Supplement

1. Material and method

Flow Cytometry Analysis of Fluorescence Intensity

HEK293T cells were transfected with EGFP or ABI1-EGFP expression plasmids using Lipo8000 Transfection Reagent (Beyotime, C0533, Shanghai China). One day post-transfection, cells were digested, resuspended in PBS, and filtered through a 40μm cell strainer (Biosharp, BS-40-XBS, Hefei, China). The filtered single-cell suspension was analyzed on a flow cytometer (CyFlow Cube 6, Sysmex Partec, Germany). Data were analyzed using FlowJo software (FlowJo_v10.8.1). Experiments were performed with three independent biological replicates, and results were plotted using GraphPad Prism software(Version 5.01).

Fluorescence Imaging

HEK293T cells were co-transfected with ABI1-EGFP and individual scaffold protein expression plasmids. At 6 hours post-transfection, ABA or vehicle (ethanol) was added as indicated. Fluorescence images were captured one day after transfection using an inverted fluorescence microscope (Olympus, IX73, Japan).

Plasmid Construction

The coding sequences for human MARCKS (NM_002356.7) and MARCKSL1 (NM_023009.7) were commercially synthesized (Hainan Shengzhe Biotech, Haikou, China). These sequences were excised from their source plasmids using EcoRI (Takara, 1611, Dalian, China) and BamHI (Takara, 1605, Dalian, China). The recipient vector fragment was amplified from a pCS2-BASP1-PYL1-HA template using the primers 5′-GTTGAAGACTGATCCGGCGGAGGCGGTAGCACACAGGACGAAT-3′ and 5′-AGTTGAAGACCAATTCGGTGGCGGATCCTGCAAAAAGAAC-3′. The PCR product was digested with BbsI (NEB, R3538S, Ipswich, MA, USA) to generate ends compatible with EcoRI and BamHI. The vector and insert fragments were ligated using T4 DNA ligase (Thermo Fisher, EL0011, Waltham, MA, USA). To generate sgRNA expression constructs, a cassette containing the U6 promoter was amplified from the pX330 vector and cloned into the pCE3 Blunt vector (Vazyme, C601, Nanjing, China) to create pCE3-sgRNA. For the PCSK9-targeting sgRNA, the oligos 5′-CACCGGTGCTAGCCTTGCGTTCCG-3′ and 5′-AAACCGGAACGCAAGGCTAGCACC-3′ were annealed and ligated into the BbsI-digested pCE3-sgRNA vector. All PCR reactions were performed using Q5 High-Fidelity DNA Polymerase (NEB, M0491, Ipswich, MA, USA), and all final constructs were verified by Sanger sequencing.

RNA Extraction and RT-PCR

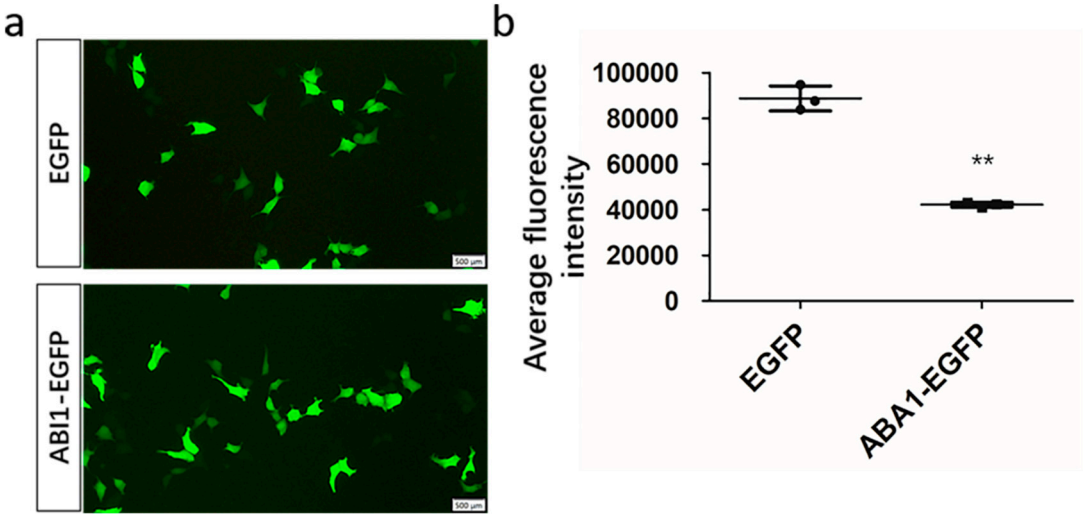
Cells cultured in 6-well plates (JETBIO-FIL, TCP011006, Guangzhou, China) were washed once with PBS (Procell, PB180327, Wuhan, China) after medium removal. Total RNA was extracted by adding 1 mL of TRIzol reagent (Ambion, 15596026, Waltham, MA, USA) per well, followed by gentle mixing and incubation for 5 min at room temperature. Lysates were transferred to microcentrifuge tubes, mixed with 100 μL chloroform (XILONG SCIENTIFIC, 13700901, Shantou, Guangdong, China) by vortexing, and incubated for 3 min. After centrifugation at 12,000× g for 15

min at 4°C, the upper aqueous phase was collected. RNA was precipitated by adding an equal volume of pre-cooled isopropanol (XILONG SCIENTIFIC, 12802501, Shantou, Guangdong, China), vortexed, and placed on ice for 10 min. The RNA pellet was collected by centrifugation at 12,000 rpm for 15 min at 4°C, washed twice with 75% ethanol (XILONG SCIENTIFIC, 12803401; 7,500×g, 5 min, 4°C), and briefly centrifuged again to remove residual ethanol. The pellet was air-dried and dissolved in 50 µL of DEPC-treated water (Biosharp, BL510A, Hefei, China). RNA concentration was measured using a Nanodrop spectrophotometer. First-strand cDNA was synthesized from 1 µg of total RNA using a reverse transcription kit (Takara, RR600A, Dalian, China) according to the manufacturer's protocol.

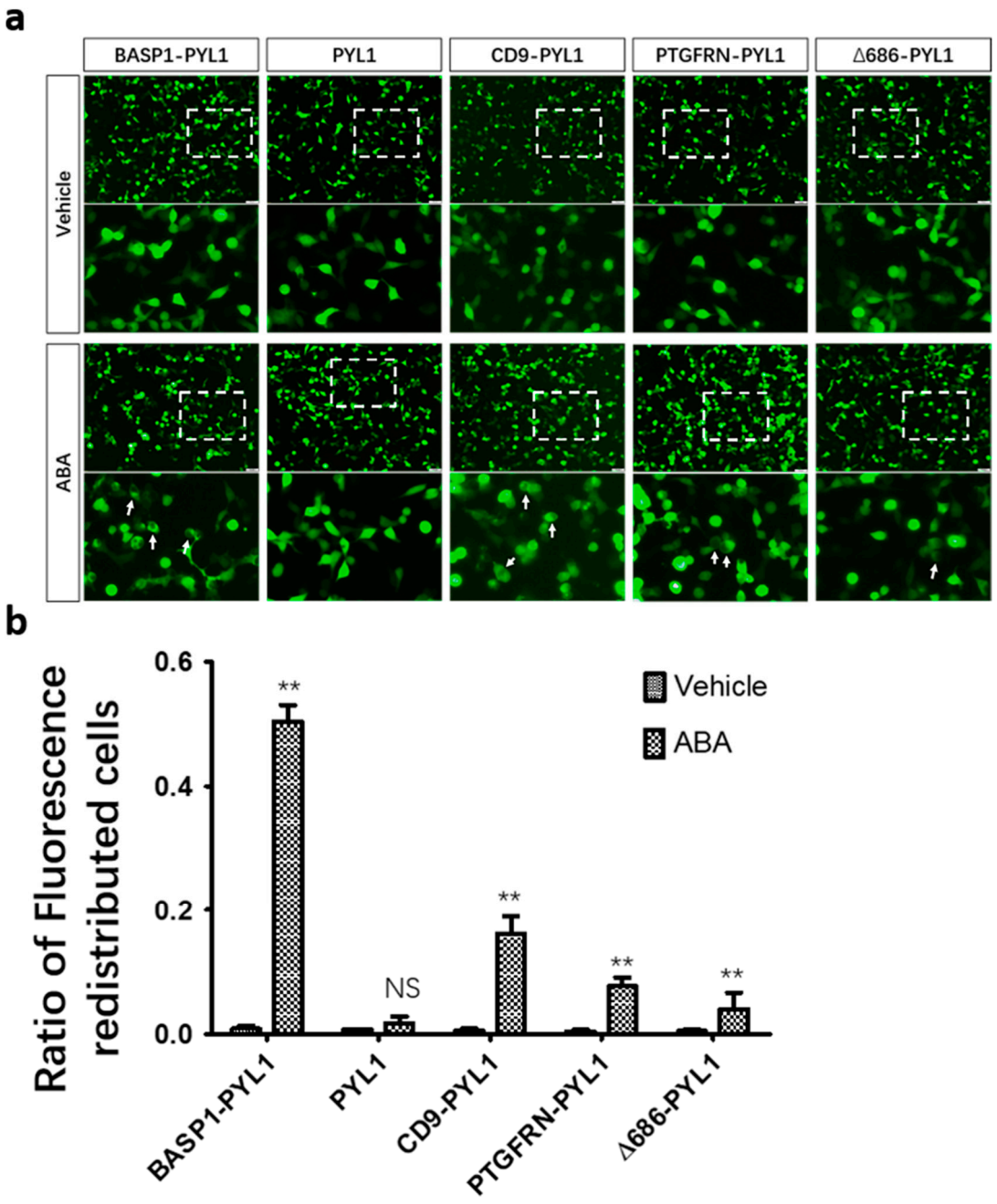
TIDE Analysis of PCSK9 Gene Editing

The genomic region surrounding the PCSK9 target site was amplified using the forward primer 5′-TCAGTTCAGGGTCTGAGCCTGGAG-3′ and the reverse primer 5′-GAAACTGAGGCCCGAGAGGAAACAG-3′. The amplicon was submitted for Sanger sequencing with the reverse primer. Sequencing traces were analyzed using the TIDE web tool according to the developer's protocol. Editing efficiencies were calculated from three independent biological replicates. Indel frequencies with a *p*-value < 0.01 were considered significant and are plotted in the figures.

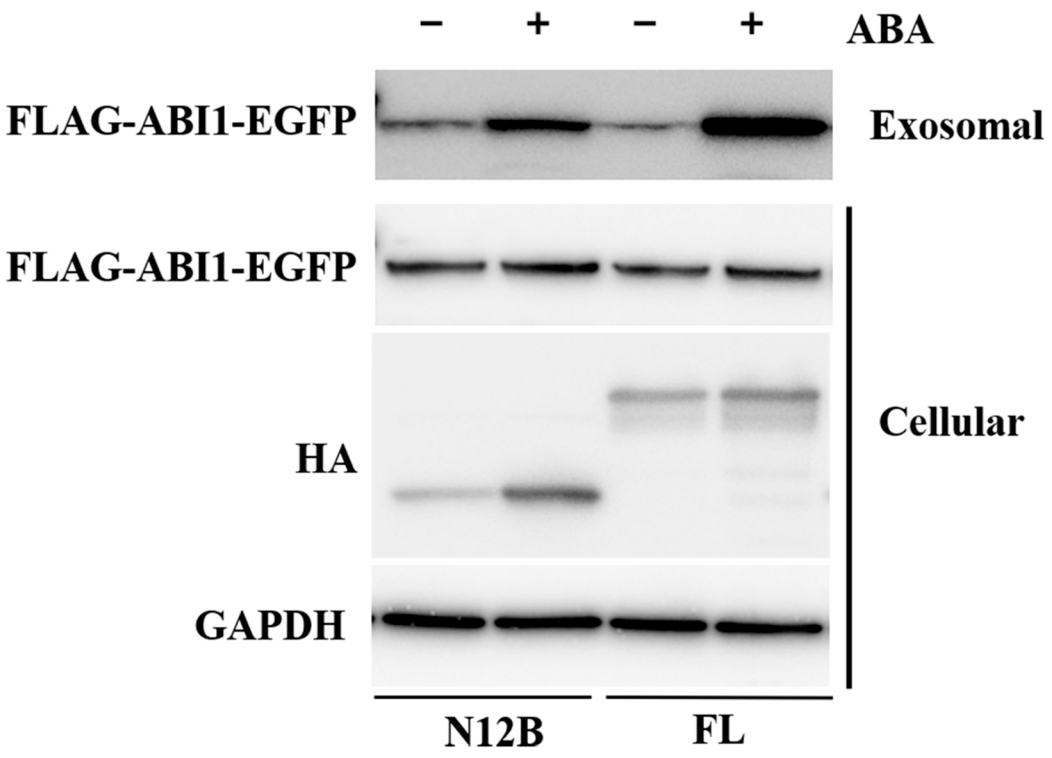
2. supplementary figures



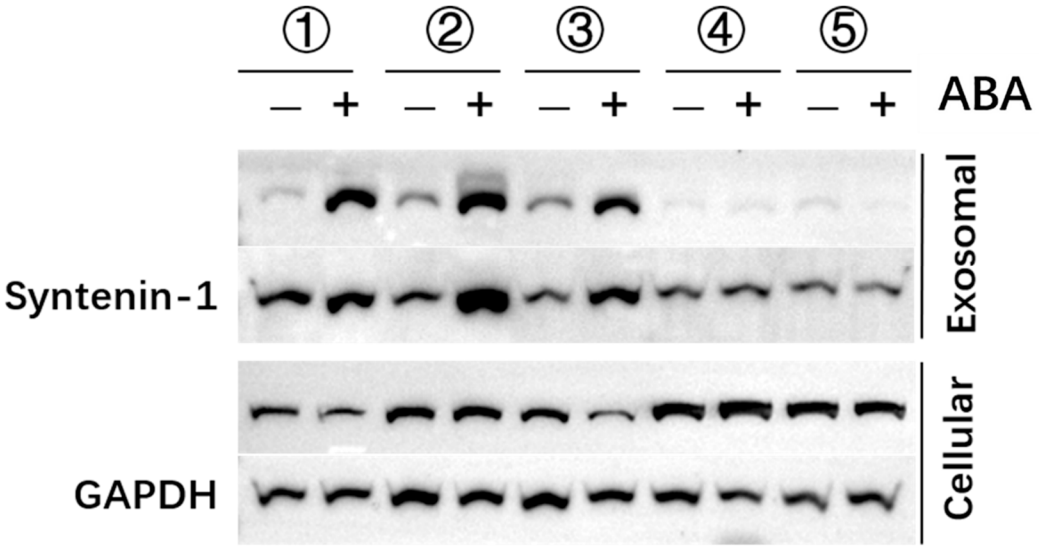
Supplementary Figure S1. Comparison of the fluorescence of ABI1-tagged EGFP versus EGFP alone. (a) Representative fluorescence microscopy images of HEK293T cells transfected either EGFP or ABI1-EGFP. (b) Flow cytometry analysis comparing the mean fluorescence intensity of cells expressing EGFP and ABI1-EGFP. Data are presented as mean ± s.d. of three independent biological replicates. Statistical significance was determined by an unpaired two-tailed Student's t-test (**p < 0.01).



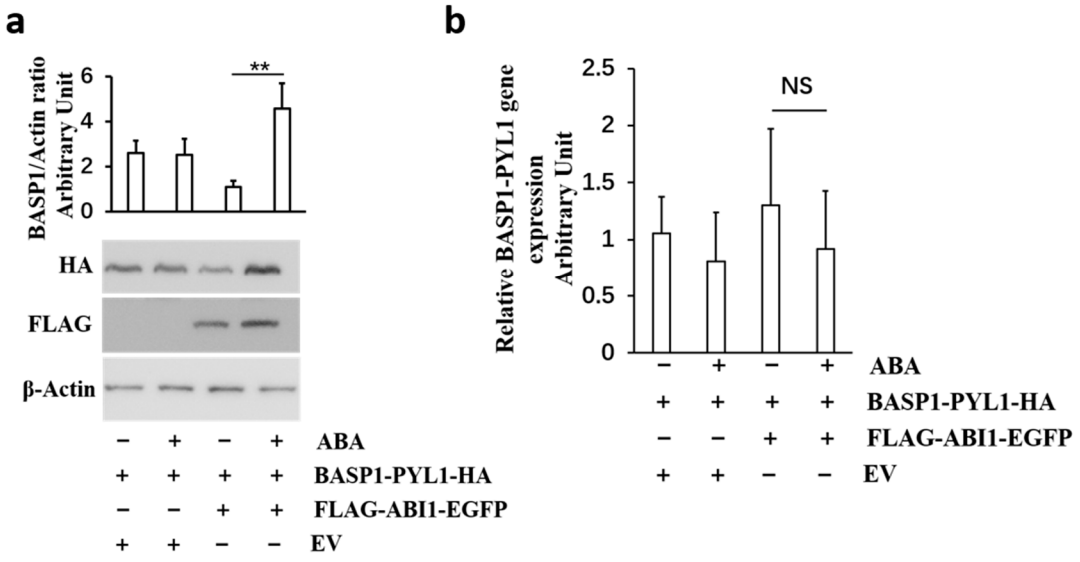
Supplementary Figure S2. EV scaffold proteins facilitate the ABA-induced cellular redistribution of ABI1-EGFP. (a) Representative fluorescence microscopy images of producer cells under the indicated conditions. The dashed rectangles in the upper panels indicate the regions magnified in the corresponding lower panels. (b) Quantification of cells exhibiting cytoplasmic redistribution of ABI1-EGFP fluorescence. Data are presented as mean ± s.d. (n=3 independent experiments). Statistical significance was determined by an unpaired two-tailed Student's t-test (**$p < 0.01$). NS, not significant.



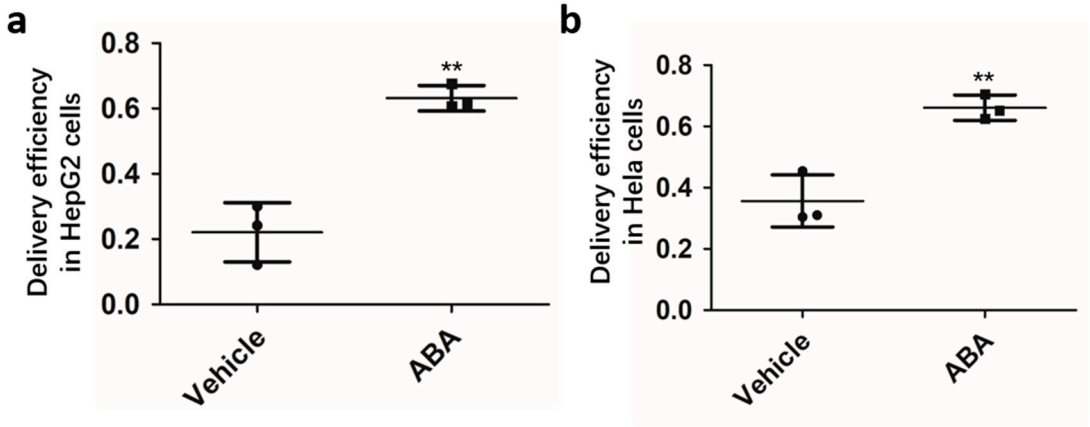
Supplementary Figure S3. Full-length BASP1 is more effective than its N-terminal peptide at promoting ABA-induced cargo encapsulation into EVs. FL, full-length BASP1; N12B, the N-terminal 12-amino-acid peptide of BASP1 containing the myristoylation site.



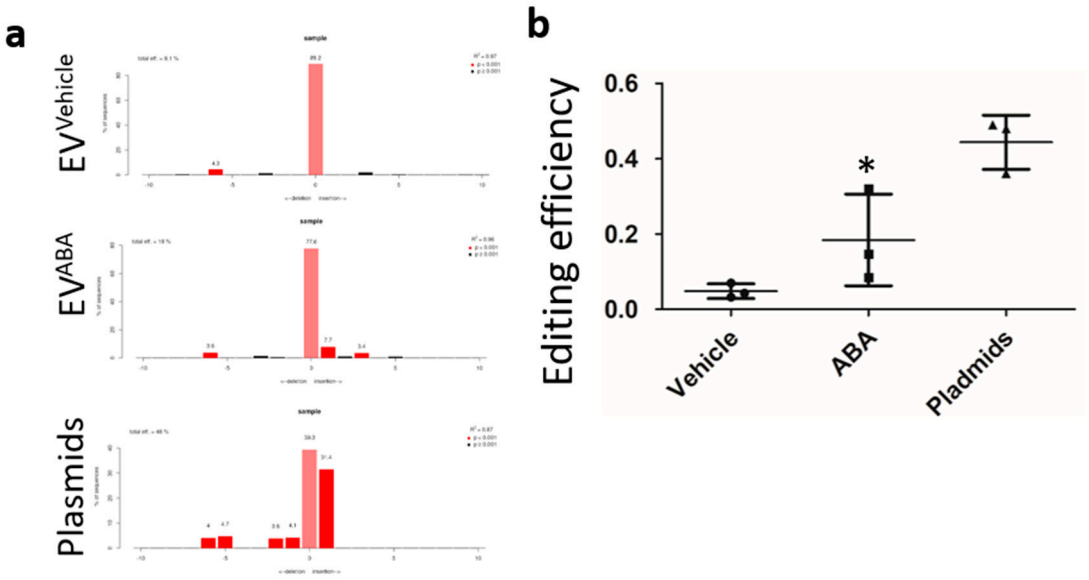
Supplementary Figure S4. Scaffold proteins MARCKS and MARCKSL1 enable ABA-inducible cargo loading into extracellular vesicles. Experimental conditions are labeled as follows: 1, BASP1-PYL1; 2, MARCKS-PYL1; 3, MARCKSL1-PYL1; 4, PYL1 alone; 5, empty vector control.



Supplementary Figure S5. ABA-induced ABI1-PYL1 interaction stabilizes BASP1-PYL1. (a) **Upper panel:** Quantification of BASP1-PYL1-HA protein levels from three independent experiments. **Lower panels:** Representative Western blot (WB) images. (b) Quantitative PCR (qPCR) analysis of *BASP1-PYL1* mRNA abundance. Data are presented as mean ± s.d. Statistical significance was determined by an unpaired two-tailed Student's t-test (ns, not significant).



Supplementary Figure S6. Delivery efficiency of ABI1-EGFP-loaded extracellular vesicles (EVs). Efficiency was quantified as the percentage of EGFP-positive recipient cells relative to the total cell count.



Supplementary Figure S7. ABA induced EV-encapsulated Cas9 can function properly. TIDE analysis was performed following delivery of a single PCSK9-targeting sgRNA and EVs containing Cas9 to recipient cells. *Vehicle*, EVs from vehicle-treated producer cells; *ABA*, EVs from ABA-treated producer cells; *Plasmid*, Cas9 expression plasmid transfection (positive control). Data are presented as mean ± s.d. (n=3). Statistical significance between Vehicle and ABA groups was determined by an unpaired, one-tailed Student's t-test (*$p < 0.05$).

3. Supplementary Table S1. Antibodies used in this study

Antibody	Company	Catalog No.	Dilution ratio
Anti-Flag	Yeasen Biotechnology	30502ES60	1: 1000
Anti-HA	Yeasen Biotechnology	30703ES60	1: 1000
Anti-CD9	Proteintech	20597-1-AP	1: 2000
Anti-CD63	Abcam	ab134045	1: 1000
Anti-Syntenin-1	Abcam	ab133267	1: 1000
Anti-Calnexin	Abcam	ab22595	1: 1000
Anti-GADPH	Yeasen Biotechnology	30202ES60	1: 1000
Anti-β Actin	ServiceBio	GB15003	1: 1000
HRP-Anti mouse IgG	Yeasen Biotechnology	33220ES	1: 5000
HRP-Anti Rabbit IgG	Yeasen Biotechnology	34201ES	1: 5000