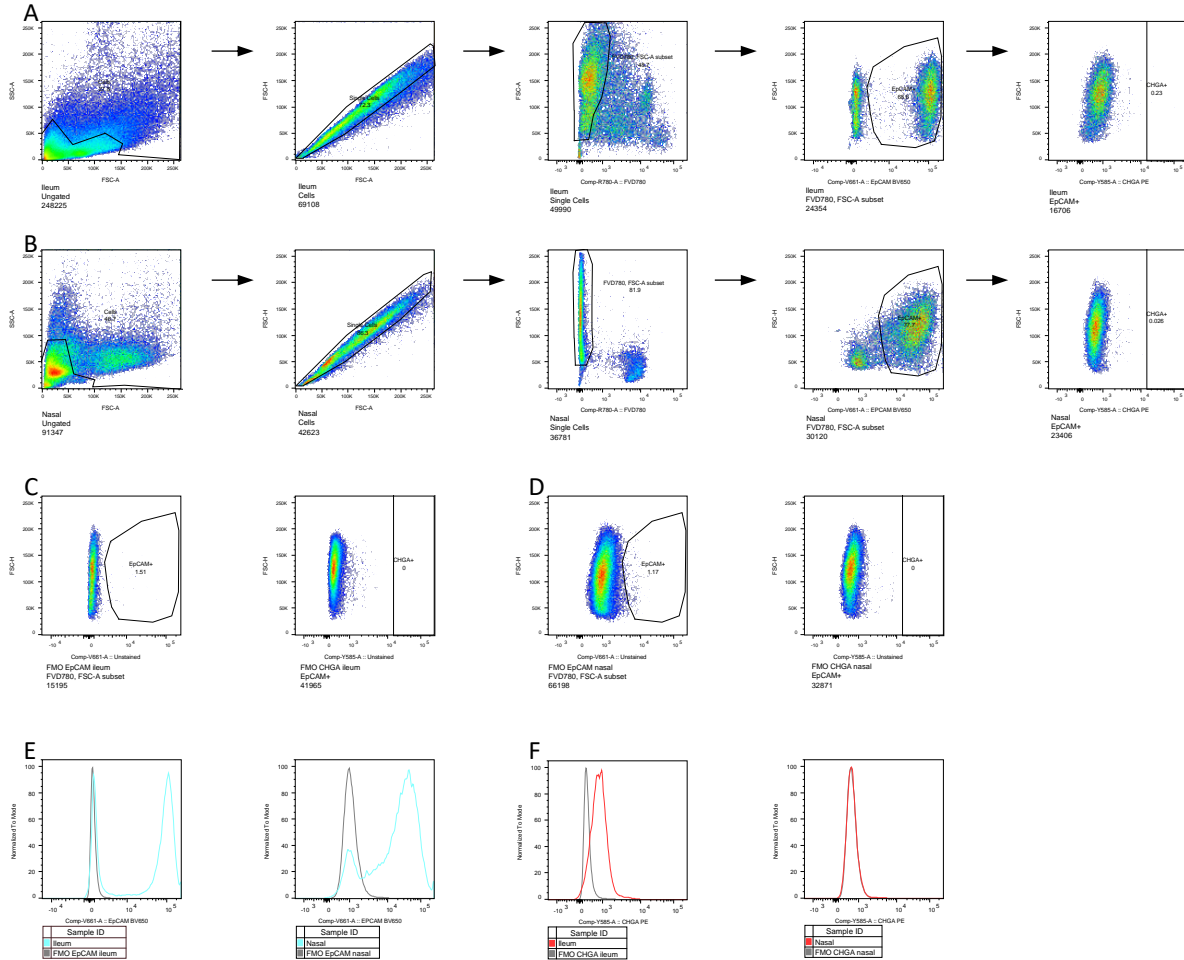# Supplementary Materials

Supplementary Figures and Tables
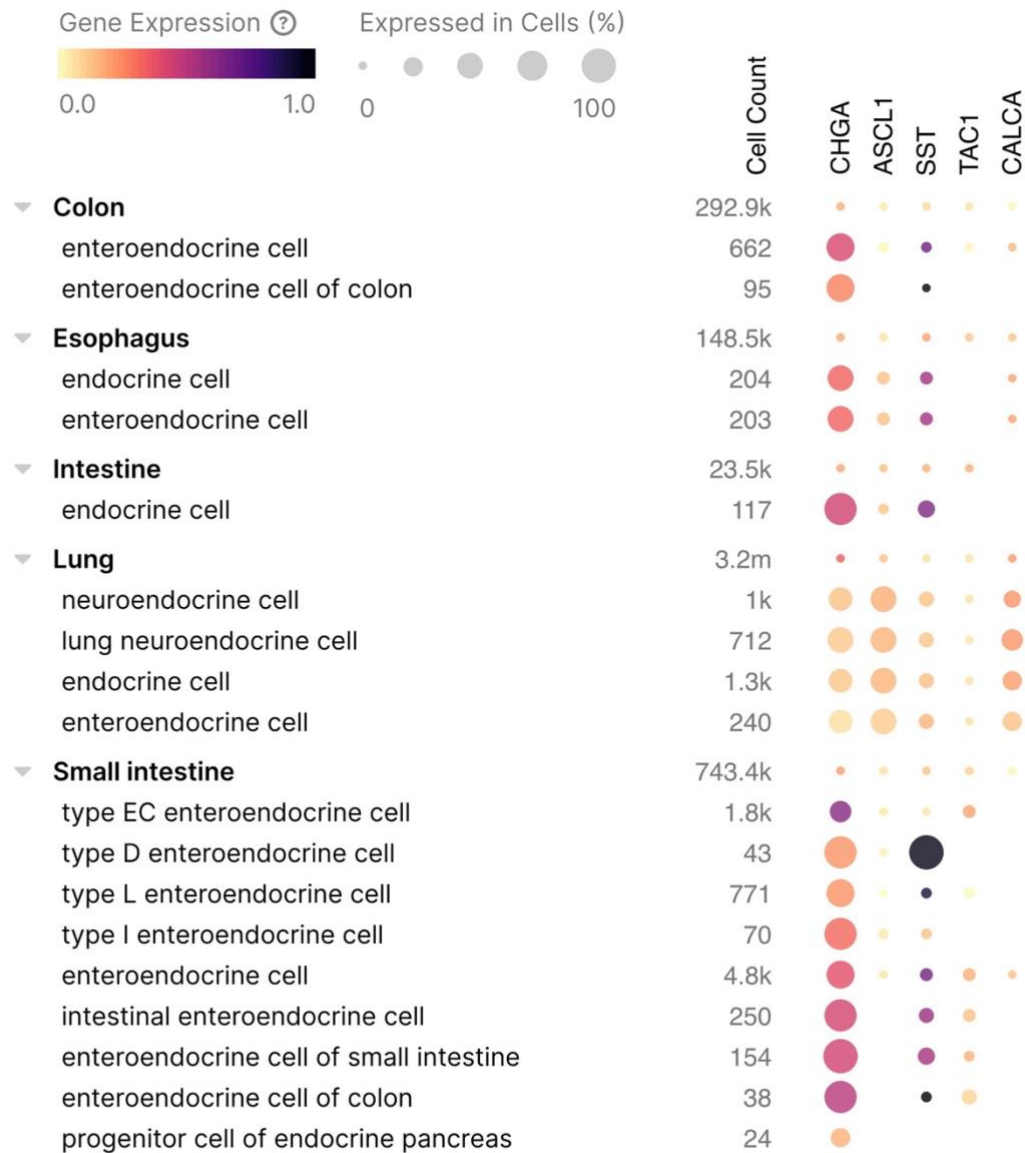
1.1   Supplementary Figures

Supplementary figure 1: Gating strategy of EpCAM- and CHGA-positive cells detected by flow cytometry. Neuroendocrine cells were identified via subsequently gating of cells, single cells and viable cells (FVD780$^-$). On the viable cell population, epithelial cells were identified as EpCAM$^+$ cells in which CHGA$^+$ cells were identified as neuroendocrine cells. Representative gaiting strategies for ileum tissue **(A)** and all nasal tissues, being control tissue, AR patient tissue and CRSwNP patient tissue **(B)**. EpCAM and CHGA gating were determined using EpCAM Fluorescence Minus One (FMO) controls and CHGA FMO controls for ileum tissue **(C)** and nasal tissue **(D)**. EpCAM (blue) and CHGA (red) expression shown as histograms compared to their respective FMO (grey) for ileum tissue **(E)** and nasal tissue **(F)**. FVD: Fixable Viability Dye eFluor™; EpPCAM: epithelial cellular adhesion molecule; CHGA: chromogranin A; AR = allergic rhinithis; CRSwNP = chronic rhinosinusitis with nasal polyps
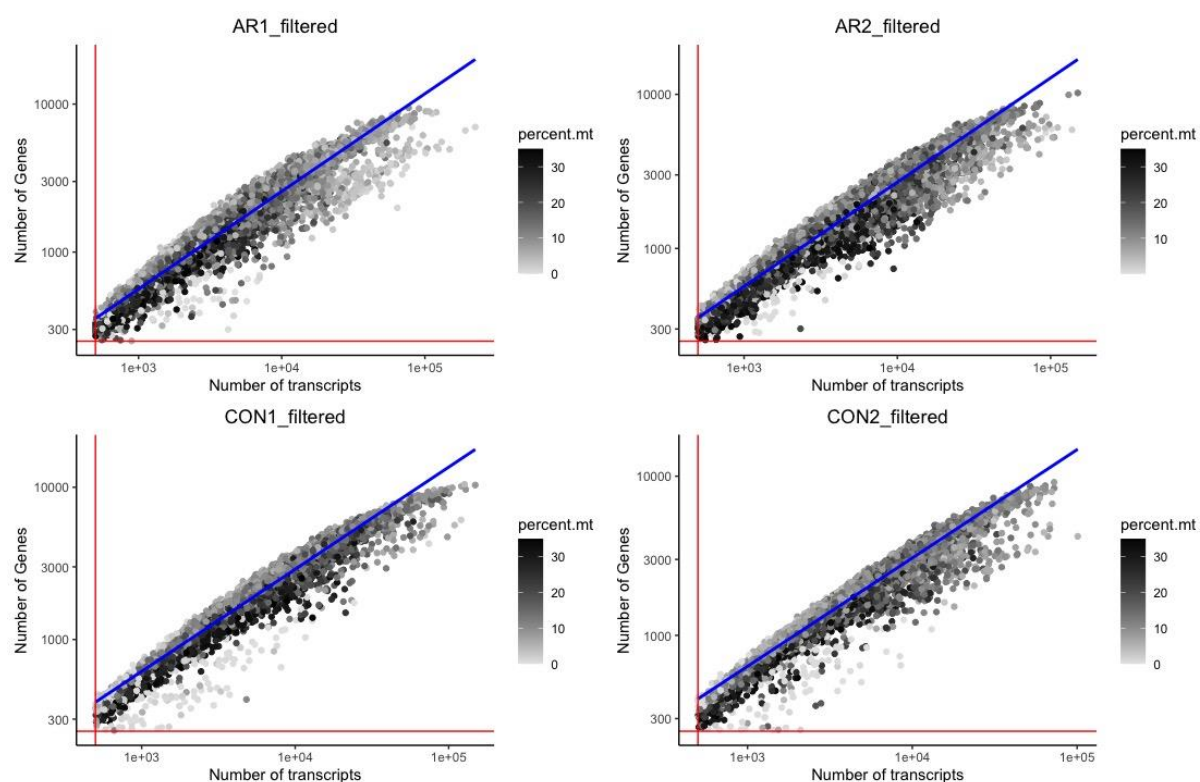
**Supplementary figure 2: Quality control of the single cell RNA-sequencing dataset.**
Graphs showing the quality of each of the samples after filtering the datasets using the
following parameters: number of genes > 250, number of transcripts > 500 and percentage of
mitochondrial DNA < 35%.
AR1 = allergic rhinitis patient 1; AR2 = allergic rhinitis patient 2; CON1 = control subject 1;
CON2 = control subject 2

	Cell Count	CHGA	ASCL1	SST	TAC1	CALCA
Colon	292.9k					
enteroendocrine cell	662					
enteroendocrine cell of colon	95					
Esophagus	148.5k					
endocrine cell	204					
enteroendocrine cell	203					
Intestine	23.5k					
endocrine cell	117					
Lung	3.2m					
neuroendocrine cell	1k					
lung neuroendocrine cell	712					
endocrine cell	1.3k					
enteroendocrine cell	240					
Small intestine	743.4k					
type EC enteroendocrine cell	1.8k					
type D enteroendocrine cell	43					
type L enteroendocrine cell	771					
type I enteroendocrine cell	70					
enteroendocrine cell	4.8k					
intestinal enteroendocrine cell	250					
enteroendocrine cell of small intestine	154					
enteroendocrine cell of colon	38					
progenitor cell of endocrine pancreas	24					

Supplementary figure 3: Dotplot of NEC markers in other human tissue based on the CZ CELLxGENE Discover database

Dotplot showing RNA expression levels of NEC markers *CHGA*, *ASLC1*, *SST*, *TAC1* and *CALCA* in human colon, human esophagus, human intestine, human lung and human small intestine tissue. Data and dotplot obtained from CZI Single-Cell Biology, BioRxiv 2023 (42).
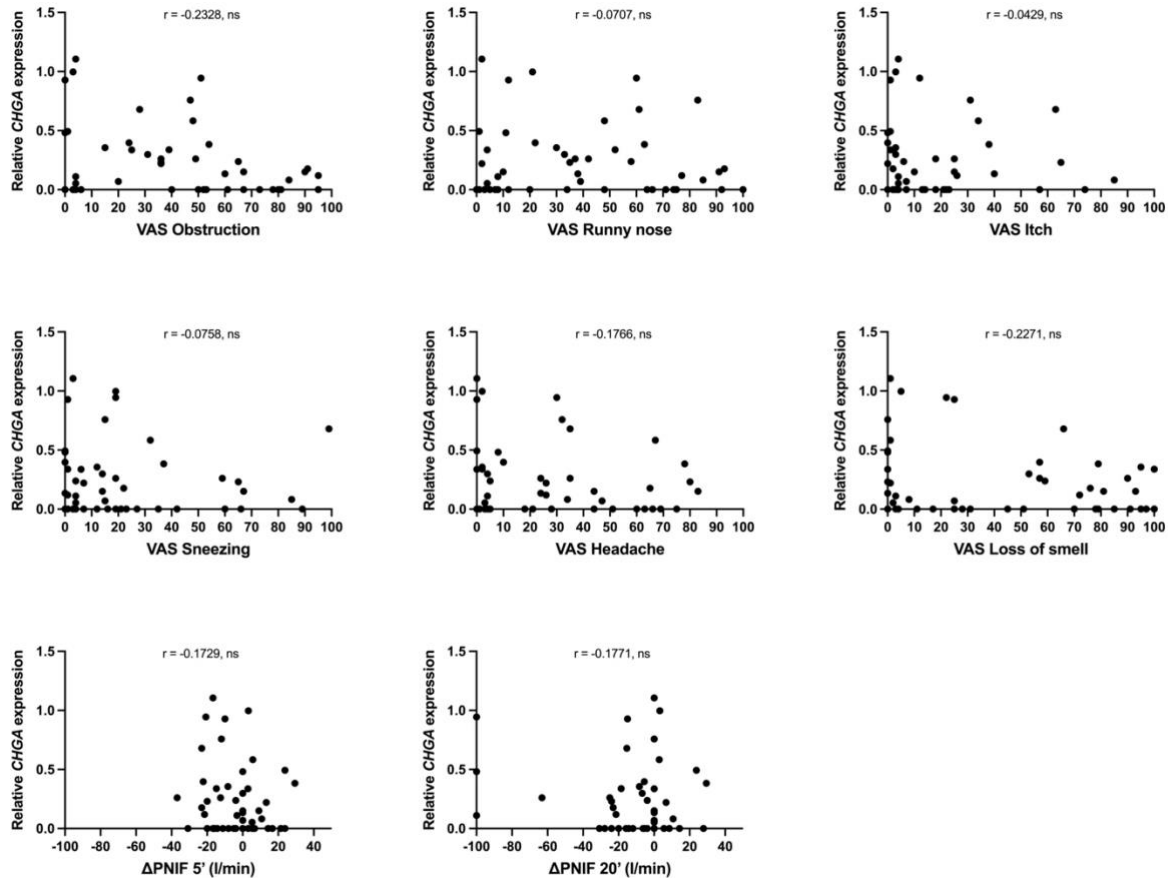
Supplementary figure 4: Correlation between the expression of the NEC marker *CHGA* and disease severity and nasal hyperreactivity

Correlation of *CHGA* mRNA expression with the VAS scores for obstruction, VAS score for runny nose, VAS score for itch, VAS score for sneezing, VAS score for headache, VAS score for loss of smell, change in PNIF 5 minutes after cold dry air provocation and change in PNIF 20 minutes after cold dry air provocation as measures of disease and NHR severity.

CHGA: chromogranin A; VAS = visual analogue score; NHR = nasal hyperreactivity; PNIF = peak nasal inspiratory flow; r = spearman r correlation value, ns = p > 0.05
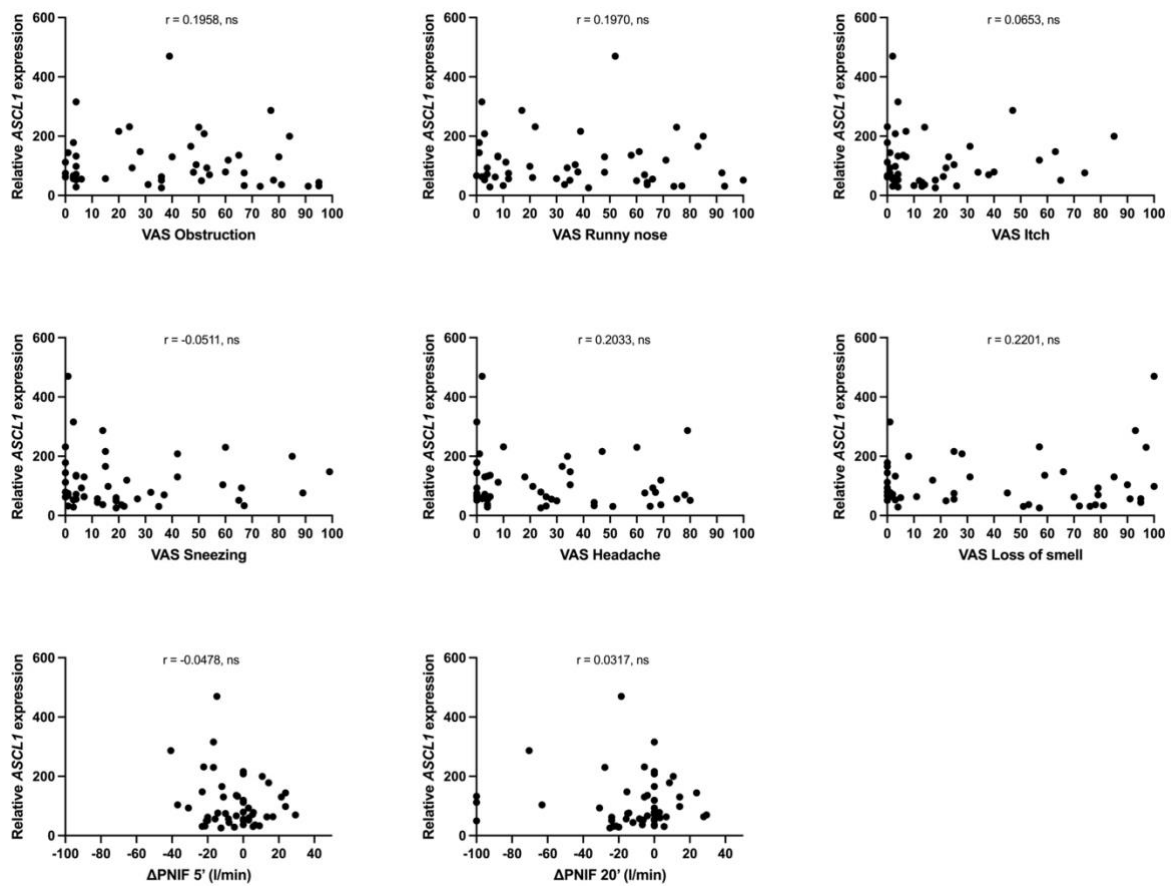
Supplementary figure 5: Correlation between the expression of the NEC marker *ASCL1* and disease severity and nasal hyperreactivity

Correlation of *ASCL1* mRNA expression with the VAS scores for obstruction, VAS score for runny nose, VAS score for itch, VAS score for sneezing, VAS score for headache, VAS score for loss of smell, change in PNIF 5 minutes after cold dry air provocation and change in PNIF 20 minutes after cold dry air provocation as measures of disease and NHR severity.

ASCL1 = Achaete-Scute Family bHLH transcription factor 1; VAS = visual analogue score; NHR = nasal hyperreactivity; PNIF = peak nasal inspiratory flow; r = spearman r correlation value, ns = p > 0.05

1.2 Supplementary Tables

Supplementary table 1: Patient's characteristics of isolated primary nasal epithelial cells

	Control	AR	CRSwNP	p value
Total	**6**	**6**	**6**	
Sex (M/F)	3/3	4/2	4/2	ns
Age	34 (18-70)	28 (20-32)	35 (27-75)	ns
Current smoker	0	0	0	ns
Asthma/COPD	0	1 (17%)	1 (17%)	ns

M = male, F = female, COPD = chronic obstructive pulmonary disease

Supplementary table 2: Patient's characteristics of nasal biopsies

	Control	AR	CRSwNP	p value
Total	**12**	**16**	**22**	
Sex (M/F)	7/5	9/7	16/6	ns
Age	36.5 (18-61)	31 (20-62)	50.5 (29-65)	AR vs CRSwNP: 0.0137
Current smoker	0 (0%)	5 (31%)	11 (50%)	0.0115
Asthma/COPD	0 (0%)	1 (6%)	3 (14%)	ns
Previous FESS	0 (0%)	1 (6%)	14 (64%)	<0,0001
Inferior conchotomy	0 (0%)	1 (6%)	3 (14%)	ns
VAS total sinonasal symptoms	0.3 ± 0.2	5.7 ± 2.0	3.8 ± 2.4	Control vs AR: <0.0001 Control vs CRSwNP: 0.0003
VAS obstructive	0.5 ± 0.7	6.1 ± 1.7	4.1 ± 3.2	Control vs AR: <0.0001 Control vs CRSwNP: 0.0028
VAS Running nose	0.6 ± 0.6	5.4 ± 3.0	4.3 ± 2.7	Control vs AR: <0.0001 Control vs CRSwNP: 0.0005
VAS Itch	0.2 ± 0.2	3.0 ± 2.4	1.6 ± 1.9	Control vs AR: 0,0001 Control vs CRSwNP: 0,0430
VAS Sneezing	0.4 ± 0.5	3.1 ± 3.0	2.6 ± 2.5	Control vs AR: 0.0016 Control vs CRSwNP: 0.0022
VAS headache	0.2 ± 0.3	3.7 ± 2.5	3.7 ± 3.0	Control vs AR: 0.0007 Control vs CRSwNP: 0.0004
VAS Smell	0.7 ± 1.9	3.8 ± 3.5	6.3 ± 3.3	Control vs CRSwNP: <0.0001
Nasal Hyperreactivity	0 (0%)	8 (50%)	9 (41%)	0.0108
ΔPNIF	-1.0 ± 12.3	-10.9 ± 14.9	-13.4 ± 17.1	ns

Exclusion criteria: use of any nasal medication, corticosteroids, antihistamines or saline lavages 1 week prior to the visit, prior use of allergen immunotherapy, relevant anatomical abnormalities in the nose, acute upper airway infection in the past 2 weeks, alcohol consumption in the past 24 hours, use of tricyclic antidepressants, intranasal drug-abuse in the past 12 months, pregnancy or breastfeeding and active malignancy

M = male, F = female, COPD = chronic obstructive pulmonary disease; FESS = functional endoscopic surgery; VAS = visual analogue scale; PNIF = peak nasal inspiratory flow

Supplementary table 3: Patient's characteristics of ileum biopsies

	Ileum
Total	**5**
CD/CRC	4/1
Sex (M/F)	0/5
Age	73 (54-96)
Current smoker	1
Asthma/COPD	0
Corticosteroid use	1

CD= Crohn's disease, CRC = colorectal cancer, M = male, F = female, COPD = chronic obstructive pulmonary disease

Supplementary table 4: Primer sequences for RT-qPCR

Target Gene	Forward primer	Reverse primer	Hydrolysis probe
CHGA	gggataccgaggtgatgaaatg	tcagaatggaaaggatccgttc	cgttgaggtcatctccgacacactttcc
ASCL1	agcaggagcttctcgacttcac	cgatcaccctgcttccaaag	ctggtcaggccctggtgcgaat
ACTB	ggacatccgcaaagacctgt	ctcaggaggagcaatgatcttgat	ctggcggcaccaccatgtaccct
RACK1	cactgtccaggatgagagcca	cataccttgaccagcttgtccc	tccgcttctcgcccaacagcag

CHGA = chromogranin A, *ASCL1* = Achaete-Scute Family BHLH Transcription Factor 1,
ACTB = actin beta, *RACK1* = Receptor For Activated C Kinase 1