

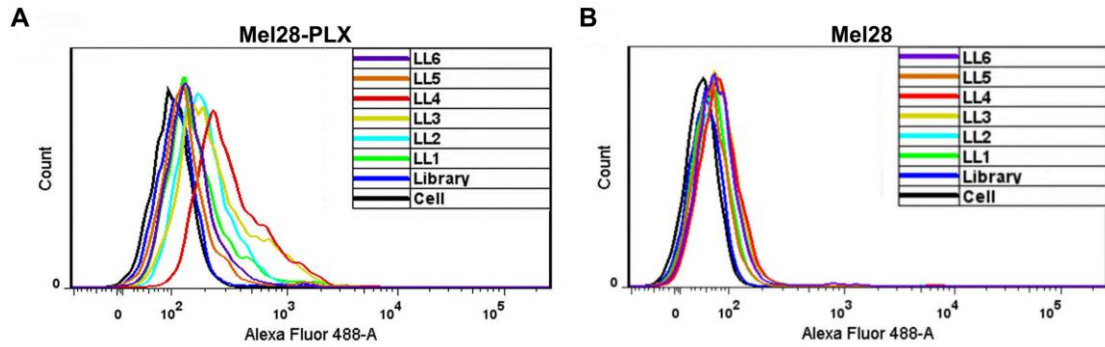
OMTN, Volume 18

Supplemental Information

**A Novel Aptamer LL4A Specifically
Targets Vemurafenib-Resistant Melanoma
through Binding to the CD63 Protein**

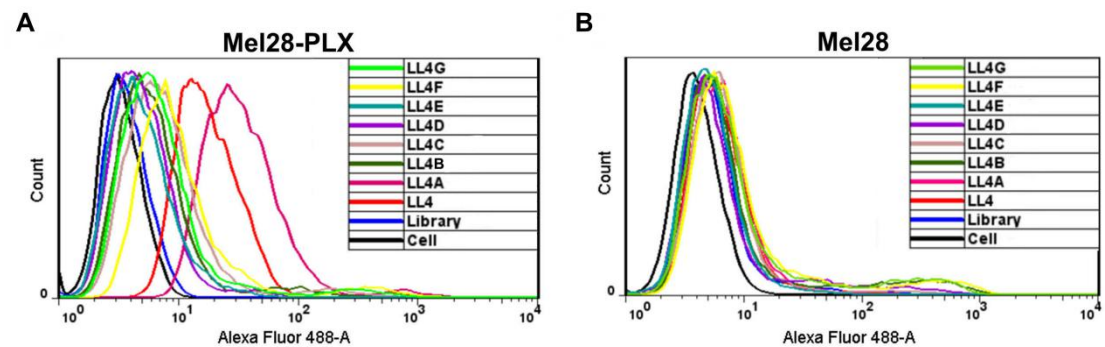
Hui Li, Juan Liu, Xiaojuan Xiao, Shuming Sun, Hui Zhang, Yibin Zhang, Weihua Zhou, Bin Zhang, Mridul Roy, Hong Liu, Mao Ye, Zi Wang, Feng Liu-Smith, and Jing Liu

Supplementary Figure S1.



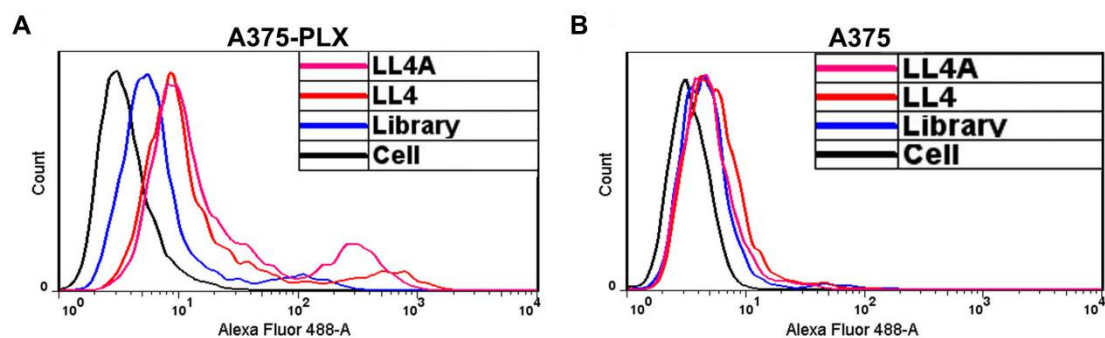
Supplementary Figure S1. Binding assays of selected aptamers with Mel28-PLX and Mel28 cells. Flow cytometry assays for the binding capacity of LL1-6 and ssDNA library with Mel28-PLX (A) and Mel28 cells (B).

Supplementary Figure S2.



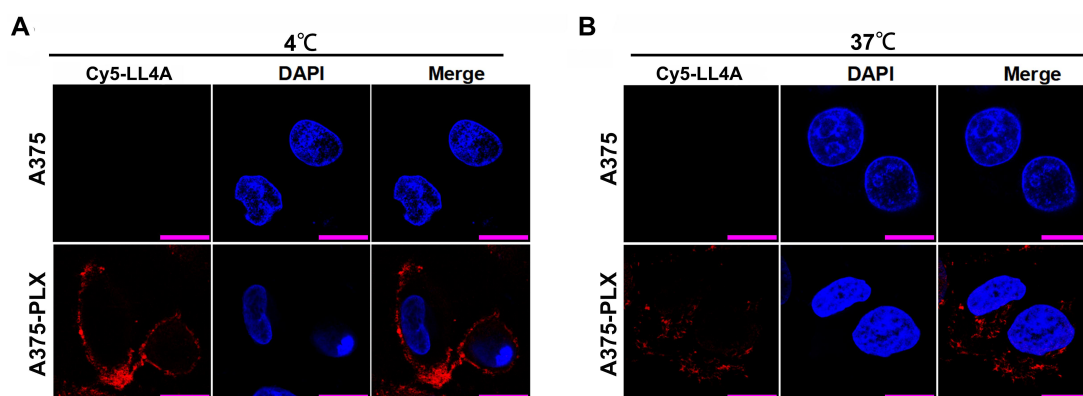
Supplementary Figure S2. Binding assays of different truncated versions of aptamer LL4 with Mel28-PLX and Mel28 cells. Flow cytometry assays for the binding capacity of seven kinds of truncated versions of LL4 (LL4A-G) with Mel28-PLX (A) and Mel28 cells (B).

Supplementary Figure S3.



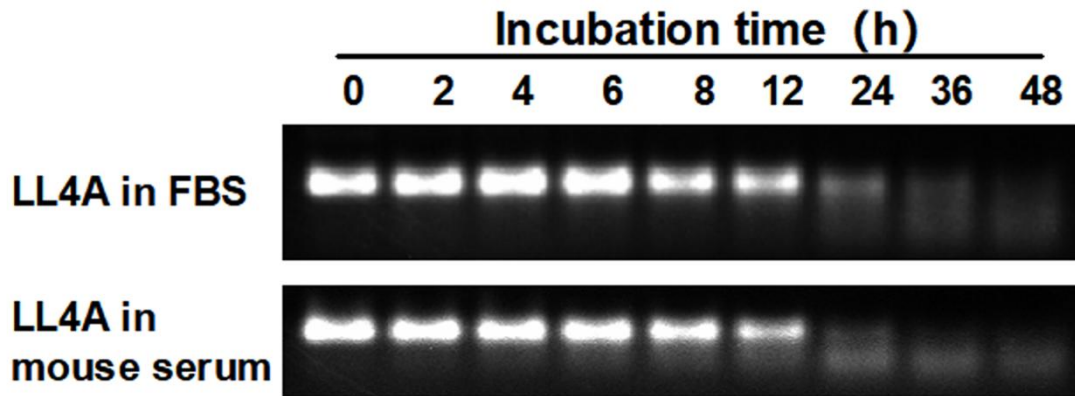
Supplementary Figure S3. Binding assays of selected aptamer LL4 and LL4A with A375-PLX and A375 cells. Flow cytometry assays for the binding capacity of LL4 and LL4A with A375-PLX (A) and A375 cells (B).

Supplementary Figure S4.



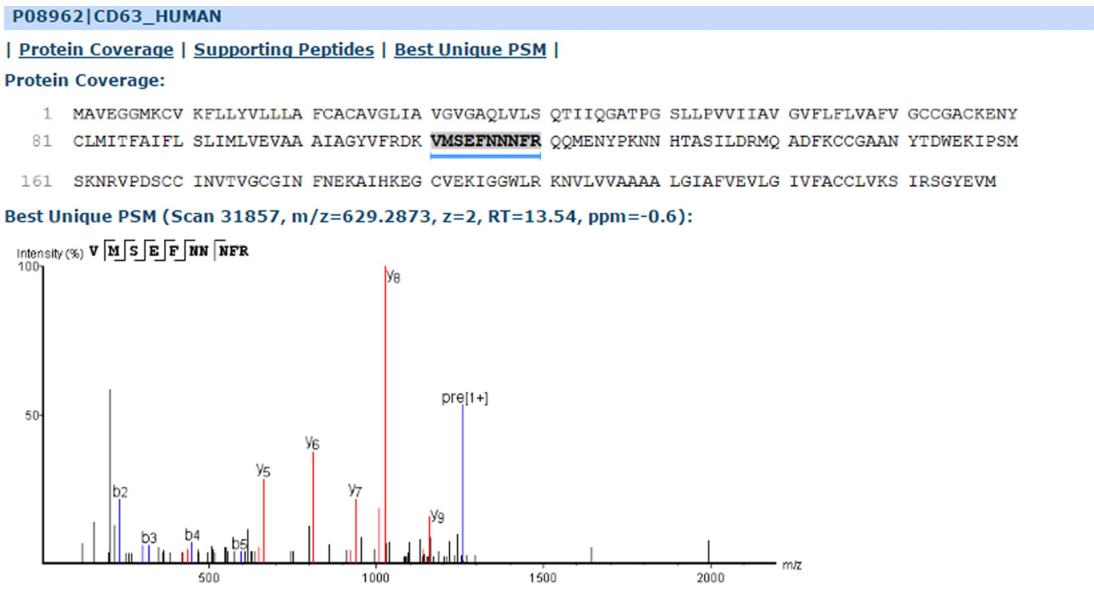
Supplementary Figure S4. Characterization of LL4A. (A) The binding site of Cy5-labeled aptamer LL4A to A375 and A375-PLX cells at 4 °C was investigated by confocal microscopy imaging, bar=25 μ m. (B) The internalization of Cy5-labeled aptamer LL4A into A375-PLX cells at 37 °C was investigated by confocal microscopy imaging, bar=25 μ m.

Supplementary Figure S5.

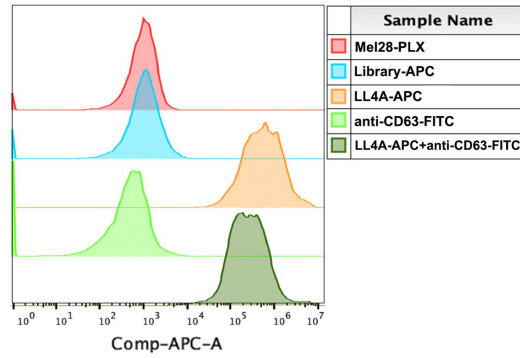


Supplementary Figure S5. The serum stability of LL4A in FBS and mouse serum. Aptamer LL4A was incubated in MEM with 10% FBS or mouse serum, and its biostability was evaluated by gel electrophoresis of the residual products at the indicated time points.

Supplementary Figure S6. LC-MS/MS QSTAR analysis for CD63.

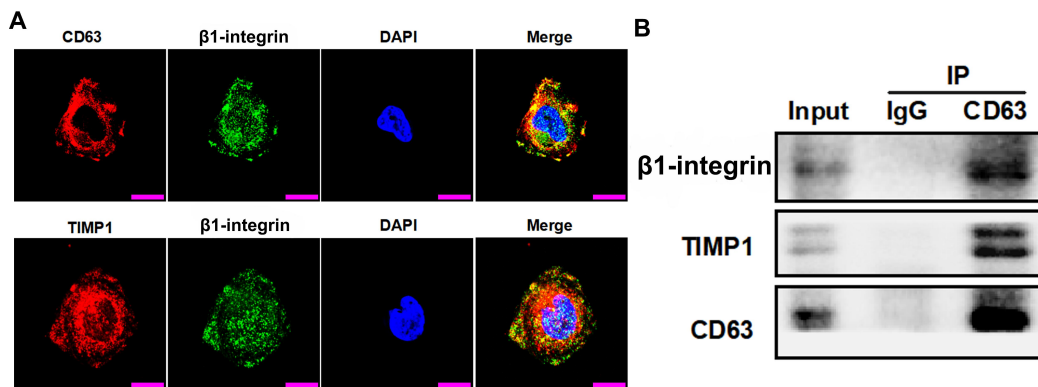


Supplementary Figure S7.



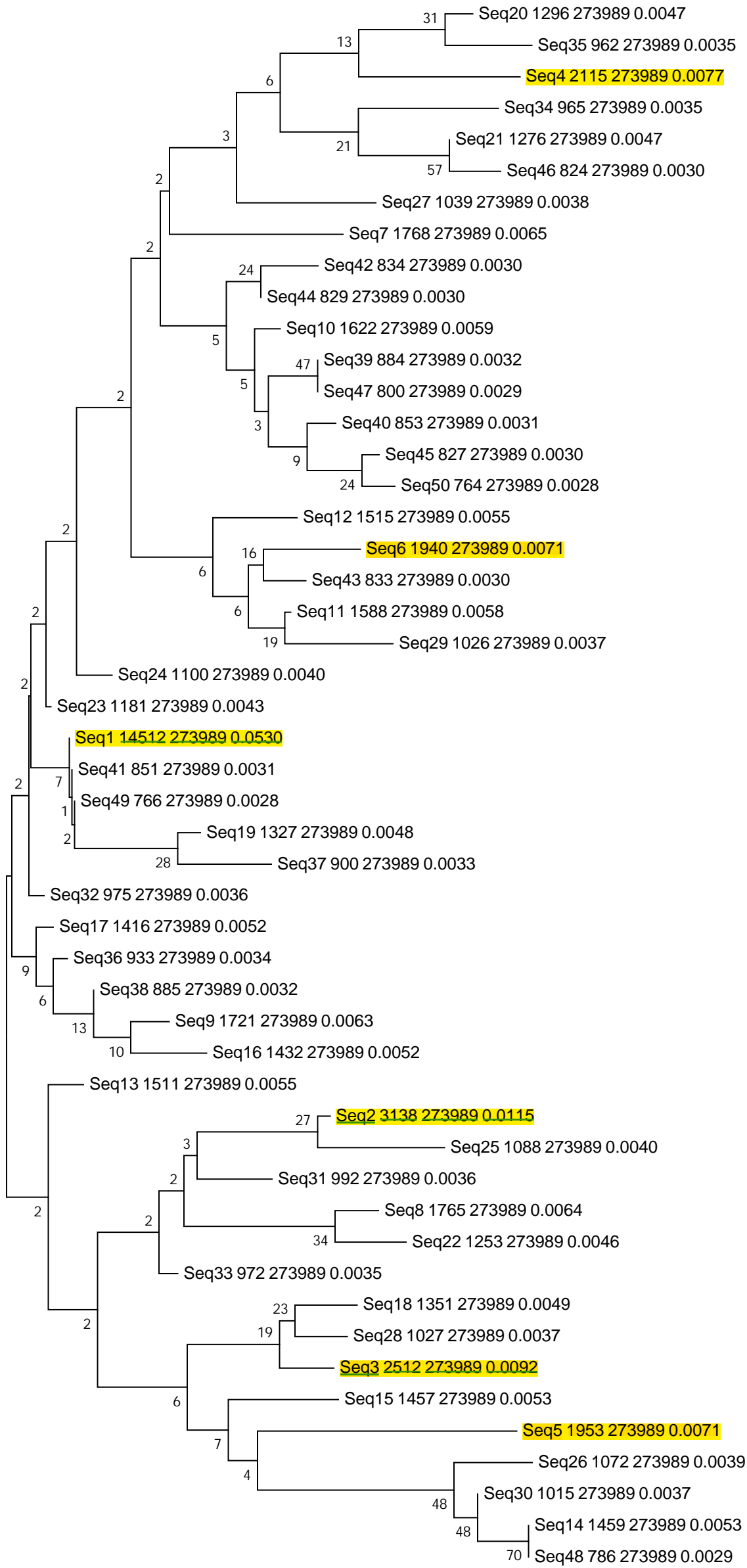
Supplementary Figure S7. Competition binding was analyzed by flow cytometry. Mel28-PLX cells were Cy5-labeled LL4A and co-incubated with FITC-labeled anti-CD63 antibody and the competition binding was analyzed by flow cytometry.

Supplementary Figure S8.



Supplementary Figure S8. The TIMP1/CD63/ β 1-integrin supramolecular complex in Mel28-PLX cells. (A) The co-localization of β 1-integrin with CD63 or TIMP1 in Mel28-PLX cells was examined by confocal microscopy imaging, bar=25 μ m. (B) Whole-cell lysates from Mel28-PLX cells were subjected to co-immunoprecipitation with a control IgG or an anti-CD63 antibody. The immunoprecipitates (TIMP1 and β 1-integrin) were detected by western blotting.

Supplementary Table S1. The biological evolutionary tree analysis data of the candidate aptamers from high-throughput sequencing.



0.2

Supplementary Table S2. The list of six representative ssDNA sequences

Name	Sequences (5' → 3')
LL1	ACCGACCGTGCTGGACTCAGGATTAAGGTGGAACTAGTG TTAATCGTCGATTTTGATTAGACTATGAGCGAGCCTGGCG
LL2	ACCGACCGTGCTGGACTCAAACCAATGTAGTACATCTACG CTTTGATTGGCAGGACTTGCGACTATGAGCGAGCCTGGCG
LL3	ACCGACCGTGCTGGACTCACGCAGTGGGTAAATTCGCCAG GACTATCAATTCACGGTCGACTATGAGCGAGCCTGGCG
LL4	ACCGACCGTGCTGGACTCACCTCGACCAGAGCCATTGGGT TTCCTAGGAAATAGGGCCTTTACTATGAGCGAGCCTGGCG
LL5	ACCGACCGTGCTGGACTCACCGGTAGTTATAAAGAGTTGT TTATTTTCCGTGTAATGGTAACTATGAGCGAGCCTGGCG
LL6	ACCGACCGTGCTGGACTCAGAGCGGAAGTCCGTTTCAGGT GGGGTTTACTGCATAGATACTATGAGCGAGCCTGGCG

Supplementary Table S3. The list of seven kinds of truncated sequences of LL4

Name	Sequences (5' → 3')
LL4A (10-80)	GCTGGACTCACCTCGACCAGAGCCATTGGGTTTCCTAGG AAATAGGGCCTTTACTATGAGCGAGCCTGGCG
LL4B (10-65)	GCTGGACTCACCTCGACCAGAGCCATTGGGTTTCCTAGG AAATAGGGCCTTTACTA
LL4C (1-65)	ACCGACCGTGCTGGACTCACCTCGACCAGAGCCATTGGG TTTCCTAGGAAATAGGGCCTTTACTA
LL4D (10-55)	GCTGGACTCACCTCGACCAGAGCCATTGGGTTTCCTAGG AAATAGG

LL4E (30-55) AGCCATTGGGTTTCCTAGGAAATAGG
 CCTCGACCAGAGCCATTGGGTTTCCTAGGAAATAGGGCC
 LL4F (20-80) TTTACTATGAGCGAGCCTGGCG
 LL4G (30-80) AGCCATTGGGTTTCCTAGGAAATAGGGCCTTTACTATGAG
 CGAGCCTGGCG

Supplementary Table S4. The list of 20 candidate proteins detected by MS.

Accession	Description
P60709 ACTB_HUMAN	Actin cytoplasmic 1
P49411 EFTU_HUMAN	Elongation factor Tu mitochondrial
Q6S8J3 POTEE_HUMAN	POTE ankyrin domain family member E
P08962 CD63_HUMAN	CD63 antigen
O15533 TPSN_HUMAN	Tapasin
O60343 TBCD4_HUMAN	TBC1 domain family member 4
P04264 K2C1_HUMAN	Keratin type II cytoskeletal 1
P07437 TBB5_HUMAN	Tubulin beta chain
P02533 K1C14_HUMAN	Keratin type I cytoskeletal 14
O96019 ACL6A_HUMAN	Actin-like protein 6A
Q14103 HNRPD_HUMAN	Heterogeneous nuclear ribonucleoprotein D0
Q8NFW8 NEUA_HUMAN	N-acetylneuraminate cytidylyltransferase
P11498 PYC_HUMAN	Pyruvate carboxylase mitochondrial
P38159 RBMX_HUMAN	RNA-binding motif protein X chromosome

P13645 K1C10_HUMAN	Keratin type I cytoskeletal 10
P68371 TBB4B_HUMAN	Tubulin beta-4B chain
Q99536 VAT1_HUMAN	Synaptic vesicle membrane protein VAT-1 homolog
O15160 RPAC1_HUMAN	DNA-directed RNA polymerases I and III subunit RPAC1
Q86UE4 LYRIC_HUMAN	Protein LYRIC
Q9NX62 IMPA3_HUMAN	Inositol monophosphatase 3

Supplementary Table S5. Information of antibodies.

Antibody names	Information
Anti-CD63	sc-365604, Santa Cruz Biotechnology
Anti-TIMP1	sc-365905, Santa Cruz Biotechnology
Anti- β 1-integrin (mouse)	sc-73610, Santa Cruz Biotechnology
Anti- β 1-integrin (rabbit)	ab-24693, abcam
Anti-NF- κ B	sc-8008, Santa Cruz Biotechnology
Anti-IKBA	sc-1643, Santa Cruz Biotechnology
Anti-p-IKBA	sc-8404, Santa Cruz Biotechnology
Anti-AKT	9272, Cell Signaling Technology
Anti-p-AKT	13038, Cell Signaling Technology
Anti-ERK	4696, Cell Signaling Technology
Anti-p-ERK	4370, Cell Signaling Technology
Anti-GAPDH	sc-365062, Santa Cruz Biotechnology

Supplementary Table S6. The list of primers and oligomers used in this study.

Name	Sequences (5' → 3')
CD63 siRNA-1 sequence	5-GGATGCAGGCAGATTTTAATT-3 (sense);
CD63 siRNA-2 sequence	5-GGATTAATTTCAACGAGAATT-3 (sense);
RNA duplex control sequence	5-UUCUCCGAACGUGUCACGUTT-3 (sense)