Pink1 and its Δψ-dependent cleavage product both localize to the outer mitochondrial membrane by a unique targeting mode*

Dorothea Becker, Judith Richter, Maja A. Tocilescu, Serge Przedborski, Wolfgang Voos

SUPPLEMENTAL FIGURE LEGENDS

FIGURE S1. **Import of ^{35}S-labeled Pink1 isoforms Pink1$_{A217D}$ and Pink1$_{G309D}$ corresponding to PD-associated mutations.** Experiments were performed as described in Fig. 1B.

FIGURE S2. **Import and submitochondrial localization of newly imported ^{35}S-labeled Pink1 in isolated SH-SY5Y mitochondria.** Experiments were performed as described in Fig. 1B, C and 2A.

FIGURE S3. **Verification of immunodecoration signals for the used Pink1 antiserum by Western blot of *in vivo* expressed C-terminally tagged Pink1-myc/His$_6$.** HeLa cells were transiently transfected with a plasmid encoding Pink1 containing a C-terminal myc/hexahistidine tag (Pink1-myc/6xHis). Isolated mitochondria from transfected cells were analyzed by Western Blot and immunodecoration with antisera against Pink1 (lane 1) and against the myc epitope (lane 2).

FIGURE S4. **Solubility of imported ^{35}S-labeled Pink1 compared to *in vivo* expressed wild-type Pink1.** (A) After import of the radiolabeled Pink1 protein, mitochondria were lysed in 0.3% triton X-100. After withdrawal of a total sample (*T*), lysates were separated into pellets (*P*) and supernatants (*S*) by centrifugation at 100 000g (lanes 1-3), 20 000g (lanes 4-6) or 12 000g (lanes 7-9), respectively. Totals and supernatant fractions were analyzed by SDS-PAGE and Western Blot. Digital autoradiography was followed by control immunodecorations against the endogenous proteins Tom40, Tim23 and aconitase 2 (*Aco2*). (B) Mitochondria isolated from Hela cells transiently expressing wild-type Pink1 were treated as described above and analyzed by SDS-PAGE and Western Blot, membranes were probed with an antibody against Pink1. Additional control immunodecorations against the endogenous proteins Tom40, Tim23 and aconitase 2 (*Aco2*) were performed.

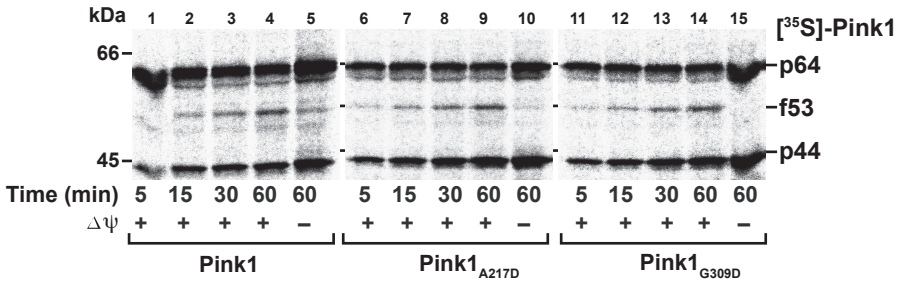
FIGURE S5. **Trypsin-resistance of full-length WT-Pink1 and Pink1$_{f53}$ in comparison to the full-length and main cleavage products of the C-terminal deletion constructs after detergent lysis.** After import of the radiolabeled WT-Pink1, Pink1$_{\Delta 516\text{-}581}$ and Pink1$_{W437X}$, mitochondria were solubilized in 0.3% TX-100 following the import reaction and incubated with the indicated amounts of trypsin as described in "EXPERIMENTAL PROCEDURES". Indicated are the respective full-length proteins (p64 or p) and the main processing fragments (f53 or PF). Control samples were analyzed by Western blot using antisera against the outer membrane receptor Tom70, the inner membrane translocase component Tim23 and the matrix protein Trap1.

FIGURE S6. **Effect of different Pink1 forms on cellular Parkin translocation to mitochondria.** Hela cells were transiently transfected with GFP-Parkin alone (A; *mock*), and together with WT-Pink1 (B) or the deletion mutants Pink1$_{\Delta 1\text{-}83}$ (C), Pink1$_{\Delta 1\text{-}111}$ (D) Pink1$_{\Delta 516\text{-}581}$ (E). Transfected cells were either treated with vehicle (EtOH) or with 1 μM valinomycin for 1-3 h. Mitochondrial colocalization of GFP-Parkin was assayed by staining of transfected cells with anti-Tom20. Images were acquired at the Leica PS5 confocal microscope. Enlarged insets show representative cells indicating mitochondrial (m), partial mitochondrial (p) and cytosolic (c) localizations of GFP-Parkin.

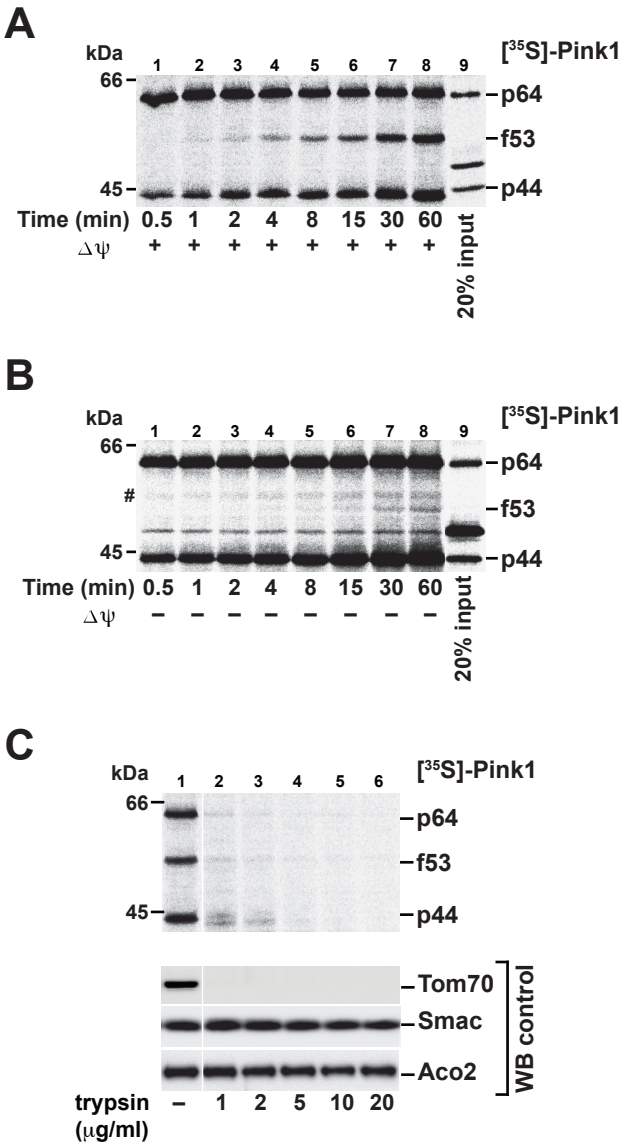
FIGURE S7. **Analysis of the N-termini of human mitochondrial Hsp60 (GroEL), Pink1 and aconitase 2 with the HeliQuest software (1).** Indicated are the net charges of the depicted N-terminal sequences as well as their helical hydrophobic moments (μH), a measure for the amphiphilicity of a helix (2).

SUPPLEMENTAL REFERENCES

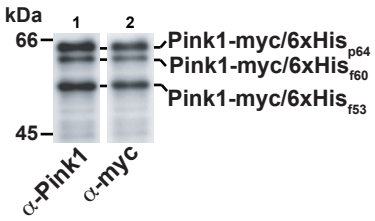
1. Gautier, C. A., Kitada, T., and Shen, J. (2008) *Proc. Natl. Acad. Sci. USA* **105**, 11364-11369
2. Eisenberg, D., Weiss, R. M., and Terwilliger, T. C. (1982) *Nature* **299**, 371-374



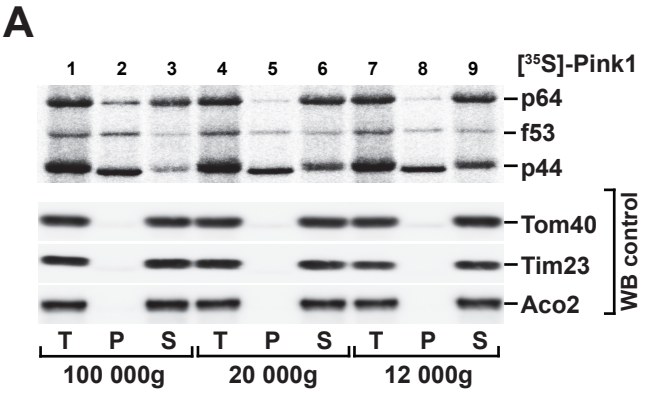
Becker et al., Figure S1

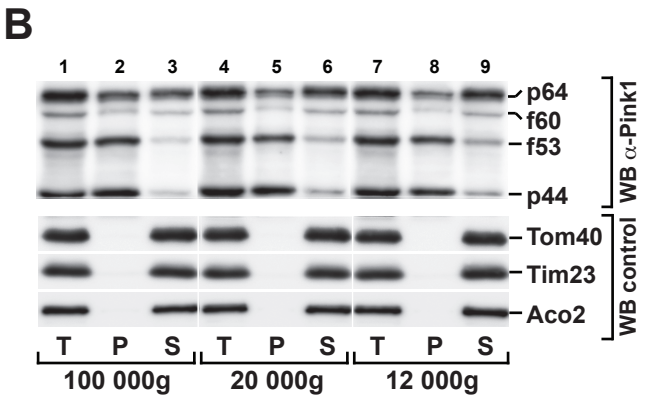


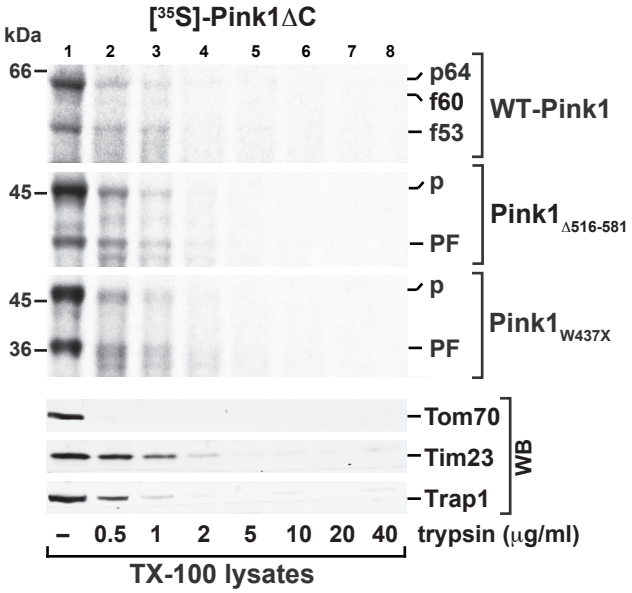
Becker et al. Figure S2



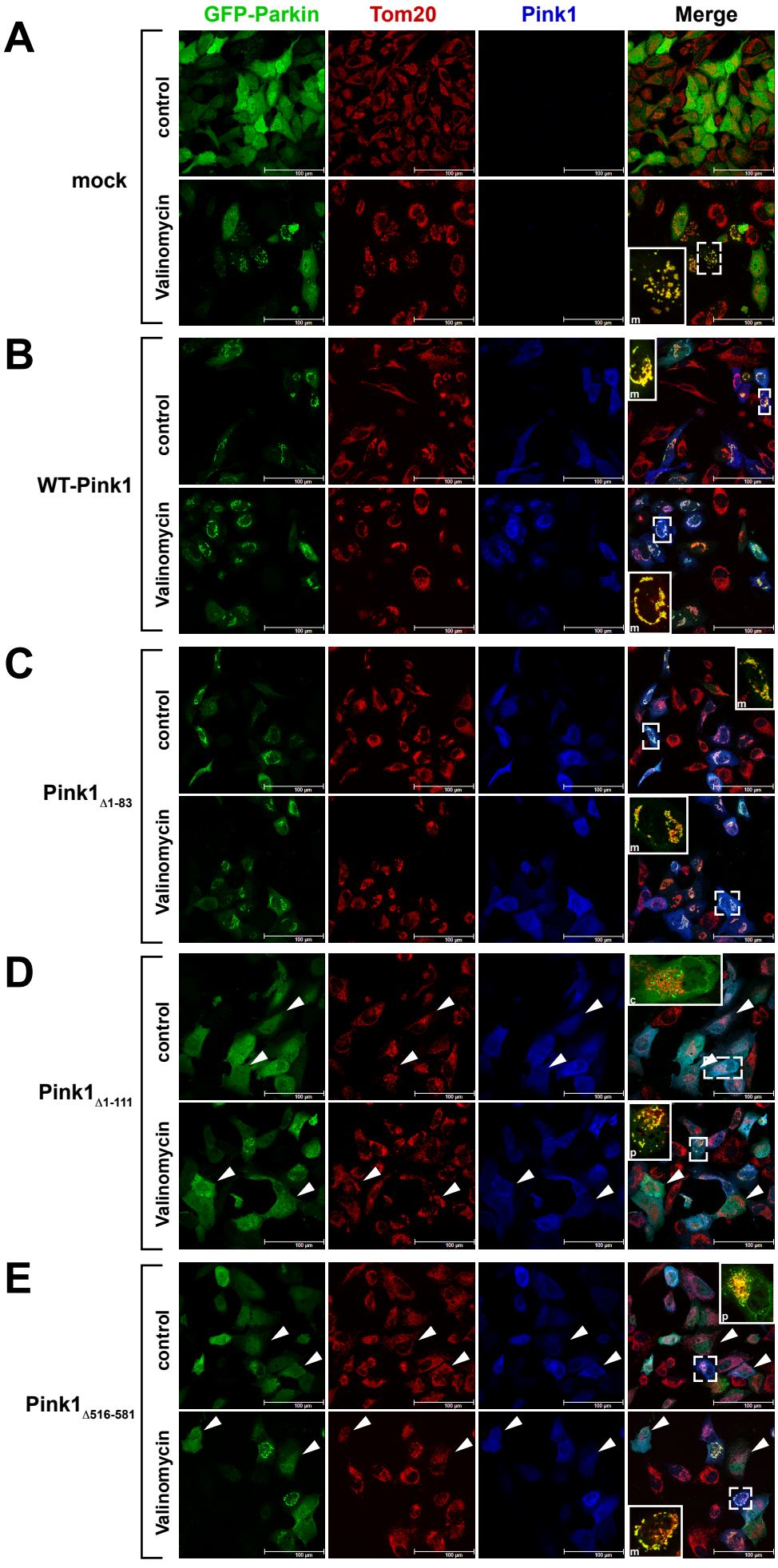
Becker et al., Figure S3


A
1 2 3 4 5 6 7 8 9
[35S]-Pink1
p64
f53
p44
Tom40
Tim23
Aco2
WB control
T P S T P S T P S
100 000g
20 000g
12 000g


B
1 2 3 4 5 6 7 8 9
p64
f60
f53
p44
WB α-Pink1
Tom40
Tim23
Aco2
WB control
T P S T P S T P S
100 000g
20 000g
12 000g

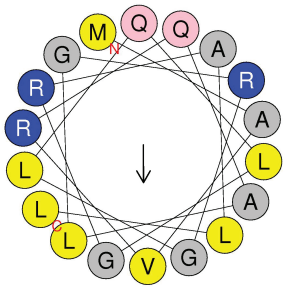


Becker et al., Figure S5



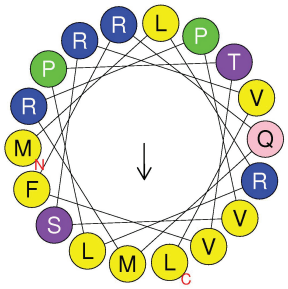
Becker et al., Figure S6

A



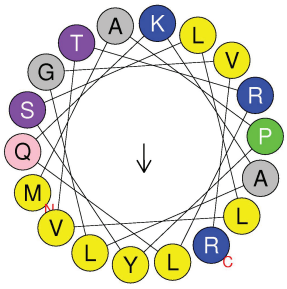
Hsp60 (GroEL), res. 1-18
net charge: +4
μH: 0.295

B



Pink1, res. 1-18
net charge: +3
μH: 0.310

C



Aconitase 2, res. 1-18
net charge: +3
μH: 0.232