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Supplemental Material to:

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KDM5B histone demethylase controls epithelial-mesenchymal transition of cancer cells by regulating the expression of the microRNA-200 family

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Supplementary Figure legends

Figure 1. Expression of KDM5B proteins in the cells infected with the retroviruses.
A549 cells (A), NMuMG cells (B) or HT29 cells (C) were infected with the control retrovirus or the retrovirus expressing FLAG-tagged wild-type (WT) KDM5B or the H499Y mutant (Mut). KDM5B proteins were detected by Western blot (WB) with anti-FLAG antibody (upper panel). As a loading control, anti-GAPDH antibody was used (lower panel).

Figure 2. Overexpression of KDM5B caused morphological changes of NMuMG mouse breast epithelial cells.
(A) Cell morphological changes of NMuMG cells induced by KDM5B. NMuMG cells infected with the control retrovirus, the control retrovirus with TGF-b treatment, or the retrovirus expressing FLAG-tagged wild-type (WT) KDM5B or the H499Y mutant (Mut), were stained with crystal violet. (B) Immunofluorescence images of cells showing the localization of E-cadherin. The panels of NMuMG cells with the same arrangement with (A) were stained with anti-E-cadherin antibody and with DAPI. (C) Fluorescence images of cells showing reorganization of actin cytoskeleton. The cells were stained with TRITC-phalloidin (indicated as Actin) and with DAPI.

Figure 3. Overexpression of KDM5B caused morphological changes of HT29 human colon cancer cells.
(A) Cell morphological changes of HT29 cells induced by KDM5B. HT29 cells infected with the control retrovirus, the control retrovirus with TGF-b treatment, or the retrovirus expressing FLAG-tagged wild-type (WT) KDM5B or the H499Y mutant (Mut), were stained with crystal violet. (B) Immunofluorescence images of cells showing the localization of E-cadherin. The panels of HT29 cells with the same arrangement with (A) were stained with anti-E-cadherin antibody and with DAPI. (C) Fluorescence images of cells showing reorganization of actin cytoskeleton. The cells were stained with TRITC-phalloidin (indicated as Actin) and with DAPI.

Figure 4. KDM5B affected the expression of EMT-related genes in NMuMG cells.
Quantitative RT-PCR analysis was performed to detect the expression of mouse *CDH1/E-cadherin* (A), *FN1/ Fibronectin* (B), *CDH2/N-cadherin* (C), *SNAI1* (D), *SNAI2* (E), *ZEB1* (F) and *ZEB2* (G) in NMuMG cells infected with the control retrovirus, the control retrovirus with TGF-b treatment, or the retrovirus expressing wild-type (WT) KDM5B or the mutant (Mut). PCR data were normalized with respect to control mouse *Actb* expression (*, $P < 0.001$ comparing to control; **, $P < 0.005$ comparing to control). (H) Western blot analysis was performed to detect the expression of E-cadherin and ZEB1 proteins using the corresponding antibodies.

Figure 5. KDM5B affected the expression of EMT-related genes in HT29 cells.
Quantitative RT-PCR analysis was performed to detect the expression of human *CDH1/E-cadherin* (A), *FN1/ Fibronectin* (B), *SNAI1*(C) and *ZEB1* (D) in HT29 cells infected with the control retrovirus, the control retrovirus with TGF-b treatment, or the retrovirus expressing wild-type (WT) KDM5B or the mutant (Mut). The expression levels of *CDH2/N-cadherin, SNAI2* and *ZEB2* were extremely low or not detected in HT29 cells. PCR data were normalized with respect to control *GAPDH* expression (*, $P < 0.001$ comparing to control; **, $P < 0.005$ comparing to control). (E) Western blot analysis was performed to detect the expression of E-cadherin and ZEB1 proteins using the corresponding antibodies.

Figure 6. Recruitment of KDM5B was not detected on the various regions upstream or around the transcription initiation site of *CDH1/E-cadherin* gene.
(A) Schematic representation of the regions upstream or around the transcription initiation site of *CDH1/E-cadherin* gene. The boxes shown on the scheme indicate the first and second exons and the dark area corresponds to the coding region. The arrow points to the transcription initiation site. The regions covered by the primer sets used for ChIP assays are shown as a to j. (B) ChIP analyses of H3K4me3, H3K27me3 and FLAG-tagged KDM5B on the regulatory regions. The occupancies of methylated histones or KDM5B protein on the regions were analyzed by quantitative PCR and presented as the percentages of enrichment over input DNA (*, $P < 0.01$ comparing to control; **, $P < 0.05$ comparing to control).

Figure 7. Recruitment of KDM5B was not detected on the various regions upstream or around the transcription initiation site of *ZEB1* gene.
(A) Schematic representation of the regions upstream or around the transcription initiation site of *ZEB1* gene. The regions covered by the primer sets used for ChIP assays are shown as a to j. (B) ChIP analyses of H3K4me3, H3K27me3 and FLAG-tagged KDM5B on the regulatory regions. The occupancies of methylated histones or KDM5B protein on the regions were analyzed by quantitative PCR and presented as the percentages of enrichment over input DNA

Figure 8. Recruitment of KDM5B was not detected on the various regions upstream or around the transcription initiation site of *ZEB2* gene.
(A) Schematic representation of the regions upstream or around the transcription initiation site of *ZEB2* gene. The regions covered by the primer sets used for ChIP assays are shown as a to j. (B) ChIP analyses of H3K4me3, H3K27me3 and FLAG-tagged KDM5B on the regulatory regions. The occupancies of methylated histones or KDM5B protein on the regions were analyzed by quantitative PCR and presented as the percentages of enrichment over input DNA.

Figure 9. KDM5B decreased the expression of miR-200a and miR-200c both in NMuMG and HT29 cells.
Quantitative RT-PCR analysis was performed to detect the expression of miR-200a, and miR-200c in NMuMG cells (A and B) and HT29 cells (C and D) infected with the control retrovirus, the control retrovirus with TGF-b treatment, or the retrovirus expressing wild-type (WT) KDM5B or the mutant (Mut). PCR data were normalized with respect to control mouse *snoRNA202* or human *U6B* expression (*, $P < 0.001$ comparing to control; **, $P < 0.005$ comparing to control).

Figure 10. ChIP experiments for the regulatory regions of *BRCA1, HOXA5* and *GAPDH* genes.
Schematic of the regulatory regions of *BRCA1* (A), *HOXA5* (C) and *GAPDH* (E) genes is presented. The boxes shown on the scheme indicate the first and second exons and the dark area corresponds to the coding region. The arrow points to the transcription initiation site. The regions covered by the primer sets used for ChIP assays are shown as a and b. ChIP analyses of H3K4me3, H3K27me3 and FLAG-tagged KDM5B on the regulatory regions of *BRCA1* (B), *HOXA5* (D) and *GAPDH* (F) genes are shown. The occupancies of methylated histones or KDM5B protein on the regions were analyzed by quantitative PCR and presented as the percentages of enrichment over input DNA (*, $P < 0.001$ comparing to control; **, $P < 0.005$ comparing to control).

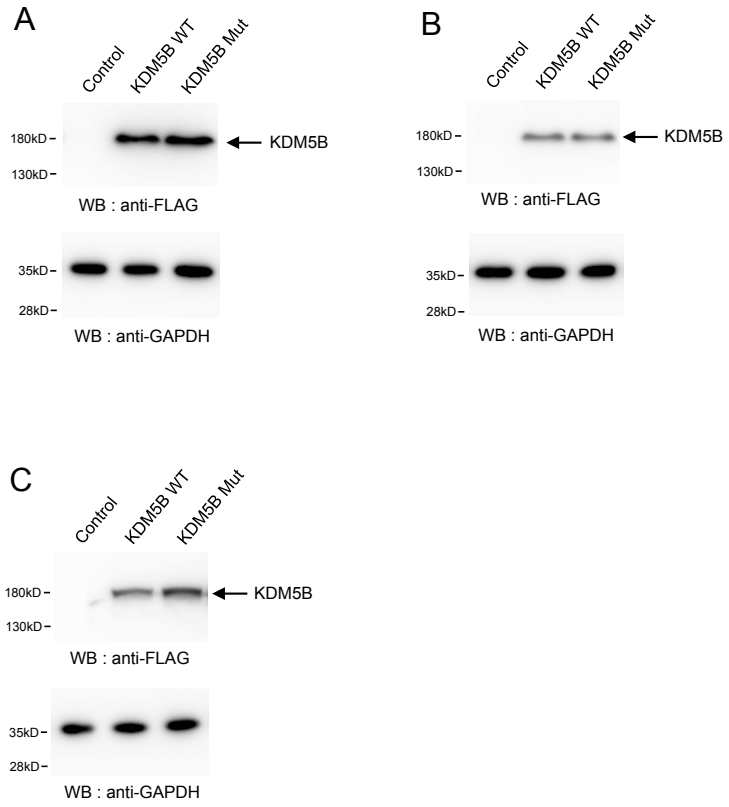
Figure 11. KDM5B-induced EMT phenotype was inhibited with the introduction of exogenous miR-200.
(A) Immunofluorescence images of cells showing the localization of E-cadherin. A549 cells infected with the control retrovirus, the retrovirus expressing wild-type KDM5B, KDM5B with miR-200a precursor and KDM5B with miR-200c precursor were stained with anti-E-cadherin antibody and with DAPI. (B) Fluorescence images of cells showing reorganization of actin cytoskeleton. The panels of A549 cells with the same arrangement with (A) were stained with TRITC-phalloidin (indicated as Actin) and with DAPI.

Figure 12. Introduction of exogenous miRNA-200 precursor did not affect the expression level of KDM5B protein.
Western blot with anti-FLAG antibody was performed to detect KDM5B proteins in A549 cells infected with the retrovirus expressing FLAG-tagged wild-type (WT) KDM5B, KDM5B with miR-200a precursor and KDM5B with miR-200c precursor. As a loading control, anti-GAPDH antibody was used.

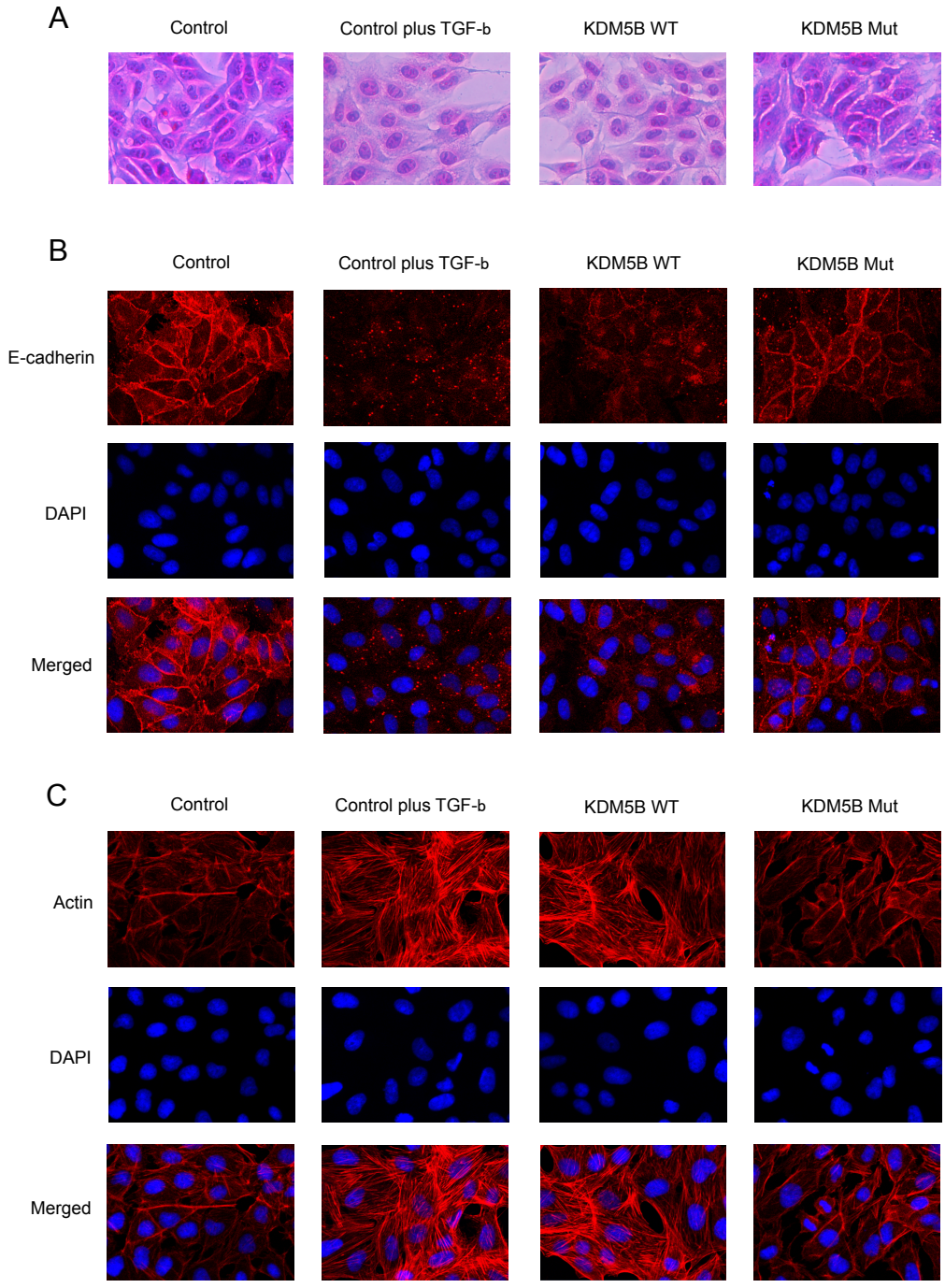
Figure 13. Knockdown of KDM5B affected the E-cadherin expression in A549 cells but did not counteract with TGF-b-induced EMT phenotype.
(A) Immunofluorescence images of cells showing the localization of E-cadherin. A549 cells were infected with retroviruses expressing control shRNA or KDM5B shRNA with or without treatment of TGF-b, and were stained with anti-E-cadherin antibody and with DAPI. (B) Fluorescence images of cells showing reorganization of actin cytoskeleton. The panels of A549 cells with the same arrangement with (A) were stained with TRITC-phalloidin (indicated as Actin) and with DAPI.

Figure 14. Knockdown of KDM5B did not affect TGF-b-induced expression changes of EMT-related genes.
Quantitative RT-PCR analysis was performed to detect the expression of *CDH1/E-cadherin* (A), *FN1/Fibronectin* (B), *CDH2/N-cadherin* (C), *ZEB1* (D) and *ZEB2* (E) in A549 cells infected with retroviruses expressing control shRNA or KDM5B shRNA with or without treatment of TGF-b (*, $P < 0.001$ comparing to control).

Figure 15. The expression of endogenous KDM5B is slightly induced by the treatment of TGF-b in A549 cells.
Quantitative RT-PCR analysis was performed to detect the expression of *KDM5B* in A549 cells before and after TGF-b treatment (12h, 24h and 48h) (*, $P < 0.001$ comparing to control; **, $P < 0.01$ comparing to control).



Enkhbaatar et al. Supplementary Figure 1



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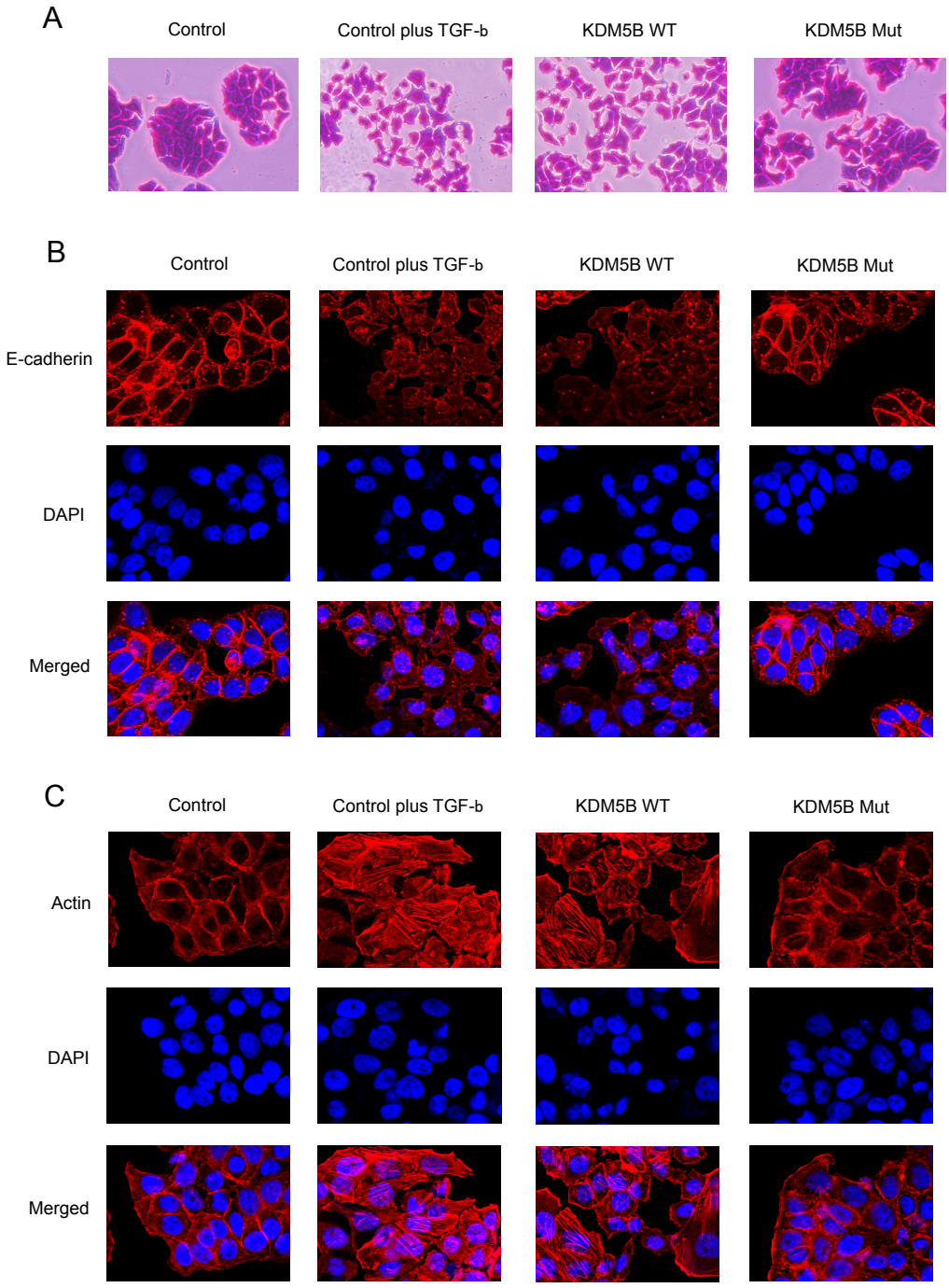

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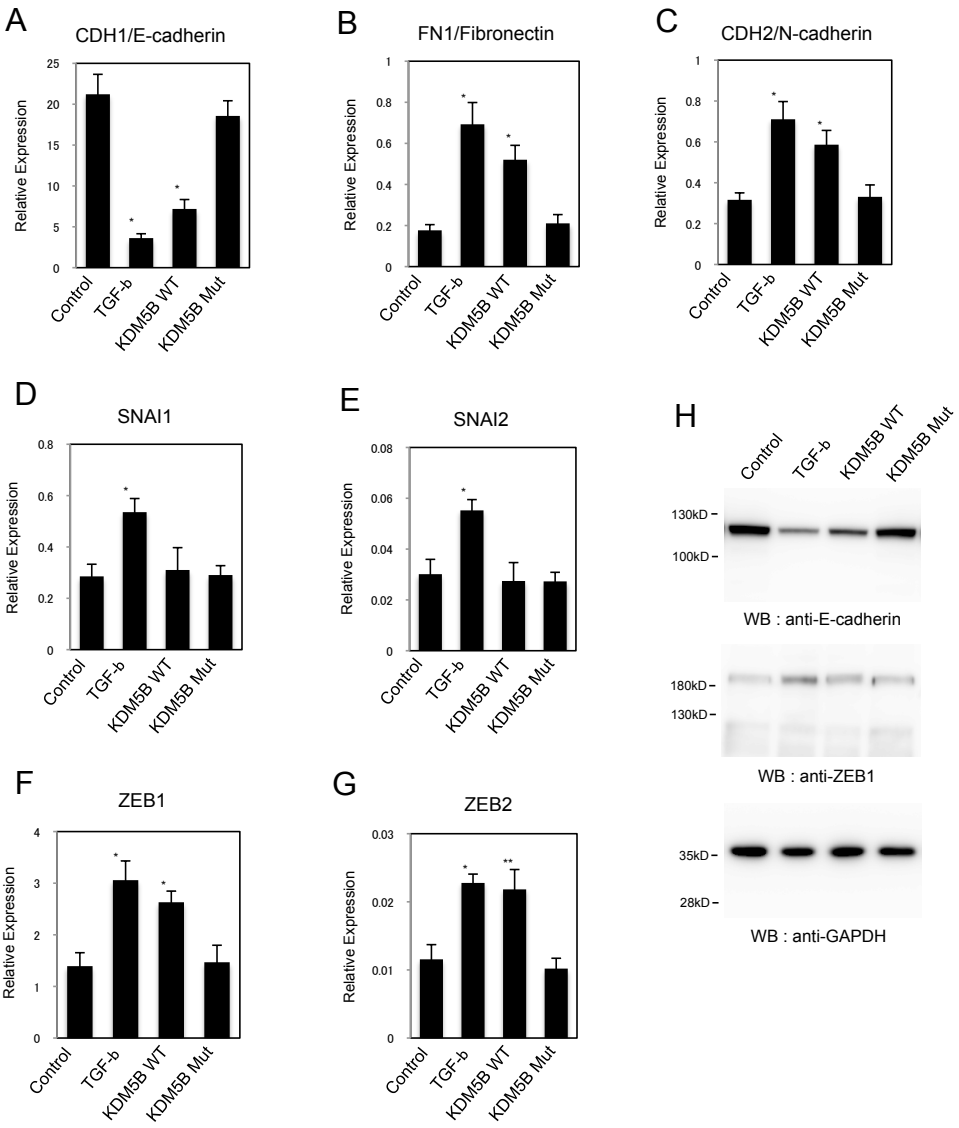

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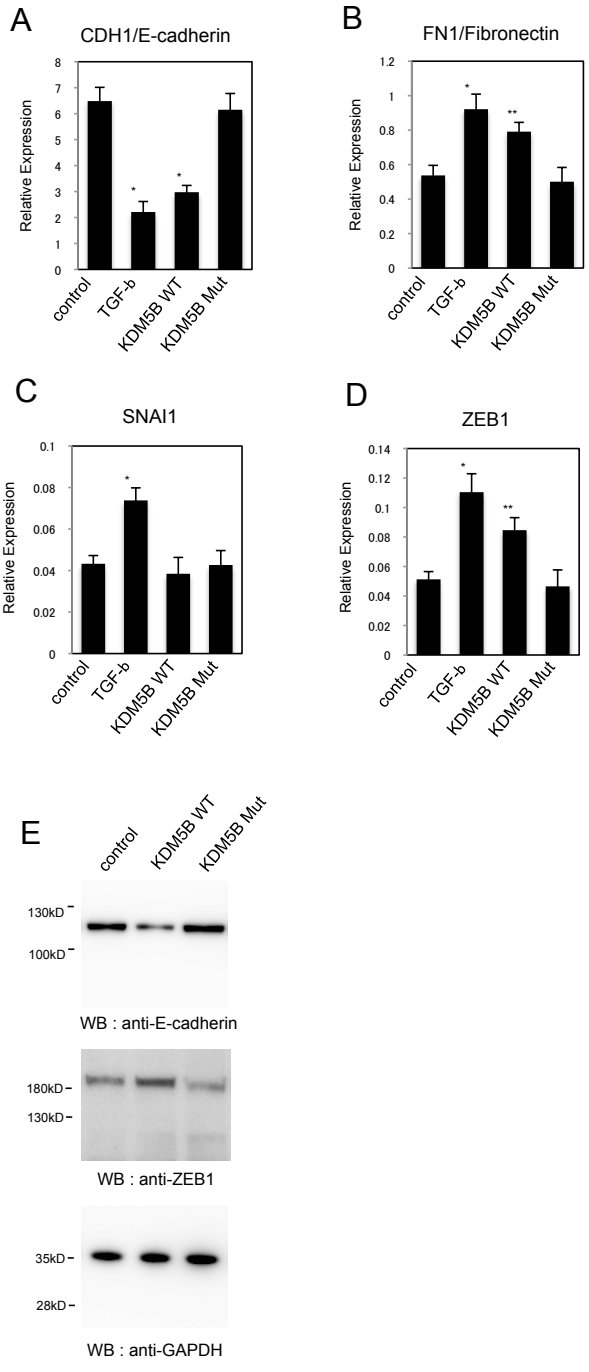

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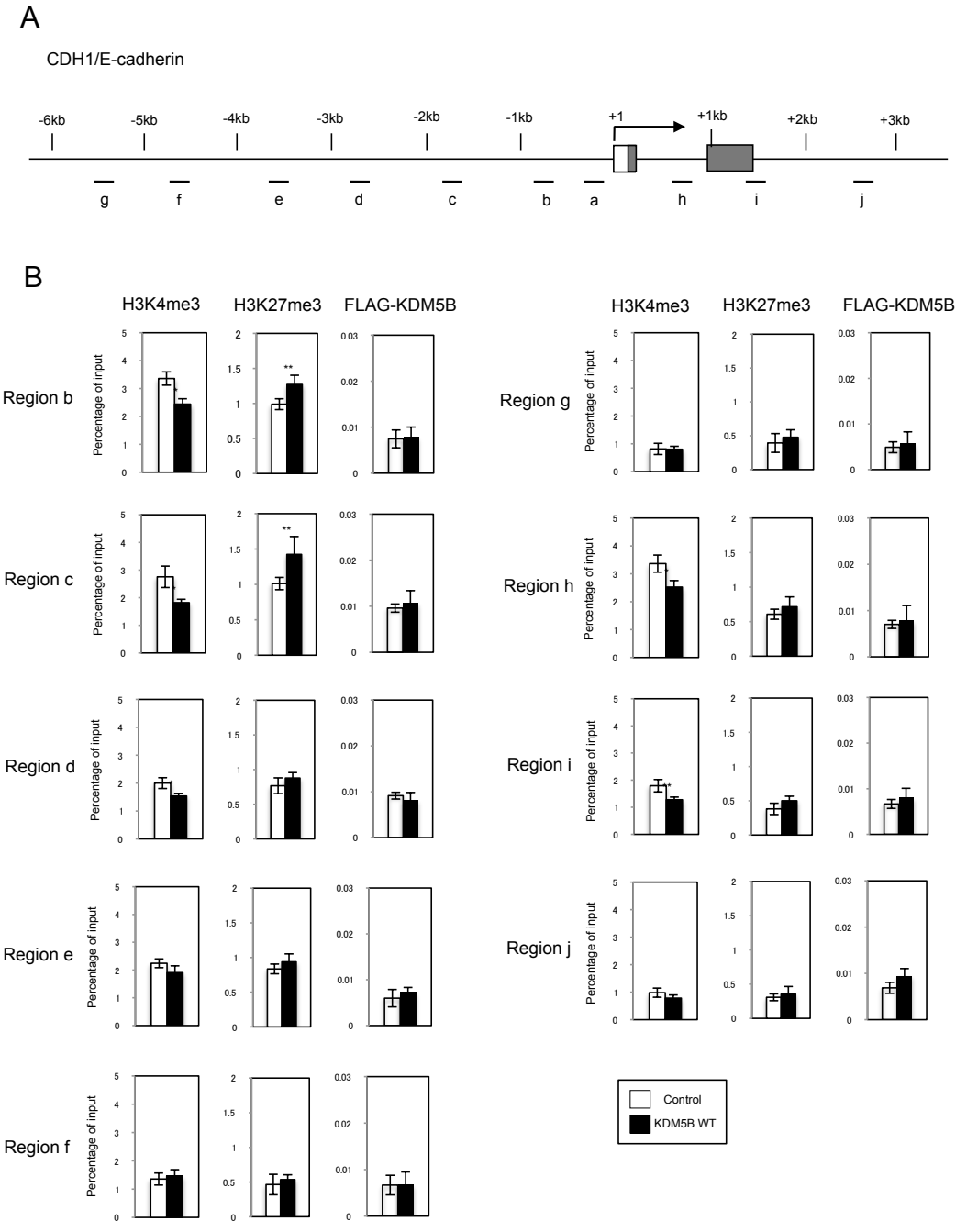

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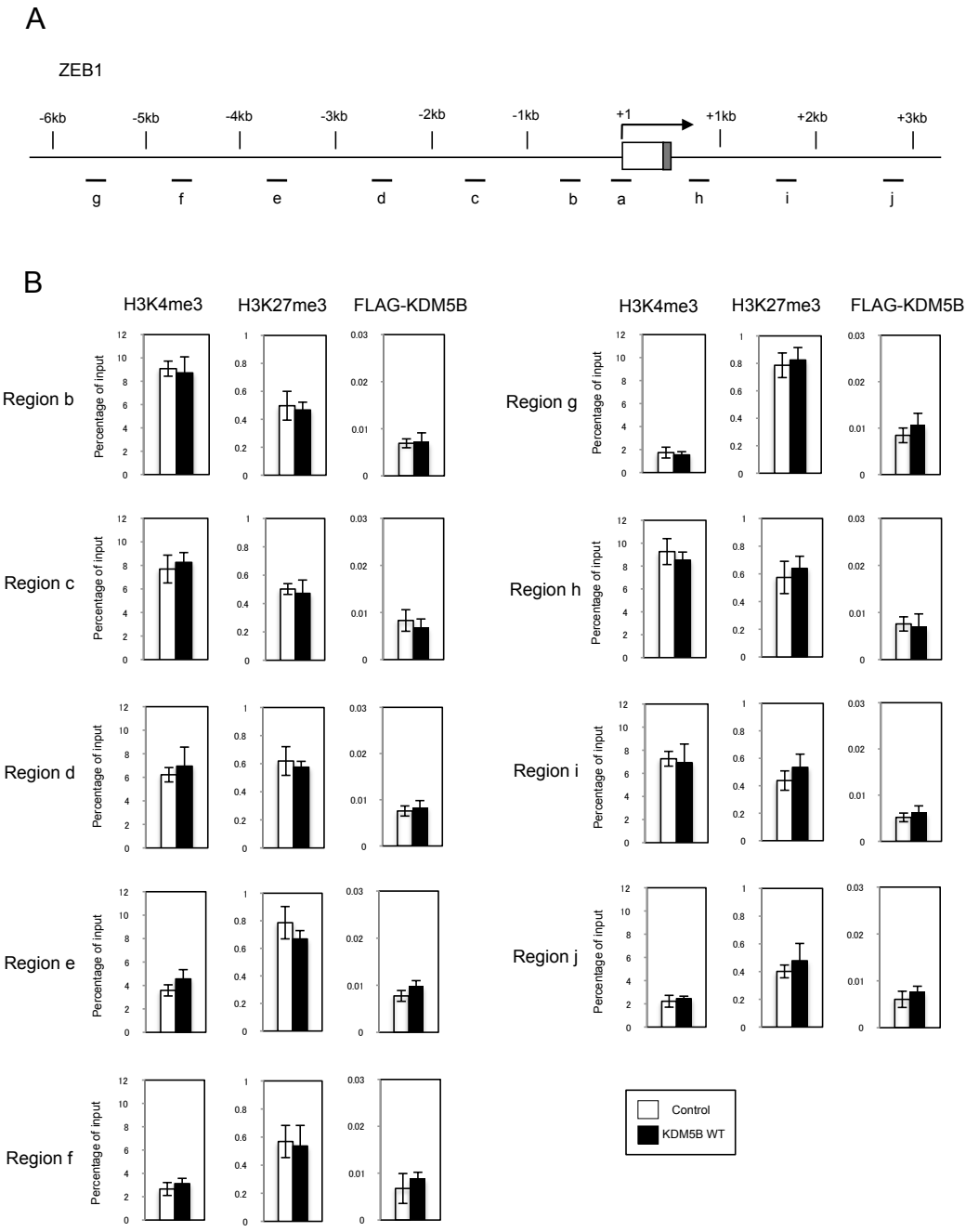

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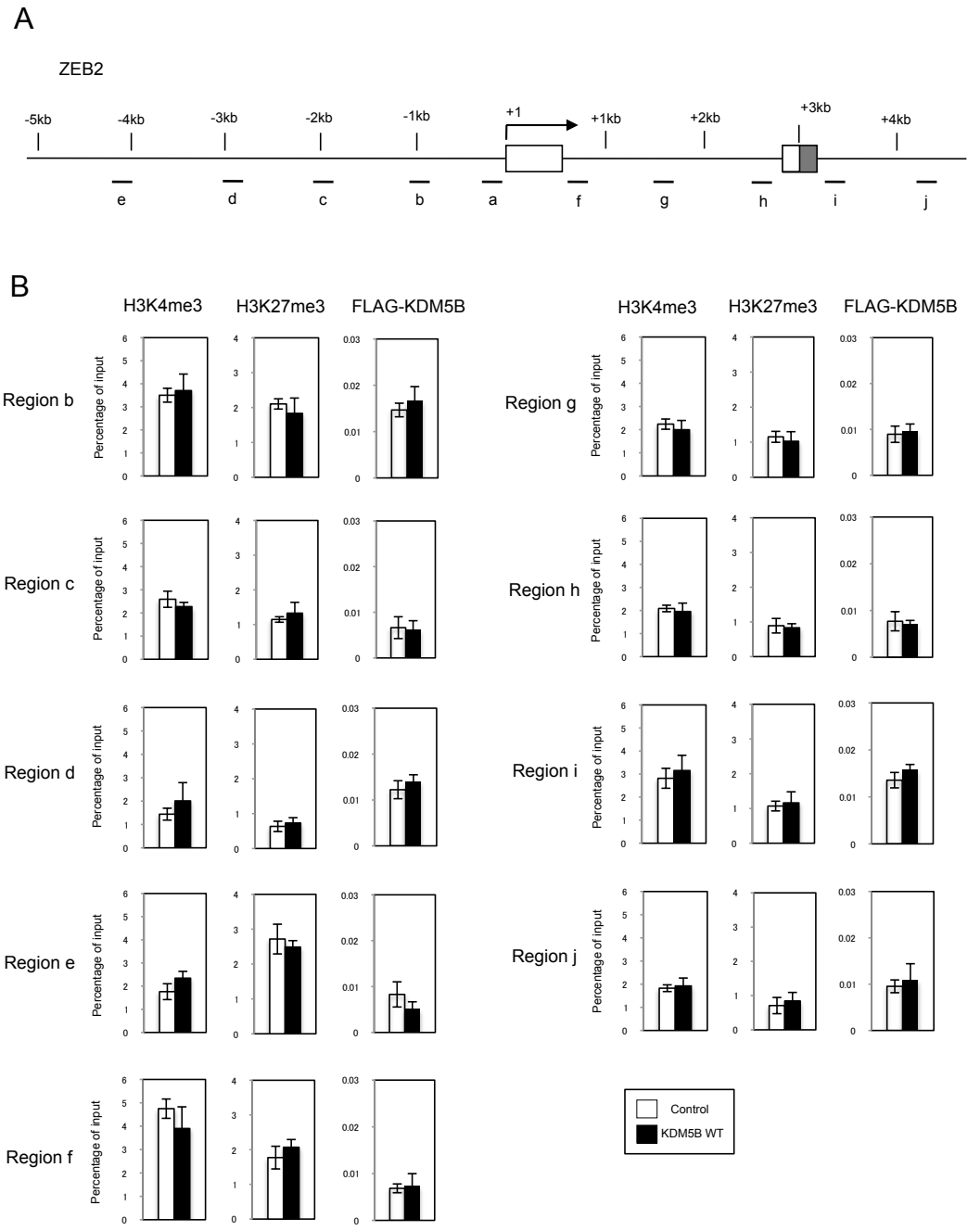

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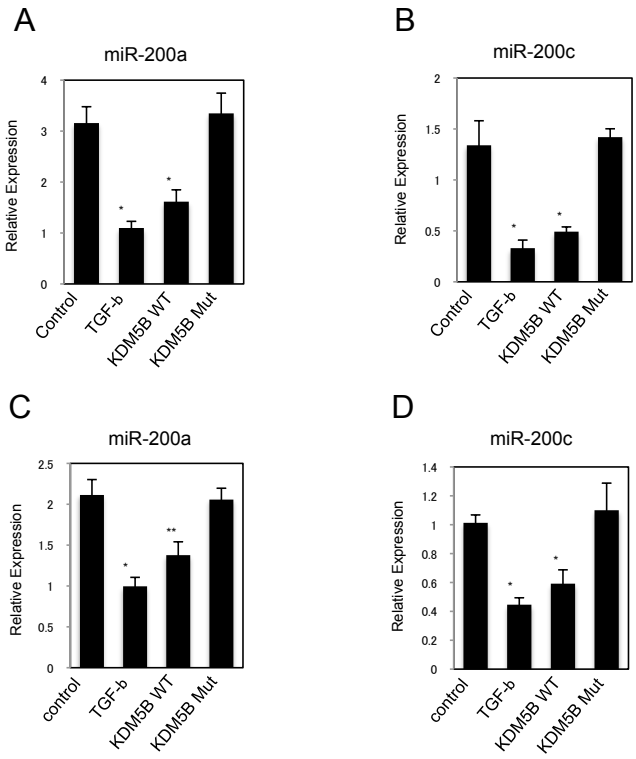

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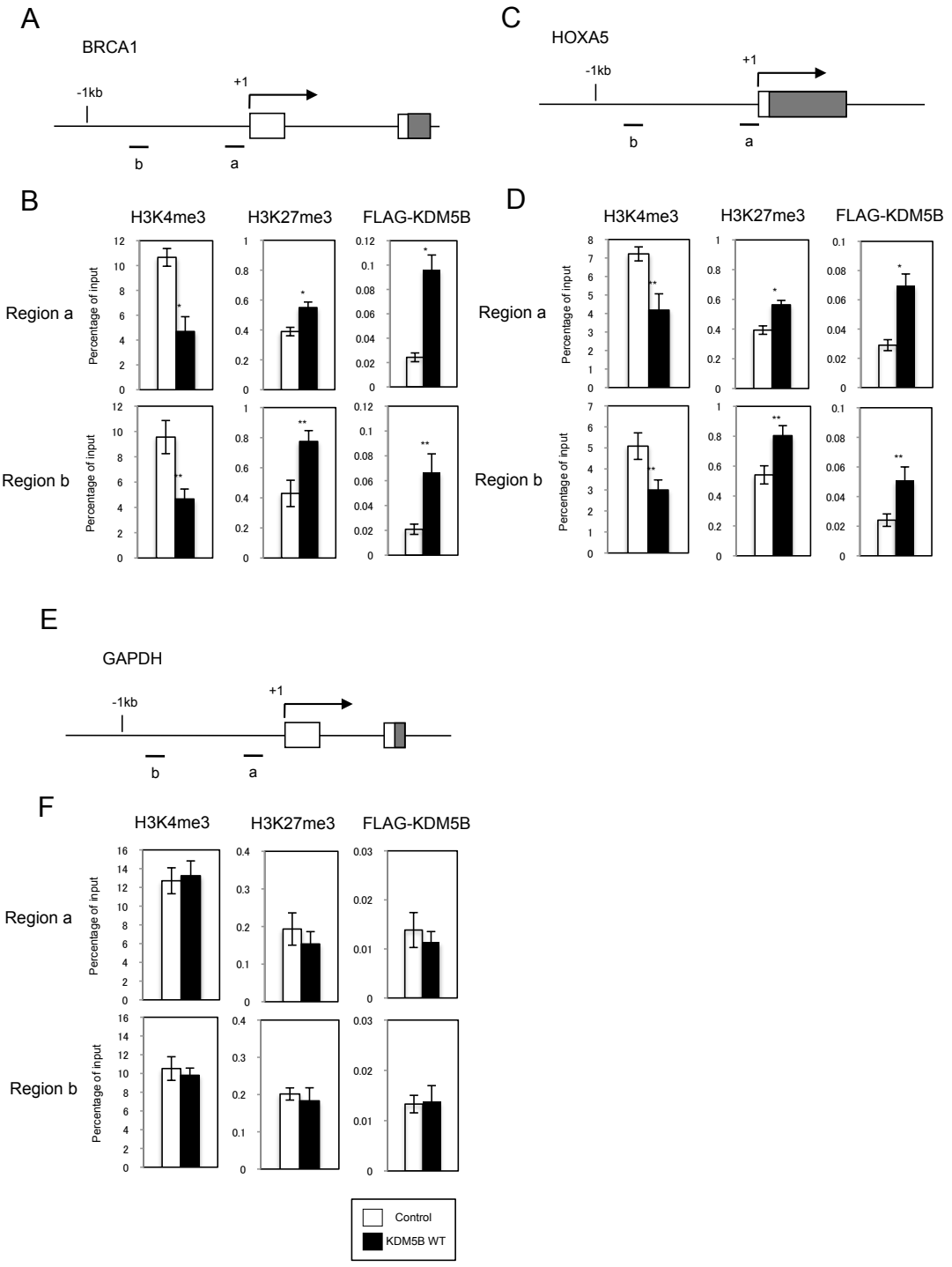

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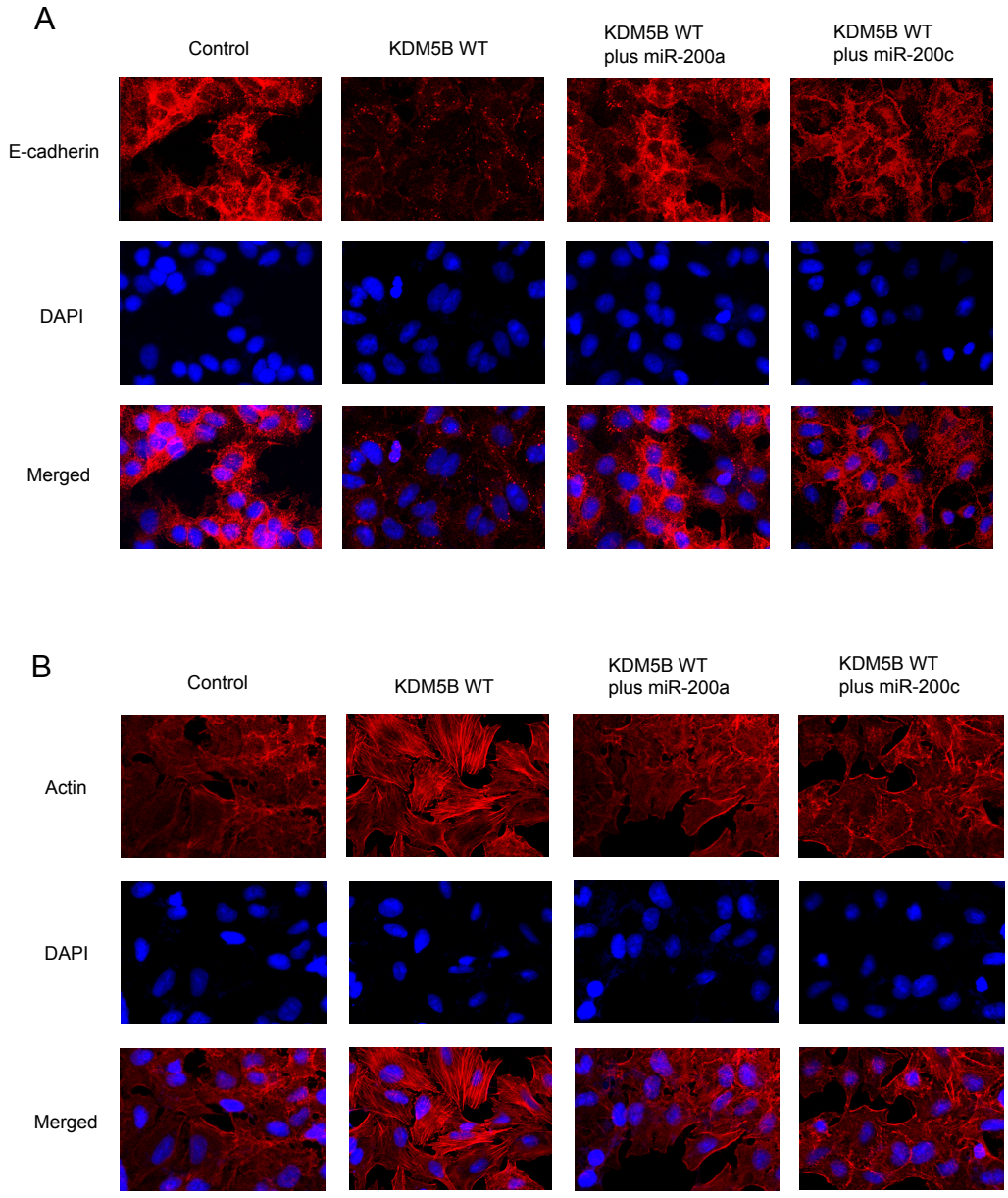

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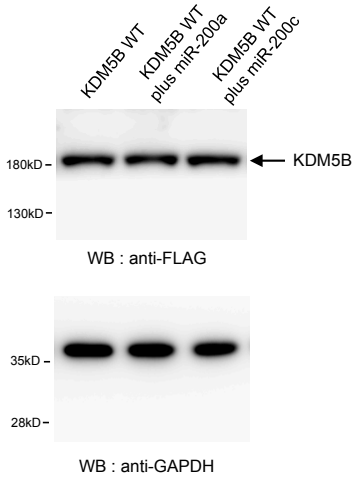

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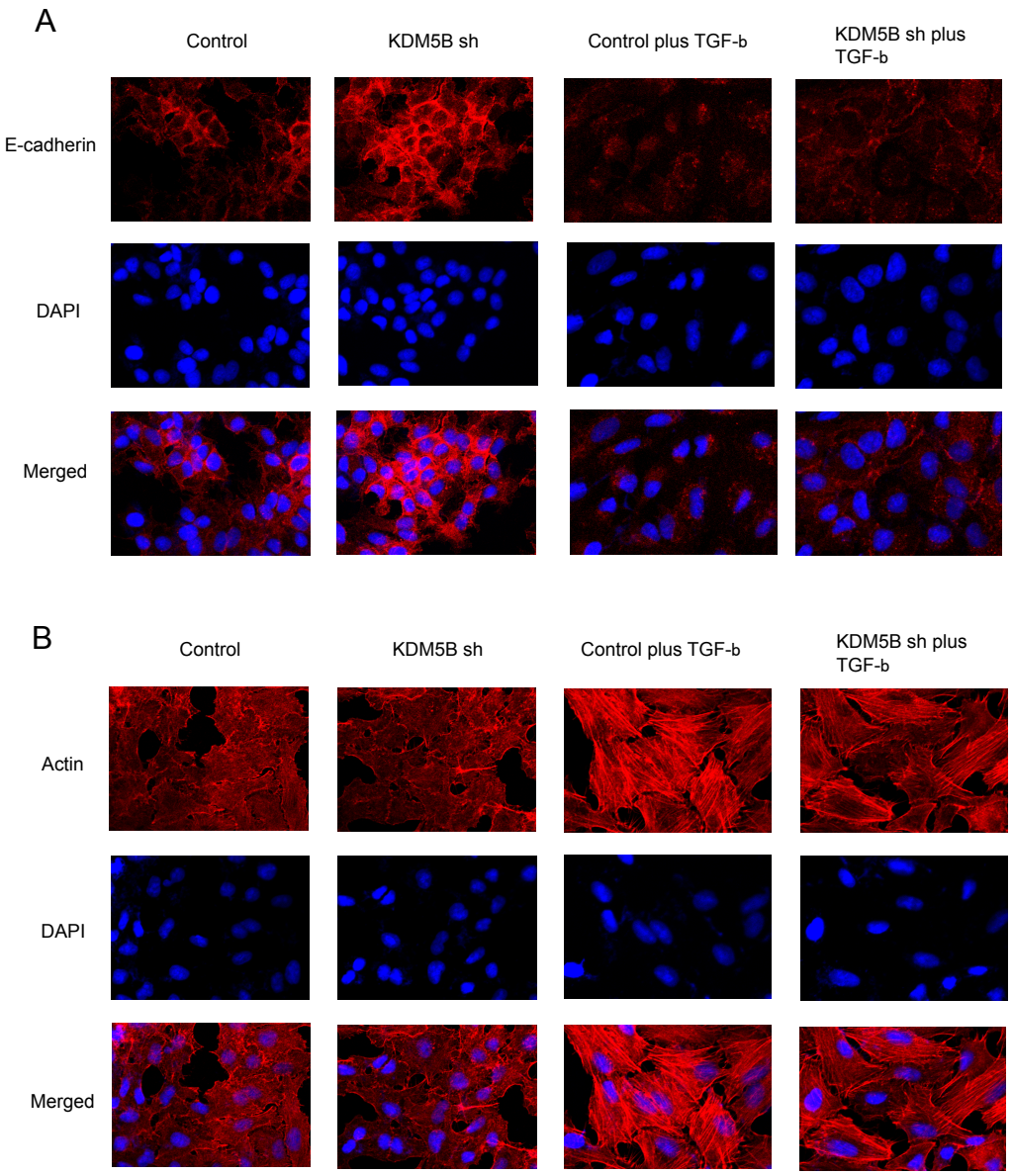

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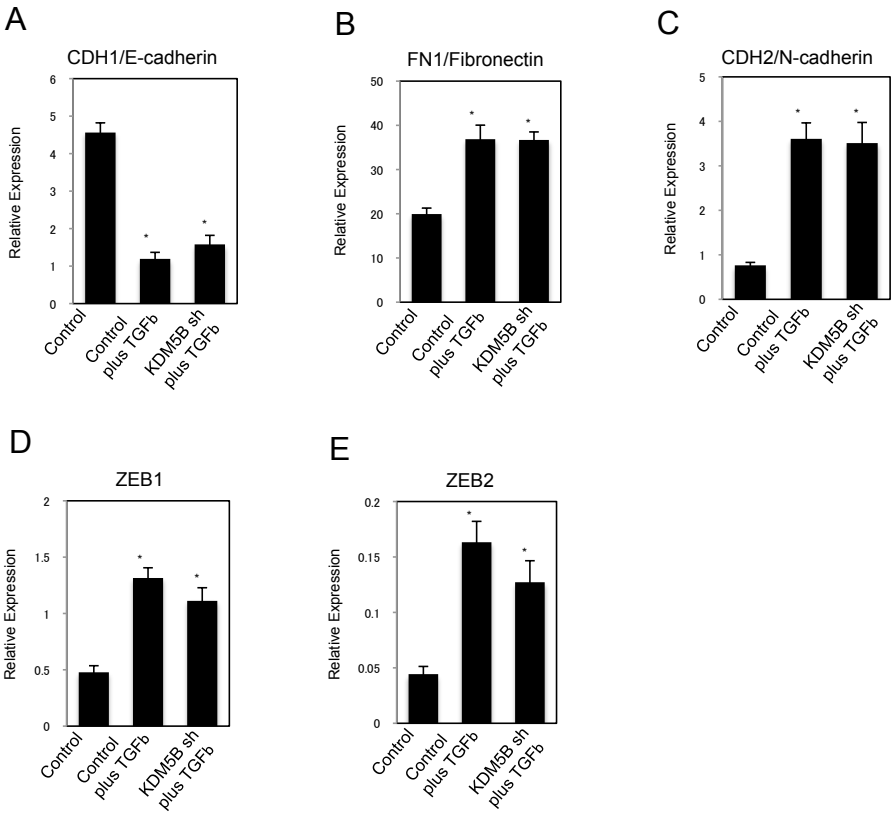

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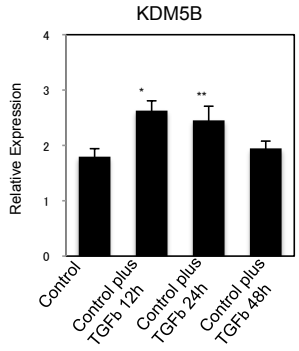

Enkhbaatar et al. Supplementary Figure 15

Table S1 Quantitative PCR primers used in this study

Gene	**Primer sequence (5' to 3')**
CDH1/*E-cadherin*	F: tgcccagaaaatgaaaaagg R: gtgtatgtggcaatgcgttc
FN1/*Fibronectin*	F: cagtgggagacctcgagaag R: tccctcggaacatcagaaac
CDH2/*N-cadherin*	F: acagtggccacctacaaagg R: ccgagatggggttgataatg
SNAI1	F: accccaatcggaagcctaact R: agatgagcattggcagcga
SNAI2	F: gcctccaaaaagccaaactaca R: gctgaggatctctggttgtggt
ZEB1	F: ttcaaacccatagtggttgct R: tgggagataccaaaccaactg
ZEB2	F: caagaggcgcaaacaagc R: ggttggcaataccgtcatcc
DDX5	F: tatggttggagtggcacaga R: ccagcaccaaacaaataggc
Mouse *Cdh1*/*E-cadherin*	F: aggagaacggtggtcaaaga R: gctggctcaaatcaaagtcc
Mouse *Fn1*/*Fibronectin*	F: ggaatggacctgcaaacctat R: catcatccagccttggtagg
Mouse *Cdh2*/*N-cadherin*	F: cattatcaaccccatctcagg R: tgcatgtgctctcaagtgaa
Mouse *Snai1*	F: cttgtgtctgcacgacctgt R: caggagaatggcttctcacc
Mouse *Snai2*	F: ctcacctcgggagcatacagc R: tgaagtgtcagaggaaggcggg
Mouse *Zeb1*	F: ttcaaacccatagtggttgct R: tgggagataccaaaccaactg

Mouse *Zeb2*	F: atgagcttcctaccgcatatgg R: tgtagtcttgtgctccatccag
Mouse *Actb/beta-Actin*	F: gctgtattcccctccatcgtg R: cacggttggccttagggttcag
CDH1 gene region g	F: aactactgttggggctgggt R: ggtgctaactgatggggtg
CDH1 gene region f	F: gatgttaggaaagcaatggg R: gtaaatgctgtccagggct
CDH1 gene region e	F: gaaacagagaagcaattcagtg R: cagtagatcaggtgtccaggg
CDH1 gene region d	F: ggctagtgagtggctgactc R: acaacttccctgtctgactcc
CDH1 gene region c	F: agtgagccaagaacacacca R: tgtgccagtctctgtgctaag
CDH1 gene region b	F: aaggcaggaggatcgcttc R: tgtagagagacaagtcggggc
CDH1 gene region a	F: ttctgatcccaggtcttagtg R: gttgctagggtctaggtgggtta
CDH1 gene region h	F: ggataagaaagtgaggtcgg R: gatgtctttattctccagtaccc
CDH1 gene region i	F: caaaccgaggctaagagagtg R: cagagatggtgcttaatggg
CDH1 gene region j	F: gctttgttccacttgactgtt R: gcctcttattgtgataccca
ZEB1 gene region g	F: ccattctgtggtaaactatgtaac R: gtgatgcagaacccacagtt
ZEB1 gene region f	F: gtatcccctaccgtttgattt R: atacagctaaagaataggggaa
ZEB1 gene region e	F: tctatgacctgattcggtag R: tatgtcaacacggtgtccttg
ZEB1 gene region d	F: acttctagcctctctttcaatcc R: agagaggctacctgacccg

ZEB1 gene region c	F: gaaagtagtgctctctgccc R: agaccaggtaagagacataacg
ZEB1 gene region b	F: gctgctgtgccaagggaaa R: aggcgactgtgcaaccacc
ZEB1 gene region a	F: gctttggttcctgcgttattt R: ttctcctaaacacgtatttcctcg
ZEB1 gene region h	F: cagcaaatggcaacttgtg R: aatgaaagcagcagacagga
ZEB1 gene region i	F: tggaatatgtctgaaggtagga R: accacacaaggtttgatgct
ZEB1 gene region j	F: gagggctcagtagtgaatagg R: ctccatgctacaatgtatctcg
ZEB2 gene region e	F: ttggagtaagttggatgcgac R: tggttttcaattccctggtg
ZEB2 gene region d	F: ttcctcaactttcacagccg R: ctgtgtgttcaagggcagaaa
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ZEB2 gene region h	F: ggagtttatcgaggcactgtc R: gacagtgtccaaagaggctta
ZEB2 gene region i	F: ggaaaagtttggttcgggc R: cttatcaatgaagcagccgat
ZEB2 gene region j	F: gagcgagaagtttcctttcc R: tgacggaggataactgagttt

miR-200b/a/429 gene region a	F: tatgggagcccaggggaca R: ctcgccttacaaggagcagtg
miR-200b/a/429 gene region b	F: gctgtgggtctgtggggtct R: tttggagcaatgaagggacc
miR-200c/141 gene region a	F: agggctcaccaggaagtgt R: ttgggtcaggcagcttcag
miR-200c/141 gene region b	F: gaaggggttaaggcagtgg R: cctccgctcttcctcctt
GAPDH gene region a	F: tactagcggttttacgggcg R: gaggctgcgggctcaattt
GAPDH gene region b	F: atcgtgaccttccgtgcaga R: catctcctggctcctggcat
BRCA1 gene region a	F: aatcagaggatgggaggga R: ctttatggcaaactcaggtagaa
BRCA1 gene region b	F: agtagtcttgtaaggtcagtggc R: taacaaacactggggctgag
HOXA5 gene region a	F: tgtgtgcttgatttgtggct R: cgtaggagggaaccaagtacat
HOXA5 gene region b	F: tgtgtagtgtttctccaaggc R: aaatcgcaaactaatgacacg

F: Forward, R: Reverse