Supplemental Figure 1

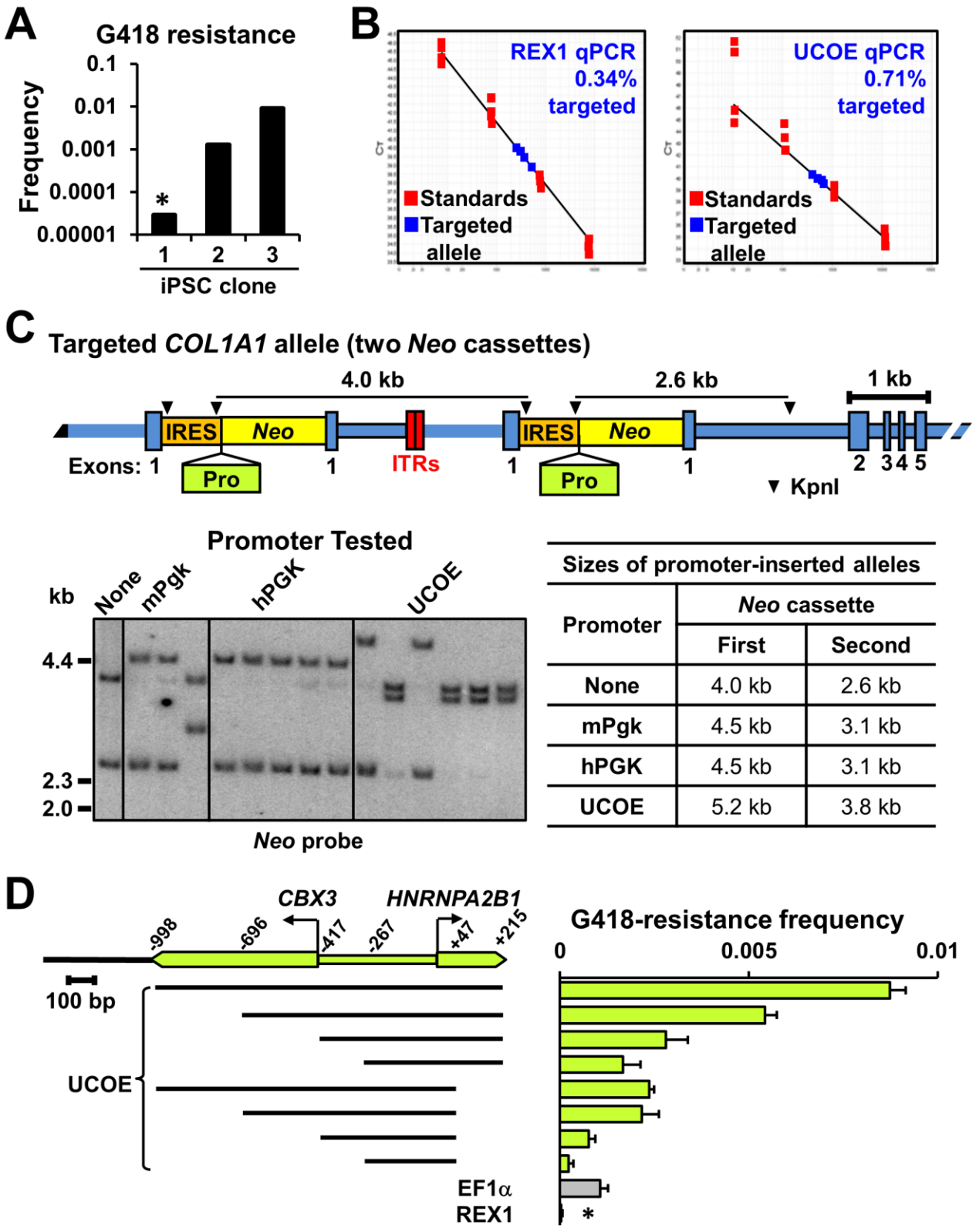


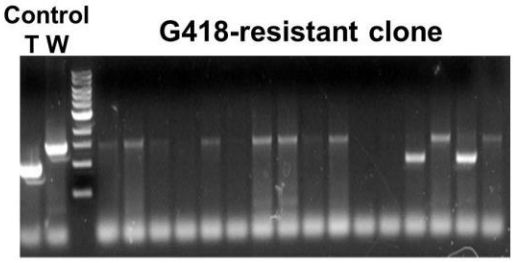
Figure S1. Targeting a silent *COL1A1*-IRES-*Neo* cassette. (**a**) Spontaneous G418 resistance frequencies of iPSC clones 1, 2 and 3 containing a silenced IRES-*Neo* cassette in *COL1A1*. *, < $2x10^{-5}$. (**b**) Examples of qPCR measurements of homologous recombination frequencies when inserting a REX1 or UCOE promoter into the *COL1A1*-IRES-*Neo* cassette of iPSC clone 1. Red squares, standard values obtained with a cloned reconstructed targeted allele mixed with a wild-type genome; blue squares, sample measurements. Primer locations are shown in Figure 1c. (**c**) Structure of the *COL1A1*-IRES-*Neo* cassette dimer in iPSC clone 1 with promoter insertion sites and the restriction enzyme sites used for Southern blot analysis. A Southern blot is shown that analyzes individual, G418-resistant subclones transduced with the indicated promoter insertion vector, allowing one to identify which *Neo* gene was targeted. The genomic DNAs were digested with KpnI and probed for *Neo* sequences. The sizes of promoter-inserted alleles are shown in the adjacent table. (**d**) The genomic structure of the UCOE promoter with its two chromosomal genes is shown (*CBX3* and *HNRNPA2B1*), along with the G418-resistance frequencies produced by rAAV vectors containing the indicated full-length and truncated UCOE promoter fragments. The boundaries of the UCOE fragments are shown relative to the transcription start site of *HNRNPA2B1*. *, < $3x10^{-5}$. Data represent mean ± SEM of three.

Figure S2

a

Wild-type *IL2RG*
Product 1.4 kb

Targeted *IL2RG*
Product 1.0 kb



PCR screening example
W, wild-type; T, targeted

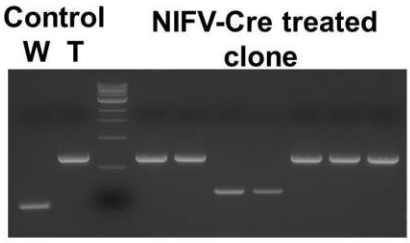
PCR analysis of G418-R clones		
Genotype	**Number of clones**	**Fraction of all infected cells**
Untargeted (Random)	15	6.9×10^{-3}
Targeted	3	1.4×10^{-3}

b

Wild-type *IL2RG*
Product 180 bp

Targeted
Product 700 bp

Cre-out *IL2RG*
Product 270 bp



PCR screening example
W, wild-type; T, targeted

PCR analysis of Cre-out clones			
Expt	**UCOE-*Neo***	**Cre-out**	**Cre-out efficiency**
1	5	2	28%
2	17	1	6%

Figure S2. *IL2RG* targeting and UCOE-*Neo* removal. (**a**) Identification of *IL2RG*-targeted clones by PCR. The structures of wild-type and targeted *IL2RG* loci are shown with primer binding sites indicated as triangles. An example of a multiplex PCR screening gel is shown with wild-type and targeted clones. (**b**) Identification of Cre-out clones. The structures of wild-type, targeted, and Cre-out *IL2RG* loci are shown with four primer binding sites indicated as triangles. Multiplex PCR was performed on clones after transducing with a non-integrated foamy virus that transiently expresses Cre recombinase. The efficiency of UCOE-*Neo* transgene removal is calculated as the number of Cre-out clones / (number of targeted clones + number of Cre-out clones) × 100.

Figure S3

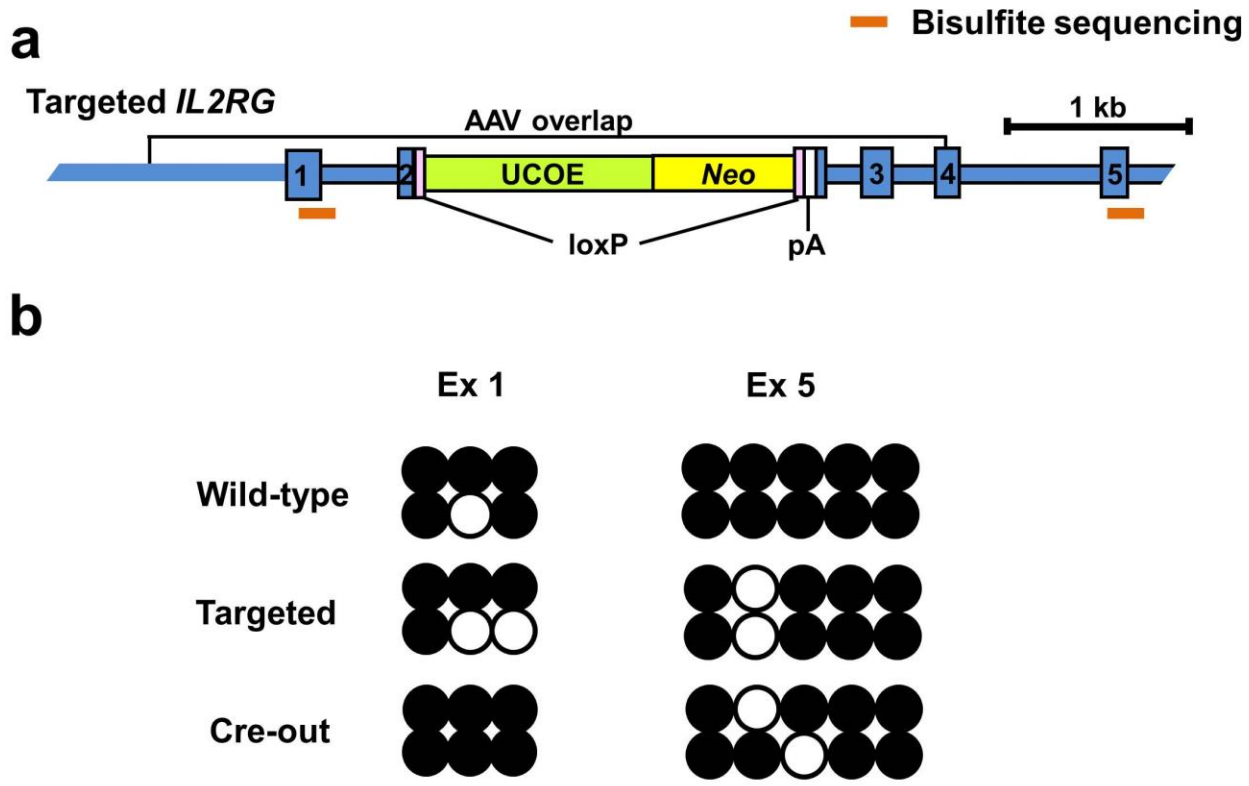

a
Bisulfite sequencing
Targeted IL2RG
AAV overlap
1 kb
1
2
UCOE
Neo
3
4
5
loxP
pA
b
Ex 1
Ex 5
Wild-type
Targeted
Cre-out

Figure S3. CpG methylation at the *IL2RG* locus. (**a**) Structure of the UCOE-*Neo* targeted *IL2RG* locus shown with rAAV overlap, loxP sites, and the locations of bisulfite sequencing fragments. The *IL2RG* locus contains no CpG islands. (**b**) Open and filled circles indicate unmethylated and methylated cytosines respectively in the CpGs assayed in exons 1 and 5.

Figure S4

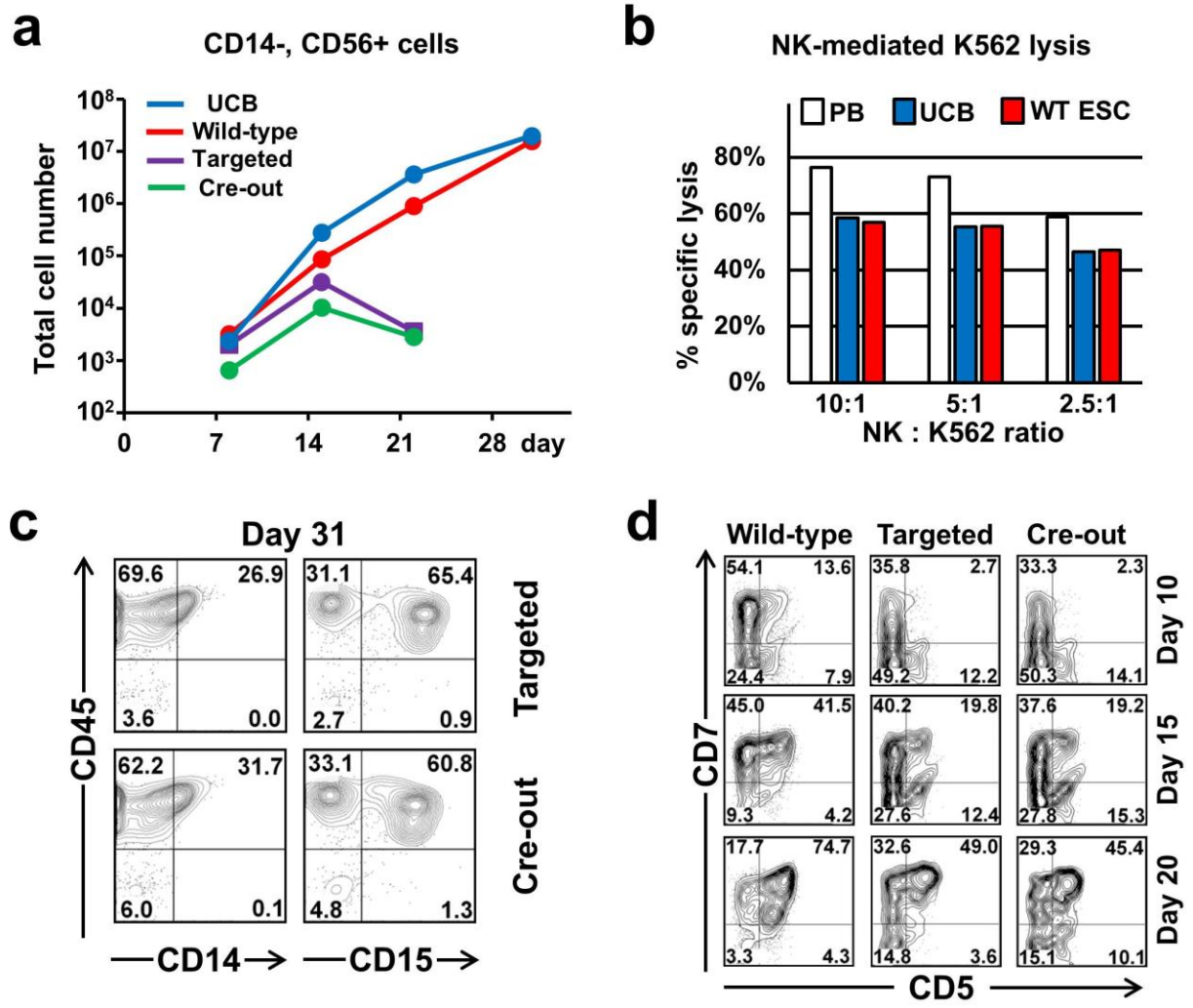

a
CD14-, CD56+ cells
UCB
Wild-type
Targeted
Cre-out
Total cell number
10^8
10^7
10^6
10^5
10^4
10^3
10^2
0
7
14
21
28 day
b
NK-mediated K562 lysis
PB
UCB
WT ESC
% specific lysis
80%
60%
40%
20%
0%
10:1
5:1
2.5:1
NK : K562 ratio
c
Day 31
CD45
CD14
CD15
Targeted
Cre-out
69.6
26.9
3.6
0.0
31.1
65.4
2.7
0.9
62.2
31.7
6.0
0.1
33.1
60.8
4.8
1.3
d
Wild-type
Targeted
Cre-out
Day 10
Day 15
Day 20
CD7
CD5
54.1
13.6
24.4
7.9
35.8
2.7
49.2
12.2
33.3
2.3
50.3
14.1
45.0
41.5
9.3
4.2
40.2
19.8
27.6
12.4
37.6
19.2
27.8
15.3
17.7
74.7
3.3
4.3
32.6
49.0
14.8
3.6
29.3
45.4
15.1
10.1

Figure S4. Characterization of T and NK cells derived from ESCs. (**a**) Total number of CD14-, CD56+ NK lineage cells produced during NK differentiation of UCB, or wild-type, *IL2RG*-targeted and Cre-out EBs. (**b**) Chromium release assays showing lysis of MHC-negative K562 cells by peripheral blood (PB)-, UCB-, or wild-type ESC-derived NK cells. NK:K562 cell ratios are indicated. (**c**) Flow cytometry analysis showing surface expression of CD45 (hematopoietic), CD14 (monocyte) and CD15 (granulocyte) markers in cells obtained after four weeks of NK differentiation from *IL2RG*-targeted and Cre-out EBs. (**d**) Flow cytometry analysis of surface expression of lymphoid markers CD5 and CD7 on different days of T cell differentiation in wild-type, *IL2RG*-targeted and Cre-out EBs.

Table S1. Primer Sequence

qRT-PCR for gene expression	
COL1A1 forward	TGCCGTGACCTCAAGATGTG
COL1A1 reverse	CTCGTGCAGCCATCGACAGT
IL2RG forward	ATGTTGAAGCCATCATTACCA
IL2RG reverse	GGCATAGTGGTCAGGAAGAA
SNX12 forward	GGCGCTACAGTGACTTTGAGTG
SNX12 reverse	CCGGGACGTAGTTCCTGTCAAT
FOXO4 forward	CATCAGCCAGGCCATTGAAAGC
FOXO4 reverse	CAGGGTTCAGCATCCACCAAGA
MED12 forward	TACCAGAGCACCCACCCTTCTA
MED12 reverse	TGCTGCTGCTGCTGCTCAGGTA
NLGN3 forward	CCCTGCAACTTCTCCAAGAATG
NLGN3 reverse	GTTGGCCTTGGTGTGAATGAAC
TEX1 forward	AACCACCTTACCTCCTTCAAG
TEX1 reverse	AGCATCTCGGGACAATGTATC
SLC7A3 forward	GCCCAGTGGTCAGTTCCATTG
SLC7A3 reverse	GAGGCAAAGCAGGCACCTTAA
GJB1 forward	CCTTCCTTCCCTGGCTACTTC
GJB1 reverse	GTCGGAGCATCCCATCTCTTG
ZMYM3 forward	TGCAGAGGCTGAGGAGTTAGA
ZMYM3 reverse	CCCAGGCTCATCCAAGACATT
NONO forward	CGGCAGCAAGAAGAAATGATG
NONO reverse	GGCACCTCTGTTGTTTATGCC
ITGB1BP2 forward	CCTGCGTTTAACTGGGTGAAG
ITGB1BP2 reverse	AGCCTTGACCAGGGAGATTTC
GAPDH forward	GAAATCCCATCACCATCTTCCAG
GAPDH reverse	ATGAGTCCTTCCACGATACCAAAG
qPCR for homologous recombination frequency	
REX1 forward	AGCCTGATTAGACCGCGTCAGT
EPCAM forward	TAGTCCTTCGGCGAGCGAGCACCTT
UCOE forward	GAGTCCGGTTCGTGTTCGTC
EF1α forward	GCCGCCAGAACACAGCTGAA
mPgk forward	TCATCTCCGGGCCTTTCGAC
hPGK forward	GGCTCCCTCGTTGACCGAATC
Neo reverse1	AGCCAACGCTATGTCCTGATA
Neo reverse2	CACGGGTAGCCAACGCTATGTCCTG
Bisulfite PCR	
CpG forward	TTTTTATTAGGATAGTATAAAAGGGGTT

CpG reverse	AAACTTCCAAAAAAACTACTTCCTTCAC
IL2RG targeting screening	
IL2RG untargeted forward	GAGGGGTAGTGGGTGAGGGA
IL2RG targeted forward	GACCGCTTCCTCGTGCTTTACGGTATCG
IL2RG reverse	ACGTCCCTAGTCACTCACAGTC
IL2RG transgene removal screening	
UCOE-Neo forward	TCGGCGTTAATTTCAAACTG
UCOE-Neo reverse	TCGGTCTTGACAAAAAGAACC
IL2RGex2 flanking forward	CCACTGACTCCCTCAGTGTTTC
IL2RGex2 flanking reverse	TCTCTGATCCAACCCACCTCTT
COL1A1 targeting CHIP	
A-forward	TGCCCTAGACCACCACTCTAA
A-reverse	GACCATGTGGCAGCAACTTGT
B-forward	CACGCATGAGCGGACGCTAA
B-reverse	AAGCGGCTTCGGCCAGTAAC
C-forward	CGGCCATTGAACAAGATGGA
C-reverse	TCGGTCTTGACAAAAAGAACC
D-forward	GTGCCGTCTTCTGCCTTTCAA
D-reverse	TGGACCTCCCAAGCTGTCTAT
IL2RG targeting CHIP	
A-forward	TAAGCACAGTGCCTGGCACAT
A-reverse	TTCTAAAGGCCCTCTGCTCTC
B-forward	TCACACTTCCTCGCCAGTCTC
B-reverse	CCCGACATCACCGTTCTATGC
C-forward	TGACCACTATGCCCACTGACTC
C-reverse	GCTGCTGTTCCAAGTGCAATTC
D-forward	CCAGCCTACCAACCTCACT
D-reverse	TCCAGAGCCTAGCCTCATC
E-forward	AAGTCCAGAAGTGCAGCCACTA
E-reverse	GGTCCTGGAGCTGAACAACAAA
F-forward	CTGGTGGGTGTTCAGGAGTAT
F-reverse	TTTCTGCCCATCCACACTAGG
Taqman qPCR probe	
Neo forward	ATCGCATCGAGCGAGCACGT