

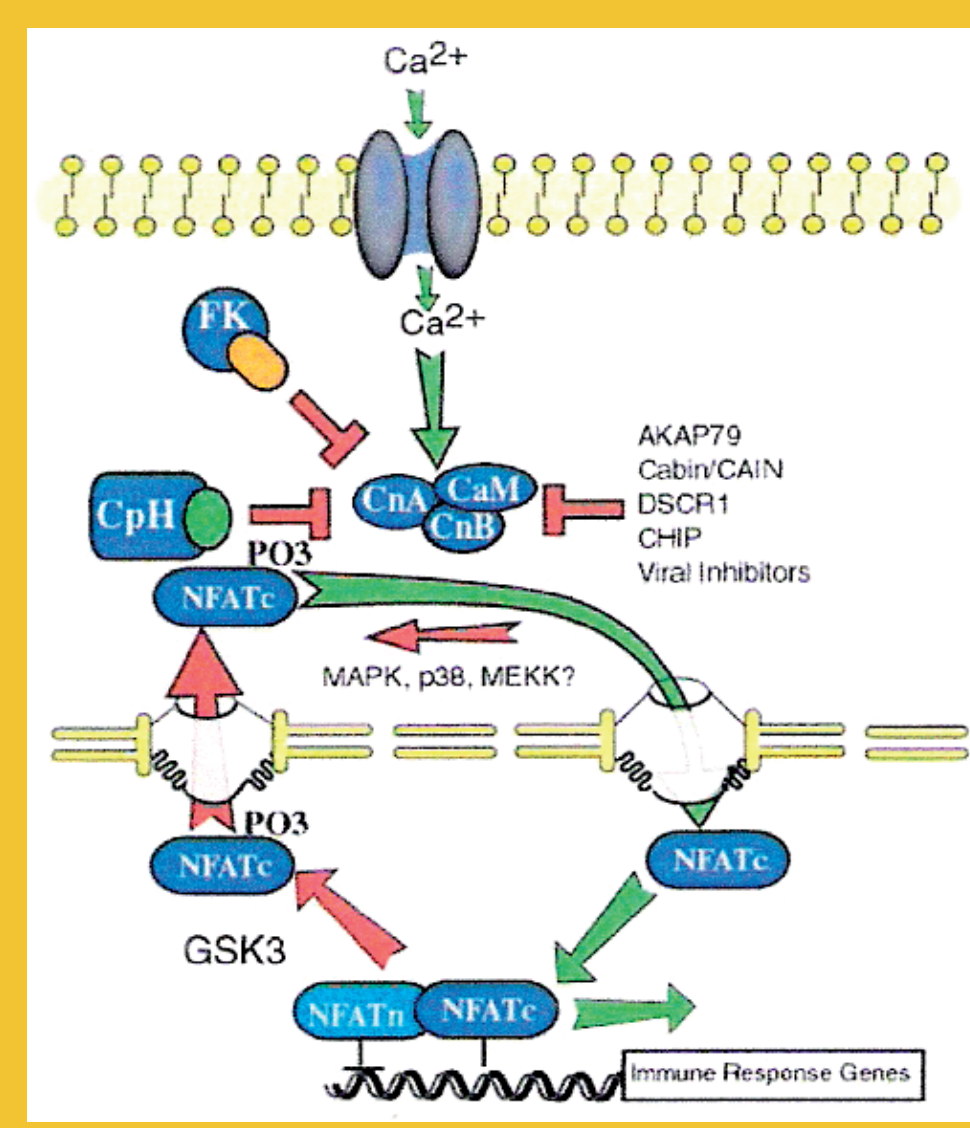
# Influence of NMR therapy on Ca<sup>2+</sup> signalling and gene expression in osteosarcoma- and chondrosarcoma cell lines

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**Introduction:** Nuclear magnetic resonance therapy (NMRT) with weak magnetic fields (up to 2.3 mT, 100 kHz) has been shown to stimulate repair processes in cartilage and to influence pain signalling. On the contrary, strong magnetic fields (3 T) used for imaging purposes are suspected to have deleterious effects on chondrocytes and cartilage repair. To assess the effect of NMRT with optimized field strength on cellular processes we used microarrays and quantitative PCR (qPCR) for expression profiling of NMRT treated chondrosarcoma and osteosarcoma cells. In addition, the Ca<sup>2+</sup> imaging technique was used to study functional effects on Ca<sup>2+</sup> influx and Ca<sup>2+</sup> release.



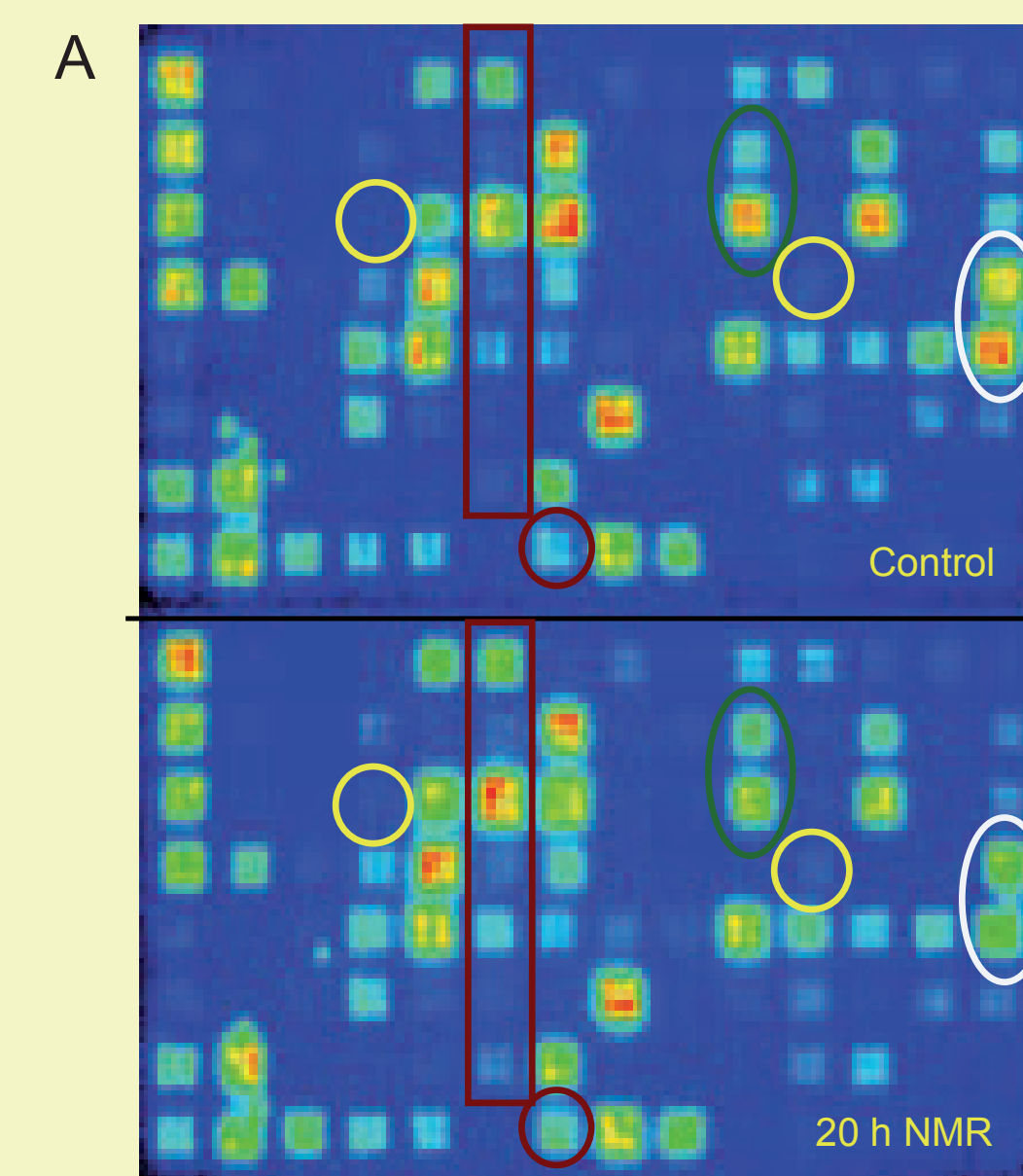
Crabtree GR, Olson EN. NFAT Signaling: Choreographing the Social Life of Cells. Cell 2002; 109: S67-S79.

**Fig. 1:** Ca<sup>2+</sup> is a key regulator of the the NFAT pathway: Activation of calcineurin (Cn) by elevated [Ca<sup>2+</sup>] results in dephosphorylation of NFATc, which is then able to enter the nucleus and acts as a transcription factor. GSK3 can phosphorylate NFAT, thus terminating the transcriptional effect of NFAT. Inhibitors of calcineurin are FK506, and other endogenous proteins but also the immunodepressant drug cyclosporine.

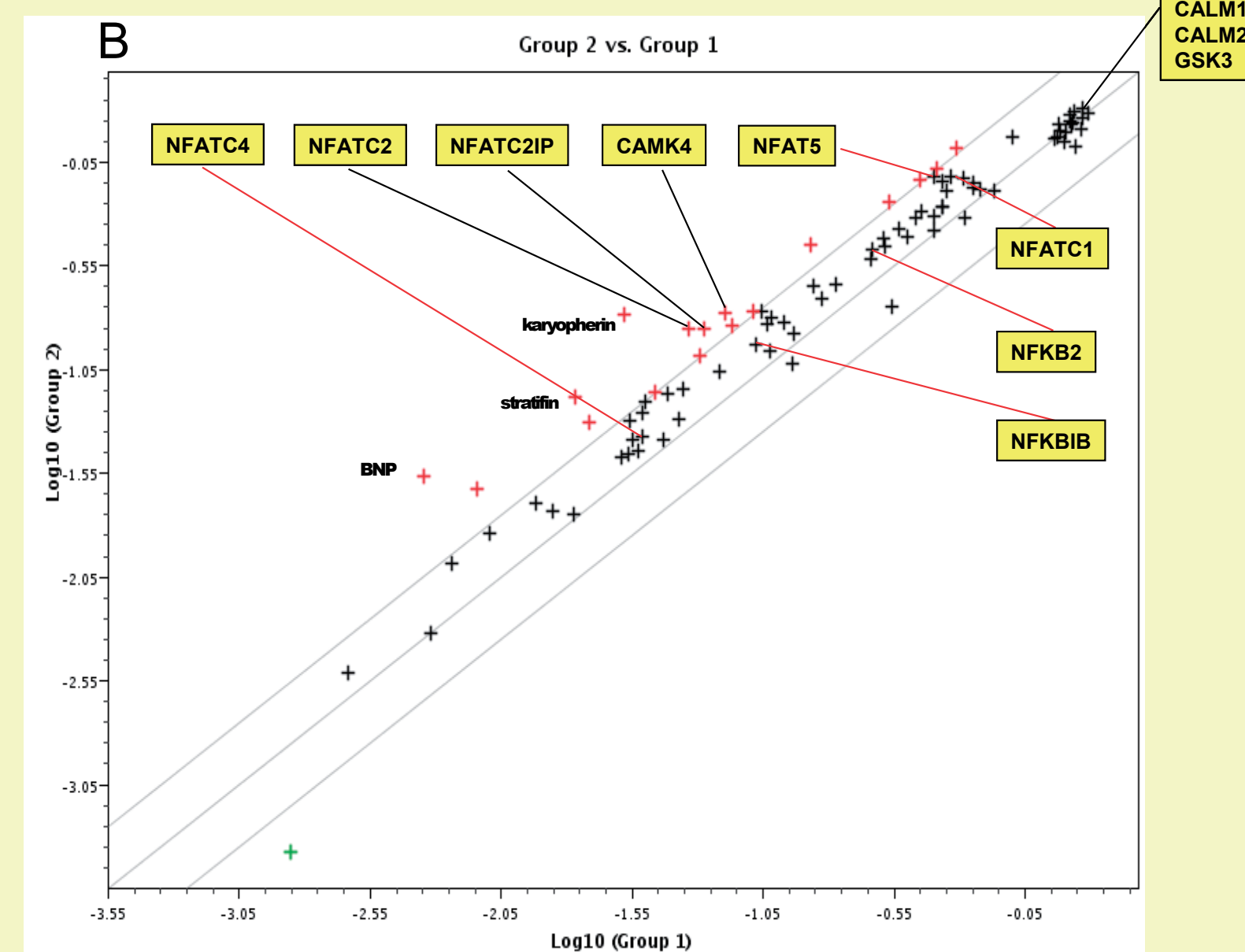
**Methods:** RNA was isolated from cells treated with an NMR therapy device (MBST®, MedTec, Wetzlar, Germany) for 20 h in 4 days. The RNA was labelled with biotin and hybridized to membranes carrying different gene markers. Bioluminescence was used to quantify the expression of specific genes. To corroborate results from gene arrays, the qPCR was used.

Free Ca<sup>2+</sup> concentration in living cells was determined fluorimetrically with cells loaded with fura 2 AM for 60 min. Ca<sup>2+</sup> release was triggered in osteosarcoma and chondrosarcoma cells by application of different concentrations of histamine. For investigation of the voltage activated Ca<sup>2+</sup> influx we used PC-12 cells, a cell line of neuronal origin. Depolarization of the cells was achieved by a solution containing a high potassium concentration.

## Quantification of Expression by Gene Arrays



**Fig. 2A:** Pseudo colour pictures of luminescent gene arrays. RNA of 8 experiments was pooled, labelled and hybridized to membranes carrying a specific gene probe. Red colour represents high levels of expression, blue is low expression. **Fig. 2B:** The luminescence values were normalized to different housekeeping genes and put into a scatter blot diagram. The 45° line represents genes with unchanged expression after NMR treatment. Red crosses show genes with enhanced expression (ratio > 1.5). **Table 1** summarizes the results of the gene array experiments.

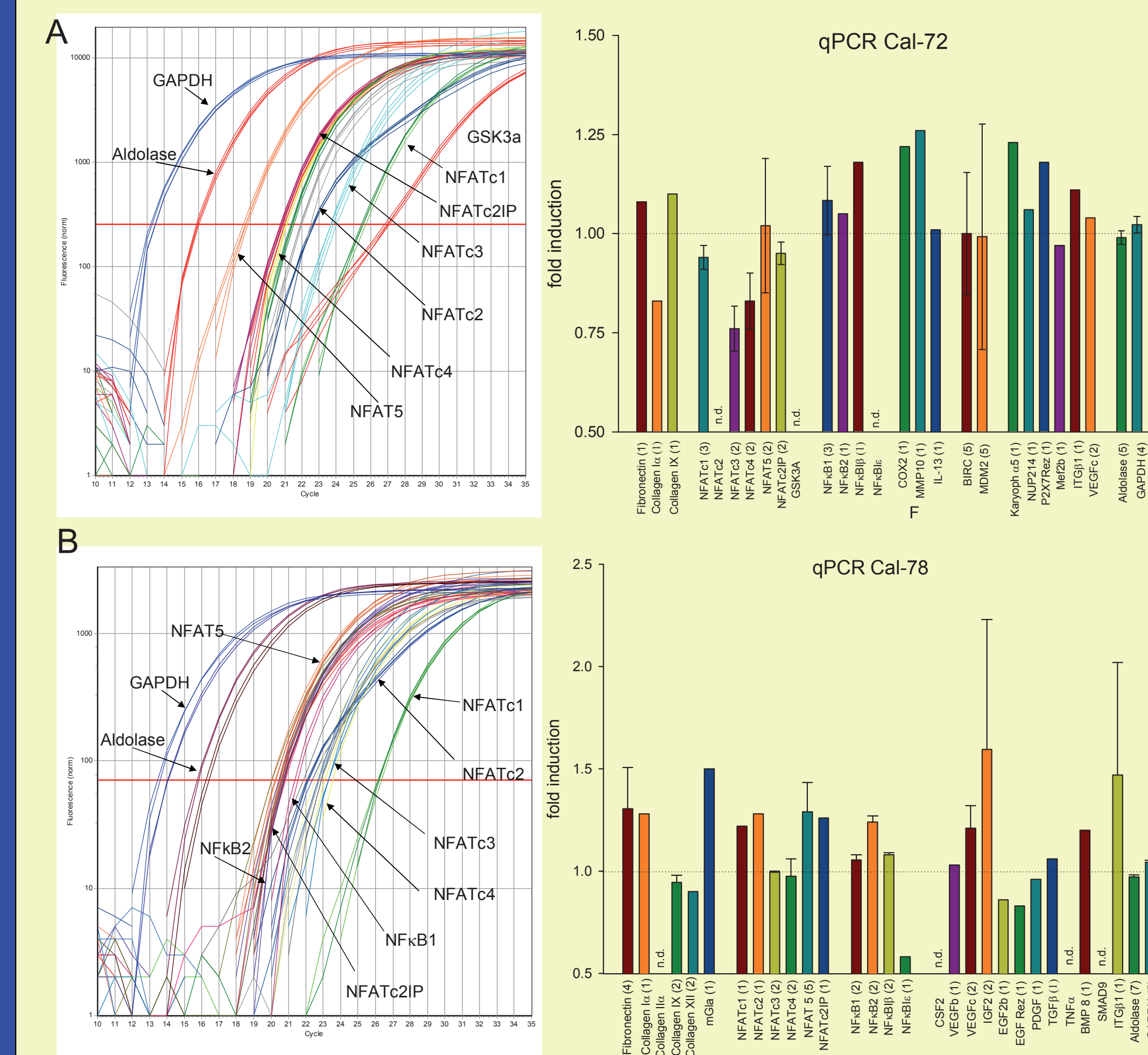


BNP: natriuretic peptide precursor B  
stratiffin: member of the 14-3-3 family of signal transduction molecules  
karyopherin: part of the nuclear pore  
NFAT: nuclear factor of activated T cells  
NFκB: nuclear factor of κ light polypeptide gene enhancer in B-cells  
NFκBIB: NFκB inhibitor, β  
Calm: Calmodulin  
GSK3: glycogen synthase kinase 3

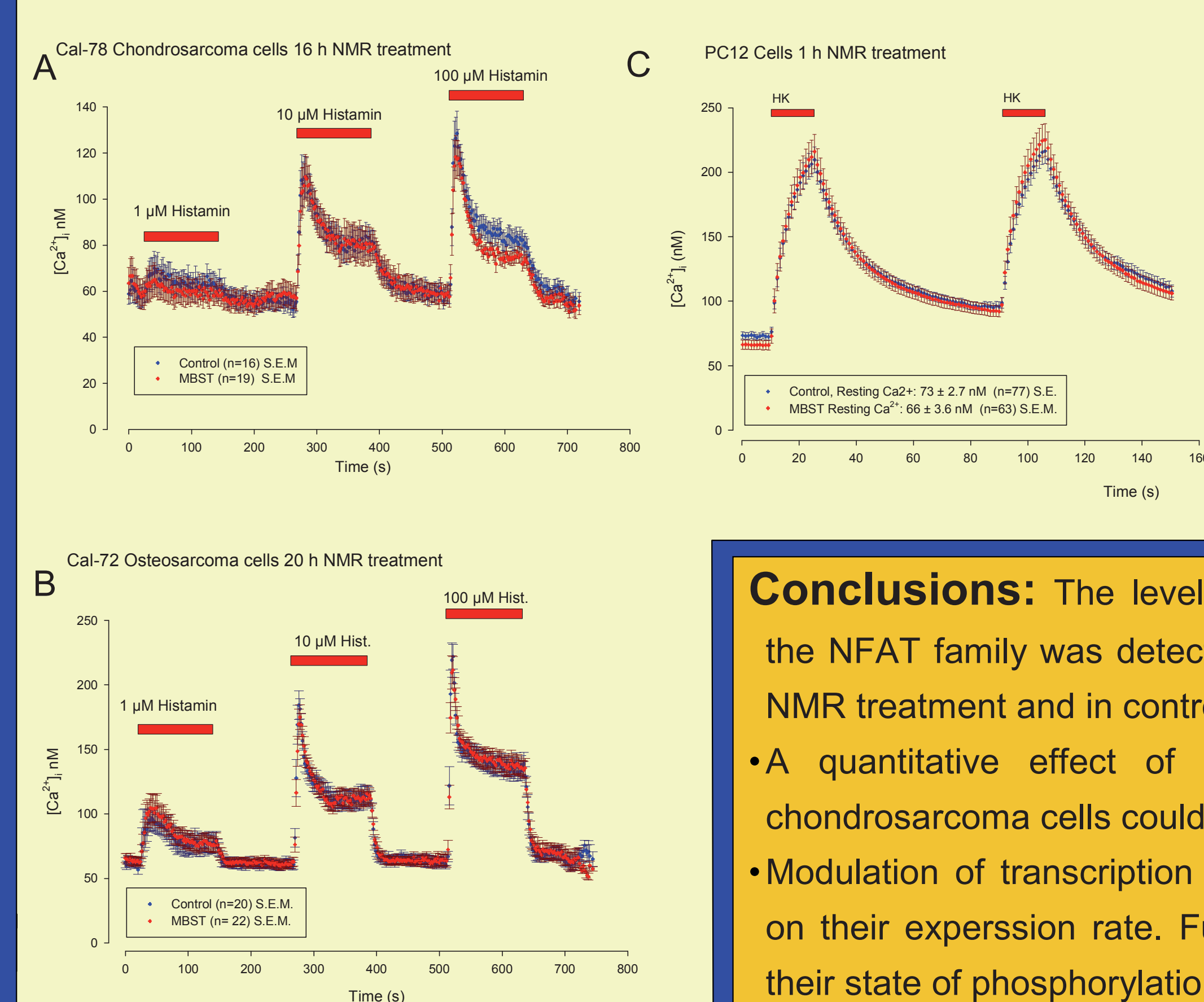
Table 1

Symbol	Description	luminescence, normalized		Group 2/Group 1
		nonstimulated avg.	stimulated avg.	
NFAT5	NFAT5	0.4055	0.7700	1.8988
NFATC1	NFATc1	0.5232	0.7544	1.4421
NFATC2	NFATc2	0.0468	0.1401	2.9914
NFATC3	NFATc3	1.2022	1.3621	1.1330
NFATC4	NFATc4	0.0310	0.0431	1.3883
NFATC2IP	NFAT2 interacting protein	0.0533	0.1408	2.6395
NFKB2	NFκB 2 (p49/p100)	0.2335	0.3431	1.4696
NFKBIB	NFκB inhibitor, beta	0.0845	0.1176	1.3918
NFKBIE	NFκB inhibitor, epsilon	0.1357	0.3577	2.6352
GSK3A	Glycogen synthase kinase 3 alpha	0.3559	0.7426	2.0866
GSK3B	Glycogen synthase kinase 3 beta	1.3324	1.4158	1.0626
CALM1	Calmodulin 1 (phosphorylase kinase, delta)	1.1601	1.1599	0.9998
CALM2	Calmodulin 2 (phosphorylase kinase, delta)	1.3947	1.0732	0.7695
CAMK4	CaM kinase IV	0.0642	0.1678	2.6124
SFN	Stratiffin	0.0172	0.0663	3.8590

## Quantification of Expression by Real Time PCR



**Fig. 3:** Quantitative PCR was performed with cDNA from isolated RNA of control and NMR treated cells. **Fig. 3A:** Cal-72 osteosarcoma cells, **Fig. 3 B:** Cal-78 chondrosarcoma cells. Left: Increase of fluorescence due to increasing concentrations of the amplicons. High amount of a specific cDNA in the sample is characterized by an early onset of fluorescence development. A two-fold difference in cDNA concentration results in a shift of one PCR cycle at a certain threshold value (CT value). For calculation of expression values, CT values of genes of interest were normalized to housekeeping genes (aldolase and GAPDH). Right: changes in expression levels due to NMR treatment.



**Fig. 4:** Time course of intracellular Ca<sup>2+</sup> concentration measured in: **A)** chondrosarcoma cells, **B)** osteosarcoma cells and **C)** in PC12 cells. NMR treatment was before the measurement. Ca<sup>2+</sup> release was triggered in Cal-72 and Cal-78 cells by application of histamine. PC12 cells were depolarized by elevation of extracellular K<sup>+</sup> ion concentration (HK) to elicit Ca<sup>2+</sup> influx through voltage dependent Ca<sup>2+</sup> channels.

**Conclusions:** The level of expression of transcription factors of the NFAT family was detected by microarrays or qPCR in cells after NMR treatment and in control cells.

- A quantitative effect of NMR treatment of osteosarcoma and chondrosarcoma cells could not be proven.
- Modulation of transcription via NFATs is not necessarily dependent on their expression rate. Functional modulation of NFATs is due to their state of phosphorylation.
- A functional modulation of NFATs by NMR has yet to be shown.