Figure S1

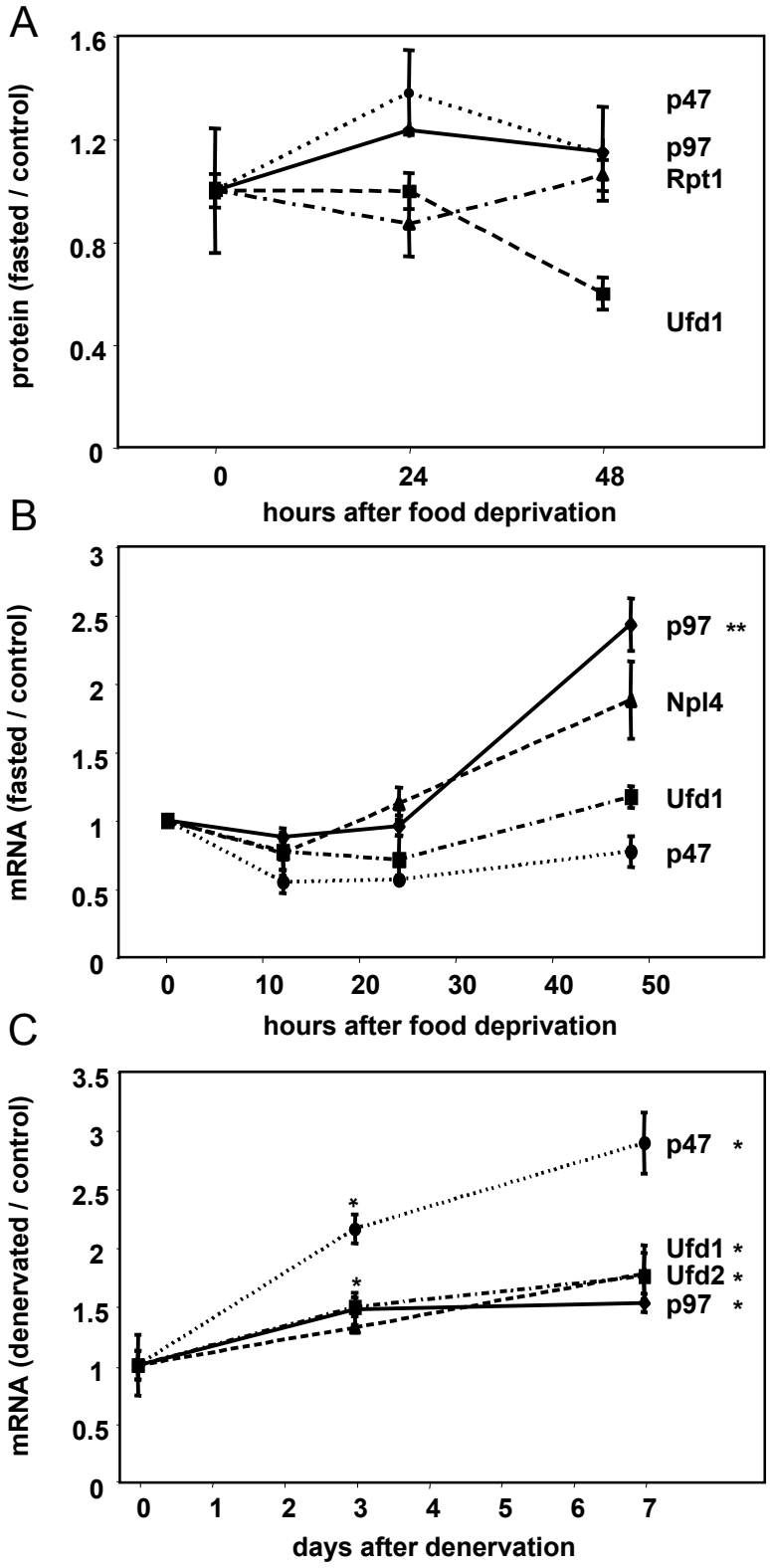


**Fig. S1. The mRNA of p97 and its cofactors are increased 10 d after denervation, while only the mRNA of p97 increases during fasting.**

**A.** Levels of p97 and its cofactors Ufd1 and p47 and the proteasomal subunit Rpt1 were measured by immunoblotting on extracts from mouse Gastrocnemius at different times after food deprivation. Equal amounts of protein were loaded per lane. Densitometry data of protein levels are plotted. Error bars indicate SEM. n = 5.

**B-C.** Expression levels of p97 and its cofactors Ufd1/Npl4, p47 and Ufd2 were measured by Real-Time PCR on extracts from mouse Gastrocnemius at different times after food deprivation (B) or after cutting the sciatic nerve (C). Equal amounts of mRNA were used. Individual samples were run in triplicates and expressed relative to control samples. Error bars indicate SEM. * $p < 0.05$, ** $p < 0.005$. n = 3.

Figure S2

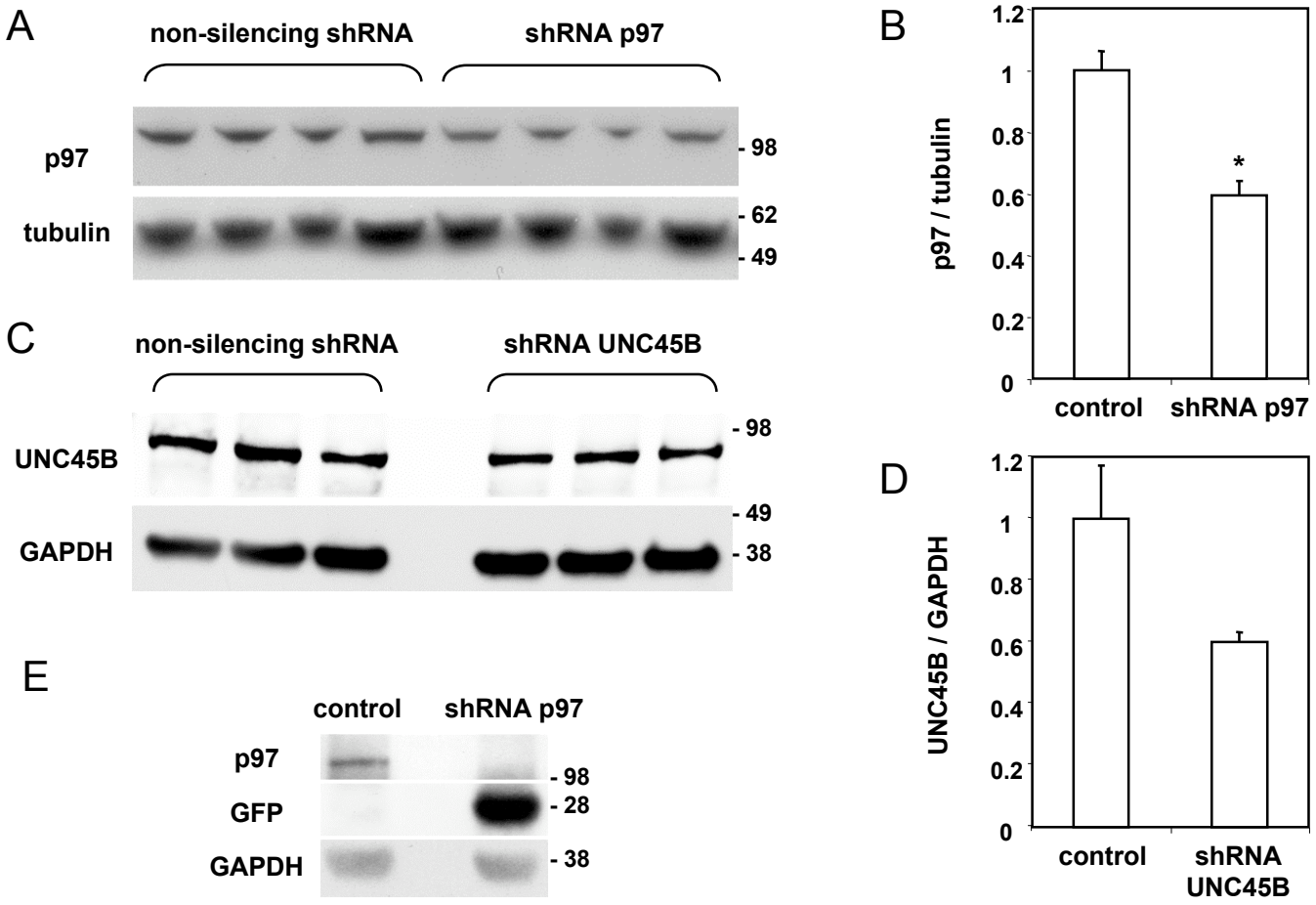


**Fig. S2. The shRNA against p97 or UNC45B partially reduce the expression of the corresponding protein.**
**A-D.** Murine neuroblastoma (A) or C2C12 myoblasts (C) were transfected for 72h with plasmids expressing a non-silencing shRNA or a shRNA against p97 or UNC45B, respectively. Tubulin and GAPDH are used as loading controls. The shRNA reduced the protein amount by 40% in both cases, as shown by quantitation of bands in SDS PAGE (B-D). Error bars indicate SEM. * $p < 0.05$.
**E.** Lysates from adult muscles electroporated with a mock vector (control) or a plasmid expressing a shRNA against p97 (that also expresses GFP) for 7 d were loaded. The great efficiency of electroporation of this muscle (indicated by the high expression of GFP) resulted in silencing of endogenous p97.

Figure S3

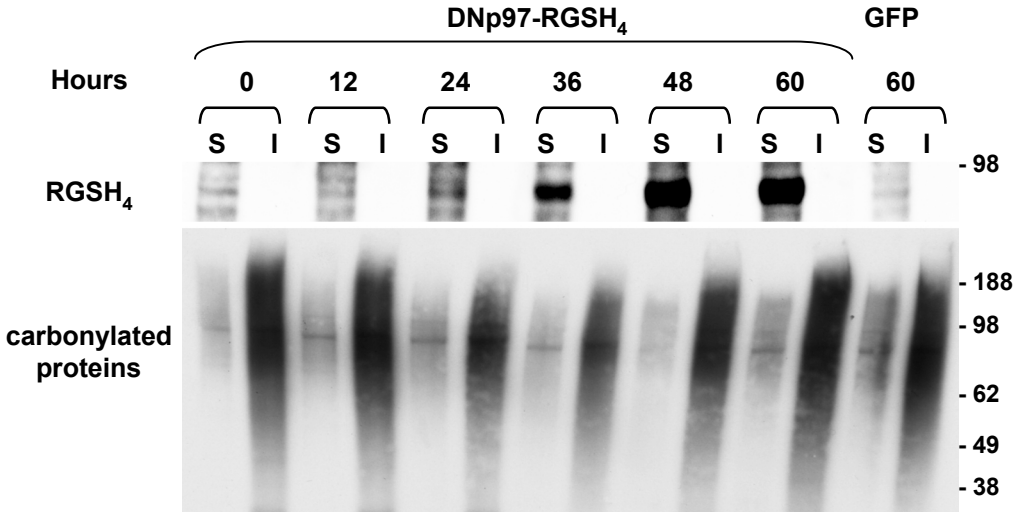


**Fig. S3. In myotubes, overexpression of DNp97 does not cause accumulation of oxidatively-damaged proteins.**

Fully differentiated myotubes were infected for the indicated times with an adenovirus expressing DNp97 or GFP. Soluble (S) and insoluble (I) fraction of these cells in lysis buffer with 1% Triton X100, 1mM DTT and 2M Urea were prepared. The presence of carbonylated proteins in equal amount of cell proteins was assayed after derivitization with DNP-hydrazine (DNPH) and then immunoblotting with an anti-DNP-hydrazone antibody.

Figure S4

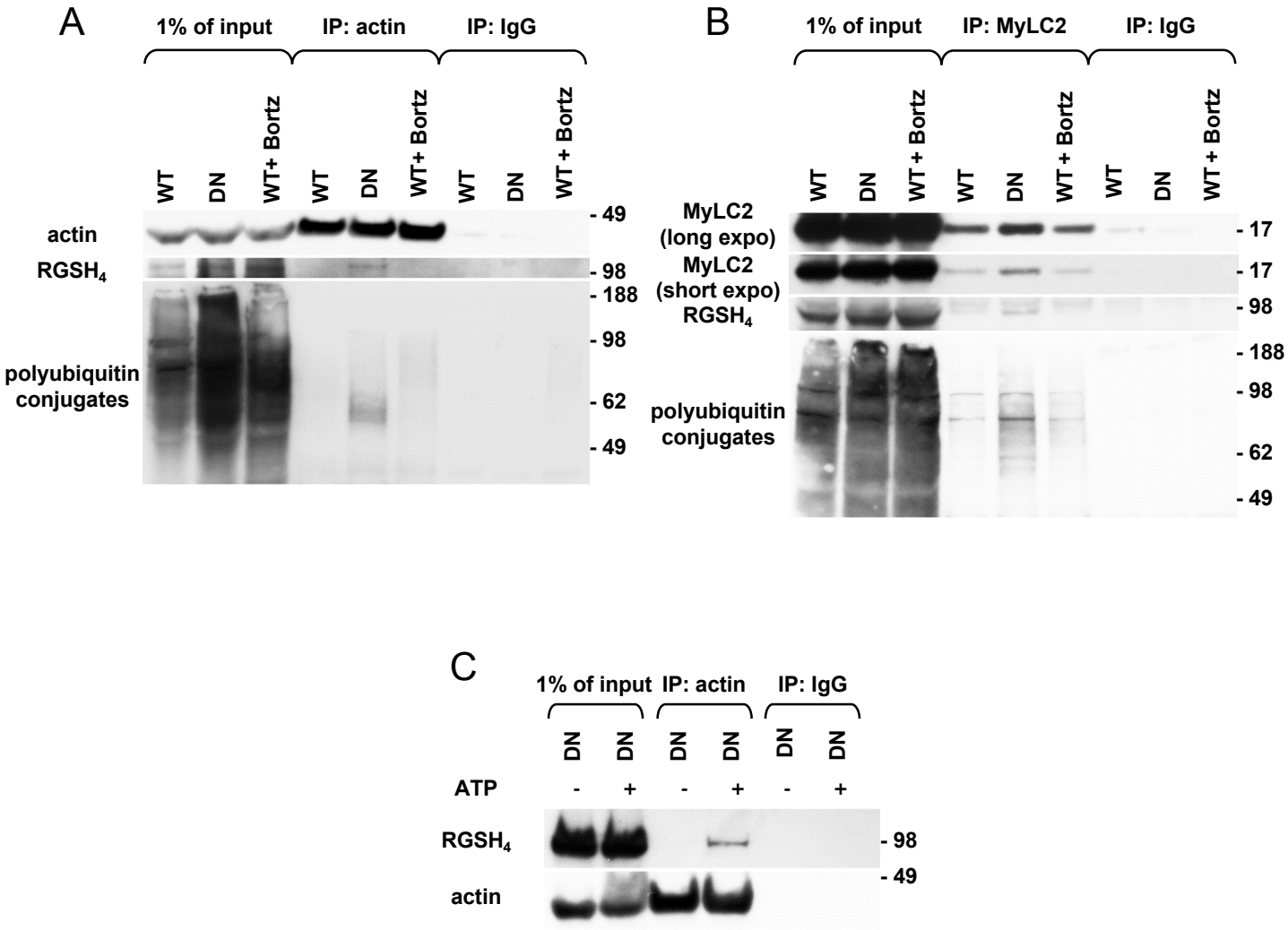


**Fig. S4. In myotubes, DNp97 binds actin or MyLC2 in an ATP-dependent way and results in accumulation of actin or MyLC2 as ubiquitinated species.**

**A-C.** Immunoprecipitation of endogenous actin (A) with and without ATP in the lysis buffer (C) or MyLC2 (B) was performed from myotubes overexpressing WTp97 or DNp97 for 72h (in presence of DMSO or 1μM Bortezomib for 3h). Actin, $RGSH_4$, polyubiquitinated proteins (FK1) and MyLC2 were immunoblotted. As control, immunoprecipitation of the same samples with appropriate IgG was carried out.

Figure S5

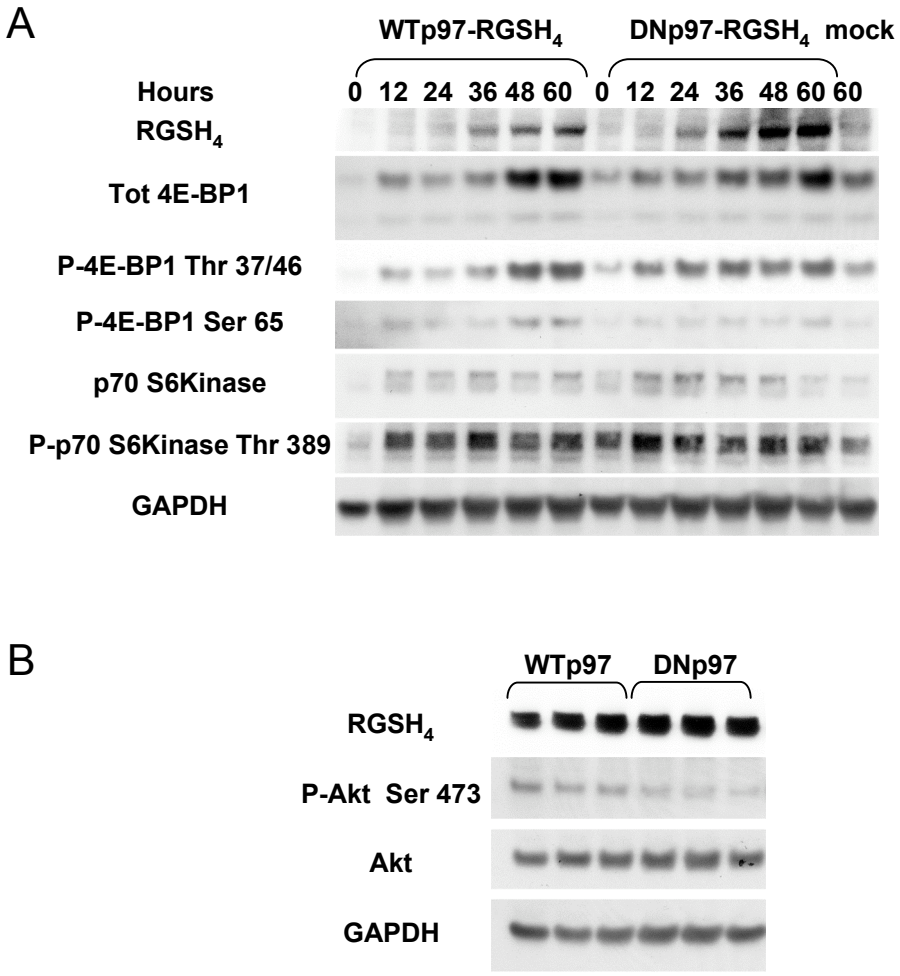


**Fig. S5. WTp97 or DNp97 overexpression does not alter the Akt/mTOR pathway in myotubes.**
**A-B.** Equal amounts of protein lysates from myotubes expressing WTp97 or DNp97 for the indicated times (A) or for 48h (B) were loaded and blotted with antibodies against: $RGSH_4$, 4E-BP1 (and its phosphorylated forms in Thr 37/46 or Ser 65), p70S6Kinase (and its phosphorylated form in Thr 389), Akt (and its phosphorylated form in Ser 473) and GAPDH as loading control.

Figure S6

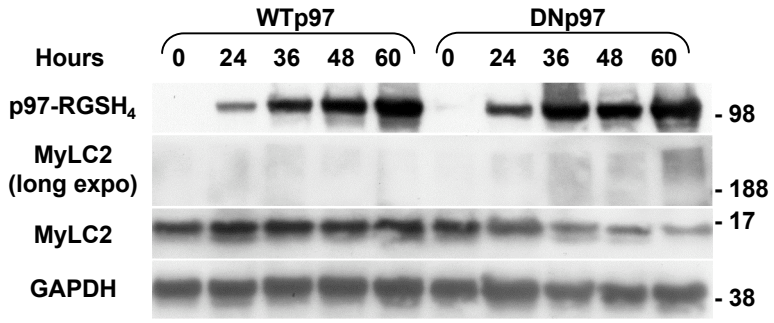


**Fig. S6. DNp97 causes accumulation of ubiquitinated MyLC2 in myotubes.**
In total extracts of myotubes expressing WTp97 or DNp97 for the indicated times, MyLC2 protein was assayed by immunoblotting. MyLC2 accumulated as ubiquitinated species upon 60h-expression of DNp97.

## SUPPLEMENTARY MATERIAL AND METHODS

For the validation of shRNA constructs, murine neuroblastoma (N1E-115) or myoblasts (C2C12) were maintained in DMEM + 10% FBS (Invitrogen) and transfected with shRNA constructs using Lipofectamine 2000 (Invitrogen). Cells were lysed 72h later, and immunoblotting was performed.

*Protein extraction and immunoblotting*
Cell lysates were prepared as indicated in Material and Methods. The following antibodies were used anti-tubulin (Rockland), anti-4E-BP1, anti-phospho-4E-BP1 (Thr 37/46), anti-phospho-4E-BP1 (Ser 65), anti-p70S6Kinase, anti-phospho-p70S6Kinase (Thr 389), anti-Akt and anti-phospho-Akt (Ser 473) (Cell Signaling).

*RNA isolation, Reverse Transcription and Quantitative Real-Time PCR*
Total RNA was isolated from muscles with TRIzol (Invitrogen). RNA concentration, purity and integrity were measured in a spectrophotometer (Ultrospec 2000, Pharmacia Biotech) and by running a denaturing agarose gel. Analysis of mRNA / μg in muscle was performed using TaqMan reverse transcription reagents (Applied Biosystems). Real-Time PCR reactions were carried out with DyNAmo HS SYBR Green qPCR kit (Finnzymes) and the appropriate primer pairs (Supplementary Table II) in an ABI 7900HT fast Real-Time PCR system (Applied Biosystems).

**Supplementary Table II**:

| **Molecule** | *Sense primer sequence (5′-3′)* | *Antisense primer sequence (5′-3′)* |
|---|---|---|
| p97 | *AACTGGCCATTCGTGAATCT* | *GCACTGGATCGTCCTCTTCT* |
| Npl4 | CTGCTAGAAGCCGTGAGGAC | CCAGGTTGGTTCATGAAGGT |
| Ufd1 | CCAATCAAGCCTGGAGACAT | CAGGTGCAATCGGATCTTTT |
| p47 | ACTGGCACTGAGGAGGACAG | CTGTGCCTCTGGACACTGAA |
| Ufd2 | GTCAAAGCTTGAGGGACGAG | CAGCAGTGCAAGTAGGGTGA |