Supplementary Materials

The proteasomal deubiquitinating enzyme PSMD14 regulates macroautophagy by controlling Golgi-to-ER retrograde transport

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Figure S1. siRNA sequences directed against human PSMD14 used for Validation Stage.

Figure S2. Primer pairs sequences used for RT-qPCR.

Figure S3. The PSMD14 DUB inhibitor CZM increases the Golgi apparatus area.

Immunofluorescence microscopy analysis of the Golgi area in parental H4 cells treated for 4 h either with the vehicle (DMSO; Control) or CZM. The Golgi marker GM130 was used to determine the region of interest in each condition. Statistical significance was determined by Student's t-test. Bars represent the mean ± SEM (n =43 cells). ***P <0.001.

Figure S4. CZM causes the accumulation of KDELR1-GFP at the Golgi apparatus.

HeLa cells expressing KDELR1-GFP were either left untreated or treated with CZM for 30, 60 or 90 min. Cells were fixed and representative confocal images were acquired.

Figure S5. Effect of CZM on proteasome activity.

Parental H4 cells were treated either with the vehicle (DMSO; Control), CZM or MG132, for 90 min. Protein extracts were used to measure *in vitro* the Chymotrypsin-like peptidase activity of the proteasome. The enzymatic activity was quantified according to the cleavage of the fluorogenic substrate Suc-LLVY-AMC to AMC, and normalized to that of control cells. The statistical significance was determined by One-Way ANOVA, followed by Tukey's test. Bars represent the mean ± SD of biological replicates (n=3). **P <0.01; n.s., not significant.

Figure S6. Effect of CZM and MG132 on basal macroautophagy.

(A) Immunofluorescence microscopy analysis of the subcellular localization of LC3 in parental H4 cells treated with either with the vehicle (DMSO; Control), CZM for 4 h or MG132 for 6 h. Cells were fixed, permeabilized and stained with a rabbit polyclonal antibody to LC3B followed by Alexa-594-conjugated donkey anti-Rabbit IgG. Scale bar 10 μm. (B) Parental H4 cells were treated as in (A) and the protein extracts were analyzed by western blot with a polyclonal antibody to LC3B. Densitometric quantification of the protein levels of LC3B were depicted as the Ratio LC3B-II/LC3B-I. The statistical significance was determined by One-Way ANOVA, followed by Tukey's test. Bars represent the mean ± SD of biological replicates (n=3). ***P <0.001; n.s., not significant.

Figure S7. Distribution of RAB1A upon CZM treatment.

Immunofluorescence analysis of endogenous RAB1A in H4 parental cells treated either with vehicle (DMSO; Control) (A-C) or CZM for 4 h (D-F). Cells were fixed, permeabilized, and double stained with a rabbit

monoclonal antibody to RAB1A (clone D3X9S) (A and D) and a mouse monoclonal antibody to GM130 (clone35/GM130) (B and E), followed by Alexa-594-conjugated donkey anti-Rabbit IgG and Alexa-488-conjugated donkey anti-Mouse IgG. Merging of the images generated the third picture (C and F). Scale bar, 10 ◎m. (G) Quantitative analysis of the fraction of RAB1A colocalizing with GM130 under CZM treatment and compared to control cells. The statistical significance was determined by Student's t-test. Bars represent the mean ± SEM of the fluorescent signal per cell area (n=173 cells). *P< 0.05.

Figure S8. ATG9A is distributed in the swollen Golgi apparatus upon CZM treatment.

Immunofluorescence analysis of endogenous ATG9A in H4 parental cells treated either with the vehicle (DMSO; Control) (A-C) or CZM for 4 h (D-F). Cells were fixed, permeabilized, and double stained with a rabbit monoclonal antibody to ATG9A (clone EPR2450(2)) (A and D) and a mouse monoclonal antibody to GM130 (clone35/GM130) (B and E), followed by Alexa-594-conjugated donkey anti-Rabbit IgG and Alexa-488-conjugated donkey anti-Mouse IgG. Merging of the images generated the third picture (C and F). Scale bar, 10 ◎m. (G) Quantitative analysis of the fraction of ATG9A colocalizing with GM130 under CZM treatment and compared to control cells. The statistical significance was determined by Student's t-test. Bars represent the mean ± SEM of the fluorescent signal per cell area (n=93 cells). **P <0.01.

	Sequence
siRNA #1	(5´-GAACAAGUCUAUAUCUCUU-3´)
siRNA #2	(5´-GGCAUUAAUUCAUGGACUA-3´)
siRNA #3	(5´-AGAGUUGGAUGGAAGGUUU-3´)
siRNA #4	(5´-GAUGGUUGUUGGUUGGUAU-3´)

Figure S1

Target	Sequence
hTBP1	f(5´-TAGTCCAATGATGCCTTACG-3´) r(5´-TGGTCAGAGTTTGAGAATGG-3´)
hPSMD14	f(5´-ACCTTAAGAGTTGTAGTTACTGACC-3´) r(5´-TTTAACAGTGCCAGGGAAGAG-3´)
hAPP	f(5´-CCTAAAGCATTTCGAGCATG-3´) r(5´-GTTTCCGTAACTGATCCTTG-3´)

Figure S2

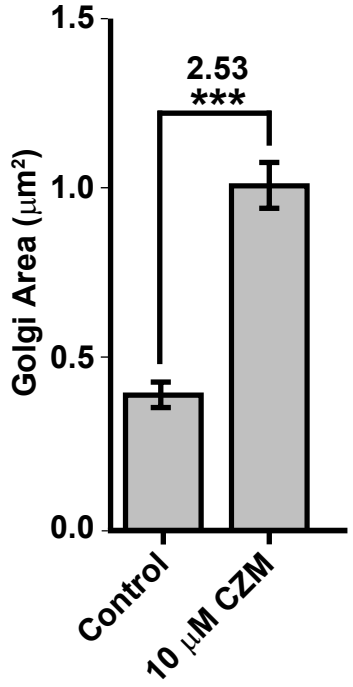

Figure S3

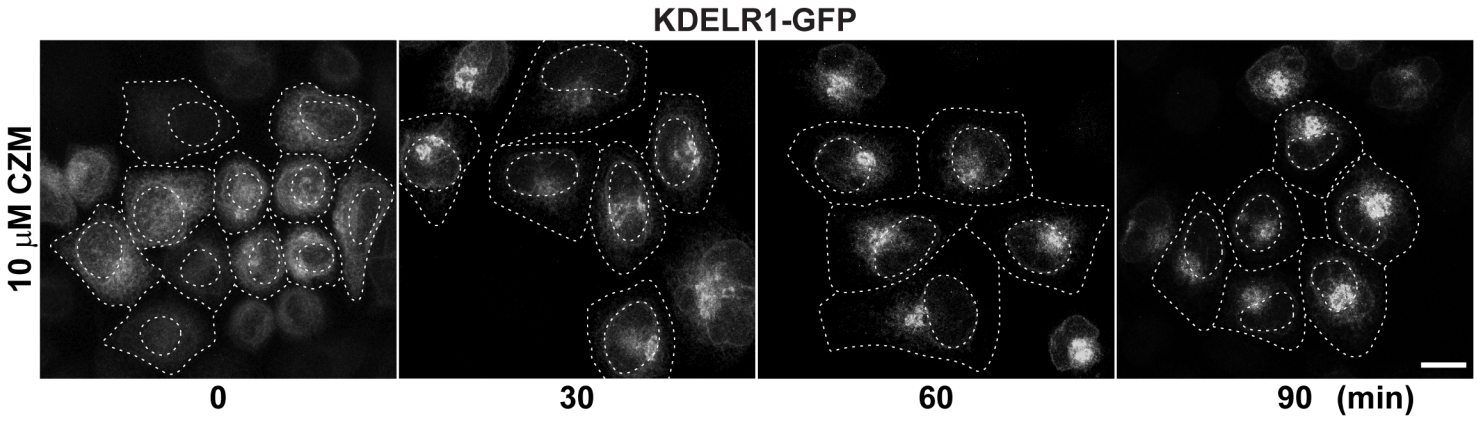

Figure S4

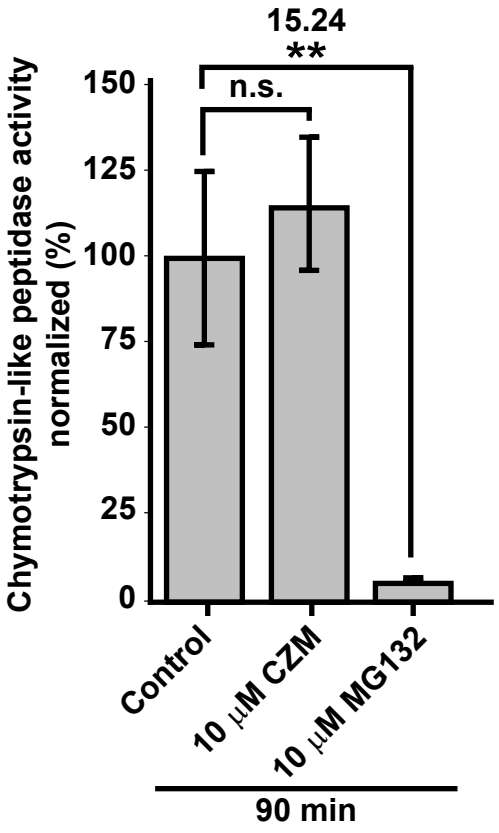
15.24
**
n.s.
Chymotrypsin-like peptidase activity normalized (%)
150
125
100
75
50
25
0
Control
10 μM CZM
10 μM MG132
90 min

Figure S5

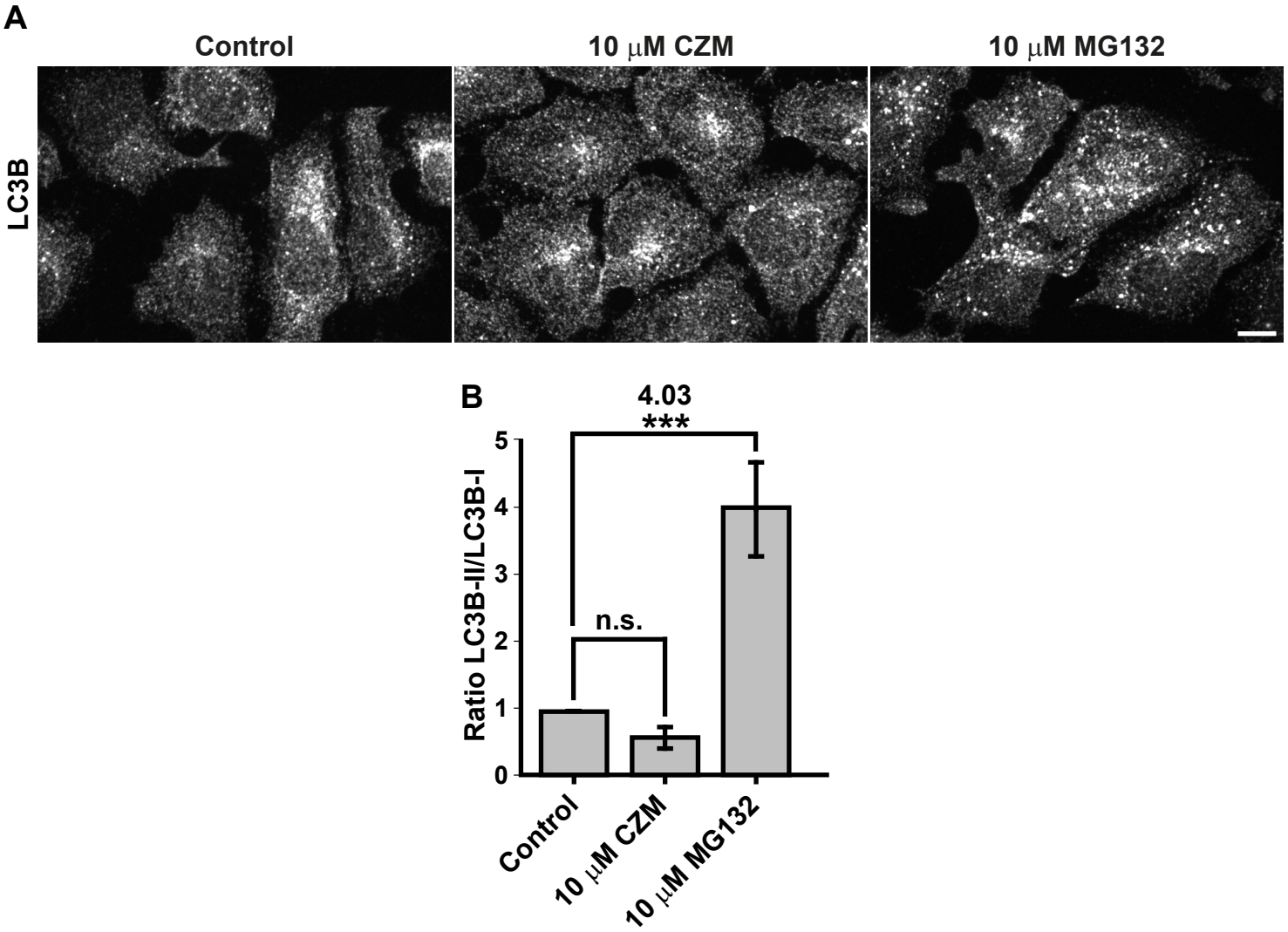
A
Control
10 μM CZM
10 μM MG132
LC3B
B
4.03
***
n.s.
Ratio LC3B-II/LC3B-I
0
1
2
3
4
5
Control
10 μM CZM
10 μM MG132

Figure S6

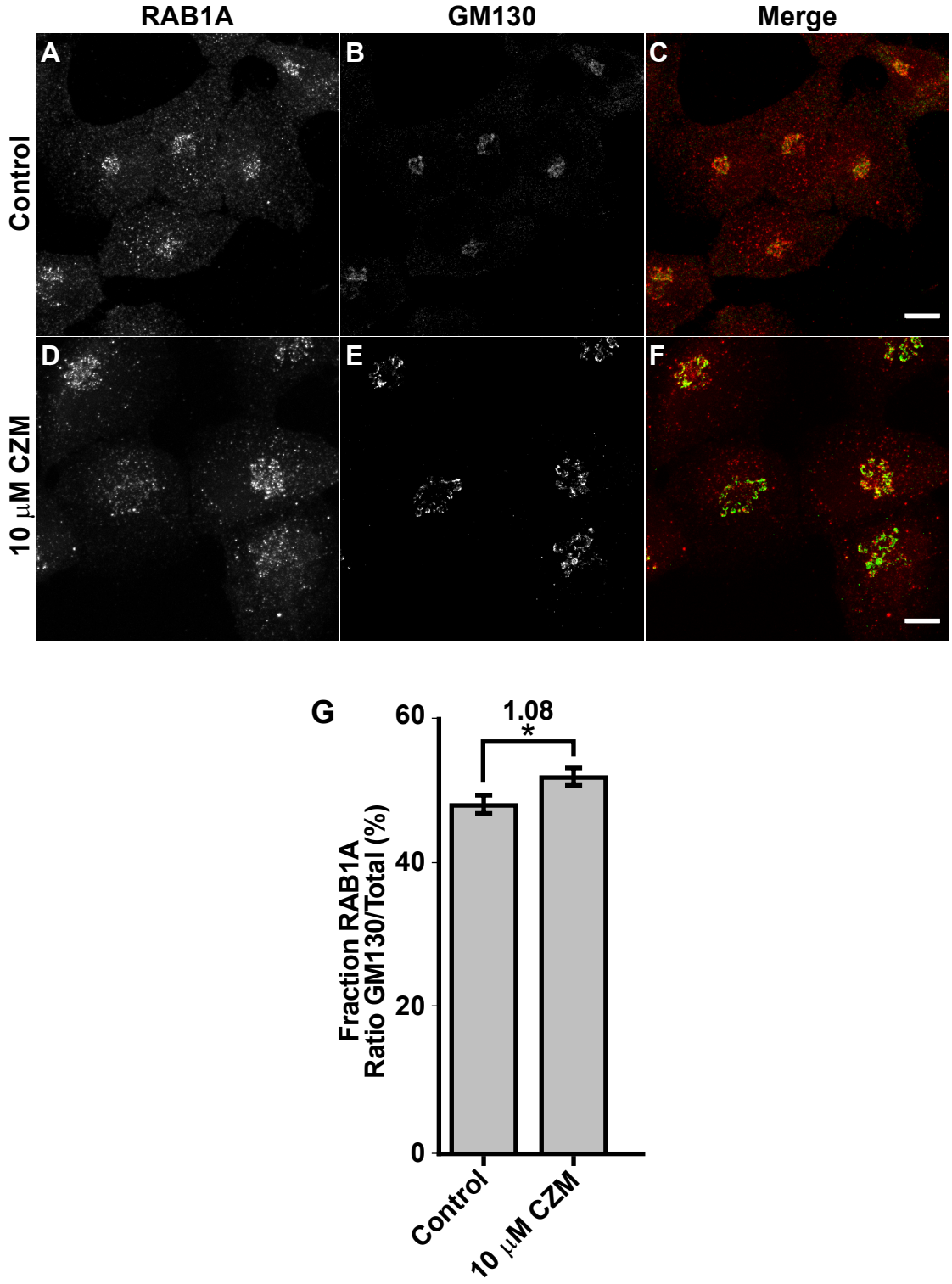
RAB1A
GM130
Merge
A
B
C
D
E
F
Control
10 μM CZM
G
1.08
*
60
40
20
0
Fraction RAB1A
Ratio GM130/Total (%)
Control
10 μM CZM

Figure S7

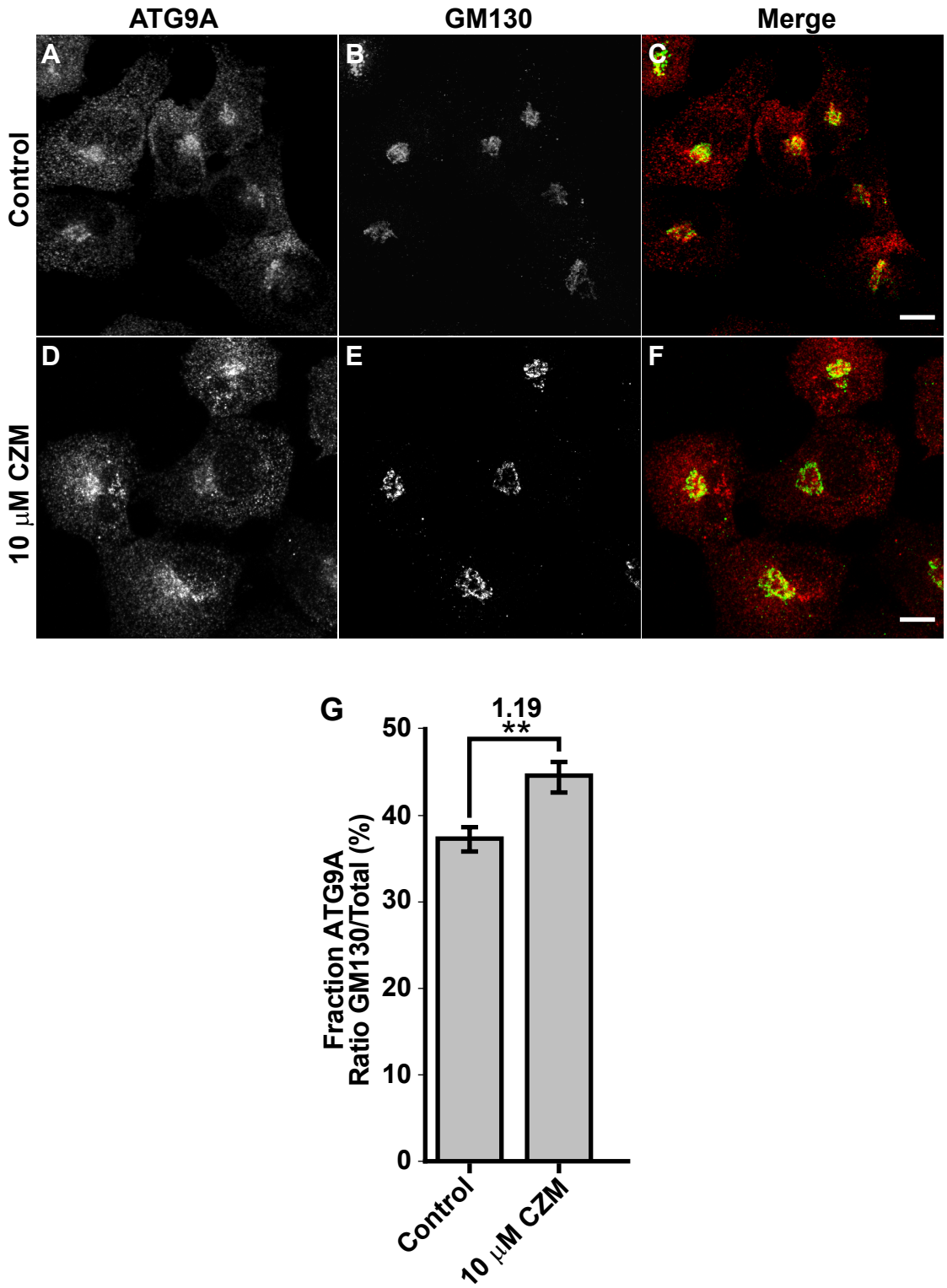
ATG9A
GM130
Merge
A
B
C
D
E
F
Control
10 μM CZM
G
1.19
**
Fraction ATG9A
Ratio GM130/Total (%)
50
40
30
20
10
0
Control
10 μM CZM

Figure S8

Target List High-Content siRNA Screening "Ubiquitinome"

Gene Symbol	NCBI Reference Sequence
PSMD14	NM_005805
PSMD7	NM_002811
KCTD3	NM_016121
UBE2E2	NM_152653
NACA	NM_005594
EPN1	NM_013333
SIAH2	NM_005067
UBA52	NM_003333
EIF3S5	NM_003754
MARK4	NM_031417
UBC	NM_021009
UBE2E3	NM_182678
RBM6	NM_005777
ZBTB12	NM_181842
PHF12	NM_020889
DNAJB2	NM_001039550
BIRC7	NM_022161
KBTBD2	NM_015483
RYBP	NM_012234
NEURL	NM_004210
HECTD3	NM_024602
ZNF592	NM_014630
FLJ25076	XM_940609
TNK2	NM_001010938
PRPF8	NM_006445
TRIM31	NM_052816
UCHL3	NM_006002
UBE2D4	NM_015983
MDM4	NM_002393
TRIM63	NM_032588
UBE2J1	NM_016021
DDI2	NM_032341
RNF146	NM_030963
CHD5	NM_015557
CISH	NM_145071
SENP8	NM_145204
FLJ43374	NM_198582
HIP2	NM_005339
UBE2V1	NM_001032288
ZNF216	NM_006007
KIAA1536	NM_020898
WDR11	NM_018117
SH3RF2	NM_152550
RAI17	NM_020338
PCGF2	NM_007144
RNF34	NM_025126
FBXO4	NM_012176
RNF141	NM_016422
BRPF3	NM_015695
CAND2	XM_944849
UBXD4	NM_181713
TRIM23	NM_033228
FAU	NM_001997
RSPRY1	NM_133368
TRIM42	NM_152616
FBXW10	NM_031456
MDM2	NM_006879
TOM1	NM_005488
SOCS3	NM_003955
FBXO44	NM_183413
RNF130	NM_018434
CREBL1	NM_004381
RNF39	NM_170770
HRC	NM_002152
VPRBP	NM_014703
FAF1	NM_131917
PSMD4	NM_002810
RNF187	XM_047499
SHFM3	NM_022039
EIF2AK4	NM_001013703
SUMO2	NM_001005849
FLJ20280	NM_017741
TCEB1	NM_005648
RAMP	NM_016448
PHF23	NM_024297
KUA-UEV	NM_003349
NICE-4	NM_014847
UBB	NM_018955
ZBTB16	NM_001018011
OTUD5	NM_017602
ASPSCR1	NM_024083
LOC652557	XM_942059
SF3B3	NM_012426
LGALS3BP	NM_005567
TRIM56	NM_030961
SAE1	NM_005500
KLHL1	NM_020866
MNAT1	NM_002431
KCTD17	NM_024681

PCF11	NM_015885
USP42	NM_032172
PHF21A	NM_016621
TRIM74	NM_198853
KLHL7	NM_001031710
RNF32	NM_030936
TRIM60	NM_152620
UBE2D2	NM_003339
UFC1	NM_016406
TA-KRP	NM_032505
UBE2J2	NM_194457
RANBP9	NM_005493
BMI1	NM_005180
PC326	NM_001017977
EIF3S3	NM_003756
CDC34	NM_004359
UBXD3	NM_152376
KCND1	NM_004979
PEX10	NM_002617
USP52	NM_014871
PLAA	NM_001031689
BMI1	NM_005180
PDZRN4	NM_013377
FLJ25555	NM_152345
BIRC2	NM_001166
STAMBPL1	NM_020799
MIB2	NM_080875
PIAS1	NM_016166
TRIM7	NM_203294
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USP9Y	NM_004654
TRIM33	NM_033020
LOC646862	XM_929820
TSG101	NM_006292
TRIM6-TRIM34	NM_001003819
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WDR26	NM_025160
SKP1A	NM_170679
CUL5	NM_003478
C13ORF22	NM_005800
TRAF7	NM_206835
RBM10	NM_152856
FBXL20	NM_032875
ASB18	NM_212556
LONRF2	NM_198461

USP6	NM_004505
KCMF1	NM_020122
VPS11	NM_021729
KRTAP5-9	NM_005553
USP26	NM_031907
USP16	NM_001001992
USP8	NM_005154
KCTD12	NM_138444
USP10	NM_005153
RPS27A	NM_002954
UBE2M	NM_003969
LOC51035	NM_015853
RKHD1	NM_203304
HACE1	NM_020771
FANCD2	NM_001018115
YAF2	NM_005748
SIP	NM_001007214
RNF44	NM_014901
ZNF151	NM_003443
ZRANB1	NM_017580
TCEB2	NM_207013
TCEB3	NM_003198
RNF186	NM_019062
LOC441061	XM_941081
BTBD14A	NM_144653
LOC646862	XM_929820
LMO7	NM_005358
FLJ10719	NM_018193
AB026190	NM_014458
UBE2NL	NM_001012989
FBXO5	NM_012177
HECTD1	NM_015382
DKFZP547C195	NM_207343
TRIM32	NM_012210
HECW1	NM_015052
AIRE	NM_000659
KCNC1	NM_004976
UNK	NM_001080419
TRIM8	NM_030912
SBBI54	NM_138334
C20ORF18	NM_031227
RNF167	NM_015528
KCNA1	NM_000217
OTUB2	NM_023112
SPSB2	NM_032641

GGA3	NM_014001
SPSB3	NM_080861
SHARPIN	NM_030974
HGS	NM_004712
TIP120A	NM_018448
UEVLD	NM_018314
ATG10	NM_031482
CHD4	NM_001273
FBXO34	NM_017943
UBQLN2	NM_013444
ZNF185	NM_007150
FBXO17	NM_148169
ANKFY1	NM_020740
USP43	XM_371015
PARC	NM_015089
HR	NM_018411
ZNF297B	NM_014007
UBL5	NM_024292
PHF19	NM_001009936
KCTD5	NM_018992
MARCH4	NM_020814
ASB14	NM_130387
FLJ34154	NM_173813
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FBXL22	NM_203373
RNF207	NM_207396
RFWD3	NM_018124
UBE2L6	NM_004223
PHF16	NM_014735
MARCH8	NM_001002266
FBXL14	NM_152441
BACH2	NM_021813
DDB1	NM_001923
TRIM2	NM_015271
RNF38	NM_194331
DTX3	NM_178502
SH3MD4	XM_293090
NSMCE1	NM_145080
PML	NM_033247
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SENP1	NM_014554
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CCL20	NM_004591
COPS7A	NM_016319

USP45	XM_371838
PHF5A	NM_032758
UBE4B	NM_006048
KLHDC5	NM_020782
KCNC3	NM_004977
RNF121	NM_194452
PARK7	NM_007262
UBE2N	NM_003348
CUEDC1	NM_017949
BARD1	NM_000465
TAB3	NM_198312
FBXL13	NM_145032
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PRPF19	NM_014502
WDR40A	NM_015397
RAB40C	NM_021168
FBXL12	NM_017703
BTBD9	NM_152733
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LRRC29	NM_001004055
USP54	NM_152586
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FBXL2	NM_012157
FBXO39	NM_153230
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LATS1	NM_004690
EEA1	NM_003566
ITCH	NM_031483
BAZ1B	NM_032408
RFP	NM_030950
TOM1L1	NM_005486
BIRC3	NM_182962
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BRE	NM_199191
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NOSIP	NM_015953
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RNF215	NM_001017981
SYVN1	NM_032431
TRIM73	NM_198924
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CPSF1	NM_013291
NEURL2	NM_080749
FBXO3	NM_033406
NLRC5	NM_032206
KCTD15	NM_024076
HIC2	NM_015094
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HOZFP	NM_152995
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SFRS2IP	NM_004719
USP3	NM_006537
DPF1	NM_004647
NEDD4	NM_006154
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TRIM36	NM_018700
PIAS4	NM_015897

MGC3123	NM_024107
FBXO11	NM_012167
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ANUBL1	NM_001128324
KLHL6	NM_130446
UBE2F	NM_080678
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RBAF600	NM_020765
SNF1LK	NM_173354
FBXL16	NM_153350
SUMO4	NM_001002255
KCNA4	NM_002233
TIF1	NM_003852
USP39	NM_006590
SOCS4	NM_080867
USP27X	XM_372213
USP7	NM_003470
FBXL17	NM_022824
DKFZP564O0463	NM_015420
ARIH1	NM_005744
USP41	XM_036729
TNFAIP3	NM_006290
FBXO43	NM_001029860
ZNF179	NM_007148
PCGF1	NM_032673
ZNF265	NM_203350
KCNA3	NM_002232
TRIM25	NM_005082
CDC20	NM_001255
DCUN1D1	NM_020640
ZBTB2	NM_020861
PDZRN3	NM_015009
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PCGF3	NM_006315
SYTL3	NM_001009991
RNF148	NM_198085
UBADC1	NM_016172
STAMBP	NM_201647
UBE1L	NM_003335
TRIM46	NM_025058
DDB2	NM_000107
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TBL1XR1	NM_024665
CUL2	NM_003591
WDSUB1	NM_152528
BMSC-UBP	NM_201265
PPIL2	NM_014337
FLJ13456	NM_024646
BTBD12	NM_032444
KCTD13	NM_178863
SKP2	NM_032637
KCNS3	NM_002252
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UBE3A	NM_130838
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HERC1	NM_003922
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IMPACT	NM_018439
UBE3B	NM_183415
KIAA0459	XM_375697
CNOT4	NM_001008225
TRIM40	NM_138700
SIK2	NM_015191
BRPF1	NM_004634
ZFPL1	NM_006782
ZNF313	NM_018683
AOF1	NM_153042
TRIM71	NM_001039111
TRAF6	NM_004620
KLHL13	NM_033495
H326	NM_015726

HERC2	NM_004667
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ZBTB39	NM_014830
RNF133	NM_139175
USP21	NM_012475
UBE3C	NM_014671
NIPBL	NM_015384
FBXO38	NM_030793
FLJ11280	NM_001040217
ASB11	NM_001012428
FLJ40411	NM_001080504
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UBE1C	NM_198197
WSB2	NM_018639
SMURF1	NM_181349
NXF2	NM_022053
FLJ46299	NM_207335
FBXO27	NM_178820
WDR21	NM_181341
D8S2298E	NM_005671
TRIP	NM_005879
RNF8	NM_183078
PCGF6	NM_032154
TRIM58	NM_015431
JARID1C	NM_004187
PHF10	NM_018288
ZNF499	NM_032792
NEDD8	NM_006156
DCUN1D5	NM_032299
TRIAD3	NM_019011
PJA1	NM_001032396
JARID1D	NM_004653
UBE2C	NM_181803
HECW2	NM_020760
KCNA7	NM_031886
UBE1DC1	NM_024818
PHF20	NM_016436
KEAP1	NM_012289
LOC441920	XM_497731
RWDD2	NM_033411
RNF157	NM_052916
KIAA0795	NM_025010
FBXO31	NM_024735

C20ORF11	NM_017896
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KIAA0644	NM_014817
TRIM45	NM_025188
ZNF547	NM_173631
LOC342931	XM_292796
ZNF294	NM_015565
UBE2G1	NM_182682
KBTBD11	NM_014867
RNF31	NM_017999
RNF169	XM_495886
MGC20470	NM_145053
CDC27	NM_001256
TRAF4	NM_145751
KCTD7	NM_153033
TULP4	NM_001007466
PIAS2	NM_173206
IPP	NM_005897
ASB10	NM_080871
UHRF2	NM_152896
SENP7	NM_020654
MOCS3	NM_014484
UBE2B	NM_003337
USP14	NM_001037334
ZNF645	NM_152577
LOC283219	NM_001029859
UBE2O	NM_022066
UBE2R2	NM_017811
DPF3	NM_012074
LOC648245	XM_942858
LOC648245	XM_942858
RAG1	NM_000448
C10ORF3	NM_018131
STUB1	NM_005861
STAM	NM_003473
SOCS1	NM_003745
IRF2BP1	NM_015649
KIAA0999	NM_025164
PHF3	NM_015153
COPS8	NM_006710
UBAP1	NM_016525
KCTD6	NM_001128214
RAD23A	NM_005053
DMRT3	NM_021240
KBTBD9	XM_496546

BIRC6	NM_016252
IBRDC2	NM_182757
MARK1	NM_018650
TANK	NM_133484
LOC196394	NM_207337
POLI	NM_007195
JARID1B	NM_006618
NDP52	NM_005831
SOCS7	NM_014598
KBTBD1	NM_001003760
KCND2	NM_012281
SENP3	NM_015670
DRE1	NM_017644
LONRF1	NM_152271
USP29	NM_020903
MKLN1	NM_013255
FLJ35834	NM_178827
DCUN1D2	NM_001014283
FBXO47	NM_001008777
CYLD	NM_015247
KLHL21	NM_014851
CXORF53	NM_001018055
SOCS6	NM_004232
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