

1 **Human repair-related Schwann cells adopt functions of antigen-presenting cells *in vitro***

2

3 Jakob Berner^{1,3,*}, Tamara Weiss^{1,2,*}, Helena Sorger^{1,4}, Fikret Rifatbegovic¹, Max Kauer¹,

4 Reinhard Windhager⁵, Alexander Dohnal¹, Peter F. Ambros¹, Inge M. Ambros¹, Peter

5 Steinberger⁶, Sabine Taschner-Mandl^{1,#}

6

7 **Affiliations:**

8 ¹ CCRI, St. Anna Children's Cancer Research Institute, Vienna, Austria

9 ² Department of Plastic, Reconstructive and Aesthetic Surgery, Medical University of Vienna

10 ³ St. Anna Children's Hospital, Vienna, Austria

11 ⁴ University for Veterinary Medicine, Vienna, Austria

12 ⁵ Department of Orthopedics and Trauma Surgery, Medical University of Vienna, Vienna, Austria

13 ⁶ Institute of Immunology, Medical University of Vienna, Vienna, Austria

14 * These authors contributed equally

15 # Corresponding author

16

17 **Running title:** Antigen-presenting functions of human repair Schwann cells

18

19 **Corresponding author information:**

20 Sabine Taschner-Mandl

21 CCRI, St. Anna Children's Cancer Research Institute

22 Zimmermannplatz 10, 1090 Vienna, Austria

23 T: +43-1-40470-4050

24 E-mail: sabine.taschner@ccri.at

25

26 **Word count:**

27 Total: 6,990

28 Abstract: 250

29 Introduction: 943

30 Material and Methods: 2,351

31 Results: 1,781

32 Discussion: 1,665

33 **Key words:** Schwann cell, immunocompetence, PD-L1, immunoregulatory, inflammation,
34 nerve injury, neuropathies, antigen presenting cell

35

36 **Main points:**

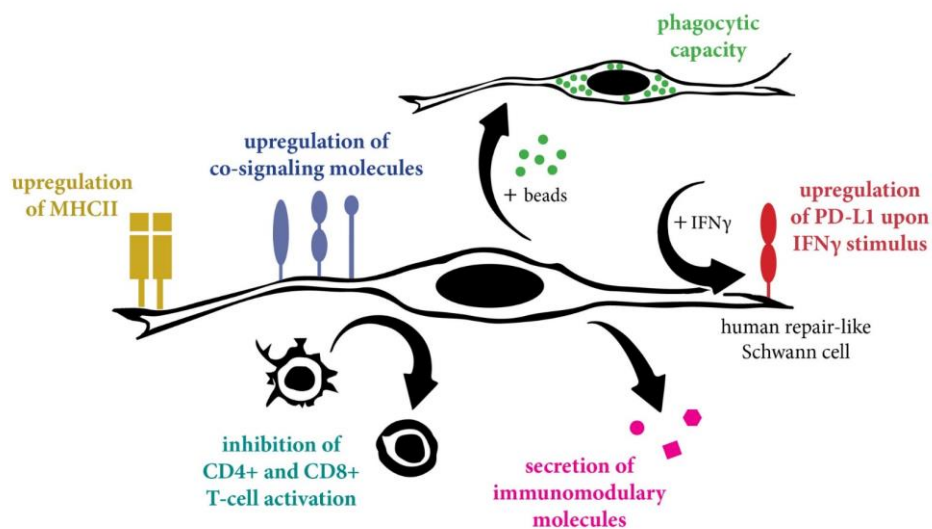
- 37 • Human repair-related Schwann cells (hrSC) function as professional antigen presenting
- 38 cells.
- 39 • HrSCs up-regulate PD-L1 upon pro-inflammatory IFN γ stimulation.
- 40 • HrSCs hamper CD4 $^{+}$ and CD8 $^{+}$ T-cell activation.

41

42 **Graphical abstract**

43

immunoregulatory features of human repair-related Schwann cells



44

45 Abstract

46 The plastic potential of Schwann cells (SCs) is increasingly recognized to play a role after nerve
47 injury and in diseases of the peripheral nervous system. In addition, reports on the interaction
48 between SCs and immune cells indicate their involvement in inflammatory processes. However,
49 data about the immunocompetence of human SCs are primarily derived from neuropathies and
50 it is currently unknown whether SCs directly regulate an adaptive immune response after nerve
51 injury.

52 Here, we performed a comprehensive analysis of the immunomodulatory capacities of human
53 repair-related SCs (hrSCs), which recapitulate SC response to nerve injury *in vitro*. We used our
54 previously established protocol for the culture of primary hrSCs from human peripheral nerves
55 and analyzed the transcriptome, secretome, and cell surface proteins for signatures and
56 markers relevant in innate and adaptive immunity, performed phagocytosis assays, and
57 monitored T-cell subset activation in co-cultures with autologous human T-cells.

58 Our findings show that hrSCs are highly phagocytic, which is in line with high MHCII expression.
59 In addition, hrSCs express co-regulatory molecules, such as CD40, CD80, B7H3, CD58, CD86,
60 HVEM, release a plethora of chemoattractants, matrix remodelling proteins and pro- as well as
61 anti-inflammatory cytokines, and upregulate the T-cell inhibiting PD-L1 molecule upon pro-
62 inflammatory stimulation with IFN γ . Furthermore, hrSC contact reduced the number and
63 activation status of allogenic CD4 $^{+}$ and CD8 $^{+}$ T-cells.

64 This study demonstrates that hrSCs possess features and functions typical for professional
65 antigen presenting cells *in vitro*, and suggest a new role of these cells as negative regulators of
66 T-cell immunity during nerve regeneration.

67 Introduction

68 Schwann cells (SCs) are glial cells of the peripheral nervous system and possess capacities that
69 go far beyond the preservation of axon integrity. Upon nerve injury, SCs undergo extensive
70 morphological and expression changes and acquire distinct repair features in a process referred
71 to as ‘adaptive cellular reprogramming’ (Jessen & Mirsky, 2016). In this dedicated repair cell
72 state, SCs re-enter the cell cycle and execute specialized functions to coordinate the multi-step
73 process of nerve regeneration, such as the recruitment of immune cells, the breakdown of
74 myelin debris, remodeling of the extracellular matrix, and the expression of neurotrophic and
75 neuritogenic factors for axon survival, regrowth, and guidance (Gomez-Sanchez et al., 2015;
76 Jang et al., 2016; Jessen & Mirsky, 2016; Nocera & Jacob, 2020; Tofaris, Patterson, Jessen, &
77 Mirsky, 2002; Weiss et al., 2016). Moreover, numerous studies support that the highly adaptive
78 cellular state of SCs plays a role in pathological conditions such as neuropathies and tumor
79 development (Azam & Pecot, 2016; Bunimovich, Keskinov, Shurin, & Shurin, 2017; Direder et
80 al., 2021; Weiss et al., 2021). We have recently shown that tumor-associated SCs in
81 neuroblastic tumors adopt a similar phenotype as upon nerve injury and exert anti-proliferative
82 and pro-differentiating effects through the release of until then unknown neurotrophins, such
83 as EGFL8 (Ambros et al., 1996; Crawford et al., 2001; Direder et al., 2021). As knowledge on the
84 involvement of SCs during regeneration and pathologies is continuously expanding, their
85 immunomodulatory potential gains increasing interest (Armati, Pollard, & Gatenby, 1990;
86 Hörste, Hu, Hartung, Lehmann, & Kieseier, 2008; Zhang et al., 2020). SCs have been
87 demonstrated as immune competent cells that contribute to inflammatory and hereditary
88 neuropathies (Ydens et al., 2013). However, less is known about the impact of human SCs on
89 the inflammatory processes during peripheral nerve regeneration (Bergsteinsdottir, Kingston,
90 Mirsky, & Jessen, 1991; Rutkowski et al., 1999; Toews, Barrett, & Morell, 1998; Weiss et al.,
91 2016).

92 Similar to any injury site in the body, injured nerves experience an early pro-inflammatory
93 response by the influx of immune cells that is followed by termination of the immune response
94 to allow tissue regeneration. Previous studies showed that SCs secrete a variety of cytokines
95 and chemokines which attract monocytes and neutrophils to the site of nerve injury
96 (Bergsteinsdottir et al., 1991; Rutkowski et al., 1999; Tofaris et al., 2002). Their expression could
97 be partially mediated by axon derived molecules recognized by SCs via toll-like receptors (TLRs)

98 (Goethals, Ydens, Timmerman, & Janssens, 2010; Kaisho & Akira, 2000; Karanth, Yang, Yeh, &
99 Richardson, 2006; Lee et al., 2006; Meyer Zu Horste et al., 2010; Meyer zu Hörste, Hu, Hartung,
100 Lehmann, & Kieseier, 2008). Within injured nerves, recruited and/or tissue resident
101 macrophages adapt a specialized regenerative phenotype with neuroprotective capacities and
102 express proteins associated with an anti-inflammatory profile (Gaudet, Popovich, & Ramer,
103 2011; La Fleur, Underwood, Rappolee, & Werb, 1996; Ydens et al., 2012). Of note, SCs might
104 be involved in polarizing macrophages towards a regenerative phenotype, but so far, the
105 underlying factors remain unknown (Stratton & Shah, 2016; Stratton et al., 2016).

106 SCs can also interact with T-cells by expressing major histocompatibility complex class II (MHCII)
107 receptors and co-signaling molecules (Armati et al., 1990; Hörste et al., 2008; Murata &
108 Dalakas, 2000). However, upregulation of MHCII on SCs was primarily reported in neuropathies
109 (Mancardi et al., 1988; Meyer Zu Horste et al., 2010; Van Rhijn, Van den Berg, Bosboom, Otten,
110 & Logtenberg, 2000) and upon treatment with IFN γ (Armati et al., 1990; Lilje & Armati, 1997;
111 Samuel, Mirsky, Grange, & Jessen, 1987), which is a potent inducer of MHCII expression in
112 antigen presenting cells (APCs). In contrast, our previous research showed that human repair-
113 related SCs highly upregulate MHCII in culture and within nerve explants independent of IFN γ
114 (Weiss et al., 2016). Furthermore, these repair-related SCs expressed genes of co-signaling
115 molecules, MHCII transcriptional co-activator *CIITA*, and other molecules involved in the
116 antigen processing and presentation machinery (Weiss et al., 2016), suggesting a biological
117 relevance of MHCII expressing SCs in response to injury.

118 APCs function as local modulators of T-cell response upon inflammatory stimulation. The
119 outcome of this modulation is dependent on the expression of sets of co-stimulatory or co-
120 inhibitory surface molecules recognized by T-cells together with MHCII. Indeed, rodent SCs
121 could be induced to activate T-cells by presenting endogenous as well as exogenous antigens
122 (Duan et al., 2007; Kingston et al., 1989; Spierings, De Boer, Zulianello, & Ottenhoff, 2000;
123 Steinhoff & Kaufmann, 1988; Wekerle, Schwab, Linington, & Meyermann, 1986). Moreover, T-
124 cell activation through MHCII expressing SCs has been associated with post-traumatic
125 inflammation and neuropathic pain in diseased peripheral nerves of rodents (Hartlehnert et al.,
126 2017). Hence, the SC function as non-professional APC has mainly focused on the promotion
127 of T-cell activation resulting in (auto-) inflammatory or infectious neuropathies, rather than
128 suppression of activated T-cells. The latter is executed by APCs to restore immune homeostasis
129 and prevent auto-immunity to self-proteins. In line with a potential T-cell inhibiting function of

130 SCs, our previous transcriptomic analyses have indicated that primary human SCs express
131 genes associated with T-cell suppression such as *PD-L1* and *DC-HIL* (Weiss et al., 2016).
132 Based on the increasing body of studies supporting the immunocompetence of SCs and their
133 recognized role in nerve injury and disease (Meyer zu Hörste et al., 2008; Weiss et al., 2021;
134 Zhang et al., 2020), we here set out to investigate immunoregulatory features of human SCs in
135 an injury condition. To this end, we cultured primary human SCs and performed phagocytosis
136 assays, analyzed the secretion of immunomodulatory mediators, and profiled their repertoire
137 of co-signaling molecules as well as upon TLR and inflammatory stimulation. We further
138 assessed the ability of SCs to modulate T-cell activation and polarization *in vitro*.

139
140

141 Methods

142 Human material

143 The collection and research use of human peripheral nerve tissues and human tumor specimen
144 was conducted according to the guidelines of the Council for International Organizations of
145 Medical Sciences and World Health Organisation and has been approved by the local ethics
146 committees of the Medical University of Vienna (EK2281/2016 and 1216/2018). Informed
147 consent has been obtained from all patients participating in this study.

148 Neuroblastoma cell lines and primary cultures are available upon request. Primary Schwann
149 cell cultures and tumor tissues are limited materials and therefore cannot be provided.

150

151 Isolation of primary human Schwann cells

152 SCs were isolated, cultured and enriched as previously described (Weiss, Taschner-Mandl,
153 Ambros, & Ambros, 2018). Briefly, peripheral nerves were cut into 2-3 cm pieces and nerve
154 fascicles were pulled out of the surrounding epineural tissue. The isolated fascicles were cut
155 into ~0.5 cm pieces and incubated in a digestion solution containing α MEM GlutaMAX™
156 (Gibco), 10% FCS (PAA), 1% Pen/strep (Pan Biotech), 1 mM sodium pyruvate (Pan Biotech), 25
157 mM HEPES (Pan Biotech), 0.125% collagenase Type IV (Gibco), 1.25 U/mL Dispase II (Sigma-
158 Aldrich) and 3 mM CaCl (Sigma-Aldrich) at 37 °C, for 20 h. The digested tissue was pelleted and
159 resuspended in SC expansion medium (SCEM) containing MEM α , 1% Pen/Strep, 1 mM sodium
160 pyruvate, 25 mM HEPES, 10 ng/mL hu FGF basic (PeproTech), 10 ng/mL hu Heregulin- β 1

161 (PeproTech), 5 ng/mL hu PDGF-AA (PeproTech), 0.5% N2 supplement (Gibco), 2 μ M forskolin
162 (Sigma-Aldrich) and 2% FCS. Cells were seeded in 0.01% Poly-L-lysine (PLL, Sigma-Aldrich) and
163 4 μ g/ml laminin (Sigma-Aldrich) coated culture dishes. Half of the medium was changed twice
164 a week. As passage 0 (p0) cultures consisted of SCs and fibroblast-like cells, SCs were enriched
165 before experimentation, by exploiting their differential adhesion potential to plastic, described
166 in (Weiss et al., 2018). As previously shown, human SCs adopt a repair-related phenotype in
167 culture (Weiss et al., 2016) and SCs are referred to as human repair-related SCs (hrSC).

168

169 **Neuroblastoma cell lines**

170 The used neuroblastoma cell lines (NB cells) are derived from biopsies or surgical resection of
171 aggressively behaving, high-risk neuroblastomas. In-house established, low passage NB cell
172 lines STA-NB-6, 7, -10 and -15 as well as the cell lines, SH-SY5Y, IMR5 and CLB-Ma (kindly
173 provided by Dr Valerie Combaret, Centre Leon Berard, France) (I M Ambros et al., 1997; Biedler,
174 Helson, & Spengler, 1973; Biedler, Roffler-Tarlov, Schachner, & Freedman, 1978; Combaret et
175 al., 1995; Fischer & Berthold, 2003; Momoi, Kennett, & Glick, 1980; Stock et al., 2008) were
176 used for experimentation and cultured in MEM α GlutaMAXTM, 1% Pen/Strep, 1 mM sodium
177 pyruvate, 25 mM HEPES and 10% FCS. NB cells are used in this study as model to reflect
178 neuronal cells.

179

180 **Primary human T-cells**

181 For the T-cell isolation, a buffy coat was obtained from the Austrian Red Cross and diluted 1:4
182 in 1x PBS. Density gradient centrifugation was performed by transferring the blood onto 20 mL
183 Lymphoprep solution (StemCell Technologies) and centrifugation for 30 min at 400g at room
184 temperature (RT) without breaks. Mononuclear cells were carefully removed from the
185 interphase layer and transferred into 50 mL 1x PBS and centrifuged at 300g for 10 min. Then,
186 the medium was removed and T-cells were isolated with the Pan T-cell Isolation Kit (Miltenyi
187 Biotec) according to the manufacturer's protocol using magnetic activated cell sorting (MACS).
188 Briefly, cells were counted and resuspended in 40 μ L of MACS buffer (PBS, pH 7.2, 0.5% bovine
189 serum albumin (BSA), 2 mM EDTA) per 10^7 cells. For each 40 μ L, 10 μ L of PAN T-Cell Biotin
190 Antibody Cocktail (Miltenyi Biotec) was added and incubated for 5 minutes at 4°C. Then 30 μ L
191 of MACS buffer and 20 μ L of Pan T-Cell Microbead Cocktail was added per each 50 μ L solution
192 and incubated for additional 10 min at 4°C. For the magnetic separation, MACS LS columns

193 (Miltenyi Biotec) were placed in the magnetic field of a MACS separator (Miltenyi Biotec) and
194 the column was rinsed with 3 mL MACS buffer. The cell suspension was added and the flow-
195 through containing the unlabelled, CD3 positive T-cells, was collected. Cells were frozen in
196 Cryostor freezing medium (Biolife Solutions) and stored in liquid nitrogen until the day of the
197 experiments.

198

199 **RNA Sequencing and data analysis**

200 RNA-sequencing datasets have been previously published and are available at the Gene
201 expression omnibus (GEO) repository under the identifiers GSE94035 (MNC, n=5), GSE90711
202 (SC, n=5), GSE90711 (NB primary cultures, n=5 over 3 patient cultures STA-NB-6, STA-NB-7 and
203 STA-NB-15). RNA isolation, library preparation and sequencing on a Illumina Hiseq 2000
204 platform were performed as previously described (Weiss et al., 2016, 2021). Short read
205 sequencing data was quality checked using FASTQC
206 (<http://www.bioinformatics.babraham.ac.uk/projects/fastqc>) and QoRTs (Hartley & Mullikin,
207 2015) and then aligned to the human genome hs37d5 (<ftp://ftp.1000genomes.ebi.ac.uk/>) using
208 the STAR aligner (Dobin et al., 2013) yielding a minimum of 11.6 million aligned reads in each
209 sample. Further analysis was performed in R statistical environment using Bioconductor
210 packages (Gentleman et al., 2004). Count statistics for Ensembl (GRCh37.75) genes were
211 obtained by the “featureCounts” function (package “Rsubread”) and differential expression
212 analysis was performed by edgeR and voom (Law, Chen, Shi, & Smyth, 2014; Ritchie et al.,
213 2015). For differential gene expression analysis only genes passing a cpm (counts per gene per
214 million reads in library) cut-off of 1 in more than two samples were included. All p-values were
215 corrected for multiple testing by the Benjamini-Hochberg method. Genes with an adjusted q-
216 value <0.05 and a log2 fold change > 1 ($|\log_2FC| > 1$) were referred to as ‘significantly regulated’
217 and used for functional annotation analysis via gene set enrichment analysis (GSEA) using
218 MSigDB according to Subramanian, Tamayo, et al. (Subramanian et al., 2005) and Mootha,
219 Lindgren, et al. (Mootha et al., 2003)

220

221 **Phagocytosis assay**

222 5×10^4 enriched p1 hrSCs were seeded per well of an 8-well chamber slide (Ibidi) coated with
223 PLL/laminin and cultured in SCEM. After 48 h, half of the medium was replaced with fresh SCEM
224 containing 1 μm big carboxylate-modified polystyrene, fluorescent yellow-green latex beads

225 (SIGMA-Aldrich) at a concentration of 8×10^6 beads/well (~ 100 beads/cell) for 15 h at 37°C.
226 Thereafter, cells were washed three times with 1x PBS and fixed with Roti-Histofix (Roth) for 10
227 min at RT. Cells were stored at 4°C in 1x PBS until multi-color immunofluorescence staining was
228 performed.

229

230 Immunofluorescence stainings

231 All antibody details, dilutions and incubation times are listed in **Supplementary Table 1**. If not
232 stated otherwise, the staining procedure was performed on RT and a washing step (3 times
233 with 1x PBS for 5 min) was performed after each antibody incubation step, except after
234 permeabilization. For extracellular staining, grown cells were blocked with 1x PBS containing
235 3% goat serum (DAKO) for 30 min at RT, followed by incubation with antibodies against
236 extracellular targets diluted in 1x PBS containing 1% BSA (Sigma-Aldrich) and 1% goat serum.
237 Cells were then incubated with secondary antibodies diluted in 1x PBS containing 1% BSA and
238 1% goat serum. For permeabilization, cells were exposed to 1x PBS containing 1% BSA, 0.3%
239 Triton-X (Sigma-Aldrich) and 3% goat serum for 10 min. Thereafter, cells were incubated with
240 primary antibodies against intracellular targets diluted in 1x PBS containing 1% BSA, 0.1%
241 Triton-X and 1% goat serum, followed by incubation with secondary antibodies diluted in 1X
242 PBS containing 1% BSA, 0.1% Triton-X and 1% goat serum. Afterwards, 2 $\mu\text{g}/\text{mL}$ 4',6-Diamidin-
243 2-phenylindol (DAPI, Sigma-Aldrich) in 1X PBS was added for 2 min followed by a final washing
244 step. Cells were embedded in Fluoromount-GTM mounting medium (Southern Biotech) and
245 stored at 4°C. Images were taken with a confocal laser scanning microscope (Leica
246 Microsystems, TCS SP8X) using Leica application suite X version 1.8.1.13759 or LAS AF Lite
247 software. Confocal images are depicted as maximum projection of total z-stacks and brightness
248 and contrast were adjusted in a homogenous manner using the Leica LAS AF software.

249

250 FACS characterization of hrSCs

251 All antibodies used for flow cytometry stainings are listed in **Supplementary Table 1**. If not stated
252 otherwise, the staining procedure was performed on 4°C. For all phenotyping experiments,
253 cells were cultured in duplicates in each condition. HrSCs were cultured in the presence of IFN γ
254 (10^3 U/mL, Bio-Techne Ltd.), LPS (10 ng/mL, Sigma-Aldrich), Poly:IC (2 $\mu\text{g}/\text{mL}$, Bio-Techne Ltd.)
255 cross-linked CD40L (500 ng/mL, Bio-Techne Ltd.) and IL-1 β (10^4 U/mL Bio-Techne Ltd.) for 24 h.
256 Cells were harvested with Accutase (Sigma-Aldrich) and transferred into FACS tubes containing

257 200 μ L FACS buffer (0.1% BSA and 0.05% natrium acides in 1x PBS). Cells were washed once
258 with FACS buffer at 1200rpm for 5 min, resuspended in 50 μ L FACS buffer and incubated with
259 50 μ L of an antibody master mix in FACS buffer for 30 min in the dark. Then, cells were washed
260 with FACS buffer and resuspended in 100 μ L of Cytofix/Cytoperm solution (BD Biosciences),
261 incubated for 20 min, and again washed with BD 1x perm buffer (BD Biosciences). Next, cells
262 were resuspended in 100 μ L 1x perm buffer containing the S100 antibody and incubated for 30
263 min in the dark. Cells were then washed in 1x perm buffer and resuspended in 100 μ L 1x perm
264 buffer with the secondary antibody for 20 min. After a washing step in 1x perm buffer, cells
265 were washed with FACS buffer and resuspended in 100 μ L FACS buffer. All samples were
266 measured with a FACSFortessa flow cytometer equipped with 5 lasers (355, 405, 488, 561 and
267 640 nm) and the FACSDiva software version 8.0 (BD Biosciences) was used.

268

269 T-cell proliferation assay

270 For all T-cell experiments, hrSCs and freshly thawed human CD3⁺ T-cells were used in various
271 conditions. For IFN γ stimulation, hrSCs were cultured in the presence of 10³ U/mL IFN γ for 24
272 h prior to the T-cell co-culture experiments. For co-culture, p1 hrSCs were harvested, counted
273 and seeded at 4x10⁴ cells per 96 well plates in duplicates. T-cells were thawed, washed once
274 with 1XPBS and centrifuged at 300g for 7 min at RT. Cells were counted and labelled with CFSE
275 (Thermo Fisher) at 1 μ L/10⁷ cells for 10 min at 37°C. Thereafter, 1 mL FCS buffer was added for
276 2 min at RT and then washed with α MEM at 300g for 7 min. Then, cells were FACS sorted for
277 intact cells using a FSC vs SSC gate using the FACS Aria instrument (BD Bioscience). The obtained
278 cells were washed with α MEM at 300g for 7 min, counted and 1x10⁵ cells were seeded to the
279 hrSCs (co-culture) or cultured alone (controls) in 96 well plates. For the T-cell stimulation, 0.25
280 μ L of anti-CD3/CD28 beads (Gibco) were added. Culture medium containing CD3/CD28 beads
281 was thoroughly replenished every 3 days by one half.

282 Cells were analysed via flow cytometry at different time points, i.e. at day 2, 4 and 10.
283 Therefore, cells were harvested with Accutase and washed once with FACS buffer. All
284 antibodies used for flow cytometry stainings are listed in **Supplementary Table 1**. Extracellular
285 staining was performed with 50 μ L of antibody mix containing all extracellular antibodies (CD3-
286 FITC, CD4-PerCP, CD8APC-Cy7, CD25-PE-Cy7) in 50 μ L of FACS buffer for 30 min at 4°C. Cells
287 were washed and incubated in Fix/Perm solution (Thermo Fisher) at 4°C for 30 min. For the
288 permeabilization, 1x perm buffer (Thermo Fisher) was added and cells were centrifuged at 300g

289 for 7 min. Then, the supernatant was discarded and the S100 antibody for intracellular staining
290 was added in 100 μ L 1x perm buffer and incubated for 20 min at RT in the dark. After this, cells
291 were washed once with 1x perm buffer, once with FACS buffer and resuspended in 100 μ L FACS
292 buffer. For exact quantification of absolute cell numbers, 10 μ L AccuCheck Counting Beads
293 (LifeTechnologies) were added to each sample prior to FACS analysis at a FACSFortessa flow
294 cytometer. For data analysis, FACSDiva software version 8.0 was used. Gating for CD4⁺ Th
295 subsets was performed in accordance to Mahnke *et al.* 2013 (Mahnke, Beddall, & Roederer,
296 2013).

297

298 **Protein array**

299 The RayBio G-Series Human Cytokine Antibody Array 4000 Kit (RayBiotech Inc.) was used to
300 assay secretomes of cell supernatants pooled from 2 independent experiments each from hrSC
301 (n=5), hrSCs co-cultured with 5 different NB cell cultures (n=5) or NB cell cultures alone (n=5).
302 A total of 274 factors were evaluated (for a complete list of factors, refer to
303 <https://www.raybiotech.com/human-cytokine-array-g4000-4/>). Arrays were processed
304 according to the manufacturer's instructions. Briefly, protein array membranes were blocked
305 with Blocking Buffer (RayBiotech Inc.) for 30 min at RT. Membranes were then incubated with
306 100 μ L of undiluted sample for 2 h. After extensive washing with Wash Buffer I and II
307 (RayBiotech Inc.) to remove unbound materials, the membranes were incubated with biotin-
308 conjugated antibodies for 2 h at RT. The membranes were then washed and incubated with
309 streptavidin-fluorescein, again for 2 hours at RT, followed by final washing steps. Finally,
310 fluorescence signals were obtained with the GenePix 4000 array scanner (Molecular Devices)
311 using the green channel (Cy3) at an excitation frequency of 532 nm and 700 PMT. The image
312 files generated in this way were aligned to respective .gal files (RayBiotech) and Gene Pix Pro 7
313 (Molecular Devices) was used to create .gpr files. Each spot was manually inspected on the .gpr
314 file images to ensure accuracy. After background correction and normalization to the internal
315 control, the mean fluorescence intensity (MFI) values were combined for all cell lines and
316 proteins that were differentially expressed ($q < 0.05$) between hrSCs and hrSCs in co-cultures,
317 compared to neuronal cells as controls, were selected for visualization using the QluCore Omics
318 Explorer V3.1 software.

319

320 **Quantification and statistical analysis**

321 If not mentioned otherwise, statistical analysis was performed with R version 3.4.2 within the
322 R studio interface including publicly available packages CRAN, GGLOT2, GGBEESWARM and
323 RESHAPE. For pair-wise comparison paired t-tests were used, for multiple comparisons two-
324 way ANOVA using a post-hoc Holm p value correction was used. P values of less than 0.05 were
325 considered significant and displayed as *, p values of less than 0.01 were displayed as **, p
326 values of less than 0.001 were displayed as ***.

327

328 **Data and code availability**

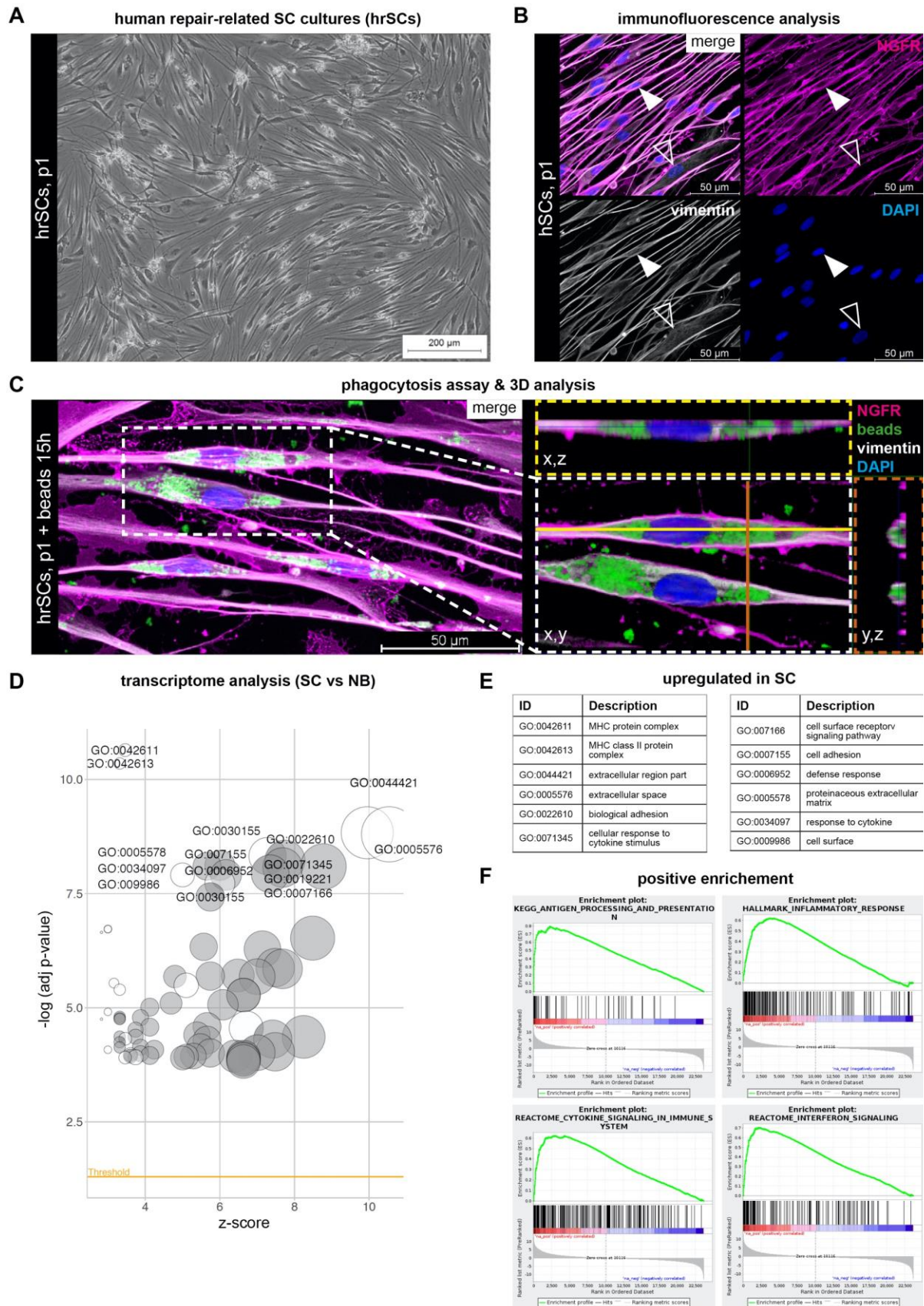
329 No original code has been generated in this study. Original/source data for all figures and
330 supplementary figures are available upon request. RNA-sequencing data are available at the
331 Gene Expression Omnibus (GEO) repository ([Home - GEO - NCBI \(nih.gov\)](#)) under the identifier
332 GSE94A035, GSE90711 and GSE90711.

333

334 **Results**

335 **Human repair-related Schwann cells show a phagocytic capacity**

336 APCs are characterized by their phagocytic ability of exogenous material to process and present
337 antigens via MHCII. To evaluate whether human SCs can take-up material different from myelin,
338 we applied our previously established protocol for the culture of primary SCs from human
339 peripheral nerves (Weiss et al., 2018). As human SCs possess a repair-like phenotype and
340 perform repair-associated functions in culture (Weiss et al., 2016), they are referred to as
341 human repair-related SCs (hrSCs) in the following. The cultured hrSCs showed the typical
342 spindle-shaped morphology with a swirled parallel alignment (**Fig. 1A**) and were characterized
343 by immunostainings for the SC marker NGFR (also known as TNFR16 or p75^{NTR}) (**Fig. 1B**). The co-
344 staining for vimentin, an intermediate filament expressed by SCs and fibroblasts, visualized a
345 straight filament network within the long hrSC processes and a more branched appearance in
346 fibroblasts (**Fig. 1B**). To obtain information about their phagocytic capacity, we challenged the
347 hrSCs with green fluorescent latex beads (1 μm diameter) for 15 hours and stained the cultures
348 for NGFR and vimentin. 3D confocal image analysis demonstrated that hrSCs were able to
349 phagocytose the beads and to accumulate them within the cell body (**Fig. 1C**).



350 Figure 1 | Phagocytosis potential and inflammatory response of hrSCs. (A) Phase contrast image of a
 351 representative passage 1 (p1) hrSC culture. (B) Immunostaining of p1 hrSCs for SC marker NGFR
 352 (magenta), intermediate filament vimentin (grey) and nuclear stain DAPI (blue); arrowheads indicate a

353 NGFR negative and vimentin positive fibroblast. **(C)** 3D confocal image analysis of hrSCs exposed to 1
354 μm in diameter green fluorescent latex beads for 15 hours. Cross sections show internalized beads
355 within the SC cytoplasm. **(D)** Gene ontology (GO) analysis of differentially expressed genes by RNA-seq
356 between hrSCs (n=5) and NB cultures (n=5 independent biological replicates of 3 donors), $-\log_{10}$ of the
357 enrichment p values (cut-off <0.05) for filtered GO categories are plotted relative to Z-scores of average
358 ratios in each category. Circle size represents the fraction of regulated genes per GO term. **(E)** Top 12
359 GO terms among genes upregulated in hrSCs vs NB cultures. **(F)** Gene set enrichment analysis (GSEA)
360 plots of hrSCs compared to NB cultures. Source data are provided in **Suppl. Tables 2-4**.

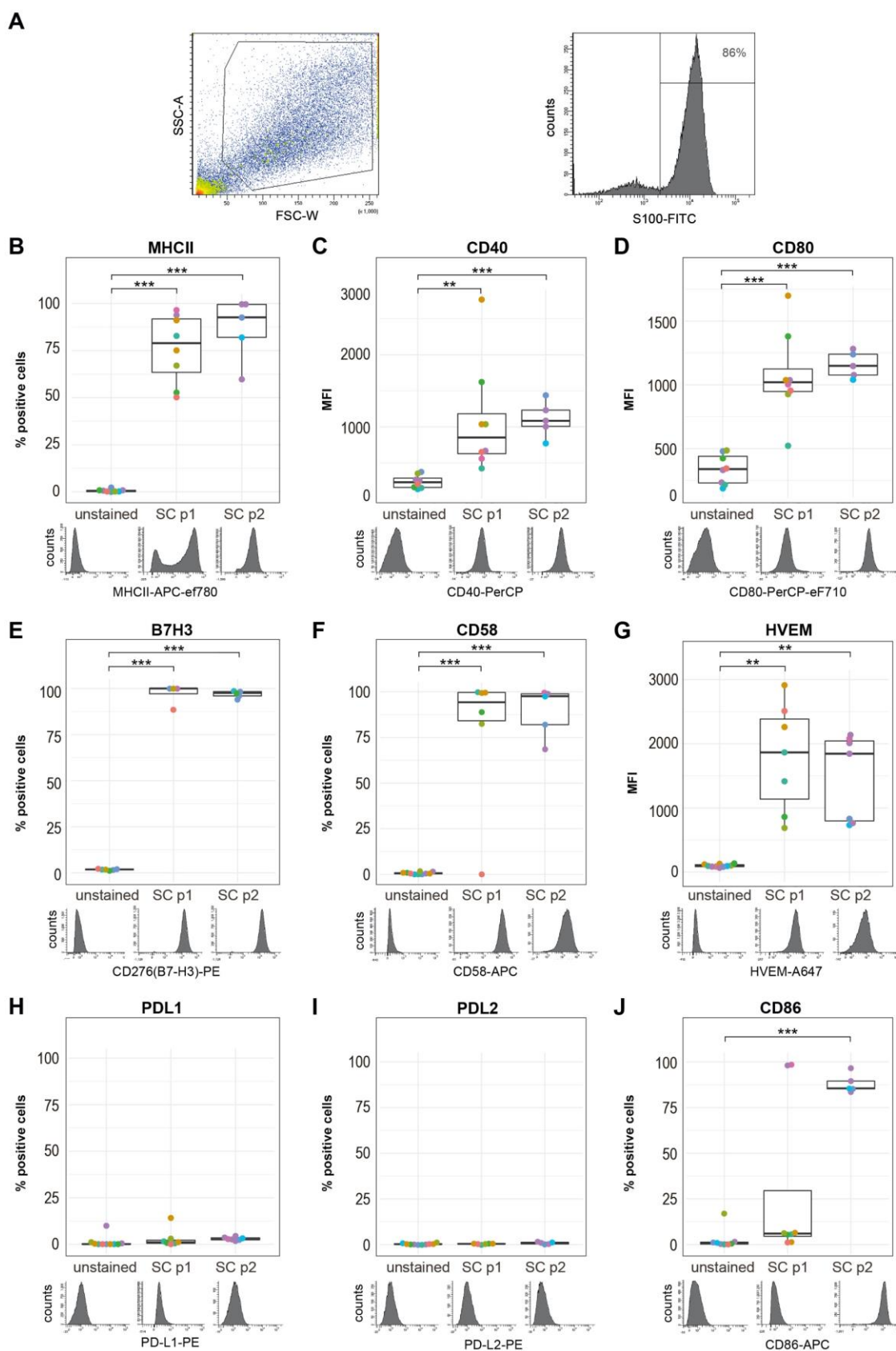
361

362 **Transcriptome profiling of human repair-related Schwann cells** 363 **revealed immunomodulatory gene signatures**

364 The phagocytic capacity of hrSCs towards cell-extrinsic material prompted us to evaluate
365 whether pathways associated with inflammation and antigen presentation were active in
366 hrSCs. To this end, we interrogated the transcriptome data generated by deep RNA-sequencing
367 (RNA-seq) of hrSC cultures (n=5) and of neuroblastic tumor cells (NB cells) (n=5), which serve
368 as models for neuronal cells. Comparison of the transcriptomes of hrSCs and NB cells revealed
369 5822 differentially expressed genes ($q\text{-value}<0.01$, $|\log_2\text{FC}>1|$), of which 3057 were
370 upregulated and 2754 were down-regulated in hrSCs (**Suppl. Table 2**). Subsequent functional
371 annotation analysis of genes unique to hrSCs demonstrated gene ontology (GO) terms
372 prominent in MHC class I and class II protein complexes, cellular response to cytokine stimulus,
373 and cytokine mediated signaling pathways (**Fig. 1D, Suppl. Table 3**). These results were further
374 supported by a gene set enrichment analysis (GSEA), which confirmed the enrichment of genes
375 associated with antigen processing and presentation alongside with cytokine signaling and an
376 inflammatory response in hrSCs (**Fig. 1E, Suppl. Table 4**). Taken together, transcriptome profiling
377 of hrSCs provides further evidence of cytokine signaling and upregulation of MHCII in response
378 to nerve damage.

379 **Human repair-related SCs express MHCII and the co-signaling**
380 **molecules CD40, CD80, CD86, CD58, HVEM and B7-H3**

381 As the primary function of APCs is to modulate T-cell activation through MHCII and the
382 expression of co-signalling molecules, we further investigated whether the latter can be found
383 on hrSCs. Therefore, we cultured hrSCs from eight different donor nerves and used flow
384 cytometry to profile the expression of MHCII and selected co-signalling molecules. As the
385 interaction of SCs with immune cells has been described as a dynamic process (Gold, Zielasek,
386 Kiefer, Toyka, & Hartung, 1996), the analysis was performed at two time points, in passage one
387 and passage two hrSC cultures. SC identity was determined by S100 expression, a well-
388 established SC marker, and showed that mean purity of hrSCs cultures was 82% in passage one
389 (p1) and 70% in passage 2 (p2) (**Fig. 2A**). The S100 negative cells, presumably nerve-associated
390 fibroblasts, were excluded from further analysis (**Fig. 2A**). In the S100 positive hrSCs, we
391 quantified the surface expression of MHCII and co-signalling molecules CD40, CD80, CD86, B7-
392 H3, HVEM, PD-L1, PD-L2 and CD58. About 76% and 87% of hrSCs were positive for MHCII in p1
393 and p2, respectively (**Fig. 2B**), which is in line with our previously published observation that
394 MHCII expression increased with prolonged culture time (Weiss et al., 2016). Further, p1 as
395 well as p2 hrSCs demonstrated expression of CD40, CD80, B7-H3, CD58, and HVEM (**Fig. 2C-G**).
396 In contrast, neither PD-L1 nor PD-L2 were detected in p1 or p2 hrSC (**Fig. 2H-I**). Interestingly,
397 most of p1 hrSCs were negative for CD86, while it was significantly upregulated in p2 cells (**Fig.**
398 **2J**). Hence, hSCs indeed express - next to MHCII - several canonical co-signalling molecules that
399 are required for T-cell activation and inhibition.



400
401
402
403

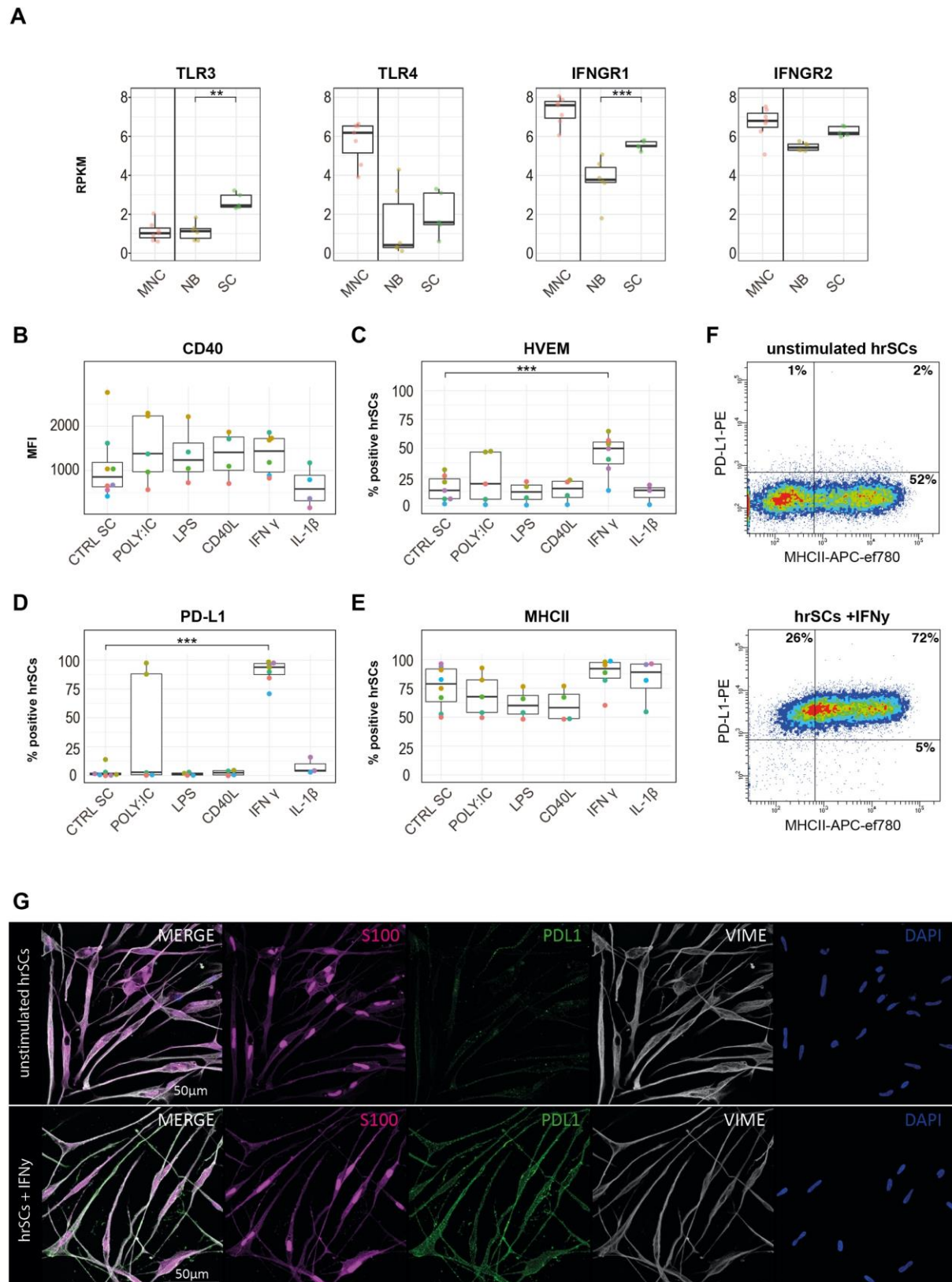
Figure 2 | Flow cytometry phenotyping of MHCII and co-signaling molecules present on human repair-related Schwann cells. (A) Gating strategy for the identification of S100 positive hrSCs illustrated for one representative experiment. Intact cells are gated in the FSC vs SSC blot and S100 positive cells were

404 selected for further analysis. **(B-J)** Box plots show the expression status of MHCII and co-signaling
405 molecules CD40, CD80, B7H3, CD58, HVEM, PD-L1, PD-L2 and CD86 of S100 positive hrSCs in passage 1
406 (p1) and p2; technical replicates (same color); biological replicates (different color). The histograms
407 underneath depict one representative experiment. Each biological replicate is conducted with hrSCs
408 isolated from a different donor nerve. **(B, E, F, H, J)** Box plots represent the percentage of positive cells
409 based on gates set in relation to unstained controls as displayed in the histograms. **(C, D, G, I)** Boxplots
410 represent the mean fluorescence intensity (MFI). Boxes contain 50% of data and whiskers the upper
411 and lower 25%; means are displayed as black horizontal lines. A two-way ANOVA using a post-hoc Holm
412 p value correction was performed. * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$

413

414 **The stimulation of TLR3 and TLR4 had no effect on the expression of** 415 **co-signaling molecules in human repair-related Schwann cells**

416 Professional as well as non-professional APCs can upregulate the expression of co-signaling
417 molecules upon toll-like receptor (TLR) ligation (Chen & Flies, 2013; Fitzgerald & Kagan, 2020;
418 Mehrfeld, Zenner, Kornek, & Lukacs-Kornek, 2018). Our RNA-seq data of hrSCs showed
419 enrichment in TLR signaling in comparison to neuronal cells (**Suppl. Table 4**), which motivated
420 us to explore the expression of co-signaling molecules in response to inflammatory stimulation.
421 First, we investigated which TLRs are expressed by hrSCs, to choose the corresponding ligands
422 for further analysis. We found elevated expression levels of TLR1, TLR3, TLR4 and TLR6 mRNA
423 (**Fig. 3A, Suppl. Fig. 1**). As TLR1 and TLR6 mainly function as heterodimers with TLR2, which was
424 not expressed, we focused on TLR3 and TLR4. We thus stimulated p1 hrSC with the TLR4 agonist
425 LPS and TLR3 agonist POLY:IC for 24 hours and subsequently analysed the expression status of
426 co-signalling molecules (**Fig. 3A, Suppl. Fig. 1**). Interestingly, neither the addition of LPS nor
427 POLY:IC caused a significant expression change of the analyzed co-signaling molecules in hrSCs
428 (**Fig. 3B-E**), which might be due to the low expression levels of TLR3 and TLR4 (**Fig.3A**). Upon
429 stimulation with POLY:IC a trend towards upregulation of CD40 and HVEM was seen, but
430 substantial donor variance was observed (**Fig. 3B-E**). CD40 is not only a co-stimulatory molecule,
431 but also a molecule that facilitates the activation of APCs upon binding of CD40-ligand (Chen &
432 Flies, 2013). CD40-ligand did, however, not affect the expression of CD40 or any other co-
433 signaling molecules tested (**Fig. 3B**). Together these data show that TLR and CD40 ligation do
434 not affect the expression of co-signaling molecules.



435
436
437
438
439

Figure 3 | Immunophenotyping of human repair-related Schwann cells upon toll-like receptor and cytokine stimulation. (A) Enrichment of toll-like receptor (TLR)-related pathways. GSEA of RNA-seq data sets of hrSCs compared to NB cultures. Box plots show the mRNA expression in reads per kilobase million (RPKM) of TLR3, IFNGR1 and IFNGR2 by RNA-seq in NB cultures (NB, n=5) and hrSCs (SC, n=5). Bone

440 marrow mononuclear cells (MNC, n=5) are shown as reference. **(B-E)** FACS analysis of p1 cultures of
441 hrSCs stimulated with POLY:IC, LPS, CD40L, IFN γ and IL-1 β for 24 h. Box plots show the MFI of CD40 (B),
442 percentage of HVEM (C), PD-L1 (D) and MHCII (E) positive hrSCs in p1 cultures stimulated with POLY:IC,
443 LPS, CD40L, IFN γ and IL-1 β . Each biological replicate is conducted with hrSCs isolated from a different
444 donor nerve. Boxes contain 50% of data and whiskers the upper and lower 25%. Means are displayed
445 as black horizontal lines. All experiments were performed in at least 4 independent biological replicates.
446 A two-way ANOVA using a post-hoc Holm p value correction was performed; * $p \leq 0.05$; ** $p \leq 0.01$;
447 *** $p \leq 0.001$. **(F)** Representative FACS plots showing PD-L1 vs. MHCII expression of p1 hrSCs either
448 unstimulated (upper plot) and after IFN γ stimulation. **(G)** Immunofluorescence image of p1 hrSCs at day
449 2 after purification without (upper panels) or with (lower panels) IFN γ stimulation. HrSC cultures are
450 stained for S100 (magenta), PD-L1 (green), vimentin (grey) and DAPI (blue).

451

452 **Human repair-related Schwann cells upregulate HVEM and PD-L1 upon** 453 **stimulation with IFN γ**

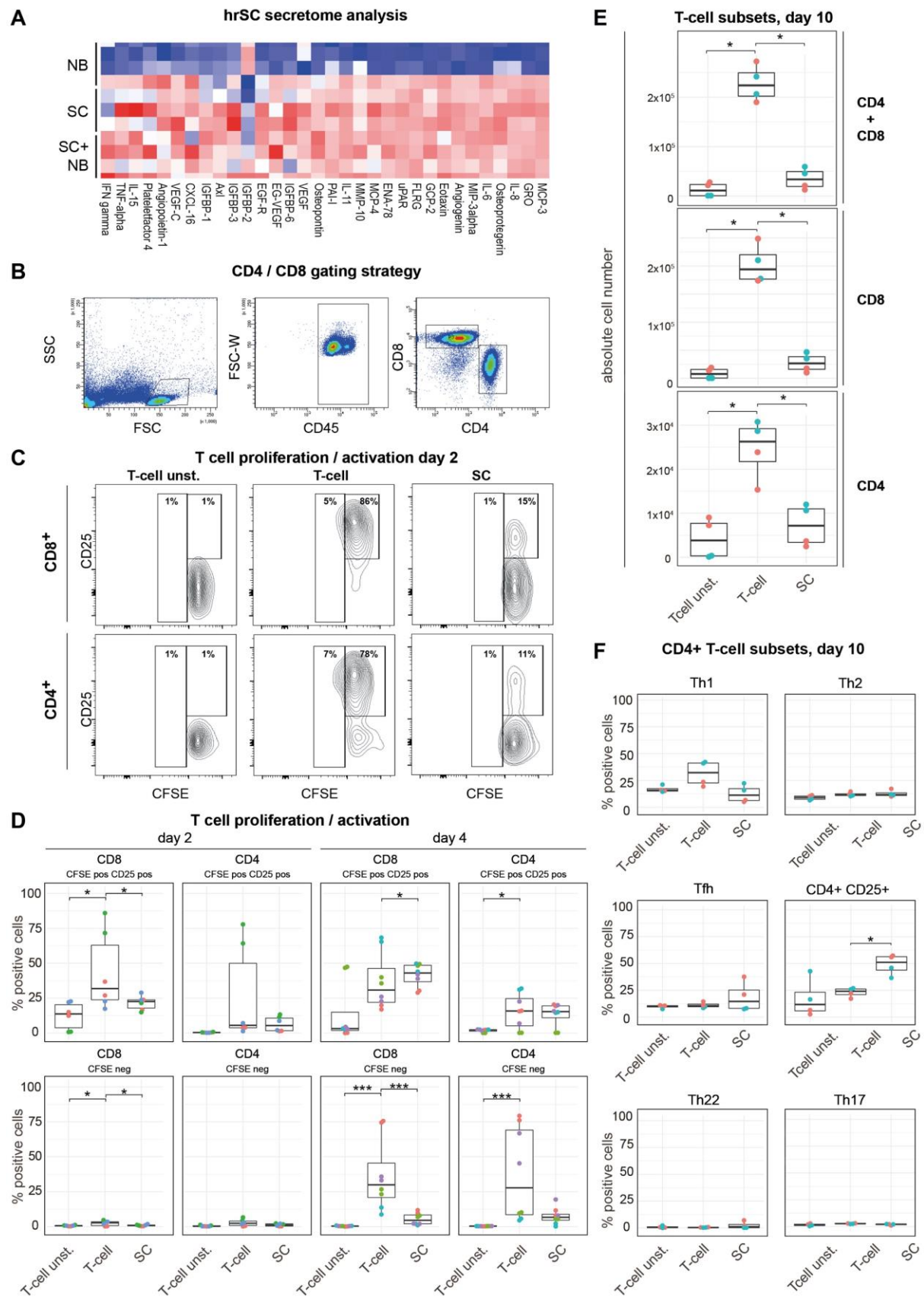
454 Inflammatory processes in peripheral nerve tissues as well as upon injury responses involve the
455 release of pro-inflammatory mediators such as IFN γ and IL-1 β by macrophages (Chiu, Von
456 Hehn, & Woolf, 2012; Yao, Graham, Akahata, Oh, & Jacobson, 2010). Notably, we found that
457 the expression of both IFN γ genes, *IFNGR1* and *IFNGR2*, was also upregulated in hrSC in
458 comparison to neuronal cells (**Fig. 3A**). We further investigated the response of hrSCs to IFN γ
459 as well as IL-1 β . While IL-1 β did not alter the expression of MHCII and any of the co-stimulatory
460 molecules tested, IFN γ led to a significant increase in HVEM expression (**Fig. 3C**). In addition,
461 IFN γ stimulation induced a profound upregulation of PD-L1 (**Fig. 3D**). PD-L1 expression in
462 response to IFN γ was further validated on stimulated hrSC using multicolor
463 immunofluorescence stainings for S100, PD-L1 and vimentin (**Fig. 3G**). Concordant with our flow
464 cytometry data, unstimulated p1 hrSC did not show a notable PD-L1 staining, whereas IFN γ
465 stimulation strongly induced PD-L1 protein expression (**Fig. 3G**). These findings show that hrSCs
466 can respond to IFN γ , but not to IL-1 β , by a significant up-regulation of the co-stimulatory
467 molecule HVEM and the immune check-point molecule PD-L1.

468

469 **Secretome analysis of human repair-related Schwann cells reveals a** 470 **broad spectrum of immunoactive mediators**

471 As the inducible expression of co-signalling molecules by hrSCs together with their well-
472 described function to recruit macrophages and neutrophils (Stratton et al., 2016; Tzekova,

473 Heinen, & Küry, 2014) points towards their active involvement in shaping a local immune
474 response upon nerve injury, we further investigated a panel of immunoregulatory molecules
475 secreted by hrSC. In order to model the *in vivo* situation following nerve injury, supernatants of
476 hrSCs cultured in the absence or presence of neuronal cells and neuronal cell control cultures
477 were analysed. Secretome analysis was performed using a protein array able to detect 274
478 different secreted factors. A total of 84 secreted molecules were unique to hrSCs and one was
479 only secreted by NB cells ($q < 0.05$). None of the 84 proteins was differentially secreted in the
480 SC-NB co-culture model as compared to SCs alone. We therefore considered these secreted
481 proteins to be derived from hrSCs and further compared those to secreted proteins obtained
482 from NB cultures (**Suppl. Table 5**). HrSC secreted factors included interleukins IL-6, IL-11, IL-15,
483 TNF α , IFN γ , molecules involved in phagocyte attraction and activation such as MCP-3, MCP-4
484 or CXCL-16, molecules for neutrophil attraction and activation such as GRO, MIP3-alpha, IL-8
485 (CXCL-8) or ENA-78 (**Fig. 4A**). Many of these molecules also directly acting on lymphocytes (**Fig.**
486 **4A**), for example IL-6 that is stimulating the proliferation of antibody producing B-lymphocytes
487 or IL-15 stimualting T-and NK- cell proliferation. Interestingly, hrSCs secreted osteopontin, a
488 molecule involved in multiple processes including the induction of IFN γ through NF- κ B
489 activation (Icer & Gezmen-Karadag, 2018). These findings demonstrate the plethora of
490 immunoactive mediators secreted by hrSCs and suggests autocrine activity as well as paracrine
491 modulation of myeloid cells and lymphocytes.



492
493
494
495

Figure 4 | HrSCs secrete immunoinhibitory mediators and inhibit allogeneic T-cell activation. (A) Secretome analysis by antibody array. Heatmap displays the top 32 differentially secreted proteins ($q < 0.05$, $|\log_2FC > 0.3$) of hrSCs ($n=5$) and SC-NB co-cultures ($n=5$) vs. NB cultures (as neuronal cell model) ($n=5$).

496 (B-F) Allogeneic CD3⁺T-cells were cultured for 2, 4 or 10 days in the absence (Tcell) or presence of hrSCs
497 (SC) and stimulated with anti-CD3/CD28 beads and analysed by flow cytometry. As control unstimulated
498 T-cells (Tcell unst.) were cultured. **(B)** Representative FACS plots show the gating strategy for CD4⁺ and
499 CD8⁺ T-cells. **(C)** Representative FACS plots showing CD25 expression against CFSE of T-cells at day 2 of
500 co-cultivation and in control cultures. **(D)** Boxplots show the absolute number of CFSE⁺/CD25⁺ and CFSE⁻
501 CD4⁺ and CD8⁺ T-cells at day 2 and day 4 based on gates set as illustrated in **(B-C)**. **(E)** Boxplots show the
502 absolute number of alive CD4⁺, CD8⁺ or combined CD4⁺and CD8⁺ cells and **(F)** percentage of CD4⁺ subsets
503 evaluated via flow cytometry at day 10 based on gates set as published by (Mahnke et al., 2013). **(D-F)**
504 Boxes contain 50% of data and whiskers the upper and lower 25% Means are displayed as black
505 horizontal lines. All experiments were performed in at least 3 independent biological replicates. A two-
506 way ANOVA using a post-hoc Holm p value correction was performed; * p ≤ 0.05; ** p ≤ 0.01; *** p ≤
507 0.001.

508

509 **Human repair-related Schwann cells reduce proliferation of allogeneic** 510 **T-cells**

511 Based on the overlapping features of hrSCs and APCs, i.e. phagocytosis, expression of MHCII,
512 co-signaling and immune checkpoint molecules, and their inducible upregulation and secretion
513 of T-cell modulatory molecules, we next asked whether hrSCs might affect T-cell activation and
514 fate in the nerve injury context. Thus, we performed co-culture assays of hrSCs and allogeneic
515 T-cells. More specifically, we used T-cells from healthy donors stimulated with anti-CD3/CD28
516 beads to simulate an inflammatory environment similarly to peripheral nerve injury. We then
517 evaluated the impact of co-culture assessing the total number of activated (CFSE⁺CD25⁺) and
518 proliferated (CFSE^{-dim}) CD4⁺ and CD8⁺ T-cells **(Fig. 4B-C)**. At day 2 stimulated T-cells showed a
519 significant increase in CFSE⁺CD25⁺ and to a minor extend proliferated (CFSE^{-dim}) CD8⁺ T-cells
520 whereas the presence of hrSCs in co-culture significantly reduced this effect **(Fig. 4D)**. At day 4,
521 stimulation of T-cells resulted in an increase of both, CD8⁺ and CD4⁺, CFSE⁺CD25⁺ as well as
522 proliferated (CFSE^{-dim}) fraction. While proliferation was significantly reduced in CD8⁺ T-cells by
523 the presence of hrSCs in co-culture, CFSE⁺CD25⁺ cells slightly increased **(Fig. 4D)**. A similar trend
524 was observed in the CD4⁺ population **(Fig. 4D)**. This effect became even more apparent at day
525 10, when the total number of CD8⁺ as well as CD4⁺ T-cells was reduced to numbers comparable
526 with those of unstimulated T-cells. **(Fig. 4E)**. Further, we interrogated the CD4⁺ T-cell population
527 for a potential shift among T-helper subpopulations, i.e. Th1, Th2, Tfh, Th17 and Th22, using a
528 13-plex flow cytometry panels as previously published by Mahnke et al (Mahnke et al., 2013).

529 Notably, there was no clear trend towards a specific CD4⁺ Th subset, yet a significantly higher
530 percentage of CD4⁺CD25⁺ cells was detected (**Fig. 4F**). In summary, hrSCs delayed proliferation
531 of CD8⁺ and CD4⁺ T-cells, while promoting the long-term survival or potential switch towards a
532 CD4⁺CD25⁺ phenotype.

533

534 Discussion

535 Building on our previous characterization of the transcriptome and proteome of human repair-
536 type SCs (Weiss et al., 2016) and studies on SCs and nerve inflammation in rodents (Hartlehnert
537 et al., 2017; Meyer zu Hörste et al., 2010), we aimed to provide novel insight into the
538 immunocompetence of human SCs in an injury condition. Using primary human repair-related
539 SCs, hrSCs, as a unique model to study their immunophenotype and associated functional
540 aspects, our study presents several layers of evidence that hrSCs possess features and functions
541 of APCs and are capable of mediating T-cell dependent immunity. We demonstrate that hrSCs
542 can express CD40, CD80, B7H3, CD58, CD86, HVEM and PD-L1 in addition to MHCII, secrete
543 numerous immunomodulatory molecules, and inhibit allogeneic T-cell activation. It is well
544 accepted that many functions of professional APCs, including the presentation of antigen via
545 MHCII, the expression of co-signalling molecules and the secretion of anti- and pro-
546 inflammatory molecules, are also carried out by non-professional APCs such as mast cells,
547 eosinophils, and non-hematopoietic cells like epithelial cells (Kambayashi & Laufer, 2014;
548 Schuijs, Hammad, & Lambrecht, 2019). With this study, we add compelling evidence that hrSCs
549 could also act as non-professional APCs that may modulate the inflammatory processes within
550 injured nerves. Studying the interaction of primary hrSCs and T-cells allowed the development
551 of a broadly applicable functional *in vitro* model that contributes to the ongoing research in the
552 field of neuroinflammatory disorders, regenerative medicine and immune oncology.

553

554 Human repair-related SCs possess features of antigen presenting cells

555 In line with our previous studies we show that hrSCs actively perform phagocytosis and express
556 MHCII on their surface (Weiss et al., 2016). It has been recently described that rodent SCs have
557 the ability to regulate MHCII dependent immunity, as the deletion of MHCII lead to a decreased
558 infiltration of CD4⁺ cells to the site of nerve injury (Hartlehnert et al., 2017). This suggests a

559 functional necessity of MHCII expression in nerve repair. The ability of SCs to present ingested
560 molecules via MHCII and shape an immune response has been suggested before (Baetas-da-
561 Cruz et al., 2009; Meyer Zu Horste et al., 2010; Steinhoff & Kaufmann, 1988; Van Rhijn et al.,
562 2000), yet more detailed studies were needed to understand the role of SCs in phagocytosis,
563 antigen presentation and immune cell modulation in human SCs.

564 It is well-established that not only classical APCs but also non-professional APCs, like mast cells
565 or epithelial cells, are capable of phagocytosis and antigen presentation via MHC II to CD4+ T-
566 cells (Kambayashi & Laufer, 2014; Schuijs et al., 2019). As non-professional APCs of non-
567 hematopoietic origin do not primarily migrate to lymph nodes, their role in priming naïve T-
568 cells may be less relevant, but their modulation of a local T-cell responses is widely accepted
569 (Kambayashi & Laufer, 2014). Thus, the expression of co-signalling molecules alongside with
570 MHCII and the secretion of other immunomodulatory molecules defines the effect of non-
571 professional APCs in different tissues and conditions. In this study, we show that hrSC are able
572 to express the co-signaling molecules CD58, CD80, and CD86 in addition to MHCII. These
573 molecules are associated with an activation of T-cells (Chen & Flies, 2013; Greenwald, Freeman,
574 & Sharpe, 2004).

575 Interestingly, a study comparing nerve biopsies of healthy patients and patients with chronic
576 inflammatory demyelinating polyneuropathy (CIDP) identified that CD58 expressing SCs were
577 only found in the latter (Van Rhijn et al., 2000). This could be due to a similarity in the role of
578 SCs after nerve injury and during the interaction with immune cells in autoimmune diseases.
579 However, Van Rhijn et al. did not observe CD86 or CD80 expressing SCs in healthy or CIDP
580 patients, which indicates that the expression of co-signaling molecules detected in our *in vitro*
581 model reflects a unique feature of hrSCs. Of note, our primary human SCs were isolated from
582 peripheral nerves obtained after amputational surgeries and knowledge on pre-existing
583 conditions such as non-diagnosed neuropathies and medication are limited. However, we
584 observed consistent and reproducible effects throughout our molecular, phenotypic and
585 functional characterization of hrSCs. Future studies should combine the knowledge derived
586 from human nerve biopsies and isolated human SCs to validate the immunomodulatory
587 capacities of this unique cell type in nerve injury and different diseases.

588 In addition to surface expression of co-signalling molecules, we found that hrSC secrete a
589 variety of immunomodulators. This is in line with previous studies on human SCs that
590 demonstrated the secretion of IL-6, IL-8, IL-15 and MCP-1 (Ozaki, Nagai, Lee, Myong, & Kim,

591 2008; Rutkowski et al., 1999). Our study enriches the repertoire of secreted hrSC molecules by
592 cytokines like IL-11 and chemoattractants like MCP-3, MCP-4, CXCL-16, GRO and MIP3 α
593 suggesting an unexpected functional diversity. In contrast to Rutkowski et al., 1999, we did not
594 detect the expression of IL-1 β in our assay (Rutkowski et al., 1999). Taken together, these
595 findings demonstrate that hrSCs express the co-signalling molecules CD58, CD80 and CD86
596 together with MHCII and provide novel insight into the repertoire of hrSC secreted molecules,
597 beyond neurotrophins, with immunoregulatory functions.

598

599 **Inhibition of allogeneic T-cell activation - evidence for an immuno-** 600 **regulatory function of human repair SCs**

601 Furthermore, we show that the exposure to hrSCs causes a delayed or even abrogated CD4⁺ T-
602 cell proliferation and activation. In line with this finding, we demonstrate that SCs express co-
603 inhibitory molecules such as B7-H3, HVEM and provide the first report that hrSC upregulate
604 PD-L1 after stimulation with IFN γ . As not only the activation, but also the termination and
605 resolution of inflammation through surface expression of inhibitory molecules is a hallmark of
606 APCs, the presence of these molecules in hrSCs is remarkable. The source for IFN γ release in
607 inflammatory tissues are mainly NK, CD4⁺ and CD8⁺ T-cells as well as macrophages. HrSCs may
608 even trigger the release of IFN γ via secreted osteopontin that has been shown to induce IFN γ
609 production in T-cells (Icer & Gezmen-Karadag, 2018). In this study we show that hrSCs are also
610 capable of IFN γ secretion. Whether the autocrine production of IFN γ by hrSCs or the paracrine
611 IFN γ released by other cell types present at the site of nerve injury induces the surface
612 expression of PD-L1 on hrSCs remains to be determined.

613 We further observed that delayed T-cell activation was not accompanied by a shift towards any
614 particular T helper subset, but rather resulted in CD4⁺ T-cells with high CD25 expression, which
615 may represent a regulatory or exhausted phenotype. The hypothesis of regulatory/exhausted
616 T-cells is supported by previous observations in rodent models (Meyer zu Horste et al., 2014;
617 Meyer zu Hörste et al., 2010; F.-J. Wang, Cui, & Qian, 2018; X. Wang et al., 2014). Similarly, it
618 has been shown that non-professional APCs like type II alveolar epithelial cells can prime
619 antigen specific CD4⁺ T-cells towards regulatory T-cells (Kambayashi & Laufer, 2014). This
620 suggests that hrSCs in their activated state might, despite high MHCII, CD40, CD80 and CD58
621 expression and secretion of pro-inflammatory cytokines IL-6, IL-8, TNF α and IFN γ , adopt an
622 antigen presenting cell phenotype similar to M2 macrophages, which tightly control and

623 terminate T-cell responses via B7-H3 and the PD-L1/PD-1 axis. Thus, a balance between the
624 initiation and termination of an immune response may be essentially controlled through repair
625 SCs during the multistep process of nerve regeneration.

626 The expression of PD-L1 by hrSCs after stimulation with IFN γ supports the idea of a time and
627 situation dependent role of repair SCs. It is tempting to speculate that repair SCs initiate the
628 termination of the inflammatory response they helped to induce as first responders to nerve
629 injury. Our data suggest that repair SCs might possess an immunoregulatory function that could
630 prevent unnecessary damage to the neuronal environment by terminating an exceeding
631 immune response, in which large amounts of IFN γ are produced by immune cells recruited to
632 the site of injury. To address this possibility, deeper phenotypic and functional characterization
633 of especially CD4+CD25+ T-cells *in vitro/ex vivo* will be required in the future. Furthermore,
634 manipulating the balance of pro- and anti-inflammatory profile of repair SCs might represent a
635 novel therapeutic target in regenerative and pathological processes.

636

637 **Implications for the field of neuro-inflammatory disorders,** 638 **regenerative medicine and immune oncology**

639 As the interaction between SCs and immune cells is of importance not only after nerve injury
640 but also regarding infectious, inflammatory and autoimmune disease of the peripheral nervous
641 system, a comprehensive understanding of this interaction is crucial. It has recently been
642 suggested that the activation of T-helper cells via MHCII by SC promotes neuropathic pain and
643 axonal loss after nerve injury in mice (Hartlehnert et al., 2017). In this regard the presented
644 panel of co-signaling molecules provides additional pharmaceutical targets to tackle this
645 interaction. In addition, it has been shown that rats with experimental autoimmune neuritis, a
646 common model for Guillain-Barré syndrome, showed a clinical improvement, reduced neuronal
647 lymphocyte infiltration and a shift towards regulatory T-cells in the peripheral blood after
648 administration of PD-L1 (Ding et al., 2016).

649 Importantly, it could be shown that SCs play a fundamental role in certain immune-oncological
650 processes. SCs with a repair-related phenotype (including MHCII expression) are attracted by
651 favorable forms of peripheral neuroblastic tumors, neuroblastomas, of a genetic subtype and
652 trigger tumor cell maturation/differentiation and apoptosis, a phenomenon which could also
653 be recapitulated in *in vitro* experiments (Weiss et al., 2021). These tumors, in comparison to
654 their malignant counterpart, frequently show prominent MHCII+ and CD3+ immune cell

655 infiltrates (Ambros et al., 1996; Weiss et al., 2021). It will be interesting to study the
656 composition of these infiltrates and whether and how SCs contribute to their recruitment and
657 modulation.

658
659 In summary, we here provide *in vitro* evidence that human SCs in an injury condition adopt
660 functions of APCs, i.e, phagocytosis, up-regulation of MHCII and co-signaling molecules,
661 secretion of an array of immunoregulatory molecules, and repression of T-cell activation. Our
662 data suggest that repair SCs can participate in the termination of the inflammatory response to
663 prevent excessive tissue damage and allow nerve regeneration. The molecules expressed and
664 secreted by hrSC presented in this study will help to understand their complex interplay with
665 immune cells after injury and in disease.

666

667 Author contributions

668 S.T.-M. conceptualized the project; J.B., T.W. and S.T.-M. planned experiments, performed
669 research, analyzed and interpreted data and wrote the manuscript; H.S. and F.R. performed
670 research and analyzed data; M.K. developed bioinformatics tools and analyzed data; A.D. and
671 P.S. provided essential reagents, planned experiments and interpreted data; R.W. provided
672 essential material; P.F.A. and I.M.A interpreted data; all authors reviewed the manuscript.

673

674 Acknowledgements

675 This study was supported by the Herzfeldersche Familienstiftung (Grants to S. Taschner-
676 Mandl), Modicell (MC-IAPP Project 285875, to S. Taschner-Mandl), FFG Visiomics (Project
677 10959423 to S. Taschner-Mandl) and FWF Liquidhope (Project I4162 to S. Taschner-Mandl) and
678 St. Anna Kinderkrebsforschung. We are also grateful to Dieter Printz and the FACS core facility
679 (Children's Cancer Research Institute) for excellent technical support.

680

681 Conflict of Interest Statement

682 The authors declare no conflict of interest.

683

684 References

- 685 Ambros, I M, Rumpler, S., Luegmayr, A., Hattinger, C. M., Strehl, S., Kovar, H., ... Ambros, P. F.
686 (1997). Neuroblastoma cells can actively eliminate supernumerary MYCN gene copies by
687 micronucleus formation--sign of tumour cell revertance? *European Journal of Cancer*
688 (Oxford, England : 1990), 33(12), 2043–2049. [https://doi.org/10.1016/s0959-](https://doi.org/10.1016/s0959-8049(97)00204-9)
689 8049(97)00204-9
- 690 Ambros, Ingeborg M., Zellner, A., Roald, B., Amann, G., Ladenstein, R., Printz, D., ... Ambros, P.
691 F. (1996). Role of Ploidy, Chromosome 1p, and Schwann Cells in the Maturation of
692 Neuroblastoma. *New England Journal of Medicine*, 334(23), 1505–1511.
693 <https://doi.org/10.1056/NEJM199606063342304>
- 694 Armati, P. J., Pollard, J. D., & Gatenby, P. (1990). Rat and human Schwann cells in vitro can
695 synthesize and express MHC molecules. *Muscle & Nerve*, 13(2), 106–116.
696 <https://doi.org/10.1002/mus.880130204>
- 697 Azam, S. H., & Pecot, C. V. (2016). Cancer's got nerve: Schwann cells drive perineural invasion.
698 *The Journal of Clinical Investigation*, 126(4), 1242–1244.
699 <https://doi.org/10.1172/JCI86801>
- 700 Baetas-da-Cruz, W., Alves, L., Pessolani, M. C. V, Barbosa, H. S., Régnier-Vigouroux, A., Corte-
701 Real, S., & Cavalcante, L. a. (2009). Schwann cells express the macrophage mannose
702 receptor and MHC class II. Do they have a role in antigen presentation? *Journal of the*
703 *Peripheral Nervous System : JPNS*, 14(2), 84–92. [https://doi.org/10.1111/j.1529-](https://doi.org/10.1111/j.1529-8027.2009.00217.x)
704 8027.2009.00217.x
- 705 Bergsteinsdottir, K., Kingston, A., Mirsky, R., & Jessen, K. R. (1991). Rat Schwann cells produce
706 interleukin-1. *Journal of Neuroimmunology*, 34(1), 15–23. [https://doi.org/10.1016/0165-](https://doi.org/10.1016/0165-5728(91)90094-N)
707 5728(91)90094-N
- 708 Biedler, J. L., Helson, L., & Spengler, B. A. (1973). Morphology and growth, tumorigenicity, and
709 cytogenetics of human neuroblastoma cells in continuous culture. *Cancer Research*,
710 33(11), 2643–2652.
- 711 Biedler, J. L., Roffler-Tarlov, S., Schachner, M., & Freedman, L. S. (1978). Multiple
712 neurotransmitter synthesis by human neuroblastoma cell lines and clones. *Cancer*
713 *Research*, 38(11 Pt 1), 3751–3757.

- 714 Bunimovich, Y. L., Keskinov, A. A., Shurin, G. V., & Shurin, M. R. (2017). Schwann cells: a new
715 player in the tumor microenvironment. *Cancer Immunology, Immunotherapy : CII*, 66(8),
716 959–968. <https://doi.org/10.1007/s00262-016-1929-z>
- 717 Chen, L., & Flies, D. B. (2013). Molecular mechanisms of T cell co-stimulation and co-
718 inhibition. *Nature Reviews. Immunology*, 13(4), 227–242.
719 <https://doi.org/10.1038/nri3405>
- 720 Chiu, I. M., Von Hehn, C. A., & Woolf, C. J. (2012). Neurogenic inflammation and the
721 peripheral nervous system in host defense and immunopathology. *Nature Neuroscience*.
722 <https://doi.org/10.1038/nn.3144>
- 723 Combaret, V., Turc-Carel, C., Thiesse, P., Rebillard, A. C., Frappaz, D., Haus, O., ... Favrot, M. C.
724 (1995). Sensitive detection of numerical and structural aberrations of chromosome 1 in
725 neuroblastoma by interphase fluorescence in situ hybridization. Comparison with
726 restriction fragment length polymorphism and conventional cytogenetic analyses.
727 *International Journal of Cancer*, 61(2), 185–191. <https://doi.org/10.1002/ijc.2910610208>
- 728 Crawford, S. E., Stellmach, V., Ranalli, M., Huang, X., Huang, L., Volpert, O., ... Bouck, N.
729 (2001). Pigment epithelium-derived factor (PEDF) in neuroblastoma: a multifunctional
730 mediator of Schwann cell antitumor activity. *Journal of Cell Science*, 114(Pt 24), 4421–
731 4428.
- 732 Ding, Y., Han, R., Jiang, W., Xiao, J., Liu, H., Chen, X., ... Hao, J. (2016). Programmed Death
733 Ligand 1 Plays a Neuroprotective Role in Experimental Autoimmune Neuritis by
734 Controlling Peripheral Nervous System Inflammation of Rats. *Journal of Immunology*
735 (*Baltimore, Md. : 1950*), 197(10), 3831–3840.
736 <https://doi.org/10.4049/jimmunol.1601083>
- 737 Direder, M., Weiss, T., Copic, D., Vorstandlechner, V., Laggner, M., Mildner, C. S., ... Mildner,
738 M. (2021). Schwann cells contribute to keloid formation. *MedRxiv*,
739 2021.08.09.21261701. <https://doi.org/10.1101/2021.08.09.21261701>
- 740 Dobin, A., Davis, C. A., Schlesinger, F., Drenkow, J., Zaleski, C., Jha, S., ... Gingeras, T. R. (2013).
741 STAR: ultrafast universal RNA-seq aligner. *Bioinformatics (Oxford, England)*, 29(1), 15–21.
742 <https://doi.org/10.1093/bioinformatics/bts635>
- 743 Duan, R.-S., Jin, T., Yang, X., Mix, E., Adem, A., & Zhu, J. (2007). Apolipoprotein E deficiency
744 enhances the antigen-presenting capacity of Schwann cells. *Glia*, 55(7), 772–776.
745 <https://doi.org/10.1002/glia.20498>

- 746 Fischer, M., & Berthold, F. (2003). Characterization of the gene expression profile of
747 neuroblastoma cell line IMR-5 using serial analysis of gene expression. *Cancer Letters*,
748 *190*(1), 79–87. [https://doi.org/10.1016/s0304-3835\(02\)00581-5](https://doi.org/10.1016/s0304-3835(02)00581-5)
- 749 Fitzgerald, K. A., & Kagan, J. C. (2020). Toll-like Receptors and the Control of Immunity. *Cell*,
750 *180*(6), 1044–1066. <https://doi.org/10.1016/j.cell.2020.02.041>
- 751 Gaudet, A. D., Popovich, P. G., & Ramer, M. S. (2011). Wallerian degeneration: gaining
752 perspective on inflammatory events after peripheral nerve injury. *Journal of*
753 *Neuroinflammation*, *8*, 110. <https://doi.org/10.1186/1742-2094-8-110>
- 754 Gentleman, R. C., Carey, V. J., Bates, D. M., Bolstad, B., Dettling, M., Dudoit, S., ... Zhang, J.
755 (2004). Bioconductor: open software development for computational biology and
756 bioinformatics. *Genome Biology*, *5*(10), R80. <https://doi.org/10.1186/gb-2004-5-10-r80>
- 757 Goethals, S., Ydens, E., Timmerman, V., & Janssens, S. (2010). Toll-like receptor expression in
758 the peripheral nerve. *GLIA*, *58*(14), 1701–1709. <https://doi.org/10.1002/glia.21041>
- 759 Gold, R., Zielasek, J., Kiefer, R., Toyka, K. V., & Hartung, H. P. (1996). Secretion of nitrite by
760 Schwann cells and its effect on T-cell activation in vitro. *Cellular Immunology*, *168*(1), 69–
761 77. <https://doi.org/10.1006/cimm.1996.0050>
- 762 Gomez-Sanchez, J. A., Carty, L., Iruarrizaga-Lejarreta, M., Palomo-Irigoyen, M., Varela-Rey, M.,
763 Griffith, M., ... Jessen, K. R. (2015). Schwann cell autophagy, myelinophagy, initiates
764 myelin clearance from injured nerves. *The Journal of Cell Biology*, *210*(1), 153–168.
765 <https://doi.org/10.1083/jcb.201503019>
- 766 Greenwald, R. J., Freeman, G. J., & Sharpe, A. H. (2004). THE B7 FAMILY REVISITED. *Annual*
767 *Review of Immunology*, *23*(1), 515–548.
768 <https://doi.org/10.1146/annurev.immunol.23.021704.115611>
- 769 Hartlehnert, M., Derksen, A., Hagenacker, T., Kindermann, D., Schäfers, M., Pawlak, M., ...
770 Meyer zu Horste, G. (2017). Schwann cells promote post-traumatic nerve inflammation
771 and neuropathic pain through MHC class II. *Scientific Reports*. London.
772 <https://doi.org/10.1038/s41598-017-12744-2>
- 773 Hartley, S. W., & Mullikin, J. C. (2015). QoRTs: a comprehensive toolset for quality control and
774 data processing of RNA-Seq experiments. *BMC Bioinformatics*, *16*(1), 224.
775 <https://doi.org/10.1186/s12859-015-0670-5>
- 776 Hörste, G. M. Z., Hu, W., Hartung, H. P., Lehmann, H. C., & Kieseier, B. C. (2008). The
777 immunocompetence of Schwann cells. *Muscle and Nerve*.

- 778 <https://doi.org/10.1002/mus.20893>
- 779 Icer, M. A., & Gezmen-Karadag, M. (2018). The multiple functions and mechanisms of
780 osteopontin. *Clinical Biochemistry*, *59*, 17–24.
781 <https://doi.org/10.1016/j.clinbiochem.2018.07.003>
- 782 Jang, S. Y., Shin, Y. K., Park, S. Y., Park, J. Y., Lee, H. J., Yoo, Y. H., ... Park, H. T. (2016).
783 Autophagic myelin destruction by Schwann cells during Wallerian degeneration and
784 segmental demyelination. *Glia*, *64*(5), 730–742. <https://doi.org/10.1002/glia.22957>
- 785 Jessen, K. R., & Mirsky, R. (2016). The repair Schwann cell and its function in regenerating
786 nerves. *The Journal of Physiology*, *594*(13), 3521–3531.
787 <https://doi.org/10.1113/JP270874>
- 788 Kaisho, T., & Akira, S. (2000). Critical roles of Toll-like receptors in host defense. *Critical*
789 *Reviews in Immunology*, *20*(5), 393–405.
- 790 Kambayashi, T., & Laufer, T. M. (2014). Atypical MHC class II-expressing antigen-presenting
791 cells: can anything replace a dendritic cell? *Nature Reviews. Immunology*, *14*(11), 719–
792 730. <https://doi.org/10.1038/nri3754>
- 793 Karanth, S., Yang, G., Yeh, J., & Richardson, P. M. (2006). Nature of signals that initiate the
794 immune response during Wallerian degeneration of peripheral nerves. *Experimental*
795 *Neurology*, *202*(1), 161–166. <https://doi.org/10.1016/j.expneurol.2006.05.024>
- 796 Kingston, A. E., Bergsteinsdottir, K., Jessen, K. R., Van der Meide, P. H., Colston, M. J., &
797 Mirsky, R. (1989). Schwann cells co-cultured with stimulated T cells and antigen express
798 major histocompatibility complex (MHC) class II determinants without interferon-
799 gamma pretreatment: synergistic effects of interferon-gamma and tumor necrosis factor
800 on MHC class II in. *European Journal of Immunology*, *19*(1), 177–183.
801 <https://doi.org/10.1002/eji.1830190128>
- 802 La Fleur, M., Underwood, J. L., Rappolee, D. A., & Werb, Z. (1996). Basement membrane and
803 repair of injury to peripheral nerve: defining a potential role for macrophages, matrix
804 metalloproteinases, and tissue inhibitor of metalloproteinases-1. *The Journal of*
805 *Experimental Medicine*, *184*(6), 2311–2326. <https://doi.org/10.1084/jem.184.6.2311>
- 806 Law, C. W., Chen, Y., Shi, W., & Smyth, G. K. (2014). voom: Precision weights unlock linear
807 model analysis tools for RNA-seq read counts. *Genome Biology*, *15*(2), R29.
808 <https://doi.org/10.1186/gb-2014-15-2-r29>
- 809 Lee, H., Jo, E. K., Choi, S. Y., Oh, S. B., Park, K., Soo Kim, J., & Lee, S. J. (2006). Necrotic

- 810 neuronal cells induce inflammatory Schwann cell activation via TLR2 and TLR3:
811 Implication in Wallerian degeneration. *Biochemical and Biophysical Research*
812 *Communications*, 350(3), 742–747. <https://doi.org/10.1016/j.bbrc.2006.09.108>
- 813 Lilje, O., & Armati, P. J. (1997). The distribution and abundance of MHC and ICAM-1 on
814 Schwann cells in vitro. *Journal of Neuroimmunology*, 77(1), 75–84.
815 [https://doi.org/10.1016/S0165-5728\(97\)00063-5](https://doi.org/10.1016/S0165-5728(97)00063-5)
- 816 Mahnke, Y. D., Beddall, M. H., & Roederer, M. (2013). OMIP-017: Human CD4+ helper T-cell
817 subsets including follicular helper cells. *Cytometry Part A*, 83 A(5), 439–440.
818 <https://doi.org/10.1002/cyto.a.22269>
- 819 Mancardi, G. L., Cadoni, A., Zicca, A., Schenone, A., Tabaton, M., De Martini, I., & Zaccheo, D.
820 (1988). HLA-DR Schwann cell reactivity in peripheral neuropathies of different origins.
821 *Neurology*, 38(6), 848–851. <https://doi.org/10.1212/wnl.38.6.848>
- 822 Mehrfeld, C., Zenner, S., Kornek, M., & Lukacs-Kornek, V. (2018). The Contribution of Non-
823 Professional Antigen-Presenting Cells to Immunity and Tolerance in the Liver. *Frontiers*
824 *in Immunology*, 9, 635. <https://doi.org/10.3389/fimmu.2018.00635>
- 825 Meyer zu Horste, G., Cordes, S., Mausberg, A. K., Zozulya, A. L., Wessig, C., Sparwasser, T., ...
826 Kieseier, B. C. (2014). FoxP3+ regulatory T cells determine disease severity in rodent
827 models of inflammatory neuropathies. *PLoS One*, 9(10), e108756.
828 <https://doi.org/10.1371/journal.pone.0108756>
- 829 Meyer Zu Horste, G., Heidenreich, H., Lehmann, H. C., Ferrone, S., Hartung, H.-P., Wiendl, H.,
830 & Kieseier, B. C. (2010). Expression of antigen processing and presenting molecules by
831 Schwann cells in inflammatory neuropathies. *Glia*, 58(1), 80–92.
832 <https://doi.org/10.1002/glia.20903>
- 833 Meyer zu Hörste, G., Heidenreich, H., Mausberg, A. K., Lehmann, H. C., ten Asbroek, A. L. M.
834 A., Saavedra, J. T., ... Kieseier, B. C. (2010). Mouse Schwann cells activate MHC class I and
835 II restricted T-cell responses, but require external peptide processing for MHC class II
836 presentation. *Neurobiology of Disease*, 37(2), 483–490.
837 <https://doi.org/10.1016/j.nbd.2009.11.006>
- 838 Meyer zu Hörste, G., Hu, W., Hartung, H.-P., Lehmann, H. C., & Kieseier, B. C. (2008). The
839 immunocompetence of Schwann cells. *Muscle & Nerve*, 37(1), 3–13.
840 <https://doi.org/10.1002/mus.20893>
- 841 Momoi, M., Kennett, R. H., & Glick, M. C. (1980). A membrane glycoprotein from human

- 842 neuroblastoma cells isolated with the use of a monoclonal antibody. *The Journal of*
843 *Biological Chemistry*, 255(24), 11914–11921.
- 844 Mootha, V. K., Lindgren, C. M., Eriksson, K.-F., Subramanian, A., Sihag, S., Lehar, J., ... Groop, L.
845 C. (2003). PGC-1 α -responsive genes involved in oxidative phosphorylation are
846 coordinately downregulated in human diabetes. *Nature Genetics*, 34(3), 267–273.
847 <https://doi.org/10.1038/ng1180>
- 848 Murata, K.-Y., & Dalakas, M. C. (2000). Expression of the co-stimulatory molecule BB-1, the
849 ligands CTLA-4 and CD28 and their mRNAs in chronic inflammatory demyelinating
850 polyneuropathy. *Brain : A Journal of Neurology*, 123 (Pt 8, 1660–1666.
851 [https://doi.org/10.1016/S0002-9440\(10\)65141-3](https://doi.org/10.1016/S0002-9440(10)65141-3)
- 852 Nocera, G., & Jacob, C. (2020). Mechanisms of Schwann cell plasticity involved in peripheral
853 nerve repair after injury. *Cellular and Molecular Life Sciences : CMLS*, 77(20), 3977–
854 3989. <https://doi.org/10.1007/s00018-020-03516-9>
- 855 Ozaki, A., Nagai, A., Lee, Y. B., Myong, N. H., & Kim, S. U. (2008). Expression of cytokines and
856 cytokine receptors in human Schwann cells. *Neuroreport*, 19(1), 31–35.
857 <https://doi.org/10.1097/WNR.0b013e3282f27e60>
- 858 Ritchie, M. E., Phipson, B., Wu, D., Hu, Y., Law, C. W., Shi, W., & Smyth, G. K. (2015). limma
859 powers differential expression analyses for RNA-sequencing and microarray studies.
860 *Nucleic Acids Research*, 43(7), e47. <https://doi.org/10.1093/nar/gkv007>
- 861 Roberts, S. L., Dun, X.-P., Doddrell, R. D. S., Mindos, T., Drake, L. K., Onaitis, M. W., ...
862 Parkinson, D. B. (2017). Sox2 expression in Schwann cells inhibits myelination in vivo and
863 induces influx of macrophages to the nerve. *Development (Cambridge, England)*,
864 144(17), 3114–3125. <https://doi.org/10.1242/dev.150656>
- 865 Rutkowski, J. L., Tuite, G. F., Lincoln, P. M., Boyer, P. J., Tennekoon, G. I., & Kunkel, S. L. (1999).
866 Signals for proinflammatory cytokine secretion by human Schwann cells. *Journal of*
867 *Neuroimmunology*, 101(1), 47–60. [https://doi.org/10.1016/s0165-5728\(99\)00132-0](https://doi.org/10.1016/s0165-5728(99)00132-0)
- 868 Samuel, N. M., Mirsky, R., Grange, J. M., & Jessen, K. R. (1987). Expression of major
869 histocompatibility complex class I and class II antigens in human Schwann cell cultures
870 and effects of infection with Mycobacterium leprae. *Clinical and Experimental*
871 *Immunology*, 68(3), 500–509.
- 872 Schuijs, M. J., Hammad, H., & Lambrecht, B. N. (2019). Professional and “Amateur” Antigen-
873 Presenting Cells In Type 2 Immunity. *Trends in Immunology*, 40(1), 22–34.

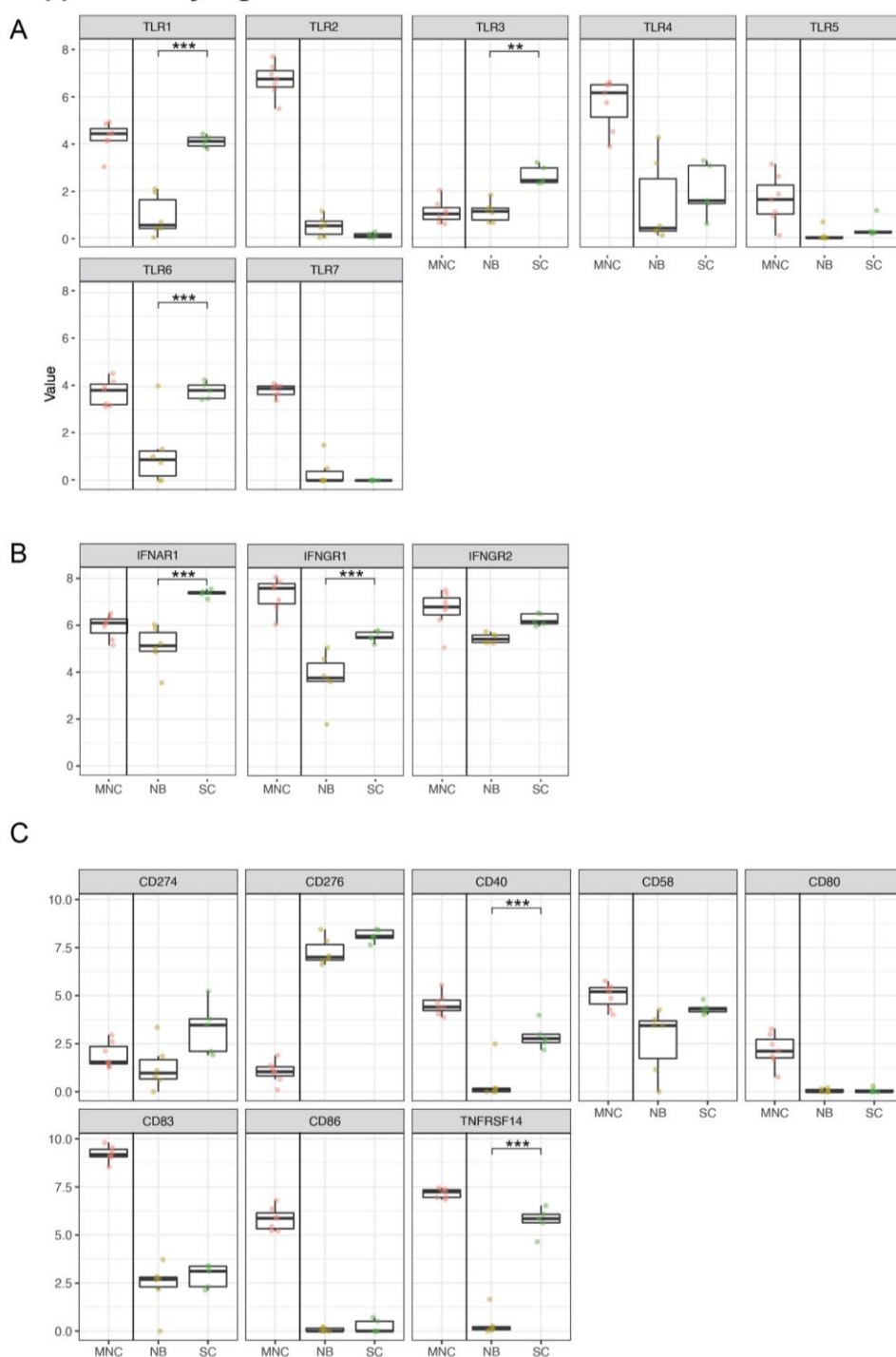
- 874 <https://doi.org/10.1016/j.it.2018.11.001>
- 875 Spierings, E., De Boer, T., Zulianello, L., & Ottenhoff, T. H. M. (2000). Novel mechanisms in the
876 immunopathogenesis of leprosy nerve damage: The role of schwann cells, T cells and
877 *Mycobacterium leprae*. *Immunology and Cell Biology*. [https://doi.org/10.1046/j.1440-](https://doi.org/10.1046/j.1440-1711.2000.00939.x)
878 [1711.2000.00939.x](https://doi.org/10.1046/j.1440-1711.2000.00939.x)
- 879 Steinhoff, U., & Kaufmann, S. H. (1988). Specific lysis by CD8+ T cells of Schwann cells
880 expressing *Mycobacterium leprae* antigens. *European Journal of Immunology*, *18*(6),
881 969–972. <https://doi.org/10.1002/eji.1830180622>
- 882 Stock, C., Bozsaky, E., Watzinger, F., Poetschger, U., Orel, L., Lion, T., ... Ambros, P. F. (2008).
883 Genes proximal and distal to MYCN are highly expressed in human neuroblastoma as
884 visualized by comparative expressed sequence hybridization. *The American Journal of*
885 *Pathology*, *172*(1), 203–214. <https://doi.org/10.2353/ajpath.2008.061263>
- 886 Stratton, J. A., & Shah, P. T. (2016). Macrophage polarization in nerve injury: do Schwann cells
887 play a role? *Neural Regeneration Research*, *11*(1), 53–57. [https://doi.org/10.4103/1673-](https://doi.org/10.4103/1673-5374.175042)
888 [5374.175042](https://doi.org/10.4103/1673-5374.175042)
- 889 Stratton, J. A., Shah, P. T., Kumar, R., Stykel, M. G., Shapira, Y., Grochmal, J., ... Midha, R.
890 (2016). The immunomodulatory properties of adult skin-derived precursor Schwann
891 cells: implications for peripheral nerve injury therapy. *The European Journal of*
892 *Neuroscience*, *43*(3), 365–375. <https://doi.org/10.1111/ejn.13006>
- 893 Subramanian, A., Tamayo, P., Mootha, V. K., Mukherjee, S., Ebert, B. L., Gillette, M. A., ...
894 Mesirov, J. P. (2005). Gene set enrichment analysis: A knowledge-based approach for
895 interpreting genome-wide expression profiles. *Proceedings of the National Academy of*
896 *Sciences*, *102*(43), 15545 LP – 15550. <https://doi.org/10.1073/pnas.0506580102>
- 897 Toews, A. D., Barrett, C., & Morell, P. (1998). Monocyte chemoattractant protein 1 is
898 responsible for macrophage recruitment following injury to sciatic nerve. *Journal of*
899 *Neuroscience Research*, *53*(2), 260–267. [https://doi.org/10.1002/\(SICI\)1097-](https://doi.org/10.1002/(SICI)1097-4547(19980715)53:2<260::AID-JNR15>3.0.CO;2-A)
900 [4547\(19980715\)53:2<260::AID-JNR15>3.0.CO;2-A](https://doi.org/10.1002/(SICI)1097-4547(19980715)53:2<260::AID-JNR15>3.0.CO;2-A)
- 901 Tofaris, G. K., Patterson, P. H., Jessen, K. R., & Mirsky, R. (2002). Denervated Schwann cells
902 attract macrophages by secretion of leukemia inhibitory factor (LIF) and monocyte
903 chemoattractant protein-1 in a process regulated by interleukin-6 and LIF. *The Journal of*
904 *Neuroscience : The Official Journal of the Society for Neuroscience*, *22*(15), 6696–6703.
905 <https://doi.org/10.1523/JNEUROSCI.22-15-06696.2002>

- 906 Tzekova, N., Heinen, A., & Küry, P. (2014). Molecules involved in the crosstalk between
907 immune- and peripheral nerve Schwann cells. *Journal of Clinical Immunology*.
908 <https://doi.org/10.1007/s10875-014-0015-6>
- 909 Van Rhijn, I., Van den Berg, L. H., Bosboom, W. M. J., Otten, H. G., & Logtenberg, T. (2000).
910 Expression of accessory molecules for T-cell activation in peripheral nerve of patients
911 with CIDP and vasculitic neuropathy. *Brain*, *123*(10), 2020–2029.
912 <https://doi.org/10.1093/brain/123.10.2020>
- 913 Wang, F.-J., Cui, D., & Qian, W.-D. (2018). Therapeutic Effect of CD4+CD25+ Regulatory T Cells
914 Amplified In Vitro on Experimental Autoimmune Neuritis in Rats. *Cellular Physiology and*
915 *Biochemistry : International Journal of Experimental Cellular Physiology, Biochemistry,*
916 *and Pharmacology*, *47*(1), 390–402. <https://doi.org/10.1159/000489919>
- 917 Wang, X., Zheng, X.-Y., Ma, C., Wang, X.-K., Wu, J., Adem, A., ... Zhang, H.-L. (2014). Mitigated
918 Tregs and augmented Th17 cells and cytokines are associated with severity of
919 experimental autoimmune neuritis. *Scandinavian Journal of Immunology*, *80*(3), 180–
920 190. <https://doi.org/10.1111/sji.12201>
- 921 Weiss, T., Taschner-Mandl, S., Ambros, P. F., & Ambros, I. M. (2018). Detailed Protocols for
922 the Isolation, Culture, Enrichment and Immunostaining of Primary Human Schwann
923 Cells. *Methods in Molecular Biology (Clifton, N.J.)*, *1739*, 67–86.
924 https://doi.org/10.1007/978-1-4939-7649-2_5
- 925 Weiss, T., Taschner-Mandl, S., Bileck, A., Slany, A., Kromp, F., Rifatbegovic, F., ... Ambros, I. M.
926 (2016). Proteomics and transcriptomics of peripheral nerve tissue and cells unravel new
927 aspects of the human Schwann cell repair phenotype. *GLIA*, *64*(12), 2133–2153.
928 <https://doi.org/10.1002/glia.23045>
- 929 Weiss, T., Taschner-Mandl, S., Janker, L., Bileck, A., Rifatbegovic, F., Kromp, F., ... Ambros, I. M.
930 (2021). Schwann cell plasticity regulates neuroblastic tumor cell differentiation via
931 epidermal growth factor-like protein 8. *Nature Communications*, *12*(1), 1624.
932 <https://doi.org/10.1038/s41467-021-21859-0>
- 933 Wekerle, H., Schwab, M., Linington, C., & Meyermann, R. (1986). Antigen presentation in the
934 peripheral nervous system: Schwann cells present endogenous myelin autoantigens to
935 lymphocytes. *European Journal of Immunology*, *16*(12), 1551–1557.
936 <https://doi.org/10.1002/eji.1830161214>
- 937 Yao, K., Graham, J., Akahata, Y., Oh, U., & Jacobson, S. (2010). Mechanism of

- 938 neuroinflammation: enhanced cytotoxicity and IL-17 production via CD46 binding.
939 *Journal of Neuroimmune Pharmacology : The Official Journal of the Society on*
940 *NeuroImmune Pharmacology*, 5(3), 469–478. [https://doi.org/10.1007/s11481-010-9232-](https://doi.org/10.1007/s11481-010-9232-9)
941 9
- 942 Ydens, E., Cauwels, A., Asselbergh, B., Goethals, S., Peeraer, L., Lornet, G., ... Janssens, S.
943 (2012). Acute injury in the peripheral nervous system triggers an alternative macrophage
944 response. *Journal of Neuroinflammation*, 9, 176. [https://doi.org/10.1186/1742-2094-9-](https://doi.org/10.1186/1742-2094-9-176)
945 176
- 946 Ydens, E., Lornet, G., Smits, V., Goethals, S., Timmerman, V., & Janssens, S. (2013). The
947 neuroinflammatory role of Schwann cells in disease. *Neurobiology of Disease*, 55, 95–
948 103. <https://doi.org/10.1016/j.nbd.2013.03.005>
- 949 Zhang, S. H., Shurin, G. V., Khosravi, H., Kazi, R., Kruglov, O., Shurin, M. R., & Bunimovich, Y. L.
950 (2020). Immunomodulation by Schwann cells in disease. *Cancer Immunology,*
951 *Immunotherapy : CII*, 69(2), 245–253. <https://doi.org/10.1007/s00262-019-02424-7>
952

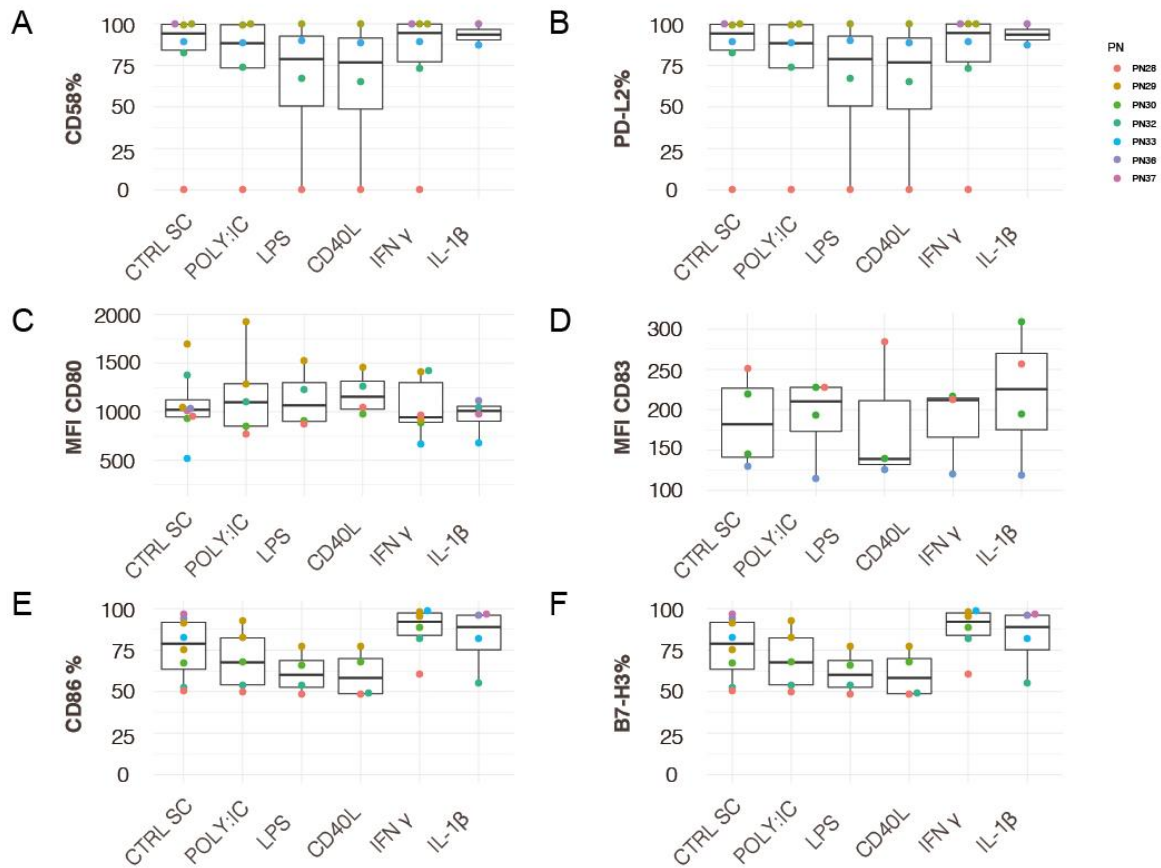
953 Supplementary Figures

Supplementary Fig1



954
955 Supplementary Figure 1 | mRNA expression of Toll-like receptors, interferon receptors and co-signaling
956 molecules. Boxplots show mRNA levels (RPKM) in hrSCs (n=5) versus NB cells (n=5). MNCs are shown as
957 reference. (A) Toll-like receptors (B) interferon receptors and (C) co-stimulatory and -inhibitory
958 molecules. Boxes contain 50% of data and whiskers the upper and lower 25% means are displayed as
959 black horizontal lines.

Supplementary Figure 2 Berner, Weiss et al, 2021



960
 961
 962
 963
 964
 965
 966
 967
 968
 969

Supplementary Figure 2 | Flow cytometry-based phenotyping of MHCII and co-signaling molecules upon inflammatory stimulation. Box plots show the expression status of CD58 (A), PDL2 (B), CD80 (C), CD83 (D), CD86 (E) and B7H3 (F) of S100 positive hrSCs after stimulation with POLY:IC, LPS, CD40L, IFN γ and IL-1 β ; n=9; technical replicates (same color); biological replicates (different color). Each biological replicate is conducted with hrSCs isolated from a different donor nerve. (A, B, E, F) Boxplots represent the percentage of positive cells based on gates set in relation to unstained controls. (C, D) Boxplots represent the mean fluorescence intensity (MFI). Boxes contain 50% of data and whiskers the upper and lower 25%; means are displayed as black horizontal lines. A two-way ANOVA using a post-hoc Holm p value correction was performed; * p \leq 0.05; ** p \leq 0.01; *** p \leq 0.001.

970 Supplementary Tables

971

| 1 st antibodies | | | | | Application | |
|----------------------------|---------|------------|-----------------|----------|----------------|--|
| Antigen | Species | catalog No | company | dilution | comment | |
| S100 | rabbit | #Z0311 | DAKO | 1:200 | 1 hr, RT, perm | |
| vimentin | chicken | #AB5733 | Merck Millipore | 1:200 | 1 hr, RT, perm | |
| NGFR | rabbit | #8238S | CellSignaling | 1:300 | o.n., 4°C | |
| PD-L1 | Mouse | #4274539 | eBioscience | 1:50 | o.n. 4°C | |
| HLA-DR-α1 | mouse | #M0746 | DAKO | 1:50 | o.n., 4°C | |

| 2 nd antibodies | | | | | Application | |
|----------------------------|---------|------------|-----------|----------|-------------|--|
| Antigen | Species | catalog No | company | dilution | comment | |
| α rb FITC | swine | #F0205 | DAKO | 1:50 | 1 hr, RT | |
| α ch AF647 | goat | #SA5-10073 | LifeTech. | 1:300 | 1 hr, RT | |
| α ms AF594 | goat | #A11032 | LifeTech. | 1:300 | 1 hr, RT | |

| directly labelled antibodies | | | | | Application | |
|------------------------------|---------|------------|---------------|----------|-------------------|--|
| Antigen | Species | catalog No | company | dilution | comment | |
| S100B-FITC* | rabbit | #Z0311 | DAKO | 1:50 | 20 min, 4°C, perm | |
| CD80-PerCP-eF710 | mouse | 46-0809-42 | eBioscience | 1:25 | | |
| CD276(B7-H3)-PE | mouse | 565829 | BD Bioscience | 1:50 | | |
| CD40-PerCP | mouse | Ab91282 | Abcam | 1:5 | | |
| MHCII-APC-ef780 | mouse | 47-9956-42 | eBioscience | 1:50 | | |
| PD-L1-PE | mouse | 557924 | BD Bioscience | 1:5 | | |
| HVEM-A647 | mouse | 564411 | BD Bioscience | 1:25 | | |
| CD86-APC | mouse | 555660 | BD Bioscience | 1:25 | | |
| CD273- PE | mouse | 565829 | BD Bioscience | 1:25 | | |
| CD58-APC | mouse | 17-0578-41 | eBioscience | 1:25 | | |

972

973 **Supplementary Table 1 | List of antibodies.**

974 perm = permeabilization necessary, RT = room temperature.

975

976 **Supplementary Table 2 | RNA-sequencing. Differential gene expression analysis of hrSCs compared to**

977 **NB cell lines.**

978 Supplementary Table 3 | RNA-sequencing. GO functional annotation of top 250 genes upregulated in
979 hrSCs versus NB cell lines.

980

981 Supplementary Table 4 | RNA-sequencing. Gene set enrichment analysis (GSEA) in hrSC versus NB cell
982 lines.

983

984 Supplementary Table 5 | Protein array. Differential protein secretion of hrSCs versus NB cell lines.