Flow cytometry

Bone marrow cell populations. They were first defined by SSC-H/CD45 scatter analysis (Supplementary Figure 6F) (granulocytes, monocytes, lymphocytes, early mixed B-progenitors, Red Blood Cell precursors and blasts) followed by specific cell staining for granulocytes (CD11b^{high}Gr1^{high}), myeloid progenitor + monocytes (CD11b^{low}Gr1^{low}), NK cells (CD11b^{low}Gr1+), immature myeloid cells (CD11b^{low}Gr1^{high}) and macrophages (F4/80^{+} CD11^{b}). In T cells lymphocyte T-CD4 (TcR $^{+}$CD4^{+}), lymphocyte T-CD8 (TcR $^{+}$CD8^{+}) and NK-T (TcR $^{+}$CD4^{-}CD8^{-}) were evaluated, and within the B-cell lineage mature lymphocyte B (IgM^{+}B220^{high}), immature B cells (IgM^{+}B220^{low}) and pre/pro-B (IgM^{-}B220^{low}) cells. In the more immature compartment global hematopoietic progenitors (CD34^{+}) were analyzed, as well as the Lin^{-}Sca1^{+}c-kit^{+} (LSK) progenitor-enriched population, essentially as previously described [1].

Lymphohematopoietic cell populations. Sp and LN B cells (B220^{+}) and T cells (CD4^{+} and CD8^{+}) were analyzed, as well their activation status by co-expression of CD69 (early marker) and CD25 (late activation marker). Finally, several cell subsets associate to macrophages/monocytes and granuloctyes (F4-80^{high}/CD11b^{+}, F4-80^{low}/CD11b^{+} and F4-80^{-}/CD11b^{+}) were monitored. In the thymus CD4^{+}, CD8^{+}, CD4^{+}CD8^{+} (double positive; DP) and CD4^{-}CD8^{-} (double negative; DN) populations were firstly analyzed. Within the DN population several subsets were evaluated (DN1: CD44^{+}CD25^{-}; DN2: CD44^{+}CD25^{+}; DN3: CD44^{-}CD25+ and DN4: CD44^{-}CD25^{-}); all thymic populations were also evaluated for the level of expression of CD5, both for positively- (TcR ^{hi}CD69^{+}) and negatively-selected (CD5^{+}CD69^{+}Casp3^{+}) thymocytes.

For caspase3 intracellular detection, 1x10^{6} cells were previously stained for superficial cell markers with specific antibodies (supplementary information 2) during 15 minutes at 4ºC in darkness, washed and then fixed/permeabilizated using CytoFix/CytoPerm solution (BD Biosciences, ref: 554714) according to the manufacturer's instructions. Anti-caspase3 antibody (Supplementary Table 1) was diluted in PermWash solution and incubated during 40 minutes at room temperature and then washed in PermWash. Cells were resuspended in PBS and analyzed in the FACSAriaIII cytometer (BD Biosciences) from the Cytometry and Fluorescence Microscopy Centre of the Complutense University of Madrid. Analyses were performed with FlowLogic software (Inivai Technologies).

Thymic epithelial cell populations. TEC subsets were analyzed from digested thymuses treated with 1U/mL of Liberase TM (Roche, ref: 05401127001) with 0.1mg/mL of DNAse I (Roche, ref: 11284932001) as previously described [2]. Cells ($1x10^6$) were resuspended in FACS buffer (PBS1x + 1%FBS + 10 mM EDTA) and stained for 15 minutes at 4 °C with specific primary antibodies or UEA-1/biotin (supplementary information 2). Cells were washed in PBS and for UEA-1 (Ulex Europaeus Agglutinin Lectin 1) detection samples were incubated with $\text{Streptavidin}^{\text{PERCP}}$ (BD Biosciences) for 15 minutes at 4ºC. Before analysis, cell suspensions were washed in PBS and resuspended in FACS buffer and analysed in a FACSAriaIII (BD Biosciences) from the Cytometry and Fluorescence Microscopy Centre of the Complutense University of Madrid. Analyses were performed with FlowLogic software (Inivai Technologies). TECs were analyzed in the total $\text{EpCAM}^{+}\text{CD45}^{-}$ epithelial cell population defining the following subsets: total cortical (c) TECs ($\text{Ly51}^{+}\text{UEA-1}^{-}$), cTEC^{lo} ($\text{UEA-1}^{-}\text{MHCII}^{\text{lo}}$), cTEC^{hi} ($\text{UEA-1}^{-}\text{MHCII}^{\text{hi}}$), total medullary (m) TECs ($\text{Ly51}^{-}\text{UEA-1}^{+}$), mTEC^{lo} ($\text{UEA-1}^{+}\text{MHCII}^{\text{lo}}$) and mTEC^{hi} ($\text{UEA-1}^{+}\text{MHCII}^{\text{hi}}$).

Immunofluorescence analysis

Small intestine samples. Tissues of Tx-induced (d5 and d7) adult (8-12 w) $\text{DSerca}^{\text{Bmi1}}$homo mice (or their control *Bmi1*-DTmt mice) were obtained, washed with PBS, included in OCT Tissue Tek (Sakura, ref: 4583) and sectioned at 10 µm. Slides were fixed with 4% paraformaldehyde and treated with PBS, 0,1% Triton-X-100 for 15 minutes to permeabilize. The slides were then treated with blocking buffer (PBS- 5%BSA) and incubated with the indicated primary antibodies (Supplementary Table 1) overnight at 4ºC. After 3 washes secondary antibodies were added for 1 hour at RT and finally samples were then incubated with DAPI (Sigma-Merck, ref: 32670) at 1:500, during 20 minutes. Finally, samples were washed and mounted with Prolong (Thermo Fisher Scientific, ref: P36930) in a cover glass. When indicated in vivo proliferation was evaluated by Ki-67 staining following the provider instructions (BD Pharmigen; ref 561126). All microscope images were taken with a multispectral confocal system Leica STELLARIS 5, with 4 laser lines, 3 Power HyD S spectral detectors with the objective Leica HC PL APO CS2 20x/0.75 DRY.

Thymus samples. Isolated thymuses were cleaned, embedded in Tissue-Tek OCT compound (Sakura, ref: 4583) and frozen in liquid nitrogen. 8μm-thick thymic cryosections were fixed in acetone for 10 minutes at room temperature and air dried. Samples were stained with anti-keratin 5, anti-Pan Cytokeratin and anti-Aire (supplementary information 2) for 1 hour at room temperature. Then, they were washed 3 times in cold PBS 1x and incubated with secondary antibody; goat anti-rabbit IgG (supplementary information 2) for 45 minutes at room temperature. Sections were washed in cold PBS three times for 5 minutes and mounted with Prolong Gold Antifade Mountant (Thermo Fisher Scientific, ref: P36930). Image acquisition was performed in a Leica SP8 confocal microscope equipped with a HC PL APO CS2 20x/0.75 DRY objective and analysed using LasX software from the Cytometry and Fluorescence Microscopy Centre of the Complutense University of Madrid. The number of Aire$^+$ cells was counted and related to the K5$^+$ medullary area in pixels^2 (Aire$^+$ cells/K5$^+$ area) using three non-overlapping sections of two different thymuses of both control and DSerca2^{Bmi1} mice. Semi-quantitative analyses were carried out using Image J software.

Supplementary information References

Luo C, Wang L, Wu G, Huang X, Zhang Y, Ma Y, Xie M, Sun Y, Huang Y, Huang Z, Song Q, Li H, Hou Y, Li X, Xu S, Chen J. Comparison of the efficacy of hematopoietic stem cell mobilization regimens: a systematic review and network meta-analysis of preclinical studies. *Stem Cell Res Ther* 2021; **12**:310. [PMID: 34051862 DOI: 10.1186/s13287-021-02379-6]

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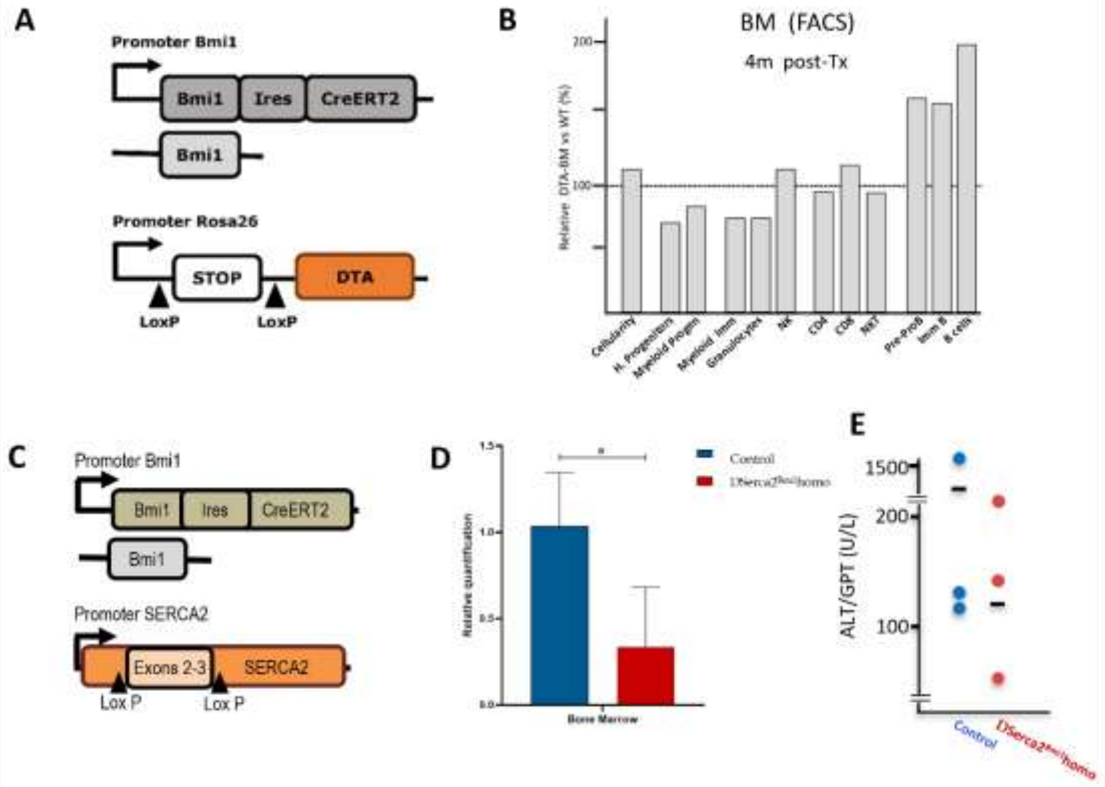
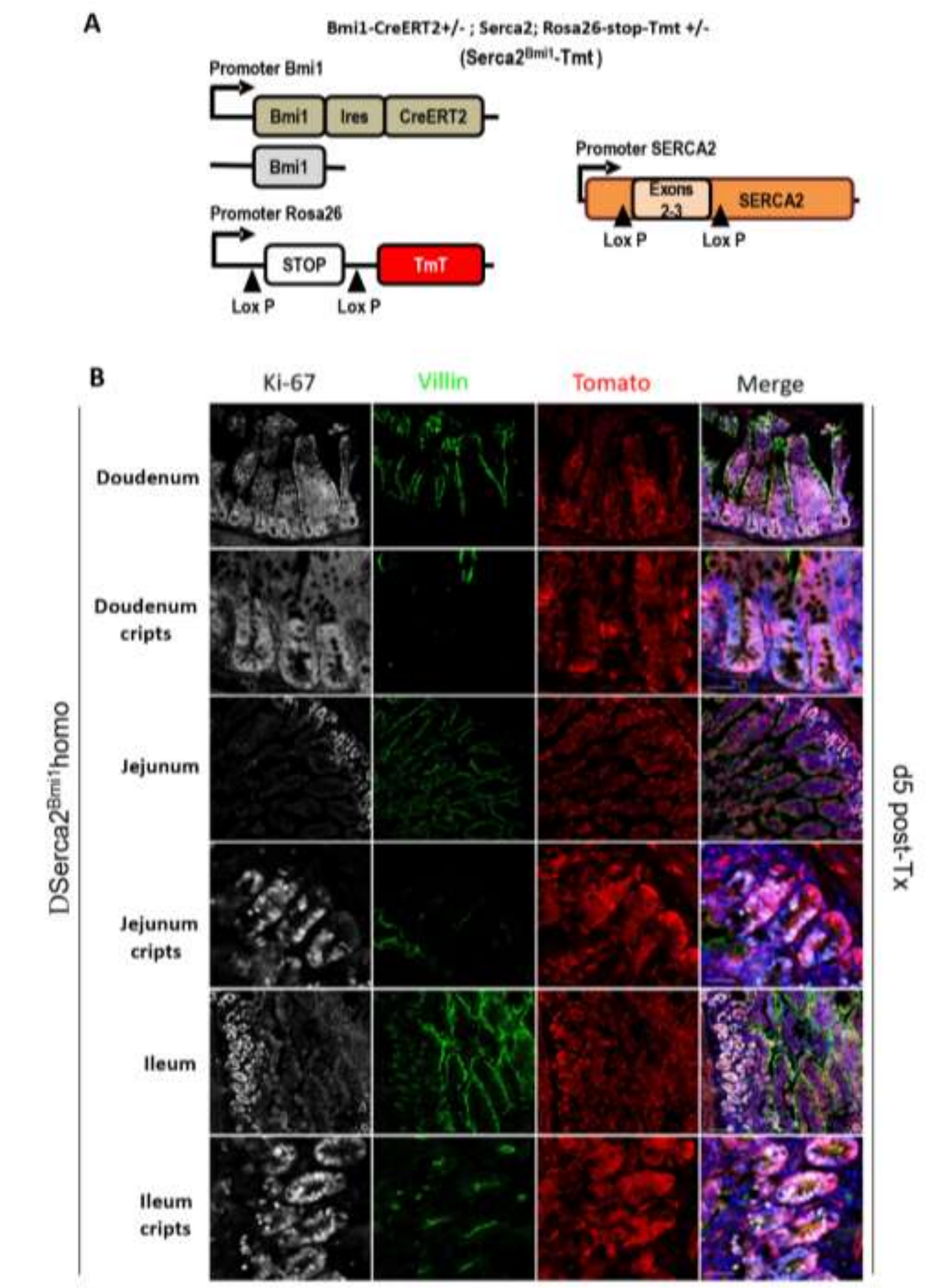
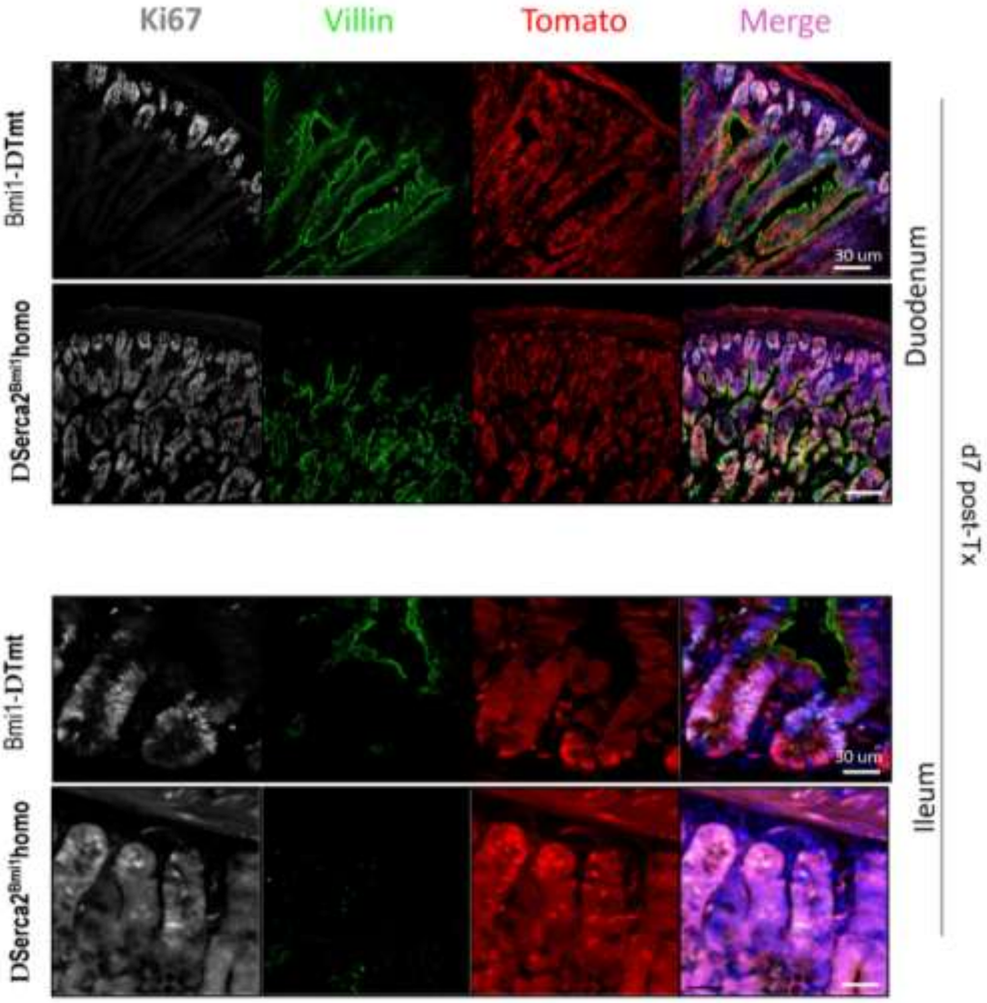
Stimulation. *Front Immunol* 2018; **9**:1020; eCollection 2018. [PMID: 29867988 DOI: 10.3389/fimmu.2018.01020]



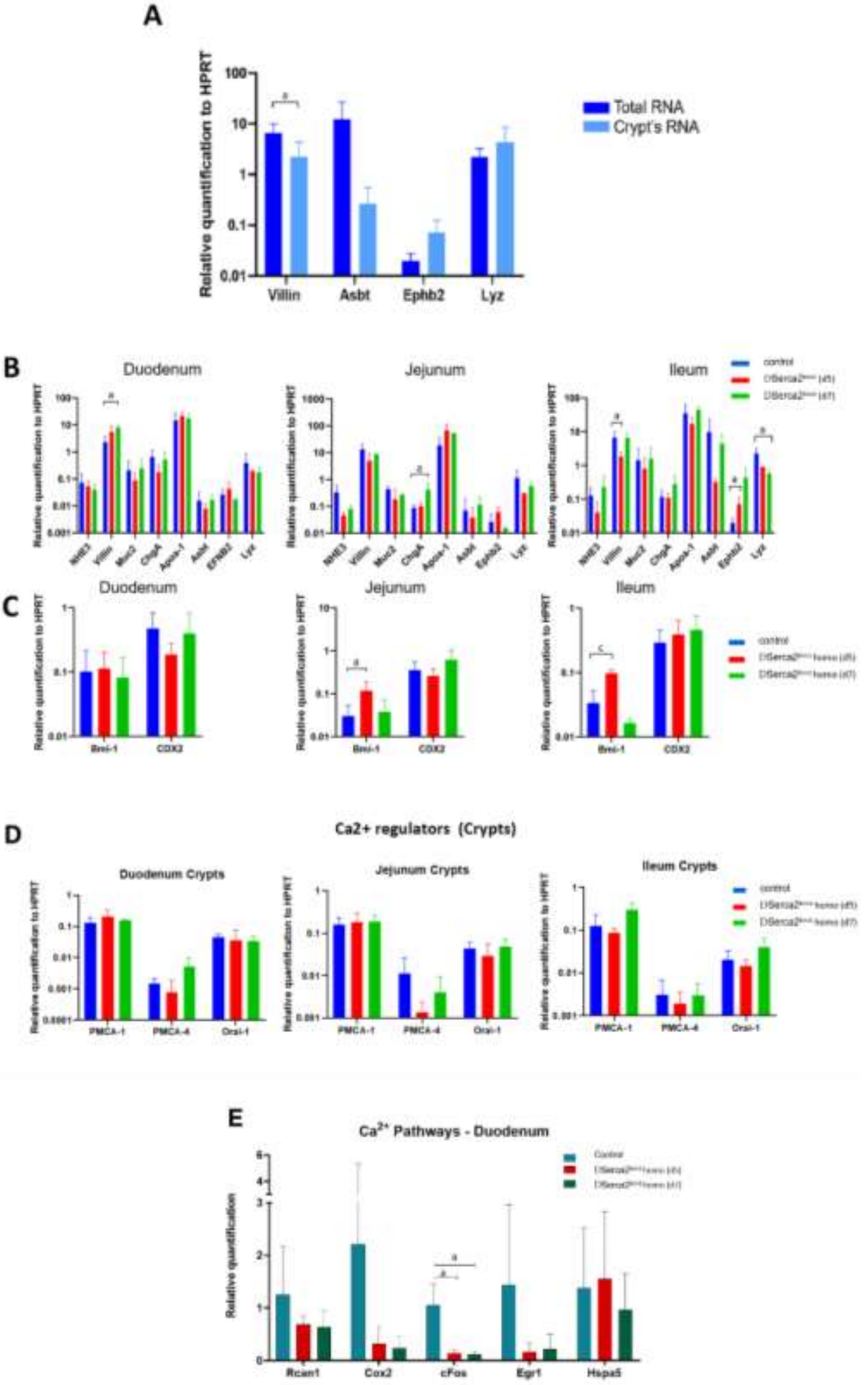
Supplementary Figure 1 Impact of *Bmi1*+ population and *Serca2* conditional depletion in bone marrow populations. A: Scheme illustrating the structure of the *Bmi1*-creER/ floxed-STOP-DTA model; B**:** Recovery of different bone marrow population 4 months post-Tx induction (initial 95% depletion of *Bmi1*+ cells), evaluated by flow cytometry; C: Scheme illustrating the structure of the *Bmi1*-creER/ floxed *Serca2* model; D**:** Relative BM cellularity in DSerca2$^{\mathrm{Bmi1}}$homo or corn oil-treated control mice (n=4, each), 10 days after Tx-induction; E**:** Comparative biochemical data of ALT/GPT in DSerca2$^{\mathrm{Bmi1}}$ (d10 post-Tx) serum and controls; (n=3, each). Data are expressed as mean ± SD; ^{a}P<0.05.



Supplementary Figure 2 Evaluation of the functional consequences of the conditional *Serca2* deletion in the *Bmi1*+ stem cell population in the different segments of the small intestine. A: Scheme illustrating the different modules, conforming the transgenic DSerca2$^{\text{Bmi1}}$homo and the control strains *Bmi1*-DTmt. DSerca2$^{\text{Bmi1}}$homo mice, upon Tx-induction deleted exons 2-3 of *Serca2* in the adult Bmi1+ lineages, with the simultaneous induction of Tomato reporter expression (Tmt); the *Bmi1*-DTmt control mice, upon Tx-induction, express Tmt in the Bmi1+ cells; B**:** Sections of the indicated segments were prepared from Tx-induce (d5) DSerca2^{Bmi1}homo mice. Samples from the different small intestine segments (duodenum; jejunum and ileum; whole tissue and crypts) were analyzed, after staining for KI-67, Villin and counterstained with DAPI (n=4; each). Scale bars: 100 µm. Scale bars in crypts: 30 µm.

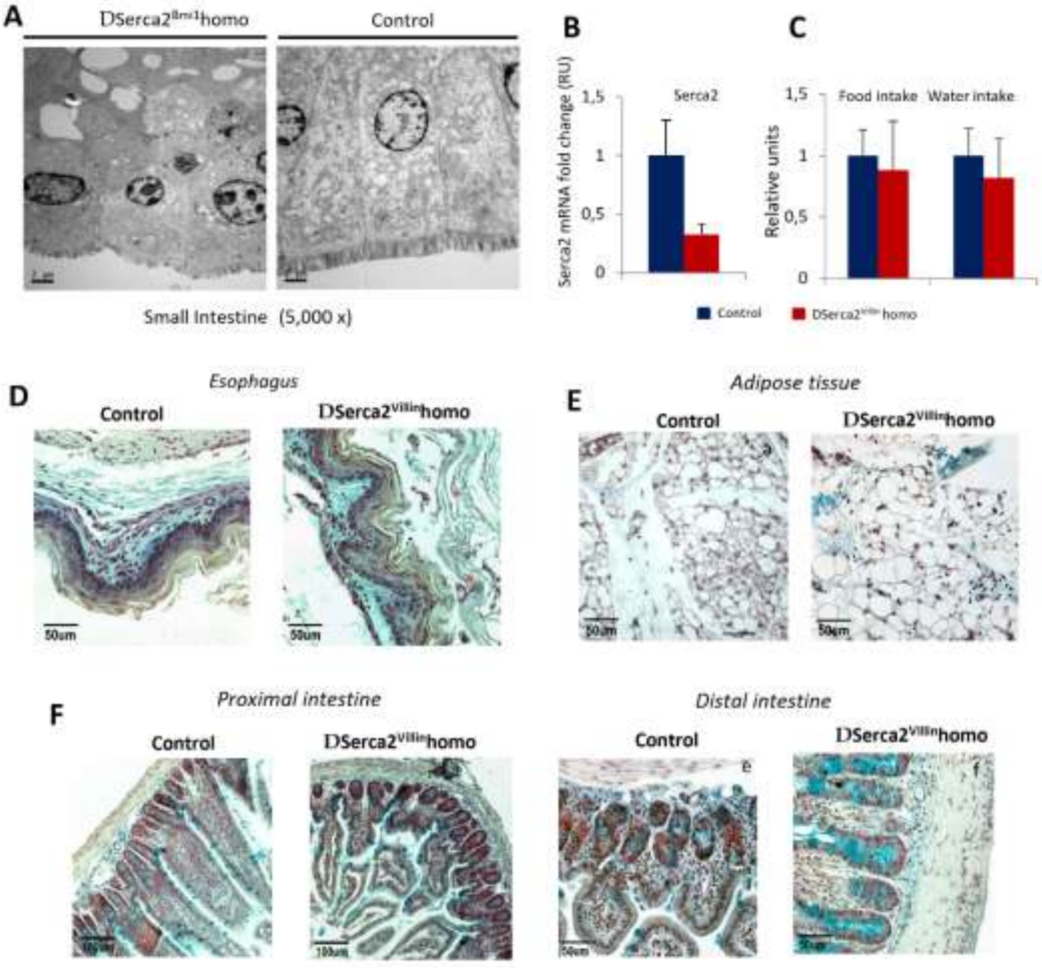


Supplementary Figure 3 Evaluation of the functional consequences of the conditional *Serca2* deletion in the *Bmi1*+ stem cell population in the different segments (Duodenum and Ileum) of the small intestine, 7 days (d7) post-Tx induction. Samples (crypts) were obtained and prepared as indicated in Figure 2, but from *Bmi1*-DTmt (controls) and DSerca2^{Bmi1}homo mice (n=3; each). Scale bars: 100 µm. Scale bars in crypts: 30 µm.

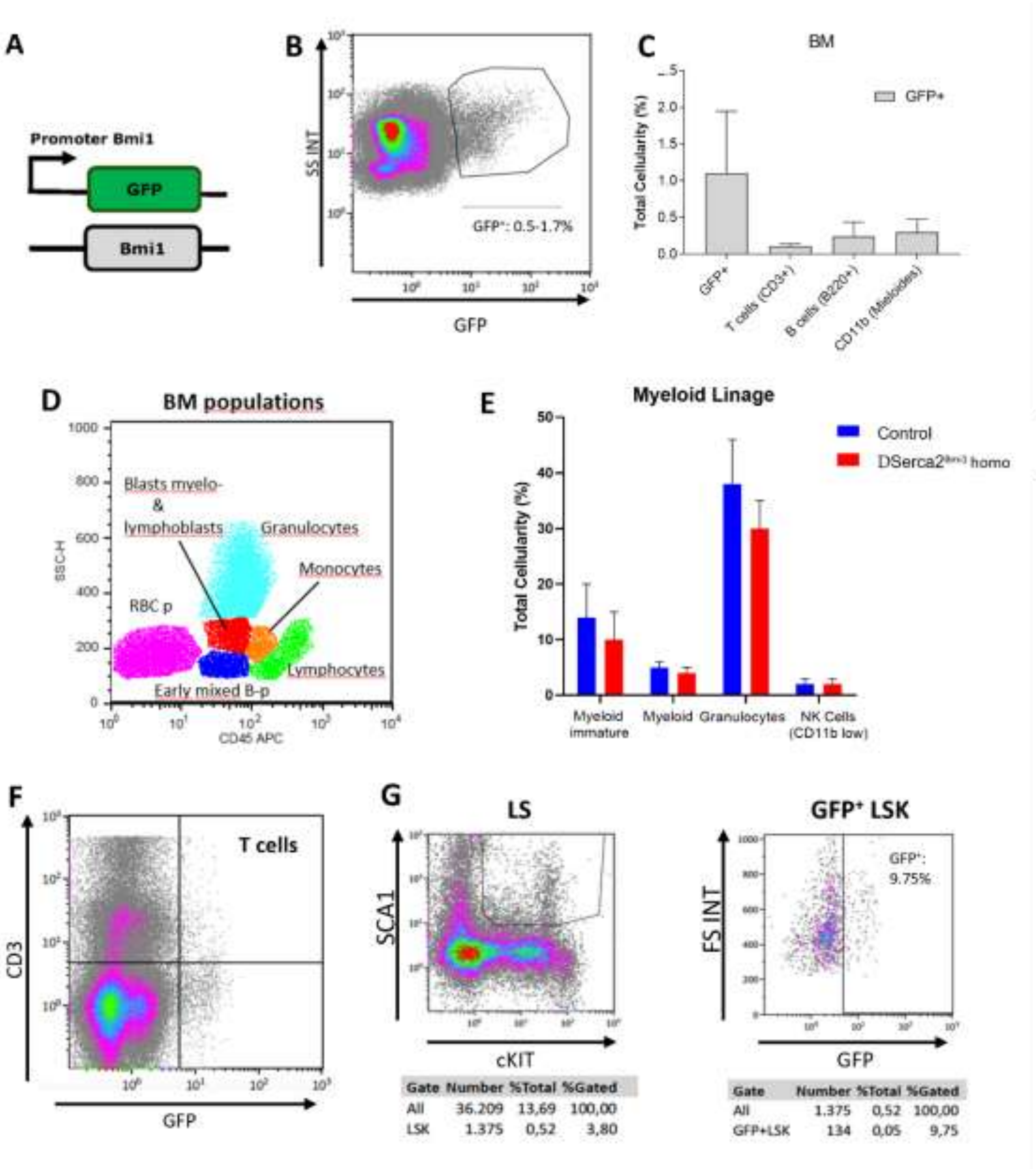


Supplementary Figure 4 Differential mRNA expression in the small intestine after the conditional deletion of *Serca2* in the *Bmi1*$^+$ stem cell compartment. A: Validation by RT-qPCR of the crypt-enrichment procedure, relative to *Hprt*; B,C**:**

Expression analysis (RT-qPCR) in total tissue of a panel of functional relevant genes in small intestine regulation; D: Expression analysis (RT-qPCR) of a panel of functional relevant genes involved in regulation of Ca2+ homeostasis in crypt-enriched fractions; E: Expression analysis (RT-qPCR) of a panel of downstream target genes involved in Ca2+ signaling pathways in duodenum; B-E: Samples were obtained in parallel from controls (*Bmi1*-DTmt) and DSerca2^{Bmi1}homo(d5 and d7 post-Tx) and normalized to *Hprt* expression (Control, n=5; DSerca2^{Bmi1}homo d5, n= 3; and DSerca2^{Bmi1}homo d7, n=3). Assays were performed three times and data are expressed as mean ± SD; Statistical significance was determined using unpaired t-tests and the Bonferroni-Dunn method with alpha = 0.05; ^{a}P<0.05; ^{c}P<0.001.



Supplementary Figure 5 Electron microscopy analysis of small intestine after conditional deletion of Serca2 in the *Bmi1*+ stem cell compartment. A**:** Samples were obtained and processed in parallel for DSerca$^{\mathrm{Bmi1}}$homo and control *Bmi1*-DTmt mice (d5 post-Tx; (n=4 each; independent animals). Representative images of DSerca$^{\mathrm{Bmi1}}$homo and control samples, at 5,000x; B**:** Confirmation of *Serca2* expression reduction in small intestine after Tx-induction (d3) of DSerca2$^{\mathrm{Villin}}$homo compared with corn-oil (control) treated mice (n= 4 each); C**:**. Relative monitorization of food- and water intake in DSerca2$^{\mathrm{Villin}}$homo mice (d3 post-induction) compared with corn-oil (control) treated mice (n= 4 each); D-F**:**. Comparative histology of different segments of the gut of DSerca2$^{\mathrm{Villin}}$homo mice (d3 after Tx-induction) compared with corn oil-treated (control) mice. Representative images correspond to esophagus (D), adipose tissue (E) and proximal and distal intestine (F) in DSerca2$^{\mathrm{Villin}}$homo samples compared with controls (n=3 each; several sections were evaluated for each mouse). Data are expressed as mean ± SD; non statistically differences were found (one-way analysis of variance followed by the Bonferroni correction for multiple comparison).

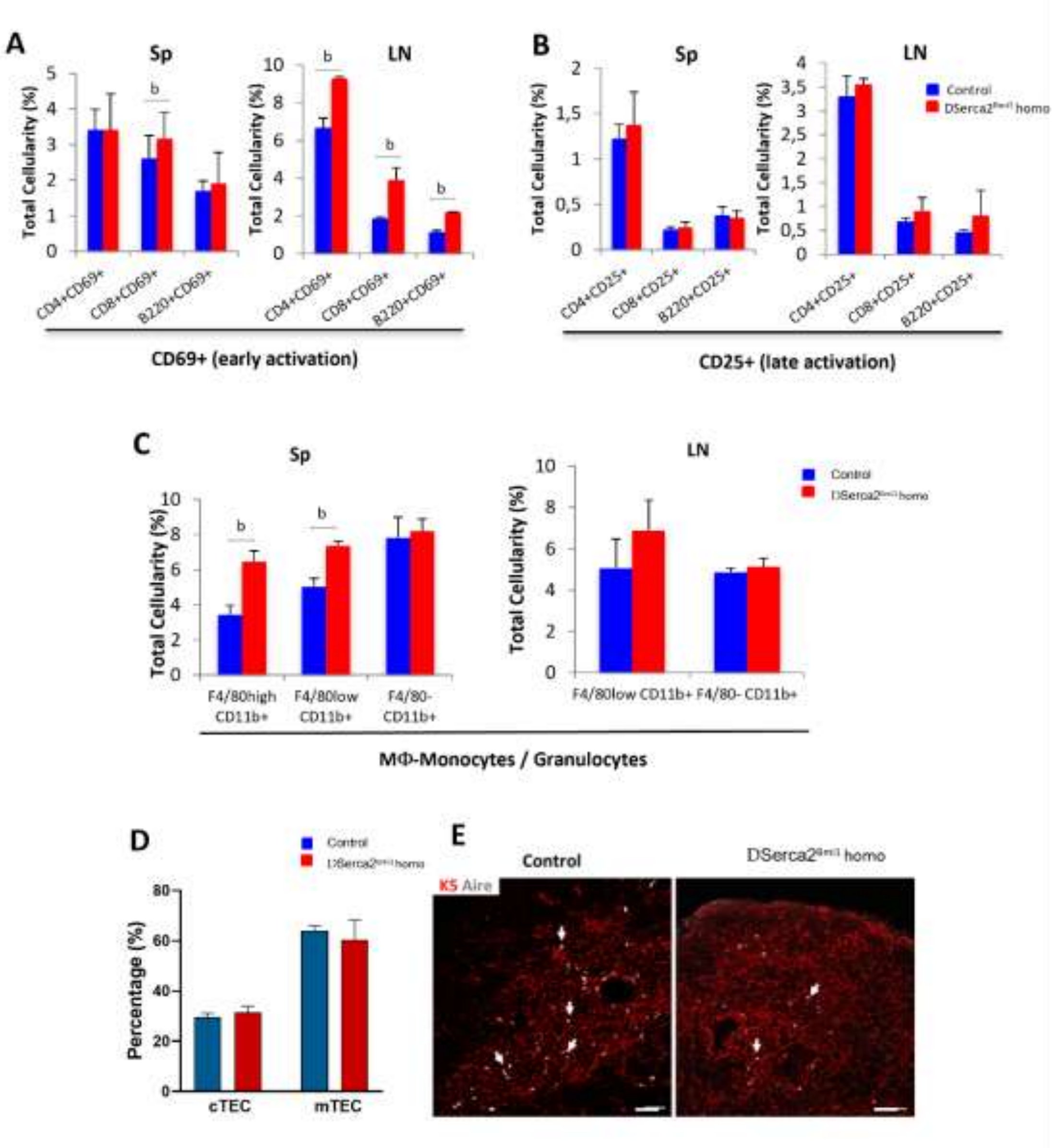


Gate	Number	%Total	%Gated
All	36.209	13,69	100,00
LSK	1.375	0,52	3,80

Gate	Number	%Total	%Gated
All	1.375	0,52	100,00
GFP+LSK	134	0,05	9,75

Supplementary Figure 6 Evaluation of the impact of Ser5ca2 deletion in the *Bmi1*+ compartment of in bone marrow. A**:** Scheme illustrating the structure of the *Bmi1*-GFP$^{+/-}$ mouse strain [29]***;*** B-D**:** Evaluation by flow cytometry of the GFP+ population (*Bmi1*-GFP$^{+/-}$) in total bone marrow (B) and in different bone marrow (*Bmi1*-GFP$^{+/-}$) subpopulations (C); Assays were performed three times and data are expressed as mean ± SD; D,E**:** Comparison by cytometry of homozygote Tx-induced (d10) DSerca2^{Bmi1}homo or corn oil-treated control mice. Bone marrow populations defined by SSC-H/CD45 scatter analysis (D) and evaluation by cytometry of the myeloid compartment (F), (n= 3; each); (F) Representative plot of GFP/CD3 population in *Bmi1*-GFP$^{+/-}$ mice; (G).

Evaluation of the LSK population in *Bmi1*-GFP$^{+/-}$ mice and the subpopulation of LSK+GFP+ (n=2; each).



Supplementary Figure 7 Evaluation of the putative alterations in several lymphohematopoietic populations as the consequence of the conditional deletion of *Serca2*. A-C: Spleen (Sp) and lymph nodes (LN) samples were obtained from DSerca2$^{\text{Bmi1}}$homo (d10 post-Tx) or corn oil-treated control mice. The indicated subpopulations were evaluated by flow cytometry for expression of the CD69 (A) and CD25 (B) activation markers (n=3; each); C: Analysis of macrophages / monocyes / granulocytes populations (n=3 each); D: Comparative percental representation of cortical and medular (c- and m-, respectively) thymic epithelial cells (TEC), both in DSerca2^{Bmi1}homo (d10 post-Tx) or corn oil-treated control mice (n=3, controls; n=2, DSerca2^{Bmi1}homo; E: Representative immunohistochemistry for the detection of Aire^{+} cells respect to K5^{+} area in thymic sections of DSerca2^{Bmi1}homo thymuses compared with controls (n=2 each). Scale bars: 50 mm. Data are expressed as mean ± SD; $^{a}P<0.05$; $^{b}P<0.02$; $^{c}P<0.002$.

Supplementary Table 1 Antibodies used in flow cytometry and immunofluorescence analyses

Antibody	Primary	Secondary / Fluorochrome
anti-Ki-67 (m)	Anti-human Ki-67 antibody BD Pharmingen (ref 561126)	Alexa Fluor 647 (conj)
anti-Villin (rab)	Polyclonal anti-Villin antibody Abcam (ref SP145) Invitrogen Molecular Probes (ref A-11034)	Goat-anti rabbit IgG AlexaFluor 488 abcam (ab150077)
anti-Gr1 (r)	Anti-Mouse Ly-6G/Ly-6C-BIOT SouthernBiotech (ref 1900-08)	APC/Cyanine7-Streptavidin BioLegend (ref 405208)
anti-B220 (r)	Anti-Mouse CD45R/B220-BIOT SouthernBiotech (ref 1665-08)	APC/Cyanine7 Streptavidin BioLegend (ref 405208)
anti-Ter119 (m)	Anti-Mouse TER-119 monoclonal antibody-BIOT ThermoFisher (ref 13-5921-82)	APC/Cyanine7 Streptavidin BioLegend (ref 405208) Invitrogene (Pacific Blue: 10328392)
anti-Sca1 (m)	Anti-Mouse Ly-6A/E (Sca-1) monoclonal antibody eBioscience (ref 17-5981-83)	APC (conj)

anti-c-Kit (r)	Anti-c-Kit monoclonal antibody Abcam (ref ab95676)	PE/Cy7 (conj)
anti-B220 (CD45R) (r)	Anti-CD45R (B220) (human/mouse) eBioscience (48-0452-82)	eFluor 450 (conj)
anti-F4/80 (m)	F4/80 Monoclonal antibody-APC eBioscience (ref 17-4801-82)	APC (conj)
anti-TCRβ (ham)	Anti-Mouse Vβ 8.3 T-Cell Receptor Antibody BD Pharmingen (ref 553664) BioLegend (ref 109207)	FITC PE (conj)
anti-CD3 (r)	Anti-mouse CD3 antibody eBioscience (ref 13-0032-82) BioLegend (ref 405208)	Biotin (conj) APC/Cyanine7-Streptavidin
anti-CD4 (r)	Anti-Mouse CD4 antibody SouthernBiotech (ref 1540-08) BioLegend (ref 100511)	APC/Cyanine7-Streptavidin BioLegend (ref 405208) PerCP
anti-CD5 (r)	Anti-mouse CD5 antibody BioLegend (ref 100602)	Biotin (conj)

anti-CD8 (m)	Anti-feline CD8 antibody SouthernBiotech (ref 8120-08) BioLegend (ref 344702)	BioLegend (Pacific Blue ; ref 405208) Invitrogene (Pacific Blue; ref 10328392)
anti-CD11b (r)	Anti-mouse CD11b antibody BioLegend (ref 101215) BD Pharmingen (ref 553309)	Biotin (conj) PE/Cyanine7 APC/Cyanine7 Streptavidin
anti-CD25 (r)	Anti-mouse monoclonal CD25 antibody ThermoFisher (AB_467177)	Biotin (conj) APC/Cyanine7 Streptavidin
anti-CD34 (r)	Anti-mouse monoclonal CD34 antibody eBioscience (ref 11-0341-85)	FITC (ocnj)
anti-CD44 (r)	Anti-mouse CD44 antibody eBioscience (ref AB_467246)	Biotin (conj) APC/Cyanine7 Streptavidin
anti-CD45 (r)	Anti-CD45 mouse antibody BioLegend (ref 103101)	APC/Cyanine7 Streptavidin
anti-CD69 (ham)	Anti-CD469 human antibody BioLegend (ref 104502)	APC

anti-EpCAM (r) CD326	Anti-CD326 human antibody BioLegend (ref 324201)	AlexaFluor488
anti-cleaved caspase 3 (rab)	Anti-mouse casp3 antibody Cell Signalling Technology (ref 9661)	AlexaFluor488
anti-Annexin V (m)	Anti-Annexin mouse antibody BioLegend (ref 640905)	FITC (conj)
anti-Ly51 (r)	Anti-Ly51 mouse antibody BioLegend (ref 108302)	AlexaFluor647 (conj) BioLegend (110720)
anti-MHCII (r)	Anti-MHCII mouse monoclonal antibody Biolegend (ref 107601)	Invitrogene (Pacific Blue 10328392)
UEA-1	Ulex Europaeus Agglutinin I Vector Labs (ref B-1065-2)	Biotin (conj)
Streptavidin (SAV)	BD Bioscience (ref 563262)	PECy7

Immunofluorescence

Antibody	Primary	Secondary
anti-PancK (m)	Anti-PancK human monoclonal antibody Sigma-Aldrich (ref C2931)	FITC

anti-Keratin 5 (rab)	Covance (polyclonal)	Goat-anti rabbit IgG AlexaFluor 488 Invitrogen Molecular Probes (ref A-11034)
anti-AIRE (r)	Anti-Aire peptide mouse antibody BD Bioscience (ref 567253)	AlexaFluor647 (conj)
anti-Rabbit IgG (g)	ThermoFisher Scientific (ref A-31556)	AlexaFluor405

Supplementary Table 2 Primers used in RT-qPCR analyses (related to results and experimental procedures)

Gene	Forward	Reverse
Abcg5	5´-TTGCGATACACAGCGATGCT-3´	5´-TGACTGCCTCTACCTTGTTGTTGT-3´
Abcg8	5´-CCGTCGTCAGATTTCCAATGA-3´	5´-GGCTTCCGACCCATGAATG-3´
Atf4	5´-CCTTCGACCAGTCGGGTTTG-3´	5´-CTGTCCCGGAAAAGGCATCC-3´
Apoa-1	5′-GGACTTCTGGGATAACCT-3′	5′-GCACCTTCTGTTTCACTT-3′
Asbt	5′-TTGCCTCTTCGTCTACACC-3′	5′-CCAAAGGAAACAGGAATAACAAG-3′
Bcl2	5´-GTACCTGAACCGGCATCTG-3´	5´-GGGGCCATATAGTTCCACAA-3´
Bmi1	5´-CGCCCGCTCAGATCGCCTC-3´	5´-ACCCTCCACACAGGACACACATT-3´
Cdx2	5´-CGAGCCCTTGAGTCCTGTGA-3´	5´-AACCCCAGGGACAGAACCA-3´
Chga	5′-CACAGCCACCAATACC-3′	5′-TCTTCCTCCTCCTCTTC-3´
Ephb2	5′-TCATCGCTGTGGTCGTCATTG-3′	5′-GTCCGCTGGTGTAGTGTTGTAG-3′

Fatp4	5´-CCAGTAGTGTGGCCAACTTCCT-3´	5´-CCACAGACCCACAAACTCATTG-3´
Gapdh	5´-TTGATGGCAACAATCTCCAC-3´	5´-CGTCCCGTAGACAAAATGGT-3´
Hprt	5´-AAGCTTGCTGGTGAAAAGGA-3´	5´-TTGCGCTCATCTTAGGCTTT-3´
Lyz1	5′-ACTCCTCCTTGCTTTCTGTC-3′	5′-GTCGGTGCTTCGGTCTC-3′
Muc2	5′-AACTACCACTGTGATGCCAATG-3′	5′-ACAATGTTGATGCCAGACTCG-3′
Nhe3	5´-GGAACAGAGGCGGAGGAGCAT-3´	5´-GAAGTTGTGTGCCAGATTCTC-3´
Orai-1	5´-ATGAGCCTCAACGAGCACT-3´	5´-GTGGGTAGTCATGGTCTG-3´
Pmca-1	5´-CGGAAAATACAGGAGAGCTATGG-3	5´-CTTTCCAAACACTGCTTCTCTTC-3´
Pmca-4	5´-TGTGGGCAGTGAGTGAGAAT-3	5´-TCAGGTTGGGTCATGAAGGT-3´
Pmaip1	5´-GAACGCGCCAGTGAACCCAA-3´	5´-CTTTGTCTCCAATCCTCCGG-3´
Serca2	5´-CACACAAAGACCGTGGAGGA-3´	5´-CTTCTTCAGCCGGCAATTCG-3´
Villin	5´-TCAAAGGCTCTCTCAACATCAC-3´	5´-AGCAGTCACCATCGAAGAAGC-3´