

Supplementary material

Bacterial transformation, plasmid purification and validation

NEB 10-beta Competent Escherichia coli (high efficiency) was used for the transformation of plenti-CRISPR-cas9-gRNA for Cav3.1/ Cav3.2 plasmids. These plasmids at the concentration of 200 ng were mixed with the competent E.coli and incubated on ice for 30 min followed by heat shock at 42°C for 30 s. After 5 min of incubation in ice, 950 µl of SOC medium was added and incubated at 37°C shaker for 60 min. The transformed bacteria with the plasmids were spread onto the warm antibiotic selection plate (LB agar +100ug/ml ampicillin) and incubated overnight at 37°C. Single colonies of bacteria expressing plasmids were isolated and inoculated in 5 ml of LB broth+100 ug/ml ampicillin and incubated in 37°C shaker. After 6 h, 100 µl from the 5 ml culture was mixed with 25 ml of LB broth +100 ug/ml ampicillin for overnight at 37°C shaker. Plasmid purification was carried out from 25 ml bacterial culture expressing the plasmid using a Qiagen plasmid midi prep kit (Qiagen Ltd, Crawley, UK) by following the manufacturer's instructions. The purified plasmid DNA was quantified using a Nanodrop and validated by restriction enzyme digestion by incubating 1µg of the plasmid with 10,000 units of MluI restriction enzyme(New England Biolabs, Herts, UK) at 37°C for an hour. Restriction digestion was confirmed by running the digest on a 1% agarose gel against 10 KB ladder (Suppl. Fig. S2a-b). The purified plasmids were also validated by Sanger sequencing with U6-5' GGACTATCATATGCTTACCG primers (Suppl. Fig. S2a-b).

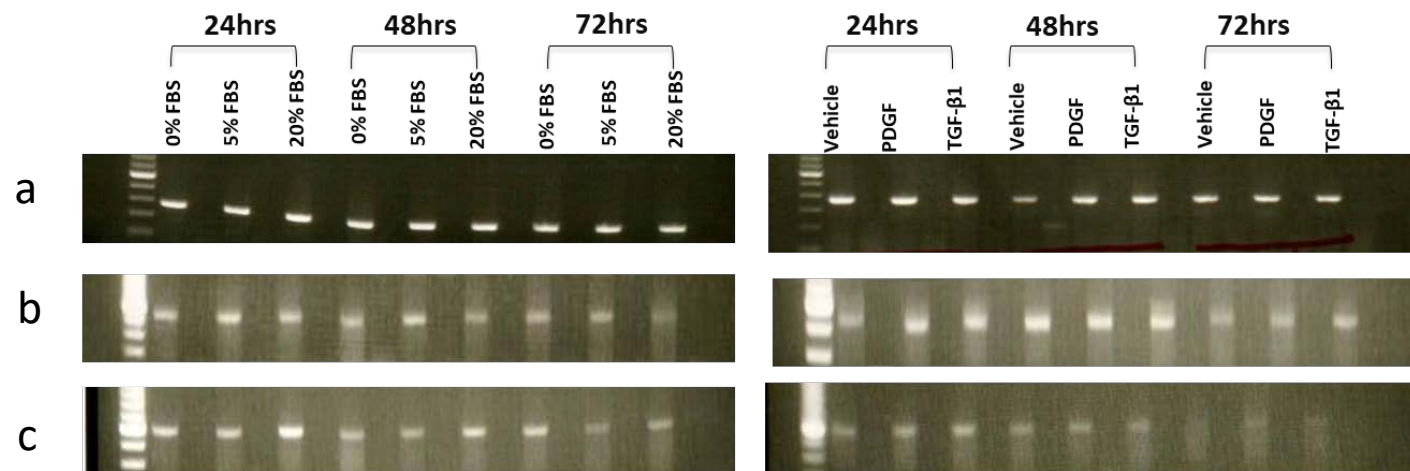
Selection and validation of single-cell clones

Around 20 single-cell clones each from Cav3.1 and Cav3.2 SKO were picked and six clones from each KO were selected and expanded for the further analysis. Whereas, only 3 clones from DKO were obtained. Six clones from each of the Cav3.1 and Cav3.2 SKO and 3 DKO clones were PCR amplified by using primers spanning the gRNA region and sent for Sanger sequencing. Out of 12 clones, clone 2 from Cav3.1, clone 3 and 6 from Cav3.2 displayed the pronounced KO effect as reflected in agarose gel (Suppl. Fig. S3a). It may be due to deletions of more than ten base pairs which are visible by the shift in the band compared to the control sample, whereas the rest of the clones did not show the shift. Hence, Sanger sequencing is essential to evaluate these clones, which can pick up even single base pair deletion. Sanger sequencing results confirmed that in comparison to control, 5 out of 6 clones of Cav3.1 KO and all six clones of Cav3.2 KO exhibited the deletion of few base-pairs. The clones of Cav3.2 knockout sequenced for Cav3.1 and Cav3.1 KO clone sequenced for Cav3.2 showed no base pair deletion, and its sequence pattern is similar to the control (Suppl. Fig. S3b). From this result, it confirmed that the KO of Cav3.1 and Cav3.2 does not have any impact on its counterpart's isoform. Sanger sequencing result of all 3 clones of DKO showed few pair deletion on both Cav3.1 and Cav3.2, confirming DKO (Suppl. Fig. S3c).

Though the sequencing result confirms the base pair deletion, the KO needs to be confirmed at the protein level. All six clones each from Cav3.1 KO and Cav3.2 KO were subjected to Western blot for Cav3.1 and Cav3.2 respectively. Similar to the sequencing result, there is a complete loss of Cav3.1 protein in all Cav3.1 clone except clone 5, which shows a faint band in Western blot in comparison to controls (WT control, clone 3 of Cav3.2 KO and positive control brain) (Suppl. Fig. S4a). This

Western blot results also confirmed that the KO of Cav3.2 does not affect its counterpart's isoform Cav3.1.

Whereas, only two of the clones: clone 3 and 4 exhibited the complete loss of Cav3.2 protein as depicted in Western blot for Cav3.2. The remaining clones show faint bands compared to the control (WT control, Cav3.1 KO and positive control HEK overexpressing Cav3.2) (Suppl. Fig. S4b). Unlike Cav3.1 Western blot, Cav3.2 Western blot showed a faint band of Cav3.1 clone 2. It is opposite to that of our sequencing result. In sequencing, the clone 2 of Cav3.1 amplified for Cav3.2 (Suppl. Fig. S3a-b) exhibited no base deletion and is similar to control. The reason for this is unknown. These results confirmed that CRISPR-cas9-Cav3.1/ Cav3.2 transfection with single-cell clonal selection allowed us to obtain cells with Cav3.1 and Cav3.2 KO.

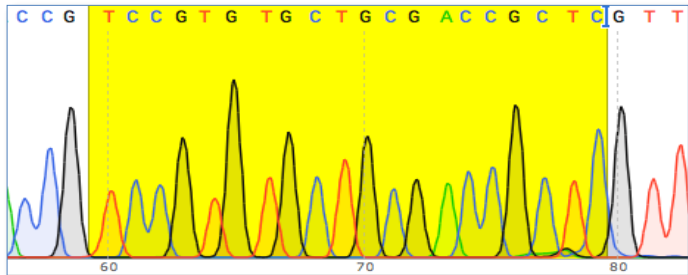
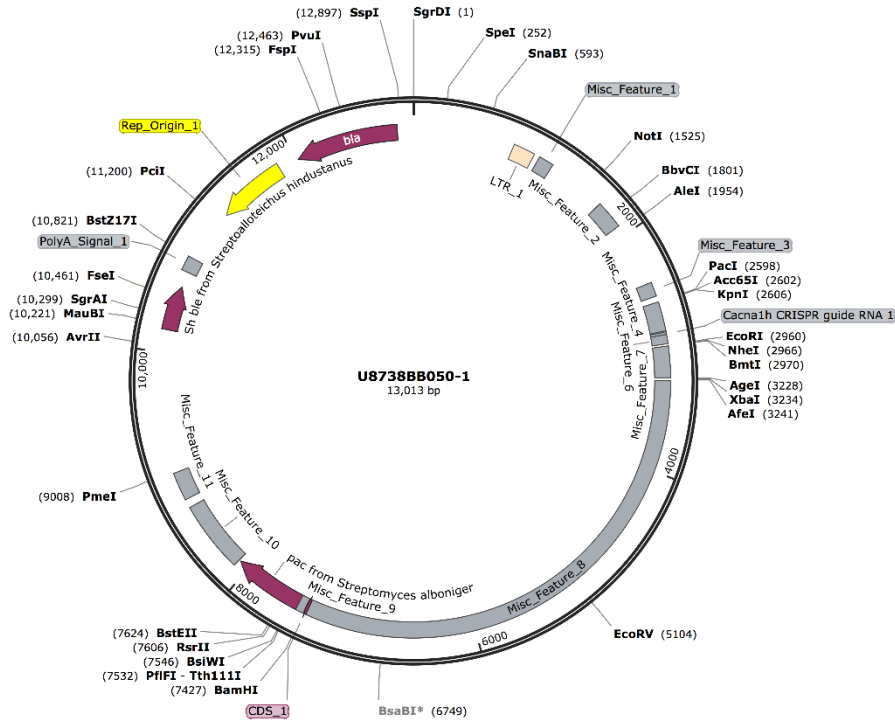


Supplementary Figure S1. Confirmation of TTCC isoform in mouse mesangial cells and modulation of its proliferation by TTCC blockers.

(a, b, c) RT-PCR for the T type calcium isoforms were detected in mouse mesangial cells (MES13). The band in each lane in the agarose gel represent the PCR product of the T type calcium isoforms *Cacna1G*, *Cacna1H* and *Cacna1I* (PCR product size: CaV3.1-376; CaV3.2-594; CaV3.3-528). mRNA of the CaV3.3 in 72 h treatment with PDGF and TGFβ1 alone shown faint band.

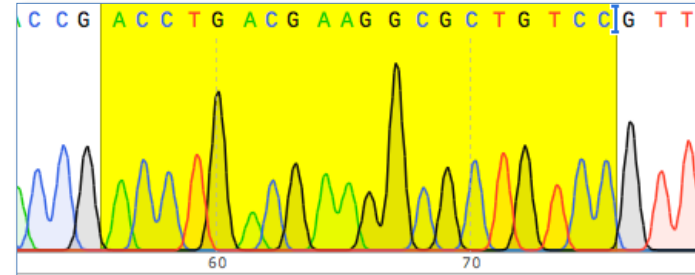
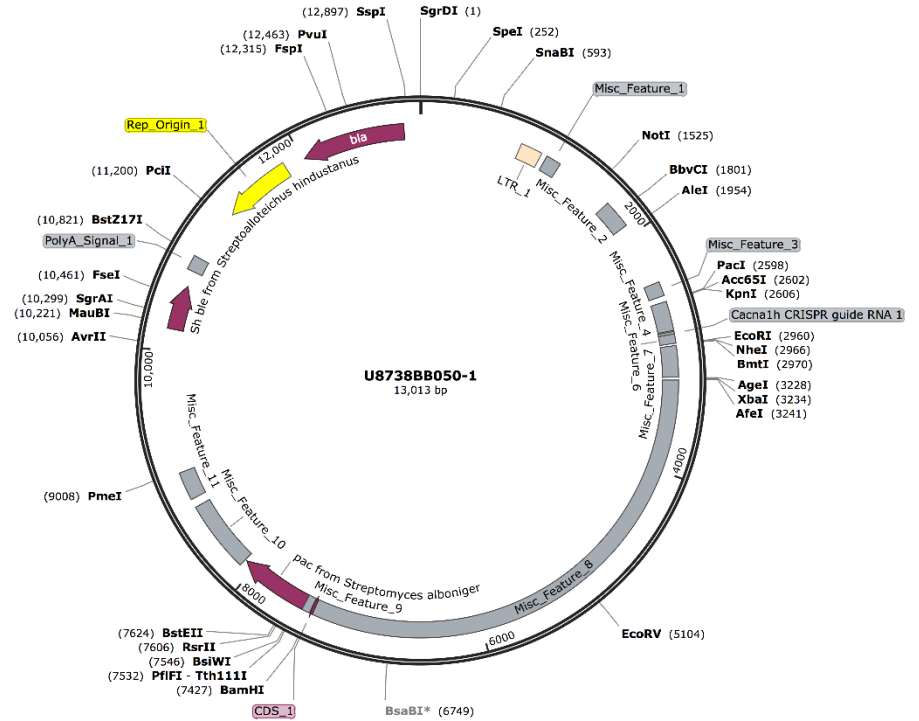
a

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b

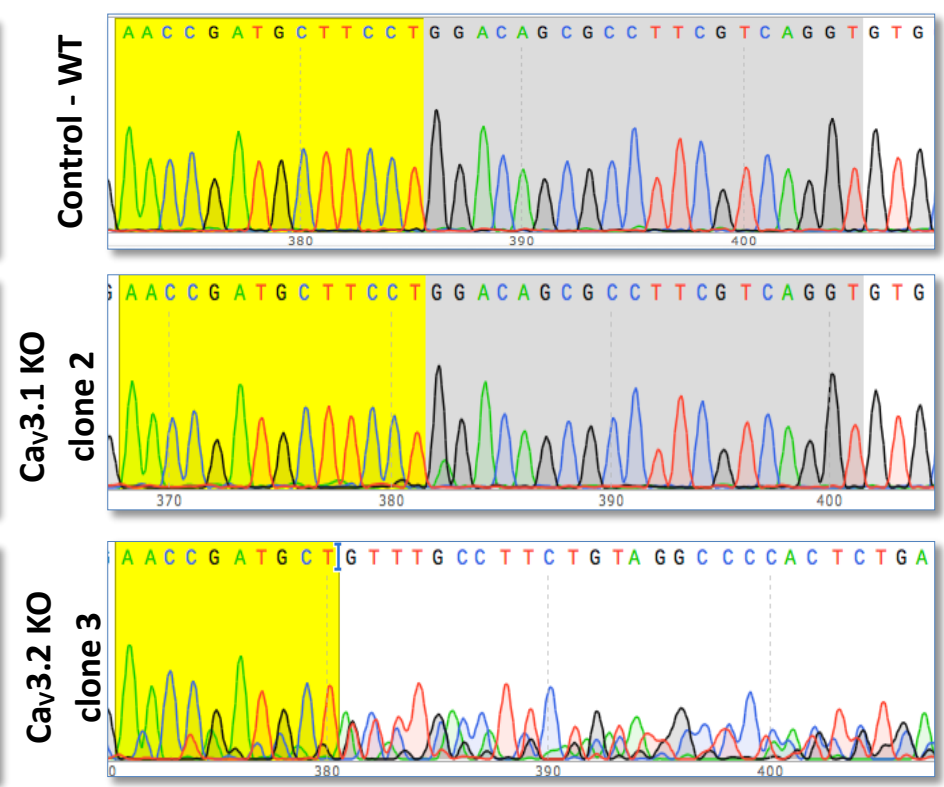
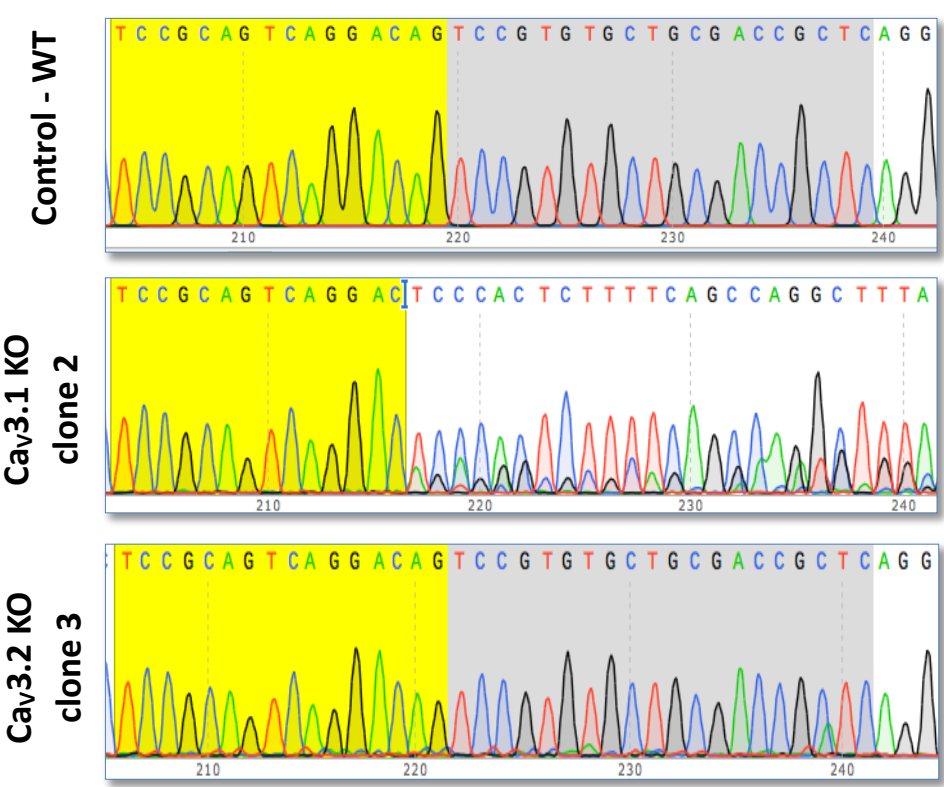
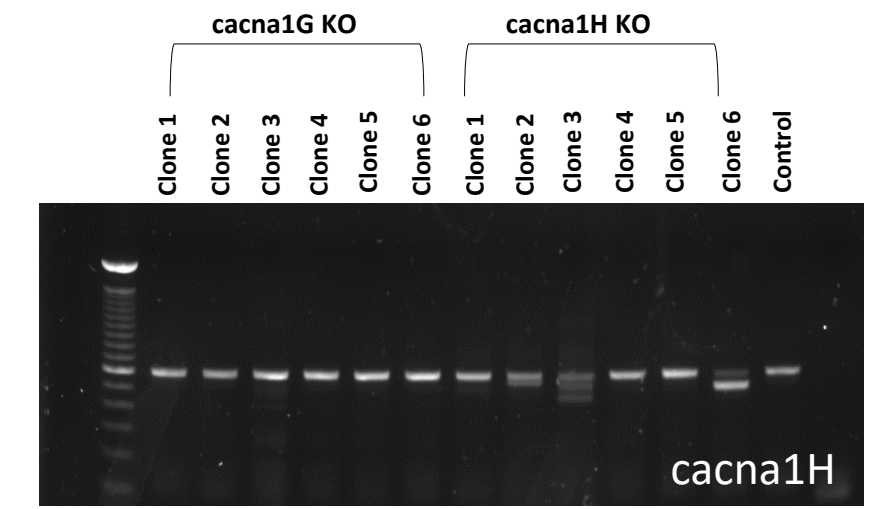
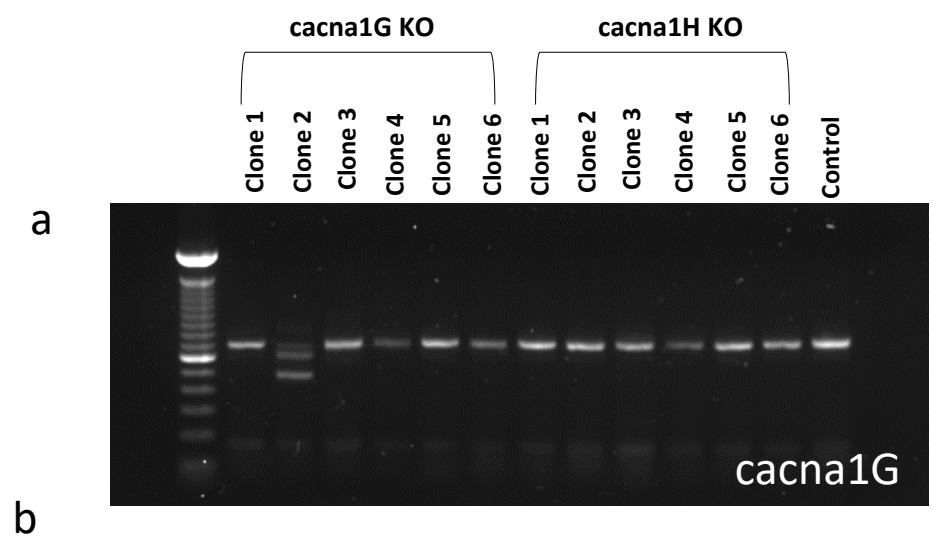
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Supplementary Figure S2. Validation of CRISPR-cas9- gRNA for cacna1G and Cacna1H.

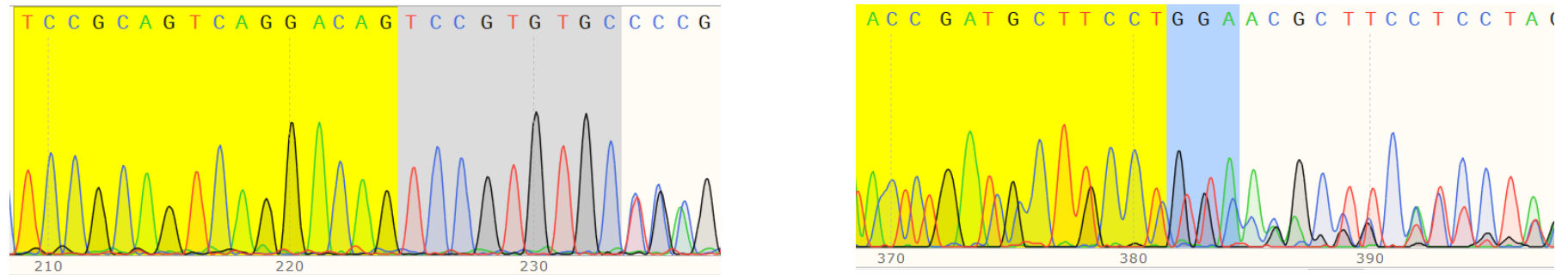
plenti-CRISPR-cas9-cacna1G a) and plenti-CRISPR-cas9-cacna1H b) vector map expressing gRNA mapping to exon 4 of 36 exons containing cacna1G gene and on exon 6 of 35 exons containing cacna1H gene under the control of U6 promoter, with selection markers ampicillin for prokaryotic and puromycin for mammalian cell selection. a) and b) plasmids were confirmed by restriction digestion by MluI restriction enzyme and by Sanger sequencing using primer designed for U6 promoter. (gRNA sequences: Cacna1G-TCCGTGTGCTGCGACCGCTC, Cacna1H- ACCTGACGAA GGCGCTGTCC).

Gel: Panel A: Lane 1-10KB ladder, Lane 2 –circular plasmid, Lane 3-No restriction enzyme control, Lane 4- plasmid digested by Mlu. Panel B: Lane 1 –circular plasmid, Lane 2-No restriction enzyme control, Lane 3- plasmid digested by Mlu, Lane 4-10KB ladder. Yellow highlight - gRNA sequence.



Ca_v3.1 and Ca_v3.2 DKO clone 1

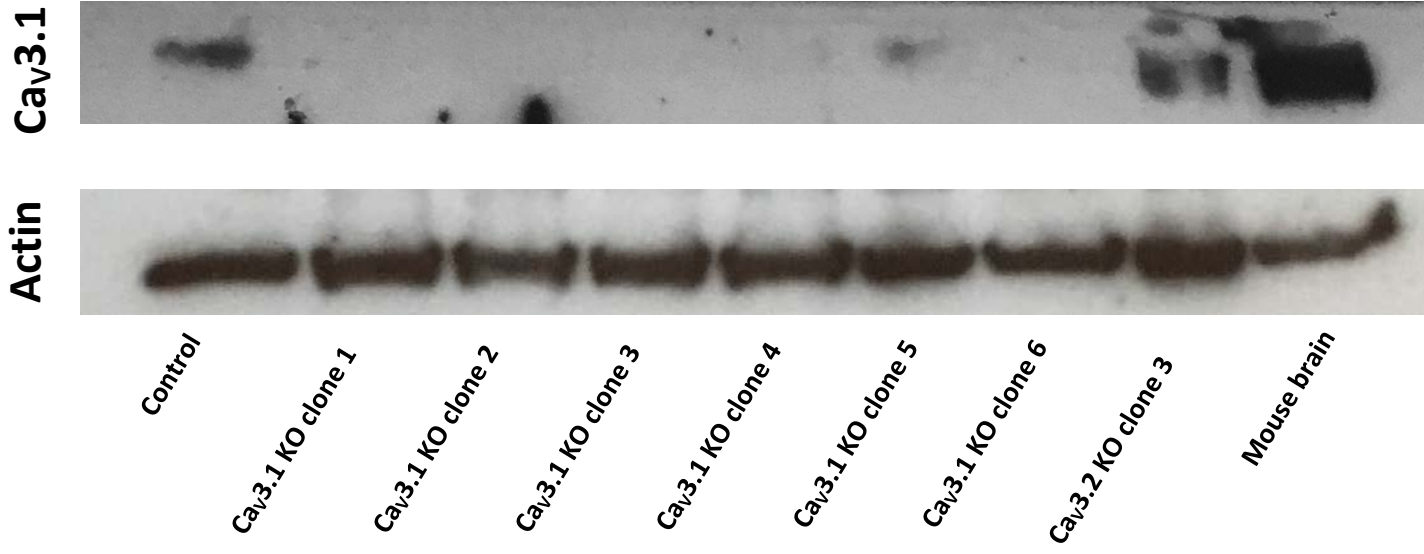
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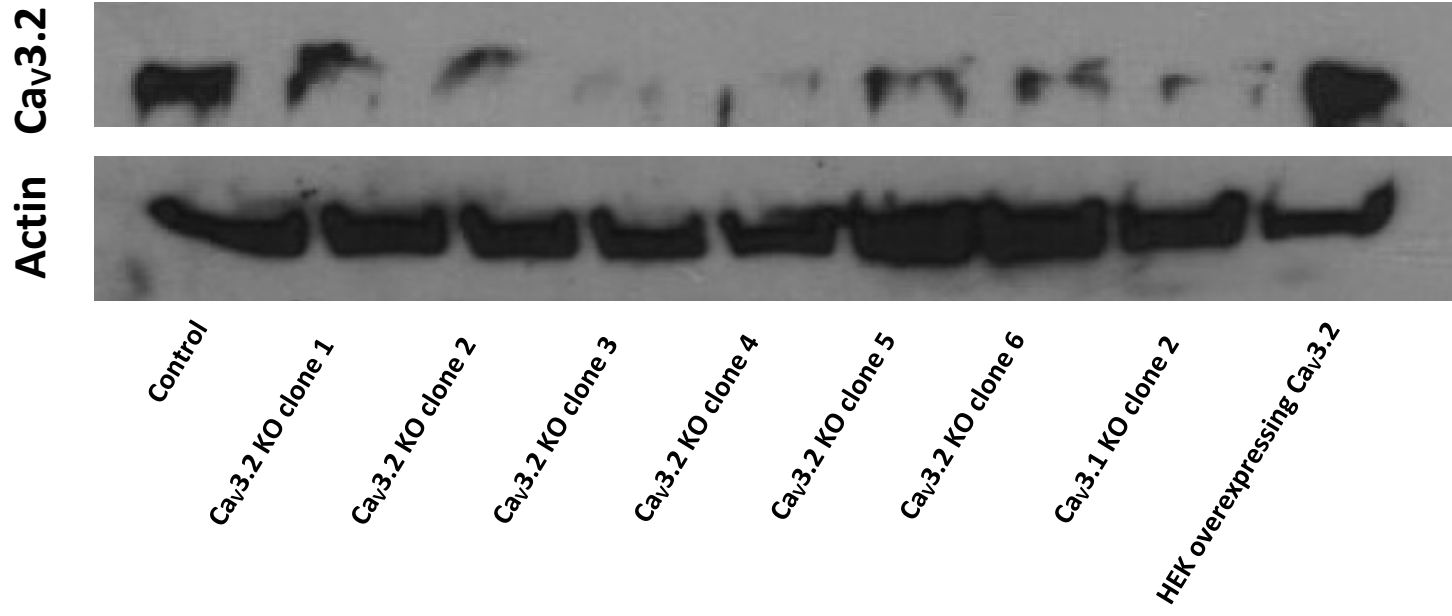
Supplementary Figure S3. Confirmation of Cav3.1 and Cav3.2 knockout at the genomic level.

a) PCR amplification for Cav3.1 (left panel) and Cav3.2 (right panel) genes in the six clones each from cacna1G and cacna1h by using primers spanning gRNA region. Cacna1G clone 2 and Cacna1H clone 3 harbouring indel mutation shows PCR product shift compare to control. b) Sequencing analysis of cacna1G and cacna1H SKO in the clone 2 of cacna1G, clone 3 of cacna1H. Sequencing results show deletion of the gRNA sequence with the frameshift mutation compare to wild type (WT) control, confirming the knockouts. c) Sequencing analysis of DKO shows frameshift mutation compare WT as in b). Right panel-sequencing for cacna1G; left panel- sequencing for cacna1H. Yellow highlight represents the sequence before gRNA in the genome and grey highlight represent the gRNA sequence.

a

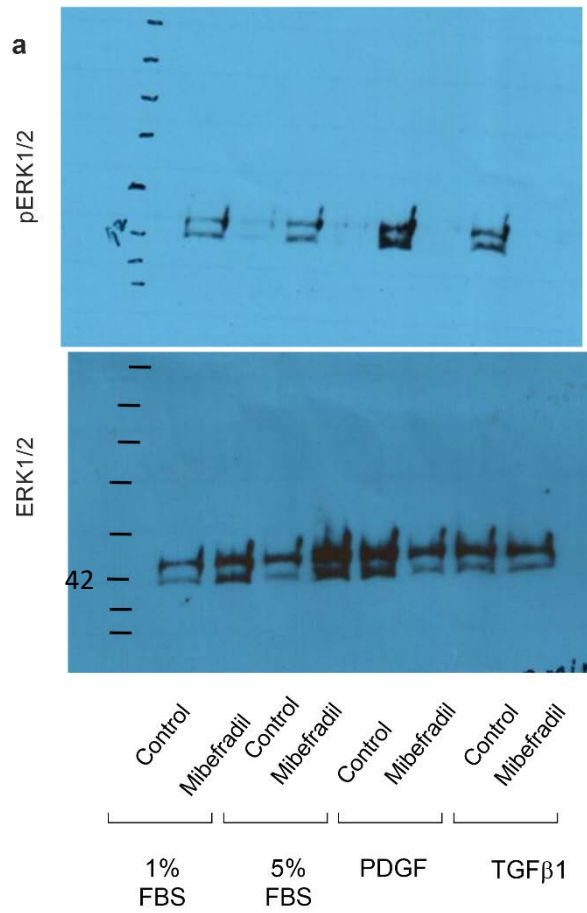


b

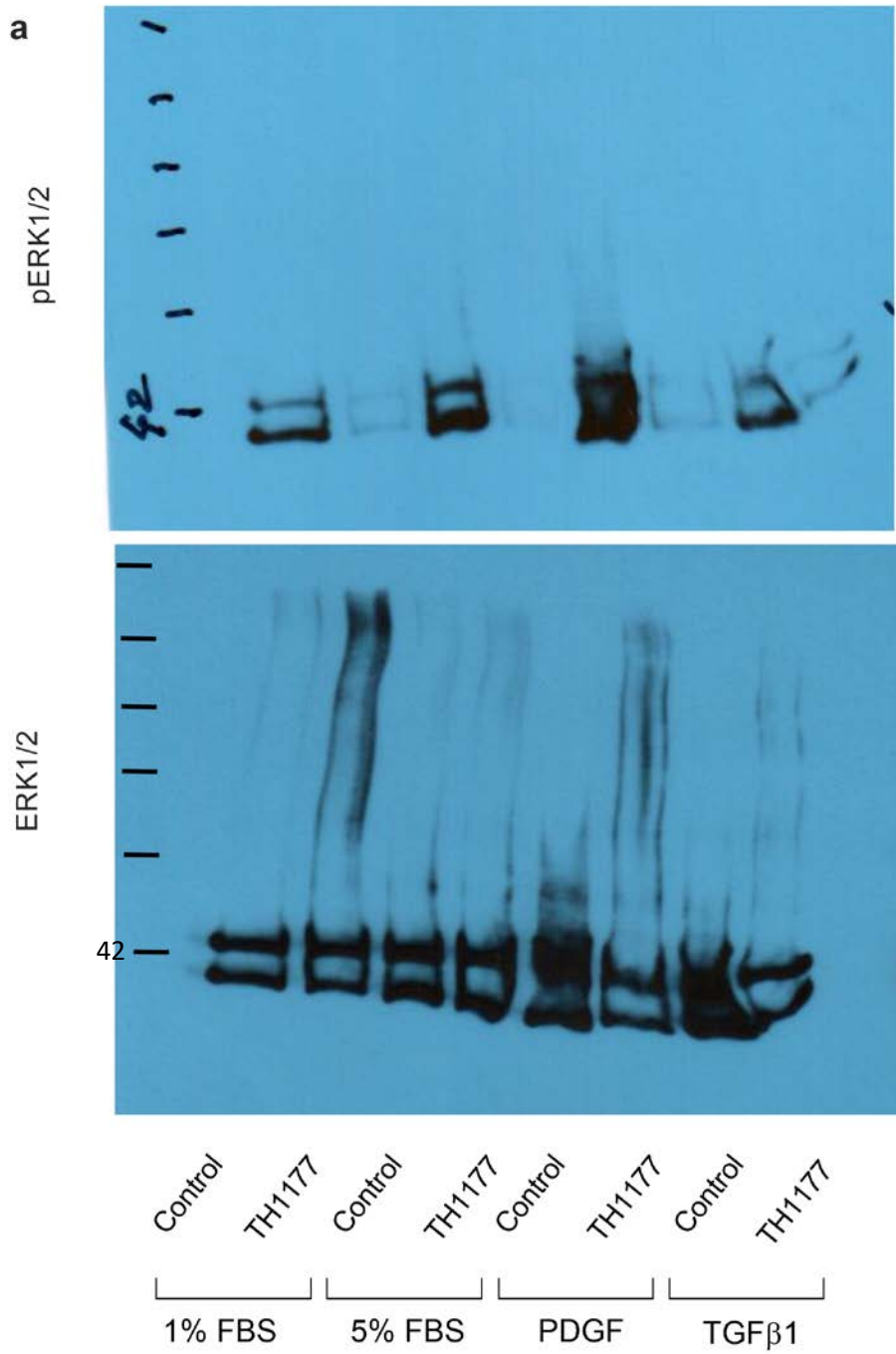


Supplementary Figure S4. Confirmation of cacna1G and cacna1H knockout at the post-translational level.

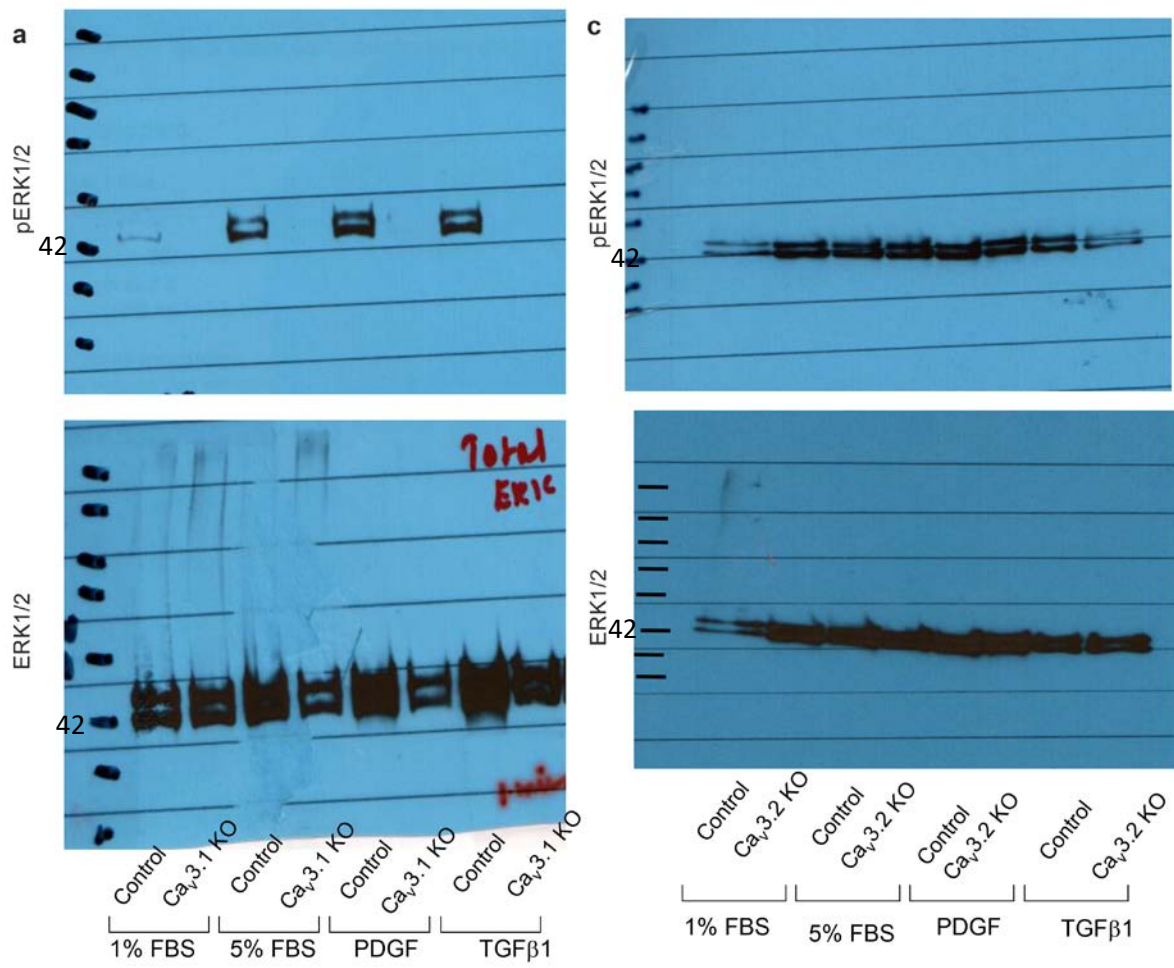
Western blot for Cav3.1 (a) protein expression in the single-cell clones of Cav3.1 knockout show absence of bands in all six Cav3.1 KO clones compared to wild type cells and the positive control mouse brain. (b) single-cell clone of Cav3.2 KO shows the absence of bands in 5 clones compared to wild type and positive control HEK cell overexpressing Cav3.2. Loading control- actin.



Supplementary Figure S5: Figure shows the full-length image of Western blot for Figure 2a described in the main text.



Supplementary Figure S6: Figure shows the full length image of Western blot for Figure 3a described in the main text.



Supplementary Figure S7: Figure shows the full length image of Western blot for Figure 4a and 4c described in the main text.

Supplementary Table S1: Primers list

Primer	Forward	Reverse
RT-PCR		
Ca _v 3.1	TTGCTGGGATGCTGGAGTAC	GGCACGCTCCTGCAGGAT
Ca _v 3.2	GAAGATAGCTGCTGCTCCG	AGTCTCCACGACCAGCTTGA
Ca _v 3.3	AGCACTGAGCGCATCTTCCT	CACAGCAACAGCATCCAATC
PCR		
Ca _v 3.1	GGAGACTGTCATTGCTTGGT	ACTGCAAGAGTTGGAAAGGG
Ca _v 3.2	CAGAAGCTGCTCTGACCTTG	ACAGTCTTAGAGGGAGGGTC