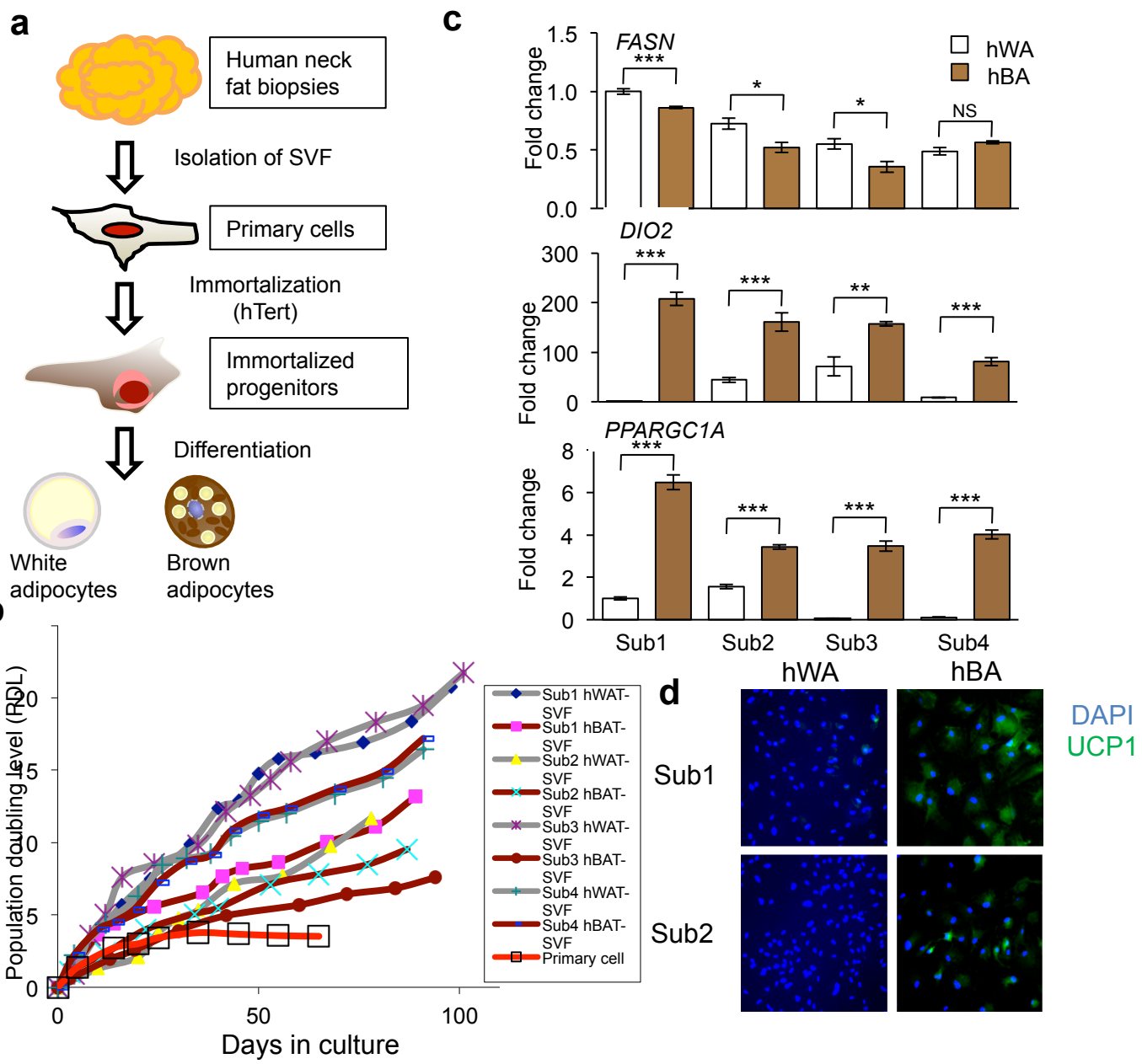
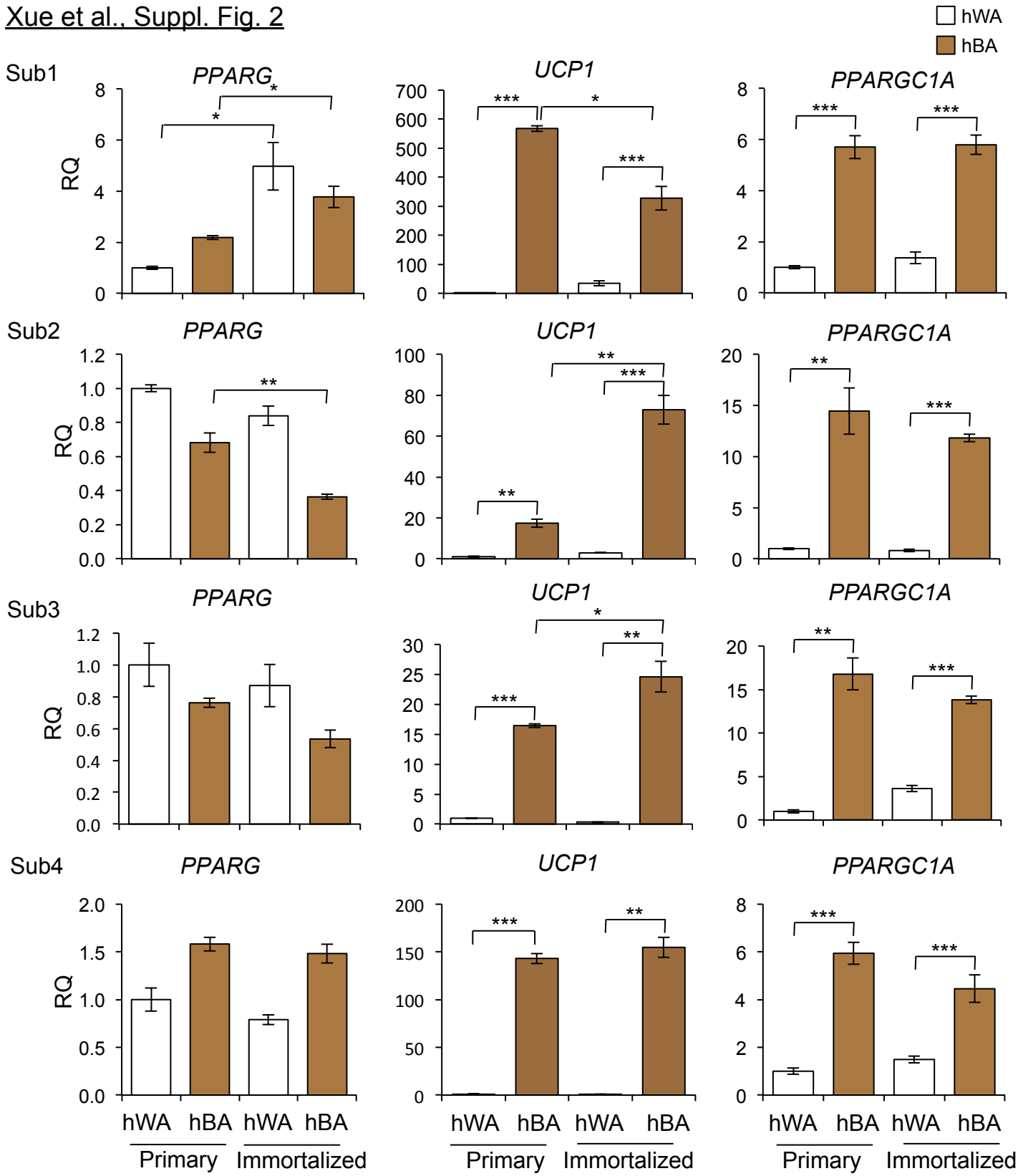


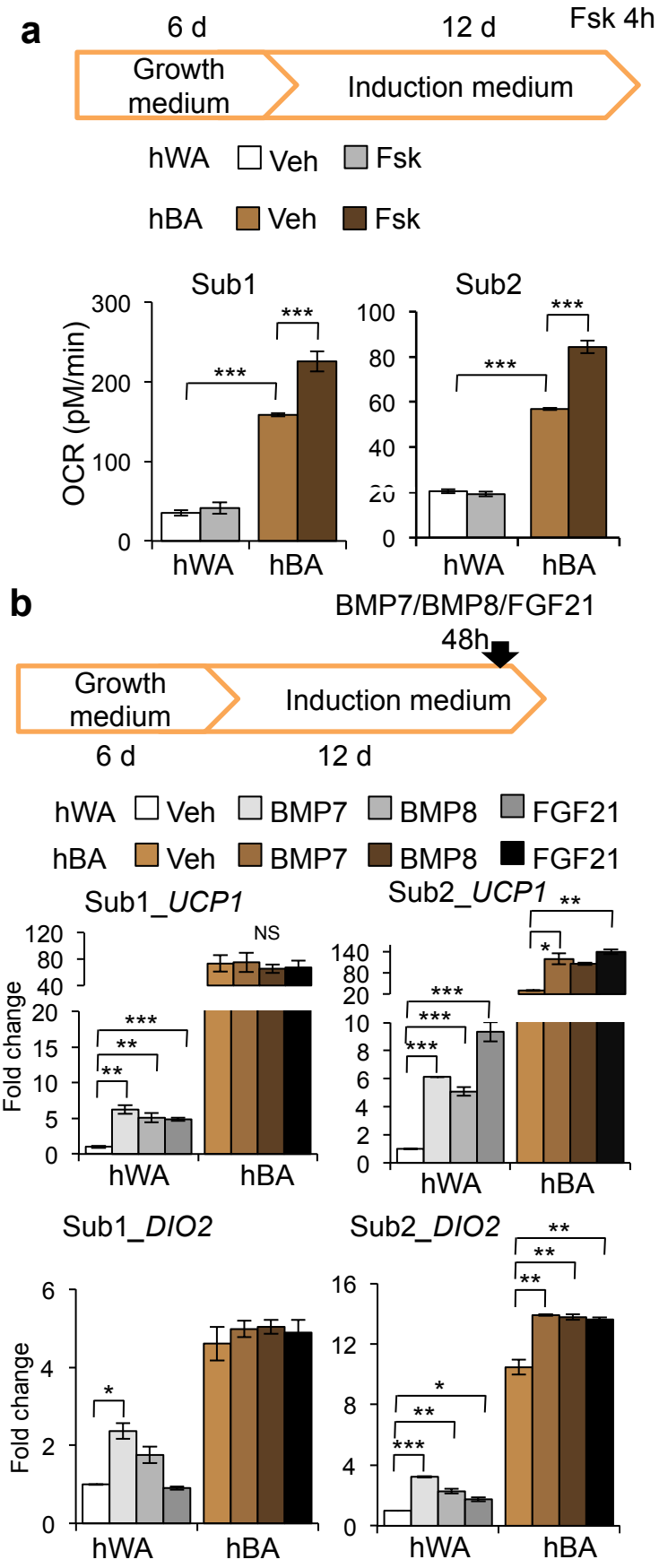
Supplementary Figure 1. Generation and characterization of immortalized human brown and white fat progenitors isolated from human neck fat depot. (a) Strategy of generating immortalized human brown and white fat progenitors from SVF isolated from BAT and WAT located in human neck fat depot. (b) Population doubling levels (PDL) of immortalized hWAT-SVF and hBAT-SVF from 4 subjects compared to primary SVF. Primary and immortalized human SVF were grown in DMEM/H supplemented with 10% FBS and 1% penicillin/streptomycin. Calculate the PDL with the following formula: ΔPDL = log(nh/ni)/log2, where ni is the initial number of cells and nh is the final number of cells at each passage. (c) Q-RT-PCR analysis for *FASN*, *DIO2* and *PPARGC1A* mRNA expression in differentiated human white fat (hWA) and brown fat (hBA) progenitors from 4 subjects. Data are presented as a fold change compared to Sub1 hWA (mean ± s.e.m., *n*=3; two-tailed Student's *t*-test; N.S: not significant, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$). (d) Immunofluorescence staining analysis of UCP1 (green) in differentiated hWA and hBA from Sub1 and Sub2. Nucleus is stained by DAPI (blue). The merge images of UCP1 (green) and DAPI (blue) are shown as indicated. The images are representative of three replicates. Scale bar, 100 μm.

Xue et al., Suppl. Fig. 1

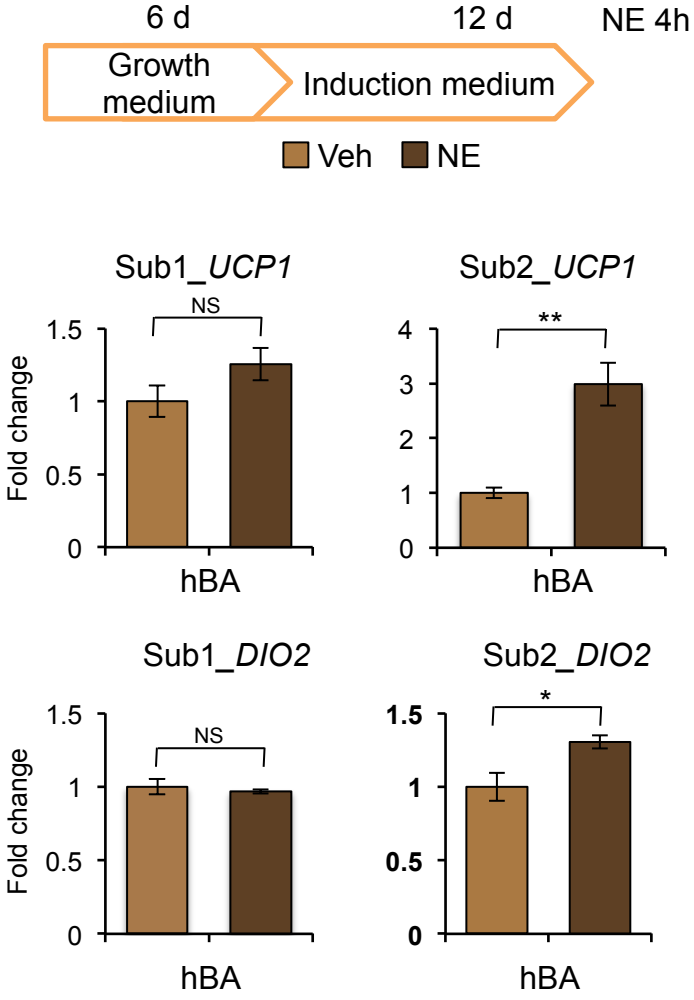
Xue et al., Suppl. Fig. 2



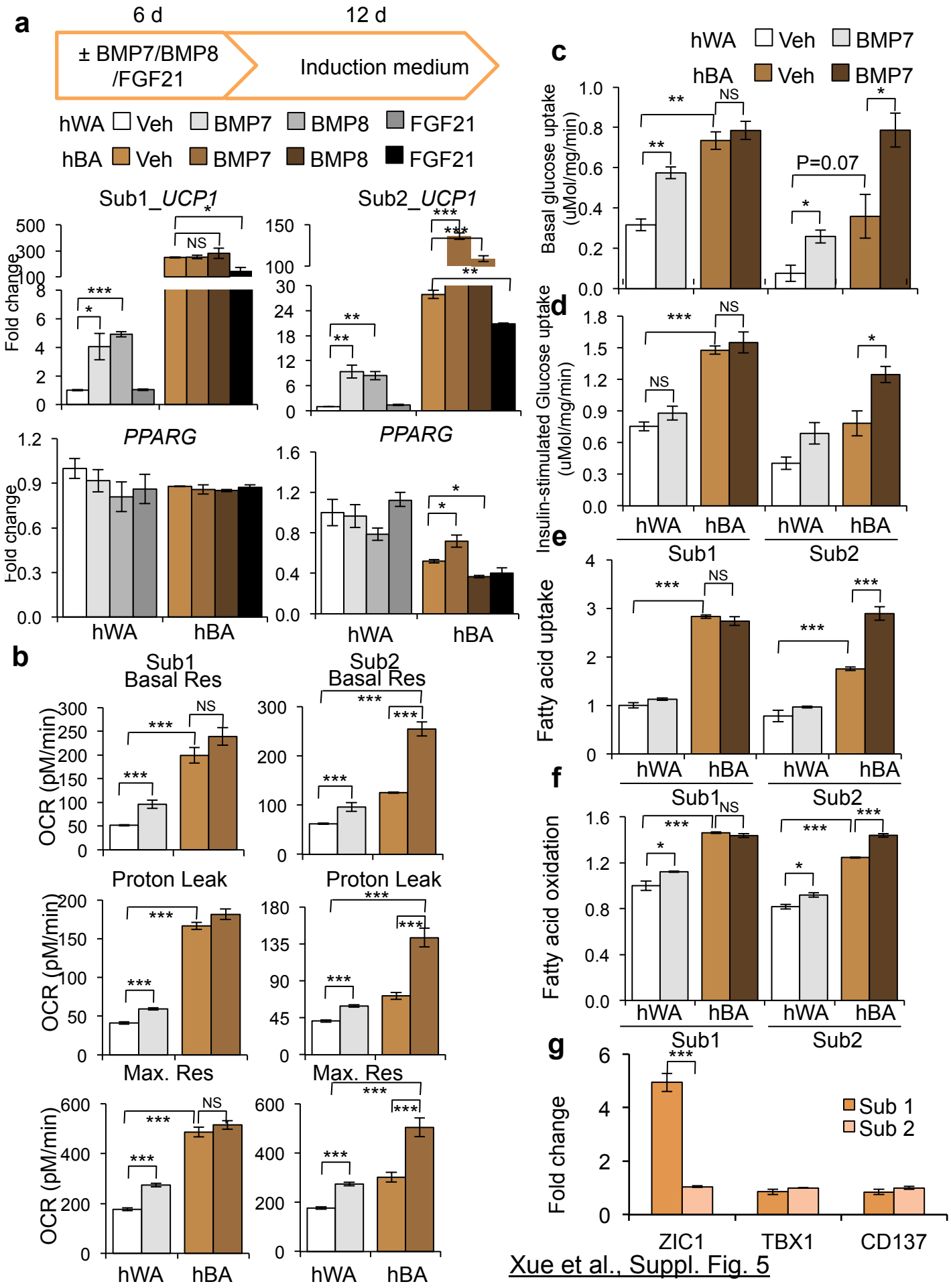
Supplementary Figure 2. Gene expression analysis of differentiated hWA and hBA derived from primary versus immortalized cells. RNA was isolated from differentiated primary and immortalized hWA and hBA from 4 subjects, and Q-RT-PCR analysis was performed for the adipocyte marker, *PPARG*, and brown-fat-specific markers, *UCP1* and *PPARGC1A*. Data are presented as a fold change compared to primary hWA in each subject (mean ± s.e.m., n=3; two-tailed Student's t-test; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$). A representative experiment from a total of three independent studies is shown.



Supplementary Figure 3. Characterization of differentiated hWA and hBA treated with forskolin and browning inducers. (a) Total cellular respiration rate (OCR) was measured using the Seahorse extracellular flux analyzer in differentiated hWA and hBA from Sub1 and Sub2 that were treated for 4 h with 10 uM forskolin (Fsk). The same number of cells was used in the assay. Data are presented as mean ± s.e.m. (*n*=10). (b) Q-RT-PCR analysis for *UCP1* and *DIO2* expression in differentiated hWA and hBA from Sub1 and Sub2 that were treated for 48 h with 3.3 nM BMP7, 3.3 nM BMP8 and 50 nM FGF21. Data are presented as a fold change compared to vehicle treatment of hWA in each subject (mean ± s.e.m., *n*=3). A representative experiment from a total of three independent studies is shown.Two-tailed Student's *t*-test was used to determine *P* values (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).



Supplementary Figure 4. Characterization of differentiated hBA treated with NE. Q-RT-PCR analysis for *UCP1* and *DIO2* expression in differentiated hBA from Sub1 and Sub2. Cells were treated for 4 h with 1 uM norepinephrine (NE). Data are presented as fold changes relative to vehicle control (mean ± s.e.m., $n=3$; two-tailed Student's *t*-test; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$). A representative experiment from a total of three independent studies is shown.

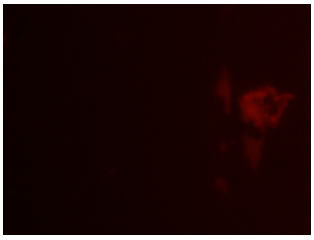
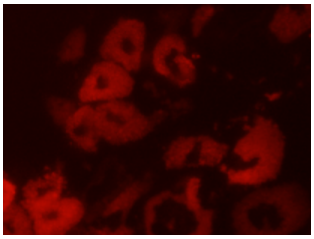
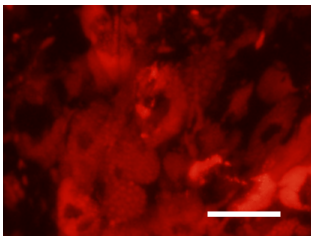


Xue et al., Suppl. Fig. 5

Supplementary Figure 5. Characterization of gene expression, oxygen consumption and fuel utilization in hWA and hBA with BMP7, BMP8, and FGF21 pretreatment and the expression of lineage markers in hBAT-SVF. (a) Q-RT-PCR analysis for *UCP1* and *PPARG* mRNA expression in differentiated hWA and hBA from Sub1 and Sub2 that were pre-treated with 3.3 nM BMP7, 3.3 nM BMP8 and 50 nM FGF21 for 6 days and then differentiation into mature adipocytes. Data are presented as a fold change compared to vehicle (Veh) treatment of hWA in each subject (mean ± s.e.m., *n*=3). A representative experiment from a total of three independent studies is shown. (b) Oxygen consumption rate (OCR) was measured in the absence (Basal Res.) and presence of oligomycin (Proton Leak) or FCCP (Max. Res.) in hWA and hBA from Sub1 (Left) and Sub2 (Right) that were pre-treated with 3.3 nM BMP7 for 6 days and then differentiation into mature adipocytes.. Data are presented as mean ± s.e.m. (*n*=10; vehicle (Veh) vs BMP7).(c-d) Using 3H 2-Deoxy-Glucose for measurement of glucose uptake in differentiated hWA and hBA from Sub1 and Sub2 that were pre-treated with 3.3 nM BMP7 or vehicle (Veh) for 6 days and then differentiation into mature adipocytes. The cells were stimulated in the absence (shown on panel c) and presence (shown on panel d) of 100 nM insulin for 30 min before assay. The same number of cells was used in the assay and data was normalized to protein content. Data are presented as mean ± s.e.m. (*n*=3). A representative experiment from a total of three independent studies is shown. (e-f) Using 14C-palmitic acid for determination of fatty acid uptake (shown on panel e) and oxidation (shown on panel f) in differentiated hWA and hBA from Sub1 and Sub2 that were pre-treated with 3.3 nM BMP7 or vehicle (Veh) for 6 days and then differentiation into mature adipocytes. Data are presented as a fold change compared to vehicle (Veh) treatment of Sub1 hWA (mean ± s.e.m., *n*=3). A representative experiment from a total of two independent studies is shown. (g) Q-RT-PCR analysis for *ZIC1*, *TBX1*, and *CD137* expression in undifferentiated (Day 0) hBAT-SVF from 2 subjects. Data are presented as fold changes relative to subject 2 (mean ± s.e.m., n=3). A representative experiment from a total of two independent studies is shown. Two-tailed Student's *t*-test was used to determine *P* values (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

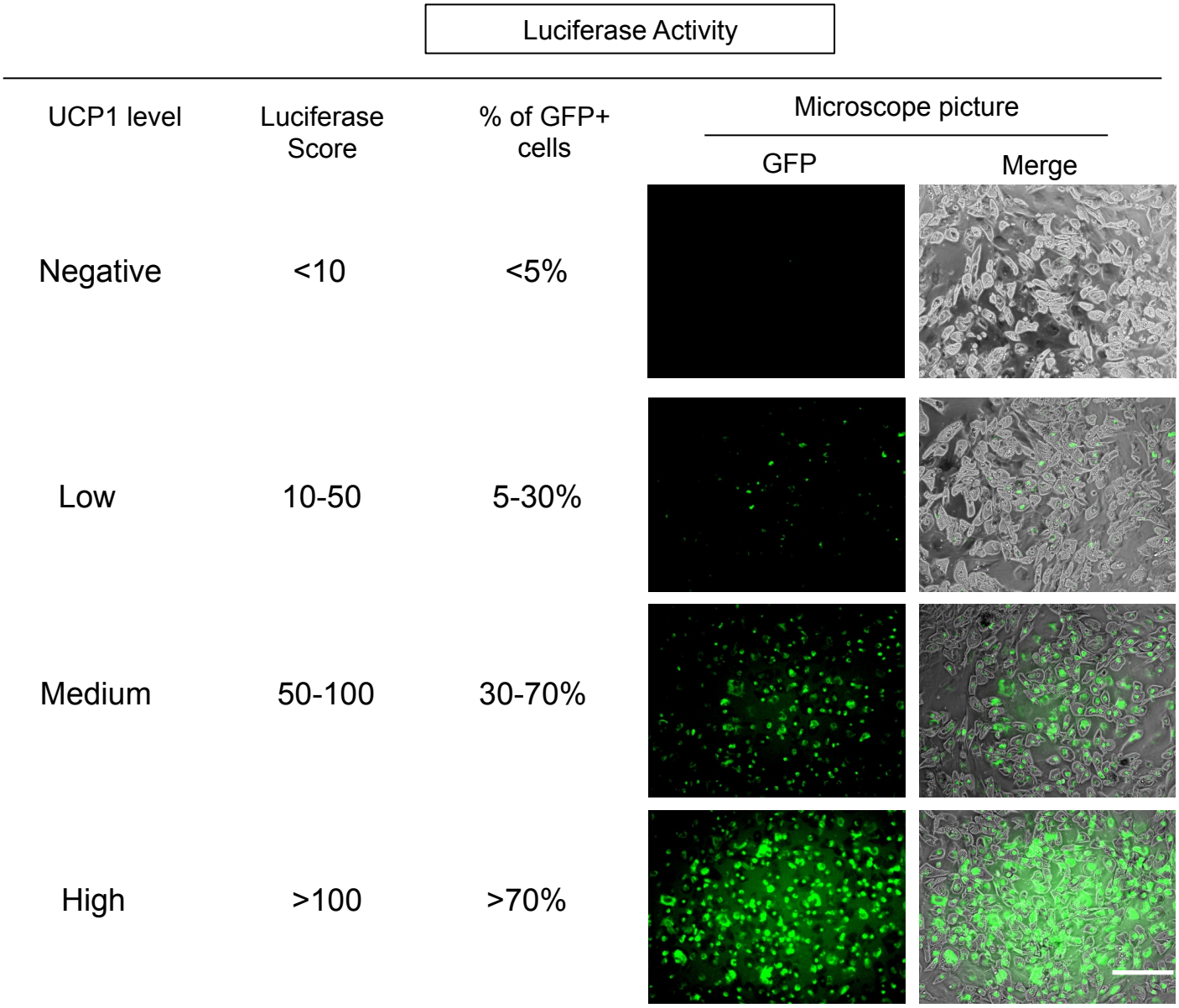
Xue et al., Suppl. Fig. 6

Nile Red Staining

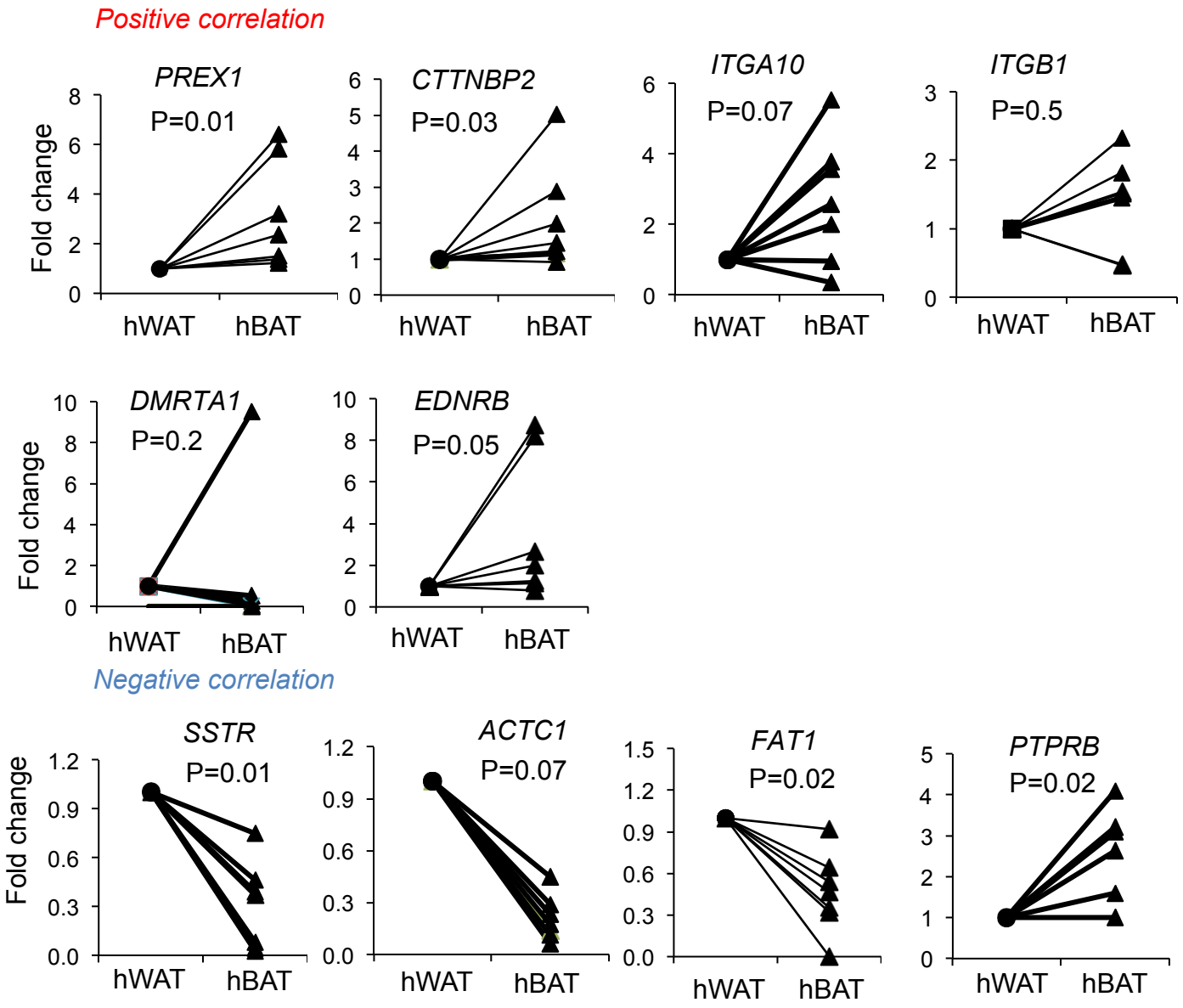
Adipogenic Capacity	Nile Red Score	% of lipid laden cells	Microscope picture
-	<0.2	<20%	
+	0.2-0.4	20-70%	
++	>0.4	>70%	

Supplementary Figure 6. Determination of adipogenic capacity in differentiated clones by Nile red staining. Fluorescence intensity of Nile red staining was detect at Ex/Em=552/636nm, and was divided into different levels (adipogenic-: fluorescence intensity < 0.2; adipogenic+: fluorescence intensity 0.2-0.4; adipogenic++: fluorescence intensity > 0.4) after normalized to protein content. Representative microscope views of Nile red staining on day 18 were shown on right. Scale bar, 100 μm.

Xue et al., Suppl. Fig. 7

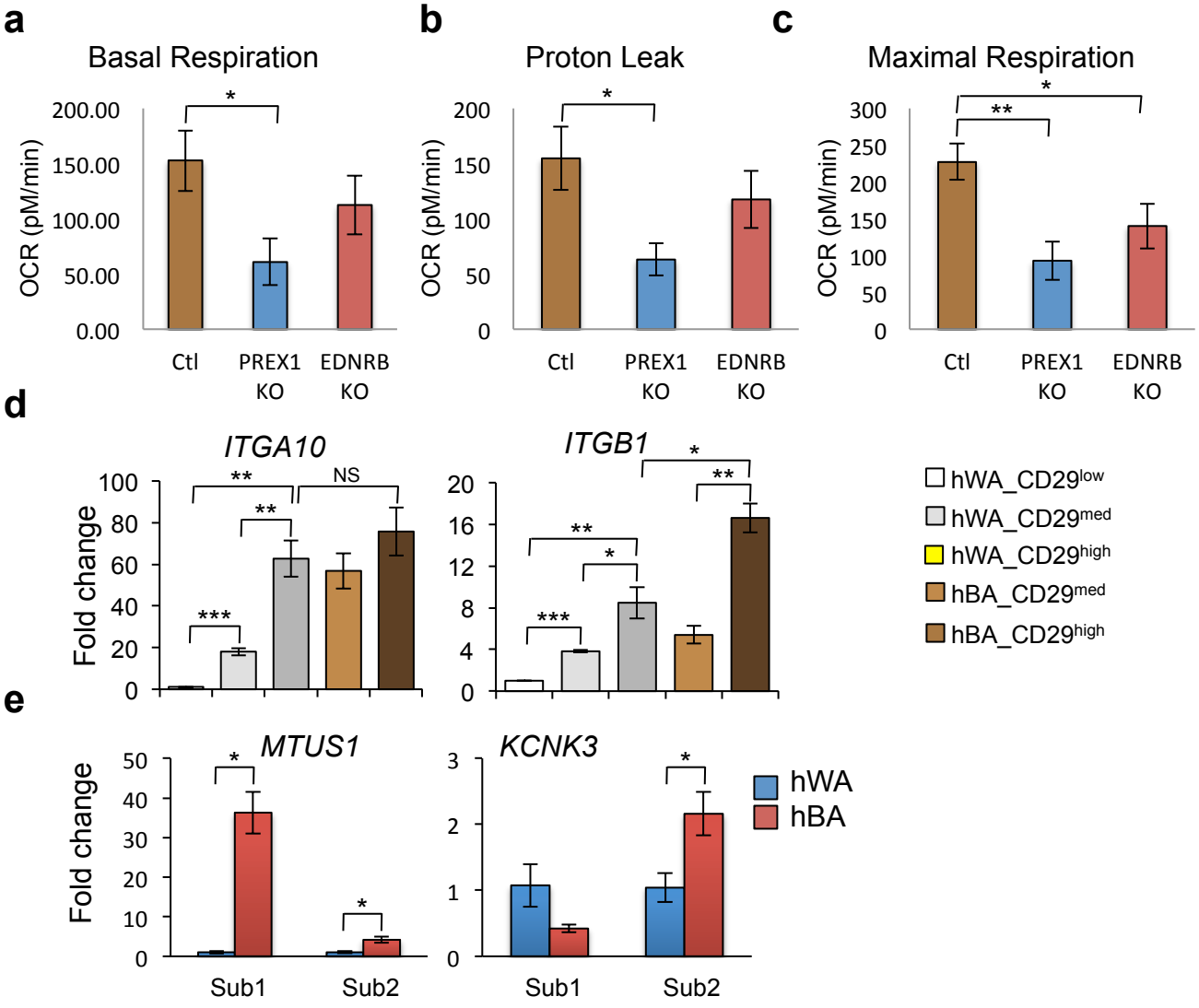


Supplementary Figure 7. Definition of differential levels of UCP1 reporters in mature human adipocytes. Luciferase activity was measured and normalized to protein content, as indicated: negative < 10; low 10-50; medium 50-100; high > 100. And the indicated luciferase activity level is consistent with GFP expression in the same clone on day 18. The microscope views of GFP and merge with light picture are shown on right panel. Scale bar, 50 μm.



Supplementary Figure 8. Expression of positively and negatively correlated candidate genes in human neck fat derived from 7 subjects. Fat was resected from 7 patients undergone neck surgery. Q-RT-PCR analysis was performed for the expression of positive and negative correlation candidate genes that were normalized to 18s. The subcutaneous fat was used as control, white adipose tissues (hWAT), and the deeper fat from each patient that had the highest expression levels of *UCP1* was chosen for the human brown adipose tissues (hBAT) sample. *P* value was analyzed using Wilcoxon matched-pairs signed-ranks test.

Xue et al., Suppl. Fig. 9



Supplementary Figure 9. Functional analysis of *PREX1* and *EDNRB* Knockout cells and expression of potential brown fat markers in hWA and hBA. Oxygen consumption rate (OCR) was measured using the Seahorse extracellular flux analyzer in wild type control, *PREX1*and *EDNRB* Knockout hBA. Equal numbers of progenitors were plated and differentiated. Quantifications of OCR in the (a) absence (Basal respiration, Basal Res.) and (b) presence of oligomycin (Proton Leak) or (c) FCCP (Maximal respiration, Max. Res.) are shown. Data were normalized to DNA content and are presented as mean ± s.e.m. (n=7). Asterisks depict statistically significant differences between wild type and each knockout. A representative experiment from three independent studies is shown. (d) mRNA expression of *ITGA10* (Left) and *ITGB1* (Right) in sorted subpopulation with different expression level of CD29 on day 0. Data are shown as a fold change compared to $CD29^{low}$ subpopulation from hWAT-SVF (mean ± s.e.m., n=3). (e) mRNA expression of *MTUS1* and *KCNK3* in the differentiated brown and white fat cells derived from pooled progenitors of Subject 1 and Subject 2. Two-tailed Student's *t*-test was used to determine *P* values (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

Supplementary Table 1a. Clinical characteristics of subjects whose neck fat were used for generation of immortalized human WAT and BAT progenitors.

Patient Code	Age (year)	Sex	BMI (kg/m^2)	hWAT[a]	hBAT[a]
Subject 1	58	M	28.0	SQ[b] SP[c]	CS[d] LC[e]
Subject 2	54	M	25.1	SQ SP	CS PV[f]
Subject 3	42	M	33.0	SQ SP	CS PV
Subject 4	63	M	33.3	SQ SP	CS LC PV

[a]These two columns indicate the anatomical locations of neck fat tissues (as described in Ref. 20) used for generation of adipose progenitors.
[b]Subcutaneous [c]Subplatysmal [d]Carotid sheath [e]Longus colli [f]Prevertebral

Supplementary Table 1b. Clinical characteristics of subjects whose neck fat were used for evaluating expression of candidate genes.

Patient Code	Age (year)	Sex	BMI (kg/m^2)
Subject 1	72	M	30.9
Subject 2	46	M	32.6
Subject 3	53	M	28.0
Subject 4	33	M	29.8
Subject 5	54	F	36.3
Subject 6	44	F	32.3
Subject 7	77	F	20.4

Supplementary Table 2. Distribution of hWAT-SVF and hBAT-SVF clones that displayed different UCP1 levels and differential responses to BMP7 pre-treatment in 4 human subjects.

hWAT-SVF Clones (*n*=67)

Patient Code	Total N	UCP1(-)[a]		UCP1(+)[b]
		BMP7 Resp(-)[c]	BMP7 Resp(+)[d]	BMP7 Resp(+)
Subject1	32	32/32	0/32	0/32
Subject2	20	20/20	0/20	0/20
Subject3	12	11/12	0/12	1/12
Subject4	3	3/3	0/3	0/3

hBAT-SVF Clones (*n*=90)

Patient Code	Total N	UCP1(-)	UCP1(+)	
		BMP7 Resp(-)	BMP7 Resp(-)	BMP7 Resp(+)
Subject1	41	2/41	29/41 (71%)	10/41
Subject2	15	0/15	5/15	10/15 (67%)
Subject3	15	3/15	3/15	9/15 (60%)
Subject4	19	0/19	14/19 (74%)	5/19

[a]UCP1 expression was considered negative in the differentiated clones, which was determined by luciferase activity on day 18. The luciferase activity score was less than 10 after normalized to protein amount.
[b]UCP1 expression was considered positive in the differentiated clones, which was determined by luciferase activity on day 18. The luciferase activity score was more than 10 after normalized to protein amount.
[c]Human fat progenitor clones were pre-treated with 3.3 nM BMP7 for 6 days and then differentiation into mature adipocytes. The fold change of luciferase activity between 0.8-1.5 compared to vehicle (Veh) treatment is defined as negative response to BMP7 stimulation (BMP7 Resp(-)).
[d]Human fat progenitor clones were pre-treated with 3.3 nM BMP7 for 6 days and then differentiation into mature adipocytes. The fold change of luciferase activity more than 1.5 compared to vehicle (Veh) treatment is defined as positive response to BMP7 stimulation (BMP7 Resp(+)).

Supplementary.Table3. Correlation of expression of known adipose markers in human preadipocytes with UCP1-reporter levels in adipocytes

Gene Name	#Cor. Coe	P-values	FDR	Reference
General preadipocyte marker				
ZNF423	0.337	0.0312*	0.232	Gupta et al., Cell Metab. 2012
PDGFRB	-0.285	0.0708	0.353	Tang et al., Science 2008
WAT marker				
TRIP (ASC-1)	-0.267	0.0921	0.402	Ussar et al., Sci Transi Me.2014
HOXC8	-0.388	0.0123*	0.138	Gesta et al., PNAS 2006; Timmons et al., PNAS 2007
HOXC9	-0.61	0.000022*	0.00309	Gesta et al., PNAS 2006; Timmons et al., PNAS 2007
Brwon/Beige fat marker				
PRDM16	0.572	0.000093*	0.00701	Seale et al., Nature 2008
EBF2	0.253	0.111	0.439	Rajakumari et al., Cell Metab 2013
EBF3	0.344	0.0277*	0.218	Wu et al., Cell 2012
FBXO31	0.37	0.0171*	0.167	Wu et al., Cell 2012
KCNK3	-0.223	0.161	0.523	Shinoda et al., Nat Med 2015
MTUS1	-0.344	0.0276	0.218	Shinoda et al., Nat Med 2015
Beige fat marker				
TBX15	-0.387	0.0124*	0.139	Gesta et al., PNAS 2006; Timmons et al., PNAS 2007
TMEM26	0.206	0.196	0.569	Wu et al., Cell 2012

Cor.Coe. = Correlation Coefficient (see methods)
'-' indicates negative correlation
'*' indicates significance (P<0.05)

Supplementary.Table4.The correlation coefficients, *P*-values and FDR for top-ranking genes

	# Cor. Coe.	P-Value	FDR	Gene Symbol
Positive Correlation	0.691	5.71E-07	0.000421	EDNRB
	0.696	4.39E-07	0.000376	ST6GALNAC3
	0.695	4.7E-07	0.000387	CTTNBP2
	0.794	5.88E-10	5.51E-06	PREX1
	0.732	5.33E-08	0.000106	S1PR3
	0.723	9.36E-08	0.000144	SVIL
	0.727	7.25E-08	0.000128	C17orf60
	0.72	1.13E-07	0.000156	MASP1
	0.691	5.61E-07	0.00042	PXK
	0.702	3.09E-07	0.000288	C10orf90
	0.728	6.97E-08	0.000128	TBC1D19
	0.732	5.39E-08	0.000106	DNASE1L1
	0.713	1.7E-07	0.0002	GPRC5A
	0.7	3.5E-07	0.00032	ITGA10
	0.72	1.11E-07	0.000156	ETFDH
	0.695	4.5E-07	0.000376	MORN4
	0.72	1.14E-07	0.000156	MRPS6
	0.802	2.82E-10	4.64E-06	SETDB2
	0.73	6.2E-08	0.000118	WRB
	0.692	5.47E-07	0.00042	SYNRG
	0.705	2.64E-07	0.000269	ANP32A
	0.82	5.62E-11	2.78E-06	DMRTA1
Negative Correlation	-0.721	1.05E-07	0.000156	TEK
	-0.764	6.15E-09	3.15E-05	CDH13
	-0.692	5.38E-07	0.00042	EPB41L3
	-0.69	6.02E-07	0.000437	KRTCAP2
	-0.705	2.72E-07	0.000269	NUCB2
	-0.711	1.93E-07	0.000222	SMYD2
	-0.755	1.15E-08	4.61E-05	PSME4
	-0.715	1.46E-07	0.000181	TJP1
	-0.741	2.98E-08	7.26E-05	ZNF518B
	-0.753	1.31E-08	4.61E-05	GRIK2
	-0.696	4.45E-07	0.000376	ANTXR1
	-0.706	2.59E-07	0.000269	SLC7A6
	-0.795	5.36E-10	5.51E-06	FAT1
	-0.692	5.51E-07	0.00042	THBS1
	-0.741	3.09E-08	7.26E-05	TOM1L1
	-0.747	1.98E-08	6.13E-05	CSRP2
	-0.81	1.48E-10	3.65E-06	STXBP6
	-0.702	3.09E-07	0.000288	ACTC1
	-0.708	2.22E-07	0.000246	SHROOM3
	-0.749	1.81E-08	5.98E-05	WNT2
	-0.718	1.24E-07	0.000166	HAPLN1
	-0.737	3.87E-08	8.31E-05	COL12A1
	-0.746	2.2E-08	6.39E-05	NALCN
	-0.739	3.44E-08	7.72E-05	PLCXD3
	-0.776	2.49E-09	1.54E-05	PTPRB
	-0.744	2.55E-08	6.64E-05	SSTR1

[#] Cor. Coe. = Correlation Coefficient (see methods), '-' indicates negative correlation

Supplementary.Table5. Quantitative Real-Time PCR primer sequences

Gene		Sequence
18s	Forward	TCAACTTTCGATGGTAGTCGCCGT
	Reverse	TCCTTGGATGTGGTAGCCGTTTCT
ACTC1	Forward	TGTCTTCCCGTCCATCGT
	Reverse	TCTCTTGCTCTGGGCTTCAT
CTTNBP2	Forward	AGAGACCTTGTCATCGAGGC
	Reverse	CACCAGCACCTGCTTCATAG
CD137	Forward	AGCTGTTACAACATAGTAGCCAC
	Reverse	TCCTGCAATGATCTTGTCCTCT
DMRTA1	Forward	GCAGAGACCGAGGCGTTAG
	Reverse	AACCTGCATCCCCGATGGTA
DIO2	Forward	AGTGCAGAAGGAGGTGACAACAGT
	Reverse	AAAGTCAAGAAGGTGGCATGTGGC
EDNRB	Forward	CAAGAACAAGTGCATGCGAA
	Reverse	ACATCTCAGCTCCAAATGGC
FABP4	Forward	ACTGGGCCAGGAATTTGACGAAGT
	Reverse	TCTCGTGGAAGTGACGCCTTTCAT
FASN	Forward	GCATCTGGACCCTCCTACCT
	Reverse	TCCTCAATTCCAATCCCTTG
FAT1	Forward	CCATTAGAGATGGCTCTGGC
	Reverse	TAGCCAATAATGGGAGGTCG
ITGA10	Forward	GACAGAAACCGATCAGGCAT
	Reverse	AATAGGCGTGGGTGATGTTC
ITGB1	Forward	ATGGGACATTTGAGTGTGGC
	Reverse	TTTCCTGCAGTAAGCATCCA
KCNK3	Forward	CTACGAGCACTGGACCTTCTT
	Reverse	CGTAAGGATGTAGACGAAGCTGA
LEP	Forward	TCTATGTCCAAGCTGTGC
	Reverse	TTGGAGGAGACTGACTGC
MTUS1	Forward	ATCTCAAGGCAGCTTTCCACG
	Reverse	TCGCTTGTTGACTTTCGACTC
PPARGC1A	Forward	AGTGGTGCAGTGACCAATCA
	Reverse	CTGCTAGCAAGTTTGCCTCA
PPARG	Forward	ACTCTGGGAGATTCTCCTATT
	Reverse	CTCCATAGTGAAATCCAGAAG
PREX1	Forward	CTCAACGAGATCTTGGGCAC
	Reverse	GACCTTGACATTCTCCTCCG
PTPRB	Forward	GAACTGTGGTCTTGCAAACAG
	Reverse	AAGGAGTCCACCAAACATGC
SSTR1	Forward	GAAGTTGTCTGAGAGAAAGCCAT
	Reverse	CTGGTCAACGTGTTTGCTGAG
TBX1	Forward	ACGACAACGGCCACATTATTC
	Reverse	CCTCGGCATATTTCTCGCTATCT
TMEM26	Forward	ATGGAGGGACTGGTCTTCCTT
	Reverse	CTTCACCTCGGTCACTCGC
ZIC1	Forward	AAGATCCACAAAAGGACGCA
	Reverse	CACGTGCATGTGCTTCTTG
UCP1	Forward	ACCGCAGGGAAAGAAACAGC
	Reverse	TCAGATTGGGAGTAGTCCCT

Xue et al., Suppl. Table 5