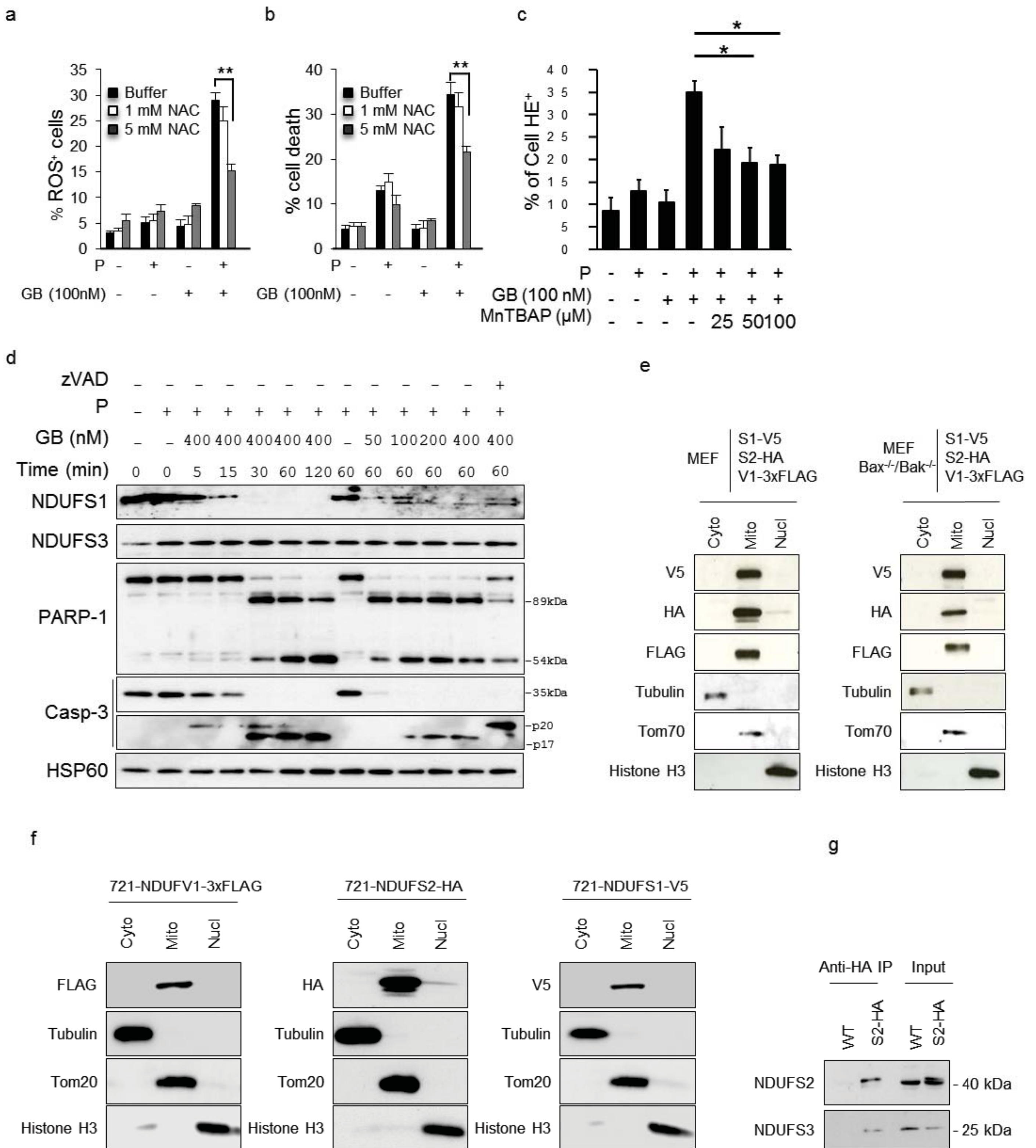
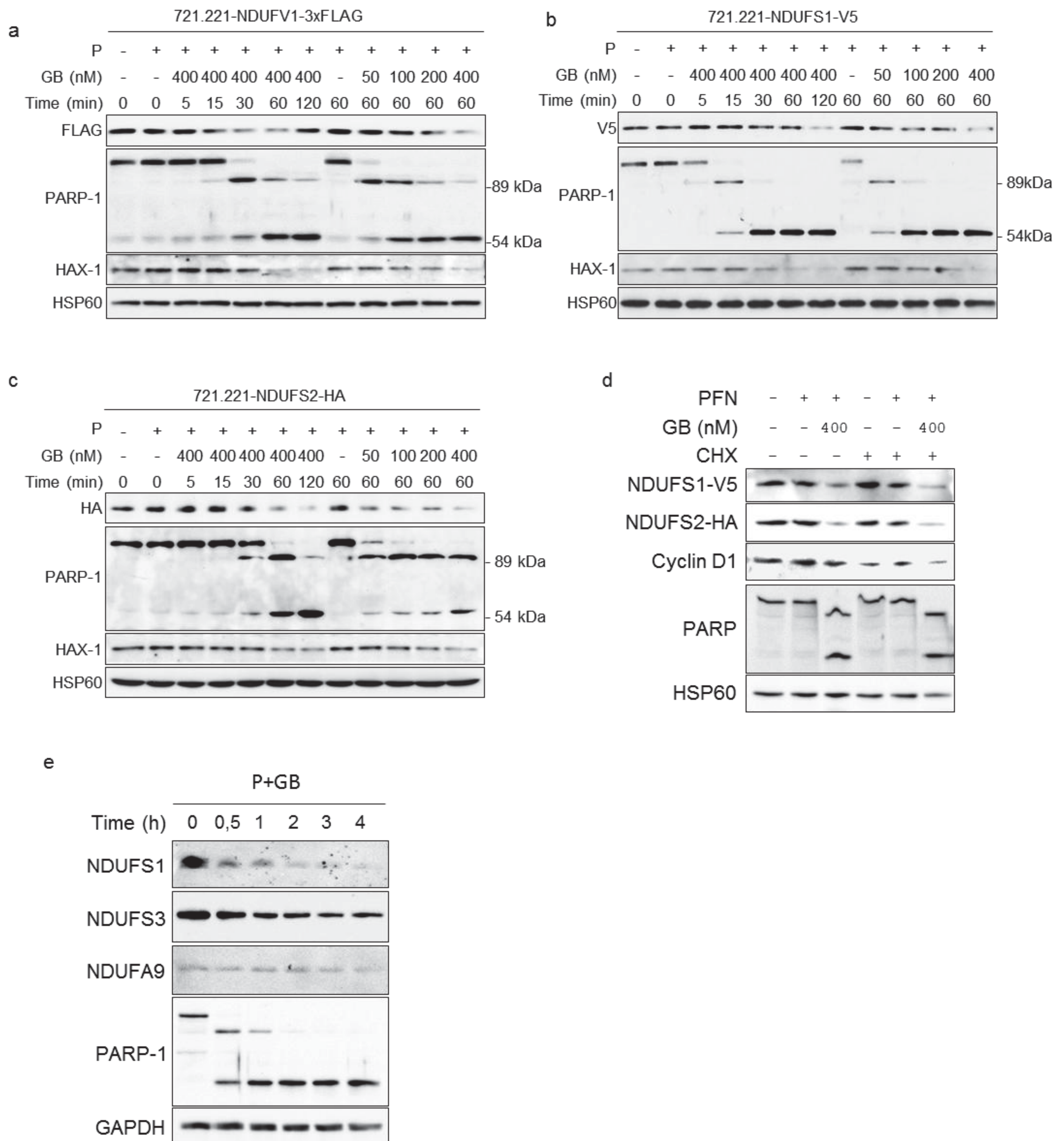


Supplemental Figure 1: GB-induced cell death is ROS-dependent. (a-b) K562 cells pre-incubated or not with NAC for 1h were treated with a sublytic dose of P +/- GB. ROS

production (a) and cell death (b) were assessed by mitoSOX and Annexin V-PI staining, respectively. (c) Same as in (a) ROS were detected with dihydroethidium (HE). Mean +/- SEM of at least 3 independent experiments is shown. $^{*}p<0.05$, $^{**}p<0.01$. (d) 721.221 cells were treated as in (a) with or without z-VAD and the status of NDUFS1, NDUFS3, PARP-1 and caspase 3 were followed by WB. Hsp60 is used as loading control. (e) WT and BAX-/-BAK-/- MEFs overexpressing tagged NDUFS1, NDUFS2 and NDUFV1 were fractionated into cytosolic (Cyto), mitochondrial (Mito) and nuclear (Nucl) fractions and localization of the overexpressed complex I subunits was assessed by immunoblot. Fractionation was verified by probing for tubulin, Tom20 and Histone H3. (f) 721.221 overexpressing tagged NDUFS1, NDUFS2 and NDUFV1 were analyzed as in (e). (g) 721.221 cells overexpressing HA-NDUFS2 were used for co-IP to show that HA-NDUFS2 associates with other complex I subunits. The results of the immunoblots are representative of at least 3 independent experiments.

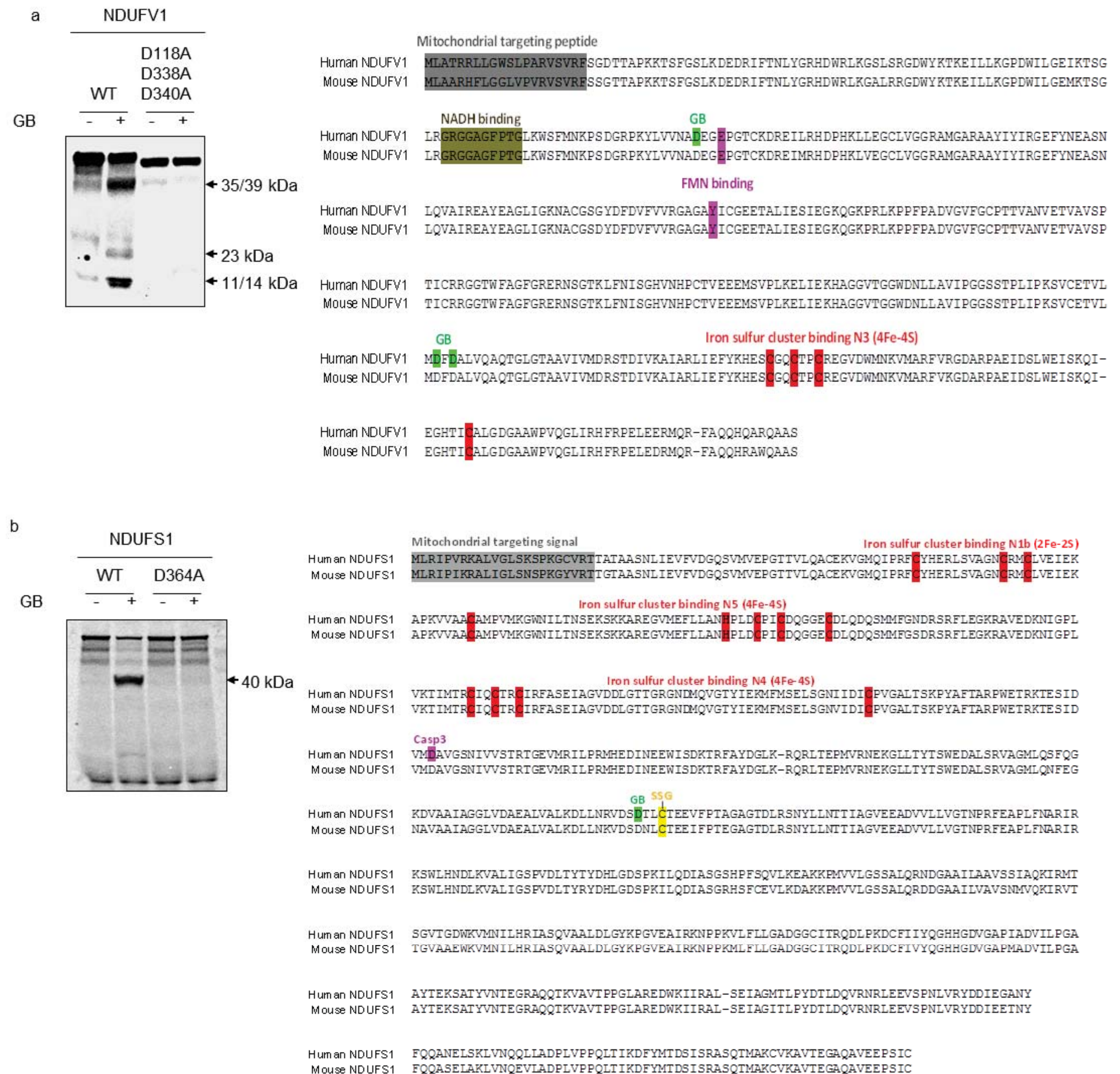
Jacquemin et al; Supplementary Fig. 2



Supplemental Figure 2: GB cleaves complex I subunits in intact cells. (a-c) 721.221 cells overexpressing NDUFV1-3xFLAG (a), NDUFS1-V5 (b) or NDUFS2-HA (c) were treated with GB and P and their cleavage relative to the GB substrates PARP-1 and

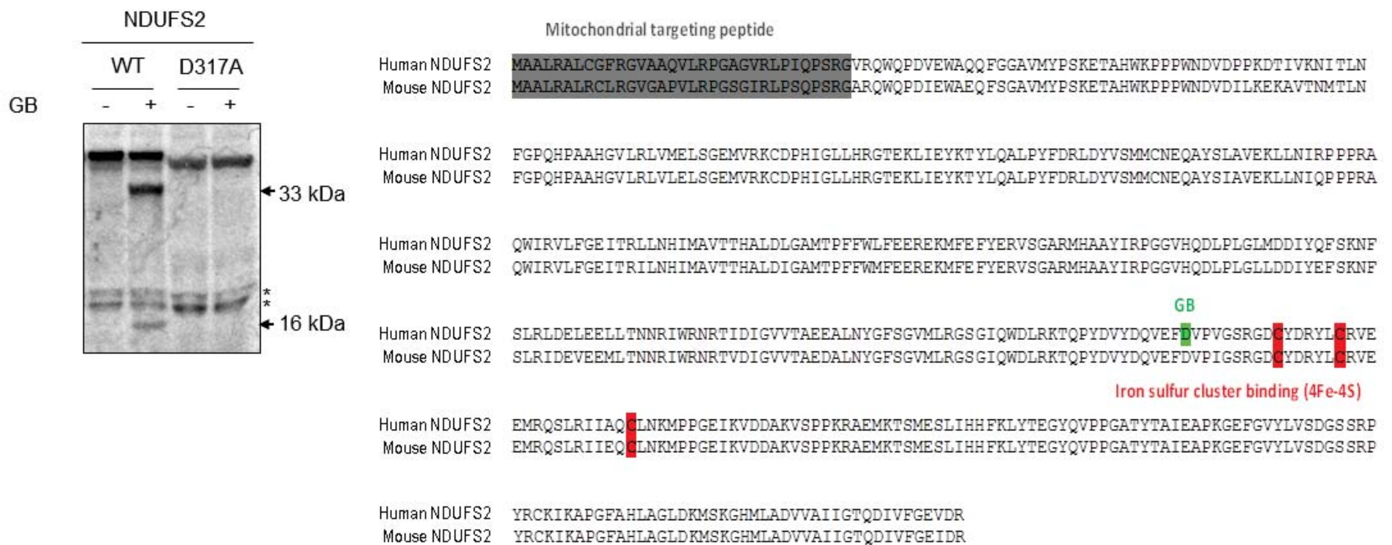
HAX-1 assessed by probing WB with anti-tag antibodies. HSP60 is a loading control. (d) 721.221 cells pretreated with 50ug/ml of cycloheximide for 30 minutes were treated with GB and P for 1 hr and the status of NDUFS1, NDUFS2, cyclin D1 and PARP-1 analyzed by WB. HSP60 is a loading control. (e) 721.221 cells were treated with GB and P as indicated and the status of NDUFS1, NDUFS3, NDUFA9 and PARP-1 analyzed by WB. GAPDH is used as loading control.

Jacquemin et al; Supplementary Fig. 3



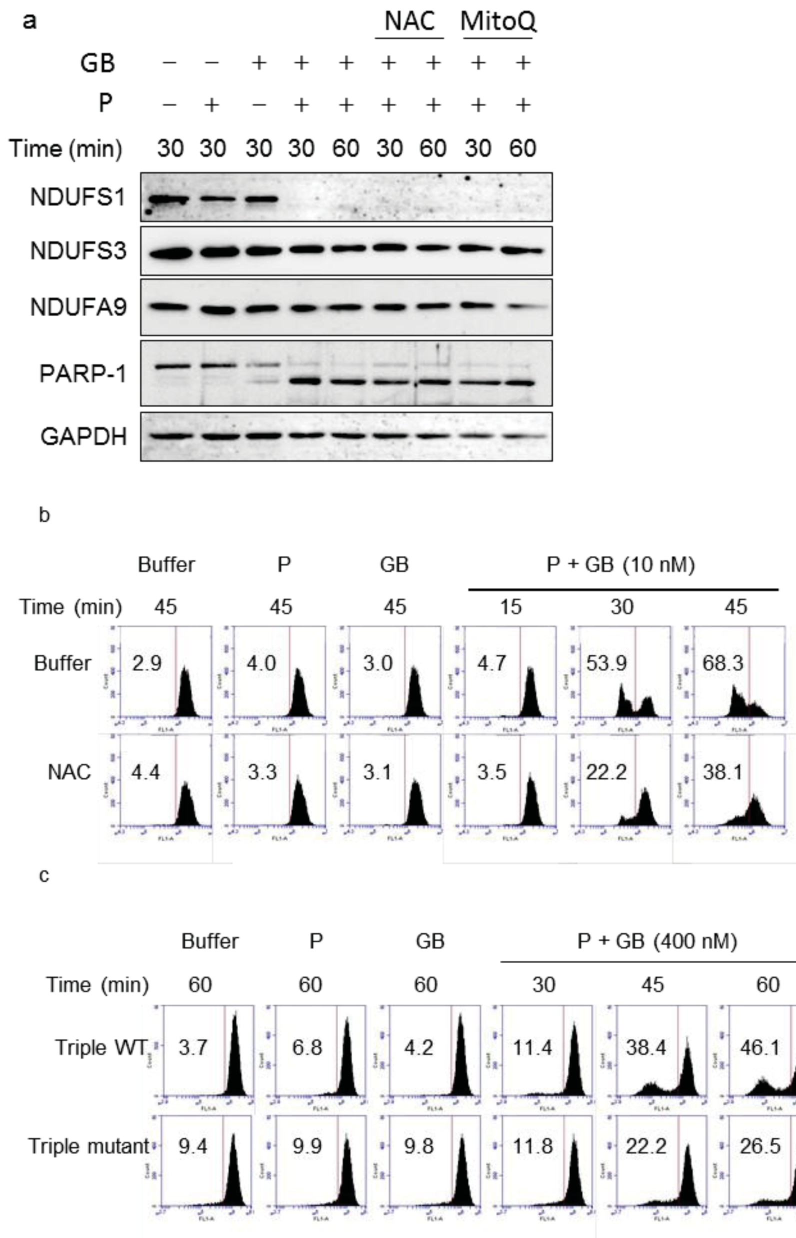
Supplemental Figure 3: Identification of GB cleavage sites of human NDUFV1 and NDUFS1. GB cleavage sites were provisionally identified based on the size of GB cleavage fragments and predicted potential cleavage sites. GB-uncleavable D-A mutants and WT complex I substrate proteins (a, NDUFV1; b, NDUFS1) were transcribed and translated *in vitro* in the presence of 35S-methionine and treated with GB before SDS-PAGE and radiography. The lack of cleavage of the mutated proteins confirms the indicated cleavage sites. Human and mouse protein sequences for each subunit are shown with the mitochondrial targeting sequence highlighted in grey, the NADH binding site in dark green, the FMN binding site in purple, the iron-sulfur cluster binding sites in red, the glutathionylation site in yellow, and casp3 and GB cleavage sites in pink and green, respectively.

Jacquemin et al; Supplementary Fig. 4



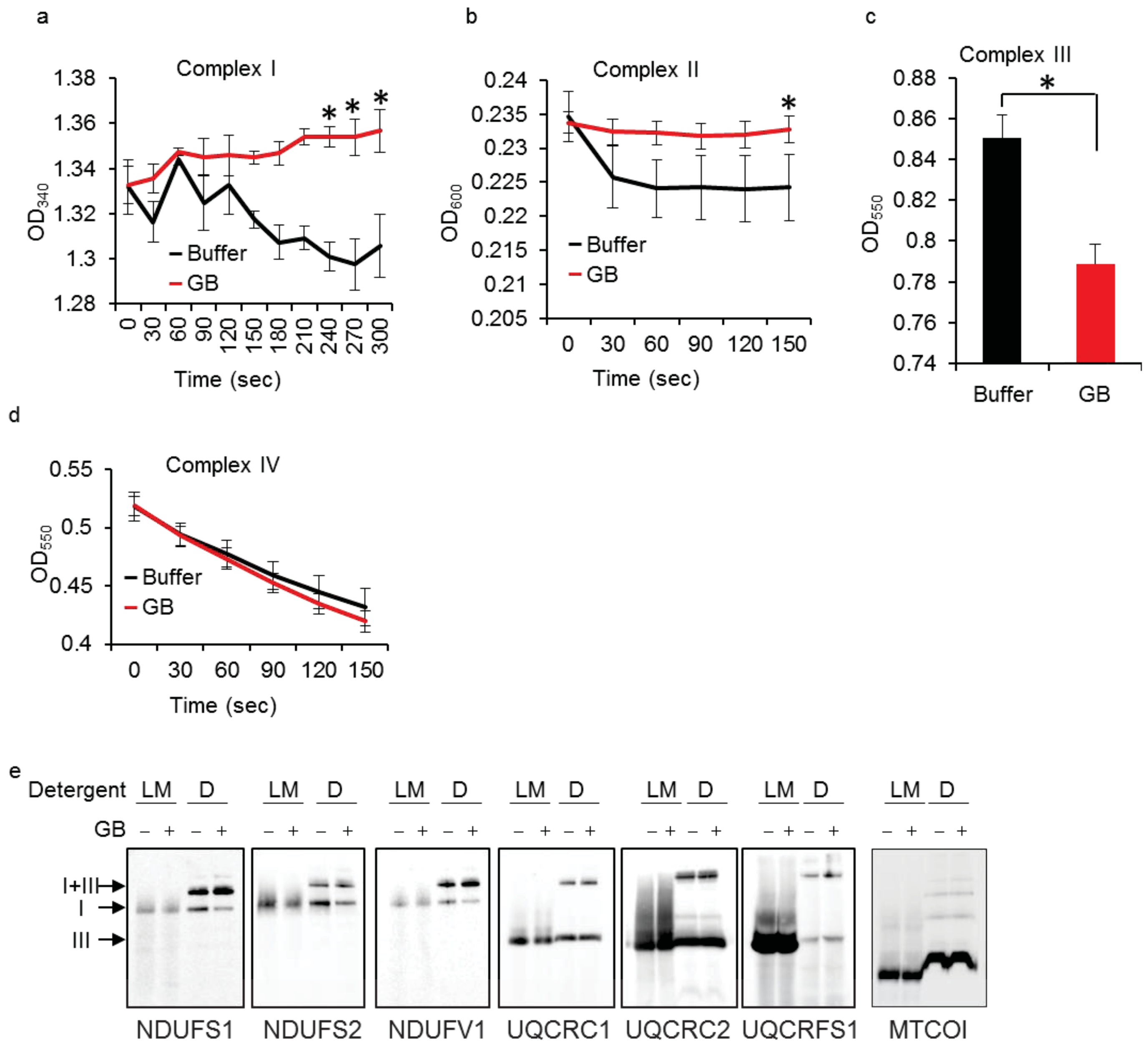
Supplemental Figure 4: Identification of GB cleavage sites of human NDUFS2
GB cleavage sites were provisionally identified based on the size of GB cleavage fragments and predicted potential cleavage sites. GB-uncleavable D-A mutants and WT NDUFS2 were transcribed and translated *in vitro* in the presence of 35S-methionine and treated with GB before SDS-PAGE and radiography. The lack of cleavage of the mutated protein confirms the indicated cleavage sites. Human and mouse protein sequences for each subunit are shown with the mitochondrial targeting sequence highlighted in grey, the iron-sulfur cluster binding sites in red and GB cleavage site in green. Asterisks on the blot indicate non-specific bands

Jacquemin et al; Supplementary Fig. 5



Supplemental Figure 5: GB-induced ROS triggers lysosomal membrane permeabilization. (a) 721.221 cells pretreated with either NAC or MitoQ were treated with GB and P as indicated and the status of NDUFS1, NDUFS3, NDUFA9 and PARP-1 analyzed by WB. GAPDH is used as loading control. (b) U937 cells treated as indicated, with or without 5 mM NAC pretreatment, were stained with Lysosensor, which only fluoresces in lysosomes that maintain acidic pH and analyzed by flow cytometry. (c) K562 cells overexpressing triple WT or triple mutant complex I subunits were treated and analyzed as in (b).

Jacquemin et al; Supplementary Fig. 6



Supplemental Figure 6: GB inactivates respiratory complex I, II and III. Mouse liver mitochondria were treated with 450 nM GB for 5 minutes and complex I, complex II, complex III and complex IV activities were followed spectrophotometrically according to (Spinazzi, M. et al 2012). (e) Isolated C57B6 mouse liver mitochondria treated with 450 nM GB for 5 min were solubilized with lauryl maltoside (LM) to resolve monomeric complexes or digitonin (D) to preserve SCs and analyzed by BNGE and immunoblot. Blots are representative of at least 4 independent experiments.