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Supplemental Material to:

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BAG3 induces the sequestration of proteasomal clients into cytoplasmic puncta: Implications for a proteasome-to-autophagy switch

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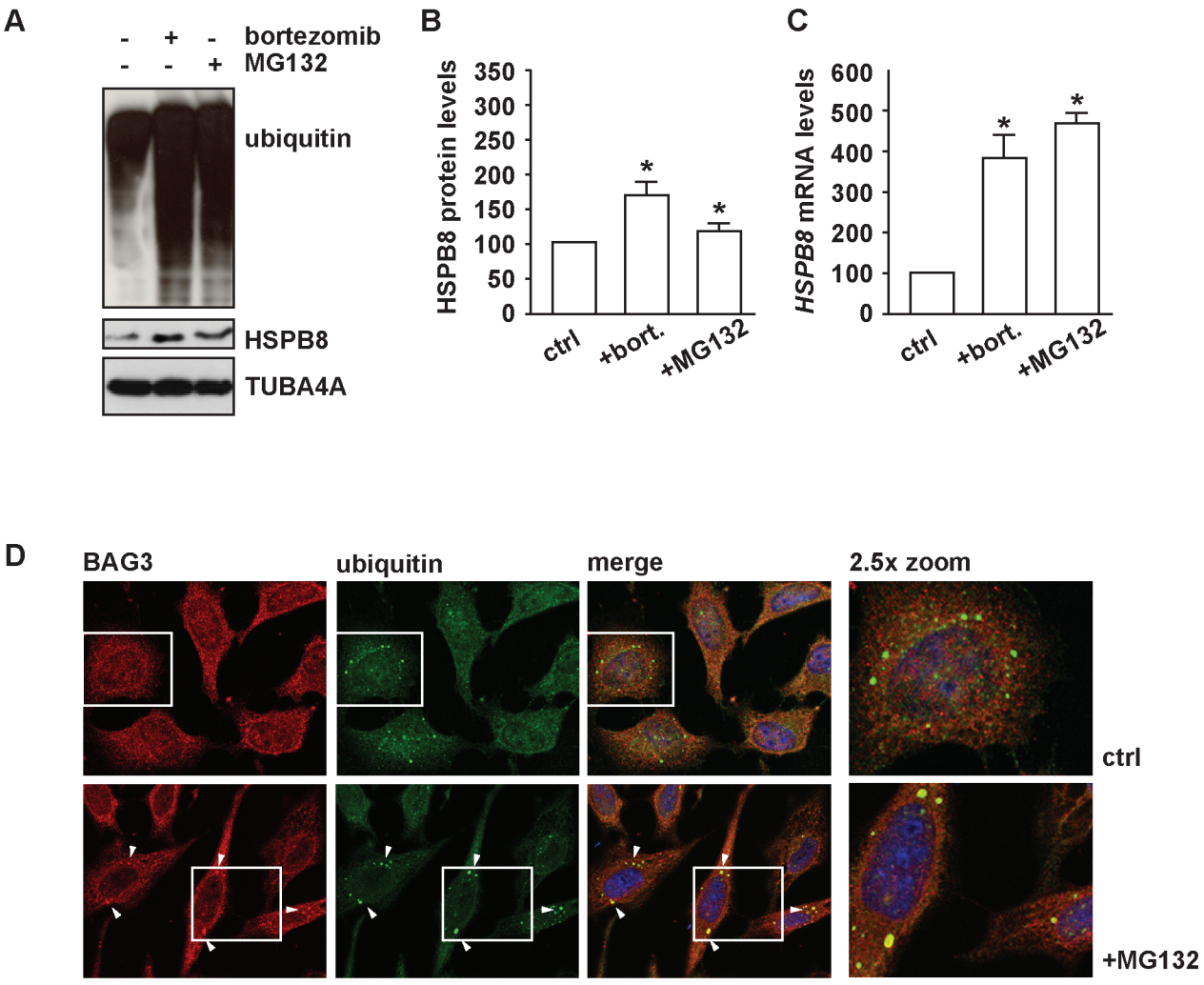
A
- + - bortezomib
- - + MG132
ubiquitin
HSPB8
TUBA4A
B
HSPB8 protein levels
350
300
250
200
150
100
50
0
*
*
ctrl
+bort.
+MG132
C
HSPB8 mRNA levels
600
500
400
300
200
100
0
*
*
ctrl
+bort.
+MG132
D
BAG3
ubiquitin
merge
2.5x zoom
ctrl
+MG132

Figure S1

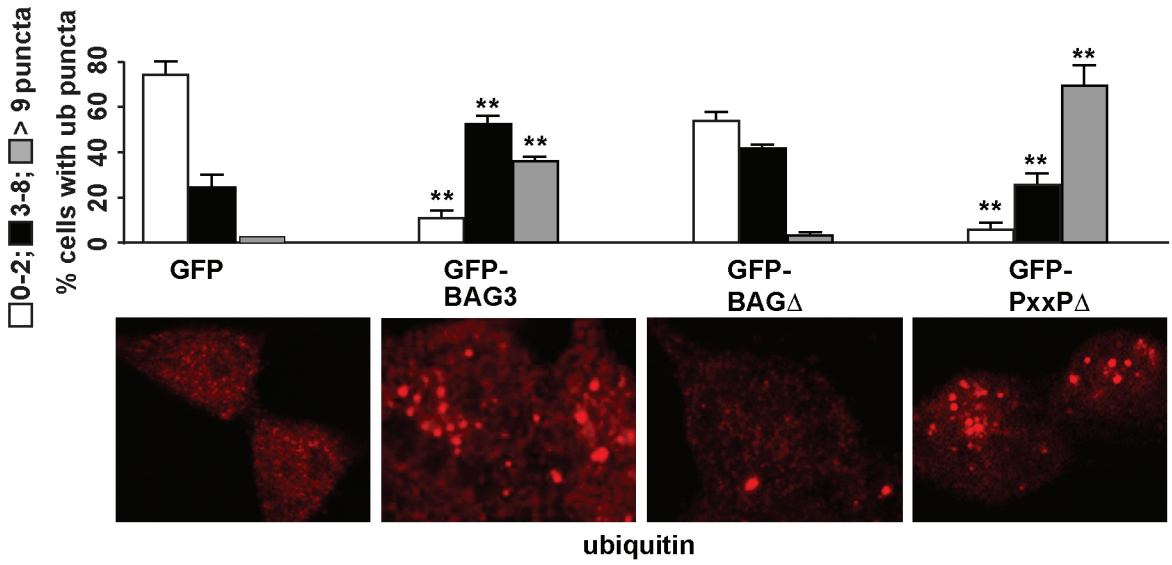
□0-2; ■3-8; ▒> 9 puncta
% cells with ub puncta
0
20
40
60
80
**
**
**
**
**
**
GFP
GFP-
BAG3
GFP-
BAGΔ
GFP-
PxxPΔ
ubiquitin

Figure S2

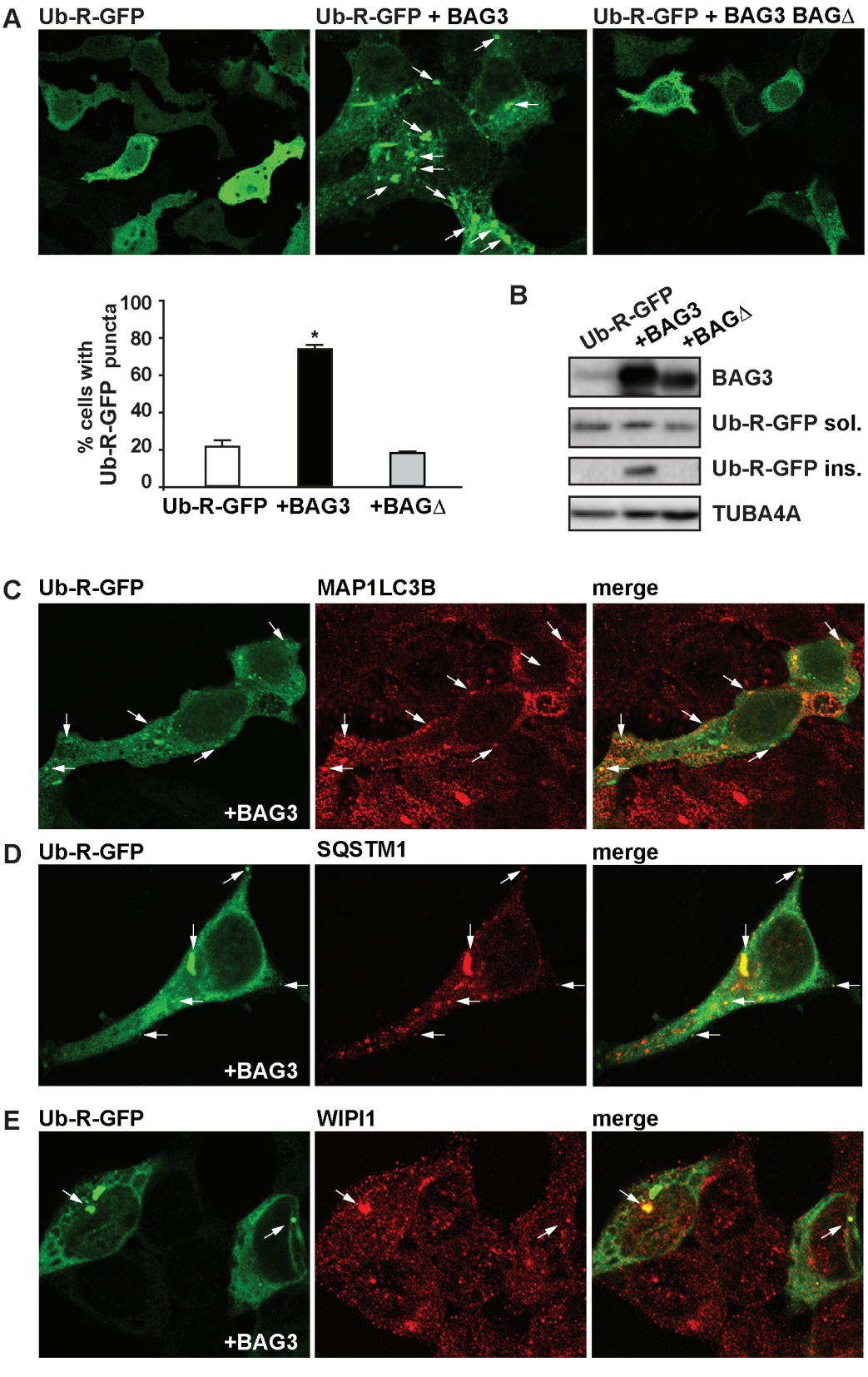
A
Ub-R-GFP
Ub-R-GFP + BAG3
Ub-R-GFP + BAG3 BAGΔ
% cells with Ub-R-GFP puncta
100
80
60
40
20
0
*
Ub-R-GFP
+BAG3
+BAGΔ
B
Ub-R-GFP
+BAG3
+BAGΔ
BAG3
Ub-R-GFP sol.
Ub-R-GFP ins.
TUBA4A
C
Ub-R-GFP
MAP1LC3B
merge
+BAG3
D
Ub-R-GFP
SQSTM1
merge
+BAG3
E
Ub-R-GFP
WIPI1
merge
+BAG3

Figure S3

Figure S1. HSPB8 is induced upon proteasome inhibition. (**A–C**) HEK293T cells were either left untreated or treated with 20 μM MG132 for 5 h or 100 nM bortezomib overnight. Total proteins (**A and B**) or mRNA (**C**) were extracted and HSPB8 protein or mRNA levels were measured (*$P <$ 0.05 compared to control; n > 3 independent samples ± sem). (**D**) HeLa cells were treated for 5 h with 20 μM MG132 and cells were fixed with 100% methanol. Subcellular distribution of endogenous BAG3 and ubiquitin was investigated by immunofluorescence using specific antibodies.

Figure S2. BAG3 induces sequestration of ubiquitin into cytoplasmic puncta. HEK293T cells were transfected with GFP, GFP-BAG3, GFP-BAGΔ or GFP-PxxPΔ encoding vectors. 48 h post-transfection cells were fixed with 100% methanol for 10 min at –20 °C and subjected to immunofluorescence to investigate ubiquitin subcellular distribution. Immunofluorescence pictures show accumulation of ubiquitin in cytoplasmic puncta in cells expressing GFP-BAG3 and GFP-PxxPΔ. The percentage of cells containing ubiquitin-positive cytoplasmic puncta is depicted (**$P <$ 0.001 compared to GFP; n = 3 to 4 independent samples ± sem).

Figure S3. BAG3 sequesters the proteasomal reporter Ub-R-GFP into cytoplasmic insoluble puncta that colocalize with SQSTM1 and canonical autophagy markers. (**A**) HEK293T cells were transfected with a Ub-R-GFP encoding vector and either an empty vector or His-tagged FL BAG3 or BAGΔ encoding vectors. Cells were fixed 24 h post-transfection. The percentage of cells containing Ub-R-GFP positive cytoplasmic puncta is depicted (**$P <$ 0.001 compared to empty vector; n = 3 to 4 independent samples ± sem). (**B**) NP-40 soluble and insoluble proteins were fractionated and accumulation of Ub-R-GFP in both fractions was analyzed by western blotting. (**C–E**) HEK293T cells were transfected with Ub-R-GFP and BAG3 encoding vectors and subjected,

24 h post-transfection, to immunofluorescence to investigate Ub-R-GFP colocalization with MAP1LC3B (**C**), SQSTM1 (**D**) and WIPI1 (**E**).