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Supplemental Information

Super-Enhancer LncRNA LINC00162

Promotes Progression of Bladder Cancer

Xin Wang, Ruirui Zhang, Shuilian Wu, Liping Shen, Meixia Ke, Yan Ouyang, Mengqi Lin, Yiting Lyu, Binuo Sun, Zhijian Zheng, Jialei Yang, Jie Yang, Wenmin Lu, Yiping Yang, Danni Li, Yunfeng Zou, Haishan Huang, and Aruo Nan

Supplemental information file

Table S1. Information of the qPCR primer sequences, Related to Figure 1.

Primer(for qPCR)	**Forward primer (5' to 3')**	**Reverse primer (5' to 3')**
LINC00162	ACTGCCTGGACTTTCAAGAGG	CCTCTTGGGTAGGGTGTTGG
RP11-152P17.2-007	AGGCTTCTGCGATTAGCTTCT	ACTCCATACTCTCCCTGGGTT
RP11-548L20.1-001	ACAGTGACAAGACCCAAGATTT	TGGAGTATCAGCAAGAACAGTG
RP11-527H14.3-001	GGGTAGCATCGCAGTCACT	TCTCTTCCTCGGTCCTTTCTG
RP11-152P17.2-001	AGGAAGTGTGCAGAGAAGATGA	AATGACTCCCTCAAGTTGTACG
PTTG1IP	CCAGTTACAAGCGTCTTGCC	CGCCTCAAAGTTCACCCAAC
ADARB1	TAGAGTCTGGTGAGGGGACG	GGAGATCCGCTGGTACATGG
UBE2G2	GGTACTGAACGACGTCCCTG	AAGATGGCAGTCTCTCCCCT
SUMO3	TCAGATTCAGGTTCGACGGG	ACAAATCGGAAGTCGCCCTG
ITGB2	GTGCGGTGAGTTCTGTGTTC	CCCGGGAACTTCTGTCTATGG
POFUT2	CGGAGGCGTTGCGATTATTG	AACCTTTGAAAGCCACCCGA
FAM207	CAGCAGGGAGAGCAACAAGC	TCCTGAAACCGGGTCCTTTC
GAPDH	ATCAATGGAAATCCCATCACCA	GACTCCACGACGTACTCAGCG

Table S2. RNA oligonucleotide sequences of siRNAs, Related to Figure 4.

RNA oligos	**Sequences**
siRNA-NC	Sense: 5'- UUCUCCGAACGUGUCACGUTT -3'
	Anti-sense: 5'- ACGUGACACGUUCGGAGAATT -3'
LINC00162 siRNA1	Sense: 5'- GCCUCUUCCUCAGACAUCUTT -3'
	Anti-sense: 5'- AGAUGUCUGAGGAAGAGGCTT -3'
LINC00162 siRNA2	Sense: 5'- CGCCUCAGUUUCAGAUUGATT -3'
	Anti-sense: 5'- UCAAUCUGAAACUGAGGCGTT -3'
PTTG1IP siRNA1	Sense: 5'- GAAGAACGUCUCCUGUCUUTT-3'
	Anti-sense: 5'- AAGACAGGAGACGUUCUUCTT-3'
PTTG1IP siRNA2	Sense: 5'- GCUGAUCAUCACCAUGUCGTT-3'
	Anti-sense: 5'- CGACAUGGUGAUGAUCAGCTT-3'
THRAP3 siRNA1	Sense: 5'- GGGAUUUCCGAGGUCACAATT-3'
	Anti-sense: 5'- UUGUGACCUCGGAAAUCCCTT-3'
THRAP3 siRNA2	Sense: 5'- GCUCAGGAACGCAAGCUUUTT-3'
	Anti-sense: 5'- AAAGCUUGCGUUCCUGAGCTT-3'

Table S3. Probe sequences of RNA FISH and pull-down assay, Related to Figure 3.

Target gene (Application)	**Sequences（5' to 3'）**
LINC00162(FISH)	GAATTTACCTCTTGAAAGTCCAGGCAGTTCAGCGG
LINC00162(pull-down) Primer F	TGAAGTGCTGACGGGTGGAGCGAC
LINC00162(pull-down) Primer R	TTGATTTCCCACTGACTTGTTATATTTCAGAAACA TTTC

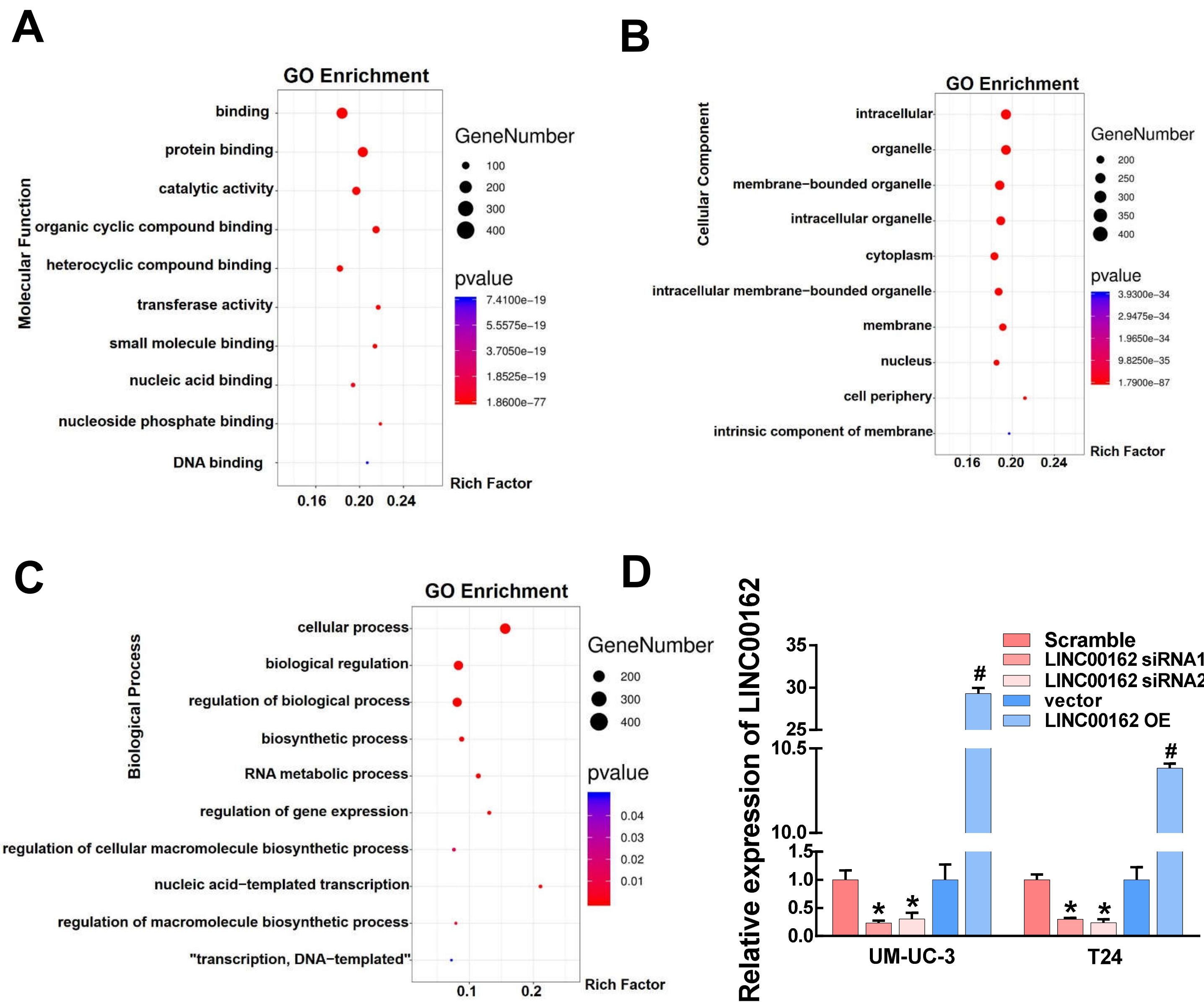

Figure S1. Analyze the potential functions of LINC00162 and silence and overexpression efficiency verification, Related to Figure 2.

(A) Molecular Function. (B) Cellular Component. (C) Biological Process. (D) Detection of the silencing and overexpression efficiency of LINC00162 in UM-UC-3 cells and T24 cells.

Figure S2

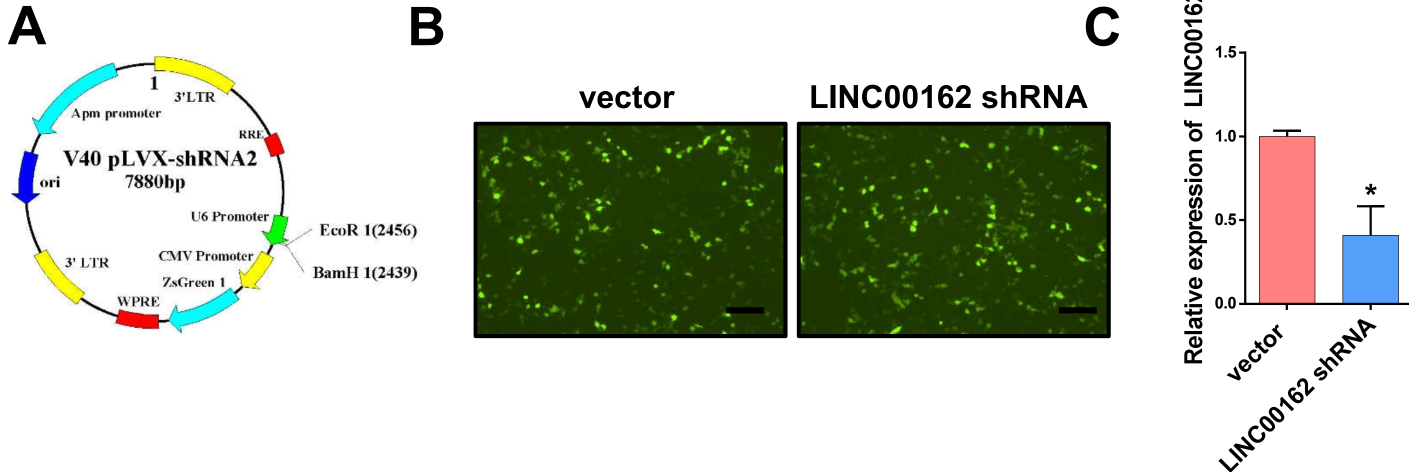

Figure S2. Construction of LINC00162 stable knockdown UM-UC-3 cell , Related to Figure 2.

(A) Structure of a stable knockdown plasmid vector. (B) Fluorescence picture of stable knockdown plasmid coated with lentivirus 6 hours after transfection in UM-UC-3 cells. (C) The silencing efficiency of the stable cell line that knocked down LINC00162 was detected by q-PCR.

Figure S3

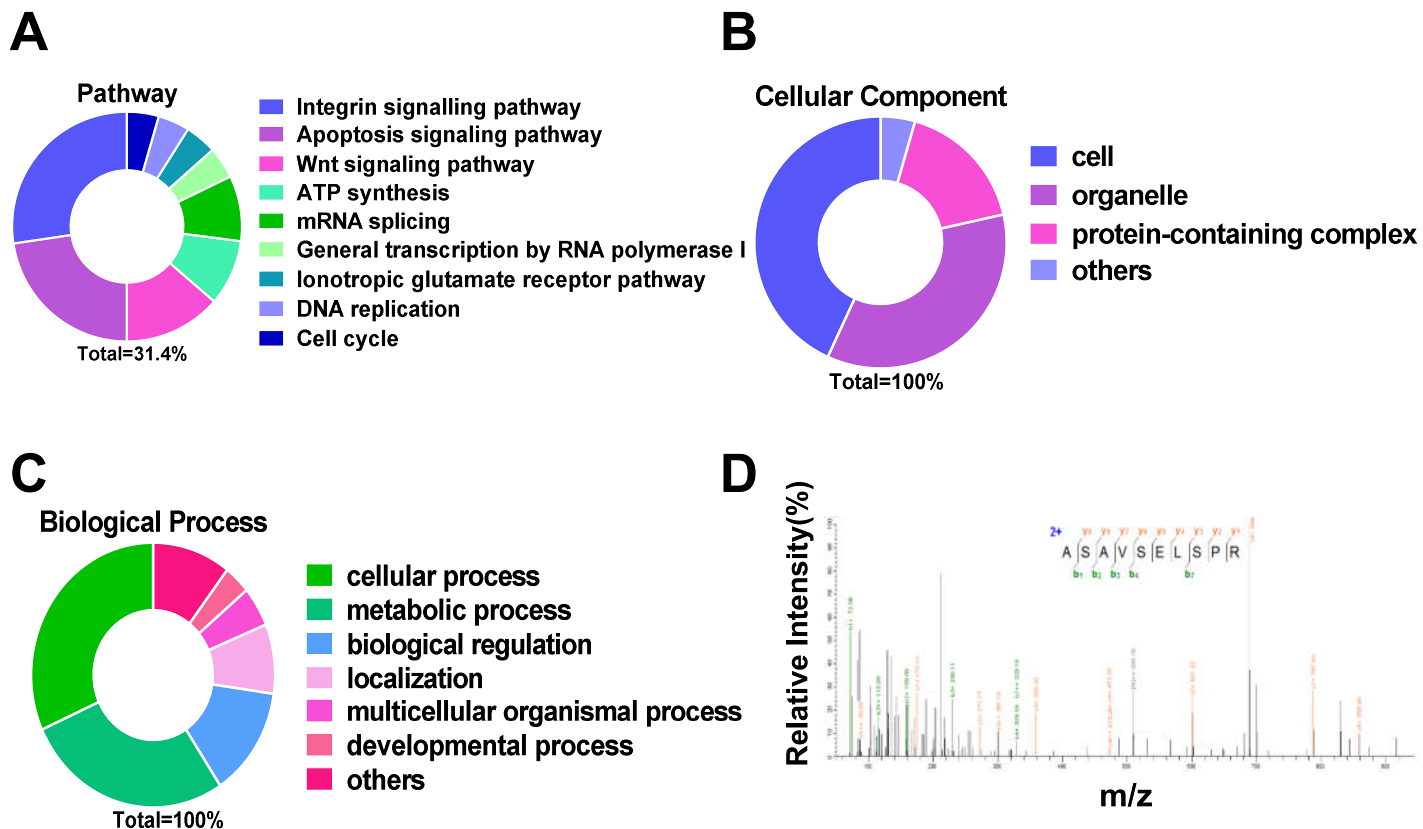

Figure S3. Potential functional analysis of pull-down proteins and mass spectrum result of THRAP3, Reltaed to Figure 5.

(A) Pathway analysis of pull-down sample proteins. (B and C) GO analysis of pull-down sample proteins.

(D) Mass spectrum of THRAP3 protein molecule

Transparent Methods

Tissue specimens and experimental animals

Thirty-two specimens of cancer and adjacent tissues from bladder cancer patients were irrespective of sex were collected from January 2018 to May 2019 in the urology department of the First Affiliated Hospital of Wenzhou Medical University. All patients underwent surgical resection and pathologically confirmed bladder cancer. Patients did not receive radiotherapy or chemotherapy before surgery. Patients were informed and signed informed consent before surgery. The research protocol was approved by the Wenzhou Medical Ethics Committee.

Four-week-old female athymic nude mice were purchased from Shanghai Slack Experimental Animal Center, and the experimental animal certificate number was SCXK (Su) 201904657. They were raised in the SPF-level experimental area of the Experimental Animal Center of Wenzhou Medical University. A total of 10 nude mice were used in the animal experiment of this study, and they were randomly divided into Vector and LINC00162 groups, with 5 nude mice in each group. All animal experiments were approved by the Animal Research Committee of Wenzhou Medical College, and animal research has been conducted in accordance with international guidelines.

Cell lines and main reagents

The human bladder cancer cell lines UM-UC-3 and T24, and the normal bladder epithelial cell line SV-HUC-1 were purchased from the ATCC cell bank. The qPCR kit was purchased from Promga, all qPCR primer sequences can be found in Table S1. TRIzol reagent was purchased from Invitrogen, and the GoScript™ Reverse Transcription System reagent was purchased from Promga.

LINC00162 overexpression vector (pcDNA3.1 (+/-)) was purchased from Guangzhou Bersinbio Company, PTTG1IP antibody was purchased from Abcam Company in the United States, THRAP3 antibody was purchased from Proteintech Company in the United States. Tubulin and GAPDH antibodies were purchased from Abway Company. Horseradish peroxidase (HRP)-labeled goat anti-mouse and rabbit IgG secondary antibodies were purchased from Cell Signaling Technology. The Luminescent Cell Viability Assay detection kit was purchased from Promega. Cell cycle detection kits were purchased from KGI. A RiboFECT CP transfection kit was purchased from

33 Guangzhou RiboBio Co., Ltd. CHIRP kits and RIP kits were purchased from the
34 Guangzhou Bersinbio Company.

35 **Cell culture and transfection**

36 The human bladder cancer cell lines UM-UC-3 and T24 and the normal bladder
37 epithelial cell line SV-HUC-1 were placed in complete medium supplemented with
38 10% fetal bovine serum, 100U/ml penicillin and 100μg/ml streptomycin, cultured in
39 an incubator at 37°C and 5% CO_2 saturation humidity. Finally, a good and stable cell
40 line was obtained for subsequent experiments. UM-UC-3 and T24 cell lines were
41 transfected with riboFECT CP transfection reagent for siRNA or scramble according
42 to the instructions, siRNAs sequence can be found in Table S2. After 48 h of
43 transfection, the transfection efficiency was determined by q-PCR.

44 **ATP and EdU assay**

45 Cell Titer-Glo 2.0 Viability Assay detection kit was used to detect cell proliferation
46 ability. Cells were seeded in 96-well plates and cultured in an incubator. Then cells
47 were treated separately in groups as described in the text. 25μL of DDW and 25μl of
48 ATP reagent were added to each well, plates were shaken in dark for five minutes and
49 allowed to stand for ten minutes, and the fluorescence value was then measured with a
50 microplate reader. The 5-ethynyl-2'-deoxyuridine (EdU) detection kit was used for
51 cell proliferation detection according to the manufacturer's instructions. The main
52 process was as follows: The EdU solution was diluted with a complete cell culture
53 medium at a ratio of 1,000:1 to prepare an appropriate amount of 50uM EdU medium.
54 100μl of 50uM EdU medium was added to each well and incubate for two hours.
55 100μL of cell fixation solution (4% paraformaldehyde in PBS) was added to each well
56 and incubate for 30 minutes at room temperature. 2mg/mL glycine was added to each
57 well and incubate for five minutes on a shaker. 100μl of 1X Apollo® Staining
58 Reaction Solution was added to each well, and incubated for 30 minutes in a dark
59 place at room temperature. Hoechst33342 reaction solution was diluted with
60 deionized water at a ratio of 100:1, and store it in the dark. 100μL of 1X Hoechst
61 33342 reaction solution was added to each well. Plates were incubated for 30 minutes
62 in dark at room temperature and shaken to remove the staining reaction solution.
63 100μL of PBS was added to each well and washed one to three times to elute Hoechst
64 33342 reaction solution. Images were acquired under a fluorescence microscope.

65 **Annexin-V-FITC / PI staining flow cytometry**

66 The cell cycle was detected on a flow cytometer using the Cycle Detection Kit
67 (KeyGen Biotech). And the cell apoptosis was detected using cell apoptosis Kit
68 (KeyGen Biotech). Both experiments were carried out in strict accordance with the
69 instructions.

70 **Western Blot (WB) assay**

71 Cell lysis buffer (10mM Tris-HCl, pH 7.4, 1% SDS and 1mM Na_3VO_4) was used to
72 prepare whole cell extracts, and proteins were extracted from cultured cells of each
73 group. A BioDrop microanalyzer was used to determine protein concentration. Equal
74 amounts of protein were separated by SDS-PAGE, transferred to a membrane,
75 blocked, and the first antibody was added, and incubated at 4 ℃ overnight. On the
76 next day, add goat anti-rabbit or mouse IgG second antibody coupled to alkaline
77 phosphatase (AP) and incubate at 4 ℃ for three hours. A Typhoon FLA 7000 (GE,
78 Pittsburgh, PA, USA) scanner was used to obtain images.

79 **Nude mouse xenograft model**

80 A vector of 3×10^6 cell density or LINC00162 shRNA UM-UC-3 stable cell line was
81 inoculated subcutaneously in female athymic nude mice to construct a subcutaneous
82 xenograft tumor model of nude mouse bladder cancer cells. Once the maximum tumor
83 size reached 1,000 mm^3, the animals were sacrificed and the tumors were dissected
84 for measurement.

85 **Chromatin Isolation by RNA Purification (CHIRP) assay**

86 Prepare cell suspension, lysate and sonicate according to the instructions of the ChIRP
87 kit (Bersinbio, Guangzhou, China), and design a LINC00162 specific probe to attach
88 to magnetic beads, probe sequence can be found in Table S3. The magnetic beads and
89 cell lysate are incubated together to pull down the target RNA while enriching the
90 DNA that interacts with it. The content of each gene in the product was detected by
91 q-PCR.

92 **RNA-FISH assay**

93 Subcellular localization of LINC00162 was performed by FISH. Design a
94 FISH-specific probe for LINC00162 (Sangon Biotech, Shanghai, China), probe
95 sequence can be found in Table S3. Cells seeded on coverslips were fixed with 4%

paraformaldehyde for 20 minutes at room temperature and digested with proteinase K (Sangon Biotech) for 5 minutes at 37 ℃. Next, the cells were fixed with 1% paraformaldehyde for 10 minutes and dehydrated in a gradient of 70%, 85%, and 100% alcohol. The probe-mixed solution was dropped on a coverslip, mounted and denatured at 73℃ for 3 minutes. In the dark, the hybridization lasted for 12-16 hours at 37 ℃. Coverslips were counted with DAPI and fixed with anti-fluorescent attenuation reagent. The cells were examined and images were acquired under a confocal microscope (Leica, Mannheim, Germany).

RNA Immunoprecipitation (RIP) assay

Refer to the instructions of the RIP Kit (Bersinbio, Guangzhou, China) to treat the cells. Use THRAP3 antibody to capture the endogenous RNA-binding protein in the cytoplasm to prevent non-specific RNA binding. Immunoprecipitation separates the RNA-binding protein and its bound RNA together. q-PCR detects RNA fragments precipitated by RIP.

RNA pull-down assay

The RNA-specific probes designed for LINC00162 were attached to magnetic beads and the cells were lysed according to the instructions of the RNA pull-down Kit (Bersinbio, Guangzhou, China). The magnetic beads were incubated with the cell lysate, and the protein interacting with LINC00162 was pulled. The pull down product was analyzed by mass spectrometry.

Immunohistochemistry (IHC)

Nude mouse transplanted tumor tissue was embedded with hard wax, sliced, dewaxed and rehydrated. After incubation with primary and secondary antibodies, it was counterstained with hematoxylin (Solarbio, China). Images were taken with microscopes at 100x and 400x magnification (Leica, Mannheim, Germany). IHC antibody: PTTG1IP (Abcam, USA).

Statistical analysis

GraphPad Prism 6 software was used for statistical analysis. Comparisons between groups were performed using two independent sample t tests. The difference was statistically significant at $P < 0.05$. And data are represented as mean ± standard deviation (SD).