Sjögren syndrome/scleroderma autoantigen 1 (SSSCA1) was first described as an autoantigen over-expressed in Sjögren’s syndrome and in scleroderma patients. SSSCA1 has been linked to mitosis and centromere association and as a potential marker candidate in diverse solid cancers. Here we characterize SSSCA1 for the first time, to our knowledge, at the molecular, structural and subcellular level. We have determined the crystal structure of a zinc finger fold, a zinc ribbon domain type 2 (ZNRD2), at 2.3 Å resolution. We show that the C-terminal domain serves a dual function as it both behaves as the interaction site to Tankyrase 1 (TNKS1) and as a nuclear export signal. We identify TNKS1 as a direct binding partner of SSSCA1, map the binding site to TNKS1 ankyrin repeat cluster 2 (ARC2) and thus define a new binding sequence. We experimentally verify and map a new nuclear export signal sequence in SSSCA1.
Sjögren syndrome/scleroderma autoantigen 1 (SSSCA1), was initially described as autoantigen p27\(^2\)\(^3\), and discovered in the late 1990s as a novel autoantigen overexpressed in Sjögren’s syndrome and/or scleroderma patients\(^5\). The human SSSCA1 gene is located on chromosome 11 (11q13.1) and encodes a small soluble protein of 21.5 kDa with a predicted N-terminal zinc ribbon domain type 2 (ZNRD2), and an unknown domain in the C terminus. The protein is predicted to be widely expressed in most normal tissues and to present an overall moderate expression level (Human Protein Atlas\(^6\) and PaxDb\(^7\)). Functionally, SSSCA1 is largely uncharacterized although it has been linked to mitosis and centromere association\(^8\).

While the function of SSSCA1 remains unknown, information about SSSCA1 has emerged in multiple studies related to the Wnt signaling pathway, diverse solid cancers, and ubiquitination. SSSCA1 has been reported for its possible implication in the Wnt signaling pathway, as identified in mass spectrometric and proteomic studies as a potential binding partner of Tankyrases 1 and 2\(^5\), as a target of the Tankyrase drugs XAV939\(^6\) and G007-LK\(^7\), and also as a target of the E3-ubiquitin ligase RNF146, which regulates Tankyrase protein levels and acts as a positive regulator of the Wnt signaling\(^8\). The Wnt signaling pathway is a crucial pathway in animals implicated in a variety of cellular processes including proliferation, differentiation, motility, survival, and apoptosis. Aberrant activation of this pathway often leads to cancer or other diseases, notably colorectal cancer for which more than 90% of the cases present an activating mutation\(^9\). In colorectal adenocarcinomas, SSSCA1 shows increased mRNA expression levels and has been identified as a key genetic marker for stroma activation and for upregulated pathway activity in colorectal adenoma-to-carcinoma progression\(^10\)\(^-\)\(^11\). Recently, SSSCA1 has been highlighted in numerous studies as a potential target gene and putative biomarker for breast cancer\(^12\)\(^-\)\(^14\). It has been associated with genomic instability at 11q and poor survival in both metastatic oral squamous cell carcinoma\(^15\) and pediatric metastatic neuroblastoma\(^16\). SSSCA1 is also indicated as a potential risk variant for type 2 diabetes\(^17\).

Finally, SSSCA1 has been linked to the ubiquitination pathway as a potential binding partner of the E3-ubiquitin ligases RNF146\(^8\), RNF166\(^18\) and HERC2\(^19\), and of RAPGEF2, a guanine nucleotide exchange factor substrate of the E3-ubiquitin ligase FBXW11\(^20\). A number of large scale studies also proposed that SSSCA1 is found ubiquitinated on four sites (K22, K64, K67, and K82)\(^21\)\(^-\)\(^24\).

Even though this multitude of publications has identified SSSCA1 in their experiments, the information is mainly obtained from large scale indirect studies. In this work, we specifically aimed at characterizing SSSCA1 for the first time, to the best of our knowledge, at the molecular, structural, and cellular level. We describe the general domain organization of SSSCA1 and identify three distinct domains including an N-terminal zinc finger domain, a disordered region and a C-terminal domain and describe that this domain organization is found in most eukaryotes and is highly conserved. We have determined the crystal structure of what we believe is a novel and unique Cys4 zinc finger domain in the N-terminal end at 2.3 Å resolution, and with structural and bioinformatic information we were able to identify putative orthologs in most animals including invertebrates and fungi. The C-terminal domain has a dual function as we here show the inclusion of an unusual nuclear export signal (NES) that overlaps with a docking site to the poly-ADP-ribosyltransferase Tankyrase 1 (TNKS1). Indeed, we identify and verify TNKS1 as a direct binding partner of SSSCA1 in three cancer cell lines. We map the binding site of SSSCA1 on TNKS1 to the ankyrin repeat cluster (ARC) 2.

**Results**

The domain architecture of SSSCA1 is evolutionary conserved. The identified homologous of SSSCA1 all encode a small soluble protein between 15 and 25 kDa composed of three distinct domains. An uncharacterized zinc-binding domain at the N terminus shows the highest degree of conservation and is annotated by the Pfam database\(^25\) as the unique Auto_anti-p27 domain belonging to the ZNRD2 family. A less conserved region with notable variations in length between species links to the C-terminal region, this domain spanning segment is predicted to be an intrinsically disordered region (IDR) high in proline content. With a disorder prediction probability above 0.5 for at least 30 consecutive residues, SSSCA1 is classified as an intrinsically disordered protein. The C-terminal domain includes a coiled-coil motif and is expected to form two consecutive α-helices from secondary structure prediction (Fig. 1a).

Several sequence databases (NCBI BLAST, Ensembl, SwissProt) were searched to identify possible SSSCA1 orthologs, and revealed potential orthologs throughout the eukaryote kingdom including vertebrates, invertebrates, protists, and fungi. Orthologs of SSSCA1 are surprisingly missing in birds (class Aves) and insects (class Insecta) (Fig. 1b, c). To further investigate SSSCA1 conservation between species, sequence alignments and disorder prediction plots were performed and showed that the distinct domain order found for SSSCA1 homologs is highly conserved from human to yeast (Fig. 1b).

SSSCA1 is a binding partner of Tankyrase 1 in cancer cells. SSSCA1 has been shown to be overexpressed in several cancers, in particular colorectal cancer. One of the major causes of colorectal cancer is an aberrant regulation of the Wnt signaling pathway and the consequent activation of TCF/LEF target genes such as the pan- Oncogene c-MYC\(^26\). As this signaling pathway and c-MYC are also altered in a variety of other cancers such breast, cervical, lung, and prostate\(^27\) cancer, we investigated the common function of SSSCA1 in three different human cancer cell lines (HeLa cervical cancer cells, SW480 colorectal cancer cells, and LnCaP prostate cancer cells).

We utilized a technique of tandem-affinity purification coupled to mass spectrometry (TAP–MS, Fig. 2a) which considerably decreases background levels and thereby increases the confidence in the interacting protein complexes\(^28\). The human gene of SSSCA1 was cloned in frame with an 8x His-GFP tag and was transiently transfected into the three cancer cell lines. Following 18 h of expression, the cells were lysed and subjected to the TAP–MS method to determine potential binding partners of SSSCA1 that would be recognized in all three cancer lines. Enrichment values were calculated for every protein co-purified with the His-GFP-SSSCA1 purification and the control purification to estimate the most abundant hits. The Venn diagram in Fig. 2b represents the enriched proteins in the different cell lines, with 93 proteins overlapping in at least two cell lines (Supplementary Data 1). DAVID\(^29\), Gene Ontology\(^30\), and STRING\(^31\) databases were searched to identify the functions of these proteins. This analysis revealed clusters of proteins related to the broad biological processes of protein binding and chaperones, microtubules and centrosomes, and RNA binding and processing. However, only five similar proteins were identified in all three cancer cell lines and these include SSSCA1 as expected, Tankyrase 1 (TNKS1), γ-tubulin (TUBG1), and the poorly characterized rRNA methyltransferase MRM2 and membrane associated VAPA (Fig. 2c). From these potential partners, TNKS1 was the most promising target as it gave the most consistent results, as it was co-purified with SSSCA1 and identified in all MS experiments, and not identified in the control
purifications. We further confirmed this interaction by immunoblotting in all three cancer cell lines (Fig. 2d, full blots in Supplementary Fig. 5b). While the Zn-binding domain and proline-rich region deletion mutants (constructs SSSCA1-ΔZn-Pro, -Δ(Zn)) pull down TNKS1, indicating that these two domains do not play a role in the interaction. The modification of the structure and functionality of H2 by mutating the leucine residues to alanine (SSSCA1-H2_Leu/Ala) also abolished the binding to TNKS1, confirming the direct interaction of SSSCA1 and TNKS1 (Fig. 3b, full blots in Supplementary Fig. 5b) with the H2 helix region. To localize the binding region of SSSCA1 on TNKS1 we performed the opposite experiment and expressed several truncated TNKS1 constructs of GFP-tagged human TNKS1 in HeLa cells (Fig. 3c). Here we observed that the TNKS1 mutant lacking the sterile alpha motif (SAM) and poly-ADP-ribose polymerase (PARP) domains still pulled down SSSCA1 (construct TNKS-Δ(SAM-PARP)), while the deletion of the ARD abolished the binding (construct TNKS-Δ(ARC1-ARC5)) (Fig. 3d, full blots in Supplementary Fig. 5c–d). More precisely, the deletion of TNKS1-specific subdomains, or ARCs, numbers 3, 4, or 5 did not affect the interaction with SSSCA1 (constructs TNKS-Δ(ARC3-PARP),

Mapping of SSSCA1 and Tankyrase 1 potential binding regions. The primary structure of SSSCA1 did not reveal any R-X-x-G-D-G consensus motif usually reported as the typical recognition motif of the TNKS1 ankyrin repeat domain (ARD) binding partners, even though deviations in the recognition motif can be tolerated. In particular RNF146 has been shown to bind TNKS1 ARD without a clear consensus motif. To locate the specific binding region, we generated a number of deletion constructs of GFP-tagged human SSSCA1 to identify the regions responsible for binding to TNKS1 (Fig. 3a). TAP from HeLa cells coupled to immunoblotting show that all SSSCA1 mutants lacking the C-terminal end helix H2 (which include constructs SSSCA1-Δ(Pro-H2), -Δ(midPro-H2), -Δ(H1–H2), -ΔH2) were not able to maintain binding to TNKS1 during the purification (Fig. 3b, full blots in Supplementary Fig. 5b). While the Zn-binding domain and proline-rich region deletion mutants (constructs SSSCA1-Δ(Zn-Pro), -Δ(Zn)) pull down TNKS1, indicating that these two domains do not play a role in the interaction. The modification of the structure and functionality of H2 by mutating the leucine residues to alanine (SSSCA1-H2_Leu/Ala) also abolished the binding to TNKS1, confirming the direct interaction of SSSCA1 and TNKS1 (Fig. 3b, full blots in Supplementary Fig. 5b) with the H2 helix region. To localize the binding region of SSSCA1 on TNKS1 we performed the opposite experiment and expressed several truncated TNKS1 constructs of GFP-tagged human TNKS1 in HeLa cells (Fig. 3c). Here we observed that the TNKS1 mutant lacking the sterile alpha motif (SAM) and poly-ADP-ribose polymerase (PARP) domains still pulled down SSSCA1 (construct TNKS-Δ(SAM-PARP)), while the deletion of the ARD abolished the binding (construct TNKS-Δ(ARC1-ARC5)) (Fig. 3d, full blots in Supplementary Fig. 5c–d). More precisely, the deletion of TNKS1-specific subdomains, or ARCs, numbers 3, 4, or 5 did not affect the interaction with SSSCA1 (constructs TNKS-Δ(ARC3-PARP),

Fig. 1 Schematic representation of the general domain organization of SSSCA1. a Domain organization of human SSSCA1, aligned with a disorder probability plot and localization of the three domains. Numbers denote amino acid positions. Zn, N-terminal zinc-binding domain (residues 20–86). Pro, proline-rich region (residues 86–148). Proline-rich α-helices H1 (150–167) and H2 (172–195). b Schematic domain organization of representative SSSCA1 orthologs. Zn, N-terminal zinc-binding domain. Pro, proline-rich region. H, C-terminal helical domain. Percentage of positive identities to human SSSCA1 is indicated. c Phylogenetic tree of SSSCA1. Orthologs’ sequences retrieved from Ensembl, Genbank, or UniProt were aligned and a phylogenetic tree was constructed using the neighbor-joining tree method. Kingdoms, major phyla, and taxonomical groups are indicated. For clarity, vertebrate and invertebrate species are shown in red and blue nuances, respectively.
TNKS-Δ(ARC4-PARP)). However, the mutants lacking ARC2 were not able to bind SSSCA1 (TNKS-Δ(ARC1-ARC4), TNKS-Δ(ARC2-PARP)) (Fig. 3d, full blots in Supplementary Fig. 5c), suggesting that this specific ARC is implicated in the direct binding to SSSCA1. Lastly, we purified human TNKS1’s ARC subdomains 1–3 (ARC1–3) from Escherichia coli (Fig. 3e) and assessed its binding to FAM-tagged peptides of human SSSCA1 by fluorescence polarization (FP) (Fig. 3f). As shown previously, it was not possible to express and purify the single ARC2 domain without the flanking ARC1 and ARC3 domains. Despite variations in solubility between the H1 and H2 peptides, we could confirm that the H2 of SSSCA1 did interact with ARC1–3 while the H1 peptide showed lower affinity for ARC1–3, compared with the H2 peptide. The binding studies indicate some interaction close to the affinity obtained for Axin1 positive control. The data accumulated for the H1 peptide were hampered by the low solubility and anisotropic polarization difference (Supplementary Data 2).

SSSCA1 belongs to a novel family of Zn-binding proteins. Human SSSCA1 N-terminal Auto_anti-p27 domain (residues 1–111, predicted MW 12.5 kDa) was successfully overexpressed in E. coli and purified over a Ni²⁺-affinity and size-exclusion chromatography (SEC). The purified SSSCA1 construct was predicted to form a dimer based on the elution time when compared with a set of common standard proteins after SEC (estimated native MW of 24.6 kDa, Fig. 4a). The Auto_anti-p27 domain was highly soluble and needed to be concentrated to more than 50 mg/ml to achieve crystallization success. The crystal structure of N-terminal SSSCA1 was determined at 2.3 Å resolution and phased using the single-wavelength anomalous dispersion method, based on the anomalous signals of two zinc ions (Fig. 4b, Table 1). The asymmetric unit contains two protomers that form an antiparallel dimer with a buried surface area of 1960 Å² (‘protein interfaces, surfaces, and assemblies’ service PISA), the interface is highly positively charged and is mainly formed by the helical part of the molecule. Each protomer binds one zinc ion coordinated by four conserved cysteines (human residues Cys53, Cys56, Cys70, and Cys73) structured around four antiparallel β-sheets (Fig. 4c). We were not able to model residues 1–12 and 78–111 in the electron density map and coincidentally, these regions correlate with the intrinsic disorder prediction plot of SSSCA1 and confirm that these residues likely are disordered in the protein crystal as well (Fig. 1a).

A systematic search for other proteins of similar architecture using available online services, the DALI server, PDBBifold revealed that SSSCA1 likely belongs to a novel Pfam family of Zn-binding ribbon domains (Auto_anti-p27, PF06677) which overall fold is not yet available in the protein data bank (PDB). However
the core antiparallel β-sheet of the zinc-binding domain shows closest structural resemblance to the E3-ubiquitin-protein ligase Smad ubiquitination regulation factor 1 (SMURF1) with an overall root-mean-square deviation (RMSD) of 2.2 Å (Fig. 4d), the neuronal protein FE65 with a RMSD of 2.3 Å (Fig. 4e), and the ubiquitin ligase NEDD4 with a RMSD of 2.4 Å (PDB ID 4N7F). The helical dimer interface has not been observed earlier for this type of Zn-binding proteins and the closest zinc-binding protein relative from the zinc ribbon domain 1 family (ZNRD1) do not share this.

Fig. 3 Mapping of SSSCA1 and TNKS1 binding. a Schematic representation of His-GFP-tagged SSSCA1 constructs. FL full length. b Different constructs of human SSSCA1 were expressed in HeLa cells. After TAP, the binding of each construct to endogenous TNKS1 was assessed by western blotting. Green stars indicate the constructs of interest. β-actin was chosen for input control because of the very low and barely detectable endogenous expression of TNKS1. c Schematic representation of His-GFP-tagged TNKS1 constructs. FL full length. d Different constructs of human TNKS1 were expressed in HeLa cells. After TAP, the binding of each construct to endogenous SSSCA1 was assessed by western blotting. Green stars indicate the constructs of interest. e TNKS1 ARC1-3 was expressed and purified from E. coli. Schematic representation of the construct and Coomassie stained SDS-gel control after purification. f TNKS1 ARC1-3 binding to FAM-tagged peptides (positive control Axin1 in black, negative control Axin1 G>R in gray, SSSCA1-H1 in pink, and SSSCA1-H2 in purple) was assessed by fluorescence polarization. Affinities of each peptide for TNKS1 ARC1-3 are indicated. n = 4; error bars, SEM; K_d error values, standard error of the fit; n.q. not quantifiable.
superimposition of the zinc-binding residues is possible, shown for the only known member of ZNRD1 found in chain I (PDB ID 4C2M) of the RNA polymerase I41 (Fig. 4f).

SSSCA1 C-terminal domain behaves as a nuclear export signal. The helix H2 in SSSCA1 C-terminal domain is predicted to be an amphipathic helix that contains motifs necessary for nuclear export. Leucine and isoleucine residues are numerous and conserved in this region across SSSCA1 orthologs (Fig. 5a). In particular, two leucine-rich NESs are predicted above the confidence level in fission yeast SSSCA1 helical domain (RIKEN Schizosaccharomyces pombe Postgenome Database). Zebrafish and worm SSSCA1 also show NES predictions with scores >0.5 in H2 (Fig. 5b, NetNES server42). In human SSSCA1, residues 173–194 display a positive Leu-rich NES prediction although below the 0.5 threshold (Fig. 5b). Similar NES motifs were first identified in PKIα and HIV-1 Rev proteins43. They consist of several closely spaced leucines or other large hydrophobic residues, and mutations of critical leucines abolish the export activity43,44. We first verified nuclear export activity by confocal microscopy in HeLa cells and show that both endogenous and overexpressed human SSSCA1 display a cytoplasmic subcellular localization (Fig. 5c).

We then generated several mutants of SSSCA1 by site-directed mutagenesis and confirmed that their localization was dependent on the presence of an intact H2 region. The deletion of H2 (SSSCA1-ΔH2, -Δ(H1–H2), -Δ(Pro-H2)) or the simultaneous mutation of all leucine residues to alanine (SSSCA1-H2Leu/Ala) lead to the ubiquitous localization of SSSCA1 constructs (Fig. 5c, Supplementary Fig. 1). Conversely, the constructs lacking one or several of the other domains (SSSCA1-ΔZn, -Δ(Zn-Pro)) still strictly localized in the cytoplasm.

**Table 1 Data collection and refinement statistics.**

| Data collection | hSSSCA1 |
|-----------------|---------|
| PETRA III       |         |
| Space group     | P 4 3 21 2 |
| Cell dimensions | 49.49, 49.49, 164.18  |
| a, b, c (Å)     | 90.0, 90.0, 90.0 |
| Wavelength (Å)  | 1.2395 |
| Resolution (Å)  | 47.2-2.3 (2.36-2.3) |
| Rsym or Rmerge  | 0.04 (100) |
| Rfree           | 0.02 (0.57) |
| CC1/2           | 1.0 (0.74) |
| I/σ             | 26.2 (1.4) |
| Completeness (%)| 99.3 (98.5) |
| Redundancy      | 9.2 (6.9) |
| Resolution (Å)  | 25.0-2.3 |
| No. reflections | 89,502 |
| No. Unique reflections | 9709 |
| Rmerge/Rsym     | 0.22/0.26 |
| No. atoms       | 1081 |
| Protein         | 1066 |
| Ligand/ion      | 8 |
| Water           | 7 |
| B-factors       | 85.6 |
| Protein         | 85.6 |
| Ligand/ion      | 105.4 |
| Water           | 70.3 |
| R.m.s. deviations | 0.006 |
| Bond lengths (Å)| 1.06 |
| Bond angles (°) | 91.3 |
| Allowed (%)     | 7.1 |
| Outliers (%)    | 1.6 |

Data collected from a single crystal.
*Values in parentheses are for highest-resolution shell.
Discussion

The human SSSCA1 gene is located on chromosome 11, one of the most gene- and disease-rich chromosomes in the genome\(^{45}\). In particular, the locus 11q13 where SSSCA1 is located has been shown to be rearranged and amplified in multiple human cancers by numerous genetic mechanisms including deletions, translocations, loss of heterozygosity and allelic amplifications\(^{46-50}\). This could be one of the reasons why SSSCA1 has been shown to be overexpressed in colorectal adenocarcinomas\(^{10,11}\), identified as a target gene in breast cancer\(^{12}\), shown to be rearranged and amplified in multiple human cancers and overexpressed in colorectal adenocarcinomas\(^{10,11}\), identified as a target gene in breast cancer\(^{12}\), shown to be rearranged and amplified in multiple human cancers and overexpressed in colorectal adenocarcinomas\(^{10,11}\), identified as a target gene in breast cancer\(^{12}\), shown to be rearranged and amplified in multiple human cancers and overexpressed in colorectal adenocarcinomas\(^{10,11}\), identified as a target gene in breast cancer\(^{12}\).

We initially set out experiments to confirm the putative binding of SSSCA1 with the human Tankyrases (TNKSs). Earlier proteomic data showed that SSSCA1 is pulled out in association with both TNKS1 and TNKS2\(^{5}\). It was however not certain whether SSSCA1 was a direct binding partner or simply part of a larger complex being pulled down with TNKS. We chose three different cancer cell lines (HeLa, SW480, and LNCaP) to increase the confidence level. However, compared with conventional immune precipitation, we made use of TAP to keep the level of false positives to a minimum and select for only the strongest binding partners (Fig. 4a). Using this method, we were able to use SSSCA1 to pull out specific binding partners. Unfortunately, the antibodies against MRM2 and VAPA gave unspecific results. The immunoblotting against γ-tubulin (TUBG1) was not conclusive (TUBG1 seemed to be pulled down by all SSSCA1 constructs and slightly by the GFP-tag alone) and the immunofluorescence showed that SSSCA1 and γ-tubulin did not co-localize at the centrosome (Supplementary Fig. 3a–b, full blots in Supplementary Fig. 5b, g). However, we cannot rule out that SSSCA1 and γ-tubulin are binding partners, for example during interphase. With this approach, we have therefore identified TNKS1 as a strong and confident binding partner of SSSCA1 in mammalian cells.

The enzyme TNKS1 and the closely related paralog TNKS2, which is less abundant, but was still identified in HeLa cells, are poly-ADP-ribose transferases involved in various processes such as telomere length regulation\(^{33}\), mitotic spindle formation\(^{34}\), proteasomal degradation\(^{55,56}\), vesicle trafficking\(^{57}\), and Wnt signaling pathway\(^{6}\). TNKS1 acts as a positive regulator of the Wnt signaling pathway by poly-ADP-riboseylation (PARylation) of
AXIN1 and AXIN2, two key components of the β-catenin destruction complex. The inhibition of TNKS1 catalytic activity antagonizes Wnt signaling and has promising implications for cancer therapy, particularly colorectal cancer and non-small cell lung cancer. Tankyrase is also crucial for embryonic development and adult homeostasis. Understanding and characterizing the mechanisms that regulate TNKS activity are therefore of particular interest.

TNKS1 and TNKS2 share three conserved domains: the ARD responsible for substrate recruitment, an SAM domain that mediates homo- and hetero-oligomerization, and a C-terminal catalytic PARP domain. The ARD is segmented into five conserved subdomains, or ARCs, which serve as a docking site for a wide range of target proteins and their subsequent scaffolding and/or PARylation. So far, most identified TNKS1 binding partners recognize various ARCs with a degenerate peptide motif, so-called Tankyrase-binding motif (TB), with the consensus sequence R-x-[G or small hydrophobic]-[D/E]-[D/E]-[D/E]. Here we have shown that the C-terminal helix H2 (residues 173–198) of SSSCA1 is crucial for the binding of Tankyrase 1. ARC domain interestingly, the primary structure of SSSCA1 does not show any conserved TB helix. The helix H2 in SSSCA1 contains a glycine (G182) and two arginine residues (R185 and R192) but these residues are not conserved among SSXCA1 orthologs and mutating these arginines to alanine residues do not alter the binding to TNKS1 (Supplementary Fig. 4, full blots in Supplementary Fig. 5c, h). This, to the best of our knowledge, could suggest that a novel binding motif between SSXCA1 and TNKS1 has been identified and that this binding mechanism needs to be investigated in the future. The protein binding between SSXCA1 and TNKS1 is likely an interaction specific to higher eukaryotes, since there are no TNKS homologs in the lower eukaryotes, like fission yeast. The interaction partner of fission yeast and other lower eukaryote SSXCA1 homologs will therefore remain unknown, but could still be an ARC like domain-containing protein, of which there are numerous.

The N-terminal zinc-binding domain shows a very high degree of conservation. We have determined the structure of this Cys4 zinc-binding domain and discovered that it belongs to a new family of undescribed zinc-binding proteins denominated Auto anti-p27 (Pfam PF06677). This type of domain is predominantly found as a zinc-binding domain and discovered that it belongs to a new family ARC like domain-containing protein, of which there are numerous homologs will therefore remain unknown, but could still be an ARC like domain-containing protein, of which there are numerous.

Methods
Phylogenetic tree and disorder prediction plots. SSXCA1 sequences (Homo sapiens ENSG00000173465, Mus musculus ENSMMUG00000017162, Bos taurus ENSBTAG0000003713, Canis lupus familiaris ENSCAFG00000013480, Rattus norvegicus ENSRNOG0000012736, Mus musculus ENSMUSG00000079974, Monodelphis domestica ENSMODG00000009665, Chrysomys picta bellia (XP_005308009.1, Strongylometra porporata XP_797294.3, Danio rerio ENSDARG00000069109, Salmo salar NP_001134714.1, Takifugu rubripes ENSTRG0000008927, Xenopus tropicalis ENSXETG00000008424, Xenopus laevis NP_001079517.1, Petromyzon marinus ENSPMAG00000009726, Bisporus pulchelus LTM625, Crassostrea gigas EKG32325, Nematostella vectensis XP_0250401, Ciona savignyi ENSCSA00000001672, Amphimedon queenslandica 1FLCF9, Ceornithabditis elegans Y930A1A.3, Dictyostelium discoideum XP_6463241.4, S. pombe NP_596406.1) were retrieved from Ensembl, Genbank or UniProt. Sequences were visualized in Jalview and aligned using T-Coffee. A phylogenetic tree was then constructed using the neighbor-joining tree method and edited in Dendroscope. Prediction of protein disorder was done using PONDR-FIT. The predictor is the newest development of the PONDR (Predictors Of Natural Disordered Regions) series and shows good accuracy.

Cell culture. HeLa “Kyoto” cells were grown in DMEM (Gibco) and LNCaP cells in RPMI, both supplemented with 10% fetal calf serum (FCS) and maintained at 37 °C, 5% CO2, SW480 cells were grown in L-15 medium (ATCC) supplemented with 10% FCS and maintained at 37 °C. For viability assays, the amount of viable cells was determined using TACS MTT Cell Proliferation Assays (Trevisgen, 4090–025 K) following the manufacturer’s recommendations.

Plasmids, constructs and transfection. Human SSXCA1 (SSXCA1 FL, residues A2-H199) was synthesized by GenScript and cloned, using the XhoI and Sulf restriction sites, into pNGFP-EU mammalian expression vector with an N-terminal 8x His and EGFP tag. The plasmids constructs SSXCA1-Δ(Zn-Pro) (residues 149–199) and SSXCA1-H2,local ΔX (L180A, L183A, I184A, L191A, L194A, L197A) were generated by GenScript from the original construct SSXCA1 FL,pNGFP-EU. The constructs SSXCA1-MAPK-H2 (mutant residues 2–34) were kindly provided by Dr. Yan Xu. The constructs SSXCA1-MAPK-H2 (ΔH1-ΔH2) (residues 2–111), SSXCA1-ΔH2 (residues 2–172) were all generated by insertion of a stop codon in the construct SSXCA1 FL,pNGFP-EU using the QuickChange Lightning Site-Directed Mutagenesis Kit (Stratagene). The constructs were introduced into the HeLa cell line using Lipofectamine 2000 (Invitrogen).
Enzyme Site-Directed Mutagenesis kit (Agilent) and the following primer pairs (Sigma-Aldrich): SSSCA1-Δ-H1 (H2) sens 5′-gcggccttcatcaaatctctcagggc-3′ and antisens 5′-ggcggccttcatcaaatctctcagggc-3′, SSSCA1-Δ-H1 (H2) sens 5′-gctgcgtcctcagggc-3′ and antisens 5′-gctgcgtcctcagggc-3′, SSSCA1-Δ-H1 (H2) sens 5′-gctgcgtcctcagggc-3′ and antisens 5′-gctgcgtcctcagggc-3′. Finally, the construct SSSCA1-Δ (residues 85–199) was generated by mutagenesis from human SSSCA1 FLP/PNGFP-EU using the QuickChange Lightning Enzyme Site-Directed Mutagenesis kit and the following primer pair (Sigma-Aldrich): sens 5′-cgagcgcagagtatttcagggcgcgggccgtggctggcgggccgtg-3′ and antisens 5′-cctgcggcagagtattttctcagggcgcgggccgtggctggcgggccgtg-3′. The human TNKS1/pEGFP-C1 construct (TNFS1 FL, residues 1–1327) was kindly provided by Prof. Harald Stenmark (The Norwegian Radium Hospital, Oslo, Norway). TNKS1 FL was cloned by GenScript, using the Xhol and Sal restriction sites, into pPNGFP-EU mammalian expression vector with an N-terminal 8x His kit and the following primer pair (Sigma-Aldrich): sens 5′-ggcgccatgacggcgtggtgggaccgggttatgagc-3′ and antisens 5′-ggtgcgtcctcagggc-3′, SSSCA1-Δ-H1 (H2) sens 5′-gctgcgtcctcagggc-3′ and antisens 5′-gctgcgtcctcagggc-3′. The human TNKS1/pEGFP-C1 construct (TNFS1 FL, residues 1–1327), TNKS1-ΔARC1-CARP (residues 1–331), TNKS1-ΔARC3-CARP (residues 1–502), and TNKS1-Δ-H1-ARC4-CARP (residues 1–502) were all generated by GenScript from human SSSCA1 FLP/PNGFP-EU. To achieve a complete lysis, cells were sonicated for 2 min at 15,000 × g. Peptides were purified and resolved by electrophoresis. Gel lanes were cut into slices and subjected to fluorescence microscopy. Hela cells were seeded in 24-well plates on glass slides 24 h before transfection. Eighteen hours post transfection, the cells were washed once in PBS and lysed for 30 min in 50 mM Tris-Cl pH 7.4, 150 mM NaCl, 5% glycerol, 0.5% NP40, 500 mM imidazole). The eluates were then incubated with PBS and washed with 10 min with PBS-50 mM NH4Cl. Fixed cells were then permeabilized for 10 min with PBS-0.25% Saponin. Cells were incubated overnight at 4°C in a wet chamber with a 1:100 dilution of a mouse anti-SSSCA1 antibody (2F5, Novus Biologicals) or a 1:200 dilution of rabbit anti-TNKS1/2 antibody (sc-8337, Santa Cruz Biotechnology) and a 1:50 dilution of mouse anti-γ-tubulin (NB100-74421, Novus Biologicals) in PBS-0.25% Saponin. Cells were washed three times with PBS-0.25% Saponin and stained for 20 min in a wet chamber with 1:200 dilution of anti-mouse-AlexaFluor 594 or anti-rabbit-AlexaFluor 680 secondary antibody (Invitrogen). After three washes with PBS-0.25% Saponin, the nuclei were stained for 8 min with 2 µg/ml Hoechst 33258 (Invitrogen). Finally, cells were washed with PBS, quickly rinsed in H2O and mounted in mowiol 4–88. Images were obtained on LSM 510 (Zeiss) microscope with a 63 × 0.55 numerical aperture oil objective. Fluorescence polarization binding assays. Human TNKS1’s ARC subdomains 1–3 (ARC1–3, residues 178–645) were cloned from TNKS1 FL/pNGFP-EU into pETM11 bacterial expression vector by GenScript, using the Ncol and XhoI restriction sites. We purified TNKS1 ARC1–3 from E. coli, following the protocol of Eisenmann et al. 72. Binding reactions were performed in a total volume of 50 µL with 50 nM 5-FAM-Ahx-conjugated peptides and the indicated concentrations of ARC1–3 protein in FP buffer (25 mM HEPES pH 8, 150 mM NaCl, 1 mM TCEP, 1.5% glycerol, and 0.05% CHAPS). Mixtures were incubated at room temperature for 15 min. After 2 min, the mixture was loaded onto the column (33% acetonitrile). FP measurements were recorded in a Jasco FP-8500 Spectrofluorometer with an excitation of 470 nm and an emission of 520 nm. Means of four replicates (n = 4) and standard error of the mean (SEM) are shown. Nonlinear regression with FP values was performed in GraphPad Prism 7.00 (GraphPad Software, La Jolla, USA) using a one-site total binding model. All conjugated peptides were purchased from GenScript: Axin1 positive control (EDAPRPVPVGE), Axin1 G-R negative control (EDAPRPVPVPEEG), SSSCA1-H1 (DVMACTQTTALLQKL, TWASAEGLSS), and SSSCA1-H2 (TSLTIQLCGILRACAEILSQQLI). Cloning, expression and purification of SSSCA1 Auto-p27. The N-terminally-heterodimeric SSSCA1 (Auto-p27 domain, M1-S111) was synthesized and codonized for E. coli expression by GenScript. Auto-p27 was cloned into a pETM11 vector using the Ncol and XhoI restriction sites, with an N-terminal 6x His tag and a tobacco etch virus (TEV) protease cleavage site. The plasmid was transformed into E. coli Rosetta 2 cells (Novagen). Colonies were inoculated in standard Luria-Bertani (LB) medium overnight (o/n) at 37°C, 200 rpm, containing 35 µg/mL chloramphenicol (Cam) and 50 µg/mL kanamycin (Kan). Fresh o/n cultures were used to inoculate 3 L LB medium containing 35 µg/mL Cam, 50 µg/mL Kan and 500 µL/L polypropylene glycol 2000 anti-foam (Sigma-Aldrich). The culture was grown at 37°C, with full airflow in a LEX HT Bioreactor (Harbinger), until OD600 reached 0.6–0.8. The culture was cooled-down on ice and expression of Auto-p27 was induced by addition of 1 mM IPTG. Protein growth continued until the OD600 was 19–20°C in the c) mass spectrometer coupled to a nano-LC system. Peptides were purified by C18 ZipTips (Millipore) before injected into an Ultimate 3000 nanoLC system (Dionex). For separation of peptides an Acclaim PepMap 100 column (50 µm x 75 µm) packed with 100 Å C18 3 µm particles (Dionex) was used. A flow rate of 300 nL/min was employed with a solvent gradient of 7–35% B in 40 min, to 50% B in 3 min and then to 80% B in 2 min. Solvent A was 0.1% formic acid and solvent B was 0.1% formic acid/90% acetonitrile. The mass spectrometer was operated in data-dependent mode and survey full scan MS spectra were acquired in the Orbitrap with 60,000 resolution at m/z 400. The method allowed sequential isolation of the seven most intense ions in the linear ion trap using collision-induced dissociation (CID). Target ions already selected for MS/MS were dynamically excluded for 60 s. Bioinformatics. MS data were acquired using Xcalibur v2.2.5 and processed using Proteowizard v3.0,5047. Mascot v2.3 was used as search engine and the data were searched against the human subset of SwissProt v2011_11 using trypsin as enzyme with one missed cleavage allowed. Variable modifications allowed included: protein N-terminal acetylation, methionine oxidation, pyro-glutamic acid for N-terminal glutamines, propionamide on cysteines, deamidation of glutamines and asparagines as well as phosphorylation of serines, threonines and tyrosines. Mascot results were imported into Scaffold v3.6 with the identification criteria: minimum two peptides per protein, peptide probability >90% and protein probability >99%. DAF values (Digital autoradiography) were calculated as described in an earlier publication (GE Healthcare). Bound proteins were eluted with an imidazole gradient from 20 to 500 mM in 1.5 mL fractions. Fractions were analyzed by SDS-PAGE gel electrophoresis. Proteins were transferred to PVDF membranes and incubated with a 1:2000 dilution of mouse anti-SSSCA1 (2F5, Novus Biologicals), rabbit anti-TNKS1/2 (1:2000, sc-8337, Santa Cruz Biotechnology), rabbit anti-γ-tubulin (1:1000, ab20722, Abcam), mouse anti-γ-tubulin (1:1000, NB1000–74421, Novus Biologicals), diluted in TBS-T-5% BSA or ~5% nonfat dry milk. Primary antibodies were visualized with secondary HRP-conjugated antibodies (Cell Signaling Technology) and Chemiluminescence Blotting substrate (Roche) on a Chemidoc Imaging System (Bio-Rad).
SDS–PAGE and target fractions were pooled. To cleave the His tag, GFP-TEV protease was added at 0.05 mg/ml and incubated for 16 h in the cold room on a magnetic stirrer. After dialysis to obtain final concentration of 20 mM imidazole, the noncleaved Auto_anti-p27 was removed by applying the protein solution to the Ni2+–column again and collecting the flow-through.

The flow-through of the second IMAC was concentrated down to 1–2 ml at 10–15 mg/ml before being applied to a HiLoad Superdex 75 16/600 PG 120 ml gel filtration column (GE Healthcare) equilibrated in separation buffer (20 mM HEPES pH 7.4, 200 mM NaCl, 5% glycerol, and 0.1 mM TCEP). The protein was eluted with 1 CV of separation buffer in fractions of 1.5 ml and the purified protein was analyzed again by SDS–PAGE. Target fractions were pooled and concentrated above 30 mg/ml using a Vivatip 50–10,000 MWCO centrifugal filter device (Sartorius Stedim Biotech). Protein concentration was measured by absorption at a wavelength of 280 nm using a calculated extinction coefficient on a NanoDrop 2000 Spectrophotometer (Thermo Fisher Scientific). The purified protein solution was aliquoted, flash frozen with liquid nitrogen and stored at −80 °C until further use.

To estimate the native molecular weight of Auto_anti-p27, the common standard proteins (MWGF200-1KT, Sigma-Aldrich) cytochrome C (12.4 kDa), carbonic anhydrase (29 kDa), and albumin serum bovine (66 kDa) were run on the HiLoad Superdex 75 16/600 PG column using the same buffer and conditions.

**Crystallization and data collection**

An aliquot of 100 µl of purified and concentrated SSSCA1 Auto_anti-p27 protein was thawed, buffer exchanged against a resolution and optimized dataset data extended to 2.3 Å on the P14 beamline at the PDB and given the PDB accession code (6HCZ).

**Statistics and reproducibility.** Unless otherwise noted, experiments were repeated at least three times. All data are presented as means ± SEM or standard deviation, as indicated for each graph. Statistical analyses between groups were determined by unpaired t test in GraphPad Prism 7.00 (GraphPad Software, La Jolla, California, USA). A P value of <0.05 was considered statistically significant.

**Reporting summary.** Further information on research design is available in the Nature Research Reporting Summary linked to this article.

**Data availability**

Data that support the findings of this study have been deposited in protein data bank with the PDB identifier code 6HCZ. Additional data are implemented as Supplementary data. All other relevant data are available from the corresponding author.

Received: 30 October 2019; Accepted: 21 February 2020; Published online: 13 March 2020

**References**

1. Yi, Y., Yamada, T., Himeno, M. & Sugimoto, K. cDNA cloning of a novel autoantigen targeted by a minor subset of anti-centromere antibodies. Clin. Exp. Immunol. **111**, 372–376 (1998).
2. Sakuma-Takagi, M. et al. Novel related cDNAs (C184L, C184M, and C184S) from developing mouse brain encoding two apparently unrelated proteins. Biochem. Biophys. Res. Commun. **263**, 737–742 (1999).
3. Urban, M. & Stredim Biotech. Towards a knowledge-based Human Protein Atlas. Nat. Biotechnol. **28**, 1248–1250 (2010).
4. Wang, M., Herrmann, C. J., Simonovic, M., Szklarczyk, D. & von Mering, C. Version 4.0 of PaxDb: protein abundance data, integrated across model organisms, tissues, and cell-lines. Proteomics **15**, 3163–3168 (2015).
5. Li, X. et al. Proteinomic Analysis of the Human Tankyrase Protein Interaction Network Reveals Its Role in Pexophagy. Cell Rep. **20**, 737–749 (2017).
6. Huang, S. M. et al. Tankyrase inhibition stabilizes axin and antagonizes Wnt signalling. Nature **461**, 614–620 (2009).
7. Voronkov, A. et al. Structural basis and SAR for G007-LK, a lead stage 1,2,4-triazole based specific tankyrase 1/2 inhibitor. J. Med. Chem. **56**, 3012–3023 (2013).
8. Yang, Y. et al. RNF146 is a poly(ADP-ribose)-directed E3 ligase that regulates axin degradation and Wnt signalling. Nat. Cell Biol. **13**, 623–629 (2011).
9. Giles, R. H., van Es, J. H. & Clevers, H. Caught up in a Wnt storm: Wnt signalling in cancer. Biochem. biophys. Acta **1653**, 1–24 (2003).
10. Sillars-Hardebol, A. H. et al. Identification of key genes for carcinogenic pathways associated with colorectal adenoma-to-carcinoma progression. *Tumor Biol.**31**, 89–96 (2010).
11. Sillars-Hardebol, A. H., Carvalho, B., van Engeland, M., Fijneman, R. J. & Meijer, G. A. The adenoma hunt in colorectal cancer screening: defining the target. *Pathol. **226**, 1–2 (2012).
12. Vendrell, J. A. et al. Candidate molecular signature associated with tamoxifen failure in primary breast cancer. Breast Cancer Res. **10**, R88 (2008).
13. Baxter, J. S. et al. Capture Hi-C identifies putative target genes at 33 breast cancer risk loci. Nat. Commun. **9**, 1028 (2018).
14. Ferreira, M. A. et al. Genome-wide association and transcriptome studies identify target genes and risk loci for breast cancer. Nat. Commun. **10**, 1741 (2019).
15. Xu, C. et al. Integrative analysis of DNA copy number and gene expression in metastatic oral squamous cell carcinoma identifies genes associated with poor survival. Mol. Cancer **9**, 143 (2010).
16. Stigliani, S. et al. High genomic instability predicts survival in metastatic high-risk neuroblastoma. *Neoplasia**14*, 823–832 (2012).
17. Xue, A. et al. Genome-wide association analyses identify 143 risk variants and putative regulatory mechanisms for type 2 diabetes. Nat. Commun. **9**, 2941 (2018).
18. Huttlin, E. L. et al. The BioPlex network: a systematic exploration of the human interactome. **Cell** **162**, 425–440 (2015).
19. Al-Hakim, A. K., Bashkurov, M., Gingras, A. C., Durocher, D. & Pelletier, L. Interaction proteomics identifies NEURL4 and the HECT E3 ligase HERC2 as novel modulators of centrosome architecture. *Mol. Cell Proteom.**11**, M111 014233 (2012).
20. Kim, T. Y. et al. Substrate trapping proteomics reveals targets of the betaTrCP2/FBXW11 ubiquitin ligase. Mol. Cell Biol. **35**, 167–181 (2015).
21. Kim, W. et al. Systematic and quantitative assessment of the ubiquitin–modified proteome. *Mol. Cell** **44**, 325–340 (2011).
22. Dinesen, J. M. et al. Mass spectrometric analysis of lysine ubiquitylation reveals promiscuity at site level. *Mol. Cell Proteom.**10**, M110 00390 (2011).
23. Udesli, N. D. et al. Methods for quantification of in vivo changes in protein ubiquitination following proteasome and deubiquitination inhibition. *Mol. Cell Proteom.**11**, 148–159 (2012).
24. Mertins, P. et al. Integrated proteomic analysis of post-translational modifications by serum enrichment. Nat. Methods **10**, 634–637 (2013).
25. Finn, R. D. et al. The Pfam protein families database: towards a more sustainable future. *Methods Mol. Biol.* **637**, 243–266 (2013).
26. Finn, R. D. et al. The Pfam protein families database: towards a more sustainable future. *Methods Mol. Biol.* **637**, 243–266 (2013).
27. Anastas, J. N. & Moon, R. T. WNT signalling pathways as therapeutic targets in cancer. *Nutr. Rev. Mol. Cell Biol.* **8**, 645–654 (2007).
28. Huang da, W., Sherman, B. T. & Lemppicki, R. A. Systematic and integrative analysis of large gene lists using DAVID biominformatics resources. *Nat. Protoc.* **4**, 44–57 (2009).
29. Ashburner, M. et al. Gene Ontology: tool for the unification of biology. *Nat. Genet.* **25**, 25–29 (2000).
30. Jensen, L. J. et al. STRING 8-a global view on proteins and their functional interactions in 630 organisms. *Nucleic Acids Res.* **37**, D412–D416 (2009).
31. Morrone, S., Cheng, Z., Moon, R. T., Cong, F. & Xu, W. Crystal structure of a Tankyrase-Axin complex and its implications for Axin turnover and Tankyrase substrate recruitment. *Proc. Natl Acad. Sci. USA* **109**, 1500–1505 (2012).
32. DaRosa, P. A. et al. Allosteric activation of the RNF146 ubiquitin ligase by a poly(ADP-ribosyl)ation signal. Nature **517**, 223–226 (2015).
33. Pollock, K., Ranes, M., Collins, I. & Guettler, S. Identifying and validating Tankyrase binding and substrates: a candidate approach. *Methods Mol. Biol.* **1608**, 445–473 (2017).
34. Krisenfeld, E. & Henrick, K. Inference of macromolecular assemblies from crystalline state. J. Mol. Biol. **372**, 774–797 (2007).
35. Holm, L. & Rosenstrom, P. Dali server: conservation mapping in 3D. *Nucleic Acids Res.* **38**, W545–W549 (2010).
37. Krissinel, E. & Henrick, K. Secondary-structure matching (SSM), a new tool for fast protein structure alignment in three dimensions. Acta Crystallogr. D. Biol. Crystallogr. 60, 2256–2268 (2004).

38. Aragon, E. et al. A Smad action turnover switch operated by WW domain readers of a phosphoserine code. Genes Dev. 25, 1275–1288 (2011).

39. Meiyanpan, M., Birrane, G. & Ladias, J. A. A structural basis for polyproline recognition by the F65E WW domain. J. Mol. Biol. 372, 970–980 (2007).

40. Qi, S., O’Hare, M. G. & Lodish, H. F. Intrinsic disorder and the canonical Wnt pathway that limits the persistence of non-small cell lung cancer cells following EGFR inhibition. Clin. Cancer Res. 23, 1531–1541 (2017).

41. Grigoryan, T., Wend, P., Klaus, A. & Birchmeier, W. Deciphering the function of canonical Wnt signals in development and disease: conditional loss- and gain-of-function mutations of beta-catenin in mice. Genes Dev. 22, 2308–2341 (2008).

42. la Cour, T. et al. Analysis and prediction of leucine-rich nuclear export signals. Protein Eng. Des. Sel. 23, 235–240 (2010).

43. Wen, W., Meinkoth, J. L., Tsien, R. Y. & Taylor, S. S. Identification of a signal for rapid export of proteins from the nucleus. Cell 82, 463–473 (1995).

44. Hwang, I. S., Woo, S. U., Park, J. W., Lee, S. K. & Yim, H. Two nuclear export signals of Cdk6 are differentially associated with CDK-mediated phosphorylation residues for cytoplasmic translocation. Biochim. et. Biophys. Acta 1843, 223–233 (2014).

45. Taylor, D. T. et al. Human chromosome 11 DNA sequence and analysis including novel gene identification. Nature 440, 497–500 (2006).

46. Koreth, J., Bakkenist, C. J. & McGee, J. O. Chromosomes, 11q and cancer: a review. J. Pathol. 187, 28–38 (1999).

47. Lammie, G. A. & Peters, G. Chromosome 11q13 abnormalities in human cancer. Cancer Cells 3, 413–420 (1991).

48. Srivatsan, E. S. et al. Fine mapping of a region of chromosome 11q13 reveals multiple independent loci associated with risk of prostate cancer. Hum. Mol. Genet. 20, 2869–2878 (2011).

49. International Chromosome Genome Sequencing, C. Sequence and comparative analysis of the chicken genome provide unique perspectives on vertebrate evolution. Nature 432, 695–716 (2004).

50. Adams, M. D. et al. The genome sequence of Drosophila melanogaster. Science 287, 2185–2195 (2000).

51. Smith, S. & de Lange, T. Tankyrase promotes telomere elongation in human cells. Curr. Biol. 10, 1299–1302 (2000).

52. Chang, P., Coughlin, M. & Michelson, T. J. Tankyrase-1 polymerization of poly(ADP-ribose) is required for spindle structure and function. Nat. Cell Biol. 7, 1133–1139 (2005).

53. Callow, M. G. et al. Ubiquitin ligase RNF146 regulates tankyrase and Axin to promote Wnt signalling. PLoS One 6, e22595 (2011).

54. Wang, Z. et al. Recognition of the iso-ADP-ribose moiety in poly(ADP-ribose) by WW domains suggests a general mechanism for poly(ADP-ribose)ylation-dependent ubiquitination. Genes Dev. 26, 235–240 (2012).

55. Chi, N. W. & Lodish, H. F. Tankyrase is a golgi-associated mitogen-activated protein kinase substrate that interacts with IRAP in GLUT4 vesicles. J. Biol. Chem. 275, 38437–38444 (2000).

56. Lau, T. et al. A novel tankyrase small-molecule inhibitor suppresses APC-mutation-driven colorectal tumor growth. Cancer Res. 73, 3132–3142 (2013).

57. Kawasaki, T. & Gouaux, E. Fluorescence-detection size-exclusion chromatography for precystalization screening of integral membrane proteins. Structure 14, 673–681 (2006).

58. Ishihama, Y. et al. Exponentially modified protein abundance index (emPAI) for estimation of absolute protein amount in proteomics by the number of sequenced peptides per protein. Mol. Cell Proteom. 4, 1265–1272 (2005).

59. Eiseeman, T. et al. Tankyrase-1 ankyrin repeats form an adaptable binding platform for targets of ADP-ribose modification. Structure 24, 1679–1692 (2016).

60. McCoy, A. J. et al. Phaser crystalllographic software. J. Appl. Crystallogr. 40, 658–674 (2007).

61. Emsley, P., Lohkamp, B., Scott, W. G. & Cowtan, K. Features and development of Coot. Acta Crystallogr. D. Biol. Crystallogr. 66, 486–501 (2010).

62. Adams, P. D. et al. PHENIX: a comprehensive Python-based system for macromolecular structure solution. Acta Crystallogr. D. Biol. Crystallogr. 66, 213–221 (2010).

63. Murshudov, G. N., Vagin, A. A. & Dodson, E. J. Refinement of macromolecular structures by the maximum-likelihood method. Acta Crystallogr. D. Biol. Crystallogr. 53, 240–255 (1997).

64. Chen, V. B. et al. MolProbity: all-atom structure validation for macromolecular crystallography. Acta Crystallogr. D. Biol. Crystallogr. 66, 12–21 (2010).

Acknowledgements H.P.D. was supported by the Norwegian Cancer Society (grant no. 4483570–2013). S.K. was supported by the Research Council of Norway (grants no. 262613 and 174938/030). We thank Jo Waaler for giving us an aliquot of the SW480 cells and protocols on how to culture them.

Author contributions H.P.D. and J.P.M. conceived and planned the experiments, and analysed the data. H.P.D. performed the experiments. J.P.M. carried out the crystal data collection and structure determination. C.P. contributed to the confocal microscopy. S.J.B. performed the sRNA and ChIP experiments. H.G. helped with the cell culture experiments. B.T. and M.A. performed the mass spectrometry study and the bioinformatic analysis. O.B., I.G.M., and S.K. provided critical feedback. H.P.D. and J.P.M. wrote the paper with input from all authors.

Competing interests The authors declare no competing interests.

Additional information Supplementary information is available for this paper at https://doi.org/10.1038/s42003-020-0851-2.

Correspondence and requests for materials should be addressed to J.P.M.

Reprints and permission information is available at http://www.nature.com/reprints

Publisher’s note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.