



Supplementary Material

10.1302/2046-3758.118.BJR-2021-0565.R1

Table i. Primer sequences in quantitative real-time polymerase chain reaction.

Name	Sequence (5'-3')	Primer length, bp	Product length, bp	Gene ID
homo RAB2A F	TCAGCCAGTGCATGACCTTA	20	244	5862
homo RAB2A R	TTCTTGCCCTGCCGTATCC	19		
homo SNTA1 F	GAGGACGTGCTGACCGTG	18	211	6640
homo SNTA1 R	CCCGGCCGCCTTTGATG	17		
homo TP63 F	CTGGAAACCAGAGATGGGCA	20	148	8626
homo TP63 R	GCTTCGTACCATCACCGTTC	20		
homo REEP1 F	GCACCCTTTACCCTGCGTATT	21	280	65055
homo REEP1 R	ACCAGACAATCATCGATTTTCCTTT	24		
homo FYCO1 F	CAGGAGGCCAAGGAGCAAAT	20	198	79443
homo FYCO1 R	CTTCAGTGCCTCAGGCGTT	20		

Table ii. Member RAS oncogene family and FYVE and coiled-coil domain autophagy adaptor 1 were amplified in clinical samples. Expression level of member RAS oncogene family in clinical samples.

Sample number	RAB2A	β -actin	Δ Ct	Blank Δ Ct mean	$-\Delta\Delta$ Ct	$2^{-\Delta\Delta$ Ct}	$2^{-\Delta\Delta$ Ct mean	SD
1	22.43	21.49	0.94	0.97	0.03	1.02	1.00	0.06
	22.41	21.51	0.90		0.07	1.05		
	22.35	21.27	1.08		-0.11	0.93		
2	22.37	22.15	0.22		0.75	1.69	1.73	0.16
	22.32	22.28	0.04		0.93	1.91		
	22.51	22.21	0.30		0.67	1.60		
3	0.00	28.16	- 28.16		29.13	0	0	0
	0.00	27.41	- 27.41		28.38	0		
	0.00	25.86	- 25.86		26.83	0		
4	21.89	22.34	-0.45		1.42	2.68	2.66	0.25
	21.80	22.09	-0.29		1.26	2.40		
	21.48	22.04	-0.56		1.53	2.90		
5	22.80	22.53	0.27		0.70	1.63	1.78	0.17
	22.69	22.53	0.16		0.81	1.76		
	22.63	22.63	0.00		0.97	1.96		
6	24.06	24.33	-0.27		1.24	2.37	2.32	0.10
	24.34	24.51	-0.17		1.14	2.21		
	24.07	24.35	-0.28		1.25	2.38		
7	22.12	23.00	-0.88		1.85	3.61	2.88	0.66
	22.20	22.65	-0.45		1.42	2.68		
	22.28	22.53	-0.25		1.22	2.34		
8	23.20	23.47	-0.27		1.24	2.37	2.26	0.21
	23.11	23.39	-0.28		1.25	2.38		
	23.04	23.08	-0.04		1.01	2.02		

SD, standard deviation.

Table iii. Expression level of FYVE and coiled-coil domain autophagy adaptor 1 in clinical samples.

Blank Δ Ct mean	FYCO 1	β -actin	Δ Ct	Blank Δ Ct mean	- $\Delta\Delta$ Ct	$2^{-\Delta\Delta$ Ct}	$2^{-\Delta\Delta$ Ct mean	SD
1	25.55	19.80	5.75	5.79	0.04	1.03	1.00	0.07
	25.91	20.00	5.91		-0.12	0.92		
	25.75	20.05	5.70		0.09	1.06		
2	25.05	20.62	4.43		1.36	2.56	2.62	0.26
	25.14	20.89	4.25		1.54	2.90		
	25.19	20.66	4.53		1.26	2.39		
3	0.00	28.11	-28.11		33.90	0	0	0
	0.00	26.03	-26.03		31.82	0		
	0.00	27.24	-27.24		33.03	0		
4	25.33	20.66	4.67		1.12	2.17	2.08	0.09
	25.10	20.37	4.73		1.06	2.08		
	25.21	20.41	4.80		0.99	1.98		
5	25.78	21.00	4.78		1.01	2.01	1.65	0.33
	26.14	21.01	5.13		0.66	1.58		
	26.43	21.08	5.35		0.44	1.35		
6	26.80	22.87	3.93		1.86	3.62	3.68	0.05
	26.59	22.70	3.89		1.90	3.72		
	26.99	23.09	3.90		1.89	3.70		
7	25.58	20.72	4.86		0.93	1.90	1.74	0.14
	25.92	20.83	5.09		0.70	1.62		
	26.09	21.08	5.01		0.78	1.71		
8	27.18	22.02	5.16		0.63	1.54	1.83	0.28
	26.58	21.86	4.72		1.07	2.09		
	26.90	22.01	4.89		0.90	1.86		

SD, standard deviation.

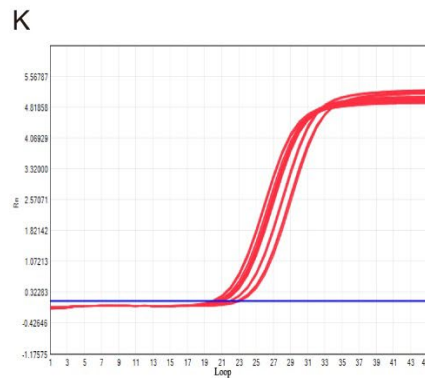
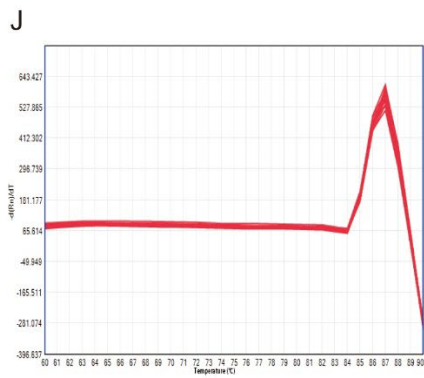
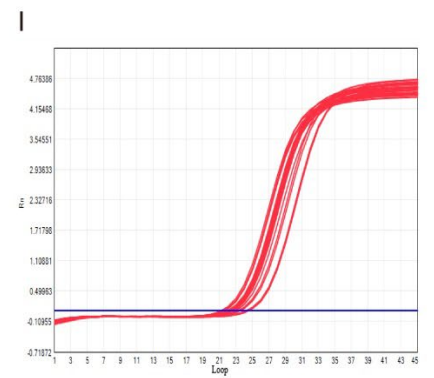
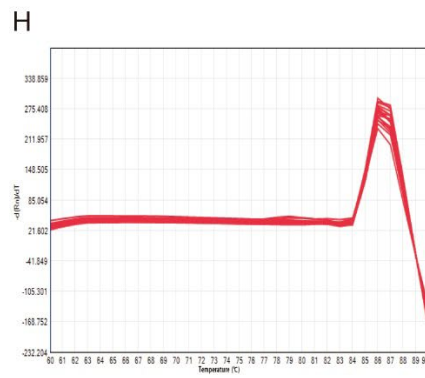
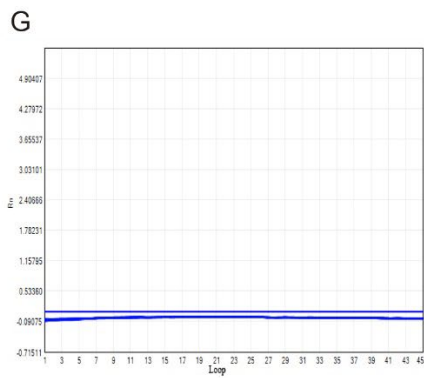
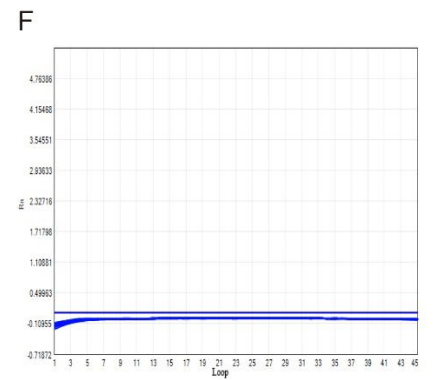
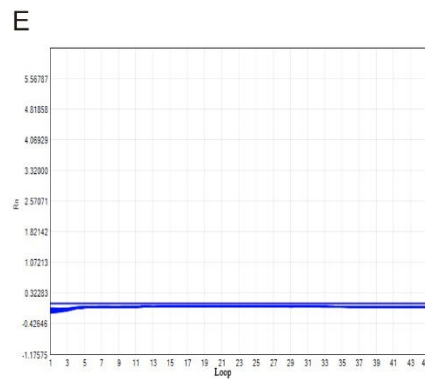
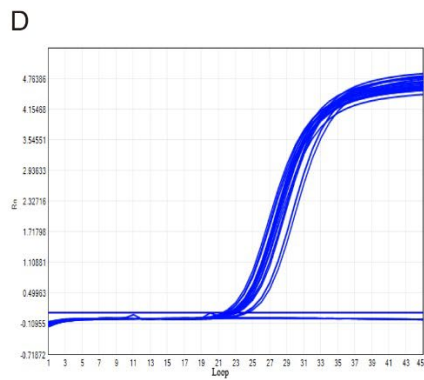
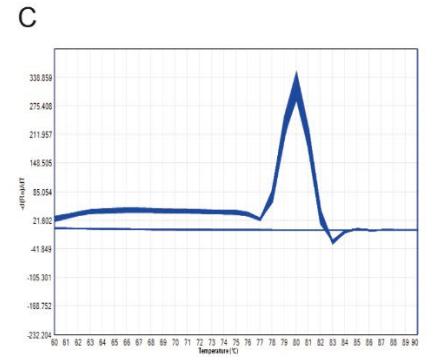
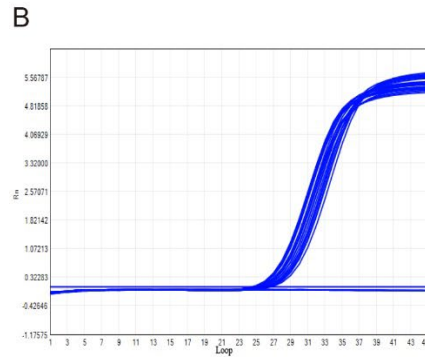
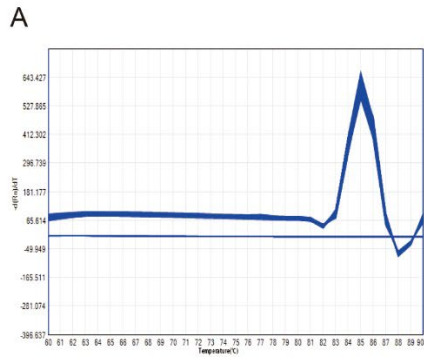


Fig a. Quantitative real-time polymerase chain reaction (qRT-PCT) analysis. With the use of the SLAN-96S fluorescence quantizer, the messenger RNA (mRNA) expression levels were quantified by qRT-PCT. The relative expression of the target genes was determined using the $2^{-\Delta\Delta CT}$ method. The diagrams show the relationship between mRNA expression level and temperature and number of cycles of genes. Correlation between (A) mRNA expression level and temperature, and (B) number of cycles of FYVE and coiled-coil domain autophagy adaptor 1 (FYCO1). Correlation between (C) mRNA expression level and temperature, and (D) number of cycles of member RAS oncogene family (RAB2A). Correlation between mRNA expression level and number of cycles of (E) receptor accessory protein 1 (REEP1), (F) Syntrophin Alpha 1 (SNTA1), and (G) tumour protein p63 (TP63). Also displayed are the correlation between (H) mRNA expression and temperature, and (I) number of cycles of β -actin 1 together with correlation between (J) mRNA expression level and temperature, and (K) number of cycles of β -actin 2.