

<u>Sup Fig. 1.</u> : X-Gal staining of  $Tshz3^{+/lacZ}$  adult diaphragm. Newly formed muscle fibers are stained, probably because of the persistence of  $\beta$ -Galactosidase activity (arrowhead in A').



Sup Fig. 2. (A-B) *Tshz3-lacZ* expression in skeletal muscle of E18.5 *Tshz3<sup>+/lacZ</sup>* embryos. X-Gal staining was detected in all the trunk and limb muscles. (A', A'') correspond to the boxed region in A, showing respectively deltoid and intercostal muscles. (B) Sagital section showing β-Gal positive cells associated with myofibers (white arrows in B). Nuclear X-Gal staining was also detected along the nerve (black arrows in A'', ie: Schawnn cell) and in muscle spindles (black arrowhead in A''). (C-K) Confocal optical views of transverse sections through Adductor Magnus muscle from *Tshz3<sup>+/lacZ</sup>* (F-H) and *Tshz3<sup>lacZ/lacZ</sup>*. (I-K) Sections of E18.5 embryos examined for expression of TSHZ3/PAX7 (C-E) or β-Gal/PAX7 (F-K). All PAX7+ cells expressed TSHZ3 or β-Gal (white arrow). (L-M) Quantitative analysis of the PAX7+ skeletal muscular progenitors of the Adductor Magnus (L) and intercostal muscles (M) in WT and *Tshz3<sup>lacZ/lacZ</sup>* E18.5 embryos. Quantification revealed no significant difference between both genotypes.



<u>Sup Fig. 3.</u> Counting details of SCs and their progeny isolated from myofibers cultures. Identification of TSHZ3 expressing cells in satellites cells (SCs) and their progeny in culture obtained from adult myofibers. Myofibers were cultured for five days and then used for immustaining. The following antibody combinations were used for triple-immunolabeling: TSHZ3/PAX7/MYOD (A), TSHZ3/MYOD/MYOG (B). The nuclei were counter-stained using DAPI. The number of cells expressing only one marker or a combination of markers was counted. Cells that were not identified by antibodies (noted as any) are most likely non myogenic. Values were calculated based on the total number of DAPI-stained nuclei. The mean percentage of cells of each category is indicated and was evaluated from five independent experiments. Noticed in A, the high proportion of the TSHZ3+ cells in the satellite compartment; defined as PAX7+ and PAX7/MYOD+ cells. 19% of TSHZ3+ cells expressed MYOD but not PAX7, indicating that TSHZ3 is also expressed in differentiating myoblasts. The expression of MYOG in activated SCs trigger final and irreversible differentiation. As indicated in B, 25.6 % of the cells coexpressed TSHZ3, MOYD and MYOG suggesting that TSHZ3 is expressed in cells committed for myogenic differentiation.



Sup Fig. 4. (A) Expression of Tshz3, Pax7, BAF57 and Myog in C2C12 myoblasts was examined by RT-qPCR. Analysis of endogenous mRNA levels in growth medium plated for 24 h and harvested or switched to differentiation medium and harvested after 24 hours, 72 hours and 120 hours. RT-qPCR analysis revealed a gradual reduction of Tshz3 and BAF57 expression level during myogenic differentiation. (B) BAF57 expression in proliferating C2C12 as shown by immunolabelling for BAF57 and TSHZ3. (C) Evaluation of the population doubling time of transfected C2C12. Cells were plated and maintained in proliferation medium. 24h, 48h and 72h after transfection cells were counted and population doubling time was calculated as (h1-h0)\*ln(2)/ln(c1/c0) with c (number of cell/dish) and h (time in hour).. (D-I) The expression level of Tshz3 and BAF57 was tested in overexpression and knock-down conditions by RT-qPCR (D, E, G, H) and Western blot (F,I). (D, G) C2C12 cells were cotransfected with pcDNA3 HA tagged Tshz3 or pcDNA3 (D) and pSG5 FLAG-tagged BAF57 or pBluescript (G) RNA were purified 24h and 48h after transfection and assay for qRT-PCR. (E, H) C2C12 cells were co-transfected with pcDNA3 HA-tagged *Tshz3* and control siRNA or si*Tshz3* (E); pSG5 FLAG-tagged BAF57 and control siRNA or siBAF57 (H). qRT-PCR indicated that the expression level of BAF57 or Tshz3 mRNAs was drastically reduced by transfecting siRNA. (F, I). Immunoblotting showing strong reduction of TSHZ3 (F) and BAF57 (I) proteins level by siRNAs. TSHZ3 and BAF57 tagged-proteins were detected with anti-HA (HA) and anti-FLAG (FLAG) antibodies respectively. ß-actin was used as control.