Macrophages with a Deletion for the Phosphoenolpyruvate Carboxykinase (Pck1) Gene Promote a More Pro-Inflammatory Phenotype

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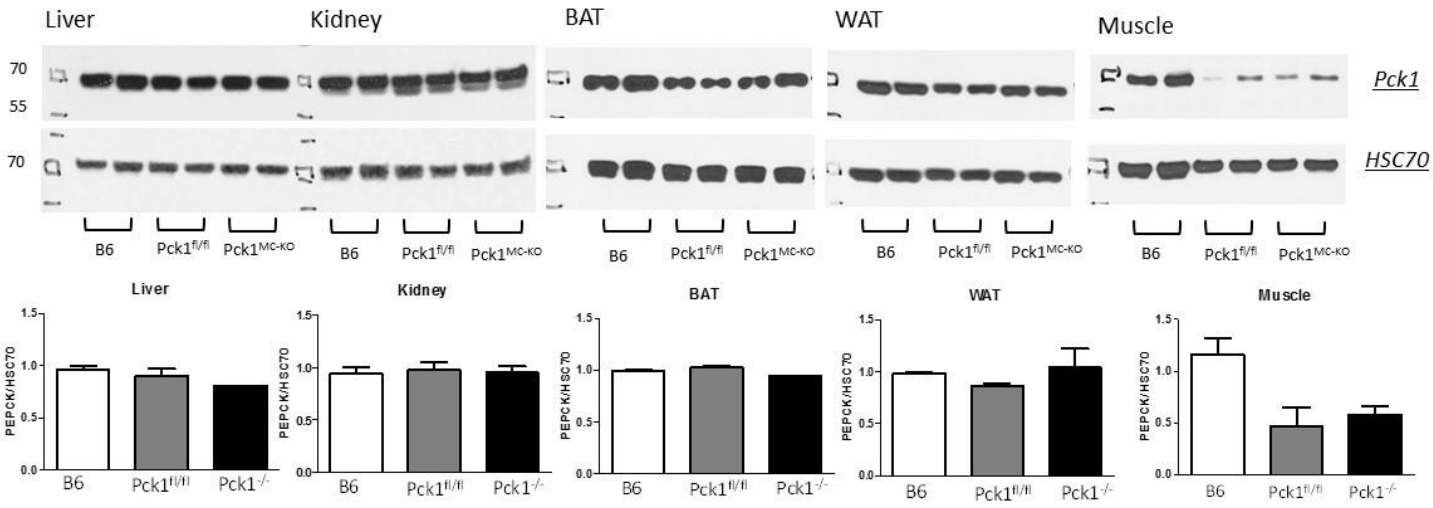
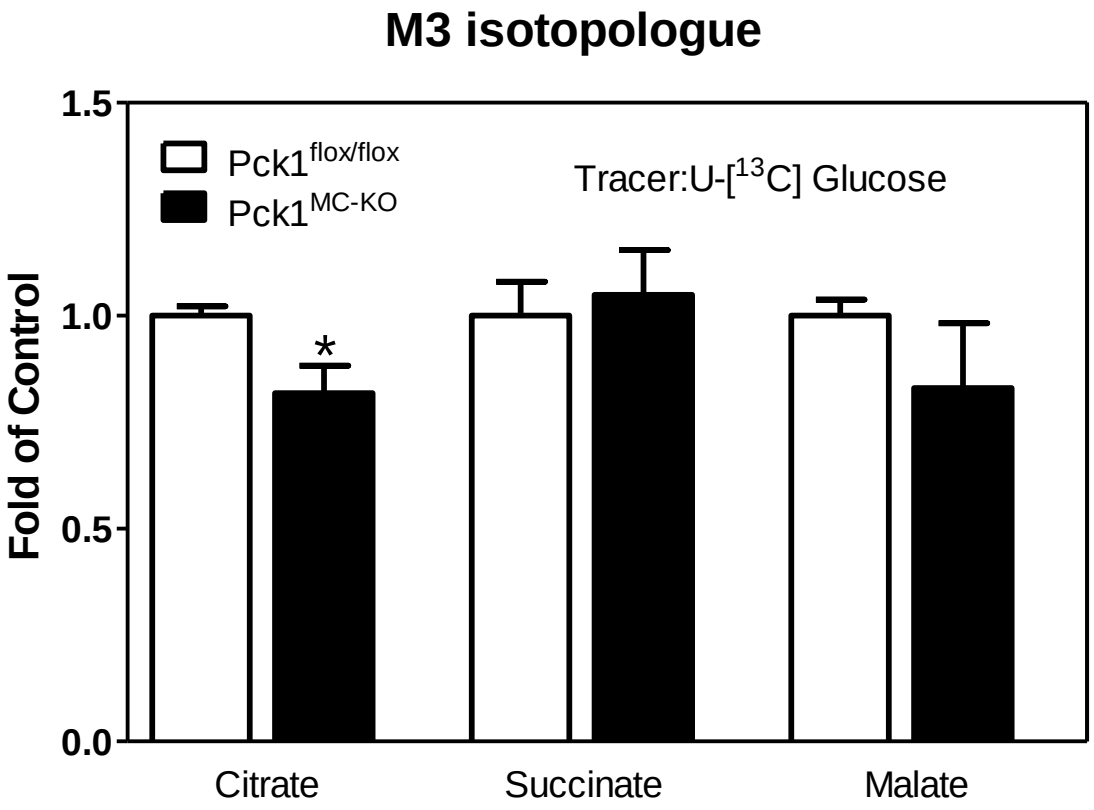
Supplementary Data Table 1 Primer Sequence

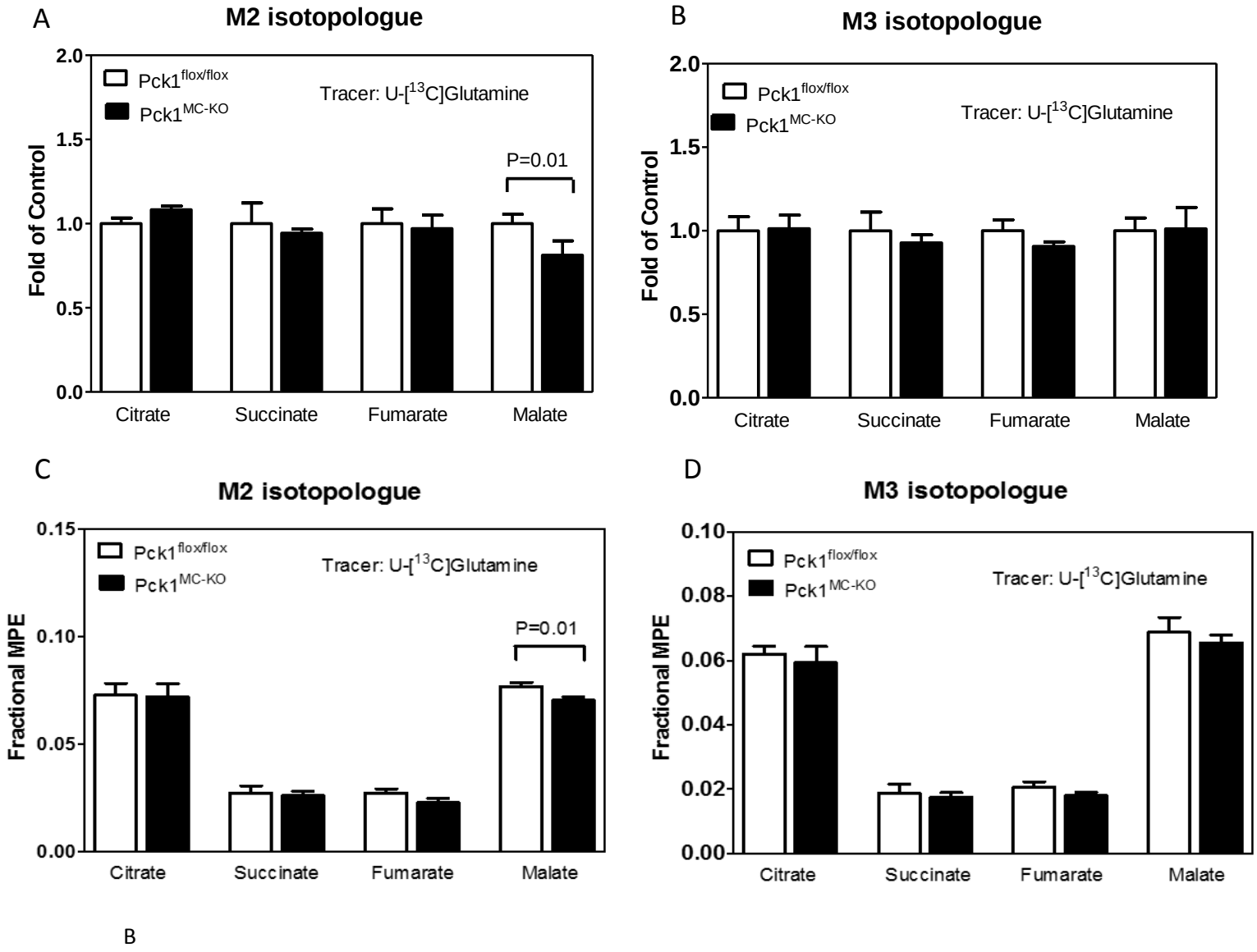
Gene	Forward Primer	Reverse Primer
ACC1 (acetyl CoA carboxylase 1)	gcctcttcctgacaaacgag	tgatctgtgctgtcctggag
ACOX (Acox1 acyl-Coenzyme A oxidase 1, palmitoyl)	ttatgcgcagacagagatgg	tatgtggcagtggtttccaa
CPT1a (carnitine palmitoyltransferase 1a, liver)	ccatgatggaccccacaaca	caggtgctggtgcttttcac
FASN (fatty acid synthase)	cccttgatgaagagggatca	caaggcgttagggttgacat
IDE (insulin degrading enzyme)	ataaccaagcaggctgcctt	ttggtgtgagcatgctcgat
IL-1β (interleukin 1 β)	caggcaggcagtatcactca	tgtcctcatcctggaaggtc
IL-4 (interleukin 4)	caaacgtcctcacagcaacg	aagcaccttggaagccctac
IL-6 (interleukin 6)	gttctctgggaaatcgtgga	cagaattgccattgcacaac
IL-10 (interleukin 10)	catgggtcttgggaagagaa	agtaggggaaccctctgagc
IL-13 (interleukin 13)	cggtgccaagatctgtgtct	ggggagtctggtcttgtgtg
iNOS (Inducible nitric oxide synthase)	gacgagacggataggcagag	gtggggttgttgctgaactt
LCAD (long-chain acyl-CoA dehydrogenase)	gagaagtgagtagagaggtctgg	aactgctgttgagagcaagtc
MCP-1 (Monocyte Chemoattractant Protein 1)	aggtccctgtcatgcttctg	cgttaactgcatctggctga
Pck1 (phosphoenolpyruvate carboxykinase 1)	aagtgcctgcactctgtgg	caggcccagttgttgacc
SREBP-1c (sterol regulatory element binding transcription factor 1)	tacttcttgtggcccgtacc	tggactgaagctggtgactg
TNFα (tumor necrosis factor α)	agcccccagtctgtatcctt	gagttggaccctgagccata



Supplementary Figure 1. Pck1 protein expression in mouse tissues. Mice were fasted and euthanized and tissues were collected. Protein expression for Pck1 was done using Western Blot analysis for the liver, kidney, brown adipose tissue (BAT), white adipose tissue (WAT) and skeletal muscle. The blots were normalized with heat shock cognate-70 (HSC70) to account for loading differences. The density of the immunoreactive bands was measured by scanning densitometry (UN-SCAN-IT, Orem, Utah). The values are the means ±SEM for n= 7 mice per group. Abbreviations: C57BL/6J (B6), $Pck1^{fl/fl}$ ($Pck1^{flox/flox}$) and LysM-Pck1 ($Pck1^{MC\text{-}KO}$) mice.



Supplementary Figure 2: BMDMs isolated from $Pck1^{flox/flox}$ and $Pck1^{MC\text{-}KO}$ mice were incubated with [U-^{13}C]glucose in the presence of LPS for 16 hours and GC-MS was performed and contribution of ^{13}C to M2-CAC citrate, succinate and malate were measured**.** Values represent the means ± SEM for n=6 per group. *P < 0.05 and **P<0.01 compared to $Pck1^{flox/flox}$ group.



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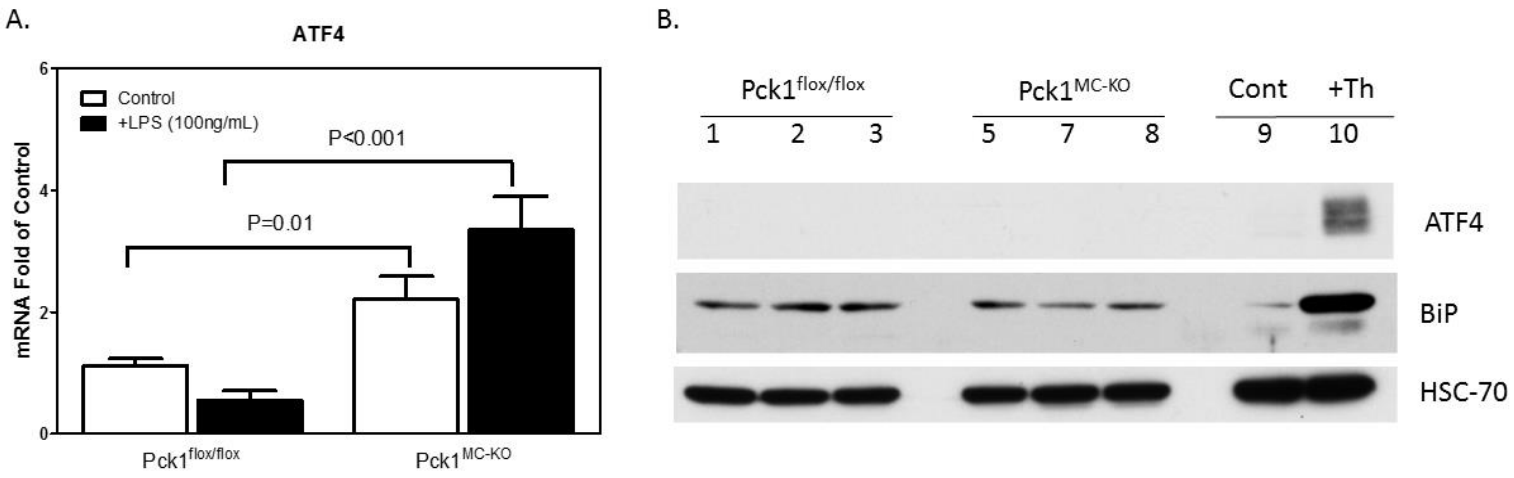
Supplementary Figure 3: BMDMs isolated from $Pck1^{flox/flox}$ and $Pck1^{MC-KO}$ mice were incubated with [U-^{13}C]glutamine in the presence of LPS for 16 hours and GC-MS was performed for contribution of ^{13}C label to A) M2-CAC and B) M3-CAC represented as fold of control and C) M2-CAC and D) M3-CAC represented as fractional MPE. Values represent the means ± SEM for n=6 per group. *P < 0.05 and **P<0.01 compared to $Pck1^{flox/flox}$ group.



Supplemental Figure 4. ATF and BiP expression in Pck1^{MC-KO} cells. BMDMs isolated and differentiated from Pck1$^{flox/flox}$ and Pck1^{MC-KO} mice (n=8). A) The cells were stimulated with LPS (100ng/ml). ATF 4 mRNA expression in macrophage treated with or without LPS were measured by qRT-PCR. B) After treatment with LPS (100ng/ml) whole cell lysates were made and immunoblotted for the indicated proteins. Control unstimulated MEF cells (Cont) and MEF cells treated with 400 nM thapsigargin (+Th). The values are the means ± SEM normalized with 18s rRNA and expressed as fold difference over Pck1$^{flox/flox}$. Abbreviations: ATF-4 (Activating transcription factor 4), BiP (GRP78/BiP) and (HSC-70 (heat shock cognate 70).