



Supplementary information, Fig S6 Mitochondrial cGAS associates with DRP1 and facilitates its oligomerization

a, Co-IP assay of the protein interaction between cGAS and DRP1. HEK293T cells were co-transfected with indicated plasmids. Cell lysates were immunoprecipitated with an anti-Flag antibody, followed by western blot analysis with antibodies against Flag and HA tags. **b**, Co-IP assay of the protein interaction between cGAS and OPA1 or ACSL4. HEK293T cells were co-transfected with Flag-EV or cGAS-Flag and HA-OPA1 or HA-ACSL4 plasmids. Cell lysates were immunoprecipitated with an anti-Flag antibody, followed by western blotting analysis with antibodies against Flag, OPA1 or ACSL4. **c**, Co-IP assay of the protein interaction between wild type cGAS or cGAS with E225A/D227A mutation and DRP1 in Hep3B cells. Hep3B cells were transfected with wild type cGAS-Flag or cGAS^{E225A/D227A}-Flag plasmid and HA-DRP1 plasmid. The cell lysates were immunoprecipitated with an anti-Flag antibody, followed by western blotting analysis with antibodies against Flag and HA tag. **d**, Pull-down assay of the protein interaction between GST-cGAS-WT (WT, wild type) or GST-cGAS^{E225A/D227A} and DRP1-His. GST and 6×His coding sequences were cloned onto the N-terminus of wild type cGAS or cGAS with E225A/D227A mutation and the C-terminus of DRP1, respectively. Recombinant GST-fused cGAS proteins or cGAS^{E225A/D227A} mutant proteins were incubated with purified 6×His-fused DRP1 protein, followed by Western blotting analysis with antibody against DRP1. **e**, PAGE distinguished oligomerization of DRP1 protein in PLC cells with endogenous cGAS knockdown, including higher- and moderate- oligomerization and monomer of DRP1. **f**, PAGE distinguished oligomerization of DRP1 protein in PLC cells with endogenous cGAS knockdown and exogenous MTS-cGAS overexpression, including higher- and moderate- oligomerization and monomer of DRP1. **g**, Measurement of mitochondrial ROS levels in PLC cells with endogenous cGAS knockdown and exogenous MTS-cGAS overexpression treated with or without Mdivi-1. Representative histogram data and statistical results are shown. **h**, Measurement of mitochondrial ROS levels in Hep3B cells with endogenous DRP1 knockdown and exogenous wild type (WT), A395D mutant or K38A mutant DRP1 overexpression. Representative histogram data

and statistical results are shown. For **(g)** and **(h)**, the data are presented as the mean \pm SD of three independent experiments. p value was calculated by two-tailed unpaired Student's t -test. *, $p \leq 0.05$; ns, not significant.