microRNA-1343 attenuates pathways of fibrosis by targeting the TGF-β receptors.
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TGFBR1 3’UTR

	site 1 nt 147-153	site 2 nt 164-171	site 3 nt 214-220
WT	CCCAGGA	CCCAGGA	CCCAGGA
MUT	CCGCGGA	CTTAAGA	CCGTCGA

TGFBR2 3’UTR

	site 1 nt 399-406
WT	CCCAGGAA
MUT	CCGCGGAA

ELMO2 3’UTR

	site 1 nt 24-30	site 2 nt 45-51	site 3 nt 381-388	site 4 nt 696-702	site 5 nt 866-873
WT	CCCAGGA	CCCAGGA	CCCAGGA	CCAGGAA	CCCAGGA
MUT	CTCGAGA	CCTAGTA	CCGCGGA	CCTAGGA	CCGCGGA

Supplementary Figure 1: Seed sites for miR-1343 within the TGFBR1, TGFBR2, and ELMO2 3’ UTRs. WT, wild type; MUT, mutant. Underlined nucleotides denote mutated bases.

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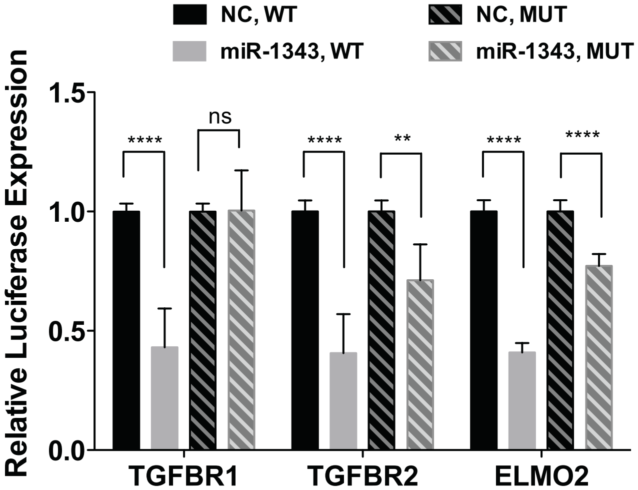
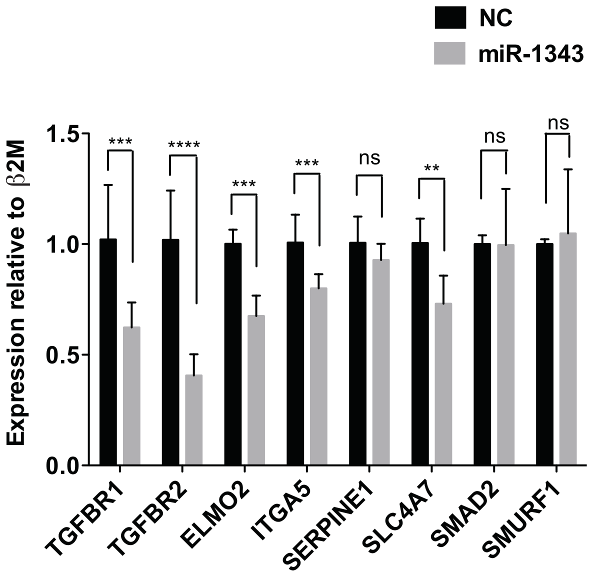


Supplementary Figure 2: Luciferase assay of Caco2 cells transiently co-transfected with either wild type (WT) or mutant (MUT) pMIR-REPORT-3' UTR constructs, and miR-1343 or negative control (NC) miRNA. Luciferase values were normalized to pMIR-β-galactosidase levels and illustrated relative to the NC. n = 3. ** $p \leq 0.01$, **** $p \leq 0.0001$, ns = not significant.



Supplementary Figure 3: Gene expression levels in 16HBE14o- cells transiently transfected with miR-1343 or negative control (NC) miRNA. RNA was extracted after 48 hours. RT-qPCR Ct values were normalized to beta-2-microglobulin (β2M). $n = 3$. ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$, ns = not significant.

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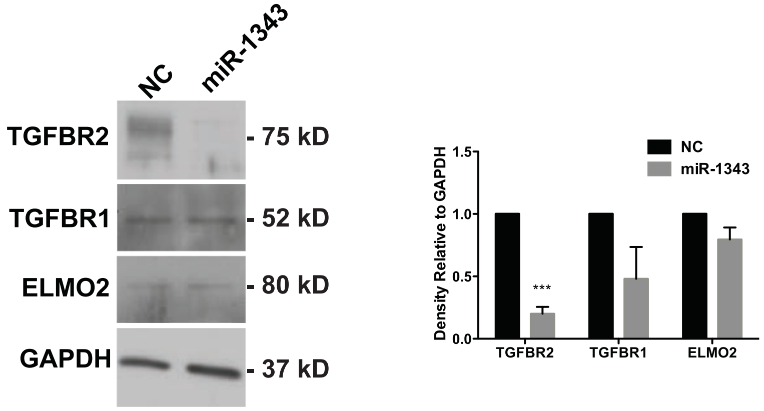

Supplementary Figure 4: Western blots of 16HBE14o- lysates transfected with miR-1343 or NC miRNA for 48 hours and probed with antibodies specific to TGFBR1, TGFBR2 and ELMO2. Proteins were quantified relative to GAPDH loading control and are expressed as fold change compared to NC. *n* = 3. *** $p \leq 0.001$.

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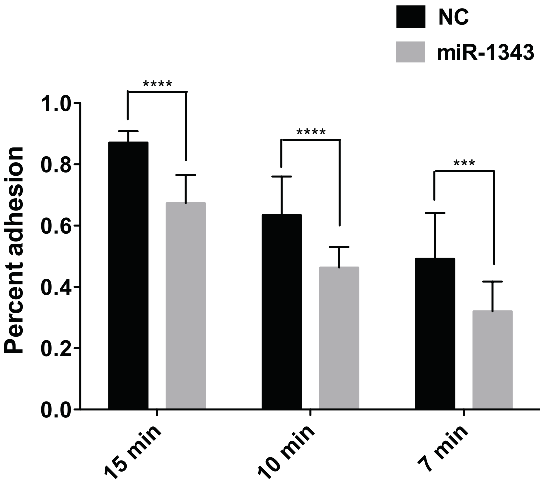

Supplementary Figure 5: Adhesion of A549 cells transiently transfected with miR-1343 or negative control (NC) miRNA for 48 hours. Graph shows the percent of total viable cells that adhered to the Collagen 1-coated surface. *n* = 4. *** p ≤ 0.001, **** p ≤ 0.0001.

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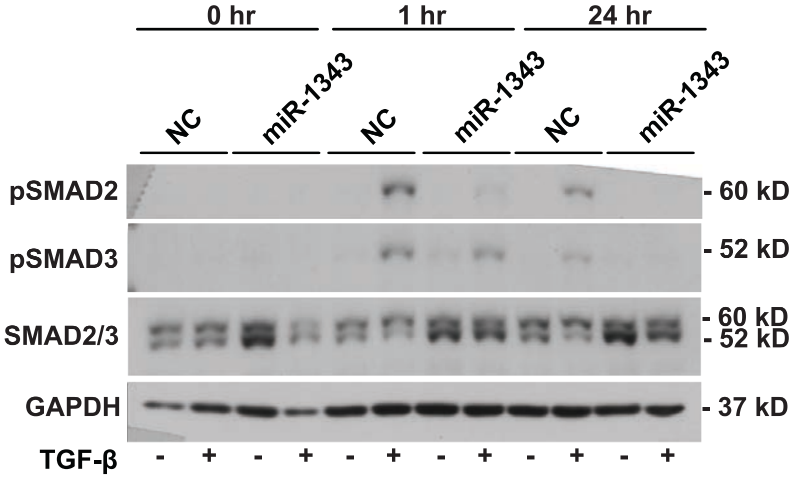

Supplementary Figure 6: miR-1343 represses phosphorylation of SMAD2/3. Western blot of lysates from primary lung fibroblasts transiently transfected with miR-1343 or NC miRNA and treated with TGF-β1 (5 ng/mL, +) or vehicle control (−) for the indicated period of time. Blots were probed with antibodies specific for pSMAD2, phosphorylated (active) SMAD2; pSMAD3, phosphorylated (active) SMAD3; total SMAD2/3, slower migrating species is SMAD2 and faster is SMAD3. GAPDH was the loading control.

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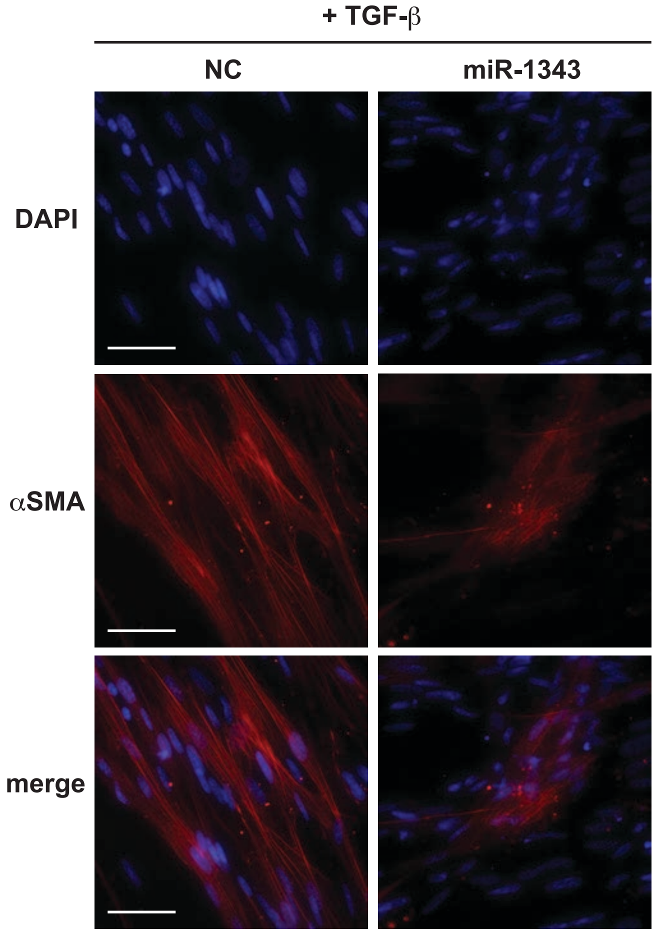

Supplementary Figure 7: miR-1343 impairs synthesis and structural organization of αSMA after TGF-β exposure. Representative images of immunofluorescence in primary lung fibroblasts transiently transfected with miR-1343 or negative control (NC) miRNA and treated with TGF-β_1 (5 ng/mL, +) or vehicle control (−) for 48 hours. Red fluorescence shows αSMA and blue is DAPI nuclear counterstain. Merge illustrates αSMA staining plus DAPI. Scale bar = 50 μm.

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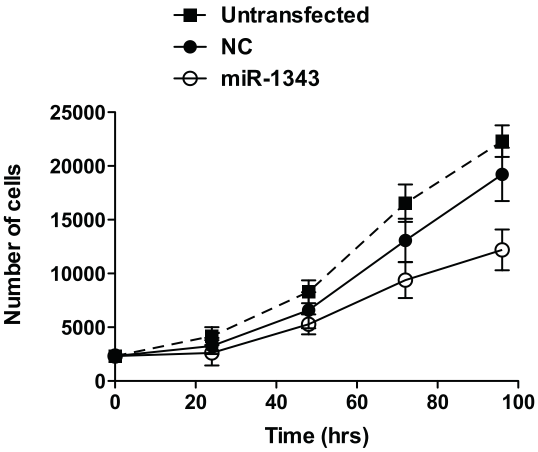

Supplementary Figure 8: Growth curve of A549 cells transiently transfected with miR-1343 or negative control (NC) miRNA by MTS assay. The number of transfected or untransfected cells was measured at 0, 24, 48, 72, and 96 hours post-transfection and compared to chemiluminescence measured from a standard curve to obtain cell number. *n* = 3.

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Term	Count	PValue
GO:0010975~regulation of neuron projection development	8	0.013006182
GO:0016023~cytoplasmic membrane-bounded vesicle	31	0.013533317
GO:0031967~organelle envelope	34	0.013696356
GO:0007265~Ras protein signal transduction	10	0.013709129
GO:0051592~response to calcium ion	7	0.014019994
GO:0031975~envelope	34	0.014124089
GO:0003779~actin binding	21	0.014916472
GO:0042127~regulation of cell proliferation	57	8.26E-07
GO:0070161~anchoring junction	21	3.16E-06
GO:0016323~basolateral plasma membrane	23	3.33E-06
GO:0040008~regulation of growth	31	6.49E-06
GO:0030055~cell-substrate junction	16	9.79E-06
GO:0005912~adherens junction	19	9.99E-06
GO:0007398~ectoderm development	22	1.08E-05
GO:0005925~focal adhesion	15	1.45E-05
GO:0005924~cell-substrate adherens junction	15	2.27E-05
GO:0008544~epidermis development	20	3.82E-05
GO:0043067~regulation of programmed cell death	52	7.77E-05
GO:0010941~regulation of cell death	52	8.59E-05
GO:0030307~positive regulation of cell growth	9	1.10E-04
GO:0031252~cell leading edge	16	1.17E-04
GO:0007010~cytoskeleton organization	33	1.18E-04
GO:0042981~regulation of apoptosis	51	1.19E-04
GO:0045927~positive regulation of growth	12	1.35E-04
GO:0043066~negative regulation of apoptosis	28	2.14E-04
GO:0005198~structural molecule activity	42	2.14E-04
GO:0001558~regulation of cell growth	19	2.38E-04
GO:0043069~negative regulation of programmed cell death	28	2.68E-04
GO:0060548~negative regulation of cell death	28	2.81E-04
GO:0045793~positive regulation of cell size	9	2.90E-04
GO:0008285~negative regulation of cell proliferation	28	2.94E-04
GO:0019899~enzyme binding	36	3.32E-04
GO:0043627~response to estrogen stimulus	13	4.01E-04
GO:0005829~cytosol	71	5.09E-04
GO:0008284~positive regulation of cell proliferation	30	5.23E-04
GO:0006928~cell motion	33	5.37E-04
GO:0001726~ruffle	10	5.79E-04
GO:0051270~regulation of cell motion	18	6.42E-04
hsa04510:Focal adhesion	19	0.001043951
GO:0042470~melanosome	11	0.001215773
GO:0048770~pigment granule	11	0.001215773
GO:0006916~anti-apoptosis	18	0.001326436
GO:0008361~regulation of cell size	18	0.001326436
GO:0015031~protein transport	45	0.001375837
GO:0030855~epithelial cell differentiation	14	0.001382116
GO:0048638~regulation of developmental growth	8	0.001386303
GO:0031090~organelle membrane	59	0.001397004
GO:0030529~ribonucleoprotein complex	33	0.001573656
GO:0045184~establishment of protein localization	45	0.001691898
GO:0009898~internal side of plasma membrane	23	0.002127484
GO:0008092~cytoskeletal protein binding	32	0.002726437
hsa05200:Pathways in cancer	25	0.002761542
GO:0051272~positive regulation of cell motion	11	0.002824672
GO:0008104~protein localization	49	0.00289929
GO:0010035~response to inorganic substance	17	0.003190437
GO:0030334~regulation of cell migration	15	0.003310487
GO:0030036~actin cytoskeleton organization	18	0.003537271
GO:0050678~regulation of epithelial cell proliferation	9	0.003972315
GO:0042060~wound healing	16	0.003994533
GO:0048514~blood vessel morphogenesis	17	0.004231839
GO:0050770~regulation of axonogenesis	8	0.004276551
GO:0001944~vasculature development	19	0.004509444
GO:0031410~cytoplasmic vesicle	37	0.00467457
GO:0031344~regulation of cell projection organization	10	0.004796092
hsa04810:Regulation of actin cytoskeleton	18	0.005386875
hsa04115:p53 signaling pathway	9	0.005452674
GO:0016477~cell migration	20	0.005554153

Supplementary Table 1: DAVID gene ontology analysis of genes down-regulated by miR-1343 compared to negative control (NC) in A549 and 16HBE14o- cells by RNA-seq.

Term	Count	PValue
GO:0051130~positive regulation of cellular component organization	15	0.006076785
hsa05222:Small cell lung cancer	10	0.006107615
GO:0055024~regulation of cardiac muscle tissue development	4	0.006234879
GO:0060043~regulation of cardiac muscle cell proliferation	4	0.006234879
GO:0055021~regulation of cardiac muscle growth	4	0.006234879
GO:0005604~basement membrane	9	0.006424346
GO:0031346~positive regulation of cell projection organization	7	0.006608186
GO:0030029~actin filament-based process	18	0.006678629
GO:0004721~phosphoprotein phosphatase activity	14	0.006870441
GO:0000165~MAPKKK cascade	15	0.006998967
GO:0032268~regulation of cellular protein metabolic process	29	0.007426734
GO:0009725~response to hormone stimulus	24	0.007503306
GO:0005739~mitochondrion	55	0.007542903
GO:0009628~response to abiotic stimulus	24	0.007746737
GO:0001568~blood vessel development	18	0.007808243
GO:0051674~localization of cell	21	0.008082914
GO:0048870~cell motility	21	0.008082914
GO:0060420~regulation of heart growth	4	0.008092409
GO:0042327~positive regulation of phosphorylation	10	0.00838081
GO:0031982~vesicle	37	0.008737037
GO:0030193~regulation of blood coagulation	6	0.009033893
GO:0004857~enzyme inhibitor activity	19	0.009308243
GO:0032535~regulation of cellular component size	19	0.00972667

Supplementary Table 1 (continued)

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Term	Count	PValue
GO:0006695~cholesterol biosynthetic process	7	9.04E-06
GO:0008202~steroid metabolic process	16	1.10E-05
GO:0006694~steroid biosynthetic process	10	4.29E-05
GO:0005768~endosome	19	5.04E-05
GO:0016126~sterol biosynthetic process	7	5.49E-05
GO:0008610~lipid biosynthetic process	18	2.26E-04
GO:0008203~cholesterol metabolic process	9	4.50E-04
hsa00100:Steroid biosynthesis	5	5.36E-04
GO:0016125~sterol metabolic process	9	8.39E-04
GO:0005783~endoplasmic reticulum	34	0.001135676
GO:0007242~intracellular signaling cascade	41	0.001534451
GO:0045638~negative regulation of myeloid cell differentiation	5	0.00267773
GO:0031090~organelle membrane	36	0.002800942
GO:0007264~small GTPase mediated signal transduction	15	0.002905681
GO:0012505~endomembrane system	28	0.00304096
GO:0005525~GTP binding	17	0.0037091
GO:0003924~GTPase activity	12	0.003732648
GO:0017157~regulation of exocytosis	5	0.00426155
GO:0019001~guanyl nucleotide binding	17	0.004743403
GO:0032561~guanyl ribonucleotide binding	17	0.004743403
GO:0032583~regulation of gene-specific transcription	9	0.004979513
GO:0010008~endosome membrane	6	0.005116388
GO:0044440~endosomal part	6	0.005116388
GO:0010552~positive regulation of specific transcription from RNA polymerase II promoter	6	0.005151719
GO:0002020~protease binding	4	0.006197341
GO:0043193~positive regulation of gene-specific transcription	7	0.007309418
GO:0045080~positive regulation of chemokine biosynthetic process	3	0.00757814
hsa00650:Butanoate metabolism	5	0.007689035
GO:0008104~protein localization	29	0.008269662
GO:0017076~purine nucleotide binding	54	0.009546325
GO:0001817~regulation of cytokine production	10	0.009611422
GO:0044459~plasma membrane part	59	0.009882343

Supplementary Table 2: DAVID gene ontology analysis of genes up-regulated by miR-1343 compared to negative control (NC) in A549 and 16HBE14o- cells by RNA-seq.

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a.

Cloned Region	Primers
TGFBR1 3’ UTR	F: 5’-GGACTAGTTTCTACAGCTTTGCCTGAACTCTC
	R: 5’-CGACGCGTCCTTCGCCTTCCTAGAAAAA
TGFBR2 3’ UTR	F: 5’-CTGCCCCTGAACTGATGCTT
	R: 5’-GGTCCAGGTAGGCAGTGGAA
ELMO2 3’ UTR	F: 5’-CCTGGAGCCAGAAACGAC
	R: 5’-CATGGTTACTGACAGGAAGC
SMAD2 3’ UTR	F1: 5’-ACTAGTGCATTGATACTGCTGGCACC
	R1: 5’-GAGCTCGCACAAAGTCTGGAAGCAAGC
	F2: 5’-ACTAGTTCTGTTGCCCAACCTGGAG
	R2: 5’-GAGCTCGAGATCACCTGTGGGTCAAGG
SMURF1 3’ UTR	F1: 5’-CCTAGGGCAACCAAAGGCAACAGAGTC
	R1: 5’-GGCGCGCCCACATAGGAACATTGGCCTGC
	F2: 5’-CCTAGGGCCTTGTCATCGGTTGTGTG
	R2: 5’-GGCGCGCCCTGCTGCCTACACACTTCCTT

c.

Gene	Primers
TGFBR1	F: 5’-CGGGGAGAAGAAGTTGCTGT
	R: 5’-CACCAACCAGAGCTGAGTCC
TGFBR2	F: 5’-AATAGGACTGCCCATCCACTG
	R: 5’-TCTCACAGATGGAGGTGATGC
ELMO2	F: 5’-GGGACATGGTTTCAATCACC
	R: 5’-TCTGGTACAGACTCTGGCTG
ITGA5	F: 5’-TTACGGCTATGTCACCATCCTT
	R: 5’-CCCCACCAGCAAGTCATCCA
SERPINE1	F: 5’-CCTCTTCCACAAATCAGACGGC
	R: 5’-CTCTTTTTCATAAGGGGCAGCA
SLC4A7	F: 5’-ATCTTGGCAAAACTAGCTCAACT
	R: 5’-CGACTCTCTTTACTAAACGGGAC
SMAD2	F: 5’-CGACACACCGAGATCCTAACA
	R: 5’-ATATCCAGGAGGTGGCGTTT
SMURF1	F: 5’-TACCAGCGTTTGGATCTATGC
	R: 5’-GCCGGTTCCTATTCTGTCTCG
β2M	F: 5’-CTCTCTCTTTCTGGCCTGGAG
	R: 5’-TCTGCTGGATGACGTGAGTA

b.

Mutated Region	Primers
TGFBR1 3’ UTR	miR-1343 site 1: 5’-GTAATAAAGTCAATTAAAAACTTCCGCGGATTTCTTTGGACCCAGGAAACAG
	miR-1343 site 2: 5’-TCCGCGGATTTCTTTGGACTTAAGAAACAGCCATGTGGGTCC
	miR-1343 site 3: 5’-GCACTATGAACGCTTCTTTCCGTCGACAGAAAATGTGTAGTCTAC
TGFBR2 3’ UTR	miR-1343 site: 5’-GAGTTCTCCAATAAAACCAATTTCCGCGGAATATTTGATGTTTTTCCTTGT
ELMO2 3’ UTR	miR-1343 site 1: 5’-GAAGGGAAGGCCAACTTCTCGAGGTCTGGAAGGCCAAAGG
	miR-1343 site 2: 5’-GGTTTTCCCTGCAGCGTTACTAGTGACTTAAGAGGGCAGGA
	miR-1343 site 3: 5’-CAGGCAGCCTTTCCGCGGCCAGCTGTTGCT
	miR-1343 site 4/5: 5’-AGAATGTAAGTGTTTCGCCTAGGCCCAAAATCCCTTCTCCGCGGTACCGTCGTTTCTGG

Supplementary Table 3: Primers used for (a) cloning of 3’ UTRs, (b) mutagenesis of miR-1343 seed sequences within cloned 3’ UTRs, and (c) SYBR Green qPCR assays.