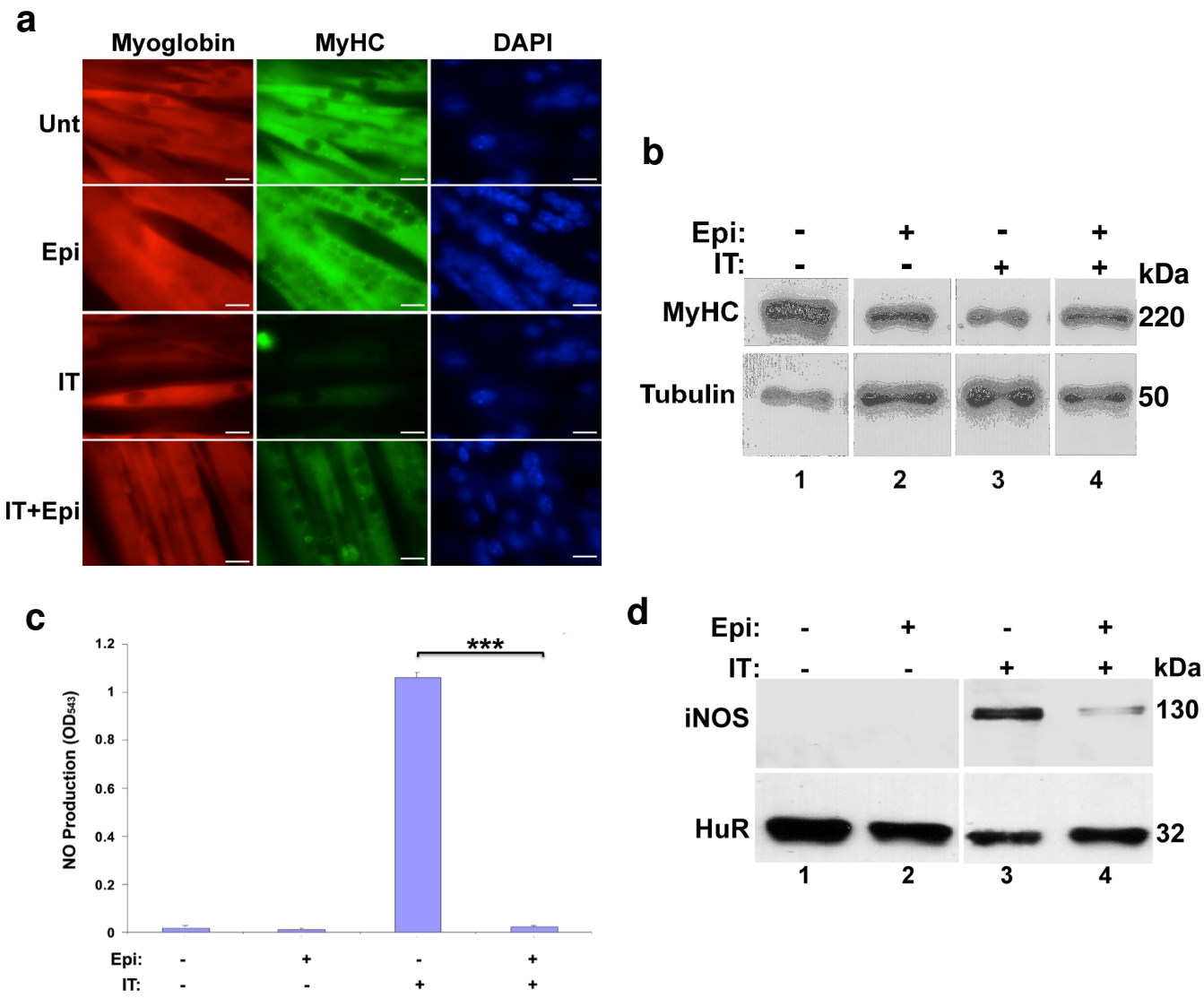
Figure S1: ***Episilvestrol, another small molecule that targets eIF4A, prevents cytokine-induced muscle loss.***



(a-b) C2C12 myofibers were treated with or without IFNγ/TNFα for 24 hours in the presence or absence of 0.05μM Epi. (a) Immunofluorescence analysis was performed using antibodies against myoglobin and MyHC. Bars, 20μm. (b) Western blot analysis of MyHC protein levels in myofibers treated with or without IFNγ/TNFα in the presence or absence of Epi. Tubulin protein levels are included as a loading control. **(c)** C2C12 myofibers were treated as described above. NO was assessed using the GREISS reagent. ***P<0.001, (Student's t-test). **(d)** Western blot analysis was performed on total protein extracts prepared from muscle fibers treated with IFNγ/TNFα as described above in the presence or absence of 0.05 mM Epi. The blot was probed with antibodies against iNOS. HuR was also probed as a loading control. All experiments shown are representative of three independent experiments except for b, which was performed twice.

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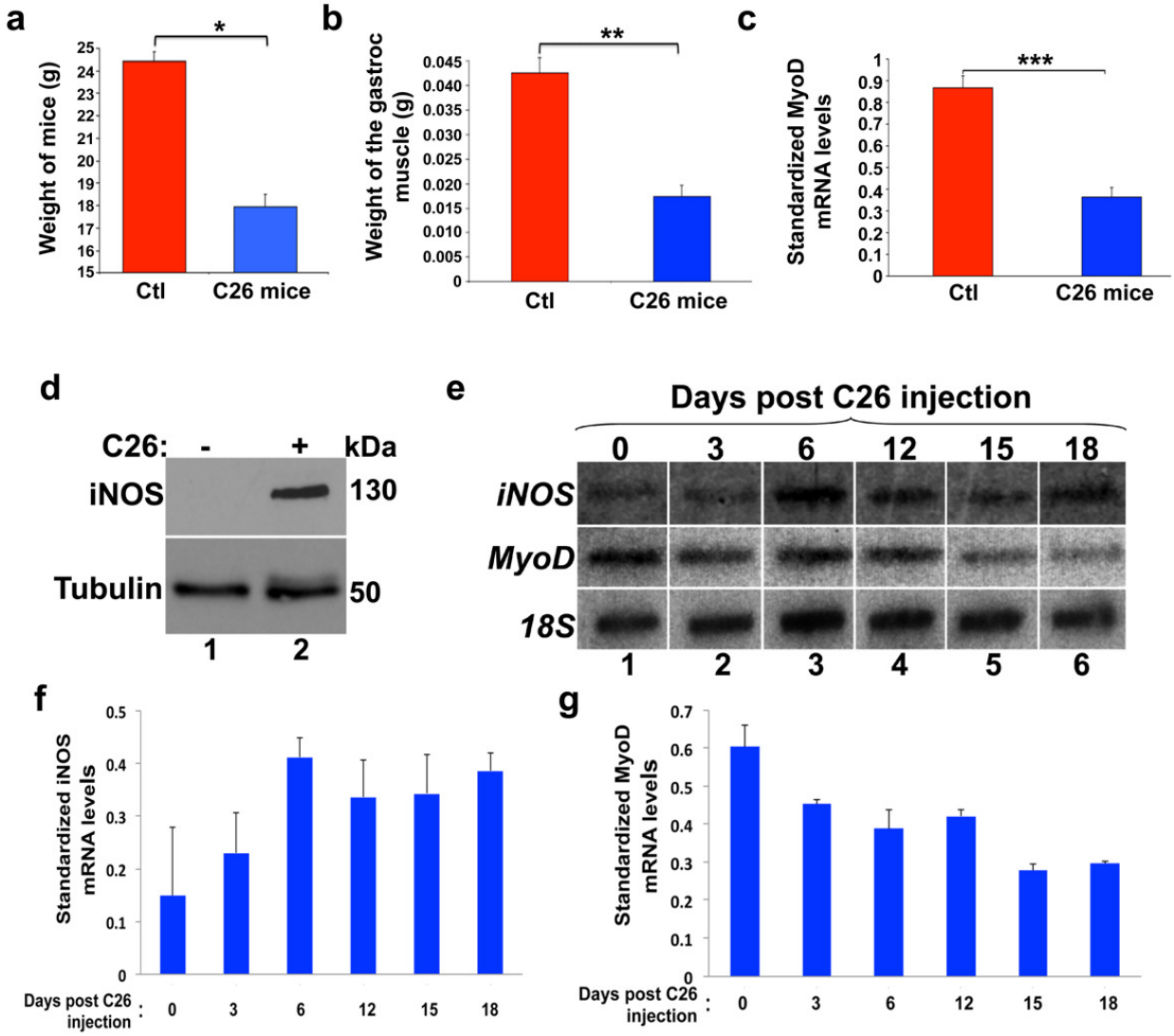
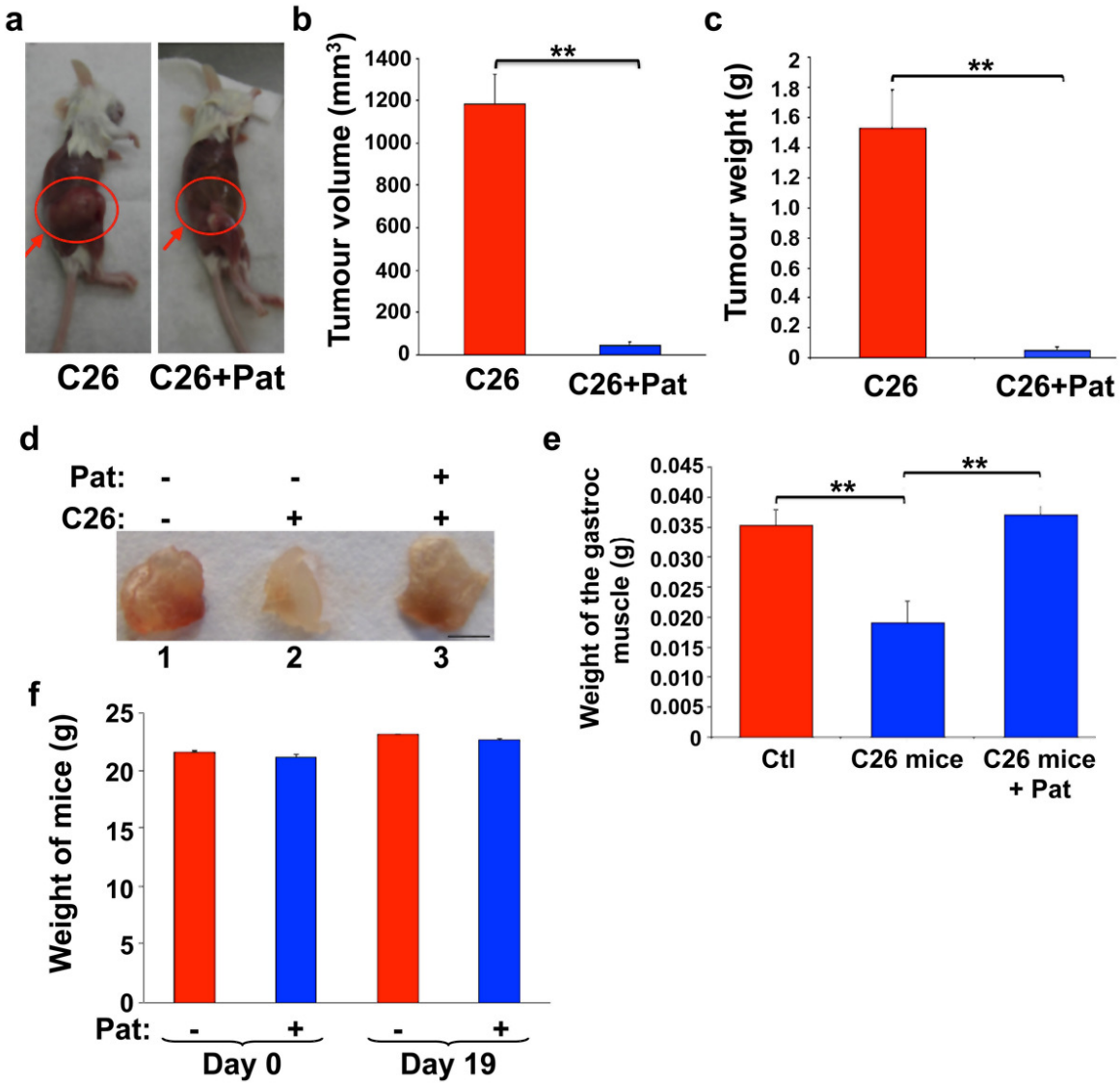
Figure S2: ***Injection of BALB/c mice with C26 adenocarcinoma cells triggers tumour growth leading to iNOS expression and the loss of MyoD mRNA.***



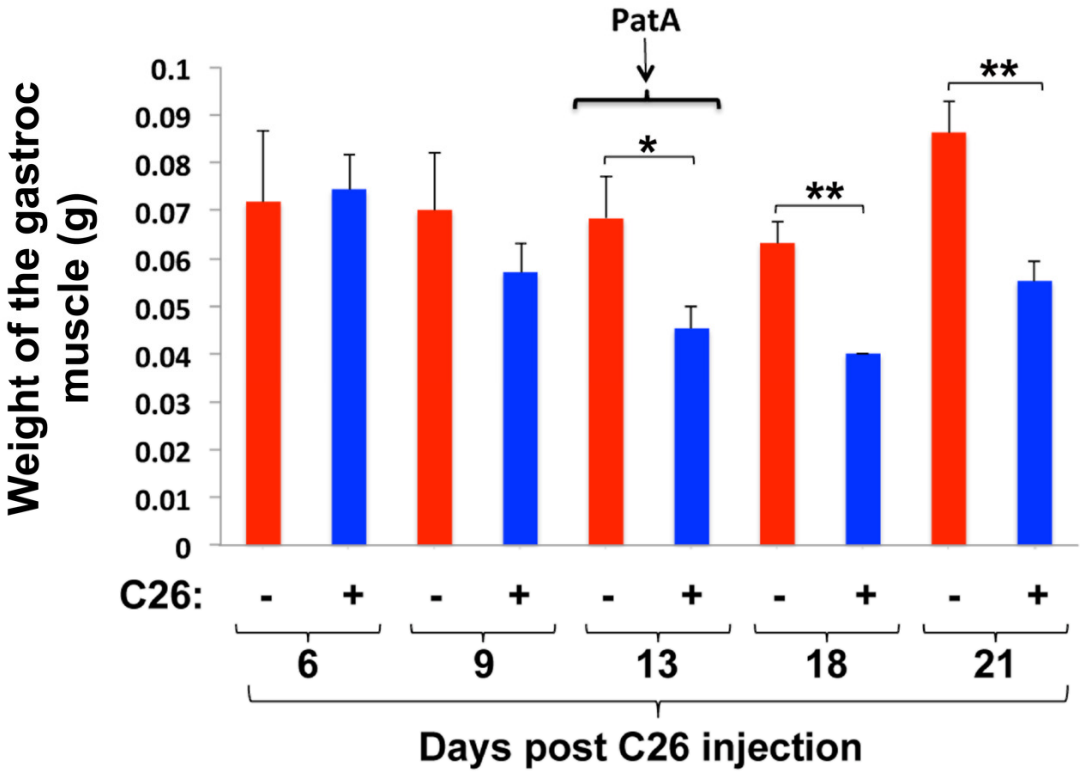
(a-e) (a) Body weight of mice (subsequent to the removal of the tumour) and the mass of the gastrocnemius (b) were determined at 19 days post-C26-injection and plotted +/- S.E.M. from 8 and 9 mice respectively for saline control and C26 injected mice. *P<0.05, **P<0.01 (Student's t-test). (c) *The expression of MyoD mRNA in the gastrocnemius muscle is decreased in C26-injected mice.* The gastrocnemius muscle was isolated from mice 19 days post injection with or without C26 cells. RNA harvested from the muscle was analyzed by slot blot to determine MyoD mRNA levels. These levels were quantified using the ImageQuant software, standardized against 18S rRNA levels and plotted +/- the S.E.M. ***P<0.001 (Student's t-test). (d) Total protein extracts from the gastrocnemius muscles of C26-injected mice were prepared and used for western blot analysis to determine the levels of iNOS protein. (e) The expression of iNOS and MyoD mRNA was determined over time using a slot blot analysis on total mRNAs prepared from the gastrocnemius muscle of BALB/c mice injected with or without C26 cells. The steady state levels of the iNOS and MyoD messages was assessed every third day until eighteen days post C26 injection. **(f-g)** The iNOS (f) and MyoD (g) mRNA levels were quantified using the ImageQuant software, standardized against 18S rRNA levels and plotted +/- the S.E.M from 3 mice per time point.

Figure S3: *50µg/kg PatA significantly reduces the growth of C26-adenocarcinoma tumours as well as C26-induced wasting of the gastrocnemius muscle.*



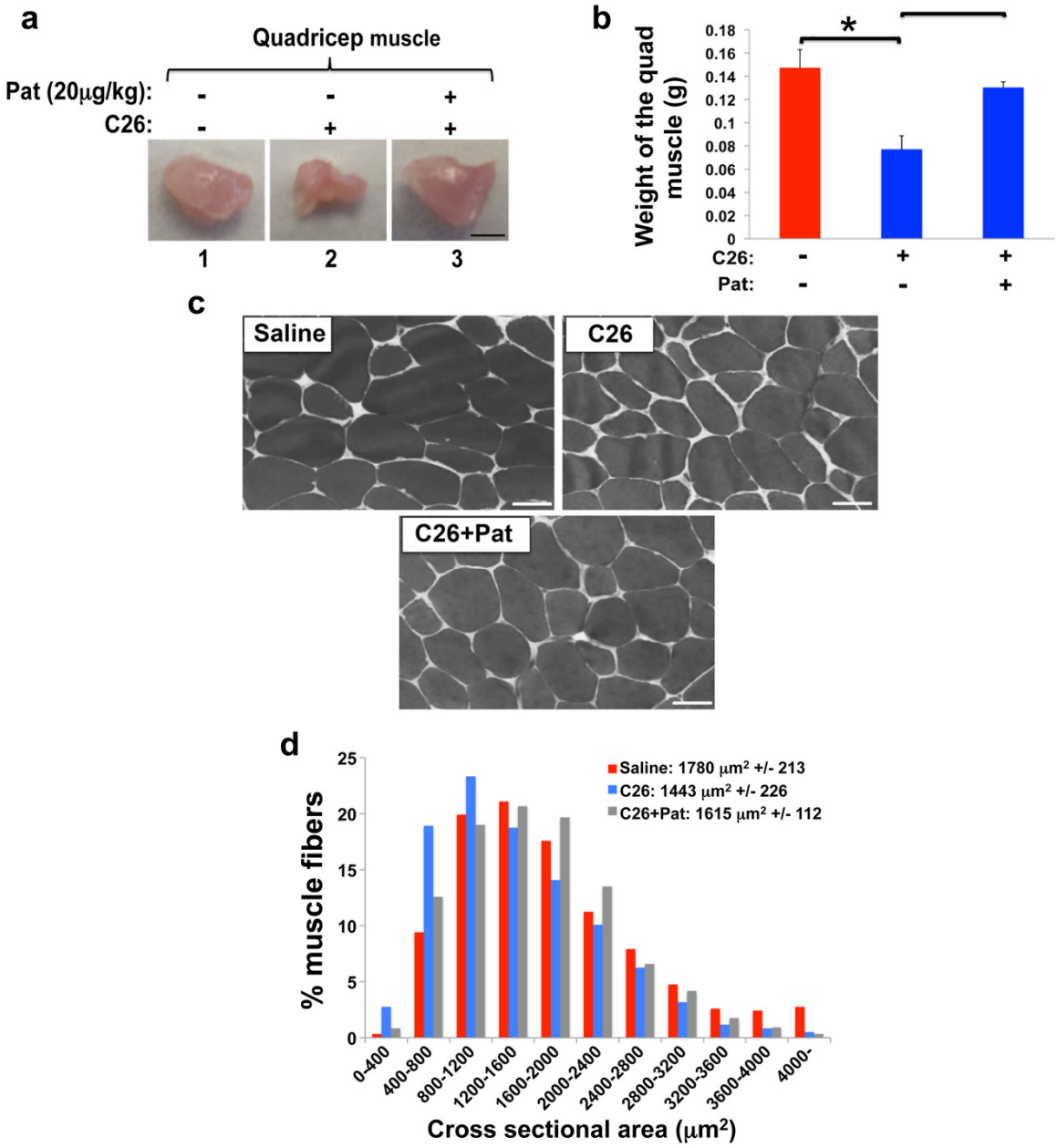
(a-e) BALB/c mice were treated with 50µg/kg of PatA and every second day starting at 6 days post-injection with the C26 cells. The mice were subsequently sacrificed 19 days after the injection with C26 cells. (a) Photograph of C26-injected mice treated with or without 50µg/kg of PatA. The tumour volume (b) and weight (c) were measured for 5 mice per group and plotted +/- S.E.M. **P<0.01 (Student's t-test). (d) Photograph of gastrocnemius muscles isolated 19 days post-C26 injection from mice treated or not with 50µg/kg of PatA as described above. Bars, 5mm. (e) The weight of gastrocnemius muscles isolated from mice treated or not with 50µg/kg PatA was determined at 19 days post-C26-injection and plotted +/- S.E.M. from 5 mice respectively. **P<0.01 (Student's t-test). **(f)** *Injection of BALB/c mice with 20 µg/kg PatA does not affect their viability over time.* BALB/c mice were injected every second day over a 19 days period with or without 20µg/kg of PatA. The weights of 4 mice obtained prior to the first injection as well as at the end of the experiment were plotted +/- S.E.M.

Figure S4: ***C26-tumours induced muscle loss in BALB/c mice starts as early as day 13 post injection of C26-adenocarcinoma cells.***



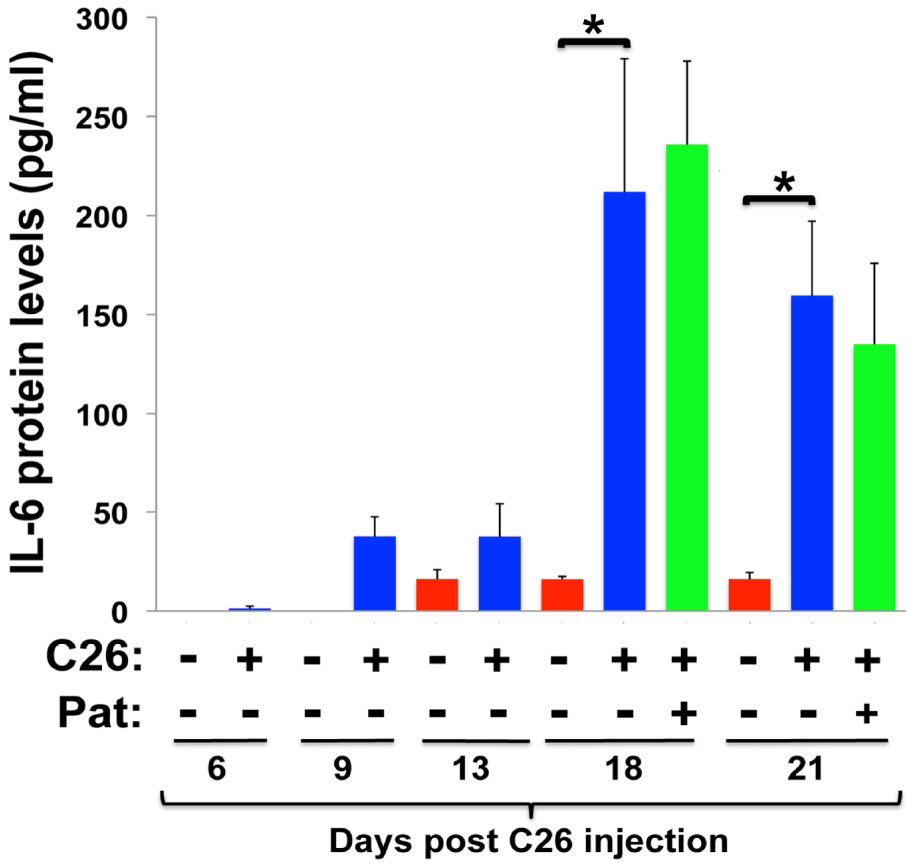
Weight of gastrocnemius muscles isolated from mice 6, 9, 13, 18 and 21 days post-C26 injection. 20 µg/kg PatA was injected into these mice on day 13 and every second day thereafter until the end of the experiment. The weight of the muscles (obtained from a range of 3-6 mice) were plotted +/- S.E.M. *P<0.05, **P<0.01 (Student's t-test).

Figure S5: ***PatA prevents the wasting of the quadriceps muscle in C26-tumour bearing mice.***



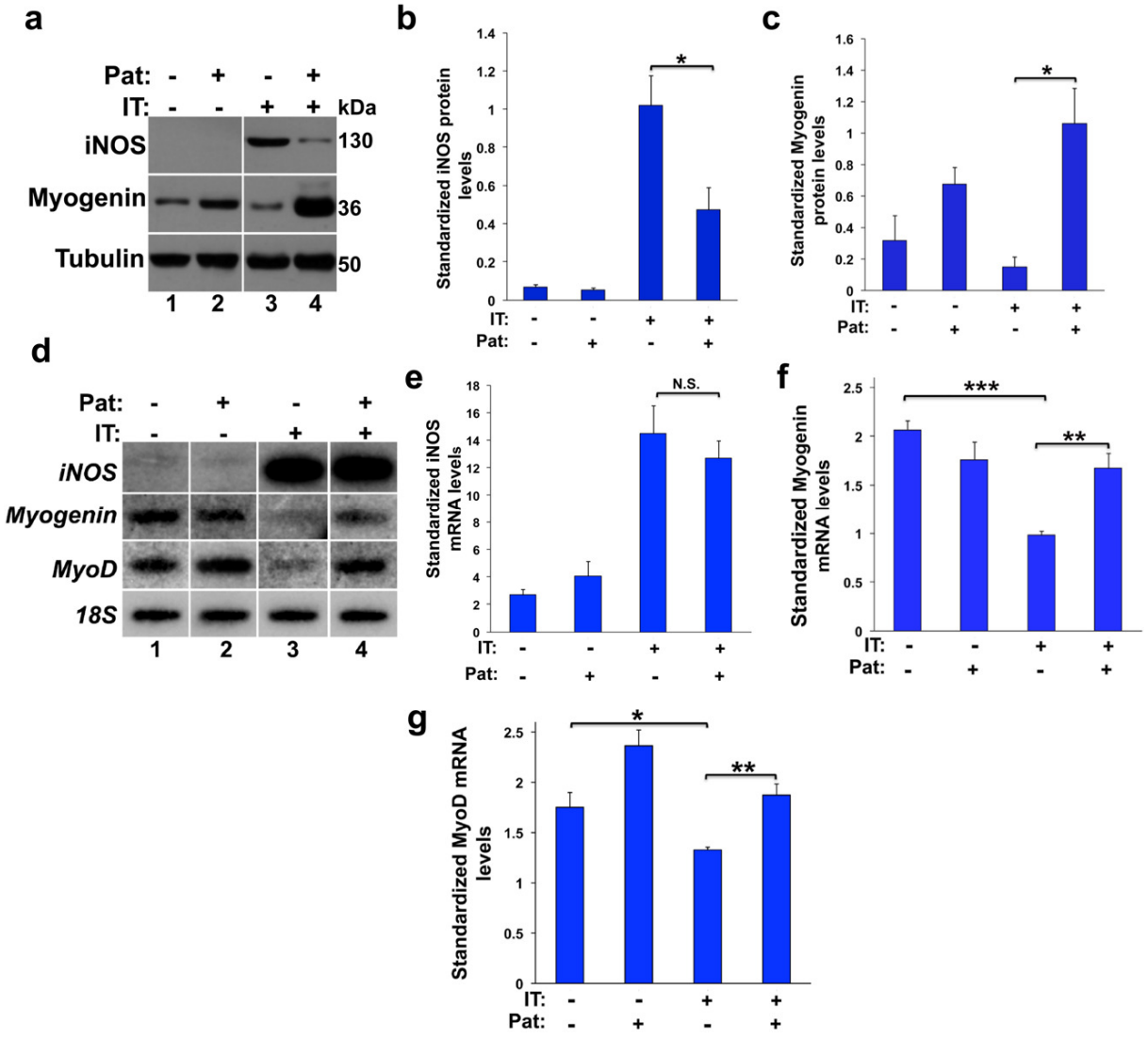
(a) Photograph and (b) weight of quadriceps muscles isolated 21 days post-C26 injection from mice treated or not on day 13 and every second day thereafter with 20μg/kg of PatA. Photograph of muscles in a are representative of n=3 mice per sample group. Bars, 5mm. Weights in b were plotted from n=3 mice +/- S.E.M. *P<0.05, (Student's t-test). (c) Immunohistochemical staining of quadricep muscle fibers isolated from saline as well as C26 tumour bearing mice treated with or without PatA as described above. Bars, 20μm. (d) The cross-sectional area of muscle fibers described in (c) are represented as a frequency histogram from n=3 mice. The mean cross-sectional area of all fibers for each sample group is indicated in the histogram +/- S.E.M.

Figure S6: ***PatA does not affect the secretion of IL-6 in C26-tumour bearing mice.***



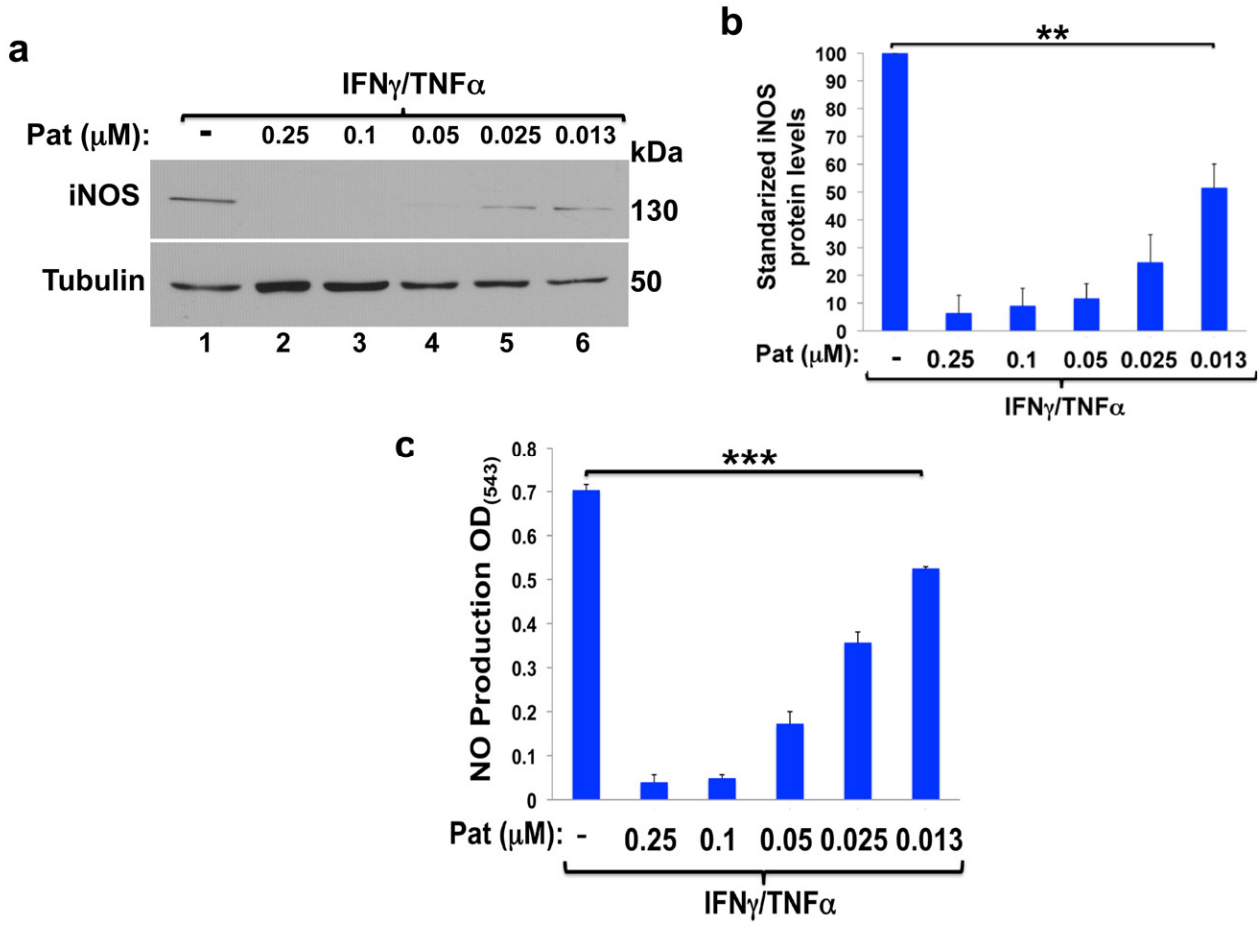
The expression of IL-6 was detected in sera obtained from mice 6, 9, 13, 18 and 21 days post-C26 injection. PatA was injected in these mice on day 13 and every second day thereafter until the end of the experiment (n=3 mice per time point). IL-6 levels were determined by ELISA and plotted +/- S.E.M. *P<0.05, (Student's t-test).

Figure S7: ***The expression levels of iNOS, Myogenin and MyoD mRNA and protein in the gastrocnemius muscles of mice treated with IFNγ/TNFα in the presence or absence of 20μg/kg of PatA.***



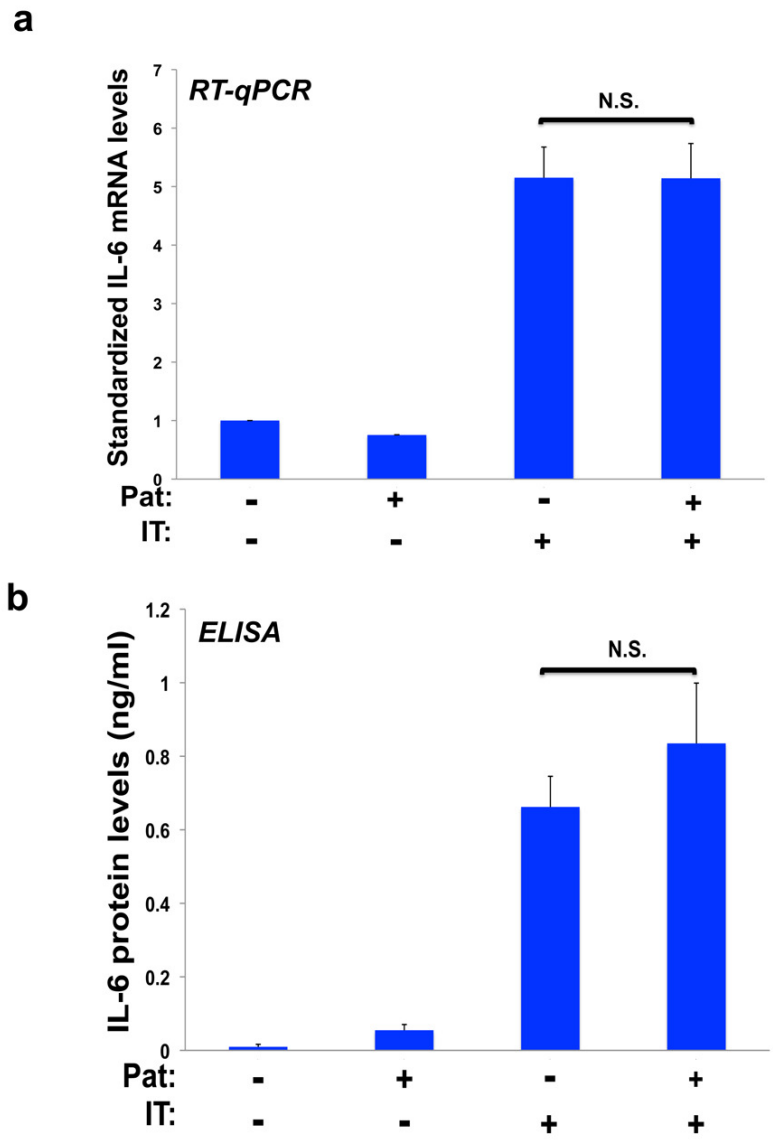
(**a**) Western blots were performed to determine the levels of Myogenin and iNOS proteins in gastrocnemius muscles isolated from mice treated with or without IFNγ/TNFα for 5 days in the presence or absence of PatA. The levels of iNOS (b) and Myogenin (c) proteins were quantified using the ImageQuant software, standardized against tubulin levels, and plotted +/- S.E.M from three different mice. *P<0.05, (Student's t-test). **(d-g)** Total mRNA was harvested from gastrocnemius muscles of mice treated as described above and analyzed by slot blot to determine the expression levels of iNOS, Myogenin and MyoD mRNAs. The levels of iNOS (e), Myogenin (f) and MyoD (g) mRNAs were quantified using the ImageQuant software, standardized against 18S rRNA levels, and plotted +/- S.E.M from three different mice. N.S, non-significance. *P<0.05, **P<0.01, ***P<0.001, (Student's t-test).

Figure S8: ***PatA significantly reduces iNOS protein levels in a dose dependent manner.***



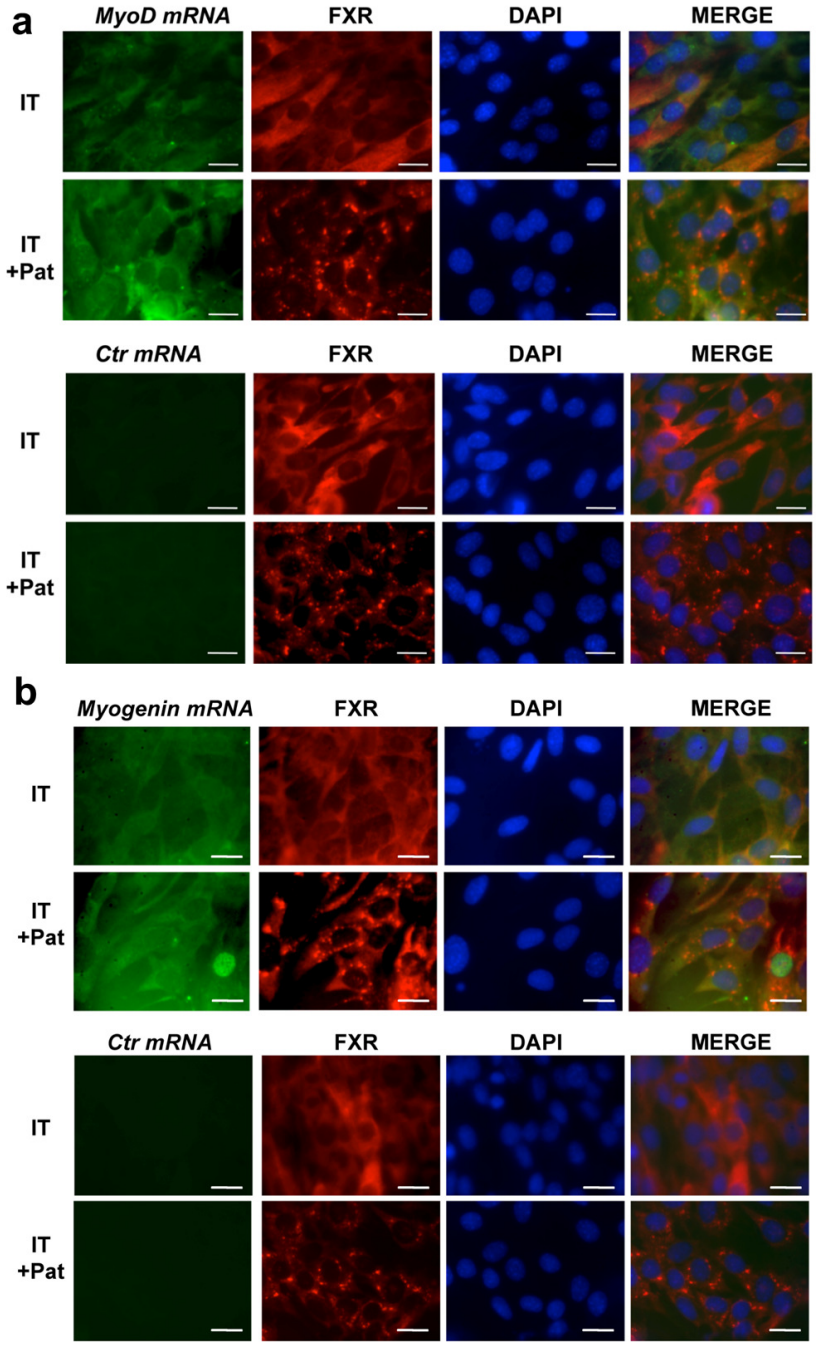
(a) Western blot analysis using an antibody detecting iNOS was performed to determine the effect of different doses of PatA ranging from 0.25μM to 0.0125μM on iNOS protein expression in cytokine treated muscle fibers. Tubulin protein levels are included as loading controls. **(b)** The iNOS protein levels above were quantified, normalized against the tubulin protein levels and plotted +/- the S.E.M from three independent experiments. **P<0.01, (Student's t-test). **(c)** *The release of nitric oxide by IFNγ/TNFα-treated myofibers is downregulated by PatA in a dose dependent manner.* Nitric oxide (NO) release by C2C12 myofibers was detected using the GREISS reagent 24 hours after treatment with or without the doses of Pat described above in the presence or absence of IFNγ /TNFα. NO levels were plotted +/- the S.E.M from three independent experiments. ***P<0.001, (Student's t-test).

Figure S9: ***PatA has no effect on the expression of IL-6 in cytokine-treated myofibers.***



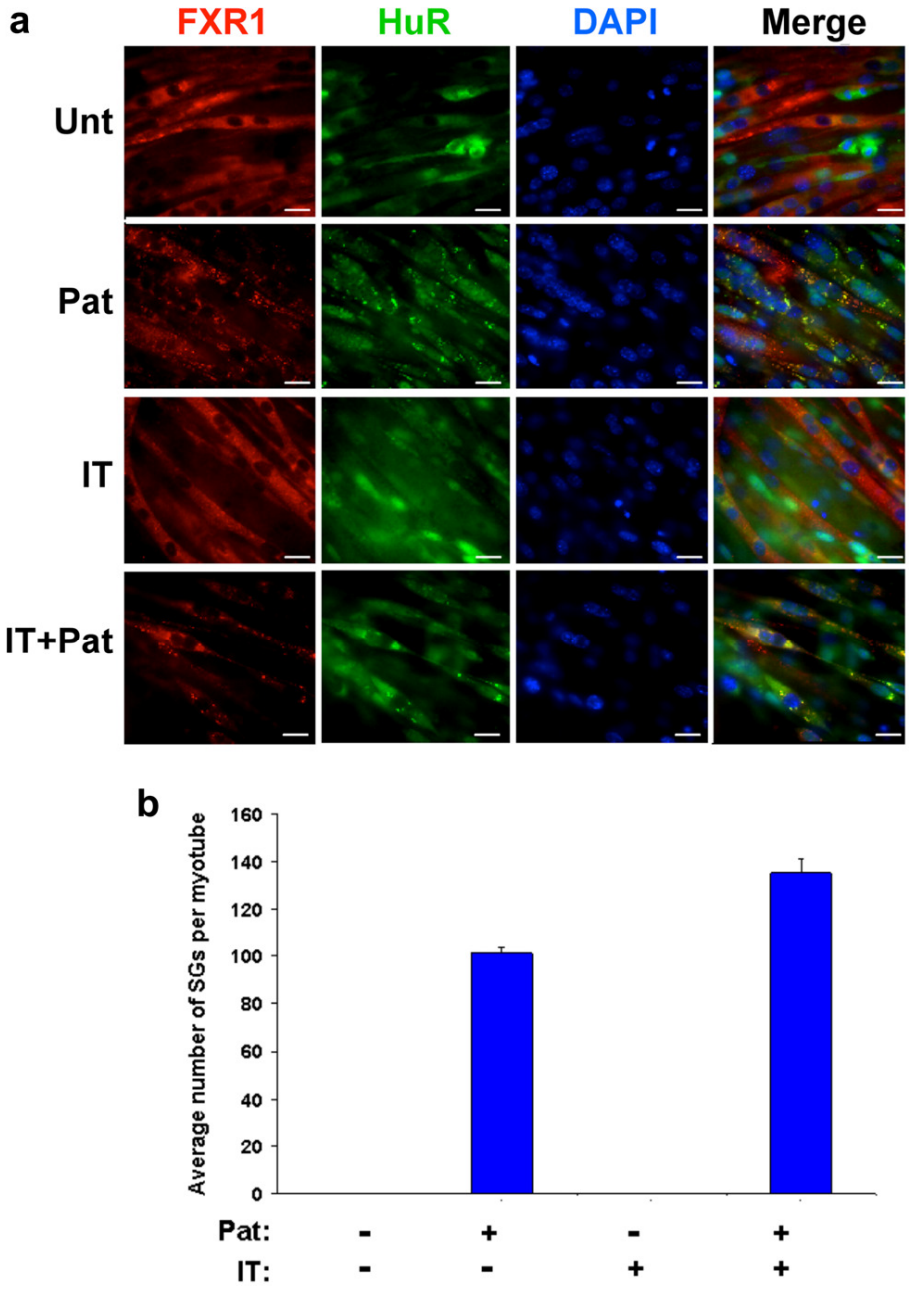
(a) Total mRNA was harvested from C2C12 myofibers treated with or without IFNγ/TNFα for 24h in the presence or absence of PatA and analyzed by RT-qPCR to determine IL-6 mRNA levels that were standardized against RPL32 mRNA levels. **(b)** IL-6 secretion was determined by ELISA Results in a,b are from N=3 experiments, +/-S.E.M.

Figure S10: ***The MyoD and Myogenin mRNAs are not recruited to SGs in*** **IFNγ/TNFα-** ***treated C2C12 cells in the presence of PatA.***



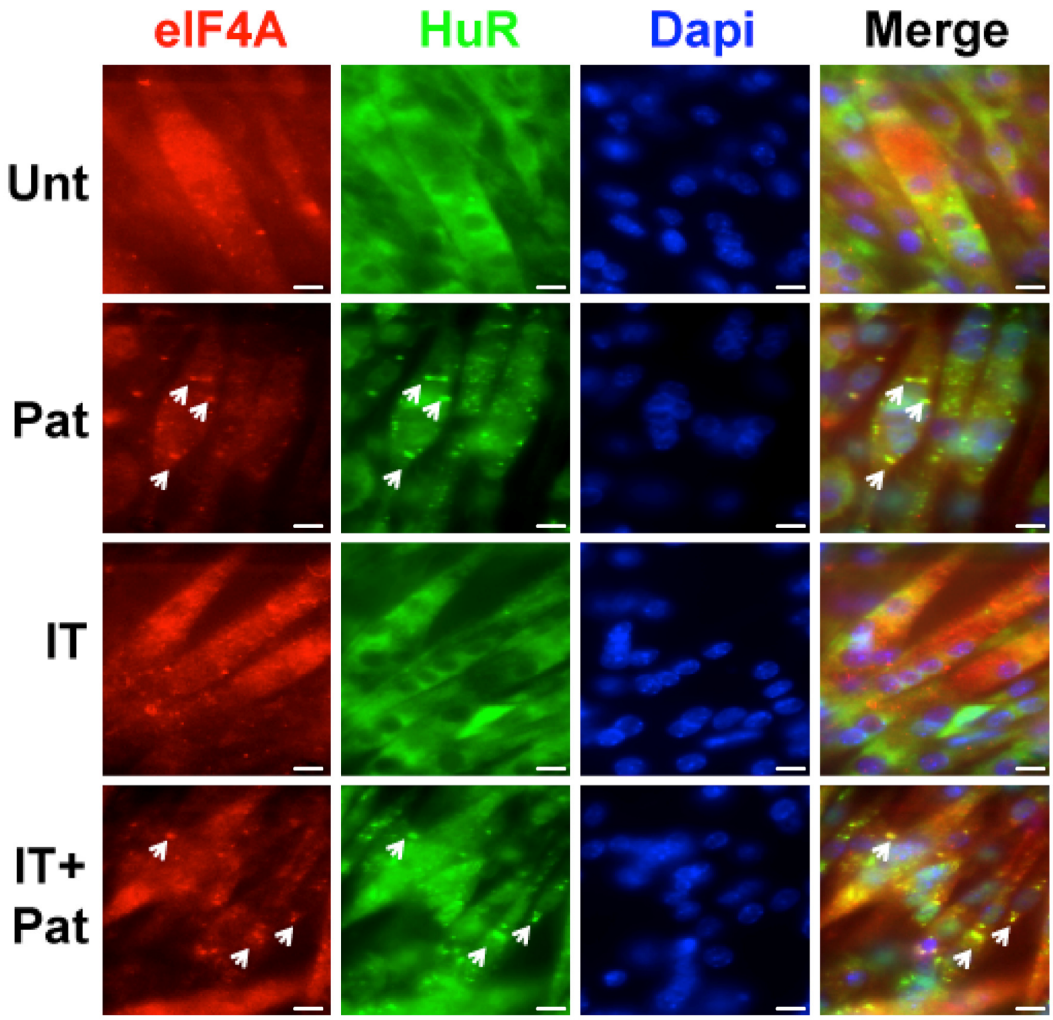
(a, b) C2C12 cells, one day post-induction of muscle cells differentiation, were treated as described in the Material and Methods section with or without PatA in the presence of IFNγ/TNFα. Twenty four hours later, the cells were fixed, permeabilized and incubated with an anti-sense or sense probe (used as a control probe) in order to detect (a) MyoD or (b) Myogenin mRNAs. DAPI staining and immunostaining against FXR1, were performed in order to visualize the nucleus and the SGs respectively. Bars, 20μm.

Figure S11: *PatA induces the formation of SGs in muscle.*



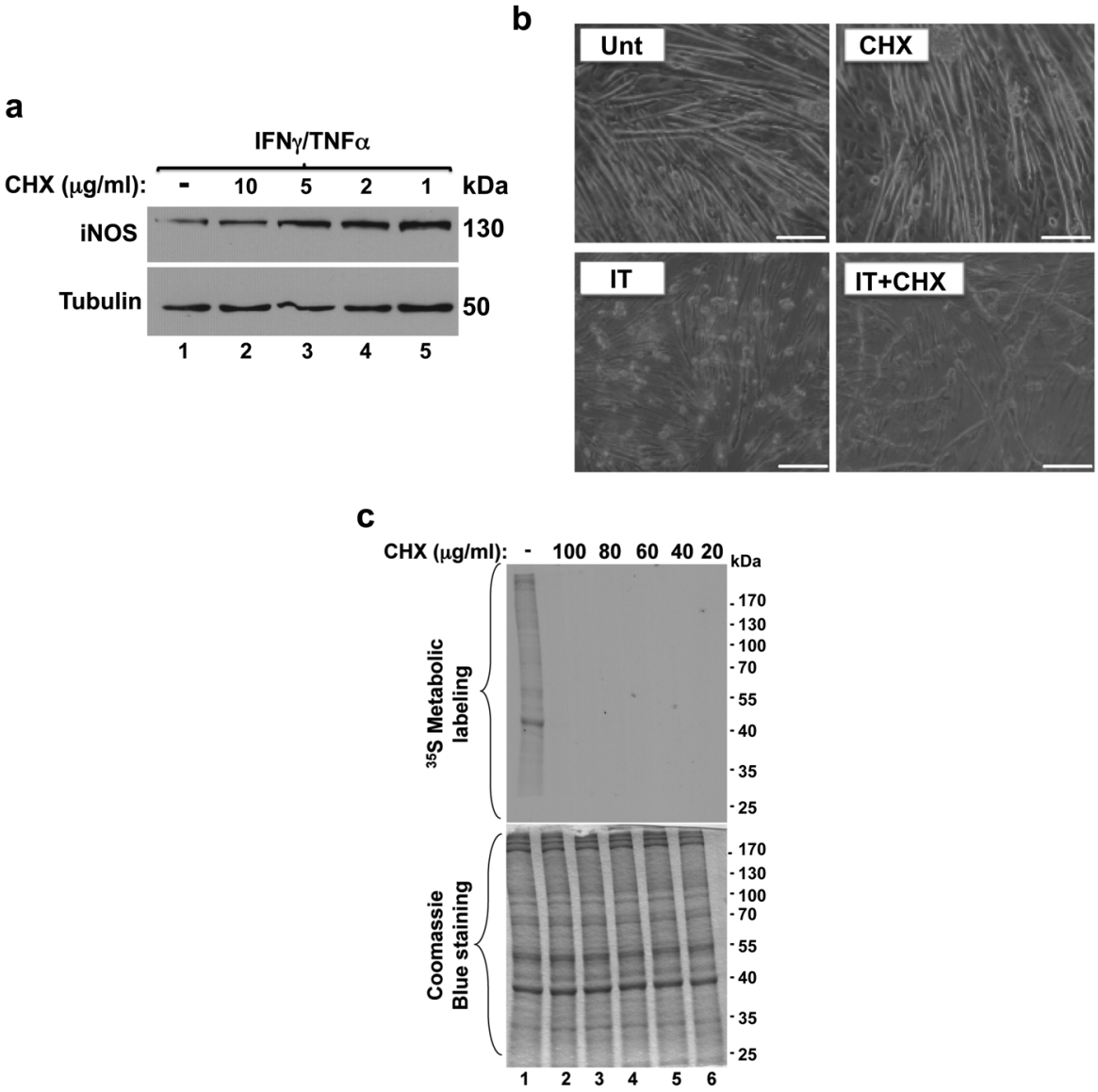
(**a**) Mature C2C12 myofibers were treated with or without Pat in the presence or absence of IFNγ/TNFα. Twenty four hours later the formation of SGs was determined by visualizing the localization of the SG markers HuR and FXR1. Bars, 20μm. (**b**) The number of SGs per myotube in (a) was determined by counting these cytoplasmic foci in 5 myofibers per field from 3 different fields. The quantified values were subsequently plotted +/- the S.E.M. Results are representative of three independent experiments.

Figure S12: ***PatA induces the localization of eIF4A to SGs in muscle fibers.***



Mature C2C12 myofibers were treated with or without Pat in the presence or absence of IFNγ/TNFα. Twenty four hours later the formation of SGs was determined by visualizing the localization of the SG markers HuR and eIF4A. White arrows point to SGs. Bars, 20μm.

Figure S13 *Low doses of Cycloheximide, an inhibitor of eukaryotic translation elongation, does not prevent iNOS protein expression in IFNγ/TNFα treated myofibers.*



(a) Western blot analyses were performed to assess the effect of different concentrations of Cycloheximide (Chx) (ranging from 10 μg/ml to 1μg/ml) on the expression of iNOS protein in IFNγ/TNFα treated muscle fibers. Tubulin protein levels are included as loading controls. **(b)** Phase contrast pictures of muscle fibers were obtained 72h post-treatment with or without IFNγ/TNFα in the presence or absence of low doses of Chx. Bars, 50μm. **(c)** Treatment of myofibers with high doses of Chx for 30 minutes prevents general translation, validating the functionality of this drug as an inhibitor of protein synthesis.

Supplementary Methods

Luciferase Reporter Experiments:

The iNOS and MyoD 5'UTRs were generated by RT-PCR from total RNA isolated from C2C12 myofibers using the following primers:
iNOS-F-BamH1-5'- CGC GGA TCC GGG ACA CAG TGT CAC TGG TT-3',
iNOS-R-NcoI 5'CAT GCC ATG GGT CTG AGA CTT TGC ACT TCT-3',
MyoD-F-BamH1 5'-CCG GGA TCC GGA CGC CCC AGG ACA CGA CT-3'
MyoD-R-NcoI 5'-CAT GCC ATG GTC CCA GTT CCT GGG TCC AGC-3'.
The PCR fragments were subsequently digested with BamH1 and NcoI and cloned into the pCREL vector (Bonnal et al., 2005) generating pCRiNOSL and pCRmyoDL. Fragments encoding the iNOS (iNOS-FLuc) and MyoD (MyoD-FLuc) 5'UTR fused to firefly luciferase were generated by PCR using the pCRiNOSL and pCRmyoDL vectors as templates and the following primers:
T7-Fluc-F 5'- TAA TAC GAC TCA CTA TAG GGA TGA ACA ATA AAC TAG GAT CC- 3'
Fluc-R 5'- TTT TTT TTT TTT TTT TTT TTT TTT AAG GGG CTT CAT GAT GTC C-3'.
The Rluc control PCR fragment was generated using the pCREL vector and the following primers:
RLuc-F 5'- TAA TAC GAC TCA CTA TAG GGC CAC TTT GCC TTT CTC TCC- 3'
RLuc-R 5'-TTT TTT TTT TTT TTT TTT TTT TTT GTG AGT CGT ATT AAT TTC GC-3'.
cRNA encoding the iNOS and MyoD 5'UTR fused to the firefly luciferase as well as the control renilla luciferase were generated from the iNOS-FLuc, MyoD-FLuc and Rluc PCR fragments respectively using the mMESSAGE mMACHINE® T7 kit (Ambion) as per the manufacturer's instructions. 0.5µg of each Fluc reporter cRNAs were co-transfected with 0.5µg control Rluc cRNA (using Lipofectamine transfection reagent, Invitrogen) into C2C12 myoblasts that were subsequently treated with or without IFNγ/TNFα and/or PatA. Luciferase activity was then measured using a luciferase assay system (Promega) with a luminometer following the manufacturer's instructions.

Primers for in situ hybridization experiments:

The iNOS, MyoD and Myogenin mRNAs were then detected by *in situ* hybridization using the following primers. The sense and anti-sense probes were generated from PCR fragments using the

following primer sequences fused to either the T7 or T3 minimal promoter: iNOS antisense 5'-AAT TAA CCC TCA CTA AAG GGG GTG TAG GAC AAT CCA CAA C-3', iNOS sense 5'-TAA TAC GAC TCA CTA TAG GGT AGA GAA CTC AAC CAC ACC T-3', MyoD antisense 5'-AAT TAA CCC TCA CTA AAG GGC ATC CGC TAC ATC GAA GGT C-3', MyoD sense 5'-TAA TAC GAC TCA CTA TAG GGC AAA GCA CCT GAT AAA TCG C-3', Myogenin antisense 5'TAA TAC GAC TCA CTA TAG GGC CCG GGC TAT GAG CGG ACT G-3', Myogenin sense 5'AAT TAA CCC TCA CTA AAG GGG CAG GAG GCG CTG TGG GAG T-3'.