

**Paracrine cross-talk between skeletal muscle and macrophages in exercise by PGC-1 α -
controlled BNP**

Regula Furrer^{1,%}, Petra S. Eisele^{1,%,&}, Alexander Schmidt², Markus Beer^{1,#}, and Christoph Handschin^{1,*}

¹Biozentrum, Division of Pharmacology/Neurobiology, University of Basel,
CH-4056 Basel, Switzerland

²Biozentrum, Proteomics Core Facility, University of Basel,
CH-4056 Basel, Switzerland

[%]shared first authorship

[&]Present address: Labor Team W, Goldach, Switzerland

[#]This manuscript is dedicated to the memory of our coworker and friend Markus Beer (deceased on Aug 24, 2015).

^{*}Materials & Correspondence: Christoph Handschin, Biozentrum, University of Basel, Klingelbergstrasse 50/70, CH-4056 Basel, Switzerland, Phone: +41 61 267 2378, Fax +41 61 267 2208, Email: christoph.handschin@unibas.ch

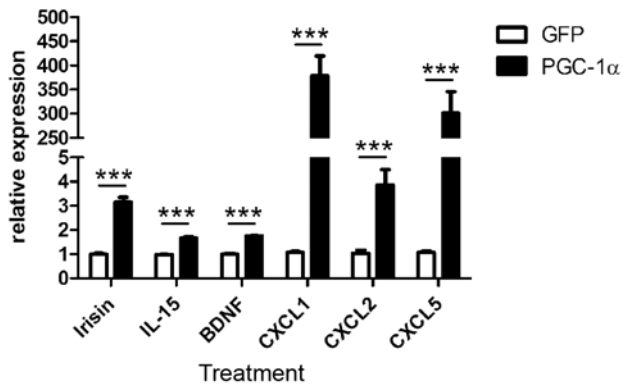
Supplemental Information

Supplemental Figures

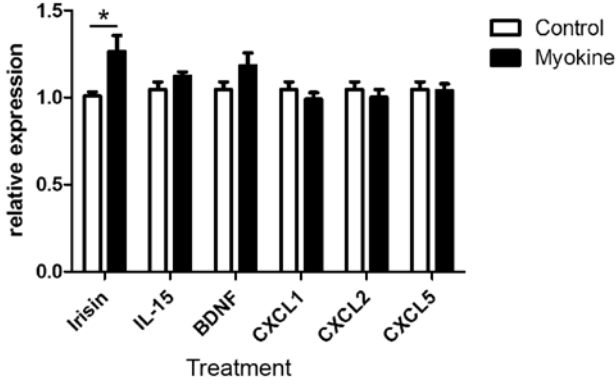
Supplemental Figure S1 related to Figure 2. *Treatment of RAW macrophages with myokines induced by PGC-1 α only mildly increased the expression of pro- and anti-inflammatory cytokines.* **A.** mRNA expression levels of myokines were measured in C2C12 myotubes adenovirally overexpressing PGC-1 α or GFP **B, C.** RAW macrophages were treated for 4h with myokines known to be induced by PGC-1 α . Relative expression of pro- (**B**) and anti-inflammatory (**C**) cytokines was determined by RT-PCR. Values represent the mean of at least 3 independent experiments +SEM. * $P<0.05$; ** $P<0.01$; *** $P<0.001$; myokine versus control.

Supplemental Figure S2 related to Figure 4. *Treatment of RAW macrophages with different BNP concentrations increased the expression of pro- and anti-inflammatory cytokines in a dose-dependent manner.* After treating RAW macrophages with PBS, 1, 2, and 4 nM BNP for 4 h, mRNA expression levels of pro- and anti-inflammatory cytokines were determined by RT-PCR. Values represent the mean of at least 3 independent experiments +SEM. * $P<0.05$; ** $P<0.01$; *** $P<0.001$; BNP treated versus control.

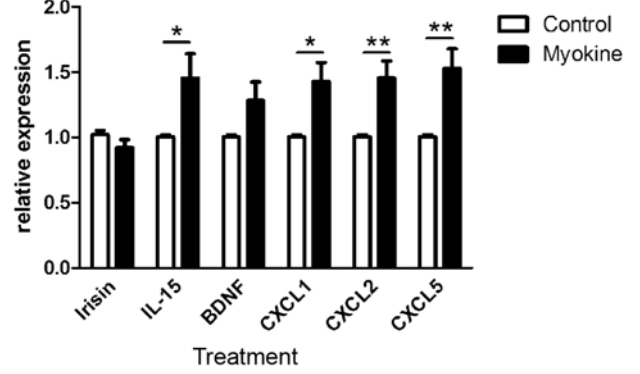
A expression in C2C12 myotubes



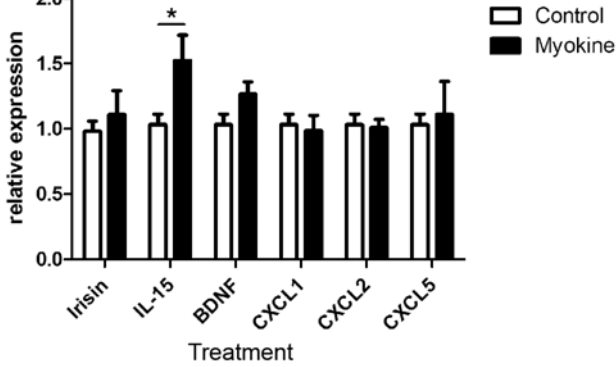
B TNFα expression



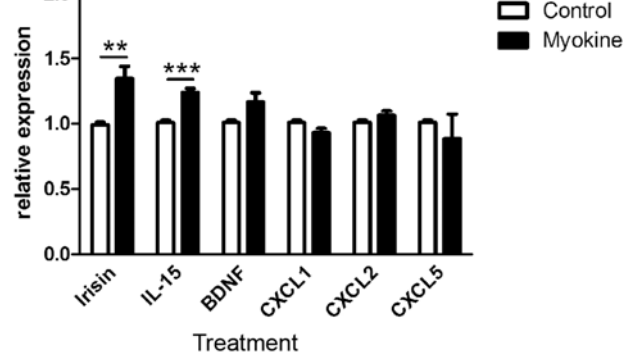
C CCL22 expression



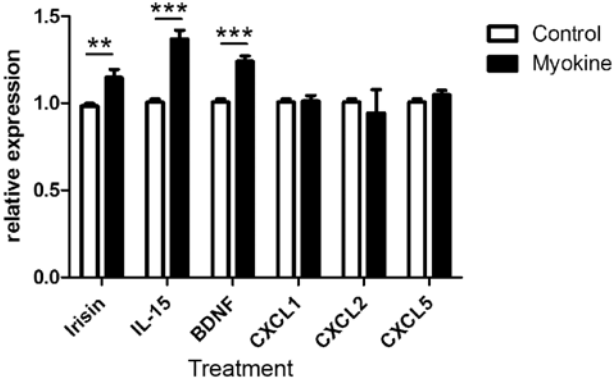
IL-6 expression



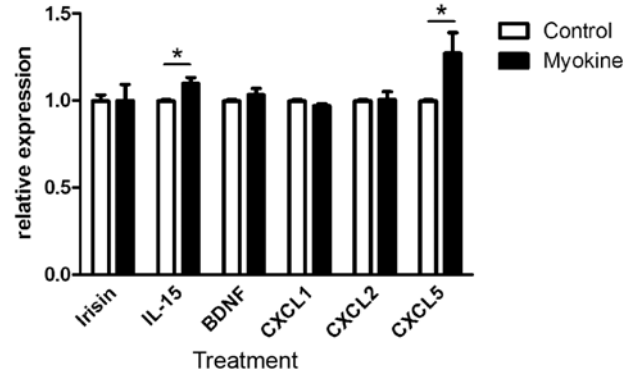
IL-1Ra expression



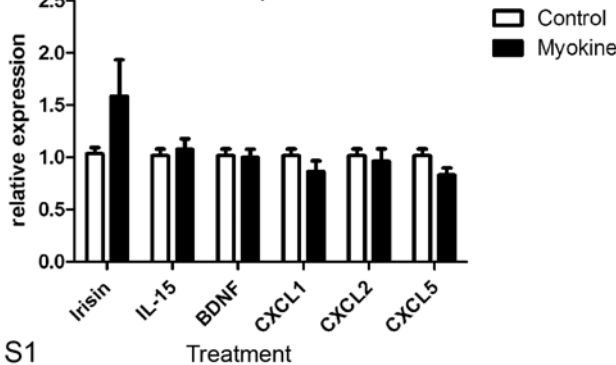
MIP-1α expression



TGFβ expression



MCP-1 expression



Il-10 expression

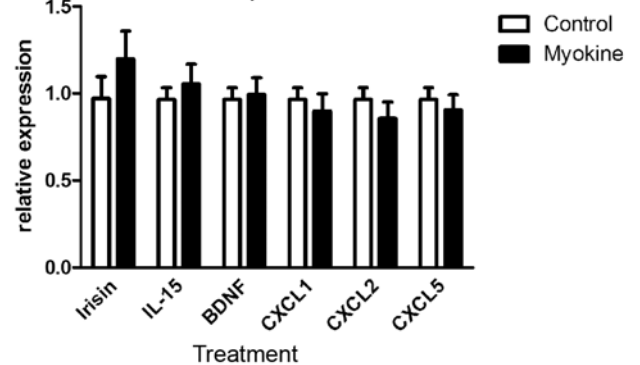


Figure S1

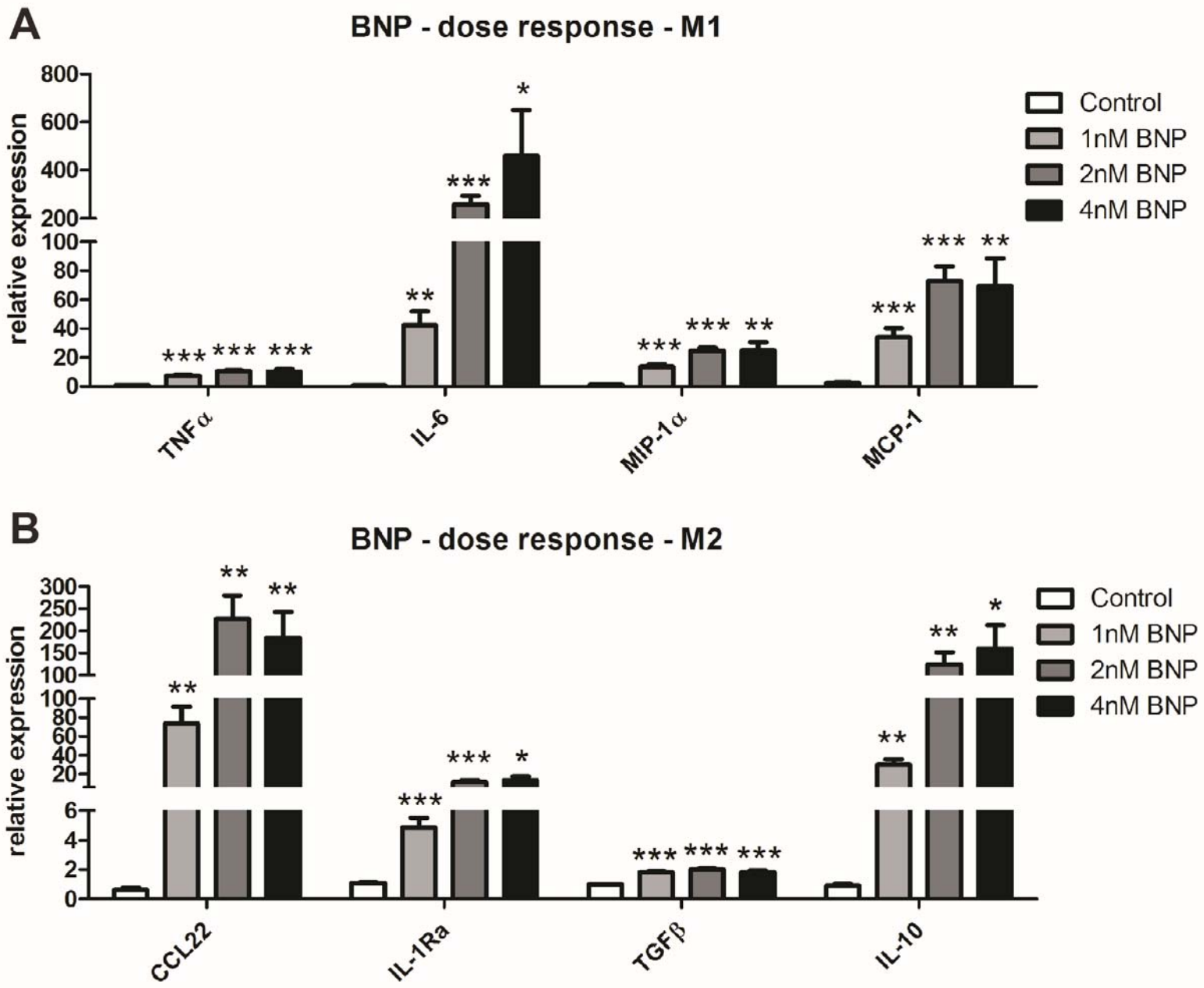


Figure S2

Supplemental Tables

Supplemental Table S1: Primer sequences for RT-qPCR.

Gene	Forward primer	Reverse primer
BDNF	CGGCGCCCATGAAAAGAAGTA	AGACCTCTCGAACCTGCCCT
BNP	GGCCTCACAAAAGAACACCC	TGCCCAAAGCAGCTTGAGAT
CCL7	GCTGCTTTCAGCATCCAAGTG	CCAGGGACACCGACTACTG
CCL22	TGGAGTAGCTTCTTCACCCA	TCTGGACCTCAAATCCTGC
CXCL1	TGAGCTGCGCTGTCAGTGCCT	AGAAGCCAGCGTTCACCAGA
CXCL2	GAGCTTGAGTGTGACGCCCCAGG	GTTAGCCTTGCCTTTGTTTCAGTATC
CXCL5	GCATTTCTGTTGCTGTTTCACGCTG	CCTCCTTCTGGTTTTTTCAGTTTAGC
CXCL10	TCTGAGTGGGACTCAAGGGA	AGGCTCGCAGGGATGATTTTC
IL-1Ra	AAATCTGCTGGGGACCCTAC	TGAGCTGGTTGTTTCTCAGG
IL-6	CCACGGCCTTCCCTACTTC	TTGGGAGTGGTATCCTCTGTGA
IL-10	CTGGACAACATACTGCTAACCG	GGGCATCACTTCTACCAGGTAA
IL-12	GCTTCTCCACAGGAGGTTT	CTAGACAAGGGCATGCTGGT
IL-15	GAGGCCAAGAAGAGTTCTGGAT	TGCCCAGGTAAGAGCTTCAA
Irisin	ATGAAGGAGATGGGGAGGAA	GCGGCAGAAGAGAGCTATAACA
LCN2	TCTGATCCAGTAGCGACAGC	CAGAAGGCAGCTTTACGATG
MCP-1	CCCAATGAGTAGGCTGGAGA	TCTGGACCCATTCTTCTTG
MIP-1α	TCCCAGCCAGGTGTCATTTT	TTGGAGTCAGCGCAGATCTG
Pcolce	GCTCCATATCGAAGACTCGG	GTTACGTGGCAAGTGAGGGT
PGC-1α	TGATGTGAATGACTTGGATACAGACA	GCTCATTGTTGTACTGGTTGGATATG
TBP	GGCCTCTCAGAAGCATCACTA	GCCAAGCCCTGAGCATAA
TGFβ	CAACCCAGGTCCTTCCTAAA	GGAGAGCCCTGGATACCAAC
TNFα	CACAAGATGCTGGGACAGTGA	TCCTTGATGGTGGTGCATGA

Supplemental Table S2: Complete proteomic analysis

See attached Excel file “Proteomics_Supplemental_Table_S1.xlsx”