Electronic Supplementary Material (ESI) for Analyst.

Supplementary Information

Combining asymmetric PCR-based enzymatic amplification with silicon photonic microring resonators for the detection of lncRNAs from low input human RNA samples

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Table S1. Fluidic handling protocol for the ring hybridization steps.

Step	Flow Rate (µL/min)	Duration (min)
Hybridization buffer	20	5
RT-PCR product cycle 30	20	13
Hybridization buffer	20	2
RT-PCR product cycle 32	20	13
Hybridization buffer	20	2
RT-PCR product cycle 34	20	13
Hybridization buffer	20	2
RT-PCR product cycle 36	20	13
Hybridization buffer	20	2
RT-PCR product cycle 38	20	13
Hybridization buffer	20	2
RT-PCR product cycle 40	20	13
Hybridization buffer	20	5

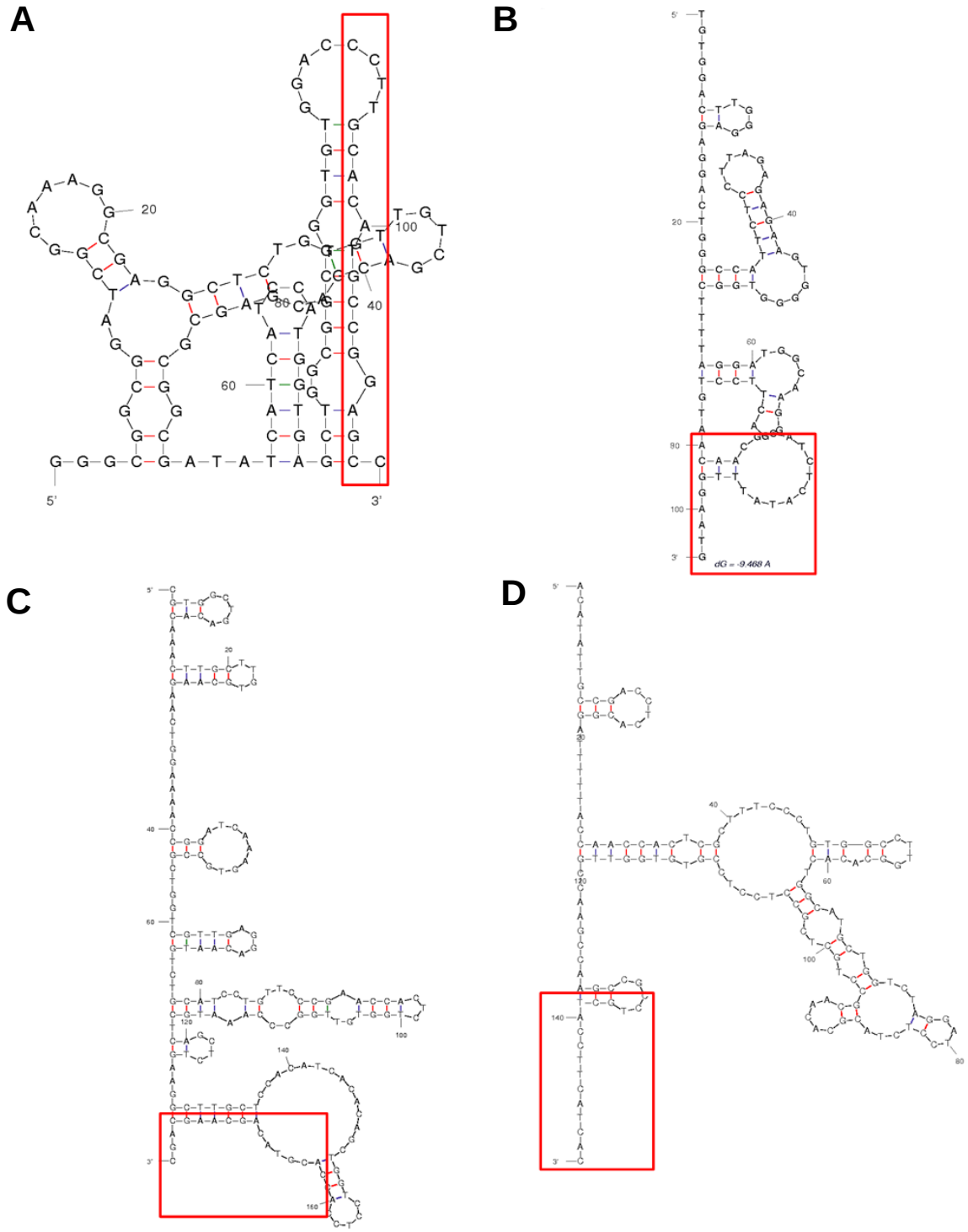

Figure S1. Secondary structures of amplified regions obtained using the DinaMelt Web Server. The region that binds to the capture probe is highlighted with a red square. (A) β-actin amplified region will not bind to complimentary capture probes due to excessive secondary structure. (B) β-actin amplified region after primer redesign that enables surface binding. (C) KIAA0495 amplified region with minimal secondary structure. (D) MALAT1 amplified region with minimal secondary structure.

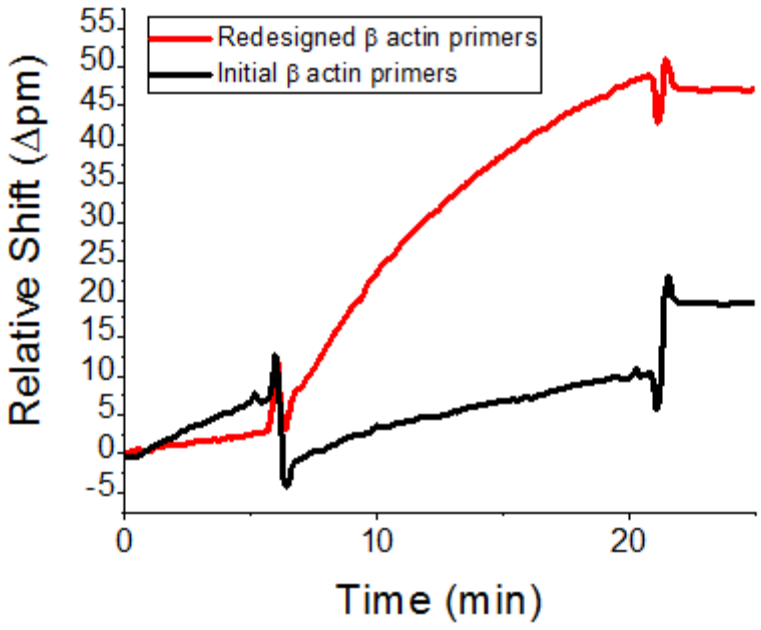

Figure S2. Comparison of β-actin PCR product binding with and without optimized primer design. The red trace shows improved binding when using the optimized primer sets (predicted structure shown in in Supplementary Figure 1B). The black trace shows data obtained using the PCR amplicon with a high degree of secondary structure (Supplementary Figure 1A). At t = 5 minutes, the solution was changed from hybridization buffer to the amplicon-containing solution, then returned to hybridization buffer at t = 20 minutes. The larger positive resonance shift confirmed improved amplification with new primer design.

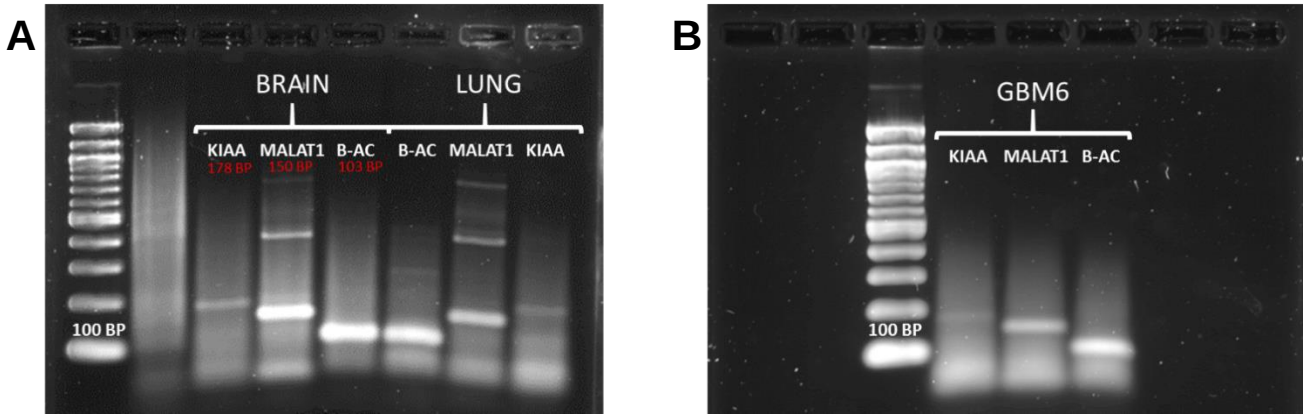

Figure S3. Agarose gel electrophoresis (2% agarose; SYBR Gold Stain) used to prove specific PCR amplification of lncRNAs and β-actin in (A) commercial brain and lung RNA samples; and (B) RNA isolated from GBM6 cells.

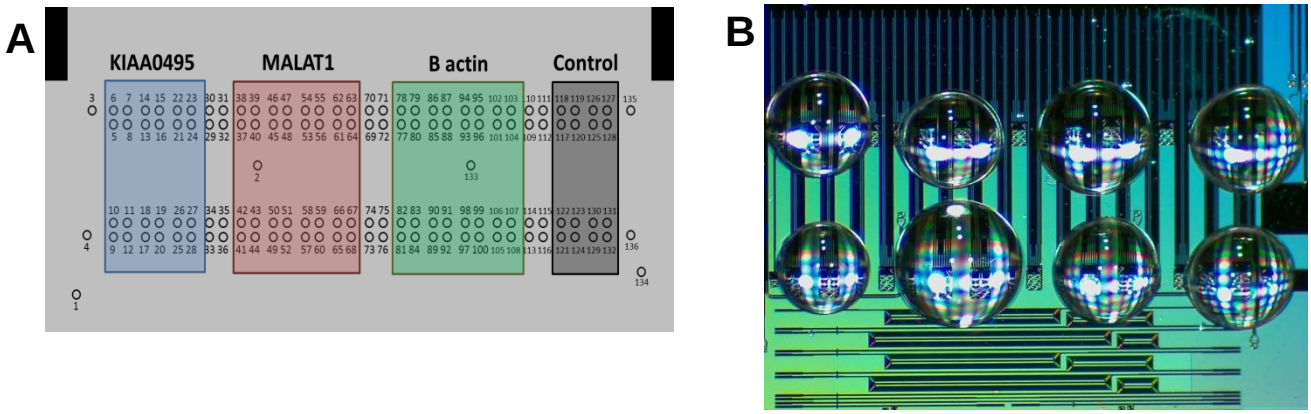

Figure S4. (A) Spotting layout of microring sensor chip showing the spatial distribution of capture probes. (B) Image of microring functionalization corresponding to the layout in Figure SI4A.

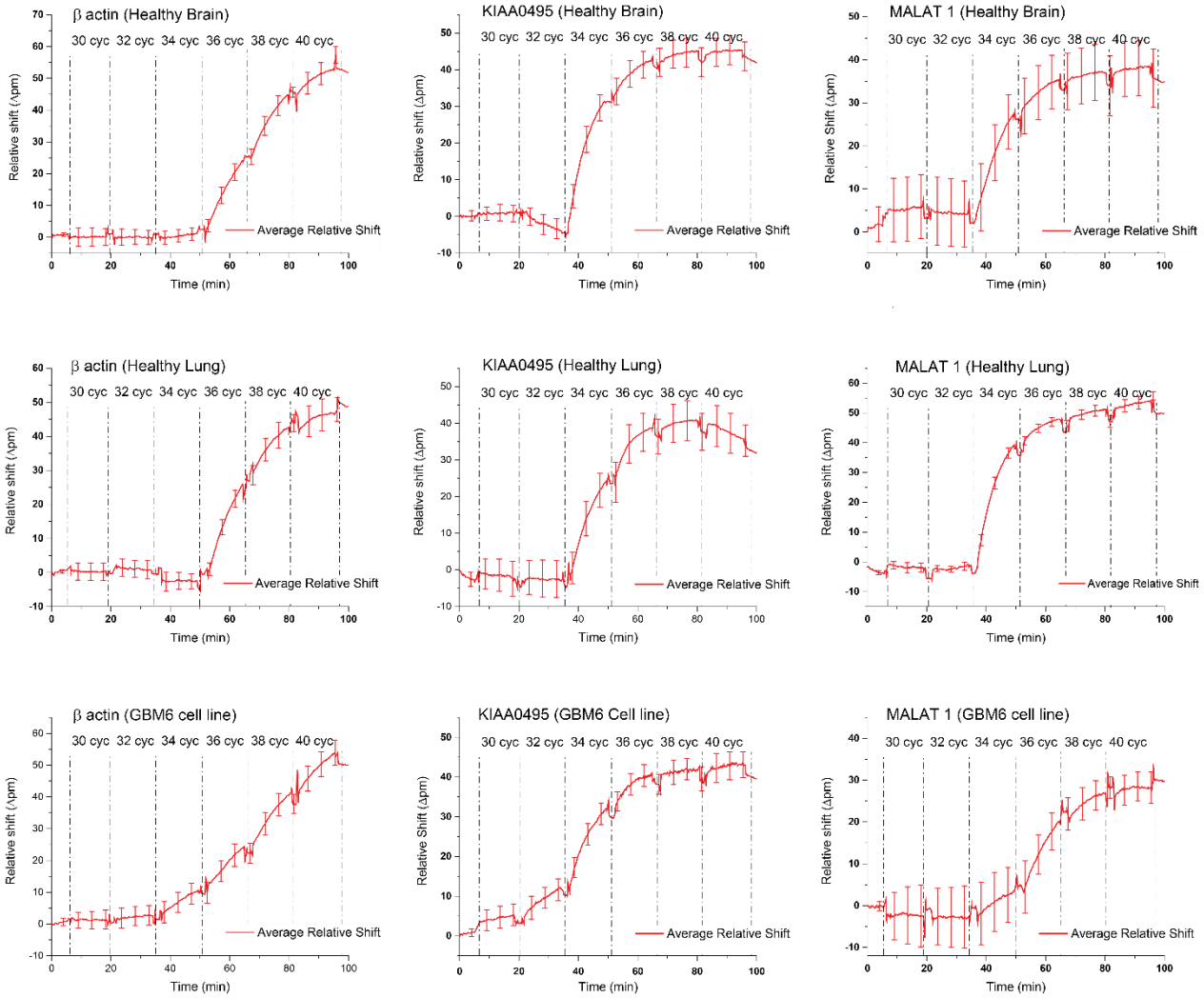

Figure S5. Microring traces used to calculate amplification plots in Figure 4. The trace signal represents the average of at least 8 replicate microrings simultaneously measure on the same chip minus the off control signal from non-specific functionalized rings. Error bars represent ± s.d. for n=8-16 replicate measurements on the same microring sensor array. University of Strathclyde, UK

Table S2. Calculated qRT-PCR and microring C(t) values from healthy brain, healthy lung and GBM6 RNA samples. The RNA input for qRT-PCR experiments was 40 ng, and the qRT-PCR experiments were completed in triplicate.

RNA	**Target**	**qRT-PCR C(t)**	**Rings C(t)**	**qPCR Fold change (log_2)****	**Rings Fold change (log_2)****
Brain	B actin	17.40	35.65	-	-
	KIAA0495	21.16	33.09	-	-
	MALAT1	17.77	33.04	-	-
Lung	B actin	17.40*	35.65*	0	0
	KIAA0495	21.57*	33.44*	- 0.41	- 0.64
	MALAT1	16.38*	33.68*	- 0.61	- 0.35
GBM 6	B actin	17.40*	35.65*	0	0
	KIAA0495	21.36*	32.72*	0.20	0.32
	MALAT1	19.05*	34.90*	-2.68	-1.81

* Corrected signal with internal control

** Healthy brain as reference; Fold Change = $C(t)_{ref} - C(t)_{sample}$