**Targeted mutation to dimerisation interface regions**

As discussed previously, disrupting the RING domain, by targeted mutation to a zinc-interacting cysteine, has a strong phenotype in vivo, characterised by a string reduction in membrane affinity. My results suggest that this phenotype may be explained by a disruption in dimerisation at the membrane. However, as C56S mutation disrupts the tertiary structure of the whole domain, rather than just the dimerisation interface, it may also have additional effects. To confirm the specific role of dimerisation, I performed targeted mutations to the dimerisation interface region of the RING domain, aiming to disrupt dimerisation in vivo whilst keeping the tertiary structure of the domain intact. In addition to L109R, I performed an additional L>R mutation to L50, which is found in the N-helix. I introduced mutations at the endogenous site by CRISPR in an mNeonGreen::PAR-2 line.

As shown in fig x, both mutations cause a strong reduction in affinity. This effect is stronger for L109R, which may suggest that L50R, whilst partially disrupting dimerisation, is insufficient to disrupt it completely. Combination of both mutants has no effect compared to L109R alone, indicating that dimerisation is completely disrupted in L109R. Interestingly, however, neither mutants reduce membrane affinity to the same extent as C56S. Assuming that dimerisation is fully disrupted by L109R, this implies that fully disrupting the domain may have additional effects compared to just disrupting dimerisation alone. One plausible explanation is that, in the case of C56S, the unfolded domain interferes with normal membrane binding, and therefore has an additional negative effect on membrane affinity. Compatible with this, a PH::RING fusion has lower overall membrane affinity that PH alone (fig x). Combining C56S and L109R has no effect compared to C56S alone, suggesting that dimerisation is completely disrupted in the C56S mutant.