Supplementary Materials

From Cell-Free Protein Synthesis to Whole-Cell Biotransformation: Screening and Identification of Novel α-Ketoglutarate-Dependent Dioxygenases for Preparative Scale Synthesis of Hydroxy-L-Lysine

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Table S1. Primer sequences

Primer	Sequence (5'→3')
PPN070	TTTAACTTTAAGAAGGAGATATACAATGTCCTCGCTGTTCCTCG
PPN071	GGTGGTGGTGGTGGTGCTCGAGTGCGCTGAAGCTGGCC
PPN072	TTTAACTTTAAGAAGGAGATATACAATGTCCTCGGTGTT
PPN073	GGTGGTGGTGGTGGTGCTCGAGTGCGGAGAAGCTGGC
PPN074	TTTAACTTTAAGAAGGAGATATACAATGCTCACCATCGAC
PPN075	GGTGGTGGTGGTGGTGCTCGAGTGCGCAGCCCCGGAT
PPN088	TTAACTTTAAGAAGGAGATATACATATGCAGCTGGGTGTGCC
PPN089	GGTGGTGGTGGTGGTGCTCGAGTGCGGTCGGCTGCGCGCT
PPN090	TTAACTTTAAGAAGGAGATATACATATGGATAATGTGAGCCAAGAAATTGTG
PPN091	GGTGGTGGTGGTGGTGCTCGAGTGCGCTAACCGGTGTAAAATCAACCAGC
PPN092	TTAACTTTAAGAAGGAGATATACATATGGGTTGGAACATTTTTACCAATGC
PPN093	GGTGGTGGTGGTGGTGCTCGAGTGCCTGCGGACGACGATCGC
PPN094	TTAACTTTAAGAAGGAGATATACATATGCATCGTATTGAAGAAGAACCGT
PPN095	GGTGGTGGTGGTGGTGCTCGAGTGCGCTGCTCAGCGGAATCGG
PPN096	TTAACTTTAAGAAGGAGATATACATATGCAGCAGCAAGGTTGG
PPN097	GGTGGTGGTGGTGGTGCTCGAGTGCATCACGCACCGGTTCCG

Table S2. Gene sequences

Encoded Protein	Gene sequence
*Caci*KDO	ATGAAGAACCTGTCTGCGTATGAAGTGTATGAATCGCCGAAGACCAGCGG AGAGTCACGTACCGAAGCCGTTTCGGAGGCCGCCTTCGAATCCGACCCCG AAGTGAGCGCAATCCTAGTCCTGACCTCCTCCGAAGCCTCGACGCTGGAGC GCGTCGCCGACCTCGTGACCGCGCACGCGCTGTATGCCGCTCACGATTTCT GTGCCCAGGCCCAGCTGGCCGCCGCCGAACTGCCGTCGCGGGTCGTTGCC CGGTTGCAGGAGTTCGCCTGGGGCGACATGAACGAGGGCCACCTTCTGAT AAAAGGCCTGCCGCAGGTCCGCTCCCTGCCACCGACACCGACGTCCAACGT GCACGCCGTCGCCGCGACGACGCCGATGTCGCGCTACCAGGCCCTCATCA ATGAGTGCGTGGGGCGCATGATTGCCTACGAGGCCGAGGGGCACGGCCA CACCTTCCAGGACATGGTCCCGAGCGCCATGAGCGCGCACTCGCAGACCA GTCTCGGCTCGGCGGTTGAGCTCGAACTGCACACCGAGCAGGCCTTCAGT CCGCTGCGTCCGGACTTCGTCAGCCTGGCCTGTCTTCGCGGCGATCCACGA GCCCTGACTTACCTGTTCTCGGCCCGGCAACTGGTCGCGACGCTGACGACA CAGGAAATCGCCATGCTGCGCGAGCCCATGTGGACCACCACCGTCGACGA GTCCTTCCTGGCGGAGGGGCGTACGTTCCTGCTCGGCTTCGAGCGCGGTC CGATCCCGATATTGAGCGGTGCCGACGACGATCCGTTCATCGTCTTCGACC AGGATCTGATGCGGGGGATCTCGGCGCCCGCGCAGGAACTCCAACAGACT GTGATCCGCGCCTACTATGCCGAGCGCGTCAGCCACTGCCTCGCACCCGGT GAGATGCTCCTCATCGACAACCGGCGCGCCGTCCACGGCAGGTCGATCTTC GCCCCGCGTTTCGACGGCGCTGACCGGTTCCTTTCCAGAAGCTTCATCGTG GCCGACGGATCGCGCAGTCGGCACGCGCGTTCTTCCTTCGGCCGCGTCGTC TCTGCGAGGTTCAGCTAA
*Krad*KDO	ATGTCCTCGCTGTTCCTCGACTCCTCCGCCCACGTGCCGACCCTGTTCGAGC TGCCCGCGCCCCAGCGGGCCGCGCTGGCCGCGCTGGGCGCGCGCCTGACC GCGGACCCGGTGACCGAGCCCGACGCCTTCGGCCGCCAGGCGCGCCTGCT GGCCCGCGAACTGTCCGTCGAGGTCACCGAGGCCCTGTGGGCGTTCGAGG AACGGGGATCGGACTCCGGGGTCCTCGTCCTGCGCGGCCTGGAGGTCGGT GAGCTGCCGCCCACCCCGGCCGACAACACCGGCGGGATCGGCGGGCGCA CCCTGCTCGCCCGCCAGCAGGCGATCGTCAGCCACGCGCTGGGGCACATG GTCGGCTACGCCGCCGAGGGCCACGGGCACCTCCTGCAGGACATGGTCCC CAACGCCAGGCTCGCCGCGACCCAGCAGTCGCAGGGCTCCCGGGTGGAGC TGGAGGCGCACACCGAGCAGTGCTTCTCCGACCTGCGCCCCGACTACGTC GTCCTGGGCTGCCTGCGCGGGGACGCCGACGCCGCCACCTACGCGTTCCG CGCCCTGGACCTGCTGGCCCACGTGGACCCCACCGACGTCATGGAGCTGTT CCGGCCGCTGTGGACGACGCTGGTCGACGAGTCCTTCGCCGACTTCCTCGA CACCCGCGAGGTGCGCGGGCCGTTCTCCATCCTCTCCGGCGACGTCGACG ACCCGACGATGCTCGTCGACCAGGACCTCATGCACGGCATCACCAAGCAC GCCCAGGCCCTGCTGGAGCGCGTGCTGGAGATCTACGTCGCCCACCGCCA CGCCGTCGTCCTCCAGCCCGGGGACGTGCTGCTGCTGGACAACCTGCGCG CCATGCACGGCCGCTCGCCGTTCGCCCCGCGCTTCGACGGCACCGACCGGT TCATCTCCCGGGGTTTCGTCGTCCGCGACCTGCGCCGCTCCCGCTTCGCCC GCCCCGGCGGGAACCGCGTCGTGCAGGCCAGCTTCAGCTGA

*Krhi*KDO

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*Cpin*KDO

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Table S3. Amino acid sequences

Protein	UniProtKB	Amino acid sequence
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*Krad*KDO	A6WF32	MSSLFLDSSAHVPTLFELPAPQRAALAALGARLTADPVTEPDAFG RQARLLARELSVEVTEALWAFEERGSDSGVLVLRGLEVGELPPTP ADNTGGIGGRTLLARQQAIVSHALGHMVGYAAEGHGHLLQDM VPNARLAATQQSQGSRVELEAHTEQCFSDLRPDYVVLGCLRGDA DAATYAFRALDLLAHVDPTDVMELFRPLWTTLVDESFADFLDTR EVRGPFSILSGDVDDPTMLVDQDLMHGITKHAQALLERVLEIYVA HRHAVVLQPGDVLLLDNLRAMHGRSPFAPRFDGTDRFISRGFVV RDLRRSRFARPGGNRVVQASFS
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*Mint*KDO	A0A1X1X0H1_9MYCO	MLTIDPLRDPQGYVRQARLLSAQLPWALRETVVDFETYGDGGLL VTGLEVGSVAATPDSPANDVTHNTPIAAASALLLACIANLVGYRA ESWGRICQSVIPTKGDASQQKSTGSRVCLECHTEQAFNLLTRPDY VALGCLRGDPHAATFMLSARALQEHLPSSVVGLLREPMFNTRID QSFIEGGVPDEVRGPIPVLSGALEDPVITFDEDLMWGNSPKHQA ALEMVKTVWADHRSSVVLKRGDVLIIDNSRAIHGRSAFRPRWD GGDRWLCRLQGVCDLTRTRDARRPQSPVIEIRGC
*Cpin*KDO	C7PLM6	MRPLDVTPTISPGAQDLPRTMHFAAEPPLQPLIIDITEEEKLEITYI GKKLKRKYKSYDDPGFISMLHLNAYTLLPERIAKVLSNFGTDFSDQ QYGAVVLRGLIEIGQDELGPTPRSWQETDHEKIMEYGFISSLLHG AVPSKPVEYFAQRKGGGLMHAIIPDENMSFTQTGSGSRTDLFVH TEDAFLHNAADFLSFLFLRNEERVPSTLYSIRSHGRPDAILQELFKPI YKCPKDANYASEEALGDDIRTSVLYGSRSAPFMRFDAAEQIYNED ANQDPEALHNLKRFWEEARKLIYNDFVPESGDLIFVNNHLCAHG RNAFLAGFREENGQLVKCERRLMLRMMSKTSLINIREVTHPENP YLIMEEHYGKVYSAHLANL

*Nkor*KDO	G8T8D0	METIIESRQRINSPGVLPPPLSPLIVDVTPKERASISNVANILLKAFG HYEHPDFISALHLNAFQLLPERIAGILSRFGTDFSRHQYGALVFRG LTEVDQEALGPTPPSWKETDYSKLVKYGFICSLLHGAIPSKPVQYY AQRKGGGLLHAVIPDEKMSHTQTGSGSRTDLFVHTEDAFLFNQA DFLSFLFLRNEEQVPSTLYSIRSHGDTNAIMAELFKPIYKCPKDANY ADDENAGEEVTTSILYGNRERPFIRFDAAEQIYNEKAGQTPEAM HNLVRFWDEAKQLIYNDFVPDSGDLIFVNNHLCAHGRNSFVAGY RNENGQLVKCERRLMLRMMSKTSLINIQSVTQLNDPYFIMEEHY GKLFHSQQ
*Fspe*KDO	J3BZS6	MSVERSAETSLTLEIPTSPLIIKITQQERNILSNVGNLLVKAFGNYE NPDYIASLHLHAFQLLPERITRILSQFGSDFSAEQYGAIVFQGLIEV DQDDLGPTPPNWQGADYGKLNKYGFICSLLHGAVPSKPVQYYA QRKGGGLLHAVIPDEKMAATQTGSGSKTDLFVHTEDAFLSNQA DFLSFLYLRNEERVPSTLYSIRSHGKMNPVMKKLFEPIYQCPKDAN YNDEDVANSGPTASVLYGNRELPFIRFDAAEQIFNENAGQTSEAL GNLMDFWDEAKTLINSDYIPNSGDLIFVNNHLCAHGRSAFIAGQ RIENGEIIKCERRQMLRMMSKTSLIHIRSVTRTDDPYFIMEEHLGK IFDLD
*Fjoh*KDO	A5FF23	MKSQSLIEDEIPVKENYAYQIPTSPLIVEVTPQERNILSNVGALLEK AFKSYENPDYIEALHLYSFQLLPERIARILSRFGTDFSADQYGAIIFR GLLEVDQDHLGPTPANWQSADYSKLNKYGFICSLLHGAVPSKPV QYYAQRKGGGILHAVIPDEKMAATQTGSGSKTNLYVHTEDAFLL HQADFLSFLYLRNEERVPSTLYSVRSHGKVNKIMEKLFDPIYQCPK DANYQEEINDGPLASVLYGNKKLPFIRFDAAEQIFNENAGQTPEA LYNLTEFWNEAKELINSDYIPDSGDVIFVNNHLCAHGRSAFTAGQ KEENGKLVPCERRQMLRMMSKTSLIHIRSMTHTDDPYFVMEEH LGKVFDQA
*Pbra*KDO	A8KCI8	MQLGVPFLKRESMSKMTGQEWAAAAPETEPGDVRAALQQRG WARFDATDMQVAVDEAADLQRLTEYARSLPVDRFGTGGRHRS YAEGILTPRRKTIAWKAGARTPDGRVEIAYVQHSEFQPEHGGVV RNFARTREDILALPLVHRLIWYDLSLTPMFDAEGDLLCGFHMIRM QATPGAVARITPDCLHQDGQPFTAVHLVERSHAEGGVNFIAPPR YTGRQFDEVPSHLLSAFVLGSPLQSYIIDDAAICHQVTAVSCSPGA SHGTRTVILIDFSPLNPASSAQPT
*Plum*KDO	A0A022PMX0_PHOLU	MGWNIFTNAPDSYHLTAAHIRNSLHQQGFATFNAADLDLSDSE KIDLISLCELSKSLPLDRFGEGGRHRSYCEGIWSWETETIDWKTGY PQPDGSVEIDYHQGSEYQPEFGGVVRKFLRMSDEILNKGLLNKLI WHDLSLTGMAEHYSHLLCGVHLIRMQALPGKPAKITPNCFHRD GQPFTAVHLIERYNVEGGATHIAPPSYTNCQLEAVPAHEITRFLLN DPLDSYIIDDAAICHYINPVICDENASVGVRTIILIDFTPLEQSDRRP Q
*Bspe*KDO	A0A1B4SJJ6	MQQQGWARFDAADLDHVEAKSHCLAQLSRYADQLPVDRHGG AGRHRSYAEGVLAPNDGHVAWKPGFVGLDGAIEIEYRQEIGFQP EYGGVHRRFLRTSDSILALPLIRKLIRFDLALTPLGELGEPLLCGLHV LRMRALPGVMSRITPDCLHQDGQPFTAVHLIDRTSAVGGVNYIA PPQYAGRQVDDVPDEQVRAFSLHVPLESYIIDDAAVSHHVTSISC APGAPCGMRTVILIDFTPLDAVVRSPSDAARPIVPTSNPEPVRD

*Bpse*KDO	Q63KU4	MHRIEEEPFSAESRAEPDAGAALRALGWMMFGMEDLRLCADE RRELAQLARYAQTLPIDGFGGNGRHRCYAEAVLSPSARTLRWKP GIVGKDGTIEVEYEQETEFQPEYGGVRRRFARASDRVLQSSLIRKLI WFDFDLIGWAGASEPLQCGFHLVRLQALPGKPSRSTPDCLHRDG QPYTAVHLVSRHDVTGGLNYIAPPRYSGRNVDELPADALTTFTLT EPLDSYIVDDAAVCHHVSAVGCAPGAASGSRTVMLIDFSPIPLSS
*Bpla*KDO	A0A0B6S2H0	MDNVSQEIVVSHESLSTDNLGIIGIRNRLSELGWARFDADDFALT AHERDELDFLARYGESLPLDRFGGDGRRRAYCEGIVDLASRQIAW RPGHRAEDGGIEIEYQQDEIYQPEYGGVVRRFRRLGSEVLETGLIN RLIWHDLDLTPLPERHETLLCGLHMICMTAIGTEPARITPDCLHRD GQPYTAAHLIRRHNAKGGVNYIAPPRFSGWRVEAVPDDQLQVF TLHEALDSYVIDDEAVSHHVTSVSCDDPALPGMRTILLVDFTPVS

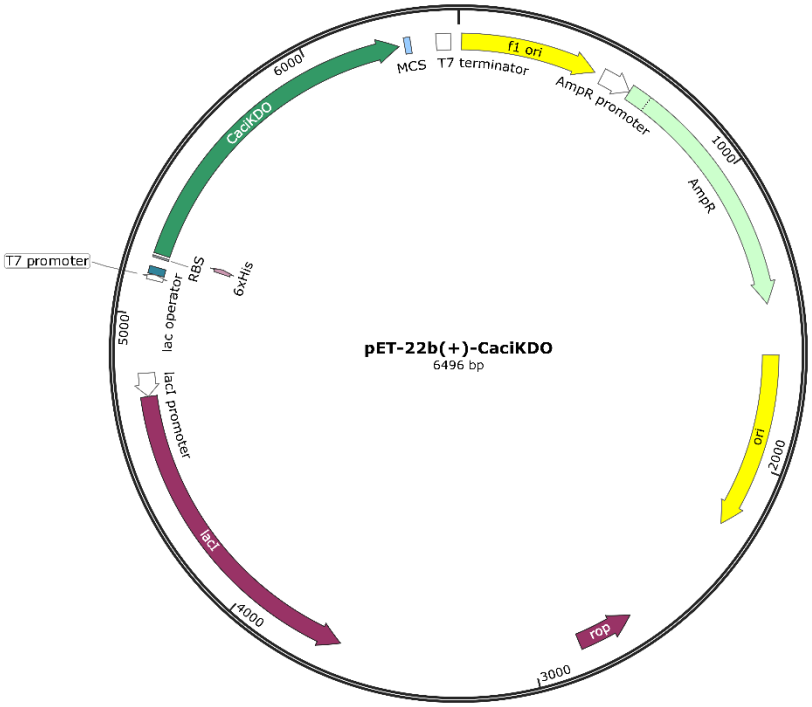

Figure S 1 Exemplary plasmid map of pET-22b(+)-CaciKDO

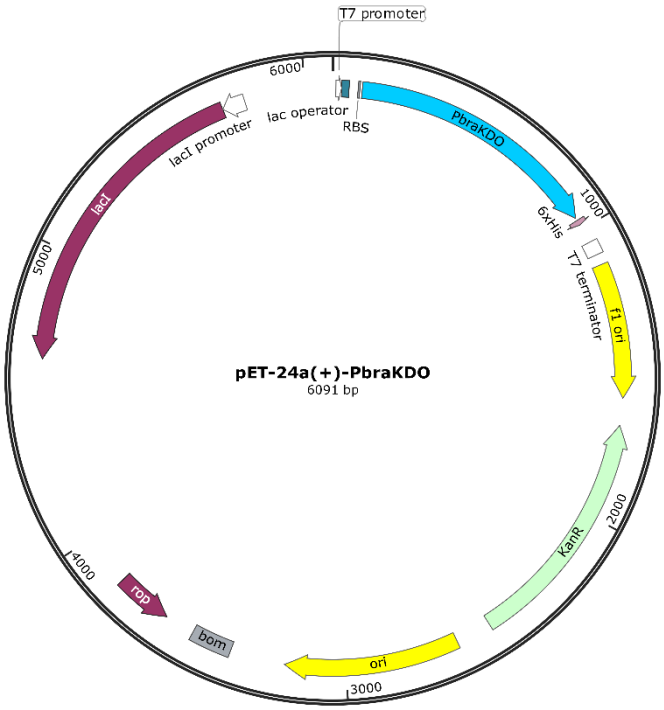

Figure S 2 Exemplary plasmid map of pET-24a(+)-PbraKDO

Results

SDS-PAGE gel images of cell-free synthesized KDOs. Reference – CFPS without chaperones, -DNA – CFPS mix without plasmid template

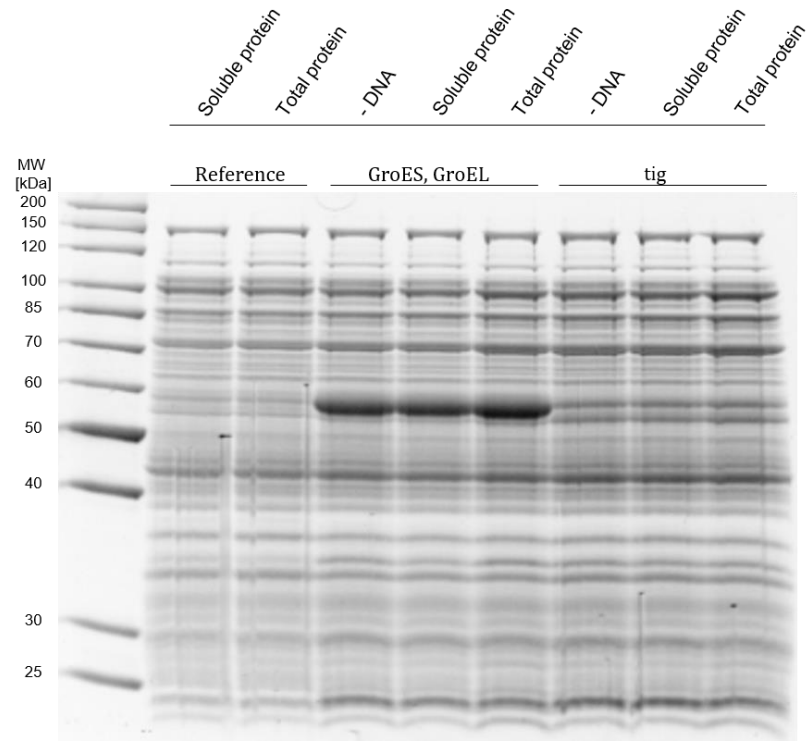

Figure S 3 Blan-KDO (31.5 kDA) + Chaperones (GroES, GroEL; tig)

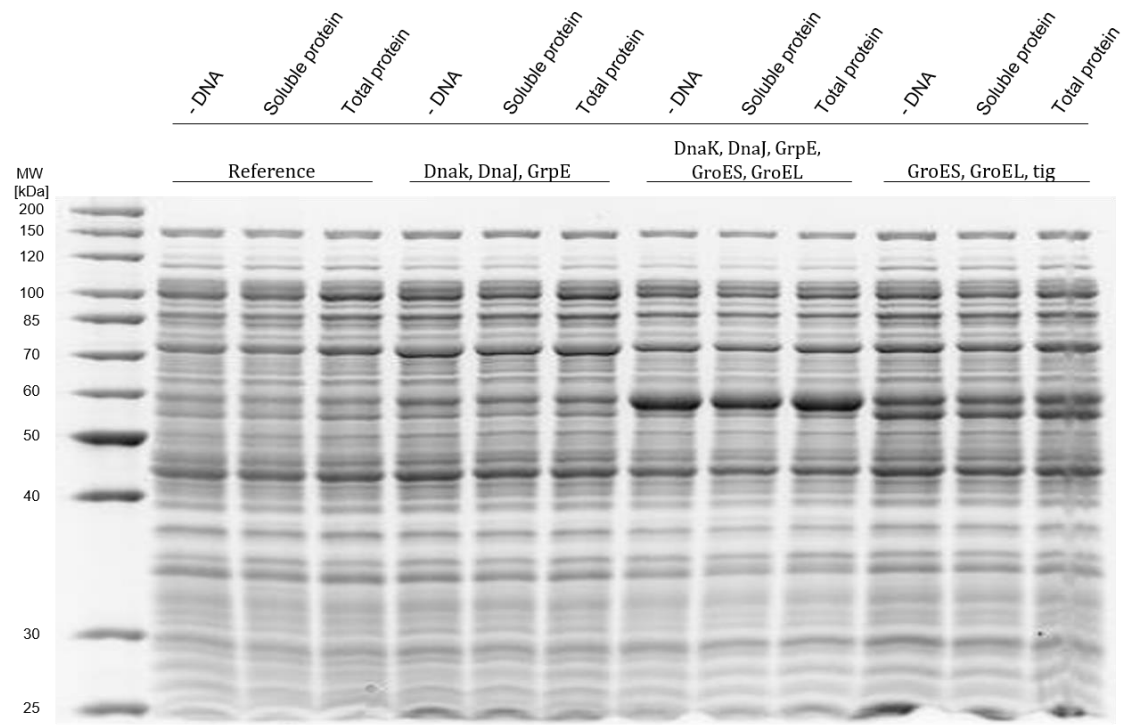

Figure S 4 Blan-KDO (31.5 kDA) + Chaperones (DnaK, DnaK, GrpE; DnaK, DnaK, GrpE, GroES, GroEL; GroES, GroEL, tig)

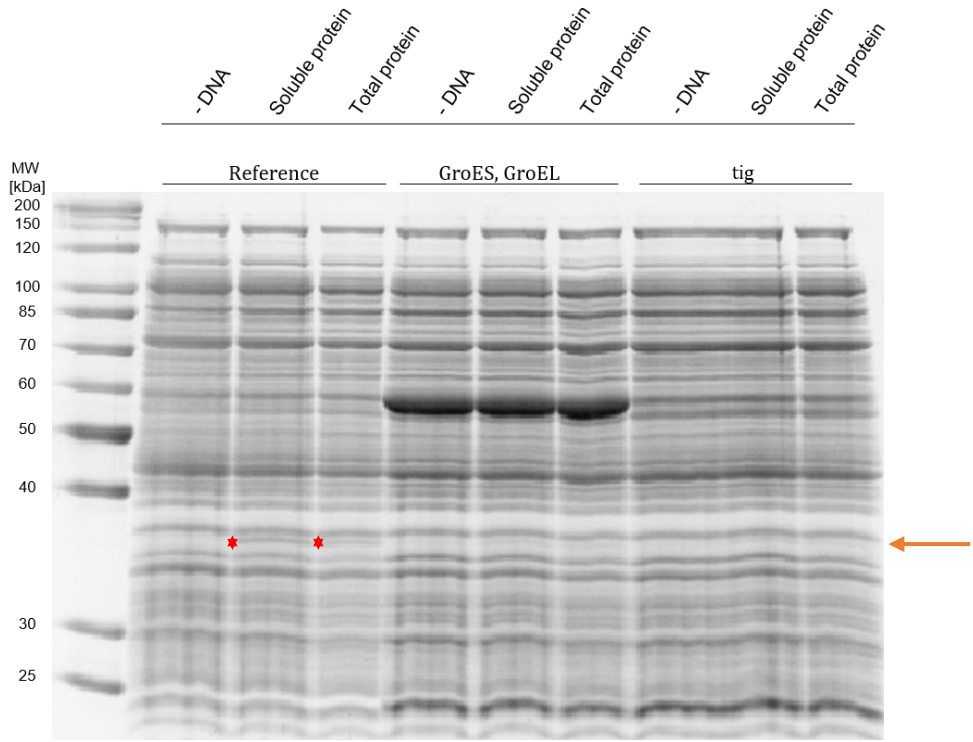

Figure S 5 Bpse-KDO (30.5 kDA) + Chaperones (GroES, GroEL; tig)

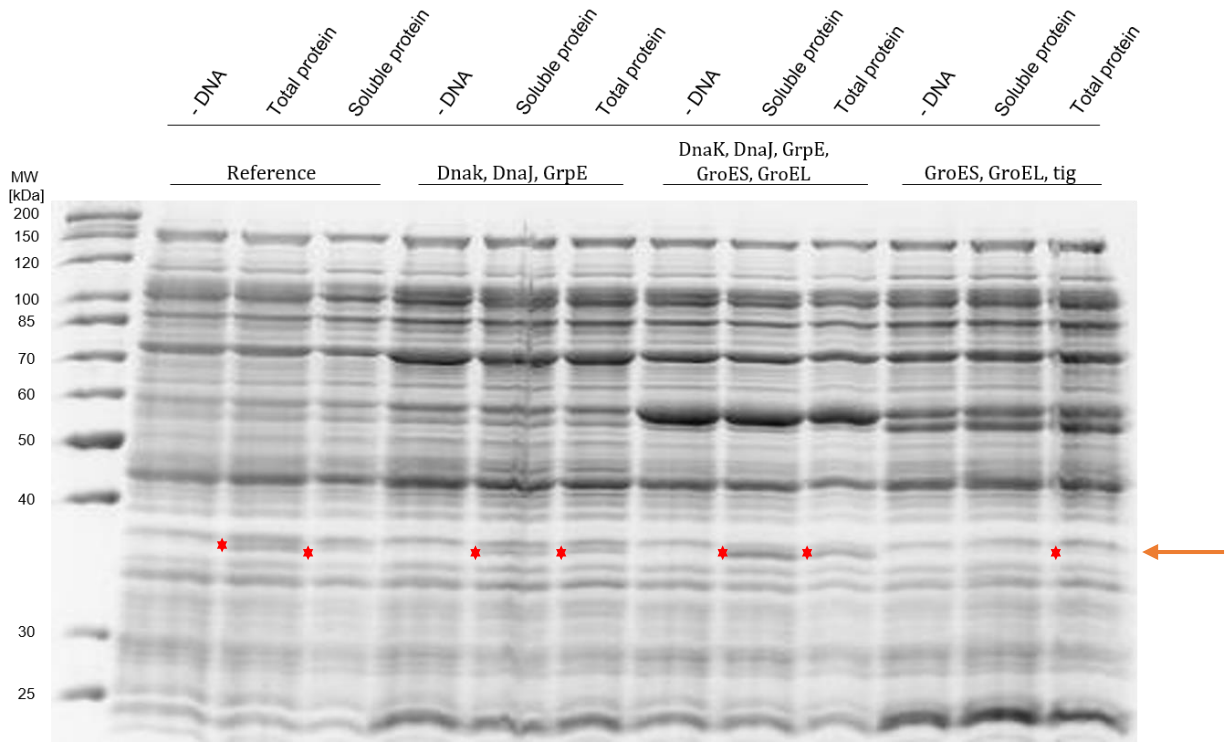

Figure S 6 Bpse-KDO (30.5 kDA) + Chaperones (DnaK, DnaK, GrpE; DnaK, DnaK, GrpE, GroES, GroEL; GroES, GroEL, tig)

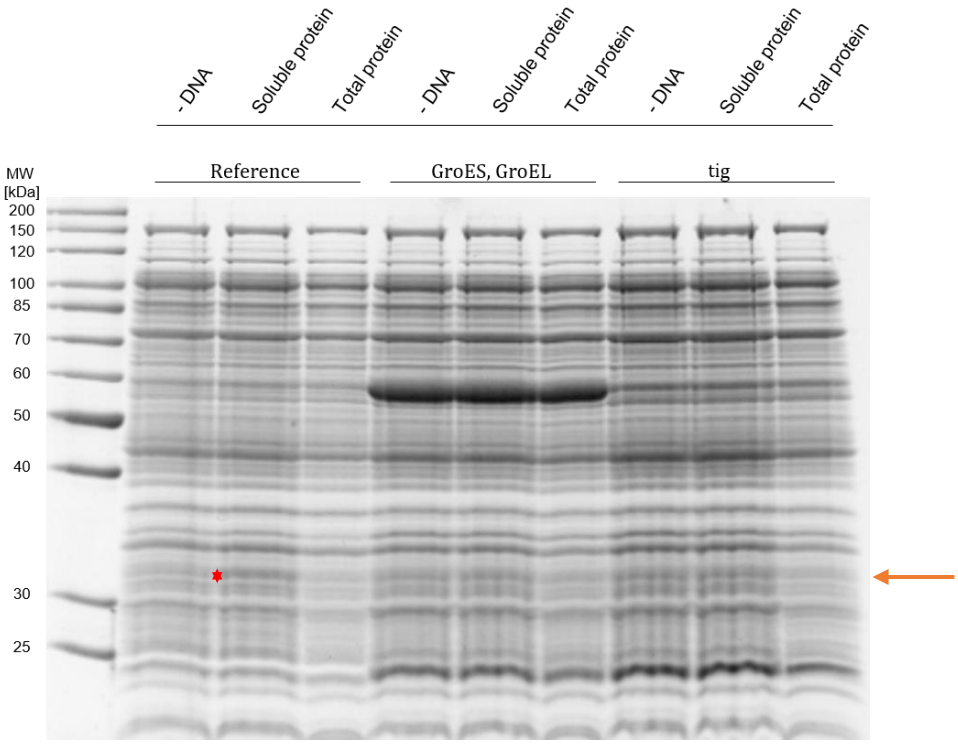

Figure S 7 Bspe-KDO (30.2 kDA) + Chaperones (GroES, GroEL; tig)

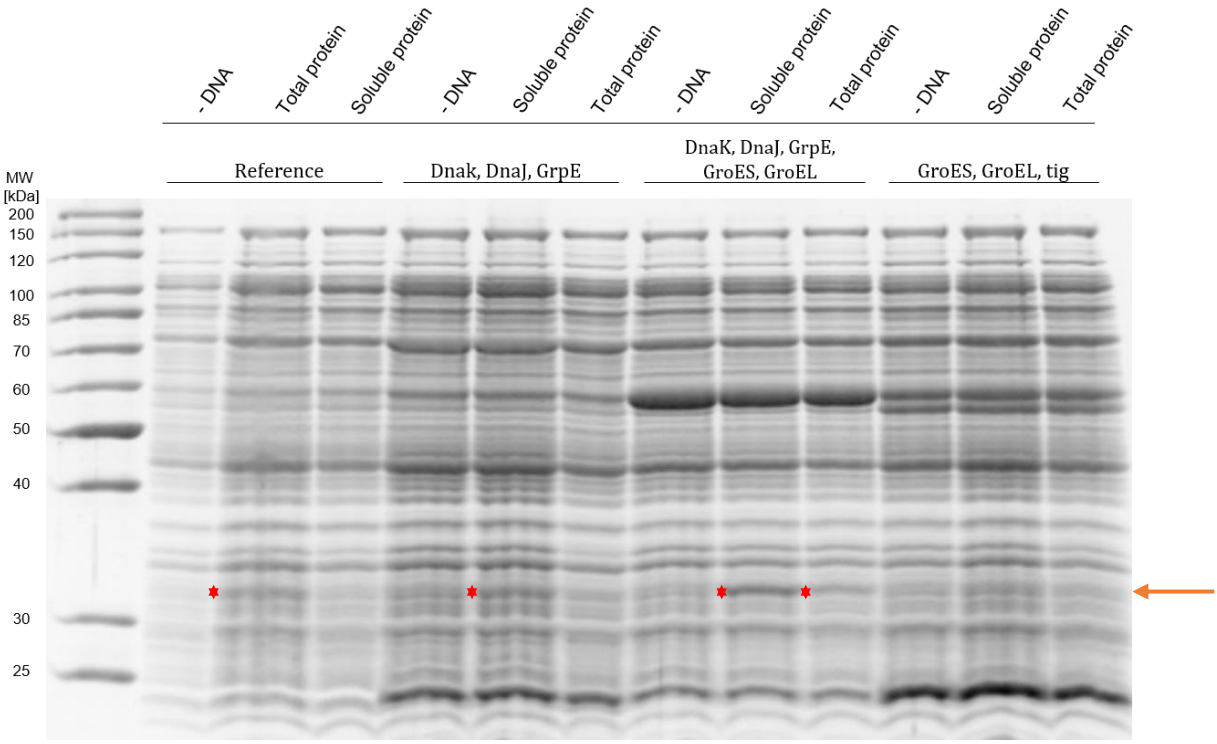

Figure S 8 Bspe-KDO (30.2 kDA) + Chaperones (DnaK, DnaK, GrpE; DnaK, DnaK, GrpE, GroES, GroEL; GroES, GroEL, tig)

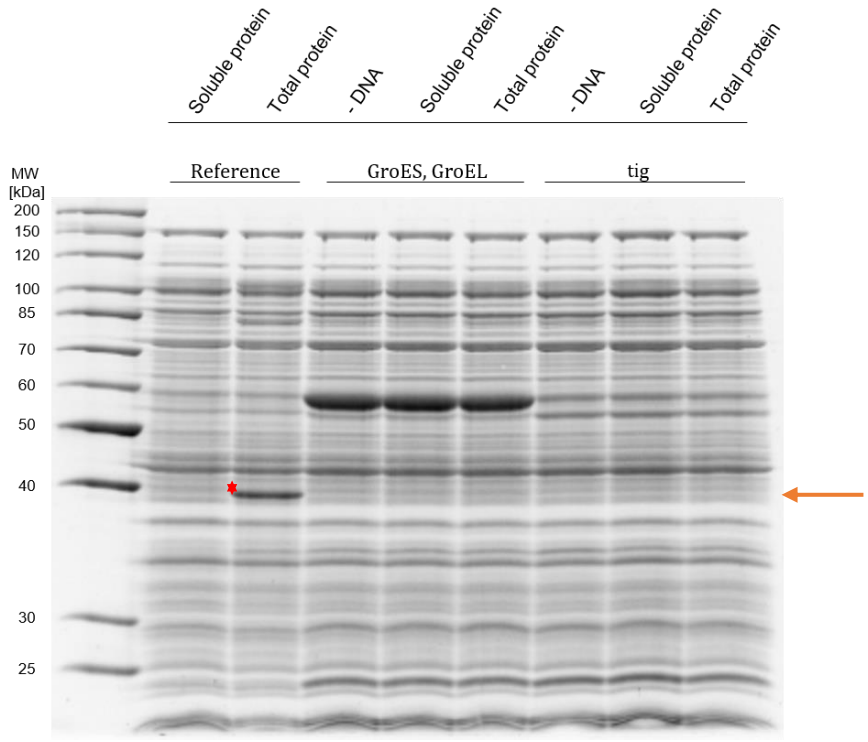

Figure S 9 Caci-KDO (40.0 kDA) + Chaperones (GroES, GroEL; tig)

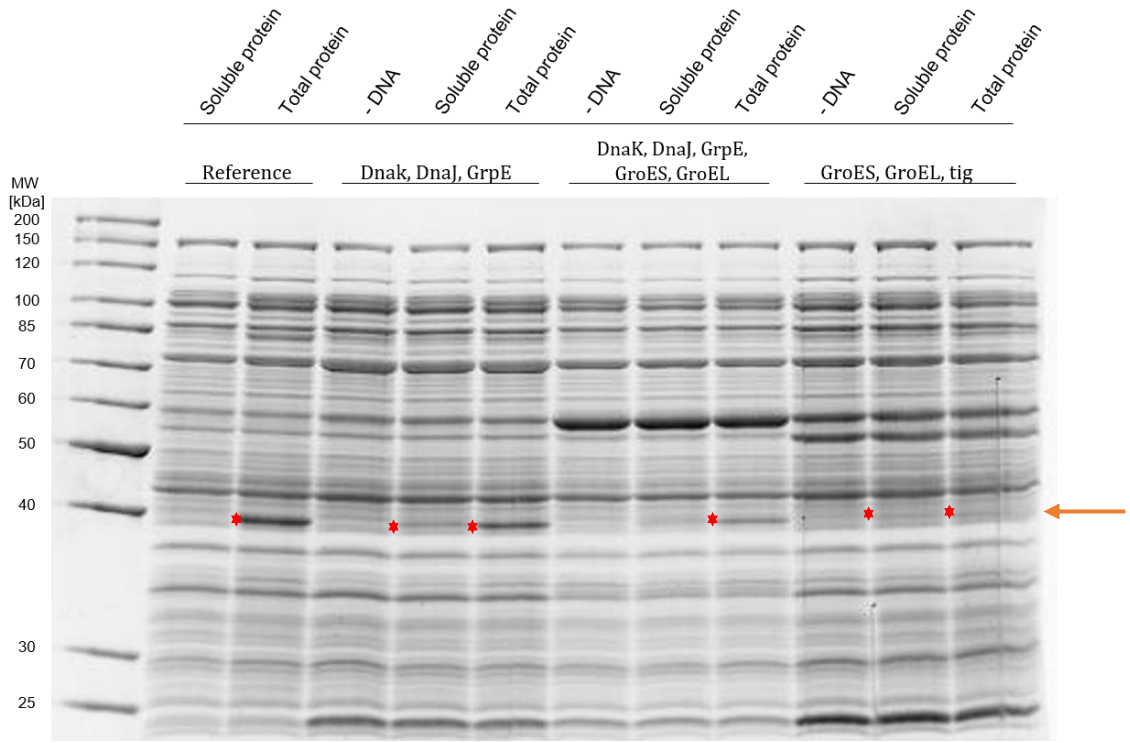

Figure S 10 Caci-KDO (40.0 kDA) + Chaperones (DnaK, DnaK, GrpE; DnaK, DnaK, GrpE, GroES, GroEL; GroES, GroEL, tig)

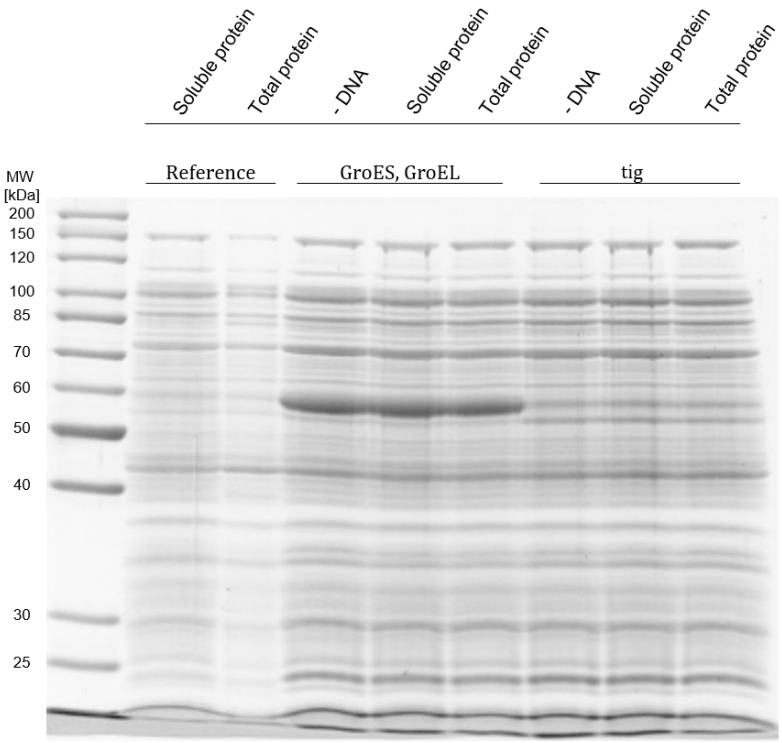

Figure S 11 Cpin-KDO (44.4 kDA) + Chaperones (GroES, GroEL; tig)

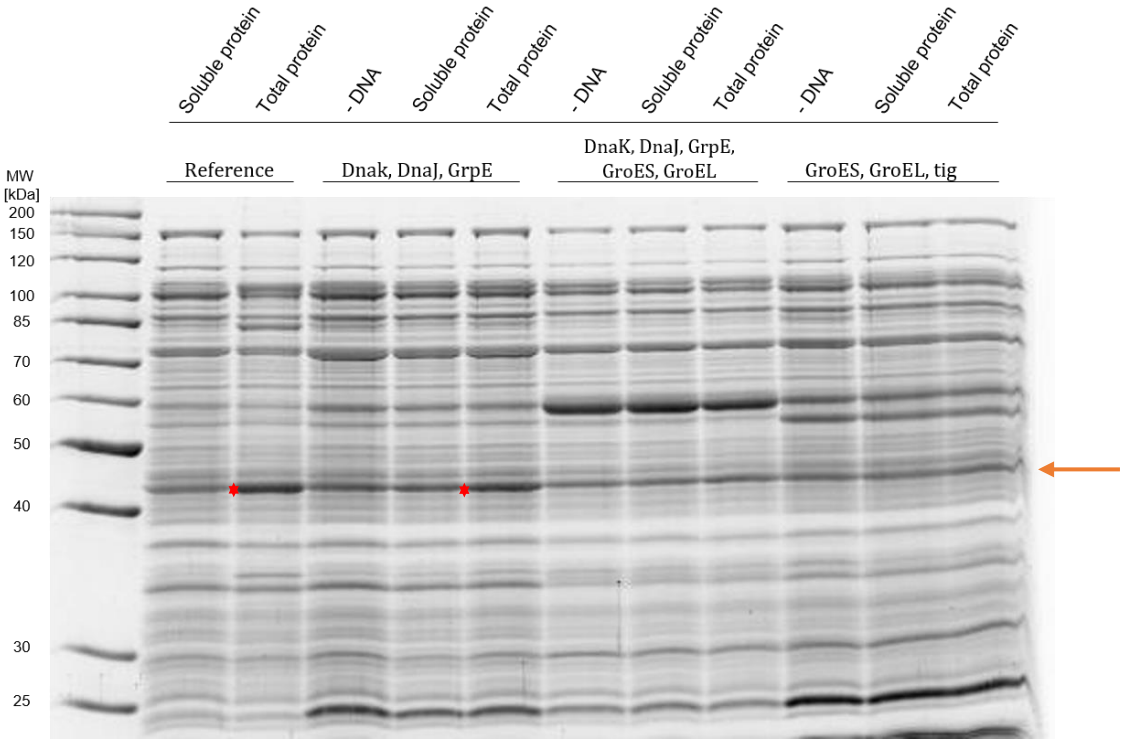

Figure S 12 Cpin-KDO (44.4 kDA) + Chaperones (DnaK, DnaK, GrpE; DnaK, DnaK, GrpE, GroES, GroEL; GroES, GroEL, tig)

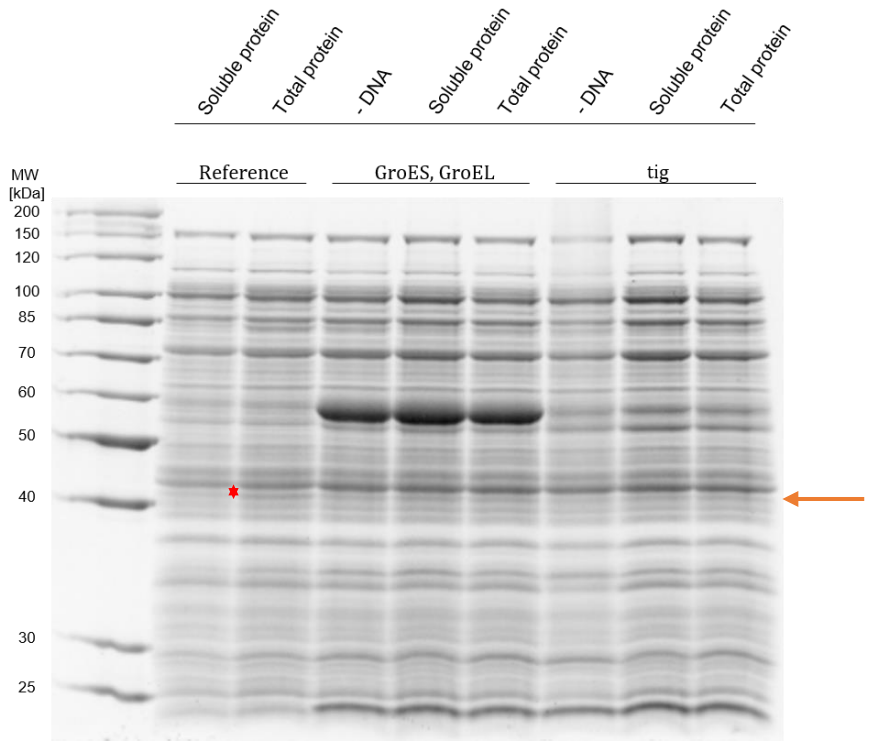

Figure S 13 Fjoh-KDO (42.8 kDA) + Chaperones (GroES, GroEL; tig)

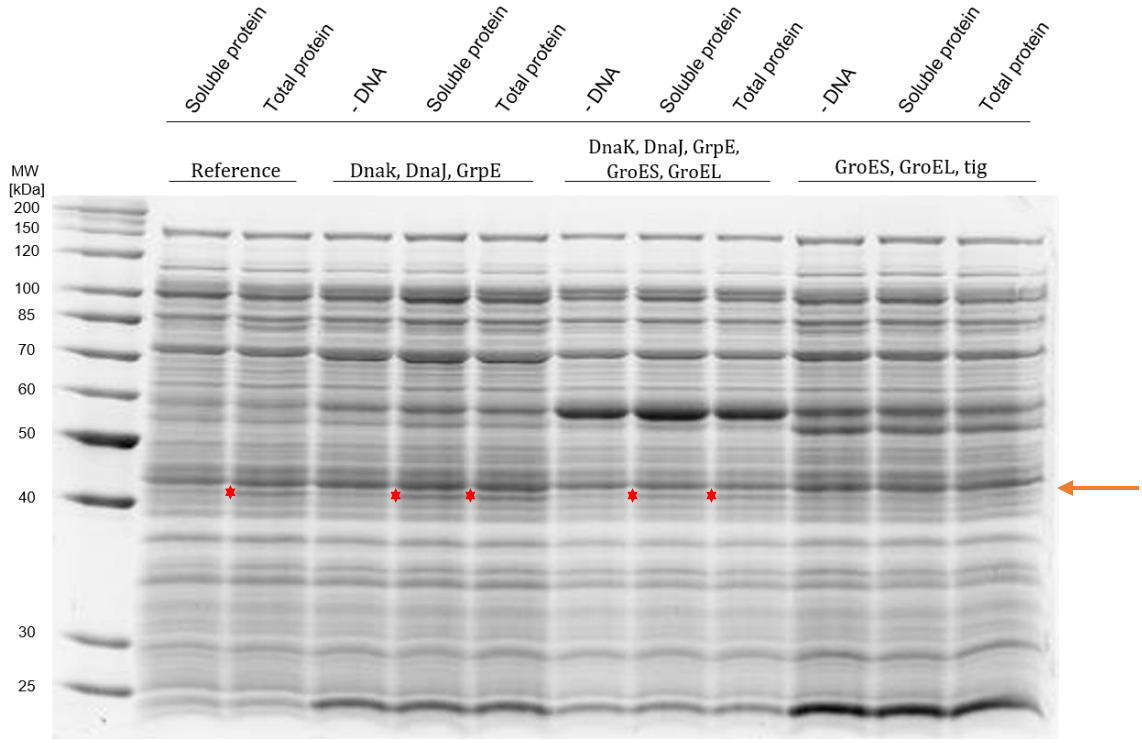

Figure S 14 Fjoh-KDO (42.8 kDA) + Chaperones (DnaK, DnaK, GrpE; DnaK, DnaK, GrpE, GroES, GroEL; GroES, GroEL, tig)

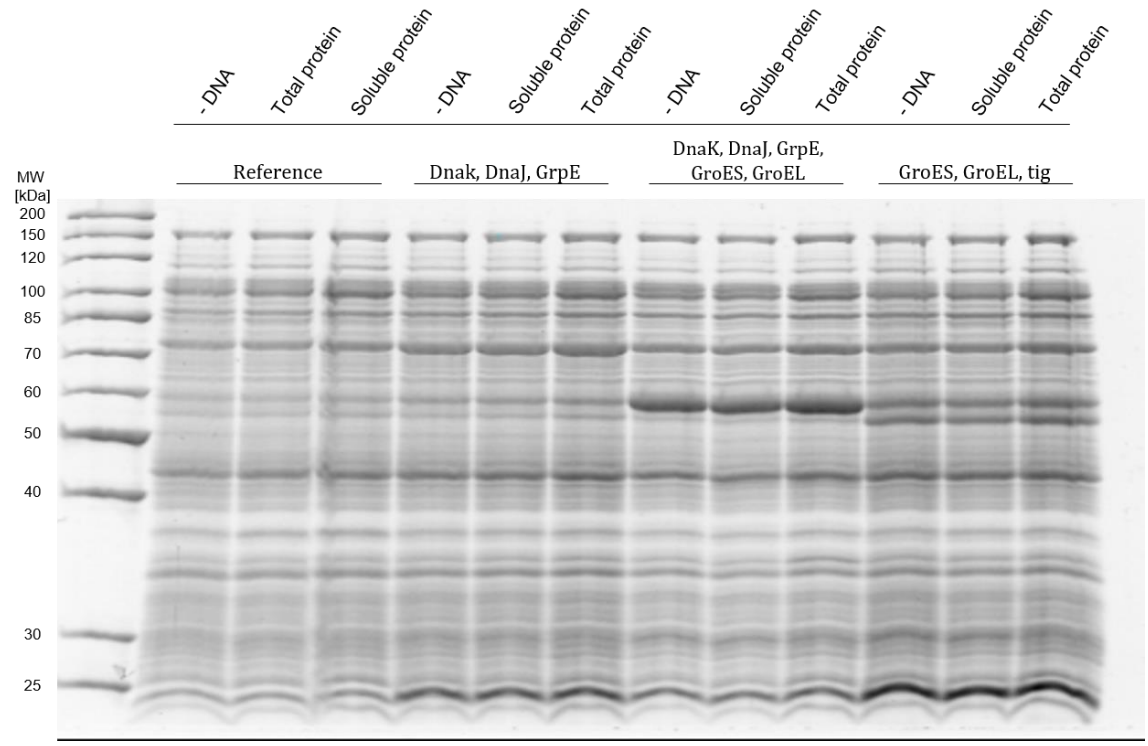

Figure S 15 Fspe-KDO (41.9 kDA) + Chaperones (DnaK, DnaK, GrpE; DnaK, DnaK, GrpE, GroES, GroEL; GroES, GroEL, tig)

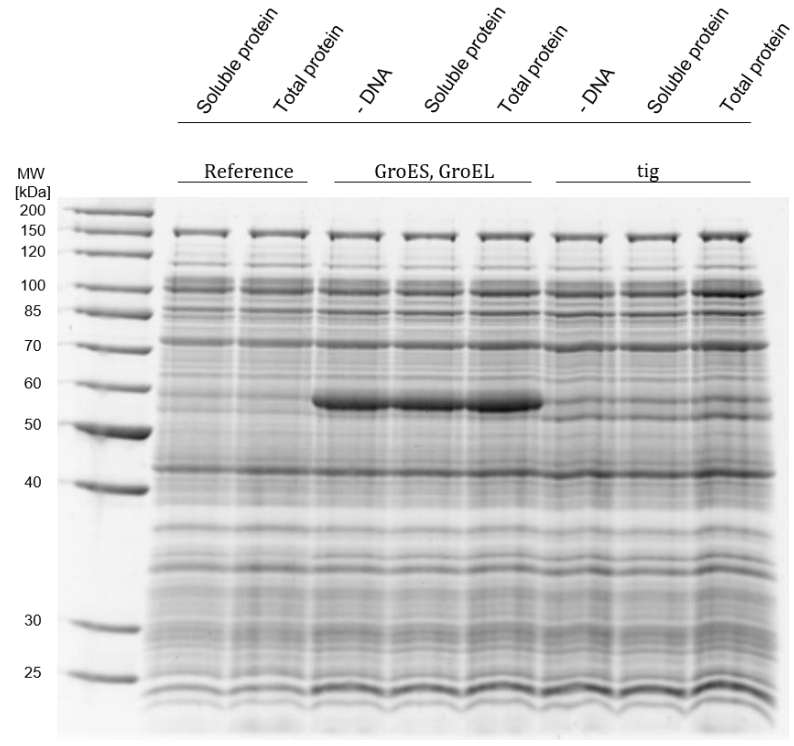

Figure S 16 Fspe-KDO (41.9 kDA) + Chaperones (GroES, GroEL; tig)

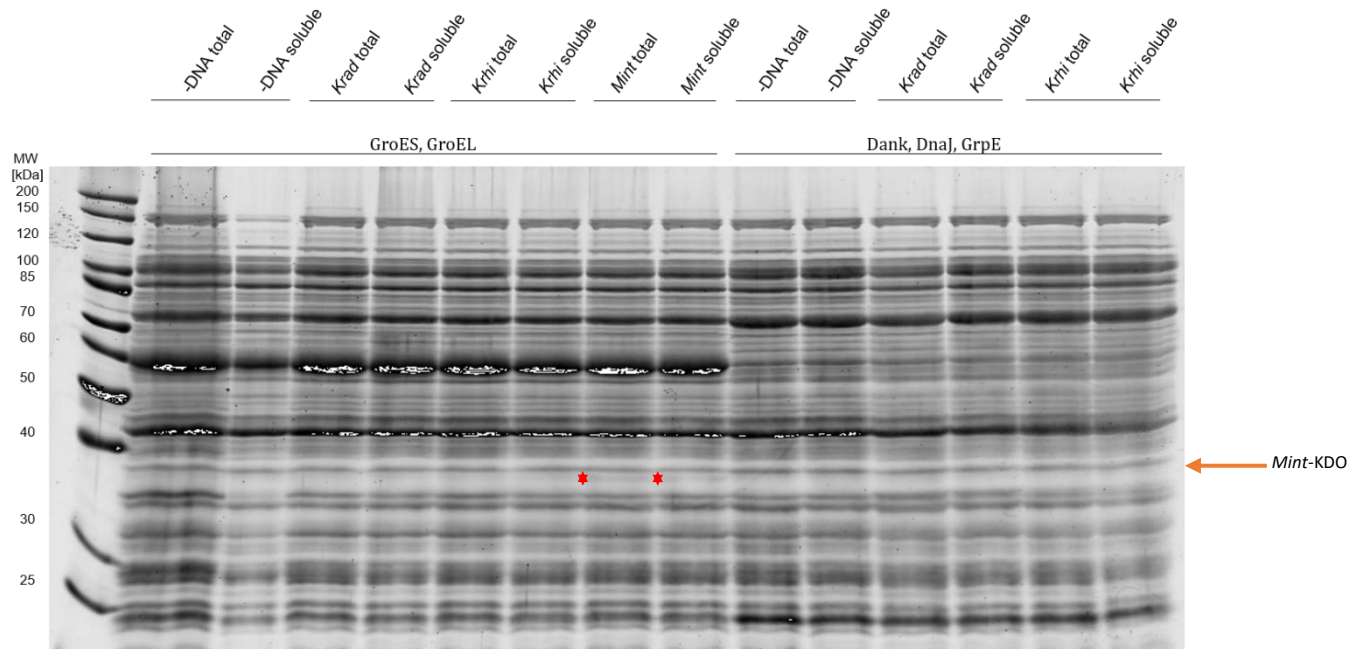

Figure S 17 Krad-(37.3 kDa), Krhi-(37.2 kDa), Mint-KDO (40.1 kDa) + Chaperones (DnaK, DnaK, GrpE; GroES, GroEL)

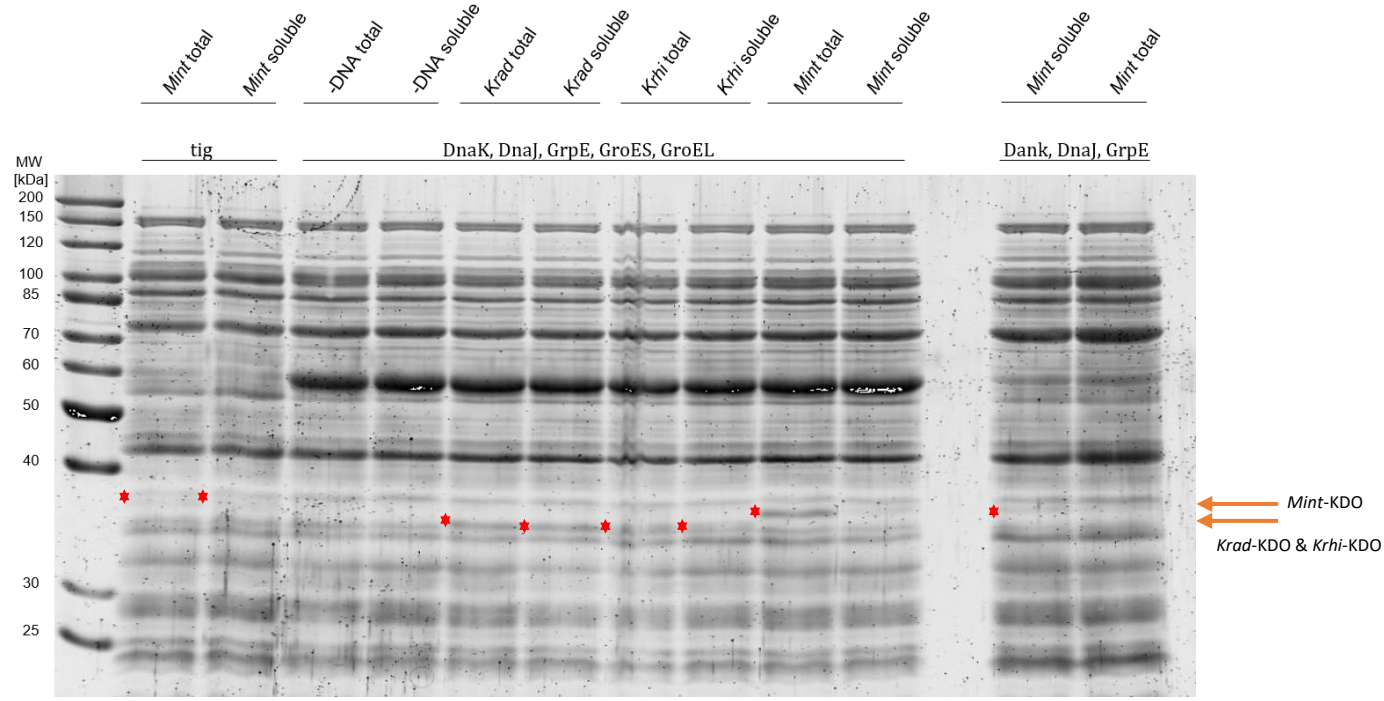

Figure S 18 Krad-(37.3 kDa), Krhi-(37.2 kDa), Mint-KDO (40.1 kDa) + Chaperones (DnaK, DnaK, GrpE, GroES, GroEL; DnaK, DnaJ, GrpE)

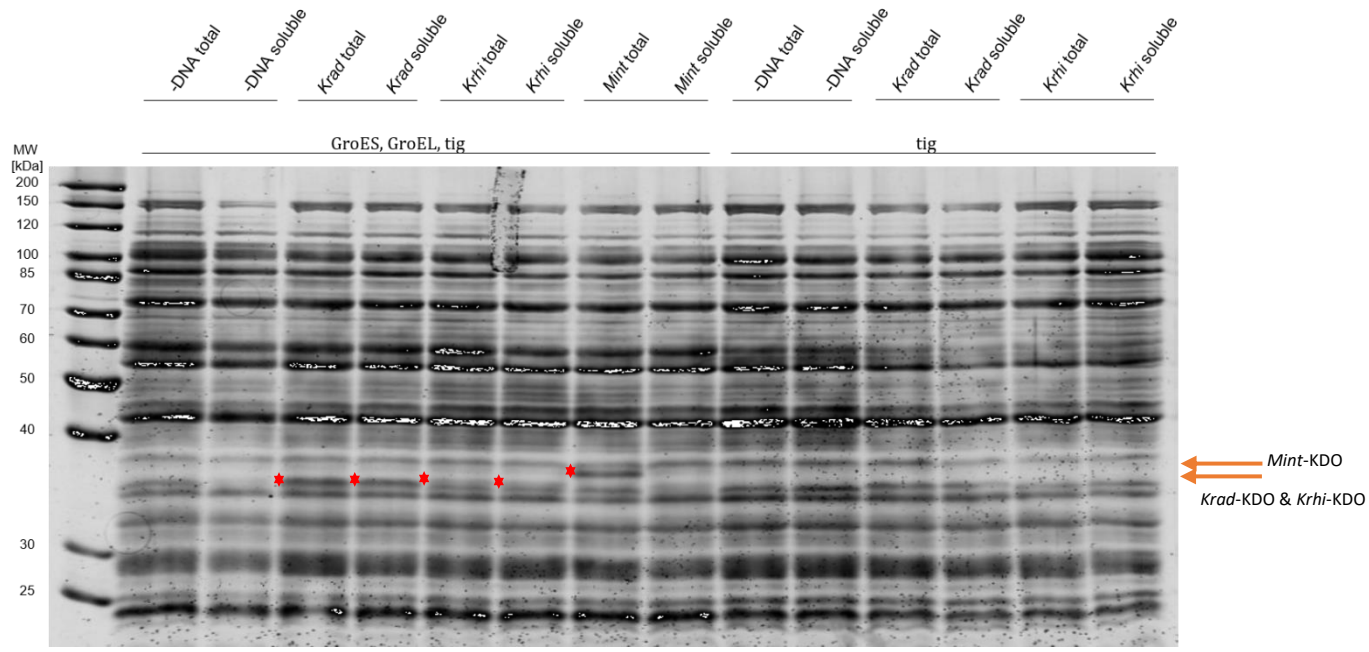

Figure S 19 Krad-(37.3 kDa), Krhi-(37.2 kDa), Mint-KDO (40.1 kDa) + Chaperones (GroES, GroEL, tig; tig)

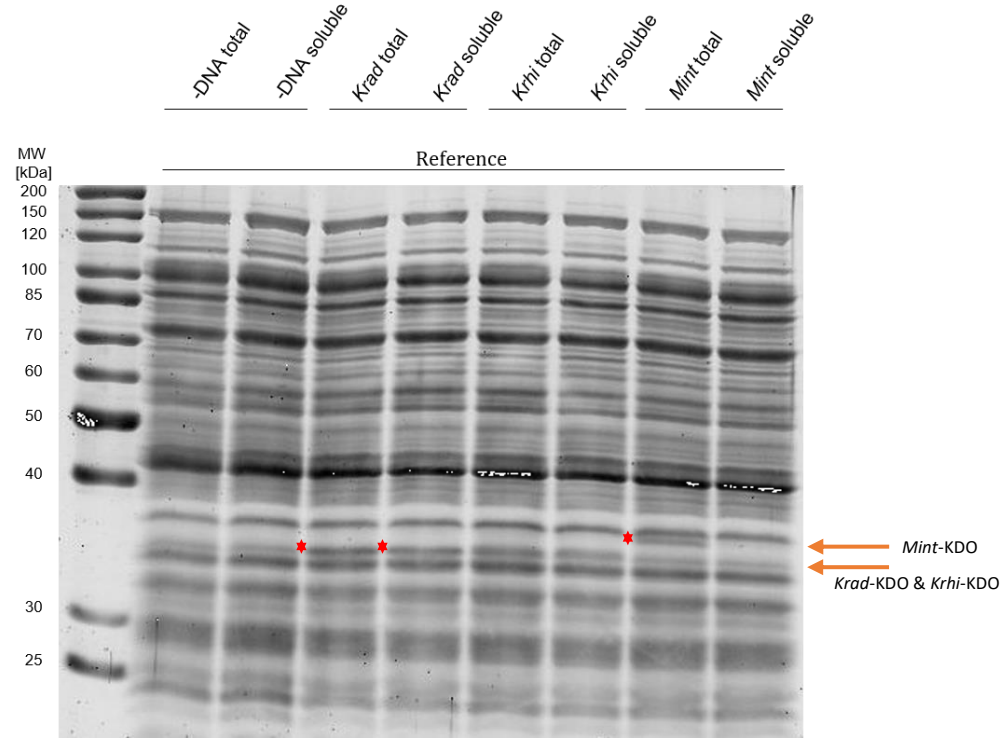

Figure S 20 Krad-(37.3 kDa), Krhi-(37.2 kDa), Mint-KDO (40.1 kDa) without chaperones

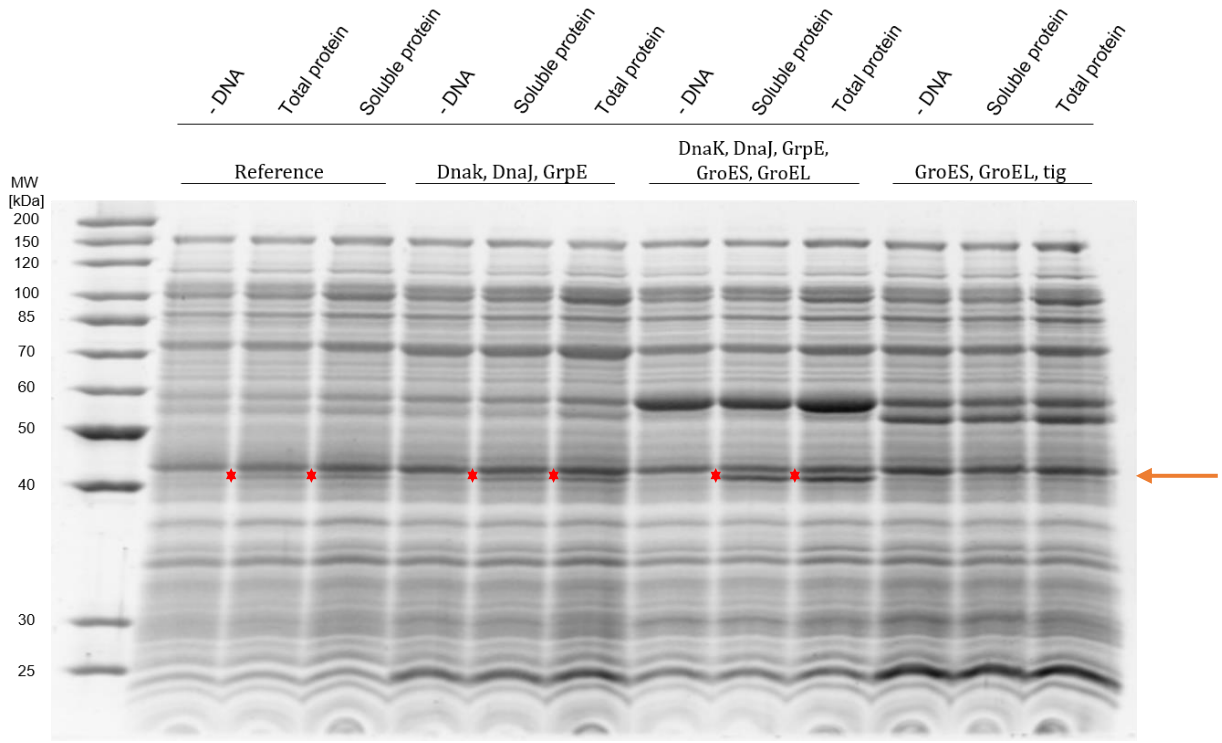

Figure S 21 Nkor-KDO (42.9 kDA) + Chaperones (DnaK, DnaK, GrpE; DnaK, DnaK, GrpE, GroES, GroEL; GroES, GroEL, tig)

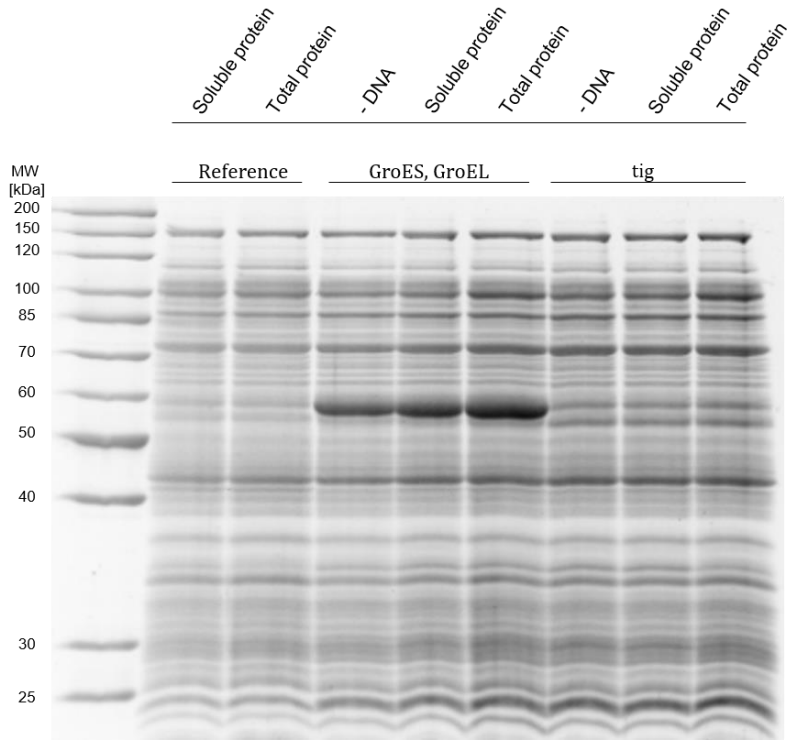

Figure S 22 Nkor-KDO (42.9 kDA) + Chaperones (GroES, GroEL; tig)

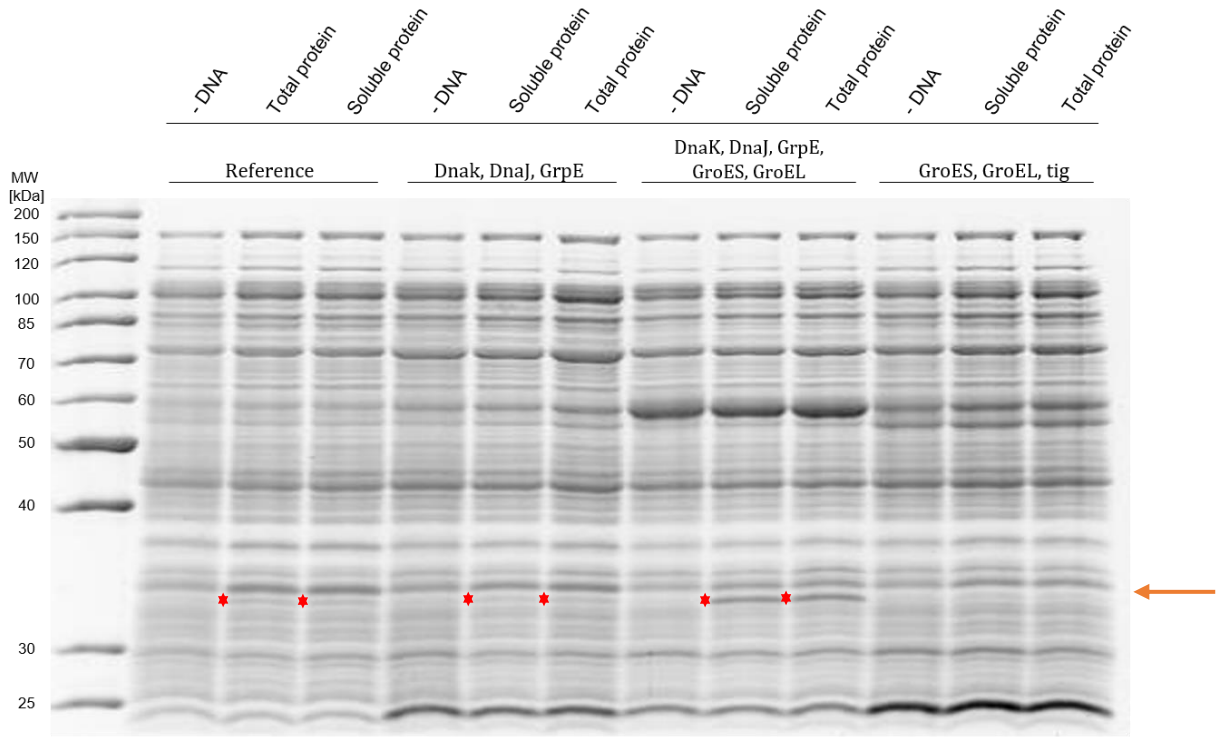

Figure S 23 Pbra-KDO (32.5 kDA) + Chaperones (DnaK, DnaK, GrpE; DnaK, DnaK, GrpE, GroES, GroEL; GroES, GroEL, tig)

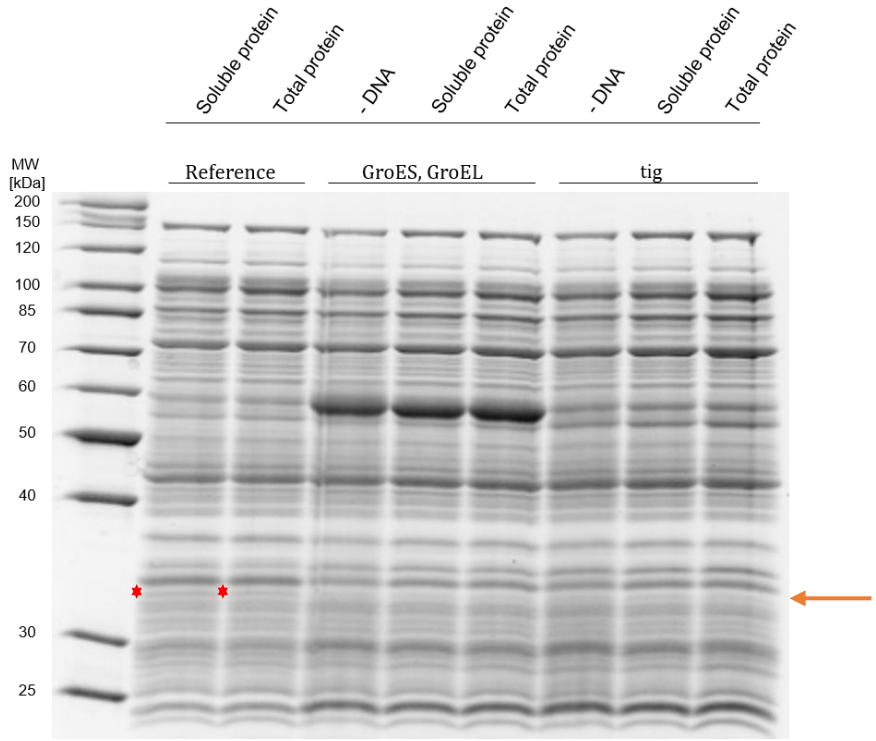

Figure S 24 Pbra-KDO (32.5 kDA) + Chaperones (GroES, GroEL; tig)

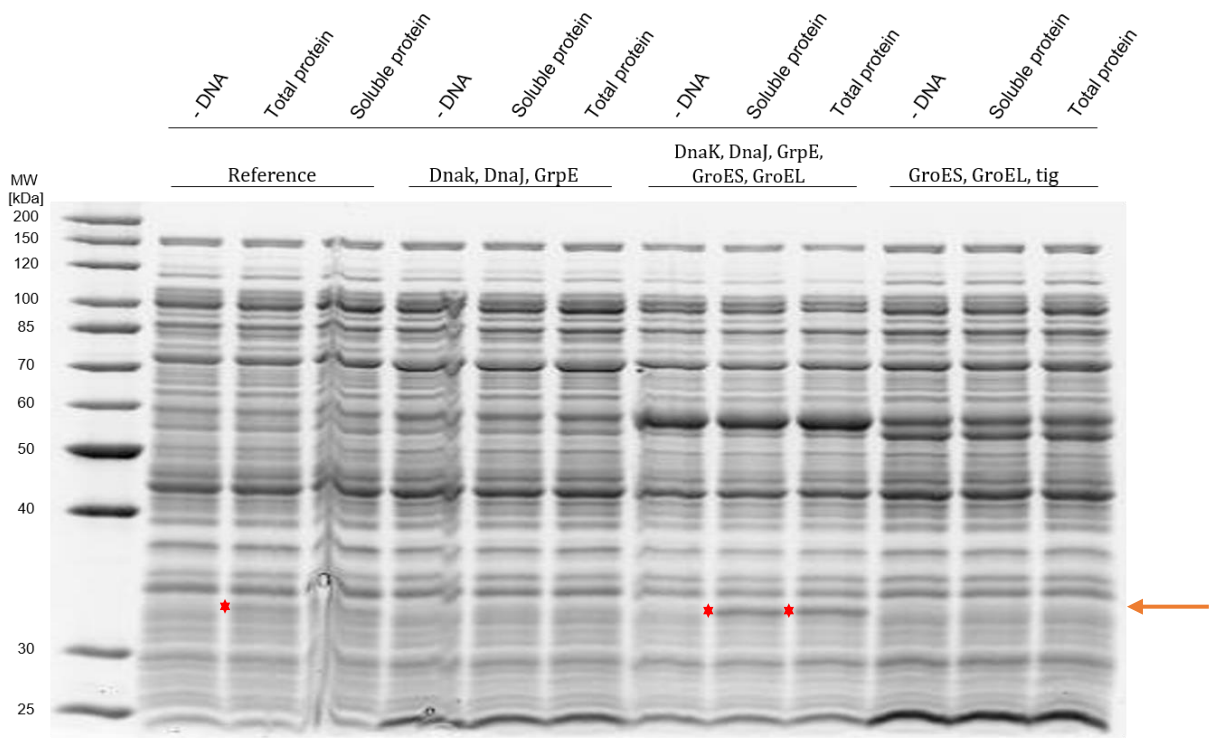

Figure S 25 Plum-KDO (31.8 kDA) + Chaperones (DnaK, DnaK, GrpE; DnaK, DnaK, GrpE, GroES, GroEL; GroES, GroEL, tig)

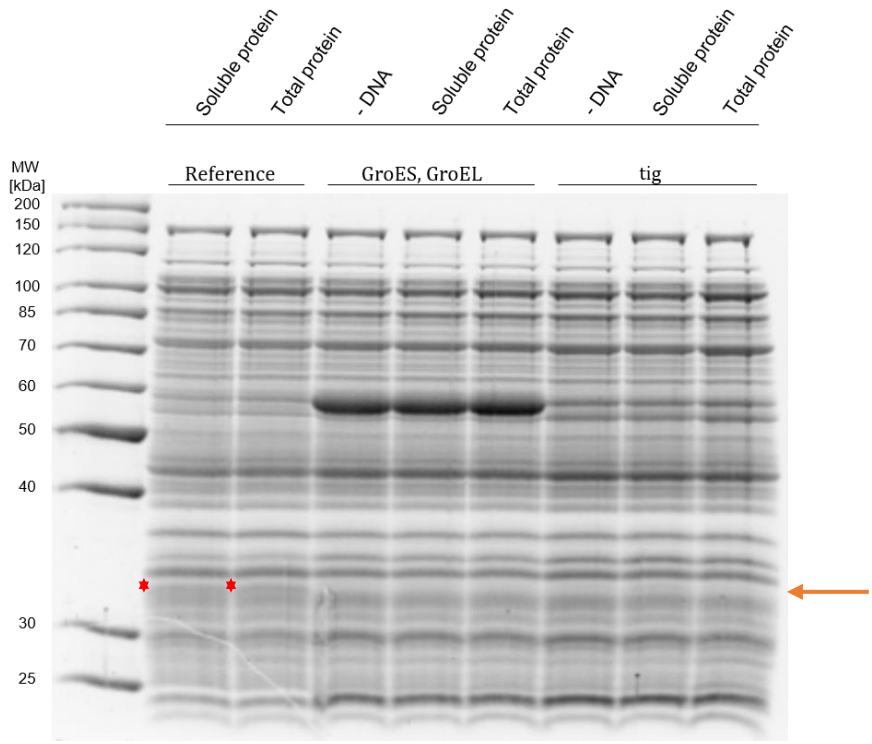

Figure S 26 Plum-KDO (31.8 kDA) + Chaperones (GroES, GroEL; tig)