# SUPPLEMENTAL MATERIALS

## Regulation of Poly(ADP-ribose) Polymerase-1-Dependent Gene Expression Through Promoter-Directed Recruitment of a Nuclear $NAD^+$ Synthase

**by Zhang *et al*., 2012**

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## 1) Supplemental Experimental Procedure

The following experimental procedures are for those assays which were used exclusively in the experiments shown in the supplemental data.

***ChIP-chip* -** LM-PCR-amplified ChIP samples were used to probe a NimbleGen high density human (HG18) promoter microarray. The array contains an isothermal probe set spanning the promoter regions (at least -3 kb to +3 kb) for all RefSeq genes. In addition, the arrays tile through all annotated CpG islands, all annotated miRNA promoters, and manually selected ENCODE regions. Multiple independent biological samples were used for array hybridization for both PARP-1 (3 replicates) and NMNAT-1 (2 replicates). Composite files derived from all replicates were used for data analysis. The raw data sets can be accessed from the NCBI GEO website (http://www.ncbi.nlm.nih.gov/projects/geo/) using accession number GSE35358.

ChIP-chip data analysis was performed as described previously (3). All data processing scripts in the statistical programming language R are available upon request. For each factor of interest, the log2 ratio data from each replicate was grouped based on probe length (50- to 75-mer) and subjected to lowess normalization within each group (4). The normalized data was then scaled based on the sum of squares of each replicate. The average values of the normalized and scaled log2 ratio data from all replicates were used to generate a composite file. The composite data were processed using a 1 kb moving window with 250 bp steps. The mean probe log2 ratio and p-value from Wilcoxon signed-rank test were calculated for each window. Significant peaks were defined as the center of three consecutive windows with positive means, the center window with a mean greater than either adjacent window, and all windows having p-values less than 0.016.

## 2) Supplemental Tables

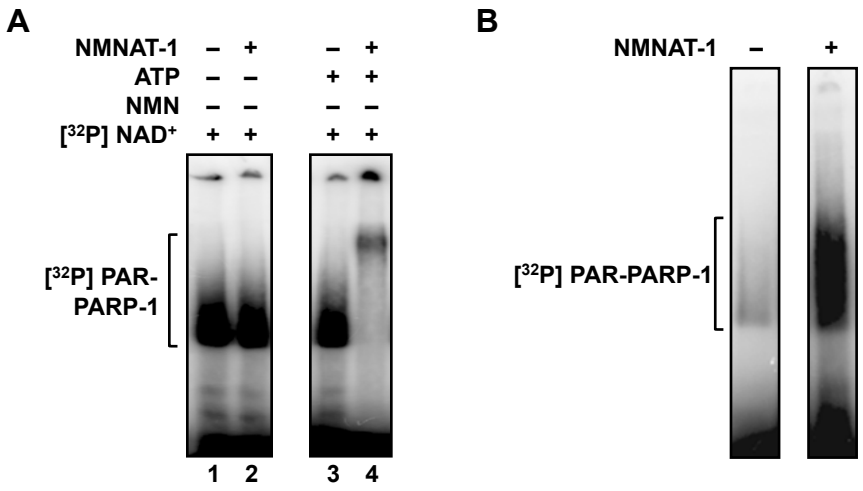
**Supplemental Table S1. Primers used for RT-qPCR.**

| | |
|---|---|
| ATXN10 forward | 5'- aagcacctttgtggatcag -3' |
| ATXN10 reverse | 5'- acagtcatttcgcacagg -3' |
| SOCS2 forward | 5'- aaggctgaagtcgcgtttta -3' |
| SOCS2 reverse | 5'- agcttggttccttcccactt -3' |
| PEG10 forward | 5'- caagccaccaccaggtagat -3' |
| PEG10 reverse | 5'- gaggcacaggttcagctttc -3' |
| TMSNB forward | 5'- tcccaacagcagatttcgac -3' |
| TMSNB reverse | 5'- gccagggaacataggtgaga -3' |
| NELL2 forward | 5'- cgggctatacagggaatg -3' |
| NELL2 reverse | 5'- ttcatcaatgtccgtttcac -3' |

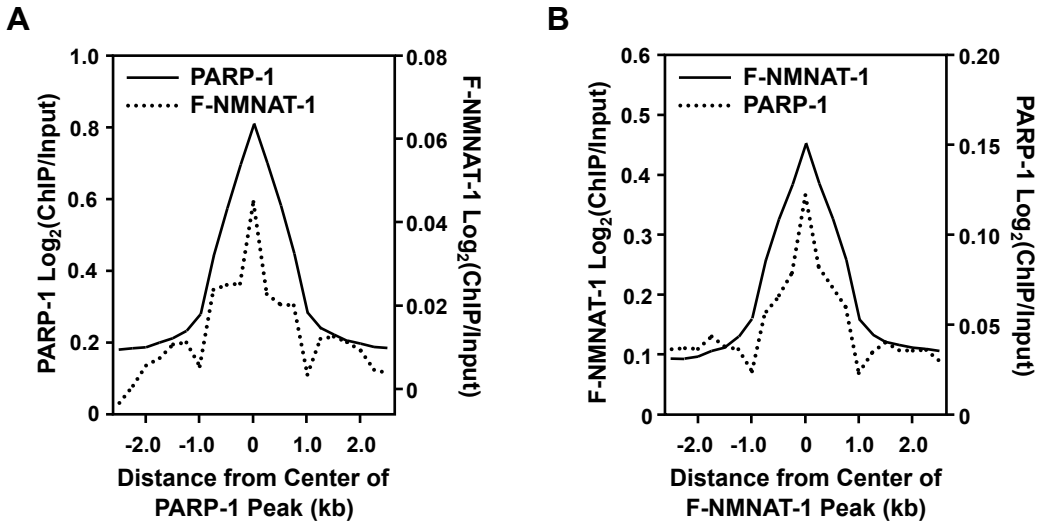
**Supplemental Table S2. Primers used for ChIP-qPCR.**

| | |
|---|---|
| ATXN10 upstream forward | 5'- tctttgctggcctttgactt -3' |
| ATXN10 upstream reverse | 5'- gtgaccccaggactctcaaa -3' |
| ATXN10 promoter forward | 5'- gacgcgcccctctttctc -3' |
| ATXN10 promoter reverse | 5'- tgtgttgatgtggccgtaaca -3' |
| SOCS2 upstream forward | 5'- caccgagagtaaaaggatgaca -3' |
| SOCS2 upstream reverse | 5'- tgatcatggtccctaccttaca -3' |
| SOCS2 promoter forward | 5'- cttcctcgcccaacttcc -3' |
| SOCS2 promoter reverse | 5'- tcactgctcgaaagcttgaa -3' |
| PEG10 upstream forward | 5'- tcttgaacatgtttacatgatagcac -3' |
| PEG10 upstream reverse | 5'- cacatagcagaaaatgatgtagcc -3' |
| PEG10 promoter forward | 5'- gagcacgctgggatttgg -3' |
| PEG10 promoter reverse | 5'- tgtaccaggagcgcagactgt -3' |
| TMSNB upstream forward | 5'- caaatccgttgctggtcttt -3' |
| TMSNB upstream reverse | 5'- aggtgcatgcagatcagaaat -3' |
| TMSNB promoter forward | 5'- tgagggtccacataccttc -3' |
| TMSNB promoter reverse | 5'- ataccggtcattcagcttgg -3' |
| NELL2 upstream forward | 5'- agactggcacccagaagaga -3' |
| NELL2 upstream reverse | 5'- gctagggattggagtgtgga -3' |
| NELL2 promoter forward | 5'- tccccggaggagcagtct -3' |
| NELL2 promoter reverse | 5'- cgcccgaacctgttgtaaag -3' |
| RAPGEF4 upstream forward | 5'- cctaagccagttgacccaga -3' |
| RAPGEF4 upstream reverse | 5'- aaatgaagccgctaggaaca -3' |
| RAPGEF4 promoter forward | 5'- gtaactcccgacgacagctc -3' |
| RAPGEF4 promoter reverse | 5'- ctgtcacagcctggaaacaa -3' |

**3) Supplemental Figures**




**Supplemental Figure S1. NMNAT-1 stimulates PAR synthesis by PARP-1 *in vitro*.**
**(A)** PARP-1 auto(ADP-ribosyl)ation assays were carried out using $^{32}$P-NAD$^{+}$ (0.67 μM) as the source of radioactive label. The reactions were carried out in the presence or absence of ATP (200 μM), but no NMN. All reactions included sheared salmon sperm DNA as an activator of PARP-1 enzymatic activity. Automodified PARP-1 was analyzed by SDS-PAGE and autoradiography.
**(B)** PARP-1 automodification reactions were carried out using $^{32}$P-NAD$^{+}$ (0.67 μM) and α-$^{32}$P-ATP (20 μM) at the same specific activity. The amount of automodified PARP-1 in the presence or absence of NMNAT-1 was analyzed by SDS-PAGE and autoradiography.

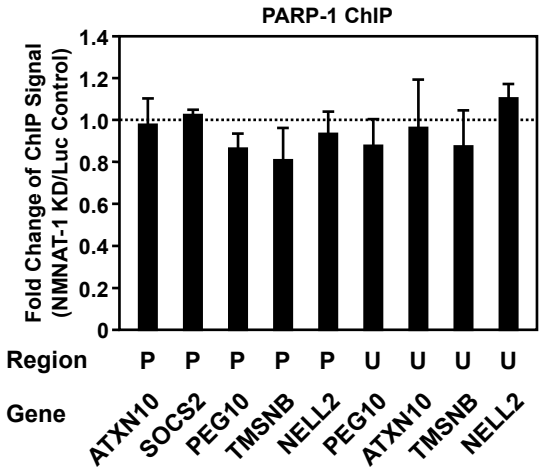



**Supplemental Figure S2. Co-enrichment of PARP-1 and NMNAT-1 across the genome in MCF-7 cells.**

ChIP-chip analyses of NMNAT-1 (FLAG) and PARP-1 in MCF-7 cells ectopically expressing FLAG-NMNAT-1 were carried out using NimbleGen promoter arrays.

**(A)** Averaging analysis of the $\log_2$ enrichment ratios for PARP-1 and F-NMNAT-1 centered on significant peaks of PARP-1. The region from -2.5 kb to +2.5 kb relative to the the center if the PARP-1 peak is shown.

**(B)** The same averaging analysis as in panel A centered around significant peaks of F-NMNAT-1.




**Supplemental Figure S3. NMNAT-1 knockdown does not affect PARP-1 association with chromatin.**

ChIP-qPCR analysis of PARP-1 at the promoter (P) and an upstream region (U, ~ 10 kb from TSS) of target genes. Results are presented as the ratio of PARP-1 ChIP signal in NMNAT-1 knockdown cells over that in luciferase (Luc) control cells. Each bar represents the mean + S.E.M. for three or more independent biological replicates.

## 4) Supplemental References

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2. Yang, T., and Sauve, A. A. (2006) *AAPS J* **8**, E632-643
3. Krishnakumar, R., Gamble, M. J., Frizzell, K. M., Berrocal, J. G., Kininis, M., and Kraus, W. L. (2008) *Science* **319**, 819-821
4. Yang, Y. H., Dudoit, S., Luu, P., Lin, D. M., Peng, V., Ngai, J., and Speed, T. P. (2002) *Nucleic Acids Res* **30**, e15