Title: Atrial Septal Defect (ASD) associated long non-coding RNA *STX18-AS1* maintains time-course of *in vitro* cardiomyocyte differentiation

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Supplementary figure captions:

This supplementary file (Online Resource 2) includes the supplementary figures and their captions supporting the main text.

Supplementary Figure 1: The eQTL analyses of *STX18-AS1* in human tissues. **a**, Linkage disequilibrium (LD) across *STX18-AS1* with SNP data from CEU population of 1000 Genomes Project. The LD map was generated with HaploView. The LD of SNP pairs is represented as r2 in white to red (0-1). The locations of the three risk SNPs identified in GWAS are labelled in red. The black triangles represent haplotype blocks (defining with confidence intervals according to Gabriel's method). **b-d**, eQTL analyses with 108 human heart right atrial appendage (RAA) samples using qPCR method for three top SNPs identified from GWAS, rs870142 (a), rs16835979(b), and rs6824295(c). The significant association between SNPs and *STX18-AS1* transcription was tested using the linear regression model, with P values between 0.038-0.039. **e-f**, The eQTL analyses of rs870142 and *STX18-AS1* transcription in internal mammary artery (IMA) samples (d) and human blood samples (e). The linear regressions are not significant for both IMA and blood eQTL analyses.

Supplementary Figure 2: Genomic location and conservation of *STX18-AS1* and nearby genes. The genomic location from UCSC genome browser surrounding *STX18-AS1* in human and mouse. *STX18* and *MSX1* are the two neighbouring genes of *STX18-AS1* in human. No annotated genes at the similar location between *STX18* and *MSX1* is identified in mouse genome. Phylop score shows the conservation score spare species. The sequences across *STX18-AS1* have lower Phylop scores

than neighbouring genes.

Supplementary Figure 3: Transcription of *STX18-AS1* in human tissues. **a**, comparison of *STX18-AS1* transcription in Adult and Foetal tissues (cDNA from Human MTC™ Panel I and Human Foetal MTC™ Panel). **b-m**, whole-mount ISH of human embryonic hearts (**b-c**, CS17; **d-e**, CS18; **f-g**, CS19) from ventral and dorsal views. **h-j**, section of all three ISH hearts (**h**, CS17 heart; **i**, CS18 heart; **j**, CS19 heart) with cuts at the view of OFT. **k-m**, section of all three ISH hearts (**k**, CS17 heart; **l**, CS18 heart; **m**, CS19 heart) with cuts at the view of AS. RA, right atrium; LA, left atrium; RV, right ventricle; LV, left ventricle; OFT, outflow tract; AS, atrial septum (yellow); AO, aorta; PA, pulmonary artery; VS, ventricular septum. *STX18-AS1* transcripts are stained blue, counterstained with eosin.; scale bars are 200μm.

Supplementary Figure 4: Neighbour gene transcription during hESC-CM differentiation in *STX18-AS1* CRISPR KO cells. **a,** Time-course expression of *STX18* during the CM differentiation from STX18-AS1 KO compared to WT hESC-CM differentiation. **b,** Time-course expression of *MSX1* during the hESC-CM differentiation from *STX18-AS1* KO compared to WT cells. *, $P< 0.05$. Two-way ANOVA test with Bonferroni adjustment is applied for generating the P values for comparisons at each time point (n=3-9).

Supplementary Figure 5: CRISPR deletions and cell properties of cells after *STX18-AS1* CRISPR transduction **a**, In CRISPR cell pool transduced with *STX18-AS1* CRISPR after puromycine selection,

around 30-40% cells contains the deletion generated by *STX18-AS1* CRISPR sgRNAs producing a short product of ~600bp in comparing with original sequence of 3.3kb. **b-d**, the sequencing results of short products with deletions from *STX18-AS1* CRISPR cell pool. Non-specific repairs were suggested by the randomised sequences after cuts surrounding two sgRNAs in both 5' (**c**) and 3' directions (**d**). **e-f,** the macroscopic cell morphology of Control CRISPR and *STX18-AS1* CRISPR transduced cells at naïve state. **g**, the proliferation rate of Control CRISPR cells and CRISPR cell pool at naïve state with MTT assay. **h-i**, the averaged apoptosis rate (**h**) of Control CRISPR and CRISPR cell pool calculated from DRAQ7 staining (**i**). **j**, Unchanged *SOX2* transcription across the hESC-CM differentiation in *STX18-AS1* CRISPR cell pool comparing to Control CRISPR. **k-n**, the pluripotency quantified by FACS with SOX2-AF647 and averaged positive rate of cells (**n**). Data are shown in Mean±S.E. *, $P<0.05$, using T-test compared to Control CRISPR (n=3). Scale bars are 100μm for **e-f** and 200μm for **i**.

Supplementary Figure 6: *STX18-AS1* KO hESC-CM differentiation. **a**, the deletion of *STX18-AS1* KO cell line. Only short products with deletions at 600bp was identified from *STX18-AS1* KO cell, while the WT cells only contained long products with original sequence at ~3kb. **b**, The sequence of *STX18-AS1* KO clone at the repaired junction of two CRISPR cuts. **c**, The transcription levels of *STX18-AS1* in WT and KO cells during CM differentiation. No transcription of *STX18-AS1* is detected in *STX18-AS1* KO cell across the full differentiation stages with qPCR. Data are shown in Mean±S.E; n=3-9.

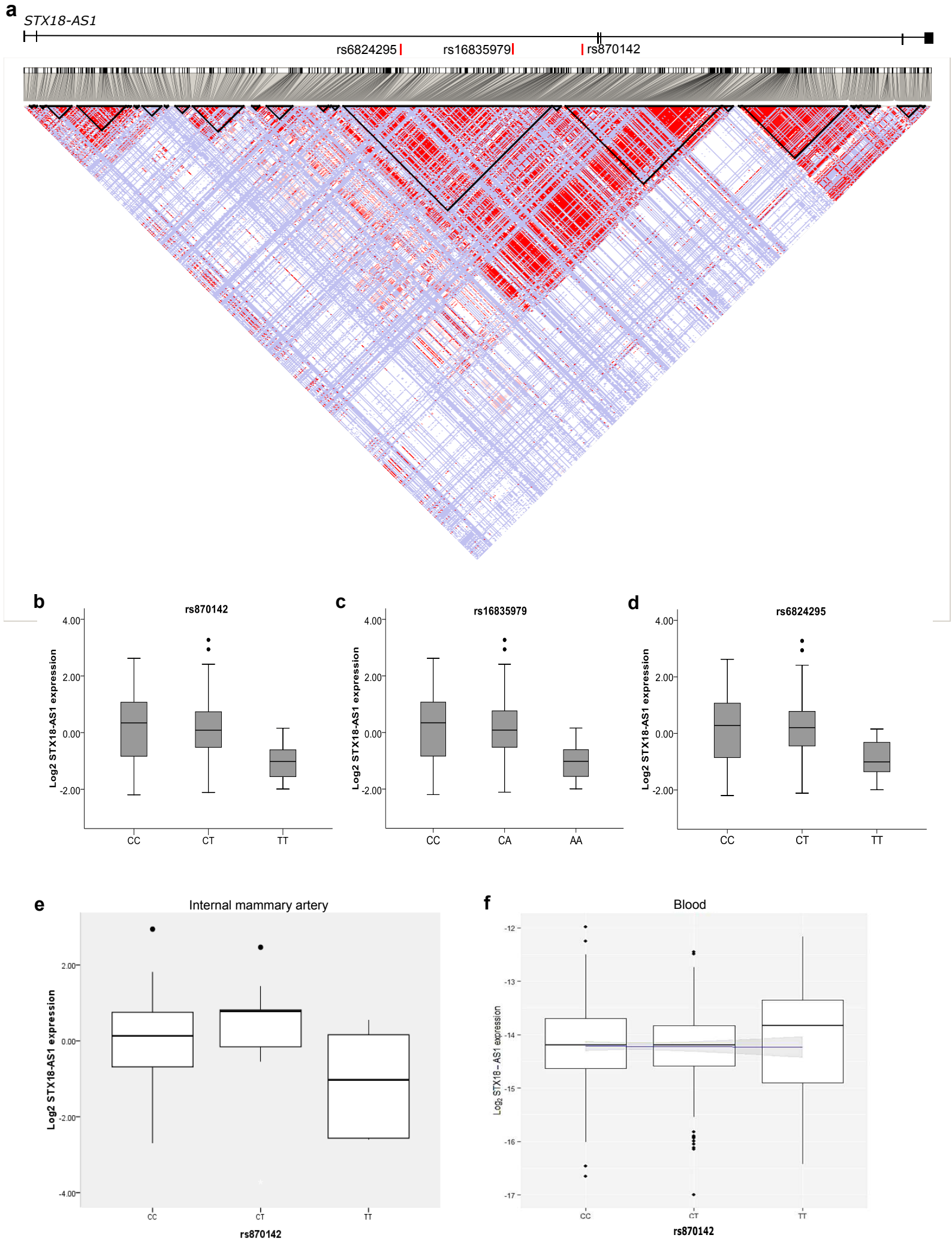
Supplementary figure 7: *STX18-AS1* CRISPR cell pool inhibits early hESC-CM differentiation. **a,**

Immunostaining of cTNT (red fluorescence, referencing to DAPI in blue) at Day 8 of differentiation in both Control CRISPR cell pool and *STX18-AS1* CRISPR cell pool derived CMs. Scale bars represent 200μm. **c-d,** the quantitative analyses of cTNT immunostaining on fluorescence density (**c**) and cTNT-positive cells (**d**). **, P< 0.01, using t-test. **e**, Time-course expression of *STX18-AS1* during the CM differentiation from Control CRISPR and *STX18-AS1* CRISPR cell pool. *, P< 0.05. Two-way ANOVA test with Bonferroni adjustment is applied for generating the P values for comparisons at each time point (n=3-9).

Supplementary Figure 8: *STX18-AS1* CRISPR HepG2 cell pool and mechanism investigation with ChIRP. HepG2 cell line expressed reasonable levels of *STX18-AS1* and *NKX2-5* but not other cardiac genes, therefore, suitable to be used as the cell context for *STX18-AS1* mechanism analyses. **a**, The deletions generated in *STX18-AS1* CRISPR HepG2 cell pool. About 70-80% cells contained deletions with short products at 600bp. **b**, the reduced *STX18-AS1* transcription in *STX18-AS1* CRISPR HepG2 cell pool. *, P<0.05, compared to Control CRISPR using T-test (n=3). **c**, NKX2-5 protein level was reduced in *STX18-AS1* CRISPR cell pool of HepG2. **d-e**, the positive control ChIRP probe antisense to U1 snRNA with successful pulldown products (**d**) and interactive protein U1A (**e**). The Input in **d** shows the total distribution of RNA populations, with the band of U1 snRNA observed around 200bp. B-actin in **e** is used as the background control. Control probe is a LacZ targeting probe as the negative control probe. RNase+ is another negative control for nonspecific signals by degrading RNAs in cell lysate. **f,** the successful pulldown of *STX18-AS1* RNA in the

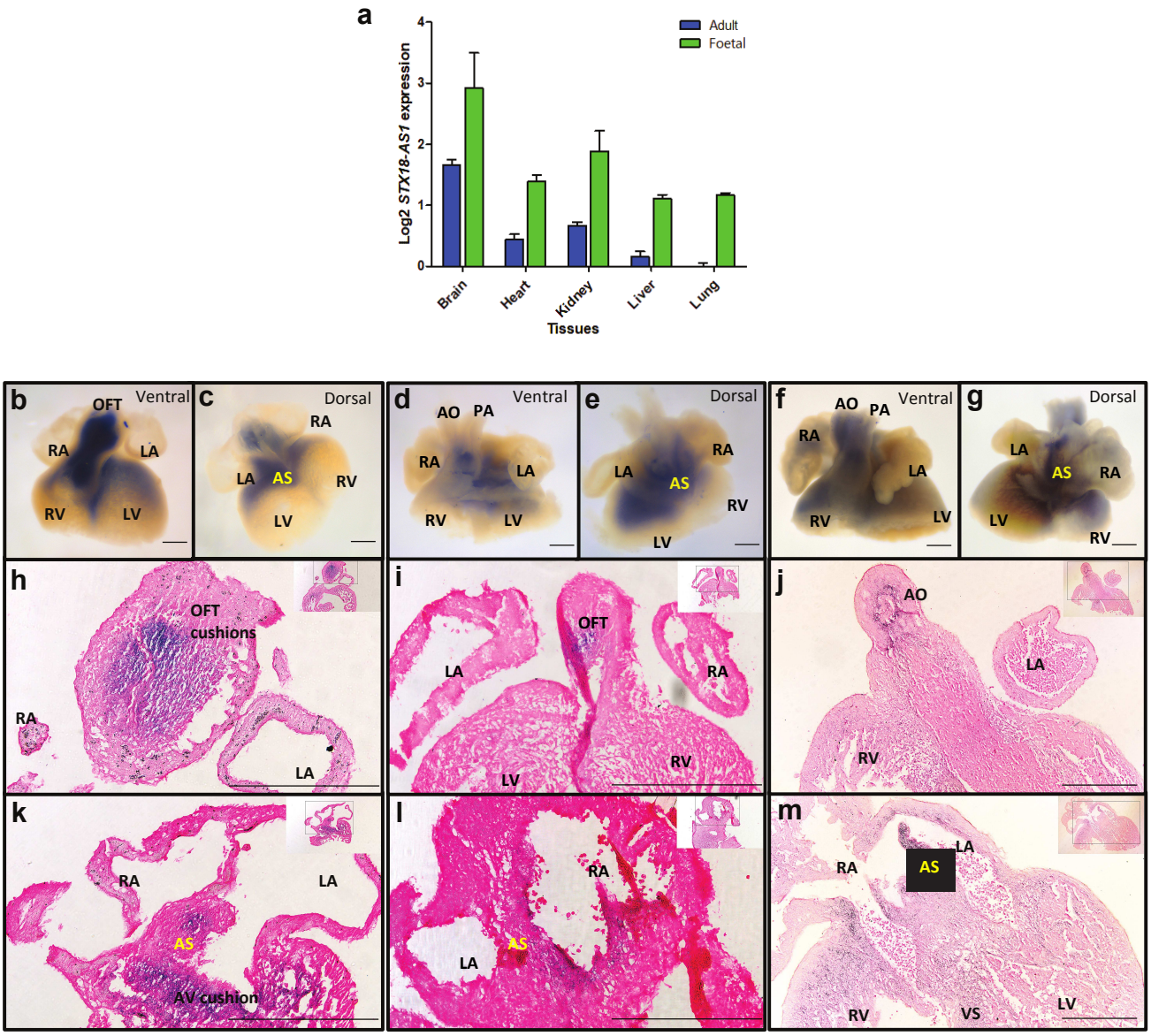
ChIRP experiment with antisense *STX18-AS1* probes.

Supplementary figure 1

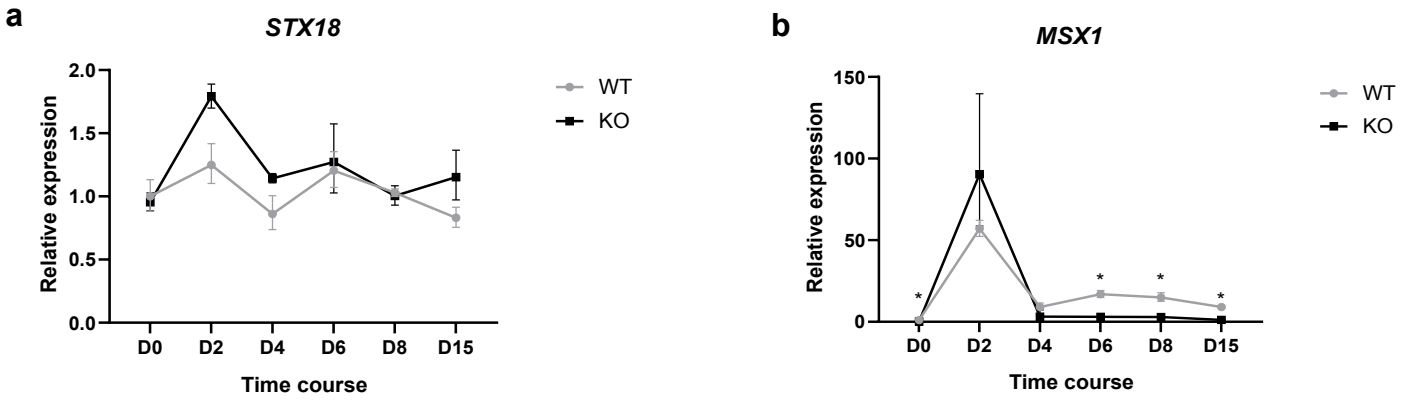


Supplementary figure 2

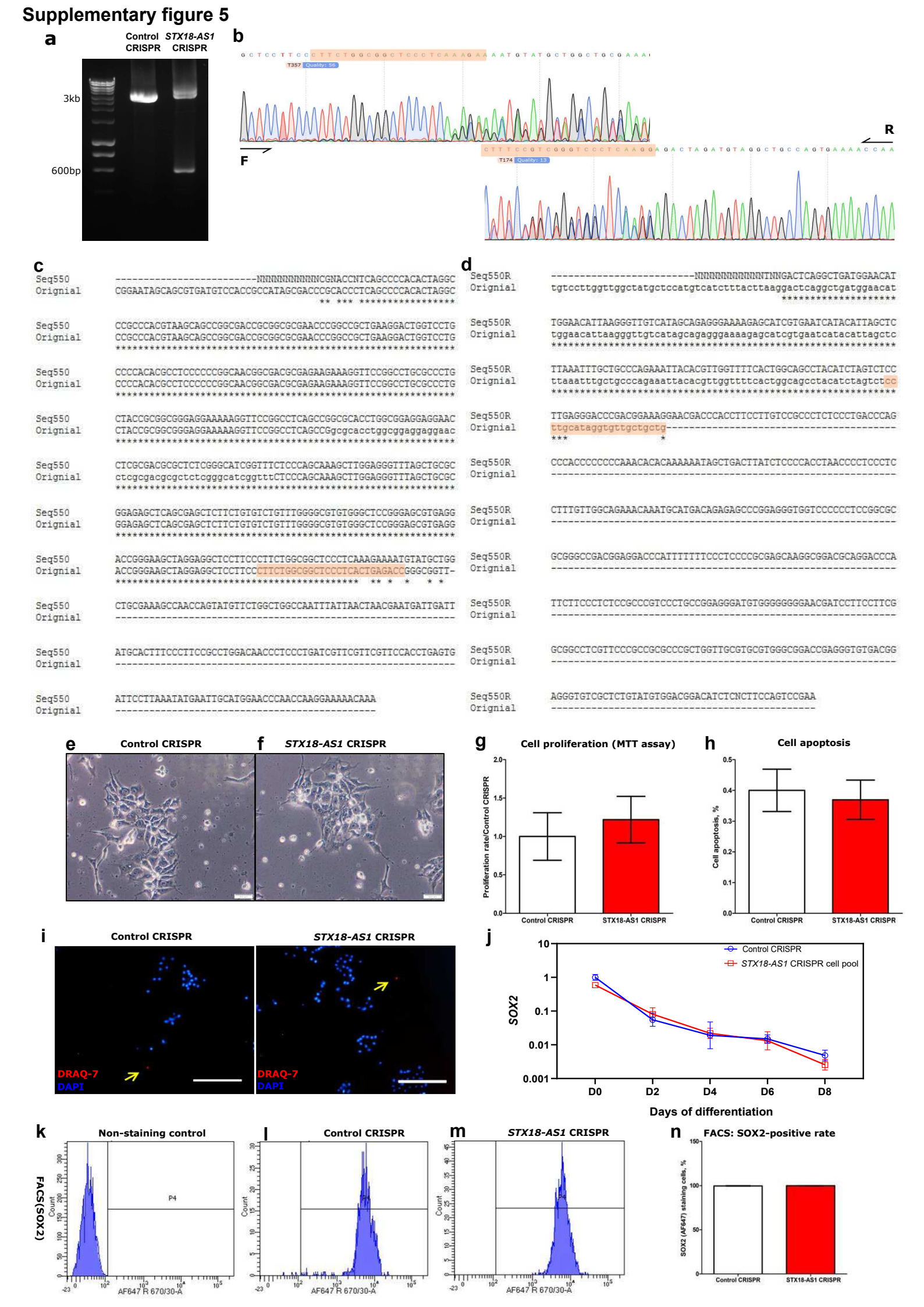
Supplementary figure 3



Supplementary figure 4

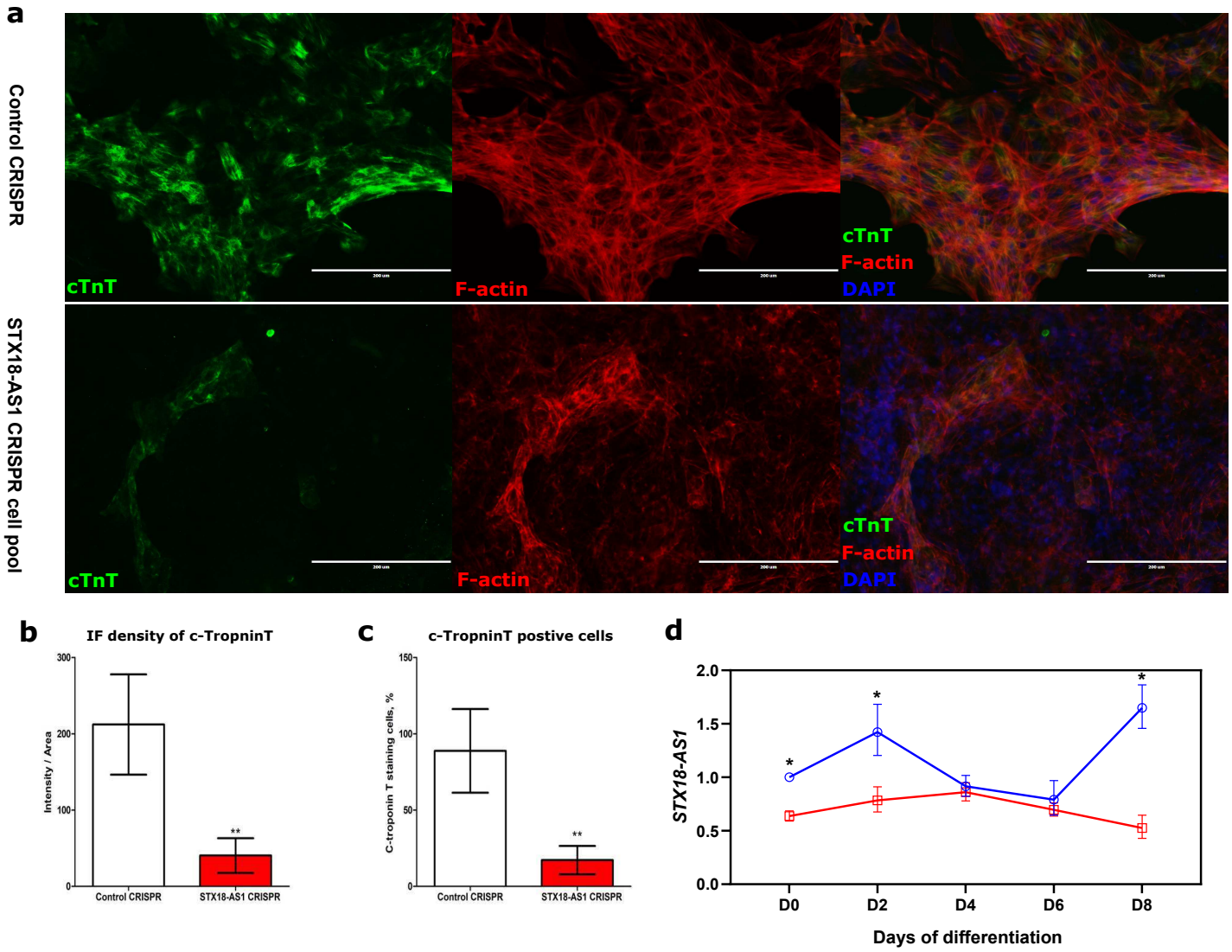


Supplementary figure 5



Supplementary figure 6

Supplementary figure 7



Supplementary figure 8