The low affinity receptor for neurotrophins p75^{NTR} plays a key role for satellite cell function in muscle repair acting via RhoA

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Supplementary Figure Legends

Suppl. Fig.1

The blockade of NGF signalling does not affect muscle differentiation

Densitometric anlysis on the western blot shown in Figure 2A. Primary satellite cells-derived myogenic precursors were constantly treated with NGF and the blocking peptide; after 12 hours of differentiation the expression of sarcomeric myosin, myogenin and PCNA was tested by western blot. The expression levels of the myogenic markers are normalized on the expression levels of the housekeeping protein GAPDH.

Suppl Fig. 2

α-actinin organization is regulated by NGF signalling

Myotubes treated for 12 hours with or without (NT) $p75^{NTR}$ blocking antibody (anti-p75), the antagonist peptide or LPA were stained for myosin (red) and for α-actinin (green). Scale bare 100 μm.

Suppl. Fig. 3

Paxillin organization is regulated by NGF signalling

Myotubes treated for 12 hours with or without (NT) $p75^{NTR}$ blocking antibody (anti-p75), the antagonist peptide or LPA were stained for sarcomeric myosin (red) and for paxillin (green). Scale bare 100 μm.

Suppl. Fig. 4

FAK organization is regulated by NGF signalling

Myotubes treated for 12 hours with or without (NT) $p75^{NTR}$ blocking antibody (anti-p75), the antagonist peptide or LPA were stained for sarcomeric myosin (red) and for FAK (green). Scale bare 100 μm.

Suppl. Fig. 5

Fusion and cytoskeleton disregulation are reverted by inhibiting RhoA activation

A-B) Satellite cells-derived myogenic precursors were constantly treated in the presence or absence of the $p75^{NTR}$ blocking antibody (p75 Ab), or the blocking peptide, in presence or absence of exoenzyme C3; after 12 hours in differentiating medium they were fixed and immunostained for sarcomeric myosin (A) in order to calculate the fusion index (B). In (B) the graph on the left shows the total fusion index, the graph on the right the percentage of syncitia with 1, 2-6, or >6 myonuclei, in the different culture conditions.

Scale bar 50 μm. Error bars indicate s.e.m. Triple asterisk $P< 0.01$ vs untreated myoblasts, double and triple single cross $P> 0.01$ or $P< 0.05$ vs treated myoblasts in absence of C3.

C) Satellite cells-derived myogenic precursors were treated after 12 hours in differentiating medium with or without (NT) the $p75^{NTR}$ blocking antibody (p75 Ab), the competing peptide or LPA, in presence or absence of C3 ; after additional 12h of treatment they were fixed and immunostained for myosin heavy chain. Scale bare 50 μm.

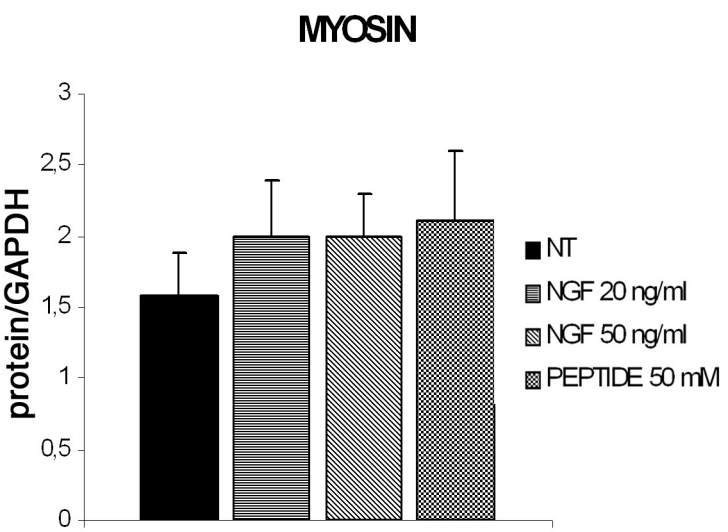
Suppl. Fig.6

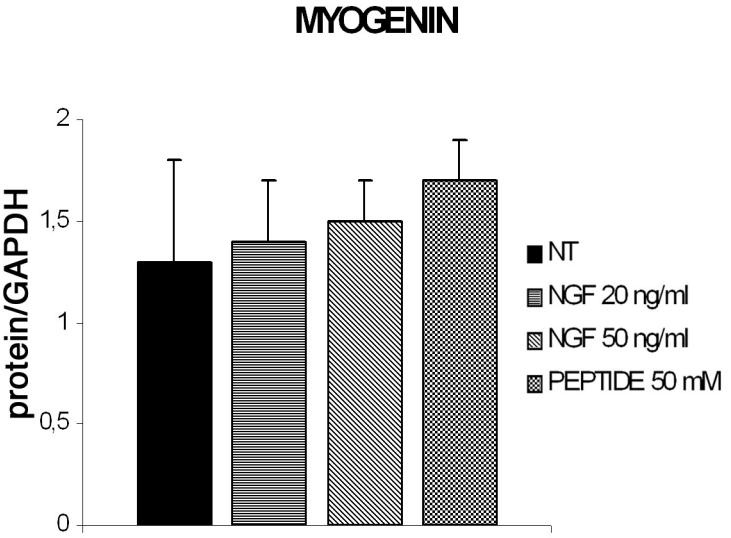
RhoA signalling participates to skeletal muscle regeneration in vivo

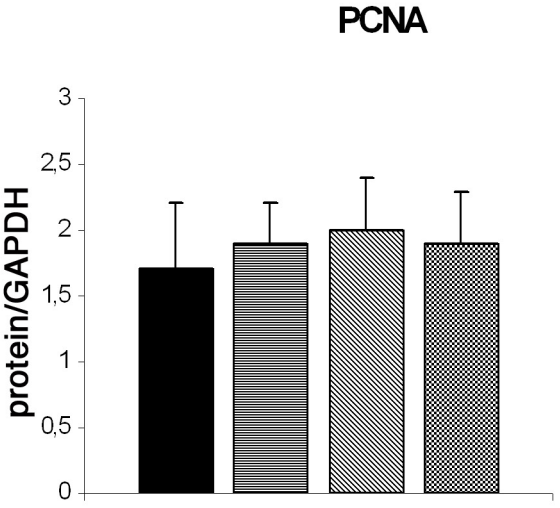
A) Anterior tibialis sections from 6 different animals (3 mock transfected controls and three pEGFP-RHOV14 transfected). Sections were stained for β-gal activity and laminin (black) in order to compare the fusion efficiency of pEGFP and pEGFP-RHOV14 transfected myoblasts. B) Quantification for 12 animals (6 mock transfected and 6 pEGFP-RHOV14 transfected) is reported in the bar graph. Numbers refer to the single animals including those for which images of the sections are shown.

Scale bare 100 μm.

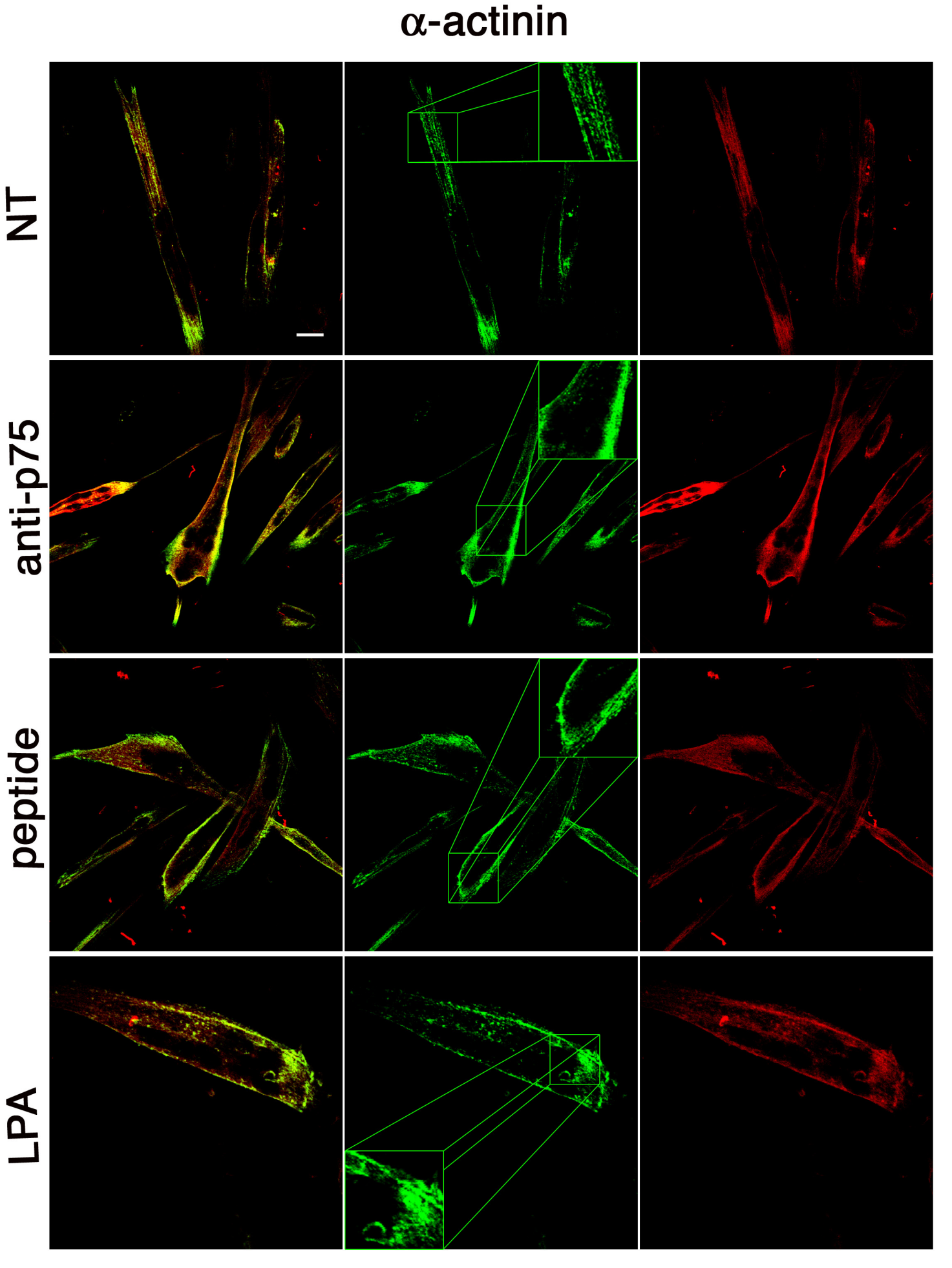
Supplementary Figure 1



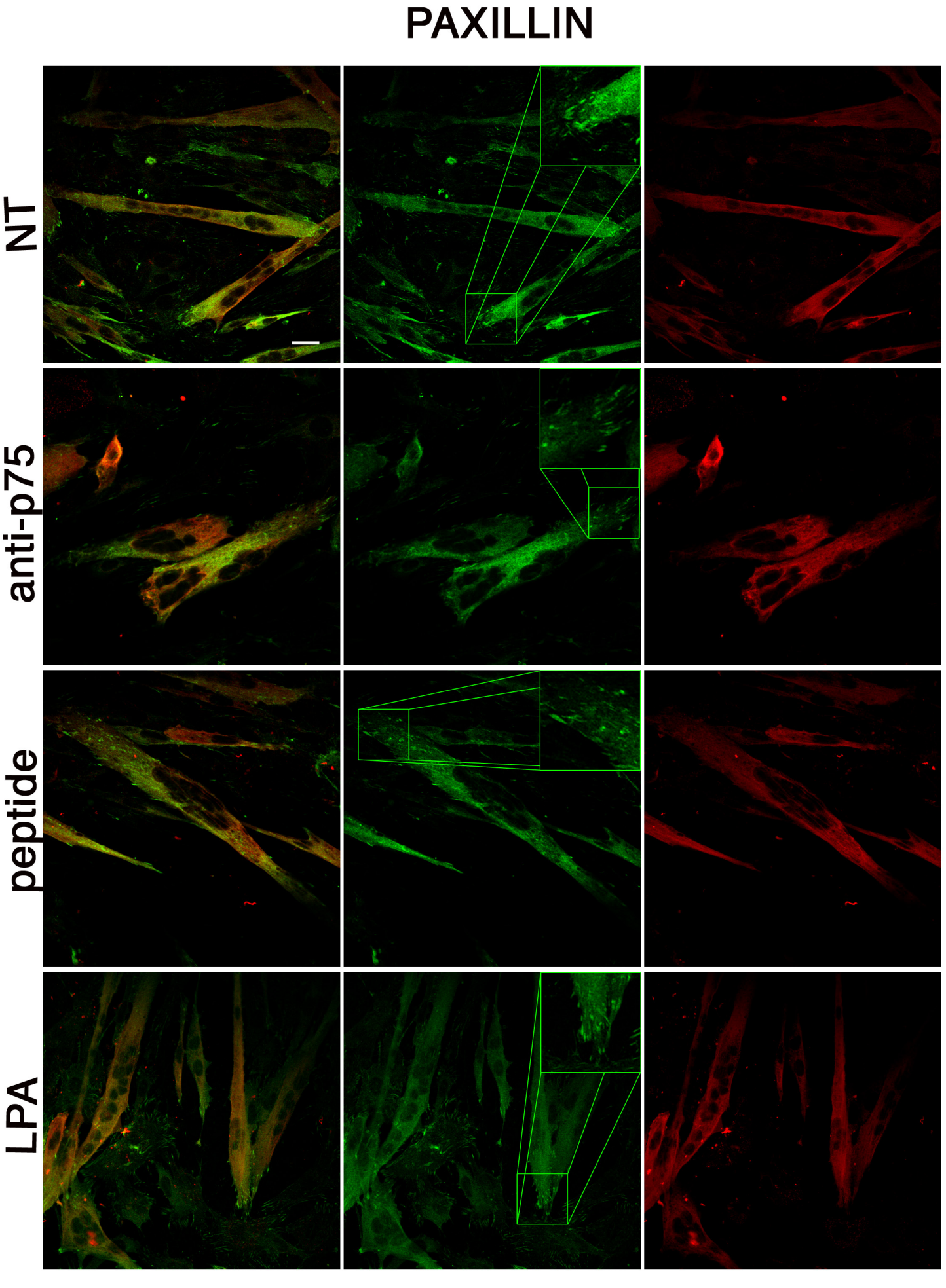




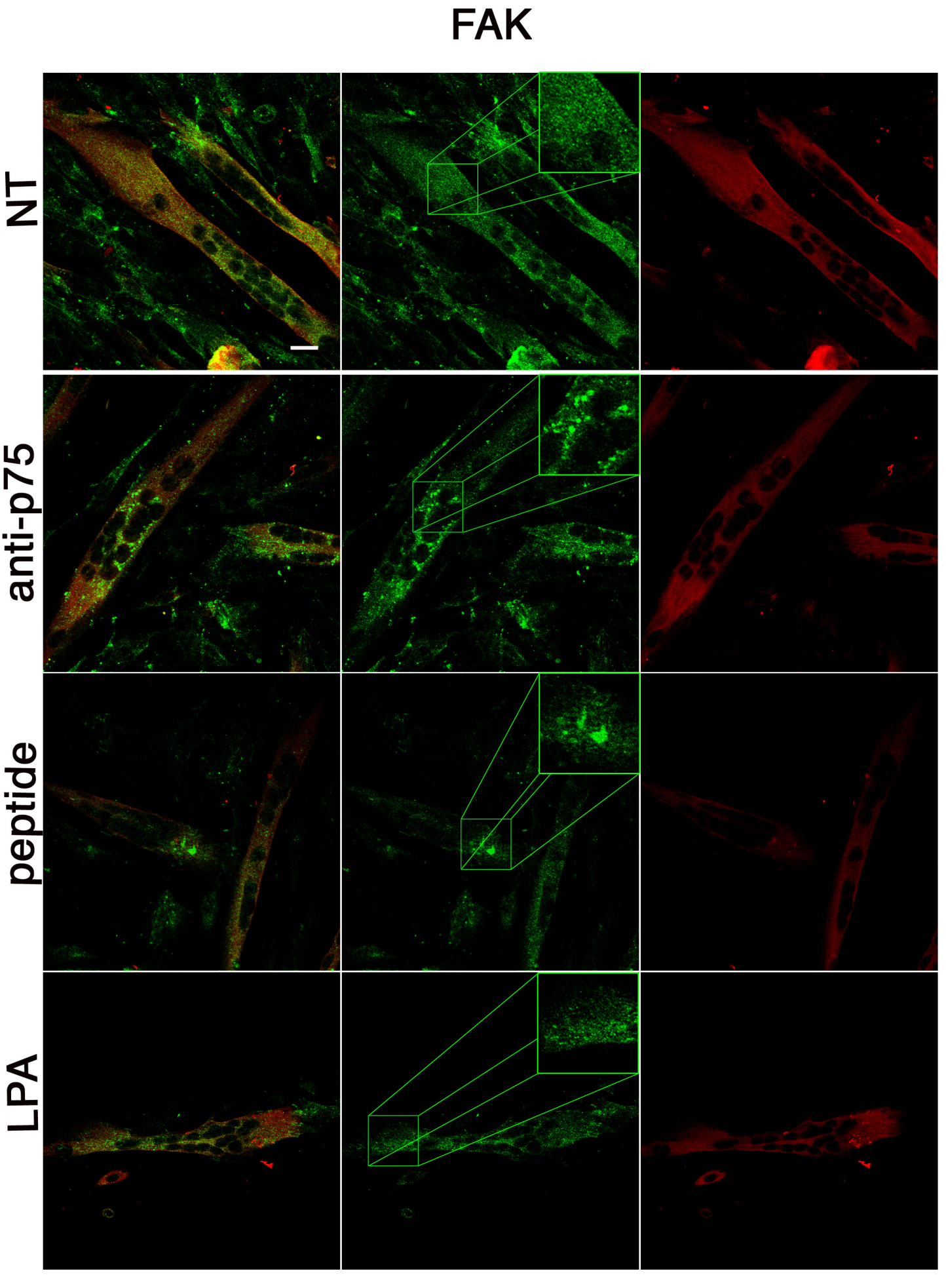
Supplementary Figure 2



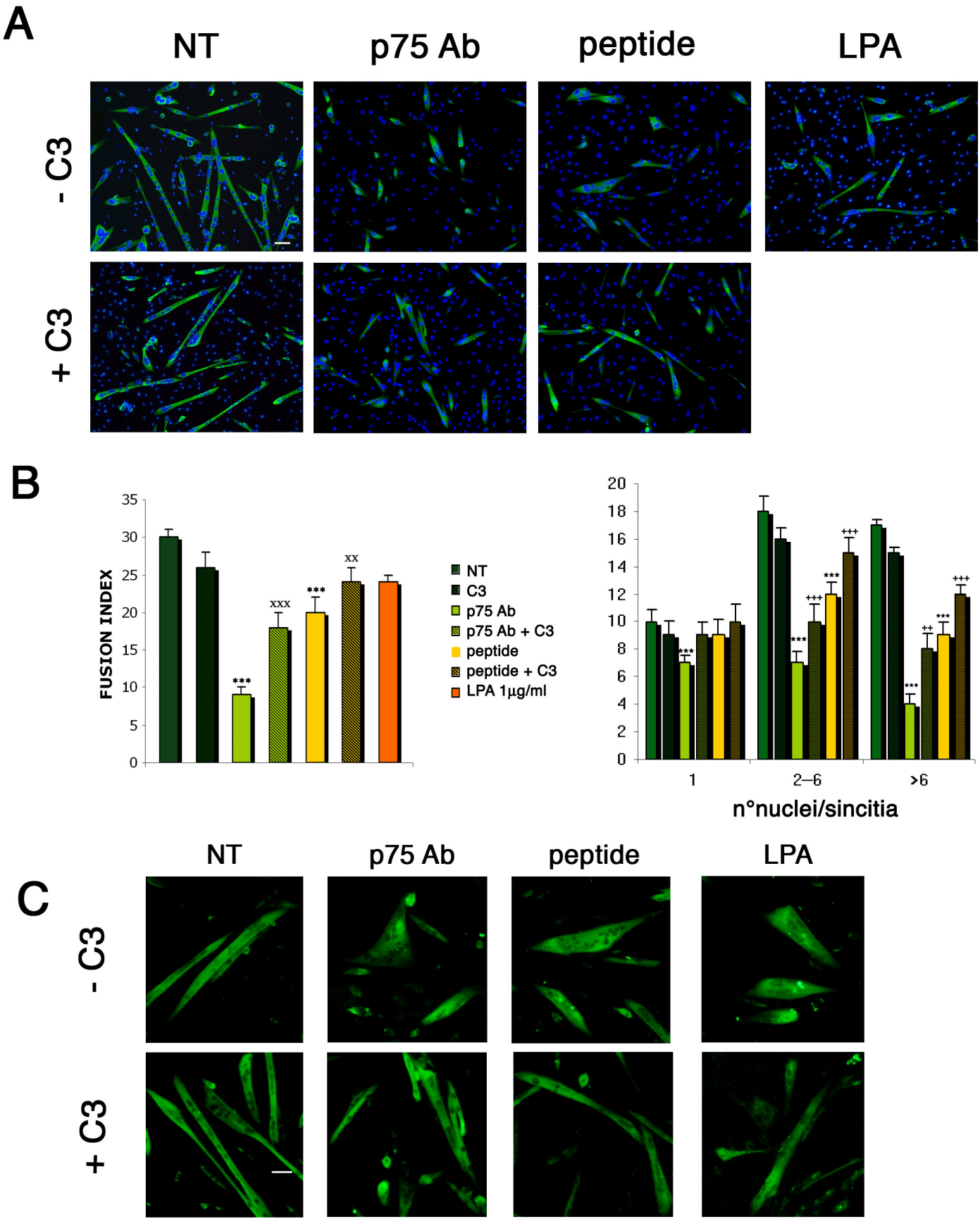
Supplementary Figure 3



Supplementary Figure 4



Supplementary Figure 5



Supplementary Figure 6