Supporting Information

Morrone et al. 10.1073/pnas.1116618109

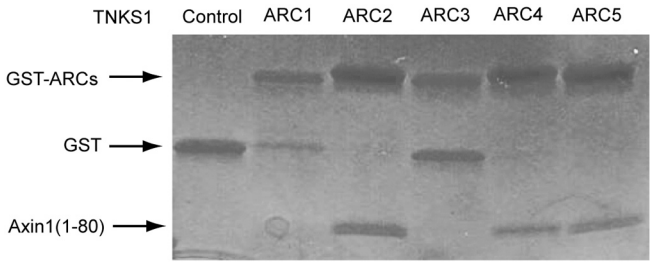


Fig. S1. Mapping of the Axin-binding domain of TNKS1. GST-tagged individual mTNKS1 ankyrin-repeat clusters (ARC) are purified using glutathione beads and then mixed with untagged mAxin1(1-80). After extensive wash, beads were boiled with SDS loading buffer, and sample are analyzed using SDS-PAGE. GST is used as a negative control. ARC2, ARC4, and ARC5, but not ARC1 and ARC3 interact with Axin1(1-80). This result is consistent with the sequence conservation in the gate-forming residues corresponding to Y372 and Y405 in ARC2. The gel was stained with Coomassie Brilliant Blue.

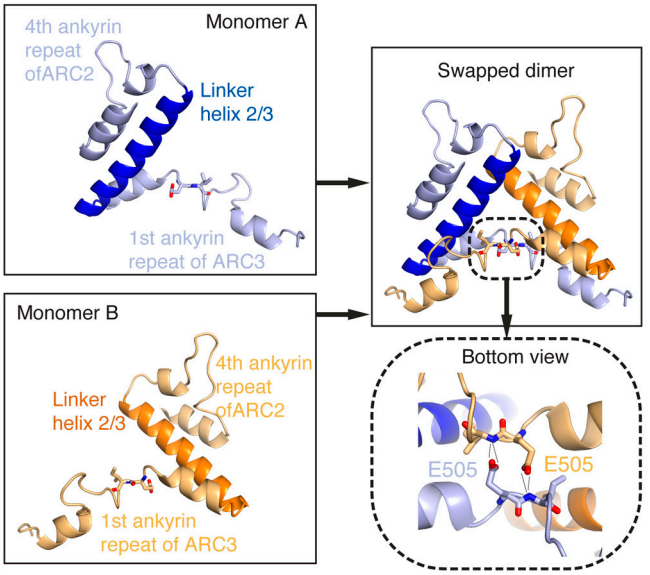
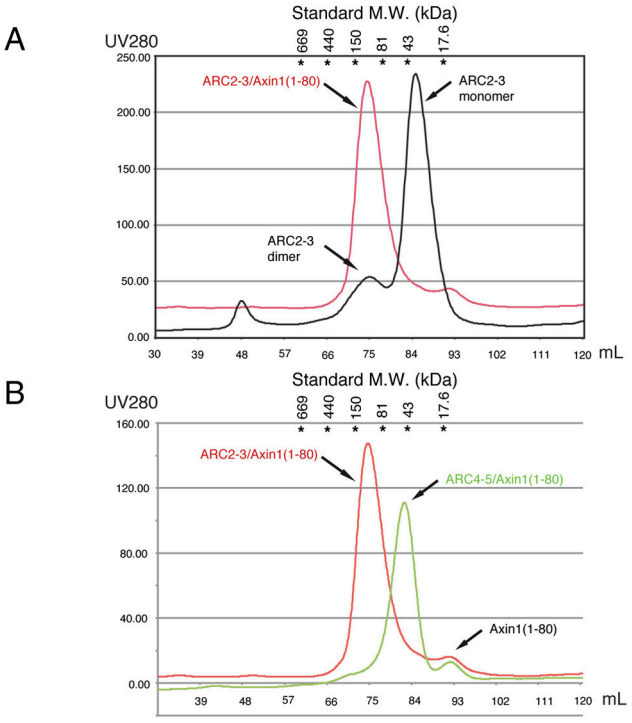


Fig. S2. Detailed TNKS structure that stabilizes domain swapping in ARC3. The linker helix 2/3 and its flanking ankyrin repeats from ARC2 and ARC3 are shown in cartoon. *Left*: Monomers are shown in light blue and light orange for ankyrin repeats, blue and orange for linker helixes. *Right*: Close-up view of the swapped dimer. Residue Glu505 in the dimer interface, which may play an important role in stabilizing the swap, is shown in stick model.



C

Summary of Dynamic Light Scattering (DLS)

Protein sample	Rh (nm)	%Poly dispersity	Measured M.W. (kDa)	Predicted M.W. (kDa) (1:1)
ARC2-3	3.98	13.6	82.5	75.6(dimer)
ARC2-3 Axin1(1-80)	4.69	14.3	123	84.1(2:1)
ARC4-5	3.15	12.6	46.8	37.8(monomer)
ARC4-5 Axin1(1-80)	3.53	18.7	61.7	46.3(1:1)

Fig. S3. Size-exclusion chromatography (SEC) and dynamic light scattering (DLS) analysis of ARC2-3 and ARC4-5, with/without mAxin1(1-80). (*A*) SEC of TNKS1 ARC2-3 and its complex with Axin1 (1-80). Individually purified proteins were mixed with excess of Axin1(1-80) and incubated for 1 h at 4 °C prior to SEC. Standard molecular weight was labeled with "*" in corresponding positions. TNKS1 ARC2-3 by itself tends to form dimer, whereas Axin1(1-80) binding stabilizes the TNKS ARC2-3 dimerization. (*B*) SEC of TNKS1 ARC2-3/Axin1(1-80) and ARC4-5/Axin1(1-80) complexes. TNKS1 ARC4-5 does not dimerize at the presence of Axin1(1-80). (*C*) DLS analysis of the above samples. The derived molecular weights of TNKS1 ARC2-3, ARC4-5, and mAxin1(1-80) are 37.8 kDa, 37.8 kDa, and 8.5 kDa, respectively. The measured molecular weight (MW) of TNKS1 ARC2-3 and its complex with mAxin1(1-80) are 82.5 and 123 kDa, which are close to the calculated MW (dimer form), indicating the dimerization tendency of TNKS1 ARC2-3 and the stabilization of the dimeric form by Axin binding. In contrast, the measured MW of ARC4-5 and its complex with mAxin1(1-80) are close to the predicted MW in monomeric form, suggesting that ARC4-5 does not form stable dimer, even in the presence of Axin1(1-80).

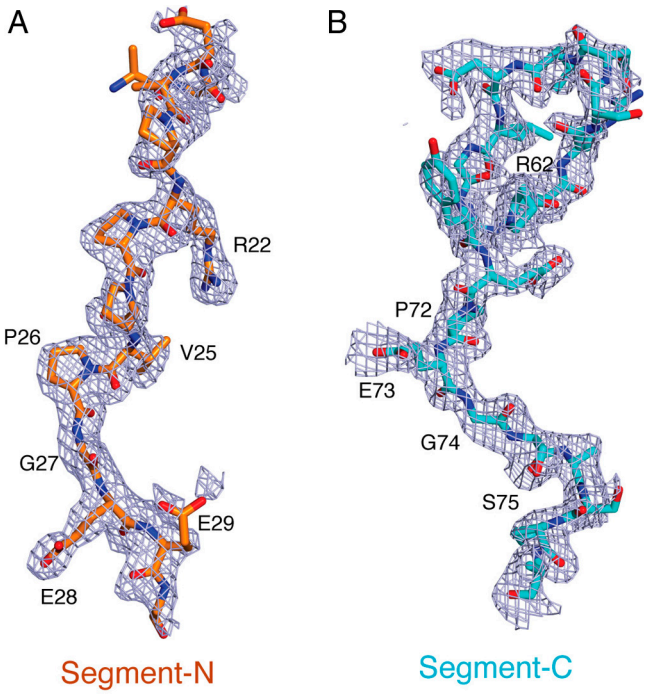


Fig. S4. Electron densities for Axin1(18–30) and Axin1(60–79). The segment-N (orange) and segment-C (cyan) of mAxin1(1-80) are shown in stick model. The corresponding F_o-F_c electron density maps (contoured at 2.0σ) are shown in light-blue mesh. Some of the key residues are labeled.

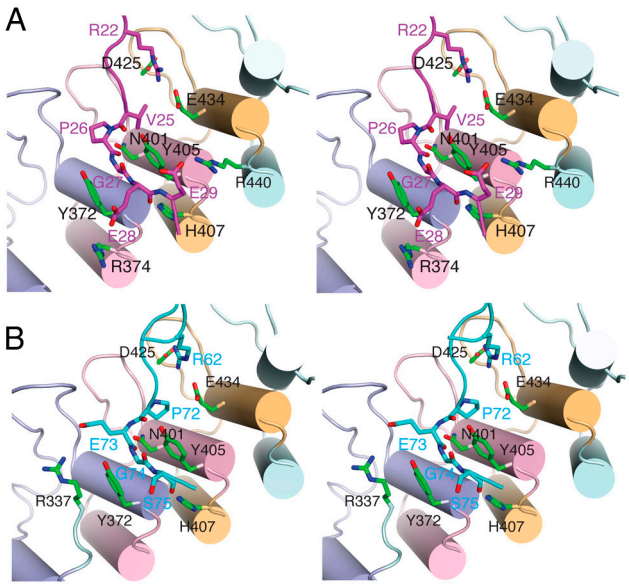


Fig. S5. Stereo view of the interactions between ARC2 and two Axin segments. The four ankyrin repeats in mTNKS1 ARC2 are shown in cylindrical cartoon with light blue, light pink, light orange, and white, respectively. Segments of mAxin are shown in stick model with magenta (segment-N in A) and cyan (segment-C in B). Key residues involved in the interface are labeled.

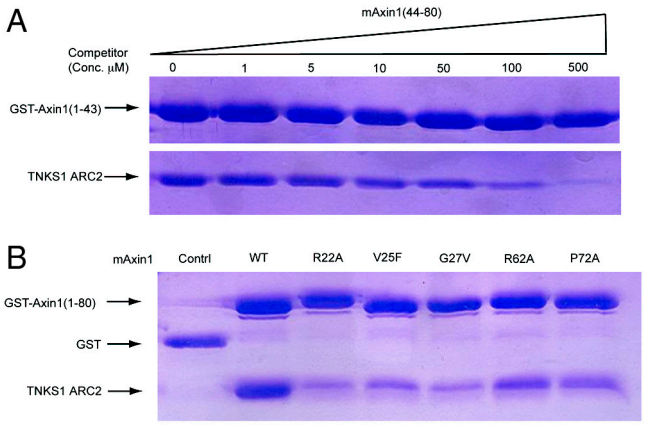


Fig. S6. Biochemical characterization of the TNKS ARC2-Axin interaction. (*A*) Competition of the TNKS ARC2-Axin1(1–43) interaction by Axin1(44–80). GST-tagged Axin1(1–43), untagged TNKS-ARC2, and increasing concentrations of untagged Axin1(44–80) were incubated together with glutathione beads for 1 h on ice. The mixture was then washed four times before boiled with SDS loading buffer for SDS-PAGE. The gel was stained with Coomassie Brilliant Blue. (*B*) Binding of Axin1(1-80) mutants to TNKS ARC2. GTS-tagged Axin1(1-80), either wild-type (WT) or mutants as labeled, is used to pull down untagged TNKS ARC2. Consistent with the presence of two TNKS binding sites in Axin1(1-80), no point mutation could completely disrupt the TNKS-Axin1(1-80) interaction. This is in sharp contrast with the mutant binding results in individual Axin segments (Fig. 3*C* and *D*).

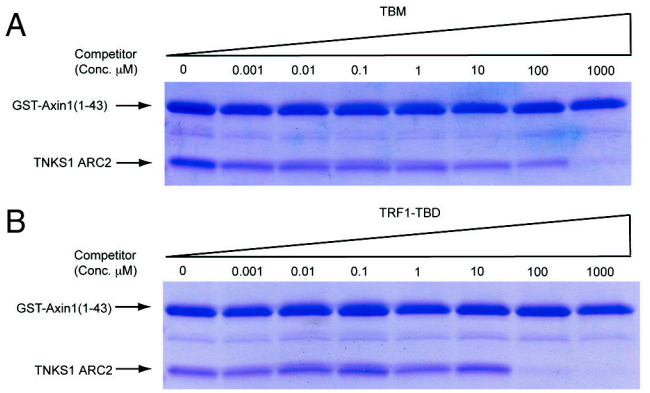


Fig. S7. Competition of the TNKS ARC2-Axin1(1–43) interaction by TBM. (*A*) Competition using a nine residue TBM peptide. (*B*) Competition using the TNKS-binding domain of human telomere repeat-binding factor 1 (TRF1-TBD; residues 1–55), which contains a TBM. GST-tagged Axin1(1–43), untagged TNKS-ARC2, and increasing concentrations of untagged TBM or TRF1-TBD were incubated together with glutathione beads for 1 h on ice. The mixture was then washed four times before boiled with SDS loading buffer for SDS-PAGE. The gel was stained with Coomassie Brilliant Blue.

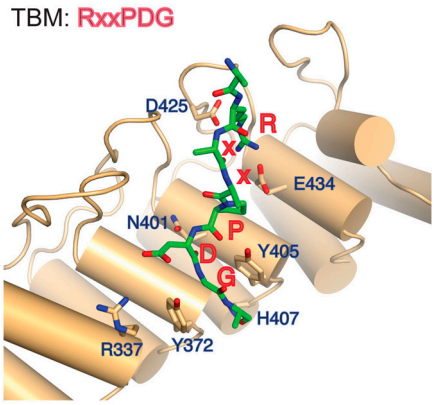


Fig. S8. A model for tankyrase binding motif (TBM) peptide binding. TNKS ARC2 is shown in light orange cylindrical cartoon. Modeled TBP peptide with a consensus sequence of RxxPDG is shown in green stick model. The conformations of TBM Rxx and PDG residues are modified from Axin segment-N and -C, respectively. Residues of TNKS ARC2 are shown in light orange stick model.

Table S1. Statistics of crystallographic analysis

Protein complex	mTNKS1(308-655)—mAxin1(1-80) complex
PDB code	3UTM
Data collection	
Space group	C2
Cell dimension, Å/°	$a = 131.67$, $b = 106.54$, $c = 73.43$, $\beta = 105.76$
Resolution, Å *	30–2.0 (2.05–2.0)
Observed reflections	127,920
Unique reflections	62,479
Redundancy	6.9
Completeness, %*	99.8 (99.9)
$\langle I/\sigma \rangle$ *	30 (2.7)
R_{sym} * †	0.059 (0.66)
Refinement	
Resolution, Å	30–2.0
R_{work}/R_{free}(%) ‡	19.84/22.77
Number of atoms	5,260
Protein	5,033
Water	227
B factors	
Protein	55
Water	49
Rms deviations	
Bond lengths, Å	0.0079
Bond angle, °	1.201

*Highest resolution shell is shown in parentheses.

†$R_{sym} = \Sigma I_j - \langle I \rangle / \Sigma I_j$, where I_j is the intensity of an individual reflection, and $\langle I \rangle$ is average intensity of that reflection.

‡$R_{work} = \Sigma ||F_{obs}\text{-}F_{calc}|| / \Sigma F_{obs} R_{free}$ is the cross-validation R factor for the test set of reflections (5% of the total) omitted in model refinement.