

# Western Blot Quantification from Muscle Tsc1 Knockout Quadriceps

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*December 14, 2015*

## Data Entry and Calculations

These data are found in /Users/davebrid/Documents/GitHub/TissueSpecificTscKnockouts/Mouse Data/Muscle Tsc1 Knockout/NCD/Protein Blots and were most recently updated on Mon Mar 4 14:29:14 2019. It reads from the input quantification file named Quantification.csv.

Table 1: Raw Quantification Data

Genotype	Wild-Type	Wild-Type	Wild-Type	Wild-Type	Wild-Type	Knockout	Knockout	Knockout	Knockout
Sample	811	867	1251	1260	1550	869	1264	1265	1265
4F2hc	11400	15600	17700	-3770	10500	18800	24700	18600	33000
Actin	296000	201000	321000	405000	317000	451000	493000	617000	520000
CD36	501000	68100	465000	686000	397000	619000	833000	1160000	1400000
CI-NDUF88	67800	72400	80900	74300	47600	60100	53500	68300	72000
CII-SDHB	174000	186000	269000	168000	118000	166000	157000	127000	170000
CIII-UQCRC2	400000	444000	465000	468000	325000	487000	440000	255000	240000
CIV-MTCO1	6180	77100	17800	2350	15900	45100	30900	25300	25000
CV-ATP5A	218000	223000	344000	283000	224000	469000	301000	198000	210000

Table 2: Summarized Ratios

Sample	811	867	1251	1260	1550	869	1264	1265	1265
Genotype	Wild-Type	Wild-Type	Wild-Type	Wild-Type	Wild-Type	Knockout	Knockout	Knockout	Knockout
pS6K.Ratio	0.0261	0.0228	0.0269	0.0219	0.0315	0.1212	0.1084	0.0801	0.0801
pS6.Ratio	1.40	1.13	11.04	1.12	17.75	21.41	32.75	28.34	28.34
pJNK.Ratio	0.641	0.580	0.588	0.708	0.671	2.039	0.946	0.897	0.897
LC3.Ratio	2.056	2.033	1.302	1.672	1.103	0.158	0.259	0.168	0.168
CD36.Ratio	0.477	0.049	0.388	0.597	0.320	0.534	0.817	0.829	0.829
NDUF88.Ratio	0.0646	0.0521	0.0674	0.0646	0.0384	0.0518	0.0525	0.0488	0.0488
SDHB.Ratio	0.1657	0.1338	0.2242	0.1461	0.0952	0.1431	0.1539	0.0907	0.0907
UQCRC2.Ratio	0.381	0.319	0.388	0.407	0.262	0.420	0.431	0.182	0.182
MTCO1.Ratio	0.00589	0.05547	0.01483	0.00204	0.01282	0.03888	0.03029	0.01807	0.01807
ATP5A.Ratio	0.208	0.160	0.287	0.246	0.181	0.404	0.295	0.141	0.141
LAT1.Ratio	0.00821	0.01453	0.01475	0.01339	0.00903	0.01164	0.01245	0.00921	0.00921
Sarcolipin.Ratio	0.1038	0.1345	0.1333	0.0254	0.1532	0.4284	0.4892	0.4921	0.4921
4F2hc.Ratio	0.01086	0.01122	0.01475	-0.00328	0.00847	0.01621	0.02422	0.01329	0.01329

Table 3: Summarized Normalized Ratios

Sample	811	867	1251	1260	1550	869	1264	1265	1265
Genotype	Wild-Type	Wild-Type	Wild-Type	Wild-Type	Wild-Type	Knockout	Knockout	Knockout	Knockout
pS6K.norm	1.011	0.882	1.041	0.846	1.220	4.689	4.197	3.098	3.098

pS6.norm	0.216	0.175	1.702	0.172	2.735	3.300	5.048	4.369	3
pJNK.norm	1.005	0.910	0.922	1.110	1.053	3.197	1.484	1.407	1
LC3.norm	2.056	2.033	1.302	1.672	1.103	0.158	0.259	0.168	0
CD36.norm	1.303	0.134	1.059	1.630	0.875	1.458	2.231	2.263	2
NDUF88.norm	1.125	0.907	1.174	1.125	0.669	0.902	0.914	0.850	0
SDHB.norm	1.083	0.875	1.465	0.955	0.622	0.935	1.006	0.593	0
UQCRC2.norm	1.084	0.909	1.103	1.158	0.746	1.195	1.228	0.518	0
MTCO1.norm	0.3232	3.0459	0.8145	0.1122	0.7041	2.1350	1.6635	0.9924	0
ATP5A.norm	0.960	0.742	1.325	1.138	0.835	1.869	1.364	0.654	0
LAT1.norm	0.685	1.213	1.231	1.118	0.754	0.971	1.039	0.769	1
4F2hc.norm	1.29	1.34	1.76	-0.39	1.01	1.93	2.88	1.58	2
Sarcolipin.norm	0.943	1.222	1.211	0.231	1.392	3.893	4.445	4.472	3

Table 4: Summarized Values

Genotype	Wild-Type	Knockout
pS6K.norm_mean	1.0	3.8
pS6.norm_mean	1.00	3.79
pJNK.norm_mean	1.00	1.67
LC3.norm_mean	1.63	0.22
CD36.norm_mean	1.00	2.45
NDUF88.norm_mean	1.00	1.08
SDHB.norm_mean	1.000	0.951
UQCRC2.norm_mean	1.000	0.851
MTCO1.norm_mean	1.00	1.15
ATP5A.norm_mean	1.0	1.1
LAT1.norm_mean	1.00	1.02
4F2hc.norm_mean	1.00	2.16
Sarcolipin.norm_mean	1.0	4.1
pS6K.norm_se	0.0663	0.3368
pS6.norm_se	0.524	0.391
pJNK.norm_se	0.0381	0.4093
LC3.norm_se	0.1912	0.0277
CD36.norm_se	0.251	0.383
NDUF88.norm_se	0.0949	0.2126
SDHB.norm_se	0.139	0.155
UQCRC2.norm_se	0.076	0.165
MTCO1.norm_se	0.527	0.353
ATP5A.norm_se	0.105	0.235
LAT1.norm_se	0.1167	0.0809
4F2hc.norm_se	0.367	0.243
Sarcolipin.norm_se	0.205	0.197

### S6K Phosphorylation

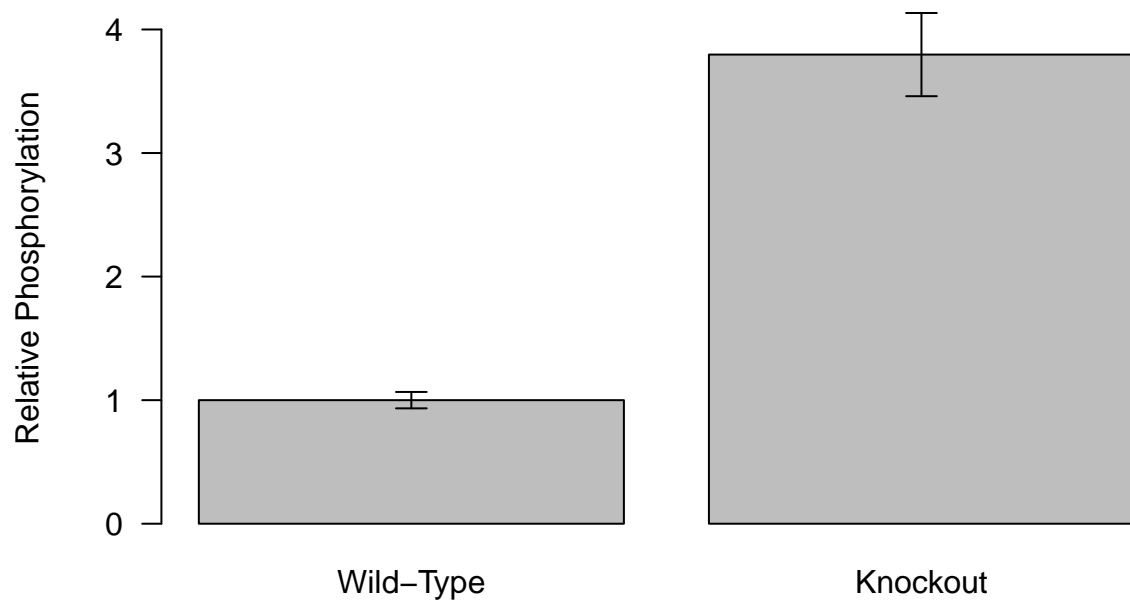


Figure 1: Normalized mTORC1 Activity in Quadriceps

### S6 Phosphorylation

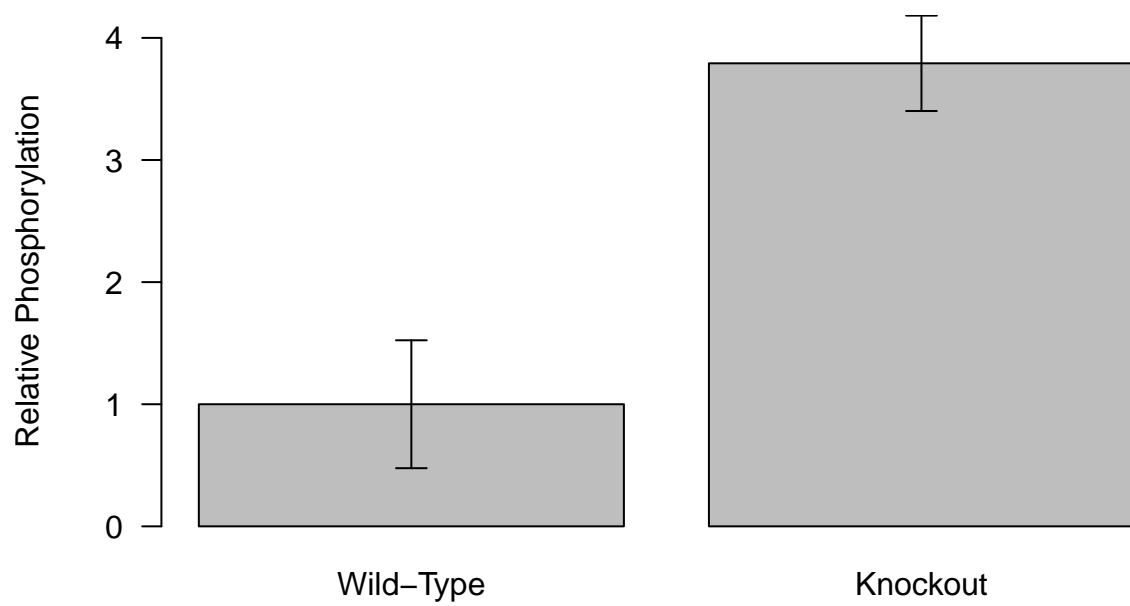


Figure 2: Normalized S6K Activity in Quadriceps

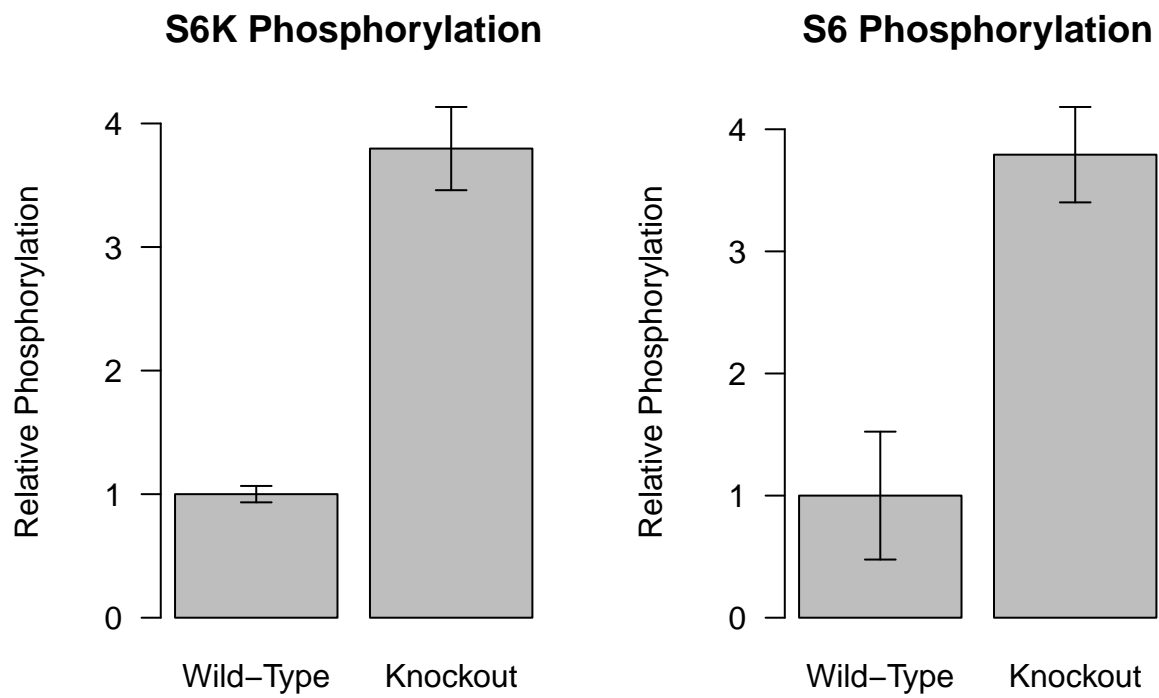


Figure 3: Normalized mTORC1 Activity in Quadriceps

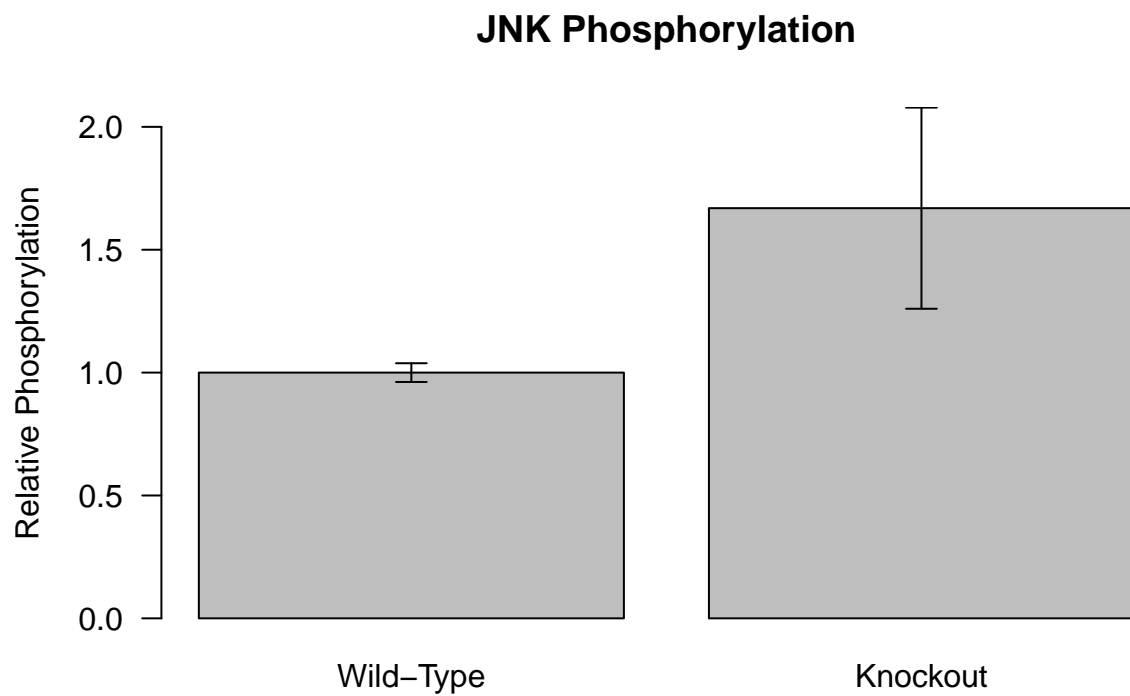


Figure 4: Normalized JNK Phosphorylation in Quadriceps

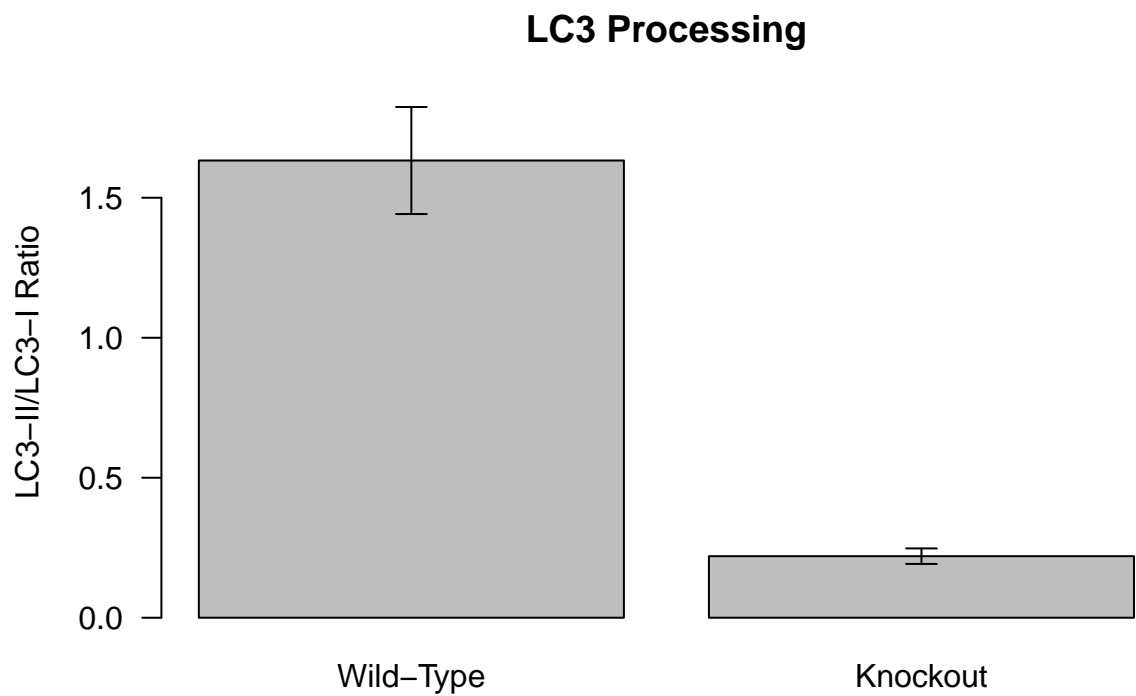


Figure 5: Normalized LC3 Processing in Quadriceps

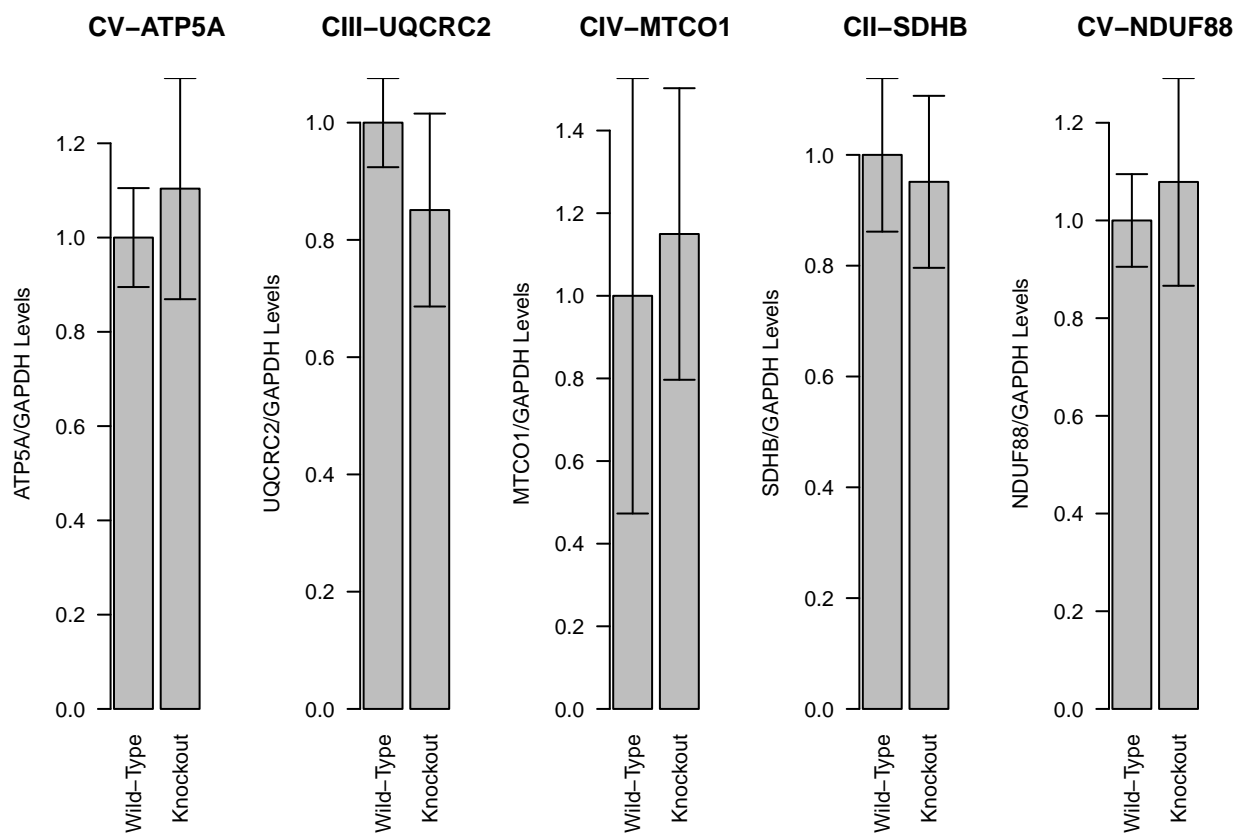


Figure 6: Normalized Mitochondrial Protein Levels in Quadriceps

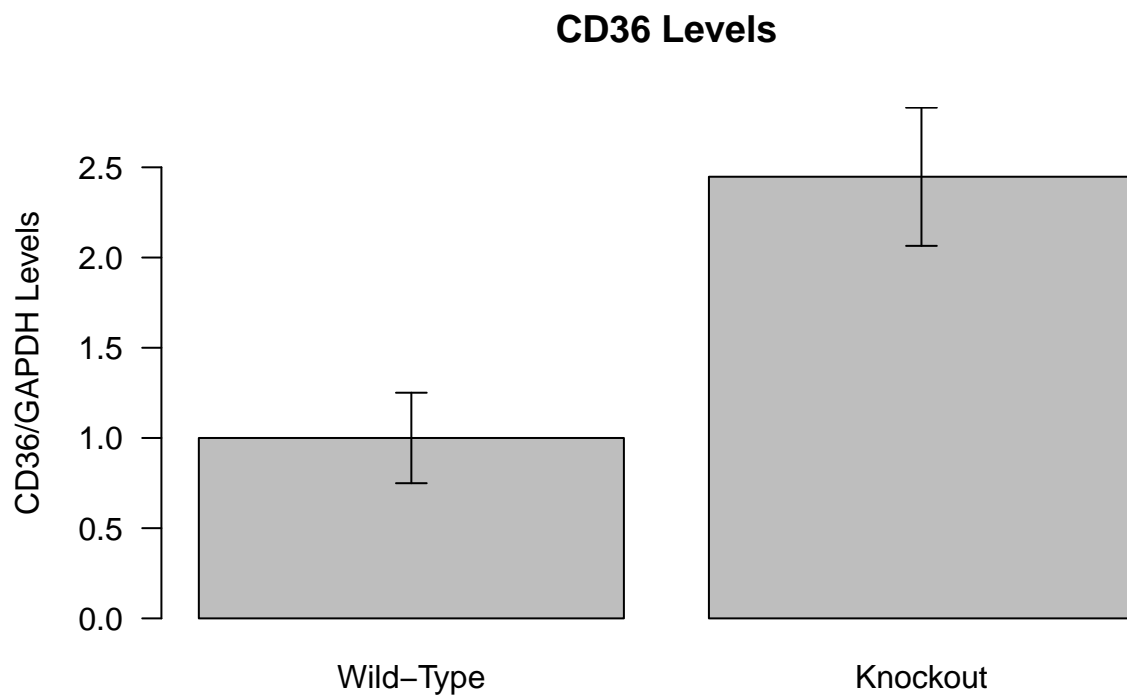


Figure 7: Normalized CD36 Levels in Quadriceps

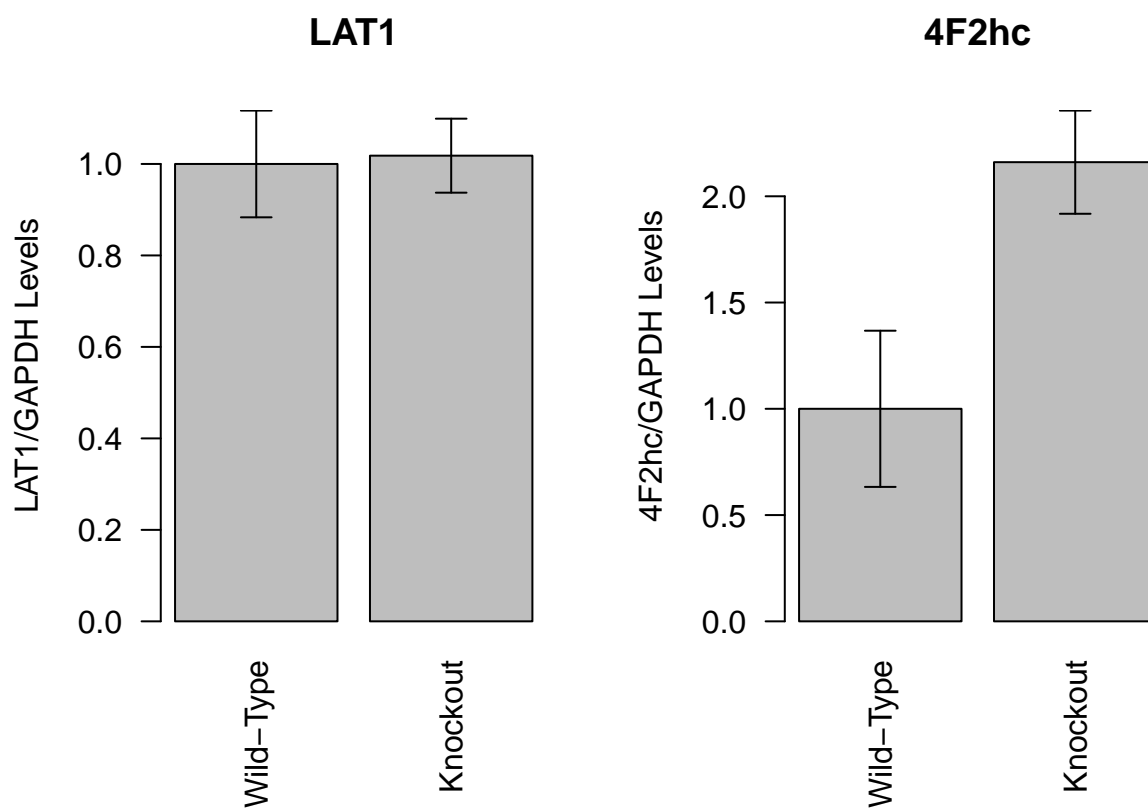
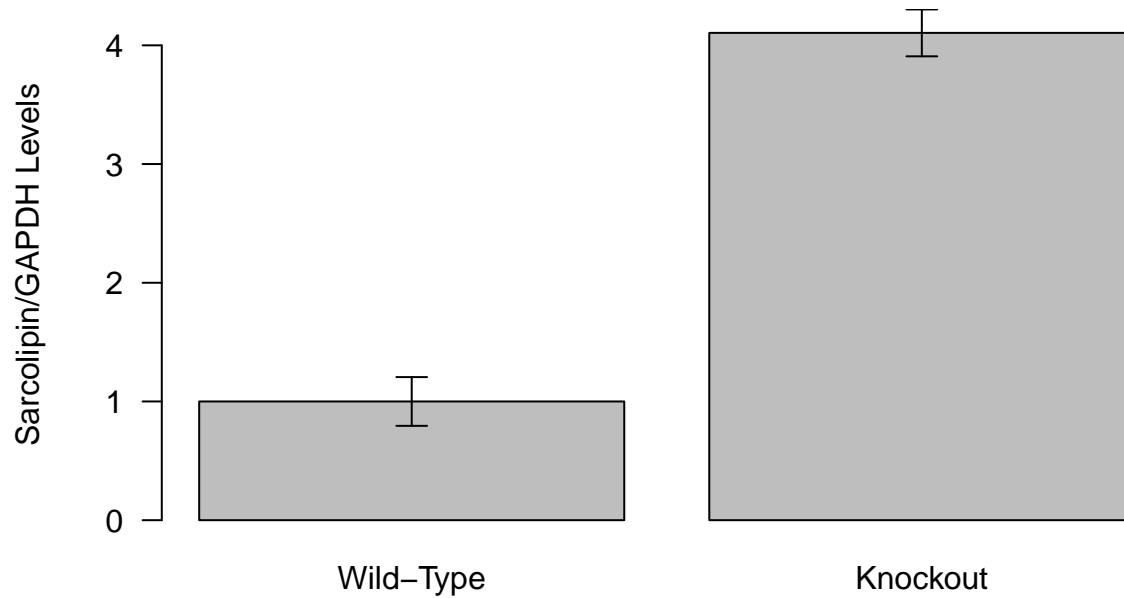


Figure 8: Normalized LAT1 Protein Levels in Quadriceps

## Sarcolipin



## Statistics

Table 5: Statistical Summary

	Shapiro-WT	Shapiro-KO	Wilcoxon	Levene	Welch	Student
pS6K	0.648	0.446	0.008	0.076	0.001	0.000
pS6	0.058	0.133	0.008	0.772	0.003	0.003
pJNK	0.603	0.158	0.151	0.206	0.178	0.142
LC3	0.395	0.605	0.008	0.015	0.002	0.000
ATP5A	0.838	0.493	1.000	0.187	0.701	0.697
UQCRC2	0.361	0.281	0.841	0.118	0.444	0.435
MTCO1	0.045	0.911	0.548	0.815	0.820	0.819
SDHB	0.885	0.596	0.841	0.849	0.821	0.821
NDUF88	0.163	0.002	0.690	0.679	0.747	0.743
Sarcolipin	0.153	0.272	0.008	0.900	0.000	0.000
CD36	0.793	0.412	0.016	0.701	0.016	0.013

Sarcolipin is upregulated 4.105 fold,  $p=0$  from a Student's  $t$ -test.

## Session Information

- R version 3.5.0 (2018-04-23), x86\_64-apple-darwin15.6.0
- Locale: en\_US.UTF-8/en\_US.UTF-8/en\_US.UTF-8/C/en\_US.UTF-8/en\_US.UTF-8
- Running under: macOS 10.14.2
- Matrix products: default

- BLAS: `/Library/Frameworks/R.framework/Versions/3.5/Resources/lib/libRblas.0.dylib`
- LAPACK: `/Library/Frameworks/R.framework/Versions/3.5/Resources/lib/libRlapack.dylib`
- Base packages: `base`, `datasets`, `graphics`, `grDevices`, `methods`, `stats`, `utils`
- Other packages: `bindrcpp` 0.2.2, `car` 3.0-2, `carData` 3.0-2, `dplyr` 0.7.8, `knitr` 1.21, `tidyr` 0.8.2
- Loaded via a namespace (and not attached): `abind` 1.4-5, `assertthat` 0.2.0, `bindr` 0.1.1, `cellranger` 1.1.0, `compiler` 3.5.0, `crayon` 1.3.4, `curl` 3.2, `data.table` 1.11.8, `digest` 0.6.18, `evaluate` 0.12, `forcats` 0.3.0, `foreign` 0.8-71, `glue` 1.3.0, `haven` 2.0.0, `highr` 0.7, `hms` 0.4.2, `htmltools` 0.3.6, `magrittr` 1.5, `openxlsx` 4.1.0, `pillar` 1.3.1, `pkgconfig` 2.0.2, `purrr` 0.2.5, `R6` 2.3.0, `Rcpp` 1.0.0, `readxl` 1.2.0, `rio` 0.5.16, `rlang` 0.3.1, `rmarkdown` 1.11, `stringi` 1.2.4, `stringr` 1.3.1, `tibble` 2.0.0, `tidyselect` 0.2.5, `tools` 3.5.0, `xfun` 0.4, `yaml` 2.2.0, `zip` 1.0.0