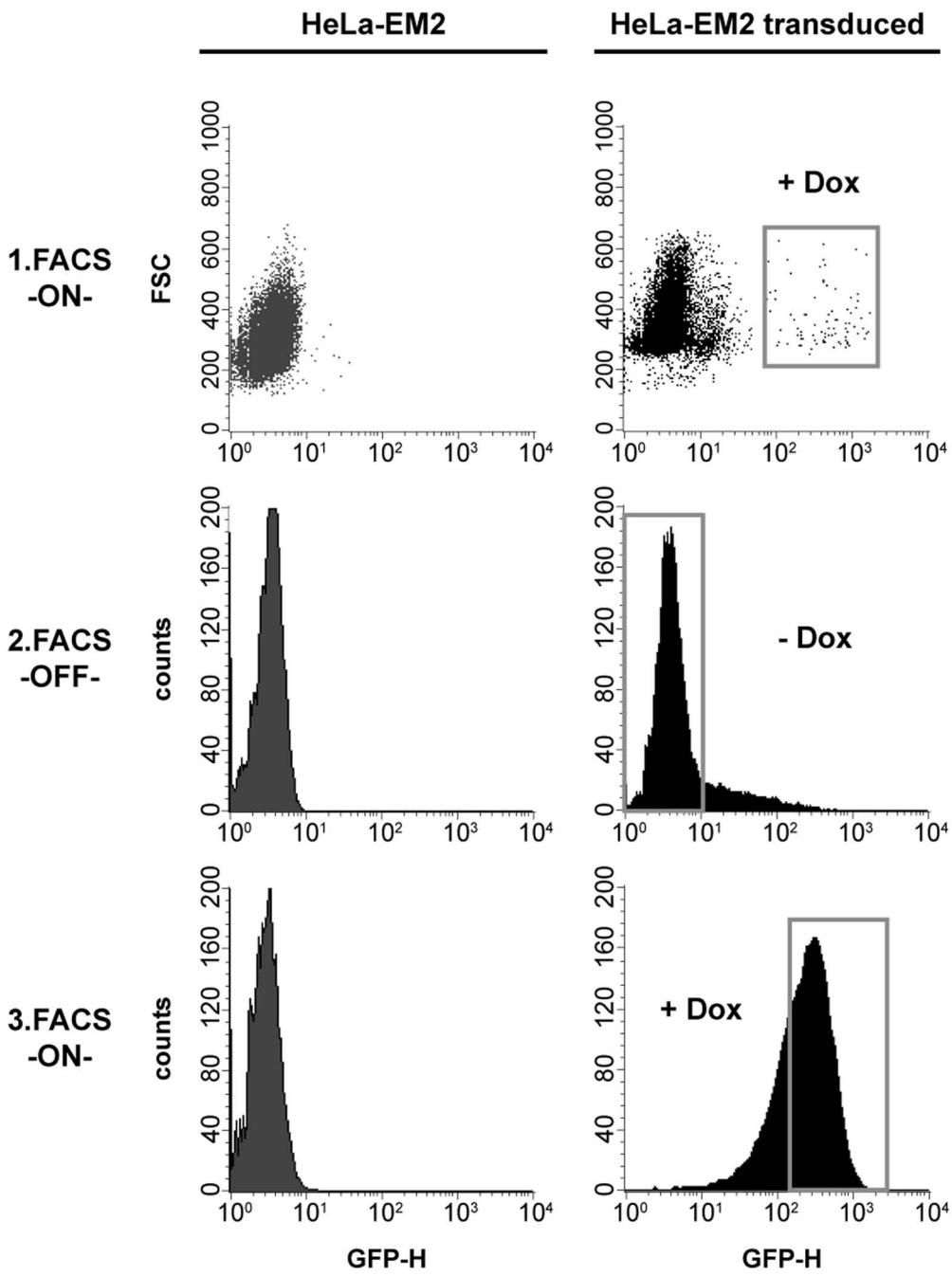
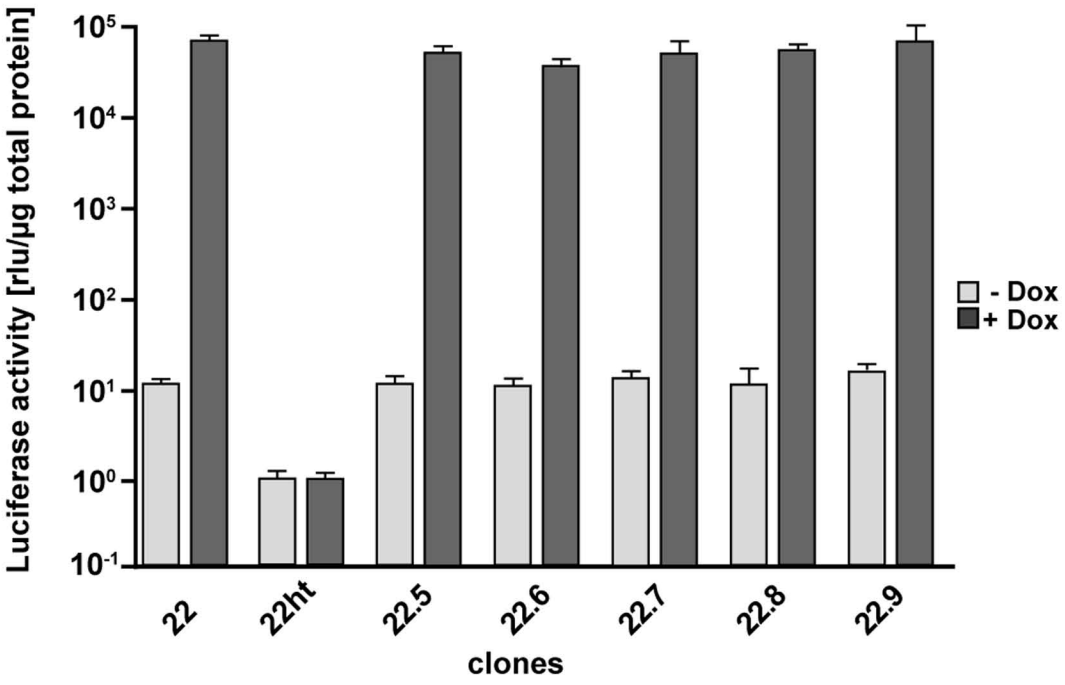


Supplementary Figure 1

FACS based strategy for harvesting silent but activatable clonal cell populations

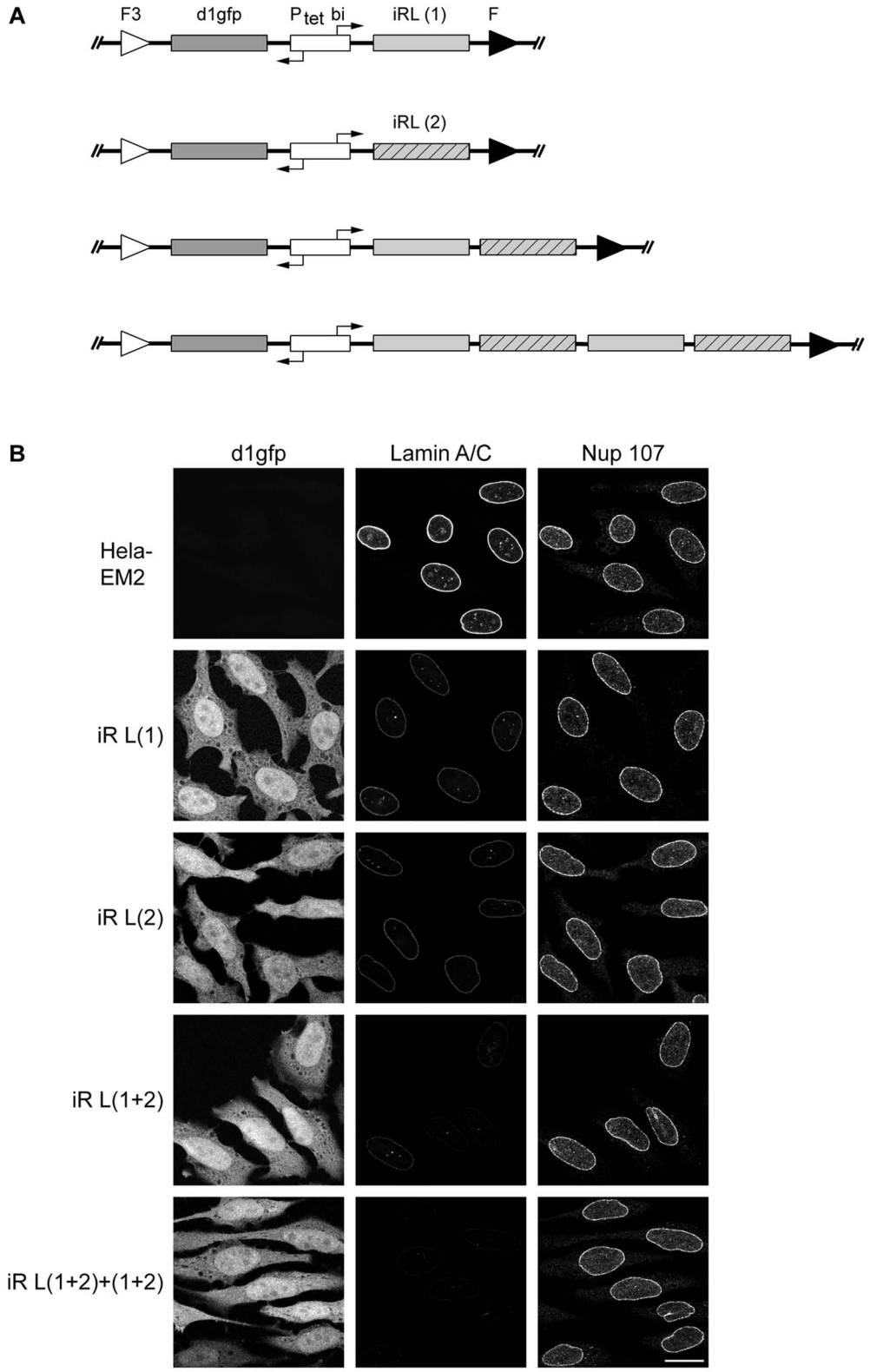
HeLa EM2 cells were transduced with the retroviral SIN vector S2f-lMGg-F3 harbouring a tetracycline inducible egfp expression cassette (Fig. 1). After induction with doxycycline (200ng/ml) EGFP positive cells were selected by fluorescence activated cell sorting (top right) and subsequently cultured in the absence of doxcycline. A second sorting step was implemented after expanding the cell population for 8 days to eliminate cells with background activity (middle right). The remaining cell population was again propagated and treated with doxycycline. Finally clonal cell lines were derived by the separation of individual EGFP positive cells in a third round of FACS.



Supplementary Figure 2

RMCE restores the original expression pattern of the HeLa EM2-22 clone

The RMCE procedure was used to introduce the bidirectional expression luc/egfp cassette (Fig. 5 A-2) in the master cell line HeLa-EM2-22ht. Five clonal RMCE derivatives (22.5 to 22.9) were analyzed by the measurement of luciferase in cell lysates of dox treated (200ng/ml for 16 hours) and untreated cells. Luciferase activity was normalized to the total protein content. Error bars represent the standard deviation between three independent measurements. The expression characteristics of the original luc/egfp containing cell line EM2-22 is shown on the left.

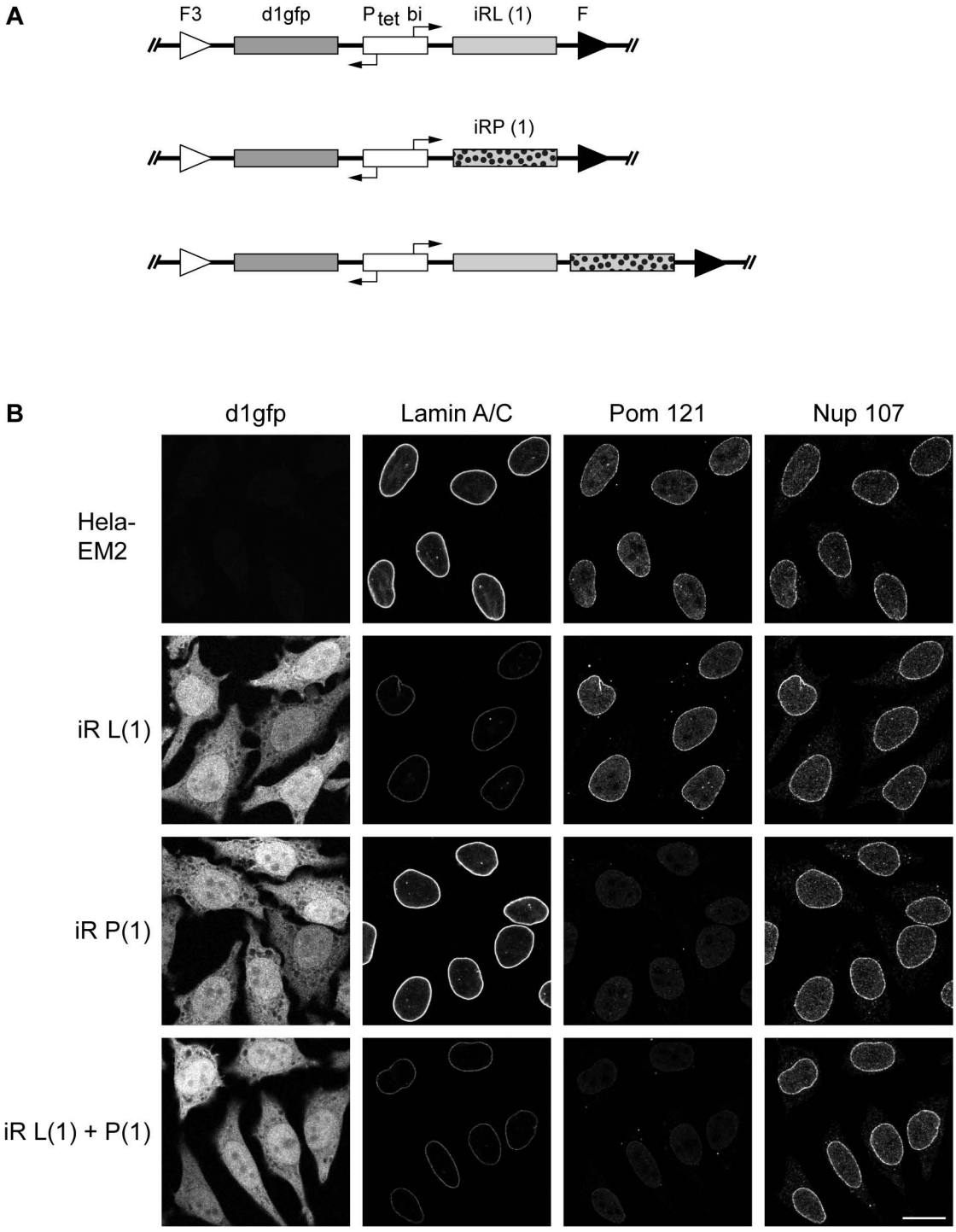


Supplementary Figure 3

Multiple miRNAs arranged in tandem mediate enhanced downregulation of endogenous lamin A/C

(A) Schematic representation of miRNA expression cassettes with single or multiple cascaded miRNAs. The bidirectional tetracycline inducible promoter (Ptet bi) is located centrally and controls the expression of destabilized green fluorescent protein (d1gfp) together with the monomeric, dimeric or tetrameric miRNA(s) against lamin A/C. The expression cassette is flanked by hetereogenous FRT sites (F3 and F) for site directed genomic integration via RMCE. iRL(1): miRNA against lamin A/C, position 608 to 628 relative to the start codon; iRL (2) miRNA against lamin A/C, position 364 and 384 relative to the start codon.

(B) Conditional knock-down of lamin A/C is demonstrated by immunofluorescence staining of stable HeLa EM2-11 cells harboring a single expression cassette with one, two and four miRNAs, respectively. Cells were seeded on cover slips and induced with 200 ng/ml of dox for 96h. Anti-Nup107 antibody served as a positive control staining nuclear pore complexes within the nuclear envelope and visualizing all stained cells. Polycistronic constructs show an enhanced downregulation of lamin A/C compared to monomeric expression constructs.



Supplementary Figure 4

Simultaneous inactivation of two genes by tandem-arrayed miRNAs

(A) Schematic representation of the Tet-regulated miRNA constructs used for the inducible knockdown of lamin A/C and Pom121 after site directed genomic integration via RMCE. Ptet bi controls the expression of d1gfp together with monomeric lamin A/C (iRL(1)) or Pom121 (iRP(1)) miRNAs. The lower drawing illustrates the expression cassette with the tandem array of iRL(1) and iRP(1).

(B) Conditional knock-down of lamin A/C and Pom121 is demonstrated by immunofluorescence staining of HeLa EM2-11 cells harboring stably integrated dimeric expression cassettes. Cells were seeded on cover slips and induced with 200 ng/ml of dox for 96h. Anti-Nup107 antibody served as a positive control staining nuclear pore complexes. The dimeric miRNA array allows the simultaneous knockdown of lamin A/C and Pom121.