

Supplementary File 3: Raw data of all experiments

Percentage of NC-derived and non-NC-derived neurons in adult Wnt1-cre:tdTomato mice

	No of neurons	% Wnt1:tdT+	% Wnt1:tdT-
Mouse 1	185	61.62	38.38
Mouse 2	276	53.62	46.38
Mouse 3	239	45.60	54.40
Mouse 4	485	54.64	45.36
Mouse 5	686	60.64	39.36
Mouse 6	345	58.26	41.74

Percentage of mesoderm-derived and non-mesoderm-derived neurons in adult Mesp1-cre:tdTomato mice

	No of neurons	% Mesp1:tdT+	% Mesp1:tdT-
Mouse 1	147	53.06	46.94
Mouse 2	137	53.28	46.72
Mouse 3	200	44.50	55.50

Percentage of tdTomato-expressing neurons of all NOS1+ neurons in adult Wnt1-cre:tdTomato mice

	NOS1+ counted	% Wnt1:tdT+	% Wnt1:tdT-
Mouse 1	248	64.04	35.96
Mouse 2	200	78.00	22.00
Mouse 3	168	76.78	23.22

Percentage of tdTomato-expressing neurons of all CGRP+ neurons in adult Wnt1-cre:tdTomato mice

	CGRP+ counted	% Wnt1:tdT+	% Wnt1:tdT-
Mouse 1	41	25.47	74.53
Mouse 2	56	23.37	76.63
Mouse 3	49	23.87	76.13

Age-wise changes in percentages of MENs in myenteric plexus neurons of Wnt1-cre:tdTomato mice

	P11 mice	
	# neurons	% MENs
1	374	2.14
2	534	10.00
3	419	0.23

	P22 mice		Mean neurons/ganglia
	# neurons	% MENs	
1	245	29.79	16.34 ± 2.07
2	175	27.43	11.67 ± 2.07
3	322	31.68	21.47 ± 1.61

	P60 mice		Mean neurons/ganglia
	# neurons	% MENs	
1	185	38.38	15.42 ± 3.59
2	276	46.38	21.23 ± 4.22
3	239	54.40	19.91 ± 3.26

	P180 mice		Mean neurons/ganglia
	# neurons	% MENs	
1	171	60.97	15.54 ± 2.52
2	239	50.39	21.73 ± 3.69
3	176	60.52	14.67 ± 1.80

	P510 mice		Mean neurons/ganglia
	# neurons	% MENs	
1	261	98.85	13.74 ± 2.22
2	360	93.22	17.14 ± 3.46
3	375	95.91	13.89 ± 1.30

Percentage of MENs in myenteric plexus of P20 Wnt1-cre:tdTomato mice dosed with and without GDNF from the P10 age

	Control			GDNF		
	# neurons	% MENs	Mean neurons/ganglia	# neurons	% MENs	Mean neurons/ganglia
1	477	22.85	22.71 ± 6.99	418	3.83	19.90 ± 4.23
2	296	20.27	12.86 ± 2.37	350	4.00	15.21 ± 3.38
3	577	34.49	26.23 ± 4.55	533	3.75	38.00 ± 4.89

Percentage of MENs in myenteric plexus of P20 Wnt1-cre:tdTomato mice dosed with and without HGF from the P10 age

	Control			HGF		
	# neurons	% MENs	Mean neurons/ganglia	# neurons	% MENs	Mean neurons/ganglia
1	325	23.69	15.48 ± 1.88	394	40.86	18.76 ± 2.51
2	439	17.77	20.90 ± 3.20	409	40.83	19.48 ± 2.88
3	454	36.12	21.62 ± 3.40	386	39.38	18.38 ± 2.71
4	336	44.05	16.00 ± 2.46	326	62.27	15.52 ± 1.75
5	416	15.38	27.73 ± 3.73	189	63.49	18.90 ± 2.01

Percentage of MENs in myenteric plexus of 17 month old mice dosed with and without GDNF

	Control			GDNF		
	# neurons	% MENs	Mean neurons/ganglia	# neurons	% MENs	Mean neurons/ganglia
1	141	88.65	15.67 ± 5.22	111	84.68	12.33 ± 4.11
2	238	93.28	21.64 ± 6.52	215	60.00	19.54 ± 5.89
3	225	89.78	20.45 ± 6.16	160	88.12	14.54 ± 4.38
4	213	84.04	18.54 ± 5.59	183	60.11	17.36 ± 5.23
5	92	88.04	11.5 ± 4.06	130	77.69	16.25 ± 5.74

Percentage of RET+ NENs in myenteric plexus of 17 month old mice dosed with and without GDNF

	Control			GDNF		
	# neurons	% RET	Mean neurons/ganglia	# neurons	% RET	Mean neurons/ganglia
1	170	8.23	17.00 ± 5.37	137	19.70	13.70 ± 4.33
2	227	6.17	20.64 ± 6.22	198	24.24	18.00 ± 5.43
3	232	5.17	21.09 ± 6.36	200	11.00	18.18 ± 5.48
4	188	9.04	18.8 ± 5.94	139	24.46	15.44 ± 5.14

Percentage of Ret-CFP+ NENs in myenteric plexus of Ret+/- mice at 9 and 16 weeks

	9 weeks			16 weeks		
	# neurons	% CFP+	Mean neurons/ganglia	# neurons	% CFP+	Mean neurons/ganglia
1	268	14.18	24.36 ± 6.27	305	10.16	16.05 ± 3.68
2	275	35.65	19.64 ± 2.28	306	13.40	13.91 ± 2.96
3	294	28.91	24.50 ± 3.40	263	11.79	12.52 ± 2.73
4				122	15.57	11.09 ± 3.34
5				231	14.75	17.77 ± 4.93

Percentage of MHCst+ MENs in myenteric plexus of Ret+/- and Ret+/+ mice at 9 and 16 weeks

Ret+/-	9 weeks			16 weeks		
	# neurons	% MENs	Mean neurons/ganglia	# neurons	% MENs	Mean neurons/ganglia
1	197	41.12	17.91 ± 2.96	217	56.68	19.73 ± 2.69
2	286	49.65	26.00 ± 3.96	218	59.17	19.82 ± 3.12
3	297	39.06	19.80 ± 3.62	316	60.44	19.75 ± 2.89
4				228	57.72	20.73 ± 4.45
5				297	38.38	18.56 ± 4.11

Ret+/-	9 weeks			16 weeks		
	# neurons	% MENs	Mean neurons/ganglia	# neurons	% MENs	Mean neurons/ganglia
1	220	44.54	20.00 ± 3.87	182	91.21	16.54 ± 2.73
2	210	69.05	19.10 ± 2.73	192	87.43	17.36 ± 1.25
3	214	62.62	13.37 ± 2.50	215	83.25	13.43 ± 2.01
4				200	70.00	18.18 ± 3.74
5				147	82.84	21.00 ± 2.40

Percent MET+ neurons

	# MET+ neurons	Wnt1-tdT+	Wnt1-tdT-
1	68	16.18	83.82
2	56	17.86	82.14
3	28	21.43	78.57

Pattern Weights of the four MEN-specific NMF patterns as represented in the post-natal mesenchymal single cell RNA sequencing data from Gut Cell Atlas

Age	Mean	Pattern 16			Mean	Pattern 27			Mean	Pattern 32			Mean	Pattern 41		
		Standard Error	Sample size	Sample size		Standard Error	Sample size	Standard Error		Sample size	Standard Error	Sample size				
Juvenile	0.0049	0.00023	5		-0.0036	0.0012	5		0.024	0.0005	5		-0.0029	0.0002	5	
Adult	0.0049	0.0006	3		0.0085	0.0021	3		0.028	0.0030	3		0.0025	0.0028	3	
Aging	0.0053	0.0007	3		0.0097	0.0019	3		0.033	0.0016	3		0.0058	0.0014	3	

Age-wise changes in whole gut transit time of Ret+/- and Ret+/- mice

Age weeks	Whole gut transit time (in minutes) of Ret +/+ mice								Whole gut transit time (in minutes) of Ret +/- mice									
	131	119	59	135	113	88	103	86	73	105	93	91	87	83	134	143	153	132
9																		
12	83	89	89	100	106	86	110	78	89	124	106	98	77	71	128	73	82	124
14	107	103	120	141	111	117	166	110	100	189	190	93	107	106	190	207	125	96
16	111	130	106	116	130	122	116	140	200	237	194	122	185	157	102	101	156	119

Fold change (transcript expression) of Gdnf in murine small intestinal LM-MP at different ages

	P30	P90
1	0.8211	0.6884
2	0.6600	0.6081
3	1.6392	0.7047
4	1.6011	0.9263
5	0.7185	0.1380
6	0.9784	0.1683

Expression levels (Fluorescence intensity) of Hgf, compared to Beta-actin, in murine small intestinal LM-MP at different ages

	P10	P30	P90
1	0.008	0.007	0.022
2	0.006	0.017	0.013
3	0.006	0.011	0.016

Expression levels (Fluorescence intensity) of Gdnf, compared to Beta-actin, in murine small intestinal LM-MP at different ages

	P10	P30	P90
1	1.372	0.069	0.069
2	1.271	0.095	0.031
3	1.392	0.053	0.053

Percent MHCst neurons that express tdTomato in Wnt1-cre:tdTomato mice

	MHCst+ neurons counted	Percent tdTomato+
1	29	0
2	30	0
3	48	0

Whole gut transit time (in min) of 17 month old mice before and after GDNF or Saline treatment

	Before Treatment		After Treatment	
	Control	GDNF	Control	GDNF
1	210	182	210	74
2	210	210	164	110
3	210	152	168	87
4	172	210	162	123
5	210	210	171	111

Effect of GDNF on MHCst neuron proportions in 17 month old mice

	Control		GDNF	
	Neurons counted	%MHCst	Neurons counted	%MHCst
1	141	88.65	111	84.68
2	238	93.27	215	60.00
3	225	89.78	160	88.12
4	213	84.03	183	60.11
5	92	88.04	130	77.69

Effect of GDNF on RET neuron proportions in 17 month old mice

	Control		GDNF	
	Fields counted	RET+	Fields counted	RET+
1	10	14	10	27
2	10	14	10	48
3	10	12	10	22
4	12	17	12	34

Effect of GDNF on neuronal numbers in 17 month old mice

	Control	GDNF
1	15.67 ± 2.63	12.33 ± 3.55
2	21.64 ± 4.18	19.54 ± 2.55
3	20.45 ± 3.19	14.54 ± 1.91
4	18.54 ± 2.84	17.36 ± 2.61
5	11.50 ± 3.81	16.25 ± 2.30

Fold change (transcript expression) of Hgf in murine small intestinal LM-MP at different ages

	P10	P30	P90
1	2.146	5.442	6.114
2	1.867	4.747	5.337
3	0.543	2.919	4.745
4	0.659	2.683	4.123
5	0.789	2.703	5.593
6	0.884	2.928	5.824

UMI per cluster (6 month data)

	Average ± SEM	# Cells
MENs	4023.91 ± 85.35	2223
NENs	3391.71 ± 386.52	77
Neuroglia	113	