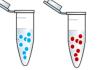


Human LCM + Bulk RNA-Seq

Mouse Bulk RNA-Seq

Mouse snRNA-Seq





Myenteric ganglia

15 donors 36 gut segments total Sequenced 3 technical reps (2 samples had 1 extra rep):

- 110 ganglia samples
- 27 muscle samples

Female (6 total)

- 4 duodenum •
- 5 ileum
- 6 colon

Male (9 total)

- 4 duodenum
- 9 ileum
- 8 colon

Β



Neurons Glia



Myenteric neurons

- 6 samples (8M, 2F)
- 2 duodenum ٠
- 2 ileum ٠ 2 colon

٠

- Myenteric glia
- 5 samples (6M)
- 2 duodenum •
- 2 ileum
- 1 colon



inDrop 🔵

Myenteric neurons

10X Genomics:

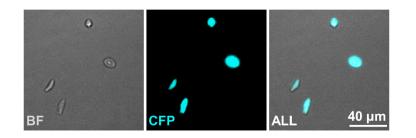
27,956 nuclei (4F, 2M) 9 samples:

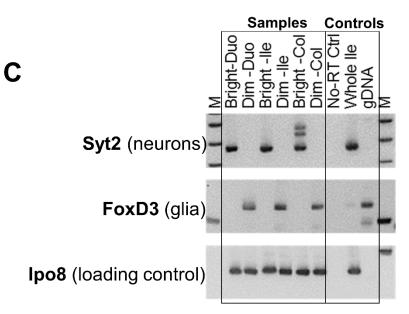
- 2 duodenum •
- 4 ileum
- 3 colon

inDrop:

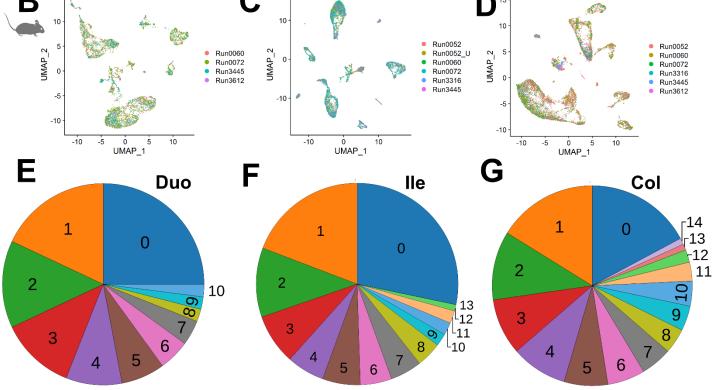
8,654 nuclei (6M)

- 6 samples
- 2 duodenum
- 2 ileum
- 2 colon





Sn-RNA-Seq run summaries	Duodenum	lleum	Colon	All
Approx. Nuclei Encapsulated	12884	24413	30551	67848
10X Total Runs	2	4	3	9
inDrop Total Runs	2	2	2	6
Total Runs	4	6	5	15
10x Total Nuclei	5394	8291	14271	27956
inDrop Total Nuclei	1756	3570	3328	8654
Total Nuclei Pre-cleaning	7150	11861	17599	36610
Total Nuclei Post-cleaning *	6217	8379	10612	25208
Total Reads 10x	7.8E+08	1.8E+09	9.5E+08	3.6E+09
Total Reads inDrop	1.7E+08	1.3E+08	2.5E+08	5.5E+08
Total Reads	9.4E+08	2.0E+09	1.2E+09	4.1E+09
Duodenum C	lleum		R 15.1	Colo
10-	. 🚷 .		D ¹⁵⁻	

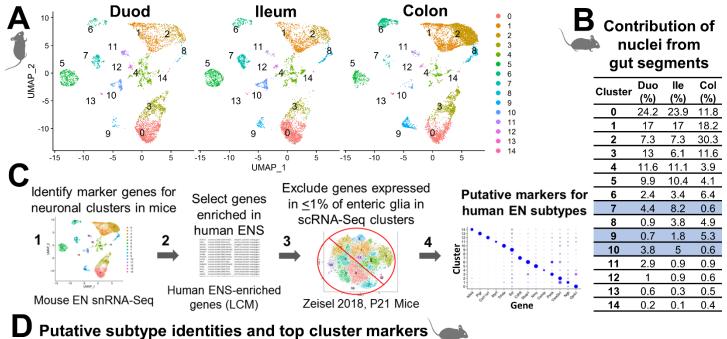


Cluster composition (% Total)

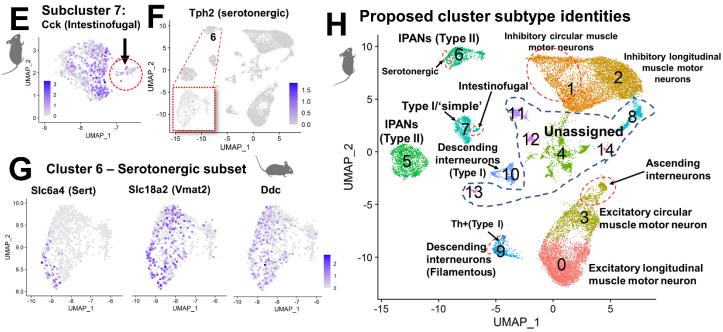
	01000			viai,
Н	Cluster	Duodenum	lleum	Colon
П	0	24.6%	28.5%	17.5%
	1	18.2%	19.0%	16.2%
	2	13.6%	11.4%	10.7%
	3	11.8%	7.8%	9.2%
	4	8.9%	6.4%	8.8%
	5	7.2%	5.9%	7.1%
	6	5.2%	5.1%	5.9%
	7	4.3%	4.6%	5.1%
	8	2.4%	4.4%	4.5%
	9	2.3%	2.3%	4.3%
	10	1.5%	1.8%	4.2%
	11	-	1.7%	3.0%
	12	-	0.6%	1.8%
	13	_	0.4%	1.3%
	14	-	-	0.6%

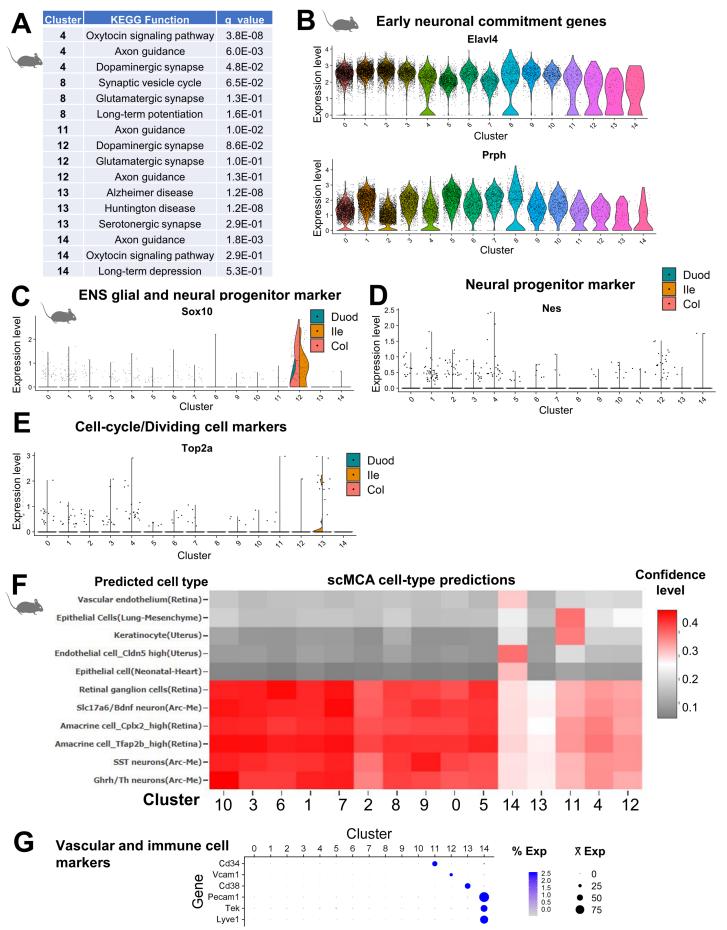
Cluster composition (Total Nuclei)

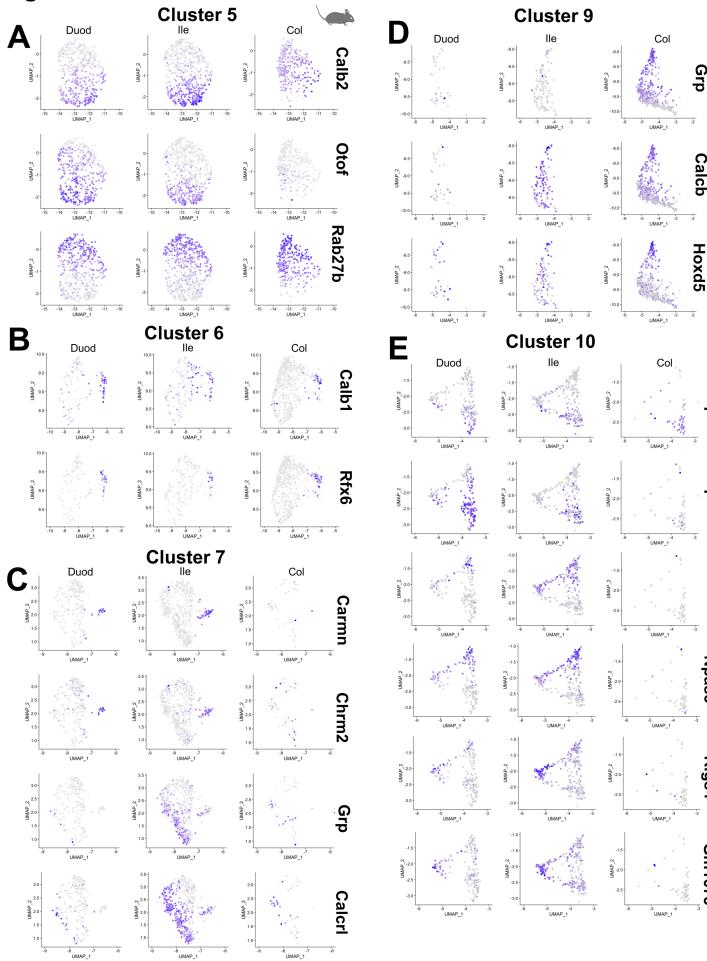
Duodenum	lleum	Colon				
1531	2388	1856				
1132	1594	1714				
848	959	1133				
731	650	972				
553	534	936				
445	491	756				
321	429	626				
265	386	536				
151	367	475				
144	195	452				
96	155	447				
-	142	320				
-	54	189				
-	35	141				
-	-	59				
	Duodenum 1531 1132 848 731 553 445 321 265 151 144	DuodenumIleum153123881132159484895973165055353444549132142926538615136714419596155-142-54				



Cluster	Proposed Subtype	Known Markers	Top 3 Mouse	Top 3 Human-Match	% Tot. (Lit)	% Obs.
0	Excitatory longitudinal MMN	Calb2/Chat/ Tac1+/-	Brinp2/Fbxw15/Specc1	Oprk1/Brinp2/Tmem132c	13% (1)	18.7%
1	Inhibitory longitudinal MMN	Nos1/Vip	Ass1/Cygb/Col25a1	Ngb/Gsg1l/Col25a1	3% (1)	~8.2% ^E
2	Inhibitory circular MMN	Nos1/Vip/Npy+/-	Gm4876/Gm16083/Dsc2	Vwa5b1/Sv2b/Oprd1	23% (1)	~26 .5% ^E
3	Excitatory circular MMN	Chat/Tac1/Calb2+/-	Tac1/Necab2/Htr2b	Penk/Sncb/Olfm3	21% (1)	7.7%
3s	Ascending IN	Chat/Tac1/Calb2+/-	Kctd16/Unc5d ^	Kctd16/Unc5d ^	4% (1)	~2.6% ^E
4	Unassigned	-	Slc24a3/Myh11/Carmn	Cemip/Kcnip1	-	8.1%
5	IPAN	Calcb/Calb2+/-	Nmu/Pcdh10/Cysltr2	Nmu/Pcdh10/Cbln2	~9%** (1)	7.5%
6	IPAN	Nefl/Calb1[sparse]	Nxph2/Cckar/Eif3h	Skap1/Nefl/Nxph2	~9%** (1)	4.0%
6s	Serotonergic (Descend. IN)	Nefl/5-HT	Slc6a4/Carpt/Grp ^	SIc6a4/Carpt/Grp ^	1% (1)	0.5%
7	Type I/'simple'	Nefl	Cdh9/Zim1/B230209E15Rik	Cdh9/Mgat4c/Klhl1	~9%** (1,2)	3.5%
7s	Intestinofugal***	Chat +/-Cck/Grp	Slc24a3/Carmn ^	Slc24a3/Carmn ^	~0.25% ^G (1,3)	0.3%
8	Unassigned	Nos1/Gal/Sst(low)	*No highly-selective markers	*No selective markers	-	3.6%
9	Descending IN (Filamentous)	Sst/Calb2/Th	Adamts1/Gm30382/Irf1	Sst/Galnt5/Pantr1	4% ^E (1)	3.0%
10	Descending IN (Type I)	Chat/Nos1/Gad2	Neurod6/AI593442/Bcr	Trhde/Bean1/Neurod6	3% (1)	2.8%
11	Unassigned	-	C3/Igfbp6/Upk3b	Myrf/Nkain4/Cldn15	-	1.4%
12	Unassigned	-	Cdh19/Apoe/Col11a1	Col11a1/Tmprss5/Car12	-	0.82%
13	Unassigned	-	Reg3b/Pigr/Epcam	Pigr/Klf5/Cdh1	-	0.45%
14	Unassigned	-	Ccl21a/Mmrn1/Pecam1	Klhl4/Arap3/Radil	-	0.03%







Grp

Calcb

≤ip

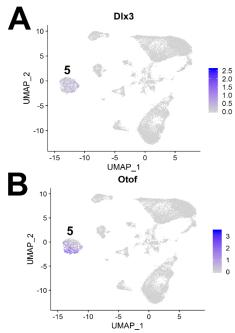
Ptprm

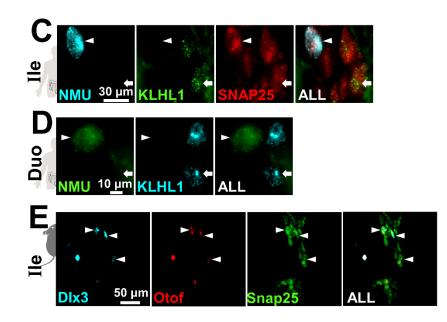
Ednra

Npas3

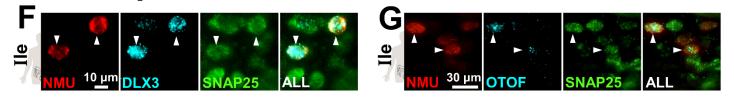
Rigs4

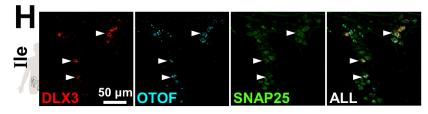
Gm1673

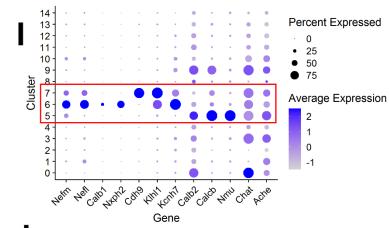


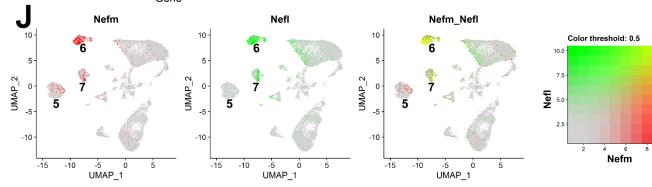


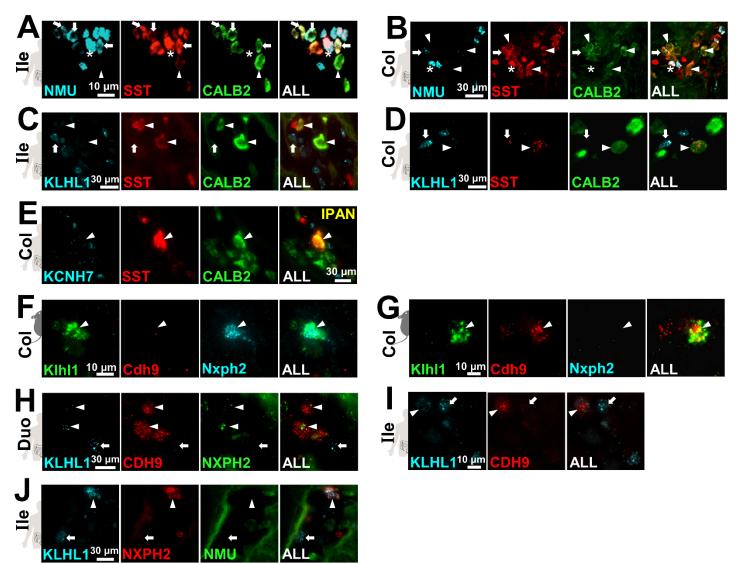
10

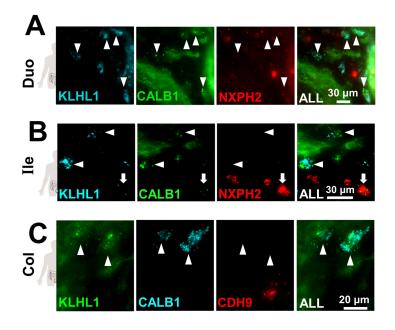


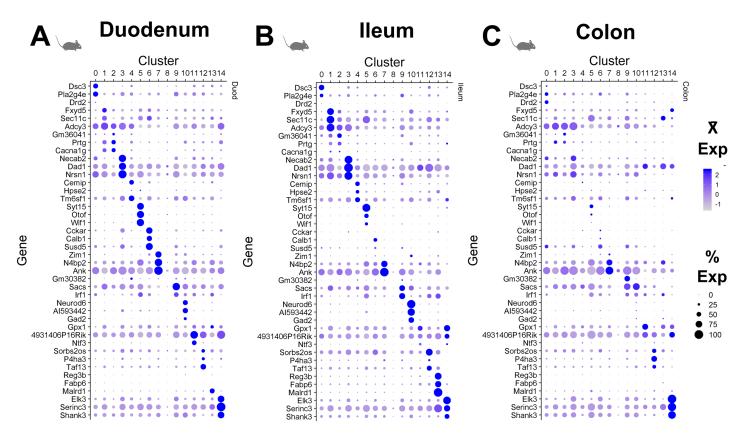


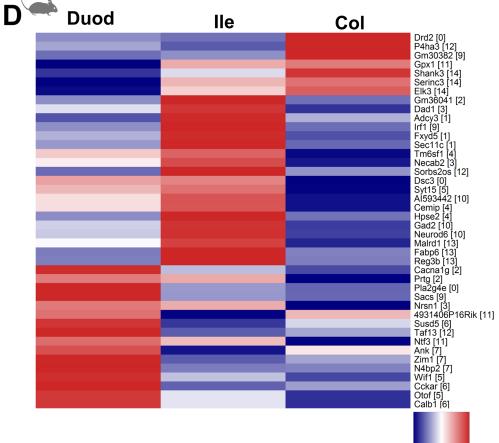






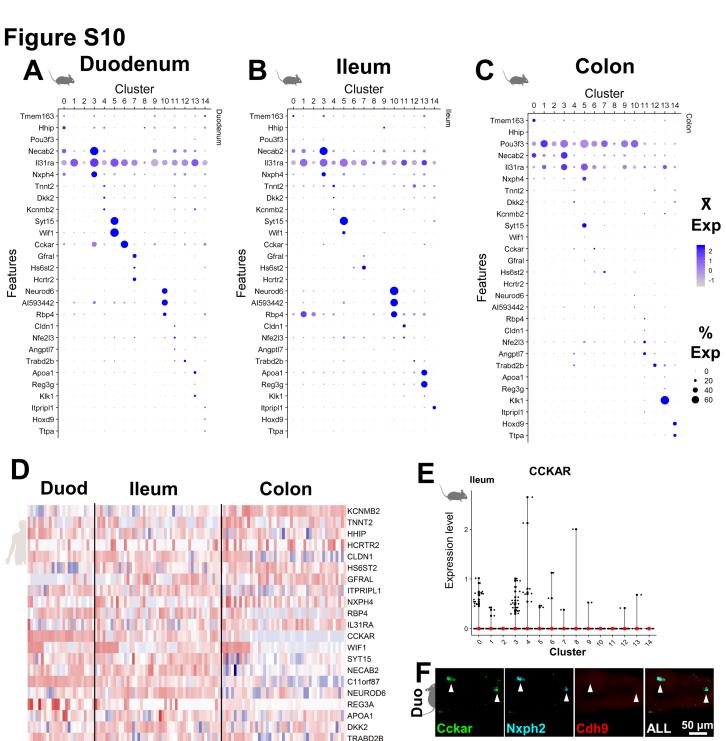


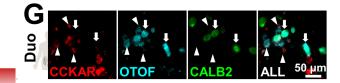




0.0 1.0

-1.0





REG3A APOA1 DKK2 TRABD2B ANGPTL7 NFE2L3 TTPA HOXD9 TMEM163 POU3F3 KLK1

The intervention of the in

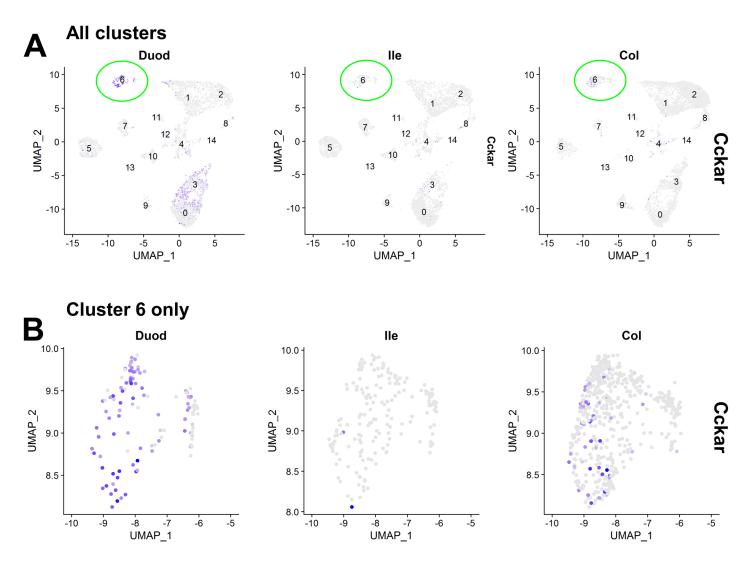
Construction Const

AVV: 0 with a second se

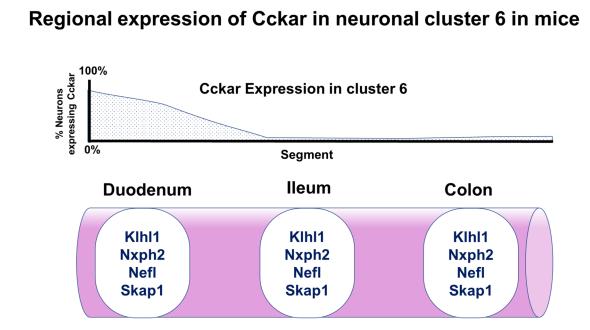
The subgroup of the subgroup o

anergia vol. an

sample voltage and second seco



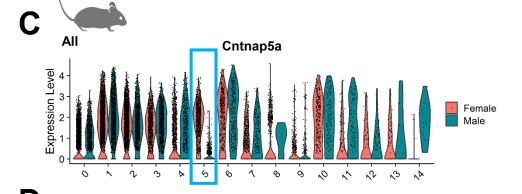
С

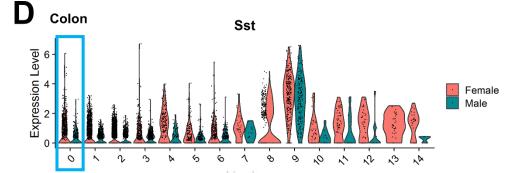


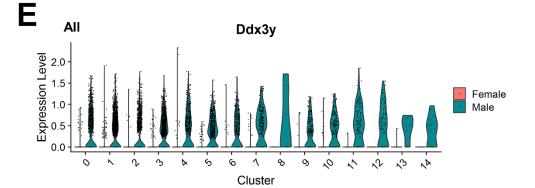
Α logFC Segment(s) Gene Sex XIST Female 5.5, 5.0, 4.7 Col, Ile, Duo AC005332.2 Female 5.1 Duo Female SLC6A14 1.3 Col TACSTD2 Female 1.2 Col LINC02529 Female 1.2 Col MUC5B Female 1.1 Col ZNF516 Female 1.1 Col 1.1 PLXNB3 Female Col RPTOR Female 1 Col RN7SL2 Female 1 Col -12.0, -10.9, -10.3 lle, Col, Duo RPS4Y1 Male EIF1AY Male -11.1, -10.4, -10.3 lle, Col, Duo DDX3Y -10.0, -9.6 Male Ile, Col, Duo NLGN4Y Male -9.3, -9.1, -8.7 lle, Duo, Col UTY -8.5, -7.8, -7.6 Male lle, Duo, Col ZFY Male -8.3, -7.7, -7.6 lle, Col, Duo KDM5D Male -7.6, -7.4, -6.7 lle, Duo, Col USP9Y Male -7.1, -7.0, -6.8 Duo, Ile, Col TMSB4Y Male -6.7, -6.3 Ile, Col, Duo **TTTY14** Male -6.3, -6.0, -5.2 Ile, Col, Duo

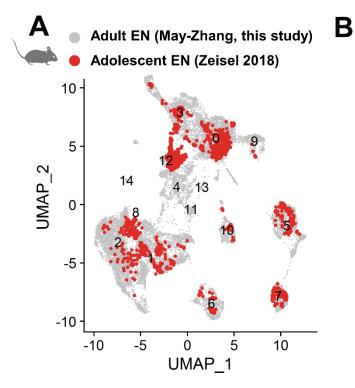
Gene	Sex	Avg logFC	Cluster(s)	Segment(s)	% Cell Exp Diff
Xist	Female	2.4	ALL	Col, Ile, Duo	98%
Tsix	Female	1	ALL	lle, Duo, Col	81%
Sst	Female	1	0	Col	10%
Cntnap5a	Female	1.7	5	Duo, Ile, Col	47%
Uty	Male	-1.1	ALL	Col, Duo, lle	-89%
Eif2s3y	Male	-0.8	ALL	lle, Duo, Col	-77%
Ddx3y	Male	-0.5	ALL	Duo, Ile, Col	-68%
Kdm5d	Male	-0.3	5, 10	Col, Duo	-62%

B









)				
)	Cluster	Proposed Identity	Adult EN (% Total)	Juvenile EN (% Total)
		Excitatory longitudinal		
	0	muscle motor neurons	18.6%	21.6%
		Inhibitory longitudinal		
	1	muscle motor neurons	17.5%	19.3%
		Inhibitory circular muscle		
	2	motor neurons	17.4%	10.9%
		Excitatory circular muscle		
	3	motor neurons	10.3%	8.4%
	4	Unassigned	8.1%	8.0%
	5	IPAN	7.5%	7.1%
	6	IPAN	4.5%	6.8%
	7	(Type I/'simple')	3.8%	6.7%
	8	Unassigned	3.5%	5.9%
1		Descending interneurons		
	9	(Filamentous)	3.0%	3.1%
		Descending interneurons		
	10	(Type I)	2.8%	2.3%
	11	Unassigned	1.4%	0.0%
	12	Unassigned	0.8%	0.0%
	13	Unassigned	0.4%	0.0%
	14	Unassigned	0.3%	0.0%