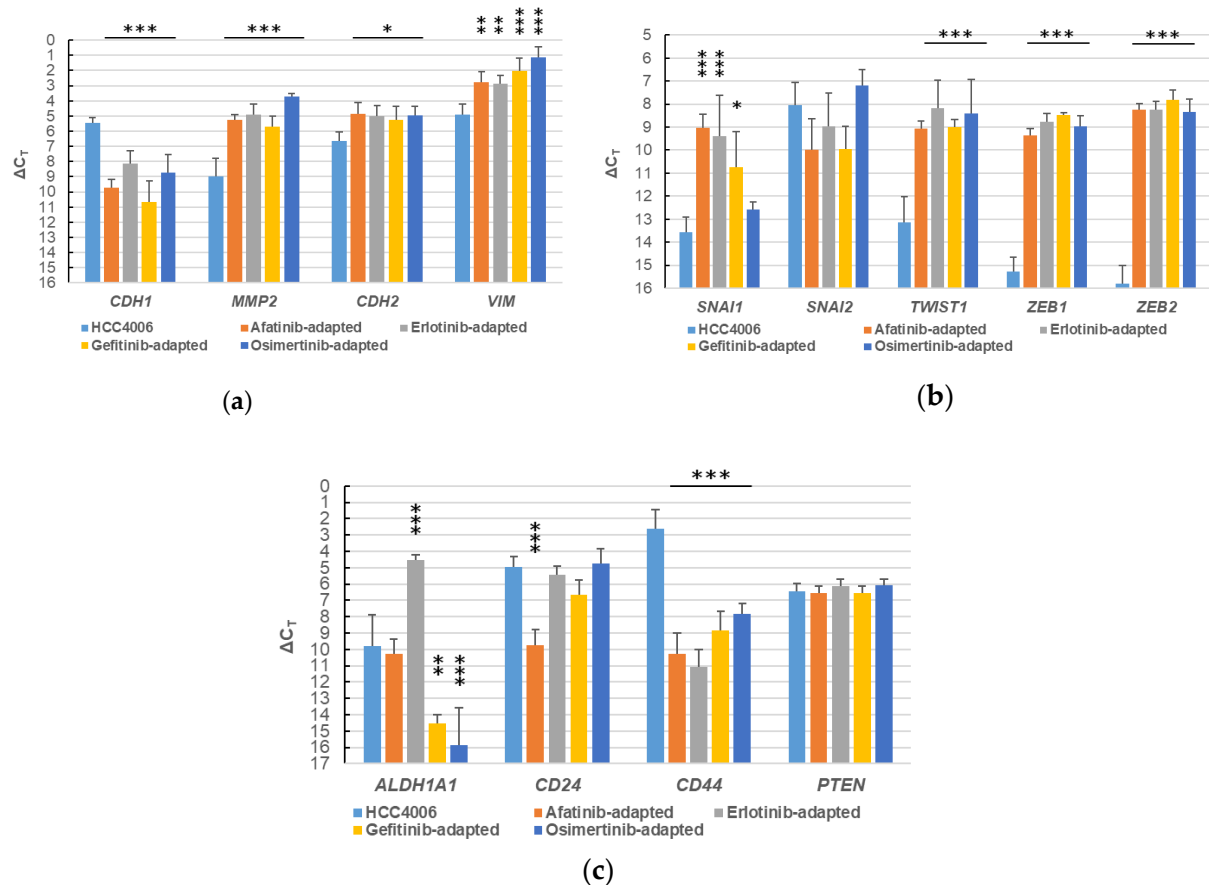


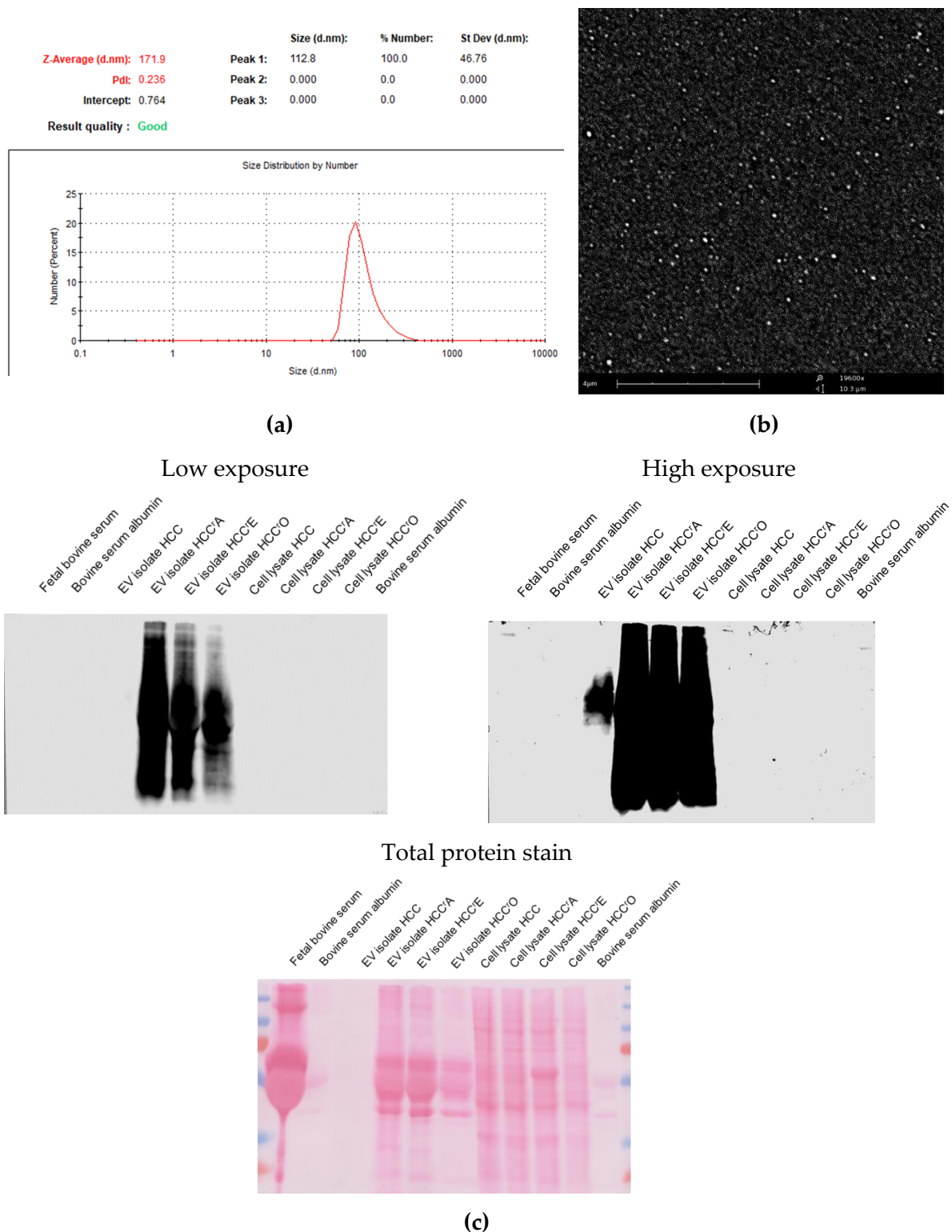
Supplementary Materials

Loss of Key EMT-Regulating miRNAs Highlight the Role of ZEB1 in EGFR Tyrosine Kinase Inhibitor-Resistant NSCLC

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Supplementary Figure S1. Epithelial-mesenchymal transition is confirmed in all resistant cell lines: (a) Gene expression of EMT-markers, (b) EMT transcription factors, and (c) stem cell markers measured by qPCR. Results are displayed as the mean + SD of the delta- C_T with $n \geq 3$. A 1-way ANOVA with Dunnett's post hoc test was used to test for statistically significant differences relative to the parental HCC4006 cell line, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.



Supplementary Figure S2: Characterisation of extracellular vesicles (EVs): (a) EVs isolated from 16HBE14o-, HCC4006 and EGFR-TKI-resistant HCC4006 cells were analysed for size and size distribution using dynamic light scattering. (b) Size and round shaped morphology of isolated EVs was confirmed by scanning electron microscopy. (c) EVs, FBS, BSA and cell extracts were analysed for protein expression of exosome-enriched marker CD63 in HCC, HCCrE and HCCrO cells. 10 µL of FBS, EV isolate, 5 µg of BSA and 30 µg of cell extract were blotted on a nitrocellulose membrane and stained with Ponceau S total protein stain or a CD63-specific antibody (n = 1).

Supplementary Table S1: List of antibodies and dilutions

Antibody	Dilution Western blotting	Dilution Immunocytochemistry
Axl (C89E7) Rabbit mAb #8861	1:1000	-
E-cadherin (24E10) Rabbit mAb #3195	1:1000	1:1600
N-cadherin (D4R1H) XP® Rabbit mAb # 13116	1:1000	1:100
Vimentin (D21H3) XP® Rabbit mAb #5741	1:1000	-
ZEB1 (E2G9Y) XP® Rabbit mAb #70512	1:1000	-
CD63 Monoclonal Antibody (Ts63) #10628D	1:250	
IRDye® 800CW Goat anti-Rabbit IgG Secondary Antibody #926-32211	1:10,000	
IRDye® 800CW Goat anti-Mouse IgG Secondary Antibody #926-32210	1:10,000	
IRDye® 680LT Goat anti-Mouse IgG Secondary Antibody #926-68020	1:20,000	
IRDye® 680LT Goat anti-Rabbit IgG Secondary Antibody #926-68021	1:20,000	

Supplementary Table S2: List of doubling times and IC₅₀ values of the parental and resistant HCC4006 cell lines

Cell line	Doubling time [h]	Inhibitor	IC ₅₀ [nM]
HCC4006	41.8 ± 7.5	Afatinib	3.601 ± 0.81
		Erlotinib	53.09 ± 2.34
		Gefitinib	26.23 ± 15.87
		Osimertinib	3.75 ± 2.02
HCC4006 ^r AFA ^{0.1}	53.7 ± 16.7	Afatinib	>100
		Osimertinib	>1000
HCC4006 ^r ERLO ^{0.5}	54.0 ± 5.5	Erlotinib	>1000
		Osimertinib	>1000
HCC4006 ^r GEFI ^{0.5}	61.0 ± 12.4	Gefitinib	>1000
		Osimertinib	>1000
HCC4006 ^r OSI ^{0.5}	66.7 ± 12.9	Afatinib	579.6
		Erlotinib	>1000
		Gefitinib	>1000
		Osimertinib	>1000

Supplementary Table S3: Primer sequences and efficiencies

Gen/miRNA/ snRNA	Forward Primer	Reverse Primer	Efficiency [%]
<i>ALDH1A1</i>	CGGGAAAAGCAATCTGAAGAGG	GATGCGGCTATACAACACTGGC	90.78
<i>ACTB</i>	CACCATTGGCAATGAGCGGTTT	AGGTCTTTGCGGATGTCCACGT	96.81
<i>CD24</i>	CACGCAGATTTATTCCAGTGAAA	GACCACGAAGAGACTGGCTGTT	90.13

CD44	CCAGAAGGAACAGTGGTTTGGC	ACTGTCCTCTGGGCTTGGTGT	90.58
CDH1	GCCTCCTGAAAAGAGAGTGAAG	TGGCAGTGTCTCTCCAAATCCG	98.83
CDH2	CCTCCAGAGTTTACTGCCATGAC	GTAGGATCTCCGCCACTGATTC	90.53
GAPDH	GTCTCCTCTGACTTCAACAGCG	ACCACCCTGTTGCTGTAGCCAA	95.35
MMP2	AGCGAGTGGATGCCGCCTTTAA	CATCCAGGCATCTGCGATGAG	97.68
PTEN	TGAGTTCCTCAGCCGTTACCT	GAGGTTTCCTCTGGTCCTGGTA	94.88
RNU6	GTCGTGAAGCGTTCCA	CAGGTCCAGTTTTTTTTTTTTTAAA	98.65
SNAI1	TGCCCTCAAGATGCACATCCGA	GGGACAGGAGAAGGGCTTCTC	91.68
SNAI2	ATCTGCGGCAAGGCGTTTCCA	GAGCCCTCAGATTGACCTGTC	92.56
TWIST1	GCCAGGTACATCGACTTCCTCT	TCCATCCTCCAGACCGAGAAGG	95.42
VIM	AGGCAAAGCAGGAGTCCACTGA	ATCTGGCGTTCAGGGACTCAT	91.15
ZEB1	GGCATAACCTACTCAACTACGG	TGGGCGGTGTAGAATCAGAGTC	100.32
ZEB2	AATGCACAGAGTGTGGCAAGGC	CTGCTGATGTGCGAACTGTAGG	96.92
miR-9-5p	GCAGTCTTTGGTTATCTAGCTGT	GTCCAGTTTTTTTTTTTTTTCATACA	86.724
miR-21-5p	TCAGTAGCTTATCAGACTGATG	CGTCCAGTTTTTTTTTTTTTCAAC	113.029
miR-34a-5p	GGCAGTGTCTTAGCTGGT	TCCAGTTTTTTTTTTTTTACAACCA	106.256
miR-99a-5p	CAGAACCCGTAGATCCGA	GTCCAGTTTTTTTTTTTTTCAACAAG	101.836
miR-126-3p	GCAGTCGTACCGTGAGTAA	CCAGTTTTTTTTTTTTTTCGCAT	109.004
miR-139-5p	GTCTACAGTGCACGTGTC	CCAGTTTTTTTTTTTTTACTGGAG	109.196
miR-155-5p	CGCAGTTAATGCTAATCGTGATA	GGTCCAGTTTTTTTTTTTTTAACC	102.858
miR-183-5p	GCAGTATGGCACTGGTAGA	GGTCCAGTTTTTTTTTTTTTAGTGA	108.645
miR-192-5p	CAGCTGACCTATGAATGACA	TCCAGTTTTTTTTTTTTTGGCT	104.902
miR-200c-3p	AGTAATACTGCCGGGTAATGA	TCCAGTTTTTTTTTTTTTCCATCA	99.441
miR-203a-3p	AGGTGAAATGTTTAGGACCAC	GTCCAGTTTTTTTTTTTTTCTAGTG	103.881
miR-205-5p	CCTTCATTCCACCGGAGT	GGTCCAGTTTTTTTTTTTTTCAGA	114.939
miR-374a-5p	GCGCAGTTATAATACAACCTGA	GGTCCAGTTTTTTTTTTTTTCACT	98.679
miR-378a-5p	TCCTGACTCCAGGTCTT	GGTCCAGTTTTTTTTTTTTTACACA	106.167
miR-451a	CGCAGAAACCGTTACCA	GTCCAGTTTTTTTTTTTTTAACTCA	101.153
miR-544a	GCAGATTCTGCATTTTAGCAAG	GGTCCAGTTTTTTTTTTTTTGAAC	83.946
Universal RT Primer	CAGGTCCAGTTTTTTTTTTTTTVN (V = A, C and G; N = A, C, G and T)		

Supplementary Table S4: Mimic and negative control mimic sequence

Mimics	Sense (5'-3')	Antisense (5'-3')
miR-205-5p	UCCUUCAUUCCACCGGAGUCUC	GACUCCGGUGGAAUGAAGGAUU
miR-205-5p NC	UUCUCCGAACGUGUCACGUTT	ACGUGACACGUUCGGAGAATT

Supplementary Table S5: Size of extracellular vesicles determined by dynamic light scattering

Cell line	Peak Size [nm]	Z-Average [nm]	Polydispersity Index
16HBE14o-	125.4	189.6	0.240
HCC4006	120.5	185.5	0.399
HCC4006 ⁺ AFA ^{0.1}	103.6	195.9	0.317
HCC4006 ⁺ ERLO ^{0.5}	58.3	121.3	0.343
HCC4006 ⁺ GEFI ^{0.5}	101.8	151.2	0.289
HCC4006 ⁺ OSI ^{0.5}	106.4	194.4	0.367

Supplementary Table S6: Size of extracellular vesicles from HCCrA determined by scanning electron microscopy

HCC4006rAFA ^{0.1}	Size [nm]	SD [nm]
	113.8	21.6