Stem Cell Reports, Volume *18*

Supplemental Information

Neuro-mesodermal assembloids recapitulate aspects of peripheral nervous system development *in vitro*

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Supplemental Figures

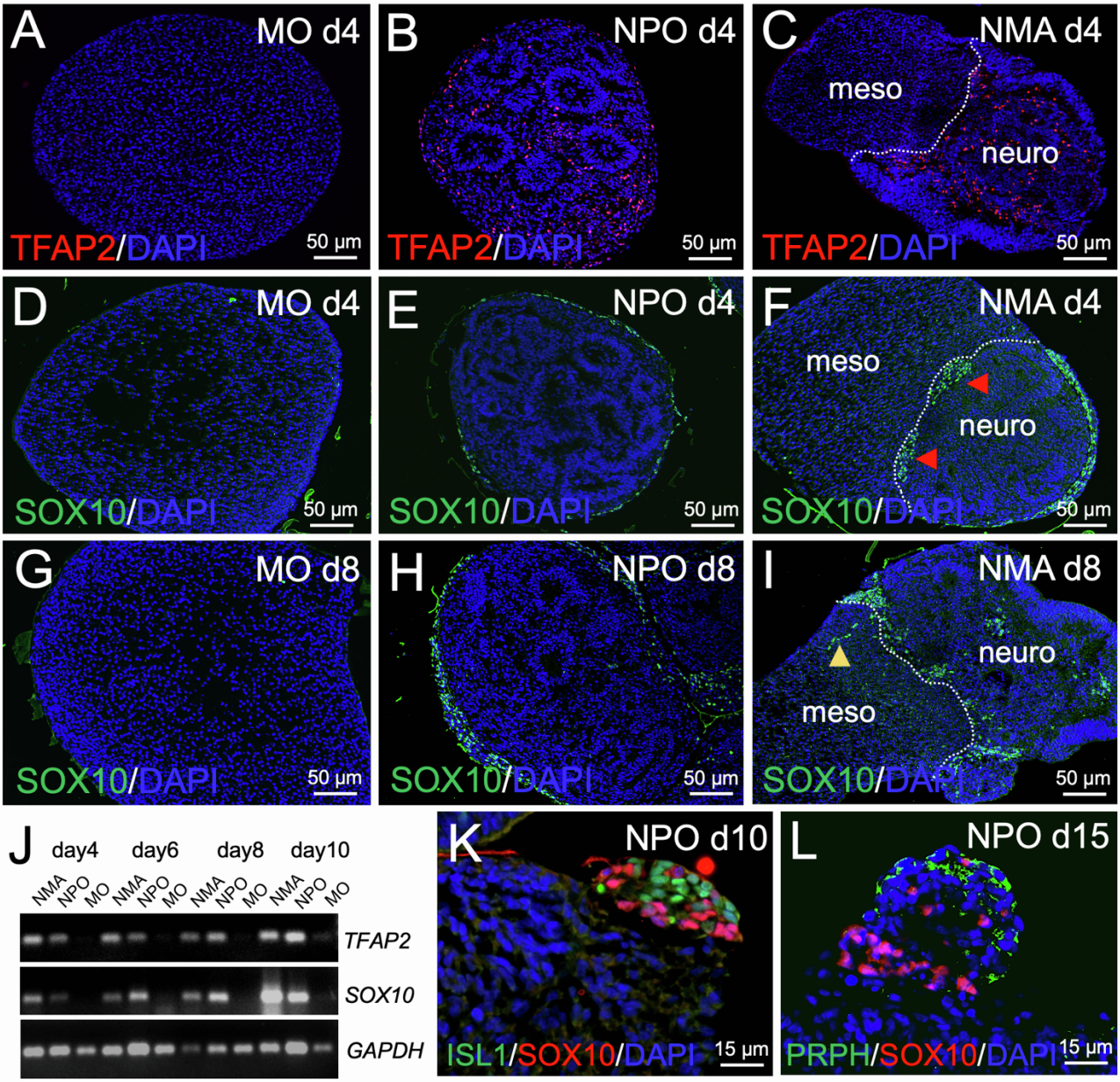

Figure S1: Neural Crest cells (NCCs) arise in neural organoids and neuro-mesodermal assembloids but not in mesodermal organoids

A-C: TFAP2^{+} cells are detectable *via* immunofluorescence microscopy in neural plate organoids (NPO) and neuro-mesodermal assembloids (NMA) after 4 days in co-culture but not in mesodermal organoids (MO) on the corresponding culture day. **D-F:** Sox10^{+} cells are detectable *via* immunofluorescence microscopy in NPOs and NMAs after 4 days in co-culture but not in MOs on the corresponding culture day. The red arrowheads in F point towards NCC clusters at the neuro-mesodermal interface which will probably give rise to sensory ganglia. **G-I:** At culture day 8, SOX10^{+} cells were found covering the complete surface of NPOs. In contrast, they were mostly recruited to the neuro-mesodermal interface in NMAs. MOs remained SOX10^{-} also at later time points in culture. The yellow arrowhead in I points towards SOX10^{+} NCCs migrating into the mesodermal part of the assembloid. **J:** Reverse transcription PCR (RT-PCR) was performed on mRNA samples collected from MOs, NPOs and NMAs at co-culture days 4, 6, 8 and 10 confirming the observations depicted in A-I. Specific primer pairs for the detection of *TFAP2* and *SOX10* were used. Detection of *GAPDH* cDNA served as loading control. **K:** NPOs at day 10 of differentiation show clearly defined clusters of cells at specific sites on the NPOs surface. These clusters consist of SOX10^{+} cells located towards the NPO and ISL1^{+} cells located towards the outside. **L:** At day 15 of differentiation, ISL1^{+} cells become partially PRPH^{+} indicating PNS neuron differentiation at defined sites within NPOs.

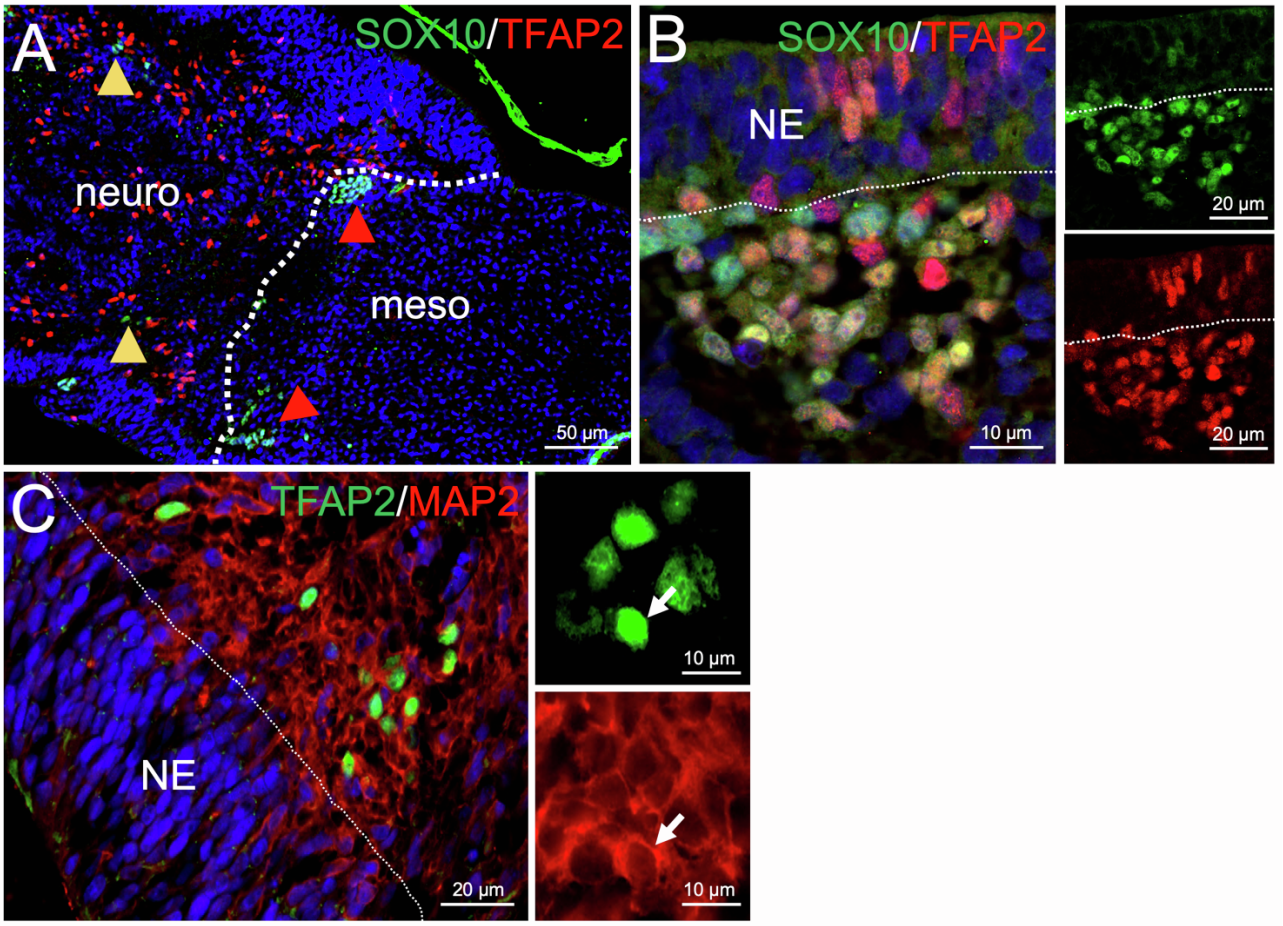

Figure S2: Immunophenotype of TFAP2⁺ cells within neuro-mesodermal assembloids.

A: TFAP2⁺ cells arise at different locations within the neural part of the organoid. Some of these cells turn TFAP2⁺/SOX10⁺ (yellow arrowhead) and start to migrate towards the neuro-mesodermal interface where they form distinct cell clusters which will give rise to sensory ganglia (red arrowhead). **B-C:** We were able to distinguish 3 different TFAP2⁺ cell types. In **B,** TFAP2⁺/SOX10⁺ cells and TFAP2⁺/SOX10⁻ cells are depicted. The TFAP2⁺/SOX10⁻ cells are located within a part of the tissue with neuroepithelial morphology (NE), while the SOX10⁺/TFAP⁺ cells are in a part of the assembloid where cells are more loosely arranged. The border between the tissue types is highlighted by the white dotted line. We conclude that TFAP2⁺/SOX10⁻ cells are neural crest precursors, while TFAP2⁺/SOX10⁺cells represent migratory neural crest cells. **C:** Besides neural crest precursors and neural crest cells a third TFAP2⁺ cell population can be detected. These cells are TFAP2⁺/MAP2⁺ and do not show SOX10 or Peripherin expression. We assume, that these cells are developing CNS neurons. The white arrow points towards a single MAP⁺/TFAP2⁺ neuron. The white dotted line marks the border between neuroepithelium (NE) and differentiated neuronal cells derived thereof.

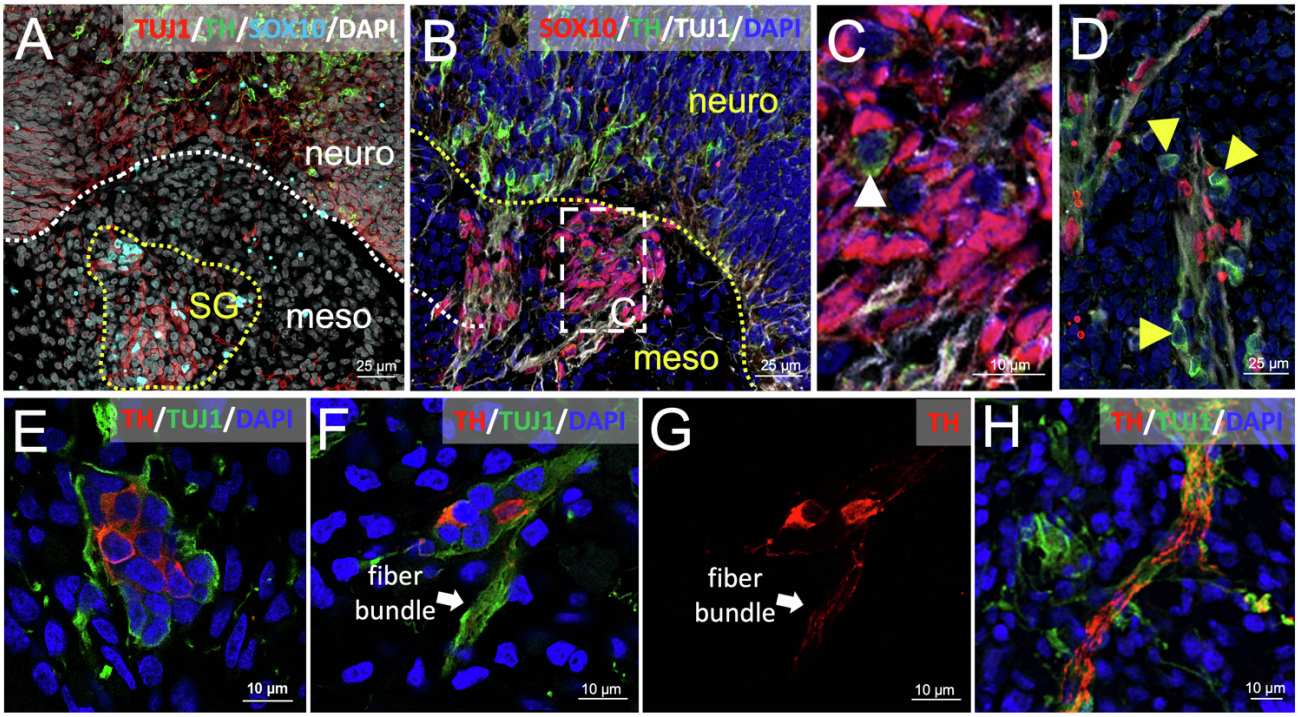

Figure S3: Tyrosine hydroxylase (TH) expression in peripheral ganglia

A: Depiction of a sensory ganglion (SG) at the neuro-mesodermal interface. TH positive neurons were detected in the neural part of the assembloid but not in the SG. The sensory ganglion was identified by the presence of $SOX10^+$ cells and its localization in the mesenchymal tissue. **B-C:** In few cases, TH^+ neurons were also found in $SOX10^+$ sensory peripheral ganglia close to the neural part of the assembloid. C shows a higher magnification of B (white dotted square). The white arrowhead in C points towards a TH^+ neuron. **D-E:** Although, rare TH^+ neurons are also present in sensory ganglia, most TH expressing neurons are found in smaller ganglion-like structures more distant from the neural part of the organoid, probably representing sympathetic ganglia. Like in the *in vivo*-situation, not all neurons in sympathetic ganglia express TH. **F-H:** TH^+ fiber bundles were detected originating from TH^+ ganglion-like cell clusters and projecting into the mesenchymal tissue.

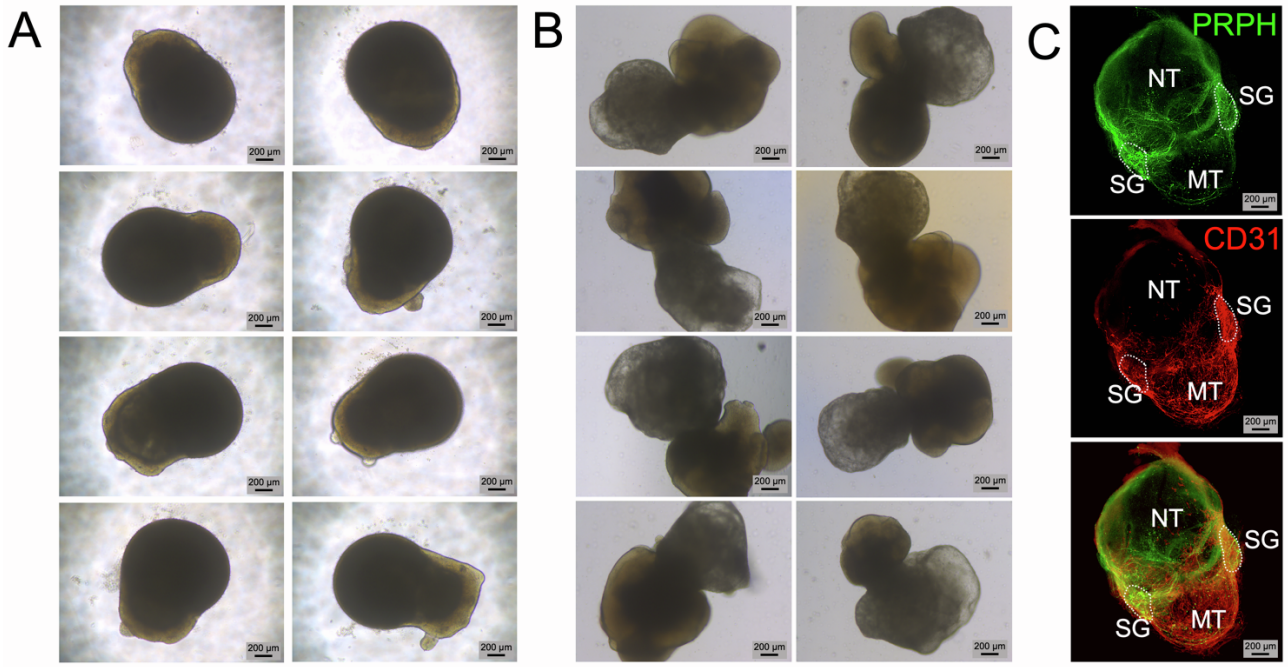

Figure S4: Reproducibility of the experimental procedure

A: 8 individual representative organoids at day 7 in co-culture are depicted. **B:** 8 representative neuro-mesodermal organoids at day 14 in co-culture are depicted. **C:** Whole mount immunofluorescence analyses using specific antibodies targeted against CD31 and Peripherin (PRPH) were performed. Images show a 3D reconstruction of confocal microscopic images from a representative tissue cleared organoid (SG: sensory ganglia, NT: neural tissue, MT: mesodermal tissue).

Supplemental Movies

Movie S1: Large sensory ganglion at the neuro-mesodermal interface

The movie shows a large sensory ganglion at the neuro-mesodermal interface. Peripheral neurons (green) were detected using specific Peripherin antibody. Neural crest cells and derivates were detected using a SOX10 antibody (red). Nuclei are stained with DAPI. The movie shows a 3D reconstruction from a z-stack of confocal images taken by laser scanning microscopy.

Movie S2: Blood vessels and peripheral nerves in the mesodermal part of the assembloid

The movie shows a hierarchically organized network of blood vessels (red) and peripheral nerve fiber bundles (green) in the mesodermal part of the assembloid. Peripheral neurons were detected using specific Peripherin antibody. Blood vessels were detected using a CD31 antibody. The movie shows a 3D reconstruction from a z-stack of confocal images taken by laser scanning microscopy.

Movie S3: Sensory ganglion cells show increased calcium levels in response to capsaicin treatment

The movie shows a calcium imaging experiment (left side). The intracellular calcium levels were visualized using Fluo4-AM. Calcium levels rise in response to capsaicin treatment. Video plays at 8x speed. After calcium imaging, the assembloid was fixed and whole mount stained using specific antibodies targeted against Peripherin (green) and SOX10 (red) to visualize the position of the peripheral ganglion (right side).