

**MicroRNA-351-5p aggravates intestinal ischemia/reperfusion
injury via targeting with MAPK13 and Sirt6**

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Table S1 The primers sequences of miRNAs in the present work.

miRNAs	Primer sequence (5'-3')
rno-miR-21-3p-F	CAACAGCAGTCGATGGGCT
mmu-miR-21-3p-F	CAACAGCAGTCGATGGGCT
rno-miR-23a-5p-F	GGGGTTCCTGGGGATGG
mmu-miR-23a-5p-F	GGGGTTCCTGGGGATGG
rno-miR-144-3p-F	CGGTACAGTATAGATGATGTACT
mmu-miR-144-3p-F	CGGTACAGTATAGATGATGTACT
rno-miR-3572-F	TTACTTGGCCCTTTTTTCCCA
mmu-miR-3572-F	TACTTGGCCCTTTTTTCCCA
rno-miR-27a-5p-F	AGGGCTTAGCTGCTTGAG
mmu-miR-27a-5p-F	AGGGCTTAGCTGCTTGAG
rno-miR-294-F	CTCAAATGGAGGCCCTATCT
mmu-miR-294-F	ACTCAAATGGAGGCCCTATCT
rno-miR-142-3p-F	GTGTAGTGTTTCCTACTTTATGGA
mmu-miR-142-3p-F	GTGTAGTGTTTCCTACTTTATGGA
rno-miR-362-3p-F	GAACACACCTGTTCAAGGATTC
mmu-miR-362-3p-F	GAACACACCTGTTCAAGGATTC
rno-miR-351-5p-F	ATCCCTGAGGAGCCCTTGA
mmu-miR-351-5p-F	ATCCCTGAGGAGCCCTTGA
U6-F	Purchased from Sangon, Shanghai
General downstream primers R	Purchased from Sangon, Shanghai
miRNAs-RT	Oligo (dT)

F: forward primer; R: reverse primer

Table S2 The primer sequences used for real-time PCR assay in the present work.

Gene	Forward primer (5'-3')	Reverse primer (5'-3')
Rat β -actin	GACGGTCAGGTCATCACTATCG	TAGTTTCATGGATGCCACAGGAT
Rat IL-1 β	CCTTGTGCAAGTGTCTGAAGC	CCCAAGTCAAGGGCTTGGAA
Rat TNF- α	TCAGTTCCATGGCCCAGAC	GTTGTCTTTGAGATCCATGCCATT
Rat ICAM-1	GCTTCTGCCACCATCACTGTGTA	ATGAGGTTCTTGCCCACCTG
Mouse β -actin	TGACGTTGACATCCGTAAG	GAGGAGCAATGATCTTGATCT
Mouse IL-1 β	ATAAGCCCACTCTACACCT	ATTGGCCCTGAAAGGAGAGA
Mouse TNF- α	TATGGCCCAGACCCTCACA	GGAGTAGACAAGGTACAACCCATC
Mouse ICAM-1	AGGTGTGATATCCGGTAGAT	CCTTCTAAGTGGTTGGAACA

Table S3 The information of the antibodies used in the present work.

Primary antibody	Source	Dilution	Company
MAPK13	Rabbit	1: 1000	Abcam, United States
p-PKD1(ser916)	Rabbit	1: 1000	CST, United States
p-PKD1(ser744/748)	Rabbit	1: 1000	CST, United States
PKD1	Rabbit	1: 500	Abcam, United States
NF-KB (p65)	Rabbit	1: 500	Proteintech Group, Chicago, USA
Sirt6	Rabbit	1: 1000	Proteintech Group, Chicago, USA
AMPK	Rabbit	1: 1000	Arigo, TaiWan
p-AMPK	Rabbit	1: 2000	Arigo, TaiWan
FoxO3 α	Rabbit	1: 1000	Proteintech Group, Chicago, USA
p-FoxO3 α	Rabbit	1: 500	Arigo, TaiWan
MnSOD	Rabbit	1: 2000	Proteintech Group, Chicago, USA
CAT	Rabbit	1: 1000	Proteintech Group, Chicago, USA
Bax	Rabbit	1: 1000	Abcam, United States
Cytc	Rabbit	1: 1000	Proteintech Group, Chicago, USA
Apaf-1	Rabbit	1: 1000	Proteintech Group, Chicago, USA
Cleaved-caspase3	Rabbit	1: 500	Proteintech Group, Chicago, USA
Cleaved-caspase9	Rabbit	1: 500	Proteintech Group, Chicago, USA
β -actin	Rabbit	1: 1000	Proteintech Group, Chicago, USA

Table S4 The up-regulated expression of miRNAs
in II/R group compare with control

Name	ID	Fold change	P-value
rno-miR-23a-5p	42638	2.734879133	0.010593737
rno-miR-300-5p	42826	3.049145173	0.043799164
rno-miR-878	42645	1.810709635	0.251360607
rno-miR-125b-1-3p	145838	6.347050188	0.195773314
rno-miR-150-5p	145678	2.048956985	0.053676579
rno-miR-3068-5p	148391	1.792258518	0.458021625
rno-miR-665	42770	1.826034186	0.225130773
rno-miR-291a-5p	31388	2.369978378	0.089644293
rno-let-7b-3p	42769	1.638152542	0.477990408
rno-miR-92b-3p	145897	2.201277299	0.086811087
rno-miR-466c-3p	148530	1.637742396	0.078294417
rno-miR-21-3p	17896	4.21429323	0.002140837
rno-miR-344b-1-3p	148429	4.323989352	0.009628705
rno-miR-450a-5p/rno-miR-450b-5p	17835	2.180524417	0.153451697
rno-miR-325-3p	42706	1.893157411	0.294497916
rno-miR-675-5p	28547	2.089371379	0.184511444
rno-miR-3560	148569	2.346516154	0.230915331
rno-miR-92b-5p	42717	9.457667571	0.259853721
rno-miR-204-3p	42502	1.849583968	0.105749063
rno-miR-1956-5p	146082	9.925134772	0.158512405
rno-miR-382-5p	145643	1.666647821	0.267543449
rno-miR-3592	148337	2.047978777	0.277944169
rno-miR-351-5p	11235	2.244891508	0.041282927
rno-miR-344b-3p	148099	1.635664289	0.069215243
rno-miR-345-5p	11231	2.193935988	0.056338601
rno-miR-874-3p	42808	1.989212151	0.039983542
rno-miR-486	32946	2.047021351	0.090361755
rno-miR-466b-5p	42933	1.593142186	0.079235952
rno-miR-329-5p	148591	2.119036389	0.123659612
rno-miR-144-3p	29802	3.98295912	0.000258416
rno-miR-377-5p	42899	1.844353658	0.197368051
rno-miR-1193-3p	46251	1.593108656	0.120137222
rno-miR-224-3p	146163	2.838629455	0.098107354
rno-miR-3572	148584	2.684151041	0.001792659
rno-miR-27a-5p	17818	26.57068226	0.01462504
rno-miR-465-3p	148207	1.679405654	0.217991186
rno-miR-344a-5p	148046	1.612540627	0.475018298
rno-miR-183-3p	17953	2.168897657	0.001978906
rno-miR-195-3p	42723	1.917443534	0.101093394
rno-miR-503-5p	14288	1.735106555	0.125060961
rno-miR-185-3p	148401	2.362145325	0.125504636
rno-miR-3552	148476	2.659550292	0.225670749
rno-miR-551b-5p	148608	1.59953467	0.460103291
rno-miR-505-5p	42490	1.528396923	0.150167688

rno-miR-32-3p	29575	1.528920254	0.14780168
rno-miR-331-5p	148385	2.660338355	0.426775751
rno-miR-1843a-5p	169394	2.111315852	0.535196933
rno-miR-294	42707	2.419531112	0.015271998
rno-miR-487b-5p	148609	1.834127169	0.275602489
rno-miR-451-5p	42866	3.975296323	7.23756E-06
rno-miR-483-3p	42500	3.293365589	0.010974439
rno-miR-330-3p	42606	1.727325115	0.283884697
rno-miR-877	30033	1.557644392	0.271784192
rno-miR-883-5p	42462	1.904958143	0.008506189
rno-miR-125b-2-3p	42845	4.484843442	0.008799017
rno-miR-381-5p	148657	1.597047995	0.176511301
rno-miR-122-3p	148334	1.550638638	0.407488795
rno-miR-466b-3p	148483	1.611954496	0.130218672
rno-miR-3573-3p	148348	1.967184769	0.002428074
rno-miR-463-3p	42881	3.264263254	0.132121601
rno-miR-485-3p	42694	3.029883153	0.044848684
rno-miR-3568	148343	1.985882646	0.145584911
rno-miR-187-5p	148512	2.770903164	0.168609146
rno-miR-490-5p	17822	1.991405501	0.011697582
rno-miR-3596c	148139	1.705739818	0.108815413
rno-miR-758-5p	148068	1.916441922	0.054187114

Table S5 The down-regulated expression of miRNAs
in II/R group compare with control

Name	ID	Fold change	P-value
rno-miR-31a-5p	11052	0.614470487	0.211697973
rno-miR-200b-3p	147186	0.646328622	0.054220355
rno-miR-138-1-3p	168937	0.524877091	0.257166426
rno-miR-207	11208	0.652378206	0.204372736
rno-miR-382-3p	169132	0.591695819	0.176712594
rno-miR-27a-3p	46483	0.566470658	0.080564681
rno-miR-181c-5p	42496	0.451581283	0.02680415
rno-miR-29b-3p	11040	0.614159735	0.078539897
rno-miR-187-3p	145637	0.665306341	0.034026333
rno-miR-3596d	148359	0.599897825	0.270921533
rno-miR-101b-3p	42953	0.656328409	0.051006129
rno-miR-128-3p	33902	0.598696936	0.064980305
rno-miR-532-5p	17624	0.607862349	0.035940214
rno-miR-542-3p	14272	0.623273096	0.118779443
rno-miR-194-5p	10988	0.595214023	0.002889869
rno-miR-347	42763	0.6359158	0.162422195
rno-miR-203a-3p	11004	0.633254657	0.00101281
rno-miR-17-1-3p	19588	0.650566765	0.007185019
rno-miR-335	11065	0.495462035	0.185665892
rno-miR-192-5p	17732	0.634386125	0.004653192
rno-miR-505-3p	42669	0.635652478	0.06872259
rno-miR-336-5p	11266	0.649015098	0.114221865
rno-miR-342-3p	32884	0.556354568	0.10093659
rno-miR-25-3p	42682	0.653439497	0.108497156
rno-miR-28-3p	145714	0.603164226	0.007451492
rno-miR-140-5p	4700	0.613948244	0.000545249
rno-miR-142-3p	10947	0.558221386	0.0009777
rno-miR-363-5p	27544	0.574993693	0.243754632
rno-miR-29c-3p	11041	0.647121125	0.001505393
rno-miR-96-5p	13147	0.660129915	0.08643274
rno-miR-375-3p	46918	0.639188667	0.071825559
rno-let-7f-5p	17752	0.583352051	0.083759491
rno-miR-215	11210	0.606772083	0.051654973
rno-miR-181b-5p	10972	0.630644786	0.209661493
rno-miR-362-3p	42474	0.606140014	0.006108155
rno-miR-664-1-5p	148397	0.579008769	0.109827806
rno-let-7a-1-3p/rno-let-7c-2-3p	17888	0.551169728	0.077217099
rno-miR-147	168968	0.558105433	0.099534387
rno-miR-322-5p	13150	0.659006143	0.103586626
rno-miR-760-5p	42741	0.634840572	0.607114818

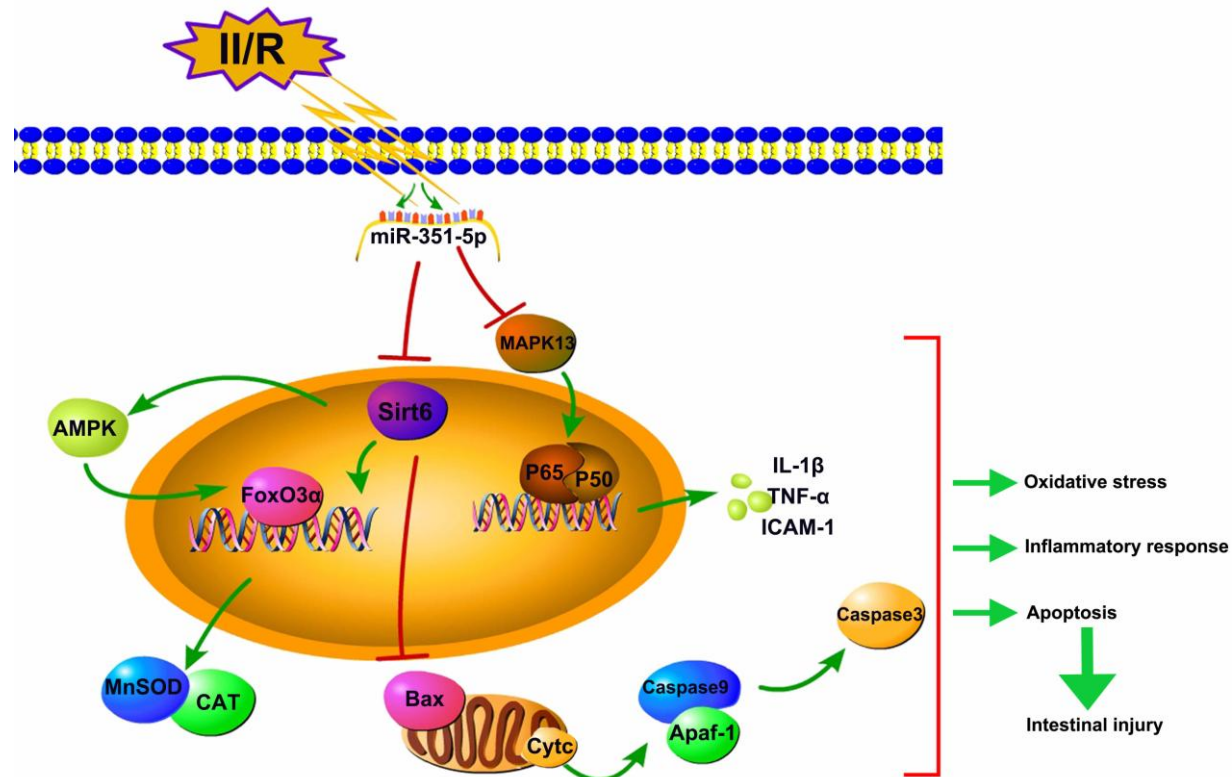


Figure S1. Proposed signaling mechanism of miR-351-5p on II/R-induced intestinal injury. miR-351-5p was selected as the target miRNA by microarrays assay, and Sirt6 and MAPK13 were the target genes of miR-351-5p. II/R significantly down-regulated the expression levels of Sirt6 and MAPK13 by increasing the level of miR-351-5p, up-regulated the levels of p-PKD1, NF- κ B(p65) and inflammatory factors including IL-1 β , IL-6, TNF- α and ICAM-1, increased p-FoxO3 α level via inhibiting AMPK, reduced MnSOD and CAT levels, and increased the levels of Bax, Cytc, Apaf-1, Cleaved- caspase3 and Cleaved-caspase9. MicroRNA-351-5p aggravated intestinal ischemia/ reperfusion injury by promoting intestinal mucosal oxidative stress, inflammation and apoptosis via targeting MAPK13 and Sirt6.