# Supplementary Figures and Tables

NatB-dependent acetylation protects procaspase-8 from UBR4-mediated degradation and is required for full induction of the extrinsic apoptosis pathway

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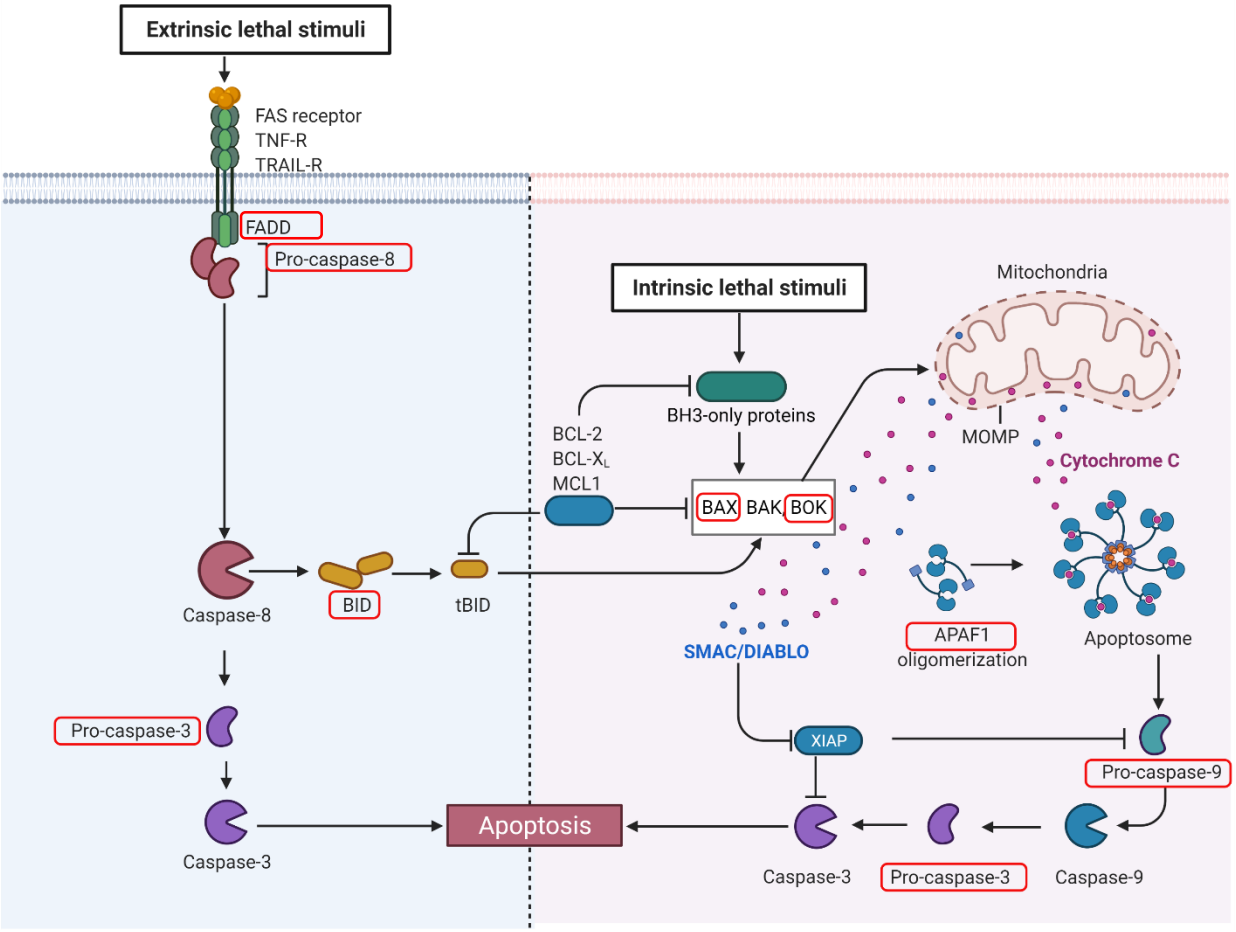


**Figure S1. Intrinsic and extrinsic apoptotic pathways.** Main components of intrinsic and extrinsic apoptotic pathways are indicated. Putative NatB substrates, as they display Asp-,Glut-, Asn- or Gln- at position two, are included in red squares. Binding of extrinsic lethal stimuli like TNF-α and Fas ligand to their receptors promotes caspase-8 activation that in type I cells can initiate apoptosis after caspase-3 proteolytic activation. In type II cells, apoptosis induction requires cleavage of BID into tBID by caspase-8 to initiate the mitochondrial apoptosis pathway. The intrinsic apoptosis pathway is initiated by several intrinsic lethal stimuli (DNA damage, ER stress, replication stress, etc.) that lead to irreversible mitochondrial outer membrane permeabilization (MOMP) controlled by pro-apoptotic (BAX, BAK, BOK) and anti-apoptotic (BCL-2, BCL-XL, MCL1) proteins with BCL2 homology (BH) domains. MOMP directly promotes the cytosolic release of cytochrome *c* (cyt *c*) and SMAC/DIABLO from the mitochondria. When in the cytosol, cytosolic cyt *c* binds to APAF1, promoting its oligomerization and assembly in a supramolecular complex known as apoptosome, which is responsible for activating procaspase-9. Activated caspase-9 can catalyzes the proteolytic activation of procaspase-3 and precipitates apoptosis (Biorender).

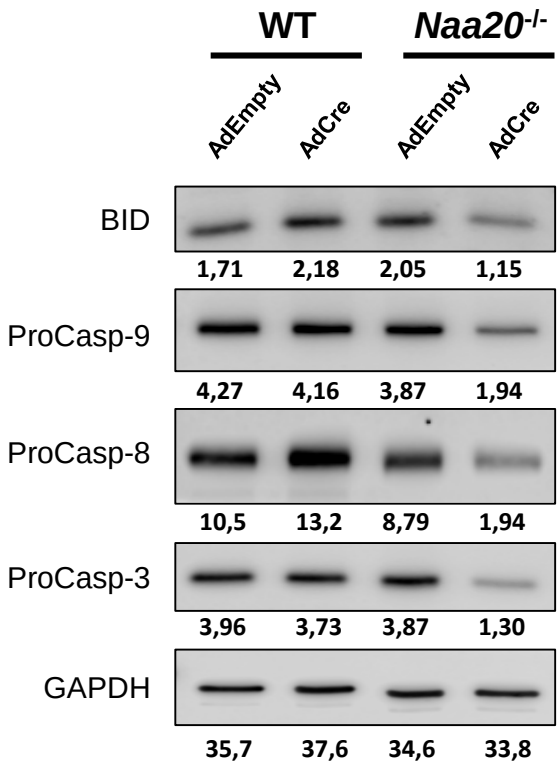


**Figure S2. Effect of Cre recombinase expression on apoptosis components in wild-type and *Naa20*$^{-/-}$ MEFs.** Representative western blot images of BID and the procaspases -8, -9 and -3 protein levels in wild type and *Naa20*$^{-/-}$ MEF cells 6 days after infection with AdEmpty or Ad5CMVCre. Each band intensity value (a.u) is indicated under each band.

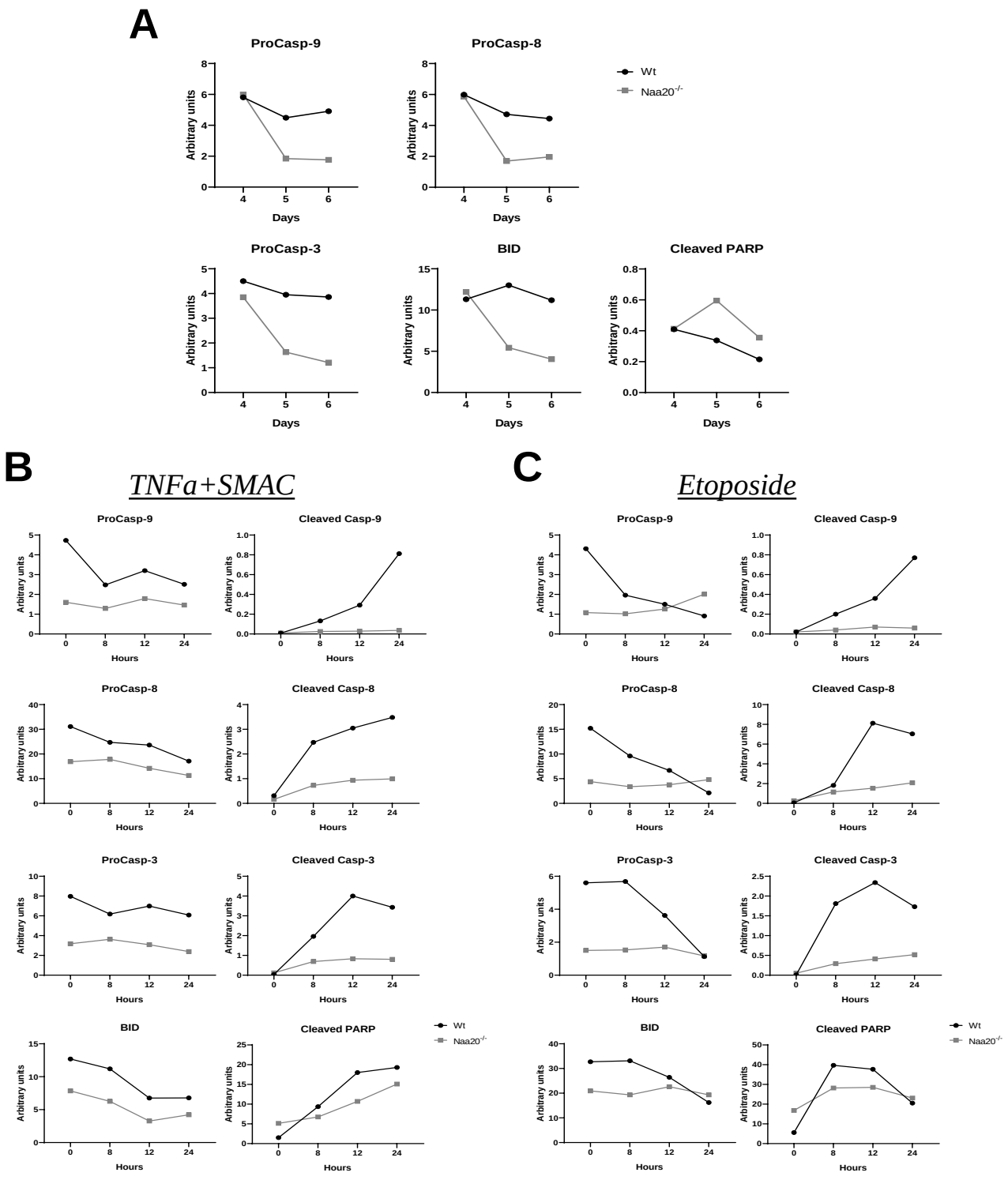


**Figure S3. Quantification of representative western blot signals from Fig. 3.** Panels (A), (B) and (C) correspond to western blot signals from Figs. 3A, 3C and 3D, respectively. Image Studio software was used for the signal quantification.

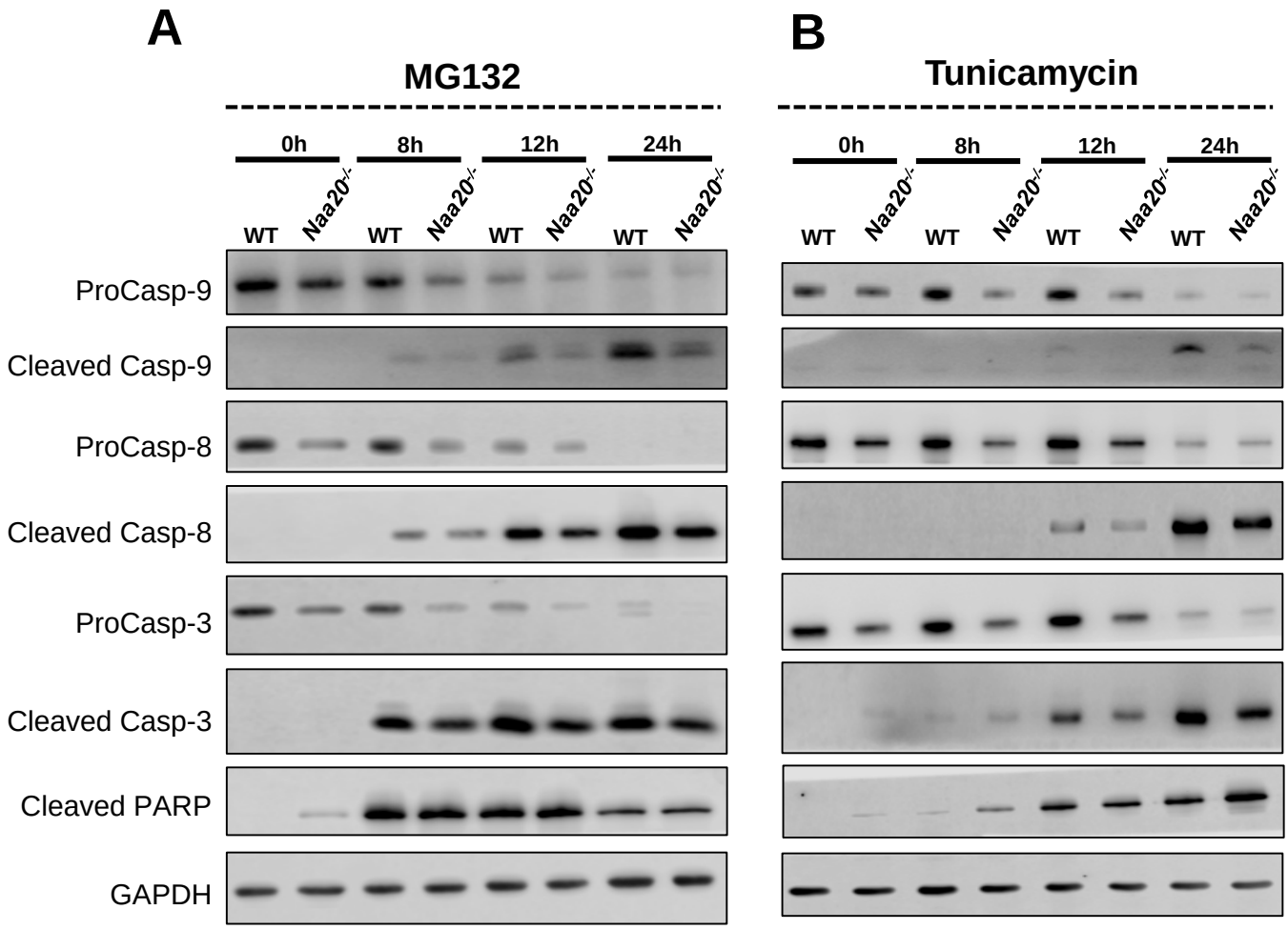


**Figure S4. Inactivation of NAA20 subunit causes a reduction in the expression levels of procaspases but this reduction was attenuated along treatment with MG132 and tunicamycin.** Representative western blot images of the main apoptotic effectors at 0, 8, 12 and 24 h after treatment with (A) 5 µM of MG132 and (B) 5 ng/µl of tunicamycin of control and *Naa20*$^{-/-}$ MEF cells, 6 days after infection with AdEmpty (WT) or Ad5CMVCre (*Naa20*$^{-/-}$).

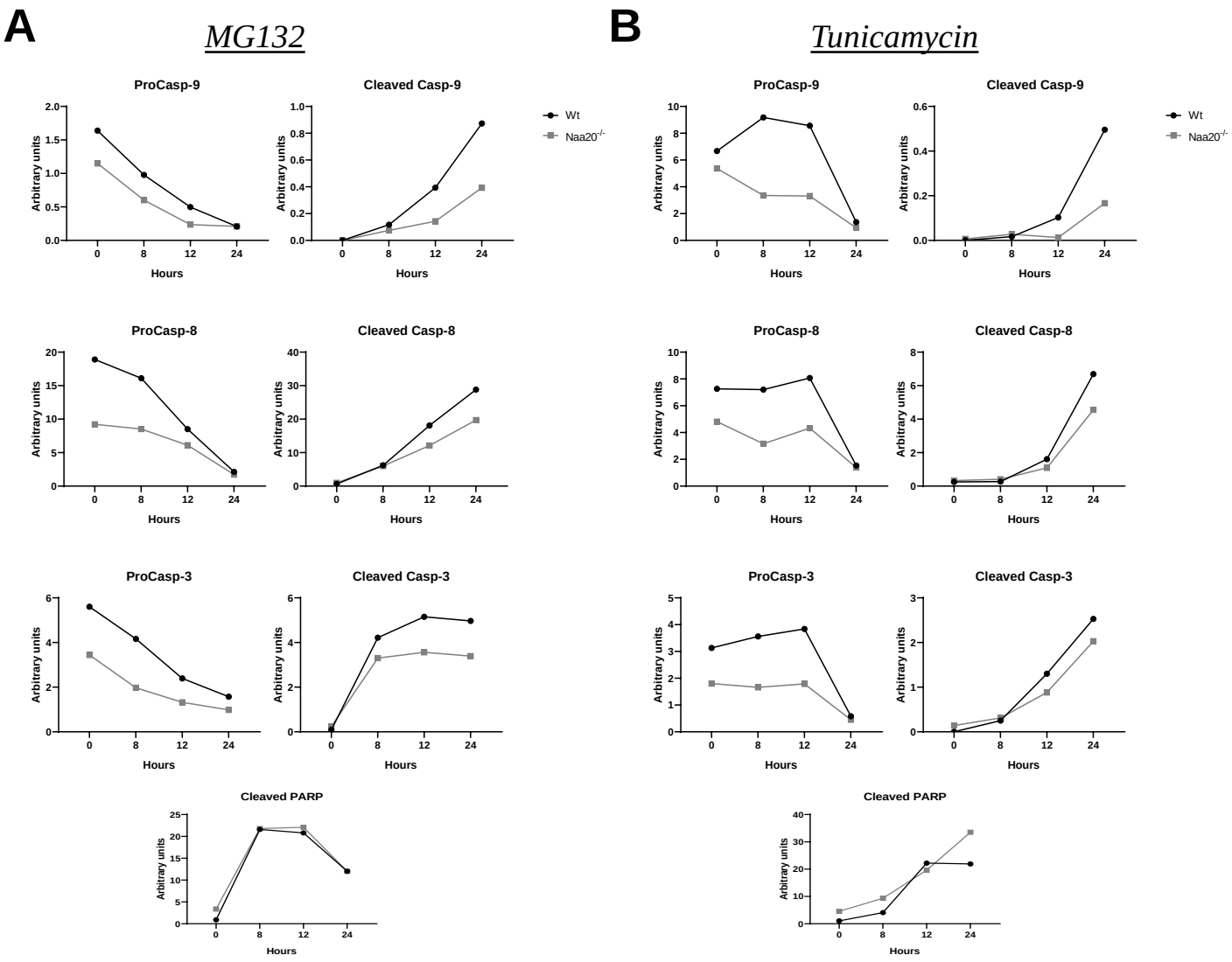


**Figure S5. Quantification of representative western blot signals from Fig. S3.** Panels (A) and (B) correspond to Supplementary Fig. 4A and 4B, respectively. Image Studio software was used for the signal quantification.

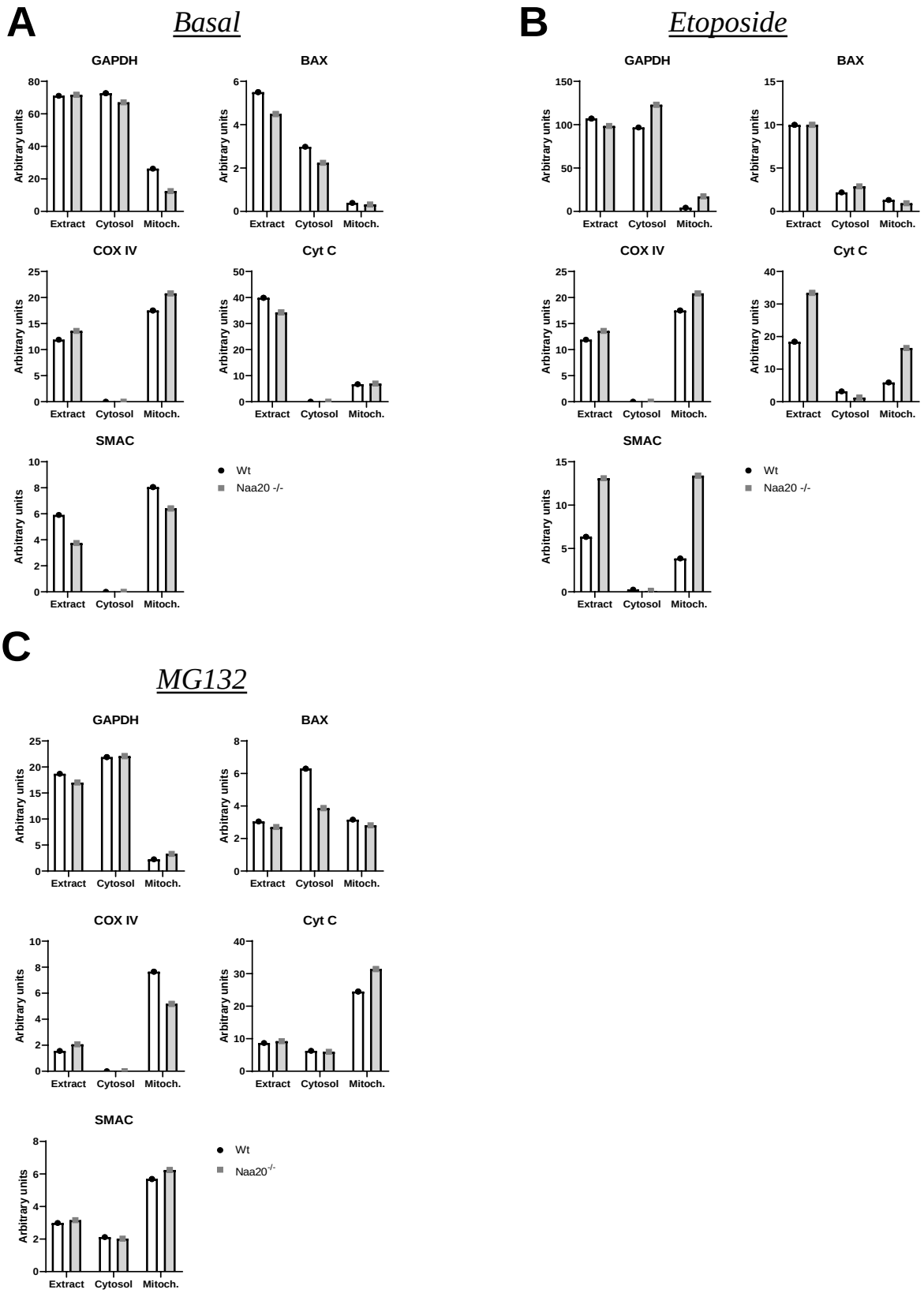


**Figure S6. Quantification of representative western blot signals from Fig. 4.** Panels (A), (B), and (C) correspond to Figs. 4A, 4B, and 4C, respectively. Image Studio software was used for the signal quantification.

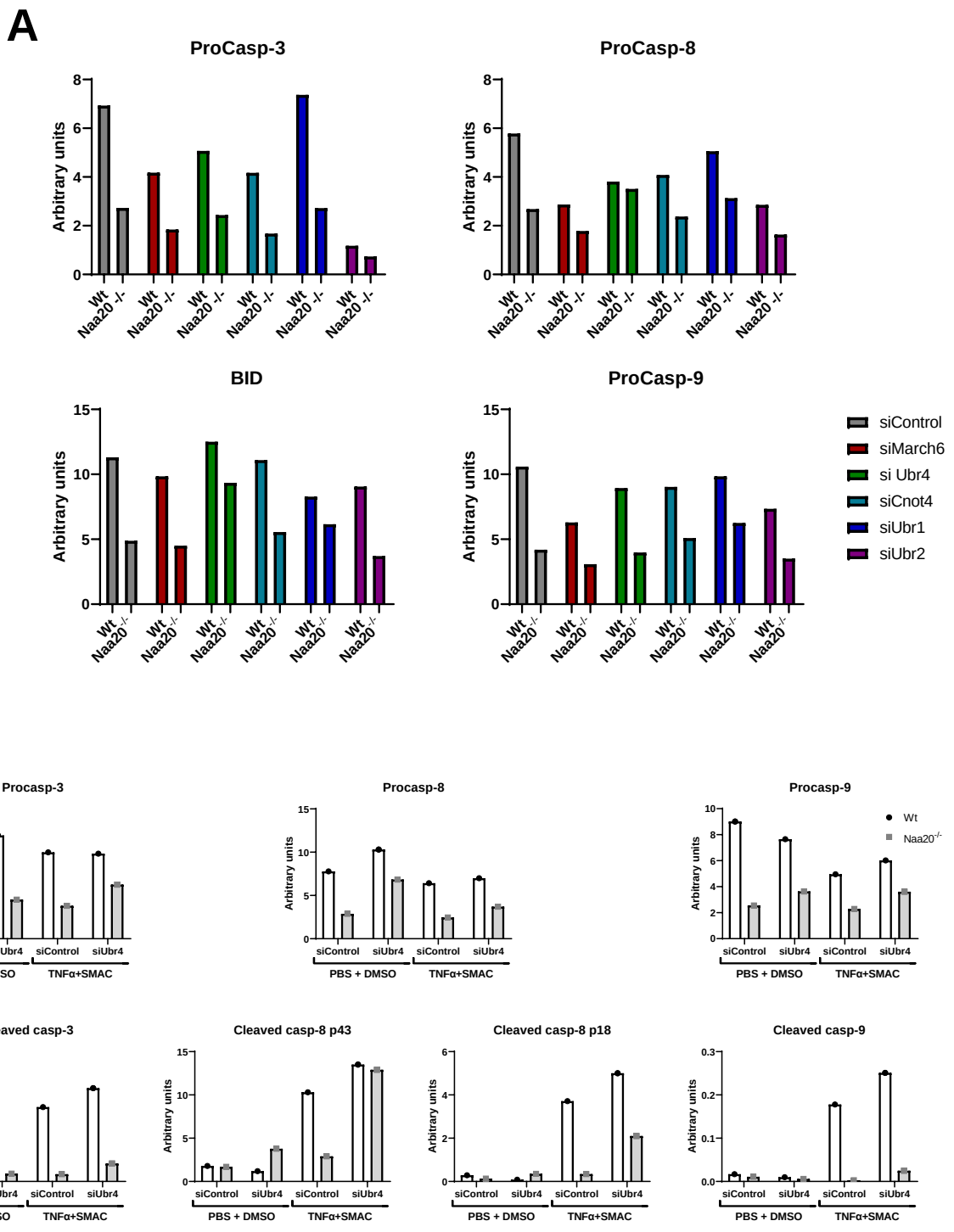


**Figure S7. Quantification of representative western blot signals from Fig. 5. P**anels (A) and (B ) correspond to Fig 5A and B, respectively. Image Studio software was used for the signal quantification.

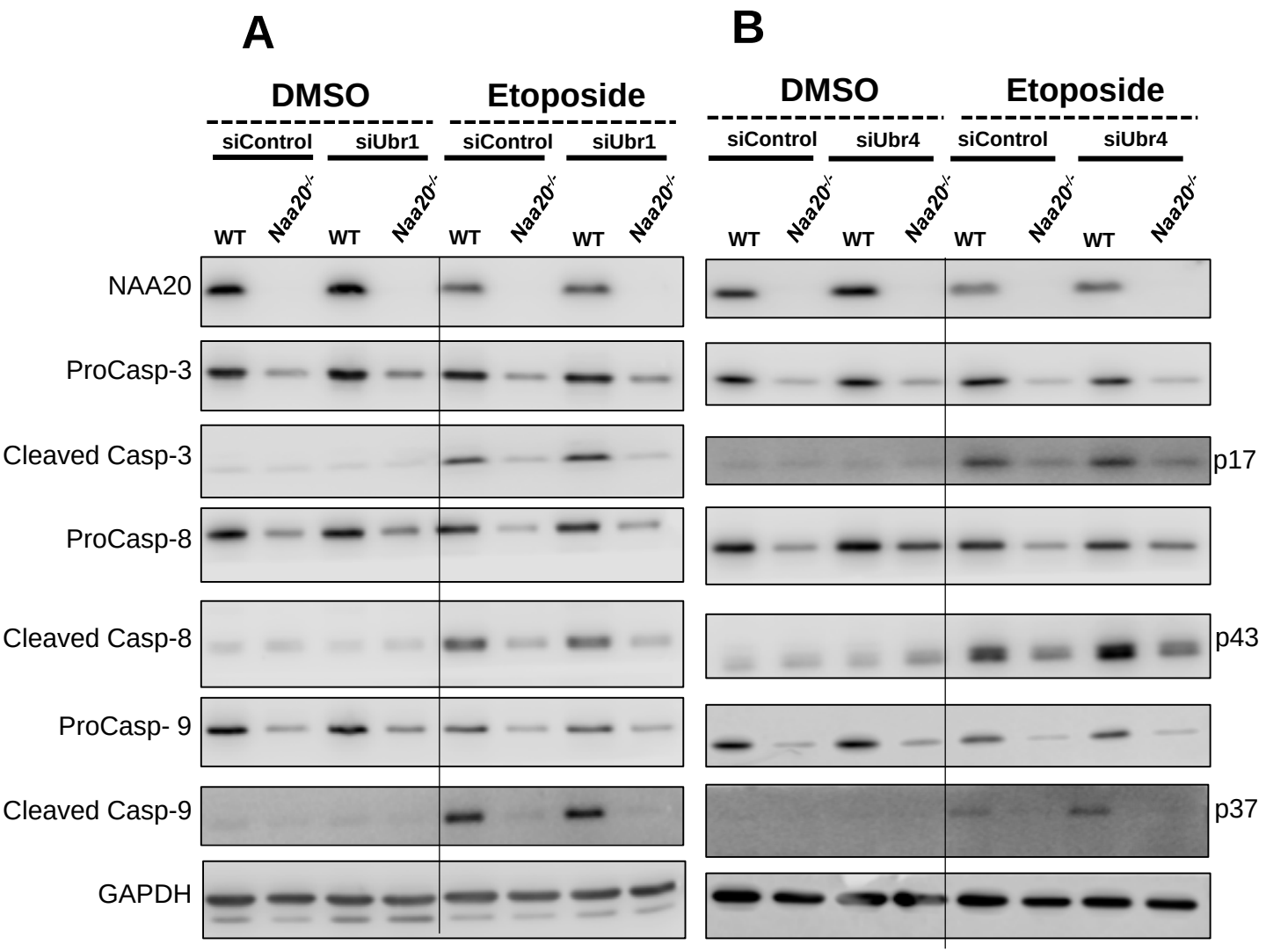


**Figure S8. Silencing of *Ubr4* and *Ubr1* is unable to restore procaspase-9 in response to etoposide in *Naa20*$^{-/-}$ MEF cells.** Representative western blot images of the procaspases and cleaved caspases-8, -9 and -3 protein levels after silencing the *Ubr1* E3 ubiquitin ligase (**A**) or *Ubr4* E3 ubiquitin ligase (**B**) in Naa20 MEF cells 6 days after infection with AdEmpty (WT) or Ad5CMVCre (-/-), before (basal conditions) and 12 h after 50 µM etoposide treatment. Vehicle (DMSO) was used as a control.

| | WT (8 replicates) | *Naa20*$^{-/-}$ (8 replicates) | Unique N-termini |
|---|---|---|---|
| **Identified N-termini** | **13149** | **11899** | |
| **Non-redundant proteoforms** | **1844** | **1675** | **1988** |
| **Quantified N-termini** | **1073** | **945** | **1191** |
| *Full acetylation (NTA > 95%)* | *734* | *586* | - |
| *Partial acetylation* | *90* | *126* | - |
| *No acetylation (NTA < 5%)* | *249* | *233* | - |
| **iMet (pos. 1)** | **244** | **181** | **258** |
| *Full acetylation (NTA > 95%)* | *194* | *99* | - |
| *Partial acetylation* | *25* | *61* | - |
| *No acetylation (NTA < 5%)* | *25* | *21* | - |
| **+NME (pos. 2)** | **688** | **632** | **769** |
| *Full acetylation (NTA > 95%)* | *528* | *478* | - |
| *Partial acetylation* | *55* | *56* | - |
| *No acetylation (NTA < 5%)* | *105* | *98* | - |
| **Processed (pos. > 2)** | **141** | **132** | **164** |
| *Full acetylation (NTA > 95%)* | *12* | *9* | - |
| *Partial acetylation* | *10* | *9* | - |
| *No acetylation (NTA < 5%)* | *119* | *114* | - |

**Supplementary Table 1. Summary of N-terminal profiling of WT and *Naa20*$^{-/-}$ MEF cells.** This table displays the number of N-termini identified and quantified in both conditions. For each sample group, 4 biological replicates were processed, every one of them being analyzed in parallel on two different mass spectrometers, an LTQ-Orbitrap Velos (Thermo Fisher Scientific) and a TIMS-ToF (Bruker), resulting in a combination of 8 replicates per sample. The quantified N-termini were sorted in 4 different categories: their total number, the ones beginning with the initiating methionine (« iMet »), those that underwent N-terminal methionine excision (« +NME ») and the matured N-termini (« Processed »). In each of these categories, the quantified N-termini were either quantified as fully acetylated (NTA yield > 95%), non-acetylated (NTA < 5%), or partially acetylated. Comparison of these numbers allowed to determine the effect of the *Naa20*$^{-/-}$ mutant on the N-terminal acetylation.