Supporting Information

Biosynthesized tumor acidity and MMP dual-responsive plant toxin gelonin for robust cancer therapy

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Figure S1. Agarose gel electrophoresis of whole plasmid PCR product for construction of recombinant plasmid pET32a-*PVGLIG-pHLIP-Gelonin*. Lane M: DNA ladder; Lane 1: whole plasmid PCR product.



Figure S2. SDS-PAGE analysis of the TPpG expression. Lane M: protein molecular weight markers; Lane 1: the supernatant of cell lysate of *E. coli* BL21 (DE3) containing empty pET32a plasmid after IPTG induction; Lane 2,3,4: total cell extract, the supernatant and pellet fraction of cell lysate of *E. coli* BL21 (DE3) containing pET32a-*PVGLIG-pHLIP-Gelonin* plasmid after IPTG induction. Lane 5: the supernatant of cell lysate of *E. coli* BL21 (DE3) containing pET32a-*PVGLIG-pHLIP-Gelonin* plasmid after IPTG induction. Lane 5: the supernatant of cell lysate of *E. coli* BL21 (DE3) containing pET32a-*PVGLIG-pHLIP-Gelonin* plasmid without IPTG induction. The red arrow indicates the target protein.



Fig. S3. Responsiveness of TPpG to MMP. TPpG was incubated with activated Collagenase IV for 2 h and was analyzed by 12% SDS-PAGE. Lane M: a protein molecular weight marker; lane 1: Collagenase IV (8 mg/mL); lane 2: purified TPpG (8 μM); lane 3: TPpG bands after 2 h incubation with collagenase IV. The red arrows indicate the two peptide fragments of TPpG after enzymatic digestion.



Figure S4. UV-Vis spectra of TPpG and FITC-TPpG.



Fig. S5. Cell viability was determined by MTT assay. MCF-7 cells (a,b) and HT1080 cells (c,d) were incubated with different concentrations of gelonin or TPpG under two different pH conditions for 48 h. And the relative cell viability was measured by MTT assay.



Fig. S6. Enlarged images of H&E staining results of tumor tissues from different groups. The scale bars are 50 µm.

Peptides	Position	Observed	Calculated
K.MIAPILDEIADEYQGK.L	38 - 53	1805.7528	1804.8917
K.EFLDANLAGSGSGHMHHHHHHSSGLVPR.G	102 - 129	3024.2117	3023.4070
K.AMGPVGLIGAAEQNPIYWAR.Y	159 - 178	2113.9429	2113.0779
K.GATYITYVNFLNELR.V	214 - 228	1773.7919	1773.7919
K.LKPEGNSHGIPLLR.K	231 - 244	1530.7686	1529.8678
R.SYFFKDAPDAAYEGLFK.N	287 - 303	1968.7976	1968.7976
K.AYRETTDLGIEPLR.I	324 - 337	1633.7336	1632.8471
R.ETTDLGIEPLR.I	327 - 337	1243.5684	1242.6456
K.KLDENAIDNYKPTEIASSLLVVIQMVSEAAR.F	342 - 372	3417.5854	3416.7861
K.LDENAIDNYKPTEIASSLLVVIQMVSEAAR.F	343 - 372	3289.5024	3288.6911
R.FTFIENQIR.N	373 - 381	1167.5397	1166.6084
R.IRPANNTISLENKWGK.L	388 - 403	1840.8678	1839.9955
R.TSGANGMFSEAVELER.A	410 - 425	1697.6671	1696.7726

 Table S1. Peptide mass fingerprinting analysis of TPpG by MALDI-TOF mass spectrometry.

Cell lines IC₅₀ (µM) Gelonin TPpG pH 7.4 pH 6.5 pH 7.4 pH 6.5 MCF-7 16.87 16.44 13.90 12.36 HT1080 13.63 13.17 7.77 5.20

Table S2. IC_{50} values of gelonin and TPpG against MCF-7 and HT1080 cells.