

Evaluation of Two Methods to Isolate Schwann Cells from Murine Sciatic Nerve.

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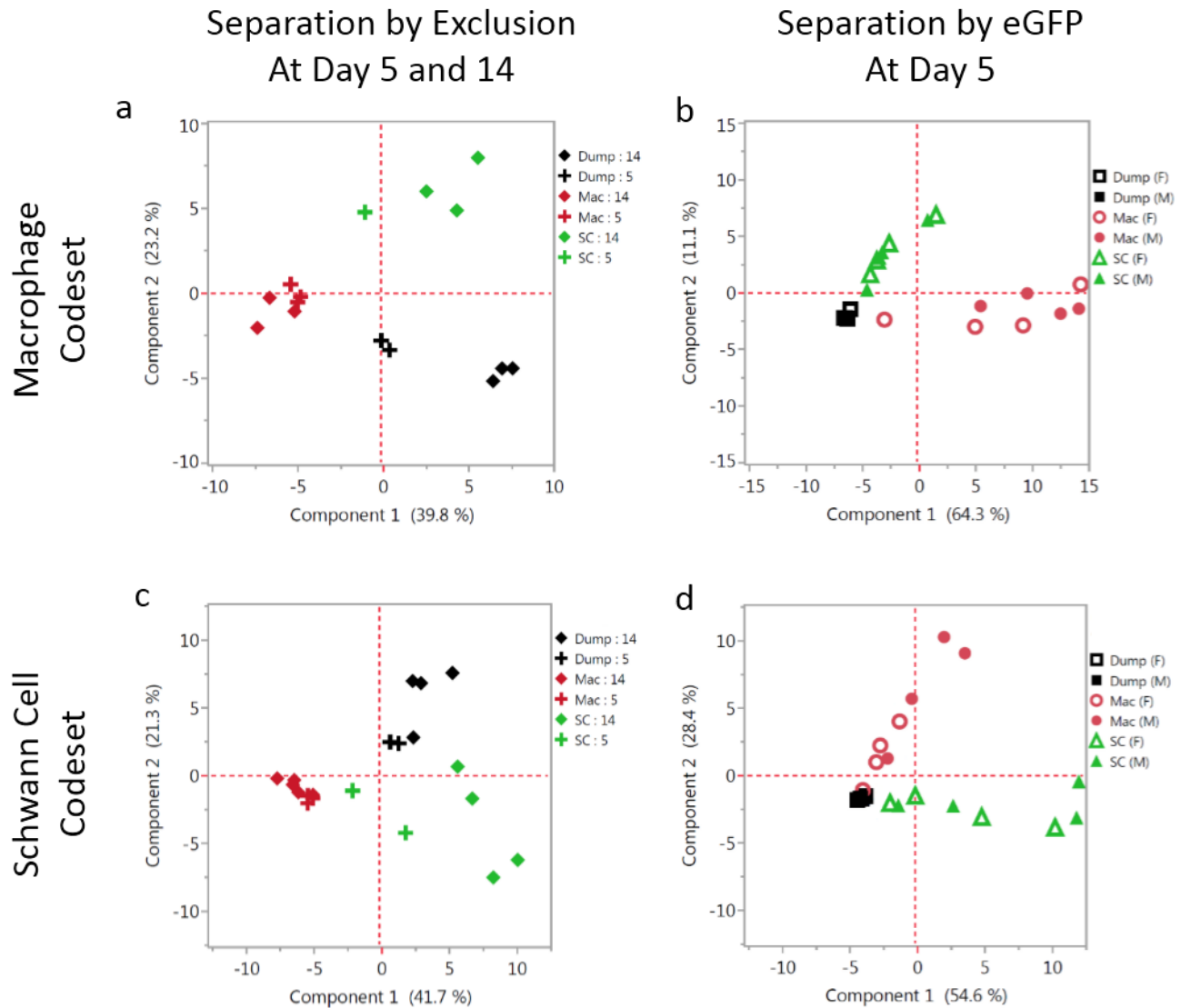


Figure S1 – Gene expression of sorted cells clusters by population more than by time:

Principal component analysis of gene expression among 3 groups of cells sorted from murine sciatic nerve either 5 or 14 days after injury (black: dump channel cells; red: macrophages; green: Schwann cells). The dump channel included eosinophils (Siglec-F+), lymphocytes (CD3e+, CD19+), neutrophils (Ly6G+), endothelial cells (CD31+), and fibroblasts (Thy-1.2+). Macrophages were F4/80+CD14+CD11b+CD16/32+ using wild type mice (a) and S100-eGFP mice (b). Presumptive Schwann cells were sorted by excluding cells positive for all of the above markers (c), or using GFP positive (d). Results indicate clustering more by population and less by the harvest timepoint (a, c). The distinct clustering of each cell type in both macrophage (b) and SC (d) codesets supports the effectiveness of the cell separation method using eGFP signal in S100-eGFP mice.

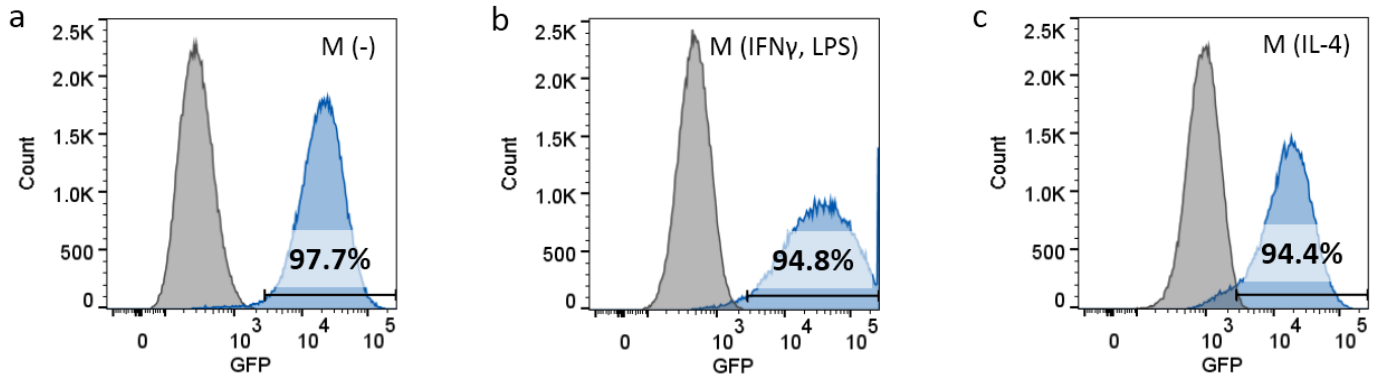


Figure S2 – Bone marrow derived macrophages from S100-eGFP transgenic mice express GFP:

Bone marrow derived macrophages were cultured under 3 stimulation conditions: no stimulation (a), IFN γ and LPS (b), or IL4 (c). Anti-F4/80 label was used to confirm differentiation into macrophages. F4/80+ cells were analyzed for GFP expression by flow cytometry. Expression of GFP in macrophages is irrespective of macrophage phenotype. Data are representative of two independent experiments (n=2-3 mice/group).

Table S1: List of genes in nanoString Codesets

CodeSet	Gene Name	HUGO Gene	CodeSet	Gene Name	HUGO Gene
Macrophage CodeSet	Actb	Actb	Schwann Cell CodeSet	F4/80	Adgre1
	F4/80	Adgre1		Ankrd27	Ankrd27
	Alox15	Alox15		Anxa1	Anxa1
	Ankrd27	Ankrd27		BDNF	Bdnf
	Areg	Areg		Bmp1	Bmp1
	Arg1	Arg1		Bmp2	Bmp2
	Birc5	Birc5		Bmp4	Bmp4
	Card11	Card11		Bmp6	Bmp6
	Ccl11	Ccl11		Bmp7	Bmp7
	Ccl17	Ccl17		ccl2	Ccl2
	Ccl2	Ccl2		ccl3	Ccl3
	Ccl22	Ccl22		Cd14	Cd14
	Ccl24	Ccl24		Cd19	Cd19
	Ccl5	Ccl5		Cd3e	Cd3e
	Ccl7	Ccl7		Cdh2	Cdh2
	Ccr2	Ccr2		Cdc2	Cdk1
	Cd14	Cd14		Cdk2	Cdk2
	Cd19	Cd19		Cdkn1b	Cdkn1b
	Cd300e	Cd300e		p16ink4a	Cdkn2a
	Cd3e	Cd3e		Clu	Clu
	Cd5l	Cd5l		CNTF	Cntf
	Chi3l3	Chi3l3		dcc	Dcc
	Chi3l1	Chil1		DHH	Dhh
	Ctnf	Cntf		Egr2	Egr2
	Col18a1	Col18a1		NSE	Eno2
	Col1a1	Col1a1		ephA	Epha1
	Col1a2	Col1a2		ephB	Ephb1
	Col3a1	Col3a1		ErbB2	ErbB2
	Ctnnb1	Ctnnb1		ErbB3	ErbB3
	Cx3cr1	Cx3cr1		BFABP	Fabp7
	Cxcl12	Cxcl12		FGF1	Fgf1
	Cxcl13	Cxcl13		FGF2	Fgf2
	Cxcl14	Cxcl14		Fn1	Fn1
	Cxcl16	Cxcl16		GAP43	Gap43
	Cxcl5	Cxcl5		Gapdh	Gapdh
	Cxcl9	Cxcl9		Gdnf	Gdnf
	Cxcr4	Cxcr4		Gfap	Gfap
	Dcstamp	Dcstamp		Gfra1	Gfra1
	IL-35	Ebi3		GMFB	Gmfb
	Fgfr1	Fgfr1		Hgf	Hgf
Gapdh	Gapdh	Hprt	Hprt		
Gdnf	Gdnf	IGF-1	Igf1		
Hexb	Hexb	IGF-2	Igf2		
Hgf	Hgf	Il10	Il10		
Ifngr1	Ifngr1	IL1B	Il1b		
Igf1	Igf1	IL6	Il6		
Il10	Il10	Jam3	Jam3		
Il10rb	Il10rb	Jun	Jun		

CodeSet	Gene Name	HUGO Gene	CodeSet	Gene Name	HUGO Gene
Macrophage CodeSet	Il12	Il12b	Schwann Cell CodeSet	Klf6	Klf6
	Il1a	Il1a		L1CAM	L1cam
	Il1b	Il1b		Lama2	Lama2
	Il4ra	Il4ra		Lama5	Lama5
	Il6	Il6		LGALS1	Lgals1
	Irf3	Irf3		Lif	Lif
	Irf5	Irf5		MAG	Mag
	Mac1 C3br C	Ilgam		MAL	Mal
	Ilgax	Ilgax		Map1b	Map1b
	Jam3	Jam3		Mapk3	Mapk3
	Jun	Jun		MBP	Mbp
	Mac2	Lgals3		Mmp17	Mmp17
	Lpar1	Lpar1		MPZ	Mpz
	Ly6a	Ly6a		NCAM1	Ncam1
	Ly6g	Ly6g		Nes	Nes
	Ly6i	Ly6i		Nf1	Nf1
	Mmp12	Mmp12		Ngf	Ngf
	Mmp13	Mmp13		p75 NTR	Ngfr
	Mmp27	Mmp27		Nos2	Nos2
	Cd206	Mrc1		NOTCH1	Notch1
	SRAI	Msr1		Nrcam	Nrcam
	Myd88	Myd88		Nrg1	Nrg1
	Nf1	Nf1		Nrp-1	Nrp1
	Nfkbiz	Nfkbiz		Ntf-5	Ntf5
	Ngfr	Ngfr		Ntn-1	Ntn1
	Nos2	Nos2		Pdgfa	Pdgfa
	Nrcam	Nrcam		cd31	Pecam1
	Nrg1	Nrg1		Pla2g6	Pla2g6
	Pak1	Pak1		PLAT gene	Plat
	Pdgfb	Pdgfb		Plp1	Plp1
	Cd31	Pecam1		Pmp22	Pmp22
	Cxcl4	Pf4		Pou3f1	Pou3f1
	Ppbp	Ppbp		Pten	Pten
	Pten	Pten		Ptn	Ptn
	Retnla	Retnla		RICTOR	Rictor
	Rictor	Rictor		Robo1	Robo1
	S100a9	S100a9		Robo2	Robo2
	Saa3	Saa3		S100b	S100b
	SiglecE	Siglece		Sema3a	Sema3a
	Socs1	Socs1		Sema3b	Sema3b
	Socs3	Socs3		slit2	Slit2
	Stat1	Stat1		Sox-10	Sox10
	Stat2	Stat2		sox2	Sox2
	Stat3	Stat3		F-spondin	Spon1
	Stat6	Stat6		Spp1	Spp1
Tbp	Tbp	Tab2	Tab2		
Tgfb1	Tgfb1	Tbp	Tbp		
Thy1	Thy1	Thy1	Thy1		
Tlr2	Tlr2	Timp2	Timp2		
Tlr4	Tlr4	Timp3	Timp3		
Tlr8	Tlr8	Tnf	Tnf		
Tnfa	Tnf	unc5h2	Unc5b		
Vegf	Vegfa	VEGFA	Vegfa		

Table S2: Number of samples collected and submitted for SC_{exclusion} experiment.

Injured sciatic nerve was harvested from 3 mice 5 days after repair, and from 5 mice 14 days after repair. Cells were isolated by FACS into three populations, dump, macrophage, and Schwann cell (SC). Sorted cell samples were submitted for gene expression on the nanoString platform using two different gene codesets: macrophage and SC. Three samples were excluded from analysis on each codeset due to high content normalization factor (NF), indicating low RNA input.

	SC codeset			Macrophage codeset		
	# of Samples Submitted	# of Samples Excluded Due to NF > 10	# of Samples Analyzed	# of Samples Submitted	# of Samples Excluded Due to NF > 10	# of Samples Analyzed
Day 5, Dump	3	1	2	3	1	2
Day 5, Macrophage	3	0	3	3	0	3
Day 5, SC	3	1	2	3	2	1
Day 14, Dump	4	0	4	3	0	3
Day 14, Macrophage	5	0	5	3	0	3
Day 14, SC	5	1	4	3	0	3

Table S3: Number of samples collected and submitted for SC_{GFP} experiment.

Injured sciatic nerve was harvested from 8 mice (4 male, 4 female) 5 days after repair. Cells were isolated by FACS into three populations, dump, macrophage, and Schwann cell (SC). Sorted cell samples were submitted for gene expression on the nanoString platform using two different gene codesets: macrophage and SC. There was enough RNA input for all the samples; so, there was no sample exclusion for gene expression analysis.

	SC codeset			Macrophage codeset		
	# of Samples Submitted	# of Samples Excluded Due to NF > 10	# of Samples Analyzed	# of Samples Submitted	# of Samples Excluded Due to NF > 10	# of Samples Analyzed
Day 5, Dump	8	0	8	8	0	8
Day 5, Macrophage	8	0	8	8	0	8
Day 5, SC	8	0	8	8	0	8