

SUPPLEMENTARY FIGURES

RAW Mφ

	+/+								-/-							
	GRP78 (total)	GRP78 (surface)	HSP70	HSP90	HSP27	eIF2α (total)	eIF2α (P-S51)	ERK2	GRP78 (total)	GRP78 (surface)	HSP70	HSP90	HSP27	eIF2α (total)	eIF2α (P-S51)	ERK2
VEH	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
AR12	77*	80*	87*	78*	87*	100	115##	100	95	99	96	98	97	97	102	100
NER	87*	84*	85*	87*	97	100	104###	100	96	99	99	99	99	100	100	100
A+N	73**	73**	80*	65**	83*	100	127###	100	94	97	97	97	97	99	105	100

Supplementary Figure 1. Deletion of Rubicon prevents the degradation of chaperones, Tau and APP in macrophages. RAW macrophages (+/+ and -/- for Rubicon) were treated with vehicle control, AR12 (2 μM), neratinib (50 nM) or the drugs in combination for 6h. Cells were fixed in place and immunostaining performed to determine the expression of GRP78 (cell surface and total), HSP70, HSP90, HSP27, eIF2α and ERK2, and the phosphorylation of eIF2α S51. (n = 3 +/-SD) * p < 0.05 less than vehicle control; ** p < 0.05 less than corresponding neratinib value; # p < 0.05 greater than vehicle control; ## p < 0.05 greater than corresponding AR12 value.

A RAW Mφ

	APP				ERK2				TAU				ERK2			
	CMV	GRP78	HSP70	HSP90	CMV	GRP78	HSP70	HSP90	CMV	GRP78	HSP70	HSP90	CMV	GRP78	HSP70	HSP90
VEH	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
AR12	86*	92	90	87*	100	100	100	100	71*	87*†	85*†	84*†	100	100	100	100
NER	89	100	94	91	100	100	100	100	80*	92	87*	85*	100	99	100	100
A+N	74**	84*	84*	83*	100	100	100	99	68*	85*†	84*†	81*†	100	100	100	100

B RAW Mφ

	APP				ERK2				TAU				ERK2			
	siSCR	siGRP78	siHSP70	siHSP90	siSCR	siGRP78	siHSP70	siHSP90	siSCR	siGRP78	siHSP70	siHSP90	siSCR	siGRP78	siHSP70	siHSP90
VEH	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
AR12	88	76†	78*	81*	100	100	100	100	81*	67†	76*	73*	100	100	100	100
NER	98	89	92	88	100	100	100	100	87*	72†	76†	74†	100	100	99	99
A+N	77*	67†	73*	75*	100	100	100	100	71*	61†	70*	71*	100	100	99	99

Supplementary Figure 2. GRP78 plays a key role in regulating Tau and APP expression after drug exposure in macrophages. (A) RAW macrophages were transfected with an empty vector plasmid or with plasmids to express GRP78, HSP70 or HSP90, and in parallel co-transfected to express Tau or APP. After 24h, cells were treated with vehicle control, AR12 (2 μM), neratinib (50 nM) or the drugs in combination for 6h. Cells were fixed in place and immunostaining performed to determine the expression of Tau, APP and ERK2. (n = 3 +/-SD) * p < 0.05 less than vehicle control; ** p < 0.05 less than corresponding AR12 value; † p < 0.05 greater than corresponding value in CMV transfected cells. (B) RAW macrophages were transfected to express APP or Tau and co-transfected with a scrambled siRNA or with an siRNA molecules to knock down the expression of GRP78, HSP70 or HSP90. After 24h, cells were treated with vehicle control, AR12 (2 μM), neratinib (50 nM) or the drugs in combination for 6h. Cells were fixed in place and immunostaining performed to determine the expression of Tau, APP and ERK2. (n = 3 +/-SD) * p < 0.05 less than vehicle control; † p < 0.05 less than corresponding value in siSCR cells.