SUPPLEMENTAL FIGURES AND LEGENDS

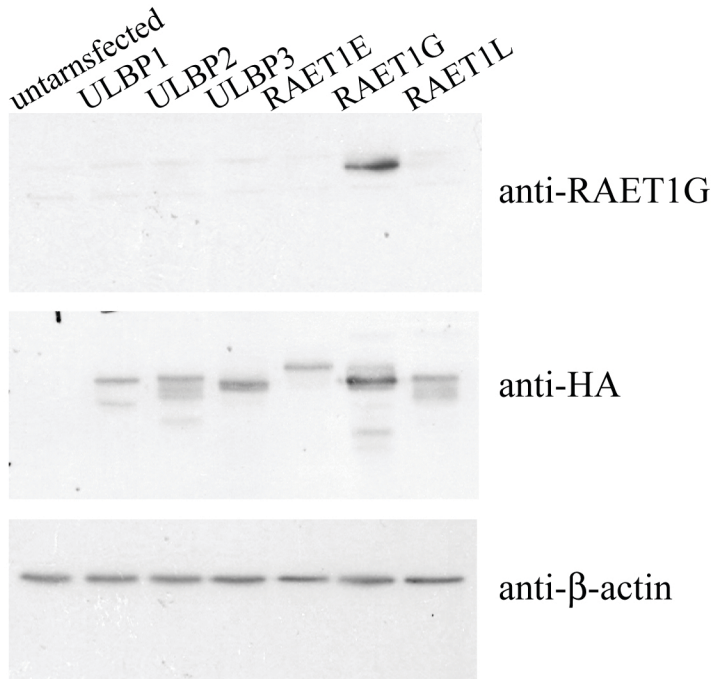
Fig. S1 **Recombinant monoclonal anti-RAET1G antibody does not cross-react with the other ULBP/RAET1 family proteins** *A,* Lysates of CHO cells transfected with recombinant ULBP1, ULBP2, ULBP3, RAET1E, RAET1G, and RAET1L were subjected to SDS 15% PAGE separation and Western blot with monoclonal anti-RAET1G antibody or anti-HA antibody. As an internal control, expression of β-actin was detected by anti-β-actin antibody. *B,* The surface expression level of ULBP/RAET1 family proteins in CHO cell lines transfected with recombinant ULBP1, ULBP2, ULBP3, RAET1E, RAET1G, and RAET1L were assessed by FACS using monoclonal anti-RAET1G or anti-HA antibody in FL4 channel.

Fig. S2 **A schematic diagram of post-translational modification of RAET1G** RAET1G is first translated as the full-length form corresponding to lower band of ~37KDa doublet in Western blotting (1) and is glycosylated in the ER corresponding to upper band of ~37KDa doublet (2). The cleavage of the C-terminus of the protein and GPI-anchoring occur simultaneously by the enzyme located in the ER, which makes RAET1G GPI-anchring form corresponding to ~28KDa band in Western blot (3). After glycosylation in the Golgi, the ~32KDa GPI-anchored RAET1G form is transported to the cell surface (4).

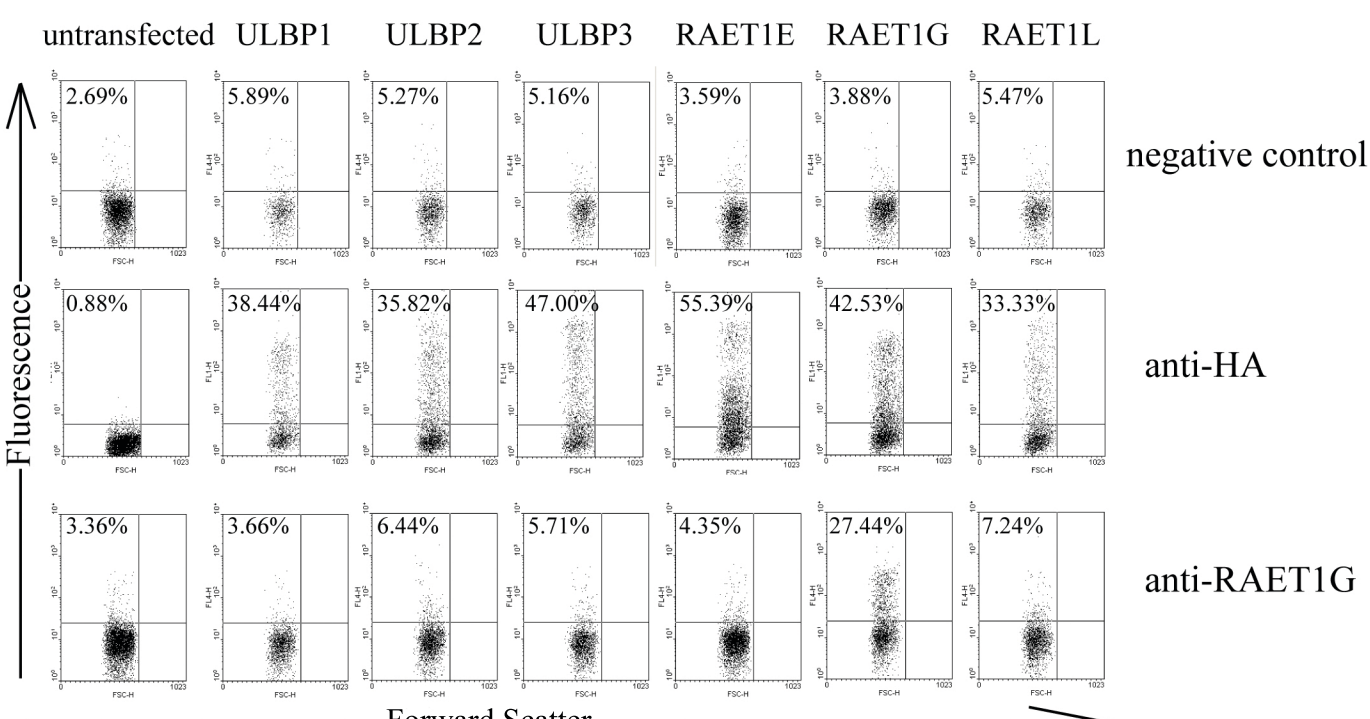
Fig. S3 **Genomic DNA encoding the transmembrane and cytoplasmic tail of RAET1G.** *A.* Schematic representation of ULBP/RAET1 family proteins. The shadowed boxes represent the hydrophobic region of ULBP1, ULBP2, ULBP3, and RAET1L proteins and transmembrane region of RAET1G protein. The underlined bars represent the region used for DNA alignment in *B. B,* The DNA alignment of the region underlined in *A.* Conserved residues are shown in red.

Fig. S1

A


untarnsfected
ULBP1
ULBP2
ULBP3
RAET1E
RAET1G
RAET1L
anti-RAET1G
anti-HA
anti-β-actin

B


untransfected
ULBP1
ULBP2
ULBP3
RAET1E
RAET1G
RAET1L
2.69%
5.89%
5.27%
5.16%
3.59%
3.88%
5.47%
negative control
0.88%
38.44%
35.82%
47.00%
55.39%
42.53%
33.33%
anti-HA
3.36%
3.66%
6.44%
5.71%
4.35%
27.44%
7.24%
anti-RAET1G
Fluorescence
Forward Scatter

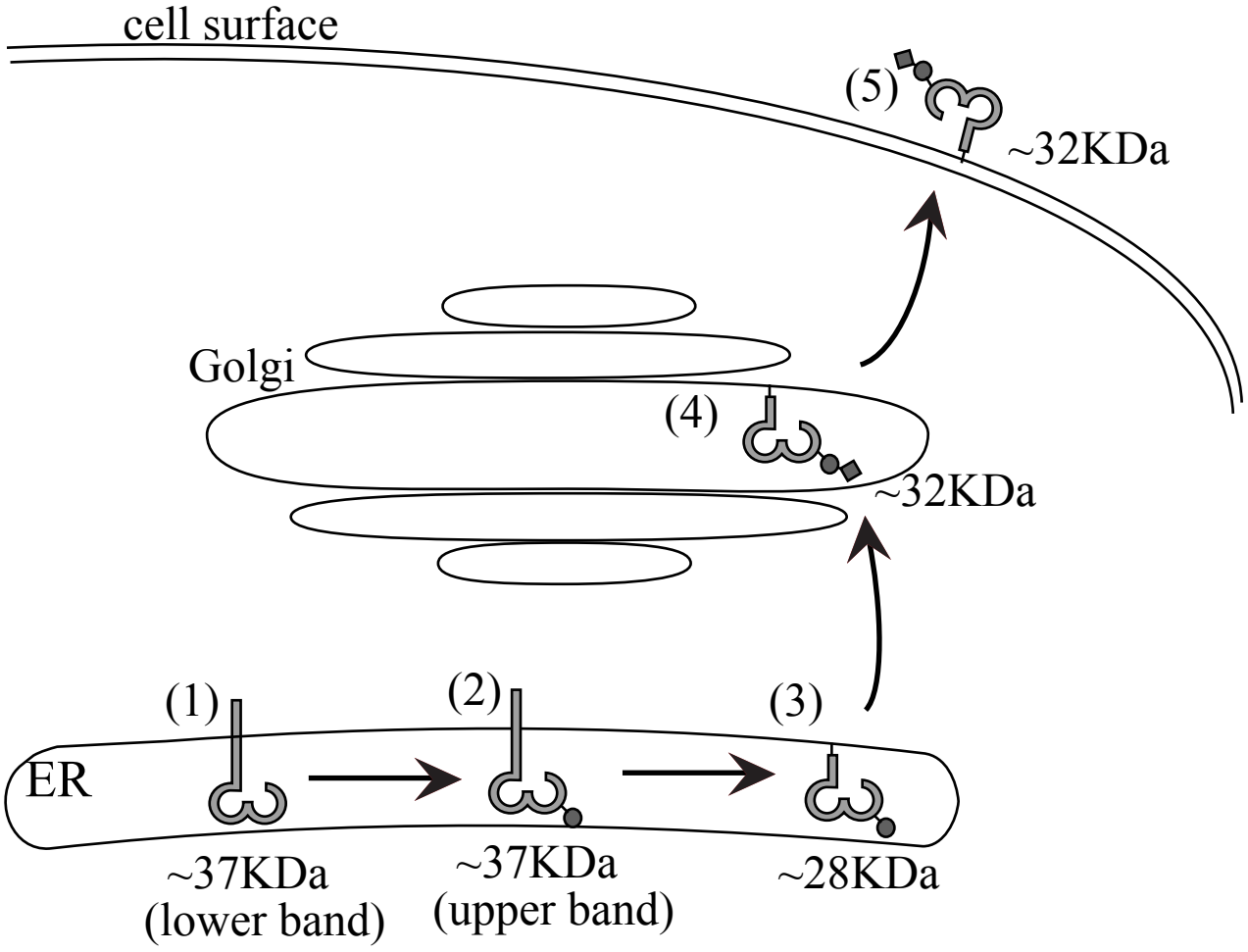
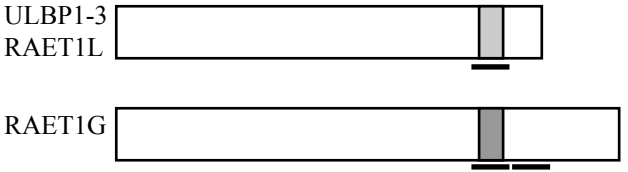

cell surface
(5)
~32KDa
Golgi
(4)
~32KDa
(1)
(2)
(3)
ER
~37KDa
(lower band)
~37KDa
(upper band)
~28KDa

Fig. S3

A



B

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ULBP2          CTCAGGCACAACCCAACTCAGGGCCACAGCCACCACCCTCATCCTTTGCTGCCTCCTCAT
RAET1L         CTCAGGCACAACCCAACTCAGGGCCACAGCCACCACCCTCATCCTTTGCTGCCTCCTCAT
ULBP1          TCCAGGCACAGTCTGACCCAAAGCCATGGCCACCACCCTCAGTCCCTGCAGCCTCCTCCT
RAET1G tail    CCCAGGCACAACCCAACCCAAGGCCATGGCCACCACCCTCAGTCCCTGGAGCCTTCTCAT
ULBP3          CCCAGGCTTAGCTCAACCCAAAGCCATAGCCACCACCCTCAGTCCCTGGAGCT---TCCT
RAET1G TM      CTCAGGCACAGCCCAACCCAGGGCCACGGCCACCACCCTCATCCTTTGCTGCCTCCTCAT
                 *****: *.   .** **..**** .*************  *  ** :**    **.*

ULBP2          CAT------CCTCCCCTGCTTCATCCTCCCTGGCATCTGA--------------------
RAET1L         CAT------CCTCCCCTGCTTCATCCTCCCTGGCATCTGA--------------------
ULBP1          CAT------CCTCCCCTGCTTCATCCTACCTGGCTGCTGAGAAGAGTCCTTTGGAGTGAC
RAET1G tail    CAT------CTTCCTCTGCTTCATTCTAGCTGGCAGATGA--------------------
ULBP3          CAT------CATCCTCTGCTTCATCCTCCCTGGCATCTGA--------------------
RAET1G TM      CATGTGTCTCCTCATATGCTCCAGGCACAGTCTGACCCAAA--------------------
               ***      * **. .**** **  *:.  *   : . .*
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