Supplemental Data:

Inter-chromosomal contact properties in live-cell imaging and in Hi-C

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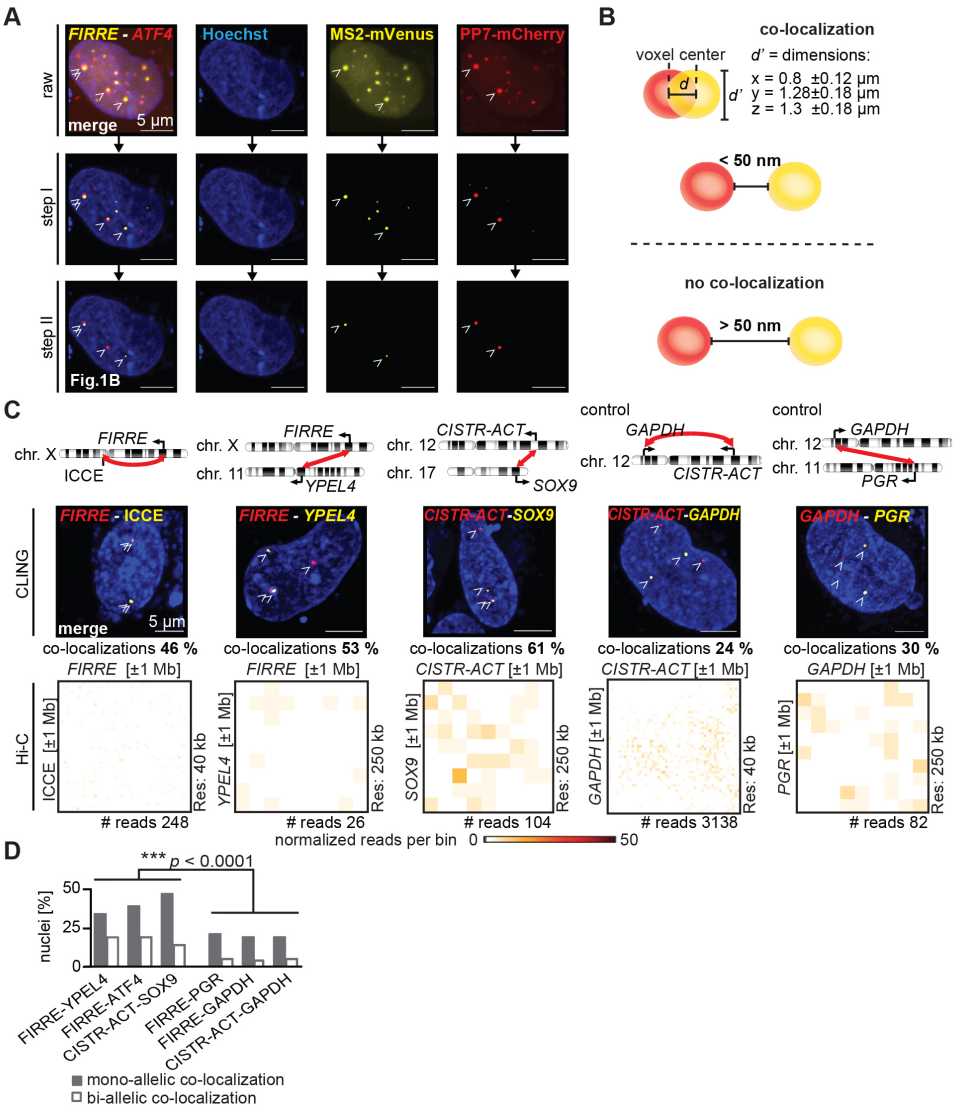
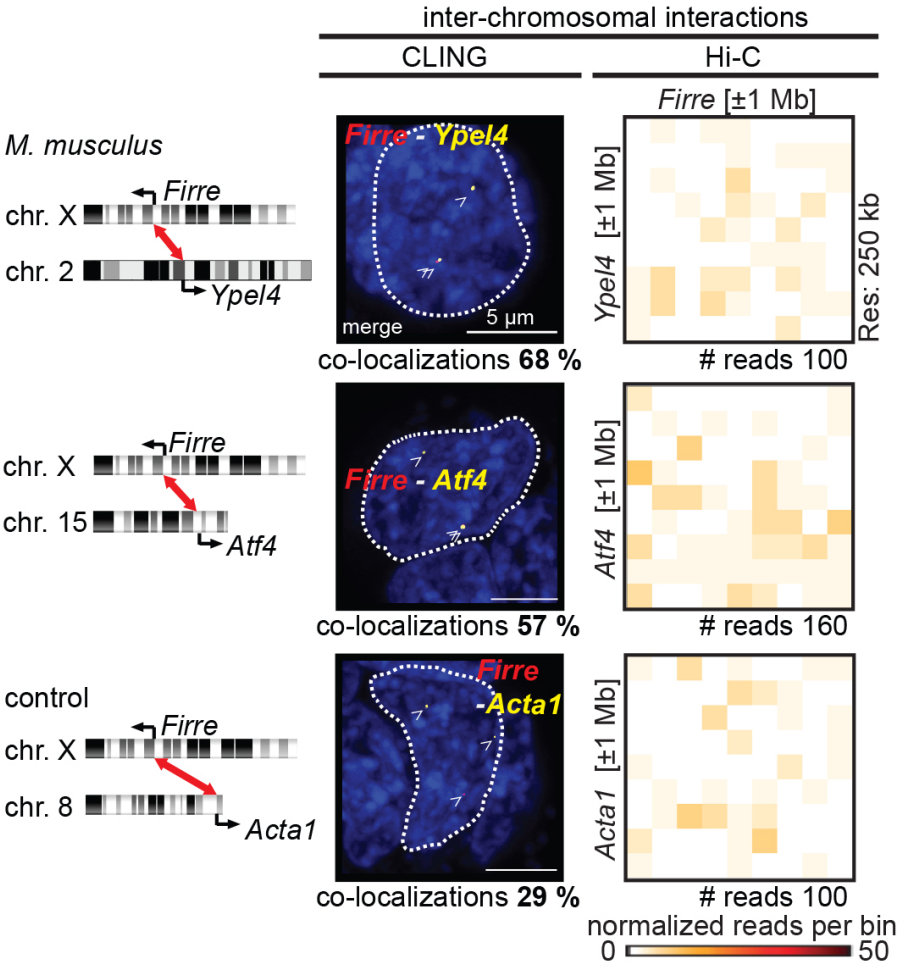
The authors provide this appendix with supplemental data to give readers additional information about their work.

Figure S1: NHCCs in Hi-C datasets, related to Figure 1.



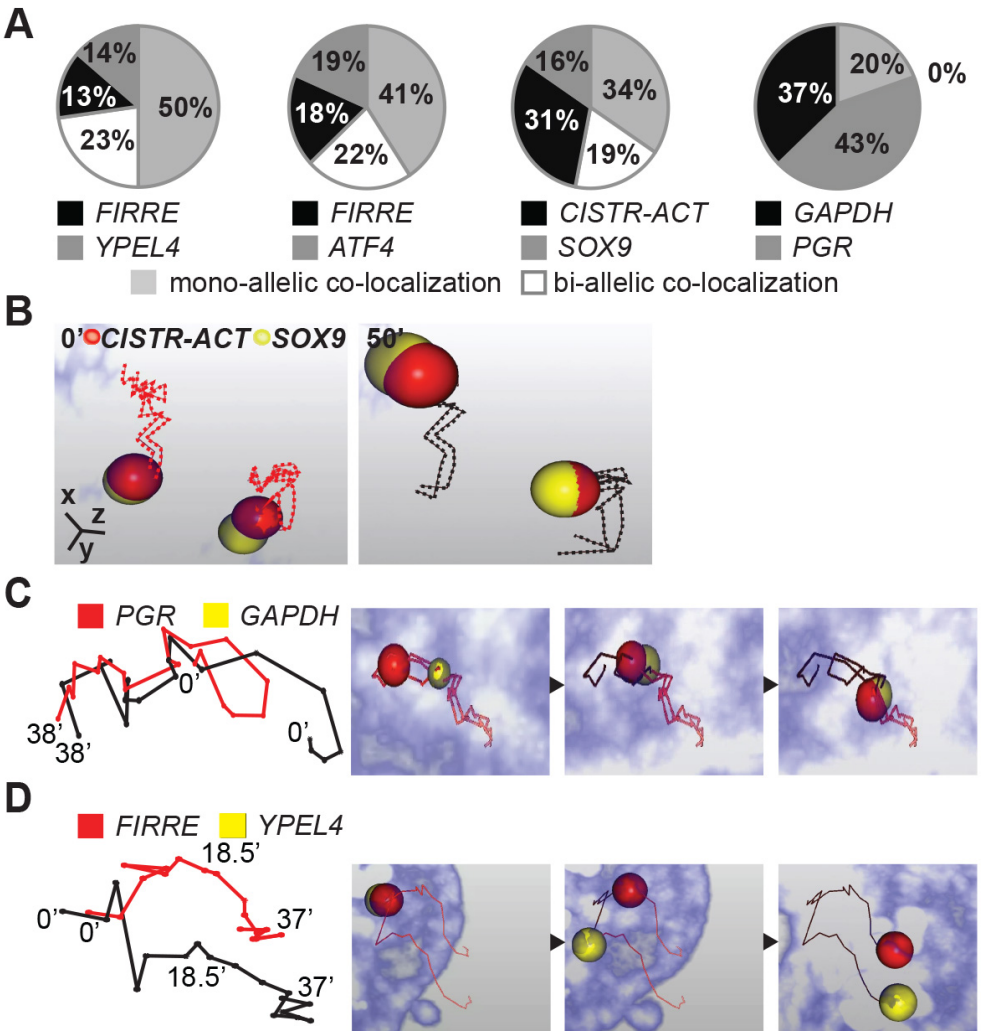
(**A**) Example of *FIRRE-ATF4* image processing. Raw CLING signals (specific signals and off-target effects) were processed in a stepwise approach to generate the shown images in the manuscript (compare with Fig. 1B). Specific foci were the brightest and largest nuclear signals and the two foci reflected the diploid female RPE-1 karyotype. (**B**) Scheme of co-localization or no co-localization measurements. Airyscan microscopy has a resolution limit of ˜130 nm beyond the diffraction limit of light (˜200 nm). Spatial distances were measured from each voxel center (*d*). If a distance of less than 50 nm occurred between the voxel border, the foci were considered as co-localized. Co-localization distances depended directly on the voxel dimensions (*d'*). Any distance above 50 nm defined distinctly separated, non-co-localized signals. (**C**) Co-localizations of *FIRRE*-ICCE (46 % of imaged nuclei), *FIRRE-YPEL4* (53 %), *CISTR-ACT- SOX9* (61 =%), *CISTR-ACT-GAPDH* (24 %), and *GAPDH-PGR* (24 %) in living RPE-1 cells (CLING: upper panels), in contrast to normalized Hi-C genomic maps and total Hi-C reads (lower panels). (**D**) Frequencies of mono-allelic or bi-allelic co-localizations of the selected NHCCs (each combination 100 nuclei, X^2-test, *** $p < 0.0001$).

Figure S2. NHCCs in mESCs, related to Figure 1.



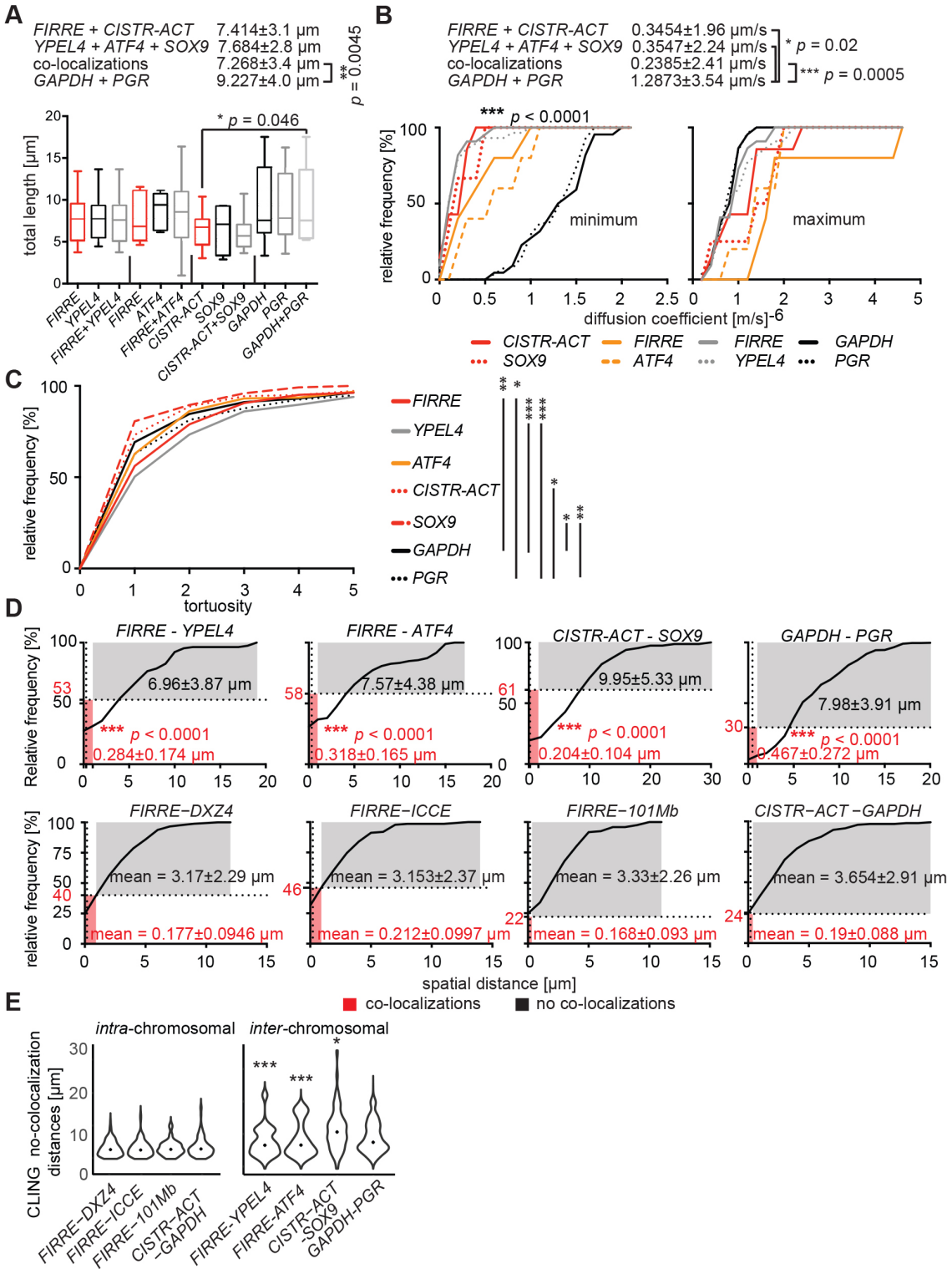
NHCCs of *Firre* interacting with *Ypel4* (68 % of nuclei), or *Atf4* (57 %), or with the control locus *Acta1* (29 %, ** $p < 0.0012$, X^2-test, each combination 100 nuclei) in male mESCs, in contrast to their interactions in normalized Hi-C genomic interaction maps (bin size = 250 kb). In mESCs, the nucleus was smaller than in RPE-1 cells: 6.44±1.26 x 10.87±2.1 µm; all measurements were obtained from in at least 30 cells.

Figure S3: 4D measurements, related to Figure 2.



(**A**) The allele frequencies that were tracked in 4D-experiments are shown either as single non-co-localized alleles, or mono-allelic, or bi-allelic co-localizations. Time properties and the number of cells that were tracked over time can be found in Table S2. (**B**) Example of 4D-imaging of a bi-allelic *CISTR-ACT-SOX9* co-localization at different time-points ('to-be-tracked-line' = red-dashed lines, traversed signals = black-dashed lines). Signals moved along the same direction and the bi-allelic interaction stayed associated over time. (**C**) Associating or dissociating NHCCs over time occurred in 6.8 % of the investigated nuclei. Association of *GAPDH* and *PGR* after six acquired time frames (~12 minutes). The scaled-up window shows the co-localized tracks of *GAPDH* and *PGR* for simpler visualization. (**D**) Both alleles of *FIRRE* and *YPEL4* interacted with each one another. After 2.5 time frames (~7 minutes), one mono-allelic NHCC of *FIRRE* and *YPEL4* dissociated, whereas the second NHCC of *FIRRE* and *YPEL4* stayed associated (see Movie S3). The dissociated *FIRRE* and *YPEL4* loci were tracked till the end of the time lapse. The data indicate that due to intra-nuclear events, NHCCs dissociate or associate in rare exemptions.

Figure S4: Loci dynamics in 4D-imaging, related to Figure 2.



(**A**) Maximal migrations of each of the signals from the first to the last time point were measured in micrometer (µm). 11 cells were tracked for *FIRRE* with *YPEL4* with an

average time of 37.68 minutes per cell. For every tracked combination the single, non-co-localized, alleles are shown next to the distances of co-localized signals. For further time and tracking properties see Table S2. 10 cells for the combination of *FIRRE* with *ATF4* were tracked with a mean time of 37.96 minutes per cell. The distances of the *CISTR-ACT* - *SOX9* combination were measured in 11 cells over 34.49 minutes in average. In the control of *GAPDH versus PGR,* 12 cells were measured with an average time of 26.4 minutes per cell. The measured distances were grouped for lncRNAs (*FIRRE, CISTR-ACT*), coding genes (*YPEL4, ATF4, SOX9*) and were compared to the control *GAPDH* and *PGR*. The tracked distances of co-localization events were significantly shorter (~2.2 µm) compared to the controls (** $p < 0.0045$, Mann-Whitney rank sum test, mean ± min or max). In comparisons of the single loci or NHCCs (bar plot), the co-localization of *CISTR-ACT* with *SOX9* was barely significant (* $p < 0.045$) when compared to *GAPDH* with *PGR*. (**B**) The diffusion coefficients of all loci across the entire time lapses (not time-matched) were measured and grouped for lncRNAs (*FIRRE, CISTR-ACT*), or coding genes (*YPEL4, ATF4, SOX9*) in minimum and maximum values. These measurements were compared to the control *GAPDH* and *PGR*. Either *FIRRE* and *CISTR-ACT* (* $p = 0.02$) or the group of *YPEL4*, *ATF4, SOX9* (* $p = 0.02$) or the co-localization events (*** $p = 0.0005$) were more slowly than the control (Mann-Whitney rank sum test, mean ± min or max). Significant differences were also found for the individual minimum diffusion coefficient measurements (*** $p < 0.0001$, Mann-Whitney rank sum test). The data suggest that the tested NHCCs, either as single loci or as co-localizations moved slower than the controls *GAPDH* or *PGR*. (**C**) Tortuosity distributions of time-matched 4D-CLING measurements of the tracked loci determined that some loci had less 3D-directional changes of their positions when compared to *GAPDH* or *PGR* (*** $p < 0.0001$, ** $p < 0.001$, * $p < 0.05$, Mann-Whitney rank sum test, mean ± SD). (**D**) Distributions and frequencies of *intra*- and *inter*-chromosomal spatial distances for co-localizations and no co-localizations. Due to the *intra*-chromosomal interactions of *FIRRE* with DXZ4, or ICCE, or the control locus at 101 Mb, we measured distances between non-co-localized signals (each combination 100 nuclei) to see how far apart they are and if both signals were located in their chromosomal territories. By using FISH, multiple publications showed that individual loci are normally found within the range of a chromosomal territory (3-4 µm). We observed non-co-localized signals being 1-4 µm apart from each one another in most nuclei. Exceptions showing distances >5 µm could derive from alleles that harbor regions of lower chromatin compaction rates enabling increased chromosomal distribution in the nucleus. For RPE-1 cells, we measured a mean nucleus size of 9.22±2.12 µm x 18.94±3.6 µm. For *inter*-chromosomal interactions, spatial distances of co-localized signals were significantly closer to each other than their homologous non-co-localized alleles. Some co-localizations were also determined for *GAPDH* and *PGR*, which were significantly further apart from each other than the co-localizations of the other tested combinations (*** $p < 0.0001$, Mann-Whitney rank sum test). (**E**) Analysis of distributions of non-co-localized *intra*- or *inter*-chromosomal contacts. *Intra*-chromosomal distances were unimodally distributed, whereas *inter*-chromosomal distances showed bimodal distribution (Hartigan's dip test, *** $p < 0.0001$, * $p < 0.05$).

Table S1: sgRNA sequences for human and mouse loci, , related to Figure 1.

locus	sgRNA	5' sequence	3' sequence
DXZ4	1	TTAAACCTGCCACCTCCAGA	TCTGGAGGTGGCAGGTTTAA
ICCE	1	GGCTGTCTCGGCTTTCACAG	CTGTGAAAGCCGAGACAGCC
	2	GCTTAACAACTACCTCCTGA	TCAGGAGGTAGTTGTTAAGC
	3	GTAGGCTGAACCTTACCCTG	CAGGGTAAGGTTCAGCCTAC
	4	TTACACTTACAGCATCCAGA	TCTGGATGCTGTAAGTGTAA
Chr.X: 101 Mb	1	TCAAAGGAGACTAAGCAGGG	CCCTGCTTAGTCTCCTTTGA
	2	AACCAGTGCTCAGAACACCT	AGGTGTTCTGAGCACTGGTT
	3	AGAGACAATGAGAAACAATG	CATTGTTTCTCATTGTCTCT
YPEL4	1	AGGAGATACGAAGAACCTGG	CCAGGTTCTTCGTATCTCCT
	2	GCTGGTGTCCAAGCTGAAAT	ATTTCAGCTTGGACACCAGC
	3	CCTTCAGGAGTATTAAACCA	TGGTTTAATACTCCTGAAGG
ATF4	1	AGTGAATCCGAACTACCCCA	TGGGGTAGTTCGGATTCACT
	2	TGTGGCCTGCGGAAACCGGG	CCCGGTTTCCGCAGGCCACA
	3	GCTGGGCTAAGGCCGCCTGG	CCAGGCGGCCTTAGCCCAGC
SOX9	1	GCTCTTGAGCAAGCGCCGCG	CGCGGCGCTTGCTCAAGAGC
	2	GCCAGGGGCGAAAGGAGCCA	TGGCTCCTTTCGCCCCTGGC
	3	GTTTCCAACTCCGAGAACCA	TGGTTCTCGGAGTTGGAAAC
PGR	1	GTAACCCAGTGGTTGTACTG	CAGTACAACCACTGGGTTAC
	2	GCTGCCCCTCCTCACCCCCA	TGGGGGTGAGGAGGGGCAGC
	3	GCAGTGAATTCAGAAACCGA	TCGGTTTCTGAATTCACTGC
GAPDH	1	TTAATGCTCTCAATGAGAAA	TTTCTCATTGAGAGCATTAA
	2	CTGCCCTTCTAGCTAAAAGC	GCTTTTAGCTAGAAGGGCAG
	3	GCTGCGCCGGGGGATATTGA	TCAATATCCCCCGGCGCAGC
FIRRE	1	ACAGCAAAGACACTTCCAGA	TCTGGAAGTGTCTTTGCTGT
	2	CTAGATGGCGAAAGAGACCT	AGGTCTCTTTCGCCATCTAG
	3	GAAATGTTGAAAACGAGCAA	TTGCTCGTTTTCAACATTTC
CISTR-ACT	1	GGTCGTCAAGACCAACCAAG	CTTGGTTGGTCTTGACGACC
	2	CATATGTACTGAGGAGACCG	CGGTCTCCTCAGTACATATG
	3	AGAAGTCCCCAAACACAAAG	CTTTGTGTTTGGGGACTTCT
Atf4	1	TAGCTCCCTGGACTCACAG	CTGTGAGTCCAGGGAGCTA
	2	CTAGCTTCTGTGCGTAACAA	TTGTTACGCACAGAAGCTAG
	3	TCAGTCACATGGTCACCTAG	CTAGGTGACCATGTGACTGA
	4	GGACTAGGCGAGAGGTCCAG	CTGGACCTCTCGCCTAGTCC
	5	TGTACCTGTCTCCCTTAGCA	TGCTAAGGGAGACAGGTACA
Acta1	1	GAACATGGAAGAATTCGGGG	CCCCGAATTCTTCCATGTTC
	2	TTCCCAGTCACTATTTCCAA	TTGGAAATAGTGACTGGGAA
	3	CATGTCTGTCCTACTCAGCA	TGCTGAGTAGGACAGACATG

Firre	1	TTATACTTAATAATAAGGCA	TGCCTTATTATTAAGTATAA
	2	GATCAAATGTAAAGAAAGCA	TGCTTTCTTTACATTTGATC
	3	ATAAATGTCTGTGTTTGCAG	CTGCAAACACAGACATTTAT

Table S2: Time properties of 4D-experiments, related to Figure 2.

Combination	Total number of acquired cells [n]	Total number of z-stacks	Mean of time interval [sec]	Total imaging time [h]	Average imaging time per cell [min]
FIRRE - YPEL4	11	200	122.7	6.91	37.68
FIRRE - ATF4	10	177	122	6.33	37.96
CISTR-ACT - SOX9	11	149	165.73	5.7	34.49
GAPDH - PGR	12	158	120	5.3	26.4