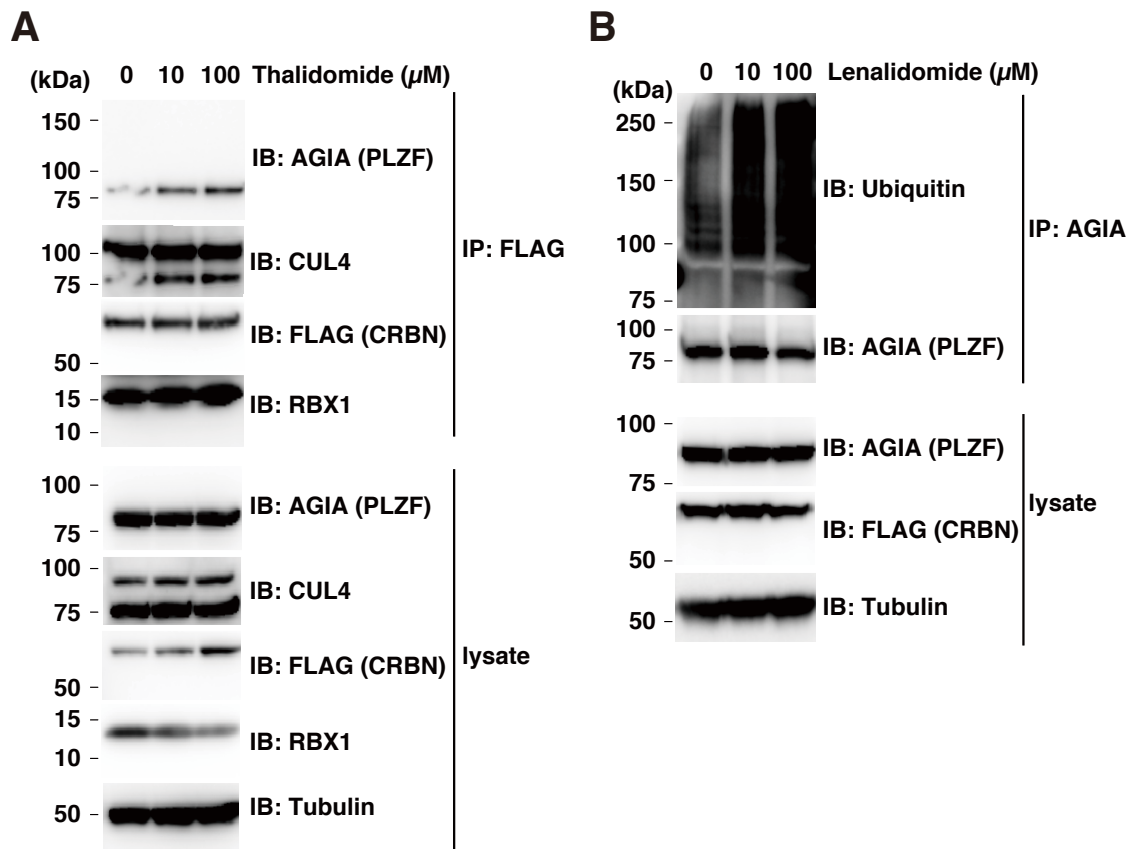


Appendix

PLZF is a CRBN substrate involved in teratogenicity by thalidomide and its metabolite, 5-hydroxythalidomide

Appendix Figure S1 ----- Page 2

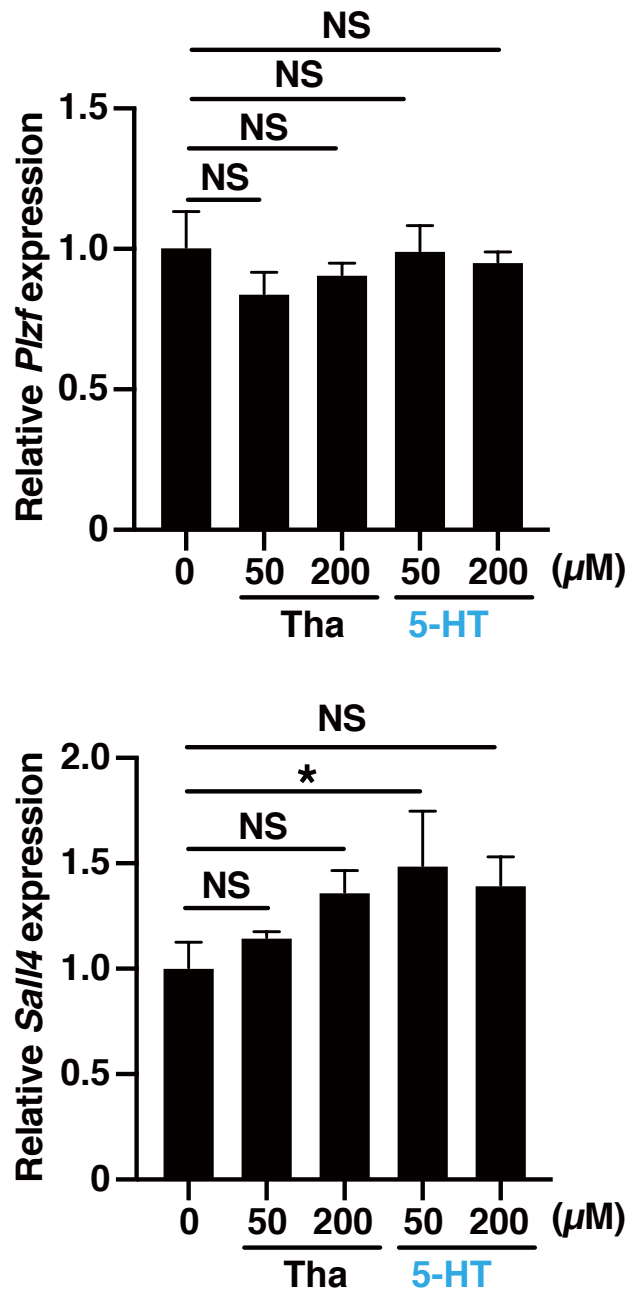
Appendix Figure S2 ----- Page 3



Appendix Figure S1. PLZF is a substrate of CUL4^{CRBN} with thalidomide and lenalidomide for E3 ubiquitin ligase

(A) Immunoprecipitation of FLAG-CRBN in FLAG-CRBN and AGIA-PLZF expressing CRBN^{-/-} HEK293T cells treated with DMSO or thalidomide in the presence of DMSO or MG132 for 8 h. Components of CUL4^{FLAG-CRBN} and AGIA-PLZF were detected using specific antibodies, as indicated.

(B) Ubiquitination of AGIA-PLZF in AGIA-PLZF and FLAG-CRBN expressing HEK293T cells treated with DMSO or lenalidomide in the presence of DMSO or MG132 for 10 h.



Appendix Figure S2. Effect of thalidomide or 5-hydroxythalidomide on mRNA expression of PLZF or SALL4 in HuH7 cells

HuH7 cells were treated with the indicated concentrations of DMSO, thalidomide (Tha), or 5-hydroxythalidomide (5-HT) for 24 h. *PLZF* or *SALL4* mRNA expression levels were measured by quantitative RT-PCR.

Relative mRNA expression used the expression level with DMSO treatment as one. Error bars denote $\pm$ standard deviation ($n = 3$). *P*-values were calculated by one-way ANOVA with Tukey's post-hoc test (NS = Not Significant, and $*P < 0.05$).