Stem Cell Reports, Volume *1*

Supplemental Information

Analysis of Induced Pluripotent Stem Cells from a *BRCA1* Mutant Family

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Figure S1. Pluripotency analysis of iPS cell lines. The iPS cell lines were immunostained for pluripotency marker expression using antibodies to the following antigens: TRA-1-60, TRA-1-81 (proteoglycans), SSEA3 (carbohydrate), SSEA4 (glycolipid), and NANOG (Stemgent, San Diego). The nuclei were stained with DAPI. The cells were also tested for mycoplasma contamination using RADIL. The iPS cell lines were thereafter grown on Matrigel substrate to avoid the use of feeders and maintained as undifferentiated cells in Nutristem growth media (Stemgent, Inc.). Representative data from one BRCA1 wild type and three BRCA1 5382insC mutant subjects are displayed. Bar = 50 microns.

Figure S1

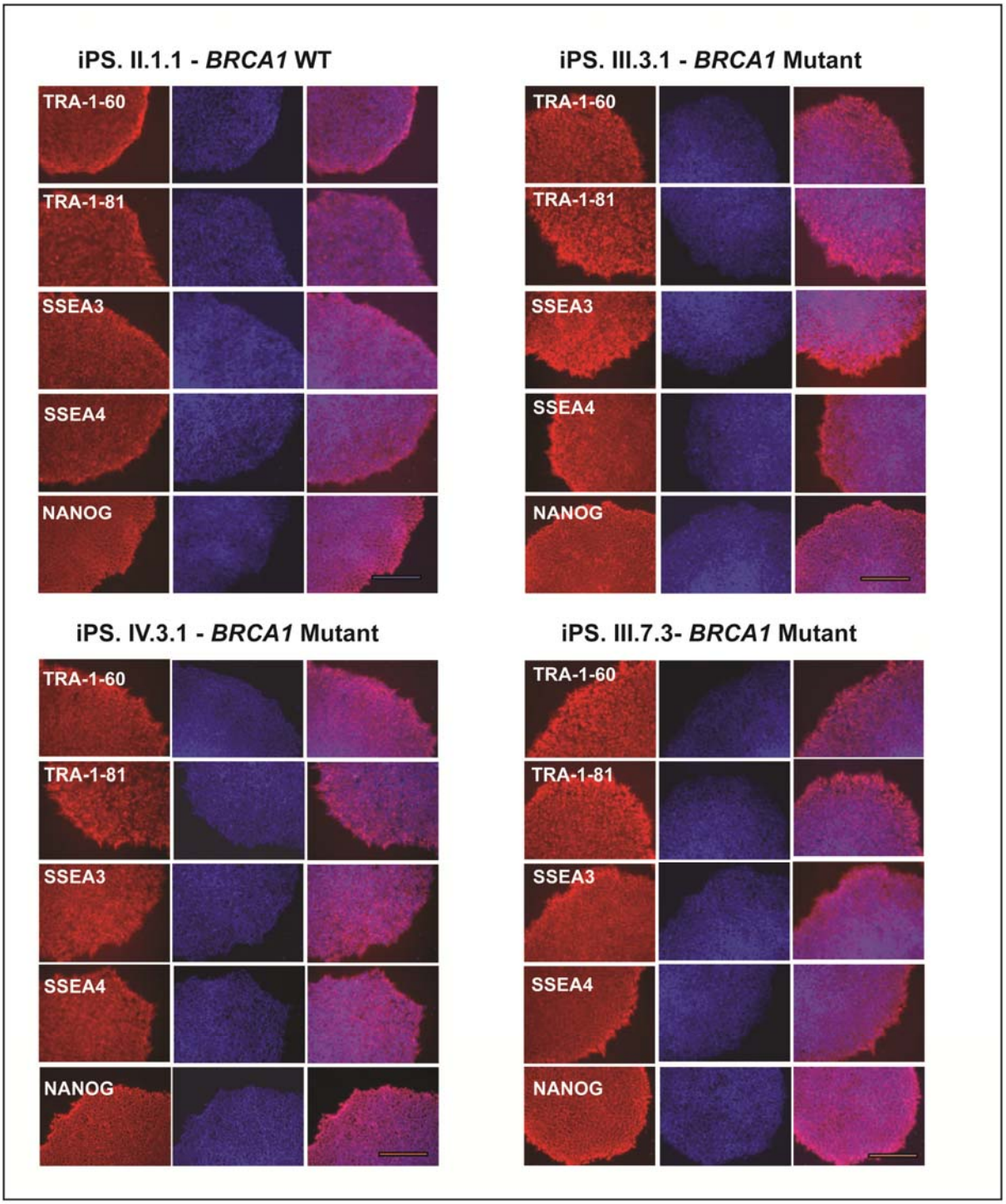
iPS. II.1.1 - *BRCA1* WT
TRA-1-60
TRA-1-81
SSEA3
SSEA4
NANOG
iPS. III.3.1 - *BRCA1* Mutant
TRA-1-60
TRA-1-81
SSEA3
SSEA4
NANOG
iPS. IV.3.1 - *BRCA1* Mutant
TRA-1-60
TRA-1-81
SSEA3
SSEA4
NANOG
iPS. III.7.3- *BRCA1* Mutant
TRA-1-60
TRA-1-81
SSEA3
SSEA4
NANOG

Figure S2. Directed differentiation and analysis of lineage-specific gene expression in iPS cell lines. *In vitro* differentiation in four of the iPS cell lines is shown. iPS cells were induced to ectoderm via dual SMAD inhibition with Noggin and SB 431542 as evidenced by PAX6 and βIII-tubulin staining. To differentiate cells towards mesoderm and endoderm, iPS cells were induced with activin A and WNT3a to the bipotential progenitor (mesendoderm) stage. On day 3 of differentiation, immunofluorescent staining for BRACHYURY (Santa Cruz) and GATA4 (R&D Systems) was performed to test for mesoderm, and staining for FOXA2 and SOX17 (R&D Systems) was performed to test for endoderm. Representative data from one BRCA1 wild type and three BRCA1 5382insC mutant subjects are displayed. Bar = 50 microns.

Figure S2

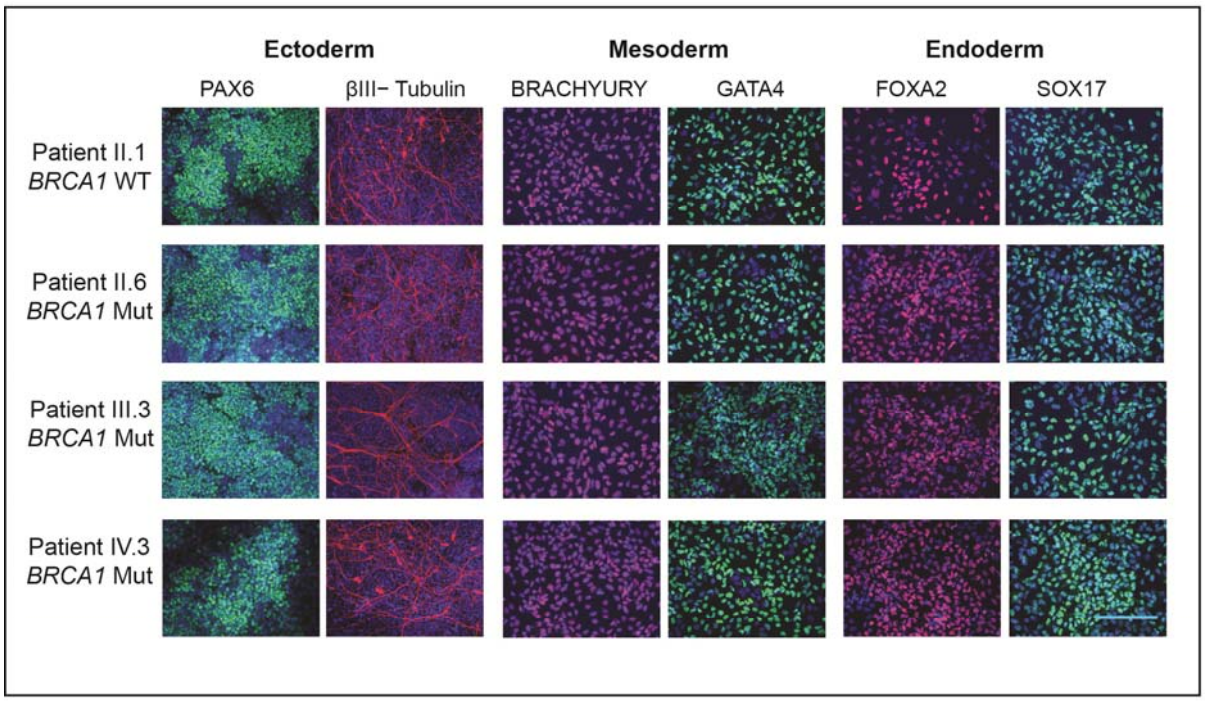
Ectoderm
Mesoderm
Endoderm
PAX6
βIII− Tubulin
BRACHYURY
GATA4
FOXA2
SOX17
Patient II.1
BRCA1 WT
Patient II.6
BRCA1 Mut
Patient III.3
BRCA1 Mut
Patient IV.3
BRCA1 Mut

Figure S3. Histologic analysis of *BRCA1* Wild Type and Mutant iPS cell line derived teratomas. For the teratoma formation assays, iPS cells (approximately 1-2 × 10^6 cells) were injected into the kidney capsules of immunodeficient NOD/SCID mice. Teratomas were harvested 10–16 weeks later. A small portion from each teratoma was extracted for RNA analysis, and the remaining tissue was fixed in PBS containing 4% paraformaldehyde and processed for histological analysis. All iPS cell lines generated teratomas with similar features that included immature and mature tissues and cystic changes. There were no distinct non-teratomatous germ cell tumors or somatic malignancies. Representative histology of (A) *BRCA1* wild type and (B) mutant iPS cell derived teratomas displayed all three germ layers: ectoderm (squamous epithelium (asterisk) and neuroepithelium (immature neuroepithelium and more mature glioneuronal tissue)), mesoderm (muscle) and endoderm (gut epithelium). Sections were stained with H&E. Bar = 50 microns.

Figure S3

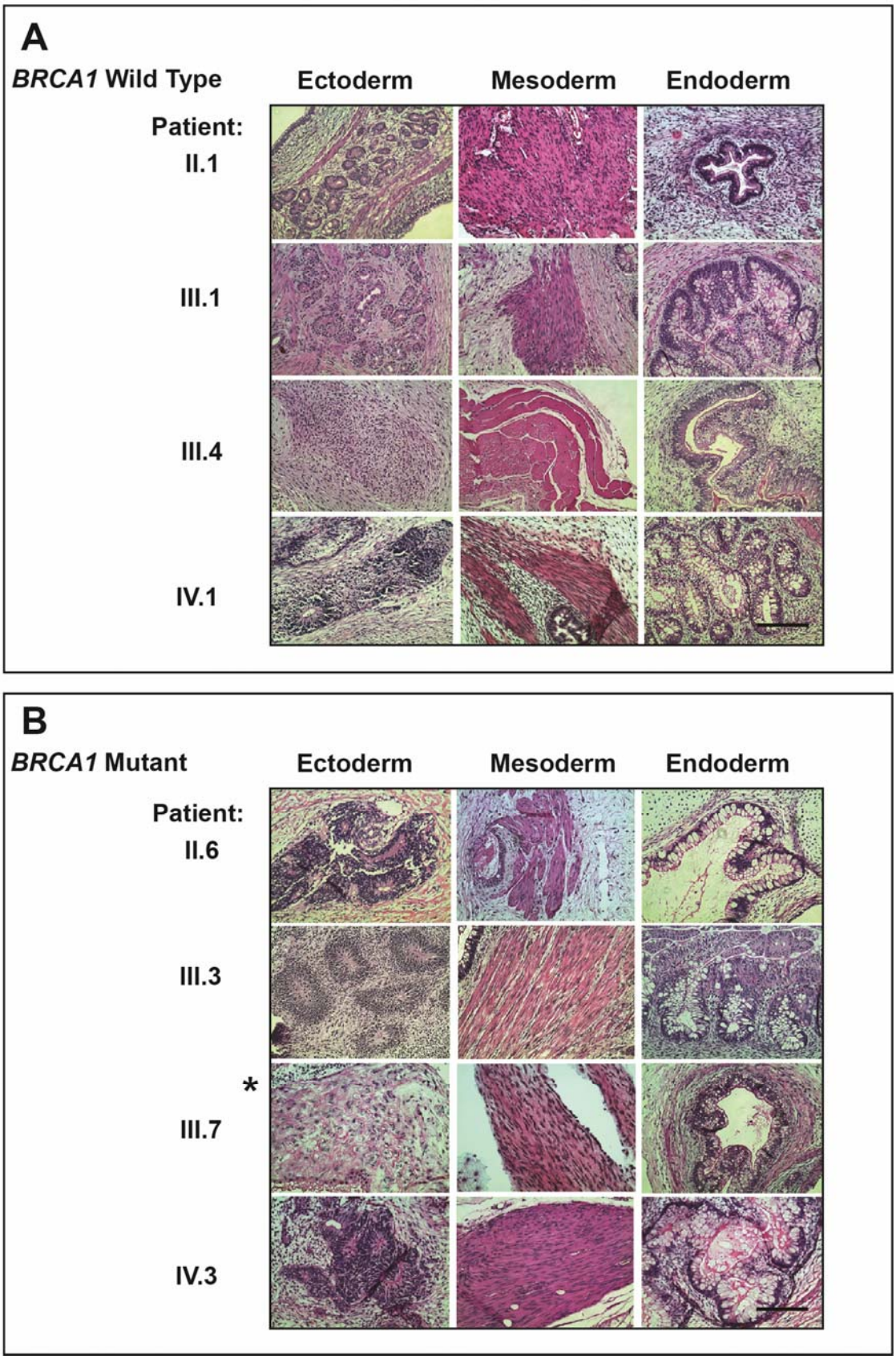
A
BRCA1 Wild Type
Ectoderm
Mesoderm
Endoderm
Patient:
II.1
III.1
III.4
IV.1
B
BRCA1 Mutant
Ectoderm
Mesoderm
Endoderm
Patient:
II.6
III.3
*
III.7
IV.3

Figure S4. Assay for wildtype and mutant BRCA1 message levels. A. Nucleotide sequence of total, wildtype, and mutant BRCA1-specific primers aligned with both wildtype (WT; top) and mutant (Mut; bottom) *BRCA1* templates. **B.** Validation of accuracy of WT and Mut primers using known ratios of *BRCA1* templates. WT and Mut *BRCA1* cDNA templates were mixed together in different ratios. The measured ratio is that obtained by real- time PCR and is consistent with the known mixture ratio. **C and D.** Amplification curve display of the specificity of WT and Mut specific primers on BRCA1 DNA templates. The Mut primer pair (blue line) shows very low efficiency of amplification of the WT template **(C)**. The WT specific primer pair (green line) has very low efficiency of amplification of the *BRCA1* 5382insC template **(D)**. **E and F** used cDNA templates from WT and mutant cells to validate the primers. The Mut primer pair (blue line) shows very low efficiency for amplification of the WT cDNA template **(E).** All three primers are able to amplify cDNA from heterozygous Mut cell cDNA (**F)**. **G and H:** Dissociation analysis was performed to analyze the specificity of amplification. The total BRCA1 and WT primer pairs yielded an amplification product from both WT and Mut cDNA at Tm 78.8°C. However, melting temperature of amplification product of Mut primer pair was 86.5°C (blue line in G) for WT cDNA and 78.8°C for Mut cDNA (blue line in H). **I:** Agarose gel analysis showed 89-bp specific band using the primer pair that is not specific to Mut or WT (the total) amplification but a 200-bp nonspecific band when the Mut-specific primer pair was used for amplification.

Figure S4

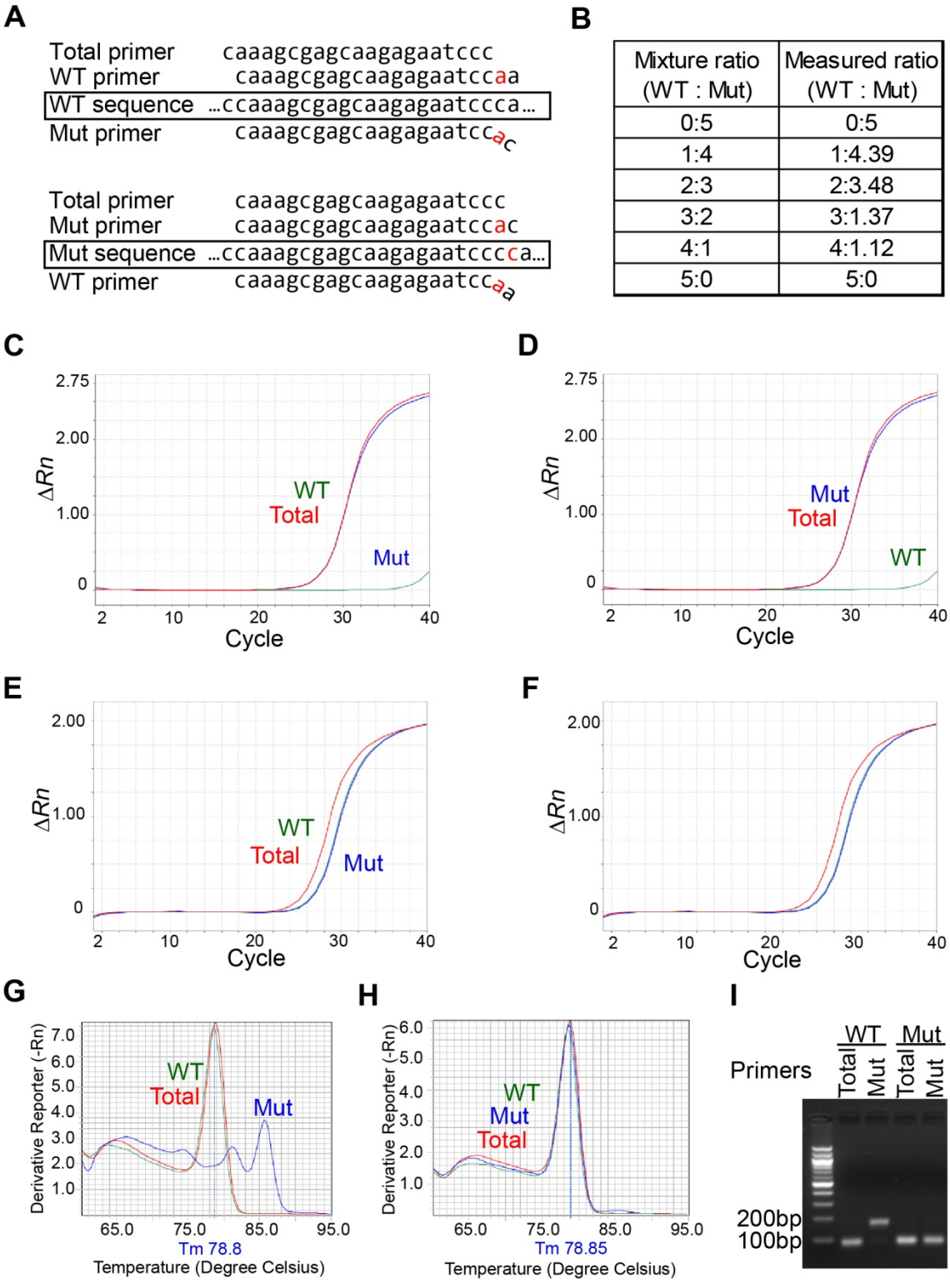
A
Total primer caaagcgagcaagagaatccc
WT primer caaagcgagcaagagaatccaa
WT sequence …ccaaagcgagcaagagaatcccca…
Mut primer caaagcgagcaagagaatccac
Total primer caaagcgagcaagagaatccc
Mut primer caaagcgagcaagagaatccac
Mut sequence …ccaaagcgagcaagagaatccccca…
WT primer caaagcgagcaagagaatccaa
B
Mixture ratio (WT : Mut)
Measured ratio (WT : Mut)
0:5 0:5
1:4 1:4.39
2:3 2:3.48
3:2 3:1.37
4:1 4:1.12
5:0 5:0
C
D
E
F
ΔRn
Cycle
WT
Total
Mut
G
H
Derivative Reporter (-Rn)
Tm 78.8
Tm 78.85
Temperature (Degree Celsius)
I
Primers
WT
Mut
Total
Mut
Total
Mut
200bp
100bp

Figure S5. Filtering whole-genome sequence for *de novo* mutations enriches for low-quality variants. To identify potential de novo mutations from all nonsynonymous mutations called in iPS cells (red bar), we excluded mutations also found in the fibroblasts and lymphoblasts from that same individual, leaving us with mutations unique to the iPS cell that are not found in the fibroblasts or lymphoblasts of that individual (green bar) and related family members (blue bar). Because de novo mutations are not likely to appear in the general population, we excluded variants observed in the 1000 Genomes Project, NHLBI Exome Variant Server, or "in-house" databases (purple bar). Finally, because *de novo* mutations are unlikely to occur in more than one cell line, we removed variants observed in more than one iPS cell line (orange bar). The majority of candidate de novo variants had quality scores <100 (dashed line). Unique de novo candidates with quality score >100 were considered *de novo* mutations.

Figure S5

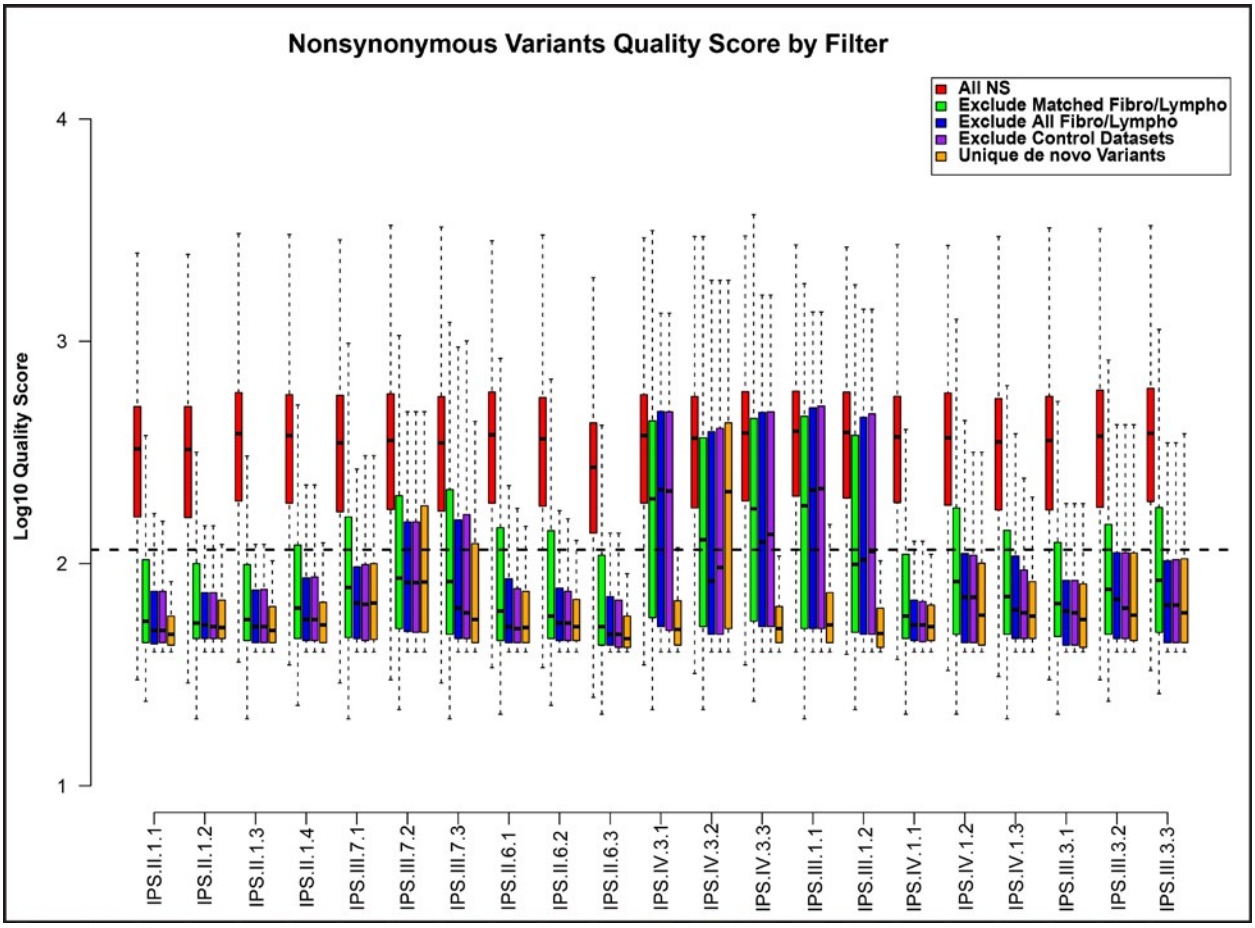
Nonsynonymous Variants Quality Score by Filter
All NS
Exclude Matched Fibro/Lympho
Exclude All Fibro/Lympho
Exclude Control Datasets
Unique de novo Variants
Log10 Quality Score
4
3
2
1
IPS.II.1.1
IPS.II.1.2
IPS.II.1.3
IPS.II.1.4
IPS.III.7.1
IPS.III.7.2
IPS.III.7.3
IPS.II.6.1
IPS.II.6.2
IPS.II.6.3
IPS.IV.3.1
IPS.IV.3.2
IPS.IV.3.3
IPS.III.1.1
IPS.III.1.2
IPS.IV.1.1
IPS.IV.1.2
IPS.IV.1.3
IPS.III.3.1
IPS.III.3.2
IPS.III.3.3

Table S1. Efficiency of Reprogramming Is Not Altered by the *BRCA1* mutation

BRCA1	Patient ID*	Round 1**	Round 2**	Round 3A*** (miRNA mix A)	Round 3B*** (miRNA mix B)
Wild Type	II.1	0	0	**25**	**14**
	III.1	0	0	**11**	**7**
	III.4	**6**	ND	ND	ND
	IV.1	0	**4**	ND	ND
BRCA1 5382insC	II.6	**10**	ND	ND	ND
	III.3	0	**6**	ND	ND
	III.7	0	**30**	ND	ND
	IV.3	**8**	ND	ND	ND

Values represent the frequency of iPS Cell Colony Formation per Number of Fibroblasts Transfected with Reprogramming Factors (iPS cell colony counts per 10,000 cells transfected). ND = no data (due to success in previous round).
***See Figure 1A for pedigree.**
****reprogramming factor mRNA transfection only.**
*****See methods for details of mRNA + miRNA mix A and mix B.**

Table S2. Expression of PKC-theta and Hormone Receptors in Breast Tumors

PKC-theta*	Total N (%)	ER/PR+ N (%)	ER/PR- N (%)	p53+ N (%)	Her2/Neu+ N (%)	Stg II N (%)	Stg III N (%)
1	20 (41)	11 (55)**	9 (28)**	8 (40)	6 (30)	11 (45)	9 (56)
2	16 (33)	4 (25)	12 (38)	7 (43)	4 (25)	5 (20)	5 (31)
3	13 (26)	2 (18)	11 (34)	8 (72)	6 (54)	8 (33)	2 (13)
2 + 3	29 (59)	6 (22)**	23 (72)**	15 (55)	10 (37)	13 (55)	7 (44)

*PKC-theta staining intensity was scored in all 49 tumors by two individuals (TSR and DR) with concordance. A score of 1 was weak, 2 intermediate, and 3 strong staining; 2+3 are all tumors with a score of 2 or 3.

** Hormone receptor positivity in breast tumors was more frequent in those tumors that were low for PKC-theta levels than in those tumors that had intermediate or strong PKC-theta staining ($P < 0.03$; two-tailed Fisher's exact test).

***There were 24 tumors classified as stage II and 16 tumors as stage III. There was no difference in PKC-theta distribution based on stage.

Table S4. Variant filtering to identify genome-wide *de novo* variants in iPS cell lines.

		iPS.II.1.1	iPS.II.1.2	iPS.II.1.3	iPS.II.1.4	iPS.II.6.1	iIPS.II.6.2	iPS.II.6.3	iPS.III.1.1	iPS.III.1.1
Nonsynonymous Mutations		7,112 (6,825)	7,104 (6,823)	7,722 (7,412)	7,636 (7,330)	7,822 (7,518)	7,662 (7,357)	6,517 (6,278)	7,989 (7,592)	7,932 (7,555)
Filtering	Exclude Matched Fibro/Lympho*	35 (26)	38 (26)	38 (30)	47 (34)	56 (49)	54 (41)	42 (40)	173 (93)	175 (97)
	Exclude All Fibro/Lympho**	24 (19)	25 (18)	22 (20)	29 (20)	31 (25)	28 (19)	21 (20)	158 (81)	150 (79)
	Exclude Control Datasets***	23 (18)	24 (17)	21 (20)	27 (19)	26 (23)	26 (17)	18 (18)	154 (78)	146 (76)
	Unique *de novo* Variants****	3 (1)	11 (5)	0 (0)	7 (0)	16 (13)	16 (7)	8 (8)	16 (7)	6 (3)

		iPS.III.3.1	iPS.III.3.2	iPS.III.3.3	iPS.III.4.1	iPS.III.4.2	iPS.III.7.1	iPS.III.7.2	iPS.III.7.3	iPS.IV.1.1	iPS.IV.1.2	iPS.IV.1.3
Nonsynonymous Mutations		7,360 (7,052)	7,498 (7,166)	7,633 (7,298)	7,766 (7,440)	7,625 (7,308)	7,543 (7,217)	7,668 (7,339)	7,580 (7,253)	7,784 (7,468)	7,669 (7,343)	7,483 (7,181)
Filtering	Exclude Matched Fibro/Lympho*	38 (27)	46 (26)	52 (35)	68 (52)	40 (30)	37 (23)	44 (31)	44 (33)	39 (29)	37 (25)	39 (30)
	Exclude All Fibro/Lympho**	12 (8)	23 (9)	20 (8)	51 (36)	17 (9)	19 (8)	27 (17)	21 (15)	16 (10)	19 (10)	17 (13)
	Exclude Control Datasets***	11 (8)	21 (9)	19 (8)	43 (28)	14 (7)	18 (7)	22 (16)	20 (14)	14 (8)	16 (8)	14 (12)
	Unique *de novo* Variants****	8 (5)	18 (7)	17 (7)	42 (28)	11 (6)	16 (7)	21 (15)	12 (7)	10 (6)	13 (6)	8 (7)

		iPS.IV.3.1	iPS.IV.3.2	iPS.IV.3.3
Nonsynonymous Mutations		7,690 (7,329)	7,452 (7,126)	7,760 (7,402)
Filtering	Exclude Matched Fibro/Lympho*	246 (177)	199 (146)	247 (182)
	Exclude All Fibro/Lympho**	165 (107)	131 (83)	160 (105)
	Exclude Control Datasets***	152 (95)	126 (78)	148 (93)
	Unique *de novo* Variants****	6 (2)	125 (77)	2 (0)

*Variants were excluded if present in the genome sequence of matched fibroblasts or lymphoblasts, or family members. Exception: IV.3 iPS cell lines had no matched lymphoblast sequence.
**Variants were excluded if present in fibroblast or lymphoblast sequences of other family members
***Variants were excluded if present in the NHLBI Exome Variant Server, the 1000 Genomes Project database, publically available whole-genome sequences provided by Complete Genomics, or an "in-house" database of inherited mutations from unrelated samples whole-genome sequenced by Complete Genomics or whole-exome sequenced using Illumina capture and sequencing technology.
****Variants present in more than one iPS cell lines were excluded.

SNPs+Indels together are shown without parentheses and SNPs alone are in parentheses.

Table S5. Number of unique *de novo* iPS cell nonsynonymous mutations			
iPS cell line	*BRCA1* status*	*De novo* SNPs+Indels**	*De novo* SNPs***
iPS.II.1.1	Wildtype	7	0
iPS.II.1.2	Wildtype	3	1
iPS.II.1.3	Wildtype	11	5
iPS.II.1.4	Wildtype	0	0
iPS.II.6.1	5382insC	16	13
iPS.II.6.2	5382insC	16	7
iPS.II.6.3	5382insC	8	8
iPS.III.1.1	Wildtype	6	3
iPS.III.1.2	Wildtype	16	7
iPS.III.3.1	5382insC	8	5
iPS.III.3.2	5382insC	18	7
iPS.III.3.3	5382insC	17	7
iPS.III.7.1	5382insC	16	7
iPS.III.7.2	5382insC	21	15
iPS.III.7.3	5382insC	12	7
iPS.IV.1.1	Wildtype	10	6
iPS.IV.1.2	Wildtype	13	6
iPS.IV.1.3	Wildtype	8	7
iPS.IV.3.1	5382insC	6	2
iPS.IV.3.2	5382insC	125	77

*Wildtype = wildtype at both *BRCA1* alleles; 5382insC = heterozygous for the *BRCA1* 5382insC mutation

***de novo* single nucleotide polymorphisms and insertions/deletions

****de novo* single nucleotide polymorphisms only

Table S6. Increased Frequency of *de novo* mutations genome-wide in iPS.IV.3.2			
iPS cell line	*BRCA1* status*	*De novo* SNPs+Indels**	*De novo* SNPs***
iPS.II.1.1	Wildtype	663	140
iPS.II.1.2	Wildtype	650	176
iPS.II.1.3	Wildtype	1224	667
iPS.II.1.4	Wildtype	655	164
iPS.II.6.1	5382insC	1568	957
iPS.II.6.2	5382insC	1523	958
iPS.II.6.3	5382insC	1292	883
iPS.III.1.1	Wildtype	1509	939
iPS.III.1.2	Wildtype	1430	828
iPS.III.3.1	5382insC	1230	699
iPS.III.3.2	5382insC	1561	907
iPS.III.3.3	5382insC	1468	922
iPS.III.7.1	5382insC	1775	1197
iPS.III.7.2	5382insC	1708	1243
iPS.III.7.3	5382insC	1594	1072
iPS.IV.1.1	Wildtype	1735	1121
iPS.IV.1.2	Wildtype	1251	819
iPS.IV.1.3	Wildtype	1229	712
iPS.IV.3.1	5382insC	922	324
iPS.IV.3.2	5382insC	19016	14328
iPS.IV.3.3	5382insC	924	310

*Wildtype = wildtype at both *BRCA1* alleles; 5382insC = heterozygous for the *BRCA1* 5382insC mutation

***de novo* single nucleotide polymorphisms and insertions/deletions

****de novo* single nucleotide polymorphisms only.