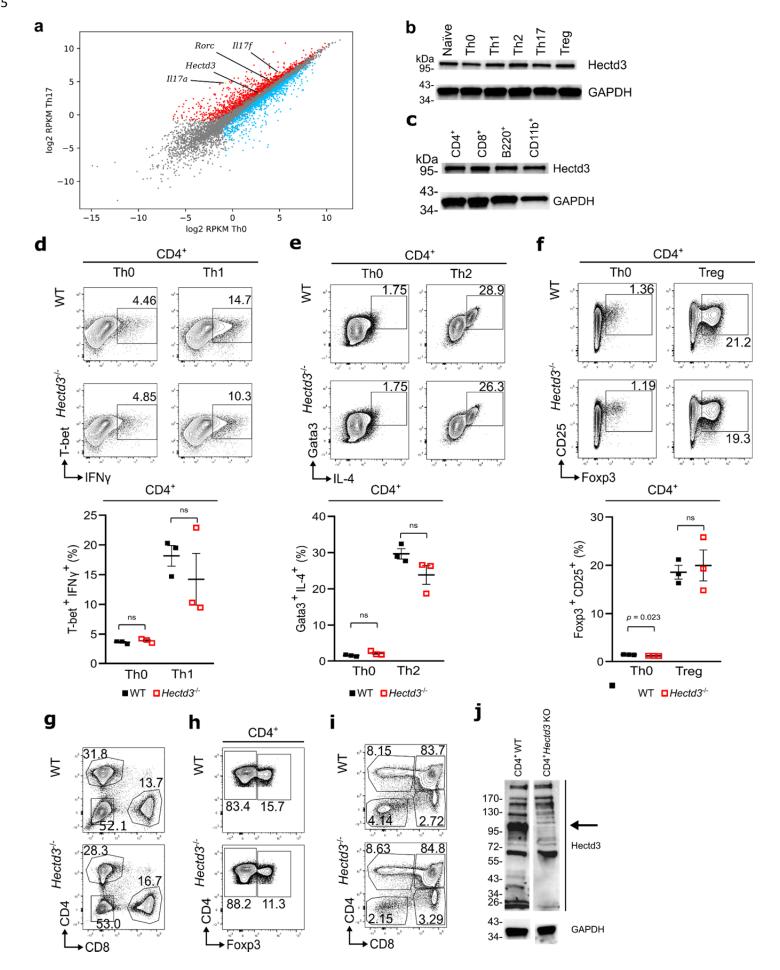
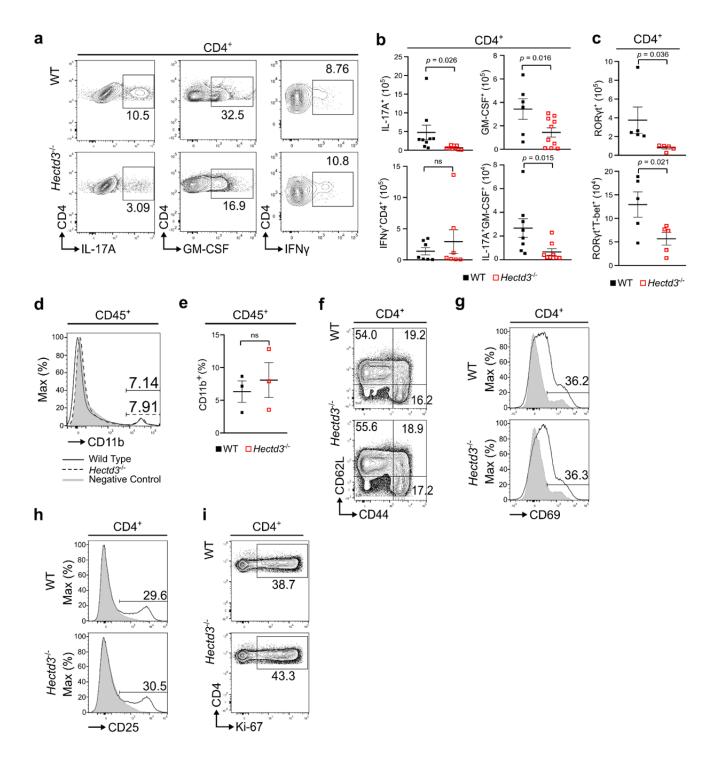
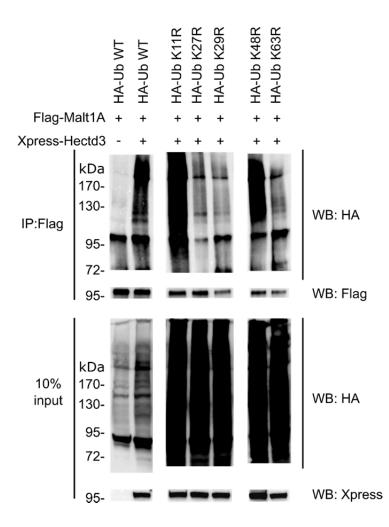
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2	Hectd3 promotes pathogenic Th17 lineage through Stat3 activation and Malt1 signaling in neuroinflammation
3	Cho et al.



Thymic and peripheral T cell populations and polarization into Th1, Th2, and Treg conditions are normal in the absence of Hectd3. a RNA-sequencing of in vitro polarized mouse wild type Th17 and Th0 cells as reported by Ciofani, et al1. Data are shown as a scatter plot of log2 of reads per kilobase per million mapped reads (RPKM). Blue dots represent >2 z-score downregulated genes, and red dots represent >2 zscore upregulated genes in Th17 cells relative to Th0 cells. Genes of interest are indicated and marked in vellow (upregulated) with the following z-scores: Hectd3 2.42, Rorc 8.93, Il-17a 6.71, Il-17f 7.05 (from 1). b Representative immunoblot of Hectd3 and GAPDH in WT naïve and in vitro polarized CD4⁺ T cells. c Representative immunoblot of Hectd3 and GAPDH in WT purified CD4⁺ T, CD8⁺ T, B220⁺, and CD11b⁺ cells. (b-c) Data are representative of two independent experiments. d-f Representative flow cytometry analysis and frequencies of T-bet and IFNy (d), Gata3 and IL-4 (e) and Foxp3 and CD25 (f) from in vitro polarized Hectd3⁻¹ and WT CD4⁺ T cells under Th0, Th1 (d), Th2 (e), and Treg (f) conditions. Purified CD4⁺ T cells were cultured and polarized as described in Materials and Methods. (d-f) Data are presented as mean ± SEM and derived from two independent experiments; p values were obtained from Student's t test. Gating strategy shown in Supplemental Figure 9. g-i Representative flow cytometry analysis of CD4⁺ and CD8⁺ T cell populations (g) and Treg cells (h) in peripheral lymph nodes, and of thymic populations (i) in Hectd3¹⁻ and WT mice at steady state. i Representative immunoblot of Hectd3 and GAPDH in purified CD4⁺ T of Hectd3^{/-} and WT CD4⁺ mice. Representative of two independent experiments. Arrow indicates specific Hectd3 band. Source data are provided as a Source Data file.

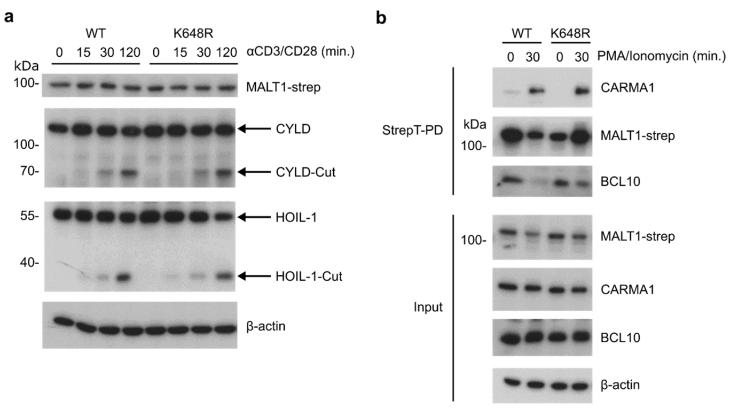


IL-17A. GM-CSF, and RORvt are diminished in Hectd3^{-/-} CD4⁺ T cells of mice induced with EAE but activation and proliferation are not altered. a Representative flow cytometry analysis of intracellular IL-17A, GM-CSF, and IFNy in CD4⁺ T cell from the draining lymph nodes dLNs of *Hectd3*^{-/-} and WT mice, 13 days following EAE induction: n=5 per group from three independent experiments. Gating strategy shown in Supplemental Figure 9. **b** Absolute numbers of IL-17A⁺, GM-CSF⁺, IFNy⁺, and IL-17A⁺GM-CSF⁺ CD4⁺ T cells in the draining lymph nodes of *Hectd3*^{-/-} and wild type mice 13 days following EAE induction. **c** Absolute numbers of RORyt⁺ CD4⁺ T cells and RORyt⁺ T-bet⁺ CD4⁺ T cells in dLNs of Hectd3^{-/-} and WT mice, 13 days following EAE induction. d-e Flow cytometry analysis of CD11b from the CNS of Hectd3^{-/-} and WT mice 22 days following EAE induction. Gating strategy was first on CD45⁺ T cells. **f** Flow cytometry analysis of CD44⁺. CD62L⁺, and CD44⁺CD62L⁺ CD4⁺ T cells from dLNs of *Hectd3*^{-/-} and WT mice 7 days following EAE induction. Gating strategy show in Supplemental Figure 9. g-h Flow cytometry analysis of surface CD69 (g) and CD25 (h) on CD4⁺ T cells from dLNs of *Hectd3*^{-/-} and WT mice 7 days following EAE induction. Gating strategy shown on Supplemental Figure 9. i Flow cytometry analysis of intranuclear Ki-67 in CD4⁺ T cells from dLNs of Hectd3^{-/-} and WT mice 7 days following EAE induction. Gating strategy shown in Supplemental Figure 9. (a,d,fi) Data (n = 6) are representative of three independent experiments. (b,c,e) Data are presented as mean \pm SEM; p values was obtained from Student's t test. Source data are provided as a Source Data file.



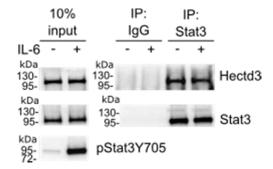
Supplementary Figure 3

Hectd3 promotes K27- and K29-linked polyubiquitin on Malt1A. Immunoblot of HA and Flag following two-step Flag immunoprecipitation of protein extracts from HEK293T cells co-transfected with the indicated HA-Ub K to R mutants, Flag-Malt1A and Xpress-Hectd3. HA-Ub K to R single mutant denote that the indicated lysine residue is mutated to arginine in the HA-Ub. Immunoblots are representative of two independent experiments. Source data are provided as a Source Data file.

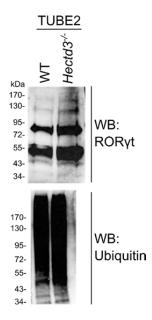


Supplementary Figure 4

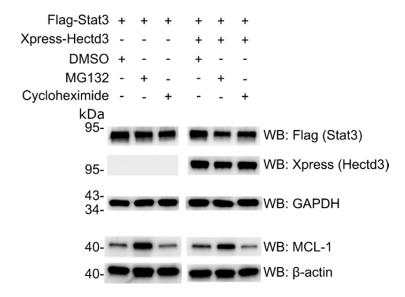
Malt1A K648R does not affect Malt1 substrate cleavage and CMB complex formation in Jurkat cells. a Immunoblot of MALT1-strep, CYLD, HOIL-1, and β-actin of protein extracts from MALT1KO Jurkat cells transduced with MSCV-Malt1A WT or MSCV-Malt1A K648R following the indicated duration of αCD3/CD28 stimulation. b Immunoblot of CARMA1, MALT1-strep, BCL10, and β-actin following StrepTactin pulldown (StepT-PD) of protein extracts from MALT1KO Jurkat cells transduced with MSCV-Malt1A WT or MSCV-Malt1A K648R. (a-b) Immunoblots are representative of two independent experiments. Source data are provided as a Source Data file.



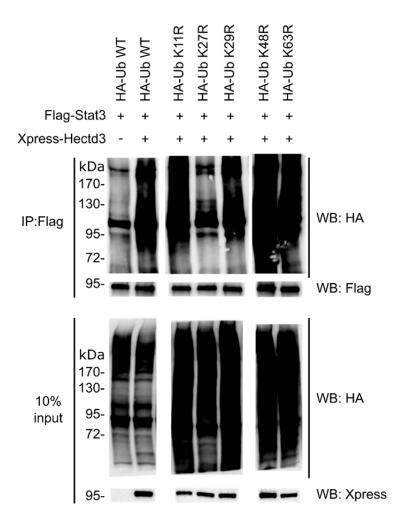
Hectd3 associates with Stat3 CD4⁺ T cells regardless of IL-6 stimulation. Immunoblot of Hectd3, Stat3 and pStat3 Y705 following Stat3 immunoprecipitation of protein extracts from WT CD4⁺ T cells stimulated or not with IL-6 (50 ng/ml) for 30 min. Representative of 3 independent experiments. Source data are provided as a Source Data file.



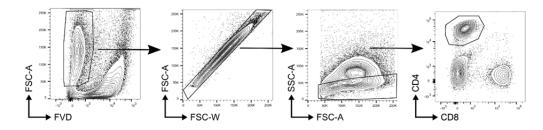
Hectd3 does not promote polyubiquitination of RORyt in EAE CD4⁺ T cells. Immunoblot for RORyt and ubiquitin following polyubiquitinated protein enrichment with TUBE2 of extracts from the dLN CD4⁺ T cells of Hectd3^{-/-} and WT mice 13 days following EAE induction. Representative of two independent experiments. Source data are provided as a Source Data file.



Hectd3 does not target Stat3 for proteasomal degradation. Immunoblot of Flag, Xpress, GAPDH, MCL-1, and β -actin of protein extracts from HEK293T cells co-transfected as indicated with Flag-Stat3 and Xpress-Hectd3 and treated for 4 hours with 20 μM MG132 or 15 μg/mL cycloheximide prior to protein extraction. Immunoblots are representative of two independent experiments. Source data are provided as a Source Data file.



Hectd3 promotes K27-linked polyubiquitin chain on Stat3. Immunoblot of HA and Flag following two-step Flag immunoprecipitation of protein extracts from HEK293T cells co-transfected as indicated with HA-Ub K to R mutants, Flag-Stat3, and Xpress-Hectd3. HA-Ub K to R single mutants denote that the indicated lysine residue is mutated to arginine in the HA-Ub. Immunoblot is representative of two independent experiments. Source data are provided as a Source Data file.



Supplementary Figure 9. Gating strategy. CD4⁺ T cells from the dLNs and CNS were gated on live, single cell lymphocytes, CD4 and CD8. Cytokines, surface markers and transcription factors were shown the CD4⁺ T cells. A similar strategy of gating was used for *in vitro* polarization on CD4+ T cells, as well as for transduced CD4⁺ T cells with retroviruses. Transduced CD4⁺ T cells were additionally gated on GFP, or CD90.1.

								Stat3						Sta	at3+Hectd3			
Protein Name	Accession	Calc PI	#AA	MW [kDA]	Coverage [%]		#Unique Peptides	Area	Sequest	Confidence (Sequest HT)		Coverage [%]	#Peptides	#Unique Peptides	Area	HT Sequest Score	Confidence (Sequest HT)	#PSMs
E3 ubiquitin-protein ligase HECTD3	Q5T447	5.64	861	97.05	0	0	0	0	0	n/a	0	14.9825784	10	10	2.80E+07	28.93	High	13
Signal transducer and activator of transcription 3	P40763	6.3	770	88	82.14286	4	1	1E+09	97.49	High	31	71.2987013	53	50	1.449E+10	1440.17	High	602

Mass spectrometry-based analysis of Stat3 and Hectd3. HEK293T cells were co-transfected with HA-Ub and Flag-Stat3 (Stat3), or co-transfected with HA-Ub, Flag-Stat3 and Xpress-Hectd3 (Stat3+Hectd3), and immunoprecipitated with anti-Flag antibodies, followed by trypsin digestion and Tandem Mass Spectrometry, as described in Material and Methods. Confidence was assigned by Sequest HT algorithm, which was used in conjunction with q-value calculator and posterior error probability (PEP) test to assign peptide confidence. Sequence coverage, number of identified peptides, number of unique peptides, sum of area of all peptides identified, sum of Sequest HT score, confidence and number of PSMs is shown per each protein in each sample. False discovery rate was 0.011% for peptide groups in Flag IP sample from HEK293T cells cotransfected Flag-Stat3 and Xpress-Hectd3 vectors (Stat3+Hectd3) and 0.01% for Flag IP sample from HEK293T cells transfected with Flag-Stat3 vector (Stat3).

Confidence Sequest HT	Sequence	Modifications	Qvality PEP	Qvality q- value	# PSMs		# Missed Cleavages	Theo. MH+ [Da]	Area	Sequest	Percolator q-Value Sequest HT	Percolator PEP Sequest HT
High	DFPAVDSVLVK		0.0099	0.0023	2	Q5T447	0	1189.64632	7630788	2.05	0.008364	0.04593
High	EQVAAMQAGLLK		0.0013	0.0003	1	Q5T447	0	1258.68238	11545471	2.41	0.000253	0.001138
High	FIAEGIIDQGGGFR		0.0121	0.0031	1	Q5T447	0	1479.75905	24512584	2.17	0.002746	0.009738
High	LEGTDPEVLYR		0.0004	0	1	Q5T447	0	1291.65286	14586199	2.23	0	0.000369
High	LVPIDTPNHLQR	1xDeamidated [N8]	0.0003	0	2	Q5T447	0	1403.76414	45346712	2.71	0	0.000261
High	RVVVYGGEGDNLKK	1xDeamidated [N11]	0.0236	0.005	1	Q5T447	2	1534.82238	unable to calculate	2.66	0.004143	0.0187
High	RVVVYGGEGDNLKK		5E-05	0	1	Q5T447	2	1533.83837	5882221	2.91	0	4.68E-05
High	TANQGNGTGEAR	1xDeamidated [N6]	0.0008	0	1	Q5T447	0	1176.52397	3043183	2.55	0	0.000653
High	VLLPVWEAEGLGLR		0.0479	0.0094	1	Q5T447	0	1551.88934	15091793	1.19	0.007354	0.03743
High	VVPQAVLDLLTWQELEK		0.0108	0.0025	1	Q5T447	0	1981.10045	1225384	2.43	0.002379	0.008753
High	WEQVVDLTYSHR		0.0006	0	1	Q5T447	0	1532.74922	3455042	2.78	0	0.00052

Mass spectrometric analysis of Hectd3 peptides. HEK293T cells were co-transfected with HA-Ub and Flag-Stat3 (Stat3), or co-transfected with HA-Ub, Flag-Stat3 and Xpress-Hectd3 (Stat3+Hectd3), and immunoprecipitated with anti-Flag antibodies, followed by trypsin digestion and Tandem Mass Spectrometry, as described in Material and Methods. XCorr values were calculated by utilizing Sequest HT algorithm and used for calculating peptide confidence. Percolator was additionally used to discriminate between correct and incorrect peptide matches. Statistical analysis by q-value and posterior error probability (PEP) were used to increase confidence in the peptide identifications at a false discovery rate of 0.011% for peptide groups.

							Stat3				5	Stat3+Hecto	13	
Peptide	Modifications	Position (residue number)	Modification	Protein Accession Number(s)	XCorr Sequest HT	Percolator PEP	Percolator q-value	Confidence (Sequest HT)	#PSMs	XCorr Sequest HT	Percolator PEP	Percolator q-value	Confidence (Sequest HT)	#PSMs
TLkSQGDMQDLNGNNQSVTR	K3(GlyGly)	178-197	K180	P40763						2.58	0.0242	0.00511	High	1
TLkSQGDMQDLnGNNQSVTR	K3(GlyGly); N12(Deamidated)	178-197	K180 ;N189	P40763						2.24	0.0124	0.00275	High	1
TLkSQGDmQDLnGnNQSVTR	K3(GlyGly); M8(Oxidation); N12(Deamidated); N14(Deamidated)	178-197	K180; M185;N189 ;N191	P40763						2.91	0.00364	0.00136	High	1
TLkSQGDMQDLNGnNQSVTR	K3(GlyGly); N14(Deamidated)	178-197	K180 ;N191	P40763						2.25	0.021	0.00492	High	1
DISGKTQIQSVEPYTK	K5(GlyGly)	627-642	K631	P40763	2.96	0.0497	0.00618	High	1					
AILSTKPPGTFLLR	K6(GlyGly)	596-609	K601	P40763	2.36	0.00015	0	High	1					

Mass spectrometry-based analysis of ubiquitinated Stat3 peptides. HEK293T cells were co-transfected with HA-Ub and Flag-Stat3 (Stat3), or co-transfected with HA-Ub, Flag-Stat3 and Xpress-Hectd3 (Stat3+Hectd3), and immunoprecipitated with anti-Flag antibodies, followed by trypsin digestion and Tandem Mass Spectrometry, as described in Material and Methods. XCorr values were calculated by utilizing Sequest HT algorithm and used for calculating peptide confidence. Percolator was additionally used to discriminate between correct and incorrect peptide matches. Statistical analysis by q-value and posterior error probability (PEP) were used to increase confidence in the peptide identifications at a false discovery rate of 0.011 % for Flag IP sample from HEK293T cells co-transfected Flag-Stat3 and Xpress-Hectd3 vectors (Stat3+Hectd3) and 0.01% for Flag IP sample from HEK293T cells transfected with Flag-Stat3 vector (Stat3).

Stat3 forward 5'-AGTCAGACTGAGATCACTCCTCCCAGGGACT-3' Stat3 forward 5'-AGTCAGACTGAGATCTCCACCATGGCTCAGTGAAACCAGCTG-3' Stat3 reverse 5'-AGTCTCAGTGCTCAGCATTATCACATGGGGGAGGTAGCACACTC-3' Stat3 K180R forward 5'-CAAAACCCTCAGGAGCCAAGGAG-3' Stat3 K180R forward 5'-AGATGCAAATCAAAGTCGTCTG-3' Malt1A K648R forward 5'-AGATGCAAATACAAGTCGTCTG-3' Malt1A K648R reverse 5'-TITTGGATCAATATCAAGTCAGTG-3' Stat3 Aaa2-130 forward 5'-CACCCAACAGCGCCCCTAG-3' Stat3 Aaa2-130 reverse 5'-CATGGCGATCGCGGCGCG-3' Stat3 Aaa131-320 forward 5'-TCGTGGTGGAGCGGCAG-3' Stat3 Aaa320-480 forward 5'-AACATGCTGACCAATAAC-3' Stat3 Aaa320-480 forward 5'-AACATGCTGACCAATAAC-3' Stat3 Aaa320-480 reverse 5'-ATTCTTCATTAAGTTTCTGAAC-3' Stat3 Aaa320-480 reverse 5'-ATTATCTTGGCCTTTGG-3' Stat3 Aaa481-574 forward 5'-AACATGCTGACCAATAAC-3' Stat3 Aaa576-679 forward 5'-AACATGCTGACCAATAAC-3' Stat3 Aaa576-679 forward 5'-AACATGCTGACCAATAAC-3' Stat3 Aaa576-679 forward 5'-AACATGCTGACCAATAAC-3' Stat3 Aaa576-679 forward 5'-AACATGCTGACCAGGATTGATGC-3' Stat3 Aaa576-679 forward 5'-AACATGCTGACCAGGATTGATGC-3' Stat3 Aaa576-679 forward 5'-AACATGCTGACCAGGATTGATGC-3' Stat3 Aaa576-679 forward 5'-AGCGGTACGCGGCCGCTC-3' Stat3 Aaa576-679 forward 5'-AGCGGTACGCGGCCGCTC-3' Stat3 Aaa576-679 forward 5'-AGCGGTACGCGGCCGCTC-3' Stat3 Aaa576-679 forward 5'-AGCGGTACGCGGCCGCTC-3' Stat3 Aaa578-679 forward 5'-GAGCTGTGCAACGGCCAC-3' Hectd3 Aaa210-397 forward 5'-GAGCTGTGCAACGGCCAC-3' Hectd3 Aaa398-511 feverse 5'-TTCCAGGCATGGTGCGCAC-3' Hectd3 Aaa398-511 feverse 5'-TTCCAGGCGTGGATATCC-3' Hectd3 Aaa512-861 forward 5'-TAGCATGAGAGCCTTGAG-3' Hectd3 Aaa512-861 forward 5'-TAGCTCAGAGCATCTC-3' Hectd3 Aaa512-861 forward 5'-TGCATGCGGCGCGCGCG-3' Hectd3 Aaa512-861 forward 5'-TGCATGCGGCGCGCGC-3' Hectd3 Aaa512-861 forward 5'-TGCATGCGGCGCGCGCG-3' Hectd3 Aaa512-861 forward 5'-TGCATGCGGCGCGCGCG-3' HA-Ub K6R reverse 5'-TGCATGCCGGCGCGCG-3' HA-Ub K6R reverse 5'-GCCCAGAACCTGAGGCCGCGCG-3' HA-Ub K6R reverse 5'-GTCCTCACGAGAACCTGACCC-3' HA-Ub K6R reverse 5'-GTCCTCACGAGAACCTCACCC-3' HA-Ub K6R reverse 5'-GTCCTCA	Name of primer	Sequence
Stat3 forward 5'-AGTCAGACTGAGATCTCCACCATGGCTCGAGAACCAGCTG-3' Stat3 reverse 5'- AGTCTCAGTGCTCGAGTTATCACATGGGGGAGGTAGCACACTC-3' Stat3 K180R forward 5'- CAAAACCCTCAGGAGCCAAGGAG-3' Stat3 K180R reverse 5'- TAGTTGAAATCAAAGTCGTCCTG-3' Malt1A K648R forward 5'- AGATGCAAATACAAAGTCGTCCTG-3' Malt1A K648R reverse 5'- TITGGATCAATATCTAGATCAAGTG-3' Stat3 Aaa2-130 forward 5'-CACCCAACAGCCGCCCGTAG-3' Stat3 Aaa2-130 reverse 5'-CATGGCGATCGCGGCGCG-3' Stat3 Aaa131-320 forward 5'-CATGGCGATCGCGGCGCCCTTG-3' Stat3 Aaa320-480 forward 5'-AACATGCTGAGCACCACTTG-3' Stat3 Aaa320-480 forward 5'-AACATGCTGACCAATAAC-3' Stat3 Aaa320-480 reverse 5'-ATTCTTCATTAAGTTTCTGAAC-3' Stat3 Aaa481-574 forward 5'-TATATCTTGGCCTTTTGG-3' Stat3 Aaa481-574 reverse 5'-ATACCACAGGATTGAG-3' Stat3 Aaa576-679 reverse 5'-ATACCACAGGATTGAAG-3' Stat3 Aaa576-679 reverse 5'-ATACTTTTCAAAGGTCC-3' Stat3 Aaa671-770 forward 5'-GAGGGTACGCGGCCCCT-3' Stat3 Aaa671-770 reverse 5'-GACCTGGCCCCTTTGG-3' Stat3 Aaa570-770 forward 5'-GAGCGTACGCGGCCCGCTC-3' Stat3 Aaa570-770 forward 5'-GAGCTGTGCCACGGCCCGCTC-3' Hectd3 Aaa2-109 reverse 5'-AGCCTGTGCAACGGCCAC-3' Hectd3 Aaa398-511 reverse 5'-GCACTGACCCAGAAGTCCTC-3' Hectd3 Aaa398-511 reverse 5'-TCCAGGGCTGGATCCCGGCCCAC-3' Hectd3 Aaa398-511 reverse 5'-TCCAGGCGTGGATCCCGGCCAC-3' Hectd3 Aaa512-861 forward 5'-TATAGGAACCCTTGGAC-3' Hectd3 Aaa512-861 forward 5'-TATAGGAACCCTTGGAC-3' Hectd3 Aaa512-861 forward 5'-TATGAGAACCCTTGGAC-3' Hectd3 Aaa512-861 forward 5'-TATGAGAAGCCCTTGGAC-3' Hectd3 Aaa512-861 forward 5'-TGCATGCCGCGCGCGCG-3' HA-Ub K6R forward 5'-GCCTGACCGGCGCGCG-3' HA-Ub K6R forward 5'-CCTGACCGGCCGCGCG-3' HA-Ub K6R forward 5'-CCTGACCGGCAGAAGACCATC-3' HA-Ub K6R forward 5'-CCTGACCGGCAGAAGACCATC-3' HA-Ub K6R,11R forward 5'-CCGAGAACGTGAGGGCCAC-3' HA-Ub K6,11R forward 5'-CCGAGAACGTGAGGGCCAC-3' HA-Ub K6,11R forward 5'-CCGAGAACGTGAGGGCCAC-3' HA-Ub K6,11R forward 5'-CCGAGAACGTGAGGCCCAC-3'	Hectd3 forward	5'-GGAAGATCTCCTCCTGCCGGCCCTGGC-3'
Stat3 reverse 5'- AGTCTCAGTGCTCGAGTTATCACATGGGGGAGGTAGCACACTC-3' Stat3 K180R forward 5'- CAAAACCCTCAGGAGCCAAGGAG-3' Stat3 K180R reverse 5'- TAGTTGAAATCAAAGTCGTCCTG-3' Malt1A K648R forward 5'- AGATGCAAATAGAGGCACACCTG-3' Malt1A K648R reverse 5'- TTTGGATCAATATCTAGATCAAGTG-3' Stat3 Aaa2-130 forward 5'- CACCCAACAGCCGCCGTAG-3' Stat3 Aaa2130 reverse 5'- CATGGCGATCGCGCGGCG-3' Stat3 Aaa131-320 forward 5'- TTCGTGGTGAGACGGCCAG-3' Stat3 Aaa320-480 forward 5'- ACACTGACCAATAAC-3' Stat3 Aaa320-480 forward 5'- ACACTGTGCCCACATAAC-3' Stat3 Aaa481-574 forward 5'- TATACTTTGGCCTTTGG-3' Stat3 Aaa481-574 reverse 5'- ATACCACAGAGTTGATGC-3' Stat3 Aaa576-679 forward 5'- AGGGGATGCCGCCCCT-3' Stat3 Aaa671-770 forward 5'- AGCGTACCGGCCGCCC-3' Stat3 Aaa671-770 forward 5'- AGCGGTACGCGCCGCTC-3' Stat3 Aaa671-770 forward 5'- AGCGGTACGCGCCCCTC-3' Stat3 Aaa671-770 forward 5'- AGCGGTACGCGCCCCTC-3' Stat3 Aaa671-770 forward 5'- AGCGGTACGCGCCCCCTC-3' Stat3 Aaa671-770 forward 5'- AGCGGTACGCGCCCCCTC-3' Stat3 Aaa671-770 forward 5'- AGCGGTACGCGGCCGCTC-3' Stat3 Aaa671-770 forward 5'- AGGCTGTGCAACGGCCAC-3' S'- AGCTGTGCAACGGCCAC-3' S'- AGCTGTGCAACGGCCAC-3' S'- AGCACTGACCCAGAAGTACTTCA-3' S'- AGCACTGACCCAGAAGTACTTAAC-3' S'- AGCACTGACCCAGAAGTACTTAAC-3' S'- AGCACTGACCCAGAAGTACTTAAC-3' S'- AGCACTGACCCAGAAGTACTTAAC-3' S'- AGCACTGACCCAGAAGTACTCA-3' S'- AGCACTGACCCAGAAGTACTCA-3' S'- AGCACTGACCCAGAAGTACTCA-3' S'- AGCACTGACCCAGAAGCCCTTGAG-3' S'- AGCACTGACCGGCAAGACCATC-3' S'- AGCACTGACCGGCAAGACCATC-3' S'- AGCACTGACCCGCCGCCG-3' S'- AGCACTGACCGGCAAGACCATC-3' S'- AGCACTGACCGGCAAGACCATCACCC-3' S'- AGCACTGACCGGCAAGACCATCACCC-3' S'- AGCACTGACCGGCAGAACCATCACCC-3' S'- AGCACTGACCGCGCCGCCGCCG-3' S'- AGCACTGACCGCGCCGCCG	Hectd3 reverse	5'- CCGCTCGAGTCACTCCTCCCAGGGACT-3'
Stat3 K180R forward 5'- CAAAACCCTCAGGAGCCAAGGAG-3' Stat3 K180R reverse 5'- TAGTTGAAATCAAAGTCGTCCTG-3' Malt1A K648R forward 5'- AGATGCAAATAGAGGCACACCTG-3' Malt1A K648R reverse 5'- TTTGGATCAATATCTAGATCAAGTG-3' Stat3 Δaa2-130 forward 5'-CACCCAACAGCCGCCGTAG-3' Stat3 Δaa131-320 forward 5'-CATGGCGATCGCGGCGGC-3' Stat3 Δaa131-320 reverse 5'-CATGGCGATCGCGCCCCTTG-3' Stat3 Δaa320-480 forward 5'-ACCATGCTGACCAATAAC-3' Stat3 Δaa320-480 forward 5'-ACCATGCTGACCAATAAC-3' Stat3 Δaa320-480 forward 5'-ACCTTTCATTAGTTTCTGAAC-3' Stat3 Δaa341-574 forward 5'-ATATCTTTGGCCCTTTGG-3' Stat3 Δaa481-574 forward 5'-ATACCACAGGATTGATGC-3' Stat3 Δaa576-679 forward 5'-AGGAGGCATTTGGAAG-3' Stat3 Δaa576-679 reverse 5'-ATACTTTTTCACAAGGTCG-3' Stat3 Δaa671-770 forward 5'-ACGCGTACGCGGCCGCTC-3' Stat3 Δaa671-770 reverse 5'-CTCCTTGGGATGTCGACCGGCAC-3' Hectd3 Δaa2-109 forward 5'-AGGAGGAGGCAGATCCTC-3' Hectd3 Δaa10-397 reverse 5'-CTCGCCAGTGGTGCACAGACATCATAC-3' Hectd3 Δaa398-511 forward 5'-TGCCTCAGGGGCAGATCCTC-3' Hectd3 Δaa398-511 forward	Stat3 forward	5'-AGTCAGACTGAGATCTCCACCATGGCTCAGTGGAACCAGCTG-3'
Stat3 K180R reverse 5'- TAGTTGAAATCAAAGTCGTCCTG-3' Malt1A K648R forward 5'- AGATGCAAATAGAGGCACACCTG-3' Malt1A K648R reverse 5'- TTTGGATCAATATCTAGATCAAGTG-3' Stat3 Δaa2-130 forward 5'-CACCCAACAGCCGCCGTAG-3' Stat3 Δaa2-130 reverse 5'-CATGGCGATCGCGGCGCG-3' Stat3 Δaa13-320 forward 5'-TTCGTGGTGGAGCGGCGC-3' Stat3 Δaa313-320 reverse 5'-GTTGGCCTGGCCCCCTTG-3' Stat3 Δaa320-480 forward 5'-AACATGCTGACCAATAAC-3' Stat3 Δaa481-574 forward 5'-TATATCTTGGCCTTTGG-3' Stat3 Δaa481-574 reverse 5'-ATACCACAGAGTTGATG-3' Stat3 Δaa526-679 forward 5'-AACATGCTGACCAATAAC-3' Stat3 Δaa576-679 reverse 5'-ATACCTCACAGAGTTGATG-3' Stat3 Δaa576-679 reverse 5'-AACATGCTGCCCTTTGG-3' Stat3 Δaa671-770 forward 5'-ACGCGTACGCGGCCCCTC-3' Stat3 Δaa671-770 reverse 5'-ACCCTTGGAATGTCGGGTAGAGG-3' Hectd3 Δaa2-109 reverse 5'-AGGAGGAGCAGTCCC-3' Hectd3 Δaa2-109 reverse 5'-AGGAGGAGCAGTCCC-3' Hectd3 Δaa310-397 forward 5'-GGCACTGACCCAGAAGTACTATAC-3' Hectd3 Δaa398-511 reverse 5'-CTCCCCAGTGGTGCGCCAC-3' Hectd3 Δaa398-511 reverse 5'-TTCCAGGCGTGGAACGCCAC-3' Hectd3 Δaa512-861 forward 5'-GACTCTCAGAAGGCTTGAG-3' Hectd3 Δaa512-861 forward 5'-GACTCTCAGAAGGCTTGAG-3' Hectd3 Δaa512-861 forward 5'-GACTCTCAGAGAGCTTGAG-3' Hectd3 Δaa512-861 forward 5'-GACTCTCAGAAGGCTTGAG-3' Hectd3 Δaa512-861 forward 5'-GACTCTCAGAAGACCTTGAG-3' Hectd3 Δaa512-861 forward 5'-GACTCTCAGAAGACCTTGAG-3' Hectd3 Δaa512-861 forward 5'-GACTCTCAGAAGACCTTGAG-3' HA-Ub K6R forward 5'-GACCTCACGAAGATCTG-3' HA-Ub K6R forward 5'-CCTGACCGGCGGCGCGCGCGCGCGCGCGCG-3' HA-Ub K6R,11R forward 5'-CCTGACCGGCAGAACCATC-3' 5'-CCTGACCGGCAGAACCATC-3' HA-Ub K6R,11R forward 5'-CAGAAACGTGaggGCCAAGATCC-3'	Stat3 reverse	5'- AGTCTCAGTGCTCGAGTTATCACATGGGGGAGGTAGCACACTC-3'
Malt1A K648R forward 5'- AGATGCAAATAGAGGCACACCTG-3' Malt1A K648R reverse 5'- TTTGGATCAATATCTAGATCAAGTG-3' Stat3 Δaa2-130 forward 5'- CACCCAACAGCCGCGCTAG-3' Stat3 Δaa21-320 forward 5'- CATGGCGATCGCGGCGGC-3' Stat3 Δaa31-320 reverse 5'- CTTGGCTGGAGCCGCCCTTG-3' Stat3 Δaa320-480 forward 5'- AACATGCTGACCAATAAC-3' Stat3 Δaa320-480 reverse 5'- ACTCTTCATTAAGTTTCTGAAC-3' Stat3 Δaa481-574 forward 5'- TATATCTTGGCCCTTTGG-3' Stat3 Δaa481-574 reverse 5'- ATACCACAGGATTGATGC-3' Stat3 Δaa576-679 forward 5'- AGGAGGGCATTTGGAAG-3' Stat3 Δaa576-679 reverse 5'- ATACTTTTCACAAGGTCG-3' Stat3 Δaa671-770 forward 5'- AGGCGTACGCGGCCGCTC-3' Stat3 Δaa671-770 reverse 5'- CTCCTTGGGAATGTCGGGTAGAGG-3' Hectd3 Δaa2-109 forward 5'- GAGCAGTGCAACGGCCAC-3' Hectd3 Δaa110-397 forward 5'- GGCACTGACCCAGAATACTATAC-3' Hectd3 Δaa110-397 reverse 5'- CTCGCCAGTGGTGCGAC-3' Hectd3 Δaa398-511 forward 5'- TATGAGAAGCCCTTGGAC-3' Hectd3 Δaa512-861 forward 5'- TATGAGAAGGCCTTGAG-3' Hectd3 Δaa512-861 forward 5'- TGACTCGAGTCTAGAGGGC-3' Hectd3 Δaa512-861 forward 5'- TGACTCGAGAGGCTTGAG-3' Hectd3 Δaa512-861 forward 5'- GACCTGACCCGCGCCGCCG-3' HA-Ub K6R forward 5'- GATCTTCGTGaggACCCTGACCGGCAAGACCATC-3' HA-Ub K6R forward 5'- CCTGACCGGCagaACCATCACCC-3' HA-Ub K6R, 11R forward 5'- CCTGACCGGCagaACCATCACCC-3' HA-Ub K6R, 11R forward 5'- CCTGACCGGCAGAGATCTG-3' HA-Ub K6R, 11R forward 5'- CCTGACAGAACGTGaggGCCAAGATCC-3' HA-Ub K6R, 11R forward 5'- CCTGACAGAACGTGaggGCCAAGATCC-3'	Stat3 K180R forward	5'- CAAAACCCTCAGGAGCCAAGGAG-3'
Malt1A K648R reverse 5'-TTTGGATCAATATCTAGATCAAGTG-3' Stat3 Δaa2-130 forward 5'-CACCCAACAGCCGCCGTAG-3' Stat3 Δaa2-130 reverse 5'-CATGGCGATCGCGGCGGC-3' Stat3 Δaa131-320 reverse 5'-GTTGGCTGGCCCCCTTG-3' Stat3 Δaa320-480 forward 5'-AACATGCTGACCAATAAC-3' Stat3 Δaa320-480 reverse 5'-ACTCTTCATTAAGTTTCTGAAC-3' Stat3 Δaa481-574 forward 5'-TATATCTTGGCCCTTTGG-3' Stat3 Δaa481-574 reverse 5'-ATACCACAGGATTGATGC-3' Stat3 Δaa576-679 forward 5'-GAGGAGGCATTTGGAAAG-3' Stat3 Δaa576-679 reverse 5'-ATACTTTTTCACAAGGTCG-3' Stat3 Δaa671-770 forward 5'-ACGCGTACGCGGCCGCTC-3' Stat3 Δaa671-770 reverse 5'-CTCCTTGGGAATGTCGGGGTAGAGG-3' Hectd3 Δaa2-109 forward 5'-GAGCGGCAGATCCTC-3' Hectd3 Δaa2-109 reverse 5'-AGGAGGAGGCAGATCCTC-3' Hectd3 Δaa110-397 reverse 5'-CTCGCCAGTGGTGCGCAC-3' Hectd3 Δaa398-511 forward 5'-TATGAGAAGCCCTTGGAC-3' Hectd3 Δaa398-511 reverse 5'-TTGCAGGCGTGGATATCG-3' Hectd3 Δaa512-861 forward 5'-GATCTCGAGTCTAGAGGG-3' Hectd3 Δaa512-861 forward 5'-GATCTTCGTGaggACCCTGACCGGCAAGACCATC-3' HA-Ub K6R forward 5'-	Stat3 K180R reverse	5'- TAGTTGAAATCAAAGTCGTCCTG-3'
Stat3 ∆aa2-130 forward 5'-CACCCAACAGCCGCCGTAG-3' Stat3 ∆aa2-130 reverse 5'-CATGGCGATCGCGGCGGC-3' Stat3 ∆aa131-320 forward 5'-TTCGTGGTGAGCGGCAG-3' Stat3 ∆aa320-480 forward 5'-ACATGCTGACCAATAAC-3' Stat3 ∆aa320-480 reverse 5'-ACATGCTGACCAATAAC-3' Stat3 ∆aa320-480 reverse 5'-ACATGCTGACCAATAAC-3' Stat3 ∆aa320-480 reverse 5'-ACATGCTGACCAATAAC-3' Stat3 ∆aa320-480 reverse 5'-ACATGTCATTAGGTTTCTGAAC-3' Stat3 ∆aa320-480 forward 5'-TATATCTTTGCCCCTTTGG-3' Stat3 ∆aa481-574 forward 5'-TATACCACAGGATTGATGG-3' Stat3 ∆aa481-574 reverse 5'-ATACCACAGGATTGATGG-3' Stat3 ∆aa576-679 forward 5'-GAGGAGGCATTTTGGAAAG-3' Stat3 ∆aa576-679 reverse 5'-ATACTTTTTCACAAGGTCG-3' Stat3 ∆aa671-770 forward 5'-ACGCGTACGCGGCCGCTC-3' Stat3 ∆aa671-770 reverse 5'-CTCCTTGGGAATGCGGGCAC-3' Hectd3 ∆aa2-109 forward 5'-GAGCTGTCAACGGCCAC-3' Hectd3 ∆aa2-109 reverse 5'-AGGAGAGCATCACCAGAAGTACTATAC-3' Hectd3 ∆aa110-397 reverse 5'-CTCGCCAGTGGTGCCAC-3' Hectd3 ∆aa398-511 reverse 5'-TTCCAGGCGTGGATATCG-3' Hectd3 ∆aa512-861 forward 5'-TGACTCGAGGC	Malt1A K648R forward	5'- AGATGCAAATAGAGGCACACCTG-3'
Stat3 ∆aa2-130 reverse 5'-CATGGCGATCGCGGCGGC-3' Stat3 ∆aa131-320 forward 5'-TTCGTGGTGGAGCGGCAG-3' Stat3 ∆aa320-480 forward 5'-AACATGCTGACCAATAAC-3' Stat3 ∆aa320-480 reverse 5'-ACTCTTCATTAAGTTTCTGAAC-3' Stat3 ∆aa481-574 forward 5'-TATATCTTGGCCTTTGG-3' Stat3 ∆aa481-574 forward 5'-AACACAGGATTGATGC-3' Stat3 ∆aa481-574 reverse 5'-ATACCACAGGATTGATGC-3' Stat3 ∆aa576-679 forward 5'-AGGGAGGCATTTGGAAG-3' Stat3 ∆aa576-679 reverse 5'-ATACTTTTCACAAGGTCG-3' Stat3 ∆aa671-770 forward 5'-ACGCGTACGCGCCGCTC-3' Stat3 ∆aa671-770 reverse 5'-CTCCTTGGGAATGTCGGGGTAGAGG-3' Hectd3 ∆aa2-109 forward 5'-AGGAGGAGGCAGTCCTC-3' Hectd3 ∆aa110-397 reverse 5'-AGGAGGAGGCAGTCCTC-3' Hectd3 ∆aa110-397 reverse 5'-CTCGCCAGTGGTGCACCAGAAGTACTATAC-3' Hectd3 ∆aa398-511 forward 5'-TATGAGAAGCCCTTGGAC-3' Hectd3 ∆aa512-861 forward 5'-TGACTCGAGGTGTGAACGCGGCGCAC-3' Hectd3 ∆aa512-861 forward 5'-TGACTCGAGAGGCTTAGAGG-3' Hectd3 ∆aa512-861 forward 5'-GACCTGACCGAGAGCTTAGAGG-3' H-Ub K6R forward 5'-GACTCTCCGCCGCGCCGCG-3' HA-Ub K6R reverse 5'-TGCATGCCGCCGCCGCCG-3' HA-Ub K6,11R forward 5'-CCTGACCGGAGAACCTTCC-3' HA-Ub K6,11R forward 5'-CCTGACCGAGAGACCTTCC-3' HA-Ub K6,11R reverse 5'-GTCCTCACGAAGATCTC-3' HA-Ub K6,11R reverse 5'-GTCCTCACGAAGATCTCC-3'	Malt1A K648R reverse	5'- TTTGGATCAATATCTAGATCAAGTG-3'
Stat3 Δaa131-320 forward Stat3 Δaa131-320 reverse 5'-GTTGGCCTGGCCCCCTTG-3' Stat3 Δaa320-480 forward 5'-AACATGCTGACCAATAAC-3' Stat3 Δaa320-480 reverse 5'-ACTCTTCATTAAGTTTCTGAAC-3' Stat3 Δaa481-574 forward 5'-TATATCTTGGCCTTTGG-3' Stat3 Δaa481-574 reverse 5'-ATACCACAGGATTGATGC-3' Stat3 Δaa481-574 reverse 5'-ATACCACAGGATTGATGC-3' Stat3 Δaa576-679 forward 5'-GAGGAGGCATTTGGAAC-3' Stat3 Δaa671-770 forward 5'-ACGCGTACGCGGCCGCTC-3' Stat3 Δaa671-770 reverse 5'-CTCCTTGGGAATGTCGGGGTAGAGGG-3' Hectd3 Δaa2-109 forward 5'-GAGGTGTGCAACGGCCAC-3' Hectd3 Δaa2-109 reverse 5'-AGGAGGAGGCAGTCCTC-3' Hectd3 Δaa110-397 forward 5'-GGCACTGACCCAGAAGTACTATAC-3' Hectd3 Δaa398-511 forward 5'-TATGAGAAAGCCCTTGGAC-3' Hectd3 Δaa398-511 reverse 5'-TTCCAGGCGTGGAATCCGGG-3' Hectd3 Δaa512-861 forward 5'-TGACTCGAGTCTAGAGGG-3' Hectd3 Δaa512-861 forward 5'-TGACTCGAGTCTAGAGGGG-3' Hectd3 Δaa512-861 forward 5'-TGACTCGAGTCTAGAGGGG-3' Hectd3 Δaa512-861 forward 5'-TGACTCGAGTCTAGAGGGG-3' Hectd3 Δaa512-861 forward 5'-TGACTCGAGTCTAGAGGGG-3' HA-Ub K6R forward 5'-GATCTTCGTGAGAAGCCCTGACCGGCAAGACCATC-3' HA-Ub K6,11R forward 5'-CCTGACCGGCAGAACCATCACCC-3' HA-Ub K6,11R reverse 5'-CTCTCACGAAACCTGAGCGTG-3' HA-Ub K6,11R reverse 5'-GTCCTCACGAAACCTG-3' HA-Ub K6,11R reverse 5'-GCGAGAACGTGAGGGGGAACCC-3'	Stat3 Δaa2-130 forward	5'-CACCCAACAGCCGCCGTAG-3'
Stat3 Δaa131-320 reverse Stat3 Δaa320-480 forward Stat3 Δaa320-480 forward Stat3 Δaa320-480 forward Stat3 Δaa320-480 reverse S'-ACTCTTCATTAAGTTTCTGAAC-3' Stat3 Δaa481-574 forward S'-TATATCTTGGCCCCTTTGG-3' Stat3 Δaa481-574 reverse S'-ATACCACAGGATTGATGC-3' Stat3 Δaa481-574 reverse S'-ATACCACAGGATTGATGC-3' Stat3 Δaa576-679 forward S'-GAGGAGGCATTTGGAAAG-3' Stat3 Δaa576-679 reverse S'-ATACTTTTTCACAAGGTCG-3' Stat3 Δaa671-770 forward S'-ACGCGTACGCGGCCGCTC-3' Stat3 Δaa671-770 reverse S'-CTCCTTGGGAATGTCGGGGTAGAGGG-3' Hectd3 Δaa2-109 forward F'-GAGCTGTGCAACGGCCAC-3' Hectd3 Δaa2-109 reverse S'-AGGAGGAGCAGATCCTC-3' Hectd3 Δaa110-397 forward F'-GGCACTGACCCAGAAGTACTATAC-3' Hectd3 Δaa398-511 forward S'-TATGAGAAGCCCTTGGAC-3' Hectd3 Δaa398-511 forward S'-TATGAGAAGCCCTTGGAC-3' Hectd3 Δaa512-861 forward S'-TCCAGGCGTGGATATCG-3' Hectd3 Δaa512-861 forward S'-GATCTCAGAGCTTAGAGGG-3' HA-Ub K6R forward S'-GATCTTCGTGaggACCCTGACCGGCAAGACCATC-3' HA-Ub K6,11R forward S'-CCTGACCGGCAGAGATCTCC-3' HA-Ub K6,11R reverse S'-GCCCTCACGAAGATCTCC-3' HA-Ub K6,11R reverse S'-GCCCTCACGAAGATCTCC-3' HA-Ub K6,11R reverse S'-GCCCTCACGAAGATCTCC-3' HA-Ub K6,11R reverse S'-GCCTCACGAAGATCTCC-3' HA-Ub K6,11,27R forward S'-CGAGAACGTGaggGCCAAGATCCC-3'	Stat3 ∆aa2-130 reverse	5'-CATGGCGATCGCGGCGGC-3'
Stat3 Δaa320-480 forward Stat3 Δaa320-480 reverse S'-ACTCTTCATTAAGTTTCTGAAC-3' Stat3 Δaa481-574 forward S'-TATATCTTGGCCCTTTGG-3' Stat3 Δaa481-574 reverse S'-ATACCACAGGATTGATGC-3' Stat3 Δaa481-574 reverse S'-ATACCACAGGATTGATGC-3' Stat3 Δaa576-679 forward S'-GAGGAGGCATTTGGAAAG-3' Stat3 Δaa576-679 reverse S'-ATACTTTTTCACAAGGTCG-3' Stat3 Δaa671-770 forward S'-ACGCGTACGCGGCCGCTC-3' Stat3 Δaa671-770 reverse S'-CTCCTTTGGAAATGTCGGGGTAGAGG-3' Hectd3 Δaa2-109 forward S'-GAGCTGTGCAACGGCCAC-3' Hectd3 Δaa2-109 reverse F'-AGGAGGAGCAGATCCTC-3' Hectd3 Δaa110-397 forward F'-GGCACTGACCCAGAAGTACTATAC-3' Hectd3 Δaa110-397 reverse F'-CTCGCCAGTGGTGCGAC-3' Hectd3 Δaa398-511 forward S'-TATGAGAAGCCCTTGGAC-3' Hectd3 Δaa512-861 forward S'-TGACTCGAGTCTAGAGGG-3' Hectd3 Δaa512-861 forward F'-GACTCTGAGAAGGCTTGAG-3' HA-Ub K6R forward F'-GATCTTCGTGaggACCCTGACCGGCAAGACCATC-3' HA-Ub K6,11R forward F'-CCTGACCGGCAGCAGCCG-3' HA-Ub K6,11R reverse F'-CTCCCCAGAAGATCTCC-3' HA-Ub K6,11R reverse F'-CCTCACCGAAGATCTCC-3' HA-Ub K6,11R reverse F'-CCTGACCGGCAAGACCCC-3' HA-Ub K6,11R reverse F'-CCGAGAACGTGAGGGCC-3'	Stat3 Δaa131-320 forward	5'-TTCGTGGTGGAGCGGCAG-3'
Stat3 ∆aa320-480 reverse 5'-ACTCTTCATTAAGTTTCTGAAC-3' Stat3 ∆aa481-574 forward 5'-TATATCTTGGCCCTTTGG-3' Stat3 ∆aa481-574 reverse 5'-ATACCACAGGATTGATGC-3' Stat3 ∆aa576-679 forward 5'-GAGGAGGCATTTGGAAG-3' Stat3 ∆aa576-679 reverse 5'-ATACTTTTCACAAGGTCG-3' Stat3 ∆aa671-770 forward 5'-ACGCGTACGCGGCCGCTC-3' Stat3 ∆aa671-770 reverse 5'-CTCCTTGGGAATGTCGGGGTAGAGG-3' Hectd3 ∆aa2-109 forward 5'-GAGCTGTGCAACGGCCAC-3' Hectd3 ∆aa2-109 reverse 5'-AGGAGGAGGCAGATCCTC-3' Hectd3 ∆aa110-397 forward 5'-GGACCTGACCCAGAAGTACTATAC-3' Hectd3 ∆aa110-397 reverse 5'-CTCGCCAGTGGTGCACC3' Hectd3 ∆aa398-511 forward 5'-TATGAGAAGCCCTTGGAC-3' Hectd3 ∆aa398-511 reverse 5'-TTCCAGGCGTGGATATCG-3' Hectd3 ∆aa512-861 forward 5'-TGACTCGAGTCTAGAGGG-3' Hectd3 ∆aa512-861 forward 5'-TGACTCGAGGCTTGAC-3' HA-Ub K6R forward 5'-GATCTTCGTGaggACCCTGACCGCAAGACCATC-3' HA-Ub K6,11R forward 5'-CCTGACCGGCAGAACCATCACCC-3' HA-Ub K6,11R reverse 5'-CTCCCCAGAAGACCCTGC-3' HA-Ub K6,11R reverse 5'-GTCCTCACGAAGACCCC-3' 5'-GCCCTCACCAGAAGACCCTGACCC-3' HA-Ub K6,11R reverse 5'-CTCACCAGAAGATCTGC-3' HA-Ub K6,11R reverse 5'-GTCCTCACGAAGATCCC-3'	Stat3 Δaa131-320 reverse	5'-GTTGGCCTGGCCCCTTG-3'
Stat3 Δaa481-574 forward Stat3 Δaa481-574 reverse 5'-ATACCACAGGATTGATGC-3' Stat3 Δaa576-679 forward 5'-GAGGAGGCATTTGGAAAG-3' Stat3 Δaa576-679 reverse 5'-ATACTTTTTCACAAGGTCG-3' Stat3 Δaa671-770 forward 5'-ACGCGTACGCGGCCGCTC-3' Stat3 Δaa671-770 reverse 5'-CTCCTTGGGAATGTCGGGGTAGAGG-3' Hectd3 Δaa2-109 forward 5'-GAGCTGTGCAACGGCCAC-3' Hectd3 Δaa110-397 forward 5'-GGCACTGACCCAGAAGTACTATAC-3' Hectd3 Δaa110-397 reverse 5'-CTCGCCAGTGGTGCACCAGAGTACTATAC-3' Hectd3 Δaa110-397 reverse 5'-CTCGCCAGTGGTGCACCAGAAGTACTATAC-3' Hectd3 Δaa398-511 forward 5'-TATGAGAAAGCCCTTGGAC-3' Hectd3 Δaa398-511 forward 5'-TATGAGAAGCCCTTGAGC-3' Hectd3 Δaa512-861 forward 5'-TGACTCGAGTCTAGAGGG-3' Hectd3 Δaa512-861 reverse 5'-CTTGTCAGAGAGGCTTGAC-3' HA-Ub K6R forward 5'-GATCTTCGTGaggACCCTGACCGGCAAGACCATC-3' HA-Ub K6R reverse 5'-TGCATGCCGCCGCCGCCG-3' HA-Ub K6,11R forward 5'-CCTGACCGGCAGAACTCTC-3' HA-Ub K6,11R reverse 5'-GTCCTCACGAAGATCTCC-3' HA-Ub K6,11R reverse 5'-GTCCTCACGAAGATCTCC-3' HA-Ub K6,11R reverse 5'-GTCCTCACGAAGATCTCC-3'	Stat3 Δaa320-480 forward	5'-AACATGCTGACCAATAAC-3'
Stat3 Δaa481-574 reverse 5'-ATACCACAGGATTGATGC-3' Stat3 Δaa576-679 forward 5'-GAGGAGGCATTTGGAAAG-3' Stat3 Δaa671-770 forward 5'-ACGCGTACGCGGCCGCTC-3' Stat3 Δaa671-770 forward 5'-ACGCGTACGCGGCCGCTC-3' Stat3 Δaa671-770 reverse 5'-CTCCTTGGGAATGTCGGGGTAGAGG-3' Hectd3 Δaa2-109 forward 5'-GAGCTGTGCAACGGCCAC-3' Hectd3 Δaa2-109 reverse 5'-AGGAGGAGCAGATCCTC-3' Hectd3 Δaa110-397 forward 5'-GGCACTGACCCAGAAGTACTATAC-3' Hectd3 Δaa110-397 reverse 5'-CTCGCCAGTGGTGCGCAC-3' Hectd3 Δaa398-511 forward 5'-TATGAGAAGCCCTTGGAC-3' Hectd3 Δaa398-511 reverse 5'-TTCCAGGCGTGGATATCG-3' Hectd3 Δaa512-861 forward 5'-TAGACTCGAGTCTAGAGGG-3' Hectd3 Δaa512-861 forward 5'-TGACTCGAGTCTAGAGGG-3' HA-Ub K6R forward 5'-GATCTTCGTGaggACCCTGACCGGCAAGACCATC-3' HA-Ub K6R reverse 5'-TGCATGCCGCCGCCGCGCG-3' HA-Ub K6,11R forward 5'-CCTGACCGGCagaACCATCACCC-3' HA-Ub K6,11R reverse 5'-GTCCTCACGAAGATCTGC-3' HA-Ub K6,11R reverse 5'-GTCCTCACGAAGATCTGC-3' HA-Ub K6,11R reverse 5'-GTCCTCACGAAGATCTGC-3' HA-Ub K6,11R reverse 5'-GTCCTCACGAAGATCTGC-3'	Stat3 Δaa320-480 reverse	5'-ACTCTTCATTAAGTTTCTGAAC-3'
Stat3 Δaa576-679 forward Stat3 Δaa576-679 reverse 5'-ATACTTTTCACAAGGTCG-3' Stat3 Δaa671-770 forward 5'-ACGCGTACGCGGCCGCTC-3' Stat3 Δaa671-770 reverse 5'-CTCCTTGGGAATGTCGGGGTAGAGGG-3' Hectd3 Δaa2-109 forward 5'-AGGAGGAGGCAGATCCTC-3' Hectd3 Δaa2-109 reverse 5'-AGGAGGAGGCAGATCCTC-3' Hectd3 Δaa110-397 forward 5'-GGCACTGACCCAGAAGTACTATAC-3' Hectd3 Δaa310-397 reverse 5'-CTCGCCAGTGGTCGACC3' Hectd3 Δaa398-511 forward 5'-TATGAGAAGCCCTTGGAC-3' Hectd3 Δaa398-511 reverse 5'-TTCCAGGCGTGGATATCG-3' Hectd3 Δaa512-861 forward 5'-TGACTCGAGTCTAGAGGG-3' Hectd3 Δaa512-861 reverse 5'-CTTGTCAGAAGGCTTGAG-3' HA-Ub K6R forward 5'-GATCTTCGTGaggACCCTGACCGGCAAGACCATC-3' HA-Ub K6,11R forward 5'-CCTGACCGGCAGGAGACCATCACCC-3' HA-Ub K6,11R forward 5'-CCTGACCGGCAGAAGATCTGC-3' HA-Ub K6,11R forward 5'-CCTGACCGGCAGAAGATCTGC-3' HA-Ub K6,11R forward 5'-CCTGACCGGCAGAGACCATC-3'	Stat3 ∆aa481-574 forward	5'-TATATCTTGGCCCTTTGG-3'
Stat3 Δaa576-679 reverse 5'-ATACTTTTCACAAGGTCG-3' Stat3 Δaa671-770 forward 5'-ACGCGTACGCGGCCGCTC-3' Stat3 Δaa671-770 reverse 5'-CTCCTTGGGAATGTCGGGGTAGAGG-3' Hectd3 Δaa2-109 forward 5'-GAGCTGTGCAACGGCCAC-3' Hectd3 Δaa2-109 reverse 5'-AGGAGGAGGCAGATCCTC-3' Hectd3 Δaa110-397 forward 5'-GGCACTGACCCAGAAGTACTATAC-3' Hectd3 Δaa110-397 reverse 5'-CTCGCCAGTGGTGCGCAC-3' Hectd3 Δaa398-511 forward 5'-TATGAGAAGCCCTTGGAC-3' Hectd3 Δaa398-511 reverse 5'-TTCCAGGCGTGGATATCG-3' Hectd3 Δaa512-861 forward 5'-TGACTCGAGTCTAGAGGG-3' Hectd3 Δaa512-861 reverse 5'-CTTGTCAGAAGGCTTGAG-3' HA-Ub K6R forward 5'-GATCTTCGTGaggACCCTGACCGGCAAGACCATC-3' HA-Ub K6R reverse 5'-TGCATGCCGCCGCCGCCG-3' HA-Ub K6,11R forward 5'-CCTGACCGGCagaACCATCACCC-3' HA-Ub K6,11R reverse 5'-GTCCTCACGAAGATCTGC-3' HA-Ub K6,11R reverse 5'-GTCCTCACGAAGATCTGC-3'	Stat3 Δaa481-574 reverse	5'-ATACCACAGGATTGATGC-3'
Stat3 Δaa671-770 forward 5'-ACGCGTACGCGGCCGCTC-3' Stat3 Δaa671-770 reverse 5'-CTCCTTGGGAATGTCGGGGTAGAGG-3' Hectd3 Δaa2-109 forward 5'-AGGAGGCAGATCCTC-3' Hectd3 Δaa2-109 reverse 5'-AGGAGGAGGCAGATCCTC-3' Hectd3 Δaa110-397 forward 5'-GGCACTGACCCAGAAGTACTATAC-3' Hectd3 Δaa110-397 reverse 5'-CTCGCCAGTGGTGCGCAC-3' Hectd3 Δaa398-511 forward 5'-TATGAGAAGCCCTTGGAC-3' Hectd3 Δaa398-511 reverse 5'-TTCCAGGCGTGGATATCG-3' Hectd3 Δaa512-861 forward 5'-TGACTCGAGTCTAGAGGG-3' Hectd3 Δaa512-861 reverse 5'-CTTGTCAGAAGGCTTGAG-3' HA-Ub K6R forward 5'-GATCTTCGTGaggACCCTGACCGGCAAGACCATC-3' HA-Ub K6R reverse 5'-TGCATGCCGCCGCCGCCG-3' HA-Ub K6,11R forward 5'-CCTGACCGGCagaACCATCACCC-3' HA-Ub K6,11R reverse 5'-GTCCTCACGAAGATCTGC-3' HA-Ub K6,11R reverse 5'-GTCCTCACGAAGATCTGC-3'	Stat3 ∆aa576-679 forward	5'-GAGGAGGCATTTGGAAAG-3'
Stat3 Δaa671-770 reverse 5'-CTCCTTGGGAATGTCGGGGTAGAGG-3' Hectd3 Δaa2-109 forward 5'-GAGCTGTGCAACGGCCAC-3' Hectd3 Δaa110-397 forward 5'-GGCACTGACCCAGAAGTACTATAC-3' Hectd3 Δaa110-397 reverse 5'-CTCGCCAGTGGTGCGCAC-3' Hectd3 Δaa398-511 forward 5'-TATGAGAAGCCCTTGGAC-3' Hectd3 Δaa398-511 reverse 5'-TTCCAGGCGTGGATATCG-3' Hectd3 Δaa512-861 forward 5'-TGACTCGAGTCTAGAGGG-3' Hectd3 Δaa512-861 reverse 5'-CTTGTCAGAAGGCTTGAG-3' HA-Ub K6R forward 5'-GATCTTCGTGaggACCCTGACCGGCAAGACCATC-3' HA-Ub K6R reverse 5'-TGCATGCCGCCGCCGCG-3' HA-Ub K6,11R forward 5'-CCTGACCGGCAGAAGACCATC-3' HA-Ub K6,11R reverse 5'-GTCCTCACGAAGATCTGC-3' HA-Ub K6,11R reverse 5'-GTCCTCACGAAGATCTGC-3' HA-Ub K6,11R reverse 5'-CGAGAACGTTGAGGGGCAAGATCC-3'	Stat3 Δaa576-679 reverse	5'-ATACTTTTCACAAGGTCG-3'
Hectd3 Δaa2-109 forward F'-GAGCTGTGCAACGGCCAC-3' Hectd3 Δaa2-109 reverse F'-AGGAGGAGGCAGATCCTC-3' Hectd3 Δaa110-397 forward F'-GGCACTGACCCAGAAGTACTATAC-3' Hectd3 Δaa110-397 reverse F'-CTCGCCAGTGGTGCGCAC-3' Hectd3 Δaa398-511 forward F'-TATGAGAAGCCCTTGGAC-3' Hectd3 Δaa398-511 reverse F'-TTCCAGGCGTGGATATCG-3' Hectd3 Δaa512-861 forward F'-TGACTCGAGTCTAGAGGG-3' Hectd3 Δaa512-861 reverse F'-CTTGTCAGAAGGCTTGAG-3' HA-Ub K6R forward F'-GATCTTCGTGaggACCCTGACCGGCAAGACCATC-3' HA-Ub K6R reverse F'-CCTGACCGCCGCCGCCG-3' HA-Ub K6,11R forward F'-CCTGACCGGCagaACCATCACCC-3' HA-Ub K6,11R reverse F'-GTCCTCACGAAGATCTGC-3' HA-Ub K6,11,27R forward F'-CGAGAACGTGaggGCCAAGATCC-3'	Stat3 Δaa671-770 forward	5'-ACGCGTACGCGGCCGCTC-3'
Hectd3 Δaa2-109 reverse 5'-AGGAGGAGGCAGATCCTC-3' Hectd3 Δaa110-397 forward 5'-GGCACTGACCCAGAAGTACTATAC-3' Hectd3 Δaa398-511 forward 5'-TATGAGAAGCCCTTGGAC-3' Hectd3 Δaa398-511 reverse 5'-TTCCAGGCGTGGATATCG-3' Hectd3 Δaa398-511 reverse 5'-TCCAGGCGTGGATATCG-3' Hectd3 Δaa512-861 forward 5'-TGACTCGAGTCTAGAGGG-3' Hectd3 Δaa512-861 reverse 5'-CTTGTCAGAAGGCTTGAG-3' HA-Ub K6R forward 5'-GATCTTCGTGaggACCCTGACCGGCAAGACCATC-3' HA-Ub K6,11R forward 5'-CCTGACCGCCGCCGCCG-3' HA-Ub K6,11R reverse 5'-GTCCTCACGAAGATCTGC-3' HA-Ub K6,11R reverse 5'-GTCCTCACGAAGATCTGC-3' HA-Ub K6,11R reverse 5'-GTCCTCACGAAGATCTGC-3'	Stat3 Δaa671-770 reverse	5'-CTCCTTGGGAATGTCGGGGTAGAGG-3'
Hectd3 Δaa110-397 forward First Structure of Structure	Hectd3 Δaa2-109 forward	5'-GAGCTGTGCAACGGCCAC-3'
Hectd3 Δaa110-397 reverse 5'-CTCGCCAGTGGTGCGCAC-3' Hectd3 Δaa398-511 forward 5'-TATGAGAAGCCCTTGGAC-3' Hectd3 Δaa398-511 reverse 5'-TTCCAGGCGTGGATATCG-3' Hectd3 Δaa512-861 forward 5'-TGACTCGAGTCTAGAGGG-3' Hectd3 Δaa512-861 reverse 5'-CTTGTCAGAAGGCTTGAG-3' HA-Ub K6R forward 5'-GATCTTCGTGaggACCCTGACCGGCAAGACCATC-3' HA-Ub K6,11R forward 5'-CCTGACCGGCAGGCAGACCATC-3' HA-Ub K6,11R reverse 5'-GTCCTCACGAAGATCTGC-3' HA-Ub K6,11,27R forward 5'-CGAGAACGTGaggGCCAAGATCC-3'	Hectd3 Δaa2-109 reverse	5'-AGGAGGAGCAGATCCTC-3'
Hectd3 Δaa398-511 forward Hectd3 Δaa398-511 reverse 5'-TTCCAGGCGTGGATATCG-3' Hectd3 Δaa512-861 forward 5'-TGACTCGAGTCTAGAGGG-3' Hectd3 Δaa512-861 reverse 5'-CTTGTCAGAAGGCTTGAG-3' HA-Ub K6R forward 5'-GATCTTCGTGaggACCCTGACCGGCAAGACCATC-3' HA-Ub K6,11R forward 5'-CCTGACCGGCGCGCCGCCG-3' HA-Ub K6,11R reverse 5'-GTCCTCACGAAGATCTGC-3' HA-Ub K6,11R reverse 5'-GTCCTCACGAAGATCTGC-3' HA-Ub K6,11,27R forward 5'-CGAGAACGTGaggGCCAAGATCC-3'	Hectd3 Δaa110-397 forward	5'-GGCACTGACCCAGAAGTACTATAC-3'
Hectd3 Δaa398-511 reverse 5'-TTCCAGGCGTGGATATCG-3' Hectd3 Δaa512-861 forward 5'-TGACTCGAGTCTAGAGGG-3' Hectd3 Δaa512-861 reverse 5'-CTTGTCAGAAGGCTTGAG-3' HA-Ub K6R forward 5'-GATCTTCGTGaggACCCTGACCGGCAAGACCATC-3' HA-Ub K6R reverse 5'-TGCATGCCGCCGCCGCG-3' HA-Ub K6,11R forward 5'-CCTGACCGGCagaACCATCACCC-3' HA-Ub K6,11R reverse 5'-GTCCTCACGAAGATCTGC-3' HA-Ub K6,11,27R forward 5'-CGAGAACGTGaggGCCAAGATCC-3'	Hectd3 Δaa110-397 reverse	5'-CTCGCCAGTGGTGCGCAC-3'
Hectd3 Δaa512-861 forward Hectd3 Δaa512-861 reverse 5'-CTTGTCAGAAGGCTTGAG-3' HA-Ub K6R forward 5'-GATCTTCGTGaggACCCTGACCGGCAAGACCATC-3' HA-Ub K6R reverse 5'-TGCATGCCGCCGCCGCCG-3' HA-Ub K6,11R forward 5'-CCTGACCGGCagaACCATCACCC-3' HA-Ub K6,11R reverse 5'-GTCCTCACGAAGATCTGC-3' HA-Ub K6,11,27R forward 5'-CGAGAACGTGaggGCCAAGATCC-3'	Hectd3 Δaa398-511 forward	5'-TATGAGAAGCCCTTGGAC-3'
Hectd3 Δaa512-861 reverse5'-CTTGTCAGAAGGCTTGAG-3'HA-Ub K6R forward5'-GATCTTCGTGaggACCCTGACCGGCAAGACCATC-3'HA-Ub K6R reverse5'-TGCATGCCGCCGCCGCGG-3'HA-Ub K6,11R forward5'-CCTGACCGGCagaACCATCACCC-3'HA-Ub K6,11R reverse5'-GTCCTCACGAAGATCTGC-3'HA-Ub K6,11,27R forward5'-CGAGAACGTGaggGCCAAGATCC-3'	Hectd3 Δaa398-511 reverse	5'-TTCCAGGCGTGGATATCG-3'
HA-Ub K6R forward 5'-GATCTTCGTGaggACCCTGACCGGCAAGACCATC-3' HA-Ub K6R reverse 5'-TGCATGCCGCCGCCGCG-3' HA-Ub K6,11R forward 5'-CCTGACCGGCagaACCATCACCC-3' HA-Ub K6,11R reverse 5'-GTCCTCACGAAGATCTGC-3' HA-Ub K6,11,27R forward 5'-CGAGAACGTGaggGCCAAGATCC-3'	Hectd3 Δaa512-861 forward	5'-TGACTCGAGTCTAGAGGG-3'
HA-Ub K6R reverse 5'-TGCATGCCGCCGCCGCCG-3' HA-Ub K6,11R forward 5'-CCTGACCGGCagaACCATCACCC-3' HA-Ub K6,11R reverse 5'-GTCCTCACGAAGATCTGC-3' HA-Ub K6,11,27R forward 5'-CGAGAACGTGaggGCCAAGATCC-3'	Hectd3 Δaa512-861 reverse	5'-CTTGTCAGAAGGCTTGAG-3'
HA-Ub K6,11R forward 5'-CCTGACCGGCagaACCATCACCC-3' HA-Ub K6,11R reverse 5'-GTCCTCACGAAGATCTGC-3' HA-Ub K6,11,27R forward 5'-CGAGAACGTGaggGCCAAGATCC-3'	HA-Ub K6R forward	5'-GATCTTCGTGaggACCCTGACCGGCAAGACCATC-3'
HA-Ub K6,11R reverse 5'-GTCCTCACGAAGATCTGC-3' HA-Ub K6,11,27R forward 5'-CGAGAACGTGaggGCCAAGATCC-3'	HA-Ub K6R reverse	5'-TGCATGCCGCCGCCG-3'
HA-Ub K6,11,27R forward 5'-CGAGAACGTGaggGCCAAGATCC-3'	HA-Ub K6,11R forward	5'-CCTGACCGGCagaACCATCACCC-3'
	HA-Ub K6,11R reverse	5'-GTCCTCACGAAGATCTGC-3'
HA-Ub K6,11,27R reverse 5'-ATGGTGTCGCTGGGCTCC-3'	HA-Ub K6,11,27R forward	5'-CGAGAACGTGaggGCCAAGATCC-3'
	HA-Ub K6,11,27R reverse	5'-ATGGTGTCGCTGGGCTCC-3'

HA-Ub K6,11,27,29R	5'-CGTGAGGGCCagaATCCAGGACA-3'
forward	
HA-Ub K6,11,27,29R	5'-TTCTCGATGGTGTCGCTG-3'
reverse	
HA-Ub K6,11,27,29,33R	5'-AATCCAGGACagaGAGGCATCCCC-3'
forward	
HA-Ub K6,11,27,29,33R	5'-CTGGCCCTCACGTTCTCG-3'
reverse	
HA-Ub K6,11,27,29,33,48R	5'-CTTCGCCGGCagaCAGCTGGAGG-3'
forward	
HA-Ub K6,11,27,29,33,48R	5'-ATCAGTCTCTGCTGGTCG-3'
reverse	
HA-Ub	5'-CAACATCCAGagaGAGAGCACCCTG-3'
K6,11,27,29,33,48,63R	
forward	
HA-Ub	5'-TAGTCGCTCAGGGTTCTG-3'
K6,11,27,29,33,48,63R	
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reverse	
	5'-GATCTTCGTGaagACCCTGACCGGCAGAACC-3'
reverse	5'-GATCTTCGTGaagACCCTGACCGGCAGAACC-3' 5'-TGCATGCCGCCGCCGCCG-3'
reverse HA-Ub K6 only forward	
reverse HA-Ub K6 only forward HA-Ub K6 only reverse	5'-TGCATGCCGCCGCCG-3'
reverse HA-Ub K6 only forward HA-Ub K6 only reverse HA-Ub K11 only forward	5'-TGCATGCCGCCGCCG-3' 5'-CCTGACCGGCaagACCATCACCC-3'
reverse HA-Ub K6 only forward HA-Ub K6 only reverse HA-Ub K11 only forward HA-Ub K11 only reverse	5'-TGCATGCCGCCGCCG-3' 5'-CCTGACCGGCaagACCATCACCC-3' 5'-GTCCTCACGAAGATCTGC-3'
reverse HA-Ub K6 only forward HA-Ub K6 only reverse HA-Ub K11 only forward HA-Ub K11 only reverse HA-Ub K27 only forward	5'-TGCATGCCGCCGCCGCCG-3' 5'-CCTGACCGGCaagACCATCACCC-3' 5'-GTCCTCACGAAGATCTGC-3' 5'-CGAGAACGTGaagGCCAGAATCC-3'
reverse HA-Ub K6 only forward HA-Ub K6 only reverse HA-Ub K11 only forward HA-Ub K11 only reverse HA-Ub K27 only forward HA-Ub K27 only reverse	5'-TGCATGCCGCCGCCGCCG-3' 5'-CCTGACCGGCaagACCATCACCC-3' 5'-GTCCTCACGAAGATCTGC-3' 5'-CGAGAACGTGaagGCCAGAATCC-3' 5'-ATGGTGTCGCTGGGCTCC-3'
reverse HA-Ub K6 only forward HA-Ub K6 only reverse HA-Ub K11 only forward HA-Ub K11 only reverse HA-Ub K27 only forward HA-Ub K27 only reverse HA-Ub K29 only forward	5'-TGCATGCCGCCGCCGCG-3' 5'-CCTGACCGGCaagACCATCACCC-3' 5'-GTCCTCACGAAGATCTGC-3' 5'-CGAGAACGTGaagGCCAGAATCC-3' 5'-ATGGTGTCGCTGGGCTCC-3' 5'-CGTGAGGGCCaagATCCAGGACA-3'
reverse HA-Ub K6 only forward HA-Ub K6 only reverse HA-Ub K11 only forward HA-Ub K11 only reverse HA-Ub K27 only forward HA-Ub K27 only reverse HA-Ub K29 only forward HA-Ub K29 only reverse	5'-TGCATGCCGCCGCCGCG-3' 5'-CCTGACCGGCaagACCATCACCC-3' 5'-GTCCTCACGAAGATCTGC-3' 5'-CGAGAACGTGaagGCCAGAATCC-3' 5'-ATGGTGTCGCTGGGCTCC-3' 5'-CGTGAGGGCCaagATCCAGGACA-3' 5'-TTCTCGATGGTGTCGCTG-3'
reverse HA-Ub K6 only forward HA-Ub K6 only reverse HA-Ub K11 only forward HA-Ub K11 only reverse HA-Ub K27 only forward HA-Ub K27 only reverse HA-Ub K29 only forward HA-Ub K29 only forward	5'-TGCATGCCGCCGCCGCG-3' 5'-CCTGACCGGCaagACCATCACCC-3' 5'-GTCCTCACGAAGATCTGC-3' 5'-CGAGAACGTGaagGCCAGAATCC-3' 5'-ATGGTGTCGCTGGGCTCC-3' 5'-CGTGAGGGCCaagATCCAGGACA-3' 5'-TTCTCGATGGTGTCGCTG-3' 5'-AATCCAGGACaagGAGGCCATCCCC-3'
reverse HA-Ub K6 only forward HA-Ub K6 only reverse HA-Ub K11 only forward HA-Ub K11 only reverse HA-Ub K27 only forward HA-Ub K27 only reverse HA-Ub K29 only forward HA-Ub K29 only forward HA-Ub K33 only forward HA-Ub K33 only reverse	5'-TGCATGCCGCCGCCGCCG-3' 5'-CCTGACCGGCaagACCATCACCC-3' 5'-GTCCTCACGAAGATCTGC-3' 5'-CGAGAACGTGaagGCCAGAATCC-3' 5'-ATGGTGTCGCTGGGCTCC-3' 5'-CGTGAGGGCCaagATCCAGGACA-3' 5'-TTCTCGATGGTGTCGCTG-3' 5'-AATCCAGGACaagGAGGCCATCCCC-3' 5'-CTGGCCCTCACGTTCTCG-3'

Primers used for PCR cloning and mutagenesis

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