Supplement 1: Discussion of FRAP normalization method

In this paper, FRAP recovery curves are normalized using the double normalization method of Phair et al. 2004[1]. This involves tracking the average intensity within three regions of interest: a "background" region of interest (ROI) with intensity $BG(t)$, a "bleach" ROI with intensity $I(t)$, and a "whole nucleolus" ROI with intensity $T(t)$, as drawn in Fig. S1. It is important to note that the "whole nucleolus" ROI is drawn to surround the same nucleolus in which the bleach ROI is drawn and FRAP is carried out. The normalized intensity is calculated as

$$I_{norm}(t) = \left(\frac{I(t) - BG(t)}{I_{pre} - BG_{pre}}\right)\left(\frac{T_{pre} - BG_{pre}}{T(t) - BG(t)}\right)$$

With this method, the normalized intensity I_{norm} is expected to return to 1 unless an "immobile fraction" is present. Illustrations of the expected recovery and accompanying recovery curves for nucleoli with and without an immobile fraction are included in Fig. S1.
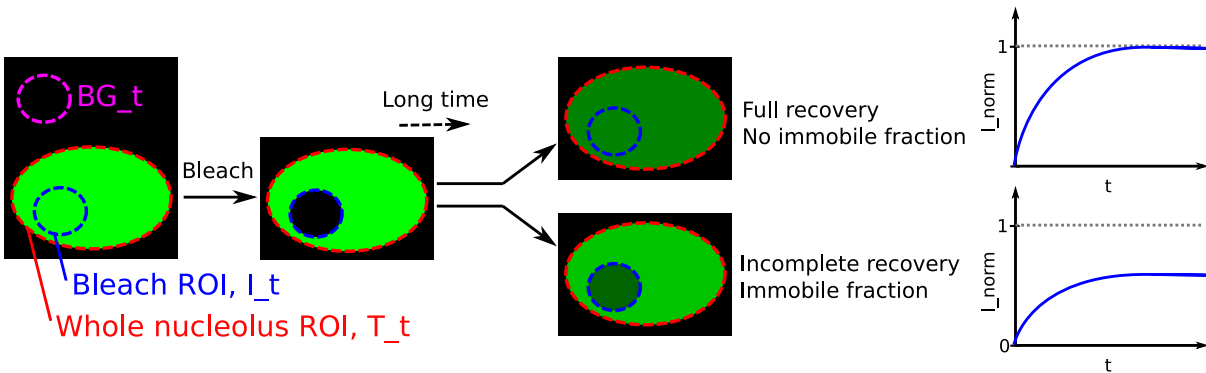

FIG S1. FRAP regions of interest and expected recovery with and without an immobile fraction.

References

[1] R.D. Phair, S.A. Gorski, and T. Misteli, in *Methods Enzymol.* (2003), pp. 393–414.