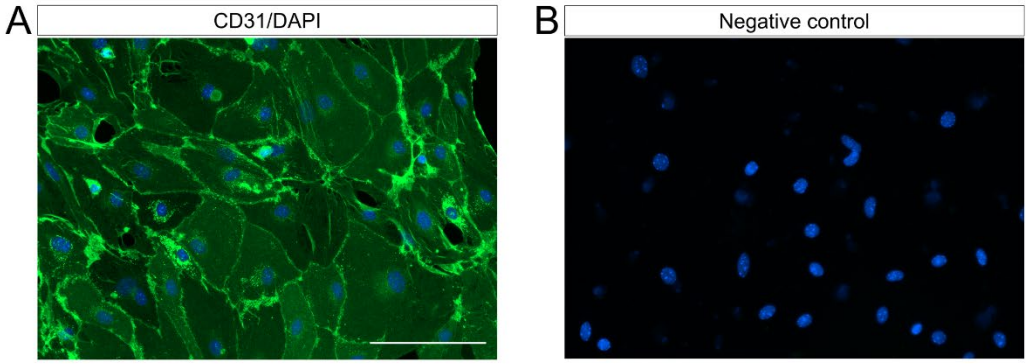
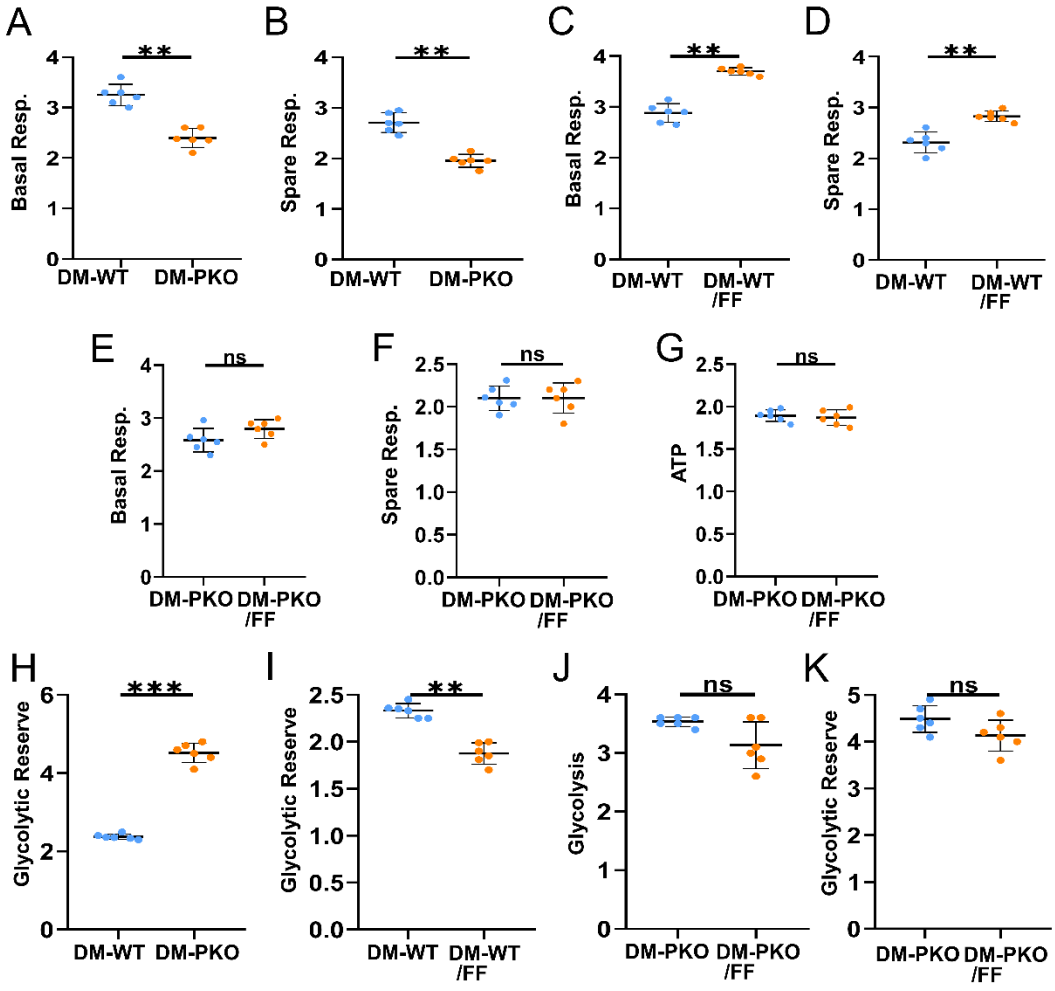
Supplemental Results



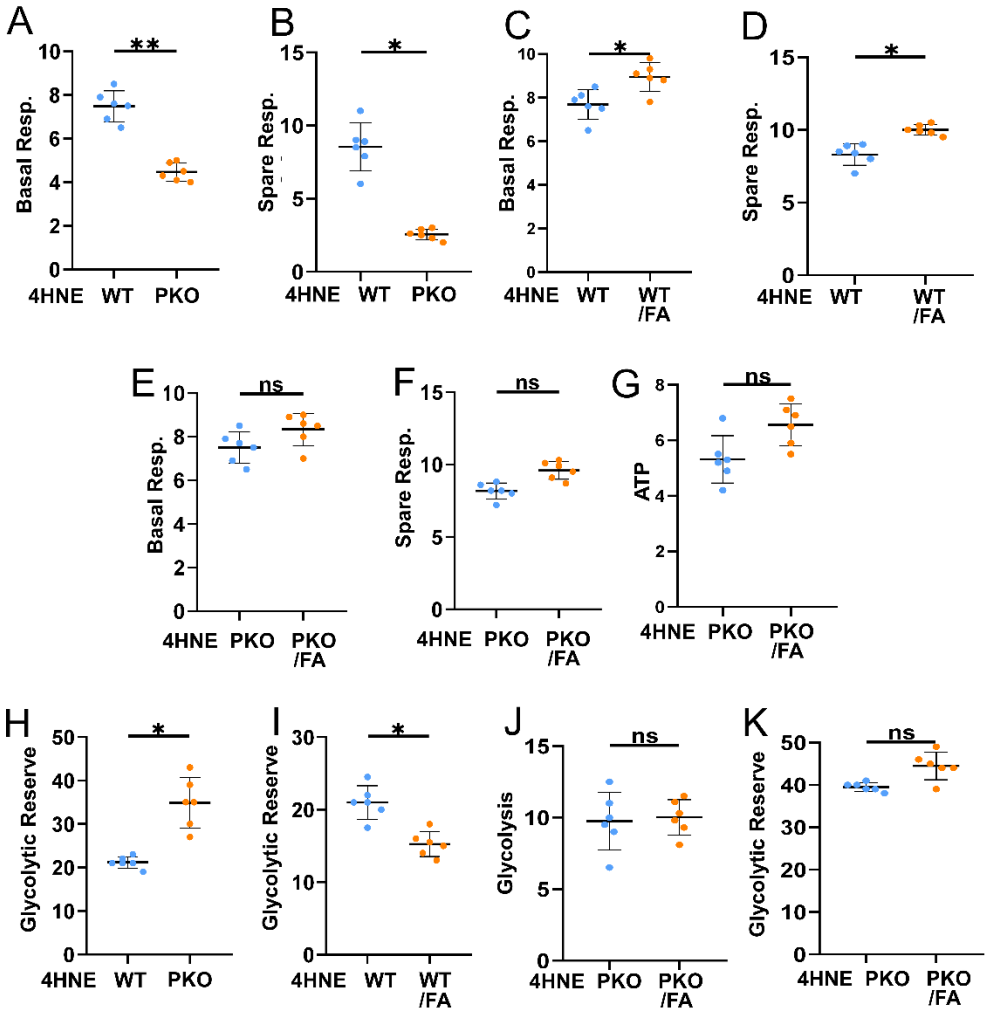
Suppl. Figure 1

Supplemental Figure 1. Verification of primary mouse capillary vascular endothelial cells. Mouse brain capillary vascular cells were isolated and seeded in collagen I-coated plates. Cells were blocked with 5% BSA with Triton X 100 following the fixation with 4% PFA. An anti-CD31 antibody (R&D) was used for the immunostaining. **A**. Representative image of CD31 staining merged with DAPI. **B**. Negative control. Scale bar: 100 µm.



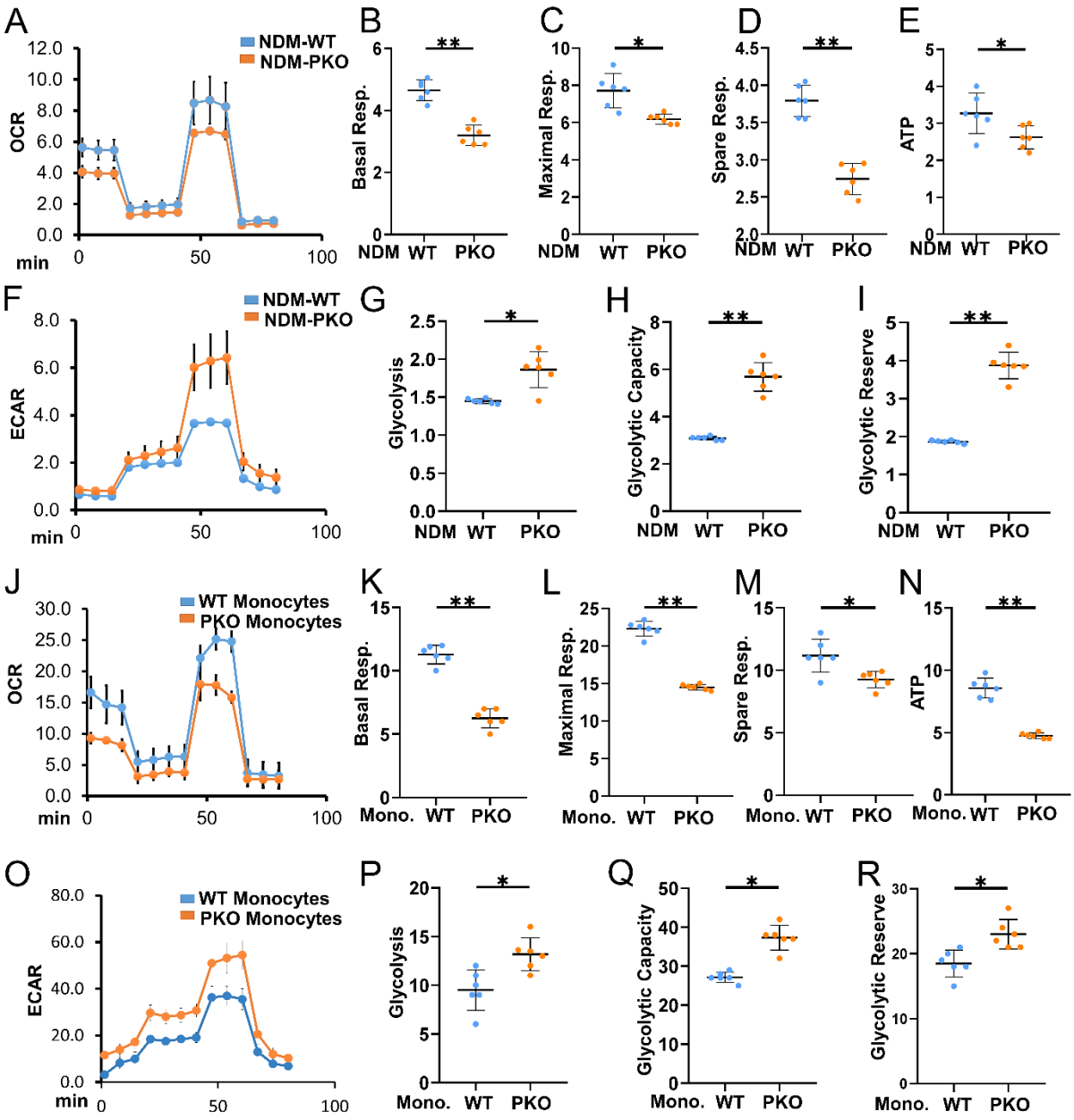
Suppl. Figure 2

Supplemental Figure 2. Decreased mitochondrial function and increased glycolysis in diabetic *PPARα*$^{-/-}$ monocytes. Monocytes isolated from diabetic wild-type (DM-WT) and diabetic *PPARα*$^{-/-}$ (DM-PKO) mice with or without fenofibrate chow (FF) were used for mitochondrial and glycolytic assays using a Seahorse XFe96 Analyzer. Monocytes from diabetic *PPARα*$^{-/-}$ mice showed a decreased basal (**A**) and spare respiration (**B**, pmol/min/1000 cells) and an increased glycolysis reserve (**H,** mpH/min/1000 cells) than monocytes from diabetic WT mice. Fenofibrate chow effectively preserved the mitochondrial function in both basal (**C**) and spare respiration capacity (**D**) and inhibited the glycolysis reserve (**I**) in diabetic WT monocytes, but not in diabetic *PPARα*$^{-/-}$ monocytes (**E-G, J, K**), suggesting that the protective effect of fenofibrate on monocytic metabolism is PPARα–dependent. All values are mean±SD, n=6; **P<0.01; ***P<0.001. n.s., no significant difference. Resp.=Respiration rate



Suppl. Figure 3

Supplemental Figure 3. PPARα prevented the metabolic profile changes induced by 4HNE in monocytes. Monocytes were isolated from *PPARα*$^{-/-}$ mice (PKO) and wild-type (WT) littermates. The cells were exposed to 4HNE with and without fenofibric acid (FA) for 6 hr and then used for assays of mitochondrial oxidation and glycolysis. Relative to WT monocytes, 4HNE induced a more prominent decrease in basal and spare respiration (**A, B**) and increased glycolysis reserve (**H**) in *PPARα*$^{-/-}$ monocytes. FA protected basal and spare (**C, D**) respiration and decreased glycolysis reserve (**I**) in 4HNE-treated WT monocytes, but not in the 4HNE-treated *PPARα*$^{-/-}$ monocytes (**E-G, J, K**). These metabolic profiles indicated that PPARα prevented the decline of mitochondrial function and the increase of glycolysis under diabetic conditions. All values are mean±SD, n=6; *P<0.05; **P<0.01. n.s., no significant difference. Resp.=Respiration rate



Suppl. Figure 4

Supplemental Figure 4. Decreased mitochondrial function and increased glycolysis in non-diabetic *PPARα*$^{-/-}$ monocytes. Monocytes were isolated from age-matched non-diabetic (NDM) *PPARα*$^{-/-}$ (PKO) mice and wild-type (WT) mice **(A-I)**. *PPARα* knockout alone caused declined mitochondrial function (**A-E**) as shown by a decreased real-time OCR value (pmol/min/1000 cells, **A**), basal, maximal, spare respiration capacity, and ATP generation (**B-E**), while elevated ECAR value (mpH/min/1000 cells, **F**), glycolysis, glycolytic and glycolytic reserve (**G-I**). Monocytes were isolated from *PPARα*$^{-/-}$ mice (PKO) and wild-type (WT) littermates **(J-R)**. *PPARα*$^{-/-}$ monocytes showed a lower OCR (**J**), basal, maximal, and spare respiration capacity, and ATP generation (**K-N**), while a higher ECAR (**O**), glycolysis, glycolytic capacity, glycolytic reserve (**P-R**) relative to WT monocytes. These results suggest that PPARα regulates the mitochondrial and glycolytic profiles in monocytes. All values are mean±SD, n=6; *P<0.05; **P<0.01.

Supplemental Table 1. Body Weight and blood Glucose of STZ-induced mice fed with Fenofibrate chow

	NDM	NDM+FF	DM	DM+FF
Body Weight (g)				
Duration of Diabetes				
0 week	24.63±0.44	24.65±0.62	26.33±0.94	27.053±0.5
1 month	27.17±0.85	27.00±1.00 n.s	26.10±1.30****	26.80±0.66****
2 months	28.83±0.90	28.95±0.59 n.s	24.17±0.69****	25.00±0.58****
3 months	30.20±1.15	29.67±0.88 n.s	23.30±0.56****	23.53±0.63****
Blood Glucose (mg/dl)				
Duration of Diabetes				
0 week	161.83±6.36	171.67±5.44 n.s	164.83±12.75	163.00±10.54
1 month	157.33±11.51	166.33±6.99 n.s	355.87±4.00****	357.00±5.20****
2 months	165.33±13.07	163.67±8.79 n.s	365.00±4.97****	361.17±5.34****
3 months	163.67±12.49	164.00±6.98 n.s	375.93±5.14****	374.17±5.15****

Values are means ± SD. ****P< 0.0001 versus NDM group, n.s., no significant difference. n=6. NDM: non diabetic mouse; DM: STZ-induced diabetic mice; FF: fenofibrate chow.

Supplemental Table 2. Body Weight and blood Glucose of STZ-induced Wild-type mice and *PPARα*$^{-/-}$ mice fed with Fenofibrate chow

	WT-DM	WT-DM+FF	PKO-DM	PKO-DM+FF
Body Weight (g)				
Duration of Diabetes				
0 week	27.17±1.07	26.83±0.69	26.67±0.75	27.00±1.00
1 month	26.62±0.61	26.53±0.69 $^{n.s}$	26.50±0.96 $^{n.s}$	26.75±0.85 $^{n.s}$
2 months	24.68±0.51	24.45±0.43 $^{n.s}$	24.03±0.48 $^{n.s}$	24.43±0.32 $^{n.s}$
3 months	23.28±0.49	23.53±0.35 $^{n.s}$	23.42±0.63 $^{n.s}$	23.93±0.86 $^{n.s}$
Blood Glucose (mg/dl)				
Duration of Diabetes				
0 week	166.17±7.20	168.50±6.95	165.50±6.45	166.50±6.24
1 month	355.83±3.62	360.50±10.47 $^{n.s}$	361.50±7.91 $^{n.s}$	355.67±5.59 $^{n.s}$
2 months	463.33±6.16	461.50±8.88 $^{n.s}$	465.17±8.55 $^{n.s}$	449.17±28.63 $^{n.s}$
3 months	456.00±13.58	454.33±7.87 $^{n.s}$	437.83±34.60 $^{n.s}$	433.83±37.93 $^{n.s}$

Values are means ± SD, n=6, WT: Wild-type mice, PKO: *PPARα*$^{-/-}$ mice. DM: STZ-induced diabetic mice; FF: fenofibrate chow. n.s., no significant difference.vs WT-DM group

Supplemental Table 3. Body Weight and blood Glucose of STZ-induced diabetic mice

	WT-DM	*PPARα*^{MCKO}-DM	*PPARα*^{MCTg}-DM
Body Weight (g)			
Duration of Diabetes			
0 week	25.97±0.78	25.75±0.89	25.33±0.94
1 month	25.75±0.85	25.25±0.63 $^{n.s}$	24.82±1.10 $^{n.s}$
2 months	23.35±0.34	23.08±0.20 $^{n.s}$	23.23±0.65 $^{n.s}$
3 months	22.57±0.50	22.22±0.85 $^{n.s}$	21.78±0.65 $^{n.s}$
Blood Glucose (mg/dl)			
Duration of Diabetes			
0 week	161.50±6.63	162.67±8.65	161.00±7.94
1 month	356.75±4.54	360.08±8.06 $^{n.s}$	361.33±10.08 $^{n.s}$
2 months	449.33±23.15	448.00±18.18 $^{n.s}$	453.00±15.83 $^{n.s}$
3 months	442.17±31.44	453.33±23.86 $^{n.s}$	435.50±42.84 $^{n.s}$

Values are means ± SD, n=6, WT: Wild-type mice. DM: STZ-induced diabetic mice. n.s., no significant difference, vs WT-DM group

Supplemental Table 4. Decreased mitochondrial function and increased glycolysis in diabetic *PPARα*$^{-/-}$ monocytes.

	GROUP 1		GROUP 2		GROUP 3		GROUP 4	
	NDM- WT	NDM-PKO	DM-WT	DM-PKO	DM-WT	DM-WT+FF	DM-PKO	DM-PKO+FF
Mito Stress Assay (OCR value, pmol/min/1000 cells)								
Basal Resp.	4.65±0.33	3.20±0.32	3.23±0.49	2.25±0.30**	2.88±0.19	3.70±0.07**	2.58±0.22	2.80±0.18 n.s
Maxi. Resp.	7.72±0.92	6.18±0.27	6.05±0.100	4.13±0.32**	5.32±0.45	8.07±0.16***	4.32±0.07	4.54±0.09 n.s
Spare. Resp.	3.79±0.21	2.74±0.21	2.71±0.19	1.95±0.13**	2.31±0.20	2.83±0.10**	2.1±0.14	2.1±0.18 n.s
ATP	3.27±0.54	2.62±0.32	2.75±0.23	1.76±0.17**	2.03±0.20	3.69±0.03***	1.89±0.07	1.87±0.09 n.s
Glycolysis Stress Assay (ECAR value, (mpH/min/1000 cells)								
Glycolysis	1.45±0.03	1.86±0.23*	2.12±0.10	3.52±0.07***	2.19±0.12	0.91±0.10***	3.53±0.08	3.28±0.32 n.s
Glyco. Cap.	3.08±0.08	5.86±0.60	4.57±0.24	7.82±0.15***	4.50±0.18	2.85±0.12**	7.95±0.15	7.48±0.50 n.s
Glyco. Res.	1.86±0.04	3.87±0.35	2.37±0.07	4.52±0.25***	2.33±0.08	1.88±0.11**	4.48±0.29	4.13±0.33 n.s

Values are means ± SD. *P< 0.05, **P< 0.01, ***P< 0.001 vs NDM-WT in GROUP 1, vs DM-WT in GROUP 2, vs DM-WT in GROUP 3, or vs DM-PKO in GROUP 4. n.s., no significant difference. n=6. Basal Resp.: Basal Respiration Rate, Maxi. Resp.: Maximal Respiration Rate, Spare Resp.: Spare mitochondrial Respiration capacity, Glyco. Cap.: Glycolytic Capacity, Glyco. Res.: Glycolytic Reserve, WT: Wild-type; PKO: *PPARα*$^{-/-}$, FF: Fenofibrate chow.

Supplemental Table 5. Decreased mitochondrial function and increased glycolysis in *PPARα*$^{-/-}$ monocytes with 4HNE treatment.

	GROUP 1		GROUP 2		GROUP 3		GROUP 4	
	WT	PKO	4HNE-WT	4HNE-PKO	4HNE-WT	4HNE-WT+FA	4HNE-PKO	4HNE-PKO+FA
Mito Stress Assay (OCR value, pmol/min/1000 cells)								
Basal Resp.	12.02±0.99	6.25±0.69**	7.48±0.71	4.47±0.41**	7.68±0.68	8.95±0.67*	7.50±0.72	8.33±0.74
Maxi.	22.28±1.01	14.52±0.37**	15.95±1.53	6.62±0.63**	16.85±1.22	18.67±1.11*	15.53±0.63	17.58±0.38 n.s
Spare.	11.17±1.21	9.22±0.68*	8.55±1.63	2.55±0.37*	8.30±0.73	10.00±0.36*	8.17±0.56	9.05±1.13 n.s
ATP	8.77±0.73	4.95±0.10**	5.67±0.64	3.03±0.20**	5.60±0.63	7.18±0.63*	5.32±0.86	6.38±0.90 n.s
Glycolysis Stress Assay ECAR value, (mpH/min/1000 cells)								
Glycolysis	9.58±2.01	13.05±1.33*	9.75±1.57	11.42±1.48	11.67±1.25	10.00±1.29*	9.75±2.02	10.02±1.24 n.s
Glyco.	28.33±0.94	35.70±4.23*	32.00±3.51	44.67±5.22	32.83±3.24	25.67±2.58*	48.17±0.75	55.17±2.91 n.s
Glyco. Res.	18.83±1.34	22.67±2.43*	21.17±1.33	34.83±5.81	21.00±2.30	15.25±1.72*	39.5±1.05	41.83±4.07 n.s

Values are means ± SD. *P< 0.05, **P< 0.01versus WT in GROUP 1, vs 4HNE-WT in GROUP 2, vs 4HNE-WT in GROUP 3, or vs 4HNE-PKO in GROUP 4. n.s., no significant difference. n=6. Basal Resp.: Basal Respiration Rate, Maxi. Resp.: Maximal Respiration Rate, Spare Resp.: Spare mitochondrial Respiration capacity, Glyco. Cap.: Glycolytic Capacity, Glyco. Res.: Glycolytic Reserve, WT: Wild-type, PKO: *PPARα*$^{-/-}$, FA: Fenofibric acid.