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Supplemental Information

Exosomal miR-208b related with oxaliplatin resistance promotes Treg expansion in colorectal cancer

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Supplemental Data Methods

Sample processing

Venous blood samples (approximately 5 mL) were collected from each participant and were processed within 1 h. Each blood sample was immediately centrifuged at 3000g for 5 min at room temperature, followed by a 5 min high-speed centrifugation step at 10,000 g at 4 °C. The supernatant serum was transferred to a fresh tube and stored at -80 °C until analysis.

TDLA technology

For the TLDA analysis, reverse transcription were performed using the TaqMan MicroRNA Reverse Transcription Kit and Megaplex RT Primers as previously described [18]. Briefly, 3 μL total RNA were added to 4.5 μL of the RT reaction mix (Megaplex RT Primers 10X, dNTPs with dTTP 100mmol, MultiScribe Reverse Transcriptase 50U/μL, 10X RT Buffer, MgCl2 25 mmol, RNase Inhibitor 20 U/μL and nuclease-free water). After incubation on ice for 5 min, reverse transcription was performed using a thermal cycler (UNO-ThermobloCk, Biometra, Göttingen, Germany). MicroRNA profiling of 739 different human miRNAs was then performed using the TaqMan Low Density Array (TaqMan Array Human MicroRNA A+B Cards Set v3.0). In order to generate enough miRNA cDNA templates for the following real-time RCR, a pre-amplification was performed after the reverse transcription. All steps were performed using a 7900 HT Fast Real-Time PCR System (Applied Biosystems, Foster City, CA) and all reactions were performed following the protocols of the manufacturer. The expression data for the miRNA were presented as threshold cycle (CT) values and normalized to an internal control recommended by the manufacturer on the calculated CT

of each miRNA (ΔCT). The relative expression levels of miRNA were calculated using the equation $2^{-\Delta\Delta CT}$.

Quantification of miRNAs by probe-based qRT-PCR

A TaqMan probe–based qRT-PCR assay was performed according to the manufacturer's instructions (7500 Sequence Detection System; Applied Biosystems), with a minor modification as described previously [18]. Briefly, the reverse transcription reaction was carried out in 10 μL mixture containing 2 μL of extract RNA from serum, 1 μL of 10 mmol/L dNTPs, 0.5 μL of AMV reverse transcriptase (TaKaRa), 1 μL of a stem-loop RT primer (Applied Biosystems), 2 μL of 5 × reverse transcription buffer and 3.5 μL of diethylpyroCarbonate (DEPC)-treated water. For synthesis of cDNA, the reaction mixtures were incubated at 16 ^{o}C for 30 min, at 42 ^{o}C for 30 min, at 85 ^{o}C for 5 min, and then held at 4 ^{o}C. Real-time PCR was then performed (1 cycle of 95 ^{o}C for 5 min, and 40 cycles of 95 ^{o}C for 15 sec and 60 ^{o}C for 1 min) with an Applied Biosystems 7500 Sequence Detection System. The reaction was performed with a final volume of 20 μL containing 1 μL of cDNA, 0.3 μL of Taq, 0.33 μL of hydrolysis probe (Applied Biosystems), 1.2 μL of 25 mmol/L MgCl2, 0.4 μL of 10 mmol/L dNTPs, 2 μL of 10 × PCR buffer, and 14.77 μL of DEPC water. All reactions, including controls no template RNA, were performed in triplicate. The resulting Ct valueswere determined using fixed threshold settings.

Supplemental Table 1

Differentially expressed miRNAs in responder serum compared to non-responders received FOLFOX determined by TaqMan Low Density Assay.

miRNA	Ct of Nonresponders	Ct of Responders	-△Ct (Nonresponders vs. Responders)
hsa-miR-210	39.81	28.87	10.94
hsa-miR-376b	39.81	30.06	9.75
hsa-miR-149	39.81	30.55	9.26
hsa-miR-605	39.68	32.10	7.59
hsa-miR-135b	39.81	32.34	7.47
hsa-miR-708	39.81	32.57	7.24
hsa-miR-381	39.81	32.64	7.17
hsa-miR-523	39.81	33.23	6.58
hsa-miR-601	39.68	33.11	6.58
hsa-miR-302d	39.68	33.15	6.54
hsa-miR-627	39.81	34.24	5.57
hsa-miR-412	39.81	34.38	5.43
hsa-miR-576-3p	39.81	34.66	5.15
hsa-miR-542-3p	34.43	40.19	-5.76
hsa-miR-199b	34.33	40.19	-5.86
hsa-miR-216b	34.32	40.19	-5.87
hsa-miR-301b	34.31	40.19	-5.88
hsa-miR-449b	34.24	40.19	-5.95
hsa-miR-190	33.98	40.19	-6.21
hsa-miR-519a	33.69	40.19	-6.50
hsa-miR-302c	33.64	40.19	-6.55
hsa-miR-208b	33.57	40.19	-6.62
hsa-miR-504	33.19	40.19	-7.00
hsa-miR-372	32.31	40.19	-7.88
hsa-miR-512-3p	32.21	40.19	-7.99
hsa-miR-597	32.15	40.19	-8.04
hsa-miR-502	32.12	40.19	-8.08
hsa-miR-517c	32.01	40.19	-8.18

Supplemental Table 2

Differentially expressed miRNAs in responder serum samples compared to non-responders received FOLFOX in testing cohort validated by quantitative qRT-PCR. The relative contents of miRNAs are presented as mean±SEM.

Regimen	miRNA	Responders (n=12)	Non-responders (n=12)	Fold change	*P* value
FOLFOX	miR-135b	0.15± 0.012	0.31± 0.024	2.07	<0.0001
	miR-208b	0.14± 0.016	0.30 ± 0.030	2.14	0.0005
	miR-372	0.11± 0.012	0.17 ± 0.017	1.54	0.032
	miR-132	0.13±0.02	0.20±0.019	1.53	0.022

Supplemental Table 3

The serum levels of miR-208b were normalized to U6. The raw Ct value of U6 from TLDA.

Array Card Name	Assay	Task	Ct	Ct	Ct
Human A v2.1	U6 snRNA	Endogenous Control	17.99837	15.03231	16.70992
Human A v2.1	U6 snRNA	Endogenous Control	17.37046	15.35241	17.9796
Human A v2.1	U6 snRNA	Endogenous Control	17.64946	15.96499	17.22582
Human A v2.1	U6 snRNA	Endogenous Control	17.15448	14.62617	16.89708
Human B v3.0	U6 snRNA	Endogenous Control	17.75492	14.75606	17.07323
Human B v3.0	U6 snRNA	Endogenous Control	17.94131	15.92483	17.42895
Human B v3.0	U6 snRNA	Endogenous Control	17.96244	15.7182	15.57702
Human B v3.0	U6 snRNA	Endogenous Control	17.9844	15.12694	17.93129

Supplemental Figure 1.

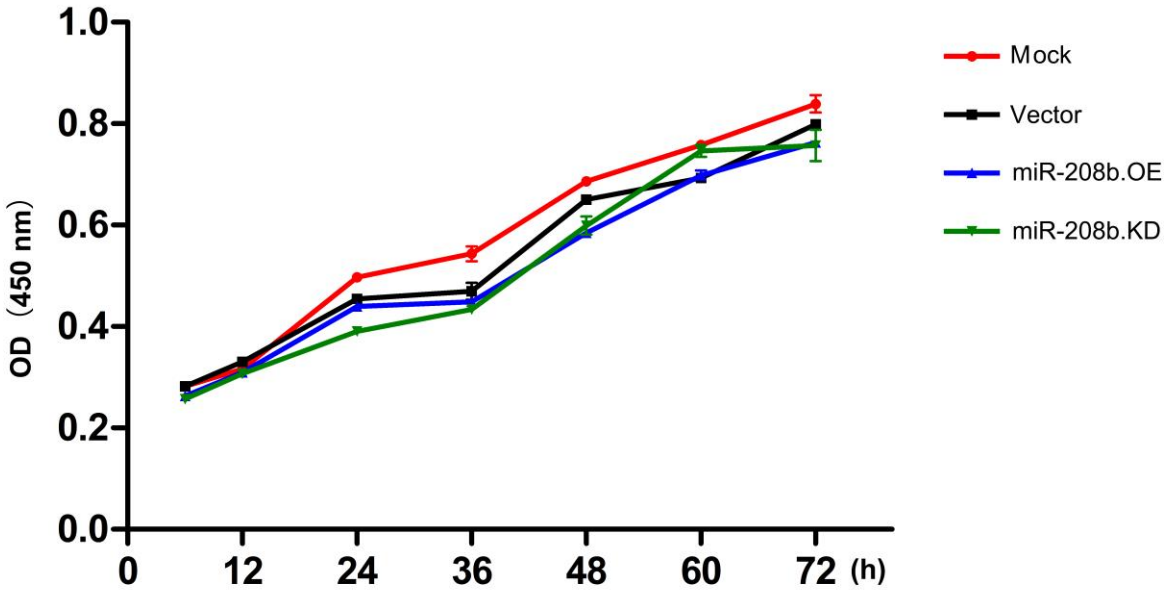

Figure S1. Effects of miR-208b on the proliferation of CT26 cells. CT26 cells were transfected with miR-208b-OE or miR-208b-KD lenti-virus, and cell viability was determined by CCK8 (n=3).

Supplemental Figure 2.

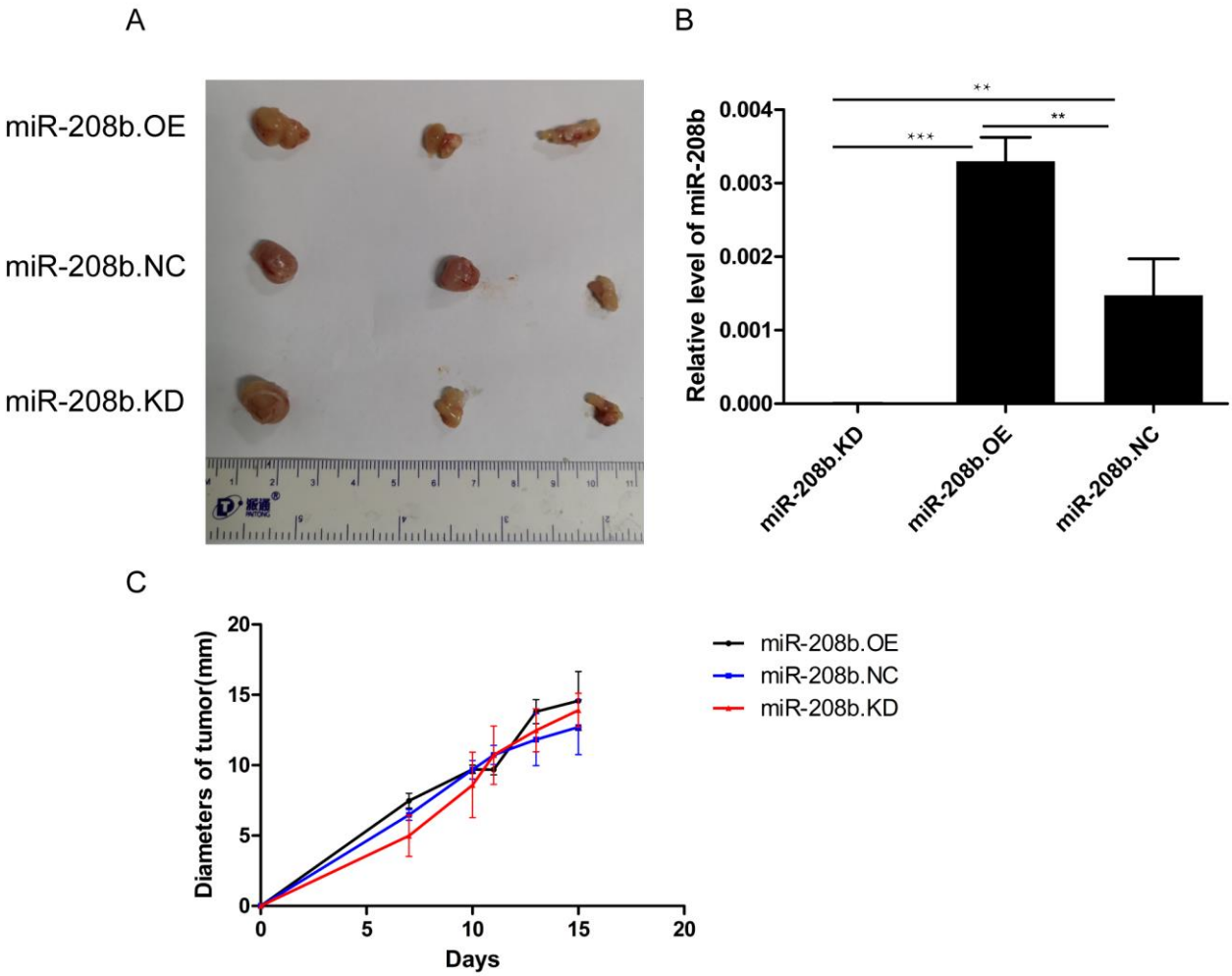

Figure S2. Effects of miR-208b on the proliferation of CT26 cells in vivo. CT26 cells were transfected with miR-208b-OE or miR-208b-KD lenti-virus. A.The comparison of the tumors taken from different groups (n=3). B. Relative levels of miR-208b in CT26-miR-208b.OE, CT26-miR-208b.NC and CT26-miR-208b.KD cell measured by RT-qPCR (n=3). C. Alterations of tumor diameters in each group (n = 3).* indicates $p < 0.05$ ** indicates $p < 0.01$and *** indicates $p < 0.001$.

Supplemental Figure 3.

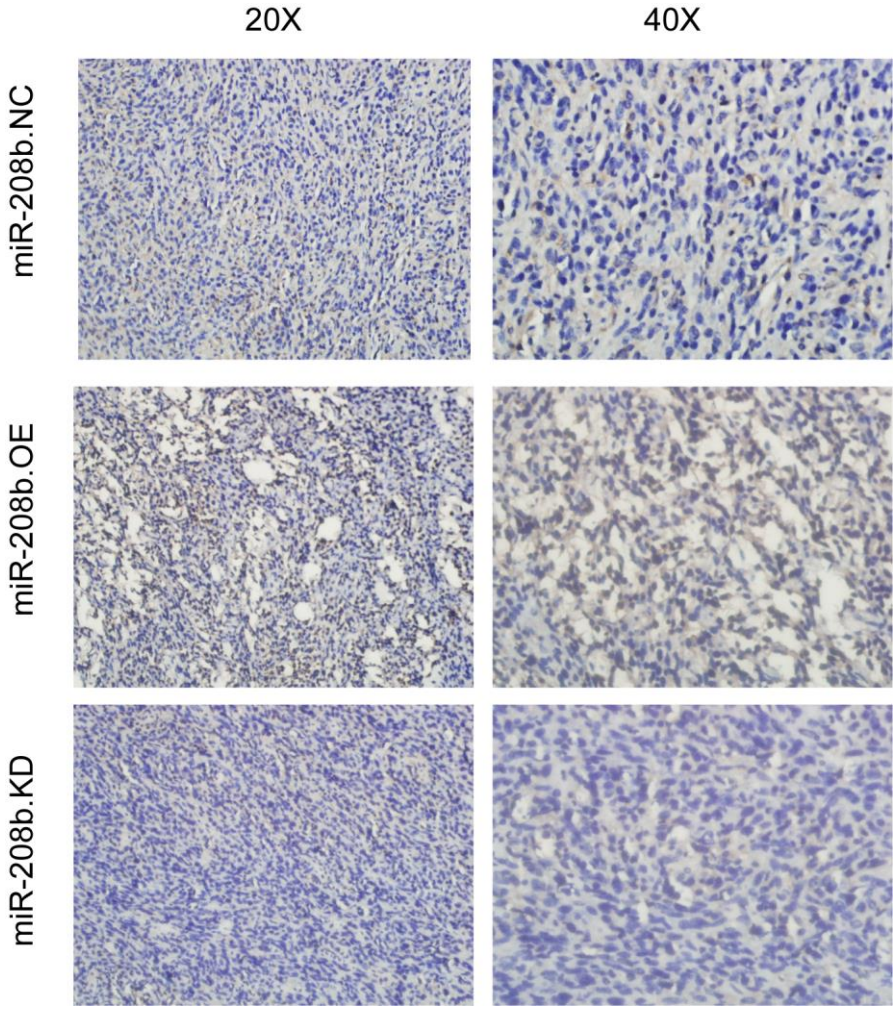

Figure S3. The tumor infiltrated Tregs in babl/c mice by Immunohistochemistry analysis. CT26 cells were transfected with miR-208b-OE or miR-208b-KD lenti-virus.