Supplementary Table 3. Overview of the interactions at the interface between AXIN1 and GSK3β for the mutated amino acids and their predicted effects.

Amino acid change in AXIN1	Located at interface	Original interaction of side chain	Predicted effect of amino acid change	Expected effect on complex formation
P385A	Yes, at edge	Cβ carbon of Proline 385 in AXIN1 makes weak vdWaals interaction with ring of Phe291 of GSK3β at 4.0 Å.	Cβ carbon of Alanine will still make the same vdWaals interaction with Phe291 of GSK3β.	None
Q386H	Not really, on the side at the surface, and points away from interface	Weak vdWaals with ring of Phe291 at 3.8 Å	Histidine can easily be accommodated on the surface	None
A389V	Yes, in hydrophobic cluster with Tyr288, Phe291, Phe293 of GSK3β	vdWaals interactions with ring of Phe291 at 3.6-3.8 Å	There is space available around the Alanine 389 sidechain to accommodate the extra carbons of Valine with only minor adjustments of the side chain positions of surrounding residues in GSK3β	Minor
E391K	On the side at the surface	vdWaals with Valine263 in GSK3β at 3.6-3.8 Å	At surface so lysine can easily be accommodated and could make same vdWaals interaction	None
H394N	No, at opposite side of helix	None, points away into solution	Polar asparagine would similarly point away into solution	None
R395H	Yes, at the side on the surface	Salt-bridge (ion-pair and hydrogen bond) with Asp264 on GSK3β	Histidine can be accommodated at surface, is also positively charged so ion-pair still possible but weaker due to shorter side chain. Hydrogen bond with Asp264 can no longer be formed.	Small effect expected due to loss of hydrogen bond at surface.
R395P	Idem	Idem	Loss of surface salt bridge with Asp264. A Proline in the middle of the AXIN1 α-helix will distort the helix and force rearrangements of atoms surrounding this region including the hydrophobic interface between AXIN1 and GSK3β	Large
R395C	Idem	Idem	Cysteine can be accommodated at the surface but the salt-bridge interaction with Asp264 will be lost	Small effect due to loss of surface salt-bridge
L396M	Yes, in hydrophobic cluster with Val267, Ile270, Pro294 and Ile296	vdWaals interactions with surrounding hydrophobic residues	There is space available in the hydrophobic cluster to accommodate the Methionine side chain which is also largely hydrophobic	Minor
R401C	Not in structure			
R401H	Not in structure			
T402K	Not in structure			