

Pulmonary microRNA changes alter angiogenesis in chronic obstructive pulmonary disease and lung cancer

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Online Data Supplement

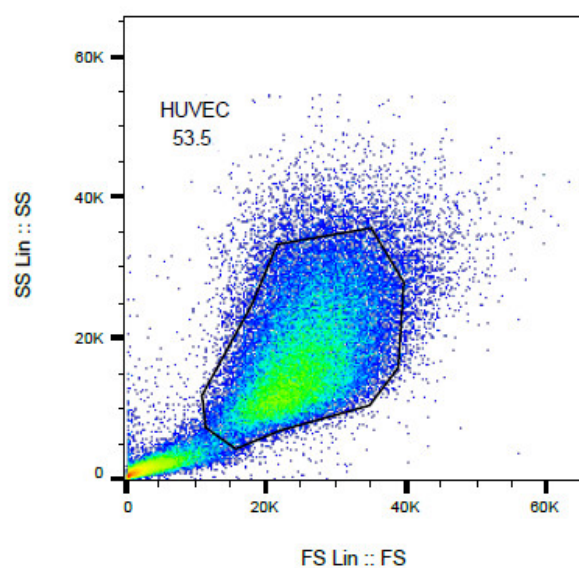
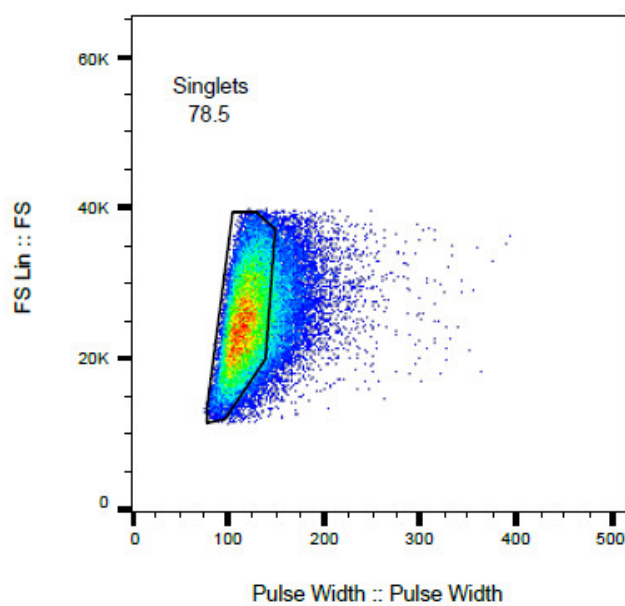
A**B**

Figure S1. Cytogram plots from the program 'FloJo' used for cell cycle analysis. (A): An initial gate was drawn to identify the main cell population. (B): A second gate was drawn to identify single cells within the main cell population. The single cell population was used for cell cycle analysis.

RESULTS

Table S1. Significantly upregulated miR in COPD.

Gene ID			
hsa-let-7f-1-3p	hsa-miR-1264	hsa-miR-146a-3p	hsa-miR-1914-5p
hsa-let-7f-2-3p	hsa-miR-1267	hsa-miR-146a-5p	hsa-miR-193b-3p
hsa-let-7i-3p	hsa-miR-1269a	hsa-miR-146b-3p	hsa-miR-193b-5p
hsa-miR-1	hsa-miR-1269b	hsa-miR-146b-5p	hsa-miR-196a-3p
hsa-miR-100-5p	hsa-miR-127-3p	hsa-miR-1470	hsa-miR-196b-5p
hsa-miR-103a-2-5p	hsa-miR-1273a	hsa-miR-148a-3p	hsa-miR-199a-5p
hsa-miR-103b	hsa-miR-1273d	hsa-miR-148a-5p	hsa-miR-199b-5p
hsa-miR-105-5p	hsa-miR-1273g-5p	hsa-miR-148b-3p	hsa-miR-19a-3p
hsa-miR-10a-3p	hsa-miR-1277-3p	hsa-miR-148b-5p	hsa-miR-19b-3p
hsa-miR-10b-5p	hsa-miR-1281	hsa-miR-149-5p	hsa-miR-200a-3p
hsa-miR-1178-5p	hsa-miR-1282	hsa-miR-150-3p	hsa-miR-200a-5p
hsa-miR-1184	hsa-miR-1283	hsa-miR-152	hsa-miR-200b-3p
hsa-miR-1185-5p	hsa-miR-1288	hsa-miR-153	hsa-miR-200c-3p
hsa-miR-1200	hsa-miR-1289	hsa-miR-1538	hsa-miR-200c-5p
hsa-miR-1204	hsa-miR-129-2-3p	hsa-miR-154-3p	hsa-miR-202-3p
hsa-miR-1206	hsa-miR-1292-3p	hsa-miR-154-5p	hsa-miR-202-5p
hsa-miR-1225-5p	hsa-miR-1293	hsa-miR-155-3p	hsa-miR-203a
hsa-miR-1226-5p	hsa-miR-1294	hsa-miR-155-5p	hsa-miR-203b-3p
hsa-miR-1228-5p	hsa-miR-1295a	hsa-miR-16-2-3p	hsa-miR-204-5p
hsa-miR-1229-3p	hsa-miR-1295b-5p	hsa-miR-17-3p	hsa-miR-206
hsa-miR-1231	hsa-miR-1296	hsa-miR-181a-5p	hsa-miR-208a
hsa-miR-1234-3p	hsa-miR-1298	hsa-miR-181b-3p	hsa-miR-208b
hsa-miR-1234-5p	hsa-miR-1303	hsa-miR-181c-5p	hsa-miR-20b-3p
hsa-miR-1238-3p	hsa-miR-1304-5p	hsa-miR-182-3p	hsa-miR-21-3p
hsa-miR-1238-5p	hsa-miR-1306-5p	hsa-miR-183-5p	hsa-miR-21-5p
hsa-miR-1245b-3p	hsa-miR-1307-3p	hsa-miR-184	hsa-miR-2110
hsa-miR-1247-5p	hsa-miR-1307-5p	hsa-miR-185-3p	hsa-miR-2114-5p
hsa-miR-1248	hsa-miR-130a-5p	hsa-miR-186-3p	hsa-miR-2115-3p
hsa-miR-1250	hsa-miR-130b-3p	hsa-miR-187-3p	hsa-miR-2115-5p
hsa-miR-1251	hsa-miR-132-3p	hsa-miR-188-3p	hsa-miR-2116-3p
hsa-miR-1252	hsa-miR-135a-5p	hsa-miR-18a-3p	hsa-miR-2117
hsa-miR-1253	hsa-miR-135b-5p	hsa-miR-18a-5p	hsa-miR-212-5p
hsa-miR-1255b-5p	hsa-miR-136-3p	hsa-miR-18b-3p	hsa-miR-216a-3p
hsa-miR-1256	hsa-miR-136-5p	hsa-miR-18b-5p	hsa-miR-216a-5p
hsa-miR-1257	hsa-miR-138-5p	hsa-miR-1909-3p	hsa-miR-218-2-3p
hsa-miR-125a-5p	hsa-miR-141-3p	hsa-miR-190a	hsa-miR-219-1-3p
hsa-miR-125b-2-3p	hsa-miR-141-5p	hsa-miR-1910	hsa-miR-219-2-3p
hsa-miR-1260a	hsa-miR-142-3p	hsa-miR-1911-3p	hsa-miR-22-5p
hsa-miR-1260b	hsa-miR-142-5p	hsa-miR-1912	hsa-miR-221-3p
hsa-miR-1262	hsa-miR-144-5p	hsa-miR-1913	hsa-miR-221-5p
hsa-miR-222-5p	hsa-miR-3140-5p	hsa-miR-33a-5p	hsa-miR-3681-3p
hsa-miR-223-5p	hsa-miR-3142	hsa-miR-33b-3p	hsa-miR-3681-5p
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hsa-miR-2277-5p	hsa-miR-3145-5p	hsa-miR-340-5p	hsa-miR-3687

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hsa-miR-2467-5p	hsa-miR-3152-5p	hsa-miR-34a-5p	hsa-miR-3689a-5p
hsa-miR-2681-3p	hsa-miR-3155a	hsa-miR-34b-5p	hsa-miR-3689d
hsa-miR-2681-5p	hsa-miR-3156-3p	hsa-miR-34c-3p	hsa-miR-3689f
hsa-miR-2682-5p	hsa-miR-3156-5p	hsa-miR-3529-3p	hsa-miR-369-3p
hsa-miR-26a-1-3p	hsa-miR-3157-3p	hsa-miR-3591-3p	hsa-miR-369-5p
hsa-miR-26b-3p	hsa-miR-3158-5p	hsa-miR-3607-3p	hsa-miR-3690
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hsa-miR-302b-3p	hsa-miR-3182	hsa-miR-363-5p	hsa-miR-376a-2-5p
hsa-miR-302c-3p	hsa-miR-3183	hsa-miR-3649	hsa-miR-376a-3p
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hsa-miR-548e	hsa-miR-5705	hsa-miR-628-3p	hsa-miR-664b-3p
hsa-miR-548f	hsa-miR-5706	hsa-miR-628-5p	hsa-miR-668
hsa-miR-548g-3p	hsa-miR-5707	hsa-miR-632	hsa-miR-671-3p
hsa-miR-548j	hsa-miR-571	hsa-miR-633	hsa-miR-6715a-3p
hsa-miR-548k	hsa-miR-573	hsa-miR-634	hsa-miR-6715b-3p
hsa-miR-548n	hsa-miR-576-3p	hsa-miR-635	hsa-miR-6716-3p
hsa-miR-548p	hsa-miR-578	hsa-miR-636	hsa-miR-6717-5p
hsa-miR-548t-5p	hsa-miR-580	hsa-miR-637	hsa-miR-6721-5p
hsa-miR-548u	hsa-miR-582-3p	hsa-miR-641	hsa-miR-6722-5p
hsa-miR-549a	hsa-miR-582-5p	hsa-miR-642a-5p	hsa-miR-675-3p
hsa-miR-550b-2-5p	hsa-miR-584-3p	hsa-miR-642b-5p	hsa-miR-675-5p

hsa-miR-550b-3p	hsa-miR-585	hsa-miR-643	hsa-miR-676-3p
hsa-miR-553	hsa-miR-588	hsa-miR-644a	hsa-miR-7-1-3p
hsa-miR-554	hsa-miR-589-3p	hsa-miR-645	hsa-miR-7-2-3p
hsa-miR-556-3p	hsa-miR-589-5p	hsa-miR-647	hsa-miR-708-5p
hsa-miR-5579-5p	hsa-miR-590-5p	hsa-miR-649	hsa-miR-758-3p
hsa-miR-5580-3p	hsa-miR-591	hsa-miR-6499-5p	hsa-miR-759
hsa-miR-5582-3p	hsa-miR-593-3p	hsa-miR-650	hsa-miR-761
hsa-miR-5583-5p	hsa-miR-595	hsa-miR-6500-5p	hsa-miR-764
hsa-miR-5589-3p	hsa-miR-597	hsa-miR-6501-5p	hsa-miR-766-5p
hsa-miR-5589-5p	hsa-miR-599	hsa-miR-6502-5p	hsa-miR-769-5p
hsa-miR-5590-5p	hsa-miR-603	hsa-miR-6503-3p	hsa-miR-877-3p
hsa-miR-5591-5p	hsa-miR-605	hsa-miR-6503-5p	hsa-miR-877-5p
hsa-miR-561-3p	hsa-miR-606	hsa-miR-6504-3p	hsa-miR-885-3p
hsa-miR-561-5p	hsa-miR-6075	hsa-miR-6504-5p	hsa-miR-888-5p
hsa-miR-563	hsa-miR-6078	hsa-miR-6505-5p	hsa-miR-890
hsa-miR-566	hsa-miR-6082	hsa-miR-6506-3p	hsa-miR-891a
hsa-miR-567	hsa-miR-6084	hsa-miR-6506-5p	hsa-miR-892c-5p
hsa-miR-568	hsa-miR-6085	hsa-miR-6507-3p	hsa-miR-9-3p
hsa-miR-5680	hsa-miR-6086	hsa-miR-6509-5p	hsa-miR-921
hsa-miR-5681a	hsa-miR-609	hsa-miR-6511a-3p	hsa-miR-92a-1-5p
hsa-miR-5681b	hsa-miR-611	hsa-miR-6512-3p	hsa-miR-92b-5p
hsa-miR-5684	hsa-miR-612	hsa-miR-6513-5p	hsa-miR-937-3p
hsa-miR-938	hsa-miR-941	hsa-miR-943	hsa-miR-944

This table demonstrates the miR that were significantly upregulated in COPD vs non-COPD in the miR microarray analyses.

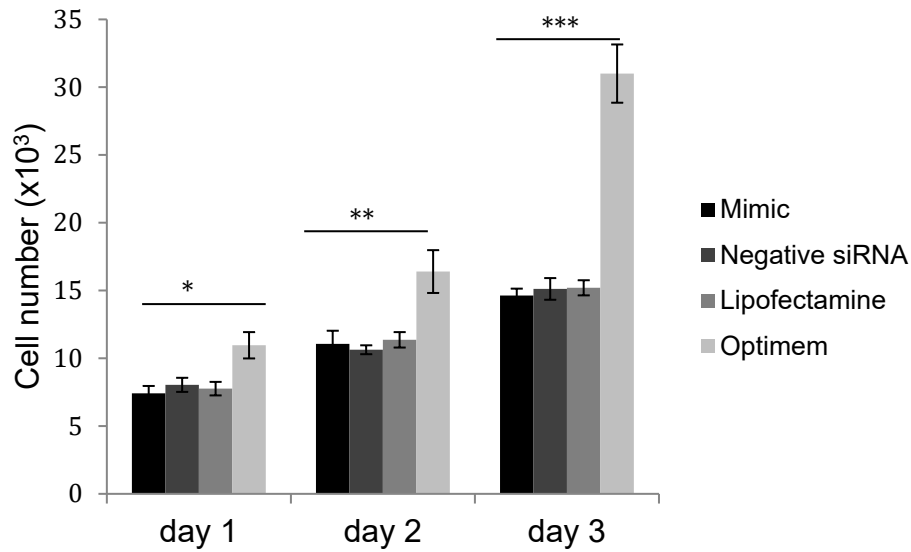


Figure S2. Cell growth assay. This figure shows the number of cells day 1, 2 and 3 after transfection in cells transfected with miR mimic, negative siRNA, lipofectamine or optimum. Numbers represent the mean from three separate experiments. There was no significant difference between mimic and negative siRNA groups suggesting that the miR mimic did not affect cell growth. The Kruskal-Wallis test was used to determine significant differences between groups. $n = 3$ in each group. Day 1; $p = 0.046$, day 2; $p = 0.001$, day 3; $op < 0.001$. * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$.

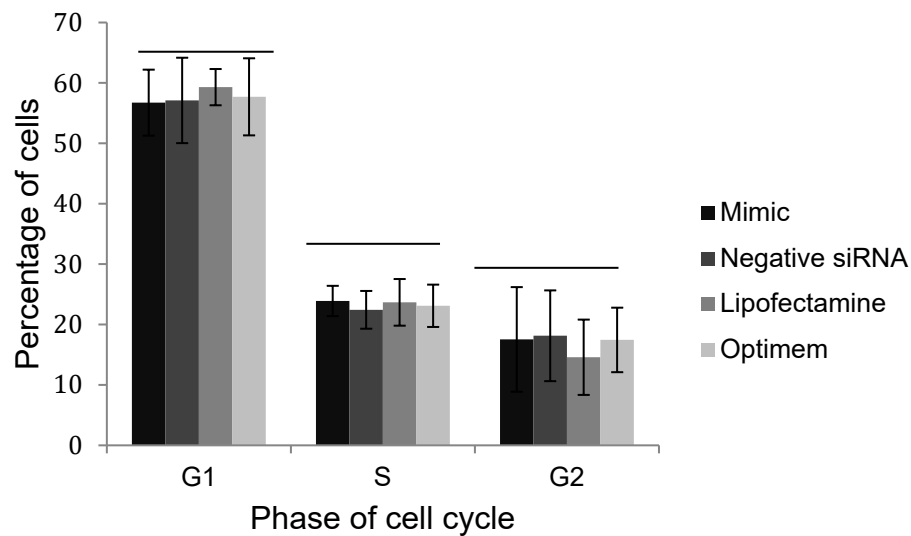


Figure S3. Cell cycle analysis according to flow cytometry. The figure shows the proportion of cells in G1, S and G2 phases of the cell cycle in cells transfected with miR mimic, negative siRNA, lipofectamine or optimum. The values represent the mean from three separate experiments. There was no significant difference between mimic and negative siRNA groups suggesting that the miR mimic did not affect the cell cycle. A one-way ANOVA test was used to determine significant differences between groups. Tukey's test was used post-hoc to look for significant differences between individual groups. There were no significant differences detected. $n = 3$ in each group.

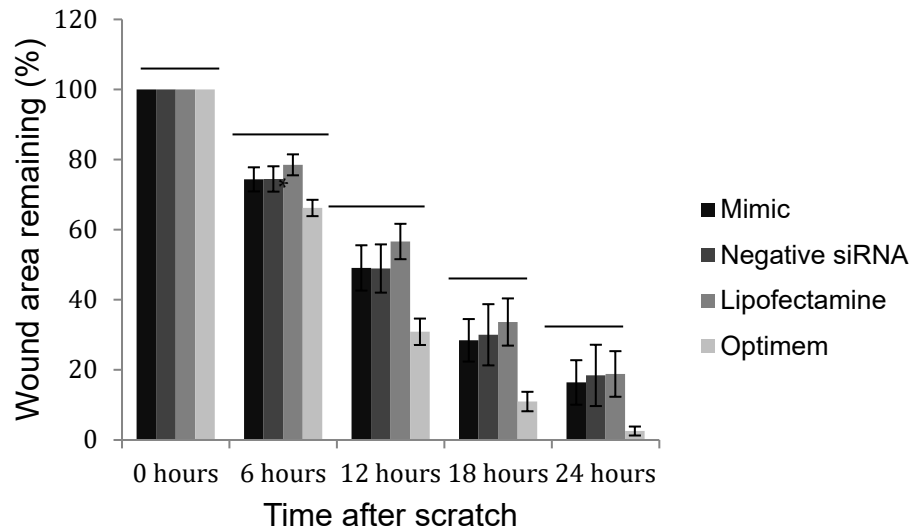


Figure S4. Percentage wound area remaining during scratch wound assay using a miR-181b-3p mimic. The average percentage wound remaining for each condition is recorded at 0, 6, 12, 18 and 24 h. The data represents the mean from three separate experiments. An ANOVA test was used to look for significance between groups. $p = 0.021$ at 12 h. $* = p < 0.05$ (ANOVA). A Tukey's test demonstrated that there was no significant difference between the mimic and negative siRNA groups suggesting that miR-181b-3p does not affect wound adhesion.

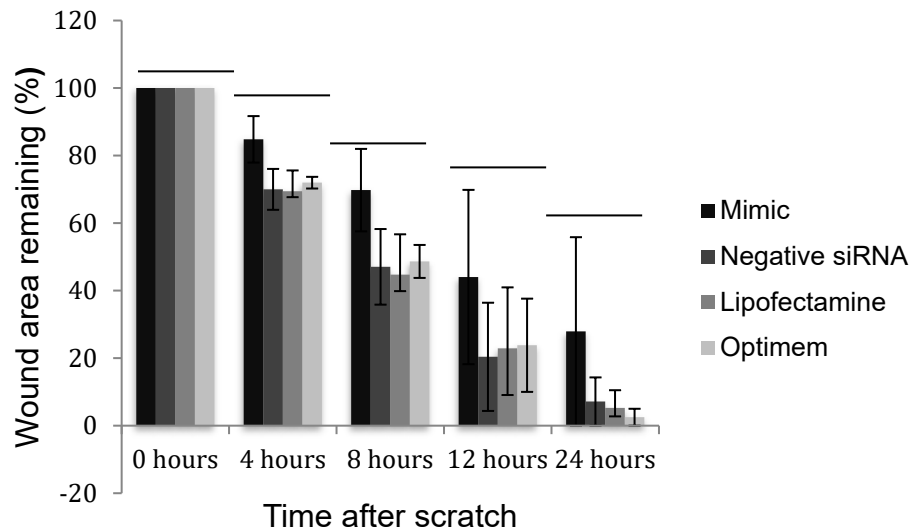


Figure S5. Percentage wound area remaining during scratch wound assay using a miR-429 mimic. The average percentage wound remaining for each condition is recorded at 0, 4, 8, 12 and 24 h. The data represents the mean from three separate experiments. An ANOVA test was used to look for significance between groups. A Tukey's test demonstrated that there was no significant difference between the mimic and negative siRNA groups suggesting that miR-429 does not affect wound adhesion.

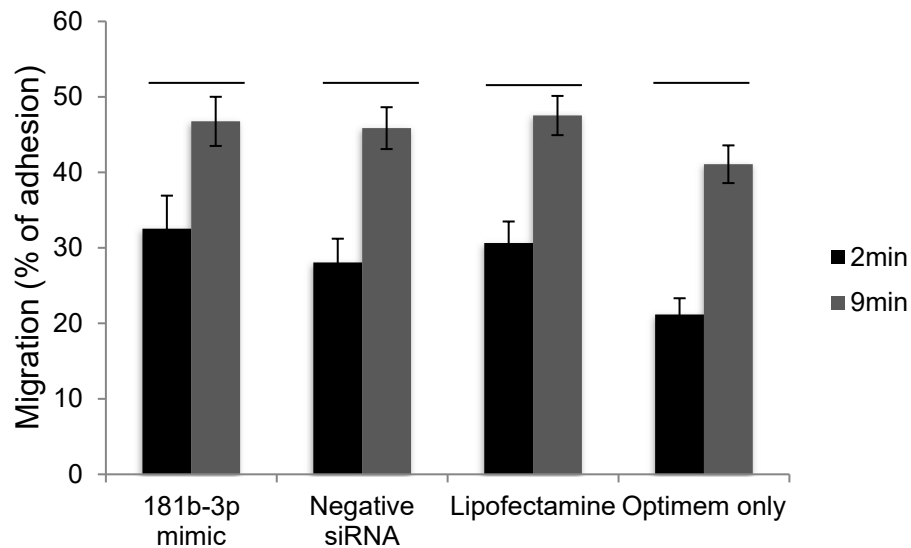


Figure S6. Static transendothelial study investigating miR-181b-3p. This chart shows the proportion of cells that have migrated during the assay at 2 and 9 min. The data represents the mean from three separate experiments. An ANOVA test was used to look for significance between groups. A Tukey's test demonstrated that there was no significant difference between the mimic and negative siRNA groups suggesting that miR-181b-3p does not affect neutrophil transmigration across pulmonary endothelium.

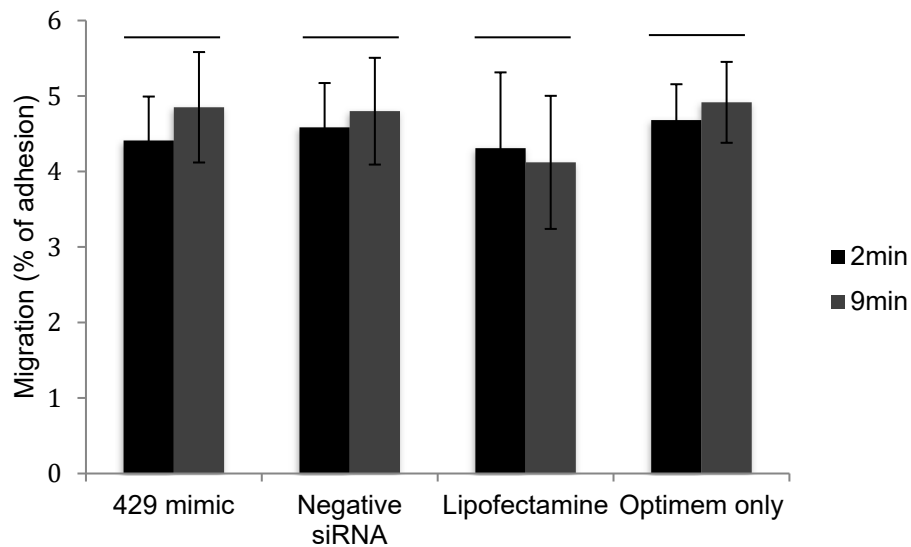


Figure S7: Static transendothelial study investigating miR-429. This chart shows the proportion of cells that have migrated during the assay at 2 and 9 min. The data represents the mean from three separate experiments. An ANOVA test was used to look for significance between groups. A Tukey's test demonstrated that there was no significant difference between the mimic and negative siRNA groups suggesting that miR-429 does not affect neutrophil transmigration across pulmonary endothelium.