

Supplementary Data

SUPPLEMENTARY FIG. S1. Amino acid alignment of Cas12a family members with SpCas9. Multiple sequence alignment of ErCa12a with homologous Cas proteins, including CRISPR-associated endonuclease Cas12b from *Alicyclobacillus acidoterrestris*, CRISPR-associated Endonuclease Cas9 from *Streptococcus pyogenes*, CRISPR-associated Endonuclease Cpf1 from *Acidaminococcus* sp. BV3L6, type V CRISPR-associated protein Cpf1 from *Lachnospiraceae* bacterium ND2006, and type V CRISPR-associated protein Cpf1 from *Francisella tularensis*. Six representative sequences were used for alignments with Clustal Omega webserver.


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AaCas12b  LERDNEEIALYFDYGAQHTG-----EFGGAK-----IQCRDQLA 489
SpCas9    YSEEVVKKKNYKOLLNAKLITQRKFDNLTKAERGLSELDKAGFIXOLWETRQIKK 929
AaCas12a  -----FNLHLYVNTGLFSFENLAKTSTI-----LNGQ-AG-----LFYKPKSRMRL-MA 798
ErCas12a  -----DLRHMVYLNLFSEENLKDIVK-----LNGE-AG-----LFFKSSKML-IT 757
LbCas12a  -----FNLHMYVNLDFDENHGGI-AG-----LSSG-AG-----LFWKASIKGDELVV 757
FnCas12a  -----FNLHLYMAGLDFENLQDVMVAG-----LNGE-AG-----LFYKASISF--K 842

AaCas12b  VMHRRRCARDVYLVSVRVQSQSEA----RGLRRR-----FYAAVFRLVGDHHRAPVHF 539
SpCas9    HVAAQLDSSR--NTKYDENDKL-IREVKVITLNSKLVSD-FRKFDFK-----V 975
AaCas12a  H--RLGKML--KXKLD-----QKTIIDTLYQELDQVYV-----H 831
ErCas12a  H--KKGSLV--KTYAAEKDOFGNIQI--VAKNIRENIYQELKRYFN-----D 801
LbCas12a  H--FANFIA--SKKFDN-----L-----V-----H 771
FnCas12a  H--FAKEAIA--KXKDN-----L-----V-----H 856

AaCas12b  DKLSDYLAEHDDKLGSEGLISLRVMSVDLGLRFSASISVTVVAKKDELKFN---- 593
SpCas9    REINNYHHAH--DAYL--NAVVTALIKKYKLEEFVYGDVYVDVRKMIKASEQEI 1029
ErCas12a  RLSHD-----LSDEARALLVIVTKVSHLIDR----- 861
FnCas12a  KSDKE-----LSDEAAKLNVVHHEAAMIVKDY--QFNGCKEDN 831
LbCas12a  -----L-----KKTTSYDQVYDK----- 786
FnCas12a  -----L-----KKEVVEYDVLIDK----- 871

AaCas12b  SKRVVFFF--FKGDN--LVAVHER--SQLLA-LPGTESKDLRAIREERQRTL 642
SpCas9    GKATAVFPYFNIMNFKTE--ITLANGEIRKRLIE-TNGET-----GEIVM 1074
AaCas12a  RFSKPPFPVPTLKYAANFSKFNQRY--NAVLEHPT--FIIIGI----- 906
ErCas12a  RYDYKVFPLMPTITKFKAN-KKGFINDRI--LQYIA-KEKDL--HVIGI----- 875
LbCas12a  RYEDVYELHPIAIKKCK-NIFKITEV--RVLLXDD-NF--YVIGI----- 830
FnCas12a  RYEDVYELHPIAIFKSS-GANKFDEI--NLLLEKANLV--HIIIGI----- 916

AaCas12b  RLRLTQAVYLRVRCSEDEVRRERSWAKLIEGVDAAN-NHMTED--VREAFVHEOK 698
SpCas9    DGGRFARV----FK-----LVSMQVRLVKK-TVQTDGFSKESILK 1113
AaCas12a  DRGENLLYITVDSK-----LDRRLMTIQ-----QFNGCKEDN 1045
ErCas12a  DRGENLLYVSVITDCGN-----IVSRSPNIVN-----GIDYQIKLQ 914
LbCas12a  DRGENLLYIVVVDGKGN-----IVQVYLAIEINNFGIRIKTDYHSLDK 877
FnCas12a  DRGENLLYVVDGKGN-----IKGDENI-----GDRMKTNYHDKLAA 959

AaCas12b  LKSLHGICSDHMDVYVESVRRVHRMCKQVRDWRKDVRSGLPKIRGVAKDVGGS 757
SpCas9    RNSDLIAKKNDDKKYK-----GFSPTVAVYLVVAVKVE 1150
AaCas12a  RKKRVAAAR-QAHSVV--G-----TIKDLRQGLVQVHEIV 979
ErCas12a  QGAGQIAR-KMKET--G-----KIKIEEGYLVVHEIS 948
LbCas12a  KKKRFEAR-QNMTSI--E-----NIEELMAGYIQVVKKIC 911
FnCas12a  IKKDDGAK-KDKKI--N-----NIEELMAGYIQVVKKIC 993

AaCas12b  IEQIEYLERQYKFLKSWFFKVSQVIRAEEKSRFAITLREHIDHAKEDRKKKADDT 816
SpCas9    K-----FKS-----FKLKVKEELG 1167
AaCas12a  D-----LMIHQAVV 989
ErCas12a  K-----MVIKNNAII 958
LbCas12a  E-----LVEKNDVAVI 921
FnCas12a  K-----LVIEENNAIV 1003

AaCas12b  INRLGVVYALDRGKQKVVAKYPPCGLILLDELSEVQFNHNR----FFSENNO-LMO 869
SpCas9    INRER--SFKKNTDF-LKAKGVKVKDLIIILPKYSLFLENGRKRRLHSAAGELG 1223
AaCas12a  VLENLNVGPKSKKGI-AKAVYQDFKMLIDLNLCLVLYYH-----AEKVGGLV 1041
ErCas12a  VMEDLSVGFKPKKFKV--EQQVYKFTMLINLNLVLFKIS-----TENGGV 1009
LbCas12a  ALDELNSGFPNSKVKV--EQQVYKFKMLIDLNLVLFKIS-----FCATGGV 972
FnCas12a  VFDLNSGFPNSKVKV--EQQVYKFKMLIDLNLVLFKIS-----FDKGGV 1054

AaCas12b  MSRRVYVEINLQAVHDLVVENH--AAFSSPARRGAGIRCRRVARCTQEHY 925
SpCas9    NRIALH-----S--KYVNFMLARHVKLGSFDNEDK-----QLFV 1259
AaCas12a  YQLDQFTSFAKHG--TSSFLFVVPAPYTSXIDPLG-----FV 1079
ErCas12a  YQLYIDDKLKNVG--NOCRCIFYVPAAYTSXIDPTFG-----FV 1047
LbCas12a  YQITNKFEFKSHS--TONGIFYIPAWLTSXIDPSPG-----FV 1010
FnCas12a  YQLAFRFETFKKHG--KGTGIYVVPAGTSSXIDPSPG-----FV 1092

AaCas12b  EFPVWLNKPVVENTLDACFLRADDLITTEGEIPVSRFSA--EGDTHQINADLN 979
SpCas9    EOKKHYLDEI-----EQIEFSKRVILANLID-KVLSAN 1295
AaCas12a  DFPVWLNKPN-----ESRKHFLERFDPLHVDKTDPT-ILHFKM 1119
ErCas12a  NIFKPKDLTVD-----AKREFIKRFDISIRDSKMLF-CFDPV 1086
LbCas12a  NLLKPKDTSIAL-----DSKFFISFDRIMVYVDEDF-EFALDK 1049
FnCas12a  NQIYKLESV-----KQSEFESFKICNLSKQVY-EFDPK 1131

AaCas12b  AONLQQRLLNSDPDISQTRIRCDHGEVDDGE----LVLIERLGRKADGSKVKVFT 1032
SpCas9    KRRD-----KPI-----RQEAENIHLTLTLNLA 1320
AaCas12a  RNLFSQ-----RLPGYMAWDIVPEKNETOPDAKGTFTYIACKIVYIEHR--F 1168
ErCas12a  NFIT-----QNTVMKSSHS--VYTGVMIKRRVYVGR--F 1118
LbCas12a  NFSR-----TDADYIKKKK-----LYSGNIRI-ERKVK--K 1079
FnCas12a  NFGD-----KA--AKKKT-----IASGSLIN-RRNSD--K 1159

AaCas12b  NTGVTVYREKCKKRRKVAQEKSE--EDELLEVEAEAR-----EKSVVLM 1079
SpCas9    FAARKYF-----DTT-----IRKRYTSKVEVDA--LH 1349
AaCas12a  TE--RYRRLYANELIALLEEKGVFRDGSNILELLENDSHAIDTMVALIRSVLW 1225
ErCas12a  SE--SDTIDTKDMEKLEMTDINWRDHDLRDID--YEIVQHIFERLWV 1172
LbCas12a  NHVDFWREVCSTSAKELFNKYGNVQGD--IRKLCQSGKAFSSSMAHLSLML 1137
FnCas12a  HNNHDFRIVYTKLEKLEKDYSEVHGECIKAAICSESKKFTAKLTSVNTILO 1218

AaCas12b  DSS-----GINRRNWT--RQKFNHVNQRIEG-----VLV 1109
SpCas9    QIIGLYERIDLS-----QLGDD----- 1368
AaCas12a  VNAA--TGDDXINSPVRDLNGVCFDSRFQ--NPEWMDADANGAVHIALKGLL 1277
ErCas12a  NLSLELDRDYDRLESPVLENMIFVDSAKA--GDALPKDADANGAVICIALKGL 1227
LbCas12a  NITGR--DQVPLISPVKNSDGFYDSRNYEAQENALPKNADANGAVHIALKGL 1194
FnCas12a  NKKK--ELEDVILIPVADVNGNFFDSRQA--KNNMQDADANGAVHIALKGL 1270

AaCas12b  KOISR-----VFLQDAGACNAGDI----- 1129
SpCas9    NHLES--KDLQNRISNOINLJAEELR-- 1306
ErCas12a  KQITENKEDKFSKIKLISKQWTFIDNRYL 1262
LbCas12a  GQFKA--EDKLDVKKIASEKWLVAATSVKH 1227
FnCas12a  GRINN--QRE--KLNVLKKEVFEVQNRN 1300

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SUPPLEMENTARY FIG. S1. (Continued).