

In silico and *in vivo* analyses of the mutated human tissue plasminogen activator (mtPA) and the antithetical effects of P19 silencing suppressor on its expression in two *Nicotiana* species

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Figure S1. Sequence alignment of the cloned sequence and the sequence with accession number I01047. Alignment was generated by MegAlign 5.00 (Lasergene 7, DNASTAR Inc., Madison, WI, USA).



Figure S2. The protein Blast of the translated cloned sequence, the first line (Query) concerns the results of the cloned sequence and the second line pertains to the sequence with accession number AAH95403.1.

Plasminogen activator, tissue [Homo sapiens]

Sequence ID: [AAH95403.1](#) Length: 562 Number of Matches: 1

Range 1: 1 to 562 [GenPept](#) [Graphics](#)

▼ Next Match ▲ Previous Match

Score	Expect	Method	Identities	Positives	Gaps
1162 bits(3005)	0.0	Compositional matrix adjust.	558/562(99%)	560/562(99%)	0/562(0%)
Query 1	MDAMKRGLCVLLLCGAVFVSPSQEIHARFRRGARSYQVICRDEKTQMIYQQHQSWLRPV	60			
Sbjct 1	MDAMKRGLCVLLLCGAVFVSPSQEIHARFRRGARSYQVICRDEKTQMIYQQHQSWLRPV	60			
Query 61	LRSNRVEYCWCNSGRAQCHSVPVKSCSEPRCFNGGTCQQALYFSDFCVCQCEGFAAGKCE	120			
Sbjct 61	LRSNRVEYCWCNSGRAQCHSVPVKSCSEPRCFNGGTCQQALYFSDFCVCQCEGFAAGKCE	120			
Query 121	IDTRATCYEDQGISYRGTWSTAESGAECTNWNSSALAQKPYSGRRPDAILGLGNHNYCR	180			
Sbjct 121	IDTRATCYEDQGISYRGTWSTAESGAECTNWNSSALAQKPYSGRRPDAILGLGNHNYCR	180			
Query 181	NPDRDSKPWCYVFKAGKYSSEFCSTPACSEGNSDCYFGNGSAYRGTHSLTESGASCLPWN	240			
Sbjct 181	NPDRDSKPWCYVFKAGKYSSEFCSTPACSEGNSDCYFGNGSAYRGTHSLTESGASCLPWN	240			
Query 241	SMILIGKVYTAQNPSAQLGLGKHNCRNPDGDAKPWCHVLKSRRLTWEYCDVPSCSTCG	300			
Sbjct 241	SMILIGKVYTAQNPSAQLGLGKHNCRNPDGDAKPWCHVLKSRRLTWEYCDVPSCSTCG	300			
Query 301	LRQYSQPQFRIKGGFLADIASHPWQAAIFAKHRRSPGERFLCGGILISSCWILSAAHCFQ	360			
Sbjct 301	LRQYSQPQFRIKGGFLADIASHPWQAAIFAKHRRSPGERFLCGGILISSCWILSAAHCFQ	360			
Query 361	ERFPPHLLTVILGRTYRVVPGEESQKFEVEKYIVHKEFDDDTYDNDIALQLKSDSSRCA	420			
Sbjct 361	ERFPPHLLTVILGRTYRVVPGEESQKFEVEKYIVHKEFDDDTYDNDIALQLKSDSSRCA	420			
Query 421	QESSVVRTVCLPPADLQLPDWTECELSGYGKHEALSPFYSERLKEAHVRLYPSSRCTSQH	480			
Sbjct 421	QESSVVRTVCLPPADLQLPDWTECELSGYGKHEALSPFYSERLKEAHVRLYPSSRCTSQH	480			
Query 481	LLNRTVTDNMLCAGDTRSGGPQANLHDACQGDSEGPLVCLNDGRMTLVGIISWGLGCGQK	540			
Sbjct 481	LLNRTVTDNMLCAGDTRSGGPQANLHDACQGDSEGPLVCLNDGRMTLVGIISWGLGCGQK	540			
Query 541	DVPGVYTKVTNYLDWIRDNMRP	562			
Sbjct 541	DVPGVYTKVTNYLDWIRDNMRP	562			

Figure S3. ERRAT plot diagram of the selected model.

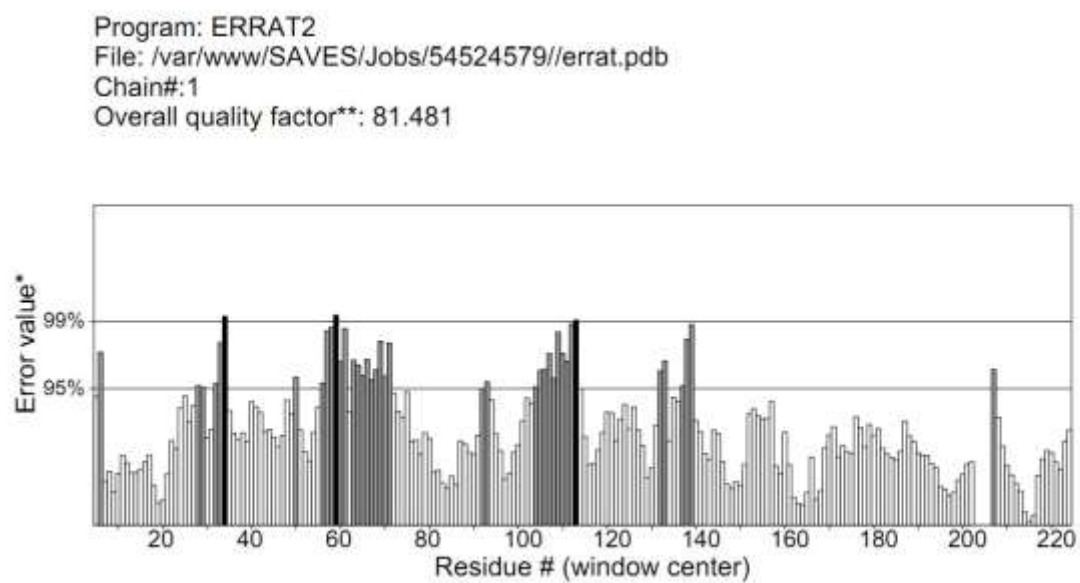


Figure S4. Graph plot of the selected model by Verify3D server.

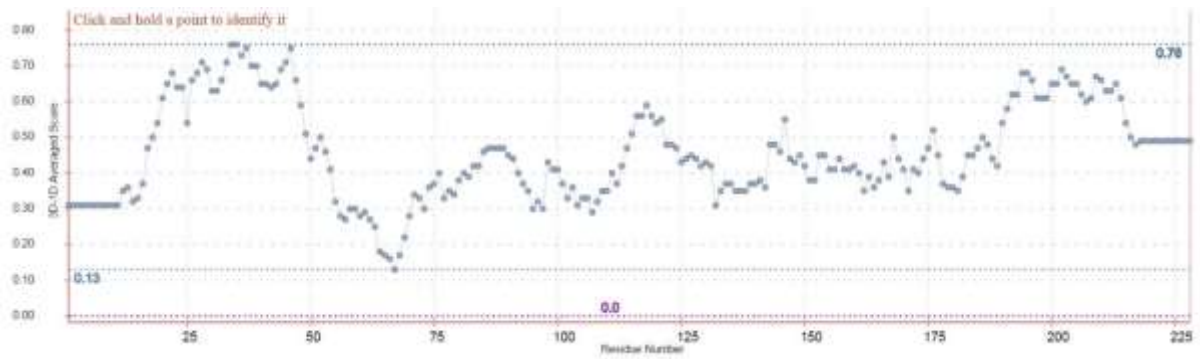


Figure S5. Full length gel of RT-PCR amplification of tissue plasminogen activator on RNA extracted from agroinjected leaves. M: 100 bp plus DNA ladder, C⁻: negative control (water template), C_R⁻: negative control (extracted RNA), C⁺: positive control (pTRAC-ERH-tPA vector), W_T and W_B: agroinjected leaves with *Agrobacterium* containing pTRA-ERH vector in *N. tabacum* and *N. benthamiana*, respectively, 1 and 2: agroinjected leaves with *Agrobacterium* harbouring pMA2 in *N. tabacum* and *N. benthamiana*, respectively. Letters were put in by Microsoft word insertion tools.

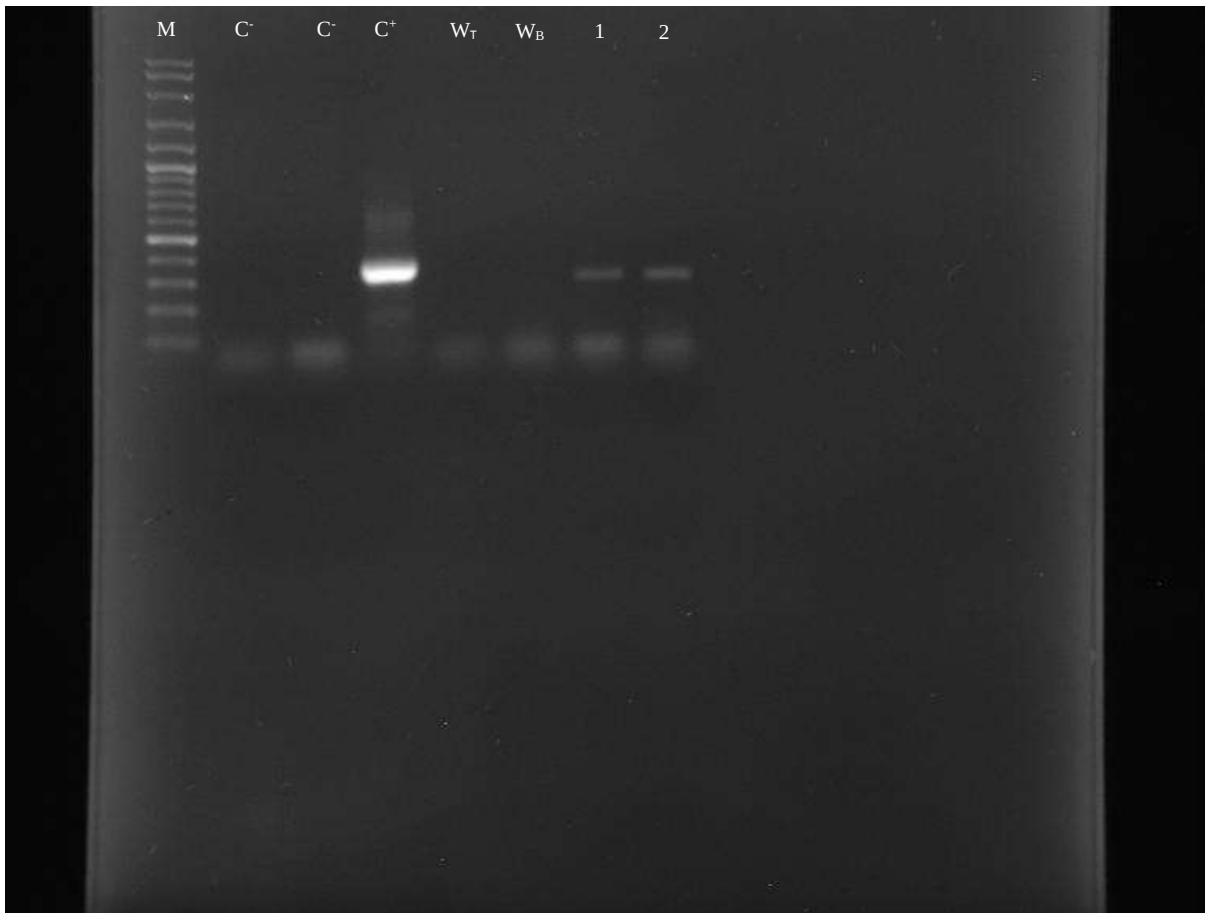
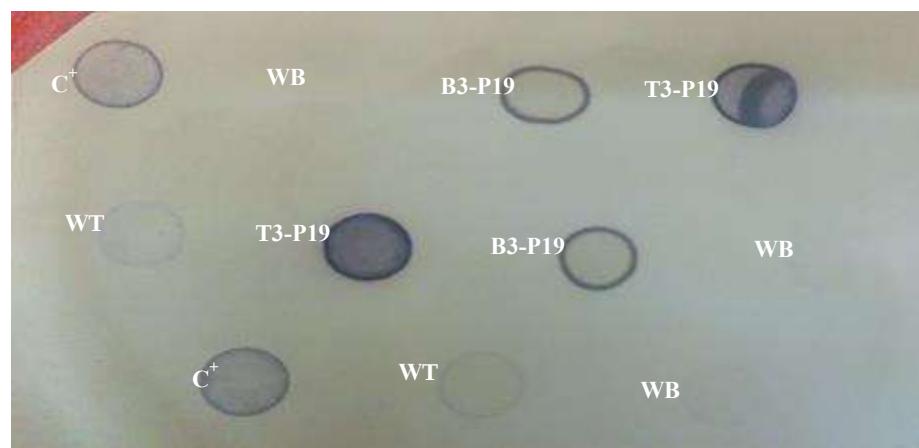
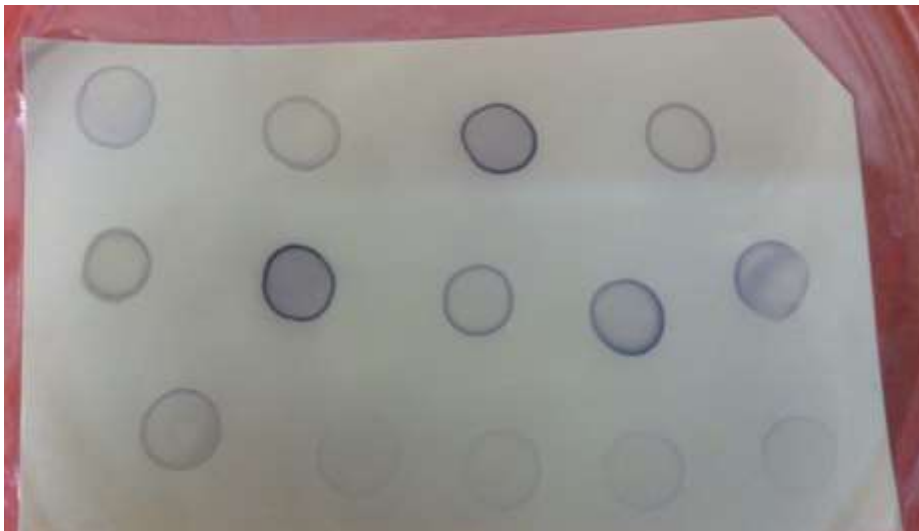
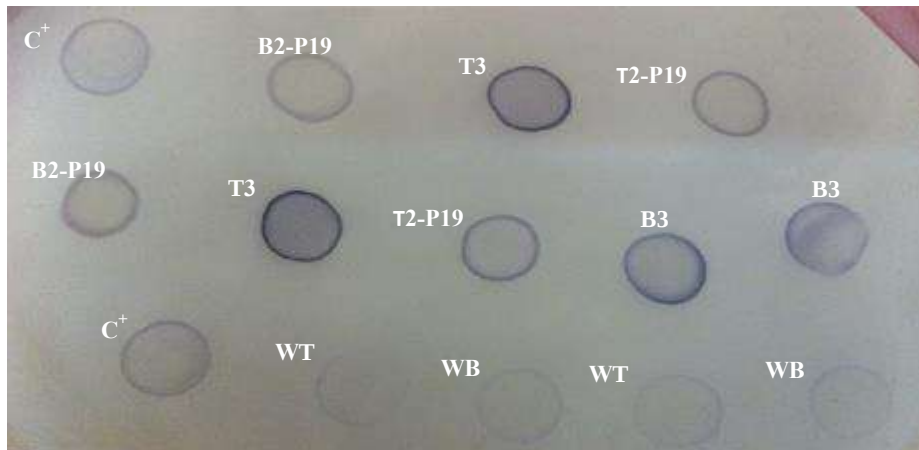


Figure S6. The original figures of dot blot analysis which are cropped in the manuscript to show the differences better; the letters were put in the blot for better understanding by Microsoft word tools, the original of every photo (without letter and more cropping) was inserted under the respective photo. C⁺: positive control (Alteplase protein), W_T and W_B: agroinjected leaves with *Agrobacterium* containing pTRA-ERH vector in *N. tabacum* and *N. benthamiana*, respectively, T2-P19 and B2-P19: tPA+P19 treatment in *N. tabacum* and *N. benthamiana* diluted to OD600=0.2, respectively. T3 and B3: tPA treatment in *N. tabacum* and *N. benthamiana* diluted to OD600=0.3, respectively, and T3-P19 and B3-P19: tPA+P19 treatment in *N. tabacum* and *N. benthamiana* diluted to OD600=0.3, respectively.



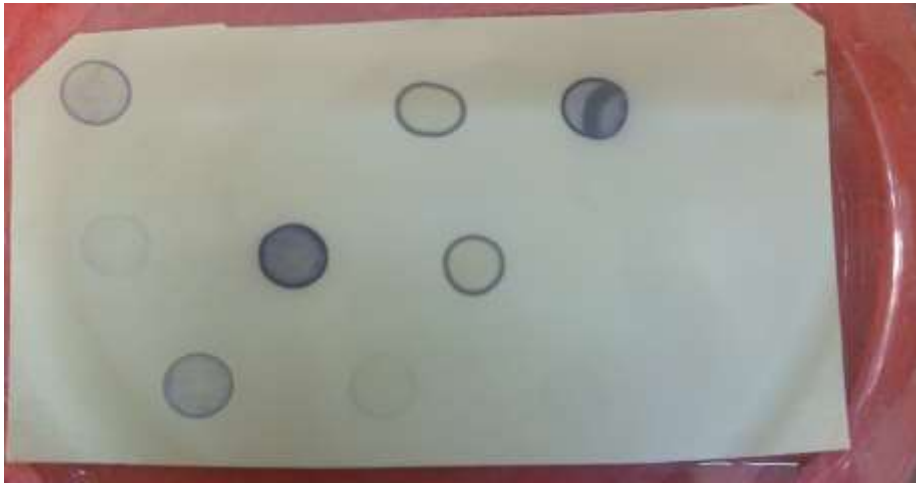


Figure S7. The original figures of western blott analysis with tPA-specific antibody which is cropped in the manuscript to show the differences better. C⁺: positive control (Alteplase protein), W_T and W_B: agroinjected leaves with *Agrobacterium* containing pTRA-ERH vector in *N. tabacum* and *N. benthamiana*, respectively, T2-P19 and B2-P19: tPA+P19 treatment in *N. tabacum* and *N. benthamiana* diluted to OD600=0.2, respectively. T3 and B3: tPA treatment in *N. tabacum* and *N. benthamiana* diluted to OD600=0.3, respectively, and T3-P19 and B3-P19: tPA+P19 treatment in *N. tabacum* and *N. benthamiana* diluted to OD600=0.3, respectively. The letters were put on the blot for better undrestanding by Microsoft word tools, the original blot (without letters) was inserted under it.

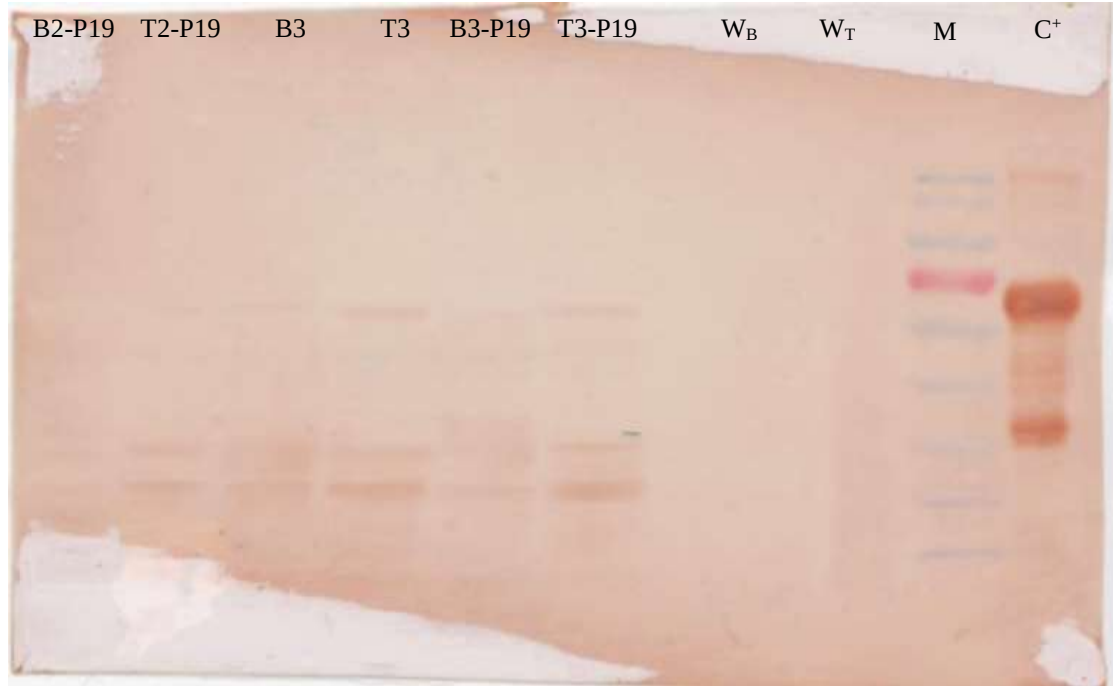


Figure S8. The original figure of Zymography assay which is cropped and its colour is changed in the manuscript to show the differences better. C⁺: positive control (Alteplase protein), W_T and W_B: agroinjected leaves with *Agrobacterium* containing pTRA-ERH vector in *N. tabacum* and *N. benthamiana*, respectively, T2-P19 and B2-P19: tPA+P19 treatment in *N. tabacum* and *N. benthamiana* diluted to OD600=0.2, respectively. T3 and B3: tPA treatment in *N. tabacum* and *N. benthamiana* diluted to OD600=0.3, respectively, and T3-P19 and B3-P19: tPA+P19 treatment in *N. tabacum* and *N. benthamiana* diluted to OD600=0.3, respectively. The letters were put on the blot for better understanding by Microsoft word tools, the original blot (without letters, color change and cropping) was inserted under it.

