

Supplementary Materials: MicroRNA-21 Plays Multiple On-cometabolic Roles in Coli-tis-Associated Carcinoma and Colorectal Cancer via the PI3K/AKT, STAT3, and PDCD4/TNF- α Signaling Pathways

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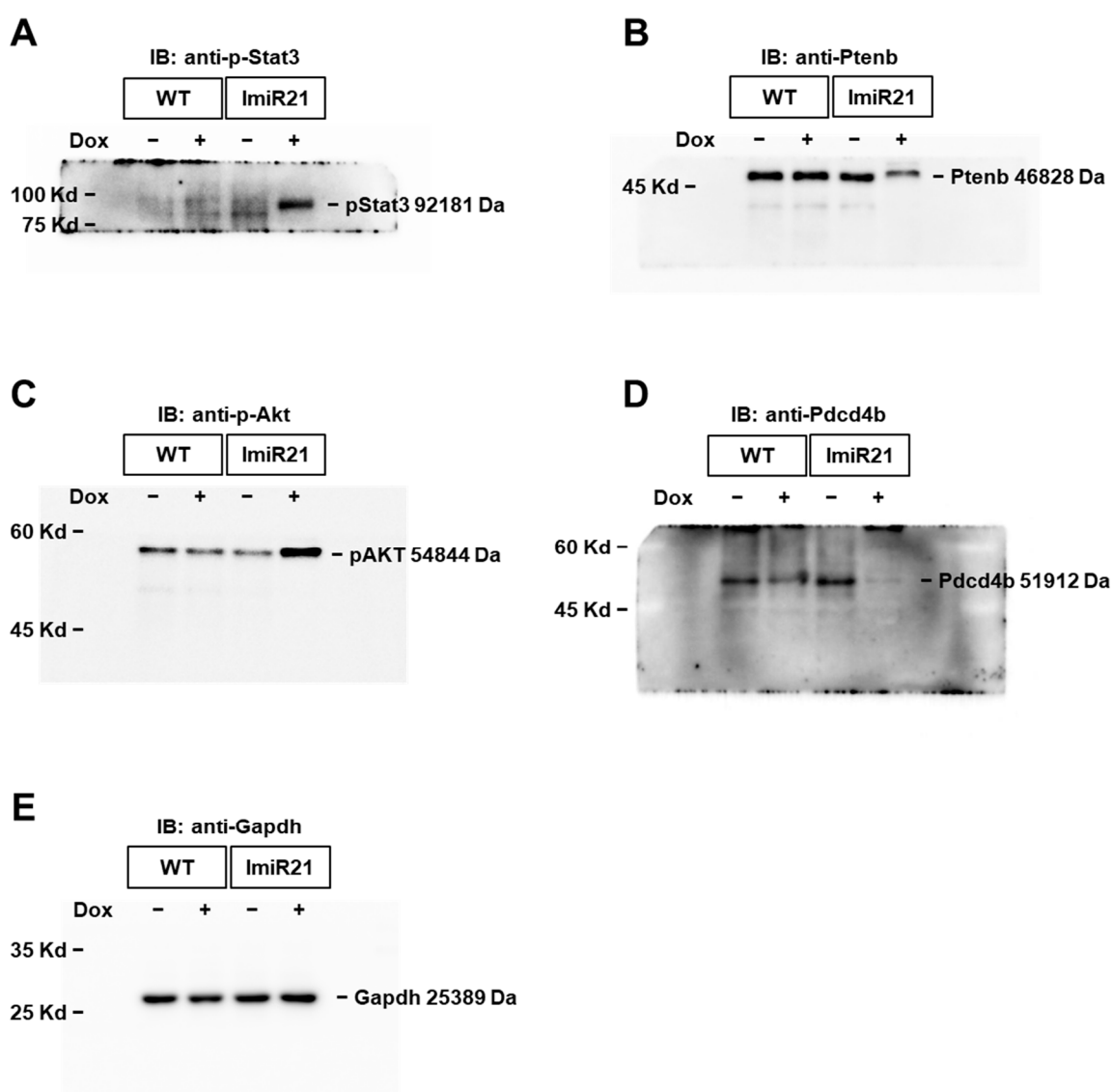


Figure S1. Raw western blots of (A) phosphorylated (p)-Stat3, (B) Ptenb, (C) p-Akt, (D) Pdcd4b, and (E) Gapdh in intestines of WT \pm Dox and ImiR-21 \pm Dox zebrafish at 6 mpf/1 mpt (months post-treatment).

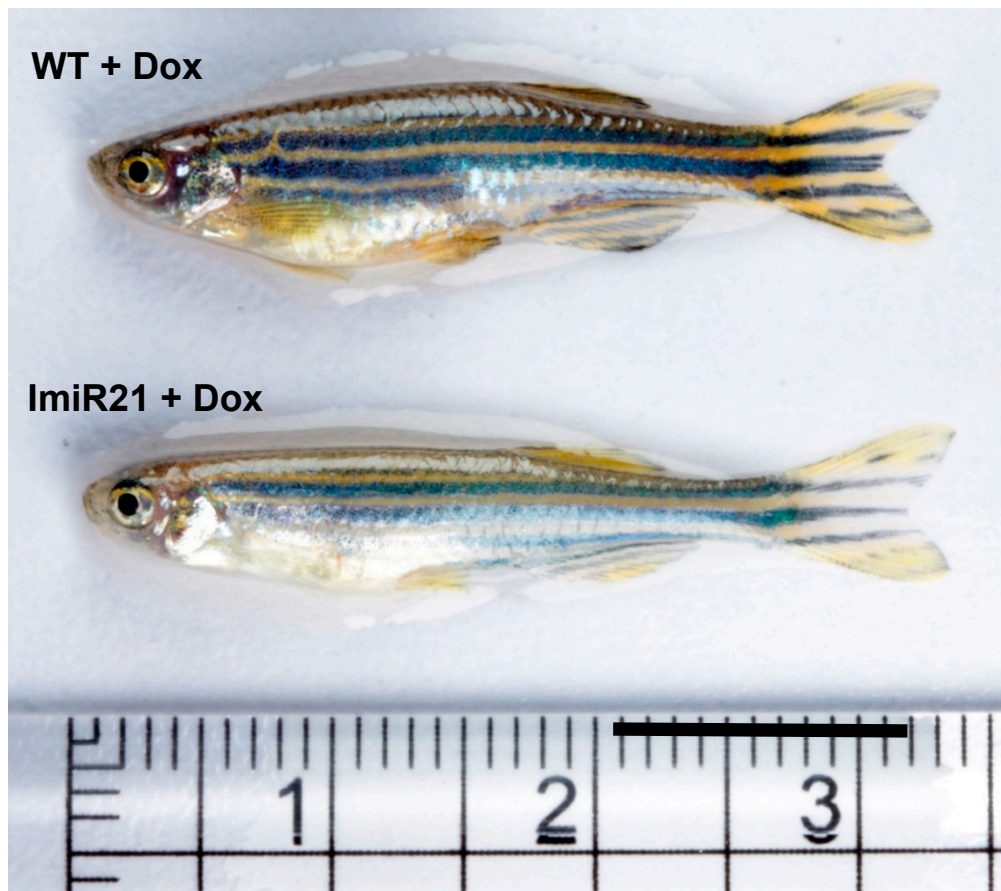
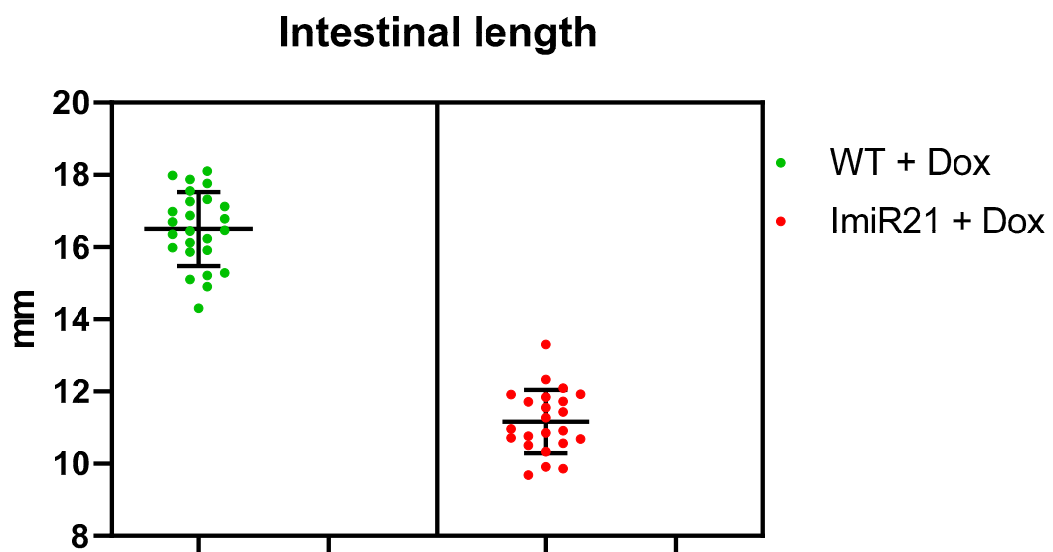
A**B**

Figure S2. Dysregulated miR-21 leads to short bowel syndrome. (A) Representative images of morphology of WT + Dox and ImiR-21 + Dox at 7 mpf/2 mpt. ImiR-21 + Dox zebrafish were weaker and more marasmic than WT + Dox. (B) Representative images of intestinal length of WT + Dox and ImiR-21 + Dox at 7 mpf/2 mpt.

Table S1. Primer sequences used for quantitative RT-PCR.

Gene.	Accession	Forward Primer	Reverse Primer
<i>gapdh</i>	NM_001115114.1	GTGGAGTCTACTGGTGTCTTC	GTGCAGGAGGCATTGCTTACA
<i>U6</i>		TTGGTCTGATCTGGCACATATAC	AAAAATATGGAGCGCTTCACG
<i>miR-21-RT</i>	NR_030020.1 and NR_030021.2	GAAAGAAGGCGAGGAGCAGATCGAGGAAGAAGACGGAA-GAATGTGCGTCTCGCCTTCTTCGCCAACAC	
<i>miR-21</i>		GCAGCTAGCTTATCAGACTGGTGT	CGAGGAAGAAGACGGAAGAAT
<i>adipoqa</i>	XM_009297486	CTGAAAGGAGAAAGTGGAGAGG	GTAAGAAGTTTTGGTGAAGCG
<i>adipor1a</i>	XM_005169284	GGAGAAGATGGAGGAGTTTGTG	AAACAGGCACGGAAGGAG
<i>adipoR1-A</i>	NM_0010024678	TGTGCATAAGGTTTGGGAGG	AGGTAGTCGTTGTCTTTAGCC
<i>adipoR1-B</i>	BC044388	GTCTGTCTGATGTGTTCTGGG	GGTAAAGTGCATTGTGGGAAC
<i>adipoR2</i>	BC154262	CGCCATCACAGTATCCCAG	CAATCATAAAGTGCAGCGTGG
<i>adipor2</i>	NM_001025506	TCTCTGTTCTCTATTTTGGCCC	CCATCAAACAGCAATAGACCAAG
<i>apelin</i>	NM_001166124	TCTTTGGGCAACCGTGAATAC	CGTTCTGACCTACTGATCCATG
<i>btg2</i>	NM_130922.2	CGCAGCTCCAGGTGTCA	AATGGTGCTAGTGTCTCTGAGA
<i>cd4-1</i>	NM_001135096.1	GTCACCTACCCAGAGAAAAAG	TGCGTTTGTCCCCTTACTG
<i>cd8a</i>	NM_001040049.1	TCGGAGGTTGTGGACTTTTC	GTGGGCATTTTCGCATGAC
<i>cyp7a1</i>	NM_201173	GATCCATTCTCCTACCATTGCTG	GGTGTAACCTTGACTGGGATC
<i>hp</i>	XM_689364.3	ACGGAAGTAGCAGTGGAAAAG	GGAAGTGGGATGGGCAATATAG
<i>il-11b</i>	XM_021468285	ACGGAATAGTGACGAATTGGG	CAATGTGGGATGGACGATCTC
<i>il-11b</i>	NM_212844	TGGCGAACGTCATCCAAG	GGAGCACTGGGCGACGCATA
<i>il-13</i>	NM_001199905	CCCAAAAGAGACAAAAGGCAAAAG	CAAATTTGACACCGGACAGG
<i>il-1b</i>	AY340959.1	TCAAACCCCAATCCACAGAG	TCACCTCACGCTCTTGGATG
<i>il-1b</i>	XM_021468285	ACGGAATAGTGACGAATTGGG	CAATGTGGGATGGACGATCTC
<i>il-23a</i>	FN869917	ATGATCAGGAATCGGCAGTG	GAGAAGCCAGGTATGAGAACAC
<i>il-23r</i>	NM_001113506	CAATGGCATTTACACCCCTG	TTCTTGGCTCAGTTCATCCC
<i>il-2rga</i>	NM_001128271	GCTGAATTGTGGCTTTACTGG	AAACTGTATGTGGTCTGACG
<i>il-2rgb</i>	NM_001123050	GAGCCGTCCTGTGATGTATG	AGATTTTCGGTTTGAGGACAGG
<i>il-6</i>	NM_001261449.1	CAGAGACGAGCAGTTTGAGAG	AGACATCTTTCCGTGCTGAAG
<i>il-8a</i>	XM_009306855.3	CATTGAAACAGAAAGCCGACG	GATCTCCTGTCCAGTTGTCATC
<i>map3k8</i>	XM_687085	AGGACACCAAAACTCGGAAG	CACAGCAAGGCCCATATAG
<i>muc2.1</i>	XM_021470771	CCAAGATGAAGGACAAGGAGAG	GGAGAAAACCGTACACAGACA
<i>nf-kb2</i>	NM_001001840.3	ATGACGAGCCTCTTTTCCTG	TCTCTTCCGTGGGTAAACAC
<i>pdc4b</i>	NM_198978.1	TCAGATGAGACGGGGCTTTG	CTTACGGCCCCGACTAGGAC
<i>smad7</i>	NM_175082.3	ACCAGACAGTTTCATCAGCAG	ATCCTCTGGAGAACAACCGG
<i>tnfa</i>	NM_212859.2	GGTGTGTTGGGATCATTGTC	GCCTTGGAAAGTGAATTGCC
<i>tpm1</i>	XM_005174375.4	TGAGCAGGCTGAGGGAGA	CGACGCTCTGGTACTCCT

Table S2. Number of zebrafish larvae for survival analysis.

<i>n</i> = 100, 3 independent experiments for every analysis					
WT-non injected	I	II	III	Average	
dpf / dpi	Death	Death	Death	Raw	Round
14 / 0	0	0	0	0.00	0
15 / 1	1	0	0	0.33	0
16 / 2	0	0	1	0.33	0
17 / 3	1	1	0	0.67	1
18 / 4	2	1	2	1.67	2
19 / 5	0	0	1	0.33	0
20 / 6	1	2	2	1.67	2
21 / 7	1	1	1	1.00	1
WT-PBS	I	II	III	Average	
dpf / dpi	Death	Death	Death	Raw	Round
14 / 0	0	0	0	0.00	0
15 / 1	2	1	2	1.67	2
16 / 2	3	3	3	3.00	3
17 / 3	2	1	1	1.33	1
18 / 4	1	0	0	0.33	0
19 / 5	1	1	0	0.67	1

20 / 6	1	0	1	0.67	1
21 / 7	1	1	0	0.67	1
WT-DSS	I	II	III	Average	
dpf / dpi	Death	Death	Death	Raw	Round
14 / 0	0	0	0	0.00	0
15 / 1	9	5	5	6.33	6
16 / 2	9	6	5	6.67	7
17 / 3	3	2	2	2.33	2
18 / 4	3	4	2	3.00	3
19 / 5	10	11	8	9.67	10
20 / 6	7	9	6	7.33	7
21 / 7	12	17	13	14.00	14
ImiR21 - Dox	I	II	III	Average	
dpf / dpi	Death	Death	Death	Raw	Round
14 / 0	0	0	0	0.00	0
15 / 1	0	1	0	0.33	0
16 / 2	1	1	0	0.67	1
17 / 3	0	2	1	1.00	1
18 / 4	0	1	0	0.33	0
19 / 5	1	1	1	1.00	1
20 / 6	0	0	1	0.33	0
21 / 7	0	2	1	1.00	1
ImiR21 + AOM	I	II	III	Average	
dpf / dpi	Death	Death	Death	Raw	Round
14 / 0	0	0	0	0.00	0
15 / 1	6	7	6	6.33	6
16 / 2	2	3	3	2.67	3
17 / 3	4	6	5	5.00	5
18 / 4	14	17	15	15.33	15
19 / 5	8	9	8	8.33	8
20 / 6	4	6	5	5.00	5
21 / 7	9	12	10	10.33	10
ImiR21 + Dox	I	II	III	Average	
dpf / dpi	Death	Death	Death	Raw	Round
14 / 0	0	0	0	0.00	0
15 / 1	0	1	0	0.33	0
16 / 2	3	6	4	4.33	4
17 / 3	6	7	7	6.67	7
18 / 4	5	4	4	4.33	4
19 / 5	2	2	3	2.33	2
20 / 6	5	4	7	5.33	5
21 / 7	6	6	7	6.33	6