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## Supplemental Information

# PINK1/Parkin Influences Cell Cycle by Sequestering TBK1 at Damaged Mitochondria, Inhibiting Mitosis

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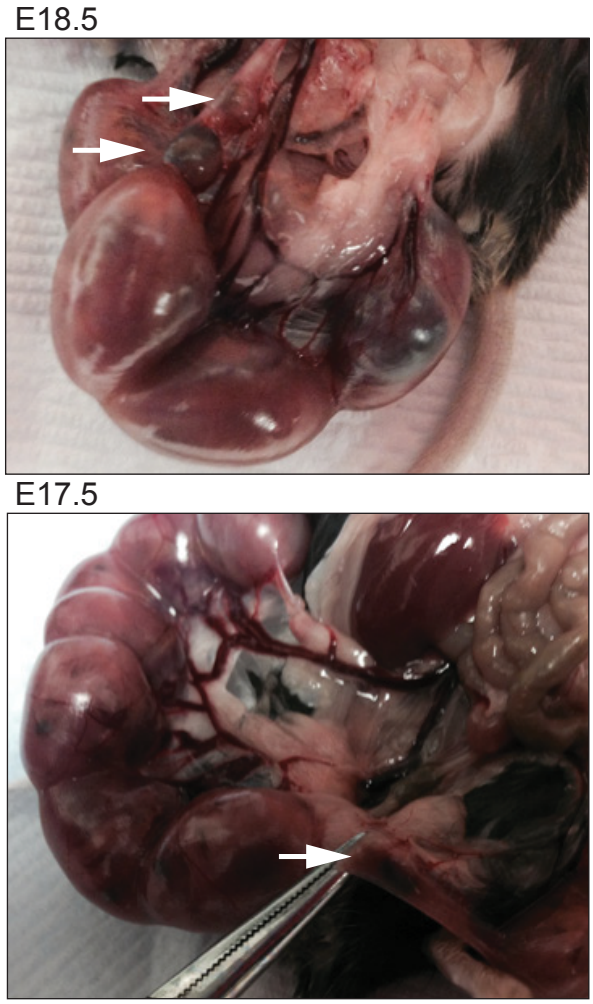
A




B

ATM+/- Parkin KO X ATM+/- Parkin KO cross

| Mother | Number of Embryos | DKO | ATM WT | ATM+/- | Absorption Sites |
|---|---|---|---|---|---|
| 1 | 9 | 0 | 1 | 8 | |
| 2 | 1 | 0 | 1 | 0 | |
| 3 | 6 | 0 | 3 | 3 | |
| 4 | 4 | 0 | 1 | 1 | 2 |
| 5 | 8 | 0 | 3 | 5 | |

E14.5-15.5

Figure S1. Absorbed Embryos from ATM+/-Parkin KO Females Mated with ATM+/-Parkin KO Males. Related to Figure 1. A) Representative photographs of pregnant mouse uteri harboring absorption sites at different days sacrificed during embryonic development. White arrowheads point to absorption sites. B) Table of genotyped embryos at E14.5-15.5 from ATM+/-Parkin KO mating crosses.

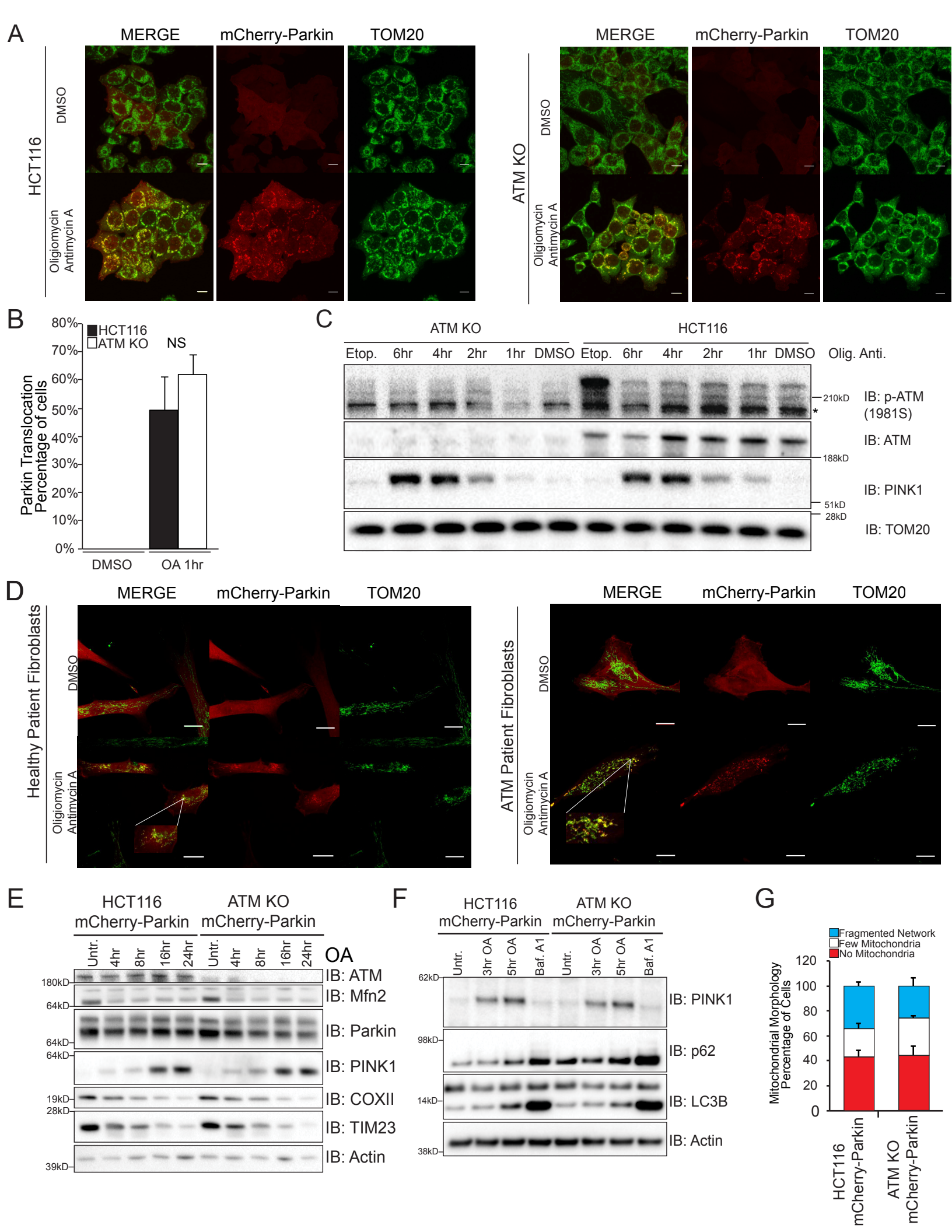

A
MERGE
mCherry-Parkin
TOM20
HCT116
DMSO
Oligiomycin
Antimycin A
ATM KO
B
HCT116
ATM KO
NS
Parkin Translocation
Percentage of cells
80%
70%
60%
50%
40%
30%
20%
10%
0%
DMSO
OA 1hr
C
ATM KO
HCT116
Etop.
6hr
4hr
2hr
1hr
DMSO
Olig. Anti.
210kD
*
IB: p-ATM
(1981S)
IB: ATM
188kD
IB: PINK1
51kD
28kD
IB: TOM20
D
Healthy Patient Fibroblasts
ATM Patient Fibroblasts
E
HCT116
mCherry-Parkin
ATM KO
mCherry-Parkin
Untr.
4hr
8hr
16hr
24hr
OA
180kD
IB: ATM
64kD
IB: Mfn2
IB: Parkin
IB: PINK1
19kD
28kD
IB: COXII
IB: TIM23
39kD
IB: Actin
F
3hr OA
5hr OA
Baf. A1
62kD
IB: PINK1
98kD
IB: p62
14kD
IB: LC3B
38kD
IB: Actin
G
Fragmented Network
Few Mitochondria
No Mitochondria
Mitochondrial Morphology
Percentage of Cells
120
100
80
60
40
20
0
HCT116
mCherry-Parkin
ATM KO
mCherry-Parkin

Figure S2. The Loss of ATM does not Affect PINK1 Accumulation or Parkin Translocation Upon Mitochondrial Damage. Related to Figure 1. A) Representative confocal images of HCT116 and ATM KO cells stably expressing mCherry-Parkin (red). Cells were treated with either DMSO or O/A for 1hr. Mitochondria were identified by immunostaining for TOM20 (green). Scale bar = 10 μm. B) Quantification of the percentage of HCT116 or ATM KO cells with mCherry-Parkin translocation to mitochondria in DMSO or O/A treated conditions after 1hr. n=3 independent experiments. C) Western blotting with a time course showing PINK1 accumulation in ATM KO and HCT116 cells. Etoposide was used as a positive control to show ATM autophosphorylation and activation. Cells were not overexpressing mCherry-Parkin. TOM20 was used as a loading control. Asterisk denotes nonspecific band. D) Representative confocal images of healthy or AT patient fibroblasts transduced with mCherry-Parkin (red). Cells were either treated with DMSO or O/A for 1hr. Mitochondria identified by immunostaining with an antibody against TOM20 (green). Scale bar = 10 μm. E) Western blotting time course with HCT116 and ATM KO cells expressing mCherry-Parkin treated with O/A. Membranes were blotted for PINK1, Parkin, ATM and the outer mitochondrial membrane protein Mfn2 and inner mitochondrial membrane proteins, TIM23 and COXII. Protein loading control is β-actin. F) Western blotting time course with HCT116 and ATM KO cells expressing mCherry-Parkin treated with O/A or Bafilomycin A1 (positive control). Membranes were blotted for PINK1, p62, and LC3B. Upper band for LC3 is nonspecific and is denoted with an asterisk. Protein loading control is β-actin. G) Quantification of the percentage of cells with differently described GRP75 phenotypes in HCT116 and ATM KO cells overexpressing mCherry-Parkin treated with O/A for 48 hrs. A pan-caspase inhibitor QVD-Oph was used. n = 3 independent experiments. B, G) Error bars represent SD and SEM, respectively.

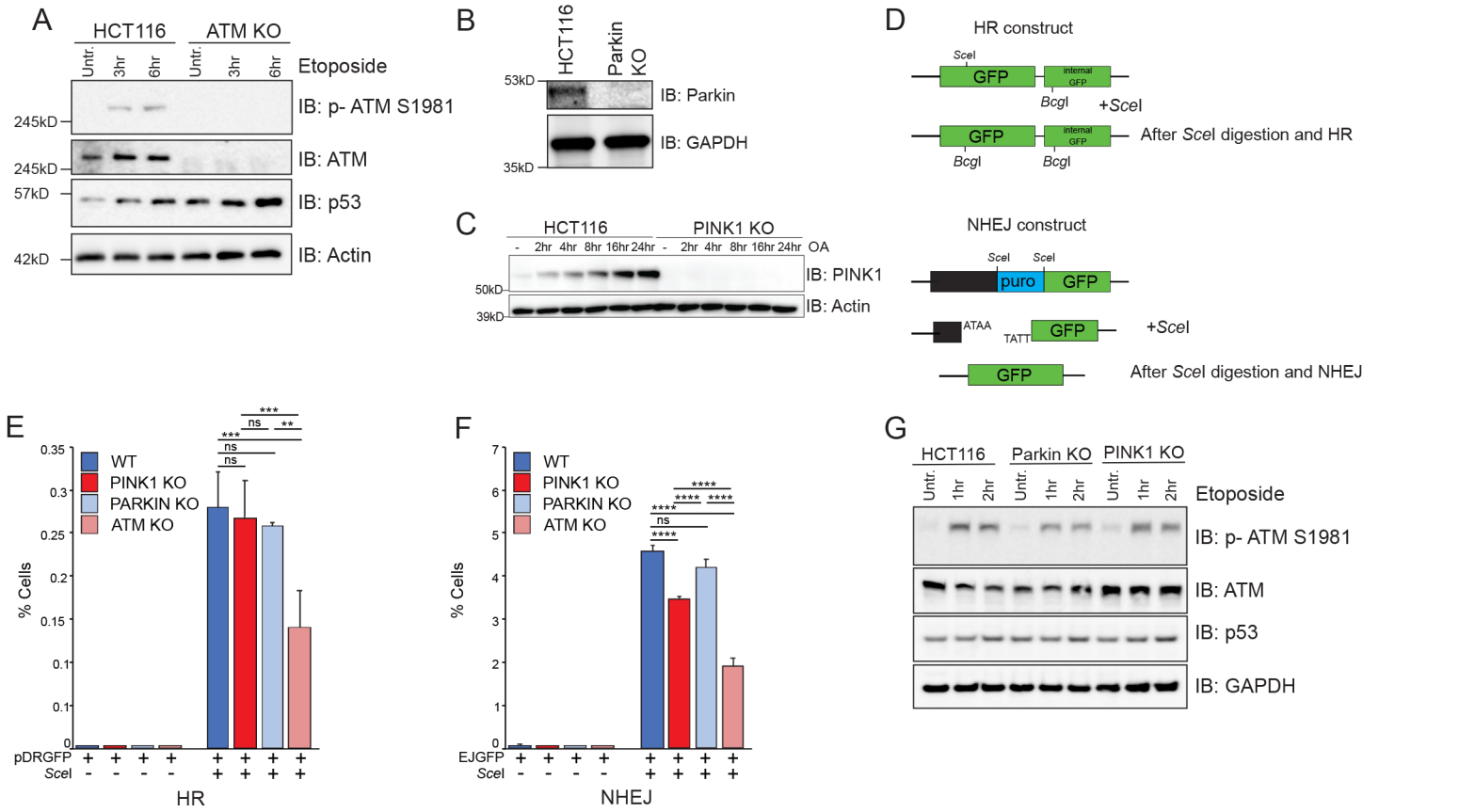



Figure S3. Characterizing PINK1 KO and Parkin KO Cells' Response to DNA Damage. Related to Figure 1 and 2. A) Western blotting with HCT116 and ATM KO cells treated with etoposide for 3 or 6hrs and blotted for p-ATM S1981, ATM or p53 antibodies. Protein loading control was β-actin. B) Western blotting with HCT116 and Parkin KO cells blotted for Parkin. Protein loading control was GAPDH. C) Western blotting with HCT116 and PINK1 KO cells blotted for PINK1 after time course with O/A treatment. Protein loading control was β-actin. D) Cartoon depiction of DRGFP construct with downstream internal GFP segment that acts as a template for homologous recombination repair (HR) after digestion with the *Sce*I endonuclease. Cartoon depiction of EJ5GFP construct with puromycin cassette flanked by two *Sce*I restriction sites as a template for non-homologous end joining repair (NHEJ) after digestion with the *Sce*I endonuclease. E) Percentage of FACS sorted cells expressing GFP that were either HCT116, PINK1 KO, ATM KO, or Parkin KO. Cells were cotransfected with mCherry-C1, DR-GFP and *Sce*I construct and analyzed 48hrs later. Statistical significance was determined by one-way ANOVA. ** p <.01, *** p<.001, NS = not significant. n = 3 independent experiments. Error bars represent SEM. F) Percentage of FACS sorted cells expressing GFP that were either HCT116, PINK1 KO, ATM KO, or Parkin KO. Cells were cotransfected with mCherry-C1, EJ5GFP and *Sce*I construct and analyzed 48 hrs later. Statistical significance was determined by one-way ANOVA. **** p<.0001, NS = not significant, n = 3 experiments. Error bars represent SEM. G) Western blotting with HCT116, Parkin KO, and PINK1 KO cells treated with etoposide for 1 or 2hrs and blotted for p-ATM S1981, ATM, or p53 antibodies. Protein loading control was GAPDH.

A

| | Genotype | Survival |
|---|---|---|
| Females | *Pink1*$^{B9/+}$; *da-G4>stg*$^{RNAi}$ | 100% |
| | *Pink1*$^{B9/+}$; *UAS-stg*$^{RNAi}$ | 100% |
| Males | *da-G4>stg*$^{RNAi}$ | 100% |
| | *UAS-stg*$^{RNAi}$ | 100% |
| | *Pink1*$^{B9}$; *da-G4>stg*$^{RNAi}$ | 100% |
| | *Pink1*$^{B9}$; *UAS-stg*$^{RNAi}$ | 80% |

B

| Genotype | Survival |
|---|---|
| *hs-G4; park*$^{25/+}$ | 100% |
| *hs-G4>CDK2*$^{RNAi}$; *park*$^{25/+}$ | 100% |
| *hs-G4; park*$^{25}$ | 100% |
| *hs-G4>CDK2*$^{RNAi}$; *park*$^{25}$ | 100% |

C

| | Genotype | Survival |
|---|---|---|
| Females | *Pink1*$^{B9/+}$; *da-G4>CDK2*$^{RNAi}$ | 100% |
| | *Pink1*$^{B9/+}$; *UAS-CDK2*$^{RNAi}$ | 100% |
| Males | *da-G4>CDK2*$^{RNAi}$ | 100% |
| | *UAS-CDK2*$^{RNAi}$ | 100% |
| | *Pink1*$^{B9}$; *da-G4>CDK2*$^{RNAi}$ | 100% |
| | *Pink1*$^{B9}$; *UAS-CDK2*$^{RNAi}$ | 100% |

D

| Genotype | Survival |
|---|---|
| *hs-G4; park*$^{25/+}$ | 100% |
| *hs-G4>CDK1*$^{RNAi}$; *park*$^{25/+}$ | 100% |
| *hs-G4; park*$^{25}$ | 100% |
| *hs-G4>CDK1*$^{RNAi}$; *park*$^{25}$ | 79.6% |

E

| | Genotype | Survival |
|---|---|---|
| Females | *Pink1*$^{B9/+}$; *da-G4>CDK1*$^{RNAi}$ | 85% |
| | *Pink1*$^{B9/+}$; *UAS-CDK1*$^{RNAi}$ | 100% |
| Males | *da-G4>CDK1*$^{RNAi}$ | 100% |
| | *UAS-CDK1*$^{RNAi}$ | 100% |
| | *Pink1*$^{B9}$; *da-G4>CDK1*$^{RNAi}$ | 100% |
| | *Pink1*$^{B9}$; *UAS-CDK1*$^{RNAi}$ | 100% |

Figure S4: *park*$^{25}$ and *Pink1*$^{B9}$ *Drosophila* Lines that Genetically Interact with CDC25, CDK1 and CDK2 Eclose Normally. Related to Figure 2. Tables showing the percentage of flies eclosed for each genotype from the crosses referred to in Figure 2. A) *Pink1*$^{B9}$/*FM7; da-G4/CyO* x *UAS-stg*$^{RNAi}$ (#29556) cross,
B) *UAS-CDK2*$^{RNAi}$ (#36128)/*CyO*; *park*$^{25}$/*TM3* x *hs-G4/CyO*; *park*$^{25}$/*TM3* cross,
C) *Pink1*$^{B9}$/*FM7*; *da-G4/CyO* x *UAS-stg*$^{RNAi}$ (#36128) cross,
D) *UAS-CDK1*$^{RNAi}$ (#36117)/*CyO*; *Park*$^{25}$/*TM3* x *hs-G4/CyO*; *Park*$^{25}$/*TM3* cross,
E) *Pink1*$^{B9}$/*FM7*; *da-G4/CyO* x *UAS-stg*$^{RNAi}$ (#36117) cross.
A-E) At least 200-300 progeny from each cross were evaluated to determine the percentage of flies that eclosed.

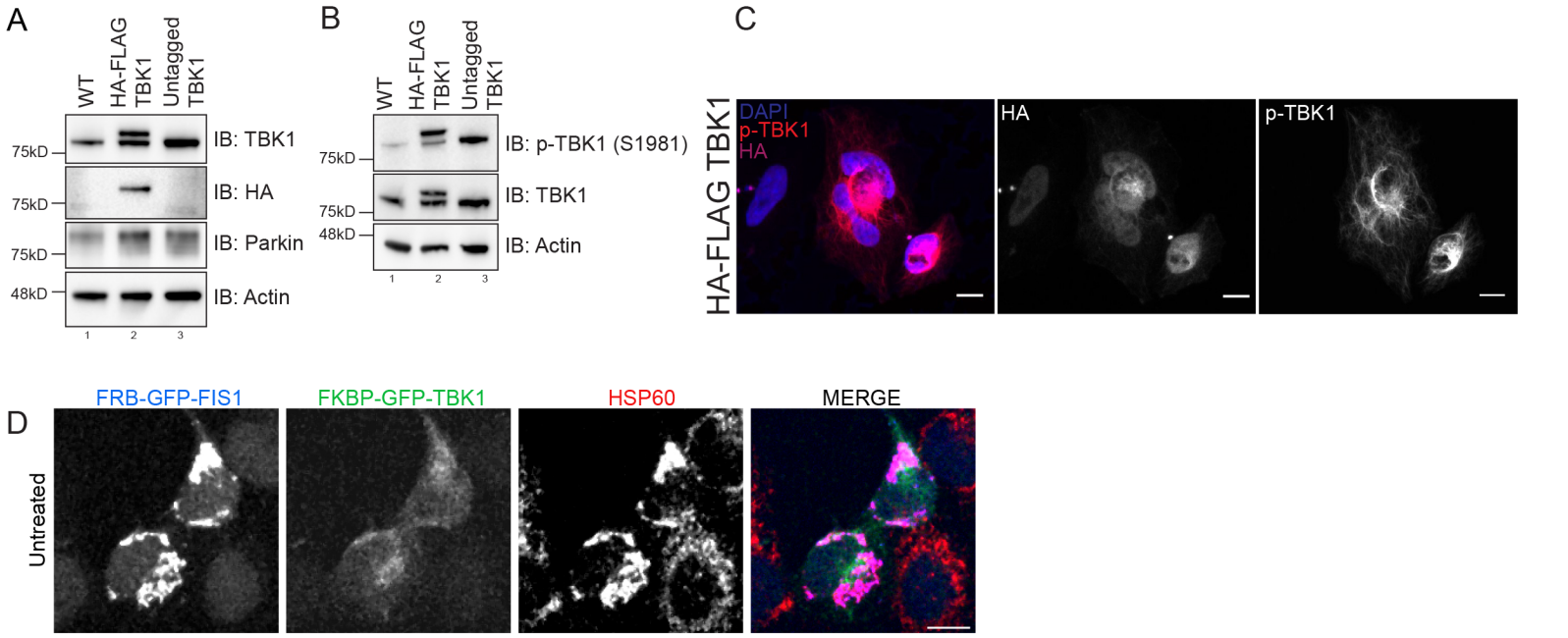



Figure S5: FKBP-GFP-TBK1 is Cytosolic at Low Levels Without Rapalog. Related to Figure 5. A) Representative western blot checking the expression of TBK1 in stably expressing HA-FLAG TBK1 and untagged TBK1 HeLa cell lines originally overexpressing YFP-Parkin. B) Representative western blot detecting p-TBK1 S1981 activation in the stably expressing HA-FLAG TBK1 and untagged TBK1 lines. C) Representative confocal images of p-TBK1 (red), HA (violet) and DAPI (blue) staining for stably expressing HA-FLAG TBK1 cells in asynchronous conditions. Scale bar = 10μm. D) Representative confocal images of stably expressing FRB-BFP-Fis1 (blue) HeLa cells in the TBK1 KO background transiently expressing FKBP-GFP-TBK1 (green) stained with HSP60 (red). Scale bar = 10μm.

| Parental Line | Protein | CRISPR sequence | Exon | Genotyping | Sequencing | Validation Methods |
|---|---|---|---|---|---|---|
| HCT116 | PINK1 | GGGAAGAAGCGGAGACGGTT | 1 | PINK1 exon 1 F - GCCCCAAGTTTGTTGTGAC<br>PINK1 exon 7 R- TGTGGTGGCTAGTGCTCCTA | Yes | absent PINK1 protein levels by western blot |
| | | GTCAATCCCTTCTACGGCCA | 7 | | | overexpressed Parkin translocation absent with OA treatment |
| HCT116 | ATM | CTCTATCATGTTCTAGTTGACGG | 2 | ATM exon 2 F - GCTACTACTGCAAGCAAGGC<br>ATM exon 2 R - GCCAAATTCATATGCAAGGCA<br>PCR-RFLP with *Hinc*II | Yes | absent ATM protein levels by western blot |
| HCT116 | Parkin | GCATTACGTGCACAGACGTCAGG | 6 | Parkin exon 6 F - TTTGGCACAAGGTCATCCGT<br>Parkin exon 6 R - TGGGCTACATGTTGTGGTGTT | Yes | validated by RT-PCR, absent Parkin protein by western blot |
| HeLa | TBK1 | AGACATTTGCAGTAGCTCCT | 2 | TBK1 exon 2 F - CATGGGGAAAATGGCATCTTGCTTT<br>TBK1 exon 2 R - GGTATAAAACCATATTCCATTAGTCTTGGACCATCA | Yes | absent TBK1 protein levels by western blotting |
| | | GGGGGATGCTAGCATGGCT | 10 | TBK1 exon 10 F - GTAGAAAAAAAAATGGATCACCCAGAATTTTACAGTTC<br>TBK1 exon 10 R - GGGATATGGTGTTAATCGTGGTTTATAACTGTAAAAT | | |

Table S1. List of CRISPRs Used to Generate Knockout Lines and the Genotyping/Methods of Verification to Validate Knockout Lines. Related to STAR Methods.

| Stock number | Information | Fly Gene | Human Gene | Chromosome |
|---|---|---|---|---|
| 36685 | PTRIP.HMS01573 | Grapes | Chk1 | 3 |
| 36117 | PTRIP.HMS01531 | CDK1 | CDK1 | 2 |
| 35350 | PTRIP.GL00262 | CDK1 | CDK1 | 3 |
| 41898 | PTRIP.GL01330 | CDK2 | CDK2 | 3 |
| 36651 | PTRIP.GL00611 | CDK2 | CDK2 | 2 |
| 36128 | PTRIP.HMS01543 | CDK2 | CDK2 | 2 |
| 62155 | PTRIP.HMC05162 | Grapes | Chk1 | 2 |
| 35267 | PTRIP.GL00162 | CDK2 | CDK2 | 3 |
| 29556 | PTRIP.JF03235 | String | CDC25 | 3 |
| 27277 | PTRIP.JF02588 | Grapes | Chk1 | 3 |
| 35287 | PTRIP.GL00189 | CDK5 | CDK5 | 3 |
| 35152 | PTRIP.GL00020/TM3 | Lok | Chk2 | 3 |
| 27517 | PTRIP.JF02667 | CDK5 | CDK5 | 3 |
| 40950 | PTRIP.HMS02212 | CDK1 | CDK1 | 3 |
| 56562 | P{UAS-stg.HA}2 | String | CDC25 | 3 |
| 56563 | P{UAS-stg.HA.mKEN}2 | String | CDC25 | 3 |
| 56564 | P{UAS-HA.stg.mKEN.mD} | String | CDC25 | 3 |
| 6789 | w[1] mei-41[D12] | Mei-41 | ATR | 1 |
| 8625 | w[*]; P{ry[+t7.2]=neoFRT}82B tefu[atm-3] e[1]/TM6B, Tb[1] | Tefu | ATM | 3 |
| 8626 | w[*]; P{ry[+t7.2]=neoFRT}82B tefu[atm-6] e[1]/TM6B, Tb[1] | Tefu | ATM | 3 |
| 8624 | P{ry[+t7.2]=neoFRT}82B tefu[atm-8] e[1]/TM3, Sb[1] | Tefu | ATM | 3 |
| 1799 | w[*]; P{w[+mC]=GAL4-Hsp70.PB}89-2-1 | | | 3 |
| 55849 | w[*]; P{w[+mW.hs]=GAL4-da.G32}2; P{w[+mW.hs]=GAL4-da.G32}UH1 | | | 2, 3 |
| 5322 | IKKepsilon[1] dpy[ov1] bw[1]/CyO | Ik2 | TBK1 | 2 |

Table S2. List of Bloomington Fly Stocks Used. Related to STAR Methods.

A

| | Genotype | Survival |
|---|---|---|
| Females | *Pink1$^{B9/+}$; da-G4>stg$^{RNAi}$* | 100% |
| | *Pink1$^{B9/+}$; UAS-stg$^{RNAi}$* | 100% |
| | *da-G4>stg$^{RNAi}$* | 100% |
| Males | *UAS-stg$^{RNAi}$* | 100% |
| | *Pink1$^{B9}$; da-G4>stg$^{RNAi}$* | 100% |
| | *Pink1$^{B9}$; UAS-stg$^{RNAi}$* | 80% |

B

| ***Genotype*** | **Survival** |
|---|---|
| *hs-G4; park$^{25/+}$* | 100% |
| *hs-G4>CDK2$^{RNAi}$; park$^{25/+}$* | 100% |
| *hs-G4; park$^{25}$* | 100% |
| *hs-G4>CDK2$^{RNAi}$; park$^{25}$* | 100% |

C

| | Genotype | Survival |
|---|---|---|
| Females | *Pink1$^{B9/+}$; da-G4>CDK2$^{RNAi}$* | 100% |
| | *Pink1$^{B9/+}$; UAS-CDK2$^{RNAi}$* | 100% |
| | *da-G4>CDK2$^{RNAi}$* | 100% |
| Males | *UAS-CDK2$^{RNAi}$* | 100% |
| | *Pink1$^{B9}$; da-G4>CDK2$^{RNAi}$* | 100% |
| | *Pink1$^{B9}$; UAS-CDK2$^{RNAi}$* | 100% |

D

| ***Genotype*** | **Survival** |
|---|---|
| *hs-G4; park$^{25/+}$* | 100% |
| *hs-G4>CDK1$^{RNAi}$; park$^{25/+}$* | 100% |
| *hs-G4; park$^{25}$* | 100% |
| *hs-G4>CDK1$^{RNAi}$; park$^{25}$* | 80% |

E

| | Genotype | Survival |
|---|---|---|
| Females | *Pink1$^{B9/+}$; da-G4>CDK1$^{RNAi}$* | 85% |
| | *Pink1$^{B9/+}$; UAS-CDK1$^{RNAi}$* | 100% |
| | *da-G4>CDK1$^{RNAi}$* | 100% |
| Males | *UAS-CDK1$^{RNAi}$* | 100% |
| | *Pink1$^{B9}$; da-G4>CDK1$^{RNAi}$* | 100% |
| | *Pink1$^{B9}$; UAS-CDK1$^{RNAi}$* | 100% |

**Table S3: *park25* and *Pink1B9 Drosophila* lines that genetically interact with CDC25, CDK1 and CDK2 eclose normally.** Related to Figure 1. Tables showing the percentage of flies eclosed for each genotype from the crosses referred to in Figure 1. A) *Pink1B9*/*FM7*; *da-G4/CyO* x *UAS-stgRNAi* (#29556) cross, B) *UAS-CDK2RNAi* (#36128)/*CyO*; *park25/TM3* x *hs-G4/CyO*; *park25/TM3* cross, C) *Pink1B9/FM7*; *da-G4/CyO* x *UAS-stgRNAi* (#36128) cross, D) *UAS-CDK1RNAi* (#36117)/*CyO*; *Park25/TM3* x *hs-G4/CyO*; *Park25/TM3* cross, E) *Pink1B9/FM7*; *da-G4/CyO* x *UAS-stgRNAi* (#36117) cross. A-E) At least 200-300 progeny from each cross were evaluated to determine the percentage of flies that eclosed.