The Proteome Signatures of Fibroblasts from Patients with Severe, Intermediate and Mild Spinal Muscular Atrophy Show Limited Overlap

Supplementary File

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Fibroblast cells used in the study:

Cell line identifier	Biopsy source	Gender	Age when biopsy taken	Clinical diagnosis	Supplier
Cell lines used in the SMA I vs age-matched controls comparison					
F012-16N-ML	skin of left upper arm, outer side	F	4 months	SMA I	Originally from University of Cologne (ID ML112) Newcastle MRC Centre Biobank for Rare & Neuromuscular Diseases
F011-16N-ML	skin of left upper arm, outer side	M	4 months	SMA I	Originally from University of Cologne (ID ML107) Newcastle MRC Centre Biobank for Rare & Neuromuscular Diseases
F010-16N-ML	skin of left upper arm, outer side	F	6 months	SMA I	Originally from University of Cologne (ID ML111) Newcastle MRC Centre Biobank for Rare & Neuromuscular Diseases
GM00232		M	7 months	SMA I	Coriell Cell Repository
GM09677		M	2 years	SMA I	Coriell Cell Repository
GM08333	foreskin	M	5 months	Control	Coriell Cell Repository
GM00302		M	10 months	Control*	Coriell Cell Repository
GM05659	chest	M	1 year	Control*	Coriell Cell Repository
GM00498		M	3 years	Control*	Coriell Cell Repository
Cell lines used in the SMA II vs age-matched controls comparison					
GM022592		M	1 year	SMA II	Coriell Cell Repository
GM03813	arm	M	3 years	SMA II	Coriell Cell Repository
F002-15N		M	3 years	SMA II	Newcastle MRC Centre Biobank for Rare & Neuromuscular Diseases
F211-14N		F	23 years	SMA II	Newcastle MRC Centre Biobank for Rare & Neuromuscular Diseases
F018-15N		M	25 years	SMA II	Newcastle MRC Centre Biobank for Rare & Neuromuscular Diseases
GM00302		M	10 months	Control*	Coriell Cell Repository
GM05659	chest	M	1 year	Control*	Coriell Cell Repository
GM00498		M	3 years	Control*	Coriell Cell Repository
F152-14N		F	27 years	Control**	Newcastle MRC Centre Biobank for Rare & Neuromuscular Diseases
Cell lines used in the SMA III vs age-matched controls comparison					
F053-15N		M	17 years	SMA III	Newcastle MRC Centre Biobank for Rare & Neuromuscular Diseases
F210-14N		F	22 years	SMA III	Newcastle MRC Centre Biobank for Rare & Neuromuscular Diseases
F055-15N		M	44 years	SMA III	Newcastle MRC Centre Biobank for Rare & Neuromuscular Diseases
F206-14N		M	66 years	SMA III	Newcastle MRC Centre Biobank for Rare & Neuromuscular Diseases
F152-14N		F	27 years	Control**	Newcastle MRC Centre Biobank for Rare & Neuromuscular Diseases
F154-14N		F	34 years	Control	Newcastle MRC Centre Biobank for Rare & Neuromuscular Diseases
F008-16N		M	39 years	Control	Newcastle MRC Centre Biobank for Rare & Neuromuscular Diseases
F011-11N		F	45 years	Control	Newcastle MRC Centre Biobank for Rare & Neuromuscular Diseases
F067-16N		F	66 years	Control	Newcastle MRC Centre Biobank for Rare & Neuromuscular Diseases

Table S1: Cell identity, source of biopsy, gender, age of patient when sampled, phenotype and source of cells are listed for the fibroblasts used in this study. *These cells lines were used as age-matched controls in the SMA I and SMA II comparisons. **This cell line was used as an age-matched control in both the SMA II and SMA III comparisons.

Summary of antibodies used for western blotting:

Target protein	Antibody	Supplier & Source	Molecular Weight (kDa)	Dilution
Survival motor neuron (SMN)	MANSMA12; Clone 2E6	Wolfson CIND; Mouse monoclonal	38	1:100
Insulin-like growth factor 2 mRNA-binding protein 1	IMP1 (D33A2); #8482	Cell Signaling Technology; Rabbit monoclonal	64	1:500
Glycogen phosphorylase, brain form	PYGB/M (17B6); sc-51923	Santa Cruz BioTechnology, Inc; Mouse monoclonal	97	1:200
Ras-related protein Rab-3B	Rab 3B (48-K2): sc-81911	Santa Cruz BioTechnology, Inc; Mouse monoclonal	25	1:50
Signal transducer and activator of transcription 1-alpha/beta	STAT1; 10144-2-AP	Proteintech; Rabbit polyclonal	84	1:500

Table S2: Details of target protein, antibody name and associated clone, supplier, source, expected molecular weight and dilution of antibodies used in this study.

A

Activation z-score

-4.017 5.840

Canonical Pathways	SMA I/Ctrl I Werdnig-Hoffman disease
EIF2 Signaling	
IL-8 Signaling	
Signaling by Rho Family GTPa...	
Integrin Signaling	
HER-2 Signaling in Breast Ca...	
CXCR4 Signaling	
Coronavirus Pathogenesis Pa...	
mTOR Signaling	
PAK Signaling	
Synaptogenesis Signaling Pat...	
RHOGDI Signaling	
Actin Cytoskeleton Signaling	
Hepatic Fibrosis Signaling Pa...	
RAC Signaling	
GP6 Signaling Pathway	
ERK/MAPK Signaling	
Reelin Signaling in Neurons	
Cardiac Hypertrophy Signaling	
Thrombin Signaling	
TEC Kinase Signaling	
Glioblastoma Multiforme Sig...	
Insulin Secretion Signaling Pa...	
Ephrin Receptor Signaling	
Natural Killer Cell Signaling	
Estrogen Receptor Signaling	
Semaphorin Neuronal Repuls...	

B

Activation z-score

-3.545 4.841

Canonical Pathways	SMA III/Ctrl III Neurologic manifestations
FAK Signaling	
Pulmonary Fibrosis Idiopathic...	
Signaling by Rho Family GTPa...	
Hepatic Fibrosis Signaling Pa...	
TEC Kinase Signaling	
RHOGDI Signaling	
Regulation of Actin-based M...	
Dendritic Cell Maturation	
Phagosome Formation	
Cardiac Hypertrophy Signalin...	
Integrin Signaling	
ERK/MAPK Signaling	
Production of Nitric Oxide an...	
Colorectal Cancer Metastasis...	
HMGB1 Signaling	
Role of Hypercytokinemia/hy...	
Fcγ Receptor-mediated Phag...	
Cholecystokinin/Gastrin-medi...	
Actin Nucleation by ARP-WA...	
RHOA Signaling	
CDC42 Signaling	
Factors Promoting Cardiogen...	
Wound Healing Signaling Pat...	
NF-κB Activation by Viruses	
Actin Cytoskeleton Signaling	
Paxillin Signaling	

C

Activation z-score

-8.059   6.226

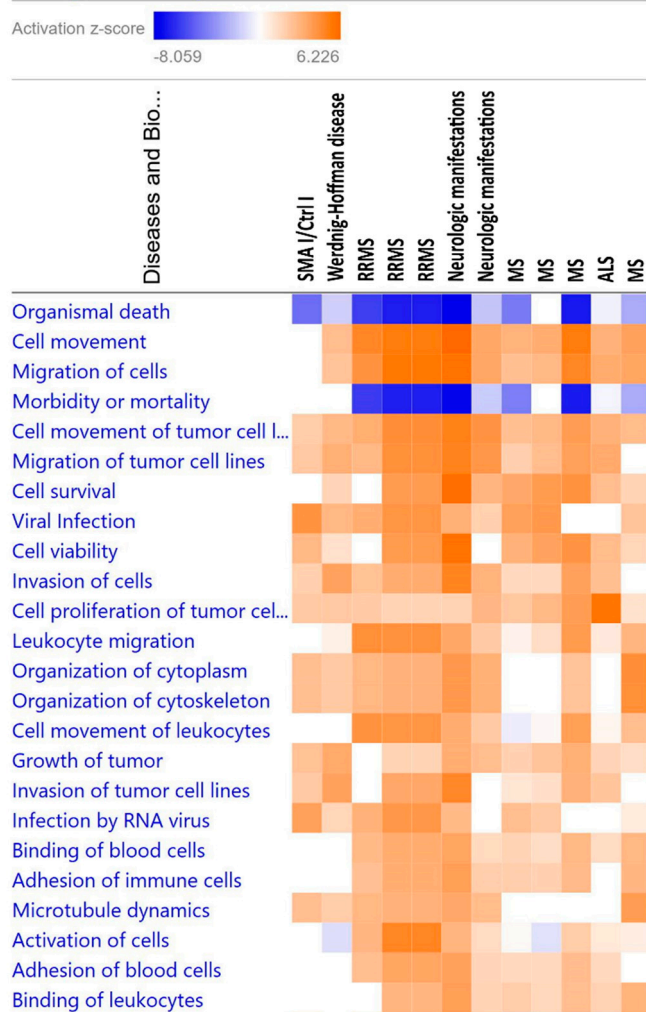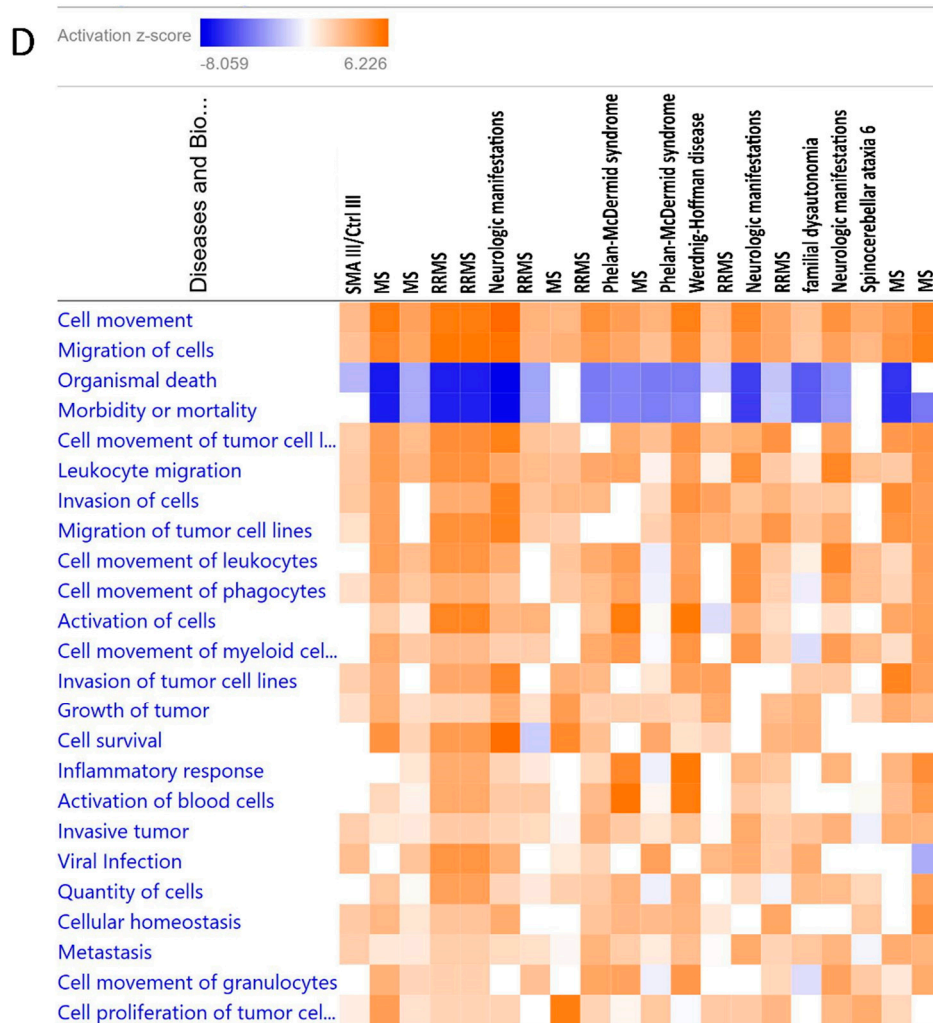| Diseases and Bio...	SMA I/Ctrl I	Werdnig-Hoffman disease	RRMS	RRMS	RRMS	Neurologic manifestations	Neurologic manifestations	MS	MS	MS	ALS	MS
Organismal death												
Cell movement												
Migration of cells												
Morbidity or mortality												
Cell movement of tumor cell l...												
Migration of tumor cell lines												
Cell survival												
Viral Infection												
Cell viability												
Invasion of cells												
Cell proliferation of tumor cel...												
Leukocyte migration												
Organization of cytoplasm												
Organization of cytoskeleton												
Cell movement of leukocytes												
Growth of tumor												
Invasion of tumor cell lines												
Infection by RNA virus												
Binding of blood cells												
Adhesion of immune cells												
Microtubule dynamics												
Activation of cells												
Adhesion of blood cells												
Binding of leukocytes												

Figure S1: Heat maps using z-scores to illustrate the degree of overlap between proteomic datasets generated in this study with previous SMA studies and studies of neurological conditions with similar outcomes to SMA identified via IPA® Analysis Match. Only signatures with a minimum positive z-score of 50 and using either skin or peripheral blood as the tissues of interest were included. Analysis Match outcomes for canonical pathways identified from (**A**) the SMA I dataset and (**B**) the SMA III dataset and Analysis Match outcomes for diseases & functions identified using (**C**) the SMA I dataset and (**D**) the SMA III dataset. MS = multiple sclerosis; RRMS = relapsing-remitting multiple sclerosis
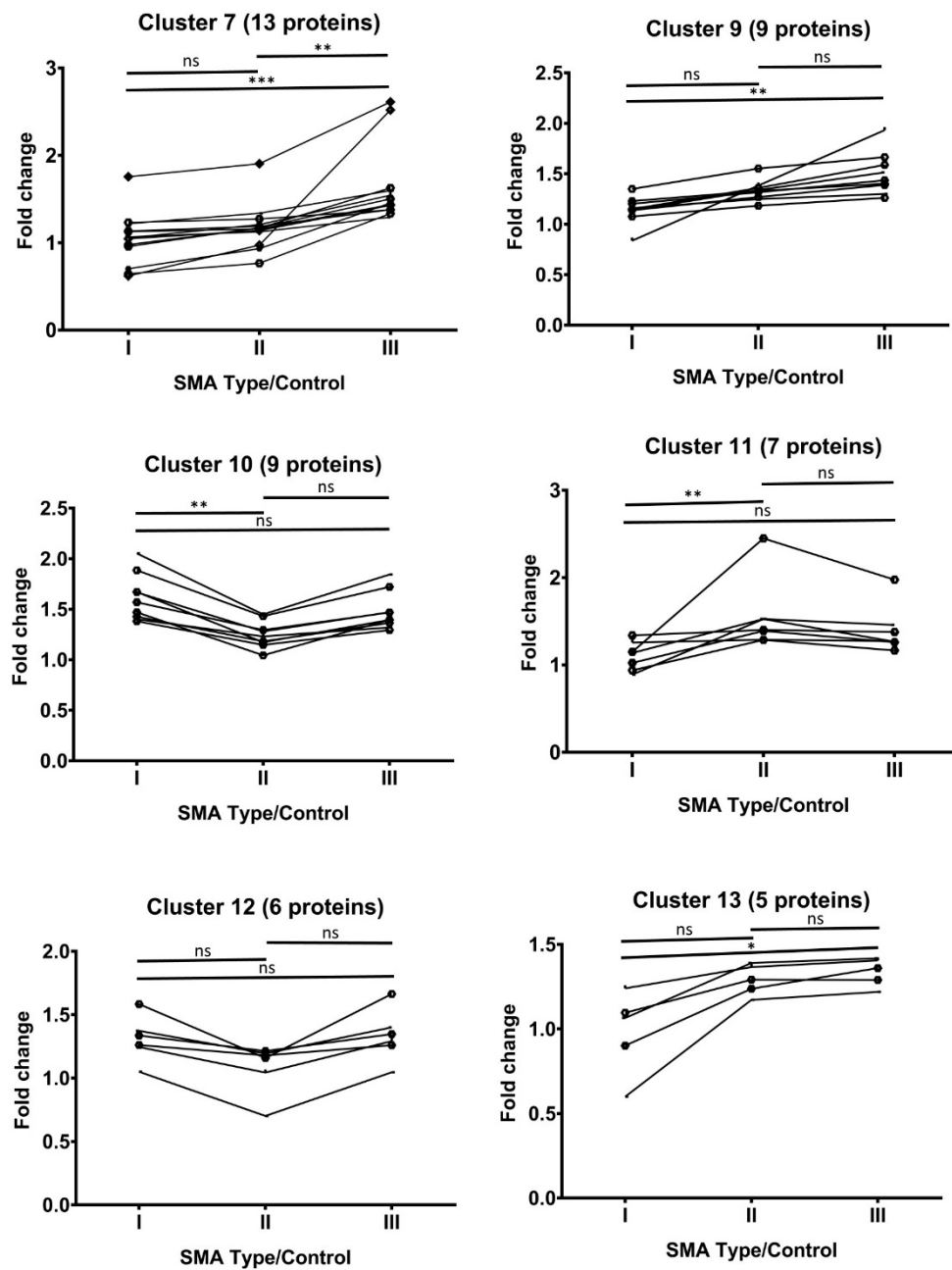
Figure S2: Clusters identified from BioLayout Express 3D analysis of protein expression trends across the three SMA types that met the criteria for differential expression in at least one SMA type (i.e., detected with 2 or more peptides; fold change ≥ 1.25 or ≤ 0.80; significant difference of $p \leq 0.05$). Each point represents a fold change in protein expression relative to age-match controls with connecting lines illustrating the trends between SMA I, II and III. Bars indicate level of significance between each SMA type with *p<0.05; **p<0.01 & ***p<0.0001.

	PYBG	STAT1	RAB3B
Type I SMA	Significantly increased	Potentially increased	Unchanged
Type II SMA	Potentially increased	Unchanged	Significantly increased
Type III SMA	Unchanged	Significantly increased	Potentially increased

Figure S3: Table illustrating how the differential expression of PYGB, STAT1 and RAB3B, as determined via SWATH mass spectrometry and confirmed via western blots, relate to SMA severity.