

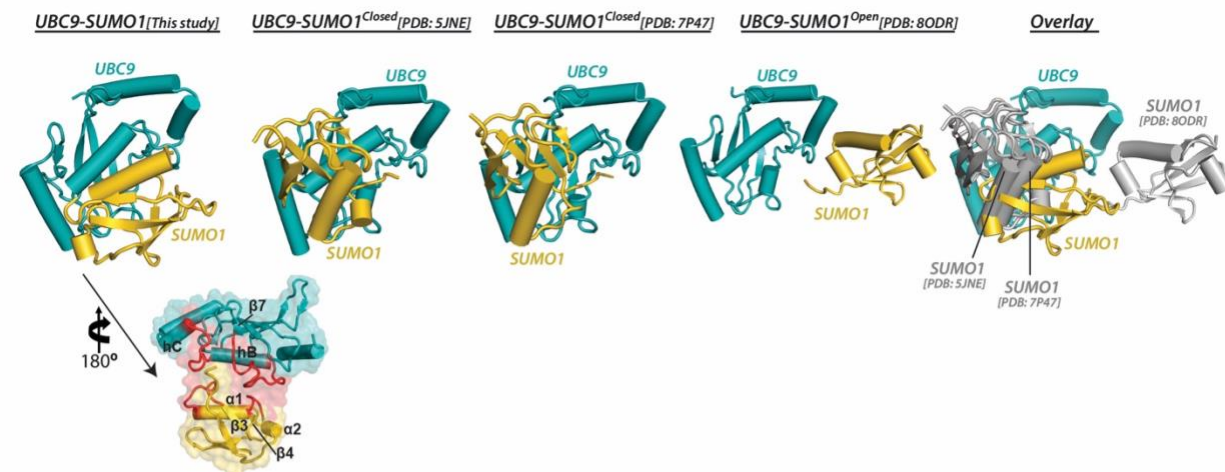
Cryo-EM structures reveal the molecular mechanism of SUMO E1–E2 thioester transfer

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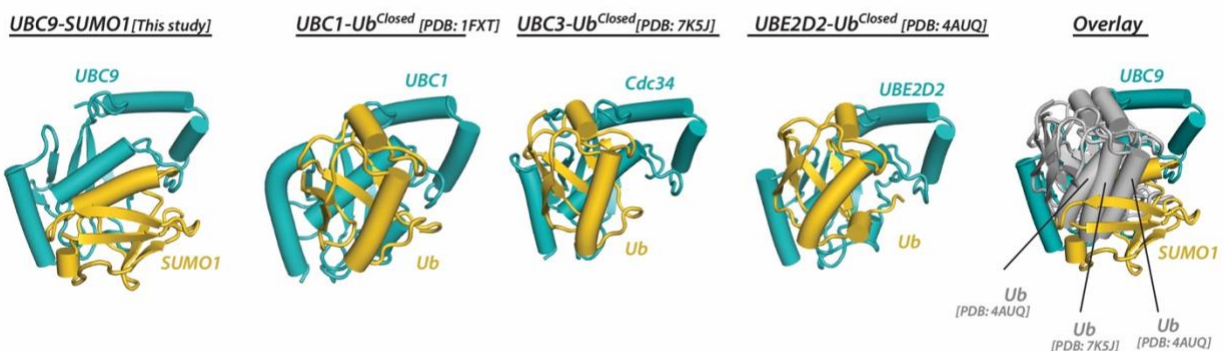
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a Comparison of UBC9-SUMO1 with other published structures

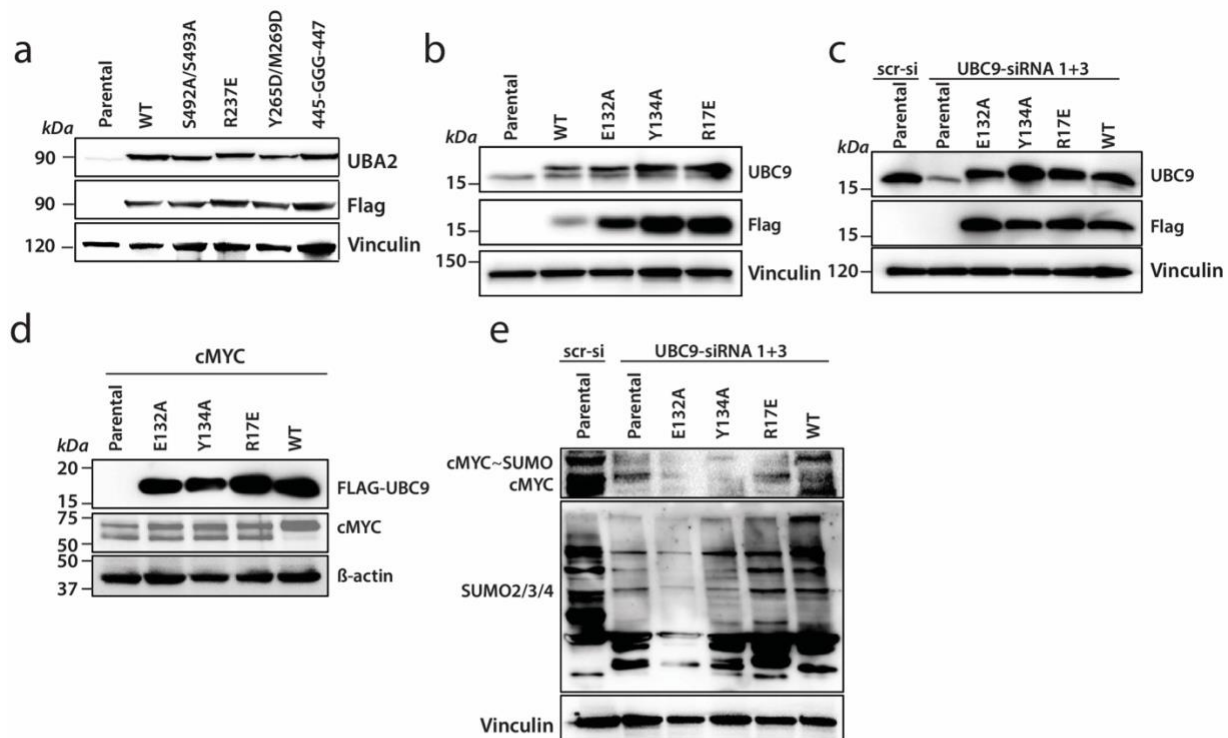


b Comparison of UBC9-SUMO1 with other E2-Ub published structures



Supplementary Fig. 1 | Comparing UBC9-SUMO1 mimetic with other published structures.

a, Top, Comparing the UBC9-SUMO1 thioester mimetic with other published UBC9-SUMO1 conjugates. Bottom, The interface shared between UBC9 and SUMO(t) shown as surface with the involving loops of UBC9 and SUMO(t) colored red. **b**, Comparing the UBC9-SUMO1 thioester mimetic with other published E2-Ub conjugates.



Supplementary Fig. 2 | Cell-based assay for SUMO E1 interfacial mutants in HCT-116 cells

a, Transfection of lentiviral plasmids resulted in stable expression of the indicated 3XFLAG-UBA2 mutants in HCT-116 cells with IPTG-induced UBA2-shRNA-1 + 2. Western blotting with UBA2 and FLAG antibodies confirmed the expression of UBA2 mutants. **b**, Transfection of lentiviral plasmids resulted in stable expression of the indicated 1XFlagUbc9 mutants in HCT-116 cells. Western blotting with UBC9 and Flag antibodies confirmed the expression of UBC9 mutants. This experiment was performed once. **c**, The efficacy of siRNA in suppressing endogenous UBC9 and expression of UBC9 mutants was verified using Western blot analysis. **d**, Transfection of lentiviral plasmids resulted in stable expression of the 1XFlag cMYC plasmid in HCT-116 cells stably expressing FLAG-UBC9 mutants. Western blotting with cMYC and FLAG antibodies confirmed the expression of UBC9 mutants and cMYC. Data shown are from a single experiment. **e**, Western blot analysis of WT and mutant UBC9 ability to rescue cMYC~SUMO2 thioester intermediate in UBC9 knockdown cells. Global SUMOylation in HCT-116 cells using UBC9 mutants and siRNA-1 + 3, tested by immunoblotting with SUMO2/3/4 antibody after 72 hours is shown in middle panel. Vinculin was used as a loading control (bottom panel). Data are representative of two independent experiments with consistent results. Uncropped blots are included in Source data file.

Supplementary Table 1 | List of primers

UBA2

S492R_S493R

F: AAAGGAACAATCCTAATAAGAAGAGAAGAGGGAGAGACGGAA

R: TTCCGTCTCTCCCTCTTCTCTTCTTATTAGGATTGTTCTTT

E494R

F: ACAATCCTAATATCTTCCAGAGAGGGAGAGACGGAAGCT

R: AGCTTCCGTCTCTCCCTCTCTGGAAGATATTAGGATTGT

N501A

F: GAGGGAGAGACGGAAGCTGCAAATCACAAGAAGTTGTCA

R: TGACAACTTCTTGTGATTTGCAGCTTCCGTCTCTCCCTC

F509A

F: CACAAGAAGTTGTCAGAAGCAGGAATTAGAAATGGCAGC

R: GCTGCCATTTCTAATTCCTGCTTCTGACAACTTCTTGTG

R176A

F: TTTCTGGCTGTACAATTGCAAACACACCTTCAGAACCT

R: AGGTTCTGAAGGTGTGTTTGCAATTGTACAGCCAGGAAA

N177E

F: CCTGGCTGTACAATTCGTGAAACACCTTCAGAACCTATA

R: TATAGGTTCTGAAGGTGTTTCACGAATTGTACAGCCAGG

N230A

F: GCCAGAGCTAGAGCATCTGCAGAAGATGGTGACATTAAA

R: TTTAATGTCACCATCTTCTGCAGATGCTCTAGCTCTGGC

D232K

F: GCTAGAGCATCTAATGAAAAAGGTGACATTAAACGTATT

R: AATACGTTTAATGTCACCTTTTTTCATTAGATGCTCTAGC

R237E

F: GAAGATGGTGACATTAAAGAAATTTCTACTAAGGAATGG

R: CCATTCCTTAGTAGAAATTTCTTTAATGTCACCATCTTC

Y265W

F: TTAAAGATGACATCAGGTGGCTGTTGACAATGGACAAA

R: TTTGTCCATTGTCAACAGCCACCTGATGTCATCTTTAAA

D270A

F: AGGTATCTGTTGACAATGGCAAACCTATGGCGGAAAAGG

R: CCTTTTCCGCCATAGTTTTGCCATTGTCAACAGATACCT

M269D

F: ATCAGGTATCTGTTGACAGATGACAAACTATGGCGGAAA

R: TTTCCGCCATAGTTTGTCTCTGTCAACAGATACCTGAT

T220A

F: GAAGCTGCCTGGGAACCAGCAGAAGCCGAAGCCAGAGCT

R: AGCTCTGGCTTCGGCTTCTGCTGGTTCCCAGGCAGCTTC

M269D_D270A

F: ATCAGGTATCTGTTGACAGATGCAAAACTATGGCGGAAAAGG

R: CCTTTTCCGCCATAGTTTTGCATCTGTCAACAGATACCTGAT

Y265A_M269D_D270A

F: AAAGATGACATCAGGGCGCTGTTGACAGATGCAAAACTATGGCGGAAAAGG

R: CCTTTTCCGCCATAGTTTTGCATCTGTCAACAGCGCCCTGATGTCATCTTT

R176A_N177A

F: TTTCTGGCTGTACAATTGCAGCAACACCTTCAGAACCTATA

R: TATAGGTTCTGAAGGTGTTGCTGCAATTGTACAGCCAGGAAA

Y265A

F: TTAAAGATGACATCAGGGCACTGTTGACAATGGACAAA

R: TTTGTCCATTGTCAACAGTGCCCTGATGTCATCTTTAAA

E160A

F: GGTGTGACCGAGTGTTATGCATGTCATCCTAAGCCGACC

R: GGTCGGCTTAGGATGACATGCATAAACTCGGTCACACC

T166A

F: GAGTGTCACTCCTAAGCCGGCACAGAGAACCTTTCCTGGC

R: GCCAGGAAAGGTTCTCTGTGCCGGCTTAGGATGAACTC

P445A

F: CCCAATTGTTATGTATGTGCACCACCACCAGAGGTGACT

R: AGTCACCTCTGGTGGTGGTGCACATACATAACAATTGGG

S446P

F: AATTGTTATGTATGTGCACCAAAGCCAGAGGTGACTGTG

R: CACAGTCACCTCTGGCTTTGGTGCACATACATAACAATT

K447P

F: TGTTATGTATGTGCAAGCCCACCAGAGGTGACTGTGCGG

R: CCGCACAGTCACCTCTGGTGGGCTTGCACATACATAACA

H124A

F: AACAGAGCTGCCCCGAAACGCAGTTAATAGAATGTGCCTG

R: CAGGCACATTCTATTAAGTGCCTTTCGGGCAGCTCTGTT

R127A

F: GCCCGAAACCATGTTAATGCAATGTGCCTGGCAGCTGA

R: ATCAGCTGCCAGGCACATTGCATTAACATGGTTTCGGGC

Y442A

F: CCTCCCAACCCCAATTGTGCAGTATGTGCCAGCAAGCCA

R: TGGCTTGCTGGCACATACTGCACAATTGGGGTTGGGAGG

A127R

F: GCCCGAAACGCAGTTAATAGAATGTGCCTGGCAGCTGA

R: TCAGCTGCCAGGCACATTCTATTAAGTGCCTTTCGGGC

A124H_A127R

F: AGAGCTGCCCCGAAACCATGTTAATAGAATGTGCCTGGCAGCTGA

R: TCAGCTGCCAGGCACATTCTATTAACATGGTTTCGGGCAGCTCT

D100A

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R: GAAAAATTCCACATTATATGCAGGGTTCATGATGCTGTC

N102A

F: ATCATGAACCCTGACTATGCAGTGGGAATTTTCCGACAG

R: CTGTCGGAAAAATTCCACTGCATAGTCAGGGTTCATGAT

R127D

F: GCCCGAAACCATGTTAATGATATGTGCCTGGCAGCTGAT

R: ATCAGCTGCCAGGCACATATCATTAAACATGGTTTCGGGC

D100A_N102A

F: GACAGCATCATGAACCCTGCATATGCAGTGGGAATTTTCCGA

R: TCGGAAAAATTCCACTGCATATGCAGGGTTCATGATGCTGTC

UBC9**R13E**

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R: GTCTTTCCTCCATGCTTTTTCTCCTGGGCGAGTCTGCT

K14A

F: AGACTCGCCCAGGAGAGGGCAGCATGGAGGAAAGACCAC

R: GTGGTCTTTCCTCCATGCTGCCCTCTCCTGGGCGAGTCT

R17E

F: CAGGAGAGGAAAGCATGGGAAAAAGACCACCCATTTGGT

R: ACCAAATGGGTGGTCTTTTTCCCATGCTTTCCTCTCCTG

K30E

F: TTCGTGGCTGTCCCAACAGAAAATCCCGATGGCACGATG

R: CATCGTGCCATCGGGATTTTCTGTTGGGACAGCCACGAA

L38A

F: CCCGATGGCACGATGAACGCAATGAACTGGGAGTGCGCC

R: GGCGCACTCCCAGTTCATTGCGTTCATCGTGCCATCGGG

D67A

F: CGGATGCTTTTCAAAGATGCATATCCATCTTCGCCACCA

R: TGGTGGCGAAGATGGATATGCATCTTTGAAAAGCATCCG

S89F

F: CACCCGAATGTGTACCCTTTCGGGACAGTGTGCCTGTCC

R: GGACAGGCACACTGTCCCGAAAGGGTACACATTCTGGGTG

Y87A

F: TTATTTCACCCGAATGTGGCACCTTCGGGGACAGTGTGC

R: GCACACTGTCCCCGAAGGTGCCACATTCTGGGTGAAATAA

Q139A

F: GCCTACACGATTTACTGCGCAAACAGAGTGGAGTACGAG

R: CTCGTACTCCACTCTGTTTGCGCAGTAAATCGTGTAGGC

R147A

F: AGAGTGGAGTACGAGAAAGCAGTCCGAGCACAAGCCAAG

R: CTTGGCTTGTGCTCGGACTGCTTTCTCGTACTCCACTCT

K154A

F: GTCCGAGCACAAGCCAAGGCATTTGCGCCCTCATAAAAG

R: CTTTTATGAGGGCGCAAATGCCTTGGCTTGTGCTCGGAC

E132F

F: CAAGACCCAGCACAAGCATTCTGCCTACACGATTTACTGC

R: GCAGTAAATCGTGTAGGCGAATGCTTGTGCTGGGTCTTG

Y134A

F: CCAGCACAAGCAGAGGCCGCAACGATTTACTGCCAAAAC

R: GTTTTGGCAGTAAATCGTTGCGGCCTCTGCTTGTGCTGG

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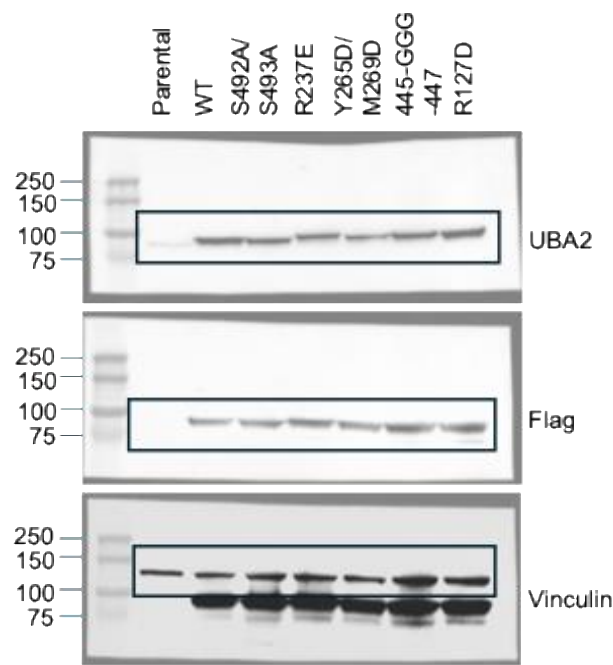
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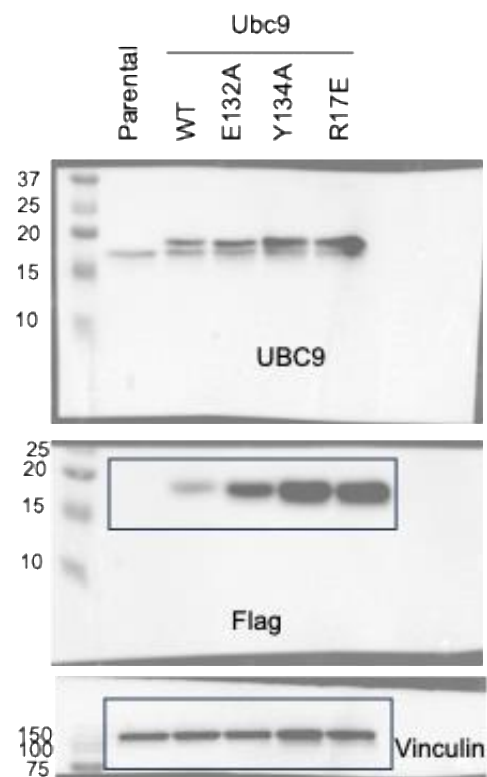
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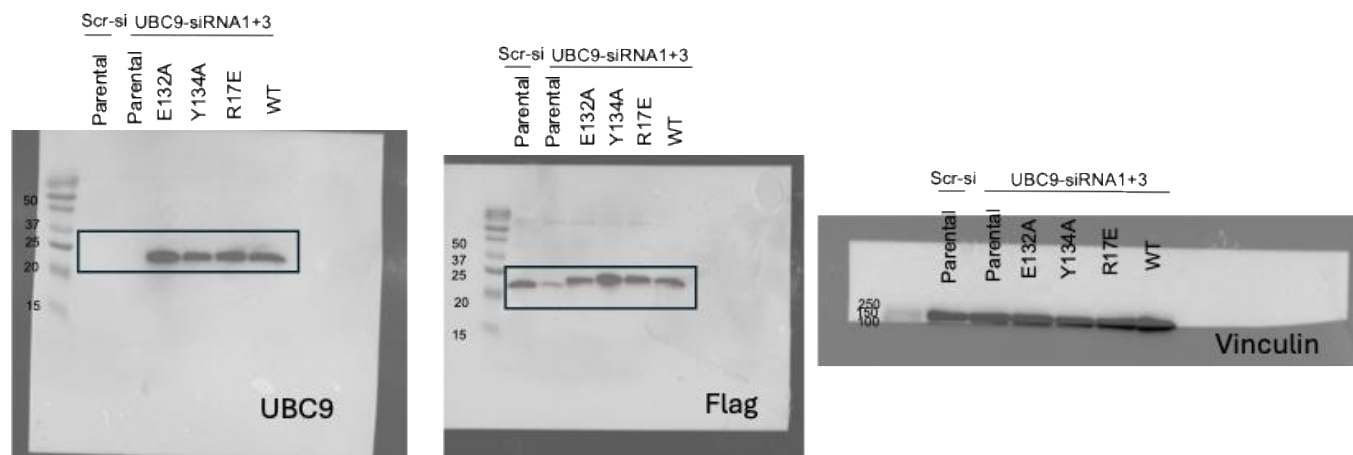
Uncropped scans of blots in the Supplementary Figure 2



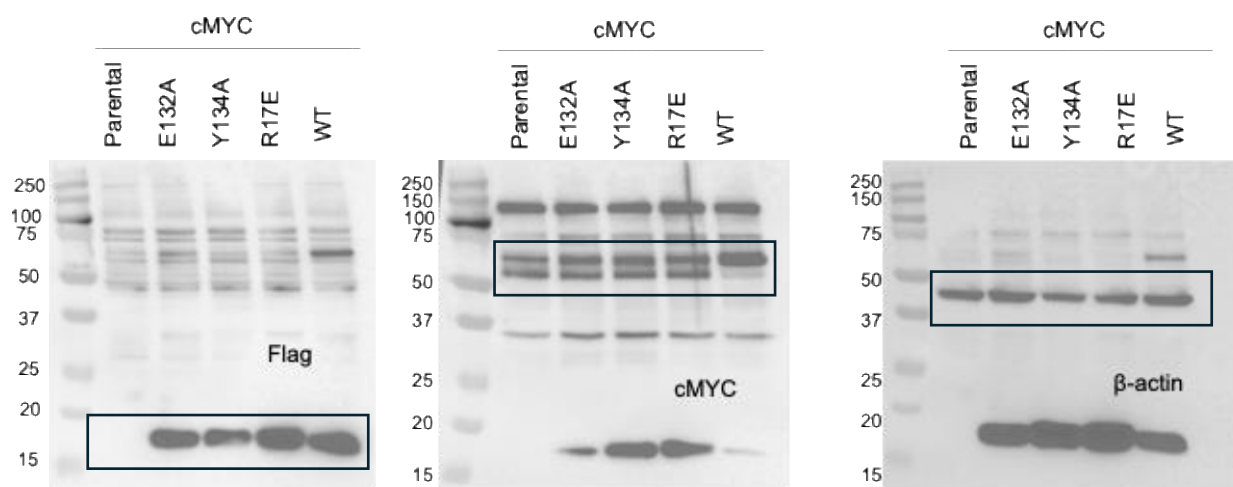
Supplementary Figure 2a



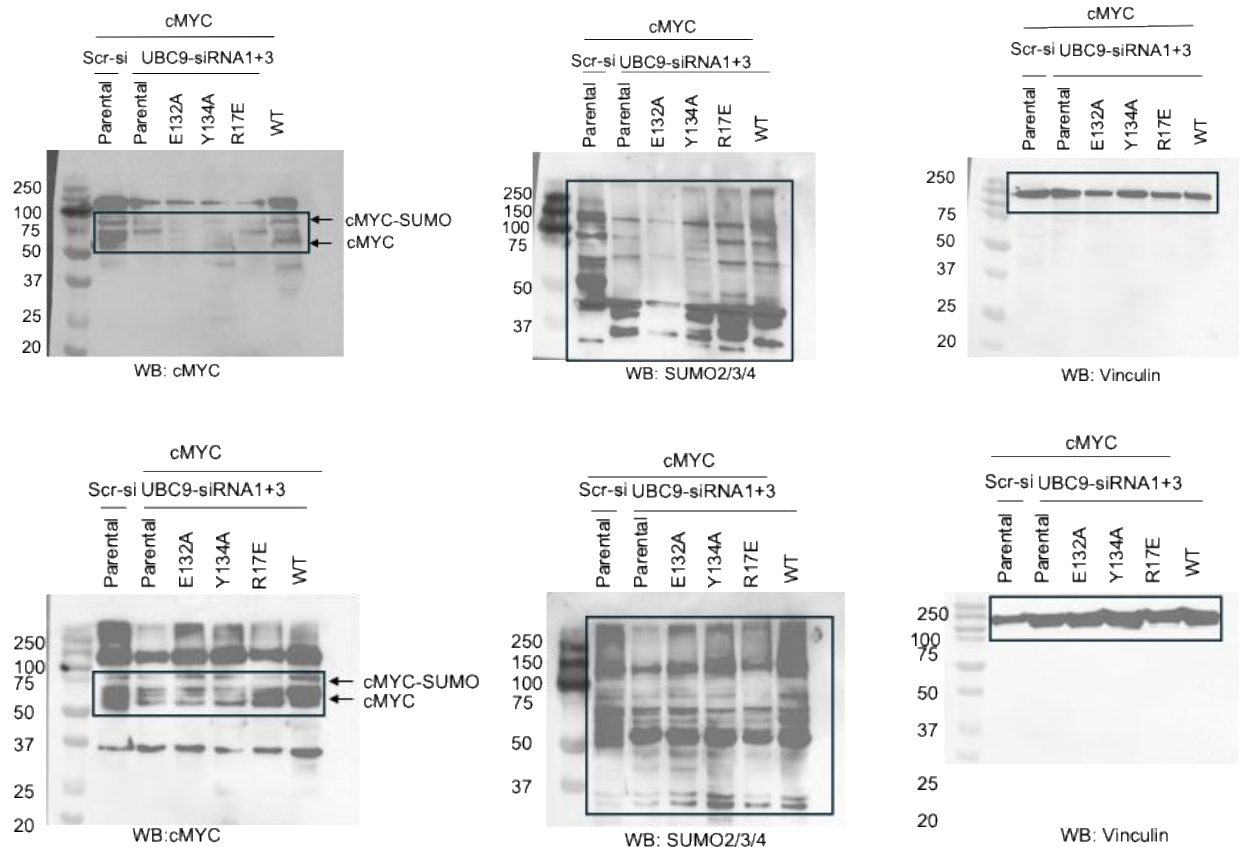
Supplementary Figure 2b



Supplementary Figure 2c



Supplementary Figure 2d



Supplementary Figure 2e