SUPPLEMENTARY FIGURE LEGENDS

Figure S1. Expression of CFP-Drp1K38E in HeLa cells. Representative confocal images of HeLa cells left untransduced (top) or infected with adenovirus encoding CFP-Drp1K38E (bottom) and fixed 24 hours post-infection. Mitochondrial morphology was visualized by endogenous TOM20 immunostaining (red, top) or localization of ectopically-expressed pOCT-DsRed2 (red, bottom). Scale bars, 50 microns.

Figure S2. Parkin does not associate with TOM20-positive MDVs. A. Quantification of the number of TOM20-positive/OCT-DsRed2-negative MDVs from GFP and GFP-parkin expressing cells from Fig. 1C. Bars represent the mean±SEM. B. HeLa cells expressing GFP-parkin (green), OCT-DsRed2 (red) and CFP-Drp1K38E were treated with 100 mU/ml glucose oxidase (GO) for two hours before fixation. Open arrowheads indicate TOM20-positive/OCT-DsRed2-negative structures that do not colocalize with GFP-parkin. Scale bars, 30 microns.

Figure S3. Loss of parkin abolishes MDV formation in COS7 cells. A. Representative immunoblot of whole-cell lysates from COS7 cells transfected with non-targeting siRNA or siRNA targeting parkin (siParkin). The asterisk indicates a non-specific band. B. Quantification of the total number of PDH E2/E3bp-positive/TOM20-negative structures from COS7 cells treated with or without 50 μM antimycin A for two hours prior to
fixation. Bars represent the mean±SEM (n = at least 45 cells over 3 experiments; ns, not significant; *, p<.05; **, p<.01; ***, p<.001).

Figure S4. Exclusion of TOM20 on MDVs does not occur through p97/VCP-dependent proteasomal degradation. A. HeLa cells transfected with siRNA targeting Drp1 and transiently expressing GFP-parkin (green) were pretreated for 30 minutes with 10 μM MG132 prior to treatment with 25 μM antimycin A (with MG132) for 90 minutes. After fixation, samples were immunostained against TOM20 (blue) and PDH E2/E3bp (red). PDH E2/E3bp-positive/TOM20-negative MDVs colocalizing with GFP-parkin (arrows) or not (circles) are indicated. Scale bars, 20 microns. B. Quantification of PDH E2/E3bp-positive/TOM20-negative structures from HeLa cells treated with antimycin A and 10 μM MG132 or 2 μM epoxomicin (epoxo); both total number (white bars) and the number colocalizing with GFP-parkin (gray bars) are indicated. Bars represent the mean±SEM. P-values are given first for GFP-/GFP-parkin-positive vesicles, then for total vesicle number (n = 61 to 73 cells in 3 experiments; ns, not significant; *, p<.05; **, p<.01; ***, p<.001). C. Quantification of the ratio of parkin-positive MDVs (PDH E2/E3bp-positive/TOM20-negative, gray bars) to fragments (PDH E2/E3bp- and TOM20-positive, white bars) of all cells quantified in B (ns, not significant). D. Representative immunoblot of whole-cell lysates from U2OS:GFP and GFP-parkin cells treated with antimycin A and proteasomal inhibitors depicting total TOM20 levels. VDAC1 is used as a mitochondrial loading control. E. Quantification of TOM20 signal intensity relative to that of actin in immunoblots from D. Bars represent the mean±SEM (n = 3 experiments). F. Representative immunoblot of whole-cell lysates from U2OS:GFP-parkin cells
transfected with non-targeting siRNA, siRNA targeting Drp1 (siDrp1) and/or p97/VCP (siVCP). G. Quantification of the total number of PDH E2/E3bp-positive/TOM20-negative structures from U2OS:GFP-parkin cells (transfected with the indicated siRNA) treated with 25 μM antimycin A for 90 minutes. Bars represent the mean±SEM (n = 48 to 62 cells in 2 experiments; ns, not significant; *, p<.05; **, p<.01; ***, p<.001).

Figure S5. Silencing of Drp1 in HeLa cells. A. Representative immunoblot of whole-cell lysates from HeLa cells transfected with non-targeting siRNA or siRNA targeting Drp1 (siDrp1). B. GFP-parkin-expressing HeLa cells transfected with siRNA targeting Drp1 were treated with 25 μM antimycin A (anti A) or DMSO (DMSO) for 90 minutes, then fixed and immunostained against PDH E2/E3bp (red) and TOM20 (blue). PDH E2/E3bp-positive/TOM20-negative MDVs colocalizing with GFP-parkin (arrows) or not (circles) are indicated. Scale bars, 20 and 5 microns.

Figure S6. Silencing of genes involved in autophagy in HeLa cells. A. Atg5+/+ and Atg5−/− mouse embryonic fibroblasts (MEFs) were transfected with GFP-parkin (green), OCT-DsRed2 (red, mtDsRed2) and CFP-Drp1K38E, treated with 40 μM antimycin A for two hours, fixed and immunostained against TOM20 (blue). Circles indicate OCT-DsRed2-positive/TOM20-negative MDVs colocalizing with GFP-parkin. Scale bars, 30 microns. B. Representative immunoblots of whole-cell lysates depicting efficiency of siRNA-mediated knockdown of autophagy-related genes in HeLa cells. The asterisk indicates a non-specific band. C. Representative images of HeLa cells transfected with GFP-LC3 (green) and siRNA targeting Drp1 and the indicated autophagy-related gene (or control),
fixed 24 hours after plasmid transfection. Boxes highlight (lack of) GFP-LC3 clustering in untreated cells.

Figure S7. Parkin-dependent mitophagy degrades mitochondria over 24 hours. A. Representative immunoblot of whole-cell lysates from U2OS:GFP-parkin cells treated with DMSO, 25 μM antimycin A (anti A), 25 μM antimycin A with 10 μM oligomycin (anti A + oligo), or 20 μM CCCP for the indicated time period. B. Quantification of mitochondrial clearance in U2OS:GFP-parkin cells treated as in A, fixed and immunostained for TRAP1. Data are shown as percentage of cells containing mitochondria, by TRAP1 staining, visualized by fluorescence microscopy. Bars represent the mean±SEM (n = 3 experiments, with at least 85 cells quantified per condition, per experiment). C. U2OS:GFP-parkin cells were treated as in A for the indicated time period, then fixed and immunostained for TRAP1 (red). Cell boundaries are delineated in single-channel images. Scale bars, 20 microns.