

Supplementary Figure 1: Amino acid sequences of AsLOV2J α and fusions with JIP used with Clustal Omega¹ alignment.

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>AsLOV2J $\alpha$ 
LATTLERIEKNFVITDPRLPDNPIIFASDSFLQLTEYSREEILGRNCRFLQGPETDRATVRKIRDAIDNQLTEVTVQLINYTEKSGKKFWNLFHLQ
PMRDQKGDVQYFIGVQLDGTGTEHVRDAAEREGVMLIKKTAENIDEAAKEL*

>AsLOV2J $\alpha$ -JIP11 = "OptoJNKi"
LATTLERIEKNFVITDPRLPDNPIIFASDSFLQLTEYSREEILGRNCRFLQGPETDRATVRKIRDAIDNQLTEVTVQLINYTEKSGKKFWNLFHLQ
PMRDQKGDVQYFIGVQLDGTGTEHVRDAAEREGVMLIKKTAENIDEAAKELSRPKRPTTLNLF*

>AsLOV2J $\alpha$ -JIP11.dsm (C450A)
LATTLERIEKNFVITDPRLPDNPIIFASDSFLQLTEYSREEILGRNARFLQGPETDRATVRKIRDAIDNQLTEVTVQLINYTEKSGKKFWNLFHLQ
PMRDQKGDVQYFIGVQLDGTGTEHVRDAAEREGVMLIKKTAENIDEAAKELSRPKRPTTLNLF*

>AsLOV2J $\alpha$ -JIP11.lsm (I539E)
LATTLERIEKNFVITDPRLPDNPIIFASDSFLQLTEYSREEILGRNCRFLQGPETDRATVRKIRDAIDNQLTEVTVQLINYTEKSGKKFWNLFHLQ
PMRDQKGDVQYFIGVQLDGTGTEHVRDAAEREGVMLIKKTAENIDEAAKELSRPKRPTTLNLF*

>AsLOV2J $\alpha$ F509R-JIP11.dsm (C450A)
LATTLERIEKNFVITDPRLPDNPIIFASDSFLQLTEYSREEILGRNARFLQGPETDRATVRKIRDAIDNQLTEVTVQLINYTEKSGKKFWNLFHLQ
PMRDQKGDVQYRIGVQLDGTGTEHVRDAAEREGVMLIKKTAENIDEAAKELSRPKRPTTLNLF*

>AsLOV2J $\alpha$ F509R-JIP11.lsm (I539E)
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PMRDQKGDVQYRIGVQLDGTGTEHVRDAAEREGVMLIKKTAENIDEAAKELSRPKRPTTLNLF*

>AsLOV2J $\alpha$ -JIP10.dsm (C450A)
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PMRDQKGDVQYFIGVQLDGTGTEHVRDAAEREGVMLIKKTAENIDEAAKELSRPKRPTTLNLF*

>AsLOV2J $\alpha$ -JIP10.lsm (I539E)
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>AsLOV2J $\alpha$ -JIP12.dsm (C450A)
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PMRDQKGDVQYFIGVQLDGTGTEHVRDAAEREGVMLIKKTAENIDEAAKELSRPKRPTTLNLF*

>AsLOV2J $\alpha$ -JIP12.lsm (I539E)
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>AsLOV2J $\alpha$ -JIP13.dsm (C450A)
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>AsLOV2J $\alpha$ -JIP13.lsm (I539E)
LATTLERIEKNFVITDPRLPDNPIIFASDSFLQLTEYSREEILGRNCRFLQGPETDRATVRKIRDAIDNQLTEVTVQLINYTEKSGKKFWNLFHLQ
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CLUSTAL O(1.2.4) multiple sequence alignment

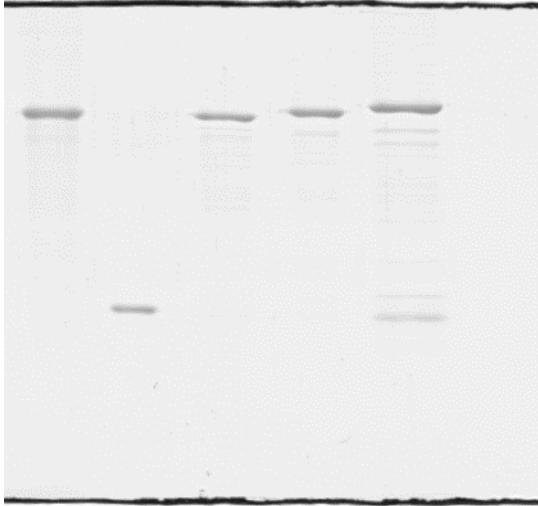
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AsLOV2Jα-JIP11.dsm(C450A) LATTLERIEKNFVITDPRLPDNPIIFASDSFLQLTEYSREEILGRNCRFLQGPETDRATV
AsLOV2Jα-JIP11.lsm(I539E) LATTLERIEKNFVITDPRLPDNPIIFASDSFLQLTEYSREEILGRNCRFLQGPETDRATV
AsLOV2JαF509R-JIP11.dsm(C450A) LATTLERIEKNFVITDPRLPDNPIIFASDSFLQLTEYSREEILGRNCRFLQGPETDRATV
AsLOV2JαF509R-JIP11.lsm(I539E) LATTLERIEKNFVITDPRLPDNPIIFASDSFLQLTEYSREEILGRNCRFLQGPETDRATV
AsLOV2Jα-JIP10.dsm(C450A) LATTLERIEKNFVITDPRLPDNPIIFASDSFLQLTEYSREEILGRNCRFLQGPETDRATV
AsLOV2Jα-JIP10.lsm(I539E) LATTLERIEKNFVITDPRLPDNPIIFASDSFLQLTEYSREEILGRNCRFLQGPETDRATV
AsLOV2Jα-JIP12.dsm(C450A) LATTLERIEKNFVITDPRLPDNPIIFASDSFLQLTEYSREEILGRNCRFLQGPETDRATV
AsLOV2Jα-JIP12.lsm(I539E) LATTLERIEKNFVITDPRLPDNPIIFASDSFLQLTEYSREEILGRNCRFLQGPETDRATV
AsLOV2Jα-JIP13.dsm(C450A) LATTLERIEKNFVITDPRLPDNPIIFASDSFLQLTEYSREEILGRNCRFLQGPETDRATV
AsLOV2Jα-JIP13.lsm(I539E) LATTLERIEKNFVITDPRLPDNPIIFASDSFLQLTEYSREEILGRNCRFLQGPETDRATV
*****

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AsLOV2Jα-JIP11    RKIRDAIDNQTEVTVQLINITYTKSGKKFWNLFHLQPMRDQKGDVQYFIGVQLDGTGTEHRDA
AsLOV2Jα-JIP11.dsm(C450A) RKIRDAIDNQTEVTVQLINITYTKSGKKFWNLFHLQPMRDQKGDVQYFIGVQLDGTGTEHRDA
AsLOV2Jα-JIP11.lsm(I539E) RKIRDAIDNQTEVTVQLINITYTKSGKKFWNLFHLQPMRDQKGDVQYFIGVQLDGTGTEHRDA
AsLOV2JαF509R-JIP11.dsm(C450A) RKIRDAIDNQTEVTVQLINITYTKSGKKFWNLFHLQPMRDQKGDVQYRIGVQLDGTGTEHRDA
AsLOV2JαF509R-JIP11.lsm(I539E) RKIRDAIDNQTEVTVQLINITYTKSGKKFWNLFHLQPMRDQKGDVQYRIGVQLDGTGTEHRDA
AsLOV2Jα-JIP10.dsm(C450A) RKIRDAIDNQTEVTVQLINITYTKSGKKFWNLFHLQPMRDQKGDVQYFIGVQLDGTGTEHRDA
AsLOV2Jα-JIP10.lsm(I539E) RKIRDAIDNQTEVTVQLINITYTKSGKKFWNLFHLQPMRDQKGDVQYFIGVQLDGTGTEHRDA
AsLOV2Jα-JIP12.dsm(C450A) RKIRDAIDNQTEVTVQLINITYTKSGKKFWNLFHLQPMRDQKGDVQYFIGVQLDGTGTEHRDA
AsLOV2Jα-JIP12.lsm(I539E) RKIRDAIDNQTEVTVQLINITYTKSGKKFWNLFHLQPMRDQKGDVQYFIGVQLDGTGTEHRDA
AsLOV2Jα-JIP13.dsm(C450A) RKIRDAIDNQTEVTVQLINITYTKSGKKFWNLFHLQPMRDQKGDVQYFIGVQLDGTGTEHRDA
AsLOV2Jα-JIP13.lsm(I539E) RKIRDAIDNQTEVTVQLINITYTKSGKKFWNLFHLQPMRDQKGDVQYFIGVQLDGTGTEHRDA
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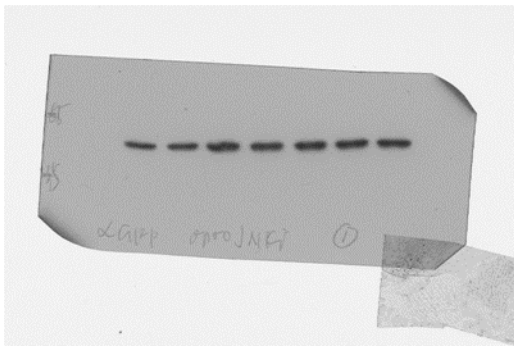
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AsLOV2Jα-JIP11.dsm(C450A) AREGVMLIKKTAENIDEAAKELSRPKRPTTLNLF*--
AsLOV2Jα-JIP11.lsm(I539E) AREGVMLIKKTAENEDEAAKELSRPKRPTTLNLF*--
AsLOV2JαF509R-JIP11.dsm(C450A) AREGVMLIKKTAENIDEAAKELSRPKRPTTLNLF*--
AsLOV2JαF509R-JIP11.lsm(I539E) AREGVMLIKKTAENEDEAAKELSRPKRPTTLNLF*--
AsLOV2Jα-JIP10.dsm(C450A) AREGVMLIKKTAENIDEAAKELSRPKRPTTLNLF*--
AsLOV2Jα-JIP10.lsm(I539E) AREGVMLIKKTAENEDEAAKELSRPKRPTTLNLF*--
AsLOV2Jα-JIP12.dsm(C450A) AREGVMLIKKTAENIDEAAKELSRPKRPTTLNLF*--
AsLOV2Jα-JIP12.lsm(I539E) AREGVMLIKKTAENEDEAAKELSRPKRPTTLNLF*--
AsLOV2Jα-JIP13.dsm(C450A) AREGVMLIKKTAENIDEAAKELSRPKRPTTLNLF*--
AsLOV2Jα-JIP13.lsm(I539E) AREGVMLIKKTAENEDEAAKELSRPKRPTTLNLF*--
*****
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Supplementary Figure 2: Whole gel and film lengths for images in Fig. 2

A.



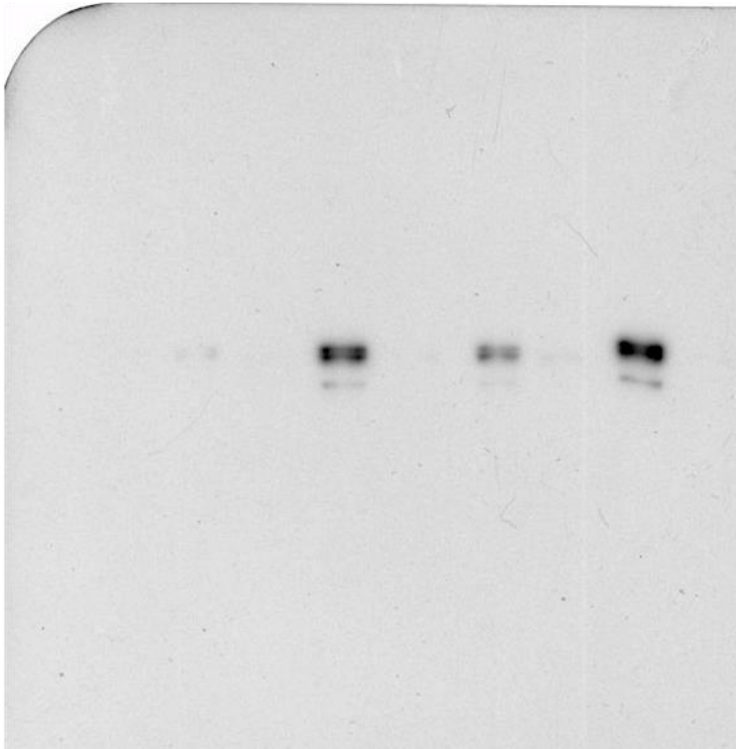
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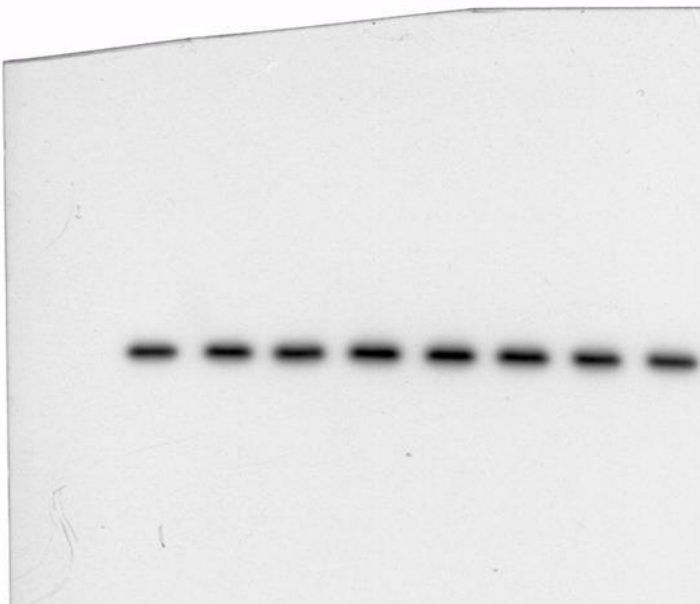
A. Whole length of gel shown corresponding to Fig. 2A

B. Whole film of blot corresponding to Fig. 2E

Supplementary Figure 3: Whole film lengths for images in Fig. 4
A



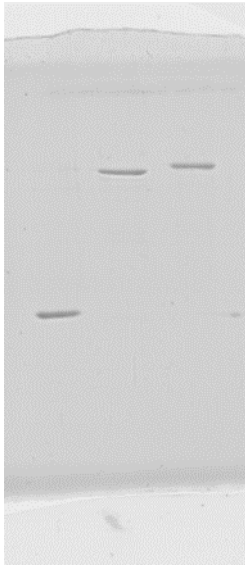
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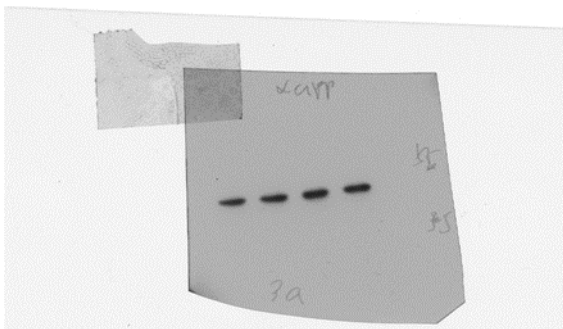
A. Film of whole length of blot shown in Fig. 5A (Phospho-Serine 73 c-Jun blot)
B. Film of whole length of blot shown in Fig. 5B (β -actin blot)

Supplementary Figure 4: Whole length of gels and films for Fig. 10

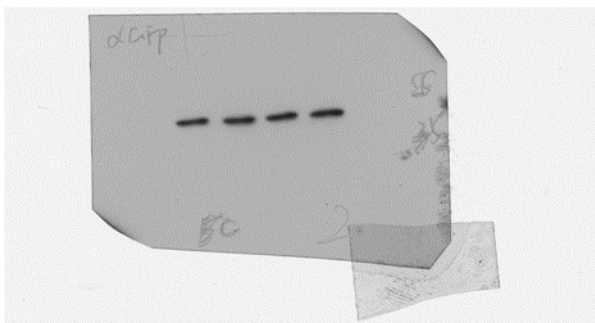
A.



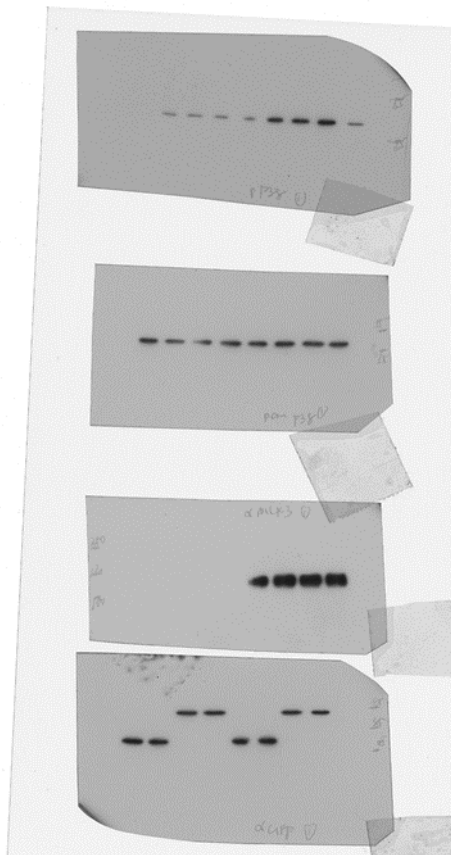
B.



C.



D.



A. Whole length of gel corresponding to Fig. 10B. Lane 1 is GST, not shown in Fig. 10B.

B. Whole film corresponding to Fig. 10H, OptoJNKi3-13F.lsm

C. Whole film corresponding to Fig. 10H, OptoJNKi5-13F.dsm

D. Whole films corresponding to Fig. 10I, from top: phospho-p38, pan-p38, MLK3, GFP.

>Optop38i(5-13F).dsm
LATTLERIEKNFVITDPRLPDNPIIFASDSFQLTEYSREEILGRNARFLQGPETDRATVRKIRDADIDNQTEVTVQLINITYKSGKKFWNLFHLQPMRDQKGDVQYFI
GVQLDGTEHVRDAAEREGVMLIKKTAENIDEAAKELSRKKDLRISCMF*

>Optop38i(5-13F).lsm
LATTLERIEKNFVITDPRLPDNPIIFASDSFQLTEYSREEILGRNCRFLQGPETDRATVRKIRDADIDNQTEVTVQLINITYKSGKKFWNLFHLQPMRDQKGDVQYFI
GVQLDGTEHVRDAAEREGVMLIKKTAENIDEAAKELSRKKDLRISCMF*

>Optop38i(5-13L).dsm
LATTLERIEKNFVITDPRLPDNPIIFASDSFQLTEYSREEILGRNARFLQGPETDRATVRKIRDADIDNQTEVTVQLINITYKSGKKFWNLFHLQPMRDQKGDVQYFI
GVQLDGTEHVRDAAEREGVMLIKKTAENIDEAAKELSRKKDLRISCLM*

>Optop38i(5-13L).lsm
LATTLERIEKNFVITDPRLPDNPIIFASDSFQLTEYSREEILGRNCRFLQGPETDRATVRKIRDADIDNQTEVTVQLINITYKSGKKFWNLFHLQPMRDQKGDVQYFI
GVQLDGTEHVRDAAEREGVMLIKKTAENIDEAAKELSRKKDLRISCLM*

>Optop38i(5-13V).dsm
LATTLERIEKNFVITDPRLPDNPIIFASDSFQLTEYSREEILGRNARFLQGPETDRATVRKIRDADIDNQTEVTVQLINITYKSGKKFWNLFHLQPMRDQKGDVQYFI
GVQLDGTEHVRDAAEREGVMLIKKTAENIDEAAKELSRKKDLRISCMV*

>Optop38i(5-13V).lsm
LATTLERIEKNFVITDPRLPDNPIIFASDSFQLTEYSREEILGRNCRFLQGPETDRATVRKIRDADIDNQTEVTVQLINITYKSGKKFWNLFHLQPMRDQKGDVQYFI
GVQLDGTEHVRDAAEREGVMLIKKTAENIDEAAKELSRKKDLRISCMV*

>Optop38i3(3-13F)
LATTLERIEKNFVITDPRLPDNPIIFASDSFQLTEYSREEILGRNCRFLQGPETDRATVRKIRDADIDNQTEVTVQLINITYKSGKKFWNLFHLQPMRDQKGDVQYFI
GVQLDGTEHVRDAAEREGVMLIKKTAENIDEAAKELSRKKDLRISCMF*

Supplementary Figure 6: List of plasmids used in this study (plasmids in bold presented here for the first time)

pGEX-6P-hJNK1 α 1
pGEX-6P-hJNK2 β 1
pGEX-6P-hJNK3 α 1
pGEX-6P1
pGEX-hp38 α tv2
pET28a-His-TAT-LOV2J α
pET28a-His-TAT-OptoJNKi
pmCherry-hMKK3b-EE
pEBG-r Δ MEKK1(1174-1493)
pEGFP-hMLK3
pYpet-C1
pLuc-C1
pLuc-mERK2
pLuc-hp38 α tv2
pLuc-hJNK1 α 1
pLuc-hJNK1 β 1
pLuc-hJNK2 α 2
pLuc-hJNK3 α 1
pLuc-hMKK3b
pCGN-GAL4-Mef2A
pcDNA3-GAL4-c-Jun(5-105)
pRL-CMV
pGL3-G5E4 Δ 38
pCMV
pH2B-Venus
pH2B-mCherry
pLifeact-Ypet
pmKeima620-NES-JIP1-277
pmKeima620-3xNLS-JIP1-277
pLuc-JIP1-277
pLuc-AsLOV2J α -JIP10.lsm
pLuc-AsLOV2J α -JIP10.dsm
pLuc-AsLOV2J α -JIP11.lsm (OptoJNKi.lsm)
pLuc-AsLOV2J α -JIP11.dsm (OptoJNKi.dsm)
pLuc-AsLOV2J α (F509R)-JIP11.lsm (OptoJNKi(F509R).lsm)
pLuc-AsLOV2J α (F509R)-JIP11.dsm (OptoJNKi(F509R).dsm)
pYpet-AsLOV2J α -JIP11.dsm (Ypet-OptoJNKi.lsm)
pYpet-AsLOV2J α -JIP11.lsm (Ypet-OptoJNKi.dsm)
pLuc-OptoJNKi
pmCherry-NLS-OptoJNKi
pmCherry-NLS-OptoJNKi.lsm
pmCherry-NES-OptoJNKi
pH2B-pmCherry-OptoJNKi
pmCherry-NLS-OptoJNKi.dsm
pLuc-AsLOV2J α -JIP12.lsm
pLuc-AsLOV2J α -JIP12.dsm
pLuc-AsLOV2J α -JIP13.lsm
pLuc-AsLOV2J α -JIP13.dsm
pLuc-Optop38i.1-13F.lsm

pLuc-Optop38i.1-13F.dsm
pLuc-Optop38i.1-10F.lsm
pLuc-Optop38i.1-10F.dsm
pLuc-Optop38i.1-13.lsm
pLuc-Optop38i.1-13.dsm
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pLuc-Optop38i.2-13F.lsm
pLuc-Optop38i.2-13F.dsm
pLuc-Optop38i.3-13.lsm
pLuc-Optop38i.3-13.dsm
pLuc-Optop38i.3-13F.lsm
pLuc-Optop38i.3-13F.dsm
pYpet-Optop38i.3-13F.lsm
pLuc-Optop38i3 (LOV2Jα.3-13F)
pLuc-Optop38i.4-13.lsm
pLuc-Optop38i.4-13.dsm
pLuc-Optop38i.4-13F.lsm
pLuc-Optop38i.4-13F.dsm
pLuc-Optop38i.5-13.lsm
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pLuc-Optop38i.5-13V.lsm
pLuc-Optop38i.5-13V.dsm
pYpet-Optop38i.5-13F.dsm

Supplementary References

1. Sievers F, Wilm A, Dineen DG, Gibson TJ, Karplus K, Li W, Lopez R, McWilliam H, Remmert M, Söding J, Thompson JD, Higgins D (2011) Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega Molecular Systems Biology (2011) 7, 539.