SUPPLEMENTARY ONLINE MATERIAL

SENP3-mediated deSUMOylation of Drp1 facilitates interaction with Mff to promote cell death

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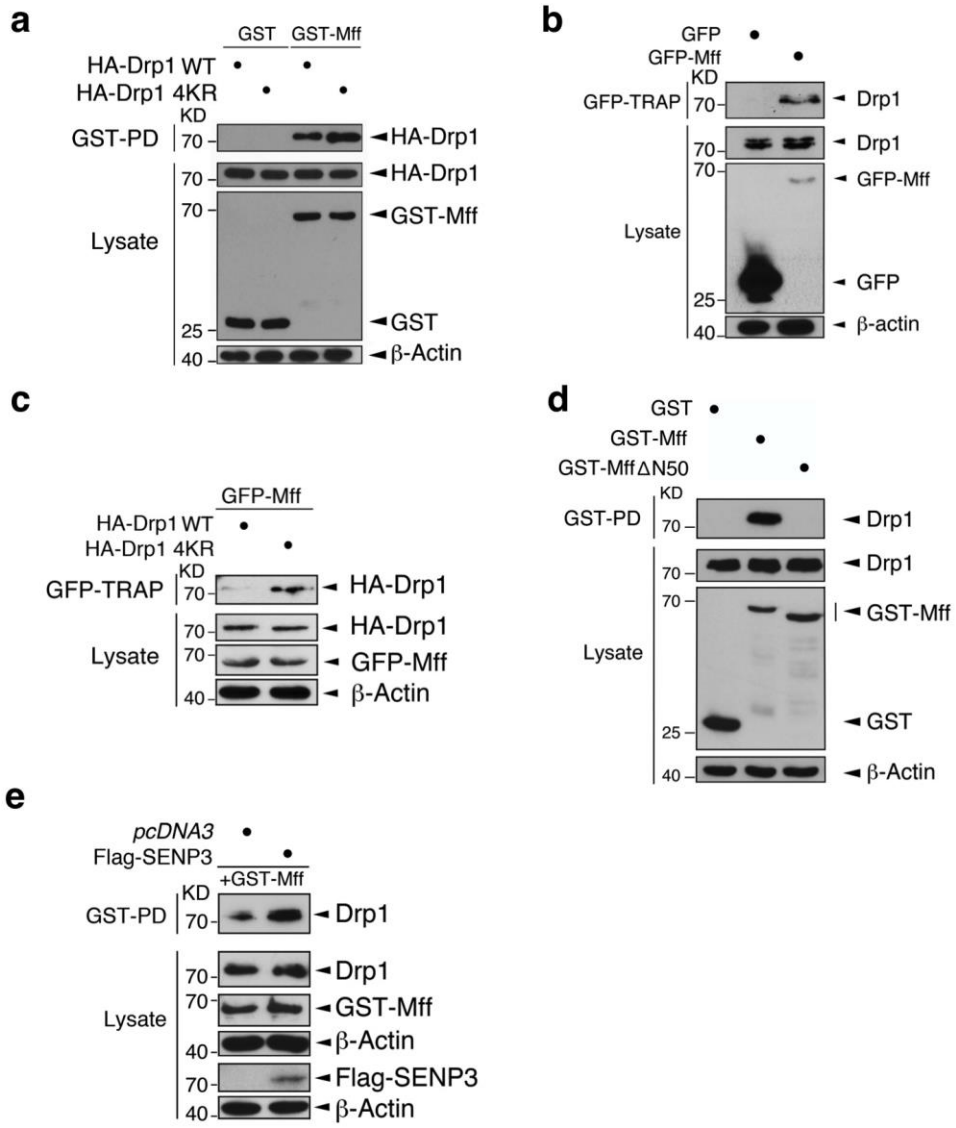
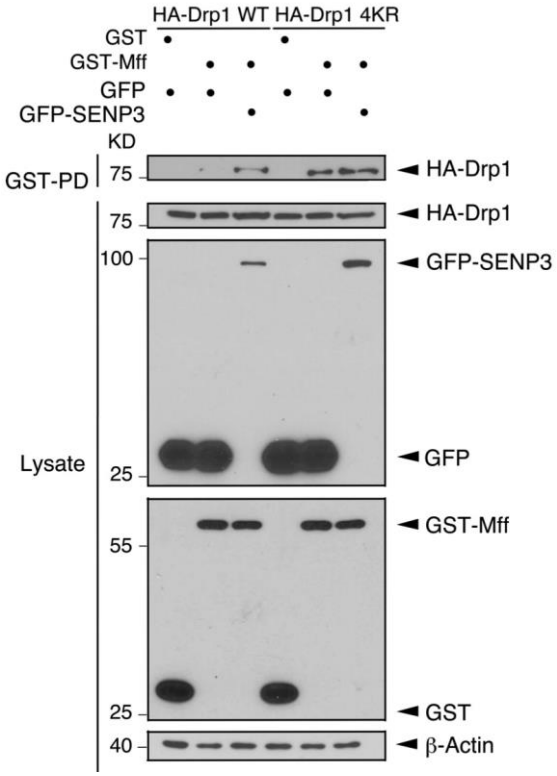
This supplement contains:

7 Supplementary Figures



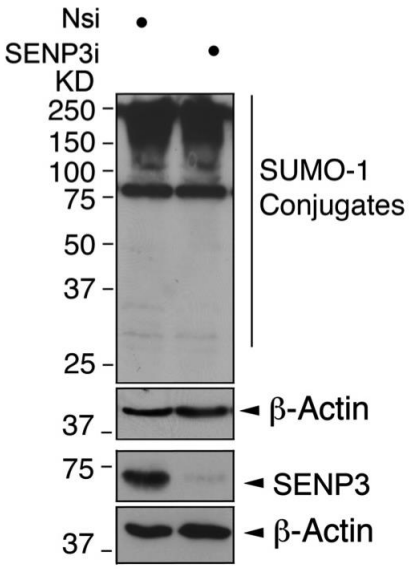
Supplementary Figure 1 DeSUMOylation of Drp1 promotes its binding to Mff.

(**a**) Non-SUMOylatable HA-Drp1 shows enhanced association with GST-Mff in HEK293 cells. Full blots of data shown in **Fig. 1a**. (**b**) Non-SUMOylatable HA-Drp1 shows enhanced association with GFP-Mff in HEK293 cells. GFP-Mff was pulled down using GFP-TRAP and blotted as shown. (**c**) Overexpressed GFP-Mff interacts with endogenous Drp1. GFP-TRAP pulldowns were blotted as shown. (**d**) GST-Mff ΔN50 does not bind Drp1 in HEK293 cells. GST-pulldowns were blotted as shown. (**e**) Overexpression of SENP3 increases the Mff-Drp1 interaction. Flag-SENP3 was transfected into HEK293 cells expressing GST-Mff. GST-pulldown and lysate samples were immunoblotted as shown.



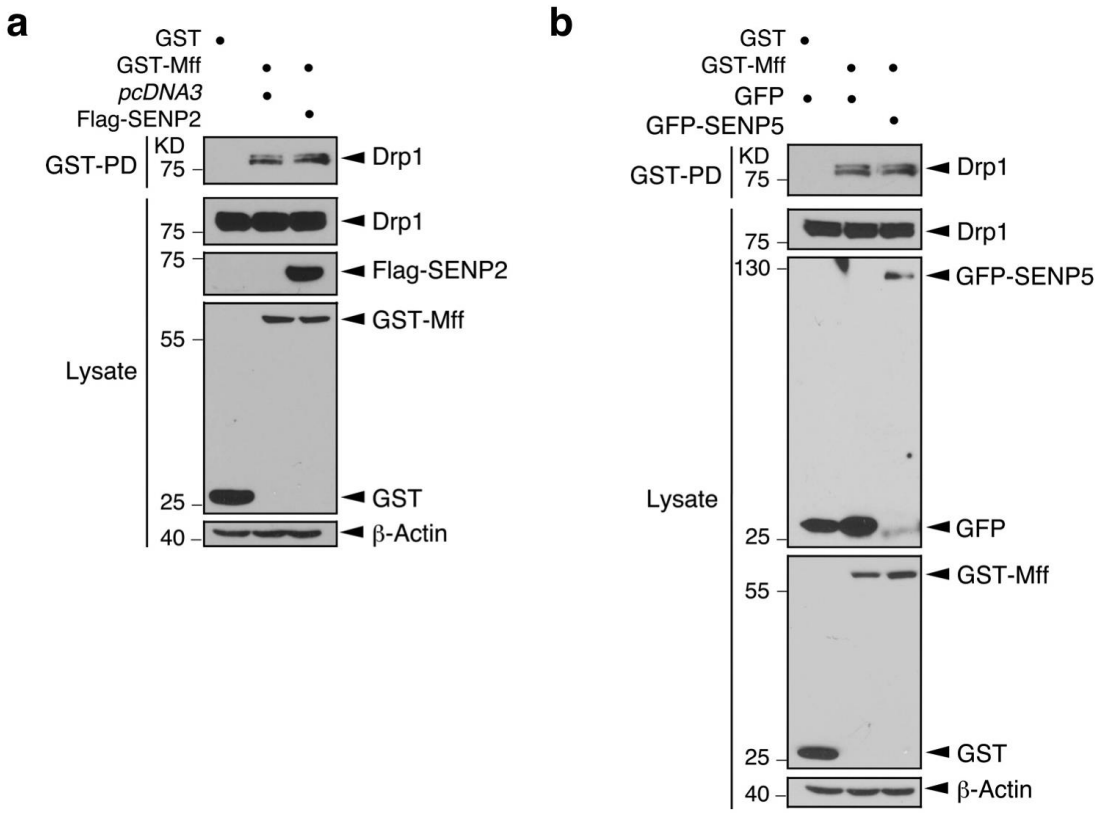
Supplementary Figure 2 SENP3 promotes binding of Drp1 but not its non-SUMOylatable mutant to Mff.

GFP-SENP3 was transfected into HEK293 cells expressing GST-Mff and HA-Drp1 WT/HA-Drp1 4KR. GST-pulldown and lysate samples were immunoblotted as shown.



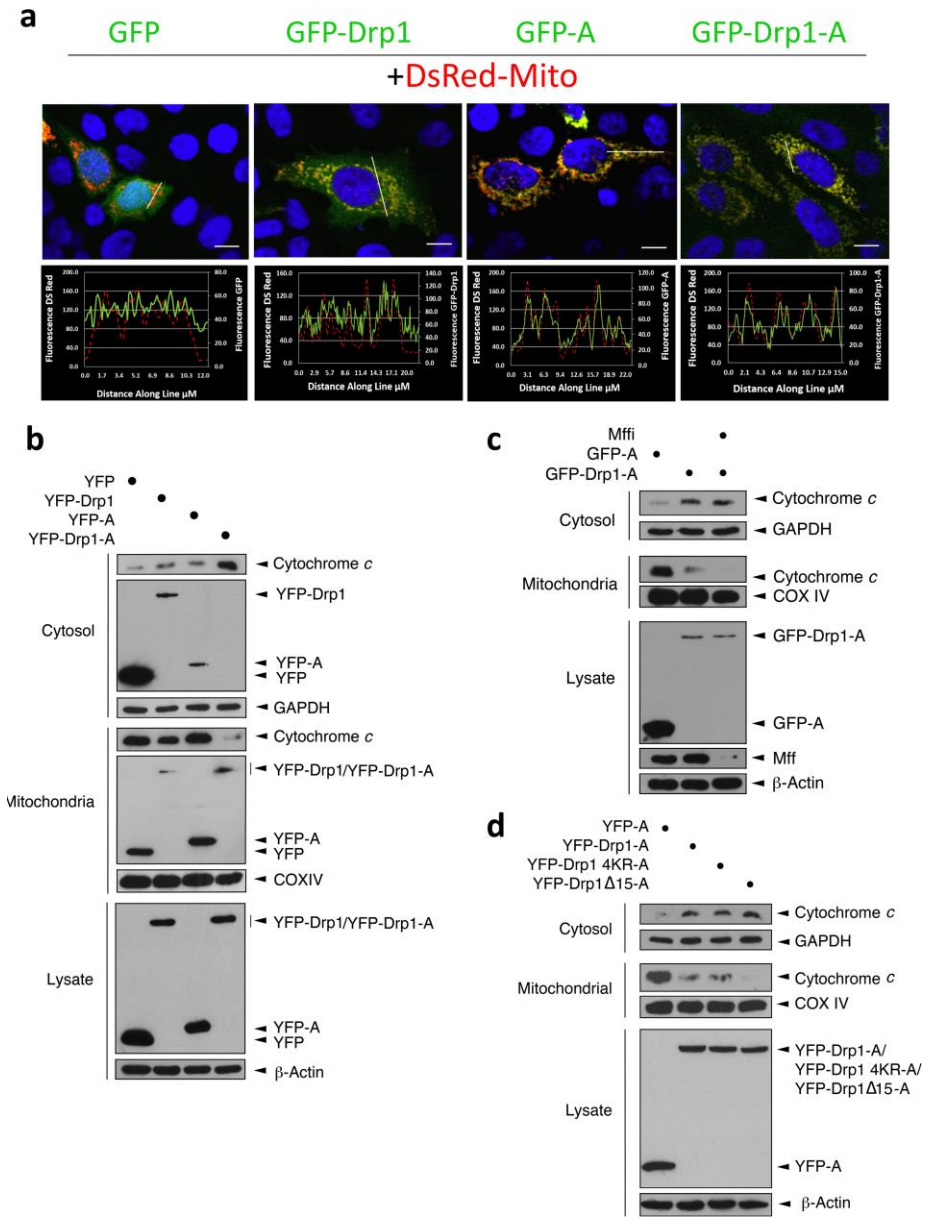
Supplementary Figure 3 SENP3 knockdown does not change global SUMO-1-ylation.

Non-specific or SENP3 knockdown constructs (Nsi or SENP3i) were transfected into HEK293 cells. Lysate samples were immunoblotted as shown.



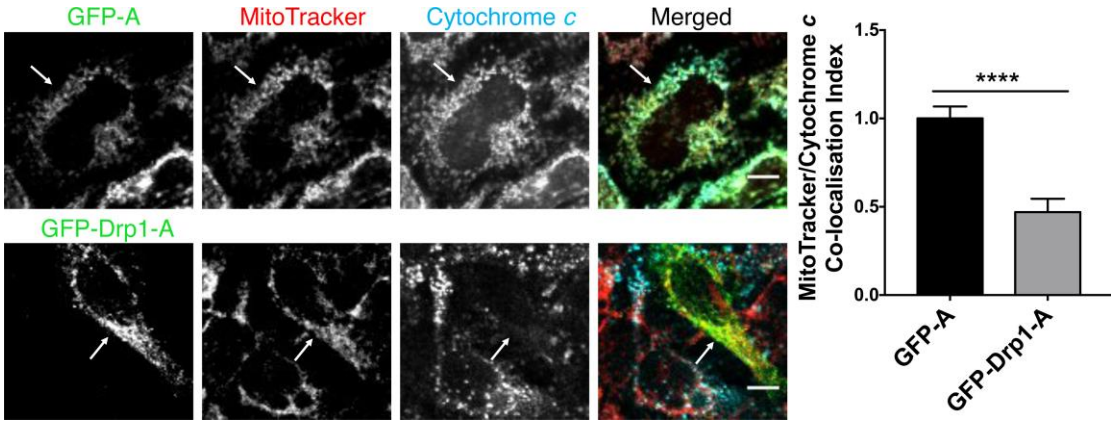
Supplementary Figure 4 Neither SENP2 nor SENP5 promotes Drp1 binding to Mff.

Flag-SENP2 or GFP-SENP5 were transfected into HEK293 cells expressing GST-Mff. GST-pulldown and lysate samples were immunoblotted as shown.



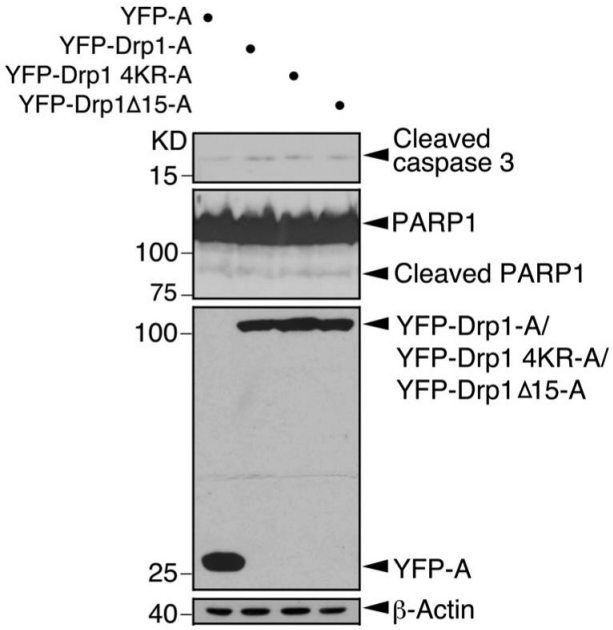
Supplementary Figure 5 Expressing Drp1-A induces Cytochrome *c* release in HEK293 cells.

(**a**) Co-localization analysis of GFP, GFP-Drp1, GFP-A, or GFP-Drp1-A with DsRed-Mito (further details for **Fig. 5a**). (**b**) Subcellular distribution of YFP, YFP-Drp1, YFP-A, or YFP-Drp1-A in HEK293 cells (complete version of **Fig. 5b**). (**c**) GFP-Drp1-A-induced cytochrome *c* release bypasses Mff. (**d**) Tethering Drp1 at mitochondria induces cytochrome *c* release independent of the SUMOylation status of Drp1 (complete version of **Fig. 5d**).



Supplementary Figure 6 Expression of GFP-Drp1-A decreases mitochondrial cytochrome *c* in HeLa cells.

GFP-A or GFP-Drp1-A was transfected into HeLa cells. Following fixation, cells were labelled with MitoTracker and immunostained to assess the mitochondrial localization of cytochrome ***c***. White arrows (Left panel) indicate GFP- A or GFP- Drp1-A expressing cells (Green: GFP-A or GFP-Drp1-A; Red: MitoTracker; Soft Cyan: cytochrome ***c***; scale bar: 10 µm). The histogram (Right panel) shows lower levels of the mitochondrial cytochrome *c* in cells expressing GFP-Drp1-A than in cells expressing GFP-A (n=40 cells for GFP- A and n=40 for GFP- Drp1-A from 3 independent experiments using different cell populations; **** p=1.2632E-06; Unpaired Student's test).



Supplementary Figure 7 Drp1-A expression does not cause cleavage of either caspase 3 or PARP1.

YFP-A, YFP-Drp1-A, YFP-Drp1 4KR-A or YFP-Drp1 Δ15-A were transfected into HEK293 cells. Lysate samples were immunoblotted as shown.