

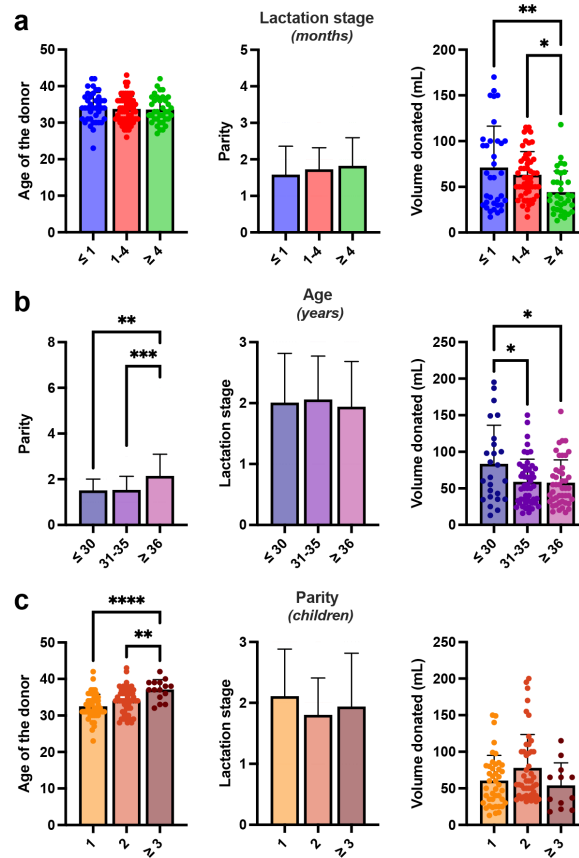
Supplementary Table S1: Primer sequences.

Gene	Forward primer	Reverse primer
<i>PROM1</i>	AGTCGGAACTGGCAGATAGC	GGTAGTGTGTACTGGGCCAAT
<i>CD24</i>	TGAAGAACATGTGAGAGGTTTGAC	GAAAACTGAATCTCCATTCCACAA
<i>KRT18</i>	GAAGAACCACGAAGAGGAAGTAA	ATCTCCAAGGACTGGACTGTA
<i>ITGA6</i>	ATTCTCGCTGGGATCTTGATG	GATCCTTACAGCATGGTATCGG
<i>KRT14</i>	GAGATGCGTGACCAGTATGAG	CTGTTCTCCAGGGATGCTTT
<i>PRLR</i>	ACCTGCCTTCTGAATGGACA	GGCCACCGGTTATGTAGTCT
<i>LTF</i>	AGGAGGAGTGTTCAGTGGTG	CATCAAGGGTCACAGCATCG
<i>LALBA</i>	TTGTCCCTCTGTTCTGGTG	TGGCTTGTGTGTCATAACCAC
<i>CSN1S1</i>	TTTCCAGCAGCTCAACCAAC	TCACCACTGTAGCATGACGT
<i>CSN2</i>	GAGAGGATGAACACCAGGATAAA	GTGCAAGAGTCTGAGGAATAGG
<i>CSN3</i>	TGGCATTAAACCCTGCCTTT	GTGCGAGGCACATATGGATTA
<i>ACTB</i>	CATGTACGTTGCTATCCAGGC	CTCCTTAATGTCACGCACGAT
<i>GAPDH</i>	TCAAGGCTGAGAACGGGAAG	CGCCCCACTTGATTTTGGAG

Supplementary Table S2: Extracted RNA quality.

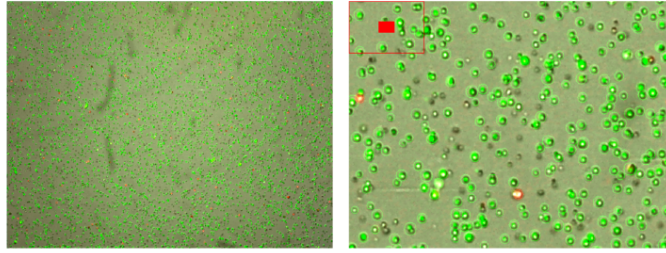
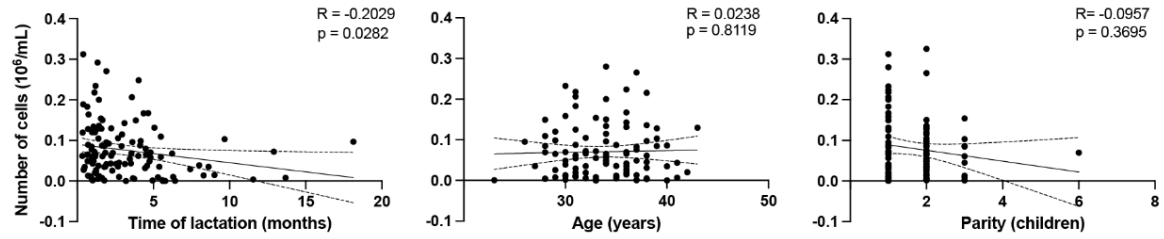
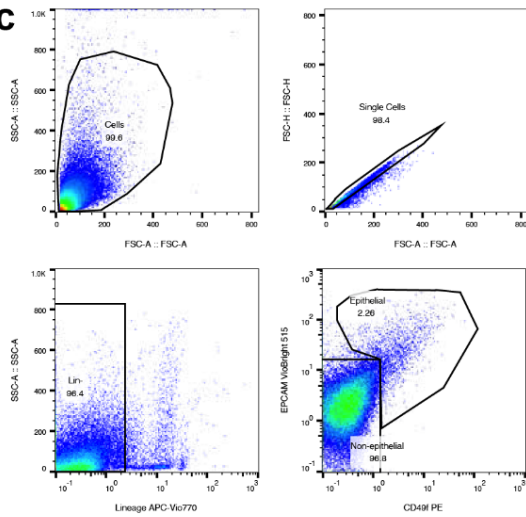
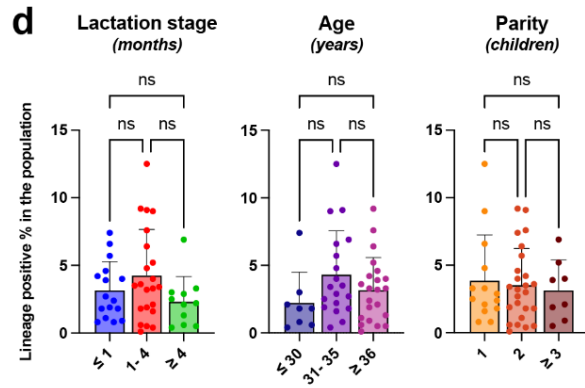
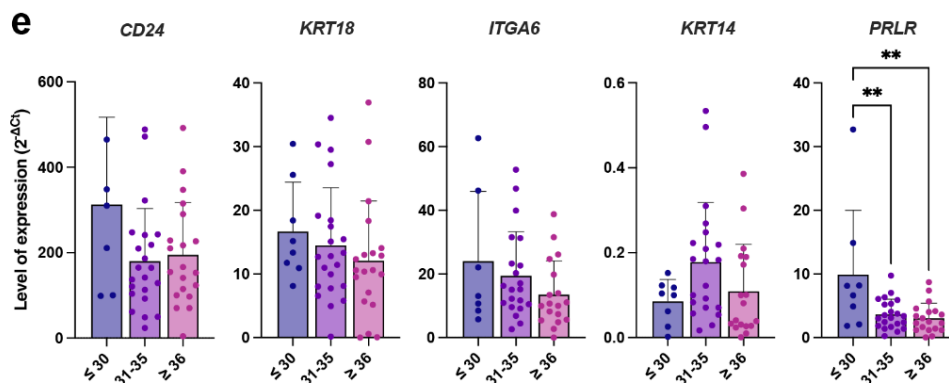
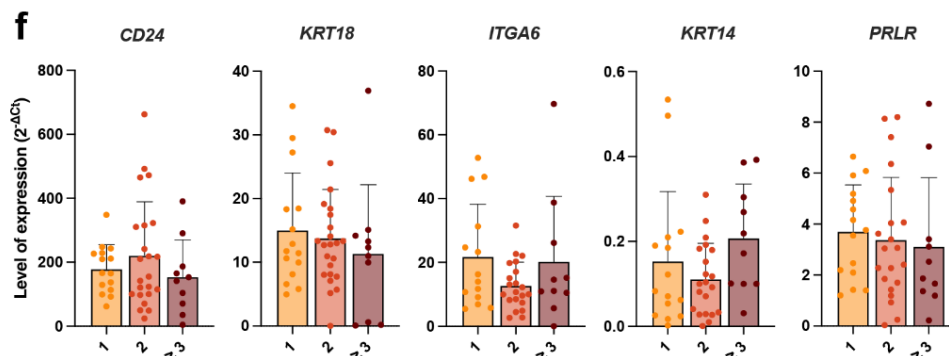
Sample	Day	A260/280 ratio	Sample	Day	A260/280 ratio	Sample	Day	A260/280 ratio
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1	d21	2,15	19	d21	2,15	39	d0	1,94
2	d0	2,17	20	d0	2,24	40	d0	2,07
3	d0	2,16	21	d0	2,07	40	d21	2,15
4	d0	2,15	22	d0	2,09	41	d0	2,36
4	d21	2,16	23	d0	2,12	41	d21	2,17
5	d0	2,15	23	d21	2,17	42	d0	1,52
6	d0	2,14	24	d0	1,89	43	d0	2,14
6	d21	2,18	25	d0	2,16	43	d21	2,02
7	d0	2,05	25	d21	2,16	44	d0	2

7	d21	2,21	26	d0	2,12	45	d0	2,12
8	d0	2,15	26	d21	2,15	46	d0	2,15
8	d21	2,14	27	d0	2,13	47	d0	2,17
9	d0	2,13	28	d0	1,88	47	d21	2,13
9	d21	2,16	29	d0	2,03	48	d0	2,13
10	d0	2,14	30	d0	2,11	48	d21	2,17
10	d21	2,15	30	d21	2,17	49	d0	2,13
11	d0	2,13	31	d0	2,11	49	d21	2,17
11	d21	2,15	31	d21	2,17	50	d0	2,15
12	d0	2,12	32	d0	1,98	50	d21	2,15
12	d21	2,16	32	d21	2,14	51	d0	2,15
13	d0	2,01	33	d0	2,12	52	d0	1,79
13	d21	1,8	34	d0	2,1	52	d21	2,11
14	d0	2,02	34	d21	2,1	53	d0	2,06
14	d21	1,72	35	d0	2,13	54	d0	2,13
15	d0	2,15	35	d21	2,15	55	d0	2,13
15	d21	2,15	36	d0	1,92	55	d21	2,15
16	d0	2,17	36	d21	2,15	56	d0	2,1
17	d0	2,03	37	d0	1,85	56	d21	2,12
17	d21	2,12	37	d21	2,11	57	d0	2,04
18	d0	1,91	38	d0	1,8	57	d0	2,06



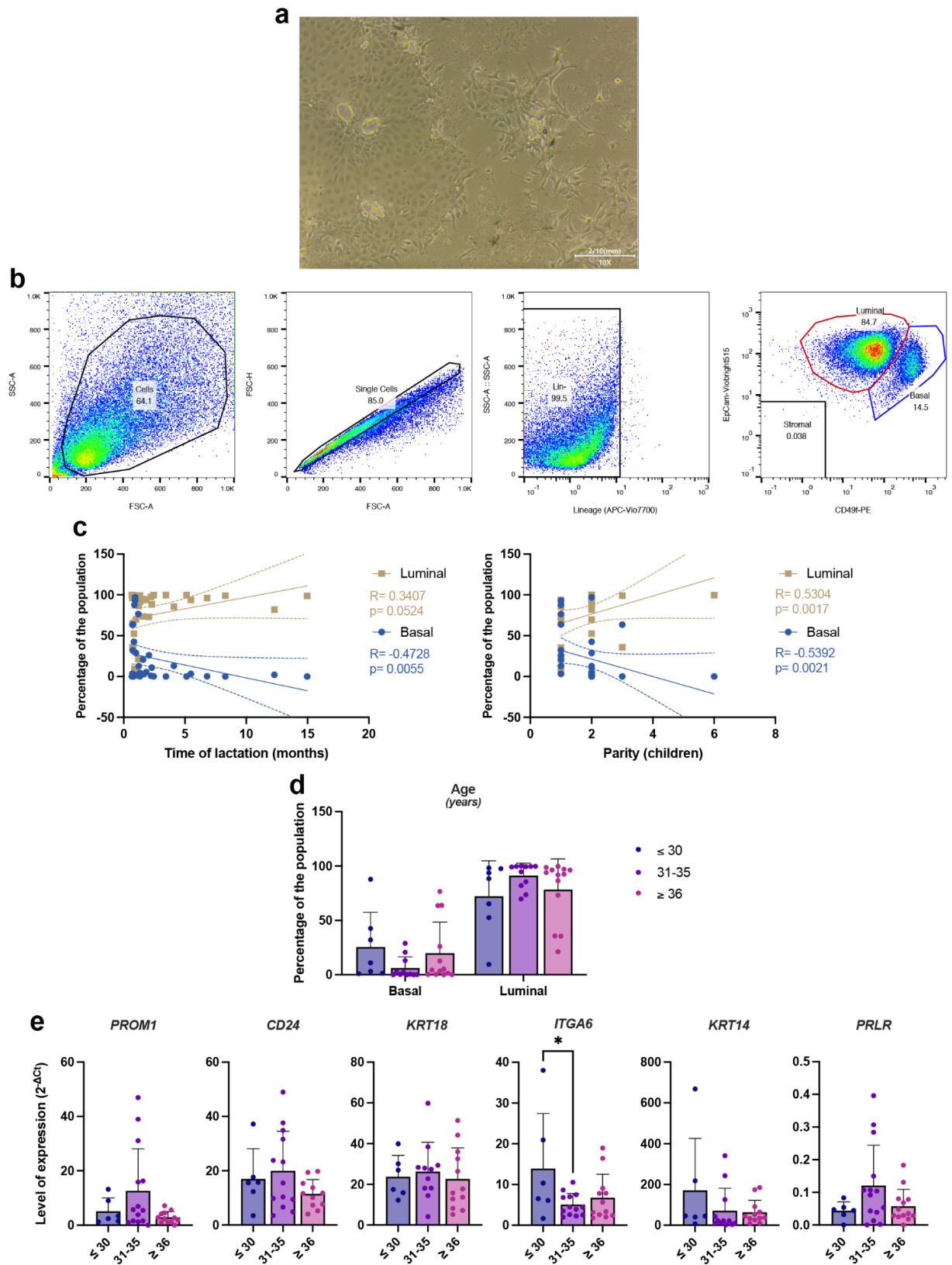
Supplementary Figure S1: Comparison of the mother's characteristics

(a) Average of the donor's age, parity and volume donated in lactation stage groups (in months). **(b)** Average of the donor's parity, lactation stage and volume donated in age groups (in years). **(c)** Average of the donor's age, lactation stage and volume donated in parity groups (number of children). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$.

a**b****c****d****e****f**

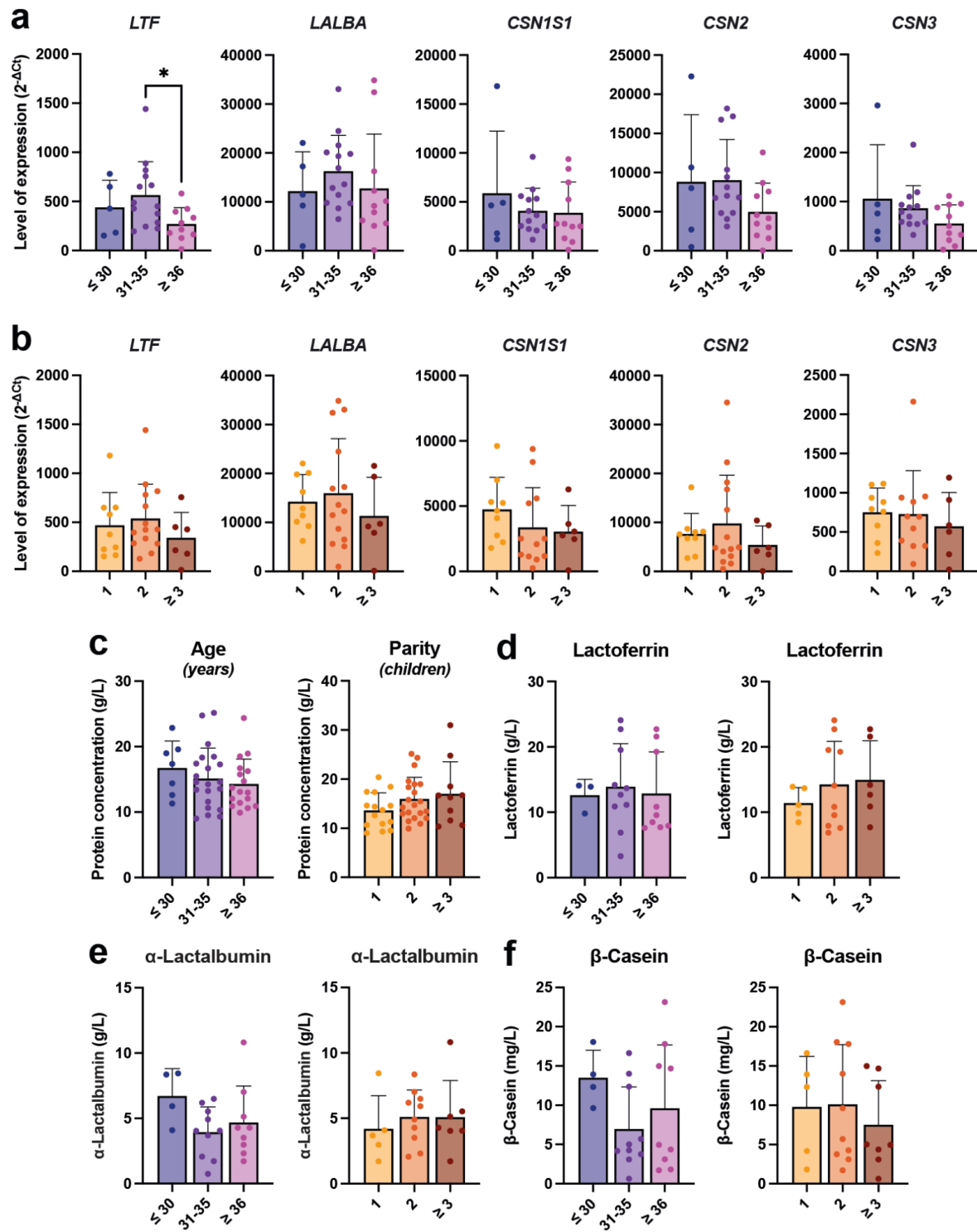
Supplementary Figure S2: Gating strategy and cell content after isolation.

(a) Representative images of cell counting with acridine orange and propidium iodide staining after cell isolation from fresh milk. Living cells are in green and dead cells are in red. Unnucleated particles are in grey. **(b)** Number of cells ($10^6/\text{mL}$) depending on time of lactation (months), age of the donor (years) and parity (number of children). Linear regression curves are represented with 95% confidence intervals in dots. Spearman correlation test was performed, and Spearman coefficient (R) and p-value (two-tailed test, $\alpha=0.05$) associated with the test is shown on the graph. **(c)** Gating strategy for milk sample after isolation. Viable cells were gated on FSC-A/SSC-A, then single cells on FSC-A/FSC-H. Lineage cells were detected thanks to APC staining (CD45, GPA, CD31). Epithelial and non-epithelial cells were gated with CD49f/ EpCam staining. **(d)** Percentage of lineage positive cells (CD31^+ , CD45^+ , GPA^+) after isolation from fresh milk determined by flow cytometry, depending on the lactation stage (in months), the age of the donor (in years) and the parity (in number of children). ns: non-significant. **(e)** Average level of expression ($2^{-\Delta\text{Ct}}$) of specific genes in cells isolated from fresh milk assessed by RT-qPCR, depending on the age of the donor (in months). **(f)** Average level of expression ($2^{-\Delta\text{Ct}}$) in cells just isolated from fresh milk, assessed by RT-qPCR, depending on the parity of the donor (number of children). * $P<0.05$, ** $P<0.01$.



Supplementary Figure S3: Cell content after culture of human milk cells.

(a) Representative image of milk-derived cells colony formed after 10 days of culture *in vitro*. Scale bar: 200µm. **(b)** Gating strategy for milk sample after 21 days of culture. Viable cells were gated on FSC-A/SSC-A, then single cells on FSC-A/FSC-H. Non-epithelial cells were detected thanks to APC staining (CD45, GPA, CD31). Epithelial (luminal and basal) and stromal cells were gated with CD49f/EpCam staining. **(c)** Percentage of luminal and basal cells depending on time of lactation (months) and parity (number of children). Linear regression curves are represented with 95% confidence intervals in dots. Spearman correlation test was performed, and Spearman coefficient (R) and p-value (two-tailed test, alpha=0.05) associated with the test is shown on the graph. **(d)** Percentage of basal and luminal cells after 21 days of culture determined by flow cytometry, depending on the age of the donor (in years). Two-way ANOVA followed by a Tukey multiple-comparisons test was performed to determine statistical significance. **(e)** Average level of expression ($2^{-\Delta Ct}$) of specific genes in cells cultured for 21 days, assessed by RT-qPCR, depending on the donor's age (in years). *P<0.05.



Supplementary Figure S4: Analysis of the milk component in the fresh milk.

(a) Average level of expression ($2^{-\Delta Ct}$) of specific lactation genes in cells just isolated from fresh milk, assessed by RT-qPCR, depending on the donor's age (in years). (b) Average level of expression ($2^{-\Delta Ct}$) of specific lactation genes in cells just isolated

from fresh milk, assessed by RT-qPCR, depending on the donor's parity (number of children). **(c)** Average protein concentration in human milk, depending on donor's age or parity. **(d)** Average Lactoferrin protein concentration detected in human milk by ELISA, depending on the age or the parity of the donor (number of children). **(e)** Average α -Lactalbumin protein concentration detected in human milk by ELISA, depending on the age or the parity of the donor. **(f)** Average β -Casein protein concentration detected in human milk by ELISA, depending on the age or the parity of the donor. *P<0.05.