

## Supplementary Information

### METHODS

**Plasmids.** pTXB3-Strep-TEV-HA-SUMO1-Intein-CBD and pTXB3-Strep-TEV-HA-SUMO3-Intein-CBD were generated by inserting a Strep-TEV-HA tag followed by SUMO1 or SUMO3 (both lacking the last Gly residue) into pTXB3 (New England Biolabs). This generated an in-frame fusion with the intein and chitin binding domain of the vector. USPL1 was amplified from HeLa cDNA and cloned via BamHI and XhoI restriction site into pcDNA3.1 HA [27]. siRNA resistant USPL1C236A and USPL1C236S were generated by site-directed mutagenesis using the HA-USPL1\_pcDNA3.1 construct as a template. YFP-USPL1 was generated in pEYFP C1 (Clontech) via XhoI and BamHI sites. USPL1cat (212-514) was amplified and cloned into NcoI and XhoI sites of pETM30 (EMBL-Heidelberg, Protein Expression Facility). Full length cDNA for USP5 (IRAU969E0578D) was obtained from the German Resource Center for Genome Research (RZPD). USP5 was cloned via BamHI and XhoI site into pET28a (Novagen). preSUMO1 and preSUMO2 were amplified by PCR and cloned into the NdeI and BamHI site of pET11a (Novagen). pEYFP C1 USPL1cat (212-514) wt and C236S were generated by PCR amplification of the fragments from pETM30 USPL1cat (212-514) wt and C236S and cloned into the XhoI and BamHI sites of pEYFP C1. Constructs for SUMO, SUMO enzymes, USP25, and Ubiquitin have been described [27-31]. Bacterial expression constructs for human untagged RanGAP1 and His-YFP-SP100 (71-480) are described in [15]. Full length C13orf22l was amplified from zebrafish cDNA and cloned via BamHI and XbaI into pCS2+ (RZPD) and pcDNA3.1-Flag. pRSFDuet1 His-MBP-TEV-C13orf22lcat (312-649) was cloned via BamHI and NotI into the first MCS of a modified vector backbone from pRSFDuet1 (Novagen), containing a N-terminal His-MBP tag, followed by TEV cleavage site. USPL1cat and C13orf22lcat variants were generated by site-directed mutagenesis. All DNA constructs were confirmed by sequencing.

**siRNA Oligos:** USPL1 siRNA-s1: 5'-GAATGAAGTTAGAGATGAA-3', USPL1 siRNA-s2: 5'-CAATAGAGGACTTGTTAAA-3'. Non-targeting siRNA was from Applied Biosystems.

**Protein purification, sumoylation and binding assays.** His-GST-TEV-USPL1cat (212-514) wt and C236S and His-MBP-TEV-C13orf22lcat (312-649) were transformed into *E. coli* Rosetta 2, induced with IPTG, grown at 16°C o/n and lysed in 50 mM Tris (pH 8.0), 100 mM NaCl, 1 mM DTT and protease inhibitors (USPL1cat wt and C236S) or 20 mM Tris pH 7.5, 200 mM NaCl, 1 mM EGTA, 1 mM DTT and protease inhibitors (C13orf22lcat). Enrichment over Glutathione-Agarose 4B (Macherey-Nagel) or amylose resin (NEB) followed standard procedures. His-GST-TEV-USPL1cat and His-MBP-TEV-C13orf22lcat were incubated with

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His-tagged TEV protease prior to final purification of untagged fragments via gel filtration (Superdex S75 or S200, GE Healthcare). His-GST-TEV-USPL1cat wt and mutants were purified as described above without TEV cleavage and gel filtration. Protocols for SUMO, Aos1/Uba2, Ubc9, GST-SENP1cat, His-Senp2cat, RanBP2 $\Delta$ FG, USP25, Ubiquitin, RanGAP1, CFP-RanGAPtail (aa 400-589), YFP-SUMO2 and His-YFP-SP100 (71-480) purifications and *in vitro* sumoylation have been described [27-30]. Purification of His-USP5 was accomplished by Nickel pulldown and gel filtration. His-SUMO3 chains generation followed the procedure described in [32], however without purification via Nickel-Agarose. All proteins were stored in TB buffer (20 mM HEPES/KOH pH 7.3, 110 mM K-acetate, 2 mM Mg-acetate, 1 mM EGTA, 1 mM DTT, supplemented with 1  $\mu$ g/ml each of aprotinin, leupeptin and pepstatin) at  $-80^{\circ}\text{C}$ . Binding assays involving SUMO and Ubiquitin immobilized on CNBr activated sepharose and recombinant USPL1cat were as previously described for USP25 [27].

**Antibodies.** Goat anti-USPL1 was raised against His-GST-TEV-USPL1cat (212-514), and affinity-purified against immobilized untagged USPL1cat (aa 212-514). Anti-Flag (M2), anti- $\beta$ -actin (Clone AC-15) and anti- $\alpha$ -tubulin mouse monoclonal antibodies were from SIGMA Aldrich, anti-HA (MMS-101P) from Covance, anti-GFP (sc-8334), anti-Ubiquitin (P4D1), anti-UBF (#sc-13125) and anti-Coilin (#sc-32860) from Santa Cruz Biotechnology. SUMO species were detected using anti-SUMO2/3 from Eurogentec or the supernatant of hybridoma cell lines SUMO1 (21C7) and SUMO2 (8A2) obtained from the Developmental Studies Hybridoma Bank at the University of Iowa. Anti-zebrafish coilin (9EA2) was kindly provided by Dr. Neugebauer [26]. Secondary antibodies were from Molecular Probes and Invitrogen.

**Immunofluorescence** analysis of untransfected and transfected HeLa cells was performed upon 3.7% formaldehyde fixation and 0.2% Triton permeabilization, using an Axioskop2 (CellObserver, Zeiss).

**Synthesis of Strep-TEV-HA-SUMO1/3-Vme.** Design, synthesis and purification of Strep-TEV-HA-SUMO1/3-Vme involved intein ligation, similar to [11]. Strep-TEV-HA-SUMO-intein-CDB was expressed in *E. coli* BL21(DE3). Cells resuspended in 20 mM HEPES pH 6.5, 50 mM NaCl and protease inhibitors (1  $\mu$ g/ml each of aprotinin, pepstatin, leupeptin and 1 mM PMSF) were lysed (EmulsiFlex C3; Avestin), and clarified lysates were loaded onto chitin beads (New England Biolabs). Bound proteins were incubated with 50 mM  $\beta$ -mercaptoethanesulfonic salt (MESNa) o/n at RT. Strep-TEV-HA-SUMO1/3-MESNa was eluted with 50 mM MESNa and purified by gel filtration (Superdex S75, GE Healthcare) in 20 mM HEPES pH 7.5. To 1 ml of Strep-TEV-HA-SUMO-MESNa (~2 mg/ml), 150  $\mu$ l N-hydroxysuccinimide (2 M) and 250  $\mu$ l glycinevinylmethylester-tosyl (1 M) were added and pH

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was adjusted to 8.0. After 2 h at 37°C, the reaction mixture was neutralized with HCl, desalted, and applied to MonoQ (GE Healthcare). Strep-TEV-HA-SUMO-Vme was stored at -80°C.

**Identification of Sumo isopeptidases using HA-SUMO1/3-Vme.** HeLa extracts (100,000 g supernatants with 10 mg/ml protein) were prepared from frozen cells in TB buffer (20 mM HEPES/KOH pH 7.3, 110 mM K-acetate, 2 mM Mg-acetate, 1 mM EGTA, 1 mM DTT, supplemented with 1 µg/ml each of aprotinin, leupeptin and pepstatin). For small scale experiments, 75-600 ng SUMO1-Vme and 250-2000 ng SUMO3-Vme were incubated with 1.5 ml cell extract for 30 min at 37°C. After centrifugation and pre-clearing with Protein-A-agarose (Roche), 20 µl anti-HA-agarose (Sigma Aldrich) was added to the supernatants for 2 h at 4°C. Bound proteins were eluted with HA peptide (0.2 mg/ml, Biomol) for 30 min at 30°C. For identification of SUMO-Vme labeled proteins by mass spectrometry, 25 ml HeLa cell extract (250 mg proteins) were first incubated with 10 µg SUMO1-Vme. After harvesting crosslinked proteins with 120 µl anti-HA-Agarose, the supernatant was incubated with 10 µg of SUMO3-Vme. Proteins bound to SUMO1-Vme and SUMO3-Vme were eluted from the beads with 250 µl HA-peptide, and separated by SDS-PAGE.

**Transfection experiments.** To study the reactivity of full length USPL1 and C13orf22l towards SUMO-VME, HEK 293T cells were transfected with pEYFP C1 USPL1 and pcDNA3.1 Flag-C13orf22l constructs using calcium phosphate. 24 hours after transfection of pEYFP C1 USPL1 and 48 hours after transfection of pcDNA3.1 Flag-C13orf22l, cells were lysed on ice in 50 mM Tris pH 7.4, 250 mM NaCl, 5 mM EDTA, 0.5% Triton X-100, supplemented with 1 mM DTT and protease inhibitors. USPL1 containing lysate (400 µg total protein) was incubated with 3 µg SUMO-Vme for 30 min at 30°C; C13orf22l lysate (100 µg total protein) was incubated with 2 µg SUMO-Vme for 30 min at 37°C. Reactions were analyzed by immunoblotting using the indicated antibodies. To study the influence of USPL1cat on cellular SUMOylation and Ubiquitination pattern, HEK 293T cells were transfected with pEYFP C1, pEYFP C1 USPL1cat (212-514) wt or C236S using jetPRIME (Polyplus transfection). After 24 h cells were washed twice with PBS supplemented with 10 mM N-ethylmaleinimide (NEM) prior to lysis in SDS sample buffer. 40 µg of total protein lysates were separated by SDS PAGE (5-20% gradient gels, 15% gels for detection of free SUMO), and analyzed by immunoblotting using the indicated antibodies.

**siRNA and siRNA/rescue experiments.** A) Proliferation upon siRNA knockdown: HeLa cells were transfected with non-targeting siRNA (mock) or Usp1l siRNA (s1 or s2) using Lipofectamine RNAimax (Invitrogen). Proliferation was measured at the indicated times with the Cell Titer-Glo® Luminescent Cell Viability Assay (Promega, Madison, WI, USA). B) Proliferation upon rescue: seven hours post transfection, cells depleted of USPL1 with siRNA

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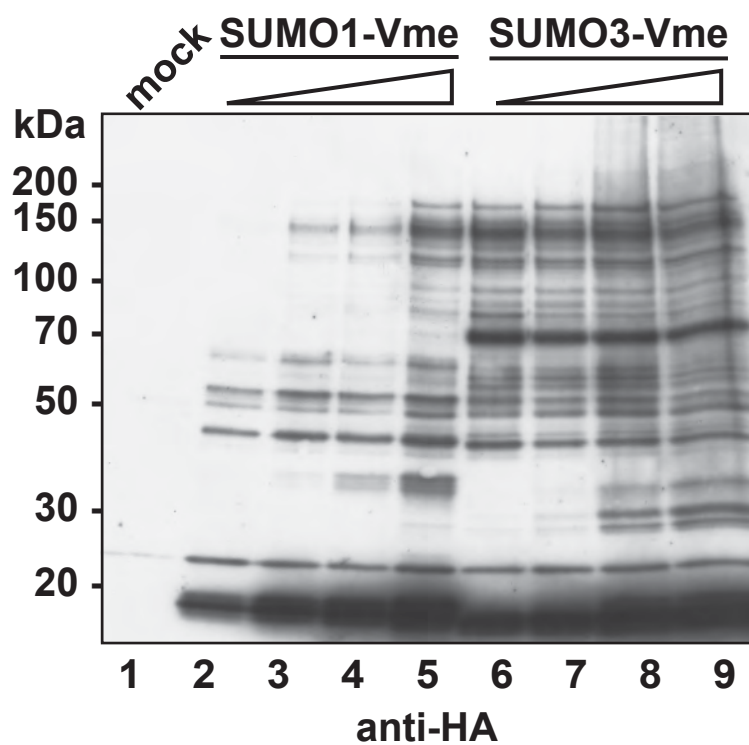
s2 were retransfected with pcDNA3.1-based plasmids encoding siRNA-resistant versions of wildtype HA-Usp11, catalytically inactive HA-Usp11 C236A or empty vector (HA-tag only) using Jetprime transfection agent (Polyplus). 24 hours after siRNA-treatment, cells were plated into 96-well plates (4000 or 8000 cells per well; five replicates per time point and condition) and relative cell numbers were determined at 48, 72 and 96 hours post siRNA-treatment using a colorimetric assay to measure dehydrogenase activity (CCK-8 cell counting kit (Dojindo). For each time point, samples were normalized to a control (a second mock transfection sample), analysed at the same time-point. Proliferation relative to this control (set to 100%) was calculated. C) Coilin distribution upon rescue: For CB observation, s2 Usp11-depleted samples were retransfected 12-24 hours post transfection with indicated rescue constructs and fixed for observation after additional 24 hours.

#### **Quantitative analysis of SUMO isopeptidase activity:**

Rates of desumoylation were analysed in a FRET-based *in vitro* assay as previously described [33]. In brief, Usp11cat and Senp2cat were added to *in vitro* generated conjugate CFP-GAPtail\*YFP-SUMO2 in 384 well plates, and fluorescence emission at 535nm and 480nm (excitation at 430 nm) was followed over time (Appliskan microplate reader; Thermo Fisher Scientific). A decrease in FRET ratio (535nm/480nm) represents desumoylation. All measurements were at 37°C in 20 mM Hepes pH 7.3, 110 mM potassium acetate, 2 mM Mg acetate, 0.5 mM EGTA, 1 mM DTT, 0.05 % Tween20 and 0.2 mg/ml ovalbumin. Substrate concentrations ranged from 50 - 600 nM, enzyme concentrations from 0.01 - 5 nM.

#### **REFERENCES not included in the main text**

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**Figure S1.** SUMO-Vme reacts with numerous proteins in HeLa cell extract. Extracts were incubated with increasing amounts of SUMO1-Vme and SUMO3-Vme, and crosslinked proteins were enriched by anti-HA-IP. Detection was by immunoblotting with anti-HA antibodies.

Human LCQWKNAYALCWLDCITLAVHSEELKNT....VTGLCSKEESIFWRLLTRKNOANTLITYTSQLSGVKDGDCKK...LTSEIFAETETLNEVRDEIFISLQPOLRCLLGD  
Mouse LCQWKNPQALCWLDCITLAVHLEVLKRT....VLEACSREECVGRFFEMVHQADELHHTHLHGVTGEDCKK...LTSEIFTEIDTGLNVRDEIFAKLQPKLRCLLGD  
Chicken LCQWRNKYNLCWLDCITLAVHLEALRFA....LVEEDDEKCLORLTKRYSQATVLLNTCKRG.....KVKDILPKABELLNEINQIFIQLOPKLCKELGK  
Xenopus KLQWRNPLFLCWLDCITLAAVHSAFNSI....VAQRVAESSIHLHLRKYKANALTKSTRRG.....RPNKLSQENITLKVRMITFDEIKPLKCLLGD  
Zebrafish SELEFNKNEENMCWLDAIMVHVCRTTGT.9..KLATVPVNDVWKLQWRVDTKTCAYLQARKKQSEDKVLRL....VPAGVIVVEARRRISALRSLVFKLLQPTLKCETGO  
Lancelet STLQWVNAHSLCWLDAAMCMLVHSRTLHDR....LSQLNGNGESTVKTTLHLAQOQAEELLSNYQKARKTPSGDRR.16.EEMICIERATELTHGVRQKVFESLRFPLKCELEK  
Lottia LPHQWQNDPAMCWLDCITLAVHSPFLQEK....AKTWANCVEGMNKLCDESVSVSDIITKTPKHHVSESKSET..5.TPMKEDIKSIARLEEDVREVWMDLQPRKLCCKEK  
Nematostella EYIOWNNSALCWLDCITLAVFNKSTLTL....TSKISSGSRATLQEVYTKALDITMHDQN.....KANNLLSDVROSVDWVLRPKMKCKLGV  
Capitella SPLOWNVDLVCWLDCITLAVHQLLSDL....MTSCSKNTVHRMTSIVSKAQVLRKGLQLNRCLDFCKR.56.LATREDQQRALWDLREDMWRLEDFSLRCMRGO  
Sea\_urchin GIOWNNESSCWLDCITLAVQFRSLRRC....HQALGATSPRKICDGVLAQEVASADVEAIKKNLSEQ.98.NSTGKLAAAVESTKTLRKLFLMELKELGCRENK  
Hydra KYPFNENDKLCLNLTSLTLNTHQKH.....IFEKRQCFVSYLCEEVAKIQSYVQTKNSYS.....HARKCQELRYTTLKFLPEARLFAKYKD

USP2 GLAGLRLNLGNTCFMNSLQOCSNTRERDYCLQRLMYRDLHHGNSAHTAVVEFAKLIQVWTSSPNDVVSPEFK..5.KAPRFVGYNQDAQDFLRFLLDGHNEINRVTLR  
USP14 LPCGLTNLGNTCKMNTQOCSRVPFLKDALKRYAGALRASGEMASQYTAALRDLFDSDKTSSSLQPLHMAP..4.EKGEQGGVQDANECWIOIMRVLQOKLEAIEDD  
USP7 GYVGLKNGCATCMNSLQTFPTNOLRKA....VYMMPTEGDSSKSVPLALQRFVYELQHSDDIPPIIKPVGTK..6.GWETLDSFMQHDVQELCRVLDLNVENKIKGTCVE  
USP21 GHVGLRLNLGNTCLNLAVALOCSSTRPLRDFCLRR.DFROEVPGGGAQELTSAFADVIGALWHPDSCSAVNPTRFR..5.YVPFSFGYSQDAQDFLKLMLERHLEINRRGR

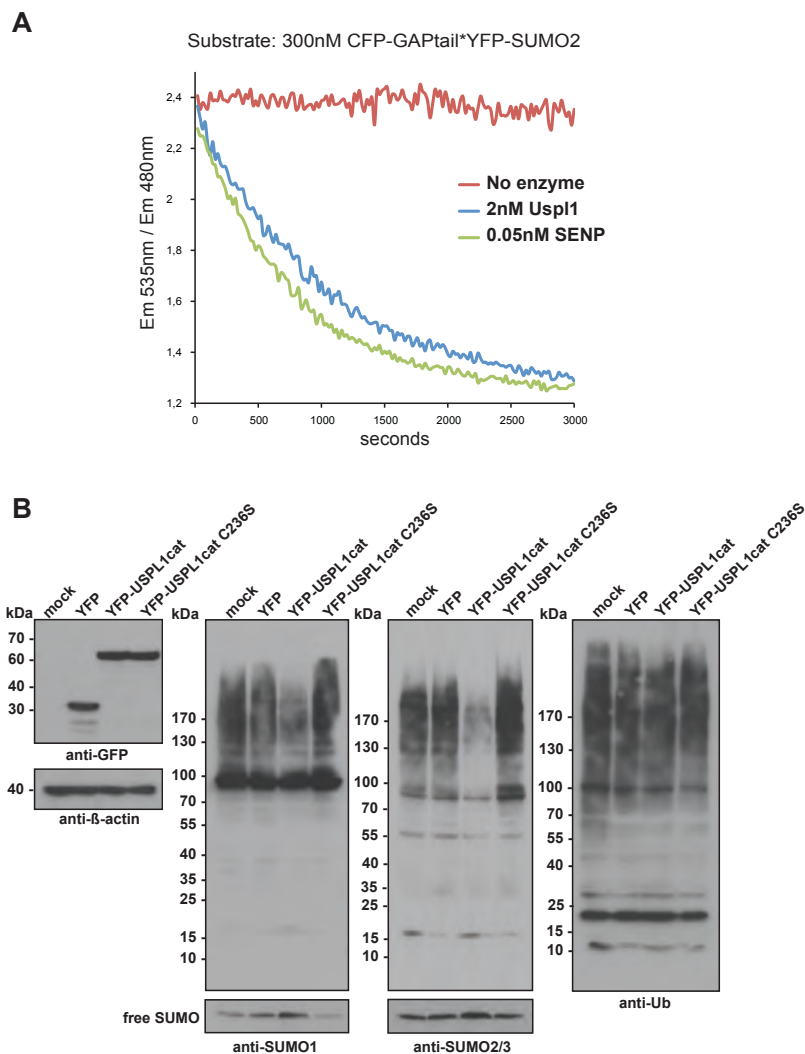
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Mouse ...MESPVFALPLLLKLEPHVSLFTYFSWNEFCSHGCHYOYQNCVKSLVTFINVIPWEHPLNAA...HFGPCNSNSKSO.IRKMVLEBRAS...PFLMLHFVEGLP...  
Chicken ...MESPVFALPLLLKQOHAELKLSHFSNKEFCMCCGKYKLDLCKRKLTFINNIIPDWHPLNAV...HVAPNNSDRSQ.RKQMLLKVPL...SILMLHFVEGLP...  
Xenopus ...MESPVFALPLLLKQVPELSCFVHSGVNEFTCKLGGYOYQHQTAVSFFKILPEWTPPLAI...HKAPCNKLCGTDQ.IFAERIKKH...D.FILHVFVEGLP...  
Zebrafish ...QEPVVFALPLLLRSKWAQDITQHTIRWBEKQPCDFVNESVERKLTLLCIVKDWHPKLAN...NRTOCNKCEHKNQ.RKMKVLEKLS...SVFALHFVEGLP...  
Lancelet ...MESPVFALPLLLRENSLITEDLRMEYDWEFSODKCGFKTDRMTRVLPFPFSVTPDFSLQAC...HLRSCFSGSPEQ.KMKMVFNRLE...PFVMLHFVGLS...  
Lottia ...NDSPVFALPLLLRESAELKMFSEIYSLVGHCAKCDVROENFKQALPSLNPVADPFSMKAPA...FVRNCFRCEAPGO.KMEKITHRMO...D.FLMLHFVGLP...  
Nematostella ...NDSPVFALPLLLRENSVTSQTLQEQYVWGHNECGSEALRKKHVVVTPNTDPTLPEAT...FVKTCPKGSHSQ.KSNLVYDKAP...SVLFLHFVEGLR...  
Capitella ...KDSPVFALPLLLNE...VEKDIALSYTRMVCORSHSHVDVPOVIVPLPSTPDSFMSQPC...FIRSCVHKQPGQ.RMNVHINRMP...ASFFVHFVEGLH...  
Sea\_urchin ...PESLISALPMLLLRGLVTEHNLRRRESYCFSCCSNVTIHRNVIASVSTSNDSMQNLTL...MHHECPQKAPGO.TSRLIYHSLP...ACPLIHFVGLSST...  
Hydra ...GEPESICLPLLLKTLRLRYEKVKVNSLQKQCNQOVGSESFQKVMITLPHADTLKFGNLS...FKRITCSNHGTO.DTTVLERYE...P.MFCHFLQGVHN...

USP2 .14.EKGRQMWKRYLFRPESRIGDLFVGLKSSLTCDGCVYCVSTVPDFPFDLSPPIAKRCRPEVTLN.15.EKPTCORGRKRRCIKFSTQRFKLLVHUKRFSRSRIR.13  
USP14 .4.TDSSASATPSSKKSLIQFGVEFETTMKCFESEEEVTKGKNOQLCFINQEVKLYFT.10.TKQSPTLQRNALY.ISSKLSRLPAYLTHQWRVPEYKEKE.58  
USP7 .....GTPKLRGKMVSYIQCKEVDVRSRREDYDYLQSLIKGKNIFESFV.11.NKYDAGEHGLQEA.EKGVKFLTLPEVLHQLMRVYDPPQ.12  
USP21 .29.DRANLWVKRYLFRPESRIGDLFVGLKSSLTCDGCVYCVSTTFEVPFDLSPPIPKKQVAGGKVS.17.NAPVDRQKTRSTKATVQRFPRILVHUNRSASRGS.11

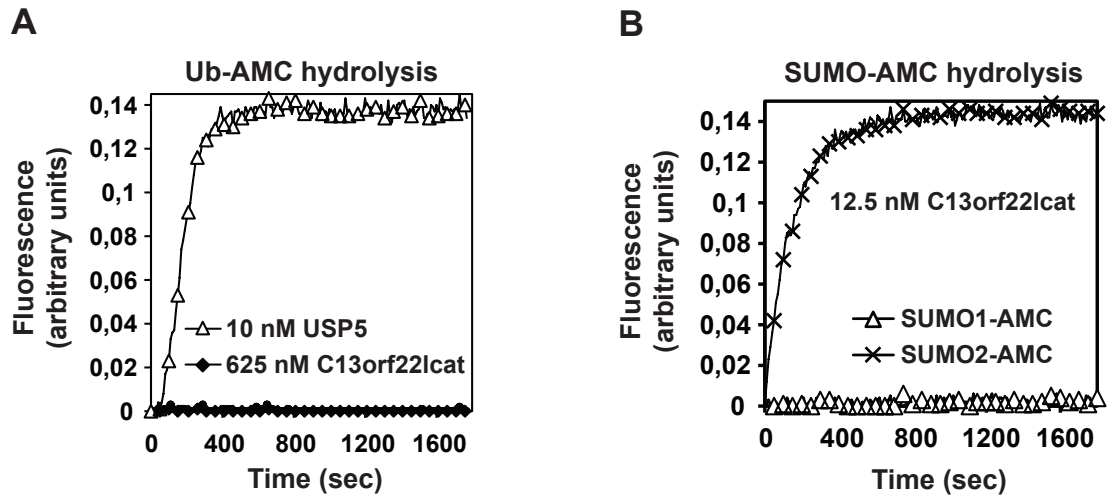
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Mouse RRDLDQHYAFHF...EGSLVQVTSVIOY...QAN.NHFTIWLFDADG.SWLECDLKGPCAK.RHVTCV...PASETHIVIEWEK  
Chicken HNKIKSYSPQL...EEDVQVTSVIOY...AVHKKHFTIWSLNPDC.TWLECDLKGPCAR.RHKRFEV...PASETHIVIEWEK  
Xenopus SNDLGMYSFHF...EGCLVQVTSVIOY...SEDFHSSWIANEDG.TWLESDDLKGYFCI.KQNCFEV...PASETHIVIEWEK  
Zebrafish RKDLTKYGFTH...QGFQVSVTSVIOY...NKHLOHFVWVWVQSNQ.FWLEDDLKHPYSP.THKRLPF...PSSEHILWEITD  
Lancelet HNSLEELCTY...QDROVRSVAVIRV...KTHPDHFVYIINDTQGNRWLKCDDLSHVPCRWQHSPPS...PESQIHIVAWELQ  
Lottia KVVYDELDFEF...EGSLVQVTSVIOY...KNNPDHFVSWLNPQCNQWMECDLKSPLCFENAPPR...PESQIHIVIEWEK  
Nematostella SEGYHNSFN...LDQPRVTOVIOY...KHNPDHFVQNTQNNQSKWMELDLKSVCVWSDIPTV...PESOVHILCEPL  
Capitella HSQTDYDFCH...GDDQVAVKGVIOF...KKCPDFVAVIRNPVENTWMECDLKGCTLRFASQPP...LPSQHILLWERQ  
Sea\_urchin TELPWLODFRH...RGQVHLSCHVIOY...VRNPNHFVWILDPSRQWLKCGLSSPVCVWWSQEPS...PSETHLVGWERQ  
Hydra QMYWKLDFSTTP...SGERKVVTOVIOY...VNKRHFLLWFSHLEKLMCADDDNDVTEYKFAIPQC...PASEVYLLWKEV

USP2 LRLDLRDFAS.3.NHAVNVAYSVHSG.TTMGGHTAACSPGTGEMHTFNDSSVTPMSSSQV.....RTSDAYLLEVELA  
USP14 QKEVYVEPFSF.7.NCGYDQAVLTHQGRSSSSGHVSVVVRKQD.EWIKPDDQVSVITVPEDILRLS..3.DWHIAYVLLYGR  
USP7 PEQLPLDPLQ.5.DPANNIHFHVLVHSG.DNHGGHYVVIINPKGDGKWKCFDDVVSRCFTEEAIEHN.11.HCINAYLVVYHRE  
USP21 LQRLSLGDFAS.3.GSPVQVYALCCHSG.SVHYGHVYALCQTC...WHVYVNDSEVSPVSNQV.....ASSEGYLVVYQLM

**Figure S2.** Multiple alignment of USPL1 catalytic domain. The catalytic domains of representative vertebrate and invertebrate USPL1 homologues were aligned and compared to four structurally characterized human USP enzymes. Invariant or conserved residues are shown on black or grey background, respectively. Some important residue groups are highlighted in color: catalytic triad (red); internal zinc finger also present in some USPs (yellow) [20, 21].

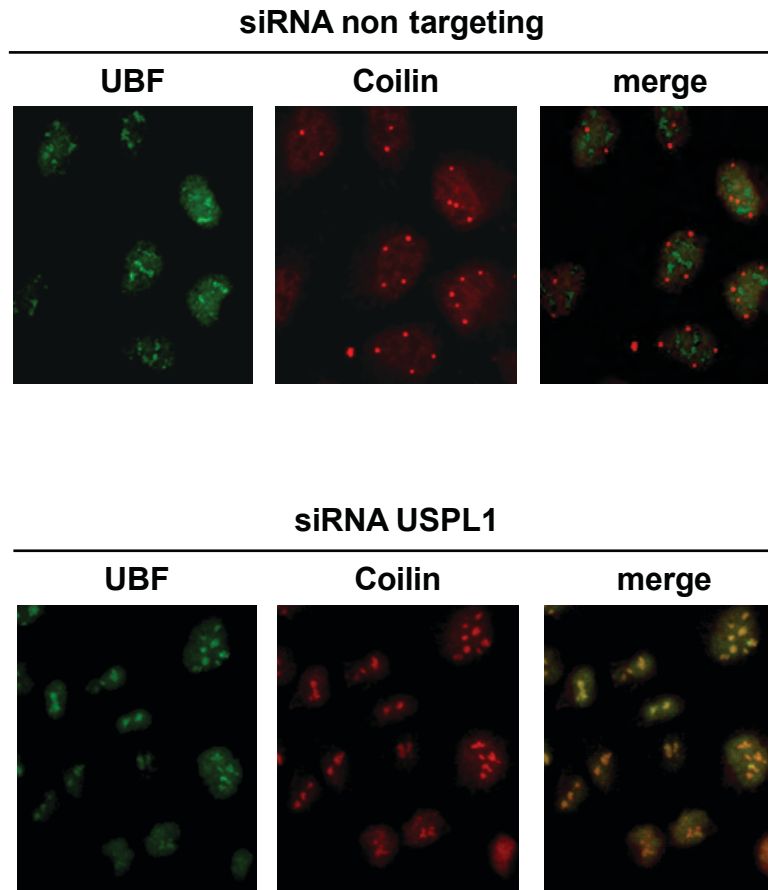


**Figure S3.** USPL1 is an isopeptidase *in vitro* and *in vivo*. (A) Comparison of USPL1 and SENP2 activity in a FRET – based *in vitro* assay. USPL1cat and His-Senp2cat were added to 300 nM CFP-GAPtail\*YFP-SUMO2 and fluorescence emission was monitored at 535 nm and 480 nm after excitation with 430 nm. Ratio of emissions at 535 nm and 480 nm was plotted over time, a decrease reflects desumoylation. Note that different enzyme concentrations were used. To compare enzyme efficiencies, we repeated measurements at different substrate and enzyme concentrations (not shown). From this, we determined  $k_{cat}/K_M$  values for USPL1 ( $4 \times 10^5 \text{ Mol}^{-1}\text{s}^{-1}$ ) and Senp2 ( $3 \times 10^7 \text{ Mol}^{-1}\text{s}^{-1}$ ), indicating that the recombinant USPL1 fragment is an efficient isopeptidase, yet two orders of magnitude less efficient than recombinant Senp2 fragment. (B) USPL1cat deconjugates SUMO but not Ubiquitin targets in cells. HEK293T cells were transfected with YFP empty vector, YFP-USPL1cat wt or C236S and lysed in 2x SDS-sample buffer 24h after transfection. 40  $\mu\text{g}$  of each cell lysate were analyzed by immunoblotting using anti-GFP, anti- $\beta$ -actin, anti-Ubiquitin, anti-SUMO1 and anti-SUMO2/3 antibodies.

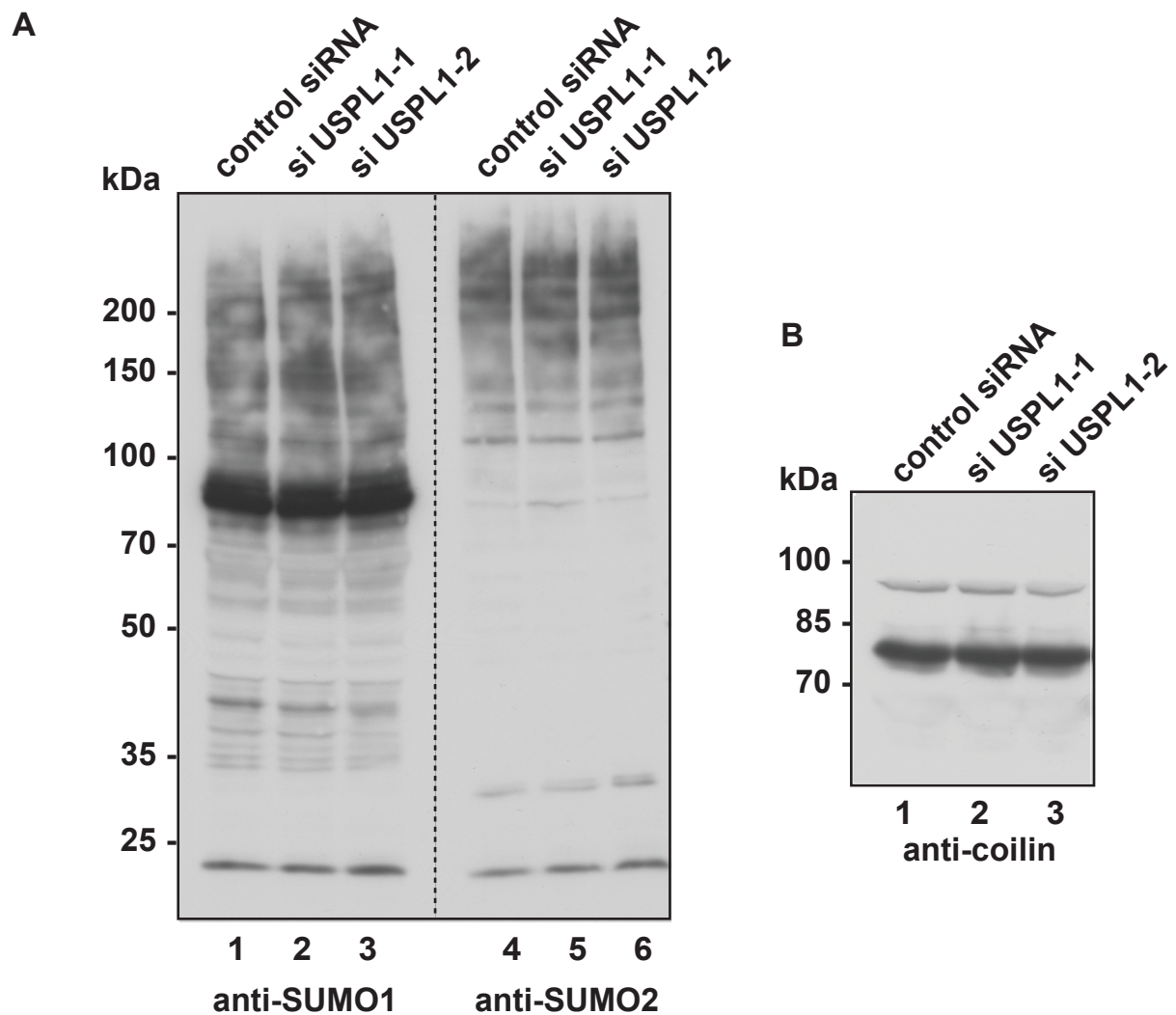


**Figure S4:** C13orf22lcat cleaves SUMO2-AMC but is inactive on Ubiquitin-AMC. (A) Indicated amounts of USP5 and C13orf22l were incubated with 0.25  $\mu$ M Ub-AMC at 30°C. Released AMC was detected by fluorescence (*excitation* at 380 nm, emission at 450 nm), using a Fluoroskan Ascent FL. (B) C13orf22lcat prefers SUMO2-AMC over SUMO1-AMC. 12.5 nM C13orf22l was incubated with 0.25  $\mu$ M SUMO1- or SUMO2-AMC. Of note, C13orf22lcat is able to cleave SUMO1 at higher concentrations (data not shown).

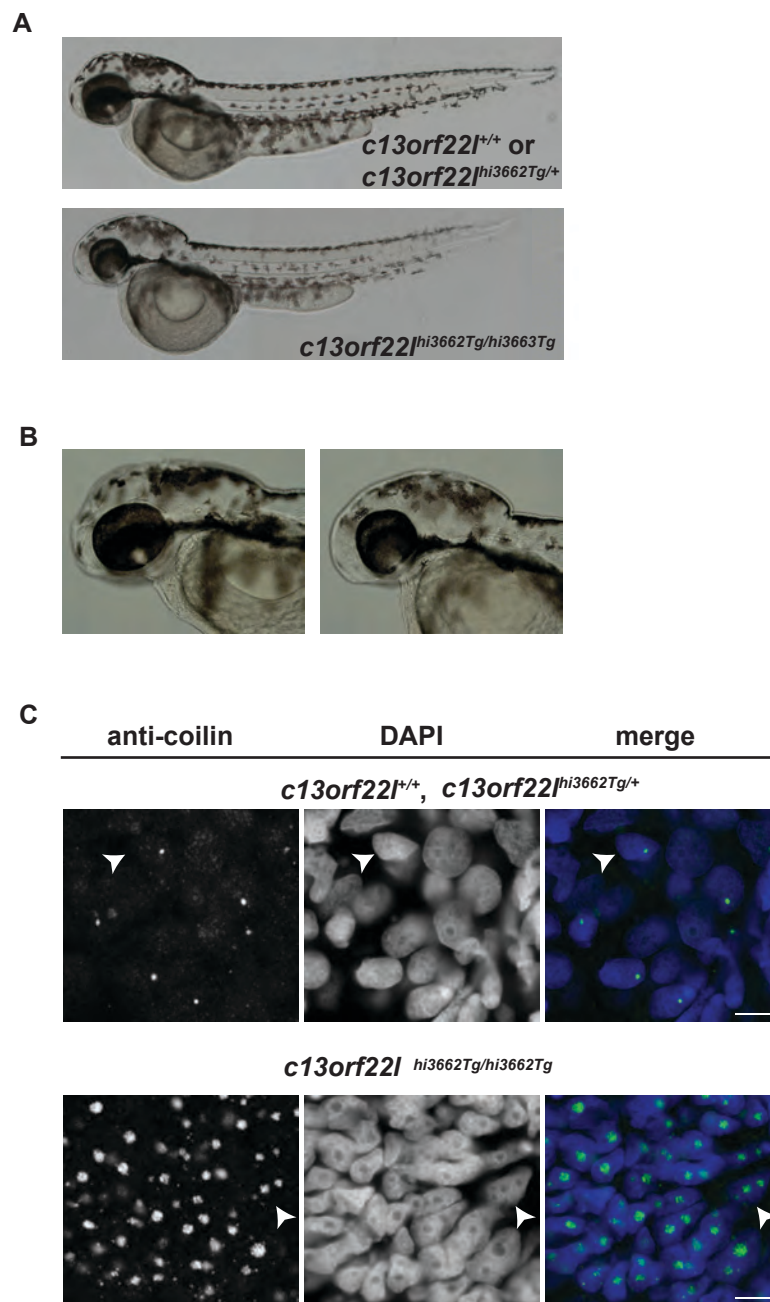




**Figure S5:** USPL1 knockdown causes relocalisation of coilin to nucleoli. HeLa cells were treated with non-targeting siRNA or USPL1 siRNA for 48 h, fixed with 4% formaldehyde in PBS/1mM MgCl<sub>2</sub>, permeabilized with 0.2% Triton X-100 and stained with mouse anti UBF and rabbit anti coilin antibodies; for visualization, secondary antibodies conjugated to Alexa488 (anti mouse) and Alexa647 (anti rabbit) were used. Samples were analysed with an LSM780 confocal microscope (Zeiss) using a Plan-Apochromat 63x/1.4 oil immersion objective, set up with Zen 2010 software.



**Figure S6.** Silencing of USPL1 does not affect global sumoylation pattern and endogenous coilin levels. HeLa cell extracts transfected for 48 h with control siRNA, or with two different siRNAs against USPL1 (si USPL1-1 and si USPL1-2) and lysates were analysed by immunoblotting with (A) anti SUMO1 and anti SUMO2 or (B) anti coilin antibodies.



**Figure S7.** Phenotype of *c13orf22l* transgenic zebrafish embryo at 2 dpf. (A) Embryos at 2 dpf, where the upper panel shows the wt sibling, *c13orf22l<sup>+/+</sup>* or *c13orf22l<sup>hi3662Tg/+</sup>*, and the lower panel the mutant, *c13orf22l<sup>hi3662Tg/hi3662Tg</sup>*. (B) Magnification of the head structure. The mutant (right) shows smaller eye phenotype and necrosis compared to the WT sibling (left). (C) Transgenic *c13orf22l* zebrafish embryos show coilin mis-localisation to the nucleolus. Labeling using anti-coilin sera (9EA2) on cryosections from the head of transgenic zebrafish mutants (*c13orf22l<sup>hi3662Tg/hi3662Tg</sup>*) and wt siblings (*c13orf22l<sup>+/+</sup>*, *c13orf22l<sup>hi3662Tg/+</sup>*) at 2 dpf.