APPENDIX

Construct	Vector	Tag	Antibiotic resistance	Insert Primers	Insert size (bp)	Protein MW (KDa)	Mutations
C9WTFL	pET23b	His6	Ampicillin	*C9SEQFWD* *C9SEQREV*	1269	46 (35+10)	None
CARD	pETGKI	GST	Kanamycin	*CARD1 FW CARD92 RV*	276	26+12,5	None
ΔCARD 4QC	pETGKI	GST	Kanamycin	*ΔCARDC9 1 FW ΔCARDC9 277 RV*	831	26+30	D304A, D315A, D330A
ΔCARDC287A	pETGKI	GST	Kanamycin	*ΔCARDC9 1 FW ΔCARDC9 277 RV*	831	26+30	C287A active site
C9C287AFL	pETGKI	GST	Kanamycin	*CARD1 FW ΔCARDC9 277 RV*	831	26+46	C287A active site
C9WTFL	pETGKI	GST	Kanamycin	*CARD1 FW ΔCARDC9 277 RV*		26 + 46 (35+10)	None
C9T125E	pETGKI	GST	Kanamycin	*CARD 1FW ΔCARDC9 277 RV*		26 + 46 (35+10)	T125E
C9S196D	GKI	GST	Kanamycin	*CARD 1FW ΔCARDC9 277 RV*		26 + 46 (35+10)	S196D
C9S310D	GKI	GST	Kanamycin	*CARD 1FW ΔCARDC9 277 RV*		26+46 (35+10)	S310D

Table 1. Caspase 9 constructs and its characteristics.

PCR caspase 9 insert	
Conditions	Mix
95 ºC – 3' **30 cycles:** **98ºC – 20''** **58 ºC – 20''** **72ºC – 1' 30''** **Final Ext: 72ºC – 5'**	5 µL buffer HiFi (Kapa Biosystems®) 0,75µL dNTPs (Kapa Biosystems®) 0,75 µL primer FWD 0,75 µL primer REV 1,5 µL DMSO 0,5 µL DNA 0,5 µL Kapa Hifi DNA polimerase (Kapa Biosystems®) 15,25 µL H_20

Table 2. PCR conditions for caspase 9 insert amplification

Name	F/R	Sequence
CARD 1 FW	Forward	CAGGGACCCGGTATGGACGAAGCGGATCGGCGGCTC
CARD92 RV	Reverse	CGAGGAGAAGCCCGGTTAGTTAGTTCGCAGAAACGAAGCCAGCATGTCC
ΔCARDC9 1 FW	Forward	CAGGGACCCGGTGGTGCTCTTGAGAGTTTGAGGGGAAATGCAGATTTG
ΔCARDC9 277 RV	Reverse	CGAGGAGAAGCCCGGTTATGATGTTTTAAAGAAAAGTTTTTTCCGGAGGAAATTAAAGCAACCAGGC
C9T125E FW	Forward	GGTTCTCAGACCGGAAGAGCCCAGACCAGTGGAC
C9T125E REV	Reverse	GTCCACTGGTCTGGGCTCTTCCGGTCTGAGAACC
C9S196D FW	Forward	GGCGTCGCTTCTCCGACCTGCATTTCATGGTG
C9S196D REV	Reverse	CACCATGAAATGCAGGTCGGAGAAGCGACGCC
C9S310D FW	Forward	GACGAGTCCCCTGGCGATAACCCCGAGCCAG
C9S310D REV	Reverse	CTGGCTCGGGGTTATCGCCAGGGGACTCGTC
PP1 $_{\alpha7\text{-}300}$ FW	Forward	CCTCAAGCCCGCCGACtAGAACAAGGGGAAGTAC
PP1 $_{\alpha7\text{-}300}$ REV	Reverse	GTACTTCCCCTTGTTCTAGTCGGCGGGCTTGAGG

Table 3. Primer used for cloning of the different caspase 9 and PP1 constructs and their nucleotide sequences.

Colony PCR for Caspase 9 constructs	
Conditions	Mix
95 ºC – 5' **30 cycles:** **95ºC – 45''** **55 ºC– 45''** **72ºC – 2'** **Ext. Final: 72ºC – 10'**	2 µL buffer B 10X (Solis Biodyne®) 2 µL $MgCl_2$ 25 mM (Solis Biodyne®) 0,4 µL dNTPs 10 mM (Kapa Biosystems®) 0,6 µL primer FWD (pGEXF) 0,6 µL primer RV (T7 terminator) → 1 colony 0,2 µL Firepol® DNA polimerase 14,2 µL H_20

***Table 4. Colony PCR conditions for CARD*, ΔCARD 4QC, ΔCARDC287A and C9C287AFL constructs in GKI vector and C9WTFL construct in pET23 vector.**

Quickchange® PCR for C9 mutants	
Conditions	Mix
95 ºC – 2' **16 cycles:** **98ºC – 20''** **66 ºC– 20''** **72ºC – 5'** **Ext. Final: 72ºC – 5'**	5 µL buffer HiFi (Kapa Biosystems®) 0,75µL dNTPs (Kapa Biosystems®) 0,75 µL primer FWD 0,75 µL primer RV 2 µL DMSO 0,5 µL DNA 0,5 µL Kapa Hifi DNA polimerasa (Kapa Biosystems®) 14,75 µL H_20

Table 5. PCR conditions for site-directed mutagenesis of C9WTFL in pETGKI vector. Mutants are C9T125E,C9 S196D and C9S310D.

Quickchange® PCR for $PP1_{\alpha 7\text{-}300}$	
Conditions	Mix
95 ºC – 2' **16 cycles:** **98ºC – 20''** **58 ºC– 20''** **72ºC – 5'** **Ext. Final: 72ºC – 5'**	5 µL buffer HiFi (Kapa Biosystems®) 0,75µL dNTPs (Kapa Biosystems®) 0,75 µL primer FWD 0,75 µL primer RV 1,5 µL DMSO 0,5 µL DNA 0,5 µL Kapa Hifi DNA polimerasa (Kapa Biosystems®) 15,25 µL H_20

Table 6. Quickchange® PCR conditions for $PP1_{\alpha 7\text{-}300}$