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SUPPLEMENTARY INFORMATION

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Breaking the chains: deubiquitylating enzyme specificity begets function

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Supplementary Table 1: Core fitness DUBs

	Hart et al. (17) *BF ≥ 6*	Broad Avana (436) *CERES <-1*	Broad Avana (436) *CERES <-0.5*	Broad GeCKO (33) *CERES <-1*	Broad GeCKO (33) *CERES <-0.5*	Wang et al. (14) *CERES <-1*	Wang et al. (14) *CS <-0.5*	McFarland RNAi (713) *DEMETER2 <-0.5*
PSMD14	94	99	100	100	100	100	100	81
COPS6	94	91	100	55	100	36	100	95
PRPF8	88	99	100	100	100	100	100	98
USP39	88	0	44	15	100	86	100	100
USP5	82	61	99	3	94	100	100	54
COPS5	82	76	100	0	15	7	93	91
USPL1	82	15	96	91	100	0	50	59
EIF3H	71	13	93	3	85	0	36	68
PSMD7	65	100	100	100	100	14	100	99
USP36	65	58	100	0	58	0	79	1
BAP1	59	1	56	3	82	0	93	5
USP7	53	9	61	36	94	0	79	24
USP37	53	0	53	0	61	0	86	1
USP8	47	6	87	0	15	0	79	21
EIF3F	47	7	94	0	76	0	36	95
High >80%								
Low 50-80%								
No <50%								

DUBs that show the most consistent core-dependency across several high quality genome wide CRISPR-Cas9 screen analysis datasets[1-5] and a combined analysis of multiple comprehensive RNAi screens (DEMETR2)[6]. Numbers of cell lines included in each analysis are indicated in brackets. Table indicates the percentage of cells that show dependency based on a threshold score shown in italics in the column header. A high degree of dependency (>80% cell lines) is shown in red. For the Hart et al. dataset, we chose the recommended strict Bayes Factor threshold score of ≥ 6. A CERES score of -1 for Broad Avana (2018Q2)[2,4] and Broad GeCKO[1] datasets is comparable to the median of all pan-essential genes. The DEMETER2 dependency score used by McFarland et al., reflects shRNA depletion values taking off-target seed effects into account, where a score of -1 is indicative of essentiality, based on negative and positive control gene sets used for scaling[6]. The CS score used in the acute myeloid leukemia-cell focused screen (Wang et al. (ref [5])), is defined as "the average log2-fold change in abundance of all sgRNAs targeting a given gene between initial and final cell populations"[5]. Genes encoding DUBs shown in bold are contained within the Core essential gene dataset (CEG2) as defined in ref. [3]. See also Figure 4 for details of cellular function of select essential DUBs.

References

1 Aguirre, A. J. *et al.* Genomic Copy Number Dictates a Gene-Independent Cell Response to CRISPR/Cas9 Targeting. *Cancer Discov* **6**, 914-929, doi:10.1158/2159-8290.CD-16-0154 (2016).

2 Doench, J. G. *et al.* Optimized sgRNA design to maximize activity and minimize off-target effects of CRISPR-Cas9. *Nat Biotechnol* **34**, 184-191, doi:10.1038/nbt.3437 (2016).

3 Hart, T. *et al.* Evaluation and Design of Genome-Wide CRISPR/SpCas9 Knockout Screens. *G3 (Bethesda)* **7**, 2719-2727, doi:10.1534/g3.117.041277 (2017).

4 Meyers, R. M. *et al.* Computational correction of copy number effect improves specificity of CRISPR-Cas9 essentiality screens in cancer cells. *Nat Genet* **49**, 1779-1784, doi:10.1038/ng.3984 (2017).

5 Wang, T. *et al.* Gene Essentiality Profiling Reveals Gene Networks and Synthetic Lethal Interactions with Oncogenic Ras. *Cell* **168**, 890-903 e815, doi:10.1016/j.cell.2017.01.013 (2017).

6 McFarland, J. M. *et al.* Improved estimation of cancer dependencies from large-scale RNAi screens using model-based normalization and data integration. *bioRxiv*, 305656 (2018).

Supplementary Table 2: Selected DUBs mutated in human disease and/or presenting attractive therapeutic targets.

DUBs mutated in disease				
	Disease setting	**Disease association**	**Genetic alterations**	**References**
BAP1	Cancer	Renal cell carcinoma, uveal melanoma, mesothelioma	Loss of function	1-3
CYLD	Cancer, innate immune signalling	Cylindromatosis	Loss of function	4
OTULIN	Innate immune signalling and inflammation	OTULIN-related autoinflammatory syndrome (ORAS)	Loss of function	5, 6
USP8	Adenomas, cancer	Cushing's Disease	Gain of function	7
USP48	Adenomas, cancer	Cushing's Disease	Gain of function	8
AMSH (STAMBP)	Developmental disorder	Microcephaly-capillary malformation	Loss of function	9
Indirect extension of druggable proteome through DUB targeting				
	Disease		**Indirect targets**	
USP1	Cancer Inhibitor sensitises cells to cisplatin		FANCD2, PCNA	10
USP7	Cancer Neuroblastoma, immunotherapy		Regulates levels of MDM2–p53, NMYC, FOXP3	11, 12
USP9X	Cancer		Reported to stabilize the anti-apoptotic protein MCL1, regulates centrosome duplication	13, 14
USP13	Cancer		Reported to stabilize the anti-apoptotic protein MCL1 and to determine sensitivity to BH3 mimetics	15
USP28	Cancer MYC-driven tumours		FBW7 ubiquitin ligase clients (MYC, Jun etc)	16-18

PSMD14	Cancer Alternative to established proteasome inhibitors	Positive regulator of proteasome activity	19
USP14	Neurodegeneration Alzheimer disease	Limits proteasomal degradation	20-23
USP30	Neurodegeneration Parkinson disease	Suppresses mitophagy	24-27
USP19	Muscle atrophy	Promotes atrophy through enhancement of glucocorticoid signalling	28

References

1 Harbour, J. W. *et al.* Frequent mutation of BAP1 in metastasizing uveal melanomas. *Science* **330**, 1410-1413, doi:10.1126/science.1194472 (2010).

2 Testa, J. R. *et al.* Germline BAP1 mutations predispose to malignant mesothelioma. *Nat Genet* **43**, 1022-1025, doi:10.1038/ng.912 (2011).

3 Pena-Llopis, S. *et al.* BAP1 loss defines a new class of renal cell carcinoma. *Nat Genet* **44**, 751-759, doi:10.1038/ng.2323 (2012).

4 Bignell, G. R. *et al.* Identification of the familial cylindromatosis tumour-suppressor gene. *Nat Genet* **25**, 160-165, doi:10.1038/76006 (2000).

5 Damgaard, R. B. *et al.* The Deubiquitinase OTULIN Is an Essential Negative Regulator of Inflammation and Autoimmunity. *Cell* **166**, 1215-1230 e1220, doi:10.1016/j.cell.2016.07.019 (2016).

6 Zhou, Q. *et al.* Biallelic hypomorphic mutations in a linear deubiquitinase define otulipenia, an early-onset autoinflammatory disease. *Proc Natl Acad Sci U S A* **113**, 10127-10132, doi:10.1073/pnas.1612594113 (2016).

7 Reincke, M. *et al.* Mutations in the deubiquitinase gene USP8 cause Cushing's disease. *Nat Genet* **47**, 31-38, doi:10.1038/ng.3166 (2015).

8 Chen, J. *et al.* Identification of recurrent USP48 and BRAF mutations in Cushing's disease. *Nat Commun* **9**, 3171, doi:10.1038/s41467-018-05275-5 (2018).

9 McDonell, L. M. *et al.* Mutations in STAMBP, encoding a deubiquitinating enzyme, cause microcephaly-capillary malformation syndrome. *Nat Genet* **45**, 556-562, doi:10.1038/ng.2602 (2013).

10 Liang, Q. *et al.* A selective USP1-UAF1 inhibitor links deubiquitination to DNA damage responses. *Nature chemical biology* **10**, 298-304, doi:10.1038/nchembio.1455 (2014).

11 van Loosdregt, J. *et al.* Stabilization of the transcription factor Foxp3 by the deubiquitinase USP7 increases Treg-cell-suppressive capacity. *Immunity* **39**, 259-271, doi:10.1016/j.immuni.2013.05.018 (2013).

12 Tavana, O. *et al.* HAUSP deubiquitinates and stabilizes N-Myc in neuroblastoma. *Nat Med* **22**, 1180-1186, doi:10.1038/nm.4180 (2016).

13 Schwickart, M. *et al.* Deubiquitinase USP9X stabilizes MCL1 and promotes tumour cell survival. *Nature* **463**, 103-107, doi:nature08646 [pii] 10.1038/nature08646 (2010).

14 Li, X. *et al.* USP9X regulates centrosome duplication and promotes breast carcinogenesis. *Nat Commun* **8**, 14866, doi:10.1038/ncomms14866 (2017).

15 Zhang, S. *et al.* Deubiquitinase USP13 dictates MCL1 stability and sensitivity to BH3 mimetic inhibitors. *Nat Commun* **9**, 215, doi:10.1038/s41467-017-02693-9 (2018).

16 Popov, N. *et al.* The ubiquitin-specific protease USP28 is required for MYC stability. *Nat Cell Biol* **9**, 765-774 (2007).

17 Diefenbacher, M. E. *et al.* The deubiquitinase USP28 controls intestinal homeostasis and promotes colorectal cancer. *J Clin Invest* **124**, 3407-3418, doi:10.1172/JCI73733 (2014).

18 Schulein-Volk, C. *et al.* Dual regulation of Fbw7 function and oncogenic transformation by Usp28. *Cell reports* **9**, 1099-1109, doi:10.1016/j.celrep.2014.09.057 (2014).

19 Li, J. *et al.* Epidithiodiketopiperazines Inhibit Protein Degradation by Targeting Proteasome Deubiquitinase Rpn11. *Cell Chem Biol*, doi:10.1016/j.chembiol.2018.07.012 (2018).

20 Lee, B. H. *et al.* Enhancement of proteasome activity by a small-molecule inhibitor of USP14. *Nature* **467**, 179-184, doi:nature09299 [pii] 10.1038/nature09299 (2010).

21 Homma, T. *et al.* Ubiquitin-specific protease 14 modulates degradation of cellular prion protein. *Scientific reports* **5**, 11028, doi:10.1038/srep11028 (2015).

22 McKinnon, C. *et al.* Prion-mediated neurodegeneration is associated with early impairment of the ubiquitin-proteasome system. *Acta neuropathologica* **131**, 411-425, doi:10.1007/s00401-015-1508-y (2016).

23 Wang, Y. *et al.* Small molecule inhibitors reveal allosteric regulation of USP14 via steric blockade. *Cell Res*, doi:10.1038/s41422-018-0091-x (2018).

24 Bingol, B. *et al.* The mitochondrial deubiquitinase USP30 opposes parkin-mediated mitophagy. *Nature* **510**, 370-375, doi:10.1038/nature13418 (2014).

25 Cunningham, C. N. *et al.* USP30 and parkin homeostatically regulate atypical ubiquitin chains on mitochondria. *Nat Cell Biol* **17**, 160-169, doi:10.1038/ncb3097 (2015).

26 Liang, J. R. *et al.* USP30 deubiquitylates mitochondrial Parkin substrates and restricts apoptotic cell death. *EMBO Rep* **16**, 618-627, doi:10.15252/embr.201439820 (2015).

27 Marcassa, E. *et al.* Dual role of USP30 in controlling basal pexophagy and mitophagy. *EMBO Rep* **19**, e45595, doi:10.15252/embr.201745595 (2018).

28 Coyne, E. S. *et al.* Knockout of USP19 Deubiquitinating Enzyme Prevents Muscle Wasting by Modulating Insulin and Glucocorticoid Signaling. *Endocrinology* **159**, 2966-2977, doi:10.1210/en.2018-00290 (2018).