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Supplemental Material

Cross-Talk in the Female Rat Mammary Gland: Influence of Aryl Hydrocarbon Receptor on Estrogen Receptor Signaling

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processes (GO= gene ontology). P-value associated with each annotation (GO) term ≥ 0.01 as determined by EASE Score (modified Fisher Exact p-value).

References

Table S1. *qPCR* Primer sequences.

gene	primer sequence
estrogen receptor α (*Esr1*)	Forward 5'-TGAAGCACAAGCGTCAGA GAGAT-3' Reverse 5'-AGACCAGACCAATCATCAGGAT-3'
estrogen receptor β (*Esr2*)	Forward 5'-CTACAGAGAGATGGTCAAAAGTGGA-3' Reverse 5'-GGGCAAGGAGACAGAAAGTAAGT-3'
aryl hydrocarbon receptor (*Ahr)*	Forward 5'-CCATGTCCATGTACCAGTGC-3' Reverse 5'-TGAGCAGCAGTCTGAAGGTG-3'
ribosomal protein S18 *(Rps18)*	Forward 5'-CGTGAAGGATGGGAAGTATAGC-3' Reverse 5'-TATTAACAGCAAAGGCCCAAAG-3'
casein beta (*Csn2*)	Forward 5'-CCCAAGCACAAACAGATGC-3' Reverse 5'-GGGGCTGAGAAGAAACCAC-3'
progesterone receptor (*Pgr*)	Forward 5'-CTACTCGCTGTGCCTTACCA-3' Reverse 5'-GGACCACCCCTTTCTGTCTT-3'
amphiregulin (*Areg*)	Forward 5'-CGGAAAAGGCAGAAGAAACA-3' Reverse 5'-CTTACGGCGGAGACAAAGAC-3'
topoisomerase (DNA) II alpha (*Top2a*)	Forward 5'-CTGCCAAAGCCAAGAACAGT-3' Reverse 5'-AAATCCCCTCACCCCTTAGA-3'
whey acidic protein (*Wap*)	Forward 5'-GCTTCATCAGCCTCGTTCTT-3' Reverse 5'-CACACTCCTCGTTGGTTTGA-3'
prolactin receptor (*Prlr*)	Forward 5'-GCATCTTTCCACCAGTTCCT-3' Reverse 5'-GCTCGTCCTCATTGTCATCA-3'
antigen identified by monoclonal antibody Ki-67 (*Mki67*)	Forward 5'-CAGTCCAGAACACCTAAAGCAA-3' Reverse 5'-CAGGCTAATCTTGGGCAGAC-3'
cyclin B1 (*Ccnb1*)	Forward 5'-CCCTACCAAAACCTGTGGAC-3' Reverse 5'-CATCGGAGAAAGCCTGACAC-3'
cyclin B2 (*Ccnb2*)	Forward 5'-TGGAGAGTGAAATACTGGAAGTCA-3' Reverse 5'-TGAGAAGCACACGATGGAAG-3'
kinesin family member 11 *(Kif11*)	Forward 5'-GTGCGGATTGCTCTTCCA-3' Reverse 5'-TCCTCCACTTTACCCTTCTCC-3'
kinesin family member 18A *(Kif18a*)	Forward 5'-AGCAGAACCGAGTGTAAAGAGG-3' Reverse 5'-CCTTCGTTGGAAATGAGGAA-3'
kinesin family member 2C *(Kif2c*)	Forward 5'-CTGTCCTCCCAGATGTCCAG-3' Reverse 5'-TCACGAAGGTCTCCAAGTCA-3'
aquaporin 5 (*Aqp5*)	Forward 5'-CTGCTCTTCCCCTCCTCTCT-3' Reverse 5'-GGGTGCTTCAAACTCTTCGT-3'
resistin (*Retn*)	Forward 5'-AGTGCGGAAGCATAGACTGG-3' Reverse 5'-ATCACCACCATCATCCCATT-3'

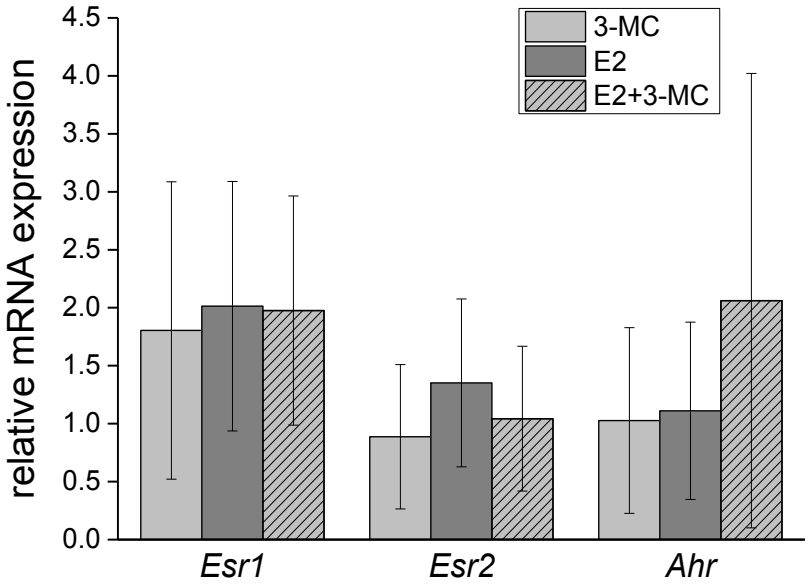

Figure S1. Expression of *Esr1, Esr2* and *Ahr* in mammary glands in ovariectomized (ovx) rats in response to E2 and 3-MC, alone and in combination.

Ovx rats were treated for three days with E2, 3-MC, E2+3-MC or vehicle control as described in methods. RNA was extracted from mammary glands as described in methods. Expression of individual genes was assessed using *qPCR* as described in methods.

Data are presented as fold change, mean ± SD for each treatment group relative to vehicle treated controls. Five to six animals per treatment group were analyzed.

Table S2. Pathway analysis of E2-regulated genes in the mammary gland of ovx rats.

Pathway[a]	E2 regulated genes involved[b]	p-value[c]
Cell cycle	↑ *Chek2, Ttk, Bub1, Bub1b, Cdc2, Ccnb2, Ccnb1, Mcm7, Pttg1, Plk1*	7.50E-05
Cell adhesion molecules (CAMs)	↑ *Cdh1, Cldn3, Cldn6, Cldn7, Cldn8, Glycam1, Mpzl1* ↓ *Pvrl3*	5.40E-03
p53 signaling pathway	↑ *Chek2, Cdc2, Ccnb1, Ccnb2, Serpinb5*	1.50E-02
Pyruvate metabolism	↓ *Acaca, Acss2, Me1, Pc*	1.80E-02
Insulin signaling pathway	↑ *Shc4, Ptpn1* ↓ *Acaca, Hk2, Pygl, Slc2a4*	4.20E-02

[a] Used pathways from the database Kyoto Encyclopedia of Genes and Genomes (KEGG) (Kanehisa and Goto 2000; Kanehisa et al. 2014).

[b] ↑↓ indicates relative expression values of genes up (↑) - or down (↓) - regulated by E2 compared to the vehicle control (fold change ≥ 1.5) as determined by a cDNA-microarray.

[c] Significance was determined by EASE Score, a modified Fisher Exact p-value.

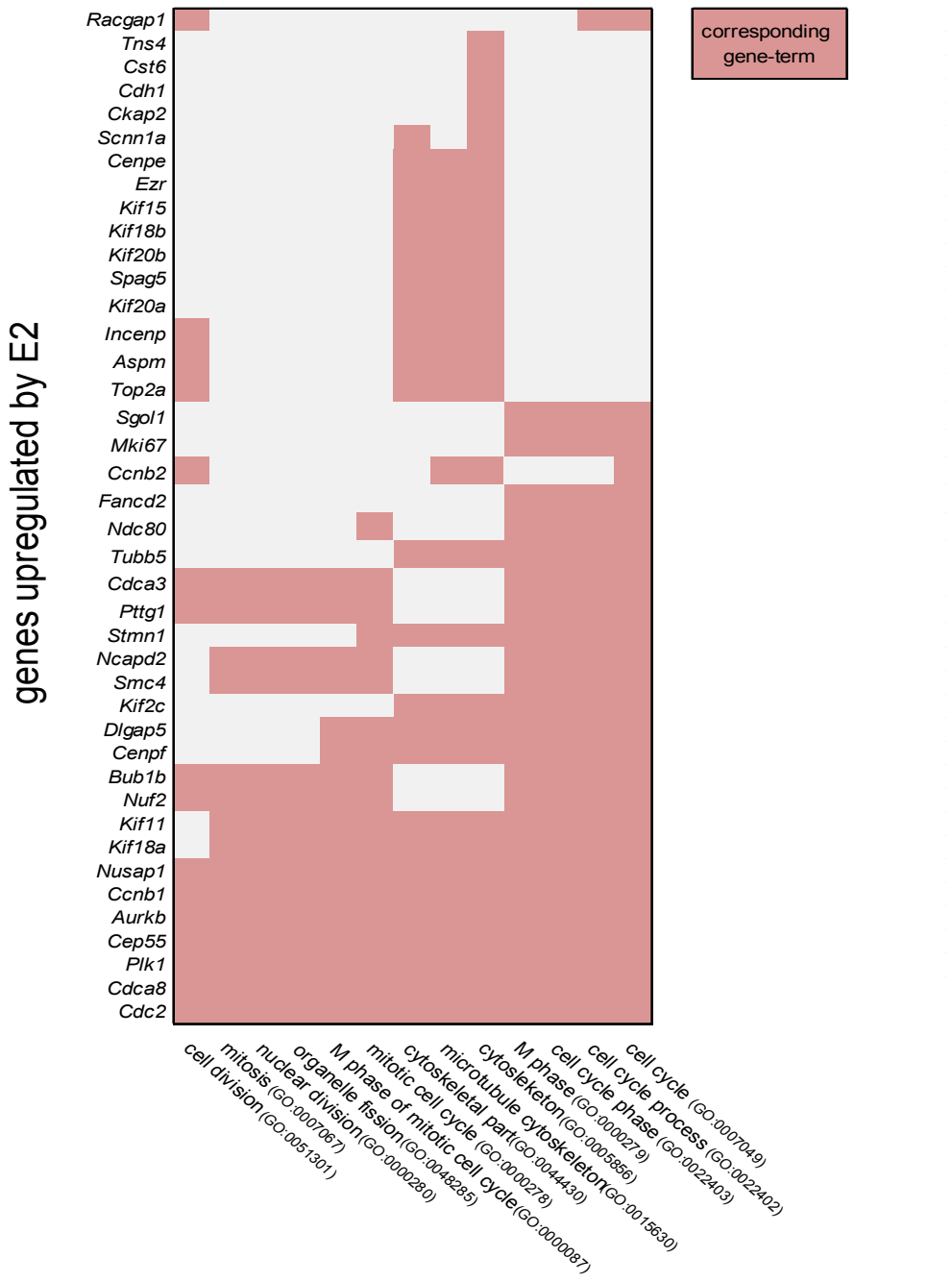

Figure S2. Functional clustering of E2-upregulated genes in the mammary gland. Functional annotation clustering was performed to assign E2-upregulated genes to associated biological processes (GO= gene ontology). P-value associated with each annotation (GO) term ≥ 0.01 as determined by EASE Score (modified Fisher Exact p-value).

References

Kanehisa, M., and S. Goto. 2000. “KEGG: Kyoto Encyclopedia of Genes and Genomes.” *Nucleic Acids Research* 28 (1): 27–30.

Kanehisa, Minoru, Susumu Goto, Yoko Sato, Masayuki Kawashima, Miho Furumichi, and Mao Tanabe. 2014. “Data, Information, Knowledge and Principle: Back to Metabolism in KEGG.” *Nucleic Acids Research* 42 (Database issue): D199–205. doi:10.1093/nar/gkt1076.