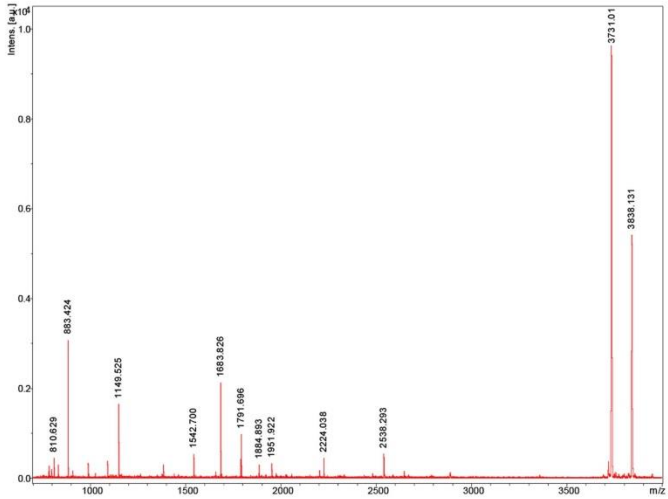
Supplementary Materials: Characterization of increased extracellular vesicle-mediated tigecycline resistance in *Acinetobacter baumannii*

Hyejin Cho, Tesalonika Sondak, and Kwang-sun Kim*

Department of Chemistry and Chemistry Institute of Functional Materials, Pusan National University, Busan, 46241, South Korea

*Correspondence: kwangsun.kim@pusan.ac.kr; Tel.: +82-51-510-2241

(a) TIG-R18



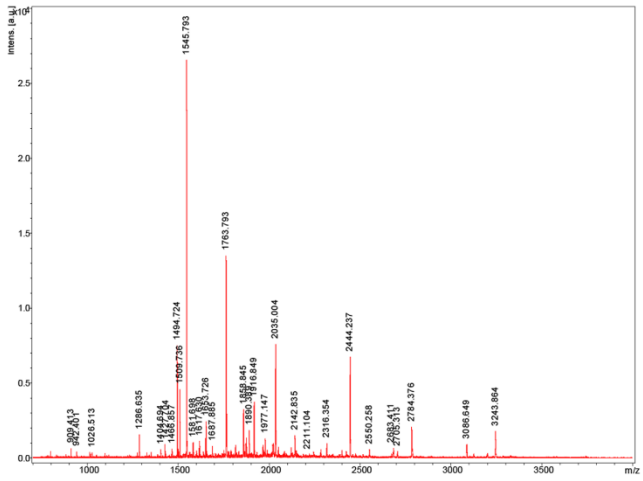
1 MKNIQKSLLA ALIVAGYAVN TQAAVTGQVD VKLNISTGCT VGGSQTEGNM 50

51 NK**FGTLNFGK** TSGTWNNVLT AEVASAATGG NISVTCDGTD PVDFTVAIDG 100

101 GERTDR**TLKN** **TASADVVAYN** **VYR**DAAR**TNL** **YVVNQPQQFT** **TVSGQATAVP** 150

151 **IFGAIAPNTG** **TPKAQGDYKD** **TLLVTVNF** 178

(b) TIG-R23



1 MKKLAIASAL LSALAVSGAA NAYQAEVGGS YNYLDPDNGS SVSK**FGVDGT** 50

51 **YYFNPVQTRN** **APLAEAAFLN** **R**ASNVNAHVN YGDNSGTK**DT** **QYGVGVEYFV** 100

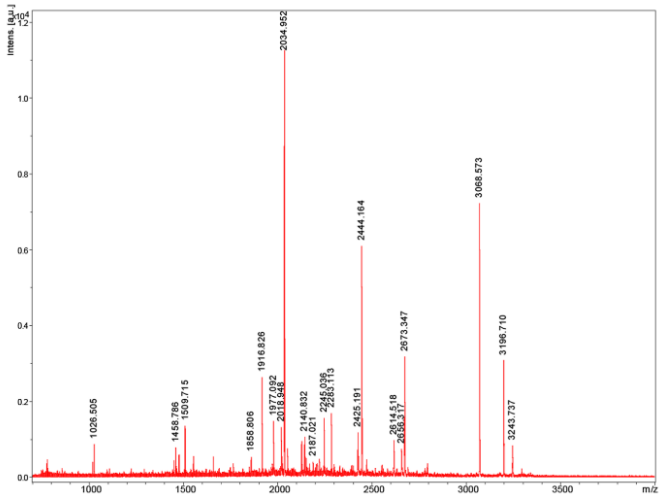
101 **PNSDFYLSGD** **VGR**NEREIDN TNIDSK**VTTY** **AAEVGYLPAP** **GLLLALGVK**G 150

151 YDEKDGKDGA DPTVRAK**YVT** **QVGQHDVNLE** **AYGAFGDLDE** **YKVR**GDYYID 200

201 K**TLSLGVDYY** **NNDLTDKDEF** **GINAKKFLNQ** **QVSVEGR**VGF GDNDNTYGVR 250

251 AAYRF 255

(c) TIG-R25



1 MKVLRVLVTT TALLAAGAAM ADEAVVHDSY AFDKNQLIPV GARAEVGTTG 50

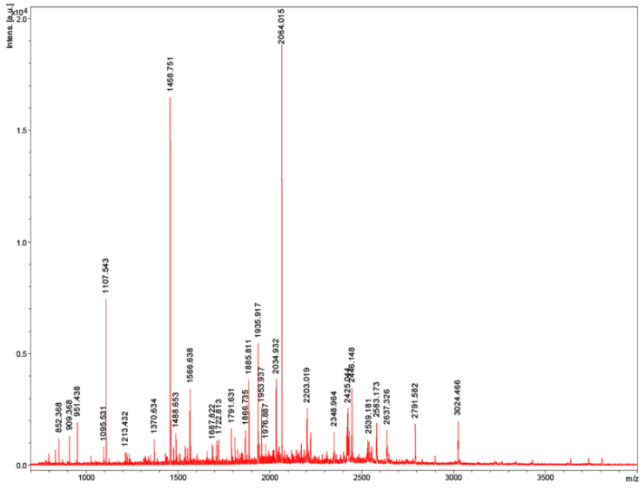
51 YGGALLWQAN PYVGLALGYN GGDISWTDDV SVNGTKYDLD MDNNNVYLNA 100

101 EIRPWGASTN PWAQGLYIAA GAAYLDNDYD LAKR**IGNGDT** **LSIDGKNYQQ** 150

151 **AVPGQEGGVR** **GKMSYKNDIA** **PYLGFGFAPK** **ISKNWGVFGE** **VGAYYTGNPK** 200

201 **VELTQYNLAP** **VTGNPTSAQD** **AVDKEANEIR** NDNKYEWMPV GKVGVNFYW 249

(d) TIG-R30



1 **MRFSQLK**VGV IAALLSVSSF AAQEFLNVSY DPTRELYTDF NK**QFGTYWKQ** 50

51 RTGQDIEFKQ SHGGSGKQAR **AVIDGLNADV** **VTLALAADID** **EIAEK**AKLLP 100

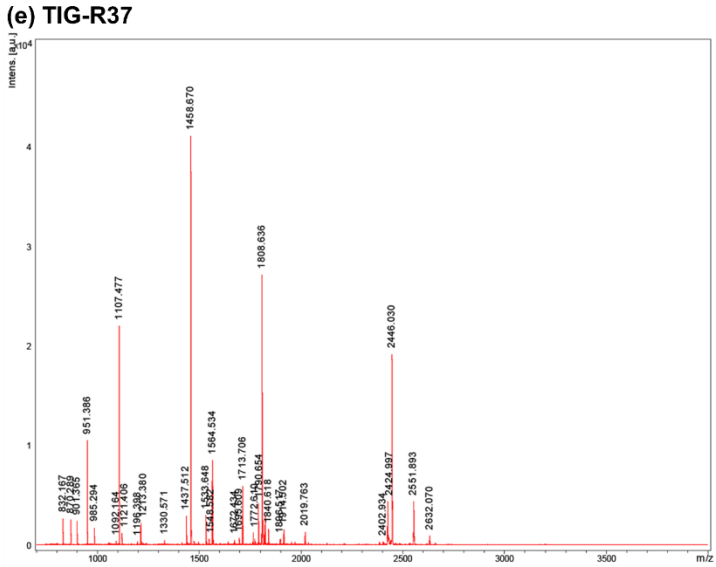
101 TDWQKK**LPQN** **STPYTSTIVF** **LVRK**GNPKQI KDWGDLIKPG VEIITPNPKT 150

151 SGGARWNYLA AWAWAKHQAG GNDAKAQEFV RQIYKHTKVL DSGARGATTT 200

201 FAER**GIGDVL** **LAWENEAHLA** **IREQPGKFEI** **VTPSLSILAE** **PPVAIVEK**NA 250

251 AKK**GNLTIAK** **GYLNYLYSPA** **GQEIAARNFY** **RPR**NAAVLK**K** **YSNVFKPLK**L 300

301 VTIDKEFGGW TKVQKQHFDN GGVFDQIVKI NSAEK 335

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1   MKLSRIALAT MLVAAPLAAA NAGVTVTPLL LGYTFQDSQH NNGGKDGNLT  50
51  NGPELQDDLF VGAALGIELT PWLGFEAEYN QVKGDVDGAS AGAEYKQKQI 100
101 NGNFYVTSDL ITKNYDSKIK PYVLLGAGHY KYDFDGVNRG TRGTSEEGTL 150
151 GNAGVGAFWR LNDALSLRTE ARATYNADEE FWNYTALAGL NVVLGGHLKP 200
201 AAPVVEVAPV EPTPVAPQPQ ELTEDLNMEL RVFFDTNKSN IKDQYKPEIA 250
251 KVAEKLSEYP NATARIEGHT DNTGPRKLNE RLSLARANSV KSALVNEYNV 300
301 DASRLSTQGF AWDQPIADNK TKEGRAMNRR VFATITGSRT VVVQPGQEAA 350
351 APAAAQ                                              356
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Figure S1. MALDI-TOF/MS spectrum and amino acid sequences of protein bands from TIG-R derived EVs. Mass spectral data (top) and full amino acids to matched proteins (bottom) of (**a**) TIG-R 18, (**b**) TIG-R23, (**c**) TIG-R25, (**d**) TIG-R30, and (**e**) TIG-R37 are shown. Band IDs are adapted from **Figure 1**. All analysis of peptide fragments were as described in *Materials and Methods*. Bold underlined sequences below the mass spectrum indicate matched amino acids to the proteins adapted from UniProt database ([26]; www.uniprot.org; accessed on February 2023).

(a)

(μg·mL^{-1})	1	2	3	4	5	6	7	8	9	10	11	12
A	AXO								MERO			
	1	2	4	8	16	32	64	128	1	2	4	8
B	CEP		POD								CIP	
	8	16	0.25	0.5	1	2	4	8	16	32	1	2
C	FOT									GEN		
	0.25	0.5	1	2	4	8	16	32	64	4	8	16
D	F/C										AMP	
	0.12/4	0.25/4	0.5/4	1/4	2/4	4/4	8/4	16/4	32/4	64/4	8	16
E	TAZ										FAZ	
	0.25	0.5	1	2	4	8	16	32	64	128	8	16
F	T/C											POS
	0.12/4	0.25/4	0.5/4	1/4	2/4	4/4	8/4	16/4	32/4	64/4	128/4	
G	IMI						P/T4					
	0.5	1	2	4	8	16	4/4	8/4	16/4	32/4	64/4	
H	FEP					FOX					NEG	
	1	2	4	8	16	4	8	16	32	64		

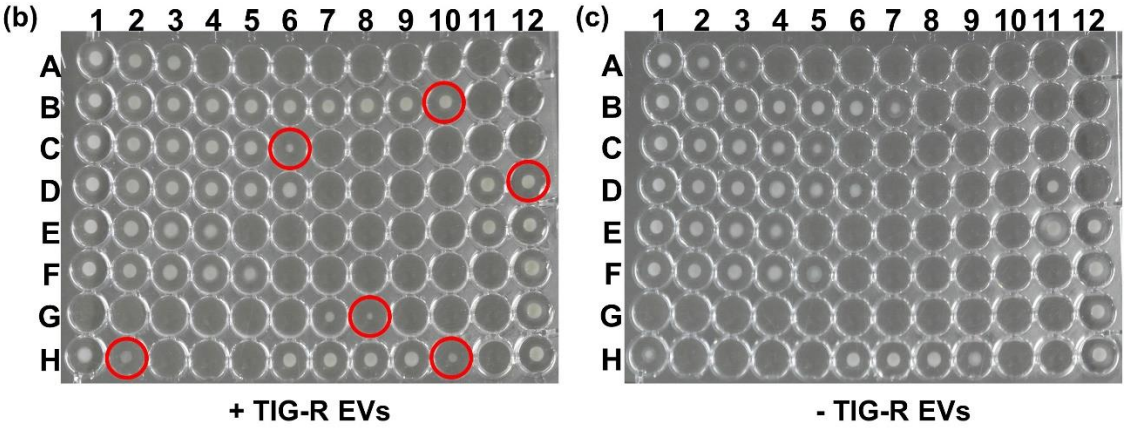

Figure S2. Confirmation of TIG-R EV-mediated resistance to other antibiotics. (**a**) Information on antibiotics and the working concentration. Sensititre™ Extended Spectrum Beta-lactamase Plate (Cat. No. ESB1F, Thermo Fisher Scientific, Waltham, MA, USA) with final concentration of individual antibiotics shown. TAZ, Ceftazidime; FAZ, Cefazolin; FEP, Cefepime; FOX, Cefoxitin; CEP, Cephalothin; POD, Cefpodoxime; FOT, Cefotaxime; AXO, Ceftriaxone; IMI, Imipenem; MERO, Meropenem; GEN, Gentamicin; AMP, Ampicillin; CIP, Ciprofloxacin; P/T4, Piperacillin/tazobactam constant 4; T/C, Ceftazidime/clavulanic acid; F/C, Cefotaxime/clavulanic acid; NEG and POS indicate the negative and positive control, respectively. (**b**, **c**) Effect of TIG-R EVs on the resistance to antibiotics. TIG-S cells were cultured in a Sensititre™ Extended Spectrum Beta-lactamase Plate with (**b**) or without (**c**) TIG-R EVs (5 μg·mL^{-1}). Red circle of the plate indicated resistance for each antibiotic. One representative from n = 3 was shown. The 96-well plates were imaged with digital camera (Samsung NX200, Suwon, Korea).

Table S1. Identification of proteins enriched or solely presented in TIG-R EVs

Band ID	Accession No.	Protein size (amino acids)	Coverage to proteins (%)	Function
TIG-R37	Q6RYW5	356	26	Outer membrane protein
TIG-R30	D0CBP2	335	42	Sulfate ABC transporter periplasmic substrate-binding protein
TIG-R25	D0CBN6	249	37	Carbapenem-associated resistance protein
TIG-R23	D0CF50	255	54	General bacterial porin
TIG-R18	A0A6F8TDQ5	178	42	Spore Coat Protein U domain protein

Band ID indicates the protein bands shown in **Figure 1**. Based on the Mass spectral data from **Figure S1** and searches of MASCOT database and UniProt database ([26]; www.uniprot.com; accessed on Fe bruary 13, 2023), proteins were identified as encoded from *Acinetobacter baumannii*. Coverage (%) was determined as the percentile of peptides overlapping to full-size proteins.

Reference

26. The UniProt Consortium. UniProt: The Universal Protein Knowledgebase in 2023. *Nucleic Acids Res.* **2023**, *51*, D523–D531. https://doi.org/10.1093/nar/gkac1052.