Supplemental Data

Identification and functional characterization of *de novo FOXP1* variants provides novel insights into the etiology of neurodevelopmental disorder.

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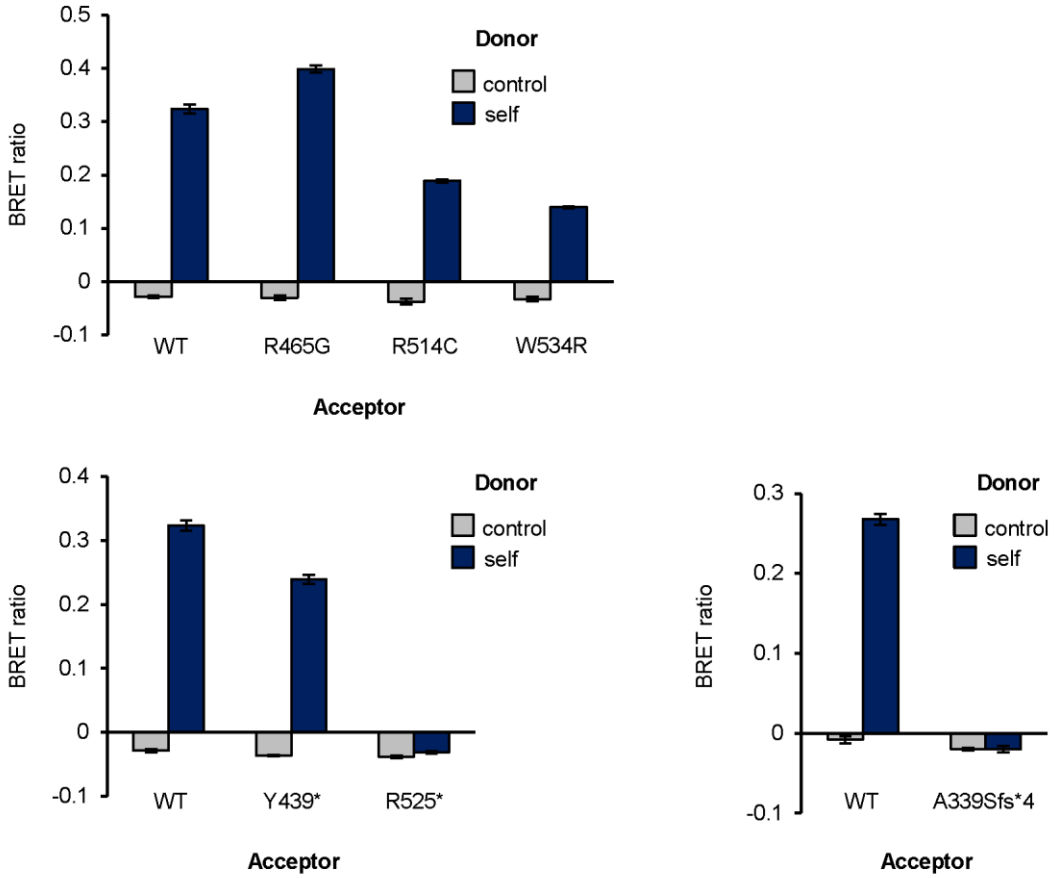
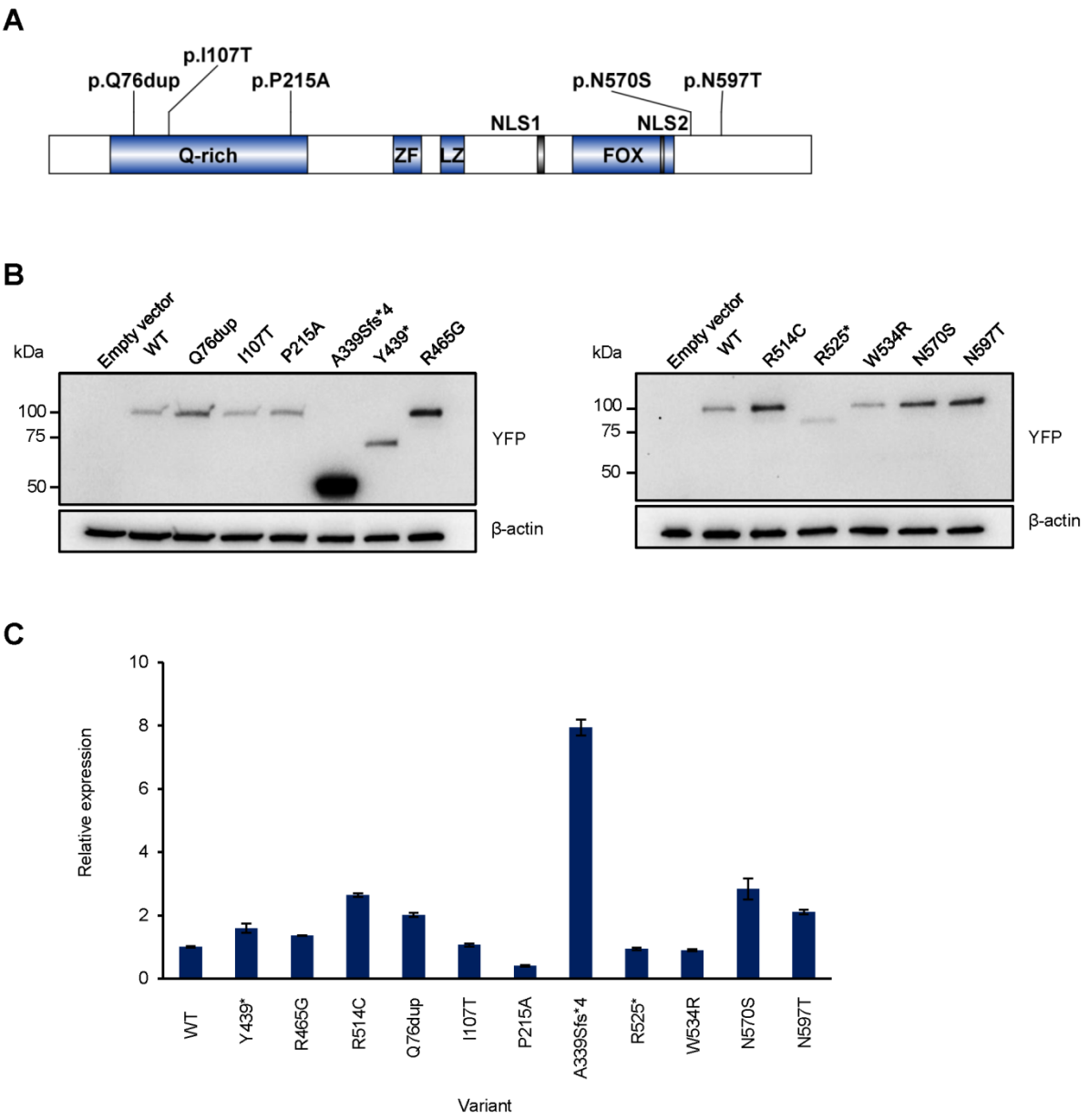
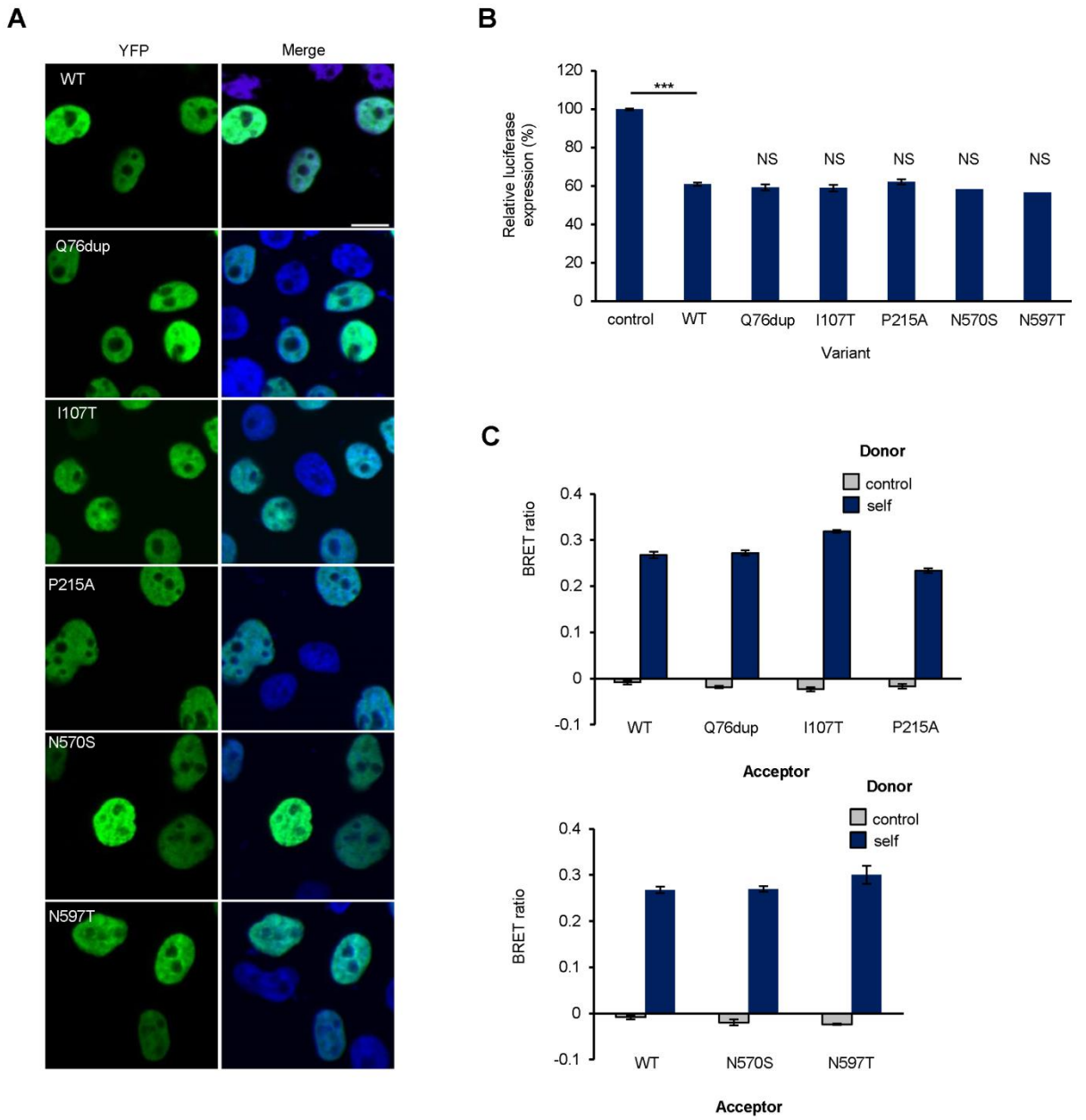


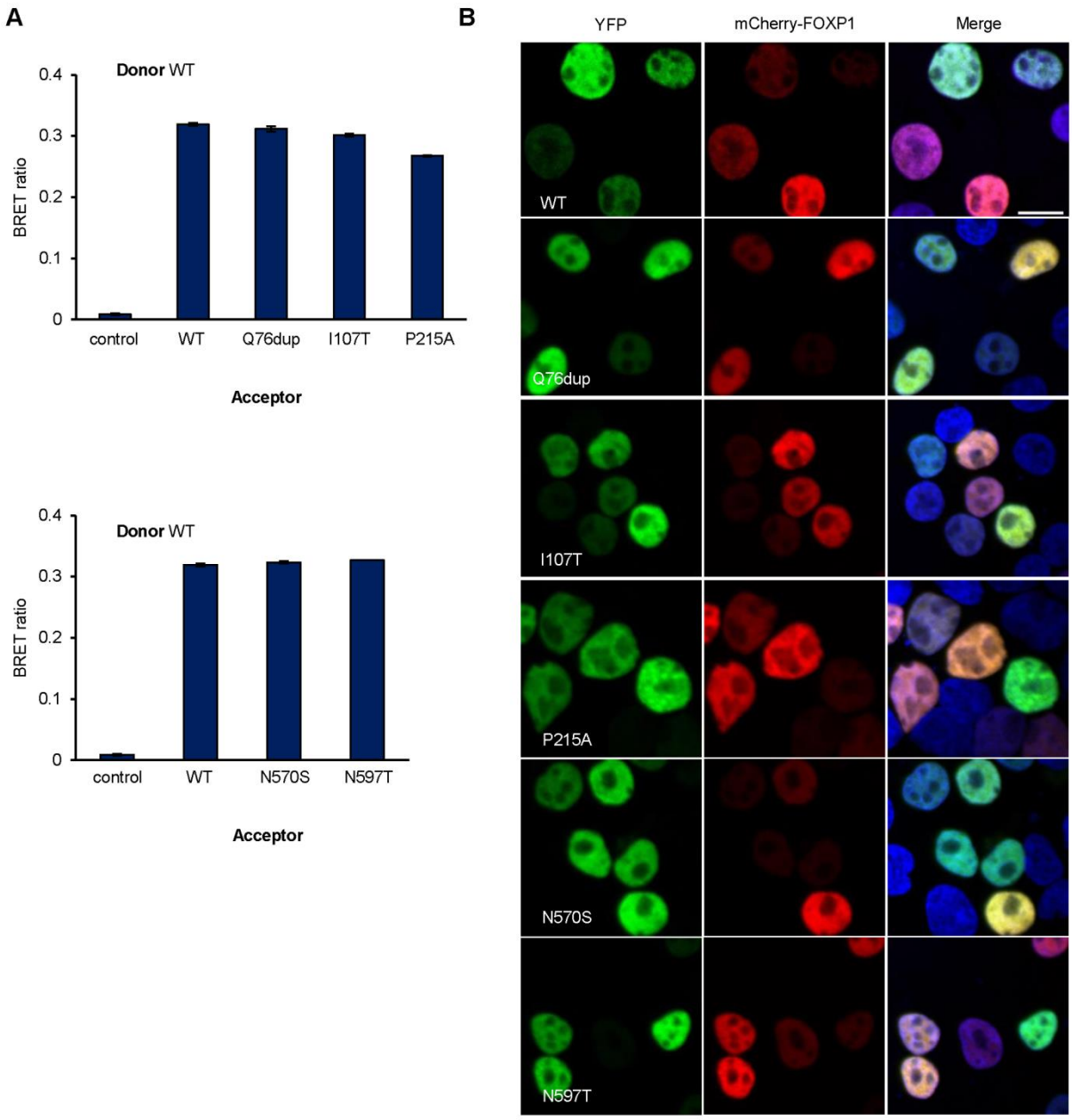
S1 Fig Effects of etiological FOXP1 variants on self-association. BRET assays for self-association of FOXP1 variants. Bars represent the corrected mean BRET ratios ±S.D. of one experiment performed in triplicate.



S2 Fig FOXP1 variants of unknown significance in neurodevelopmental disorder. (**A**) Schematic representation of the FOXP1 protein indicating variants of unknown significance found in cases of ID or CAS. (**B**) Immunoblot of whole-cell lysates of cells expressing FOXP1 variants probed with anti-EGFP antibody. Blots were stripped and re-probed with anti-β-actin antibody to confirm equal loading. (**C**) Relative expression of FOXP1 protein variants in live cells as assessed by YFP fluorescence (average of three experiments ±S.D.).

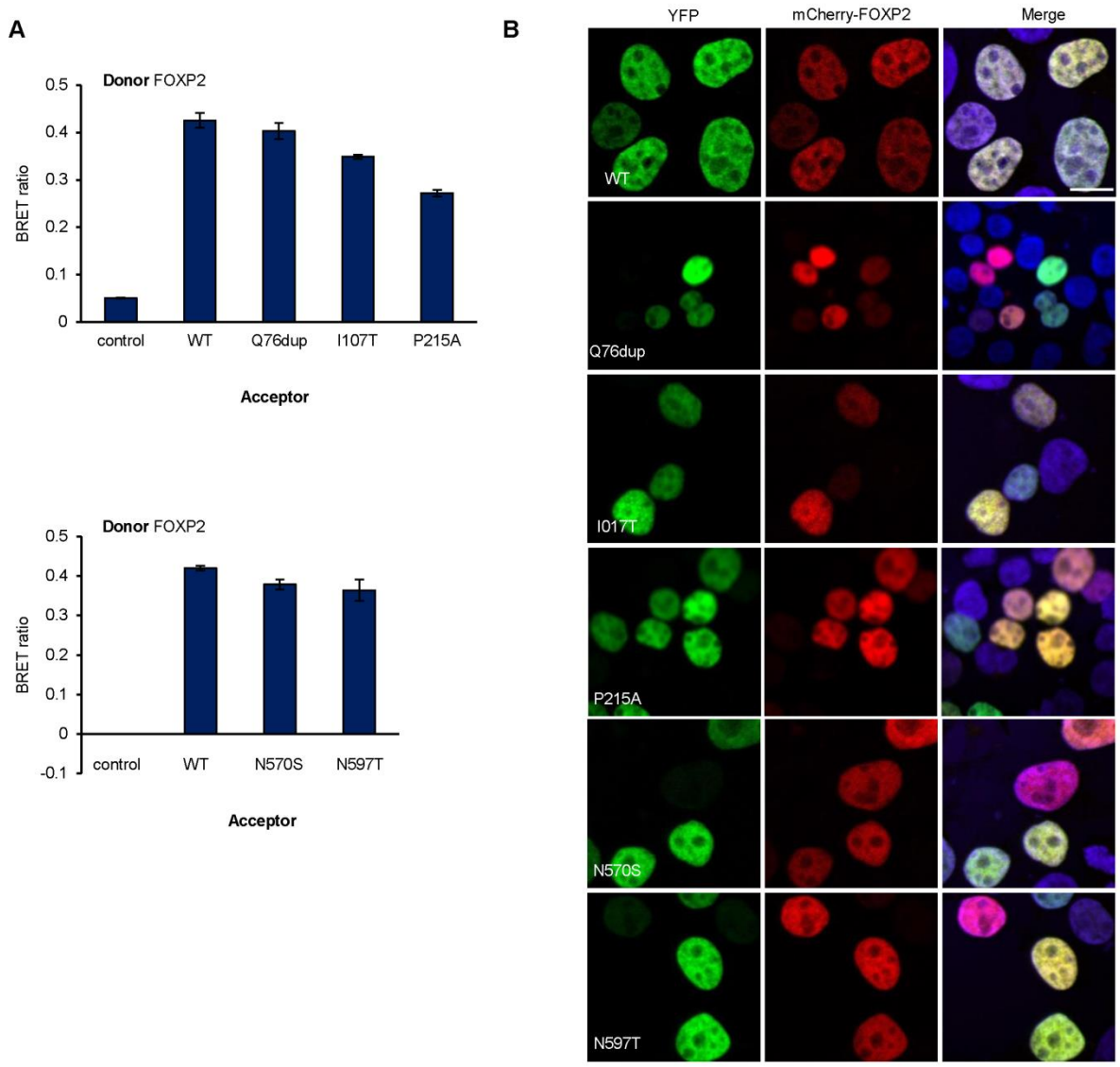


S3 Fig Functional characterization of FOXP1 variants of unknown significance. (A) Fluorescence imaging of cells expressing YFP-tagged FOXP1 variants (green). Nuclei were stained with Hoechst 33342 (blue). Scale bar, 10 μm. (**B**) Luciferase reporter assays using the SV40 promotor. The mean ±S.E.M. of three independent experiments is shown. Values are expressed relative to the control. WT FOXP1 was significantly different to the control (***P<0.001), but not to the FOXP1 variants. (**C**) BRET assays for self-association of FOXP1 variants. Bars represent the corrected mean BRET ratios ±S.D. of one experiment performed in triplicate.

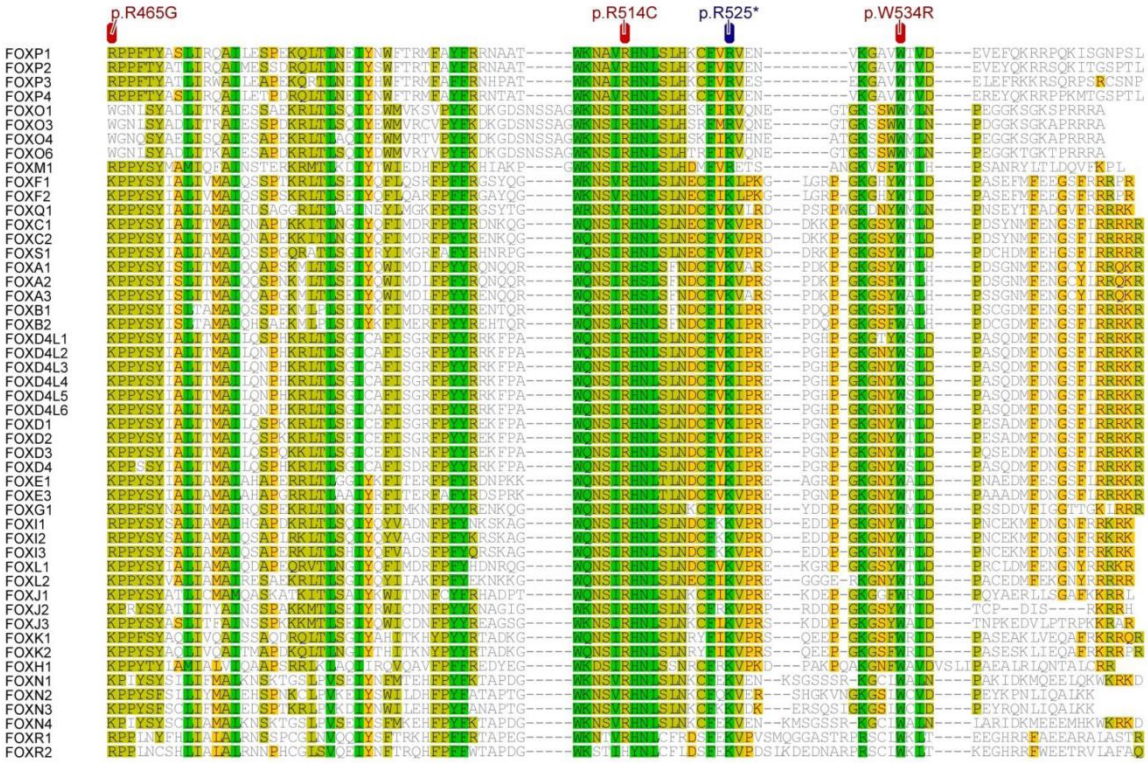


S4 Fig Effects of FOXP1 variants of unknown significance on interaction with WT FOXP1. (**A**) BRET assays for interaction between WT FOXP1 and FOXP1 variants. Bars represent the corrected mean BRET ratios ±S.D. of one experiment performed in triplicate. (**B**) Fluorescence imaging of cells co-transfected with WT FOXP1 and FOXP1 variants. FOXP1 variants fused to YFP are shown in green (left panel) and WT FOXP1 fused to

mCherry is shown in red (middle panel). Nuclei were visualized using Hoechst 33342 (blue). Scale bar, 10 μm.



S5 Fig Effects of FOXP1 variants of unknown significance on interaction with WT FOXP2. (**A**) BRET assays for interaction between WT FOXP2 and FOXP1 variant proteins. Bars represent the corrected mean BRET ratios ±S.D. of one experiment performed in triplicate. (**B**) Fluorescence imaging of cells co-transfected with WT FOXP2 and FOXP1 variants. FOXP1 variants fused to YFP are shown in green (left panel) and WT FOXP2 fused to mCherry is shown in red (middle panel). Nuclei were visualized using Hoechst 33342 (blue). Scale bar, 10 μm.



S6 Fig Protein sequence alignment of FOX domains from human FOX transcription factors. FOXP1 variants arising from *de novo* missense mutations, including the ones reported in this study, are indicated in red, whereas as the variant resulting from a *de novo* nonsense mutation is indicated in blue. UniProt accession numbers: FOXP1 (Q9H334), FOXP2 (O15409), FOXP3 (Q9BZS1), FOXP4 (Q8IVH2), FOXO1 (Q12778), FOXO3 (Q43523), FOXO4 (P98177), FOXO6 (A8MYZ6), FOXM1 (Q08050), FOXF1 (Q12946), FOXF2 (Q12947), FOXQ1 (Q9C009), FOXC1 (Q12948), FOXC2 (Q99958), FOXS1 (O43638), FOXA1 (P55317), FOXA2 (Q9Y261), FOXA3 (P55318), FOXB1 (Q99853), FOXB2 (Q5VYV0), FOXD4L1 (Q9NU39), FOXD4L2 (Q6VB85), FOXD4L3 (Q6VB84), FOXD4L4 (Q8WXT5), FOXD4L5 (Q5VV16), FOXD4L6 (Q3SYB3), FOXD1 (Q16676), FOXD2 (O60548), FOXD3 (Q9UJU5), FOXD4 (Q12950), FOXE1 (O00358), FOXE3 (Q13461), FOXG1 (P55316), FOXI1 (Q12951), FOXI2 (Q6ZQN5), FOXI3 (A8MTJ6), FOXL1 (Q12952), FOXL2 (P58012), FOXJ1 (Q92949), FOXJ2 (Q9P0K8), FOXJ3

(Q9UPW0), FOXK1 (P85037), FOXK2 (Q01167), FOXH1 (O75593), FOXN1 (O15353), FOXN2 (P32314), FOXN3 (O00409), FOXN4 (Q96NZ1), FOXR1 (Q6PIV2), FOXR2 (Q6PJQ5).

Table S1. Primers used to generate *FOXP1* variants by site directed mutagenesis

Variant	Forward primer (5' to 3')	Reverse primer (5' to 3')
c.1393A>G (p.R465G)	atatgtaaatggtggtccaacttctgcgttcttataa aattcttggtt	aaccaagaattttataagaacgcagaagttggaccacc atttacatat
c.1540C>T (p.R514C)	tgaagactaagattatgacacactgcattcttccac gtg	cacgtggaagaatgcagtgtgtcataatcttagtcttca
c.1317C>G (p.Y439*)	gttgtatttgtctgattaccgcctgcggatggg	cccatccgcaggcggtaatcagacaaatacaac
c.1573C>T (p.R525*)	ccctttaacgttttctactcacacaaaacacttgtga agac	gtcttcacaagtgttttgtgtgagtagaaaacgttaaagg g
c.1600T>C (p.W534R)	acttcatccactgtccgtactgccccttaacgtt	aacgttaaaggggcagtacggacagtggatgaagt
c.226_228dup (p.Q76dup)	cagcagcaacagcagcagcagcaagttagtgg attaaaa	ttttaatccactaacttgctgctgctgctgttgctgctg
c.320T>C (p.I107T)	gctatgatgacacctcaagttaccactccccagca a	ttgctggggagtggtaacttgaggtgtcatcatagc
c.643C>G (p.P215A)	ggcagcctgcccttgcccttcaacc	ggttgaagggcaagggcaggctgcc
c.1709A>G (p.N570S)	cgcctactgcacacctctcagtgcagctttac	gtaaagctgcactgagaggtgtgcagtaggcg
c.1790A>C (p.N597T)	cccactctgggcaccttagccagcgca	tgcgctggctaaggtgcccagagtggg