Modulation of STIM1 and capacitative Ca^{2+} entry by the endoplasmic reticulum luminal oxidoreductase ERp57

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STIM1 is an endoplasmic reticulum (ER) membrane Ca^{2+} sensor responsible for activation of store-operated Ca^{2+} influx. We discovered that STIM1 oligomerization and store-operated Ca^{2+} entry (SOC) are modulated by the ER oxidoreductase ERp57. ERp57 interacts with the ER luminal domain of STIM1, with this interaction involving two conserved cysteine residues, C⁴⁹ and C⁵⁶. SOC is accelerated in the absence of ERp57 and inhibited in C⁴⁹ and C⁵⁶ mutants of STIM1. We show that ERp57, by ER luminal interaction with STIM1, has a modulatory role in capacitative Ca^{2+} entry. This is the first demonstration of a protein involved in ER intraluminal regulation of STIM1.

Keywords: calcium signalling; oxidoreductase; endoplasmic reticulum; STIM

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INTRODUCTION

Intracellular Ca^{2+} is an important second messenger in processes as varied as muscular contraction, gene transcription and apoptosis. Ca^{2+} is released from the endoplasmic reticulum (ER) into the cytoplasm and the loss of ER Ca^{2+} stores necessitates refilling by store-operated Ca^{2+} entry (SOC; Putney & McKay, 1999; Hogan *et al*, 2010), mediated by STIM1, a transmembrane ER protein that senses the Ca^{2+} filling state of the ER (Roos *et al*, 2005), and Orai1, a plasma membrane Ca^{2+} channel that, on stimulation by STIM1, allows Ca^{2+} to enter the cell from the extracellular medium (Soboloff *et al*, 2006). STIM1 has an amino-terminal, ER luminal portion containing a Ca^{2+} -sensing EF-hand motif (Stathopoulos & Ikura, 2009). At rest, Ca^{2+} is bound to the EF hand of STIM1 in the lumen of ER and the protein is held as an inactive dimer (Covington *et al*, 2010; Hogan *et al*, 2010). In response to Ca^{2+} depletion in the ER, Ca^{2+} dissociates from STIM1, leading to oligomerization of STIM1 and its translocation to subplasmalemmal punctae (Liou *et al*, 2005; Roos *et al*, 2005; Muik *et al*, 2008; Covington *et al*, 2010). Although STIM1 and

Orai1 alone can reconstitute SOC activity, other associated proteins might be involved, including SERCA (Sampieri *et al*, 2009; Manjarres *et al*, 2010), EB1 (Grigoriev *et al*, 2008) and CRACR2A (Srikanth *et al*, 2010).

Here, we discovered that ERp57, an ER luminal oxidoreductase, modulates STIM1 function in the lumen of the ER. ERp57 bound to STIM1, depending on the presence of two conserved cysteine residues (C⁴⁹ and C⁵⁶) in the ER luminal domain of STIM1. SOC was accelerated in the absence of ERp57 and inhibited in C⁴⁹ and C⁵⁶ mutants of STIM1. This is the first demonstration of a protein involved in ER intraluminal modulation of STIM1.

RESULTS

ERp57 interacts with the luminal domain of STIM1

Our initial goal was to identify molecules that bind to the ER luminal domain of STIM1, and thus we used surface plasmon resonance (BIAcore) to screen for ER-resident proteins that might interact with STIM1 within the ER lumen. The recombinant ER luminal domain of STIM1, encompassing the EF-hand and sterile- α motif domains (Fig 1A), was immobilized to BIAcore sensor chips to test whether ER-luminal-resident proteins including ERp57, protein disulphide isomerase, calreticulin, calsequestrin and the ER luminal domain of calnexin would bind to STIM1. Of all tested proteins, only ERp57 bound to the immobilized STIM1 luminal domain (Fig 1B). We concluded that the oxidoreductase ERp57 interacts *in vitro* with the STIM1 ER luminal domain.

ERp57 is a 58-kDa thiol oxidoreductase and a member of the protein disulphide isomerase-like family (Coe & Michalak, 2010) that promotes disulphide bond formation and isomerization (Frickel *et al*, 2004). ERp57 is widely expressed in mouse tissues and has been most studied for its role in quality control in the secretory pathway (Coe & Michalak, 2010). The protein is a required component of the peptide-loading complex of major histocompatibility complex class I molecules (Wearsch & Cresswell, 2007), affects STAT3 signalling (Coe *et al*, 2010) and modulates SERCA function (Li & Camacho, 2004).

To further analyse the interaction between ERp57 and STIM1, we carried out fluorescence resonance energy transfer (FRET) analysis.

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Fig 1 | Analysis of interactions between ERp57 and the STIM1 endoplasmic reticulum (ER) luminal domain. (A) A linear model of STIM1 and the ER luminal domain of STIM1. The ER luminal domain of STIM1 (amino-acid residues 23–213) was used for BIAcore analysis. The locations of the two conserved cysteines (C⁴⁹ and C⁵⁶) are indicated. ER luminal and cytoplasmic domains of the protein are also indicated: EF1 and EF2, EF-hand domains 1 and 2; SAM, sterile- α motif domain; TM, transmembrane domain; and CC1 and CC2, coiled-coil domains. The locations of proline/serine- and lysine-rich domains are also indicated. (B) ERp57 was injected over immobilized recombinant STIM1 ER luminal domain (amino-acid residues 23–213). The ER luminal domain of STIM1 was coupled to a BIAcore chip and exposed to different ER luminal proteins as ligands. Inset, SDS-PAGE of recombinant proteins used for BIAcore analysis. CRT, calreticulin; CSQ, calsequestrin; GST, glutathione *S*-transferase; PDI, protein disulphide isomerase; sCNX, soluble calnexin. (C) *STIM1*^{-/-} cells were transfected with expression vectors encoding yellow fluorescent protein (YFP)-STIM1 and ERp57-cyan fluorescent protein (CFP). Panels show: YFP signal (i), CFP signal (ii), a merger of YFP and CFP signals (iii) and fluorescence resonance energy transfer (FRET) signal (iv). Scale bar, 16 µm. Bleed-through values were 0.05 for YFP and 0.6 for CFP.

We generated *STIM1*^{-/-} mice and mouse embryonic fibroblasts (MEFs), as well as *ERp57*^{-/-} cell lines. *STIM1*^{-/-} MEFs were transfected with expression vectors encoding yellow fluorescent protein (YFP)-tagged STIM1 (YFP-STIM1) and cyan fluorescent protein (CFP)-tagged ERp57 (ERp57-CFP). In resting cells, YFP-STIM1 was distributed throughout the ER and colocalized with ERp57-CFP (Fig 1C, iii). Coexpression of YFP-STIM1 and ERp57-CFP produced a robust FRET signal under resting conditions (Fig 1C, iv). FRET signal remained present after thapsigargin-induced store depletion (supplementary Fig S1 online). These observations supported our *in vitro* observations (Fig 1B) and indicated that ERp57 and STIM1 form complexes *in vivo* and *in vitro*.

Enhanced SOC in the absence of ERp57

We next tested whether binding of ERp57 to STIM1 had an effect on the ability of STIM1 to activate SOC. To do this, we used ERp57-deficient MEFs and Fura2 imaging of intracellular Ca²⁺ dynamics (Coe *et al*, 2010). We used thapsigargin, a SERCA inhibitor, to deplete ER Ca²⁺ stores, followed by addition of extracellular Ca²⁺ to initiate SOC. As expected, there was robust SOC in wild-type cells, whereas *STIM1*^{-/-} MEFs showed minimal SOC (Fig 2A,B; extent of SOC: wild type: 233 ± 10 nM, *n* = 3; *STIM1*^{-/-}: 140 ± 6 nM, *n* = 4; *P* = 0.004). In contrast, *ERp57*^{-/-} MEFs showed enhanced SOC when compared with wild-type cells (extent of SOC: wild type: 233 ± 10 nM, *n* = 3; *ERp57*^{-/-}:

Fig 2 | Store-operated Ca²⁺ entry in *ERp57*^{-/-} cells. (A) Representative traces of Fura2 fluorescence in wild-type (wt), *STIM1*^{-/-}, *ERp57*^{-/-} and *ERp57*^{-/-} cells expressing recombinant *ERp57* (*ERp57*^{-/-} + *ERp57*). Endoplasmic reticulum (ER) store Ca²⁺ depletion was induced with 2 μ M thapsigargin (TG), followed by activation of store-operated Ca²⁺ entry (SOC) with the addition of 2 mM Ca²⁺. Ca²⁺ concentrations were calculated by calibration steps as described in supplementary methods online. (B) Maximum extent of SOC for wild-type (WT), *STIM1*^{-/-}, *ERp57*^{-/-}, and *ERp57*^{-/-} cells expressing recombinant *ERp57* (*ERp57*^{-/-} + *ERp57*). Inset, western blot analysis of wild-type, *ERp57*^{-/-}, and *ERp57*^{-/-} cells expressing recombinant *ERp57* probed with anti-*ERp57* antibodies. (C) Yellow fluorescent protein (YFP)-STIM1 punctae formation in *STIM1*^{-/-} cells, *ERp57*^{-/-} cells, and *ERp57*^{-/-} cells expressing recombinant *ERp57*. Under resting conditions, YFP-STIM1 shows a reticular pattern when expressed in *STIM1*^{-/-} cells (i). Under resting conditions in *ERp57*^{-/-} cells, in addition to reticular distribution of YFP-STIM1, there were also several punctae forming as indicated by the arrows (ii), with this abolished in *ERp57*^{-/-} cells expressing recombinant *ERp57* (iii). Thapsigargin-dependent depletion of Ca²⁺ stores induced YFP-STIM1 relocation to subplasmalemmal punctae in all cells investigated. Scale bar, 16 μ m. (D) YFP-STIM1 punctae formation in *STIM1*^{-/-} cells without (i–iv) or with (v–viii) concomitant expression of *ERp57*-cyan fluorescent protein (CFP). Three minutes after thapsigargin-induced depletion of Ca²⁺ stores, there was evidence of punctae formation in cells without *ERp57*-CFP but none in cells overexpressing *ERp57*-CFP (compare ii and vi). After 6 min, extensive punctae can be seen in cells without *ERp57*-CFP (iii) but minimal punctae are present in cells overexpressing *ERp57*-CFP (vii). Even 10 min after store depletion, most YFP-STIM1 signal remains non-punctate in cells overexpressing *ERp57*-CFP (viii).

336 ± 22 nM, $n=3$; $P=0.02$; Fig 2A,B), indicating that STIM1-dependent activation of SOC was enhanced in the absence of Erp57. Wild-type and *Erp57*^{-/-} cells had comparable rates of basal Ca²⁺ entry (that is, without store depletion), further supporting a role for Erp57 in SOC (data not shown). Next, we expressed recombinant Erp57 in *Erp57*^{-/-} cells (Coe et al, 2010) to test for the specificity of the Erp57 effect on SOC. *Erp57*^{-/-} cells expressing recombinant Erp57 showed significantly reduced SOC to a similar extent to that seen in wild-type cells (extent of SOC: *Erp57*^{-/-}: 336 ± 22 nM, $n=3$; *Erp57*^{-/-} + Erp57: 232 ± 26 nM, $n=3$; $P=0.05$; Fig 2A,B), demonstrating the specificity of the effect of Erp57 on SOC. Interestingly, *Erp57*^{-/-} cells had lower thapsigargin-releasable ER Ca²⁺ stores than did wild-type cells (wild-type: 427 ± 26 nM, $n=3$; *Erp57*^{-/-}: 283 ± 1 nM, $n=3$; $P=0.03$; Fig 2A; supplementary Fig S2 online). This could have contributed to altered SOC dynamics in Erp57-deficient cells. However, the expression of recombinant Erp57 in *Erp57*^{-/-} cells did not return thapsigargin-releasable ER Ca²⁺ stores to wild-type levels (*Erp57*^{-/-}: 283 ± 1 nM; *Erp57*^{-/-} + Erp57: 281 ± 14 nM; Fig 2A; supplementary Fig S2 online), supporting a role for Erp57 on SOC by interaction with STIM1 rather than its effects on the size of ER Ca²⁺ stores.

Triggering of SOC is intimately linked to the oligomerization and movement of STIM1 to punctae (Stathopoulos et al, 2006). Therefore, we examined whether increased SOC in the absence of Erp57 was accompanied by STIM1 punctae formation. *Erp57*^{-/-} cells were transfected with a YFP-STIM1 expression vector, followed by confocal microscopy. In wild-type cells under resting conditions, YFP-STIM1 was primarily localized in a reticular ER-like distribution (Fig 2C, i). In *Erp57*^{-/-} cells, in addition to reticular distribution of YFP-STIM1, we also observed several constitutively formed STIM1 containing punctae (Fig 2C, ii, arrows). This was not observed in *Erp57*^{-/-} cells expressing recombinant Erp57 (Fig 2C, iii). To further investigate how Erp57 regulates STIM1 movement to punctae, we compared cells expressing YFP-STIM1 alone with cells coexpressing YFP-STIM1 and Erp57-CFP (Fig 2D), where cells also expressing Erp57-CFP represent a condition with high levels of Erp57 expression. When YFP-STIM1 was expressed without Erp57 overexpression, punctae were visible 3 min after store depletion (Fig 2D, ii) and translocation to punctae was complete by 6 min after store depletion (Fig 2D, iii). In contrast, in the presence of excess Erp57, YFP-STIM1 translocation to punctae was significantly slowed: the first few punctae were not visible until after 6 min after store depletion (Fig 2D, vii), with large amounts of YFP-STIM1 remaining away from punctae even after 10 min after store depletion (Fig 2D, viii). These results indicated that Erp57 expression inhibited the movement of STIM1 to punctae.

To further verify that altered Ca²⁺ dynamics in Erp57-deficient cells was related to SOC, we used small interfering RNA (siRNA) to knock down STIM1 and recorded Ca²⁺ measurements (Fig 3A,B). Knockdown of STIM1 significantly reduced the extent of SOC in *Erp57*^{-/-} cells (wild type: 315 ± 41 nM; STIM1 knockdown: 135 ± 7 nM, $P=0.048$). Transfection with non-STIM1 targeting siRNA had no significant effect on SOC (wild type: 315 ± 41 nM; negative siRNA: 305 ± 36 nM, $P=0.849$). This further supported a role for Erp57 in SOC. Western blotting confirmed that STIM1 levels were lower in STIM1 knockdown cells (Fig 3C). Altered SOC in *Erp57*^{-/-} cells is not solely attributable to different ER

Fig 3 | Store-operated Ca²⁺ entry in *Erp57*^{-/-} cells with knockdown of STIM1. (A) Representative traces of Fura2 fluorescence in wild-type (WT) cells, *Erp57*^{-/-} cells and in *Erp57*^{-/-} cells transfected with small interfering RNA (siRNA) targeting STIM1, or scrambled siRNA (sc siRNA). Endoplasmic reticulum (ER) store Ca²⁺ depletion was induced with 2 μ M thapsigargin (TG), followed by activation of store-operated Ca²⁺ entry (SOC) with the addition of 2 mM Ca²⁺. Ca²⁺ concentrations were calculated by calibration steps as described in supplementary methods online. (B) Extent of SOC in wild-type, *Erp57*^{-/-} cells and *Erp57*^{-/-} cells transfected with siRNA targeting STIM1, or scrambled siRNA. (C) Western blot (WB) analysis of *Erp57*^{-/-} cells treated with siRNA using anti-STIM1 and anti-GAPDH antibodies (loading control). GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

Ca²⁺ store content, substantiating a direct role for Erp57 binding to STIM1 (supplementary Fig S2 online). We concluded that Erp57, by binding to STIM1 in the lumen of ER, might serve as a brake on the initiation of SOC.

ER luminal cysteines of STIM1 form a disulphide bond

Erp57 is an ER luminal oxidoreductase; therefore, we identified two highly conserved cysteine amino-acid residues (C⁴⁹ and C⁵⁶)

Fig 4 | Fluorescence resonance energy transfer (FRET) analysis of STIM1 mutants. (A) *STIM1*^{-/-} cells were transfected with expression vectors encoding yellow fluorescent protein (YFP)-STIM1-C^{49/56}A and ERp57-cyan fluorescent protein (CFP). Upper panel (basal) and lower panel (after store depletion) show distribution of yellow fluorescent protein (YFP)-STIM1-C^{49/56}A and ERp57-CFP in resting cells and thapsigargin-treated cells. Each panel shows YFP signal (i, v), CFP signal (ii, vi), a merger of YFP and CFP signals (iii, vii), and FRET signal (iv, viii). (i, ii, iii, iv) FRET between ERp57-CFP and YFP-STIM1-C^{49/56}A in resting cells. (v, vi, vii, viii) FRET between ERp57-CFP and YFP-STIM1-C^{49/56}A in thapsigargin-treated cells. The arrows indicate the cell expressing ERp57-CFP only with no FRET. Scale bar, 16 μm. (B) N_{FRET} values for FRET between ERp57-CFP and either YFP-STIM1 or YFP-STIM1-C^{49/56}A. N_{FRET} was significantly lower with STIM1-C^{49/56}A as compared with wild-type STIM1 (N_{FRET} for wild type: 0.274 ± 0.011 , $n = 70$; N_{FRET} for C^{49/56}A: 0.182 ± 0.007 , $n = 64$; $P = 9.02 \times 10^{-8}$). Bleed-through values were 0.05 for YFP and 0.6 for CFP.

upstream of the EF-hand Ca²⁺-binding domain as potential ERp57-binding sites. We carried out site-specific mutagenesis of these residues and SDS-PAGE analysis of STIM1 and STIM1 mutants under reducing and non-reducing conditions to examine whether C⁴⁹ and C⁵⁶ form an intramolecular disulphide bond in the ER luminal domain of STIM1. Under non-reducing conditions, wild-type YFP-STIM1 migrated more slowly than under reducing conditions, supporting the presence of an intramolecular disulphide bond in STIM1 (supplementary Fig S3A online). In contrast, the YFP-STIM1-C^{49/56}A mutant showed the same mobility in SDS-PAGE (supplementary Fig S3A online), indicating that ER luminal C⁴⁹ and C⁵⁶ form a disulphide bond in STIM1. Analysis of YFP-STIM1 expressed in *Erp57*^{-/-} cells indicated that ERp57 was not necessary for disulphide bond formation (supplementary Fig S3B online). Similar experiments using lysates from wild-type and *Erp57*^{-/-} cells expressing Orai1-YFP showed shifts in apparent size, indicating the lack of a role for ERp57 on Orai1 itself (supplementary Fig S3C online).

Cysteines of STIM1 are required for ERp57 binding

To test the role of STIM1 ER luminal domains and luminal cysteines, we used our YFP-STIM1-C^{49/56}A mutant for analysis of punctae formation, FRET interactions between YFP-STIM1 and ERp57-CFP, and SOC activity. The YFP-STIM1-C^{49/56}A mutant expressed in *STIM1*^{-/-} cells showed ER-like reticular localization with no visible punctae (Fig 4A, i). Importantly, thapsigargin-dependent Ca²⁺ store depletion did not result in oligomerization and punctae formation by the YFP-STIM1-C^{49/56}A mutant (Fig 4A, v, after store depletion). We concluded that ER luminal C⁴⁹ and C⁵⁶ have a role in STIM1 subplasmalemmal punctae localization.

We next examined whether STIM1 ER luminal cysteines are involved in ERp57 binding to STIM1. *STIM1*^{-/-} cells were co-transfected with expression vectors encoding YFP-STIM1-C^{49/56}A and ERp57-CFP, followed by FRET analysis. Fig 5A shows that YFP-STIM1-C^{49/56}A and ERp57-CFP produced robust FRET at rest (Fig 4A, iv and viii), although the signal was at a

Fig 5 | Store-operated Ca^{2+} entry (SOC) in cells expressing STIM1 mutants. (A) Fura2 fluorescence in STIM1-deficient cells (STIM1^{-/-}), and STIM1-deficient cells expressing yellow fluorescent protein (YFP)-STIM1 or YFP-STIM1-C^{49/56}A. Cells were treated with 2 μM thapsigargin (TG), followed by addition of 2 mM Ca^{2+} . Fura2 ratios were calibrated by addition of excess Ca^{2+} and ionomycin to obtain a maximum Fura2 ratio, which was arbitrarily set to equal 1 in all cases. (B) Maximum rate of SOC achieved after Ca^{2+} addition in STIM1^{-/-} cells and STIM1^{-/-} cells expressing YFP-STIM1 and YFP-STIM1-C^{49/56}A.

lower level than that observed between ERp57-CFP and wild-type YFP-STIM1. Although ER luminal C⁴⁹ and C⁵⁶ might have a role in ERp57 binding to STIM1, the FRET signal was unaffected by depletion of ER Ca^{2+} stores (Fig 4A, after store depletion). When ERp57^{-/-} cells were co-transfected with the same expression vectors, we again observed robust FRET signal between YFP-STIM1 and ERp57-CFP (supplementary Fig S4A online, i-iv) and reduced FRET between YFP-STIM1-C^{49/56}A and ERp57-CFP (supplementary Fig S4A online, v-viii).

To quantify, we calculated N_{FRET} values for FRET between ERp57-CFP and YFP-STIM1 or YFP-STIM1-C^{49/56}A to account for differences in relative expression of YFP and CFP fluorophores (Xia & Liu, 2001). N_{FRET} was significantly reduced (33%) with YFP-STIM1-C^{49/56}A as compared with wild-type YFP-STIM1

(N_{FRET} for wild type: 0.274 ± 0.011 , $n = 70$; N_{FRET} for C^{49/56}A: 0.182 ± 0.007 , $n = 64$; $P = 9.02 \times 10^{-8}$; Fig 5B). Expression of ERp57-CFP and YFP-STIM1 or YFP-STIM1-C^{49/56}A in ERp57^{-/-} cells yielded similar results, with a decrease in N_{FRET} by 28% for YFP-STIM1-C^{49/56}A, significant at $P = 1 \times 10^{-6}$ (supplementary Fig S4B online). We concluded that STIM1 ER luminal cysteines were involved in binding to ERp57.

STIM1 ER luminal cysteines are critical for SOC entry

Finally, we tested whether mutations of cysteine amino-acid residues affected SOC. STIM1^{-/-} cells were co-transfected with expression vectors encoding Orai1-dsRed and YFP-STIM1 or YFP-STIM1-C^{49/56}A. Cells were treated with thapsigargin to empty ER Ca^{2+} stores, followed by addition of Ca^{2+} to initiate SOC. Cells expressing YFP-STIM1 (or YFP-STIM1-C^{49/56}A) and Orai1-dsRed were identified by fluorescence microscopy followed by monitoring of Fura2 fluorescence. Fig 5 shows robust SOC observed in cells coexpressing wild-type YFP-STIM1 and Orai1-dsRed. In cells co-expressing YFP-STIM1-C^{49/56}A and Orai1-dsRed, the extent of SOC was severely inhibited, as with STIM1^{-/-} cells expressing Orai1-dsRed alone (Fig 5), indicating that C⁴⁹ and C⁵⁶ of STIM1 were critical for the activation of SOC. In summary, site-specific mutation of C⁴⁹ and C⁵⁶ not only reduced ERp57 binding, but also made STIM1 unable to translocate to punctae or stimulate SOC activity.

DISCUSSION

In this study, we have identified two unique aspects of STIM1 function that are separate but overlapping. We discovered that ERp57, an ER-resident oxidoreductase, interacts with the ER luminal domain of STIM1 and affects SOC. Although ERp57^{-/-} cells show reduced ER Ca^{2+} stores, expression of ERp57 in ERp57^{-/-} cells inhibits SOC without restoring Ca^{2+} stores, supporting a role for ERp57 on SOC by interaction with STIM1 rather than its effects on the size of ER Ca^{2+} stores. ERp57/STIM1 interaction involves two conserved cysteines (C⁴⁹ and C⁵⁶), forming a disulphide bridge upstream from the EF-hand domain. The mutation of these residues to eliminate the disulphide bond rendered STIM1 incapable of proper punctae translocation. As the conserved C⁴⁹ and C⁵⁶ are located in close proximity to the EF-hand Ca^{2+} sensor in STIM1, these results indicated that the conformation of the disulphide-containing region of STIM1 might affect that of the EF hand (and vice versa). Indeed, extensive *in vitro* data indicate that the oligomerization behaviour of STIM1 is highly dependent on the degree of folding and unfolding of its EF-S-sterile- α motif domain, as modulated by Ca^{2+} (Stathopoulos *et al*, 2006, 2008; Huang *et al*, 2009).

While this work was in preparation for publication, Hawkins *et al* (2010) reported that oxidative stress promotes STIM1 redistribution and punctae formation. On induction of oxidative stress, STIM1 ER luminal C⁵⁶ undergoes S-glutathionylation, resulting in decreased Ca^{2+} binding to STIM1, its oligomerization and SOC activation (Hawkins *et al*, 2010). In contrast, we did not observe constitutive Ca^{2+} entry in cells expressing STIM1-C^{49/56}A (Fig 5). The discrepancy between this study and that of Hawkins *et al* (2010) might represent differences in cellular systems used and/or differences in the way store-operated Ca^{2+} was measured, but both studies strongly support the notion that STIM1 is regulated by redox state. Importantly, if the cysteine residues

of STIM1 are modified, for example, by S-glutathionylation, it might not bind to ERp57. Modification of cysteine residues might be one way that STIM1, on oxidative stress, escapes ERp57-dependent inhibition.

Interestingly, Ca^{2+} release by inositol (1,4,5)-trisphosphosphate receptor is inhibited by another ER-resident oxidoreductase, ERp44 (Higo et al, 2005). We showed that ERp57 has a role in Ca^{2+} homeostasis by affecting STIM1 function and consequently SOC, suggesting that ER-resident oxidoreductases are critical regulators of ER Ca^{2+} homeostasis. The ER is the intersection of many signalling pathways and communication between different cellular organelles (Burns et al, 1994; Berridge et al, 2003; Higo et al, 2005; Rutkowski & Kaufman, 2007; Coe et al, 2010). Ca^{2+} is an important signalling molecule in the lumen of the ER (Corbett & Michalak, 2000; Hogan et al, 2010), and regulation of STIM1 by ERp57 might be involved in converging Ca^{2+} -dependent information from the lumen of the ER to the cytoplasm.

METHODS

Expression and purification of recombinant proteins. Complementary DNA encoding the ER luminal domain of STIM1 (amino acids 23–213) was generated by polymerase chain reaction-driven amplification and cloned into pBAD/gIII *Escherichia coli* expression vector. See the supplementary information online for details on recombinant protein expression and purification.

Surface plasmon resonance (BIAcore) analysis and FRET analysis. Surface plasmon resonance measurements were recorded using BIAcore. FRET was quantified as described (Mitra et al, 1996). See the supplementary information online for details.

Cell culture, immunoblotting, plasmid DNA, siRNA and site-specific mutagenesis. See the supplementary information online for details.

Ca^{2+} flux measurements. Ca^{2+} measurements on cells in suspension or for a single-cell Ca^{2+} imaging were recorded using Fura2-AM. See the supplementary information online for details.

Supplementary information is available at EMBO reports online (<http://www.emboreports.org>).

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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