

Anatomical characterisation of somatostatin-expressing neurons belonging to the anterolateral system

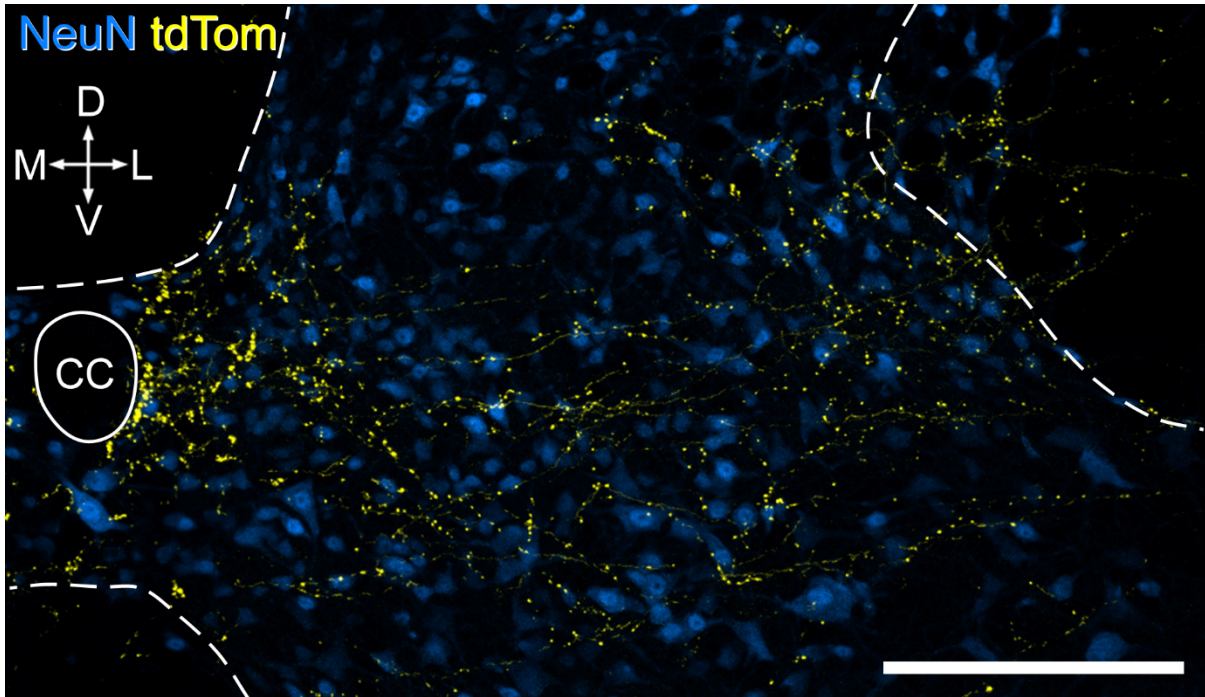
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Supplementary Figures 1-5

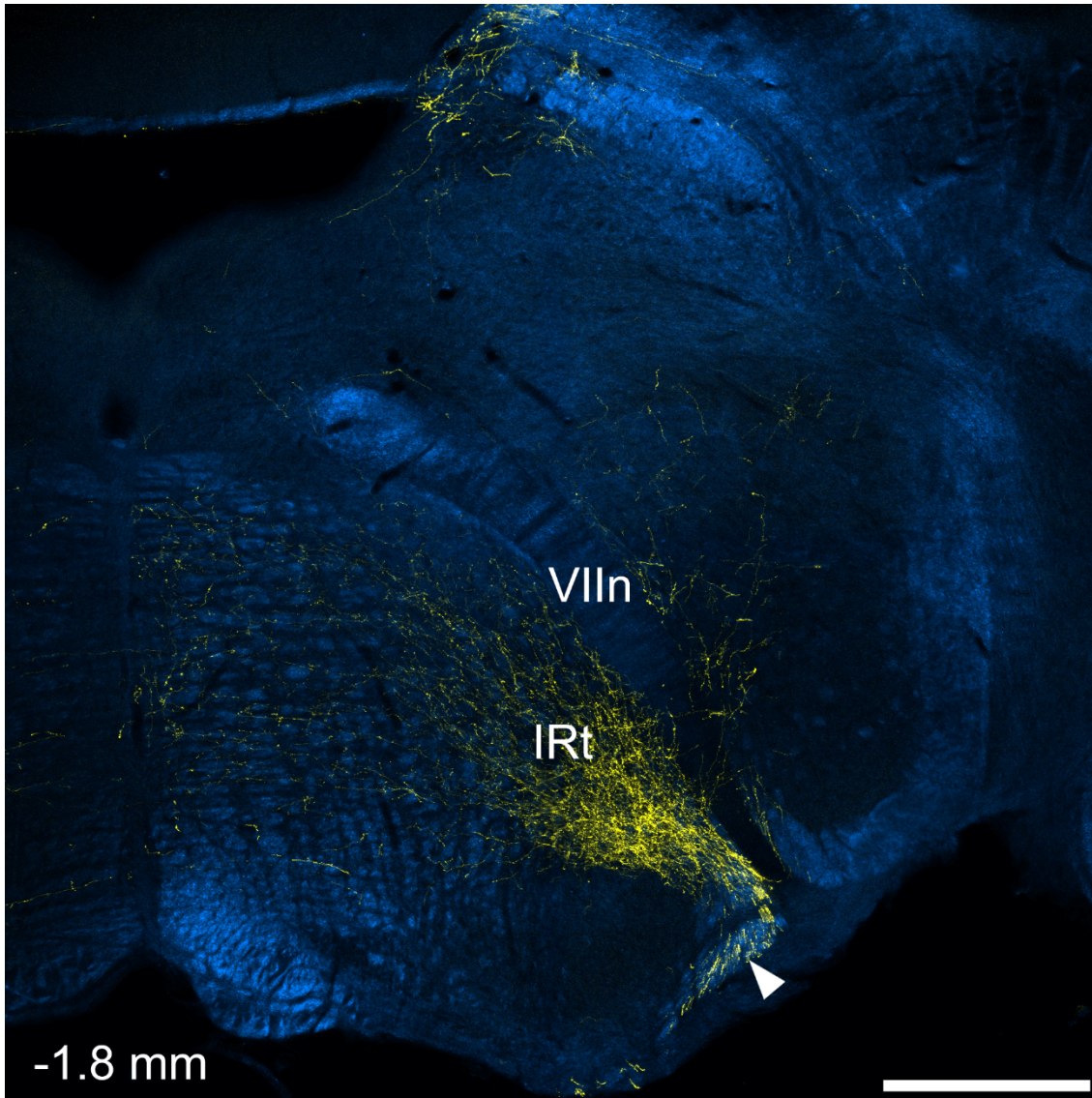
Supplementary Tables 1-2

Figure S1



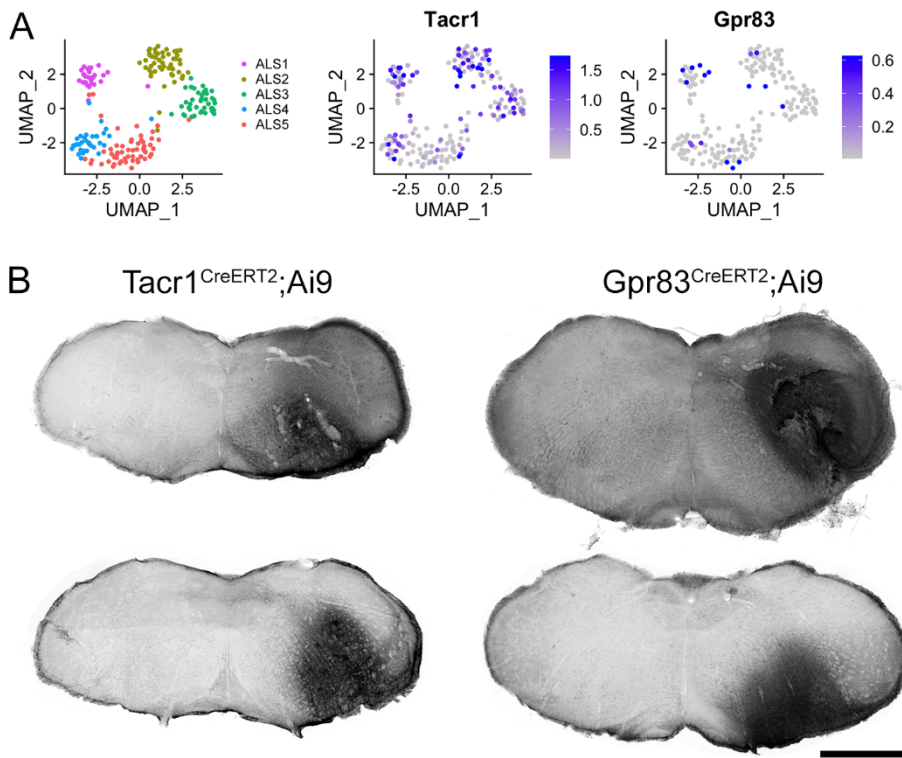
Axon collaterals in the cervical spinal cord. TdTomato labelling (yellow) on the ipsilateral side in the C7 spinal cord segment of a Sst^{Cre} mouse that had received intraspinal injection of AAV.Cre^{ON}.tdTomato into the L3, L4 and L5 segments on the right side 5 weeks previously (#3 in Table 1). NeuN immunostaining is shown in blue. Note the presence of numerous tdTomato-labelled axons and boutons in the deep dorsal horn and the area around the central canal (cc). The dashed line represents the grey/white matter border. Orientation markers: D, dorsal; V, ventral; L, lateral; M, medial. Scale bar = 200 μ m.

Figure S2



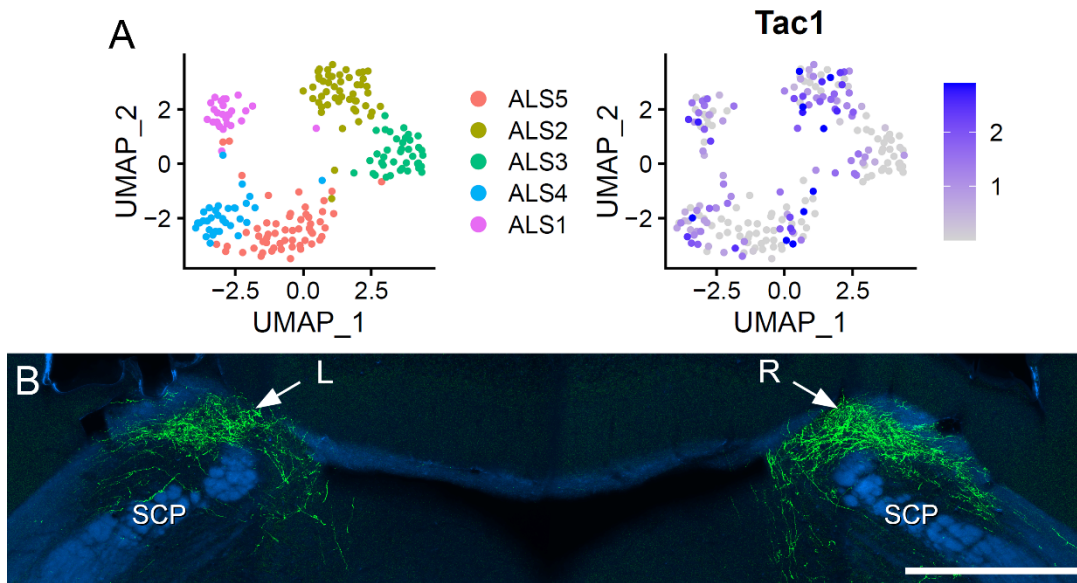
TdTomato labelling in a coronal section through the ipsilateral side of the brainstem of a Sst^{Cre} mouse that had received an injection of AAV.Cre^{ON}.tdTomato into the L3 spinal segment on the right (#8 in Table 1). The section is located at approximately 1.8 mm caudal to the interaural line. The main ascending bundle of labelled fibres on the ventral surface of the brainstem is indicated with an arrowhead. This gives rise to a dense collection of axonal branches with boutons that lies on the ventromedial aspect of the facial nerve (VIIIn) and these arborise mainly within the intermediate reticular nucleus (IRt). Scale bar = 500 μ m.

Figure S3



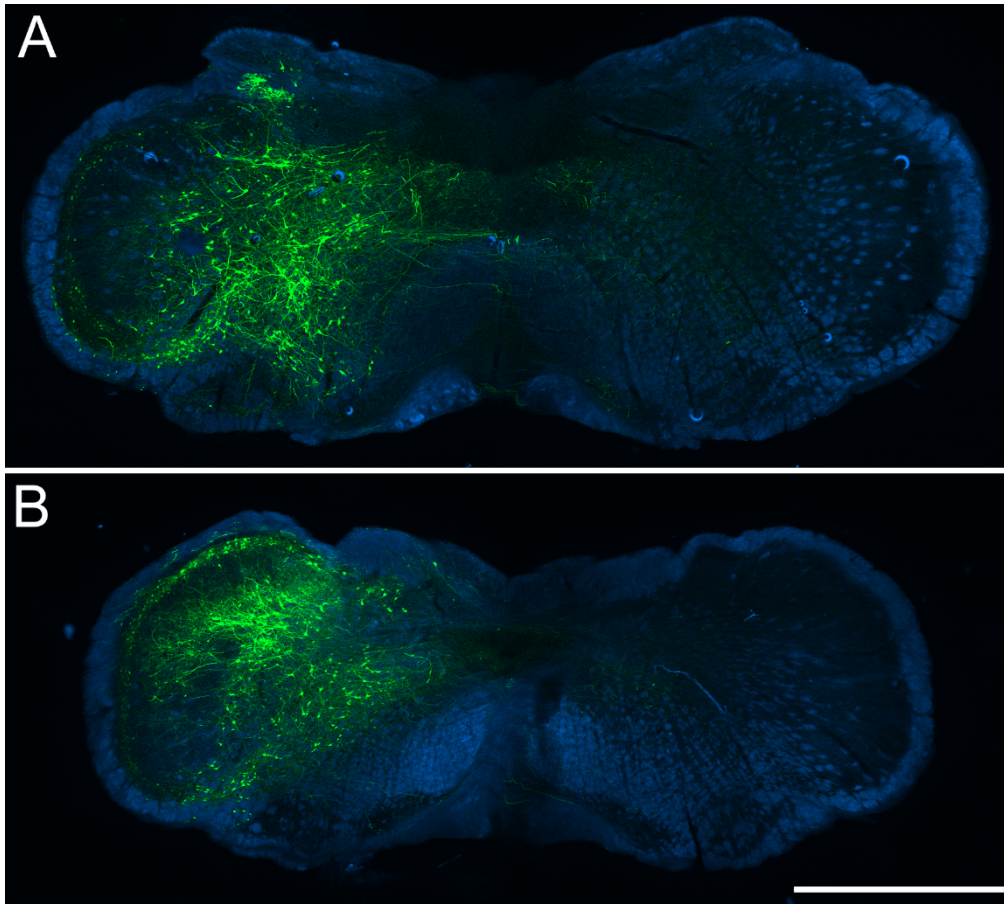
Expression of *Tacr1* and *Gpr83* by cells in ALS4. **A:** UMAP plot showing the distribution of cells in the ALS1-5 clusters, together with plots for expression of *Tacr1* and *Gpr83*. Note the presence of many *Tacr1*-positive cells, and a few *Gpr83*-positive cells, in the ALS4 cluster. **B:** CTb injection sites in the four mice that were used to analyse expression of tdTomato in projection neurons in lateral lamina V. Scale bar = 1 mm.

Figure S4



Expression of *Tac1* by cells in ALS4. **A**: UMAP plot showing the distribution of cells in the ALS1-5 clusters, together with a plot for expression of *Tac1*. Note the presence of many *Tac1*-positive cells in the ALS4 cluster. **B**: anterograde labelling following injection of AAV.Cre^{ON}.GFP into the L5 segment on the right side of a *Tac1*^{Cre} mouse. Immunolabelling for GFP (green) is superimposed on a dark-field image (blue). There is strong bilateral labelling with GFP (arrows) in the dorsalmost part of the lateral parabrachial area, corresponding to the PBil. SCP, superior cerebellar peduncle. Scale bar = 500 μ m.

Figure S5



Injection sites for retrograde tracing of Sst-expressing ALS neurons. Sections through the medulla show the expression of GFP (green), superimposed on dark-field images (blue) following injection of AAV2r.Cre^{ON}.GFP into two Sst^{Cre} mice. Note that GFP will only be expressed in Sst-expressing cells near the injection site. Scale bar = 1 mm.

Table S1 Viruses used in this study

						Details of injection	
	Construct(s)	Serotype	Promoter	Catalogue number	Source	Number of GCs	Volume
AAV.Cre ^{ON} .tdTom	tdTomato	AAV1	CAG	v167-1	VVF Zurich	9.48×10^7	300 nl
AAV2r.Cre ^{ON} .GFP	GFP	AAV2r	shortCAG	v158-retro	VVF Zurich	2.15×10^9	500 nl
AAV.Flp	Flp	AAV9	shortCAG	v453-9	VVF Zurich	1.24×10^9	400 nl
AAV.Cre ^{ON} /Flp ^{ON} .GFP	GFP	AAV1	hEF1 α	vHW33-1	VVF Zurich	$1.62\text{-}8.1 \times 10^7$	300 nl

The values in columns 7 and 8 refer to individual injections. GCs, gene copies; GFP, green fluorescent protein.

Table S2 Antibodies used in this study

Antibody	Species	Source	Catalogue #	Dilution	RRID
GFP	Chicken	Abcam	ab13970	1:1000	RRID:AB_300798
mCherry*	Rat	Invitrogen	M11217	1:1000	RRID:AB_2536611
CTb	Goat	List Biological	703	1:5000†	RRID:AB_10013220
NK1r	Rabbit	Sigma-Aldrich	S8305	1:1000	RRID:AB_261562
NeuN-Alexa647	Rabbit	Abcam	ab190565	1:1000	RRID:AB_2732785

*The mCherry antibody also recognises tdTomato

†The CTb antibody was used at 1:200,000 for immunoperoxidase staining of brain injection sites