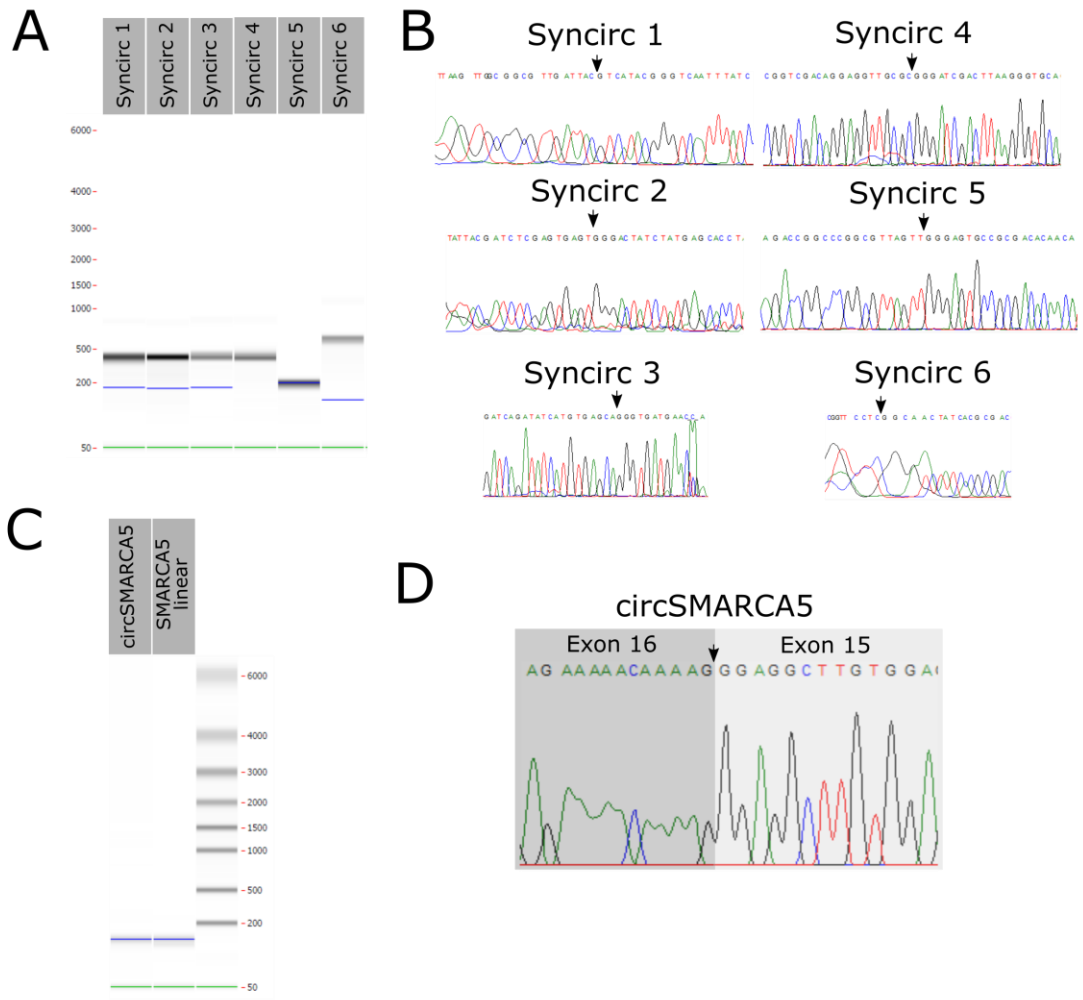
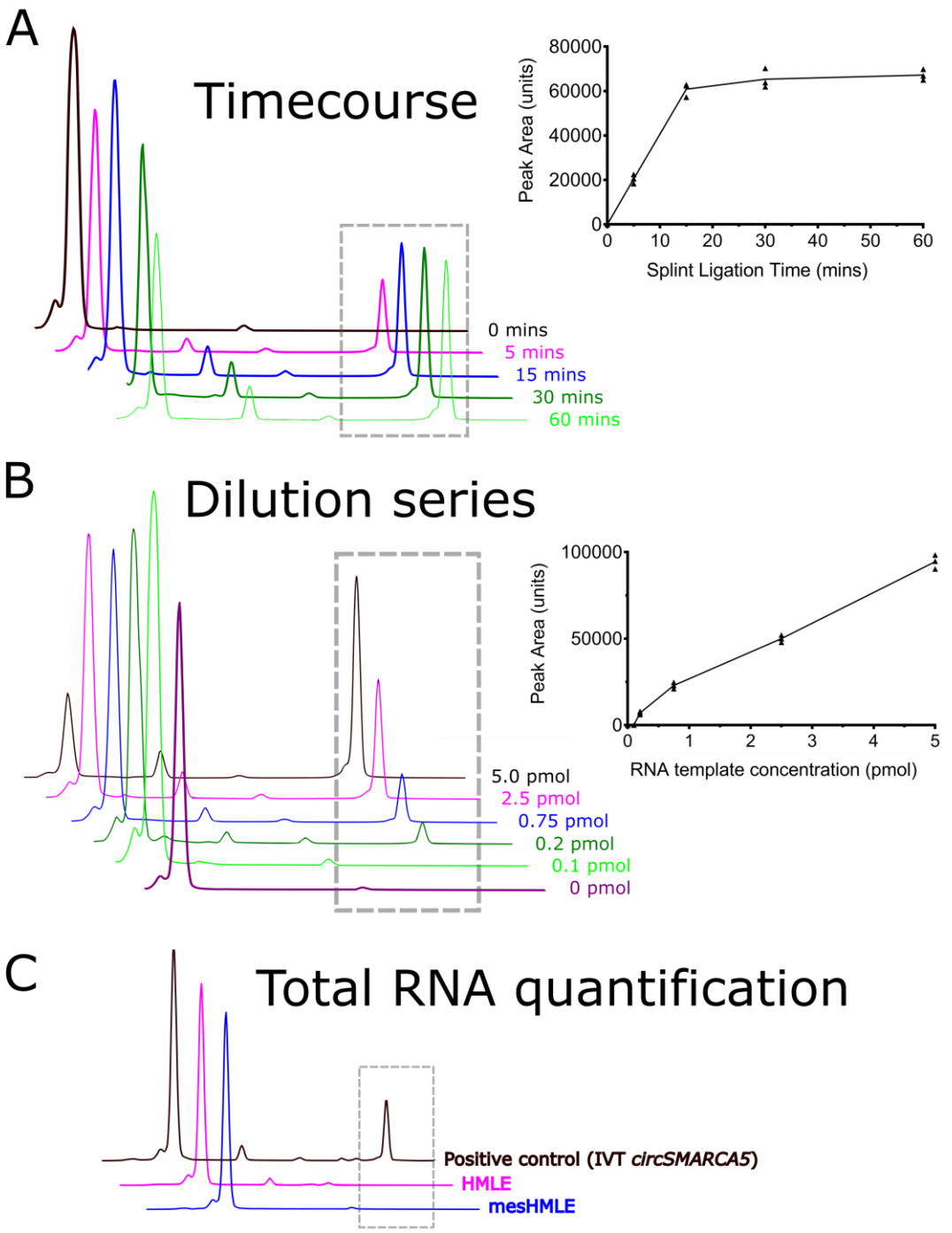
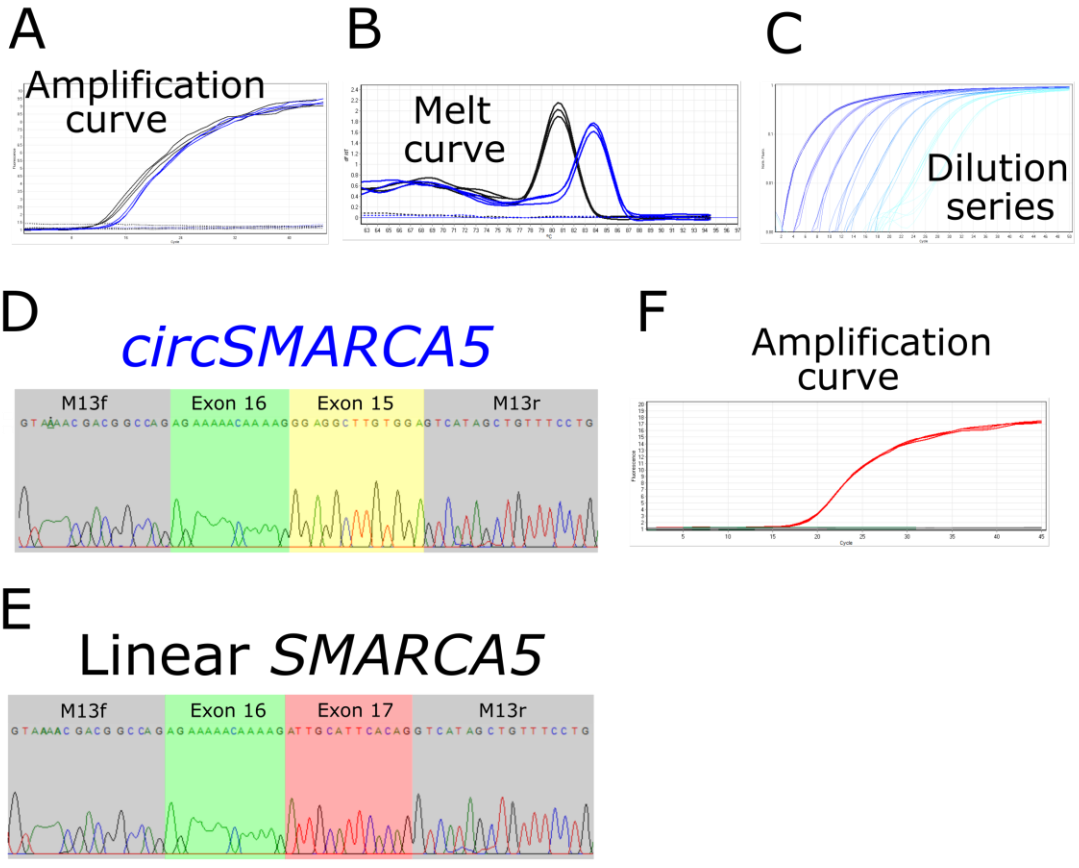
Supplementary Figures



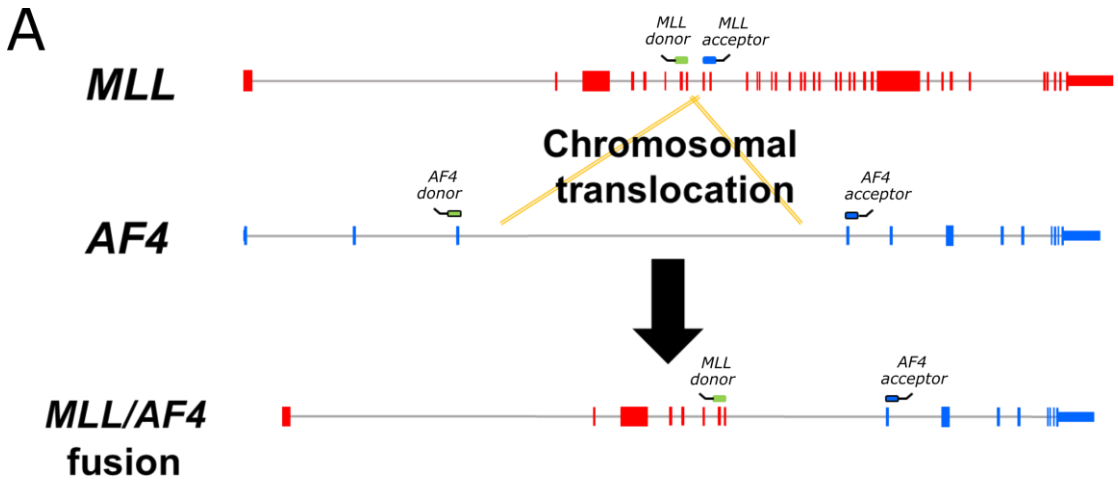
Supplemetary Figure S1: LabChip and Sanger sequencing chromatograms for IVT RNAs
A) Synthetic circRNAs #1-6 separated by LabChip HT with RNA pico ladder (nt). Expected syncircRNA sizes: #1 (402nt), #2 (403nt), #3 (400nt), #4 (395nt), #5 (203nt) and #6 (584nt).
B) Sanger sequencing of qRT-PCR products for synthetic circRNAs. Arrows show backsplice sites.
C) Linear and circular RNA made by IVT for *SMARCA5*. Expected sizes of 161nt (*circSMARCA5*) and 163nt (linear *SMARCA5* RNA).
D) Sanger sequencing of qRT-PCR product of *circSMARCA5*. Arrow shows backsplice site between exon 16 and 15.



Supplementary Figure S2: SplintQuant timecourse and dilution series for *circSMARCA5*. **A)** Timecourse and **B)** dilution series of SplintQuant targeting *circSMARCA5*, using 6-FAM labeled *donor* DNA oligonucleotide. Ligated product within grey dotted box, with conditions of reaction listed. *Inset*, graph showing peak area (arbitrary units) for ligated product, n = 3 biological replicates and line through mean of each time/concentration. **C)** *circSMARCA5* detection in 250ng total RNA from HMLE or mesHMLE cells, or positive control IVT *circSMARCA5* RNA (1.0 fmol)



Supplementary Figure S3: SplintQuant real time PCR validation for *SMARCA5*.
A) Amplification curves, **B)** melt curves following SplintQuant-qPCR of *circSMARCA5* (blue line) and *SMARCA5* linear (black line). Non-template controls (dotted lines) comprising no oligonucleotides, no SplintR ligase, or no template did not amplify signal. **C)** Dilution series of *circSMARCA5* with SplintQuant-qPCR over 8-logs (5-50,000,000 copies per reaction).
D) Sanger sequencing of SplintQuant qRT-PCR targeting circSMARCA5$^{Exon15-16}$
E) Sanger sequencing of SplintQuant qRT-PCR targeting linear SMARCA5$^{Exon16-17}$
F) Absence of off-target products as shown by no SplintQuant amplification in HMLE RNA when combining all 12 splint oligos for synthetic circRNAs (green) or synthetic linear RNAs (grey). Positive sample (red) comprising syncirc1 was included to show successful SplintQuant ligation.



Supplementary Figure S4: SplintQuant analysis of *MLL*, *AF4* and *MLL/AF4* in human cell lines **A)** Schematic of *MLL*, *AF4* with *donor* (green) and *acceptor* (blue) oligonculetoide positions highlighted above gene locus diagram. Chromosomal rearrangement of *MLL/AF4* in MV4;11.

Supplementary Tables

Supplementary Table S1: List of oligonucleotides used in this study

Supplementary Table S2: Absolute quantification of *circSMARCA5*, *circDOCK1, circMLL* (exons9-10) and *circAF4* (exons 3-4) presented as circRNA copies per cell in HMLE and mesHMLE cells calculated by SplintQuant and qRT-PCR. Normalisation was done against *GAPDH* as determined by qRT-PCR. Mean ± SD. n = 3 biological replicates, in technical duplicates.

Target	qRT-PCR			SplintQuant		
	HMLE	mesHMLE	Fold-change	HMLE	mesHMLE	Fold-change
circSMARCA5	42.1 ± 3.8	273.9 ± 37.2	6.5	9.8 ± 0.8	36.3 ± 3.7	3.7
circDOCK1	33.2 ± 4.2	3.3 ± 0.47	-10	16.2 ±1.2	3.2 ± 0.45	-5
circMLL$^{e9\text{-}10}$	3.4 ± 0.2	3.5 ± 0.3	1.0	2.4 ± 0.1	1.9 ± 0.3	0.8
circAF4$^{e3\text{-}4}$	5.1 ± 0.9	14.6 ± 1.8	2.9	2.4 ± 0.6	5.2 ± 1.0	2.1