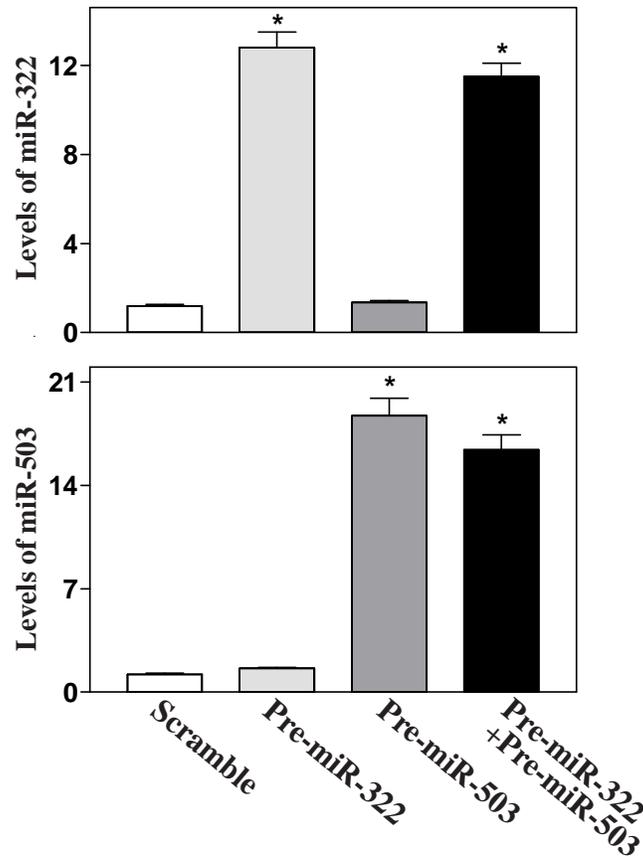


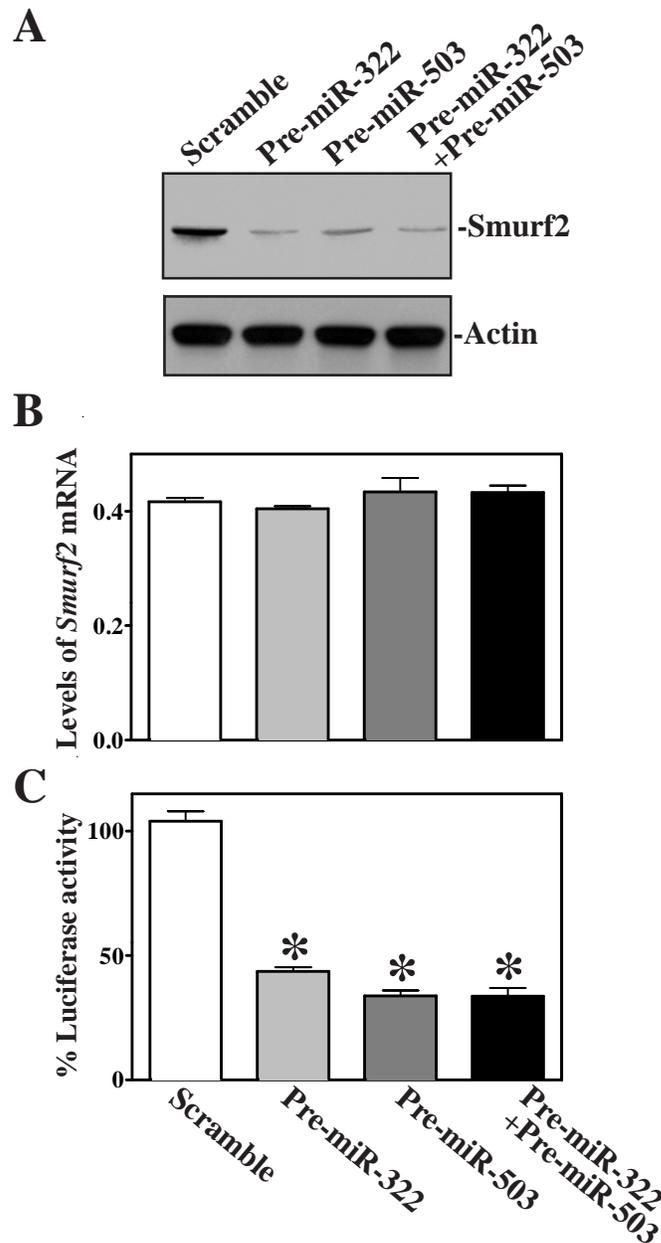
Supplemental Materials

Molecular Biology of the Cell

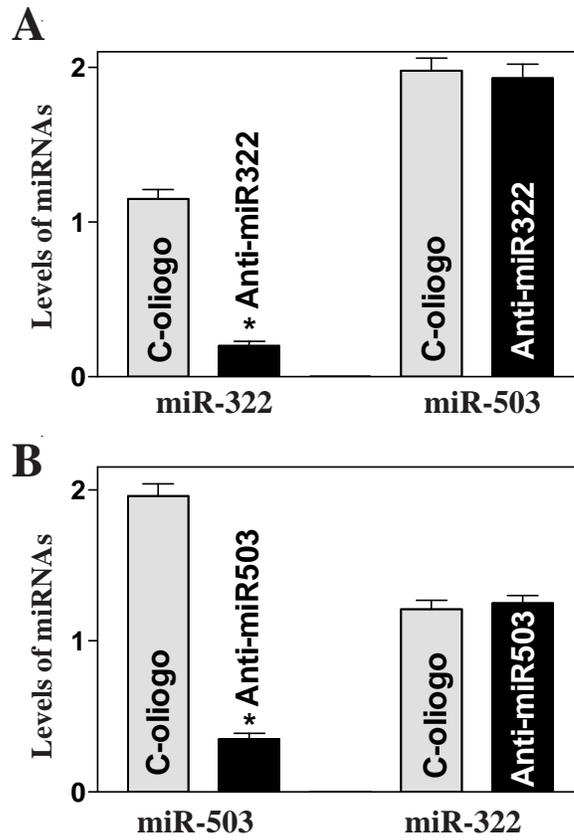
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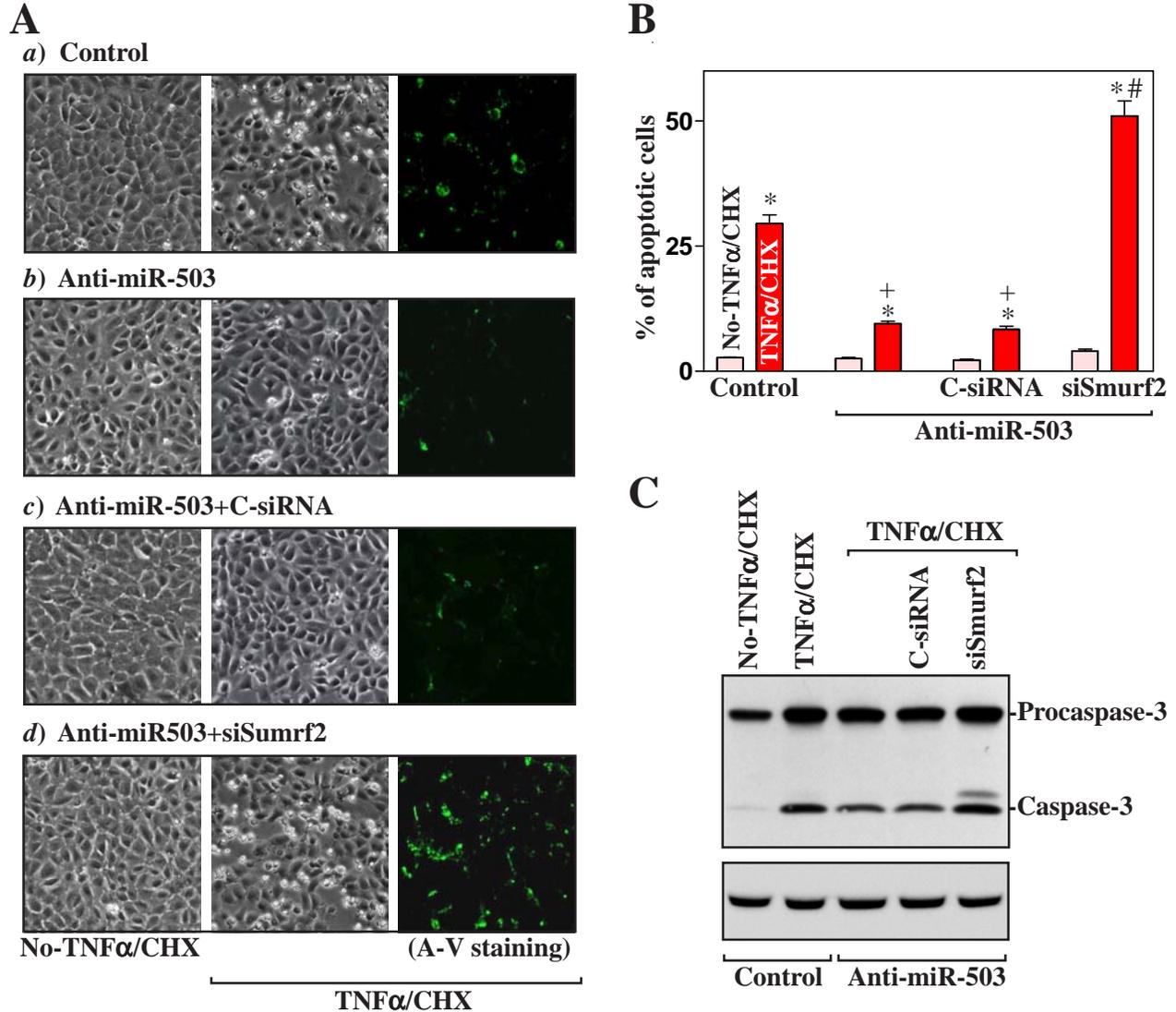
Supplementary Figure 1: Changes in the levels of miR-322 (*top*) and miR-503 (*bottom*) after co-transfection with pre-miR-322 and pre-miR-503. Forty-eight hours after cotransfecting cells with pre-miR-322 and pre-miR-503, the levels of miR-322 and miR-503 were measured by Q-PCR analysis. Values are means \pm SEM from three separate experiments. * $P < 0.05$ compared with scrambled oligo.



Supplementary Figure 2. Effect of increased levels of both miR-322 and miR-503 on Smurf2 expression. (A) Forty-eight h after co-transfection with pre-miR-322 and pre-miR-503, the expression of Smurf2 in cells was measured by immunoblot analysis. (B) Levels of *Smurf2* mRNA as examined by Q-PCR analysis in cells described in (A). (C) Levels of reporter activities after ectopic overexpression of both miR-322 and miR-503. The results are expressed as the means \pm SEM data from three separate experiments. * $P < 0.05$ compared with cells transfected with scrambled RNA.



Supplementary Figure 3: Changes in miR-322/503 levels in cells transfected with anti-miR-322 or anti-miR-503 oligomers. After transfecting IECs with anti-miR-322 oligomers (**A**) or with anti-miR-503 (**B**) for 48 h, levels of miR-322/503 were measured by Q-PCR analysis. Values are means \pm SEM from three separate experiments. * $P < 0.05$ compared with cells transfected with control oligo (C-oligo).



Supplementary Figure 4. Increased levels of endogenous Smurf2 by miR-503 silencing protect IECs against apoptosis. **(A)** TNF- α /CHX-induced apoptosis after various treatments. Forty-eight hours after transfecting cells with anti-miR-503 or control oligo (Control), cells were treated with TNF- α /CHX for 4 followed by measurement of apoptosis. Original magnification $\times 150$. **(B)** Percentages of apoptotic cells after different treatments as described in **(A)**. Values are means \pm SEM of data from 3 experiments. * $P < 0.05$ compared with cells untreated with TNF- α /CHX; + $P < 0.05$ compared with control cells exposed to TNF- α /CHX; # $P < 0.05$ compared with cells co-transfected with anti-miR-503 and C-siRNA and then exposed to TNF- α /CHX. **(C)** Changes in levels of caspase-3 in cells described in **(A)**.

Supplemental Table 1. Alignment of the Smurf2 sequences with miR-322/503

Binding site 1 3721-3774	AGGUUUUG--UACUUAACGACGAC miR-322
	:
	CATCAGAACAATGCACTGTTGTTT Smurf2
	:
	GACGUCAUGACAAG-GGCGACGAU miR-503
Binding site 2 3974-3998	AGGUU----UUGUA---CUUAACGACGAC miR-322
	TGCAGTG—GTATCGAGATGCTGCTT Smurf2
	GACGUCAUGACAAGG-----CGACGAU miR-503
Binding site 3 4919-4921	AGGUU-UUGUACUUAACGACGAC miR-322
	GAGCAATAAAATGAAT--GCTGTTT Smurf2
	GACGUCAUGACAAGGGCGACGAU miR-503

Supplemental Table-2: Primers for Smurf2 luciferase reporter

name	sequence
Smurf2 CR sense	<i>GCTAGCGCCTGACAGTACTCTGTGCAA</i>
Smurf2 CR anti-sense	<i>GTCGACACTCCACAGCAAAGCCACA</i>
Smurf2 3'UTR sense	<i>GCTAGCCGCTGGAGTACAGGACAACA</i>
Smurf2 3'UTR anti-sense	<i>GTCGACTTGTGACAGTGCAAACAGCA</i>
Smurf2 3' UTR-F1 sense	<i>GCTAGCAGGACTCACCCACAGCTCTT</i>
Smurf2 3' UTR-F1 anti-sense	<i>GTCGACTAGCTCAGGCTGGCCTTAAA</i>
Smurf2 3' UTR-F2 sense	<i>GCTAGCTGAAACTGAGGAGTTCGTGAGA</i>
Smurf2 3' UTR-F2 anti-sense	<i>GTCGACGATGGGATTACGGGTGTCAG</i>
Smurf2 3' UTR-F3 sense	<i>GCTAGCGGCAGAAGGATCAGCAAGTC</i>
Smurf2 3' UTR-F3 anti-sense	<i>GTCGACAGGCAAGTCCAGGTTCTGTG</i>
Smurf2 3' UTR-F4 sense	<i>GCTAGCCATTTACAGGCCAGAGCACA</i>
Smurf2 3' UTR-F4 anti-sense	<i>GTCGACTTGTGACAGTGCAAACAGCA</i>
Smurf2 3' UTR-F3 mutation sense	CAGGGATGCAGTGGTATCGAAATACTACTTGCAGTGGTTTTCCGTGC
Smurf2 3' UTR-F3 mutation anti-sense	GCACGGAAAACCACTGCAAGTAGTATTTTCGATACCACTGCATCCCTG