McLelland *et al*. SUPPLEMENTARY MATERIAL

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-Drp1^{K38E} (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-Drp1^{K38E} 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-$Drp1^{K38E}$, 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.