Figure S1. **TIP47 localization in human cell types.** (A–C) Human fibroblasts (A–C), the monocytic cell line MonoMac (D–F), and the liver cell lines Huh-7 (G–I) and HepG2 (J–L) were incubated for 16 h in the presence of oleic acid and processed for immunofluorescence of the indicated proteins. Note that in all cell types examined, TIP47 could not be found to colocalize with MPR46 as indicated by the lack of any yellow color. In contrast, TIP47 labeled the surface of lipid droplets as indicated by inclusion of Bodipy 493/503 in the lumen of TIP47-positive structures (compare images in B, E, H, and K). The lipid droplet localization was further confirmed by partial colocalization with the known lipid droplet marker protein ADRP (Adipophilin). Note that ADRP and TIP47 can decorate different lipid droplet populations depending on the cell type analyzed. Identical results were obtained in several cell types from other species (mouse fibroblasts, rat normal rat kidney, hamster CHO, bovine Madin-Darby bovine kidney, porcine LLC-PK1, and monkey COS-7 cells; not depicted). Bar, 15 µm.
Figure S2. **TIP47 is not detectable on structures of the biosynthetic and endocytic pathways.** Double immunofluorescence of HeLa cells after overnight incubation with oleic acid. The TIP47-positive structures (A–F, green) were clearly distinct from early endosomes (A, EEA1), endocytosed transferrin (B), the endoplasmic reticulum (C, protein disulfide isomerase [PDI]), the Golgi (D, GM130), and lysosomes (E, Lamp1). Significant colocalization was only observed with the known lipid droplet protein ADRP (F) as indicated in yellow. Bar, 15 µm.
Figure S3. **Specificity of TIP47 antibodies.** (A) Coomassie stain of recombinant wild-type TIP47 and a truncated version [residues 178–434] purified from *E. coli.* Both proteins were used as antigens for the immunization of rabbits. (B) Characterization of a rabbit anti–wild-type TIP47 serum. A single protein is recovered from metabolically labeled HeLa cells (lane 1), and a single band is visible in Western blots of HeLa and TIP47 KD cell extracts (lanes 2 and 3). In HeLa cells transfected with GFP-TIP47, two bands of the expected molecular mass with similar intensities are detected. (C) Immunoprecipitation (IP) of TIP47 with an affinity-purified rabbit antibody (Ab1) was donated by S. Pfeffer (Stanford University, Palo Alto, CA; lane 1). The same antibody detected a single protein of the same size in a Western blot of a HeLa cell lysate (lane 2). An identical detection pattern was obtained when using a commercially available anti-TIP47 guinea pig serum (lane 3, Ab2), indicating that both antibodies detect a single protein, TIP47. This was further confirmed by using the rabbit serum raised against wild-type TIP47 for immunoprecipitation (Ab3; A and B) followed by detection of the precipitated protein by Western blotting using the guinea pig serum. As shown in lane 4, the guinea pig serum recognized the protein immunoprecipitated by the rabbit antibody, unequivocally demonstrating that both antibodies are specific for TIP47. Identical results were obtained with the rabbit serum against TIP47 178–434 (not depicted).

Values on blots are given in kilodaltons. White lines indicate that intervening lanes have been spliced out.
**Figure S4. YFP-TIP47 is recruited to lipid droplets.** HeLa cells were transiently transfected with YFP-TIP47 and processed for immunofluorescence after 24 h of culture. Cells in B and D had been incubated overnight with oleic acid before fixation. In images A–C, the YFP-TIP47–positive cells were marked with asterisks. YFP-TIP47 (green in all images) exhibited a faint cytoplasmic staining at steady state (A and C) but decorated lipid droplets (blue) after incubation of cells in fatty acid rich medium (B and D). No overlap with endogenous MPR46 (A and B, red) was detectable (compare A and B, A′–A‴, and B′–B‴), but significant colocalization with endogenous ADRP was evident in cells incubated with oleic acid (D and D′–D‴). Most YFP-TIP47–positive structures incorporated neutral lipid dyes, clearly identifying them as lipid droplets (D and D′–D‴). (C) Note that ADRP was undetectable in cells at steady state. The boxed areas in A, B, and D mark regions that are shown at a higher magnification in A′–D‴. Bars, 15 µm.
Figure S5. **Time course of TIP47 recruitment to LDs.** HeLa cells were incubated for 24 h in medium with 2% serum to deplete the cells of neutral lipid stores (control). Then they were incubated for the indicated time periods in medium with 400 µM oleic acid before fixation and staining of TIP47 (red) and LDs (green). Note that TIP47 becomes visible in dotlike structures within minutes after fatty acid feeding, whereas incorporation of the neutral lipid dye Bodipy 493/503 is delayed. Bar, 15 µm.