

Figure S1: FXYD3 gene silencing changes the expression of Na,K-ATPase isozymes during the cell differentiation process. (A-H) Western blot analysis of Na,K-ATPase isozymes. Fifty μ g proteins of cell lysates were subjected to SDS-PAGE, transferred onto nitrocellulose membranes, and the total Na,K-ATPase pool was immunodetected with an antibody recognizing all Na,K-ATPase α isoforms (A). After stripping, the nitrocellulose membrane was probed with a Na,K-ATPase $\alpha 1$ isoform (C) and a $\alpha 3$ isoform-specific (E) antibody and with a $\beta 1$ isoform-specific antibody (G). 1: siRNA2-treated Caco-2 cells at 80% confluency, 2: wild type cells at 80% confluency, 3: siRNA1-treated Caco-2 cells at 80% confluency, 4: siRNA2-treated Caco-2 cells, 21 days after confluency, 5: wild type cells, 21 days after confluency, 6: siRNA1-treated Caco-2 cells, 21 days after confluency. (B, D, F, H) Quantification of data shown in A, C, E, G.

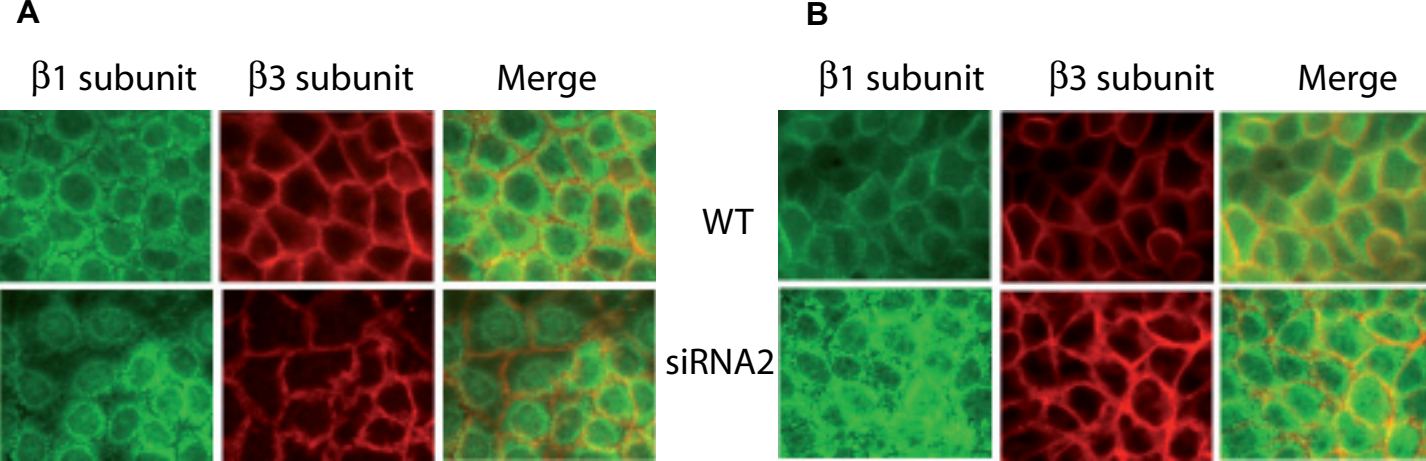


Figure S2: Double immunostaining of Caco-2 cells at 80% confluency (A) and 21 days after confluency (B) with a polyclonal $\beta 1$ isoform-specific and a monoclonal $\beta 3$ isoform-specific antibody. After merging, co-localization is indicated in yellow.

Table S1 : Identification of protein up-regulation in wild type and siRNA2-treated cells by a combination of 2D-DIGE and mass spectrometry.

Dye	Vol Ratio	Intensity	Area	Up-regulation in cells treated with siRNA2					
				Vol	Protein	AC SWISSprot	Theoretical mass	Analysis done	Score
Cy5	4.8	8249	4.0	7117.2	Carbamoyl-phosphate synthase	CPSM	165975	M	101
Cy5	4.6	11401	4.1	11136.8	Carbamoyl-phosphate synthase	CPSM	165975	M	200
Cy5	1.5	1657	4.6	2748.9	Lamin-A/C	LMNA	74380	M	168
Cy5	1.5	2279	5.7	6747.9	Heterogeneous nuclear ribonucleoprotein K	HNRPK	51230	M	281
Cy5	1.5	3325	8.0	11439.1	Retinal dehydrogenase 1	AL1A1	55454	M	218
Cy5	1.9	14761	13.0	59895.1	Ornithine aminotransferase	OAT	48846	M	237
Cy5	1.7	3769	7.4	9532.8	Ornithine aminotransferase	OAT	48846	M	67
Cy5	1.7	4694	4.6	8163.0	Argininosuccinate synthase	ASSY	46786	M	88
					Isocitrate dehydrogenase [NADP]	IDHP	51333		69
Cy3	4.5	3864	3.7	3415.9	Carbamoyl-phosphate synthase	CPSM	165975	M	180
Cy3	7.0	7092	4.2	8664.6	Carbamoyl-phosphate synthase	CPSM	165975	M	143
Cy3	7.4	11101	4.8	14473.0	Carbamoyl-phosphate synthase	CPSM	165975	M	100
Cy3	1.5	3284	6.7	7793.2	Proliferation-associated protein 2G4	PA2G4	41228	M	82
Cy3	2.5	14626	12.4	50871.0	Ornithine aminotransferase	OAT	48846	M	221
Cy3	1.7	2656	7.0	8346.3	Ornithine aminotransferase	OAT	48846	M	87
Cy3	1.9	3261	5.2	6985.4	Isocitrate dehydrogenase [NADP]	IDHP	51333	M	70
Cy3	1.5	3838	9.9	12515.7	Isocitrate dehydrogenase [NADP]	IDHP	51333	M	118
Cy3	1.7	2281	7.2	5442.2	Medium-chain specific acyl-CoA dehydrogenase	ACADM	47015	M	124
Cy3	1.7	2454	6.3	6555.1	Methionine aminopeptidase 1	AMPM1	44100		126
					L-2-hydroxyglutarate dehydrogenase	L2HDH	51068	M	98
Cy3	1.6	421	8.5	2233.7	PDZ domain-containing protein GIPC1	GIPC1	36141	LC	148
					Mitochondrial 28S ribosomal protein S22	RT22	41425		117
					NAD-dependent deacetylase siruin-2	SIRT2	43782		111
					Heterogeneous nuclear ribonucleoprotein H3	HNRH3	36960		72

Dye	Vol Ratio	Intensity	Area	Up-regulation in Wild type cells					
				Vol	Protein	AC SWISSprot	Theoretical mass	Analysis done	Score
Cy3	1.6	5144	21.7	27601.5	Cleavage and polyadenylation specificity factor subunit 5	CPSF5	26268	M	112
Cy3	1.8	2282	13.8	10238.0	Glutathione S-transferase A2	GSTA2	25648	M	112
Cy3	1.6	623	18.1	5748.8	Galectin-3	LEG3	26229	M	84
Cy3	2.0	3022	32.7	26825.5	Calmodulin	CALM	16827	M	69
Cy3	1.7	1741	7.2	4087.8	Protein-glutamine gamma-glutamyltransferase 2	TGM2	78420	M	200
Cy3	1.9	828	5.0	1182.2	Phosphofructokinase, platelet	Q5VSR8	86454	LC	703
					Nucleolin	NUCL	76625		123
					Heterogeneous nuclear ribonucleoprotein Q	HNRPQ	69788		48
Cy3	1.5	1075	5.1	1897.6	Meprin A subunit alpha precursor	MEP1A	85398	M	75
Cy3	1.6	2443	3.5	2679.3	Lamin-A/C	LMNA	74380	M	88
Cy3	1.6	8426	11.5	19421.7	Very long-chain specific acyl-CoA dehydrogenase	ACADV	70745	M	516
					Carnitine O-acetyltransferase	CACP	71336		184
Cy3	1.6	2358	8.2	5100.2	ERO1-like protein alpha precursor	ERO1A	55213	M	573
Cy3	1.5	3220	5.2	4087.4	Very long-chain specific acyl-CoA dehydrogenase	ACADV	70745	M	333
Cy3	2.0	6852	13.4	18286.4	Prolyl 4-hydroxylase subunit alpha-1 precursor	P4HA1	61296	M	70
Cy3	1.6	1353	3.7	1725.1	Dihydroxyacetone kinase	DAK	59282	LC	319
					KDEL motif-containing protein 2 precursor	KDEL2	58934		66
					T-complex protein 1 subunit eta	TCPH	59842		54
Cy3	1.6	20094	10.5	60416.5	Keratin, type II cytoskeletal 8	K2C8	53671	M	269
Cy3	1.7	3619	8.1	11461.8	Keratin, type II cytoskeletal 8	K2C8	53671	M	110
Cy3	1.5	1217	7.3	2537.6	NADPH:adrenodoxin oxidoreductase	ADRO	54259	M	94
Cy3	1.6	4172	6.1	9713.0	Pyruvate dehydrogenase E1 component subunit alpha	ODPA	43952	M	172

Cy5	1.5	2015	20.0	11945.7	Annexin A5	ANXA5	35971	M	122
Cy5	1.8	1723	12.3	7023.6	Pyroline-5-carboxylate reductase	A6NFM2	33548	M	69
Cy5	1.5	1991	6.8	6254.1	Adenylate kinase isoenzyme 4	KAD4	25366	M	96
Cy5	1.9	4955	14.7	22482.8	Cleavage and polyadenylation specificity factor subunit 5	CPSF5	26268	M	136
Cy5	1.7	1458	15.1	7886.4	Glutathione S-transferase A2	GSTA2	25648	M	39
Cy5	1.8	1802	3.4	1947.5	Pyruvate carboxylase, mitochondrial precursor	PYC	130293	M	92
Cy5	1.6	854	6.1	2398.9	Meprin A subunit alpha precursor	MEP1A	85398	M	156
Cy5	1.6	404	5.5	1715.1	Protein disulfide-isomerase A4 precursor	PDIA4	73229	LC	146
					NADH-ubiquinone oxidoreductase 75 kDa subunit,	NDUS1	80443		110
					Leucine-rich PPR motif-containing protein	LPPRC	159003		95
					Nucleolin	NUCL	76625		92
Cy5	1.6	1683	6.8	3066.4	Very long-chain specific acyl-CoA dehydrogenase	ACADV	70745	M	130
Cy5	1.8	3993	14.8	13301.9	Prolyl 4-hydroxylase subunit alpha-1 precursor	P4HA1	61296	M	199
Cy5	1.6	2398	5.9	4651.3	Protein disulfide-isomerase A3 precursor	PDIA3	57146	M	96
Cy5	1.6	21571	11.2	70050.6	Keratin, type II cytoskeletal 8	K2C8	53671	M	290
Cy5	1.6	4512	6.9	15493.7	Keratin, type II cytoskeletal 8	K2C8	53671	M	89
Cy5	1.8	1973	11.7	8610.5	Protein NDRG1	NDRG1	43264	M	65
Cy5	2.5	6845	14.5	23841.5	Creatine kinase B-type	KCRB	42902	M	234

Proteins from each cell lysate were labelled separately with CyDye DIGE fluor Cy3 or Cy5 dye. Only spots showing a volume ratio greater than 1.5 were considered as significant. Theoretical mass was calculated from the sequence. Score : MASCOT score, M: MALDI-MS/MS, LC: LC-MS/MS.