

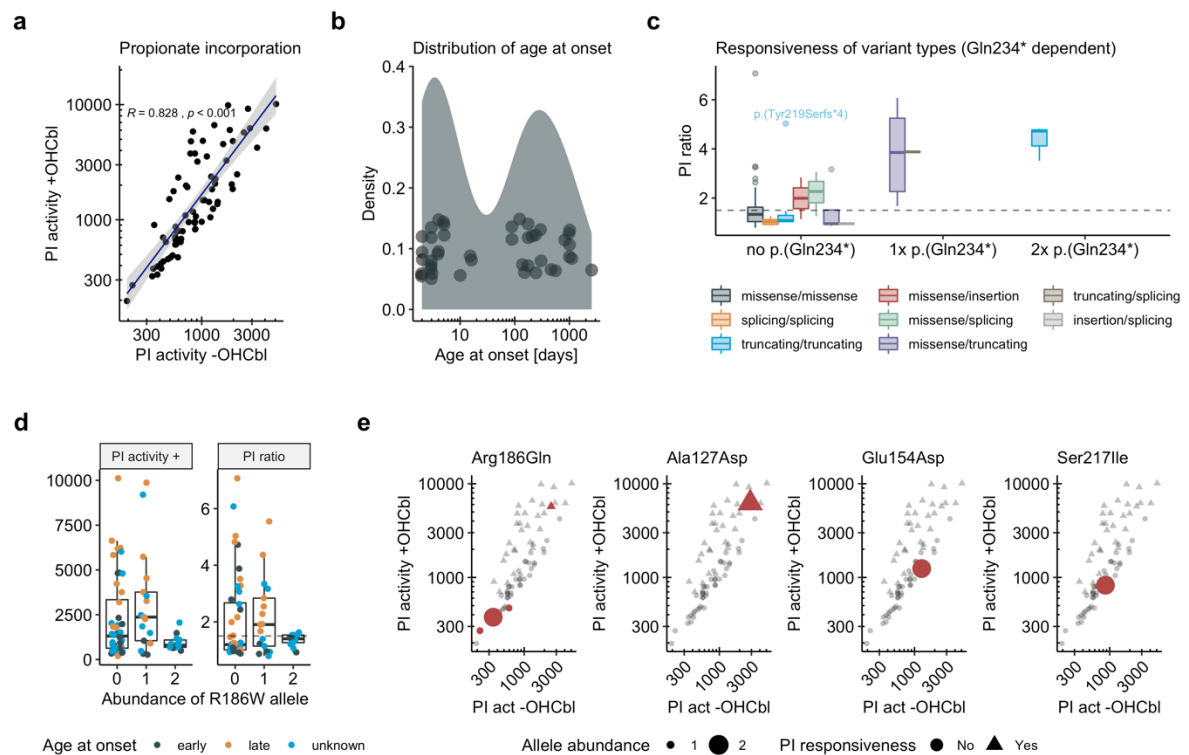
Spectrum and characterization of bi-allelic variants in *MMAB* causing *cb1B*-type methylmalonic aciduria

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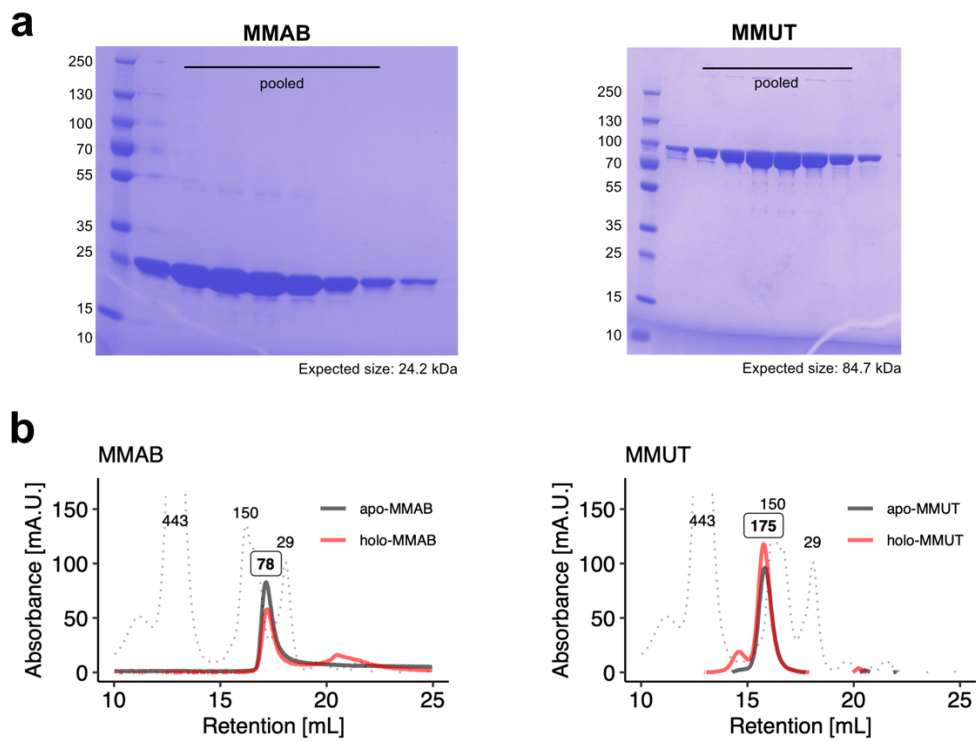
Supplementary Information

Supplementary Figure 1



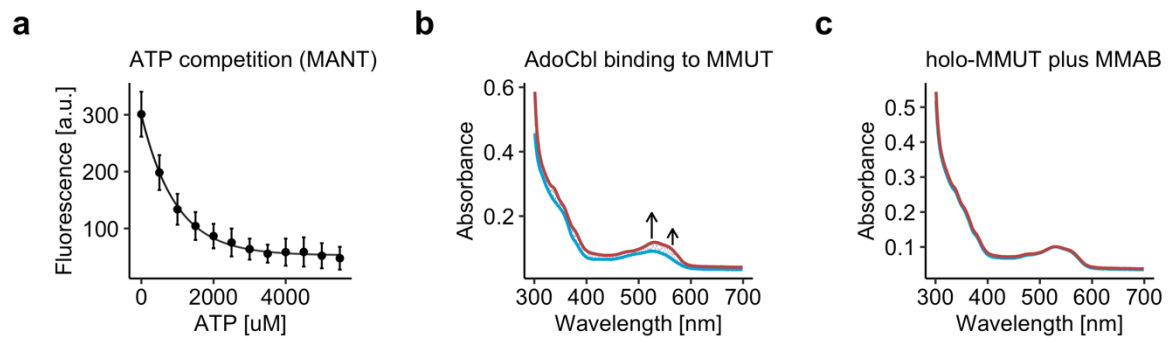
Suppl. Fig. 1. (a) Scatter plot of PI activity with and without supplementation of OHcbl; linear regression calculated by Pearson correlation. (b) Density distribution plot of the age of onset of patients with available information; dots represent individual patients; y axis jitter was added for better visibility. (c) PI ratios according to allele combinations, grouped based on the abundance of the p.(Gln234*) allele. (d) PI activity supplemented with OHcbl and PI ratio of all samples, grouped based on the abundance of the p.(Arg186Trp) allele. (e) Scatter plot of PI activity with and without supplementation of OHcbl; specific alleles (plot title) are highlighted in red; triangles indicate in vitro responsiveness, dot or triangle size indicate the abundance of the specific allele (plot title).

Supplementary Figure 2



Suppl. Fig. 2. (a) SDS-PAGE gels indicating high purity (>95%) after protein purification for MMAB, MMUT, and MMAA. (b) Size-exclusion chromatograms with indicated peaks for the respective proteins (MMAB as homotrimer, MMUT as homodimer); dotted curve represents a standard sample allowing calculation of protein size.

Supplementary Figure 3



Suppl. Fig. 3. (a) Increasing ATP concentrations lead to exchange for MANT-ATP at the binding site of MMAB. (b) Absorbance spectrum of AdoCbl under increasing concentrations of MMUT (blue line, 0 μ M of MMUT; red line, 80 μ M of MMUT added). (c) Increasing concentration of MMAB [0-60 μ M] were added to holo-MMUT, revealing no change of the absorbance spectrum.

Supplementary Table 1

Exon	Direction	Oligonucleotide sequence	PCR product size (bp)
1	Forward	ACC AAT AAA AAT TGA AAC CTG AGC G	365
1	Reverse	AAC AGC GTG GAG ACG TCA CCT GAC	
2	Forward	AGA TGA CCA TCT AAG CAG TAA GG	304
2	Reverse	CGC GCC TGG TAT ATT ATA CTT TA	
3	Forward	GGA GGT CAA GAC TAG CTC GG	521
3	Reverse	CAA TAA GGG CTG ACA ACC	
4	Forward	GAC TGG AAA TAA TGA GCA TCC GA	357
4	Reverse	TAA CTG GTG GCT GGA TGC TGA GT	
5	Forward	CAC CTC CAA CTC TGG GGA TCA CA	339
5	Reverse	TGG GAT CCC AGA TGG TGA CCC TA	
6	Forward	CAC GGG TGG GTC AGT TAG CCT TGC T	386
6	Reverse	TTG GGG AAC TGG AGG ACA GCG TCT	
7	Forward	ATC CCT CTT TTC ACA GTG ACA GA	483
7	Reverse	GTC TGT TGA TGT GTG GCT GGA TG	
8	Forward	CCA GGT GGC TCC CTC AAC TCA	563
8	Reverse	CAG CAG CCT CTC CCA ATA AGA	
9	Forward	GCA GAA GAG CTG ACA GCG TA	1101
9	Reverse	GGT GGC AAG AGG TGT AGA TCA	

Suppl. Table 1. Sequences of primers used in Sanger sequencing.