**Targeted mutation to putative linchpin site**

A key site in many RING E3 ligases is an arginine or lysine residue immediately downstream of the final zinc-coordinating cysteine, which is known as the linchpin site. <what does this site do?>. 46% of RING domains have an arginine at this site linchpin, whereas 14% have lysine (Stewart). Typically, the choice of residue at this site regulates a trade-off between ubiquitination activity and E2 specificity. RINGs with a K at this site typically show lower ubiquitination activity in vitro (REF). Stewart showed for the protein <> that mutating this site from an arginine to a lysine increases ubiquitination activity but reduces E2 specificity (CHECK). However, this isn’t a universal mechanism, and many functional RING E3 ligases have other residues at this site <examples, refs>. This suggests that other mechanisms of <> must exist. Currently this is poorly understood.

Notably, however, C elegans does have a lysine at this site, suggesting a potential role as a linchpin. Alignment of Caenorhabditis PAR-2 RING domain sequences shows that this site is largely conserved. There are, however, a few exceptions in some of the more distantly related (?) species. C. bovis, castelli and monodelphis have neither an arginine nor a lysine at this site. The fact that this site isn’t universally conserved may argue against an important role for ubiquitination activity, could suggest that PAR-2 does play a role as a ubiquitin ligase, but doesn’t rely on the linchpin, or could suggest that these other species have evolved alternative strategies.

To test the potential role of linchpin-mediated autoubiquitination for PAR-2 membrane binding affinity, I used CRISPR to perform targeted mutation to this site, turning it into an A. <similar approach used in other studies>. As shown in fig x, this has no detectable effect on membrane affinity. Whilst this cannot rule out a role for ubiquitination in vivo, the result argues against a model in which linchpin mediated autoubiquitination is a driver of membrane affinity.