Supplementary Information

Probing the modularity of megasynthases by rational engineering of a fatty acid synthase (FAS) type I

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Author affiliations

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Table of contents:

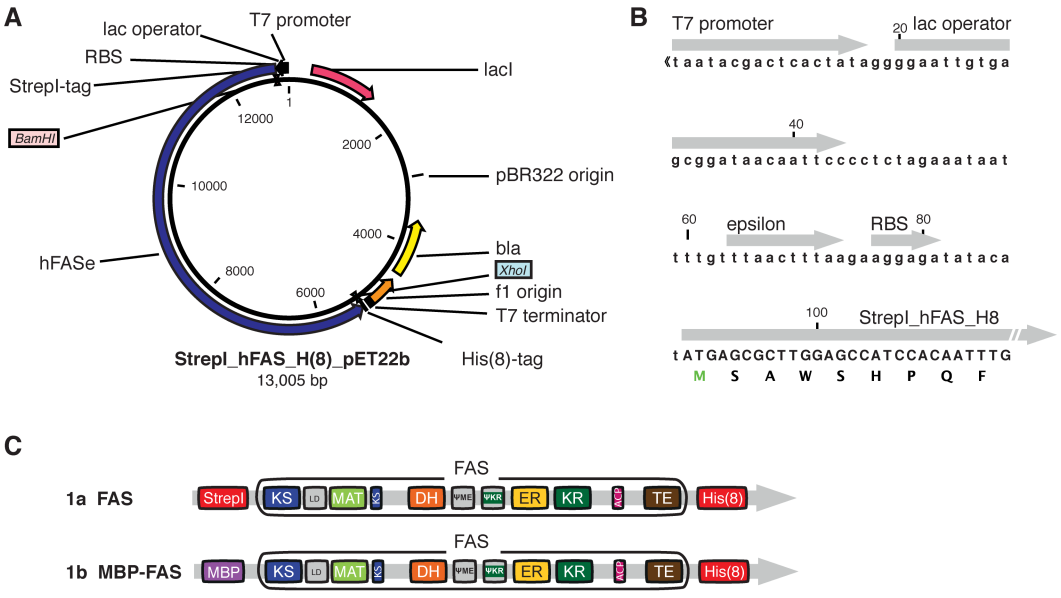


Figure S1: Cloning of human FAS expression constructs in *E. coli*. (A) Representative vector map of a pET22b derived expression vector generating N-terminally StrepI- and C-terminally His-tagged constructs. Abbreviations: lacI, Lac repressor protein; bla, β-lactamase; RBS, ribosome binding site. (B) Sequence of the 5' regulatory region of the multiple cloning site of pET22b and the 5' part of the N-terminally Strep-tagged hFAS. (C) Domain organization of FAS constructs containing different tags. Abbreviations as introduced in the main text. MBP, referring to the maltose binding protein, is fused to the N-terminus for increased protein solubility.

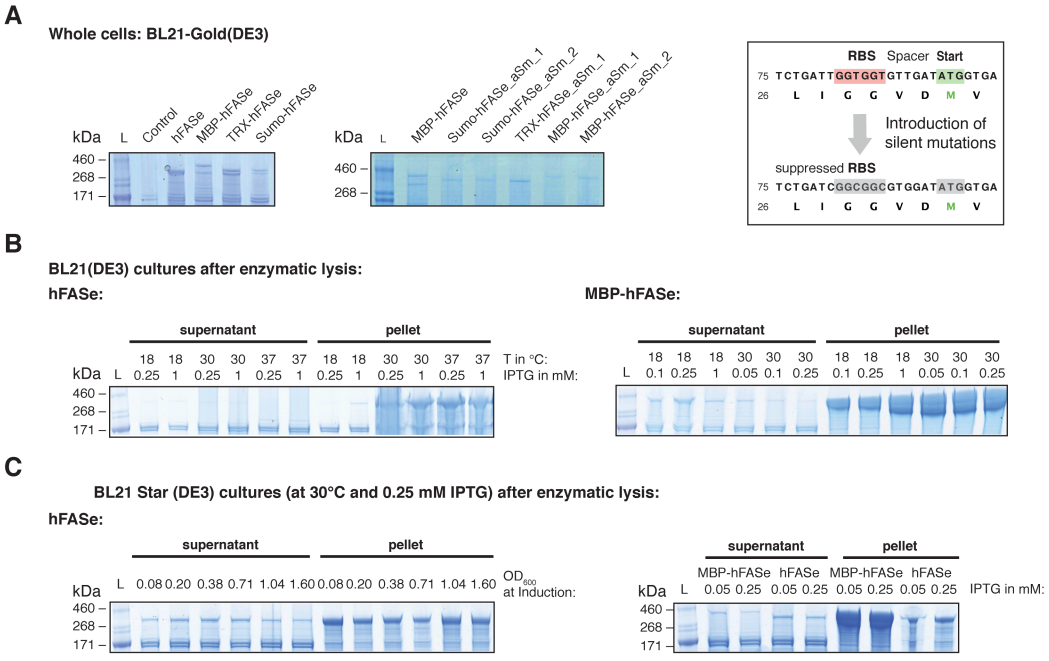


Figure S2: Screening of expression conditions for human FAS in *E. coli*. SDS-PAGE was performed with NuPage 4-12 % Bis-Tris gradient gels. The MBP-tag was used for increasing protein solubility. (A) Initial test-expressions of different fusion constructs of hFASe (hFAS expressed from a codon optimized gene for *E. coli*) in BL21-Gold(DE3) cells. Whole cells were loaded on the SDS-PAGE gel to analyze total expression yields (left and middle panels). Due to the appearance of a second band for all constructs, an alternative translation start was identified and suppressed by introducing silent mutations in the RBS (aSm; middle and right panel). The alternative start codon, referring to M32, is highlighted in green. (B) Influence of temperature and IPTG inducer concentrations on the expression of hFASe (left panel) and MBP-hFASe (right panel) in BL21(DE3). hFASe refers to a sequence optimized gene. Supernatants and pellets after centrifugation of enzymatically lysed cells were loaded. (C) Influence of the cell density at induction on the expression of hFASe (left panel) and MBP-hFASe (right panel) in BL21 Star (DE3) cells. Supernatants and pellets after centrifugation of enzymatically lysed cells were loaded.

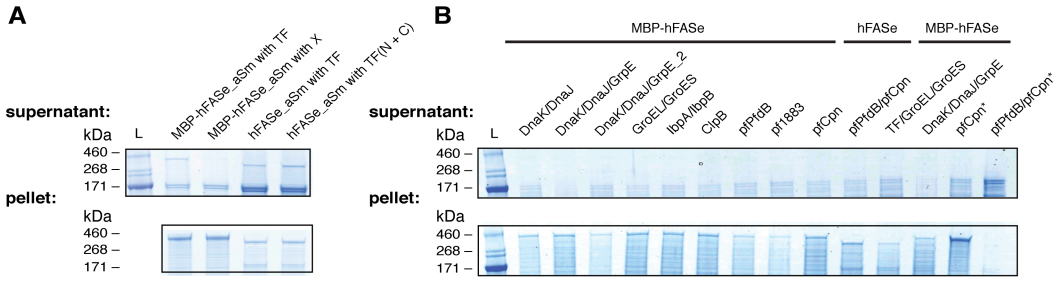


Figure S3: Co-expression of hFAS with chaperones encoding genes. SDS-PAGE was performed with NuPage 4-12 % Bis-Tris gradient gels. (A) Co-expression of MBP-hFASe and hFASe in BL21-Gold(DE3) cells with trigger factor (TF), truncated trigger factor (TF(N+C)) and a control dodecin (X). Coomassie-stained SDS-PAGE of the supernatant and pellet after enzymatic lysis of 50 mL cultures (4 h/30 °C) are shown. TF(N + C) refers to a truncated version containing the fused N- and C- terminal part of the TF. (B) Co-expression of MBP-hFASe and hFASe with various chaperones from *E. coli* and *P. furiosus* in BL21-Gold(DE3) cells. Coomassie-stained SDS-PAGE of the supernatant and pellet after enzymatic lysis of 50 mL cultures (4 h/30 °C) are shown. Asterisks indicates co-expression at 47 °C for potential activity of enzymes from *P. furiosus*.

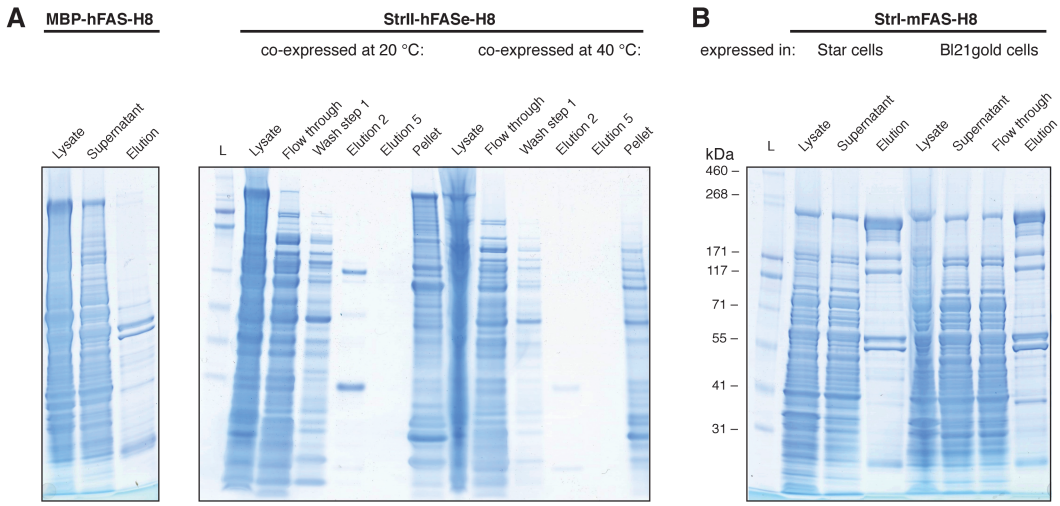
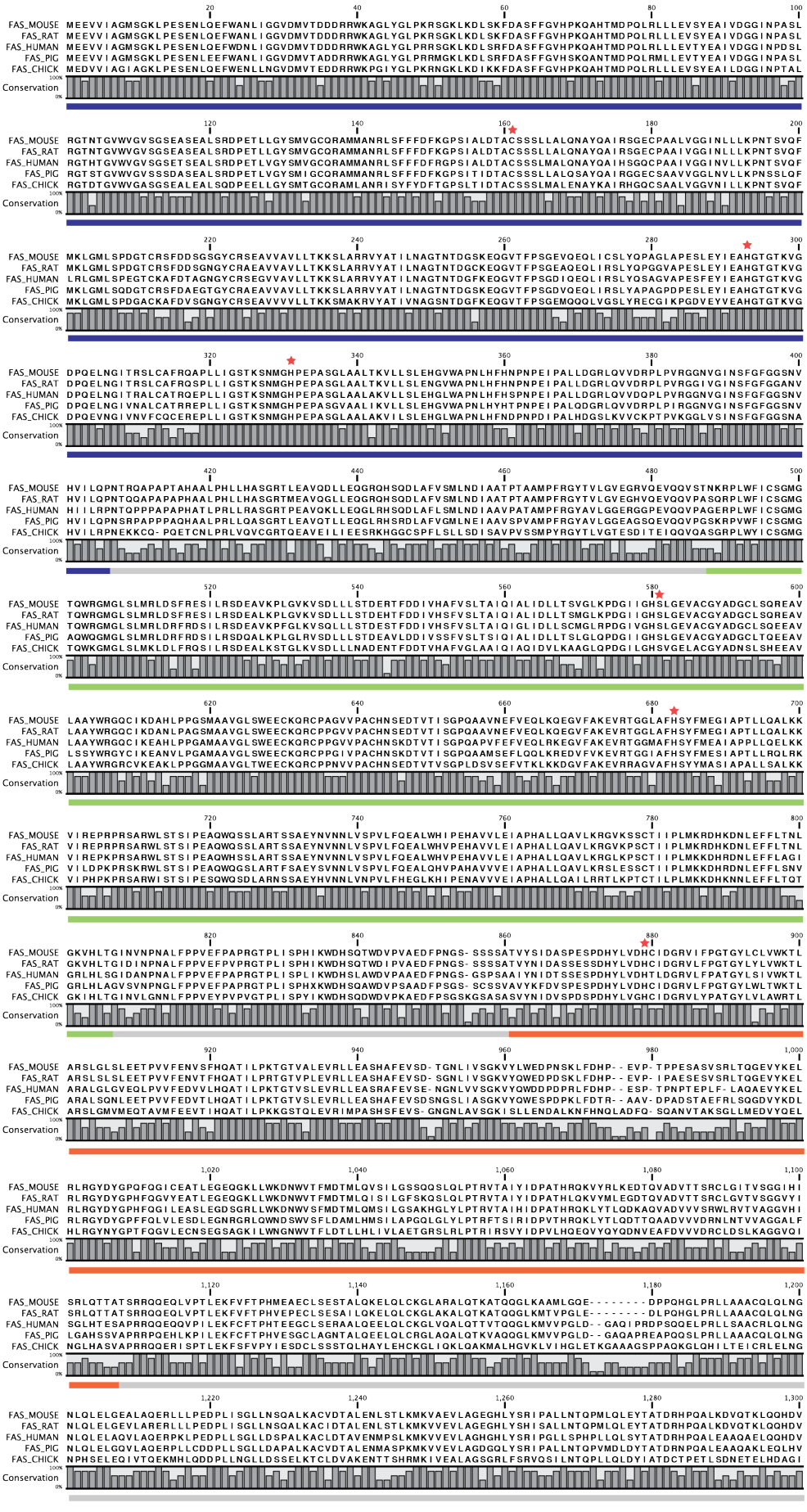


Figure S4: Expression of MBP-hFAS, hFASe and mFAS constructs in large scale (1-2 L expression cultures). (A) Expression of MBP-hFAS in a 2 L BL21-Gold(DE3) culture and co-expression of hFASe with chaperones (pfuPfdA, pfuPfdB and pfuCpn bearing a mutation to increase activity at lower temperatures)[1] in 1 L cultures at 20 °C and 40 °C. (B) Expression and purification of mFAS, expressed in 2 L cultures of BL21 Star (DE3) or BL21-Gold(DE3) cells. Coomassie-stained SDS-PAGE of the lyzed cells (French press) and crudely purified protein (Ni-chelating affinity chromatography) is shown.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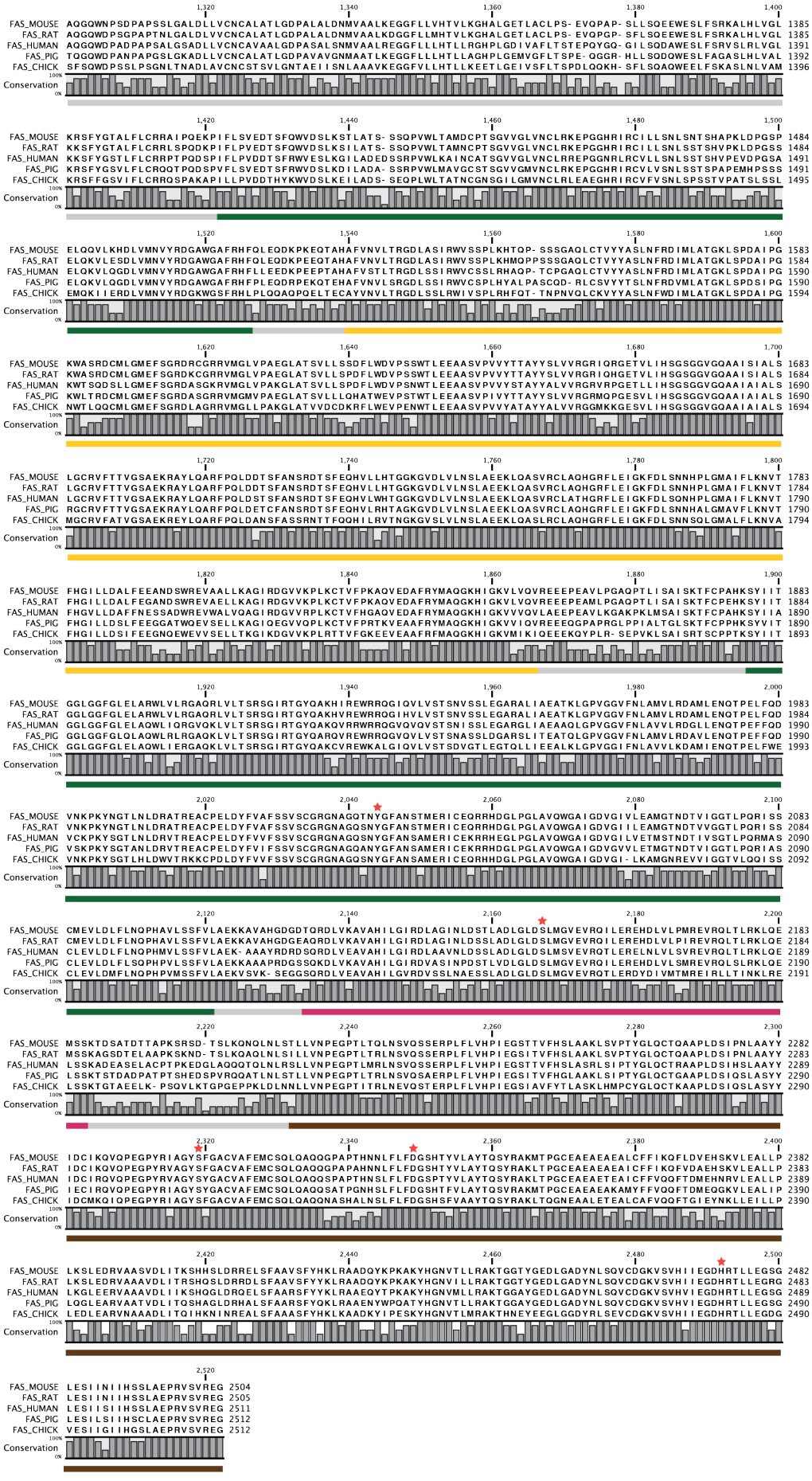
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Conservation
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Conservation
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100%
Conservation
0%
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100%
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0%
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FAS_HUMAN FHGVLLDAFFNESSADWREVWALVQAGIRDGVVRPLKCTVFHGAQVEDAFRYMAQGKHIGKVVVQVLAEEPEAVLKGAKPKLMSAISKTFCPAHKSYIIA 1890
FAS_PIG FHGILLDSLFEEGGATWQEVSELLKAGIQEGVVQPLKCTVFPRTKVEAAFRYMAQGKHIGKVVIQVREEEQGPAPRGLPPIALTGLSKTFCPPHKSYVIT 1890
FAS_CHICK FHGILLDSIFEEGNQEWEVVSELLTKGIKDGVVKPLRTTVFGKEEVEAAFRFMAQGKHIGKVMIKIQEEEKQYPLR- SEPVKLSAISRTSCPPTKSYIIT 1893
100%
Conservation
0%
1,920 1,940 1,960 1,980 2,000
FAS_MOUSE GGLGGFGLELARWLVLRGAQRLVLTSRSGIRTGYQAKHIREWRRQGIQVLVSTSNVSSLEGARALIAEATKLGPVGGVFNLAMVLRDAMLENQTPELFQD 1983
FAS_RAT GGLGGFGLELARWLVLRGAQRLVLTSRSGIRTGYQAKHVREWRRQGIHVLVSTSNVSSLEGARALIAEATKLGPVGGVFNLAMVLRDAMLENQTPELFQD 1984
FAS_HUMAN GGLGGFGLELAQWLIQRGVQKLVLTSRSGIRTGYQAKQVRRWRRQGVQVQVSTSNISSLEGARGLIAEAAQLGPVGGVFNLAVVLRDGLLENQTPEFFQD 1990
FAS_PIG GGLGGFGLQLAQWLRLRGAQKLVLTSRSGIRTGYQARQVREWRRQGVQVLVSTSNASSLDGARSLITEATQLGPVGGVFNLAMVLRDAVLENQTPEFFQD 1990
FAS_CHICK GGLGGFGLELAQWLIERGAQKLVLTSRSGIRTGYQAKCVREWKALGIQVLVSTSDVGTLEGTQLLIEEALKLGPVGGIFNLAVVLKDAMIENQTPELFWE 1993
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Conservation
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FAS_MOUSE VNKPKYNGTLNLDRATREACPELDYFVAFSSVSCGRGNAGQTNYGFANSTMERICEQRRHDGLPGLAVQWGAIGDVGIVLEAMGTNDTVIGGTLPQRISS 2083
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FAS_HUMAN VCKPKYSGTLNLDRVTREACPELDYFVVFSSVSCGRGNAGQSNYGFANSAMERICEKRRHDGLPGLAVQWGAIGDVGILVETMSTNDTIVSGTLPQRMAS 2090
FAS_PIG VSKPKYSGTANLDRVTREACPELDYFVIFSSVSCGRGNAGQANYGFANSAMERICEKRRHDGLPGLAVQWGAIGDVGVVLETMGTNDTVIGGTLPQRIAS 2090
FAS_CHICK VNKPKYSGTLHLDWVTRKKCPDLDYFVVFSSVSCGRGNAGQSNYGFANSAMERICEQRHHDGLPGLAVQWGAIGDVGI-LKAMGNREVVIGGTVLQQISS 2092
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FAS_MOUSE CMEVLDLFLNQPHAVLSSFVLAEKKAVAHGDGDTQRDLVKAVAHILGIRDLAGINLDSTLADLGLDSLMGVEVRQILEREHDLVLPMREVRQLTLRKLQE 2183
FAS_RAT CMEVLDLFLNQPHAVLSSFVLAEKKAVAHGDGEAQRDLVKAVAHILGIRDLAGINLDSSLADLGLDSLMGVEVRQILEREHDLVLPIREVRQLTLRKLQE 2184
FAS_HUMAN CLEVLDLFLNQPHMVLSSFVLAEK- AAAYRDRDSQRDLVEAVAHILGIRDLAAVNLDSSLADLGLDSLMSVEVRQTLERELNLVLSVREVRQLTLRKLQE 2189
FAS_PIG CLEVLDLFLSQPHPVLSSFVLAEKKAAPRDGSSQKDLVKAVAHILGIRDVASINPDSTLVDLGLDSLMGVEVRQILEREHDLVLSMREVRQLSLRKLQE 2190
FAS_CHICK CLEVLDMFLNQPHPVMSSFVLAEKVSVK- SEGGSQRDLVEAVAHILGVRDVSSLNAESSLADLGLDSLMGVEVRQTLERDYDIVMTMREIRLLTINKLRE 2191
100%
Conservation
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FAS_MOUSE MSSKTDSATDTTAPKSRSD- TSLKQNQLNLSTLLVNPEGPTLTQLNSVQSSERPLFLVHPIEGSTTVFHSLAAKLSVPTYGLQCTQAAPLDSIPNLAAYY 2282
FAS_RAT MSSKAGSDTELAAPKSKND- TSLKQAQLNLSILLVNPEGPTLTRLNSVQSSERPLFLVHPIEGSITVFHSLAAKLSVPTYGLQCTQAAPLDSIPNLAAYY 2283
FAS_HUMAN LSSKADEASELACPTPKEDGLAQQQTQLNLRSLLVNPEGPTLMRLNSVQSSERPLFLVHPIEGSTTVFHSLASRLSIPTYGLQCTRAAPLDSIHSLAAYY 2289
FAS_PIG LSSKTSTDADPATPTSHEDSPVRQQATLNLSTLLVNPEGPTLTRLNSVQSAERPLFLVHPIEGSITVFHGLAAKLSIPTYGLQCTGAAPLDSIQSLASYY 2290
FAS_CHICK LSSKTGTAEELK- PSQVLKTGPGEPPKLDLNNLLVNPEGPTITRLNEVQSTERPLFLVHPIEGSIAVFYTLASKLHMPCYGLQCTKAAPLDSIQSLASYY 2290
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Conservation
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FAS_CHICK IDCMKQIQPEGPYRIAGYSFGACVAFEMCSQLQAQQNASHALNSLFLFDGSHSFVAAYTQSYRAKLTQGNEAALETEALCAFVQQFTGIEYNKLLEILLP 2390
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Conservation
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FAS_MOUSE LKSLEDRVAASVDLITKSHHSLDRRELSFAAVSFYHKLRAADQYKPKAKYHGNVTLLRAKTGGTYGEDLGADYNLSQVCDGKVSVHIIEGDHRTLLEGSG 2482
FAS_RAT LKSLEDRVAAAVDLITRSHQSLDRRDLSFAAVSFYYKLRAADQYKPKAKYHGNVILLRAKTGGTYGEDLGADYNLSQVCDGKVSVHIIEGDHRTLLEGRG 2483
FAS_HUMAN LKGLEERVAAAVDLIIKSHQGLDRQELSFAARSFYYKLRAAEQYTPKAKYHGNVMLLRAKTGGAYGEDLGADYNLSQVCDGKVSVHVIEGDHRTLLEGSG 2489
FAS_PIG LQGLEARVAATVDLITQSHAGLDRHALSFAARSFYQKLRAAENYWPQATYHGNVTLLRAKTGGAYGEDLGADYNLSQVCDGKVSVHVIEGDHRTLLEGSG 2490
FAS_CHICK LEDLEARVNAAADLITQIHKNINREALSFAAASFYHKLKAADKYIPESKYHGNVTLMRAKTHNEYEEGLGGDYRLSEVCDGKVSVHIIEGDHRTLLEGDG 2490
100%
Conservation
2,520
FAS_MOUSE LESIINIIHSSLAEPRVSVREG 2504
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FAS_HUMAN LESIISIIHSSLAEPRVSVREG 2511
FAS_PIG LESILSIIHSCLAEPRVSVREG 2512
FAS_CHICK VESIIGIIHGSLAEPRVSVREG 2512
100%
Conservation
0%

Figure S5: Sequences of animal type I FAS. The sequences were obtained from NCBI (http://www.ncbi.nlm.nih.gov; UniProt accession codes: murine FAS: P19096; rat FAS: P12785; human FAS: P49327; porcine FAS: A5YV76 and chicken FAS: P12276. Primary sequences were aligned using Clustal Omega. Sequence numbering based on murine FAS (mFAS) used in the construct design. The colored bars indicate the different domains according to the following color code: KS, blue; MAT, light green; DH, orange; KR, dark green; ER, yellow; ACP, magenta; TE, brown. Catalytic residues are indicated by asterisks. Amino acid conservation within set of presented sequences is given in percent.

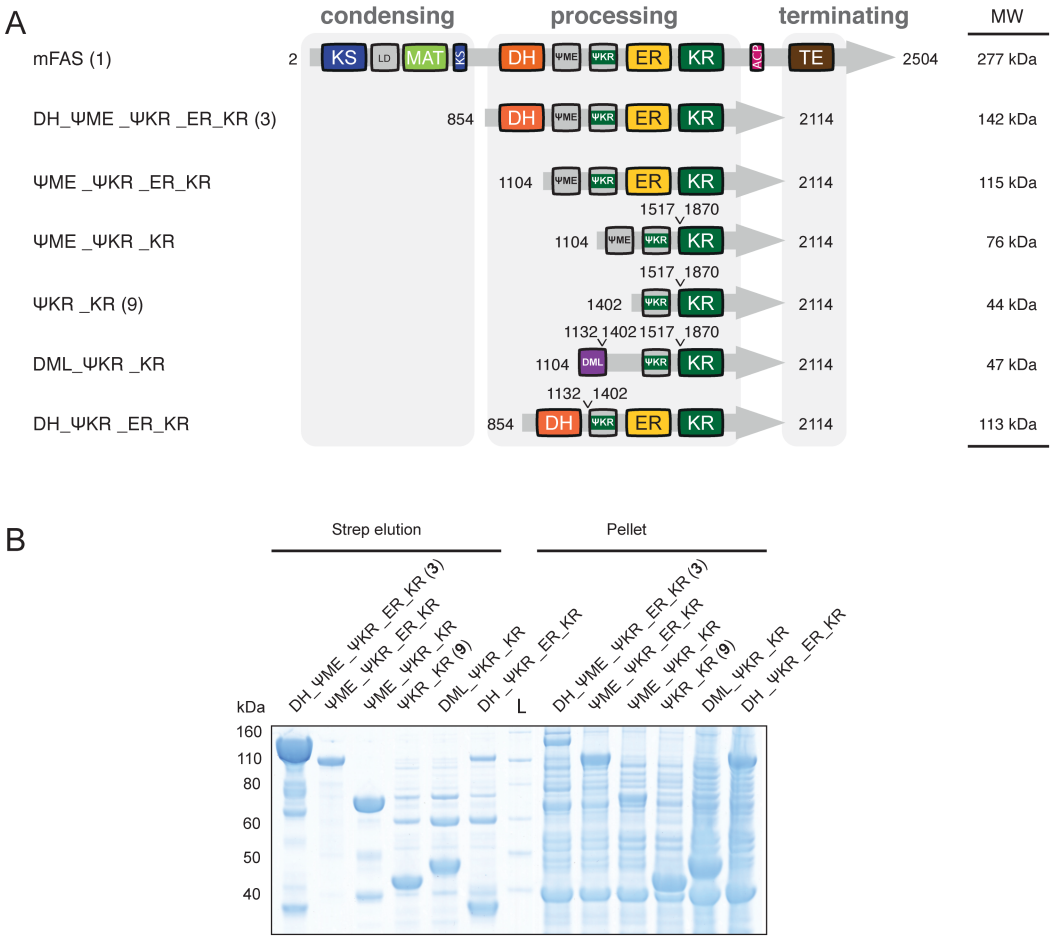

Figure S6: Deconstruction of the processing part of mFAS. (A) Domain organization of mFAS and constructs of the processing part. Construct number are given in brackets. All amino acid positions/numbers refer to the wild type mFAS. Molecular weights refer to proteins without the N-terminal methionine. The abbreviation DML refers to the linker between the DH and ΨME domain. (B) Comparison of soluble and aggregated fractions of various truncated constructs of the processing part. Constructs were solely purified with a Strep-Tactin columns, which explains the relatively high degree of contamination.

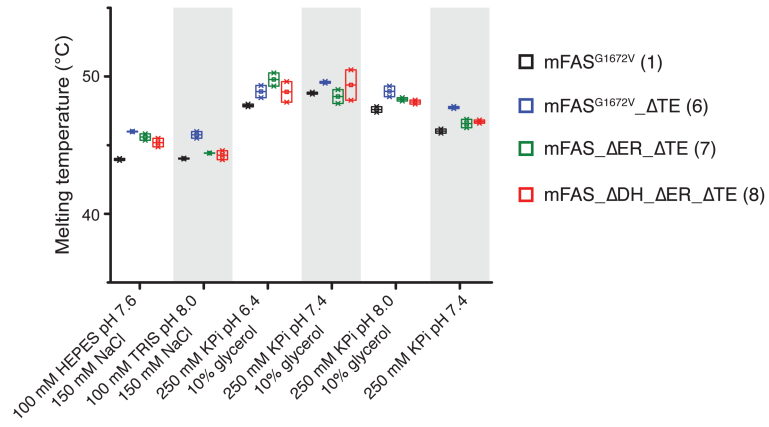

Figure S7: **Stability of PKS-like reducing modules.** Melting temperatures were determined by a thermal shift assay as described in the Methods section. The four constructs were tested in different buffer conditions. In general, phosphate buffers and glycerol have a stabilizing effect on these proteins and melting temperatures were in between 44-50 °C. Two replicates are shown as bar plot.

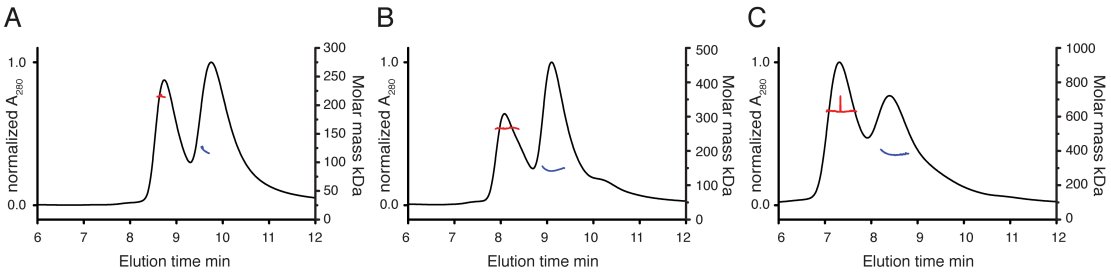

Figure S8: **Confirmation of the oligomeric state of select constructs.** HPLC-MALS analysis (multiangle light scattering) of select constructs with absorption normalized to the highest peak. (A) KS–MAT–ACP (**17**): the first and the second peaks correspond to 215 (216) and 121 (108) kDa. (B) KS–MAT–ACP–TE (**22**): the first and the second peaks correspond to 266 (284) and 149 (142) kDa. (C) LD–MAT–ACP–mFAS (**25**): the first and the second peaks correspond to 632 (674) and 390 (337) kDa. The calculated masses confirm the two oligomeric states, peak 1 and peak 2 referring to monomeric and dimeric states of the samples, respectively. The theoretical masses are given in brackets.

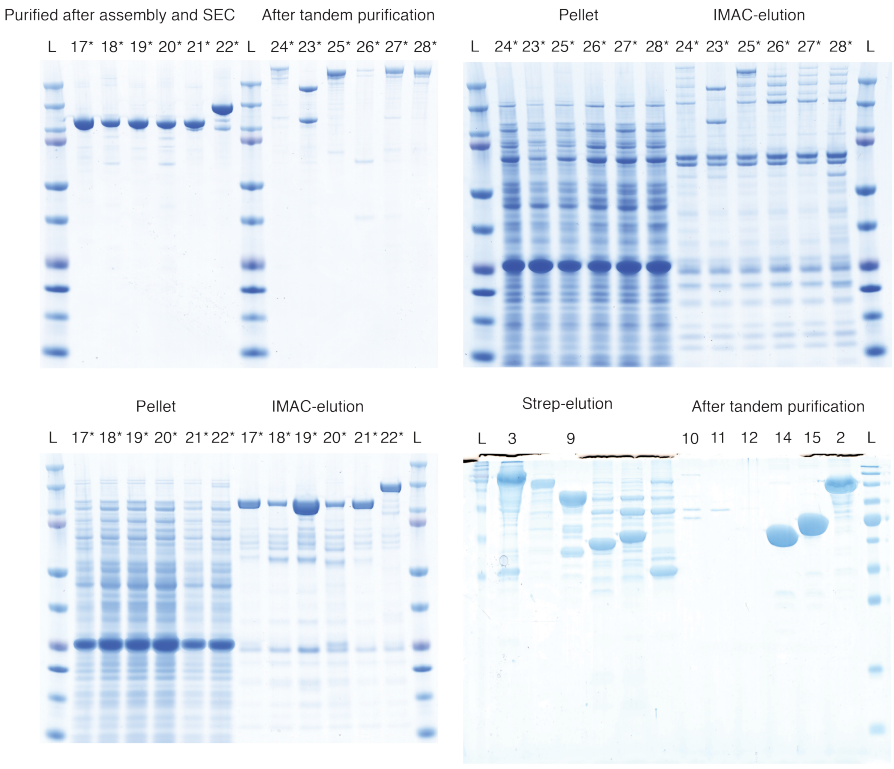

Figure S9: **Original pictures of relevant presented gels.**

Table S1: List of plasmids

Number	Important Construct	Name
pAR018	**1**; mFAS	pAR18_StrepI_mFASm_H8_pET22b
pAR069	**2**; KS-MAT; condensing part	pAR69_STRI_m(KS_MAT)_H8_pET22b
pAR236	**3**; processing part	pAR236_StrepI_m(DH_ps_ER_KR)_H8_pET22b
pAR100	**4**; apo-ACP	pAR100_StrepII_mACP_H8_pET22b
pAR194	**5**; TE	pAR194_mTEI_H8_pET22b
pAR088	**6**; mFAS_ΔTE	pAR88_StrepI_mFASm_ΔTE_H8_pET22b
pAR162	**7**; mFAS_ΔER_ΔTE	pAR162_StrepI_mFASm_ΔER_ΔTE_H8_pET22b
pAR163	**8**; mFAS_ΔDH_ΔER_ΔTE	pAR163_StrepI_mFASm_ΔDH_ΔER_ΔTE_H8_pET22b
pAR239	**9**; ΨKR _KR	pAR239_StrepI_m(psKR_KR)_H8_pET22b
pAR243	**10**; KSt_{runc}	pAR243_STRI_mKS_H8_pET22b
pAR244	**11**; KS-LD	pAR244_STRI_m(KS_LD)_H8_pET22b
pAR245	**12**; MAT_{trunc}	pAR245_StrepI_mMAT_H8_pET22b
pAR309	**13**; MAT_{Ave}	pAR309_StrepI_mMAT(Structure)_H8_pET22b
pAR246	**14**; LD-MAT	pAR246_StrepI_m(LD_MAT)_H8_pET22b
pAR247	**15**; LD-MAT-pAT	pAR247_STRI_m(LD_MAT_postATL)_H8_pET22b
pAR327	**16**; MAT_{Ave}-MBP	pAR327_StrepI_mMAT(Structure)_GGGS_MBP_H8_pET22b-1
pAR127	**17**; KS-MAT-ACP	pAR127_StrepI_m(KS_MAT)_mouseL_ACP_H8_pET22b
pAR125	**18**; KS-MAT-AM3L-ACP	pAR125_StrepI_m(KS_MAT)_AM3L_ACP_H8_pET22b
pAR151	**19**; KS-MAT-AM3L2-ACP	pAR151_StrepI_m(KS_MAT)_AM3L2_ACP_H8_pET22b
pAR126	**20**; KS-MAT-AM11L-ACP	pAR126_StrepI_m(KS_MAT)_AM11L_ACP_H8_pET22b
pAR152	**21**; KS-MAT-AM11L2-ACP	pAR152_StrepI_m(KS_MAT)_AM11L2_ACP_H8_pET22b
pAR128	**22**; KS-MAT-ACP-TE	pAR128_StrepI_m(KS_MAT)_mouseL_ACP_TE_H8_pET22b
pAR168	**23**; KS-MAT-ACP-m(KMA)	pAR168_StrepI_m(KS_MAT_mL_ACP)_mL_m(KS_MAT_mL_ACP)_H8_pET22b
pAR167	**24**; KS-MAT-ACP-mFAS	pAR167_StrepI_m(KS_MAT_mL_ACP)_mL_mFASm_H8_pET22b
pAR292	**25**; LD-MAT-ACP-mFAS	pAR292_StrepI_m(LD_MAT_mACP)_mFASm_H8_pET22b
pAR306	**26**; $(AT0\text{-}ACP0)_{AVES}$-mFAS	pAR306_StrepI_AVES(AT0_ACP0)_mFASm_H8_pET22b
pAR307	**27**; $(AT0\text{-}ACP0)_{DEBS}$-mFAS	pAR307_StrepI_DEBS(AT0_ACP0)_mFASm_H8_pET22b
pAR340	**28**; MAT_{Ave}-ACP-mFAS	pAR340_StrepI_mMAT(Str)_GGS_mACP_GGS_mFASm_H8_pET22b
pAR291	**29**; LD-MAT-mFAS	pAR291_StrepI_m(LD_MAT)_mFASm_H8_pET22b
pAR357	Sfp	pAR357_SFP_pCDF-1b

Number	Constructs (Sup. Info)	Name
pAR001	MBP-hFASe	pAR01_StrepI_MBP_hFASe_H8_pET22b
pAR002	TRX-hFASe	pAR02_StrepI_Trx_hFASe_H8_pET22b
pAR003	Sumo-hFASe	pAR03_StrepI_SUMO3_hFASe_H8_pET22b
pAR010	hFASe_aSm	pAR10_StrepI_hFASe(aSTARTm)_H8_pET22b
pAR011	MBP-hFASe_aSm	pAR11_StrepI_MBP_hFASe(aSTARTm)_H8_pET22b
pAR012	TRX-hFASe_aSm	pAR12_StrepI_Trx_hFASe(aSTARTm)_H8_pET22b
pAR013	Sumo-hFASe_aSm	pAR13_StrepI_SUMO3_hFASe(aSTARTm)_H8_pET22b
pAR017	hFAS	pAR17_StrepI_hFASh_H8_pET22b
pAR026	StrII-hFASe-H8	pAR26_StrepII_hFASe(aSTARTm)_H8_pET22b
pAR036	TF(N+C)+hFASe	pAR36b_TF(N+C)_RBS_StrepII_hFASe(aSTARTm)_H8_pET22b
pAR038	MBP-hFAS	pAR38_MBP_hFASh_H8_pMAL-c5G
pAR237	ΨME _ΨKR _ER_KR	pAR237_StrepI_m(ps_ER_KR)_H8_pET22b
pAR238	ΨME _ΨKR _KR	pAR238_StrepI_m(ps_KR)_H8_pET22b
pAR240	DML_ΨKR _KR	pAR240_StrepI_m(PKSL_psKR_KR)_H8_pET22b
pAR241	DH _ΨKR _ER_KR	pAR241_StrepI_m(DH_psKR_ER_KR)_H8_pET22b

Number	Chaperones (Sup. Info)	Name
pAR32A	TF	pAR32_TF_pETcoco-1
pAR32B	TF(N+C)	pAR32b_TF(N+C)_pETcoco-1-1
pAR033	X	pAR33_mycdodecin_pETcoco
pAR035	DnaK-/DnaJ	pAR35_DnaK_RBS_DnaJ_pETcoco
pAR039	pfPfdB	pAR39_pfuPre(b)_pETcoco
pAR043	GroEL/GroES	pAR43_GroES_RBS_GroEL_pETcoco
pAR044	DnaK-/DnaJ/GrpE	pAR44_DnaK_RBS_DnaJ_RBS_GrpE_pETcoco
pAR045	prefoldin (pfuPfdB)	pAR45_pfuPre(b)_RBS_pfuPre(a)_pETcoco
pAR046	pf1883	pAR46_PF1883_pETcoco
pAR047	pfCpn	pAR47_PfCPN_pETcoco
pAR048	IbpA/IbpB	pAR48_IbpA_RBS_IbpB_pETcoco
pAR049	ClpB	pAR49_ClpB_pETcoco

Table S2: List of primers for the cloning of respective plasmids

Number	Name	Length	Orientation	T_m [°C]*	Sequence (5'3')	Construct Template
AR37	Step_mFASm_infusion	41	forward	55.4	GTTCGAAAAAGGCGCCGGATCCgaggaggtggtgatagccg	**1**
AR38	mFASm_His_infusion	36	reverse	55.4	GGTGATGATGCTCGAGgccctcccgtacactcactc	**IRAVp968**
AR163	hMAT_His_for	36	forward	53.2	caggttccccctcagccCATCATCACCACCACCACC	**2**
AR148	mMAT_hDH_rev	36	reverse	55.9	ctgaggggggaacctgagccgtttgggaagtcctcag	**pAR18**
AR422	StrI_mDH_for	36	forward	55.9	CGAAAAAGGCGCCGGATCCagctcctcctctgctac	**3**
AR204	ΔmACPTE_His_rev	33	reverse	57.2	GTGATGATGCTCGAGgtccccatgggccacagc	**pAR18**
AR176	STRII_mACP_for	30	forward	56.2	TCGAAAAAGGCGCCGgggacggggacaccc	**4**
AR227	mACP_H8_rev	35	reverse	57.9	GTGATGATGCTCGAGgggggctgtcgtgtcagtag	**pAR18**
AR349	pET22b_mTEI_for	38	forward	56.7	AGAAGGAGATATACATATGtccaggagtgacacgtctc	**5**
AR38	mFASm_His_infusion	36	reverse	55.4	GGTGATGATGCTCGAGgccctcccgtacactcactc	**pAR18**
AR37	Step_mFASm_infusion	41	forward	55.4	GTTCGAAAAAGGCGCCGGATCCgaggaggtggtgatagccg	**6**
AR203	ΔmTE_His_rev	36	reverse	56.3	GTGATGATGCTCGAGtgtcgtgtcagtagccgagtc	**pAR18**
AR311	ΔmER_for	35	forward	55.9	gagcaggacaagccctccaagaccttctgcccagc	**7**
AR312	ΔmER_rev	21	reverse	56.3	gggcttgtcctgctctaactg	**pAR88**
AR313	ΔmDH_for	19	forward	55.4	tcacggcggcagcaagaac	**8**
AR314	ΔmDH_rev	33	reverse	54.9	ttgctgccgccgtgaagaggaggagctggagcc	**pAR88**
AR424	StrI_mpsKR_for	41	forward	54.8	CGAAAAAGGCGCCGGATCCccacaggagaaacctatcttcc	**9**
AR204	ΔmACPTE_His_rev	33	reverse	57.2	GTGATGATGCTCGAGgtccccatgggccacagc	**pAR162**
AR429	m(LD_MAT)del_for	41	forward	55.9	cagcccaacacacggGGCGGCcgagggactcctctcatctc	**10**
AR430	m(LD_MAT)del_rev	19	reverse	57.6	ccgtgtgttgggctggagg	**pAR18**
AR431	mMATdel_for	39	forward	57.4	caagaagtgcagcaaagctgcaccatcattcccttgatg	**11**
AR432	mMATdel_rev	22	reverse	56.7	ttgctgcacttcttggacacgg	**pAR18**
AR433	STRI_mMAT_for	39	forward	56.3	GAAAAAGGCGCCGGATCCctctggttcatctgctcaggg	**12**
AR434	mMAT_H8_rev	34	reverse	57.6	GTGATGATGCTCGAGgcctcgcttcaggacagcc	**pAR18**
AR512	STRI_mMAT2_for	37	forward	55.4	GAAAAAGGCGCCGGATCCtccaccaacaagcgcccac	**13**
AR513	mMAT2_H8_rev	34	reverse	55.4	GTGATGATGCTCGAGgatgcctgtgaggtgcacc	**pAR18**
AR435	STRI_mLD_for	34	forward	56.2	GAAAAAGGCGCCGGATCCgcccctgcgcccactg	**14**
AR436	mLD_H8_rev	36	reverse	56.3	GTGATGATGCTCGAGaggtgggaacaaggcgttagg	**pAR18**
AR435	STRI_mLD_for	34	forward	56.2	GAAAAAGGCGCCGGATCCgcccctgcgcccactg	**15**
AR148	mMAT_hDH_rev	36	reverse	55.9	ctgaggggggaacctgagccgtttgggaagtcctcag	**pAR18**
AR202	LEHis_for	21	forward	56.3	CTCGAGCATCATCACCACCAC	**16**
AR537	mMATstr_GGS_rev	43	reverse	55.4	CGATCCACCGCCAGAACCTCCACCgatgcctgtgaggtgcacc	**pAR309**
AR539	GGS_MalE_for	44	forward	53.7	GTTCTGGCGGTGGATCGAAAATCGAAGAAGGTAAACTGGTAATC	**16**
AR540	MalE_H8_rev	35	reverse	55.9	GTGATGATGCTCGAGAGTCTGCGCGTCTTTCAGGG	**pMAL-c5G**
AR272	mMAT_mouseL_for	37	forward	56.3	cagcatcgacgccagtgcagagaagaaagctgtggcc	**17**
AR273	mouseL_mACP_rev	21	reverse	56.3	actggcgtcgatgctgtagac	**pAR88**
AR267	mACP_for	16	forward	56.2	ggggacggggacaccc	**18**
AR266	mKS_W_rev	20	reverse	55.9	ccaagtctgactgtggtccc	**pAR88**
AR268	mMAT_AM3L_for	37	forward	54.9	ggaccacagtcagacttggCTCCAACCACCCGGCAAG	**18**
AR269	AM3L_mACP_rev	35	reverse	55.4	gggtgtccccgtcccccACGAGGACATCACGCAGC	**DSM46492**
AR267	mACP_for	16	forward	56.2	ggggacggggacaccc	**19**
AR299	mKS_G_mouseL_rev	39	reverse	55.9	cctcagcaaccgggacatcccaagtctgactgtggtccc	**pAR88**
AR297	mMAT_AM3L2_for	48	forward	57.2	gtcccggttgctgaggacttcccaaacggcCGGAGCGAAGGCCGTGAG	**19**
AR269	AM3L_mACP_rev	35	reverse	55.4	gggtgtccccgtcccccACGAGGACATCACGCAGC	**DSM46492**
AR267	mACP_for	16	forward	56.2	ggggacggggacaccc	**20**
AR266	mKS_W_rev	20	reverse	55.9	ccaagtctgactgtggtccc	**pAR88**
AR270	mMAT_AM11L_for	36	forward	54.3	ggaccacagtcagacttggCTGGATGTGGAGGGGGC	**20**
AR271	AM11L_mACP_rev	32	reverse	56.2	gggtgtccccgtcccccGGCGAGGCAGTCGCGC	**DSM46492**
AR267	mACP_for	16	forward	56.2	ggggacggggacaccc	**20**
AR299	mKS_G_mouseL_rev	39	reverse	55.9	cctcagcaaccgggacatcccaagtctgactgtggtccc	**pAR88**
AR298	mMAT_AM11L2_for	47	forward	54.3	gtcccggttgctgaggacttcccaaacggcGTCTCTGGGCAGTGGGG	**20**
AR271	AM11L_mACP_rev	32	reverse	56.2	gggtgtccccgtcccccGGCGAGGCAGTCGCGC	**DSM46492**
AR272	mMAT_mouseL_for	37	forward	56.3	cagcatcgacgccagtgcagagaagaaagctgtggcc	**22**
AR273	mouseL_mACP_rev	21	reverse	56.3	actggcgtcgatgctgtagac	**pAR18**
AR317	mFASm_for	19	forward	55.4	gaggaggtggtgatagccg	**23**
AR428	StrepGS_rev	19	reverse	55.4	GGATCCGGCGCCTTTTTCG	**pAR127**
AR37	Step_mFASm_infusion	41	forward	55.4	GTTCGAAAAAGGCGCCGGATCCgaggaggtggtgatagccg	**23**
AR318	mACP_mL_mFAS_rev	40	reverse	56.7	ggctatcaccacctcctcagacgtgtcactcctggacttg	**pAR127**
AR317	mFASm_for	19	forward	55.4	gaggaggtggtgatagccg	**24**
AR428	StrepGS_rev	19	reverse	55.4	GGATCCGGCGCCTTTTTCG	**pAR18**
AR37	Step_mFASm_infusion	41	forward	55.4	GTTCGAAAAAGGCGCCGGATCCgaggaggtggtgatagccg	**24**
AR318	mACP_mL_mFAS_rev	40	reverse	56.7	ggctatcaccacctcctcagacgtgtcactcctggacttg	**pAR127**
AR494	Not1_mLD_for	36	forward	54.3	GTTCGAAAAAGCGGCCGCAcccactgcacacgctgc	**25**
AR496	mACP_Not1_rev	39	reverse	56.7	acctcctcTGCGGCCGCagacgtgtcactcctggacttg	**pAR127**
AR467	Not1_mFAS_for	34	forward	55.4	GAAAAAGCGGCCGCAgaggaggtggtgatagccg	**25**
AR468	Not1_Strep1_rev	30	reverse	56.3	cTGCGGCCGCTTTTTCGAACTGCGGGTGGC	**pAR18**
AR508	Not1_AVES(AT0)_for	34	forward	54.3	GTTCGAAAAAGCGGCCCAGAGGATGGACGGCGGG	**26**
AR509	AVES(ACP0)_Not1_rev	32	reverse	56.2	cacctcctcTGCGGCCGCCGTGCCTCCGTGGC	**DSM46492**
AR467	Not1_mFAS_for	34	forward	55.4	GAAAAAGCGGCCGCAgaggaggtggtgatagccg	**26**
AR468	Not1_Strep1_rev	30	reverse	56.3	cTGCGGCCGCTTTTTCGAACTGCGGGTGGC	**pAR18**
AR510	Not1_DEBS(AT0)_for	37	forward	56.3	GTTCGAAAAAGCGGCCGACCTGTCAAAGCTCTCCGAC	**27**
AR511	DEBS(ACP0)_Not1_rev	34	reverse	57.2	cacctcctcTGCGGCCGGCGCCGCTTCGTTGGTC	**pBP144**
AR467	Not1_mFAS_for	34	forward	55.4	GAAAAAGCGGCCGCAgaggaggtggtgatagccg	**27**
AR468	Not1_Strep1_rev	30	reverse	56.3	cTGCGGCCGCTTTTTCGAACTGCGGGTGGC	**pAR18**
AR522	Not1_mMAT_for	38	forward	55.4	GTTCGAAAAAGCGGCCGCAtccaccaacaagcgcccac	**28**
AR537	mMATstr_GGS_rev	43	reverse	55.4	CGATCCACCGCCAGAACCTCCACCgatgcctgtgaggtgcacc	**pAR18**
AR560	GGS_mACP_for	46	forward	56.2	GGTTCTGGCGGTGGATCGGCAGGTGGCTCTggggacggggacaccc	**28**
AR561	mACP_GGS_rev	62	reverse	53	CAGAGCCACCTCCGCTCGATCCACCGCCAGAACCTCCACCggaggacatttcctgaagtttc	**pAR18**
AR562	GGS_Not1_rev	56	reverse	57.2	cacctcctcTGCGGCCGCAGAGCCCCCAGATCCACCCCCAGAGCCACCTCCGCTCG	
AR467	Not1_mFAS_for	34	forward	55.4	GAAAAAGCGGCCGCAgaggaggtggtgatagccg	**28**
AR468	Not1_Strep1_rev	30	reverse	56.3	cTGCGGCCGCTTTTTCGAACTGCGGGTGGC	**pAR18**
AR494	Not1_mLD_for	36	forward	54.3	GTTCGAAAAAGCGGCCGCAcccactgcacacgctgc	**29**
AR495	postAT_Not1_rev	38	reverse	54.4	acctcctcTGCGGCCGCactgtggtcccacttgatgtg	**pAR127**
AR467	Not1_mFAS_for	34	forward	55.4	GAAAAAGCGGCCGCAgaggaggtggtgatagccg	**29**
AR468	Not1_Strep1_rev	30	reverse	56.3	cTGCGGCCGCTTTTTCGAACTGCGGGTGGC	**pAR18**
AR589	pCDF1b_for	20	forward	53.8	TTAACCTAGGCTGCTGCCAC	**Sfp**
AR590	pCDF1b_rev	29	reverse	53	catGGTATATCTCCTTATTAAAGTTAAAC	**pCDF-1b**
AR591	pCDF1b_SFP_for	43	forward	52.1	AATAAGGAGATATACCatgaagatttacggaatttatatggac	**Sfp**
AR592	SFP_pCDF1b_rev	41	reverse	54.4	CAGCAGCCTAGGTTAAttatataaagctcttcgtacgagacc	**SFP_*bsub***

AR01	Strep_MBP_SLIC	54	forward	49.2	GGAGCCACCCGCAGTTCGAAAAAGGCGCCGGAAAAATCGAAGAAGGTAAACTGG	**MBP-hFASe**
AR02	MBP_hFASE_SLIC	47	reverse	51.1	TCATACCTGCAATAACAACTTCTTCGGATCCGTAGTGTGCCGCACCC	**pMAL-c5G**
AR03	Strep_TRX_SLIC	57	forward	52.8	GGAGCCACCCGCAGTTCGAAAAAGGCGCCGGAagcgataaaattattcacctgactg	**TRX-hFASe**
AR04	TRX_hFASE_SLIC	49	reverse	54.9	TCATACCTGCAATAACAACTTCTTCGGATCCgccagaaccagaaccggc	**pET-32b(+)**
AR05	Strep_SUMO3_SLIC	54	forward	53	GGAGCCACCCGCAGTTCGAAAAAGGCGCCGGAaatgaccacatcaacctgaagg	**Sumo-hFASe**
AR06	SUMO3_hFASE_SLIC	49	reverse	54.9	TCATACCTGCAATAACAACTTCTTCGGATCCggatccaccggtctgctg	**pET28M_sumo3**
AR18	hFASe_aSTARTm_for	36	forward	55.3	GATCGGCGGCGTGGATATGGTGACCGATGATGATCG	**hFASe_aSm**
AR19	hFASe_aSTARTm_rev	36	reverse	53.2	CACGCCGCCGATCAGATTATCCCAAAATTCTTGCAG	**pVR01**
AR18	hFASe_aSTARTm_for	36	forward	55.3	GATCGGCGGCGTGGATATGGTGACCGATGATGATCG	**MBP-hFASe_aSm**
AR19	hFASe_aSTARTm_rev	36	reverse	53.2	CACGCCGCCGATCAGATTATCCCAAAATTCTTGCAG	**pAR001**
AR18	hFASe_aSTARTm_for	36	forward	55.3	GATCGGCGGCGTGGATATGGTGACCGATGATGATCG	**TRX-hFASe_aSm**
AR19	hFASe_aSTARTm_rev	36	reverse	53.2	CACGCCGCCGATCAGATTATCCCAAAATTCTTGCAG	**pAR002**
AR18	hFASe_aSTARTm_for	36	forward	55.3	GATCGGCGGCGTGGATATGGTGACCGATGATGATCG	**Sumo-hFASe_aSm**
AR19	hFASe_aSTARTm_rev	36	reverse	53.2	CACGCCGCCGATCAGATTATCCCAAAATTCTTGCAG	**pAR003**
AR35	Strep_hFASh_infusion	43	forward	57.9	AGTTCGAAAAAGGCGCCGGATCCgaggaggtggtgattgccgg	**hFAS**
AR36	hFASh_His_infusion	34	reverse	59.4	GGTGATGATGCTCGAGgccctcccgcacgctcac	**IRAK110**
AR57	pET22b_STRItoII_for	33	forward	54.9	ATACATATGAGCGCTTGGAGCCACCCGCAGTTC	**StrII-hFASe-H8**
AR58	pET22b_STRItoII_rev	24	reverse	54	AGCGCTCATATGTATATCTCCTTC	**pVR01**
AR81	pET22b_Xba1_for	31	forward	56.4	GGATAACAATTCCCCTCTAGAAATAATTTTG	**TF(N+C)+hFASe**
AR82	tig_RBS_rev	43	reverse	55.4/58.6	CAAAATTATTTCTAGCGCGGAtcaCGCCTGCTGGTTCATCAGC	**pAR32B**
AR87	MBP_hFASh_infusion_for	40	forward	57.9	TCGTCGACGGATCCGAATTcgaggaggtggtgattgccgg	**MBP-hFAS**
AR40	His_pMAL_infusion_rev	36	reverse	55.4	CGTTTTATTTGAAGCTtTCAGTGGTGGTGGTGGTGG	**pAR26**
AR423	StrI_mpsMT_for	38	forward	55.4	CGAAAAAGGCGCCGGATCCtcacggcggcagcaagaac	**ΨME _ΨKR _ER_KR**
AR204	ΔmACPTE_His_rev	33	reverse	57.2	GTGATGATGCTCGAGgtccccatgggccacagc	**pAR18**
AR311	ΔmER_for	35	forward	55.9	gagcaggacaagccctccaagaccttctgcccagc	**ΨME _ΨKR _KR**
AR312	ΔmER_rev	21	reverse	56.3	gggcttgtcctgctctaactg	**pAR237**
AR409	mFAS_psMTdel_for	22	forward	54.8	ccacaggagaaacctatcttcc	**DML_ΨKR _KR**
AR410	mFAS_psMTdel_rev	35	reverse	55.9	aggtttctcctgtgggctctcagacaggcactcag	**pAR238**
AR409	mFAS_psMTdel_for	22	forward	54.8	ccacaggagaaacctatcttcc	**DH _ΨKR _ER_KR**
AR410	mFAS_psMTdel_rev	35	reverse	55.9	aggtttctcctgtgggctctcagacaggcactcag	**pAR236**
AR75	pETcoco_tig_for	41	forward	56.4	ggagatataagcatgCAAGTTTCAGTTGAAACCACTCAAGG	**TF**
AR76	tig_pETcoco_rev	34	reverse	55.4	agcagccggatctcaCGCCTGCTGGTTCATCAGC	**Prof. Hartl**
AR73	pETcoco-1_lin_for	22	forward	54.8	tgagatccggctgctaacaaag	**TF**
AR74	pETcoco-1_lin_rev	30	reverse	54.8	catgcttatatctccttcttaaagttaaac	**pETcoco-1**
AR75	pETcoco_tig_for	41	forward	56.4	ggagatataagcatgCAAGTTTCAGTTGAAACCACTCAAGG	**TF(N+C)**
AR76	tig_pETcoco_rev	34	reverse	55.4	agcagccggatctcaCGCCTGCTGGTTCATCAGC	**Prof. Hartl**
AR73	pETcoco-1_lin_for	22	forward	54.8	tgagatccggctgctaacaaag	**TF(N+C)**
AR74	pETcoco-1_lin_rev	30	reverse	54.8	catgcttatatctccttcttaaagttaaac	**pETcoco-1**
AR77	pETcoco_mycdodecin_for	39	forward	57.4	ggagatataagcatgAGCAATCACACCTACCGAGTGATC	**X**
AR78	mycdodecin_pETcoco_rev	34	reverse	55.4	agcagccggatctcaGGAATCCTCCAGGCGGAAG	**mtDod**
AR73	pETcoco-1_lin_for	22	forward	54.8	tgagatccggctgctaacaaag	**X**
AR74	pETcoco-1_lin_rev	30	reverse	54.8	catgcttatatctccttcttaaagttaaac	**pETcoco-1**
AR84	pETcoco_DnaK_infusion_for	39	forward	55.2	ggagatataagcatgGGTAAAATAATTGGTATCGACCTG	**DnaK-/DnaJ**
AR83	Dnak_RBS_rev	54	reverse	56.9	CAAAATTATTTCTAGCGCGGATCATTTTTTGTCTTTGACTTCTTCAAATTCAGC	**Prof. Hartl**
AR85	Dnak_RBS_DnaJ_for	51	forward	54.5	GACAAAAAATGATCCGCGCTAGAAATAATTTTGTTTAACTTTAAGAAGGAG	**DnaK-/DnaJ**
AR86	DnaJ_pETcoco_infusion_rev	33	reverse	54.9	ggctggcttgcggccTCAGCGGGTCAGGTCGTC	**Prof. Hartl**
AR73	pETcoco-1_lin_for	22	forward	54.8	tgagatccggctgctaacaaag	**DnaK-/DnaJ**
AR74	pETcoco-1_lin_rev	30	reverse	54.8	catgcttatatctccttcttaaagttaaac	**pETcoco-1**
AR88	pETcoco_pfuPre_for	40	forward	57.7	gaaggagatataagcatgCAAAACATTCCACCCCAAGTCC	**pfPfdB**
AR89	pfuPre_pETcoco_rev	35	reverse	55.9	ggctggcttgcggccTCATCCAGCGGTTGGAGGTC	**DSM3638**
AR97	pETcoco_GroES_for	41	forward	54.8	gaaggagatataagcatgAATATTCGTCCATTGCATGATCG	**GroEL/GroES**
AR98	GroES_RBS_GroEL_rev	58	reverse	54.4	CCTTAAATTCGTATGTTCAGTGTCGTGCGCGGATTACGCTTCAACAATTGCCAGAATG	**Prof. Hartl**
AR99	RBS_GroEL_for	60	forward	54.4	CGACACTGAACATACGAATTTAAGGAATAAAGATAATGGCAGCTAAAGACGTAAAATTCG	**GroEL/GroES**
AR100	GroEL_pETcoco_rev	36	reverse	54.9	gttagcagccggatctcaCATCATGCCGCCCATGCC	**Prof. Hartl**
AR73	pETcoco-1_lin_for	22	forward	54.8	tgagatccggctgctaacaaag	**GroEL/GroES**
AR74	pETcoco-1_lin_rev	30	reverse	54.8	catgcttatatctccttcttaaagttaaac	**pETcoco-1**
AR84	pETcoco_DnaK_infusion_for	39	forward	55.2	ggagatataagcatgGGTAAAATAATTGGTATCGACCTG	**DnaK-/DnaJ/GrpE**
AR83	Dnak_RBS_rev	54	reverse	56.9	CAAAATTATTTCTAGCGCGGATCATTTTTTGTCTTTGACTTCTTCAAATTCAGC	**Prof. Hartl**
AR85	Dnak_RBS_DnaJ_for	51	forward	54.5	GACAAAAAATGATCCGCGCTAGAAATAATTTTGTTTAACTTTAAGAAGGAG	**DnaK-/DnaJ/GrpE**
AR101	DnaJ_RBS_rev	46	reverse	54.9	CGCAGCATTCATCTCGCGTAAGCGCGGATCAGCGGGTCAGGTCGTC	**Prof. Hartl**
AR102	RBS_GrpE_for	64	forward	54.8	CGCGAGATGAATGCTGCGAAAAAAACGCGGAGAAATTCATGAGTAGTAAAGAACAGAAAACGCC	**DnaK-/DnaJ/GrpE**
AR103	GrpE_pETcoco_rev	42	reverse	55.7	gttagcagccggatctcaCGCTTTTGCTTTCGCTACAGTTAC	**Prof. Hartl**
AR73	pETcoco-1_lin_for	22	forward	54.8	tgagatccggctgctaacaaag	**DnaK-/DnaJ/GrpE**
AR74	pETcoco-1_lin_rev	30	reverse	54.8	catgcttatatctccttcttaaagttaaac	**pETcoco-1**
AR88	pETcoco_pfuPre_for	40	forward	57.7	gaaggagatataagcatgCAAAACATTCCACCCCAAGTCC	**prefoldin (pfuPfdB)**
AR72	pET22b_rev	30	reverse	53.4	CATATGTATATCTCCTTCTTAAAGTTAAAC	**pAR39**
AR105	RBS_pfuPreA_for	55	forward	54.1	GTTTAACTTTAAGAAGGAGATATACATATGGAAAACAATAAGGAATTGGAAAAGG	**prefoldin (pfuPfdB)**
AR106	pfuPreA_pETcoco_rev	42	reverse	56.7	gttagcagccggatcTCACTTCTTAAGCTTGAAGCTCATTGC	**DSM3638**
AR109	pETcoco_PfCPN_for	34	forward	55.4	gaaggagatataagcatgGCCCAGTTAGCAGGCC	**pfCpn**
AR110	PfCPN_pETcoco_rev	39	reverse	55.7	gttagcagccggatctcaGTCTAGATCACTGCTGAAGTC	**DSM3638**
AR73	pETcoco-1_lin_for	22	forward	54.8	tgagatccggctgctaacaaag	**pfCpn**
AR74	pETcoco-1_lin_rev	30	reverse	54.8	catgcttatatctccttcttaaagttaaac	**pETcoco-1**
AR111	pETcoco_IbpA_for	40	forward	54	gaaggagatataagcatgCGTAACTTTGATTTATCCCCGC	**IbpA/IbpB**
AR112	IbpA_RBS_rev	53	reverse	56.7	GCGAGTAAGTACCTGCAAATCCGAAGATTTCAGTTGATTTCGATACGGCGCGG	***E. coli* DNA**
AR113	RBS_IbpB_for	23	forward	57.1	CGGATTTGCAGGTACTTACTCGC	**IbpA/IbpB**
AR114	IbpB_pETcoco_rev	40	reverse	57.1	gttagcagccggatctcaGCTATTTAACGCGGGACGTTCG	***E. coli* DNA**
AR104	pETcoco_ClpB_for	45	forward	57.5	gaaggagatataagcatgCGTCTGGATCGTCTTACTAATAAATTC	**ClpB**
AR105	RBS_pfuPreA_for	55	forward	54.1	GTTTAACTTTAAGAAGGAGATATACATATGGAAAACAATAAGGAATTGGAAAAGG	**E. coli DNA**

Supporting Note 1. Expression of human FAS in *E. coli*

Most of the recent kinetic and biochemical data was collected on rat FAS, expressed in *Spodoptera frugiperda* (*Sf*-9) insect cells in the Smith laboratory.[2] As we aimed at cheap and quick access to recombinant protein, we did not follow this strategy, but instead searched for expressible animal FAS constructs in *E. coli*. The initial focus was on human FAS (hFAS). hFAS is a member of the animal FAS family and hence suited to investigate the relationship to PKS. It also serves as a potential drug target due to its relevance in the therapy of several diseases.[3,4]

The project was initiated by cloning human FASN genes into bacterial expression plasmids. Two different genes were used, a synthetic, codon optimized gene (GeneArt, ThermoFisher) and a verified cDNA clone (Source BioScience). Both genes were cloned into a pET22b (Novagen) derived expression vector, generating a N-terminal StrepI-tag and a C-terminal His-tag (hFASe, synthetic gene encoding human FAS; hFASh, native sequence) (see Fig. S1A-C). Additionally, both genes were cloned into the vector pMAL-c5G (New England BioLabs) generating a N-terminal MBP-fusion and a C-terminal His-tag according to Jayakumar *et al*..[5] Contrary to Jayakumar's report, all attempts to express the human FAS in *E. coli* were either unsuccessful or yielded aggregated material, as also stated elsewhere.[6] Different expression strategies were tested; using different fusion constructs, *E. coli* cell lines, expression temperatures, concentration of inducer IPTG to different cell densities for induction.

<u>A summary of expression is as following:</u>

The first expression of four different constructs of hFASe (N-terminal StrepI-tag, MBP-, Trx- and Sumo-fusion) in BL21-Gold (DE3) cells was analyzed via SDS-PAGE, performed on whole cells (see Fig. S2A). Every lane showed a faint band at the expected construct sizes (hFASe: 278 kDa, MBP-hFASe: 320 kDa; Trx-hFASe 290 kDa; Sumo-hFASe 287 kDa) indicating expression of full-length constructs. Interestingly, a second prominent band appeared at a slightly smaller construct size in all lanes indicating truncated protein. Since all different N-terminal fused constructs resulted in the same truncated protein it is clear that either a prominent proteolytic cleavage site or an alternative

translation start codon exists at the beginning of hFAS coding sequence. Indeed, a methionine at position 32 was identified with an appropriate strong translation initiation site. Two silent mutations in the alternative RBS abolished the production of truncated FAS (see Fig. S2A, right panel).

Based on the finding that *E. coli* is capable of translating the human FASN gene into a full-length polypeptide chain regardless of a N-terminal fusion, we then investigated whether hFAS is properly folded. Test-expressions in medium scale (50 mL) were performed to simultaneously screen the impact of different expression conditions. Cells were lysed mildly by lysozyme treatment in a stabilizing buffer, and the insoluble fraction was separated by centrifugation. Both fractions (inclusion bodies were dissolved in 8 M urea) were analyzed by SDS-PAGE. Unfortunately, most of the hFAS was expressed insolubly independent of the tested expression conditions. N-terminal fusion of MBP did not increase the fraction of soluble protein significantly (see Fig. S2B). Interestingly, the total amount of produced protein was dramatically increased by the MBP-fusion, but only when the plasmid pAR19 was used for expression (see Fig. S2B middle panel). This plasmid is based on the vector pMAL, which contains the whole regulatory region of *malE* under a TAC promoter. Furthermore, this plasmid does not have a N-terminal tag and directly starts with the *malE* sequence.

In order to increase the fraction of soluble protein, we tested several approaches. First, the improved *E. coli* BL21 strain BL21 Star (DE3) was used, which offers a better mRNA stability due to a mutation in the *rne131* gene. Additionally, the cell density at induction with IPTG was varied (see Fig. S2C). From the Coomassie-stained SDS-PAGE, it seems that expression in BL21 Star (DE3) cells resulted in a slightly increased soluble fraction, especially, when cells were induced at an OD_{600} between 0.2-0.7. Again, a fusion with MBP did not increase the soluble fraction of protein.

A last attempt to obtain a soluble expression of hFAS in *E. coli* was tried by co-expression with molecular chaperones as they are known to assist the folding of polypeptide chains and the assembly of the macromolecule. Different chaperone systems were reported to facilitate heterologous expression of especially mammalian proteins in *E. coli*.[7,8] Indeed, a potential role of the human chaperonin TRiC/CCT was assigned to the folding process

of hFAS.[9,10] We decided to test a broad variety of chaperones from *E. coli*: Trigger factor (TF), DnaK-/DnaJ/GrpE, GroEL/GroES, ClpB and IbpA/IbpB plus chaperones of the thermophilic organism *Pyrococcus furiosus*: prefoldin (pfuPfdB), chaperonin (Cpn) and PF1883.

We decided to use the pETcoco system (Merck Biosciences) as co-expression vector, as it allows regulating copy number in the cell from low to medium copy. Every chaperone system consisting of more than one gene was organized polycistronically. In general, the 5' regulatory region of pET22b from the XbaI restriction site was used as translation initiation site between the genes. The effect of co-expression was again tested in 50 mL test cultures with subsequent separation of the soluble and insoluble fraction (see Fig. S3A and B).

Neither a chaperone system from *E. coli* nor from *P. furiosus* was able to prevent aggregation of hFAS. Hardly any beneficial effect on the fraction of soluble protein was observed. Only co-expression with TF seems to result in the appearance of small amounts of hFAS in the soluble fraction (see Fig. S3A). Though hFAS is one of the largest proteins in *E. coli* but we observed only 'thin' band(s) at ca. 171 kDa which may mean perhaps only a low amount of protein was loaded onto the gel. Therefore, it is difficult to draw a final conclusion, but a major effect of these chaperone systems on hFAS folding can be excluded.

Nevertheless, due to the appearance of small bands in the soluble fractions of our test expressions, we decided to test this expression on a larger scale. The scale of a bacterial culture may influence expression yields and also Jayakumar *et al.* used optimal conditions of a fermenter (15 L scale) to express hFAS.[5,11]

It can clearly be seen that MBP-hFAS is expressed in full-length in *E. coli* (see Fig. S4A-C). Comparing the lane with lysate to the supernatant shows a dramatic decrease of protein after centrifugation reflecting the expected high portion of insoluble protein. A supernatant fraction indicating soluble protein could not be purified by Ni-chelating affinity chromatography. This finding implies that either the C-terminal His-tag was inaccessible or that hFAS was unfolded, either as not expressed in its native conformation or denatured

during protein preparation. hFAS without a N-terminal fusion domain behaved similarly and again no beneficial effect of chaperone co-expression was seen.

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