

### **Supplementary data**

S1, S2 and S3 show histograms of lifetime distributions for different combinations of proteins measured in the nucleus or at the PM. Each histogram shows the combined distributions of 5 representative cells for that combination. Taking 10 instead of 5 experiments did not change the value for the average lifetime; therefore 5 were taken for the calculations. On the horizontal axis the lifetimes are depicted, on the vertical axis the number of pixels per lifetime. In Figure S1 from top to bottom all the experiments performed with combinations of CDC48A at the PM are listed. The double combinations of CDC48A (2<sup>th</sup>, 3<sup>th</sup> and 4<sup>th</sup> histogram should be compared with the donor alone in the 1<sup>th</sup> histogram. In the right panel the experiments with CDC48A in the nucleus are depicted. Again the double combinations of CDC48A (2<sup>th</sup>, 3<sup>th</sup> and 4<sup>th</sup> histogram) should be compared with the donor alone in the 1<sup>th</sup> histogram. In Figure S2 the experiments performed with CDC48C proteins are depicted and these should be compared in the same way. In Figure S3 the CDC48A<sup>N-D1</sup> double combination should be compared with the CDC48A<sup>N-D1</sup>-donor alone.