*Supplementary Materials*

# Bioluminescent RIPoptosome Assay for FADD/RIPK1 Interaction Based on Split Luciferase Assay in a Human Neuroblastoma Cell Line SH-SY5Y

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**Table S1.** Primers used for the amplification of tags.

| Primer | Sequences [a] | Strategy |
|---|---|---|
| F-pEntry3C-*SacI* | CGGCCGCACTCGAGATAGAGCTCTAGACCCAGCTTTCTTGTAC ' | Site directed mutagenesis |
| R-pEntry3C-*SacI* | GTACAAGAAAGCTGGGTCTAGAGCTCTATCTCGAGTGCGGCCG | Site directed mutagenesis |
| F-pEntryR2L3-*SacII* | CAACTTTCTT-GTACAAAGTGGCCGCGGTTAAAGGAACAAATTCAGTCG | Site directed mutagenesis |
| R-pEntryR2L3-*SacII* | CGACTGAATTGGTTCCTTTAACCGCGGCCACTTTGTACAA-GAAAGTTG | Site directed mutagenesis |
| F-mRIPK1-pEntry3C | AACCAATTCAGTCGACCATGCAACCAGACATGTCCTTGG | CloneEZ |
| R-mRIPK1-pEntry3C | GAAAGCTGGGTCTAGGCTCTGGCTGGCACG | CloneEZ |
| F-mFADD-pEntry3C | TTAGGATCCGCCATGGACCCATTCCTGGTGC | Ligation (*BamHI*) |
| R-mFADD-pEntry3C | GCGCTCGAGAGGGTGTTTCTGAGGAAGACACAG | Ligation (*XhoI)* |
| F-NLuc-linker-pEntryR2L3 | TAACCGCGGAAGGTGGTTCTGGATCTATGGAAGACGCCA AAAACATAAAGAAAGGCCCG | Ligation (*SacII*) |
| R-NLuc-pEntryR2L3 | ATACTCGAGCTAATCCTTGTCAATCAAGGCGTTGGTCGCTT CCG | Ligation (*XhoI)* |
| F-CLuc-linker-pEntryR2L3 | TAACCGCGGAAGGTGGTTCTGGATCTATGATTATGTCCG GTTATGTAAACAATCCGGAAGCGACC | Ligation (*SacII*) |
| R-CLuc- pEntryR2L3 | ATACTCGAGCTACACGGCGATCTTTCCGCCCTTCTTGGCC | Ligation (*XhoI*) |
| F-Ubiquitin promoter-pEntryL4R1 | ATAGGGATCCGGCCTCGCGCCGGGTTTGGGC | Ligation (*BamHI*) |
| R-Ub promoter-pEntryL4R1 | AGATCTCGAGGTCTAACAAAAAAGCC | Ligation (*XhoI)* |
| F-R1PK1 $^{K612R}$ | GGACTGAAAGAGAGAGTTTACCAAATGCTTC | Site directed mutagenesis |
| R-RIPK1 $^{K612R}$ | GAAGCATTTGGTAAACTCTCTCTTTCAGTCC | Site directed mutagenesis |

[a] Underline sequence represents the restriction endonuclease site used for cloning and mutation sites.

**Table S2.** PCR programs of the used genes.

| Gene | Plasmid | Initial denaturalization | Denaturalization | Annealing | Extension | Final extension |
|---|---|---|---|---|---|---|
| mRIPK1 | pEntry3C | 95℃ (5 min) | 95℃ (45 Sec) | 64℃ (30 Sec) | 72℃ (150 Sec) | 72℃ (7 Min) |
| mFADD | pEntry3C | 95℃ (5 min) | 95℃ (45 Sec) | 60℃ (30 Sec) | 72℃ (60 Sec) | 72℃ (7 Min) |
| Ub promoter | pEntryL4R1 | 95℃ (5 Min) | 95℃ (45 Sec) | 65℃ (30 Sec) | 72℃ (90 Sec) | 72℃ (7 Min) |
| NLuc | pEntryR2L3 | 95℃ (5 Min) | 95℃ (45 Sec) | 65℃ (30 Sec) | 72℃ (90 Sec) | 72℃ (7 Min) |
| CLuc | pEntryR2L3 | 95℃ (5 Min) | 95℃ (45 Sec) | 66℃ (30 Sec) | 72℃ (45 Sec) | 72℃ (7 Min) |
| R1PK1 $^{K612R}$ | pLenti6-R1C $^{K612R}$ | 98 (3 Min) | 98 (10 Sec) | 55 (5 Sec) | 72 (615 Sec) | 72 (10 Min) |

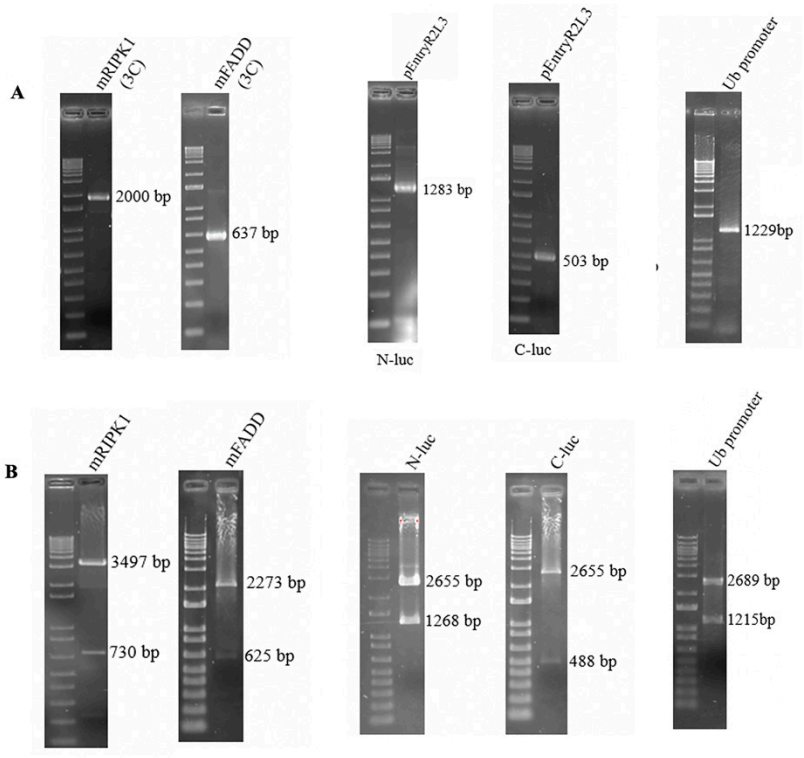



**Figure S1.** (**A**) agarose gel electrophoresis (1%) of PCR product of (mFADD and mRIPK1), (CLuc and NLuc) and Ub promotor for ligation in pEntry3C, pEntryR2L3 and pEntryL4R1 vectors, respectively. (**B**) double digestion of constructs for validation of cloning containing mFADD- pEntry3C (*BamHI* and *XhoI*) and mRIPK1- pEntry3C (*PvuII*), Nluc- pEnrtyR2L3 (*SalI* and *SacI*), CLuc- pEnrtyR2L3 (*SalI* and *SacI*) and Ub promoter- pEntryL4R1 (*BamHI* and *XhoI*); M (10 Kb).

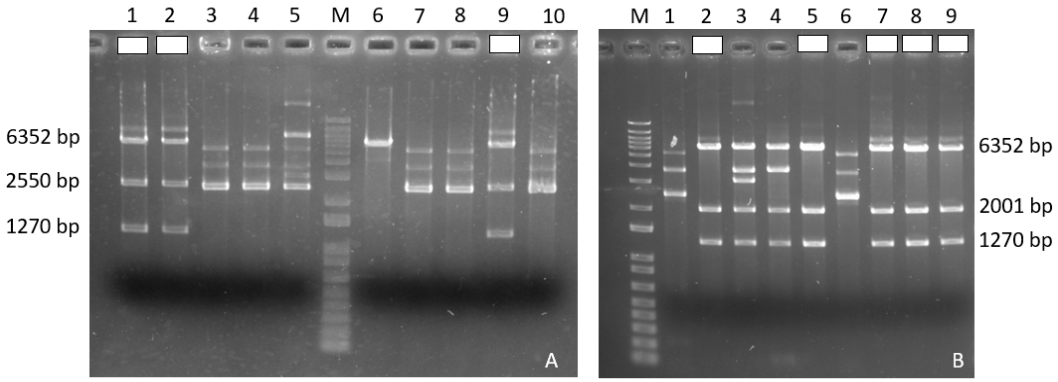



**Figure S2.** Digestion patterns of some final constructs using *EcoRV*, white marked clones are positive based on the band size (**A**) R1C; (**B**) FN; M (10 Kb).

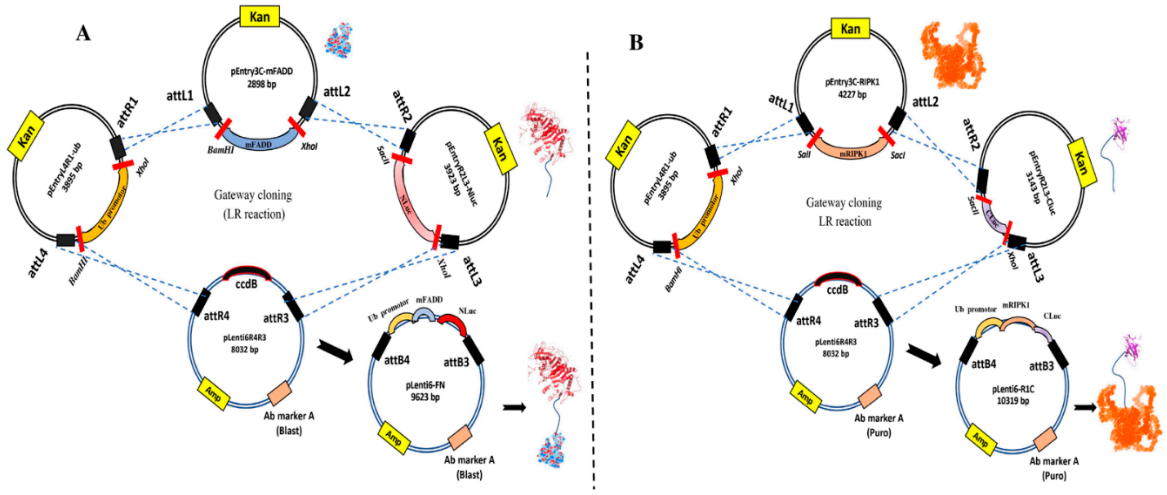



**Figure S3.** The Gateway cloning procedures for generating Luciferase-tagged proteins based on split luciferase assay using the PLenti6R4R3 destination vector. Firstly, the fragments encoding the Ub, mFADD, mRIPK1, NLuc and CLuc is inserted into an pEntry vector. Three pEntry vectors were described in this study: pEntry3C which used for mFADD and mRIPK1; pEntryL4R1 which used for Ub and pEntryR2L3 which used for luciferase fragments. In the end, the constructs in the pEntry vectors are recombined into pLenti6R4R3 vectors and generate (**A**) pLenti6-FN and (**B**) pLenti6-R1C.