Supplementary Table S1.

Comprehensive physicochemical, synthetic, and quality-control profiles of candidate peptide constructs.

Detailed specifications of all parental constructs (PIP1-1 through PIP12-2), optimized chemical derivatives (cyclized, palmitoylated, truncated), and negative control comparators used in in vitro and in vivo studies. Synthesized via Fmoc-SPPS and validated by RP-HPLC and ESI-LC/MS.

Peptide Construct	Modifications (N / C-term)	Amino Acid Sequence (PIP – Spacer – CPP)	Synthesis Method & Manufacturer	Lot / Batch & Counter-ion	Purity (%) & Method	Observed M.W. (Da)	Stock Vehicle (Formulation)	Stock Conc. (mg/mL / µM)	Storage & Freeze-Thaw	Solubility & Stability Profile
PART A. Lead Optimization Scaffolds, Chemical Modifications, and Control Constructs (In Vivo & In Vitro Efficacy Cohorts)										
PIP4-1 (Lead)	N: Acetyl C: Amide	YRLNVDGVEIY GG CKSKRRRRRRSKRKD	Fmoc-SPPS (Peptron/GL)	Lot #P41-2025B TFA salt	95.84% (RP-HPLC)	3494.01	Sterile PBS (pH 7.4)	2.0 mg/mL (572.6 µM)	-80℃ lyoph. F/T ≤ 2 cycles	Soluble >10 mg/mL in PBS; stable >48 h at 37℃ in NBE
PIP8-1 (Lead)	N: Acetyl C: Amide	HVQIPVMLMPDDF GG CKSKRRRRRRSKRKD	Fmoc-SPPS (Peptron/GL)	Lot #P81-2025B TFA salt	96.58% (RP-HPLC)	3695.36	Sterile PBS (pH 7.4)	2.0 mg/mL (541.3 µM)	-80℃ lyoph. F/T ≤ 2 cycles	Soluble >8 mg/mL in PBS; stable >48 h at 37℃ in NBE
PIP4-1 cyclization 1 (N-term to mid Cys)	Cyclic (disulfide) C: Amide	C YRLNVDGVEIY C GG YKSKRRRRRRSKRKD	Fmoc-SPPS / Air-ox. (Peptron/GL)	Lot #P41C1-25 TFA salt	96.07% (RP-HPLC)	3716.29	Sterile PBS (pH 7.4)	2.0 mg/mL (538.2 µM)	-80℃ lyoph. F/T ≤ 1 cycle	Soluble >5 mg/mL; enhanced proteolytic stability (>72 h)
PIP8-1 cyclization 1 (N-term to mid Cys)	Cyclic (disulfide) C: Amide	C HVQIPVMLMPDDF C GG YKSKRRRRRRSKRKD	Fmoc-SPPS / Air-ox. (Peptron/GL)	Lot #P81C1-25 TFA salt	95.21% (RP-HPLC)	3917.64	Sterile PBS (pH 7.4)	2.0 mg/mL (510.5 µM)	-80℃ lyoph. F/T ≤ 1 cycle	Soluble >5 mg/mL; enhanced proteolytic stability (>72 h)
PIP4-1 cyclization 2 (Terminal loop)	Cyclic (end-to-end) Disulfide bridge	C YRLNVDGVEIY GG YKSKRRRRRRSKRKD C	Fmoc-SPPS / Air-ox. (Peptron/GL)	Lot #P41C2-25 TFA salt	96.42% (RP-HPLC)	3717.27	Sterile PBS (pH 7.4)	2.0 mg/mL (538.0 µM)	-80℃ lyoph. F/T ≤ 1 cycle	Soluble >5 mg/mL in PBS; stable >72 h at 37℃
PIP8-1 cyclization 2 (Terminal loop)	Cyclic (end-to-end) Disulfide bridge	C HVQIPVMLMPDDF GG YKSKRRRRRRSKRKD C	Fmoc-SPPS / Air-ox. (Peptron/GL)	Lot #P81C2-25 TFA salt	95.23% (RP-HPLC)	3918.62	Sterile PBS (pH 7.4)	2.0 mg/mL (510.4 µM)	-80℃ lyoph. F/T ≤ 1 cycle	Soluble >5 mg/mL in PBS; stable >72 h at 37℃
PIP4-1 palmitoylated	N: Palmitoyl (C16) C: Amide	YRLNVDGVEIY GG CKSKRRRRRRSKRKD	Fmoc-SPPS (Peptron/GL)	Lot #P41Palm-25 TFA salt	97.45% (RP-HPLC)	3690.38	Sterile PBS (0.05% Tween-20)	2.0 mg/mL (541.9 µM)	-80℃ lyoph. Avoid repeat F/T	Lipophilic; solubilized with trace surfactant; stable >48 h
PIP8-1 palmitoylated	N: Palmitoyl (C16) C: Amide	HVQIPVMLMPDDF GG CKSKRRRRRRSKRKD	Fmoc-SPPS (Peptron/GL)	Lot #P81Palm-25 TFA salt	97.01% (RP-HPLC)	3891.73	Sterile PBS (0.05% Tween-20)	2.0 mg/mL (513.9 µM)	-80℃ lyoph. Avoid repeat F/T	Lipophilic; solubilized with trace surfactant; stable >48 h
PIP4-1 truncated (Shortened form)	N: Acetyl C: Amide	RLNVDGVEIY GG YKSKRRRRRRSKRKD	Fmoc-SPPS (Peptron/GL)	Lot #P41Sh-25 TFA salt	97.05% (RP-HPLC)	3390.87	Sterile PBS (pH 7.4)	2.0 mg/mL (589.8 µM)	-80℃ lyoph. F/T ≤ 2 cycles	Highly soluble (>15 mg/mL); stable in PBS
PIP8-1 truncated (Shortened form)	N: Acetyl C: Amide	HVQIPVMLMPD GG YKSKRRRRRRSKRKD	Fmoc-SPPS (Peptron/GL)	Lot #P81Sh-25 TFA salt	95.75% (RP-HPLC)	3493.13	Sterile PBS (pH 7.4)	2.0 mg/mL (572.5 µM)	-80℃ lyoph. F/T ≤ 2 cycles	Highly soluble (>15 mg/mL); stable in PBS
PIP4 Scrambled (Negative Control)	N: Acetyl C: Amide	NKDSRKR DRGVKGIRRVCRERYKYGRLS	Fmoc-SPPS (Peptron/GL)	Lot #P4Scr-25 TFA salt	95.45% (RP-HPLC)	3494.01	Sterile PBS (pH 7.4)	2.0 mg/mL	-80℃ lyoph.	Soluble >10 mg/mL in PBS; stably

								(572.6 µM)	F/T ≤ 2 cycles	maintained control
PART B. Parental PIP Screening Panel — RMAD1 CPP Conjugates (Series -1; Carrier: CKSKRRRRRRSKRKD)										
PIP1-1	N: Ac / C: Amide	PIP1 domain GG CKSKRRRRRRSKRKD	Fmoc-SPPS (Peptron)	Lot #P011-24 / TFA	>95% (HPLC)	4190	1 mM HOAc in PBS (pH 7.0)	1.0 mg/mL (238.7 µM)	-80℃; F/T ≤2	Soluble in mildly acidic PBS
PIP2-1	N: Ac / C: Amide	PIP2 domain GG CKSKRRRRRRSKRKD	Fmoc-SPPS (Peptron)	Lot #P021-24 / TFA	>95% (HPLC)	4198	Sterile PBS (pH 7.4)	2.0 mg/mL (476.4 µM)	-80℃; F/T ≤2	Freely soluble in PBS
PIP3-1	N: Ac / C: Amide	PIP3 domain GG CKSKRRRRRRSKRKD	Fmoc-SPPS (Peptron)	Lot #P031-24 / TFA	>95% (HPLC)	4071	Sterile PBS (pH 7.4)	2.0 mg/mL (491.3 µM)	-80℃; F/T ≤2	Freely soluble in PBS
PIP4-1 (Screen)	N: Ac / C: Amide	YRLNVDGVEIY GG CKSKRRRRRRSKRKD	Fmoc-SPPS (Peptron)	Lot #P041-24 / TFA	95.84% (HPLC)	3493	Sterile PBS (pH 7.4)	2.0 mg/mL (572.6 µM)	-80℃; F/T ≤2	Freely soluble; lead candidate
PIP5-1	N: Ac / C: Amide	PIP5 domain GG CKSKRRRRRRSKRKD	Fmoc-SPPS (Peptron)	Lot #P051-24 / TFA	>95% (HPLC)	4026	Sterile PBS (pH 7.4)	2.0 mg/mL (496.8 µM)	-80℃; F/T ≤2	Freely soluble in PBS
PIP6-1	N: Ac / C: Amide	PIP6 domain GG CKSKRRRRRRSKRKD	Fmoc-SPPS (Peptron)	Lot #P061-24 / TFA	>95% (HPLC)	3736	Sterile PBS (pH 7.4)	2.0 mg/mL (535.3 µM)	-80℃; F/T ≤2	Freely soluble in PBS
PIP7-1	N: Ac / C: Amide	PIP7 domain GG CKSKRRRRRRSKRKD	Fmoc-SPPS (Peptron)	Lot #P071-24 / TFA	>95% (HPLC)	4190	Sterile PBS (pH 7.4)	2.0 mg/mL (477.3 µM)	-80℃; F/T ≤2	Freely soluble in PBS
PIP8-1 (Screen)	N: Ac / C: Amide	HVQIPVMLMPDDF GG CKSKRRRRRRSKRKD	Fmoc-SPPS (Peptron)	Lot #P081-24 / TFA	96.58% (HPLC)	3694	Sterile PBS (pH 7.4)	2.0 mg/mL (541.3 µM)	-80℃; F/T ≤2	Freely soluble; lead candidate
PIP9-1	N: Ac / C: Amide	PIP9 domain GG CKSKRRRRRRSKRKD	Fmoc-SPPS (Peptron)	Lot #P091-24 / TFA	>95% (HPLC)	4703	1 mM HOAc in PBS (pH 7.0)	2.0 mg/mL (425.3 µM)	-80℃; F/T ≤2	Soluble in mildly acidic PBS
PIP10-1	N: Ac / C: Amide	PIP10 domain GG CKSKRRRRRRSKRKD	Fmoc-SPPS (Peptron)	Lot #P101-24 / TFA	>95% (HPLC)	4673	Sterile PBS (pH 7.4)	2.0 mg/mL (428.0 µM)	-80℃; F/T ≤2	Freely soluble in PBS
PIP11-1	N: Ac / C: Amide	PIP11 domain GG CKSKRRRRRRSKRKD	Fmoc-SPPS (Peptron)	Lot #P111-24 / TFA	>95% (HPLC)	4217	Sterile PBS (pH 7.4)	2.0 mg/mL (474.3 µM)	-80℃; F/T ≤2	Freely soluble in PBS
PIP12-1	N: Ac / C: Amide	PIP12 domain GG CKSKRRRRRRSKRKD	Fmoc-SPPS (Peptron)	Lot #P121-24 / TFA	>95% (HPLC)	5155	Sterile PBS (pH 7.4)	2.0 mg/mL (388.0 µM)	-80℃; F/T ≤2	Freely soluble in PBS
PART C. Parental PIP Screening Panel — RMMR1 CPP Conjugates (Series -2; Alternative Carrier Conjugates)										
PIP1-2	N: Ac / C: Amide	PIP1 domain GG RMMR1 carrier	Fmoc-SPPS (Peptron)	Lot #P012-24 / TFA	>95% (HPLC)	3857	1 mM HOAc in PBS (pH 7.0)	1.0 mg/mL (259.3 µM)	-80℃; F/T ≤2	Soluble in mildly acidic PBS

PIP2-2	N: Ac / C: Amide	PIP2 domain GG RMMR1 carrier	Fmoc-SPPS (Peptron)	Lot #P022-24 / TFA	>95% (HPLC)	3865	Sterile PBS (pH 7.4)	2.0 mg/mL (517.5 µM)	-80℃; F/T ≤2	Freely soluble in PBS
PIP3-2	N: Ac / C: Amide	PIP3 domain GG RMMR1 carrier	Fmoc-SPPS (Peptron)	Lot #P032-24 / TFA	>95% (HPLC)	3737	Sterile PBS (pH 7.4)	2.0 mg/mL (535.2 µM)	-80℃; F/T ≤2	Freely soluble in PBS
PIP4-2	N: Ac / C: Amide	PIP4 domain GG RMMR1 carrier	Fmoc-SPPS (Peptron)	Lot #P042-24 / TFA	>95% (HPLC)	3160	Sterile PBS (pH 7.4)	2.0 mg/mL (633.0 µM)	-80℃; F/T ≤2	Freely soluble in PBS
PIP5-2	N: Ac / C: Amide	PIP5 domain GG RMMR1 carrier	Fmoc-SPPS (Peptron)	Lot #P052-24 / TFA	>95% (HPLC)	3692	1 mM HOAc in PBS (pH 7.0)	1.0 mg/mL (270.8 µM)	-80℃; F/T ≤2	Soluble in mildly acidic PBS
PIP6-2	N: Ac / C: Amide	PIP6 domain GG RMMR1 carrier	Fmoc-SPPS (Peptron)	Lot #P062-24 / TFA	>95% (HPLC)	3403	Sterile PBS (pH 7.4)	2.0 mg/mL (587.7 µM)	-80℃; F/T ≤2	Freely soluble in PBS
PIP7-2	N: Ac / C: Amide	PIP7 domain GG RMMR1 carrier	Fmoc-SPPS (Peptron)	Lot #P072-24 / TFA	>95% (HPLC)	3856	Sterile PBS (pH 7.4)	2.0 mg/mL (518.6 µM)	-80℃; F/T ≤2	Freely soluble in PBS
PIP8-2	N: Ac / C: Amide	PIP8 domain GG RMMR1 carrier	Fmoc-SPPS (Peptron)	Lot #P082-24 / TFA	>95% (HPLC)	3361	1 mM HOAc in PBS (pH 7.0)	1.0 mg/mL (297.5 µM)	-80℃; F/T ≤2	Soluble in mildly acidic PBS
PIP9-2	N: Ac / C: Amide	PIP9 domain GG RMMR1 carrier	Fmoc-SPPS (Peptron)	Lot #P092-24 / TFA	>95% (HPLC)	4369	1 mM HOAc in PBS (pH 7.0)	2.0 mg/mL (457.8 µM)	-80℃; F/T ≤2	Soluble in mildly acidic PBS
PIP10-2	N: Ac / C: Amide	PIP10 domain GG RMMR1 carrier	Fmoc-SPPS (Peptron)	Lot #P102-24 / TFA	>95% (HPLC)	4339	1 mM HOAc in PBS (pH 7.0)	1.0 mg/mL (230.5 µM)	-80℃; F/T ≤2	Soluble in mildly acidic PBS
PIP11-2	N: Ac / C: Amide	PIP11 domain GG RMMR1 carrier	Fmoc-SPPS (Peptron)	Lot #P112-24 / TFA	>95% (HPLC)	3884	Sterile PBS (pH 7.4)	2.0 mg/mL (515.0 µM)	-80℃; F/T ≤2	Freely soluble in PBS
PIP12-2	N: Ac / C: Amide	PIP12 domain GG RMMR1 carrier	Fmoc-SPPS (Peptron)	Lot #P122-24 / TFA	>95% (HPLC)	4281	Sterile PBS (pH 7.4)	2.0 mg/mL (467.2 µM)	-80℃; F/T ≤2	Freely soluble in PBS

Abbreviations: Ac, acetyl; Amide, C-terminal -CONH2; CPP, cell-penetrating peptide; F/T, freeze-thaw cycles; Fmoc-SPPS, fluorenylmethoxycarbonyl solid-phase peptide synthesis; HOAc, acetic acid; HPLC, high-performance liquid chromatography; M.W., molecular weight (daltons); NBE, Neurobasal/N2/B27/EGF/bFGF complete culture medium; PBS, phosphate-buffered saline; PIP, phosphatidylinositol phosphate-binding motif; RMAD1 / RMMR1, polybasic arginine-rich carrier motifs; TFA, trifluoroacetic acid salt.

Note: Analytical purity determined by reverse-phase HPLC (C18 column, linear gradient acetonitrile/water containing 0.1% TFA, detection at 214/220 nm). Observed molecular weights validated via electrospray ionization liquid chromatography-mass spectrometry (ESI-LC/MS). Freeze-thaw stability evaluated by HPLC peak retention and visual dissolution assessment.

Supplementary Table S2

Patient-derived and murine model panel with PTEN and PIP4K2A protein status and experimental use

Model	Model type	Species and context	PTEN protein status	PIP4K2A protein status	Experimental use in this study
NHA	Normal human astrocytes	Human; nonmalignant reference	Detected	Detected	Immunoblot reference
PDC1	Patient-derived glioblastoma culture	Human glioblastoma	Not detected	Not detected	Initial 2D and 3D peptide screening; concentration-response assays; peptide–temozolomide testing
PDC2	Patient-derived glioblastoma culture	Human glioblastoma	Detected	Not detected	PTEN/PIP4K2A molecular characterization
PDC3	Patient-derived glioblastoma culture	Human glioblastoma	Detected	Not detected	PTEN/PIP4K2A molecular characterization
PDC4	Patient-derived glioblastoma culture and xenograft	Human glioblastoma	Not detected	Not detected	Fixed-dose peptide testing; peptide optimization; peptide–temozolomide testing; subcutaneous xenograft pilot
PDC5	Patient-derived glioblastoma culture	Human glioblastoma	Not detected	Detected	PTEN/PIP4K2A molecular characterization
PDC6	Patient-derived glioblastoma culture	Human glioblastoma	Detected	Weakly detected	PTEN/PIP4K2A molecular characterization
PDC7	Patient-derived glioblastoma culture	Human glioblastoma	Not detected	Weakly detected	Fixed-dose peptide-response experiment; included in the Figure 2A immunoblot
GL26	Murine glioma cell line	Mouse glioma	PTEN-deficient	Not evaluated	Exploratory PTEN-differential peptide and temozolomide viability comparison
GL261	Murine glioma cell line	Mouse glioma	PTEN-expressing	Not evaluated	Exploratory PTEN-differential peptide and temozolomide viability comparison
HaCaT	Keratinocyte cell line	Human; nonmalignant	Not evaluated	Not evaluated	Preliminary cellular tolerability assessment
Normal human liver organoid	Primary organoid model	Human; nonmalignant	Not evaluated	Not evaluated	Preliminary viability and albumin-based tolerability assessment

Notes. PTEN and PIP4K2A entries for NHA and PDC1–PDC7 are descriptive interpretations of the immunoblot shown in Figure 2A. ‘Not detected’ indicates that no band was detected under the evaluated immunoblot conditions and does not establish complete biological absence. ‘Weakly detected’ denotes a low-intensity band and is descriptive. ‘Not determined’ indicates that the model was not included in the displayed immunoblot; ‘Not evaluated’ indicates that the relevant assay was not performed. X-682T was not included in Figure 2A. PIP4K2A status was not evaluated in GL26 or GL261. Additional molecular variables, including IDH status, MGMT promoter methylation, and MGMT expression, were not available in the supplied records and were not inferred. GL26 and GL261 are biologically distinct murine glioma models and should not be described as a genetically matched pair.