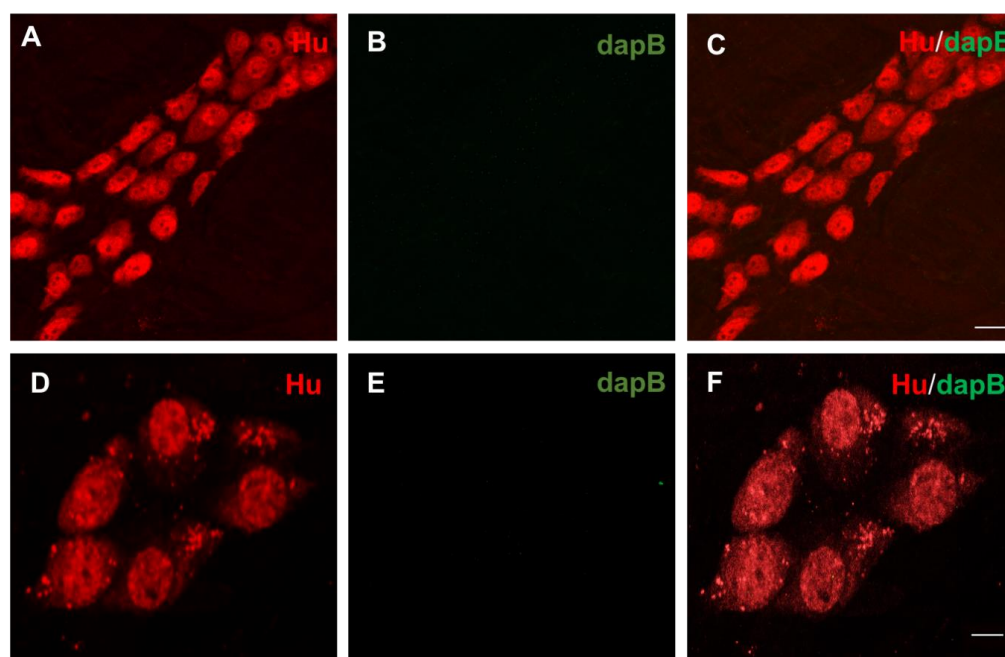


# Supplementary Materials

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**Figure S1. Negative control experiments for the myenteric plexus and the submucosal plexus.** Myenteric neurons labelled with: **(A)** Hu pan neuronal marker and **(B)** the ACDBio RNAscope universal negative control probe (dapB) do not show detectable labelling. **C:** Merge. Submucosal neurons labelled with **(D)** Hu **(E)** dapB; **F:** merge. Scale bar=50μm.

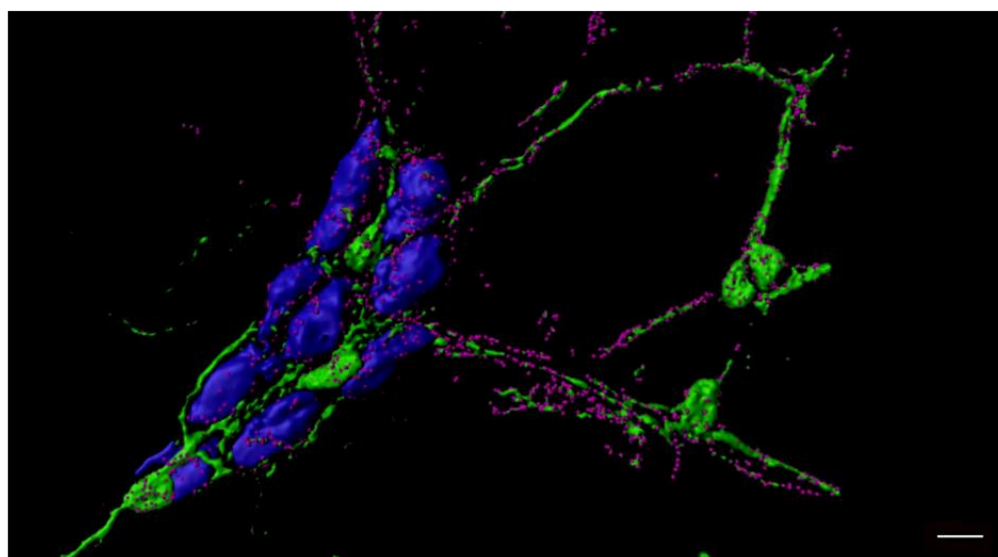
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**Figure S2. *Nlgn3* mRNA expression in submucosal neurons, glia and glial fibers**

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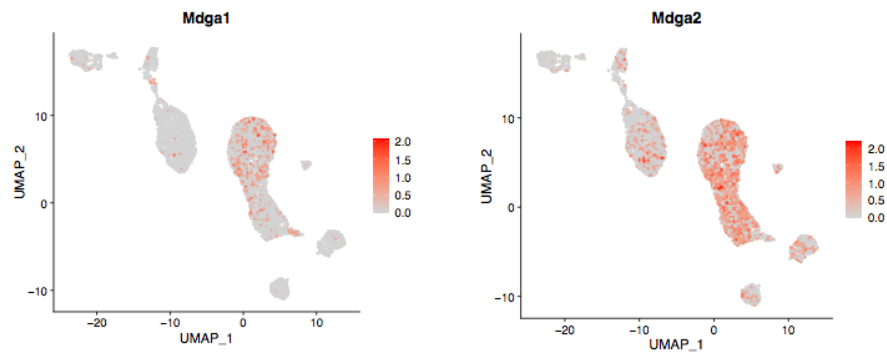
3D reconstructed image of a mouse ileal submucosal ganglion showing *Nlgn3* mRNA expression in glial fibers. Blue: Hu pan neuronal marker, green: S100 glial marker, magenta: *Nlgn3* mRNA, scale bar=50μm.

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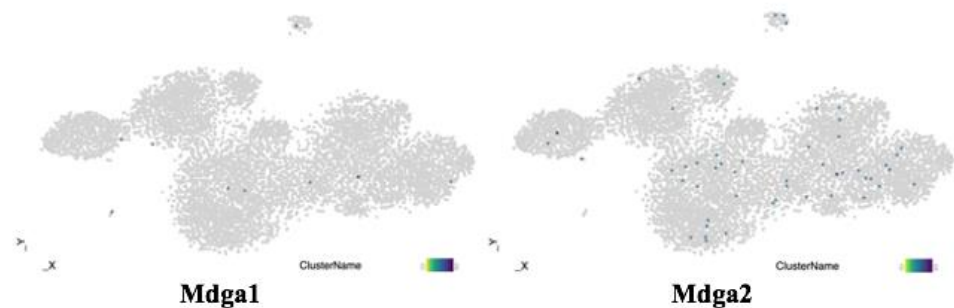
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**Figure S3. Expression of NLGN3 potential binding partners MDGA1 and 2 in mouse myenteric neurons.** *Mdga1* was present in fewer than 50% of cells in ENC1 and 2 (excitatory motor neurons). *Mdga2* was expressed in a significant proportion of cells in ENC clusters 1-4 (excitatory motor neurons) and in a lower number of neurons belonging to clusters ENC8,9 (inhibitory motoneurons) and ENC12 (likely an heterogeneous cell group). Scale bar refers to maximum and minimum expression levels within ENCs (refer to Figure 4 for legend).



**Figure S4. Expression of NLGN3 potential binding partners MDGA1 and 2 in mouse enteric glia.** Scale bar refers to maximum and minimum expression levels within ENTGs and ENMFB (refer to Figure 5 for legend).