

Supplementary File for Figure 1

Supplementary Material to *in silico* screen: The *in silico* screen that lead to NFAT5a and alternative potentially lipid-modified transcription factors

Supplementary Table 1 and Supplementary Figure 1.1: Taxonomic distribution of NFAT5 isoforms and their sequence conservation in the N-terminal region of human NFAT5a

Supplementary Figure 1.2: Sequences of all five human NFAT5a mRNA transcripts in the regions used for qPCR

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The *in-silico* screen

Protein sequence hits from Hidden Markov Model (HMM) searches ¹ with protein domain models taken from PFAM ² and SMART ³ annotated as nucleic acid binding domains against the non-redundant protein sequence database were scored for the presence of an N-terminal myristoylation signal ^{4,5}, a prenylation site ^{6,7} or the GPI lipid anchor attachment motif ⁸. The resulting list of a few thousand hits was to be further

reduced with the goal to come up with singular targets for an experimental follow up. By excluding entries with doubtful sequence termini, we wished to exclude cases of technically correct prediction but possibly non-existing proteins. Considerations of accessibility of cDNA and cell lines restricted the search to protein entries from well-studied model organisms. The requirement for conservation of the predicted lipid anchor sites in other species enhances the reliability of prediction and the possibility to find biological results of more general value.

Finally, the restriction to experimentally verified transcription factor (TF) domains ensures that the candidate under investigation has truly the potential to act as TF. NFAT5a was finally selected as candidate protein for studying the feasibility of nuclear import of lipid-modified TFs because there is experimental evidence for the existence of this isoform⁹⁻¹². Equally important, knowing NFAT5 as the osmotic stress response TF provided us initial hope that testing isoform a for nuclear import upon salt stress is an accessible functional assay^{13,14}.

About other TFs with predicted lipid anchors

The most interesting question for general biology is whether lipid-modified TFs that are imported into the nucleus in a regulated manner are a more general phenomenon and whether reversible palmitoylation as a mobilization mechanism might be found in contexts other than NFAT5a.

It appears impossible to get an exact overview with regard to lipid modified TFs from a renewed *in silico* screen. Despite more than a decade of availability of “complete genomes”, new releases of genome assemblies remain accompanied with addition/losses of thousands of proteins, not to speak about isoforms. Nevertheless, several more years of sequencing have improved the data situation since 2004 when we first aggressively unselected hits to come up with the single, hopefully easy-to-test example NFAT5a. Frequently, termini of proteins are described more reliably. The likelihood to find homologues in genomes of alternative species has increased and this improves chances to test the conservation of posttranslational modification sites. Therefore, cases that we *a priori* omitted previously might become worthwhile to be investigated now. For example, the human protein Q8WYA1 (named MOP9, CLIF or BMAL2), a transcriptional regulator with functions in circadian and hypoxia pathways ¹⁵, was considered as a potential candidate alongside with NFAT5a. Only two of its isoforms were known in 2004 but eight isoforms are described in the database today. Isoforms 6, 7 and 8 are predicted targets of myristoylation; yet, they do not have an obvious acylation site.

The following five examples all have at least one isoform reported to have an N-terminal glycine after the leading methionine as well as sequentially close cysteines. The proteins are predicted targets for myristoylation at the N-terminal glycines (with NMT/MyrPS ⁴) and the cysteines in their vicinity are likely palmitoylation sites ¹⁶. The sequence features are conserved in neighbouring species. (1) The human protein BTBD7 (Q9P203) and (2) the protein BAC20790 from *Oryza sativa* are predicted to possess a BTB/POZ domain (PFAM PF00651) which is known to occur in TFs ¹⁷. Similarly, (3) the CAD60697

(*Podospora anserine*) is a predicted classical zinc finger protein. (4) The human proteins LZTS1/FEZ1 (Q9Y250, isoform 1) and (5) LZTS2/LAPSER1 (isoform AAK31577) are annotated as leucine zipper putative tumor suppressor 1 and 2, respectively. They harbour a Fez1 domain (PFAM PF06818) which contains a leucine-zipper region with similarity to the DNA-binding domain of the cAMP-responsive activating-transcription factor 5¹⁸. There is evidence that Fez1 regulates mitosis and inhibits cancer cell growth¹⁹. As the accuracy of the protein sequences in the proteomes, especially of the isoforms, improves in the future, more protein examples might pass the selection criteria.

Supplementary Table 1: Taxonomic distribution of NFAT5 isoforms and their sequence conservation in the N-terminal region of human NFAT5a

This part contains Supplementary Table 1 (a list of sequence database entries with orthologues of NFAT5 isoforms) and Supplementary Figure 1.1 (an alignment figure of NFAT5 protein isoform sequence segments).

The Supplementary Table 1 lists the entries in non-redundant protein database or UniProt that carry sequences homologous to NFAT5. Generally, protein isoforms are not well studied and it is not surprising that explicit NFAT5a entries are available only for a few species at the time of writing (July 2011); yet, their number has grown since 2004 when the authors have first done this survey.

Supplementary Table 1

Species	NFAT5: isoform a	NFAT5: other isoforms
<i>Homo sapiens</i> (human)	NP_619728.2	AAD48441.1 (isoform b) O94916 (isoform c) NP_001106649.1 (isoform d) NP_619727.2 BAA74850.2 [#]
<i>Pan troglodytes</i> (chimpanzee)		XP_001168930.1
<i>Macaca mulatta</i> (rhesus monkey)		XP_001093880.2
<i>Callithrix jacchus</i> (white-tufted-ear marmoset)		XP_002807800.1
<i>Equus caballus</i> (horse)		XP_001497345.2
<i>Ailuropoda melanoleuca</i> (giant panda)		XP_002923698.1
<i>Canis familiaris</i> (dog)		XP_546854.2
<i>Bos taurus</i> (cattle)		XP_002694885.1
<i>Oryctolagus cuniculus</i> (rabbit)		XP_002711712.1
<i>Mus musculus</i> (house mouse)	NP_598718.2	NP_061293.2 AAF31405.1 Q9WV30.1
<i>Rattus norvegicus</i> (Norway rat)	EDL92475.1	NP_001100895.1 EDL92477.1
<i>Monodelphis domestica</i> (gray short-tailed opossum)		XP_001378219.1
<i>Ornithorhynchus anatinus</i> (platypus)		XP_001510046.1
<i>Gallus gallus</i> (red jungle fowl)	BAG70407.2	XP_414226.2
<i>Xenopus (Silurana)</i> <i>tropicalis</i> (western clawed frog)		XP_002937623.1

[#] This sequence is annotated as KIAA0827 protein having a length of 1608 AAs. It is an N-terminally prolonged isoform c-type sequence that does not start with methionine. The 77 additional AAs are rich in P (27.9%), R (19.7%), and S (19.7%). Possibly, this is just a hypothetical translation with no real protein equivalent.

Supplementary Figure 1.1

The alignment figure below (created with CLUSTALX; ²⁰⁻²²) shows that the protein sequence region in the environment of the human NFAT5a N-terminus is strongly conserved over a wide range of species. The species is encoded with a two-letter abbreviation in accordance with Supplementary Table 1. The N-terminal positions Gly2 and Cys5 are remarkably preserved throughout species.

Supplementary Figure 1.2: Sequences of all five human NFAT5a mRNA transcripts in the regions used for qPCR

The alignment figure below (created with CLUSTALX; ²⁰⁻²²) shows the mRNA sequences of all five human transcripts in the region of the segments 2|B, -|X and 4|D used for quantitative RT-PCR.

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isoform_c|CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC|1684
isoform_d|DDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDD|1738
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