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IRE1 α Cleaves Select microRNAs During ER Stress to Derepress Translation of Proapoptotic Caspase-2

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Summary

Protein misfolding stimulates a signaling pathway involving noncoding RNAs to promote cell death.

The endoplasmic reticulum (ER) is the primary organelle for folding and maturation of secretory and transmembrane proteins. Inability to meet protein-folding demand leads to “ER stress,” and activates IRE1 α , an ER transmembrane kinase-endoribonuclease (RNase). IRE1 α promotes adaptation through splicing *Xbp1* mRNA or apoptosis through incompletely understood mechanisms. Here we found that sustained IRE1 α RNase activation caused rapid decay of select microRNAs (miRs -17, -34a, -96, -125b) that normally repress translation of *Caspase-2* mRNA, and thus sharply elevates protein levels of this initiator protease of the mitochondrial apoptotic pathway. In cell-free systems, recombinant IRE1 α endonucleolytically cleaved microRNA precursors at sites distinct from DICER. Thus, IRE1 α regulates translation of a proapoptotic protein through terminating microRNA biogenesis, and noncoding RNAs are part of the ER stress response.

Various physiological events (e.g., secretory cell differentiation, polypeptide hormone production) and pathological insults (e.g., hypoxia, ischemia, changes in intracellular pH) increase protein-folding demand on the secretory pathway to trigger ER stress (1). The tripartite unfolded protein response (UPR) signaling arms of IRE1 α , PERK, and AFT6 α

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attempt to resolve ER stress through complimentary homeostatic mechanisms, which include inhibiting cap-dependent protein translation, increasing ER chaperones, and enhancing ER-associated protein degradation (ERAD) (2). However, if ER stress cannot be remedied through these mechanisms, the UPR induces apoptosis through the mitochondrial BAX/BAK-dependent pathway (1). Excessive ER stress-induced cell loss contributes to numerous human degenerative diseases, including diabetes, neurodegeneration, and cardiovascular disease (1, 3, 4)

Severe ER stress activates the protease CASPASE-2 (CASP2) as an early apoptotic switch upstream of mitochondria (3-7). Once activated, CASP2 cleaves the BH3-only protein BID, which then localizes to mitochondria to induce BAX/BAK-dependent apoptosis (8,9). However, the molecular events leading from the detection of upstream ER stress to CASP2 activation remain unknown. To address this question, we challenged wild-type (WT) and apoptosis-resistant *Bax*^{-/-}*Bak*^{-/-} (DKO) mouse embryonic fibroblasts (MEFs) with brefeldin A (BFA), a drug that retards protein trafficking in the secretory pathway to cause ER stress. CASP2 protein, which is normally expressed at low levels in these cells, increased dramatically within 2hrs of BFA treatment and rose steadily over the next 12-18hrs (Fig. 1A,B, S1). CASP2 then underwent internal cleavage at ~18hrs, concomitant with entry of WT—but not DKO—MEFs into the apoptotic pathway, as evidenced by activation of the downstream executioner CASP3 and Annexin-V staining (Fig. 1A-C). CASP2 upregulation and activation was preserved in DKO MEFs, which suggests that it occurs upstream of the mitochondrial apoptotic pathway (Fig. 1B,C). Furthermore, CASP2 was efficiently induced by ER stress in *Atf6a*^{-/-} and *Perk*^{-/-}, but not in *Ire1a*^{-/-} MEFs (Fig. 1D, E); and CASP2-dependent proteolytic activation of BID in response to ER stress was absent in *Ire1a*^{-/-} MEFs (Fig. S2). Thus, IRE1 α may represent the upstream ER sensor used by cells to upregulate CASP2 protein. Consistent with this notion, *Ire1a*^{-/-} MEFs were resistant to BFA-induced apoptosis, and provision of IRE1 α reconstituted apoptosis in a BAX/BAK-dependent manner (Fig. S3 and S4).

IRE1 α consists of an N-terminal sensor domain within the ER that detects misfolded proteins, a transmembrane region, and a cytosolic tail containing two distinct catalytic activities—a serine/threonine kinase and an endoribonuclease (RNase) (10, 11) (Fig. S7). Accumulation of misfolded proteins within the ER leads to IRE1 α oligomerization and subsequent trans-autophosphorylation, which allosterically activates its RNase. Isogenic T-REx-293 cell lines have been generated that express either WT or various mutant forms of IRE1 α under doxycycline (Dox) control (12). Because activation of IRE1 α requires oligomerization in the ER membrane, this process can be driven by mass action in the absence of ER stress (12). Activation of WT-IRE1 α was sufficient for both robust upregulation of CASP2 and its subsequent cleavage, similar to what occurs under irremediable ER stress (Fig. 2A, S5 and S6).

To determine the contribution of the kinase and/or RNase activity of IRE1 α in CASP2 upregulation, we tested various IRE1 α mutants (Fig S7). A kinase-active/RNase-dead variant of IRE1 α (K907A) was unable to upregulate CASP2 (Fig. 2B). We employed a chemical-genetic tool that can selectively activate IRE1 α 's RNase, IRE1 α (I642G) (12). The IRE1 α (I642G) mutant is deficient in phosphotransfer activity (12, 13), but in the presence of 1NM-PP1 undergoes a conformational change that activates its RNase (12,13). Dox-induced expression of IRE1 α (I642G) alone (RNase 'OFF') did not increase CASP2, but the addition of 1NM-PP1 (RNase 'ON') increased CASP2 as efficiently as WT-IRE1 α (Fig 2B). Unlike under WT-IRE1 α activation, there was no CASP2 cleavage after 1NM-PP1 activation of IRE1 α (I642G). Thus, CASP2 is subject to multi-step regulation downstream of IRE1 α , and activating the RNase of IRE1 α (I642G) with 1NM-PP1 selectively activates the initial step in the process. Consistent with this notion, forcible

activation of IRE1 α (I642G) does not cause apoptosis (12). IRE1 α -mediated CASP2 upregulation occurred independently of its classical target, XBP1 (Fig. 2C, D, and S8).

While *Casp2* mRNA levels remained stable in response to BFA or WT-IRE1 α (Fig. S9), poly-ribosome-associated *Casp2* mRNA significantly increased within 1hr of BFA in *Ire1a*^{+/+} but not *Ire1a*^{-/-} MEFs (Fig. 3A and S10). Moreover, CASP2 increased in response to BFA even when transcription was blocked with Actinomycin D (Fig. 3B), but not when translation was blocked with cycloheximide (Fig. S11). Thus, IRE1 α 's RNase appears to upregulate CASP2 expression post-transcriptionally. A bioinformatic analysis of *Casp2* mRNA revealed several high confidence matches for binding sequences of known microRNAs (miRs) within its 3'-untranslated region (3'-UTR), including miR-17, miR-34a, miR-96 and miR-125b (Fig. S12). These 4 miRNAs rapidly and significantly decreased in *Ire1a*^{+/+} but not *Ire1a*^{-/-} MEFs upon BFA treatment (Fig. 3C); while an unrelated miRNA, let-7a, did not. We could mimic this regulation using our chemical-genetic tools: WT-IRE1 α or 1NM-PP1 activation of IRE1 α (I642G) also caused rapid decreases in miR-17, -34a, -96, and -125b (Fig. 3D).

To test whether IRE1 α -mediated reduction of select miRNAs can increase translation of a target mRNA, we devised a reporter system with an mCherry gene construct containing defined miRNA binding sites within its 3'-UTR. As such, mCherry expression is low in the presence of the matching miRNA, but becomes upregulated if the specific miRNA decreases sufficiently. The mCherry reporters for miR-17, -34a, -96, and -125b (but not let-7a) in T-REx-293 cells were all upregulated following Dox-induction of WT-IRE1 α or IRE1 α (I642G) plus 1NM-PP1 (Fig. S13 A, B, G-H). BFA caused similar increases in these miRNA reporters in *Ire1a*^{+/+} but not *Ire1a*^{-/-} MEFs (Fig. S13, C-F). To examine the role of these miRNAs in regulating *Casp2* translation, we transfected T-REx-293 cells with specific anti-miRNA oligonucleotides (14-16). Anti-miR-17, -34a, -96, or -125b each modestly increased CASP2, but all 4 together led to CASP2 levels similar to those seen with BFA (Fig. 4A). Conversely, single anti-*Casp2* miRs (-17, -34a, -96, -125b) partially reduced and the combination of all four effectively prevented CASP2 upregulation by IRE1 α (Fig. 4B). To determine if the binding sequences for miR-17, -34a, 96- and -125b within the 3'-UTR of *Casp2* mRNA are critical for translational control, we introduced the complete 3'-UTR sequence into a dual Firefly (FLuc) and *Renilla* (RLuc) luciferase reporter system in the T-REx-293 cells (17). Similar to the endogenous *Casp2* mRNA, Dox-induction of WT-IRE1 α or I642G-IRE1 α plus 1NM-PP1 activated 3'-UTR-dependent translation as indicated by increases in the RLuc/FLuc ratio, but not when the binding sequences for the anti-*Casp2* miRNAs were mutated (Fig. S14). Thus, IRE1 α controls *Casp2* translation via downregulating these select anti-*Casp2* miRNAs.

To explore the mechanism through which IRE1 α downregulates these miRNAs, we examined the biogenesis of miR-17. IRE1 α activation did not affect pri-miR-17, but significantly reduced both pre-miR-17 and mature miR-17 (Fig. 4C), suggesting that IRE1 α regulates its biogenesis at the precursor step. Indeed, incubation of radiolabeled pre-miR-17 with recombinant human WT IRE1 α or 1NM-PP1 activated IRE1 α (I642G) (but not the K907A RNase mutant) resulted in two prominent fragments (~40 nt and ~17 nt), as well as a slightly less abundant ~49nt fragment (Fig. 4D). These IRE1 α cleavage products all mapped to positions distinct from DICER sites but with some similarity to IRE1 α 's scission sites in *Xbp1* (Fig. 4E, F). Thus, IRE1 α appears to cleave select pre-miRNAs directly to prevent proper DICER processing of their mature forms, a mechanism recently described for how another ribonuclease, MCPIP1, terminates miRNA biogenesis (18).

Hyperactivated WT IRE1 α --but not 1NM-PP1 activated IRE1 α (I642G)--endonucleolytically degrades hundreds of ER-localized mRNAs, which further compromises

ER protein-folding capacity and hastens cell demise (12). While activated WT-IRE1 α and IRE1 α (I642G) both decreased anti-*Casp2* miRNAs to induce expression of pro-CASP2, only the former triggered its subsequent proteolytic processing, suggesting that ER-localized mRNA decay may be a critical “second signal” for full CASP2 activation and apoptosis. Unlike mRNAs directed to the ER membrane through a signal peptide leader sequence, it is currently unclear how pre-miRNAs gain proximity to IRE1 α under conditions of ER stress. Possibilities include alterations in miRNA localization structures (e.g., P-bodies, stress granules), the involvement of miRNA-binding proteins, or the loss of other miRNA protective factors (19-21). Alternatively, because the outer nuclear membrane is continuous with the ER, IRE1 α may become exposed to pre-miRNAs as they transit through the nuclear pore.

There have been previous hints of a connection between miRNAs and ER stress signaling. For instance, the apoptotic proteins BIM, BAK and PUMA are known to increase during ER stress-induced apoptosis (7, 22, 23), and contain putative miRNA binding sites within their 3'-UTRs. Furthermore, we recently found that IRE1 α posttranscriptionally stabilizes the mRNA encoding the pro-oxidant TXNIP in part through reducing miR-17 to activate the NLRP3 inflammasome in pancreatic beta cells under ER stress (24). Here we provide evidence that the action of IRE1 α on miRNA biogenesis is direct and that it antagonizes classical processing by DICER to derepress a translational block to entry into apoptosis. Thus, endonucleolytic cleavage of miRNA precursors by IRE1 α adds to a growing list of extra-Xbp1 mRNA splicing functions controlled by this UPR sensor. Given their potential to alter expression of multiple mRNA targets simultaneously, non-coding RNAs are well-suited to govern complex cellular remodeling in response to ER stress signaling.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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REFERENCES

1. Shore GC, Papa FR, Oakes SA. Signaling cell death from the endoplasmic reticulum stress response. *Curr Opin Cell Biol.* 2011; 23:143–149. [PubMed: 21146390]
2. Ron D, Walter P. Signal integration in the endoplasmic reticulum unfolded protein response. *Nat Rev Mol Cell Biol.* 2007; 8:519–529. [PubMed: 17565364]
3. Yoshida H. ER stress and diseases. *Febs J.* 2007; 274:630–658. [PubMed: 17288551]
4. Reyes NA, et al. Blocking the mitochondrial apoptotic pathway preserves motor neuron viability and function in a mouse model of amyotrophic lateral sclerosis. *J Clin Invest.* 2010; 120:3673–3679. [PubMed: 20890041]
5. Upton JP, et al. Caspase-2 cleavage of BID is a critical apoptotic signal downstream of endoplasmic reticulum stress. *Mol Cell Biol.* 2008; 28:3943–3951. [PubMed: 18426910]

6. Nutt LK, Buchakjian MR, et al. Metabolic control of oocyte apoptosis mediated by 14-3-3zeta-regulated dephosphorylation of caspase-2. *Dev Cell*. 2009; 16(6):856–866. [PubMed: 19531356]
7. Nutt LK, Margolis SS, et al. Metabolic regulation of oocyte cell death through the CaMKII-mediated phosphorylation of caspase-2. *Cell*. 2005; 123(1):89–103. [PubMed: 16213215]
8. Bonzon C, Bouchier-Hayes L, Pagliari LJ, Green DR, Newmeyer DD. Caspase-2-induced apoptosis requires bid cleavage: a physiological role for bid in heat shock-induced death. *Mol Biol Cell*. 2006; 17:2150–2157. [PubMed: 16495337]
9. Wei MC, et al. Proapoptotic BAX and BAK: a requisite gateway to mitochondrial dysfunction and death. *Science*. 2001; 292:727–730. [PubMed: 11326099]
10. Tirasophon W, et al. A stress response pathway from the endoplasmic reticulum to the nucleus requires a novel bifunctional protein kinase/endoribonuclease (Ire1p) in mammalian cells. *Genes & Development*. 1998; 12:1812–1824. [PubMed: 9637683]
11. Wang X-Z, et al. Cloning of mammalian Ire1 reveals diversity in the ER stress responses. *EMBO J*. 1998; 17:5708–5717. [PubMed: 9755171]
12. Han D, et al. IRE1alpha kinase activation modes control alternate endoribonuclease outputs to determine divergent cell fates. *Cell*. 2009; 138:562–575. [PubMed: 19665977]
13. Papa FR, et al. Bypassing a kinase activity with an ATP-competitive drug. *Science*. 2003; 302:1533–1537. [PubMed: 14564015]
14. Krutzfeldt J, et al. Silencing of microRNAs in vivo with ‘antagomirs’. *Nature*. 2005; 438:685–689. [PubMed: 16258535]
15. Vermeulen A, et al. Double-stranded regions are essential design components of potent inhibitors of RISC function. *Rna*. 2007; 13:723–730. [PubMed: 17400817]
16. Bartel DP. MicroRNAs: target recognition and regulatory functions. *Cell*. 2009; 136:215–233. [PubMed: 19167326]
17. Yekta S, et al. MicroRNA-Directed Cleavage of HOXB8 mRNA. *Science*. 2004; 304:594–596. [PubMed: 15105502]
18. Suzuki, Hiroshi I.; Arase, M., et al. MCPIP1 Ribonuclease Antagonizes Dicer and Terminates MicroRNA Biogenesis through Precursor MicroRNA Degradation. *Molecular Cell*. 2011; 44(3): 424–436. [PubMed: 22055188]
19. Almeida MI, Reis RM, et al. MicroRNA history: Discovery, recent applications, and next frontiers. *Mutat Res*. 2011; 30:30.
20. Long D, et al. Potent effect of target structure on microRNA function. *Nat Struct Mol Biol*. 2007; 14:287–294. [PubMed: 17401373]
21. Leung AK, Sharp PA. MicroRNA functions in stress responses. *Mol Cell*. 2010; 40:205–215. [PubMed: 20965416]
22. Puthalakath H, et al. ER Stress Triggers Apoptosis by Activating BH3-Only Protein Bim. *Cell*. 2007; 129:1337–1349. [PubMed: 17604722]
23. Kieran D, Woods I, et al. Deletion of the BH3-only protein puma protects motoneurons from ER stress-induced apoptosis and delays motoneuron loss in ALS mice. *Proc Natl Acad Sci U S A*. 2007; 104(51):20606–20611. [PubMed: 18077368]
24. Lerner AG, et al. IRE1 α induces thioredoxin-interacting protein to activate the NLRP3 inflammasome and promote programmed cell death under irremediable ER stress. *Cell Metab*. 2012; 16(2):250–264. [PubMed: 22883233]
25. Yoon A, et al. Impaired control of IRES-mediated translation in X-linked dyskeratosis congenita. *Science*. 2006; 312:902–906. [PubMed: 16690864]

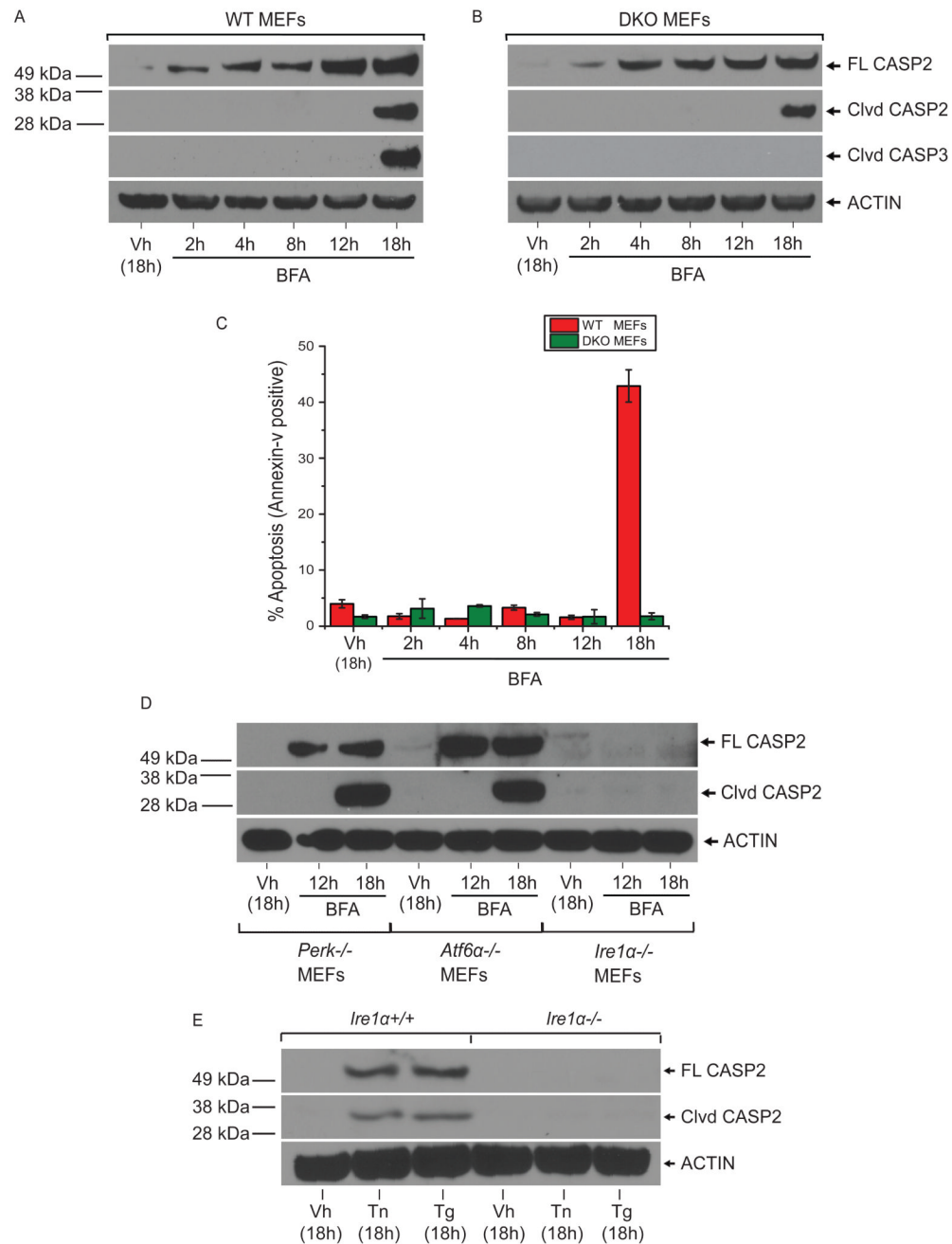


Fig. 1. IRE1 α is necessary and sufficient for CASP2 upregulation. (**A** and **B**) Immunoblot for full length (FL) CASP2 and cleaved (Clvd) CASP2 in WT and DKO MEFs after BFA treatment. (**C**) Annexin-V directed FACS analysis of WT and DKO MEFs treated with BFA. (**D**) CASP2 immunoblot in UPR sensor deficient MEFs treated with BFA. (**E**) CASP2 immunoblot of *Ire1a*^{+/+} and *Ire1a*^{-/-} MEFs treated with tunicamycin (Tn) or thapsigargin (Tg). Each data point represents the mean value \pm SD from three independent experiments.

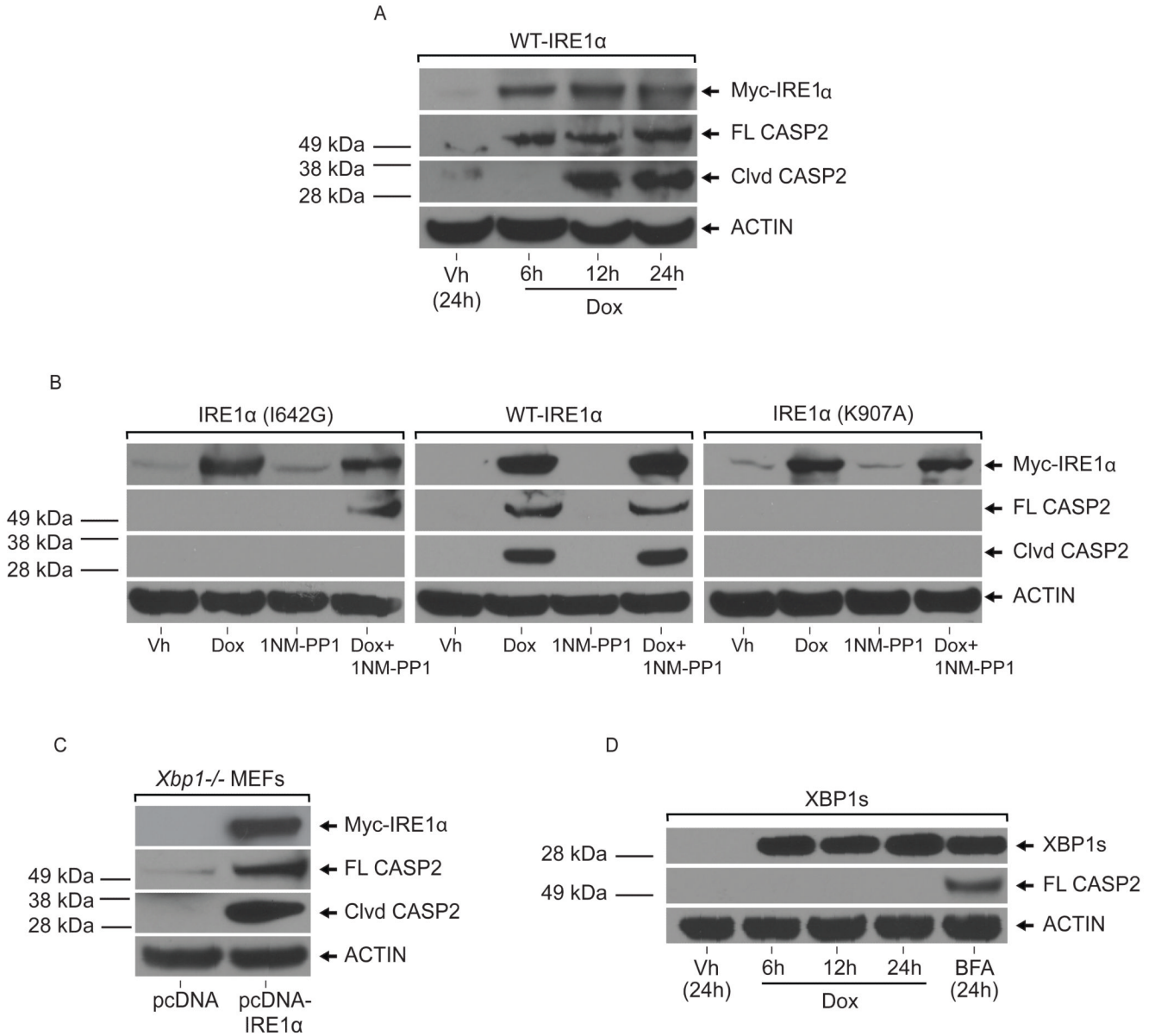


Fig. 2. The RNase activity of IRE1 α upregulates CASP2 independently of XBP1. **(A)** CASP2 immunoblot upon Dox-induction of WT-IRE1 α in T-REx-293 cells. **(B)** CASP2 immunoblot in cells that over-express various IRE1 α forms **(C)** CASP2 immunoblot in *Xbp1*^{-/-} MEFs transfected with pcDNA5-WT-IRE1 α . **(D)** CASP2 immunoblot in T-REx-293 cells before and after Dox-induction of XBP1s.

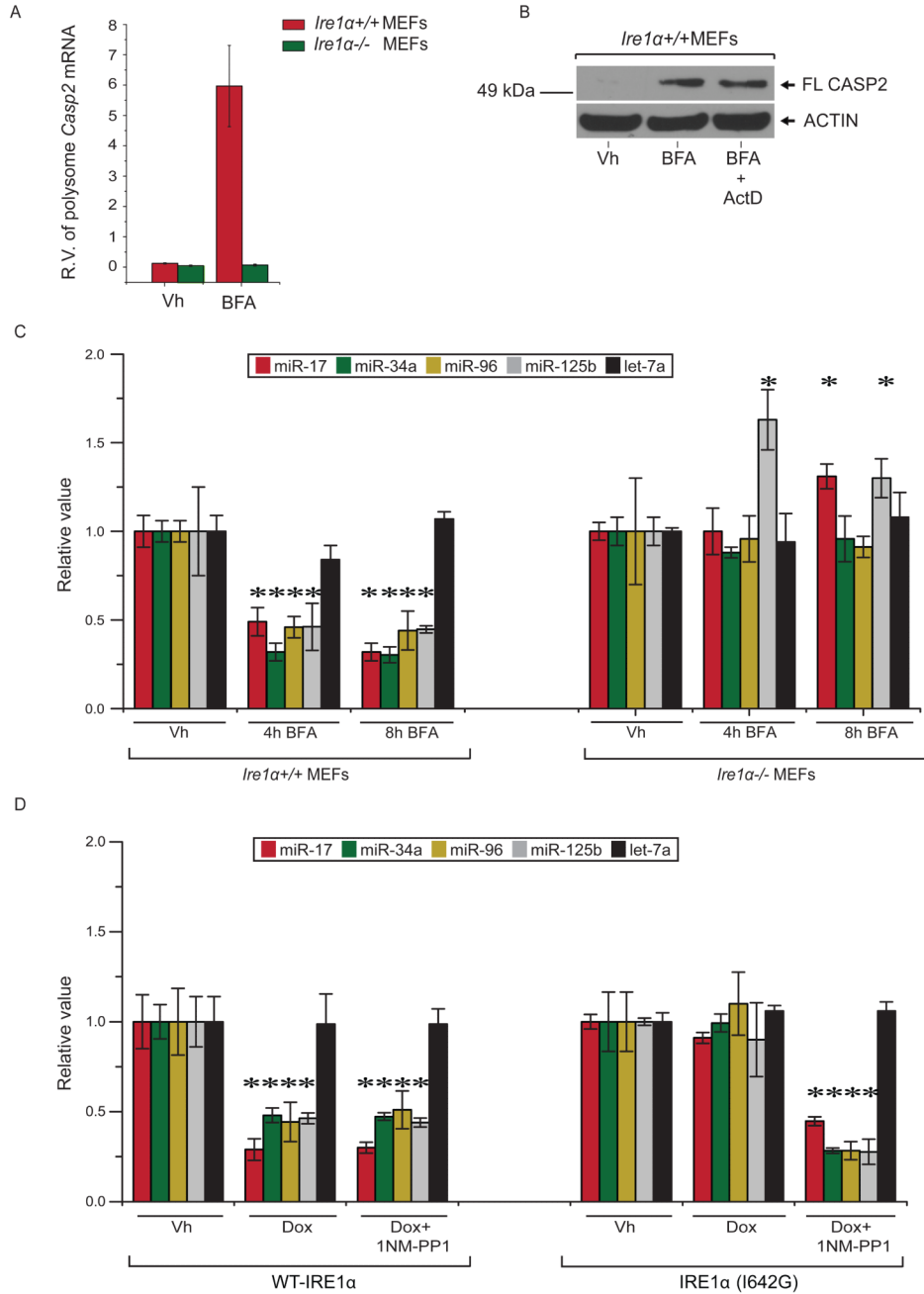


Fig. 3. Anti-*Casp2* miRNAs decrease in IRE1 α -dependent manner. (A) qPCR on poly-ribosome associated *Casp2* mRNA derived from *Ire1 α ^{+/+}* and *Ire1 α ^{-/-}* MEFs before and after BFA treatment (B) CASP2 Immunoblot of *Ire1 α ^{+/+}* MEFs treated with BFA plus/minus pre-treatment with actinomycin A (ActD). qPCR of select miRNAs from (C) *Ire1 α ^{+/+}* and *Ire1 α ^{-/-}* MEFs after BFA treatment and (D) T-REx-293 cells after over-expression of WT-IRE1 α or 1NM-PP1 activation of IRE1 α (I642G). Each data point represents the mean value \pm SD from three independent experiments. Asterisks indicate a statistically significant change from the vehicle treated controls ($p < 0.05$).

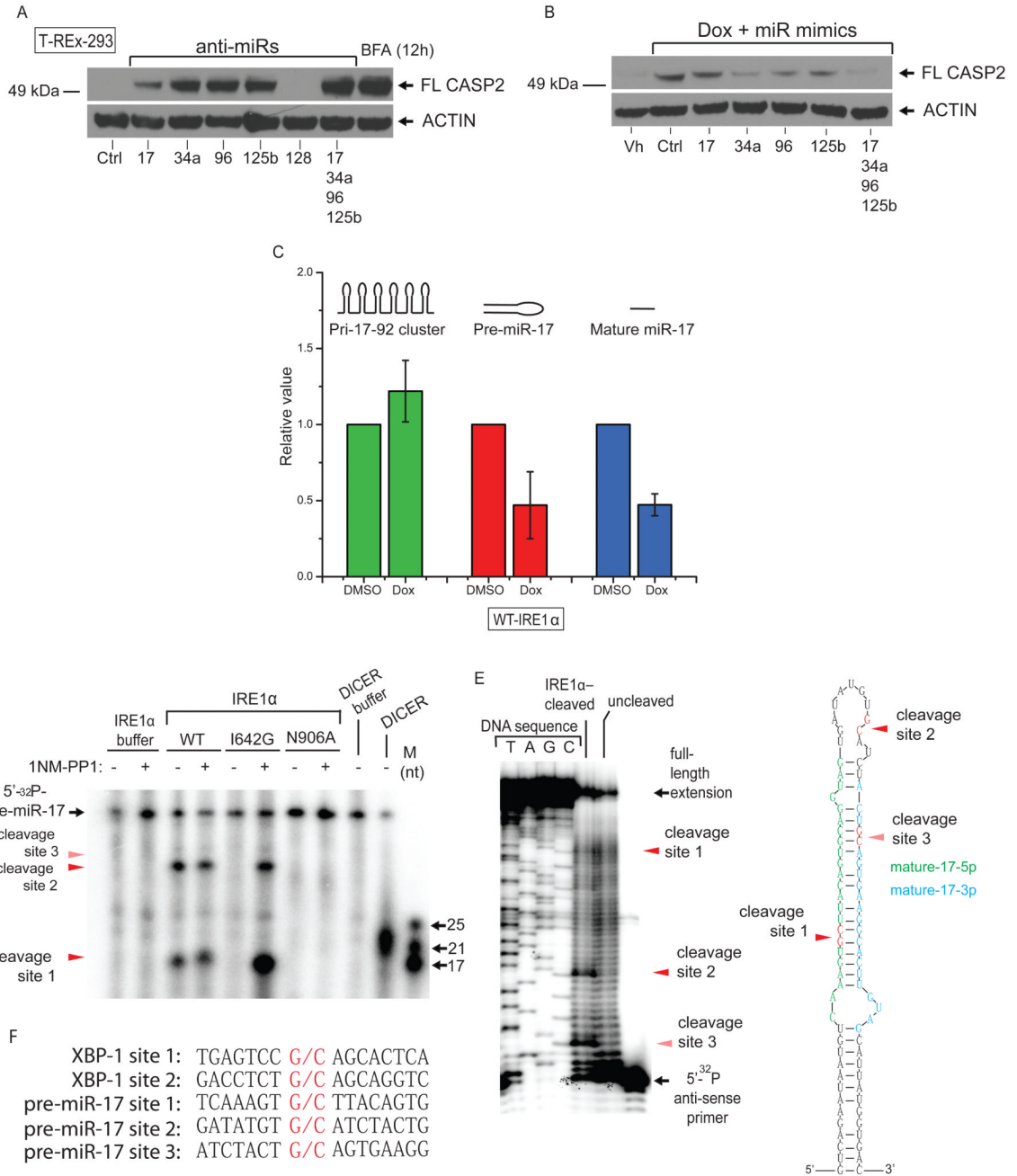


Fig. 4. WT-IRE1α directly cleaves pre-miR-17. CASP2 immunoblot of T-REx-293 cells transfected with indicated (A) anti-miRNAs or (B) miRNA mimics after Dox-induction of WT-IRE1α. (C) qPCR of pri-, pre-, and mature miR-17 after IRE1α activation in WT-IRE1α T-REx-293 cells. (D) Radioblot of ³²P-labeled pre-miR-17 digestion products after incubation with indicated recombinant IRE1α proteins. (E) Mapping of IRE1α cleavage sites in pre-miR-17. (F) Illustration of the IRE1α cleavage sites within pre-miR-17. Each data point represents the mean value ± SD from three independent experiments.