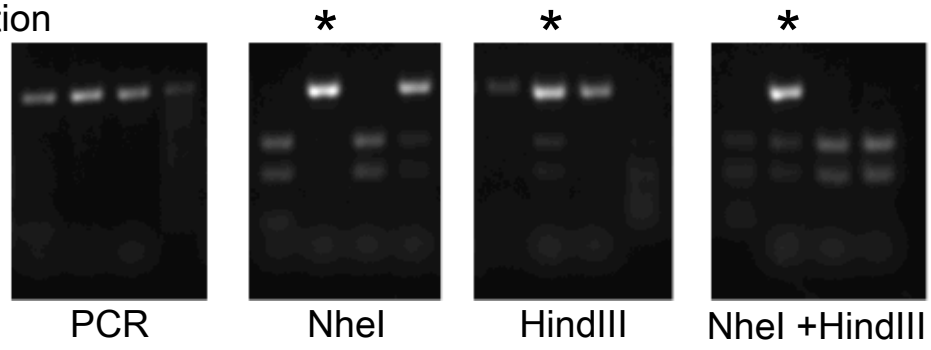
Hi-C
PCR control – Biotin integration

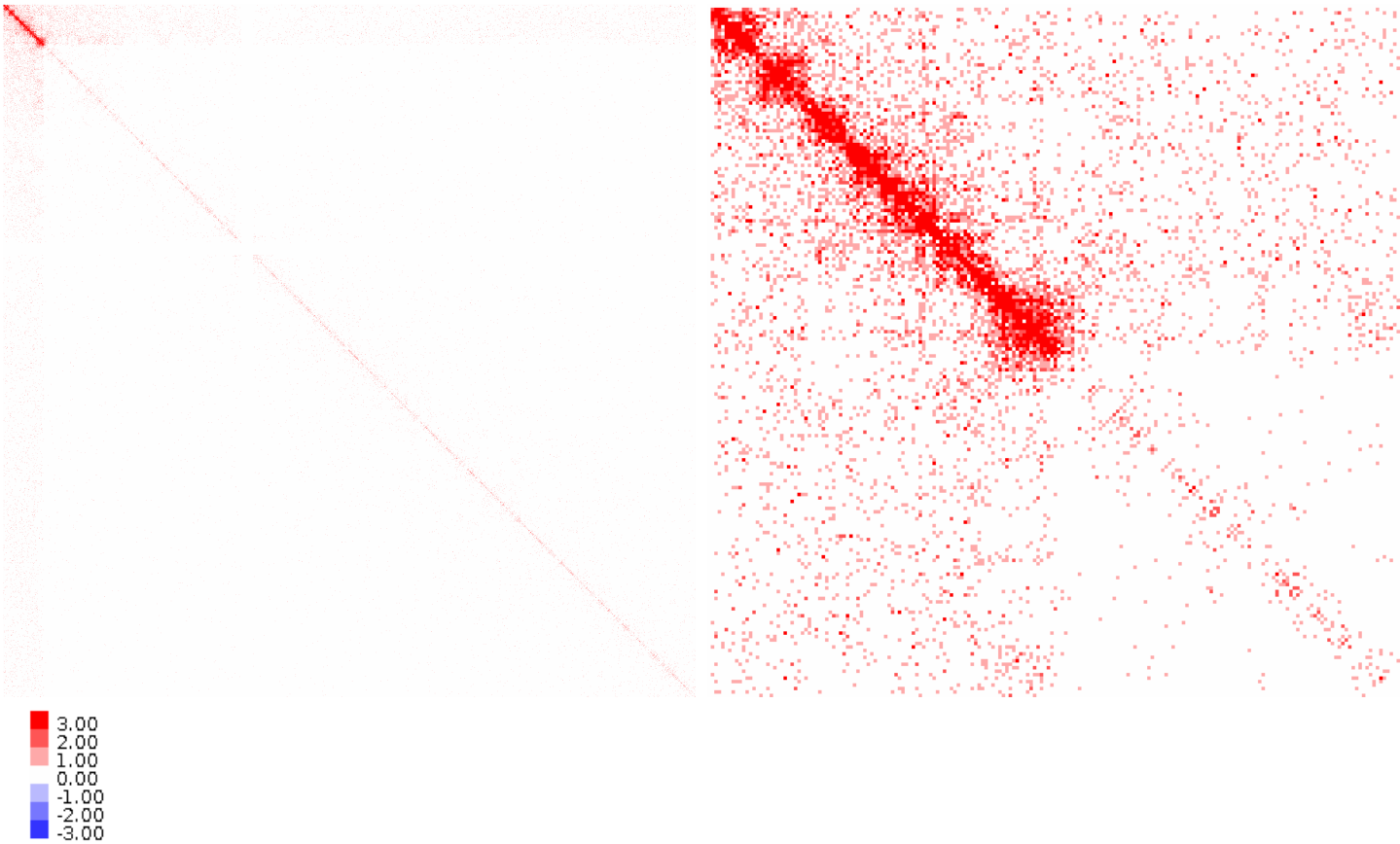
Comparison to * 3C control (digested only by HindIII).

Successful ligation = NheI digestion



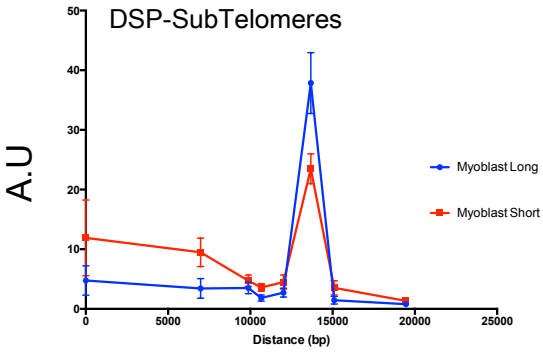
Chr. 6

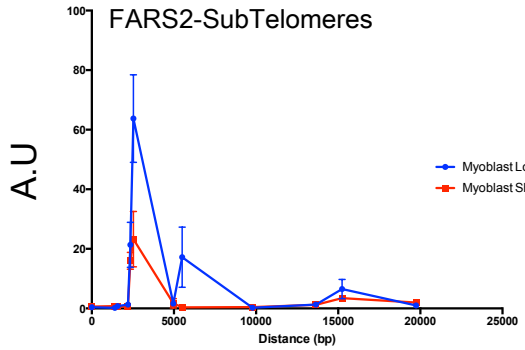
Chr. 6p – 20Mb from telomere end

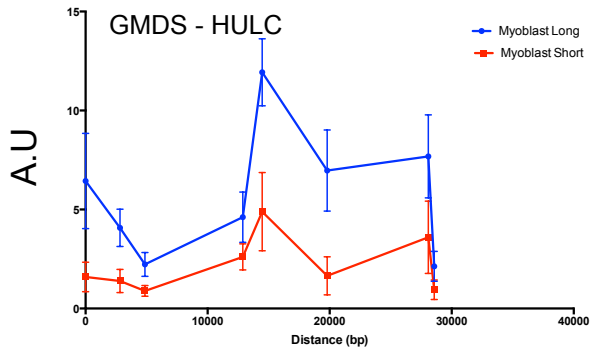


Supplementary FigS1. Hi-C quality controls.
Using biotinylated probes, the Hi-C technique can be used to investigate in an unbiased fashion specific regions of the genome (in our case, the first 10 Mbs of 6p).PCR ligation control (top) successful biotin integration will result in the creation of a NheI restriction site instead of an HindIII site. the vast majority of the mapped reads are located on chromosome 6, among them the majority are in the first 10Mb of 6p,demonstrating the efficiency of the capture done.
Heat-map representation of the treated paired end sequencing, mapped to the chromosome 6 (bottom left), and the first 20Mbs of the chromosome (bottom right)

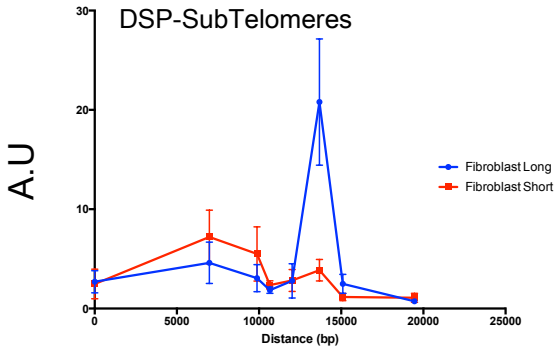
Myoblast

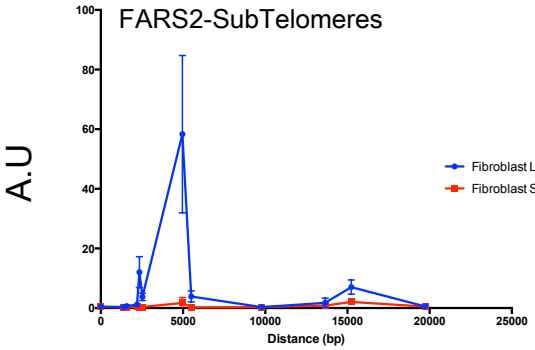


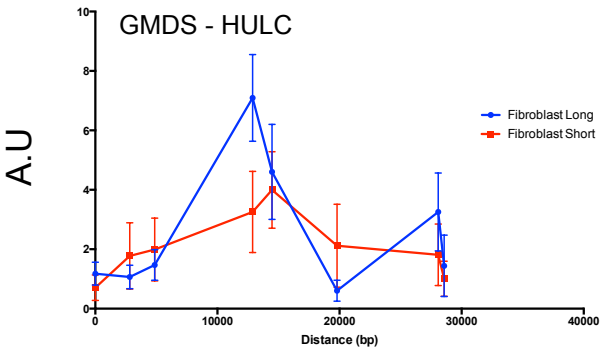




Fibroblast (BJ)

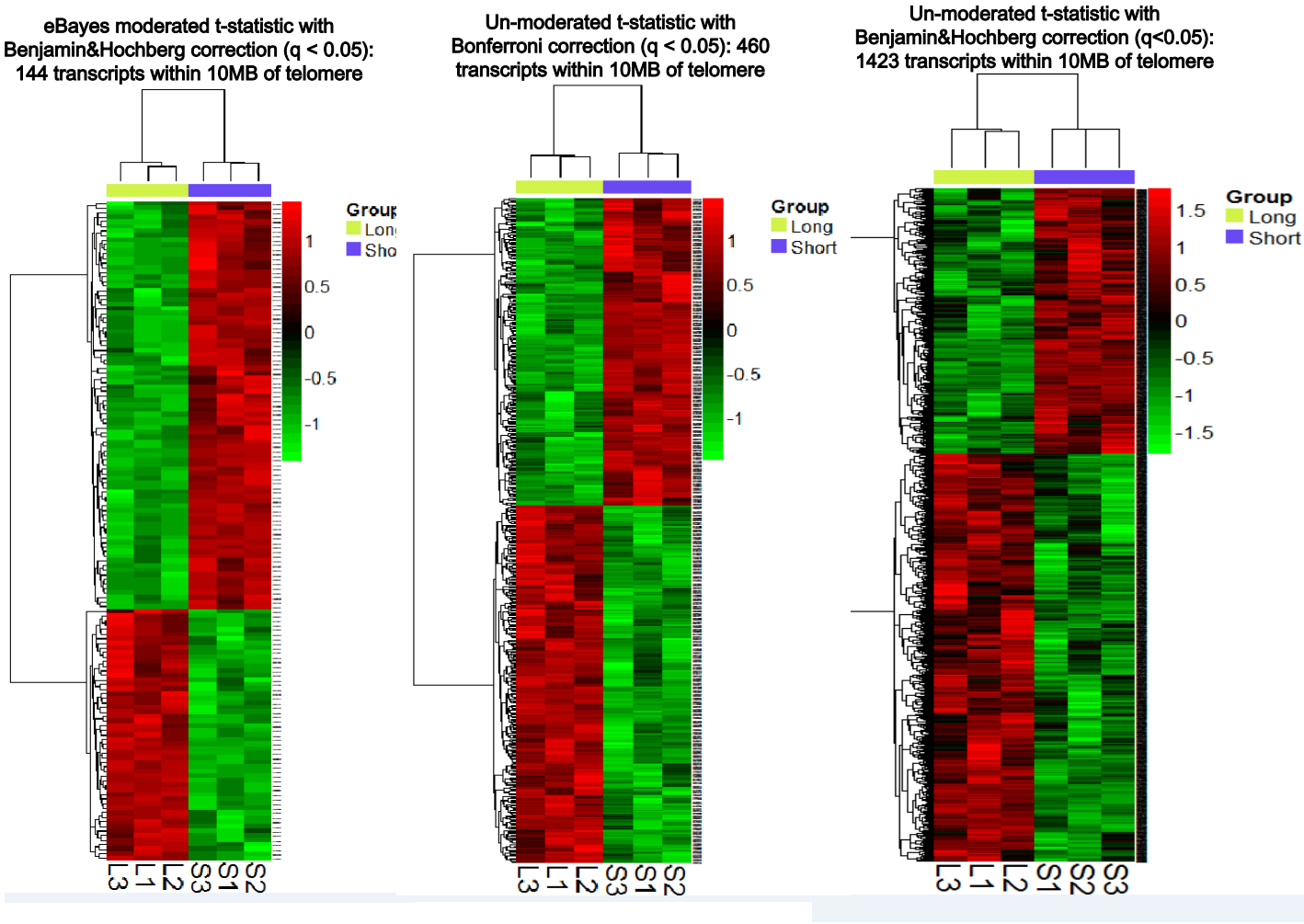




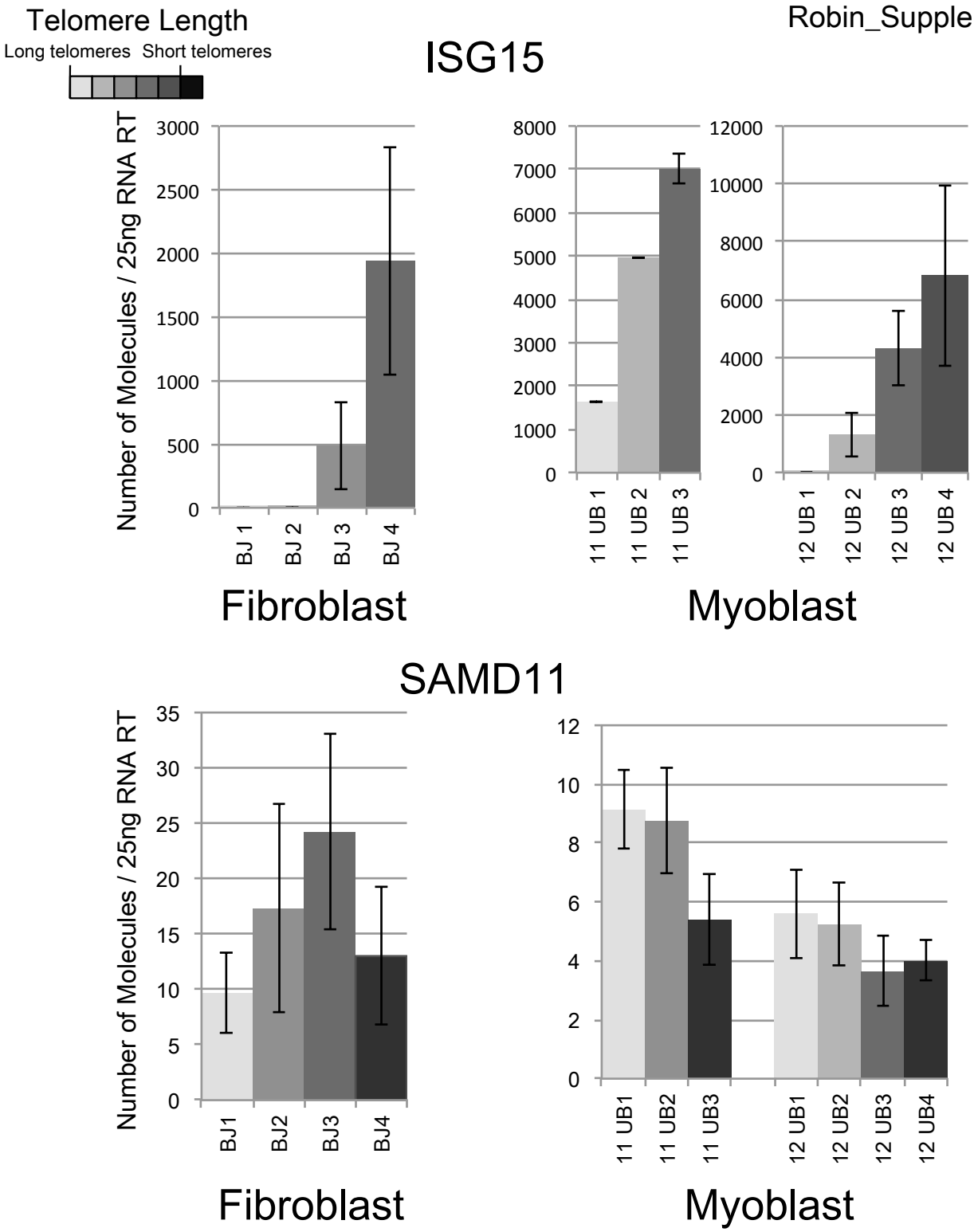


Supplementary FigS2. 3C quality validation.
Subtelomeric Interactions in cells with long and short telomeres (myoblast, left; fibrobast, right). For all interaction tested, a common primer adjacent to a HindIII site was used (in subtelomeric regions) with a variety of reverse primers adjacent to HindIII sites in DSP, FARS2 or GMDS gene (from top to bottom). Following crosslinking, digestion with HindIII, ligation and reversal of crosslinks, the DNA was amplified with the different primer pairs. In all quantification experiments we observed that sample with short telomere differs, with a decreased of signal towards the center of interaction.

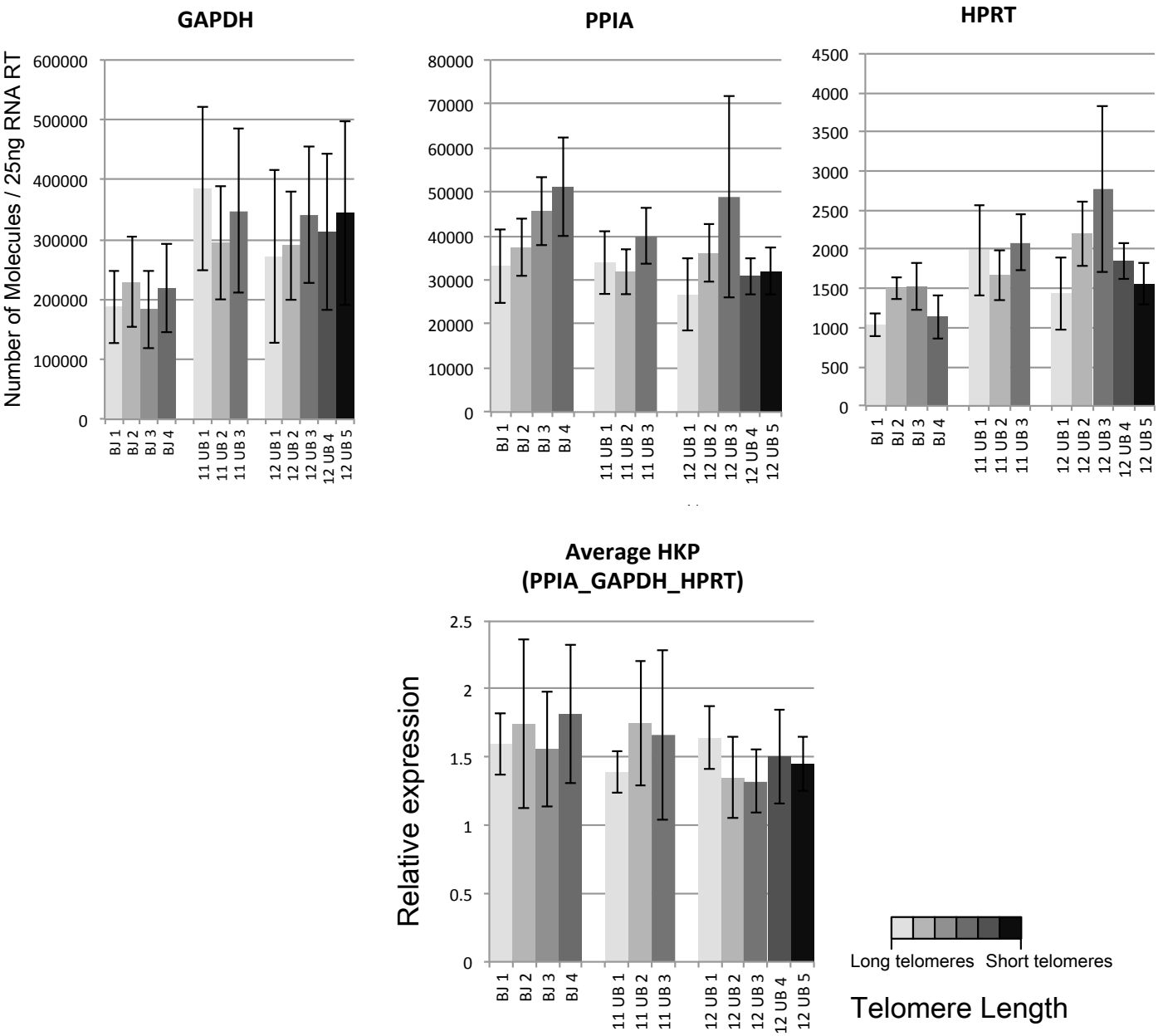
To normalized 3C interactions and allow quantification between samples, an average of 11 points was made. The 11 points were made using a set of primers picked surrounding successive HindIII restriction sites in Enr313, a gene that showed no looping interactions. One primer was constant. The interactions detected represent non-specific proximity effects in the absence of true looping. All values are a result of 3 independent cross linked sample combined with a technical duplicate (total of 6 measure per data point).



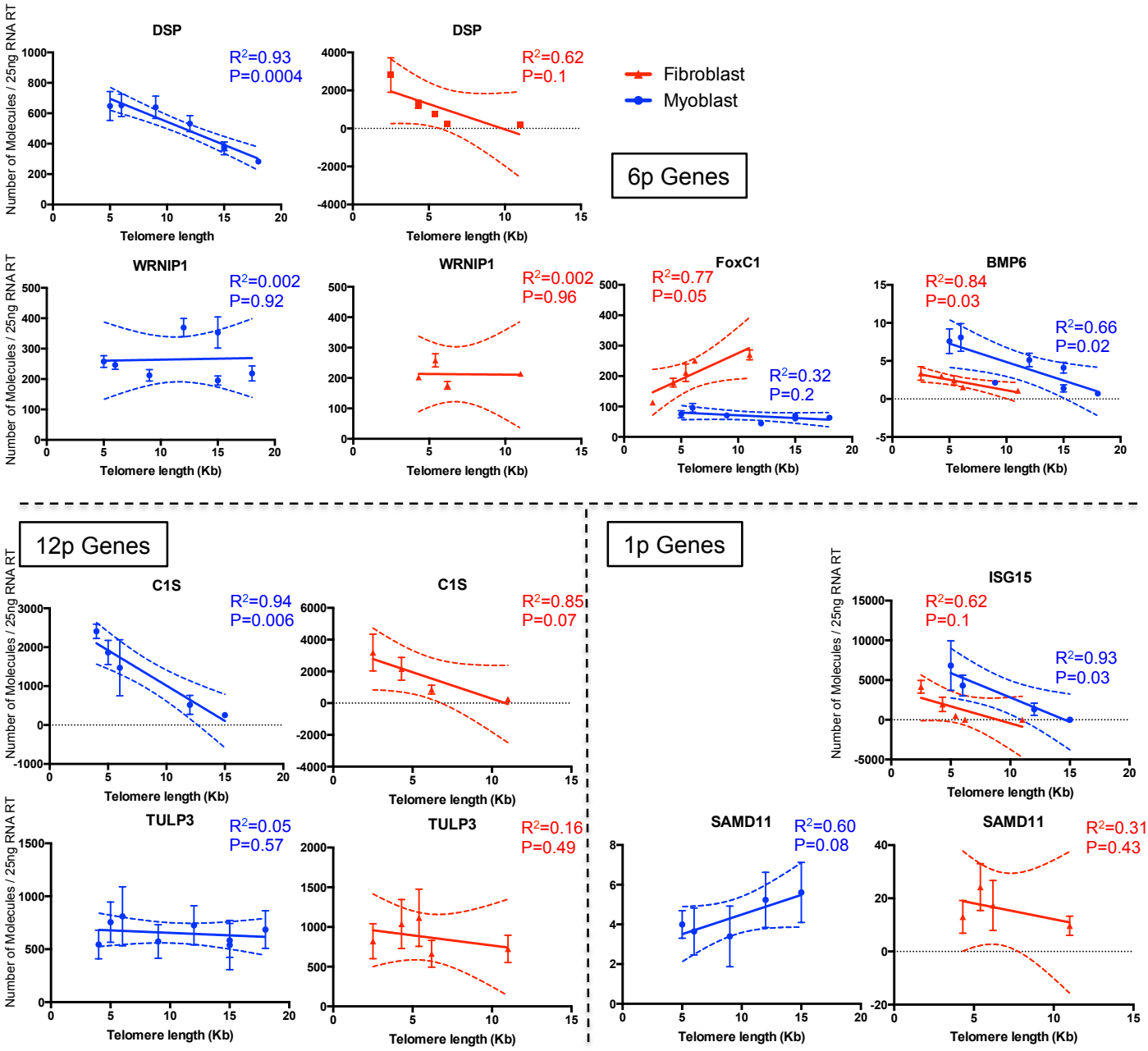
Supplementary Fig S3.
Heatmap representation of gene expression between myoblast with long (L1, L2, L3) and short telomeres (S1, S2, S3) [biological triplicates for each] after filtering and normalization of the data (see supplementary methods). Genes and samples were clustered using average hierarchical clustering with Euclidean distance. Red samples represent up regulated transcripts whereas green samples represent down regulated transcripts. We compare moderated and un-moderated t-statistics with either Bonferroni or BH p-value adjustment. The three methods are shown from left to right in order of decreasing stringency. The most restrictive method resulting in 144 transcripts was used in all subsequent analysis, as all of these transcripts in the most restrictive analysis were also present in the other less restricted methods (all 144 transcripts met the q-value criteria of 0.05 regardless of which method was employed).



Supplementary Fig S4. ISG15 gene expression.
Telomere shortening was monitored following the excision of hTERT. Two series of clones were isolated from 2 different primary myoblasts cultures (11UB, 12UB), and one series of fibroblast clones (BJ). All clones are analyzed at least 30PDs prior senescence. ddPCR was used to analyze the expression of ISG15 (a genes with no changes observed in the array but known to be affected by TPE) and a gene located between ISG15 and the 1p telomere. Expression in myoblast and fibroblast with long telomeres (light grey) are compared to a series of isogenic clones with shorter telomeres (grey scale). ISG15 expression varies with length, while any changes in SAMD11 do not appear to be significant.

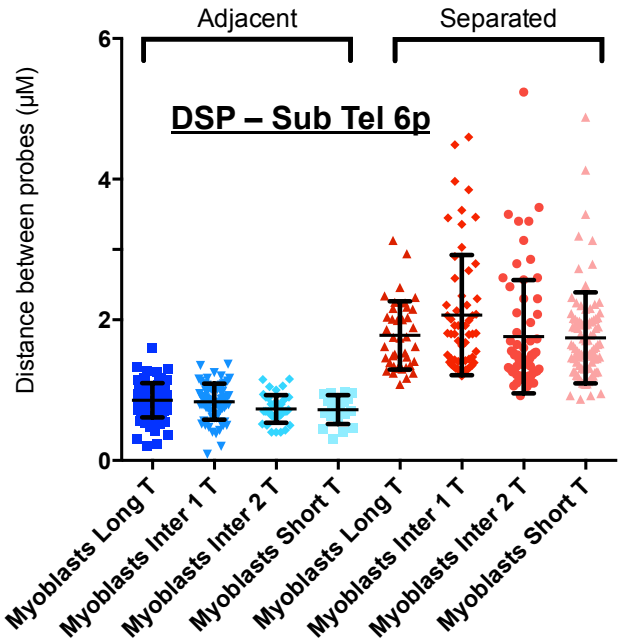


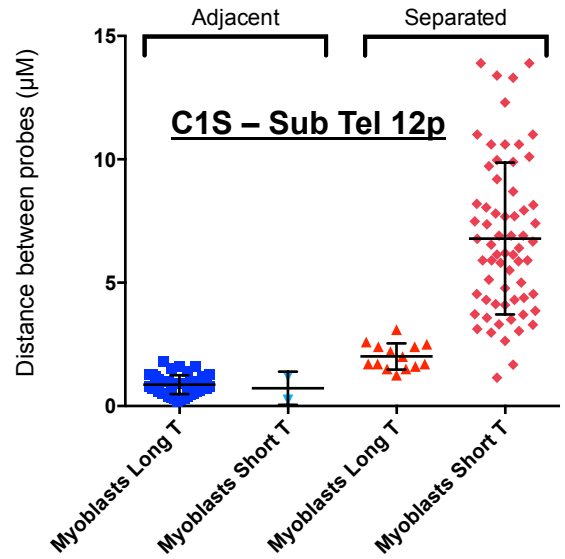
Supplementary Fig S5.
Progressive expression changes in myoblast and fibroblast Isogenic clones.
ddPCR analysis of three common housekeeping genes (GAPDH, PPIA and HPRT). Expression in myoblasts and fibroblasts with long telomeres (light grey) are compared to a series of isogenic clones with shorter telomeres (grey scale).
Each assay was performed in biological triplicate and technical duplicate
No differences were observed in the gene expression of any of these genes.

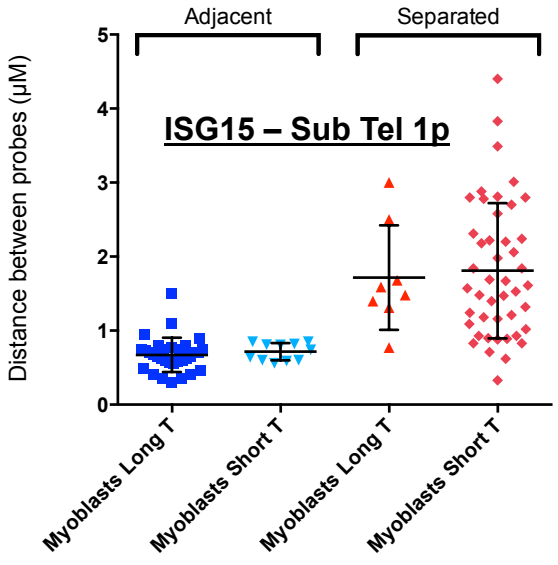


Supplemental Figure S6.

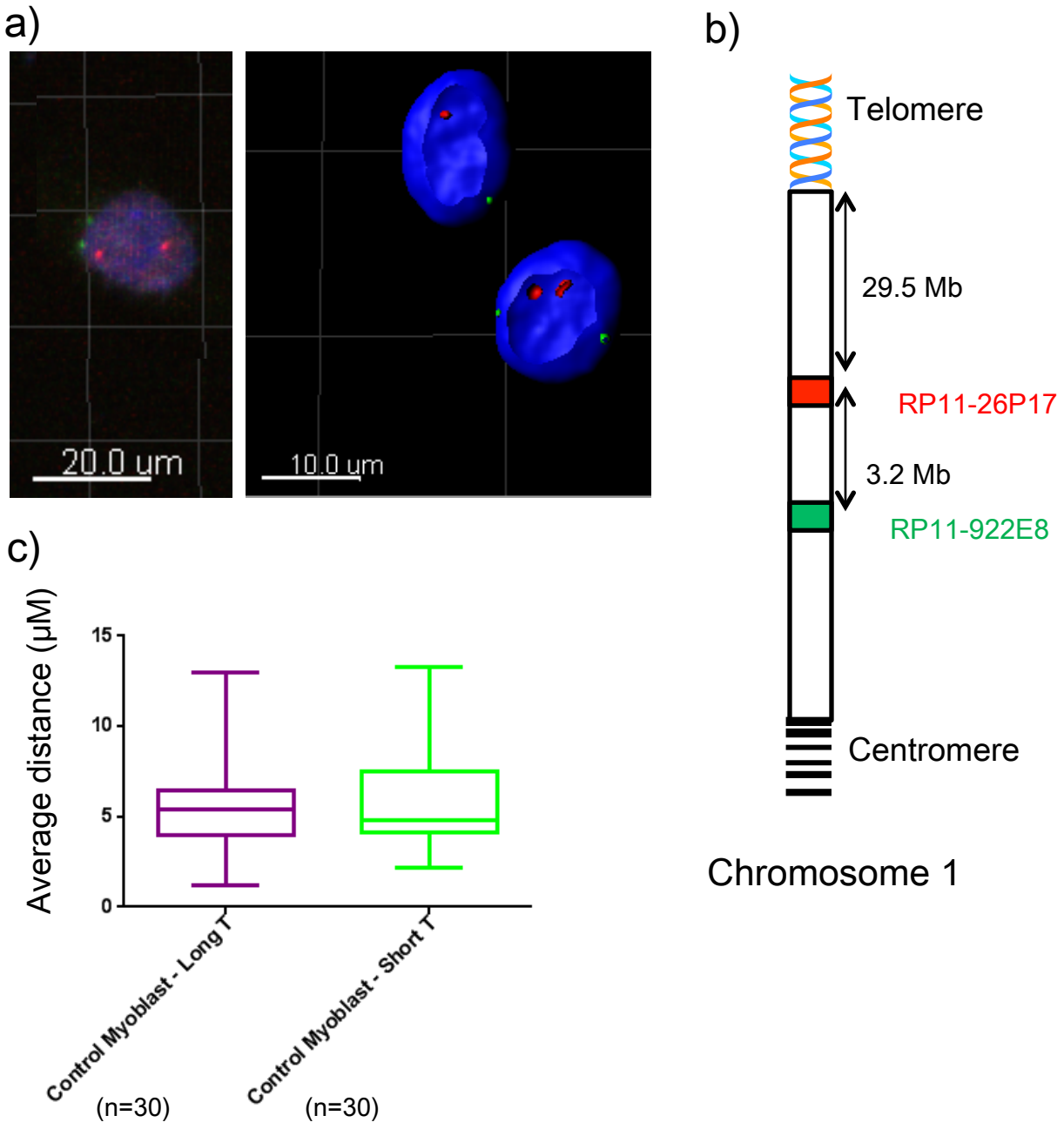
Gene expression of TPE-OLD genes correlate with telomere length. Representation of the gene expression analysis panel from Figure 4. and 5. Telomere length of each sub-clone isolated from fibroblasts (red) and myoblasts (blue) are reported as function of gene expression. We present in detail the TPE-OLD and their respective controls (WRINP1, SAMD11 and TULP3). For each assay, we report the R^2 and P value associated with the goodness of fit of the linear regression curve. The plots reports also the 95% confidence interval of the linear regression. In brief, for all TPE-OLD genes R^2 associated are above 0.62; and most are significant. For fibroblast, tests are based on only few samples, hence associated P are close to significance in some cases (DSP associated P value= 0.1; ISG15=0.1; C1S=0.07).



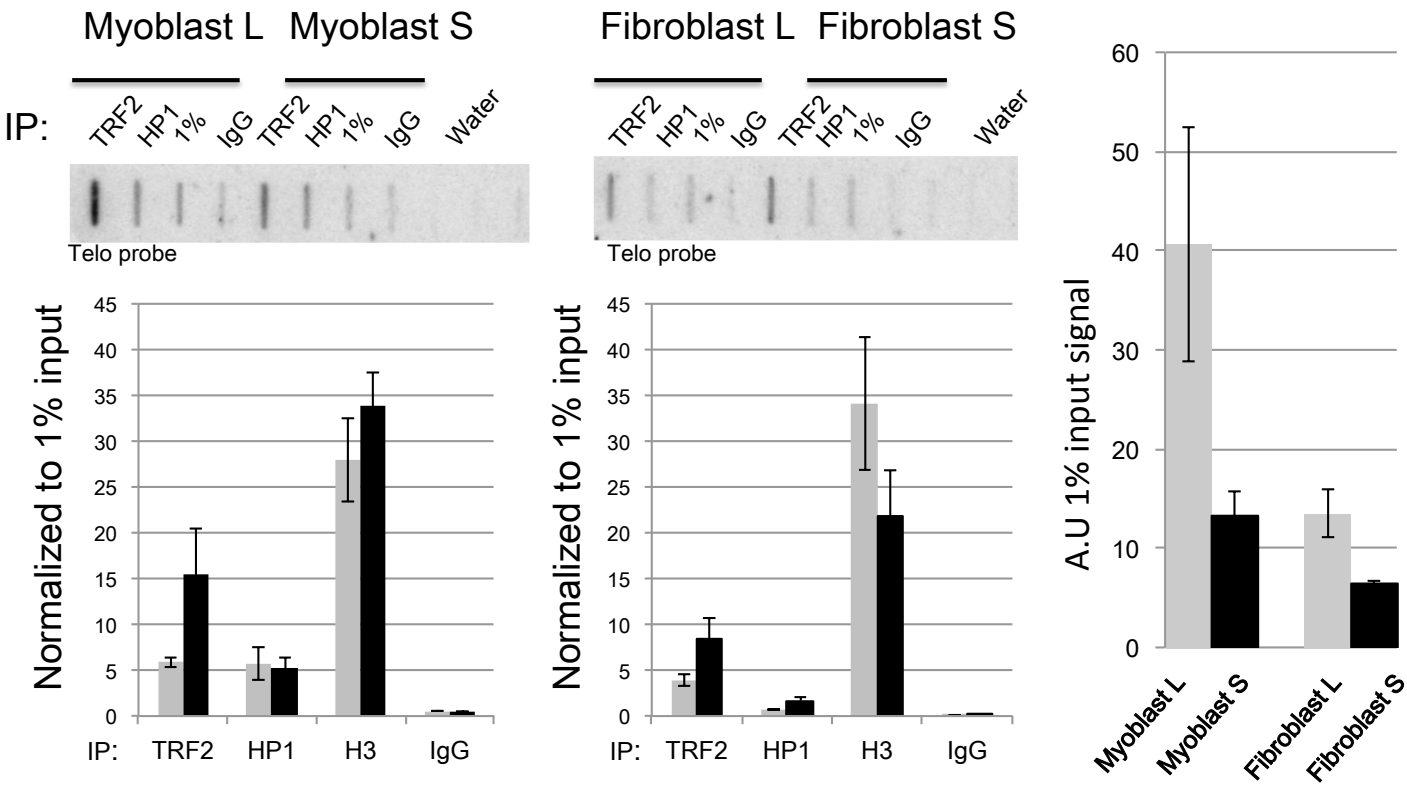




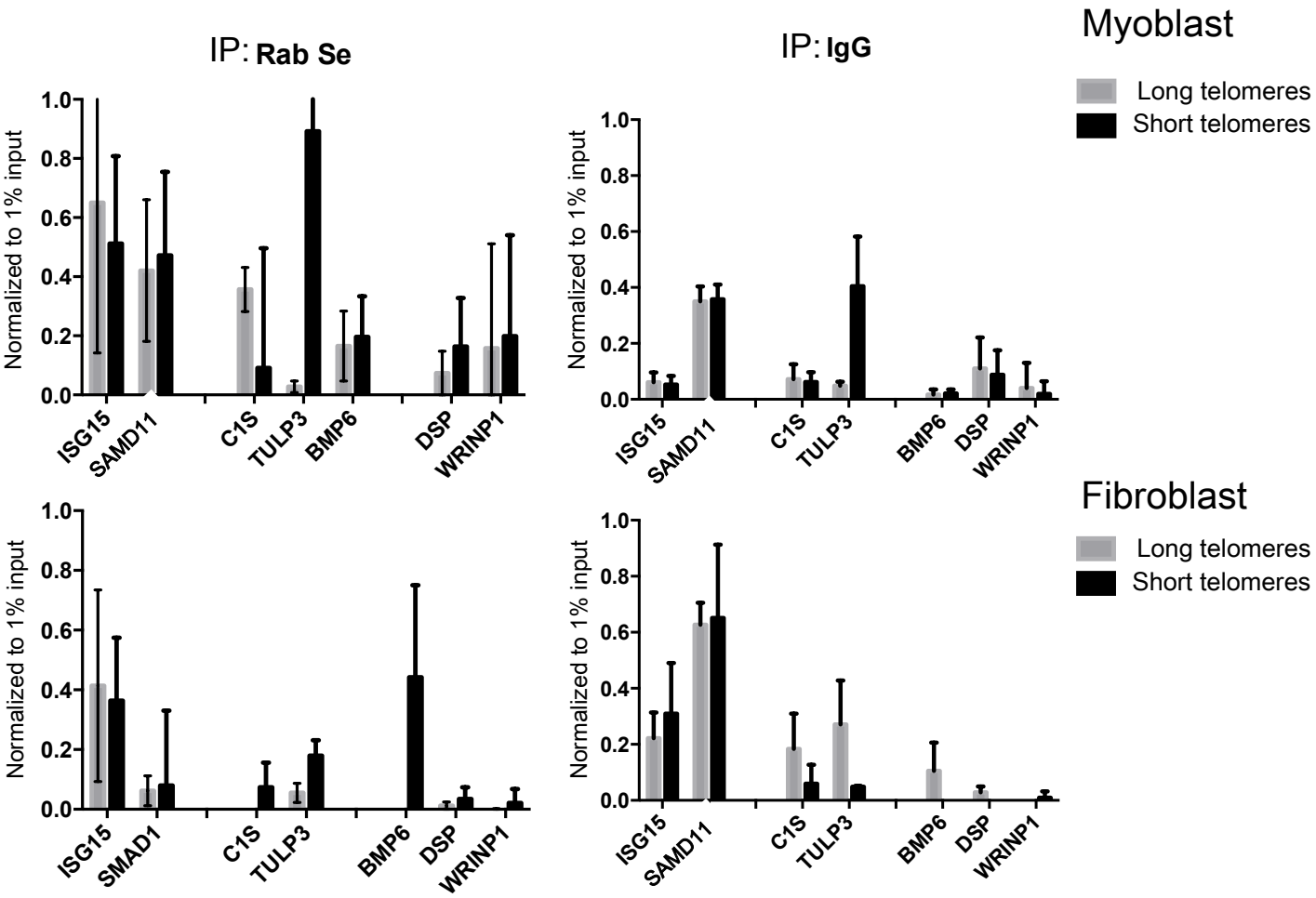
Supplementary Fig S7.
Single Cell representation of distance between probes in 3D-FISH experiments.
Representation of the averages distance measured in the presented 3D-FISH experiments (Figure 2 and 6). Single measurement of distance between the probes are plotted respectively for each chromosome ends testes (6p, 12p and 1P in respective order). Each point represent a single measure and allows to appreciate homogeneity of measures. Means and SD values are reported in Figure 2 and 6.



Supplementary Fig S8.
Differences of conformation are restricted to telomeric region.
In order to observe if the differences seen in subtelomeric region could be due to a more broad DNA compaction related phenomenon we performed the following experiment, mimicking the strategy from Fig.2 and Fig.6 with probes further away from the telomeres (30Mb).a) *In situ* analysis. Cells were hybridized to fosmid probes targeting respectively HDAC1 (green) and SRSF4 (red) two genes located roughly 30 Mb from the telomeres of chromosome 1p as shown in b). An average of 30 con-focal nuclear z-stacks were analyzed per condition (myoblast with long and short telomere) and processed with Imaris for quantification (right image). Results of the distances measured between the probes are plotted in c) the box and whiskers format allows to appreciate than no differences of mean and variances can be found between the two conditions (respective mean value : 5.4 and 5.8 µM) .



Supplementary Fig S9.
Slot blot of ChIP samples using a telomeric radioactive probe in myoblast (left) and fibroblasts (right) with long and short telomeres. No differences of enrichment were observed between long and short telomeres samples with the exception of TRF2. We observe a significant increaseof telomere detection with a TRF2 IP in cells with short telomeres. An explanation that can be related to either an increase in TRF2 protein levels or an increase in the binding of TRF2 towards telomeric sequences (higher density of TRF2 biding, resulting to higher detection). On the far right we show the detection levels of the telomeric probe in the 1% inout used for the IPs as expected, cells with long telomeres contained more telomeric repeats.



Supplementary Fig S10.
Chromatin immuno precipitation representative controls. Enrichments of promoter sites after IP using Rabbit serum or IgG. All ddPCR assays are ranging below 1 after normalization to 1% IP. ChIP was performed using biological duplicates, and triplicates for ddPCR analysis. All results are normalized to 1% input signal.

	#genes changed on the array	# genes in the array	#total genes identified	# change if all genes were on the array	% genes changed	Name	Distance from telomere (in Mb)	P value	Log fold change	Fold change
0-10 Mb	12	133	288	24	9	TEAD4	3.07	0.014	-0.56	↓ 1.8x
						CCND2	4.38	0.004	0.72	↑ 2.0x
						C12orf5	4.43	0.051	0.32	↑ 1.4x
						CD9	6.31	0.046	-0.38	↓ 1.5x
						TAPBL	6.56	0.012	0.58	↑ 1.8x
						C1S	7.10	0.002	1.00	↑ 2.7x
						C1R	7.19	0.002	0.81	↑ 2.2x
						C1RL	7.25	0.013	0.51	↑ 1.7x
						CD163L1	7.50	0.021	0.71	↑ 2.0x
						MFAP5	8.79	0.018	-0.45	↓ 1.6x
						microRNA 1244-1	9.39	0.043	-0.45	↓ 1.6x

Table S1. Array analysis of Chromosome 12p.
The number of changes (increased or decreased) that reached $p<0.05$ is shown in comparison with the number of 12p genes present on the array and the total number of genes within 0-5 and 5-10 Mb identified by the USC genome browser. Genes In green were confirmed by ddPCR gene. The distance from the telomere, p values and fold-changes are shown.