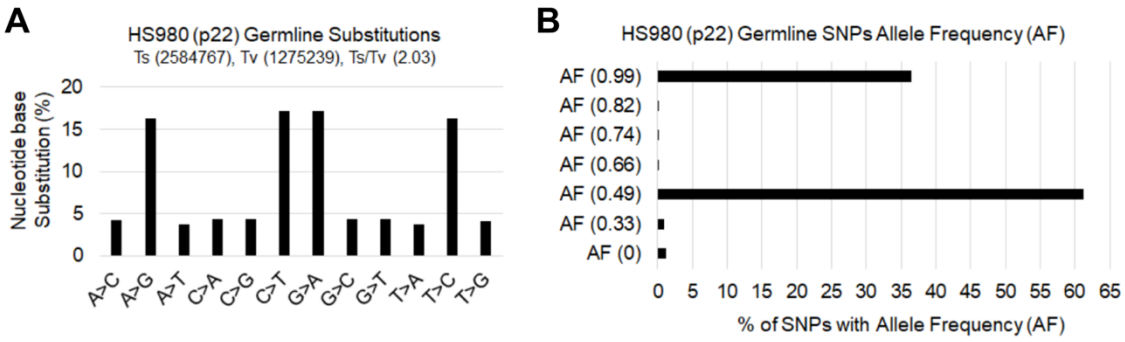
# SUPPLEMENTAL INFORMATION

# Preclinical safety studies of human embryonic stem cell-derived retinal pigment epithelial cells for the treatment of age-related macular degeneration

**Petrus-Reurer et al.**

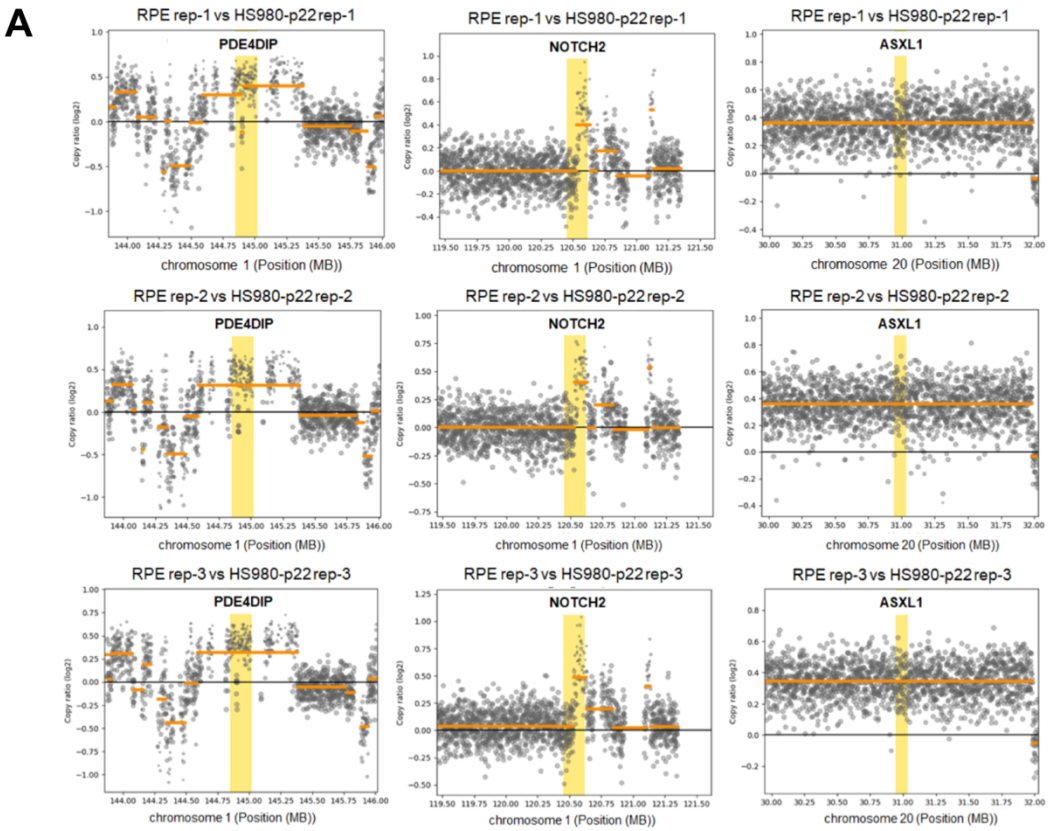
**Supplemental Figures S1 – S5**

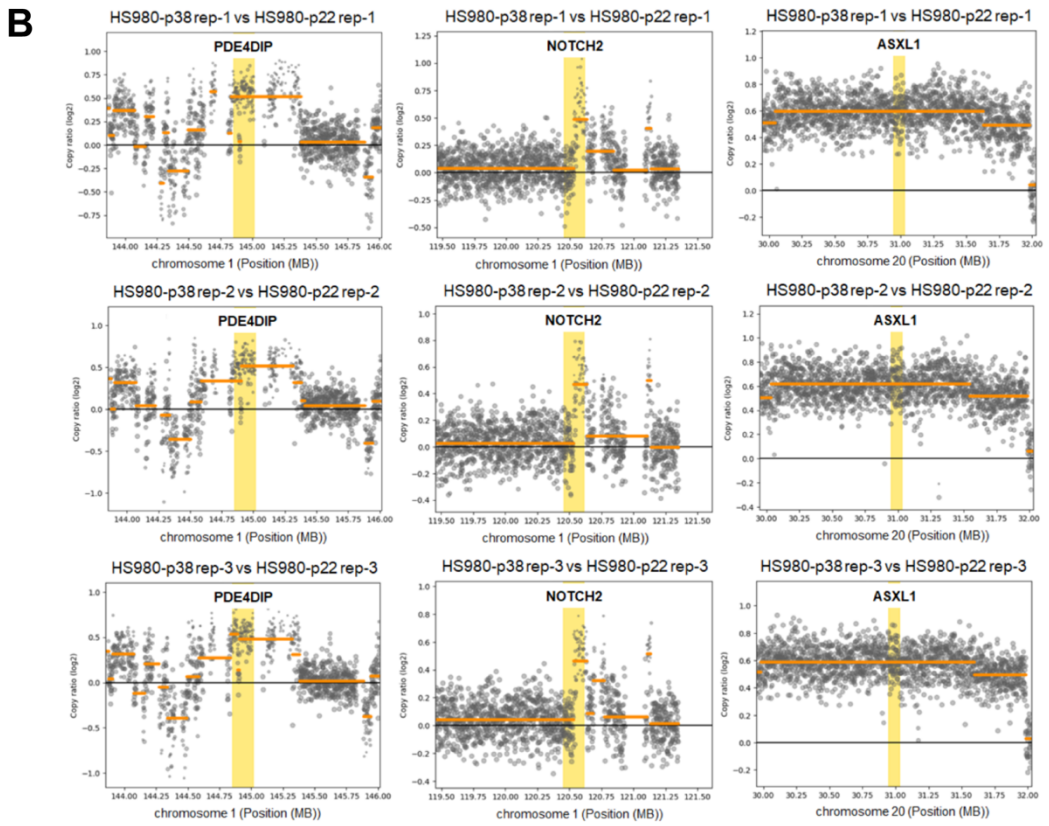
**Supplemental Tables S1 – S7**




**SUPPLEMENTAL FIGURE S1. Annotation of germline SNVs.**

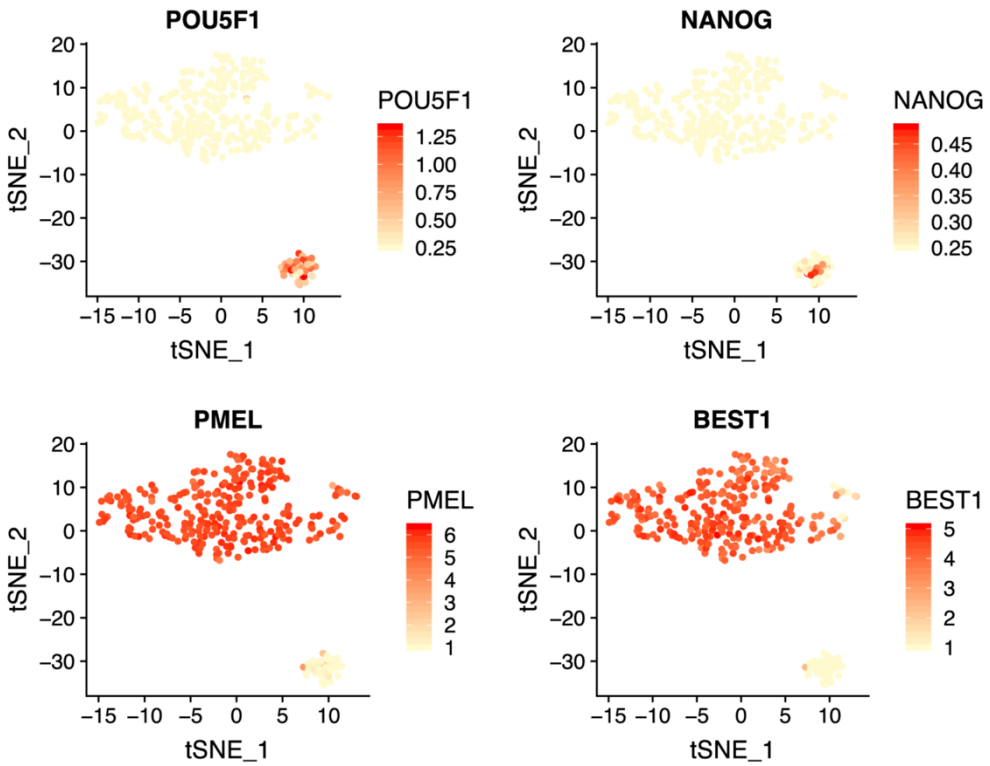
**(A)** Bar charts showing the relative percentage of mutational subtypes for the germline SNVs in HS980 (p22) sample. **(B)** Bar charts showing the relative percentage of germline SNP allele frequency for the HS980 (p22) sample.


A
RPE rep-1 vs HS980-p22 rep-1
PDE4DIP
NOTCH2
ASXL1
RPE rep-2 vs HS980-p22 rep-2
PDE4DIP
NOTCH2
ASXL1
RPE rep-3 vs HS980-p22 rep-3
PDE4DIP
NOTCH2
ASXL1
Copy ratio (log2)
chromosome 1 (Position (MB))
chromosome 20 (Position (MB))


B
HS980-p38 rep-1 vs HS980-p22 rep-1
PDE4DIP
NOTCH2
ASXL1
HS980-p38 rep-2 vs HS980-p22 rep-2
PDE4DIP
NOTCH2
ASXL1
HS980-p38 rep-3 vs HS980-p22 rep-3
PDE4DIP
NOTCH2
ASXL1
Copy ratio (log2)
chromosome 1 (Position (MB))
chromosome 20 (Position (MB))

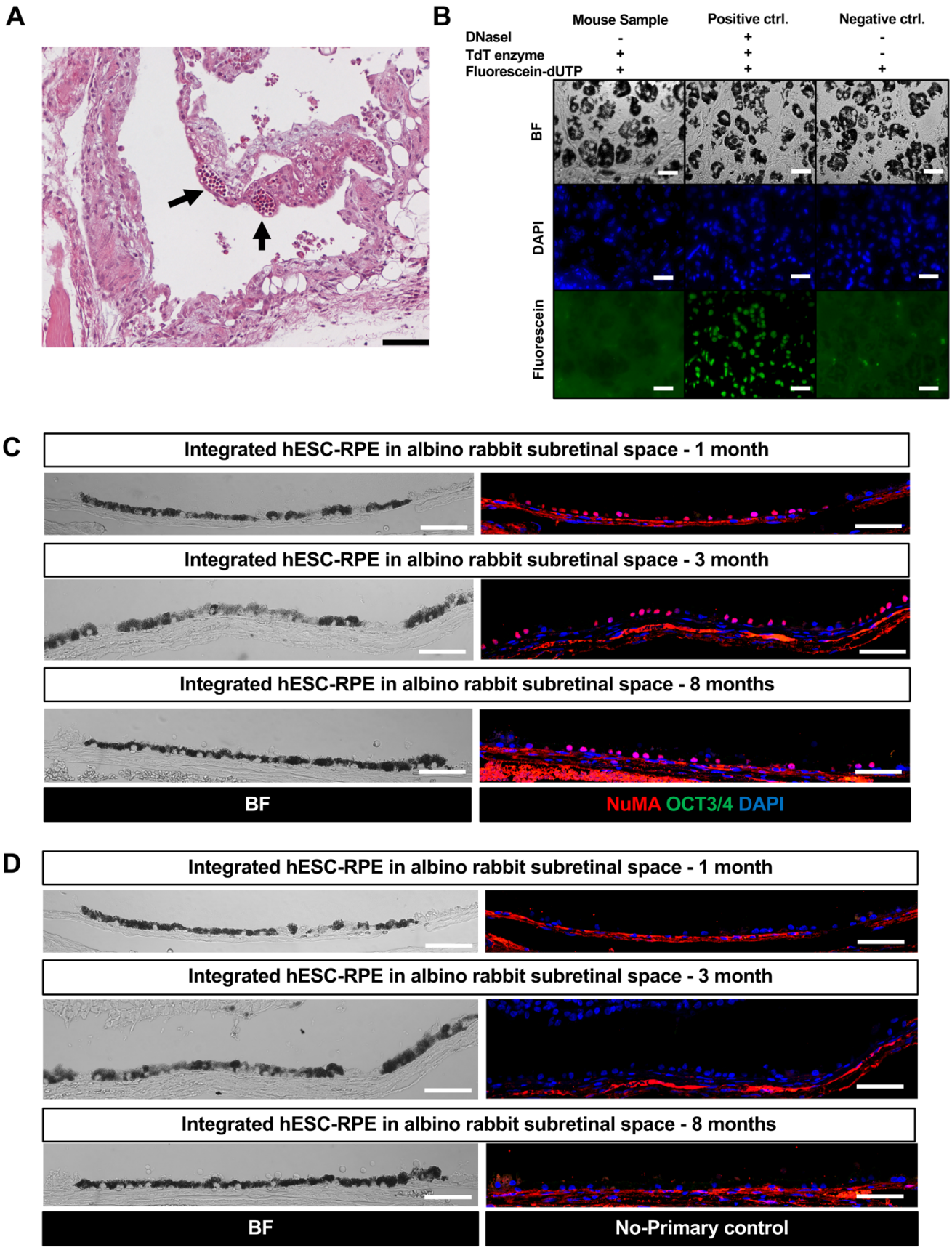
**SUPPLEMENTAL FIGURE S2. Copy number profile for cancer-driver genes (*PDE4DIP, NOTCH2 and ASXL1*) in hESC-RPE and HS980 (p38) samples.**

**(A)** Plots showing copy number ratio by CNVkit for cancer-driver genes *PDE4DIP, NOTCH2* and *ASXL1* for hESC-RPE samples. X-axis represents log2 copy ratio of hESC-RPE samples compared with their respective HS980 (p22) control samples and Y-axis represents genomic position. **(B)** Plots showing copy number ratio by CNVkit for *PDE4DIP, NOTCH2* and *ASXL1* genes for HS980 (p38) samples. X-axis represents log2 copy ratio of HS980 (p38) samples compared with their respective HS980 (p22) control samples and Y-axis represents genomic position.




**SUPPLEMENTAL FIGURE S3. Single cell RNA sequencing analysis of hESC-RPE and hESC.**

tSNE plots showing expression of selective hESC and RPE markers in hESC and hESC-RPE cell clusters.

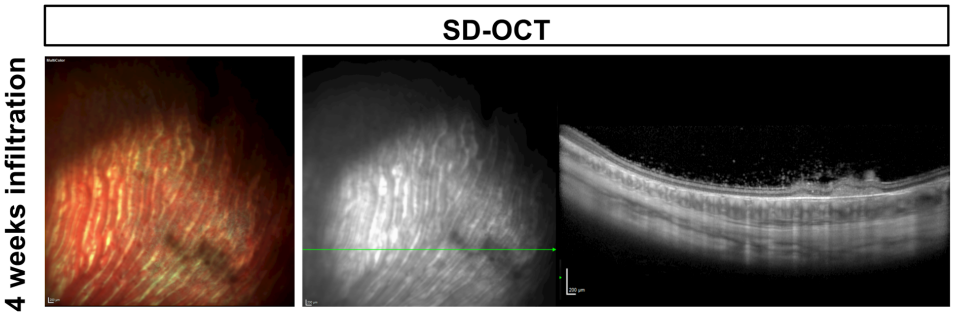

A
B
Mouse Sample
Positive ctrl.
Negative ctrl.
DNaseI
TdT enzyme
Fluorescein-dUTP
BF
DAPI
Fluorescein
C
Integrated hESC-RPE in albino rabbit subretinal space - 1 month
Integrated hESC-RPE in albino rabbit subretinal space - 3 month
Integrated hESC-RPE in albino rabbit subretinal space - 8 months
BF
NuMA OCT3/4 DAPI
D
Integrated hESC-RPE in albino rabbit subretinal space - 1 month
Integrated hESC-RPE in albino rabbit subretinal space - 3 month
Integrated hESC-RPE in albino rabbit subretinal space - 8 months
BF
No-Primary control

**SUPPLEMENTAL FIGURE S4. Evaluation of hESC-RPE injected subcutaneously into NOG mice or subretinally into albino rabbits.**

**(A)** HE staining image showing dilated vascular profiles densely packed with nucleated cells with an intensely eosinophilic cytoplasm representing primitive hematopoiesis. Cells with hepatoid features border these yolk sac blood islands. **(B)** Representative BF and immunofluorescence images showing TUNEL-negative (alive) collected hESC-RPE cells 7 months after subcutaneous transplantation into NOG mice. **(C)** Representative BF and immunofluorescent images of NuMA and OCT3/4 staining of integrated hESC-RPE in the rabbit subretinal space at 1, 3 and 8 months after transplantation. **(D)** No-primary control stainings of the same specimens showed in Figure S4C.

Scale bars: (A, B) = 100 µm; (C, D) = 50 µm

**A**

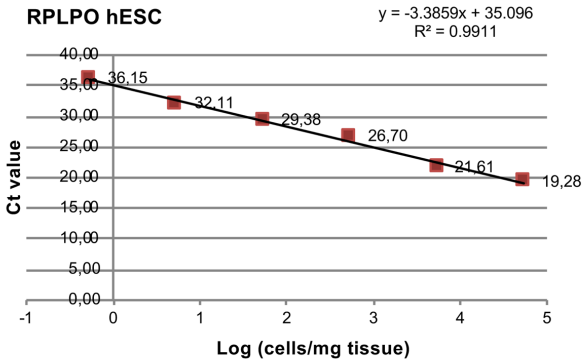


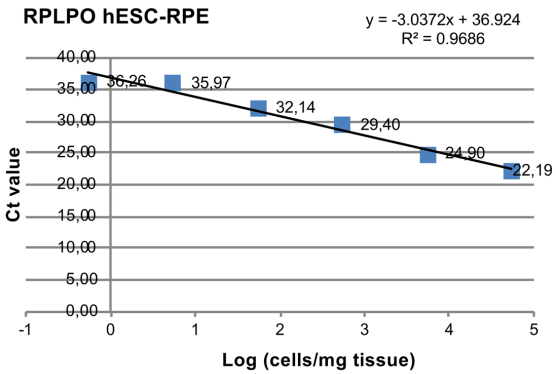

**B**

| Nr hESC | Cells/mg | Log (Cells/mg) | Ct RPLPO (Human) |
|---|---|---|---|
| Rb Ki_10 | 0.54 | -0.27 | 36.15 |
| Rb Ki_100 | 5.4 | 0.73 | 32.11 |
| Rb Ki_1000 | 54 | 1.73 | 29.38 |
| Rb Ki_10000 | 540 | 2.73 | 26.70 |
| Rb Ki_100000 | 5400 | 3.73 | 21.61 |
| Rb Ki_1000000 | 54000 | 4.73 | 19.28 |

**C**

| Nr hESC-RPE | Cells/mg | Log (Cells/mg) | Ct RPLPO (Human) |
|---|---|---|---|
| Rb Ki_10 | 0.54 | -0.27 | 36.26 |
| Rb Ki_100 | 5.4 | 0.73 | 35.97 |
| Rb Ki_1000 | 54 | 1.73 | 32.14 |
| Rb Ki_10000 | 540 | 2.73 | 29.40 |
| Rb Ki_100000 | 5400 | 3.73 | 24.90 |
| Rb Ki_1000000 | 54000 | 4.73 | 22.19 |



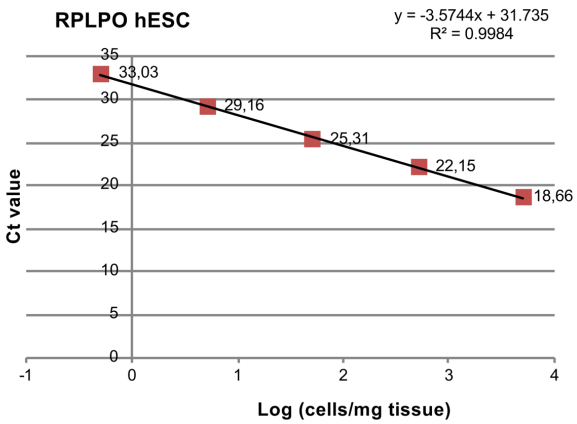

**Detection limit: 1 cell in 1.85mg of rabbit tissue**

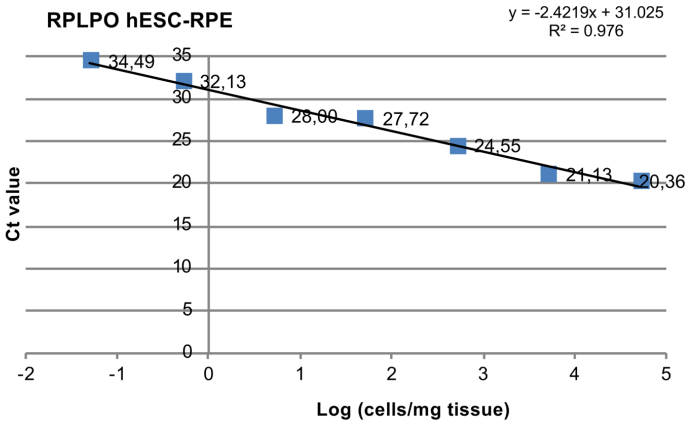
**D**

| Nr hESC | Cells/mg | Log (Cells/mg) | Ct RPLPO (Human) |
|---|---|---|---|
| Ms WT Li_10 | 0.5 | -0.30 | 33.03 |
| Ms WT Li_100 | 5 | 0.69 | 29.16 |
| Ms WT Li_1000 | 50 | 1.69 | 25.31 |
| Ms WT Li_10000 | 500 | 2.69 | 22.15 |
| Ms WT Li_100000 | 5000 | 3.69 | 18.66 |

**E**

| Nr hESC-RPE | Cells/mg | Log (Cells/mg) | Ct RPLPO (Human) |
|---|---|---|---|
| Ms WT Li_1 | 0.05 | -1.30 | 34.49 |
| Ms WT Li_10 | 0.5 | -0.30 | 32.13 |
| Ms WT Li_100 | 5 | 0.69 | 28 |
| Ms WT Li_1000 | 50 | 1.69 | 27.72 |
| Ms WT Li_10000 | 500 | 2.69 | 24.55 |
| Ms WT Li_100000 | 5000 | 3.69 | 21.13 |
| Ms WT Li_1000000 | 50000 | 4.69 | 20.36 |




**Detection limit: 1 cell in 20mg of mouse tissue**

**SUPPLEMENTAL FIGURE S5. hESC and hESC-RPE cell spiking in rabbit and mouse tissues for biodistribution studies**.

**(A)** SD-OCT image showing infiltration in the subretinal space 4 weeks after injection of hESC-RPE cells. **(B)** Detection of RPLPO transcripts by qPCR after serial dilutions of hESC in rabbit (Rb) kidney (Ki) tissue. **(C)** Detection of RPLPO transcripts by qPCR after serial dilutions of hESC-RPE in rabbit kidney tissue. **(D)** Detection of RPLPO transcripts by qPCR after serial dilutions of hESC in wild-type (WT) mouse (Ms) liver (Li) tissue. **(E)** Detection of RPLPO transcripts by qPCR after serial dilutions of hESC-RPE in wild-type mouse liver tissue.

Nr: Number

Scale bars: (A) = 200 µm

**Supplemental Table S1. Tumorigenicity studies design. Relation of injected cell type, number of cells and monitoring time in each group of NOG mice.**

| Mice group | Number of mice | Cell type | Number of cells | Monitoring period |
|---|---|---|---|---|
| 1 | 10 | hESC | $1x10^1$ | 4 months |
| 2 | 10 | hESC | $1x10^2$ | 4 months |
| 3 | 10 | hESC | $1x10^3$ | 4 months |
| 4 | 10 | hESC | $1x10^4$ | 4 months |
| 5 | 10 | hESC | $1x10^5$ | 4 months |
| 6 | 10 | hESC | $1x10^6$ | 4 months |
| 7 | 10 | 3-week EBs | $1x10^7$ | 7 months |
| 8 | 10 | 5-week EBs | $1x10^7$ | 7 months |
| 9 | 10 | hESC-RPE | $1x10^7$ | 7 months |

**Supplemental Table S2. Whole-genome DNA sequence alignment statistics for HS980 (p22), hESC-RPE and HS980 (p38) samples.**

| Sample | Total Reads (paired-end) | Aligned Reads (both in pairs) | Coverage (mean) | Non-duplicated (read pairs) |
|---|---|---|---|---|
| HS980 p22 rep-1 | 824,883,240 | 812,036,402(98.44%) | 38.24 | 352,832,529(84.7%) |
| HS980 p22 rep-1 | 839,393,930 | 832,313,573(99.16%) | 39.27 | 360,164,708(85.3%) |
| HS980 p22 rep-1 | 787,107,006 | 782,885,654(99.46%) | 36.91 | 362,620,161(91.9%) |
| hESC-RPE rep-1 | 834,164,276 | 793,489,002(95.12%) | 37.56 | 364,737,787(83.6%) |
| hESC-RPE rep-2 | 768,238,398 | 764,986,043(99.58%) | 36.26 | 354,434,223(92.1%) |
| hESC-RPE rep-3 | 836,031,077 | 830,641,424(99.36%) | 39.12 | 376,953,431(89.1%) |
| HS980 p38 rep-1 | 831,868,835 | 824,536,555(99.12%) | 38.96 | 335,881,565(80.3%) |
| HS980 p38 rep-2 | 788,437,294 | 783,554,320(99.38%) | 37.06 | 362,663,677(91.7%) |
| HS980 p38 rep-3 | 775,465,729 | 771,450,994(99.48%) | 36.63 | 359,163,976(92.4%) |

**Supplemental Table S3. HS980 (p22) Germline SNV overlapping to ClinVar Database.**

**# HS980 p22 combined all three replicates germline SNVs**
# SNVs overlapping to ClinVar clinical Databases (release 29th October 2017)
**# SNVs reported as CLNSIG (clinical significance) ="Pathogenic"**
# CLNDN; Description="ClinVar's preferred disease name for the concept specified by disease identifiers in CLNDISDB"
# GMAF = Global Minor Allele Frequency
# Common Variant (minor allele frequency (GMAF) of >= 0.01 in at least one 1000 Genomes Phase III major population with at least two individuals from different families having same minor allele)

| Chr_No | Position | dbSNP_rs-ID | ClinVar-ID | REF | ALT | GMAF | Population Status | Gene-ID | CLNSIG | CLNDN |
|---|---|---|---|---|---|---|---|---|---|---|
| chr1 | 55504650 | rs2479409 | 440703 | G | A | 0.3986 | Common Variant | PCSK9 | Benign/Pathogenic | Familial_hypercholesterolemia |
| chr1 | 198796120 | rs12406470 | 427753 | C | T | 0.4816 | Common Variant | MIR181A1HG | Pathogenic | Acute_myeloid_leukemia_with_maturation |
| chr1 | 198867678 | rs60639710 | 427754 | G | T | 0.2043 | Common Variant | MIR181A1HG | Pathogenic | Acute_myeloid_leukemia_with_maturation |
| chr1 | 198868084 | rs10800597 | 427755 | G | A | 0.2045 | Common Variant | MIR181A1HG | Pathogenic | Acute_myeloid_leukemia_with_maturation |
| chr1 | 198869514 | rs10800598 | 427756 | T | C | 0.1799 | Common Variant | MIR181A1HG | Pathogenic | Acute_myeloid_leukemia_with_maturation |
| chr4 | 6295693 | rs6446482 | 4528 | C | G | 0.2788 | Common Variant | WFS1 | Pathogenic | Diabetes_mellitus,_noninsulin-dependent |
| chr4 | 187158034 | rs3733402 | 12037 | G | A | 0.3954 | Common Variant | KLKB1 | Pathogenic | Prekallikrein_deficiency |
| chr5 | 176520243 | rs351855 | 16326 | G | A | 0.2995 | Common Variant | FGFR4 | Pathogenic | Cancer_progression_and_tumor_cell_motility |
| chr8 | 11606312 | rs3735819 | 433016 | T | C | 0.1284 | Common Variant | GATA4 | Pathogenic | Congenital_heart_disease |
| chr8 | 11606364 | rs10503425 | 433017 | G | C | 0.0493 | Common Variant | GATA4 | Pathogenic | Congenital_heart_disease |
| chr8 | 11614769 | rs12156163 | 433023 | C | T | 0.1142 | Common Variant | GATA4 | Pathogenic | Congenital_heart_disease |
| chr8 | 11615695 | rs745379 | 433015 | A | G | 0.2702 | Common Variant | GATA4 | Pathogenic | Congenital_heart_disease |
| chr8 | 11616836 | rs804290 | 433024 | G | A | 0.117 | Common Variant | GATA4 | Pathogenic | Congenital_heart_disease |
| chr8 | 11617240 | rs12458 | 433026 | A | T | 0.4 | Common Variant | GATA4 | Pathogenic | Congenital_heart_disease |
| chr10 | 54531235 | rs1800450 | 14350 | C | T | 0.122 | Common Variant | MBL2 | Pathogenic | Mannose-binding_protein_deficiency |
| chr11 | 48145375 | rs1566734 | 8690 | A | C | 0.1899 | Common Variant | PTPRJ | Pathogenic | Carcinoma_of_colon |
| chr16 | 16291858 | rs12929920 | 433383 | G | C | 0.2686 | Common Variant | ABCC6 | Benign/Pathogenic | Pseudoxanthoma_elasticum |
| chr16 | 16291871 | rs9940089 | 433382 | G | C | 0.2121 | Common Variant | ABCC6 | Benign/Pathogenic | Pseudoxanthoma_elasticum |
| chr17 | 32579788 | rs1024611 | 14207 | A | G | 0.3636 | Common Variant | CCL2 | Pathogenic | Coronary_artery_disease |
| chr20 | 23618427 | rs1064039 | 5635 | C | T | 0.2123 | Common Variant | CST3 | Pathogenic | Age-related_macular_degeneration_11 |

Highlighted in grey: genes with some clinical relevance reported for AMD in ClinVar

**Supplemental Table S4A. HS980 (p22) Germline SNV overlapping to COSMIC Cancer Gene Census.**

# HS980 p22 Germline SNVs common to three replicates
# SNVs reported in COSMIC database (Catalog of Somatic Mutation in Cancer - release v83; Coding; 07-11-2017)
# SNVs with "FATHMM" prediction as "Pathogenic"
**# SNVs within Cancer Driver Genes (COSMIC Cancer Gene Census)**
# GMAF in population = Global Minor Allele Frequency
# Common Variant (minor allele frequency (GMAF) of >= 0.01 in at least one 1000 Genomes Phase III major population)

| Chr_No | Position | dbSNP_rs-ID | COSMIC-ID | REF | ALT | GMAF | Population Status | FATHMM SCORE | Gene-ID | BASE CHANGE | AA CHANGE | Substitution Type |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| chr2 | 29416481 | rs1881420 | COSM1130802 | T | C | 0.4151 | Common Variant | 0.92214 | ALK | c.4472A>G | p.K1491R | Missense |
| chr2 | 29940529 | rs2246745 | COSM4416269 | A | T | 0.4107 | Common Variant | 0.9054 | ALK | c.702T>A | p.P234P | coding silent |
| chr2 | 141116447 | rs35546150 | COSM5019952 | G | T | 0.0449 | Common Variant | 0.86876 | LRP1B | c.11200C>A | p.Q3734K | Missense |
| chr2 | 141274576 | rs4954672 | COSM4001201 | T | C | 0.6424 | Common Variant | 0.93919 | LRP1B | c.8031A>G | p.Q2677Q | coding silent |
| chr2 | 212587119 | rs77309171 | COSM4583147 | T | C | 0.002 | Common Variant | 0.86171 | ERBB4 | c.882A>G | p.P294P | coding silent |
| chr3 | 37053568 | rs1799977 | COSM1131469 | A | G | 0.1296 | Common Variant | 0.80196 | MLH1 | c.655A>G | p.I219V | Missense |
| chr3 | 128204951 | rs2335052 | COSM445531 | C | T | 0.2328 | Common Variant | 0.91046 | GATA2 | c.490G>A | p.A164T | Missense |
| chr4 | 55152040 | rs2228230 | COSM22413 | C | T | 0.2404 | Common Variant | 0.88315 | PDGFRA | c.2472C>T | p.V824V | coding silent |
| chr4 | 55979558 | rs2305948 | COSM1131107 | C | T | 0.1526 | Common Variant | 0.96877 | KDR | c.889G>A | p.V297I | Missense |
| chr4 | 187630590 | rs3733415 | COSM4416272 | G | A | 0.2614 | Common Variant | 0.95514 | FAT1 | c.392C>T | p.A131V | Missense |
| chr5 | 56177443 | rs702689 | COSM4003565 | G | A | 0.4764 | Common Variant | 0.98239 | MAP3K1 | c.1927G>A | p.D643N | Missense |
| chr5 | 56177743 | rs832582 | COSM4416191 | G | A | 0.7083 | Common Variant | 0.76719 | MAP3K1 | c.2227G>A | p.V743I | Missense |
| chr5 | 112162854 | rs2229992 | COSM1432175 | T | C | 0.51 | Common Variant | 0.83456 | APC | c.1458T>C | p.Y486Y | coding silent |
| chr5 | 112176559 | rs866006 | COSM6475354 | T | G | 0.333 | Common Variant | 0.71742 | APC | c.5268T>G | p.S1756S | coding silent |
| chr7 | 55249063 | rs1050171 | COSM1451600 | G | A | 0.4327 | Common Variant | 0.95009 | EGFR | c.2361G>A | p.Q787Q | coding silent |
| chr7 | 116340262 | rs33917957 | COSM5020653 | A | G | 0.0329 | Common Variant | 0.86365 | MET | c.1124A>G | p.N375S | Missense |
| chr7 | 128845511 | rs111694017 | COSM1738148 | G | A | 0.0024 | Common Variant | 0.90625 | SMO | c.808G>A | p.V270I | Missense |
| chr7 | 138556013 | rs776449063 | COSM5434577 | G | A | 0.00022(ExAC) | NOT Common | 0.84266 | KIAA1549 | c.4441C>T | p.R1481W | Missense |
| chr8 | 69020496 | rs4260880 | COSM3763384 | T | C | 0.4798 | Common Variant | 0.85007 | PREX2 | c.2868T>C | p.S956S | coding silent |
| chr9 | 117846570 | rs2274836 | COSM3763575 | C | T | 0.4641 | Common Variant | 0.72876 | TNC | c.2049G>A | p.E683E | coding silent |
| chr9 | 135985796 | rs3761824 | COSM4163477 | C | T | 0.2486 | Common Variant | 0.97073 | RALGDS | c.372G>A | p.V124V | coding silent |
| chr11 | 102201848 | rs17878663 | COSM3998183 | G | A | 0.0699 | Common Variant | 0.70352 | BIRC3 | c.1200G>A | p.Q400Q | coding silent |
| chr12 | 25362777 | rs1137282 | COSM3753105 | A | G | 0.1755 | Common Variant | 0.76767 | KRAS | c.519T>C | p.D173D | coding silent |
| chr12 | 121416650 | rs1169288 | COSM430522 | A | C | 0.2985 | Common Variant | 0.91018 | HNF1A | c.79A>C | p.I27L | Missense |
| chr12 | 121435342 | rs2259820 | COSM3931546 | C | T | 0.3167 | Common Variant | 0.9731 | HNF1A | c.1375C>T | p.L459L | coding silent |
| chr12 | 121435427 | rs2464196 | COSM4984989 | G | A | 0.3177 | Common Variant | 0.82717 | HNF1A | c.1460G>A | p.S487N | Missense |
| chr13 | 21562948 | rs558614 | COSM432208 | G | A | 0.3704 | Common Variant | 0.96847 | LATS2 | c.971C>T | p.A324V | Missense |
| chr13 | 28624294 | rs1933437 | COSM5019176 | G | A | 0.5587 | Common Variant | 0.92594 | FLT3 | c.680C>T | p.T227M | Missense |
| chr14 | 38061742 | rs7144658 | COSM3753953 | C | T | 0.417 | Common Variant | 0.83972 | FOXA1 | c.247G>A | p.A83T | Missense |
| chr14 | 105239894 | rs1130233 | COSM3765730 | C | T | 0.3225 | Common Variant | 0.71625 | AKT1 | c.726G>A | p.E242E | coding silent |
| chr16 | 9943666 | rs2229193 | COSM3999994 | C | T | 0.2258 | Common Variant | 0.73792 | GRIN2A | c.1275G>A | p.L425L | coding silent |
| chr19 | 42799049 | rs1052023 | COSM3756833 | C | T | 0.1198 | Common Variant | 0.78474 | CIC | c.4533C>T | p.I1511I | coding silent |
| chr20 | 40714479 | rs2016647 | COSM3758574 | G | A | 0.144 | Common Variant | 0.91263 | PTPRT | c.3927C>T | p.Y1309Y | coding silent |
| chrX | 44938563 | rs20539 | COSM1179848 | G | A | 0.2217 | Common Variant | 0.88609 | KDM6A | c.3111G>A | p.Q1037Q | coding silent |
| chrX | 153629155 | rs4909 | COSM4590446 | A | G | 0.6689 | Common Variant | 0.81036 | RPL10 | c.605A>G | p.N202S | Missense |

Highlighted in grey: genes with SNV non-common to population

**Supplemental Table S4B. HS980 (p22) Germline SNV overlapping to Bailey MH et al, Cancer Driver Genes.**

# HS980 p22 Germline SNVs common to three replicates
# SNVs reported in COSMIC database (Catalog of Somatic Mutation in Cancer - release v83; Coding; 07-11-2017)
# SNVs with "FATHMM" prediction as "Pathogenic"
**# SNVs within Cancer Driver Genes (Bailey MH et al.; Cell-2018; PMID: 29625053).**
# GMAF = Global Minor Allele Frequency
# Common Variant (minor allele frequency (GMAF) of >= 0.01 in at least one 1000 Genomes Phase III major population)

| Chr_No | Position | dbSNP_rs-ID | COSMIC-ID | REF | ALT | GMAF | Population Status | FATHMM Score | Gene-ID | BASE Change | AA Change | Substitution Type |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| chr2 | 29416481 | rs1881420 | COSM1130802 | T | C | 0.4151 | Common Variant | 0.92214 | ALK | c.4472A>G | p.K1491R | Missense |
| chr2 | 29940529 | rs2246745 | COSM4416269 | A | T | 0.411 | Common Variant | 0.9054 | ALK | c.702T>A | p.P234P | coding silent |
| chr2 | 212587119 | rs77309171 | COSM4583147 | T | C | 0.002 | Common Variant | 0.86171 | ERBB4 | c.882A>G | p.P294P | coding silent |
| chr3 | 37053568 | rs1799977 | COSM1131469 | A | G | 0.1296 | Common Variant | 0.80196 | MLH1 | c.655A>G | p.I219V | Missense |
| chr4 | 55152040 | rs2228230 | COSM22413 | C | T | 0.2404 | Common Variant | 0.88315 | PDGFRA | c.2472C>T | p.V824V | coding silent |
| chr4 | 187630590 | rs3733415 | COSM4416272 | G | A | 0.2614 | Common Variant | 0.95514 | FAT1 | c.392C>T | p.A131V | Missense |
| chr5 | 112162854 | rs2229992 | COSM1432175 | T | C | 0.51 | Common Variant | 0.83456 | APC | c.1458T>C | p.Y486Y | coding silent |
| chr5 | 56177443 | rs702689 | COSM4003565 | G | A | 0.4764 | Common Variant | 0.98239 | MAP3K1 | c.1927G>A | p.D643N | Missense |
| chr5 | 56177743 | rs832582 | COSM4416191 | G | A | 0.7083 | Common Variant | 0.76719 | MAP3K1 | c.2227G>A | p.V743I | Missense |
| chr5 | 112176559 | rs866006 | COSM6475354 | T | G | 0.333 | Common Variant | 0.71742 | APC | c.5268T>G | p.S1756S | coding silent |
| chr7 | 55249063 | rs1050171 | COSM1451600 | G | A | 0.4327 | Common Variant | 0.95009 | EGFR | c.2361G>A | p.Q787Q | coding silent |
| chr7 | 116340262 | rs33917957 | COSM5020653 | A | G | 0.0329 | Common Variant | 0.86365 | MET | c.1124A>G | p.N375S | Missense |
| chr12 | 25362777 | rs1137282 | COSM3753105 | A | G | 0.1755 | Common Variant | 0.76767 | KRAS | c.519T>C | p.D173D | coding silent |
| chr13 | 21562948 | rs558614 | COSM432208 | G | A | 0.37 | Common Variant | 0.96847 | LATS2 | c.971C>T | p.A324V | Missense |
| chr13 | 28624294 | rs1933437 | COSM5019176 | G | A | 0.5587 | Common Variant | 0.92594 | FLT3 | c.680C>T | p.T227M | Missense |
| chr14 | 38061742 | rs7144658 | COSM3753953 | C | T | 0.417 | Common Variant | 0.83972 | FOXA1 | c.247G>A | p.A83T | Missense |
| chr14 | 105239894 | rs1130233 | COSM3765730 | C | T | 0.3225 | Common Variant | 0.71625 | AKT1 | c.726G>A | p.E242E | coding silent |
| chr19 | 42799049 | rs1052023 | COSM3756833 | C | T | 0.1198 | Common Variant | 0.78474 | CIC | c.4533C>T | p.I1511I | coding silent |
| chrX | 44938563 | rs20539 | COSM1179848 | G | A | 0.2217 | Common Variant | 0.88609 | KDM6A | c.3111G>A | p.Q1037Q | coding silent |
| chrX | 32380996 | rs1801187 | COSM4999535 | C | T | 0.4652 | Common Variant | 0.96068 | DMD | c.1211G>A | p.R404H | Missense |

## Supplemental Table S4C. HS980 (p22) Germline SNV overlapping to Shibata Cancer Driver Genes.

# HS980 p22 Germline SNVs common to three replicates.
# SNVs reported in COSMIC database (Catalog of Somatic Mutation in Cancer - release v83; Coding; 07-11-2017)
**# SNVs with "FATHMM" prediction as "Pathogenic"**
**# SNVs within Cancer Driver Genes (Shibata Cancer driver gene list based on an article (Cancer Research 72:636-644, 2012)).**
# GMAF = Global Minor Allele Frequency
# Common Variant (minor allele frequency (GMAF) of >= 0.01 in at least one 1000 Genomes Phase III major population)

| Chr_No | Position | dbSNP_rs-ID | COSMIC-ID | REF | ALT | GMAF | Population Status | FATHMM SCORE | Gene-ID | BASE CHANGE | AA CHANGE | Substitution Type |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| chr2 | 29416481 | rs1881420 | COSM1130802 | T | C | 0.4151 | Common Variant | 0.92214 | ALK | c.4472A>G | p.K1491R | Missense |
| chr2 | 29940529 | rs2246745 | COSM4416269 | A | T | 0.411 | Common Variant | 0.9054 | ALK | c.702T>A | p.P234P | Coding silent |
| chr3 | 37053568 | rs1799977 | COSM1131469 | A | G | 0.1296 | Common Variant | 0.80196 | MLH1 | c.655A>G | p.I219V | Missense |
| chr4 | 55979558 | rs2305948 | COSM1131107 | C | T | 0.1526 | Common Variant | 0.96877 | KDR | c.889G>A | p.V297I | Missense |
| chr4 | 55152040 | rs2228230 | COSM22413 | C | T | 0.2404 | Common Variant | 0.88315 | PDGFRA | c.2472C>T | p.V824V | Coding silent |
| chr5 | 112162854 | rs2229992 | COSM1432175 | T | C | 0.51 | Common Variant | 0.83456 | APC | c.1458T>C | p.Y486Y | Coding silent |
| chr5 | 112176559 | rs866006 | COSM6475354 | T | G | 0.333 | Common Variant | 0.71742 | APC | c.5268T>G | p.S1756S | Coding silent |
| chr7 | 55249063 | rs1050171 | COSM1451600 | G | A | 0.4327 | Common Variant | 0.95009 | EGFR | c.2361G>A | p.Q787Q | Coding silent |
| chr7 | 128845511 | rs111694017 | COSM1738148 | G | A | 0.0024 | Common Variant | 0.90625 | SMO | c.808G>A | p.V270I | Missense |
| chr7 | 116340262 | rs33917957 | COSM5020653 | A | G | 0.0329 | Common Variant | 0.86365 | MET | c.1124A>G | p.N375S | Missense |
| chr12 | 25362777 | rs1137282 | COSM3753105 | A | G | 0.1755 | Common Variant | 0.76767 | KRAS | c.519T>C | p.D173D | Coding silent |
| chr12 | 121435342 | rs2259820 | COSM3931546 | C | T | 0.3167 | Common Variant | 0.9731 | HNF1A | c.1375C>T | p.L459L | Coding silent |
| chr12 | 121416650 | rs1169288 | COSM430522 | A | C | 0.2985 | Common Variant | 0.91018 | HNF1A | c.79A>C | p.I27L | Missense |
| chr12 | 121435427 | rs2464196 | COSM4984989 | G | A | 0.3177 | Common Variant | 0.82717 | HNF1A | c.1460G>A | p.S487N | Missense |
| chr13 | 28624294 | rs1933437 | COSM5019176 | G | A | 0.5587 | Common Variant | 0.92594 | FLT3 | c.680C>T | p.T227M | Missense |
| chr14 | 105239894 | rs1130233 | COSM3765730 | C | T | 0.3225 | Common Variant | 0.71625 | AKT1 | c.726G>A | p.E242E | Coding silent |
| chr19 | 42799049 | rs1052023 | COSM3756833 | C | T | 0.1198 | Common Variant | 0.78474 | CIC | c.4533C>T | p.I1511I | Coding silent |
| chrX | 44938563 | rs20539 | COSM1179848 | G | A | 0.2217 | Common Variant | 0.88609 | KDM6A | c.3111G>A | p.Q1037Q | Coding silent |

**Supplemental Table S5. Exonic and Spliced somatic SNVs for hESC-RPE samples compared with respective HS980 (p22) samples.**

# Somatic SNVs Identified using GATK-MuTect2 (HS980 p22 vs RPE)
# Combined Non-redundant SNVs (replicate-1, 2 & 3)
# ANNOVAR annotation of SNVs
# SNVs with Missence, Frameshift and Silent mutation annotation
# Average Expression (RPKM) of gene from single-cell experiment (hES cells & RPE cells)

| Chr_No | Start | End | Ref | Alt | dbSNP_rs-ID | Gene-ID | Annotation | Amino Acid Change | Avg. Exp. hESC | Avg. Exp. RPE |
|---|---|---|---|---|---|---|---|---|---|---|
| chr1 | 17086940 | 17086941 | AC | - | rs748266310 | MST1L | Frameshift Deletion | c.383_384del:p.128_128del | 0.80 | 0.03 |
| chr11 | 56143716 | 56143719 | TGTT | - | rs760647141 | OR8U1 | Frameshift Deletion | c.617_620del:p.206_207del | 0.00 | 0.00 |
| chr1 | 145349584 | 145349584 | T | A | rs370191373 | NBPF10 | Non-synonymous | c.T6999A:p.D2333E, | 0.03 | 1.14 |
| chr1 | 152186042 | 152186042 | A | G | rs12751022 | HRNR | Non-synonymous | c.T8063C:p.L2688S | 0.05 | 0.01 |
| chr12 | 10588530 | 10588530 | C | G | rs34195537 | KLRC2 | Non-synonymous | c.G56C:p.R19P | 0.00 | 0.00 |
| chr16 | 21623970 | 21623970 | A | T | . | METTL9 | Non-synonymous | c.A170T:p.Y57F | 73.69 | 114.00 |
| chr19 | 54745682 | 54745682 | C | T | rs111666280 | LILRA6 | Non-synonymous | c.G419A:p.R140Q | 0.00 | 0.00 |
| chr2 | 113147370 | 113147370 | G | A | rs202082997 | RGPD5 | Non-synonymous | c.C3152T:p.T1051I | 0.00 | 0.00 |
| chr22 | 24579049 | 24579049 | G | A | rs35660748 | SUSD2 | Non-synonymous | c.G101A:p.R34H | 0.24 | 0.80 |
| chr3 | 47030858 | 47030858 | C | T | rs764816462 | NBEAL2 | Non-synonymous | c.C460T:p.R154C | 0.00 | 0.14 |
| chr6 | 30954694 | 30954694 | C | A | rs112415706 | MUC21 | Non-synonymous | c.C742A:p.P248T | 0.00 | 0.00 |
| chr7 | 100639618 | 100639618 | C | T | rs111933539 | MUC12 | Non-synonymous | c.C5774T:p.T1925M | 0.14 | 0.07 |
| chrX | 57618621 | 57618621 | T | C | rs113289397 | ZXDB | Non-synonymous | c.T140C:p.L47P | 1.46 | 2.16 |
| chr1 | 201180222 | 201180222 | G | A | rs28465285 | IGFN1 | Synonymous | c.G6201A:p.E2067E | 0.00 | 0.02 |
| chr11 | 1092684 | 1092684 | C | T | rs201269049 | MUC2 | Synonymous | c.C4503T:p.S1501S | 0.00 | 3.88 |
| chr11 | 117077034 | 117077034 | A | G | rs28590104 | PCSK7 | Synonymous | c.T2037C:p.T679T | 6.60 | 4.13 |
| chr17 | 15539560 | 15539560 | C | T | rs9911397 | TRIM16 | Synonymous | c.G639A:p.A213A | 0.89 | 4.66 |
| chr17 | 39197593 | 39197593 | G | T | rs145621540 | KRTAP1-1 | Synonymous | c.C57A:p.T19T | 0.00 | 0.00 |
| chr18 | 14542867 | 14542867 | C | T | rs200779556 | POTEC | Synonymous | c.G279A:p.T93T | 0.00 | 0.00 |
| chr20 | 60904081 | 60904081 | G | A | rs544096167 | LAMA5 | Synonymous | c.C4266T:p.N1422N | 4.91 | 11.93 |
| chr4 | 367291 | 367291 | T | C | . | ZNF141 | Synonymous | c.T1065C:p.N355N | 17.45 | 17.16 |
| chr9 | 43628651 | 43628651 | A | C | rs2261119 | SPATA31A6 | Synonymous | c.T291G:p.L97L | 0.00 | 0.00 |
| chrX | 140785790 | 140785790 | T | C | rs139363903 | SPANXD | Synonymous | c.A126G:p.L42L | 0.00 | 0.00 |
| chr1 | 16890602 | 16890602 | T | G | rs2990551 | NBPF1 | Unknown | UNKNOWN | 0.34 | 2.32 |
| chr1 | 148017582 | 148017582 | A | C | rs879951427 | NBPF8 | Unknown | UNKNOWN | 0.05 | 2.86 |

Highlighted in grey: genes with some clinical relevance reported in COSMIC/ClinVar

**Supplemental Table S6. Exonic and Spliced somatic SNVs for HS980 (p38) samples compared with respective HS980 (p22) samples.**

# Somatic SNVs Identified using GATK-MuTect2 (HS980 p22 vs HS980 (p38))
# Combined Non-redundant SNVs (replicate-1, 2 & 3)
# ANNOVAR annotation of SNVs
# SNVs with Missence, Frameshift and Silent mutation annotation
# Average Expression (RPKM) of gene from single-cell experiment (hES cells)

| Chr | Start | End | Ref | Alt | dbSNP_rs-ID | Gene-ID | Annotation | Amino Acid Change | Avg. Expression (hESc) |
|---|---|---|---|---|---|---|---|---|---|
| chr1 | 17086940 | 17086941 | AC | - | rs748266310 | MST1L | Frameshift Deletion | c.383_384del:p.128_128del | 0.80 |
| chr1 | 229738614 | 229738615 | AA | - | . | TAF5L | Frameshift Deletion | c.299_300del:p.100_100del | 1.30 |
| chr8 | 143958453 | 143958453 | - | GGAA | . | CYP11B1 | Frameshift Insertion | c.580_581insTTCC:p.H194fs | 0.00 |
| chr1 | 145349584 | 145349584 | T | A | rs370191373 | NBPF10 | Non-synonymous | c.T6999A:p.D2333E | 0.03 |
| chr11 | 1018012 | 1018012 | T | G | rs10751676 | MUC6 | Non-synonymous | c.A4789C:p.K1597Q | 0.00 |
| chr14 | 105417313 | 105417313 | T | C | rs80275639 | AHNAK2 | Non-synonymous | c.A4475G:p.K1492R | 0.98 |
| chr15 | 82635194 | 82635194 | T | C | rs1610794 | GOLGA6L10 | Non-synonymous | c.A1376G:p.E459G | 0.00 |
| chr18 | 14542791 | 14542791 | C | T | rs201849570 | POTEC | Non-synonymous | c.G355A:p.A119T | 0.00 |
| chr19 | 4511746 | 4511746 | A | T | rs62115192 | PLIN4 | Non-synonymous | c.T2184A:p.N728K | 0.16 |
| chr19 | 54745682 | 54745682 | C | T | rs111666280 | LILRA6 | Non-synonymous | c.G419A:p.R140Q | 0.00 |
| chr2 | 113147370 | 113147370 | G | A | rs202082997 | RGPD5,RGPD8 | Non-synonymous | c.C3152T:p.T1051I | 0.00 |
| chr20 | 30231249 | 30231249 | G | C | . | COX4I2 | Non-synonymous | c.G290C:p.R97P | 0.72 |
| chr21 | 45994014 | 45994014 | C | T | rs13051603 | KRTAP10-4 | Non-synonymous | c.C379T:p.P127S | 0.00 |
| chr5 | 79747478 | 79747478 | C | A | . | ZFYVE16 | Non-synonymous | c.C3557A:p.A1186E | 7.01 |
| chr6 | 17602910 | 17602910 | G | A | rs144013791 | FAM8A1 | Non-synonymous | c.G802A:p.V268I | 2.11 |
| chr6 | 32549402 | 32549402 | C | T | rs3205588 | HLA-DRB1 | Non-synonymous | c.G584A:p.R195Q | 0.00 |
| chr7 | 75124676 | 75124676 | G | A | rs28422159 | SPDYE5 | Non-synonymous | c.G242A:p.G81D | 0.11 |
| chr7 | 100645807 | 100645807 | G | A | rs112404953 | MUC12 | Non-synonymous | c.G11963A:p.G3988D | 0.14 |
| chr9 | 43915893 | 43915893 | G | C | rs200215881 | CNTNAP3B | Non-synonymous | c.G3741C:p.M1247I | 0.15 |
| chr11 | 1643096 | 1643096 | G | A | rs59587741 | KRTAP5-4 | Synonymous | c.C228T:p.G76G | 0.00 |
| chr11 | 62296299 | 62296299 | G | A | . | AHNAK | Synonymous | c.C5590T:p.L1864L | 0.98 |
| chr11 | 117077034 | 117077034 | A | G | rs28590104 | PCSK7 | Synonymous | c.T2037C:p.T679T | 6.60 |
| chr12 | 125397475 | 125397475 | C | A | rs11537760 | UBC | Synonymous | c.G843T:p.G281G | 134.65 |
| chr18 | 14542867 | 14542867 | C | T | rs200779556 | POTEC | Synonymous | c.G279A:p.T93T | 0.00 |
| chr19 | 1881263 | 1881263 | G | A | . | ABHD17A | Synonymous | c.C303T:p.C101C | 0.44 |
| chr2 | 234358746 | 234358746 | C | T | rs754569132 | DGKD | Synonymous | c.C2007T:p.F669F | 1.90 |
| chr20 | 60904081 | 60904081 | G | A | rs544096167 | LAMA5 | Synonymous | c.C4266T:p.N1422N | 4.91 |
| chr3 | 195452689 | 195452689 | C | T | rs141077164 | MUC20 | Synonymous | c.C702T:p.D234D | 0.00 |
| chr3 | 195508986 | 195508986 | G | A | rs2948679 | MUC4 | Synonymous | c.C9465T:p.V3155V | 0.00 |
| chr6 | 31964664 | 31964664 | G | A | rs12526327 | C4A,C4B | Synonymous | c.G3735A:p.P1245P | 0.00 |
| chr12 | 40880507 | 40880507 | A | G | . | MUC19 | Unknown | UNKNOWN | 0.02 |
| chr7 | 26246132 | 26246132 | T | G | . | CBX3 | Splicing | exon3:c.167+2T>G | 84.05 |

Highlighted in grey: genes with some clinical relevance reported in COSMIC/ClinVar

**Supplemental Table S7. Clinical significance analysis of 11 normal participants from personal genome project UK.**

| Sample-ID | A | B | C | D | E | F | G | H |
|---|---|---|---|---|---|---|---|---|
| **HS980 (p22) common to three replicates** | 4355521 | 2.05 | 20 | 20 | 2160 | 35/34 | 20/20 | 18/18 |
| **01_uk35C650** | 4105831 | 2.07 | 28 | 27 | 2102 | 33/32 | 30/29 | 15/15 |
| **02_uk2E2AAE** | 4133496 | 2.07 | 24 | 24 | 2039 | 31/31 | 19/19 | 18/18 |
| **03_uk2DF242** | 4128205 | 2.06 | 23 | 21 | 2026 | 31/31 | 21/21 | 21/20 |
| **04_uk740176** | 4248904 | 2.07 | 31 | 30 | 2084 | 29/28 | 18/17 | 12/11 |
| **05_uk33D02F** | 4188095 | 2.07 | 24 | 22 | 2109 | 25/25 | 17/16 | 12/12 |
| **06_uk0C72FF** | 4112224 | 2.07 | 24 | 24 | 2069 | 40/39 | 28/28 | 18/17 |
| **07_uk1097F9** | 4137176 | 2.07 | 18 | 16 | 2150 | 30/29 | 18/17 | 11/10 |
| **08_uk174659** | 4175392 | 2.06 | 16 | 14 | 2067 | 46/45 | 29/29 | 24/24 |
| **09_uk85AA3B** | 4179265 | 2.06 | 25 | 23 | 2049 | 28/28 | 21/21 | 15/15 |
| **10_uk481F67** | 4901623 | 2.07 | 28 | 28 | 1794 | 44/42 | 27/26 | 14/13 |
| **11_uk4CA868** | 3897315 | 2.07 | 23 | 22 | 2072 | 38/37 | 24/23 | 21/20 |

A: Total Germline SNVs
B: Transitions/Transversions (ts/tv)
C: SNVs with clinical significance (CLNID) as “Pathogenic” in ClinVar-20171029 database release
D: ClinVar annotated “Pathogenic” SNVs reported as COMMON in dbSNP (minor allele frequency (MAF) of >= 0.01 in at least one 1000 Genomes Phase III major population with at least two individuals from different families having the same minor allele)
E: SNVs with FATHMM prediction as “Pathogenic” in COSMIC (COSMIC Coding v-83) database
F: COSMIC annotated “Pathogenic” SNVs within COSMIC Cancer Gene Census / SNVs COMMON in dbSNP
G: COSMIC annotated “Pathogenic” SNVs within Bailey MH et al. Cancer Driver Genes / SNVs COMMON in dbSNP
H: COSMIC annotated “Pathogenic” SNVs within Shibata Cancer Driver Genes / SNVs COMMON in dbSNP