Supplementary material

Method S1. Details for construction of $pKB \triangle C_{His}$ vector.

To construct pBaMV△CMCS-1, by truncating BaMV CP and inserting the multiple cloning sites (ClaI/HidIII/SpeI), a two-step cloning strategy was used as described below. In the first step, the DNA fragment of downstream coding sequence of BaMV TGBp3 was amplified with a plasmid pBaMV as template using specific primer pairs B-8 plus LNK-3 containing HidIII restriction enzyme site. The amplified PCR fragment was digested with *Ppu10I* and *Hid*III and ligated into a pBaMV vector restricted with the same enzyme to generate the recombinant plasmid pBaMV (LINK-3). In the second step, the DNA fragment of coding sequence of BaMV 3'UTR was amplified with a plasmid pBaMV as the template using specific primer pairs 6223SH, containing Spel and HidIII, plus B21N, containing SacI restriction enzyme sites. The amplified PCR fragment was digested with SpeI and SacI and ligated into the pBaMV (LINK-3) vector restricted with the same enzyme to generate the recombinant plasmid pBaMV \triangle CMCS-1, which was further subcloned into a pCass vector to generate pCB \triangle CMCS-1.To construct pCBACMCS-2 containing putative BaMV CP promoter region, The DNA fragment of the downstream coding sequence of BaMV TGBp3 was amplified with a plasmid pKB as template using specific primer pairs B-8 plus MSNS LIK, containing a series of multiple cloning sites (*MluI/StuI/NotI/SpeI*) and the 5'-terminal 15 nts of BaMV CP coding sequence, of which start codon was mutated. The amplified PCR fragment was digested with Spel and Nsil and ligated into a pCB△CMCS-1 vector restricted with the same enzyme to generate the recombinant plasmid pCBACMCS-2, which was further subcloned into a pKn binary vector to generate pKB∆CMCS-2.

To construct pKB Δ C_{His} containing 6xHis tag for purification, DNA fragment of BaMV 3'UTR was amplified with a plasmid pkB as the template using specific primer pairs SH-6223, containing coding sequence of six repeats of His and *Spe*I restriction enzyme site, plus B21N, containing the *SacI* restriction site. The amplified PCR fragment was digested with *Spe*I and *SacI*, and ligated into a pKB Δ CMCS-1 vector restricted with the same enzymes to generate the recombinant plasmid pKB Δ CHis.

Method S2. Expression of recombinant mIFN_Y in *E. coli*.

The coding region of mIFN γ was amplified with the plasmid pKBmIFN γ as a template using specific primer pairs F-*MluI*-*NcoI*-mIFN γ , 5' GCACGCGTCCATGGGCTGTTACTGCC AGGACC-3' plus R-*NotI*-TGA-His-IFN γ CGGCGGCCGCTCAGTGGTGGTGGTGGTGGTGGTGGTG The amplified PCR fragment was digested with *MluI* and *NotI* and cloned into a plasmid pET28a. The plasmid was then transformed into *E. coli* BL21(DE3) cell for overexpression. Recombinant mIFN γ derived from E. coli was induced by 1 mM IPTG and purified as described the manufacturer's instructions.

Primer	Sequence (5' - 3')
F-IFNγ- <i>Mlu</i> I	GC <u>ACGCGT¹</u> ATGAAATATACAAGTTATATC
F:MluI-ATG-IFNγC-1/C-2	GC <u>ACGCGT¹</u> ATGAAGTACACGAGT
F-MluI-NcoI-mIFNγ	GC <u>ACGCGTCCATGG¹</u> GCTGTTACTGCCAGGACC
F:MluI-NcoI-mIFNyC-1	GC <u>ACGCGTCCATGG¹</u> GTTGTTATTGCCAACAT
F:MluI-NcoI-mIFNyC-2	GC <u>ACGCGTCCATGG¹</u> GTTGTTATTgCCAAGAT
R-SpeI-IFNγ	CC <u>ACTAGT¹</u> CTGGGATGCTCTTCGACC
R-NotI-TGA-His-IFNγ	CG <u>GCGGCCGC¹</u> TCAGTGGTGGTGGTGGTGGTGGTG
F-P19-DraIII	GCC <u>CACGCGGTG¹</u> ATGGAACGAGCTATACAAGG
R-P19-DraIII	GCG <u>CACATGGTG¹</u> TTACTCGCTTTCTTTTGA
F-BaMV4102	CCACTACCAAACAATCAG
R-DraIII-p28	GCCA <u>CATGGTGT¹</u> CAAGTGGTCTGGCCAGATG
F-P38 DraIII	GCC <u>CACGCGGTG¹</u> ATGGAAAATGATCCTAGAGT
R-P38 DraIII	GCG <u>CACATGGTG¹</u> CTAAATTCTGAGTGCTTGCC
R-SpeI-SEKDEL-IFNγ	GCACTAGT ¹ GAGCTCATCCTTCTCAGA CTGGGATGCTCTTCG
F-SP-SpeI	GCACTAGT ¹ TCACCCTCTCCAAGCCCTTCCCCATCGCCTAGTCCCTCACCATCC
R-SP-SpeI	CG <u>ACTAGT'</u> TGGGCTGGGAGAAGGGGATGGTGAGGGACTAGGCGA
F-SS-MluI	${\rm GC}\underline{{\rm ACGCGT}^1}{\rm ATGGGGGAAAATGGCTTCTCTATTTGCCACTCTTCTAGTAGTTTTAGTGTCA}$
R-SS-MluI	CG <u>ACGCGT¹</u> TGCTGAGCTTTCAGAAGCTAAGCTAAGTGACACTAAAACTACTAGAAGAGT
R-BaMV5703	ATCCACTGCTAAGTGTTTGC
F-BCPN	AGGCATCCTATATAATATAC
R-BaMV6366	TGGAAAAAACTGTAGAAACCAAAAGG

Table S1. List of sense (F) and antisense (R) oligonucleotides used for PCR amplifications.

¹ Nucleotides underlined indicate restriction enzyme recognition site.

IFNy	ATGA	AA	TAT	ACA	AGT	TAT	ATO	TT	GGC	ттт	TCA	AGO	TT	TG	AT	CGT	ттт	GG	GT	тст	CTT	GGC
IFNy-1	ATGA	AA	TAT	ACA	AGT	TAT	ATO	TTO	GGC	TTT	TCA	AGO	TT	TG	AT	CGT	TTT	GG	GT	тст	CTT	GGC
IFNy-2	ATGA	AG	TAC	ACG	AGT	TAC	ATO	CTO	GGC	TTT	TCA	AAC	TT	TG	TAT	CGT	СТТ	GG	GT	тст	CTT	GGA
	****	* *	**	**	***	**	***	* *:	***	***	***	k \$	***	**	**	***	**	***	**	***	***	**
	Μ	Κ	Y	Т	S	Y	Ι	L	Α	F	(2	L	С	Ι	V	L	(Ĵ	S	L	G
IFNy	TGTT	AC	TGC	CAG	GAC	CCA	TAT	TGT/	AAA	AGA	AGO	AG	JAA	AA	CT	TAA	GAA	AT	AT	ттт	AAT	GCA
IFNy-1	TGTT	AC	TGC	CAG	GAC	CCA	TAT	TGT/	AAA	AGA	AGO	AG	5AA	AA	CT	TAA	GAA	AT	AT	TTT	AAT	GCA
IFNy-2	TGTT	TAT	TGC	CAA	GAT	CCA	TAT	TGT/	AAA	GGA	AGO	AG	JAG	AA	CT	TAA	GAA	AT	AC	TTC	AA	GCA
	****	* 28:	***	**	**	***	***	***	***	**	***	***	s 280	***	***	***	***	***	*	**	**	***
	C	Y	С	Q	D	Р	Y	V	K	E	L A	A	E	Ν	L	K	K		Y	F	Ν	A
IFNy	GGTO	AT	TCA	GAT	GTA	GCG	GAT	TAAT	TGG.	AAC	TCT	ТТ	TC	TT	AGG	CAT	ттт	GA	AG	AAT	TGO	AAA
IFNy-1	GGTO	AT	TCA	GAT	GTA	GCT	GAT	TAAT	TGG.	AAC	тст	ТТ	TC	CT	GG	CAT	ттт	GA	AG	AAT	TGO	AAA
IFNy-2	GGTO	AT	TCA	GAT	GTT	GCT	GAT	TAAT	TGG.	AAC	TCT	ГТТ	TC	TT/	GG	TAT	TTT	GA	AG	AAC	TGO	AAA
	****	**	***	***	**	**	***	***	***	***	***	***	**	*	**	**	***	***	**	**	***	***
	G	Н	S	D	V	A	D	Ν	G	Т		L	F	L	G	1	1	_	K	Ν	W	K
IFNy	GAGO	AG	AGT	GAC	AGA	AAA	ATA	AAT	SCA	GAG	CCA	AAA	TT	GTO	TC	СТТ	TTA	ACT	TC	AAA	CTT	TTT
IFNy-1	GAGO	AG	AGT	GAC	AGA	AAA	ATA	AAT	SCA	GAG	CCA	AAA	TT	GTO	TC	CTT	TTA	ACT	TC	AAA	CTT	TTT
IFNy-2	GAGO	AA	AGT	GAT	CGT	AAG	ATA	AAT	SCA	AAG	CCA	AAA	TT	GTO	AG	TTT	СТА	TT	TC	AAG	CTO	TTT
3	****	e 28:	***	**	*	**	***	***	***	**	***	***	***	***	R	**	**	* *	**	**	**	***
	E	Е	S	D	R	K	Ι	Μ	Q	S		Q	Ι	V	S]	F '	Y	F	Κ	L	F
IFNy	AAAA	AC	ттт	AAA	GAT	GAC	CAC	SAG	CAT	CCA	AAA	AGA	GT	GT	5GA	GAC	CAT	ICA	AG	GAA	GAC	ATG
IFNy-1	AAAA	AC	TTT	AAA	GAT	GAC	CAC	SAG	AT	CCA	AAA	AGA	GT	GTO	GA	GAC	CAT	ICA	AG	GAA	GAG	ATG
IFNy-2	AAGA	AC	TTT	AAA	GAC	GAC	CAG	SAG	CAT	TCA	GAA	AT	CC	GTO	GA	GAC.	AAT	TA	AG	GAA	GAG	ATG
	** *	***	***	***	**	***	***	***	***	**	**	k		***	***	***	**	* *	**	***	***	***
	K	Ν	F	Κ	D	D	Q	S	Ι	Q	K	5	S	V	Е	Т	Ι	ŀ	Χ	E	D	Μ
IFNy	AATO	TC	AAG	TTT	TTO	AAT	AG	CAA	CAA	AAA	GAA	AAC	GA	GAT	GA	CTT	CGA	AAA	AG	СТО	ACT	TAAT
IFNy-1	AATO	TC	AAG	TTT	TTO	AAT	AGO	CAA	CAA	AAA	GAA	AAA	GG	GAT	GA	CTT	CGA	AAA	AG	ста	ACT	TAAT
IFNy-2	AATO	TG	AAA	TTC	TTT	AAT	TCO	CAA	TAA	GAA	GAA	AAC	GA	GAT	GA	TTT	TGA	GA	AA	CTA	ACC	AAT
	****	- 180	**	**	**	***		***	**	**	***	**	*	***	***	**	**	* *	*	**	**	***
	N	V	K	F	F	N	S	N	K	K	K	C	R	D	D	F	Е	F	ζ	L	Т	Ν
IFNy	TATT	CG	GTA	ACT	GAC	TTO	AAT	TGT	CCA	ACG	CAA	AAG	iCA	AT/	ACA	TGA	ACT	ICA	TC	CAA	GTO	ATG
IFNy-1	TATT	TCT	GTA	ACT	GAC	TTO	AAT	TGT	CA	AAG	GAA	AAG	ICA	ATA	ACA	TGA	ACT	ICA	TC	CAA	GTO	ATG
IFNy-2	TATT	CA	GTT	ACT	GAT	СТО	AAT	TGT	CA	GAG	GAA	AAG	SCA	ATA	ACA	TGA	ACT	ICA	TT	CAG	GT	ATG
	****	1 18:	**	***	**	**	***	***	**	*	**	***	**	***	***	***	***	***	*	**	**	***
	Y	S	V	Т	D	L	Ν	V	Q	R	ŀ	Χ	А	Ι	Н	Е	L		Ι	Q	V	Μ
IFNy	GCTO	5AA	CTG	TCG	CCA	GCA	GCT	TAA/	AAC	AGG	GAA	AGO	GA	AAA	AAG	GAG	TCA	AGA	TG	CTG	TTT	CAA
IFNy-1	GCTO	AA	CTG	тст	CCA	GCA	GCT	TAA	AAC	AGG	TAA	AGA	GG	AAA	AAG	GAG	TCA	AGA	TG	ста	TTT	CAA
IFNy-2	GCTO	AA	TTG	TCA	CCT	GCC	GCT	TAA	SAC	TGG	GAA	AAC	GG	AA	AG	GTC	TCA	AGA	TG	TTA	TTT	CAA
	****	***	**	**	**	**	***	***	**	**	**	k	*	**	**	*	***	***	**	*	***	***
	Α	Е	L	S	Р	A	Α	K	Т	G	i I	K	R	K	R	S	0		Μ	L	F	0
IFNV	GGTO	GA	AGA	GCA	TCC	CAG	ACT	TAG	TCA	CCA	CCA	ACC	AC	CAO	CA	CTG	A					
IFNy-1	GGTA	GG	AGA	GCA	TCC	CAG	ACT	TAG	TCA	CCA	CCA	ACC	AC	CAG	CA	CTG	A					
IFNy-2	GGCA	GA	AGA	GCC	TCT	CAA	ACT	LAG	TCA	CCA	CCA	ACC	AC	CAG	CA	CTG	A					
	**	*	***	**	**	**	***	***	***	***	***	***	**	**	***	***	*					
	G	R	R	Α	S	Q	Т	S	Η	Η	F	Ŧ	Η	Н	Η	*						

Figure S1. Alignment of codon-optimized nucleotide sequence of IFN γ -1 and -2 with native IFN γ sequence. The codon-optimized IFN γ -1 sequence (14-nucleotide substitutions) was generated based on codon usage table of *N. benthamiana*. Another codon-optimized IFN γ -2 sequence (87-nucleotide substitutions) was generated from the service provided by Integrated DNA technologies (https://sg.idtdna.com/CodonOpt). The codon-optimized IFN γ -1 and IFN γ -2 sequences were aligned to the reference native IFN γ sequence (GenBank accession no. AY121833.1) using Multiple Sequence Alignment. The codon-optimized sites for IFN γ -1 and IFN γ -2 sequences were highlighted by the orange and yellow blocks, respectively.



Figure S2. Analysis of preference of different primary antibodies against mIFN γ or IFN γ . *N. benthamina* leaves were infiltrated with A. tumefaciens harboring pKBmIFN γ and pKBIFN γ . Total proteins were analyzed SDS-PAGE, stained by CBS (**A**), and analyzed by immunoblot following the same protocol described in Materials & Methods using different primary antibodies specific to mIFN γ (**B**), N-terminal 1-100 amino acids of native IFN γ (abcam, ab133566) (**C**), and 6X His tag (GeneTex, GTX115045) (**D**). Total protein extracts of IFN γ (2°) and mIFN γ (2°) were prepared from infiltrated leaf tissue at 5DPI. In order to verify whether the primary antibodies exhibited preferences against IFN γ or mIFN γ , the mIFN γ protein extract was 2-fold serially diluted. The goat-anti-rabbit IgG horseradish peroxidase conjugate was used as secondary antibody for all immunoblot assays. The signal intensities of mIFN γ or IFN γ in each blot were quantified by densitometry (Image Reader LAS-4000). The numbers below each lane indicate the band intensities of mIFN γ or IFN γ protein derived from E. coli (100 ng for CBS and 10 ng for IB).











С

Figure S3. The influence of CP C-terminal coding sequence on BaMV replication and IFNY production. (A). Schematic representation of BaMV-based vectors in which various lengths of 3'-terminal nucleotides (positions 40-250) of CP coding region were retained between target protein (TP, mIFNy) and BaMV 3' untranslated region (UTR) to generate the following constructs: 1. pKBmIFNγ, 2. pKBΔCmIFNγCP40, 3. pKBΔCmIFNγCP60, 4. pKB Δ CmIFN γ CP100, 5. pKB Δ CmIFN γ CP150, 6. pKB Δ CmIFN γ CP200, and 7. pKB△CmIFNγCP250. (B). Analysis of TP expression in inoculated leaves. Total protein extracts were prepared from infiltrated leaf tissue at 3 and 5 DPI and analyzed by SDS-PAGE, followed by staining with CBS and IB analysis with anti-mIFNy as primary antibody and goat-anti-rabbit IgG alkaline phosphatase conjugate as secondary antibody. M, Marker; H, Healthy leaf; P, Positive control, purified mIFNy protein derived from E. coli (250 ng for CBS and 25 ng for IB). (C). Northern blot analysis of wild-type or chimeric BaMV RNA in infiltrated leaves at 3 and 5 DPI. BaMV genomic RNA, and the subgenomic RNAs for triple gene block proteins (TGPsgRNA) and CP (CPsgRNA) were detected with a BaMV-specific probe (vector, pKB Δ CHis as control). The bottom panel shows the amount of rRNA in each sample, stained with ethidium bromide as the loading control.