Statistical significance of quantitative PCR: Additional File 1

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Additional Tables

Additional Table1: Primer sequences and qPCR dataset

Gene	Forward primer	Reverse primer	Amplicon size	Samples	Dilutions
Cav	TGATGAGTGAACCTCCAGGGA	GCAGTCTCGGTTTAGCAGCC	57	4	4
CTGF	CATTAAGAAGGGCAAAAAGTGCA	ACAGGCTTGGCGATTTTAGGT	51	4	5
Eln	TCTTTGTGTTTCGCTGTGATAGATC	CAAACATCATCCCCAAATATCCA	72	4	4
FN	GCAAGGAAAGTCACCCAGACA	TGCAGGTCAGATGGCAAAAG	51	4	5
L27	GCCAAGCGATCCAAGATCAA	GCTGGGTCCCTGAACACATC	120	8	5
Perl	GGAGGCCCGTCTTGTCTCA	TGTTGACCGCCACATTAGGAC	149	4	4
PAI-1	CCCAATAGCGAGCCTTCTCC	TGTGGGTCGAAAAAAGCTGTG	100	4	4

Target genes, primer sequences and amplicon size (in base pair, primers included). The number of independent biological samples used for each primer set and number of dilutions used in the serial dilution assay is indicated. Each dilution was measured in 5 replicates PCR. The size of the messenger RNA is indicated only for the genes analyzed in the Northern blot assay.

Additional Table 2: Average efficiencies measured for each set of primers

Primer set	Efficiency	SEM	n
Cav	1.676	0.0088	80
CTGF	1.843	0.0055	97
Eln	1.753	0.0062	80
FN	1.827	0.0064	116
L27	1.911	0.0040	211
Perl	1.753	0.0111	76
PAI-1	1.896	0.0080	100

Efficiencies were determined using the LinReg method for each target cDNA using the dataset described in Add. Table 1, and they are expressed as the average, standard deviation of the mean (SEM), and number of values used for each determination

Additional Table 3: TGF-β induction of extracellular matrix gene expression in NIH-3T3 fibroblasts as assessed from 10 replicate assays

Gene	PAI-1			FN			CTGF		
	$(\text{Savrg E})^{Ct}$	$(\text{Pavrg E})^{Ct}$	ΔCt	$(\text{Savrg E})^{Ct}$	$(\text{Pavrg E})^{Ct}$	ΔCt	$(\text{Savrg E})^{Ct}$	$(\text{Pavrg E})^{Ct}$	ΔCt
Induction	8.01	14.75	14.11	1.60	1.08	1.08	23.15	43.53	40.96
SD	1.12	2.09	1.98	0.21	0.14	0.14	3.21	6.46	6.01
CV	14.04	14.17	14.04	12.93	13.37	13.25	13.84	14.83	14.68
p-value	<0.0001	<0.0001	<0.0001	0.03	0.384	0.389	<0.0001	<0.0001	<0.0001

The expression of the PAI1, FN and CTGF genes was measured and normalized to the L27 mRNA using the $(SavrgE)^{Ct}$, $(PavrgE)^{Ct}$ and the ΔCt models. Average induction ratios (e.g. induced over non-induced cDNA ratios) were determined over the entire set of 10 replicate assays and they are shown alongside with the standard deviation and coefficient of variation (CV) values. A t-test was performed on the normalized gene expression to test whether expression levels are statistically different between the induced and non-induced state. A p-value below 0.05 indicates that gene expression levels are statistically different.

Additional Table 4: TGF-β induction of extracellular matrix gene expression in NIH-3T3 fibroblasts as assessed from 3 replicate assays

Gene	PAI-1			FN			CTGF		
	$(\text{Savrg E})^{Ct}$	$(\text{Pavrg E})^{Ct}$	ΔCt	$(\text{Savrg E})^{Ct}$	$(\text{Pavrg E})^{Ct}$	ΔCt	$(\text{Savrg E})^{Ct}$	$(\text{Pavrg E})^{Ct}$	ΔCt
Induction	23.64	13.74	12.94	2.38	1.07	1.09	44.01	34.23	44.12
SD	3.84	2.22	2.05	0.26	0.11	0.12	4.95	3.86	5.29
CV	16.25	16.17	15.86	10.75	10.59	11.25	11.25	11.29	11.99
p-value	<0.0001	<0.0001	<0.0001	<0.0001	0.263	0.236	<0.0001	<0.0001	<0.0001

Expression of 3 genes was measured and normalized with L27 using the $(SavrgE)^{Ct}$, $(PavrgE)^{Ct}$ and the ΔCt models, and results are represented as described in the footnote to Additional Table 3, except that only the first three measurements were taken into account.