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Supplemental Information

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Claudia Ribeiro de Almeida[1*], Somdutta Dhir[1], Ashish Dhir[1], Amin E. Moghaddam[1], Quentin Sattentau[1], Anton Meinhart[2], Nicholas J. Proudfoot[1,3*]

[1]Sir William Dunn School of Pathology, University of Oxford, South Parks Road, OX1 3RE Oxford, United Kingdom
[2]Department of Biomolecular Mechanisms, Max-Planck-Institute for Medical Research, Jahnstrasse 29, 69120 Heidelberg, Germany
[3]Lead contact

*Correspondence:
claudia.ribeirodealmeida@path.ox.ac.uk or nicholas.proudfoot@path.ox.ac.uk

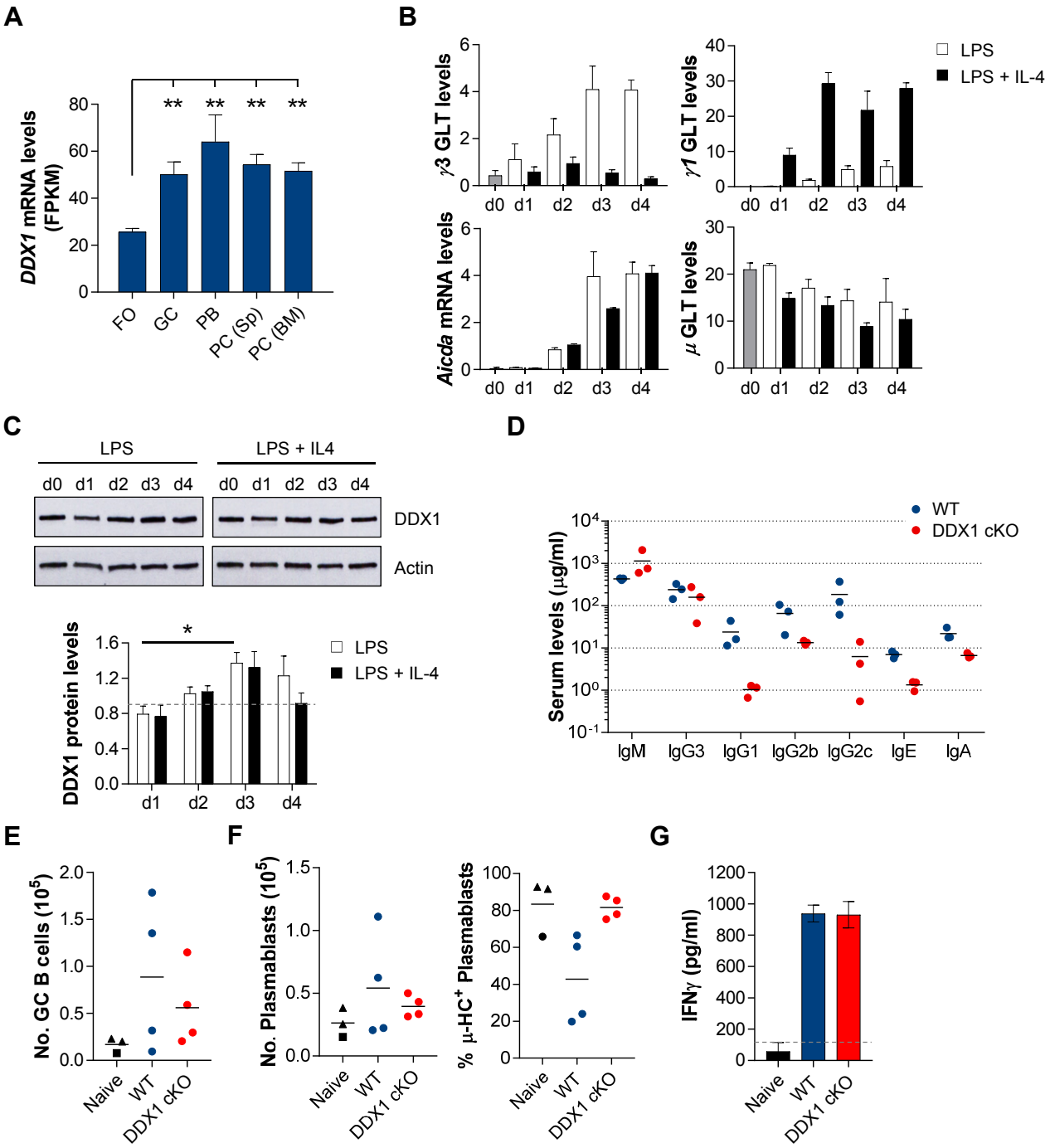


Figure S1. Related to Figure 1. (A) *DDX1* mRNA levels in mature B cell subsets: naïve follicular (FO) B cells, germinal centre (GC) B cells, plasmablasts (PB) and plasma cells (PC) either from spleen (Sp) or bone-marrow (BM). Expression levels (FPKM, mean ± SD) were obtained from published RNA-Seq datasets (Brazao et al., 2016). (B) Quantitative PCR analysis of γ3 GLT, γ1 GLT, μ GLT and *Aicda* mRNA levels in total RNA from splenic B cells stimulated with LPS or LPS plus IL-4 for 1-4 days or unstimulated cells (day 0). Values were normalized to *β-actin* mRNA levels (n=3, mean ± SD). (C) Western blot in WT B cells stimulated with LPS or LPS plus IL-4 for 1-4 days. DDX1 protein levels from 3 replicates were normalized to Actin loading control and set to 1 in unstimulated B cells (dashed line). (D-G) Mice were immunized with ovalbumin (OVA) antigen emulsified in complete Freund's adjuvant and boosted 4 weeks after. (D) Serum Ig concentrations in WT and DDX1 cKO mice at week 2 post-immunization. (E-F) Flow cytometric analysis in WT and *DDX1* cKO mouse spleens at week 2 post-immunization. (E) Numbers of germinal centre B cells per spleen identified as $CD19^+CD95^+PNA^+$ cells. (F) Numbers of plasmablasts per spleen identified as $B220^{low}CD19^+CD138^+$ cells. The percentage of non-switched plasmablasts (identified as positive for intracellular μ-heavy-chain (μ-HC) expression) is shown on the right. Each symbol represents individual mice and small horizontal lines indicate the mean. Naïve (non-immunized) WT (square) and *DDX1* cKO (triangles) mice were used as controls. (G) *In vitro* recall responses of splenocytes from WT and *DDX1* cKO mice at week 10 post-immunization (2-3 mice, mean ± SD). Splenocytes were stimulated *in vitro* with ovalbumin antigen and IFNγ concentration was determined by ELISA in the supernatant. Levels of secreted IFNγ in cultures from naïve (non-immunized) mice are shown as a negative control. The dashed line represents average levels of secreted IFNγ in cultures without ovalbumin antigen.

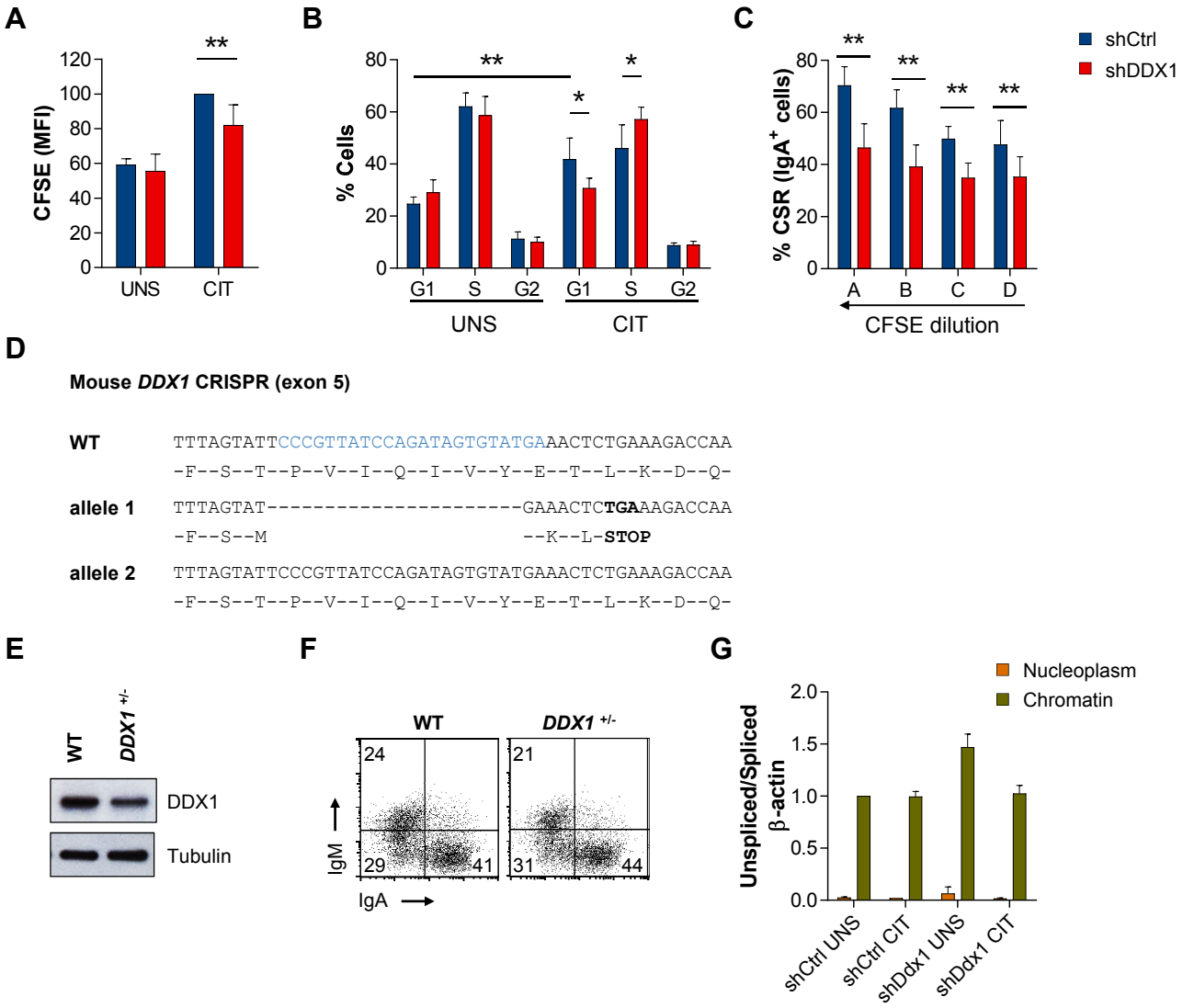


Figure S2. Related to Figure 2. (A-C) CH12 cells transduced with shCtrl or shDDX1 were cultured for 72 hr in unstimulated (UNS) or CIT stimulated conditions. (A) Proliferation analysis measured as dilution of the cell-tracking dye CFSE mean fluorescence intensity (MFI) by flow cytometry (values were normalized to shCtrl CIT; n>3, mean ± SD). (B) Percentage of cells in different cell-cycle stages analysed using BrdU and PI staining and flow cytometry (n≥3, mean ± SD). (C) Quantification of CSR as a function of cell proliferation. Cells were divided into approximate quartile gates on the basis of CFSE dilution (A – high CFSE, low proliferation; D - low CFSE, high proliferation) and the percentage of IgA^+ cells in each gate is shown for CIT cultures (n≥3, mean ± SD). (D-F) CRISPR/Cas9-mediated targeting of mouse *DDX1* in CH12 cells. (D) Genomic sequence of mouse *DDX1* exon 5 alleles in $DDX1^{+/-}$ CH12 cell line are depicted below the WT sequence (guide RNA sequence highlighted in blue); corresponding protein sequences are also shown. Allele 1 shows a 22 bp deletion that creates a frameshift mutation leading to a premature stop codon. Allele 2 contains the WT sequence. (E) Western blot for DDX1 and Tubulin loading control, in $DDX1^{+/-}$ and parental (WT) CH12 cell lines. (F) Flow cytometric analysis for surface expression of IgM and IgA in $DDX1^{+/-}$ and WT CH12 cell lines cultured in UNS and CIT stimulated conditions for 72 hr. (G) Quantitative PCR analysis of chromatin and nucleoplasm fractions of nuclear RNA from shCtrl or shDDX1 CH12 cells after 24 hr in UNS or CIT stimulated conditions. Unspliced over spliced *β-actin* gene expression levels are shown (values were normalized to chromatin fraction shCtrl UNS; n=2, mean ± SD).

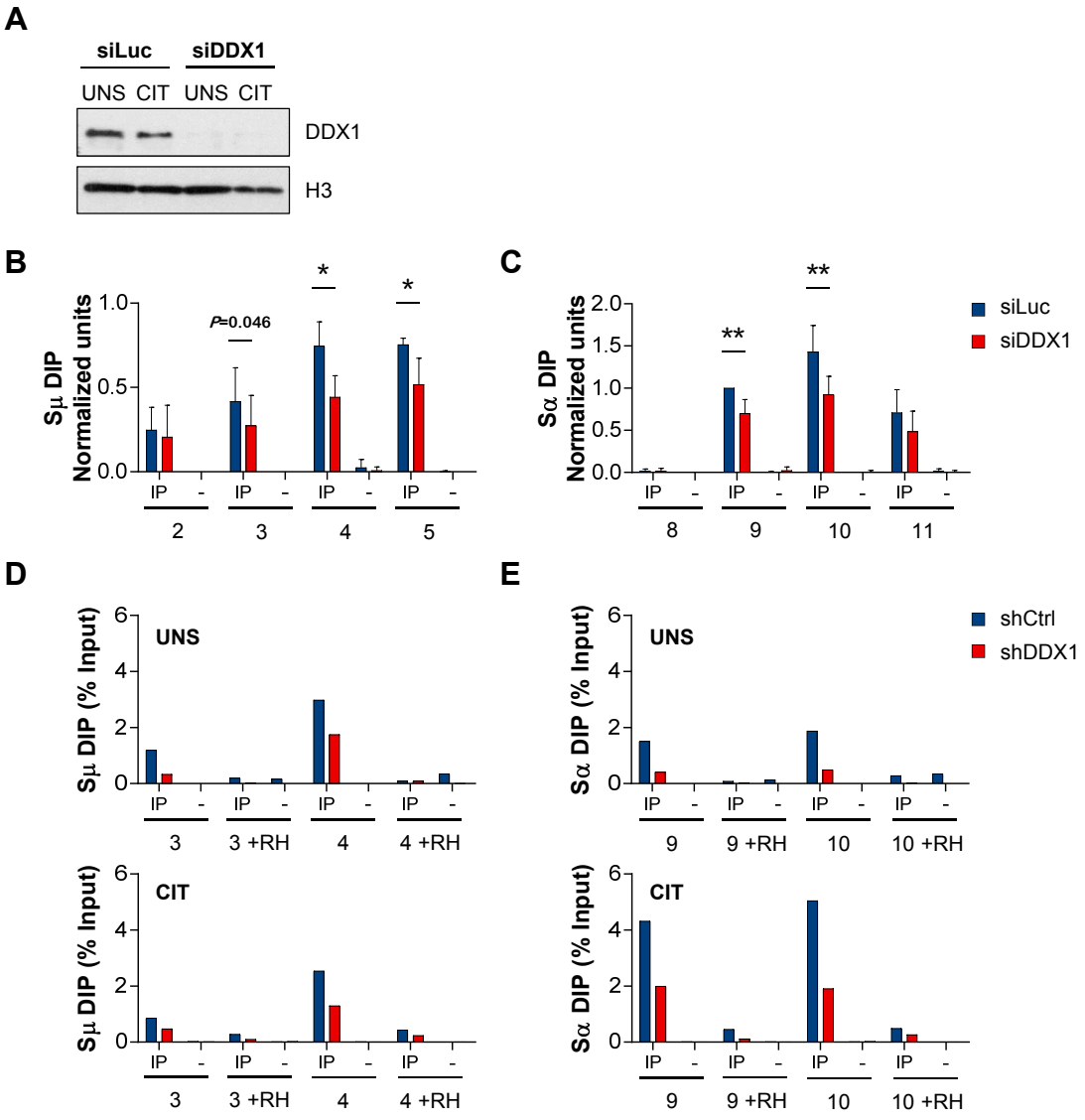


Figure S3. Related to Figure 3. (A-C) CH12 cells were transfected with siRNA against Luciferase (siLuc) or mouse DDX1 (siDDX1) and unstimulated (UNS) or CIT stimulated cells analysed after 24 hr. (A) Western blot for DDX1 and Histone H3 loading control. (B-C) DIP analysis of siRNA treated CH12 cells using the S9.6 antibody (IP) or no antibody control (-), after 24 hr in CIT stimulated conditions. DIP signals were measured across Sμ (B) and Sα (C) regions and values were normalized to probe 9 in siLuc CIT cells in each experiment (n=5, mean ± SD). (D-E) CH12 cells transduced with shCtrl or shDDX1 were cultured in UNS or CIT stimulated conditions for 24 hr. To confirm the specificity of the S9.6 antibody for RNA:DNA hybrids, samples were treated with recombinant RNaseH (RH) before DIP analyses. DIP signals upstream Sμ region, probes 3 and 4 (D) and Sα region, probes 9 and 10 (E) were strongly reduced after RH treatment both in shCtrl and shDDX1 samples. The positions of the probes used are indicated in the schematic diagrams on Figure 3B and C.

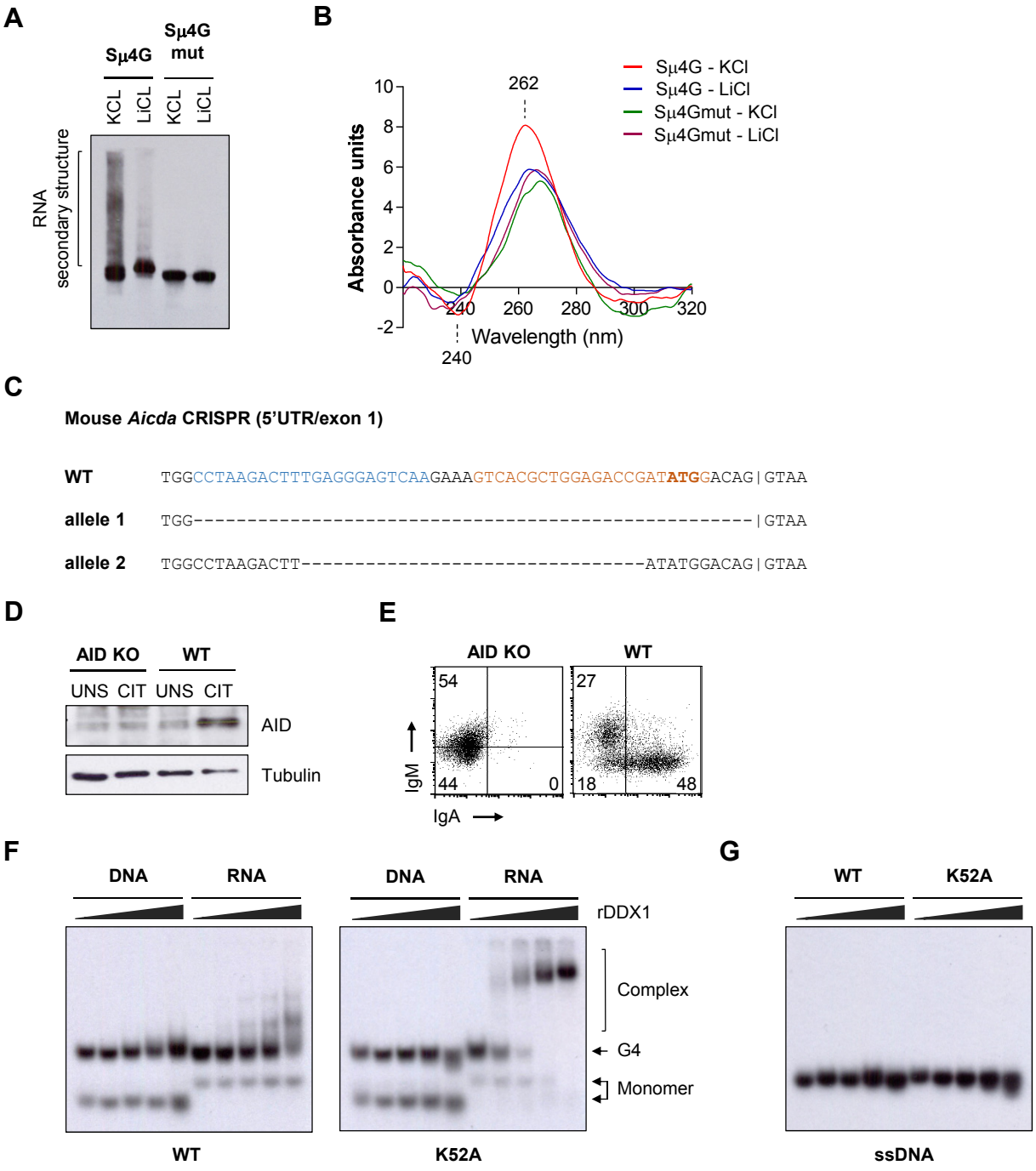


Figure S4. Related to Figure 4. (A) Native gel electrophoresis of biotinylated Sμ4G and Sμ4Gmut oligonucleotides folded in the presence of either KCl or LiCl. Sμ4G-KCl migrate as a high molecular weight smear, denoting of higher-order RNA structures. (B) Circular dichroism spectrum of Sμ4G and Sμ4Gmut oligonucleotides folded in the presence of either KCl or LiCl. Sμ4G-KCl oligonucleotides show an absorbance spectrum characteristic of a parallel G4 structure with a positive signal at 262 nm and a negative signal at 240 nm. (C-E) CRISPR/Cas9-mediated targeting of mouse *Aicda* in CH12 cells. (C) Two guide RNAs (sequences highlighted in blue and orange) were targeted to *Aicda* promoter region/exon 1 by Cas9 D10A nickase. Genomic sequence of *Aicda* alleles in AID KO CH12 cell line are depicted below the WT sequence. Allele 1 has a 52 bp deletion which includes exon 1 coding sequences and 5'UTR and allele 2 has a 32 bp deletion in 5'UTR. *Aicda* exon 1-5' splice site is denoted as a vertical bar (|). (D) Western blot for AID and Tubulin loading control, in *Aicda*$^{-/-}$ (AID KO) and parental (WT) CH12 cell lines. Note that background antibody signal is observed for WT UNS and AID KO samples. (E) Flow cytometric analysis for surface expression of IgM and IgA. AID KO and WT CH12 cell lines were cultured in CIT stimulated conditions and analysed after 72 hr. Native electrophoretic mobility shift assays (EMSA) using (F) ^{32}P-labelled tetramolecular G4 RNA or G4 DNA or (G) ^{32}P-labelled single-stranded DNA and recombinant human DDX1 (rDDX1) proteins (0.125, 0.25, 0.5 or 1 μg). Both WT DDX1 (rDDX1) and an ATPase mutant (rDDX1-K52A) were used. Data shown in (F) is representative of 2 independent assays.

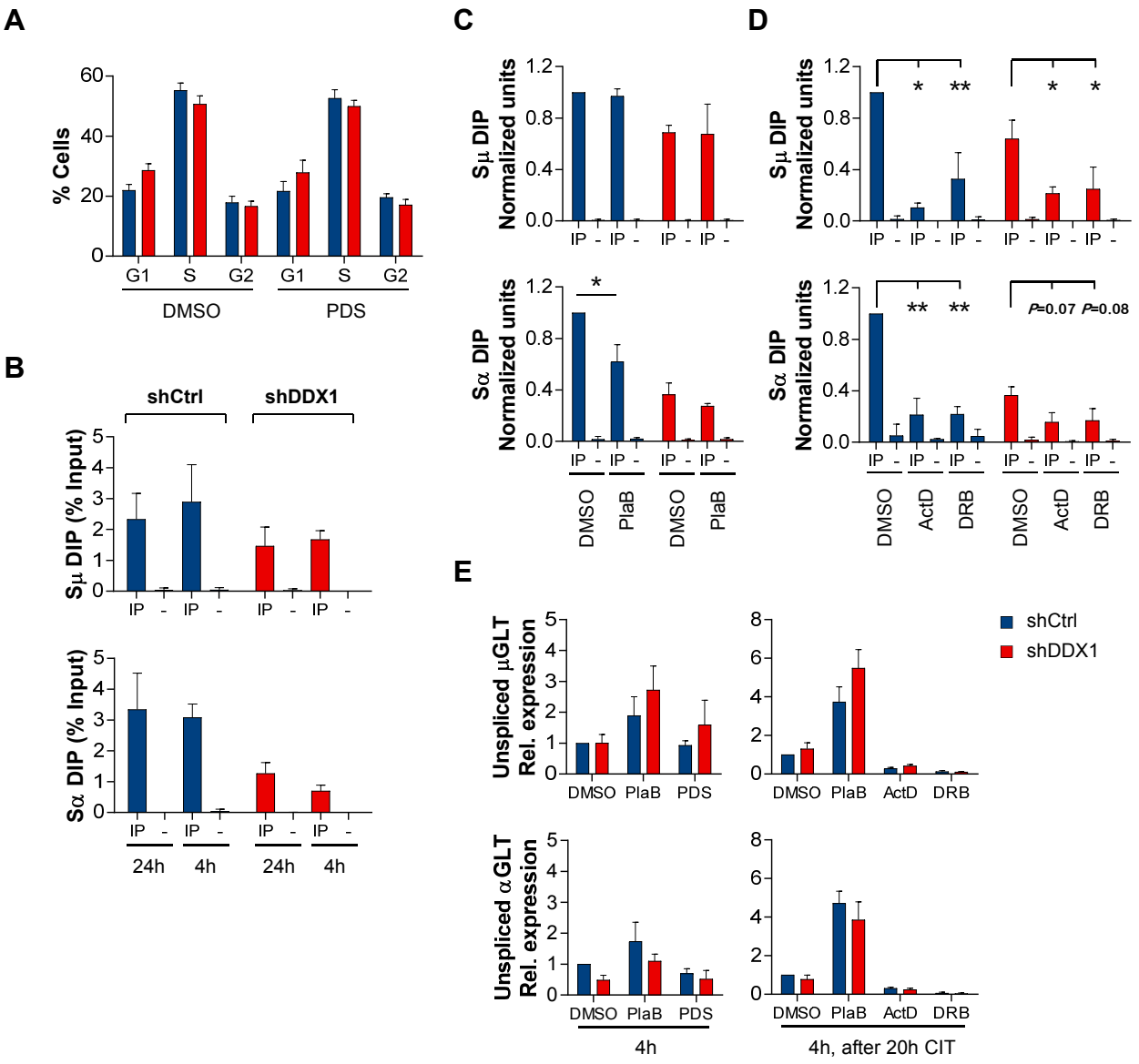


Figure S5. Related to Figure 5. (A) Cell cycle analysis of CH12 cells transduced with shCtrl or shDDX1 and cultured under CIT stimulation in the presence of DMSO and pyridostatin (PDS, 10 μM) for 4 hr (n=3, mean ± SD). Percentage of cells in different cell-cycle stages was analysed by flow-cytometry using BrdU and PI staining. (B-D) CH12 cells transduced with shCtrl or shDDX1 were analysed by DIP with the S9.6 RNA:DNA hybrid-specific antibody (IP) or no antibody control (-). DIP signals upstream Sμ region (probe 4) and Sα region (probe 10) were evaluated (positions of the probes used are indicated in the schematic diagrams on Figure 5). (B) Cells cultured in CIT stimulated conditions for 24 hr or 4 hr. Values are expressed as percentage of Input material (n≥2, mean ± SD). (C-D) Cells cultured in CIT stimulated conditions for 20 hr and subsequently with CIT and (C) Pladienolide (PlaB, 1 μM) for 4 hr or (D) the transcription inhibitors ActinomycinD (ActD, 5 μg/mL) or 5,6-Dichloro-1-β-D-ribofuranosylbenzimidazole (DRB, 150 μM) for 4 hr. DMSO treated cells were used as a control. Values were normalized to shCtrl DMSO (n≥2, mean ± SD). (D) Quantitative PCR analysis of unspliced μGLT and αGLT expression levels in total RNA from CH12 cells transduced with shCtrl or shDDX1 and treated as indicated. Values were normalized to 18S rRNA and shCtrl DMSO (n≥3, mean ± SD).

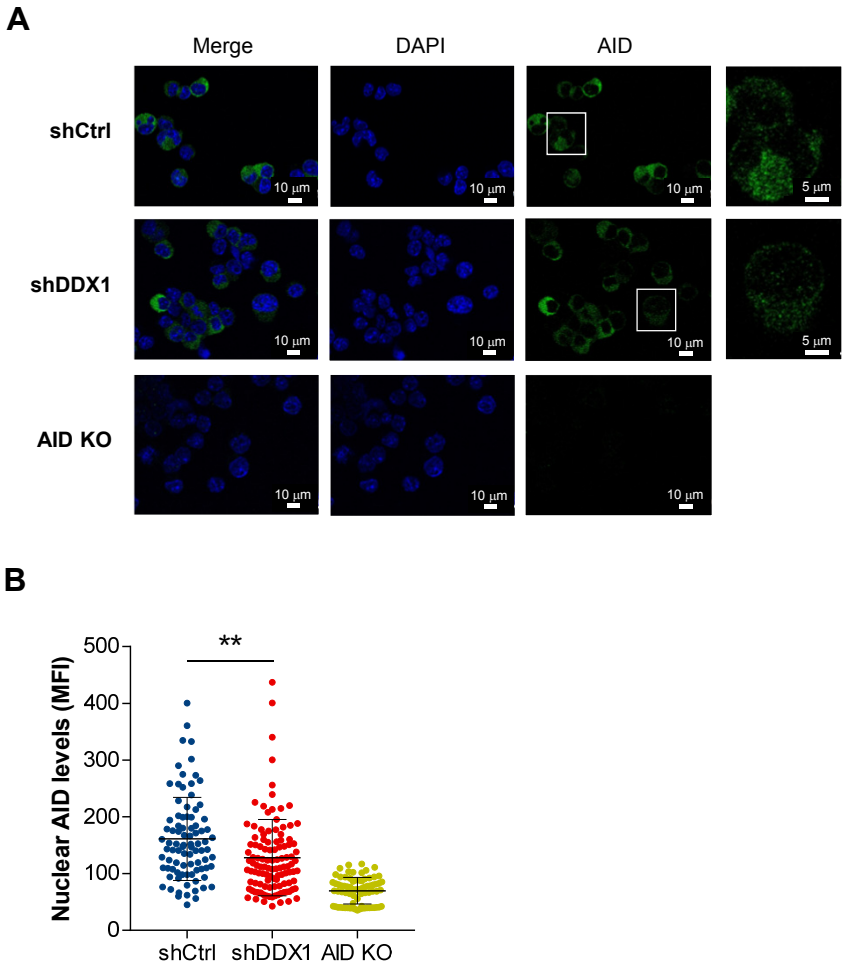
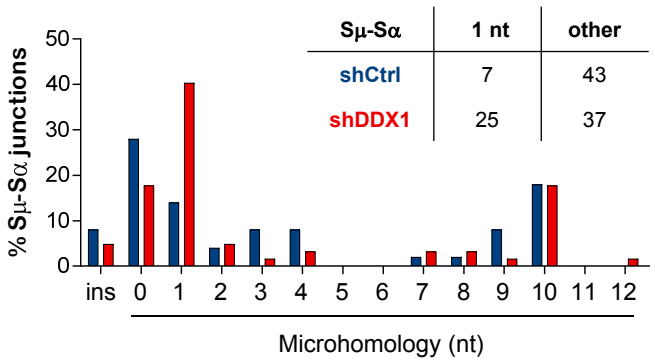


Figure S6. Related to Figure 6. (A) Immunofluorescence analysis of DAPI (blue) and AID (green) in CH12 cells transduced with shCtrl or shDDX1 and cultured under CIT stimulation for 24 hr (scale bar, 10 μm). AID KO CH12 cells were used as a negative control. A larger magnification of AID immunofluorescence is shown on the right to facilitate visualization of nuclear AID signal (scale bar, 5 μm). (B) Nuclear AID levels were quantified from cells in five different fields and expressed as mean fluorescence intensity (MFI). Each symbol represents individual nuclei (shCtrl, 88 nuclei, shDDX1, 113 nuclei and AID KO, 74 nuclei; mean ± SD).

A

Exp	No. Sμ Mutations / bp		% Sμ Mutation	
	shCtrl	shDDX1	shCtrl	shDDX1
#1	55/4385	49/7208	1.25	0.68
#2	22/3959	36/8059	0.56	0.45
#3	25/5336	16/3987	0.47	0.40
#4	34/3315	29/5946	1.03	0.49

C



Sμ-Sα	1 nt	other
shCtrl	7	43
shDDX1	25	37

B

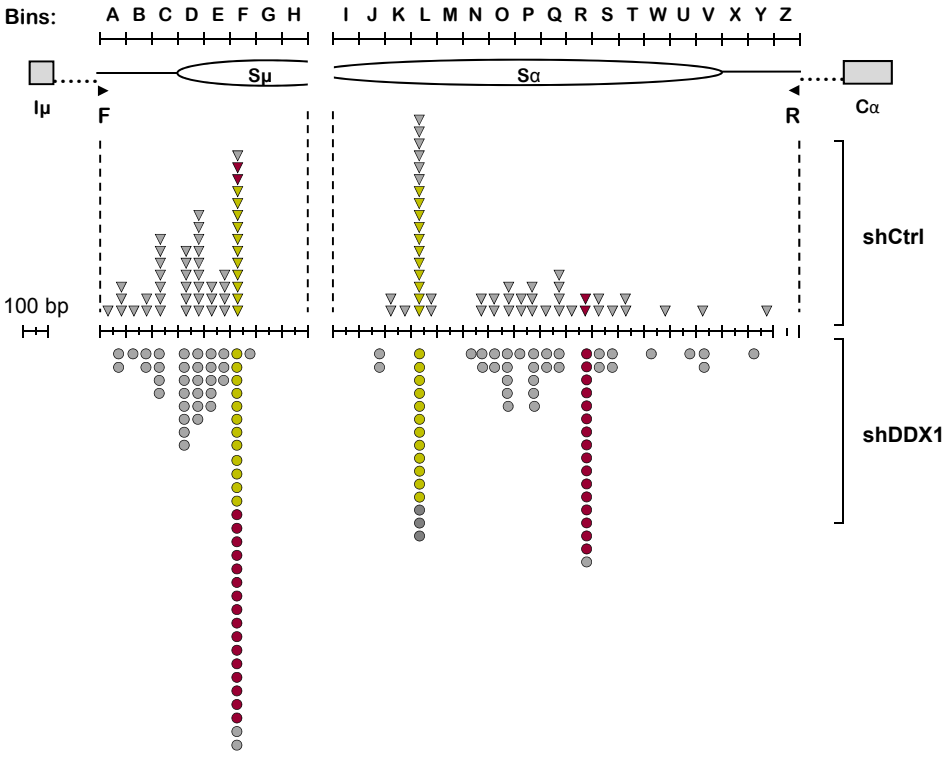


Figure S7. Related to Figure 6. Genomic DNA extracted from CH12 cells transduced with shCtrl or shDDX1 and cultured in CIT stimulated conditions for 72 hr was evaluated for: (A) Number of mutations per total number of bp analysed and percentage of mutation in recombined Sμ DNA sequences in each experiment (relative to Figure 6F); (B) Position of Sμ and Sα recombination breakpoints relative to PCR primers and grouped in bins of 100 bp (A to Z) to facilitate visualization. Recombination occurs between same coloured triangles for shCtrl or circles for shDDX1 between Sμ and Sα, see Table S2 and S3; (C) Percentage of Sμ–Sα junctions with nucleotide insertions (ins) or the indicated length of microhomology (MH) measured as the number of consecutive nucleotides (nt) with perfect homology. Table on the top refers to the number of Sμ–Sα junctions in shCtrl and shDDX1 cells with 1 nt or other length (ins, 0 or 2-12 nt) of MH. The difference in the number of junctions with 1 nt of MH observed between shCtrl and shDDX1 cells is statistically significant (Fisher's exact test, ***P*=0.003).

Table S2. Related to Figure 6. Sμ-Sα recombination breakpoints in shCtrl CH12 cells. Microhomology at junctions (black) was measured as the number of consecutive nucleotides with perfect homology between germline Sμ (blue) and Sα (red). Insertions were defined as nucleotides at the breakpoints with no homology.

Exp 2	AGCCTAACTCAGCTCGCACCAGCCCAGT
shDDX1_31	AGCCTAACTCAGCTCAAGCCCAGCTTTG
4 nt	ACTTCATTTTGGCTCAAGCCCAGCTTTG
Exp 3	CTATTCCATCTCATTCCAGCTCAGCTCA
shDDX1_1	CTATTCCATCTCATTCCAGTTCATTACA
10 nt	CGGCCCAGCTCATTCCAGTTCATTACA
Exp 2	CCCTAAGTCTAGCTCAGCTCAATCCATT
shDDX1_2	CCCTAAGTCTAGCTATTCCAGTTCATTA
0 nt	AGCGGCCCAGCTGATTCCAGTTCATTA
Exp 3	GTCTCATCCCAGCTTAGTTTATCCTAGT
shDDX1_10	GTCTCATCCCACATTCCAGTTCATTACA
0 nt	GCGGCCCAGCTCATTCCAGTTCATTACA
Exp 1	CCCAGCCTAGTCTAGCTCAGCCAGCCC
shDDX1_6	CCCAGCCTAGTGTATTGGCTCATGTCG
0 nt	AAGTTCATTCCAGTTTGGCTCATCTCG
Exp 1	GTTCAGCCTAACCTAGCTCACACCAGC
shDDX1_5	GTTCAGCCTAACCTTAGCTCTATTCAAC
1 nt	TAGGCAGTAGAGTTTAGCTCTATTCAAC
Exp 1	AGCTCAGCTCAGACAACCCTGTCTAAC
shDDX1_27	AGCTCAGCTCATTTAGCTCTATTCAACC
0 nt	AGGCAGTAGAGTTTAGCTCTATTCAACC
Exp 3	CTCATTCCAGTTCAGCTCAGCTCAGCT
shDDX1_16	CTCATTCCAGTTCATCTCAGCTCAGAA
3 nt	CTCACCCCAGCTCATCTCAGCTCAGAA
Exp 3	AGCCCATTTCAGCCTAGCTTAGCTCAG
shDDX1_7	AGCCCATTTCAGCCAGCTCATCTCAGC
2 nt	GCTGAGCTCACCCCAGCTCATCTCAGC
Exp 1	TCAGCTCAGCTCAGCTCAGCTCAGCTC
shDDX1_25	TCAGCTCAGCTCAGTACCCCAGGTCAT
1 nt	AGCATAGCTGAGCTCACCCCAGCTCAT
Exp 1	AGCTTAGCTCAGTTTAGCGCAGCTCAG
shDDX1_2	AGCTTAGCTCAGTTCAACACAGCGTAA
0 nt	AGATCAGCTCACCCCAACACAGCGTAG
Exp 2	CAGCTCAGCTCAGCTCAGCTCAGCTCA
shDDX1_19	CAGCTCAGCTCAGCCAACACAGCGTAA
1 nt	AGATCAGCTCACCCCAACACAGCGTAG
Exp 2	AGCCCAGACTAACTCAGCCTAGCCCAG
shDDX1_14	AGCCCAGACTAACTCACCCCAACACAG
4 nt	CATTTCAGATCAGCTCACCCCAACACAG
Exp 3	CCAGCTCAGTTCAGCCTAACCTAGCT
shDDX1_5	CCAGCTCAGTTCACCCCAACACAGCG
3 nt	TCAGATCAGCTCACCCCAACACAGCG
Exp 2	CACACCAGCCAGCCCAGCCTATTCCAT
shDDX1_10	CACACCAGCCAGCCCAGTTCAGTTCAC
9 nt	AGTCTAGCTCAGCCCAGCTCAGCTCAC
Exp 1	CAGCCCATTTCAGCCTAGCTTAGCTCA
shDDX1_17	CAGCGCATTTCATAGTAGGTTAGCTCA
ins	AGTCTAGCTCAGCCCAGCTCAGCTCA
Exp 4	AGCTCAGCTCAGCTCAGCTCAGCTCA
shDDX1_4.5	AGCTCAACTCAGCAGCTCAGCCCAGT
0 nt	CTCAGCTCAGTCTAGCTCAGCCCAGC
Exp 3	GTTTATACTAGTTCAGCTCAACCCAGC
shDDX1_11	GTTTATACTACCTCATTCAGCTCAGTC
ins	TCAGCTCACCCTAGCTCAGCTCAGTC
Exp 3	CCTGTCTCATCCCAGCTTAGTTTATCC
shDDX1_23	CCTGTCTCATCCCCACCCTAGTTCAG
0 nt	ACCCAGCTCAGCTCACCCTAGCTCAG
Exp 3	CTCATTCCAGTACAGCCTAGCCAGACA
shDDX1_21	CTCATTCCAGTATCAGCTCACCCTAAC
0 nt	GCTCACCCAGCTCAGCTCACCCTAGC
Exp 3	AGCTCATTCCAGCTCAGCCCAGCCTA
shDDX1_15	AGCTCATTCCAGCACCCAGTTCAGCT
1 nt	CTTAGCTCAGCTCACCCAGCTCAGCT
Exp 1	GCTCATTCCAGCTCAGCCCAGCCAGTCT
shDDX1_11	GCTCATTCCAGCTCAGCTCACCCAGCTC
8 nt	CCCCAGCTTAGCTCAGCTCACCCAGCTC
Exp 3	CTTAGGCCAGCTCAGACAGCACAGCTT
shDDX1_13	CTTAGGCCAGCTCAGTTCACCCAGCTC
7 nt	CCCAGCTTAGCTCAGCTCACCCAGCTC
Exp 2	CAGTCCAGCCTGTCTCATCCCAGCTTA
shDDX1_24	CAGTCCAGCCTGTCTCAGCTCACCCAG
4 nt	CACCCCAGCTTAGCTCAGCTCACCCAG
Exp 2	CTCAGCTCAGCTCAGCCTAACCCAGCT
shDDX1_30	CTCAGCTCAGCTCAGCCCAGCTCACCC
0 nt	CTCACCCCAGCTTAGCTCAGCTCACCC
Exp 2	GCCCAGTCAGTTCATCCCATCTCATCC
shDDX1_23	GCCCAGTCAGTTCACAGCTTAGCTCAG
0 nt	AGCTCAGCTCACCCCAGCTTAGCTCAG
Exp 1	TTCAGCCCAGTTCAGAAAGCCCATTCC
shDDX1_7	TTCAGCCCAGTTTTCCCCAGCCTAGGT
3 nt	CTCAGCTCAGCTCACCCCAGCTTAGCT
Exp 2	GACAGCACAGCTTGCCTAGGTCAGCTC
shDDX1_21	GACAGCACAGCACCCCAGCTTAGCTCA
1 nt	CAGCTCAGCTCACCCCAGCTTAGCTCA
Exp 1	GCCCAGTCCAGCCCAGCTCAGGCCAT
shDDX1_19	GCCCAGTCCAGCCCATCAGCCAAGTT
0 nt	CCAGCTCACCCTAGCTCAGCCAAGCT
Exp 1	CTCAGCTCAGCCTAACTCAGCTCGCAC
shDDX1_13	NTCAGCTNAGCCTAACAGCCCAGGTCA
1 nt	CAGCTCACCCCAGCTCAGCCCAGCTCA
Exp 2	CAATCCAGCAAAGCTCAGGCTAGAACT
shDDX1_15	CAATCCAGCAAACTCTCAGTCCAGCTC
ins	CAGCTCACCCCAGCTCAGCCCAGCTC
Exp 1	TCAGCTCAGCTCAGCCTAACCCAGCTC
shDDX1_8	TCAGNTCAGNTCAGCCCAGATCAGCCC
2 nt	TCAGCTCAGCTCACCCCAGCTCAGCCC
Exp 3	TCAGCTCAGCTCAGCTCAGCCTAACCC
shDDX1_3	TCAACTCAGCTCATCCCCCCAGGTCA
0 nt	CAGCTCAGCTCAGCTCACCCCAGCTCA
Exp 1	AGCCCAGTTCAGCTCAGCTCATTCCAG
shDDX1_1	AGCCCAGTTCAGCTCCAGCTCAGCTCA
3 nt	CAGCTCAGCTCACTCCAGCTCAGCTCA
Exp 3	GTTTATACTAGTTCAGCTCAACCCAGC
shDDX1_17	GTTTATACTAGTTCACAGCTCAGCTCA
0 nt	ACCCCAGCTCAGCCCAGCTCAGCTCA
Exp 4	GCTCACACCAGCTGAGCCCAACCTATT
shDDX1_20	GCTCACCCCAGCTCAGCTCAGTTCACT
0 nt	CCCCAGCTCAGCCCAGCTCAGCTCACT
Exp 1	TCTCCTCTCATTCCAGTTCAGCTCAGC
shDDX1_4	TCTCCTCTCATTCCAGCTCACCCCAGC
4 nt	CCCAGCTCAGCCCAGCTCACCCCAGC
Exp 1 ▽	AGCTCAGCTCAGCTCAGCCTAACCCA
shDDX1_29	AACTCAGCTCAGCTCAGCCCAGCTCA
10 nt	AGCTCAGCCCAGCTCAGCCCAGCTCA
Exp 2 ▽	AGCTCAGCTCAGCTCAGCCTAACCCA
shDDX1_4	AACTCAGCTCAGCTCAGCCCAGCTCA
10 nt	AGCTCAGCCCAGCTCAGCCCAGCTCA
Exp 2 ▽	CTCAGCTCAGCTCAGCCTAACCCAGC
shDDX1_5	CTCAGCTCAGCTCAGCCCAGCTCACC
10 nt	CTCAGCCCAGCTCAGCCCAGCTCACC
Exp 2 ▽	GCTCAGCTCAGCTCAGCCTAACCCAG
shDDX1_9	ANTCAGCTCAGNTCAGNCAGCTCAC
10 nt	CTTCAGCCCAGCTCAGCCCAGCTCAC
Exp 3 ▽	AGCTCAGCTCAGCTCAGCCTAACCCA
shDDX1_2	AACTCAGCTCAGCTCAGCCCAGCTCA
10 nt	AGCTCAGCCCAGCTCAGCCCAGCTCA
Exp 4 ▽	CAGCTCAGCTCAGCTCAGCCTAACCCAG
shDDX1_4.4	CAACTCAGCTCAGCTCAGCCCAGCTCAC
10 nt	CAGCTCAGCCCAGCTCAGCCCAGCTCAC
Exp 4 ▽	AGCTCAGCTCAGCTCAGCCTAACCCAGC
shDDX1_8	AACTCAGCTCAGCTCAGCCCAGCTCACC
10 nt	AGCTCAGCCCAGCTCAGCCCAGCTCACC
Exp 4 ▽	CTCAGCTCAGCTCAGCCTAACCCAGCTC
shDDX1_9	CTCAGCTCAGCTCAGCCCAGCTCACCCC
10 nt	CTCAGCCCAGCTCAGCCCAGCTCACCCC
Exp 3 ▽	GCTCAGCTCAGCTCAGCTCAGCCTAAC
shDDX1_9	ACTCAGCTCAGCTCAGCCCAGCTCACC
9 nt	GCTCAGCCCAGCTCAGCCCAGCTCACC
Exp 3 ▽	GCTCAGCTCAGCTCAGCTCAGCCTAAC
shDDX1_10	ACTCAGCTCAGCTCAGCCCAGCTCACC
9 nt	GCTCAGCCCAGCTCAGCCCAGCTCACC
Exp 4 ▽	CAGCTCAGCTCAGCTCAGCTCAGCTCA
shDDX1_11	CAGGTCAGCTCAACTCAGCCCAGCTCA
9 nt	CAGCTCAGCCCAGCTCAGCCCAGCTCA
Exp 3 ▼	TCTAGGTCTGCCCGGTCTAGGTAAGCT
shDDX1_22	TCTAGGTCTGCCCGATCAGCCCAGCTC
1 nt	CAGCTCAGCCCAGCTCAGCCCAGCTC
Exp 4 ▼	CCGGTCTAGGTAAGCTCAGCCTTGT
shDDX1_19	CCGGTCTAGGTTCAGCTCAGCTCAAC
ins	CTCAGCTCACCCCAGCTCAGCCCAGC

Table S3. Related to Figure 6. Sμ-Sα recombination breakpoints in shDDX1 CH12 cells. Microhomology at junctions (black) was measured as the number of consecutive nucleotides with perfect homology between germline Sμ (blue) and Sα (red). Insertions were defined as nucleotides at the breakpoints with no homology.

Exp 4	TCATTCCAGCTCAGCTCAGCCTAACTCA	
shDDX1_5.1	TCATTCCAGCTCAACTTCATTTTGGCTCA	
0 nt	TTCATTACAGTCTACTTCATTTTGGCTCA	
Exp 3	GCCTATTCCAGTCTAGTTCAGCCCATC	
shDDX1_23	GCCTATTCCAGTTACAGTCTACTTCATT	
1 nt	ATTCCAGTTCATTACAGTCTACTTCATT	
Exp 2	ATCCTAGTCCATCCCAGCTTAGCCCAGT	
shDDX1_62	ATCCTAGTCCAGTGTAGGCAGTAATGAA	
ins	TTTAGCTCTATTCAACCTAGATTAATGAA	
Exp 3	CTCAGCCTAACTCAGCTCGCACCAGCC	
shDDX1_18	CTCAGCCTAACTCAATTCAACCTAGATT	
0 nt	TAGAGTTTAGCTCTATTCAACCTAGATT	
Exp 3	TAAGCTCAGCCTTGTTCAGCCCATTCCA	
shDDX1_6	TCAGCTCAGCTCAACTCAGAATTTAACT	
ins	GTCCAGTGTAGGCAGTAGAGTTTAGCT	
Exp 3	CCTAGTCCATCCCAGCTTAGCCCAGTTC	
shDDX1_13	CCTAGTCCATCCCATAGATGAGCTCACC	
2 nt	ACACAGCGTAGCATAGCTGAGCTCACC	
Exp 1	AGTGTAGCCTAGCTTTGTCCAGCTCTGC	
shDDX1_29	AGTGTAGCCTAGCCCCAACACAGCGTAT	
1 nt	CAGATCAGCTCACCCCAACACAGCGTAG	
Exp 2	TCTTAGGCCAGCTCAGACAGCACAGCT	
shDDX1_70	TCTTAGGCCAGCTCACCCCAACACAGC	
7 nt	TTTCAGATCAGCTCACCCCAACACAGC	
Exp 2	ATCCTAGTCCATCCCAGCTTAGCCCAG	
shDDX1_8	ATCCTAGTCCATCAGCTCAACCCAACA	
3 nt	TCTCATTTCAGATCAGCTCACCCCAACA	
Exp 2	GCCCATCCCAGCTCATTCCAGCTCAGC	
shDDX1_59	GCCCATCCCAGCTTTCAGCTCACCCCA	
1 nt	TAGCTCAGCCCAGCTCAGCTCACCCCA	
Exp 2	GCTCAACCCAGCTCATTCCAGCTCAGC	
shDDX1_13	GCTCAACCCAGCTAGTTCAGCCCAGTT	
2 nt	CTCAGCTCAGTCTAGCTCAGCCCAGCT	
Exp 3	CTCAGCTCAGCTCAGCTCAGCTCAGCT	
shDDX1_4	CTCAGCTCAGTTCAGCTCAGTCTAGCT	
12 nt	CTCACCCTAGCTCAGCTCAGTCTAGCT	
Exp 1	TCACACCAGCCAGCCCAGCCTATTCCA	
shDDX1_1	TCACACCAGCCAGCAGCTCAGTCTAGC	
1 nt	GCTCACCCTAGCTCAGCTCAGTCTAGC	
Exp 1	GCTCAGCTCAGCCTAACCCAGCTCACA	
shDDX1_16	GNTCAGCTCAGCCCAGCTCAGTNNANN	
0 nt	GCTCACCCTAGCTCAGCTCAGTCTAGC	
Exp 2	GTTCATCCCATCTCATCCCATCCCATCC	
shDDX1_19.1	GTTCATCCCATCTCAGTTCAGCTCAGTC	
1 nt	GCTCAGCTCACCCTAGCTCAGCTCAGTC	
Exp 1	CCAGCCTAGTCTAGCTCAGCCAGCCCT	
shDDX1_3	CCAGCCTAGTCTAGTTCAGCTCACCCT	
8 nt	CTCAGCTCACCCAGCTCAGCTCACCCT	
Exp 4	ATCCCATCCCATCCCATCCCATCCCAT	
shDDX1_3	ATCCCATCCCTCACCCTAGTTCAGCTC	
1 nt	CCAGCTCAGCTCACCCTAGCTCAGCTC	
Exp 2	TTAGCTCAGTTTAGCGCAGCTCAGCCT	
shDDX1_19.2	TTAACTCAGTTTCCCCAGCTCAGCTCA	
1 nt	TTAGCTCAGCTCACCCAGCTCAGCTCA	
Exp 3	ATCCTAGTCCATCCCAGCTTAGCCCAG	
shDDX1_7	ATCCTAGTTTAGCACCCAGCTCAGCTC	
1 nt	CTTAGCTCAGCTCACCCAGCTCAGCTC	
Exp 3	GACAGCACAGCTTGCCTAGGTCAGCTC	
shDDX1_20	GACAGCACAGCTCAGCTCACCCAGTTC	
4 nt	CCCAGCTTAGCTCAGCTCACCCAGCTC	
Exp 2	TGCTCAGCCCATTTCAGCCTAGCTTAGC	
shDDX1_2	TGCTCAGCCCATTTCAGTCAGCTCACCC	
0 nt	GCTCACCCCAGCTTAGCTCAGCTCACCC	
Exp 2	CATCTTAGGCCAGCTCAGACAGCACAGC	
shDDX1_20	CATCTTAGGCCAGCTAGCTCAGCTCACC	
1 nt	AGCTCACCCCAGCTTAGCTCAGCTCACC	
Exp 1	CCTAGTCTAGCTCAGCCAGCCCTTCCAG	
shDDX1_28	CCTAGTCTAGCTCAGCTTAGCTCAGCTC	
4 nt	CTCAGCTCACCCCAGCTTAGCTCAGCTC	
Exp 1	TTTCAGCCTAGCTTAGCTCAGTTTAGCG	
shDDX1_6	TTTCAGCCTAGCTTAGTCACCCTAGTTC	
0 nt	CCAGCTCAGCCCAGCTCACCCTAGCTC	
Exp 2	CAGCTCAGACAGCACAGCTTGCCTAGG	
shDDX1_88	CAGCTCAGACATCACCCTAGTTCAGCCA	
0 nt	CTCAGCCCAGCTCACCCTAGCTCAGCCA	
Exp 2	AGCTCCTCTCCTCTCCTCTCCTCTCCT	
shDDX1_75	AGCTCCTNTCCAGCTCAGCTCACCCCC	
ins	CTCACCCCAGCTCAGCCCAGCTCACCC	
Exp 2	CATCCTAGTTCAGCTCAGTTTAGCCCAT	
shDDX1_64	CATCCTAGTTCAGCTCACCCCAGCTCAG	
8 nt	AGCTCAGCTCAGCTCACCCCAGCTCAG	
Exp 3	GCTCAACCCAGCTCATTCCAGCTCAGC	
shDDX1_2	GCTCAACCCAGCTCACCCCAGCTCAGC	
7 nt	GCTCAGCTCAGCTCACCCCAGCTCAGC	
Exp 2	AGTACAGCCTAGCCAGACAGTGCAGT	
shDDX1_6	AGTACAGCCTAGCCACATCTCAGTTTA	
0 nt	CAGCTCACCCCAGCTCATCCCAGCTTA	
Exp 3	AGCCTAGCTCAGCTCACACCAGCCAGC	
shDDX1_11	AGCCTAGCTCAGTCAGCTCAGTTCACC	
0 nt	GCTCACTCCAGCTCAGCTCAGCTCACC	
Exp 4	TAACTCAGCCTAGCCCAGACTAACTC	
shDDX1_7.1	TAACTCAGCCTAGTCAGCCCAGCTCA	
0 nt	AGCTCACCCCAGCTCAGCCCAGCTCA	
Exp 2	CTTTGTCCAGCTCTGCTCAGCCCATTT	
shDDX1_7	CTTTGTCCAGCTCTGAGCTCAGCCCAG	
0 nt	GCCCAGCTCACCCCAGCTCAGCCCAG	
Exp 3	CTCAGCTCAGCTCAGCCTAACCCAGCT	
shDDX1_4	CTCAGCTCAGCTCAGCCCAGCTCACCC	
10 nt	CTCAGCCCAGCTCAGCCCAGCTCACCC	
Exp 1	GCTCAGCTCAGCTCAGCCTAACCCAGC	
shDDX1_11	ACTCAGNTCAGNTCAGCCCAACTCACC	
10 nt	GCTCAGCCCAGCTCAGCCCAGCTCACC	
Exp 1	AGCTCAGCTCAGCTCAGCCTAACCCAG	
shDDX1_36	AACTCAGCTCAGCTCAGCCCAGCTCAC	
10 nt	AGCTCAGCCCAGCTCAGCCCAGCTCAC	
Exp 2	CAGCTCAGCTCAGCTCAGCCTAACCCA	
shDDX1_40	CAACTCAGCTCAGCTCAGCCCAGCTCA	
10 nt	CAGCTCAGCCCAGCTCAGCCCAGCTCA	
Exp 2	GCTCAGCTCAGCTCAGCCTAACCCAGC	
shDDX1_51	ACTCAGCTCAGCTCAGCCCAGCTCACC	
10 nt	GCTCAGCCCAGCTCAGCCCAGCTCACC	
Exp 2	TCAGCTCAGCTCAGCTCAGCCTAACCC	
shDDX1_3	TCAACTCAGCTCAGCTCAGCCCAGCTC	
10 nt	CCAGCTCAGCCCAGCTCAGCCCAGCTC	
Exp 2	GCTCAGCTCAGCTCAGCCTAACCCAGC	
shDDX1_1	ACTCAGCTCAGCTCAGCCCAGCTCACC	
10 nt	GCTCAGCCCAGCTCAGCCCAGCTCACC	
Exp 3	GCTCAGCTCAGCTCAGCCTAACCCAGC	
shDDX1_8	ACTCAGCTCAGCTCAGCCCAGCTCACC	
10 nt	GCTCAGCCCAGCTCAGCCCAGCTCACC	
Exp 3	GCTCAGCTCAGCTCAGCCTAACCCAGC	
shDDX1_14	ACTCAGCTCAGCTCAGCCCAGCTCACC	
10 nt	GCTCAGCCCAGCTCAGCCCAGCTCACC	
Exp 4	AGCTCAGCTCAGCTCAGCTCAGCCTAA	
shDDX1_1.1	AACTCAGCTCAGCTCAGCCCAGCTCAC	
9 nt	AGCTCAGCCCAGCTCAGCCCAGCTCAC	
Exp 1	CTCAGCTCAGCTCAGCTCAGCCTAACCC	
shDDX1_13	CTCAANTCAGNTCAGNTCAGCCCAGCTC	
10 nt	CCCAGCTCAGCCCAGCTCAGCCCAGCTC	
Exp 2	AGCTCAGCTCAGCTCAGCCTAACCCAGC	
shDDX1_10	AACTCAGCTCAGCTCAGCCCAGCTCACC	
10 nt	AGCTCACCCCAGCTCAGCCCAGCTCAGC	
Exp 4	GCCCGGTCTAGGTAAGCTCAGCCTTGTT	
shDDX1_7.2	GCCCGGTCTAACTCAGCTCAGCCTAACT	
2 nt	CCCCAGCTCAGCCCAGCTCAGCCCAGCT	
Exp 1	TCCCAGCTTAGTTTATCCTAGTCCATCC	
shDDX1_4	TCCCAGCTTAGTTGTTCAACTCAGCTCA	
0 nt	CACCCCAGCTCAGCCCAGCTCAGCCCA	
Exp 1	CTGCCCGGTCTAGGTAAGCTCAGCCTT	
shDDX1_14	CTGCCCGGTCTAACTCAGCTCAGCTCA	
1 nt	CAGCTCAGCTCACCCCAGCTCAGCCCA	
Exp 1	CTGCCCGGTCTAGGTAAGCTCAGCCTT	
shDDX1_26	CTGCCCGGTCTAACTCAGCTCAGCTCA	
1 nt	CAGCTCAGCTCACCCCAGCTCAGCCCA	
Exp 1	CTGCCCGGTCTAGGTAAGCTCAGCCTT	
shDDX1_27	CTGCCCGGTCTAACTCAGCTCAGCTCA	
1 nt	CAGCTCAGCTCACCCCAGCTCAGCCCA	
Exp 1	CGGTCTAGGTAAGCTCAGCCTTGTTC	
shDDX1_31	CGGTCTAACTCAACTCAGCTCAACTC	
1 nt	CAGCTCACCCCAGCTCAGCCCAGCT	
Exp 2	TCTGCCCGGTCTAGGTAAGCTCAGCCT	
shDDX1_46	TCTGCCCGGTCTAACTCAGCTCAGCTC	
1 nt	CCAGCTCAGCTCACCCCAGCTCAGCCC	
Exp 2	TCTGCCCGGTCTAGGTAAGCTCAGCCTT	
shDDX1_17	TCTGCCCGGTCTAACTCAGCTCAGCTCA	
1 nt	CAGCTCAGCTCACCCCAGCTCAGCCCA	
Exp 3	GGTCTGCCCGGTCTAGGTAAGCTCAGC	
shDDX1_10	GGTCTGCCCGGTCTAACTCAGCTCAGC	
1 nt	CCCCAGCTCAGCTCACCCCAGCTCAGC	
Exp 4	TCTGCCCGGTCTAGGTAAGCTCAGCCTT	
shDDX1_1.2	TCTGCCCGGTCTAANTCAGCTCAGCTCA	
1 nt	CCAGCTCAGCTCACCCCAGCTCAGCCCA	
Exp 4	TGCCCGGTCTAGGTAAGCTCAGCCTTGT	
shDDX1_2.1	TGCCCGGTCTAACTCAGCTCAGCTCAAC	
1 nt	AGCTCAGCTCACCCCAGCTCAGCCCAG	
Exp 4	TGCCCGGTCTAGGTAAGCTCAGCCTTG	
shDDX1_2.2	TGCCCGGTCTAACTCAGCTCAGCTCAA	
1 nt	AGCTCAGCTCACCCCAGCTCAGCCCAG	
Exp 4	CCGGTCTAGGTAAGCTCAGCCTTGTTCA	
shDDX1_5.2	CCGGTCTAACTCAGCTCAGCTCAACTCA	
1 nt	TCAGCTCACCCCAGCTCAGCCCAGCTCA	
Exp 4	AGGTCTGCCCGGTCTAGGTAAGCTCAG	
shDDX1_10	AGGTCTGCCCGGTCTAACTCAGCTCAG	
1 nt	CTCAGAGCTCAGCTCACCCCAGCTCAG	
Exp 4	AGGTCTGCCCGGTCTAGGTAAGCTCAG	
shDDX1_17	AGGTCTGCCCGGTCTAACTCAGCTCAG	
1 nt	ACCCCAGCTCAGCTCACCCCAGCTCAG	
Exp 4	CCCGGTCTAGGTAAGCTCAGCCTTGTT	
shDDX1_16	CCCGGTCTAACTCAGCTCAGCTCAACT	
1 nt	CTCAGCTCACCCCAGCTCAGCCCAGCT	
Exp 4	GTCTGCCCGGTCTAGGTAAGCTCAGCC	
shDDX1_4	GTCTGCCCGGTCTAACTCAGCTCAGCT	
1 nt	CCCAGCTCAGCTCACCCCAGCTCAGCC	
Exp 1	CCCAGGCTAGCTCACTGTCAAGTTTGG	
shDDX1_23	CCCAGGCTAGCTCACCCAGNTCAGCTC	
1 nt	TCAGCTCAGCTCACCCCAGCTCAGCTC	

Table S4. Primer sequences. Related to STAR Methods.

qPCR Gene-specific primers		**Sequence**
unspliced β-actin	forward	AGACTCCCAGCACACTGAACTTAG
	reverse	CAGAAGAAAGACAATTGAGAAAGGG
spliced β-actin	forward	TGCGTGACATCAAAGAGAAG
	reverse	CGGATGTCAACGTCACACTT
unspliced μGLT	forward	CTCTGGCCCTGCTTATTGTTG
	reverse	ATTGGTTAACAGGCAACATTTTTCTTTTAC
unspliced αGLT	forward	GATTTAAGCAGGCCTGGGGTG
	reverse	CTAGTTCAGGCCACTCCATG
spliced μGLT	forward	CTCTGGCCCTGCTTATTGTTG
	reverse	AATGGTGCTGGGCAGGAAGT
spliced αGLT	forward	CCAGGCATGGTTGAGATAGAGATAG
	reverse	GAGCTGGTGGGAGTGTCAGTG

DIP/ChIP primers		**Sequence**
CH12 VDJ promoter (probe 1)	forward	AGCCTACATGCAGCTCAGCA
	reverse	CAGTAGTCAAAGTAGTACCCCCAGC
3'JH4 (probe 2)	forward	CATCCAGGGACTCCACCAAC
	reverse	AGAATGGCCTCTCCAGGTCT
IμEx (probe 3)	forward	AAGGGCTTCTAAGCCAGTCC
	reverse	CACAACCATACATTCCCAGGT
Sμ (probe 4)	forward	GCTAAACTGAGGTGATTACTCTGAGGTAAG
	reverse	GTTTAGCTTAGCGGCCCAGCTCATTCCAGT
DownSμ (probe 5)	forward	GCTGACATGGATTATGTGAGG
	reverse	CCTACACCAGATCATCCAGTACAGCT
Cμ_secpA (probe 6)	forward	AGCTGGAGGAATCGCATGTT
	reverse	ACACCCTGCATACTTGCCTC
IgM +1Kb (probe 7)	forward	CCAGCATCCCAGGGTAACAA
	reverse	TCTAGTGGGTAGCTGCAGGA
IαEx -0.5Kb (probe 8)	forward	CTGACCACATGGGCCTTGAT
	reverse	CTGTTGCTCTGGCTCCTTGA
IαEx (probe 9)	forward	GTGATTCAGGGAGCAAGAGC
	reverse	TCTAGCCTGGGAGTCTCCTG
UpSα (probe 10)	forward	GGGCTAGGCTGAGCAAATCTA
	reverse	CCCGCCCAATCTAACCTAGC
DownSα (probe 11)	forward	TGAAAAGACTTTGGATGAAATGTGAACCAA
	reverse	GATACTAGGTTGCATGGCTCCATTCACACA
Cα_secpA (probe 12)	forward	CGTGGCATCTTCTTCCCAGT
	reverse	AAGGGTAGCACCATCAAGGC
IgA +1Kb (probe 13)	forward	TCAGGCCTTAGTGACGAGGA
	reverse	TCTACTGCGGCACCTACAAC

Human DDX1 primers		**Sequence**
hDDX1 cDNA	forward	CGGAGGACGGGGTGAAGAT
	reverse	AAGAAGGTTCTGAACAGCTGGTTAG

Sμ-Sα junction primers		**Sequence**
UpSμ	forward	CGCTAAACTGAGGTGATTACTCTG
DownSα	reverse	GATACTAGGTTGCATGGCTCCATTCACACA

Genotyping primers		**Sequence**
DDX1 wild-type/loxP alleles	forward	AGTTCATGCAGGCTTCCTCC
	reverse	CCTTCCTGTTGGTCTTTCAGAGT
DDX1 deleted allele	forward	AGTTCATGCAGGCTTCCTCC
	reverse	GAACTGATGGCGAGCTCAGA
Aicda-Cre	forward	CGTTTTCTGAGCATACCTGGA
	reverse	ATTCTCCCACCGTCAGTACG