Supplementary Files

CHIP/STUB1 Ubiquitin Ligase Functions as a Negative Regulator of ErbB2 by Promoting Its Early Post-Biosynthesis Degradation

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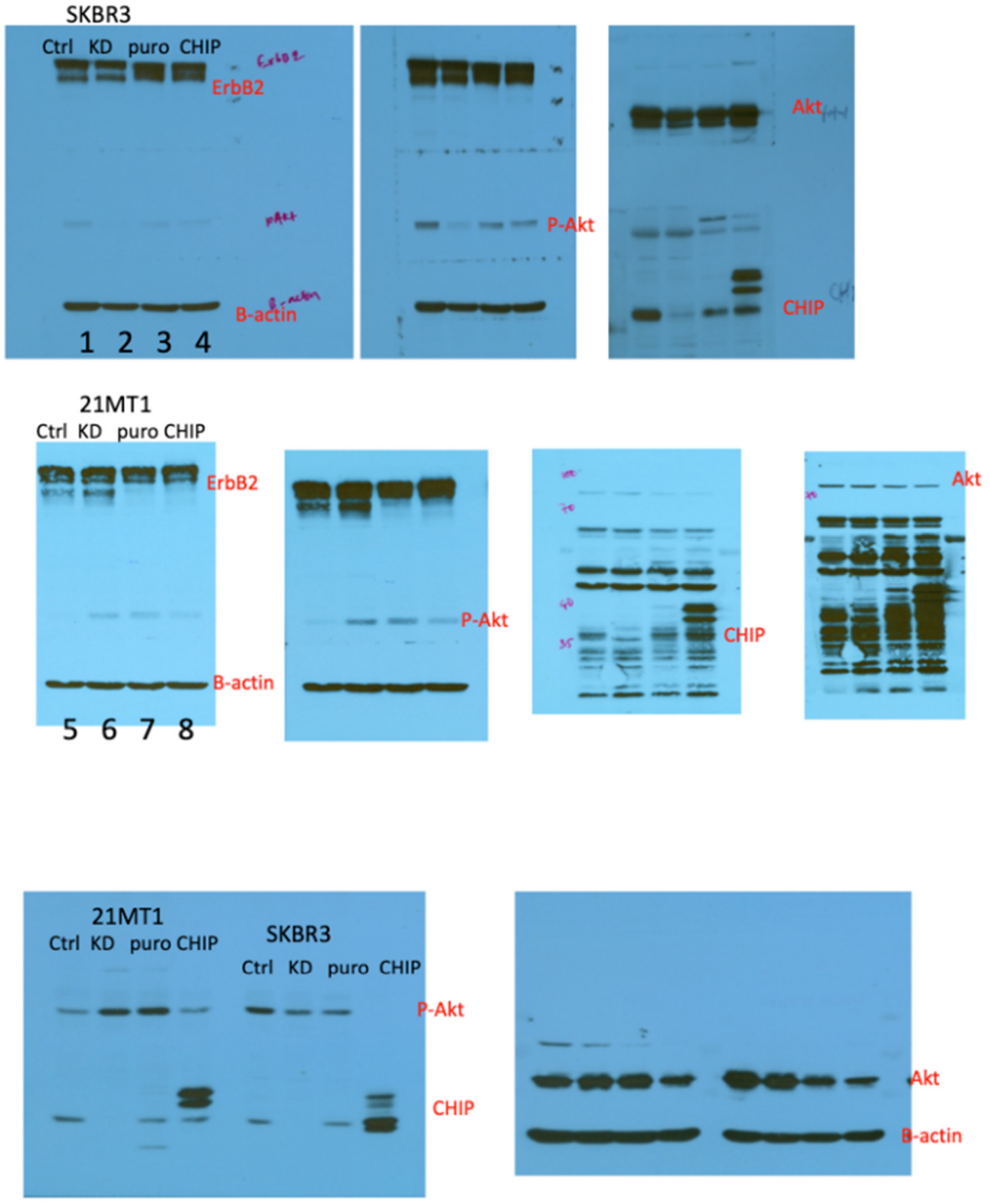
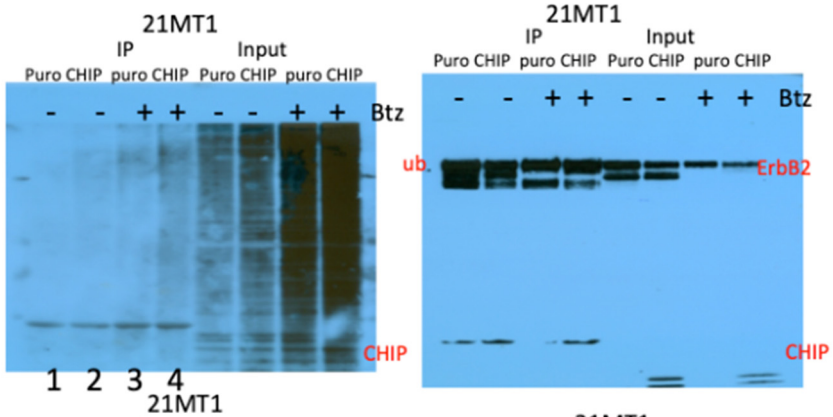
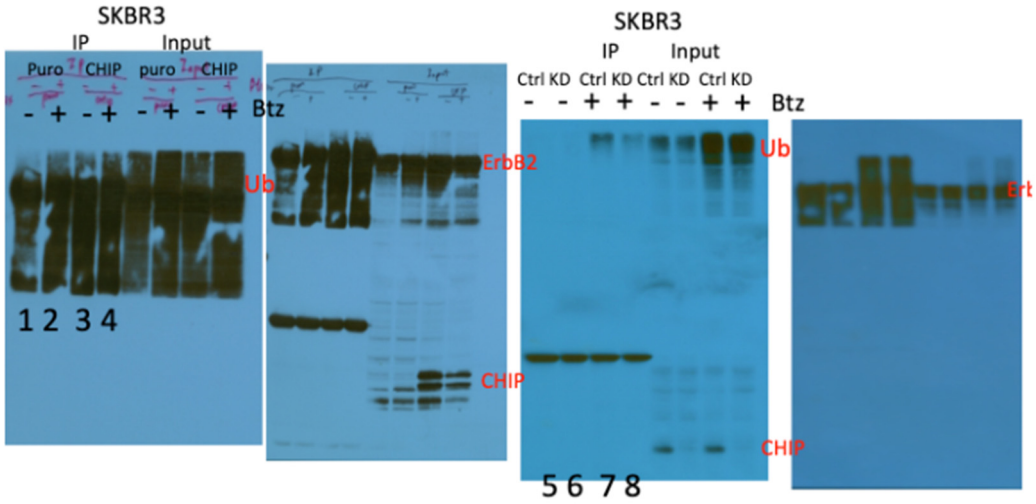


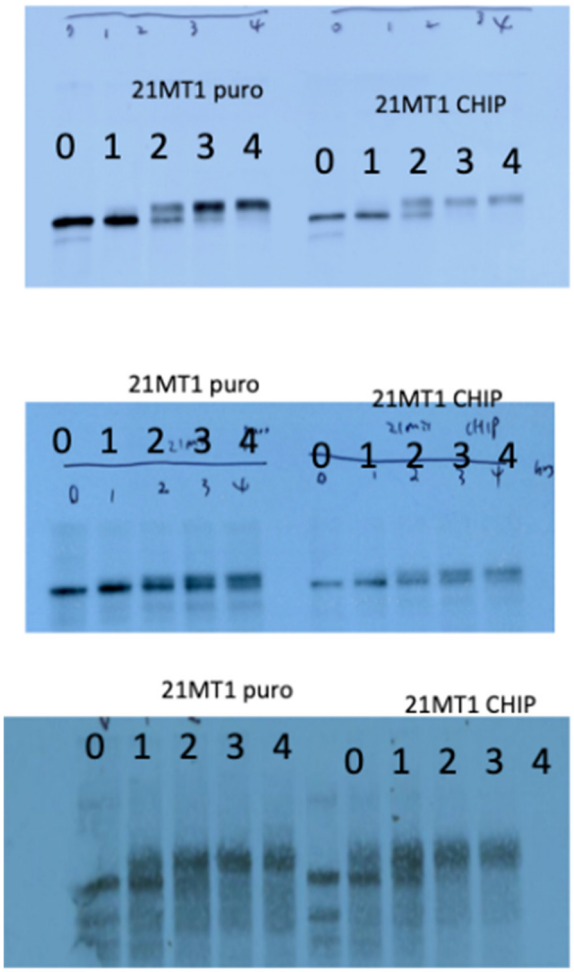
Figure S1. CHIP regulates cell surface ErbB2 levels. Original blots of western blot analysis of SKBR3 and 21MT1 cell lyScheme 2. phospho-Akt and total Akt levels, and β-actin as a loading control.

(**A**)

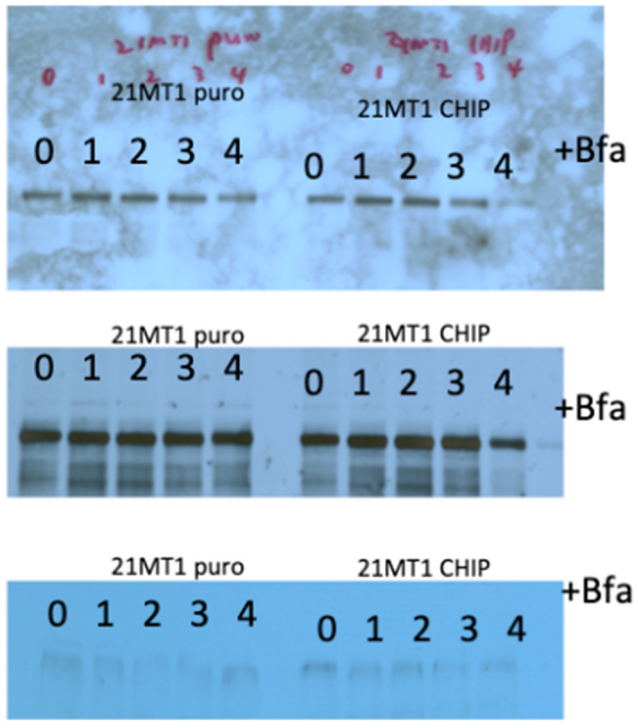

21MT1
IP
Input
Puro CHIP puro CHIP Puro CHIP puro CHIP
- - + + - - + + Btz
CHIP
1 2 3 4
21MT1
IP
Input
Puro CHIP puro CHIP Puro CHIP puro CHIP
- - + + - - + + Btz
ub.
ErbB2
CHIP
21MT1
IP
Input
Ctrl KD Ctrl KD Ctrl KD Ctrl KD
- - + + - - + + Btz
ub
CHIP
5 6 7 8
21MT1
IP
Input
Ctrl KD Ctrl KD Ctrl KD Ctrl KD
- - + + - - + + Btz
ErbB2


SKBR3
IP
Input
Puro CHIP puro CHIP
- + - + - + - + Btz
Ub
1 2 3 4
ErbB2
CHIP
SKBR3
IP
Input
Ctrl KD Ctrl KD Ctrl KD Ctrl KD
- - + + - - + + Btz
Ub
CHIP
5 6 7 8
Erb

(**B**)


21MT1 puro
0 1 2 3 4
21MT1 CHIP
0 1 2 3 4
21MT1 puro
0 1 2 3 4
21MT1 CHIP
0 1 2 3 4
21MT1 puro
0 1 2 3 4
21MT1 CHIP
0 1 2 3 4

(**C**)


21MT1 puro
0 1 2 3 4
21MT1 CHIP
0 1 2 3 4
+Bfa
21MT1 puro
0 1 2 3 4
21MT1 CHIP
0 1 2 3 4
+Bfa
21MT1 puro
0 1 2 3 4
21MT1 CHIP
0 1 2 3 4
+Bfa

(**D**)

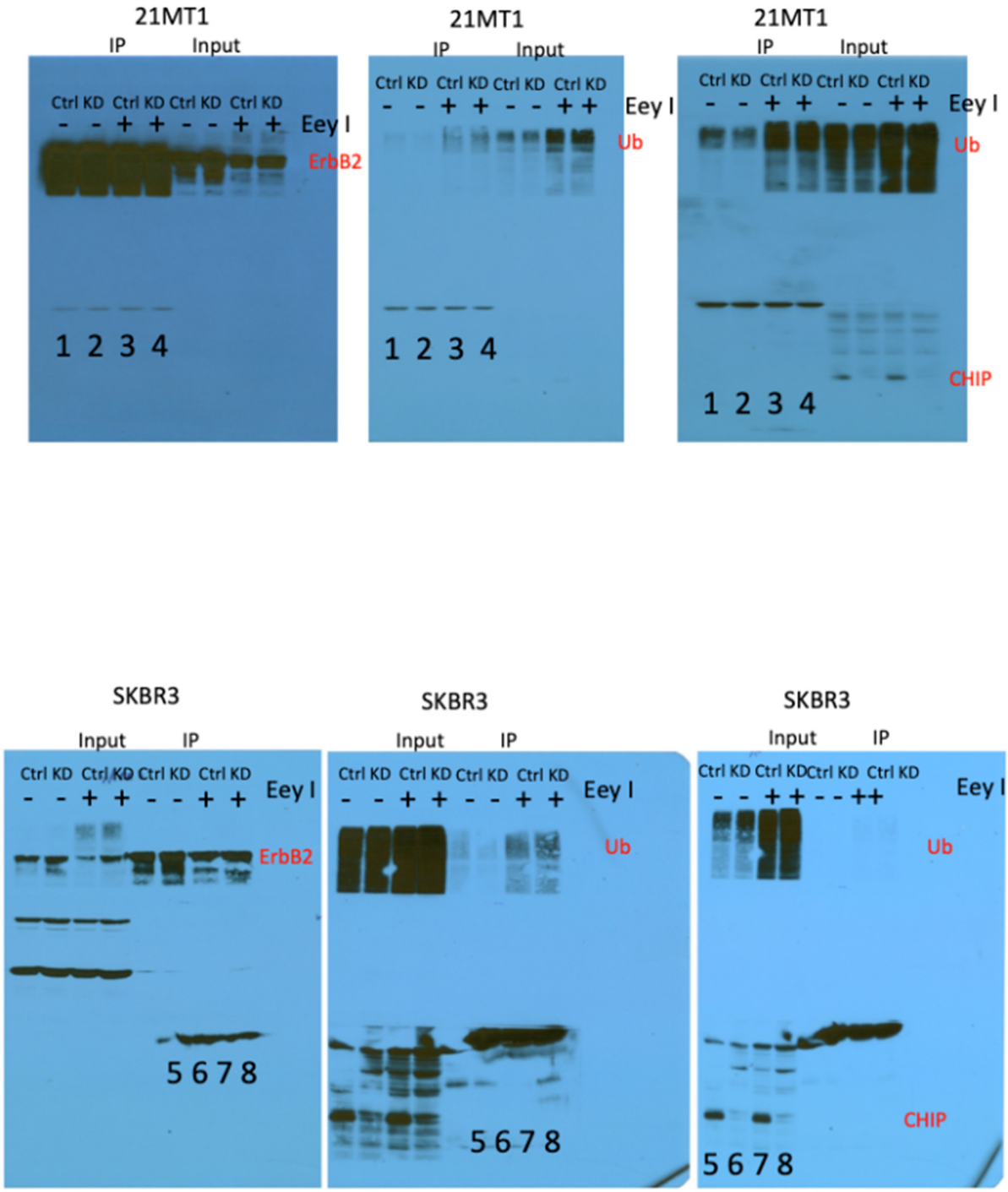


Figure S2. CHIP elevates the basal ubiquitination of ErbB2 during its maturation. (**A**) Original blots of immunoprecipitation of ErbB2 in 21MT1 and SKBR3 cells treated with Bortezomib (1 μM) for 4h. (**B**) Original blots of [^{35}S]-methionine/cysteine chase of ErbB2 in 21MT1. (**C**) Original blots of [^{35}S]-methionine/cysteine chase of ErbB2 in 21MT1 with Brefeldin A (1 μg/mL). (**D**) Original blots of immunoprecipitation of ErbB2 in 21MT1 and SKBR3 cells treated with Eeyarestatin I (1 μM) for 4 h.

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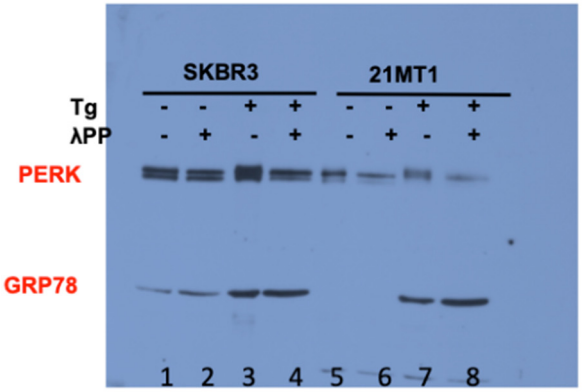
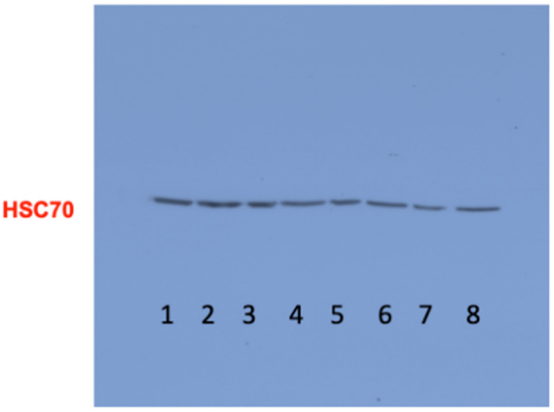
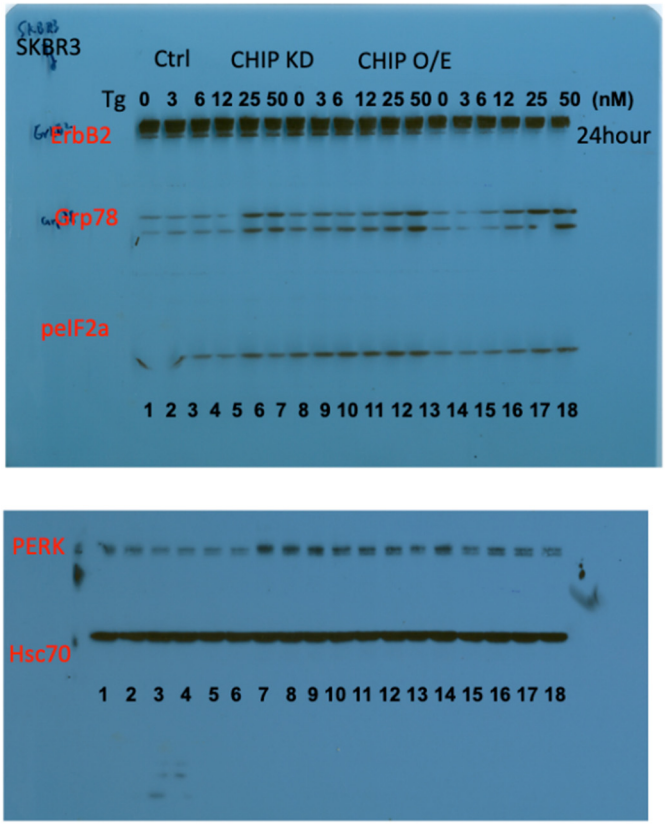
(**A**)





(**B**)


SKBR3
Ctrl
CHIP KD
CHIP O/E
Tg 0 3 6 12 25 50 0 3 6 12 25 50 0 3 6 12 25 50 (nM)
ErbB2
24hour
Grp78
peIF2a
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
PERK
Hsc70
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18

(**C**)

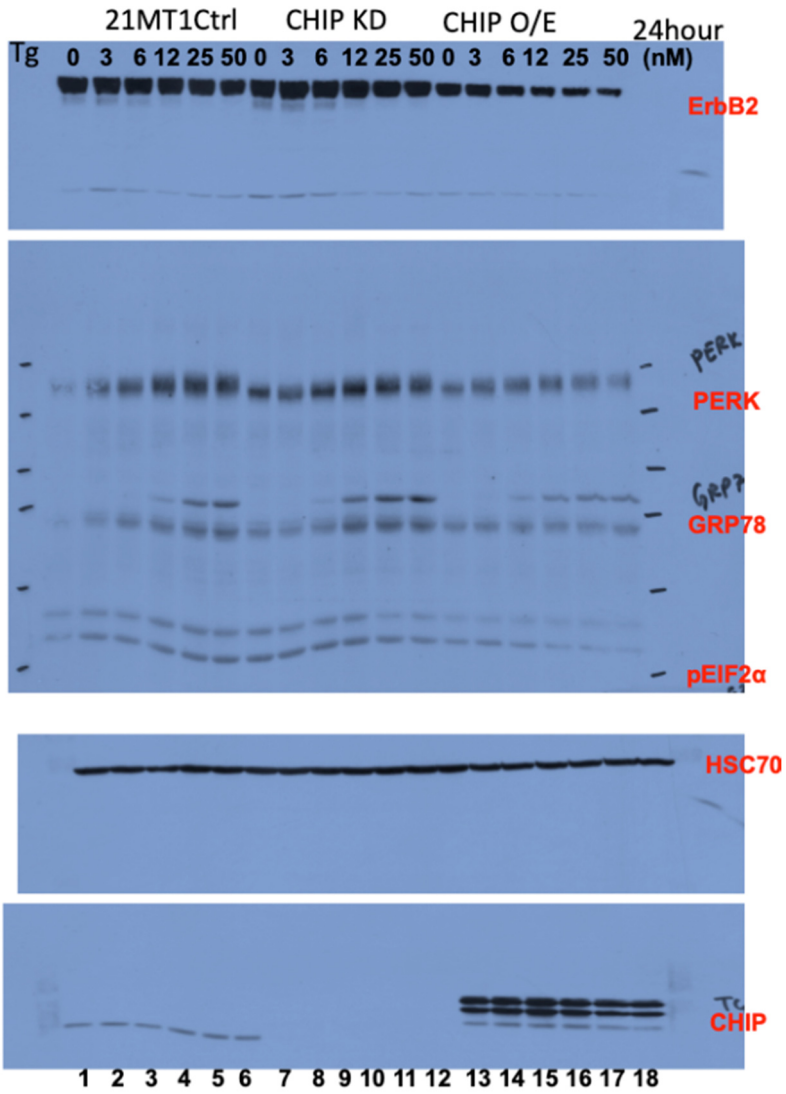
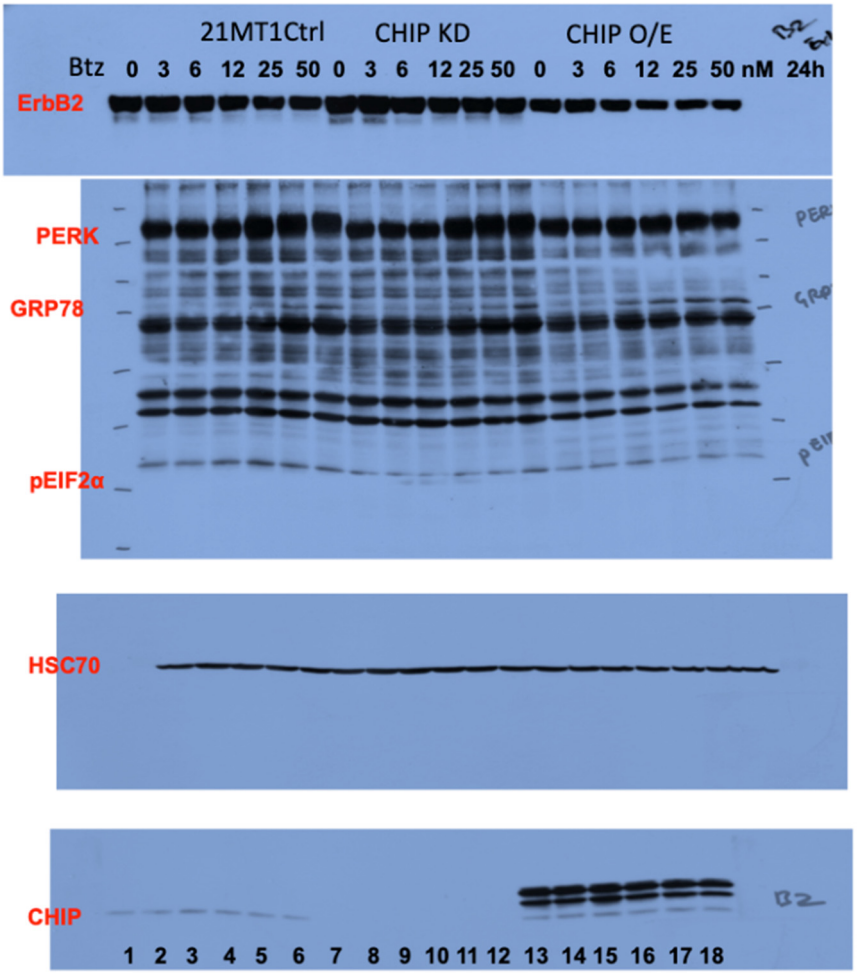


Figure S3. Reduced expression of CHIP sensitizes ErbB2-overexpressing breast cancer cell lines to ER stress. (**A**) Original blots of western blot analysis of SKBR3 and 21MT1 cell treated with thapsigargin (50 nM) for GRP78 and PERK. (**B**) Original blots of western blot analysis of SKBR3 cell with thapsigargin treatment for ER stress markers. (**C**) Original blots of western blot analysis of 21MT1 cell treated with thapsigargin for ER stress markers.

(**A**)


21MT1Ctrl
CHIP KD
CHIP O/E
Btz 0 3 6 12 25 50 0 3 6 12 25 50 0 3 6 12 25 50 nM 24h
ErbB2
PERK
GRP78
pEIF2α
HSC70
CHIP
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18

(**B**)

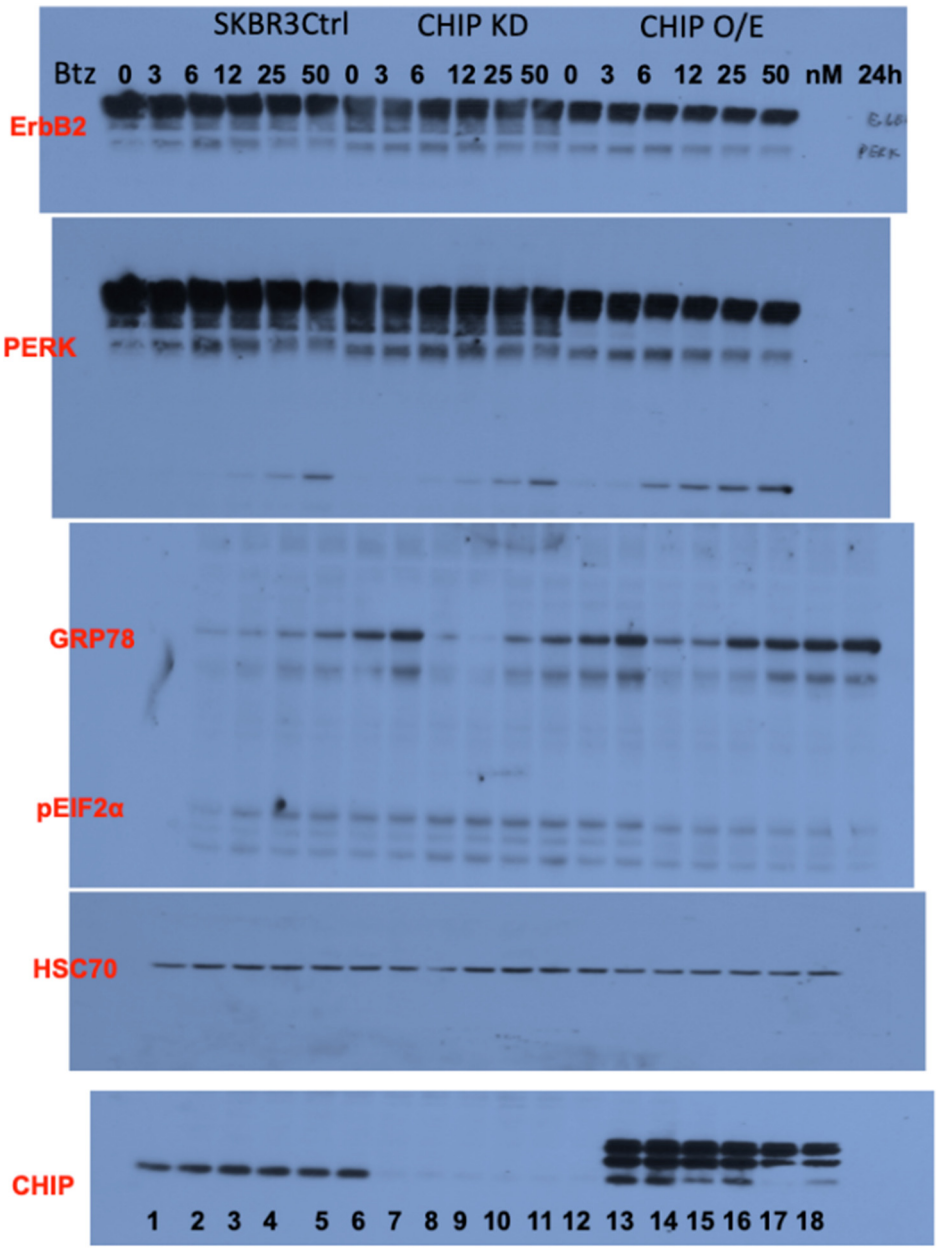


Figure S4. Bortezomib induces ER stress in ErbB2-overexpressing breast cancer cell lines. (**A**) Original blots of western blot analysis of 21MT1 cell treated with Bortezomib treatment for ER stress markers. (**B**) Original blots of western blot analysis of 21MT1 cell treated with Bortezomib treatment for ER stress markers.