Supplemental Figure 1. Phenotypic characterization of mesenchymal stem cells isolated from bone marrow. (A) Cell surface markers were characterized by FACS flow cytometer. Values are mean ± SEM of 15 individual experiments. (B) Representative FACS results of 1 out of 15 individual experiments. (C) Representative staining results of osteogenic (ARS and ALP staining) and adipogenic differentiation (Oil Red staining) of 1 out of 15 individual experiments. MSC were cultured for 14 days in the specific differentiation media. (D) Expression of 2 specific bone markers after 14 days in osteogenic medium. Runx2 and OCN expression were quantified by qPCR using the ΔΔCT method. Values were normalized for HPRT1 expression and are expressed as the ΔΔCT compared to D0. Results are means ± SEM of 5 individual experiments. ** $p < 0.01$vs D0.

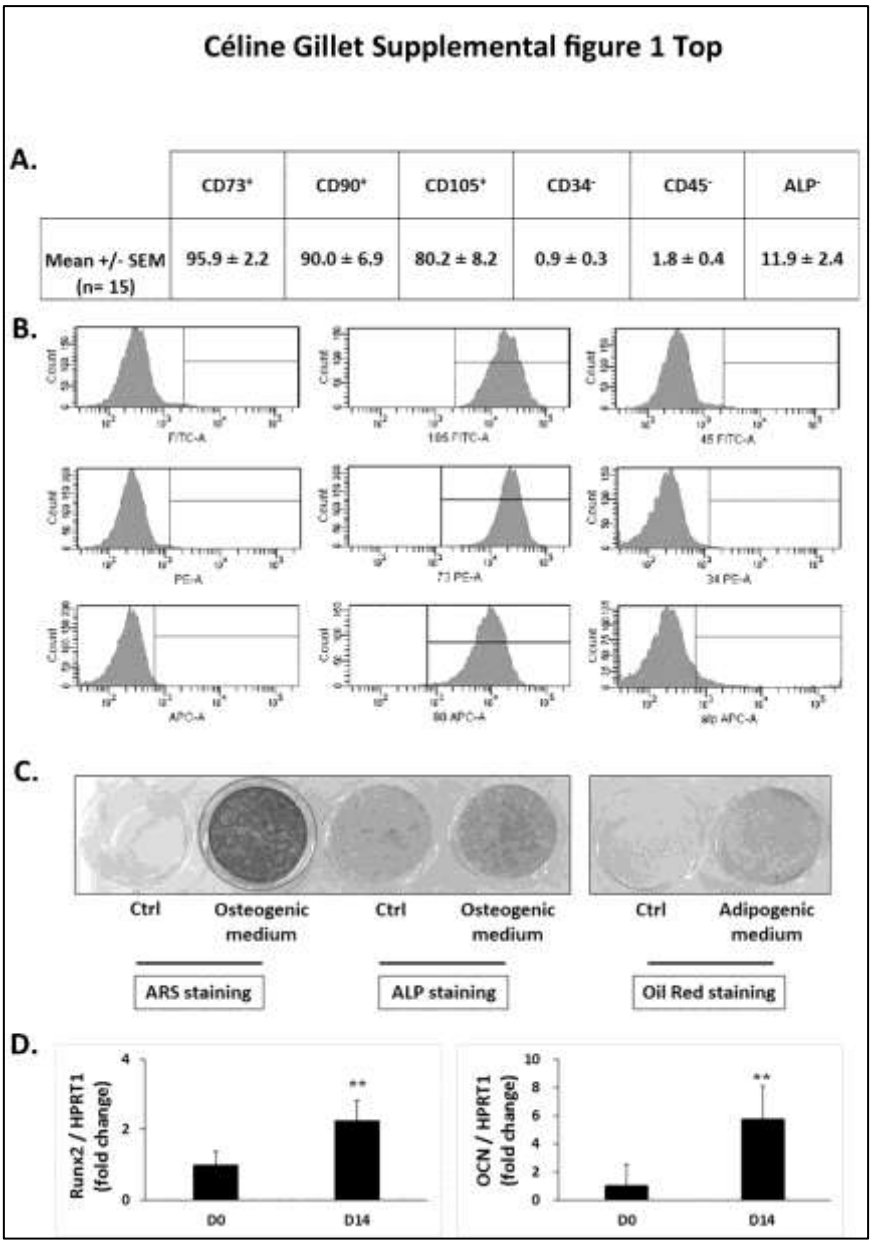
Supplemental Figure 2. Oleate suppressed palmitate-induced cytotoxicity in SaOS-2 and Ob. SaOS-2 (A,B) and Ob (C) were treated with increasing concentrations of Palm (0.125 mM to 0.50 mM) ± Ole (0.05 mM or 0.25 mM) for the indicated times. (A) Cell viability was quantified by nuclear staining with Hoechst and propidium iodide. Values are mean ± SEM of 5 individual experiments. (B,C) Caspases-3/7 activity was measured using the Caspases-3/7 Glo assay. Values are expressed relative to Ctrl and are mean ± SEM of 5 individual experiments for SaOS-2 (B) and 4 individual experiments for Ob (C). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ vs appropriate control (Ctrl, 24, 48 or 72 h); #$p < 0.05$ vs 0.25 mM Palm.

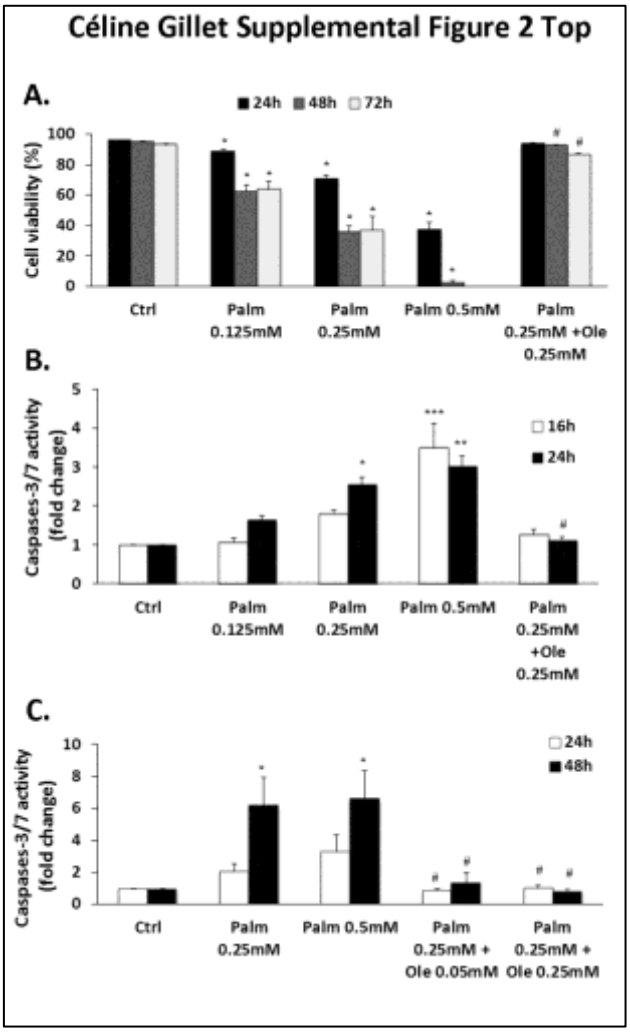
Supplemental Figure 3. Oleate blocked palmitate-induced activation of ERK. SaOS-2 were treated for 16 h with 0.25 mM Palm ± 0.25 mM Ole. ERK phosphorylation was evaluated by western blotting and normalized for total ERK. The figure is representative of 1 out of 3 individual experiments. Values are mean ± SEM. *$p < 0.05$ vs Ctrl, #$p < 0.05$ vs 0.25 mM Palm.

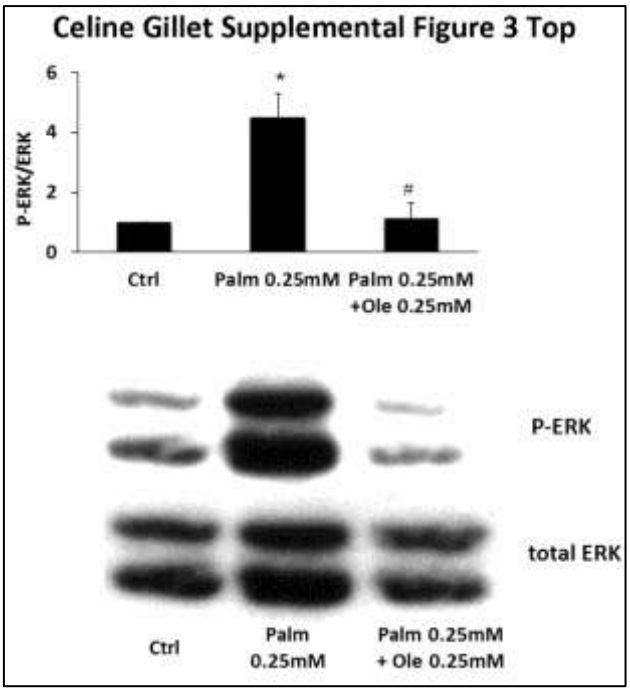
Supplemental Figure 4. Oleate prevented palmitate activation of NF-κB. MSC were treated for 48 h with 0.50 mM Palm or 0.25 mM Palm plus 0.25 mM Ole. NF-κB activity was regularly evaluated by ELISA. Values are expressed relative to Ctrl and are means ± SEM of 6 individual experiments.

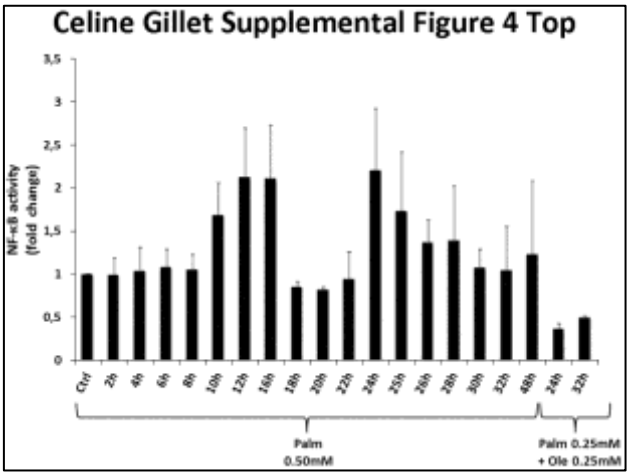
Supplemental Figure 5. Oleate suppressed palmitate-induced cytotoxicity in WJ-MSC. Cells were treated with increasing concentrations of Palm (0.125 mM to 0.50 mM) ± 0.25 mM Ole for the indicated times. Cell viability was quantified by nuclear staining with Hoechst and propidium iodide.

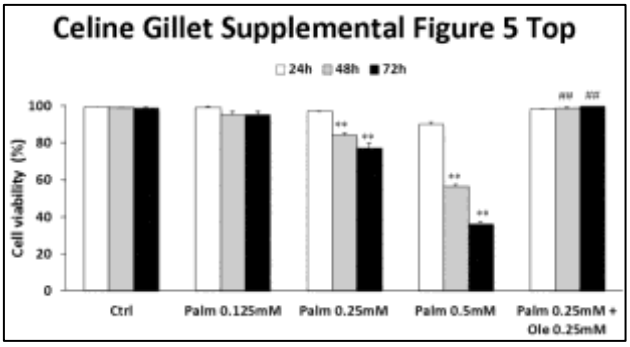
Values are mean ± SEM of 3 individual experiments. **p < 0.01 vs appropriate control (24, 48 or 72 h); ##p < 0.01 vs 0.25 mM Palm.




Céline Gillet Supplemental Figure 2 Top
A.
24h
48h
72h
Cell viability (%)
100
80
60
40
20
0
Ctrl
Palm 0.125mM
Palm 0.25mM
Palm 0.5mM
Palm 0.25mM +Ole 0.25mM
B.
Caspases-3/7 activity (fold change)
5
4
3
2
1
0
16h
24h
Ctrl
Palm 0.125mM
Palm 0.25mM
Palm 0.5mM
Palm 0.25mM +Ole 0.25mM
C.
Caspases-3/7 activity (fold change)
10
8
6
4
2
0
24h
48h
Ctrl
Palm 0.25mM
Palm 0.5mM
Palm 0.25mM + Ole 0.05mM
Palm 0.25mM + Ole 0.25mM


Celine Gillet Supplemental Figure 3 Top
P-ERK/ERK
6
4
2
0
Ctrl
Palm 0.25mM
Palm 0.25mM +Ole 0.25mM
P-ERK
total ERK
Ctrl
Palm 0.25mM
Palm 0.25mM + Ole 0.25mM





Supplemental material

Supplemental Table 1 Primer sequences for standard PCR

Name	Sequence	Product size (bp)
HPRT1-For	5’-TTCCTCCTCCTGAGCAGTCA-3’	564
HPRT1-Rev	5’-ATCCAACACTTCGTGGGGTC-3’	
TLR4-For	5’-ACAGAAGCTGGTGGCTGTG-3’	291
TLR4-Rev	5’-TCTTTAAATGCACCTGGTTGG-3’	
XBP1-For	5’-TCATGGCCTTGTAGTTGAGAAC-3’	289 or 263
XBP1-Rev	5’-GGCATTTGAAGAACATGACTGG-3’	

Supplemental Table 2 Primer sequences for real-time PCR

Name	Sequence	Product size (bp)
ATF4-For	5’-CTCCAGCGACAAGGCTAAGG-3’	180
ATF4-Rev	5’-GGCATGGTTTCCAGGTCATC-3’	
BiP-For	5’-ACCAATTATCAGCAAACTCTATGGAA-3’	74
BiP-Rev	5’-CATCTTTTTCTGCTGTATCCTCTTCA-3’	
CHOP-For	5’-TGGAAGCCTGGTATGAGGAC-3’	123
CHOP-Rev	5’-AAGCAGGGTCAAGAGTGGTG-3’	
CCL2-For	5’-AGCAAGTGTCCCAAAGAAGC-3’	93
CCL2-Rev	5’-CATGGAATCCTGAACCCACT-3’	
HPRT1-For	5’-GGCGTCGTGATTAGTGATGAT-3’	189
HPRT1-Rev	5’-CTTGAGCACACAGAGGGCTAC-3’	
IL6-For	5’-AGCCACTCACCTCTTCAGAACGAA-3’	122
IL6-Rev	5’-CAGTGCCTCTTTGCTGCTTTCACA-3’	
IL8-For	5’-GGACCACACTGCGCCAACACAG-3’	98
IL8-Rev	5’-TCCACAACCCTCTGCACCCAGTT-3’	
OCN-For	5’-GTGCAGCCTTTGTGTCCAAG-3’	157
OCN-Rev	5’-TCAGCCAACTCGTCACAGTC-3’	
Runx2-For	5’-GATGACACTGCCACCTCTGA-3’	118
Runx2-Rev	5’-ATGAAATGCTTGGGAACTGC-3’	
TLR4-For	5’-GAGAACTTCCCCATTGGACA-3’	165
TLR4-Rev	5’-CCGCAAGTCTGTGCAATAAA-3’	
XBP1-For	5’-TTGTCACCCCTCCAGAACATC-3’	74
XBP1-Rev	5’-CAGGATATCAGACTCTGAATCTGAAGA-3’	