

Supplementary Table S1: Antibodies

Name	Clone	Application	Product information
Anti-human/mouse NEDD8	Rabbit monoclonal [Y297] IgG	WB	Abcam/ab81264
Anti-human HSPC150/UBE2T	Rabbit monoclonal [EPR9446] IgG	WB	Abcam/ab140611
Anti-human/mouse NAE1/APPBP1	Rabbit IgG, AG6672	WB	Proteintech/14863-1-AP
Anti-human GPX4	Rabbit monoclonal [EPNCIR144] IgG	WB	Abcam/ab125066
Anti-human NEDP1	Polyclonal antibody	WB	Thermo Fisher Scientific /PA5-31033
Anti-human Ubiquitin	Monoclonal antibody [GT7811]	WB	Thermo Fisher Scientific /MA5-42345
Anti-human Cullin 1	Monoclonal antibody [2H4C9]	WB	Thermo Fisher Scientific /32-2400
Anti-human CDT1	Rabbit polyclonal antibody	WB	Proteintech 29766-1-AP-20UL
Anti-Rabbit IgG HRP-linked Antibody	Goat IgG	WB	Cell Signaling Technology/7074S
Anti-mouse IgG HRP-linked-Antibody	Horse IgG	WB	Cell Signaling Technology/7076S
Anti-human/mouse Vinculin	nVin-1, mouse IgG1	WB	Sigma Aldrich/V9131
Anti-human/mouse β -Actin (ACTBD11B7)	Mouse monoclonal IgG	WB	Santa Cruz Biotechnology/sc-81178
Anti-CRISPR-Cas9 antibody [7A9-3A3]	Mouse monoclonal IgG1	WB	ABCAM/ab191468
Anti-GAPDH–Peroxidase antibody	GAPDH-71.1, mouse IgM	WB	Sigmaaldrich/G9295
OPDIVO (nivolumab) anti-PD-1	Human monoclonal IgG4	In vitro block	Bristol-Myers Squibb
IMFINZI (durvalumab) anti-PD-L1	Human monoclonal IgG1, k	In vitro block	AstraZeneca
Anti-human HLA-ABC	W6/32, mouse IgG2a, k	In vitro block	Biolegend/311428
FITC anti-human CD38	HIT2, mouse IgG1, k	FACS	Biolegend/303504
Brilliant Violet 650 anti-human CD45	HI30, mouse IgG1, k	FACS	Biolegend/304044
APC anti-human CD55	JS11, mouse IgG1, k	FACS	Biolegend/311311
APC anti-human HLA-ABC	W6/32, mouse IgG2a, k	FACS	Biolegend/311410
PE anti-human CD119 (IFN- γ R α chain)	GIR-208, mouse IgG1, k	FACS	Biolegend/308606
FITC anti-human CD274 (B7-H1,PD-L1)	MIH2, mouse IgG1, k	FACS	Biolegend/393606
APC/Cyanine 7 anti-human HLA-DR	L-243, mouse IgG2a, k	FACS	Biolegend/307618
PerCp-Cy5.5 anti-human B2M	2M2, mouse IgG1, k	FACS	Biolegend/316315
APC anti-human CD3	HIT3a, mouse IgG2a, k	FACS	Biolegend/300312
PE anti-human CD4	OKT4, mouse IgG2b, k	FACS	Biolegend/317410
PerCp-Cy5.5 anti-human CD8	RPA-T8, mouse IgG1, k	FACS	Biolegend/301032
PE-Cy7 anti-human CD56	HCD56, mouse IgG1, k	FACS	Biolegend/318318
APC-Cy7 anti-human CD25	BC96, mouse IgG1, k	FACS	Biolegend/302614
PE anti-mouse CD4	RM4, rat IgG2a, k	FACS	Biolegend/100512
PE-Cy7 anti-mouse CD25	PC61, rat IgG1, λ	FACS	Biolegend/102016
PE anti-mouse TNF alpha	MP6-XT22, rat IgG1, k	FACS	Thermo Fisher Scientific/12-7321-81
PerCP-Cy5.5 anti-mouse CD3	17A2, rat IgG2b, k	FACS	Biolegend/100218
Brilliant Violet 650 anti-mouse CD8a	53-6.7, rat IgG2a, k	FACS	Biolegend/100742
PE-Fire 700 anti-mouse CD45	30-F11, rat IgG2b, k	FACS	Biolegend/103178
APC-Cy7 anti-mouse Ly6G	1A8, rat IgG2a, k		Biolegend/127624

PE-Dazzle 594 anti-mouse/human CD11b	M1/70, rat IgG2b, k	FACS	Biolegend/101256
APC anti-mouse F4/80	BM8, rat IgG2a, k	FACS	Biolegend/123116
PE-Cy7 anti-mouse CD206	C068C2, rat IgG2a k	FACS	Biolegend/141720
Anti-Mo CD16/CD32	93, rat IgG2a, λ	FACS , FcR blocker	Thermo Fisher Scientific/14-0161-85
Anti-mouse PD-1 (CD279)	RMP1-14, rat IgG2a, k	In vivo block	BioXcell/BE0146
Rat IgG2a isotype9	2A3, rat IgG2a k	In vivo block	BioXcell/BE0089
Anti-mouse CD8a	2.43, rat IgG2b, k	In vivo block	BioXcell/BE0061
Rat IgG2b isotype	LTF-2, rat IgG2b, k	In vivo block	BioXcell/BE0090

Supplementary Table S2: other reagents

Name	Application	Product information
QIAmp DNA Blood Maxi kit	DNA isolation	QIAGEN/51194
Lymphoprep	Cell isolation	StemCell/07851-07861
SepMate tubes	Cell isolation	StemCell/85450
CD14+ positive selection kit	Cell isolation	StemCell/17858
Red blood cell lysis buffer	Cell isolation	Biolegend/420301
RNeasy Mini kit	mRNA isolation	Qiagen/74104
Neon transfection system kit	CRISPR KO	Invitrogen/MPK10025
Alt-R® S.p. Cas9 Nuclease V3	CRISPR KO	IDT/1081058
TracrRNA	CRISPR KO	IDT/1072534
IDTE buffer	CRISPR KO	IDT/11-01-02-02
Nuclease free duplex buffer	CRISPR KO	IDT/11-01-03-01
IFN γ ELISA Kit	ELISA	Biolegend/430104
ELISA Flex: Human IFN- γ (HRP)	ELISA	MabTech/3220-1H-20
Granzyme B ELISA Kit	ELISA	MabTech/3485-1H-20
Blocker BSA (10%) in PBS	ELISA	Thermo Scientific/37525
1-Step ultra TMB-ELISA	ELISA	Thermo Scientific/34028
ELISA stop solution	ELISA	Thermo Fisher Scientific/SS04
Tween 20	ELISA/WB	Fisher bioreagents/9005-64-5
FoxP3/transcription factor staining buffer set	FACS	eBioscience/00-5523-00
Aqua fixable live/dead marker	FACS	Invitrogen by Thermo Fisher Scientific/L34966A
CellTrace violet	FACS	Invitrogen by Thermo Fisher Scientific/C34557
Tumor dissociation kit, mouse	Tissue digest	Miltenyi Biotech/130-096-730
GentleMacs C-tubes	Tissue digest	Miltenyi Biotech/130-093-237
MACS smartstrainers	Tissue digest	Miltenyi Biotech/130-110-916
Mycoplasma detection kit	Cell culture	Lonza/LT07-218
IMDM medium	Cell culture	Thermo Fisher Scientific /12440-053
Heat inactivated FBS	Cell culture	Thermo Fisher Scientific /10500-064
PenStrep	Cell culture	Thermo Fisher Scientific /15140-122
Versene	Cell culture	Thermo Fisher Scientific /15040-033
PBS	Cell culture	Thermo Fisher Scientific /20012-027
Trypan Blue Solution, 0.4%	Cell counting	VWR/K940
WB gels (NuPAGE 4-12% Bis-Tris Gel)	WB	Invitrogen by Thermo Fisher Scientific/NP0321BOX
Protein Ladder	WB	Thermo Scientific/26619
iBlot 2NC Regular Stacks	WB	Invitrogen by Thermo Fisher Scientific/IB23001
MOPS SDS Running Buffer (20x)	WB	Novex by life technologies/NP0001
Transfer Buffer (20x)	WB	Novex by life technologies/NP0006-1
LDL Sample buffer (4x)	WB	Novex by life technologies/B0007
SKIM Milk POWDER	WB	OXOID/LP0033
Ponceaus S solution for electrophoresis (0.2%)	WB	Serva/33427.01
Pico substrate	WB	Thermo Scientific/34580
Femto substrate	WB	Thermo Scientific/34095
RIPA Buffer	WB	Thermo Scientific/89900
BCA Protein Assay Kit	WB	Thermo Scientific/23225
Protease and Phosphatase Inhibitor Cocktail	WB	Thermo Scientific/78446
Dimethyl Sulfoxide (DMSO)	In vitro	Fisher bioreagents/67-68-5
ImmunoCult Human CD3/CD28 T Cell Activator	In vitro stim	Stemcell/10971
rhIFN γ	In vitro	Peprotech/AF-300-02
rhIL2	In vitro	Peprotech/AF-200-02
Pevonedistat (MLN4924)	Inhibitor	MedChemExpress/HY-70062/CS-0348
TAS4464 (hydrochloride)	Inhibitor	MedChemExpress/HY-128586A/CS-0095953

TAK-243 (MLN7243)	Inhibitor	Selleck Chemicals/S8341-2MG
Fludarabine	Inhibitor	MedChemExpress/21679-14-1
Doxorubicin (Andriamycin) HCl	Inhibitor	Selleckchem/S1208
PYR-41	Inhibitor	MedChemExpress/418805-02-41
Paclitaxel	Inhibitor	Cell signaling/9807S
pHAGE-EF1-dCas9-KRAB	Plasmid	Addgene #50919
psPAX2	Plasmid	Addgene #12260
pCMV-VSVG	Plasmid	Addgene #8454
FuGENE 6	Transfection	Promega/E2691
BsrGI	Buffer	New England BioLabs/R0575S

Supplementary Table S3: sequences

Name	Sequence
Hu NEDD8 crRNA4	<i>TGACATTGAACCTACAGACA</i>
Hu B2M crRNA1	<i>CGTGAGTAAACCTGAATCTT</i>
Hu B2M crRNA2	<i>AAGTCAACTTCAATGTCGGA</i>
Mo NEDD8 crRNA5	<i>TCTAAAGGTGGAGCGAATCA</i>
Mo NEDD8 crRNA8	<i>AGACATCGAACCCACAGACA</i>
Carrier DNA	<i>CCAGCAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCCGACAACCACT ACCTGAGCACCCAGTCCGCCCTGAGCAAAGACCCCAACGAGA</i>
NEDD8trunc gBlock in bold	<i>GTGTGGTGGAAATTCTGCAGATATCAACAAGTTTGTACgccaccATGCTAATTAAAGT GAAGACGCTGACCGGAAAGGAGATTGAGATTGACATTGAACCTACAGACAAG GTGGAGCGAATCAAGGAGCGTGTGGAGGAGAAAGAGGGAATCCCCCACA CAGCAGAGGCTCATCTACAGTGGCAAGCAGATGAATGATGAGAAGACAGCAG CTGATTACAAGATTTAGGTGGTTCAGTCCTTCACCTGGTGTGGCTCTGAGA TGAGTACAAAGTGGTTGATATCCAGCACAGTGGCG</i>
Hu NEDP1 crRNA1	<i>CCCCGTAGTCTTGAGTTACA</i>
Hu NEDP1 crRNA2	<i>GTTACATGGACAGTCTACTG</i>
Hu NEDP1 crRNA3	<i>CATCAATGATAACTCCAACC</i>
Hu NAE1/APBP1 crRNA3	<i>TCAAAGAAGCAGTATCGGCA</i>

Supplementary Table S4: Detected peptides from the proteomics assay

Sample	No. Identified proteins
Ctrl-1	2554
Ctrl-2	2520
Ctrl-3	2305
Ctrl-4	2378
NEDD8 KO-5	2295
NEDD8 KO-6	2363
NEDD8 KO-7	2383
NEDD8 KO-8	2364