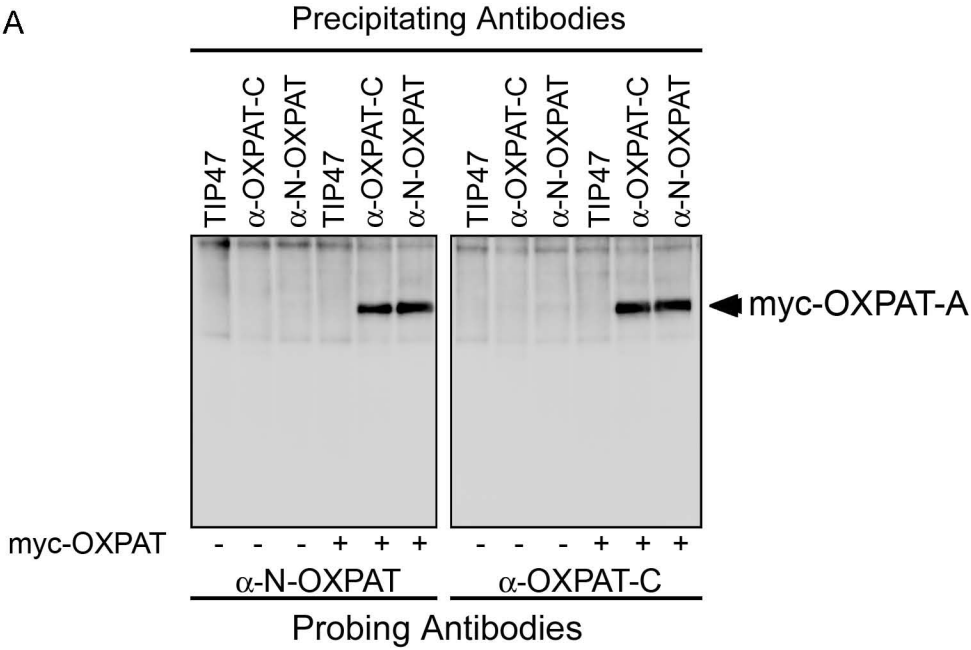
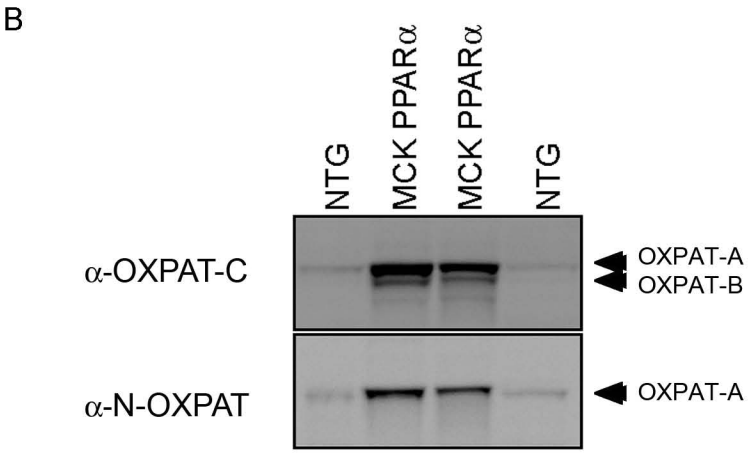
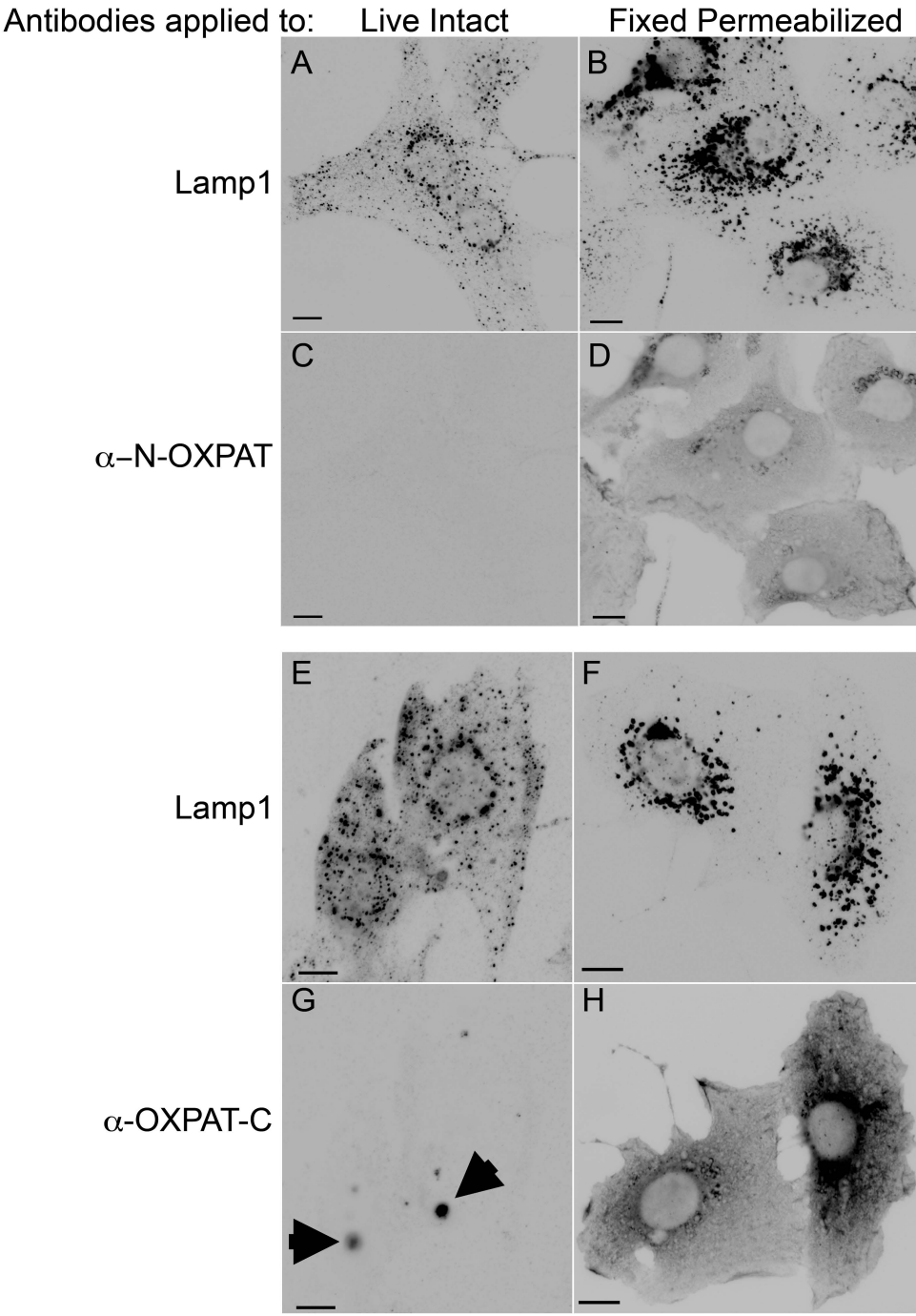
Supplemental Figure 1

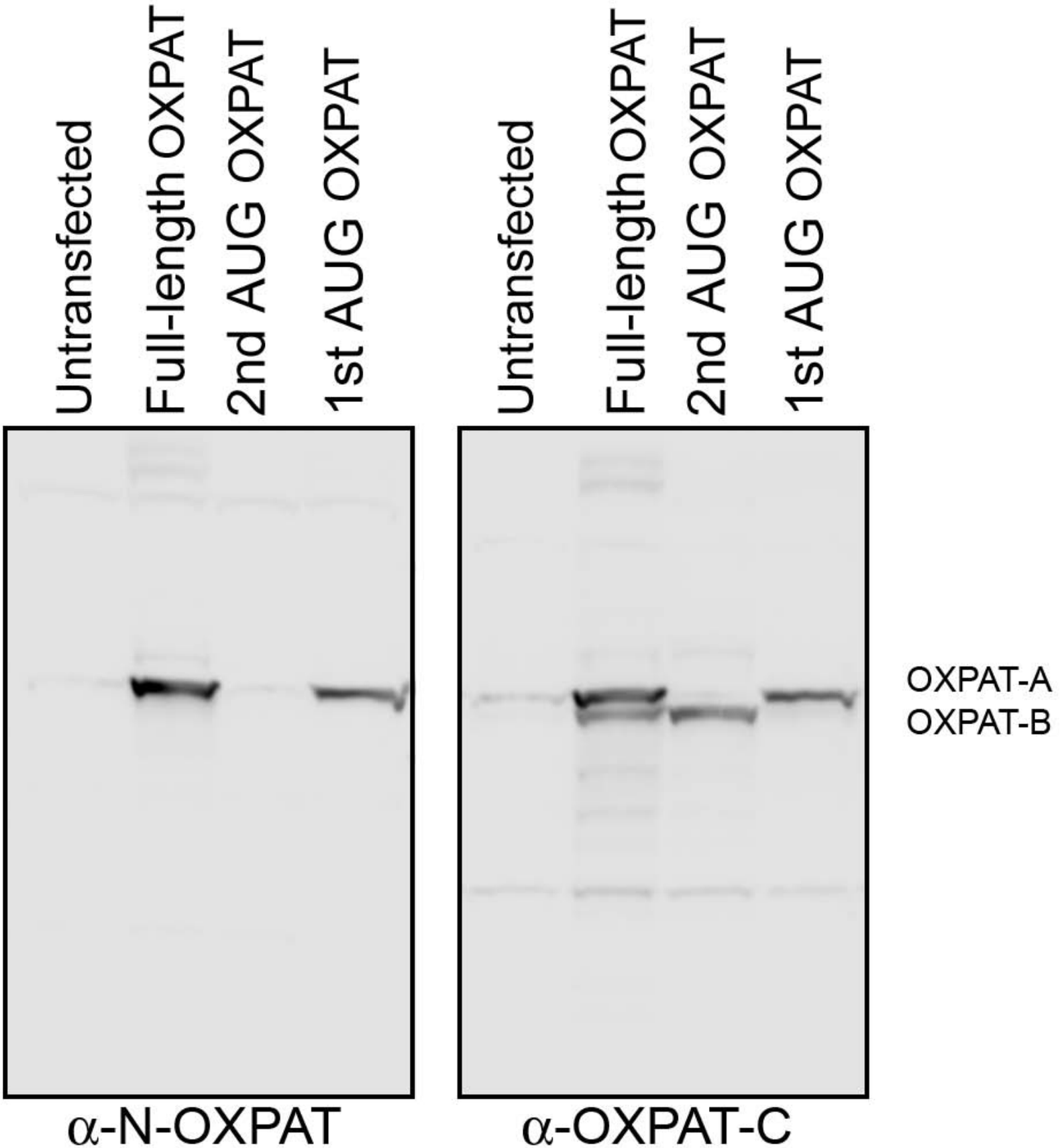




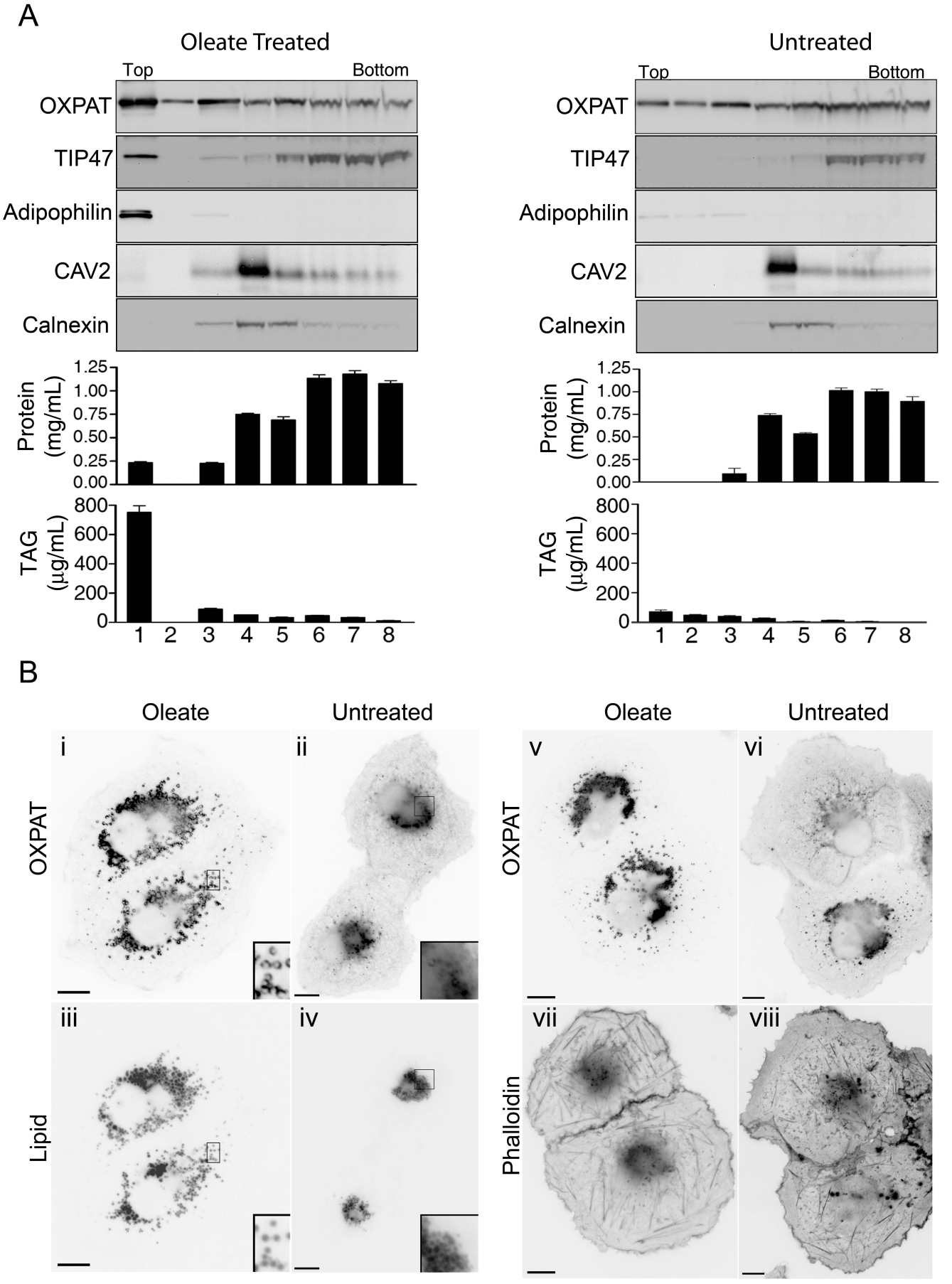
Supplemental Figure 2



Supplemental Figure 3



Supplemental Figure 4



SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure 1. α-N-OXPAT and α-OXPAT-C bind to both native and denatured OXPAT protein. As discussed in the main text, antibodies, α-N-OXPAT and α-OXPAT-C, were raised to the unique termini of mouse OXPAT and characterized by immunoprecipitation and Western blot. [A] Immunoblots of immunoprecipitated proteins are shown. Proteins were extracted with HNETC from $10^{7.3}$ COS-7 cells or $10^{7.3}$ COS-7 myc-OXPAT cells. Proteins were captured with 3 μg of the indicated antibodies. One sixth of the immunoprecipitates were loaded into the lanes as indicated. After transfer, the membrane was cut into pieces and one piece was probed with α-N-OXPAT diluted to 700 ng/ml and the other piece was probed with α -OXPAT-C at 400 ng/ml. Both of these antibodies also work in immunofluorescence microscopy (data not shown). [B] Immunoblotted proteins from gastrocnemius muscle of high fat fed mice were probed with α-N-OXPAT and α-OXPAT-C. Proteins were extracted from non-transgenic (NTG) or MCK-PPARα transgenic mice as indicated. Both these antibodies recognized a ~ 50 kDa protein. The intensities of the signals for the ~ 50 kDa protein recognized by each antibody track between samples in a parallel fashion, thereby allowing for the unambiguous identification of OXPAT in mouse tissues.

Supplemental Figure 2. OXPAT termini are not accessible to antibodies applied to intact cells. To determine if the termini of OXPAT are extracellular, live, intact or fixed, permeabilized cells were treated with antibodies to the termini of OXPAT (α-N-OXPAT and α -OXPAT-C) and with an antibody to an extracellular epitope of Lamp1. Shown are

immunofluorescent micrographs of OP9 OXPAT cells. Cells were grown on coverslips**.** Primary antibodies were applied to live, intact cells for 1 h on ice and then 20 min at 37^{o}C or applied to fixed, permeabilized cells for 1 h at room temperature as indicated in the figure. The live, intact cells were then fixed and permeabilized, and all cells were stained with secondary antibodies. The following four sets of 2 panels (A and C, B and D, E and G, and F and H) show separate channels of the same field of cells. Primary antibody concentrations applied to intact cells were as follows: Lamp1 50 μg/ml, α-N-OXPAT 10 μg/ml, and α -OXPAT-C 10 μg/ml. Primary antibody concentrations applied to fixed, permeabilized cells were as follows: Lamp1 1 μg/ml, α-N-OXPAT 0.5 μg/ml and α-OXPAT-C 0.5 μg/ml. Arrows point to strongly staining cellular debris. This strong staining is likely due to the fact that in this debris, access to OXPAT is not impeded by the cell membrane. Bars = 10 μm.

As expected, all 3 antibodies bound to the permeabilized, fixed cells. Also, as expected, the antibody to the extracellular domain of Lamp1 was endocytosed with the Lamp1 that cycled to the plasma membrane of living cells. Unlike Lamp1, α-N-OXPAT and α-OXPAT-C did not interact with the intact OXPAT OP9 cells, but both bound strongly to the permeabilized, fixed OXPAT OP9 cells. These data show that the α-N-OXPAT and α-OXPAT-C epitopes are not accessible in intact cells.

Supplemental Figure 3. OXPAT mRNA is translated predominately from the first in frame AUG. As discussed in main text, the recently deposited mRNA sequence to PAT-1 is identical to OXPAT. However, this record reports translation starting at the second in frame AUG rather than the first in frame AUG. To determine where cells begin

translation of the OXPAT mRNA sequence, we transfected the following three constructs into COS-7 cells and extracted the cellular protein: [1] pCMV-SPORT6-OXPAT with the complete OXPAT coding and untranslated regions (full-length OXPAT), [2] pcDNA3.1/V5-His-TOPO encoding OXPAT with the first in frame AUG excised and a Kozak sequence imposed around the second AUG (2^{nd} AUG OXPAT); this imposed starting methionine is labeled with an asterisk in Figure 1B, and [3] pcDNA3.1/V5-His-TOPO encoding OXPAT with a Kozak sequence imposed around the first in frame AUG (1^{st} AUG OXPAT); this imposed starting methionine is the first residue in the protein sequence shown in Figure 1B (OXPAT-A).

Immunoblotting shows that α-N-OXPAT binds a single major protein in lysate from cells expressing full-length OXPAT or 1^{st} AUG OXPAT, and showed little reactivity against 2^{nd} AUG OXPAT lysate. α-OXPAT-C bound 2 PAGE resolvable proteins from full-length OXPAT lysates. The more abundant, slower migrating species migrated with 1^{st} AUG OXPAT, and the faster migrating, less abundant species migrated with 2^{nd} AUG OXPAT. This demonstrates that translation can start at either in frame AUG, usually starting at the first AUG. This preference for the first in frame AUG is also seen in mouse tissues (Main text Figure 2B and Supplementary Figure 1B).

Supplemental Figure 4. OXPAT coats nascent TAG. COS-7 myc-OXPAT-A cells were incubated in standard media supplemented (Oleate) or not (Untreated) with 900 μM albumin-bound oleate for 3 h (Oleate). **[A]** Oleate-treated and untreated COS-7 myc-OXPAT-A cells were fractionated by flotation on a density gradient. Immunoblots loaded with 30 μl of each fraction were probed with the indicated antibodies at the following

concentrations: α-OXPAT-C (400 ng/ml), α-TIP47-C (410 ng/ml), α-human-adipophilin mouse hybridoma supernatant (1:10 dilution), α-caveolin-2 (333 ng/ml), and α-calnexin (250 ng/ml). The bar graphs below the immunoblots show the protein and TAG concentrations of each fraction. **[B]** COS-7 myc-OXPAT-A cells were supplemented or not with oleate as indicated and fixed. The top panels (i, ii, v, and vi) show OXPAT staining (α-OXPAT-C 500 ng/ml). The same microscopic fields are shown in the bottom panels. Panels iii and iv show the cellular lipids labeled with fluorescent fatty acid. Panels vii and viii show polymerized actin visualized by staining cells 1 h with 6 units/ml phalloidin. Bars = 10 μm. Insets are magnified 3x.

Methods for Supplemental Figure 4:

Cell fractionation: Four confluent 150 mm plates of COS-7 myc-OXPAT-A cells were treated as described in the Supplemental Figure 4 legend. The cells were washed well with PBS and then pelleted. The pellet was resuspended in 1.5 ml of hypotonic lysis buffer (10 mM HEPES and 1 mM EDTA, pH 7.3) and allowed to swell for 10 min. The swollen cells were sheared by 6 passages through a 27 gauge needle. Nuclei and cell debris were removed by centrifugation (10 min at 500 x *g*). Then 1 ml of the resulting supernatant was brought to 42% sucrose with 70% sucrose. 2.5 ml of this solution were then transferred into a 5 ml centrifuge tube and overlayed with the following sucrose layers: 1 ml 40%, 1 ml 15%, and 0.5 ml 0%. All sucrose solutions were dissolved in lysis buffer on a weight/weight basis. The gradients were centrifuged in a swing-bucket rotor with increasing forces: 5 min at 2700 x g, 10 min at 10,700 x g, 20 min at 43,000x g, 60 min at 172,000 x g. The floating fractions were collected with a tube slicer (Beckman

Coulter, Inc., Fullerton, CA) by slicing the tube just below the top of the gradient. The floating fraction was brought to 650 μl with lysis buffer. Then 7 additional 650 μl fractions were collected. No pellet was evident at the bottom of the tube; however, the bottom of the tube was scraped in order to resuspend any pelleted material.

Labeling cells with fluorescent fatty acids: COS-7 myc-OXPAT cells were incubated 18 h in 12 μM green fluorescent fatty acid (BODIPY® FL C_{12} (Invitrogen, Carlsbad, CA: catalog no. D3822)).

Supplemental Table 1: quantitative RT-PCR primers for mouse genes

Gene	Accession Number	Sequence
OXPAT	BC024138	fwd: GTGATCAGACAGCTCAGGACCCT rev: CGATTCACCACATTCTGCTGG
Acadm	NM_007382	fwd: GGAAATGATCAACAAAAAAAGTATTT rev: ATGGCCGCCACATCAGA
Acadvl	NM_017366	fwd: ATCTCTGCCCAGCGACTTT rev: TTCTGGCTTGTCCAGAACTG
Cox2	DQ106413	fwd: TGAAGACGTCCTCCACTCATG rev: CCCTGGTCGCTTTGATGTTA
Cox4	NM_009941	fwd: TACTTCGGTGTGCCTTCGA rev: TGACATGGGCCACATCAG
Sdha	NM_023281	fwd: GTTGGCGCAGTTTCGAGGCT rev: GCCGCAGGTCTGTTTTTGGA

Ucp1	NM_009463	fwd: CGACTCAGTCCAAGAGTACTTCTCTTC
		rev: GCCGGCTGAGATCTTGTTTC
36B4	NM_007475	fwd: GCAGACAACGTGGGCTCCAAGCAGAT
		rev: GGTCCTCCTTGGTGAACACGAAGCCC

Supplemental Table 2: quantitative RT-PCR primers for human genes

Gene	Accession Number	Sequence
OXPAT	not available	fwd: AGCTGGTGGATCACTTCCTG rev: CCTGCTGTCTCCTCTGATCC
Perilipin	BC031084	fwd: CCTGCCTTACATGGCTTGTT rev: CCTTTGTTGACTGCCATCCT
S3-12	XM_375558	fwd: TGCTGCAGAATGAGTTGGAG rev: GACCCAGGTCACCTAAACGA
TIP47	NM_005817	fwd: GCTGGACAAGTTGGAGGAGA rev: CCGACACCTTAGACGACACA
18S	X03205	fwd: TTCGAACGTCTGCCCTATCAA rev: ATGGTAGGCACGGCGACTA