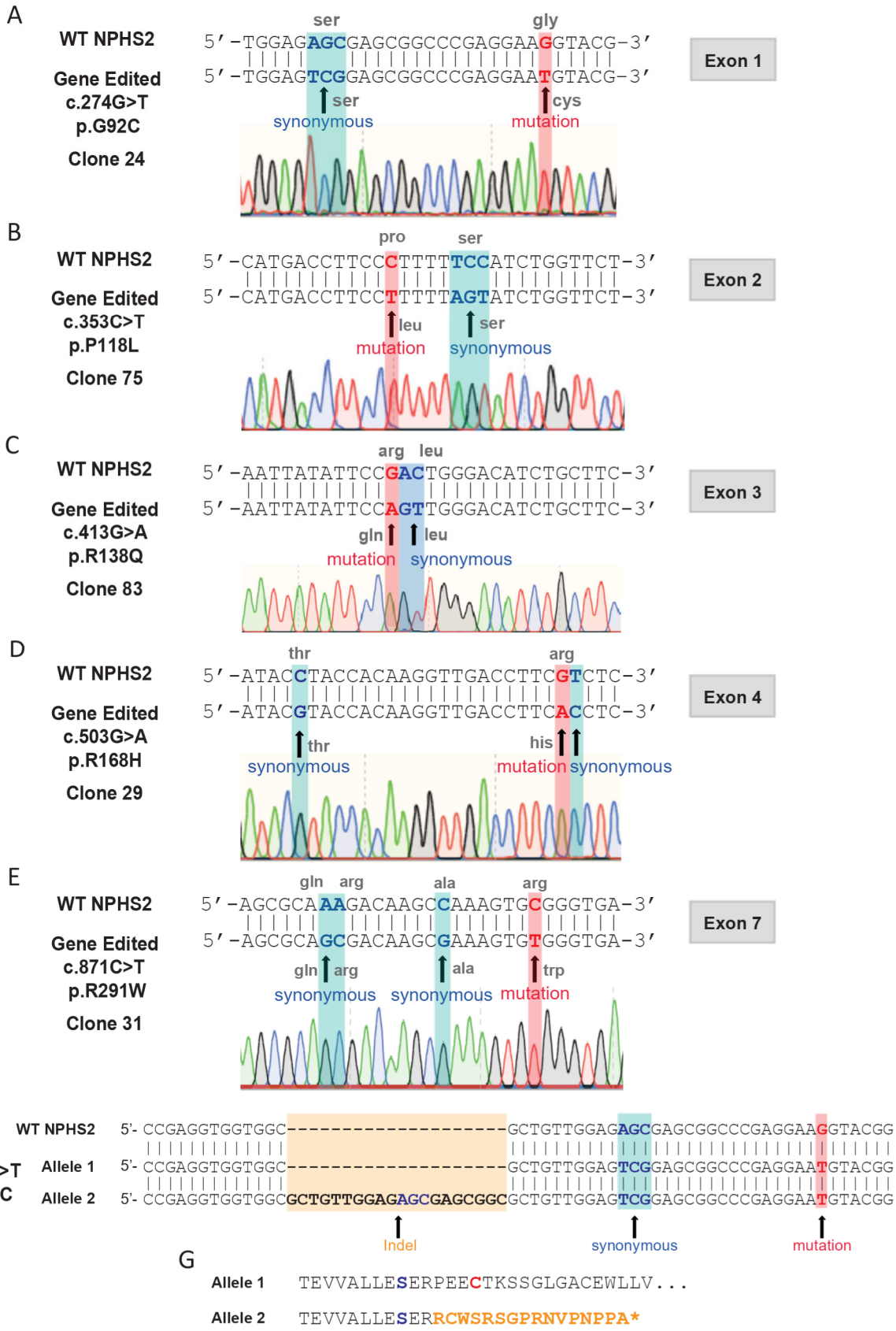
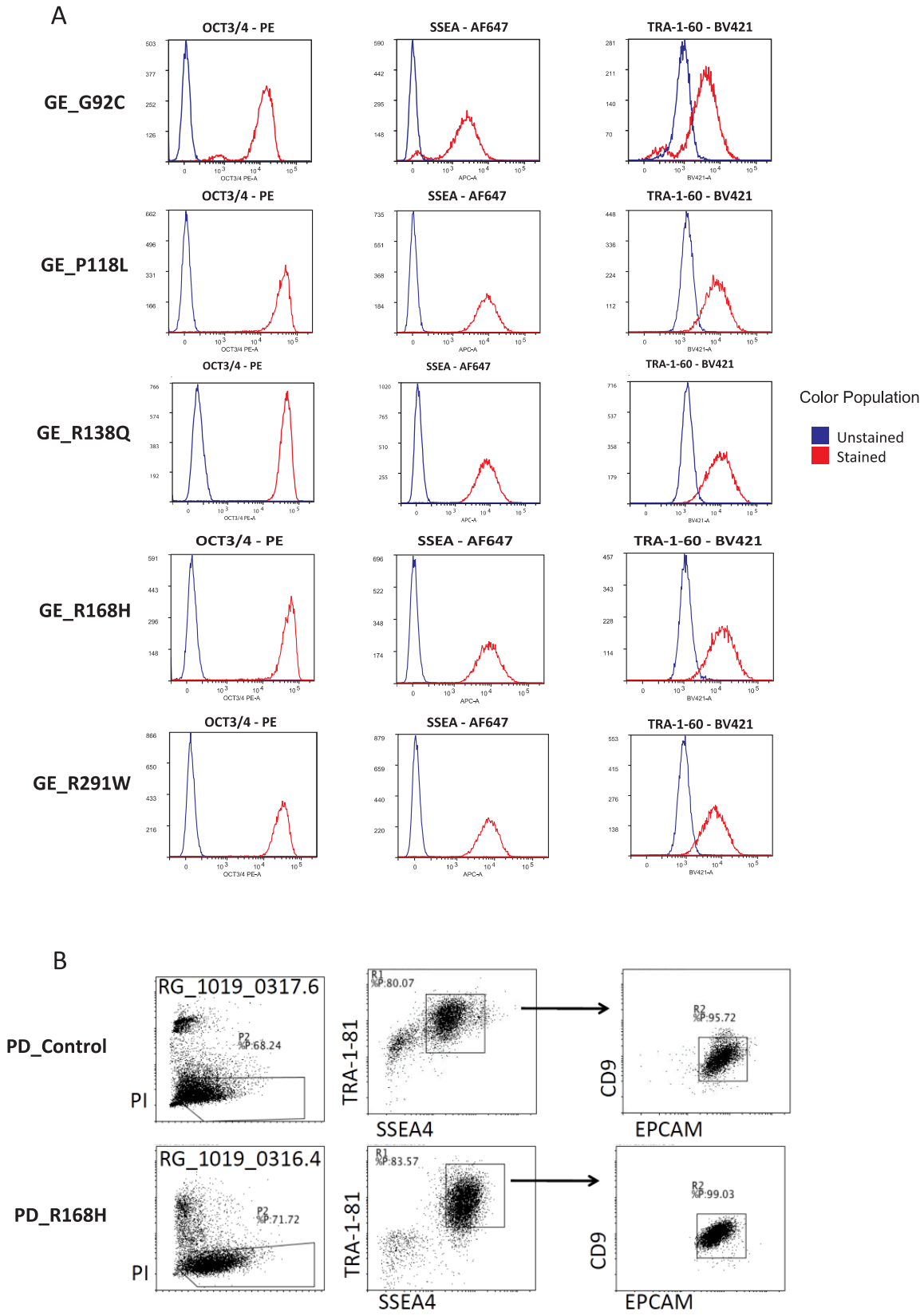
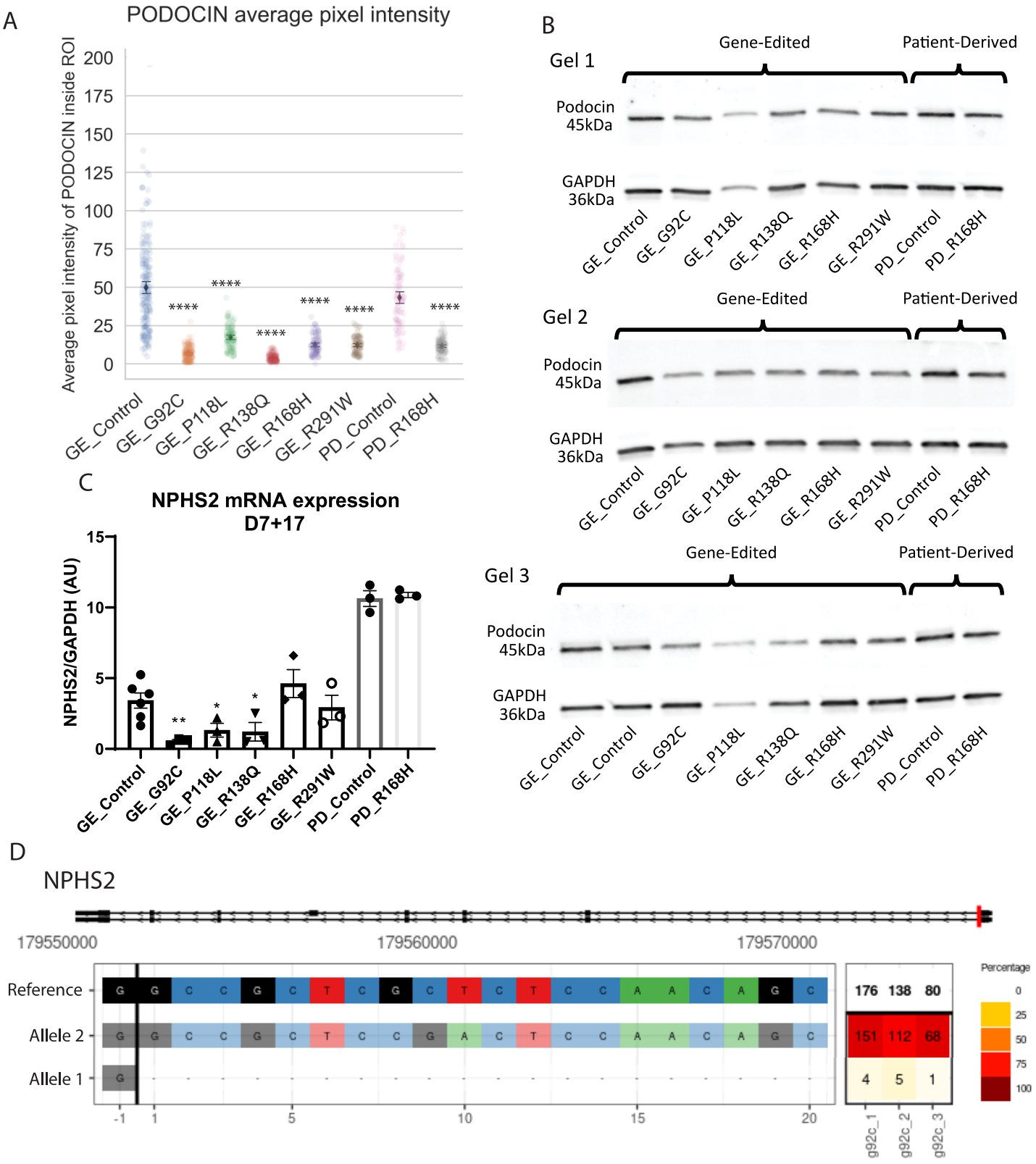
Supplemental table of content



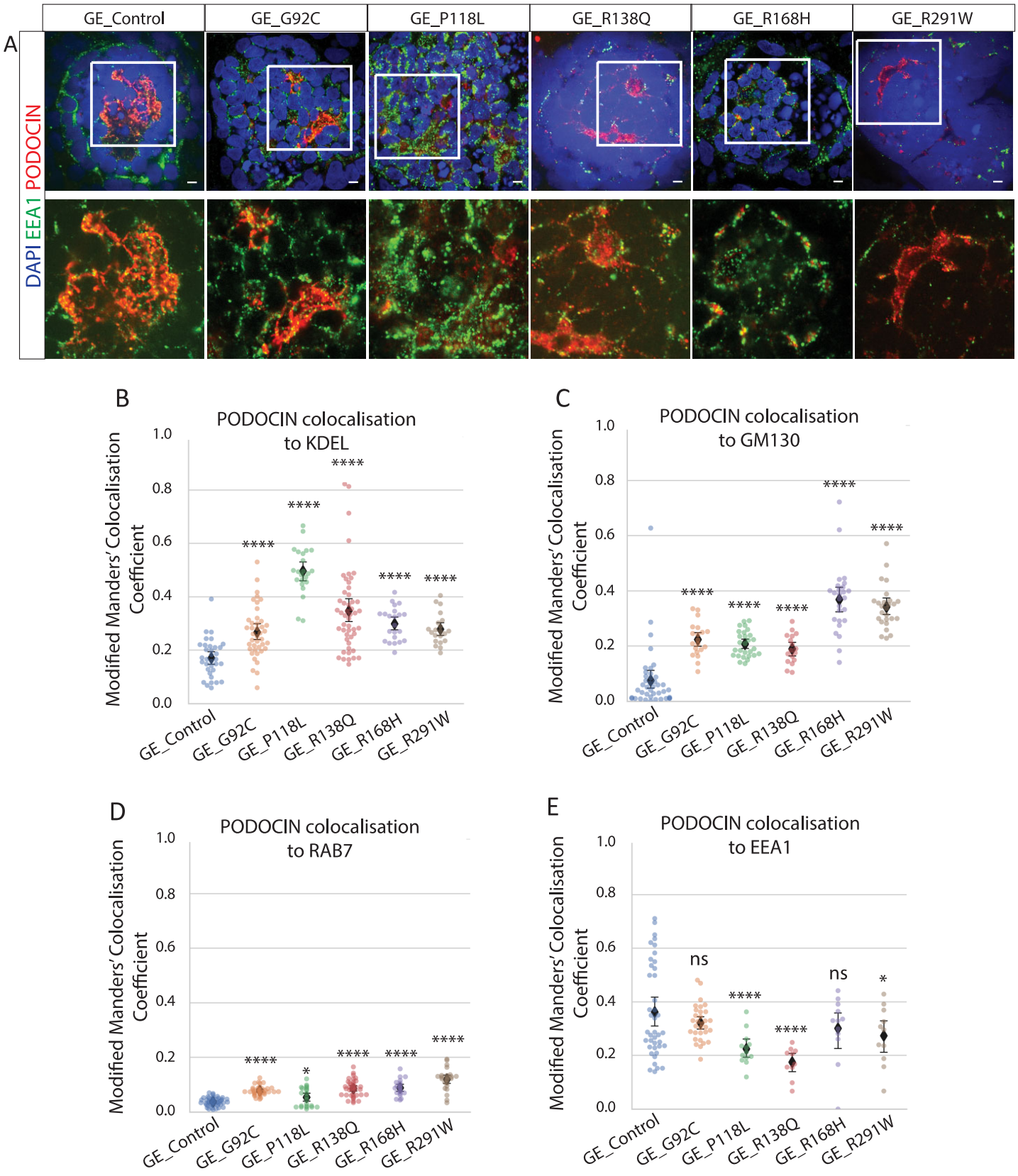
Supp Figure 1. (A-E) Sanger sequencing of gene edited iPSC lines aligned with WT NPHS2 showing homozygous knock-in of the intended variant and corresponding amino acid change in grey: c.274G>T p.G92C (A); c.353C>T p.P118L (B); c.413G>A p.R138Q (C); c.503G>A p.R168H (D); c.871C>T p.R291W (E). Synonymous base pair changes are highlighted in blue and intended variants in red. (F) DNA sequence alignement of WT NPHS2 and the variant alleles of c.274G>T p.G92C showing a 20bp indel (5'-GCTGTTGGAGAGCGAGCGGC) upstream of the synonymous and intended variants on one of the alleles. The 20bp indel is highlighted in yellow, the synonymous base pair change in blue and the intended variant in red. (G) Protein translation of the DNA sequence (F). The indel on allele 2 causes a frame shift variant and premature termination, highlighted in orange.



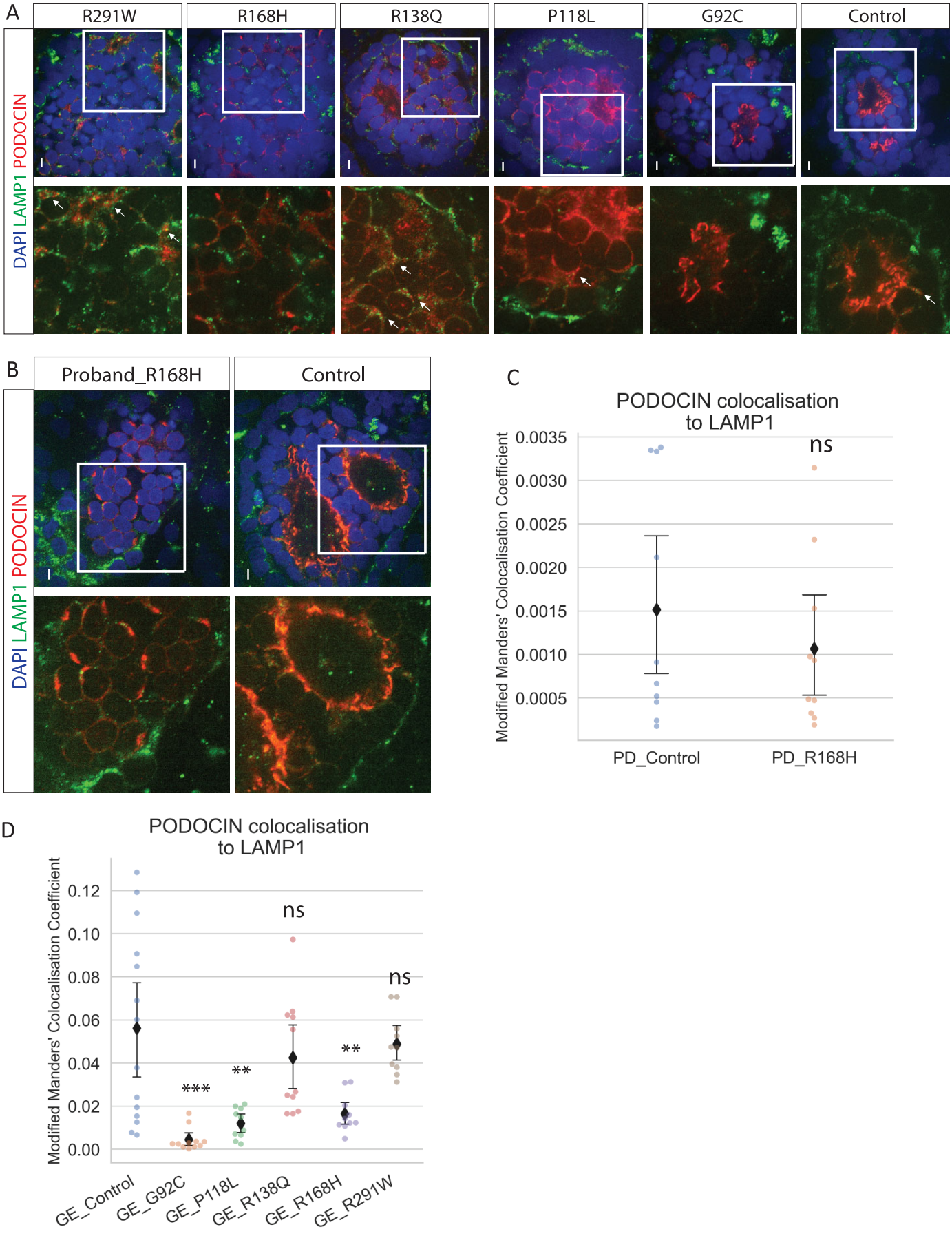
Supp Figure 2. (A) Flow cytometry analysis of NPHS2 variant iPSC lines showing the expression of the pluripotency markers OCT3/4, SSEA and TRA-1-60. (B) Flow cytometry analysis of patient (PD_R168H) and unaffected relative (PD_Control) derived iPSC lines showing the expression of pluripotent markers SSEA4, TRA-1-81 and CD9.



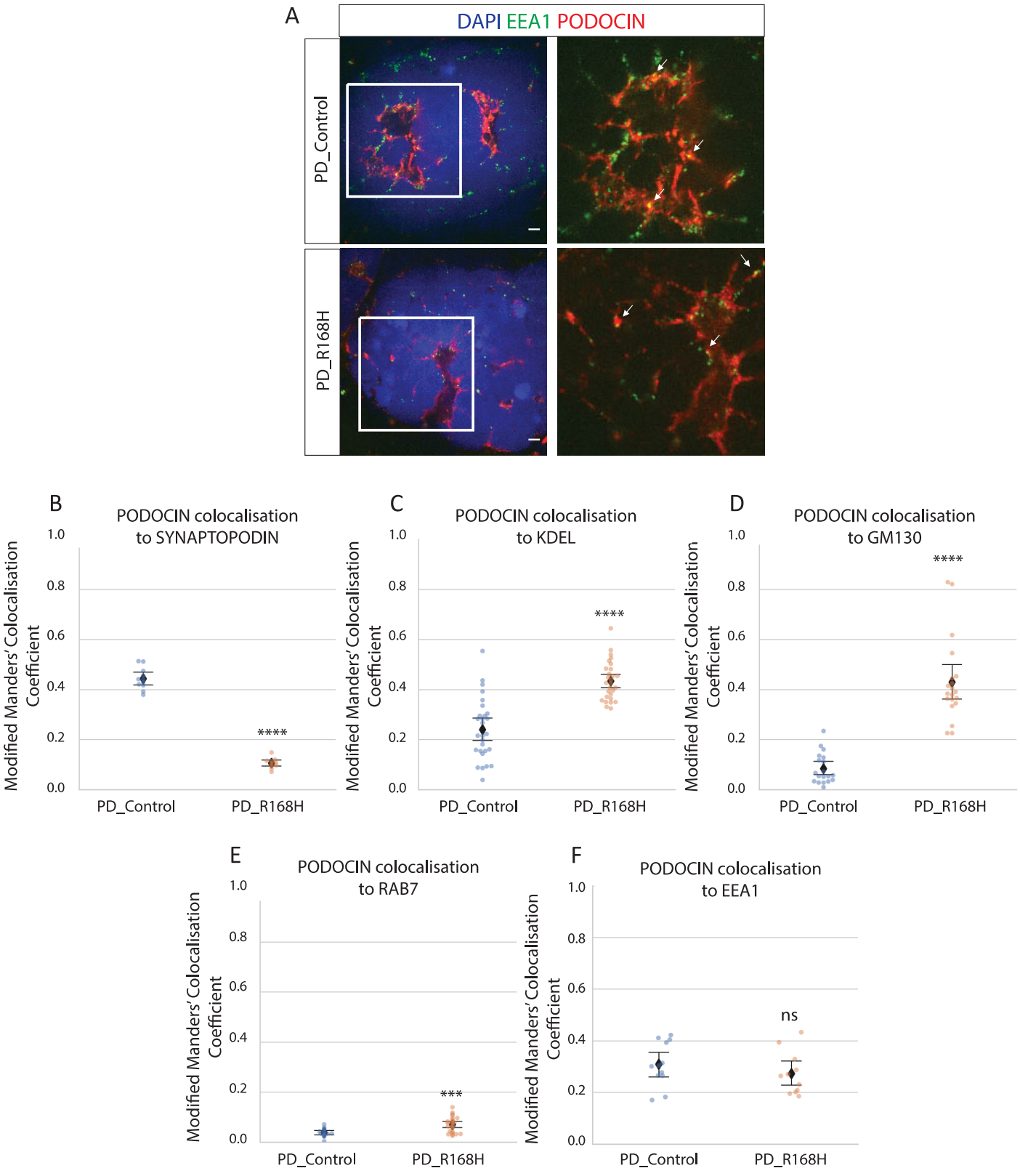
Supp Figure 3. (A) Quantification of PODOCIN protein expression in glomeruli stained by immunofluorescence in gene-edited and patient-derived kidney organoids. (B) Western blot showing PODOCIN protein expression levels at D7+18 in all isolated glomeruli samples. (C) NPHS2 mRNA expression levels at D7+17 measured by qPCR in isolated glomeruli. (D) NPHS2 transcript sequence distribution over the indel region measured in isolated glomeruli bulk RNA sequencing data for the GE_G92C samples. The red bar shows the position of the indel sequence in the genome. The "Reference" line is the reverse complement to the indel sequence with one additional base to the left. The wildtype allele (allele 1) which does not contain the indel sequence displays the indel sequence as a deletion (-). The table to the right indicates the number of reads spanning the region of interest. Remaining not displayed reads are other transcript variants. *p<0.05; **p<0.01; ****p<0.0001 vs respective Controls.



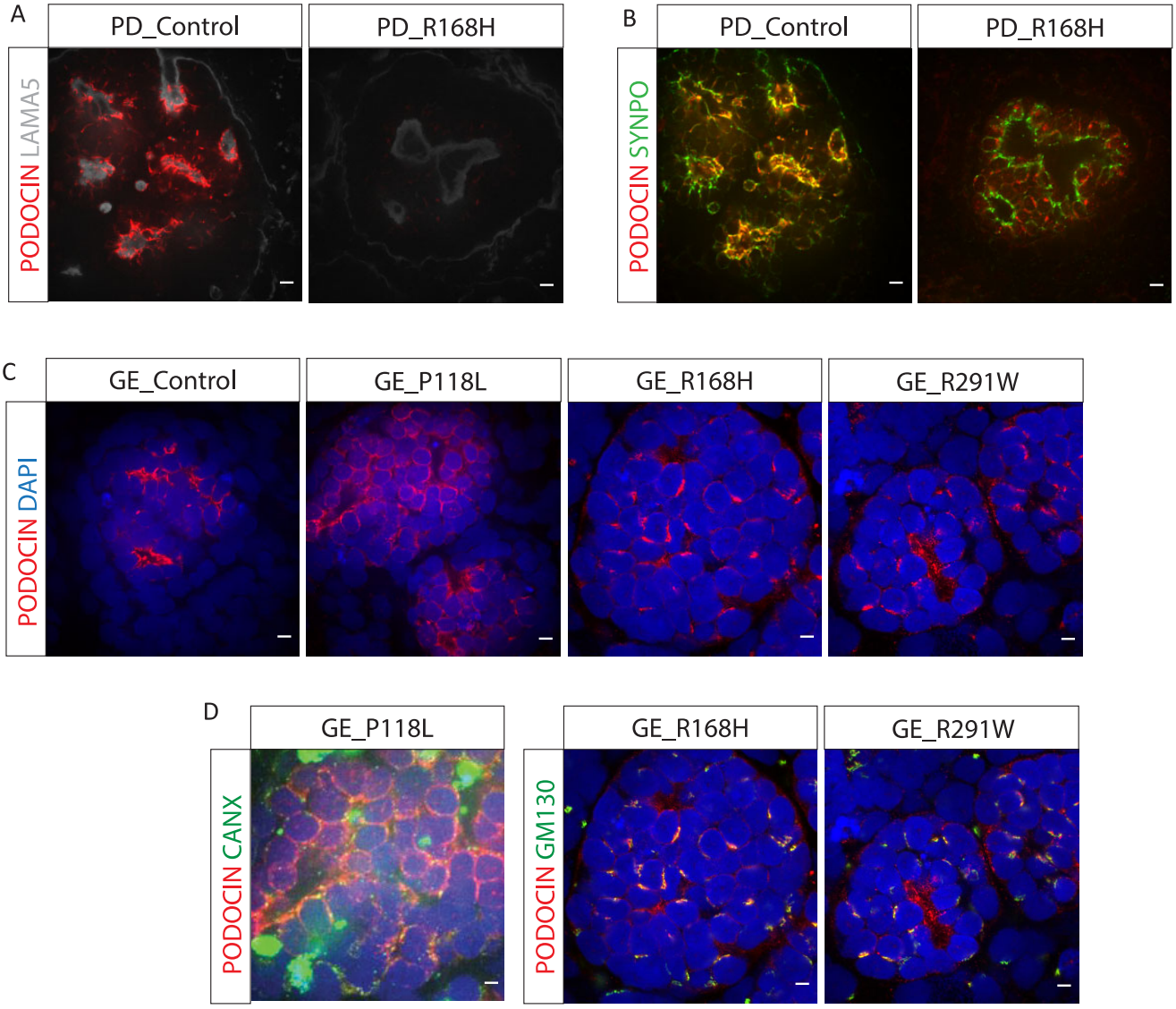
Supp Figure 4. (A) Kidney organoids stained for PODOCIN and the early endosome marker EEA1 showing partial co-localisation with this subcellular structure for all cell lines at D7+17 (Dapi=nuclei, scale bar 5um, images are adjusted to maximum value). (B-E) Quantification for subcellular markers KDEL (B), GM130 (C), Rab7 (D) and EEA1 (E). *p<0.05; ****p<0.0001 vs Control.



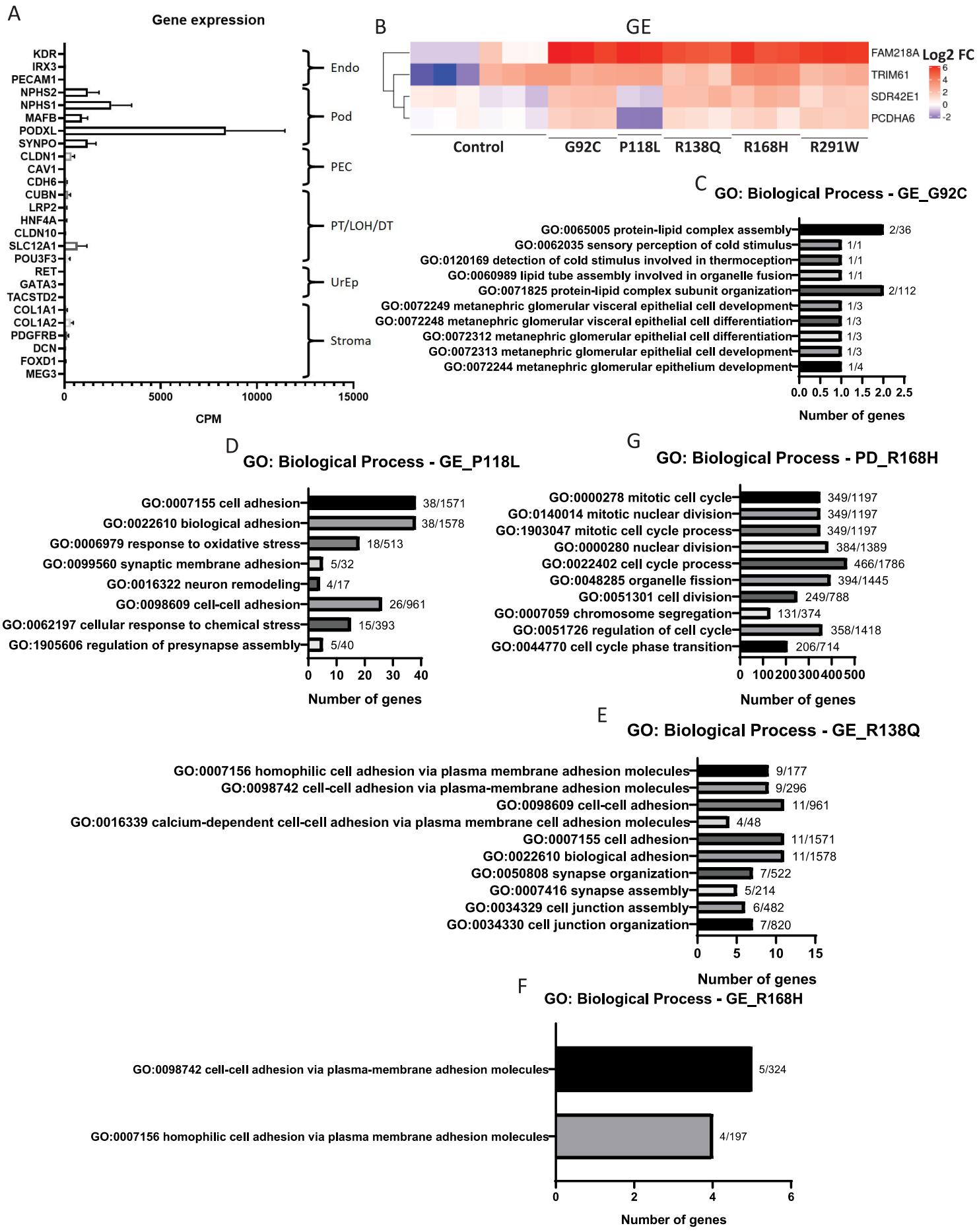
Supp Figure 5. Kidney organoids stained for PODOCIN and the lysosome marker LAMP1 showing no increased co-localisation with this subcellular structure in variant lines compared to control at D7+17 (DAPI=nuclei, scale bar 5um, images adjusted to maximum value). **p<0.01; ***p<0.001 vs Controls.



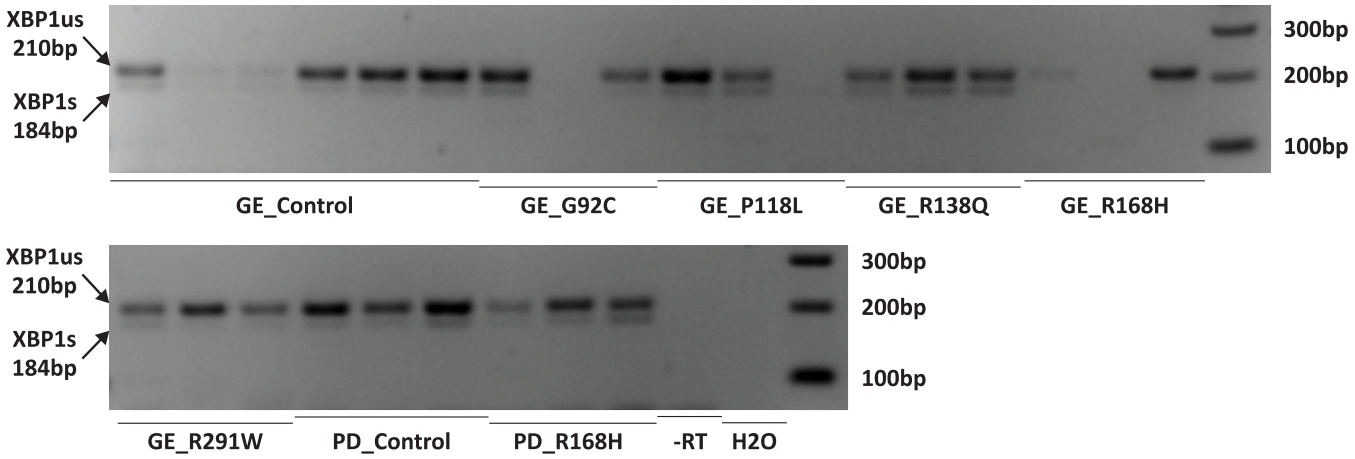
Supp Figure 6. (A) Kidney organoids stained for PODOCIN and the early endosome marker EEA1 showing limited variant localisation outside of Golgi apparatus at D7+17 (Dapi=nuclei, scale bar 5um, images are adjusted to maximum value). (B-F) Quantification for subcellular markers SYNPO (B), KDEL (C), GM130 (D), Rab7 (E) and EEA1 (F). *p<0.05; ***p<0.001; ****p<0.0001 vs Control.



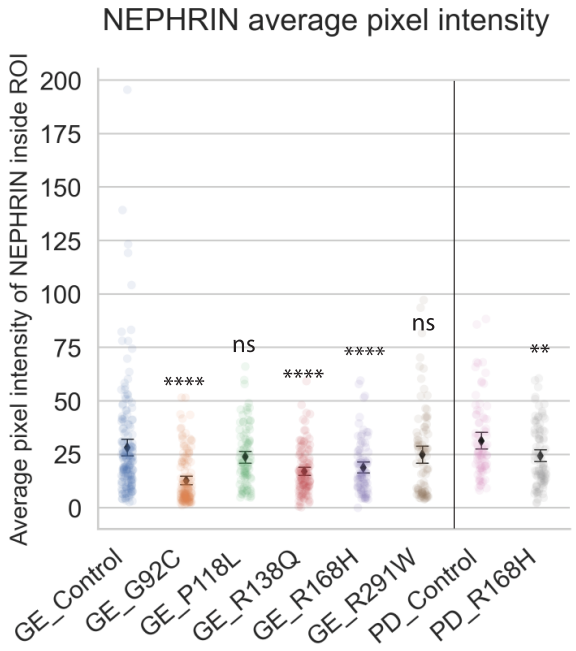
Supp Figure 7. (A-B) Kidney organoids stained for PODOCIN, the glomerular basement membrane marker LAMININ alpha 5 (LAMA5) and the slit-diaphragm marker SYNAPTOPODIN (SYNPO) showing altered PODOCIN level and pattern in patient-derived variant at D7+14. (C-D) Kidney organoids stained for PODOCIN, the endoplasmic reticulum marker CALNEXIN (CANX) and the Golgi marker GM130 showing PODOCIN mislocalisation in gene-edited variant lines at D7+14. (Dapi=nuclei, scale bar 5um, images are adjusted to maximum value). Panel A, images are scaled at the same range to show relative PODOCIN levels. Panels B-D, images were adjusted to maximum value to illustrate location and not relative protein levels.



Supp Figure 8. (A) Kidney genes expression in bulk RNA sequencing of organoid glomeruli isolated at D7+14 showing a significant enrichment of podocyte specific-genes. (B) Heatmap of differentially expressed genes in all gene-edited glomeruli compared to control. (C-G) Pairwise analysis of variant and respective control samples displayed as a list of the top 10 biological process gene ontology terms for differentially expressed genes in each comparison. GO terms are displayed in a p-value increasing order with significance assumed for $p<0.05$. No Biological Process GO terms were identified for GE_R291W.



Supp Figure 9. Electrophoresis of XBP1 PCR showing limited XBP1 mRNA splicing in all organoid glomeruli at D7+17. XBP1us, XBP1 unspliced; XBP1s, XBP1 spliced.



Supp Figure 10. Quantification of NEPHRIN protein expression in gene-edited (GE) and patient-derived (PD) kidney organoids at D7+17. The black diamond represents the mean. **p<=0.01; ****p<=0.0001 vs respective Controls.

Supplementary file 1. Knock-in template sequences (olinucleotide or gene block) used for gene-editing of NPHS2 variant iPSC lines. Introns are shown in lowercase and exons in uppercase. Knock-in variants are shown in red and synonymous variants introduced to facilitate the identification of correctly edited clones and prevent re-cutting by CRISPR/Cas9 complex are shown in blue.

c.274G>T p.G92C

Oligonucleotide sequence:

GTCCGAGGCTCCGGCGAGGAGGGCACCGAGGTGGTGGCGCTGTTGGAGTCGGAGCGGCCCGAGGAATg
tacggattcagcaccactatctgctacttttccaggtggtaactaaggggcg

c.353C>T p.P118L

Gene block sequence cloned into a pSMART-HCKan plasmid vector:

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c.413G>A p.R138Q

Gene block sequence cloned into a pSMART-HCKan plasmid vector:

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c.503G>A p.R168H

Gene block sequence cloned into a pSMART-HCKan plasmid vector:

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c.871C>T p.R291W

Gene block sequence cloned into a pSMART-HCKan plasmid vector:

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