Figure EV1. The Oncomine database analyses of FAM189A1, ENTREP/FAM189A2, and FAM189B expression in various types of cancer.

Number indicates the number of datasets of gene expression analyses.

### Analysis Type by Cancer

| Cancer Type         | FAM189A1 Cancer vs. Normal | FAM189A2 Cancer vs. Normal | FAM189B Cancer vs. Normal |
|---------------------|---------------------------|---------------------------|---------------------------|
| Bladder Cancer      | 6                         | 3                         | 1                         |
| Brain and CNS Cancer| 12                        | 1                         | 2                         |
| Breast Cancer       | 8                         | 8                         | 1                         |
| Cervical Cancer     | 1                         | 1                         |                           |
| Colorectal Cancer   | 3                         |                           |                           |
| Esophageal Cancer   |                           | 1                         |                           |
| Gastric Cancer      | 3                         |                           |                           |
| Head and Neck Cancer|                           |                           |                           |
| Kidney Cancer       |                           | 1                         |                           |
| Leukemia            |                           | 1                         |                           |
| Liver Cancer        |                           |                           |                           |
| Lung Cancer         | 1                         | 12                        | 1                         |
| Lymphoma            |                           |                           |                           |
| Melanoma            |                           |                           |                           |
| Myeloma             |                           |                           |                           |
| Other Cancer        | 1                         |                           |                           |
| Ovarian Cancer      | 2                         |                           |                           |
| Pancreatic Cancer   | 1                         |                           |                           |
| Prostate Cancer     | 1                         | 1                         |                           |
| Sarcoma             |                           |                           | 2                         |

**Significant Unique Analyses**: 2, 16, 51, 6

**Total Unique Analyses**: 415, 432, 426

Gene rank percentile for the analyses.
Figure EV2. Ubiquitination of ENTREP.

A Sample preparation for the ubiquitin-AQUA/PRM analysis. The immunoprecipitated samples using anti-FLAG antibody under the denaturing condition were separated by the SDS-PAGE and served for the immunoblot analyses and the Coomassie Brilliant Blue (CBB) gel staining. The gel area corresponding to the band #A, #B, and #C of the immunoblot were excised from the CBB-stained gel and used for ubiquitin-AQUA/PRM and shotgun MS analyses. The immunoblot image of FLAG IP/FLAG IB is the same with that of Fig 3B. We analyzed samples from three biological replicates (#1-3 of the control samples and #4-6 of ENTREP samples). The raw data of the ubiquitin-AQUA/PRM analysis are listed on Appendix Table S2 and the result of the shotgun MS is in Appendix Fig S3.

B The cycloheximide chase assay. Twenty-four hours after transfection, HEK293T cells were incubated with 50 mg/ml cycloheximide for the indicated time periods and served for the immunoblot analyses. The immunoblot analyses were carried out using six independent samples, and their blot bands were semi-quantified using ImageJ software. The relative FLAG-ENTREP expression levels were calculated as a ratio of GAPDH-adjusted FLAG-ENTREP at each time points and presented as a mean ± SD from six biological replicates. * P < 0.05. Note that, in the immunoblot analyses, the volume of Halo-ITCH transfected samples applied was a half of Halo-transfected samples applied, as indicated by GAPDH.

Figure EV3. The ITCH WW domain responsible for the association with ENTREP and CXCR4.

A Schema of the expression vectors of ITCH deletion mutants.

B, C The immunoprecipitation analysis. FLAG-ENTREP wild cyt co-precipitated with Halo-ITCH delWW123 but not with delWW124, whereas HA-CXCR4DD-DsRed co-precipitated equally with either Halo-ICTH delWW123 or delWW124. Data shown are representative of at least two independent experiments.
Figure EV4. Comparison of NDFIP1, N4BP1, and FAM189 family.

A The Oncomine database analysis of NDFIP1, NDFIP2, and N4BP1 expression in various types of cancer. Number indicates the number of datasets of gene expression analyses.

B Phylogenetic analysis of FAM189A1, ENTREP/FAM189A2, FAM189B, NDFIP1, and N4BP1. The coding DNA sequences of these genes were analyzed using Phylogeny.fr software (https://www.phylogeny.fr/). Analyzed sequences were as follows: human FAM189A1, NM_015307.1; mouse Fam189a1, NM_183087.4; chicken Fam189a1, XM_025154007.1; dog Fam189a1, XM_025438066.1; human FAM189A2, NM_001127608.2; mouse Fam189a2, NM_001114174.1; chicken Fam189a2, XM_424828.6; dog Fam189a2, XM_022414281.1; human FAM189B, NM_006589.3; mouse Fam189b, NM_001014995.2; chicken Fam189b, XM_025143513.1; dog Fam189b, XM_005622739.2; human N4BP1, NM_153029.1; mouse N4bp1, NM_030563.2; chicken N4bp1, NM_001030570.1; dog N4bp1, XM_02241581.1; human NDFIP1, NM_030571.4; mouse Ndfp1, NM_001355749.1; chicken Ndfp1, XM_414658.5; dog Ndfp1, XM_022408863.1.

C The comparison of PPxY motif. FAM189A1 and FAM189B contain the overlapped PPxY sequences which are separated with PYY by 40 aa and 28 aa, respectively.