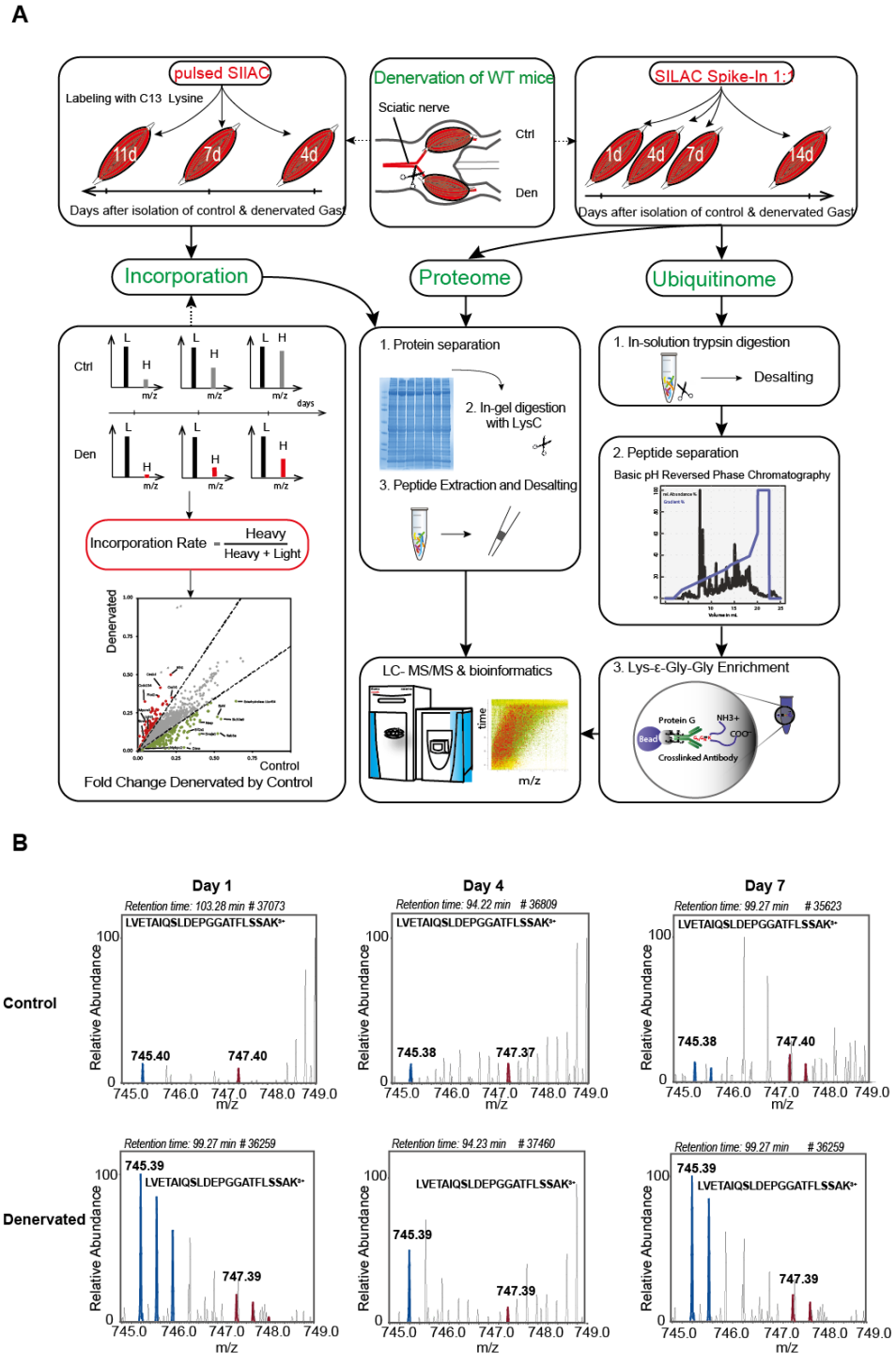
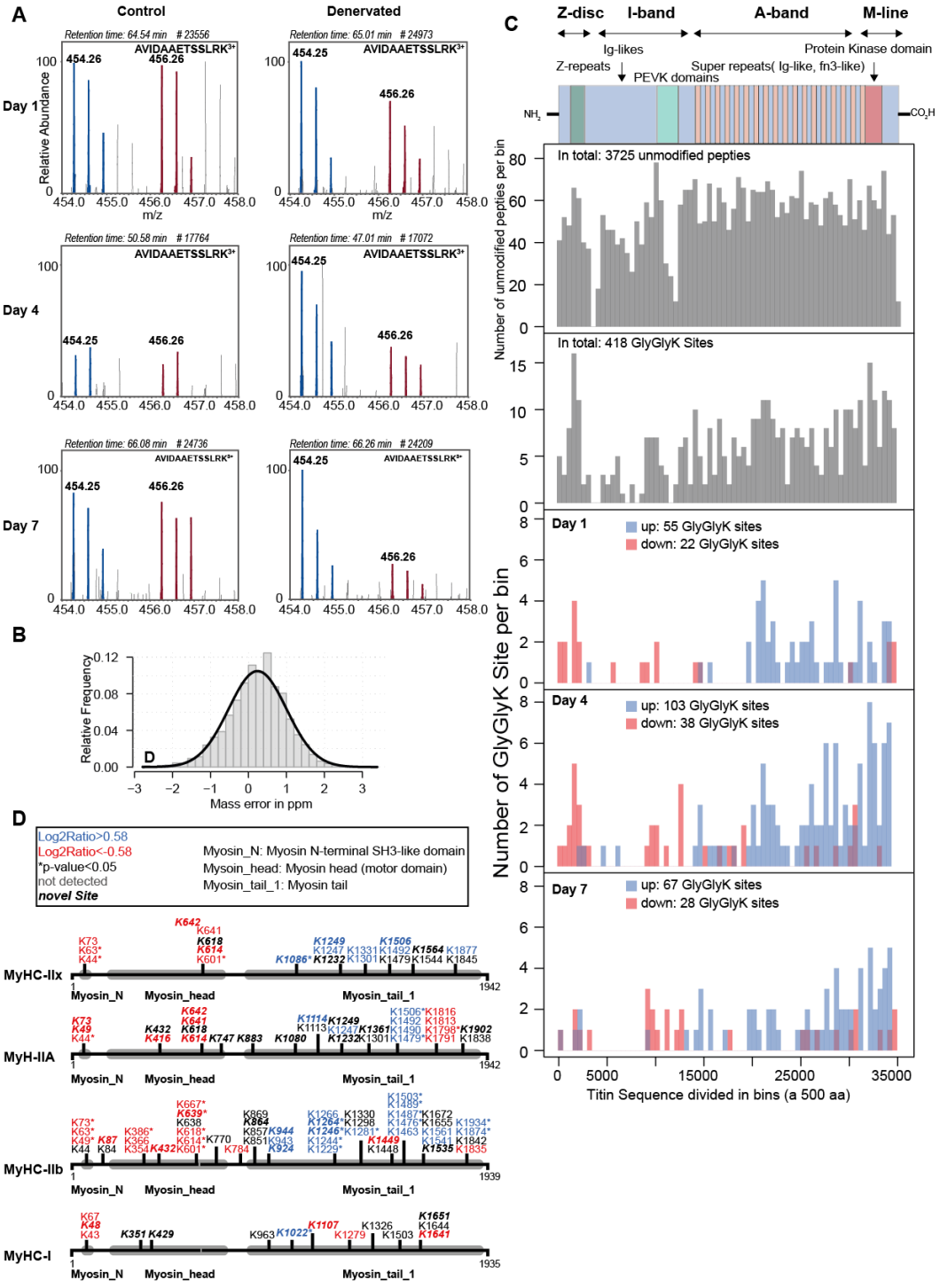


Supplementary Figure 1: Mass spectrometric workflow for the multi-layered proteomics analysis of denervated muscles.

(**A**) Control (right side) and denervated sample (left side) originating from the same animal. Relative protein quantification was achieved by the SILAC spike-in approach using completely

Lys6-labelled muscle tissue from the SILAC mouse (right upper panel). After separation by 1D-SDS-PAGE and Coomassie staining, proteins were digested with the protease LysC. Peptides were extracted, desalted and analysed by LC-MS/MS. Analysis of the RAW data was performed with MaxQuant and Perseus. To measure protein dynamics after denervation, a mouse diet containing Lys6 was administrated for 4, 7, and 11 days after section of the sciatic nerve (left site). The incorporation of Lys6 into newly synthesized proteins is shown by schematic MS spectra (left site, lower panel). The enrichment of diglycine remnants was achieved by immunoaffinity using an anti-K-ε-GG antibody (right panel). After in-solution digestion with the protease trypsin, peptides were separated by high pH reverse phase chromatography. Enriched diglycine containing peptides were measured by LC-MS/MS. (**B**) Selected SILAC pairs for TRIM63/MURF1 peptides under control and denervated conditions.



Supplementary Figure 2: Accurate detection of ubiquitination sites

(**A**) Selected SILAC pairs for TRIM25 peptides under control and denervated conditions. (**B**) The histogram indicates the mass error < 2ppm for more than 97% of the Gly-Gly-K sites. (**C**) Diglycine sites on titin (**D**) Quantified diglycine peptides of MYH1 (MYHC IIx), MYH2 (MYHC IIa), MYH4 (MYHC IIb) and MYH7 (MyHC I).

Supplementary Table 1: Protein list of control and denervated muscles

Sheet 1 Proteome data: Profiling of protein abundance changes using SILAC quantification 1, 4, 7 and 14 days after denervation

Sheet 2 Incorporation data: Lys6 incorporation in control and denervated GAST at 4, 7 and 11 days

Sheet 3 Overlap of protein expression data and Lys6 incorporation

Sheet 4 Diglycine remnant data: Profiling of diglycine sites using SILAC quantification 1, 4 and 7 days after denervation

Sheet 5 Overlap normalized diglycine sites to protein levels and Lys6 Incorporation rates

Click here to Download Table S1

Supplementary Table 2: Log2 Protein expression fold changes of fast and slow marker proteins after denervation and area under the curve (AUC) of Lys6 incorporation profiles in control muscle. *Sheet 1* Slow (soleus) marker proteins; *Sheet 2* Fast (EDK) marker proteins

Click here to Download Table S2

Supplementary Table 3: Overlap of protein expression data with different mRNA sets from https://agoldberg.med.harvard.edu/muscledatabase

Sheet 1 Overlap with dataset from Sacheck et al. and Lecker et al.
Sheet 2 Overlap with mRNA data 3 days after denervation

Click here to Download Table S3

Supplementary Table 4: Random forest for identification of atrogene-like proteins

Sheet 1 Training Set: Proteins known to be regulated following denervation

Sheet 3 Random forest analysis

Click here to Download Table S4

Supplementary Table 5: Overlap of diglycine remnants with published sites from the phosphosite.org database

Click here to Download Table S5