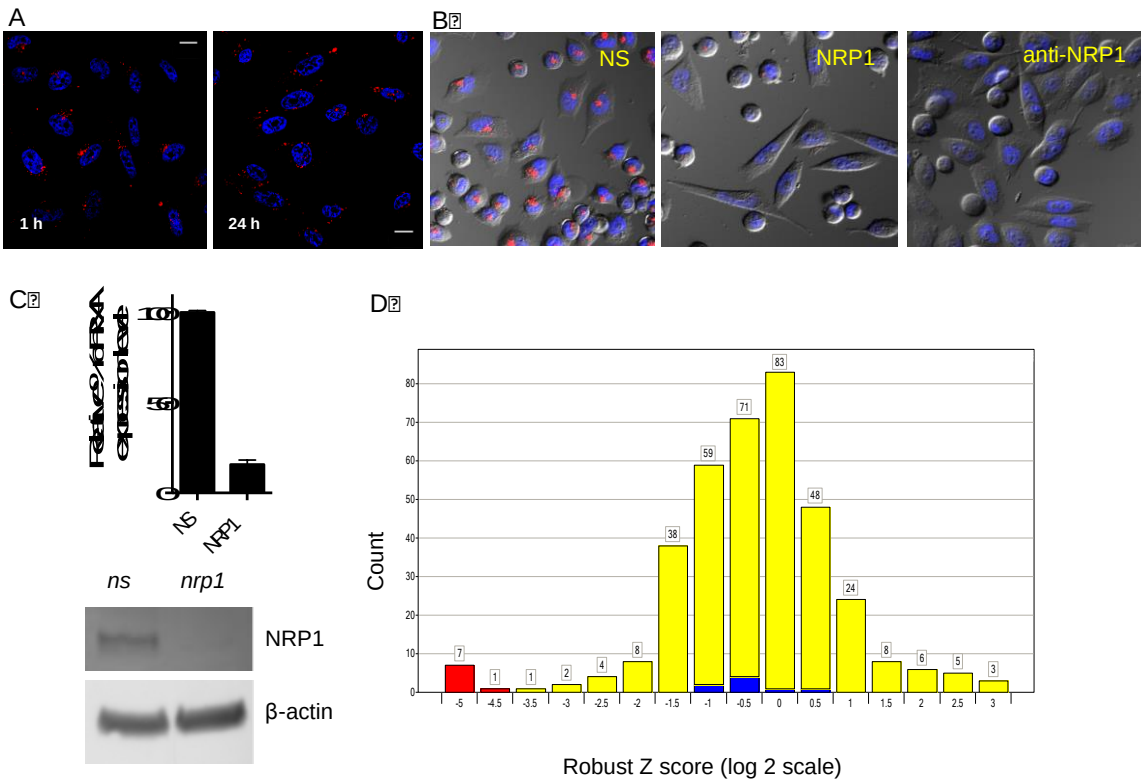
SUPPLEMENTARY FIGURES

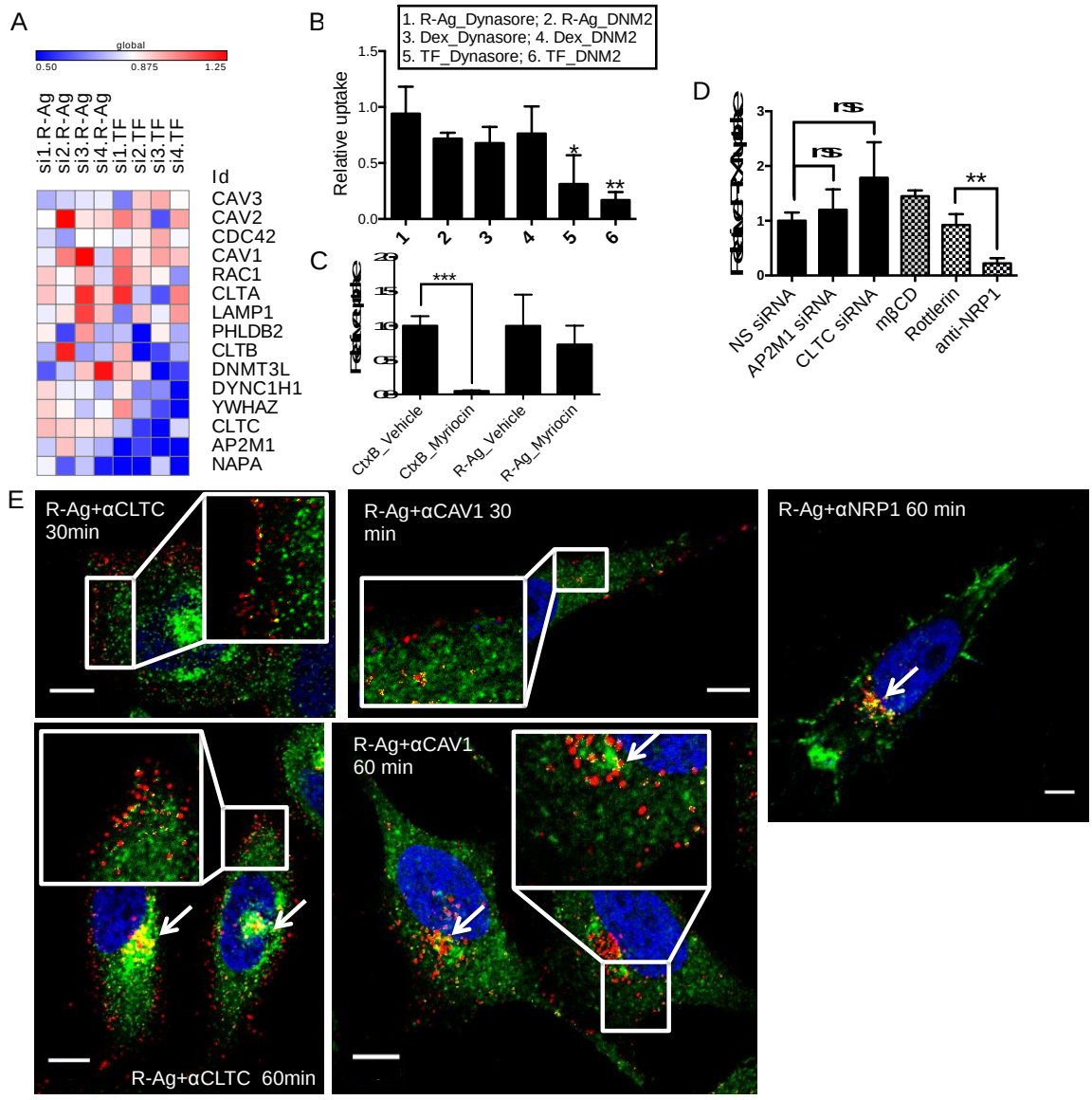


Supplementary Fig. 1. Optimization of screening assay

(A) *The fluorescence signal of R-Ag internalized into cells is stable.* PPC1 cells were incubated with 5 pM R-Ag for 1 h at 37°C. After etching, some wells were fixed with 4% PFA, while the rest were incubated for another 23 h before fixation. The fluorescence signal of internalized R-Ag was captured with confocal microscopy. Representative images are shown. Scale bar, 15 μm.

(B) *Effect of knocking down NRP1 on R-Ag uptake into cells*. PPC1 cells were treated with NRP1 siRNA or NS siRNA as described in Methods, or incubated with NRP1 blocking antibody (anti-NRP1) 10 min prior to addition of R-Ag. Representative images for these three conditions are shown (R-Ag, red; nuclei, blue).

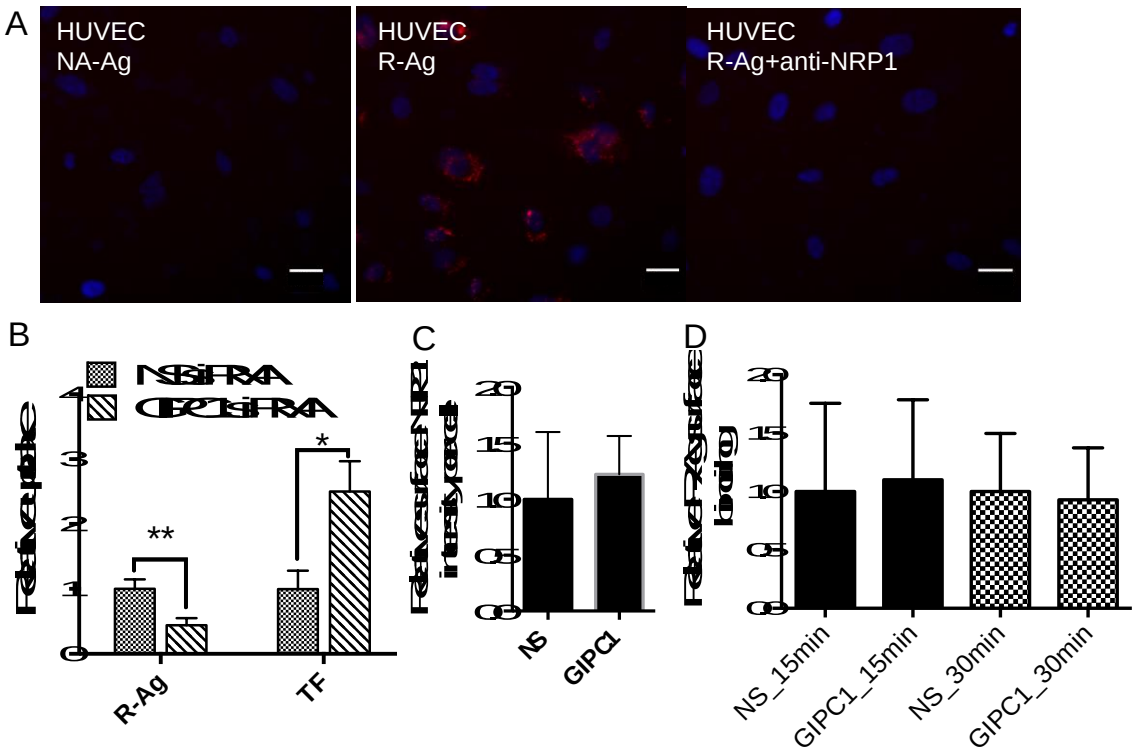
(C) *The efficacy of siRNA treatment at the mRNA and protein levels*. After treatment with NRP1 siRNA or NS siRNA, PPC1 cells were lysed to extract cellular mRNAs for qPCR analysis, or cellular proteins were separated on SDS-PAGE. Upper panel shows the knockdown efficiency at mRNA level (error bars, SEM, 3 replicates). Bottom panel: immunoblotting using anti-NRP1 and anti-β-actin antibodies (loading control).
(D) *R-Ag uptake after knockdown of randomly selected genes*. The assay was performed as described in Supplementary Results and the robust Z-score for each gene was calculated as described in Methods. Z-scores were divided into different zones and the values along the x-axis indicate the lowest values for each zone. The number of genes in each Z-score zone is shown on the y-axis. The negative (NS siRNA) and positive (NRP1 siRNA) controls are shown in blue and red, respectively, and the selected genes are in yellow.



Supplementary Fig. 2. CendR endocytosis is mechanistically distinct from known endocytic pathways.

(A) *Effect of knocking down selected genes known to be involved in endocytic pathways on the uptake of R-Ag and TF*. Four individual siRNA for each gene from genomic library were tested separately and the values represent relative probe uptake normalized to that of negative controls (which is 1, Supplementary File 2). The heat map was generated using Gene-E (Broad Institute).

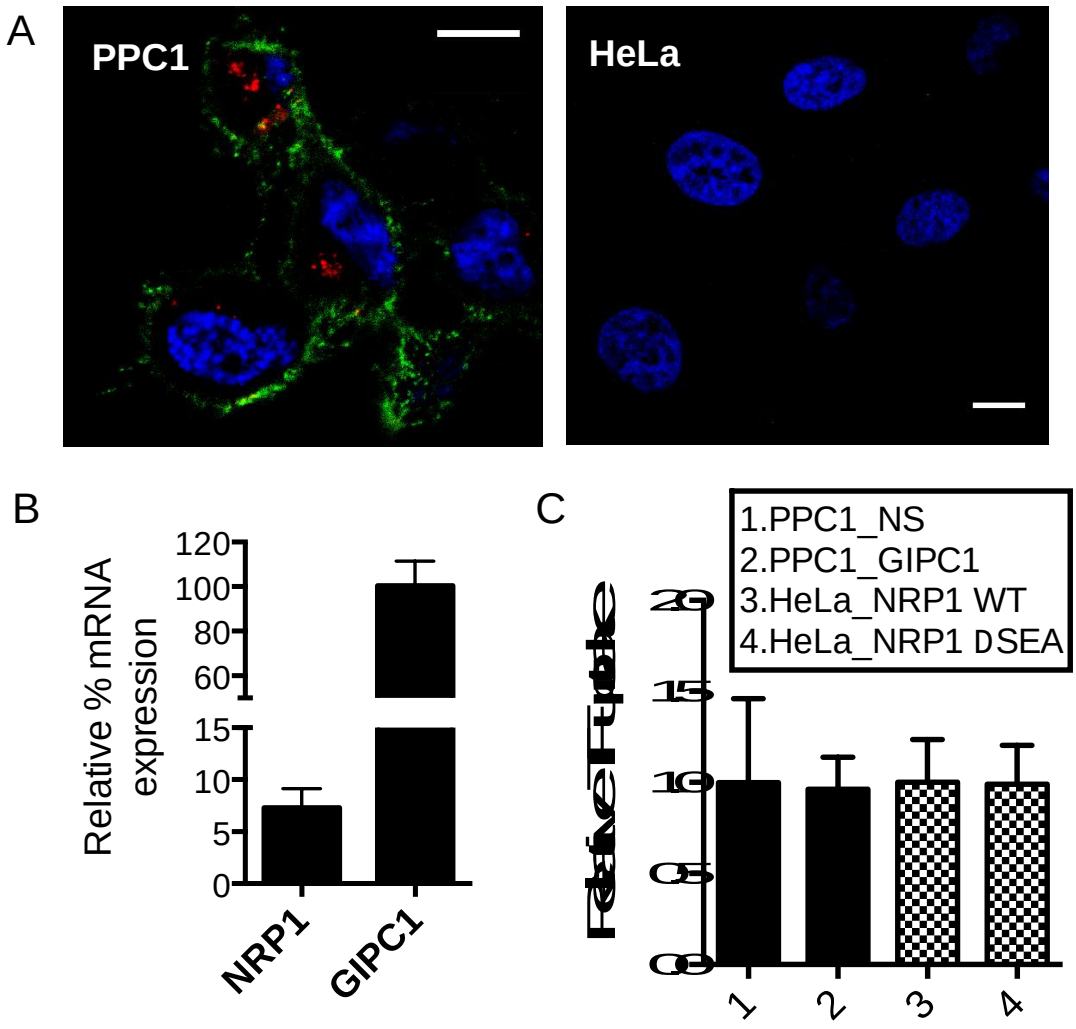
(B) *R-Ag uptake is independent of DNM2*. PPC1 cells were treated with Dynasore (20 μM in DMSO) for 1 h or DNM2 siRNA (DNM2), followed by testing for R-Ag, TF and Dex uptake as described in Methods. The fluorescence intensity of the probes per cell was quantified and normalized to the average of the corresponding negative controls (vehicle alone or NS siRNA) as relative uptake (y-axis). *P<0.05 and **P<0.01 in comparison to the corresponding negative controls (Student's t-test).
(C) *R-Ag uptake is resistant to Myriocin treatment*. PPC1 cells were treated with vehicle (DMSO alone) or Myriocin (20 μM) for 48 h. Cells were then incubated with CtxB or R-Ag, for internalization testing as described in Methods. The relative probe uptake was quantified as described in (B). ***P<0.001 (Student's t-test).
(D) *R-NA uptake depends little on known endocytic machineries*. PPC1 cells were treated with indicated siRNAs or inhibitors followed by testing for R-NA uptake as described in Methods. The relative R-NA uptake was quantified as described in (B) (negative controls: NS siRNA for siRNA treated ones; vehicle alone for inhibitor/anti-NRP1 blocking antibody treated ones). ns, not significant; **P<0.01 (Student's t-test).
(E) *Analysis of R-Ag colocalization with CLTC, CAV1 and NRP1 at later stages of cell entry and subcellular transport.* PPC1 cells were incubated with R-Ag (red) for 30 or 60 min, and then etched, washed and fixed. After permeabilization, CLTC, CAV1 and NRP1 proteins were detected with rabbit anti-CLTC and anti-CAV1 antibodies, or mouse anti-NRP1, followed by staining with the corresponding secondary antibody (green). Nuclei were labeled with Hoechst 33342 (blue). Representative images are shown. The colocalization events between R-Ag and CLTC/CAV1/NRP1 were identified with the "colocalization highlighter" macro for Image J and shown in yellow. Arrows point at colocalization events around the nucleus. Scale bar, 10 μm.
Error bars indicate SEM (3-4 replicates).



Supplementary Fig. 3. GIPC1/synectin is needed for CendR uptake.

(A) *HUVEC uptake of R-Ag is NRP1-dependent.* HUVEC cells were incubated with 5 pM R-Ag or NA-Ag for 3 h at 37°C. To block the activity of NRP1, an anti-NRP1 blocking antibody (20 µg ml^{-1}) was added to cells 10 min prior to addition of R-Ag. Representative images are shown (red, silver particles; blue, nuclei). Scale bar, 10 µm.
(B) *The effect of GIPC1/synectin depletion on R-Ag and TF uptake into HUVEC cells*. The cells were treated with a GIPC1/synectin siRNA and then incubated with R-Ag or TF for 3 h at the concentration as described in Methods. The intensity of probe signal was normalized to the average of negative controls (NS siRNA treated) as relative uptake (y-axis).
(C) *Surface NRP1 expression following knockdown of GIPC1/synectin*. PPC1 cells were treated with NS siRNA or a GIPC1/synectin siRNA as described in Methods. After fixation, surface NRP1 was stained with a mouse anti-human NRP1 antibody and visualized by confocal microscopy. The average intensity of NRP1 staining per cell was quantified by ImageJ and normalized to that of NS siRNA-treated cells (y-axis).

(D) *GIPC1/synectin knockdown does not affect R-Ag binding at the cell surface*. PPC1 cells were treated with NS or a GIPC1/synectin siRNA as described in Methods. R-Ag was then added and incubated for the indicated time before washing without etching. After fixation, the fluorescence intensity of R-Ag per cell was quantified and normalized to the average of NS sample at the corresponding time point as relative R-Ag surface binding (y-axis).
Error bars indicate SEM (3-4 replicates).



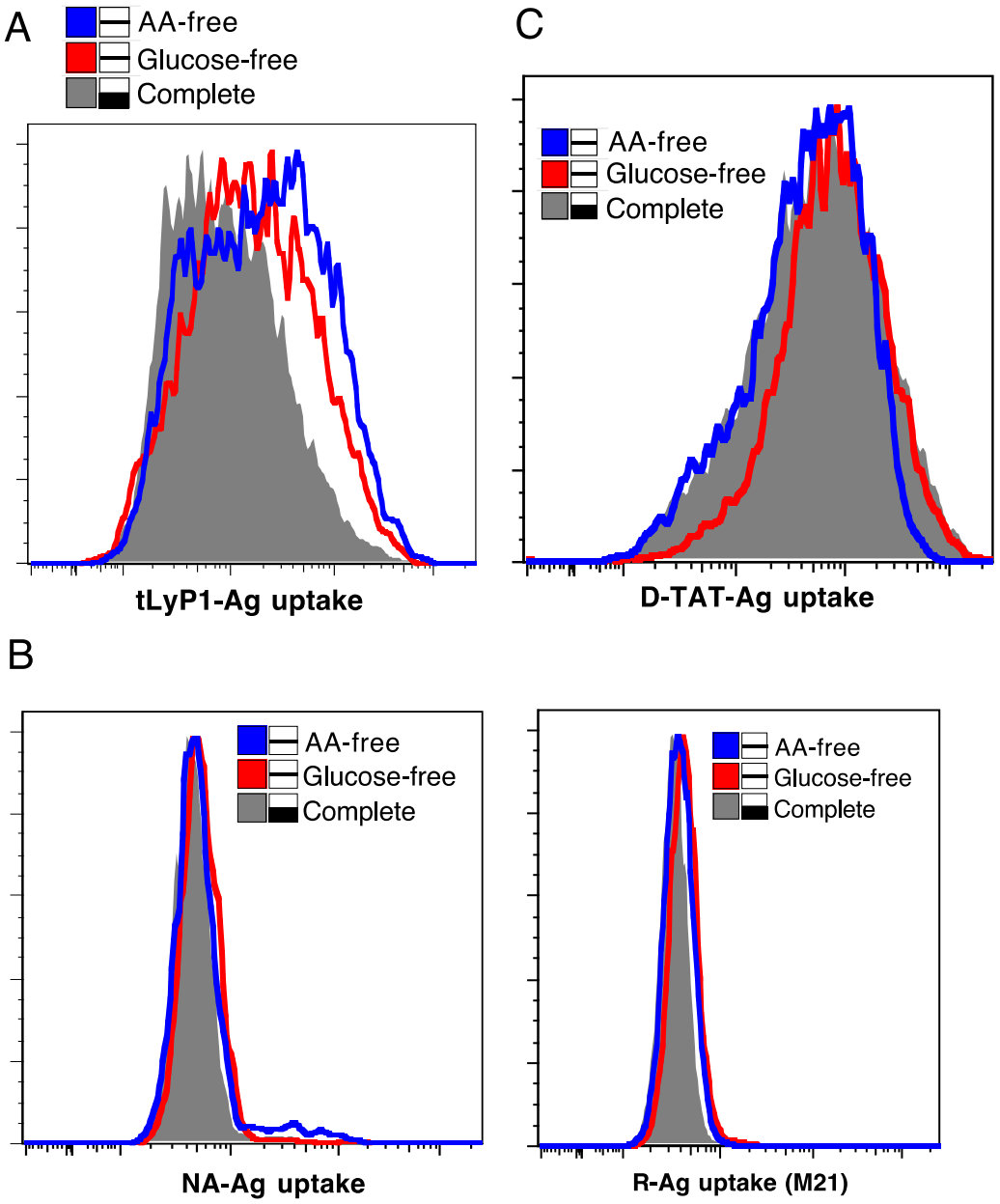
Supplementary Fig. 4. NRP1-GIPC1/synectin interaction is required for CendR uptake

(A) *HeLa cells express no detectable NRP1 at the cell surface and are not permissive for CendR uptake.* PPC1 and HeLa cells were incubated with 5 pM R-Ag (red) for 1 h at 37°C before etching and fixation. Cell surface NRP1 was stained using mouse anti-human NRP1 antibody (green). Nuclei were stained with Hoechst 33342 (blue). Fluorescence images were acquired using confocal microscopy and representative ones are shown. Scale bar, 10 µm.

(B) *Expression of NRP1 and GIPC1/synectin mRNAs*. Total mRNA was extracted from PPC1 and HeLa cells, respectively, and the mRNA levels per cell were quantified with

qPCR as described in Methods. The expression level of each gene (x-axis) in HeLa cells was further normalized to that of PPC1 cells as its relative mRNA expression level (y-axis).

(C) *GIPC1/synectin does not regulate TF uptake*. PPC1 cells were treated with NS or GIPC1/synectin siRNA, and HeLa cells were transfected with plasmids expressing WT or ΔSEA NRP1. The cells were then incubated with TF-594 for 1 h before washing and fixation. The fluorescence intensity of TF-594 per cell (per NRP1-positive cells for HeLa) was quantified and normalized to the average of the corresponding negative controls (NS for PPC1 samples, WT NRP1 for HeLa samples) as relative uptake (y-axis). Error bars indicate SEM (3-4 replicates). *P<0.05 and **P<0.01 (Student's t-test).



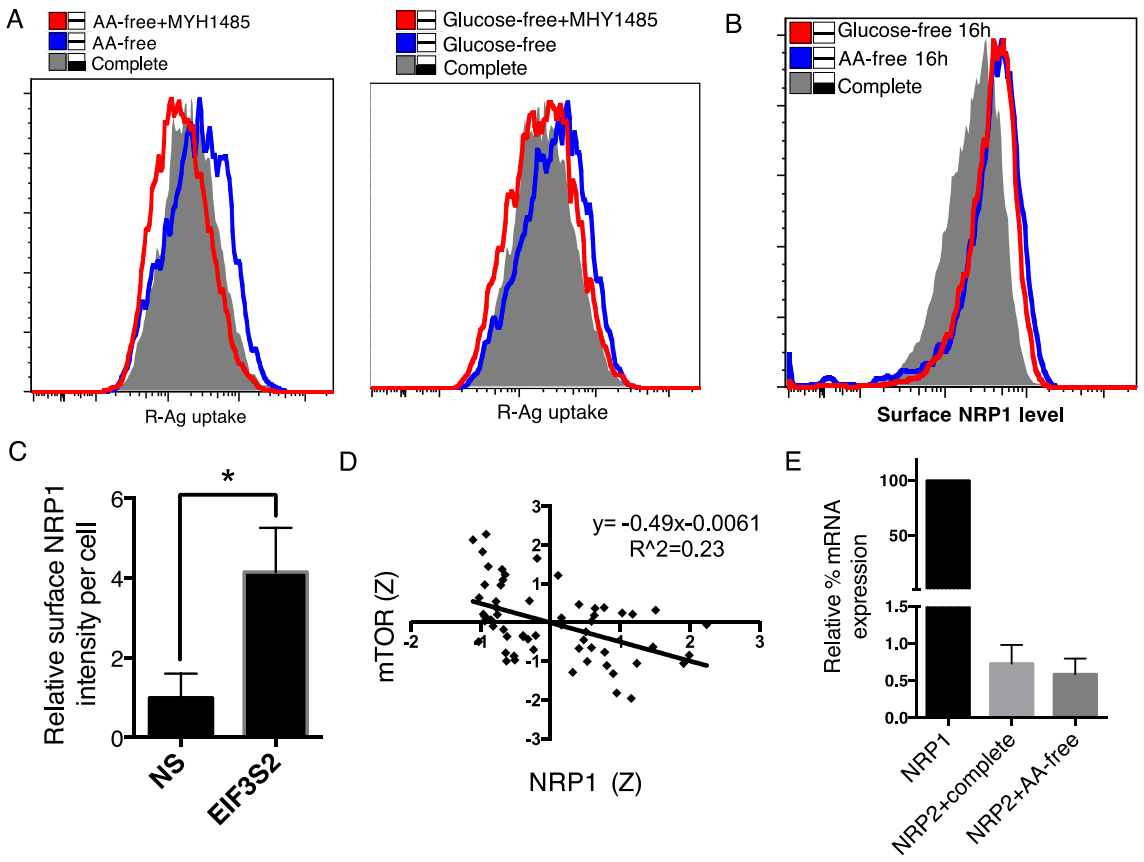
Supplementary Fig. 5. CendR pathway responds to nutrient deprivation.

(A) *Cellular uptake of tLyP1-Ag is enhanced by nutrient deprivation*. PPC1 cells were incubated with the indicated media for 16 h and silver nanoparticles coated with a CendR peptide different from RPARPAR (tLyP1-Ag; tLyP-1 sequence, CGNKRTR) (5 pM) was added for another hour incubation. After etching and fixation, the fluorescence intensity of internalized tLyP1-Ag was quantified by flow cytometry, and the curves were

overlaid using FlowJo. The experiments were performed three times and a representative result is shown.

(B) *CendR response to nutrient deprivation is not nonspecific engulfment*. Left panel: PPC1 cells were first incubated with indicated media for 16 h before NA-Ag (5 pM) was added for another 1-h incubation. Right panel: M21 cells, which do not express NRP1, were similarly starved before R-Ag (5 pM) was added for 1-h incubation. After etching and fixation, the fluorescence intensity of internalized particles was quantified by flow cytometry, and the curves were overlaid using FlowJo. The experiments were performed three times and a representative result is shown.

(C) *Cellular uptake of a cell-penetrating peptide lacking CendR activity does not respond to nutrient deprivation*. PPC1 cells were first incubated with indicated media for 16 h before D-TAT coated onto silver particles (D-TAT-Ag; 5 pM) was added for another 1-h incubation. After etching and fixation, the fluorescence intensity of internalized particles was quantified by flow cytometry, and the curves were overlaid using FlowJo. The experiments were performed three times and a representative result is shown.



Supplementary Fig. 6. CendR response to nutrient conditions occurs via mTOR signaling.

(A) *mTOR activation reverses CendR response to nutrient deprivation*. PPC1 cells were first incubated with the indicated media for 16 h, with or without the mTOR activator (MHY1485) at a final concentration of 10 μM. R-Ag (5 pM) was added for 1-h internalization. After etching and fixation, the fluorescence intensity of internalized particles was quantified by flow cytometry, and the curves were overlaid using FlowJo. The experiments were performed three times and a representative result is shown.
(B) *Surface NRP1 level in response to nutrient starvation.* PPC1 cells were incubated in the indicated media for 16 h before fixed and stained for surface NRP1 as described in Methods. The intensity of surface NRP1 staining was quantified by flow cytometry, and the curves were overlaid using FlowJo. The experiments were performed three times and a representative result is shown.

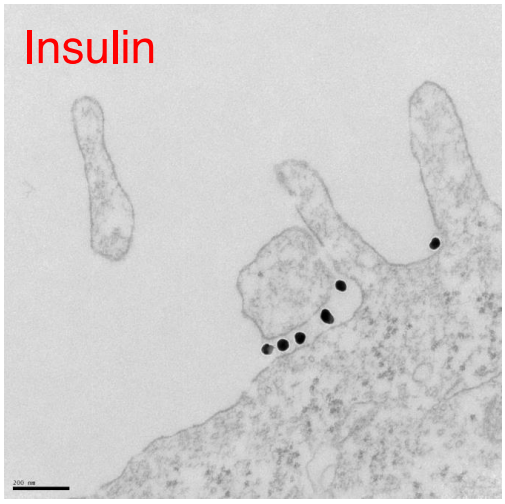
(C) *Surface NRP1 expression following knockdown of EIF3S2*. PPC1 cells were treated with an EIF3S2 siRNA as described in Methods. After fixation, surface NRP1 was stained with a mouse anti-human NRP1 antibody and visualized by confocal microscopy. The average intensity of NRP1 staining per cell was quantified by ImageJ and normalized to that of NS siRNA-treated cells.

(D) *The expression of mTOR and NRP1 mRNA is inversely correlated in various cancer cell lines*. The relative mRNA expression levels (Z) of mTOR and NRP1 in various cell lines were retrieved from CellMiner™ (NIH) and listed in Supplementary Data 3. Fitting into linear correlation was performed using Prism software, and the relevant equation is shown.

(E) *NRP2 expression and its response to nutrient depletion*. The total mRNA was extracted from PPC1 cells cultured in complete or AA-free medium for 16 h, and qPCR was performed as described in Methods. The NRP2 mRNA level in samples obtained under the indicated conditions was normalized to that of NRP1 in PPC1 cells cultured in complete medium.

Error bars indicate SEM (3 replicates). *P<0.05 (Student's t-test).

A



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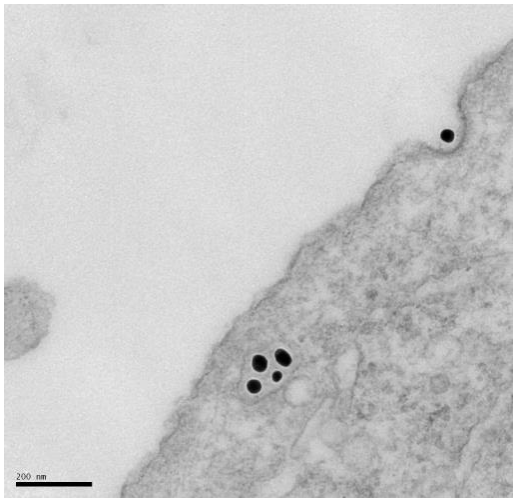

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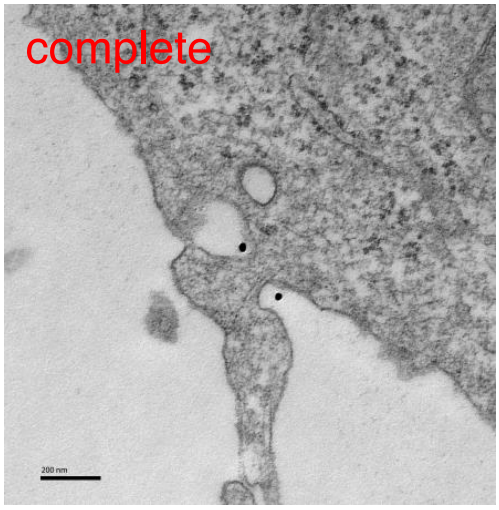

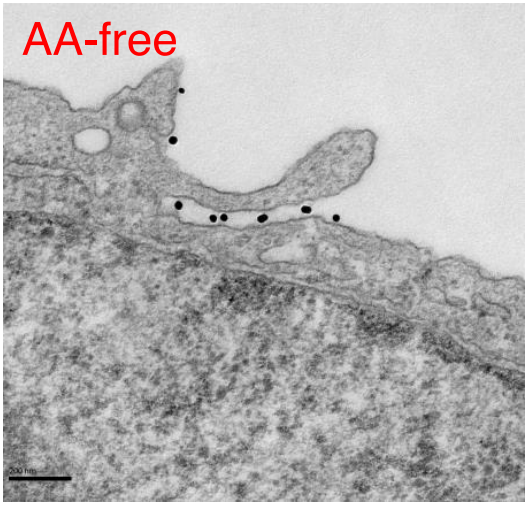

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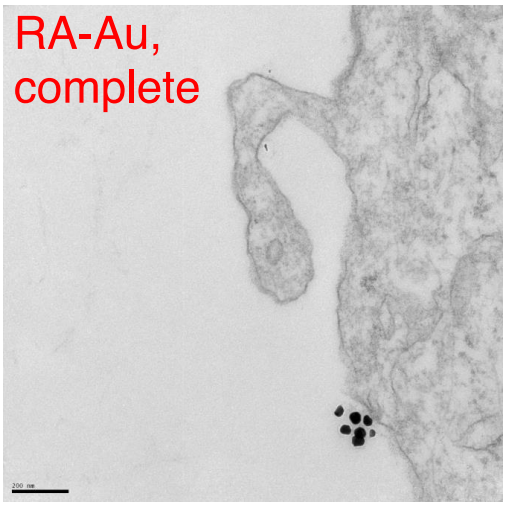
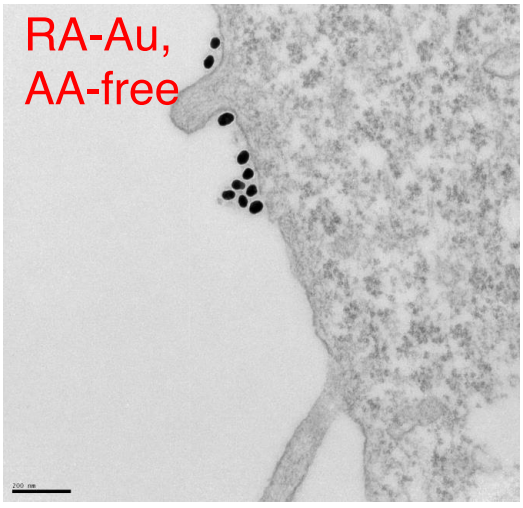
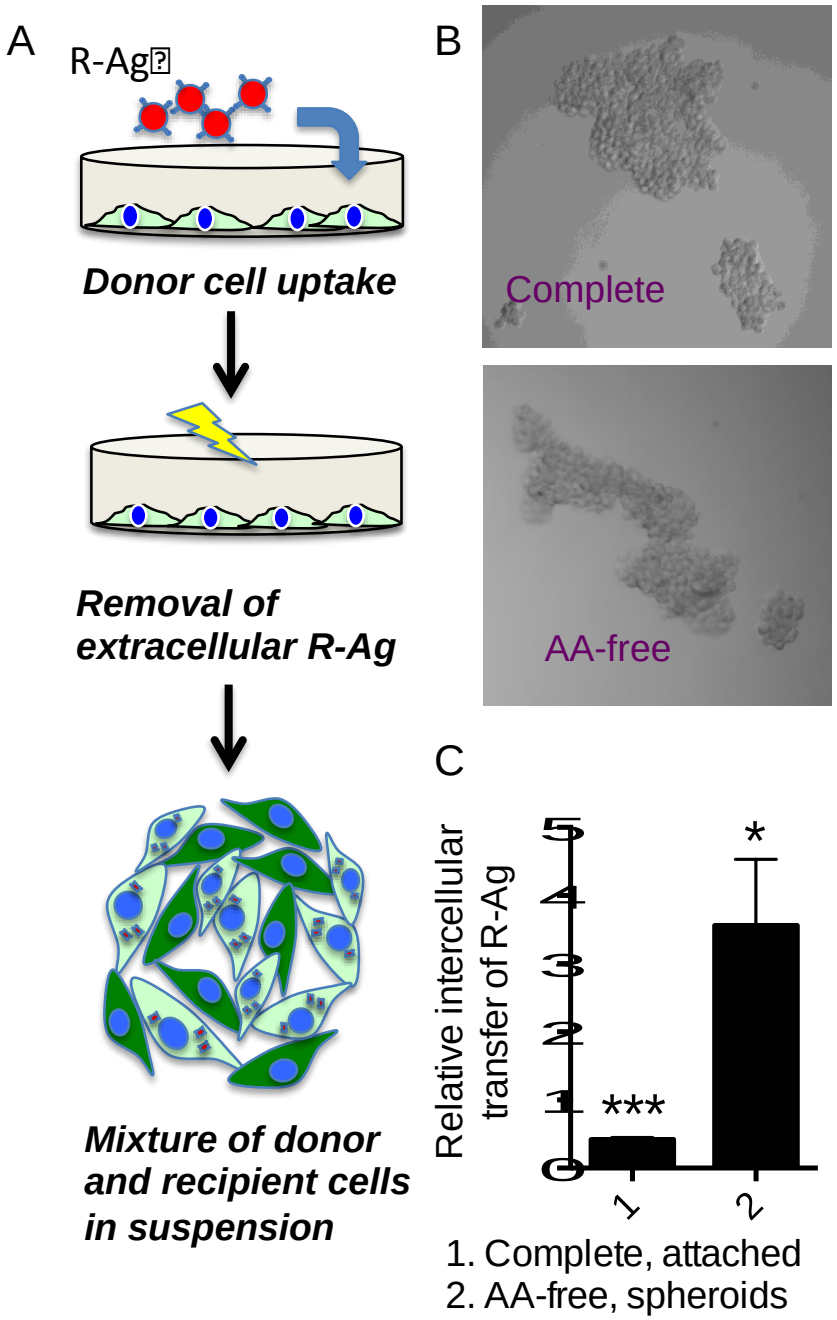




Supplementary Fig. 7. CendR peptide initiates an NRP1-dependent endocytosis resembling macropinocytosis.

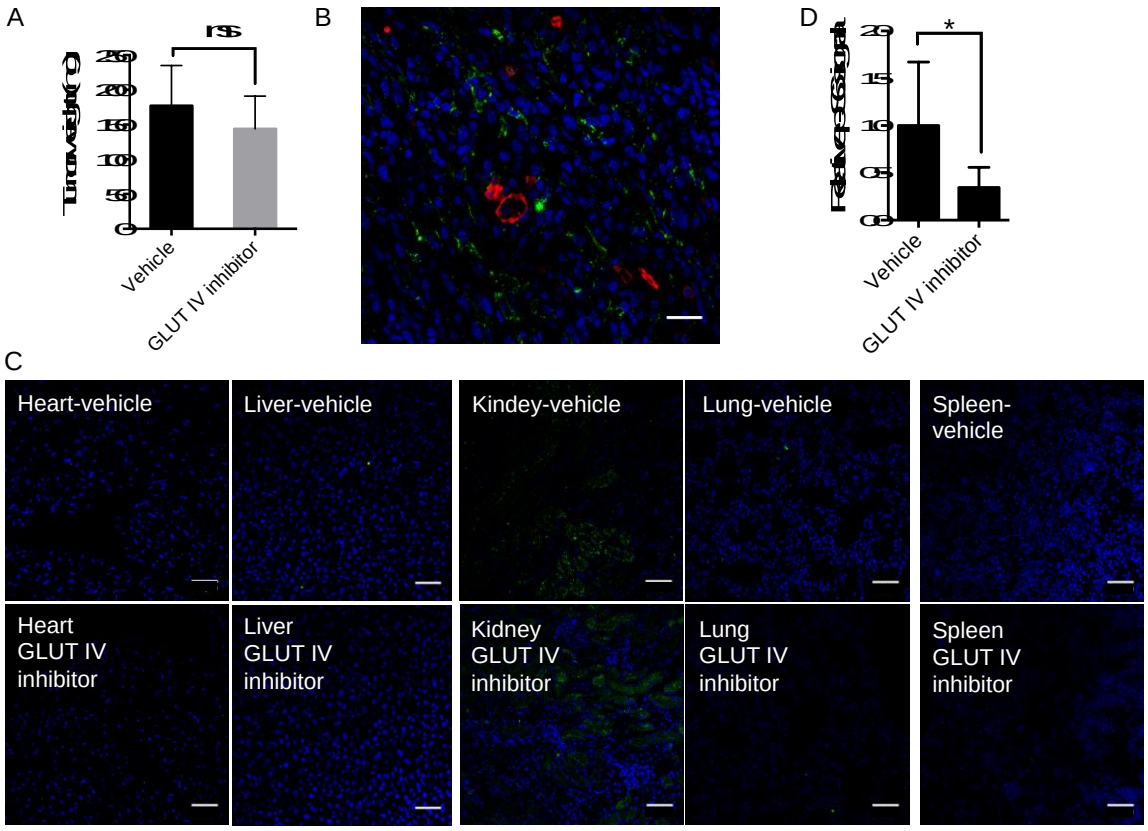
(A) PPC1 cells were treated with insulin as described in Methods before incubation with R-Au (50 nm) for 30 min, followed by fixation and TEM. Shown is representative TEM image of R-Au (dense dots) at the plasma membrane.
(B) PPC1 cells were cultured in regular medium before incubation with R-Au (50 nm) for 30 min, followed by fixation and TEM. Shown is representative image of R-Au engulfment in endocytic vesicles that are smaller and resemble clathrin-coated vesicles.
(C) PPC1 cells were cultured in complete and AA-free medium as described in Methods before incubation with smaller R-Au (17 nm) for 30 min, followed by fixation and TEM. Shown are representative TEM images of cell engulfment of R-Au (dense dots).
(D) A control peptide with an inactive CendR motif fails to initiate endocytosis. PPC1 cells were cultured in complete or AA-free medium for 16 h before incubation with RA-Au (50 nm) for 30 min, followed by fixation and TEM. Shown are representative TEM images of RA-Au (dense dots) at the plasma membrane.
Scale bar, 200 nm.



Supplementary Fig. 8. Intercellular transport of CendR cargo and its response to nutrient availability

(A) *Scheme for the assay to monitor intercellular transport of R-Ag*. The color of the donor (light green) and recipient (dark green) cells illustrate the fact that fluorescent labeling was used to distinguish the donor and recipient cell populations.

(B) *Examples of cell spheroids formed in suspension after 16-h incubation*. PPC1 cells were suspended in complete or AA-free media as described in Methods and imaged with white-field microscopy. Representative images are shown.
(C) *CendR transfer to HUVEC cells is stimulated by nutrient deprivation*. PPC1-GFP cells were incubated with R-Ag for 2 h. After etching, cells were harvested (donor cells) and mixed with unlabeled HUVEC cells (recipient cells) in PBS (pH=7.4). The cell mixture was then seeded as a monolayer in complete medium (complete, attached), or as spheroids in complete (complete, spheroids) or amino acid-free (AA-free, spheroids) medium. After 16 h, cells were harvested, fixed and analyzed using flow cytometry. The percentage of recipient cells becoming positive for R-Ag was quantified using FlowJo and normalized to that of “complete spheroids”, which was set as 1. The experiments were performed three times and error bars indicate SEM. *P<0.05 and ***P<0.001 (Student’s t-test) in comparison to that of complete spheroid condition.



Supplementary Fig. 9. CendR response to nutrient availability *in vivo*

(A) *GLUT IV inhibitor treatment does not significantly alter tumor weight.* Mice bearing 4T1 tumors were treated with intratumoral injections of vehicle only or GLUT IV inhibitor, and the tumors were excised and weighed. The experiment was carried out twice with at least three mice per group. There was no statistically significant difference (ns; Student's t-test) between the two groups.

(B) *iRGD localization outside blood vessels.* FAM-labeled iRGD was detected by staining with anti-FITC antibody (green), blood vessels were detected with anti-CD31 antibody (red) and nuclei were stained with DAPI (blue). A representative image is shown. Scale bar, 25 μm.

(C) *GLUT IV inhibitor treatment does not affect iRGD distribution in normal tissues.* The indicated organs were excised from the mice bearing 4T1 tumors described in Fig. 7D. FAM-labeled iRGD was detected by anti-FITC antibody (green) and nuclei were stained with DAPI (blue). Representative images for each organ are shown. Scale bar, 50 μm.

(D) *mTOR activity is suppressed by glucose restriction*. Tumor sections from mice treated with vehicle or GLUT IV inhibitor were stained with anti-phospho-S6 (Ser235/236) antibody. Nuclei were detected by DAPI. The average signal intensity per cell was normalized to vehicle-treated tumors as relative uptake (y-axis). The experiment was conducted twice with at least three mice per group. *P<0.05 (Student's t-test).

Supplementary Table 1

AA-free medium (100 X)

Inorganic Salts	**MW**	**mg/L**	**mM**
Calcium Chloride ($CaCl_2$•$2H_2O$)	111 200	1.8	
Ferric Nitrate ($Fe(NO_3)_3$•$9H_2O$)	404	0.1	0.000248
Magnesium Sulfate ($MgSO_4$) (anhyd.)	120	97.67	0.814
Potassium Chloride (KCl)	75	400 5.33	
Sodium Bicarbonate ($NaHCO_3$)	84	3700	44.05
Sodium Chloride (NaCl)	58	4750	81.9
Sodium Phosphate monobasic (NaH_2PO_4-H_2O)	138 125	0.906	
Other Components			
D-Glucose (Dextrose)	180 4500	25	
HEPES	238 5958	25.03	

Fetal Bovine Serum (FBS) was dialyzed using cassette (MWCO=3,500) into 1X PBS to remove amino acids and glucose. 10% dialyzed FBS was added to 1X AA-free medium and used in this study.

Gene symbol	Forward	Reverse
TBP	CCACTCACAGACTCTCACAAC	CTGCGGTACAATCCCAGAACT
NRP1	TGTTGGCCCTCACATTGGGCG	TGCTATCGCGCTGTCGGTGT
Rab5a	GCGGTCTCAGGTTTCTTTACCTCC	GTTGCGCCTCGACTAGCCATGT
Rab7a	TACAAAGCCACAATAGGAGCTG	GCAGTCTGCACCTCTGTAGAAG
Rab11a	ACACAGCAGGGCAAGAGCGA	AGGAACTGCCCTGAGATGACGTA
VEGFR2	CGGCACCACTCAAACGCTGA	ACAGCTTGGCTGGGCTCGTT
GIPC1	AAGCTCACGGAGCCTCGCAA	AAGGCAGAGGGCAGATCCTCCA
EIF3S2	AGGACCCTATCGTCAATGTATGG	CACACAGCTCCGGTATGGC
AP2M1	AGATCAGCGAGGAAAACATCAAG	TTCTGTGGGTAGCCAAAGTCT
NRP2	CCCTCACTTTGAAATCGAGAAGC	CTGCGGATTCACTGTCCCC