Supplementary Information

In vitro and in vivo RNA inhibition by CD9-HuR functionalized exosomes encapsulated with miRNA or CRISPR/dCas9

Zhelong Li[1,2,#], Xueying Zhou[1,2,#], Mengying Wei[2,#], Xiaotong Gao[2,3], Lianbi Zhao[1,2], Ruijing Shi[1,2], Wenqi Sun[1,2], Yunyou Duan[1], Guodong Yang[2,*], Lijun Yuan[1,*]

[1]Department of Ultrasound Diagnostics, Tangdu Hospital, Fourth Military Medical University, Xi'an, 710038, People's Republic of China

[2]The State Laboratory of Cancer Biology, Department of Biochemistry and Molecular Biology, Fourth Military Medical University, Xi'an, 710032, People's Republic of China

[3]Department of Hematology, Tangdu Hospital, Fourth Military Medical University, Xi'an, 710038, People's Republic of China

# These authors contributed equally to this article.

* Correspondence authors:

Lijun Yuan, Department of Ultrasound Diagnostics, Tangdu Hospital, Fourth Military Medical University, Xinsi Road NO.569th, 710038, Xi'an, China, email: yuanlj@fmmu.edu.cn; Tel: +862984777471, Fax: +862984777471.

Guodong Yang, The State Laboratory of Cancer Biology, Department of Biochemistry and Molecular Biology, Fourth Military Medical University, Changlexi Road NO.169th, 710032, Xi'an, email: yanggd@fmmu.edu.cn; Tel: +862984774516, Fax: +862984774516.

Table S1 Sequences of the miRNA mimics, inhibitors, and PCR primers used in the study.

Name	**Sequence**	
mimics/inhibitors		
miR328	5’-GGGGGCUGGUGGGGCUCAG-3’	
miR155	5’-UUAAUGCUAAUUGUGAUAGGGGU-3’	
antimiR155-AREs	5’-ACCCCUAUCACAAUUAGCAUUAAUAUUUAUUUAUUUAU-3’	
qPCR		
miR328	Forward	5’-GGGGGCAGGAGGGGCTCAG-3’
	Reverse	Provided in the kit
miR155（mouse）	Forward	5’-TTAATGCTAATTGTGATAGGGGT-3’
	Reverse	Provided in the kit
miR155（human）	Forward	5’-TTAATGCTAATCGTGATAGGGGT-3’
	Reverse	Provided in the kit
AntimiR-155	Forward	5’-ACCCCTATCACAATTAGCATTAA-3’
	Reverse	Provided in the kit
T6	Forward	5’-CTCGCTTCGGCAGCACA-3’
	Reverse	Provided in the kit
C/ebpα	Forward	5’-GATGGTTTCGGGTCGCTGGAT-3’
	Reverse	5’-ACCACGGCCTGACTCCCTCAT-3’
dCas9	Forward	5'-GTGGACGCTATCGTGCCTCAG-3'
	Reverse	5'-GCCGCCAGTAGTTCTTCATCTT-3'
Col1a1	Forward	5'-GCTCCTCTTAGGGGCCACT-3'
	Reverse	5'-ATTGGGGACCCTTAGGCCAT-3'
Tnfα	Forward	5’-GACGTGGAACTGGCAGAAGAG-3’
	Reverse	5’-TTGGTGGTTTGTGAGTGTGAG-3’
Mcp1	Forward	5’-CCTGCTGTTCACAGTTGCC-3’
	Reverse	5’-ATTGGGATCATCTTGCTGGT-3’
β-actin（mouse）	Forward	5’-CTGTCCCTGTATGCCTCTG-3’
	Reverse	5’-ATGTCACGCACGATTTCC-3’
β-actin（human）	Forward	5’-CATGTACGTTGCTATCCAGGC-3’
	Reverse	5’-CTCCTTAATGTCACGCACGAT-3’
Cloning		
C/ebpα gRNA	sense	5’-CACCGCTATAAAAGCGGTCCCCGCG-3’
	antisense	5’-AAACCGCGGGGACCGCTTTTATAGC-3’
CD9	Forward (Nhe1)	5’-GGGCTAGC ACCATGCCGGTCAAAGGAGGCACCA-3’
	Reverse (Xho1)	5’-GGCTCGAG GACCATCTCGCGGTTCCTG-3’
HuR	Forward (Xho1)	5’-GGCTCGAGATGTCTAATGGTTATGAAGA-3’
	Reverse (EcoR1)	5’-CCGAATTCTTATTTGTGGGACT TGTTGGT-3’
CD9-HuR	Forward (Pac1)	5’-GGTTAATTAA

sub-cloning		ACCATGCCGGTCAAAGGAGGCACCA-3’
	Reverse (Pac1)	5’-GGTTAATTAA TTATTTGTGGGACTTGTTGGT-3’
dCas9	Forward (Pac1)	5’-GGTTAATTAAACCATGGAC AAGAAGTACAGCATCGG-3’
	Reverse (BstB1)	5’-CCTTCGAATTATCAAGCGGC CGCCACCTTCCTCTT-3’
AREs	Forward (BstB1)	5’-CGAAATTTACCCATTTACCCATTTAC CCATTTACCCATTTACCCATTTATT-3’
	Reverse (BstB1)	5’-CGAATAAATGGGTAAATGGGTAAATG GGTAAATGGGTAAATGGGTAAATTT-3’

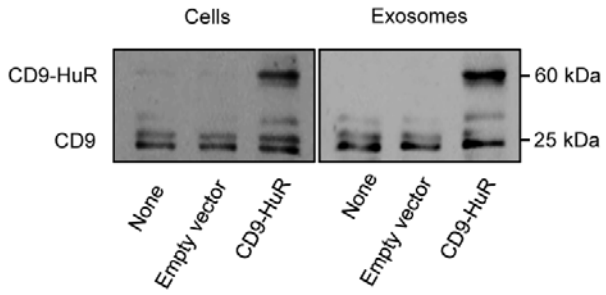

Figure S1. Expression of fusion protein CD9-HuR in both the parental cells and derived exosomes. Western blot analysis of the expression of fusion protein CD9-HuR in both the parental cells and derived exosomes with indicated treatments. The membrane was incubated with anti-CD9 antibody.

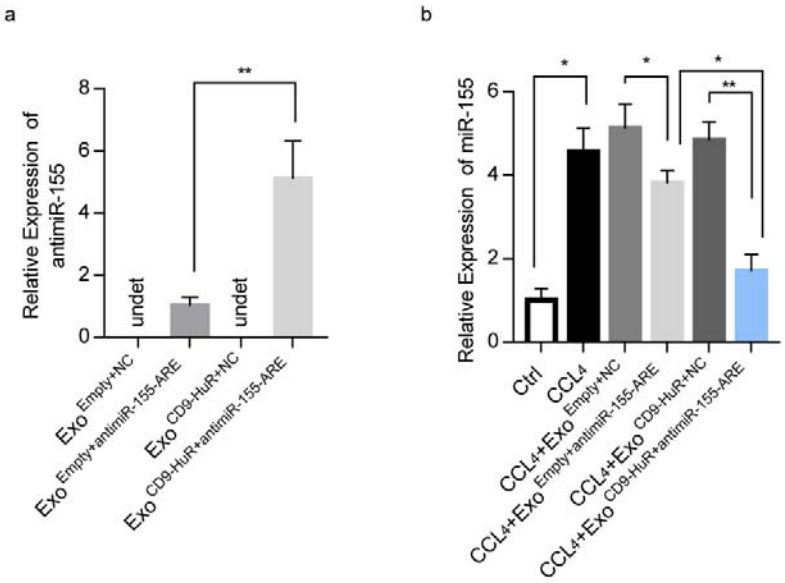

Figure S2. Effects of CD9-HuR functionalized exosomes on antimiR-155-ARE encapsulation and endogenous miR155 knockdown. (a) qPCR analysis of antimiR-155 expression in the indicated exosomes. (b) qPCR analysis of endogenous miR-155 expression in the liver of mice after receiving injection of indicated exosomes twice a week for one week. U6 served as internal control. Data are expressed as mean±SEM of three different experiments. *, $p < 0.05$; **, $p < 0.01$ vs indicated groups.