

Additional file 8 – Comparison of predicted subcellular locations with experimental data for both known and hitherto unknown PTS1 and PTS2-targeted proteins

A. Comparison of LOCATE summaries and integrated prediction results with experimentally supported localization of proteins

Protein	LOCATE SCL Summary	Individual subcellular location (SCL) predictions intergrated in LOCATE										Experimentally supported localization (this study or from literature)
		CELLO		pTarget		Proteome Analyst		WoLF PSORT		MultiLoc		
PTS1-containing proteins with hitherto unknown peroxisomal localization												
KBTBD10 [1]	PM, Cyt, Chs (DB); soluble, non-secreted	Cyt	2.94	Cyt	87.60	Cyt	1.00	Cyt	23.00	Per	0.80	Cyt [this study], Cyt, PM (pseudopodia) of FBR fibroblast cell line [2,3]
Gab2 [1]	No SCL data; soluble, non-secreted	Nuc	4.50	Nuc	100.00	Cyt	0.92	Nuc	28.50	Nuc	0.88	Cyt, PM, End of EGF-induced hepatocytes [4]
Scrab1 [1]	No SCL data; multipass-membrane	PM	2.24	Lys	81.40	PM	0.93	ER	16.00	ExR	0.77	PM (caveolae) of endothelial cells [5]
						ER	0.01	PM	9.00	ER	0.11	
						Gol	0.00	Per	4.00	Lys	0.08	
Recently identified PTS1-containing proteins with known peroxisomal localization												
Zadh2 [1]	No SCL data; soluble, non-secreted	Mit	1.85	Mit	87.60	Cyt	0.99	Cyt	14.00	Mit	0.71	Per [this study], Per of CHO cells and rat liver peroxisomal fraction [6,7]
		Chl	1.36			ER	0.00	Cyt-Nuc	11.00	Per	0.27	
						Mit	0.00	Mit	7.00	Cyt	0.01	
Tysnd1 [1]	No SCL data; soluble, non-secreted	PM	2.44	Lys	100.00	ExR	0.98	Mit	14.00	PM	0.41	Per [6,7] of CHO cells and rat liver peroxisomal fraction
						Cyt	0.00	Cyt-Nuc	7.33	Per	0.30	
						ER	0.00	Cyt	7.00	Mit	0.11	

Protein	LOCATE SCL Summary	Individual subcellular location (SCL) predictions intergrated in LOCATE										Experimentally supported localization (this study or from literature)
		CELLO		pTarget		Proteome Analyst		WoLF PSORT		MultiLoc*		
PTS2-containing proteins with hitherto unknown peroxisomal localization												
Galk2	No SCL data; soluble, non-secreted	Cyt	1.92	Cyt	100.00	Cyt	0.86	Cyt	14.50	Cyt	0.98	Cyt [this study]
						ER	0.00	Cyt-Mit	9.00	PM	0.00	
						Nuc	0.00	PM	7.00	Per	0.00	
Qpctl	No SCL data; multipass-membrane	PM	1.37	PM	50.10	ExR	0.03	ER	6.00	ER	0.39	Cyt [this study]
		Mit	1.34			Cyt	0.00	Per	5.50	Gol	0.36	
						ER	0.00	Cyt-Nuc	5.00	PM	0.06	
Syt13	No SCL data; soluble, non-secreted	Nuc	3.87	Cyt	87.60	Cyt	1.00	Nuc	18.00	Cyt	0.84	Cyt [this study], Dev of pancreatic beta cell lines (beta HC9 and MIN6) [8] and neuronal PC12 cell line [9]
						PM	0.00	Cyt-Nuc	17.00	Mit	0.05	
						Nuc	0.00	Cyt	14.00	PM	0.03	
Fut8	Gol (Lit); type II membrane	Cyt	2.59	Gol	93.90	Gol	1.00	ER	11.50	Gol	0.83	Cyt structures in proximity to Nuc [this study], Gol (Golgi-rich fraction of rat liver) [10]
						ER	0.27	ER-Gol	8.50	ER	0.09	
						Cyt	0.00	ExR	7.00	Lys	0.03	
Ppp3ca	Cyt; Nuc (Lit); soluble, non-secreted	Cyt	2.59	Cyt	87.60	Cyt	0.99	Cyt	20.50	Cyt	0.96	Cyt and Nuc of skeletal muscle, COS7 cell line [11], and aortic myocytes [12] ; translocation from Cyt to Nuc in keratinocytes upon intracellular Ca ²⁺ increase [13]; mainly Cyt; minority Nuc [this study]
						ER	0.00	Cyt_PM	11.00	Per	0.01	
						Nuc	0.00	Nuc	5.00	PM	0.01	

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		CELLO		pTarget		Proteome Analyst		WoLF PSORT		MultiLoc*		
PTS2-containing proteins with hitherto unknown peroxisomal localization												
Ppp3cb	Cyt; Nuc (Lit); soluble, non-secreted	PM	1.61	Cyt	56.30	Cyt	0.99	Cyt	9.50	Cyt	0.92	Cyt aortic myocytes [12]; upon PDGF induction translocalization from Cyt to Nuc in aortic myocytes [12], translocation from Cyt to Nuc in keratinocytes upon intracellular Ca ²⁺ increase [13]
		Nuc	1.07			ER	0.00	Cyt-Nuc	8.50	PM	0.04	
						Nuc	0.00	Nuc	6.50	Per	0.02	
Zmiz1	Cyt (DB); soluble, non-secreted	Cyt	4.11	Cyt	100.00	Nuc	1.00	Nuc	13.00	Nuc	0.96	Nuc in monkey kidney cell line CV-1, prostate cancer cell line PC13 [14]
						Cyt	0.00	Cyt	9.00	Cyt	0.02	
						ER	0.00	PM	5.00	Per	0.01	
Wdr6	No SCL data; soluble, non-secreted	Cyt	3.04	Cyt	75.10	Cyt	0.99	Cyt	19.00	Cyt	0.82	Cyt in embryo kidney cell line HEK293T [15]
						Nuc	0.02	Nuc	8.00	Per	0.10	
						ER	0.00	ExR	2.00	ER	0.05	
Adhfe1	No SCL data; soluble, non-secreted	Mit	2.33	Mit	93.90	Cyt	0.37	Mit	29.00	Mit	0.99	Mit in COS cells [16]
						Mit	0.00			ExR	0.01	
						ER	0.00			Per	0.00	
Pgm2	No SCL data; soluble, non-secreted	Cyt	2.42	Cyt	81.40	Cyt	1.00	Cyt	21.00	Cyt	0.69	Cyt ; mouse liver cytosolic fraction; identified by MALD-TOF mass spectrometry [17]
						ER	0.79	Mit	11.00	Per	0.26	
						Gol	0.00			Mit	0.03	

Protein	LOCATE SCL Summary	Individual subcellular location (SCL) predictions intergrated in LOCATE										Experimentally supported localization (this study or from literature)
		CELLO		pTarget		Proteome Analyst		WoLF PSORT		MultiLoc*		
PTS2-containing proteins with known peroxisomal localization												
Pex11c	Per (DB); CP (Exp.;Hela cells) Multipassmem.	PM	2.33	Per	100.0	NA	NA	Mit	16.00	Mit	0.51	Per in fibroblasts [18]
								Cyt-Nuc	8.33	Per	0.26	
								Mit-Per	8.33	Cyt	0.12	
Phyh	Per (DB); soluble, non-secreted	Per	2.99	Per	50.10	Per	0.99	Mit	27.00	Mit	0.71	Per ; liver subcellular fraction [19]
						ER	0.00	Cyt	4.00	Cyt	0.09	
						Cyt	0.00			ER	0.08	
Acaal	Per (Lit; DB); soluble, non-secreted	Per	1.47	Per	68.90	Mit	0.35	Mit	13.00	Mit	0.98	Per ; liver subcellular fraction [20] and CVH-Px110 cell line (derived from CV-1) [21]
		Chl	1.26			Per	0.00	Nuc	7.50	Per	0.01	
						Cyt	0.00	Cyt-Nuc	7.50	Cyt	0.00	
Acaalb	No SCL data; soluble, non-secreted	Per	2.04	Lys	81.40	Mit	0.35	Mit	11.00	Mit	0.98	Per in CVH-Px110 cell line [21]
						Per	0.00	Cyt-Nuc	8.50	Per	0.01	
						Cyt	0.00	Cyt	7.50	Cyt	0.00	
Mvk	Cyt, Per (DB); soluble, non-secreted	Cyt	1.82	Cyt	1.00	Cyt	0.99	ExR	11.00	Cyt	0.42	Per in CV1 cells, skin fibroblasts, liver peroxisomal subfraction; Cyt and Per in liver by electron microscopy [22], Cyt in Hek293, CV1 and fibroblasts subcellular fractions [23], Per in hepatoblastoma HepG2 cell line [24]
						Per	0.38	Mit	8.50	ExR	0.27	
						ER	0.01	ER	5.00	Mit	0.16	

Protein	LOCATE SCL Summary	Individual subcellular location (SCL) predictions intergrated in LOCATE										Experimentally supported localization (this study or from literature)
		CELLO	pTarget	Proteome Analyst		WoLF PSORT		MultiLoc*				
PTS2-containing proteins with hitherto unknown peroxisomal localization												
Agps	Per (DB); soluble, non- secreted	Per	2.21	Per	87.60	Per	1.00	Cyt	19.50	Cyt	0.79	Per ; liver peroxisomal subfraction [25]
						ER	0.20	Cyt-Nuc	14.83	Per	0.17	
						Mit	0.15	Cyt-PM	11.16	Mit	0.03	

For each protein the integrated LOCATE result derived from five subcellular localization prediction methods is shown. The predicted localization is accompanied by a score that is specific for the method. Details are described in the FAQ of LOCATE (<http://locate.imb.uq.edu.au/>). Abbreviations. DB: database annotation; Lit: Literature; Exp: experimental; Cyt: cytoplasm; CP: cytoplasmic puncta; Dcv: dense core vesicles; Nuc: nucleus; Per: peroxisomes; Mit: mitochondria; End: endosomes; ER: endoplasmic reticulum; Lys: lysosome; PL: plasma membrane; ExR: extracellular region; Chl: chloroplast; Chs: cytoskeleton; Gol: Golgi apparatus; NA: not assigned; *MultiLoc was not trained to detect PTS2 signals; Gab2, Scrab1, and KBTBD10 are candidates that were identified in a previous study [1]. The localization of Sytl3, an effector of Rab27a-dependent membrane trafficking [26] the observed localization appears to depend on the ontogeny of the cells. The localization to dense core vesicles cannot be predicted by any of the programs as they are not trained for this location.

The LOCATE accessions and their corresponding GenPept or ENSEMBL accessions are: KBTBD10: hPA13085.1, NP_006054; Gab2: mPA18842.1, AAD05166; Scrab1: mPA17109.1, AAH04656; Zadh2: mPA10238.1, BAC38389; Tysnd1: mPA1614.1, BAC38389; Galk2: mPA11864.1,

AAH79843; Qpctl: mPA19634.1, BAC29114; Sytl3: mPA8872.1, BAB41086; Fut8: mPA4396.3, BAE22175 ; Ppp3ca: mPA13948.1, ENSMUSP00000053101; Ppp3cb: mPA6713.2, AAH66000; Zmiz1: mPA6589.1, AAH65120; Wdr6: mPA22875.1, NP_001020546; Adhfe1: mPA421.1, BAC30151; Pgm2: mPA14944.1; BAE28266; Pex11c: mPA21256.1, AAH62135; Phyh: mPA11499.1; AAH02018; Acaa1: mPA22546.1, AAP31668; Acaa1b: mPA23310.1, ENSMUSP00000010795; Mvk: mPA16137.2, AAH05606; Agps: mPA12066.1, AAH63086.

Selection criteria. All known mammalian PTS2-targeted proteins are represented by six mouse sequences. The other sequences represent ten of 13 PTS2-targeted candidates that were predicted in this study (see also Additional File 4). Three candidates were excluded from the comparison because of problems with the original cDNA clone or its sequence (see Additional File 4; Footnote 6). Five of the ten candidates were shown to locate in this and other studies to non-peroxisomal locations. The other five candidates were not experimentally tested by us (low priority). Therefore the comparison of the predicted location relies only on experiments reported in the literature.

B. Comparison of PTS1Prowler/PProwler and PTS1 Predictors predictions with experimentally supported localization of proteins

Protein	GenPept Accession	PTS1 Prowler/ PProwler		PTS1 Predictor		PeroxisomeDB PTS1 Predictor (Blimps Search)		Experimentally supported localization (this study or from literature)
PTS1-containing proteins with hitherto unknown peroxisomal localization								
KBTBD10 [1]	NP_006054	Per Oth Mtp Sp	0.02 0.02 0.96 0.00	Per	2.052 <i>P</i> FP 0.14%	NA	E-value 0.32	Cyt [this study], Cyt , PM (pseudopodia) of FBR fibroblast cell line [2,3]
Gab2 [1]	AAD05166	Per Oth Mtp Sp	0.46 0.96 0.02 0.01	Per	8.628 <i>P</i> FP 1.7e-04	NA	E-value 0.12	Cyt , PM , End of EGF-induced hepatocytes [4]
Scrab1 [1]	AAH04656	Per Oth Mtp Sp	0.05 0.10 0.01 0.90	Per	3.627 <i>P</i> FP 8.8e-04	NA	E-value 1	PM (caveolae) of endothelial cells [5]
Recently identified PTS1-containing proteins with known peroxisomal localization								
Zadh2 [1]	BAC38389	Per Oth Mtp Sp	0.99 0.24 0.76 0.00	Per	2.734 <i>P</i> FP 0.11%	NA	E-value 0.0063	Per [this study], Per of CHO cells and rat liver peroxisomal fraction [6,7]

Protein	GenPept Accession	PTS1 Prowler/ PProwler		PTS1 Predictor		PeroxisomeDB PTS1 Predictor (BlimpsSearch)		Experimentally supported localization (this study or from literature)
Recently identified PTS1-containing proteins with known peroxisomal localization								
Tysnd1 [1]	BAB23793	Per	0.98	Per	16.264	NA	E-value	Per [6,7] of CHO cells and rat liver peroxisomal fraction
		Oth	0.96		<i>P</i> FP 5.4e-06		0.045	
		Mtp	0.04					
		Sp	0.00					

Selection criteria: Four mouse (Gab2, Scrab1, Zadh1, and Tsynd1) proteins and human KBTBD10 were previously predicted by us as strong PTS1-targeted candidates [1]. Two of the candidates, Tysnd1 [6; 7] and Zadh [this study; 7] were experimentally confirmed by us [6] and other [7] as PTS1-targeted. Abbreviations. Per: peroxisome; Oth: others; Mtp: mitochondrial protein; Sp: secreted protein. *P* FP: probability of false positive; NA: not applicable; Blimps provides all possible hits over the entire sequence length, together with alignment scores and E-values based on the position-specific scoring matrix of a twelve amino acids PTS1 profile. The decision making whether the protein of interest might be targeted to peroxisomes is left to the user.

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