SUPPLEMENTAL ITEMS

1. Supplemental Figures:
    - Figure S1 (related to Figure 1) shows frequency of transmission in the transwell, co-culture, and conditioned media experiments.
    - Figure S2 (related to Figure 2) shows DPR transmission to microglia and mature oligodendrocytes through conditioned media from GFP-DPR expressing NSC34.
    - Figure S3 (related to Figure 3) shows additional immunoblotting analysis of isolated exosomes and frequency of transmission through conditioned media and isolated exosomes from GFP-DPR expressing NSC34.
    - Figure S4 (related to Figure 4) shows a schematic of our iPS cells differentiation protocol and PCR expression profile of critical motor neuron genes over time.
2. Supplemental Experimental Procedures
3. Supplemental References

SUPPLEMENTAL FIGURES

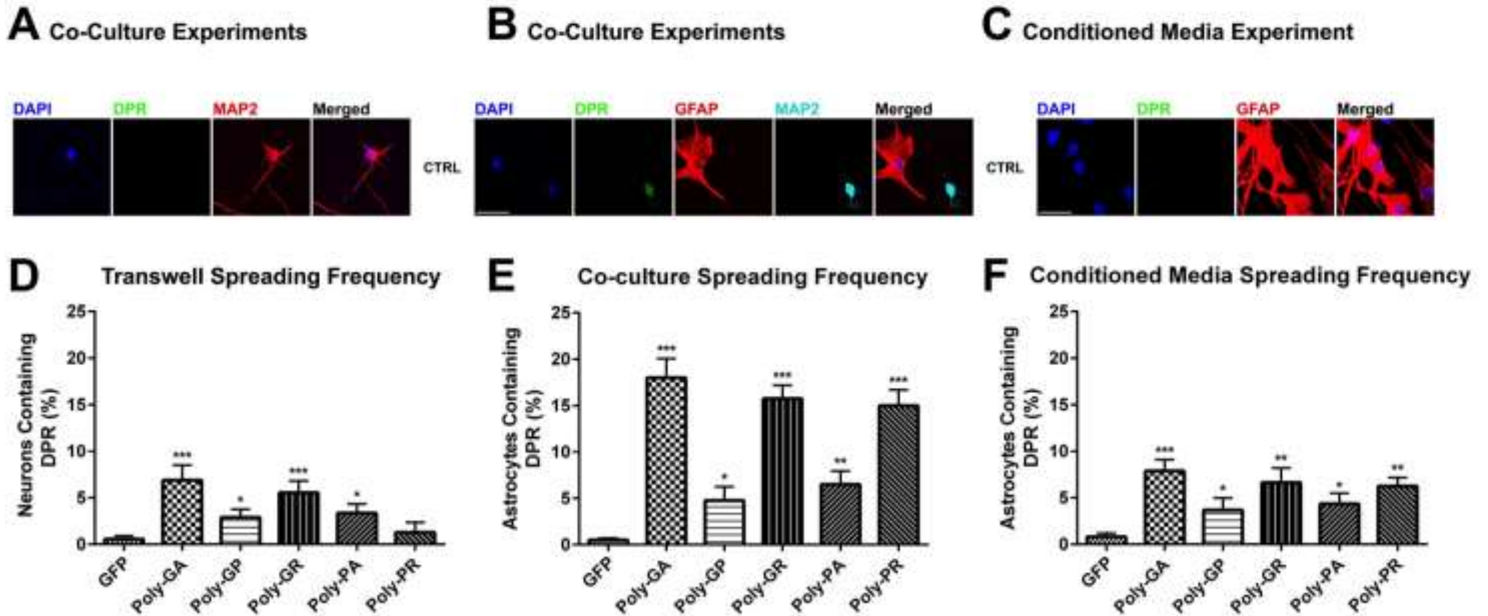


Figure S1. Frequency of transmission in the transwell, co-culture, and conditioned media experiments. (A) Representative confocal images show no transmission for control GFP-FLAG from motor neuron-like NSC34 to primary cortical neurons. Nuclei were stained with DAPI (blue), green represents GFP-FLAG, red represents MAP2-positive cortical neurons. **(B)** Representative confocal images show no transmission for control GFP-FLAG from primary cortical neurons to primary astrocytes. Nuclei were stained with DAPI (blue), green represents GFP-FLAG, red represents GFAP-positive astrocytes, and cyan represents neurons stained by MAP2. **(C)** Representative images show no transmission of control GFP-FLAG to astrocytes from collected media of transfected cortical neurons. Nuclei were stained with DAPI (blue), green represent GFP-FLAG, and red represents GFAP in astrocytes. Bars = 50 μm. **(D)** Transmission frequency for all DPRs in the transwell experiments. 50 neurons were counted per condition in each experimental group; n = 5 experiments. All DPRs, besides PR, had significantly higher transmission that the control groups (*$p<0.05$; **$p<0.01$; ***$p<0.001$; one-way ANOVA with Bonferroni correction). **(E)** Transmission frequency for all DPRs in the co-culture experiments. 50 astrocytes were counted per condition in each experimental group; n = 5 experiments. All DPRs had significantly higher transmission frequency than the control groups (*$p<0.05$; **$p<0.01$; ***$p<0.001$; one-way ANOVA with Bonferroni correction). **(F)** Transmission frequency for all DPRs in the conditioned media experiments. 50 astrocytes were counted per condition in each experimental group; n = 5 experiments. All DPRs had significantly higher transmission frequency than the control groups (*$p<0.05$; **$p<0.01$; ***$p<0.001$; one-way ANOVA with Bonferroni correction).

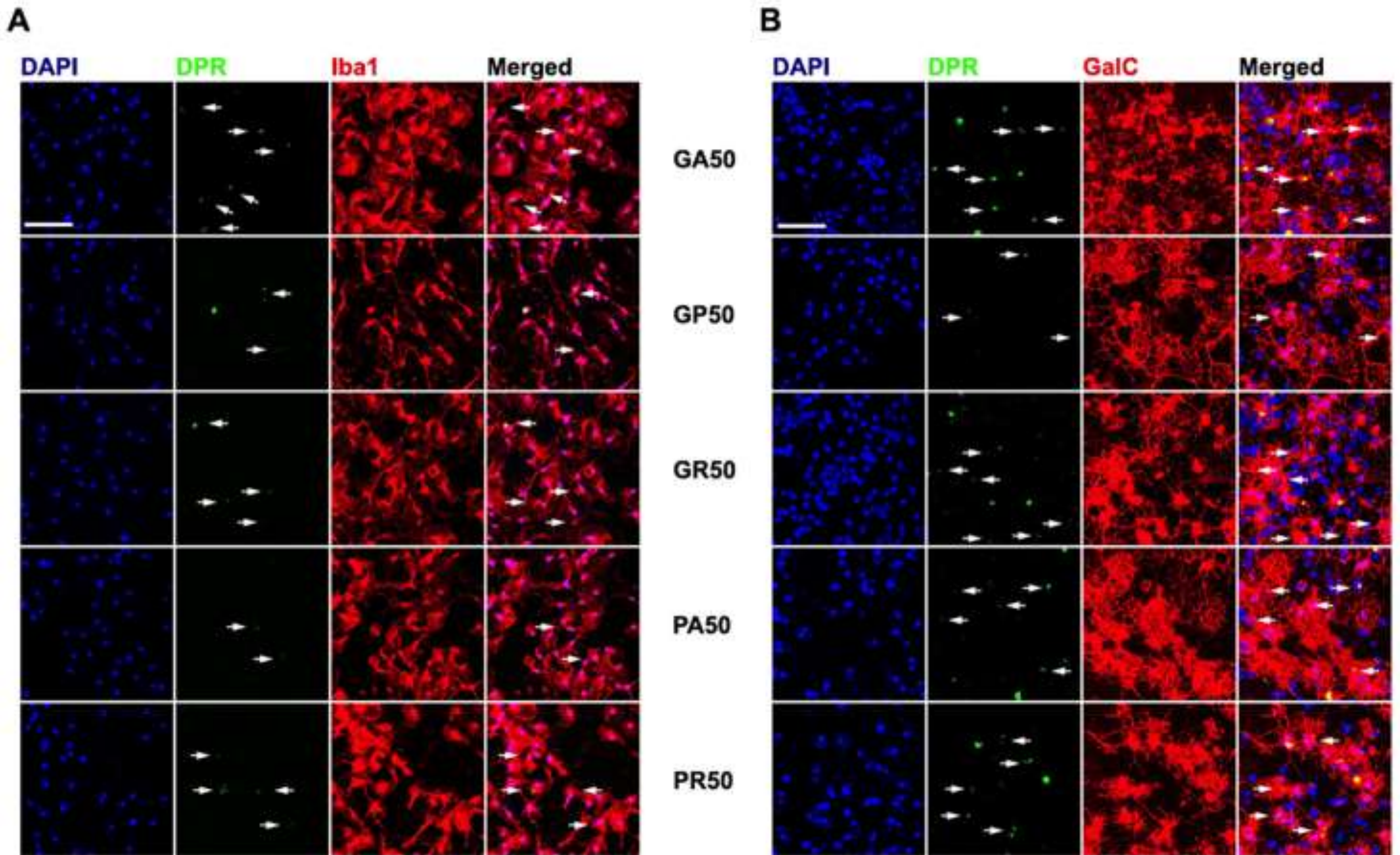


Figure S2. DPRs transmit to microglia and mature oligodendrocyte cultures through conditioned media. (A) Representative images show transmission of all DPR species to primary microglia cultures through conditioned media from GFP-DPR expressing NSC34 cells. Nuclei were stained with DAPI (blue), green represents DPRs, and red represents $Iba1^{+}$ microglia. **(B)** Representative images show transmission of all DPR species to mature oligodendrocyte cultures. Nuclei were stained with DAPI (blue), green represents DPRs, and red represents $GalC^{+}$ mature oligodendrocytes. Small or low fluorescent intracellular aggregates are marked by arrows. Representative images from at least 3 experiments. Bars = 100 μm.

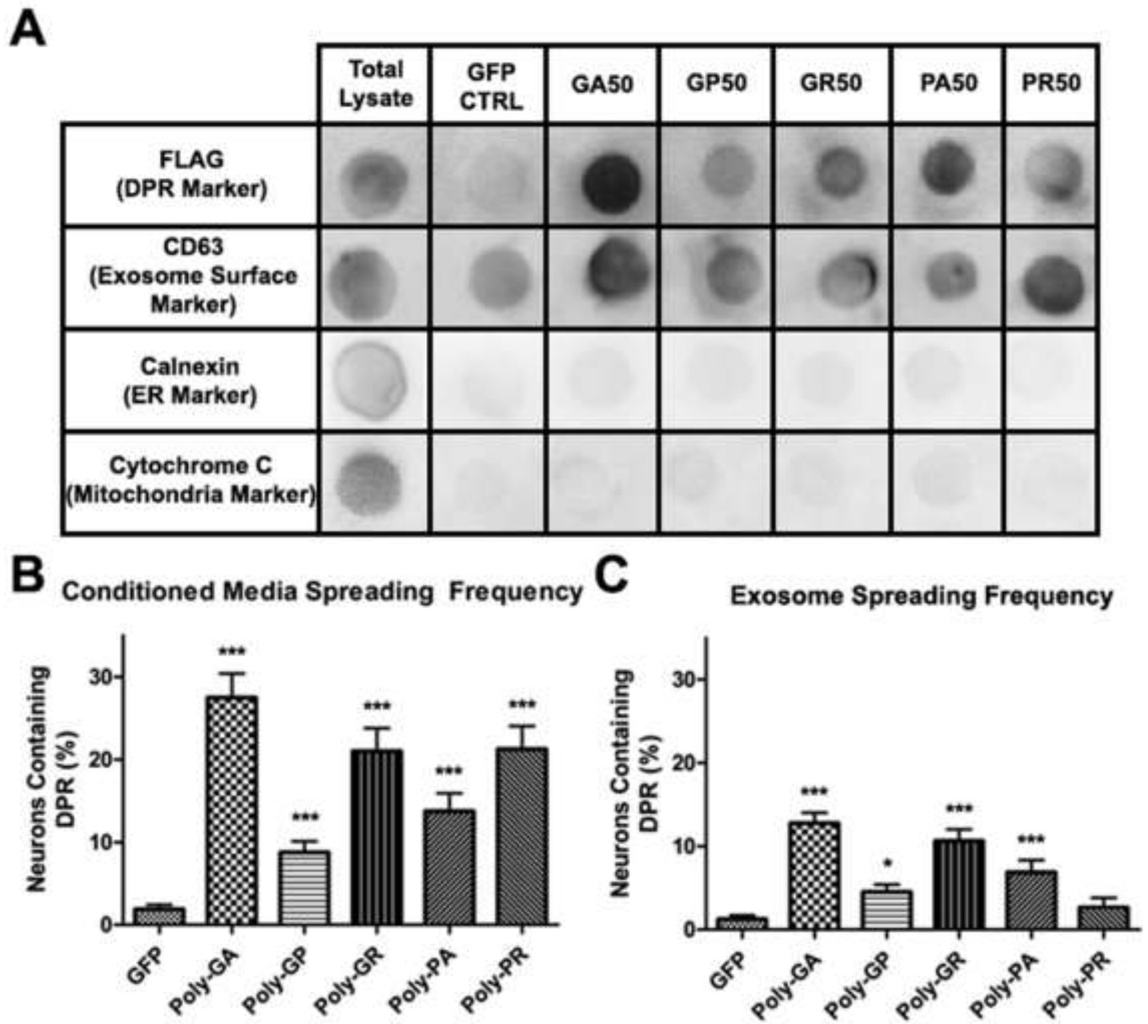
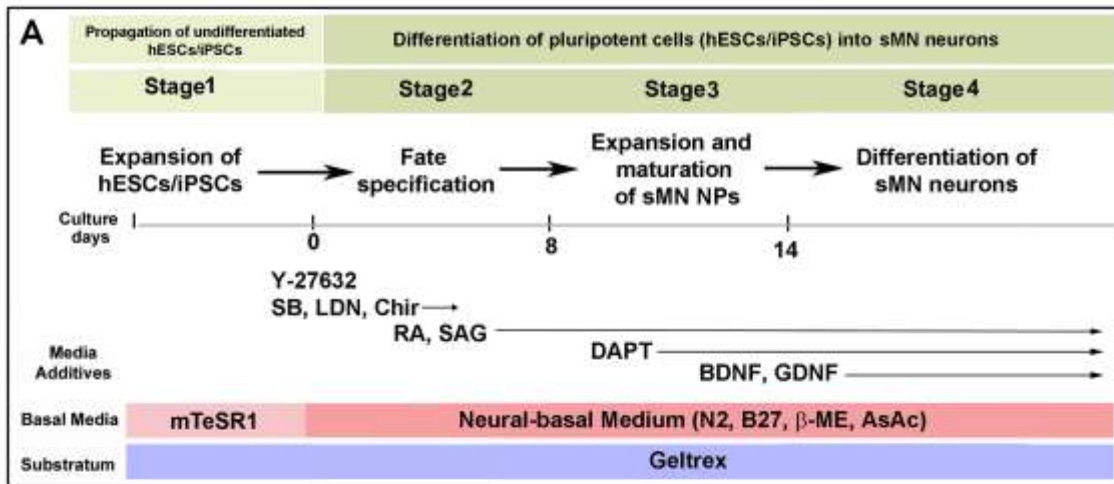


Figure S3. Additional immunoblotting analysis of isolated exosomes and frequency of transmission through conditioned media and isolated exosomes from GFP-DPR expressing NSC34. (A) Immunoblotting of NSC34 transfected with DPR-encoding constructs and their corresponding isolated exosomes isolated from conditioned media. DPRs were detected using a FLAG antibody. CD63 was used as an exosome marker. Calnexin and Cytochrome C were used as a negative staining for other vesicle types. **(B)** Transmission frequency for all DPRs in the conditioned media experiments. 50 neurons were counted per condition in each experimental group; n = 4 experiments. All DPRs had significantly higher transmission that the control groups (*p<0.05; **p<0.01; ***p<0.001; one-way ANOVA with Bonferroni correction). **(C)** Transmission frequency for all DPRs in the isolated exosome experiments. 50 neurons were counted per condition in each experimental group; n = 4 experiments. All DPRs, besides PR, had significantly higher transmission that the control groups (*p<0.05; **p<0.01; ***p<0.001; one-way ANOVA with Bonferroni correction).



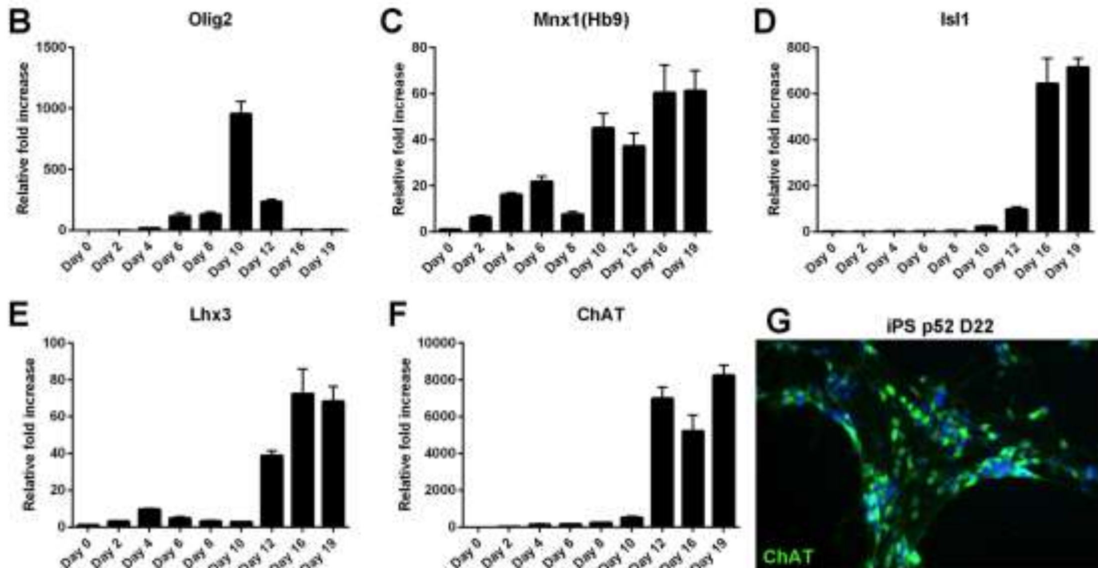


Figure S4. Motor neuron differentiation from induced pluripotent stem cells (iPSCs). (A) Experiment paradigm of motor neuron differentiation protocol (modified from (Maury et al., 2015) used to derive motor neurons from iPSCs (IMR90 clone 4, Wicell) in culture. **(B-F)** Time course of expression of progenitor and mature motor neuron markers by real time PCR. Day 0 undifferentiated iPS cells were used as control. **(G)** ChAT immunostaining at Day22 of differentiation reveals that nearly all live cells (large Dapi^{+} nuclei) are ChAT^{+} motor neurons.

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Cell cultures and transfections

Transfection of cortical neurons with plasmids encoding poly(GA)$_{50}$, poly(GP)$_{50}$, poly(GR)$_{50}$, poly(PA)$_{50}$, and poly(PR)$_{50}$ and FLAG-GFP was performed as previously reported (Wen et al., 2014). In co-culture experiments, cortical rat astrocytes (Vega et al., 2006) were plated onto cortical neurons (200,000 cells/well). In experiments with conditioned media, astrocytes were plated at a density of 300,000 cells/well on 12 mm coverslips and half conditioned medium changes were done.

In transwell experiments, NSC34 cells were plated 24 hours before transfection (3,000 cells/mesh insert with 0.4 μm pores). For exosome isolation or in conditioned media experiments, NSC34 cells were plated 24 hours before transfection (300,000 cells/6-well plates). Medium was changed to DMEM (1% FBS) and NSC34 cells transfected with Lipofectamine 2000, washed (2x) and medium replaced 24 hours after transfection to remove remaining Lipofectamine particles.

Human iPSC were differentiated into sMNs using a modified protocol previously described (Maury et al., 2015). C9orf72 iPSC lines were acquired through Target ALS (ID: TALS9-9.3 and TALS 9-9.5) and control iPSC lines were generated from the IMR-90 cell line (Wicell). The lentivirus carrying CMV driven GFP to identify control sMNs was packaged in 293FT cells using pCDH-CMV-MCS-EF1-GFP (System Biosciences) and three helper plasmids, pLP-1, pLP-2, and pLP/VSVG (Qiagen). C9orf72 and control sMNs were co-cultured on day 15 (when ChAT and Hb9 levels are high). Conditioned media from C9orf72 sMNs was collected every other day after day 15, and a half media exchange was done in the control sMNs.

Immunocytochemistry and confocal microscopy

Cells were fixed in 4% PFA in PBS (15 min., room temperature), permeabilized in 0.3% PBST (15 min.), blocked in 1% BSA in PBST (1 hour, room temperature), incubated with primary antibodies (overnight, 4°C), secondary antibodies (1 hour, room temperature), and mounted with anti-DAPI prolong-diamond anti-fade mounting media. Cells were then washed with PBS three times between each step and imaged using confocal microscopy (Leica SP8) at a 0.3 μm step-size. An average of 30 fields (2-5 neurons and 20 non neuronal cells/field) were analyzed per experimental paradigms. Images shown are representative from at least 3 experiments. Anti-MAP2 antibody (Millipore #AB5622) was used at 1:500 dilution.

Primary Cortical Oligodendrocyte Cultures

Primary oligodendrocyte precursor cell (OPC) cultures were isolated from day 4 postnatal Sprague-Dawley rat pups bred in house, with slight modifications from previously described protocols (Jensen et al., 2015; See et al., 2004). Cortical cell suspensions generated under standard protocols were plated onto poly-D-lysine coated 10-cm petri dishes in Neurobasal medium with B27 supplement and grown overnight at 37°C with 5% CO_2. Cultures were then switched into growth medium containing Neurobasal medium with B27, bFGF (10 ng/mL), PDGF-AA (2 ng/mL), and neurotrophin-3 (1 ng/mL). After growing to confluency, cells were purified to between 91-98% OPCs and 2-9% astrocytes using a wash-down procedure, and sub-cultured onto poly-D-lysine coated coverslips.

To assess mature oligodendrocytes, growth medium was replaced with differentiation medium consisting of DMEM/F12 1:1, with glutamine (2 mM), Penn/Strep, glucose (0.3%),

transferrin (50 μg/mL), insulin (12.5 μg/mL), putrescine (5 μg/mL), L-Thyroxine (0.4 μg/mL), biotin (10 ng/mL), progesterone (3 ng/mL), and selenium (2.6 ng/mL).

DPR Media Treatment of oligodendrocytes

Conditioned medium from DPR-expressing NSC34 cells was added to maturing oligodendrocytes, which had already undergone differentiation for 48 hours. Twenty-four hours following application of this treatment, cells were immunostained for oligodendrocyte lineage surface markers A2B5 (OPC) or Galactocerebroside (GalC, for maturing oligodendrocytes), fixed, mounted, and imaged (Leica SP8 confocal microscope) per standard protocols (Jensen et al., 2015; See et al., 2004).

Oligodendrocyte lineage cells on coverslips were processed for staining of well-characterized antigens for specific stages of oligodendrocyte maturation as previously described (Jensen et al., 2015; See et al., 2004). Prior to fixation, live cells were labelled for cell surface antigen α-GalC (mouse hybridoma supernatant). Rhodamine-conjugated secondaries from Jackson Immunoresearch were utilized. Cells were then fixed with ice-cold methanol for 10 minutes. For mounting and staining of all nuclei, Vectashield with DAPI was used.

Dot Blots

For detection of DRPs and exosome-associated proteins, isolated exosomes were placed in RIPA buffer and applied to a nitrocellulose membrane with 0.2 μm pore size. The following primary antibodies were used at indicated dilutions: anti-GFP (Clontech #632381, 1:3,000), anti-FLAG (Sigma #F1804, 1:500), anti-Flotillin (Santa Cruz #sc-74566, 1:200), anti-CD63 (Santa Cruz #sc-15363, 1:200), anti-TSG101 (Abcam #ab83, 1:200), anti-GM130 (Santa

Cruz #sc-55591, 1:200), anti-Calnexin (Abcam #ab22595, 1:200), and anti-Cytochrome C (Santa cruz #sc-7159, 1:200).

SUPPLEMENTAL REFERENCES

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