

SUPPLEMENTARY DATA

Supplementary Table 1. Body weight and body composition were examined in male wild-type (WT), control (CON: GLUT4 LoxP^{+/-}, GLUT4 LoxP^{+/+} and Cre⁺), muscle-specific GLUT4 heterozygous (mGLUT4 HET) and muscle-specific GLUT4 knockout (mGLUT4 KO) mice at 11-12 weeks old. Plantaris muscle hypertrophy was induced by unilateral synergist ablation of the distal two-thirds of the gastrocnemius and soleus muscles. The contralateral limb was sham-operated and served as the control. After 5 days, plantaris muscles were excised and weighed. Statistical significance was defined as P<0.05 and denoted by ‘a’ vs sham, and ‘b’ vs WT/CON. N=8-17 mice or muscles/ group.

Genotype	WT/CON	mGLUT4 HET	mGLUT4 KO
Pre-Surgery Body Weight – Fed State (g)	28.9 ± 0.7	30.6 ± 0.6	28.1 ± 0.8
Body Composition – Fat Mass (g)	4.6 ± 0.2	5.5 ± 0.5	4.2 ± 0.7
– Lean Mass (g)	21.1 ± 0.6	21.7 ± 0.5	20.8 ± 0.5
– Fat Mass (%)	15.9 ± 0.7	18.0 ± 1.5	14.5 ± 2.0
– Lean Mass (%)	72.7 ± 0.8	71.0 ± 1.4	74.2 ± 1.7
Pre-Tissue Collection Body Weight – Fasted State (g)	25.8 ± 0.7	26.7 ± 0.7	24.4 ± 0.8
Plantaris Muscle Wt – Sham (mg)	17.2 ± 0.4	16.6 ± 0.6	16.4 ± 0.5
– Overload (mg)	23.8 ± 0.8 ^a	22.4 ± 1.0 ^a	22.1 ± 0.5 ^a
Plantaris Muscle Wt : Body Wt – Sham (mg/g)	0.677 ± 0.011	0.621 ± 0.017	0.675 ± 0.023
– Overload (mg/g)	0.940 ± 0.027 ^a	0.840 ± 0.039 ^{ab}	0.909 ± 0.024 ^a
Percent Change in Muscle Wt (%)	38.8 ± 3.6	35.0 ± 3.5	35.2 ± 3.2

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Supplementary Table 2. Characteristics of Mice and Skeletal Muscles from Phloridzin Inhibitor Experiments. Plantaris muscle hypertrophy was induced in female CD-1 mice by unilateral synergist ablation of the distal two-thirds of the gastrocnemius and soleus muscles. The contralateral limb was sham-operated and served as the control. After 5 days, mice were weighed, and plantaris muscles were excised and weighed. Statistical significance was defined as $P < 0.05$ and denoted by 'a' vs sham. $N = 6$ mice or muscles/group.

Treatment	EtOH (0.1%)	Phloridzin (10 $\mu\text{mol/L}$)	Phloridzin (100 $\mu\text{mol/L}$)
Body Wt (g)	23.3 \pm 0.5	22.9 \pm 0.6	23.5 \pm 0.3
Plantaris Muscle Wt - Sham (mg) - Overload (mg)	11.6 \pm 0.5 24.0 \pm 0.8 ^a	10.2 \pm 0.5 22.4 \pm 1.5 ^a	10.2 \pm 0.5 22.1 \pm 1.1 ^a
Muscle Wt :Body Wt - Sham (mg/g) - Overload (mg/g)	0.501 \pm 0.026 1.031 \pm 0.047 ^a	0.434 \pm 0.024 0.969 \pm 0.081 ^a	0.429 \pm 0.022 0.939 \pm 0.054 ^a
Percent Change in Muscle Weight (%)	108.2 \pm 11.0	120.1 \pm 22.8	118.9 \pm 14.0

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Supplementary Table 3. Characteristics of Mice and Skeletal Muscles from Cytochalasin B Inhibitor Experiments. Plantaris muscle hypertrophy was induced in female CD-1 mice by unilateral synergist ablation of the distal two-thirds of the gastrocnemius and soleus muscles. The contralateral limb was sham-operated and served as the control. After 5 days, mice were weighed, and plantaris muscles were excised and weighed. Statistical significance was defined as $P < 0.05$ and denoted by 'a' vs sham. $N = 5$ mice or muscles/group.

Treatment	DMSO (0.2%)	Cytochalasin B (10 μ mol/L)
Body Wt (g)	26.1 \pm 1.2	26.9 \pm 1.0
Plantaris Muscle Wt - Sham (mg) - Overload (mg)	13.8 \pm 0.4 19.8 \pm 1.0 ^a	13.9 \pm 0.3 20.0 \pm 0.9 ^a
Muscle Wt :Body Wt - Sham (mg/g) - Overload (mg/g)	0.532 \pm 0.035 0.766 \pm 0.054 ^a	0.521 \pm 0.024 0.747 \pm 0.020 ^a
Percent Change in Muscle Weight (%)	44.1 \pm 5.2	44.3 \pm 7.0

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Supplementary Table 4A. Characteristics of Mice and Skeletal Muscles from Sugar Competition Experiment 1. Plantaris muscle hypertrophy was induced in female CD-1 mice by unilateral synergist ablation of the distal two-thirds of the gastrocnemius and soleus muscles. The contralateral limb was sham-operated and served as the control. After 5 days, mice were weighed, and plantaris muscles were excised and weighed. Statistical significance was defined as $P < 0.05$ and denoted by 'a' vs sham. $N = 4$ mice or muscles/group.

Treatment	L-glucose (35 mM)	D-Glucose (35 mM)	D-Fructose (35 mM)	D-Galactose (35 mM)
Body Wt (g)	23.9 ± 0.9	24.8 ± 0.5	24.3 ± 0.9	23.8 ± 0.3
Plantaris Muscle Wt - Sham (mg) - Overload (mg)	13.3 ± 0.5 20.9 ± 1.0 ^a	13.3 ± 0.4 20.2 ± 1.8 ^a	12.8 ± 0.7 20.5 ± 1.8 ^a	13.0 ± 0.7 19.2 ± 1.5 ^a
Muscle Wt :Body Wt - Sham (mg/g) - Overload (mg/g)	0.559 ± 0.019 0.880 ± 0.051 ^a	0.535 ± 0.010 0.811 ± 0.069 ^a	0.532 ± 0.045 0.856 ± 0.101 ^a	0.544 ± 0.025 0.808 ± 0.062 ^a
Percent Change in Muscle Weight (%)	56.9 ± 4.2	51.2 ± 10.7	59.4 ± 6.8	50.5 ± 17.3

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Supplementary Table 4B. Characteristics of Mice and Skeletal Muscles from Sugar Competition Experiment 2. Plantaris muscle hypertrophy was induced in female CD-1 mice by unilateral synergist ablation of the distal two-thirds of the gastrocnemius and soleus muscles. The contralateral limb was sham-operated and served as the control. After 5 days, mice were weighed, and plantaris muscles were excised and weighed. Statistical significance was defined as $P < 0.05$ and denoted by 'a' vs sham. $N = 6$ mice or muscles/group.

Treatment	L-glucose (35 mM)	D-Xylose (35 mM)
Body Wt (g)	22.5 ± 0.3	22.3 ± 0.5
Plantaris Muscle Wt - Sham (mg) - Overload (mg)	11.8 ± 0.4 16.3 ± 1.3 ^a	12.1 ± 0.4 18.0 ± 0.8 ^a
Muscle Wt :Body Wt - Sham (mg/g) - Overload (mg/g)	0.523 ± 0.017 0.726 ± 0.060 ^a	0.544 ± 0.018 0.811 ± 0.051 ^a
Percent Change in Muscle Weight (%)	38.5 ± 9.9	50.3 ± 11.6

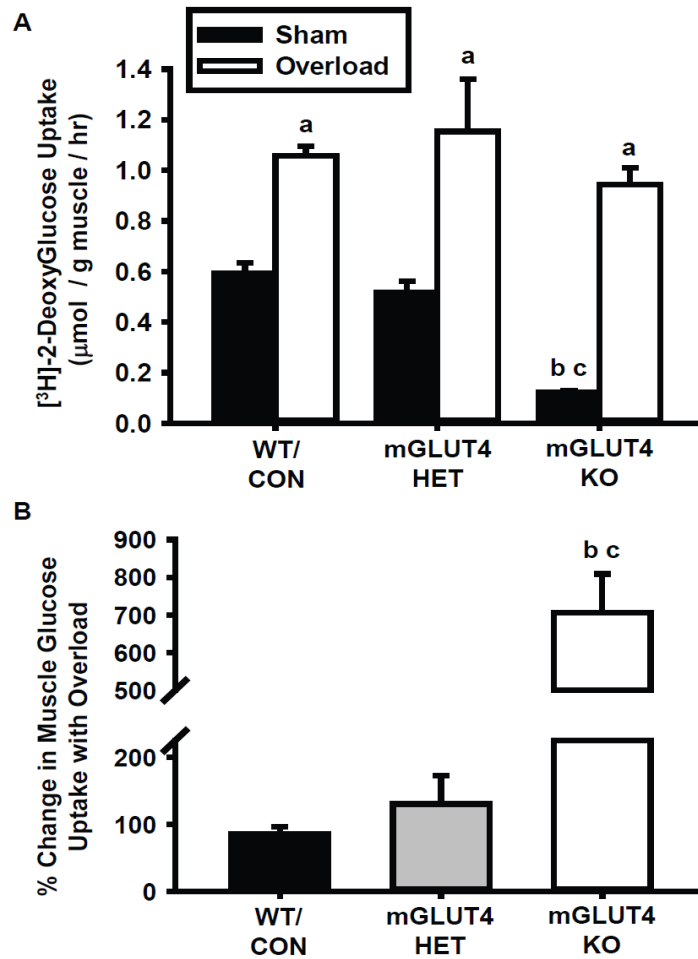
SUPPLEMENTARY DATA

Supplementary Table 5. Characteristics of Mice and Skeletal Muscles from Overload Time Course Experiments. In female wild-type CD-1 mice at 6-8 weeks old, plantaris muscle hypertrophy was induced by unilateral synergist ablation of the distal two-thirds of the gastrocnemius and soleus muscles. The contralateral limb was sham-operated and served as the control. After 1, 3, and 5 days, plantaris muscles were excised and weighed. Statistical significance was defined as $P < 0.05$ and denoted by 'a' vs sham, and 'b' vs Day 1, 'c' vs Day 3. $N = 6-7$ mice or muscles/group.

Treatment	Day 1	Day 3	Day 5
Body Weight (g)	26.6 ± 0.5	28.6 ± 0.8	27.6 ± 0.9
Plantaris Muscle Wt – Sham (mg) Overload (mg)	12.9 ± 0.2 16.4 ± 0.6 ^a	12.6 ± 0.6 18.1 ± 1.0 ^a	13.1 ± 0.7 23.9 ± 1.7 ^{abc}
Muscle Wt :Body Wt - Sham (mg/g) - Overload (mg/g)	0.49 ± 0.01 0.62 ± 0.03 ^a	0.44 ± 0.02 0.63 ± 0.03 ^a	0.47 ± 0.01 0.88 ± 0.08 ^{abc}
Percent Change in Muscle Wt (%)	27.0 ± 4.5	43.3 ± 2.2	86.1 ± 19.3 ^{bc}

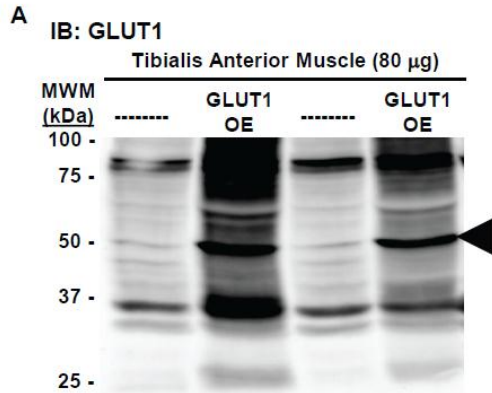
SUPPLEMENTARY DATA

Supplementary Figure 1. Muscle-specific loss of GLUT4 does not impair overload-induced skeletal muscle glucose uptake in male mice. At 11-12 wks old, male wild-type/control (WT/CON), muscle-specific GLUT4 heterozygous (mGLUT4 HET), and muscle-specific GLUT4 knockout (mGLUT4 KO) mice underwent unilateral synergist ablation surgery to induce plantaris muscle hypertrophy. After 5 days, muscles were excised and [³H]-2-deoxyglucose uptake assessed *ex vivo*. (A) Rate of muscle glucose uptake. (B) Percent change in glucose uptake relative to the contralateral, sham-operated control muscle. Statistical significance was defined as P<0.05 and denoted by ‘a’ vs sham-operated controls, ‘b’ vs WT/CON, and ‘c’ vs mGLUT4 HET. N=5-10 muscles/group.



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Supplementary Figure 2. Validation of commercially available GLUT antibodies for detection of specified mouse GLUT isoform. GLUT isoform antibodies were validated using mouse tissues known to express that isoform and/or GLUT isoform overexpression samples as indicated in the figure panels. Immunoblotting conditions and representative blots are provided above.



Immunoblotting Conditions:

Blocking: 5% non-fat milk in 1x TBST; 1 hr at RT

1° antibody: 1:2000 anti-GLUT1, Millipore (cat#AB1344), in 5% BSA in 1x TBST+0.01% NaN₃; overnight at 4°C

2° antibody: 1:2000 anti-rabbit-HRP, ThermoScientific (cat#PI31460), in 5% BSA in 1x TBST; 1 hr at RT

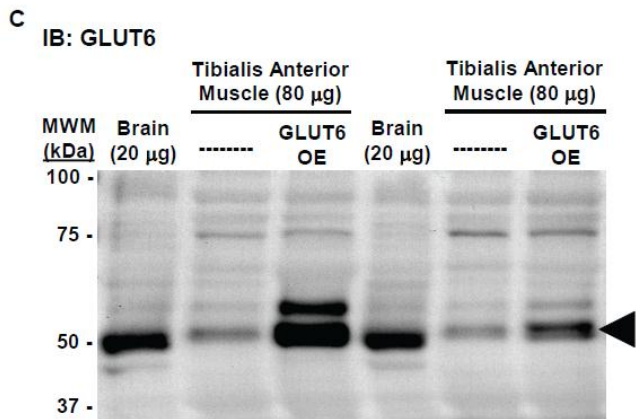


Immunoblotting Conditions:

Blocking: 5% non-fat milk in 1x TBST; 1 hr at RT

1° antibody: 1:2000 anti-GLUT3, Millipore (cat#AB1344), in 5% BSA in 1x TBST+0.01% NaN₃; overnight at 4°C

2° antibody: 1:2000 anti-rabbit-HRP, ThermoScientific (cat#PI31460), in 5% BSA in 1x TBST; 1 hr at RT

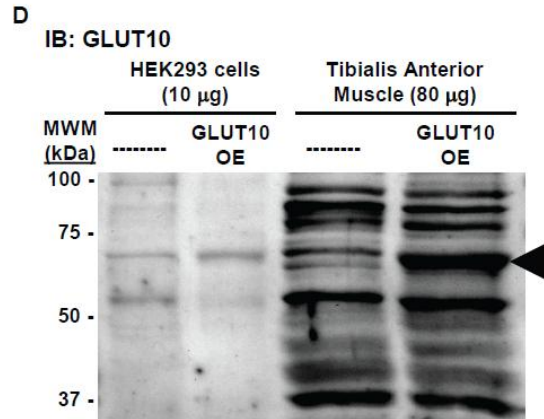


Immunoblotting Conditions:

Blocking: 5% BSA in 1x TBST; 1 hr at RT

1° antibody: 1:500 anti-GLUT6, Abcam (cat#ab118025), in 5% BSA in 1x TBST+0.01% NaN₃; overnight at 4°C

2° antibody: 1:5000 anti-mouse-HRP, Millipore Corp (cat#12-349), in 5% BSA in 1x TBST; 1 hr at RT



Immunoblotting Conditions:

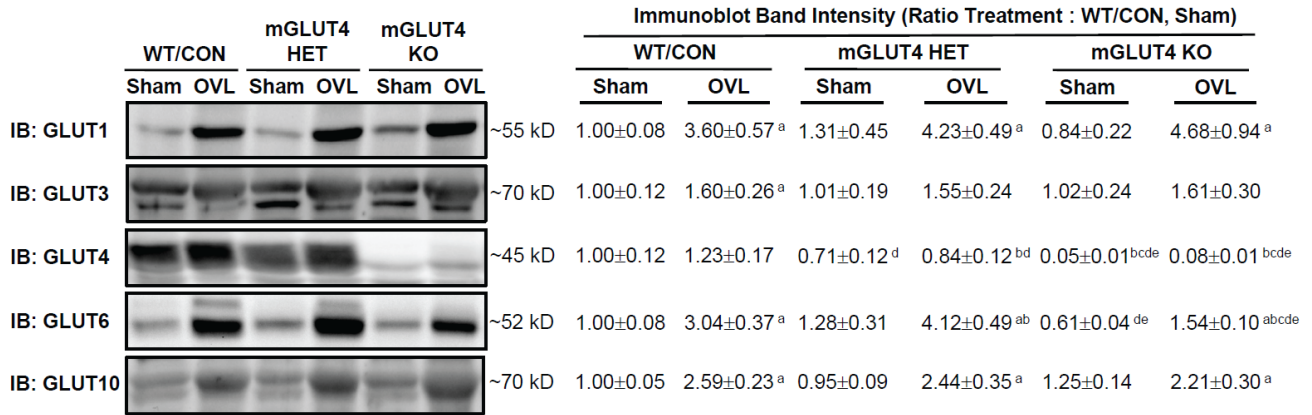
Blocking: 5% non-fat milk in 5% BSA in 1x TBST; 1 hr at RT

1° antibody: 1:500 anti-GLUT10 (L-20), Santa Cruz (cat#sc-21635), in 5% BSA in 1x TBST+0.01% NaN₃; overnight at 4°C

2° antibody: 1:5000 anti-goat-HRP, Promega Corp (cat#V8051), in 5% BSA in 1x TBST; 1 hr at RT

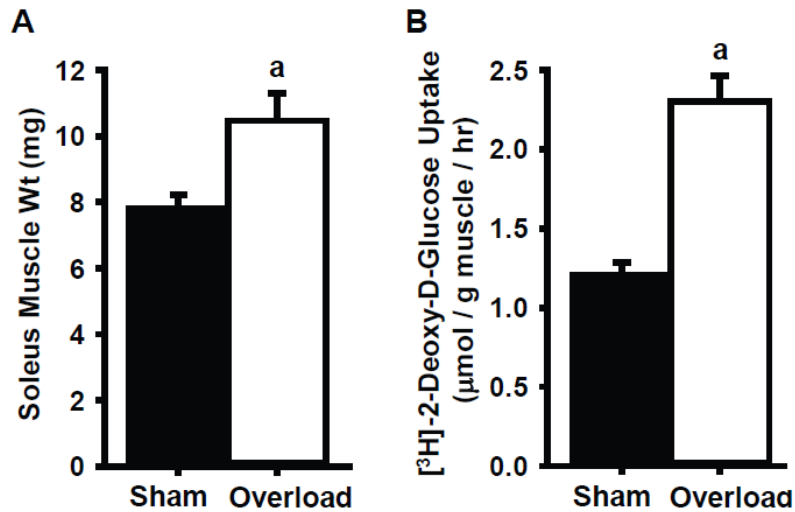
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Supplementary Figure 3. Effects of muscle-specific loss of GLUT4 on overload-induced changes in glucose transporter isoform protein levels in male mice. At 11-12 wks old, male wild-type/control (WT/CON), muscle-specific GLUT4 heterozygous (mGLUT4 HET), and muscle-specific GLUT4 knockout (mGLUT4 KO) mice underwent unilateral synergist ablation surgery to induce plantaris muscle hypertrophy. After 5 days, muscles were excised and processed to assess GLUT transporter isoform protein expression by immunoblot (IB) analysis. Representative blots and quantification provided above. Statistical significance was defined as $P < 0.05$ and denoted by ‘a’ vs sham, ‘b’ vs WT/CON, ‘c’ vs mGLUT4 HET, ‘d’ genotype main effect vs WT/CON, ‘e’ genotype main effect vs mGLUT4 HET. N=4-6 muscles/group.



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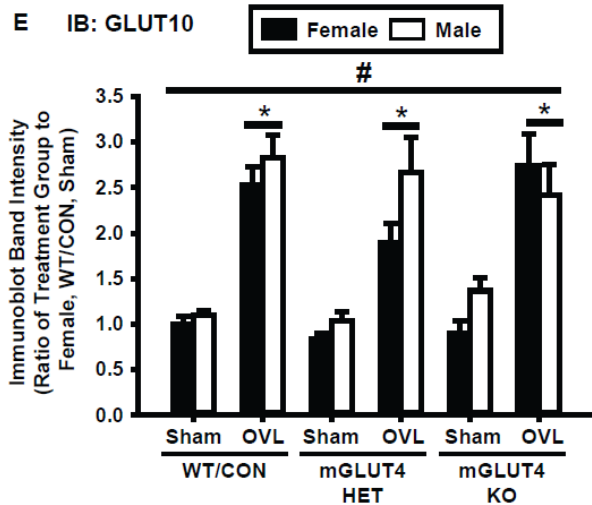
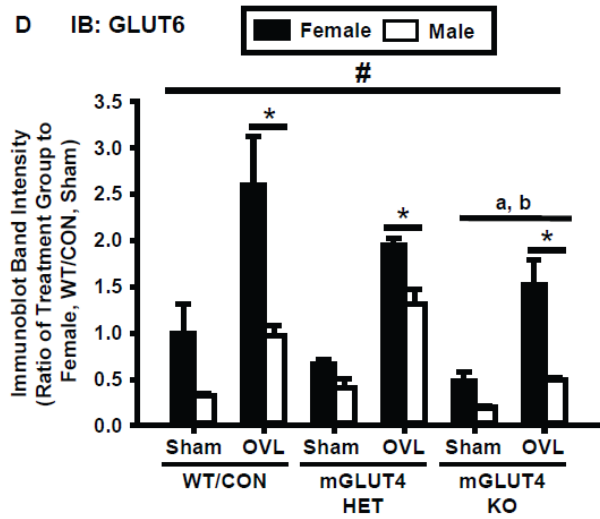
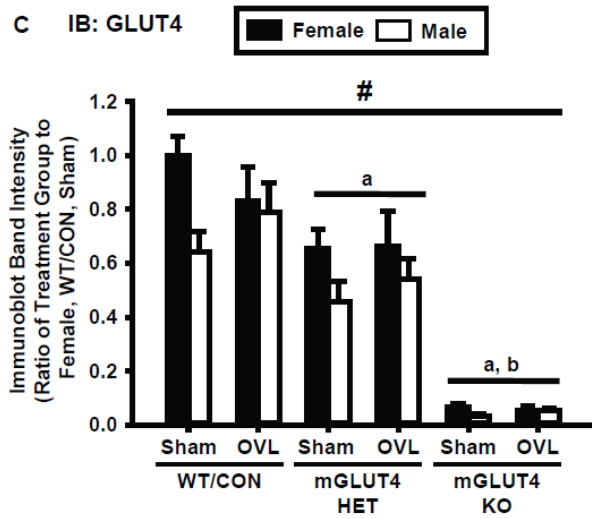
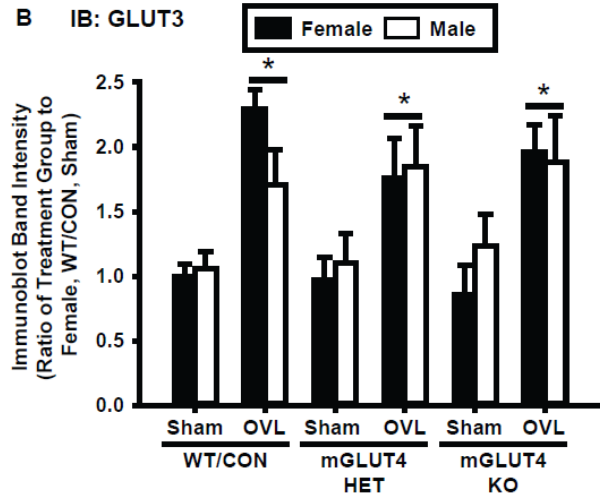
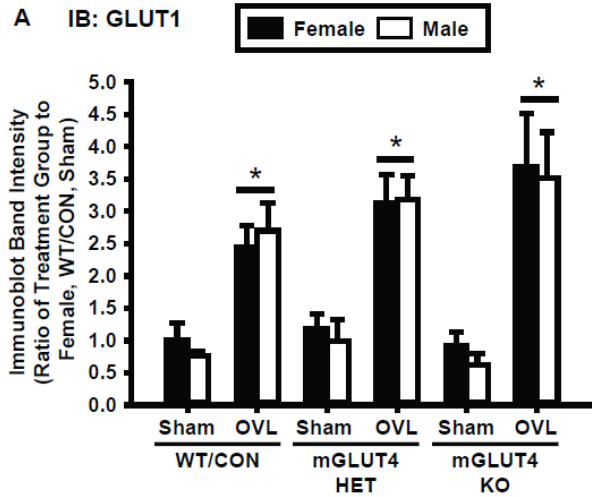
Supplementary Figure 4. Overload increases glucose uptake in mouse soleus muscle. At 8-9 wks old, female wild-type CD-1 mice underwent unilateral synergist ablation surgery of the distal two-thirds of the gastrocnemius muscle to induce soleus muscle hypertrophy. After 5 days, muscles were excised and [³H]-2-deoxy-D-glucose uptake assessed *ex vivo*. Statistical significance was defined as P<0.05 and denoted by 'a' vs sham-operated controls. N=6 muscles/group.



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Supplementary Figure 5. Differential effects of gender on glucose transporter (GLUT) isoform protein levels. At 11-12 weeks old, male and female, wild-type/control (WT/CON), muscle-specific GLUT4 heterozygous (mGLUT4 HET), and muscle-specific GLUT4 knockout (mGLUT4 KO) mice underwent unilateral synergist ablation surgery to induce plantaris muscle hypertrophy. After 5 days, muscles were excised and processed to assess GLUT isoform protein levels by immunoblot (IB) analysis. (A) GLUT1; (B) GLUT3; (C) GLUT4; (D) GLUT6; (E) GLUT10. (F) Statistical significance was determined by Three-Way Analysis of Variance and Student-Newman-Keuls post-hoc analysis, and P-values provided for comparisons. Within figures, statistically significant ($P < 0.05$) main effects was denoted by ‘*’ vs. sham; ‘a’ vs. WT/CON; ‘b’ vs. mGLUT4 HET; and ‘#’ vs. female. N=4-7 muscles/group.

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Three Way Analysis of Variance Student Newman-Keuls Post-Hoc Analysis			
	Gender (Male vs Female)	Genotype (WT/CON vs mGLUT4 KO)	Muscle Treatmt (Sham vs Overload)
GLUT1	P=0.691	P=0.276	P<0.001
GLUT3	P=0.858	P=0.975	P<0.001
GLUT4	P=0.019	P<0.001	P=0.804
GLUT6	P<0.001	P=0.011	P<0.001
GLUT10	P=0.049	P=0.978	P<0.001