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Lab Resource: Multiple Cell Lines

Generation of heterozygous and homozygous hESC H9 sublines carrying inactivating mutations in *RB1*

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A B S T R A C T

Inactivation of the tumor suppressor gene *RB1* is causal for development of retinoblastoma, a tumor of the neural retina arising in children under the age of five. In addition, secondary *RB1* mutations are found in many other tumor types. To investigate retinoblastoma formation in vitro, stem cells with inactivated *RB1* can be differentiated into neural retina. To enable such studies, two sublines of hESC line H9 carrying mutations in *RB1* exon 3 in heterozygous or homozygous state were generated and characterized. Homozygous mutation led to loss of RB1 protein expression.

Resource table

Unique stem cell lines identifier	WAe009-A-12 WAe009-A-13
Alternative names of stem cell lines	C7 (homozygous deletion, WAe009-A-12) G12LS (heterozygous deletion, WAe009-A-13)
Institution	University Hospital Essen, University Duisburg-Essen, Essen, Germany
Contact information of distributor	Dr. Laura Steenpass, laura.steenpass@uni-due.de Dr. Deniz Kanber, deniz.kanber@uni-due.de
Type of cell lines	ESC
Origin	Human
Cell Source	Human ESC line H9 purchased from WiCell
Clonality	Clonal
Method of reprogramming	N/A
Multiline rationale	clones selected for deletion in heterozygous and homozygous state
Gene modification	YES
Type of modification	Indels in *RB1* exon 3
Associated disease	Retinoblastoma
Gene/locus	*RB1*, chromosome 13q14.2
Method of modification	CRISPR/Cas9 nuclease
Name of transgene or resistance	N/A
Inducible/constitutive system	N/A
Date archived/stock date	C7 12.05.2018 G12LS 12.05.2018
Cell line repository/bank	N/A
Ethical approval	Approval obtained from the Robert-Koch Institute, Berlin, Germany (Az.3.04.02/0101) and from the local Ethical Review Board University Duisburg-Essen (16–7215-BO)

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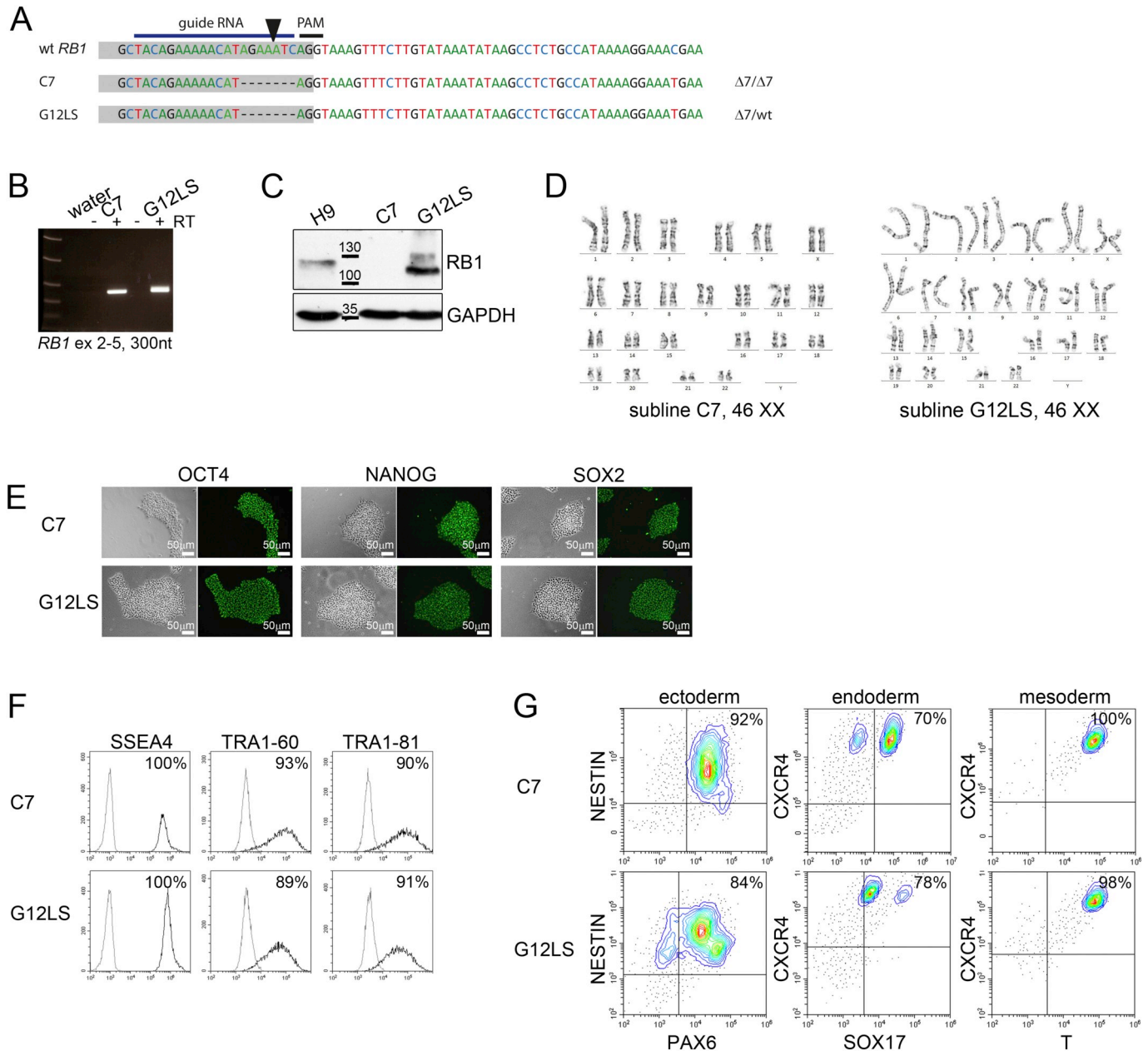

Fig. 1. Generation and characterization of RB1 modified H9 sublines C7 (WAe009-A-12) and G12LS (WAe009-A-13).

Table 1
Summary of lines.

hESC line names	Abbreviation in figures	Gender	Age	Ethnicity	Genotype of locus	Disease
H9_RB1ex3_C7 (WAe009-A-12)	C7	female	blastocyst	NA	RB1 −/−	Retinoblastoma
H9_RB1ex3_G12LS (WAe009-A-13)	G12LS	female	blastocyst	NA	RB1 +/−	Retinoblastoma

Resource utility

With 3D organoid models, modelling development of retinoblastoma in human neural retina cells in vitro has become feasible. Retinoblastoma is caused by inactivation of the tumor suppressor *RB1*. To generate appropriate starting cells for such experiments, the *RB1* gene was inactivated by insertion of indels into exon 3 using CRISPR/Cas9.

Resource details

H9 hESCs were modified in *RB1* exon 3 by insertion of random indel mutations using the CRISPR/Cas9 nuclease system (Fig. 1A, positions of guide RNA and PAM sequence are shown above the sequence alignment, grey box indicates 3′-end of *RB1* exon 3). 288 clonal cell lines were screened using GeneScan fragment length analysis. In total 88 clones showed modifications and eight sublines with heterozygous, two with compound heterozygous and one with homozygous mutations were