

Systematic and quantitative analysis of stop codon readthrough in Rett syndrome nonsense mutations

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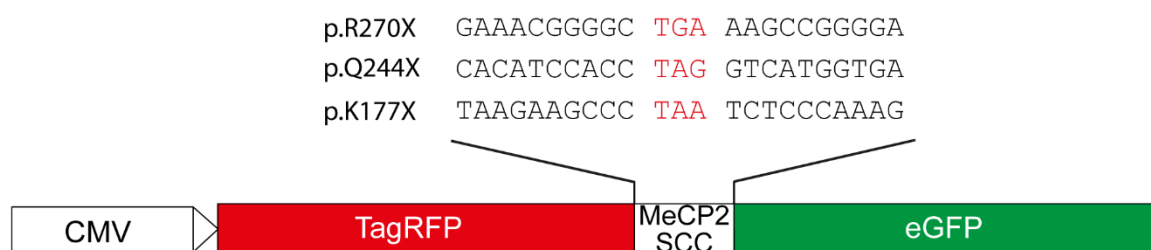
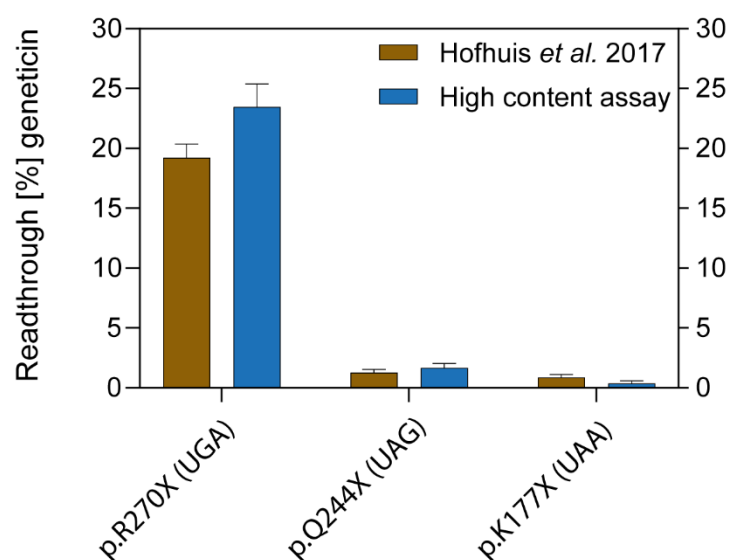
Supplemental Figures

Supplementary Table 1: Dual reporter plasmids generated in this study, the corresponding plasmid numbers (PST), and used oligonucleotides. Pathogenic mutation leading to a PTC is marked in red.

Plasmid PST	Specification	Oligonucleotide 1		Oligonucleotide 2	
		OST	Sequence	OST	Sequence
1596	STOP absent				
1600	p.R198X	1419	GTCACCGCGGCACCACG TGA CCCAAGGCGGT	1420	CCGGACCGCCTTGGGTC ACGT GGTGCCGCG
1601	p.S49X	1421	GTCACCGCGTGCAGCCAT AA GCCCACCACTT	1422	CCGGAAGTGGTGGGCT TATGG CTGCACGCG
1602	p.Q406X	1423	GTCACCGCCCTGAGCC CTAG GACTTGAGCAT	1424	CCGGATGCTCAAGTCCT AGGG CTCAGGGCG
1603	p.R294X	1425	GTCACCGGTCTTCTAT CTGA TCTGTGCAGGT	1426	CCGGACCTGCACAGATC AGAT AGAAGACCG
1739	p.R168X	3014	GTCACCGCCCCTCCCG TGA GAGCAGAACT	3015	CCGGAGTTTCTGCTCTC ACCG GGAGGGGCG
1740	p.R270X	1579	GTCACCGGAAACGGGG TGA AAGCCGGGGAT	1580	CCGGATCCCCGGCTTTC AGCC CCGTTTCCG
1741	p.Y141X	3085	GTCACCGGTGATTGCGT AG TTCGAAAAGGT	3086	CCGGACCTTTTCGA CTACGC AATCAACCG
1742	p.Q170X	1583	GTCACCGCCGGCGAG AGTAG AAACCACCTAT	1584	CCGGATAGGTGGTTTCT ACTC TCGCCGGCG
1743	p.R255X	1585	GTCACCGCGGCAGGA AGTGA AAAGCTGAGGT	1586	CCGGACCTCAGCTTTTCA CTT CCTGCCGCG
1744	p.S204X	1587	GTCACCGGGCGGCCACG TGA GAGGGTGTGCT	1588	CCGGAGCACACCTCT CACGT GGCCGCCCG
1745	p.Q244X	1589	GTCACCGCACATCCAC CTAG GTCATGGTGAT	1590	CCGGATCACCATGACCT AGGT GGATGTGCG
1746	p.K175X	3087	GTCACCGACCACCTA AGTAG CCCAAATCTCT	3088	CCGGAGAGATTTGGGCT ACTT AGGTGGTCG
1747	p.S68X	1593	GTCACCGATCAGAAGGG TGA GGCTCCGCCCT	1594	CCGGAGGGCGGAGCCT CACCC TTCTGATCG
1748	p.K177X	1595	GTCACCGTAAGAAGCC TAA TCTCCCAAAGT	1596	CCGGACTTTGGGAGATT AGGG CTTCTTACG
1749	p.K363X	3089	GTCACCGCTCACCC CTAG AAGGAGCACCT	3090	CCGGAGGTGCTCCTTCT AGGG GGGTGAGCG

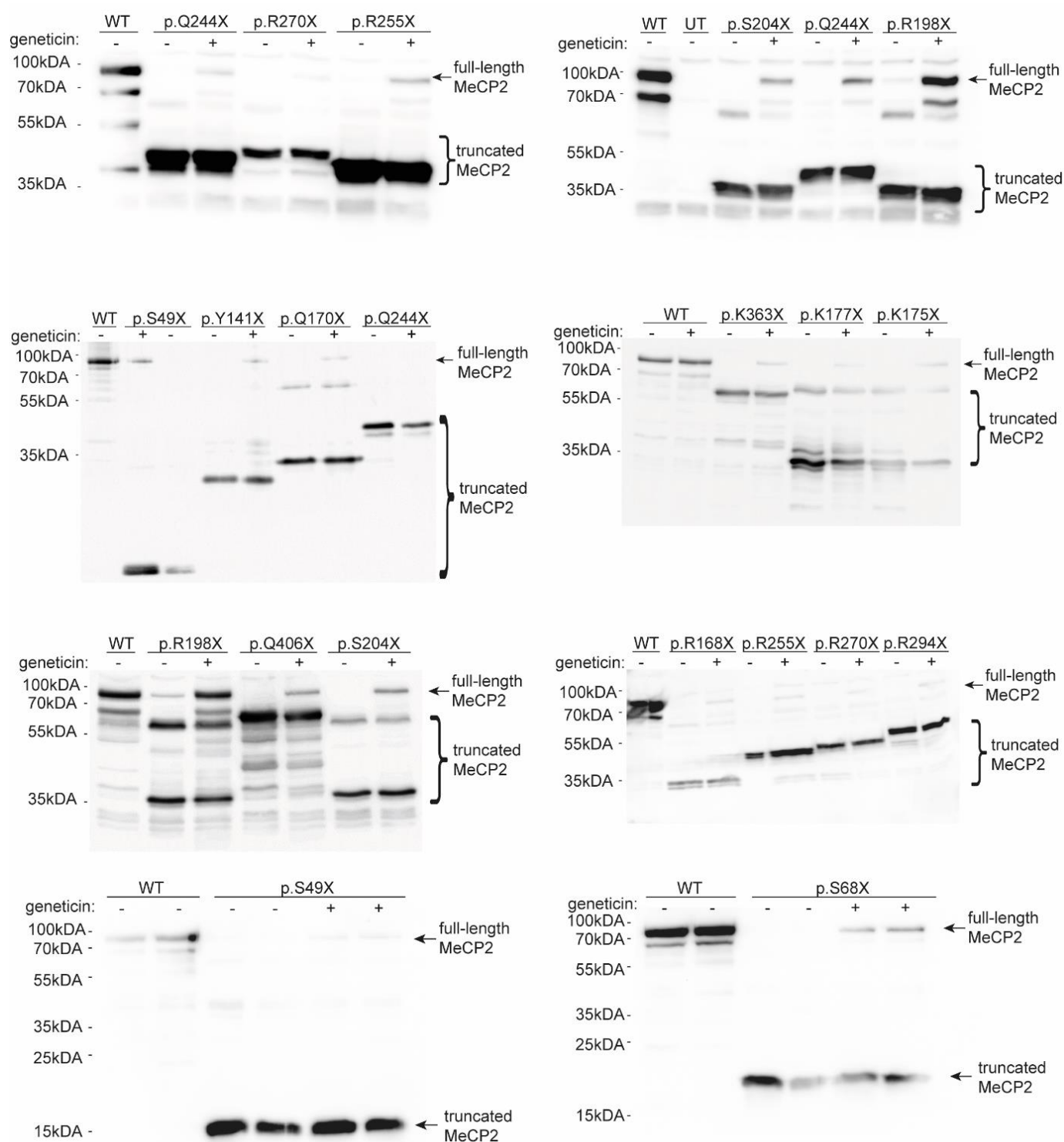
Supplementary Table 2: Full-length MeCP2 plasmids generated in this study, the corresponding plasmid numbers (PST), and used oligonucleotides for mutagenesis. Mutated nucleotide leading to a PTC is marked in red.

Plasmid PST	Specification	Oligonucleotide 1		Oligonucleotide 2	
		OST	Sequence	OST	Sequence
1810	Wildtype MeCP2 cDNA				
1814	c.423C>G, p.Y141X	1619	CTCTAAAGTGGAGTTGATTG CGTA ^G TCGAAAAGGTAGGC GACAC	1620	GTGTCGCCTACCTTTTCGAA ^C TAC GCAATCAACTCCACTTTAGAG
1815	c.508C>T, p.Q170X	1621	GCCCCTCCCGGCGAGAG ^T AG AAACCACCTAAGAAGC	1622	GCTTCTTAGGTGGTTTCT ^A CTCTC GCCGGGAGGGGC
1816	c.611C>G, p.S204X	1623	GACCCAAGGCGGCCACGT ^G A GAGGGTGTGCAGGTG	1624	CACCTGCACACCCTCT ^C ACGTGGC CGCCTTGGGTC
1817	c.730C>T, p.Q244X	1625	GGGCCACCACATCCAC ^T AG GTCATGGTGATCAAACGC	1626	GCGTTTGATCACCATGACCT ^A GGT GGATGTGGTGGCCC
1818	c.523A>T, p.K175X	1627	GAGAGCAGAAACCACCTAAG ^T AGCCCAAATCTCCCAAAGC TCC	1628	GGAGCTTTGGGAGATTGGGCT ^A C TTAGGTGGTTTCTGCTCTC
1819	c.203C>G, p.S68X	1629	GCAGAGACATCAGAAGGGT ^G AGGCTCCGCCCCGGCTG	1630	CAGCCGGGGCGGAGC ^C TCACCCTT CTGATGTCTCTGC
1820	c.529A>T, p.K177X	1631	GCAGAAACCACCTAAGAAGC CCT ^A AATCTCCCAAAGCTCCA GG	1632	CCTGGAGCTTTGGGAGATT ^A GGGC TTCTTAGGTGGTTTCTGC
1821	c.1087A>T, p.K363X	1633	GCGCCTCCTCACCCCC ^T AG AAGGAGCACCACCACC	1634	GGTGGTGGTGCTCCTTCT ^A GGGGG GTGAGGAGGCGC
1847	c.146C>A, p.S49X	1710	GCCCGTGCAGCCAT ^A AGCCC ACCACTCTGC	1711	GCAGAGTGGTGGGCT ^T ATGGCTGC ACGGGC
1848	c.1216C>T, p.Q406X	1712	GCCCCCTGAGCCC ^T AGGAC TTGAGCAGCAG	1713	CTGCTGCTCAAGTCCT ^A GGGCTCA GGGGGGC
1849	c.592A>T, p.R198X	1714	GGAGCGGCACCACG ^T GACCC AAGGCGGCC	1715	GGCCGCCTTGGGT ^C ACGTGGTGCC GCTCC

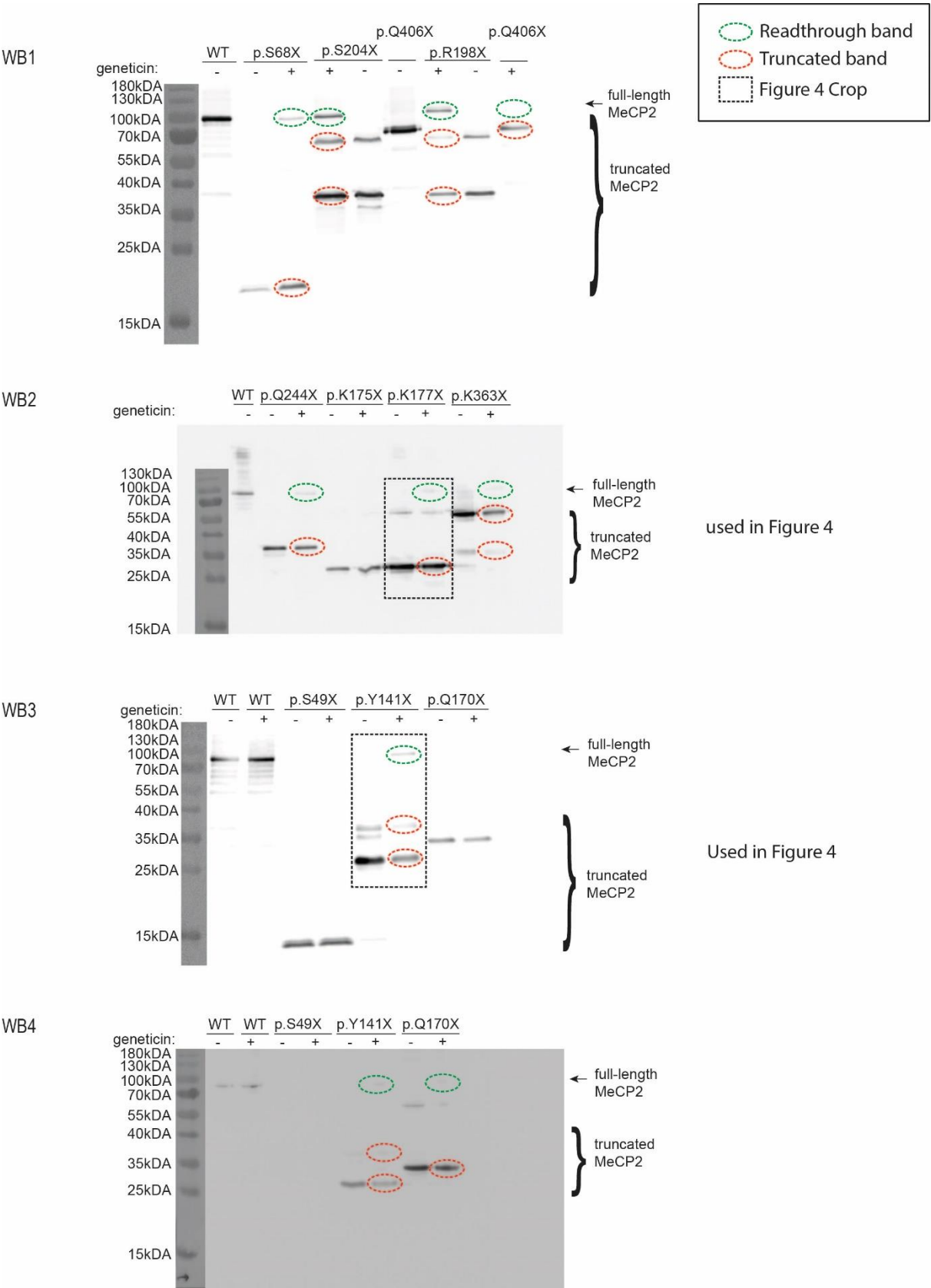
A**B**

Supplementary Figure 1: Assessment of high-content dual reporter TR assay. (A)

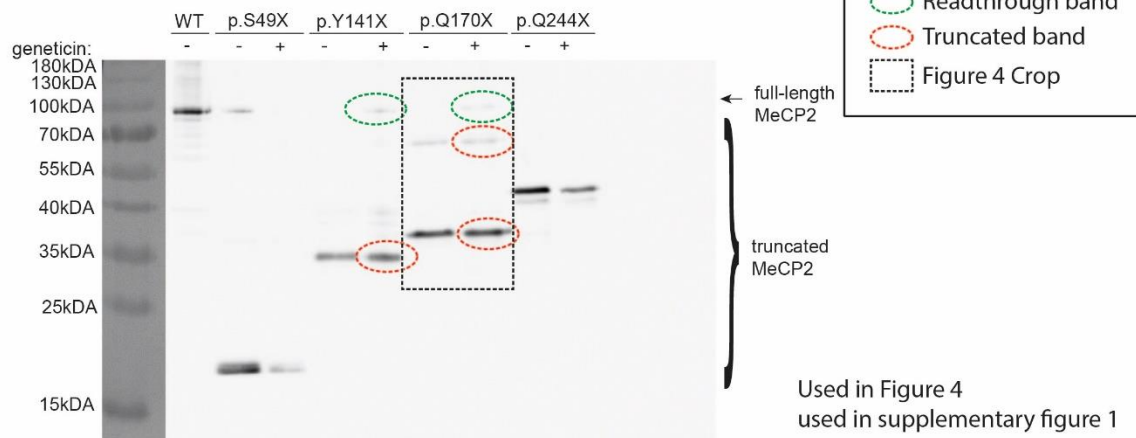
Three reporter constructs with different stop codons were used for the comparison between both assays. **(B)** TR measurement of the reporters in both assays using transfected HeLa cells



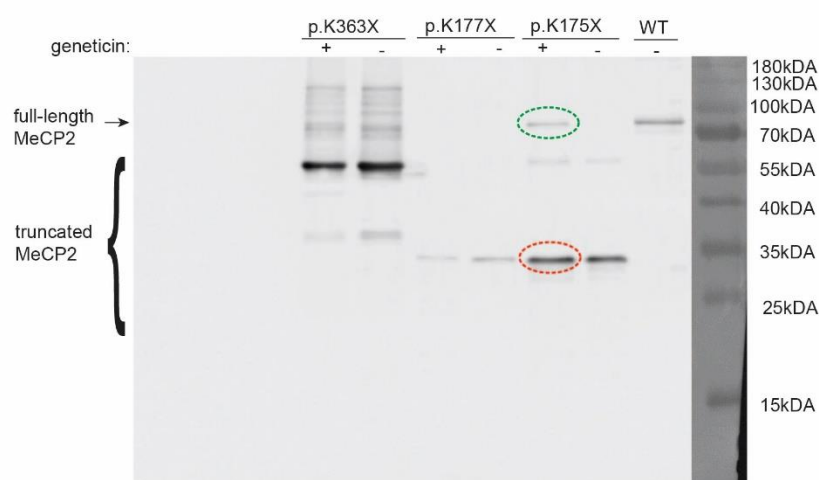
Supplementary Figure 2: Western Blots of wildtype and nonsense-mutated MeCP2 before and after geneticin treatment.



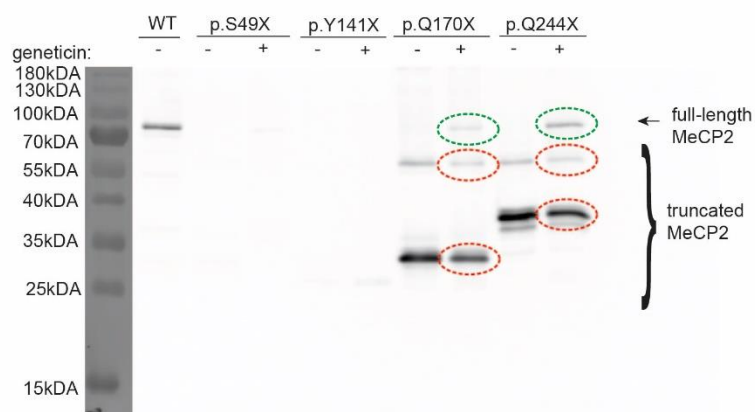
WB5

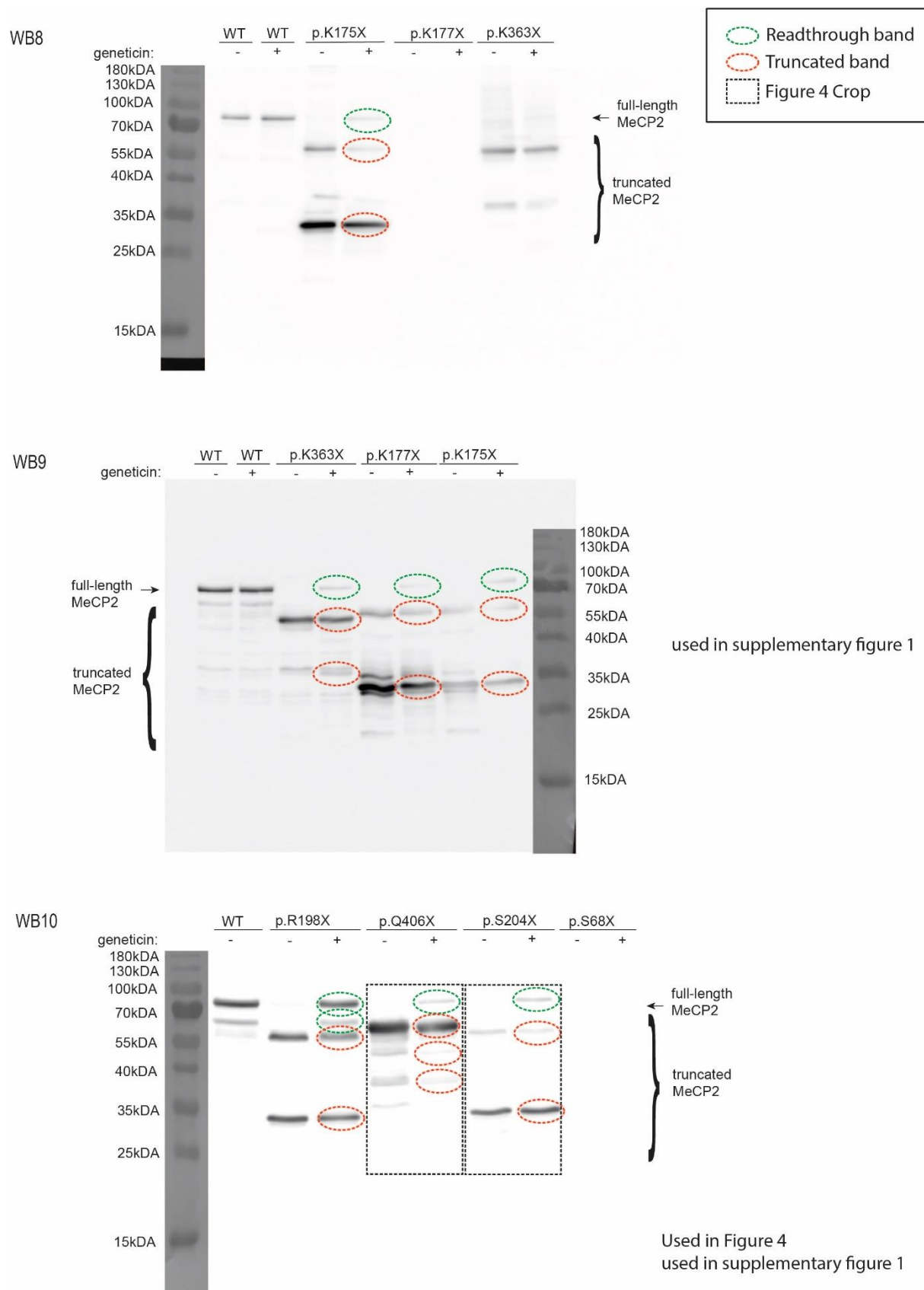


WB6

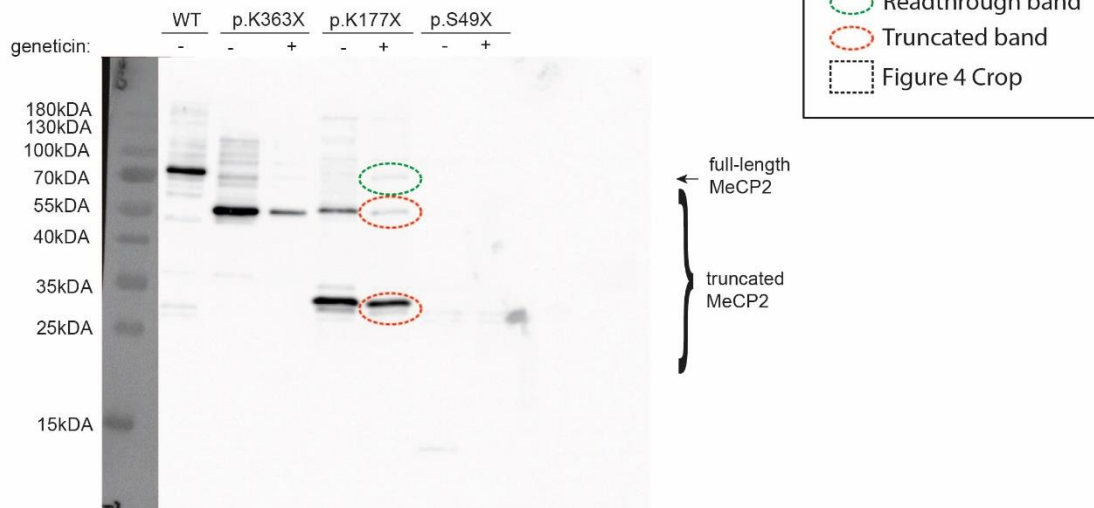


WB7

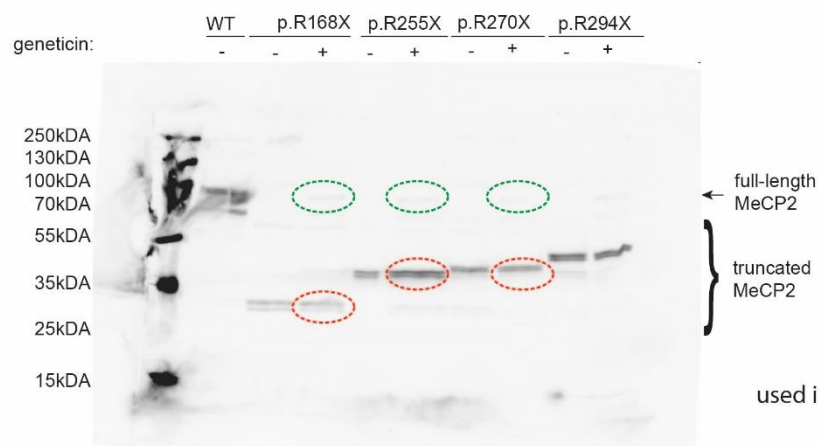




WB11

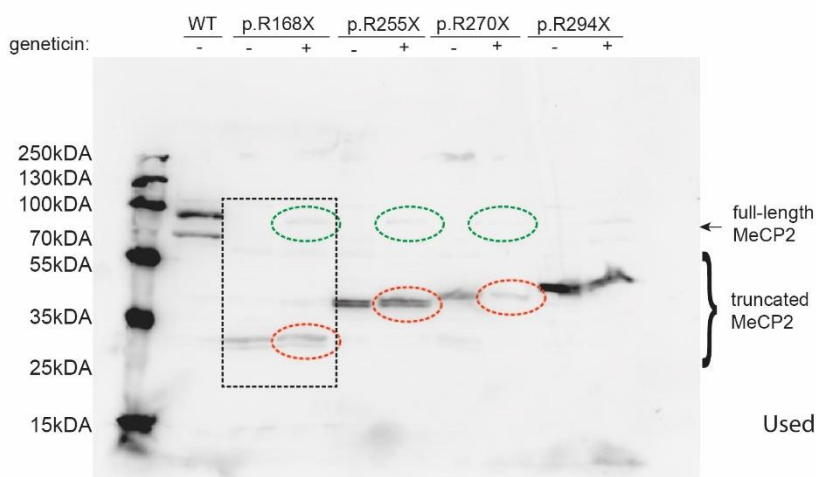


WB12



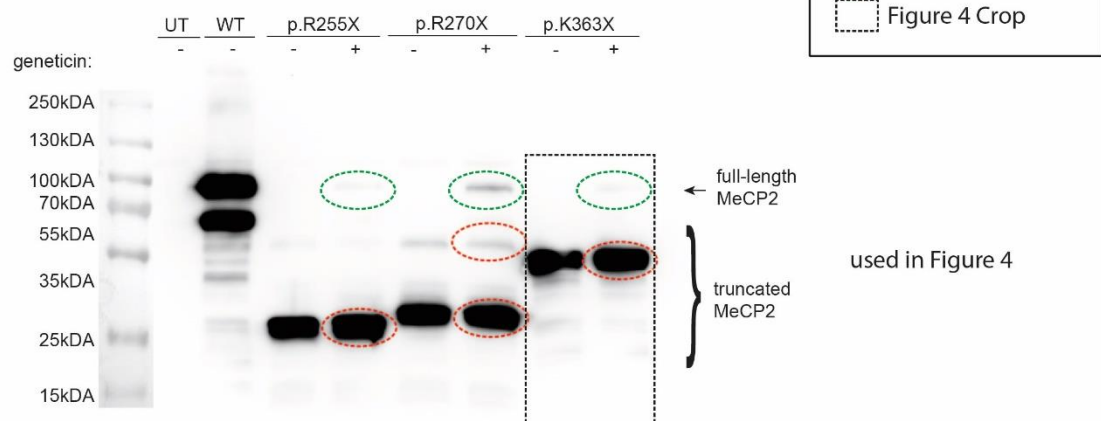
used in supplementary figure 1

WB13

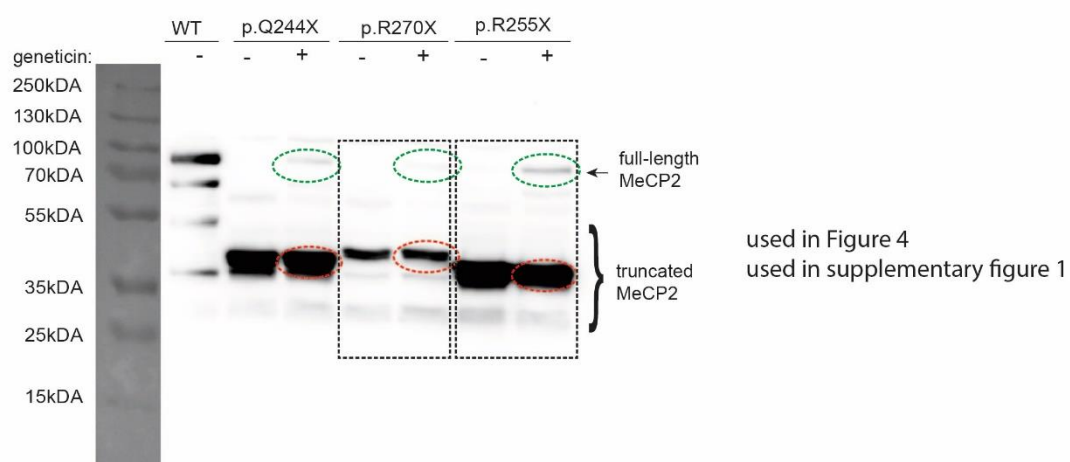


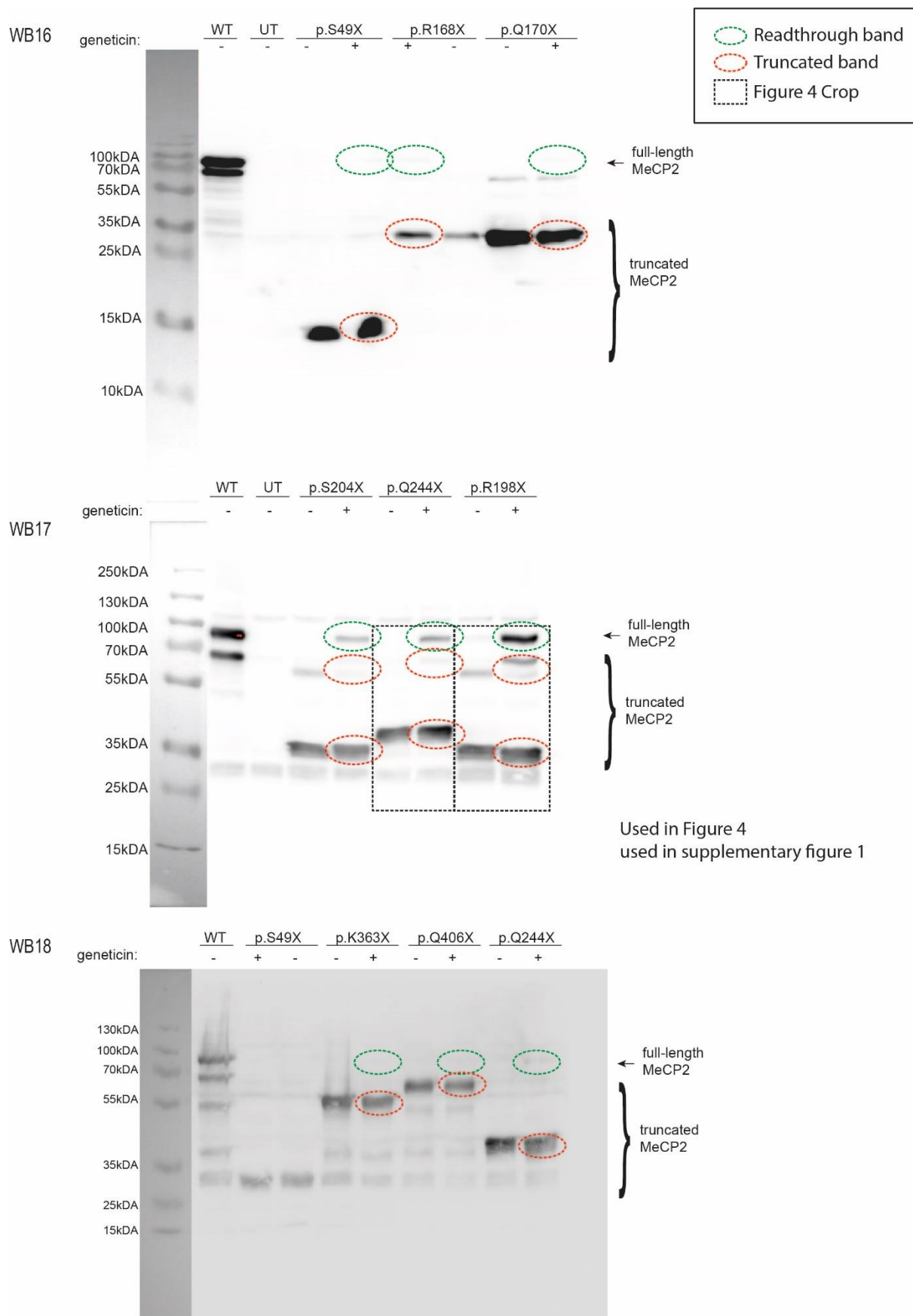
Used in Figure 4

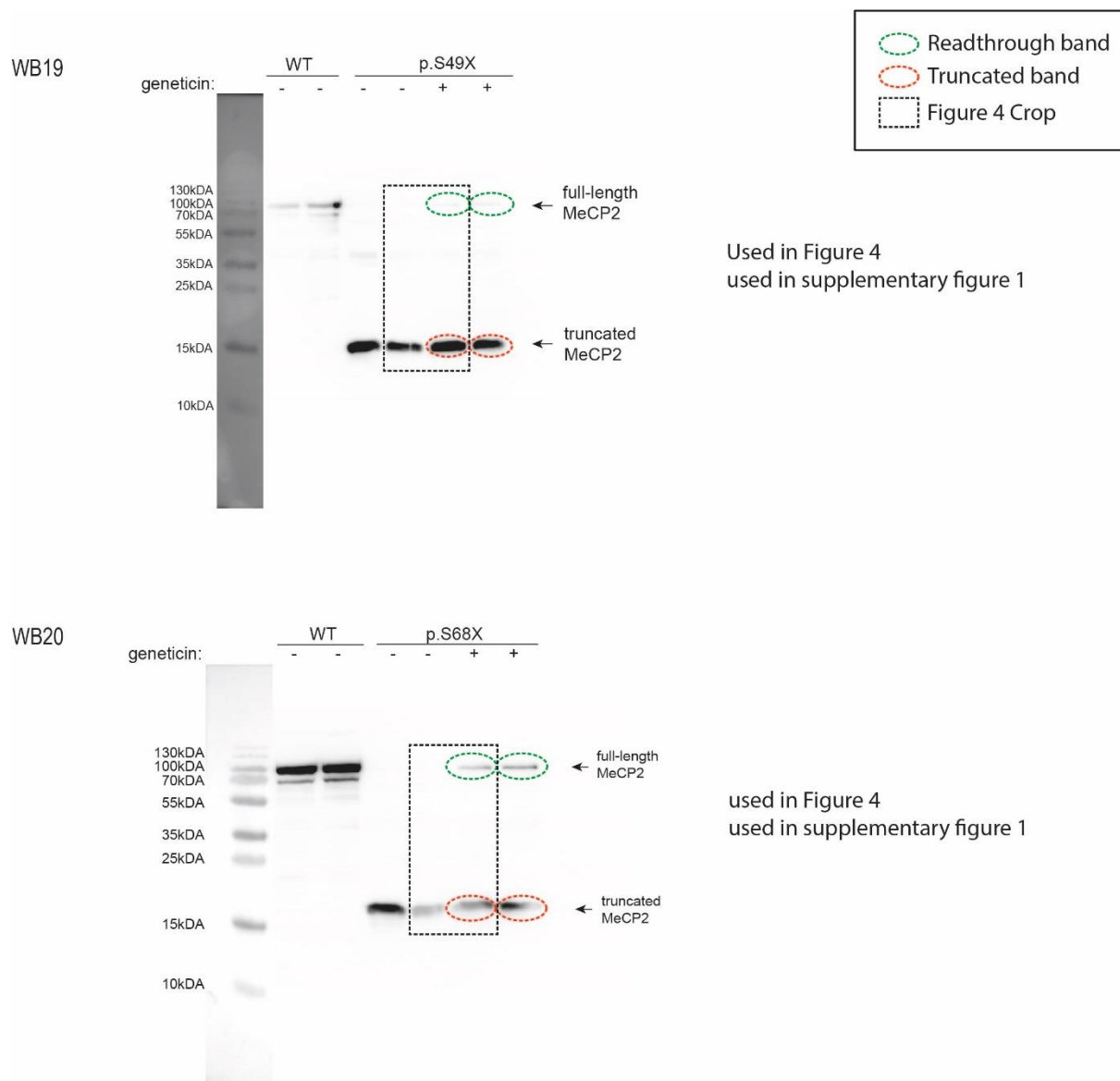
WB14



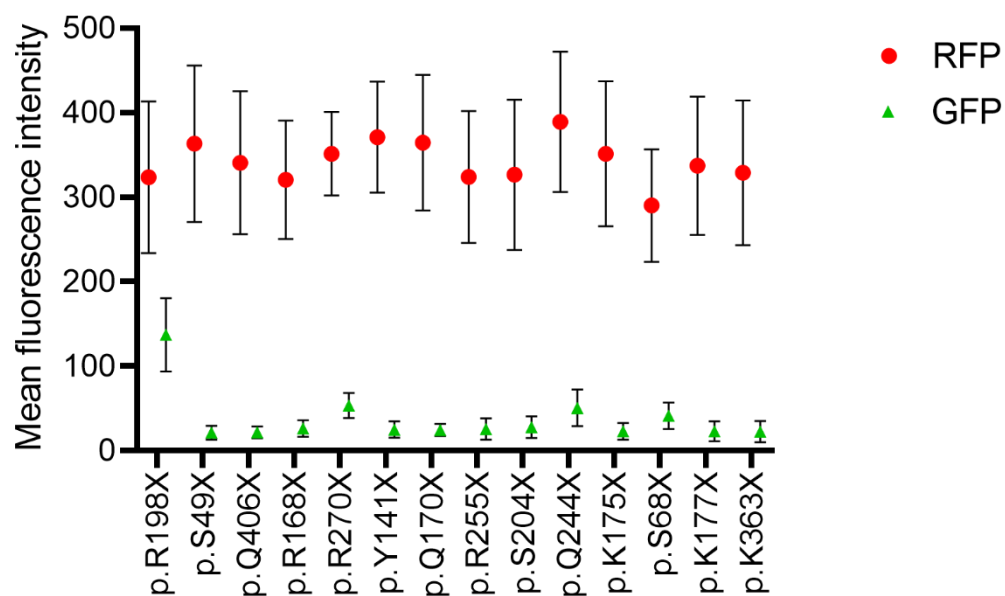
WB15







Supplementary Figure 3: Full unedited Western Blots (WB) 1-20 for Figure 4 and Supplementary Figure 2. Cropped areas for Figure 4 are marked in black dotted squares. All used bands for densitometric analyses are encircled in green for readthrough bands and red for truncated bands. Readthrough quantification was determined as the readthrough band intensity (green) normalized on the readthrough plus truncated band intensity.



Supplementary Figure 4: Mean of all absolute fluorescence intensities of all constructs measured by the dual reporter system. A non-significant difference between all RFP intensities indicates stable expression of all reporter constructs. ($n = 9$, $n = 6$ for Q244X). Dots indicate mean, error bars indicate s.d. One-way ANOVA