# EGR1 knock-out uterus displays abnormal hyperproliferation and suppressed immune response after estrogen treatment

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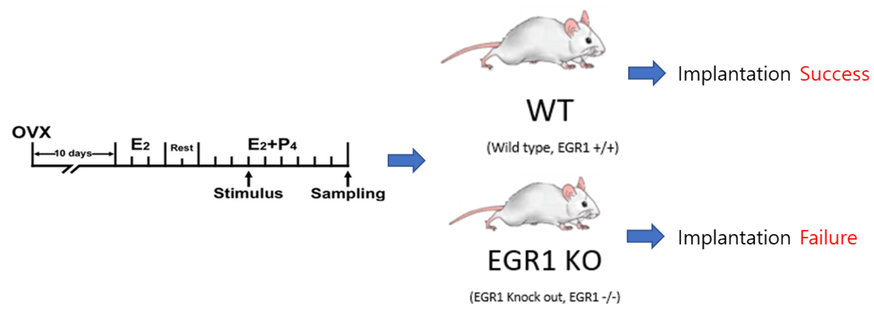
## Abstract

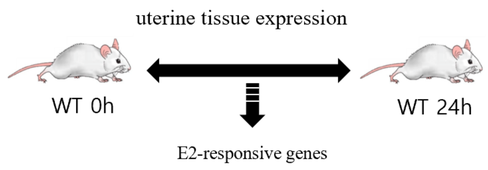
Early Growth Response protein 1 (EGR1) is a transiently and rapidly activated transcription factor involved in various cellular processes such as differentiation, mitogenesis and neuronal activity. EGR1 knock-out (KO) mice has been reported to exhibit an implantation failure, and an impaired hormone signaling in uterine epithelium. Because EGR1 is an immediate early response transcription factor, and estrogen is a major hormone at the very beginning of the period between ovulation and implantation, we scrutinized how the absence of EGR1 alters the early transcriptional response in the uterine tissue upon estrogen exposure. When comparing EGR1 knock-out mice and wild type mice at each elapsed time, we could identify only a small number of differentially expressed genes. In contrast, estrogen-responsive gene sets of each group were significantly different, and the difference was increased more over time. EGR1 knock-out mice exhibited an enhanced proliferation and suppressed immune response compared with EGR1 wild type mice, and these phenotypes have a significant overlap with the estrogen response of the pre-puberty uterus. We also found that the enrichment of poly-ADP ribosylation target genes among estrogen-induced genes, which was transient in wild-type mice, was prolonged and increased in the EGR1 knock-out mice.

## Introduction

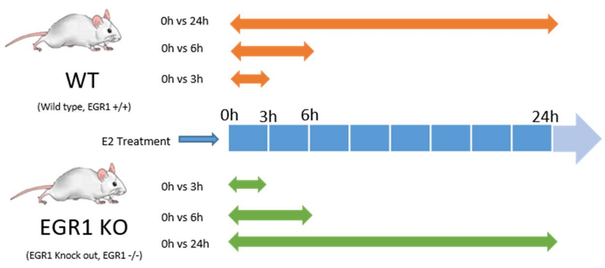



## Methods

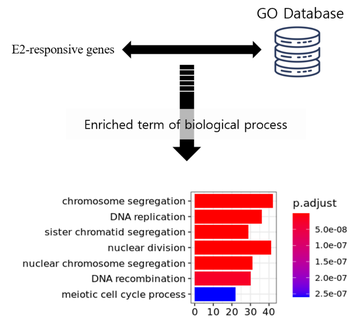
### Differentially Expressed Gene (DEG) analysis



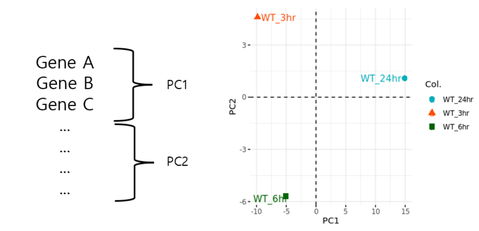

### E2-responsive gene analysis design




### Gene Ontology (GO) term enrichment analysis



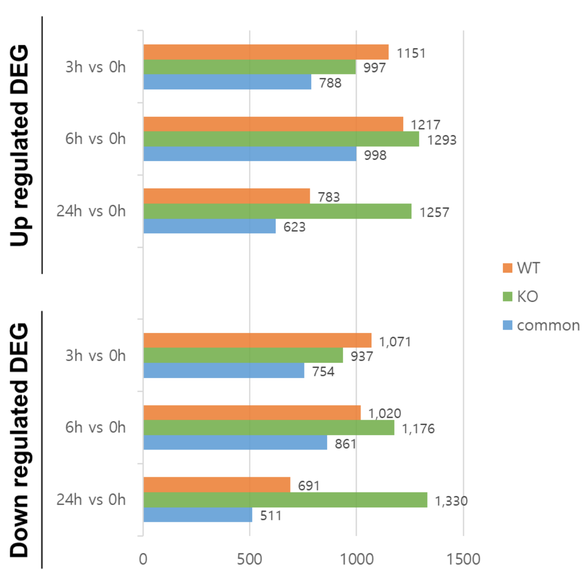

### Principal Components Analysis (PCA)

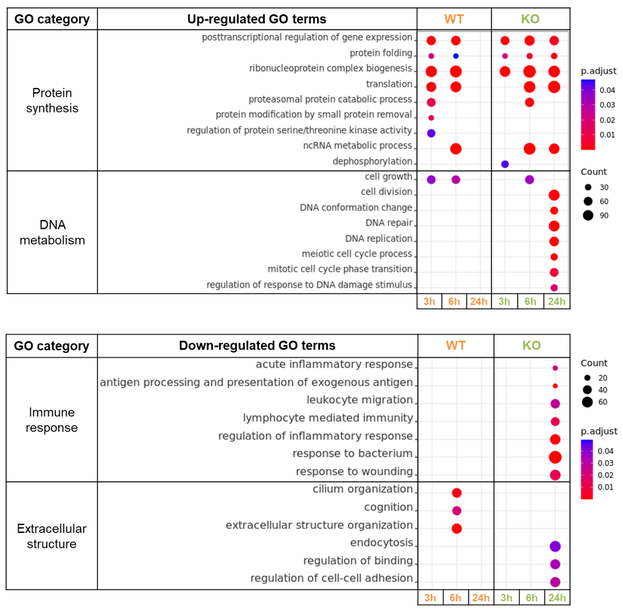



Using the constructed PC1 and PC2, the samples are placed on the coordinate plane.

## Results

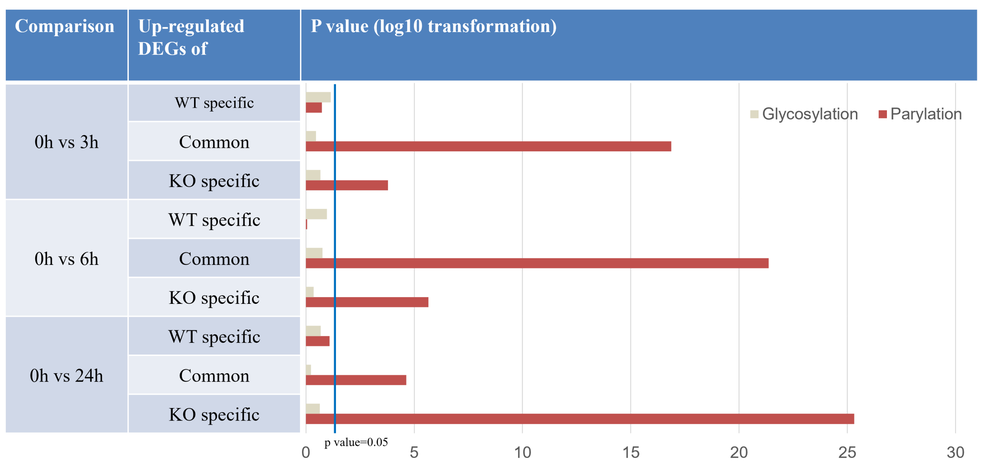
### E2-responsive gene analysis and GO term enrichment analysis



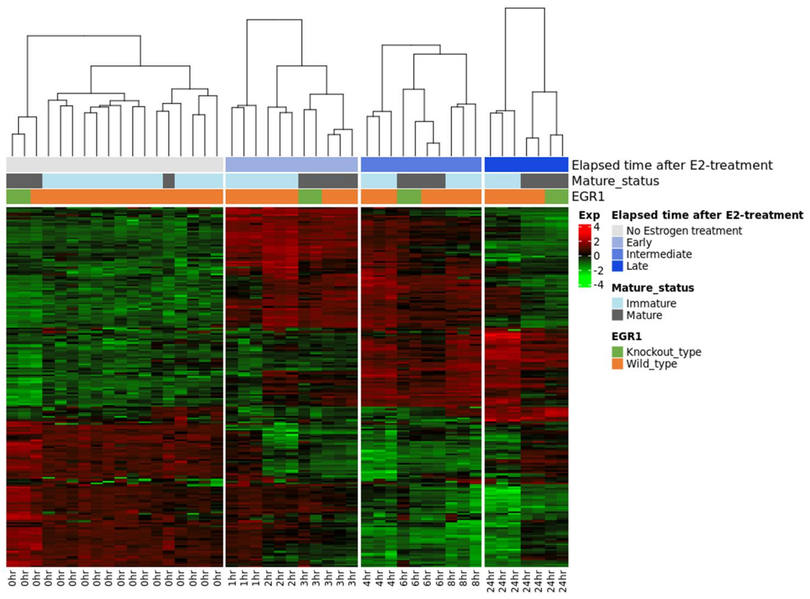

In DEG analysis of wild-type mice, the number of DEGs was decreased over time, and in KO mice, the opposite trend was observed. In GO analysis, the biological processes involved in protein synthesis transiently appeared in wild-type mice, but they were continuously observed in KO mice. In addition, DNA metabolism was activated and immune response were repressed at 24h in EGR1 KO mice.

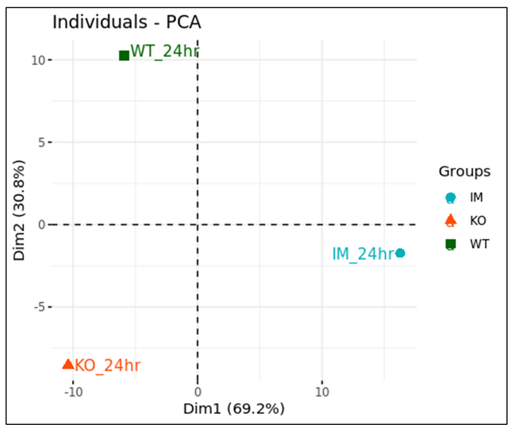
### Poly-ADP ribosylation target gene enrichment in E2-responsive gene

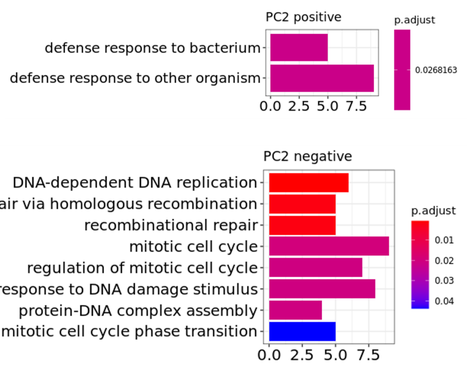



Protein synthesis and DNA metabolism, which were significantly different in EGR1 KO mice, are known to be affected by poly-ADP(pADP) ribosylation. In results of Fisher's exact test, the pADP targets were relatively highly enriched in up-regulated DEGs of the KO mice. This trend was increased over time, and PARP1 gene was also up-regulated in KO mice.

### Similarity of E2-response pattern between EGR1 KO and Immature (IM) mouse after 24hr of estrogen treatment




When we investigated the similarity between samples through PCA. PC1 discriminated our data from immature (IM) data. However, it seemed to be caused by different batch effects. PC2 showed the similarity between KO and IM. GO enrichment results showed that PC2 was related to DNA metabolism and proliferation, and biological processes related to immune response were also found.

## Conclusion

- EGR1 KO is characterized by sustained protein synthesis, enhanced DNA metabolism, and suppressed immune response in response to estrogen.
- The enrichment of poly-ADP ribosylation target genes among E2-responsive genes, which was transient in WT, was prolonged and increased in KO. In addition, it was confirmed that the expression level of PARP1 was Up-regulated in the EGR1 KO.
- EGR1 KO and IM showed similarity in E2-responsive pattern 24 hours after estrogen treatment. According to PCA and GO term analysis results, similarities between the two groups are formed due to genes involved in DNA metabolism and proliferation.

## Acknowledgement

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