ARTICLE

Received 5 Feb 2014 | Accepted 23 Jun 2014 | Published 28 Jul 2014

DOI: 10.1038/ncomms5487

Receptor-interacting protein 140 attenuates endoplasmic reticulum stress in neurons and protects against cell death

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Inositol 1, 4, 5-trisphosphate receptor (IP\(_3\)R)-mediated Ca\(^{2+}\) release from the endoplasmic reticulum (ER) triggers many physiological responses in neurons, and when uncontrolled can cause ER stress that contributes to neurological disease. Here we show that the unfolded protein response (UPR) in neurons induces rapid translocation of nuclear receptor-interacting protein 140 (RIP140) to the cytoplasm. In the cytoplasm, RIP140 localizes to the ER by binding to the IP\(_3\)R. The carboxyl-terminal RD4 domain of RIP140 interacts with the carboxyl-terminal gate-keeping domain of the IP\(_3\)R. This molecular interaction disrupts the IP\(_3\)R’s ‘head–tail’ interaction, thereby suppressing channel opening and attenuating IP\(_3\)R-mediated Ca\(^{2+}\) release. This contributes to a rapid suppression of the ER stress response and provides protection from apoptosis in both hippocampal neurons \textit{in vitro} and in an animal model of ER stress. Thus, RIP140 translocation to the cytoplasm is an early response to ER stress and provides protection against neuronal death.

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Ca\(^{2+}\) release mediated by the inositol 1, 4, 5-trisphosphate receptor (IP\(_3\)R) is critical to cellular signal propagation\(^1\). However, uncontrolled Ca\(^{2+}\) release from the endoplasmic reticulum (ER) triggered by IP\(_3\)R dysfunction or ER stress elevates intracellular Ca\(^{2+}\) concentration ([Ca\(^{2+}\)\(_{\text{cyt}}\)]\(_i\)) resulting in toxicity and cell death\(^2\). Thus, regulation of IP\(_3\)R activity is vital to Ca\(^{2+}\) homeostasis and cell survival. Disruption of ER Ca\(^{2+}\) homeostasis contributes to cell death in the progression of neurological disease\(^3,4\). Depletion of ER Ca\(^{2+}\) stores results in ER stress that evokes the unfolded protein response (UPR). Deregelation of the UPR elevates expression of CCAAT/enhancer-binding protein homologous protein (CHOP) resulting in ER-dependent apoptosis\(^5\). Conversely, blockade of ER Ca\(^{2+}\) release by dantrolene and xestospongin C, inhibitors of ryanodine receptor (RYRs) and IP\(_3\)Rs, respectively, prevents prion and amyloid-beta(A\(_{\beta}\))-induced neuronal death\(^6,7\). Furthermore, inhibition of IP\(_3\)R-mediated Ca\(^{2+}\) release attenuates ER stress and improves cell survival following sarcoendoplasmic reticulum calcium transport ATPase inhibition\(^8\).

In mammals, there are three IP\(_3\)R subtypes: IP\(_3\)R1, 2 and 3. IP\(_3\)R1 is the predominant subtype expressed in the cerebellum and hippocampus\(^9,10\). IP\(_3\)R activity is regulated by proteins residing in the ER lumen or the cytosol\(^11\). Similar to other Ca\(^{2+}\) channels, the functional IP\(_3\)R exists as a tetramer. Its primary structure is divided into three domains: the NH\(_2\)-terminal ligand-binding domain, the coupling domain and the carboxyl-terminal channel gate domain. ER Ca\(^{2+}\) release through the IP\(_3\)R channel is regulated by the actions of accessory modulating proteins that can interact with the large cytosolic regions of the ligand-binding domain and the channel gate domain under both normal and certain pathologic conditions\(^12,13\). The positive modulating proteins include neuronal calcium sensor 1, huntington and cytochrome c, which enhance channel opening and Ca\(^{2+}\) release\(^14,15\). In contrast, the IP\(_3\)R-binding protein released with IP\(_3\) is a pseudo-ligand that suppresses the activity of IP\(_3\)R by competing with IP\(_3\) binding\(^16\). Moreover, the anti-apoptotic protein Bcl-2 inhibits IP\(_3\)-dependent channel opening and Ca\(^{2+}\) release by interacting with the channel gate domain and the coupling domain of the IP\(_3\)R\(^17\). Blocking Ca\(^{2+}\) transfer from the ER to mitochondria by IP\(_3\)R-binding proteins, GIT1 and GIT2, attenuates cell death\(^18\). However, IP\(_3\)R is negatively regulated, which can be neuroprotective, following channel overactivation remains unclear.

Receptor-interacting protein 140 (RIP140), a wide-spectrum transcription co-regulator, is highly expressed in multiple tissues including the brain\(^19\). Its primary physiological action is to negatively regulate hormonal control of gene activity by recruiting repressive chromatin remodelling machinery in the presence of hormones, thereby triggering hormone-elicited gene suppression\(^20,21\). Interestingly, the expression of RIP140 decreases with aging\(^22\). RIP140 knockout mice exhibit learning and memory deficits and increased stress responses\(^23\). Additionally, elevated RIP140 levels in the hippocampi of patients with Down syndrome\(^24\) suggest physiological or pathological relevance of RIP140 in the brain. But the action of RIP140 in neurons remains unclear. In dissecting the molecular mechanisms underlying RIP140’s functions, we have systemically examined this protein in various cellular contexts and found that it is extensively modified by post-translational modifications in a context-dependent manner. These include various forms of phosphorylation\(^25\), lysine and arginine methylation\(^26,27\), lysine-vitamin B conjugation\(^27\), lysine acetylation and sumoylation\(^28,29\), and ubiquitination\(^30\). Most of these post-translational modifications modulate RIP140’s regulation of gene expression/ chromatin remodelling in the nucleus, which is consistent with its principal activity as a transcriptional co-regulator. However, a novel signalling pathway rapidly stimulates the export of RIP140 from the nucleus to the cytoplasm where it then becomes a cytosolic regulator. This pathway is triggered by specific serine phosphorylation on Ser\(^102\) and Ser\(^1063\) followed by arginine methylation on Arg\(^160\), Arg\(^650\) and Arg\(^948\) (ref. 30). In adipocytes, cytosolic RIP140 can interact with AS160, an Akt substrate, to suppress glucose transporter 4 and adiponectin vesicle trafficking, thereby reducing glucose uptake and adiponectin secretion\(^31\).

Considering the high levels of neuronal RIP140 expression, the behavioural phenotypes of RIP140 knockout mice, and the gradual decline of RIP140 in aging brain, we hypothesized that RIP140 is important for the proper function of neurons. In this study, we observed rapid stress-induced export of RIP140 from the nucleus to the cytosol in neurons. The cytosolic RIP140 is an early, neuroprotective IP\(_3\)-R-interacting protein. By binding to IP\(_3\)R to suppress its channel opening, cytosolic RIP140 helps to attenuate IP\(_3\)-R-mediated Ca\(^{2+}\) release. We also demonstrate the physiological relevance of this mechanism in ER stress models in vitro and in vivo.

## Results

**ER stress triggers RIP140 nuclear export and ER localization.** RIP140 is exported from the nucleus to the cytoplasm of adipocytes following sequential post-translational modifications initiated by PKCe-dependent phosphorylation\(^32\). We found that in neurons, the UPR triggered the translocation of RIP140 to the cytoplasm. We applied several in vitro ER stress inducers to hippocampal neurons. Thapsigargin (Tg) depletes ER Ca\(^{2+}\) by blocking ER membrane Ca\(^{2+}\) pumps; dithiothreitol is a reducing agent; brefeldin A induces ER stress by collapse of the Golgi into the ER; and tunicamycin inhibits N-linked glycosylation.

Treating mouse hippocampal neural cells (HT22 cells) with Tg, dithiothreitol or brefeldin A increased cytoplasmic RIP140 levels while simultaneously decreasing nuclear RIP140 levels (Fig. 1a–c). A\(_{\beta}\) neurotoxicity involves elevated ER stress\(^33\). Interestingly, RIP140 translocation can also be triggered by aggregated A\(_{\beta}\) peptide (A\(_{\beta}_{1-40}\) and A\(_{\beta}_{1-42}\)) in hippocampal neurons. As shown in Fig. 1d, using lentivirus carrying green fluorescent protein (GFP)–RIP140, we detected GFP–RIP140 mainly in the nucleus, which was translocated to the cytoplasm following A\(_{\beta}\) treatment for 24 h. To rule out the potential translational effect on the elevation of RIP140 in the cytoplasm, we applied a translational inhibitor cycloheximide. Fig. 1e,f shows that pretreatment of cells with cycloheximide did not block the increase of RIP140 in the cytoplasm. Since nuclear export of RIP140 could be triggered by PKCe-initiated phosphorylation of RIP140, we examined the effects of Tg on RIP140 (CN), a mutant previously shown defective in nuclear export in adipocytes\(^34\). We also employed a PKC inhibitor to examine whether this PKC pathway was involved in nuclear export of RIP140 in the stressed neurons. Figure 1g confirms that the nuclear export-deficient mutant RIP140 failed to translocate out of the nucleus in Tg-treated cells. Figure 1h further supports this notion because the PKC inhibitor blocks nuclear export of RIP140 in Tg-treated cells. These experiments demonstrate that in stressed hippocampal neurons, RIP140 is exported from the nucleus to the cytoplasm via a PKC-mediated nuclear export pathway.

Next, to study the distribution of cytoplasmic RIP140, we measured the endogenous RIP140 levels in different subcellular fractions derived from HT22 cells following UPR induced by Tg, dithiothreitol and tunicamycin. These data all show that both endogenous RIP140 (detected by western blot using anti-RIP140 antibody) and exogenous RIP140 (transfected by lentivirus and detected by GFP fluorescence) target ER following ER stress (Fig. 2).
Cytoplasmic RIP140 interacts with the IP₃R. Since the elevated cytosolic RIP140 increasingly accumulated on ER following UPR, we then determined the cytoplasmic protein that might bind RIP140 by performing a yeast two-hybrid screen. Using RIP140 as the bait, we identified IP₃R as a RIP140-interacting protein. We then examined the nature of RIP140 interaction with IP₃R by monitoring its localization to the ER. Figure 3a shows that RIP140 was detected in the ER fraction but was no longer detectable in the proteinase K-treated ER fraction, in contrast to RIP140 that contains RD4 (RD3-4), the formation of endogenous RIP140/IP₃R complex, as indicated by the reciprocally precipitated RIP140 or IP₃R, was inhibited. Since RD4 of RIP140 is essential and sufficient to mediate the interaction of RIP140 with IP₃R, we next evaluated the potential function of RD4 motif in response to ER stress. Transfected flag-tagged full-length RIP140 (RD1-4) and RD4 were detected by immunofluorescence assay using flag antibody. As shown in Supplementary Fig. 2, RD4 was initially detected only when lystate was co-precipitated with the C-terminal gate-keeping domain of IP₃R (CT), indicating that CT is the target of RIP140 binding (Fig. 3c). A series of truncated mutants of RIP140 were examined for their interaction with IP₁₃R-CT in HT22 cells. As show in Fig. 3d, RIP140 mutant lacking RD4 (RD1-3) failed to interact with IP₁₃R-CT, suggesting that RD4 of RIP140 is essential and sufficient for its binding to IP₁₃R-CT. This finding was further confirmed by in vitro direct protein interaction assays (Supplementary Fig. 1). We then hypothesized that the interaction between RIP140 and IP₁₃R might be disrupted by the specific RD4 motif of RIP140. To test this hypothesis, we employed a competitive co-IP assay by overexpressing RD4 in HT22 cells and monitoring the formation of endogenous RIP140/IP₁₃R complex using an antibody specific for the N terminus of RIP140 and the IP₁₃R antibody (Fig. 3e). In the presence of exogenously provided RD4 or RIP140 that contains RD4 (RD3-4), the formation of endogenous RIP140/IP₁₃R complex, as indicated by the reciprocally precipitated RIP140 or IP₁₃R, was inhibited. Since RD4 of RIP140 is essential and sufficient to mediate the interaction of RIP140 with IP₁₃R, we next evaluated the potential function of RD4 motif in response to ER stress. Transfected flag-tagged full-length RIP140 (RD1-4) and RD4 were detected by immunofluorescence assay using flag antibody. As shown in Supplementary Fig. 2, RD4 was initially homogenously distributed in nuclei and cytoplasm, and remained evenly distributed following ER stress. As predicted, full-length RIP140 was translocated to cytoplasm and interacted with IP₁₃R on ER stress. These results show that RD4 can interact with IP₁₃R, but it lacks the signal for translocation in response to ER stress. Finally, we used an in situ proximity ligation assay (PLA) to examine the kinetics of endogenous IP₁₃R-RIP140 complex formation during Tg-induced ER stress. This assay employed two antibodies, anti-RIP140 and anti-IP₁₃R, followed by the addition of specific oligonucleotides that could be amplified into fluorescent signals. Only when the two antibodies existed in close proximity (<40 nm) could amplification be successful as indicated by fluorescent signals. Thus, the appearance of fluorescent signal indicates the formation and location of the molecular complex containing specific components recognized by IP₁₃R and RIP140 antibodies. As shown in Fig. 3f, Tg rapidly
were loaded with fura-2 and then [Ca2+](i) was recorded using digital imaging. Basal [Ca2+](i), remained stable in non-expressing neurons and in neurons expressing RIP140 or DsRed2 at 79 ± 3 nM, 68 ± 12 nM and 40 ± 4 nM, respectively. After recording stable basal [Ca2+](i) for 1 min, Ca2+ influx via voltage-gated Ca2+ channels was evoked by applying a 15-s depolarizing stimulus (50 mM K+). The depolarization-induced increase in [Ca2+](i), was 722 ± 123 nM, 858 ± 366 nM and 606 ± 182 nM for non-expressing neurons, neurons expressing RIP140 and DsRed2, respectively. This transient increase in the [Ca2+](i) showed that RIP140 expression did not affect voltage-gated Ca2+ channel function or Ca2+ release mechanisms and also served to uniformly load ER Ca2+ stores.[37]. After returning to basal [Ca2+](i), the group I metabotropic glutamate receptor agonist DHPG (30 μM) was superfused for 30 s. Activation of group I metabotropic glutamate receptors activates phospholipase C to generate IP3 and mobilize ER Ca2+ in hippocampal neurons[38]. As shown in Fig. 4, DHPG evoked a rapid increase in [Ca2+](i) that quickly returned to basal [Ca2+](i) in the maintained presence of the agonist. A secondary response was observed in some cells, which is consistent with IP3-evoked [Ca2+](i) oscillations.

Figure 2 | UPR induces translocation of RIP140 to the ER. (a–c) Western blot analyses of RIP140 levels in nuclear (Nuc), endoplasmic reticulum (ER) and mitochondrial (Mit) fractions of HT22 cells at different time points following treatment with 1 μM thapsigargin (Tg) (panel a), 2 μM dithiothreitol (DTT) (panel b) or 50 μM tunicamycin (Tm) (panel c). The relative protein level was analysed as a percentage of lamin A/C for nuclear, calnexin for ER and COX4 for Mit. The RIP140 level in Nuc of control group was calculated as 100%. (d) Representative confocal microscopy images indicating RIP140 cytoplasmic translocation and localization to ER in HT22 cells after treatment with 1 μM Tg, 2 μM DTT and 50 μM Tm. Green fluorescence indicates infected cells with lentivirus carrying GFP–RIP140. Red fluorescence shows ER tracker. Nuclei are labelled with 4',6-diamidino-2-phenylindole (DAPI) (blue). Scale bars, 20 μm. (e) Percentage of nuclear and cytoplasmic GFP fluorescence or red fluorescence intensity from three independent experiments (43 cells were included in each group), showing RIP140 and ER intensity, respectively, in treated cells compared with control cells. Results are presented as means ± s.e.m. **P<0.01 compare with control group as determined by Student’s t-test.

induced the formation of endogenous RIP140/IP3R complex, which was attenuated by pretreating cells with the PKC inhibitor, CHE. The co-IP in Fig. 3g shows the time-dependent formation of the RIP140/IP3R complex following Tg treatment, in agreement with the time-dependent association described in the PLA assay. These results show that stress promotes RIP140 export from the nucleus to the cytoplasm where it localizes to the ER and binds the gate-keeping domain of IP3R via RIP140’s RD4 domain.

RIP140 attenuates ER Ca2+ release in neurons. Because RIP140 translocated to the ER in response to stress and interacted with the IP3R, we suspected that RIP140 might regulate IP3 function. To examine this possibility, primary hippocampal neurons were transfected with plasmids encoding DsRed2 plus RIP140 (n = 7 coverslips, 8 cells) or DsRed2 only (n = 4 coverslips, 4 cells) as an expression control. Non-expressing neurons (n = 11 coverslips, 686 cells) in the same imaging field served as a control (Fig. 4a–e). Twenty-four hours after transfection, neurons were loaded with fura-2 and then [Ca2+](i) was recorded using digital imaging. Basal [Ca2+](i), remained stable in non-expressing neurons and in neurons expressing RIP140 or DsRed2 at 79 ± 3 nM, 68 ± 12 nM and 40 ± 4 nM, respectively. After recording stable basal [Ca2+](i) for 1 min, Ca2+ influx via voltage-gated Ca2+ channels was evoked by applying a 15-s depolarizing stimulus (50 mM K+). The depolarization-induced increase in [Ca2+](i), was 722 ± 123 nM, 858 ± 366 nM and 606 ± 182 nM for non-expressing neurons, neurons expressing RIP140 and DsRed2, respectively. This transient increase in the [Ca2+](i) showed that RIP140 expression did not affect voltage-gated Ca2+ channel function or [Ca2+](i) clearance mechanisms and also served to uniformly load ER Ca2+ stores[37]. After returning to basal [Ca2+](i), the group I metabotropic glutamate receptor agonist DHPG (30 μM) was superfused for 30 s. Activation of group I metabotropic glutamate receptors activates phospholipase C to generate IP3 and mobilize ER Ca2+ in hippocampal neurons[38]. As shown in Fig. 4, DHPG evoked a rapid increase in [Ca2+](i) that quickly returned to basal [Ca2+](i) in the maintained presence of the agonist. A secondary response was observed in some cells, which is consistent with IP3-evoked [Ca2+](i) oscillations.
described for these cells. Expression of RIP140 attenuated DHPG-evoked Ca\(^{2+}\) release relative to non-expressing neurons or neurons expressing DsRed2. To confirm that DHPG was evoking Ca\(^{2+}\) release via activation of the IP\(_3\)R, we applied DHPG in the presence of the IP\(_3\)R antagonist, xestospongin C (10 \(\mu M\)). DHPG-evoked Ca\(^{2+}\) release was blocked by xestospongin C (n = 5 coverslips, 168 cells) relative to untreated control (n = 3 coverslips, 155 cells; data not shown). We next examined the role of RIP140 in regulating the release of Ca\(^{2+}\) from ER stores in the HT22 cell line that was used for the preceding translocation and biochemical studies. Tg-evoked Ca\(^{2+}\) release was recorded from HT22 cells expressing RIP140 (n = 3 coverslips, 114 cells) or a RIP140 mutant (RIP140 RD4-neg) (n = 4 coverslips, 169 cells) that lacks the IP\(_3\)R-binding domain (Fig. 4fg). Non-expressing cells (n = 12 coverslips, 779 cells) served as a control. Basal [Ca\(^{2+}\)]\(_i\), remained stable in non-expressing cells and cells expressing RIP140 or RIP140 (RD4-neg) at 85 ± 2 nM, 101 ± 2 nM and 109 ± 5 nM, respectively. After recording stable basal [Ca\(^{2+}\)]\(_i\), for 5 min, ER Ca\(^{2+}\) release was evoked by applying Tg (1 \(\mu M\)). Tg evoked a transient increase in [Ca\(^{2+}\)]\(_i\) that returned to basal levels in the maintained presence of the drug. Expression of RIP140 attenuated Tg-evoked Ca\(^{2+}\) release relative to non-expressing cells or cells expressing RIP140 (RD4-neg). Taken together, these data indicate that RIP140 suppresses the 'head-tail' interaction of IP\(_3\)R. It is well established that binding of IP\(_3\) to the IP\(_3\)R promotes a 'head–tail'
interaction in which the cytoplasmic amino- and carboxyl-termini interact, resulting in channel opening[6]. Since RIP140 bound to the C-terminal gate-keeper domain of IP3R and inhibited IP3-induced Ca2+ release without altering IP3 binding to IP3R (Supplementary Fig. 3), we hypothesized that RIP140 might inhibit the ‘head–tail’ interaction of the IP3R. We first tested this notion using recombinant proteins in in vitro competition assays, and asked if RD4 domain could prevent IP3R-NT from binding to IP3R-CT. As shown in Fig. 5a, immunoprecipitation of HA-tagged IP3R-CT pulled down flag-tagged IP3R-NT. Adding RD4 domain (in various concentrations, + and ++ ) increasingly abolished IP3R-CT interaction with IP3R-NT. On the contrary, adding the same amount of RIP140 RD1-3 domain failed to inhibit IP3R-CT binding to IP3R-NT. Thus, RD4 of RIP140 can inhibit IP3R-CT binding to IP3R-NT in a dose-dependent manner. Since RIP140 cytoplasmic translocation is the early response to ER stress, cytoplasmic localized RIP140 may act to disrupt IP3R-CT and NT interaction. This was confirmed by co-IP assay in HT22 cells that, overexpressing mutant RIP140 (CN), which cannot translocate to cytoplasm, failed to inhibit IP3R-CT and -NT interaction (Fig. 5b). To examine the ‘head–tail’ interaction of endogenous IP3R, we overexpressed or silenced endogenous RIP140, and employed in situ PLA assay to follow the endogenous IP3R’s ‘head–tail’ interaction using antibodies against the N-terminal and the C-terminal gate region of IP3R. As shown in Fig. 5c,d, in both control transfections (SiCtrl and Vector), m-3M3FBS (m-3M3), which increases IP3 levels by activating phospholipase C, increased the number of fluorescent puncta indicative of positive ‘head–tail’ interaction resulting from the activation of endogenous IP3R. The increased fluorescent puncta were further enhanced following RIP140 knockdown (Fig. 5c), but were blocked on RIP140 overexpression (Fig. 5d). These data show that RIP140 negatively regulates IP3R-mediated channel opening by interfering with the ‘head–tail’ interaction of IP3R.

RIP140-IP3R interaction protects neurons against ER stress. Considering that stress promotes translocation of RIP140 to the cytoplasm to interact with the IP3R and that overexpressing RIP140 attenuates IP3R-mediated Ca2+ release, we speculated that the cytoplasmic RIP140 might protect cells from ER stress. To examine this notion, we inhibited the interaction of RIP140 with IP3R and then measured the ER stress response in HT22 cells. Several strategies were used to inhibit RIP140 interaction with IP3R including: siRNA-mediated knockdown of RIP140 (SiRIP140), expressing a mutant RIP140 that is deficient in nuclear export (CN) or expressing a truncation mutant of RIP140 that lacks the RD4 domain (RD1-3). Figure 6a shows that Tg induced ER stress in the control cell (SiCtrl) as indicated by upregulated expression of Bip, ATF4, CHOP and phosphorylated eIF2a. These stress-induced changes were enhanced by

Figure 4 | RIP140 attenuates ER Ca2+ release in neurons. [Ca2+], was recorded using fura-2-based digital imaging (Methods). (a) Representative image (n = 1 of 8) shows a neuron expressing RIP140 (yellow arrows) and DsRed2 in a field of neurons loaded with fura-2 (white arrows) (scale bar, 100 μm). Neuronal processes are not visible in the fura-2 image because it was focused to the centre of the soma above the focal plane of the dendrites. (b) Representative trace shows [Ca2+]i for the non-expressing (black) and RIP140-expressing (red) neurons identified by the arrows in a. Neurons were superfused with 50 mM K+ and 30 μM DHPG at the times indicated by the horizontal bars in b. (c) Pseudocolour images were scaled as shown in b. Images were collected at the times indicated by the lowercase numerals annotating the traces in b. (d) Representative traces show DHPG-evoked Ca2+ release from non-expressing neurons (black) and neurons expressing DsRed2 (dark grey) or RIP140 (red). (e) Bar graph shows net [Ca2+]i increase evoked by DHPG in non-expressing neurons (black) and neurons expressing DsRed2 (dark grey) or RIP140 (red). ***P < 0.001 relative to non-expressing control. ###P < 0.01 relative to RIP140 as determined by one-way analysis of variance with three levels followed by Tukey’s post test for multiple comparisons. (f) Representative traces show Ca2+ release evoked by 1 μM thapsigargin from non-expressing (black) HT22 cells and from HT22 cells expressing RIP140 (red) or RIP140 (RD4-neg) (light grey). (g) Bar graph shows net [Ca2+]i increase evoked by Tg in non-expressing (black) HT22 cells and HT22 cells expressing RIP140 (red) or RIP140 (RD4-neg) (light grey). ***P < 0.0001 relative to control. ####P < 0.0001 relative to RIP140 as determined by one-way analysis of variance with three levels followed by Tukey’s post test for multiple comparisons.
knockdown of RIP140 (SiRIP140). In consistence with this observation, treatment of cultured hippocampal neurons with aggregated Aβ peptide increased the expression of CHOP, and the level of CHOP was further elevated in RIP140-deficit neurons (Supplementary Fig. 4). These results indicate that RIP140 plays a protecting role against Aβ neurotoxicity. To validate the neuroprotective effect of RIP140 against stress-induced apoptosis, we monitored cell apoptosis by pSIVA–propridium iodide (PI) double staining. pSIVA detects cell surface phosphatidylserine, an early marker for apoptosis, and PI labels the nuclei of dead cells. Figure 6b,c show that knockdown of RIP140 increased ER stress resulting in an increased number of cells labelled for pSIVA and PI. Importantly, Fig. 6d shows that expressing the mutant RIP140 (CN- and RD4-deleted RIP140) increased the extent of ER stress relative to expressing wild-type RIP140 (RIP140-WT), as indicated by the elevation of ER stress markers phosphorylated eIF2α (p-eIF2α) and CHOP. Furthermore, compared with wild-type RIP140, mutant RIP140 (RIP140-CN) failed to protect cells from ER stress-induced apoptosis (Fig. 6e,f). These results demonstrate that RIP140 protects cells from ER stress.

Loss of RIP140 enhances ER stress-induced neuronal death. To validate the functional role for RIP140 in protecting neurons from ER stress in vivo, we employed an animal model of ER stress induced by intraperitoneal injection of Tg41. Lentivirus carrying shRNA against RIP140 was injected stereotaxically to knock down endogenous RIP140 in the CA1 region of the hippocampus as depicted in Fig. 7a. Figure 7b (top) and Supplementary Fig. 5 confirmed the reduction in RIP140 protein level in animals injected with shRNA against RIP140 (shRIP140). ER stress was elicited 3 or 18 days after lentivirus delivery of shRNA, and analyses of the hippocampus were conducted 2 days later.

Figure 5 | RIP140 interrupts ‘head–tail’ interaction of IP3R. (a) In vitro competition assay using RD4 to compete out the binding of IP3R1-NT to IP3R1-CT. RIP140 RD1-3 was used as a negative control. Inputs correspond to 10% of flag-labelled protein as indicated. The binding of IP3R1-NT to IP3R1-CT was significantly blocked by an increasing concentration (+ + +) of RD4. (b) Coimmunoprecipitation examining the effect of cytoplasmic RIP140 on IP3R1 ‘head–tail’ interaction. HT22 cells were transfected with HA-tagged C terminus (CT) and flag-tagged N terminus (NT) of IP3R1 and flag-tagged wild-type RIP140 (WT) or mutant RIP140 (CN), which is localized in the nucleus. Only expressing WT RIP140, but not the mutant (CN), blocked IP3R1-CT and NT interaction. (c,d) The ‘head–tail’ interaction of endogenous IP3R1 was examined in HT22 cells. In situ PLA assay was used to monitor the interaction of endogenous IP3R1’s ‘head’ (N-terminal-suppressing domain) and ‘tail’ (C-terminal gate-keeper domain) using antibodies specific to C or N terminus of IP3R1. Red puncta show positive ‘head–tail’ interaction of endogenous IP3R1. ‘Head–tail’ interaction was increased by applying the PLC activator m-3M3FBS (10 µM; m-3M3). The increased ‘head–tail’ interaction was further enhanced following RIP140 knockdown (c) and was abolished on RIP140 overexpression (d). The statistic results of ‘head–tail’ interaction from three independent experiments (seven different fields from each experiment) are presented as means ± s.e.m., **P < 0.01 compare with control group; ##P < 0.01 relative to SiCtrl or Vector as determined by one-way analysis of variance. Scale bar, 20 µm.
Figure 7b (panels 2–6) show elevated ER stress-related proteins, including Bip, phosphorylated eIF2α (p-eIF2α), ATF4 and CHOP in Tg-treated control (without silencing RIP140) animals. The RIP140-silenced animals (shRIP140, 5 days post lentivirus injection), even without Tg induction, exhibited elevated ER stress markers that were enhanced by treatment with Tg. Cells from RIP140 knockdown mice (20 days post lentivirus injection) exhibited increased apoptosis on Tg treatment (Fig. 7c,d). These animal studies validate a functional role for RIP140 in neuroprotection against ER stress in vivo.

Discussion

RIP140 is highly expressed in metabolic tissues and the brain. The physiological role for RIP140 in metabolism is primarily mediated by its nuclear receptor co-regulator activity that regulates insulin sensitivity and inflammatory responses in adipocytes and macrophages. How RIP140 may function in the brain has not been determined. This study reveals rapid nuclear export of RIP140 to the cytoplasm in ER-stressed hippocampal neurons. The study also reports a new functional role for cytoplasmic RIP140 in neurons. RIP140 attenuates ER stress by interacting with IP₃R through the carboxyl-terminal RD4 domain of RIP140 and the C-terminal gate-keeping domain of the IP₃R. This interaction interferes with IP₃R’s ‘head–tail’ interaction, thereby suppressing Ca²⁺ release and preventing neuronal death. Finally, the physiological relevance of this novel protective mechanism against Aβ neurotoxicity and ER stress was demonstrated in hippocampal neuron cultures and an animal model.

Our previous studies showed a specific signalling pathway regulating the phosphorylation of RIP140, which triggers nuclear export of RIP140 in adipocytes. The signalling pathway is initiated by activated nuclear PKCe, which specifically phosphorylates Ser¹⁰² and Ser²⁰³ of RIP140, followed by arginine methylation on Arg⁴¹⁰, Arg⁶⁵⁰ and Arg⁹⁴⁸ (ref. 34). PKCe is broadly expressed in neuronal tissue and is regarded as a stress sensor. Increased cytosolic Ca²⁺ causes PKCe activation, which plays protective roles against hypoxia and excitatory neurotoxicity. Interestingly, activation of PKCe protects neurons from ER stress-induced apoptosis. In the present study, hippocampal neurons appear to engage the PKCe-dependent pathway to stimulate RIP140's nuclear export on ER stress. Both inhibiting PKCe and mutating RIP140 to abrogate its PKCe-elicited nuclear export can abolish the protective function of RIP140 in stressed neurons. Of significance is the observation that acute ER stress readily triggers nuclear export of RIP140 in hippocampal neurons, which can be a new signalling pathway...
mediating PKCε action as a stress sensor to protect neurons from ER stress-induced cell death. This may represent a fundamental, stress-elicited physiological mechanism to rapidly resolve acute ER stress in neurons. However, it remains to be determined whether this may provide any protection during chronic ER stress. The fate of the exported RIP140 following its binding to the IP₃R also awaits further study.

With respect to the molecular mechanism, it is known that IP₃R activity is controlled by the direct association of its head and tail in both homo- and hetero-tetrameric complexes. Interestingly, this can be regulated by RIP140 via a direct interaction with the IP₃R C-terminal gate-keeper domain, which disrupts the ‘head–tail’ interaction and then suppresses channel opening and Ca²⁺ release. Importantly, a simple RD4 epitope is sufficient to reduce the formation of endogenous RIP140/IP₃R complex. Thus, the RIP140 RD4 domain interaction with the C-terminal gate-keeper domain of the IP₃R may serve as a potential therapeutic target in diseases related to ER stress. ER homeostasis is regulated by Ca²⁺ uptake and efflux through various transporters and ion channels on the ER membrane that are modulated by intracellular signalling cascades. Depleting the ER Ca²⁺ store by blocking ER membrane Ca²⁺ pumps or through uncontrolled ER Ca²⁺ release results in ER stress and cell death, which is believed to contribute to the development of neurodegenerative disease. Activating IP₃Rs is known to trigger pathological processes, including apoptosis. A recent study showed that loss of IP₃R1 enhances neuronal vulnerability to ER stress via a mitochondria-dependent pathway in an animal model. Furthermore, stimulation of ER Ca²⁺ release channels accelerates Tg-induced ER depletion and apoptosis and reducing Ca²⁺ release from ER by pharmacologically blocking IP₃R-mediated Ca²⁺ release following treatment with a serendipitous reticulum calcium transport ATPase inhibitor-attenuated ER stress and improved cell survival. These studies demonstrate complicated regulation of IP₃R in normal and stressed conditions. Despite an in-depth understanding of IP₃R activation by its binding partner, much less is known about the negative regulation of the IP₃R. The present study describes a new physiological pathway, initiated by ER stress, in which nuclear export of RIP140 provides an immediate negative regulatory mechanism to attenuate IP₃R activity and the subsequent stress response. While RIP140 does not seem to affect IP₃ binding to IP₃R (Supplementary Fig. 3), it remains to be determined whether and how RIP140 may affect the action of other IP₃R regulatory molecules implicated in the regulation of IP₃R-mediated Ca²⁺ release, such as cytochrome c and bcl-2 (refs. 55, 56).

IP₃R-mediated Ca²⁺ release is regulated by IP₃R modulatory proteins that can act on either cytosolic or luminal sites. Intraluminal proteins, such as ERP44 and chromogranin, are typically involved in long-term chronic stress. Ca²⁺-dependent apoptosis is regulated by ER oxidase 1-a that disrupts the interaction between ERP44 and IP₃R in prolonged ER stress. Interaction of the IP₃R with cytosolic proteins, like cytochrome c and bcl-2, represents a delayed response following ER stress. RIP140 is a different type of cytosolic regulator of the IP₃R because it acts by rapidly attenuating the IP₃R ‘head–tail’ interaction immediately following stress induction.
newly identified mechanism may be integrated with other IP$_3$R modulatory events to maintain the homoeostasis of IP$_3$R activity remains to be determined.

**Methods**

**Cell cultures.** Primary hippocampal neurons were isolated as previously described$^1$. Briefly, the hippocampal tissues from embryonic (E16-18) mice brain were dissected on a glass tray and cultured on poly-d-lysine-coated plates in neurobasal medium supplemented with B27. The experiments were carried after 12–14 days of culture (DIV 12–14).

HT22 cells (from Salk Institute) were maintained with Dulbecco’s modified eagle medium supplemented with 10% fetal bovine serum and differentiated in neurobasal medium with N2 supplement for 3 days before treatment.

**[Ca$^{2+}$]$^2$ imaging.** Intracellular Ca$^{2+}$ concentration ([Ca$^{2+}$]$^2$) was recorded as previously described$^2$ with minor modifications. Cells (DIV12-14) were loaded with fura-2 by incubation in hepes-buffered Hanks’ salt solution containing 5 mM fura-2 acetoxymercurinal ester in 0.04% pluronic for 30 min at 37°C followed by washing in the absence of indicator for 10 min. Coverslips containing fura-2-loaded cells were transferred to a recording chamber, placed on the stage of an Axioskop microscope (Zeiss) with the stage of IX71 microscope (Melville, NY, USA), and viewed through a ×20 objective. Excitation wavelength was selected with a galvanometer-driven monochromator (8-nm slit width) coupled to a 75-W Xenon arc lamp (Optoscan; Cairn Research). [Ca$^{2+}$]$^2$ was monitored using sequential excitation of fura-2 at 340 and 380 nm; image acquisition every 1 s. For experimental recordings, cells were superfused at a rate of 1.2–1.4 ml min$^{-1}$ with hepes-buffered Hanks’ salt solution for 2 min followed by a 15-s perfusion of 50 mM K$^+$ to depolarize the neurons and equally load the ER with Ca$^{2+}$.$^+$ release from IP$_3$-sensitive stores was evoked by the manufacturer. Briefly, cells were fixed and incubated with 4% polyvinylalcohol and incubated overnight at 4°C. To detect the endogenous interaction of RIP140 with IP$_3$R, anti-RIP140 (sc-8997; Santa Cruz; 1:200) and anti-IP$_3$R (sc-271197; Santa Cruz; 1:200) were used. The IP$_3$R and N-terminal association was detected using antibodies recognize C(407140, Calbiochem; 1:300) and N-terminal(C-121863, Lifespan; 1:300) of the IP$_3$R. Images were acquired by Olympus FluovView 1000 IX2 upright confocal microscope. The fluorescent puncta represent protein complex from different fields of individual experiment was counted with ImageJ image processing freeware (http://rsbweb.nih.gov/ij/), and the result was analysed using SPSS17.0 software. Uncropped images of all blots are shown in Supplementary Figs 5–14.

**Plasmids and siRNAs.** Flag- or HA-tagged full-length and truncated constructs of 

**Cell transfection.** Mouse primary hippocampal neurons were transfected between 12–14 days in vitro using a modification of a calcium phosphate protocol. Briefly, hE12 rat cultures were incubated for 30 min in Dulbecco’s modified eagle medium supplemented with 1 mM kynurenic acid, 10 mM MgCl$_2$ and 5 mM HEPES. A DNA/calcium phosphate precipitate containing 1 µg plasmid DNA per well was prepared, allowed to form for 30 min at 21°C then added to the culture. Following 90-min incubation, cells were washed once with Dulbecco’s modified eagle medium supplemented with MgCl$_2$ and HEPES and then returned to conditioned media. HT22 cells were transfected with siRNA to knockdown RIP140 using HiPeFect transfect reagent (301707, QIAGEN) according to the manufacturer’s instruction. The plasmid DNA was transfected into HT22 cells using Lipofectamine 2000 Transfection Reagent(11668019, Invitrogen) according to the manufacturer’s instruction.

**Western blotting.** For western blotting, the cell lysates were prepared by sonication in RIPA buffer with protease and phosphatase inhibitor cocktails. Proteins (60–80 µg) were separated by 8–15% SDS–polyacrylamide gel electrophoresis gel electrophoresis and transferred to polyvinylidene difluoride membranes, blocked in (5% milk) and incubated overnight in primary antibodies (information is provided in Supplementary Table 1) followed by respective anti-IgG secondary antibodies. Membranes were developed for visualization and photography using ECL reagent (Thermo). Optical band densities were quantified using Pro-Gel Image software and the result was analysed using SPSS17.0 software. Uncropped images of all blots are shown in Supplementary Figs 5–14.

**In situ proximal ligation assay.** In situ proximal ligation assay was performed using Duolink PLA assay kit (Olink Bioscience) according to the protocol provided by the manufacturer. Briefly, the cell cultures were fixed and incubated with 4% polyvinylalcohol and incubated overnight at 4°C. To detect the endogenous interaction of RIP140 with IP$_3$R, anti-RIP140 (sc-8997; Santa Cruz; 1:200) and anti-IP$_3$R (sc-271197; Santa Cruz; 1:200) were used. The IP$_3$R and N-terminal association was detected using antibodies recognize C(407140, Calbiochem; 1:300) and N-terminal(C-121863, Lifespan; 1:300) of the IP$_3$R. Images were acquired by Olympus FluovView 1000 IX2 upright confocal microscope. The fluorescent puncta represent protein complex from different fields of individual experiment was counted with ImageJ image processing freeware (http://rsbweb.nih.gov/ij/), and the result was analysed using SPSS17.0 software.

**Subcellular fractionation.** Subcellular fractions including ER, mitochondria and nucleus from cell or hippocampus were isolated using ER and mitochondria isolation kit (ER 0100 and MTOISO2, Sigma) according to the protocol.

**Detection of apoptosis.** Cell apoptosis was detected using CytoGLO SIVA–INABD kit (IMG-6701K, IMGENEX) and CF594 terminal dUTP nick end labeling (TUNEL) apoptosis detection kit (30064, Biotium) according to the protocol. Images were acquired by Olympus FluovView 1000 IX2 upright confocal microscope. The fluorescence intensity representing pSIVA, PI and TUNEL-positive cell number from different fields was counted using ImageJ and quantified.

**Aβ aggregation and cell treatment.** The aggregation of peptide was described as previously described$^3$. Briefly, synthetic Aβ$_{1–40}$ and Aβ$_{1–42}$ (American Peptide, 62-0-86 and 62-0-80) were dissolved to 0.5 mg ml$^{-1}$ in neurobasal medium and incubated for 4 days at 37°C. Cultured hippocampal neurons were incubated with 10 µM of aggregated peptide for 24 h and then immunofluorescence was performed.

**In vitro competition assay.** HA-tagged IP$_3$R-NT was synthesized in vitro using a coupled reticulocyte lysate system (TNT, promega) and incubated overnight at 4°C with the presence of flag-tagged IP$_3$R-CT. Sequential reactions were performed with increasing amounts of flag-tagged RDI4 synthesized in a TNT system present in reaction. Flag-tagged RDI4-1 was added to the reaction as negative control. In vitro pull-down assay was then performed and the protein levels of IP$_3$R-CT, RDI4 and RDI1–3 were determined by western blot.

**Animal experiments.** Male adult C57BL/6 mice (8–9 weeks old), from Charles River Laboratories, were maintained and experimental procedures were conducted according to NIH guidelines and approved by the University of Minnesota Institutional Animal Care and Use Committee (Protocol number 1007866322). Lentivirus carrying RIP140–shRNA was delivered to hippocampus using stereotactic apparatus at anteroposterior 2.0 mm, medial-lateral 1.2 mm and dorsoventral 1.6 mm as previously described$^4$. RIP140–protein levels following virus injection were detected by immunofluorescence and western blot as indicated in the figure legend. The ER stress animal models were generated following intraperitoneal injection of Tg (1 µg per ml$^{-1}$) or normal saline(control) after 3 or 18 days of virus injection. Then the animals were anaesthetised 2 days later and brain tissues were subjected to immunofluorescence staining or protein extraction. To detect the apoptotic cells, TUNEL staining of brain sections was performed using CF594 TUNEL apoptosis detection kit (30064, Biotium). Images were acquired by

**NATURE COMMUNICATIONS | DOI: 10.1038/ncomms5487**

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Olympus Fluoview 1000 IX2 upright confocal microscope. The TUNEL-positive cell number versus total cell number indicated by 4,6-diamidino-2-phenylindole from the lentivirus-infected areas was counted and quantified. Background was adjusted using ImageJ before counting.

**Statistical analyses.** Statistical significance for multiple comparisons was determined by Student’s t-test or analysis of variance as indicated in the figure legend using SPSS17.0 software and summarized as the mean ± s.e.m. of repeated measures. P < 0.05 was considered statistically significant at the 95% level.

For [Ca2+]i imaging studies, an individual experiment (n = 1) was defined as the change in DHIP-evoked or Tg-evoked Ca2+ release from a single cell on a single coverslip. Changes in DHIP-evoked or Tg-evoked Ca2+ release are presented as mean ± s.e.m. Each experiment was replicated using at least three separate coverslips from at least two separate cultures. Significant differences were determined by one-way analysis of variance with three levels followed by Tukey’s post hoc test for multiple comparisons (OriginPro v8.5).

**References**

1. Taylor, C. W. & Tovey, S. C. IP3 receptors: toward understanding their activation. Cold Spring Harb. Perspect. Biol. 2, 22 (2010).
2. Narayanan, R., Dougherty, K. I. & Johnston, D. Calcium store depletion induces persistent perisomatic increases in the functional density of h channels in hippocampal pyramidal neurons. Neuron 68, 921–935 (2010).
3. Makadi, D., Bult, G., Parys, J. B., De Smedt, H. & Missiaen, L. Endoplasmic-ribosome calcium depletion and disease. Cold Spring Harb. Perspect. Biol. 3, 1–12 (2011).
4. Bodaia, A., Li, H. & Jackson, M. F. Loss of endoplasmic reticulum Ca2+ homeostasis: contribution to neuronal cell death during cerebral ischemia. Acta. Pharmacol. Sin. 34, 49–59 (2013).
5. Tabas, I. & Ron, D. Integrating the mechanisms of apoptosis induced by endoplasmic reticulum stress. Nat. Cell. Biol. 13, 184–190 (2011).
6. Ferreiro, E., Resende, R., Costa, R., Oliveira, C. R. & Pereira, C. M. An endoplasmic-reticulum-specific apoptotic pathway is involved in prison and amyloid-beta peptides neurotoxicity. Neurobiol. Dis. 23, 669–678 (2006).
7. Ferreiro, E. et al. Involvement of mitochondria in endoplasmic reticulum stress-induced apoptotic cell death pathway triggered by the prion peptide PrP(106-126). J. Neurochem. 104, 766–776 (2008).
8. Dorr, V. et al. Glucose and endoplasmic reticulum calcium channels regulate HIF-1bata via presynaptic in pancreatic beta-cells. J. Biol. Chem. 283, 9909–9916 (2008).
9. Cai, W. et al. Activity-dependent expression of inositol 1,4,5-trisphosphate receptor type 1 in hippocampal neurons. J. Biol. Chem. 279, 23691–23698 (2004).
10. Mikoshita, K. IP3 receptor/Ca2+ channel: from discovery to new signaling concepts. J. Neurochem. 102, 1426–1446 (2007).
11. Choe, C. U. & Ehrlich, B. E. The inositol 1,4,5-trisphosphate receptor (IP3R) and its regulators: sometimes good and sometimes bad teamwork. Stem. STK. 2006, re15 (2015).
12. Uchida, K., Tsai, N. P., Lin, Y. P., Higgins, L. & Wei, L. N. Vitamin B6 deficiency suppresses receptor interacting protein 140 gene expression. Methods 45, 137–144 (2008).
13. Cai, W. et al. Activity-dependent expression of inositol 1,4,5-trisphosphate receptor type 1 in hippocampal neurons. J. Biol. Chem. 279, 23691–23698 (2004).
53. Luciani, D. S. et al. Roles of IP3R and RyR Ca2+ channels in endoplasmic reticulum stress and beta-cell death. *Diabetes* **58**, 422–432 (2009).
54. Taylor, C. W. & Laude, A. J. IP3 receptors and their regulation by calmodulin and cytosolic Ca2+. *Cell Calcium* **32**, 321–334 (2002).
55. Chen, R. et al. Bel-2 functionally interacts with inositol 1,4,5-trisphosphate receptors to regulate calcium release from the ER in response to inositol 1,4,5-trisphosphate. *J. Cell. Biol.* **166**, 193–203 (2004).
56. Boehning, D., van Rossum, D. B., Patterson, R. L. & Snyder, S. H. A peptide inhibitor of cytochrome c/inositol 1,4,5-trisphosphate receptor binding blocks intrinsic and extrinsic cell death pathways. *Proc. Natl Acad. Sci. USA* **102**, 1466–1471 (2005).
57. Higo, T. et al. Subtype-specific and ER luminal environment-dependent regulation of inositol 1,4,5-trisphosphate receptor type 1 by ERp44. *Cell* **120**, 85–98 (2005).
58. Hattori, M., Higo, T. & Mikoshiba, K. [Subtype-specific and ER luminal environment-dependent regulation of IP3 receptor type 1 by ERp44]. *Tanpakushitsu Kakusan Koso* **50**, 1292–1296 (2005).
59. Li, G. et al. Role of ERO1-alpha-mediated stimulation of inositol 1,4,5-trisphosphate receptor activity in endoplasmic reticulum stress-induced apoptosis. *J. Cell. Biol.* **186**, 783–792 (2009).
60. Boehning, D., van Rossum, D. B., Patterson, R. L. & Snyder, S. H. A peptide inhibitor of cytochrome c/inositol 1,4,5-trisphosphate receptor binding blocks intrinsic and extrinsic cell death pathways. *PNAS* **102**, 1466–1471 (2005).
61. Fath, T., Ke, Y. D., Gunning, P., Gotz, J. & Ittner, L. M. Primary support cultures of hippocampal and substantia nigra neurons. *Nat. Protoc.* **4**, 78–85 (2009).
62. Li, Y., Popko, J., Krogh, K. A. & Thayer, S. A. Epileptiform stimulus increases Homer 1a expression to modulate synapse number and activity in hippocampal cultures. *J. Neurophysiol.* **109**, 1494–1504 (2013).
63. Tiscornia, G., Singer, O. & Verma, I. M. Production and purification of lentiviral vectors. *Nat. Protoc.* **1**, 241–245 (2006).
64. Nicholson, A. M. & Ferreira, A. Increased membrane cholesterol might render mature hippocampal neurons more susceptible to beta-amyloid-induced calpain activation and tau toxicity. *J. Neurosci.* **29**, 4640–4651 (2009).
65. Keith, B. J. & Franklin, G. P. in *Stereotaxic Coordinates* (Academic Press, 2008).

**Acknowledgements**

This work was supported by NIH grants DK54733 and DK60521, the Dean’s commitment and Distinguished McKnight Professorship of University of Minnesota (L.-N.W.) and by DA07304 (S.A.T.). We thank technical assistance from X. Chan, P.-C. Ho, Shawna Persaud, Y.-F. Kam and David Lin. A National Institute on Drug Abuse Training Grant (DA007097) supported Kelly Krogh.

**Authors contributions**

L.-N.W. and X.F. designed the study; X.F., K.A.K., C.-Y.W., Y.-W.L. and H.-C.T. performed experiments; X.F. and K.A.K. collected and analysed data; X.F., wrote the manuscript; L.-NW. and S.A.T. provided reagents, technical support and conceptual advice.

**Additional information**

**Supplementary Information** accompanies this paper at http://www.nature.com/naturecommunications

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**How to cite this article:** Feng, X. et al. Receptor-interacting protein 140 attenuates endoplasmic reticulum stress in neurons and protects against cell death. *Nat. Commun.* 5:4487 doi: 10.1038/ncomms5487 (2014).