Supplementary Information

Supplementary figure 1. FLIM measurement of HeLa cells co-producing H2B-SCFP1 and mTurquoise2-NES. Cells were measured (A-C) live, or fixed and mounted in (D-F) TBS or (G-I) Mowiol. The panels show the fluorescence intensity (A,D,G), modulation lifetime (B,E,H) and corresponding histogram (C,F,I).
Supplementary figure 2. FLIM and lifetime-unmixing of HeLa cells co-producing 3xNLS-SGFP2(T65G) and SGFP2-3xNES. Transfection of HeLa cells was performed by a single plasmid encoding 3xNLS-SGFP2(T65G)-T2A-SGFP2-3xNES with two different GFP lifetime variants. Frequency domain FLIM was performed on live cells (A-F) or fixed cells mounted in TBS (G-L). The figure represents the fluorescence intensity (A,G), false-color map of the combined GFP and GFP contributions (B,H) extracted from dual lifetime unmixing of GFP (C,I) and GFP (D,J). Recorded phase- (E,K) and modulation (F,L) lifetime images with corresponding histograms.