Target Identification

RESEARCH ARTICLE

Identification of a Primary Target of Thalidomide Teratogenicity

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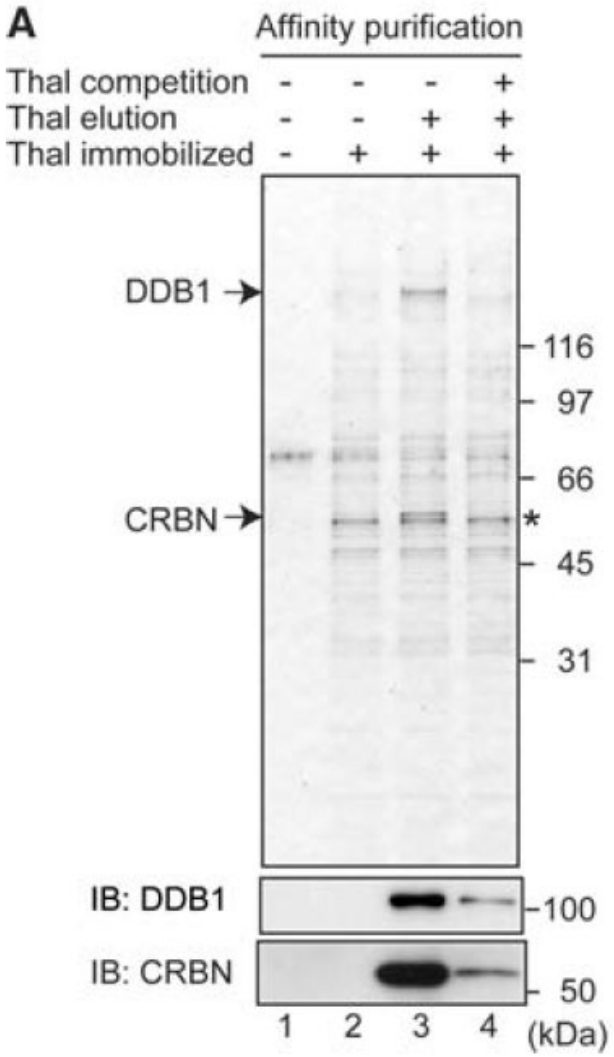
Half a century ago, thalidomide was widely prescribed to pregnant women as a sedative but was found to be teratogenic, causing multiple birth defects. Today, thalidomide is still used in the treatment of leprosy and multiple myeloma, although how it causes limb malformation and other developmental defects is unknown. Here, we identified cereblon (CRBN) as a thalidomide-binding protein. CRBN forms an E3 ubiquitin ligase complex with damaged DNA binding protein 1 (DDB1) and Cul4A that is important for limb outgrowth and expression of the fibroblast growth factor Fgf8 in zebrafish and chicks. Thalidomide initiates its teratogenic effects by binding to CRBN and inhibiting the associated ubiquitin ligase activity. This study reveals a basis for thalidomide teratogenicity and may contribute to the development of new thalidomide derivatives without teratogenic activity.

During the late 1950s and early 1960s, thalidomide was sold as a sedative in over 40 countries and was often prescribed to pregnant women. It was found to be teratogenic, causing multiple birth defects (*1*–*3*). Today, thalidomide is still used in the treatment of leprosy and multiple myeloma, although how it causes limb malformation and other developmental defects is unknown. Elucidation of the molecular mechanism of thalidomide is desirable for wider applications of this potentially useful drug. It is important to

Binding of thalidomide to CRBN and DDB1. To purify thalidomide-binding proteins, we performed affinity purification using ferrite-glycidyl methacrylate (FG) beads (*9*). The carboxylic thalidomide derivative FR259625 was covalently conjugated to the beads (fig. S1) and incubated with human HeLa cell extracts (*10*). After extensive washing, bound proteins were eluted with free thalidomide, and the eluate fractions were subjected to SDS gel electrophoresis and silver staining. Two polypeptides were specifically eluted (Fig. 1A, lane 3). When free thalidomide was added to extracts before incubation with the beads, the yields of these proteins were reduced (Fig. 1A, lane 4), which suggested that these proteins specifically interact with thalidomide. The 127- and 55-kD proteins were therefore subjected to proteolytic digestion and tandem mass spectrometry and were identified as CRBN and damaged DNA binding protein 1 (DDB1), respectively (table S1). Identities of these proteins were confirmed by immunoblotting (Fig. 1A). CRBN and DDB1 were isolated similarly as thalidomide-

Relevant for exam: Figures 1 and 2

Figure 1a



- Analysis of proteins:

  Proteins were analyzed on an SDS-PAGE gel. How are the protein visualized? Color? Western Blot?

- Purification of proteins:

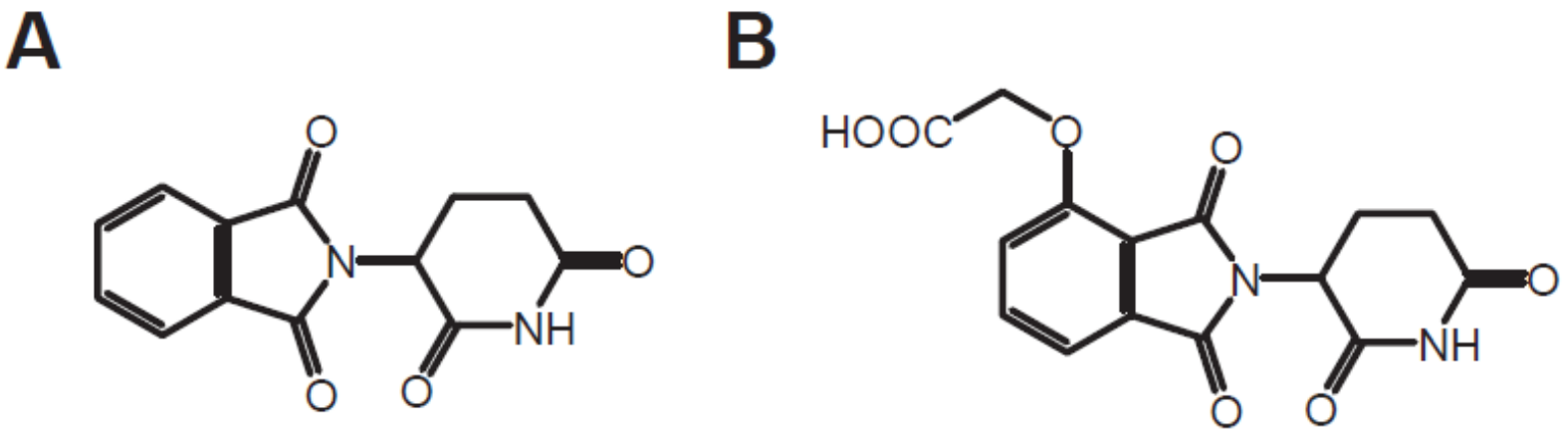
  The proteins were obtained from HeLa cells. How were they purified?

- Thalidomide immobilization:

  How was thalidomide immobilized?

Supplementary Figures (no need to prepare)

SI Figure 1a and 1b



SI Figure 1c

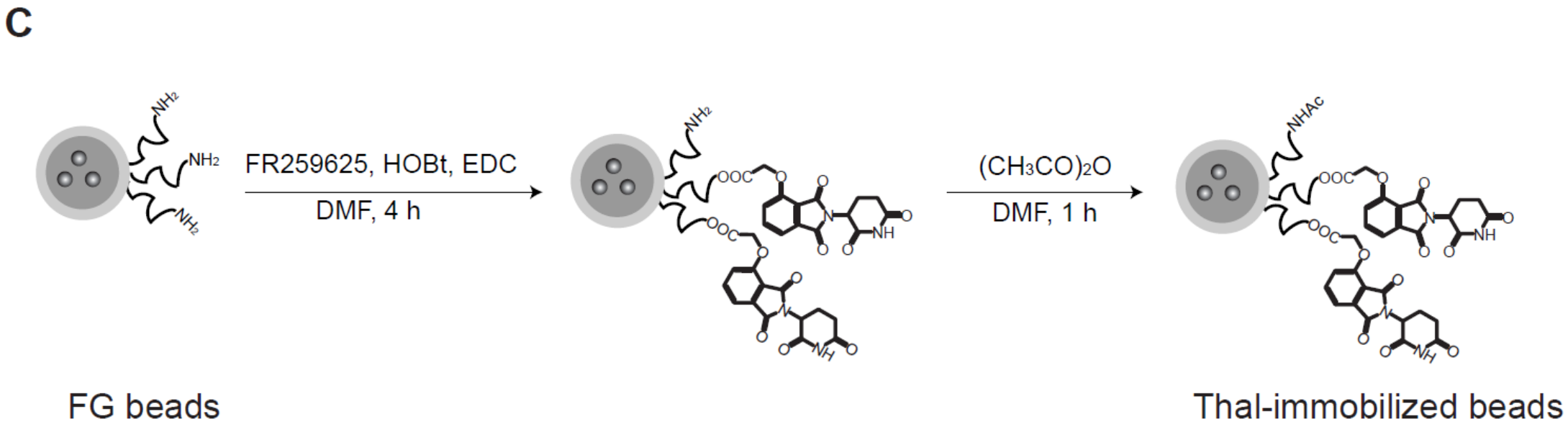
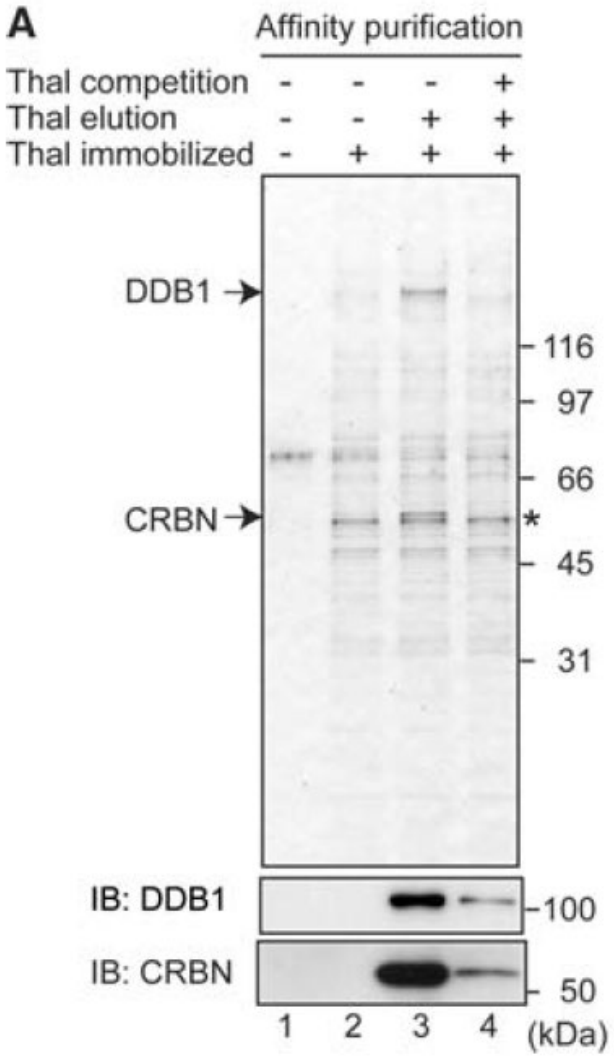

c
NH2
NH2
NH2
FR259625, HOBt, EDC
DMF, 4 h
OOC
NH
(CH3CO)2O
DMF, 1 h
NHAc
FG beads
Thal-immobilized beads

Figure 1a



- Controls:

  Lanes 1, 2 and 4 are controls. Why were they performed?

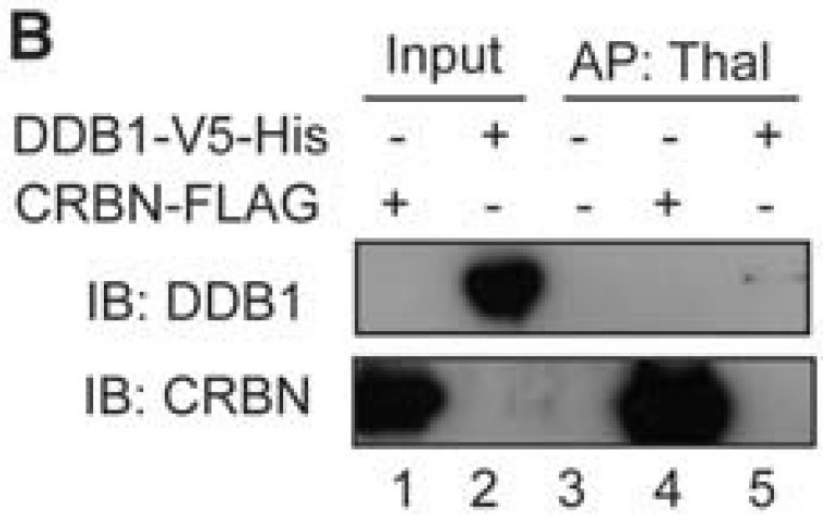
- Thalidomide binders:

  Which bands are selectively purified with thalidomide? How were they identified?

- Western blot:

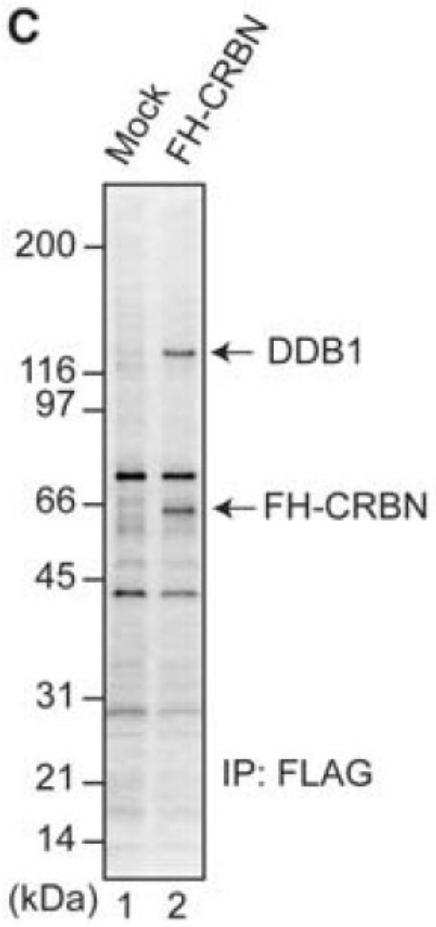
  Why? How?

Figure 1b



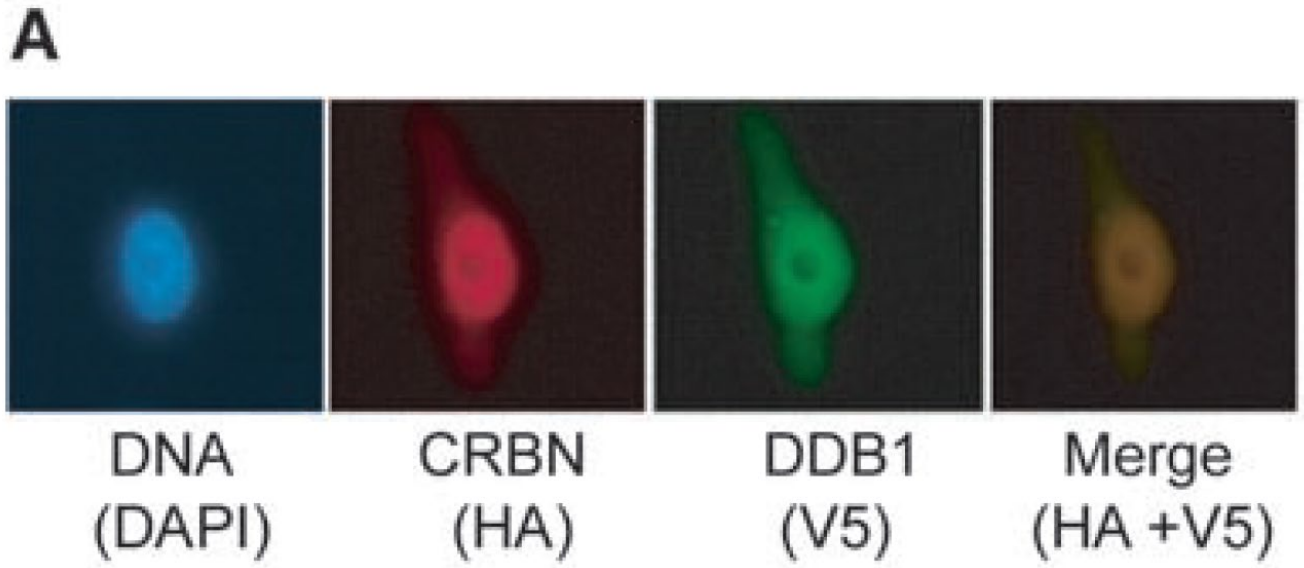
- Which question was addressed by this experiment?
- What is V5? His? FLAG? IB?
- «Input»: Why is this control performed?
- «AP: Thal»: What is this?
- What came out in this experiment?

Figure 1c



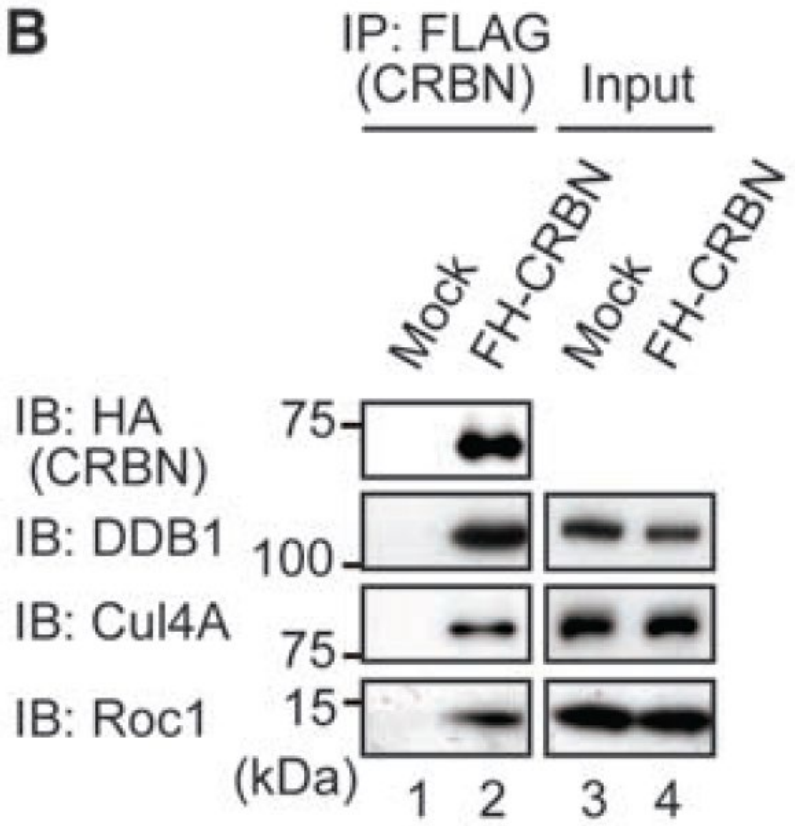
- Which question was addressed by this experiment?
- What is FH? FH-CRBN?
- How was this experiment performed (immunoprecipitation)?
- What is the «Mock» control?
- What came out in this experiment?

Figure 2a



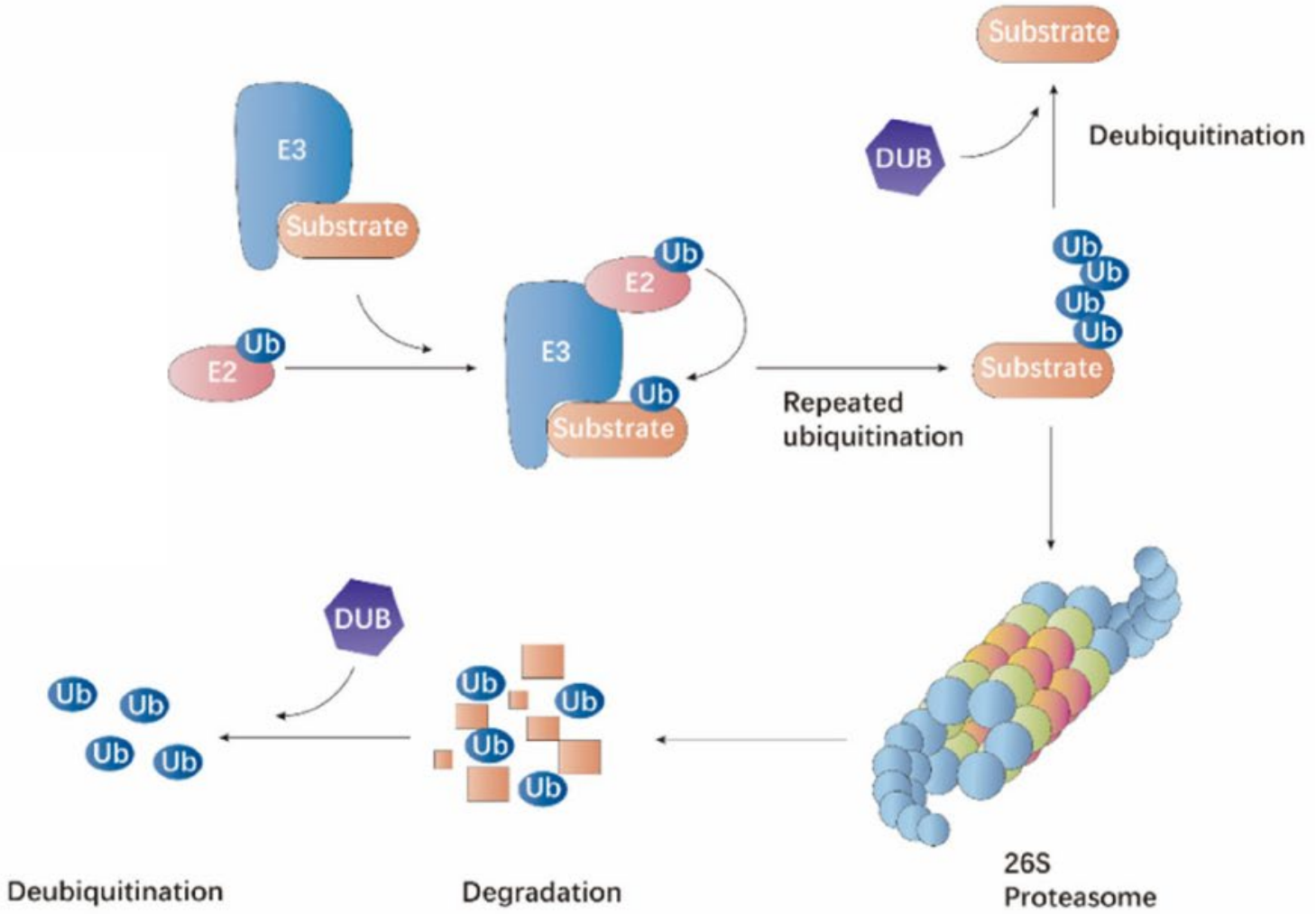
- Which question was addressed by this experiment?
- How was this experiment performed? What is DAPI? HA? V5? Merge?

Figure 2b



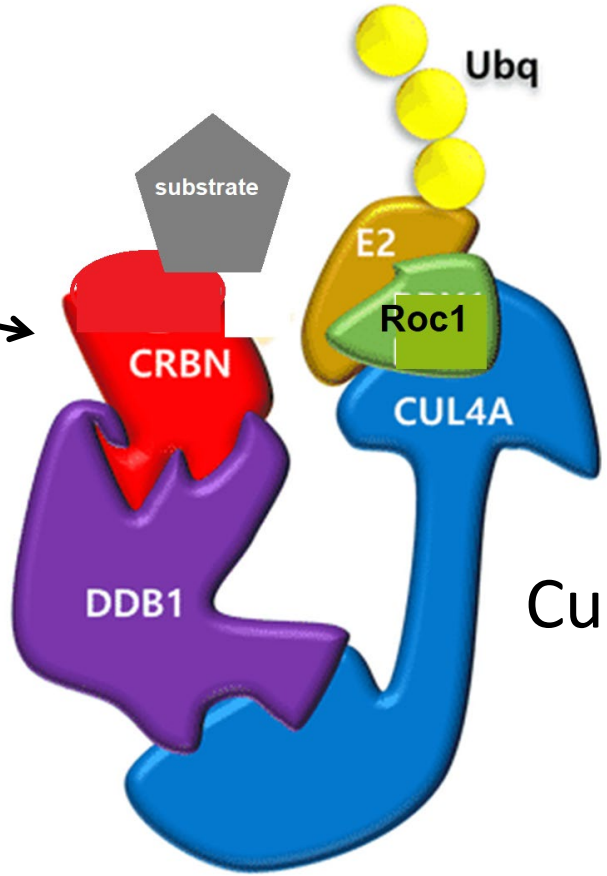
- Which question was addressed by this experiment?
- How was this experiment performed (immunoprecipitation)?
- What is the «Mock» control?
- What came out in this experiment?

E3 ligase: background information



E3 ligase: background information

substrate receptor?



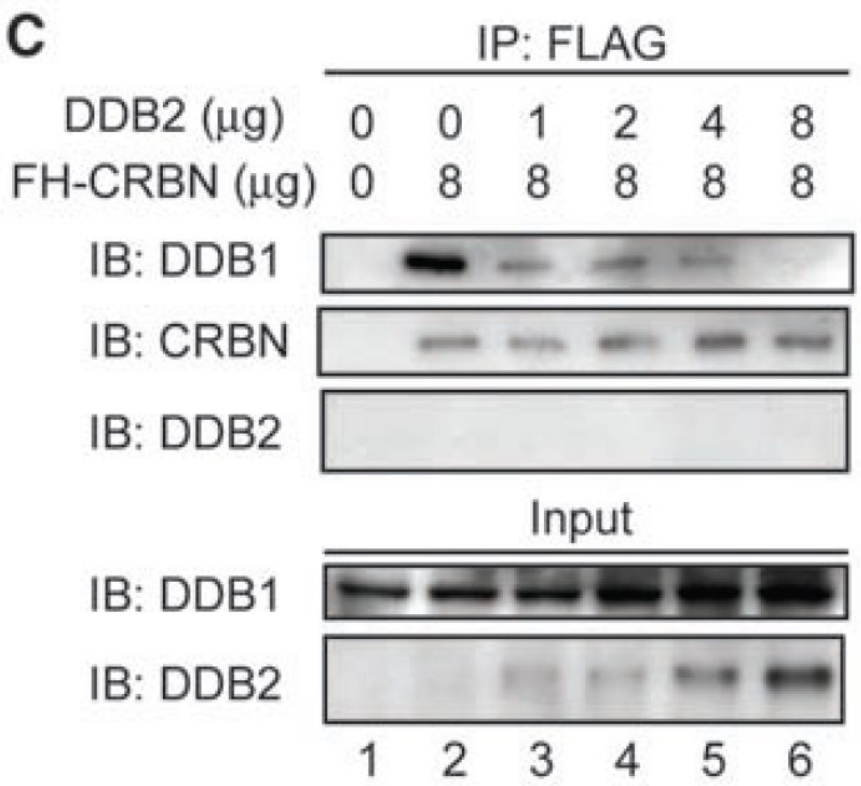
E2: ubiquitin ligase

Roc1: regulator of cullin 1

Cullin 4: scaffold function

DDB1: known component of E3 ligase complex

Figure 2c



substrate receptor

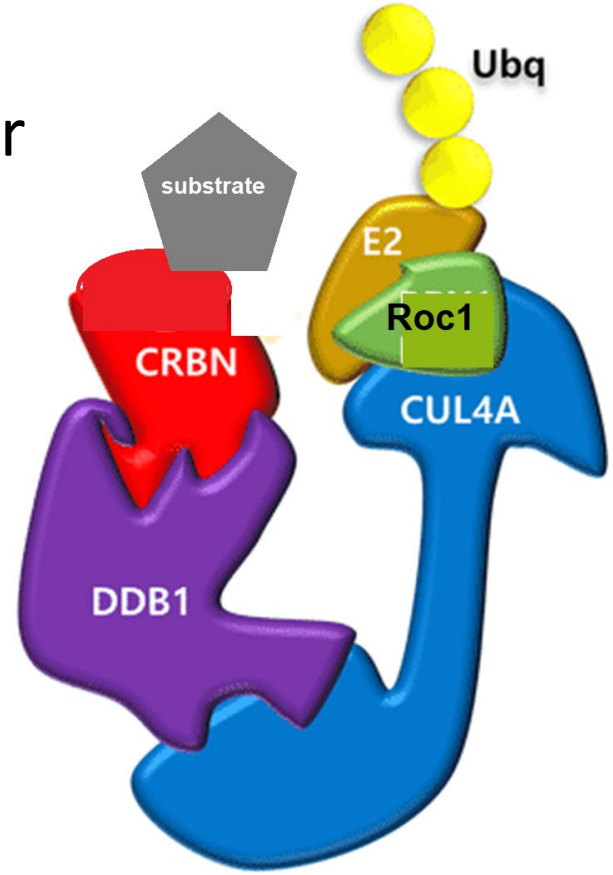
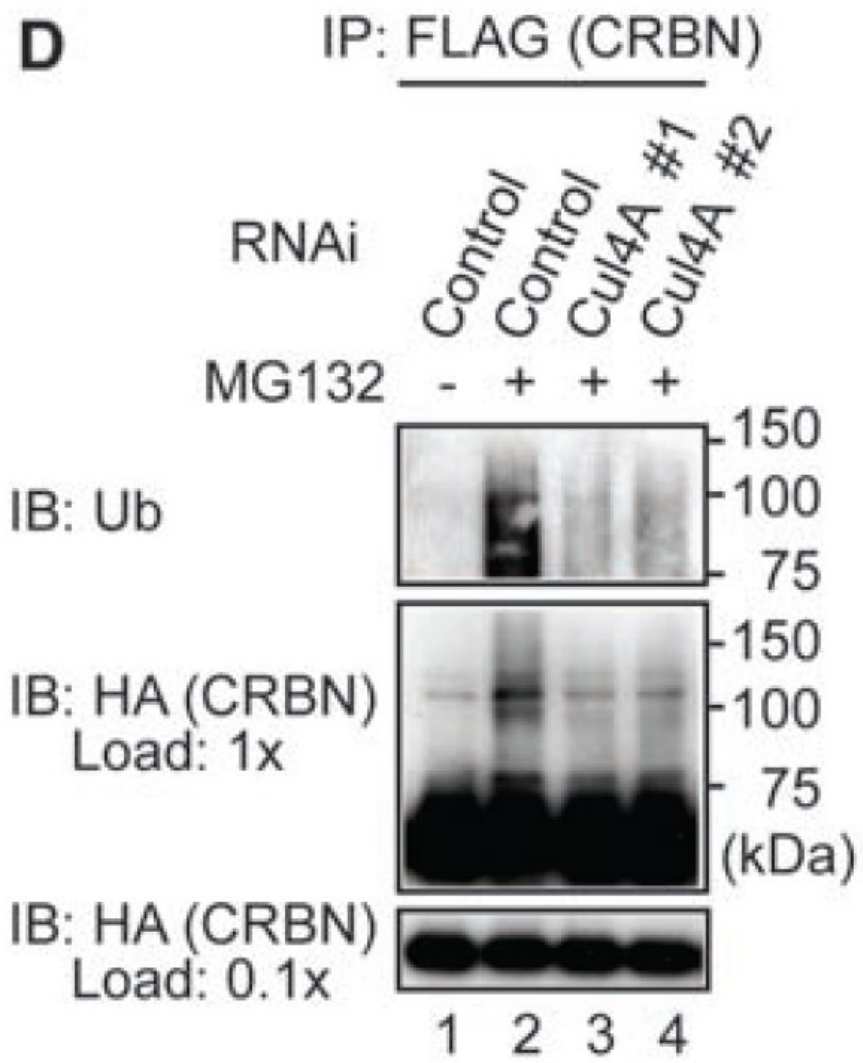
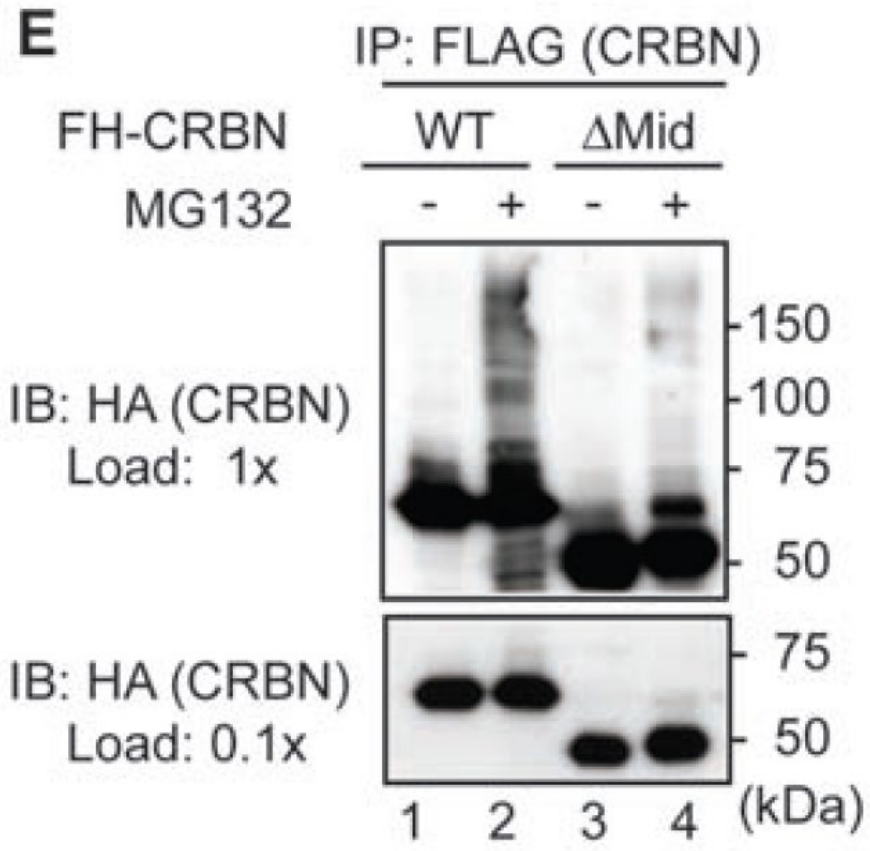


Figure 2d

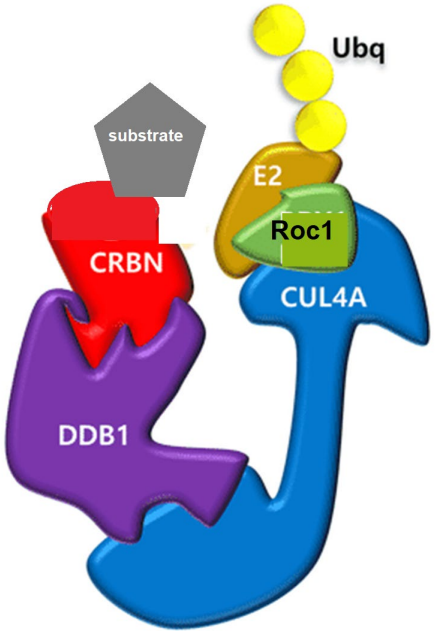


- Which question was addressed by this experiment?
- How was this experiment performed? What is MG132?
- What came out in this experiment?

Figure 2e



- Which question was addressed by this experiment?
- What is Δmid?

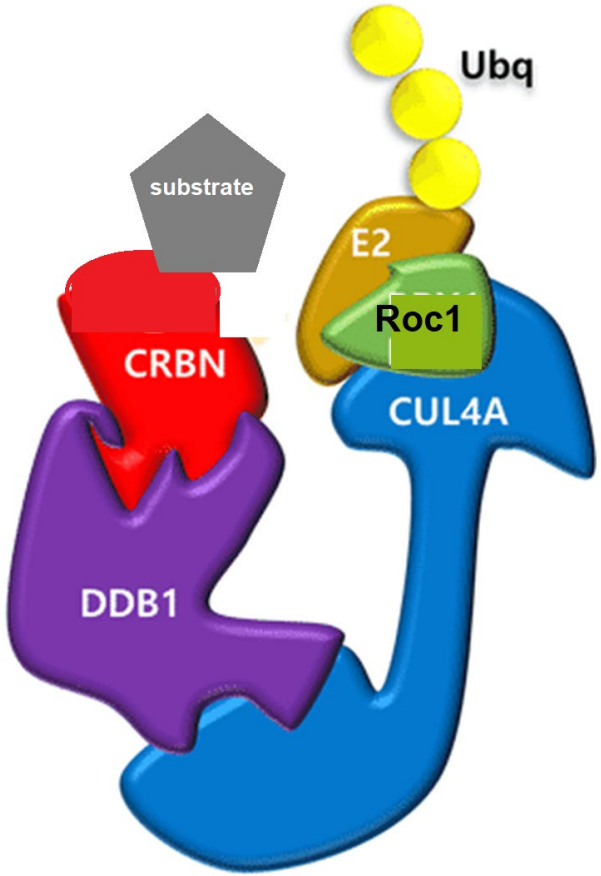


Key conclusions of study

- Thalidomide binds cereblon
- Thalidomide inhibits autoubiquitination
- Teratogenicity of thalidomide is likely mediated via cereblon binding

Open questions after the study

- What is the substrate of cereblon (that gets ubiquitinated)
- Molecular mechanism by which thalidomide inhibits ubiquitination



Impact

- Thalidomide is widely used as E3 ligase ligands for PROTAC development!

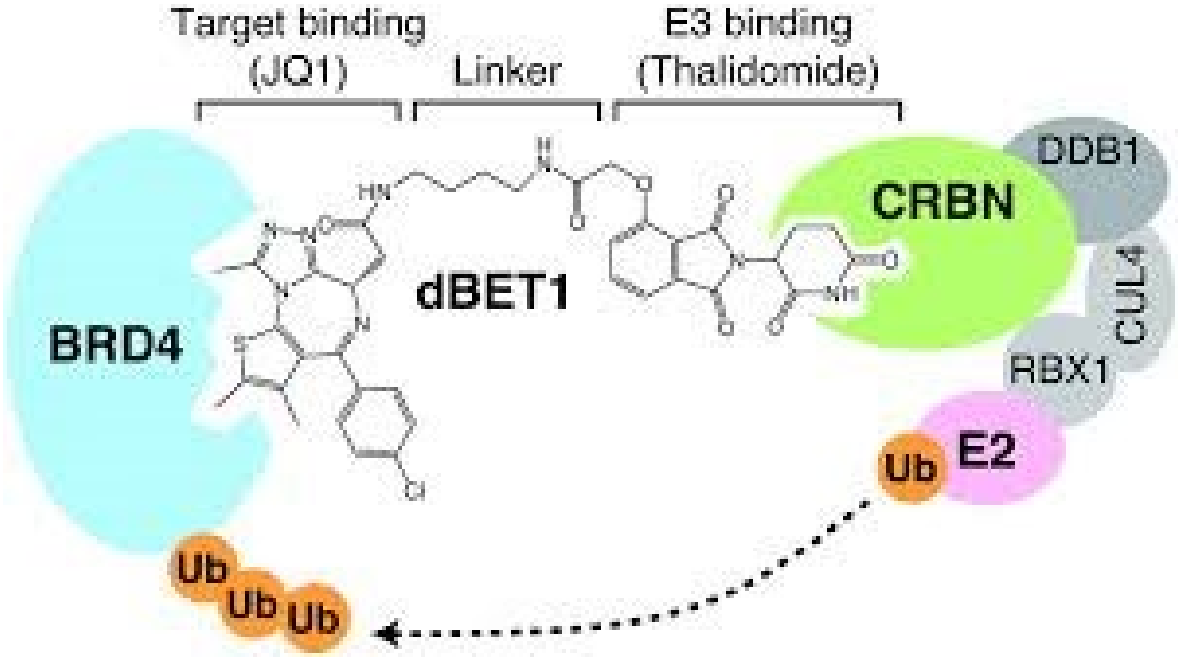
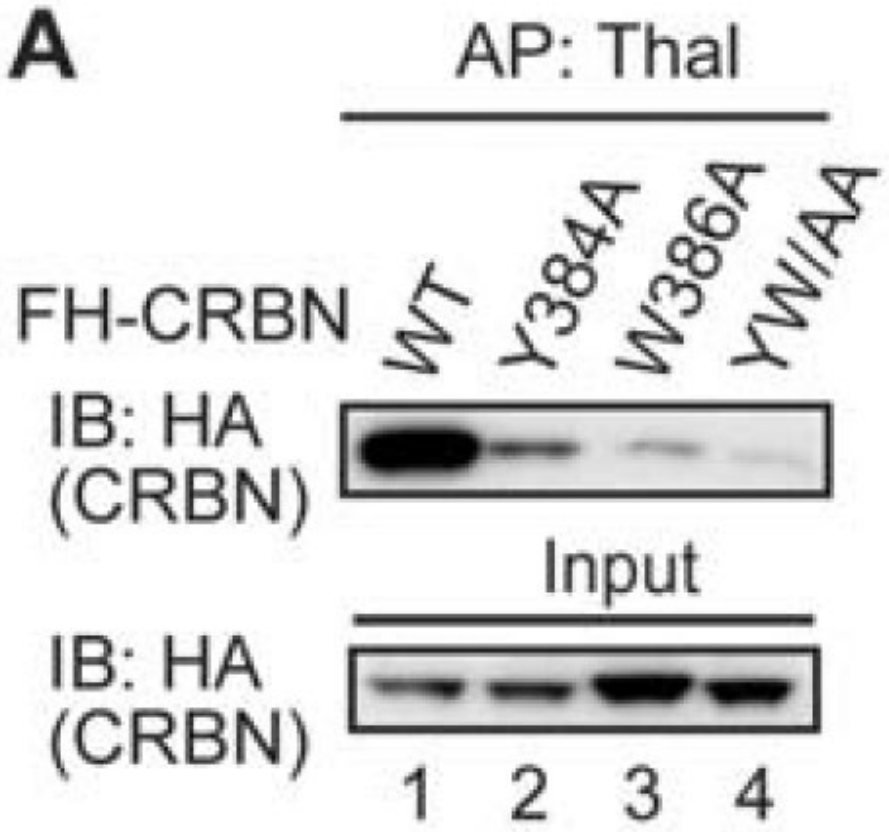
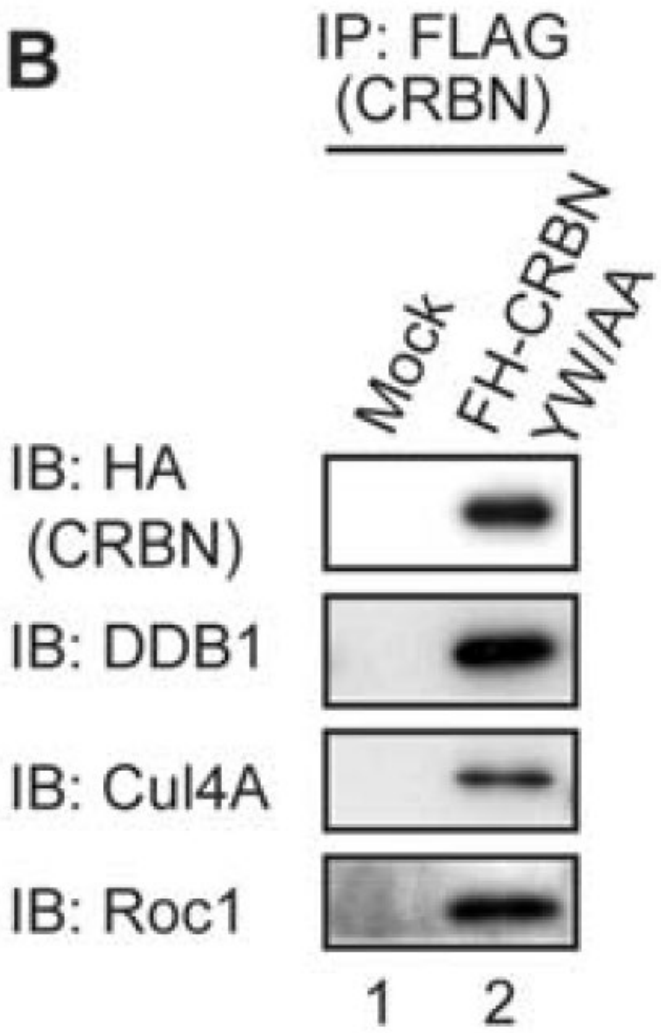


Figure 3a



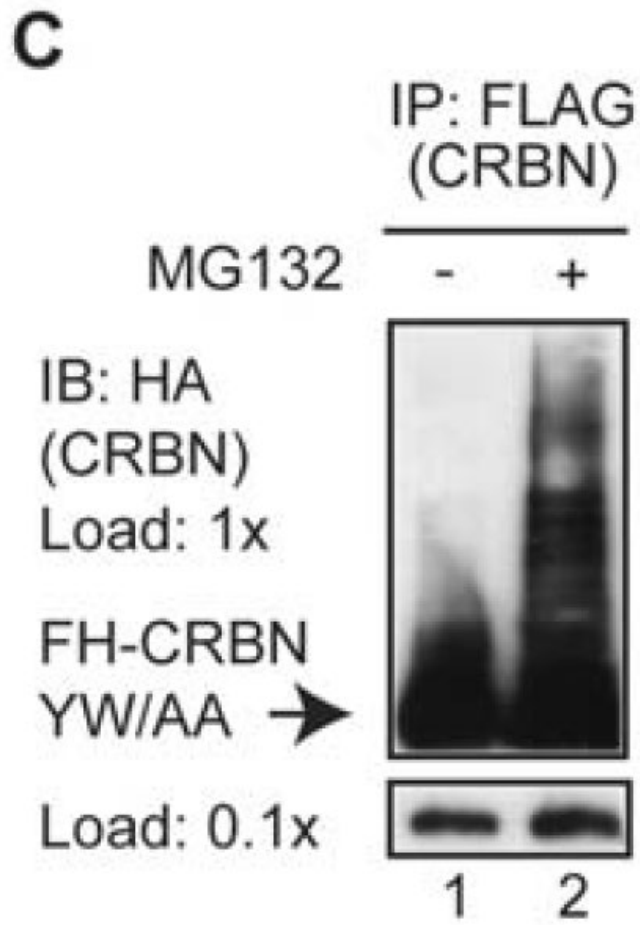
- What are the mutants Y384A and W386A?
- Why are they of interest?
- What was shown with this experiment?

Figure 3b



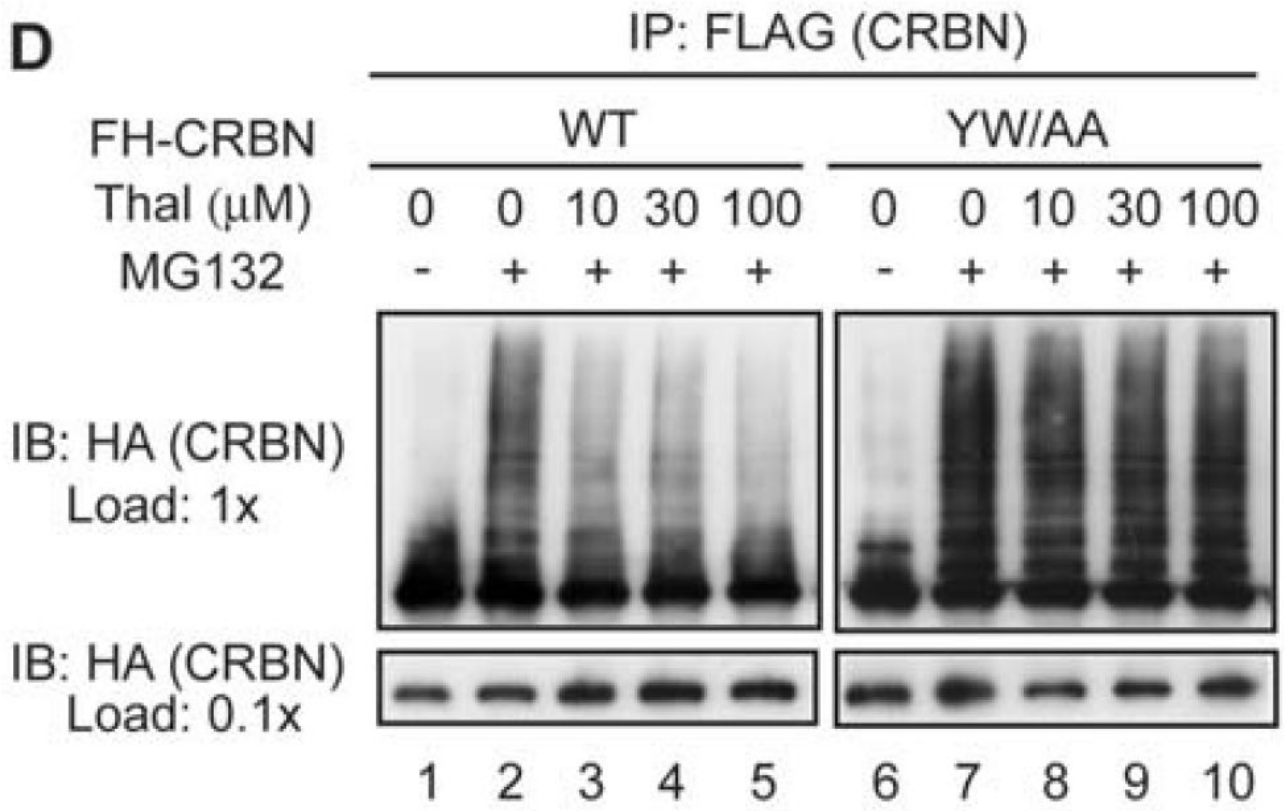
- What was shown with this experiment?

Figure 3c



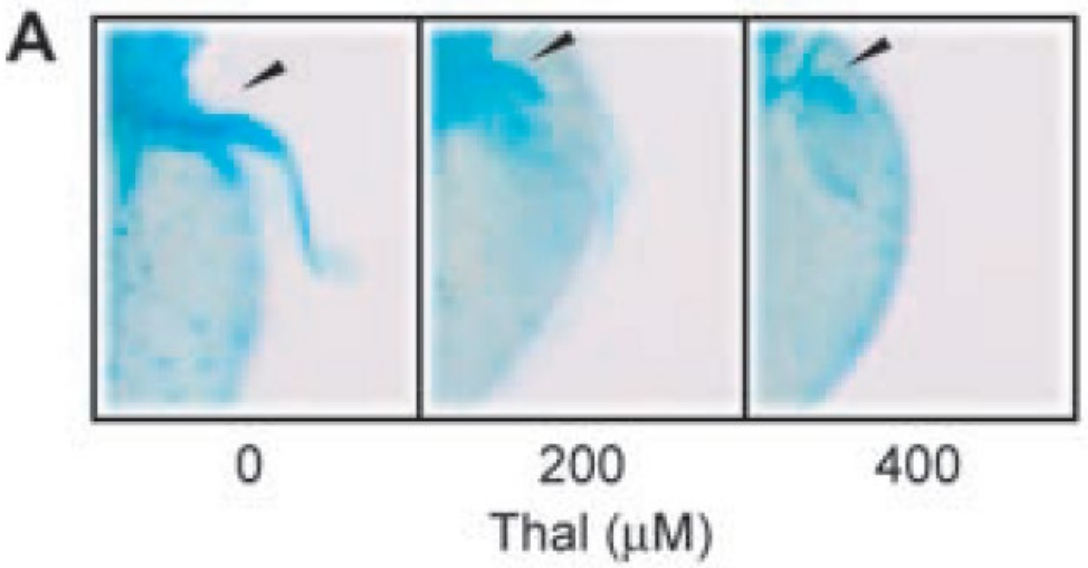
- What was shown with this experiment?

Figure 3d



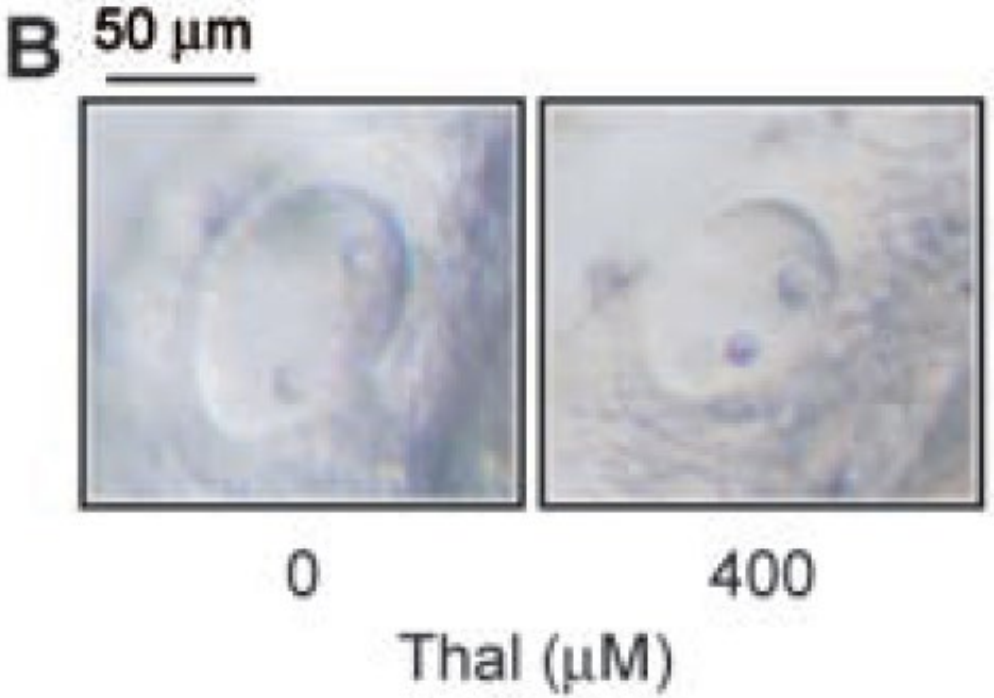
- What was shown with this experiment?

Figure 4a



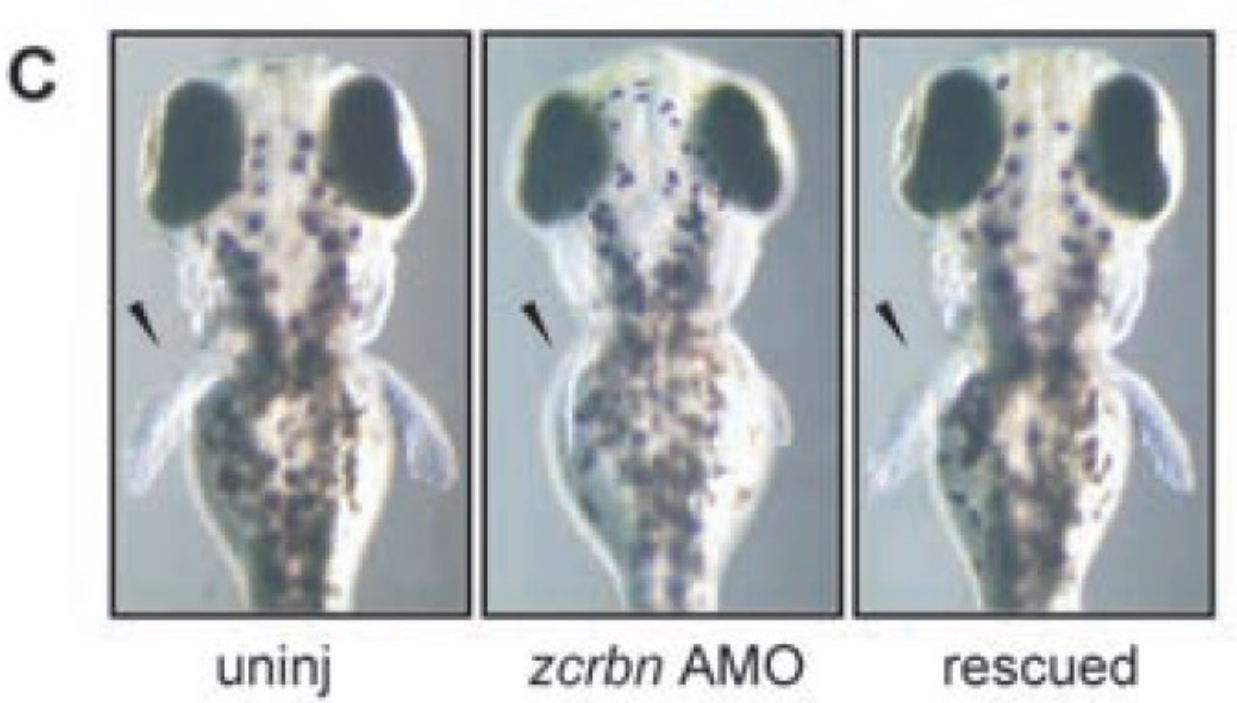
- What was done in this experiment?
- What effect did thalidomide have on the pectoral fins?

Figure 4b



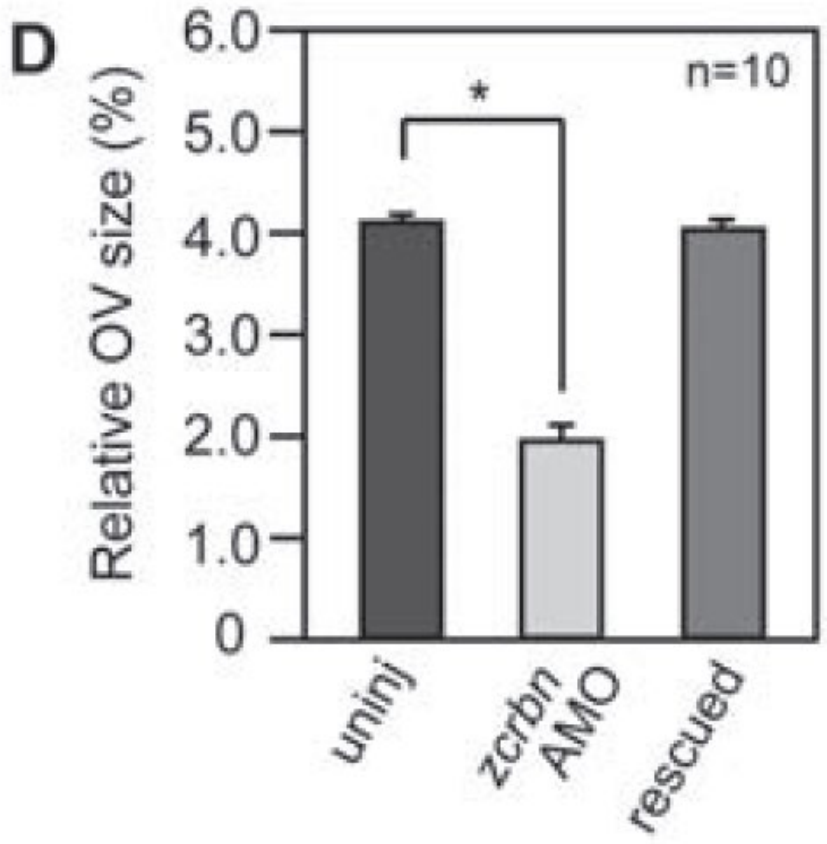
- What was done in this experiment?
- What effect did thalidomide have on the otic vesicles?

Figure 4c



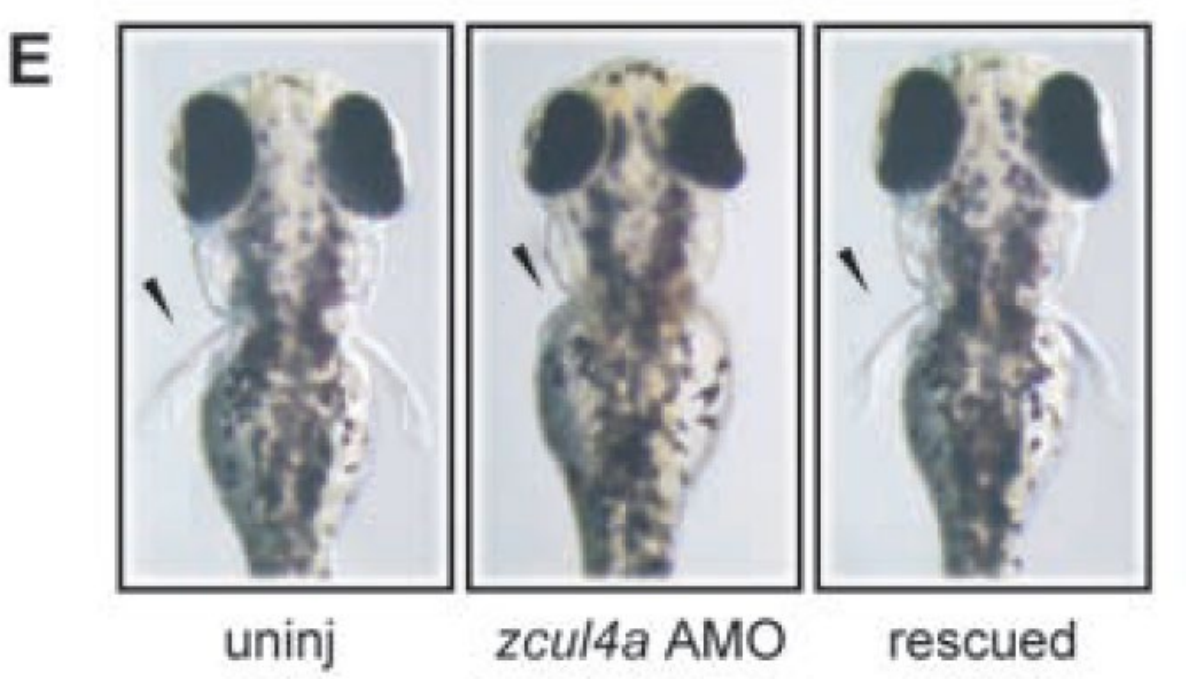
- What was done in this experiment?
- What is AMO?

Figure 4d



- What does this bar graph show?
- What is «rescued»?

Figure 4e



- What is different in this experiment compared to Fig. 4c?

Figure 4f

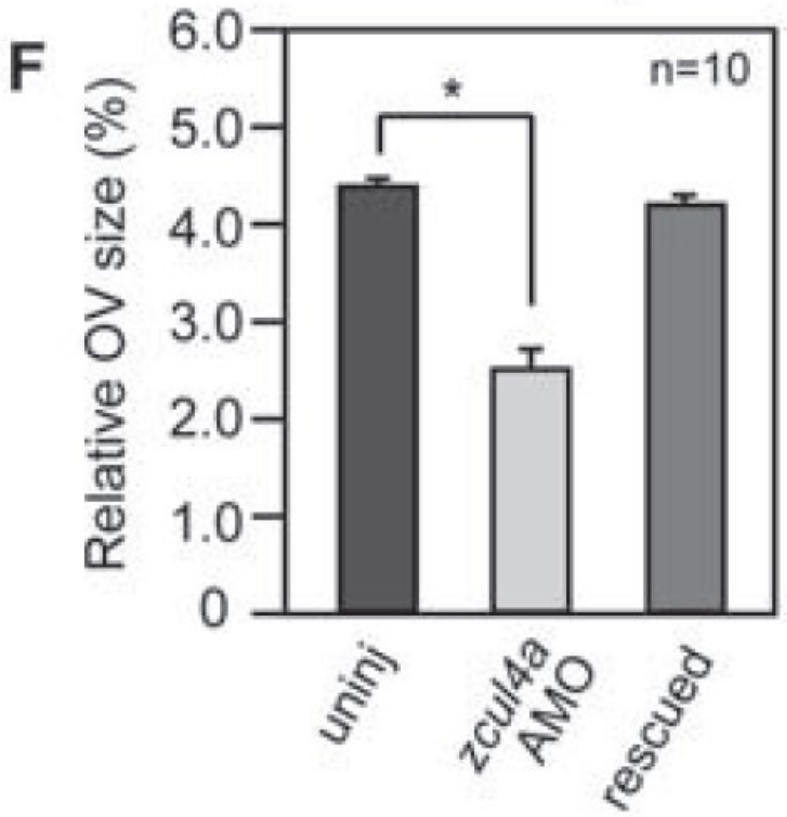

F
Relative OV size (%)
6.0
5.0
4.0
3.0
2.0
1.0
0
*
n=10
uninj
zcul4a AMO
rescued

Figure 5a

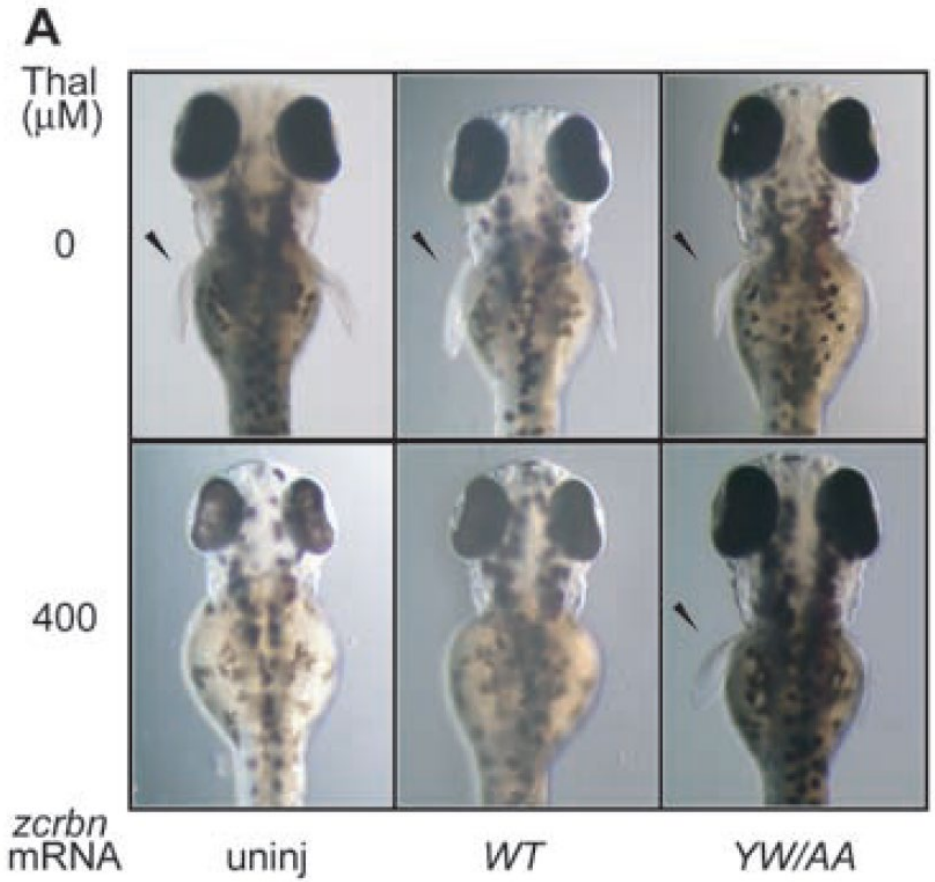


Figure 5b

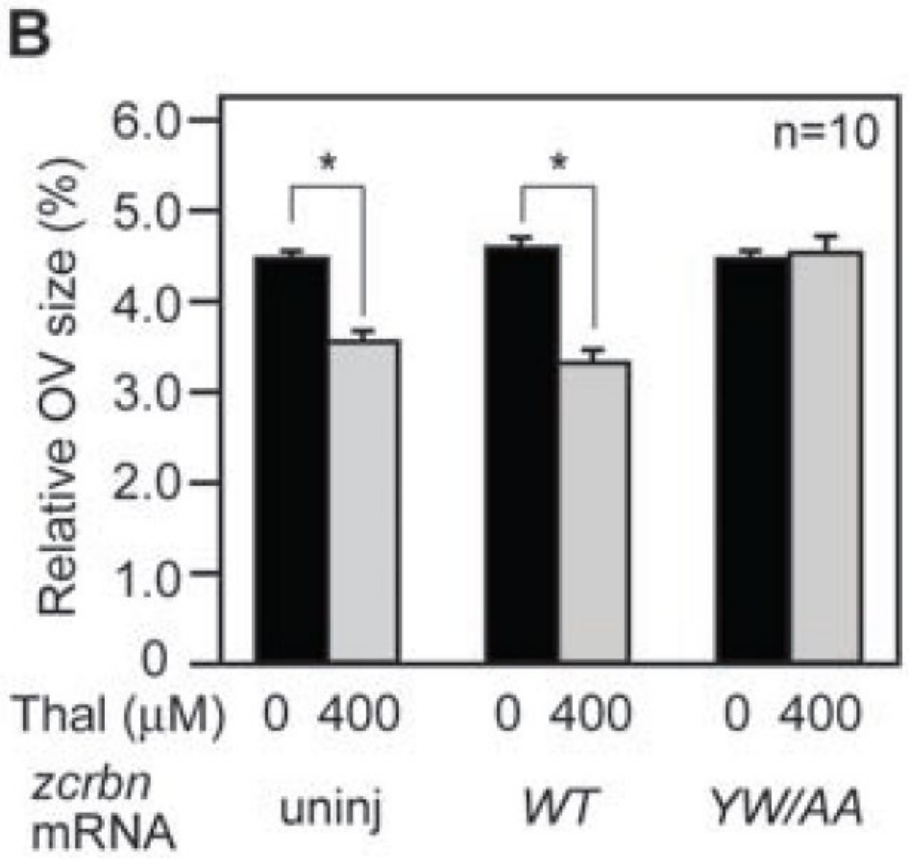

B
Relative OV size (%)
6.0
5.0
4.0
3.0
2.0
1.0
0
n=10
*
*
Thal (μM) 0 400 0 400 0 400
zcrbn mRNA
uninj
WT
YW/AA

Figure 5c

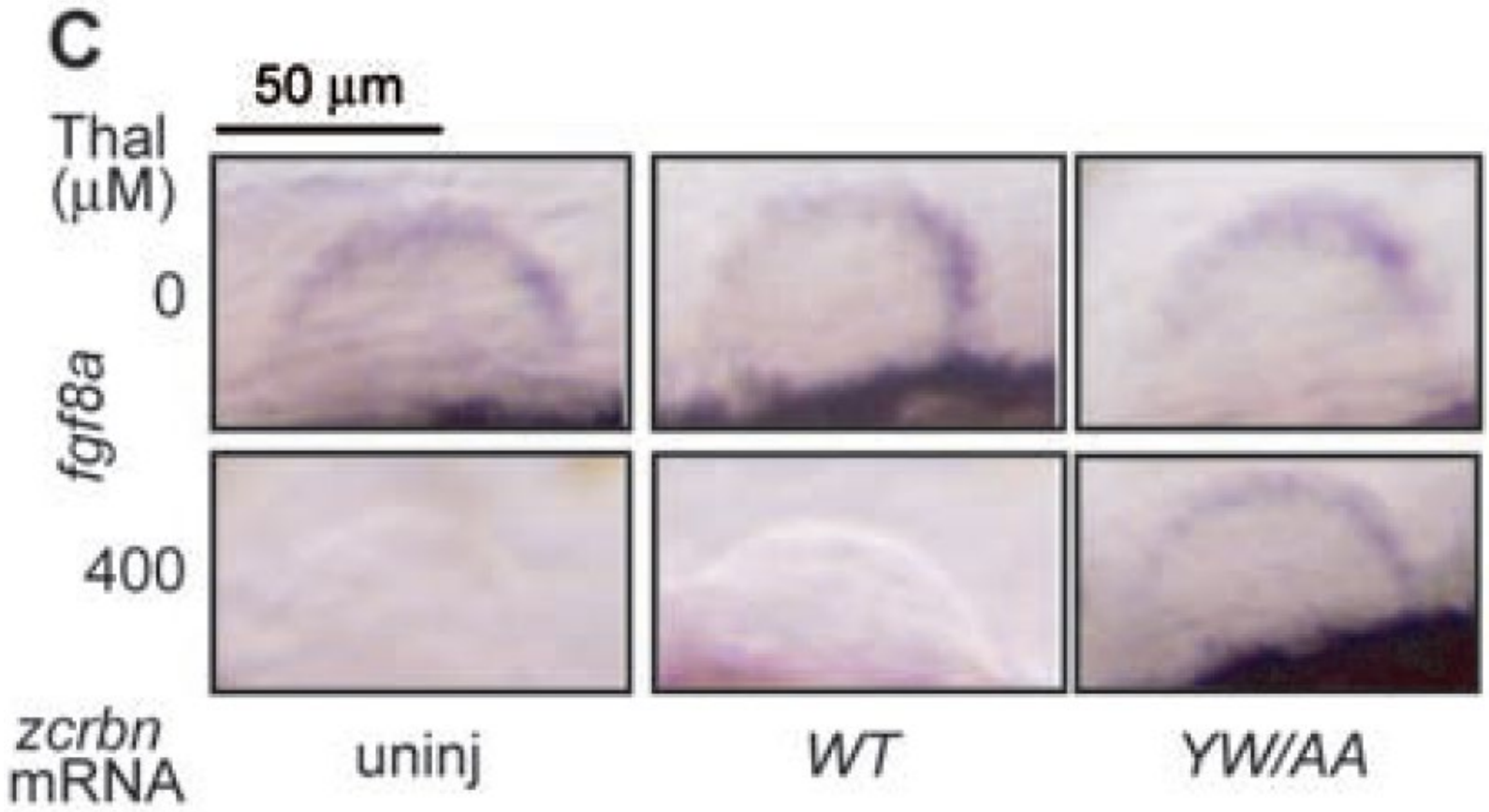


Figure 5d

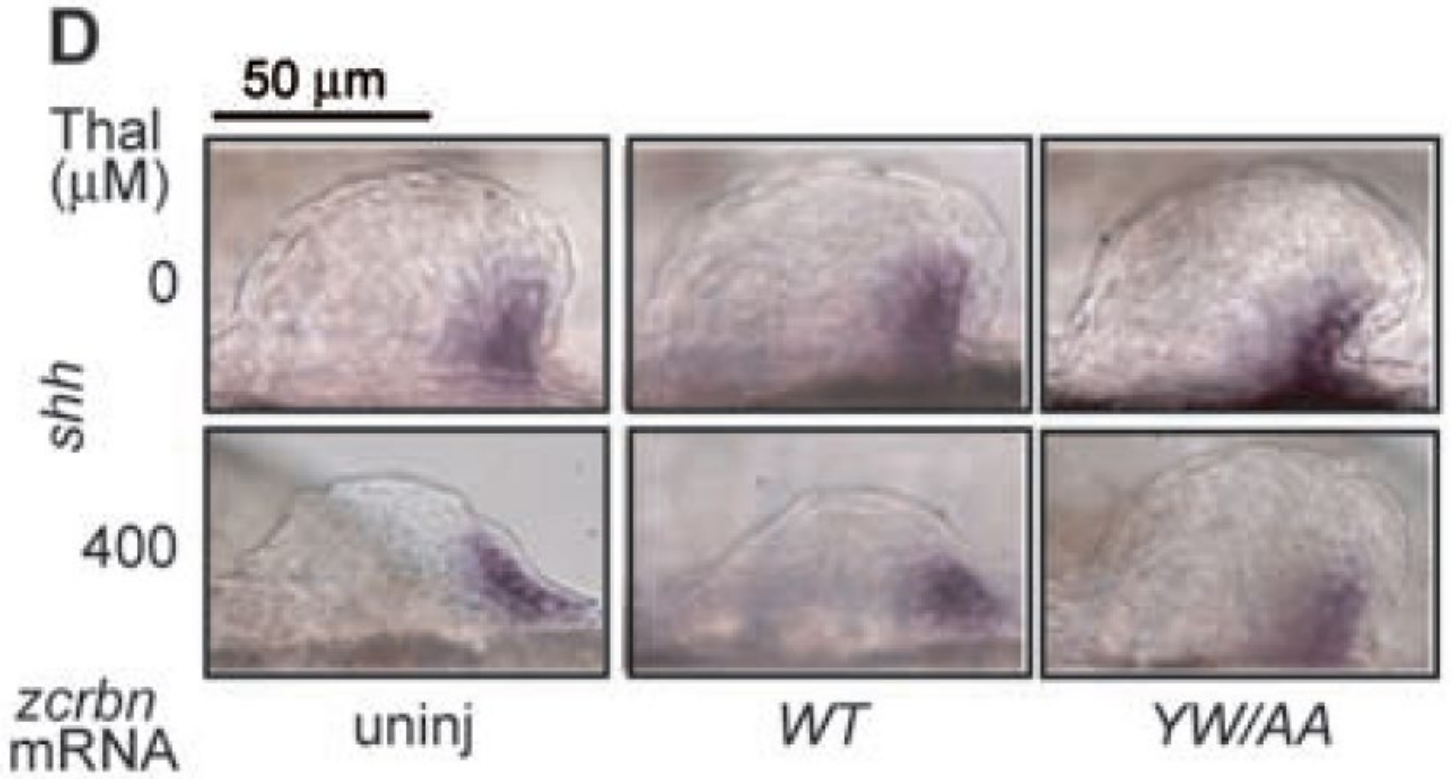


Figure 6a

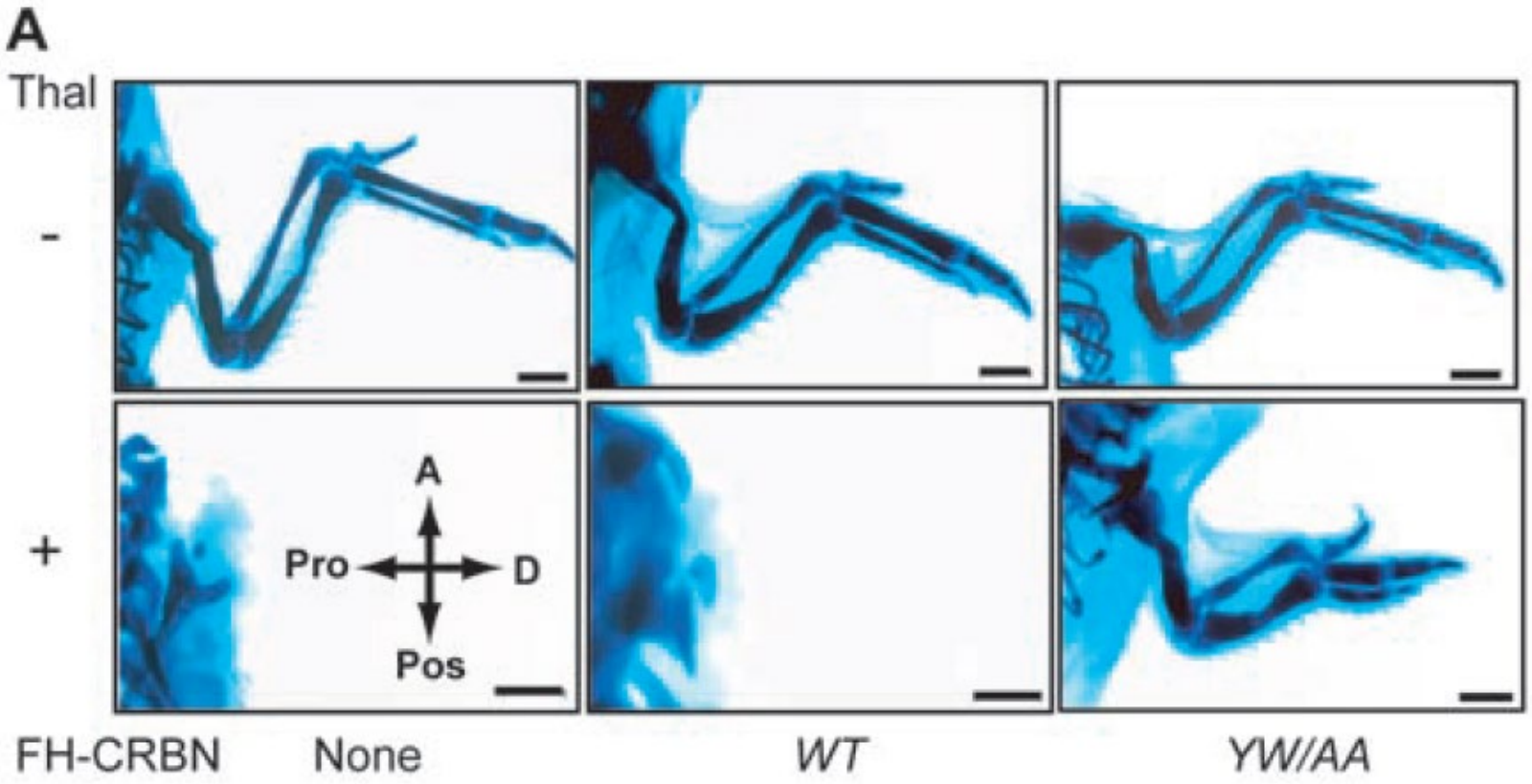


Figure 6b