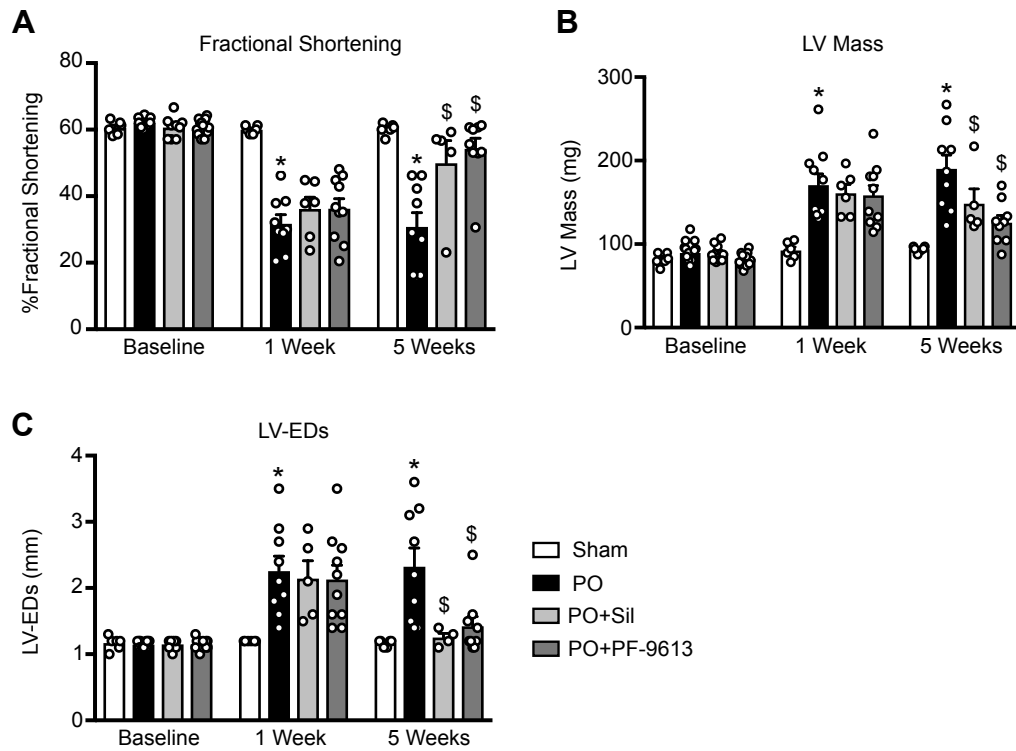


Supplemental Material for “Marked Disparity of MicroRNA Modulation by cGMP-selective PDE5 or PDE9 Inhibitors in Heart Disease”

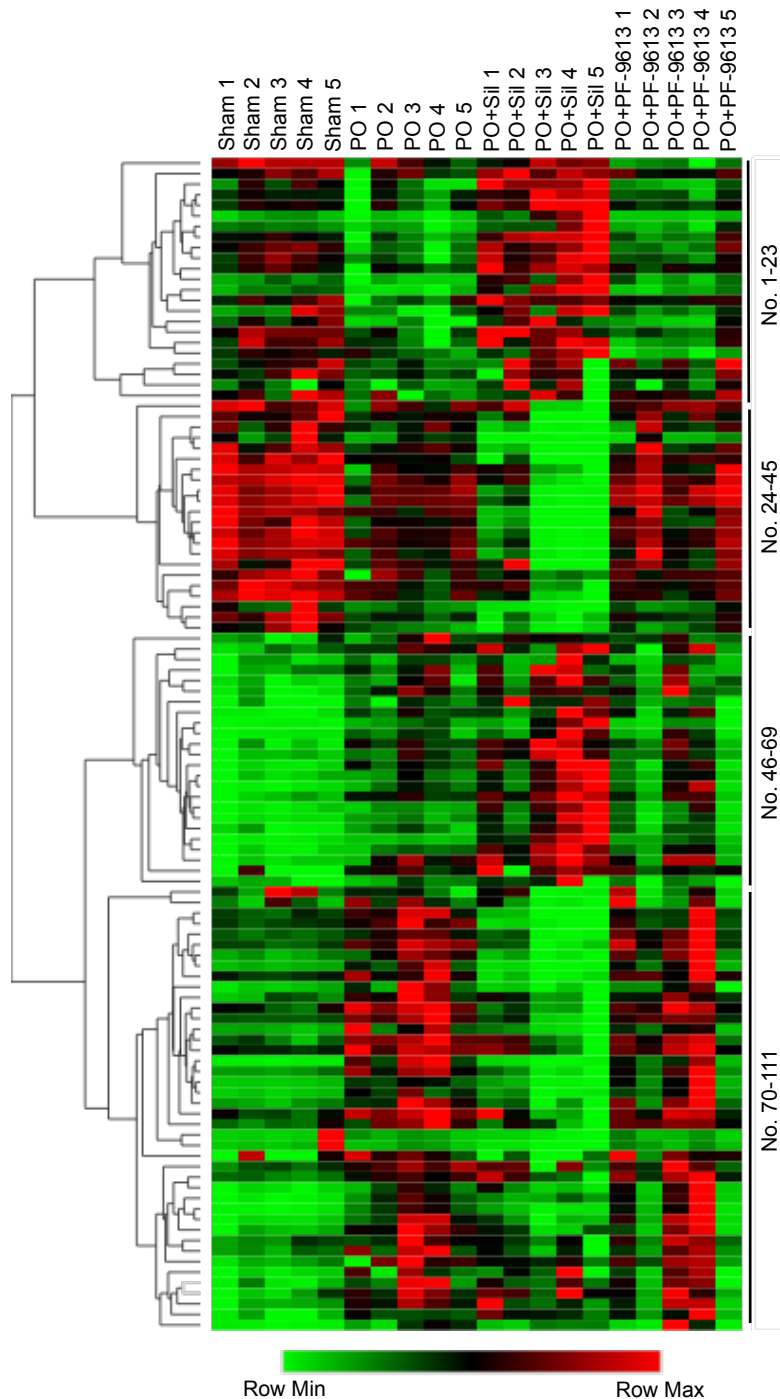
This file includes:

Supplemental Figures 1-4

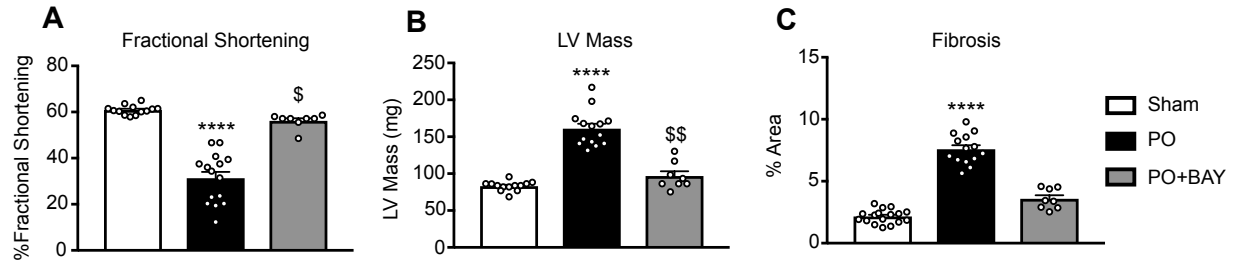
Supplemental Tables 1-4



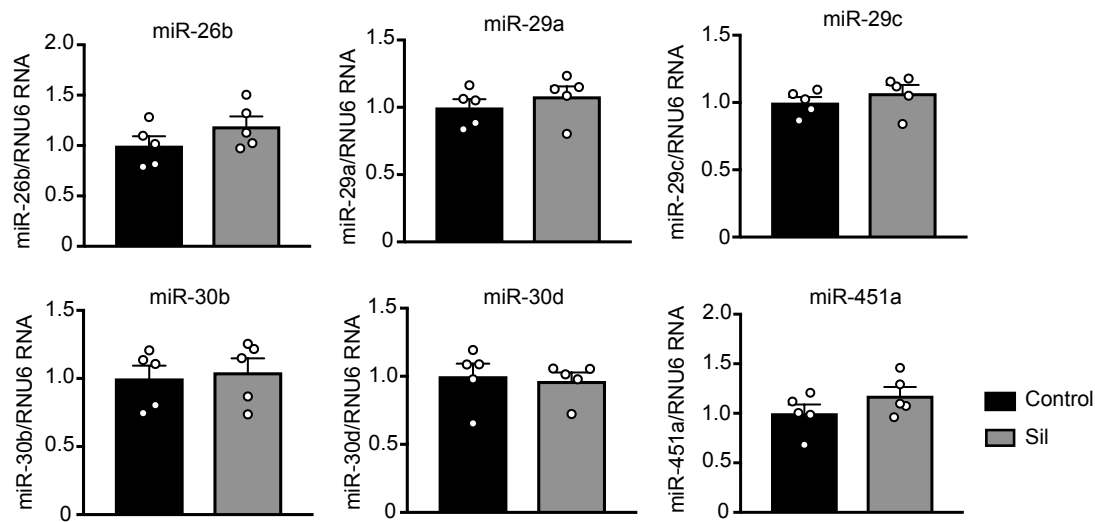
Supplemental Figure 1. C57Bl6/J mice were studied as previously described (14). Left ventricular tissue was subsequently used for miRNA-seq analysis. A) Fractional shortening. B) Left ventricular mass. C) Left ventricular end-systolic dimension as a measure of dilation. All parameters were significantly improved by treatment with either Sil (PDE5-I) or PF-9613 (PDE9-I). * p<0.05 versus PO group baseline measurements. \$ p<0.05 versus PO measurement as same time point. N=5-9 per group. Data is presented as mean ± SEM. Data were analyzed using multiple t-tests.



Supplemental Figure 2. Heatmap of all miRNAs changed significantly with PO for all treatment groups, clustered only by rows (miRNAs). Row labels can be found in Table S4. C57Bl6/J mice were studied as previously described for Figure 1 and S1, with left ventricular tissue assayed by miRNA-seq.



Supplemental Figure 3. C57Bl6/J mice were subjected to sham or PO surgeries. PO mice were subsequently treated with either vehicle or BAY 60-2770 (GC-1 activator) and followed for 3 weeks(19). **A)** Left ventricular mass. **B)** Fractional shortening. **C)** Fibrosis as quantified by Masson's trichrome stain. All parameters were significantly improved by treatment with BAY 60-2770. **** p<0.0001 versus sham. \$ p<0.05 versus PO, \$\$ p<0.01 versus PO. N=8-15 per group. Data is presented as mean ± SEM. Data was analyzed using a Kruskal-Wallis test with Dunn's post hoc test.



Supplemental Figure 4. qRT-PCR was performed for various miRNAs in lung tissue from normal, non-stressed C57Bl6/J mice treated with PDE5-I (Sil) for 5 weeks (n=5 per group). No significant changes were seen with PDE5-I treatment. Data is presented as mean \pm SEM. Data was analyzed using two-tailed unpaired t-tests.

Supplemental Table S1. miRNAs differentially expressed in PO as compared to sham. Left ventricle myocardium from mice subjected to sham or PO surgery and subsequently given either vehicle, PDE5-I (Sil), or PDE9-I (PF-9613) was subjected to miRNA sequencing and subsequent differential expression analysis (n=5 per group). The log2 fold change and p value are provided for each miRNA, with table sorted by p value.

miRNA ID	log2FoldChange (vs. sham)	p value
mmu-miR-208b-3p	4.177277275	4.15E-69
mmu-miR-455-3p	2.566344072	6.01E-23
mmu-miR-1247-5p	2.245542141	1.16E-19
mmu-miR-199-5p	1.987698664	3.13E-19
mmu-miR-34c-5p	2.081491061	4.76E-18
mmu-miR-21a-5p	1.778120295	1.50E-17
mmu-miR-214-3p	1.846356429	1.62E-15
mmu-miR-214-5p	1.803377612	2.47E-13
mmu-miR-199-3p/mmμ-miR-199b-3p	1.531757504	8.96E-13
mmu-miR-146b-5p	1.66286642	1.41E-11
mmu-miR-21a-3p	1.486612606	6.22E-10
mmu-miR-127-3p	1.228474661	1.78E-08
mmu-miR-224-5p	1.282069615	1.17E-07
mmu-miR-199b-5p	1.25544462	2.33E-07
mmu-miR-140-3p	1.149507292	2.39E-07
mmu-miR-34b-5p	1.713965516	1.26E-06
mmu-miR-411-5p	1.093259429	1.40E-06
mmu-miR-1247-3p	2.407271182	6.26E-06
mmu-miR-9-5p	-1.15246671	7.28E-06
mmu-miR-455-5p	-1.125269179	7.29E-06
mmu-miR-298-5p	1.121706324	7.94E-06
mmu-miR-501-5p	1.857763818	2.48E-05
mmu-miR-27b-3p	0.85679754	3.09E-05
mmu-miR-300-3p	0.985183431	4.55E-05
mmu-miR-499-5p	-0.777585635	0.000147302
mmu-miR-410-3p	0.89245254	0.000161098
mmu-miR-497a-5p	0.776615594	0.000174491
mmu-miR-379-5p	0.982619158	0.000271363
mmu-miR-541-5p	0.853822266	0.000285637
mmu-miR-133a-5p	-0.796649968	0.000305674
mmu-miR-92b-3p	0.883212541	0.000384535
mmu-miR-222-3p	0.744534001	0.000496055
mmu-miR-431-5p	0.882820558	0.000496317
mmu-miR-140-5p	0.883350398	0.000507314

mmu-miR-24-3p	0.76897668	0.000551962
mmu-miR-338-3p	-0.837017936	0.000575003
mmu-miR-574-3p	0.798361265	0.000606652
mmu-miR-125b-1-3p	0.812749712	0.000614638
mmu-miR-582-5p	-1.01850343	0.000615216
mmu-miR-135-5p	-0.950045305	0.000621182
mmu-miR-23b-3p	0.719158479	0.000643536
mmu-miR-195-5p	0.723228506	0.000698554
mmu-miR-10a-5p	-0.769965209	0.000984668
mmu-miR-29c-3p	-0.709761981	0.00102143
mmu-miR-329-3p	1.094665852	0.001190208
mmu-miR-30c-1-3p	-0.765860358	0.001248326
mmu-miR-30c-5p	-0.676552634	0.001334222
mmu-miR-874-3p	-0.793623431	0.001440808
mmu-miR-136-5p	0.792994603	0.001450841
mmu-miR-30e-5p	-0.599468785	0.001594309
mmu-miR-30e-3p	-0.605251844	0.001700235
mmu-miR-136-3p	0.863419406	0.002005438
mmu-miR-34b-3p	0.945263719	0.002109153
mmu-miR-99-3p	0.791626989	0.002221577
mmu-miR-10b-5p	0.651387841	0.00242168
mmu-miR-221-3p	0.668772841	0.002702869
mmu-miR-144-3p	-0.791623865	0.002710719
mmu-miR-149-5p	-0.647036271	0.002778003
mmu-miR-208a-3p	-0.658540111	0.002872092
mmu-miR-1a-3p	-0.57678815	0.002904741
mmu-miR-27b-5p	0.677970616	0.00345167
mmu-miR-30c-2-3p	-0.643612613	0.003849693
mmu-miR-1960	-0.875994383	0.003898051
mmu-miR-210-3p	0.64683885	0.004630607
mmu-miR-667-3p	1.204853494	0.004877288
mmu-miR-30b-5p	-0.612743013	0.004952484
mmu-miR-133b-3p	-0.595539655	0.006280098
mmu-miR-483-3p	-0.730210151	0.007147573
mmu-miR-582-3p	-0.601335273	0.008068592
mmu-miR-1964-3p	-0.641278554	0.008245143
mmu-miR-301a-3p	0.60618848	0.008330173
mmu-miR-139-3p	-0.697509549	0.008483423
mmu-miR-195-3p	0.640570655	0.00867885
mmu-miR-184-3p	0.718421494	0.00988326
mmu-miR-200c-3p	-1.144613357	0.010961819

mmu-miR-219-5p	-0.951127563	0.011887967
mmu-miR-154-5p	1.009780507	0.014955031
mmu-miR-132-3p	0.502716542	0.014970729
mmu-miR-99b-5p	0.519732556	0.01554761
mmu-miR-145-3p	-0.510560397	0.015906786
mmu-miR-26b-3p	-0.621098994	0.01596098
mmu-miR-181a-1-3p	-0.466649623	0.018218181
mmu-miR-3098-5p	-0.920489101	0.018330095
mmu-miR-3098-3p	-0.929468531	0.018787963
mmu-miR-501-3p	0.62960178	0.018971356
mmu-miR-328-3p	-0.511540878	0.019431341
mmu-miR-1198-5p	-0.54981481	0.020920779
mmu-miR-30a-3p	-0.441725334	0.02179809
mmu-miR-212-5p	0.593630296	0.0218344
mmu-miR-133a-3p	-0.449577112	0.021989004
mmu-miR-871-3p	-1.15694682	0.022021046
mmu-miR-99-5p	0.53007521	0.022479183
mmu-miR-341-3p	0.716012717	0.02354315
mmu-miR-378a-3p	-0.425598087	0.024202638
mmu-miR-486a-3p	-0.451965148	0.02424837
mmu-miR-134-5p	0.683384586	0.025274159
mmu-miR-331-5p	-0.71529655	0.025670549
mmu-miR-499-3p	-0.614728415	0.025868154
mmu-miR-574-5p	0.564633788	0.027924415
mmu-let-7d-5p	-0.482804262	0.029207826
mmu-miR-532-5p	0.529975705	0.034580381
mmu-miR-100-5p	0.494306994	0.034768324
mmu-miR-141-3p	-0.577602833	0.034852473
mmu-miR-24-1-5p	0.556695732	0.036921798
mmu-miR-152-5p	0.548148402	0.039963989
mmu-miR-409-3p	0.539785248	0.04177907
mmu-miR-187-3p	-0.549552419	0.043913063
mmu-miR-324-5p	0.522237481	0.044598747
mmu-miR-144-5p	-0.51562852	0.047280829
mmu-miR-874-5p	-0.663785168	0.04760991
mmu-miR-185-5p	-0.446271837	0.047679759

Supplemental Table 2. miRNAs differentially expressed in PO+PDE5-I as compared to PO. Left ventricle myocardium from mice subjected to sham or PO surgery and subsequently given either vehicle, PDE5-I (Sil), or PDE9-I (PF-9613) was subjected to miRNA sequencing and subsequent differential expression analysis (n=5 per group). The log2 fold change and p value are provided for each miRNA, with table sorted by p value.

miRNA ID	log2FoldChange (vs. PO)	p value
mmu-miR-101a-3p	-4.575787696	2.18E-25
mmu-miR-208b-3p	-4.259710819	1.93E-24
mmu-miR-142a-5p	-4.720138559	5.59E-24
mmu-miR-21a-5p	-4.292238301	1.29E-22
mmu-miR-144-3p	-5.079271251	7.17E-20
mmu-miR-136-5p	-4.821509198	1.39E-15
mmu-miR-1a-3p	-3.593613103	5.60E-15
mmu-let-7a-1-3p/mmu-let-7c-2-3p	-4.305815692	1.01E-14
mmu-miR-101b-3p	-3.606449179	4.26E-14
mmu-miR-208a-3p	-3.520515306	9.09E-14
mmu-miR-301a-3p	-3.226350129	1.01E-13
mmu-miR-153-3p	-5.244158568	1.37E-13
mmu-miR-32-5p	-4.019856028	2.90E-13
mmu-miR-126a-5p	-3.466656304	2.95E-13
mmu-miR-148a-3p	-2.994665951	1.62E-12
mmu-miR-190a-5p	-3.733256642	5.83E-12
mmu-miR-340-5p	-3.067304799	5.96E-12
mmu-miR-142a-3p	-3.265370345	5.96E-12
mmu-miR-215-5p	-4.043439658	1.25E-11
mmu-miR-98-3p	-3.786218782	1.30E-11
mmu-miR-455-5p	-3.88221935	2.60E-11
mmu-miR-19-3p	-4.331984183	5.15E-11
mmu-miR-136-3p	-4.061067822	2.93E-10
mmu-miR-450b-5p	-3.780780242	3.25E-10
mmu-miR-322-5p	-2.91301546	5.93E-10
mmu-miR-542-3p	-3.517618215	1.12E-08
mmu-miR-19b-3p	-3.37716808	1.71E-08
mmu-miR-144-5p	-3.185355157	5.68E-08
mmu-miR-145-3p	-2.782872281	7.21E-08
mmu-miR-499-5p	-2.570804953	1.93E-07
mmu-miR-143-3p	-2.574191179	3.80E-07
mmu-miR-29b-3p	-2.783979064	3.84E-07
mmu-miR-15b-3p	-3.274581748	4.08E-07
mmu-miR-199-3p/mmu-miR-199b-3p	-2.258928351	4.19E-07
mmu-miR-30d-3p	-2.819186253	4.36E-07

mmu-miR-335-5p	-3.073154998	4.91E-07
mmu-miR-192-5p	-2.478450318	6.64E-07
mmu-miR-147-3p	-3.445852507	1.61E-06
mmu-miR-329-3p	-2.619721833	2.86E-06
mmu-let-7f-1-3p	-3.073407632	3.04E-06
mmu-miR-29c-3p	-2.297479573	3.32E-06
mmu-miR-146b-5p	-2.297222027	3.65E-06
mmu-miR-201-5p	-3.550553371	4.15E-06
mmu-miR-223-3p	-2.615866479	4.39E-06
mmu-miR-199b-5p	-2.504635063	5.19E-06
mmu-miR-199-5p	-1.763126499	1.15E-05
mmu-miR-1a-1-5p	-3.39147674	1.29E-05
mmu-miR-582-5p	-3.364000211	1.49E-05
mmu-miR-21a-3p	-2.167642629	1.63E-05
mmu-miR-101a-5p	-2.733260511	2.03E-05
mmu-miR-195-5p	-2.148722392	2.58E-05
mmu-miR-135-5p	-3.142603222	2.66E-05
mmu-let-7c-1-3p	-2.510702219	3.54E-05
mmu-miR-411-5p	-1.80305691	4.03E-05
mmu-miR-15-5p	-2.146684268	5.18E-05
mmu-miR-26a-2-3p	-4.061562476	5.30E-05
mmu-miR-451a	-2.15864161	5.35E-05
mmu-miR-30e-5p	-2.153355798	6.19E-05
mmu-miR-3068-3p	-2.165959958	6.52E-05
mmu-miR-488-3p	-2.302146104	6.70E-05
mmu-miR-362-3p	-2.810144748	7.47E-05
mmu-miR-376c-3p	-4.311137398	9.59E-05
mmu-miR-30b-5p	-1.975135773	9.66E-05
mmu-miR-872-5p	-2.407065203	0.000119065
mmu-miR-335-3p	-2.422673274	0.000129716
mmu-miR-26b-5p	-1.940699675	0.00013046
mmu-miR-7a-1-3p	-2.877452256	0.000139076
mmu-miR-218-5p	-2.330485724	0.0001522
mmu-miR-27a-3p	-1.69596849	0.000156031
mmu-miR-24-2-5p	-1.830541263	0.000171641
mmu-miR-483-3p	1.205677818	0.000201253
mmu-miR-676-5p	-2.382599291	0.000254347
mmu-miR-140-5p	-2.06288526	0.000259883
mmu-miR-98-5p	-2.032506534	0.000287761
mmu-miR-16-1-3p	-3.109524923	0.0003418
mmu-miR-27b-3p	-1.742733471	0.0003486

mmu-miR-34b-5p	-2.304392315	0.000361079
mmu-miR-10b-5p	-1.767827241	0.000483953
mmu-miR-34c-5p	-1.607260613	0.000679358
mmu-miR-450a-5p	-2.017319595	0.00089246
mmu-miR-547-3p	-2.324212207	0.001522837
mmu-let-7f-5p	-1.74726319	0.001620898
mmu-miR-582-3p	-1.803396153	0.001629021
mmu-miR-152-3p	-1.798922482	0.001724373
mmu-miR-32-3p	-3.01248029	0.002335911
mmu-miR-376b-5p	-1.934067313	0.002376858
mmu-miR-299-3p	-2.983870311	0.002768105
mmu-miR-106b-5p	-1.856559443	0.003448093
mmu-miR-203-3p	-1.928163717	0.004164814
mmu-miR-3068-5p	-2.051851585	0.004832613
mmu-miR-423-5p	1.427573041	0.005622449
mmu-miR-598-3p	-2.790289114	0.005771312
mmu-miR-1247-3p	1.078058216	0.005816182
mmu-miR-328-3p	1.346733766	0.006049766
mmu-miR-30a-5p	-1.543528495	0.006297692
mmu-miR-148b-5p	-1.389338531	0.006305273
mmu-miR-219-5p	-3.034733728	0.007325161
mmu-miR-501-3p	1.546412839	0.007537389
mmu-miR-744-3p	-1.77447997	0.007649749
mmu-miR-182-5p	-1.465409276	0.007755117
mmu-miR-874-5p	1.249901366	0.008429325
mmu-miR-350-3p	-1.585452909	0.008543687
mmu-miR-152-5p	-1.365001436	0.008651479
mmu-miR-16-2-3p	-1.801419981	0.009177028
mmu-miR-381-3p	-1.521641225	0.009260401
mmu-miR-18a-5p	-2.235746329	0.00999135
mmu-miR-208a-5p	1.342510232	0.01018137
mmu-miR-455-3p	-0.839591689	0.010910983
mmu-miR-411-3p	-1.594941657	0.01213073
mmu-miR-380-3p	-2.819319513	0.013275558
mmu-miR-423-3p	1.263230963	0.013490059
mmu-miR-340-3p	-1.421705584	0.01514416
mmu-miR-150-5p	1.044478629	0.015674139
mmu-miR-125-5p	1.175742371	0.015696524
mmu-let-7d-3p	1.090460392	0.018232638
mmu-miR-26a-5p	-1.317301178	0.018334224
mmu-miR-7236-3p	-2.70124277	0.018645667

mmu-miR-214-5p	-0.967135919	0.018901065
mmu-miR-338-3p	-1.260644787	0.020013995
mmu-miR-148a-5p	-1.394021505	0.020430572
mmu-miR-9-5p	-1.231035575	0.021472458
mmu-miR-744-5p	1.255979317	0.02479594
mmu-miR-93-3p	0.985683796	0.025067739
mmu-miR-92a-3p	1.127560438	0.025076345
mmu-miR-379-5p	-1.558712488	0.025156207
mmu-miR-16-5p	-1.40711933	0.026162994
mmu-miR-490-5p	1.247672271	0.027533408
mmu-miR-7a-5p	-2.331704002	0.02844339
mmu-miR-200c-3p	1.13668089	0.030329734
mmu-let-7g-5p	-1.237526729	0.032344629
mmu-miR-149-5p	0.942728598	0.033806779
mmu-miR-99-3p	-1.167097642	0.034474809
mmu-miR-194-5p	-1.577767761	0.036915266
mmu-miR-141-3p	-1.849075308	0.037001253
mmu-miR-410-3p	-1.135707973	0.038459548
mmu-miR-1981-5p	1.093683482	0.039078004
mmu-miR-24-1-5p	-1.062738184	0.039385136
mmu-miR-31-3p	-2.613782686	0.039462493
mmu-miR-24-3p	-0.981127044	0.040437228
mmu-miR-125b-2-3p	-0.985936937	0.043431362
mmu-miR-330-3p	1.173939541	0.047306434
mmu-miR-15-3p	-1.582116176	0.047921565

Supplemental Table 3. miRNAs differentially expressed in PO+PDE9-I as compared to PO. Left ventricle myocardium from mice subjected to sham or PO surgery and subsequently given either vehicle, PDE5-I (Sil), or PDE9-I (PF-9613) was subjected to miRNA sequencing and subsequent differential expression analysis (n=5 per group). The log2 fold change and p value are provided for each miRNA, with table sorted by p value.

miRNA ID	log2FoldChange (vs. PO)	p value
mmu-miR-486a-3p	-0.852288229	0.000759706
mmu-miR-1247-5p	-0.829164673	0.005017167
mmu-miR-133b-3p	-0.602267066	0.007479807
mmu-miR-208b-3p	-0.726931451	0.012992257
mmu-miR-483-3p	0.678439682	0.017241916
mmu-miR-3098-3p	0.853667319	0.021434169
mmu-miR-144-3p	-0.476077278	0.022872759
mmu-miR-146b-5p	-0.734020656	0.024039032
mmu-miR-501-3p	0.462887415	0.038742158

Supplemental Table 4. Row legends (i.e. miRNA IDs) for the heatmap presented in **Figure 1D** and **Supplemental Figure 2**. miRNAs differentially expressed in PO as compared to sham were clustered using Morpheus (Broad Institute) according to rows (miRNA) and columns (samples), or only rows (miRNA) by the one minus Spearman rank coefficient method.

Row Number	miRNA ID
1	mmu-miR-133b-3p
2	mmu-miR-187-3p
3	mmu-miR-1198-5p
4	mmu-miR-133a-3p
5	mmu-miR-149-5p
6	mmu-miR-328-3p
7	mmu-miR-139-3p
8	mmu-miR-874-3p
9	mmu-miR-30c-1-3p
10	mmu-miR-30c-2-3p
11	mmu-let-7d-5p
12	mmu-miR-874-5p
13	mmu-miR-1964-3p
14	mmu-miR-200c-3p
15	mmu-miR-331-5p
16	mmu-miR-1960
17	mmu-miR-378a-3p
18	mmu-miR-181a-1-3p
19	mmu-miR-486a-3p
20	mmu-miR-3098-3p
21	mmu-miR-3098-5p
22	mmu-miR-483-3p
23	mmu-miR-871-3p
24	mmu-miR-185-5p
25	mmu-miR-141-3p
26	mmu-miR-144-5p
27	mmu-miR-144-3p
28	mmu-miR-208a-3p
29	mmu-miR-219-5p
30	mmu-miR-133a-5p
31	mmu-miR-30c-5p
32	mmu-miR-145-3p
33	mmu-miR-30b-5p
34	mmu-miR-1a-3p
35	mmu-miR-135-5p
36	mmu-miR-29c-3p

37	mmu-miR-30e-5p
38	mmu-miR-499-5p
39	mmu-miR-499-3p
40	mmu-miR-26b-3p
41	mmu-miR-30a-3p
42	mmu-miR-30e-3p
43	mmu-miR-455-5p
44	mmu-miR-338-3p
45	mmu-miR-9-5p
46	mmu-miR-212-5p
47	mmu-miR-134-5p
48	mmu-miR-409-3p
49	mmu-miR-184-3p
50	mmu-miR-132-3p
51	mmu-miR-23b-3p
52	mmu-miR-1247-3p
53	mmu-miR-140-3p
54	mmu-miR-1247-5p
55	mmu-miR-298-5p
56	mmu-miR-221-3p
57	mmu-miR-222-3p
58	mmu-miR-127-3p
59	mmu-miR-125b-1-3p
60	mmu-miR-214-3p
61	mmu-miR-195-3p
62	mmu-miR-574-5p
63	mmu-miR-92b-3p
64	mmu-miR-324-5p
65	mmu-miR-501-3p
66	mmu-miR-99b-5p
67	mmu-miR-574-3p
68	mmu-miR-501-5p
69	mmu-miR-667-3p
70	mmu-miR-10a-5p
71	mmu-miR-10b-5p
72	mmu-miR-136-3p
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74	mmu-miR-195-5p
75	mmu-miR-140-5p
76	mmu-miR-199b-5p
77	mmu-miR-21a-5p

78	mmu-miR-301a-3p
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81	mmu-miR-152-5p
82	mmu-miR-27b-3p
83	mmu-miR-146b-5p
84	mmu-miR-24-3p
85	mmu-miR-99-5p
86	mmu-miR-208b-3p
87	mmu-miR-411-5p
88	mmu-miR-199-3p/mm-miR-199b-3p
89	mmu-miR-99-3p
90	mmu-miR-34b-5p
91	mmu-miR-379-5p
92	mmu-miR-24-1-5p
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98	mmu-miR-224-5p
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107	mmu-miR-210-3p
108	mmu-miR-431-5p
109	mmu-miR-541-5p
110	mmu-miR-532-5p
111	mmu-miR-455-3p