SUPPLEMENTAL MATERIAL

Detailed Methods

Animals: WT FVB and Abcg2 deficient[1] (cat. #002767) male mice (8 weeks old) were purchased from Taconic and bred in our animal facility. All animal studies were performed according to the guidelines of the Harvard Medical School, the Longwood Medical Area's Institutional Animal Care and Use Committee (IACUC) and the National Society for Medical Research.

CSP cell isolation and expansion: CSP isolation was performed as previously described.[2] Briefly, mouse hearts were enzymatically and mechanically digested with a mixture of 0.1% collagenase B (Roche), 2.4U/ml dispase II (Roche) and 2.5mmol/L CaCl2. Mononuclear cells were stained with Hoechst 33342 (Sigma Cat #B2261) (5µg/10^6 cells/ml) at 37°C for 90min. Verapamil (50 mmol/L) (Sigma, #V4629) was used as a negative control. Cells were further incubated with fluorochrome-conjugated monoclonal rat anti-mouse antibodies against Sca-1, CD31 and CD45 (Pharmigen). 7-Aminoactinomycin-D (7-AAD) was used for dead cell detection. $Sca1^+/CD31^-/CD45^-$ CSP were used for all experiments. Sorted CSP cells were cultured in expansion medium (α-MEM, 20% FBS HI, 2 mM L-Glutamine and 1% penicillin/streptomycin). All experiments were performed with CSP cells from passages 4-6.

Propidium iodide staining (PI): PI staining and analysis were performed as previously described.[3] Briefly, CSP cells were fixed in 70% ethanol and incubated with PI staining solution (0.1% Triton-X100, 2µg/ml PI, 400ng/ml RNase A) for one hour at 37°C protected from light. Cells were analyzed by flow cytometry and data processing was performed by the ModFit Lt software (Verity House).

Flow cytometric analysis and sorting: Flow cytometric analysis was performed with the DXP11 analyzer (Cytek) and the Accuri C6 analyzer (BD Biosciences). FACS was performed with a FACSAria sorter (BD Biosciences). Hoechst 33342 dye was excited by an UV (355-nm) laser. Data were analyzed by the FACSDIVA software (BD Biosciences) and FlowJo (Tree Star).

Cell Cycle Analysis Using the Fluorescence Ubiquitination Cell Cycle Indicator (FUCCI) Lentiviral System: CSP cells were infected with lentiviruses expressing the FUCCI reporter system and selected by FACS.[4] Cells were synchronized in G1 (24 hours in α-MEM, 0.1% FBS HI, 2 mM L-Glutamine and 1% pen/strep) and cultured in CSP expansion medium for up to 40 hours. Sampling of the cells was performed at several time points and cells were analyzed by flow cytometry with the DXP11 analyzer (Cytek) or by confocal live imaging (Zeiss LSM 510 inverted live-cell confocal system) for Cdt1 and geminin reporters. Data were analyzed with the FlowJo and LSM510 software programs respectively. For cell cycle analysis, expression of the Cdt1 reporter in G1 phase, geminin reporter in S-G2-M cell cycle phases, expression of both protein reporters during the G1-S transition, and lack of expression of either reporter shortly after cytokinesis and in early G1 were monitored, as previously described.[4] T_{50} values were calculated according to a "plateau followed by exponential decay" model.[5] The T_{50} value represents the required time for G1-residing CSP cells to be decreased by 50% from their initial value.

Abcg2 shRNA: shRNA lentiviral constructs were generated according to the instructions provided by Addgene. Scramble (5'CCGGCCTAAGGTTAAGTCGCCCTCGCTCGAGCGAG GGCGACTTAACCTTAGGTTTTTG3') or Abcg2 (5'CCGGGCAACACTTCTCATGACAATCC

TCGAGGATTGTCATGAGAAGTGTTGCTTTTTG3) shRNA sequences were cloned in the pLKO.1-puro lentiviral vector under the control of the U6 promoter. Infected CSP selection was achieved through selection with puromycin (4µg/ml).

Cell cycle-focused RT-PCR gene array: mRNA from scramble and sh-Abcg2 expressing CSP cells was used to synthesize cDNA (RT^2-First strand kit, SABiosciences). Cell cycle gene expression profile was established using the Cell Cycle RT-PCR array (Cat #PAMM-020, SABiosciences) and the MyiQ cycler (Bio-Rad) according to the manufacturer's guidelines. Data were obtained from three independent experiments. Data analysis was carried out according to the manufacturer's instructions and as previously described.[3] The full list of the analyzed genes is provided in table 1.

Asymmetric division assessment: Unsynchronized CSP cells on coverslips were fixed with 4% paraformaldehyde, permeabilized with methanol followed by blocking with 1% BSA. Cells were then incubated with antibodies against phospho-Histone H3 (pH3) (1/200, Abcam Cat #ab32107) and α-adaptin[6] (1/50, Santa Cruz cat # sc-17771) followed by secondary antibodies (1/200, anti-rabbit Alexa-488 and anti-mouse Alexa-555, Molecular probes). Coverslips were mounted on slides with DAPI-containing mounting medium (Vector Vectashield). Visualization was performed with an epi-fluorescent microscope (Zeiss, Axiovert 200M). Staining for numb[6, 7] was performed in CSP cells fixed with 4% paraformaldehyde and permeabilized with BD Perm/Wash solution (BD biosciences) according to the manufacturer's instructions. Cells were then blocked with 1% BSA and incubated with anti-numb antibody (1/500, Abcam Cat #ab4147) followed by a secondary goat-anti-rabbit 488 antibody (Molecular probes). Data were acquired with the Accuri C6 analyzer.

Statistical analysis: Statistical differences were evaluated using one-way ANOVA analysis and Student's unpaired t-test, using GraphPad Prism (Version 5.03). Data are presented as mean ± s.e.m. A p-value ≤ 0.05 was considered statistically significant.

Online Table I: Cell cycle-focused RT-PCR array of scramble and sh-Abcg2 expressing CSP cells

Description	Symbol	(ΔCt)		Up-Down Regulation (comparing to Scramble)
		Scramble	sh-Abcg2	
C-abl oncogene 1, receptor tyrosine kinase	Abl1	11.106178	11.420862	-1.2437
Adenylate kinase 1	Ak1	1.8289	2.147363	-1.247
Amyloid beta (A4) precursor protein-binding, family B, member 1	Apbb1	8.367457	8.377834	-1.0072
Ataxia telangiectasia mutated homolog (human)	Atm	5.850675	5.30991	1.4547
Breast cancer 1	Brca1	7.734002	6.825685	1.8769
Breast cancer 2	Brca2	6.90382	6.069443	1.7831
Calcium/calmodulin-dependent protein kinase II alpha	Camk2a	8.726875	8.514801	1.1584
Calcium/calmodulin-dependent protein kinase II, beta	Camk2b	11.106178	11.635309	-1.4431
Caspase 3	Casp3	4.375578	3.339269	2.051
Cyclin A1	Ccna1	11.106178	11.635309	-1.4431
Cyclin A2	Ccna2	4.476715	3.571836	1.8724
Cyclin B1	Ccnb1	3.212888	2.579624	1.5511
Cyclin B2	Ccnb2	5.263591	3.677749	3.0018
Cyclin C	Ccnc	6.091513	7.270975	-2.2649
Cyclin D1	Ccnd1	1.426526	2.148219	-1.6491
Cyclin E1	Ccne1	7.093276	7.731017	-1.5559
Cyclin F	Ccnf	4.612443	3.817572	1.7349
Cell division cycle 25 homolog A (S. pombe)	Cdc25a	4.979034	4.111663	1.8243
Cyclin-dependent kinase 2	Cdk2	5.539315	5.067542	1.3868
Cyclin-dependent kinase 4	Cdk4	2.786019	1.978295	1.7504
CDK5 regulatory subunit associated protein 1	Cdk5rap1	7.099793	7.425954	-1.2537
Cyclin-dependent kinase inhibitor 1A (P21)	Cdkn1a	1.11014	1.840963	-1.6596
Cyclin-dependent kinase inhibitor 1B	Cdkn1b	6.03447	4.990849	2.0614
Cyclin-dependent kinase inhibitor 2A	Cdkn2a	2.639825	3.229998	-1.5054
Checkpoint kinase 1 homolog (S. pombe)	Chek1	6.8862	6.34775	1.4524
CDC28 protein kinase 1b	Cks1b	3.106571	2.649848	1.3724
DNA-damage inducible transcript 3	Ddit3	2.194981	3.402252	-2.309
DnaJ (Hsp40) homolog, subfamily C, member 2	Dnajc2	3.320207	3.546802	-1.1701
Dystonin	Dst	4.176003	4.096243	1.0568
E2F transcription factor 1	E2f1	7.65494	6.996614	1.5783
E2F transcription factor 2	E2f2	11.106178	11.635309	-1.4431
E2F transcription factor 3	E2f3	6.080322	6.247647	-1.123
E2F transcription factor 4	E2f4	3.772541	3.754677	1.0125
Growth arrest and DNA-damage-inducible 45 alpha	Gadd45a	4.521487	5.196044	-1.5961
G protein-coupled receptor 132	Gpr132	11.106178	11.635309	-1.4431
Hus1 homolog (S. pombe)	Hus1	6.020265	6.168142	-1.1079
Inhibin alpha	Inha	7.611555	7.451969	1.117

Integrin beta 1 (fibronectin receptor beta)	Itgb1	-1.705923	-1.351013	-1.2789
Microtubule-actin crosslinking factor 1	Macf1	2.085609	2.332797	-1.1869
MAD2 mitotic arrest deficient-like 1 (yeast)	Mad2l1	4.865167	4.094512	1.706
Minichromosome maintenance deficient 2 mitotin (S. cerevisiae)	Mcm2	5.947158	4.838795	2.156
Minichromosome maintenance deficient 3 (S. cerevisiae)	Mcm3	4.530528	3.72946	1.7424
Minichromosome maintenance deficient 4 homolog (S. cerevisiae)	Mcm4	2.64628	2.084709	1.4759
Transformed mouse 3T3 cell double minute 2	Mdm2	2.738524	1.620668	2.1702
Antigen identified by monoclonal antibody Ki 67	Mki67	3.514161	2.721685	1.732
Meiotic recombination 11 homolog A (S. cerevisiae)	Mre11a	6.648424	5.72085	1.9021
MutS homolog 2 (E. coli)	Msh2	4.539862	3.850688	1.6124
Mdm2, transformed 3T3 cell double minute p53 binding protein	Mtbp	8.635183	8.600524	1.0243
Myeloblastosis oncogene	Myb	11.106178	11.635309	-1.4431
NIMA (never in mitosis gene a)-related expressed kinase 2	Nek2	11.106178	10.252296	1.8074
Nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 1	Nfatc1	3.728189	3.840408	-1.0809
Notch gene homolog 2 (Drosophila)	Notch2	8.373932	6.396327	3.9384
Nucleophosmin/nucleoplasmin 2	Npm2	11.106178	11.350593	-1.1846
Proliferating cell nuclear antigen	Pcna	2.143046	1.263756	1.8395
Pescadillo homolog 1, containing BRCT domain (zebrafish)	Pes1	4.101918	4.226599	-1.0903
Polycystic kidney disease 1 homolog	Pkd1	4.02754	4.328398	-1.2319
Peripheral myelin protein 22	Pmp22	2.233625	2.111629	1.0882
Protein phosphatase 1D magnesium-dependent, delta isoform	Ppm1d	5.607756	5.029499	1.493
Protein phosphatase 2 (formerly 2A), regulatory subunit B", alpha	Ppp2r3a	10.805114	9.865458	1.9181
Protein phosphatase 3, catalytic subunit, alpha isoform	Ppp3ca	3.788133	4.481805	-1.6174
Protamine 1	Prm1	11.106178	11.635309	-1.4431
RAD17 homolog (S. pombe)	Rad17	6.058783	6.062272	-1.0024
RAD21 homolog (S. pombe)	Rad21	4.681669	4.442202	1.1806
RAD51 homolog (S. cerevisiae)	Rad51	9.395378	8.334477	2.0862
RAD9 homolog (S. pombe)	Rad9	5.869122	5.696741	1.1269
RAN, member RAS oncogene family	Ran	-1.819387	-1.503806	-1.2445
Retinoblastoma-like 1 (p107)	Rbl1	7.246548	6.439949	1.7491
Retinoblastoma-like 2	Rbl2	6.16683	5.920503	1.1862
Sestrin 2	Sesn2	6.770173	7.372871	-1.5186
Stratifin	Sfn	10.925972	11.333543	-1.3265
Src homology 2 domain-containing transforming protein C1	Shc1	3.27843	3.011812	1.203
S-phase kinase-associated protein 2 (p45)	Skp2	6.762598	7.075956	-1.2426
Schlafen 1	Slfn1	11.106178	11.635309	-1.4431

Structural maintenance of chromosomes 1A	Smc1a	4.750962	3.845469	1.8732
Stromal antigen 1	Stag1	5.081462	4.207143	1.8331
SMT3 suppressor of mif two 3 homolog 1 (yeast)	Sumo1	2.225602	2.041863	1.1358
TAF10 RNA polymerase II, TATA box binding protein (TBP)-associated factor	Taf10	2.538463	3.137654	-1.5149
Telomeric repeat binding factor 1	Terf1	6.846584	5.840404	2.0086
Transcription factor Dp 1	Tfdp1	1.425901	1.350236	1.0538
Proteasome (prosome, macropain) assembly chaperone 2	Psmg2	6.058063	5.13353	1.8981
Transformation related protein 53	Trp53	3.339588	2.5548	1.7228
Transformation related protein 63	Trp63	11.106178	11.635309	-1.4431
Tumor susceptibility gene 101	Tsg101	5.633285	6.251244	-1.5347
WEE 1 homolog 1 (S. pombe)	Wee1	5.28807	5.570576	-1.2163

Supplemental References

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Online Video I: Representative video of time-lapse live imaging (40X) monitoring of Fucci^{+} WT CSP cells 18-40 hours following synchronization.

Online Video II: Representative video of time-lapse live imaging (40X) monitoring of Fucci^{+} Abcg2-KO CSP cells 18-40 hours following synchronization.