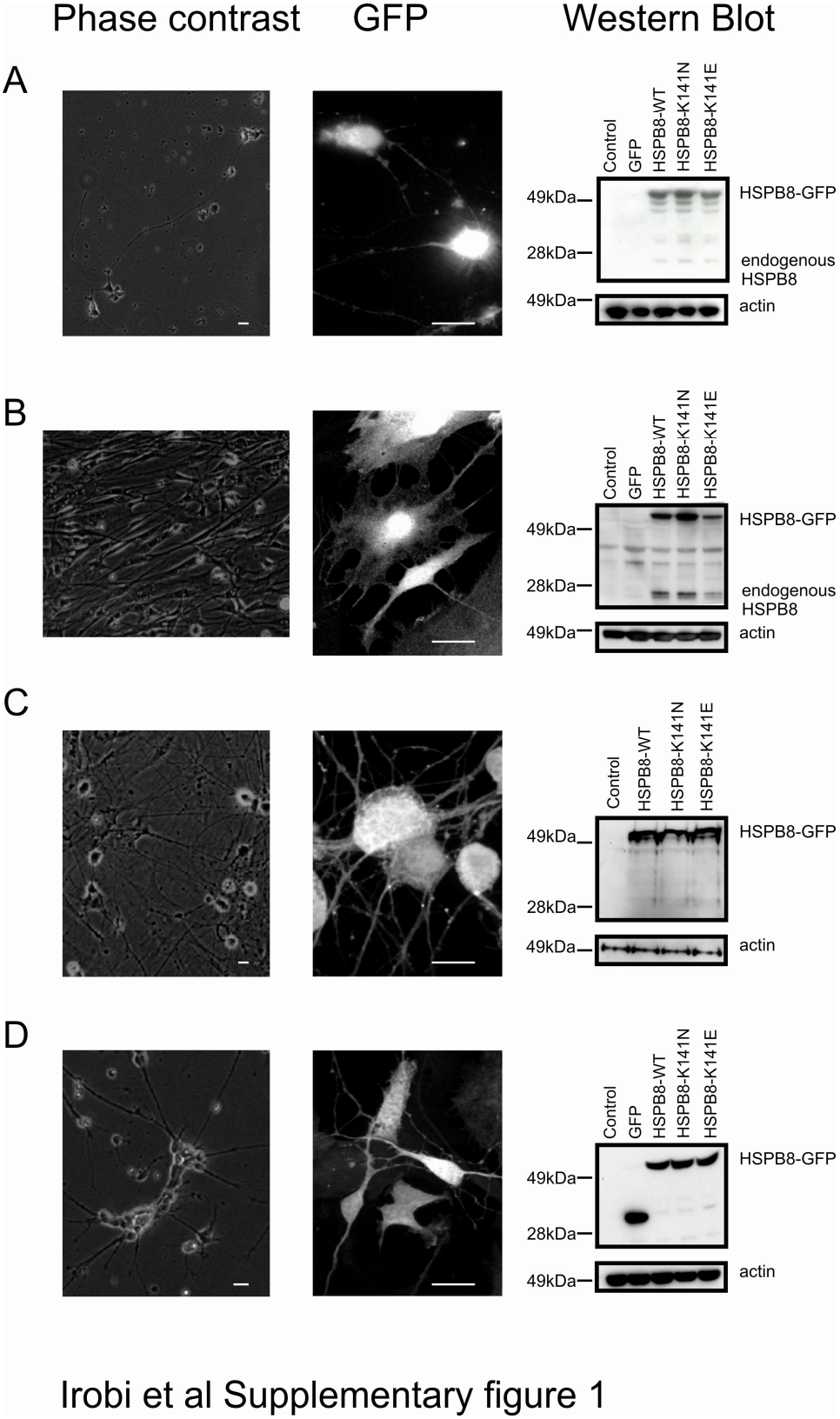
Supplementary figure 1: Isolation and transduction of primary motor neurons, glial cells, sensory and cortical neurons.

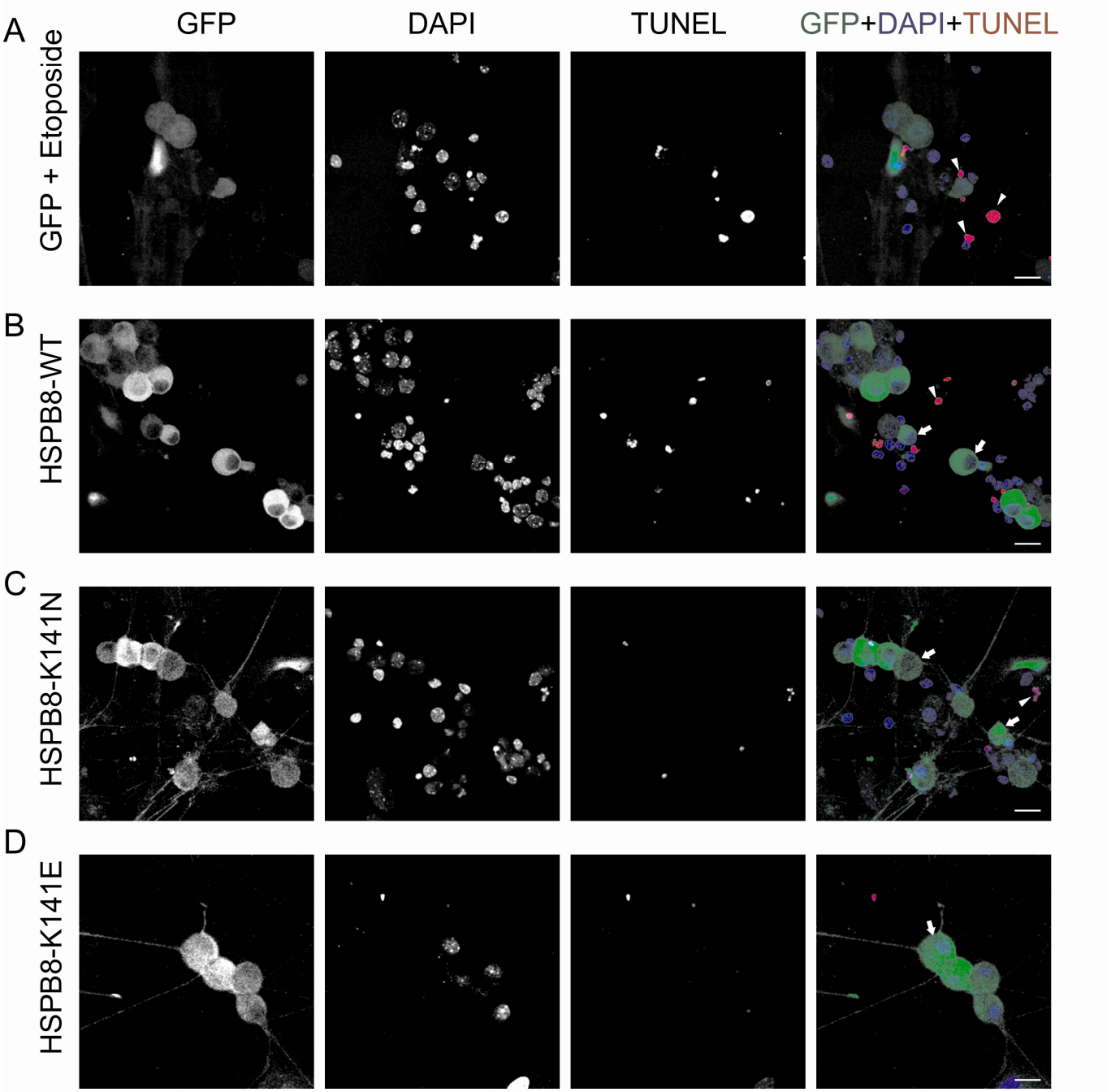
Motor neurons (**A**) and glial cells (**B**) were isolated from Wistar rat E14 embryos; sensory neurons (**C**) and cortical neurons (**D**) were isolated from E13 and E15 mouse embryos respectively. Cells were transduced with pLenti-GFP, pLenti-WT-HSPB8-GFP or mutant pLenti-K141N/K141E-HSPB8-GFP constructs at DIV3. For each cell type, a representative phase contrast morphology image of the culture at DIV3 and expression of HSPB8-WT by GFP fluorescence at DIV7 is shown. All cell types could be transduced with efficiencies higher than 70%. The Western blots shows similar expression levels in wild type versus mutant HSPB8 protein soluble lysates in the different cell types. Since HSPB8 monoclonal antibody (Abcam, 2H5, cat. no. ab15896) was no longer commercially available, we used GFP antibody JL8 in **D**. Normalization was performed with ß-actin. Scale bar = 10µm.



Irobi et al Supplementary figure 1

Supplementary Figure 2: Analysis of apoptosis in sensory neurons expressing mutant HSPB8.

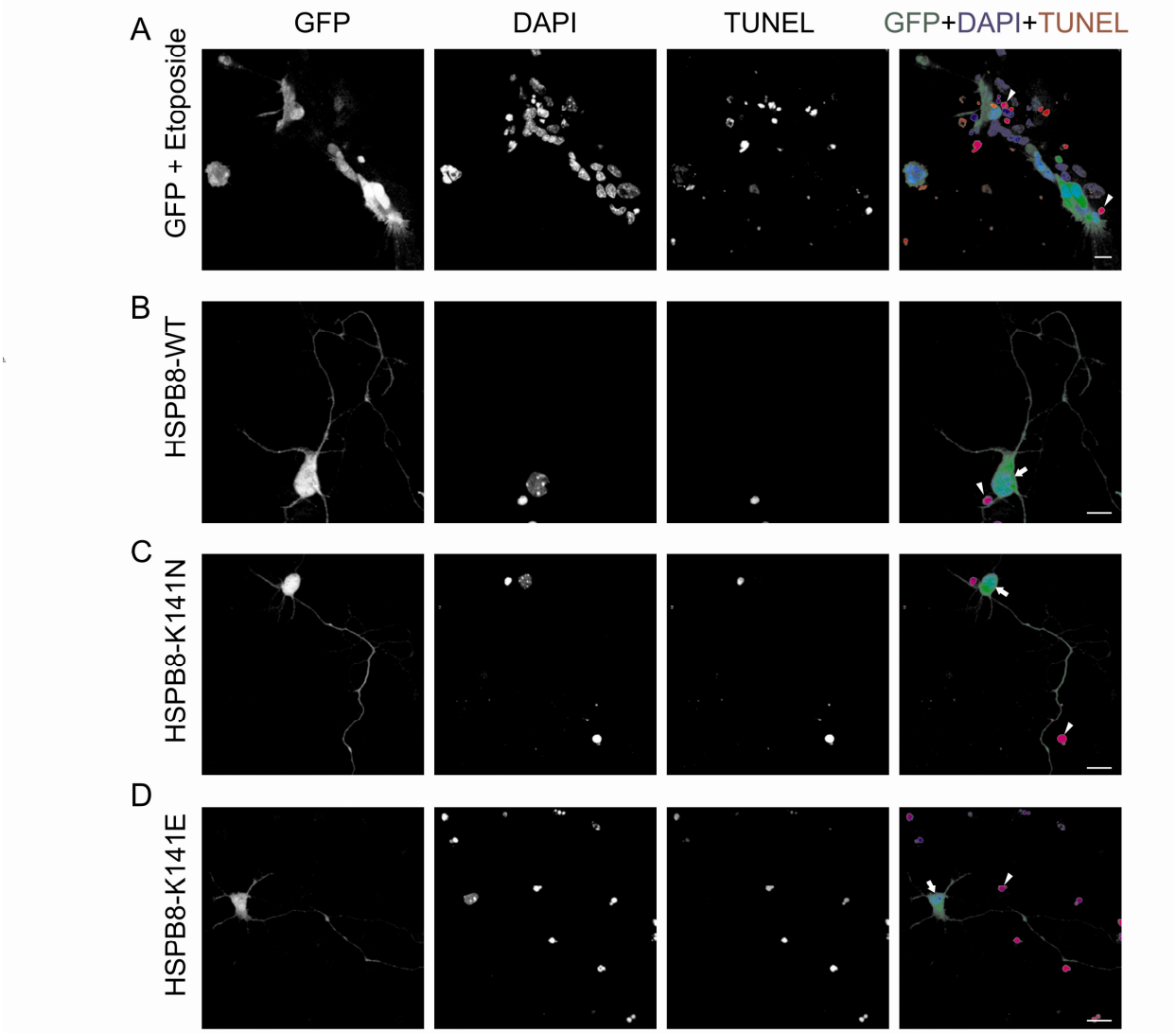
Mouse sensory neurons were transduced with pLenti-GFP, pLenti-WT-HSPB8-GFP or mutant pLenti-K141N/K141E-HSPB8-GFP constructs at DIV6 and analyzed for the activation of apoptosis at DIV10 with the TUNEL assay which detects DNA breaks in the nuclei of apoptotic cells. Confocal micrographs are shown of GFP expression (green in merge), nuclei of all cells (apoptotic and non-apoptotic, DAPI, blue in merge) and nuclei of apoptotic cells (TUNEL, red in merge). Treating the sensory neuron cultures with 50µM etoposide for 2h was used a positive control for cell death activation (**A**). Expression of the different constructs did not lead to detectable TUNEL staining (**B-D**). An additional internal positive control was provided by the TUNEL-positive nuclei resulting from dead cells always present in the primary culture (arrowheads). Arrowheads indicate TUNEL-positive cells and arrows indicate GFP or HSPB8-positive TUNEL-negative cells. Scale bar = 10µm.



Irobi et al Supplementary figure 2

Supplementary Figure 3: Analysis of apoptosis in cortical neurons expressing mutant HSPB8.

Mouse cortical neurons were transduced with pLenti-GFP, pLenti-WT-HSPB8-GFP or mutant pLenti-K141N/K141E-HSPB8-GFP constructs at DIV3 and analyzed for the activation of apoptosis at DIV7 with the TUNEL assay which detects DNA breaks in the nuclei of apoptotic cells. Confocal micrographs are shown of GFP expression (green in merge), nuclei of all cells (apoptotic and non-apoptotic, DAPI, blue in merge) and nuclei of apoptotic cells (TUNEL, red in merge). Treating the cortical neuron cultures with 50µM etoposide for 2h was used a positive control for cell death activation (**A**). Expression of the different constructs did not lead to detectable TUNEL staining (**B-D**). An additional internal positive control was provided by the TUNEL-positive nuclei resulting from dead cells always present in the primary culture (arrowheads). Arrowheads indicate TUNEL positive cells and arrows indicate GFP or HSPB8-positive TUNEL-negative cells. Scale bar = 10µm.



Irobi et al Supplementary figure 3

The support figures are for the reviewers information.

Support figure 1: Phase contrast images of OptiPrep™ iodixanol cushion seperation of different cell types from ventral horn spinal cord.

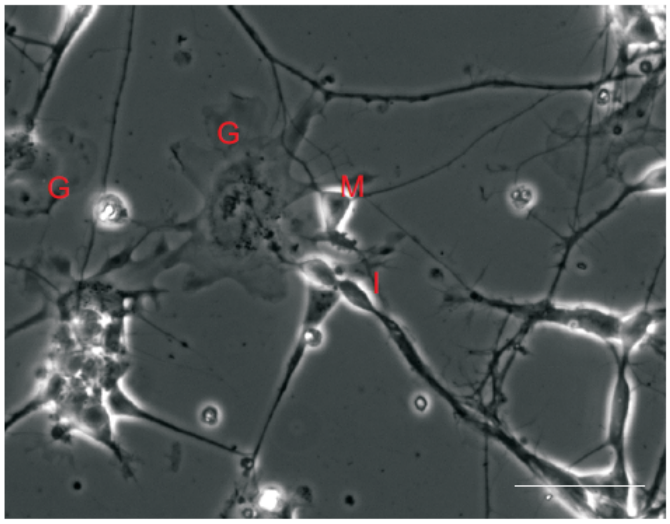
A. Ventral horn spinal cord was dissociated to obtain ventral horn single cell suspension comprising of mixed population of glial cells, motor neurons and other small neurons.

We prepared motor neuron enriched monocultures by layering the ventral horn single cell suspension on a OptiPrep™ iodixanol cushion to generate two fractions (F1 interphase and F2 cell pellet). G = glial cells, I = inter neurons, M = motor neurons.

B. shows the F2 cell pellet fraction consisting of predominant glial cells and small neurons.

C. shows the large motor neuron in the F1 interphase. Scale bar = 50µm

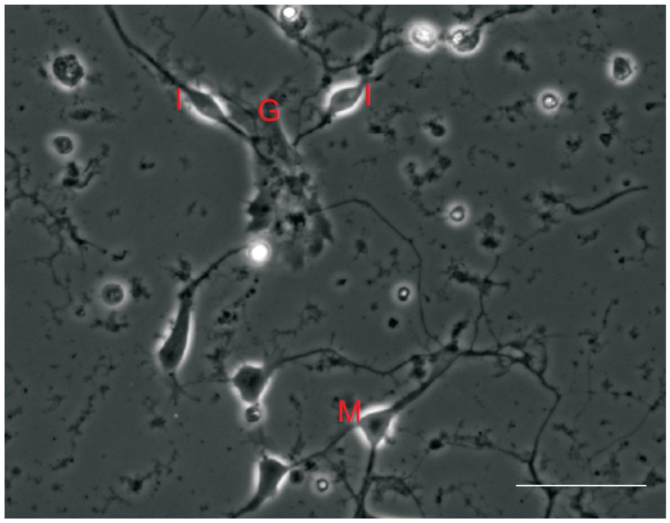
A



Before Optiprep cushion

Ventral horn coculture

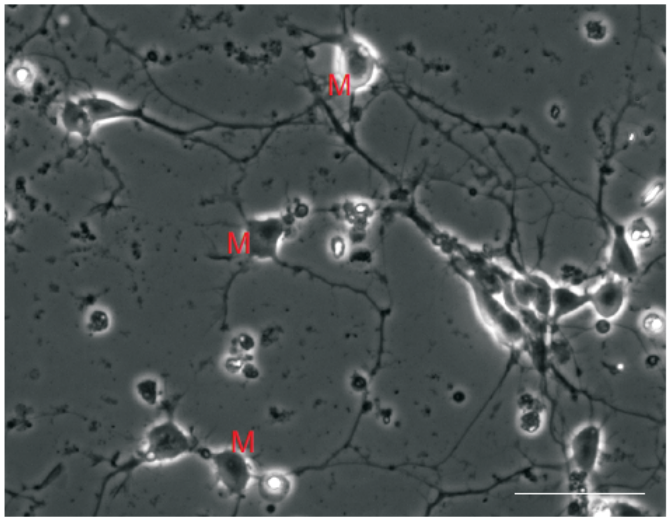
B



After Optiprep cushion

F2 pellet fraction: predominant glial cells and small neurons

C

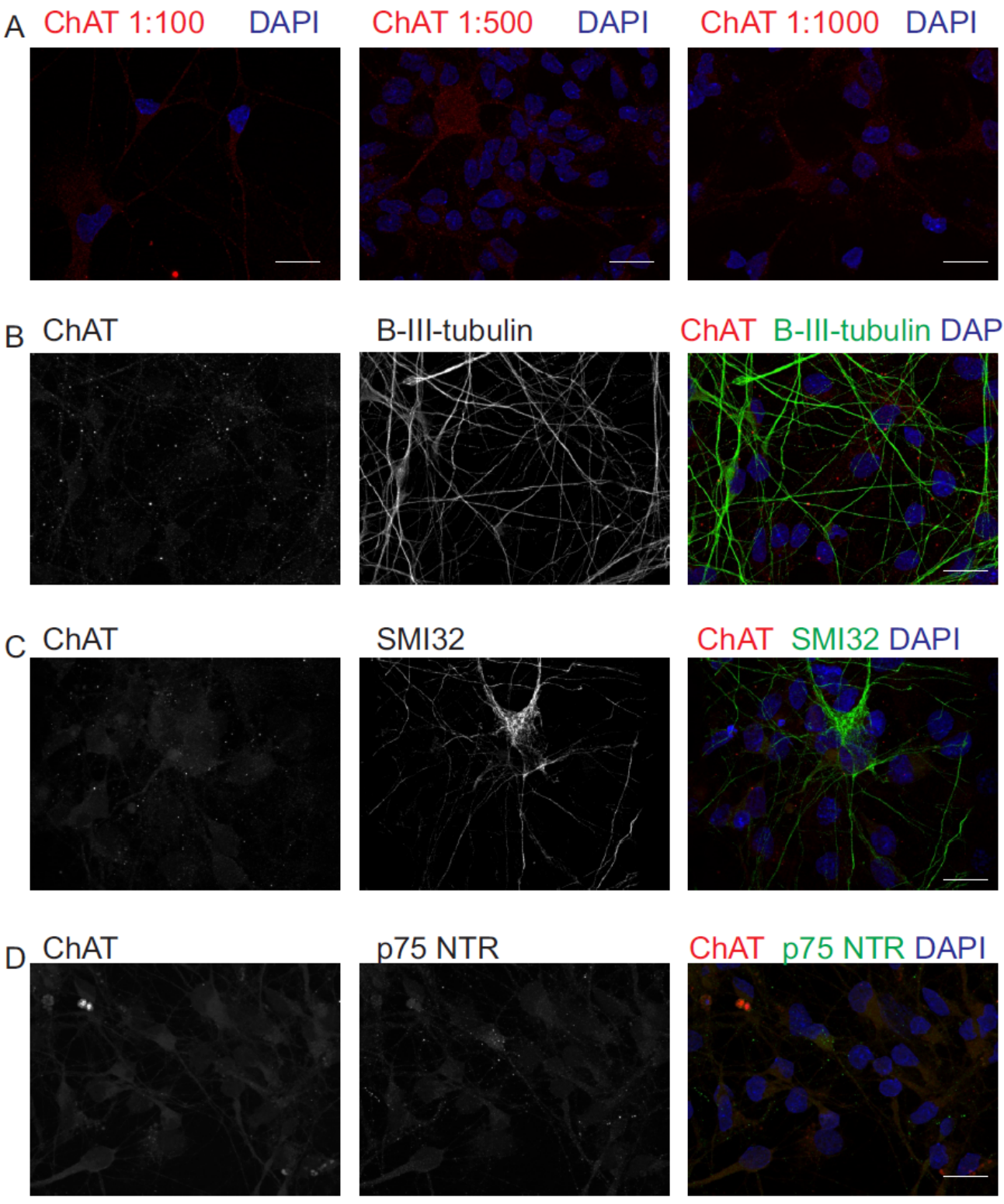


F1 interphase fraction: enriched motor neuron monoculture

Support Fig. 1

Support figure 2: Validation of the presence of motor neurons in our culture system using different motor neuron markers

Immunostaining using different dilutions (1/100 to 1/1000) of the choline acetyl transferase (ChAT) antibody showed non-specific immunoreactivity to both neuronal and non-neuronal cells (**A**). Comparison of immunostainings using both ChAT and beta-3 tubulin (**B**), ChAT and SMI32 non-phosphorylated neurofilament heavy isoform (**C**), ChAT and 75-kDa low-affinity neurotrophin receptor (p75NTR) (**D**) shows that beta-3 tubulin and SMI32 specifically stain neuronal cells, while in our hands, staining specificity was insufficient when ChAT or p75NTR was used. Scale bar = 20µm.



Support Fig. 2