Title: Ivermectin promotes peripheral nerve regeneration during wound healing

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Running Title: Ivermectin promotes peripheral nerve regeneration

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SUPPLEMENTARY INFORMATION

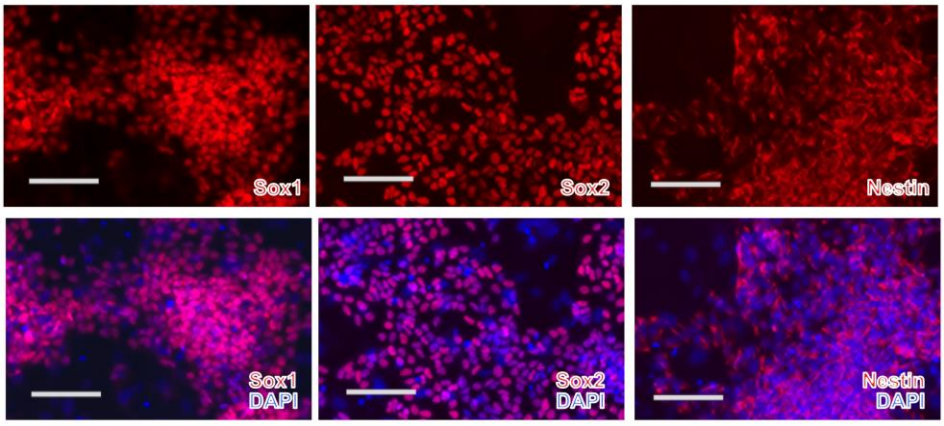


Figure S1. Characterization of proliferating hiNSCs. Proliferating hiNSCs express high levels of neural stem cell markers Sox1, Sox2 and Nestin. Scale bar, 100 µm.

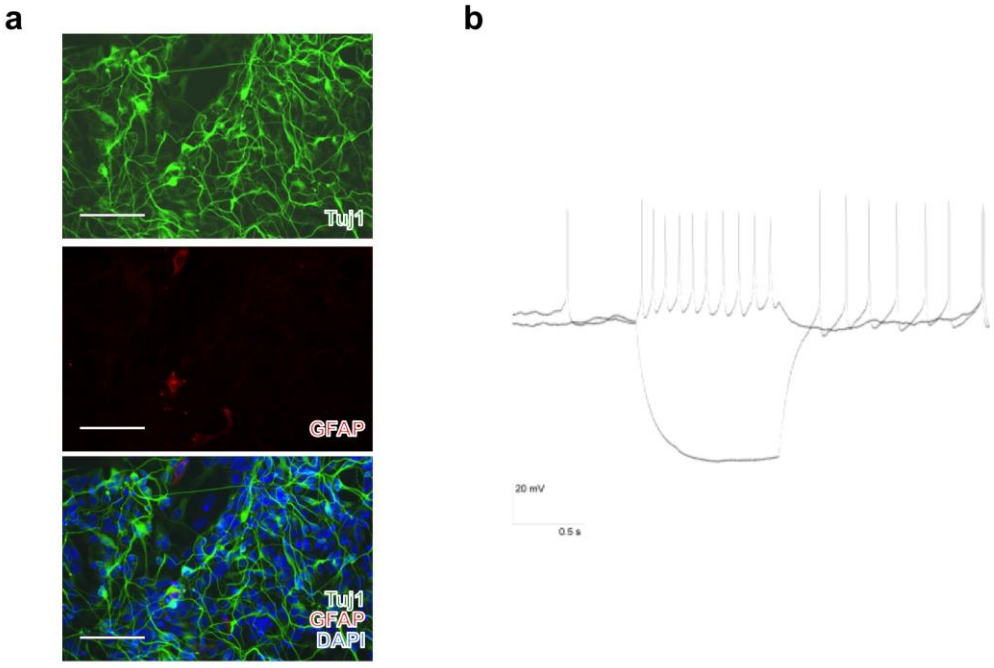


Figure S2. Characterization of predifferentiated hiNSCs. a) hiNSCs pre-differentiated on gelatin-coated plates for one week express high levels of neuronal marker beta III tubulin (~97% Tuj1+ cells) and low levels of glial fibrillary acidic protein (~2% GFAP+ cells). Scale bar, 100 µM. b) Mature hiNSCs are functional neurons as demonstrated by patch-clamp electrophysiology results, which indicate that differentiated hiNSCs elicit current-induced action potentials.

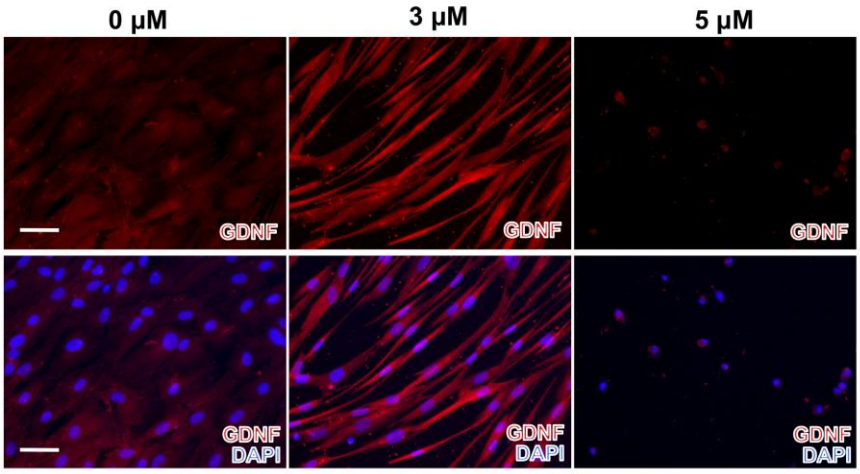
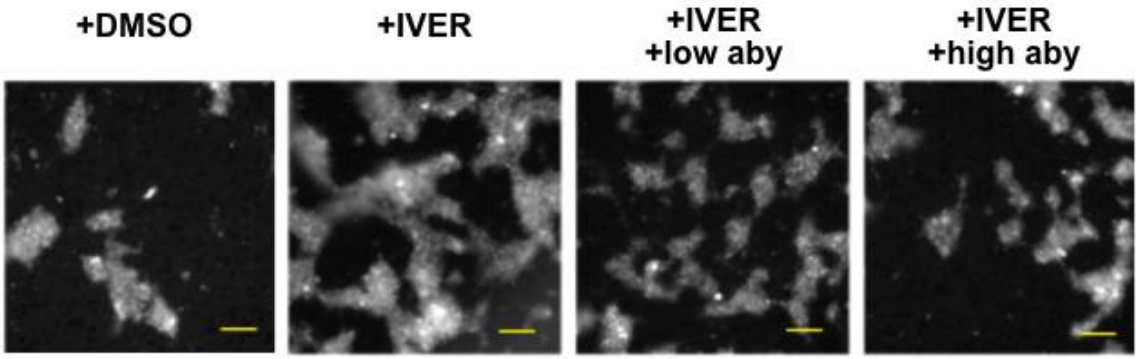
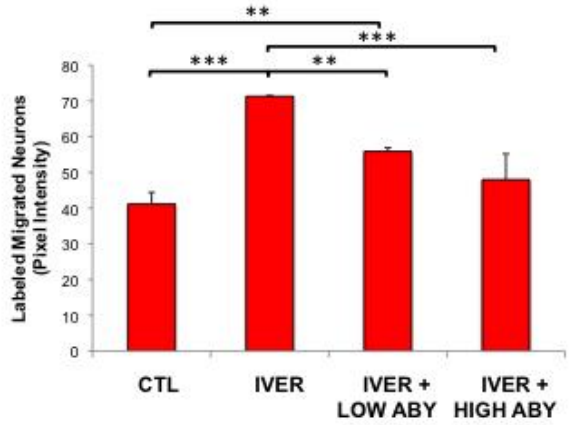


Figure S3. Toxicity of ivermectin in vitro. Human dermal fibroblasts (hDFs) were treated with various concentrations of ivermectin for one week then subjected to immunostaining against glial derived neurotrophic factor (GDNF). At a concentration of 3µM, ivermectin results in a visible increase in GDNF expression, however, at higher levels of 5µM, ivermectin results in substantial cell death. Scale bar = 100 µM.

a


+DMSO
+IVER
+IVER
+low aby
+IVER
+high aby

b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Labeled Migrated Neurons
(Pixel Intensity)
80
70
60
50
40
30
20
10
0
CTL
IVER
IVER +
LOW ABY
IVER +
HIGH ABY

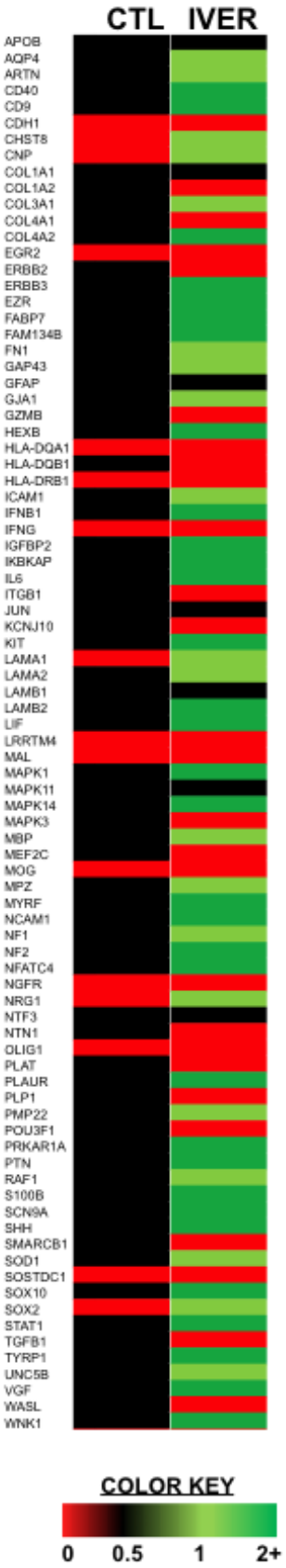

Figure S4. Functionally blocking ivermectin-induced GDNF upregulation by hDFs prevents migration of differentiated neurons. Human dermal fibroblasts were seeded into the bottom of cell culture plates, subsequently treated with or without ivermectin, and washed repeatedly to remove the drug. Differentiated DiD-labeled neurons were seeded onto coated transwells (8µM pore size), which were placed into the wells containing fibroblasts. Cells were cultured in low serum media with or without a functionally blocking antibody against GDNF, and the relative number of cells migrating to the bottom of transwells was quantified. a) Images of fluorescently labeled neurons that migrated to the bottom of transwells upon co-culture with dermal fibroblasts pre-treated with or without ivermectin and in the presence or absence of anti-GDNF, scale bar: 100 µM. b) Quantification of migrated cells. *P≤0.05, **P≤0.01, ***P≤0.001; as determined by two tailed t-test. Error bars show mean±s.d.

Figure S5. Treatment of dermal fibroblasts with ivermectin causes an upregulation in multiple genes involved in Schwann cell differentiation and maintenance, peripheral nerve regeneration and extracellular matrix (ECM) synthesis. Human dermal fibroblasts (hDFs) were treated with or without 1 µM ivermectin for 4 or 8 days, then subjected to GeneQuery Human Schwann Cell Biology qPCR Array Kit (ScienCell Research Laboratories, Carlsbad, CA). Samples receiving the same treatment were pooled from Day 4 and Day 8 time points. Multiple Schwann cell-associated genes were found to be upregulated in hDFs in response to ivermectin treatment.

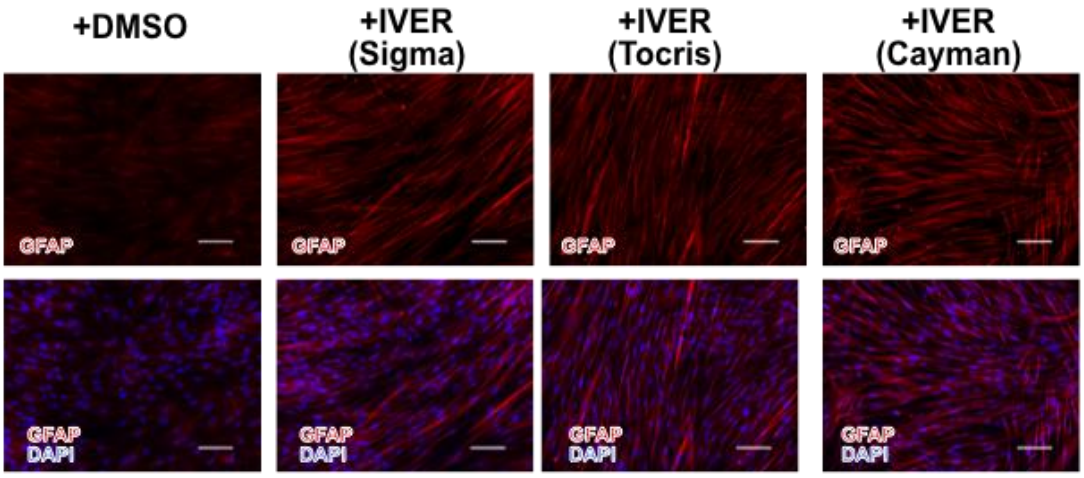

Figure S6. Different formulations of ivermectin produce similar results in hDFs in vitro. Human dermal fibroblasts (hDFs) were treated with 1μM ivermectin for 8 days then subjected to immunostaining against glial fibrillary associated protein (GFAP). All forms of ivermectin tested resulted in a visible increase in GFAP expression, as well as the phenotypic elongated morphology reminiscent of Schwann cells. Scale bar = 100 μM.

Gene	Accession No.	Sequence 1 (5' → 3')	Sequence 2 (5' → 3')
GAPDH	NM_002046.5	ATTGCCCTCAACGACCACT	ATGAGGTCCACCACCCTGT
BDNF	NM_001143805	AGCCCATTCAGCCCATTT	TGCTTATCCCTCACCCTACT
NGF	NM_002506	CTGAGCTTGGGTCCAGCAT	AGAGAGCGCTGGGAGCC
GDNF	NM_000514	TCCATGACATCATCGAACTGA	CCGAAGACCGCTCCCTC
GFAP	NM_001131019.2	ACTGGCAGAGCTTGTTAGTG	AGTGACAGGAAGAGGTGAGA

Table S1. List of primer sequences used for qRT-PCR analysis.

Host	Antigen	Vendor	Catalog #
Rabbit	KI67	Abcam	ab15580
Rabbit	GDNF	Abcam	ab18956
Rabbit	GFAP	Sigma	G9269
Rabbit	PGP9.5	Millipore	AB1761-I
Goat (Alexa 594 conjugated)	Rabbit IgG	Invitrogen	A-11072

Table S2. List of antibodies used for immunofluorescence analysis.