

SUPPLEMENTAL FIGURES LEGEND

Supplemental Figure 1. Palmitate increased PUMA mRNA and protein levels. A, WT Huh-7 cells were treated with palmitic acid (PA; 800 μ M) for 0, 8 or 16 h and total RNA analyzed for PUMA expression by real-time PCR. PUMA is expressed in relation to 18S. Data are expressed as mean and standard error for three experiments, * $p < 0.01$. B, Whole cell lysate was prepared from WT Huh-7 cells treated with PA at 800 μ M for 0, 2, 4, 8, 12 or 16 h. Immunoblot analysis was performed for PUMA and β -actin was used as a control for protein loading. This immunoblot up to time 12 h was originally published in Journal of Biological Chemistry. Cazanave SC, Mott JL, Elmi NA, Bronk SF, Werneburg NW, Akazawa Y, Kahraman A, Garrison SP, Zambetti GP, Charlton MR, Gores GJ. JNK1-dependent PUMA expression contributes to hepatocyte lipoapoptosis. 2009, 284(39):26591-602. © the American Society for Biochemistry and Molecular Biology, and is depicted with the additional 16 h time point from the same immunoblot.

Supplemental Figure 2. The non-toxic unsaturated FFA, oleate, did not altered miR-296-5p levels. WT Huh-7 cells were treated with vehicle (Veh) or oleic acid (OA; 800 μ M) for 24 h and total RNA analyzed for miR-296-5p expression. miR-296-5p is expressed in relation to the small housekeeping RNA, Z30. Data are expressed as mean and standard error for three experiments.

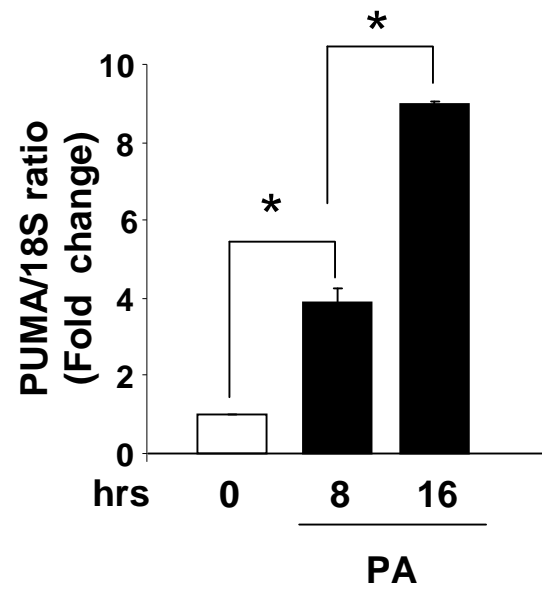
Supplemental Figure 3. Palmitate treatment did not modify miR-296-5p's half-life. WT Huh-7 cells were treated with vehicle (Veh) or palmitic acid (PA; 800 μ M) in the presence of Actinomycin D (2 μ g/mL) for the indicated time. Total RNA was analyzed for miR-296-5p expression and was expressed as fold change compared to time 0. Half-life ($t_{1/2}$) was calculated based on the decay constant.

Supplemental Figure 4. Oleate prevented miR-296-5p decrease and PUMA mRNA induction by palmitate. *A*, WT Huh-7 cells were treated with vehicle (Veh) or 800 μ M palmitate (PA) plus 400 μ M oleate (OA) for 16 h. Total RNA was analyzed for miR-296-5p expression. miR-296-5p is expressed in relation to the small housekeeping RNA, Z30. *B*, WT Huh-7 were treated as in panel *A*, and total RNA analyzed for *PUMA* expression by real-time PCR. PUMA is expressed in relation to 18S. Data are expressed as mean and standard error for three experiments.

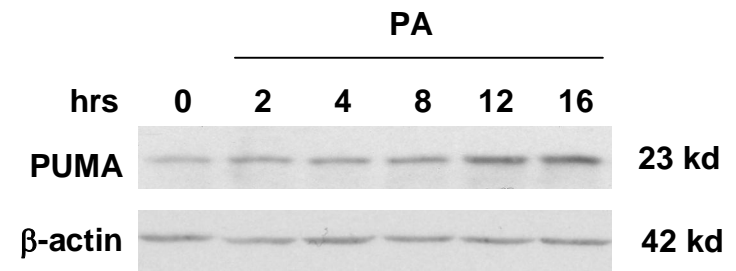
Supplemental Figure 5. miR-221, miR-222 and miR-483-3p levels are not decreased by palmitate treatment. WT Huh-7 cells were treated with vehicle (Veh) or palmitic acid (PA; 800 μ M) for 24 h and total RNA analyzed for miR-221 (*A*), miR-222 (*B*) or miR-483-3p (*C*) expression. MicroRNAs levels are normalized to Z30 levels. Data are expressed as mean and standard error for three experiments, * $p < 0.01$.

Supplemental Fig.1

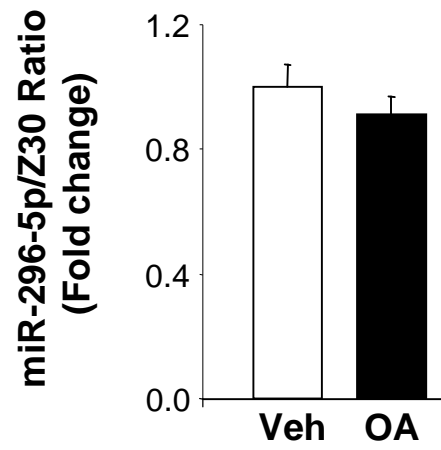
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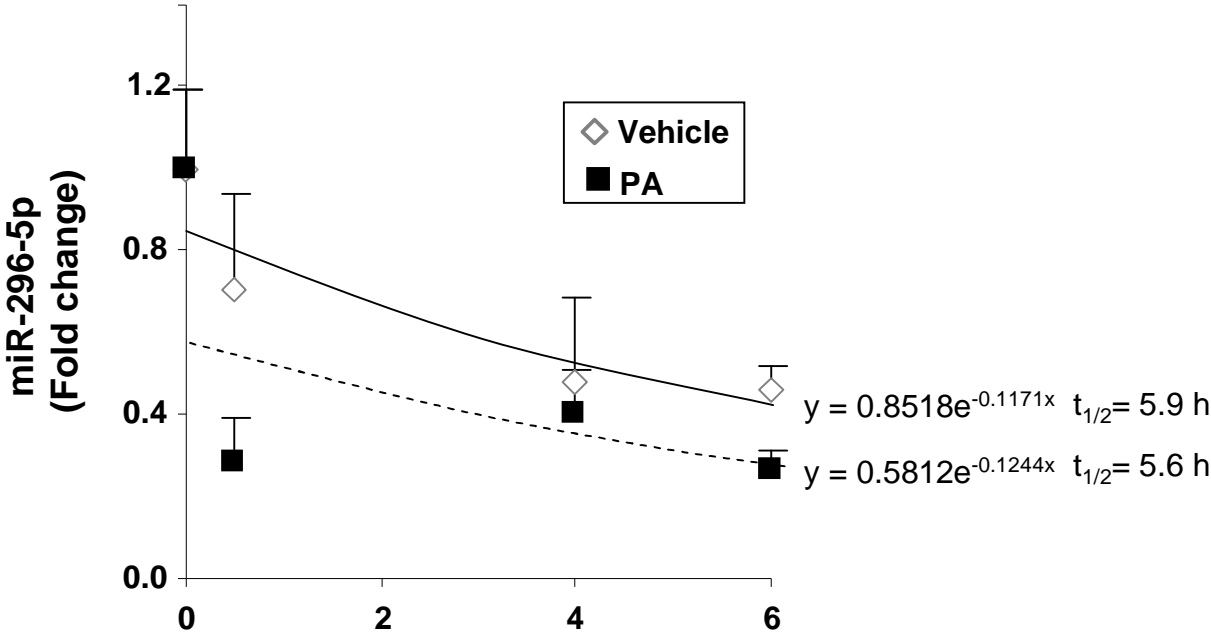
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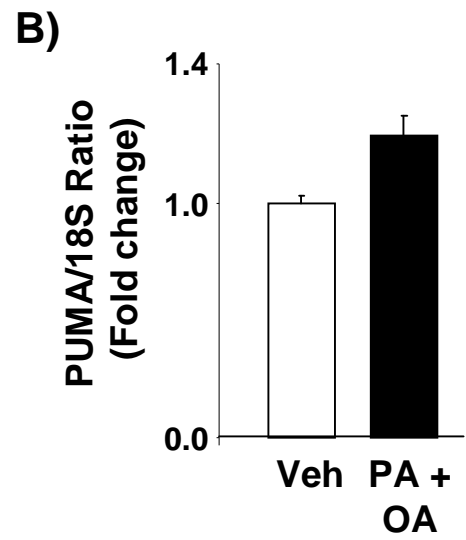
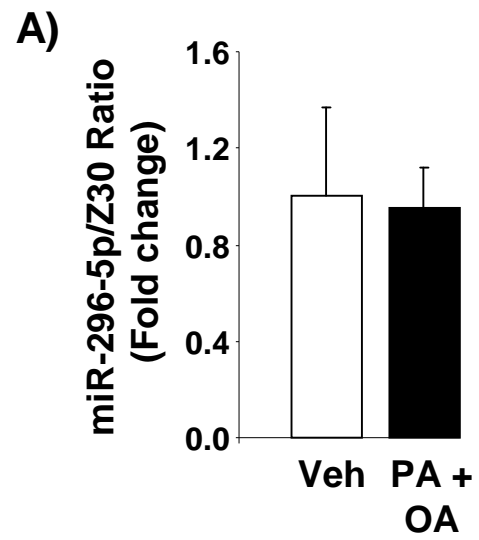
Supplemental Fig.2



Supplemental Fig.3



Supplemental Fig.4



Supplemental Fig.5

