To test whether the RING domain can dimerise when enriched on the membrane, used a PH membrane anchor to tether it to the membrane. Should this construct be capable of dimerisation, we would expect some degree of dimerisation with endogenous PAR-2, end therefore preferential enrichment in the posterior of the cell. Whereas PH alone is uniform on the membrane, tethering the PAR-2 RING domain causes clear enrichment in the posterior, indicating an interaction, presumably a dimerisation reaction, with endogenous PAR-2. A construct with a C56S mutant, by contrast, shows a small degree of polarity, but this is greatly reduced compared to wild type.

The above results suggest that the construct is polarising by responding to polarised endogenous PAR-2. Therefore, it is expected that disrupting polarity of endogenous PAR-2 might disrupt this. To test this, I aimed to disrupt endogenous polarity using RNAi. In conditions of par-2 RNAi, endogenous PAR-2 is expected to be entirely absent from the cell. Under these conditions, as shown in figure x, the construct loses enrichment in the posterior, and overall polarity is low. Similarly, in conditions of par-6 RNAi, where endogenous PAR-2 is expected to be uniform on the cortex, albeit less weakly concentrated, the construct also loses the ability to enrich in the posterior. Whilst we might expect overall affinity to be higher in the par-6 RNAi condition, as there is some level of endogenous PAR-2 on the cortex, this doesn’t appear to be the case. This may imply a concentration dependent effect, whereby the construct is only able to respond to endogenous PAR-2 that’s highly concentrated, or could be a product of inaccurate quantification at very low signal levels.