

Electronic supplementary material (ESM)

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**Growth hormone replacement therapy regulates microRNA-29a and targets involved in insulin
resistance**

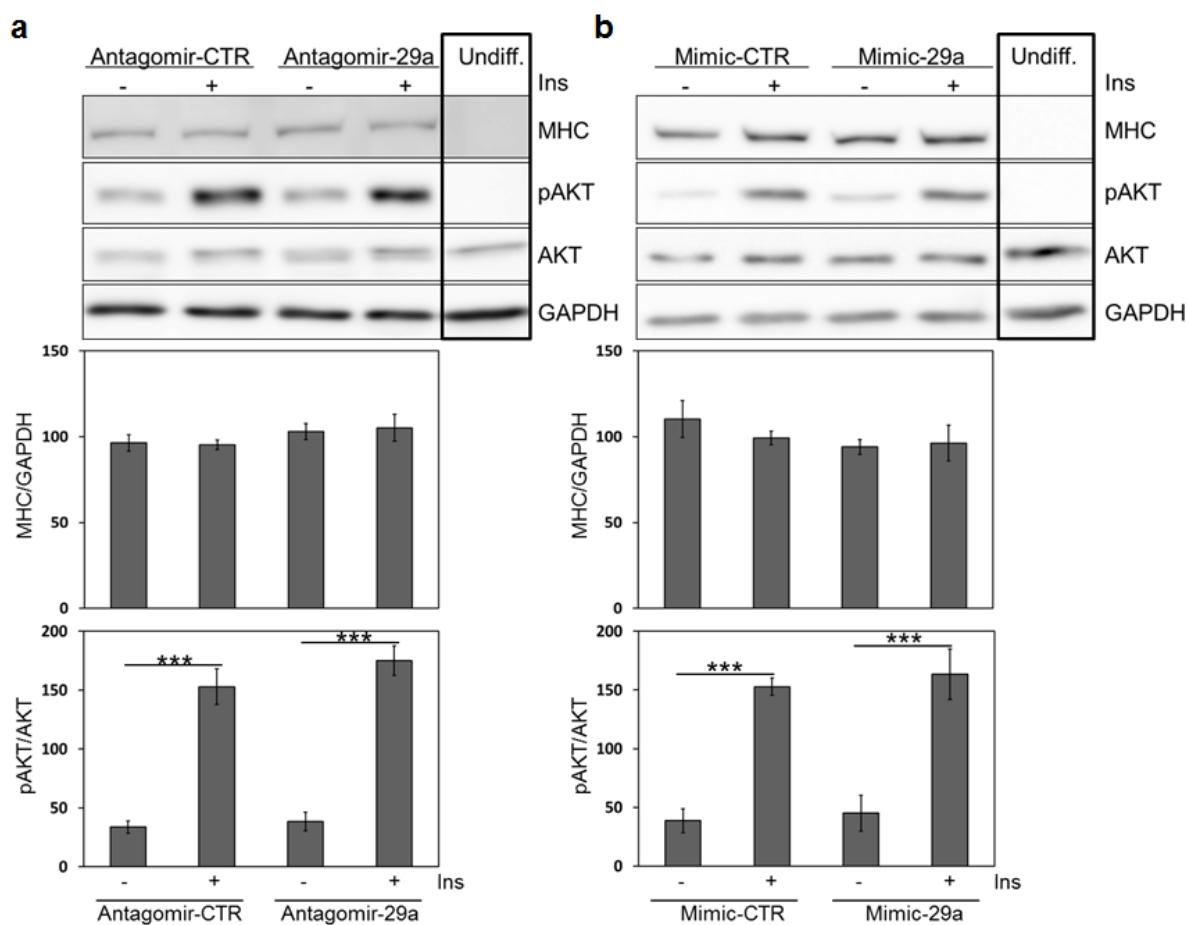
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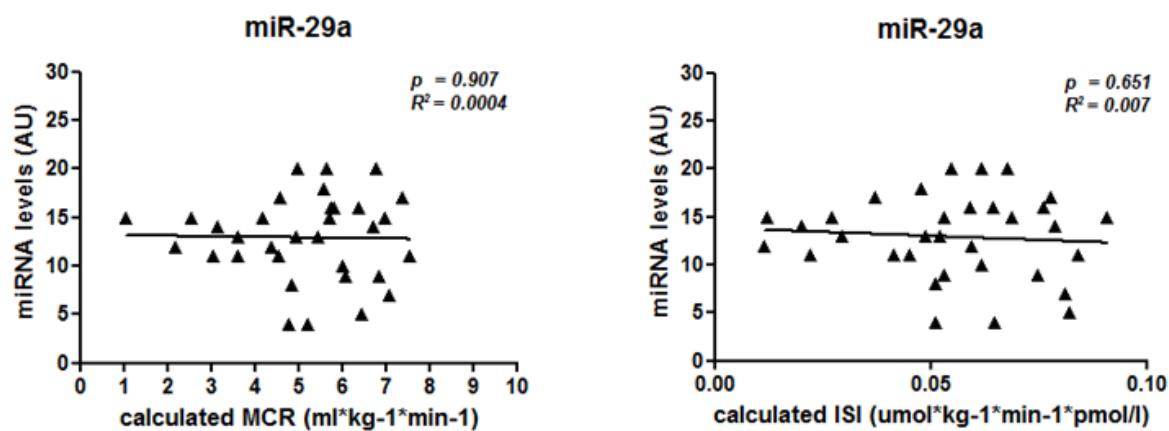
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Supplementary Figure 1. Changes in miRNA-29a expression do not influence the differentiation state or insulin sensitivity of human myotubes. Human myotubes were transfected with miR-29a mimics, miR-29a antagonists or the respective scrambled control oligonucleotides. After 48 h cells were stimulated with or without 100 nM insulin (Ins) for 15 min before harvesting. AKT phosphorylation at serine 473 (pAKT), total AKT, adult myosin heavy chain (MHC) and GAPDH amounts were determined by western blotting. Proliferating undifferentiated myoblasts (Undiff.) were used to demonstrate specificity of MHC staining for the differentiated state. Shown are densitometry results for MHC normalized for GAPDH and for pAKT normalized for total AKT in arbitrary units (AU), n=4-6. Changes were analyzed using one-way ANOVA and Tukey's post test comparing all samples. ***, p<0.001

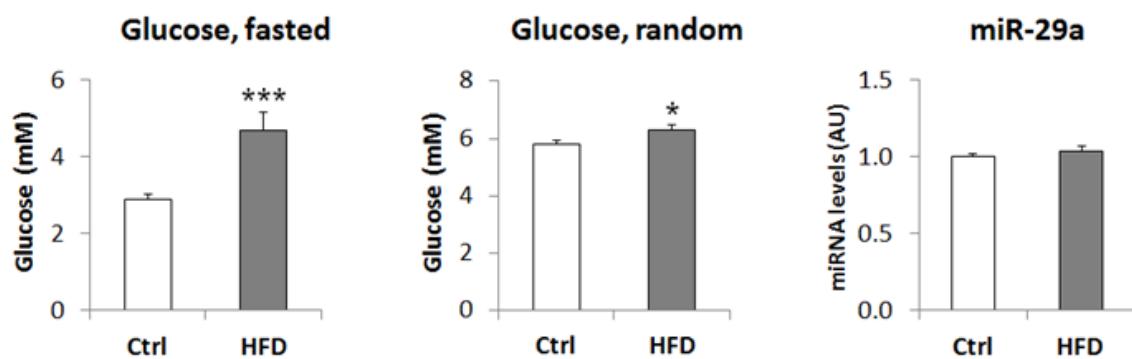


Supplementary Figure 2. MiR-29a levels in skeletal muscle do not correlate with insulin sensitivity in patients with prediabetes. OGTTs were performed in 33 subjects with prediabetes and the metabolic clearance rate (MCR) or the insulin sensitivity index (ISI) calculated as described in Methods. miR-29a levels were measured in muscle biopsies (M.vastus lateralis) and normalized to miR-let-7 and shown as arbitrary units (AU). miRNA levels were plotted against MCR or ISI, respectively.

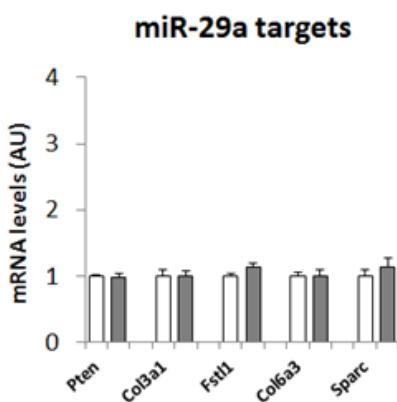


Supplementary Figure 3. Unchanged miR-29a levels and miR-29a target gene expression in skeletal muscle after HFD in mice. (A) C57Bl6 mice were subjected to HFD diet over 20 weeks and glucose values measured in the fasted or non-fasted state. miR-29a levels in tibialis anterior muscle were normalized to miR-let-7 and shown as arbitrary units (AU). (B) qRT-PCR analysis of miR-29a target genes in skeletal muscle after HFD (grey) or normal chow (white). Results for mice after HFD and normal chow (control) are each n=7 (male mice). mRNA was normalized to 18S RNA. Results for HFD versus control were analyzed using student's t test. *: p<0.05, ***: p<0.001.

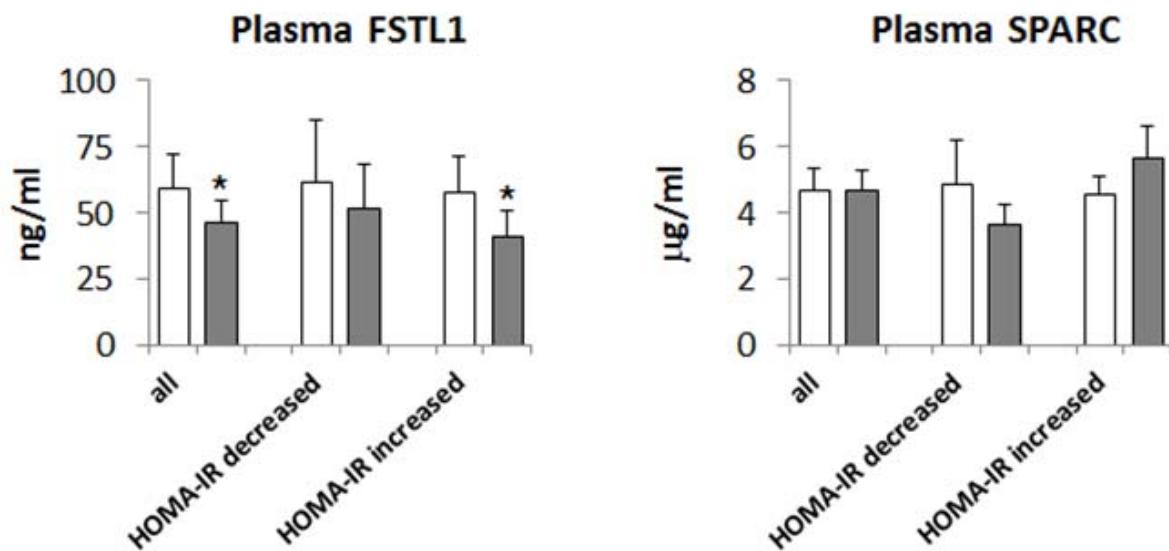
a



b



Supplementary Figure 4. Plasma levels of FSTL1 and SPARC in GHD patients before and after 4 months of GHRT. 10 GHD patients as described in Fig.4 were subjected to GHRT for 4 months. Plasma FSTL1 and SPARC levels were measured in fasted blood samples using ELISA. Values are shown for the whole cohort (all, n=10) or separately analyzed for individuals with improved IS (HOMA-IR decreased), n=5, or worsened IS (HOMA-IR increased), n=5, after GHRT. Baseline values are shown in white, values after GHRT shown in grey.*; p<0.05 (paired student's t test).



Supplementary Table 1. RNA transcript regulation in skeletal muscle from GHD mice after GHRT. GHD mice were treated with PBS or GH over 3 weeks. Total RNA was isolated from the tibialis anterior muscle and analyzed using RNA deep sequencing (n=3, male mice). Shown are all genes that were considered significantly regulated based on a q value < 0.05 as provided by the manufacturer.

Supplementary Table 2. All upregulated predicted miR-29a targets in skeletal muscle from GHD mice after GHRT. Shown are all predicted miR-29a targets that are conserved between mouse and human according to the Targetscan database and that are upregulated in the gene set as shown in Suppl.Table 1.

Supplementary Table 3. ≥2-fold upregulated predicted miR-29a targets in skeletal muscle from GHD mice after GHRT. Shown are all predicted miR-29a targets as shown in Suppl.Table 2 that are upregulated at least 2-fold.

FPRM						
nearest_re	gene	PBS	GH	log2(fold)	p_value	q_value
NM_007729	Col11a1	0.40	2.89	2.86	5.00E-005	8.65E-004
NM_008970	Pthlh	0.83	2.09	1.34	2.00E-004	2.93E-003
NM_008590	Mest	1.05	6.27	2.58	1.50E-004	2.26E-003
NM_010728	Lox	1.20	2.54	1.08	5.00E-005	8.65E-004
NM_008815	Etv4	1.43	5.14	1.84	5.00E-005	8.65E-004
NM_010234	Fos	1.60	4.25	1.41	5.00E-005	8.65E-004
NM_0012041	C1qtnf6	1.87	13.36	2.84	5.00E-005	8.65E-004
NM_026122;	Hmgn3	2.36	5.01	1.08	1.80E-003	1.70E-002
NM_008695	Nid2	7.14	17.51	1.29	5.00E-005	8.65E-004
NM_007737	Col5a2	8.69	39.75	2.19	5.00E-005	8.65E-004
NM_016919	Col5a3	10.01	34.59	1.79	5.00E-005	8.65E-004
NM_015734	Col5a1	12.13	51.63	2.09	5.00E-005	8.65E-004
NM_181395	Pxdn	12.94	26.28	1.02	5.00E-005	8.65E-004
NM_007993	Fbn1	16.34	48.22	1.56	5.00E-005	8.65E-004
NM_008610	Mmp2	23.93	48.10	1.01	5.00E-005	8.65E-004
NM_146007	Col6a2	23.94	89.58	1.90	5.00E-005	8.65E-004
NM_009930	Col3a1	24.12	190.65	2.98	5.00E-005	8.65E-004
NM_008047	Fstl1	30.68	76.50	1.32	5.00E-005	8.65E-004
NM_009928	Col15a1	35.87	111.30	1.63	5.00E-005	8.65E-004
NM_0012430	Col6a3	37.65	135.31	1.85	5.00E-005	8.65E-004
NM_0011110	Serpinh1	69.01	151.46	1.13	5.00E-005	8.65E-004
NM_009932	Col4a2	84.60	170.54	1.01	5.00E-005	8.65E-004
NM_009931	Col4a1	96.66	270.61	1.49	5.00E-005	8.65E-004
NM_007743	Col1a2	116.65	1'324.84	3.51	5.00E-005	8.65E-004
NM_009242	Sparc	184.85	915.32	2.31	5.00E-005	8.65E-004

Supplementary Table 4. Regulation of myokines in skeletal muscle from GHD mice after GHRT.

GHD mice were treated with PBS or GH over 3 weeks. Total RNA was isolated from the tibialis anterior muscle and analyzed using RNA deep sequencing (n=3, male mice) as described in Suppl.Table 1. Shown are the results for the transcripts of known myokines [38,41,42].

	test_id	nearest_regene	locus	FPKM		log2(fold)	p_value	q_value	significance
				GHPBS	GHT				
Il6	XLOC_01525NM_031168	Il6	chr5:30339	0.00	0.05	inf	1.00E+000	1.00E+000	no
Il7	XLOC_01316NM_008371	Il7	chr3:75720	0.11	0.13	0.23	1.00E+000	1.00E+000	no
Il8	Cxcl18, not found in RNA seq data								
Il15	XLOC_02072NM_008357	Il15	chr8:84855	8.39	6.75	-0.31	1.33E-001	3.40E-001	no
Lif	XLOC_00240NM_0010395	Lif	chr11:4157	0.20	0.32	0.67	1.00E+000	1.00E+000	no
Mstn	XLOC_00010NM_010834	Mstn	chr1:53116	22.01	19.56	-0.17	1.72E-001	3.97E-001	no
Bdnf	XLOC_01112NM_0010481	Bdnf	chr2:10951	0.20	0.22	0.15	1.00E+000	1.00E+000	no
Angptl4	XLOC_00892NM_020581	Angptl4	chr17:3391	13.10	5.13	-1.35	5.00E-005	8.65E-004	<u>yes</u>
Fgf21	XLOC_01919NM_020013	Fgf21	chr7:52869	0.00	0.40	inf	1.00E+000	1.00E+000	no
Mcp-1	XLOC_00287NM_011333	Ccl2	chr11:8184	0.77	0.99	0.36	1.00E+000	1.00E+000	no
Myonectin	Ctrp15, not found in RNA seq data								
Pai-1	XLOC_01632NM_008871	Serpine1	chr5:13753	0.83	1.40	0.75	1.00E+000	1.00E+000	no
Pedf	XLOC_00361NM_011340	Serpinf1	chr11:7522	107.77	217.50	1.01	5.00E-005	8.65E-004	<u>yes</u>
Fstl1	XLOC_00761NM_008047	Fstl1	chr16:3777	30.68	76.50	1.32	5.00E-005	8.65E-004	<u>yes</u>
Irsin	XLOC_01416NM_027402	Fndc5	chr4:12881	43.22	42.51	-0.02	8.32E-001	9.27E-001	no
Vegf	XLOC_00905NM_0010252	Vegfa	chr17:4615	64.85	61.45	-0.08	4.96E-001	7.25E-001	no
Meteorin-like	XLOC_00322NM_144797	Metrnl	chr11:1215	3.20	5.63	0.82	1.00E+000	1.00E+000	no
Sparc	XLOC_00342NM_009242	Sparc	chr11:5520	184.85	915.32	2.31	5.00E-005	8.65E-004	<u>yes</u>

Supplementary Table 5. Regulation of Myh transcripts in skeletal muscle from GHD mice after

GHRT. GHD mice were treated with PBS or GH over 3 weeks. Total RNA was isolated from the tibialis anterior muscle and analyzed using RNA deep sequencing (n=3, male mice) as described in Suppl.Table 1. Shown are the results as FPKM for the transcripts of all Myh genes that are represented in the RNAseq data.

test_id	nearest_regene	locus	GHPBS	GHT	log2(fold)	p_value	q_value	significan
XLOC_00265NM_030679	Myh1	chr11:6701	4'390.08	4'651.06	0.08	8.84E-001	9.48E-001	no
XLOC_00265NM_0010395	Myh2	chr11:6698	126.90	61.36	-1.05	5.00E-005	8.65E-004	yes
XLOC_00265NM_0010996	Myh3	chr11:6689	4.46	2.28	-0.97	1.00E+000	1.00E+000	no
XLOC_00265NM_010855	Myh4	chr11:6705	36'724.80	36'202.40	-0.02	9.89E-001	9.94E-001	no
XLOC_00635NM_0011641	Myh6	chr14:5556	1.41	1.14	-0.30	1.00E+000	1.00E+000	no
XLOC_00635NM_080728	Myh7	chr14:5558	2.07	6.26	1.59	5.00E-005	8.65E-004	yes
XLOC_01141NM_0010853	Myh7b	chr2:15543	11.34	7.01	-0.69	2.42E-001	4.85E-001	no
XLOC_00265NM_177369	Myh8	chr11:6709	2.41	3.59	0.58	1.20E-003	1.24E-002	yes
XLOC_00714NM_022410	Myh9	chr15:7759	66.92	82.04	0.29	9.20E-003	5.84E-002	no
XLOC_00265NM_175260	Myh10	chr11:6850	6.30	7.28	0.21	1.12E-001	3.05E-001	no
XLOC_00780NM_0011617	Myh11	chr16:1419	54.80	43.74	-0.33	4.60E-003	3.50E-002	yes
XLOC_00265NM_0010812	Myh13	chr11:6714	13.86	8.57	-0.69	5.00E-005	8.65E-004	yes
XLOC_01915NM_028021	Myh14	chr7:51861	53.67	49.37	-0.12	2.98E-001	5.45E-001	no
XLOC_00765NM_0011662	Myh15	chr16:4905	0.03	0.02	-0.84	1.00E+000	1.00E+000	no

Supplementary Table 6. Primer sequences used.

Mouse Primers

mPten-F	TCGTTAGCAGAAACAAAAGGA
mPten-Re	TCTGCAGGAAATCCCATAGC
mCol3a1-F	GGAACCTGGTTCTTCACC
mCol3a1-Re	TAGGACTGACCAAGGTGGCT
mFstl1-F	GAGCACGATGTGGAAACGA
mFstl1-Re	CGCAGATCTTGGATTGCTT
mCol6a3-F	GGCAGCACACCGAGCATCCAGTTC
mCol6a3-Re	AGTGCATAGTAACCTGAAGTC
mSerpinH1-F	GCCGAGGTGAAGAAAACCCC
mSerpinH1-Re	CATCGCCTGATATAGGCTGAAG
mSparc-F	TTCAGACCGCCAGAACTCTT
mSparc-Re	CCAGGCAAAGGAGAAGAACG
m18S-F	GACACGGACAGGATTGACAGATTG
m18S-Re	AAATCGCTCCACCAACTAAGAACG

Human Primers

HU_PTEN-F	TGAAGGGGTATACAGGAACAAT
HU_PTEN-Re	CGGTGTCATAATGTCCTTCAGC
HU_COL3A1-F	AGGGGAGCTGGCTACTTCTC
HU_COL3A1-Re	AGGAAGTGACCAAGATGGGAA
HU_FSTL1-F	CCAGACACCGATGTGGAAAC
HU_FSTL1-Re	GGCACAGATCTTGGATTGC
HU_COL6A3-F	TAATTGAATCGAGGAGCCA
HU_COL6A3-Re	AAGTGCCGATGTTCTCAT
HU_SERPINH1-F	AGAGTAGAACATCGTGTGCGGG
HU_SERPINH1-Re	CATGCCAGGAAGTGGTTT
HU_SPARC-F	CTTCAGACTGCCGGAGA
HU_SPARC-Re	GAAAGAAGATCCAGGCCCTC
HU_18S-F	GTAACCCGTTGAACCCATT
HU_18S-Re	CCATCCAATCGGTAGTAGCG