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Supplementary information

Xist spatially amplifies SHARP/SPEN recruitment to balance chromosome-wide silencing and specificity to the X chromosome

In the format provided by the authors and unedited

SUPPLEMENTARY NOTE

Xist localization across the X and chromosome-wide silencing

Previous studies have shown that there are between 60-200 copies of Xist within each nucleus[18,19]. This level of expression is sufficient to drive chromosome-wide silencing across the >1500 genes encoded on the X. Based on these numbers, Xist cannot simultaneously localize to each gene within each cell because there are not enough Xist molecules present; it must instead mediate silencing over several genes at once (**Extended Data Fig. 5a**). As such, Xist localization within individual cells must be heterogenous such that in one cell it localizes at a subset of genes but in another cell, it localizes at a different subset of genes.

Based on ensemble measurements, we know that Xist does not preferentially accumulate at specific sequences (e.g., promoters) but instead localizes broadly across the chromosome (**Extended Data Fig. 5b**). This means that the Xist RNA molecules within each cell must localize randomly at distinct positions spread across the >167 megabases of the chromosome.

Using this information, we can simulate the expected occupancy of Xist across the X within single cells in a manner that would explain the ensemble pattern (**Extended Data Fig. 5b**). We find that the likelihood that Xist is present over any given gene within an individual cell is extremely low (on average <5% of genes per cell would be covered by Xist) (**Extended Data Fig. 5c**). For example, Xist would be expected to localize over any region of Pgk1 in only ~7% of cells (**Extended Data Fig. 5c**). As such, if Xist-mediated silencing was solely dependent on such localization, we would expect that this gene would remain active in >90% of individual cells. However, using our single cell measurements we observe that this gene is silenced in >87% of single cells (**Extended Data Fig. 4c**). Therefore, these single cell measurements allow us to measure chromosome-wide silencing when focusing on a subset of X chromosome genes.

SUPPLEMENTARY TABLES

Table S1. List of plasmids generated and used in this study

Plasmid Name	Addgene number (if applicable)	Description
Cas9-nickase-eGFP (Cas9n-eGFP)	48140	eGFP-tagged Cas9n backbone into which four different SHARP-targeting gRNAs were inserted to generate SHARP-KO mESCs
SHARP-targeting gRNA plasmid	-	Cas9n-eGFP backbone containing four different SHARP-targeting gRNAs
FL-SHARP entry clone	-	Entry clone of full-length SHARP sequence used for Gateway cloning
ΔRRM-SHARP entry clone	-	Entry clone of SHARP sequence lacking its RRMs (deletion of amino acids 2-590) used for Gateway cloning
ΔIDR-SHARP entry clone	-	Entry clone of SHARP sequence lacking its IDRs (deletion of amino acids 639-3460) used for Gateway cloning
PB-TAG-ERN	80476	PiggyBac destination vector that was modified prior to Gateway cloning
PB-HALO-IRES-NGFR destination vector	-	PiggyBac destination vector used for Gateway cloning containing HALO and NGFR
PB-eGFP-IRES-NGFR destination vector	-	PiggyBac destination vector used for Gateway cloning containing eGFP and NGFR
FUS-mCherry plasmid	101223	Plasmid from which FUS-mCherry fusion was derived to create PB-FUS-mCherry-IRES-NGFR
PB-FUS-mCherry-IRES-NGFR	-	PiggyBac destination vector used for Gateway cloning containing FUS-mCherry fusion and NGFR
HALO-FL-SHARP rescue construct	-	FL-SHARP entry clone inserted into PB-HALO-IRES-NGFR destination vector
HALO-ΔRRM-SHARP rescue construct	-	ΔRRM-SHARP entry clone inserted into PB-HALO-IRES-NGFR destination vector
HALO-ΔIDR-SHARP rescue construct	-	ΔIDR-SHARP entry clone inserted into PB-HALO-IRES-NGFR destination vector
eGFP-FL-SHARP rescue construct	-	FL-SHARP entry clone inserted into PB-eGFP-IRES-NGFR destination vector
eGFP-ΔRRM-SHARP rescue construct	-	ΔRRM-SHARP entry clone inserted into PB-eGFP-IRES-NGFR destination vector
eGFP-ΔIDR-SHARP rescue construct	-	ΔIDR-SHARP entry clone inserted into PB-eGFP-IRES-NGFR destination vector
FUS-mCherry-ΔIDR-SHARP rescue construct	-	ΔIDR-SHARP entry clone inserted into PB-FUS-mCherry-IRES-NGFR destination vector
turboGFP plasmid	69072	Non-targeting plasmid co-transfected with SHARP rescue constructs to enrich for successfully transfected cells
eGFP-EED construct	-	EED entry clone inserted into PB-eGFP-IRES-NGFR used for imaging in HEK293T cells
eGFP-Ptbp1 construct	-	Ptbp1 entry clone inserted into PB-eGFP-IRES-NGFR used for imaging in HEK293T cells

Table S2. List of oligo sequences used in this study (guide RNAs and primers)

gRNA	**Target**	**Top Strand**	**Bottom Strand**
gRNA 1	SHARP	TCTGGAGTCAGGTGAGACGC	GCGTCTCACCTGACTCCAGA
gRNA 2	SHARP	GTGAGTGTTTGCTTACACCG	CGGTGTAAGCAAACACTCAC
gRNA 3	SHARP	CTCGGTTCTTACACAGCTCC	GGAGCTGTGTAAGAACCGAG
gRNA 4	SHARP	TCTTTGAGCAAGACTCCAAG	CTTGGAGTCTTGCTCAAAGA
Method	**Target**	**Sequence_Fw**	**Sequence_Rv**
gDNA PCR 1	SHARP WT	ACACACACGCAATCACACAA	ATGAGTCTCCGGCTCTTCCT
gDNA PCR 2	SHARP KO	ACACACACGCAATCACACAA	TGAAGCCCTGCATTTAGGAG
gDNA PCR 3	SHARP KO	AGGCCTATTCCGTCCTGGTA	ATGAGTCTCCGGCTCTTCCT
RT-qPCR	Xist 1	GCCTCTGATTTAGCCAGCAC	GCAACCCCAGCAATAGTCAT
RT-qPCR	Xist 2	AGCCAGCACTGATCTCAAGC	GCAACCCCAGCAATAGTCAT
RT-qPCR	GAPDH	CATGGCCTTCCGTGTTCCTA	GCCTGCTTCACCACCTTCTT

Table S3. List of RNA-FISH probes used in this study and corresponding design ID

Probe Target	**Probe Type**	**Fluorescent Label**	**Affymetrix Design ID**
Xist	4	Alexa Fluor 488	VB4-19746
Xist	6	Alexa Fluor 647	VB6-10824
Kdm5c	1	Alexa Fluor 546	VPYMJG7-01
Kdm5c	4	Alexa Fluor 488	VB4-20659
Kdm6a	1	Alexa Fluor 546	VPZTD24-01
Pgk1	1	Alexa Fluor 546	VB1-6000089
Atrx	6	Alexa Fluor 647	VPNKRFV-06
Gpc4	4	Alexa Fluor 488	VB4-17177
MeCP2	4	Alexa Fluor 488	VPH49TK

SUPPLEMENTARY VIDEO LEGENDS

Supplementary Video 1.

Live imaging of HEK293T cell expressing FL-SHARP labeled with eGFP representing fluorescent intensity over time.

Supplementary Video 2.

Live imaging of HEK293T cell expressing FL-SHARP labeled with eGFP representing 3D surface reconstructions based on fluorescent intensities over time, volume color coded.

Supplementary Video 3.

Live imaging of HEK293T cell expressing ΔIDR-SHARP labeled with eGFP representing fluorescent intensity over time.