Fig. S1

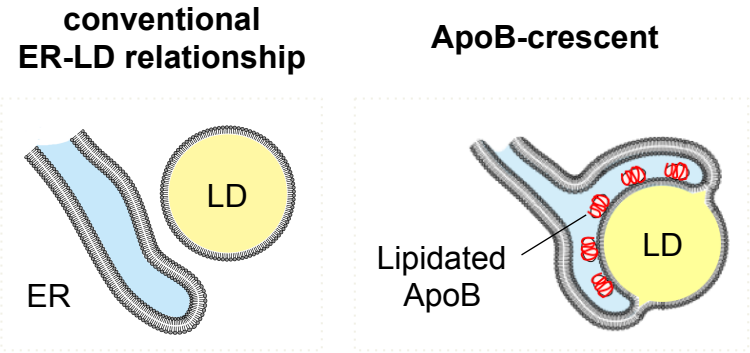

Figure S1. Schematic diagrams showing the structural relationship between the ER and LD. The ER and LD often approximate each other, but membrane continuity is not observed between them. In the ApoB-crescent, the phospholipid monolayer of the LD surface is continuous with the two leaflets of the ER membrane, forming the LD–ER amalgamation structure. This structure can be regarded as an LD intercalated between the two leaflets of the ER membrane. Lipidated ApoB binds tightly to the LD surface facing the ER lumen (Ohsaki et al., 2008).

Fig. S2

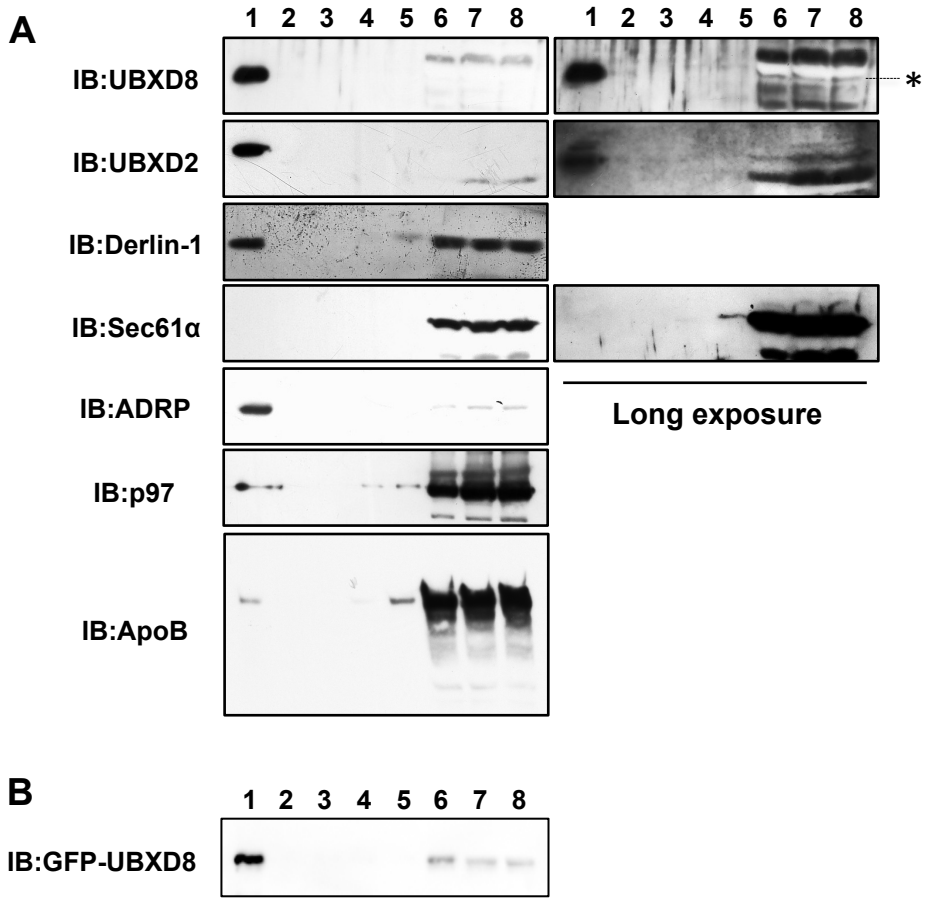

Figure S2. Western blotting of subcellular fractions obtained from Huh7 cells.
Huh7 cells were subjected to sucrose density-gradient centrifugation and eight fractions were obtained from the top. The LD was recovered in the lightest fraction (#1) almost exclusively, whereas most membrane and cytosolic proteins were in dense fractions (#6–8).
(A) Huh7 cells in the normal culture medium. A longer exposure version of Figure 1A is presented to show the amount of UBXD8 and UBXD2 in the bottom fractions. Weak bands of UBXD8 (*) that were pushed downward by overloaded proteins are seen in fractions #6–8.
(B) Huh7 cells expressing GFP-UBXD8 in the normal culture medium. Most GFP-UBXD8 was recovered in the LD fraction, but some was found in the bottom fractions.

Fig. S3

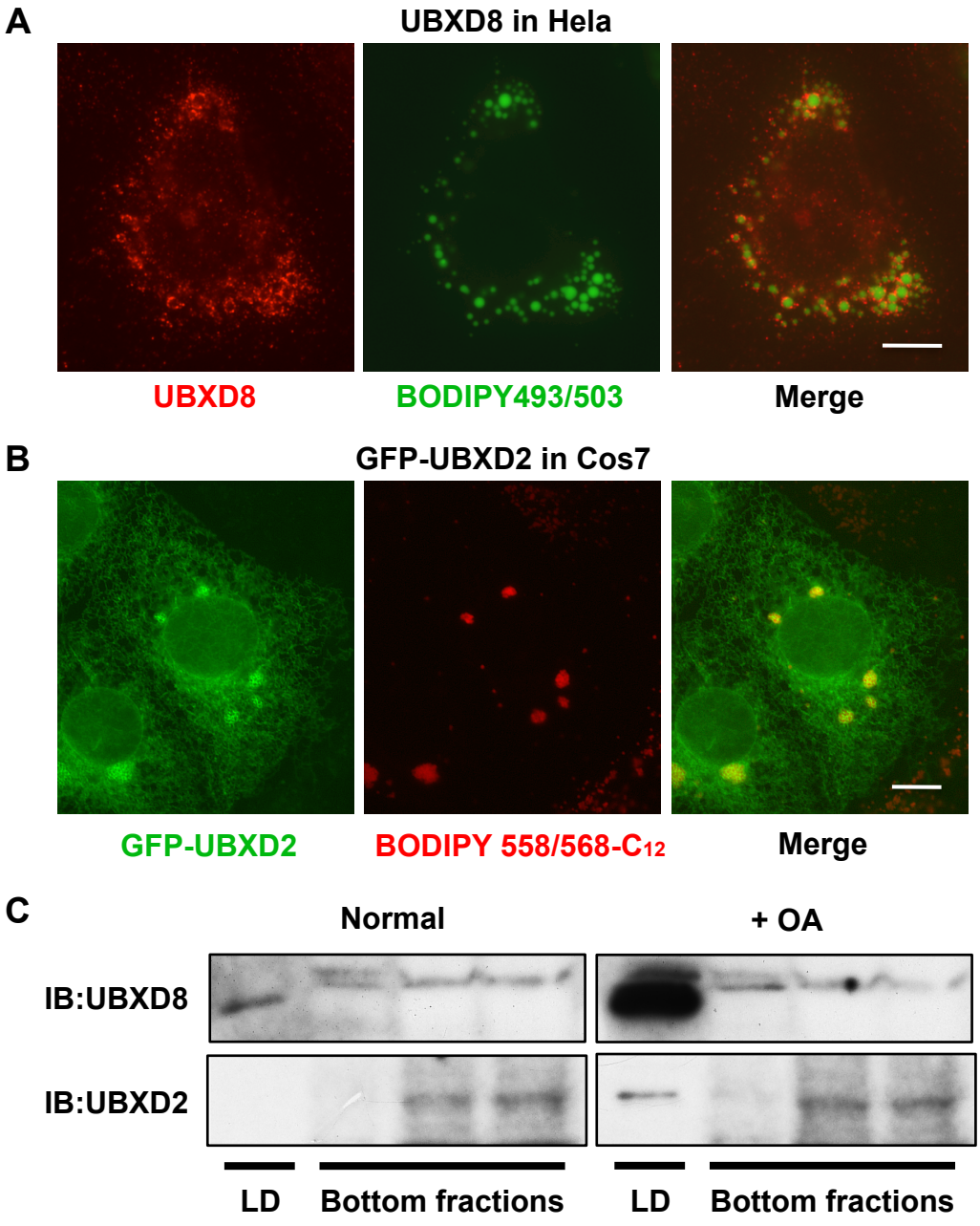

Figure S3. Distribution of UBXD8 and UBXD2 in non-hepatocytes.
(A) Endogenous UBXD8 in HeLa cells. The cells were treated with 0.4 mM OA for 24 hr to induce LD formation. UBXD8 (red) was conspicuous around LDs that were stained with BODIPY493/503 (green), but weak filamentous labeling was also seen, which probably corresponded to the ER. Bar, 10 μm.
(B) GFP-UBXD2 in Cos7 cells. GFP-UBXD2 (green) showed a network pattern with some concentration around LDs stained with BODIPY558/568-C_{12} (red). The network was likely to represent ER. Bar, 10 μm.
(C) Western blotting of subcellular fractions obtained from HeLa cells cultured in the normal medium or treated with 0.4 mM OA for 12 hr. UBXD8 was detected intensely in the LD fraction in both conditions, whereas most UBXD2 was in bottom fractions and only a small amount was present in LDs even after OA loading.

Fig. S4

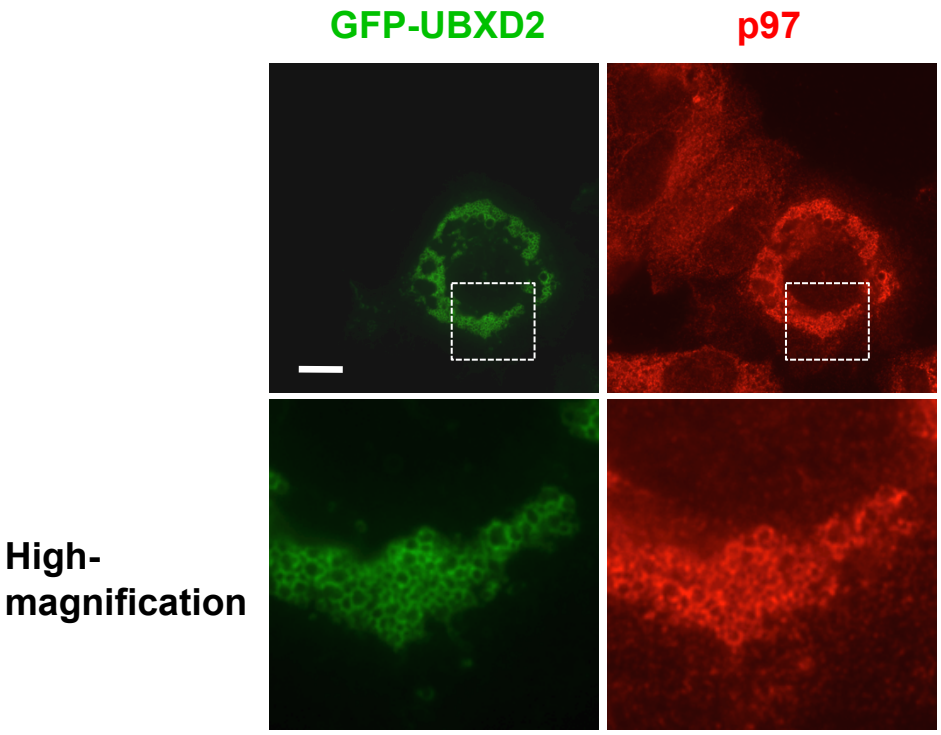

Figure S4. Recruitment of p97 to LDs by GFP-UBXD2.
Huh7 cells expressing GFP-UBXD2 were labeled for endogenous p97. LDs that harbor GFP-UBXD2 (green) showed intense labeling for p97 (red), indicating that UBXD2 can recruit endogenous p97 to LDs. The area in the rectangle of the upper figures is magnified in the lower figures. Bar, 10 μm.

Fig. S5

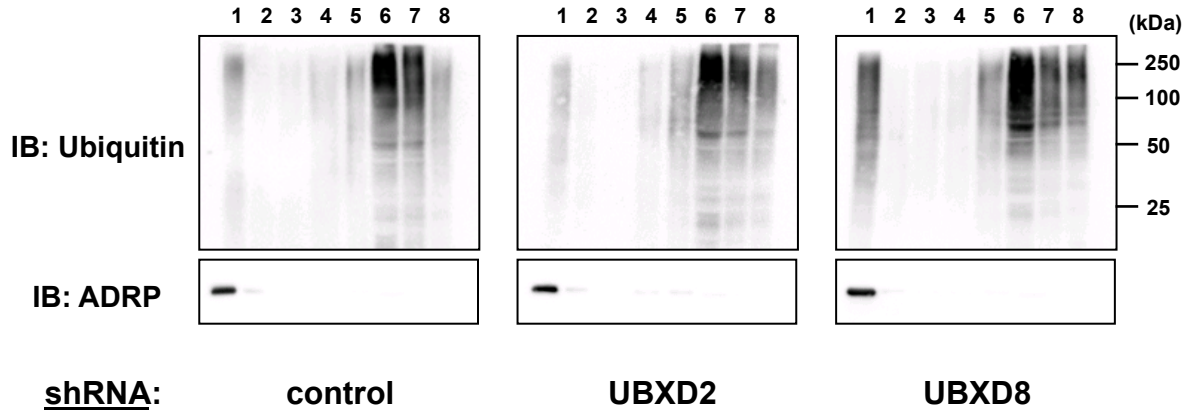

Figure S5. UBXD8 knockdown increased ubiquitinated proteins in the LD fraction. Huh7 cells stably harboring control, UBXD2, and UBXD8 shRNAs were fractionated by sucrose density-gradient ultracentrifugation and probed with an anti-ubiquitin antibody. Ubiquitinated proteins in the LD fraction increased significantly in cells with UBXD8 shRNA than in cells with control or UBXD2 shRNA. The ADRP reaction verified that the amount of LDs was comparable in the three samples.