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Differential localisation and anabolic responsiveness of mTOR complexes in human skeletal muscle in response to feeding and exercise

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Abbreviations

mTOR – Mechanistic target of rapamycin

LAMP2 – Lysosomal associated membrane protein 2

WGA – Wheat Germ Agglutinin

Raptor – Regulatory associated protein of mTOR

Rictor – Rapamycin insensitive companion of mTOR

GβL - G protein beta subunit-like

PRAS40 - Proline-rich AKT1 substrate 1 of 40kDa

DEPTOR - DEP domain-containing mTOR-interacting protein

mSIN1 - Mammalian stress-activated protein kinase interacting protein 1

Protor – Protein observed with Rictor

MPS – Muscle protein synthesis

MPB – Muscle protein breakdown

S6K1 – Ribosomal protein S6 Kinase 1

AKT - RAC serine/threonine-protein kinase/ Protein kinase B

CASA – Chaperone assisted selective autophagy

Rheb – Ras homolog enriched in brain

eIF3F - Eukaryotic translation initiation factor 3 subunit F

Abstract

Mechanistic target of rapamycin (mTOR) resides as two complexes within skeletal muscle. mTOR complex 1 (mTORC1–Raptor positive) regulates skeletal muscle growth, whereas mTORC2 (Rictor positive) regulates insulin sensitivity. To examine the regulation of these complexes in human skeletal muscle, we utilised immunohistochemical analysis to study the localisation of mTOR complexes prior to and following protein-carbohydrate feeding (FED) and resistance exercise plus protein-carbohydrate feeding (EXFED) in a unilateral exercise model. In basal samples, mTOR and the lysosomal marker LAMP2 were highly co-localized and remained so throughout. In the FED and EXFED states, mTOR/LAMP2 complexes were redistributed to the cell periphery (WGA positive staining) (time effect; p=.025), with 39% (FED) and 26% (EXFED) increases in mTOR/WGA association observed 1h post-feeding/exercise. mTOR/WGA colocalisation continued to increase in EXFED at 3h (48% above baseline) whereas colocalisation decreased in FED (21% above baseline). A significant effect of condition (p=.05) was noted suggesting mTOR/WGA co-localization was greater during EXFED. This pattern was replicated in Raptor/WGA association, where a significant difference between EXFED and FED was noted at 3h post-exercise/feeding (p=.014). Rictor/WGA colocalization remained unaltered throughout the trial. Alterations in mTORC1 cellular location coincided with elevated S6K1 kinase activity, which rose to a greater extent in EXFED compared to FED at 1h post-exercise/feeding (p<.001), and only remained elevated in EXFED at the 3h time point (p=.037). Collectively these data suggest that mTORC1 redistribution within the cell is a fundamental response to resistance exercise and feeding, whereas mTORC2 is predominantly situated at the sarcolemma and does not alter localisation.

Introduction

Resistance exercise and protein ingestion are potent anabolic stimuli, elevating muscle protein synthesis (MPS) (5, 20) resulting in a positive net protein balance (NPB) (5). Such elevations in MPS are underpinned by the activation of the conserved serine/threonine kinase, mechanistic target of rapamycin (mTOR). This kinase can both augment MPS (17) and offset muscle protein breakdown (MPB) (19). In skeletal muscle, mTOR resides in two distinct complexes distinguishable by the composition of proteins within each. For example, complex 1 (mTORC1) contains mTOR, RAPTOR, GβL, PRAS40 and DEPTOR (4), believed to activate protein synthetic machinery (1), whereas complex 2 (mTORC2) is comprised of mTOR, RICTOR, DEPTOR, GβL, Sin1 and Protor, and is implicated in insulin sensitivity and actin cytoskeleton dynamics (4). Due to the critical role mTORC1 plays in regulating protein synthesis, this complex has received the most detailed examination in relation to resistance exercise and protein feeding. Acute resistance exercise, protein ingestion, or combinations of such stimuli are consistently reported to elevate mTORC1 activity (5, 20), with effects maintained for up to 24 hours (6). Furthermore, the acute inhibition of mTORC1 with rapamycin administration ablates any effect of anabolic stimuli on MPS (8, 9).

As mTORC1 activity seems to be directly implicated in the stimulation of MPS, research has focussed on understanding the mechanism by which mTORC1 is activated. Sancak et al.(21), identified the interaction of mTORC1 with the lysosome to be of particular importance to the activation of the kinase complex *in vitro*. A similar mechanism has also been reported in rodent skeletal muscle, where eccentric contractions of the tibialis anterior muscle induce mTOR-lysosome colocalisation (13) in parallel to increases in mTORC1 activity (inferred by the phosphorylation of $S6K1^{Thr389}$). Together these data infer an importance of mTOR-lysosome colocalisation in the activation of molecular pathways implicated in protein synthesis. Recently, however, Korolchuk et al. (15) reported that the cellular localisation of

these mTOR/lysosomal complexes play a pivotal role in mTOR activation. In support of this hypothesis, we recently reported that a single bout of resistance exercise initiated mTOR/lysosome translocation to the cell periphery, and occurred in parallel to an increase in mTOR activity and interaction between mTOR and proteins involved in translation initiation (23).

Whilst the use of immunofluorescence approaches allowed us to study the cellular localisation of mTOR, this approach did not enable us to distinguish between mTOR complexes. Consequently, we were unable to conclude whether the movement of mTOR following anabolic stimuli was mTORC1 or mTORC2 specific. Further, given the parallel group design we employed (23), we were unable to assess whether mTOR translocation was amplified by feeding. Therefore, the aim of the current study was to evaluate whether mTOR translocation following resistance exercise and/or protein-carbohydrate feeding is specific to mTORC1. In addition, we utilised a within-subject design to evaluate whether a synergistic effect of exercise and feeding exists. We hypothesised that exercise plus protein-carbohydrate feeding would elicit a greater mTOR/LAMP2 translocation to the cell periphery compared to feeding alone. Further, we hypothesised this translocation would be specific to mTORC1.

Methods

Subjects. Eight young, healthy, recreationally active males (age=22.5±3.1y, BMI=24.6±2.2kg/m^2, body fat=17.6±4.8%) volunteered to partake in the study. Potential participants were informed about all experimental procedures to be undertaken and any risks involved before written informed consent was obtained. The study was approved by the Hamilton Integrated Research Ethics Board (REB 14-736) and adhered to the ethical standards outlined by the Canadian tri-council policy statement regarding the use of human

111 participants in research as well as the principles according to the Declaration of Helsinki as
112 revised in 2008.

113 *Experimental design*. Following initial assessment for 1 repetition maximum (1-RM) on leg
114 extension 7 d previously, participants reported to the laboratory at ~7.00am after a 10-h
115 overnight fast. Participants then rested in a semi-supine position on a bed and an initial
116 skeletal muscle biopsy was taken from the *vastus lateralis* using a modified bergstrom
117 needle. Following this biopsy, participants performed 4 sets of unilateral leg extension
118 (Atlantis, Laval, QC, Canada) at 70% 1RM until volitional failure interspersed by 2 min
119 recovery. Immediately following the cessation of the final set of leg extension all participants
120 consumed a commercially available beverage (Gatorade Recover®, Chicago, IL, USA) that
121 provided 20, 44, and 1g of protein, carbohydrate, and fat respectively. Subsequent bilateral
122 skeletal muscle biopsies were obtained from the *vastus lateralis* at 1h and 3h after beverage
123 ingestion to examine mTORC1-related signalling and associated localisation.

124 *Skeletal muscle immunohistochemistry*. Skeletal muscle immunohistochemical preparation
125 and staining was conducted as described previously (23). All samples from each subject were
126 sectioned onto the same slide, in duplicate, to ensure accurate comparisons between time
127 points could be made.

128 *Antibodies*. The mouse mono-clonal anti-mTOR (#05-1592) antibody was purchased from
129 Merck Chemical Ltd. (Nottingham, UK). The corresponding conjugated secondary antibody
130 to this was Goat anti-mouse IgGγ1 Alexa®594 (#R37121, ThermoFisher, UK). Antibodies
131 targeting LAMP2 (#AP1824d, Abgent, USA), Rictor (CST#53A2, Cell Signalling
132 Technologies, USA) and Raptor (#ab40768, Abcam, Cambridge, UK) were visualised using
133 Goat anti-rabbit IgG(H+L) Alexa®488 secondary antibodies (#A11008, ThermoFisher, UK).

Finally, wheat germ agglutinin (WGA-350, #11263, ThermoFisher, UK) was used to identify the sarcolemmal membrane of muscle fibres.

Antibody Validation. The specificity of Rictor (CST#53A2) and Raptor (Abcam#ab40768) primary antibodies were tested utilising skeletal muscle samples from the gastrocnemius of muscle-specific knockout (mKO) mice for each protein respectively (3). Wild-type, littermate muscle samples for each mouse model were used as controls. Primary antibodies were also omitted from a subset of samples on slides to examine any background staining from the secondary antibody utilised. The fluorescence intensity of each image was then calculated using ImageJ software (Version 1.51 for Windows).

Image capture. Prepared slides were imaged as described previously (23). DAPI UV (340–380 nm) filter was used to view WGA-350 (blue) signals and mTOR proteins tagged with Alexa Fluor 594 fluorophores (red) were visualised under the Texas red (540–580 nm) excitation filter. The FITC (465–495nm) excitation filter was used to capture signals of mTOR-complex proteins and LAMP2, which were conjugated with Alexa Fluor 488 fluorophores. On average, 8 images were captured per section, and each image contained ~8 muscle fibres such that around 120 fibres per time point (per subject) were used for analysis. Image processing and analysis was undertaken on ImagePro Plus 5.1 (Media Cybernetics, MD., USA.) and all factors i.e. exposure time and de-speckling, were kept constant between all images on each individual slide. Image signals generated by WGA were used to estimate cell membrane borders, which were merged with the corresponding target protein images to identify the association between the protein of interest and the plasma membrane. Pearson's correlation coefficient (Image-Pro software) was used to quantify colocalization with the plasma membrane and mTOR-associated proteins. This process was also completed to quantify the localization of mTOR with complex-associated proteins (Raptor & Rictor) and a marker of the lysosomal membrane (LAMP2).

AKT and S6K1 Kinase Activity Assays. At each time point during the experimental trial, a separate piece of muscle tissue was blotted and freed from any visible adipose or connective tissue. The tissue was then frozen in liquid nitrogen and stored at -80ºC. The kinase activity of AKT and S6K1 was determined via [-γ-32P] ATP kinase assays following immuno-precipitation of the target protein, as previously described (18).

Statistical Analysis. All statistical analysis was conducted on SPSS version 22 for Windows (SPSS Inc., Chicago, IL, USA). Differences in staining intensity between mKO, wild type (WT) and primary omitted (CON) muscle sections were analysed using a one-way analysis of variance (ANOVA). Differences in kinase activity, fluorescence intensity and staining colocalisation were analysed using a two-factor mixed-model ANOVA with two within subject factors (time; three levels – PRE.vs.1h.vs.3h and condition; two levels – FEDvs.EXFED), with Bonferonni correction for multiple comparisons. Pairwise comparisons were conducted when a significant main/interaction effect was found. Significance for all variables analysed was set at $p \leq .05$. Data are presented as means±SEM unless otherwise stated.

Results

Rictor and Raptor antibodies are specific to their target proteins. Rictor protein staining intensity in Rictor mKO tissue was significantly lower than that in littermate WT controls ($p < .001$, Fig.1B). Furthermore, the staining intensity in this tissue was comparable to when the primary antibody was omitted in both mKO and WT tissue ($p > .999$, Fig.1B). Raptor protein staining intensity in Raptor mKO tissue was also significantly lower than that noted in littermate WT controls ($p < .001$, Fig.1C), with this staining intensity again similar to when the primary antibody was omitted in either tissue ($p > .999$, Fig.1C). Therefore, we take this as

evidence that the Rictor (CST#53A2) and Raptor (Abcam#ab40768) antibodies are specific to their target protein.

S6K1 and AKT kinase activity. A significant condition by time effect was observed for S6K1 activity ($p<.001$). S6K1 activity rose above baseline in both conditions at 1h post-exercise/feeding (FED-$p=.015$, EXFED-$p<.001$), and kinase activity at this time point was 165% greater in the EXFED condition ($p<.001$, Fig.1D). At 3h post-exercise/feeding, kinase activity only remained above baseline values in the EXFED condition (52.8% greater than baseline, $p=.037$, Fig.1D). A significant main effect for time was noted for AKT kinase activity ($p=.023$, Fig.1E). Pairwise comparisons displayed a trend toward an increase in AKT kinase activity 1h post-intervention, when conditions were combined, compared to 3h post-intervention ($p=.073$).

Lysosomal content and colocalisation with mTOR. LAMP2 fluorescence intensity was unchanged from baseline in either condition, however a significantly greater intensity was noted in the EXFED condition, compared to FED, at 3h post-exercise/feeding ($p=0.41$, Fig.2B). A significant condition × time effect was observed for mTOR-Lamp2 colocalisation ($p=.004$). Consistent with our previous work (23), mTOR and LAMP2 were highly localised in basal skeletal muscle (Fig.2C). The colocalisation of these two proteins did not change from baseline in either condition over the 3h post-exercise/feeding period. However, at the 3h time point, the colocalisation of the proteins was greater in the FED condition compared to the EXFED condition (0.51(FED)vs.0.47(EXFED), $p=.011$, Fig. 2C).

mTOR/lysosome translocation to the cell membrane. Significant main effects of condition ($p=.05$) and time ($p=.025$) were observed for mTOR colocalisation with the cell membrane (WGA positive staining). The significant main effect of condition suggests that, when all time points are combined, mTOR-WGA was greater in the EXFED condition compared to

206 the FED condition. Subsequent pairwise comparisons also display that when both conditions
207 were combined, mTOR colocalisation with the cell membrane was greater at 3h post-
208 exercise/feeding compared to baseline values (p=.008, Fig.2C). Further comparisons also
209 displayed a trend toward a difference between mTOR-WGA colocalisation between
210 conditions at the 3h time point (0.16(FED) vs. 0.19(EXFED), p=.085). This pattern of
211 colocalisation was mirrored when analysing LAMP2-WGA colocalisation (main effect of
212 time, p=.031, data not shown.), reiterating the constant colocalisation of mTOR and the
213 lysosome.

214 *Rictor colocalisation with mTOR and WGA*. Significant main effects of group (p=.046) and
215 time (p=.035) were noted for Rictor colocalisation with mTOR proteins (Fig.3B). Overall,
216 there was a greater colocalisation of these two proteins in the EXFED condition compared to
217 the FED condition. Following pairwise comparisons, there was no difference in the
218 colocalisation between Rictor and mTOR between any time points (p>.05, Fig.3B).
219 Furthermore, Rictor colocalisation with WGA did not change from baseline at any time point
220 in either condition (Fig.3C), suggesting post exercise translocation is specific to mTORC1.

221 *Raptor colocalisation with mTOR and WGA*. The colocalisation of Raptor and mTOR
222 proteins did not change in either group, at any time point, suggesting any alterations in sub-
223 cellular location of either protein occurred concurrently (Fig.4B). A significant condition x
224 time effect was observed for Raptor colocalisation with WGA (p=.029). Here, Raptor
225 colocalisation with WGA rose to a similar extent to the previously reported increase in
226 mTOR-WGA colocalisation at 1h post-exercise/feeding in both conditions. At the 3h time
227 point, Raptor-WGA colocalisation in the FED group dropped below baseline and 1h post-
228 ex/feeding levels (p=.007, Fig.4C), and colocalisation at this time point was greater in the
229 EXFED condition (0.12(FED) vs. 0.17(EXFED), p=.014, Fig.4C).

Discussion

Utilising a within-subject design, we report that a combination of unilateral resistance exercise and protein-carbohydrate feeding elicits a greater mTOR translocation toward the cell membrane than feeding alone. This observation is consistent with previous findings from our laboratory in which we reported that mTOR associates with the lysosome in basal skeletal muscle, with mTOR/lysosomal complexes translocating to the cell periphery following mTOR activation (23). Utilising immunofluorescent approaches to distinguish between mTORC1 and mTORC2, the present study extends this observation, suggesting that mTORC1 seems to be the predominant mTOR complex translocating in human skeletal muscle following anabolic stimuli, with mTORC2 in constant association with the cell membrane.

In addition to mTORC1 translocation to the cell periphery, we report a greater colocalisation of mTOR and LAMP2 in the FED condition, compared to the EXFED condition, at the 3h time point. This finding was unexpected and contrasted our previous research using a parallel group design (23). The greater association of mTORC1 with lysosomes in the FED condition would infer greater mTORC1 activity in this leg (13, 21); however, this was not apparent in our S6K1 kinase activity data. A possible explanation for this difference is the increased lysosomal content (LAMP2 fluorescence intensity) noted in the EXFED condition at this time point (Fig.2B). It is possible that the acute resistance exercise bout may have elicited an increase in chaperone assisted selective autophagy as a stress response to the strenuous exercise, as previously reported (24). This may have increased the free-lysosomal pool (24) and altered the ratio of mTOR-LAMP2 association. As this is only a proxy measure of lysosomal content, further research directed towards lysosomal biogenesis in response to physiological stimuli would be needed to address this mechanism.

Previous research from our laboratory has shown an elevation in mTOR association with the cell membrane in response to resistance exercise, in both the fed and fasted state (23). This association coincided with an increase in S6K1 kinase activity, suggesting that mTOR trafficking is associated with an increase in intrinsic mTOR activity. Consistent with this hypothesis, here we report that mTORC1-cell membrane association increased 1h post-intervention, in both FED and EXFED conditions, and the increment was similar to that noted in our previous work (23). However, in contrast to our previous results, mTOR-WGA colocalisation in the FED condition returned close to baseline values at 3h and colocalisation in the EXFED condition displayed a continued elevation. In addition to the main effects of time ($p=.025$) and condition ($p=.05$) apparent here, a trend toward greater colocalisation in the EXFED condition ($p=.085$) was noted at the 3h time point. This greater colocalisation is suggestive of retention of mTOR at the cell periphery when resistance exercise is followed with protein/carbohydrate ingestion, inferring a synergistic effect of resistance exercise and protein-carbohydrate feeding, an observation previously reported for MPS (5).

The mTORC1 and mTORC2 protein complexes are involved in varying metabolic signalling processes in skeletal muscle, and as such are suggested to reside in distinct cellular locations (4). As mTOR-lysosome translocation has been previously associated with mTORC1 activation in response to amino-acids *in vitro* (15), we sought to determine whether mTORC1 is the principal mTOR complex translocating in human skeletal muscle as we have previously reported (23).The colocalisation of Raptor with WGA increased at 1h post-intervention in both conditions, and to a similar extent to that noted in mTOR-WGA colocalisation, suggesting that mTORC1 is a spatially regulated mTOR complex in human skeletal muscle. Further to this notion, a disparity between conditions became apparent at the 3h time point, with Raptor-WGA colocalisation enhanced in the EXFED condition ($p=.014$). This is in agreement with the data regarding mTOR-WGA colocalisation where a trend toward EXFED

eliciting greater membrane colocalisation compared to FED is reported. Raptor colocalisation with mTOR itself was not altered at any time point, or between conditions, however we did observe a reduction in raptor association with WGA at 3h in both FED and EXFED. We are currently unable to explain this result, however, it could be due to an increase in free Raptor content (14) or increased Raptor degradation (12), both potential mechanisms proposed to regulate mTORC1 activity. In contrast, co-staining of Rictor with WGA, suggested that mTORC2 localises with the cell membrane in basal tissue, with this colocalisation unaffected by resistance exercise or protein/carbohydrate ingestion. This finding has also been replicated using *in vitro* models, where a large proportion of mTORC2 activity was noted at the plasma membrane of HEK293 cells (10).

Our data are congruent with both *in vitro* (15) and *in vivo* (23) studies suggesting that mTORC1 cellular colocalisation is linked to mTORC1 activity. Whilst this observation is in contrast to previous *in vitro* studies (21, 25), where mTOR translocation to the lysosome is deemed essential, we believe the increase in autophagy/MPB in post-absorptive skeletal muscle prevents the disassociation of mTOR and the lysosome noted in previous *in vitro* studies, where a complete amino acid withdrawal protocol is utilised. Further, many physiological mechanisms occur at the cell periphery suggesting the redistribution of mTORC1/lysosomal complexes to the cell periphery is physiologically relevant. mTORC1 is known to stimulate MPS which, through the use of the SUnSET technique (22) and immunohistochemical staining methods, is purported to occur primarily in peripheral regions of muscle fibres (11). Consistent with this, we previously identified mTOR to interact with Rheb, eIF3F and the microvasculature at the cell periphery following resistance exercise in the fed state (23). Collectively this data therefore suggests that both upstream regulators and downstream substrates of mTORC1 are membrane-associated in skeletal muscle (11). Further, given we observed that mTORC1 association with the cell periphery was prolonged

with feeding [a scenario of heightened MPS], we propose that maintaining mTORC1 at the cell periphery may provide an mechanistic explanation as to why exercise in the fed state results in prolonged increases in MPS in human skeletal muscle compared to exercise or feeding in isolation (6).

In summary, our data show that mTOR-lysosome translocation in response to resistance exercise and feeding is driven primarily by mTORC1, and occurs in parallel to increases in S6K1 activity. Further, we report that resistance exercise combined with protein-carbohydrate feeding sustains this response, compared to feeding alone, suggesting a synergistic effect of these two stimuli. Collectively, these data add further support to the importance of spatial regulation of mTORC1 in response to anabolic stimulation. Further research should now examine the relevance of mTORC1 colocalisation in clinical scenarios, i.e ageing (7) or obesity (2). Finally, the tools described herein to study mTORC2 localisation could be used to examine the regulation of skeletal muscle glucose uptake and insulin sensitivity, factors thought to be under the direct control of mTORC2 (16).

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Author Contributions: NH and AP conceived the study. NH analysed data presented in Figures 1-4. CM, SO and SMP conducted human experiments reported in Figures 2-4. SJ, ZS and DLH performed experiments and analysis presented in Fig 1. MAR generated the Raptor and Rictor mKO mice used in figure 1. NH and AP interpreted results of experiments. NH conducted statistical analysis and prepared figures. NH and AP drafted the manuscript, with all authors approving the final version of the manuscript.

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Figure headings

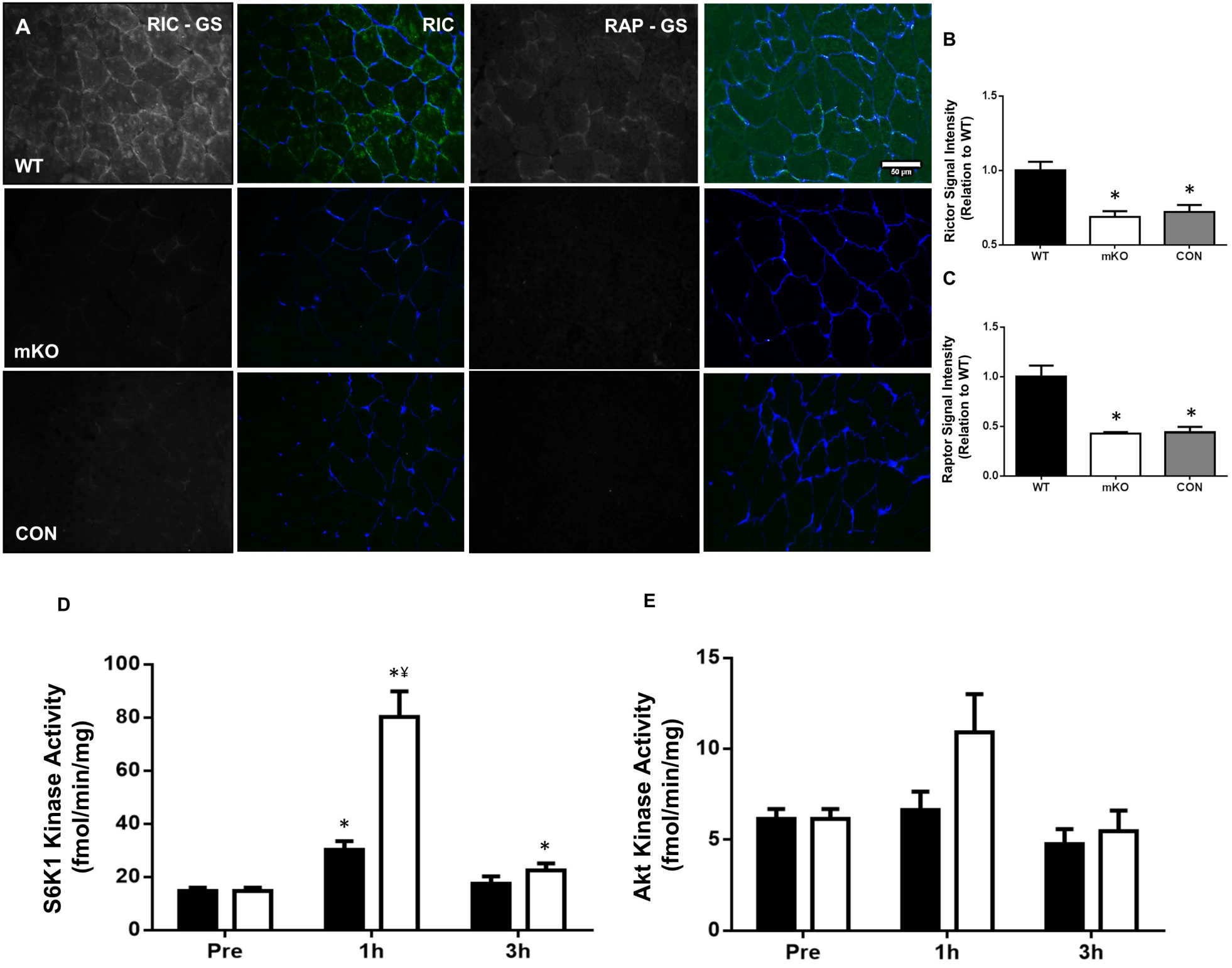
Figure 1. Rictor and Raptor antibody validation and S6K1 and AKT kinase activity. Immuno-fluorescent staining of each protein was performed in mKO and littermate WT samples, in addition to staining of each sample with primary antibodies omitted (CON). Rictor/Raptor is displayed in green and WGA (cell membrane) is stained in blue. Representative images of staining in each condition are displayed (A) alongside the corresponding quantifications for Rictor (B) and Raptor (C). Scale bars are 50μm. Data presented as mean±SEM. *Significantly different WT ($p<.001$). S6K1 (D) and AKT (E) kinase activity following unilateral resistance exercise and/or protein-carbohydrate feeding. Black bars denote FED condition and open bars denote EXFED condition. Data presented as Mean±SEM. *Significantly different to baseline ($p<.05$), ¥significant difference between conditions at this time point ($p<.001$).

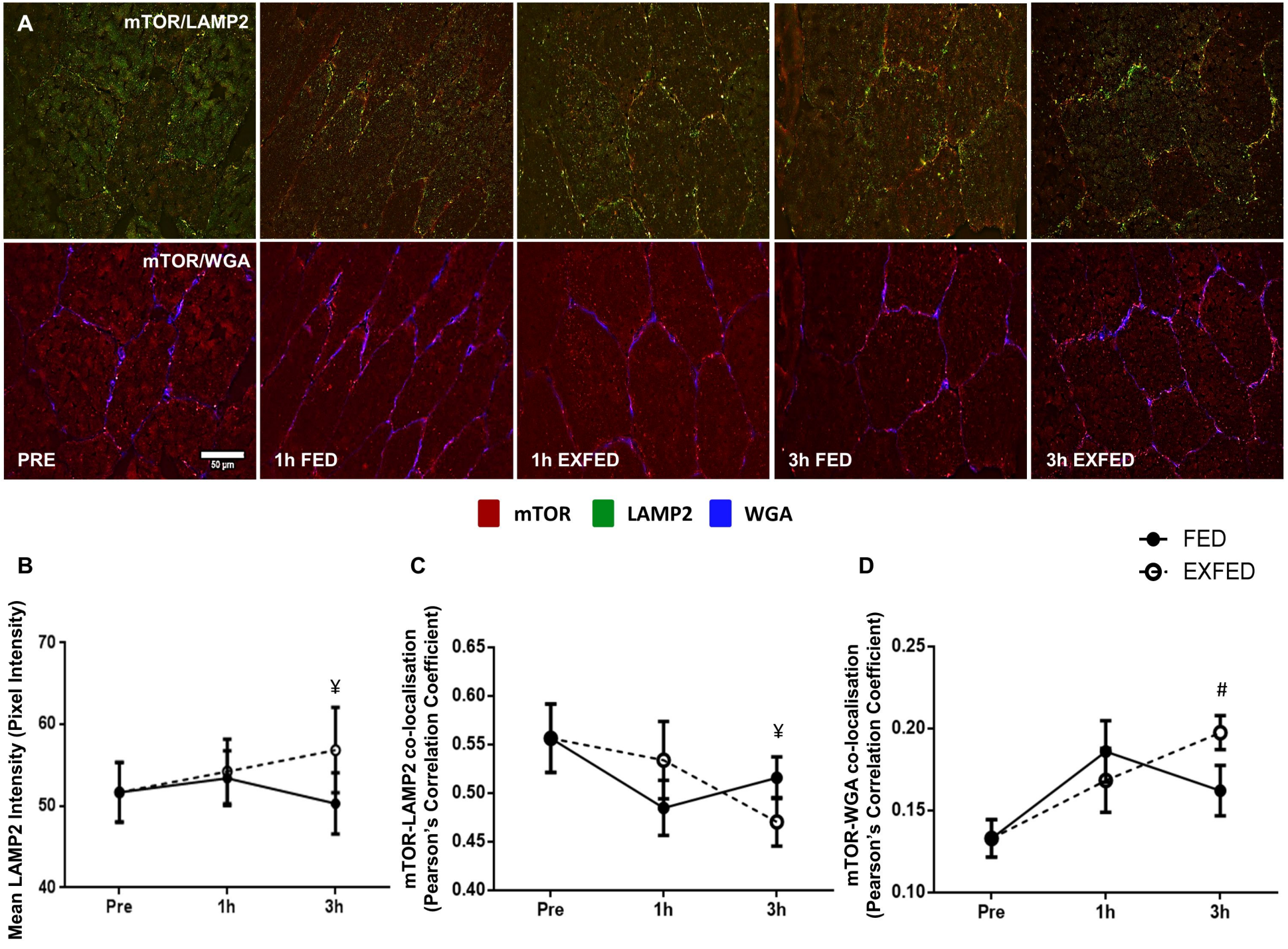
Figure 2. The effect of resistance exercise and/or protein carbohydrate feeding on mTOR-LAMP2 and mTOR-WGA colocalisation. Representative images of mTOR-LAMP2 and mTOR-WGA co-localisation at rest, and following resistance exercise and/or protein-carbohydrate feeding (A). Orange/yellow regions denote areas of mTOR localisation with the marker of the lysosome in images on the top row. mTOR-positive staining is shown in red, LAMP2-positive in green and WGA-positive in blue. Quantification of LAMP2 fluorescence intensity (B), mTOR-LAMP2 colocalisation (C) and mTOR-WGA (D) co-localisation at each time point. Scale bars are 50μm. Data presented as Mean±SEM. ¥Significant difference between conditions at this time point ($p<.05$), #significantly difference compared to baseline when conditions combined ($p=.008$).

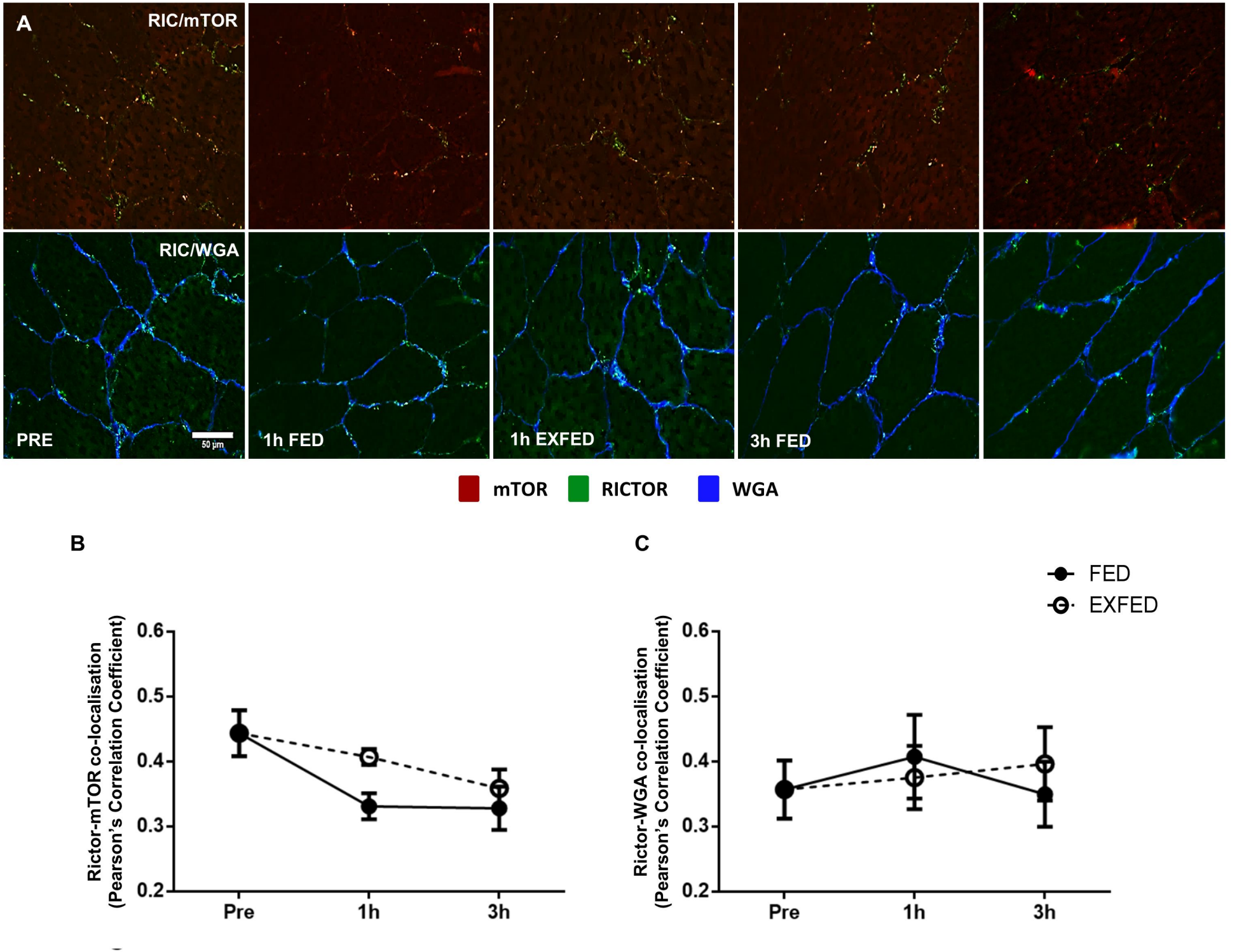
Figure 3. The effect of resistance exercise and/or protein carbohydrate feeding on Rictor-mTOR and Rictor-WGA colocalisation. Representative images of Rictor-mTOR

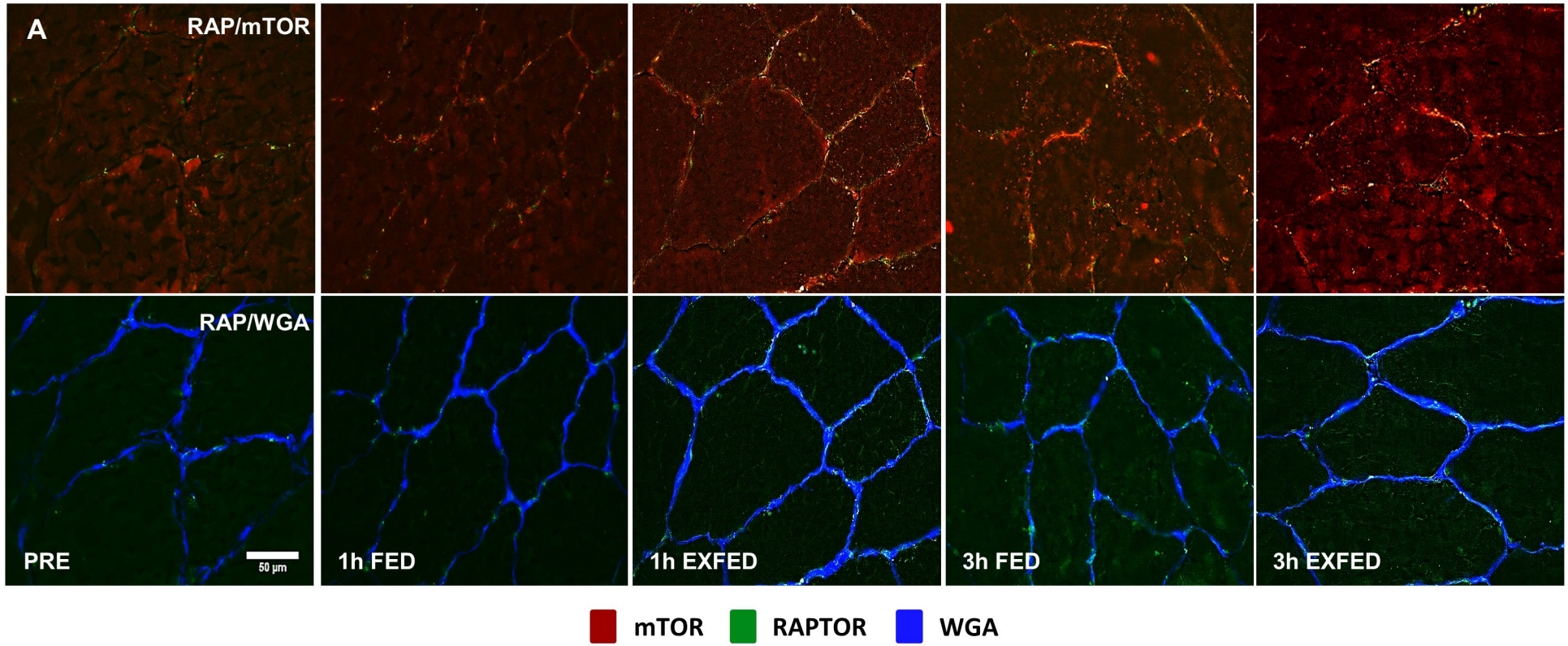
and Rictor-WGA co-localisation at rest, and following resistance exercise and/or protein-carbohydrate feeding (A). Orange/yellow regions denote areas of Rictor localisation with mTOR on top row. mTOR-positive staining is shown in red, Rictor-positive in green and WGA-positive in blue Quantification of Rictor-mTOR (B) and Rictor-WGA (C) co-localisation at each time point. Scale bars are 50μm. Data presented as Mean±SEM.

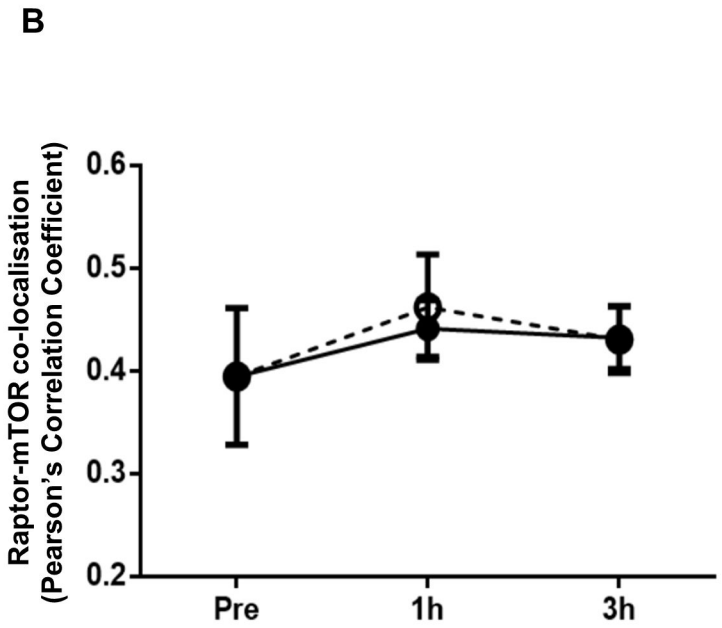
Figure 4. The effect of resistance exercise and/or protein carbohydrate feeding on Raptor-mTOR and Raptor-WGA colocalisation. Representative images of Raptor-mTOR and Raptor-WGA co-localisation at rest, and following resistance exercise and/or protein-carbohydrate feeding (A). Orange/yellow regions denote areas of Raptor localisation with mTOR. mTOR-positive staining is shown in red, Raptor-positive in green and WGA-positive in blue Quantification of Raptor-mTOR (B) and Raptor-WGA (C) co-localisation at each time point. Scale bar is 50μm. Data presented as Mean±SEM. [¥]Significant difference between conditions at this time point (p=014), [#]significantly difference compared to baseline when conditions combined (p=.007).

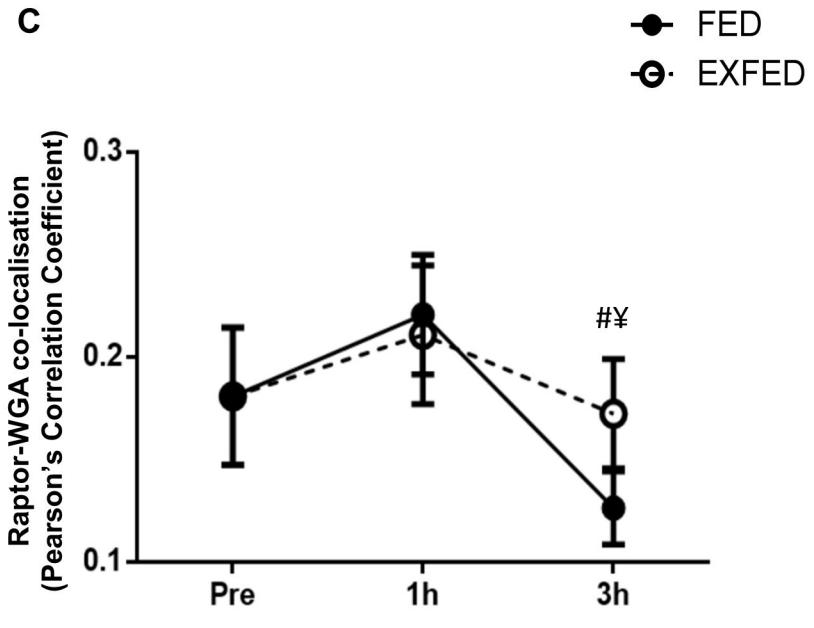

A
RIC - GS
RIC
RAP - GS
WT
mKO
CON
50 μm
B
Rictor Signal Intensity
(Relation to WT)
1.5
1.0
0.5
*
*
WT
mKO
CON
C
Raptor Signal Intensity
(Relation to WT)
1.5
1.0
0.5
0.0
*
*
WT
mKO
CON
D
S6K1 Kinase Activity
(fmol/min/mg)
100
80
60
40
20
0
*
*¥
*
Pre
1h
3h
E
Akt Kinase Activity
(fmol/min/mg)
15
10
5
0
Pre
1h
3h


A
mTOR/LAMP2
mTOR/WGA
PRE
50 µm
1h FED
1h EXFED
3h FED
3h EXFED
mTOR
LAMP2
WGA
B
Mean LAMP2 Intensity (Pixel Intensity)
70
60
50
40
Pre
1h
3h
¥
C
mTOR-LAMP2 co-localisation (Pearson's Correlation Coefficient)
0.65
0.60
0.55
0.50
0.45
0.40
Pre
1h
3h
¥
D
FED
EXFED
mTOR-WGA co-localisation (Pearson's Correlation Coefficient)
0.25
0.20
0.15
0.10
Pre
1h
3h
#


A
RIC/mTOR
RIC/WGA
PRE
50 µm
1h FED
1h EXFED
3h FED
mTOR
RICTOR
WGA
B
Rictor-mTOR co-localisation
(Pearson's Correlation Coefficient)
0.6
0.5
0.4
0.3
0.2
Pre
1h
3h
C
FED
EXFED
Rictor-WGA co-localisation
(Pearson's Correlation Coefficient)
0.6
0.5
0.4
0.3
0.2
Pre
1h
3h


A
RAP/mTOR
RAP/WGA
PRE
50 µm
1h FED
1h EXFED
3h FED
3h EXFED
mTOR
RAPTOR
WGA


B
Raptor-mTOR co-localisation
(Pearson's Correlation Coefficient)
0.6
0.5
0.4
0.3
0.2
Pre
1h
3h


C
FED
EXFED
Raptor-WGA co-localisation
(Pearson's Correlation Coefficient)
0.3
0.2
0.1
#¥
Pre
1h
3h