H23年度3年実習レポート図一覧

平成23年10月4日から13日にかけて、斉藤担当の実習「DNA塩基配列の 決定とタンパク質相互作用の解析」が行われました。

実習では3つの目標達成を目指しました。

1:DNA塩基配列の決定法の原理を学び、実際に塩基配列を解析する

2:タンパク質の相互作用の解析の原理を学び、Bead halo法を経験する

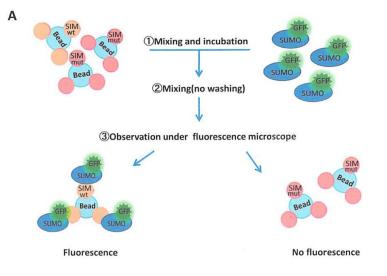
3:英語でレポートを作成する

1と2の目標達成は実習期間中にお互いで確認しました。

さて、以下に、皆さんから提出してもらったレポートの図を公開します。お 互いの図を比較しながら、皆さんが目標3も達成したことをお互いに確認しま しょう。

実習とレポート作成、大変ご苦労様でした。 斉藤 寿仁

PS: 皆さんの図がそれぞれ異なって、どれ一つ同じものが無いことに は驚きました。どれも大変上手に、見やすくまとまっていました。



B GST-MCAF1-SIM(Wt) GVIDLTMDDEE GST-MCAF1-SIM(Mut) GVAHLTMDDEE

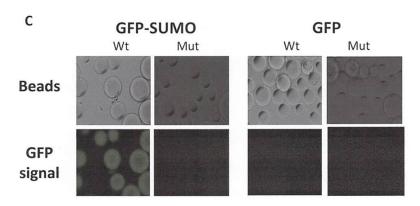
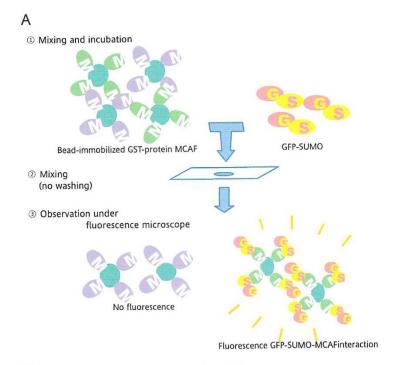


Fig Legend

- A Schematic representation of the procedure for the real-time SUMO binding assay.
- B Comparison of sequence of amino acid between SIMwt and SIMmut.
- C Bacterial Tysate containing GFP-SUMO(1st and 2nd columns) or GFP (3rd and 4 th columns). Lower panels show the phase-contrast images of the Sepharose beads.

 A GFP signal localized to the beads indicates a positive SUMO-SIM interaction(upper panel).

Aさん: バランスがよく、Cの図がモノクロなの も落ち着いた感じでいいですね。



B GST-MCAF1-SIMwt (GVAHLTMDDEE) GST-MCAF1-SIMmut (GVIDLTMDDEE)

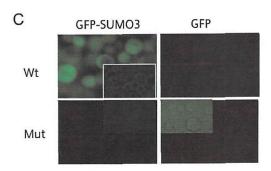


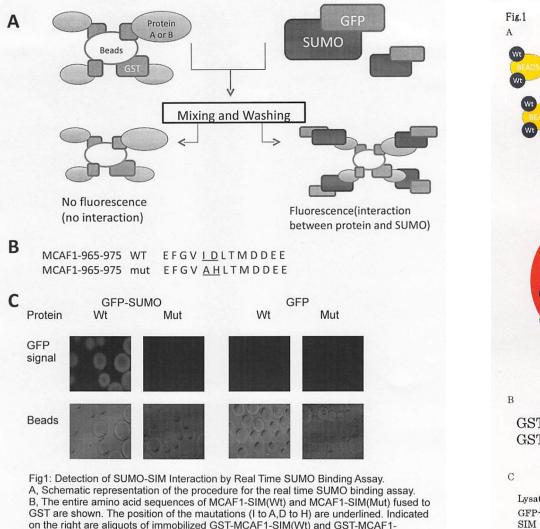
Fig.1. Detection of SUMO-SIM Interaction by Real-Time SUMO Binding Assay.

A,Schematic representation of the procedure for the real-time SUMO binding assay.

B,Comparison of sequence of amino acids between the GST-MCAF-SIMwt and the GST-MCAF-SIMmut.

C,Bacterial lysate containing GFP-SUMO-3 or GFP was mixed with bead-immobilized GST-MCAF1-SIMwt or the bead-immobilized GST-MCAF1-SIMmut. A GFP signal localized to the beads indicates a positive SUMO-SIM interaction. Smaller panels show the phase-contrast images of the Sepharose beads.

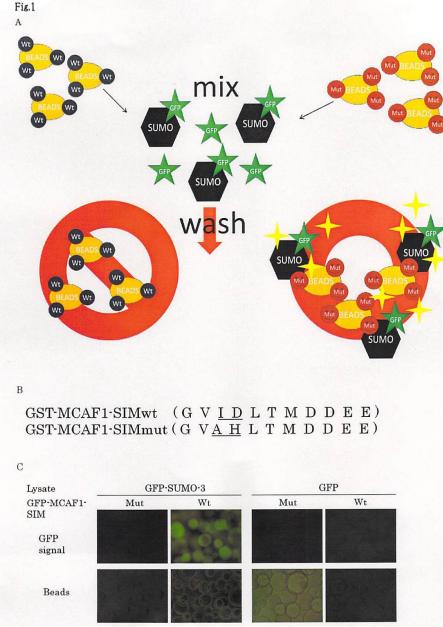
Aさん:わかりやすいです。Cの図も他の人にはないレイアウトで印象的です。



on the right are aliquots of immobilized GST-MCAF1-SIM(Wt) and GST-MCAF1-SIM(Mut).

C, Bacterial lysate containing of GFP-SUMO-3 or GFP was mixed with bead immobilized GST-MCAF1-SIM(Wt) or the bead immobilized GST-MCAF1-SIM(Mut). An aliquot of recombinant GFP-SUMO-3 or GFP resolved by PBS is shown. A GFP signal localized to the beads indicates a positive SUMO-SIM interaction (upper panel), Lower panels show the phase-contrast images of the Glutathione sepharose beads.

Eさん: コントラストがきれいです。とても見や すく仕上がっています。



Hさん:かなりのインパクトです(特にAは)。 ただ、Fig. Legendが無いのが残念です。

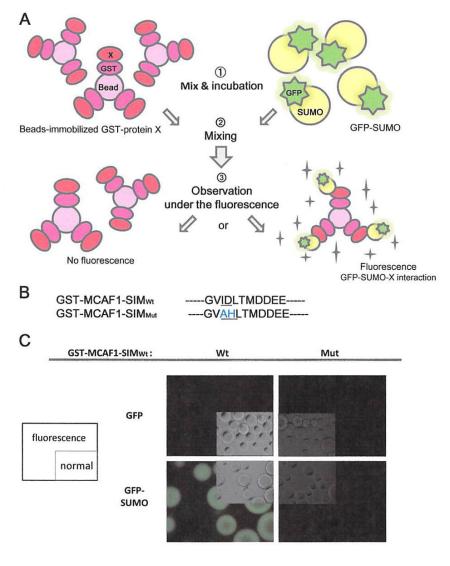


Fig. 1 Detection of SUMO-SIM Interaction by Real-Time SUMO Binding Assay. A:Schematic representation of the procedure for the real-time SUMO binding assay. B:Comparison of sequence of amino acids between the GST-MCAF1-SIMwt and GST-MCAF1-SIMMut. The position of the mutation(I to A and D to H) is underlined. C:Bacterial lysate containing of GFP-SUMO or GFP was mixed with bead-immobilized GST-MCAF1-SIMwt or the bead-immobilized GST-MCAF1-SIMMut. A GFP -SUMO localized to the beads indicates a positive SUMO-SIM interaction.

Wash Wash fluorescence No fluorescence No fluorescence If fluorescence is observed, GFP-SUMO is binding with SIM. Then, the SIM is wild type protein. В. Sample A: ···FGVIDLTHDDEE* I (isoleucine) → A (alanine) Sample B: ···F G V A H L T H D D E E* D (aspartic acid) → H (histidine) C. WT Mut **GFP-SUMO GFP GFP-SUMO GFP** Beads **GFP** signal Fig.1 A, Schematic representation of the bead halo assay. B, Amino acids sequences of DNA sample A and B decided by the result of DNA sequence. The position of the mutation (I to A, D to H) is red. The right of figure B shows mutational amino acid. C, The micrographs showing the result of the bead halo assay. The 4 pictures of left side are beads immobilized GST-SIMmt. The others are beads immobilized GST-SIMmut. The 2 pictures of left side in 4 pictures are beads contained GFP-SUMO and the others are beads contained GFP. The beads immobilized GST-SIMwt (the left of lower panel) only showed fluorescence.

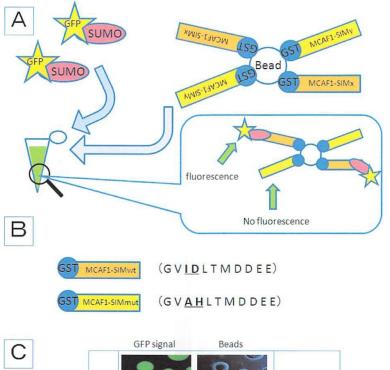
Bead

2min

GFP SUMO

Kさん:AとBでは色の違いを上手に使ってます。Kさん:結果を詳しく、わかりやすく示してくれ Cの見せ方にも工夫がありますね。

ています。よくまとまってます。



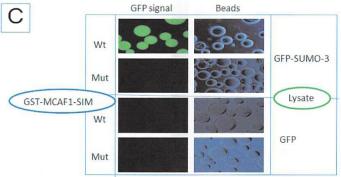


Fig 1. Detection of SUMO-SIM Interaction by Real-Time SUMO Binding Assay.

(A) Schematic representation of the procedure for the real-time SUMO binding assay. (B) The entire amino acid sequences of MCAF1-SIMwt and MCAF1-SIMmut fused to GST are shown. The position of the mutation (I to A) is underlined. Indicated on the right are aliquots of immobilized GST-MCAF1-SIMwt and GST-MCAF1-SIMmut, as analyzed by SDS-PAGE and stained with Coomassie Brilliant Blue (CBB). Note that equal amounts of GST-MCAF1-SIMwt and GST-MCAF1-SIMmut, to be used for assay in B, were immobilized on the beads. (C) Bacterial lysate containing 0.01 mg/ml of GFP-SUMO·3 (1st and 2nd columns) or GFP (3nd and 4th columns) was mixed with bead immobilized GST-MCAF1-SIMwt (1st and 3nd columns) or the bead-immobilized GST-MCAF1-SIMmut (2nd and 4th columns). An aliquot of recombinant GFP-SUMO·3 or GFP resolved by SDS-PAGE and stained with CBB is shown. A GFP signal localized to the beads indicates a positive SUMO-SIM interaction (upper panel). Lower panels show the phase-contrast images of the Sepharose beads.

Mさん:見せ方にずいぶん工夫がありますね。 全体のバランスもいいです。

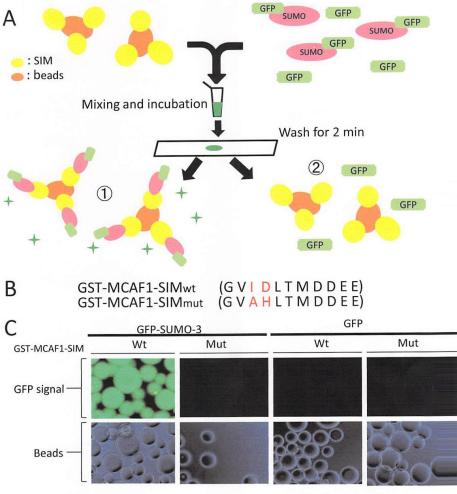
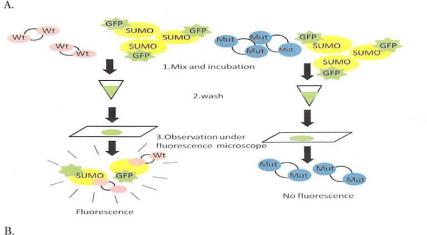


Fig1 **A**: Schematic representation of the procedure for the real-time SUMO binding assa—Observation under fluorescence microscope, GFP combined with SUMO show fluorescence(①) but GFP uncombined with SUMO is not shown(②). **B**: The amino a—sequences of MCAF1-SIMwt and MCAF1-SIMmut fused to GST are shown. The position of the mutation (I to A, D to H) are shown red. **C**: A GFP signal localized to the bead—indicates a positive SUMO-SIM interaction in upper panels. Lower panels show the phase-contrast images of the beads.

Mさん:ピンクを基調に見やすい図に仕上がっています。







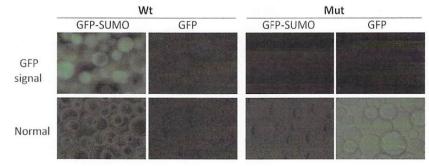
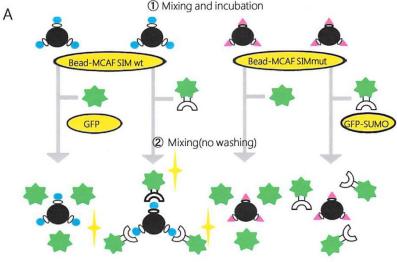


Fig1.

A: Schematic representation of the procedure for the real-time SUMO binding assay. B: The entirest amino acid sequences of MCAF1-SIMwt and MCAF1-SIMmut fused to GST are shown. The position of the mutation(I to A and D to H) is underlined. C: Bacterial lysate containing GFP-SUMO or GFP was mixed with bead-immobilized GST-MCAF1-SIMwt or the bead-immobilized GST-MCAF1-SIMmut. Assigned to the beads indicates a positive SUMO-SIM interaction (upper panel). Lower—panels show the phase-contrast images of the Sepharose beads.

Nさん: 反応系が見やすく描かれています。バランスもとてもいいと思います。



3 Observation under fluorescence microscope

MCAF SIM wt GVIDLTMDDEE MCAF SIM mut GVAHLTMDDEE

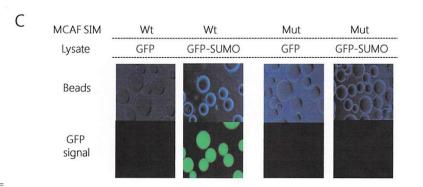


Fig 1. Delection of SUMO-SIM Interaction by Real-Time SUMO Binding Assay.

В

- A. Schematic representation of the procedure for the real-time SUMO binding assay.
- B. The entire amino acid sequences of MCAF-SIMwt and MCAF-SIMmut fused to GST are shown. Upper lane is sequence of amino acids of the wild type. Lower lane is that of the mutated. The position of the mutation (I to A and D to H) is red.
- C. GFP-SUMO or GFP was mixed with bead-immobilized GST·MCAF SIMwt or the bead-immobilized GST·MCAF SIMmut. A GFP signal localized to the beads indicates a positive SUMO·SIM interaction (upper panel). Lower panels show the phase-contrast images of the sepharose beads.

Nさん:Aの図が幾何学的ですね。全体としてまとまりもいいです。

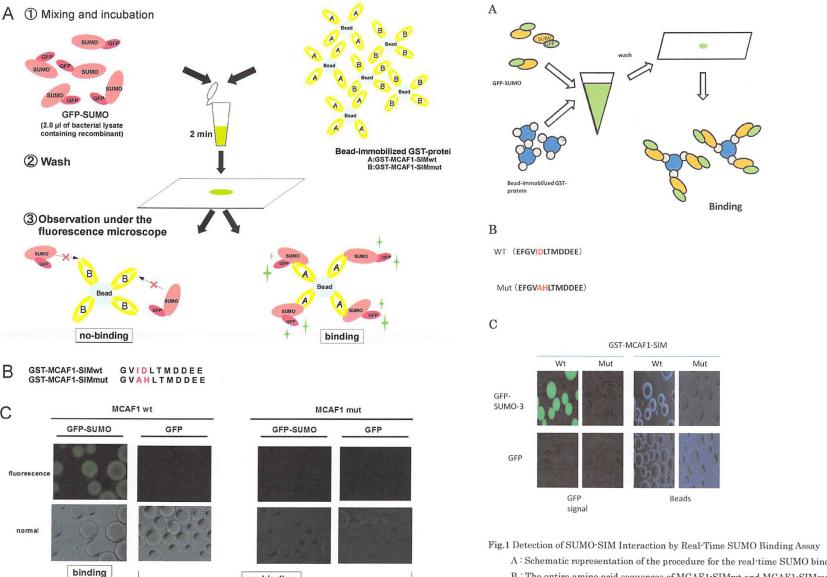


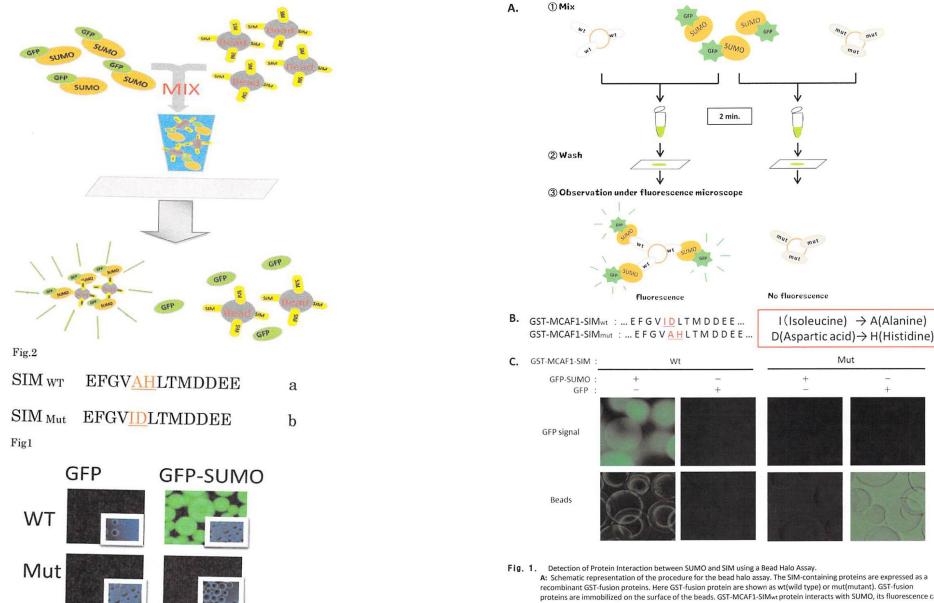
Fig.1. Detection of SUMO-SIM Interaction by Real-Time SUMO Binding Assay. A, Schematic representation of the procedure for the real-time SUMO binding assay. B, Comparison of sequence of amino acids between the GST-MCAF-SIMwt and the GST-MCAF-SIMmut. C, Bacterial lysate containing GFP-SUMO-3 or GFP was mixed with bead-immobilized GST- MCAFI-SIMwt or GST- MCAFI-SIMmut, A GFP signal localized to the beads indicates a positive SUMO-SIM interaction. Lower panels show the phase-contrast images of the Sepharose beads.

no-binding

Rさん:Aの図を丁寧に書いてくださいました。 分かりやすいと思います。

- A: Schematic representation of the procedure for the real-time SUMO binding assay.
- B: The entire amino acid sequences of MCAF1·SIMwt and MCAF1·SIMmut fused to GST are shown.
- C: Bacterial lysate containing GFP-SUMO·3 or GFP was mixed with bead-immobilized GST-MCAF 1-SIMwt or the bead-immobilized GST-MCAF1-SIMmut.

Uさん: すっきりとした図で、わかりやすくまとめ ています。



recombinant GST-fusion proteins. Here GST-fusion protein are shown as wt(wild type) or mut(mutant). GST-fusion proteins are immobilized on the surface of the beads. GST-MCAF1-SIMwt protein interacts with SUMO, its fluorescence can be detected under the fluorescence microscope. B: A part of amino acid sequences of GST-MCAF1-SIMwt and GST-MCAF1-SIMmut are shown. The position of the mutation Fig3 (I to A, D to H) is underlined and changed the color. The mutation of amino acid is picked out on the right.

C: Bacterial lysate containing GFP or GFP-SUMO was mixed with bead-immobilized GST-MCAF1-SIMwt or GST-MCAF1 SIMmut. Upper panels show a GFP signal. The signal localized to the beads indicates positive protein interaction between

Mut

Yさん:全体が丁寧に描かれています。アミ ノ酸の情報も入れてくれました。

Yさん:Aの図でMixingの画を丁寧に書いてくだ さいました。分かりやすいです。Legendは?

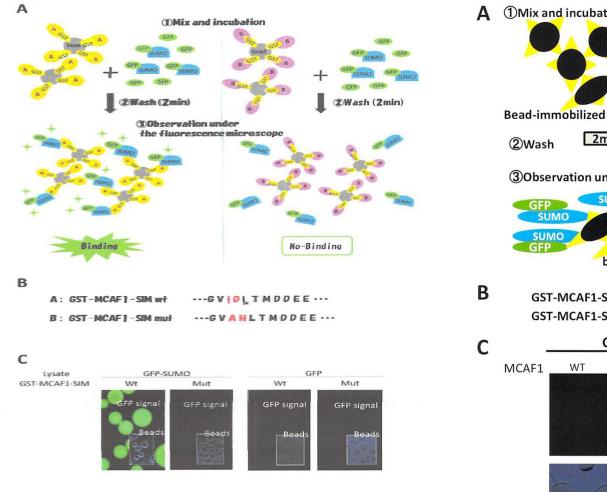


Fig.1 *1 Detection of SUMO-SIM Interaction by Real-Time SUMO Binding Assay.

- A. Schematic represention of the procesure for real-time SUMO binding assay.
- B. The entire amid acid sequences of MCAF1-SIMwt and MCAF1-SIMmut fused to GST are shown The position of the mutation (I to A and D to H) is red.
- C. Bacterial lysate containing GFP-SUMO or GFP was mixed with bead immobilized GSTMCAF1-SIM GSTMCAF1-SIMmut. A GFP signal localized to the beads indicates a positive SUMO-SIM interactio (widder panels). Narrower panels shows the phase contrast images of the Sepharose beads.

Yさん:Cに工夫が見られます。A, Bも上手にま とめていますね。

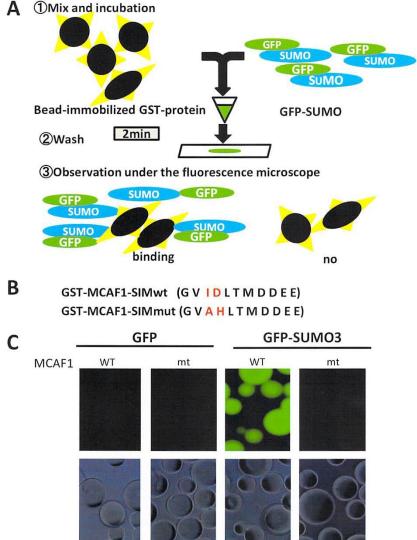


Fig.1. Detection of SUMO-SIM Interaction by Real-Time SUMO Binding Assay.

A, Schematic representation of the procedure for the real-time SUMO binding assay. B, The entire amino acid sequences of MCAF1-SIMwt and MCAF1-SIMmt fused to GST are shown. The position of the mutation is red.

C, Bacterial lysate containing 0.01mg/ml of GFP-SUMO-3 or GFP was mixed with bead immobilized GST-MCAF1-SIMwt or the bead-immobilized GST-MCAF1-SIMmt. A GFP signal located to the beads indicates a positive SUMO-SIM

interaction. Lower panels show the phase-contrast images of the Sepharose beads.

Fさん、全体が明るく見えますね。Cの写真も綺麗です。

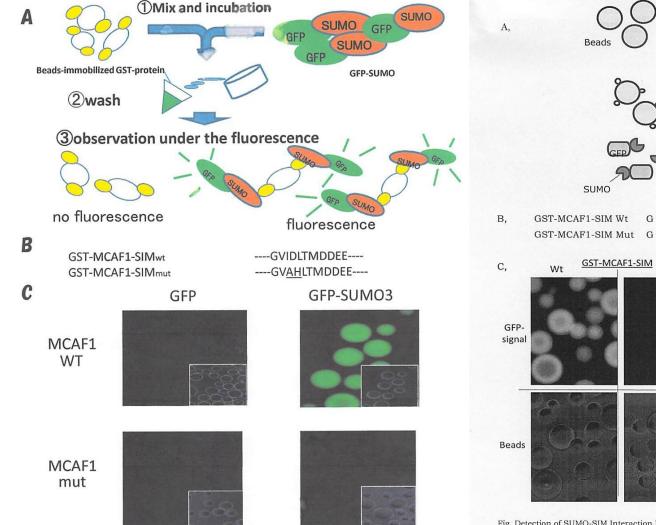


Fig1 Detection of SUMO-SIM Interaction by Real-Time SUMO Binding Assay.

A) Schematic representation of the procedure for the real-time SUMO binding assay. B) Comparison of sequence of amino acids between GST-MCAF1-SIMwt and GST-MCAF1-SIMmut. Top lane is sequence of amino acids of GST-MCAF1-SIMwt. Bottom lane is that of GST-MCAF1-SIMmut. The Position of the mutation(I to A and D to H) is underlined. C) Bacterial lysate cantaining GFP-SUMO3 or GFP was mixed bead-immobilized GST-MCAF1-SIMwt or the bead-immobilized GST-MCAF1-SIMmut. A GFP signal localized to the beads indicates a positive SUMO-SIM interaction(each panel). Each image of the lower right is the phase-contrast images of the Sepharose beads.

Kさん、手際良く図とレポートを作成してくれました。 図Cのinsetのビーズの写真がgoodです。

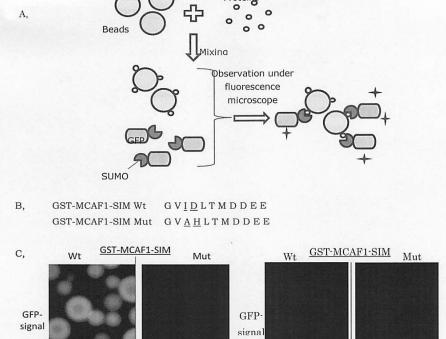


Fig. Detection of SUMO-SIM Interaction by Real-Time SUMO Binding Assay

- A, Schematic representation of the procedure for the real-time SUMO binding assay.
- B, The entire amino acid sequences of MCAF1-SIMWt and MCAF1-SIMMut fused to GST are shown. The position of the mutation (I to A and D to H) is underlined. Note that equal amounts of GST-MCAF1-SIMWt and GST-MCAF1-SIMMut, to be used for the assay in B, were immobilized on the beads.

Beads

C, Bacterial lysate containing 0.01 mg/ml of GFP-SUMO-3 (1st and 2nd columns) or GFP (3rd and 4th columns) was mixed with bead-immobilized GST-MCAF1-SIMWt or the bead-immobilized GST-MCAF1-SIMMut (2nd and 4th columns). A GFP signal localized to the beads indicates a positive SUMO-SIM interaction (upper panel). Lower panels show the phase-contrast images of the Sepharose beads.

Sさん、図Aのビーズとタンパク質のバランスがいい です。濃淡のコントラストでよくまとまってます。

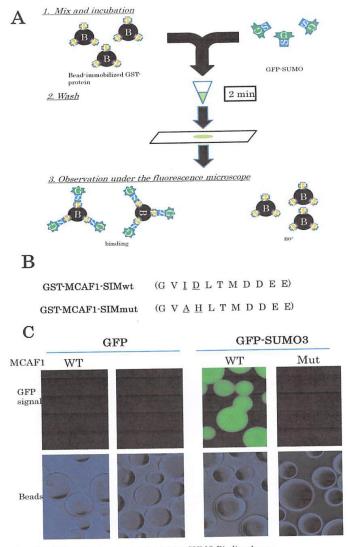
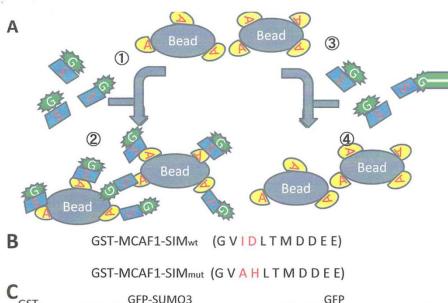


Fig. 1. Detection of SUMO-SIM Interaction by Real-Time SUMO Binding Assay.

A, Schematic representation of the procedure for the real-time SUMO binding assay. B, The entire amino acid sequences of MCAF1-SIMwt and MCAF1-SIMmut fused to GST are shown. The position of the mutation (I to A and D to H) is underlined. Indicated on the right are aliquots of immobilized GST-MCAF1-SIMwt and GST-MCAF1-SIMmut, to be used for the assay in B, were immobilized on the beads. C, A GFP signal localized to the beads indicates a positive SUMO-SIM interaction (upper panel)—Lower panels show the phase-contrast images of the Sepharose beads.

Tさん、とても見やすい図です。GFPタンパク質や SUMOをGとSであらわしたのは言い工夫ですね。



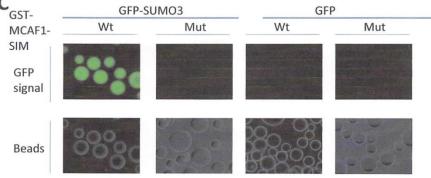
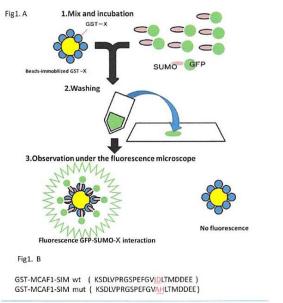


Fig , A , SUMO indicates an interaction to be to protein A (it is SIM this time) combined with the bead(①), and GFP combined with SUMO by joining together show fluorescence(②). Since it does not join together unless protein A and SUMO show an interaction(③), fluorescence is not shown(④). B , The amino acid which constitutes the protein of a wild type and a mutant type shows the variation of arrangement. C , The SUMO-SIM interaction using bead halo assay .

Tさん、図Aが力強いですね。おそらく矢印が聞いているのでしょう。変異アミノ酸を朱色にした工夫は分かりやすいです。



GST-MCAF1-SIM wt
TCGGATCTGGTTCCGCGTGGATCCCCGGAATTCGGTGTCATTGATCTCACAATGGATGATGAAGAGTGA
GST-MCAF1-SIM mut
TCGGATCTGGTTCCGCGTGGATCCCCGGAATTCGGTGTCGCACATCTCACAATGGATGATGAAGAGTGA

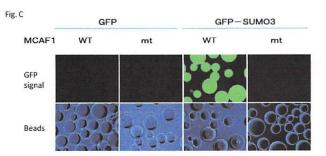


Fig. 1

A, Detection of SUMO -SIM Interaction by Bead Halo assay.

B, The entire amino acid and nucleic acide sequences of MCAF-SIMwt and MCAF1-SIMmut fused to GST are shown.

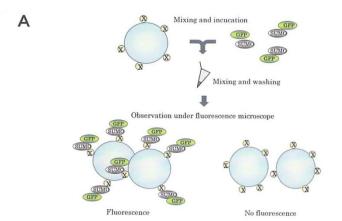
The protein of the mutation(TD to AH)

C, Bacterial lysate containing GFP·SUMO3 or GPT was mixed with bead immobilized GST·MCAF1·SIMwt or bead immobilized GST·MCAF1·SIMmut.

A GFP signal localized to the beads indicates a positive SUMO-SIM interaction(upper panel).

Lower panels show the phase contrast images of the beads.

Aさん、図Aの結合状態が分かりやすく描かれてます。 DNA塩基配列も示されて、手堅い感じにまとまってます。





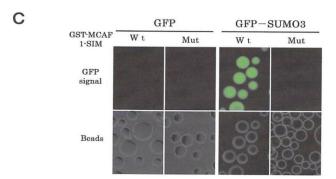
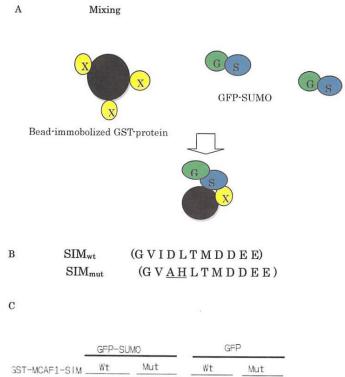


Fig.1

- A. Achematic representation of the procedure for the bead halo assay.
- B. The entireamino acid sequences of MCAF1·SIMwt and MCAF1·SIMMut fused to GST are shown. T
- C. Bacterial lysate containing GFP-SUMO·3 or GFP was mixed with bead·immobilized GST-MCAF1·SIMwt or the bead·immobilized GST-MCAF1·SIMmut. A GFP signal localized to the bead-indicates a positive SUMO·SIM interaction (upper panel). Lower panels show the phase·contra_images of the Sepharose beads.

Mさん、ビーズが本物っぽくていい感じですね。大変見やすいです。



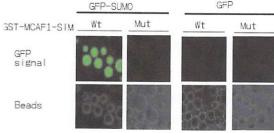
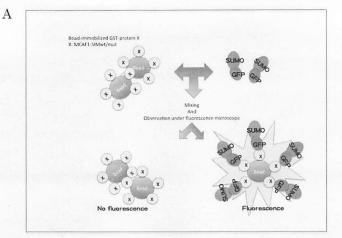


Fig.1. Detection of SUMO-SIM Interaction by Real-Time SUMO Binding Assay.

A, Schematic representation of the procedure for the real-time SUMO binding assay. B, The entire amino acid sequences of MCAF1-SIMwt and MCAF1-SIMMt fused to GST are shown. The position of the mutation is underlined. Indicated on the right are aliquots of immobilized GST-MCAF1-SIMwtMt, as analyzed by SDS-PAGE and stained with Coomassie Brilliant blue(CBB). Note that equal amounts of GST-MCAF1-SIMwtMt, to be used for the assay in B, were immobilized on the on the beads. C, Bacterial lysate containing GFP-SUMO-3 or GFP was mixed with bead-immobilized GST-MCAF-SIMwtMt. An aliquot of recombinant GFP-SUMO-3 or GFP resolved by SDS-PAGE and stained with CBB is shown. A GFP signal localized to the beads indicates a positive SUMO-SIM interaction. Lower panels show the phase-contrast images of the Sepharose beads.

Mさん、要点がはっきりしていて見やすいですね。



B
GST-MCAF1-SIMwt
GVIDLTMDDEE
GST-MCAF1-SIMmut
GVAHLTMDDEE

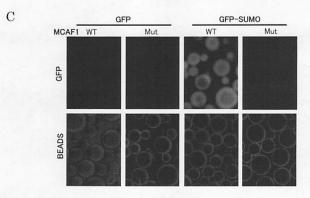
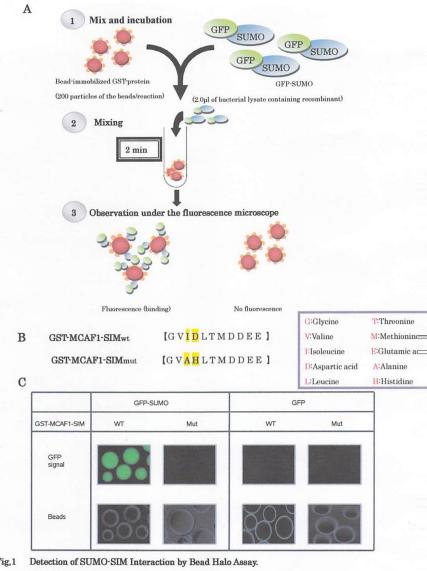


Fig.1. Detection of SUMO-SIM Interaction by Bead Halo Assay.

A, Schema shown the procedure for the bead halo method. B, The entire amino acid sequences of MCAF1-SIMwt and MCAF1-SIMmt fused to GST are shown. Shaded letters shows the position of the mutation occurred. C, Fluorescence microscope images are shown the detection of GFP-SUMO-SIM interaction. A GFP signal localized to the beads indicates a positive SUMO-SIM interaction. The images in the second row show the phase-contrast images of the sepharose beads.

Aさん、結合状態の画が印象的ですね。変異のポイントをしっかり示しているのもいいですね。



- A: Schematic representation of the procedure for the bead halo assay.
- B: The entire amino acid sequences of GST-MCAF1-SIMwt and GST-MCAF1-SIMmut fused to GST are shown. The position of mutation (I to A and D to H) is underlined.
- C:GFP-SUMO or GFP was mixed with bead-immobilized GST-MCAF1-SIMwt or the bead-immobilized GST-MCAF1-SIMmut.

Sさん、アミノ酸を示しているのが特徴ですね。画 が凝ってますね。見やすく仕上がってます。

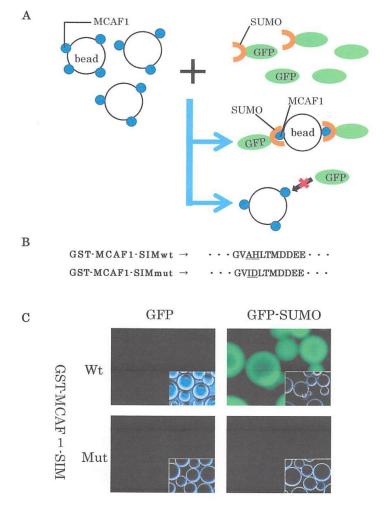
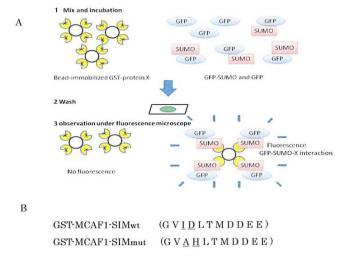


Fig.1. Detection of SUMO-SIM Interaction by Real-Time SUMO Binding Assay.

A, Schematic representation of the procedure for the real-time SUMO binding assay. B, The entire amino acid sequences of MCAF1-SIMwt and MCAF1-SIMmut fused to GST are shown. Positions of the mutation (I to A, D to H) are underlined. Note that equal amounts of GST-MCAF1-SIMwt and GST-MCAF1-SIMmt, to be used for the assay in B, were immobilized on the beads. C, Bacterial lysate containing 0.01 mg/ml of GFP-SUMO (two columns of right) or GFP (two columns of left) was mixed with bead-immobilized GST-MCAF1-SIM_{Wt} (upper two columns) or the bead-immobilized GST-MCAF1-SIM_{Mut} (lower two columns). A GFP signal localized to the beads indicates a positive SUMO-SIM interaction (upper right column). The panel of the lower right corner of each column shows the phase-contrast images of the Sepharose beads.

Aさん、青を基調として、図も写真も見やすいです ね。



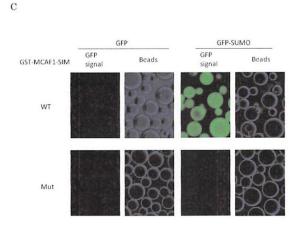
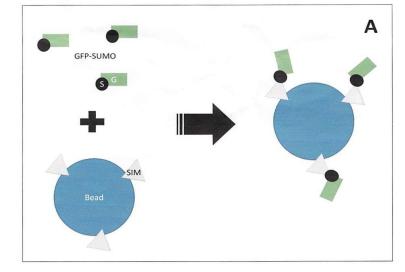


Fig.1. Detection of SUMO-SIM Interaction by Real-Time SUMO Binding Assay.

A, Schematic representation of the procedure for the real-time SUMO binding assay. B, The entire amino acid sequences of MCAF1-SIMwt and MCAF1-SIMmut fused to GST are shown. The position of the mutation (ID to AH) is underlined. C, Bacterial lysate containing GFP-SUMO or GFP was mixed with bead-immobilized GST-MCAF1-SIMwt or the bead-immobilized GST-MCAF1-SIMmut. A GFP signal localized to the beads indicates a positive SUMO-SIM interaction (upper panel). Lower panels show the phase-contrast images of the Sepharose beads.

Mさん、やわらかな色調で、見やすいですね。全体のバランスもいいです。



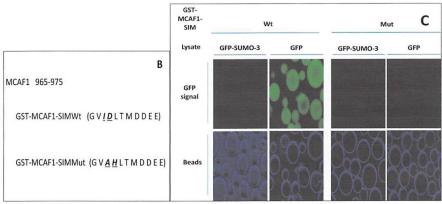


Fig1. Investigation of SUMO-SIM Interaction by Beads Halo Assay.

- A, Schematic representation of the procedure for beads halo assay.
- B, The entire amino acid sequences of MCAF1·SIMwt and MCAF1·SIMMut fused to GST are shown. The position of the mutation (I to A and D to H) are underlined.
- C, Bacterial lysate conteining 0.01 mg/ml of GFP·SUMO·3 (1st and 3rd columns) or GFP (2nd and 4th columns) was mixed with bead-immobilized GST·MCAF1·SIMWt (1st and 2nd columns) or GST·MCAF1·SIMMut (3rd and 4th columns). An aliquot of recombinant GFP·SUMO·3 or GFP resolved by SDS·PAGE and stained with CBB is shown. A GFP signal localized to the beads indicates a positive SUMO·SIM interaction (upper panels). Lower panels show the phase-contrast images of the Sepharose beads.

Yさん、シンプルな図で、ビーズが大きく描かれているところが写実的です。BとCの並べ方も工夫が見られます。