

# Supplementary Material

## Ligand induced activation of human TRPM2 requires the terminal ribose of ADPR and involves Arg 1433 and Tyr 1349

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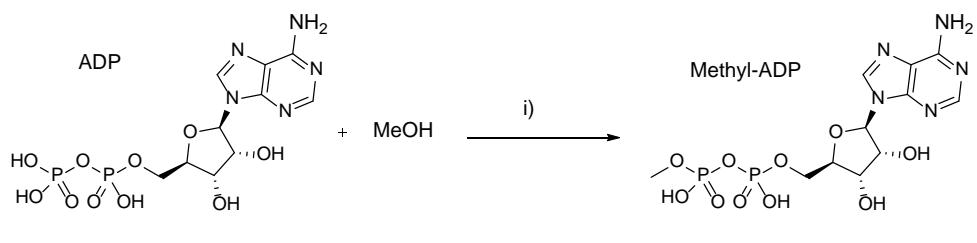
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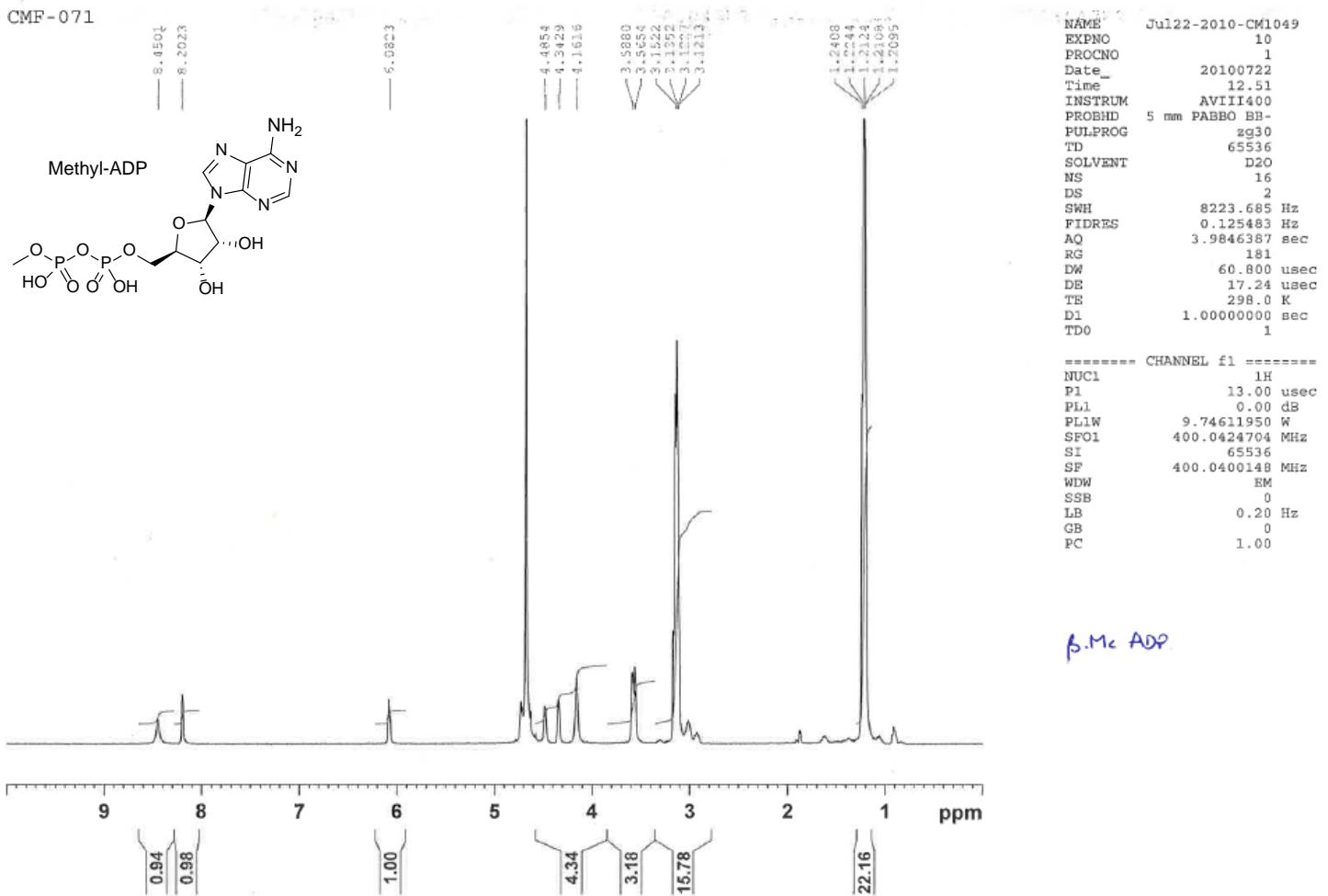
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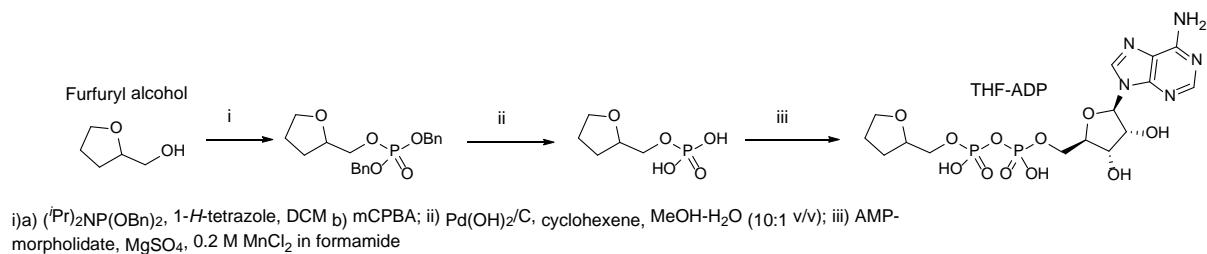
**Supplementary Figure S1.** Synthesis of methyl-ADP and  $^1\text{H}$ -NMR data.



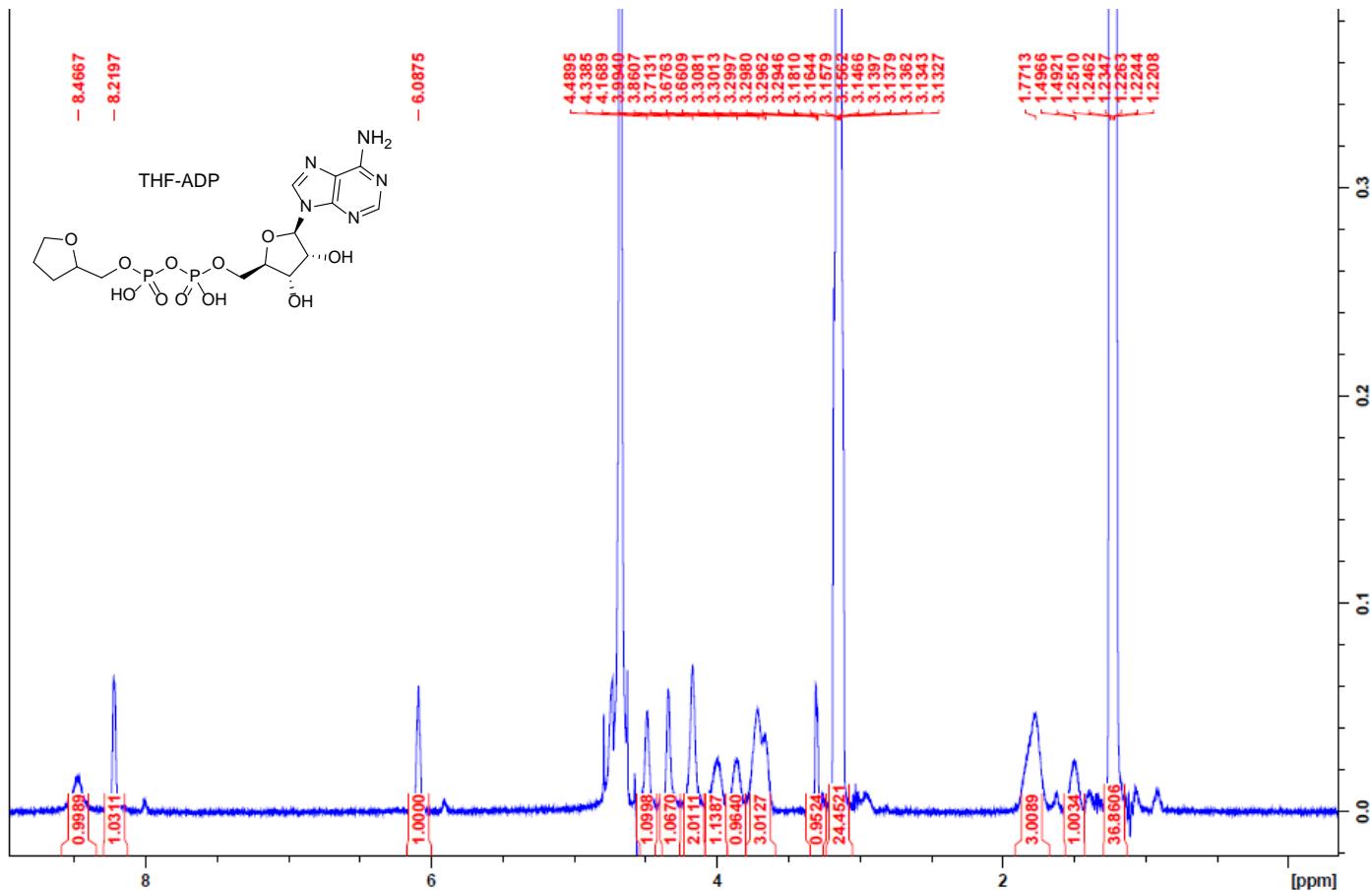
i) *N,N'*-Dicyclohexylcarbodiimide (DCC),



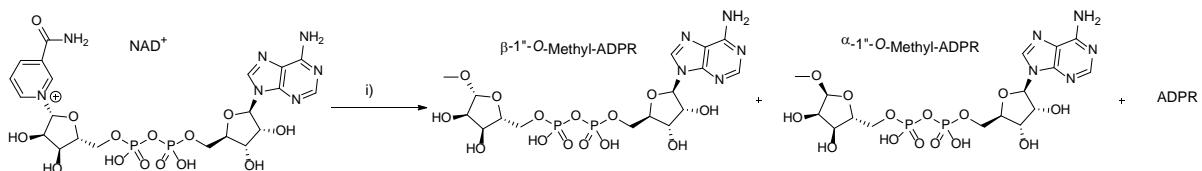
**Supplementary Figure S2.** Synthesis of THF-ADP and  $^1\text{H}$ -NMR data.



i)a)  $(^{\text{Pr}})_2\text{NP(OBn)}_2$ , 1-*H*-tetrazole, DCM b) mCPBA; ii)  $\text{Pd}(\text{OH})_2/\text{C}$ , cyclohexene,  $\text{MeOH-H}_2\text{O}$  (10:1 v/v); iii) AMP-morpholidate,  $\text{MgSO}_4$ , 0.2 M  $\text{MnCl}_2$  in formamide



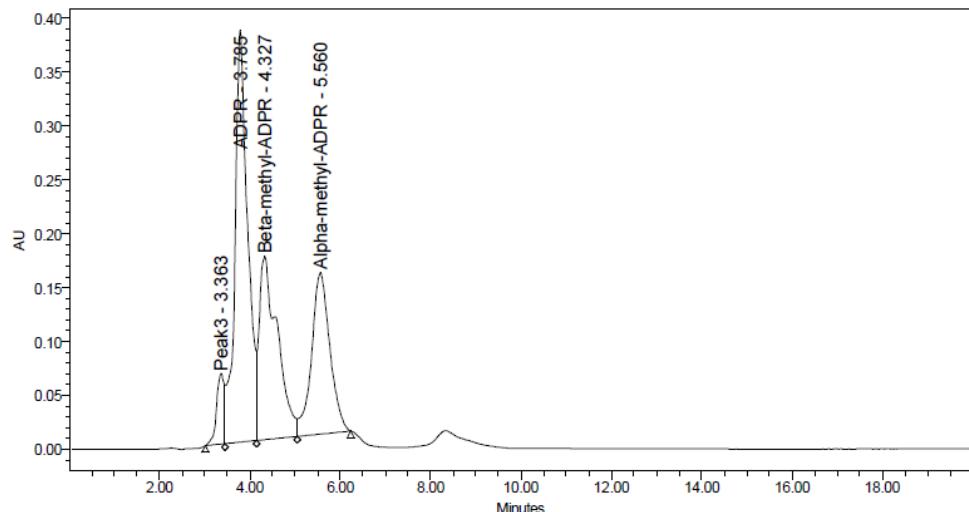
**Supplementary Figure S3.** Synthesis and separation of  $\alpha$ -1''-O-methyl-ADPR and  $\beta$ -1''-O-methyl-ADPR and chromatographic data.



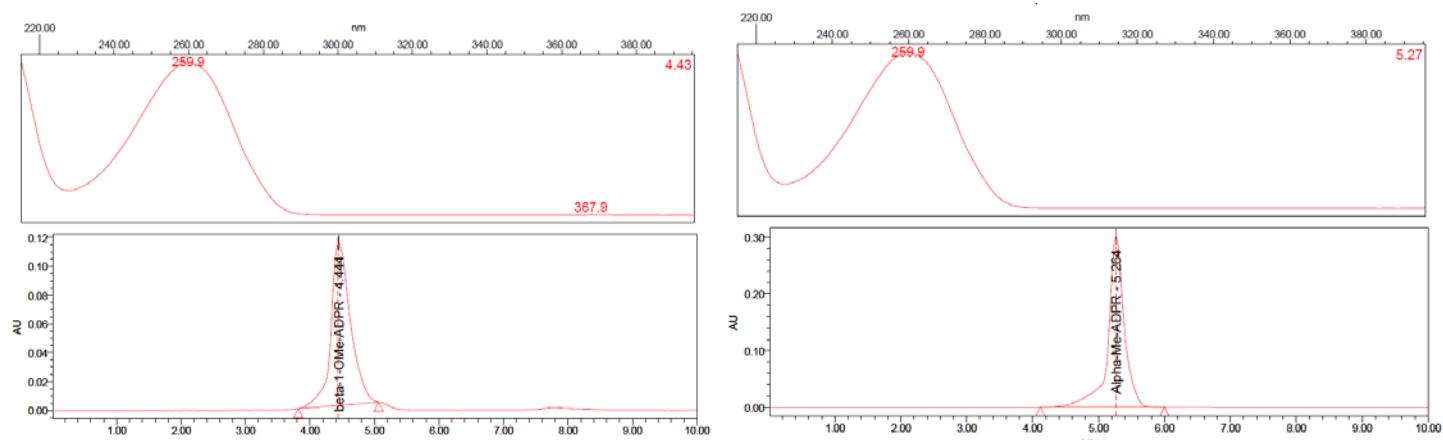
i) MeOH-Na<sub>2</sub>HPO<sub>4</sub> (0.05 M, aq.) (1:2 v/v, 3mL), 60 °C

HPLC of crude mixture containing  $\alpha$ -1''-O-methyl-ADPR,  $\beta$ -1''-O-methyl-ADPR and ADPR.

SAMPLE INFORMATION	
Sample Name:	JMS 1077 NMR
Sample Type:	Unknown
Vial:	10
Injection #:	1
Injection Volume:	10.00 $\mu$ l
Run Time:	20.0 Minutes
Sample Set Name:	JMS 1077 isocratic
Acquired By:	Joanna
Date Acquired:	10/02/2015 1:25:55 PM
Acq. Method Set:	RP18 LC
Date Processed:	07/09/2016 2:51:41 PM
Processing Method:	Methyl Tiazo Riboside Phosphat
Channel Name:	WvinCh1
Proc. Chnl. Descr.:	PDA 259.1 nm



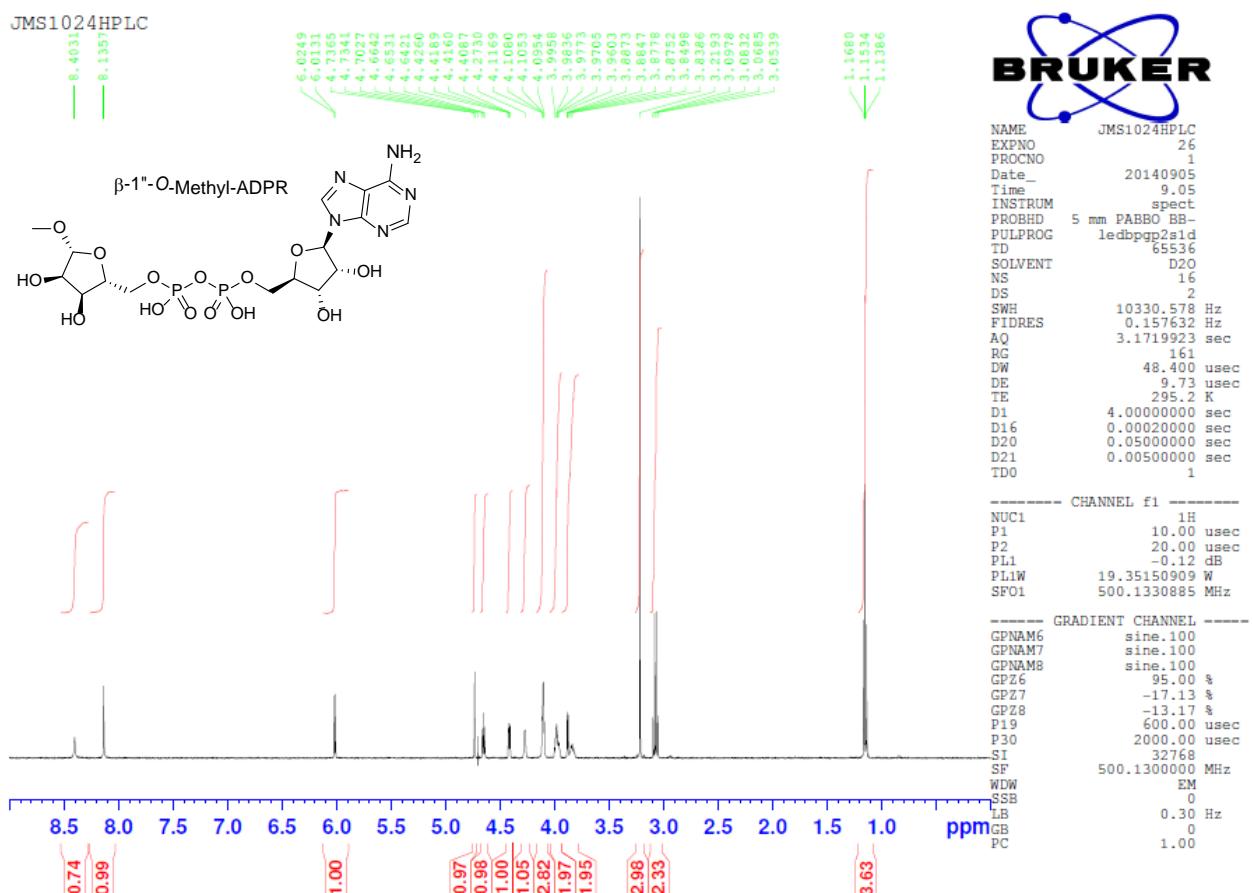
Separation of the desired products after semi-preparative HPLC:

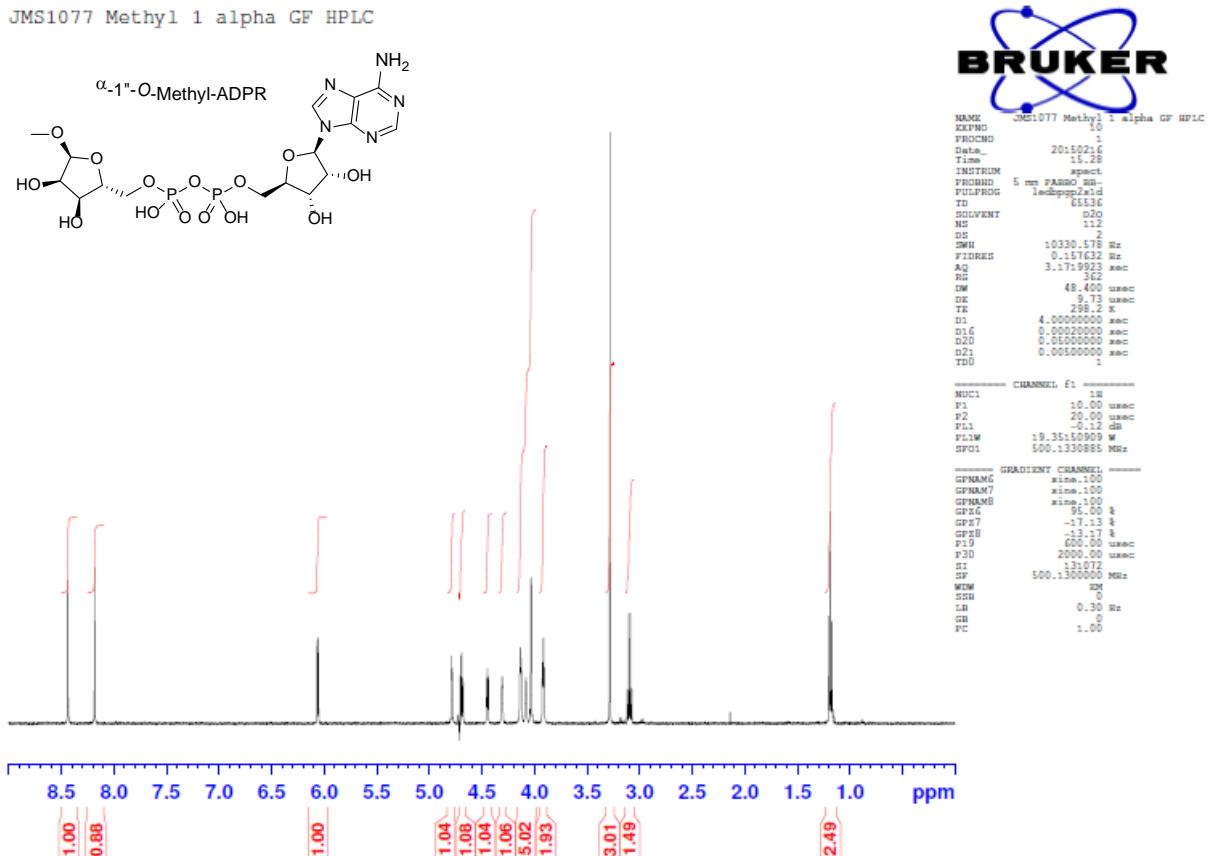


PDA Result Table									
Name	RT	Area	Purity (#1) Angle	Purity (#1) Threshold	Purity Flag	Match (#1) Spect. Name	Match (#1) Angle	Match (#1) Threshold	PDA Match Flag
1 beta-1'-O-Me-ADPR	4.444	2381448			No				No
2 Alpha-Me-ADPR	5.294				No				No

Name	RT	Area	Purity (#1) Angle	Purity (#1) Threshold	Purity Flag	Match (#1) Spect. Name	Match (#1) Angle	Match (#1) Threshold	PDA Match Flag
1 Alpha-Me-ADPR	5.294	5188645			No				No

**Supplementary Figure S4.**  $^1\text{H}$ -NMR data for  $\alpha$ -1''-O-methyl-ADPR and  $\beta$ -1''-O-methyl-ADPR.





**Supplementary Table S1.** Possible templates for modelling ADPR into the TRPM2 Nudix domain binding site.

PDB Structure	Species	Protein	Reference
1G9Q	<i>Escherichia coli</i>	ADPRase	[1]
1MK1	<i>Mycobacterium tuberculosis</i>	ADPRase	[2]
1V8L	<i>Thermus thermophilus</i>	ADPRase	[3]
2QJO	<i>Synechocystis</i> sp. PCC6803	NMN adenyltransferase/ADPRase	[4]
3GZ8	<i>Shewanella oneidensis</i>	Transcriptional Regulator	[5]

#### References

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- 2 Kang, L.-W., Gabelli, S. B., Cunningham, J. E., O'Handley, S. F. and Amzel, L. M. (2003) Structure and mechanism of MT-ADPRase, a nudix hydrolase from *Mycobacterium tuberculosis*. *Structure* **11**, 1015-23.
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- 4 Huang, N., Sorci, L., Zhang, X., Brautigam, C. a., Li, X., Raffaelli, N., Magni, G., Grishin, N. V., Osterman, A. L. and Zhang, H. (2008) Bifunctional NMN Adenylyltransferase/ADP-Ribose Pyrophosphatase: Structure and Function in Bacterial NAD Metabolism. *Structure* **16**, 196- 209.
- 5 Huang, N., De Ingeniis, J., Galeazzi, L., Mancini, C., Korostelev, Y. D., Rakhmaninova, A. B., Gelfand, M. S., Rodionov, D. a, Raffaelli, N. and Zhang, H. (2009) Structure and function of an ADP-ribose-dependent transcriptional regulator of NAD metabolism. *Structure*, Elsevier Ltd **17**, 939-51.

**Supplementary Table S2.** Primers used for QuikChange mutagenesis and sequencing of the coding sequence for TRPM2

**Mutagenesis primers**

T1347V for	TTCGGACCAACCACGTGCTGTACCCATGGTC
T1347V rev	GACCATGGGTACAGCACGTGGTGGTCCGAA
Y1349F for	CCCAACCACACGCTGTTCCCCATGGTCACGCGG
Y1349F rev	CCGCGTGACCATGGGAACAGCGTGTGGTGGG
L1381I for	GTGGTGAAGCTCCCTATCTCGAGCACTGGGCC
L1381I rev	GGCCCAGTGCTCGGAGATAAGGAGCTTCACCAC
R1433M for	TACATGGATGACCCGATGAACACGGACAATGCC
R1433M rev	GGCATTGTCCGTGTTCATGGGTACATCCATGTA
Y1485F for	CGCATCCCACTCTCGCGAACCAAGACC
Y1485F rev	GGTCTTGTGGTTCGCGAAGAGTGGGATGCG

**Sequencing primers**

CMVfor	CGCAAATGGCGGTAGCGTG
TRPM2.5	GCTCATCACCATGGAGTCGC
TRPM2.7	CACCTTGCTCTACCTGTACGA
TRPM2.9	CTCTGCCTGTTCGCCTACGTG
TRPM2.11	CTGCAGCTCTCATCAAGAGG
TRPM2.13	CAGGCCGGGTTGCCCTGAAC
TRPM2.15	GGGAGACACCCTGGAGCCAC