

#### Supplemental Table 1:

Ingenuity Pathway Analysis (IPA) shows the top 20 enriched canonical pathways in ESR1-regulated gene sets. Genes with >1.2-fold difference in transcript abundance in *ESR1* siRNA-treated versus control siRNA-treated HESC were selected for analysis. The *left* y-axis corresponds to the blue bars representing the negative logarithm of Benjamini-Hochberg *P* value (B-H *P* value) of the enrichment analysis. The threshold was set at a B-H *P* value of 0.05. The *right* y-axis corresponds to the line graph (yellow squares) representing the ratio of the number of molecules in a given pathway divided by total number of molecules that contribute to that pathway. This figure was generated using IPA software.

#### Supplemental Table 2:

IPA analysis showing the list of top five associated network functions enriched in ESR1 regulated gene sets and related scores. Genes with >1.2-fold difference in transcript levels in *ESR1* siRNA treated samples compared to non-targeting siRNA treated controls in three independent microarrays were selected for analysis.

#### Supplemental Table 3:

IPA analysis showing the list of top five molecular and cellular functions enriched in ESR1 regulated gene sets, related p-values, and number of molecules in each functional category. Genes with >1.2-fold difference in transcript levels in *ESR1* siRNA treated samples compared to non-targeting siRNA treated controls in three independent microarrays were selected for analysis.

#### Supplemental Table 4:

Cell cycle associated genes enriched in ESR1 regulated gene sets in differentiating HESC. Mean fold changes represent the mean ratio of the normalized expression level of each gene in ESR1 depleted cells compared to non-targeting siRNA treated control cells from three independent microarray analysis.

#### Supplemental Table 5:

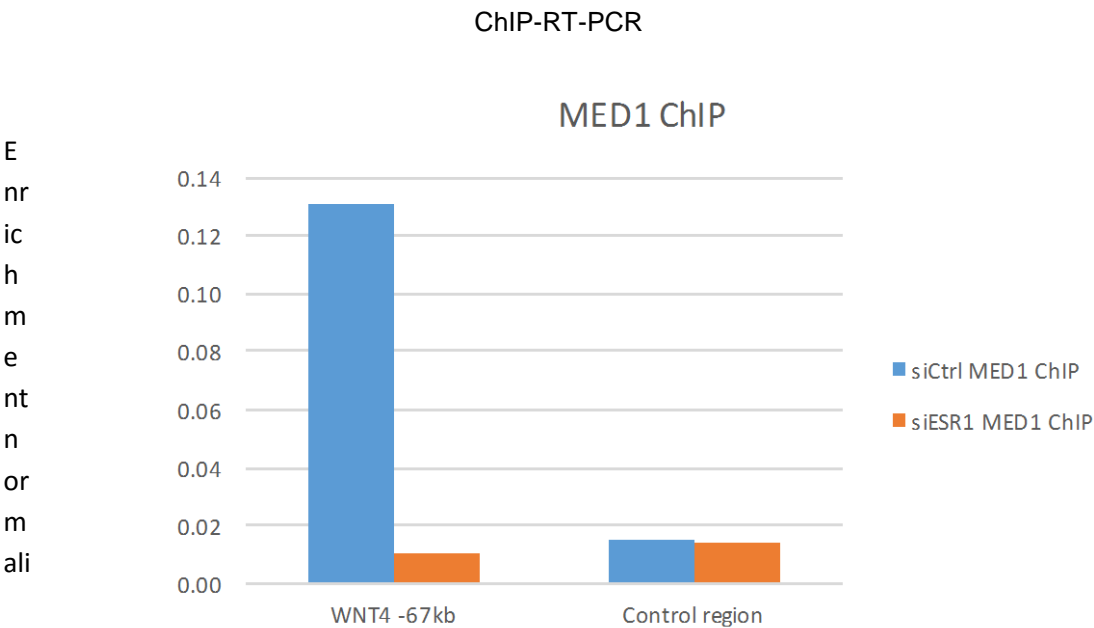
List of peptides and corresponding proteins identified by mass spectrometry as potential targets of PKA during HESC differentiation.

#### Supplemental Figure 1:

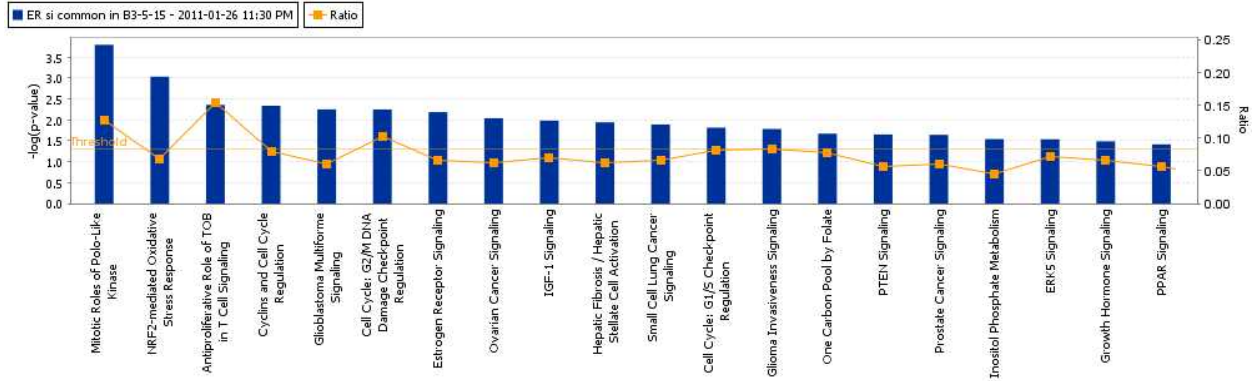
HESC were transfected with control (scrambled) siRNA or siRNA targeting ESR1. 48 h after transfection, cells were treated with differentiation cocktail for 1 h. MED1 ChIP was performed.

Chromatin enrichment was quantified by real-time RT-PCR using primers flanking the potential ESR1 binding site located 67kb upstream of WNT4 gene and a control region (chr13:109144684-109145891) which does not contain a consensus ERE. To calculate the enrichment of chromatin, the resulting signals were normalized to 1% input DNA.

Supplemental Figure 1



Supplemental Table 1



Supplemental Table 2

<b>ID</b>	<b>Associated Network Functions</b>	<b>Score</b>
1	Cell Cycle, DNA Replication, Recombination, and Repair, Cellular Assembly and Organization	48
2	Cell Morphology, Genetic Disorder, Lipid Metabolism	45
3	DNA Replication, Recombination, and Repair, Cell-To-Cell Signaling and Interaction, Cancer	39
4	Organ Morphology, Tissue Morphology, Cell Signaling	38
5	Carbohydrate Metabolism, Lipid Metabolism, Molecular Transport	34

Supplemental Table 3

<b>Molecular and Cellular Functions</b>	<b>p-value</b>	<b># Molecules</b>
Cell Cycle	2.89E-19 - 6.49E-03	94
Cellular Assembly and Organization	1.27E-09 - 6.49E-03	46
DNA Replication, Recombination, and Repair	1.27E-09 - 7.40E-03	71
Cellular Growth and Proliferation	1.72E-08 - 7.40E-03	139
Cellular Development	3.23E-08 - 7.28E-03	98

Supplemental Table 4

Symbol	Entrez Gene Name	fold change (mean)
AURKA	Aurora kinase A	1.6
AURKB	Aurora kinase B	1.8
BUB1	Budding Uninhibited By Benzimidazoles 1	1.8
BUB1B	budding uninhibited by benzimidazoles 1 homolog beta (yeast)	2.0
CCNA2	cyclin A2	1.8
CCND3	cyclin D3	1.3
CCNE2	cyclin E2	1.7
CDC20	cell division cycle 20 homolog ( <i>S. cerevisiae</i> )	1.8
CDK1	cyclin-dependent kinase 1	1.9
CDK2	cyclin-dependent kinase 2	1.3
CKS2	CDC28 protein kinase regulatory subunit 2	1.5
E2F8	E2F transcription factor 8	1.3
ESPL1	extra spindle pole bodies homolog 1 ( <i>S. cerevisiae</i> )	1.4
HSP90AA1	heat shock protein 90kDa alpha (cytosolic), class A member 1	1.3
KIF23	kinesin family member 23	1.5
NEK2	NIMA (never in mitosis gene a)-related kinase 2	1.8
NUSAP1	nucleolar and spindle associated protein 1	1.8
PLK4	polo-like kinase 4	1.9
SKP2	S-phase kinase-associated protein 2 (p45)	1.4
SLK	STE20-like kinase	1.5
TFDP1	transcription factor Dp-1	1.2
CDKN1C	Cyclin dependent kinase inhibitor 1C	-
GAS1	Growth arrest specific 1	-

Supplemental Table 5

Protein Identification	Peptide Sequence
Coil-coil domain protein	<b>R.DEQDFR.N</b>
Non-muscle myosin 9	<b>K.ALSLAR.A, R.VVFQEFR.Q, R.VEEEEERCQHLQAEK.K, K.ANLQIDQINTDLNLER.S</b>
Brain 4 Transcription factor/ MED1	<b>K.TDTSCHDL.-/ K.DNPAQDFSTLYGSSPLER.Q</b>
MED1	K.AQGETEESEK.L
Phospholipase C	<b>K.EAAEPR.T</b>
Vimentin	<b>R.SSVPGVR.L, R.SYVTTSTR.T, R.QQYESVAAK.N, R.ISLPLPNFSSLNLR.E</b>
Peroxisome biogenesis factor	K.GMMKELQTK.Q
MED1	K.VTSLPAMTDR.L, K.AQGETEESEK.L
Skeletal tropomyosin	<b>R.AELSEGQVR.Q, R.IQLVEEELDR.A</b>