Supplemental Information

A Renewable Source of Human Beige Adipocytes for Development of Therapies to Treat Metabolic Syndrome

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Figure S1, related to Figure 1. Generation of splanchnic mesoderm using commercial and in house medium. (A) qPCR analysis of skin-derived iPSCs differentiated with STEMdiff MIM. Data are expressed as mean ± SD of 3 replicates shown. (B) Western blot analysis of skin-derived iPSCs differentiated with STEMdiff MIM over 5 days. (C) A directed screen using previously described factors known to give rise to mesoderm tissue were used to determine medium compositions that give rise to FOXF1+ mesoderm. BMP4 in combination with CHIR99021 (CHIR, 1.5 µM) resulted in increased expression of FOXF1, whereas Activin A (ACT-A) or VEGFA (VEGF) with CHIR had no response. Further combinations of BMP4 with ACT-A and VEGF demonstrated that BMP4 and VEGF in combination with CHIR lead to the highest expression of FOXF1. Data are expressed as mean ± SD of 3 replicates shown. (D) Increasing the concentration of CHIR to 5µM in combination with BMP4 and VEGF in an independent assay caused a decrease in the expression of FOXF1. Data are expressed as mean ± SD of 3 replicates shown. (E) 1.5 µM CHIR in combination with VEGF and BMP4 lead to a significant increase in FOXF1 protein compared to StemDiff mesoderm induction medium (MIM). While 5µM CHIR (+ BMP4 + VEGF) lead to similar levels of FOXF1 protein compared to Stemdiff MIM, this concentration of CHIR also induced expression of PDGFRα, a marker of paraxial mesoderm. Inhibition with the TGFβ inhibitor SB431542 (SB) blocked protein expression of FOXF1 and increased expression of the somatic marker IRX3. (F) Transcription of the somatic mesoderm marker IRX3 was low or absent from mesoderm produced by in house or StemDiff mesoderm induction mediums, however, additional inhibition with SB431542 induced transcription of the somatic mesoderm marker IRX3 and inhibited FOXF1 protein expression (Fig S1E). Data are expressed as mean ± SD of 3 replicates shown. (G) Model showing that the presence or absence TGFβ signaling can specify either splanchnic or somatic mesoderm subcompartments, respectively.
Figure S2, related to Figure 4. Comparisons of iPS-derived adipocytes with adipocytes derived from primary tissues. (A) qPCR analysis of UCP1 expression in human subcutaneous white preadipocytes differentiated with the beige adipogenic cocktail, with or without SB431542. Data are expressed as mean ± SD of 3 replicates. (B) qPCR analysis of iPSC-beige adipocytes (day 9) and primary beige (day 9) or brown adipocytes (day 7). Data are expressed as mean ± SD of 3 replicates. (C) Mass spectrometry analysis of myosin proteins with increased expression in interscapular brown adipose tissue. Data are normalized relative to expression in iPSC-beige adipocytes (N = 3 adipocyte lysates per group). P values less than 0.001 are indicated by an asterisk.
**Figure S3, related to Figure 4.** Derivation of beige adipocytes from CD34+ cord blood. iPSCs derived from cord blood were differentiated into beige adipocytes using the method described in the text. (A) Flow cytometry analysis of MSC and perivascular markers (red) of iPSCs differentiated into FD-MSCs (pass 6) with MesenCult-ACF. Isotype controls shown in blue. (B) Fluorescence microscopy of BODIPY stained FD-MSCs (left) or FD-MSCs differentiated into adipocytes (right) for 12 days with 5µM SB431542 (SB) during pretreatment and induction. Representative images are shown. Scale bar = 100µm. (C) qPCR analysis of common brown/beige adipogenic transcription factors and markers enriched in beige or brown adipocytes (day 12). Data are expressed as mean ± SD of 3 replicates. (D) Western blot analysis showing timecourse of adipocytes differentiated from FD-MSCs with the brown/beige adipogenic cocktail.
Figure S4, related to Figure 4. Derivation of beige adipocytes from urine derived iPSCs. iPSCs were differentiated into beige adipocytes using the method described in the text. (A) Primary urine-derived cells (top panel) were expanded and reprogrammed into Tra-1-60+ iPSCs (bottom panel). (B) Flow cytometry analysis of MSC and perivascular markers (red) of iPSCs differentiated into FD-MSCs (pass 6) with MesenCult-ACF. Isotype controls shown in blue. (C) Primary urine-derived cells (left panels) and FD-MSCs derived from urine-derived iPSCs (right panels) induced with the brown/beige adipogenic cocktail of factors for 12 days. Phase contrast (upper) and immunofluorescence of BODIPY stained (lower) images shown. Results with primary urine-derived cells demonstrate that reprogramming is necessary to generate beige adipocytes. Scale bar = 100µm. (D) qPCR analysis of common brown/beige adipogenic transcription factors and markers enriched in beige or brown adipocytes. Data are expressed as mean ± SD of 3 replicates. (E) Western blot analysis of primary urine-derived cells and FD-MSCs induced with the brown/beige adipogenic cocktail of factors (day 12 shown).
Figure S5, related to Figure 4. IL-4 increases expression of beige adipogenic precursor markers in cooperation with TGFβ inhibition. qPCR analysis of FD-MSCs treated with SB (5µM), IL-4 (10nM), and SB + IL-4 for 2 days and analyzed for transcripts associated with adipogenic precursors, including (A) PDGFRα, (B) EBF2 and (C) PPARG isoforms. Data are expressed as mean ± SD of 3 replicates.
Figure S6, related to Figure 6. Generation of iPSC-beige adipocytes from type 2 diabetic adipogenic precursors (76 years old). (A) qPCR analysis time course of common brown/beige adipogenic transcription factors and markers enriched in beige or brown adipocytes for T2 primary subcutaneous adipocytes (top) and T2 iPSC-beige adipocytes (bottom). Data are expressed as mean ± SD of 3 replicates. (B) UCP1 western blot analysis of T2 primary subcutaneous adipocytes and T2 iPSC-beige adipocytes following 12 days of differentiation. Two additional normal primary subcutaneous beige adipocytes are included as comparisons. β-tubulin serves as a loading control.
Figure S7, legend on the next page.
Figure S7, related to Figure 6. Generation of iPSC-beige adipocytes from type 2 diabetic adipogenic precursors. (A) Cumulative chart of all primary preadipocytes and their donor characteristics. (B) Flow cytometry analysis of PDGFRα surface expression in primary type 2 diabetic subcutaneous and omental adipogenic precursors. Isotype controls shown in blue. (C) Fluorescence microscopy of BODIPY stained T2 primary adipocytes and iPSC-beige adipocytes following 12 days of differentiation. Representative images are shown. Scale bar = 100µm. (D) Quantitation of lipid accumulation (as shown in (C)) by image J software as measured by relative integrated density expressed as mean ± SD (n = 3, 20X images). (E) Quantitation of PPARγ2+ stained primary beige adipocytes and iPSC-beige adipocytes following 12 days of differentiation expressed as ± SD, (n = 3, 20X images). (F) UCP1 qPCR analysis of T2 primary adipocytes and iPSC-beige adipocytes following 12 days of differentiation. Data are expressed as mean ± SD of 3 replicates. (G) Western blot analysis of T2 primary subcutaneous adipocytes (34 years old) and T2 iPSC-beige adipocytes (34 years old) following 12 days of differentiation. Two additional normal primary subcutaneous beige adipocytes are included for comparison. β-tubulin serves as a loading control. (H) Western blot analysis of T2 primary omental adipocytes (63 years old) and T2 iPSC-beige adipocytes (63 years old) following 12 days of differentiation. Non-diabetic primary subcutaneous beige adipocytes are included for comparison. β-tubulin serves as a loading control. (I) Quantitative summary of Seahorse XF analyzer profile of live T2 primary subcutaneous adipocytes (34 years old) and T2 iPSC-beige adipocytes (day 14). Data are expressed as mean of 3 time point measurements ± SD. **P<0.01 using Student’s t test. (J) Quantitative summary of Seahorse XF analyzer profile of live T2 primary omental adipocytes (63 years old) and T2 iPSC-beige adipocytes (day 14). Data are expressed as mean of 3 time point measurements ± SD. **P<0.01 and *P<0.05 using Student’s t test.
Figure S8, related to Figure 7. iPSC-derived beige adipocytes from a 34 year old patient secrete factors that improve insulin sensitivity and glucose uptake in the primary subcutaneous adipocytes. (A) Western blot analysis of phospho-AKT in T2 subcutaneous adipocytes (34 years old) treated with insulin in the presence of T2 subcutaneous adipocyte conditioned medium (lanes 4-6) or T2 iPSC-beige adipocyte conditioned medium (lanes 10-12). (B) Quantitation of phospho-AKT protein expression (shown in (A)). Data are normalized to β-actin protein. Data are expressed as mean ± SD. (C) Glucose uptake analysis of T2 subcutaneous adipocytes (34 years old) treated with insulin in the presence of T2 subcutaneous adipocyte conditioned medium or T2 iPSC-beige adipocyte conditioned medium. Data are expressed as mean ± SD and values from Student's t test shown.
**Table S1.** Oligonucleotide sequences used for qPCR in the study, Related to STAR Methods.

| Oligonucleotides | Gene       | Forward (5' to 3') | Reverse (5' to 3') |
|------------------|------------|--------------------|--------------------|
|                  | UCP1       | AGTTTCTCACCGCAGGGAAAGA | GTAGCGAGGTTTGATTCCGTGG |
|                  | CEBPA      | AGGAGGATGAAGCCAACAGCT | AGTGCGCGATCTGGAACCTGAG |
|                  | CEBPB      | AGAAGACCGTGGAACACACAG | CTCCAGGACCTTGTGCTGCT |
|                  | EBF2       | GAGCAAGAAGCCTTGACCACATC | CCAACACAACTGGAGACATC |
|                  | PGC1α      | CCAAAAGGATGCGCTCTGAATCA | CGGTGTCTGTAGTGCTGCTT |
|                  | PRDM16     | CAGCCAATCTCACCAGACACCT | GTGGCACTTGAAAGGCTTTC |
|                  | PPARγ      | CGAGGACACCAGGAGAGG | TGTGTATTAGTGCTGCTT |
|                  | TMEM26     | GCAGTTGCCACTTGACCTGGCA | GAAGACGCTGATCCGATGTT |
|                  | CITED1     | CCACTAGCTCTCCTGGATCAG | AGCCCTTGTACCTGCTAT |
|                  | CD137      | TCTTCTCACGCTCCCTTTCATC | TGGAAATCGTGACCTACAGCCA |
|                  | ZIC1       | GATGTGCGACAAGTGCTCTACACAG | TGGAGGATTCTAGCCAGAGCT |
|                  | PPARγ1     | CGAGGACACCAGGAGAGG | TGTGTTTATGTTGGCTT |
|                  | PPARγ2     | TTTTAACCGGATTTGATCTTTTGC | AGGAGTGAGGATTGGCTT |
|                  | PPARγ3     | TTCTGCTTAATTCCCTTTCAG | AGGAGTGAGGATTGGCTT |
|                  | BAPX1      | CCGCTTCCAAAGACCTAGAGGA | ACCGTCGTCTCGGTCCTT |
|                  | HOX11      | TGTGCCAGGCTTCTGGAGAGG | CTCCGCACCTGCTGGGACTT |
|                  | FOXF1      | AGCCAGCCGTATCTGCAACAGAA | CTCCCTTGCTGTCACATGCT |
|                  | IRX3       | CTCGGCACCTGCTGGAAGACTTC | CTCCACCTCCAAGGCACTACAG |
|                  | T (brachyury) | CTTTCAGCAAGACCTAGCCTAC | TGAAGCTGCTGTCAGGGAAGCA |
|                  | MIXL1      | CCCGACATCCACTTGCAGCAG | GGAAGGATTCCCACCTGAGC |
|                  | SOX1       | GAGTGGAGGGTGATGTGGGAGG | CTTCTTGAGCAGCGTCTTGG |
|                  | SOX17      | ACGCTTTGATGATGTTGGCTAAG | GTACGCGCTTCCACAGACTTG |
|                  | VEGFR2     | GGAACCTCACTATCCGGAGAGG | CCAAGTTTCATTCTCTGCTG |
|                  | OSR1       | CCTAACCTGTGACATCTGCCA | GTGAGTGTAGCGCTTGGAGC |
| Gene   | Forward Primer                        | Reverse Primer                        |
|--------|--------------------------------------|---------------------------------------|
| ACTA1  | AGGTCATCACCATCGGCAACGA               | GCTGTTTAGGTGGTCTCGTGA                 |
| αSMA   | CTATG CCTCTGGACGCAA CA              | CAGATCCAGACGCATGATGGCA                |
| SM22α  | TCCAGGTCTGGC TGAAGAATGG             | CTGCT CACATCTGT GCTTG AAGACC          |
| TGFβ1  | TACCTGAACCCTGGTCGTCCTCTC          | GTTGCTGAGGTATCGCCAGGAA                |
| TGFβ2  | AAGAAGCGTGCTTTGGATGCGG            | ATGCTCCAGCAGAAAGTTGGGC               |
| TGFβ3  | C TAA GCGGAATGAGCAGAGG GATC       | TCTCAA CAGCCAC T CACGCACA            |
| TGFβR1 | GACAACGTCA GGTTCGCTGCTCA          | CCGCA CTTTCTCC TCCAAACT              |
| TGFβR2 | GTC TTGGATGACCTGGCTAAC         | GACATCGGGTC TGTGCTTG AAGGAC          |
| FGF21  | CTGCAGCTGAAAGCCTTGAAGC          | GTATCCGTCTCAAAGAAGCAGC               |
| NRG4   | TGTGGCATTGGCCGGTCCTAGTA       | ACTGCTCGTCTCA GGTGGTG               |
| IL6    | AGACAGCCACTCACCTTCCAG       | TTCTGCGATGCTCT TTTGGCTG             |
| ADIPOQ | CAGGCCGTGATGGCAGAGATG     | GGTTCACCAGATGTCTCCCTT TAG            |
Culture of iPSCs

Cell plating and culture
1. Thaw ES cell qualified Matrigel on ice, dilute 1:25 in cold RPMI and coat plates prior to cell seeding. Coat each well of a 6 well plate with 1 ml of Matrigel. Distribute the Matrigel over the entire surface of the well and allow plate to sit covered and undisturbed in a laminar flow cabinet for cell culture for at least 1 hour.

2. Prior to cell plating, remove Matrigel from wells using a sterile pipette tip connected to a vacuum aspirator. Immediately wash well surface with PBS without calcium or magnesium. Aspirate PBS and add desired volume of cell colonies suspended in 2ml of NutriStem hPSC XF Medium containing 10 μM Y-27632 Rho-kinase inhibitor to each well. Agitate plate to spread cell colonies evenly on well surface. Incubate plate at 37°C in a 5% CO2 incubator for expansion.

Note: Only use Y-27632 stock solutions that have been resuspended in DMSO. We have found that water based versions lose significant inhibitor activity. Conduct Mesoderm induction of iPSC in a laminar flow cabinet.

3. Expand cells in NutriStem hPSC XF Medium overnight and change 100% of the medium without Y-27632 every 24 hours in a laminar flow cabinet for cell culture. When changing media, tilt plate up and allow media to pool at the bottom of wells. Aspirate the pooled medium without touching the surface of the plate. Replace with medium warmed to room temperature by pipetting 2 ml down the side of the wells.

Cell passaging
1. Expand cell colonies until they have reached 70-80% confluence in high-density areas of the wells. High-density accumulation can occur along the edges of the well as well as the middle. Cells permitted to reach high density will lose their potential for differentiation after multiple passages.

2. Prior to cell passage, warm cell wash buffer and NutriStem hPSC XF Medium to room temperature. Conduct cell passage in a laminar flow cabinet.

3. Aspirate NutriStem hPSC XF Medium from wells with vacuum aspirator and wash with PBS without calcium or magnesium. Remove PBS and add 1 ml of ReLeSR reagent to side of well. Agitate plate to allow ReLeSR reagent to spread evenly on the surface.

4. Aspirate ReLeSR reagent with vacuum aspirator after 30 seconds, which will leave behind a thin film of liquid. Incubate plate at 37°C for 5-8 minutes undisturbed.

5. Add fresh NutriStem hPSC XF Medium to wells and gently remove the cell colonies from the plate. Check that most cells have been dislodged from the plate surface with a light microscope. If not, rewash with more medium as needed.

6. Transfer cell colony suspension in NutriStem hPSC XF Medium to Matrigel coated culture plates for further expansion (see Cell plating and culture above).
Generation of iPSC derived MSCs

Mesoderm induction of iPSC
1. When iPSCs have reached 70-80% confluence, they will need to be passaged as single cells rather than colonies prior to mesoderm induction (day 0).

2. Prepare for passage by warming cell wash buffer and NutriStem hPSC XF medium to room temperature and add Y-27632 Rho kinase inhibitor to medium at a final concentration of 10 μM. Heat TrypLE cell dissociation reagent to 37°C.

3. Aspirate NutriStem hPSC XF medium from wells and wash once with PBS. Remove PBS and add 500 μl of pre-warmed TrypLE reagent to each well of a 6 well plate.

4. Incubate culture plate at 37°C for 5 minutes. Check that most cells have been dislodged from the plate surface with a light microscope. If not, extend incubation time in 1-minute increments until noticeable cell detachment.

5. Add cell wash buffer to each well and gently resuspend cells from the plate by washing with a pipette. Check that most cells have been dislodged from the plate and that colonies are dissociated to small clusters of cells (≈5 cells per cluster) with a light microscope. If not, pipette up and down as needed.

6. Transfer cells to an appropriate tube and centrifuge at 300 g for 5 minutes. Check for the appearance of a cell pellet at the bottom of the tube.

7. Remove the supernatant and resuspend cells in an appropriate amount of NutriStem hPSC XF Medium containing 10 μM Y-27632 Rho kinase inhibitor. Plate cells on Matrigel coated culture plates (see Cell plating and culture above). Note: Because different lines of iPSCs proliferate at different rates, it is best to titrate one 6 well worth of iPSCs across 3 wells of a 6 well plate (ex. 1:2 (50%), 1:4 (25%), 1:8 (12.5%) for mesoderm induction (Figure M1). This will ensure that at least 1 well will have the appropriate cell density after the mesoderm induction period.

8. Twenty-four hours after cell plating (day 1), aspirate NutriStem hPSC XF medium containing 10 μM Y-27632 Rho kinase inhibitor and wash once with PBS. Remove PBS and add 2 ml of STEMdiff Mesoderm Induction Medium (MIM) to each well of a 6 well plate. Change 100% of the medium each day until day 5.

![Figure M1. Strategy to ensure the appropriate cell density for mesoderm induction.](image-url)
Generation of MSCs from mesoderm
1. On day 5 of mesoderm induction (see Mesoderm induction of iPSC above), choose wells from the dilution series that are approximately 25-50% confluent, aspirate MIM and wash once with PBS. Remove PBS and replace with 2 ml of MesenCult-ACF Plus medium by gently pipetting down the side of the wells. Change media 100% every day until 100% confluent (usually between day 7 and 12). On day 7-12, cells must be passaged for further expansion and characterization. Cells passaged before growth for at least 2 days in MesenCult-ACF Plus medium will adhere poorly after plating.

2. Prior to passage, coat plates with MesenCult-ACF attachment substrate. Dilute MesenCult-ACF attachment substrate 1:300 in PBS without calcium or magnesium and add 1 ml/well for 6 well plates. Agitate plate to ensure that the coating is distributed over the entire surface of the wells. Allow plate to sit covered and undisturbed in a laminar flow cabinet for cell culture for at least 2 hours at room temperature.

3. To passage, warm cell wash buffer, ACF enzymatic dissociation solution, ACF enzymatic inhibition solution, and MesenCult-ACF Plus medium to room temperature.

4. Aspirate MesenCult-ACF Plus medium from cell culture plates and wash once with PBS. Remove PBS and add 500 μl of ACF enzymatic dissociation solution to each well of a 6 well plate. Note: Other enzymatic dissociation solutions such as trypsin, EDTA or TrypLE will not work as well for harvesting cells.

5. Place cell culture plate in a 37°C incubator for 5 minutes undisturbed. Following 5 minutes, remove plate from incubator and tap side of plate firmly on hard surface several times such as bench countertop to sheer cells from plate surface (Figure M2). Check that cells have dislodged from the well surface under a light microscope. If not, extend incubation time in 1-minute increments and tap again until noticeable detachment.

6. Add 500 μl of ACF enzymatic inhibition solution to each well of a 6 well plate.

7. Further suspend cells in 2 ml of cell wash buffer and gently remove cells from the plate by washing with a pipette. Check that most cells have been dislodged from the plate surface with a light microscope. If not, rewash with more cell wash buffer as needed.

Figure M2. Strategy to release attached MSCs from culture plate.
8. Transfer cell suspension to an appropriate tube and centrifuge at 300 g for 5 minutes. Check for the appearance of a cell pellet at the bottom of the tube.

9. Remove the supernatant and resuspend pelleted cells in an appropriate amount of MesenCult-ACF Plus medium (4 ml per each well passaged). Cells will be passaged 1:2.

10. Prior to cell plating, aspirate MesenCult-ACF attachment substrate in PBS without calcium or magnesium from wells. Wash once with PBS and add cells suspended in MesenCult-ACF Plus medium (2 ml each well) to wells coated with MesenCult-ACF attachment substrate. Agitate plate to spread cells evenly on well surface and incubate plate at 37°C in a 5% CO2 incubator undisturbed.

11. Change 100% of the culture medium daily. After reaching 90% confluence, continue to passage cells 1:2 for further expansion until they are >95% positive for CD105, CD73, CD90, CD146, and PDGFRβ cell surface markers as determined by flow cytometry. This usually occurs between 20 and 30 days. After full maturation of the MSC phenotype as determined by flow cytometry, cells can be passaged at lower densities (1:4-1:6 split). After their maturation, cells should be split when the high-density regions in the wells reach approximately 80% confluence. Growing MSCs to higher densities results in decreased differentiation potential over time.

Differentiation of MSCs into mature cells

Recipe for beige differentiation mediums
Beige adipogenic precursor induction medium:

| Factor           | stock concentration | working concentration | for 10 ml of medium |
|------------------|---------------------|-----------------------|--------------------|
| MesenCult - ACF  | 1X                  | 1X                    | 10 ml              |
| SB431542         | 10 mM               | 5 μM                  | 5 μl               |
| IL-4             | 10 μM               | 10 nM                 | 10 μl              |

Beige adipocyte induction medium:

| Factor            | stock concentration | working concentration | for 10 ml of medium |
|-------------------|---------------------|-----------------------|--------------------|
| EGM-2             | 1X                  | 1X                    | 10 ml              |
| Insulin (10 μg/ml=1.7 mM) | 170 nM | 1 μl |
| T3                | 1 μM                | 2 nM                  | 20 μl              |
| Rosiglitazone     | 5 mM                | 1 μM                  | 2 μl               |
| SB431542          | 10 mM               | 5 μM                  | 5 μl               |
| IBMX              | 500 mM              | 0.5 mM                | 10 μl              |
| Dexamethasone     | 5 mM                | 5 μM                  | 10 μl              |
| Indomethacin      | 125 mM              | 125 μM                | 10 μl              |

Beige adipocyte maintenance medium:

| Factor            | stock concentration | working concentration | for 10 ml of medium |
|-------------------|---------------------|-----------------------|--------------------|
| EGM-2             | 1X                  | 1X                    | 10 ml              |
| Insulin           | 1.7 mM              | 170 nM                | 1 μl               |
| T3                | 1 μM                | 2 nM                  | 20 μl              |
| Rosiglitazone     | 5 mM                | 1 μM                  | 2 μl               |
| SB431542          | 10 mM               | 5 μM                  | 5 μl               |
Methods S1 cont.

Note: EGM-2 medium can be replaced with DMEM containing 10% FBS for both beige induction and beige maintenance medium. It is often better to use DMEM containing 10% FBS when comparing iPSC-derived beige cells directly to primary cells, since primary cells will not differentiate properly in EGM2 medium.

Generation of beige adipocytes
1. Prior to beige adipogenic precursor induction, MSCs grown in culture plates must be at approximately 90-100% confluence. Conduct beige adipogenic induction in a laminar flow cabinet.

2. Aspirate MesenCult-ACF Plus medium and replace with MesenCult-ACF Plus medium containing SB431542 (5 μM) and IL-4 (10 nM) in the following amounts based upon well size.
   - 96 well plates – 100 μl/well
   - 24 well plates – 500 μl/well
   - 6 well plates – 2000 μl/well

   Allow cells to stay in MesenCult-ACF Plus medium containing SB431542 and IL-4 for 48 hours.

3. Following 48 hours (cells should now be 100% confluent), aspirate MesenCult-ACF Plus medium containing SB431542 and IL-4 and wash once with PBS without calcium or magnesium. Remove PBS and replace with beige adipocyte induction medium in the following amounts based upon well size.
   - 96 well plate – 150 μl/well
   - 24 well plates – 1000 μl/well
   - 6 well plates – 3000 μl/well

4. After 72 hours of induction treatment, aspirate beige adipocyte induction medium from wells and replace with beige adipocyte maintenance medium in the following amounts based upon well size.
   - 96 well plate – 150 μl/well
   - 24 well plates – 1000 μl/well
   - 6 well plates – 3000 μl/well

5. Change 100% of beige adipocyte maintenance medium every 72 hours for 9 days.