SUPPLEMENTARY FILE

# Unveiling the Nexus: Cellular Metabolomics Unravels the Impact of Estrogen on Nicotinamide Metabolism in Mitigating Rheumatoid Arthritis Pathogenesis

**Swati Malik** [1,2], **Debolina Chakraborty** [1,2], **Prachi Agnihotri** [1,2], **Vijay Kumar** [3]
**and Sagarika Biswas** [1,2,*]

[1]  Department of Integrative and Functional Biology, CSIR- Institute of Genomics and
     Integrative Biology, Mall Road, Delhi 110007, India; swati.malik@igib.res.in (S.M.);
     debolina@igib.res.in (D.C.); prachi.igib22a@acsir.res.in (P.A.)

[2]  AcSIR- Academy of Scientific and Innovative Research, Ghaziabad 201002, India

[3]  Department of Orthopaedics, AIIMS- All India Institute of Medical Sciences,
     Ansari Nagar, New Delhi 110029, India; vkgene@aiims.edu

[*]  Correspondence: sagarika.biswas@igib.res.in or sagarika.zollu@gmail.com;
     Tel.: +91-11-27662581

**Table S1**. The clinical demography characteristics of patients with RA

| S. No. | Patient's characteristics | RA patient (n=6) |
|---|---|---|
| 1 | Age (yrs.) | 55± 10 |
| 2 | Sex (Female, Male) | F |
| 3 | ESR (mm/hr) | 35 ± 5 |
| 4 | RF (+ve/-ve) | +ve |
| 5 | CRP (mg/L) | 80±15 |
| 6 | Tender Joint | 20±6 |
| 7 | Swollen joints | 10±4 |
| 8 | DAS-28 score | 6 ± 0.5 |
| 9 | Disease duration yrs. | 15 ± 5 |
| 10 | Medication (Yes/No) | Yes |

*RA: Rheumatoid arthritis; ESR: Erythrocyte sedimentation rate; RF: Rheumatoid factor; CRP: C-reactive protein; DAS-28: Disease Activity Score-28. The values are expressed as Mean ±SD

**Table S2:** Primer Name and Sequence

| S.no. | Primer | Forward | Reverse |
|---|---|---|---|
| 1. | STAT1 | 5' TGACTTCCATGCGGTTGAAC 3' | 5' TCCTTTGGCCTGGAGTAATACT 3' |
| 2. | MMP3 | 5' CACTGGGATAGGAGGGGATG 3' | 5' TCTGTGGGTGCCATTTCTGT 3' |
| 3. | MMP9 | 5' TGCGTGGAGAGTCGAAATCT 3' | 5' GGTGATGTTGTGGTGGTGC |
| 4. | MAPK14 | 5' CCCGAGCGTTACCAGAACC 3' | 5' TCGCATGAATGATGGACTGAAT 3' |

**Table S3:** The significant metabolic pathways of 109 annotated metabolites from KEGG pathway library analysed by MetaboAnalyst software

| S.no. | Pathway Name | p-value |
|---|---|---|
| 1. | Phenylalanine, tyrosine and tryptophan biosynthesis | 0.001 |
| 2. | Valine, leucine and isoleucine biosynthesis | 0.00452 |
| 3. | Phenylalanine metabolism | 0.00452 |
| 4. | Glutathione metabolism | 0.0054 |
| 5. | Arginine biosynthesis | 0.014 |
| 6. | Nicotinate and nicotinamide metabolism | 0.01602 |
| 7. | Pantothenate and CoA biosynthesis | 0.02785 |

**Table S4:** The 8 significant metabolites based on the p-value detailing the m/z value, Fold change and HDMB ID

| S.no. | m/z | HMDB ID | Metabolite Name | Fold change | p-value |
|---|---|---|---|---|---|
| 1. | 512.3068 | HMDB0010379 | LPC 14:0 | 0.689485 | 0.000623 |
| 2. | 137.0705 | HMDB0000699 | 1-Methylnicotinamide | 1.627562 | 0.00547 |
| 3. | 169.0633 | HMDB0001112 | Glyceraldehyde, 3-(dihydrogen phosphate) | 1.678601 | 0.00728 |
| 4. | 277.0928 | HMDB0030791 | Dihydromethysticin | 1.419794 | 0.00763 |
| 5. | 381.2486 | HMDB0253945 | Lactobionic acid | 1.970484 | 0.011718 |
| 6. | 664.1139 | HMDB0000902 | beta-Nicotinamide adenine dinucleotide | 1.188219 | 0.021411 |
| 7. | 305.9473 | HMDB0029093 | Trp-Thr | 1.67495 | 0.031722 |
| 8. | 248.0668 | HMDB0032983 | 1-hydroxy-10-methylacridone | 1.736537 | 0.049748 |

**Table S5:** The significant metabolic pathways of 8 significant metabolites from KEGG pathway library analysed by MetaboAnalyst software

| S.no. | Pathway Name | p-Value |
|---|---|---|
| 1. | Nicotinate and nicotinamide metabolism | 2.53E-04 |
| 2. | Fructose and mannose metabolism | 0.037637 |
| 3. | Pentose phosphate pathway | 0.0432 |
| 4. | Glycolysis / Gluconeogenesis | 0.048741 |
| 5. | Inositol phosphate metabolism | 0.056096 |

**Table S6:** The matched common proteins obtained from Pharmmapper targets of metabolite 1-methylnicotinamide and RA associated gene targets with their GDA score

| S.no. | Gene | Gene ID | UniProt | Gene Full Name | GDA Score |
|-------|------|---------|---------|----------------|-----------|
| 1 | STAT1 | 6772 | P42224 | signal transducer and activator of transcription 1 | 0.4 |
| 2 | MAPK14 | 1432 | Q16539 | mitogen-activated protein kinase 14 | 0.1 |
| 3 | MMP3 | 4314 | P08254 | matrix metallopeptidase 3 | 0.1 |
| 4 | MMP9 | 4318 | P14780 | matrix metallopeptidase 9 | 0.1 |
| 5 | PARP1 | 142 | P09874 | Poly (ADP-ribose) polymerase 1 | 0.09 |
| 6 | MMP8 | 4317 | P22894 | matrix metallopeptidase 8 | 0.08 |
| 7 | CASP1 | 834 | P29466 | caspase 1 | 0.07 |
| 8 | GSTM2 | 2946 | P28161 | glutathione S-transferase mu 2 | 0.05 |
| 9 | DPEP1 | 1800 | P16444 | dipeptidase 1 | 0.03 |
| 10 | CDK2 | 1017 | P24941 | cyclin dependent kinase 2 | 0.02 |
| 11 | GSK3B | 2932 | P49841 | glycogen synthase kinase 3 beta | 0.02 |
| 12 | INSR | 3643 | P06213 | insulin receptor | 0.02 |
| 13 | PDPK1 | 5170 | O15530 | 3-phosphoinositide dependent protein kinase 1 | 0.01 |
| 14 | PDE4D | 5144 | Q08499 | phosphodiesterase 4D | 0.01 |
| 15 | LCK | 3932 | P06239 | LCK proto-oncogene, Src family tyrosine kinase | 0.01 |
| 16 | TGFB2 | 7042 | P61812 | transforming growth factor beta 2 | 0.01 |
| 17 | XIAP | 331 | P98170 | X-linked inhibitor of apoptosis | 0.01 |
| 18 | F10 | 2159 | P00742 | coagulation factor X | 0.01 |
| 19 | EIF4E | 1977 | P06730 | eukaryotic translation initiation factor 4E | 0.01 |

**Table S7**: The gene targets associated with 1-methylnicoyinamide (1-MNA) obtained from Pharmmapper database (**In excel file**)

**Table S8**: The Gene Ontology (GO) analysis depicting the 186 entries of Biological process associated with obtained 19 common gene targets of 1-methylynicotinamide and Rheumatoid arthritis pathogenesis (**In excel file**)

**Table S9**: The Gene Ontology (GO) analysis depicting the 14 entries of Molecular functions associated with obtained 19 common gene targets of 1-methylynicotinamide and Rheumatoid arthritis pathogenesis (**In excel file**)

**Table S10**: The KEGG pathways associated with 19 common gene targets of 1-methylynicotinamide and Rheumatoid arthritis pathogenesis (**In excel file**)

**Tabel S11**: The Reactome pathways associated with 19 common gene targets of 1-methylynicotinamide and Rheumatoid arthritis pathogenesis (**In excel file**)