The FASEB Journal

FASEB
Federation of American Societies for Experimental Biology

RESEARCH ARTICLE

Integrated Transcriptomic and Proteomic Analysis of Omentin-1 Effects on Primary Anterior Pituitary Cells From Different Pig Breeds: An In Vitro Study

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Received: 27 June 2025 | **Revised:** 27 August 2025 | **Accepted:** 17 September 2025

Funding: This work was supported by Narodowe Centrum Nauki (NCN), 2020/37/B/NZ9/01154.

Keywords: adipokines | endocrine system | omentin | pituitary gland | proteomics | swine | transcriptome

ABSTRACT

Omentin-1 (OMNT1) is a metabolically active adipokine implicated in endocrine regulation; however, its role in the anterior pituitary (AP) remains unknown. We hypothesized that OMNT1 modulates the endocrine function of AP cells through gene- and protein-level mechanisms, with effects depending on the animal's metabolic background. To investigate this hypothesis, AP cells isolated from two pig breeds with distinct metabolic profiles, Large White (LW; normal weight) and Meishan (MS; genetically obese), were treated with OMNT1. Transcriptomic and proteomic analyses were performed alongside assessments of hormone expression and secretion. Transcriptomic profiling revealed 13 310 and 13 272 expressed genes in LW and MS pigs, respectively. Differentially expressed gene (DEG) analysis revealed 655 DEGs in LW pigs and 420 in MS pigs. Integrated transcriptomic and proteomic analyses revealed that OMNT1 modulates pathways involved in cellular signaling, cytoskeleton dynamics, responses to stimuli, intracellular protein transport, and post-translational modifications. We further examined the mRNA expression and secretion of tropic hormones (GH, PRL, TSH, ACTH, LH, and FSH) and selected adipokines (adiponectin, leptin, chemerin, apelin, visfatin, resistin, and vaspin), along with their specific receptors. OMNT1 increased LH secretion and decreased FSH levels in a breed-dependent manner. Additionally, in MS pigs, OMNT1 reduced adiponectin and increased leptin secretion. These findings highlight the role of OMNT1 as a key modulator of pituitary endocrine activity, integrating metabolic signals through breed-specific molecular responses at the transcriptional, proteomic, and functional levels.

1 | Introduction

The anterior pituitary (AP) is a central regulator of mammalian endocrine function and secretes tropic hormones such as growth hormone (GH), prolactin (PRL), thyroid-stimulating hormone (TSH), adrenocorticotropic hormone (ACTH), and the gonadotropins: luteinizing hormone (LH) and follicle-stimulating hormone (FSH), which regulate metabolism, growth, stress responses, and reproduction [1, 2]. While traditionally considered under hypothalamic control, emerging evidence indicates that

peripheral metabolic signals, including adipokines, also directly modulate AP activity [3]. Several adipokines and their receptors, including adiponectin, leptin, chemerin, apelin, visfatin, resistin, and vaspin, are expressed in the AP and may exert direct effects on AP cells [3, 4]. Adipokines represent a crucial hormonal link between energy metabolism and endocrine axes [3, 5]. However, the role of omentin-1 (OMNT1), also referred to as intelectin-1 (ITLN1), in the AP cells has not yet been investigated in any species. OMNT1 is an adipokine originally identified in Paneth cells of the mouse small intestine [6]. It is encoded by the *ITLN1* gene located on chromosome 1q21.3 and contains 313 amino acids, with a molecular weight of 34 kDa [7]. It is primarily secreted by visceral adipose tissue (particularly by the stromal-vascular fraction) and is known to increase insulin sensitivity and maintain metabolic homeostasis [8, 9]. OMNT1 is known for its anti-inflammatory properties and role in maintaining blood–brain barrier integrity [10]. While its specific receptor remains unidentified, recent studies suggest that OMNT1 may act through integrin receptors, particularly αvβ3 and αvβ5, to influence intracellular signaling cascades [11]. Additionally, OMNT1 enhances insulin sensitivity and glucose uptake in human adipocytes and skeletal muscle cells, and it may modulate the phosphorylation of the insulin receptor and insulin-like growth factor 1 receptor in human granulosa cells [12]. OMNT1 can also promote inflammation in primary human adipocytes through activation of extracellular signal-regulated kinase (ERK1/2) [13]. Our recent findings demonstrated the presence of OMNT1 in the AP of pigs with different metabolic statuses [14]. This previous study demonstrated that OMNT1 was expressed in most AP cell types, such as somatotrophs, lactotrophs, thyrotrophs, and gonadotrophs. OMNT1 expression varied during the estrous cycle, suggesting hormonal regulation. Moreover, hypothalamic–pituitary signals, including gonadotropin-releasing hormone (GnRH), LH, and FSH, modulated OMNT1 levels in cultured AP cells in a dose- and estrous cycle phase and breed-dependent manner [14]. These results indicate that OMNT1 may function both as a target and a modulator of pituitary activity, linking metabolic signals to hormonal regulation. In this study, the experimental model was pigs with different genetic backgrounds: Large White (LW) and Meishan (MS) breeds. LW pigs represent a normal-weight breed with a relatively delayed onset of puberty, whereas MS pigs are characterized by a genetic predisposition to obesity and early sexual maturation [15–17]. Metabolic and endocrine profiling of these breeds has revealed significant differences in adipokine dynamics [4, 18]. Compared with LW pigs, MS pigs present lower OMNT1 levels in white adipose tissue and in circulation [19]. Interestingly, our recent studies have shown that MS pigs exhibit increased *ITLN1* expression in the AP gland [14]. These animal models provide a valuable framework for integrating molecular changes such as gene and protein expression with physiological effects such as hormonal secretion and reproductive performance, thereby advancing our understanding of how metabolic status shapes endocrine regulation. Therefore, we hypothesized that OMNT1 modulates AP endocrine function through gene and protein regulatory mechanisms that differ between pig breeds with different metabolic profiles. For both pig breeds, normal-weight LW and obese MS, we investigated the impact of OMNT1 on the transcriptome in AP cells and performed functional analyses on the basis of the proteome. Furthermore, we determined the impact of OMNT1 on the mRNA expression and secretion of tropic hormones (GH, PRL, TSH, ACTH, LH, and FSH), as well as selected adipokines (adiponectin, leptin, chemerin, apelin, visfatin, resistin, and vaspin) and gene expression-specific receptors for both tropic hormones (*GHRHR, TRHR, CRHR1*, and *GNRHR*) and adipokines (*ADIPOR1, ADIPOR2, LEPR, CMKLR1, CCRL2, GPR1, APLNR, TLR4*, and *HSPA5*). Additionally, we conducted a study using pharmacological inhibitors of ERK1/2 and protein kinase C (PKC), which are key regulators of hormone secretion, to assess the involvement of OMNT1 in the activation of intracellular signaling pathways related to secretion processes in AP cells.

2 | Materials and Methods

2.1 | Sample Collection and In Vitro Culture of AP Cells

Pituitary glands were obtained as by-products from pig slaughter at INRAE (Nouzilly, France) and a Polish slaughterhouse, in compliance with Directive 2010/63/EU and the Polish Animal Protection Act (Dz.U. 2015, item 266). Ethics committee approval was not required, as the material was collected post-mortem from animals slaughtered for research or commercial purposes. The study used sexually mature female pigs aged 5–8 months from two breeds: LW (mean body weight: 91.76 ± 8.2 kg) and MS (30.62 ± 5.8 kg); these pig breeds differ in their genetic profiles [15]; MS pigs are characterized by greater backfat thickness as well as selected metabolic and reproductive parameters, as described previously [4, 14, 19]. The Orfrasiere Animal Physiology Experimental Unit and Pig Phenotyping and Innovative Breeding Facility routinely collect zootechnical data (https://doi.org/10.15454/1.5572415481185847E12). Pituitary glands were collected from pigs in the middle luteal phase of the cycle (days from 10 to 12 of the estrous cycle, fully active corpora lutea), as assessed on the basis of ovarian morphology (substantial, centrally enclosed pink corpora lutea, yielding to gentle pressure, containing an internal cavity filled with luteal tissue and visible surface blood vessels; in addition, follicles of middle size ranging from 4.0 to 6.0 mm in diameter are present), as are the plasma profiles of estradiol and progesterone [20, 21]. The stage of the estrous cycle was chosen according to previous studies on the phase-dependent expression and regulation of OMNT1 in APs [14].

To investigate the effects of OMNT1 on the transcriptome and proteome, as well as on the mRNA expression and secretion of tropic hormones, selected adipokines, and their receptors, all experiments were performed on primary AP cell cultures (passage 0; refers to primary AP cells immediately after isolation, which were seeded directly into culture without any prior passaging). For both breeds, the same isolation protocol, seeding density, culture conditions, and treatment schemes were applied (Figure 1). Replicates for each experimental setup were prepared within a comparable time frame and using reagents from the same batches to ensure full methodological and biological comparability between breeds. Only the AP gland was used for cell isolation as previously described [14, 21], avoiding contamination with the posterior lobe. Therefore, after dissection, the AP gland was processed into small fragments (1–2 mm) and separated in Dulbecco's Modified Eagle's Medium (DMEM; Sigma-Aldrich, USA) supplemented with 0.1% bovine serum albumin, 100 U/mL penicillin, 100 μg/mL streptomycin, and 2.5 μg/mL amphotericin B (cat. no. 15240062, Thermo Fisher Scientific, USA). Single-cell suspensions were obtained by repeated digestion with 0.2% collagenase type V (cat. no. C9263,

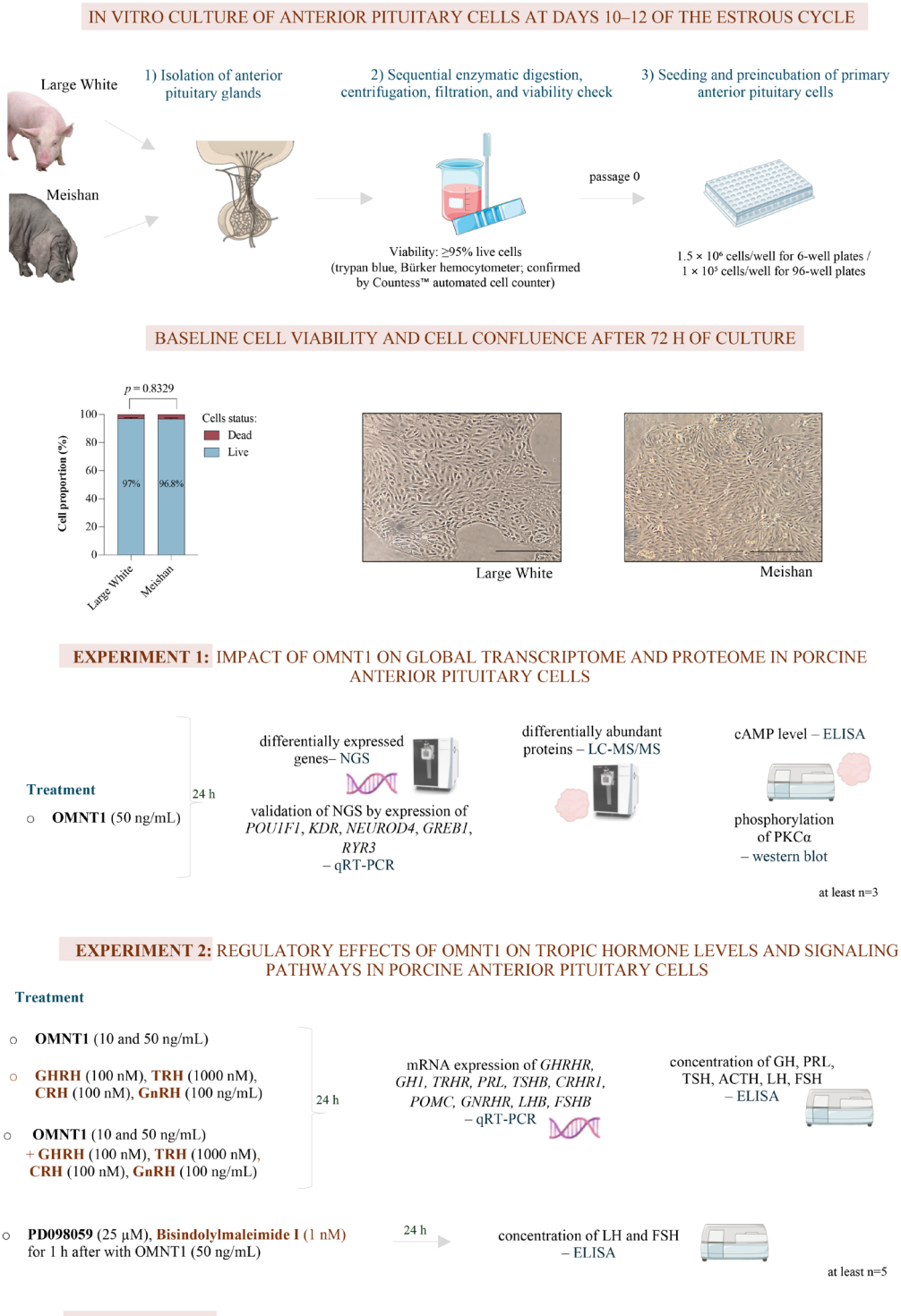


FIGURE 1 | Legend on next page.

FIGURE 1 | Baseline viability, morphology, and experimental workflow of primary anterior pituitary cells from Large White and Meishan pigs. Anterior pituitary cells were isolated by sequential enzymatic digestion, centrifugation, and filtration. Post-isolation viability was assessed by Trypan blue exclusion and manual counting in a Bürker hemocytometer and further confirmed using a Countess automated cell counter (Invitrogen, USA). Data from five replicates (mean ± SEM) showed ≥ 95% viable cells with no significant differences between breeds. Representative phase-contrast micrographs at 72 h post-isolation (20× objective) illustrate comparable monolayers with 60%–70% confluence in both breeds (scale bar = 100 μm). The schematic also presents experimental workflow, including culture conditions and treatments applied in both breeds. The presence of all endocrine cell types was confirmed by immunohistochemistry [14]. *ACTH*, adrenocorticotropic hormone; *ADIPOQ*, adiponectin; *ADIPOR1/ADIPOR2*, adiponectin receptor 1/2; *APLN*, apelin; *APLNR*, apelin receptor; *CCRL2*, C–C chemokine receptor-like 2; *CMKLR1*, chemokine-like receptor 1; CRH, corticotropin- releasing hormone; *CRHR1*, corticotropin-releasing hormone receptor 1; ELISA, Enzyme-Linked Immunosorbent Assay; *FSHB*, follicle-stimulating hormone beta subunit; *GH*, growth hormone; GHRH, growth hormone-releasing hormone; *GHRHR*, growth hormone-releasing hormone receptor; GnRH, gonadotropin-releasing hormone; *GNRHR*, gonadotropin-releasing hormone receptor; *GPR1*, G protein-coupled receptor 1; *GREB1*, growth regulation by estrogen in breast cancer 1; *HSPA5*, heat shock protein family a member 5; *KDR*, kinase insert domain receptor; LC–MS/MS, Liquid Chromatography–Tandem Mass Spectrometry; *LEP/LEPR*, leptin/leptin receptor; *LHB*, luteinizing hormone beta subunit; *NAMPT*, nicotinamide phosphoribosyltransferase; *NEUROD4*, neuronal differentiation 4; NGS, Next Generation Sequencing; *OMNT1*, omentin–1; PKCα, protein kinase C alpha; *POM*C, proopiomelanocortin; *POU1F1*, POU class 1 homeobox 1; *PRL*, prolactin; qRT-PCR, quantitative Reverse Transcription Polymerase Chain Reaction; *RARRES2*, retinoic acid receptor responder protein 2; *RETN*, resistin; *RYR3*, ryanodine receptor 3; *SERPINA12*, serpin family a member 12; *TLR4*, toll-like receptor 4; *TRHR*, thyrotropin-releasing hormone receptor; TRH, thyrotropin-releasing hormone; *TSHB*, thyroid-stimulating hormone beta subunit.

Sigma-Aldrich, USA) and/or 0.25% pancreatin (cat. no. P1750, Sigma-Aldrich, USA) in DMEM for 10 min at 37°C. The AP cells separated by several centrifugations at 170 × g for 10 min were filtered through a nylon mesh (70 μm) to exclude undigested tissue fragments. Cell viability was assessed immediately after isolation, before to seeding, using 0.4% Trypan blue (cat. no. 15250061, Thermo Fisher Scientific, USA) and counted in a Bürker hemocytometer. Results were additionally verified with a Countess automated cell counter (Invitrogen, USA). For each independent isolation, the percentage of live cells was calculated. Only cultures with viability ≥ 95% were used in experiments. Cultures were maintained in McCoy's 5A medium supplemented with 10% horse serum (cat. no. H1270, Sigma-Aldrich, USA), 1% antibiotic cocktail, 0.1% minimum essential medium (MEM) vitamins (cat. no. 11120052, Thermo Fisher Scientific, USA), and 0.01% MEM non-essential amino acids (cat. no. 11140050, Thermo Fisher Scientific, USA). Cells were seeded immediately after isolation, without prior subculturing, preserving the original cellular composition and phenotype. They were plated in 6 well plates at 1.5×10^6 cells/well or in 96 well plates at 1×10^5 cells/well, and preincubated for 72 h at 37°C in a humidified atmosphere with 5% CO_2 and 95% air. After preincubation, cultures were maintained in McCoy's 5A medium containing 1% horse serum, with or without the tested substances under specific experimental conditions. In both breeds, cells were treated with OMNT1 (cat. no. 9137-IN-050, Bio-Techne, USA) at concentrations of 10 and/or 50 ng/mL, alone or in combination with the following hypothalamic-releasing hormones: GHRH (100 nM; cat. no. G4535, Sigma-Aldrich, USA), TRH (1000 nM; cat. no. P1319, Sigma-Aldrich, USA), CRH (100 nM; cat. no. C3042, Sigma-Aldrich, USA), and GnRH (100 ng/mL; cat. no. L8008, Sigma-Aldrich, USA). Also, we assessed the role of OMNT1in hormone secretion via ERK1/2 and PKC signaling pathways, using their pharmacological inhibitors: PD098059 (25 μM; cat. no. 1213, Tocris, UK) and Bisindolylmaleimide I (1 nM; cat. no. 203290, Sigma-Aldrich, USA). Doses of all hormones and pharmacological inhibitors were selected on the basis of our previous data and the available literature [14, 21–25]. After 24 h of incubation, the culture medium and cells were stored at −70°C for further analyses, including transcriptome profiling (next-generation sequencing, NGS; $n = 3$ biological replicates per group), proteomic analysis (liquid chromatography-tandem mass spectrometry, LC–MS/MS; $n = 4$), gene expression analysis (reverse transcription quantitative real-time polymerase chain reaction, RT-qPCR; $n = 5$), protein expression (Western blot; $n = 3$), intracellular cyclic adenosine monophosphate (cAMP) levels ($n = 5$), and hormone concentrations (enzyme-linked immunosorbent assay, ELISA; $n = 5$).

2.2 | RNA Isolation, Library Preparation, and High-Throughput Sequencing Procedure

Total RNA was extracted via phenol-chloroform RNA extraction according to the manufacturer's protocol (cat. no. EM30, CytoGen, South Korea). The concentration (A260) and purity (A260/A280 ratio) of the isolated RNA were assessed spectrophotometrically via a NanoDrop spectrophotometer (Peqlab Biotechnologie GmbH, Germany). Furthermore, RNA integrity was evaluated with a Bioanalyzer 2100 system (Agilent Technologies, USA). Only RNA samples with a purity (A260/A280) > 1.8 and an RNA integrity number (RIN) > 7.0 were considered suitable for RNA-Seq and quantitative qPCR validation. RNA library preparation and sequencing were performed as a service by Macrogen (RRID:SCR_014454; South Korea). Libraries were prepared via the TruSeq mRNA-Stranded Kit with poly(A) selection according to the manufacturer's protocol. High-throughput sequencing was carried out on the NovaSeq X platform (Illumina, USA) with a 2 × 150 bp configuration, with a minimal throughput of 40 million reads per sample.

2.3 | Raw Reads Pre-Processing, Mapping to a Reference Genome, and Differentially Expressed Genes Processing

Raw sequencing reads were quality-checked using FastQC (ver. 0.12.1; RRID:SCR_014583) [26]. Adapter sequences and low-quality fragments were trimmed using Trimmomatic (ver. 0.39; RRID:SCR_011848) [27], and reads were clipped to equal lengths. For adapter removal, a maximum of two mismatches was allowed in the adapter sequence, and an adapter was

removed if its alignment score fell below 20. Quality filtering was performed using a sliding window approach, where a 4 base window was scanned, and regions with an average quality score (QPhred) below 20 were removed. Additionally, bases with a quality score below 20 were trimmed from both the 5′ and 3′ ends of each read to improve sequence reliability. Reads shorter than 36 bases after trimming were discarded to ensure sufficient read length for accurate alignment. The processed reads were then re-evaluated for quality and residual adapter content using FastQC. The filtered reads were mapped to the *Sus scrofa domestica* reference genome (assembly: Sscrofa11.1), obtained from the Ensembl database (RRID:SCR_002344) [28], using STAR (ver. 2.7.11a; RRID:SCR_004463) [29] on the Galaxy platform (ver. 2.7.11a + galaxy1; RRID:SCR_006281) [30]. The mapping parameters allowed up to two mismatches per read, required a minimum of eight bases for detecting splice junctions to optimize exon–intron boundary accuracy, and implemented a two-pass mapping strategy to enhance splice junction discovery. Post-mapping quality control was performed using SAMtools (ver. 1.18; RRID:SCR_002105), assessing mapping efficiency, read distribution, and duplication rates. Transcript assembly was conducted using StringTie (ver. 2.2.3; RRID:SCR_016323) [31, 32] in reference-guided mode, available on the Galaxy platform. Transcript expression levels were quantified using featureCounts (ver. 2.0.1; RRID:SCR_012919) [33] within the Galaxy platform. Gene expression normalization and differential gene expression analysis were performed in the R environment (ver. 4.1.1; RRID:SCR_001905) [34] using the DESeq2 package (ver. 1.40.2; RRID:SCR_000154) [35]. Normalization was performed using the median ratio method to correct for sequencing depth and RNA composition bias. For further analysis, only genes with a count per million (CPM) value ≥ 1 in at least three samples were retained. Differential expression analysis was conducted using a negative binomial generalized linear model (GLM) with dispersion estimated via empirical Bayes shrinkage [36]. Statistical significance was determined using the Wald test with Benjamini–Hochberg correction. DEGs were primarily defined using a fold-change threshold (≤ -1.2 or ≥ 1.2) combined with raw p values ($p < 0.05$), to capture subtle, physiologically relevant changes that may not necessarily reach significance after multiple testing correction but could still indicate meaningful pathway-level effects of OMNT1 treatment. For transparency, the numbers of DEGs passing false discovery rate (FDR)–adjusted thresholds (adj $p < 0.05$ and 0.1) are reported in the Results and Supplementary Files 2 and 3.

2.4 | Protein Isolation, TMT Labeling, and LC–MS/MS Analysis

For proteomic analysis, AP cells were lysed in 20% 2,2,2-trifluoethanol (50 μL/well; cat. no. T63002, Sigma-Aldrich, USA) in 100 mM triethylammonium bicarbonate (cat. no. 18597, Sigma-Aldrich, USA), harvested, and incubated at 60°C for 30 min to denature proteins and inactivate proteases. Samples were additionally sonicated for 45 min in an ultrasonic bath, and total protein concentration was measured using the bicinchoninic acid assay (cat. no. 23225, Thermo Fisher Scientific, USA). Lysates were stored at −70°C until analysis. From this point on, each sample was individually processed and analyzed at the Mass Spectrometry Laboratory, Institute of Biochemistry and Biophysics, Polish Academy of Sciences. Protein digestion was performed using a modified Single-Pot Solid-Phase-enhanced Sample Preparation (SP3) method [37] with a 1:1 mixture of hydrophilic and hydrophobic Sera-Mag Carboxyl magnetic beads (Cytiva, USA), which were pre-washed in MS grade water and resuspended to 10 μg/μL working concentration. Lysates were supplemented with 1% SDS and 10 mM tris(2-carboxyethyl)phosphine (TCEP) and incubated for 60 min at 60°C, followed by alkylation with 30 mM methanethiosulfate for 15 min to prevent disulfide bond formation. Proteins were bound to the beads in the presence of 80% acetonitrile and 1% formic acid, washed three times with 85% ethanol and once with acetonitrile, and then digested overnight at 37°C with trypsin (Promega, USA). The resulting peptides were eluted, quantified with the Pierce Colorimetric Peptide Assay (Thermo Fisher Scientific, USA), and labeled with TMTpro 16plex reagents (Thermo Fisher Scientific, USA). Labeling efficiency was verified, quenched with 5% hydroxylamine, and peptides were desalted using Oasis HLB columns (Waters, Poland). Labeled peptides were separated by high-pH reversed-phase chromatography (HpRP) using an XBridge Peptide BEH C18 column (Waters, Poland) yielding 48 fractions which were pooled into 24 final fractions. Mass spectrometry was performed using an Orbitrap Exploris 480 instrument (Thermo Fisher Scientific, USA) coupled to an Evosep One LC system (Evosep Biosystems, Denmark). Data were acquired in data-dependent acquisition mode (DDA) with TurboTMT settings, at a resolution of 60 000 for MS1 and 30 000 for MS2, with normalized collision energy of 30% and dynamic exclusion of 20 s. Peptides were separated on a C18 analytical column (Dr. Maisch GmbH) using the Evosep Extended method (15 samples/day). Raw MS data were processed in MaxQuant (version 2.5; RRID:SCR_014485) using the *Sus scrofa domestica* reference proteome from UniProt (UP000008227; RRID:SCR_002380) with trypsin/P specificity, fixed carbamidomethylation (Cys), oxidation (Met) as a variable modification, maximum two missed cleavages, and peptide-spectrum match and protein FDR set to 0.01. Data were further analyzed in Perseus (version 1.6.15; RRID:SCR_015753). Contaminants, reverse hits, and proteins lacking sufficient data were excluded. The data were $\log_2$-transformed, missing values imputed from a normal distribution, and batch effects corrected using Limma (RRID:SCR_010943). Differentially abundant proteins (DAPs) were identified using a two-sample t-test with a fold change threshold of ≤ -1.2 or ≥ 1.2, consistent with physiological relevance in hormonal studies [38].

2.5 | Functional Analysis of the Transcriptome and Proteome

Gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG; RRID:SCR_018145) pathway enrichment analyses were performed using the Functional Annotation Tool of the DAVID Knowledgebase (version 2024q2) [39, 40], with *Sus scrofa domestica* as the reference organism. Additionally, pathway analysis was performed using the protein analysis through evolutionary relationships (PANTHER) Classification System (version 19.0; RRID:SCR_004869) [41, 42], which used an overrepresentation test on Reactome pathways (version 86; RRID:SCR_003485) [43]. For insights into the functional associations of the DEGs and DAPs, the Gene Multiple

Association Network Integration Algorithm (GeneMANIA; RRID:SCR_005709) [44] prediction server was utilized. The genes and proteins were analyzed against the human interactome, including genetic interactions, co-expression, co-localization, physical interactions, shared protein domains, pathways, and predicted interactions. The functional annotations derived from these interactions were also assigned. A statistically significant threshold was set at a p value < 0.05 for all analyses performed. Data visualization was performed via R Studio (R version 4.4) and Python (version 3.12; RRID:SCR_008394).

2.6 | Concordance Analysis

To evaluate the concordance of gene and protein regulation between LW and MS pigs, we compared the results of differential expression and differential abundance analyses for both breeds. The analysis included genes and proteins that met the criteria of a fold change of ≤ -1.2 or ≥ 1.2 and a p value of ≤ 0.05 in both data sets. Concordance was defined as the proportion of overlapping genes or proteins exhibiting the same direction of regulation in both breeds, while discordance corresponded to those regulated in opposite directions. The results were visualized using scatter plots for the two breeds, generated in R (R version 4.4) with the ggplot2 and ggrepel packages. Complementary Venn diagrams were generated to illustrate the overlap of DEGs and DAPs between breeds.

2.7 | Reverse Transcription Quantitative Real-Time Polymerase Chain Reaction (RT-qPCR)

Gene expression was assessed via TaqMan probes and specific primers (Supporting Information File 1), with sample preparation performed according to the manufacturer's guidelines. TaqMan gene expression assays (Thermo Fisher Scientific, USA) were used to validate genes from NGS and measure the mRNA content of tropic hormones and their receptors. The RNA isolation and cDNA synthesis followed the TaqMan Gene Expression Cells-to-CT Kit protocol (cat. no. AM1728, Applied Biosystems, USA), as previously described [45]. Due to the lack of specific TaqMan probes, primers were used to examine the mRNA expression of the genes encoding adipokines and their receptors. The RNA extraction from AP cells was performed via QIAzol lysis reagent (cat. no. 79306, Qiagen, Germany) according to the manufacturer's instructions, as previously described [14]. For cDNA synthesis, 1 μg of total RNA was reverse transcribed (at 37°C for 60 min, total volume of 20 μL). The reaction mixture included deoxynucleotide triphosphates, 10× reverse transcription buffer, oligodeoxythymidylic acid, a recombinant ribonuclease inhibitor, and Moloney murine leukemia virus reverse transcriptase (cat. no. U1515, A3561, C1101, N2515, and M1705, respectively, Promega, USA). TaqMan probes and specific primers for reference genes *PPIA* (cyclophilin A), *ACTB* (β-actin), and *GAPDH* (glyceraldehyde 3 phosphate dehydrogenase), unaffected by breed, doses of OMNT1, and hormones released from the hypothalamus, were used for qPCR. The reaction mixtures for the TaqMan probes and primers were prepared following previously described protocols [14, 45]. Relative gene expression was calculated via the comparative cycle threshold $2^{-\Delta\Delta Ct}$ method [46]. The negative control used RNase-free water instead of cDNA.

2.8 | Western Blot

Western blot analysis was conducted to validate the proteomic results for selected proteins, including zinc finger protein 24, Ras-related GTP-binding protein D, SIX homeobox 1, NAD(+) hydrolase, voltage-dependent anion channel 3, and the signaling proteins protein kinase B (AKT) and ERK1/2. The detailed proteomic data are presented at http://doi.org/10.6019/PXD061269. In this study, the time-dependent phosphorylation of PKCα kinase in OMNT1-treated LW breed pigs was investigated. Lysates from cultured AP cells were obtained as previously described [14]. Protein samples (20 μg per lane) were mixed with Laemmli buffer (cat. no. 38733, Sigma-Aldrich, USA), heated at 95°C for 5 min, and separated on manually prepared 8% sodium dodecyl sulfate-polyacrylamide gels in an electrophoresis chamber. Proteins were then transferred to polyvinylidene fluoride membranes (cat. no. IPVH00010, Sigma-Aldrich, USA) and blocked (for 60 min at 25°C) with 5% bovine serum albumin and Tris-buffered saline containing 0.1% Tween 20. The membranes were incubated overnight at 4°C with primary antibodies against phosphor-PKCα and total PKCα (cat. no. ab180848, RRID:AB_2783796, and ab32376, RRID:AB_777294, respectively, Abcam, UK) at a 1:300 dilution and then washed. Next, the membranes were incubated for 60 min with an anti-rabbit antibody (cat. no. 7074, RRID:AB_2099233; Cell Signaling Technology, USA) conjugated with horseradish peroxidase diluted 1:1000. Protein detection was carried out via chemiluminescence via the Immobilon Western Chemiluminescent HRP Substrate (cat. no. WBKLS0500, Millipore, USA) and visualized with the ChemiDoc imaging system (Bio-Rad, USA). The protein levels were quantified through densitometric analysis via ImageJ software (RRID:SCR_003070; National Institutes of Health, USA).

2.9 | Enzyme-Linked Immunosorbent Assay (ELISA)

The intracellular level of cAMP and the concentrations of tropic hormones and adipokines in cell cultures were determined via porcine-specific ELISA kits according to the manufacturers' instructions. The analysis included cAMP (ADI-900-067A, Enzo Biochem Inc., USA), the tropic hormones: GH, PRL, TSH, ACTH, LH, and FSH (cat. no. EP0064, EP0139, EP0310, EP0003, EP0105, and EP0060, FineTest, China), and the adipokines: adiponectin, leptin, chemerin, apelin, resistin (cat. no. EP0006-HS, EP0103, EP0169, EP0140, EP0185, FineTest, China), visfatin, and vaspin (cat. no. CK-bio-24 553 and CK-bio-24 552, Shanghai Coon Koon Biotech Co. Ltd., China). The intra-assay coefficient of variation was less than 7%, whereas the inter-assay variability was less than 10%. Absorbance readings for tropic hormones and adipokines were obtained at 450 nm, whereas the absorbance for cAMP was measured at 405 nm via a Varioskan LUX multimode microplate reader (Thermo Fisher Scientific, USA). Standard

curves were generated via Curve Expert software (Hyams Development, USA), with all curve fits validated by a coefficient of determination (R^2) greater than 0.99.

2.10 | Statistical Analyses

All experimental data are presented as the mean ± standard errors of the mean (SEM) from at least three biologically independent experiments per group. The normality of data distribution was assessed using the Shapiro–Wilk test. Homogeneity of variances was evaluated with Levene's test where applicable. For cell viability data, differences between breeds were analyzed using an unpaired the Student's *t*-test with Welch's correction. For comparisons between OMNT1 (50 ng/mL) and control, a two-tailed the Student's *t*-test was applied. Two-way analysis of variance (ANOVA), followed by Tukey's post hoc test, was used to compare hormone levels across breeds (LW and MS) and across OMNT1 doses or in combination with hypothalamic hormones. One-way ANOVA was used to assess the effects of OMNT1 with or without pharmacological inhibitors. Statistical analysis was performed using GraphPad Prism software (RRID:SCR_002798). Statistical significance was set at $p < 0.05$. Significance is indicated by different lettering or by asterisks: * ($p < 0.05$), ** ($p < 0.01$), **** ($p < 0.0001$).

3 | Results

3.1 | Baseline Characterization and Culture Conditions of Primary AP Cells From Both Breeds

Primary AP cells from LW and MS pigs were derived at passage 0 and seeded at identical densities for each plate format to ensure comparable starting conditions. Post-isolation viability was high in both breeds (LW: 97.0 ± 1.581%, MS: 96.8 ± 1.304%; $p > 0.05$, Figure 1). Phase-contrast microscopy at 72 h revealed monolayers with similar morphology, confluence (estimated 60%–70%), and attachment patterns in both breeds (Figure 1). The presence of all AP endocrine cell types was previously confirmed by immunohistochemistry, including GH-positive somatotrophs, PRL-positive lactotrophs, ACTH-positive corticotrophs, TSH-positive thyrotrophs, and LH-/FSH-positive gonadotrophs [14]. The experimental workflow illustrates that both breeds were handled using identical protocols (Figure 1).

3.2 | Overall Metrics of RNA-Seq, Reads Mapping, and DEG Analysis

The RNA-Seq generated an average of 42.54 million raw reads per sample, with an average Q20 (percentage of bases with a sequencing error probability ≤ 1%) of 98.22% and an average Q30 (percentage of bases with a sequencing error probability ≤ 0.1%) of 95.29%. These results confirm high RNA-Seq sequencing quality and an adequate read depth. Mapping efficiency was evaluated using RNA-Seq mapping metrics, with an average of 91.63% of reads uniquely mapped to the reference genome, indicating high-sequencing quality, specificity, and efficient mapping. On average, only 1.88% of the reads mapped to multiple loci, suggesting that the majority of the sequences were unique. DEG analysis revealed a total of 13 310 genes in LW pigs and 13 272 genes in MS pigs. For further analysis, only genes meeting a fold-change ≤ −1.2 or ≥ 1.2 and *p* values ≤ 0.05 were selected. On the basis of these thresholds, 655 DEGs were identified in LW pigs (Supporting Information File 2), and 420 DEGs were identified in MS pigs (Supporting Information File 3), indicating significant differential expression between the treatment and control groups. Among these genes, 293 were downregulated and 362 genes were upregulated in LW pigs, whereas 260 genes were downregulated and 160 genes were upregulated in MS pigs (Figure 2A). For transparency, at FDR-adjusted thresholds, 39 (adj $p < 0.05$) and 53 (adj $p < 0.1$) DEGs were detected in LW pigs, whereas no DEGs passed these thresholds in MS pigs (Supporting Information Files 2 and 3).

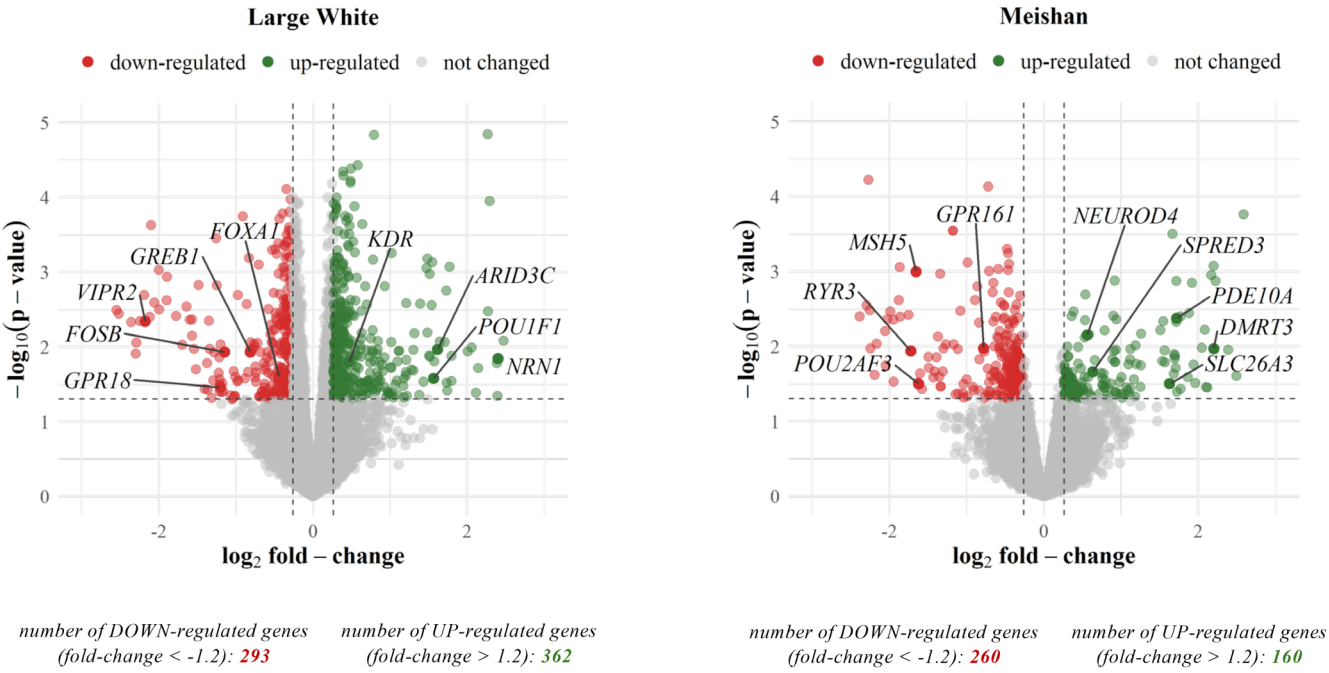
To assess the concordance of differential expression and abundance results between breeds, only nine common genes and ten common proteins met the filtering criteria (fold change ≤ −1.2 or ≥ 1.2 and *p* value ≤ 0.05) in both breeds (Supporting Information File 4). In the transcriptomic data set, approximately 66.7% concordance was observed (six out of nine genes exhibited the same direction of change), while three genes (33.3%) were regulated in opposite directions. In the proteomic data set, nine proteins (90%) showed the same direction of change, and only one protein (10%) exhibited opposite regulation. This suggests a higher level of concordance at the protein level than in the transcriptome; however, the small number of overlapping genes and proteins limits the ability to draw broader conclusions. The overlap between breeds is further illustrated by scatter plots and complementary Venn diagrams (Supporting Information File 4).

The results of the NGS analysis were confirmed via RT-qPCR validation of five selected DEGs. As shown in Figure 2B, OMNT1 (50 ng/mL) treatment significantly increased the expression of POU class 1 homeobox 1 (*POU1F1*), kinase insert domain receptor (*KDR*) in LW pigs, and neuronal differentiation 4 (*NEUROD4*; $p < 0.05$) in MS pigs, while reducing the expression of growth regulating estrogen receptor (ESR) binding 1 (*GREB1*) in LW pigs and ryanodine receptor 3 (*RYR3*) in MS pigs compared with the control group ($p < 0.05$).

3.3 | Gene Ontology Analyses

To investigate the biological relevance of OMNT1-modulated genes, GO enrichment analysis was performed with the DAVID Functional Annotation Tool for both pig breeds. The results of the functional enrichment analysis for the LW and MS pigs were categorized into three GO groups: “biological processes”, “cellular components”, and “molecular function” (Figure 3A,B, $p < 0.05$). In LW pigs, most DEGs were predominantly related to the extracellular space and collagen-containing extracellular matrix, as well as plasma membrane components. Biological processes highlighted extracellular matrix (ECM) organization, collagen fibril assembly, and calcium-mediated signaling, while molecular functions were mainly associated with protein binding, calcium ion binding, and GTPase activity ($p < 0.05$, Figure 3A; Supporting Information File 2). In MS pigs, enriched cellular components included the endoplasmic reticulum, microtubules, and extracellular matrix. Biological processes and molecular functions

(A) IDENTIFIED GENES



number of DOWN-regulated genes (fold-change < -1.2): ***293***

number of UP-regulated genes (fold-change > 1.2): ***362***

number of DOWN-regulated genes (fold-change < -1.2): ***260***

number of UP-regulated genes (fold-change > 1.2): ***160***

(B) VALIDATION

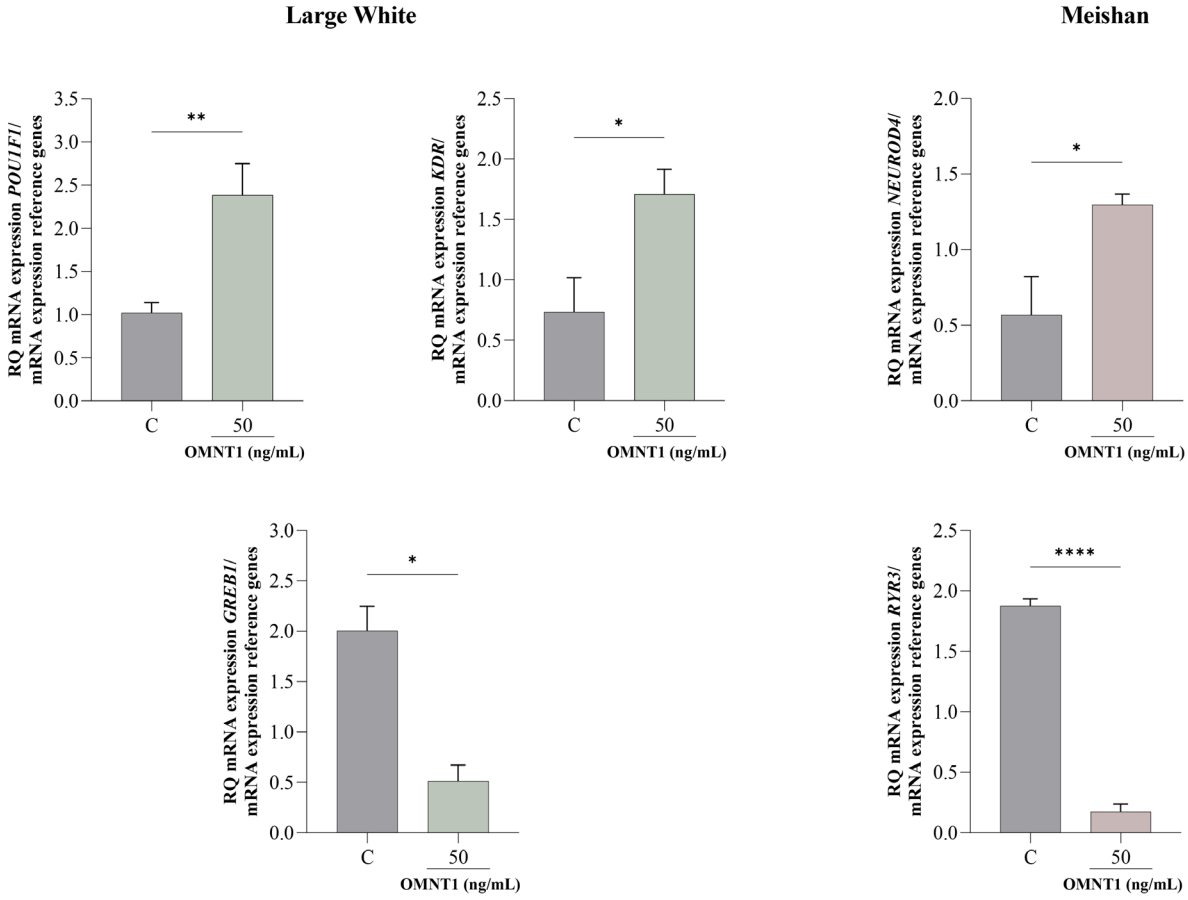


FIGURE 2 | Legend on next page.

FIGURE 2 | Analysis of the transcriptome of anterior pituitary cells affected by omentin-1 (OMNT1; 50 ng/mL) depends on the breed of Large White and Meishan pigs. (A) Volcano plots of differentially expressed genes in the presence of OMNT1. Genes with a fold change ≤ −1.2 or ≥ 1.2 were considered statistically significant. Red dots indicate significantly downregulated proteins, green dots indicate significantly upregulated genes, and gray dots indicate proteins whose expression did not significantly differ. (B) Validation of next sequencing generation results by reverse transcription quantitative polymerase chain reaction for differentially expressed genes affected by OMNT1 (50 ng/mL) in anterior pituitary cells. The results of at least three independent replicates are presented as the mean ± SEM for each group. The bars represent statistical significance * ($p < 0.05$), ** ($p < 0.01$), **** ($p < 0.0001$). *GREB1*, growth regulating estrogen receptor binding 1; *KDR*, kinase insert domain receptor; *NEUROD4*, neuronal differentiation 4; *POU1F1*, POU class 1 homeobox 1; *RYR3*, ryanodine receptor 3.

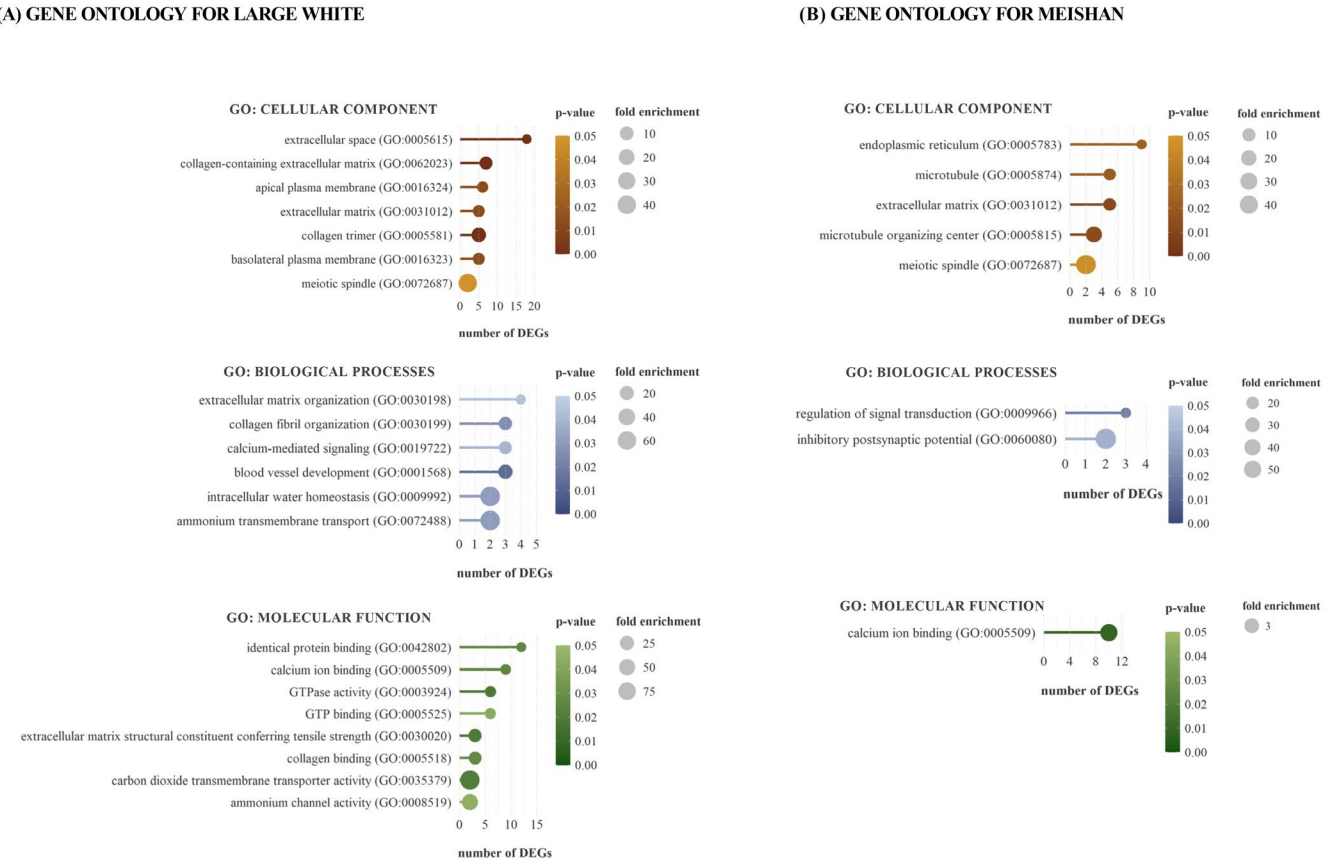


FIGURE 3 | Gene ontology (GO) classification in terms of the cellular component, biological process, and molecular function of differentially expressed genes (DEGs) in the presence of omentin-1 (50 ng/mL) in (A) Large White and (B) Meishan pigs. Statistical significance (p value) and fold enrichment are shown.

were related to signal transduction regulation, synaptic potential, and calcium ion binding ($p < 0.05$, Figure 3B; Supporting Information File 3). Detailed gene ontology associations are provided in Supporting Information Files 2 and 3 for readers interested in specific genes.

3.4 | Signaling Pathways and Gene Interaction Networks

Pathway enrichment analysis of OMNT1-modulated DEGs was performed using the PANTHER overrepresentation test with Reactome pathway annotation. In LW pigs, nine enriched pathways were identified, mainly related to cellular communication and cytoskeleton dynamics, with the largest group of DEGs linked to collagen formation and fibril assembly ($p < 0.05$, Figure 4A). Interaction networks generated with GeneMANIA revealed a densely interconnected network, with a compact core dominated by ECM components, adhesion molecules, and plasma membrane receptors. Functional enrichment analysis corroborated these findings, pointing to pathways related to collagen metabolism, adhesion complexes, growth factor binding, and receptor-mediated signaling. Taken together, these results suggest that in LW pigs, OMNT1 primarily acts through ECM-receptor-associated mechanisms, supporting both structural integrity and receptor-level communication. Additionally, 11 pathways were enriched for genes involved in the regulation of cellular processes in response to stimuli, most significantly signaling by receptor tyrosine kinases. The network again displayed a predominance of physical interactions and a relatively dense architecture. Strong links connected genes involved in Ras protein signal transduction, protein kinase activity, angiogenesis, and mitochondrial organization. This profile suggests that OMNT1

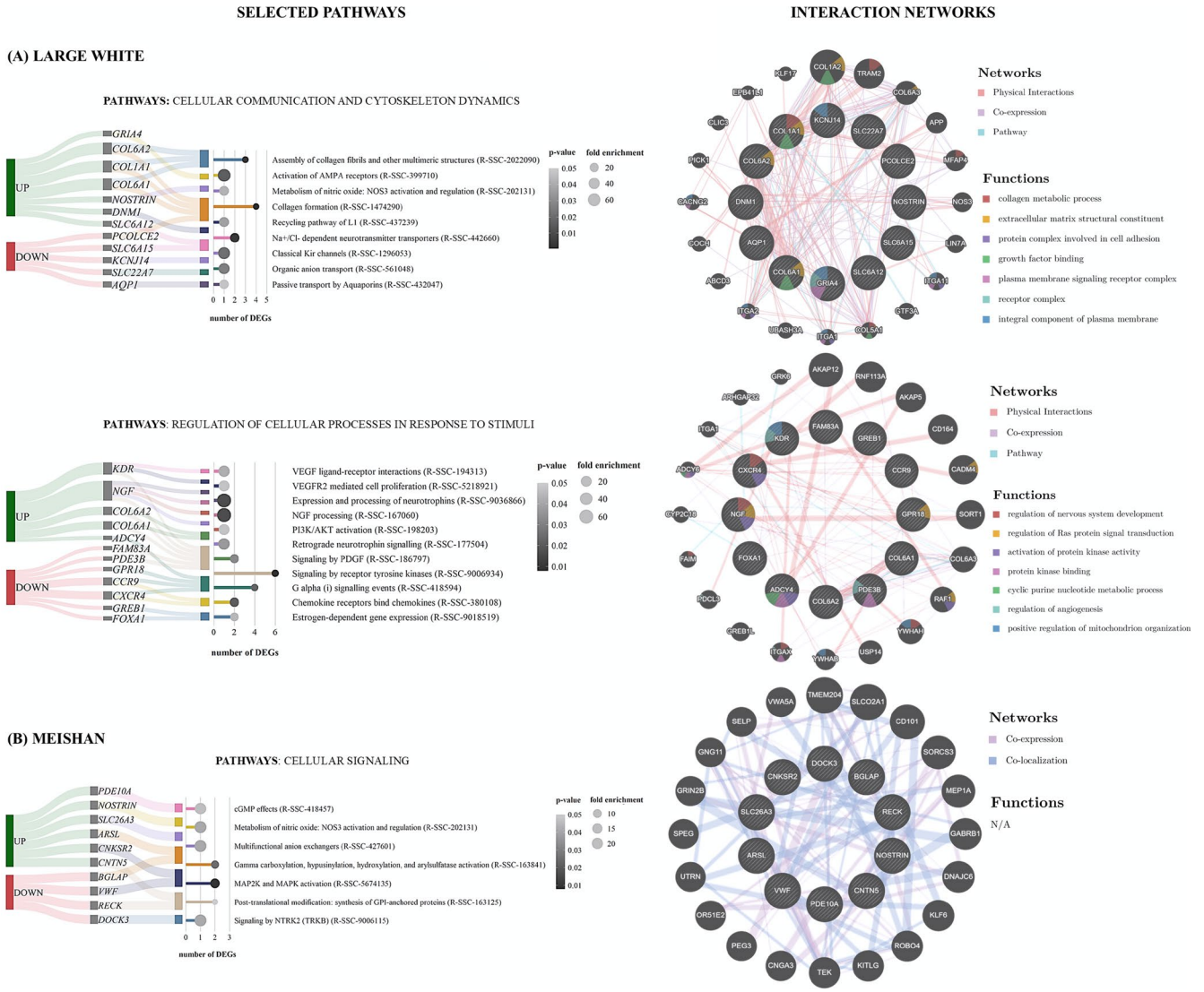
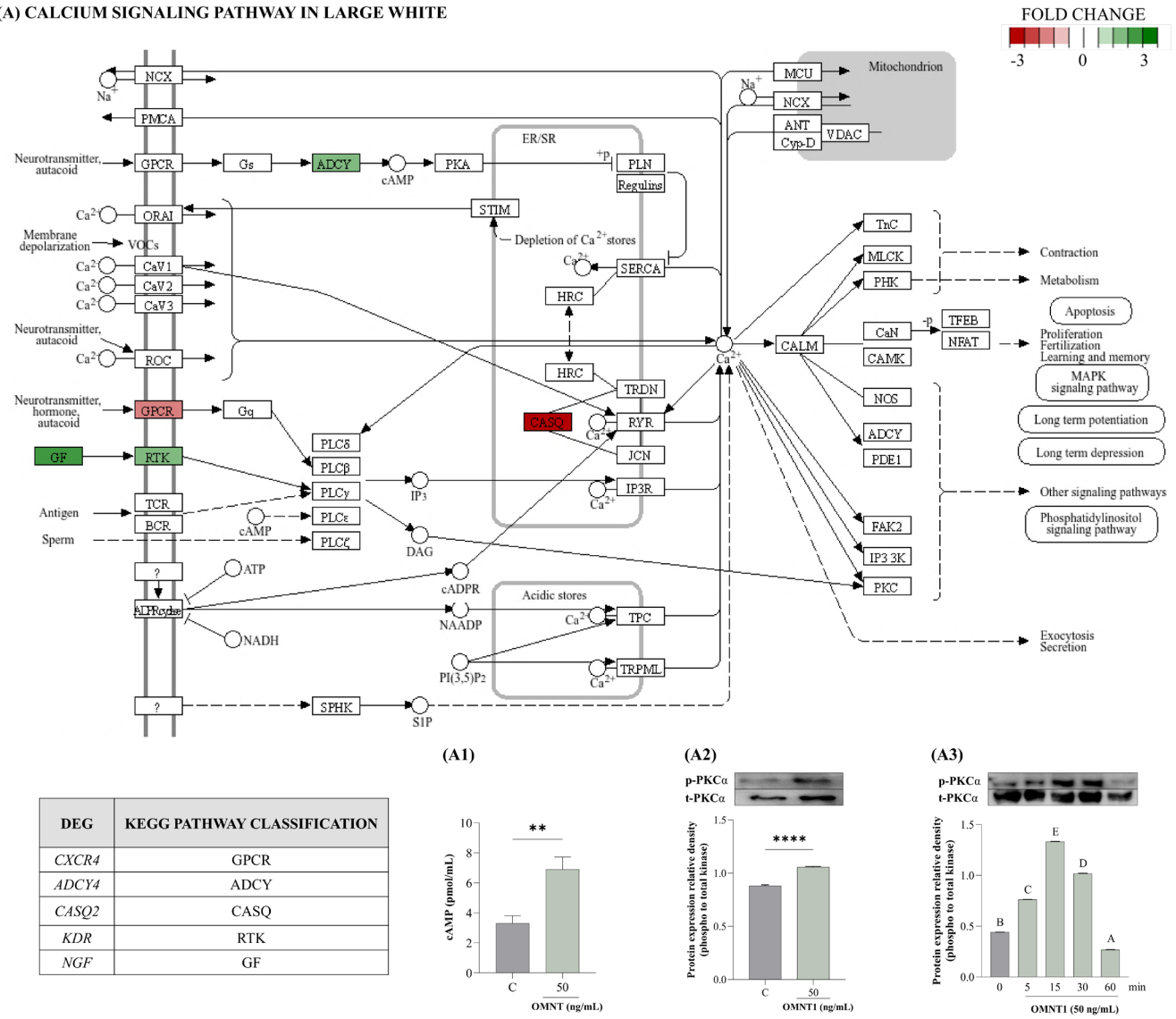


FIGURE 4 | A Sankey diagram illustrates the differentially expressed genes (DEGs) identified in the anterior pituitary cells of (A) Large White and (B) Meishan pigs treated with omentin-1 (50 ng/mL). The diagram shows upregulated and downregulated genes linked to Reactome pathways. The GeneMANIA interaction network shows the interaction strength (edge thickness), interaction type (bottom-right color), and major functions of the proteins. Statistical significance (p value) and fold enrichment are shown.

activates intracellular cascades, with signaling integration in LW pigs depending largely on direct protein–protein interactions rather than transcriptional co-regulation (Figure 4A; Supporting Information File 5).

In MS pigs, seven enriched pathways were detected, mainly related to cellular signaling, with most DEGs linked to gamma carboxylation, hypusynylation, hydroxylation, arylsulfatase activation, mitogen-activated protein kinase (MAPK) and MAP2K activation, and post-translational modification of GPI-anchored proteins ($p < 0.05$, Figure 4B). The interaction network included both co-expression and co-localization links, indicating that the OMNT1 response in MS pigs is mainly coordinated at the transcriptional level and within defined cellular contexts, rather than through direct protein–protein interactions. Thicker edges reflected stronger associations and were concentrated among central nodes, whereas peripheral genes were connected by thinner edges, suggesting weaker or more indirect relationships. Overall, the network indicates that regulation is spread across many genes, but central nodes act as key connectors (Figure 4B; Supporting Information File 5).

Additionally, KEGG enrichment analysis performed with the DAVID Functional Annotation Tool in LW pigs revealed DEGs associated with the calcium signaling pathway ($p < 0.05$, Figure 5A). In LW pigs, OMNT1 (50 ng/mL) increased the expression of genes involved in cAMP signaling, consistent with elevated cAMP levels after 24 h ($p < 0.01$, Figure 5A1). It also upregulated nerve growth factor (*NGF*; KEGG: GF) and *KDR* (KEGG: RTK), linked to PKC pathway regulation, which was supported by increased PKCα protein levels ($p < 0.05$, Figure 5A2) and its time-dependent phosphorylation pattern, with activation observed at 5 min and peaking at 15 min ($p < 0.05$, Figure 5A3). In contrast, OMNT1 reduced the expression of C–X–C motif chemokine receptor 4 (*CXCR4*; KEGG:



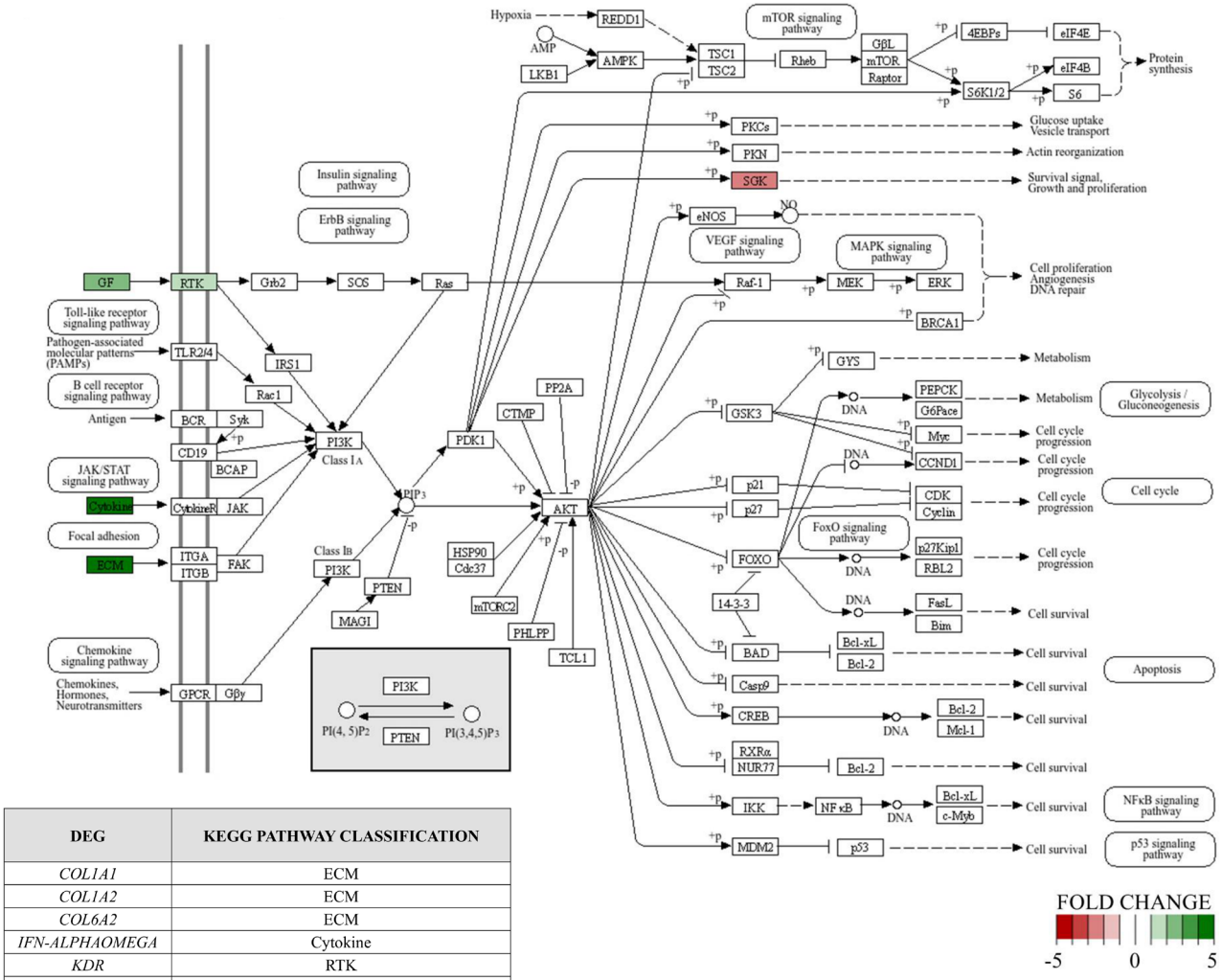
DEG	KEGG PATHWAY CLASSIFICATION
CXCR4	GPCR
ADCY4	ADCY
CASQ2	CASQ
KDR	RTK
NGF	GF

FIGURE 5 | Kyoto encyclopedia of genes and genomes (KEGG) enrichment analysis differentially expressed genes (DEGs) analyzed in the anterior pituitary cells treated with omentin-1 (OMNT1, 50 ng/mL) was performed. (A) The calcium signaling pathway enriched by five DEGs and functional analysis of (A1) cyclic adenosine monophosphate (cAMP) after 24 h and (A2 and A3) time-dependent phosphorylation of protein kinase c alpha (PKCα; 77 kDa). (B) Phosphoinositide 3-kinase/protein kinase B (PI3K/AKT) signaling pathway enriched with five DEGs. The red box shows downregulated genes, and the green box represents upregulated genes. The results of at least three independent replicates are presented as the mean ± SEM for each group. The bars represent statistical significance according to different superscripts and asterisks ** ($p < 0.01$), **** ($p < 0.0001$).

GPCR) and calsequestrin 2 (*CASQ2*; KEGG: CASQ; $p < 0.05$, Figure 5A). In addition, KEGG enrichment indicated modulation of the phosphoinositide 3-kinase (PI3K)/AKT signaling pathway ($p < 0.05$, Figure 5B). OMNT1 upregulated genes activating the MAPK signaling cascade, which regulates cell proliferation, angiogenesis, and DNA repair, while also enhancing the expression of the cytokine interferon alphaomega (*IFN-ALPHAOMEGA*) and ECM-related genes involved in PI3K activation. Conversely, serum/glucocorticoid-regulated kinase 2 (*SGK2*), associated with survival and growth signaling, was downregulated ($p < 0.05$, Figure 5B; Supporting Information File 2). We retained the detailed DEG pathway associations in the Supporting Information Files 2 and 3 for readers interested in specific genes.

3.5 | Signaling Pathways and Protein Interaction Networks

Our proteomic analyses revealed that in MS pigs, OMNT1 (50 ng/mL) modulated the expression of proteins involved in pathways related to protein modifications, their transport and maturation, cytoskeleton dynamics, and the regulation of cellular processes in response to stimuli ($p < 0.05$, Figure 6). In pathways of protein modifications, their transport, and maturation, six enriched pathways were identified. The interaction networks were enriched for proteins involved in the secretory granule lumen, protein processing, and steroid metabolism. The network was dominated by co-expression links, forming a moderately dense architecture with edges of mixed thickness.



DEG	KEGG PATHWAY CLASSIFICATION
COL1A1	ECM
COL1A2	ECM
COL6A2	ECM
IFN-ALPHAOMEGA	Cytokine
KDR	RTK
NGF	GF
SGK2	SGK
VWF	VWF

FIGURE 5 | (Continued)

Functional annotation highlighted protein folding, enzyme inhibitor activity, and hemostasis. This configuration indicates that OMNT1 modulates the coordinated expression of secretory and metabolic proteins. Additionally, we identified eight enriched pathways related to cytoskeleton dynamics. The network grouped proteins associated with cytoskeleton dynamics, especially Ras and Rho GTPase signaling. Here, pathway interactions predominated, showing a relatively scattered but topologically coherent structure. Thicker edges were concentrated around central regulators such as RAS homolog family member A (RHOA), actin-binding proteins, and tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein beta family members, indicating coordinated GTPase-driven signaling and cytoskeletal remodeling. Furthermore, we identified five enriched pathways related to the regulation of cellular processes in response to stimuli. The network included proteins linked to stress responses, steroid binding, and organelle remodeling. The majority of edges reflected shared domain associations, resulting in a looser structure with less clustering. Nevertheless, enriched functions such as multivesicular body organization, sterol transfer, and protein complex remodeling suggest that OMNT1 influences cellular adaptation processes that extend beyond transcriptional or receptor-level regulation (Figure 6; Supporting Information File 6).

The detailed proteomic data are presented in a separate manuscript entitled 'Role of omentin-1 in the global proteome of porcine pituitary cells: anti-proliferative and anti-apoptotic effects,' currently under review. The corresponding data set has been deposited and is available as indicated in the data availability statement (https://doi.org/10.6019/PXD061269).

3.6 | Effects of OMNT1 Alone or in Combination With Hormone Release on Tropic Hormone Levels and Its Impact on Signaling Pathways Related to Hormone Secretion

Our results demonstrated greater gene expression of *GHRHR* (Figure 7A1) and greater GH secretion (Figure 7A5,A6) in LW

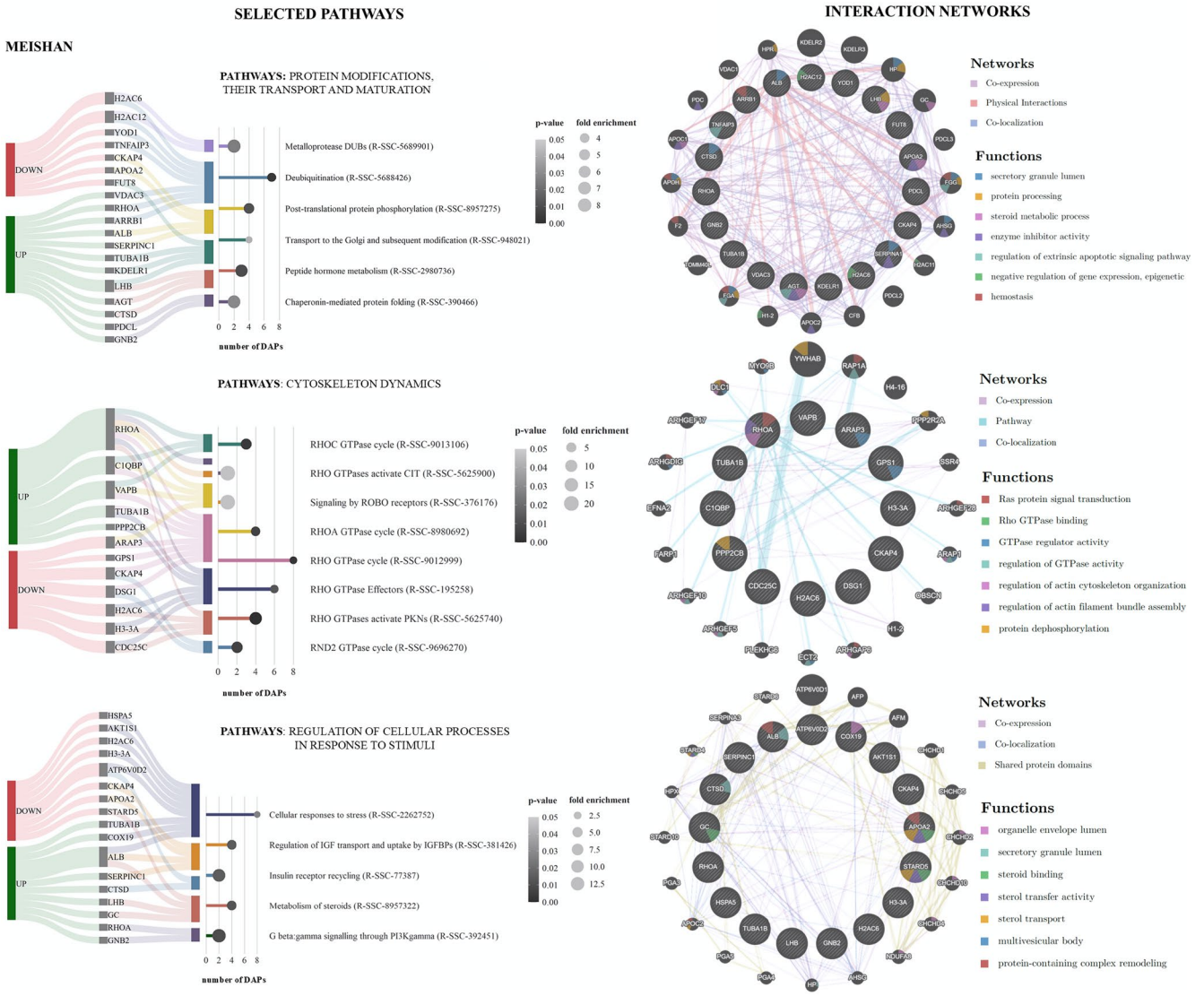


FIGURE 6 | A Sankey diagram illustrates the differentially abundant proteins (DAPs) identified in the anterior pituitary cells of Meishan pigs treated with omentin-1 (50 ng/mL). The diagram shows upregulated and downregulated proteins linked to Reactome pathways. The GeneMANIA interaction network shows the interaction strength (edge thickness), interaction type (bottom-right color), and major functions of the proteins. Statistical significance (p value) and fold enrichment are shown.

pigs than in MS pigs ($p < 0.0001$); a significant reduction in GH secretion was observed only following OMNT1 (10 and 50 ng/mL) and GHRH (100 nM) co-treatment, compared with GHRH alone, in MS pigs ($p < 0.0001$, Figure 7A6). In response to GHRH, *GHRHR* expression was upregulated only in MS pigs and was not significantly affected by OMNT1 ($p > 0.05$, Figure 7A2). Compared with the control, OMNT1 at a dose of 10 ng/mL increased *GH1* gene expression in LW pigs ($p < 0.001$, Figure 7A3). Interestingly, compared with GHRH treatment alone, the addition of OMNT1 inhibited the stimulatory effect of GHRH on *GH1* expression in both breeds ($p < 0.0001$, Figure 7A4).

Analysis of the receptor for TRH stimulating both PRL and TSH revealed that OMNT1 modulates *TRHR* expression in a dose- and breed-dependent manner. Compared with TRH alone, co-treatment with OMNT1 (50 ng/mL) and TRH (1000 nM) reduced *TRHR* expression in MS pigs, while OMNT1 at 10 ng/mL in combination with TRH decreased *TRHR* expression in LW pigs ($p < 0.05$, Figure 7B2); however, no statistically significant differences were detected following OMNT1 alone in both breeds ($p > 0.05$, Figure 7B1). OMNT1 alone, at doses of 10 and 50 ng/mL, upregulated *PRL* expression and at a dose of 10 ng/mL increased *TSHB* expression in LW pigs compared with that in the control groups of LW pigs ($p < 0.01$, Figure 7B3). In contrast, its co-treatment with OMNT1 and TRH reduced *PRL* expression compared with that of TRH alone in both breeds ($p < 0.01$, Figure 7B4,C1). Moreover, a decrease in PRL secretion was detected in LW pigs following treatment with OMNT1 combined with TRH compared with those following treatment with TRH alone ($p < 0.001$, Figure 7B6). In both breeds, no statistically significant differences were detected in PRL and TSH secretion following treatment with OMNT1 alone ($p > 0.05$, Figure 7B5,C3) or in *TSHB* expression and TSH secretion after OMNT1 and TRH co-treatment ($p > 0.05$, Figure 7C2,C4). However, greater basal PRL secretion was demonstrated in LW pigs, whereas MS pigs presented greater TSH secretion ($p < 0.0001$, Figure 7B5,C3).

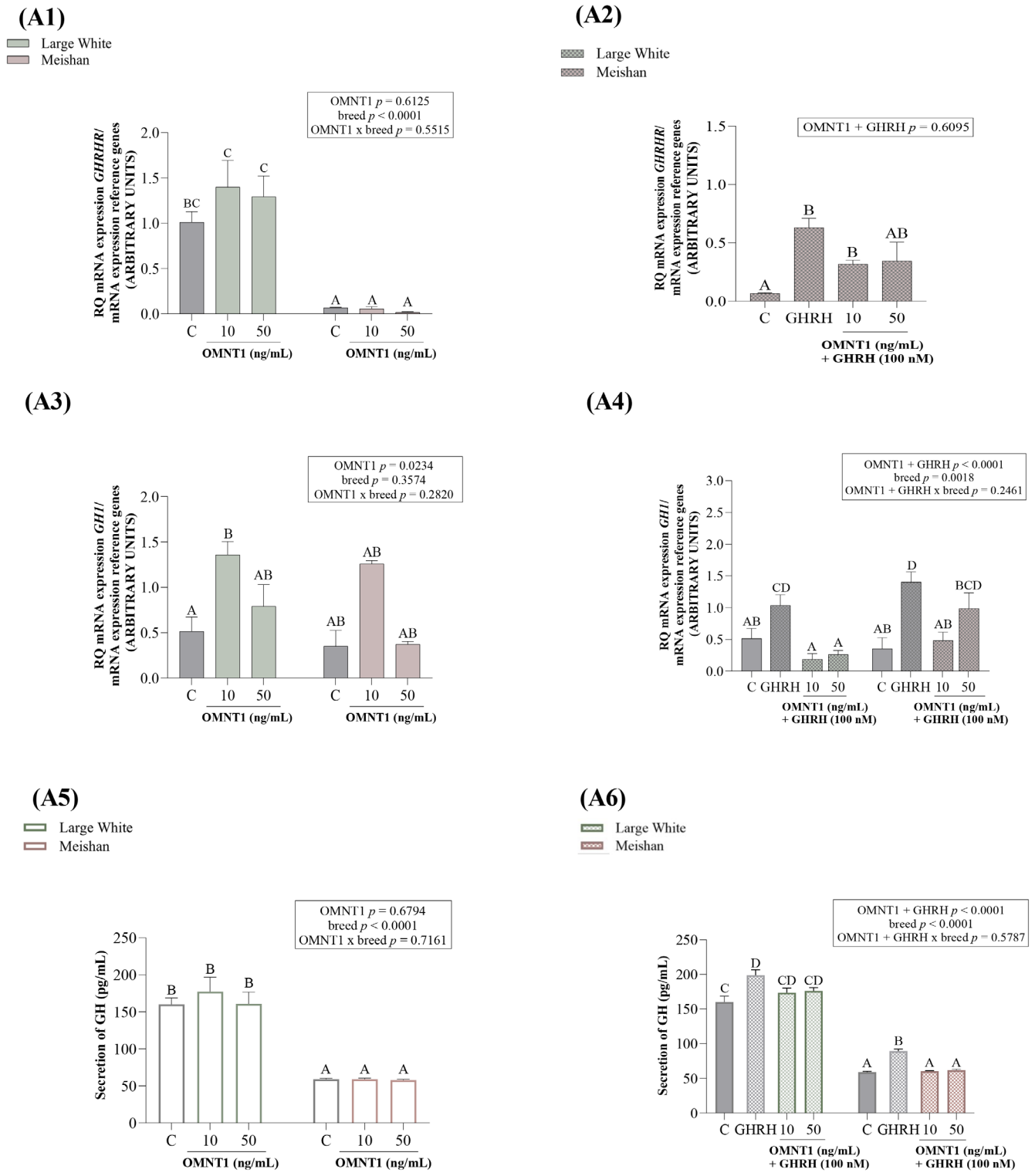


FIGURE 7 | Effects of omentin-1 (OMNT1; 10 and 50 ng/mL) alone or in combination with hypothalamic releasing hormones on the mRNA expression of tropic hormone receptors (A1, A2, B1, B2, D1, D2, E1, E2) and on both the mRNA expression and secretion of growth hormone (GH; A3–A6), prolactin (PRL; B3–B6), thyroid-stimulating hormone (TSH; C1–C4), adrenocorticotropic hormone (ACTH; D3–D6), luteinizing hormone (LH; E3–E6), and follicle-stimulating hormone (FSH; F1–F4) in anterior pituitary cells of Large White and Meishan pigs. The involvement of OMNT1 (50 ng/mL) in the extracellular signal-regulated kinase 1/2 (pharmacological inhibitor: PD098059, 25 μM) and protein kinase C alpha (pharmacological inhibitor: Bisindolylmaleimide I, 1 nM) signaling pathways was also tested for LH (E7) and FSH (F5) secretion. The results of at least five independent replicates are presented as the mean ± SEM for each group. The bars represent statistical significance according to different superscripts.

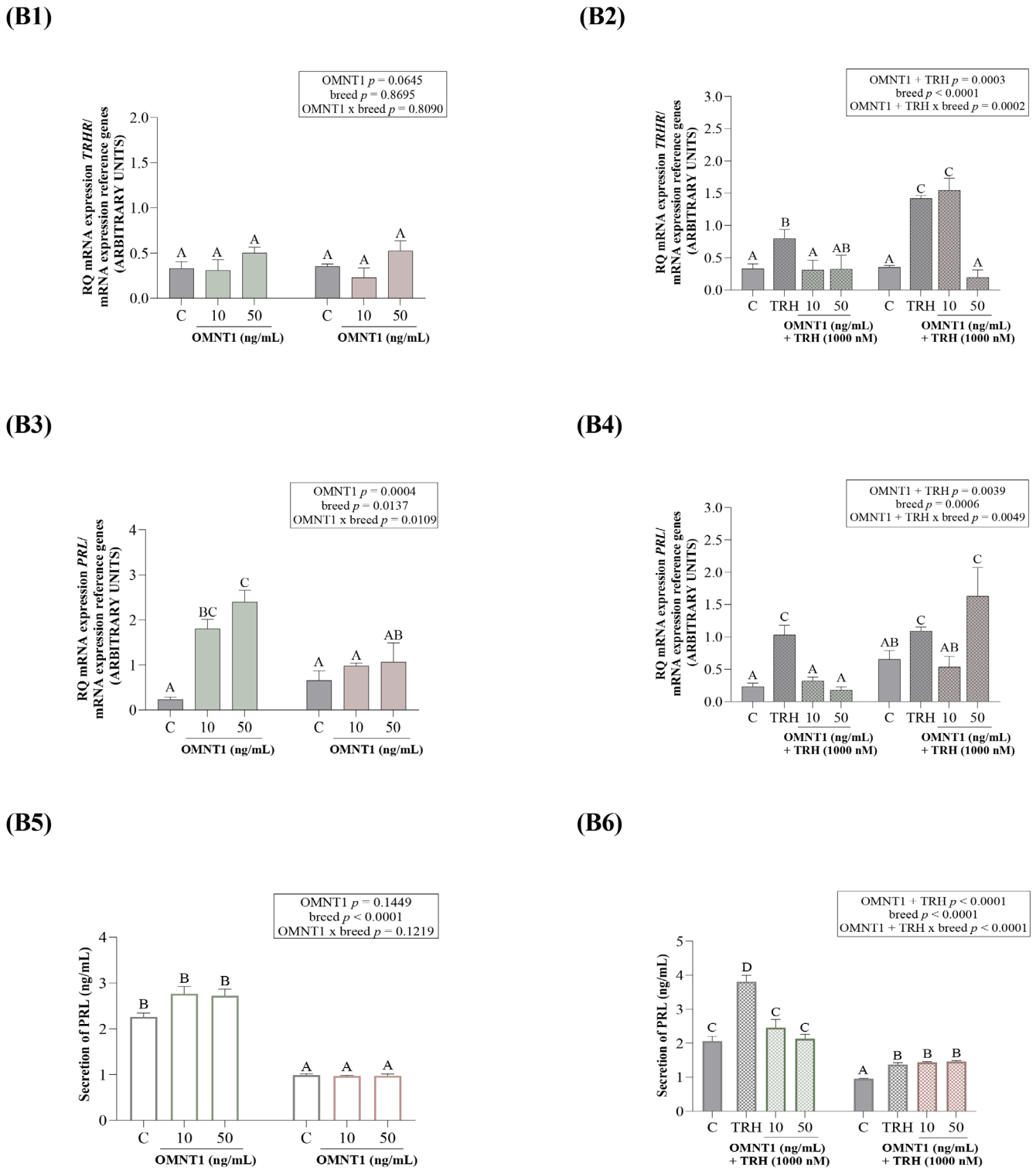


FIGURE 7 | (Continued)

Furthermore, we observed lower *CRHR1* and higher *POMC* expression in MS pigs than in LW pigs ($p < 0.0001$, Figure 7D1,D3). OMNT1 in combination with CRH (100 nM) downregulated *CRHR* expression in both breeds compared with CRH alone ($p < 0.05$, Figure 7D2). The results revealed an increase in *POMC* expression in LW pigs after OMNT1 treatment at doses of 10 and 50 ng/mL ($p < 0.001$, Figure 7D3). Compared with CRH alone, OMNT1 at all doses reduced *POMC* expression in LW pigs ($p < 0.05$, Figure 7D4), whereas in MS pigs, OMNT1 (10 ng/mL) decreased ACTH secretion ($p < 0.0001$, Figure 7D6). No differences in ACTH secretion were observed following OMNT1 treatment or between the pig breeds ($p > 0.05$, Figure 7D5).

OMNT1 or breed had no effect on *GNRHR* expression ($p > 0.05$, Figure 7E1). Interestingly, in both breeds, a decrease in *GNRHR* expression was noted following treatment with OMNT1 in combination with GnRH (100 ng/mL) compared with that following treatment with GnRH alone ($p < 0.05$, Figure 7E2). Following

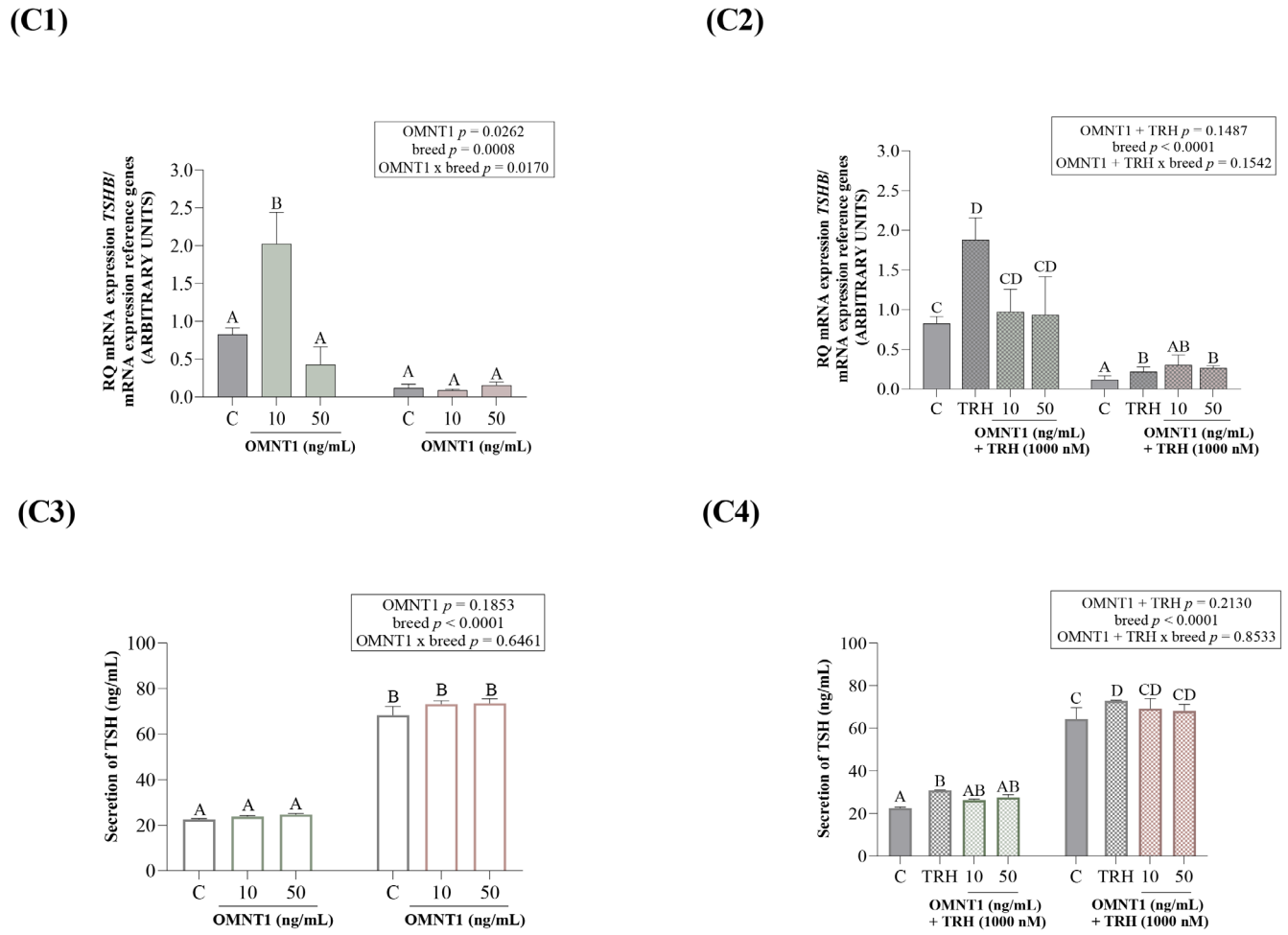


FIGURE 7 | (Continued)

treatment with OMNT1 at a dose of 50 ng/mL, we observed increased *LHB* expression in LW pigs, as well as upregulation of *FSHB* expression and LH secretion in both breeds compared with the control ($p < 0.05$, Figure 7E3,E5,F1). In both breeds, OMNT1 in combination with GnRH had no significant effect on *FSHB* expression compared with that in combination with GnRH ($p > 0.05$, Figure 7F2). In contrast, compared with GnRH alone, OMNT1 combined with GnRH reduced both *LHB* mRNA content and LH secretion in LW ($p < 0.05$, Figure 7E4,E6). Moreover, OMNT1 (50 ng/mL) decreased FSH secretion in LW pigs. When combined with GnRH, this inhibitory effect on FSH secretion was observed at 10 ng/mL in LW pigs and at 50 ng/mL in MS pigs ($p < 0.05$, Figure 7F3,F4). The application of pharmacological inhibitors of ERK1/2 kinase and PKCα together with OMNT1 (50 ng/mL) abolished the stimulatory effect of OMNT1 on LH secretion and its suppressive effect on FSH secretion, restoring hormone levels to control values in LW pigs ($p < 0.05$, Figure 7E7,F5).

3.7 | Effects of OMNT1 on Adipokine Levels and Signaling Pathways Related to Hormone Secretion

Notably, MS pigs exhibited higher levels of adiponectin, *CCRL2*, *GPR1*, and chemerin compared to LW pigs ($p < 0.05$, Figure 8A4,C2,C3,C5). In contrast, leptin levels were higher in LW pigs than in MS pigs ($p < 0.0001$, Figure 8B3). Our results revealed increased expression of the *ADIPOR1* gene following treatment with 50 ng/mL OMNT1 in LW pigs ($p < 0.05$, Figure 8A1). Interestingly, in MS pigs, a reduction in adiponectin secretion was demonstrated following OMNT1 treatment at a dose of 50 ng/mL ($p < 0.0001$, Figure 8A4). Compared with the control, in MS pigs, the application of a pharmacological ERK1/2 kinase inhibitor in combination with OMNT1 (50 ng/mL) abolished the inhibitory effect of OMNT1, restoring the response to control levels. However, after the use of a pharmacological PKCα inhibitor, an increase in adiponectin secretion was observed in MS pigs ($p < 0.05$, Figure 8A5). Moreover, OMNT1 (50 ng/mL) increased leptin secretion in MS pigs ($p < 0.05$, Figure 8B3). After treatment with pharmacological inhibitors of ERK1/2 and PKC kinases, no changes in leptin secretion were observed compared with that of the control in MS pigs ($p > 0.05$, Figure 8B4). In LW pigs, an increase in *CMKLR1* expression was observed, whereas in MS pigs, the opposite effect was noted following treatment with OMNT1 at a dose of 50 ng/mL ($p < 0.0001$, Figure 8C1). Moreover, OMNT1 at all the tested doses reduced *RARRES2* expression in MS pigs ($p < 0.05$, Figure 8C4). A decrease in *NAMPT* expression was observed in LW pigs after treatment with OMNT1 (50 ng/mL; $p < 0.05$, Figure 8E1). As shown in Figure 8, the effects of OMNT1 on the mRNA levels of *ADIPOR2* (A2), *ADIPOQ* (A3), *LEPR* (B1), *LEP* (B2), *CCRL2* (C2), *GPR1* (C3), *APLNR* (D1), *APLN* (D2), *TLR4* (F1), *RETN* (F2), *HSPA5* (G1), and *SERPINA12* (G2) were statistically

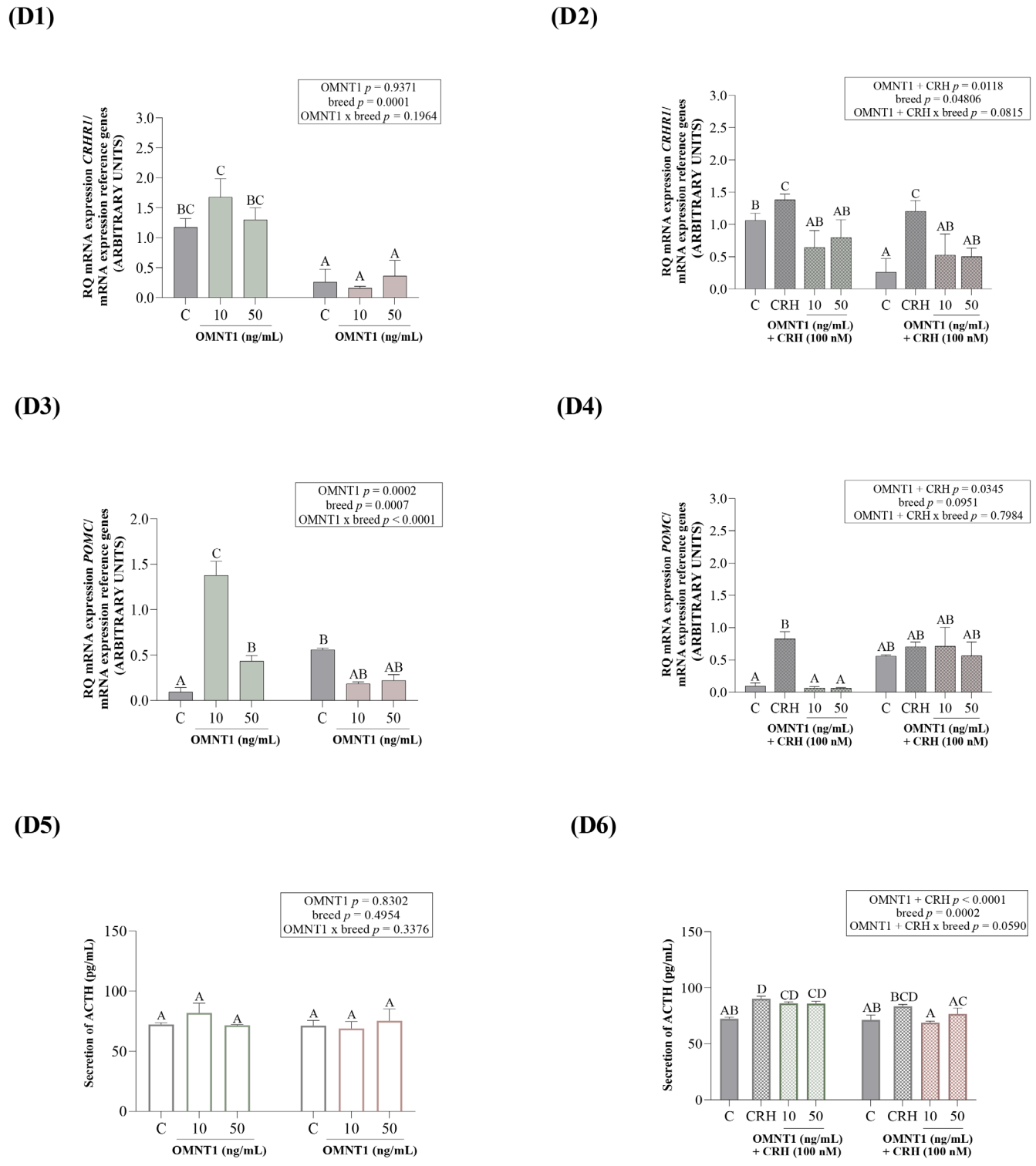


FIGURE 7 | (Continued)

insignificant in both breeds ($p > 0.05$). In addition, in both breeds, OMNT1 had no effect ($p > 0.05$) on the secretion of chemerin (C5), apelin (D3), visfatin (E2), resistin (F3), or vaspin (G3).

4 | Discussion

This in vitro study provides the first comprehensive analysis of the impact of OMNT1 on the transcriptome and proteome of mammalian AP cells, revealing a complex and breed-specific regulatory role. Using two genetically distinct pig breeds, LW and MS, we demonstrated that OMNT1 modulates endocrine function through coordinated transcriptional, proteomic, and functional changes, which are likely shaped by differences in metabolic status and hormonal environment. Transcriptomic changes were most pronounced in LW pigs; OMNT1-modulated DEGs were associated mainly with cellular communication, cytoskeleton dynamics, and calcium and PI3K/AKT signaling.

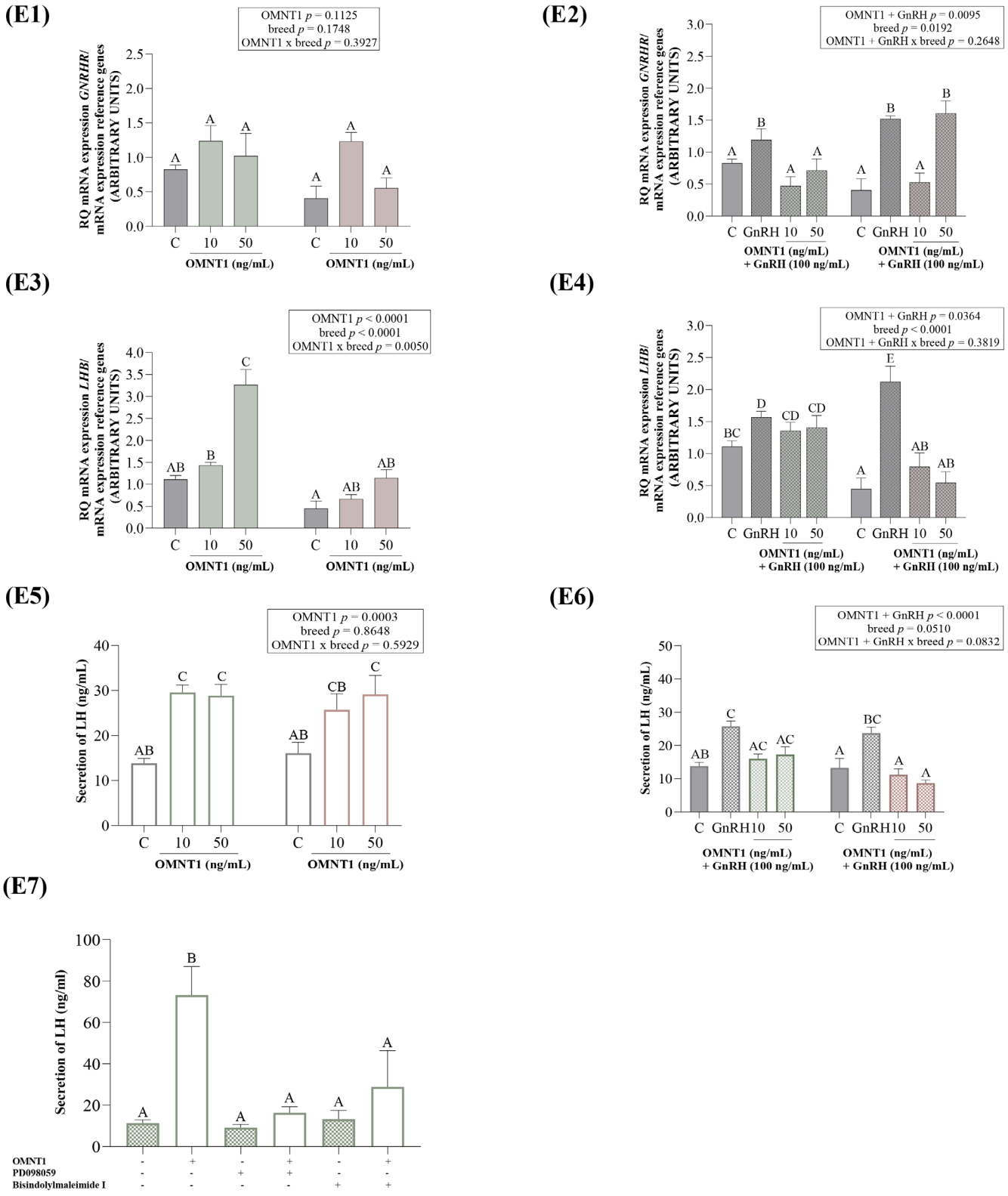


FIGURE 7 | (Continued)

Proteomic modifications were strongest in MS pigs, confirming the observations and highlighting DAPs involved in similar pathways, including protein modification, transport, and maturation. These results suggest a functional role for OMNT1 in pituitary endocrine activity.

Presented interaction networks revealed clear differences in the mode of OMNT1 action between breeds. In LW pigs, networks corresponding to physical protein–protein interactions formed compact modules of ECM proteins, adhesion molecules, and receptor complexes. This indicates OMNT1 primarily strengthens

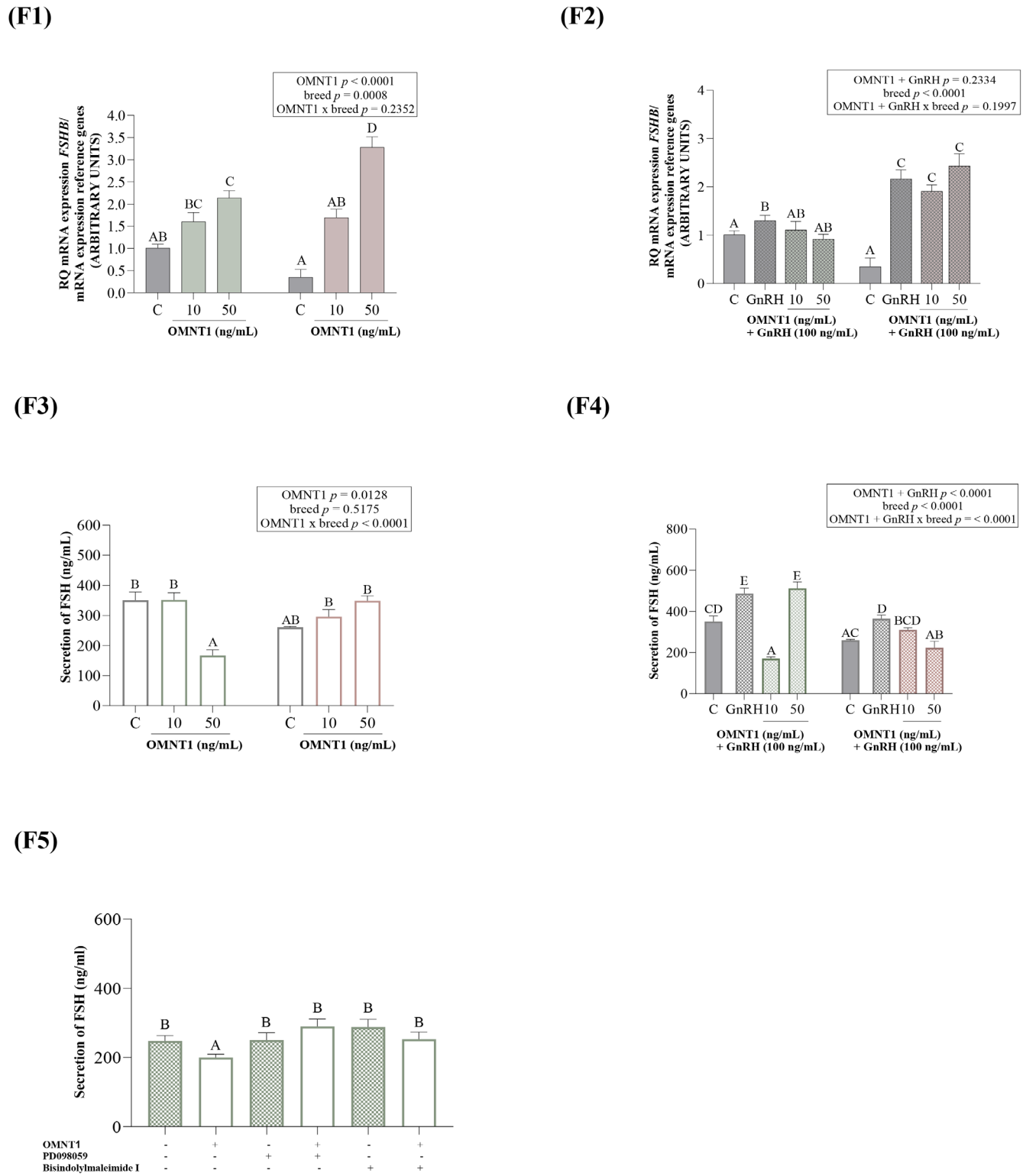


FIGURE 7 | (Continued)

structural and receptor-mediated signaling, with integration of well-established intracellular cascades such as Ras/MAPK and kinase activity pathways. In contrast, in MS pigs, both transcriptomic and proteomic networks were characterized by a predominance of co-expression, pathway, and co-localization links. This pattern suggests that OMNT1 activity is mediated mainly through coordinated expression of multiple genes and proteins, rather than by assembling stable protein complexes. Proteomic networks further highlighted OMNT1-driven regulation of secretory activity, cytoskeletal remodeling via Rho GTPase signaling, and organelle adaptation, consistent with a broader cellular plasticity.

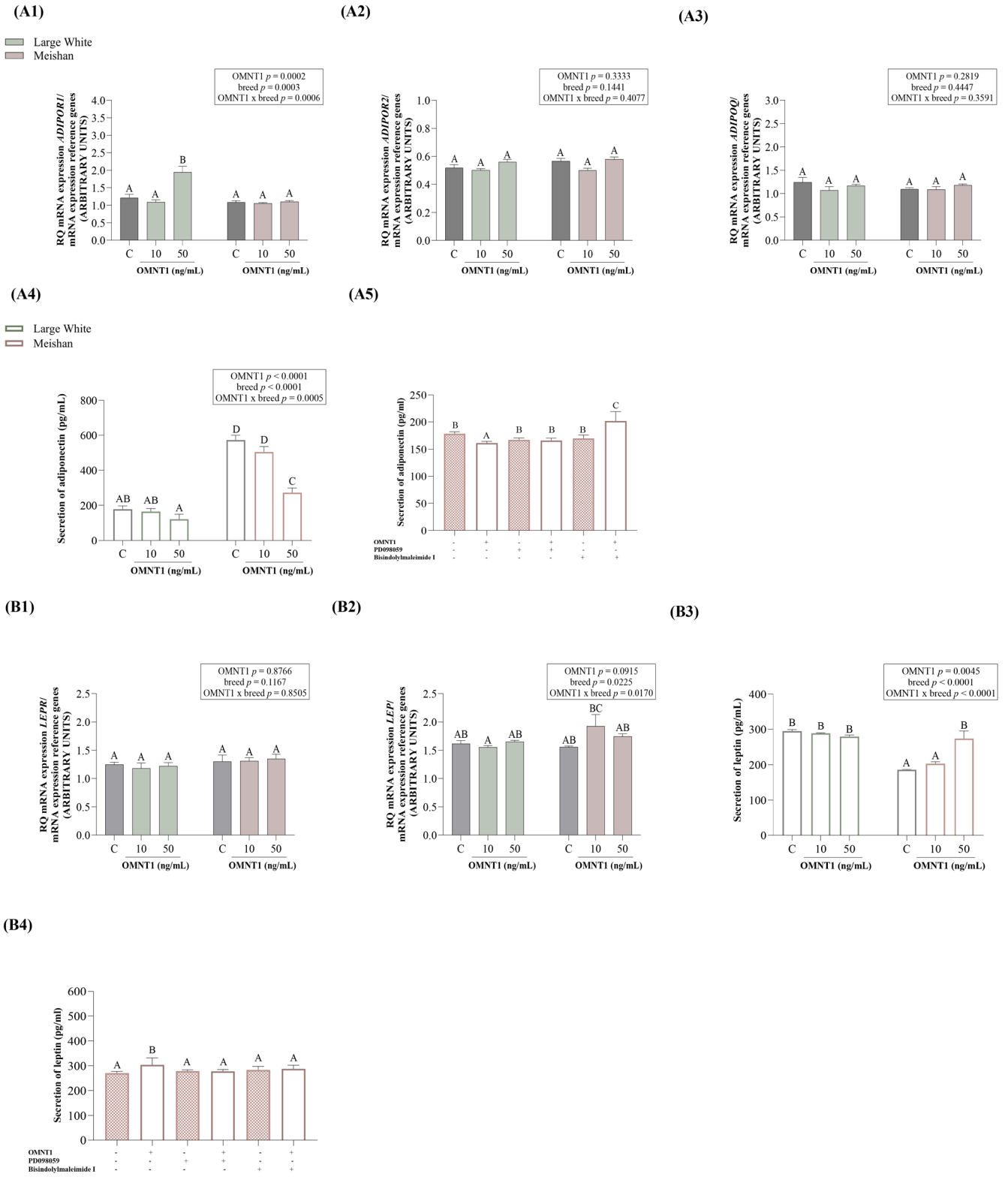


FIGURE 8 | Effects of omentin-1 (OMNT1; 10 and 50 ng/mL) on the mRNA expression of adipokine receptors (A1, A2, B1, C1, C2, C3, D1, F1, G1) and on both the mRNA expression and secretion of adiponectin (A3, A4), leptin (B2, B3), chemerin (C4, C5), apelin (D2, D3), visfatin (E1, E2), resistin (F2, F3), and vaspin (G2, G3) in anterior pituitary cells of Large White and Meishan pigs. The involvement of OMNT1 (50 ng/mL) in the extracellular signal-regulated kinase 1/2 (pharmacological inhibitor: PD098059, 25 μM) and protein kinase C alpha (pharmacological inhibitor: Bisindolylmaleimide I, 1 nM) signaling pathways was also tested for adiponectin (A5) and leptin (B4) secretion. The results of at least five independent replicates are presented as the mean ± SEM for each group. The bars represent statistical significance according to different superscripts.

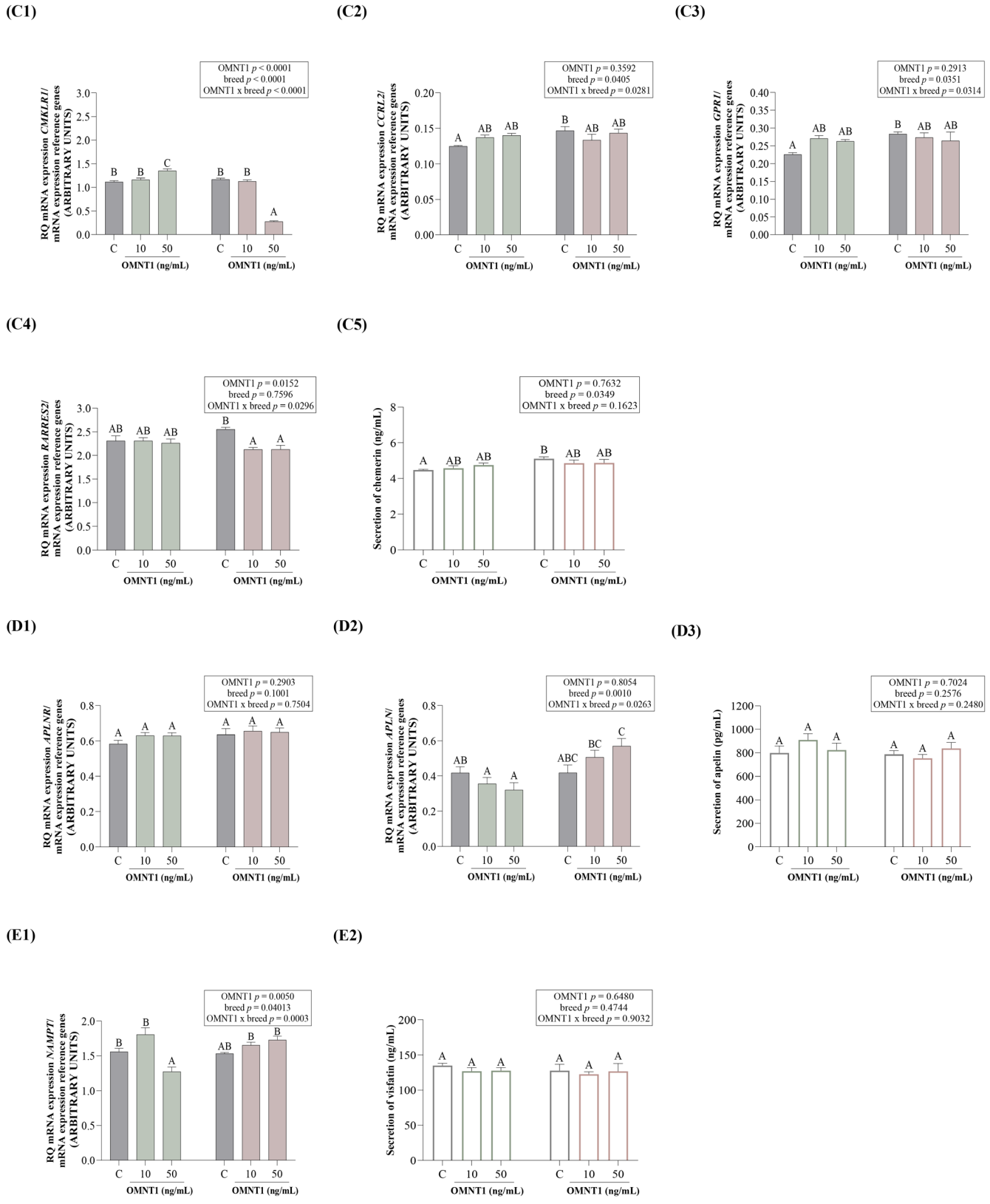


FIGURE 8 | (Continued)

Previous studies have indicated that OMNT1 has anti-inflammatory, anti-atherosclerotic, and insulin-sensitizing effects by multiple signaling pathways, including PI3K/AKT and 5′-AMP-activated protein kinase pathways [47, 48]. This is the first report demonstrating the impact of OMNT1 on calcium signaling in AP cells. Other adipokines modulate this pathway: adiponectin increases intracellular calcium to stimulate GH release [49], while resistin promotes GH secretion via the ADCY/cAMP/

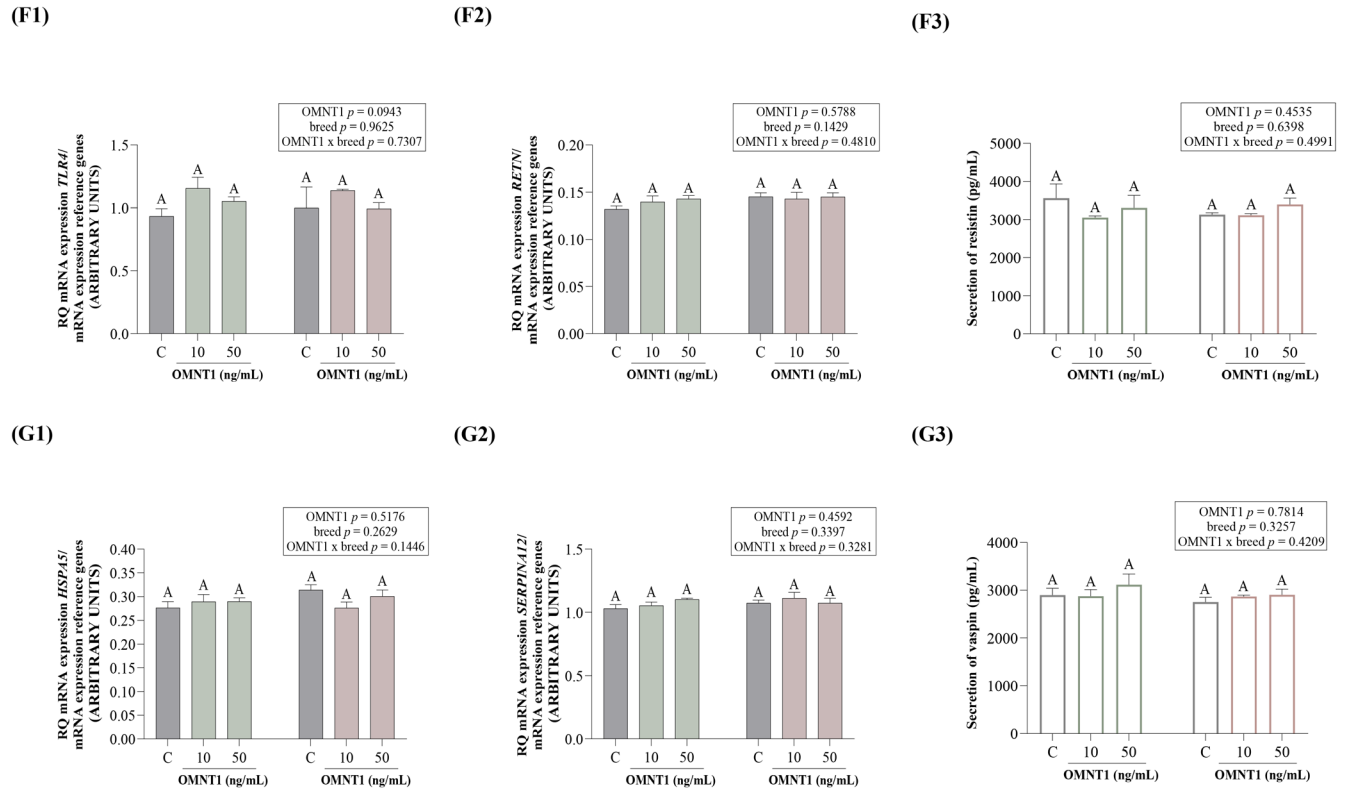


FIGURE 8 | (Continued)

protein kinase A (PKA) pathway and L-type calcium influx [34]. In LW pigs, OMNT1 modulates calcium-signaling genes (*ADCY4*, *NGF*, *KDR*, *CASQ2*, and *CXCR4*), essential for hormone secretion via voltage-gated channels [50]. As an enzyme converting ATP to cAMP [51], ADCY4 is regulated by hypothalamic peptides that affect both cytosolic calcium and its activity in AP cells [52]. cAMP amplifies GnRH-induced LH secretion without initiating its immediate release [53, 54]. Consistent with this, OMNT1 increases both *ADCY4* expression and intracellular cAMP levels, suggesting a GnRH-like mechanism in promoting LH secretion. In contrast, FSH secretion is less responsive to cAMP [55], implying that OMNT1 may suppress FSH via alternative mechanisms, such as PKC and ERK1/2 signaling. In LW pigs, as OMNT1 seems to modulate FSH and LH secretion via PKC, we further examined this pathway. We demonstrated time-dependent phosphorylation of PKCα; moreover, transcriptomic analyses revealed the activation of genes, including *NGF* and *KDR*, at the early stage of the PKC pathway. *NGF* stimulates calcium uptake in rat adrenal cell lines and neurons via PKC, and this process is modulated by intracellular calcium [56–58]. In neurons, KDR-mediated calcium influx occurs through tyrosine kinase signaling [59, 60]. The involvement of OMNT1 in this signaling pathway is strongly supported by our proteomic analysis. In MS pigs, OMNT1 downregulates tyrosine-protein phosphatase non-receptor type 2, a known negative regulator of insulin signaling [61–63]. Proteomic data supporting the findings of this study have been deposited in the ProteomeXchange Consortium via the PRIDE partner repository with the data set identifier PXD061269 (https://doi.org/10.6019/PXD061269). On the other hand, the *CASQ2* gene encodes a calcium-binding protein that functions as both a storage and regulatory component in cardiac calcium signaling [64], suggesting a similar role in the pituitary. In LW pigs, OMNT1-induced downregulation of *CASQ2* may reduce calcium storage in organelles, potentially enhancing cytoplasmic calcium influx. AP cells may compensate by upregulating other calcium-binding proteins or modulating calcium channels. Given OMNT1's role in calcium signaling, we found reduced *RYR3* expression in AP cells of MS pigs. RYR3, which participates in GnRH signaling, is regulated by gonadal steroids—its expression is downregulated by ESR and upregulated by progesterone [65]. It functions as a calcium-induced calcium release channel that is activated by high intracellular calcium ion levels and cyclic ADP-ribose [66]. In LW pigs, we also observed decreased expression of *CXCR4*, a receptor expressed in somatotrophs and gonadotrophs [67, 68], critical for pituitary development. Dysregulation of the CXCL12/CXCR4 axis affects GH secretion and somatotroph proliferation [67, 69]. Its overexpression is linked to pituitary adenomas [70, 71], suggesting a role for OMNT1 in pituitary homeostasis.

Our analyses revealed a complex role of OMNT1 in regulating the ERK1/2 pathway. Increased *NGF* expression activates the RAS/ERK cascade, a calcium- and calmodulin-dependent process in which calmodulin controls Raf-1 activation [72]. In MS pigs, OMNT1 downregulates von Willebrand factor (*VWF*) and upregulates connector enhancer of kinase suppressor of RAS 2 (*CNKSR2*), both involved in MAP2K and MAPK activation. In MS pigs, it also modulates RHOA, a key component of RAS signal transduction. The upregulation of *CNKSR2* and RHOA suggests enhanced signaling through the RAS/RAF/MAPK2/ERK cascade [73, 74]. VWF is known to promote ERK2 phosphorylation via a pathway involving protein kinase G, p38 MAPK, and ERK, culminating in integrin activation [11, 46]. OMNT1 may act via integrin receptors [11], and our results

provide strong evidence that OMNT1 inhibits ERK1/2 activation both indirectly at the gene expression level and directly at the protein level. On the other hand, we observed an increase in the expression of *COL6A1*, which regulates glucose uptake via integrin-dependent signaling pathways [75]. Further research is necessary to determine whether OMNT1 mediates its effects through integrin receptors. Although the gene and protein expression profiles suggest ERK1/2 inhibition by OMNT1, the use of a pharmacological ERK1/2 inhibitor in AP cells indicates that OMNT1 may still be involved in secretion regulation via this pathway. This is supported by previous studies on pituitary showing that calcium and PKC-dependent pathways mediate ERK1/2 activation in response to hormonal stimulation [76]. In LW pigs, we observed reduced *GREB1* expression, suggesting an effect of OMNT1 on estrogen signaling. *GREB1* is also involved in PRL and gonadotrophin regulation in the pituitary [77, 78]. In LW pigs, OMNT1 upregulated *PRL* expression, whereas OMNT1 combined with TRH suppressed *PRL* in both breeds, indicating possible crosstalk between OMNT1 and TRH in lactotroph regulation. Furthermore, increased *NEUROD4* expression in MS pigs indicates a role for OMNT1 in pituitary development [79]. Additionally, in LW pigs OMNT1 led to upregulation of *POU1F1*, a transcription factor essential for GH, PRL, and TSH production, provides strong evidence for OMNT1 involvement in hormone regulation [80, 81]. Our proteomic analyses revealed that in MS pigs OMNT1 increases the expression of G protein subunit beta 2 (GNB2), a key regulator of gonadotropin subunit gene expression in pituitary tropic hormone production [82].

Numerous studies have shown that OMNT1 signals via the PI3K/AKT pathway in various tissues [83, 84], which aligns with our transcriptomic data from LW pigs, where OMNT1 modulated DEGs associated with this pathway. Given the known interaction between PI3K and calcium signaling [85, 86], OMNT1 may integrate these pathways in AP cells. OMNT1 influenced the expression of *COL6A1*, a PI3K/AKT inhibitor in pituitary cells [87]. Our proteomic analysis revealed that OMNT1 reduced the expression of *phosphodiesterase 3B*, a downstream target of AKT [88, 89], and *SGK2*, a PI3K-dependent kinase [90, 91]. Since SGK3 can reactivate mTORC1 independently of AKT and cross-talks with the RAS/RAF/ERK pathway [91–93], its regulation by OMNT1 may indicate broader pathway interactions. Reduced *GREB1* and miRNA-324 expression indicate that OMNT1 may suppress proliferation via PI3K/AKT and MAPK signaling [94, 95]. Although both breeds responded to OMNT1, transcriptomic analysis revealed a greater number of DEGs in LW pigs, whereas proteomic data indicated stronger effects in MS pigs. These breed-specific responses may reflect differences in post-transcriptional regulation, including mRNA stability and protein renewal. Genetic differences between LW and MS pigs, particularly in pathways related to endocrine function and metabolism, likely underlie the observed divergence in molecular responses.

In this study, the transcriptome revealed that OMNT1 plays a role in hormone regulation. Although RNA-seq analysis did not reveal direct changes in tropic hormones gene expression, RT-qPCR identified significant alterations in selected targets. This variation may result from the more highly selective statistical thresholds applied in RNA-seq analyses and the higher sensitivity of RT-qPCR, particularly for low-abundance transcripts. No significant changes were observed in the expression of hormone-releasing receptors following OMNT1 treatment alone, suggesting that OMNT1 acts downstream of receptor activation, modulating intracellular signaling in cells with functional receptors. In both breeds, OMNT1 combined with TRH, CRH, or GnRH reduced expression of their corresponding receptors (*TRHR*, *CRHR*, and *GNRHR*), possibly reflecting a negative feedback mechanism. *GHRHR* expression was induced by GHRH stimulation only in MS pigs, while LW pigs exhibited higher baseline levels, potentially indicating receptor desensitization due to constitutive overexpression. These findings highlight breed-specific differences in GHRH receptor regulation and sensitivity.

Pituitary hormone secretion is essential for regulating homeostasis, growth, metabolism, and reproduction [82]. Adipokines have been shown to modulate tropic hormone levels locally by activating signaling pathways, regulating receptor expression, and influencing transcription factor activity in AP cells [96, 97]. For example, deletion of LEPR in somatotrophs reduces GH and GHRHR expression via the JAK/STAT3 pathway in mice [98–100]. In primate AP cells, leptin and adiponectin stimulate PRL, while resistin increases ACTH via ADCY/PKA and PI3K signaling [97]. Conversely, adiponectin inhibits GH secretion in rats and primates [96, 97]. Knowledge about the role of OMNT1 in neuroendocrine regulation is limited. It increases norepinephrine secretion and reduces CRH expression in the rat hypothalamus [101], potentially also acting indirectly via mitochondrial regulation [102]. This is the first study to identify how OMNT1 influences hormone release in AP cells. We first assessed the direct effects of OMNT1, followed by its combined effects with hypothalamic hormones. While OMNT1 alone did not affect GH, PRL, ACTH, or TSH secretion, we observed baseline higher GH and PRL levels in LW pigs and higher TSH in MS pigs, reflecting breed-specific differences in AP cell activity and endocrine demands. OMNT1 alone increased LH secretion in both breeds and reduced FSH in LW pigs. Notably, OMNT1 combined with GnRH reduced LH secretion in MS pigs, suggesting modulation of gonadotroph sensitivity under breed-specific metabolic conditions. Current knowledge indicates that treatment of bovine gonadotrophs with leptin alone does not affect gonadotropin secretion but enhances LH when combined with GnRH [93, 94]. In contrast, adiponectin with GnRH inhibits LH secretion and GnRHR expression in rats [35]. The breed-specific effects of OMNT1 on gonadotropin expression may be influenced by genetic differences in promoter regulation. Previous studies demonstrated that single nucleotide polymorphisms within the GNRHR promoter create unique transcription factor binding sites in MS pigs, leading to enhanced promoter activity compared with the LW pigs [103, 104]. While these polymorphisms may contribute to baseline differences in gonadotroph function, our data indicate that OMNT1 primarily modulates the LHB/LH axis. Thus, genetic variation in regulatory elements likely shapes the transcriptional landscape of gonadotrophs, upon which OMNT1 and GnRH exert their effects. Adipokines secreted by adipose tissue modulate each other's levels and, together with other signals, regulate pituitary hormone activity [105]. Therefore, we examined how OMNT1 affects their expression and secretion. In MS pigs, OMNT1 significantly decreased adiponectin and increased leptin secretion, similar to effects previously observed in porcine granulosa cells [106].

Categories	Large White	Meishan
TRANSCRIPTOMIC ANALYSIS	➢ Broader set of DEGs; pathways related to communication, cytoskeleton, calcium, PI3K/AKT; *POU1F1* ↑, *KDR* ↑, *GREB1* ↓	➢ Fewer DEGs; consistent with proteomic shifts; *NEUROD4* ↑, *RYR3* ↓)
PROTEOMIC ANALYSIS	➢ ECM proteins, adhesion molecules, receptor complexes; Ras/MAPK, PI3K/AKT signaling	➢ Stronger proteomic changes; GNB2 ↑; secretory activity, Rho GTPase, ERK1/2, PKC pathways
SIGNALING PATHWAYS	➢ Calcium signaling (*ADCY4* ↑, *CASQ2* ↓, *KDR* ↑, *NGF* ↑, *CXCR4* ↓), PI3K/AKT (*SGK2*↓, *PDE3B*↓), PKC (PKCα phosphorylation), Ras/MAPK	➢ ERK1/2 (*VWF*↓, *CNKSR2*↑), Rho GTPase (*RHOA*↑), secretory adaptation
RELEASING HORMONES RECEPTORS	➢ *GHRHR* ↑ (baseline, not further induced by GHRHR+OMNT1); *TRHR*, *CRHR*, *GNRHR* ↓ with releasing hormone+OMNT1	➢ *GHRHR* ↑ with GHRH+OMNT1; *GNRHR* ↓ with GnRH+OMNT1; *TRHR*, *CRHR* ↓ with releasing hormone +OMNT1
TROPIC HORMONES	➢ GH ↑, PRL ↑ (baseline); FSH ↓; LH ↑ with OMNT1 via ERK1/2 and PKC pathways; *PRL* ↓ with TRH+OMNT1	➢ TSH ↑ (baseline); LH ↑ (with OMNT1), ↓ (with OMNT1+GnRH); *PRL* ↓ with TRH+OMNT1
ADIPOKINES	➢ *ADIPOR1* ↑	➢ Adiponectin ↓ , leptin ↑ via ERK1/2 and PKC pathways; *APLN* ↑ , *RARRES2* and *CMKLR1* ↓

FIGURE 9 | Summary of omentin-1 (OMNT1) effects in anterior pituitary cells of Large White and Meishan pigs. Arrows indicate direction of change: ↑ increase, ↓ decrease. *ADCY4*, adenylate cyclase 4; *ADIPOR1*, adiponectin receptor 1; *APLN*, apelin; *CASQ2*, calsequestrin 2; *CMKLR1*, chemokine-like receptor 1; *CNKSR2*, connector enhancer of kinase suppressor of Ras 2; *COL6A1*, collagen type VI alpha 1 chain; CRH, corticotropin-releasing hormone; *CRHR*, corticotropin-releasing hormone receptor; *CXCR4*, C–X–C chemokine receptor type 4; DEGs, differentially expressed genes; ECM, extracellular matrix; FSH, follicle-stimulating hormone; GH, growth hormone; *GHRHR*, growth hormone-releasing hormone receptor; *GNB2*, G protein subunit beta 2; GnRH, gonadotropin-releasing hormone; *GnRHR*, gonadotropin-releasing hormone receptor; *GREB1*, growth regulation by estrogen in breast cancer 1; *KDR*, kinase insert domain receptor (vascular endothelial growth factor receptor 2, VEGFR2); *KDR*, kinase insert domain receptor; LH, luteinizing hormone; *NEUROD4*, neuronal differentiation 4; *NGF*, nerve growth factor; *PDE3B*, phosphodiesterase 3B; PI3K/AKT, phosphoinositide 3-kinase/protein kinase B signaling pathway; PKC, protein kinase C; *POU1F1*, POU class 1 homeobox 1; PRL, prolactin; *RARRES2*, retinoic acid receptor responder protein 2 (chemerin); Ras/MAPK, Ras/mitogen-activated protein kinase signaling pathway; *RHOA*, Ras homolog family member A; *RYR3*, ryanodine receptor 3; *SGK2*, serum/glucocorticoid-regulated kinase 2; *TRHR*, thyrotropin-releasing hormone receptor; TRH, thyrotropin-releasing hormone; *VWF*, von Willebrand factor.

These changes in AP cells appear to be mediated via the ERK1/2 and PKC pathways. At present, no specific receptor for OMNT1 has been identified, although studies suggest that it may act via integrins [11] or by enhancing insulin receptor phosphorylation [12]. The breed-specific differences observed here may therefore reflect differential sensitivity or activity of these receptor

systems in AP cells. Interestingly, the observed breed-specific effects of OMNT1 on adipokine secretion in AP cells may be related to the different baseline levels of this peptide and adipokines between MS and LW pigs. Our previous studies showed that MS pigs have lower circulating OMNT1 concentrations, and reduced OMNT1 levels in visceral white adipose tissue, as well as during the mid-luteal phase, decreased OMNT1 protein expression in the AP glands compared with LW pigs [14, 19]. This may lead to AP cells in MS pigs being more sensitive to exogenous OMNT1, resulting in pronounced changes in adiponectin and leptin secretion. In contrast, LW pigs, characterized by higher OMNT1 protein expression in AP glands [14], may exhibit a state of relative desensitization to OMNT1, which could explain the weaker response in AP cells. No changes were found in *ADIPOQ* or *LEP* gene expression, though *ADIPOR1* was upregulated in LW pigs. Previous studies report that OMNT1 levels correlate positively with adiponectin and negatively with leptin [107, 108]. In humans, pharmacological doses of leptin reduce OMNT1 levels, whereas physiological doses have no effect [109]. OMNT1 has also been shown to upregulate adiponectin, promoting lipid metabolism and insulin sensitivity [110]. Thus, the reduction of adiponectin by OMNT1 in AP cells also may reflect a local regulatory mechanism, influencing energy balance and metabolic signaling. In MS pigs, OMNT1 also increased apelin transcripts, while downregulating *RARRES2* and *CMKLR1*. Chemerin is linked to insulin resistance and inflammation, and OMNT1 shows the opposite association [111, 112]. These findings underscore a complex interplay between adipokines and metabolic regulation. Interestingly, OMNT1-induced upregulation of *nitric oxide synthase* trafficking protein may link OMNT1 to nitric oxide signaling in pituitary cells, suggesting a role in vascular function, metabolic homeostasis, and endocrine regulation [113, 114].

The results of this study indicate that OMNT1 exerts a regulatory role in AP cells of both MS and LW pigs, influencing tropic hormone secretion, expression of their receptors, and adipokine release. Nevertheless, the underlying molecular mechanisms differ between breeds. In MS, OMNT1 action is linked primarily to proteomic remodeling, ERK1/2 and PKC signaling, and regulation of adipokine secretion, whereas in LW it involves broader transcriptomic changes, modulation of calcium and PI3K/AKT pathways, and regulation of structural and receptor-mediated networks (Figure 9). These breed-specific differences likely reflect genetic polymorphisms, variation in receptor expression and sensitivity, and post-transcriptional regulation.

In conclusion, elucidating OMNT1's interactions with metabolic regulators provides novel insights into its role in endocrine adaptation and the coordination of metabolic and reproductive functions. Collectively, these findings suggest that OMNT1 acts as a breed-specific modulator of pituitary signaling and hormone secretion, linking metabolic state with reproductive regulation. Future studies may help identify therapeutic targets for the treatment of metabolic and endocrine disorders.

Author Contributions

N.R.-D.: Conceptualization, methodology, validation, formal analysis, investigation, data curation, visualization, writing original draft preparation, writing review, and editing. K.P., J.C., and C.R.: Investigation. E.R., B.Ś., and A.M.: Formal analysis and data curation. J.D.: Investigation, resources, writing review and editing. T.K. and N.S.: Writing review and editing. A.R.: Conceptualization, methodology, writing review and editing, supervision, project administration, funding acquisition.

Acknowledgments

The authors thank the team of the porcine experimental unit and the slaughterhouse of INRAE Centre Val de Loire, BioRender, and Servier Medical Art for the figure.

Ethics Statement

This study did not require ethics committee approval for animal experimentation, as the slaughtering of animals, collection of biological material, and transport to the laboratory were conducted in accordance with the European Communities Council Directive 2010/63/EU of September 22, 2010, as well as the Polish Act on the Protection of Animals Used for Scientific or Educational Purposes of January 15, 2015 (Journal of Laws Dz.U. 2015, item 266).

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are openly available at the NCBI BioProject repository (https://www.ncbi.nlm.nih.gov/bioproject/1227421) under the dataset identifier PRJNA1227421, and at the ProteomeXchange Consortium via the PRIDE partner repository [115] with the dataset identifier PXD061269 and https://doi.org/10.6019/PXD061269.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Supplementary File 1.** List of TaqMans and specific primers used for RT-qPCR. **Supplementary File 2**. Identification and enrichment functional analysis of differentially expressed genes (DEGs), categorized into up- and downregulated groups, in Large White (LW) pigs. **Supplementary File 3**. Identification and enrichment functional analysis of differentially expressed genes (DEGs), categorized into up- and downregulated groups, in Meishan (MS) pigs. **Supplementary File 4**. Direction concordance between breeds: Large White and Meishan pigs—transcriptome and proteome. Common differentially expressed genes and differentially abundant proteins with direction concordance, illustrated by Venn diagrams. **Supplementary File 5**. GeneMANIA interaction network analysis of cellular communication and cytoskeleton dynamics, regulation of cellular processes in response to stimuli, and cellular signaling in Large White and Meishan pigs. **Supplementary File 6**. GeneMANIA interaction network analysis of protein modifications, their transport and maturation, cytoskeleton dynamics, and regulation of cellular processes in response to stimuli in Meishan pigs.