1 **SUPPLEMENTAL MATERIAL**
2
3 **Title: TGFβ promotes low IL10-producing ILC2 with pro-fibrotic ability involved in**
4 **skin fibrosis in systemic sclerosis.**
5
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37 France (ASF).

38 **METHODS**

39

40 **Study population**

41 Patients were included in the context of the VISS (Vasculopathy and Inflammation in
42 Systemic Sclerosis) biomedical research project founded in 2012 and approved by the
43 institutional ethical committee (CPP, 2012-A00081-42, Aquitaine). All participants provided
44 written informed consent before inclusion. All patients satisfied the classification criteria
45 proposed by the American College of Rheumatology (ACR) and the European League
46 Against Rheumatism (EULAR) 2013[1]. Punch biopsy specimens (3-4 mm) of affected mid
47 forearm skin were obtained for some patients. Clinical features are described in Table 1.

48

49 **Clinical features in the study population**

50 Age- and sex-matched healthy donors (HDs) were recruited at the local Blood Transfusion
51 Centre (University Hospital, Bordeaux) for blood tests. For the control skin samples, biopsy
52 specimens were isolated from skin that had been discarded during plastic surgery
53 (brachioplasty). None of the HDs had dermatological disorders or treatment with
54 immunosuppressant agents and/or glucocorticoids. For each patient, a disease- and organ-
55 specific questionnaire was completed by the clinician in charge of the patient and then
56 centralized by investigators. Clinical features (scleroderma form, sex, age at Raynaud's
57 phenomenon (RP) onset, age at onset of the first non-RP manifestation, disease duration, and
58 symptoms of skin, articular, heart, lung, kidney, and gastrointestinal involvement),
59 immunologic test results (antinuclear antibodies, anti-Scl70 antibodies, and anticentromere
60 antibodies), imaging and functional exams (thorax CT scans, respiratory function tests,
61 cardiac ultrasonography and right heart catheterization) and treatments were recorded. For the
62 modified Rodnan skin thickness score (mRSS) and Right Ventricle Systolic Pressure, the

63 highest values from the medical history were registered for each patient. Interstitial lung
64 disease was diagnosed when pulmonary function tests showed a restrictive defect with
65 decreased diffusion capacity (DLCO) associated with several types of lesions on thorax CT
66 scans. Lung fibrosis was diagnosed based on specific lesions observed on thorax CT scans,
67 i.e., honeycomb cysts and reticular septal thickening.
68
69 **Animal model**
70 Six-week-old female BALB/c mice were purchased from Janvier Laboratory (Le Genest Saint
71 Isle, France). Animals received human care in compliance with the European Union
72 guidelines (European Directive 2010/63/UE) and approved by the local ethics
73 committee: *Comité d'éthique pour l'expérimentation animale de Bordeaux* (CE50) [project
74 n°15544]. All mice were housed in ventilated cages with sterile food and water provided *ad*
75 *libitum*.
76 Mice were randomly distributed into experimental and control groups (10 mice/group). SSc
77 was induced according to the protocol described by Kavian *et al.*[2]. A total of 300 µl of
78 HOCl solution was prepared extemporaneously by adding NaClO solution (9.6% active
79 chlorine) to 100 mM of KH_2PO_4 (pH 6.2) and injected intradermally into the shaved backs of
80 the mice (one injection of 150 µl on either side of the tail) using a 27-gauge needle every day
81 for 6 weeks (HOCl-mice). Control mice received injections of 300 µl of sterilized PBS (PBS-
82 mice). The mice were sacrificed at weeks 6 (apex of the fibrotic phase). Fibrosis was assessed
83 by dermal thickness, histopathological analysis and measurement of the hydroxyproline
84 content in the skin[2]. Among the SSc-induced mice, one group received a daily flank
85 injection of recombinant IL10 (500ng/ml, Miltenyi Biotec), one group received daily
86 pirfenidone gavage (300mg/kg, Roche), and one group received both treatments daily.
87

88 **Isolation of ILC**

89 In human experiments, blood samples (5 mL) were incubated with red blood cell lysis buffer
90 (Miltenyi Biotec) for 10 min at room temperature before staining. Skin biopsies from
91 Systemic Sclerosis (SSc) patients and healthy donors were digested with collagenase (5
92 mg/ml, Roche) and liberase (40 µg/mL, Roche) in HBSS at 37°C for 3 hours in a shaker and
93 filtered through a 0.70-µm nylon mesh.

94 ILCs were defined as $CD45^+Lin^-CD127^+$ cells. A cocktail of antibodies against CD3, CD5,
95 CD11c, CD14, CD16, CD19, CD31, CD34, CD56, CD94, CD123, CD303, TCR-αβ-γδ,
96 αSMA and FcεR1 defined lineage. ILC1 were defined as $CD45^+Lin^-CD127^+CD117^-CRTH2^-$
97 cells; ILC2 were defined as $CD45^+Lin^-CD127^+CRTH2^+$ cells; and ILC3 were defined as
98 $CD45^+Lin^-CD127^+CD117^+CRTH2^-$ cells[3]. All the gates were setup using Fluorescence
99 Minus One controls and were then copied/pasted for analysis of the data on FlowJo software.

100 In the mouse experiments, cell suspensions from collected skin was taken from the back
101 region of mice with a 6-mm-diameter punch (3 punches per mouse). Then, 1 ml of Dulbecco's
102 modified Eagle's medium (DMEM) containing 1% nonessential amino acids, 1% L-
103 glutamine, 1% sodium pyruvate, 50 units/ml penicillin, 50 mg/ml streptomycin, and 10% fetal
104 calf serum (cDMEM, GIBCO) was added to each well of 6-well plates, and a mixture of
105 collagenase (12.5 mg/mL, Roche) and liberase (100 µg/mL, Roche) was added to each well.
106 After digestion for 5 hours at 37°C, the remaining tissue was passed through a 70-µm strainer
107 (VWR) and washed with cDMEM. After centrifugation, the pellet was suspended in cold
108 DMEM and passed through a 40-µm strainer. Cells were first gated for small/non-granular
109 (FSC^{low}/SSC^{low}) and live (viability fixable dye positive) leukocytes ($CD45^+$). ILC2s were
110 identified as Lin^-, $CD127^+$ and CD25 $(IL2\text{-}R\alpha)^+$.

111 ILC2 were quantified as the percentage of cells with lymphoid morphology $CD45^+$ Lin^- and
112 $CRTH2^+$ cells. Absolute values of ILC2 (for blood) were calculated as the % of ILC2 *

113 absolute lymphocyte count (G/L) / 100. Cells were analyzed using a Fortessa flow cytometer
114 with FACSDiva (BD), and the data analysis was performed with FlowJo 10.1.
115

116 **Purification, expansion and activation of ILC2**

117 Peripheral blood mononuclear cells (PBMCs) were isolated from HD blood (buffy coat
118 concentrate) by Ficoll. After NK enrichment (NK cell isolation kit, Miltenyi Biotec), ILC2
119 were sorted using ARIA FACS (BD). Once purified, the cells were cultured in NK MACS
120 medium (Miltenyi) with 50 units/ml penicillin, 50 mg/ml streptomycin, and 5% human serum
121 in a 96-well round-bottom plate, with 200µl of medium/well, in the presence of IL1β (R&D
122 Systems) and IL2 (Miltenyi Biotec), both at a concentration of 10 ng/ml, as previously
123 described[4]. The cells were duplicated every couple of days by dividing one well in two
124 wells and adding 100µl of fresh medium with cytokines. The ILC2 were expanded for
125 approximately 20 days and the purity was evaluated by cytofluorimetric analysis. This
126 phenotype demonstrated a purity > 95% of viable ILC2 cells (online supplemental figure 3B).
127 Then, ILC2 were harvested, and extensively washed. 50 000 ILC2 were activated for 48
128 hours with either IL33 (Miltenyi Biotec), TGFβ (R&D Systems), IL4 (Miltenyi Biotec), TSLP
129 (R&D Systems) or IL25 (Miltenyi Biotec). All these cytokines were used at 10 ng/ml, in NK
130 MACS medium complemented with 5% human serum and IL2 at 10ng/ml. The 48-hour
131 supernatants (SNs) were harvested while the cells were used for cytofluorimetric analysis.
132

133 **Extraction and incubation of fibroblasts**

134 Fibroblasts were obtained from skin biopsy samples from HDs[5]. Briefly, skin biopsy
135 specimens were digested with 0.1% type IA collagenase at 37°C for 2 hours. Adherent cells
136 were grown in cDMEM. Fibroblasts were used between the third and sixth passages. Then,
137 3.10^4 fibroblasts were cultured in duplicate in a 96-well flat-bottom plate and incubated with

138 non-stimulated ILC2 SNs or TGFβ-stimulated ILC2 SNs for 24 hours (50% SNs; 50%
139 cDMEM). TGFβ (10 ng/ml) was used as a positive control. rIL10 (10 ng/ml) was added to the
140 TGFβ-stimulated ILC2 SNs and then incubated with fibroblasts. Blocking IL10 antibody
141 (Miltenyi Biotec, 10 ng/ml) was incubated with non-stimulated ILC2 SNs and then added to
142 the fibroblasts. Blocking TGFβ antibody (R&D Systems, 10 ng/ml) was incubated with
143 TGFβ-stimulated ILC2 SNs and then added to the fibroblasts. After 24 hours, the SNs were
144 harvested and placed at -80°C, and the fibroblasts were collected with a lysis buffer.
145
146 **Tissue processing and immunofluorescence**
147 All samples were fixed in 3.8% formalin, dehydrated with graded concentrations of alcohol
148 and finally embedded in paraffin, as described previously[6]. The tissue blocks were cut into
149 3-µm thin sections with a microtome (Leica RM2255). Sections were floated in a 37°C water
150 bath, mounted on electrostatically charged adhesion slides (SuperFrost Plus, Thermo
151 Scientific) and dried on a heating plate for 2 hours at 56°C. Sections were dewaxed with
152 xylene and rehydrated in decreasing concentrations of ethanol. Using a PT link instrument
153 (Dako), a heat-induced antigen retrieval (HIER) step was performed by immersion of the
154 samples into a citrate buffer at pH 6 (PT Module Buffer 1, Thermo Scientific) for 20 min at
155 97°C. Then, in a humidified incubation box, the sections were saturated with a blocking
156 buffer (PBS 10 mM pH 7.4, 0.1% Triton X-100, 5% Normal Goat Serum (Thermo Scientific))
157 for 1 hour at room temperature. The sections were subsequently incubated overnight at 4°C
158 with the following antibodies diluted in blocking buffer: rabbit anti-human CRTH2 (GPR44,
159 Novus Biologicals), mouse anti-human CD3 (PS1, Abcam), mouse anti-human CD11b
160 (CL1719, Novus Biologicals), and mouse anti-human FcER1 (9E1, Thermo Fisher). Negative
161 controls were systematically obtained by omitting the primary antibodies. After washing
162 thoroughly with PBS, secondary antibodies coupled to fluorescent dyes (goat anti-rabbit

163 AF488, Invitrogen and goat anti-mouse AF568, Invitrogen) were applied to the tissue sections
164 for 1.5 hours at room temperature in the dark, and then the nuclei were counterstained with
165 DAPI. The slides were mounted with ProLong Gold Antifade Mountant (ThermoFisher
166 Scientific). Whole tissue slices were scanned using a NanoZoomer 2.0HT (Hamamatsu), and
167 3-D acquisitions were achieved with a confocal microscope SP5 (Leica). Mouse tissue stains
168 (hematoxylin and eosin H&E, PAS, Alcian Blue) were performed using the LEICA ST5020-
169 CV5030 automated stainer (Leica Biosystems, Wetzlar, Germany).
170
171 **Image extraction and cell count**
172 Image acquisition was obtained with a NanoZoomer from Hamamatsu. For each acquired
173 image, the user must visualize the ndpi files using "NDP.view2" software (Hamamatsu).
174 Interesting parts of the virtual slides were manually selected using regions of interests (ROIs).
175 The following steps were developed using QuantaCell custom-made software solutions
176 (MATLAB scripts and C++/OpenCV programs). Virtual images were automatically cropped
177 inside the user-defined region and exported into jpg files at a 20x resolution. A semiautomatic
178 sample detection was performed as follows: a gray image (named im_gray) was calculated
179 from the original images (named im_orig), im_gray was filtered with morphological
180 mathematics to make it homogeneous, and the automatic threshold value (named th) was
181 calculated on im_gray using the Otsu method. Manual correction of th was possible with user
182 intervention. A binary mask (named mask_sample) was calculated using th on im_gray. In
183 mask_sample, small objects <7500 µm² were removed, and semiautomatic epidermal
184 detection was performed. Green images (named im_green) were extracted from im_orig, and
185 im_green was filtered with morphological mathematics to make it homogeneous. The
186 automatic threshold value (named th_green) was calculated on im_green using the Otsu
187 method, and manual correction of th_green was possible with user intervention. A binary

188 mask (named mask_epiderma) was calculated using th_green on im_green (mask_epiderma
189 was restricted inside mask_sample). If several objects were present in mask_epiderma, then
190 the object closest to the sample border was kept (to separate the epiderma from the internal
191 green islet). A manual procedure was sometimes necessary to manually redraw
192 mask_epiderma. It was useful for very inhomogeneous epidermis.
193 Automatic cell quantification was performed on the processed images. Then, blue images
194 (named im_blue) were extracted from im_orig, and automatic nuclei detection was performed
195 to individualize each nuclei from im_blue. This process was achieved using image smoothing
196 (to remove noise), binarization, a watershed strategy to separate contiguous nuclei, and
197 rejection of nuclei that were too small. The obtained image of the nuclei was named
198 nuclei_mask. A donut shape with a width of 3.5 µm was generated around the nuclei to define
199 the cytoplasmic area. The cell mask (named cell_mask) was the union of nuclei_mask and the
200 donut-shaped mask. Intensity statistics using the averages among the pixel intensities were
201 calculated for each cell in the whole cell (nuclei + cytoplasm) and for each fluorescent
202 channel; for each cell, the closest distance to the epidermis was calculated, and the position in
203 the tissue was recorded (in the epidermis, "epidermis islet" or dermis). Cell classification was
204 performed according to 3 parameters: (i) whether the cell was in the epidermis or dermis; (ii)
205 whether the cell was positive in red or negative in red and/or green (red positivity threshold
206 was calculated as the median value for all cells of the sample + 10 fluorescent intensity units,
207 and green positivity threshold was calculated as the median value for all cells of the sample +
208 50 fluorescent intensity units); and (iii) whether the cell was positive in green or negative in
209 green. The following class counts were measured: cell count in epidermis, green positive cell
210 count in epidermis, red negative cell count in epidermis, green positive and red negative count
211 in epidermis, cell count in dermis, green positive cell count in dermis, red negative cell count
212 in dermis, and green positive and red negative count in dermis. Statistics were exported in

213 Excel files for further exploitation, and montage images were automatically generated to
214 represent the sample boundaries, epidermis and cell classes.
215 Cells were considered positive ILC2 when they had a nucleus surrounded by green
216 fluorescence without any red.
217
218 **Quantitative-Reverse Transcription-PCR (Q-RT-PCR)**
219 RNA was purified from ILC subsets and fibroblasts using an RNeasy Plus Micro Kit
220 (Qiagen), and the RNA concentration and purity were assessed using a Spectrophotometer
221 DS11 (Denovix). The RNA integrity number (RIN) was assessed using an Agilent 2200
222 TapeStation (Agilent Technologies). All procedures were performed according to the
223 manufacturer's instructions.
224 Total RNA was converted to cDNA using GoScript Reverse Transcription (Promega^{TM}).
225 qPCR was performed using GoTaq Master Mix (all reagents were purchased from
226 Promega^{TM}). The following targets were analyzed: *Tbet*, *GATA3*, *RORγt*, *COL1A1*, *COL1A2*,
227 *FN1*, *IL10*, *LTC4S* and MMP1. The mRNA levels were normalized to *18S* rRNA and *RPLP0*.
228 Samples were distributed in duplicate in a 384-well plate using an Epmotion 5073 automated
229 pipetting system (Eppendorf). Real-time quantitative PCR was performed using a CFX384
230 thermocycler (Bio-Rad^{TM}).
231 The data were analyzed using Bio-Rad^{TM} CFX Manager software (Bio-Rad^{TM}), and
232 differential expression was evaluated according to the ΔΔCt method.
233 For the reverse transcription quantitative PCR (RTqPCR) analysis of transcription factors,
234 samples from 6 different donors were mixed at the same time. After sorting, the different
235 subtypes of ILCs were separated into two fractions: one part was for the study of transcription
236 factors at day 0 (D0) while the second part was expanded for 20 days for the study of
237 transcription factors at day 20 (D20). The primers used in this study are listed in Table E1.

238

239 **Collagen ELISA**

240 The collagen production by dermal fibroblasts from HDs were assessed with the DuoSet®
241 ELISA "Human Pro-Collagen I α1" (R&D Systems). Culture conditions were prepared as
242 described by Dufour *et al*.[7]. Fibroblasts were used at passage 5–8 and cultured in DMEM
243 containing 10% FCS, 1% non-essential amino acids, 1% l-glutamine, 1% sodium pyruvate, 50
244 U/ml penicillin, and 50 μg/ml streptomycin. Twenty thousand cells/well were seeded in 96-
245 well plates for 24 hours, then starved for 16 hours in the absence of FCS, followed by
246 stimulation with 50% of ILC2 supernatants in DMEM containing 1% FCS, 25 μg/ml L-
247 ascorbic acid, 3.4 μg/ml α-ketoglutaric acid, and 50 μg/ml β-amino propionitrile to favor
248 collagen maturation, as well as 50 U/ml penicillin and 50 μg/ml streptomycin. When used,
249 cytokines or antibodies were added for 30 minutes to ILC2 supernatants before stimulation.
250 Culture supernatants were harvested after 48 hours.

251

252 **Average growth rate**

253 The proliferation of fibroblasts was followed by live-cell imaging (Incucyte® ZOOM, Essen
254 BioScience) for 48h. The average growth rate was calculated from the slope of the growth
255 curves. For this assay, 2000 cells/well were seeded in 96-well plates for 24 hours, then starved
256 for 16 hours in the absence of FCS, followed by stimulation with 50% of ILC2 supernatants
257 in DMEM containing 0% FCS and 50 U/ml penicillin and 50 μg/ml streptomycin. When used,
258 cytokines or antibodies were added for 30 minutes to ILC2 supernatants before stimulation.

259

260 **Myofibroblasts differentiation**

261 The differentiation of fibroblast into myofibroblasts was evaluated 72h post stimulation by
262 flow cytometry analysis from fixed and permeabilized-cells stained with human alpha-

263 Smooth Muscle Actin APC-conjugated antibody (R&D Systems). For this assay, 20 000
264 cells/well were seeded in 24-well plates until the confluence was reached. Then, cells were
265 starved for 24 hours in the absence of FCS, followed by stimulation with 50% of ILC2
266 supernatants in DMEM containing 0% FCS and 50 U/ml penicillin and 50 µg/ml
267 streptomycin. When used, cytokines or antibodies were added for 30 minutes to ILC2
268 supernatants before stimulation.
269

270 **RNA sequencing (RNAseq)**

271 5.10^4 ILC2 were incubated at 37°C for 48 hours with or without recombinant TGFβ (R&D
272 systems) at 10 ng/mL. After incubation, cells were washed with PBS and lysed using
273 QIAGEN RNeasy micro kit plus®. RNA were subsequently extracted using the
274 manufacturer's instructions. After RNA extraction, RNA concentrations were obtained using
275 nanodrop or a fluorometric Qubit RNA assay (Life Technologies, Grand Island, New York,
276 USA). The quality of the RNA (RNA integrity number) was determined on the Agilent 2100
277 Bioanalyzer (Agilent Technologies, Palo Alto, CA, USA) as per the manufacturer's
278 instructions.
279 To construct the libraries, 800 ng of high quality total RNA sample (RIN >8) was processed
280 using TruSeq Stranded mRNA kit (Illumina) according to manufacturer instructions. Briefly,
281 after purification of poly-A containing mRNA molecules, mRNA molecules are fragmented
282 and reverse- transcribed using random primers. Replacement of dTTP by dUTP during the
283 second strand synthesis will permit to achieve the strand specificity. Addition of a single A
284 base to the cDNA is followed by ligation of Illumina adapters.
285 Libraries were quantified by qPCR using the KAPA Library Quantification Kit for Illumina
286 Libraries (KapaBiosystems, Wilmington, MA) and library profiles were assessed using the
287 DNA High Sensitivity LabChip kit on an Agilent Bioanalyzer. Libraries were sequenced on

288 an Illumina Nextseq 500 instrument using 75 base-lengths read V2 chemistry in a paired-end
289 mode.
290

291 **RNASeq analysis**

292 Illumina paired-end reads were quality filtered with fastp 0.20.0[8]. Mapping against the
293 human GRCh38.primary assembly genome was performed with STAR 2.7.1a[9] by using the
294 ENCODE options. "--quantMode GeneCounts" option was used to make STAR count the
295 number of reads per gene while mapping. Differential expression study was performed by
296 using R package DESeq2 (v1.14.1 with R version 3.3.2)[10]. Pre-filtering was carried out on
297 the total counting matrix in order to keep only the genes having at least 10 reads detected
298 (here : 18,726 genes). Differentially expressed genes between non activated cells and
299 activated with TGFβ were filtered at a threshold of the adjusted pvalue (padj) < 0.01 (here :
300 3,134 genes). These differentially expressed genes were plotted as a heatmap with the R
301 pheatmap library (version 1.0.12). {https://CRAN.R-project.org/package=pheatmap}.
302 Subsequently, these genes were subjected to an enrichment analysis using the R Gprofiler2
303 library (version 0.1.8) {https://CRAN.R-project.org/package=gprofiler2} on the latest
304 available versions of the following databases : Gene Ontology Molecular Function, Biological
305 Process, Cellular Component ; Biological Pathways : KEGG ; Reactome ; WikiPathways ;
306 Regulatory motifs in DNA : TRANSFAC , miRTarBase ; Protein databases : Human Protein
307 Atlas, CORUM ; Human phenotype ontology. The results were filtered at a threshold of the
308 adjusted pvalue (padj) < 0.05.
309

310 **Statistical analysis**

311 Statistical analyses were performed using GraphPad Prism (La Jolla, CA). For distributions
312 that satisfied the Kolmogorov–Smirnov normality test, a two-tailed Student t-test for unpaired

313 or paired samples and one-way repeated-measures ANOVA followed by Bonferroni
314 correction were used to compare the different populations according to the experimental
315 design. When the normality test was not satisfied, the Mann-Whitney, Wilcoxon and Kruskal-
316 Wallis tests were used. Correlations were analyzed using the Spearman test. A P-value < 0.05
317 indicated statistical significance.

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346

347 **FIGURES**

348

349 **Online supplemental figure 1. Absolute values of ILCs in the blood of patients with**
350 **systemic sclerosis (SSc) and healthy donors (HDs).**
351 (A) FMOs and gating strategy: the FMO for the lineage negative cells (FMO Lin) was gated
352 on $CD45^+$ cells from the peripheral blood. The scale allows the visualization of the
353 dimensions of the gates and the proper set of the gate for ILCs. The FMO for CRTH2 (FMO
354 CRTH2) and CD117 (FMO CD117) are gated on ILCs. Then, ILCs subsets are shown
355 according to the FMO of CRTH2 and CD117. (B) The whole set of cells identified by our
356 gating strategy as ILCs show a similar distribution, making it a homogeneous population. (C)
357 Absolute values of ILCs in HD and SSc blood. (D) Absolute values of ILC subsets in HD and SSc
358 blood. (E) Percentage of $KLRG1^+$ ILC2 in HD and SSc blood. (F) Absolute number of circulating
359 ILC2 in patients with limited (Rodnan <10) and diffuse (Rodnan >10) SSc. Bar graphs show
360 data as the mean ± SEM (n=59 and 73 for HDs and SSc patients respectively). Comparison
361 between groups was calculated using Mann-Whitney test. ***, P < 0.001.

362

363 **Online supplemental figure 2. Characterization of ILCs in the skin of patients with**
364 **systemic sclerosis (SSc) and healthy donors (HDs).**
365 (A) FMOs and gating strategy. (B) Percentage of ILC1 in HD and SSc skin. (C) Correlation
366 between percentage of cutaneous ILC1 and the extent of fibrosis (mRSS). (D) Percentage of
367 ILC2 per total cell count in the dermis of HD and SSc skin. Bar graphs show data as the mean
368 ± SEM (n=17 for HD and SSc in flow cytometry and n=17 and 30 for HD and SSc,
369 respectively for immunofluorescence). Comparison between groups was calculated using
370 Mann-Whitney test. ***, P < 0.001.

371

372 **Online supplemental figure 3. Characterization of ILC2 after sorting and 20 days of**
373 **amplification.**
374 (A) Representative dot blot of ILC2 ($CD45^+Lin^-CD127^+CRTH2^+$) sorting. (B) Verification of
375 ILC2 phenotype after 20 days of amplification with IL2 and IL1β. (C) RTqPCR analysis of
376 ILC2 for *Tbet*, *GATA3* and *RORγt* after amplification. Relative expressions of *Tbet*, *GATA3*
377 and *RORγt* were represented using 1/ΔCt. Bar graphs show data as the mean ± SEM (n=3).

378

379 **Online supplemental figure 4. Heat map of gene expression between TGFβ-stimulated**
380 **ILC2 and unstimulated ILC2.**
381 (A) Heat map of 2840 genes differentially expressed (p adjusted value < 0.01) between
382 TGFβ-stimulated ILC2 (n=3) and unstimulated ILC2 (n=3). (B) Q-RT-PCR analysis of *IL10*
383 and *LTC4S* mRNA expression (n=4). Comparison between groups was calculated using
384 Mann-Whitney test. *, P < 0.05.

385

386 **Online supplemental figure 5. Characterization of ILCs in skin of HOCL-treated mice**
387 **and control mice.**
388 (A) Absolute count of ILC2 and (B) ILCs in skin of control and HOCl mice at day 42. (C)
389 Percentage of ILC2 among $CD45^+$ lymphoid cells in control and HOCl skin mice at day 21.
390 (D) Absolute count of ILC2 in skin of control and HOCl mice at day 21. (E) Skin thickness
391 and (F) collagen assay in skin of control and HOCl mice. Bar graphs show data as the mean ±
392 SEM (n=10 for control mice and n=9 to 10 for HOCl mice respectively in D21 and D42).
393 Comparison between groups was calculated using Mann-Whitney test. **, P < 0.01; ***, P <
394 0.001; ****, P < 0.0001.

395 **TABLES**

396

397 **Table E1. Primers used in the study.**

mRNA	***PRIMER***	***TM (SA)***	**Amplicon**
MMP1	**F:** GGAGGAAAAGCAGCTCAAGAAC	62,1°C	50pb
	R: TCCAGGGTGACACCAGTGACT	63,2°C	
COL1A1	**F:** CCCTCCTGACGCACGG	58,4°C	66pb
	R: GTGATTGGTGGGATGTCTTCGT	62,1°C	
COL1A2	**F:** CTGTAAGAAAGGGCCCAGCC	62,5°C	50pb
	R: GACCCCTTTCTCCACGTGG	61,6°C	
FN1	**F:** GGGAGAATAAGCTGTACCATCG	59°C	129pb
	R: TCCATTACCAAGACACACACACT	59°C	
IL10	**F:** TGCCTTCAGCAGAGTGAAGA	59°C	103pb
	R: GCAACCCAGGTAACCCTTAAA	60°C	
LTC4S	**F:** ACCATGAAGGACGAGGTAGC	59°C	77pb
	R: TGCAGGGAGAAGTAGGCTTG	60°C	
RORγt	**F:** CTGGGCATGTCCCGAGATG	62.2°C	133pb
	R: GAGGGGTCTTGACCACTGG	61.3°C	
GATA3	**F:** GCCCCTCATTAAGCCCAAG	65,6°C	80pb
	R: TTGTGGTGGTCTGACAGTTCG	66,3°C	
Tbet	**F:** GTCCAACAATGTGACCCAGAT	60°C	75pb
	R: ACCTCAACGATATGCAGCCG	62.4°C	
HOUSEKEEPING G.	***PRIMER***	***TM (SA)***	**Amplicon**
18s	**F:** TGCCATCACTGCCATTAAG	65.9°C	61pb
	R: TGCTTTCCTCAACACCACATG	66°C	
RPLP0	**F:** GCAGCATCTACAACCCTGAAG	60.7°C	87pb
	R: CACTGGCAACATTGCGGAC	62°C	

398

399 **Table E2. Reagents and products used in this study.**

REAGENT or RESSOURCE	SOURCE	IDENTIFIER
Antibodies		
anti-human CD3 APC	Miltenyi	BW264/56
anti-human CD5 APC	Miltenyi	UCHT2
anti-human CD14 APC	Miltenyi	TUK4
anti-human CD16APC	Miltenyi	REA423
anti-human CD19APC	Miltenyi	LT19
anti-human CD31APC	Miltenyi	AC128
anti-human CD34APC	Miltenyi	AC136
anti-human CD45APC	Miltenyi	5B11
anti-human CD56APC	Miltenyi	AF12-7H3
anti-human CD94APC	Miltenyi	REA113
anti-human CD123 APC	Miltenyi	AC145
anti-human CD303APC	Miltenyi	AC144
anti-humanTCRabAPC	Miltenyi	BW242/412
anti-humanTCRgdAPC	Miltenyi	11F2
anti-human CD117Pe-Vio770	Miltenyi	A3C6E2
anti-humanCD127Pe-Vio665	Miltenyi	MB15-18C9
anti-humanCLAFITC	Miltenyi	HECA-452
anti-humanCCR10PerCP/Cy5.5	BD	1B5
anti-humanCCR6 BV786	BD	11A9
anti-humanHLA-DRPerCP/Cy5.5	BD	L243
anti-humanOX40L PE	Abcam	ANC10G1
anti-humanTSLPR PE	BD	1F11
anti-humanCRTH2BV421	BD	BM16
Anti-human FcεR1 APC	eBioscience	AER-37
anti-humanKLRG1FITC	eBioscience	13F12F2
anti-humanaSMAAPC	R&D Systems	1A4
anti-human CD1aAPC	Beckman Coulter	BL6
anti-human CD3 APC	Beckman Coulter	UCHT1
anti-human CD11c APC	Beckman Coulter	BU15
anti-human CD14APC	Beckman Coulter	RMO52
anti-human CD16APC	Beckman Coulter	3G8
anti-human CD19APC	Beckman Coulter	J3-119
anti-human CD34APC	Beckman Coulter	581
anti-human CD94APC	Beckman Coulter	HP-3B1
anti-human CD123APC	Beckman Coulter	AC145
anti-human CD45KO	Beckman Coulter	J33
anti-human CD117PE/Cyy5.5	Beckman Coulter	104D2D1
anti-human CD127APC/AF700	Beckman Coulter	R34.34
anti-humanCRTH2 FITC	Beckman Coulter	BM16
anti-human CD5 APC	BD	UCHT2
anti-human FcεR1 APC	eBioscience	AER-37
anti-humanCD31APC	Miltenyi	AC128
Anti-mouse ST2	Biolegend	DIH9
anti-mouse CD117	Miltenyi	REA791

Anti-mouse CD25 PE	Miltenyi	REA568
Anti-mouse CD45	Miltenyi	REA737
Anti-mouse KLRG1	Miltenyi	REA1016
Anti-mouse CD127	Miltenyi	REA680
anti-mouse CD5 Biotin	Miltenyi	REA421
Anti-mouse CD3e Biotin	Miltenyi	17A2
anti-mouse LY6G/C Biotin	Miltenyi	REA526
anti-mouse CD45R Biotin	Miltenyi	REA755
anti-mouse CD11b Biotin	Miltenyi	REA592
anti-mouse Ter-119 Biotin	Miltenyi	TER119
Rabbit anti-human CRTH2	Novus Biologicals	GPR44
Human anti-human CD3	Abcam	PS1
Human anti-human CD11b	Novus Biologicals	CL1719
Human anti-human FcεR1	Thermo Fisher	9E1
Chemicals, Peptides, and Recombinant Proteins		
Human recombinant IL33	Miltenyi	
Human recombinant TGFb	R&D System	
Human recombinant IL4	Miltenyi	
Human recombinant TSLP	R&D System	
Human recombinant IL25	Miltenyi	
Human recombinant IL2	Miltenyi	
Human recombinant IL1b	R&D System	
Human recombinant IL10	Miltenyi	
Mouse recombinant IL10	Miltenyi	
Pirfenidone	Hospital of Bordeaux	
KH_2PO_4	Sigma-Aldrich	
NaClO	Sigma-Aldrich	

400

401 **Table E3. Gene Ontology analysis. List of the first 50-upregulated biological processes.**

source	term_name	term_id	padj
GO:BP	positive regulation of GTPase activity	GO:0043547	2.64E-06
GO:BP	regulation of GTPase activity	GO:0043087	2.64E-06
GO:BP	regulation of signaling	GO:0023051	3.49E-06
GO:BP	regulation of response to stimulus	GO:0048583	7.43E-06
GO:BP	regulation of cell communication	GO:0010646	8.02E-06
GO:BP	regulation of signal transduction	GO:0009966	1.06E-05
GO:BP	myeloid cell differentiation	GO:0030099	1.06E-05
GO:BP	leukocyte differentiation	GO:0002521	1.35E-05
GO:BP	regulation of hemopoiesis	GO:1903706	1.35E-05
GO:BP	immune system development	GO:0002520	2.29E-05
GO:BP	hemopoiesis	GO:0030097	4.30E-05
GO:BP	regulation of leukocyte differentiation	GO:1902105	7.23E-05
GO:BP	negative regulation of response to stimulus	GO:0048585	7.23E-05
GO:BP	hematopoietic or lymphoid organ development	GO:0048534	1.42E-04
GO:BP	T cell activation	GO:0042110	1.42E-04
GO:BP	lymphocyte activation	GO:0046649	1.98E-04
GO:BP	regulation of cell-cell adhesion	GO:0022407	2.23E-04
GO:BP	immune system process	GO:0002376	2.26E-04
GO:BP	T cell differentiation	GO:0030217	2.26E-04
GO:BP	positive regulation of hydrolase activity	GO:0051345	2.55E-04
GO:BP	regulation of lymphocyte differentiation	GO:0045619	2.63E-04
GO:BP	regulation of B cell proliferation	GO:0030888	3.43E-04
GO:BP	intracellular signal transduction	GO:0035556	3.43E-04
GO:BP	leukocyte activation	GO:0045321	4.06E-04
GO:BP	cell activation	GO:0001775	4.13E-04
GO:BP	positive regulation of cell-cell adhesion	GO:0022409	4.13E-04
GO:BP	regulation of cell activation	GO:0050865	4.13E-04
GO:BP	positive regulation of cell adhesion	GO:0045785	4.31E-04
GO:BP	regulation of T cell activation	GO:0050863	4.86E-04
GO:BP	myeloid leukocyte differentiation	GO:0002573	5.85E-04
GO:BP	positive regulation of leukocyte differentiation	GO:1902107	6.45E-04
GO:BP	osteoclast differentiation	GO:0030316	6.45E-04
GO:BP	positive regulation of hemopoiesis	GO:1903708	6.45E-04
GO:BP	regulation of cell adhesion	GO:0030155	6.45E-04
GO:BP	regulation of alpha-beta T cell activation	GO:0046634	6.69E-04
GO:BP	regulation of leukocyte activation	GO:0002694	6.91E-04
GO:BP	granzyme-mediated programmed cell death signaling pathway	GO:0140507	7.23E-04
GO:BP	negative regulation of transferase activity	GO:0051348	7.37E-04
GO:BP	regulation of lymphocyte activation	GO:0051249	7.81E-04
GO:BP	regulation of T cell differentiation	GO:0045580	8.71E-04
GO:BP	negative regulation of signaling	GO:0023057	9.46E-04
GO:BP	regulation of osteoclast differentiation	GO:0045670	1.11E-03
GO:BP	negative regulation of immune response	GO:0050777	1.11E-03
GO:BP	regulation of leukocyte cell-cell adhesion	GO:1903037	1.11E-03
GO:BP	cell adhesion	GO:0007155	1.17E-03
GO:BP	negative regulation of signal transduction	GO:0009968	1.18E-03
GO:BP	leukocyte cell-cell adhesion	GO:0007159	1.21E-03
GO:BP	regulation of tumor necrosis factor superfamily cytokine production	GO:1903555	1.21E-03
GO:BP	regulation of intracellular signal transduction	GO:1902531	1.21E-03
GO:BP	negative regulation of tumor necrosis factor production	GO:0032720	1.21E-03

402

403 **Table E4. Gene Ontology analysis. List of upregulated cellular components.**

source	term_name	term_id	padj
GO:CC	plasma membrane	GO:0005886	3.41E-05
GO:CC	cell periphery	GO:0071944	3.41E-05
GO:CC	side of membrane	GO:0098552	2.02E-04
GO:CC	external side of plasma membrane	GO:0009897	4.01E-04
GO:CC	specific granule membrane	GO:0035579	5.54E-04
GO:CC	membrane microdomain	GO:0098857	9.49E-04
GO:CC	membrane raft	GO:0045121	9.49E-04
GO:CC	microvillus	GO:0005902	1.04E-03
GO:CC	cytoplasmic vesicle	GO:0031410	1.19E-03
GO:CC	intracellular vesicle	GO:0097708	1.19E-03
GO:CC	intrinsic component of plasma membrane	GO:0031226	1.22E-03
GO:CC	focal adhesion	GO:0005925	1.22E-03
GO:CC	protein complex involved in cell adhesion	GO:0098636	1.22E-03
GO:CC	cell-substrate junction	GO:0030055	1.90E-03
GO:CC	cell surface	GO:0009986	2.13E-03
GO:CC	integral component of plasma membrane	GO:0005887	3.57E-03
GO:CC	Golgi apparatus	GO:0005794	4.45E-03
GO:CC	condensed chromosome kinetochore	GO:0000777	4.72E-03
GO:CC	bounding membrane of organelle	GO:0098588	5.46E-03
GO:CC	integrin complex	GO:0008305	5.46E-03
GO:CC	ruffle membrane	GO:0032587	5.97E-03
GO:CC	endosome	GO:0005768	6.94E-03
GO:CC	ruffle	GO:0001726	6.94E-03
GO:CC	condensed chromosome, centromeric region	GO:0000779	8.41E-03
GO:CC	condensed nuclear chromosome kinetochore	GO:0000778	8.41E-03
GO:CC	plasma membrane raft	GO:0044853	8.90E-03
GO:CC	specific granule	GO:0042581	9.61E-03
GO:CC	cytoplasmic vesicle membrane	GO:0030659	9.61E-03
GO:CC	protein serine/threonine phosphatase complex	GO:0008287	9.67E-03
GO:CC	phosphatase complex	GO:1903293	9.67E-03
GO:CC	histone locus body	GO:0035363	1.00E-02
GO:CC	condensed nuclear chromosome outer kinetochore	GO:0000942	1.07E-02
GO:CC	vesicle membrane	GO:0012506	1.07E-02
GO:CC	integrin alphaM-beta2 complex	GO:0034688	1.23E-02
GO:CC	cytoplasm	GO:0005737	1.23E-02
GO:CC	growth cone	GO:0030426	1.35E-02
GO:CC	tertiary granule	GO:0070820	1.42E-02
GO:CC	adherens junction	GO:0005912	1.42E-02
GO:CC	nuclear body	GO:0016604	1.42E-02
GO:CC	recycling endosome	GO:0055037	1.61E-02
GO:CC	cell leading edge	GO:0031252	1.89E-02
GO:CC	midbody	GO:0030496	1.91E-02
GO:CC	protein phosphatase type 2A complex	GO:0000159	1.92E-02
GO:CC	site of polarized growth	GO:0030427	1.93E-02
GO:CC	kinetochore	GO:0000776	2.01E-02
GO:CC	nucleoplasm	GO:0005654	2.01E-02
GO:CC	fibrillar center	GO:0001650	2.41E-02
GO:CC	cytosol	GO:0005829	2.41E-02
GO:CC	endomembrane system	GO:0012505	2.44E-02
GO:CC	actin-based cell projection	GO:0098858	2.64E-02
GO:CC	cleavage furrow	GO:0032154	2.67E-02
GO:CC	anchoring junction	GO:0070161	2.67E-02
GO:CC	perinuclear region of cytoplasm	GO:0048471	3.01E-02
GO:CC	transcription factor AP-1 complex	GO:0035976	3.47E-02
GO:CC	cell projection membrane	GO:0031253	3.49E-02

GO:CC	vesicle	GO:0031982	3.71E-02
GO:CC	phagolysosome	GO:0032010	3.71E-02
GO:CC	myosin complex	GO:0016459	3.71E-02
GO:CC	F-actin capping protein complex	GO:0008290	3.76E-02
GO:CC	immunological synapse	GO:0001772	4.01E-02
GO:CC	early endosome	GO:0005769	4.01E-02
GO:CC	tertiary granule membrane	GO:0070821	4.01E-02
GO:CC	**heteromeric SMAD protein complex**	**GO:0071144**	**4.01E-02**
GO:CC	axonal growth cone	GO:0044295	4.16E-02
GO:CC	beta-catenin destruction complex	GO:0030877	4.16E-02
GO:CC	protein kinase 5 complex	GO:0016533	4.21E-02
GO:CC	endosome membrane	GO:0010008	4.22E-02
GO:CC	condensed chromosome	GO:0000793	4.22E-02
GO:CC	cytoplasmic side of dendritic spine plasma membrane	GO:1990780	4.22E-02
GO:CC	transcription elongation factor complex	GO:0008023	4.27E-02
GO:CC	**SMAD protein complex**	**GO:0071141**	**4.45E-02**
GO:CC	muscle cell projection	GO:0036194	4.61E-02
GO:CC	muscle cell projection membrane	GO:0036195	4.61E-02
GO:CC	centrosome	GO:0005813	4.61E-02

404

405 **Table E5. Gene Ontology analysis. List of the first 50-upregulated molecular function.**

source	**term_name**	**term_id**	**padj**
GO:MF	GTPase regulator activity	GO:0030695	1.17E-07
GO:MF	GTPase activator activity	GO:0005096	1.18E-06
GO:MF	nucleoside-triphosphatase regulator activity	GO:0060589	1.18E-06
GO:MF	enzyme binding	GO:0019899	4.02E-04
GO:MF	enzyme regulator activity	GO:0030234	6.60E-04
GO:MF	enzyme activator activity	GO:0008047	8.88E-04
GO:MF	kinase activity	GO:0016301	4.11E-03
GO:MF	C-C chemokine binding	GO:0019957	5.60E-03
GO:MF	kinase regulator activity	GO:0019207	5.64E-03
GO:MF	cell adhesion molecule binding	GO:0050839	9.72E-03
GO:MF	GTPase activity	GO:0003924	9.72E-03
GO:MF	**SMAD binding**	**GO:0046332**	**9.72E-03**
GO:MF	protein kinase regulator activity	GO:0019887	9.72E-03
GO:MF	phosphatidylinositol 3-kinase binding	GO:0043548	9.72E-03
GO:MF	kinase binding	GO:0019900	9.72E-03
GO:MF	**I-SMAD binding**	**GO:0070411**	**9.72E-03**
GO:MF	chemokine binding	GO:0019956	9.72E-03
GO:MF	protein kinase binding	GO:0019901	9.72E-03
GO:MF	kinase inhibitor activity	GO:0019210	9.72E-03
GO:MF	death receptor activity	GO:0005035	1.08E-02
GO:MF	actin filament binding	GO:0051015	1.08E-02
GO:MF	phosphotransferase activity, alcohol group as acceptor	GO:0016773	1.08E-02
GO:MF	protein kinase inhibitor activity	GO:0004860	1.10E-02
GO:MF	protein tyrosine/threonine phosphatase activity	GO:0008330	1.11E-02
GO:MF	immune receptor activity	GO:0140375	1.28E-02
GO:MF	cytokine receptor activity	GO:0004896	1.30E-02
GO:MF	C-C chemokine receptor activity	GO:0016493	1.58E-02
GO:MF	coreceptor activity	GO:0015026	1.58E-02
GO:MF	G protein-coupled chemoattractant receptor activity	GO:0001637	1.58E-02
GO:MF	chemokine receptor activity	GO:0004950	1.58E-02
GO:MF	cadherin binding	GO:0045296	1.88E-02
GO:MF	tumor necrosis factor-activated receptor activity	GO:0005031	1.88E-02
GO:MF	phosphoric ester hydrolase activity	GO:0042578	2.03E-02
GO:MF	15-hydroxyprostaglandin dehydrogenase (NAD+) activity	GO:0016404	2.03E-02
GO:MF	UDP-N-acetylglucosamine-lysosomal-enzyme N-acetylglucosaminephosphotransferase activity	GO:0003976	2.03E-02
GO:MF	C-X-C chemokine receptor activity	GO:0016494	2.03E-02
GO:MF	inorganic phosphate transmembrane transporter activity	GO:0005315	2.09E-02
GO:MF	spectrin binding	GO:0030507	2.09E-02
GO:MF	phosphatidylinositol-4,5-bisphosphate phosphatase activity	GO:0106019	2.09E-02
GO:MF	transferase activity, transferring phosphorus-containing groups	GO:0016772	2.09E-02
GO:MF	protein domain specific binding	GO:0019904	2.18E-02
GO:MF	cytokine binding	GO:0019955	2.62E-02
GO:MF	MAP kinase phosphatase activity	GO:0033549	2.79E-02
GO:MF	actin binding	GO:0003779	2.79E-02
GO:MF	GTP binding	GO:0005525	3.79E-02
GO:MF	C-X-C chemokine binding	GO:0019958	3.79E-02
GO:MF	glycerophosphodiester phosphodiesterase activity	GO:0008889	3.79E-02
GO:MF	guanyl nucleotide binding	GO:0019001	3.79E-02
GO:MF	guanyl ribonucleotide binding	GO:0032561	3.79E-02
GO:MF	polyamine oxidase activity	GO:0046592	3.79E-02
GO:MF	virus receptor activity	GO:0001618	3.85E-02
GO:MF	purine ribonucleoside binding	GO:0032550	4.10E-02
GO:MF	exogenous protein binding	GO:0140272	4.10E-02
GO:MF	ubiquitin-like protein ligase binding	GO:0044389	4.27E-02

GO:MF	ribonucleoside binding	GO:0032549	4.27E-02
GO:MF	purine nucleoside binding	GO:0001883	4.27E-02
GO:MF	ubiquitin protein ligase binding	GO:0031625	4.27E-02
GO:MF	phosphatidylinositol phosphate 4-phosphatase activity	GO:0034596	4.30E-02
GO:MF	MAP kinase tyrosine/serine/threonine phosphatase activity	GO:0017017	4.30E-02
GO:MF	phosphatidylinositol-3,4,5-trisphosphate binding	GO:0005547	4.35E-02
GO:MF	cytoskeletal protein binding	GO:0008092	4.48E-02
GO:MF	growth factor activity	GO:0008083	4.71E-02
GO:MF	nucleoside binding	GO:0001882	4.71E-02
GO:MF	AP-1 adaptor complex binding	GO:0035650	4.71E-02

406

407 **Table E6. miRTarBase analysis.**

source	term_name	term_id	padj
MIRNA	hsa-miR-519d-3p	MIRNA:hsa-miR-519d-3p	4.17E-02

408

409 **Table E7. Transfac analysis. List of the first 50-upregulated transcription factors.**

source	term_name	term_id	padj
TF	Factor: E2F-3; motif: GGCGGGN; match class: 1	TF:M02089_1	2.17E-16
TF	Factor: Sp1; motif: NGGGGGCGGGGCCNGGGGGGGG; match class: 1	TF:M10071_1	3.79E-16
TF	Factor: ETF; motif: GVGGMGG; match class: 1	TF:M00695_1	1.81E-14
TF	Factor: BTEB1; motif: GGGGGCGGGGCNGSGGGNGS; match class: 1	TF:M09723_1	2.29E-14
TF	Factor: GKLF; motif: GCCMCRCCCNNN; match class: 1	TF:M01588_1	3.07E-14
TF	Factor: SP2; motif: GNNGGGGGCGGGGSN; match class: 1	TF:M03807_1	5.65E-14
TF	Factor: sp4; motif: NNGNARGRGGCGGRGCNNRR; match class: 1	TF:M10072_1	5.65E-14
TF	Factor: Sp2; motif: NYSGCCCCGCCCCCY	TF:M03567	7.44E-14
TF	Factor: KLF3; motif: NNNNNNGGGCGGGGCNNGN	TF:M09970	8.30E-14
TF	Factor: WT1; motif: CGCCCCCNCN; match class: 1	TF:M02036_1	9.64E-14
TF	Factor: WT1; motif: GNGGGGGCGGGG; match class: 1	TF:M03893_1	1.79E-13
TF	Factor: Sp1; motif: GGGGCGGGGC; match class: 1	TF:M00931_1	1.79E-13
TF	Factor: Sp1; motif: GGGGCGGGGT; match class: 1	TF:M00008_1	2.66E-13
TF	Factor: Sp1; motif: NNGGGGCGGGGNN; match class: 1	TF:M00932_1	2.82E-13
TF	Factor: SP1; motif: NCCCCKCCCCC; match class: 1	TF:M07226_1	3.11E-13
TF	Factor: KLF3; motif: NNNNNNGGGCGGGGCNNGN; match class: 1	TF:M09970_1	3.11E-13
TF	Factor: Sp1; motif: NGGGGCGGGGN; match class: 1	TF:M07395_1	5.26E-13
TF	Factor: BTEB2; motif: RGGGNGKGGN; match class: 1	TF:M07277_1	6.14E-13
TF	Factor: EKLF; motif: NGGGYGKGGCNNGG; match class: 1	TF:M09969_1	8.45E-13
TF	Factor: Sp1; motif: NGGGGGCGGGGCCNGGGGGGGG	TF:M10071	8.91E-13
TF	Factor: sp4; motif: NNNGCYCCGCCCCCY; match class: 1	TF:M10530_1	9.25E-13
TF	Factor: BTEB2; motif: RGGGNGKGGN	TF:M07277	9.68E-13
TF	Factor: Sp2; motif: GGSNNGGGGGCGGGGCCNGNGS	TF:M09658	1.13E-12
TF	Factor: AP-2rep; motif: NGGGGCGGGGC	TF:M09967	1.40E-12
TF	Factor: GKLF; motif: NNCCMCRCCCN; match class: 1	TF:M12173_1	1.43E-12
TF	Factor: Sp1; motif: NGGGGGCGGGGYN; match class: 1	TF:M00196_1	1.61E-12
TF	Factor: BTEB3; motif: CCNNSCCNSCCCCKCCCCC; match class: 1	TF:M09826_1	1.87E-12
TF	Factor: CPBP; motif: GNNRGGGHGGGGNNGGGRN; match class: 1	TF:M09973_1	1.87E-12
TF	Factor: AP-2; motif: GSCCSCRGGCNRNRNN; match class: 1	TF:M00800_1	1.87E-12
TF	Factor: TIEG1; motif: NCCCNSNCCCCGCCCCC; match class: 1	TF:M12351_1	2.05E-12
TF	Factor: AP-2; motif: MKCCCSCNGGCG; match class: 1	TF:M00189_1	2.21E-12
TF	Factor: SP2; motif: GNNGGGGGCGGGGSN	TF:M03807	3.75E-12
TF	Factor: BTEB1; motif: GGGGGCGGGGCNGSGGGNGS	TF:M09723	4.16E-12
TF	Factor: Sp1; motif: NGGGGGCGGGGYN	TF:M00196	4.64E-12
TF	Factor: BTEB2; motif: GCCCCRCCCH	TF:M07409	6.95E-12
TF	Factor: Sp2; motif: NYSGCCCCGCCCCCY; match class: 1	TF:M03567_1	7.16E-12
TF	Factor: Sp2; motif: GGGGCGGGG	TF:M10435	7.61E-12
TF	Factor: Sp1; motif: GGGGCGGGGC	TF:M07063	7.61E-12
TF	Factor: Sp1; motif: NGGGGCGGGGN	TF:M07395	8.20E-12
TF	Factor: Sp2; motif: GYCCCGCCYCYNNNN	TF:M07129	9.60E-12
TF	Factor: SP1; motif: NRGKGGGCGGGGCN; match class: 1	TF:M09765_1	1.11E-11
TF	Factor: BTEB2; motif: GCCCCRCCCH; match class: 1	TF:M07409_1	1.11E-11
TF	Factor: Sp1; motif: CCCCGCCCCN; match class: 1	TF:M00933_1	1.15E-11
TF	Factor: Egr-1; motif: GCGGGGGCGG; match class: 1	TF:M01873_1	1.18E-11
TF	Factor: SP1; motif: NRGKGGGCGGGGCN	TF:M09765	1.38E-11
TF	Factor: Sp1; motif: NNGGGGCGGGGNN	TF:M00932	1.77E-11
TF	Factor: Egr-1; motif: GCGGGGGCGG; match class: 1	TF:M07354_1	1.77E-11
TF	Factor: Sp3; motif: GGGGCGGGGSNN	TF:M07615	2.55E-11
TF	Factor: Egr-1; motif: GCGGGGGCGG	TF:M07354	2.91E-11
TF	Factor: AP-2rep; motif: NGGGGCGGGGC; match class: 1	TF:M09967_1	2.93E-11

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411 **Table E8. List of the first 60 upregulated genes (red) and downregulated genes (green).**

Gene name	log2FoldChange	padj	Gene name	log2FoldChange	padj
QPCT	5.13	1.52E-121	*ST6GALNAC1*	-3.92	8.64E-53
CA10	4.18	2.46E-52	***IL10***	**-3.71**	**1.36E-51**
PRICKLE1	3.97	3.93E-63	*ARG2*	-3.23	2.06E-48
PMEPA1	3.95	1.61E-176	*SOCS3*	-3.16	1.44E-79
NKD1	3.91	2.58E-82	*NHSL2*	-2.91	5.21E-52
RGS16	3.83	1.64E-132	*GZMA*	-2.87	2.96E-48
CSPG5	3.83	8.25E-55	*AL121933.2*	-2.81	7.12E-67
AFAP1	3.35	5.71E-33	*SELL*	-2.67	1.32E-31
SH2D4A	3.10	4.02E-66	*AIRE*	-2.62	1.86E-17
SLC6A4	2.87	7.09E-19	*PAPSS2*	-2.52	1.03E-24
CDK5R1	2.86	3.48E-57	*SH3RF3*	-2.48	1.55E-29
ATP10D	2.77	4.07E-33	*AXIN2*	-2.47	5.65E-18
INAVA	2.76	2.24E-20	*ROPN1L*	-2.42	1.13E-34
GPR35	2.75	1.55E-22	*LINC01943*	-2.38	8.35E-24
TBC1D16	2.73	8.80E-179	*SULT1B1*	-2.37	5.27E-13
PNMA8C	2.71	6.68E-27	*RAP1GAP2*	-2.37	9.14E-52
LTC4S	**2.71**	**9.89E-22**	*MGC16275*	-2.32	1.58E-15
IER3	2.58	3.16E-19	*CCL3*	-2.30	2.39E-15
CLIC3	2.54	7.22E-207	*C5AR2*	-2.30	6.92E-38
SMAD7	2.48	7.13E-177	*SMIM35*	-2.21	4.05E-44
AC006033,2	2.47	8.08E-51	*SCAMP5*	-2.17	1.31E-89
NCR2	2.45	7.11E-15	*SEMA3G*	-2.16	1.94E-10
NKILA	2.45	2.82E-17	*CCR1*	-2.15	6.71E-46
CCL20	2.45	6.97E-14	*VEPH1*	-2.14	8.86E-11
SIAH3	2.44	1.77E-13	*CTLA4*	-2.13	7.23E-25
TINCR	2.38	6.31E-21	*TRABD2A*	-2.12	1.36E-22
LMO7	2.36	1.52E-52	*TNIP3*	-2.12	3.33E-11
SARDH	2.35	4.18E-13	*RPS27AP2*	-2.11	1.54E-12
RFX2	2.35	1.33E-170	*CSF2*	-2.10	2.48E-17
IL4I1	2.34	1.81E-16	*ITGA6*	-2.10	9.78E-33
LRRC2	2.34	1.68E-56	*FAM30A*	-2.06	8.34E-10
LINC00996	2.32	4.70E-21	*IGFBP7*	-2.05	1.03E-11
RASGRP3	2.32	1.14E-66	*RCN3*	-2.04	3.03E-11
GPR34	2.32	8.14E-27	*CREG2*	-2.04	1.57E-09
CPNE5	2.32	4.04E-25	*MUC1*	-2.03	1.78E-25
KCNH4	2.32	6.07E-19	*FAAHP1*	-2.03	5.49E-14
SKIL	2.30	7.28E-58	*WDR86-AS1*	-2.02	1.51E-21
FSCN1	2.28	2.04E-14	*EEPD1*	-2.00	4.88E-43
NXPH4	2.28	8.56E-14	*AC068279.2*	-1.99	4.20E-09
JAG2	2.27	1.42E-35	*PTGDS*	-1.98	5.46E-10
CYS1	2.24	4.05E-20	*AP003469.2*	-1.94	3.82E-13
FAM241A	2.21	8.62E-44	*AC061992.2*	-1.94	1.27E-08
UBE2QL1	2.21	3.48E-13	*HK3*	-1.93	1.51E-08
RGS1	2.20	6.30E-23	*NKG7*	-1.92	2.15E-52
TRGV8	2.20	6.33E-26	*BFSP1*	-1.91	2.59E-37
HS3ST1	2.19	2.27E-15	*SLC22A20P*	-1.90	1.89E-11
TRGV7	2.17	9.95E-33	*LINC00484*	-1.88	4.31E-11
CHST15	2.16	1.49E-10	*AC087645.2*	-1.86	2.72E-08
CCDC170	2.16	1.25E-10	*AC008894.2*	-1.85	5.31E-09
VASH1	2.15	4.89E-51	*RGS6*	-1.83	8.93E-10
CPNE7	2.14	5.19E-18	*HILPDA*	-1.83	1.22E-08
LTF	2.13	3.18E-10	*ITGA9*	-1.83	1.99E-10
PLEK2	2.11	4.23E-10	*AL355581.1*	-1.83	3.51E-10
KIF13A	2.09	8.07E-17	*GNG4*	-1.82	3.37E-09
TRO	2.07	1.89E-20	*CRISPLD2*	-1.82	1.01E-07
CYP4F22	2.05	7.93E-50	*SLC26A11*	-1.81	5.54E-14
PGLYRP2	2.04	5.08E-13	*WSCD2*	-1.79	4.52E-09
SMOX	2.01	3.85E-24	*SYNC*	-1.79	2.14E-07
DPY19L2	2.01	5.30E-10	*TMEM38A*	-1.78	1.54E-71
ATRNL1	2.01	3.04E-16	*HRH2*	-1.78	2.21E-07

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