

<i>Xist</i> mutants	5'end /3'end sgRNAs	Primers to confirm deletion (F/R)	Primers to confirm loss of WT sequence (F/R)
<i>Xist</i> Δ F+B+C	TCACGCAGAAGCCATAATGG/ CTTGAGAGATGATACCTCCA	CTGCTGATCGTTTGGTGCTG/ CAGACCTGTGTTTGCCCCTT	CTGCTGATCGTTTGGTGCTG/ ATCAAGGCCGAATCCCGCAAC
<i>Xist</i> Δ F+B	TCACGCAGAAGCCATAATGG/ AGGGCTGGACTGGATTGGGT	TGGTGCTGTGTGAGTGAACC/ TTAGCACTGAATCAATGAAGA	CTGCTGATCGTTTGGTGCTG/ ATCAAGGCCGAATCCCGCAAC
<i>Xist</i> Δ B+C	TATAACAGTAAGTCTGATAG/ GTGTATCTTGATTAACATGA	ATGACTGGATGTCAGGAGTA/ CAGACCTGTGTTTGCCCCTT	ATGACTGGATGTCAGGAGTA/ CTGAGTCTTGAGGAGAATCT
<i>Xist</i> Δ B+1/2C	TATAACAGTAAGTCTGATAG/ CATACTGACTTCTAGAGTCA*	ATGACTGGATGTCAGGAGTA/ CAGACCTGTGTTTGCCCCTT	ATGACTGGATGTCAGGAGTA/ CTGAGTCTTGAGGAGAATCT
<i>Xist</i> Δ B	TATAACAGTAAGTCTGATAG/ CTCTAAGTAGAAGTGGGCTT	ATGACTGGATGTCAGGAGTA/ TTAGCACTGAATCAATGAAGA	ATGACTGGATGTCAGGAGTA/ CTGAGTCTTGAGGAGAATCT
<i>Xist</i> Δ C	CTCTAAGTAGAAGTGGGCTT/ GTGTATCTTGATTAACATGA	CCAGGCCAGATACTTTCAG/ CAGACCTGTGTTTGCCCCTT	TCCATGGACAAGTAAACAAAGA A/ TGTTTGCCCCTTGTCTAAAT

Appendix Table 1 – List of gRNA sequences and primers used for *CRISPR/Cas9* editing of the different *Xist*-TetOP mutants

Primer sequences to confirm deletion and loss of wild-type (WT) allele are displayed for each *Xist* mutant; * highlights for the fact that the 3'end gRNA used to generate Δ B+1/2C was designed to a unique region within the repeat C, but several sequences with only 1 or 2 mismatched can be found within this repeat and are likely to be targeted to generate different types of Δ B+1/2C mutants.

RT-PCR analysis	Sequences (F/R)
<i>Xist</i> exon 1- exon 3	GCTGGTTCGTCTATCTTGTGGG / CAGAGTAGCGAGGACTTGAAGAG
<i>Xist</i> before repeat B	ATGACTGGATGTCAGGAGTA / CTGAGTCTTGAGGAGAATCT
<i>Xist</i> before repeat C	TCCATGGACAAGTAAACAAAGAA / TGTTTGCCCCTTTGCTAAAT
<i>Gapdh</i>	AACTTTGGCATTGTGGAAGG / ACACATTGGGGGTAGGAACA
18S	GGGAGCCTGAGAAACGGC / GGGTCGGGAGTGGGTAATTT

Appendix Table S2- Primer sequences for RT-PCR analysis of *Xist* mutants (used in Fig. EV1B)

Exon 1 - 5' end Set	Oligo-probes
1	gtctgataacagacctgtgt
2	tfaatggccaatgccttgaa
3	gcagagggttttgctgaaa
4	agggtagtattaggacctg
5	aggggactgaacaactgca
6	gtatgagggtatgggatctt
7	agagaagtggctcattggt
8	gtgcacacaacaggcaca
9	tccaatgcttaggaagaggg
10	tctcaccagatgcagatta
11	gcacaactgtggacatgaga
12	gtacaggagtcctgatctaa
13	gcttatattagtggacctc
14	tcttacctgaaggaccatt
15	agtttgaggaaggggttca
16	gcctactacaatcagtcatt
17	gttccttctgtagtgaaca
18	aagggtacatgttatggcca
19	tgagagtaggatcgtatcca
20	gagtggaaggaggggacag
21	tcatgtcctgttatataca
22	gcaggagtgaagagataca
23	attaatggtccactagcagg
24	gcatacctgatatagtga
25	aaagaatgcggcctgttga
26	atcctttcaagtgcacagag
27	ctctttctgctttaaagcgg
28	attaatatgcctctggtgct
29	cagggtcatatgtgaagggt
30	gtaggcatttcagaaccttt
31	ctcagcaacctctgcaataa
32	tgctattaagagtcccaa
33	ataaacatccagaactgcc
34	aaactagcaggggatttgct
35	ctctccttaatgatgggt
36	aggcaaaagggtatggcatga
37	agtcaagagaagggcttgc
38	aactgagacactgtagccat
39	catagggtgtttgggtaat
40	tgtaaaggaaagcccaagt
41	aaagtgctaagcttctct
42	taactttctggcagttggt
43	ttgcaatccaaatgcctttc
44	tcagtacactgagacactgc
45	agaaggcttaggtcatcttc
46	ttgccagagttaatggtgg
47	ttctcagtaatgctgggaga
48	ctgcactggatgagttactt

Exon 7 Set	Oligo-probes
1	acaggcaccagagaaagtga
2	gagccatagctagtgaagac
3	agattatttctcagggcagt
4	gaccggacggaatgatgtat
5	acttcagagccactgaaac
6	tcacagggtcctgtagaaa
7	tatgcttgacttagctcag
8	ctttatgggcaatggcaaca
9	agaccttgcacaatactat
10	caaatggctaagaccagtt
11	gatttagtccgtctcaagt
12	tatgcatgcttattcttagg
13	aggatggcagtatgcatatc
14	tttcttagatagcctgaca
15	tagaaaaccataccctgctc
16	actactatgagcagggagtt
17	tcagggtactagtctgatgc
18	tcagggtgatcaagtaca
19	ttcatacgttctctggttac
20	ggaagttacagtaggcttca
21	gcatgttattcctagagcaa
22	cctattttataggcagcttt
23	gtgcatgctgggataaaaag
24	acaagctccacacagatct
25	gtggtgttaggtgataagca
26	tctatgtgttcgctcaacac
27	tttggctagatttctacc
28	taacatttagcacactgcct
29	ggaagttatcctagacagcat
30	tttactggcaaggtgtttgt
31	tcactctggcctataaaaac
32	aggacctaggttagcatatt
33	aggaagtctccagtttatgt
34	ctgagccaggcaatgaacaa
35	ctgtagtctcaaggtgtgac
36	tgttttccagctaaagtg
37	ggaggggagcaactttttat
38	agtagcatctctgcaatgt
39	ctgtaagtcacactgagtgc
40	ttcaacctctgaggcaact
41	tcctgtagaacaggcgaac
42	agggtctgttaactttagg
43	cttaactgtttcattccca
44	agctcaaggcaacttggat
45	gtaacacttttgatgccaa
46	gaaatgtaagccattccta
47	gatgatggttaggatgtgctt
48	cagttggtgggaagatgact

Appendix Table S3 – Sequences of the two sets of Stellaris RNA FISH oligo-probes