Supplementary data

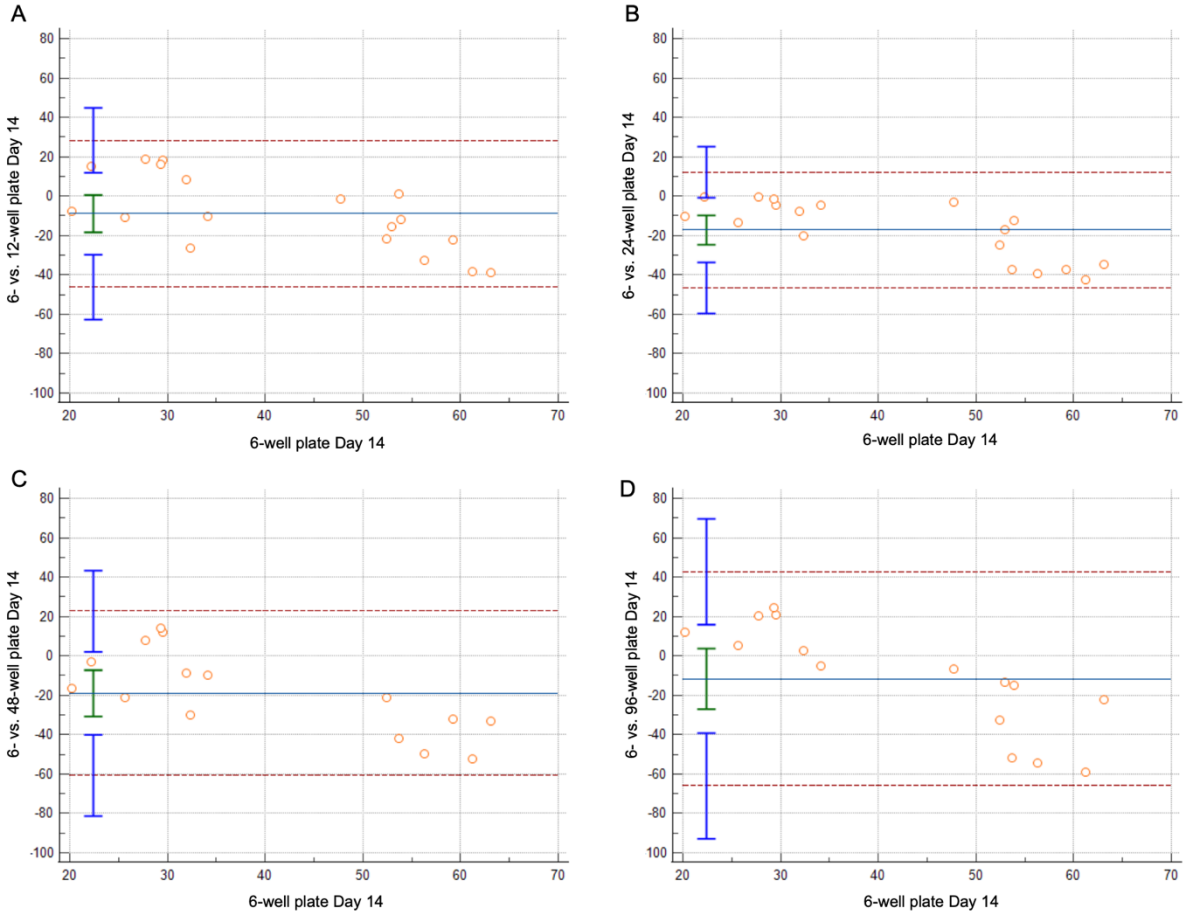

Fig. S1 Bland-Altman plots illustrating the comparison between the well sizes using flow cytometry on day 14. **a** 6- vs. 12-well plate, **b** 6- vs. 24-well plate, **c** 6- vs. 48-well plate and **d** 6- vs. 96-well plate. The two horizontal red dotted lines represent the limits of agreement (upper and lower) (mean difference ± 1.96), the horizontal blue line represents the bias (mean difference; smaller well size minus 6-well plate) between the two methods and the error bars (blue and green vertical lines) represent the 95% confidence intervals for the limit of agreement and mean difference, respectively.

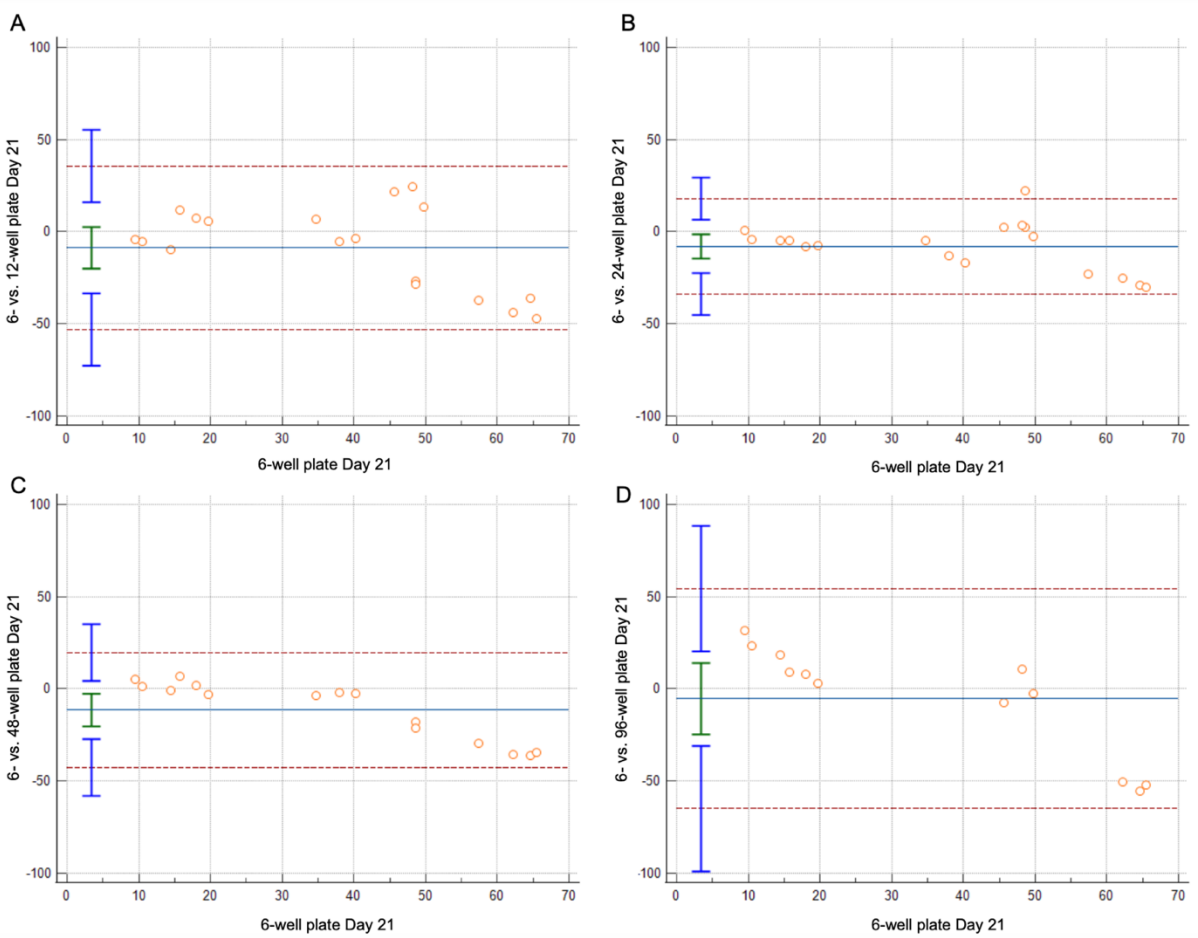

Fig. S2 Bland-Altman plots illustrating the comparison between the well sizes using flow cytometry on day 21. **a** 6- vs. 12-well plate, **b** 6- vs. 24-well plate, **c** 6- vs. 48-well plate and **d** 6- vs. 96-well plate. The two horizontal red dotted lines represent the limits of agreement (upper and lower) (mean difference ± 1.96), the horizontal blue line represents the bias (mean difference; smaller well size minus 6-well plate) between the two methods and the error bars (blue and green vertical lines) represent the 95% confidence intervals for the limit of agreement and mean difference, respectively.

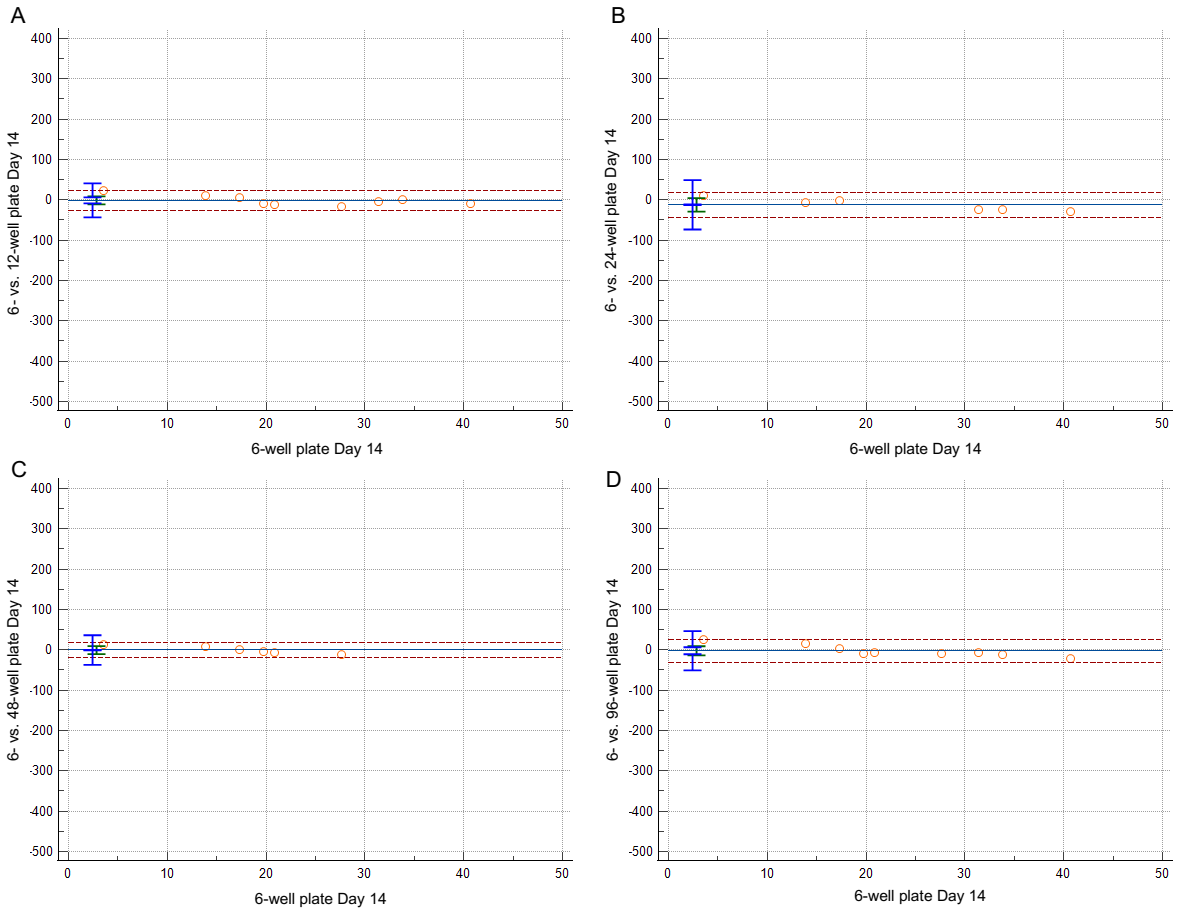

Fig. S3 Bland-Altman plots illustrating the comparison between the well sizes for spectrophotometry on day 14. **a** 6- vs. 12-well plate, **b** 6- vs. 24-well plate, **c** 6- vs. 48-well plate and **d** 6- vs. 96-well plate. The two horizontal red dotted lines represent the limits of agreement (upper and lower) (mean difference ± 1.96), the horizontal blue line represents the bias (mean difference; smaller well size minus 6-well plate) between the two methods and the error bars (blue and green vertical lines) represent the 95% confidence intervals for the limit of agreement and mean difference, respectively.

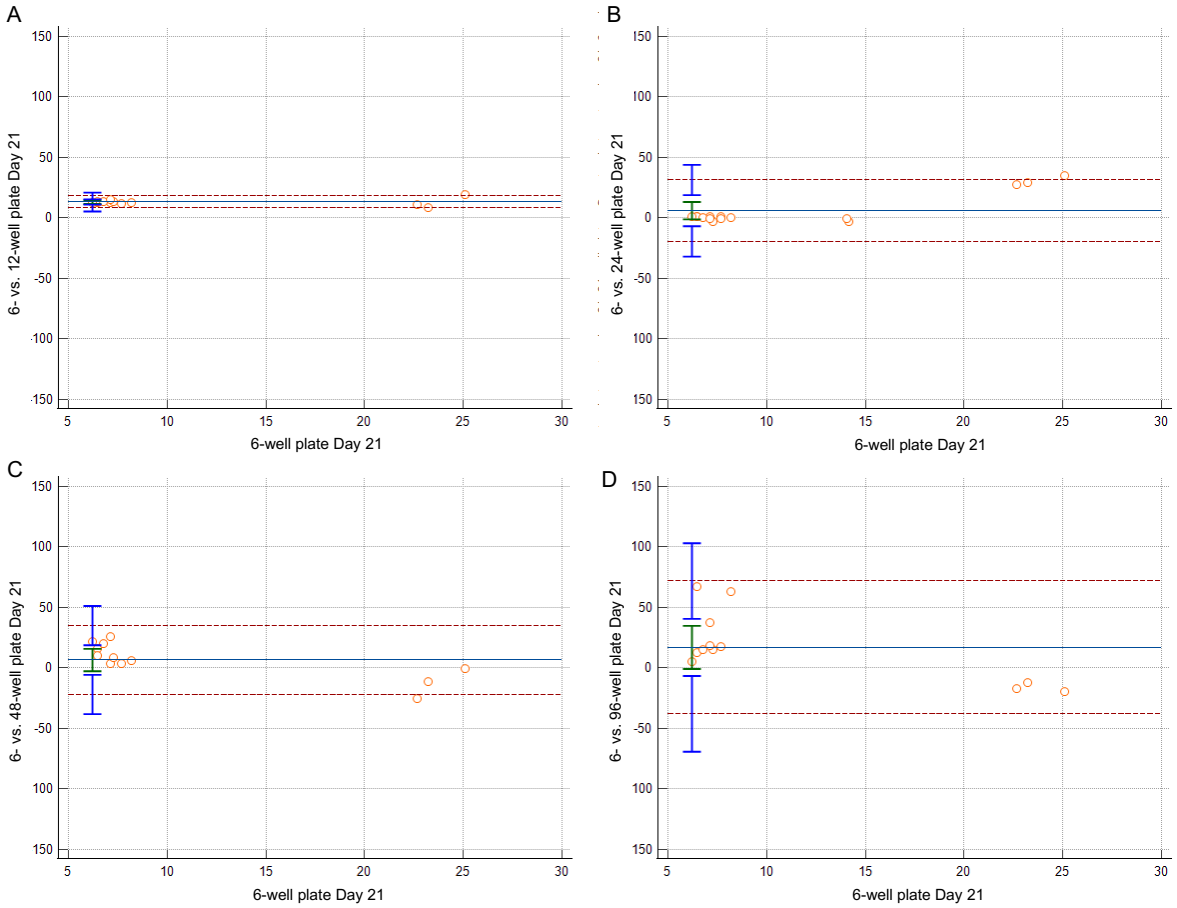

Fig. S4 Bland-Altman plots illustrating the comparison between the well sizes for spectrophotometry on day 21. **a** 6- vs. 12-well plate, **b** 6- vs. 24-well plate, **c** 6- vs. 48-well plate and **d** 6- vs. 96-well plate. The two horizontal red dotted lines represent the limits of agreement (upper and lower) (mean difference ± 1.96), the horizontal blue line represents the bias (mean difference; smaller well size minus 6-well plate) between the two methods and the error bars (blue and green vertical lines) represent the 95% confidence intervals for the limit of agreement and mean difference, respectively.

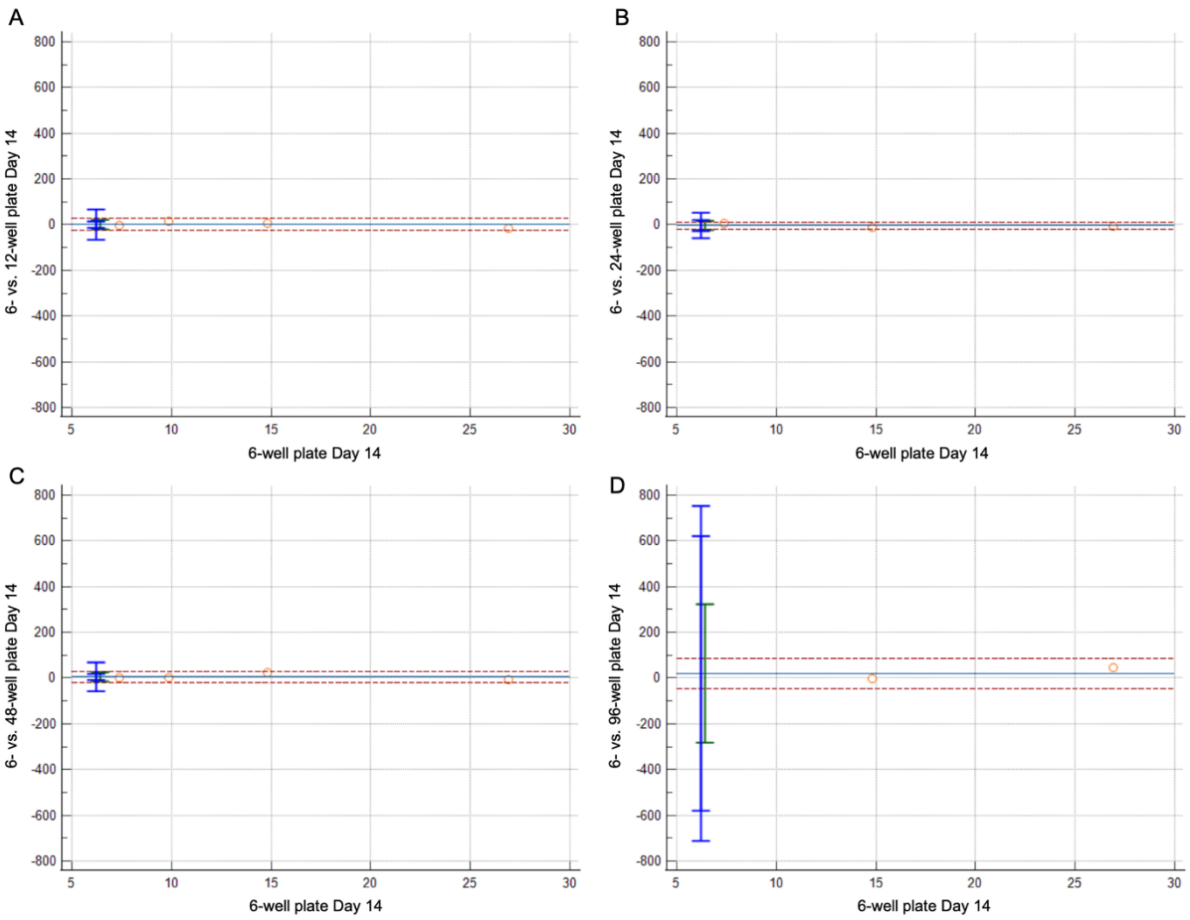

Fig. S5 Bland-Altman plots illustrating the comparison between the well sizes for *PPARG* expression using RT-qPCR on day 14. **a** 6- vs. 12-well plate, **b** 6- vs. 24-well plate, **c** 6- vs. 48-well plate and **d** 6- vs. 96-well plate. The two horizontal red dotted lines represent the limits of agreement (upper and lower) (mean difference ± 1.96), the horizontal blue line represents the bias (mean difference; smaller well size minus 6-well plate) between the two methods and the error bars (blue and green vertical lines) represent the 95% confidence intervals for the limit of agreement and mean difference, respectively.

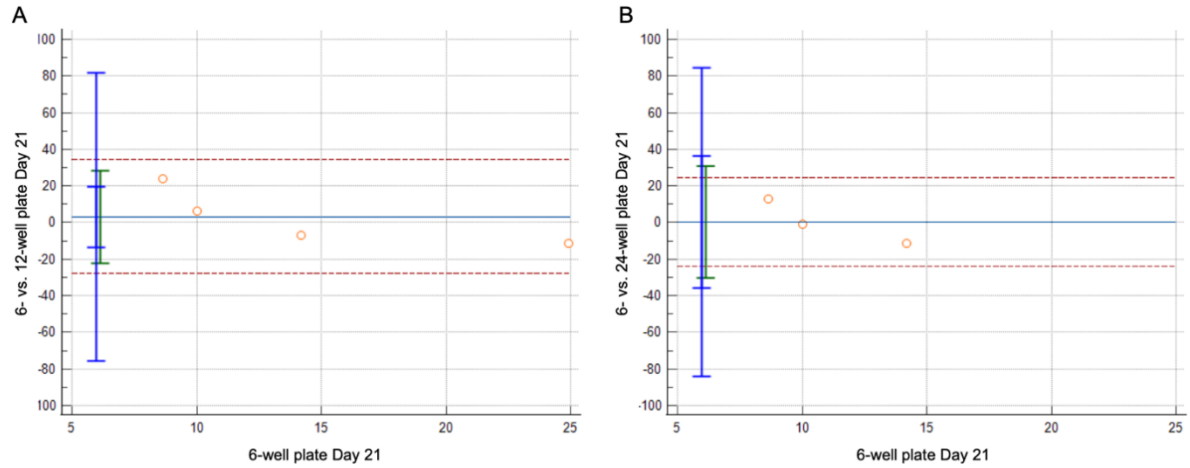

Fig. S6 Bland-Altman plots illustrating the comparison between the well sizes for *PPARG* expression using RT-qPCR on day 21. **a** 6- vs. 12-well plate and **b** 6- vs. 24-well plate. The two horizontal red dotted lines represent

the limits of agreement (upper and lower) (mean difference ± 1.96), the horizontal blue line represents the bias (mean difference; smaller well size minus 6-well plate) between the two methods and the error bars (blue and green vertical lines) represent the 95% confidence intervals for the limit of agreement and mean difference, respectively.

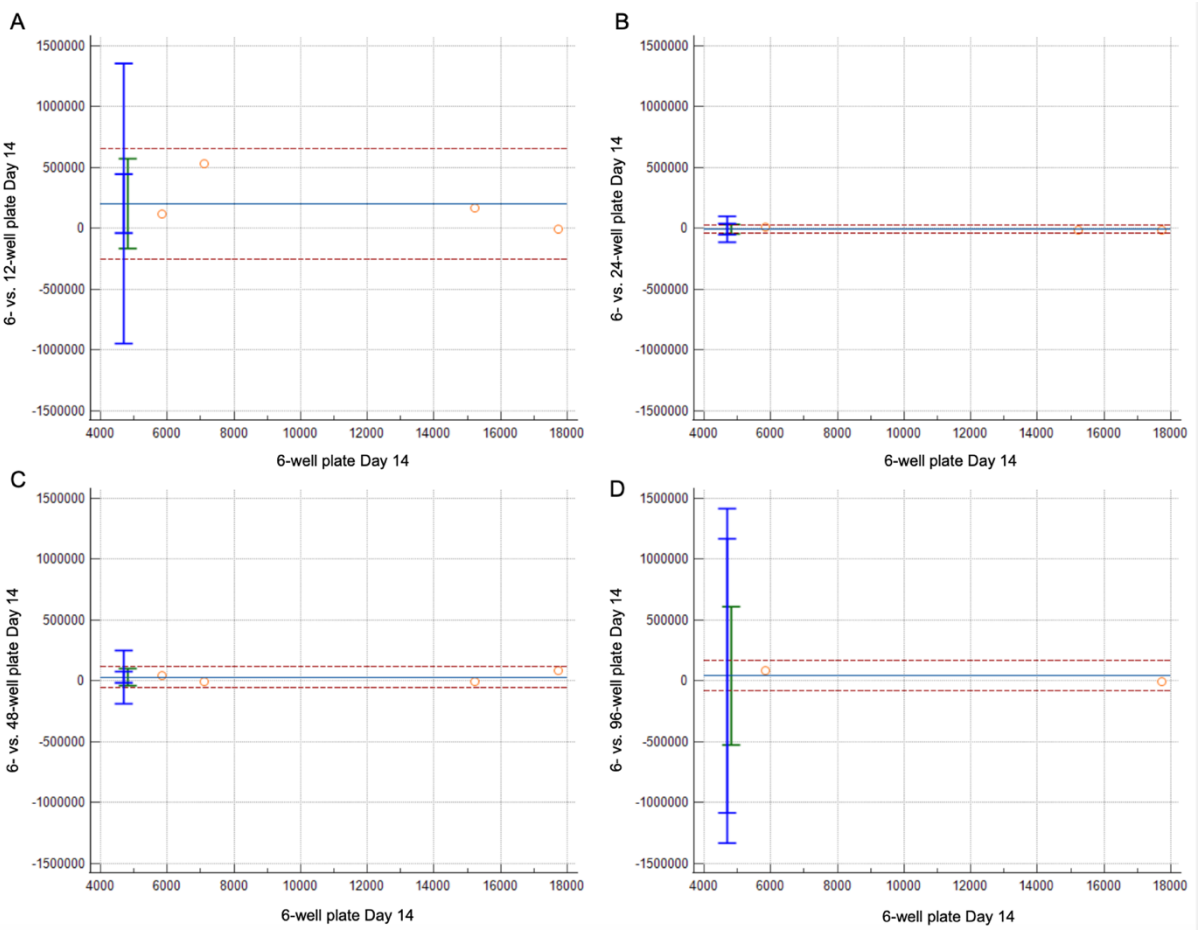

Fig. S7 Bland-Altman plots illustrating the comparison between the well sizes for *FABP4* expression using RT-qPCR on day 14. **a** 6- vs. 12-well plate, **b** 6- vs. 24-well plate, **c** 6- vs. 48-well plate and **d** 6- vs. 96-well plate. The two horizontal red dotted lines represent the limits of agreement (upper and lower) (mean difference ± 1.96), the horizontal blue line represents the bias (mean difference; smaller well size minus 6-well plate) between the two methods and the error bars (blue and green vertical lines) represent the 95% confidence intervals for the limit of agreement and mean difference, respectively.

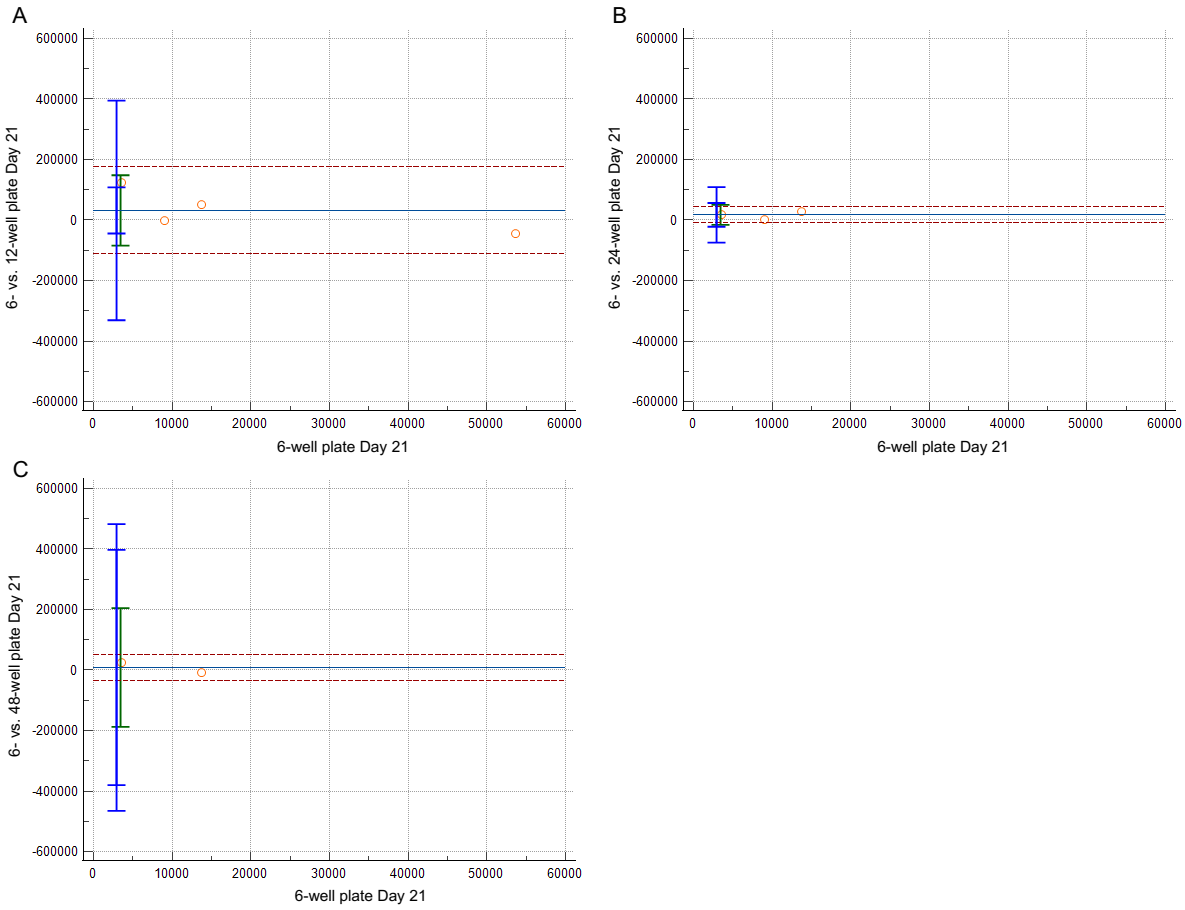

Fig. S8 Bland-Altman plots illustrating the comparison between the well sizes for *FABP4* expression using RT-qPCR on day 21. **a** 6- vs. 12-well plate, **b** 6- vs. 24-well plate and **c** 6- vs. 48-well plate. The two horizontal red dotted lines represent the limits of agreement (upper and lower) (mean difference ± 1.96), the horizontal blue line represents the bias (mean difference; smaller well size minus 6-well plate) between the two methods and the error bars (blue and green vertical lines) represent the 95% confidence intervals for the limit of agreement and mean difference, respectively.

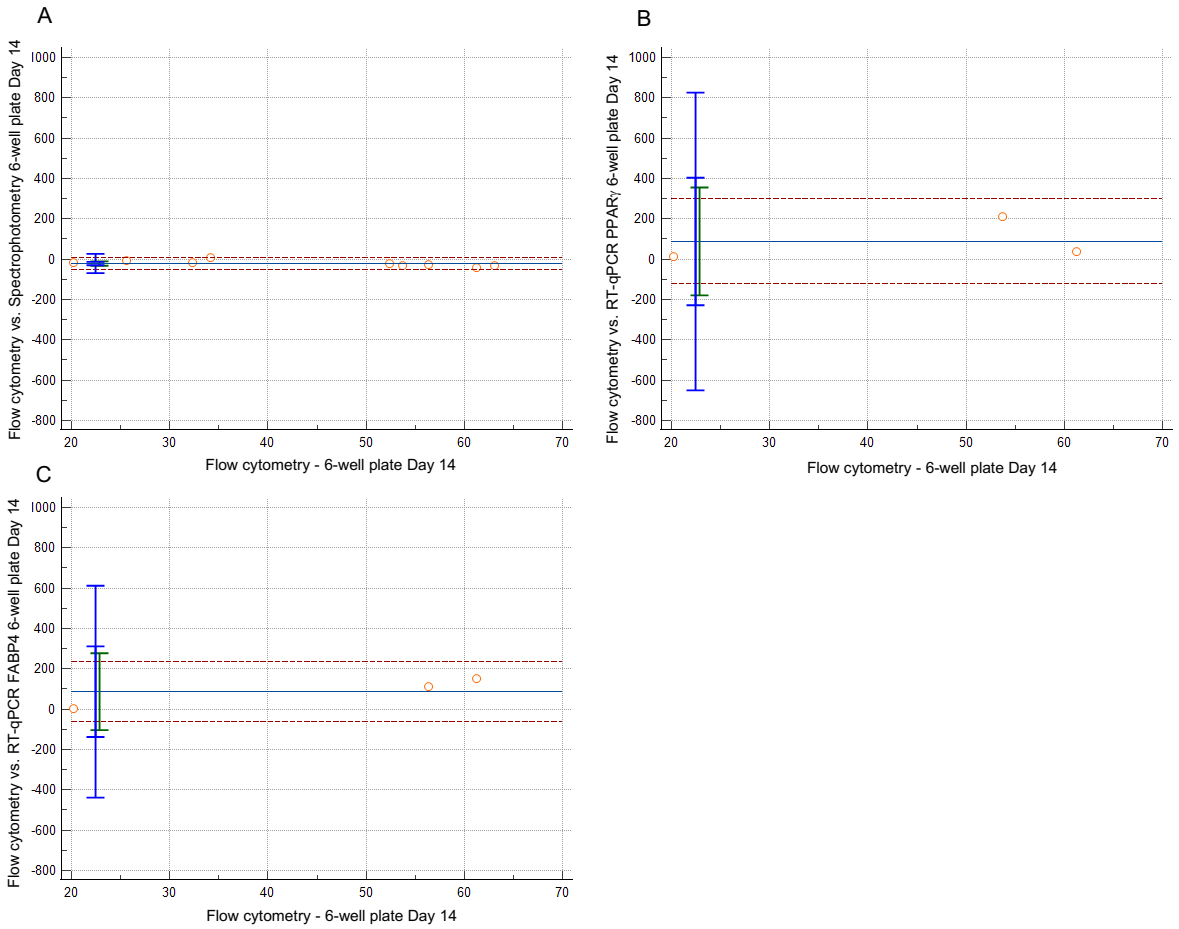

Fig. S9 Bland-Altman plots illustrating the comparison between the methods used in this study using flow cytometry as the reference method in the 6-well plate on day 14. **a** Flow cytometry vs. spectrophotometry, **b** Flow cytometry vs. RT-qPCR *PPARg* and **c** Flow cytometry vs. RT-qPCR *FABP4.* The two horizontal red dotted lines represent the limits of agreement (upper and lower) (mean difference ± 1.96), the horizontal blue line represents the bias (mean difference; other method minus flow cytometry) between the two methods and the error bars (blue and green vertical lines) represent the 95% confidence intervals for the limit of agreement and mean difference, respectively.

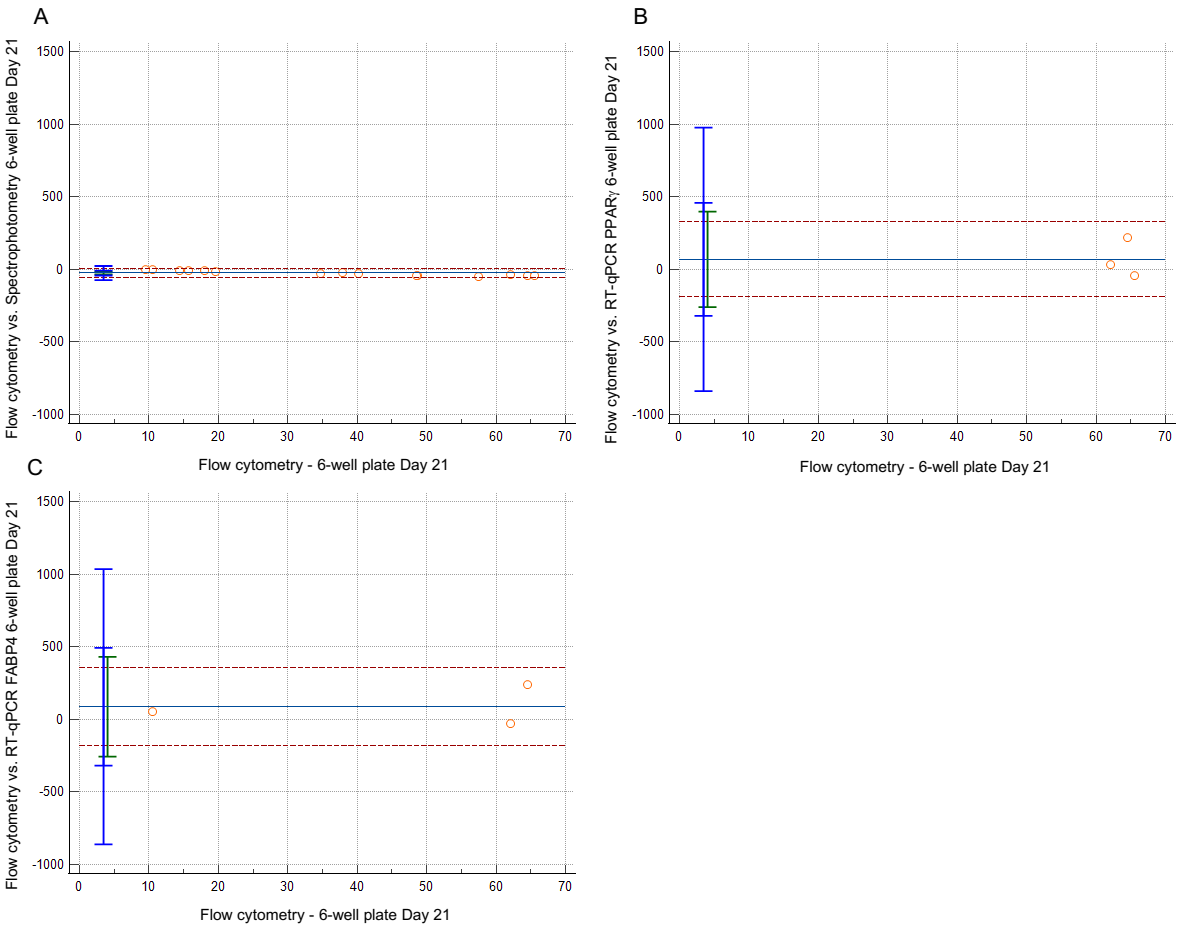

Fig. S10 Bland-Altman plots illustrating the comparison between the methods used in this study using flow cytometry as the reference method in the 6-well plate on day 21**. a** Flow cytometry vs. spectrophotometry, **b** Flow cytometry vs. RT-qPCR *PPARg* and **c** Flow cytometry vs. RT-qPCR *FABP4*. The two horizontal red dotted lines represent the limits of agreement (upper and lower) (mean difference ± 1.96), the horizontal blue line represents the bias (mean difference; other method minus flow cytometry) between the two methods and the error bars (blue and green vertical lines) represent the 95% confidence intervals for the limit of agreement and mean difference, respectively.

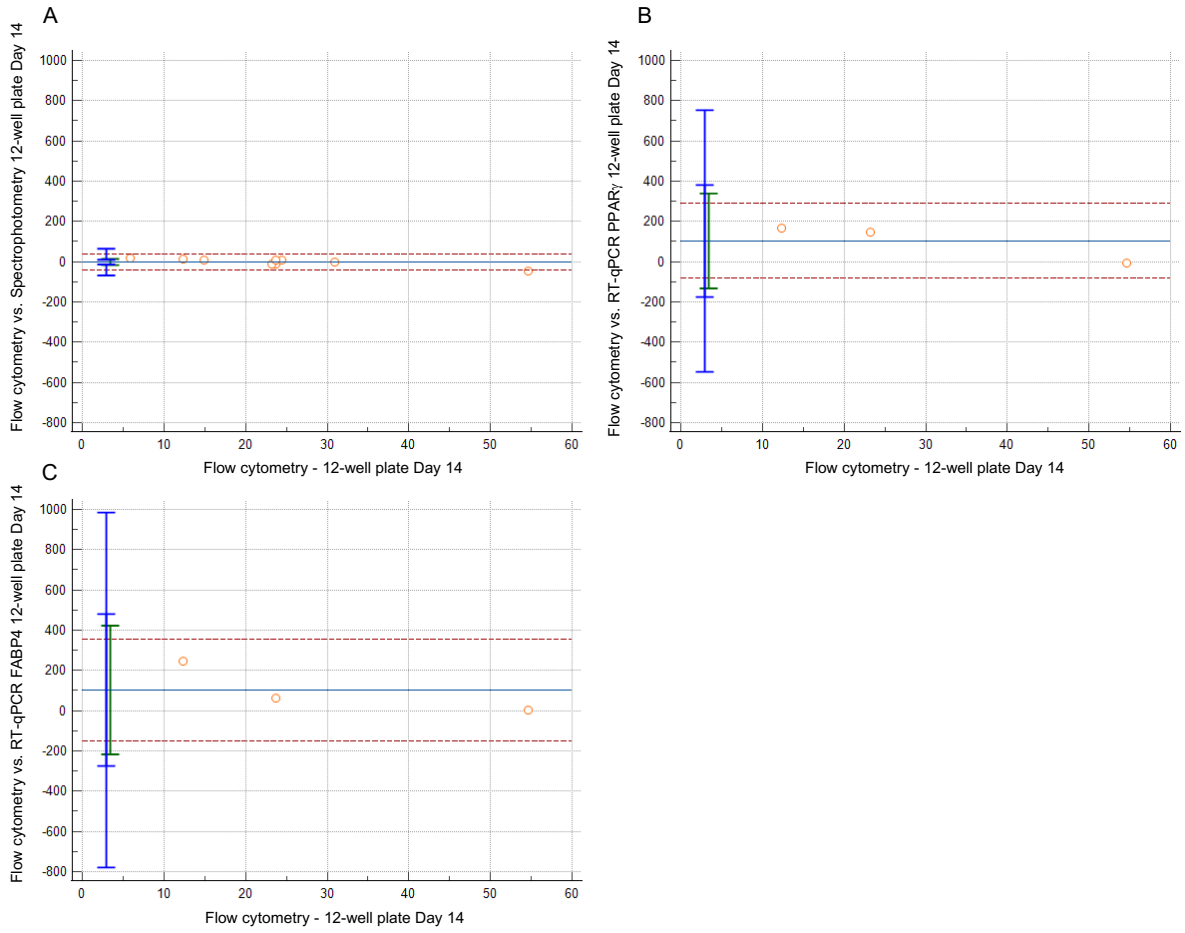

Fig. S11 Bland-Altman plots illustrating the comparison between the methods used in this study using flow cytometry as the reference method in the 12-well plate on day 14. **a** Flow cytometry vs. spectrophotometry, **b** Flow cytometry vs. RT-qPCR *PPARg* and **c** Flow cytometry vs. RT-qPCR *FABP4*. The two horizontal red dotted lines represent the limits of agreement (upper and lower) (mean difference ± 1.96), the horizontal blue line represents the bias (mean difference; other method minus flow cytometry) between the two methods and the error bars (blue and green vertical lines) represent the 95% confidence intervals for the limit of agreement and mean difference, respectively

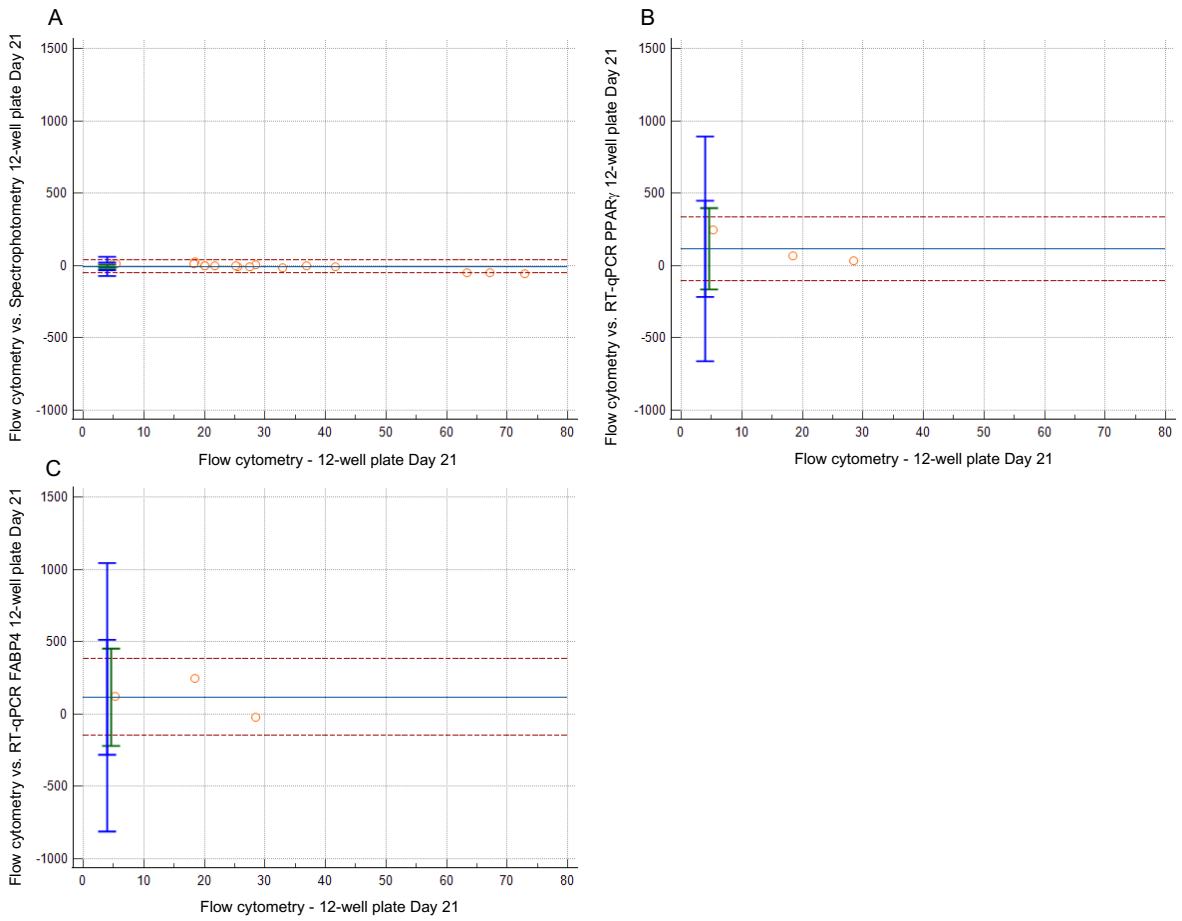

Fig. S12 Bland-Altman plots illustrating the comparison between the methods used in this study using flow cytometry as the reference method in the 12-well plate on day 21. **a** Flow cytometry vs. spectrophotometry, **b** Flow cytometry vs. RT-qPCR PPARg and **c** Flow cytometry vs. RT-qPCR FABP4. The two horizontal red dotted lines represent the limits of agreement (upper and lower) (mean difference ± 1.96), the horizontal blue line represents the bias (mean difference; other method minus flow cytometry) between the two methods and the error bars (blue and green vertical lines) represent the 95% confidence intervals for the limit of agreement and mean difference, respectively.

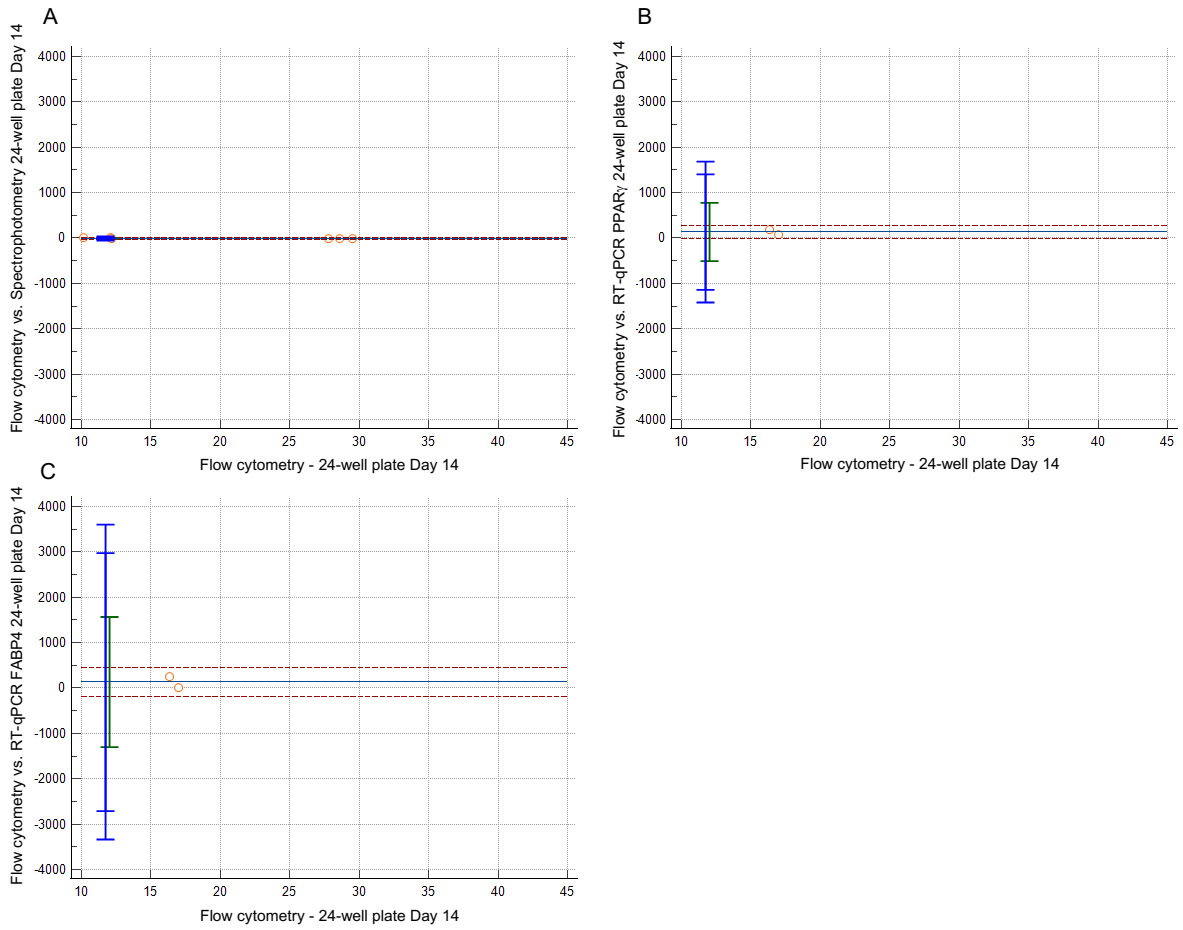

Fig. S13 Bland-Altman plots illustrating the comparison between the methods used in this study using flow cytometry as the reference method in the 24-well plate on day 14. **a** Flow cytometry vs. spectrophotometry, **b** Flow cytometry vs. RT-qPCR *PPARg* and **c** Flow cytometry vs. RT-qPCR *FABP4*. The two horizontal red dotted lines represent the limits of agreement (upper and lower) (mean difference ± 1.96), the horizontal blue line represents the bias (mean difference; other method minus flow cytometry) between the two methods and the error bars (blue and green vertical lines) represent the 95% confidence intervals for the limit of agreement and mean difference, respectively.

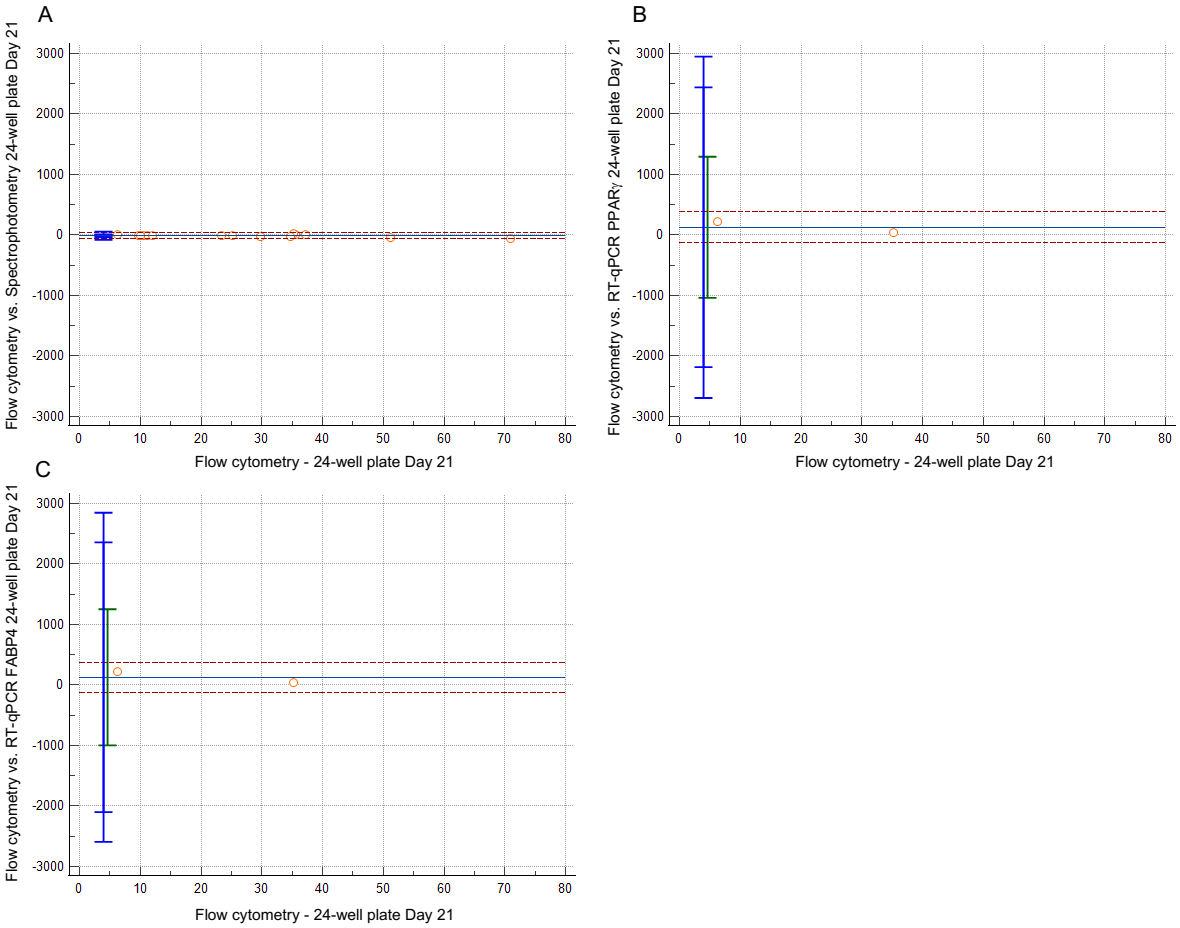

Fig. S14 Bland-Altman plots illustrating the comparison between the methods used in this study using flow cytometry as the reference method in the 24-well plate on day 21. **a** Flow cytometry vs. spectrophotometry, **b** Flow cytometry vs. RT-qPCR *PPARg* and **c** Flow cytometry vs. RT-qPCR *FABP4*. The two horizontal red dotted lines represent the limits of agreement (upper and lower) (mean difference ± 1.96), the horizontal blue line represents the bias (mean difference; other method minus flow cytometry) between the two methods and the error bars (blue and green vertical lines) represent the 95% confidence intervals for the limit of agreement and mean difference, respectively.

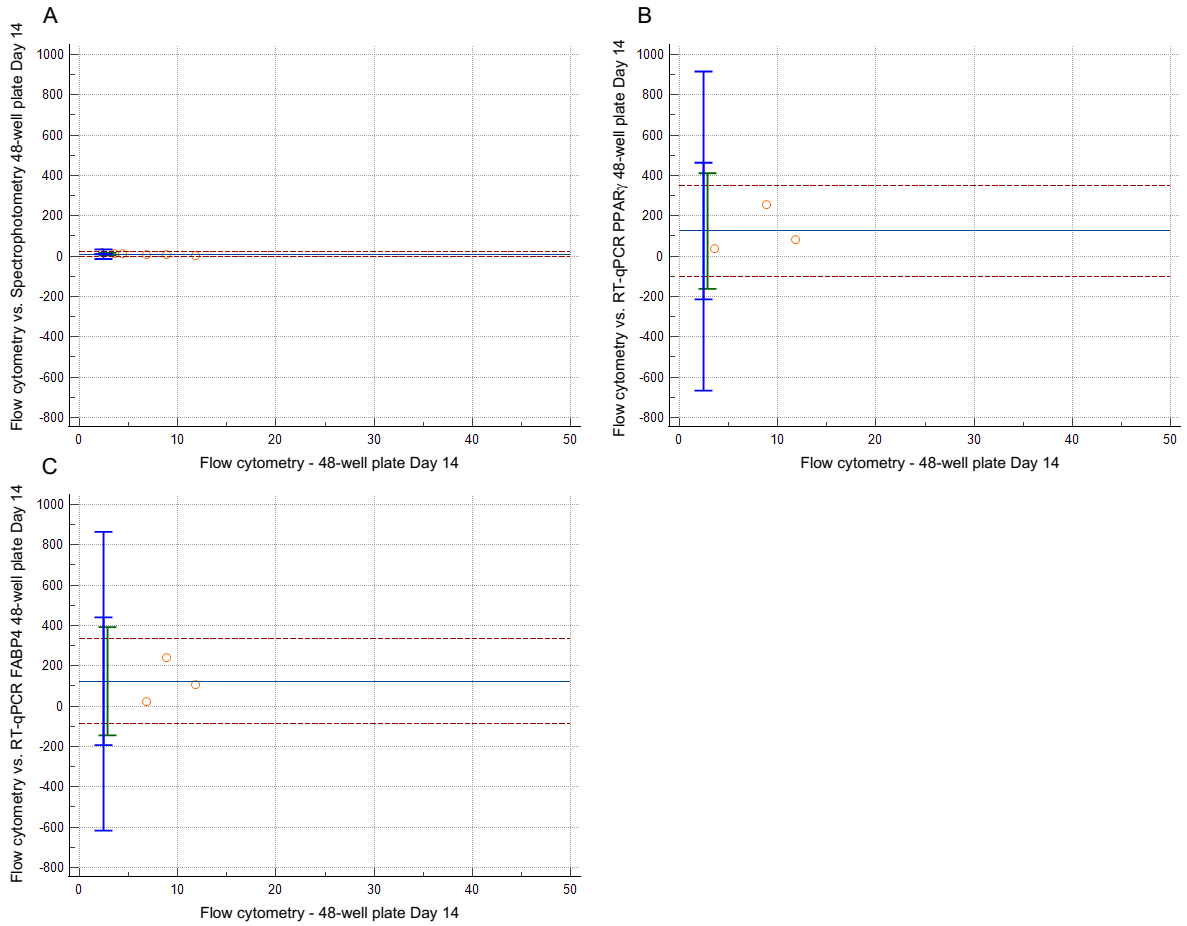

Fig. S15 Bland-Altman plots illustrating the comparison between the methods used in this study using flow cytometry as the reference method in the 48-well plate on day 14. **a** Flow cytometry vs. spectrophotometry, **b** Flow cytometry vs. RT-qPCR *PPARg* and **c** Flow cytometry vs. RT-qPCR *FABP4.* The two horizontal red dotted lines represent the limits of agreement (upper and lower) (mean difference ± 1.96), the horizontal blue line represents the bias (mean difference; other method minus flow cytometry) between the two methods and the error bars (blue and green vertical lines) represent the 95% confidence intervals for the limit of agreement and mean difference, respectively.

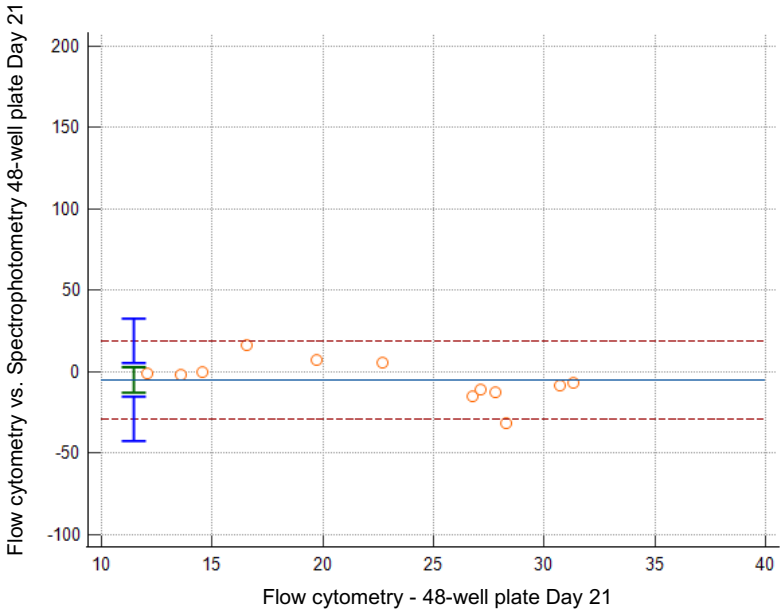

Fig. S16 Bland-Altman plot illustrating the comparison between flow cytometry and spectrophotometry using flow cytometry as the reference method in the 48-well plate on day 21. The two horizontal red dotted lines represent the limits of agreement (upper and lower) (mean difference ± 1.96), the horizontal blue line represents the bias (mean difference; other method minus flow cytometry) between the two methods and the error bars (blue and green vertical lines) represent the 95% confidence intervals for the limit of agreement and mean difference, respectively.

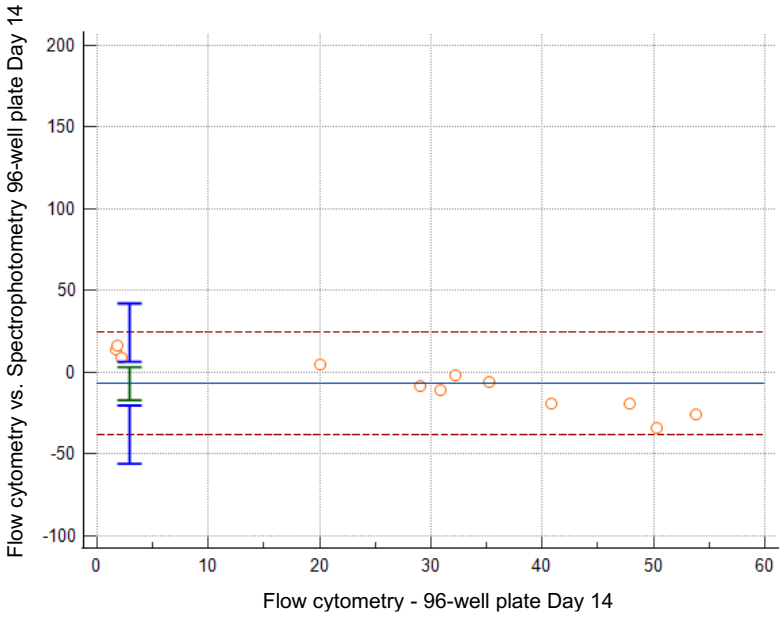

Fig. S17 Bland-Altman plot illustrating the comparison between flow cytometry and spectrophotometry using flow cytometry as the reference method in the 96-well plate on day 14. The two horizontal red dotted lines represent the limits of agreement (upper and lower) (mean difference ± 1.96), the horizontal blue line represents the bias (mean difference; other method minus flow cytometry) between the two methods and the error bars (blue and green vertical lines) represent the 95% confidence intervals for the limit of agreement and mean difference, respectively.

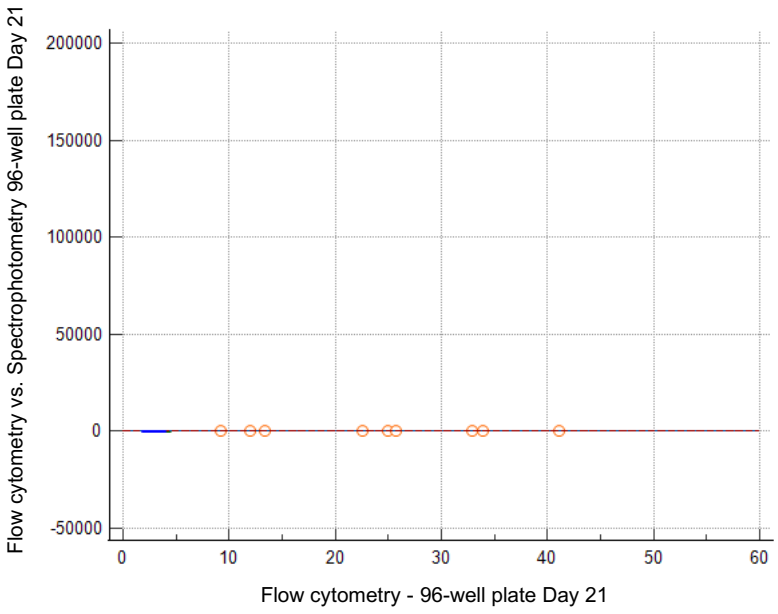

Fig. S18 Bland-Altman plot illustrating the comparison between flow cytometry and spectrophotometry using flow cytometry as the reference method in the 96-well plate on day 21. The two horizontal red dotted lines

represent the limits of agreement (upper and lower) (mean difference ± 1.96), the horizontal blue line represents the bias (mean difference; other method minus flow cytometry) between the two methods and the error bars (blue and green vertical lines) represent the 95% confidence intervals for the limit of agreement and mean difference, respectively.

Table S1 Costs associated with screening 100 compounds (in triplicate) for the most effective well size (48-well plate) for flow cytometry and spectrophotometry

Reagent	**Quantity**	**Supplier**	**Volume/number of reagents/consumables required**	**Number of units required**	**Price per unit (excl. VAT)**	**Total price (excl. VAT)**
48-well plate	100 units	Greiner	8 plates	1	R2 951 ($157.89)	R2 951 ($157.89)
CGM						
DMEM	500 mL	Gibco™	450 mL	1	R2 147.20 ($114.88)	R2 147.20 ($114.88)
FBS	500 mL	Gibco™	50 mL	1	R3 572.80 ($191.16)	R3 572.80 ($191.16)
Pen/strep	100 mL	Gibco™	10 mL	1	R237.60 ($12.71)	R237.60 ($12.71)
Trypsinization						
Trypsin-EDTA	500 mL	Gibco™	39 mL	1	R1 770.56 ($94.73)	R1 770.56 ($94.73)
Adipogenic induction medium						
DMEM	500 mL	Gibco™	562.5 mL	2	R2 147.20 ($114.88)	R4 294.40 ($229.77)
FBS	500 mL	Gibco™	62.5 mL	1	R3 572.80 ($191.16)	R3 572.80 ($191.16)
Pen/strep	100 mL	Gibco™	12.5 mL	1	R237.60 ($12.71)	R237.60 ($12.71)
IBMX	1 g	Sigma Aldrich	0.06875 g	1	R2 921 ($156.29)	R2 921 ($156.29)
Insulin	5 mL	Gibco™	1.5625 mL	1	R1 100 ($58.86)	R1 100 ($58.86)
Indomethacin	5 g	Sigma Aldrich	0.04475 g	1	R1 706 ($91.28)	R1 706 ($91.28)
***Preparation of dexamethasone**						
Dexamethasone	100 mg	Sigma Aldrich	19 mg	1	R1 132 ($60.57)	R1 132 ($60.57)

Ethanol	500 mL	Sigma Aldrich	10 mL	1	R465 ($24.88)	R465 ($24.88)
Total						**R26 108 ($1 396.90)**
Flow cytometry						
CD36	0.5 mL	Beckman Coulter	768 µL	2	R2 958.94 ($158.32)	R5 917.88 ($316.63)
Nile Red	50 mg	Sigma Aldrich	768 µL	1	R1 601 ($85.66)	R1 601 ($85.66)
DAPI	10 mg	Invitrogen	384 µL	1	R3 210 ($171.75)	R3 210 ($171.75)
96-well U bottom plate (used for 48- and 96-well plate analysis)	100 units	Beckman Coulter	4 plates	1	R2 398 ($128.30)	R2 398 ($128.30)
Total						**R13 126.88 ($702.35)**
***Grand total**						**R39 234.88 ($2 099.24)**
Spectrophotometry						
4% formaldehyde	100 mL	Sigma Aldrich	96 mL	1	R302 ($16.16)	R302 ($16.16)
Oil Red O	25g	Sigma Aldrich	96 mL	1	R415 ($22.20)	R415 ($22.20)
DAPI	10 mg	Invitrogen	384 µL	1	R3 210 ($171.75)	R3 210 ($171.75)
PBS	500 mL	Merck		1	R403 ($21.56)	R403 ($21.56)
96-well flat bottom plate	100 units	Lasec	4	1	R560 ($29.96)	R560 ($29.96)
Total						**R4 890 ($261.64)**
***Grand total**						**R30 998 ($1 658.53)**

Prices are based on quotes received in 2021
*Grand total includes the 48-well plate consumables plus the method total
General flow cytometry consumables were not included (Sheath fluid, FlowClean cleaning agent, and CytoFLEX Daily QC beads) as very small volumes will be required
*A stock solution of 5mM of dexamethasone was prepared and stored at -20°C. The same stock solution was used for all well sizes

Dollar rate taken on 5 April 2024; $1 equal to R18.69

Table S2 Costs associated with screening 100 compounds (in triplicate) for the most effective well size (12-well plate) for RT-qPCR

Reagent	**Quantity**	**Supplier**	**Volume/number of reagents/consumables required**	**Number of units required**	**Price per unit (excl. VAT)**	**Total price (excl. VAT)**
12-well plate	100 units	Greiner	26 plates	1	R2 925.97 ($156.55)	R2 925.97 ($156.55)
CGM						
DMEM	500 mL	Gibco™	1 462.5 mL	3	R2 147.20 ($114.88)	R6 441.60 ($344.65)
FBS	500 mL	Gibco™	162.5 mL	1	R3 572.80 ($191.16)	R3 572.80 ($191.16)
Pen/strep	100 mL	Gibco™	32.5 mL	1	R237.60 ($12.71)	R237.60 ($12.71)
Trypsinization						
Trypsin-EDTA	500 mL	Gibco™	98.25 mL	1	R1 770.56 ($94.73)	R1 770.56 ($94.73)
Adipogenic induction medium						
DMEM	500 mL	Gibco™	1 800 mL	4	R2 147.20 ($114.88)	R8 588.80 ($459.54)
FBS	500 mL	Gibco™	200 mL	1	R3 572.80 ($191.16)	R3 572.80 ($191.16)
Pen/strep	100 mL	Gibco™	40 mL	1	R237.60 ($12.71)	R237.60 ($12.71)
IBMX	1 g	Sigma Aldrich	0.22 g	1	R2 921 ($156.29)	R2 921 ($156.29)
Insulin	5 mL	Gibco™	5 mL	1	R1 100 ($58.86)	R1 100 ($58.86)
Indomethacin	5 g	Sigma Aldrich	0.1432 g	1	R1 706 ($91.28)	R1 706 ($91.28)
Preparation of dexamethasone						
Dexamethasone	100 mg	Sigma Aldrich	19 mg	1	R1 132 ($60.57)	R1 132 ($60.57)
Ethanol	500 mL	Sigma Aldrich	10 mL	1	R465 ($24.88)	R465 ($24.88)
Total						**R34 671.73 ($1 855.10)**

RT-qPCR						
RNeasy Plus Micro Kit	50 reactions	Qiagen	103 reactions	3	R9 994 ($534.72)	R29 982 ($1 604.17)
RNA ScreenTape Ladder	112 samples	Agilent Technologies	103 samples	1	R1 378 ($73.73)	R1 378 ($73.73)
RNA ScreenTape Sample Buffer	600 µL	Agilent Technologies	515 µL	1	R1 621 ($86.73)	R1 621 ($86.73)
SensiFast cDNA synthesis kit	250 reactions	SensiFast™	103 reactions	1	R15 571.11 ($833.12)	R15 571.11 ($833.12)
Light Cycler 480 SYBR Green I Master Mix	500 reactions	Roche	133 reactions	1	R6 618.29 ($354.11)	R6 618.29 ($354.11)
Light Cycler 96 well plate	50 plates	Roche	2 plates	1	R5 661.46 ($302.91)	R5 661.46 ($302.91)
Forward primer 1	20 000 reactions	Whitehead Scientific (Pty) Ltd.	133 reactions	1	R244.96 ($13.11)	R244.96 ($13.11)
Reverse primer 1	20 000 reactions	Whitehead Scientific (Pty) Ltd.	133 reactions	1	R272.17 ($14.56)	R272.17 ($14.56)
Reference gene forward primer 1	20 000 reactions	Whitehead Scientific (Pty) Ltd.	133 reactions	1	R244.96 ($13.11)	R244.96 ($13.11)
Reference gene reverse primer 1	20 000 reactions	Whitehead Scientific (Pty) Ltd.	133 reactions	1	R258.57 ($13.83)	R258.57 ($13.83)
Reference forward primer 2	20 000 reactions	Whitehead Scientific (Pty) Ltd.	133 reactions	1	R299.39 ($16.02)	R299.39 ($16.02)
Reference gene reverse primer 2	20 000 reactions	Whitehead Scientific (Pty) Ltd.	133 reactions	1	R244.96 ($13.11)	R244.96 ($13.11)
Reference gene forward primer 3	20 000 reactions	Whitehead Scientific (Pty) Ltd.	133 reactions	1	R272.17 ($14.56	R272.17 ($14.56)
Reference gene reverse primer 3	20 000 reactions	Whitehead Scientific (Pty) Ltd.	133 reactions	1	R272.17 ($14.56)	R272.17 ($14.56)
Total						**R62 941 ($3 367.63)**

***Grand total**						**R97 612.73 ($5 222.72)**

Prices are based on quotes received in 2021
*Grand total includes the 48-well plate consumables plus the method total

Calculations for the well size yielding the most accurate results (screening 100 compounds)

Calculations are based on volumes used in Methods (Flow cytometry Table 2 and Spectrophotometry Table 4)

1. 48-well plate for flow cytometry and spectrophotometry

The 48-well plate was determined as the smallest well size which yielded an acceptable level of agreement for flow cytometry and spectrophotometry.

Table S3 Number of wells and plates to screen 100 compounds (in triplicate) in a 48-well plate

	Number of wells
Positive control **Negative control** **Non-induced control**	3 3 3
Total	**9**
48-well plate minus controls **Compounds per plate** **100 compounds (in triplicate)**	48 wells - 9 = 39 wells 39 wells/3 (triplicate) = 13 compounds per plate 100 x 3 = 300 wells
Total number of wells	39 + 300 = **339**
Number of plates required to screen 100 compounds (in triplicate)	100 compounds/13 compounds per plate = 7.69 plates (rounded to 8 plates = 384 wells)

Number of ASCs required to seed 8 x 48-well plates

48-well plate x 8 plates x 1250 [surface area (1 cm^2) multiplied by seeding density (1250 cells/cm^2)] = 480 000 cells.

To determine the number of flasks required to achieve the number of cells needed, it was assumed that a T75 flaks will yield an average of one million cells (based on previous findings at the ICMM). Therefore, 1 flask is required.

Table S4 The number of flasks/wells and volume of medium required to seed, expand and maintain ASCs in a 48-well plate over a 21-day period to screen 100 compounds (in triplicate)

	Number of flasks/wells	**Expansion time**	**Number of medium changes**	**Volume of medium**	**Total volume of medium**	**Volume of trypsin**
T75 flask	1 flask	2 weeks	4	7 mL	7 mL x 4 = 28 mL CGM	3 mL x 1 flask = 3 mL Neutralizing trypsin: 3 mL CGM
48-well plate	384 wells	2 weeks	4	0.25 mL per well	384 wells x 0.25 mL x 4 = 384 mL CGM	384 wells x 100 µL = 38.4 mL Neutralizing trypsin: 38.4 mL CGM
Day 14	**Non-induced:** 24 wells **Induced:** 360 wells	14 days post induction	4	0.25 mL per well	**Non-induced:** 24 wells x 0.25 mL x 4 = 24 mL CGM **Induced:** 360 wells x 0.25 mL x 4 = 360 mL adipogenic induction medium	
Day 21	Non-induced: 24 wells Induced: 360 wells	21 days post induction	2	0.25 mL per well	**Non-induced:** 24 wells x 0.25 mL x 2 = 12 mL CGM **Induced:** 360 wells x 0.25 mL x 2 = 180 mL adipogenic induction medium	

Total volume of medium					CGM: **389.4 mL** Adipogenic induction medium: **540 mL**	

To prepare CGM make up a total volume of 500 mL (Table S5) and adipogenic medium make up a total volume of 625 mL (1 x 125 mL and 500 mL; Table S6).

Table S5 Standard volumes used at the ICMM to prepare complete growth medium

	125 mL	**250 mL**	**500 mL**
DMEM	112.5 mL	225 mL	450 mL
FBS (10%)	12.5 mL	25 mL	50 mL
Pen/strep (2%)	2.5 mL	5 mL	10 mL

Table S6 Standard volumes used at the ICMM to prepare adipogenic induction medium

	125 mL	**250 mL**	**500 mL**
IBMX	0.01375 g	0.0275 g	0.055 g
Insulin	0.3125 mL	0.625 mL	1.25 mL
Indomethacin	0.00895 g	0.0179 g	0.0358 g
Dexamethasone	25 µL	50 µL	100 µL

Calculations to prepare dexamethasone in ethanol:
Stock: 5 mM
Amount used: 100 µL
Molar mass: 392.46 g/mol

(5 mM)(392.46 g/mol)(0.01L)
= 0.019623 g
Therefore, dissolve 19 mg in 10 mL ethanol

Flow cytometry reagents:

Table S7 Volume of reagents used for flow cytometry to screen 100 compounds (in triplicate) in the 48-well plate

	Volume required	**Number of wells**	**Total volume/number of plates**
CD36	2 µL	384	2 µL x 384 wells = 768 µL
Nile Red	2 µL	384	2 µL x 384 wells = 768 µL
DAPI	1 µL	384	1 µL x 384 wells = 384 µL
96-well U bottom plate			4 plates

Therefore, two vials of CD36 (2 x 0.5 mL) and one vial/unit of Nile Red, DAPI and 96-well U bottom plates are required.

Spectrophotometry reagents

Table S8 Volume of reagents used for spectrophotometry to screen 100 compounds (in triplicate) in the 48-well plate

	Volume required	**Number of wells**	**Total volume/number of plates**
4% formaldehyde	250 µL	384	250 µL x 384 wells = 96 mL
Oil Red O	250 µL	384	250 µL x 384 wells = 96 mL
DAPI	1 µL	384	1 µL x 384 wells = 384 µL
PBS	250 µL	384	250 µL x 384 wells = 96 mL
96-well flat bottom plate			4 plates

Therefore, one vial of each reagent is required.

2. 12-well plate for RT-qPCR

The 12-well plate was determined as the smallest well size which yielded an acceptable level of significance.

Table S9 Number of plates required to screen 100 compounds (in triplicate) using RT-qPCR in the 12-well plate

	Number of wells
Positive control	3
Negative control	3
Non-induced control	3
Total	**9** (one plate)

Compounds per plate	12 wells/3 (triplicate) = 4 compounds per plate
Number of plates required to screen 100 compounds (in triplicate)	100 compounds/4 compounds per plate = 25 plates and an extra plate for the controls Therefore, 26 plates

*only one plate of controls were included as the cost would increase dramatically if 1 plate per compound was used.

Number of ASCs required to seed 26 x 12-well plates:

12-well plate x 26 plates x 19 500 [surface area (3.9 cm^2) multiplied by seeding density (5 000 cells/cm^2)] = 6 084 000 cells

Therefore, 7 flasks are required.

Table S10 The number of flasks/wells and volume of medium required to seed, expand and maintain ASCs in a 48-well plate over a 21-day period to screen 100 compounds (in triplicates)

	Number of flasks/wells	**Expansion time**	**Number of medium changes**	**Volume of medium**	**Total volume of medium**	**Volume of trypsin**
T75 flask	7 flasks	2 weeks	4	7 mL	7 flasks x 7 mL x 4 = 196 mL CGM	3 mL x 7 flasks = 21 mL Neutralizing trypsin: 21 mL CGM
12-well plate	309 wells	2 weeks	4	1 mL per well	309 wells x 1 mL x 4 = 1 236 mL CGM	309 wells x 250 µL = 77.25 mL Neutralizing trypsin: 77.25 mL CGM
Day 14	**Non-induced:** 3 wells **Induced:** 306 wells	14 days post induction	4	1 mL per well	**Non-induced:** 3 wells x 1 mL x 4 = 12 mL CGM **Induced:** 306 wells x 1 mL x 4 = 1 224 mL adipogenic induction medium	

Day 21	Non-induced: 3 wells Induced: 306 wells	21 days post induction	2	1 mL per well	**Non-induced:** 3 wells x 1 mL x 2 = 6 mL CGM **Induced:** 306 wells x 1 mL x 2 = 612 mL adipogenic induction medium	
Total volume of medium					CGM: **1 548.75 mL** Adipogenic induction medium: **1 836 mL**	

The total volume of CGM needed is 1 625 mL (1 x 125 mL and 3 x 500 mL; Table S5). The total volume of adipogenic medium needed is 2 000 mL (4 x 500 mL; Table S6).

RT-qPCR reagents

For RT-qPCR the wells were pooled, therefore 100 compounds = 100 reactions

Addition of positive, negative and induced control = 3 reactions

Therefore, 103 reactions

= 3 x RNeasy Plus Micro Kit (50 reactions per kit).

1. RNA integrity

Table S11 Number of samples that can be analyzed using one RNA ScreenTape sample buffer and one RNA ScreenTape Ladder to determine RNA integrity

	Volume/samples of reagent	**Volume used per sample**	**Number of samples that can be analyzed per kit**
RNA ScreenTape sample buffer	600 µL	5 µL	600 µL /5 µL = 120 samples
RNA ScreenTape Ladder	112 samples	1	112 samples

Therefore, one vial of sample buffer and one box of ScreenTape Ladders are required to screen 100 compounds.

2. cDNA synthesis

SensiFast cDNA synthesis kit includes 1 mL TransAmp buffer and 250 µL reverse transcriptase which can be used for 250 reactions

Therefore, one SensiFast cDNA synthesis kit is required.

3. RT-qPCR

A Standard, reference genes (4), non-induced control and induced sample (including positive and negative control) in triplicate are included. This comes to 133 reactions.

Table S12 SYBR Green Master Mix for Roche LightCycler

Reagent	**[Stock]**	**Quantity** **1x reaction**	**Volume (µL)** **1x reaction**
SYBR Green Master Mix	2x	1x	5 µL x 133 reactions = **665 µL**
***F Primer**	10 µM	400 nM	0.4 µL x 133 reactions = **53.2 µL**
***R Primer**	10 µM	400nM	0.4 µL x 133 reactions = **53.2 µL**

*Primers are made up in 800 µL Tris-EDTA buffer solution. Primers are then diluted to make a 1:10 dilution, thus yielding a 8000 µL working solution. Per reaction, 0.4 µL of primer is used and thus approximately 20 000 reactions can be used per primer vial.

Therefore, 1 vial of SYBR green master mix, forward and reverse primer is required.

Nile Red preparation

1 mg in 1 mL = 50 mg Nile Red in 50 mL ethanol

DAPI preparation

10 mg DAPI in 2 mL dH_2O

Oil Red O preparation

200 mg Oil Red O in 40 mL propanol

Table S13 Example of the spectrophotometry calculations normalized to average cell count in the 6-well plate day 0

6-well plate						
	OD value	**Minus blank (0.037333)**	**x10^7**	**Average cell count**	**Corrected to cell count (x10^7/average cell count)**	**Average corrected to cell count**
Day 0	0.11 0.135 0.127	0,072667 0,097667 0,089667	726666,7 976666,7 896666,7	130779,608	5,556422 7,468035 6,856319	6,626925