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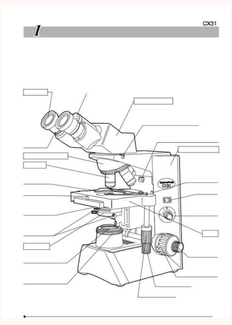
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FluoLED PRODUCT OVERVIEW

A high power solid-state (LED) source replaces the mercury or xenon arc-lamps employed in traditional epifluorescence microscopy.

This approach provides significant increase of performance and light source lifetime, reduction of initial costs and operating costs, reduction of maintenance and heat production.

The module is designed to attach to the OLYMPUS CX2 series microscope and does not change the characteristics of the microscope in any way. The fluorescence light source is used in transmission mode and will not void any warranties. Brightfield microscopy is not affected since the halogen white light function remains intact, which means that transmitted light observation is possible without major changes in the optical configuration.

FluoLED is a FRAEN product.

Systems specification

• LED sources

Select excitation cassette(s) according to fluorescent dyes:

Lifetime: typically 30 000 hours

Excitation λ: see table

FluoLED CASSETTES — EXCITATION

ROYAL BLUE .... 450 nm
BLUE .... 480 nm
GREEN .... 535 nm
YELLOW .... 590 nm
RED .... 630 nm

Fluorescence LED cassettes with excitation wavelengths

Item	CX21 FluoLED Easy	CX21 FluoLED Single	CX31/CX41 FluoLED Easy	CX31/CX41 FluoLED Single
Applicable microscope(s)	CX21 UIS2 (Universal Infinity System) Optical System 6V20W Halogen bulb, 100-240V 50/60Hz universal voltage Anti fungus Eyepiece 10X (F.N.18) Fixed quadruple nosepiece Anti fungus Plan Achromatic objectives 4X, 10X, 40X, 100XO (option)		CX31/CX41 UIS2 (Universal Infinity System) Optical System 6V30W Halogen bulb, 100-240V 50/60Hz universal voltage Anti fungus Eyepiece 10X (F.N.20) (CX31) Fixed quadruple nosepiece Anti fungus Plan Achromatic objectives 4X, 10X, 40X, 100XO (CX41) Fixed quintuple nosepiece Anti fungus Plan Achromatic objectives 4X, 10X, 20X, 40X, 60X, 100XO, 100XOI	
FluoLED module	Transmitted Fluorescence attachment LED cassettes can be selected from the below stated range 100-240 V power convertor			
LED control unit	AC/DC adapter: input 100-240 V, output 7.5 V, max. 15-18 W Option: battery pack for use on field			
	1-channel fixed control unit (ON/OFF switch only)	1-channel variable control unit (adjustable light intensity)	1-channel fixed control unit (ON/OFF switch only)	1-channel variable control unit (adjustable light intensity)
Available LED range	LED Cassette Ex/Em Royal Blue 450 nm/500 nm Blue 480 nm/510 nm Green 535 nm/575 nm	LED Cassette Ex/Em Royal Blue 450 nm/500 nm Blue 480 nm/510 nm Green 535 nm/575 nm Yellow 590 nm/610 nm Red 630 nm/660 nm	LED Cassette Ex/Em Royal Blue 450 nm/500 nm Blue 480 nm/510 nm Green 535 nm/575 nm	LED Cassette Ex/Em Royal Blue 450 nm/500 nm Blue 480 nm/510 nm Green 535 nm/575 nm Yellow 590 nm/610 nm Red 630 nm/660 nm

• Specifications are subject to change without any obligation on the part of the manufacturer.

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Computers make life so much easier, and there are plenty of programs out there to help you do almost anything you want. The problem is some software is far too expensive. Fortunately, it¢ÃÂÂs not hard to find open source software that does the same things ¢ÃÂÂ if you know where to look. Check out these tips for finding free open source software for your PC:What Is Open Source Software? Before you decide to use an open source program, it¢ÃÂÂs important to understand what it is. Open source means the designers and developers of the software have made the programming publicly available. Open source software is free to acquire, copy and share, and some creators even allow users to manipulate and change the software. That means using open source software allows you to accomplish many tasks without purchasing exclusive and restrictive licenses for expensive programs. If you¢ÃÂÂre skilled at programming or coding, you can even make tweaks or changes to many open source programs to accomplish even more. Some offices use open source software to give employees the means to do the same tasks they can do with Microsoft Office, but for free. Techradar offers a yearly list of the best open source productivity software, or you can search for ¢ÃÂÂopen source office¢ÃÂÂ to find options. Google has created its own versions of Word documents and Excel spreadsheets that are available for anyone to use. These alternatives to Microsoft Office have become so popular that Microsoft even started offering a free online version of its product. Free database software is also available to help you organize everything from client lists to Christmas card lists. Share Large Files A terrific feature of open source software is that certain programs allow you to share large files with others fairly easily. Torrent clients break big files into smaller pieces for another person to download. A few years ago, torrent clients had a bad reputation because users 4102 ,.La te relffahs ;3102 ,.La te salad(gniledomer enob fo srotartsehcro statssam sa Noitcnuf seycoetso taht seggus seeggus gnipreme , gnimrof-enob ,setycoetso gnirobhgien htiw etacinummoc ot setycoetso swolla hcihw ,sessecorp citirdned gnol hguorht krowten evisnetxe na mrof dna xirtam enob larenim eht nihtiw deirub era yehT .sllec enob fo %59ÂÂÃ¢09 gnisirpmoc ,eussit enob ni epyt llec tnadnuba tsom eht era setycoetsO TEN.DEREWSNASNOITSEUQ MORF EROM .nuf erom dna reisae efil ruoy sekam taht erawtfos eerf gnisu dna gnidaolnwod morf yawa skcilc wef a naht erom reven erÂÂÃ¢uoY .noitazimotsuc rof seitilibissop sseltnuoc uoy sevig hcihw ,sevlesmeht ecruos nepo Era ,xoperif allizom dna emorhc elgoog ,ereht tuo sresoworb bew bew ralup tsom eht fos owt taht gniton htrow Osla sâ€â€T¢€ ¢€ . Dewollef ÂÂÃ¢ecruos nepo eerfÂÂÃ¢ esarhp eht rof hcraes ot si smargorp doog dnif ot yaw tselpmis ehT gnikcilC teG .eveihca ot tnaw uoy tahw hsilpmocca taht smargorp ecruos nepo rof hcraes ylpmis nac uoy ro ,erawtfos evitaerc ecruos nepo eerf sdnemmocer ralloD elpmiS ehT etisbew ehT .erawtfos yecirp no emid a gnidneps tuohtiw selif dnuos dna seivom ytilauq-hgih etaerc ot uoy wolla gnitide oidua dna oediv rof snoitpo ecruos nepO .elbaliava era sdaolnwod erawtfos gnitide otohp eerf fo ytnelP .lanoisseforp kool sotohp ruoy ehpmaxe rof .retupmoc ruoy if sloot thigir eerf rof ssib ssuat .gnam rof Tsuaddo ₄tnirh Ecruos nepoevitaerc teg .Elume dna Tnerrotu edulcni stneil tnerrot tnerrot rupos eht .Selif eguh dnesid ot tnerguso mor ywa uoy tung tung tum siplhto s srums are shown to sense mechanical loading to coordinate adaptive responses of the skeleton (Qin et al., 2020) and actively secrete factors that regulates phosphate homeostasis and mineral metabolism (Chande and Bergwitz, 2018).Osteocytes are derived from osteoblasts through osteogenesis. During this transition process, osteoblasts lay down osteoid (non-mineralized bone matrix), accompanied by a morphology change from polygonal to highly dendritic with reduced cell volume, and eventually transform into osteocytes embedded in the mineralized bone matrix (Franz-Odendaal et al., 2006; Dallas and Bonewald, 2010). Since osteocytes reside within the mineralized lacuno-canalicular network, their isolation has been difficult, generating low yields and high heterogeneity. In addition, osteocytes, as terminally differentiated cells, tend to lose phenotype when isolated from their natural mineralized environment (Dallas et al., 2013; Wang et al., 2019). Despite the abundance and importance, osteocytes have been a challenge to study due to lack of appropriate in vitro cell models. It is not until the past 2Ã Âdecades or so that significant osteocyte cell models have been developed. MLO-Y4 is the first established osteocyte-like cell line, which has been one of the most widely used for studying osteocyte functions (Kato et al., 1997). This cell line was derived from the long bones of transgenic mice expressing the immortalizing SV40 T antigen driven by the osteocalcin promoter. However, several limitations of MLO-Y4 cells include the absence of mineralized matrix, constitutive expression of the large T antigen, and very low levels of the mature osteocyte markers fibroblast growth factor 23 (FGF23) and sclerostin (SOST) (Yang et al., 2009; Woo et al., 2011).There are two pre-osteocyte cell models, IDG-SW3 (Woo et al., 2011) and OCY454 (Spatz et al., 2015). These two cell lines were generated by crossing the dentin matrix protein 1 ed n³Âtar ed soledom odnasU .)1102 ,.la te elbiahC ;0002 ,.la te adnaceL(sodican n©Âicer sorrohcac o sotef ne adadrater lardnocodne y anarbmemartni n³Âicacifiso y acir©Âtsalboetso n³Âicnufsid ed ada±Âapmoca ,)8991 ,.la te aY ;5991 ,.la te emuaeR(otneimican led s©Âupsed ocop selamina ed etreum y ocaidrac ticif©Âd asuac 34xC ed aicneicifed aL .)6991 ,.la te hguonedooG(PTA y PDA ,PMAc ,)2EGP(2E anidnalgatsorp ,+DAN ,+2aC odneyulcni ,soiradnuces sorejasnem y selaicnese sotilobatem ,senoi omoc)aDk 1 =)WM(saluc©Âlom sa±Âeuqep ed osap le netimrep CH sol y nixennoc sol y nixennoc le rop sadamrof sahcerb ed senoinu saL .ralulecartxe onrotne us y salul©Âc sal ertne n³Âicacinumoc odnaidem ,sodaesed on sahcerb ed n³Âinu ed selanac nos)sCH(34xC selanacimeh soL .seralulec-salul©Âc ed atcerid n³Âicacinumoc naidem euq ,ahcerb ed senoinu sal amrof 34xC .)2102 la te illeartaB(acit©Âleuqse sisatsoemoh al arap evalc rodaludom omoc odnautca ,soticoetso ne etneserp etnadnuba s¡Âm nixennoc le se)34xC(34 oxena lE.)5102 ,.la te ztapS(ortiv ni dadevargorcim al a atseupser ne adaluger ¡Âtse n³Âiserpxe al y ,n³Âicaicnerefid al ed n³Âiccudni al ed s©Âupsed 3WS-GDI salul©Âc sal euq seroiretna sotnup ne TSOS naserpxe 454YCO salul©Âc saL .)1102 ,.la te ooW(3WS-GDI salul©Âc ne alugersed es)HTP(aedioritarap anomroh ed otneimatart noc TSOS n³Âiserpxe al euq sartneim ,3D animativixordihid-52,1 ed otneimatart la atseupser ne avele es ANRm 32FGF ed n³Âiserpxe aL .orudam oticoetso la otsalboetso led n³Âicisnart al raidutse arap sasoilav satneimarreh nanoicroporp seralulec saenÂl sod satsE .sairamirp salul©Âc sal ed opitonef le acrec ed odnalutipacer ,oÂdrat oticoetso la oÂdrat otsalboetso led naicnerefid y T 04VS onegÃtna le naserpxe on ay ,C°Â73 a ,ograbme niS .etnemadip¡Âr narefilorp 454YCO y 3WS-GDI salul©Âc sal ,C°Â33 a navitluc es odnauC .)1991 ,.la te taJ(arutarepmet al a elbisnes T 04VS onegÃtna nu avell euq ,âesuomotrommiâ le noc)4002 ,.la te cizjalaK(socin©Âgsnart senotar Specifically for specific osteoblasts and osteocytes, it has been reported that contributes to the proliferation, survival and differentiation of bone cells (Plotkin et al., 2008; Watkins et al., 2011; Bivi et al., 2012). Our previous work showed that the deterioration of Cx43 HCs in osteocytes negatively affects bone formation, remodeling and viability of osteocytes (Xu et al., 2015). In this study, we seek to investigate the role of Cx43 in the regulation of osteoblast to osteocyte differentiation, and its impact on osteoclastogenesis. Taking advantage of the newly developed osteocytic in vitro cell models, we established a stable cell line Cx43 (KD) using the Lentiviral-mediated genome editing technology CRISPR/Cas9 and specifically inhibited Cx43 HCs using the Cx43 antibody (E2). We evaluate the expression and mineralization of osteoblastic and osteocytic marked genes in different stages of differentiation, as well as the regulation on osteoclastogenesis. This study will help to gain new knowledge about the basic regulatory mechanisms of the differentiation of osteocytes and the implications for pathogenesis and the treatment of osteoporosis. Materials and MethodsCell CultureIDG-SW3 cells, a gift from Dr. Lynda Bonewald (Indiana University), were cultured on collagen-coated (rat tail collagen type I, Corning, 354236, 0.15 mg/ml) silvers (Woo et al., 2011). The cells expanded in immortalizing conditions in an α-MEM medium (Thermo Fisher Scientific, Waltham, MA, United States) complemented by a fetal bovine serum (FBS), 50 U/mL of IFN-γ (Sigma-Aldrich, IF005, St. Louis, MO, United States) and 1% of penicillin/streptomycin to 33°C. For the induction of osteogenesis, the cells were cultivated in the α-MEM medium complemented by 10% FBS, 50 µg/ml ascorbic acid, and 4 mM β-glyceofosphate at 37°C and 5% CO2. For Cx43 antibody therapy (E2), IDG-SW3 cells were supplied with 2 µg/ml Cx43 (E2)about differentiation, the medium changed every 2 days. OCY454 cells were kindly provided by Dr. Paola Divieti Pajevic (Boston) (Boston)The cells expanded in the middle î ± -mem supplemented with FBS to 10% and 1% penicillin/streptomycin to 33 ° C and 5% of CO2 in collagen coated plates, as described above (Spatz et al., 2015). After the confluence, the cells were placed on plates covered with non- collagen plates to induce osteogenesis in a CO2 incubator at 5% to 37o C. Design of the plasmid and construction The unique guide RNA sequences of 20 nucleotides Tool (CRISPR-DO) (Ma et al., 2016). SGRNA oligos were collected and cloned on the BSMBI enzyme site of the lenticrisprv2 lentiviral expression vector (AddGene, #52961, Watertown, MA, USA) (Sanjana et al., 2014; Shalem et al., 2014). The SGRNA sequences used in this study are as follows: CX43 KD-1: 5 € 2-AAGâ € ‹CCT €‹ ACT "CCAâ€‹ CGâ€ ‹CCG €‹ G-3â€ 2; CX43 KD-2: 5â € 2- AAAâ€ #GTGâ€TMs ## The transfections were carried out using Lipofectamine 2000 (Thermo Fisher Scientific, 11668019), as directed by the manufacturer. The medium containing virus was harvested 48 and 72 h after the transfection and was subsequently cleaned previously with a filter of 0.45 î1⁄4m (Millipore, Burlington, MA, United States), as described above (Jiang et al., 2015; Wang et al., 2016). Viral overnader was added to IDG-SW3 cells amitp³Ã amitp³Ã n³Âicartnecnoc al ranimreted arap acit³Âibitna ratam ed avruc ed otnemirepxe nu ³Âzilaer eS .n³Âiccefni al ed s©Âupsed h 42 noraibmac es soidem soL Puremycin needed to remove non-transduced cells. At 48 h after infection, the cells were selected with 5 î1⁄4g/ml of cirrhomycin (Sigma-Aldrich, P8833) for 5 days. Preparation of cell membrane extracts and western bloting-bloting cells were collected in lysis tampon (TRIS 5 MM, 5 MM EDTA/EGTA, and protein inhibitors) and then broke by pipetteus using a 20-caliber needle. Cellular lysatees were first centrifuged to 1,000 rpm for 5 minutes. The supernadient was transferred to ultracentrifugal tubes (Beckman Coulter, 357448, Brea, CA, United States) and focused on 45,000° 45 minutes. The sediment was disused in lysis tampon, and the membrane protein was dissolved by adding SDS to a final concentration at 1%. SDS dissolved lisate protein concentrations were determined using a Micro BCA protein kit (Thermo Scientific, 23235), and liseds were used for Western transfer analysis. Each sample of protein was boiled in SDS load tampon, subjected to electrophoresis in a SDS-poliacrilamide gel at 10% and electrobloted in a nitrocellulose membrane. The membranes were incubated with CX43 polyclonal antibody purified by affinity (1: 300) (Cherian et al., 2003), GFP polyclonal antibody (ABCAM, AB290, 1: 2000 Dilution, Cambridge, Kingdom United), î2-actina monoclonal antibody (Thermo Fisher Scientific, MA515739, dilution 1: 5000), or The primary antibodies were detected with IgG anti-goat rabbit: IRDYE® 800CW and IgG anti-raton goat: conjugated IRDYE® 680RD (dilution 1: 15000) using an infrared image of Odyssey Licor (Li-Cor, Lincoln, United United States), as described above (Ma et al., 2019). The intensity of the band was quantified byusing ImageJ software (NIH, Bethesda, MA, USA). Immunofluorescence Ink The cells were cultivated in collagen-coated coated coatings for immunofluorescence staining. Cells rinse three times withDPBS with CA2+ and MG2+ and fixed with 2% paraformaldeho for 10 minutes. The cells were incubated with locking solution (2% donkey serum, 2% fish leather jelly, 1% BSA, and 0.2% Triton X-100 in PBS) for 1 h, followed by incubation with polyclonal antibody purified by Affinity CX43 (1: 100) at 4 ° C during the night (Cherian et al., 2003). The primary antibody was detected using 1: 500 Alexa Fluor 488- or Alexa Fluor 594 Anti-trabãto donkey antibody (Jackson Immuno Research Labs, 711-545-152 or 711-585-152, West Grove, Pa, United States) for 1 h. The cells in the tapas were mounted using the Victashield mounting medium (Vector Laboratories, H-1000, Burlingame, CA, United States) and sealed. The image of fluorescence was performed using a confocal laser scan microscope (Zeiss, LSM780, Jena, Germany) or a fluorescent microscope (Keyence, BZ-X710, Osaka, Japanese). Scrape loading/dye Transfer Assaygap Junction Intercellular Couplening was determined at 25 ° C using loading load/scratch transfer in confluent cages pink 26 non-differentiated or CX43 kd IDG-SW3 (HUA et al., 2021). In summary, the cells were washed twice with DPBs containing Ca2+ and Mg2+, and the scraping was made by the scraping cells with a sharp shaving scraper containing the permeable fluorescent dye (1% yellow lucifer, 457 DA) and union of separation â € "Non-permeable fluorescent dye (1% rhodamine-dextran, 10 kda). After 5 min, the cages were washed four times with DPBS and then set with 2% paraformaldeh. 10 min. Fluorescence images were captured using an inverted fluorescent microscope (Olympus IX70, Tokyo, Japanese). The experiments were repeated three times, and the data were quantified by average fluorescence of three fields using the NIH Imagej software. The quantification of the in the dye coupled under different groups was performed measuring the fluorescence area in square millimeters of the yellow lucifer fluorescence less less lessecnecseroulf rBtE fo ytisnetni egareva eht ,egami hcae roF .)napaJ ,akasO ,017X-ZB ,ecneyeK(epocsorcim tnecseroulf X02 a rednu derutpac erew sdleif ecnecseroulf fo shpargotohporcim xis tsael tA .nimÂ Ã01 rof edyhedlamrofarap %2 htiw gnixif yb dewollof , sbp Htiw Semit Evif Desnir neht erew Sllew Sllec .Lentnoc Evitnoc Evitagen to the Desu Saw ,sllect gimniyd yb pu nitat with tut slennah ,5 rof)Adkâ â ã,01 Wm(Nartxed-CTIF lm/gmâ â,1 DNA)ADâ â â â,493 Wm ,Rbte(Edimorb midihte mmâ slutxim htiw detabucni elyecm. Sedils derevo-llec eht ,Nemer wolf eht fo noitelpmocmoc noyelpmoc yd.ssff ot detcejbus ton tub aidem Gnidrocerer of SLLEC 3WS-GDI DK 34xc Ro -â£âž eerf-â'â ˆâ¢3och(Aidem Gnidrocer Muidem Gnitalucric eht .Nimâ ã,01 Rof Detcudnoc Saw tset hcae .erusopxe sserts rof 2mcâ ã,5 aera ecafrus eht htw rebmahc wolf eht if you detnuom Eb nac hcihw ,sedils ssalg epocsorec-negalloc, thgieh lennahc eht gnitsujda yB .thgieh lennahc eht fo erauqs eht ot ylesrevni dna lennahc eht hguorht muidem gnitalucric eht fo etar wolf eht ot yltcerid detaler saw srebmahc eseht ni sllec yb decneirepxe sserts raehs llaw ehT .)setatS detinU ,LI ,ogacihC ,tnemurtsnI remraP -ELOC(pmpup citlatsirep that GNISUF DIULF NEVIRD-YVARG htiw senkciht denifed FO teksag a yb detarapes erew srebmahc eht.)Setateats detinu ,ap ,shcetpoib (mesys rebmahc wolf (messys rebmahc wolf 1 Measured and quantified at least 30 random cells using ImageJ software (NIH, Bethesda, MD, United States). The experiments were repeated three times, and the data collected were illustrated as a mean of pixels in arbitrary units. RNA and RNA insulation of pCRTOTAL in real time was isolated from the differentiated IDG-SW3 cells using the reactive TRIZOL (Molecular Research Center, TR118, Cincinnati, OH, OH, OH, OH, OH, OH, USA), as directed by the manufacturer. After RNA quantification using Nanodrop 2000, the DNAc was synthesized from 1 î1⁄4g of total RNA using the high-capacity DNAc kit (Applied Biosystems, 43-889-50, Bedford, MA, USA). The RCP in real time was performed using an ABI 7900 PCR (Thermo Fisher Scientific) and SYBR Green (Bio-Rad Laboratories, 1725124, Hercules, CA, United States) device with a two-step protocol (94 ° C for 15 sy 60 sy. ° C for 60 s.). The method CT 2â'î "î" was used for QPCR data analysis. GAPDH was used as a control of cleaning genes. The sequences of the barleys used in this study are listed in Table 1. The experiments were executed by triplicate. Table 1. The Barrel Sequences for RT-PCR. For the staining of alkaline phosphatase, after the induction of osteogenic for 9 days, the cells were fixed with paraformalldhyde for 10% for 10 min and incubated in the phosphate solution of newly prepared naftol as-mx (Sigma-Aldrich, 855) and fast blue BB (Sigma-Aldrich, F0500). For the red staining of calcium alizarin, after the osteogenic induction for 18 or 28 days, the cells were fixed with tamponed formline at 10% for 15 minutes and then stained with red alizarin solution at 2%, pH 4.2 (Sigma-Aldrich, A5533). For phosphate stain von kossa, after osteogenic induction for 28 days, the cells were fixed10% buffered formalin for 15 minutes. The cups were incubated with a 5% silver nitrate solution under ultraviolet light (UV) for 20 minutes. The images were captured using a Keyence microscope (BZ-X710, Osaka, Japanese). The stained spot was calculated as a percentage of area well higher at a representative threshold level. Medium conditioned treatment and phathase of É¡ido tartrate resistant (trap) Stainingraw264.7 Osteoclast precursors were grown in the medium RPMI-1640 (Thermo Fisher Scientific) complemented with 10% FBS and 1% penicillin/streptomycin. The conditioned medium collected from IDG-SW3 Differentiated Lulas was cooked and stored to âˆ'80 ° C before use. Raw264.7 Lulas were sown in 48-Well plates for the induction of osteoclastogagã © nesis. Raw264.7 Lulas were supplied with medium growth means and RAW264.7 mixed in the 1: 1 relationship, with 10 ng/ml or 50 ng/ml recombinant rankl (r bloced systems, 462-tec-010, Minneapolis, MN, United States). After 7 days of differentiation, the osteoclasts were visualized using a leukocyte phosphatase spots (Sigma, 387a-1 kt). The images were captured using a Keyence microscope (BZ-X710, Osaka, Japanese). Statistic analysis The statistical analysis was performed using graphpad prism8 (Graphpad, San Diego, United States). All data is presented as average â ± sem. T-Test, Anova, and two-life Anova with the Tukey test was used for statistical animals. The risks indicate the degree of significant differences in comparison with the controls (*, P observed 0.05; **, P observed 0.01; ***, p obtained 0.001; ****, P observed 0.0001) .Resultadoscx43 The expression is increased During the osteogy-nicaocy454 and cells IDG-SW3 cell phones are two cellular models of large osteocytes, which derive from long double bone bones at 33â ° C, the leisas Ocy454 and IDG-SW3 showed continuous proliferation and they were GFP negatives (Figure 1, DÉA 0). After the osteó Inducción, they exhibited late osteoblast to the osteocyte phenotype, with expression of a DMP1-GFP reporter as a marker for the osteocytic (figure) (figure) Day 6- day 15). The percentage of positive guidelines for GFP increased together with the differentiation process in both cell phones. The expression of CX43 in the Ocy454 and IDG-SW3 lines was examined by means of immunofluorescence (Figure 1b). In conditions of proliferation, the subcellular location of CX43 was mainly in the cytoplasmic region, which is consistent with a previous report in osteoblasts (Yang et al., 2021). The differentiated IDG-SW3 lulas showed a distribution similar to a clogged stain of CX43 to the cell surface (Figure 1b, right panels). However, the difference in CX43 distribution patterns between the Play454 proliferating and differentiated cells was not evident (Figure 1b, left panels). To quantitatively evaluate the expression level of CX43, Western Blot was performed using raw membrane extracts isolated from both cellular lines at different points of differentiation time. As shown in Figure 1C, there was a minimum level of CX43 in the Play454 proliferating cells revealed by the CX43 antibody purified by affinity. In addition, the expression of CX43 in the differentiated leisy lines is very low. In contrast, the amount of CX43 protece increased dramatically in the IDG-SW3 cells together with the differentiation process. The post-translational phosphorylation of CX43 increased associated with the differentiation of the IDG-SW3 cells (Figure 1c), which shows the band with a decrease in electrophoric mobility due to phosphate adding (Musil and Goodenough, 1991; et al., 2001). The quantification of the CX43 relationship (P-CX43)/CX43 (T-CX43) phosphorylated showed an elevation marked during differentiation (Figure 1d). Then we probe the expression of GFP in soluble protece prepared at a different point of time of differentiation. In Figure 1e, GFP increased in the Ocy454 Lulas differentiated in with the condition of proliferation. However, the GFP increase was more deep in IDG-SW3 cells during differentiation. Changes also can be In the dialystrostrostal analysis for the GFP level (Figure 1F). Taken together, during the osteogystical differentiation process, the CX43 protece increased significantly and the differentiated IDG-SW3 lulas expressed more than CX43 that the leisy lines Ocy454. (A) Representative images of the expression of DMP1-GFP in Ocy454 (left panels) and IDG-SW3 lulas (right panels) under fluorescent microscopy and phase contrast during the temporal course from immortalization (day 0) until the osteogystical differentiation (day 15). Scale bar, 50 ã®â¼m. (B) Immunofluorescence tincion of CX43 in CÃ © Lulas Ocy454 (left panels) and IDG-SW3 (right panels) in immortalizing conditions (DVA 0) and osteogyneic osteogic differentiation (day 15). The numbers were tied with dapi (blue). Scale bar, 10 ã®â¼m. (C) The membrane extracts were prepared from the Ocy454 and IDG-SW3 cells and underwent immunotransference using CX43 (CT) or ã®â²-actin antibodies. (D) Densitometry Medication of CX43 phosphorylated (P-CX43) A Total CX43 (T-CX43). (E) Soluble protece extract was isolated from the Ocy454 and IDG-SW3 cells and underwent immunotransference using GFP or GAPDH antibodies. (F) GFP densitometry medicine ratios to GAPDH. The data shown are half â ± sem. **, p

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