Supporting Information

Coupling of glucose metabolism with mitophagy via O-GlcNAcylation of PINK1

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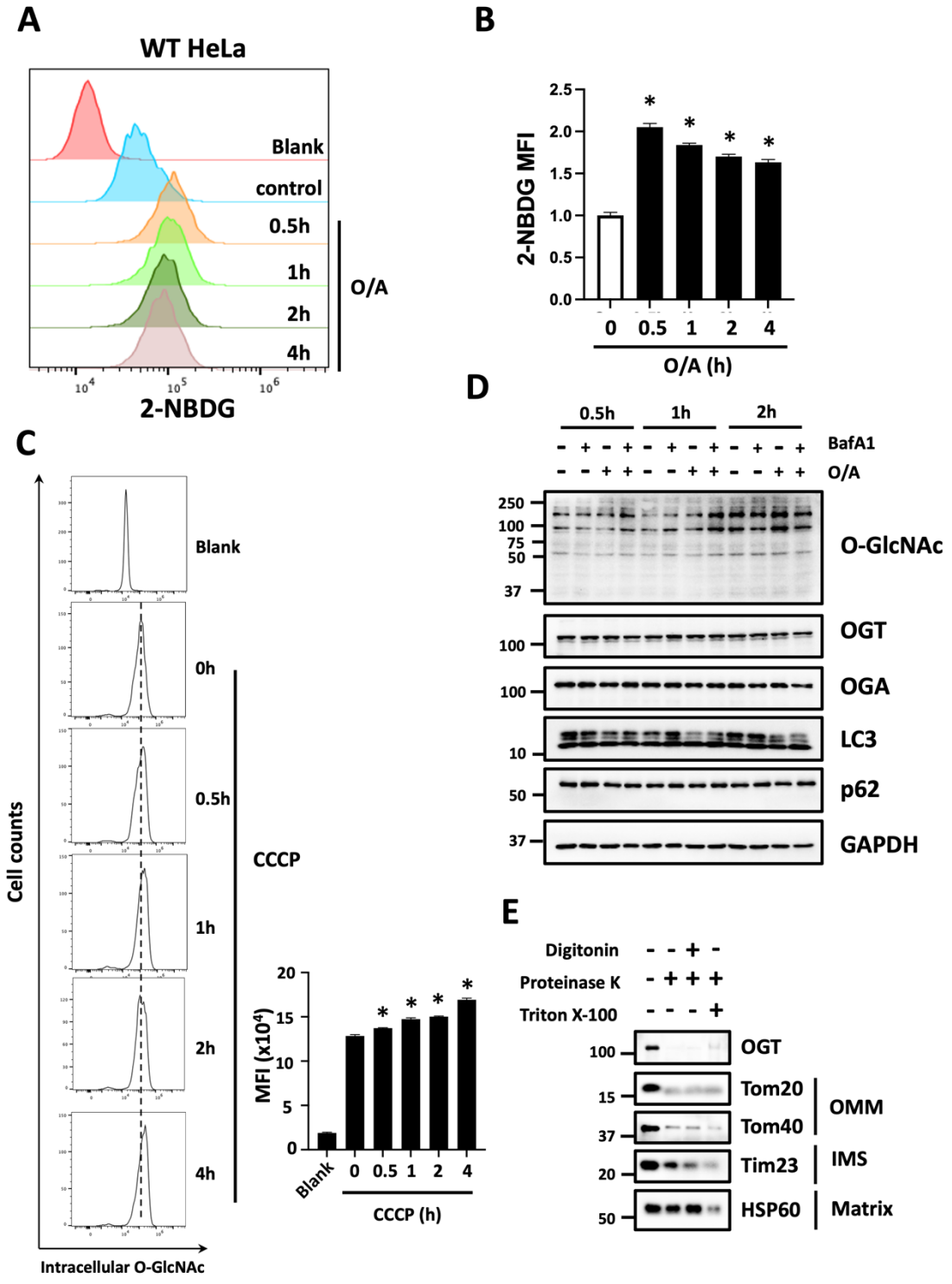


Figure S1. Increased glucose uptake and O-GlcNAcylation in WT HeLa cells following O/A-induced mitophagy. (**A and B**) Flow cytometry assay of glucose uptake by WT HeLa cells in the 2-NBDG assay. (**C**) Flow cytometric analysis of intracellular O-GlcNAc levels in YFP-HeLa treated with CCCP (20 μM) for 0.5 to 4 h. (**D**) YFP-HeLa cells were pretreated with or without Bafilomycin A1 (BafA1,100nM) for 1 h. Subsequently, the cells were treated with or without O/A (1μM and 1μM) for 0.5, 1, 2h

and subjected to western blotting analysis with the indicated antibodies. (**E**) Topology assay showing OGT localization at the OMM. Purified mitochondria were isolated from YFP-Parkin-HeLa cells and treated with the indicated reagents of proteinase K and digitonin. *$p < 0.05$.

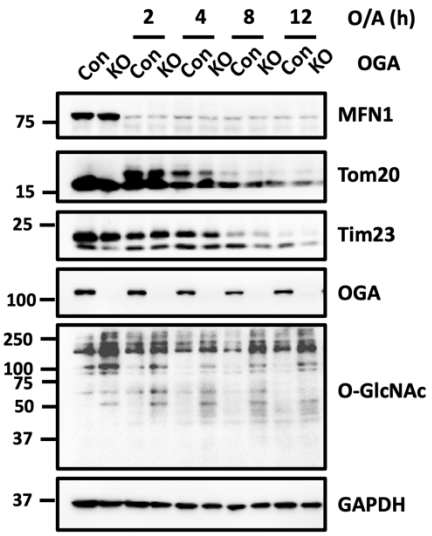


Figure S2. OGA KO did not regulate mitophagy. Mcherry-Parkin expressing control and OGA KO cells HeLa cells treated with 1 μM O/A, then harvested at the indicated time points for western blotting analysis with the indicated antibodies.

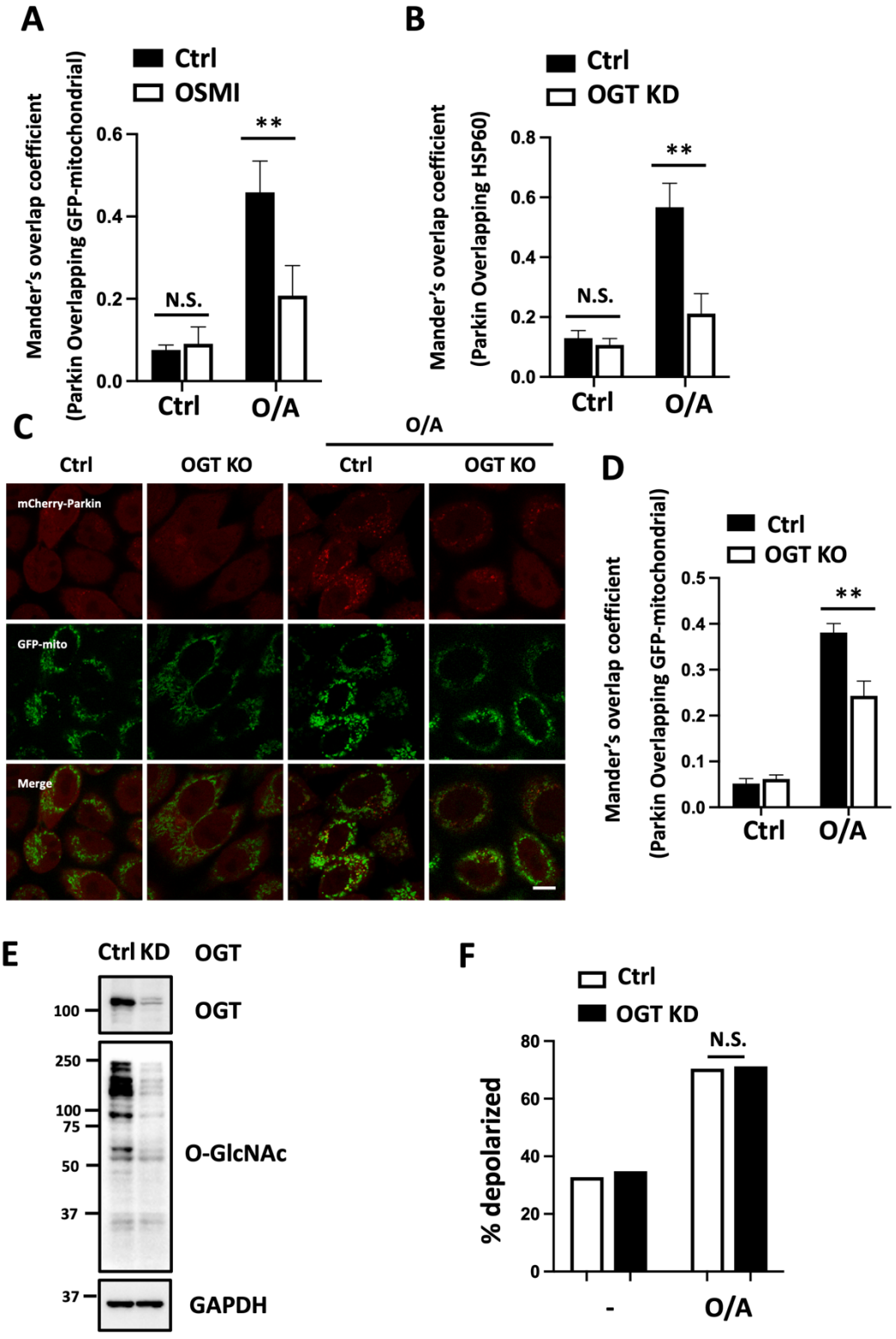


Figure S3. OGT KO reduced Parkin mitochondrial translocation. (**A and B**) Colocalizations between mCherry-Parkin and GFP-mitochondrial, YFP-Parkin and mitochondrial marker HSP60 as in (Fig 3D and 3F) were analyzed by Mander's overlap coefficient. (**C**) Control and OGT-KO HeLa stably expressing mCherry-Parkin upon treatment with or without O/A for 2h. Representative immunofluorescence images of WT and OGT-KO cells. Scale bar=10μm. (**D**) Colocalization between mCherry-Parkin

and GFP-mitochondrial as in (Fig S3C). (**E**) Control or OGT KD cells were lysed for western blotting analysis to confirm the knockdown effects of OGT. (**F**) JC-1 staining and quantification of Ctrl and OGT KD HEK293T treated with 1 μM O/A or DMSO for 6h. **, p < 0.01, N.S., no significance.

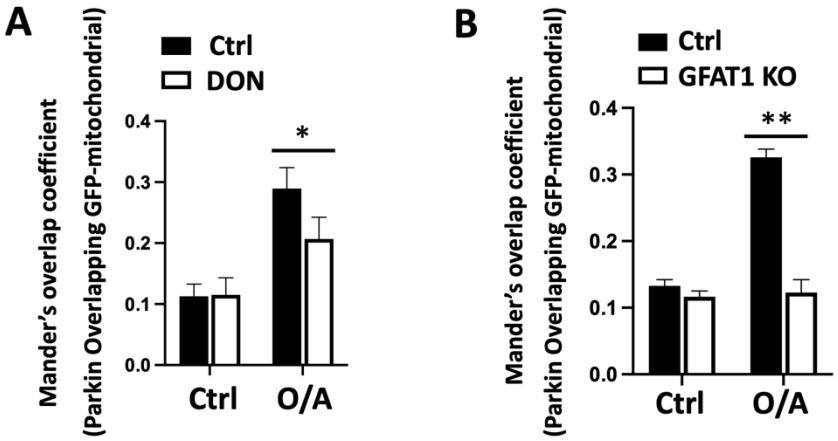


Figure S4. Colocalization between mcherry-Parkin and GFP-mitochondrial as in (Fig 4B and 4D) was analyzed by Mander's overlap coefficient. (N.S., no significance; *, P < 0.1, **, P < 0.01; Two-way ANOVA with Sidak's multiple comparisons test).

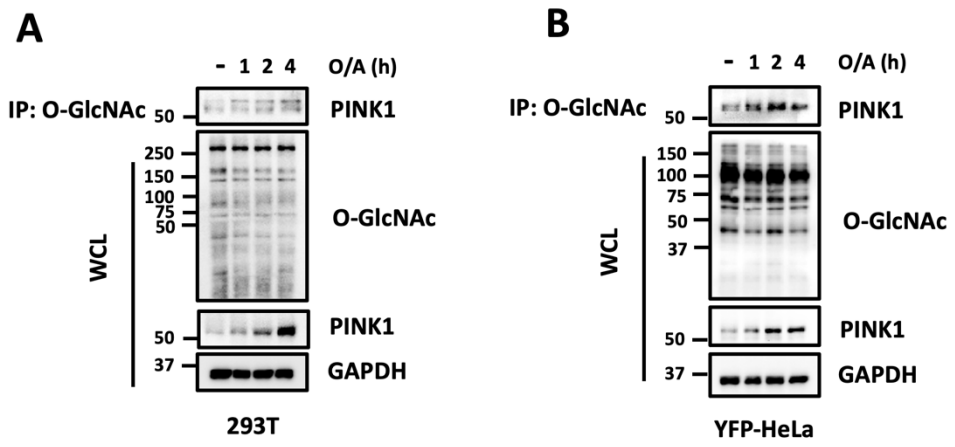
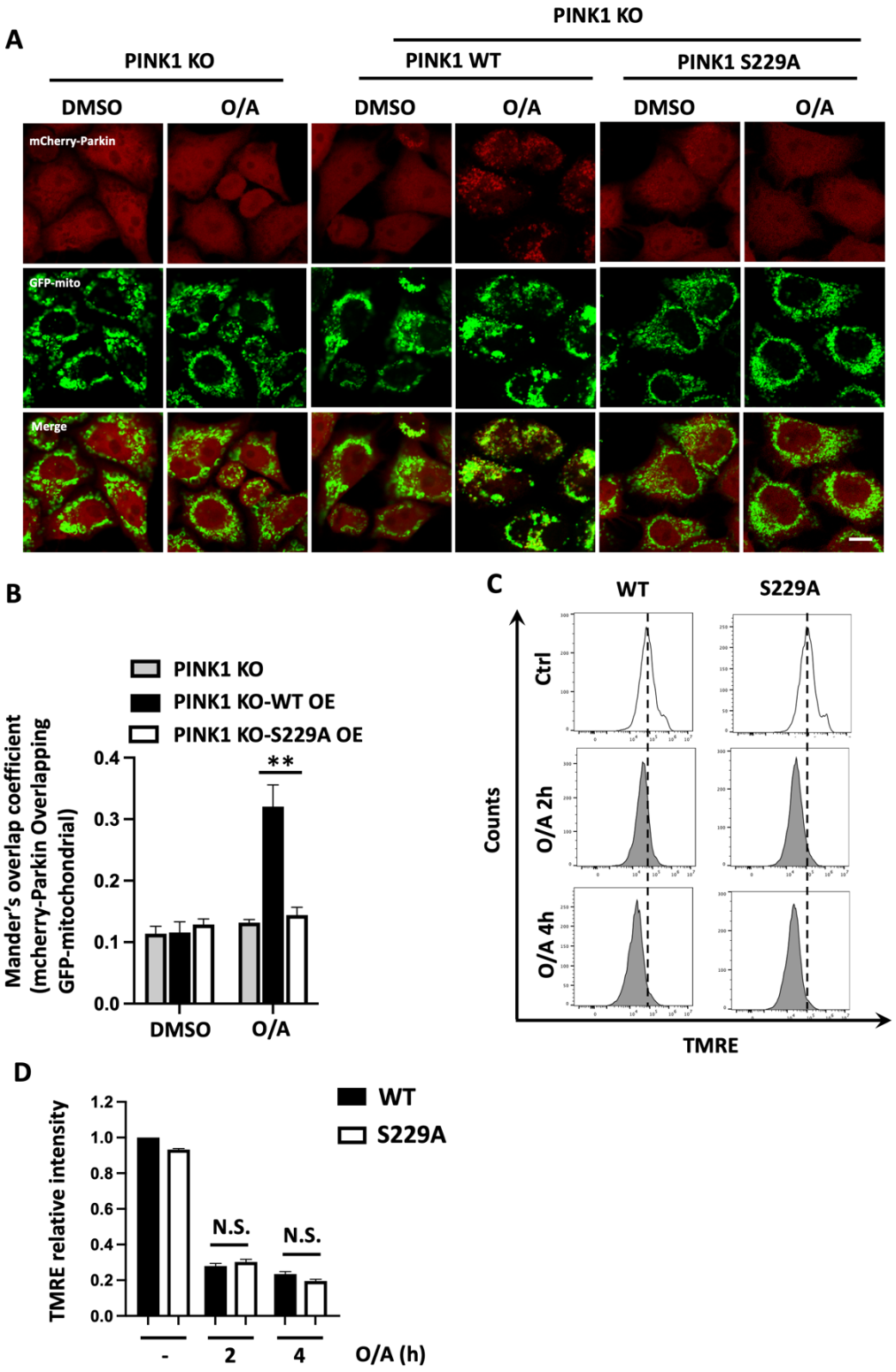


Figure S5. PINK1 undergoes O-GlcNAcylation. (**A and B**) HEK293T and YFP-HeLa cells following O/A for the indicated time were subjected to immunoprecipitation with anti-O-GlcNAc antibody. Then the immunoprecipitation was blotted with PINK1 antibody and lysates were subjected to O-GlcNAc antibody respectively.

SeqName	Residue		O-GlcNAc result	Potential (o-glcnac)
NP_115785.1	22	T	+++	0.2932
NP_115785.1	73	S	+++	0.3181
NP_115785.1	118	S	+++	0.2733
NP_115785.1	123	S	+++	0.5152
NP_115785.1	133	T	+++	0.3832
NP_115785.1	136	S	+++	0.3192
NP_115785.1	145	T	+++	0.3103
NP_115785.1	161	S	+++	0.2817
NP_115785.1	167	S	+++	0.2708
NP_115785.1	174	T	+++	0.2371
NP_115785.1	177	T	+++	0.2923
NP_115785.1	185	T	+++	0.4256
NP_115785.1	187	S	+++	0.2909
NP_115785.1	188	T	+++	0.2929
NP_115785.1	198	T	+++	0.4757
NP_115785.1	199	S	+++	0.3143
NP_115785.1	225	S	+++	0.3586
NP_115785.1	228	S	+++	0.3546
NP_115785.1	229	S	+++	0.3112
NP_115785.1	230	S	+++	0.3480
NP_115785.1	236	T	+++	0.3678
NP_115785.1	238	S	+++	0.3788
NP_115785.1	245	S	+++	0.3743
NP_115785.1	257	T	+++	0.4696
NP_115785.1	261	S	+++	0.2329
NP_115785.1	282	T	+++	0.5008
NP_115785.1	283	S	+++	0.5747
NP_115785.1	284	S	+++	0.3839
NP_115785.1	301	S	+++	0.2168
NP_115785.1	313	T	+++	0.2176
NP_115785.1	324	T	+++	0.3166
NP_115785.1	333	T	+++	0.3340
NP_115785.1	335	S	+++	0.2933
NP_115785.1	365	S	+++	0.2254
NP_115785.1	393	S	+++	0.2999
NP_115785.1	401	S	+++	0.5954
NP_115785.1	402	S	+++	0.3498
NP_115785.1	419	S	+++	0.7224
NP_115785.1	420	T	+++	0.4713
NP_115785.1	432	S	+++	0.3413
NP_115785.1	463	S	+++	0.2361
NP_115785.1	465	S	+++	0.2813
NP_115785.1	477	S	+++	0.4912
NP_115785.1	495	S	+++	0.5014
NP_115785.1	499	S	+++	0.2598
NP_115785.1	510	S	+++	0.1658
NP_115785.1	535	S	+++	0.3341
NP_115785.1	538	T	+++	0.2207
NP_115785.1	545	T	+++	0.3223
NP_115785.1	552	T	+++	0.2053
NP_115785.1	566	T	+++	0.2546
NP_115785.1	576	S	+++	0.3408

Figure S6. The predicted sites of PINK1 could be modified by O-GlcNAcylation. Computational O-GlcNAcylation sites of PINK1 predicted via YinOYang prediction program. This prediction program characterizes PINK1 O-GlcNAcylation over 50 different sites, where its prediction strengths are marked with high [“+++”], ranging from low to high [“+” to “++++”] in the program. The prediction strength depends on the sequence condition and conformation.


A
PINK1 KO
PINK1 KO
PINK1 WT
PINK1 S229A
DMSO
O/A
DMSO
O/A
DMSO
O/A
mCherry-Parkin
GFP-mito
Merge
B
PINK1 KO
PINK1 KO-WT OE
PINK1 KO-S229A OE
Mander's overlap coefficient (mcherry-Parkin Overlapping GFP-mitochondrial)
0.4
0.3
0.2
0.1
0.0
**
DMSO
O/A
C
WT
S229A
Ctrl
O/A 2h
O/A 4h
Counts
TMRE
D
WT
S229A
TMRE relative intensity
1.2
1.0
0.8
0.6
0.4
0.2
0.0
N.S.
N.S.
-
2
4
O/A (h)

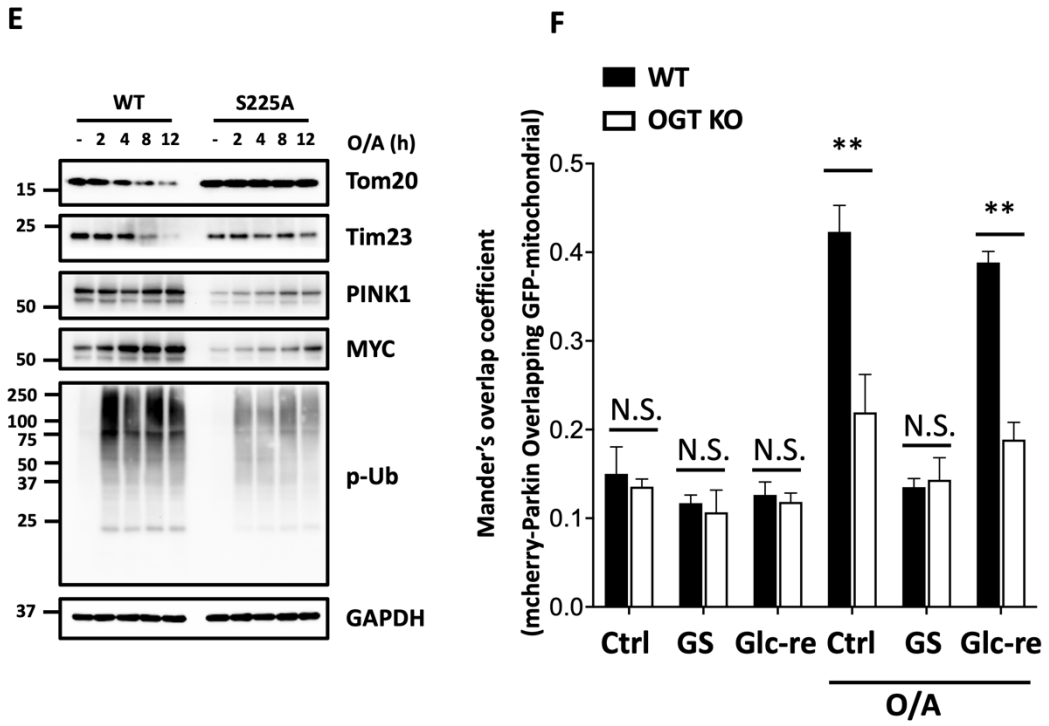


Figure S7. S229A and S225A mutant suppress PINK1-Parkin-mediated mitophagy. (**A**) PINK1 KO cells transfected with WT PINK1 or S229A mutant were treated with or without O/A for 2h. (**B**) Colocalization between mCherry-Parkin and GFP-mitochondrial as in (Fig S7A) was analyzed. (**C and D**) TMRE staining and quantification of WT PINK1 and S229A mutant overexpressing YFP-HeLa PINK1 KO cells treated with 1 μM O/A or DMSO for 2 and 4 h. (**E**) PINK1 WT and S225A were transfected in HeLa PINK1−/− stably expressing mCherry-Parkin cells, time course for mitochondrial proteins detection upon treatment with O/A. *p < 0.05. (**F**) Colocalization between mcherry-Parkin and GFP-mitochondrial as in (Fig 7C) was analyzed by Mander's overlap coefficient. (N.S., no significance; **, P < 0.01; Two-way ANOVA with Sidak's multiple comparisons test. GS, glucose starvation, Glc-re, glucose re-supplement). Scale bar=10μm.