Buffalo Fibronectin Type III Domain Proteins

Subjects: Agriculture, Dairy & Animal Science

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FN-III proteins are widely distributed in mammals and are usually involved in cellular growth, differentiation, and adhesion. The FNDC5/irisin regulates energy metabolism and is present in different tissues (liver, brain, etc.). In large mammals, the regulation of energy homeostasis under metabolic shifts is the foremost challenge to keep normal physiological and molecular functioning. Fibronectin type III domain containing 5 (FNDC5) was initially designated as a critical factor that causes cellular differentiation of skeletal muscle. Principally, it was detected in peroxisomes. Irisin is a myokine involved in higher energy expenditure through stimulation of white adipose tissues. Keeping in view the physiological roles of FN-III proteins (particularly FNDC-5), it is imperative to characterize these proteins at the genomic level to better understand their structure and putative functions in the buffalo.

buffalo FN-III proteins FNDC5/irisin receptors binding affinities

1. Introduction

Buffalo (*Bubalus bubalis*) is a unique livestock species with peculiar productive performance, predominantly found in Asia including China, India, and Pakistan [1][2]. Buffaloes are renowned for their unique ability to consume roughages and convert them into valuable products such as meat and milk. Additionally, buffalo can tolerate harsh weather conditions, perform better under poor feeding resources, and provide draught power [3]. Buffalo milk is relished owing to its peculiar taste with a higher protein, fat, and solid content [4][5][6]. Mainly, buffalo in the Mediterranean and South-Eastern region of Asia serves as an important economic component in the agriculture sector [1][7][8]. Despite having excellent production potential, the productivity of the buffalo is jeopardized due to its poor reproductive efficiency. Buffalo as a dairy animal is known as a poor breeder mainly due to major challenges such as a higher rate of infertility, poor estrus expression [9], poor reproductive efficiency [10], distinct seasonal reproductive pattern [11][12], delayed sexual maturity, prolonged calving intervals [13], and low calf survival rates [14]. It is challenging to improvise the buffalo reproductive and energy metabolism efficiency through finding some biological molecular chaperon that could target reproduction-related signaling receptors to improve the reproductive ability of the buffalo.

In animals, fibronectin proteins are widely dispersed in an extracellular matrix with a variety of functions including cellular growth, migration, differentiation, and adhesion. These proteins are involved in important processes such as healing and the replacement of damaged tissues and embryogenesis [15][16].

In large mammals, the regulation of energy homeostasis under metabolic shifts is the foremost challenge to keep normal physiological and molecular functioning [17]. Fibronectin type III domain containing 5 (FNDC5) was initially designated as a critical factor that causes cellular differentiation of skeletal muscle. Principally, it was detected in peroxisomes [18]. Irisin is a myokine involved in higher energy expenditure through stimulation of white adipose tissues. Firstly, the irisin hormone proteolytically dissociates from its precursor FNDC5, which enhances the circulating irisin levels, subsequently reducing insulin resistance while improving glucose homeostasis [19]. Irisin is mainly secreted from subcutaneous, visceral adipose tissue and skeletal muscles [20][21], but a recent study also reported its presence in other tissues including the spleen, liver, brain, stomach, and testis [22]. The regulatory, molecular, and physiological role of FNDC5/irisin has not yet been fully described and various contradictory findings have been documented in this regard. Thus, there is a dire need to explore the mechanism of FNDC5/irisin functioning in mammals.

2. Identification of *FN-III* Gene Family and Their Physiochemical Properties

In this study, a comprehensive strategy was applied to characterize the *FN-III* gene family in the buffalo genome. A total of 29 *FN-III* genes, widely distributed over different chromosomes of buffalo, harboring variable exons, were detected by using cattle and human as a query sequence and their physiochemical features are presented in **Table 1**. The FN-III protein isoform's functional diversity in buffalo was realized from their total number of amino acids ranging from 205 (FNDC5) to 3490 (IGFN1) and MW ranged between 20 kDa and 258 kDa (**Table 1**). Moreover, according to the instability index, all the members of the *FN-III* family are unstable except FANK1, LRFN5, IGFN, FLRT1, and FLRT2 which are stable. The isoelectric point indicated that most of the FN-III proteins are acidic (pI < 7), while basic FN-III proteins were also found (pI > 7), as shown in **Table 1**. Additionally, all of the FN-III proteins have AI values greater than 65 exhibiting thermostable abilities except IGFN1 and FNDC1 having lower AI values (<65), which are seen as thermo-unstable proteins. Furthermore, all of the FN-III proteins behaved as hydrophilic owing to their lower GRAVY values, but FNDC7 and FNDC10 were hydrophobic in nature due to their higher GRAVY values (**Table 1**).

Table 1. Physiochemical properties of the fibronectin gene family in *Bubalus bubalis.*

Gene	Chr.	Exon Count	MW (Da)	A.A	pI	AI	II	GRAVY
Fibronectin 1 (FN1)	2	46	258,641.53	2354	5.28	69.74	40.09	−0.487
Fibronectin type III domain containing 5 (FNDC5)	2	6	22,869.33	205	6.44	92.68	52.30	−0.218
Fibronectin type III domain containing 3B (FNDC3B)	1	31	127,736.34	1160	5.91	69.91	53.98	−0.434
Fibronectin type III and ankyrin repeat domains 1 (FANK1)	23	14	38,413.93	345	8.51	89.51	33.76	−0.334

Gene	Chr.	Exon Count	MW (Da)	A.A	pI	AI	II	GRAVY
Fibronectin type III and SPRY domain containing 1 like (FSD1L)	3	16	58,607.09	521	6.32	75.93	46.15	−0.574
Leucine-rich repeat and fibronectin type III domain containing 1 (LRFN1)	18	8	82,023.66	770	7.89	90.16	49.73	−0.066
Leucine-rich repeat and fibronectin type III domain containing 5 (LRFN5)	20	8	52,122.68	466	6.60	95.47	35.44	−0.141
Fibronectin type III and SPRY domain containing 1 (FSD1)	9	13	55,768.58	662	4.96	77.88	48.72	−0.380
Fibronectin type III domain containing 3A (FNDC3A)	13	31	133,632.56	1217	6.44	71.27	46.88	−0.412
Fibronectin type III domain containing 1 (FNDC1)	10	23	205,865.78	1905	9.66	59.01	59.92	−0.799
Leucine-rich repeat and fibronectin type III domain containing 3 (LRFN3)	18	5	72,450.76	679	9.38	87.05	59.78	−0.246
Fibronectin type III and SPRY domain containing 2 (FSD2)	20	15	84,755.73	747	4.81	69.69	47.20	−0.593
Fibronectin type III domain containing 7 (FNDC7)	6	13	85,949.11	811	6.53	77.69	45.18	0.046
Ankyrin repeat and fibronectin type III domain containing 1 (ANKFN1)	3	20	120,567.79	1068	6.52	80.73	58.15	−0.467
Immunoglobulin like and fibronectin type III domain containing 1 (IGFN1)	5	26	347,525.99	3490	6.49	55.35	34.98	−0.590
Fibronectin type III domain containing 4 (FNDC4)	12	7	24,753.16	230	7.66	88.87	55.08	−0.252
Fibronectin type III domain containing 8 (FNDC8)	3	4	34,298.93	312	5.29	80.93	46.44	−0.370
Leucine-rich repeat and fibronectin type III domain containing 4 (LRFN4)	5	3	66,839.10	636	6.70	94.14	42.55	−0.028
Fibronectin type III domain containing protein 3C1-like (LOC102393884)	X	27	157,320.54	1433	6.79	71.84	45.92	−0.439
Fibronectin leucine-rich transmembrane protein 2 (FLRT2)	11	4	73,773.40	660	7.89	94.18	36.58	−0.185
EGF like, fibronectin type III and	19	23	112,751.54	1032	6.53	74.46	41.70	−0.325

Gene	Chr.	Exon Count	MW (Da)	A.A	pI	AI	II	GRAVY
laminin G domains (EGFLAM)								
Fibronectin type III domain containing 9 (FNDC9)	9	2	25,342.98	227	5.65	85.99	54.56	−0.055
Leucine-rich repeat and fibronectin type III domain containing 2 (LRFN2)	2	2	87,694.08	820	6.59	90.88	44.73	−0.097
Fibronectin leucine-rich transmembrane protein 3 (FLRT3)	14	3	73,171.75	649	7.56	94.18	44.53	−0.296
Fibronectin leucine-rich transmembrane protein 1 (FLRT1)	5	2	74,144.68	677	6.15	96.88	32.12	−0.122
Fibronectin type III domain containing 11 (FNDC11)	14	4	38,198.37	333	6.81	96.34	53.23	−0.280
Fibronectin type III domain containing 10 (FNDC10)	5	3	24,097.32	225	9.11	87.33	66.15	0.124
Extracellular leucine-rich repeat and fibronectin type III domain containing 2 (ELFN2)	4	4	90,363.67	824	7.78	81.78	48.76	−0.295
Extracellular leucine-rich repeat and fibronectin type III domain containing 1 (ELFN1)	24	3	87,687.60	808	8.82	79.43	61.89	−0.351

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e 1). The
ANKFN1,
otifs were
0, 50, 41, 50, and 16 amino acids, respectively, were annotated as Leucine-rich repeat domain, while motif 3 and 10 were annotated as fn3 domain after the Pfams search (**Table 2**). The CDD BLAST was used to confirm the predicted conserved domains in buffalo *FN-III* genes (**Figure 1**C). Additionally, the domain of SPRY, PRY_1SPD1_BBC, Ig_Contactin_hnrnp, PRK04537, PRK07764, PHA03247, Laminin_G_2, LamG_EGF_CA_SPRY, PK12704, DUF5581, DUF4808, DUF5579, PRK15370, PCC, TPKR_C2, Ig_2Ank_2, and ANKYR superfamily has also been dredged up in the buffalo *FN-III* gene family (**Figure 1**C).

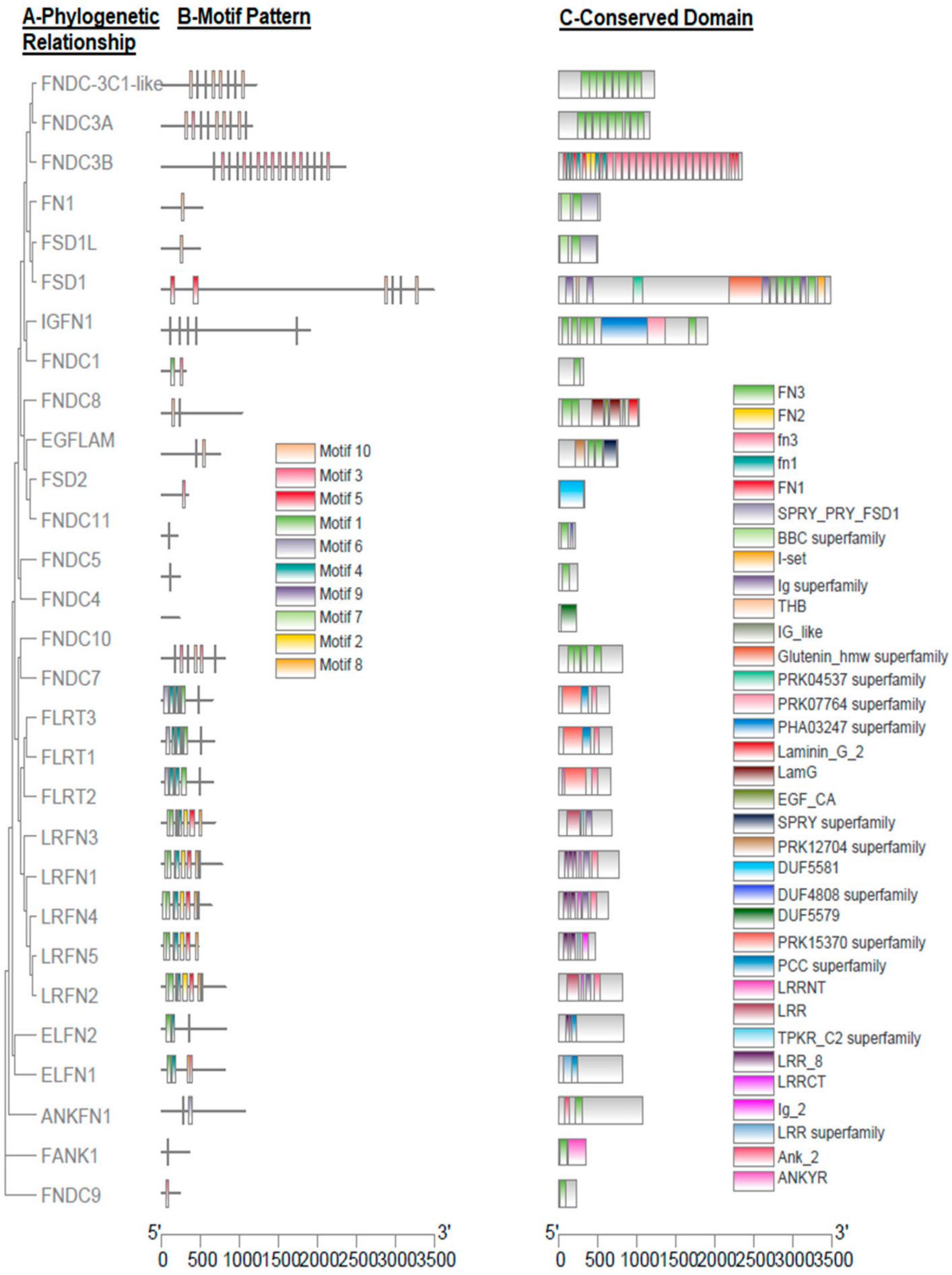
A-Phylogenetic Relationship
B-Motif Pattern
C-Conserved Domain
FNDC-3C1-like
FNDC3A
FNDC3B
FN1
FSD1L
FSD1
IGFN1
FNDC1
FNDC8
EGFLAM
FSD2
FNDC11
FNDC5
FNDC4
FNDC10
FNDC7
FLRT3
FLRT1
FLRT2
LRFN3
LRFN1
LRFN4
LRFN5
LRFN2
ELFN2
ELFN1
ANKFN1
FANK1
FNDC9
Motif 10
Motif 3
Motif 5
Motif 1
Motif 6
Motif 4
Motif 9
Motif 7
Motif 2
Motif 8
FN3
FN2
fn3
fn1
FN1
SPRY_PRY_FSD1
BBC superfamily
I-set
Ig superfamily
THB
IG_like
Glutenin_hmw superfamily
PRK04537 superfamily
PRK07764 superfamily
PHA03247 superfamily
Laminin_G_2
LamG
EGF_CA
SPRY superfamily
PRK12704 superfamily
DUF5581
DUF4808 superfamily
DUF5579
PRK15370 superfamily
PCC superfamily
LRRNT
LRR
TPKR_C2 superfamily
LRR_8
LRRCT
Ig_2
LRR superfamily
Ank_2
ANKYR
5'
3'
0 500 1000 1500 2000 2500 3000 3500
5'
3'
0 500 1000 1500 2000 2500 3000 3500

Figure 1. Phylogenetic relationships, motif patterns, and conserved domain regions of buffalo FN-III proteins. (**A**) Phylogenetic relationship of 29 amino acid sequences of FN-III proteins. (**B**) Motif pattern. (**C**) Conserved domain regions. Buffalo ten putative motifs of FN-III proteins are indicated in different colored boxes and details of motifs are enlisted in **Table 2**.

Table 2. Ten differentially conserved motifs detected in *FN-III* genes family in Buffalo.

Motif	Protein Sequence	Length	Pfam Domain
MEME-1	DNFIAAIPRRDFANMTGLVDLTLSRNTISHIEAGAFDDLENLRALHLDNN	50	LRR_8
MEME-2	NPLHCNCELLWLRRLAREDDLETCASPPGLTGRYFWSVPEEEFLCEPPLI	50	LRRCT
MEME-3	LTNLEPDTTYRLCVQALNSAG	21	fn3
MEME-4	MVNLETLRLDHNLIDTIPPGAFSELHKLARLDLTSNRLQKL	41	LRR_8
MEME-5	HWVAPDGRLVGNSSRTRVYPNGTLDILITTSGDSGAFTCIASNAAGEATA	50	I-set
MEME-6	CPSVCRCDRGFIYCNDRGLTSIPAGIPEDATTLYLQNNQINNAGIPADLK	50	LRRNT
MEME-7	CPKRCICQNLSPSLSTLCAKKGLLFVPPNIDRRTVELRL	39	Toxin_11
MEME-8	WPVQRPAPGIRMYQIQYNSSADDTLVYRM	29	-
MEME-9	LEDLDLSYNNLESIPW	16	LRR_4
MEME-10	GTEYRFRVRACNEAGEGPLSEPYTVTTPP	29	fn3

4. Collinearity Analysis of *FN-III* Gene Family

Collinearity analysis showed that genes of the *FN-III* family in buffalo were distributed over 18 chromosomes, while these genes were present over 21 chromosomes in cattle. Mostly, the buffalo *FN-III* genes were distributed on proximal or terminal ends of the chromosomes as presented in **Figure 2**.

[LRR_8, Leucine-rich repeat; LRRCT, Leucine-rich repeat C-terminal domain; fn3, Fibronectin type III domain; I-set, Immunoglobulin I-set domain; LRRNT, Leucine-rich repeat N-terminal domain; Toxin_11, Spasmodic peptide gm9a conotoxin from Conus species; LRR_4, Leucine-rich repeats (2 copies)].

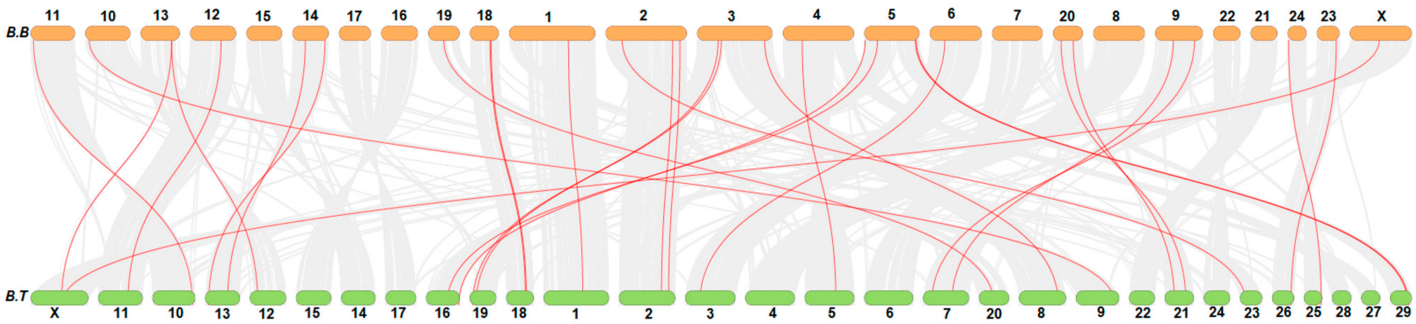

Figure 2. Collinearity analysis of *FN-III* genes family in buffalo (B.B) and *Bos taurus* (B.T).

5. Structural Configuration of FNDC5 Protein

For comparative structural configuration, three-dimensional protein models for FNDC5 were also predicted in humans, and different buffalo and cattle breeds (**Figure 3**). It was observed that FNDC5 protein structures in all species varied with a different number of amino acid residues ranging between 181 and 250. Indeed, there was variation in amino acid residues, but the FNDC5 structure in human, *Mediterranean buffalo* and cattle was quite similar to each other (**Figure 3**). Moreover, secondary structural elements including α-helix, β-sheets, transmembrane helix (TM), and degree of disorder also varied in all species. The α-helix was absent in Murrah buffalo and *Bos taurus*, while human and *Mediterranean buffalo* breeds shared an approximately similar proportion of β-sheets and TM helix. Furthermore, protein in cattle was mainly comprised of α-helix and a higher degree of disorder was observed in buffalo breeds.

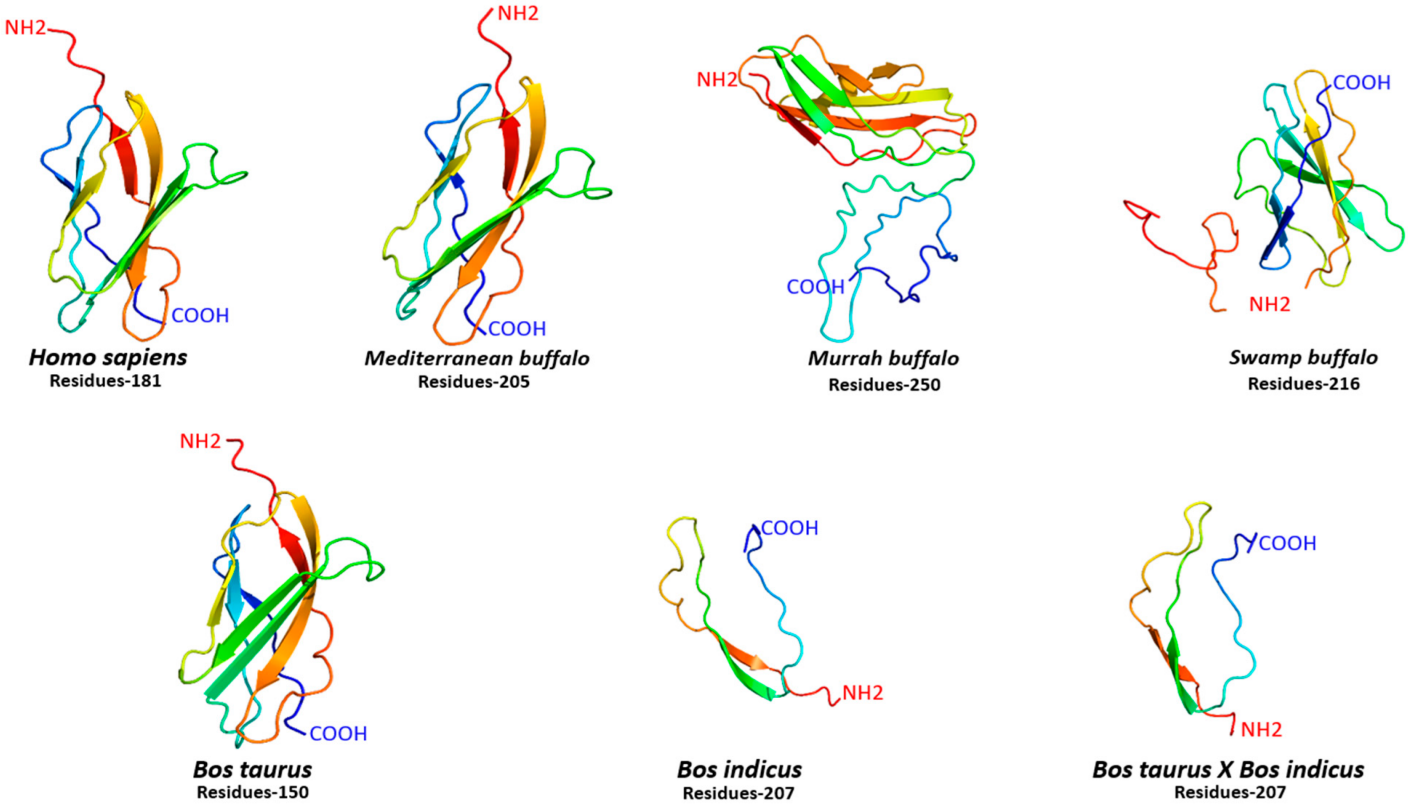

Figure 3. Three-dimensional protein configuration of FNDC5 in humans, cattle, and buffalo.

6. Multiple Sequence Alignment Analysis of Irisin

The comparative amino acid analysis of irisin peptide revealed conserved nature from human to cattle except for *Bos indicus* and hybrid cattle. *Bos indicus* and hybrid cattle exhibited a long deletion of 44 amino acids toward the NH_2-terminal end. Only a single amino acid variation D106 > G along with 6 amino acid deletion was also observed in *Bos taurus* at COOH-end. Furthermore, in comparison to humans, all the buffalo breeds had conserved irisin peptide sequences with 100% amino acid sequence homology (**Figure 4**).

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                        1        10          20          30          40          50
                Human   DSPSAPVNVT VRHLKANSAV VSWDVLEDEV VIGFAISQQK KDVRMLRFIQ
Mediterranean_buffal    DSPSAPVNVT VRHLKANSAV VSWDVLEDEV VIGFAISQQK KDVRMLRFIQ
      Murrah_buffalo    DSPSAPVNVT VRHLKANSAV VSWDVLEDEV VIGFAISQQK KDVRMLRFIQ
       Swamp_buffalo    DSPSAPVNVT VRHLKANSAV VSWDVLEDEV VIGFAISQQK KDVRMLRFIQ
          Bos_taurus    DSPSAPVNVT VRHLKANSAV VSWDVLEDEV VIGFAISQQK KDVRMLRFIQ
         Bos_indicus    .......... .......... .......... .......... ....MLRFIQ
Bos_taurusxBos_indic    .......... .......... .......... .......... ....MLRFIQ
                        51       60          70          80          90         100
                Human   EVNTTTRSCA LWDLEEDTEY IVHVQAISIQ GQSPASEPVL FKTPREAEKM
Mediterranean_buffal    EVNTTTRSCA LWDLEEDTEY IVHVQAISIQ GQSPASEPVL FKTPREAEKM
      Murrah_buffalo    EVNTTTRSCA LWDLEEDTEY IVHVQAISIQ GQSPASEPVL FKTPREAEKM
       Swamp_buffalo    EVNTTTRSCA LWDLEEDTEY IVHVQAISIQ GQSPASEPVL FKTPREAEKM
          Bos_taurus    EVNTTTRSCA LWDLEEDTEY IVHVQAISIQ GQSPASEPVL FKTPREAEKM
         Bos_indicus    EVNTTTXSCA LWDLEEDTEY IVHVQAISIQ GQSPASEPVL FKTPREAEKM
Bos_taurusxBos_indic    EVNTTTRSCA LWDLEEDTEY IVHVQAISIQ GQSPASEPVL FKTPREAEKM
                        101       112
                Human   ASKNKDEVTM KE
Mediterranean_buffal    ASKNKDEVTM KE
      Murrah_buffalo    ASKNKDEVTM KE
       Swamp_buffalo    ASKNKDEVTM KE
          Bos_taurus    ASKNKG.... ..
         Bos_indicus    ASKNKDEVTM KE
Bos_taurusxBos_indic    ASKNKDEVTM KE
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Figure 4. Comparative irisin peptide amino acid analysis of FNDC5 protein in humans, buffalo, and cattle.

7. Molecular Docking Analysis of FNDC5/Irisin

The FNDC5/irisin protein with a molecular weight of 22,869.33 (Dalton) was docked against six receptors to find out the binding affinities. All of the targeted receptors exhibited significant interactions as well as high docking scores ranging from −256.63 to −311.40 (**Table 3**). A total of 36 hydrogen bonds were detected, which were capable of interacting with the N-terminal portion of all the receptors, except for nuclear receptor subfamily 3 group C member 1 (**Table 3**, **Figure 5** and **Figure 6**). The FNDC5/irisin also exhibited a strong binding potential with different residues of the selected receptor molecules, where the amino acid residues 36 to 41 were mostly bonded with AR, DCAF6, and ERR-γ (**Table 3**, **Figure 5**A,B,D and **Figure 6**A,B,D). Furthermore, the irisin pocket with amino acid residues ranged between 72 and 91, and showed strong binding potential with ERR-β and KLF15 (**Table 3**, **Figure 5**C,E,F and **Figure 6**C,E,F). The superimposition of FNDC5/irisin (ligand) with all the receptors and their interactions are presented in **Figure 5** and **Figure 6**.

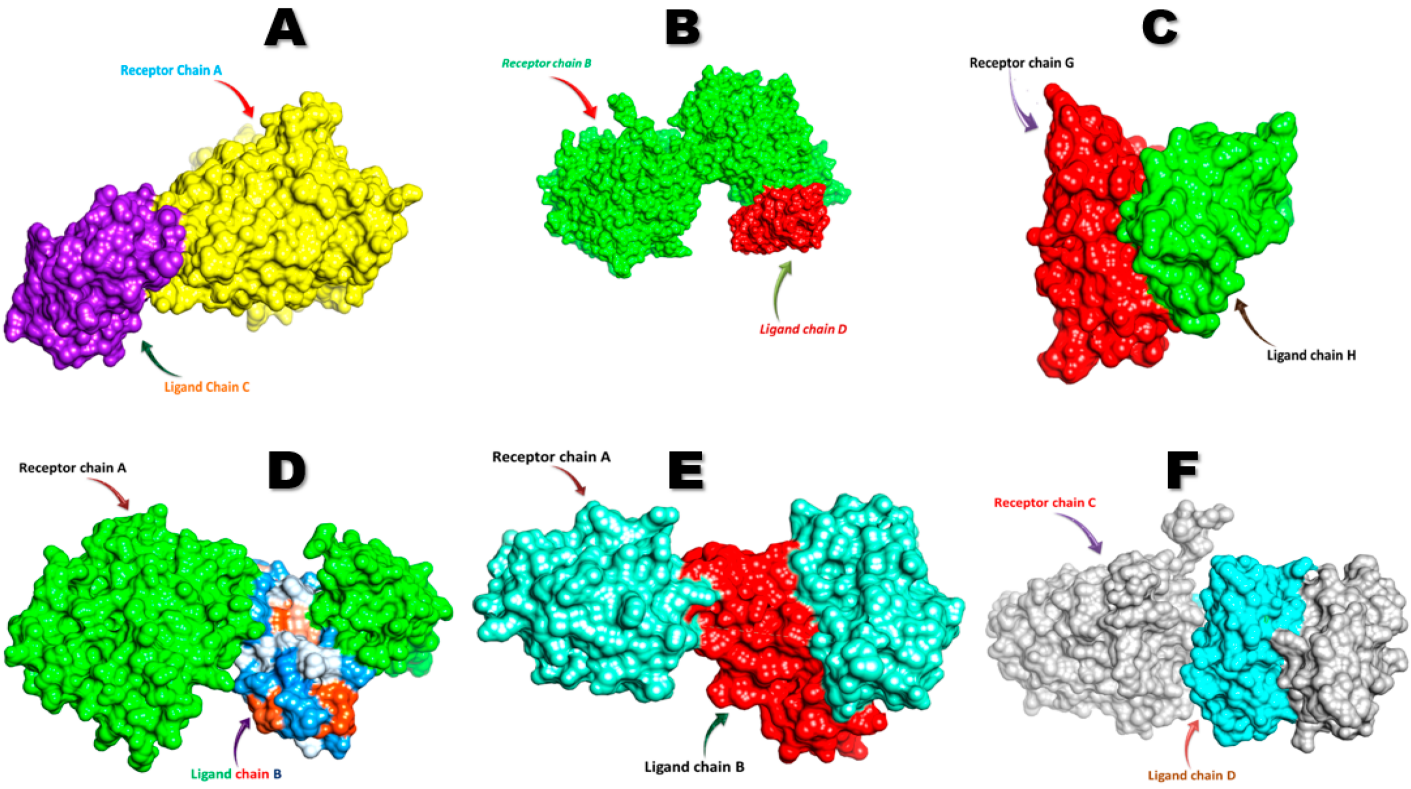

Figure 5. The superimposition of FNDC5 or irisin (ligand) and the receptors (**A**) Androgen (**B**) DDB1 and CUL4 associated factor 6 (**C**) Estrogen-related receptor β (**D**) Estrogen-related receptor γ (**E**) Krüppel-like factor 15 (**F**) Nuclear receptor subfamily 3 group C member 1.

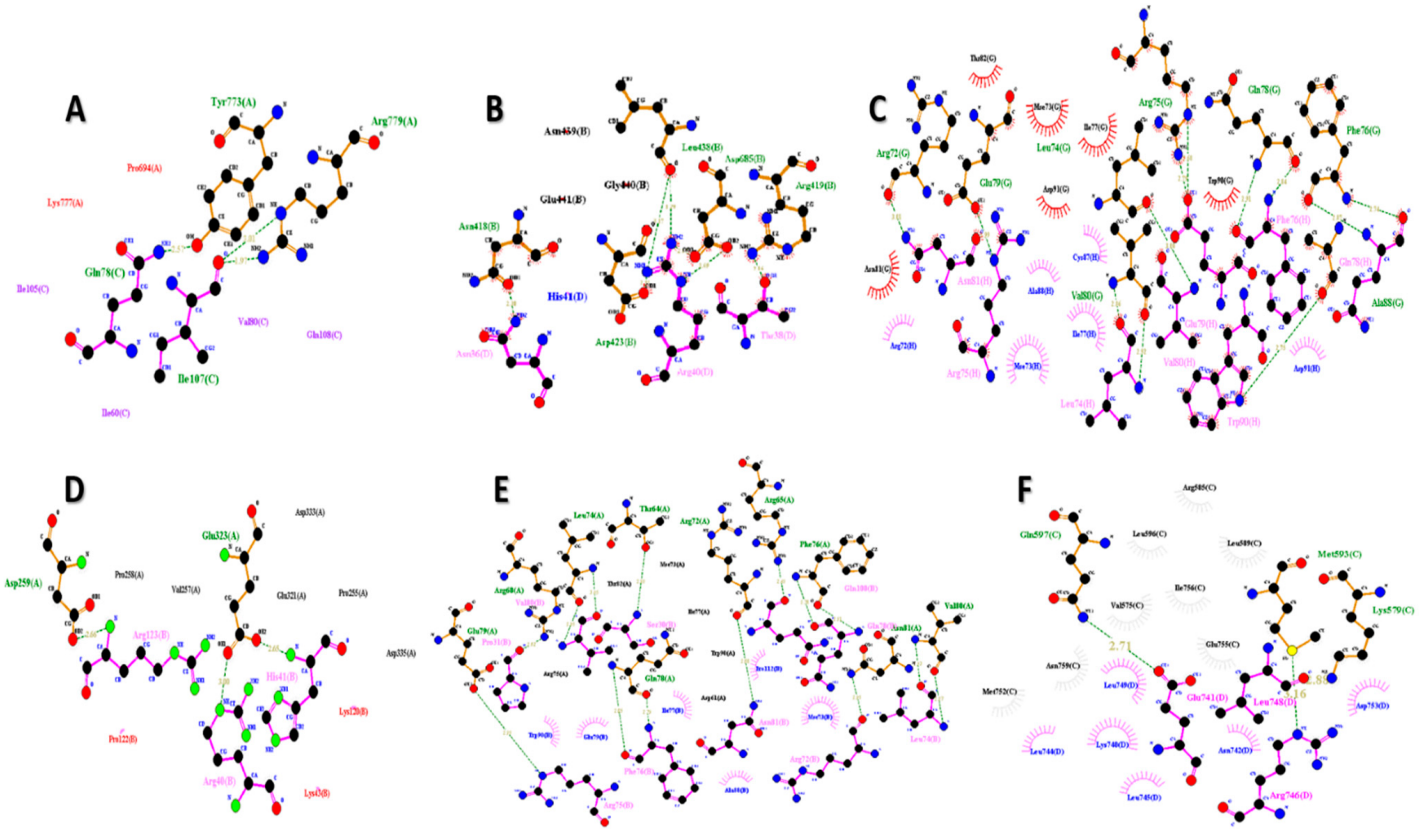

Figure 6. The FNDC5 or irisin (pink; ligand residues) amino acid residues interacting with receptors (**A**) Androgen (**B**) DDB1 and CUL4 associated factor 6 (**C**) Estrogen-related receptor β (**D**) Estrogen-related receptor γ (**E**) Krüppel-like factor 15 (**F**) Nuclear receptor subfamily 3 group C member 1 (all the receptors interacting residues are in green color).

Table 3. Molecular docking results of ligand (FNDC5 or Irisin) binding affinity with different receptors.

Sr. No.	Receptor	Docking Score	Ligand RMSD (A0)	Ligand Interacting Residues
1	Androgen	−311.40	86.42	Asn36, Thr38, Arg40
2	DDB1 and CUL4 associated factor 6	−256.63	79.76	Asn36, Thr38, Arg40, His41
3	Estrogen-related receptor β	−295.57	108.96	Arg72, Mse73, Leu74, Arg75, Phe76, Ile77, Gln78, Glu79, Val80, Asn81, Cys87, Ala88, Trp90, Asp91
4	Estrogen-related receptor γ	−256.63	79.76	Arg40, His41, Lys43, Lys120, Pro122, Arg123
5	Krüppel-like factor 15	−260.71	81.85	Ser30, Pro31, Arg72, Mse73, Leu74, Arg75, Phe76, Ile77, Gln78, Glu79, Asn81, Ala88, Trp90, Gln108, Pro112, Val180
6	Nuclear receptor subfamily 3 group C member 1	−308.59	108.34	Lys740, Glu741, Asn742, Leu744, Leu745, Arg746, Leu748, Leu749, Asp753

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