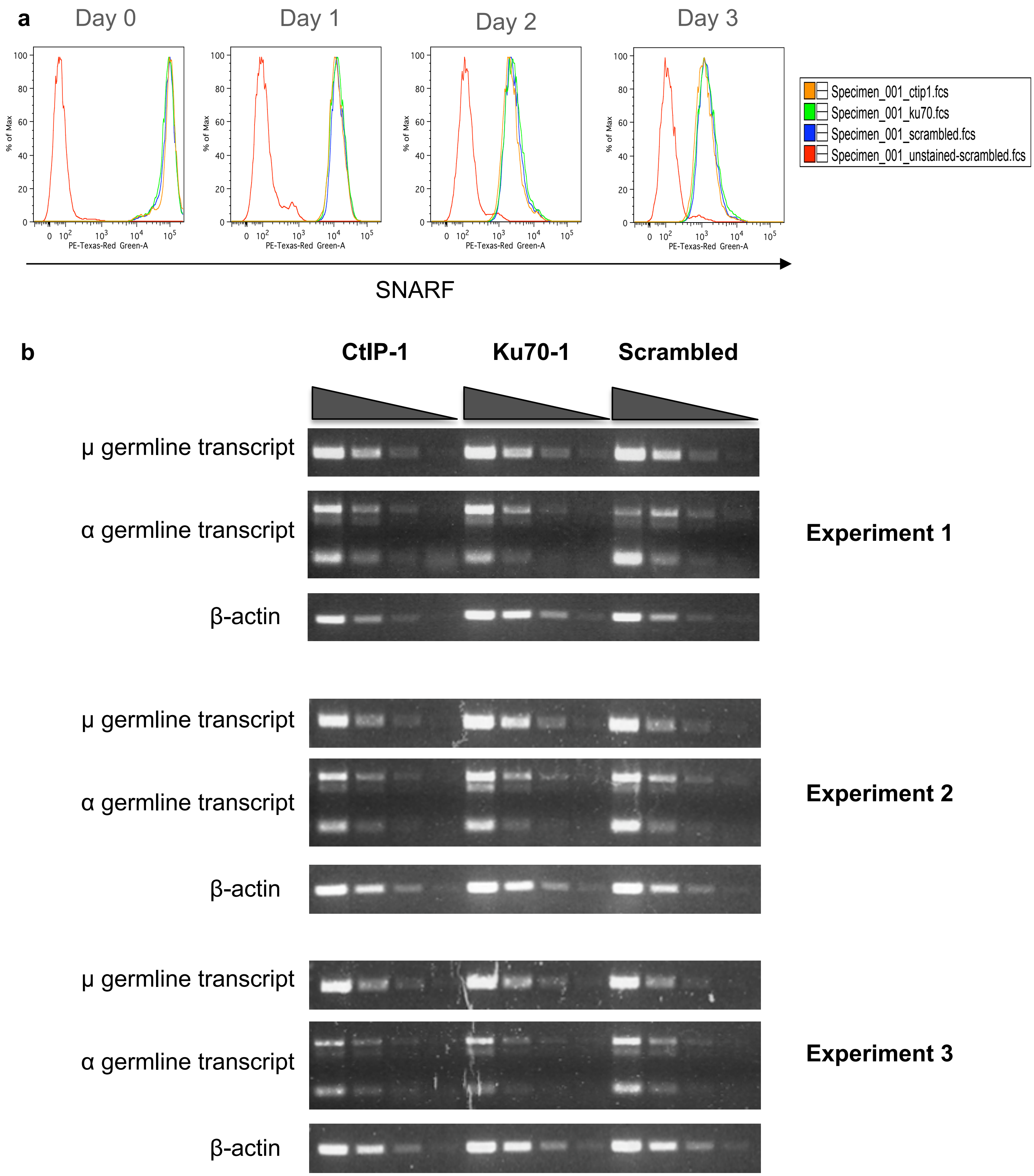
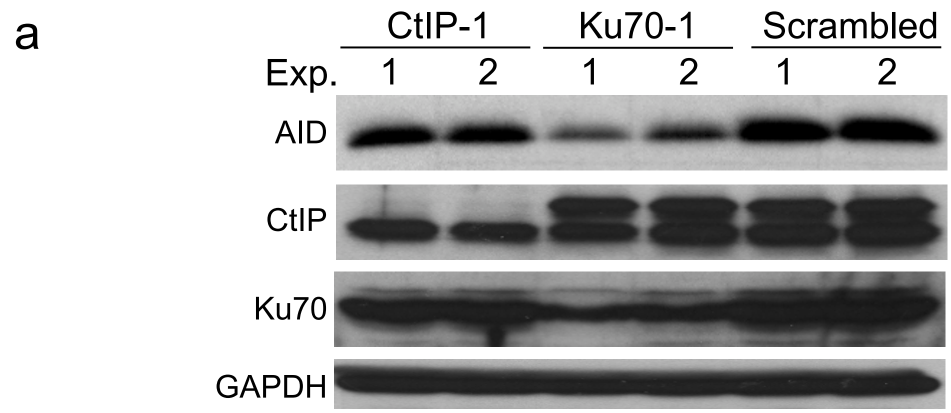
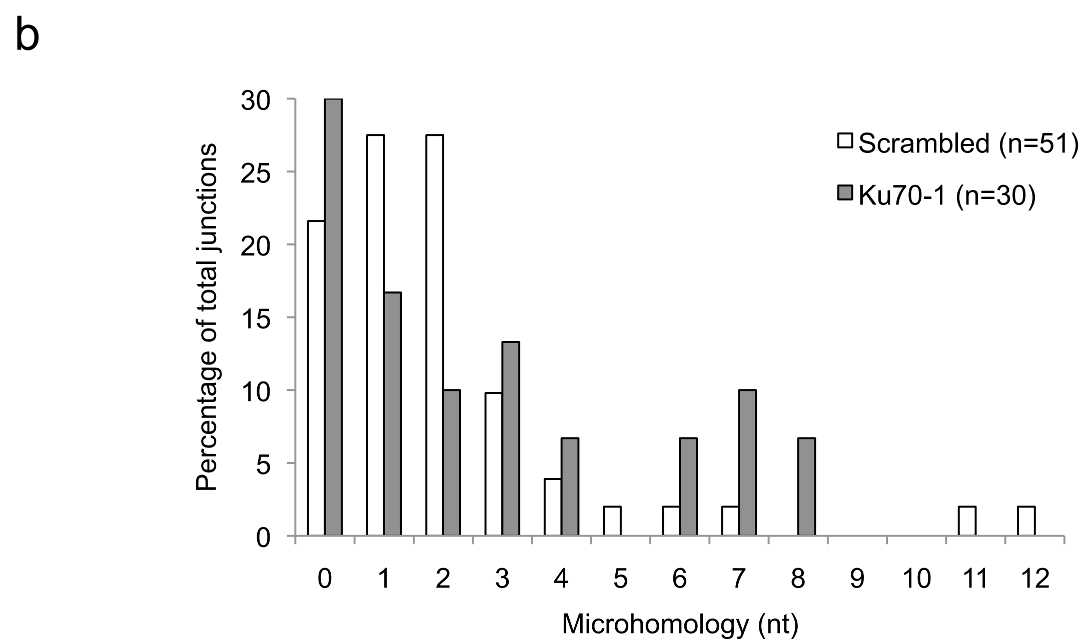


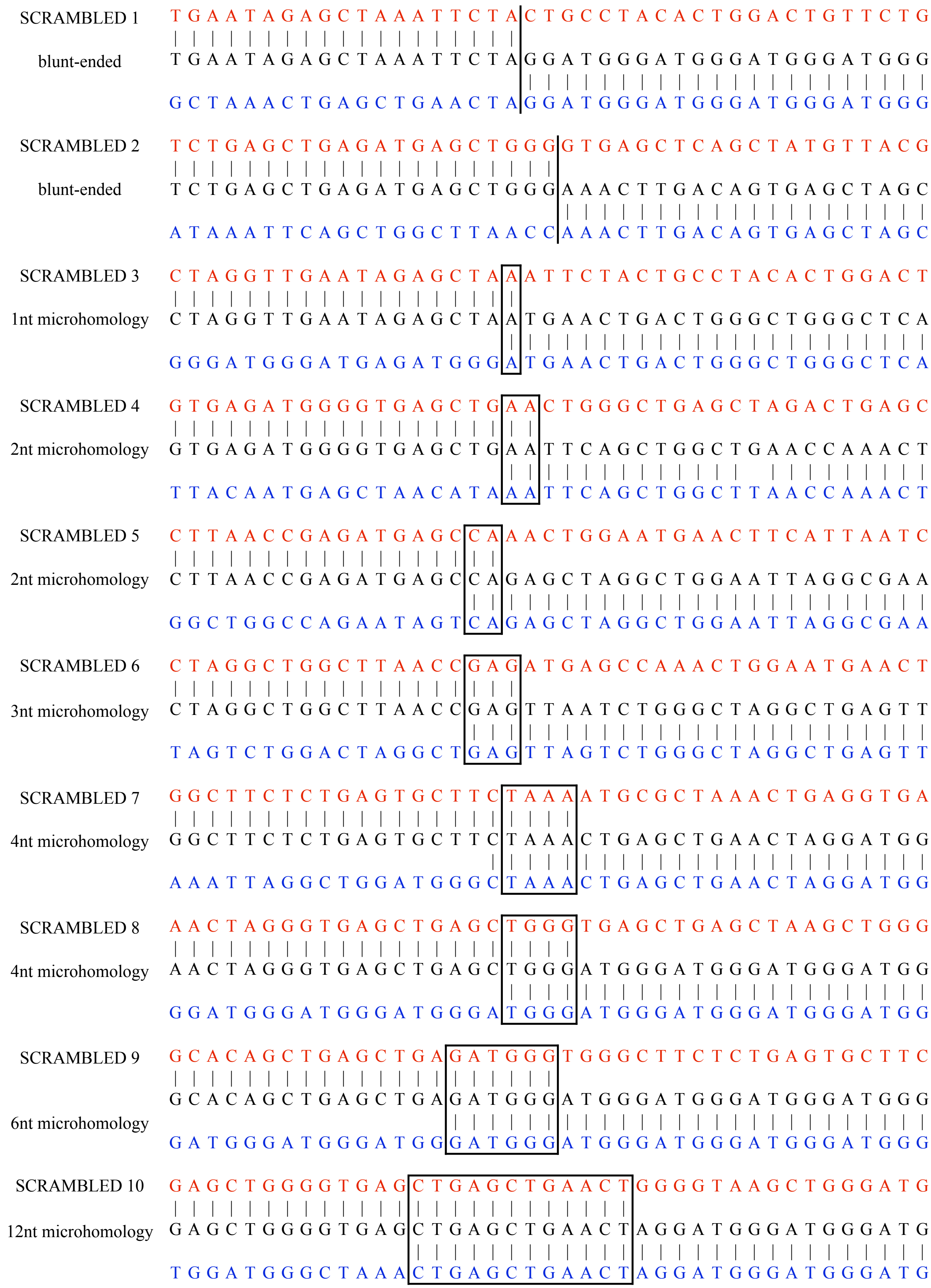
Supplementary Figure 1. Proliferation and germline transcription are not affected in CtIP and Ku70 knock-down cells. a, Knock-down and control cells were labeled with SNARF and stimulated with α-CD40, IL-4 and TGF-β (CIT). Cells were harvested at the indicated time points following SNARF labeling and analyzed by flow cytometry. The data are representative of three independent experiments. **b,** Knockdown and control cells were stimulated for 48 hours, cDNA generated from isolated RNA and amplified for α and μ germline transcripts and β-actin transcripts.





Supplementary Figure 2. shRNAs against CtIP and Ku70 reduce AID expression and alter switch junctions. a, Stimulated CH12 cells expressing CtIP, Ku70 or scrambled shRNAs in two independent experiments were analyzed by western blots using indicated antibodies. **b,** Sμ-Sα junctions from control or Ku70-depleted cells were cloned, sequenced and the percentage of junctions with indicated lengths of microhomology was plotted.

a



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blunt-ended
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SCRAMBLED 3
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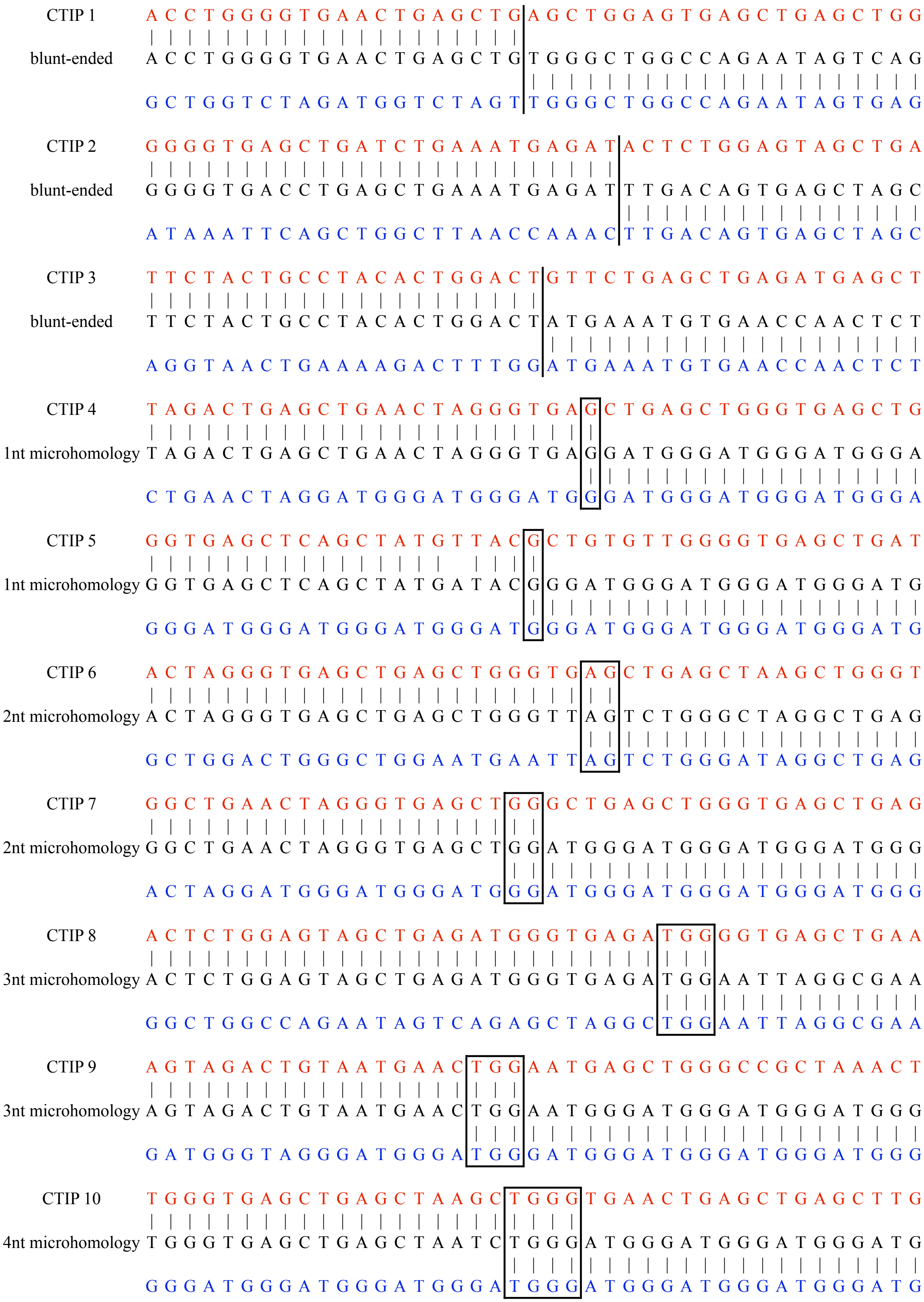
b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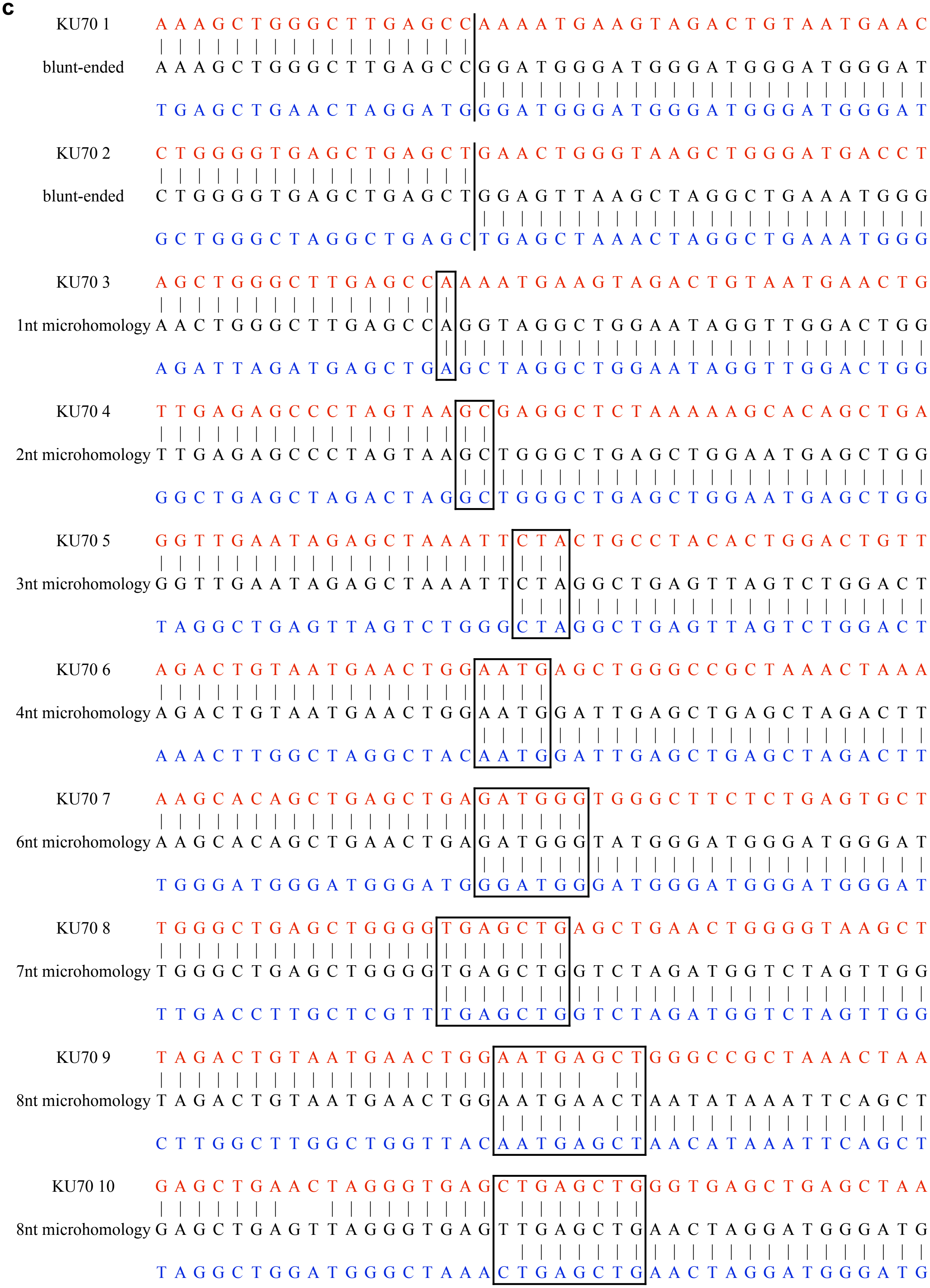

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KU70 2
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blunt-ended
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d

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KU70-SCRAMB 2        TAAACTAAACTAGGCTGGCT[T]CTACTGCCTACACTGGACTG
1nt microhomology    TAAACTAAACTAGGCTGGCT[T]GAACTGGGCTAAGCTGGGAT
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KU70-SCRAMB 3        GAGCTAAATTCTACTGCC[TA]CACTGGACTGTTCTGAGCTGA
2nt microhomology    GAGCTAAATTCTACTGTC[TA]TAAACTTGGCTAGGCTACAAT
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KU70-SCRAMB 4        GCTGGCTTAACCGAGATG[AGC]CAAACTGGAATGAACTGGCA
3nt microhomology    GCTGGCTTAACCGAGATG[AGC]TGGGATGGACTAGGATAAAC
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KU70-SCRAMB 5        ACTGGGCTGAGCTAGACTGA[GCTG]AACTAGGGTGAGCTGAG
4nt microhomology    ACTGGGCTGAGCTAGACTGA[GCTG]GACTGCAGGAGGAAGAC
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KU70-SCRAMB 10       GGGCTGAGCTGGGG[TGAGCTGGGCTG]AGTTGAGCTGAGTTG
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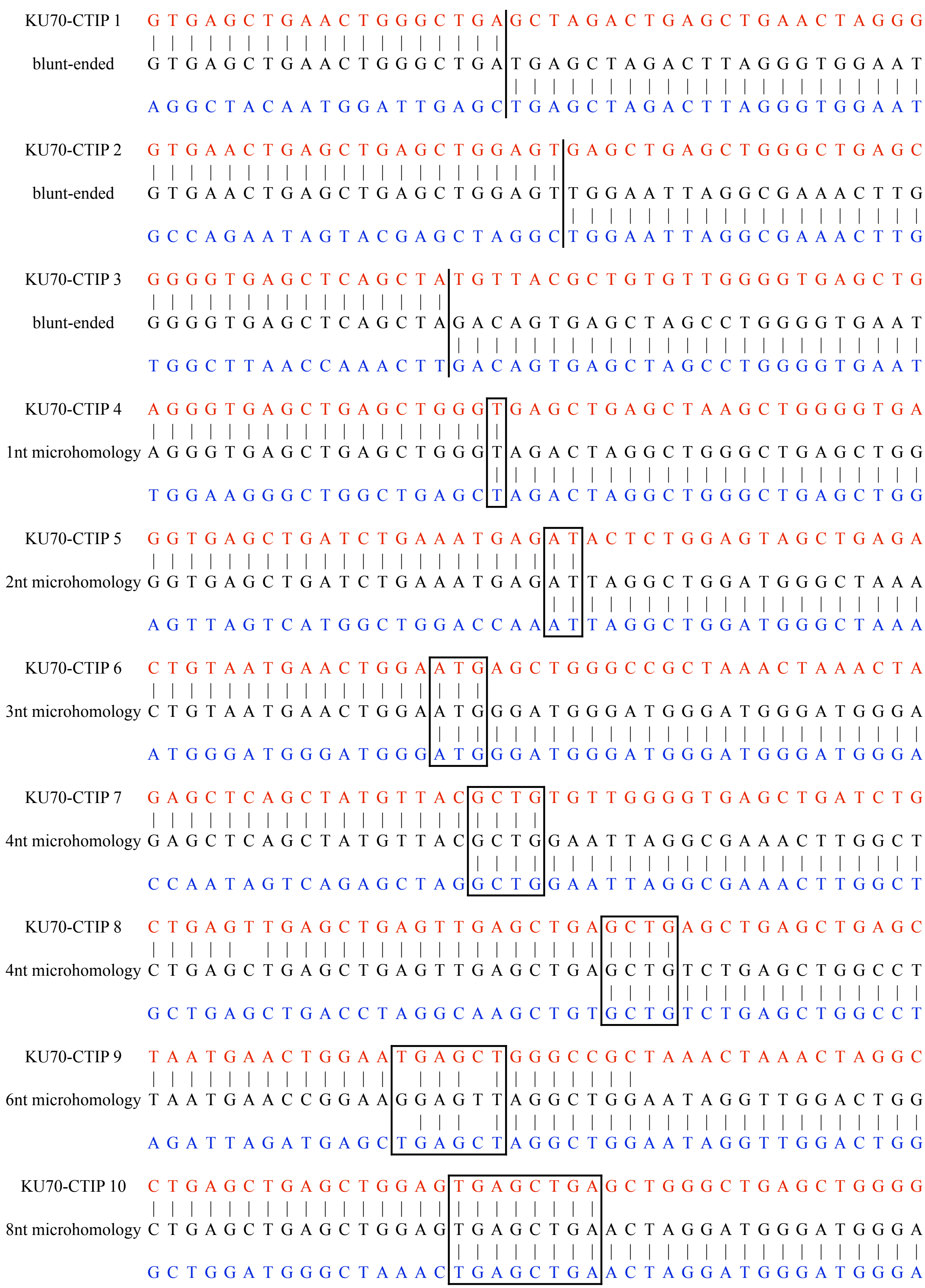
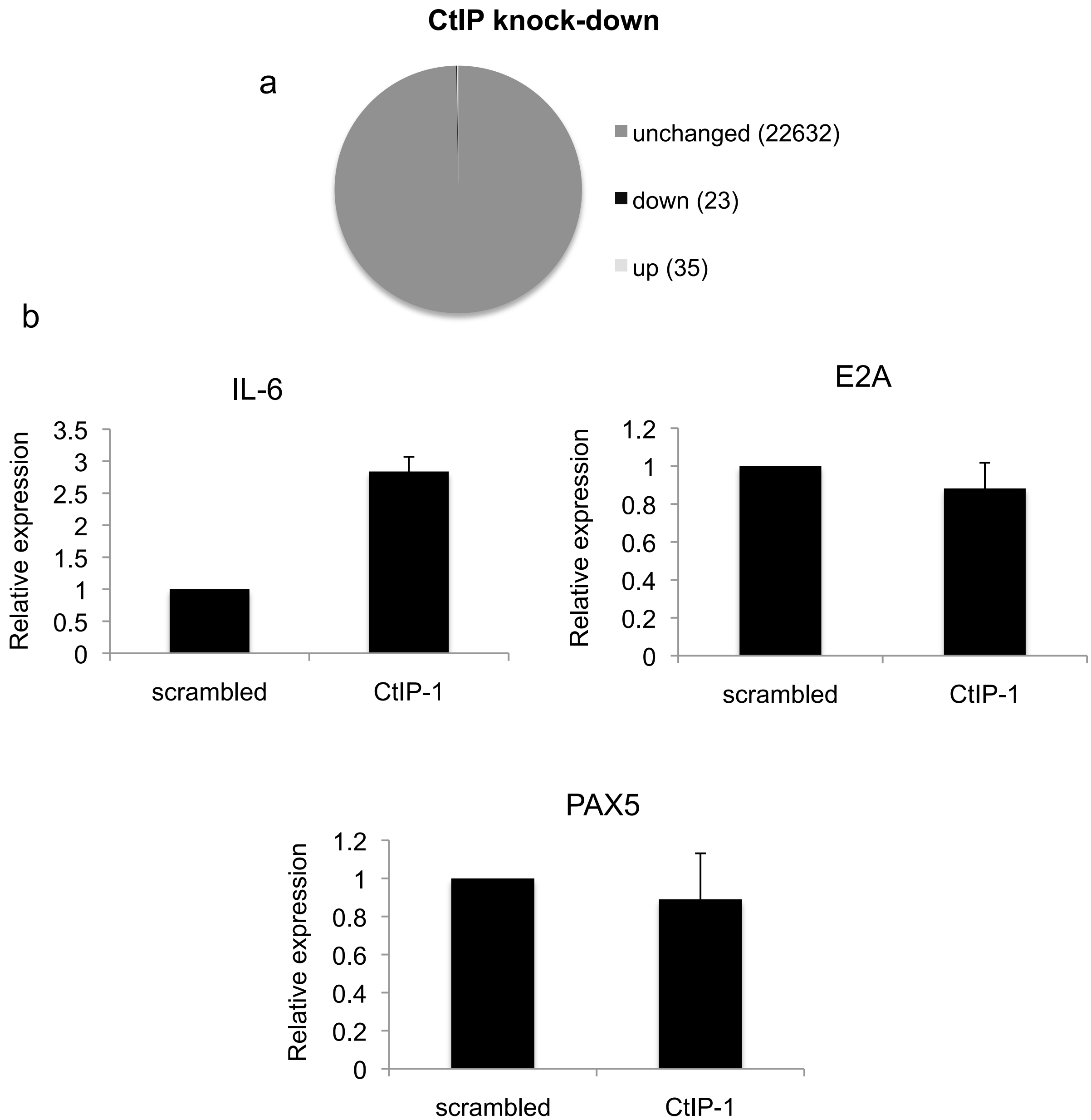
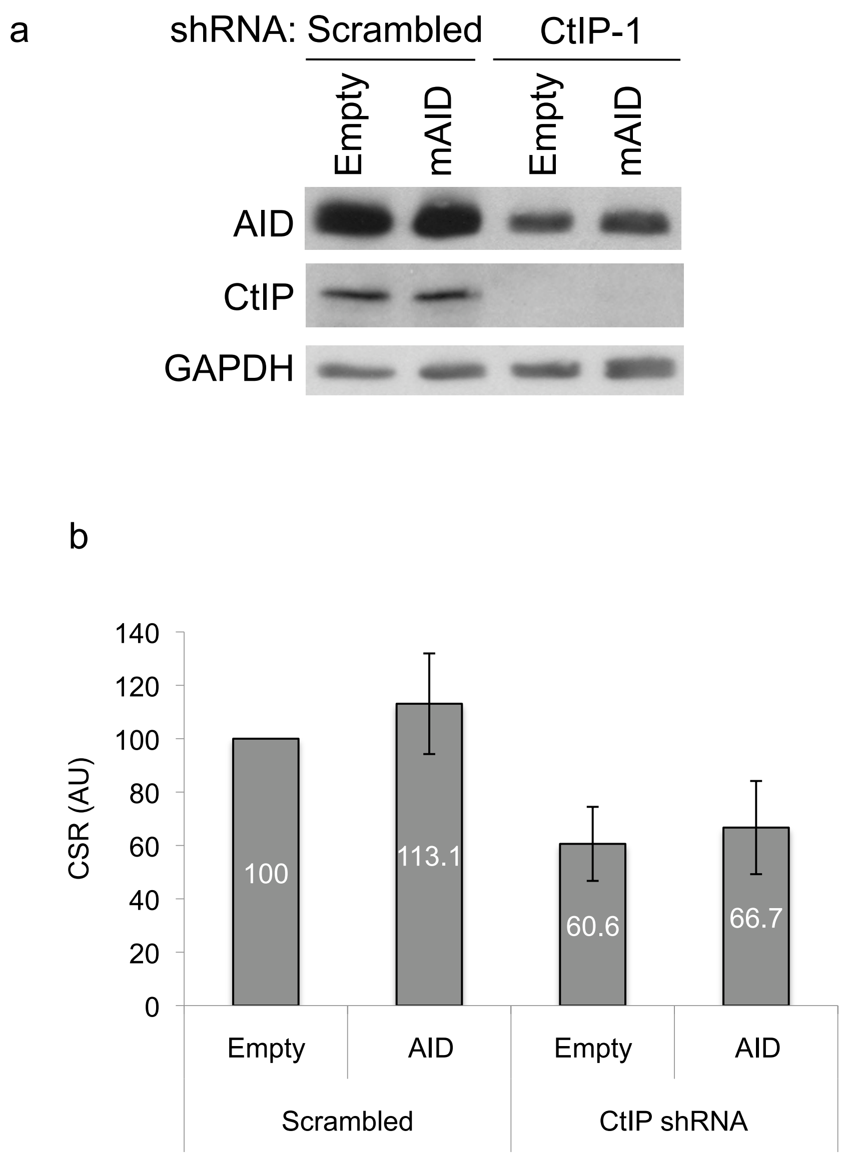
e



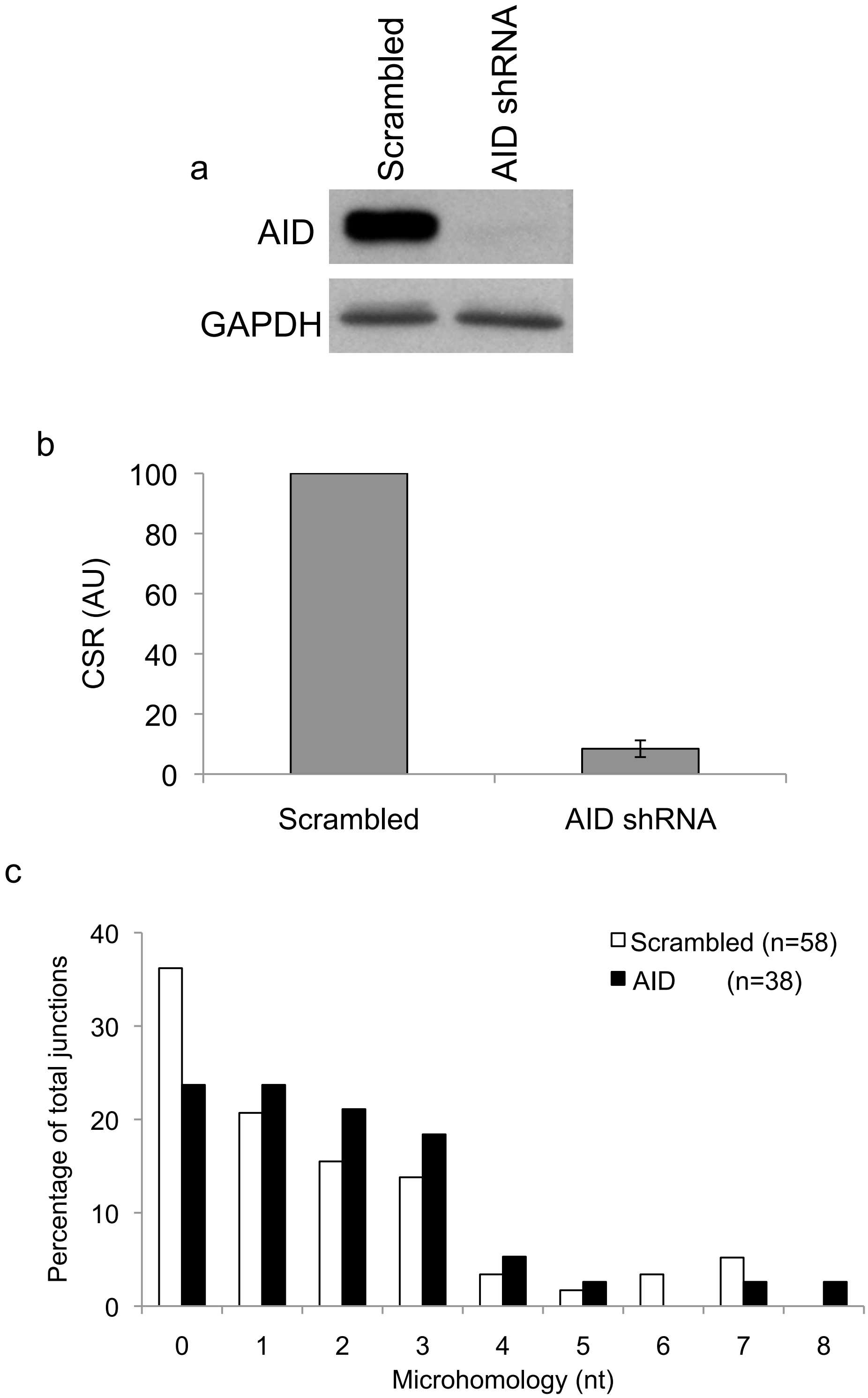
Supplementary Figure 3 (a-e). Representative Sμ-Sα junctions. Microhomology at junctions (boxed) was identified as the longest match to both germline Sμ (red) and Sα sequences (blue). Vertical lines indicate blunt-ended junctions.



Supplementary Figure 4. **Gene expression profile of CtIP knock-down cells. a, Microarray analysis: CtIP knock-down does not affect global transcription.** Control and CtIP knock-down cells were stimulated with CIT for 72 hours and total RNA subjected to a microarray analysis. The pie chart shows the numbers of genes whose expression was unchanged (dark grey), down-regulated (black) or up-regulated (light grey) in CtIP depleted cells compared to control scrambled cells**. b, Analysis of a select set of genes by real-time PCR.** Transcript levels of IL-6, E2A, and PAX5 were amplified from control or knock-down cells stimulated with CIT for 48 hours from 3 independent experiments. The comparative Pfaffl method was used to determine fold change in expression using β-actin and GAPDH as reference genes and values obtained from scrambled shRNA samples as the controls.



Supplementary Figure 5. Enforced expression of AID in CtIP knock-down cells. a, Murine AID (AID) cDNA or empty vector was introduced into CtIP knock-down cells via lentiviral transduction and analyzed for protein expression by western blots. Cells transduced with empty vector served as controls. **b,** Cells were stimulated with CIT and assayed for CSR to IgA by flow cytometry. CSR frequency in cells expressing scrambled shRNA and transduced with empty vector was assigned an arbitrary unit (AU) of 100.



Supplementary Figure 6. Decrease in AID levels does not affect the nature of the junctions. **a,** Whole cell extracts of CIT-stimulated control and AID knock-down cells were analyzed for AID and GAPDH expression by western blot analysis. **b,** CSR to IgA in CIT-stimulated AID knock-down or control cells was determined by flow cytometry. CSR frequency was normalized to that of scrambled shRNA infected cells that was assigned an arbitrary value of 100. The data are the mean of three independent experiments with error bars representing standard deviation from the mean. **c,** Sμ-Sα junctions were sequenced from AID knock-down and control cells.

Experiment	Scrambled	CtIP-1
1	33.7	41.8
2	33.6	17.5
3	19.5	11.3
4	34.3	28.1
5	16.9	7.94
6	24.7	12.7
7	24.5	6.4
8	11	7
9	11.45	9.4
10	25	13.4
11	15.2	5.9
12	29.4	16.1
13	20.8	12.4
14	20.6	10.9

Supplementary Table 1. Percentage of cells undergoing CSR to IgA in control (scrambled) or CtIP knock-down CH12 cells.

shRNA	Sequence
CtIP-1	CCGGGCAAGGTTTACAAGTCAAAGTCTCGAGACTTTGACTTGTAAACCTTGCTTTTTG
CtIP-2	CCGGGCATCCAATGACTTCAAGGAACTCGAGTTCCTTGAAGTCATTGGATGCTTTTTG
Ku70-1	CCGGCCGACACAGGTGGAGAATATACTCGAGTATATTCTCCACCTGTGTCGGTTTTT
Ku70-2	CCGGGCTCACTGTACCTACACTGAACTCGAGTTCAGTGTAGGTACAGTGAGCTTTTT
AID	CCGGCATGACCTTCAAAGACTATTTCTCGAGAAATAGTCTTTGAAGGTCATGTTTTTG

Application	Name	Forward	Reverse
germline transcription	GLT μ	5′-ctc tgg ccc tgc tta ttg ttg-3′	5′-gag aca ttt ggg aag gac tga ct-3′
	GLT α	5′-cct ggc tgt tcc cct atg aa-3′	5′-gag ctg gtg gga gtg tca gtg-3′
ChIP	Sμ	5’-tag taa gcg agg ctc taa aaa gca t-3’	5’-aga aca gtc cag tgt agg cag tag a-3’
	Iμ promoter	5’-gct cag cct gga ctt tcg gtt tgg t-3’	5’-gga gtc aag atg gcc gat cag aac c-3’
	Sγ1	5’-tat gat gga aag agg gta gca ttc acc-3’	5’-ctc ctt ccc aat ctc ccg tg-3’
	p53	5’-tat act cag agc cgg cct-3’	5’-cag cgt ggt ggt aac ctt at-3’
Sμ-Sα junctions	Sμfor3, Sαrev3	5’-ttg aga gcc cta gta agc gag gct cta-3’	5’-gaa ctg tga ata agt cca gtc atg cta at-3’
reference genes	β-actin	5’-tgc gtg aca tca aag aga ag -3’	5’-cgg atg tca acg tca cac tt-3’
	GAPDH	5’-agc ctc gtc ccg tag aca a-3’	5’-aat ctc cac ttt gcc act gc-3’
real-time PCR	AID	5’-gcc acc ttc gca aca agt ct -3	5’-ccg ggc aca gtc ata gca c-3’
	IL-6	5’-agt tgc ctt ctt ggg act ga-3’	5’-tcc acg att tcc cag aga ac-3’
	tcfe2a	5’-ttg acc cta gcc gga cat ac-3’	5’-tgc caa cac tgg tgt ctc tc-3’
	Pax5	5’-gcc cac agt cct acc cta ttg tca-3’	5’-ggg ttg ggg aac ctc caa gaa tc-3’

Supplementary Table 2. Sequence encoding knock-down shRNAs and primers used for analysis of knock-down cells are shown.