

## **Irisin – still chasing shadows**

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**Supplementary Table S 1.** Characterization of human serum samples analyzed in this study.

Subject/ Samples	Use	Exercise* (Sampling time after exercise)	Irisin level (ELISA)**	
			Phoenix (ng/mL)	Adipogen (µg/mL)
HS 1	Fig. 3	acute exercise (120 min)	125.9	2.44
HS 2a	Fig. 4	acute exercise (120 min)	168.0	3.91
HS 2b	Tab. 2	combined training (0 min)	102.6	4.18
HS 3	Fig. 4	acute exercise (120 min)	163.6	2.55
HS 4a	Tab. 2	No exercise (baseline)	124.0	2.30
HS 4b	Fig. 5A	combined training (0 min)	114.5	1.96
HS 5a	Tab. 2	No exercise (baseline)	109.2	3.17
HS 5b	Fig. 5A, Tab. 2	combined training (0 min)	130.3	2.97
HS 6a	Fig. 5C	combined training + acute exercise (120 min)	99.9	3.18
HS 6b	Fig. 6	combined training (0 min)	105.3	3.16
HS 7a	Fig. 5C	combined training + acute exercise (120 min)	182.7	2.77
HS 7b	Fig. 6, Tab. 2	combined training + acute exercise (0 min)	236.8	2.96
HS 7c	Fig. S 3, Tab. 2	combined training (0 min)	188.9	2.96

\* Acute exercise: A 45-min bicycle test at 70 % of VO<sub>2</sub>max was performed.

Combined training: Combined strength and endurance training for 12 weeks, including two endurance bicycle sessions (60 min) and two whole body strength training sessions (60 min) per week (Norheim et al. [5]).

\*\* Irisin ELISAs: Phoenix (Cat.-#: EK-067-52; Phoenix Pharmaceuticals, Burlingame, USA; Norheim et al. [5])

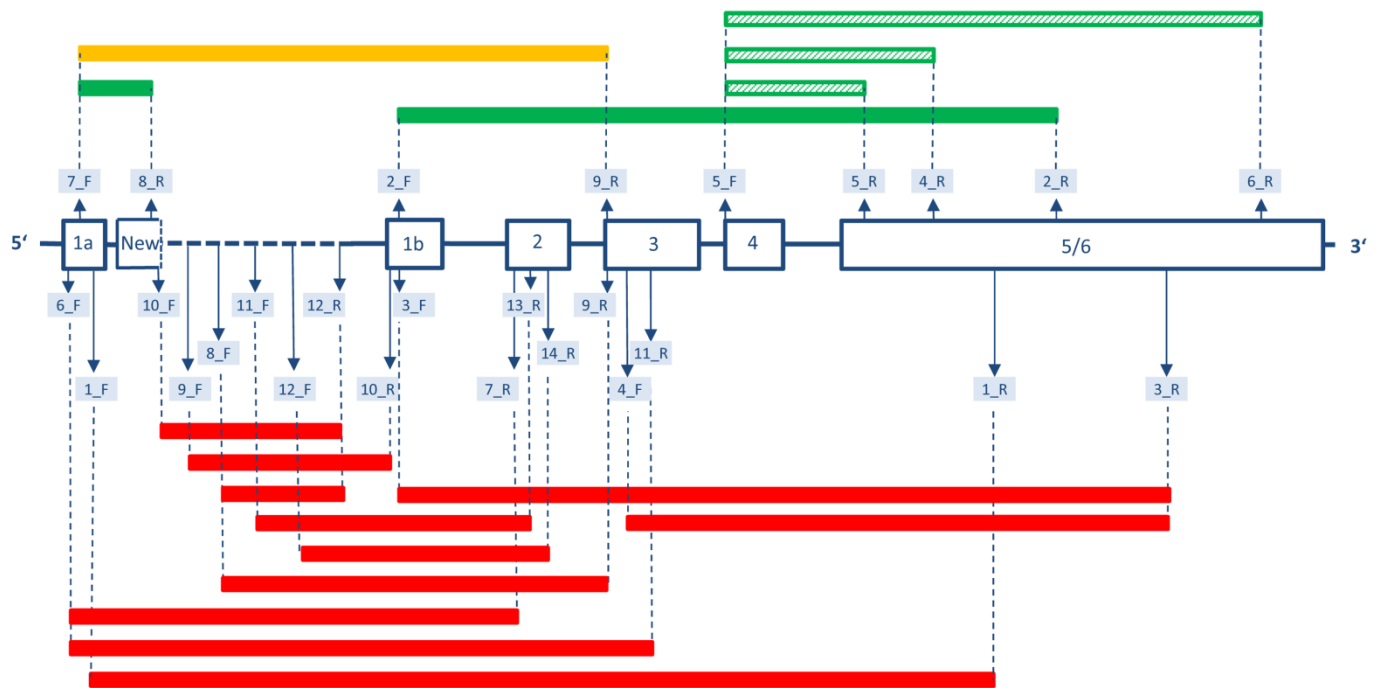
Adipogen (Cat.-#: AG-45A-0046Y; Adipogen Life Sciences, Liestal, Switzerland; Albrecht et al. [6])

**Supplementary Table S 2.** Primers for cDNA-PCR used in this study.

Primer	T <sub>a</sub> [°C]	Amplicon length (bp)	Sequence 5'-3'	GenBank Accession no.
HS_FNDC5_1_F HS_FNDC5_1_R	57	670	GTC GAG GTC CCA GCT GAG CTG TCG CCA TGC CAG TCC	NM_001171941.2
HS_FNDC5_2_F HS_FNDC5_2_R	57	671	GAG CCA CCA TAC ACC CCG TGA GGG CAA GCA CTG AAA AG	NM_153756.2
HS_FNDC5_3_F HS_FNDC5_3_R	57	557	GAG CCA CCA TAC ACC CCG GCA GTC ACG CTT CAA TGA TGT CA	NM_001171940.1
HS_FNDC5_4_F HS_FNDC5_3_R	60	250	TAC ATA GTC CAC GTG CAG GC GCA GTC ACG CTT CAA TGA TGT CA	NM_001171940.1
HS_FNDC5_5_F* HS_FNDC5_4_R*	60	292	CCT CCA AGA ACA AAG ATG AGG TAC CAG AGC ATG AGG CAC AG	NM_001171941.2
HS_FNDC5_5_F* HS_FNDC5_5_R*	60	247	CCT CCA AGA ACA AAG ATG AGG TTT CAT ATC TTG CTG CGG AGA	NM_153756.2
HS_FNDC5_5_F* HS_FNDC5_6_R*	60	160	CCT CCA AGA ACA AAG ATG AGG ACA GGC AGT CAC GCT TCA AT	NM_001171940.1
HS_FNDC5_6_F HS_FNDC5_7_R	57/60	160	AGA AAA GAG AGA GAG AGG TGC T CTC CAG AAC ATC CCA GCT CA	NM_001171941.2
HS_FNDC5_7_F HS_FNDC5_8_R	57/60	218	GGC TCT TCT CCC AAA CGG GGT CTT CCT CTC CAT CCC C	NM_001171941.2
HS_FNDC5_8_F HS_FNDC5_9_R	57/60	177	CCC GGC CAA TTC TAG AGT CT GCA CAT CCT TCT TCT GCT GG	NM_153756.2; NM_001171940.1
HS_FNDC5_9_F HS_FNDC5_10_R	57/60	239	TCC ACC TTG TTT CTC TGG CT GGG GTG TAT GGT GGC TCC	NM_001171941.2
HS_FNDC5_7_F HS_FNDC5_9_R	57	177	GGC TCT TCT CCC AAA CGG GCA CAT CCT TCT TCT GCT GG	NM_001171941.2
HS_FNDC5_6_F HS_FNDC5_11_R	57	306	AGA AAA GAG AGA GAG AGG TGC T GCC TGC ACG TGG ACT ATG TA	NM_001171941.2
HS_FNDC5_8_F HS_FNDC5_12_R	57	206	CCC GGC CAA TTC TAG AGT CT GGC CGC AAA GTC ATA CTC TG	NM_153756.2
HS_FNDC5_10_F HS_FNDC5_12_R	57	395	TTT CGG ATA GTC GTG GGA GG GGC CGC AAA GTC ATA CTC TG	NM_001171941.2
HS_FNDC5_11_F HS_FNDC5_13_R	57	366	GCT TTG GGA GTC AGA TCA GC ATC CTC CAG AAC ATC CCA GC	NM_001171941.2
HS_FNDC5_12_F HS_FNDC5_14_R	57	364	TGT TCA AAT CCT GGC TCT GC GGC AAA TCC GAT GAC AAC CT	NM_001171941.2
MM_Fndc5_1a_F MM_Fndc5_1a_R	53	250	GAG CCG ATA TGC AGG C CTG ACC ACG GCA GAG TTG	XM_006503212.3
MM_Fndc5_1b_F MM_Fndc5_1b_R	57	189	GTG GCT AGG CTG CGT CTG TCC TGA ATG AAC CGG AGC AT	NM_027402.4
MM_Fndc5_2_F MM_Fndc5_2_R	57	245	TGC CAT CTC TCA GCA GAA GA TCC TCC CCA TCT CCT TCA TG	NM_027402.4
MM_Fndc5_3_F MM_Fndc5_3_R	57	304	TGA AGG AGA TGG GGA GGA AC GCA TGT TGG ACA ATC ACC GT	NM_027402.4

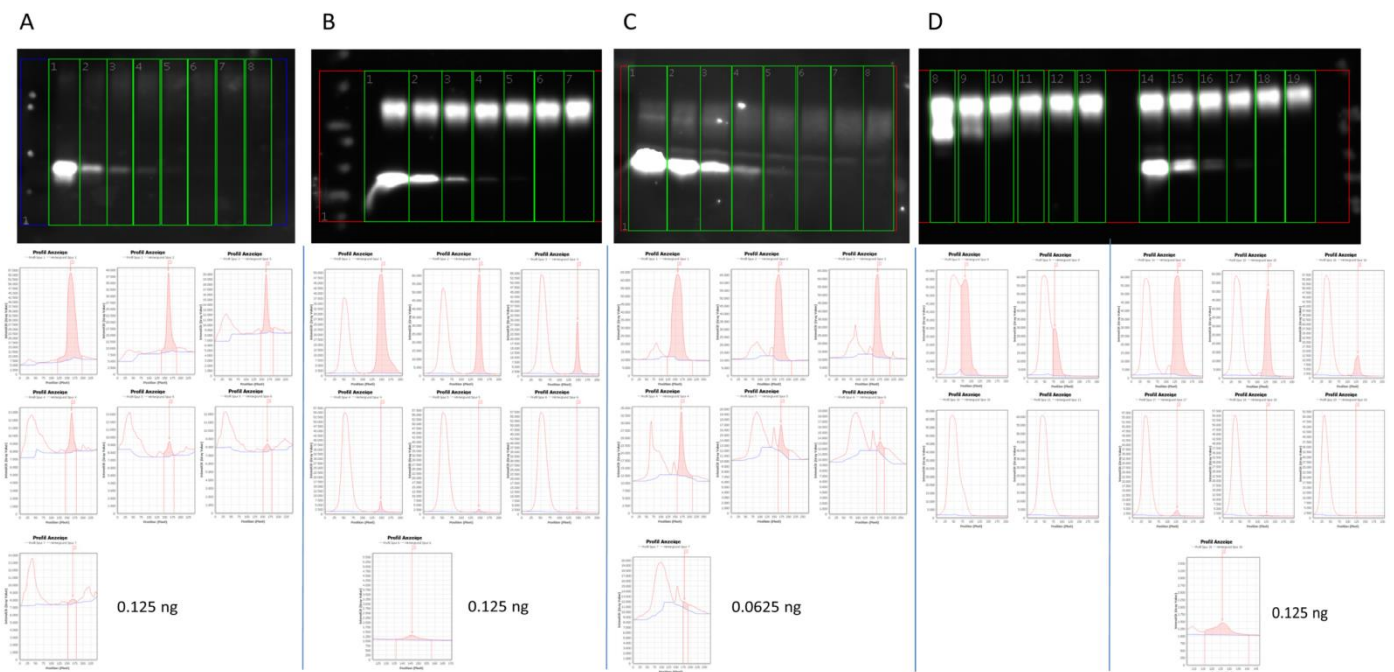
HS: Homo sapiens; MM: Mus musculus

\* Primer pairs from Kim et al. [30]



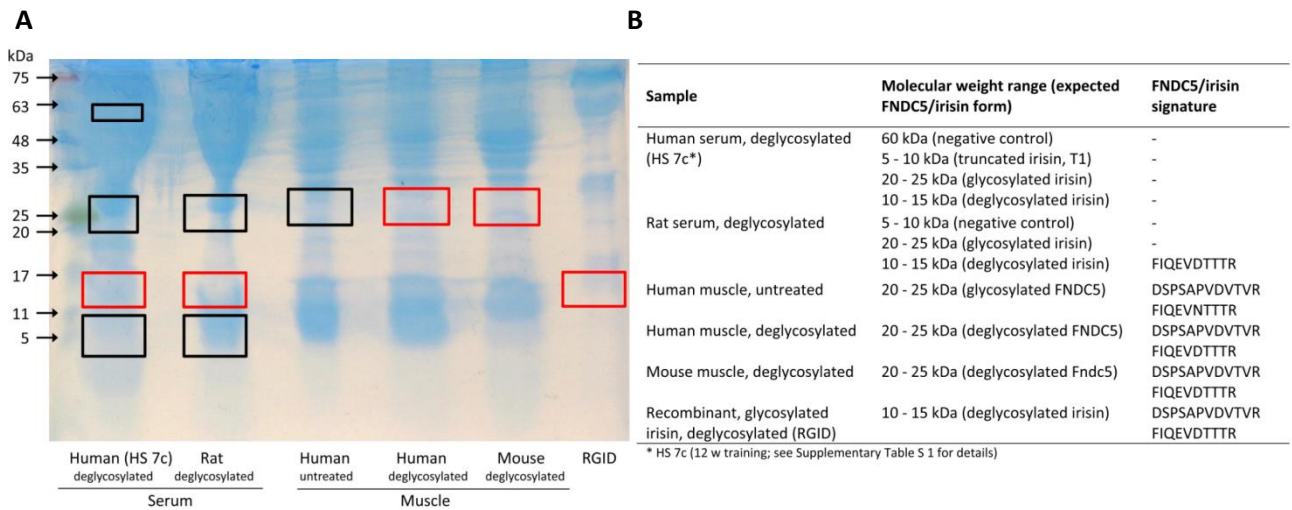
**Supplementary Figure S 1.** Primer combinations for *FNDC5* transcript analysis in human muscle.

The boxes refer to exons in the human *FNDC5* locus (compare Figure 1). The dashed line indicates a region annotated as intron but suspected to be expressed. Tested primer pairs are marked by arrows and the numbering relates to Supplementary Table S 2. Green bars: Successfully amplified and sequenced. Hatched green bars denote amplicons based on the primers published by Kim et al. [29]. Yellow bar: PCR product observed but no sequence derived. Red bars: Amplification failed.



**Supplementary Figure S 2.** Image analysis of dilution series of recombinant irisin species on western blots.

**A** Recombinant irisin diluted in albumin-depleted bovine plasma. Irisin amounts per lane: 20, 4, 2, 1, 0.5, 0.25, 0.125, and 0.062 ng. Detection with antibody A. **B** Irisin amounts per lane: 4, 2, 1, 0.5, 0.25, 0.125, and 0.062 ng. Detection with antibody B. **C** Irisin amounts per lane: 4, 2, 1, 0.5, 0.25, 0.125, 0.062, and 0.031 ng. Detection with antibody C. **D** Glycosylated irisin amounts per lane: 2, 1, 0.5, 0.25, 0.125, and 0.062 ng (left panel). Same irisin amounts after deglycosylation with Protein Deglycosylation Mix II (NEB; right panel).



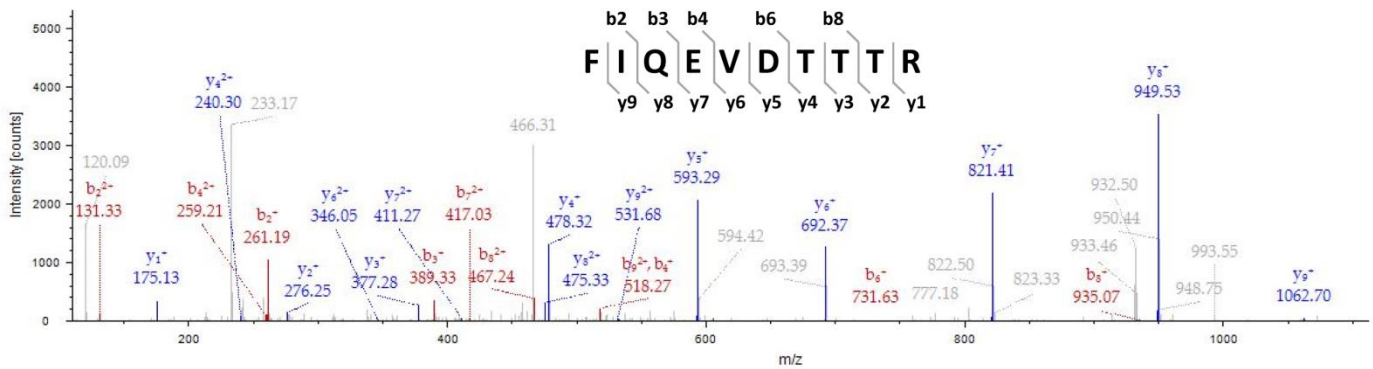
**Supplementary Figure S 3.** Detection of FNDC5/irisin by targeted mass spectrometry (DDA mode).

**A** Coomassie-stained gel with boxed regions of interest cut-out for targeted mass spectrometry in data dependent acquisition (DDA) mode. Red boxes sign areas where FNDC5/irisin was expected.

**B** Results of mass spectrometry with the detected peptides given in the last column.

## Predicted Fragmentation Pattern

#1	b <sup>+</sup>	b <sup>2+</sup>	Seq.	y <sup>+</sup>	y <sup>2+</sup>	#2
1	148.07569	74.54148	F			10
2	261.15975	131.08352	I	1062.54258	531.77493	9
3	389.21833	195.11280	Q	949.45851	475.23290	8
4	518.26092	259.63410	E	821.39994	411.20361	7
5	617.32934	309.16831	V	692.35734	346.68231	6
6	732.35628	366.68178	D	593.28893	297.14810	5
7	833.40396	417.20562	T	478.26199	239.63463	4
8	934.45164	467.72946	T	377.21431	189.11079	3
9	1035.49932	518.25330	T	276.16663	138.58695	2
10			R	175.11895	88.06311	1



**Supplementary Figure S 4.** MS/MS spectrum of the deglycosylated, recombinant irisin peptide with b- and y-ion series m/z values.