Glucagon-Like Peptide-1 Receptor Activation Stimulates PKA-Mediated Phosphorylation of Raptor and this Contributes to the Weight Loss Effects of Liraglutide

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ABSTRACT

Objective: Glucagon-like peptide 1 (GLP-1) receptor (GLP-1R) agonists are approved for obesity management, yet surprisingly little is known about the signaling mechanisms mediating GLP-1R agonist-induced anorexia and weight loss. We have shown that GLP-1R activation in the ventromedial hypothalamus (VMH) stimulates mechanistic Target of Rapamycin Complex 1 (mTORC1) activity, and this is necessary for the ability of VMH GLP-1R agonism to reduce food intake and body weight. Here, we aim to define the signaling mechanisms by which GLP-1R activation induces mTORC1 activity to regulate body weight. Although mTORC1 is classically activated by Akt, previous studies show that the canonical target of the GLP-1R, Protein Kinase A (PKA), stimulates mTORC1 signaling by phosphorylating the mTOR-regulating protein Raptor at Ser^{791} following β-adrenergic stimulation. Thus, we tested whether GLP-1R agonists similarly stimulate mTORC1 activity via PKA phosphorylation of Raptor at Ser^{791} and whether this phosphorylation event is relevant to the weight loss effect of the therapeutic GLP-1R agonist liraglutide.

Methods: We treated Chinese Hamster Ovary (CHO-K1) cells stably expressing the human GLP-1R (*hGLP1R*) with the GLP-1R agonist liraglutide in combination with either of the PKA inhibitors H89 or KT5720 and measured phosphorylation of the mTORC1 signaling target ribosomal protein S6. To test whether PKA phosphorylates Raptor at Ser^{791} in response to liraglutide, we expressed myc-tagged wild-type (WT) Raptor or a PKA-resistant (Ser^{791}Ala) Raptor mutant in *hGLP1R*-stably expressing CHO-K1 cells, treated them with vehicle or liraglutide, immunoprecipitated myc-WT or myc-Ser^{791}Ala Raptor, and immunoblotted for phosphorylation of the PKA substrate RRXS*/T* motif, as previously described by Liu *et al.*[1]. To assess the physiological relevance of GLP-1R agonist-induced PKA phosphorylation of Raptor, we measured the body weight response to vehicle or liraglutide in WT mice and mice with a targeted knock-in of PKA-resistant Ser^{791}Ala Raptor.

Results: In CHO-K1 cells stably expressing the *hGLP1R*, liraglutide significantly increased phosphorylation of S6 and PKA phosphorylation of myc-tagged WT Raptor, both of which were blocked by PKA inhibition. Liraglutide also failed to stimulate phosphorylation of the PKA-targeting motif in cells expressing myc-Ser^{791}Ala Raptor. In addition, knock-in mice expressing Ser^{791}Ala Raptor were partially resistant to GLP-1R agonist-induced weight loss.

Conclusion: Our results demonstrate that PKA activation downstream of the GLP-1R leads to phosphorylation of Raptor at Ser^{791}, which activates mTORC1 signaling in a non-canonical fashion to facilitate the weight lowering effects of liraglutide.

Key Words: Glucagon-like peptide 1 receptor, Protein Kinase A, mechanistic Target of Rapamycin, Raptor, food intake, weight loss

Abbreviations: GLP-1R, Glucagon-like peptide 1 receptor; PKA, Protein Kinase A; mTORC1, mechanistic Target of Rapamycin Complex 1

1. INTRODUCTION

Obesity and its associated comorbidities have become some of the largest public health challenges across the world in recent decades.[2,3] Glucagon-like peptide-1 (GLP-1) receptor (GLP-1R) agonists not only stimulate insulin secretion but also act on several regions of the brain to reduce food intake and body weight.[4–8] As such, GLP-1R agonists are among a handful of pharmacological options currently approved for weight loss.[9] However, their high cost, injectable delivery, and adverse effects such as nausea limit their access and use. Our current understanding of GLP-1R signaling with regards to mechanisms that regulate feeding behavior and body weight is limited. Elucidating the cellular mechanisms mediating the weight-lowering effects of GLP-1R agonists could enhance the therapeutic potential of these compounds or identify novel targets for anti-obesity drug development.

Our lab has shown that the GLP-1R agonist Exendin 4 (Ex4) stimulates mechanistic Target of Rapamycin (mTOR) Complex-1 (mTORC1) signaling in Chinese Hamster Ovary (CHO-K1) cells stably expressing the human GLP-1R (*hGLP1R*).[10,11] We have also demonstrated that inhibition of mTORC1 signaling in the ventromedial hypothalamus (VMH) blocks the reduction in food intake and body weight induced by GLP-1R activation in this brain nucleus.[11] The goal of this study is to better define the mechanism(s) by which GLP-1R agonism stimulates mTORC1 signaling and thereby reduces body weight.

mTOR is a Ser/Thr protein kinase exerting its biological activities by forming two structurally and functionally distinct complexes: mTORC1 and mTOR complex 2 (mTORC2).[12] The rapamycin-sensitive mTORC1 has unique accessory proteins including Raptor (regulatory associated protein of mTOR).[13] mTORC1 activity is stimulated by a number of cellular signals such as growth factors, nutrients, hormones, and hypoxia. Classically, growth factors and insulin activate mTORC1 by promoting PI3K/Akt-mediated phosphorylation of tuberous sclerosis complex 1/2 (TSC1/2) and subsequent activation of a small GTPase Rheb (Ras homolog enriched in brain).[12] The GLP-1R,

on the other hand, is a $G_{\alpha}s$-coupled receptor that canonically signals *via* cyclic AMP production and subsequent activation of protein kinase A (PKA),[14] making activation of mTORC1 downstream of the GLP-1R unlikely. However, upon activation of another $G_{\alpha}s$-coupled receptor, the β-adrenergic receptor, it has been shown that PKA can phosphorylate Raptor to activate mTORC1 activity.[1] This raises the possibility that published findings from our lab showing activation of mTORC1 signaling in response to GLP-1R activation[10,11] may be mediated by PKA phosphorylation of Raptor.

In this study, we demonstrate that GLP-1R activation induces phosphorylation of Raptor at Ser^{791} by PKA, which is in turn required for the stimulatory effect of GLP-1R agonism on mTORC1 activity. Importantly, we show that interference with phosphorylation of Raptor by PKA reduces the ability of the therapeutic GLP-1R agonist liraglutide to suppress food intake and body weight. These findings reveal a novel GLP-1R-PKA-mTORC1 axis responsible for facilitating the weight-lowering effects of GLP-1R agonists such as liraglutide.

2. MATERIALS AND METHODS

2.1 Cell culture

Chinese Hamster Ovary (CHO-K1) cells expressing *hGLP-1R* (DiscoverX, Fremont, CA) were proliferated and maintained at 37°C (5% CO2, humidified) in Ham's F12 media containing 10mM glucose, 1mM pyruvate, and 1mM glutamine (Cat. No. 11765054, Gibco) supplemented with 10% fetal bovine serum (FBS; Cat. No. 16140071, Gibco), 10ml/l penicillin-streptomycin (Cat. No. 91, Hyclone, Rockford, IL), and 0.8mg/ml Geneticin G418 Sulfate (Cat. No. 10131027, Gibco) to preserve the selection of *hGLP-1R*-expressing cells. β-TC3 cells[15] were a generous gift from Dr. Richard O'Brien and cultured in DMEM media containing 25mM glucose, 1mM pyruvate, and 1mM glutamine (Cat. No. 11995065, Gibco) supplemented with 15% FBS, 2% horse serum (Cat. No. 26050088, Gibco) and 10ml/l penicillin-streptomycin. Prior to treatment, *hGLP-1R*-expressing CHO-K1 cells were washed twice with and maintained in serum-free Ham's F12 media and β-

TC3 cells in serum-free DMEM media containing 5.5 mM glucose for at least 3 hours. Cells were treated with vehicle (1X PBS), liraglutide (Victoza, Novo Nordisk, Plainsboro, NJ), or semaglutide (Ozempic, Novo Nordisk, Plainsboro, NJ) for 1 hour with or without a 30-min pre-treatment with vehicle (DMSO), either of the PKA inhibitors – H89 (Cat. No. 2910, Tocris Bioscience, Minneapolis, MN) or KT5720 (Cat. No. 1288, Tocris Bioscience, Minneapolis, MN), or either of the Akt inhibitors – Akti-1/2 (Cat. No. 5773, Tocris Bioscience, Minneapolis, MN) or MK-2207 (Cat. No. S1078, Selleck Chemicals LLC, Houston, TX) at concentrations stated in the figure legends.

2.2 Immunoblotting

Traditional Western Blotting. Treated cells were washed once with ice-cold 1X PBS and lysed in RIPA buffer (Cat. No. R0278, Sigma-Aldrich) containing 1X protease and phosphatase inhibitors. Supernatant was collected after centrifugation of cell homogenates at 17,000xg for 10 min at 4°C. Protein concentration was determined by performing the BCA protein assay (Pierce BCA Protein Assay Kit, Cat. No. 23225, Thermo Scientific). Unless otherwise indicated, total protein (20 ug/lane) was loaded and run in 4%–20% Tris-glycine gels (Invitrogen) at 200V for 45 min and transferred onto PVDF membranes (Bio-Rad) at 100 V for 60 min at 4°C. Blots were blocked in a blocking buffer containing 5% bovine serum albumin (BSA, Cat. No. A9418, Sigma-Aldrich) in 1X Tris-Buffered Saline and 0.1% Tween® 20 Detergent (TBS-T) for 1 hr and then incubated in corresponding primary antibodies (phospho-S6 $Ser^{235/236}$, Cat. No. 4858; total S6, Cat No. 2317; phospho-p70S6K Thr^{389}, Cat. No. 9205; total p70S6K, Cat. No. 9202; p-CREB, Cat. No. 9198; total CREB, Cat. No. 9104; phospho-Akt Thr^{308}, Cat. No. 9275; phospho-Akt Ser^{473}, Cat. No. 4060; total Akt, Cat. No. 9272; and myc-tag, Cat. No. 2276; all from Cell Signaling Technology) overnight at 4°C and secondary antibodies (IRDye 800CW Goat anti-Rabbit IgG, Cat. No. 926-32211, and IRDye® 680RD Goat anti-Mouse IgG, Cat. No. 926-68070, LI-COR Biosciences) for 1 hr at room temperature. Blots were imaged with an Odyssey Imaging System and full lanes were analyzed using ImageJ.

In-Cell Western Blotting. *hGLP-1R*-expressing CHO-K1 cells were seeded at approximately 1.5×10^4 cells per well on 96-well plates in Ham's F12 media as described above. Prior to treatment, cells were washed twice with and maintained in serum-free Ham's F12 media for at least 3 hours. Cells were then treated with vehicle (DMSO) or either H89 or KT5720 followed by treatment with vehicle (1X PBS) or liraglutide for 1 hr at concentrations stated in the figure legend. Medium was discarded and cells fixed with 4% formaldehyde in PBS (50 µl/well) for 15 min at room temperature. Cells were washed three times for 5 minutes each with room temperature 1X PBS (100 µl/well) and then blocked in 1X PBS containing 5% normal goat serum (Cat. No. 5425, Cell Signaling Technology) and 0.3% Triton X-100 (Blocking Buffer) for 1 hr at room temperature (50 µl/well). Blocking buffer was then replaced by respective primary antibodies (see above) diluted in 1X PBS containing 1% normal goat serum (Cat. No. 5425, Cell Signaling Technology) and 0.3% Triton X-100 (total volume 50 µl/well). Plates were covered and incubated overnight at 4°C. Cells were washed three times for 5 minutes each with 1X PBS and incubated in diluted secondary antibodies (IRDye 800CW Goat anti-Rabbit IgG and IRDye® 680RD Goat anti-Mouse IgG) for 1 hr at room temperature in the dark. Cells were washed three times with 1X PBS for 5 min each and plates were imaged by scanning simultaneously with an Odyssey Imaging System.

2.3 Immunoprecipitation

Myc-tagged wild-type (WT) Raptor and PKA-resistant mutant Raptor vectors were obtained and generated as described by Liu *et al.*[1] CHO-K1 cells stably expressing *hGLP-1R* were transfected with myc-tagged WT Raptor or myc-tagged Ser^{791}Ala Raptor plasmids using lipofectamine 3000 (Invitrogen) according to the instructions provided. Forty-eight hr post transfection, cells were washed 2X and maintained in serum-free medium for at least 3 hr. Cells were treated with DMSO, H89 or MK-2207 for 30 min and then treated with 1X PBS, liraglutide, FSK, or insulin for 1 hr at concentrations stated in the figure legend. Treated cells were washed once with ice-cold 1X PBS

and lysed in lysis buffer (25mM Tris HCl, 131 150mM NaCl, 1µM EDTA, 1µM EGTA, 2.5mM sodium pyrophosphate, 100mM sodium fluoride, 1mM sodium orthovanadate) containing 1X protease and phosphatase inhibitors. Supernatant was collected after centrifugation of cell homogenates at 17,000xg for 10 min at 4°C. Protein concentration was determined by performing the BCA protein assay (Pierce BCA Protein Assay Kit, Cat. No. 23225, Thermo Scientific). 200 ug total protein was incubated with anti–c-myc affinity gel (EZview™ Red Anti-c-Myc Affinity Gel, Cat. No. E6654, Sigma-Aldrich) overnight. Beads were washed thoroughly in lysis buffer. Protein phosphorylation by PKA was measured by immunoblotting as described above using a PKA substrate monoclonal antibody (phospho-RRXS*/T* motif antibody; Cat. No. 9624, Cell Signaling Technology).

2.4 Animals

All mice were housed in the Vanderbilt University Medical Center animal vivarium and kept on a standard 12 hr/12 hr light/dark cycle. They had *ad libitum* access to distilled water and were maintained on a chow diet (57.9% calories provided by carbohydrates, 28.7% protein, 13.4% fat; 3.36 kcal/g; 5L0D, LabDiet, St. Louis, MO) from the time of weaning. All mice were 12–16 weeks old at the time of testing. Experimental procedures were approved by the Institutional Animal Care and Use Committee at Vanderbilt University.

Generation of mice expressing Ser^{791}Ala Raptor in all tissues. Ser^{791}Ala Raptor knock-in mice were originally generated by inGenious Targeting Laboratory Inc. (Ronkonkoma, NY, USA) using an inversion and deletion approach, and will be described in detail in a forthcoming manuscript from the Collins lab. Mice expressing Ser^{791}Ala Raptor in all tissues were generated by crossing heterozygous Ser^{791}Ala Raptor knock-in mice and CMV-*Cre* mice (JAX 006054, The Jackson Laboratory, Bar Harbor. ME). Tail DNA genotyping and cDNA sequencing were performed to confirm successful expression of Ser^{791}Ala Raptor in place of endogenous Raptor.

Liraglutide experiments. Wild-type and Ser^{791}Ala Raptor knock-in mice on a C57BL/6J background were maintained on a diet containing 70% calories provided by carbohydrates, 20% by protein, and 10% by fat (3.85 kcal/g, D12492J, Research Diets, Inc., New Brunswick, NJ) for 4 weeks prior to testing to ensure consistent response to subsequent liraglutide treatment. Mice were then administered vehicle (0.9% saline) or liraglutide (200 ug/kg body weight) subcutaneously twice a day for 14 days. Body weight and food intake were collected daily. At the start and end of the treatment period, 4-hr fasting blood glucose levels were measured using a Contour Next EZ blood glucose meter (Cat. No. BD15789, Bayer) and body composition measurements (fat mass, lean mass, and free body fluid) obtained by NMR (Minispec 235 LF90II-TD NMR Analyzer, Bruker, Billerica, MA).

2.5 Data Analysis

Data were analyzed using GraphPad Prism 9 Software (GraphPad Software, Inc., La Jolla, CA). One-way ANOVA or mixed-effects analysis followed by Holm-Sidak's multiple comparisons was used when appropriate and indicated in the figure legends.

3. RESULTS

3.1 GLP-1R agonists activate mTORC1 in a PKA- and Akt 1/2-dependent manner

To define the signaling molecules mediating mTORC1 stimulation following GLP-1R agonism, we employed a series of pharmacological tools to test whether PKA, a canonical downstream target of GLP-1R signaling, and/or Akt 1/2, a well-known mediator of mTORC1 signaling, are required for GLP-1R agonists to induce phosphorylation of the mTORC1 signaling target S6 ribosomal protein. We utilized two inhibitors to target each of these pathways, the PKA inhibitors H89 and KT5720 and the Akt inhibitors MK-2206 and Akti-1/2 and performed drug titration experiments to obtain their optimal working concentrations (Supp. Fig. 1A and 1B). We show that like Ex4,[10] the GLP-1R agonists currently approved for weight loss liraglutide and semaglutide increase

phosphorylation of S6 in a dose-dependent manner (Supp. Fig. 2A). Liraglutide consistently increases phosphorylation of S6 in CHO-K1 cells transiently or stably expressing the *hGLP1R* (Fig. 1A and 1B respectively) as well as in β-TC3 cells, an immortalized beta cell line endogenously expressing the GLP-1R (Supp. Fig. 3A). Treatment with the mTORC1 inhibitor rapamycin blocks the ability of liraglutide to increase phosphorylation of S6 (Fig. 1A). Treatment with the PKA inhibitor H89 blocks S6 phosphorylation by liraglutide or the adenylyl cyclase/PKA activator forskolin in CHO-K1 cells expressing the *hGLP1R* (Fig. 1B). H89 also inhibited the ability of liraglutide and forskolin to stimulate S6 phosphorylation in β-TC3 cells (Supp. Fig. 2A). It is important to note that H89 has been shown to inhibit p70S6K activity[16] and prevent the dephosphorylation of Akt and p70S6K independent from its effects on PKA and other known H89-targeting kinases[17]. We therefore employed the PKA inhibitor KT5720 with minimal off-target effects on mTORC1 signaling[16]. Similar to H89, inhibition of PKA by KT5720 significantly reduces liraglutide-induced phosphorylation of S6 (Fig. 1C). It is important to note that the concentration of KT used here did not fully block PKA, as evident by the retention of p-CREB in response to liraglutide and Fsk, suggesting that the residual phosphorylation of S6 is likely due to incomplete PKA inhibition. These findings support our hypothesis that PKA is required for liraglutide to stimulate mTORC1 signaling.

While treatment with the pan-Akt inhibitor MK-2206 does not significantly interfere with the ability of liraglutide to stimulate mTORC1 activity (Fig. 1D), treatment with the Akt 1/2 inhibitor Akti-1/2 does diminish liraglutide-induced mTORC1 signaling (Fig. 1E). Inhibition of Akt did not affect stimulation of p-CREB (Supp. Fig. 3B). Notably, the activating effect of GLP-1R activation on PKA activity, as measured by phosphorylation of CREB at Ser^{133} (Fig. 1B and 1C), is much more robust than on Akt activity, as measured by phosphorylation of Akt at Ser^{473} (Fig. 1D and 1E). Furthermore, the stimulatory effect of liraglutide on phosphorylation of Akt is markedly less than the effect of insulin (Fig. 1D and 1E).

3.2 GLP-1R activation induces PKA phosphorylation of Raptor at Ser^{791}

Liu *et al.*[1] identified three evolutionarily conserved PKA phosphorylation sites on mTOR (Ser^{1276}, Ser^{1288}, Ser^{2112}) and one on Raptor (Ser^{791}) and showed that the catalytic subunit of PKA directly phosphorylates mTOR and Raptor at these corresponding sites. While mTOR is a key component of both the mTORC1 and mTORC2 complexes, Raptor is an mTORC1-defining subunit. To examine the mechanism by which PKA facilitates liraglutide-induced stimulation of mTORC1, we focused the PKA phosphorylation site on Raptor and tested the hypothesis that GLP-1R activation promotes phosphorylation of Raptor at Ser^{791} by PKA. To do this, we expressed a plasmid containing myc-tagged wild-type (WT) Raptor or myc-tagged mutant Raptor with an alanine in place of a serine at the 791st position (Ser^{791}Ala Raptor) in *hGLP-1R*-expressing CHO-K1 cells. We then treated these cells with PBS or liraglutide with or without pre-treatment with the PKA inhibitor H89, immunoprecipitated myc-WT Raptor and myc-Ser^{791}Ala Raptor from the cell lysates, and immunoblotted for phosphorylation targets of PKA using an antibody against the PKA-binding motif RRXS*/T*. As shown in Fig. 2A and 2B, liraglutide and forskolin significantly increase phosphorylation of the PKA target motif on myc-WT Raptor, and this was blocked by H89. In addition, liraglutide fails to increase phosphorylation of the PKA target motif on myc-Ser^{791}Ala Raptor (Fig. 2A and 2B). This was also observed in β-TC3 cells (Supp. Fig. 3B). Taken together, these results strongly support the hypothesis that GLP-1R activation leads to phosphorylation of Raptor at Ser^{791} by PKA.

Given our finding that Akt may also play a role in mediating mTORC1 stimulation by liraglutide and the possibility that the anti-phospho-PKA substrate antibody may recognize other -3 arginine-bearing phospho-Ser/Thr peptides, we tested whether Akt phosphorylates Raptor at Ser^{791} in response to GLP-1R activation. *hGLP-1R*-expressing CHO-K1 cells transfected with myc-WT Raptor were treated with PBS or liraglutide with or without pre-treatment with the pan-Akt inhibitor MK-2207. Akt inhibition did not affect the ability of liraglutide to induce phosphorylation of the PKA

target motif on Raptor in CHO-K1 cells expressing the *hGLP1R* (Fig. 2A and 2B) and in β-TC3 cells (Supp. Fig. 3B). This further suggests that GLP-1R activation promotes phosphorylation of Raptor at Ser^{791} specifically *via* activation of PKA.

3.3 PKA phosphorylation of Raptor at S791 mediates GLP-1R agonist-induced weight loss

PKA phosphorylation of Ser^{791} is an important component of beta-adrenergic signaling in adipose tissue.[1] To examine the physiological role of PKA phosphorylation of Raptor at Ser^{791} in response to GLP-1R activation, we tested the body weight and glycemic response to liraglutide treatment in wild-type (WT) mice and novel knock-in mice in which endogenous Raptor is replaced by a PKA-resistant Raptor expressing Ala in place of Ser at the 791st position (Ser^{791}Ala Raptor knock-in mice). Expression of this mutant is driven by the CMV promoter such that the Ser^{791}Ala Raptor mutation is expressed in all tissues. At baseline, WT and Ser^{791}Ala Raptor knock-in mice exhibit similar body composition (Supp. Fig. 4A-D) and fasting blood glucose (Supp. Fig. 4E). When treated with liraglutide for 14 days, Ser^{791}Ala Raptor knock-in mice were partially resistant to the weight lowering effects of liraglutide compared to WT mice (Fig. 3A and 3B). Body composition measurements before and after treatment revealed that this protection from weight loss is largely due to preservation of fat mass in liraglutide-treated Ser^{791}Ala Raptor knock-in mice compared to their WT counterparts (Fig. 3D). Changes in free body fluid was also preserved in liraglutide-treated Ser^{791}Ala Raptor knock-in mice; however, their absolute contribution to the overall weight difference between genotypes was minimal (Fig. 3F). Changes in lean mass (Fig. 3E) and fasting blood glucose (Fig. 3G) are similar between WT and Ser^{791}Ala Raptor knock-in mice in response to GLP-1R agonist treatment. Taken together, these data suggest that phosphorylation of Raptor at Ser^{791} is required for the full body weight-lowering effect of liraglutide.

4. DISCUSSION

The GLP-1R agonists liraglutide and semaglutide are among few medications currently approved for weight loss. However, their arguably modest effect (5-15% weight loss) and significant inter-

individual variability in weight lowering potential pose significant opportunities for improvement. Given the complexity of signaling mechanisms downstream of the GLP-1R,[18] the ability to assign specific pathways to a desirable phenotype, e.g. decreased food intake and weight loss, is crucial to our progress towards more effective weight loss therapeutics. Here, we present findings supporting a novel GLP-1R-PKA-mTORC1 axis that mediates the weight lowering effects of liraglutide.

Our lab has previously reported that GLP-1R agonism leads to mTORC1 stimulation and that inhibition of mTORC1 signaling in the VMH abrogates the anorexic effect of the GLP-1R agonist exendin-4 in this brain nucleus.[11] These findings are in line with previous studies showing that activation of mTORC1 in the hypothalamus reduces food intake and body weight and mediates the weight-lowering effect of other anorectic hormones such as leptin.[19–21] Elucidating mechanisms that activate hypothalamic mTORC1 signaling may, therefore, provide insight into potential new approaches for weight loss therapies. In the current study, we identify PKA as an important mediator of GLP-1R-mediated activation of mTORC1 signaling. We show that pharmacological inhibition of PKA significantly reduces mTORC1 activity induced by GLP-1R activation. We further demonstrate that PKA phosphorylates Raptor, a critical component of mTORC1, specifically at Ser^{791} in response to activation of the GLP-1R. Importantly, using a novel PKA-resistant Raptor knock-in mouse model, we show that PKA-mediated phosphorylation of Raptor at Ser^{791} is responsible for approximately 50% of the weight loss induced by liraglutide administration. These findings suggest that PKA-mediated activation of mTORC1 is a key component of the weight-lowering effect of GLP-1R agonists.

While PKA phosphorylation of Raptor at Ser^{791} upon activation of the $G\alpha_S$ protein-coupled β-adrenergic receptor has been reported,[1,22] our study is the first to describe this PKA-mTORC1 interaction along with its physiological relevance in the context of GLP-1R signaling. Although we observe a PKA-dependent increase in mTORC1 signaling following GLP-1R activation, it is

notable that PKA has been reported to have either stimulatory[1] or inhibitory[22] effects on mTORC1 activity. Similar to our study, Liu *et al.* observed a PKA-mediated increase in mTORC1 signaling in a nutrient-deficient condition where cells were incubated in serum-free media prior to and during drug treatment.[1] This is in contrast to the use of serum-supplemented media in experiments conducted by Jewell *et al.*[22], where a decrease in mTORC1 activity was reported following PKA activation by either forskolin or by an $G\alpha_S$ protein-coupled receptor agonist. This is indeed consistent with experiments by Lawrence and colleagues which showed that insulin is a powerful activator of mTOR in adipocytes, but that in the presence of a cAMP elevating agent such as forskolin, the insulin-mediated effect is markedly reduced.[23] Given the role of mTORC1 as a cellular energy sensor, it is possible that PKA exerts differential actions on mTORC1 activity depending on the nutrient availability and energy status of the cell.

One limitation of our *in vivo* studies is that the PKA-resistant Raptor in our novel knock-in line of mice is expressed in all tissues. Therefore, we cannot ascribe a role for PKA phosphorylation of Raptor in mediating the effect of liraglutide to a specific tissue or cell population. It is well accepted that the brain is a key target for the weight-lowering effects of GLP-1R agonists such as liraglutide.[7,24,25] While mTORC1 is widely expressed in the brain, its activity, particularly with regards to its role in regulation of energy balance, has been reported to be most critical in specific regions of the hypothalamus such the arcuate (ARC), paraventricular hypothalamus (PVH), VMH, and suprachiasmatic nucleus (SCN).[21,26,27] Importantly, the GLP-1R is highly expressed in all of these hypothalamic regions.[21,25,27–30] Our finding that mTORC1 activation is necessary for the anorectic effect of Ex4 targeted to the VMH suggests that this may be a relevant region for this signaling event.[31] However, in those studies, Ex4 was targeted directly to the VMH, but systemically-delivered GLP-1R agonists, such as liraglutide, do not appreciably engage the VMH directly.[29] A more likely target region in the hypothalamus that is directly engaged by systemic liraglutide and mediates its weight-lowering effect is the ARC.[32] Besides the hypothalamus,

hindbrain regions such as the nucleus tractus solitarius (NTS) can also be directly engaged by systemic liraglutide and mediate its weight-lowering effects.[33] Interestingly, inhibition of PKA signaling in the hindbrain attenuates the anorectic effect of Ex4 targeted to the 4th ventricle in rats.[34] Future studies utilizing the Ser^{791}Ala Raptor knock-in mice to replace endogenous Raptor with Ser^{791}Ala Raptor in a time-dependent and/or tissue/cell-type specific manner will enable identification of specific brain region(s) and/or neuronal population(s) where the GLP-1R-PKA-mTORC1 signaling pathway is engaged by GLP-1R agonists such as liraglutide to reduce food intake and body weight.

Although we focus here on the canonical GLP-1R target PKA as a regulator of mTORC1 signaling, Akt may also play a role in liraglutide-induced mTORC1 activation. While PKA activity is well-known to be increased by GLP-1R agonists, a direct effect of GLP-1R signaling on Akt has not been clearly demonstrated. Interestingly, inhibition of phosphoinositide 3-kinase, a mediator of Akt activity, in either the hindbrain[35] or the hypothalamus[36] attenuates the anorectic effect of Ex4. In our study, inhibition of Akt1/2 by Akti-1/2 partially diminishes S6 phosphorylation following GLP-1R activation, yet liraglutide fails to appreciably induce phosphorylation of Akt at Ser^{473} in *hGLP1R*-expressing CHO cells (Fig. 1D and 1E) and only slightly increases phosphorylation of Akt at Ser^{473} in β-TC3 cells (Supp. Fig. 2A). Therefore, a potential role for Akt as a mediator of the anorectic effects of GLP-1R agonists via mTORC1 activation requires further study.

AMP-activated protein kinase (AMPK) is also implicated in facilitating the metabolic actions of GLP-1 and GLP-1R agonists. In nutrient-deficient states, AMPK suppresses mTORC1 activity by phosphorylating TSC2[37] or Raptor[38]. Prior studies from our lab and others suggest that AMPK may mediate the anorectic effects of GLP-1R activation.[11,31,34,39,40] We show that Ex4 inhibits AMPK and that the AMPK activator AICAR delivered intracerebroventricularly (ICV) [28] or stereotaxically to the VMH[11] blunts the anorectic effect of Ex4. This suggests that GLP-1R agonists reduce food intake and body weight, at least in part, by inhibiting AMPK. Since AMPK,

in turn, inhibits mTORC1, this raises the possibility that GLP-1R activation can stimulate mTORC1 signaling by reducing AMPK activity. Interestingly, AMPK can also be inhibited by mTORC1 signaling,[41] so it is possible that the inhibition of AMPK we observe in response to GLP-1R activation is downstream of mTORC1.

Given the variability in response and adverse effects (e.g. nausea) of current GLP-1 based therapies, much attention is being given to the potential development of improved, 'biased' agonists that elicit distinct cellular signaling profiles downstream of the GLP-1R. Here, we define a PKA-mTORC1 pathway engaged by the GLP-1R agonist liraglutide that contributes to the weight loss effect of this drug in mice. Our findings will allow us to gain a better understanding of the cellular mechanisms underlying the food intake and weight lowering effects induced by GLP-1R activation and thereby aid in the development of more effective and targeted anti-obesity therapies. In addition, given the ubiquitous expression of mTORC1 and PKA, results from our study provide a framework for future investigation into the significance of the GLP-1R-PKA-mTOR axis not only to the anorexic and weight loss properties of GLP-1R agonists via brain regions but also to beneficial metabolic effects of GLP-1R agonists mediated in other tissues such as hormone secretion from the endocrine pancreas.

5. ACKNOWLEDGEMENTS

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REFERENCES

1. Liu, D. *et al.* Activation of mTORC1 is essential for β-adrenergic stimulation of adipose browning. *J. Clin. Invest.* **126**, 1704–1716 (2016).

2. Hales, C. M., Carroll, M. D., Fryar, C. D. & Ogden, C. L. *Prevalence of Obesity Among Adults and Youth: United States, 2015-2016 Key findings Data from the National Health and Nutrition Examination Survey*. (2015).

3. Flegal, K. M., Kruszon-Moran, D., Carroll, M. D., Fryar, C. D. & Ogden, C. L. Trends in Obesity Among Adults in the United States, 2005 to 2014. *JAMA* **315**, 2284 (2016).

4. Kinzig, K. P., D'Alessio, D. A. & Seeley, R. J. The diverse roles of specific GLP-1 receptors in the control of food intake and the response to visceral illness. *J. Neurosci.* **22**, 10470–6 (2002).

5. Hayes, M. R., Bradley, L. & Grill, H. J. Endogenous Hindbrain Glucagon-Like Peptide-1 Receptor Activation Contributes to the Control of Food Intake by Mediating Gastric Satiation Signaling. *Endocrinology* **150**, 2654–2659 (2009).

6. Secher, A. *et al.* The arcuate nucleus mediates GLP-1 receptor agonist liraglutide-dependent weight loss. *J. Clin. Invest.* **124**, 4473–4488 (2014).

7. Sisley, S. *et al.* Neuronal GLP1R mediates liraglutide's anorectic but not glucose-lowering effect. *J. Clin. Invest.* **124**, 2456–2463 (2014).

8. Astrup, A. *et al.* Effects of liraglutide in the treatment of obesity: a randomised, double-blind, placebo-controlled study. *Lancet (London, England)* **374**, 1606–16 (2009).

9. Van Gaal, L. & Dirinck, E. Pharmacological Approaches in the Treatment and Maintenance of Weight Loss. *Diabetes Care* **39 Suppl 2**, S260-7 (2016).

10. Brown, J. D. *et al.* Oleoylethanolamide modulates glucagon-like peptide-1 receptor agonist signaling and enhances exendin-4-mediated weight loss in obese mice. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **315**, R595–R608 (2018).

11. Burmeister, M. A. *et al.* The Glucagon-Like Peptide-1 Receptor in the Ventromedial Hypothalamus Reduces Short-Term Food Intake in Male Mice by Regulating Nutrient Sensor Activity. *Am. J. Physiol. - Endocrinol. Metab.* ajpendo.00113.2017 (2017). doi:10.1152/ajpendo.00113.2017

12. Saxton, R. A. & Sabatini, D. M. mTOR Signaling in Growth, Metabolism, and Disease. *Cell* **168**, 960–976 (2017).

13. Kim, D. H. *et al.* mTOR Interacts with Raptor to Form a Nutrient-Sensitive Complex that Signals to the Cell Growth Machinery. *Cell* **110**, 163–175 (2002).

14. Thorens, B. Expression cloning of the pancreatic beta cell receptor for the gluco-incretin hormone glucagon-like peptide 1. *Proc. Natl. Acad. Sci. U. S. A.* **89**, 8641–8645 (1992).

15. Efrat, S. *et al.* Beta-cell lines derived from transgenic mice expressing a hybrid insulin gene-oncogene. *Proc. Natl. Acad. Sci. U. S. A.* **85**, 9037–9041 (1988).

16. Davies, S. P., Reddy, H., Caivano, M. & Cohen, P. Specificity and mechanism of action of some commonly used protein kinase inhibitors. *Biochem. J.* **351**, 95–105 (2000).

17. Melick, C. H. & Jewell, J. L. Small molecule H89 renders the phosphorylation of S6K1 and AKT resistant to mTOR inhibitors. *Biochem. J.* **477**, 1847 (2020).

18. Rowlands, J., Heng, J., Newsholme, P. & Carlessi, R. Pleiotropic Effects of GLP-1 and

Analogs on Cell Signaling, Metabolism, and Function. *Front. Endocrinol. (Lausanne).* **9**, 672 (2018).

19. Cota, D., Matter, E. K., Woods, S. C. & Seeley, R. J. The Role of Hypothalamic Mammalian Target of Rapamycin Complex 1 Signaling in Diet-Induced Obesity. *J. Neurosci.* **28**, 7202–7208 (2008).

20. Blouet, C., Ono, H. & Schwartz, G. J. Mediobasal Hypothalamic p70 S6 Kinase 1 Modulates the Control of Energy Homeostasis. *Cell Metab.* **8**, 459–467 (2008).

21. Cota, D. *et al.* Hypothalamic mTOR signaling regulates food intake. *Science* **312**, 927–930 (2006).

22. Jewell, J. L. *et al.* GPCR signaling inhibits mTORC1 via PKA phosphorylation of raptor. *Elife* **8**, (2019).

23. Scott, P. H. & Lawrence, J. C. Attenuation of Mammalian Target of Rapamycin Activity by Increased cAMP in 3T3-L1 Adipocytes. *J. Biol. Chem.* **273**, 34496–34501 (1998).

24. Adams, J. M. *et al.* Liraglutide Modulates Appetite and Body Weight Through Glucagon-Like Peptide 1 Receptor-Expressing Glutamatergic Neurons. *Diabetes* **67**, 1538–1548 (2018).

25. Varin, E. M. *et al.* Distinct Neural Sites of GLP-1R Expression Mediate Physiological versus Pharmacological Control of Incretin Action. *Cell Rep.* **27**, 3371-3384.e3 (2019).

26. Proulx, K., Cota, D., Woods, S. C. & Seeley, R. J. Fatty acid synthase inhibitors modulate energy balance via mammalian target of rapamycin complex 1 signaling in the central nervous system. *Diabetes* **57**, 3231–3238 (2008).

27. Inhoff, T. *et al.* Novel insight in distribution of nesfatin-1 and phospho-mTOR in the arcuate nucleus of the hypothalamus of rats. *Peptides* **31**, 257–262 (2010).

28. Jensen, C. B. *et al.* Characterization of the Glucagonlike Peptide-1 Receptor in Male Mouse Brain Using a Novel Antibody and In Situ Hybridization. *Endocrinology* **159**, 665–675 (2018).

29. Salinas, C. B. G. *et al.* Integrated Brain Atlas for Unbiased Mapping of Nervous System Effects Following Liraglutide Treatment. *Sci. Reports 2018 81* **8**, 1–12 (2018).

30. Ast, J. *et al.* Super-resolution microscopy compatible fluorescent probes reveal endogenous glucagon-like peptide-1 receptor distribution and dynamics. *Nat. Commun. 2020 111* **11**, 1–18 (2020).

31. Burmeister, M. A. *et al.* The glucagon-like peptide-1 receptor in the ventromedial hypothalamus reduces short-term food intake in male mice by regulating nutrient sensor activity. *Am. J. Physiol. - Endocrinol. Metab.* **313**, E651–E662 (2017).

32. Secher, A. *et al.* The arcuate nucleus mediates GLP-1 receptor agonist liraglutide-dependent weight loss. *J. Clin. Invest.* **124**, 4473–4488 (2014).

33. Fortin, S. M. *et al.* GABA neurons in the nucleus tractus solitarius express GLP-1 receptors and mediate anorectic effects of liraglutide in rats. *Sci. Transl. Med.* **12**, (2020).

34. Hayes, M. R. *et al.* Intracellular signals mediating the food intake-suppressive effects of hindbrain glucagon-like peptide-1 receptor activation. *Cell Metab.* **13**, 320–330 (2011).

35. Rupprecht, L. E. *et al.* Hindbrain GLP-1 receptor-mediated suppression of food intake requires a PI3K-dependent decrease in phosphorylation of membrane-bound Akt. *Am. J. Physiol. Endocrinol. Metab.* **305**, (2013).

36. Yang, Y. *et al.* Exendin-4 reduces food intake via the PI3K/AKT signaling pathway in the hypothalamus. *Sci. Reports 2017 71* **7**, 1–7 (2017).

37. Inoki, K., Zhu, T. & Guan, K. L. TSC2 Mediates Cellular Energy Response to Control Cell Growth and Survival. *Cell* **115**, 577–590 (2003).

38. Gwinn, D. M. *et al.* AMPK Phosphorylation of Raptor Mediates a Metabolic Checkpoint. *Mol. Cell* **30**, 214–226 (2008).

39. Burmeister, M. A., Ayala, J., Drucker, D. J. & Ayala, J. E. Central glucagon-like peptide 1 receptor-induced anorexia requires glucose metabolism-mediated suppression of AMPK and is impaired by central fructose. *Am. J. Physiol. - Endocrinol. Metab.* **304**, (2013).

40. Sandoval, D. *et al.* The Anorectic Effect of GLP-1 in Rats Is Nutrient Dependent. *PLoS One* **7**, e51870 (2012).

41. Dagon, Y. *et al.* p70S6 kinase phosphorylates AMPK on serine 491 to mediate leptin's effect on food intake. *Cell Metab.* **16**, 104–112 (2012).

Figure 1: The GLP-1R agonist liraglutide increases mTOR activity in a PKA- dependent manner. (A) Immunoblot of pS6, S6, pCREB, and CREB and quantification of pS6 expression (pS6 / S6) in CHO-K1 cells transfected with the a vector expressing the *hGLP1R* and treated with DMSO or the mTORC1 inhibitor rapamycin (100 nM) for 30 min followed by treatment with 1X PBS (Veh) or liraglutide (Lira, 10 nM) for 1 hr, (B-C) Immunoblot of pS6, S6, pCREB, and CREB and quantification of pS6 expression (pS6 / S6) in CHO-K1 cells stably expressing the *hGLP1R* treated with DMSO (Veh) or either of the PKA inhibitors, KT5720 (10µM) (B) or H89 (10µM) (C), for 30 min followed by treatment with vehicle (PBS), Lira (10 nM), forskolin (Fsk, 10 µM), or insulin (Ins, 10 mg/mL) for 1 hr. (D-E) Immunoblot of pS6, S6, pAkt, and Akt and quantification of pS6 expression (pS6 / S6) in CHO-K1 cells stably expressing the *hGLP1R* treated with DMSO or either of the Akt inhibitors, the Akt 1 and 2 inhibitor Akti-1/2 (10 µM) (B) or the pan-Akt inhibitor MK-2206 (20 µM) (C), for 30 min followed by treatment with vehicle (PBS), Lira (10 nM), Fsk (10 µM), or Ins (10 mg/mL) for 1 hr. Analysis was done using one-way ANOVA followed by Holm-Sidak's multiple comparisons. Data are normalized to the respective control (Veh or Veh and inhibitor) and shown as mean ± SEM, ns not significant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$, n = 3-4 biological replicates.

Figure 2: PKA, not Akt 1/2, phosphorylates Raptor at S791 upon GLP-1R activation. (A) Immunoblot and quantification of phospho-PKA substrate and myc in CHO-K1 cells stably expressing the *hGLP1R* transfected with myc-wild-type (WT) Raptor or myc-Ser^{791}Ala Raptor and treated with vehicle (DMSO) or H89 (10 µM) for 30 min followed by treatment with Lira (10 nM), forskolin (Fsk, 10 µM), or insulin (Ins, 10 mg/mL) for 1 hr. (B) Immunoblot and quantification of phospho-PKA substrate and myc in CHO-K1 cells stably expressing the *hGLP1R* transfected with myc-wild-type (WT) Raptor and treated with vehicle (DMSO) or MK-2206 (20 µM) for 30 min followed by treatment with Lira (10 nM), Fsk (10 µM), or Ins (10 mg/mL) for 1 hr. Analysis was

done using one-way ANOVA followed by Holm-Sidak's multiple comparisons. Data are shown as mean ± SEM, ns not significant, *** $p < 0.001$, n = 2-4 biological replicates.

Figure 3. PKA phosphorylation of Raptor at S791 facilitates the weight-lowering effects of Lira. (A-B) Relative (A) and absolute (B) body weight change from baseline of wild-type (WT) and Ser^{791}Ala Raptor knock-in mice given vehicle (PBS) or liraglutide (Lira, 200 µg/kg) subcutaneously twice a days for 14 days. (C-G) Changes in body composition including total mass (C), fat mass (D), lean mass (E), free body fluid (F), and fasting blood glucose (G) of WT and Ser^{791}Ala Raptor knock-in mice at the start and end of the treatment period. Analysis was done using mixed-effects analysis followed by Holm-Sidak's multiple comparisons between Lira-treated WT and LKO (A-B) or one-way ANOVA followed by Holm-Sidak's multiple comparisons as shown in graphs (C-G). Data are shown as mean ± SEM, ns not significant, * $p < 0.05$, ** $p < 0.01$, **** $p < 0.0001$, n = 9-12 mice per group (A-F) or 5-8 mice per group (G).

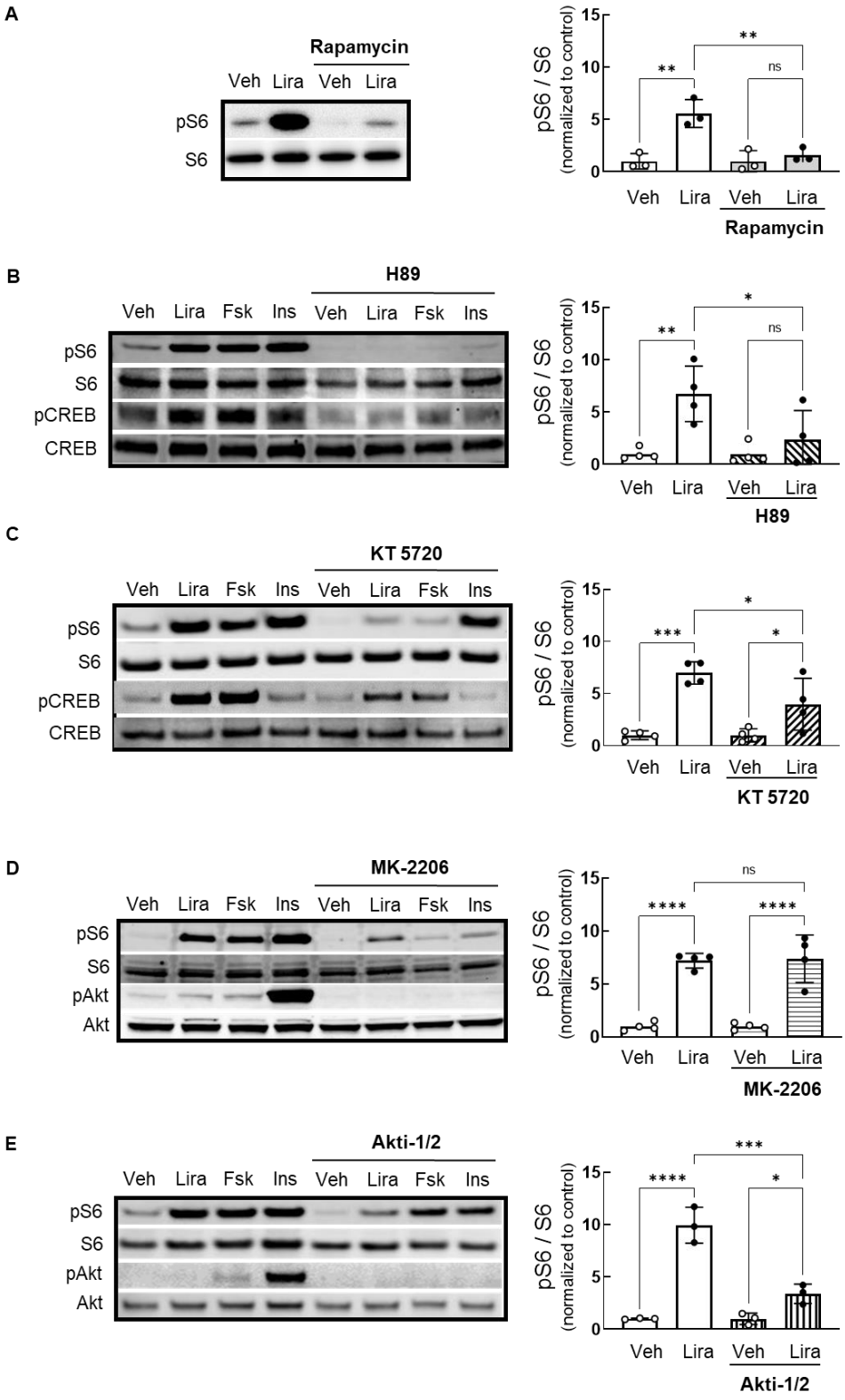
Supplemental Figure 1. The PKA inhibitors, H89 and KT5720, and the Akt inhibitors, MK-2206 and Akti-1/2, block liraglutide-induced phosphorylation of S6 in a dose-dependent manner. (A) Representative In-Cell Western blots for pS6 (fluorescent green) and S6 (fluorescent red) in *hGLP-1R*-stably expressing CHO-K1 cells treated with DMSO or either H89 (1 or 10 µM) or KT5720 (1 or 10 µM) for 30 min followed by treatment with 1X PBS (Veh) or liraglutide (Lira) at increasing concentrations (1 pM, 10 pM,100 pM, 1nM, 10 nM, and 100 nM) for 1 hr. (B) Immunoblot of pS6, S6, pAkt, and Akt in *hGLP-1R*-stably expressing CHO-K1 cells treated with DMSO or either MK-2206 (5 or 20 µM) or Akti-1/2 (1 or 10 µM) for 30 min followed by treatment with Veh or Lira (10 nM) for 1 hr.

Supplemental Figure 2. The GLP-1R agonists liraglutide and semaglutide increase mTORC1 activity in a dose-dependent manner. Immunoblot of pS6 and S6 in *hGLP-1R*-stably expressing CHO-K1 cells treated with PBS (Veh), liraglutide (Lira), or semaglutide at increasing concentrations (1 pM, 10 pM,100 pM, 1nM, and 10 nM).

Supplemental Figure 3. PKA mediates GLP-1R-induced mTORC1 activity and phosphorylates Raptor at Ser^{791} in a pancreatic beta-cell line. (A) Immunoblot of pS6, S6, pCREB, CREB, pAkt, and Akt (G) in β-TC3 cells endogenously expressing the GLP-1R treated with DMSO, H89 (10 µM), or MK-2206 (20 µM) followed by a 1-hr treatment with PBS, Lira, Fsk, or Ins. (B) Immunoblot and quantification of phospho-PKA substrate and myc in *hGLP-1R*-CHO-K1 cells transfected with myc-wildtype (WT) Raptor or myc-Ser^{791}Ala Raptor and treated with vehicle (DMSO) or MK-2206 (10 µM) for 30 min followed by treatment with Lira (10 nM), forskolin (Fsk, 10 µM), or insulin (Ins, 10 mg/mL) for 1 hr. Analysis was done using one-way ANOVA followed by Holm-Sidak's multiple comparisons. Data are shown as mean ± SEM, ** $p < 0.01$, n = 3 biological replicates.

Supplemental Figure 4. Wildtype (WT) and Ser^{791}Ala Raptor knock-in mice display similar body composition and fasting blood glucose at baseline. (A-D) Total (A), fat (B), lean (C), and free body fluid (D) measurements in WT and Ser^{791}Ala Raptor knock-in mice prior to vehicle or liraglutide administration. (E) 5-hr fasting blood glucose in WT and Ser^{791}Ala Raptor knock-in mice before drug treatment. Analysis was done using one-way ANOVA followed by Holm-Sidak's multiple comparisons. Data are shown as mean ± SEM, ns not significant, n = 5-8 mice per group.

Figure 1


A
Rapamycin
Veh Lira Veh Lira
pS6
S6
pS6 / S6 (normalized to control)
15
10
5
0
**
**
ns
Veh Lira Veh Lira
Rapamycin
B
H89
Veh Lira Fsk Ins Veh Lira Fsk Ins
pS6
S6
pCREB
CREB
*
**
ns
H89
C
KT 5720
Veh Lira Fsk Ins Veh Lira Fsk Ins
pS6
S6
pCREB
CREB
***
*
*
KT 5720
D
MK-2206
Veh Lira Fsk Ins Veh Lira Fsk Ins
pS6
S6
pAkt
Akt
ns
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****
MK-2206
E
Akti-1/2
Veh Lira Fsk Ins Veh Lira Fsk Ins
pS6
S6
pAkt
Akt
***
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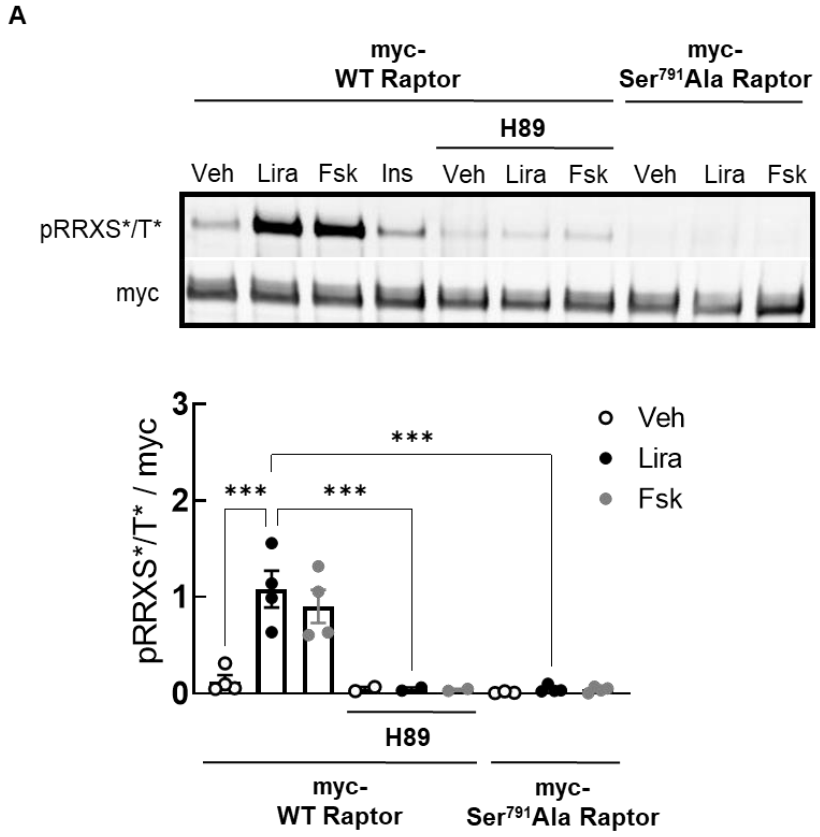
*
Akti-1/2

Figure 2


A
myc-
WT Raptor
myc-
Ser791Ala Raptor
H89
Veh Lira Fsk Ins Veh Lira Fsk Veh Lira Fsk
pRRXS*/T*
myc
pRRXS*/T* / myc
3
2
1
0
***
***
***
Veh
Lira
Fsk
H89
myc-
WT Raptor
myc-
Ser791Ala Raptor

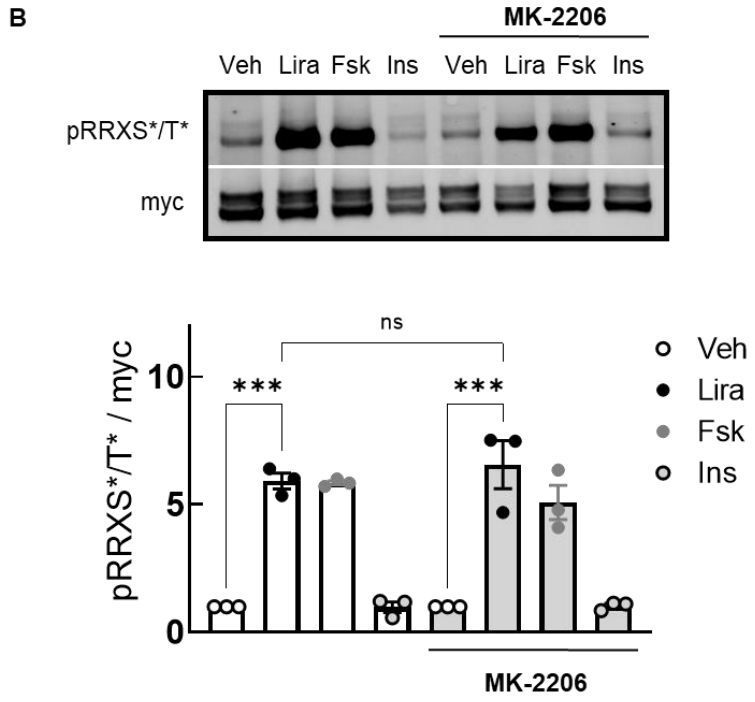
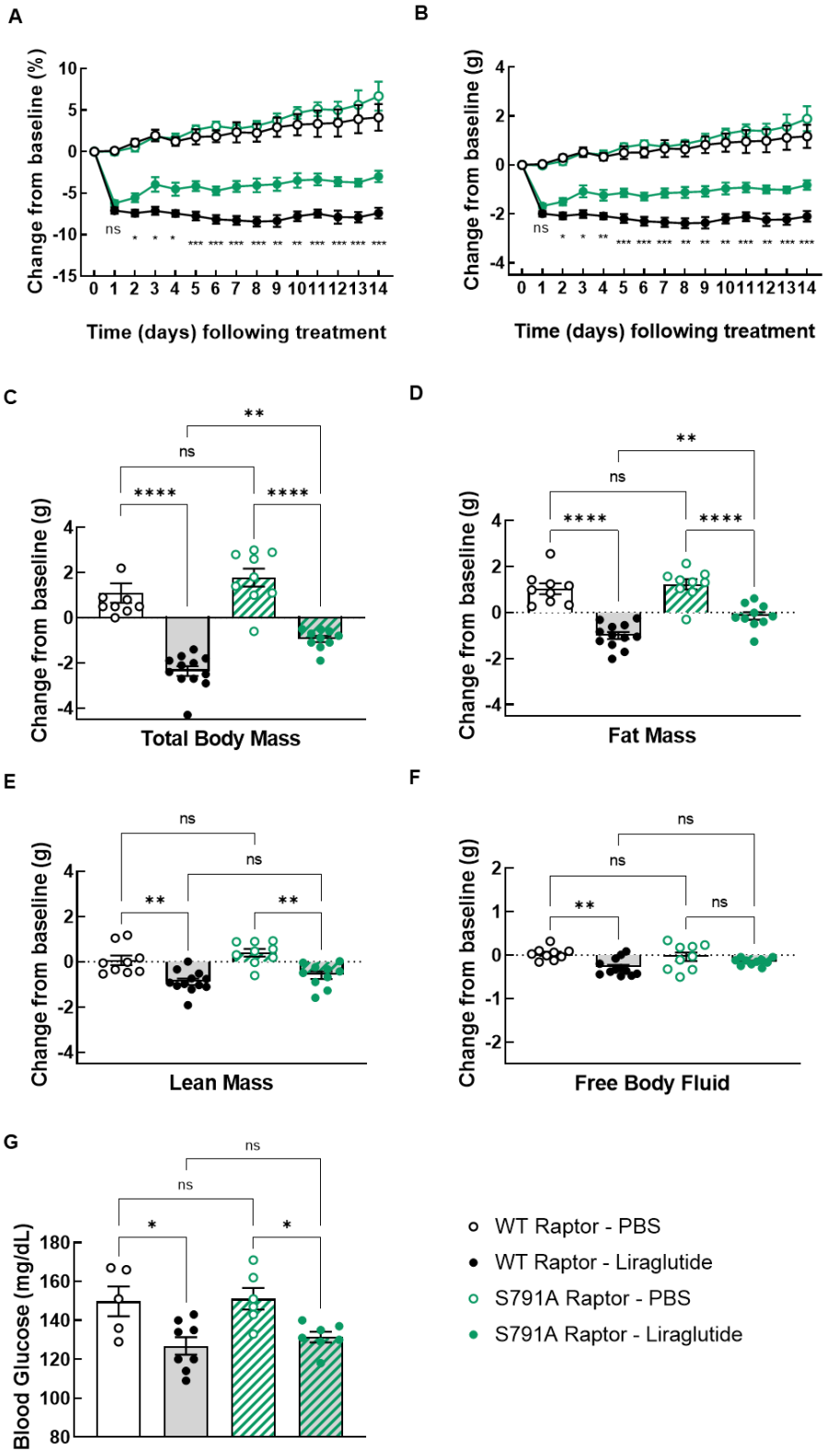
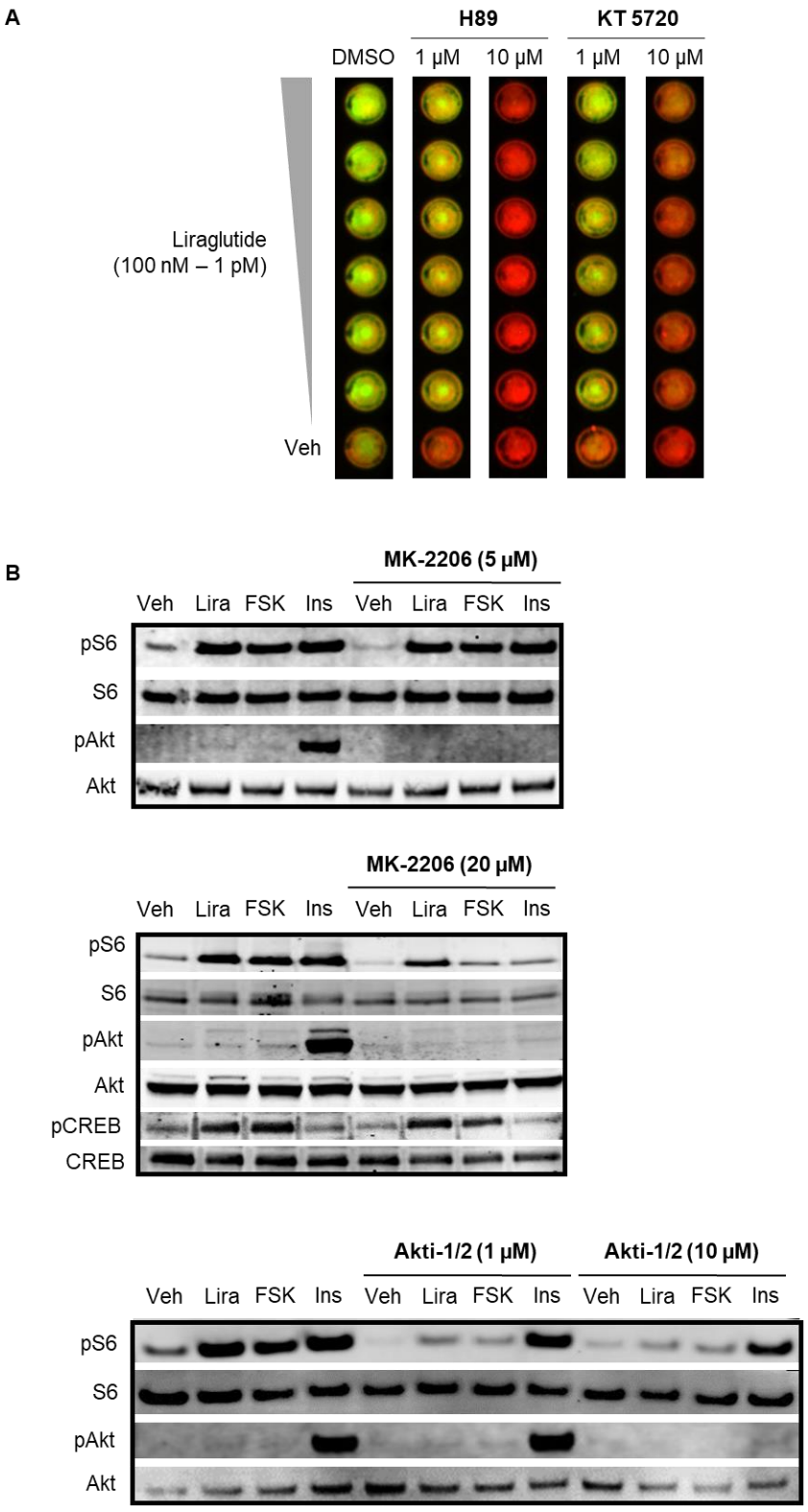

B
MK-2206
Veh Lira Fsk Ins Veh Lira Fsk Ins
pRRXS*/T*
myc
pRRXS*/T* / myc
10
5
0
ns
***
***
Veh
Lira
Fsk
Ins
MK-2206

Figure 3


A
Change from baseline (%)
Time (days) following treatment
B
Change from baseline (g)
Time (days) following treatment
C
Change from baseline (g)
Total Body Mass
D
Change from baseline (g)
Fat Mass
E
Change from baseline (g)
Lean Mass
F
Change from baseline (g)
Free Body Fluid
G
Blood Glucose (mg/dL)
WT Raptor - PBS
WT Raptor - Liraglutide
S791A Raptor - PBS
S791A Raptor - Liraglutide

Supplemental Figure 1



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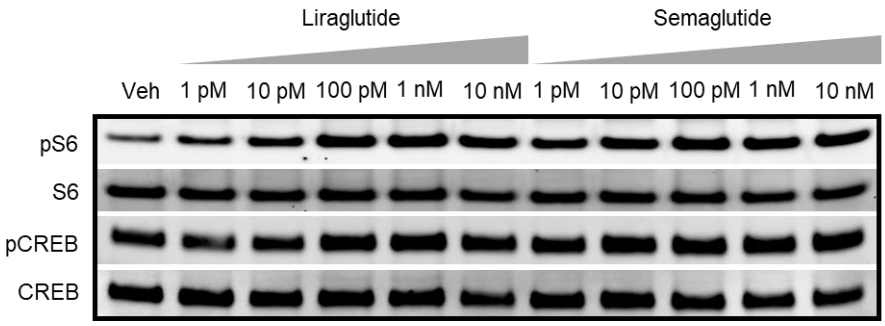
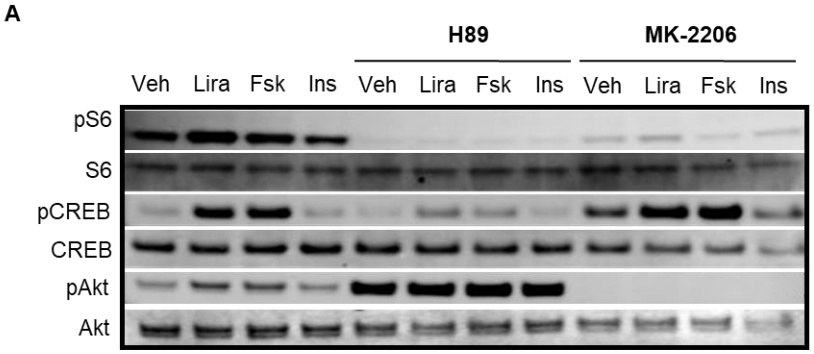
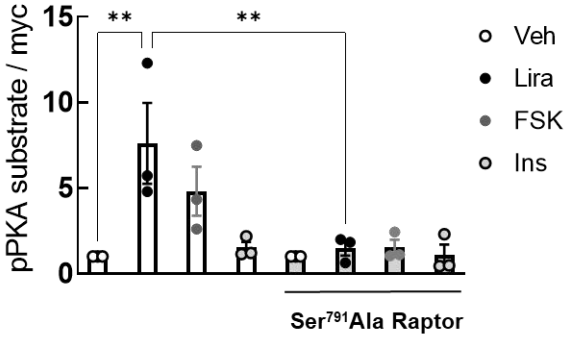
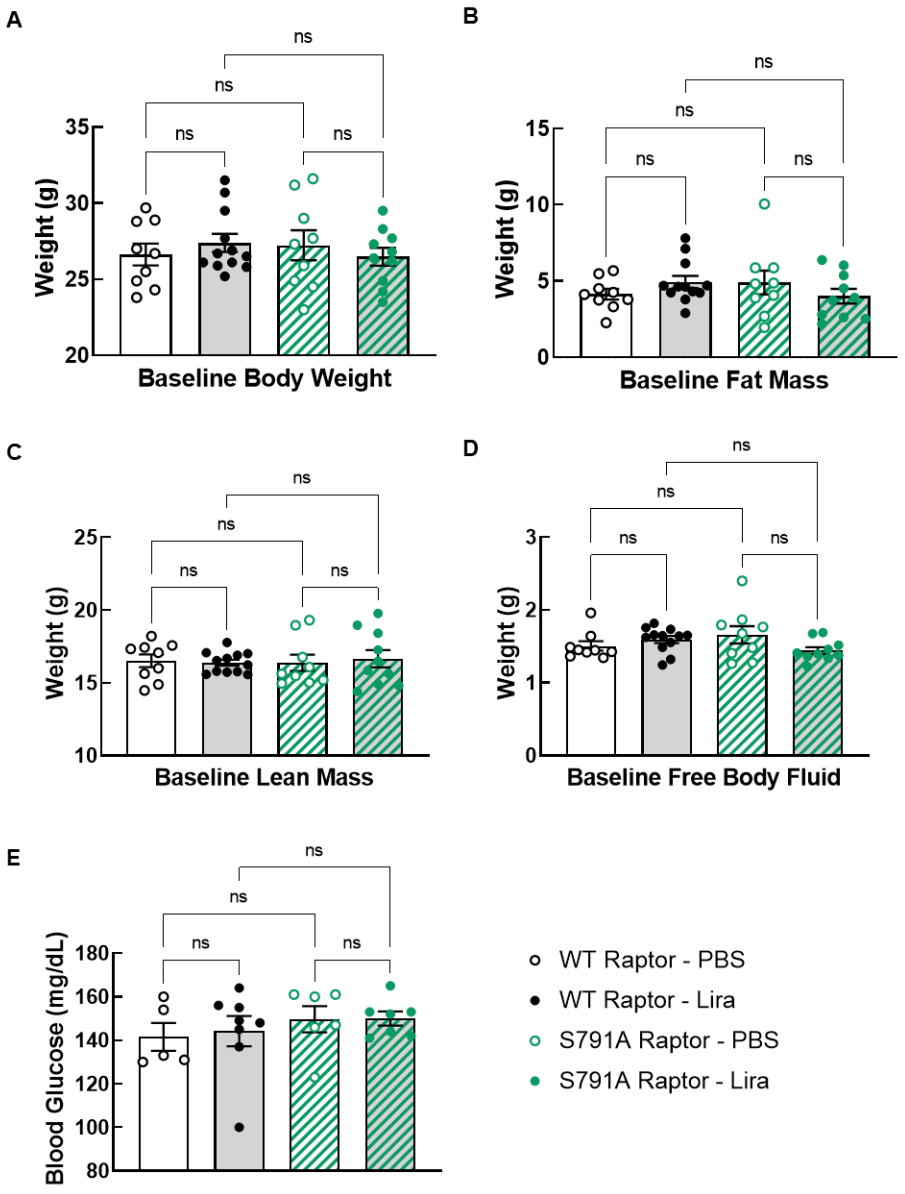


Supplemental Figure 3





Supplemental Figure 4