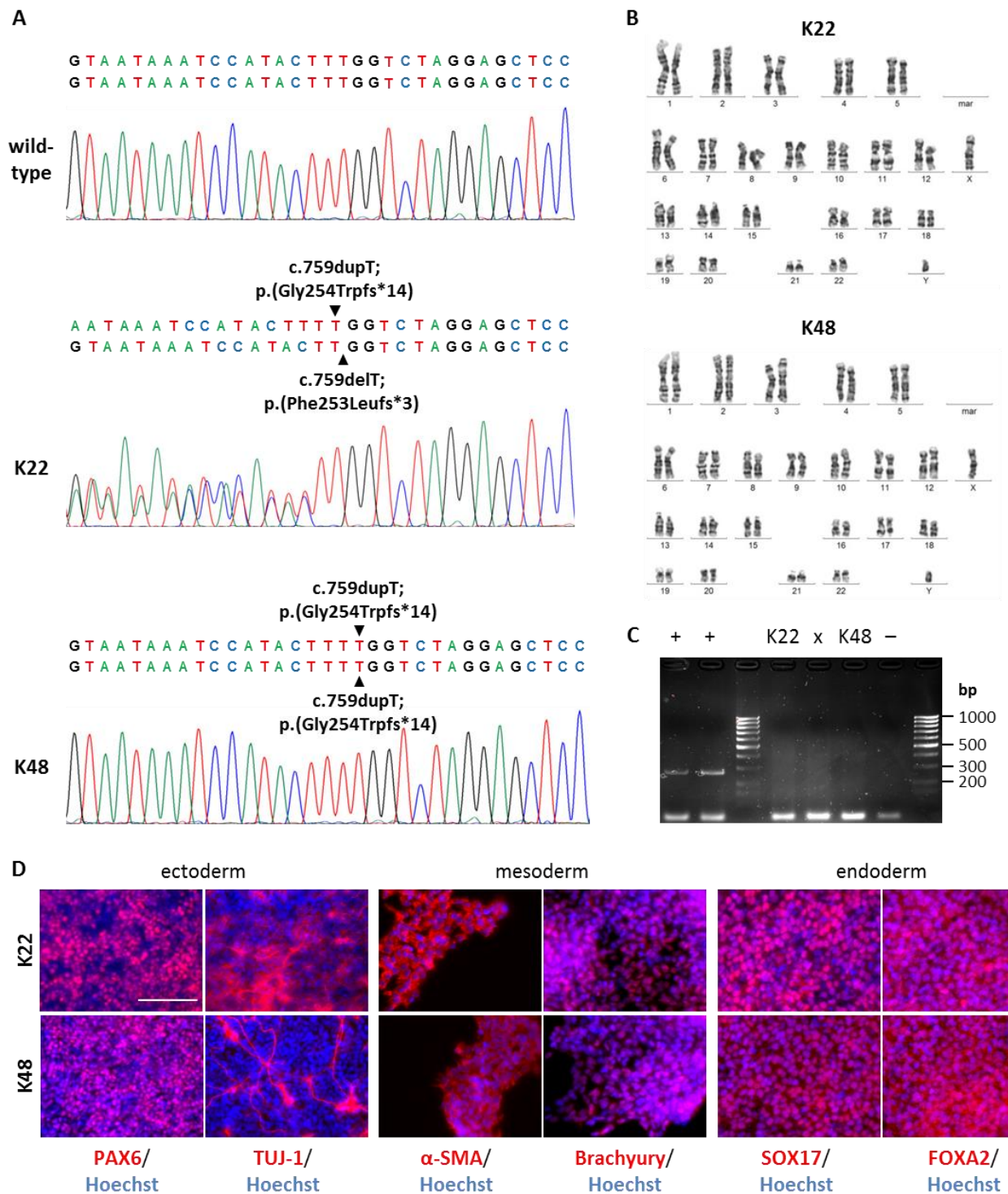
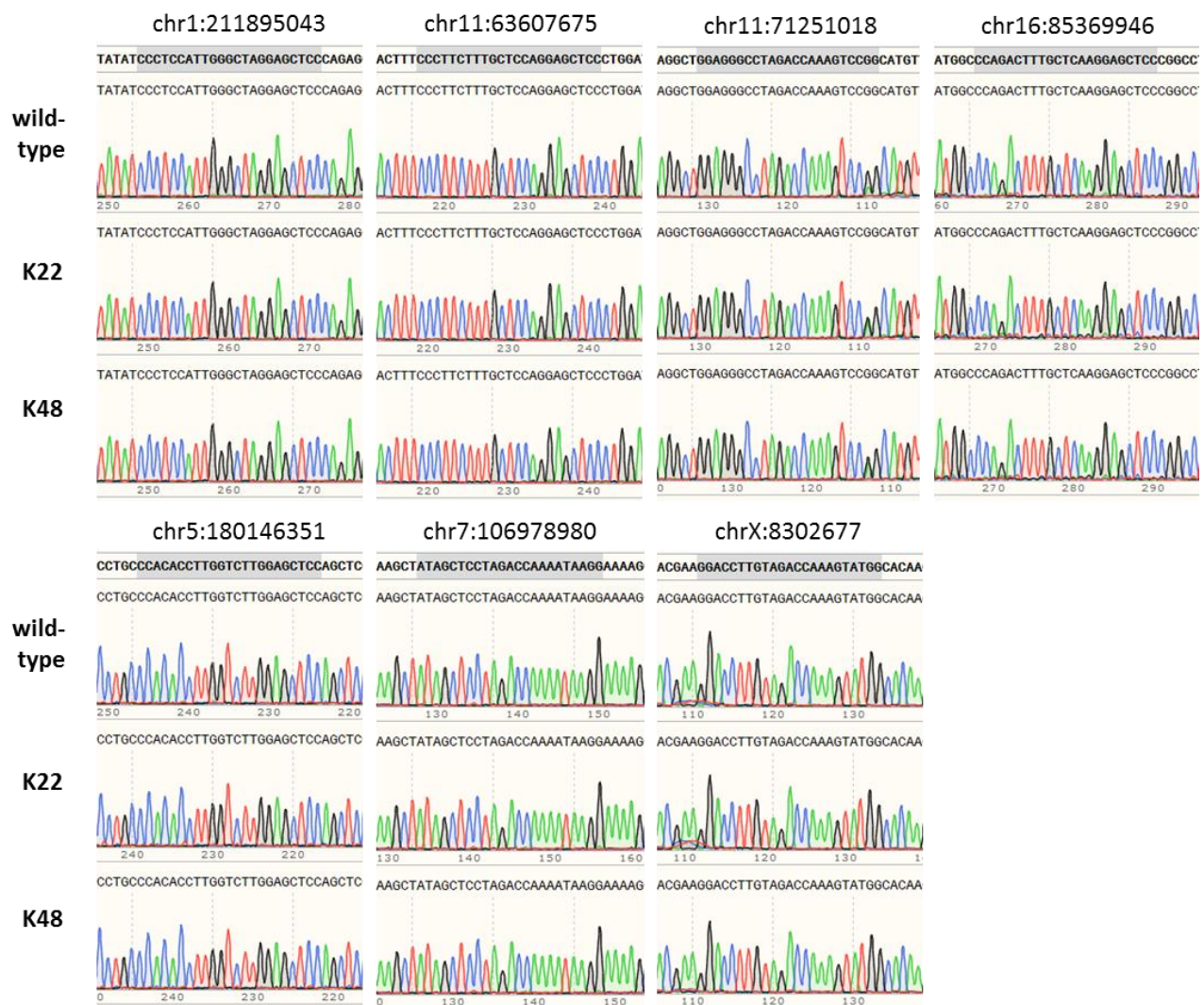


Supplementary Material

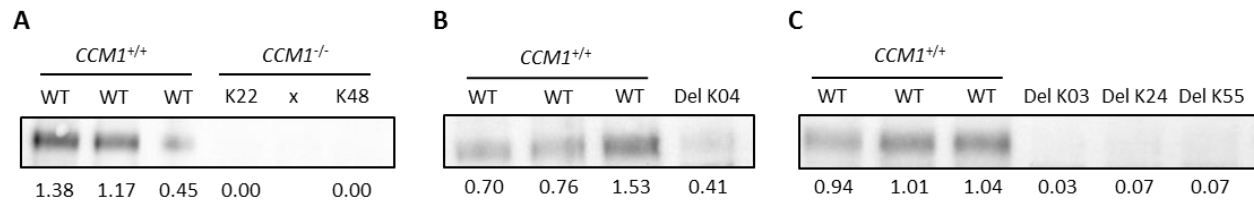
1 Supplementary Figures



Supplementary Figure 1. Quality control analyses for AICS-0023-derived *CCM1*^{-/-} iPSC clones. **(A)** Sanger sequences showing the CRISPR/Cas9-induced variants. **(B)** Karyotyping by GTG staining. A composite karyotype from 20 metaphase spreads is given. **(C)** PCR test for mycoplasma contamination (mycoplasma-specific band: 270 bp; + = positive controls, – = negative control; x = clone not included in this article). **(D)** Immunofluorescence analyses for ectoderm markers PAX6 and TUJ-1, mesoderm markers α -SMA and Brachyury, and endoderm markers SOX17 and FOXA2 after differentiation into the three germ layers (scale = 100 μ m).



Supplementary Figure 2. Sanger sequencing of seven top off-target sites for *CCM1*^{-/-} AICS-0023 iPSCs. Potential off-target sites are highlighted in grey.



Supplementary Figure 3. Independently performed western blot analyses for *CCM1*^{-/-} AICS-0023 iPSCs (**A**), for HEK293T clone Del K04 (**B**), and for *CCM1*^{del/del} AICS-0023 iPSCs (**C**). Total protein served as reliable loading control (Tin et al., 2018). Expression levels normalized to the wild-type (WT) control group are given. x = clone not included in this article. Reference: Tin, A., Li, Y., Brody, J.A., Natile, T., Chu, A.Y., Huffman, J.E., et al. (2018). Large-scale whole-exome sequencing association studies identify rare functional variants influencing serum urate levels. *Nat Commun* 9(1), 4228. doi: 10.1038/s41467-018-06620-4.

2 Supplementary Tables

Supplementary Table 1. Oligonucleotides used for CRISPR/Cas9 editing, PCR and quantitative PCR analyses.

analysis	target	sequence (5'-3') / assay
PCR/sequencing	<i>CCM1</i> (Cerebral Cavernous Malformations 1)	for: CCACTGTCGTTCCCTAATCA rev: TCGGGTAAGAGTTGAATGAA
PCR/sequencing for deletion region	<i>CCM1</i>	for: CAGGCGGAGGCTGATGTG rev: CCTCCTGTTTCCCGAAGGAC
crRNA guide sequence (combination i)	<i>CCM1</i>	GCGCGGGGGAACCCTCAAGA/ ATGAGCCGGGCTTGACCGCG
crRNA guide sequence (combination ii)	<i>CCM1</i>	GCGCGGGGGAACCCTCAAGA/ AGGCGGCGCAGCGGCCGGAG
crRNA guide sequence (combination iii)	<i>CCM1</i>	GCGCGGGGGAACCCTCAAGA/ CGGCGAGGCTCAGACTGGGT
sgRNA guide sequence	<i>CCM1</i>	GGAGCTCCTAGACCAAAGTA
qPCR	<i>RPLP0</i> (Ribosomal Protein Lateral Stalk Subunit P0)	for: TCGACAATGGCAGCATCTAC rev: ATCCGTCTCCACAGACAAGG
qPCR	<i>CCM1</i>	Hs.PT.56a.600880 (Integrated DNA Technologies)
qPCR	<i>ANKIB1</i> (Ankyrin Repeat And IBR Domain Containing 1)	Hs.PT.58.27551849 (Integrated DNA Technologies)

qPCR	<i>KLF2</i> (Kruppel Like Factor 2)	Hs.PT.58.39339409 (Integrated DNA Technologies)
qPCR	<i>KLF4</i> (Kruppel Like Factor 4)	Hs.PT.58.45542593 (Integrated DNA Technologies)
qPCR	<i>THBS1</i> (Thrombospondin 1)	Hs.PT.58.45510824 (Integrated DNA Technologies)
qPCR	<i>NOS3</i> (Nitric Oxide Synthase 3)	Hs.PT.58.21447620 (Integrated DNA Technologies)
qPCR	<i>HEY2</i> (Hes Related Family BHLH Transcription Factor With YRPW Motif 2)	Hs.PT.58.582440 (Integrated DNA Technologies)
Mycoplasma Test		for: GGGAGCAAACAGGATTAGATACCCT rev: TGCACCATCTGTCACTCTGTTAACCTC
PCR/sequencing for off-target sites	chr1:211895043 (GRCh37/hg19)	for: GCTCTGCAGATTTCTGGGGT rev: GAAACAGCGGACTGAAGGGA
PCR/sequencing for off-target sites	chr11:63607675 (GRCh37/hg19)	for: TCCACCTCTCCCTTCGTCTC rev: ATTGGAACCCCAAACGCAAC
PCR/sequencing for off-target sites	chr11:71251018 (GRCh37/hg19)	for: TGGGCTTCTCTCCCCTACAA rev: TGCCATCCTGTGAGAAGGTG
PCR/sequencing for off-target sites	chr16:85369946 (GRCh37/hg19)	for: GAGATATGGAGGAACAGGGCG rev: ATCCTGCACCTCTGATTCTGC

PCR/sequencing for off-target sites	chr5:180146351 (GRCh37/hg19)	for: TATGGGTGGTCCCCAATCCA rev: AGGTGAAAACAACGGCCAGA
PCR/sequencing for off-target sites	chr7:106978980 (GRCh37/hg19)	for: ACCAAGGCTACAAAATGGGGA rev: CTCTCTGTTGTTTCCTCTACTGC
PCR/sequencing for off-target sites	chrX:8302677 (GRCh37/hg19)	for: TGTCAGCATGGGCTTTACCAA rev: GACCAGTATTGCGTCCCGTG

Supplementary Table 2. Antibodies used in immunofluorescence and western blot analyses.

antibody (target)	clone/catalog number, RRID	vendor	dilution
primary antibodies			
Mouse Anti-SSEA4 (Stage-Specific Embryonic Antigen 4)	A24866, RRID:AB_2651001	Thermo Fisher Scientific	1:100
Rabbit Anti-OCT4 (Octamer-Binding Transcription Factor 4)	A24867, RRID:AB_2650999	Thermo Fisher Scientific	1:200
Rat Anti-SOX2 (SRY-Box Transcription Factor 2)	A24759, RRID:AB_2651000	Thermo Fisher Scientific	1:100
Mouse Anti-TRA-1-60 (T Cell Receptor Alpha Locus)	A24868, RRID:AB_2651002	Thermo Fisher Scientific	1:100
Mouse Anti-Human PAX6 (Paired Box 6)	561462, RRID:AB_10715442	BD Biosciences	1:200

Mouse Anti-Beta-III Tubulin (Tubulin Beta 3 Class III)	MAB1195, RRID:AB_357520	R&D Systems	1:100
Goat Anti- Human/Mouse Brachyury (T-Box Transcription Factor T)	AF2085, RRID:AB_2200235	R&D Systems	1:20
Mouse Anti-Alpha Smooth Muscle Actin [1A4] (Actin Alpha 2, Smooth Muscle)	ab7817, RRID:AB_262054	Abcam	1:200
Goat Anti-Human SOX17 (SRY-Box Transcription Factor 17)	AF1924, RRID:AB_355060	R&D Systems	1:20
Goat Anti-Human HNF-3 beta/FoxA2 (Forkhead Box A2)	AF2400, RRID:AB_2294104	R&D Systems	1:20
Mouse Anti-CD31 (PECAM-1) (89C2) (Platelet And Endothelial Cell Adhesion Molecule 1)	3528, RRID:AB_2160882	Cell Signaling	1:800
Rabbit Anti-VE- Cadherin (D87F2) XP (Cadherin 5)	2500, RRID:AB_10839118	Cell Signaling	1:400

Rabbit Anti-GAPDH (Glyceraldehyde-3- Phosphate Dehydrogenase)	PA1-16777, RRID:AB_568552	Thermo Fisher Scientific	1:500
Rabbit Anti-KRIT1 [EPR16560] (Krev Interaction Trapped Protein 1)	ab196025	Abcam	1:200
secondary antibodies			
Alexa Fluor 555 Goat Anti-Mouse IgG H+L	ab150114, RRID:AB_2687594	Abcam	1:500 / 1:200
Alexa Fluor 555 Donkey Anti-Rabbit	A24869, RRID:AB_2651006	Thermo Fisher Scientific	1:250
Alexa Fluor 555 Goat Anti-Rat IgG H+L	A-21434, RRID:AB_2535855	Thermo Fisher Scientific	1:500
Alexa Fluor 555 Goat Anti-Mouse IgM	A24871, RRID:AB_2651009	Thermo Fisher Scientific	1:250
Alexa Fluor 555 Donkey Anti-Goat IgG H+L	ab150130, RRID:AB_10894526	Abcam	1:200
Alexa Fluor 555 Goat Anti-Rabbit IgG H+L	A-21429, RRID:AB_2535850	Thermo Fisher Scientific	1:500
Goat Anti-Rabbit IgG H+L (HRP)	ab205718, RRID:AB_2819160	Abcam	1:400