Supplemental Materials for

Dynamics of protein expression reveals primary targets and secondary messengers of estrogen receptor alpha signaling in MCF-7 breast cancer cells

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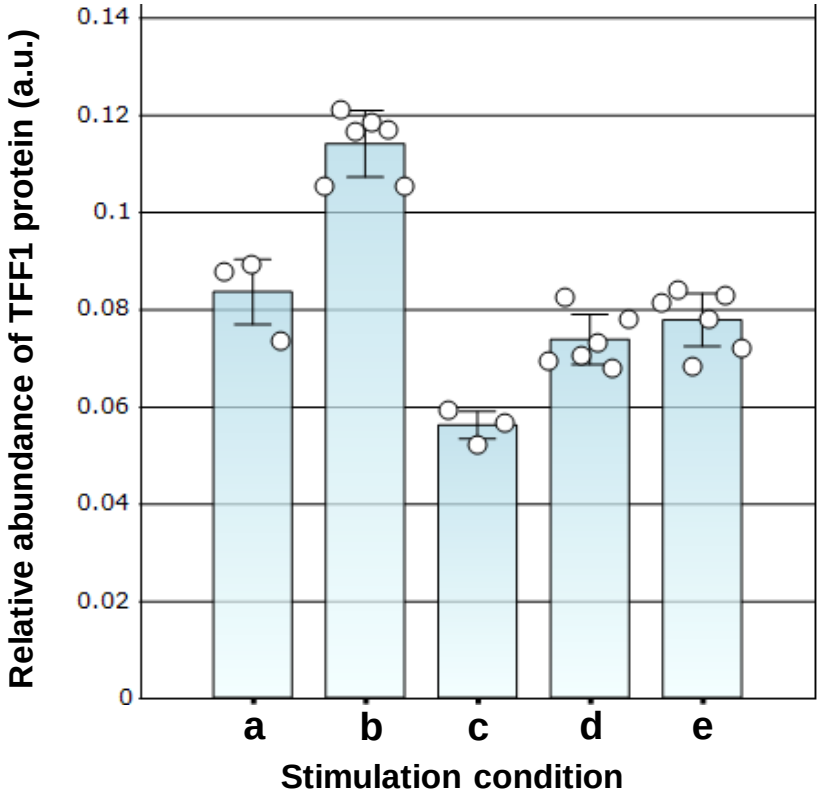
Supplemental Figure S1

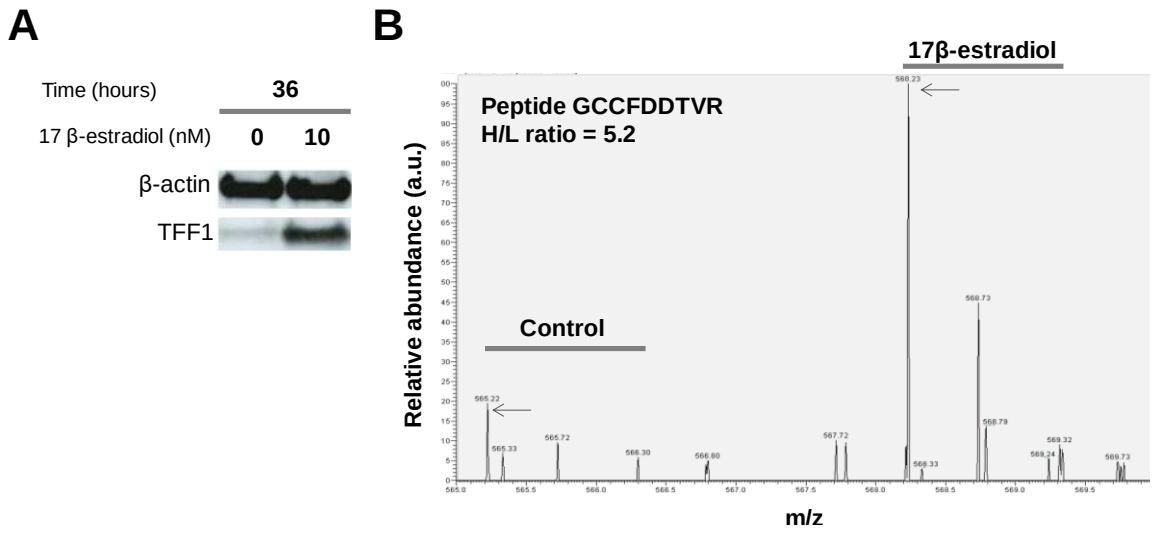


Supplemental Figure S1. Optimization of cell culture conditions based on levels of trefoil factor 1, as measured by SRM. MCF-7 cells were first grown in RPMI-1640 media supplemented with 10% FBS to reach ~80% confluence. Cells were then transferred to 6-well plates and grown for 24 hours in the FBS-free RPMI-1640 media, to synchronize the cell cycle. Following that, the media was replaced with RPMI-1640 supplemented with 10% dextran-coated charcoal-treated FBS. Cells were either stimulated right away or grown for additional 24 hours (to facilitate cell attachment and accommodate cells to 10% dextran-coated charcoal-treated FBS). Thus, the following conditions were tested:

Growth condition	**(a)**	**(b)**	**(c)**	**(d)**	**(e)**
24 hours in RPMI-1640 with 10% dextran-coated charcoal-treated FBS before stimulation	(-)	(-)	(+)	(+)	(+)
Estradiol concentration, nM	0	10	0	10	30
Growth after stimulation, hours	24	24	24	24	24

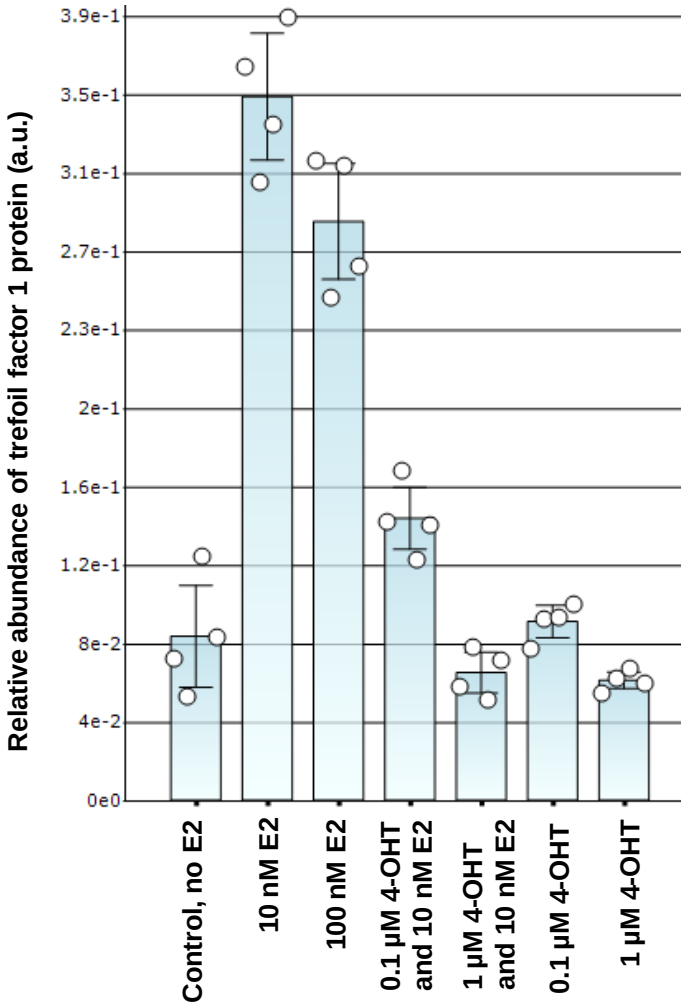
Abundance of trefoil factor 1 relative to beta-actin was calculated using SRM areas. Since no substantial differences were observed for (a) and (b) versus (c) and (d), and since stimulation with 30 nM (f) versus 10 nM (e) estradiol resulted in a marginal increase of TFF1 expression, we selected conditions (c) and (d) for further experiments. a.u., arbitrary units.

Supplemental Figure S2



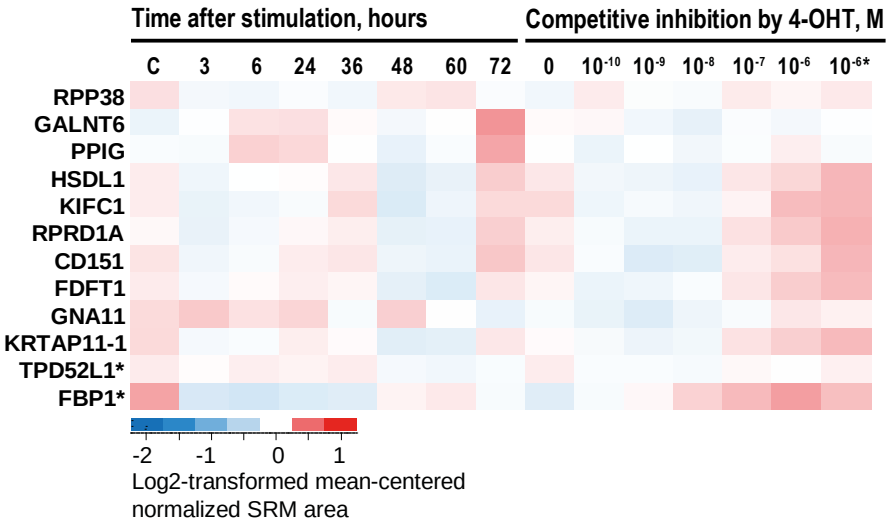
Supplemental Figure S2. Expression of trefoil factor 1 (TFF1) upon stimulation with 10 nM 17β-estradiol. Expression was measured by Western blotting in regular MCF-7 cells (A) or by shotgun mass spectrometry in the mixture of lysates of SILAC-labeled light (Control) and heavy (17β-estradiol) MCF-7 cells at 36 hours. a.u., arbitrary units.

Supplemental Figure S3



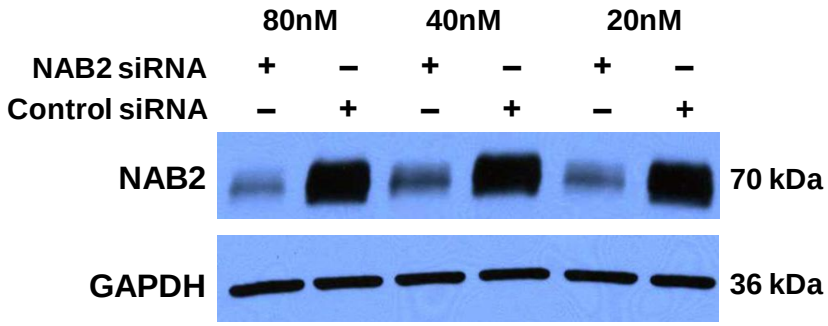
Supplemental Figure S3. Optimization of concentrations of 17β-estradiol and 4-hydroxytamoxifen based on levels of trefoil factor 1, as measured by SRM. E2, 17β-estradiol; 4-OHT, 4-hydroxytamoxifen; a.u., arbitrary units.

Supplemental Figure S4



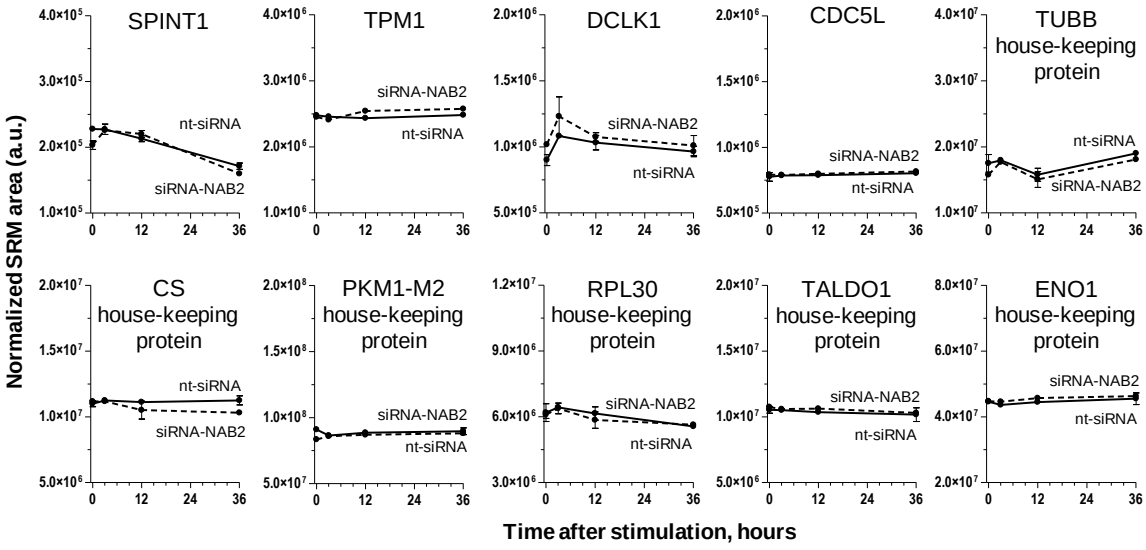
Supplemental Figure S4. False-positive proteins, as validated by SRM assay. Even though presented proteins had substantial fold changes in some single replicates of SILAC data, SRM validation revealed no response to estradiol stimulation or 4-OHT treatment. Thus, these proteins were denoted as false positives. 4-OHT, 4-hydrohytamoxifen. * identified in our previous work (13), but not significant in the present SILAC dataset.

Supplemental Figure S5



Supplemental Figure S5. Optimization of siRNA silencing of NAB2 in MCF-7 cells. Cells were transfected for 24 hours with different concentrations of siGENOME human NAB2 siRNA pool or vehicle control (20-80 nM non-targeting siRNA pool). GAPDH and NAB2 proteins were measured by Western blotting. Concentrations of 40 nM of siRNA pool and vehicle control were chosen for all subsequent stimulation experiments.

Supplemental Figure S6



Supplemental Figure S6. Levels of putative EGR3-regulated proteins SPINT1, TPM1, DCLK1 and CDC5L upon transcfection with siRNA-NAB2 or non-targeting siRNA and stimulation with 10 nM 17β-estradiol. No differential expression was observed for either SPINT1, TPM1, DCLK1 and CDC5L or house-keeping proteins (in addition to ACTB, **Fig. 6C**). nt-siRNA, non-targeting siRNA; a.u., arbitrary units.