

Supplementary Materials for

**Profiling of mature-stage human breast milk cells identifies six unique lactocyte subpopulations**

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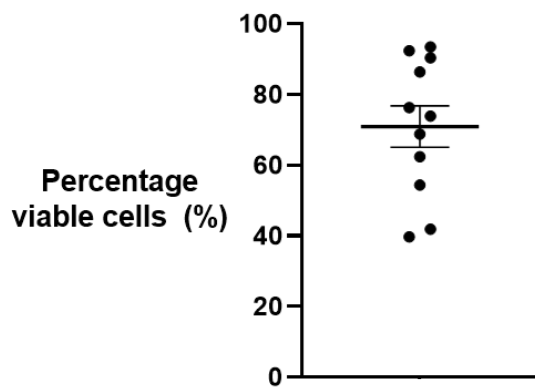
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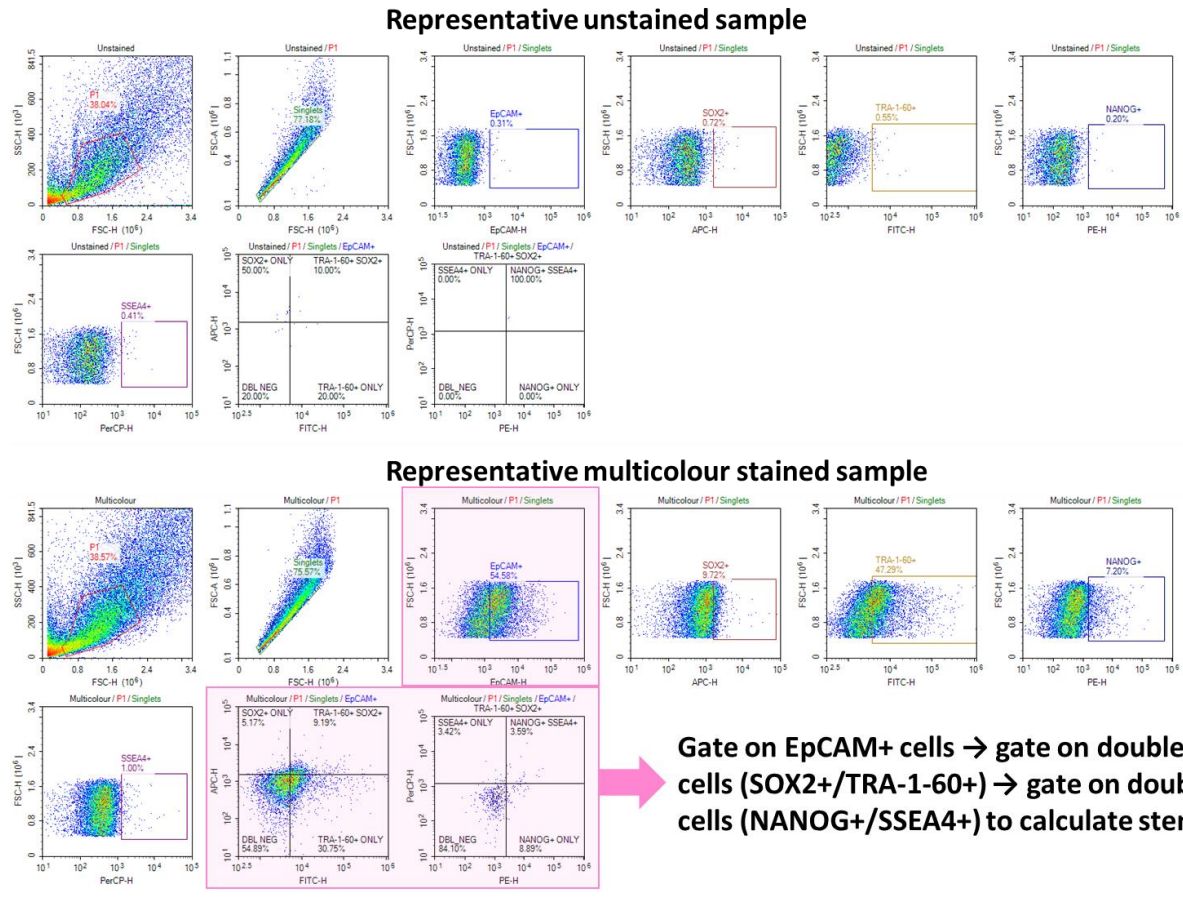
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Figs. S1 to S7  
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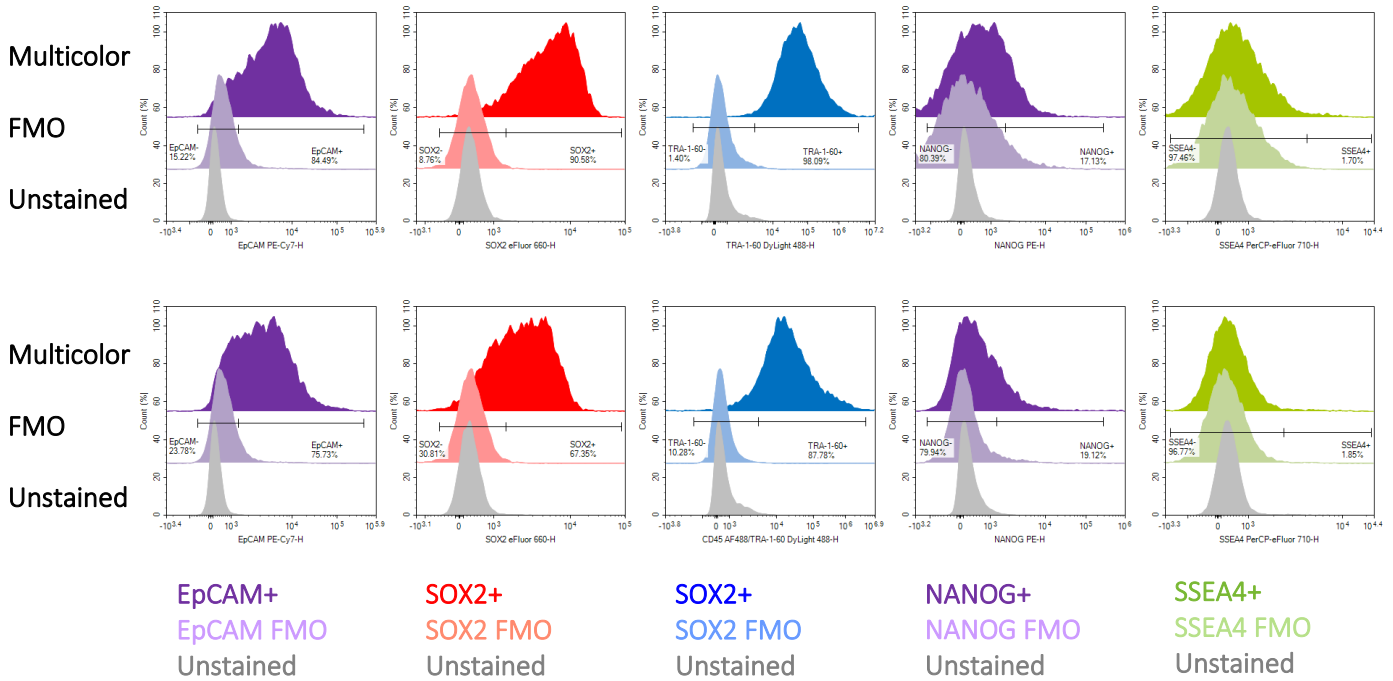
**Fig. S1** – Cells isolated from the breastmilk of 12 unique donors were 40 to 95% viable with an average viability of 70%. Viability was measured using flow cytometry LIVE/DEAD™ Fixable Yellow stain ( $79.2\% \pm 6.9\%$ ; N = 10).



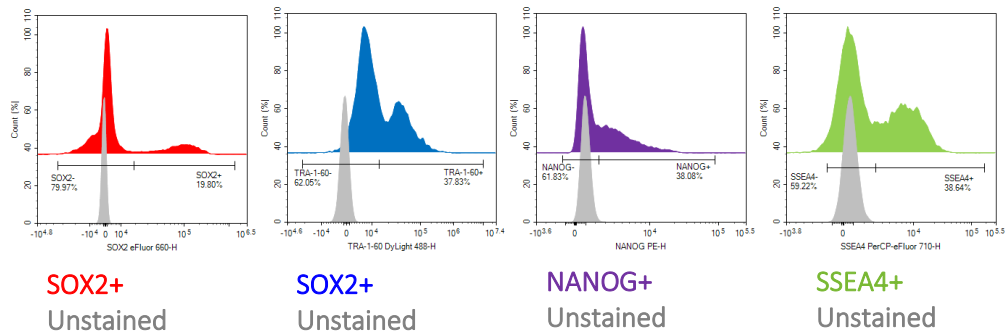
**Fig. S2** – Representative gating strategy for multicolor flow cytometry analysis. Top panel shows the gates selected for unstained samples, the middle panel shows the gating in the presence of multicolor antibodies. The gating strategy selects for singlets and then selects for EpCAM+ cells. Of these, they are gated for double positive initially of SOX2+ and TRA-1-60+, and these double positive gated cells are subsequently gated for double positive NANOG+ and SSEA4+.



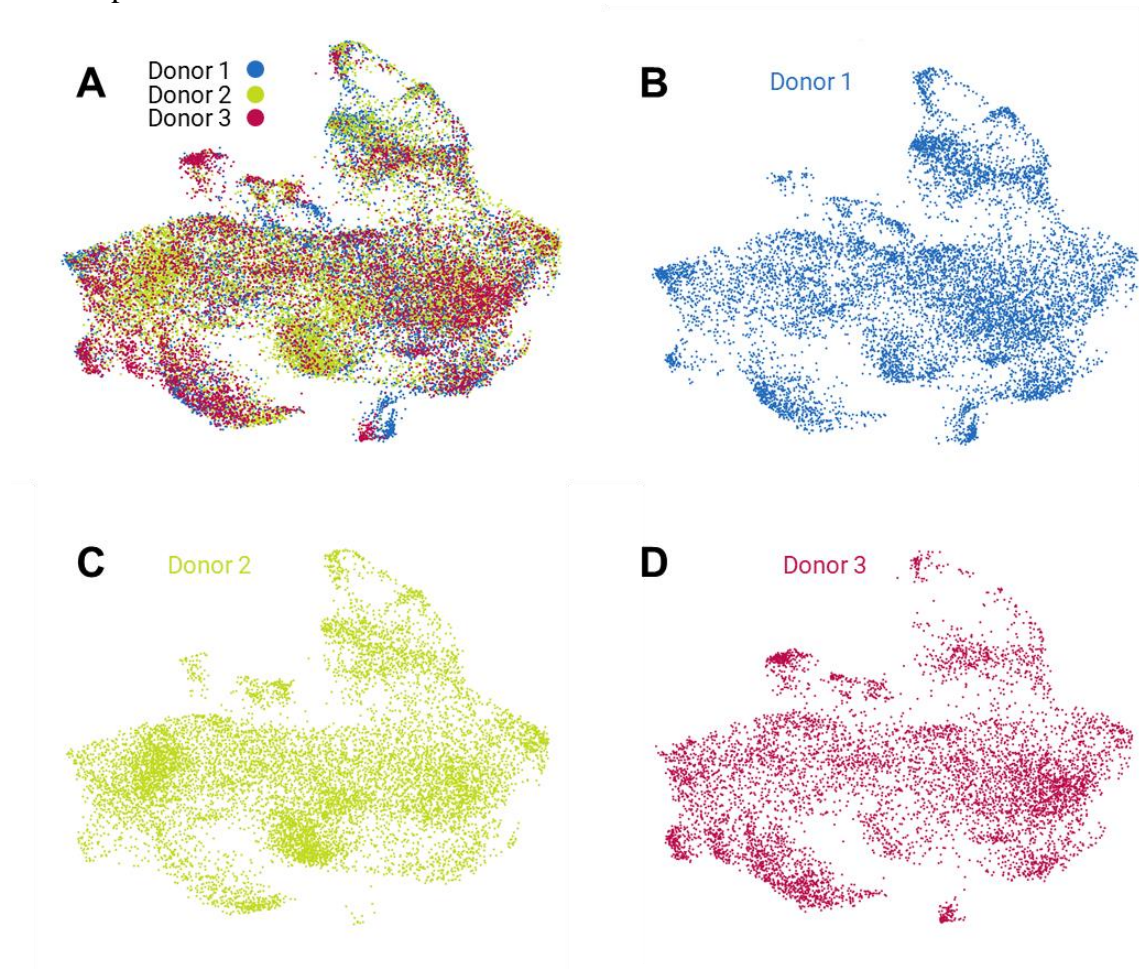
**Fig. S3** – Representative fluorescence minus one (FMO) multicolor flow cytometry analysis on human breastmilk cells (n=2). Each sample contained either all of the antibodies in the panel (multicolor), removal of the antibody of interest (FMO), or no fluorescent antibodies (unstained).



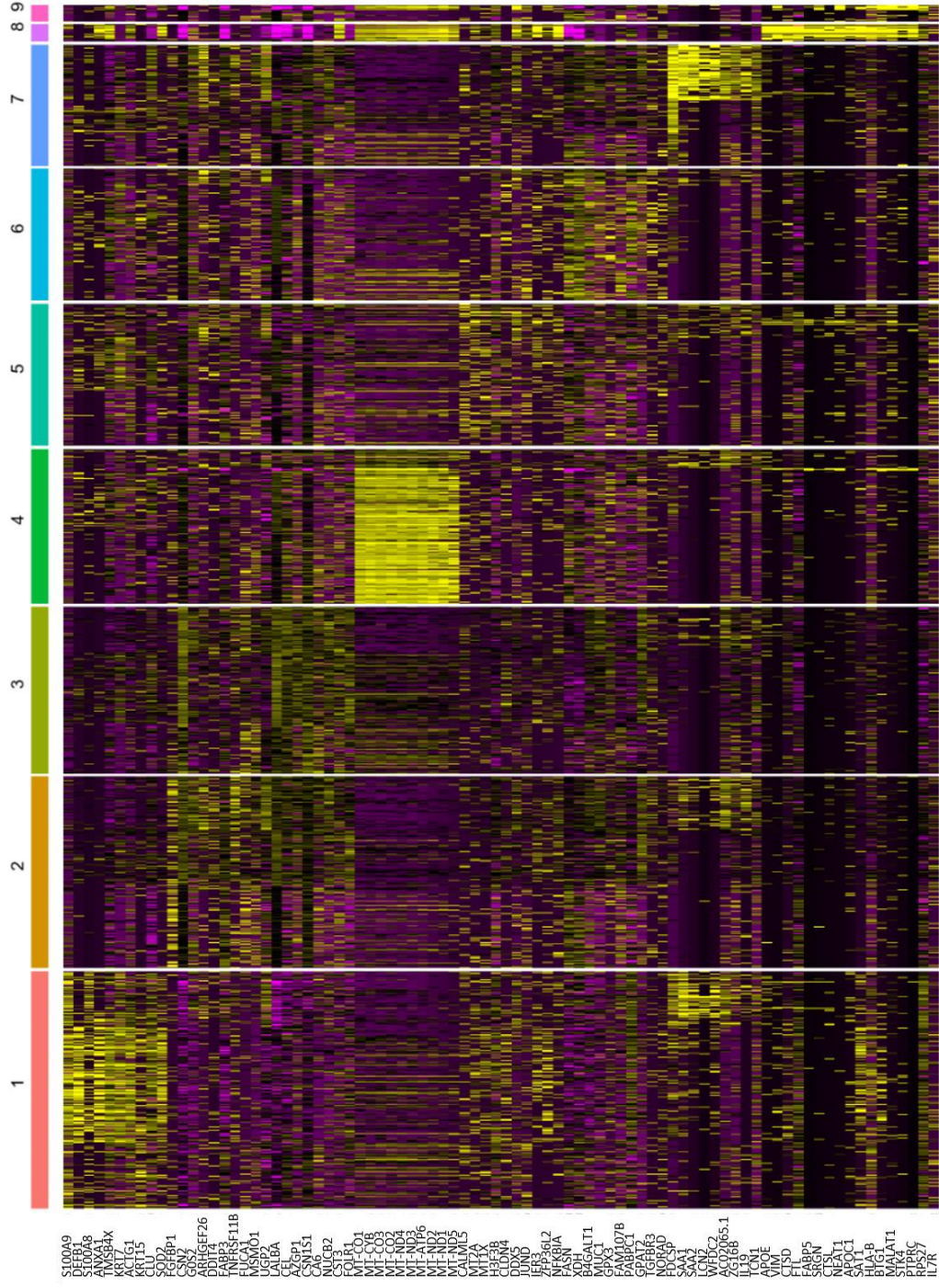
**Fig. S4** – Representative validation of stem cell fluorescent flow cytometry markers in induced pluripotent stem cells (iPSC)



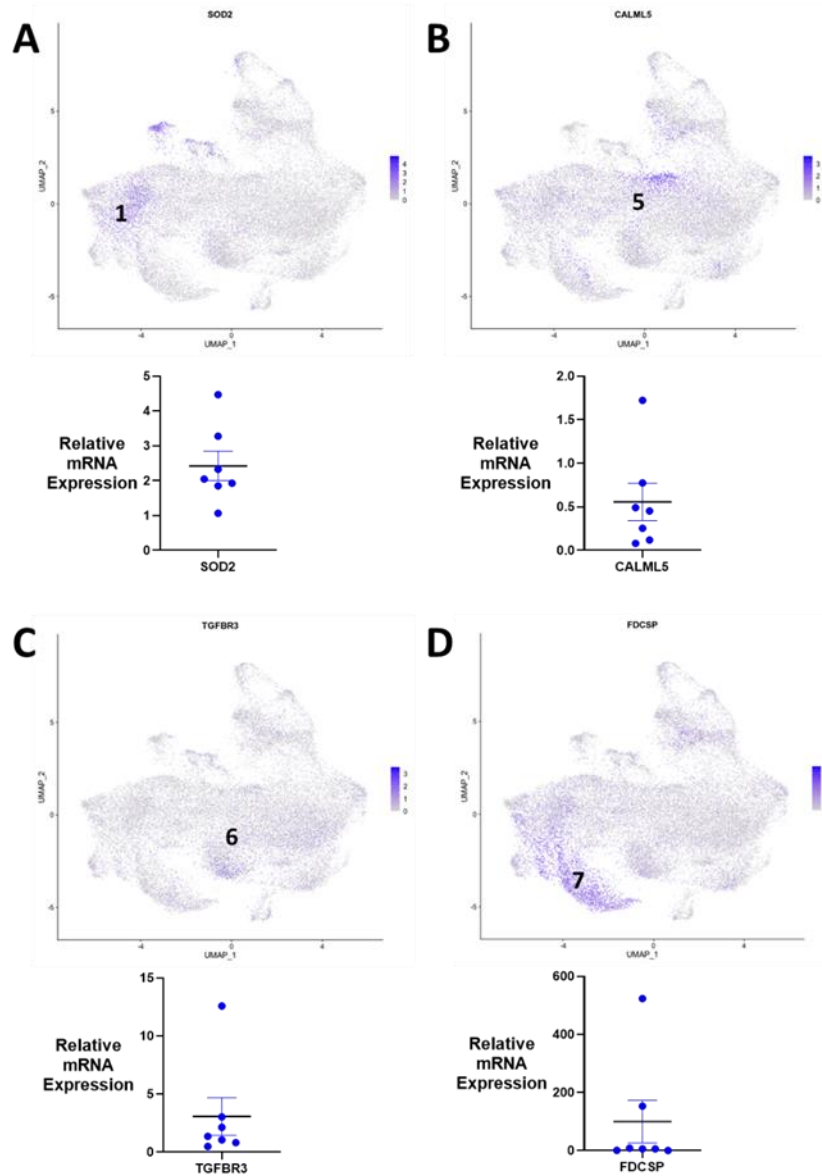
**Fig. S5** – Single cell RNAseq data generated a UMAP plot in Fig. 4C/D. The UMAP cluster is consistent across donors with some variations shown below in individual donor UMAP plots.



**Fig. S6** – Higher quality cluster heatmap shown in Fig. 4E.



**Fig. S7** – Cluster marker mRNA expression in a larger donor pool is relatively consistent (A) UMAP localization and corresponding mRNA expression of epithelial cell marker, progenitor cells (*SOD2*; cluster 1/5) and differentiating cell marker (*CALML5*; cluster 5) are consistently expressed across multiple donor samples. Markers for specialized subtypes are enriched in specific donors (C; *PTPRF*+ lactocytes) and (D; chemotactic epithelial cells). UMAP analysis (N = 3) and RT-qPCR (N = 7).





**Table S1.** Donor characteristics and experimental groups

Donor ID	Weeks Postpartum	Health status	Viability assay	Cell type flow cytometry	Cell type qPCR	Stem cell flow cytometry	Stem cell qPCR	scRNAseq	RNAseq qPCR
2.1	18	H		Y	Y	Y	Y		Y
2.2	28	H		Y		Y			
2.3	31	H	Y					Y	
4	38	H		Y		Y			
5	83	H	Y	Y		Y			
6.1	12	H		Y	Y	Y	Y		Y
6.2	19	H		Y		Y			
6.3	20	H	Y					Y	
8.1	39	H	Y	Y		Y			
8.2	44	H		Y		Y			Y
9	21	M	Y	Y		Y			
11	11	M	Y	Y	Y	Y	Y		Y
12.1	29	H		Y		Y			
12.2	32	H		Y		Y			
12.3	33	H	Y					Y	
13	92	H	Y	Y	Y	Y	Y		Y
17	6	S	Y	Y					
18	5	H		Y	Y	Y	Y		Y
19	36	M		Y					
21	40	H	Y	Y	Y	Y	Y		Y

Health status: H = healthy mother and infant, M = recent mastitis infection, and S = infant currently sick.

**Table S2.** Flow cytometry antibodies used in analysis

Marker	Fluorophore	Company	Cat. No
EpCAM	PE-Cy7	eBioscience	25-9326-42
CD45	Alexa Fluor® 488	R&D Systems	FAB1430G
Vimentin	APC	Invitrogen	MA528601
CK18	FITC	Invitrogen	MA110327
aSMA	Alexa Fluor® 594	R&D Systems	IC1420T-100
SOX2	eFluor® 660	eBioscience	50-9811-82
TRA-1-60	DyLight® 488	Invitrogen	MA1023D488X
Nanog	PE	Invitrogen	PA546891
SSEA4	PerCP-eFluor® 710	eBioscience	46-8843-42
CD49f	eFluor® 450	eBioscience	48-0495-82

**Table S3.** Primer sequences for RT-qPCR

<b>Gene</b>	<b>Forward</b>	<b>Reverse</b>
<i>EEF1A</i>	ATCTCAGGCTGACTGTGCTG	AGTGTGTAAGCCAGAAGGGC
<i>EPCA M</i>	GCTGGCCGTAAACTGCTTTG	ACATTTGGCAGCCAGCTTTG
<i>PTPRC</i>	TGAAATGTGTATGCACCTATTG AAA	TGGGGCCTGTAAAAGTGTCC
<i>VIM</i>	CGCACATTCGAGCAAAGACA	ACAAGAGCGCCCCTAAGTTT
<i>KRT18</i>	AAAGCCTGAGTCCTGTCCCTTC	GGTGAAGCTCATGCTGTCCG
<i>FUT4</i>	GGGGGTTCTTCCTCACCTTG	ATATGGCCTGTGGCAGATGG
<i>PODXL</i>	TCAGACCGTGGTCGTCAAAG	TTCATGTCACTGACCCCTGC
<i>SOX2</i>	TACAGCATGATGCAGGACCA	CCGTTTTCATGTAGGTCTGCGA
<i>NANO G</i>	ACATGAGTACTGCTTTAGTTGG T	TCCACCCCAACCAAAAATTTA ACA
<i>LALBA</i>	AGCCAGGTCCCTCAGTCAA	ATGGGCCAACCAGTAGTCAA
<i>CSN2</i>	GCTCTTGCAAGGGAGACCAT	TCTGTAATAGATTCCTCACTGC TTG
<i>FTH1</i>	AGAACTACCACCAGGACTCAG A	GTCAAAGTAGTAAGACATGGA CAG
<i>SPP1</i>	AACGCCGACCAAGGAAAAC	TGTTGTGGAGGGGTAGGTACA
<i>LYZ</i>	AGGTGTGAGTTGGCCAGAAC	ACACATCCAGTTTGCTAGGC
<i>TMSB1 0</i>	ACGAGACTGCACGGATTGTTT	TCCGCTTCTCCTGCTCAATG
<i>FABP3</i>	ACTACATGAAGTCACTCGCTCA TA	TGAGGGTAGGGGGAAGGTTA
<i>FGFBP 1</i>	CAGCCTGGCTCCTGTTGAAT	GGCGTTCACCTTGTTCTGAG
<i>CALML 5</i>	TACGAGGAGTTCGCGAGGAT	AGAGTCCCAGCACAAAAGCA
<i>FDCSP</i>	TTTCGCCACTTCCACCAAT	AGGTGACCAGGTTTATCGTGA C
<i>SOD2</i>	AAACCTCAGCCCTAACGGTG	CACGTTTGATGGCTTCCAGC
<i>TGFBR 3</i>	CACTAAGCCCCTTGCTGTGA	CCAGACCATGGAAAATTGGTG G
<i>CSN1S 1</i>	CTCACCTGTCTTGTGGCTGT	TGTTTCTCTGCCTGTTTCATACC