagagggg	tgaaggaagactccactca
38	caactcaagcacatggcttc	tcttggtaaattatgtgcc	tacaagtctctgcaggtcaa
39	ctgactgacaacgcatctc	agtccggatgtgattacat	gttaagggatcagggtagg
40	cccattcaacgggataaaaa	ggactccaggaagttagaat	tggaatggctccagagtaag
41		ggcaggtacgaatggaactt	agtggagtgaaaggcttctc
42		tccaggacaatgcaggtaat	cttctgttccaaggaaga
43		tacagagaagaactgtgcc	gcgtgaagacatctcacttt
44		agccattgtgcaaacctagg	gtctctgacagaacactgga
45		agtggaggacagtgttagt	agcattgggcaaatagagca
46		catccagcttcgcaagtaa	ctgggcagaaacagtaagag
47		cttggctctgtcaagttaa	gagtgcaagtgttaagggc
48		gaaacctccatattcttga	aaaacagcccaacccttaa

Probe	Sox10	Pdgfra
1	tgaatcctcaccaccaaca	taagagctggcaggagatga
2	gggagtgacgctgatggact	tctcgttgggagatagag
3	tcgtcctgaggaaagtggaaa	gagtggtgttagtagcaa
4	acctctgataggtcttgttc	gactaaggaatcggtcatcc
5	cacacggggaacttgtcatc	agattcagttctgacgttc
6	acacatgaaggcgttcacg	agtgacaccagttgatgg
7	ttgtggaggtgagggactg	ctgactctgcaagttgac
8	tagtgtcttgctgagctcag	tctcggtgagattctctatc
9	ttgtcacttctgttcagcaa	atttgcctctgatacctgtt
10	tctcagcctcctcaatgaag	atgggtgtcatctacgagat
11	ttttgtgctgcatccggag	cagatcatccagtcgatttc
12	cgaggttggtactttagtc	agacattgctggccaaaact
13	ttgtagtgagcctgaatagc	aacgctgaggtgttctttg
14	tgctcttgggggtgttgag	tgagttcagatcgagagtg
15	acgttgccgaagtcgatgtg	caatcaccaacagcaccaac
16	atgttgacattacctctgtg	caaatgaccaccaggagat
17	agctcagtcacatcaaaggt	aaatgcaccggatcccaaaa
18	atctgggaagtggacggctg	aaatgggacctgacttgggtg
19	gcagactgagggaaagtgtag	cccatagaagcagattctg
20	aaactggggctgtagatgg	tggtggctcatgaagctatc
21	agggctgatggtcagaatag	caatccaaagatgtccaggt
22	tgcatggccataatagggtc	acatactgtgtggtatcagc
23	cagagatggcaggttagagg	aaacctcttcccttcaagc
24	tggtgggactgtggattg	cgccgaaaggagggttttg
25	ctaaggtcgggatagatcg	cgtagttggaatcgtgcatg
26	fgggagagccaccaaacttc	acaccgatgtacgcattatc
27	tcatttctggggaagggtgg	caagtcctcttcaacttt
28	tctcaggtcctgggatagag	ccggagaggagagttaacac
29	cttgctccaaggagataa	tactcacggtggtagttt
30	aggcaatgacacagtagcac	tacttacctctgctctcta
31	gttcaaagctgtcctcagtg	cttattcaacagcaccatt
32	ccacctgtctttacaaag	caaatctctggggcaaaggt
33	ggcggagaaggatcagagt	gactattcaagagcaccagg
34	tcccacattcaggtgaagag	aaattcaaagttgccctcc
35	tgacacggaactggatctgg	tcaaggttaggaatagctgc
36	tcagttaggcaaggatgag	ggaagatgttccaagtcaa
37	gagaagaaggctaggtggat	atggagctggtgcacaaaag
38	gggcttgaggctgacagaag	cccatgttcattcatacag
39	cggtccatgctaactctgag	agattatgccagtaggtagt
40	acatggcaggggttagtgt	aagggaaagttggggtttgc
41	gtgcaaggcaaaggtagact	tcagtcttcacctactgt
42	taaggtatcgctggagcaaa	gccacgagtctagaagacg
43	gagacagagctacgtacttt	gtatcatccaacttcaggt
44	fgggctgcagacaggagatg	attgaatctcagagggtgggt
45	cagcgtatgtttacatgtgg	cattttgtacaggtggcag
46	acatggcgactatggagata	tacgtccctctcacaata
47	ctggagtagctagaggata	gtcatacacattttggcagt
48	aggcaaccagaagcattgat	catctgtattggaagacc

<b>Table S4. Related to Figure 7. qPCR primer sequences (5' -&gt; 3')</b>				
Gene	Accession No.	Sense	Antisense	Length (bp)
ALDH1A1	NM_000689	TGTTAGCTGATGCCGACTTG	CTGGCCCTGGTGGTAGAATA	71
CORIN	NM_006587	CATATCTCCATCGCCTCAGTTG	GGCAGGAGTCCATGACTGT	106
EN1	NM_001426	CGTGGCTTACTCCCCATTTA	TCTCGCTGTCTCTCCCTCTC	117
FOXA2	NM_021784	TTCAGGCCCGGCTAACTCT	AGTCTCGACCCCCACTTGCT	67
GAPDH	NM_002046	GAAGGTGAAGGTCGGAGTCA	TTGAGGTCAATGAAGGGGTC	117
LMX1A	NM_177398	GATCCCTCCGACAGGGTCTC	GGTTTCCCACTCTGGACTGC	175
NANOG	NM_024865	ACAAGTGGCCGAAGAATAGCA	GGTCCCAGTCGGGTTC	111
NR4A2	NM_006186	CAGCTCCGATTCTTAACTCCAG	GGTGAGGTCCATGCTAACTTGA	52
POU5F	NM_002701	AGGGCCCCATTTTGGTACC	TCAGTTTGAATGCATGGGAGAGC	136
SOX1	NM_005986	GCGGAGCTCGTCGCATT	GCGGTAACAACACAAAAAATTG	76
TH	NM_199292	ACTGGTTCACGGTGGAGTTC	TCTCAGGCTCCTCAGACAGG	117