Table S1. Key stages and molecular mechanisms of macropinocytosis

Stage	Cellular event	Key molecules	Function and mechanism	References
Initiation and formation	Membrane ruffling and microdomain formation	PI3K, PIP3, PTEN	PI3K promotes PIP3 accumulation and local PTEN exclusion, thereby establishing stable lipid-signaling microdomains that provide a spatiotemporal platform for effector recruitment.	[29]
	Small GTPase activation	RhoG, CDC42, RAC1, GEF complexes	RhoG and CDC42 recruit and activate RAC1 through GEF complexes, such as ELMO/DOCK, thereby initiating actin reorganization.	[31]
	Membrane protrusion and actin polymerization	SCAR/WAVE, Arp2/3, Ena/VASP	RAC1 activates SCAR/WAVE-Arp2/3 to drive branched actin nucleation and cooperates with Ena/VASP to promote membrane protrusion.	[32]
	Cup closure	Formins, RhoC, RhoA, ROCK-MLC	Formins stabilize the cup base. RhoC facilitates closure initiation, whereas RhoA-ROCK-MLC-mediated actomyosin contraction seals the macropinosome.	[11]
Maturation	Phosphoinositide conversion and Rab switching	PIP3, VPS34, Rab5, Rab7	PIP3 is converted to PI(3,4)P_2 and PI(3)P. Rab5 recruits VPS34 to promote PI(3)P	[37-40, 42]

			generation, whereas Rab7 replacement marks the transition to the late endosomal stage.	
	Shrinkage and acidification	TPC1/2, ASOR, V-ATPase	TPC1/2-mediated Na^+ efflux and ASOR-mediated Cl^- efflux drive osmotic water loss and macropinosome shrinkage, while V-ATPase supports luminal acidification.	[36]
Recycling and degradation	Sorting and recycling	Rab4, Rab11, WASH, SNX-Retromer	WASH supports actin-dependent membrane recycling, Rab4 and Rab11 regulate recycling routes, and SNX-retromer machinery participates in selective cargo retrieval.	[16, 44, 45]
	Degradation	PIKfyve, $PI(3,5)P_2$, TRPML1	PIKfyve generates $PI(3,5)P_2$ to regulate fusion with lysosomes, whereas TRPML1 maintains lysosomal calcium homeostasis and acidity to support efficient proteolysis.	[46, 47]

Table S2. Representative macropinocytosis-based delivery strategies

Strategy	Representative system	Payload	Disease/cell model	In vivo validation model	Macropinocytosis-related mechanism	Evidence for macropinocytic involvement	Quantitative metrics	Clinical status	References
Albumin	DOX-ALB	DOX	PDAC (MIA PaCa-2 and BxPC-3 cells; KRAS/FcRn-stratified models)	MIA PaCa-2 and BxPC-3 cell-derived xenografts	Albumin conjugation exploits KRAS-enhanced macropinocytic uptake and reduced FcRn-mediated recycling to improve intracellular DOX delivery.	EIPA dose-dependent inhibition (75 μM); 30 min pretreatment; flow cytometry	Approximately 10-fold wider therapeutic window than free DOX in KRAS-mutant, FcRn-low PDAC models	Preclinical	[106]

	Alb-vc-MMAE	MMAE	ATC	Orthotopic ATC	Constitutively elevated macropinocytosis supporting uptake of albumin conjugates in a MAPK/ERK-dependent context.	70 kDa dextran/albumin colocalization; EIPA (100 μM, 5 h pretreatment); 4 h albumin uptake; fluorescence microscopy	62% lower albumin uptake with EIPA; albumin tracer accumulation of 8.8% ID/g in tumors	Preclinical	[181]
	nab-(pro-β-lap)	β-lapachone	PDAC	Subcutaneous KPC xenograft	KRAS-enhanced macropinocytosis and reduced FcRn-mediated recycling promote the uptake of albumin nanoparticles.	70 kDa FITC-dextran (1.5 mg/mL) colocalization; EIPA inhibition; 4 h uptake; confocal microscopy	2–5-fold higher uptake in KRAS-mutant cells; 3-fold higher MTD; 58% lower tumor volume	Preclinical	[180]

	DF-HSA	HBD2	PDAC	MIA PaCa-2 xenograft	Enhanced macropinocytosis in KRAS-mutant PDAC promotes uptake of the HBD2-HSA fusion protein.	70 kDa TMR-dextran (1 mg/mL) colocalization; EIPA (25 and 75 μM); 2 h uptake; confocal microscopy	IC_{50}: 1.0 ± 0.08 μM in MIA PaCa-2 cells versus 18.7 ± 1.21 μM in BxPC-3 cells; 56.6–81.2% TGI at 10–30 mg/kg in MIA PaCa-2 xenografts	Preclinical	[182]
	DF_2-HSA	HBD2	PDAC	MIA PaCa-2 xenograft	Enhanced macropinocytosis in KRAS-mutant PDAC promotes uptake of the HBD2-HSA fusion protein.	EIPA (75 μM, 2 h co-treatment); confocal microscopy and flow cytometry	2.2-fold lower IC_{50} in KRAS-mutant cells; tumor retention for more than 14 d; 64.8% TGI alone and 88.4% with GEM	Preclinical	[183]

	Pal-cyclic-(D-Arg)$_{12}$/HSA	Various cargo	HeLa	Not evaluated	HSPG/CXCR4 engagement with PKC-PI3K-JNK-mTOR-driven macropinocytic uptake of albumin complexes.	70 kDa TMR-dextran (0.5 mg/mL); EIPA (100 μM); 30 min co-treatment; confocal microscopy	Approximately 90% lower uptake with HSPG inhibition; approximately 20% lower uptake with CXCR4 inhibition	Preclinical	[184]
	Fv-LDP-D3	LDP	PDAC	AsPC-1 xenograft	EGFR-mediated recognition plus macropinocytic uptake of albumin-fusion carriers.	70 kDa TMR-dextran (5 μM); EIPA (50 μM); 30 min uptake; confocal microscopy and flow cytometry	Tumor fluorescence retained for 360 h; 58% TGI	Preclinical	[185]
	F-HSA-LDP-AE	AE/LDP	PDAC	AsPC-1 xenograft	Folate-receptor targeting plus macropinocytic uptake of albumin-fusion carriers.	70 kDa TMR-dextran; EIPA (50 μM); 2 h co-treatment; fluorescence microscopy	Tumor retention for 360 h; 91.2% and 94.8% TGI at 0.5 and 1 mg/kg, respectively	Preclinical	[186]

Lipoprotein	ApoE-rHDL	siRNA targeting ATF5	GBM/GICs	Intracranial C6/GIC GBM	ApoE3-reconstituted HDL exploits Ras-associated macropinocytosis for siRNA delivery; the ApoE3-containing carrier may also enhance macropinocytic uptake.	70 kDa FITC-dextran (1 mg/mL); amiloride (5 mM) and EIPA (25 μM, 30 min pretreatment); 1 h uptake; confocal microscopy and flow cytometry	82.5% dextran colocalization; approximately 80% lower uptake with amiloride or EIPA; Tumor-cell apoptosis rates of 54% and 43% in C6 and patient-derived GIC xenografts, respectively	Preclinical	[193]
	PLCNP	Porphyrin/ CaO_2	GBM/GICs	GIC-derived orthotopic GBM	Lipoprotein-coated nanoparticles exploit macropinocytic uptake by GBM cells for intracellular delivery of porphyrin and calcium peroxide.	70 kDa FITC-dextran (1 mg/mL, 1.5 h); EIPA (150 μM, 1 h pretreatment); confocal microscopy and flow cytometry	37.67% dextran colocalization; approximately 70% lower cellular uptake with EIPA; 73.83% lower GBM volume	Preclinical	[194]

	FH38-HDL	miR-34a	GICs	Patient-derived GIC orthotopic GBM	FH38-HDL exploits CXCR4-triggered macropinocytosis to enhance uptake in GICs.	70 kDa FITC-dextran (1 mg/mL, 1.5 h); EIPA (150 μM, 1 h pretreatment); confocal microscopy and flow cytometry	Approximately 3-fold higher uptake of FH38-DiI-SLNPs than DiI-LNPs in GICs after 3.5 h; more than 65% lower uptake with EIPA; 30–36% SOX2-positive GIC capture	Preclinical	[195]
	pGpC-rHDL	pCBD/pGEM	PDAC/KPC1199	Orthotopic PDAC	4F-rHDL exploits PDAC nutrient-scavenging and macropinocytic uptake to enhance drug delivery.	FITC-dextran colocalization (2 h); amiloride and EIPA (0.5 h pretreatment); 2 h uptake; fluorescence microscopy and HCS analysis	Approximately 50% lower uptake with amiloride or EIPA; 3.5-fold higher tumor accumulation at 24 h; 84.5% lower tumor volume	Preclinical	[196]

	R837-αOVA-ApoE3-HNP	R837/α-OVA	BMDCs/B16-OVA melanoma	B16-OVA lung metastasis and subcutaneous tumor models	ApoE3-facilitated macropinocytic internalization by dendritic cells.	FITC-dextran colocalization (2 h); amiloride and EIPA (5 mM, 30 min pretreatment); fluorescence microscopy and flow cytometry	0.66 dextran colocalization coefficient; 54.9% and 75.5% lower uptake with amiloride and EIPA; 7.56-fold higher antigen-specific $CD8^+$ T cells; lung metastases in only 2/6 mice	Preclinical	[197]
Exosome	iExosomes	KRAS-targeting siRNA	PDAC/PANC-1	Orthotopic PANC-1, KPC/KTC GEMMs	CD47-dependent persistence/immune evasion with macropinocytosis-facilitated uptake enables efficient delivery of KRAS-targeting RNA cargo in PDAC.	TMR-dextran (1 mg/mL, 30 min); EIPA (5, 25, and 75 μM, 30 min pretreatment); fluorescence microscopy and ImageJ analysis	Approximately 85–90% lower exosome uptake with 75 μM EIPA; 100% mouse survival at day 87	Phase I/II, recruiting (NCT03608631)	[206, 207]

	M-LIP-CLT	Celastrol	PDAC/PANC-1	Subcutaneous PANC-1 xenograft	KRAS-associated macropinocytosis.	EIPA (50 μM, 30 min pretreatment); 2 h uptake; flow cytometry and confocal microscopy	More than 4-fold higher PANC-1 uptake; 2.44-fold higher tumor accumulation at 48 h; 50% mouse survival at day 110	Preclinical	[214]
	HeLa-derived exosomes	EGF/Saporin	A431/MIA PaCa-2	Not evaluated	EGF/EGFR-stimulated macropinocytic uptake of exosomes.	70 kDa Texas Red/FITC-dextran (0.5 mg/mL, 24 h); EIPA (20 μM, 30 min pretreatment); confocal microscopy and flow cytometry	Approximately 27-fold higher exosome uptake with EGF stimulation; approximately 14-fold higher uptake in MIA PaCa-2 than BxPC-3 cells	Preclinical	[61]

	K4-modified exosomes	Saporin	BC/MDA-MB-231	Not evaluated	EGFR clustering-induced macropinocytic uptake.	EIPA (100 μM, 1 h co-treatment); rhodamine-phalloidin F-actin staining (20 min); flow cytometry and confocal microscopy	Approximately 2-fold higher exosome uptake in E3-EGFR-expressing cells; approximately 20% higher uptake in wild-type cells	Preclinical	[208]
	Stearyl-R8-modified microvesicles	Saporin	HeLa/A431 cells	Not evaluated	Stearyl-R8 induces syndecan-4/PKCα-associated actin remodeling and macropinocytosis, enhancing EV uptake.	70 kDa FITC/Texas Red-dextran (0.5 mg/mL, 24 h); EIPA (100 μM, 30 min pretreatment); flow cytometry and confocal microscopy	1.22-fold higher dextran uptake and 190-fold higher MV uptake with stearyl-R8	Preclinical	[211]

Liposome	RGD-LP	DOX	HRT-18	Subcutaneous HRT-18 xenograft	RGD modification enhances cell association, with macropinocytosis reported to contribute to subsequent liposome internalization.	Wortmannin (30 μg/mL, 4 h co-treatment); flow cytometry	1.47-fold lower IC_{50} than LP/DOX	Preclinical	[218]
	89WP-LP	si-c-Myc	GBM	Orthotopic U87 GBM	89WP-modified lipoplexes associate with necrotic cell debris and undergo macropinocytic co-internalization by glioma cells.	Wortmannin (200 nM) and amiloride (0.5 mM, 2 h pretreatment); 4 h co-treatment; flow cytometry and confocal microscopy	Approximately 5-fold higher cellular uptake; 3-fold higher glioma accumulation; 4-fold higher brain siRNA accumulation; 2-fold lower tumor c-Myc expression	Preclinical	[219]

	LPP	mRNA	Dendritic cells	B16-OVA lung metastasis	Macropinocytic internalization by dendritic cells enables mRNA delivery.	Amiloride (0.2 mM, 4 h co-treatment); cytochalasin D (4 h co-treatment); fluorescence microscopy	70% and 80% lower uptake with amiloride and cytochalasin D; 96% fewer pulmonary tumor nodules	Preclinical	[222]
Virus	Ad-PPCBA	VEGF shRNA	U87/MCF-7	Subcutaneous U87 xenograft	Acid-triggered activation with macropinocytosis-mediated internalization.	NH_4Cl (0.5 and 1 mM, 30 min pretreatment); 2 h co-treatment; fluorescence microscopy and flow cytometry	475.2-fold higher transduction in CAR-negative MCF-7 cells at pH 6.0; 91.1% and 65.0% TGI after intratumoral and intravenous administration	Preclinical	[224]

Peptide	MPD3	Docetaxel	PDAC/TNBC	Orthotopic MDA-MB-436 xenograft / lung metastasis	Albumin hitchhiking plus KRAS/PTEN-associated macropinocytosis.	FITC-albumin (10 μg/mL, 24 h); EIPA (10 μM, 4 h pretreatment); flow cytometry and confocal microscopy	64.6% TGI with MPD3 and 118.8% with olaparib/MPD3 in MDA-MB-436 xenografts; approximately 91% lower lung metastatic BLI with the combination	Preclinical	[233, 235]

	MPD1 (PDC)	DOX	PDAC/CRC/NSCLC/TNBC	Multiple subcutaneous xenografts	Albumin hitchhiking with KRAS-enhanced albumin metabolism and macropinocytic uptake.	FITC-BSA (10 µg/mL, 24 h); EIPA (50 µg/mL, 4 h pretreatment); confocal microscopy and flow cytometry	21-fold higher albumin-positive cells; approximately 140-fold higher intratumoral free DOX in MIA PaCa-2 versus BxPC-3 xenografts after MPD1 treatment; 100% and 113% TGI at 5 and 10 mg/kg, respectively, in MIA PaCa-2 xenografts	Preclinical	[234]
	KGF-ELP/$(KLAKLAK)_2$-ELP nanoparticles	$(KLAKLAK)_2$	NSCLC/A549	Not evaluated	KGF/KGFR-mediated stimulation of macropinocytosis.	Rhodamine-dextran (100 µg/mL, 1 h); amiloride (20 µM) and wortmannin (10 µM); flow cytometry	70% and 75% lower uptake with amiloride and wortmannin; 21-fold higher uptake with KGF-ELP; 50–70% cell death in KGFR-high cells	Preclinical	[237]

	SN21-LK15	siRNA/proteins	HeLa	Not evaluated	CXCR4-associated macropinocytosis and endosomal escape.	70 kDa Dex70-FL (1 mg/mL, 30 min); EIPA (100 μM) and wortmannin (500 nM); flow cytometry and confocal microscopy	Approximately 75% higher dextran uptake with SN21; cytosolic IgG delivery in 61% of cells	Preclinical	[240]
	LK15-oxP4A	Proteins	HeLa	Not evaluated	P4A-driven macropinocytosis via disulfide-dependent conformational activation; CXCR4-independent.	70 kDa TMR-dextran (0.5 mg/mL, 30 min); EIPA (100 μM) and wortmannin (500 nM, 30 min pretreatment); flow cytometry and live-cell imaging	Approximately 2-fold higher dextran uptake; 68% nuclear delivery of NLS-GFP; 46% functional Cre delivery	Preclinical	[241]

	stearyl-oxP4A	mRNA	HeLa	Not evaluated	Membrane-anchored P4A-driven macropinocytic enhancement for extracellular vesicle uptake.	70 kDa TMR-dextran (0.5 mg/mL, 30 min); EIPA (80 μM) and wortmannin (500 nM, 30 min pretreatment); flow cytometry	2.7-fold higher sEV uptake	Preclinical	[242]
Nutrient	DEX-TP	Triptolide	PDAC/MIA PaCa-2, PANC-1, BxPC-3	Subcutaneous / orthotopic PDAC	Macropinocytic uptake of 70 kDa dextran enabling KRAS-selective delivery.	70 kDa FITC-dextran; EIPA (50 μM, 30 min pretreatment); 30 min uptake; flow cytometry and confocal microscopy	7-fold higher dextran uptake with $KRAS^{G12V}$; 3–10-fold higher IC_{50}-derived DEX-TP cellular deposition efficiency in KRAS-mutant versus wild-type cells; more than 3-fold higher tolerated TP dose	Preclinical	[253]

	Gln-IDS	V9302 / BSO	PDAC/MIA PaCa-2	Subcutaneous MIA PaCa-2 xenograft; in situ/metastatic MIA PaCa-2 model	Gln modification promotes nutrient-associated uptake, while V9302-induced Gln deprivation activates compensatory macropinocytosis and further enhances IDS internalization.	70 kDa FITC-dextran uptake; EIPA (50 μM, 6 h co-treatment); macropinocytic cup formation; imaging flow cytometry, confocal microscopy and TEM	10.7-fold higher uptake with V9302 loading; approximately 66% lower in vivo tumor-cell uptake with EIPA; approximately 71% lower endpoint tumor volume	Preclinical	[254]
Inorganic	Mesoporous silica nanorods	HCQ/DOX	MCF-7	Not evaluated	Nanorod geometry and HCQ-mediated suppression of clathrin-mediated endocytosis favor delivery through cancer-cell macropinocytosis.	DOX-NR-Cy5.5 uptake (24 h); amiloride inhibition; fluorescence microscopy	Approximately 75% lower nuclear DOX with amiloride; approximately 80% MCF-7 cell killing after 72 h	Preclinical	[257]

Note: The evidence summarized here reflects the methods used in the original studies. NH_4Cl is a broad inhibitor of endosomal/lysosomal acidification rather than a macropinocytosis-specific antagonist. Wortmannin is a pan-PI3K inhibitor that can also affect other endocytic processes and autophagy. Given these methodological

limitations and the lack of standardized criteria discussed in Section 6.4, mechanistic conclusions based solely on chemical inhibition should be interpreted cautiously, particularly when genetic or fluid-phase colocalization evidence is unavailable. TGI values follow the definitions used in the original studies.

Abbreviations: ATC: anaplastic thyroid carcinoma; BLI: bioluminescence imaging; BMDCs: bone marrow-derived dendritic cells; BNCT: boron neutron capture therapy; BSO: buthionine sulfoximine; CLSM: confocal laser scanning microscopy; GEM: gemcitabine; GEMMs: genetically engineered mouse models; HBD2: human β-defensin 2; HCS: high-content screening; IC_{50}: half-maximal inhibitory concentration; MTD: maximum tolerated dose; TGI: tumor growth inhibition; TMR: tetramethylrhodamine; %ID/g: percentage of injected dose per gram of tissue.

Delivery systems: ApoE-rHDL: apolipoprotein E-reconstituted high-density lipoprotein; DEX-TP: dextran-triptolide conjugate; DF-HSA: fusion protein of human beta-defensin 2 and human serum albumin; DF_2-HSA: fusion protein containing tandem human beta-defensin 2 and human serum albumin; F-HSA-LDP-AE: folate-targeted construct comprising human serum albumin, lidamycin apoprotein, and an enediyne toxin; FH38-HDL: FH38 peptide-modified high-density lipoprotein; Gln-IDS: glutamine-modified iron-delivery system; LPP: lipopolyplex; M-LIP-CLT: DC2.4 cell membrane-fused celastrol-loaded PEGylated liposome; MPD1: albumin-binding caspase-3-cleavable peptide-doxorubicin conjugate; MPD3: albumin-binding caspase-3-cleavable peptide-docetaxel conjugate; pGpC-rHDL: pGEM/pCBD-loaded 4F-modified reconstituted high-density lipoprotein; PLCNP: porphyrin-lipoprotein-coated calcium peroxide nanoparticle.

Table S3. Comparison of macropinocytosis-based delivery strategies

Delivery strategy	Design rationale	Major advantages	Major limitations	Suitable tumor contexts
Albumin delivery	Exploits the demand of tumors for extracellular protein uptake	High biocompatibility, low immunogenicity, and relatively strong translational potential	Can be diverted to TAMs, CAFs/PSCs, and normal tissues, limiting tumor cell selectivity	Tumors with high extracellular protein demand and active macropinocytosis, especially PDAC with KRAS mutations
Lipoprotein delivery	Uses lipoprotein-based carriers to exploit tumor-associated macropinocytosis, with functional modifications that can further enhance uptake	Suitable for hydrophobic cargo, easy surface modification, and potential for blood-brain barrier penetration in some systems	Often involves receptor-mediated endocytosis and other pathways, with strong cell-type dependence	Tumors with active macropinocytosis and high lipid demand, such as GBM/GIC
Exosome delivery	Exploits the high uptake of extracellular vesicles by tumor cells	Natural membrane structure, good biocompatibility, and suitability for nucleic acids and biological macromolecules	Multiple uptake routes, uptake by non-tumor cells, and limited standardization of source and preparation	Tumors with active macropinocytosis and high exosome uptake, with current evidence mainly in PDAC with KRAS alterations

Liposome delivery	Uses carrier modification or external stimuli to exploit or enhance macropinocytic uptake	Mature formulation technology, broad cargo compatibility, and tunable size and surface properties	Lacks an intrinsic preference for macropinocytosis and usually requires additional modification or stimulation	Tumors with active macropinocytosis, or those responsive to modifications or stimuli that enhance this uptake route.
Viral delivery	Exploits natural viral entry mechanisms and, in some cases, actively induces macropinocytosis	High cellular entry and gene transduction efficiency, with potential integration into gene therapy or oncolytic therapy	Only some viruses rely on macropinocytosis and delivery may be limited by native receptors, immune clearance, and infection of off-target tissues	Tumor contexts in which viral entry can exploit or induce macropinocytosis
Peptide delivery	Uses peptides to bind albumin, stimulate macropinocytosis, or promote co-internalization of nearby cargo	Simple structure, easy synthesis and functionalization, with potential integration of targeting and macropinocytosis induction	Induction efficiency is cell-dependent, while in vivo stability and membrane safety remain concerns	Tumors with active macropinocytosis or responsiveness to macropinocytosis-inducing peptides
Nutrient-camouflaged delivery strategies	Exploits tumor nutrient stress by mimicking accessible nutrient sources	Can simultaneously exploit metabolic demand and macropinocytic activity to enhance uptake	Highly dependent on tumor nutrient status and may also involve other uptake pathways	Tumors under high nutrient stress that depend on extracellular material uptake, especially some tumors with RAS/KRAS

				alterations
Inorganic nanomaterial delivery	Uses physicochemical properties to improve particle compatibility with macropinocytic uptake	High structural stability, tunable physicochemical properties, and easy integration with imaging or physical treatment functions	Uptake lacks pathway specificity, with additional concerns regarding in vivo accumulation and long-term safety	Solid tumors in which macropinocytosis contributes to particle uptake and can be further influenced by particle properties