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Supplemental Information

An Atlas of Vagal Sensory Neurons and Their Molecular Specialization

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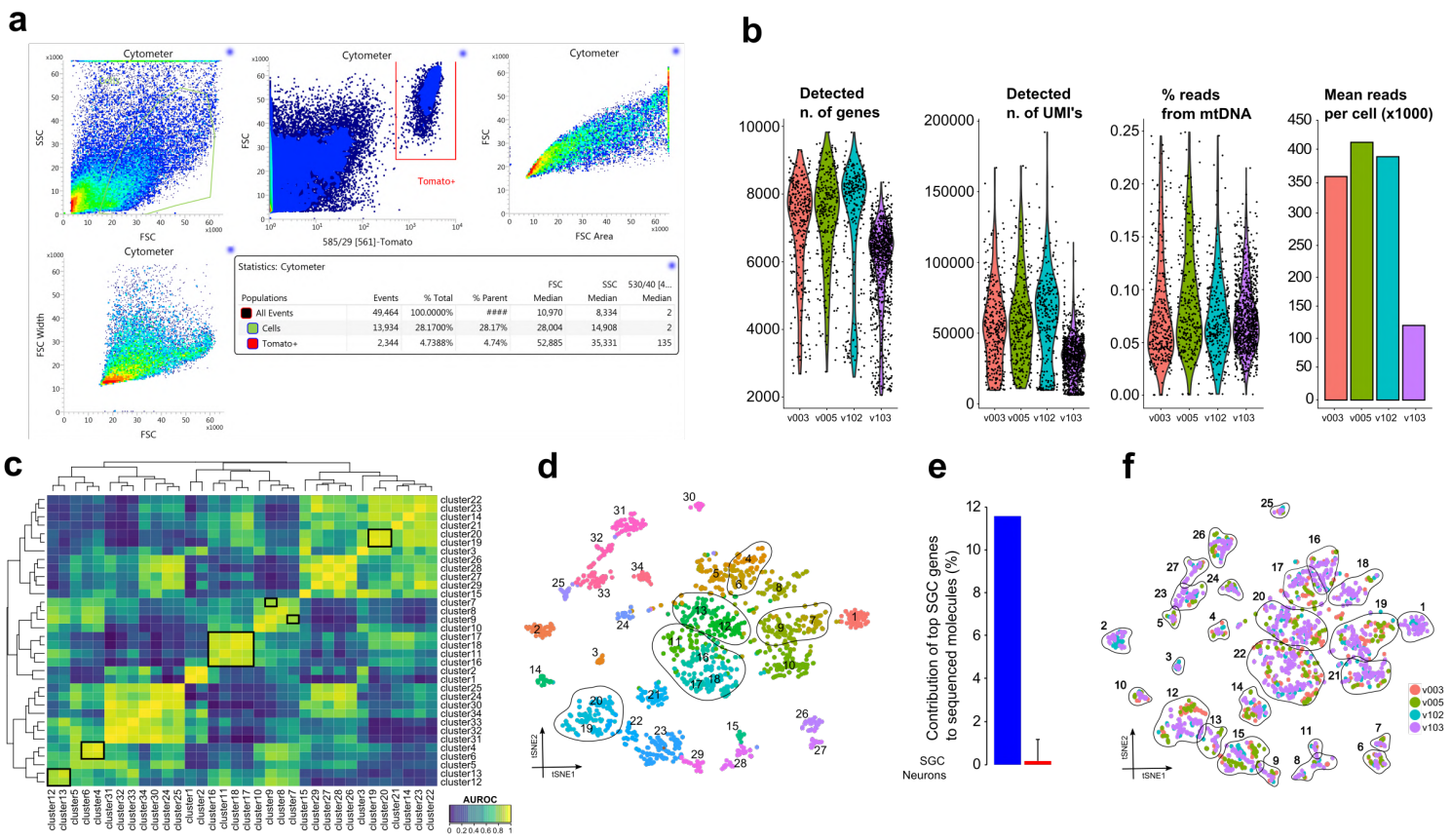


Figure S1. Sample preparation and clustering QC related information (related to Figure 1)
(a) Information from the Vglut2Cre^{Tomato} vagal cell suspension FACS. **(b)** Detected numbers of genes, UMIs, the percentage of mt-DNA derived gene expression across the different batches, and mean reads per cell. Sample v103 captured significantly more cells compared to the other three (>900 vs. ~300) resulting in lower counts of UMIs and lower sequencing depth; however the mt-DNA% remained the same. **(c)** Heatmap from Metaneighbor analysis comparing clusters from the first round of clustering (34 clusters) against each other. Highly similar types without clear distinguishing markers (indicated by rectangles) were merged together to yield the final 27 clusters. **(d)** tSNE showing the original 34 clusters; the merged clusters are indicated. **(e)** Percentage contribution of the ten most highly SGC enriched genes to total RNA molecule counts in SGCs and sensory neuron clusters (average of all neuron clusters). Error bar displays sem. **(f)** tSNE demonstrating how cells originating from different batches distribute among the final clusters. Each sensory neuron cluster (4-27) was formed from cells derived from all biological replicates. Abbreviations: Endo, endothelial cells; SGC, satellite glial cells; Symp, sympathetic neurons.

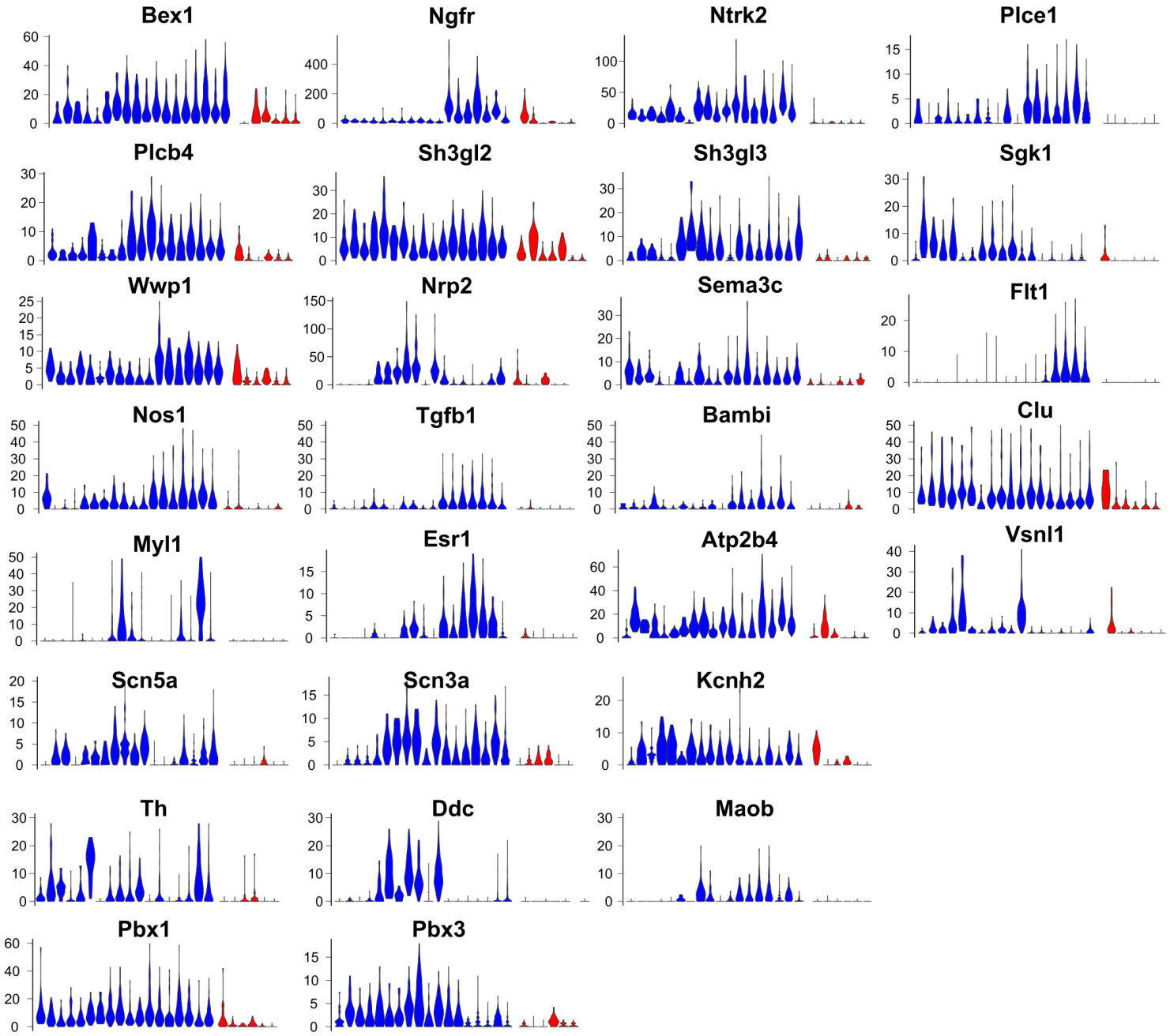


Figure S2. Nodose enriched genes from STRING-analysis (related to Figure 2)
Violin plots for the expression of genes that showed protein-protein interactions in nodose ganglion sensory neurons. Blue and red color indicate nodose and jugular clusters, respectively. Y-axis indicates the number of raw UMI counts.

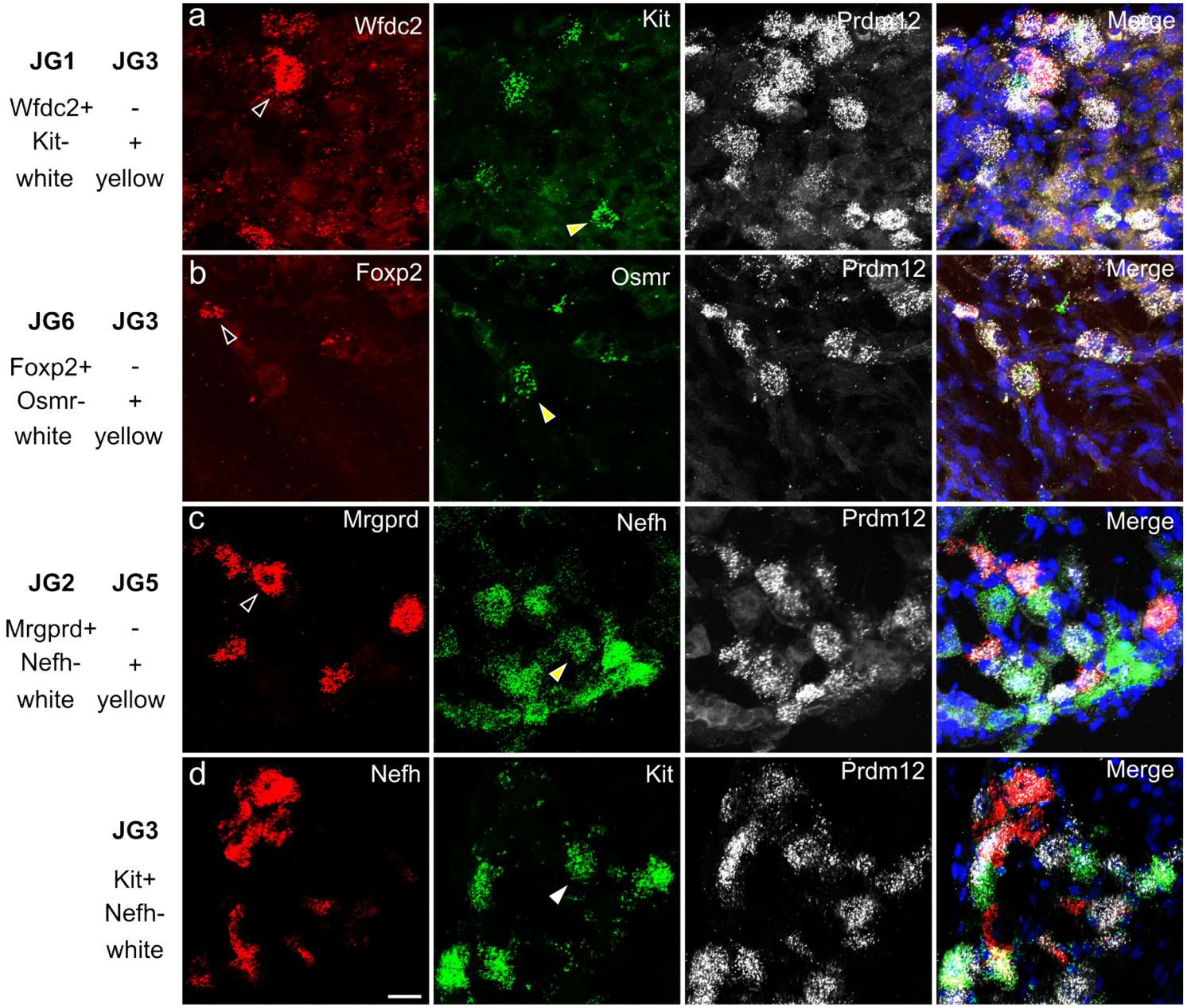
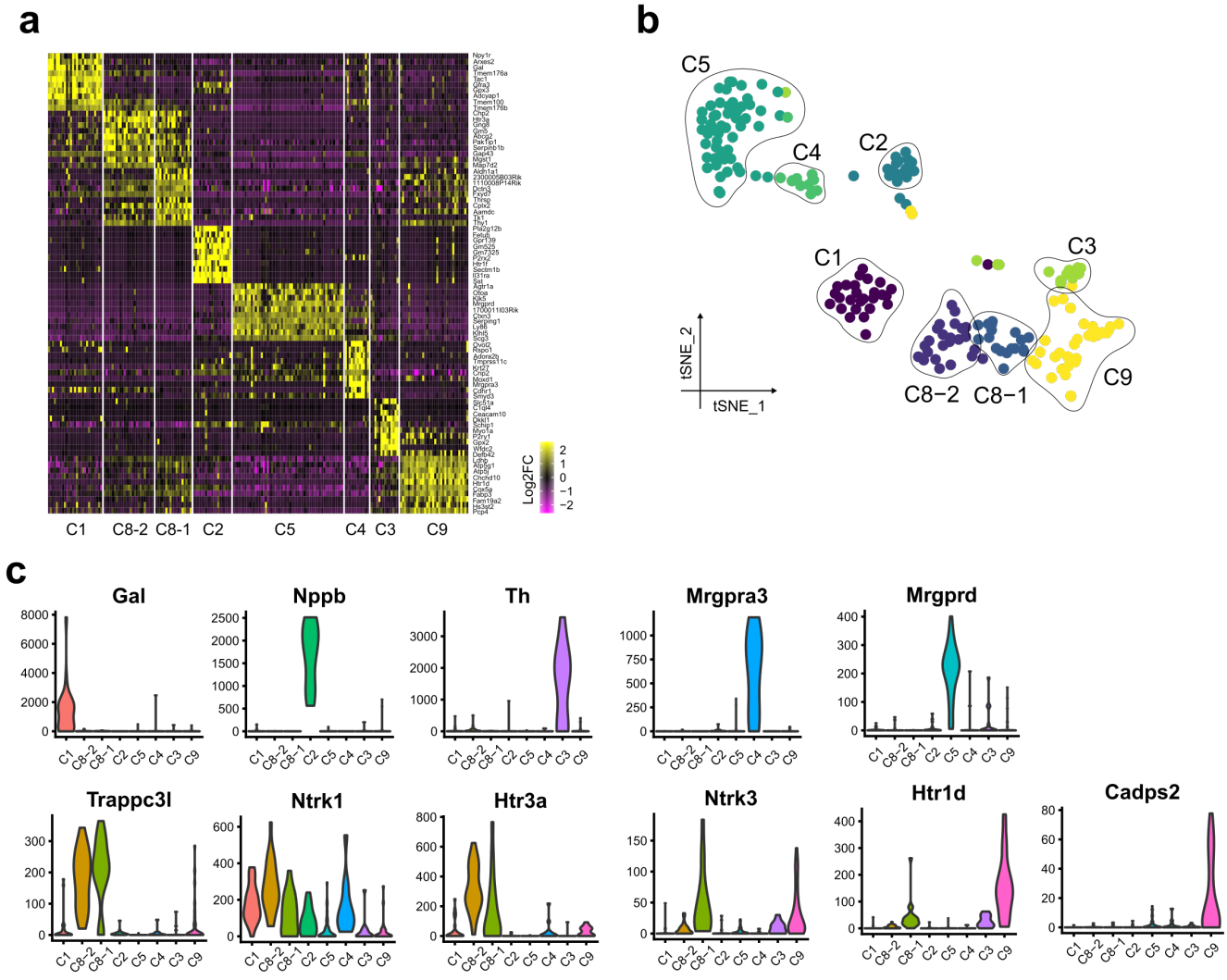


Figure S3. Jugular marker expression *in vivo* (related to Figure 3)
(a-d) Low magnification images from *in vivo* confirmation of jugular neuron types; the cells shown in the main figure are highlighted with arrowheads. Legends of the left indicate presence/absence of gene-expression ($^{+}$/-); white and yellow indicate the color of the arrowheads. Panel is read left to right. Scale bar represents 10 μm.



d

	NF1	NF2/3	NF4	PEP2	TH	PEP1	NP1	NP2	NP3	TRPM8
C9	0.955	0.924	0.953	0.759	0.789	0.312	0.190	0.204	0.277	0.714
C8-1	0.871	0.920	0.913	0.913	0.613	0.495	0.195	0.238	0.339	0.576
C3	0.709	0.588	0.653	0.319	0.916	0.097	0.587	0.344	0.321	0.562
C8-2	0.696	0.850	0.839	0.930	0.331	0.767	0.126	0.256	0.433	0.617
C1	0.184	0.382	0.427	0.641	0.039	0.967	0.345	0.460	0.728	0.712
C4	0.180	0.161	0.135	0.395	0.293	0.578	0.742	0.860	0.858	0.228
C5	0.159	0.162	0.147	0.157	0.633	0.151	0.979	0.774	0.787	0.171
C2	0.079	0.087	0.163	0.316	0.137	0.618	0.694	0.887	0.997	0.370

e

	JG1	JG2	JG3	JG4	JG5	JG6
C3	0.933	0.629	0.262	0.158	0.527	0.761
C9	0.853	0.253	0.200	0.449	0.873	0.851
C8-1	0.717	0.107	0.230	0.651	0.953	0.696
C5	0.585	0.997	0.715	0.065	0.158	0.234
C8-2	0.421	0.046	0.316	0.837	0.915	0.601
C4	0.231	0.757	0.877	0.504	0.275	0.086
C2	0.088	0.703	0.985	0.512	0.155	0.164
C1	0.055	0.192	0.569	0.977	0.563	0.596

f

	NG1	NG2	NG3	NG4	NG5	NG6	NG7	NG8	NG9	NG10	NG11	NG12	NG13	NG14	NG15	NG16	NG17	NG18
C9	0.953	0.969	0.862	0.854	0.863	0.772	0.800	0.716	0.847	0.595	0.882	0.230	0.225	0.172	0.196	0.152	0.404	0.444
C8-1	0.912	0.880	0.945	0.875	0.854	0.698	0.661	0.436	0.727	0.437	0.763	0.402	0.334	0.269	0.319	0.250	0.524	0.559
C8-2	0.809	0.779	0.750	0.660	0.672	0.533	0.563	0.280	0.607	0.207	0.640	0.446	0.451	0.448	0.578	0.366	0.473	0.547
C3	0.802	0.805	0.775	0.735	0.724	0.649	0.564	0.657	0.699	0.761	0.693	0.374	0.302	0.361	0.244	0.326	0.549	0.635
C5	0.524	0.401	0.754	0.522	0.383	0.250	0.162	0.404	0.291	0.773	0.186	0.764	0.171	0.703	0.192	0.718	0.895	0.943
C2	0.293	0.183	0.497	0.180	0.196	0.087	0.287	0.225	0.171	0.328	0.143	0.717	0.597	0.906	0.633	0.793	0.607	0.722
C4	0.271	0.115	0.505	0.387	0.251	0.272	0.164	0.387	0.164	0.568	0.144	0.780	0.586	0.784	0.534	0.764	0.809	0.809
C1	0.269	0.209	0.433	0.141	0.239	0.237	0.412	0.180	0.228	0.203	0.330	0.666	0.557	0.897	0.775	0.707	0.433	0.528

Figure S4. Comparisons between DRG and vagal neuron types (related to figures 4 and 6)
(a) Heatmap showing the ten most specific marker genes (by lowest p-adj) for each DRG cluster after re-clustering Li et al. (2016) data using Seurat. We examined our clusters using the cluster-defining markers from the original publication by Li et al., (2016) allowing us to identify the corresponding clusters in that study. Thus, the cluster names are according to Li et al., (2016). Clusters C6, C7, and C10 did not separate as individual clusters in our analysis. **(b)** A tSNE of the clusters. **(c)** Violin plots showing examples of defining markers (from Li et al. 2016). Note the relation of marker expression and cluster name. Units shown are FPKM. **(d-f)** Heatmaps showing mean AUROC scores from Metaneighbor comparisons between the Li et al., (2016) dataset and **(d)** DRG neurons from Zeisel et al (2018), **(e)** jugular, and **(f)** nodose neurons.

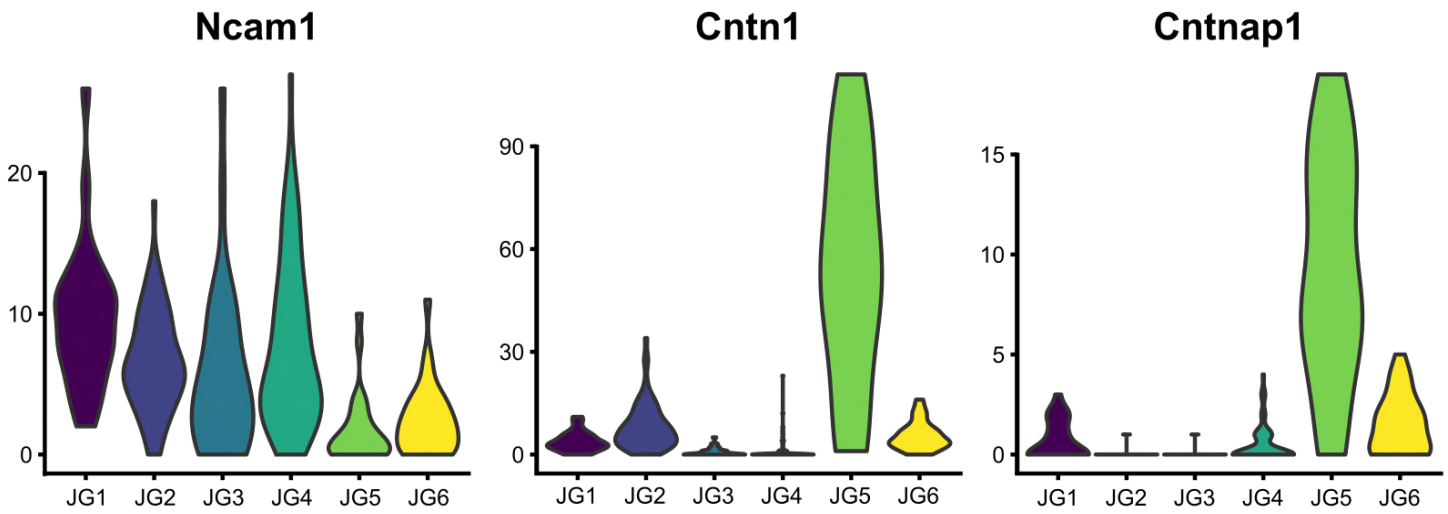


Figure S5. Expression of myelination related markers in the jugular ganglion clusters (related to Figure 4)
Violins depicting expression of indicated genes in the jugular ganglion neuron types, demonstrating that JG5 is myelinated and that JG6 could be lightly myelinated, consistent with their relation to DRG neurons obtained by the Metaneighbor analysis. Y-axis indicates raw UMI counts.

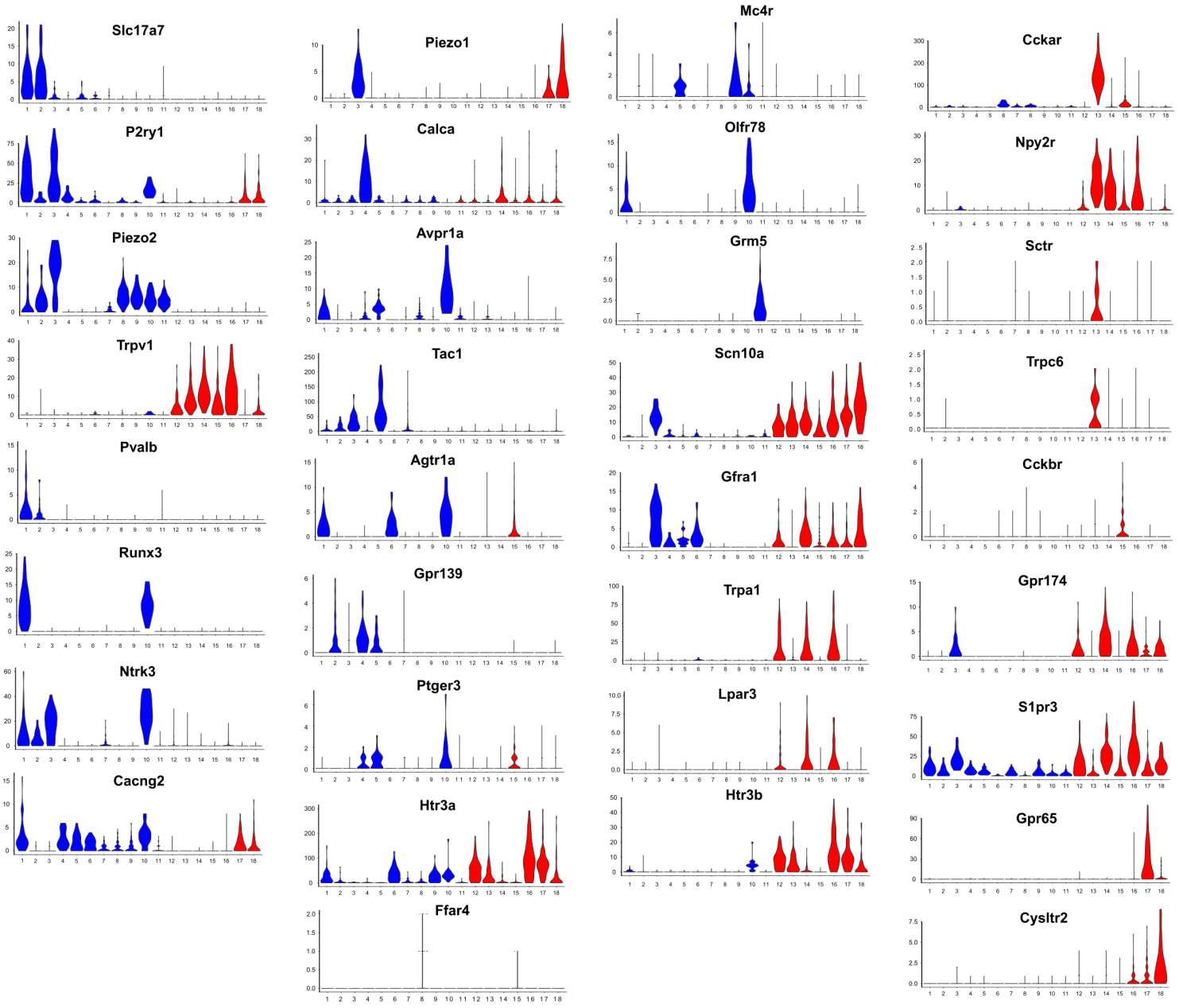
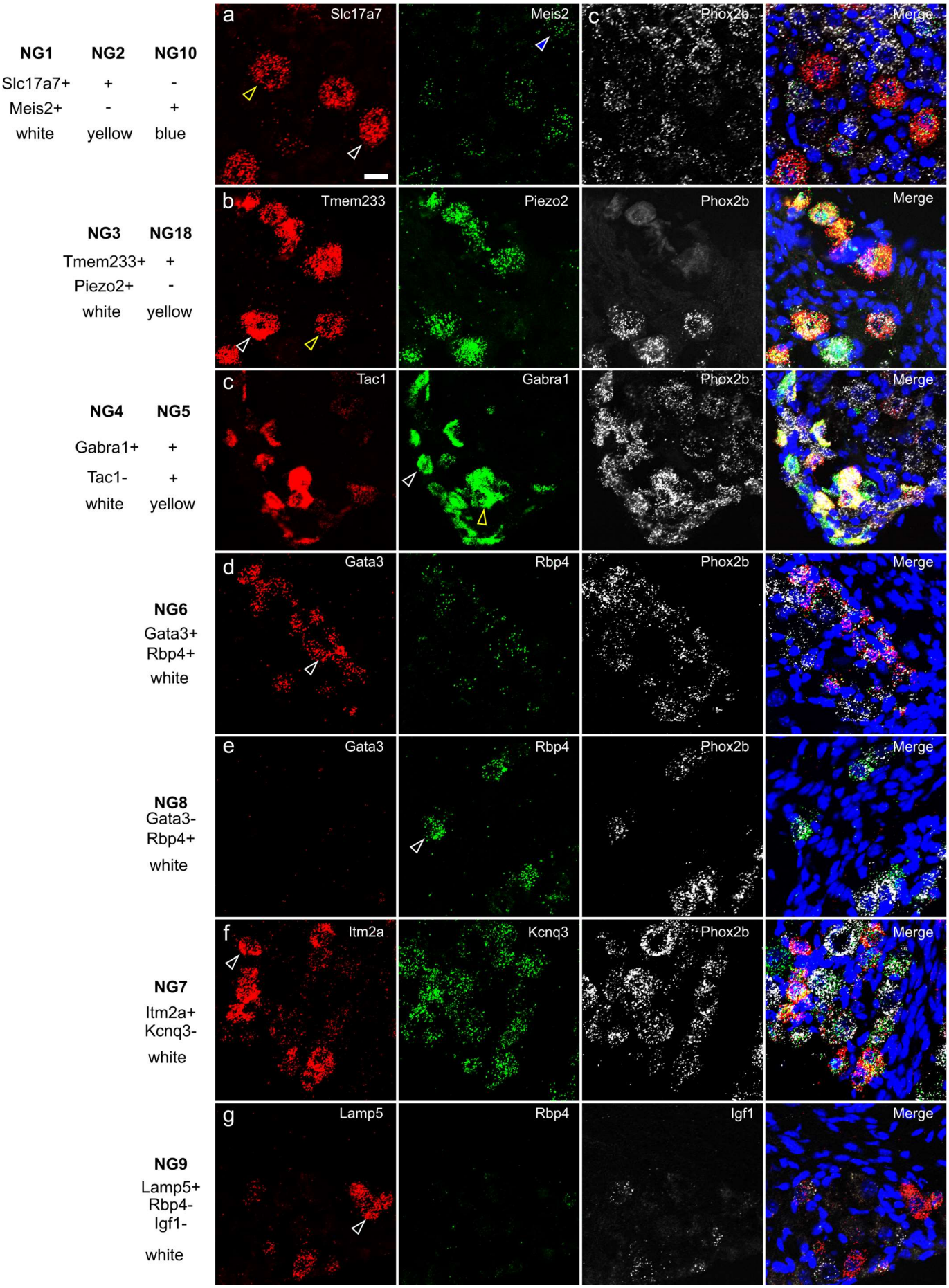
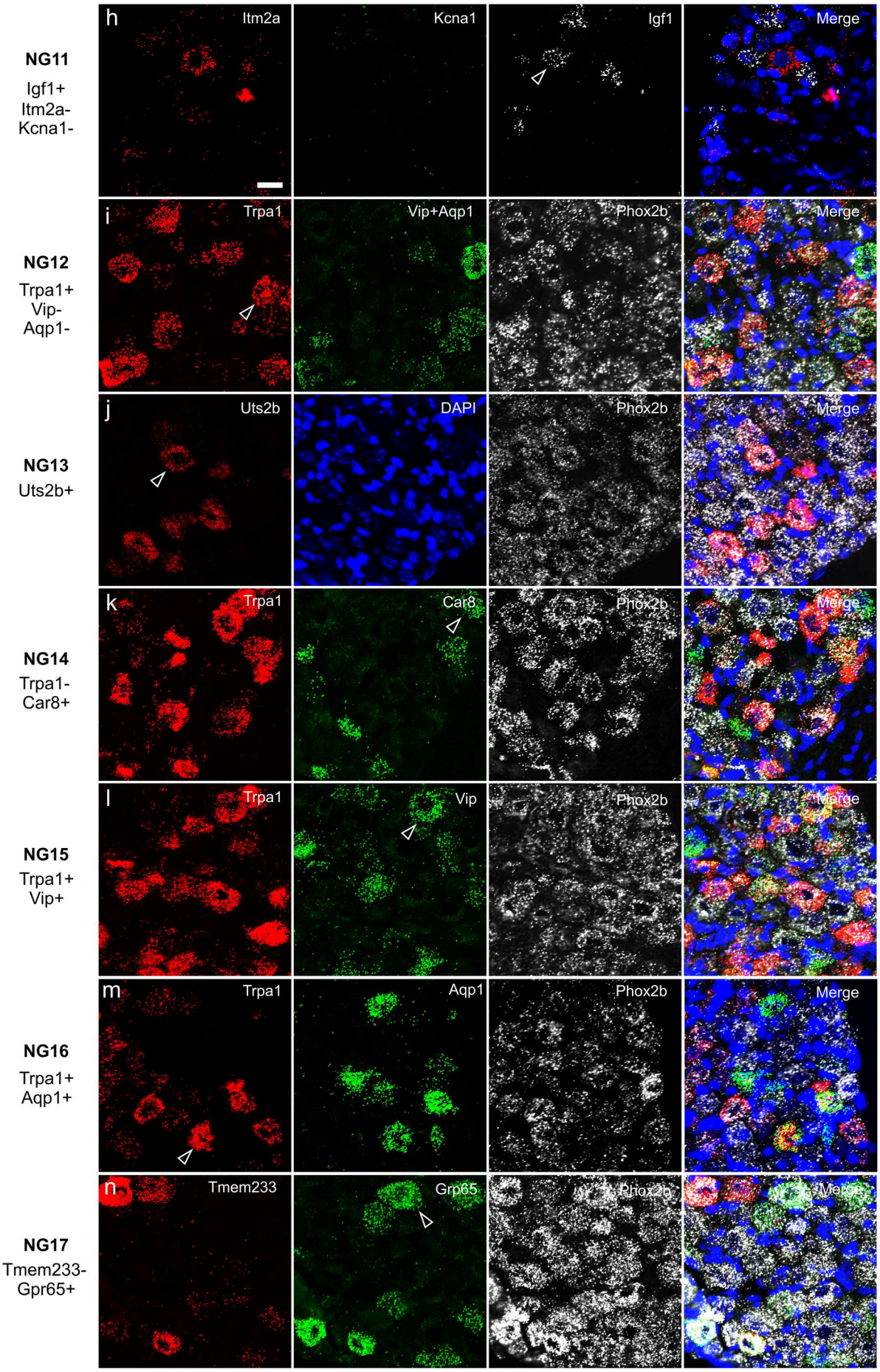


Figure S6. Expression of selected genes across the nodose clusters (related to Figure 7)
Violin plots showing the expression of genes shown in Figure 7 across nodose clusters. Y-axis indicates detected raw UMI counts. Blue and red color distinguish NG1-11 and NG12-18 clusters, respectively.



Data S1. Nodose marker expression *in vivo* (related to Figure 5). *Data S1 continues on the next page.*



Data S1. Nodose marker expression *in vivo* (related to Figure 5)
Low magnification images from *in vivo* confirmation of nodose neuron types (a-n). Legends on the left indicate presence/absence of gene-expression ($^{+}$/-) in the neuron types; white, yellow, and blue distinguish the color of the arrowheads that identify examples of the specific neuron types in the images. Panel is read left to right. Scale bar indicates 10 μm.