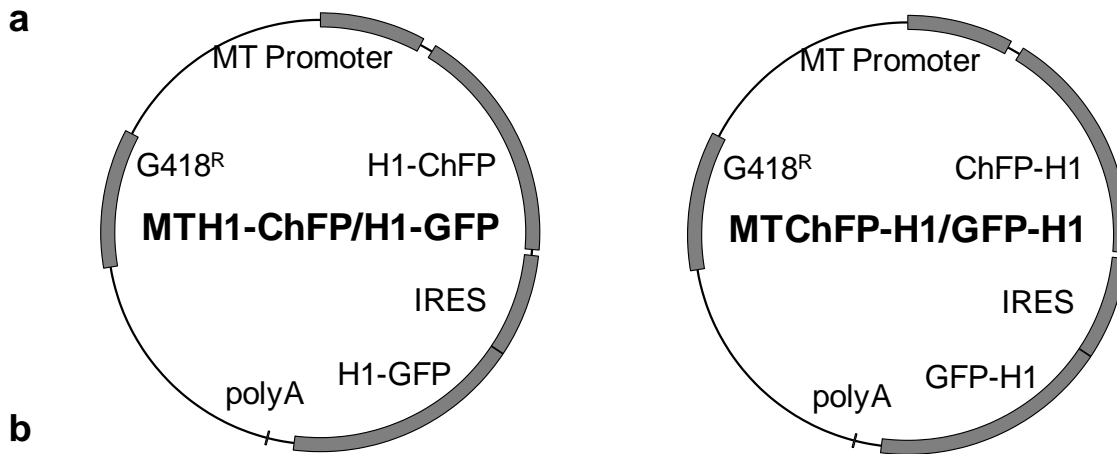## Supplemental Fig. S1

a

b

H1^0-ChFP
**MTENSTSAPAAKPKRAKASKKSTDHPKYSDMIVAAIQAEKNRAGSSRQSIQKYIKSHYKVGENADSQIKLSIKRLVTT
GVLKQTKGVGASGSFRLAKGDEPKRSVAFKKTKKEVKKVATPKKAAKPKKAASKAPSKKPKATPVKKKKKPAATPKKA
KKPKVVKVKPVKASKPKKAKTVKPKAKSSAKRGSKKK**AMVSKGEEDNMAIIKEFMRFKVHMEGSVNGHEFEIEGEGEG
RPYEGTQTAKLKVTKGGPLPFAWDILSPQFMYGSKAYVKHPADIPDYLKLSFPEGFKWERVMNFEDGGVVTVTQDSSL
QDGEFIYKVKLRGTNFPSDGPVMQKKTMGWEASSERMYPEDGALKGEIKQRLKLKDGGHYDAEVKTTYKAKKPVQLPG
AYNVNIKLDITSHNEDYTIVEQYERAEGRHSTGGMDELYKSGLRSRPRRQRTAFLNIKGMG

H1^0-GFP
**MTENSTSAPAAKPKRAKASKKSTDHPKYSDMIVAAIQAEKNRAGSSRQSIQKYIKSHYKVGENADSQIKLSILVTTGV
LKQTKGVGASGSFRLAKGDEPKRSVAFKKTKKEVKKVATPKKAAKPKKAASKAPSKKPKATPVKKAKKKPAATPKKAK
KPKVVKVKPVKASKPKKAKTVKPKAKSSAKRGSKKK**MVSKGEELFTGVVPILVELDGDVNGHKFSVSGEGEGDATYG
KLTLKFICTTGKLPVPWPTLVTTLTYGVQCFSRYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDT
LVNRIELKGIDFKEDGNILGHKLEYNYNSHNVYIMADKQKNGIKVNFKIRHNIEDGSVQLADHYQQNTPIGDGPVLLP
DNHYLSTQSALSKDPNEKRDHMVLLEFVTAAGITLGMDELYKSGLRSRPRRQRTAFLNIKGMG
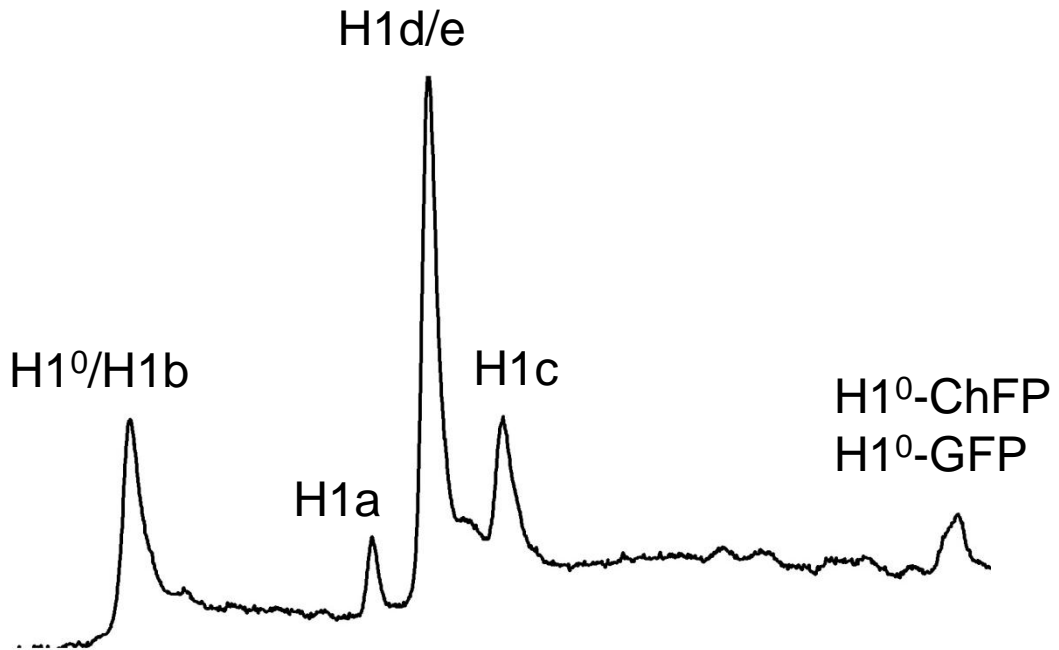
ChFP-H1^0
MVSKGEEDNMAIIKEFMRFKVHMEGSVNGHEFEIEGEGEGRPYEGTQTAKLKVTKGGPLPFAWDILSPQFMYGSKAYV
KHPADIPDYLKLSFPEGFKWERVMNFEDGGVVTVTQDSSLQDGEFIYKVKLRGTNFPSDGPVMQKKTMGWEASSERMY
PEDGALKGEIKQRLKLKDGGHYDAEVKTTYKAKKPVQLPGAYNVNIKLDITSHNEDYTIVEQYERAEGRHSTGGMDELY
KSGLRSRPRRQRTAFLNIAMA**TENSTSAPAAKPKRAKASKKSTDHPKYSDMIVAAIQAEKNRAGSSRQSIQKYIKSHY
KVGENADSQIKLSIKRLVTTGVLKQTKGVGASGSFRLAKGDEPKRSVAFKKTKKEVKKVATPKKAAKPKKAASKAPSK
KPKATPVKKAKKKPAATPKKAKKPKVVKVKPVKASKPKKAKTVKPKAKSSAKRGSKKK**

GFP-H1^0
MVSKGEELFTGVVPILVELDGDVNGHKFSVSGEGEGDATYGKLTLKFICTTGKLPVPWPTLVTTLTYGVQCFSRYPDH
MKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNYNSHNVYIMAD
KQKNGIKVNFKIRHNIEDGSVQLADHYQQNTPIGDGPVLLPDNHYLSTQSALSKDPNEKRDHMVLLEFVTAAGITLGM
DELYKSGLRSRPRRQRTAFLNIAMA**TENSTSAPAAKPKRAKASKKSTDHPKYSDMIVAAIQAEKNRAGSSRQSIQKYI
KSHYKVGENADSQIKLSIKRLVTTGVLKQTKGVGASGSFRLAKGDEPKRSVAFKKTKKEVKKVATPKKAAKPKKAASK
APSKKPKATPVKKAKKKPAATPKKAKKPKVVKVKPVKASKPKKAKTVKPKAKSSAKRGSKKK**

Supplemental Fig. S1. Constructs used in this study. (a) Representative maps of expression vectors. Transcription is driven by the mouse metallothionine I promoter. The IRES sequence was derived from pIRES2AcGFP (Clontech). (b) Sequences of expressed hybrid proteins. Histone coding sequences are in bold. GFP and ChFP are appropriately colored.

a

MTH1^{0}ChFP

ChFP GFP Merge

b

MTH1^{0}GFP

ChFP GFP Merge

Supplemental Fig. S2. Independence of ChFP and GFP signals. (a) A construct expressing only H1^0-ChFP was stably introduced into fibroblasts. Cells were imaged under the conditions used for dual-color FRAP. No signal was seen in the GFP channel. (b) A construct expressing only H1^0-GFP was stably introduced into fibroblasts. Cells were imaged under the conditions used for dual-color FRAP. No signal was seen in the ChFP channel.
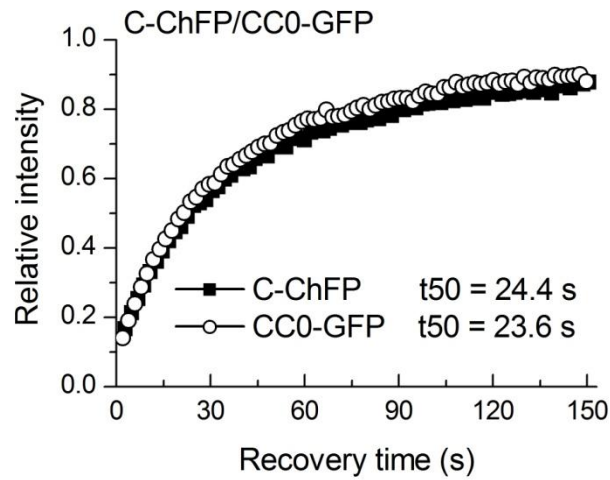
Supplemental Fig. S3

Supplemental Fig. S3. HPLC separation of H1 proteins. H1 proteins were extracted from a cell line expressing $H1^0$-ChFP and $H1^0$-GFP and separated by HPLC as previously described (16). The peak containing the tagged proteins is not seen in control cells (16, data not shown).
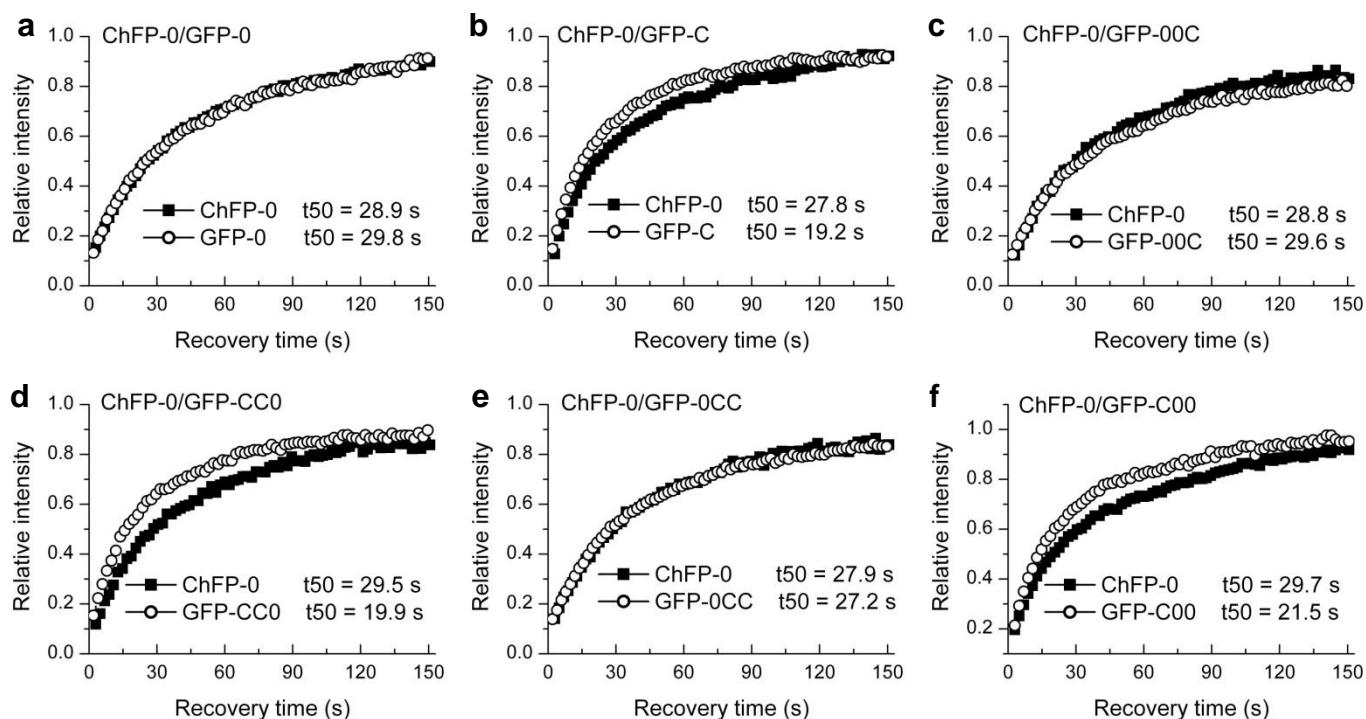
Supplemental Fig. S4
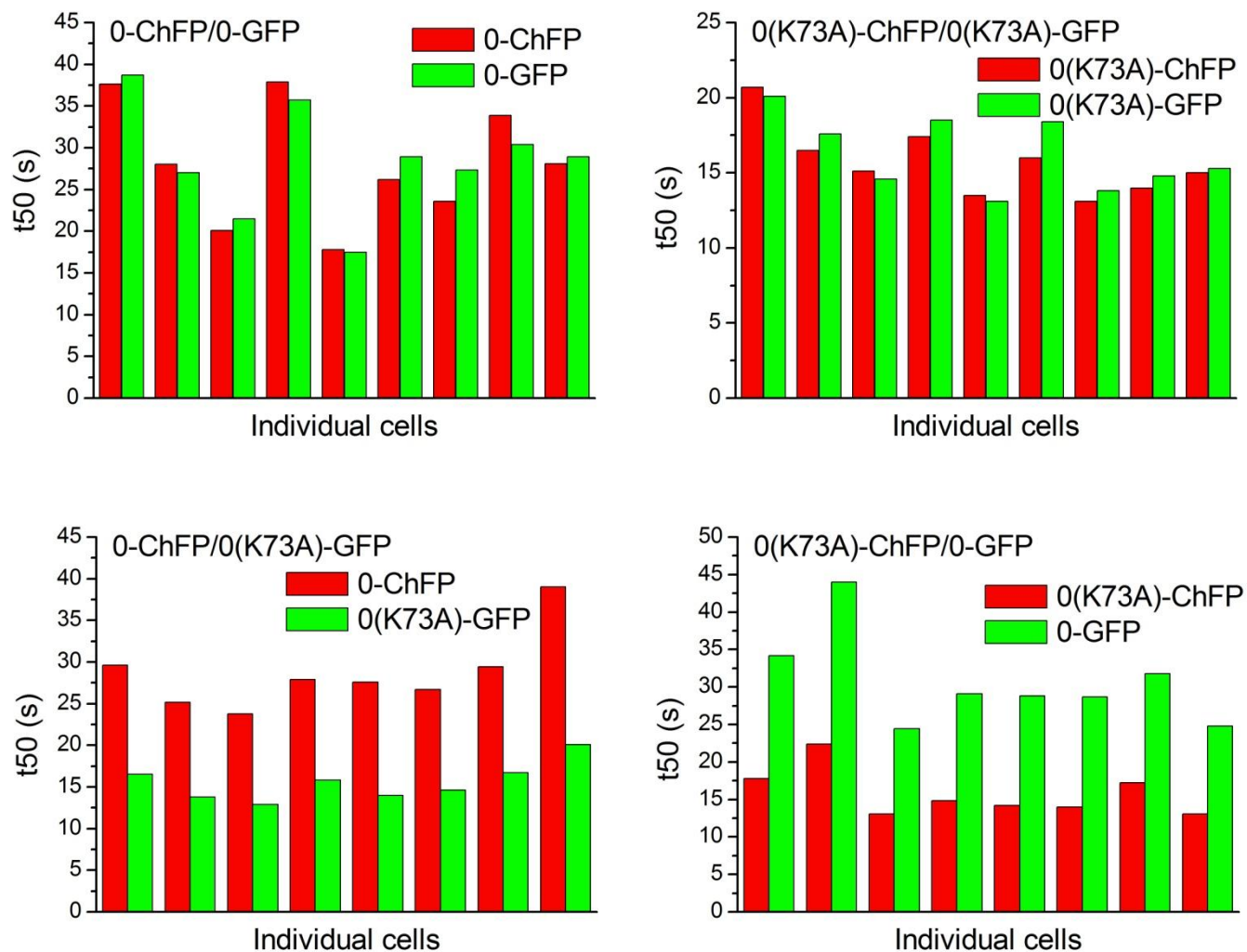
Supplemental Fig. S4. Comparison of t_{50} values for individual cells. Grouped pairs indicate the values obtained for the ChFP-tagged and GFP-tagged H1 proteins within a single cell. Data for averaged recovery curves are shown in Fig. 2

Supplemental Fig. S5

Supplemental Fig. S5. FRAP analysis of C-ChFP/CC0-GFP. FRAP analysis with cells co-expressing WT H1c-ChFP and CC0-GFP. Values for the half time of recovery (t_{50}) were determined as previously described (35) and represent means $\pm$ S.D. of at-least 8 independent measurements from a pool of three stable cell lines. Error bars are omitted from the plots for clarity.

Supplemental Fig. S6. Quantitative analysis of the relative binding kinetics of N-tagged $H1^0$ and H1c and effects of swapping their terminal domains. FRAP analyses with cells co-expressing **(a)** ChFP-$H1^0$ and GFP-$H1^0$ **(b)** ChFP-$H1^0$ and GFP-H1c show faster recovery kinetics for H1c than $H1^0$. Simultaneous recovery curves of the C-terminal switch mutants **(c)** GFP-00C relative to ChFP-$H1^0$ and **(d)** GFP-CC0 relative to ChFP-$H1^0$ and of the N-terminal switch mutants **(e)** GFP-0CC relative to ChFP-$H1^0$ and **(f)** GFP-C00 relative to ChFP-$H1^0$. Values for the half time of recovery (t_{50}) were determined as previously described (35) and represent means $\pm$ S.D. of at-least 6 independent measurements from a pool of three stable cell lines. Error bars are omitted from the plots for clarity. Supplemental Table 1 provides the corresponding statistical analyses.