SUPPLEMENTAL DATA

Identification of a new fatty acid synthesis-transport machinery at the peroxisomal membrane

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Supplementary Table 1. Peptides of ALDP and PMP70 specific binding partners (ACLY, FASN) identified by subsequent LCMS analysis.

Reference	**Peptide**	**MH+**	**z**	***P* (pro)**	**Protein Score**
Scans				*P* (pep)	Xscore
gi|603074|gb|AAB60340.1|ACLY				**2,80E-09**	**266,24**
1015-1017	R.AVQGMLDFDYVCSR.D	1660,74573	2	2,80E-09	4,70
1087	K.LGLVGVNLTLDGVK.S	1397,83618	2	5,84E-09	4,06
959	R.EAYPEEAYIADLDAK.S	1697,79053	2	1,13E-08	4,10
882	-.SGGMSNELNNIISR.-	1491,72205	2	4,00E-07	3,66
1092	-.LGLVGVNLTLDGVK.-	1397,83618	2	7,12E-07	3,76
965	R.KHPEVDVLINFASLR.S	1737,96460	3	3,01E-06	3,83
1139	-.TIAIIAEGIPEALTR.-	1567,90540	2	3,28E-06	4,32
1012	K.MIVVLGEIGGTEEYK.I	1637,84546	2	4,04E-06	4,71
1003	K.DGVYVLDLAAK.V	1163,63062	2	7,63E-06	3,04
958	-.KHPEVDVLINFASLR.-	1737,96460	3	1,04E-05	4,18
773	R.EGDYVLFHHEGGVDVGDVDAK.A	2258,03613	2	1,81E-05	4,00
447	K.HLLVHAPDDKK.E	1272,70593	2	4,09E-05	3,15
819	-.AIVWGMQTR.-	1061,55603	2	1,55E-04	2,53
1417	K.DLVSSLTSGLLTIGDR.F	1646,89600	2	2,38E-04	4,74
872	-.GVTIIGPATVGGIKPGCFK.-	1872,04114	2	2,53E-04	3,52
749	R.SGGM*SNELNNIISR.T	1507,72142	2	2,53E-04	3,72
744	R.SAYDSTMETM*NYAQIR.T	1896,81472	2	4,21E-04	3,51
482	-.RGGPNYQEGLR.-	1246,62866	2	1,19E-03	3,60
1000	-.DGVYVLDLAAK.-	1163,63062	1	1,59E-03	2,72
468	K. AKPAM*PQDSVPSPR.S	1496,75707	2	1,75E-03	2,74
1447	-.DLVSSLTSGLLTIGDR.-	1646,89600	2	2,35E-03	3,68
997	K.IGNTGGMLDNILASK.L	1503,78357	2	5,33E-03	4,24

717	R.TTDGVYEGVAIGGDR.Y	1509,71802	2	1,14E-02	3,84
744	-.SAYDSTM*ETMNYAQIR.-	1896,81472	2	2,02E-02	2,74
1018	R.DEPSVAAMVYPFTGDHK.Q	1863,85815	2	3,18E-02	2,52
1280	K.ILIIGGSIANFTNVAATFK.G	1950,10583	2	5,90E-02	3,47
1182	-.WGDIEFPPPFGR.-	1417,68994	2	6,16E-02	3,29
580-582	K.LYRPGSVAYVSR.S	1367,74304	3	6,42E-02	3,41
gi|68533031|dbj|BAE06070.1|FASN				**1,77E-08**	**238,23**
786	R.FPQLDSTSFANSR.D	1469,70190	2	1,77E-08	3,54
756	R.QGVQVQVSTSNISSLEGAR.G	1960,00940	2	2,85E-07	4,62
849	K.VLQGDLVMNVYR.D	1406,74609	2	3,01E-07	3,88
749	K.VLQGDLVM*NVYR.D	1422,74545	2	3,28E-06	3,64
911	K.VVQVLAEEPEAVLK.G	1622,93640	2	3,41E-06	3,66
726	R.LQVVDQPLPVR.G	1263,74194	2	1,52E-05	3,65
761	-.SLLVNPEGPTLM*R.-	1442,77166	2	1,64E-05	2,96
763	R.AFEVSENGNLVVSGK.V	1549,78564	2	2,18E-05	3,18
561	K.ADEASELACPTPK.E	1388,63623	2	3,96E-05	3,04
893	R.SEGVVAVLLTK.K	1115,66699	2	2,01E-04	3,82
917	-.VVVQVLAEEPEAVLK.-	1622,93640	3	2,24E-04	4,45
569	K.VVEVLAGHGHLYSR.I	1536,82813	2	4,11E-04	3,05
527	-.DPSQQELPR.-	1069,52722	2	4,98E-04	2,95
854	K.MVVPGLDGAQIPR.D	1352,73547	2	5,54E-04	2,76
828	-.VAAAVDLIIK.-	1012,64014	2	5,59E-04	2,95
653	-.GNAGQSNYGFANSAMER.-	1773,76086	2	7,46E-04	4,19
668	K.EDGLAQQQTQLNLR.S	1613,82422	2	7,55E-04	4,12
691	R.GTHTGVWVGVSGSETSEALSR.D	2117,02588	3	3,13E-03	3,78
856	R.SLLVNPEGPTLMR.L	1426,77222	2	3,91E-03	3,15
791	.-FPQLDSTSFANSR.-	1469,70190	2	3,92E-03	3,23
499	.-VSVHVIEGDHR.-	1247,64917	2	9,72E-03	2,43
673	.-EDGLAQQQTQLNLR.-	1613,82422	2	5,44E-02	3,19
574	K.AQVADVVVSR.W	1043,58435	2	8,21E-02	3,10
823	R.VYATILNAGTNTDGFK.E	1684,85413	2	2,42E-01	3,45

Peptides of ACLY and FASN obtained by LCMS are listed including the molecular weight of charged peptides MH+, charge state z of peptides, probability value of the protein *P* (pro) and of the peptides *P* (pep), and the cross correlation scores X (Xscore).

Supplementary Table 2. BRET ratios derived from PPI studies among ALDP, PMP70, ACLY, FASN, FATP4, and ACC1.

PPI	protein 1	protein2	experiment1	experiment 2	mean
ALDP-FASN	Rluc-ALDP	YFP-FASN	0.11	0.09	0.10
	Rluc-ALDP	FASN-YFP	0.05	0.05	0.05
	ALDP-Rluc	YFP-FASN	0.07	0.08	0.08
	ALDP-Rluc	FASN-YFP	0.04	0.03	0.04
	Rluc-FASN	YFP-ALDP	0.02	0.02	0.02
	Rluc-FASN	ALDP-YFP	0.02	0.02	0.02
	FASN-Rluc	YFP-ALDP	0.11	0.08	0.09
	FASN-Rluc	ALDP-YFP	0.05	0.00	0.03
PMP70-FASN	Rluc-PMP70	YFP-FASN	0.08	0.14	0.11
	Rluc-PMP70	FASN-YFP	0.04	0.03	0.03
	PMP70-Rluc	YFP-FASN	*low signal*	*low signal*	
	PMP70-Rluc	FASN-YFP	*low signal*	*low signal*	
	Rluc-FASN	YFP-PMP70	-0.01	-0.02	-0.01
	Rluc-FASN	PMP70-YFP	0.00	-0.03	-0.02
	FASN-Rluc	YFP-PMP70	0.05	0.08	0.07
	FASN-Rluc	PMP70-YFP	0.00	0.01	0.00
ALDP-ACLY	Rluc-ALDP	YFP-ACLY	0.11	0.09	0.10
	Rluc-ALDP	ACLY-YFP	0.15	0.11	0.13
	ALDP-Rluc	YFP-ACLY	0.13	0.10	0.11
	ALDP-Rluc	ACLY-YFP	0.17	0.12	0.14
	Rluc-ACLY	YFP-ALDP	0.04	0.03	0.03
	Rluc-ACLY	ALDP-YFP	0.05	0.03	0.04
	ACLY-Rluc	YFP-ALDP	0.05	0.03	0.04
	ACLY-Rluc	ALDP-YFP	0.04	0.07	0.06
PMP70-ACLY	Rluc-PMP70	YFP-ACLY	0.14	0.18	0.16
	Rluc-PMP70	ACLY-YFP	0.18	0.19	0.19
	PMP70-Rluc	YFP-ACLY	0.28	*low signal*	0.28
	PMP70-Rluc	ACLY-YFP	0.38	0.39	0.39
	Rluc-ACLY	YFP-PMP70	0.02	0.04	0.03
	Rluc-ACLY	PMP70-YFP	0.04	0.02	0.03
	ACLY-Rluc	YFP-PMP70	0.03	0.08	0.05

	ACLY-Rluc	PMP70-YFP	0.02	0.03	0.02
FASN-ACLY	Rluc-FASN	YFP-ACLY	0.06	0.06	0.06
	Rluc-FASN	ACLY-YFP	0.05	0.05	0.05
	FASN-Rluc	YFP-ACLY	0.12	0.15	0.14
	FASN-Rluc	ACLY-YFP	0.14	0.19	0.17
	Rluc-ACLY	YFP-FASN	0.06	0.05	0.05
	Rluc-ACLY	FASN-YFP	0.03	0.02	0.02
	ACLY-Rluc	YFP-FASN	0.06	*low signal*	0.06
	ACLY-Rluc	FASN-YFP	0.02	*low signal*	0.02
ALDP-PMP70	Rluc-ALDP	YFP-PMP70	0.06	0.05	0.06
	Rluc-ALDP	PMP70-YFP	0.04	0.03	0.04
	ALDP-Rluc	YFP-PMP70	0.07	0.05	0.06
	ALDP-Rluc	PMP70-YFP	0.04	0.03	0.04
	Rluc-PMP70	YFP-ALDP	0.13	0.12	0.12
	Rluc-PMP70	ALDP-YFP	0.09	0.02	0.05
	PMP70-Rluc	YFP-ALDP	*low signal*	*low signal*	
	PMP70-Rluc	ALDP-YFP	*low signal*	*low signal*	
PMP70-PMP70	Rluc-PMP70	YFP-PMP70	0.12	0.10	0.11
	Rluc-PMP70	PMP70-YFP	0.02	0.04	0.03
	PMP70-Rluc	YFP-PMP70	*low signal*	*low signal*	
	PMP70-Rluc	PMP70-YFP	*low signal*	*low signal*	
ALDP-ALDP	Rluc-ALDP	YFP-ALDP	0.09	0.076	0.09
	Rluc-ALDP	ALDP-YFP	0.10	0.095	0.10
	ALDP-Rluc	YFP-ALDP	0.16	0.206	0.16
	ALDP-Rluc	ALDP-YFP	0.53	0.496	0.51
ACLY-ACLY	Rluc-ACLY	YFP-ACLY	0.19	0.16	0.17
	Rluc-ACLY	ACLY-YFP	0.18	0.16	0.17
	ACLY-Rluc	YFP-ACLY	0.73	0.67	0.70
	ACLY-Rluc	ACLY-YFP	0.41	0.42	0.42
FASN-FASN	Rluc-FASN	YFP-FASN	0.06	0.06	0.06
	Rluc-FASN	FASN-YFP	0.01	0.02	0.02
	FASN-Rluc	YFP-FASN	0.11	0.11	0.11
	FASN-Rluc	FASN-YFP	0.03	0.05	0.04
ALDP-FATP4	Rluc-ALDP	YFP-FATP4	0.08	0.08	0.08
	Rluc-ALDP	FATP4-YFP	0.04	0.02	0.03

	ALDP-Rluc	YFP-FATP4	0.11	0.12	0.11
	ALDP-Rluc	FATP4-YFP	0.06	0.07	0.07
	Rluc-FATP4	YFP-ALDP	0.06	0.02	0.04
	Rluc-FATP4	ALDP-YFP	0.05	0.00	0.02
	FATP4-Rluc	YFP-ALDP	0.07	*low signal*	0.07
	FATP4-Rluc	ALDP-YFP	0.06	*low signal*	0.06
PMP70-FATP4	Rluc-PMP70	YFP-FATP4	0.19	0.11	0.15
	Rluc-PMP70	FATP4-YFP	0.07	0.06	0.07
	PMP70-Rluc	YFP-FATP4	*low signal*	*low signal*	
	PMP70-Rluc	FATP4-YFP	*low signal*	*low signal*	
	Rluc-FATP4	YFP-PMP70	0.05	-0.01	0.02
	Rluc-FATP4	PMP70-YFP	0.05	0.04	0.05
	FATP4-Rluc	YFP-PMP70	0.07	*low signal*	0.07
	FATP4-Rluc	PMP70-YFP	0.04	-0.03	0.00
FASN-FATP4	Rluc-FASN	YFP-FATP4	0.03	0.03	0.03
	Rluc-FASN	FATP4-YFP	0.02	0.01	0.01
	FASN-Rluc	YFP-FATP4	0.04	0.10	0.07
	FASN-Rluc	FATP4-YFP	0.02	0.02	0.02
	Rluc-FATP4	YFP-FASN	0.05	0.04	0.04
	Rluc-FATP4	FASN-YFP	0.03	0.02	0.02
	FATP4-Rluc	YFP-FASN	0.10	0.10	0.10
	FATP4-Rluc	FASN-YFP	0.03	0.02	0.02
ACLY-FATP4	Rluc-ACLY	YFP-FATP4	0.02	0.03	0.03
	Rluc-ACLY	FATP4-YFP	0.02	0.02	0.02
	ACLY-Rluc	YFP-FATP4	0.02	0.02	0.02
	ACLY-Rluc	FATP4-YFP	0.00	0.00	0.00
	Rluc-FATP4	YFP-ACLY	0.05	0.04	0.05
	Rluc-FATP4	ACLY-YFP	0.04	0.04	0.04
	FATP4-Rluc	YFP-ACLY	0.06	0.06	0.06
	FATP4-Rluc	ACLY-YFP	0.07	0.09	0.08
FATP4-FATP4	Rluc-FATP4	YFP-FATP4	0.14	0.09	0.14
	Rluc-FATP4	FATP4-YFP	0.03	0.02	0.03
	FATP4-Rluc	YFP-FATP4	0.19	0.10	0.19
	FATP4-Rluc	FATP4-YFP	0.05	0.04	0.05
ALDP-ACC	Rluc-ALDP	YFP-ACC	0.01	0.02	0.02

	Rluc-ALDP	ACC-YFP	0.00	0.02	0.01
	ALDP-Rluc	YFP-ACC	0.01	0.03	0.02
	ALDP-Rluc	ACC-YFP	0.01	0.02	0.01
	Rluc-ACC	YFP-ALDP	0.01	0.03	0.02
	Rluc-ACC	ALDP-YFP	0.00	0.01	0.01
	ACC-Rluc	YFP-ALDP	0.01	0.02	0.02
	ACC-Rluc	ALDP-YFP	0.00	0.02	0.01
PMP70-ACC	Rluc-PMP70	YFP-ACC	0.06	0.03	0.05
	Rluc-PMP70	ACC-YFP	0.03	0.03	0.03
	PMP70-Rluc	YFP-ACC	*low signal*	*low signal*	
	PMP70-Rluc	ACC-YFP	*low signal*	*low signal*	
	Rluc-ACC	YFP-PMP70	0.01	0.02	0.01
	Rluc-ACC	PMP70-YFP	0.01	0.02	0.01
	ACC-Rluc	YFP-PMP70	*low signal*	*low signal*	
	ACC-Rluc	PMP70-YFP	*low signal*	0.02	0.02
ACLY-ACC	Rluc-ACLY	YFP-ACC	0.01	0.03	0.02
	Rluc-ACLY	ACC-YFP	0.02	0.03	0.02
	ACLY-Rluc	YFP-ACC	0.01	0.03	0.02
	ACLY-Rluc	ACC-YFP	0.02	0.03	0.02
	Rluc-ACC	YFP-ACLY	0.01	0.03	0.02
	Rluc-ACC	ACLY-YFP	0.01	0.03	0.02
	ACC-Rluc	YFP-ACLY	*low signal*	0.19	0.17
	ACC-Rluc	ACLY-YFP	0.18	0.19	0.19
FASN-ACC	Rluc-FASN	YFP-ACC	0.01	0.03	0.02
	Rluc-FASN	ACC-YFP	0.02	0.03	0.02
	FASN-Rluc	YFP-ACC	0.03	0.01	0.02
	FASN-Rluc	ACC-YFP	0.02	0.03	0.02
	Rluc-ACC	YFP-FASN	0.03	0.03	0.03
	Rluc-ACC	FASN-YFP	0.00	0.02	0.01
	ACC-Rluc	YFP-FASN	*low signal*	*low signal*	
	ACC-Rluc	FASN-YFP	*low signal*	0.06	0.06
FATP4-ACC	Rluc-FATP4	YFP-ACC	0.02	0.03	0.03
	Rluc-FATP4	ACC-YFP	0.01	0.03	0.02
	FATP4-Rluc	YFP-ACC	0.04	0.05	0.04
	FATP4-Rluc	ACC-YFP	0.02	0.02	0.02

	Rluc-ACC	YFP-FATP4	0.04	0.07	0.05
	Rluc-ACC	FATP4-YFP	0.01	0.02	0.02
	ACC-Rluc	YFP-FATP4	*low signal*	*low signal*	
	ACC-Rluc	FATP4-YFP	*low signal*	0.02	0.02
ACC-ACC	Rluc-ACC	YFP-ACC	0.10	0.1162	0.10
	Rluc-ACC	ACC-YFP	0.02	0.0321	0.02
	ACC-Rluc	YFP-ACC	*low signal*	*low signal*	
	ACC-Rluc	ACC-YFP	*low signal*	*low signal*	
ALDP-IL10RB	Rluc-ALDP	YFP-IL10RB	0.01	-0.02	-0.01
	Rluc-ALDP	IL10RB-YFP	-0.01	-0.02	-0.01
	ALDP-Rluc	YFP-IL10RB	0.01	0.00	0.00
	ALDP-Rluc	IL10RB-YFP	-0.01	-0.01	-0.01
	Rluc-IL10RB	YFP- ALDP	0.02	0.00	0.01
	Rluc-IL10RB	ALDP-YFP	-0.01	-0.01	-0.01
	IL10RB-Rluc	YFP-ALDP	0.02	*low signal*	0.02
	IL10RB-Rluc	ALDP-YFP	-0.01	*low signal*	-0.01
ALDP-ACADS	Rluc-ALDP	YFP-ACADS	0.01	-0.01	0.01
	Rluc-ALDP	ACADS-YFP	*low signal*	0.01	0.01
	ALDP-Rluc	YFP-ACADS	0.05	*low signal*	0.05
	ALDP-Rluc	ACADS-YFP	0.01	0.03	0.02
	Rluc-ACADS	YFP- ALDP	-0.01	-0.03	-0.02
	Rluc-ACADS	ALDP-YFP	-0.01	-0.03	-0.02
	ACADS-Rluc	YFP-ALDP	-0.02	0.01	0.00
	ACADS-Rluc	ALDP-YFP	*low signal*	-0.02	-0.02

BRET ratios tested in 8 possible tag combinations are listed for 2 independent experiments. BRET ratios above the threshold for a positive PPI are highlighted in grey.

Supplementary Table 3. BRET saturation experiments.

	ALDP - ALDP	PMP70 - PMP70	PMP70 - ALDP	PMP70 - FASN	ALDP - FASN	PMP70 - ACLY	ALDP - ACLY	ACLY - FASN
BRET_{max}	0.16 ± 0.02	0.33 ± 0.01	0.25 ± 0.04	0.35 ± 0.02	0.27 ± 0.03	0.50 ± 0.01	0.49 ± 0.01	0.47 ± 0.01
P-value		< 0.001	< 0.001		< 0.01		ns	-
BRET_{50}	0.85 ± 0.19	1.22 ± 0.16	2.05 ± 0.60	1.81 ± 0.33	2.02 ± 0.46	0.59 ± 0.02	4.25 ± 0.2	3.83 ± 0.29
P-value		ns	< 0.01		ns		< 0.001	-

BRET saturation experiments were performed to determine relative binding affinities ($BRET_{50}$, acceptor to donor ratio at half-maximum BRET ratio) and maximal BRET ratios as a measure of the distance of BRET tags ($BRET_{max}$). Calculation of the maximal BRET ratios at saturation level ($BRET_{max}$) and the relative affinity index ($BRET_{50}$) derived from BRET saturation experiments, given as mean ± corresponding standard error of the mean (SEM). Significance for the difference between the homomeric ALDP transporter, the homomeric PMP70 or heteromeric PMP70-ALDP transporter, as well as for the interaction between PMP70 or ALDP with FASN and for PMP70 or ALDP with ACLY, respectively, were calculated by one-way ANOVA and a Dunnett`s post test (ns, not significant). $BRET_{50}$ and $BRET_{max}$ values are displayed as means ± SD of n = 3 independent experiments.

Supplementary Table 4. PPI network of ALDP, PMP70, ACLY, FASN and FATP4 and first order interactions.

	BIOGRID	**HPRD**	**Manually recurated**	**PMID**
FATP4	GT197		GT197	20562859
	S1R		S1R	17353931
PMP70	ALDP	ALDP	ALDP	10551832
		PMP70	PMP70	10551832
		ALDR	ALDR	10551832
	PEX19	PEX19	PEX19	10777694
	USP50			
	USP32			
	CLN3		CLN3	20562859
	BRF-2		BRF-2	17353931
	mPR		mPR	17353931
	PRL-R		PRL-R	17353931
	PELO		PELO	17353931
	MLF2		MLF2	17353931
	MEST		MEST	17353931
		COL6A2		
	RIP5		RIP5	17353931
ALDP		ALDP	ALDP	10551832
	PMP70	PMP70	PMP70	10551832
	PEX19	PEX19	PEX19	10777694
	ALDR	ALDR	ALDR	10551832
ACLY		ACLY	ACLY	9116495
		PKA C-a		
	AMPKb		AMPKb	17353931
	GSK-3b	GSK-3b	GSK-3b	17353931

	NDKB		NDKB	17353931
	Hrs		Hrs	19019082
	IkBKE		IkBKE	17353931
	bHLHe33		bHLHe33	17353931
FASN	FASN	FASN	FASN	11248039
	EWS	EWSR1	EWS	16189514
		DRG-1	DRG-1	17220478

Interaction partners of ALDP, PMP70, ACLY, FASN and FATP4 identified by database analysis (HPRD, BioGRID) with subsequent manual recuration are listed and corresponding PubMed identification numbers (PMID) are given.

Supplementary Table 5. Oligonucleotide sequences used for construction of plasmids.

Name	**Sequence (5´- 3´)**	**Restriction enzyme**
C-term. ALDP for.2	ttt*gaattc*aatactcagagtcagatgcagaggcc	EcoRI
ALDPStop-NotI rev	taa*gcggccgc*tcaggtggaggcaccctggaggcc	NotI
ALDP up	tta*ctcgag*caatgccggtgctctccaggccccgg	XhoI
ALDP down	tttaagctttcaggtggaggcaccctggaggcc	HindIII
C-term. PMP70 for	ttaggatccgatttgtctcatcctcgacatctc	Bam HI
PMP70Not I rev	ttagcggccgcctaagagccaaactcaactgtatc	NotI
PMP70 up	taa*tccgga*atggcggccttcagcaagtacttg	BspEI
PMP70 down	ttaggatccctaagagccaaactcaactgtatc	Bam HI
ACLY for	tta*gaattc*aaatgtcggccaaggcaatttc	EcoRI
ACLY rev	taa*gcggccgc*ttacatgctcatgtgttcc	NotI
FATP4myc for	ttagaattcatgctgcttggagcctctctg	EcoRI
FATP4myc rev	taaaagcttttcagcttctcctcgcctgcc	HindIII
FATP4 attB1 for	ggggacaagtttgtacaaaaaagcaggctttatgctgcttggagcctctctg	
FATP4 attB1 rev	ggggaccactttgtacaagaaagctgggttcagcttctcctcgcctgcc	
ACLY attB1 for	ggggacaagtttgtacaaaaaagcaggctacaccatgtcggccaaggcaatttcag	
ACLY attB2 rev	ggggaccactttgtacaagaaagctgggtacatgctcatgtgttccggaa	
ALDP attB1 for	gggacaagtttgtacaaaaaagcaggcttgacatgccggtgctctccaggcc	
ALDP attB2 rev	gggaccactttgtacaagaaagctgggtgaattccggtggaggcaccctggagg	

Sequences are shown from 5´ to 3´ end. Restriction sites in the sequence of the cloning oligonucleotides are shown in italic and restriction enzymes are listed.