Supplemental Materials

Molecular Biology of the Cell

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Supplemental Materials and Methods

Microarray analysis

Control (ctrl) THP-1 and ECSIT^{KD} THP-1 cells were treated with or without addition of LPS (100 ng/ml)for different lengths of time (1 h, 3 h, 5 h, 7h, and 9 h). Total RNA was extracted using Trizol (Invitrogen) and purified using RNeasy columns (Qiagen) according to the manufacturers' protocol. Microarray analysis and processing of raw intensity data was performed, as described (Kim, 2014; Kim, 2012).

Quantitative RT-PCR

Isolation of total RNA and cDNA synthesis were performed following the protocols provided along with the kit (Qiagen). All primers used in this study was purchased from Qiagen. The qRT-PCR analysis was performed using Roter-GeneQ (Qiagen), according to the manufacturers' protocol.

References

Kim, S.Y., Jeong, S., Jung, E., Baik, K.H., Chang, M.H., Kim, S.A., Shim, J.H., Chun, E., and Lee, K.Y. (2012). AMP-activated protein kinase-α1 as an activating kinase of TGF-β-activated kinase 1 has a key role in inflammatory signals. Cell Death Dis *3,* e357

Kim, S.Y., Baik, K.H., Baek, K.H., Chah, K.H., Kim, K.A., Moon, G., Jung, E., Kim, S.T., Shim, J.H., Greenblatt, M.B., Chun, E., and Lee, K.Y. (2014), S6K1 negatively regulates TAK1 activity in the toll-like receptor signaling pathway. Mol Cell Bio*. **3,*** 510-521.

Figure S1: Microarray analysis of control (ctrl) THP-1 and ECSIT-knockdown THP-1 cells treated with or without LPS.

A and B. Ctrl THP-1 (A) and ECSIT-knockdown THP-1 (B) cells were treated with or without LPS (100 ng/ml) for different times, as indicated, and then microarray analysis was performed. The up- or down-gene expressions were represented. **C.** Ctrl THP-1 and ECSIT-knockdown THP-1 cells were treated with or without LPS for different times, as indicated, and the time dependent gene expression patterns were compared between ctrl and ECSIT-knockdown THP-1 cells. Boxes indicated the down-regulated genes in the ECSIT-knockdown THP-1 cells as compared with that of ctrl THP-1 cells.

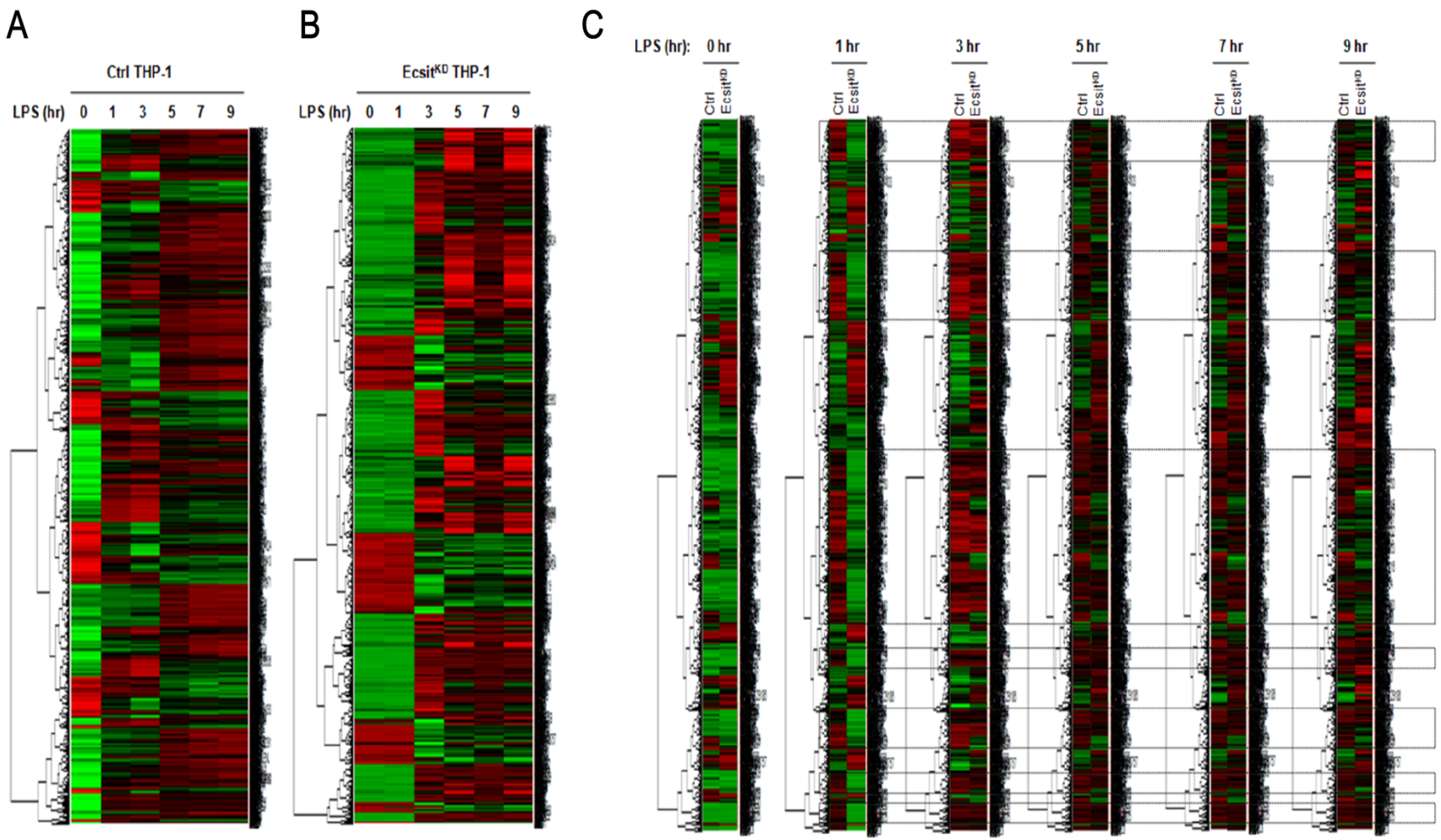
Figure S2: ECSIT-knockdown THP-1 cells exhibit the sever impairment of induction of electron transfer related gene expression in mitochondria.

A. Ctrl THP-1 and ECSIT-knockdown THP-1 cells were treated with or without LPS (100 ng/ml) for different times, 0 h (1), 1 h (2), 3 h (3), 5 h (4), 7 h (5), and 9 h (6) as indicated, and the time dependent gene expression related electron transfer related gene expression in mitochondria were compared between ctrl and ECSIT-knockdown THP-1 cells; lane 1, wt ECSIT^{KD} THP-1/ ctrl THP-1; lane 2, ECSIT^{KD} THP-1 + LPS (1hr) / ctrl THP-1 + LPS (1hr); lane 3, ECSIT^{KD} THP-1 + LPS (3hr) / ctrl THP-1 + LPS (3hr); lane 4, ECSIT^{KD} THP-1 + LPS (5hr) / ctrl THP-1 + LPS (5hr); lane 5, ECSIT^{KD} THP-1 + LPS (7hr) / ctrl THP-1 + LPS (7hr); lane 6; ECSIT^{KD} THP-1 + LPS (9hr) / ctrl THP-1 + LPS (9hr)). **B.** Among them as shown in A, four different genes, *CYP4B1, CYBAC3, NCF1, and NDUFA9*, were selected and confirmed by quantitative real-time PCR (qRT-PCR).

Figure S3: ECSIT-knockdown THP-1 cells exhibit the sever impairment of mesoderm development related genes

A. Ctrl THP-1 and ECSIT-knockdown THP-1 cells were treated with or without LPS (100 ng/ml) for different times, 0 h, 1 h, 3 h, 5 h , 7 h, and 9 h, and the time dependent gene expression related mesoderm development related genes were compared between ctrl and ECSIT-knockdown THP-1 cells; lane 1; ctrl THP-1 + LPS (1hr) / ctrl THP-1; lane 2, ctrl THP-1 + LPS (3hr) / ctrl THP-1; lane 3, ctrl THP-1 + LPS (5hr) / ctrl THP-1; lane 4, ctrl THP-1 + LPS (7hr) / ctrl THP-1; lane 5, ctrl THP-1 + LPS (9hr) / ctrl THP-1; lane 6, ECSIT^{KD} THP-1 + LPS (1hr) / ECSIT^{KD} THP-1; lane 7, ECSIT^{KD} THP-1 + LPS (3hr) / ECSIT^{KD} THP-1; lane 8, ECSIT^{KD} THP-1 + LPS (5hr) / ECSIT^{KD} THP-1; lane 9, ECSIT^{KD} THP-1 + LPS (7hr) / ECSIT^{KD} THP-1; lane 10, ECSIT^{KD} THP-1 + LPS (9hr) / ECSIT^{KD} THP-1, lane 11, ECSIT^{KD} THP-1/ ctrl THP-1; lane 12, ECSIT^{KD} THP-1 + LPS (1hr) / ctrl THP-1 + LPS (1hr); lane 13,

ECSIT^{KD} THP-1 + LPS (3hr) / ctrl THP-1 + LPS (3hr); lane 14, ECSIT^{KD} THP-1 + LPS (5hr) / ctrl THP-1 + LPS (5hr); lane 15, ECSIT^{KD} THP-1 + LPS (7hr) / ctrl THP-1 + LPS (7hr); lane 16; ECSIT^{KD} THP-1 + LPS (9hr) / ctrl THP-1 + LPS (9hr)). **B**. Among them as shown in A, five different genes as indicated were selected and confirmed by quantitative real-time PCR (qRT-PCR).



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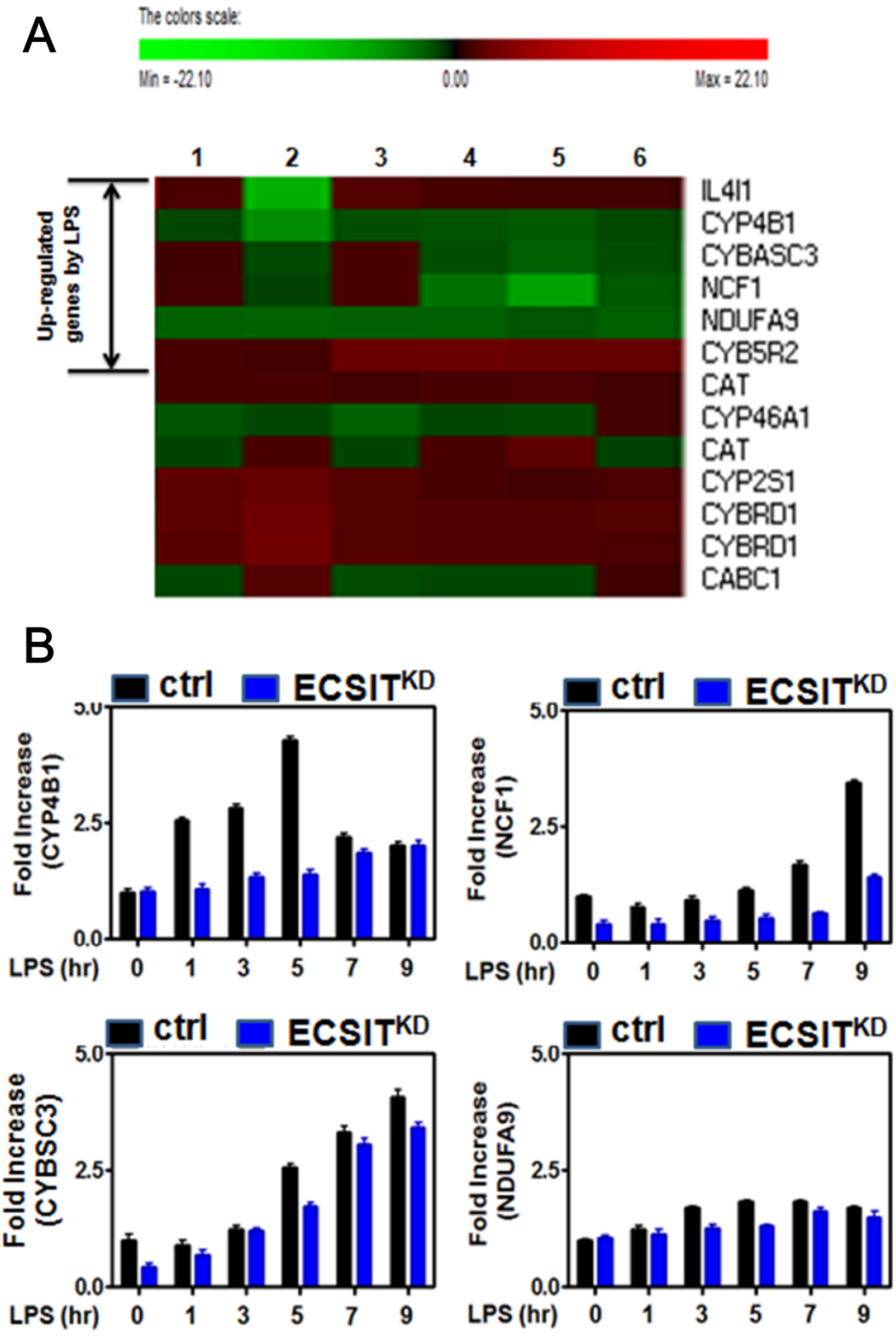
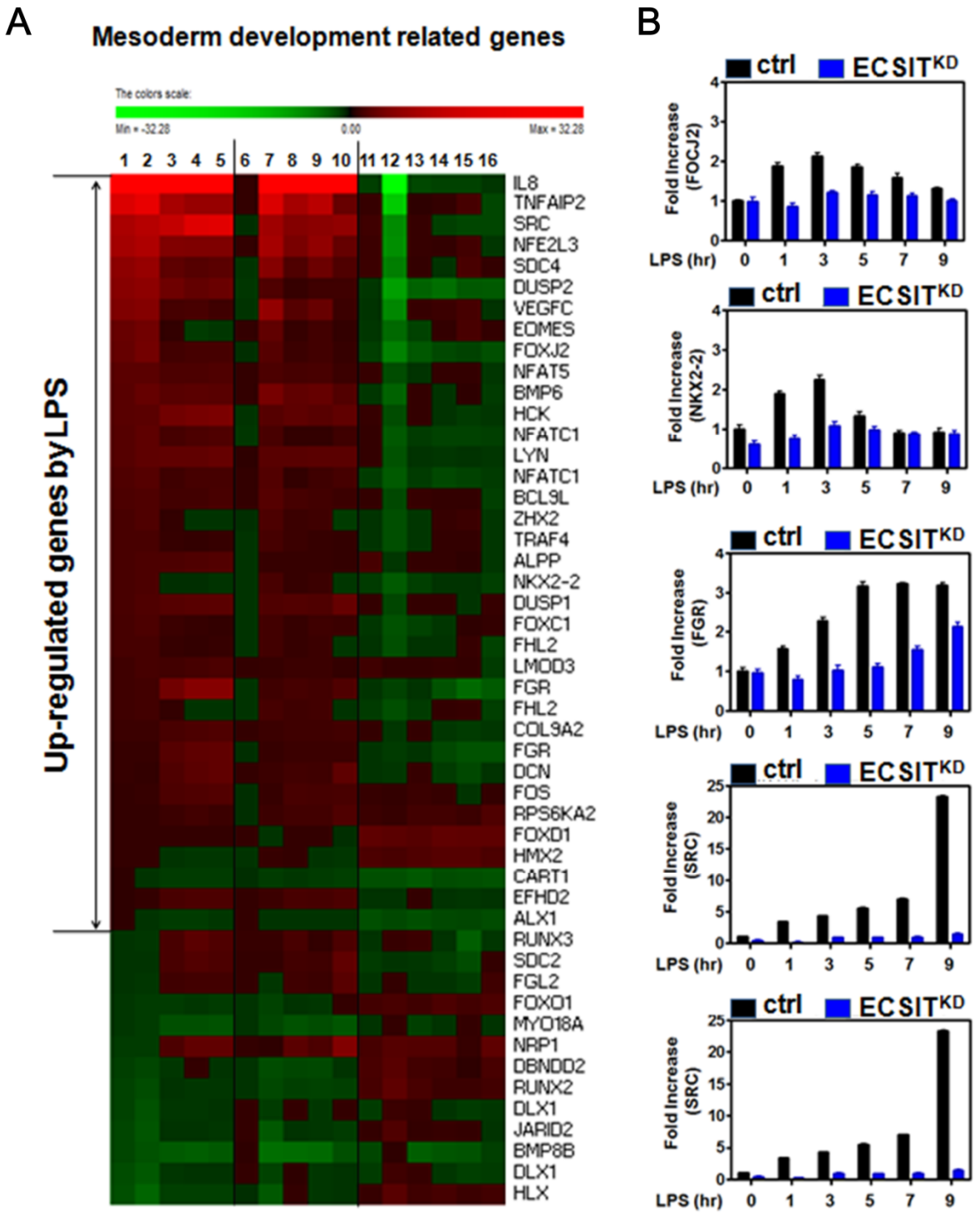


Wi et al., Figure S2



Wi et al., Figure S3