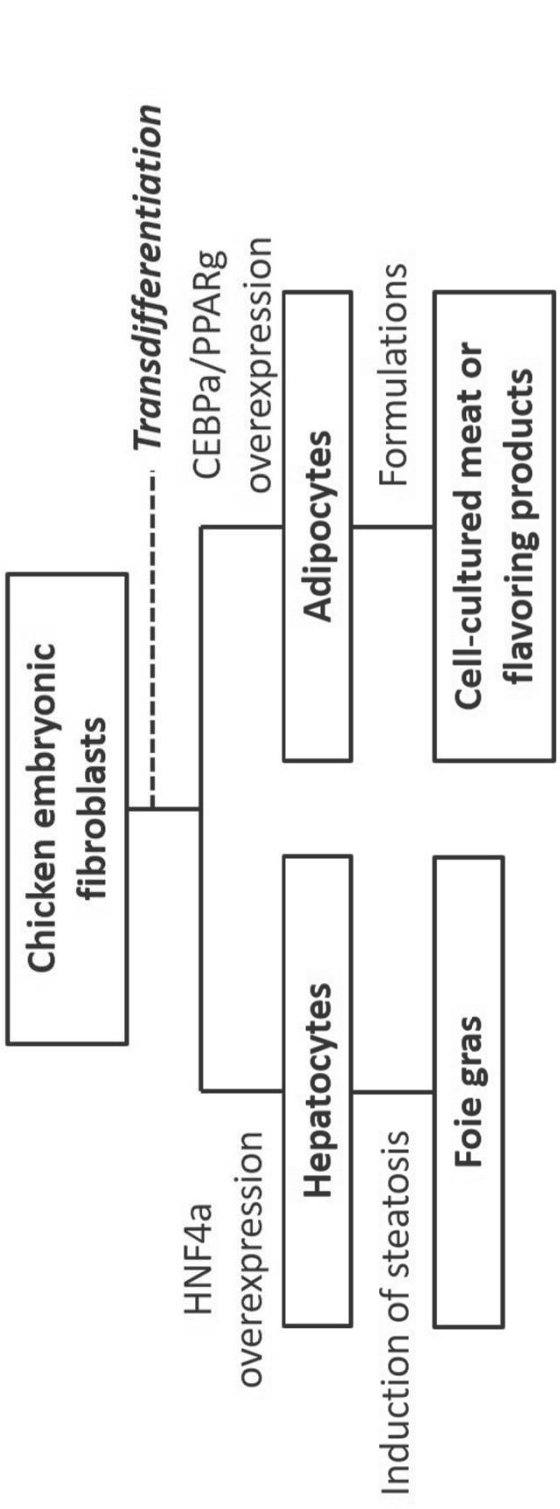
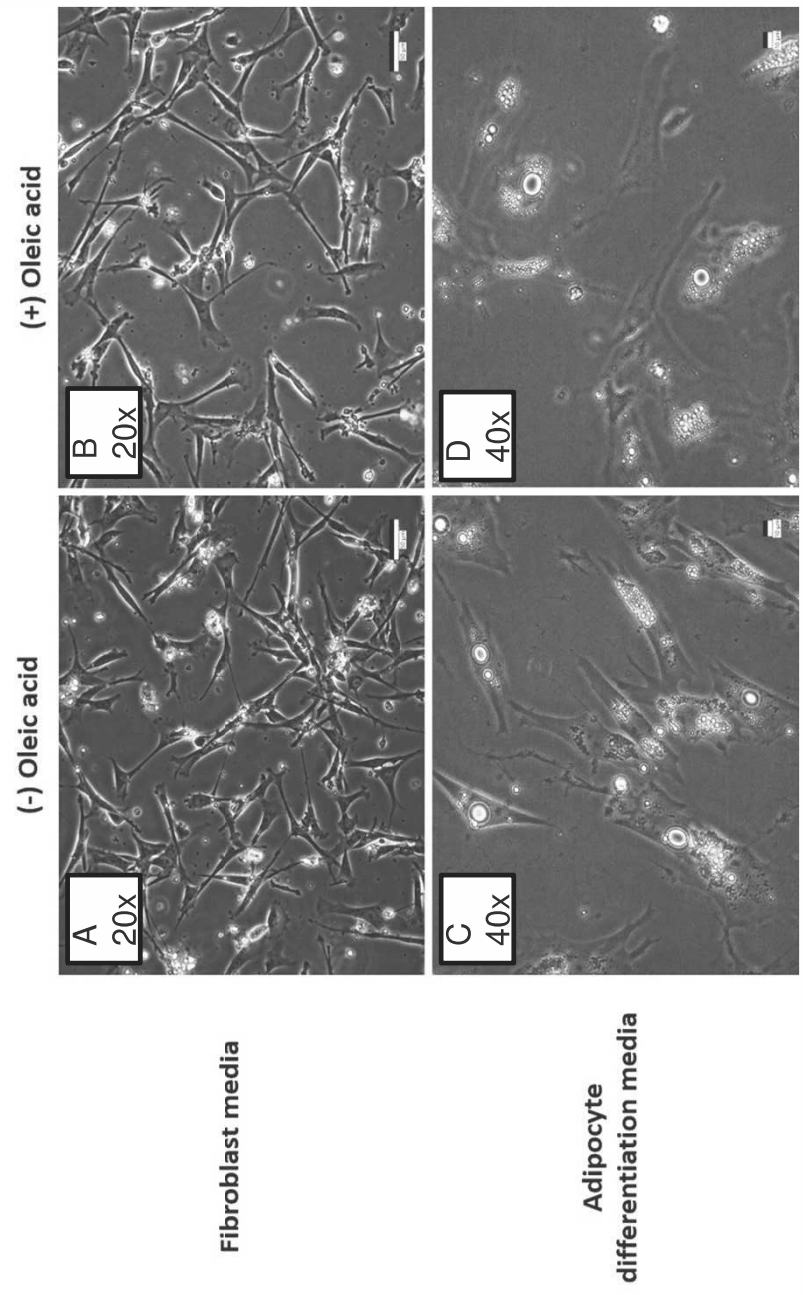

Chicken embryonic fibroblasts
Transdifferentiation
CEBPa/PPARg overexpression
Adipocytes
Formulations
Cell-cultured meat or flavoring products
HNF4a overexpression
Hepatocytes
Induction of steatosis
Foie gras

Figure 1

Figure 2


(-) Oleic acid
(+) Oleic acid
A 20x
B 20x
C 40x
D 40x
Fibroblast media
Adipocyte differentiation media

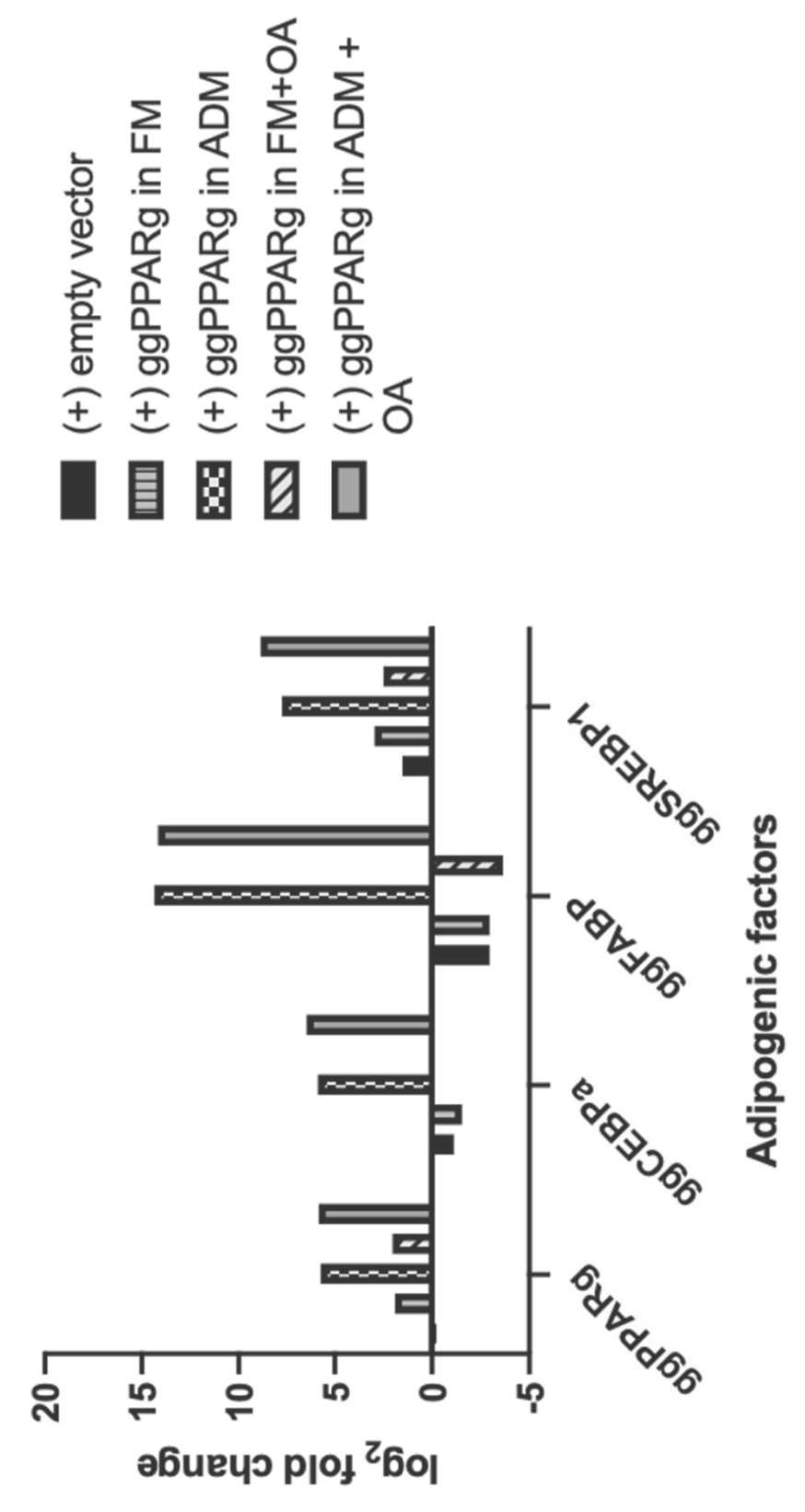

(+) empty vector
(+) ggPPARg in FM
(+) ggPPARg in ADM
(+) ggPPARg in FM+OA
(+) ggPPARg in ADM + OA
log2 fold change
20
15
10
5
0
-5
ggPPARg
ggCEBPa
ggFABP
ggSREBP1
Adipogenic factors

Figure 3

Figure 4

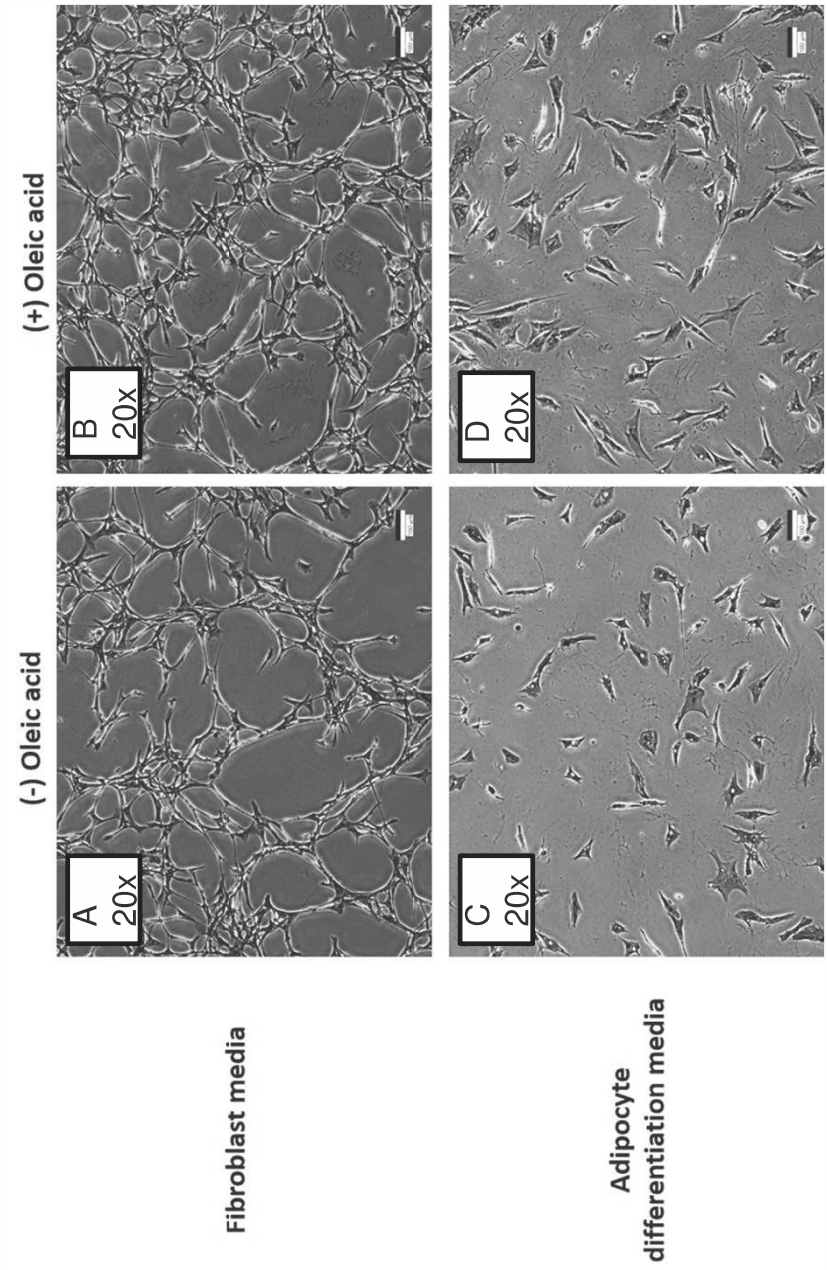
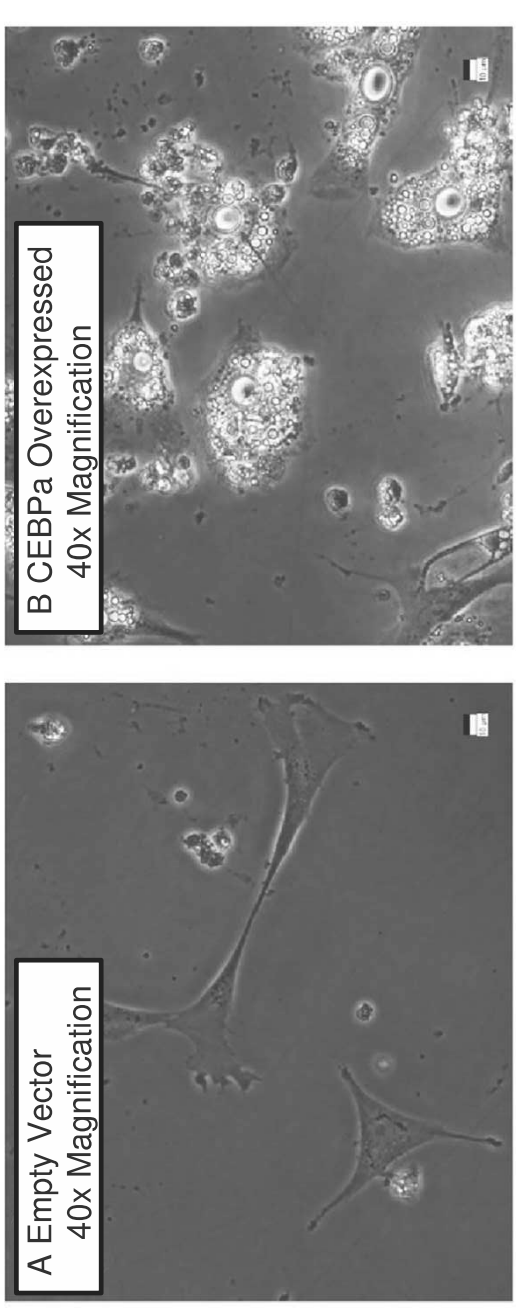

(-) Oleic acid
(+) Oleic acid
A 20x
B 20x
C 20x
D 20x
Fibroblast media
Adipocyte differentiation media

Figure 5


A Empty Vector
40x Magnification
B CEBPa Overexpressed
40x Magnification

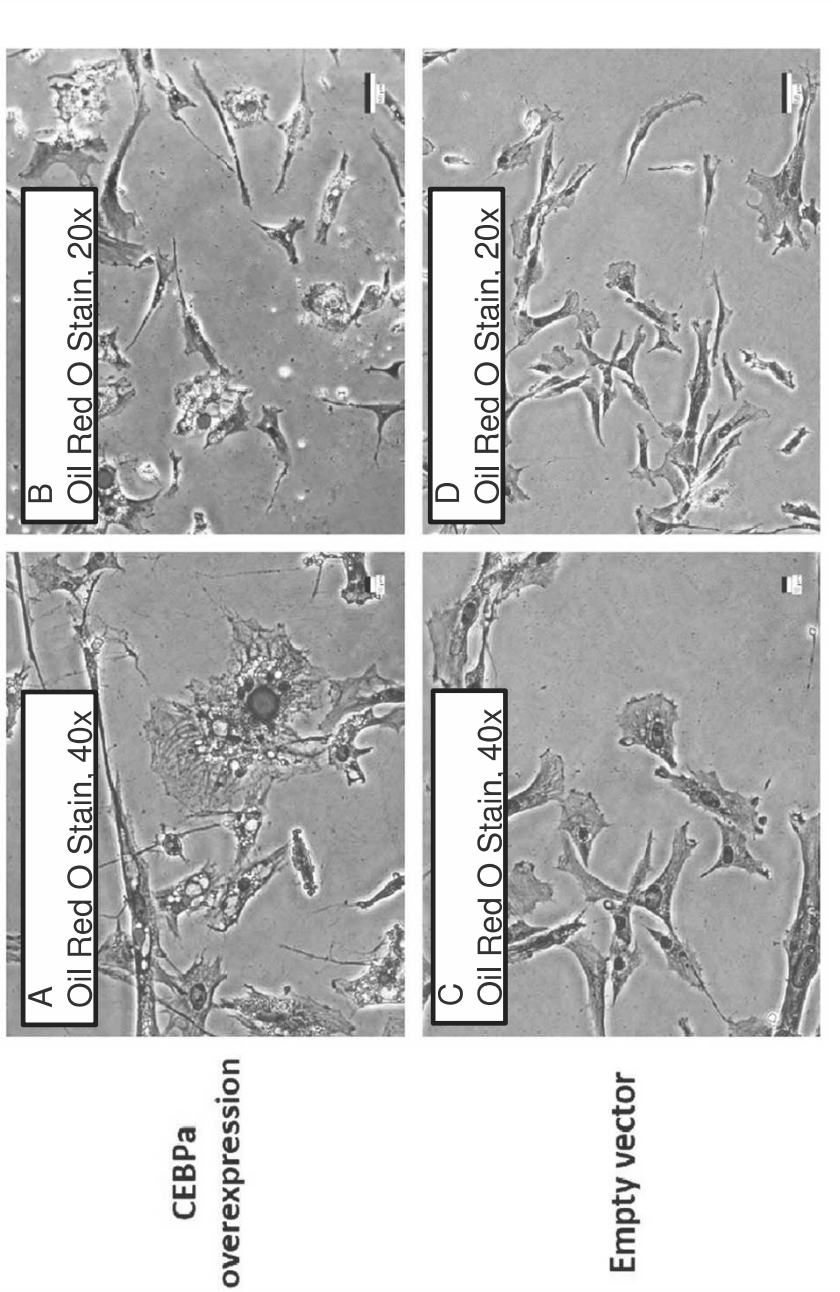
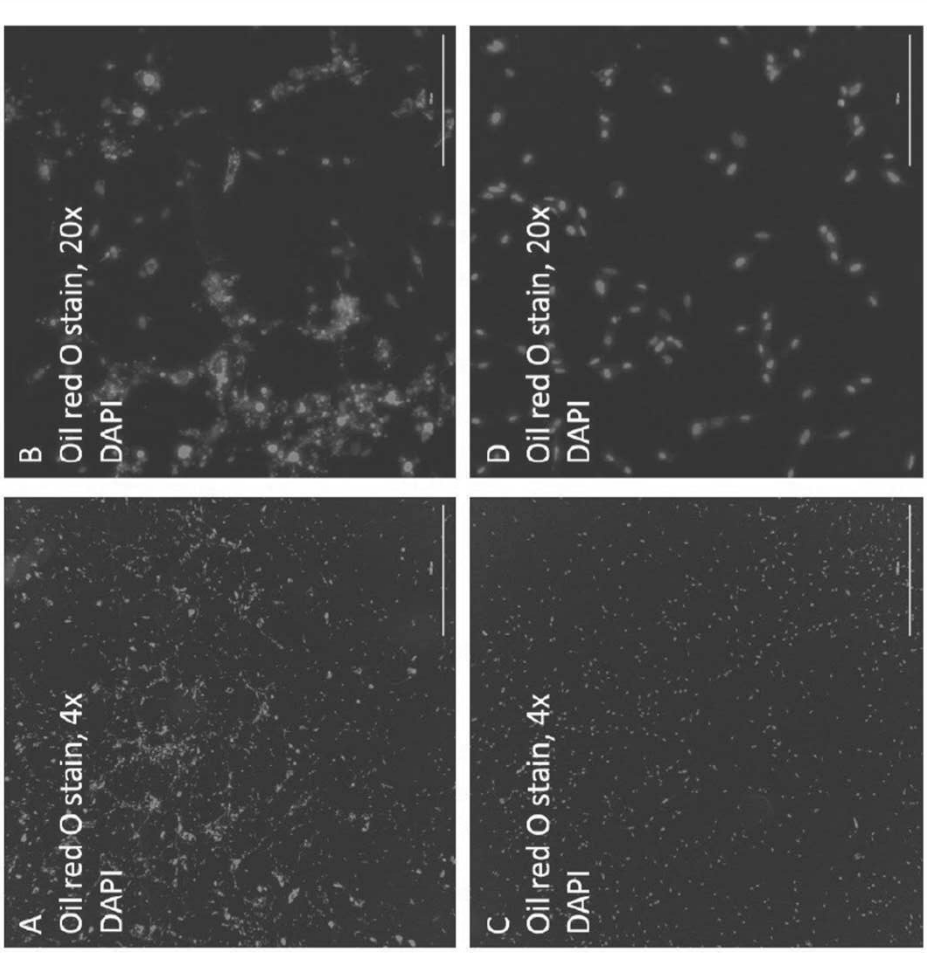

A
Oil Red O Stain, 40x
B
Oil Red O Stain, 20x
C
Oil Red O Stain, 40x
D
Oil Red O Stain, 20x
CEBPa overexpression
Empty vector

Figure 6

Figure 7


A
Oil red O stain, 4x
DAPI
B
Oil red O stain, 20x
DAPI
C
Oil red O stain, 4x
DAPI
D
Oil red O stain, 20x
DAPI
CEBPa overexpression
Empty vector

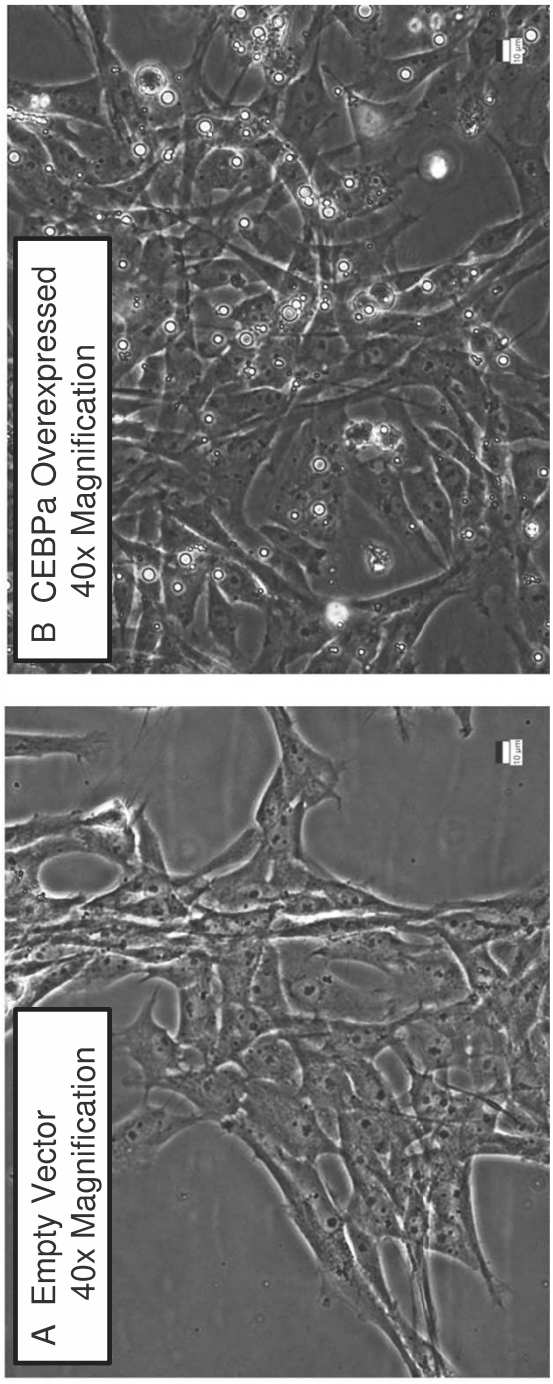

A Empty Vector
40x Magnification
B CEBPa Overexpressed
40x Magnification

Figure 8

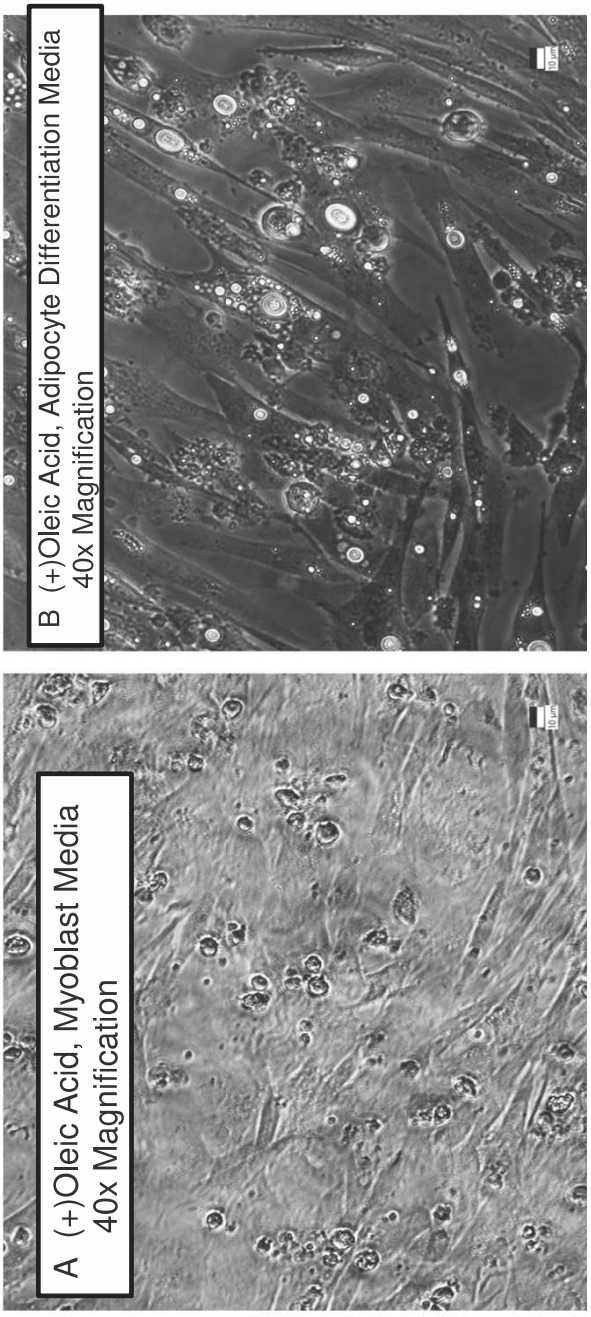

A (+)Oleic Acid, Myoblast Media
40x Magnification
B (+)Oleic Acid, Adipocyte Differentiation Media
40x Magnification

Figure 9

Figure 10

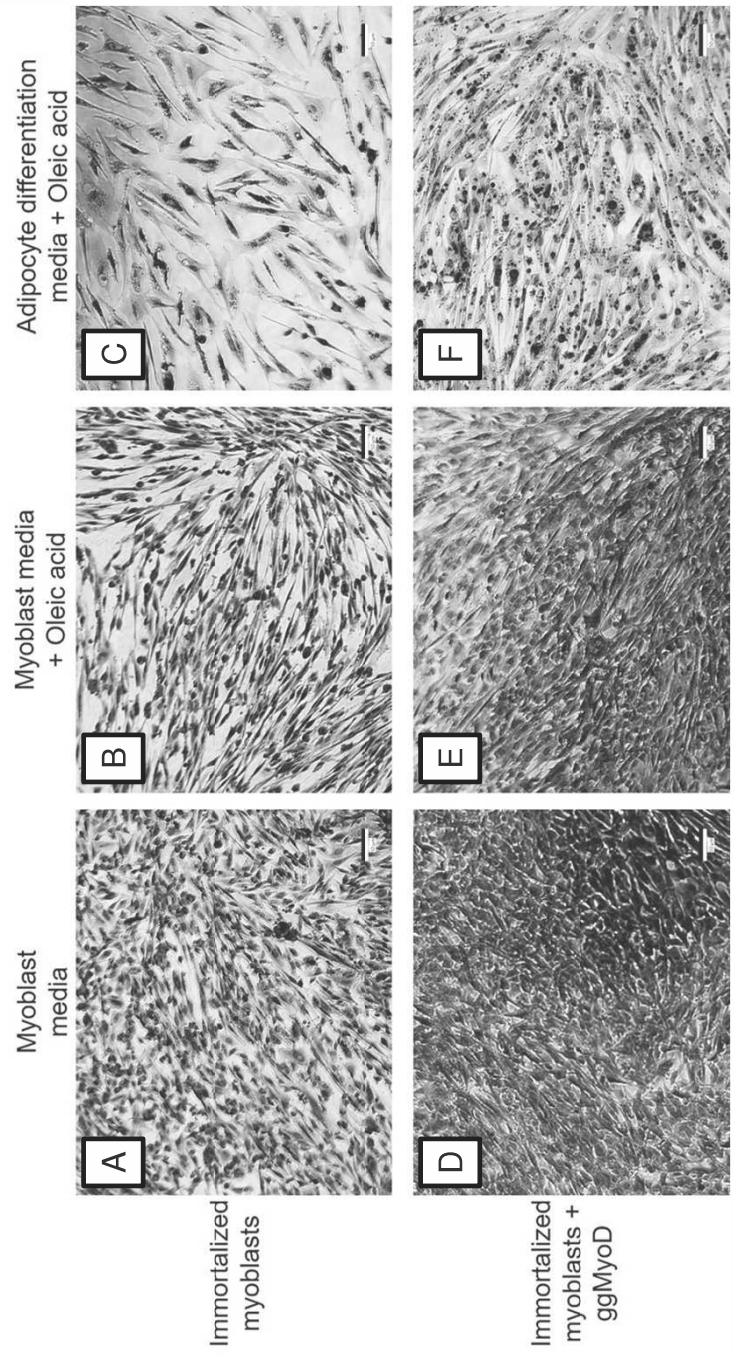
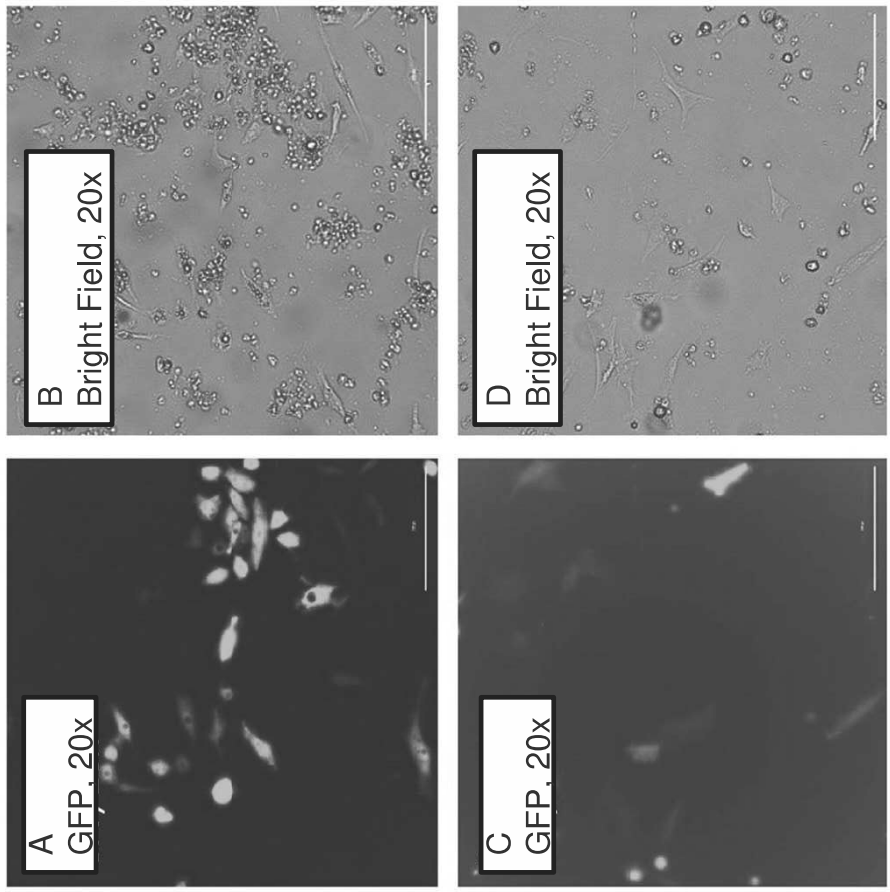

Myoblast media
Myoblast media + Oleic acid
Adipocyte differentiation media + Oleic acid
A
B
C
D
E
F
Immortalized myoblasts
Immortalized myoblasts + ggMyoD

Figure 11


A
GFP, 20x
B
Bright Field, 20x
C
GFP, 20x
D
Bright Field, 20x
CEBPa overexpression
Empty vector

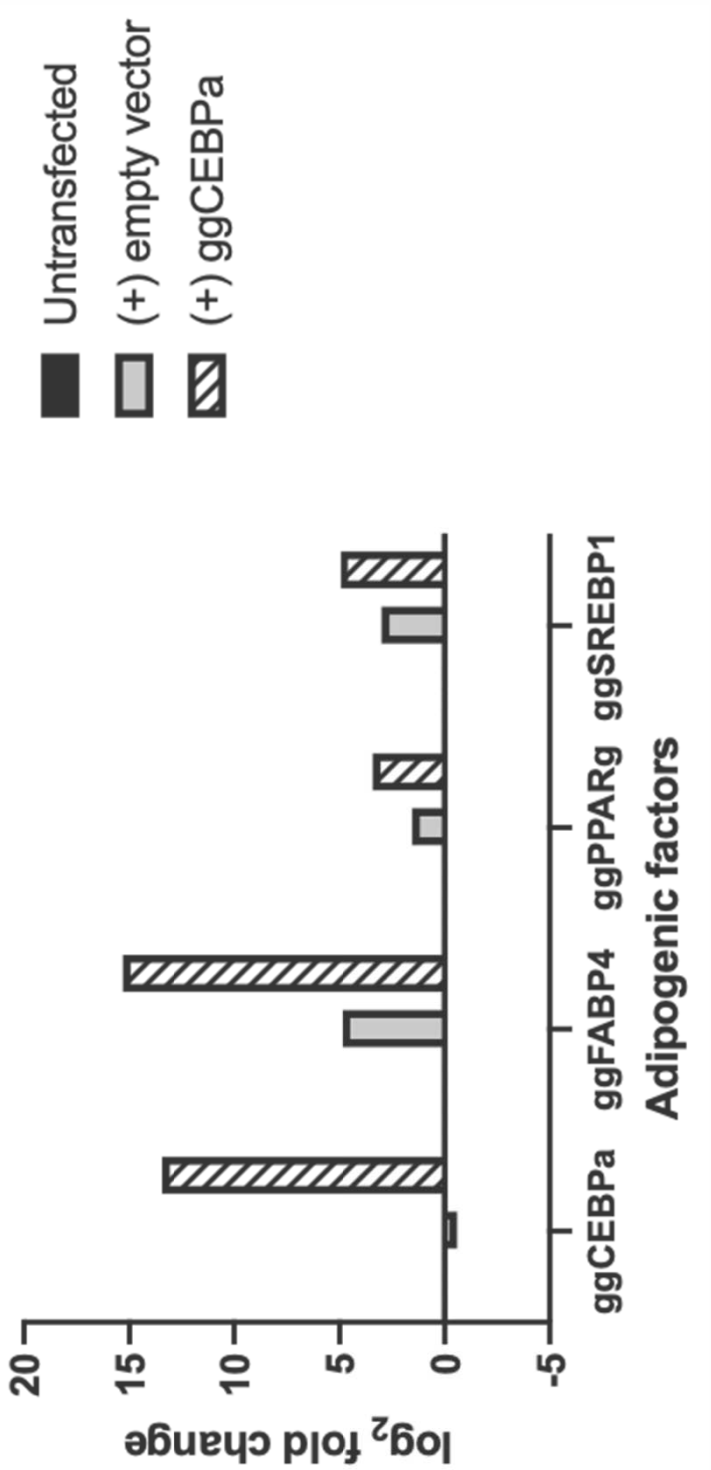

Untransfected
(+) empty vector
(+) ggCEBPa
log2 fold change
20
15
10
5
0
-5
ggCEBPa
ggFABP4
ggPPARg
ggSREBP1
Adipogenic factors

Figure 12

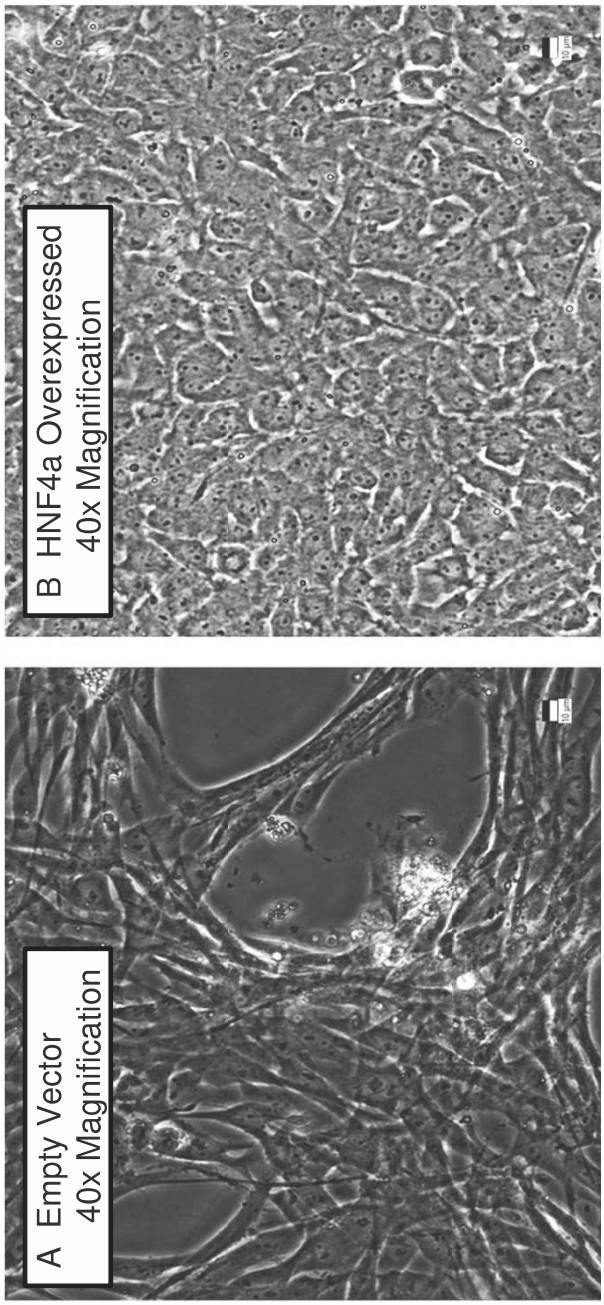

A Empty Vector
40x Magnification
B HNF4a Overexpressed
40x Magnification

Figure 13

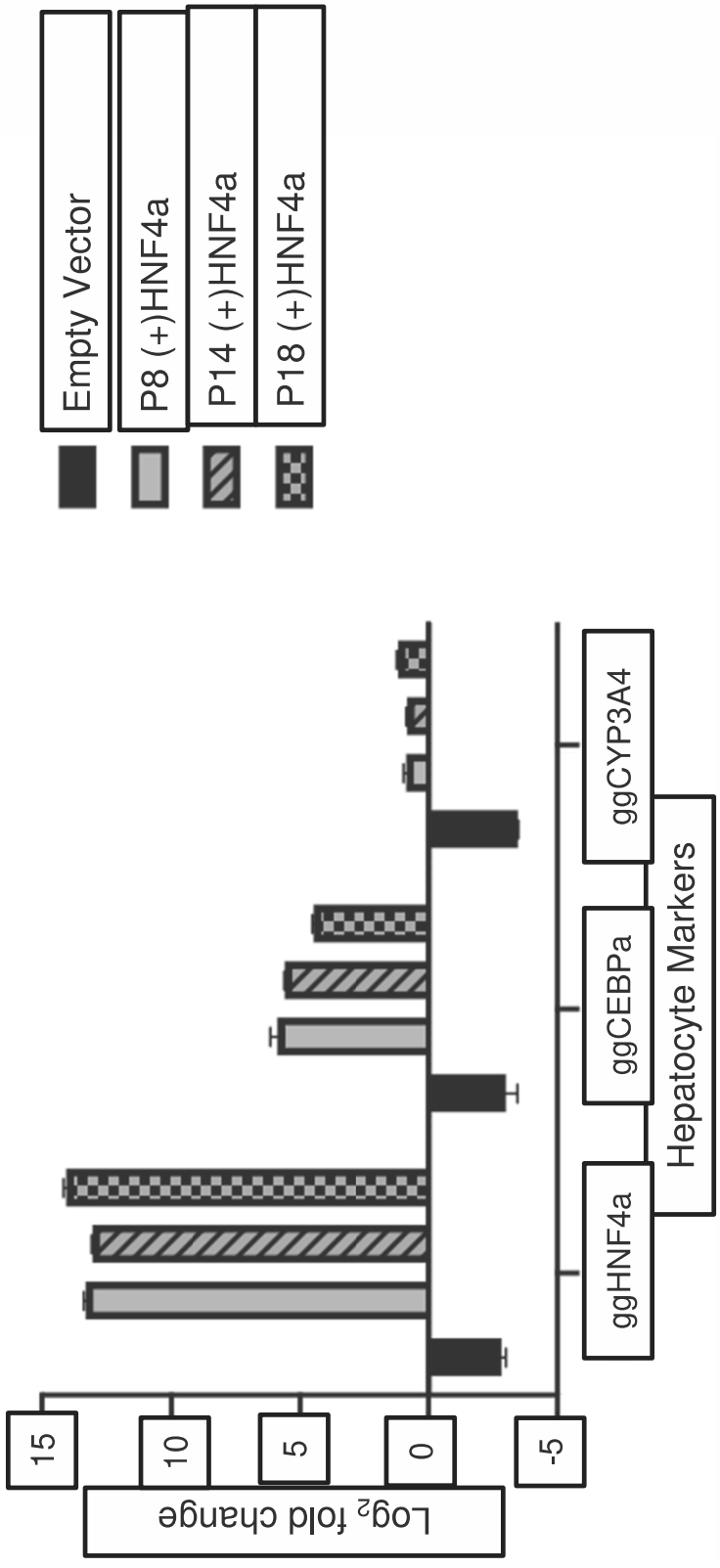

Empty Vector
P8 (+)HNF4a
P14 (+)HNF4a
P18 (+)HNF4a
Log2 fold change
15
10
5
0
-5
ggHNF4a
ggCEBPa
ggCYP3A4
Hepatocyte Markers

Figure 14

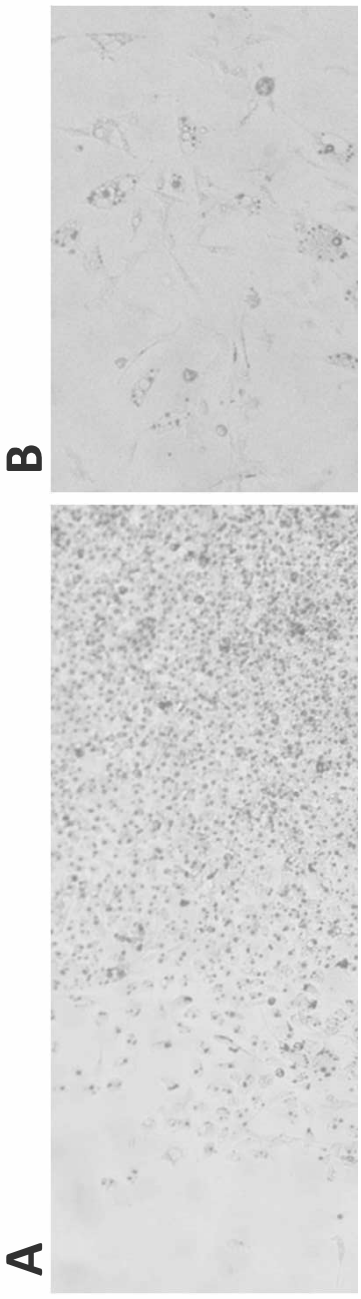


Figure 15

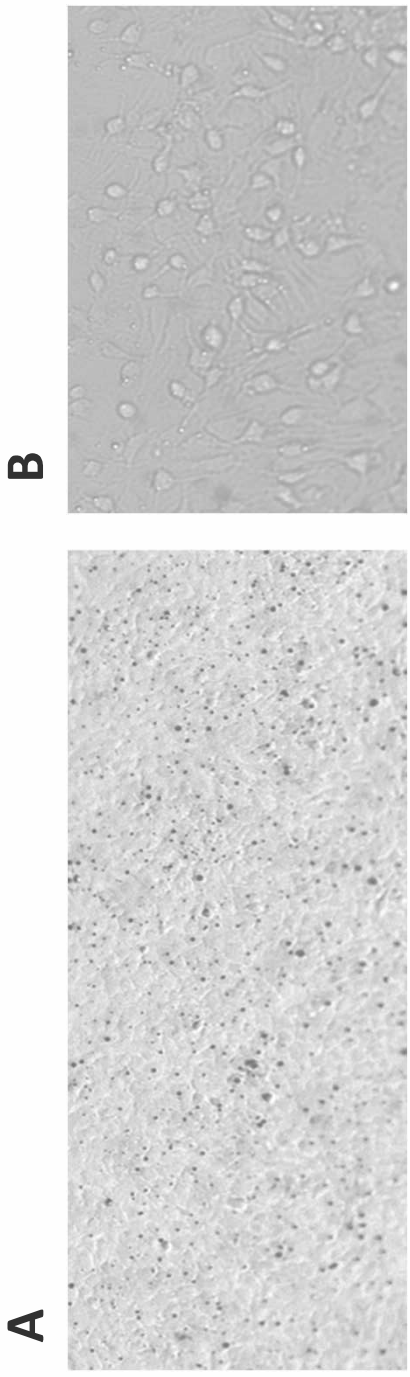


Figure 16

Figure 17

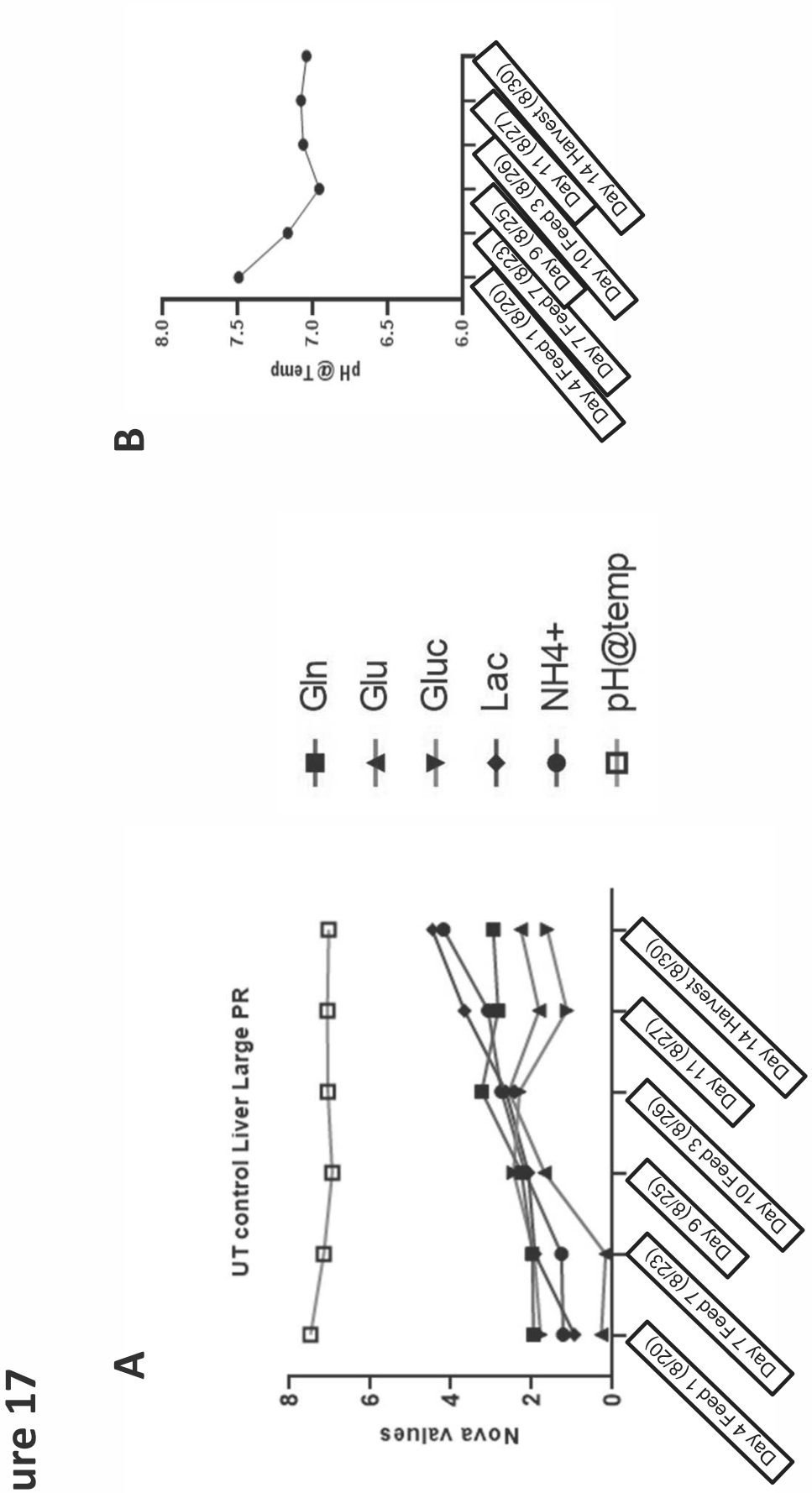

A
UT control Liver Large PR
Nova values
8
6
4
2
0
Gln
Glu
Gluc
Lac
NH4+
pH@temp
Day 4 Feed 1 (8/20)
Day 7 Feed 7 (8/23)
Day 9 (8/25)
Day 10 Feed 3 (8/26)
Day 11 (8/27)
Day 14 Harvest (8/30)
B
pH @ Temp
8.0
7.5
7.0
6.5
6.0
Day 4 Feed 1 (8/20)
Day 7 Feed 7 (8/23)
Day 9 (8/25)
Day 10 Feed 3 (8/26)
Day 11 (8/27)
Day 14 Harvest (8/30)

Figure 18

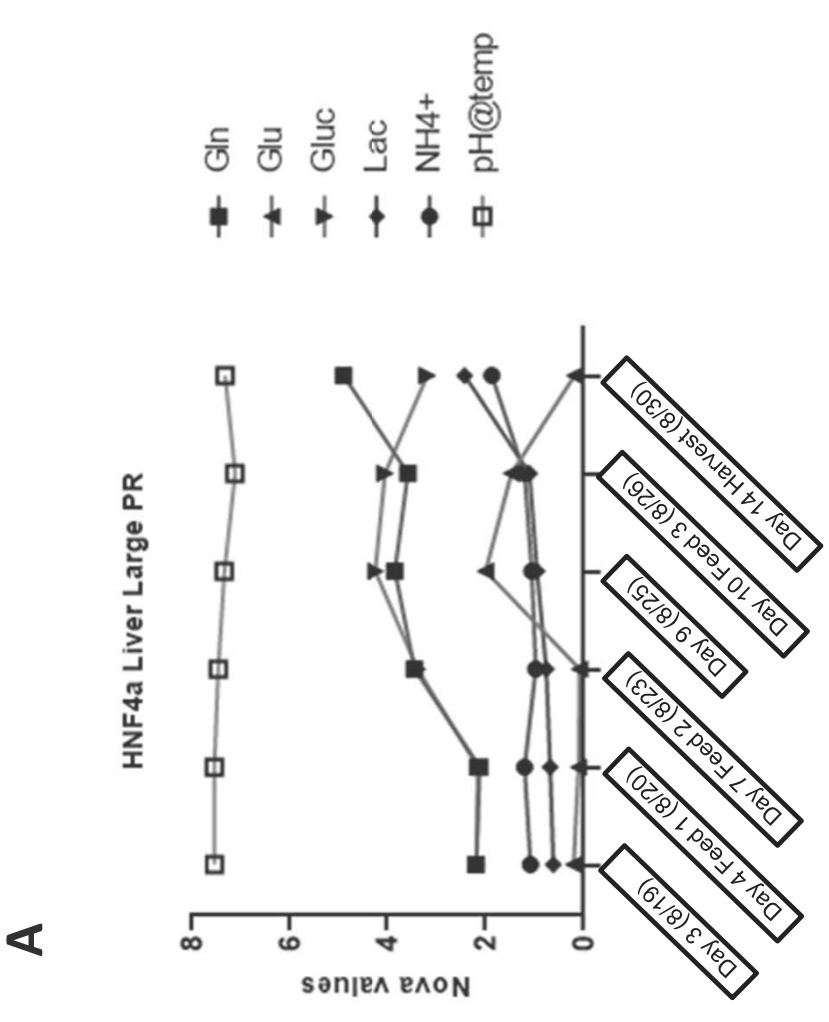

A
HNF4a Liver Large PR
Nova values
8
6
4
2
0
Gln
Glu
Gluc
Lac
NH4+
pH@temp
Day 3 (8/19)
Day 4 Feed 1 (8/20)
Day 7 Feed 2 (8/23)
Day 9 (8/25)
Day 10 Feed 3 (8/26)
Day 14 Harvest (8/30)
B
pH @ Temp
8.0
7.5
7.0
6.5
6.0
Day 3 (8/19)
Day 4 Feed 1 (8/20)
Day 7 Feed 2 (8/23)
Day 9 (8/25)
Day 10 Feed 3 (8/26)
Day 14 Harvest (8/30)

Figure 19

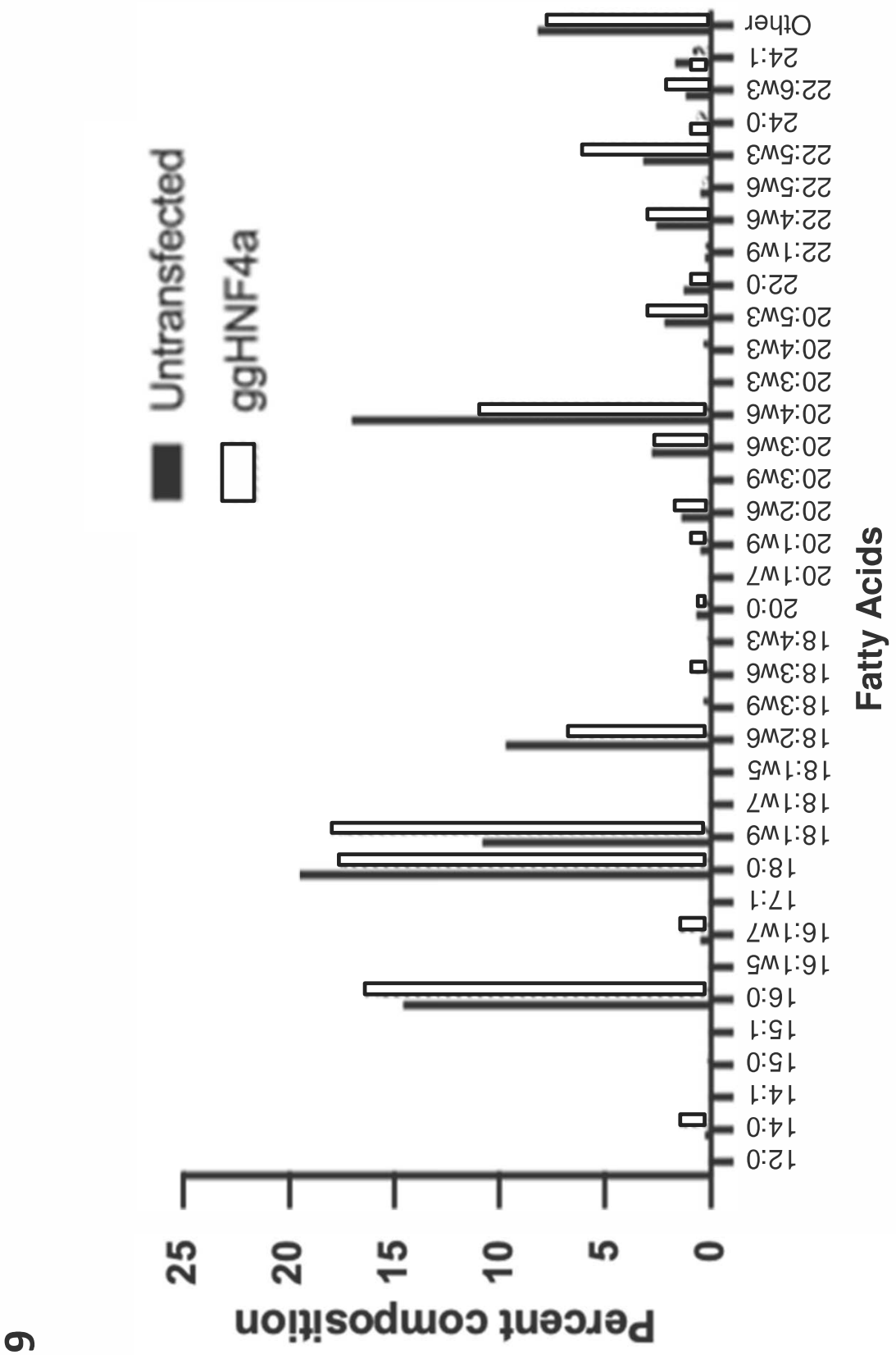

Untransfected
ggHNF4a
Percent composition
0
5
10
15
20
25
12:0
14:0
14:1
15:0
15:1
16:0
16:1w5
16:1w7
17:1
18:0
18:1w9
18:1w7
18:1w5
18:2w6
18:3w9
18:3w6
18:4w3
20:0
20:1w7
20:1w9
20:2w6
20:3w9
20:3w6
20:4w6
20:3w3
20:4w3
20:5w3
22:0
22:1w9
22:4w6
22:5w6
22:5w3
24:0
22:6w3
24:1
Other
Fatty Acids







Figure 20

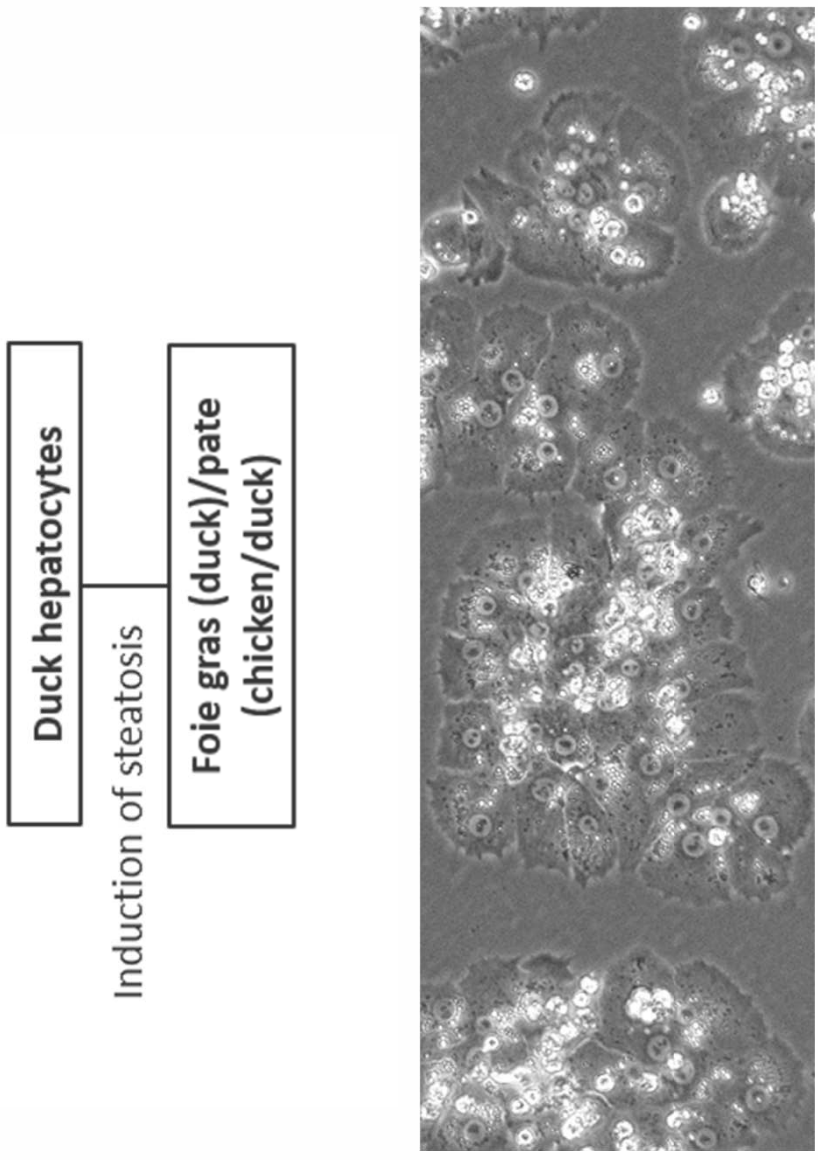

Duck hepatocytes
Induction of steatosis
Foie gras (duck)/pate
(chicken/duck)

Figure 21

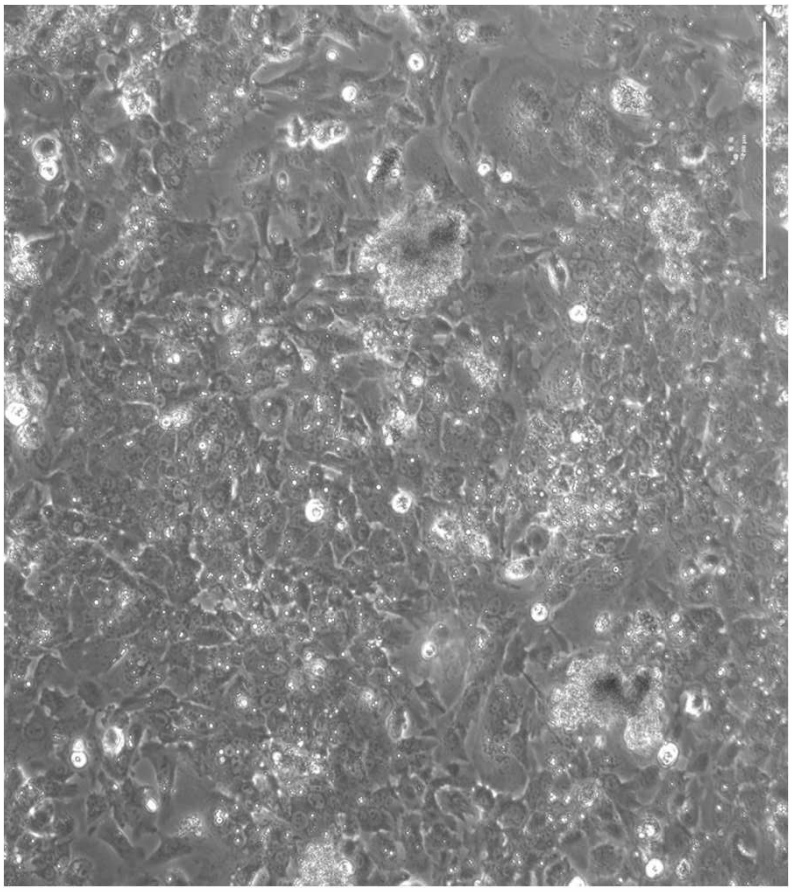


Figure 22

Figure 23

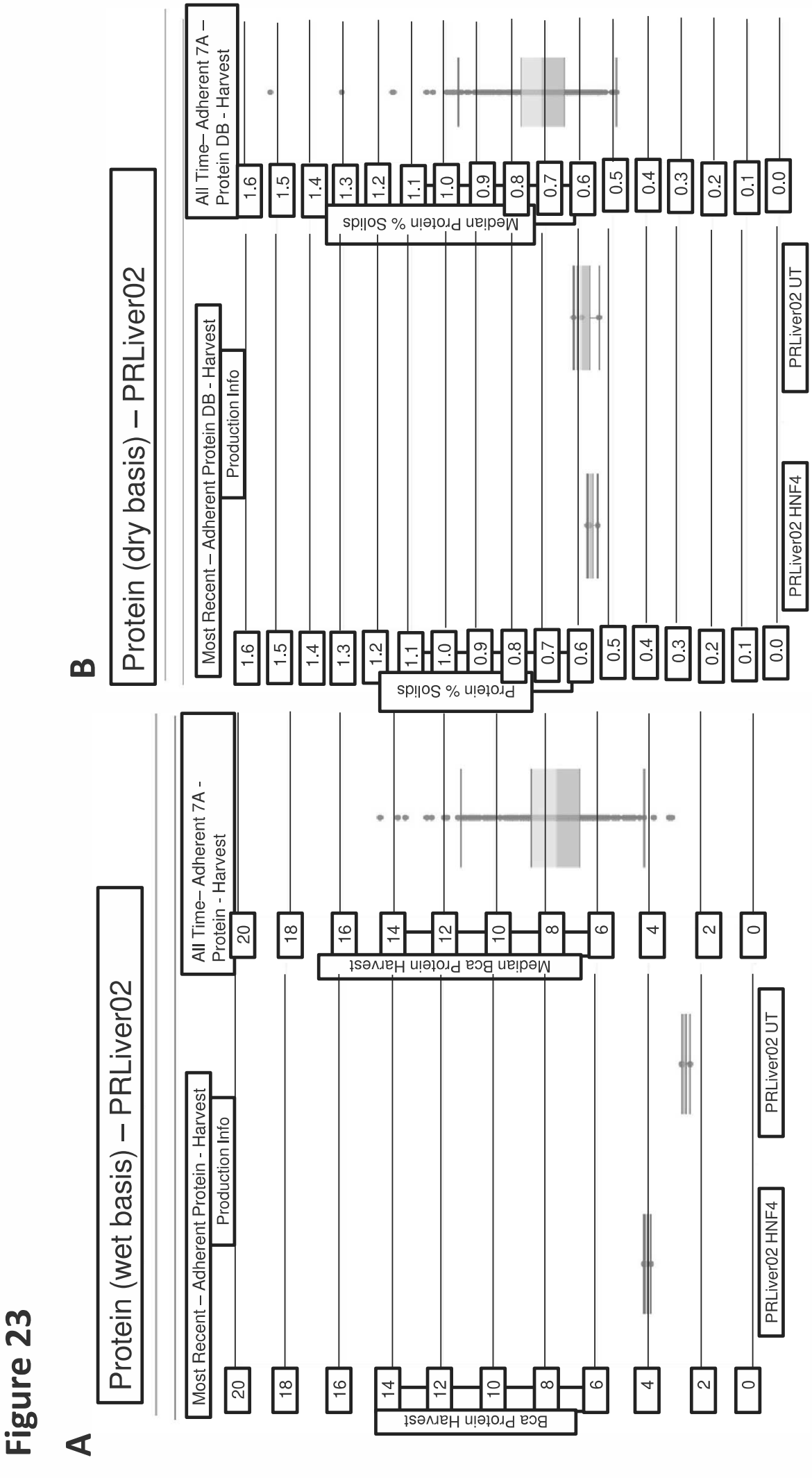
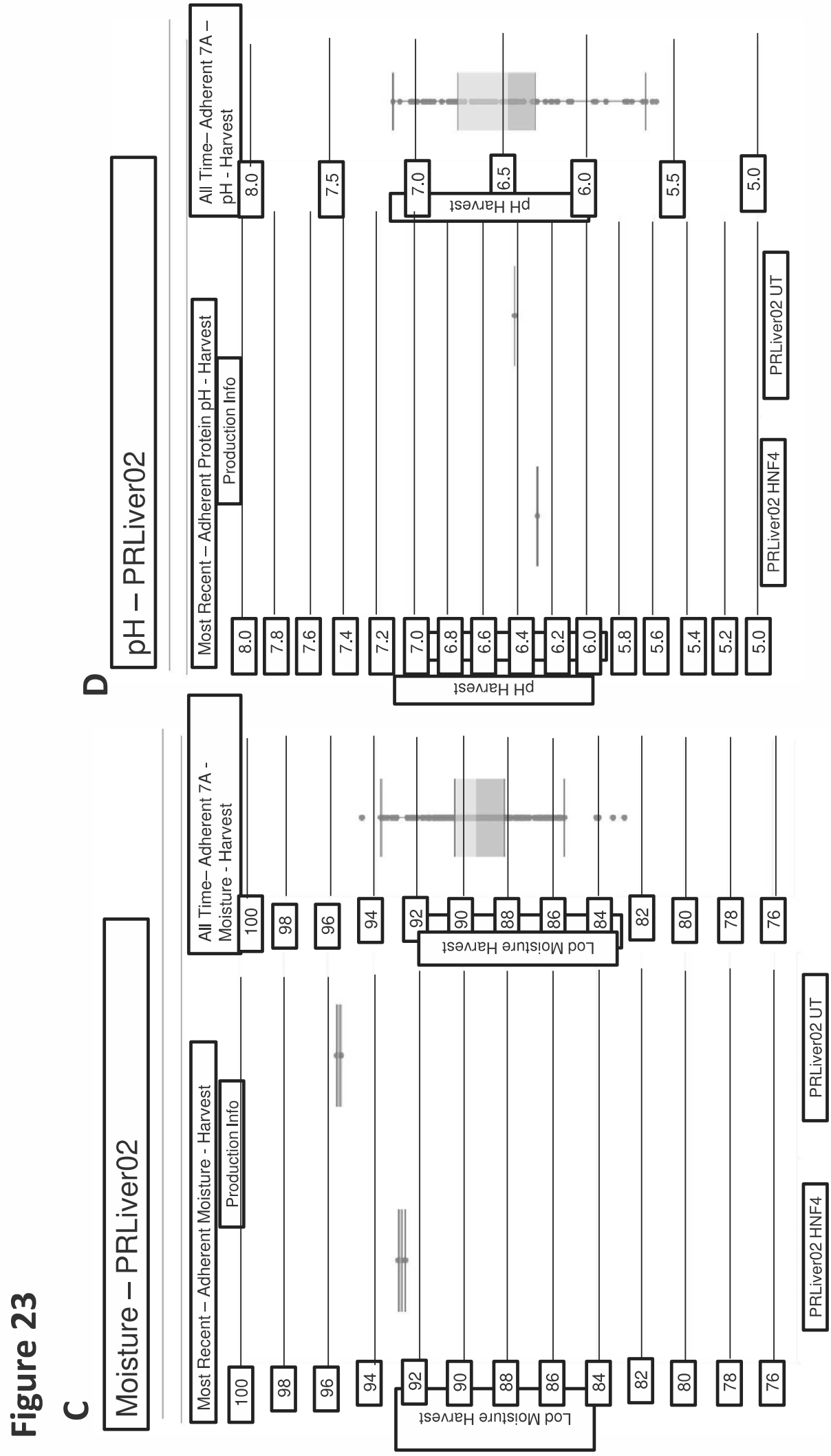

A
Protein (wet basis) – PRLiver02
Most Recent – Adherent Protein - Harvest
Production Info
All Time– Adherent 7A - Protein - Harvest
Bca Protein Harvest
Median Bca Protein Harvest
PRLiver02 HNF4
PRLiver02 UT
B
Protein (dry basis) – PRLiver02
Most Recent – Adherent Protein DB - Harvest
Production Info
All Time– Adherent 7A – Protein DB - Harvest
Protein % Solids
Median Protein % Solids
PRLiver02 HNF4
PRLiver02 UT

Figure 23


C
Moisture – PRLiver02
Most Recent – Adherent Moisture - Harvest
Production Info
All Time– Adherent 7A - Moisture - Harvest
Lod Moisture Harvest
PRLiver02 HNF4
PRLiver02 UT
D
pH – PRLiver02
Most Recent – Adherent Protein pH - Harvest
Production Info
All Time– Adherent 7A – pH - Harvest
pH Harvest
PRLiver02 HNF4
PRLiver02 UT

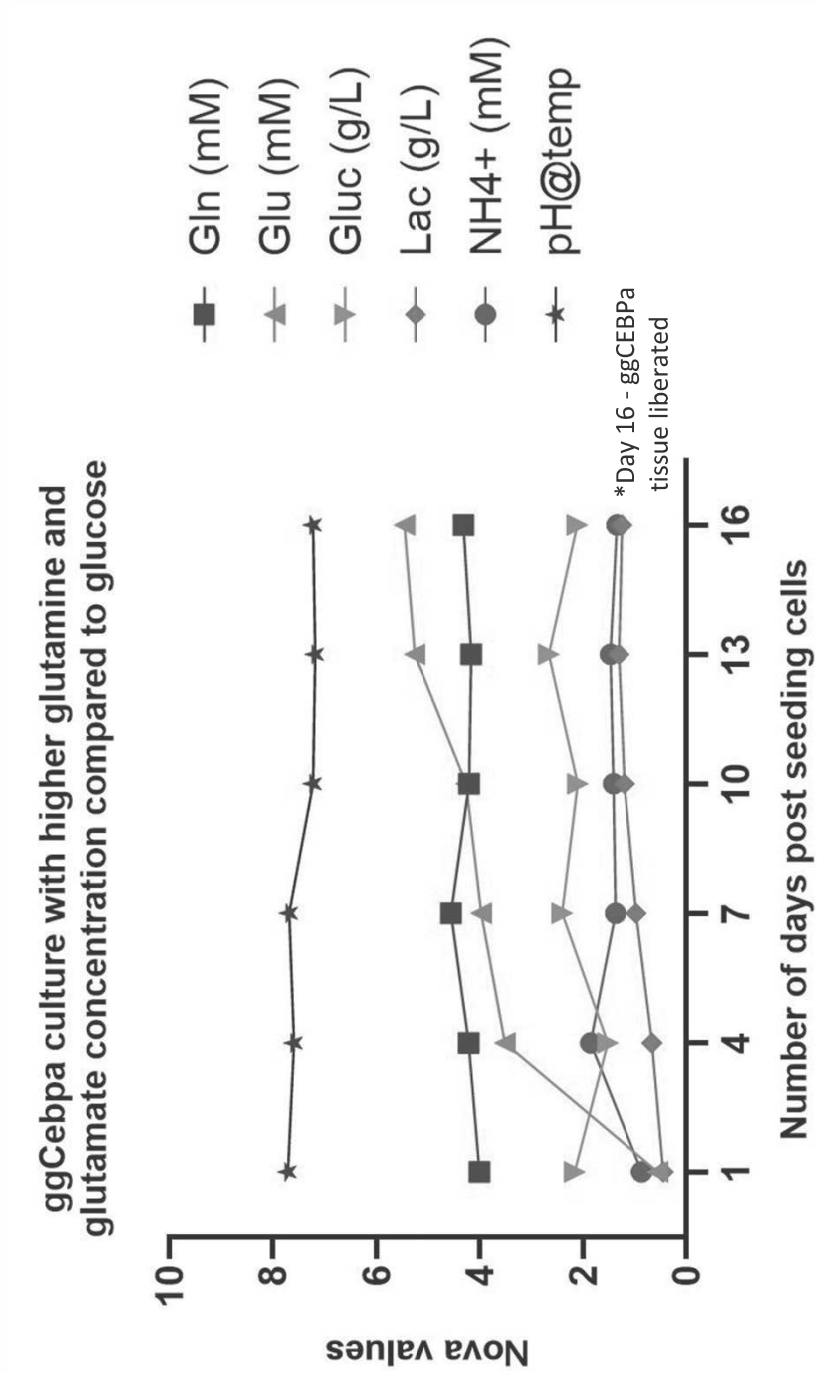

ggCebpa culture with higher glutamine and glutamate concentration compared to glucose
Gln (mM)
Glu (mM)
Gluc (g/L)
Lac (g/L)
NH4+ (mM)
pH@temp
*Day 16 - ggCEBPa tissue liberated
Nova values
0
2
4
6
8
10
1
4
7
10
13
16
Number of days post seeding cells

Figure 24

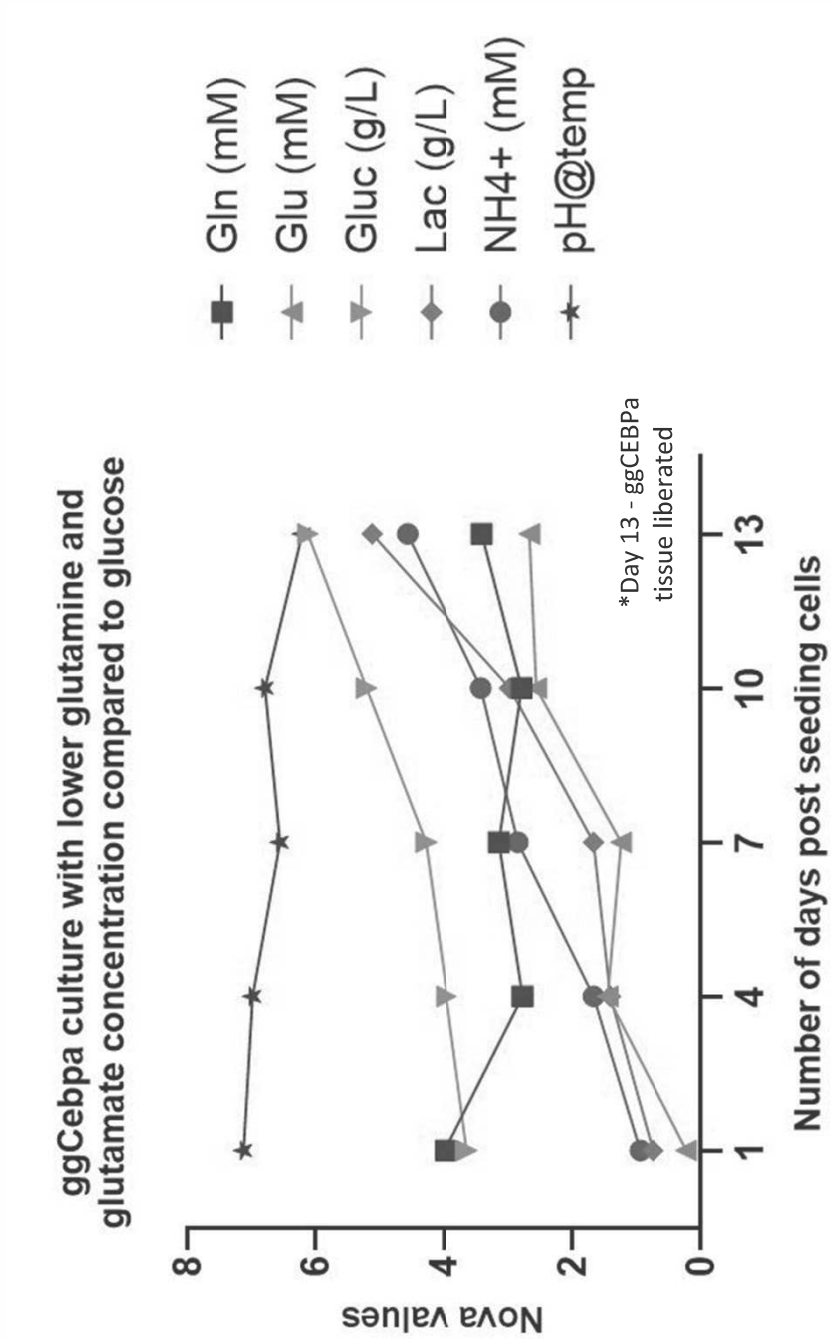

ggCebpa culture with lower glutamine and glutamate concentration compared to glucose
Gln (mM)
Glu (mM)
Gluc (g/L)
Lac (g/L)
NH4+ (mM)
pH@temp
Nova values
0
2
4
6
8
1
4
7
10
13
Number of days post seeding cells
*Day 13 - ggCEBPa tissue liberated

Figure 25

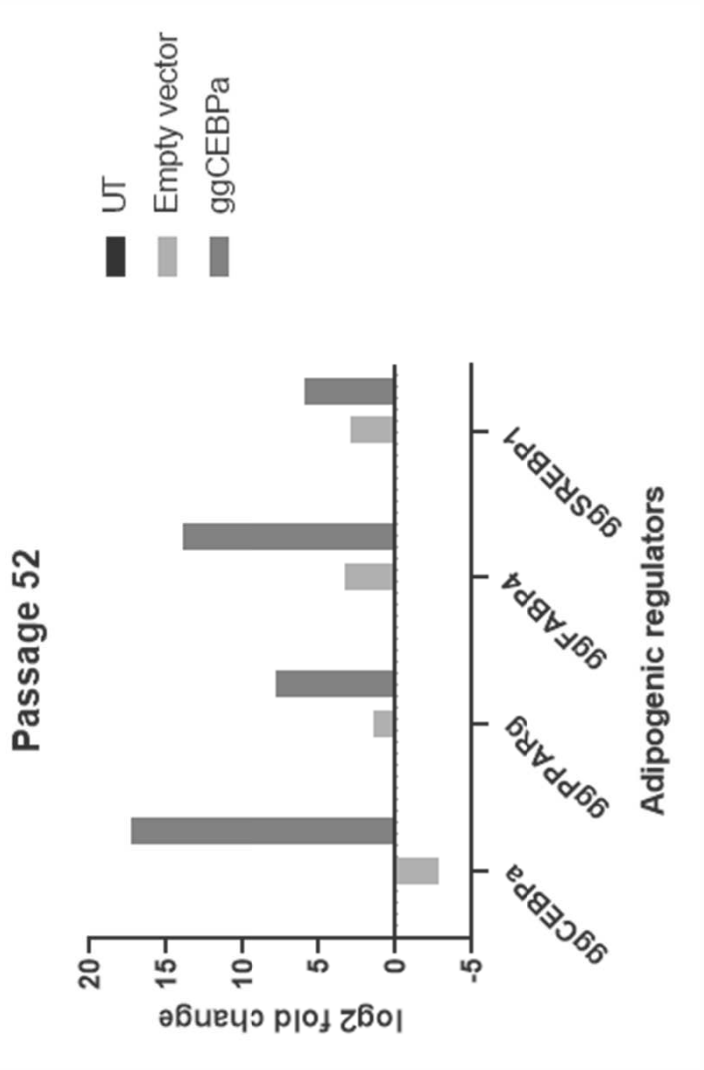

UT
Empty vector
ggCEBPa
Passage 52
log2 fold change
20
15
10
5
0
-5
ggCEBPa
ggPPARg
ggFABP4
ggSREBP1
Adipogenic regulators

Figure 26

Figure 27

Passage number	Passaging cells at **80-90%** confluence	Passaging cells 2 days past **100%** confluence
	Viability %	**Viability %**
46	99.39	85.54
47	98.34	85.78
48	99.45	86.11
49	98.95	91.79
50	99.21	83.43
51	99.45	74.48
52	97.35	76.17
53	99.67	88.58