Supplemental figure legends

Fig. S1. Localization of the chimeric IL-2Rα including the hydrophobic amino acid cluster of IL-2Rβ to the plasma membrane. (A) Structures of wild-type IL-2Rα and its chimeric receptors. The signal sequence, transmembrane region, the cytoplasmic tail (residues 269-551) and hydrophobic amino acid cluster (residues 365-369) of IL-2Rβ are indicated. (B) MEF cells transiently expressing wild-type IL-2Rα and the indicated chimeric receptors were grown on coverslips and the cell surface receptors were bound by an anti-IL-2Rα antibody (H-31) at 0°C, followed by treatment with a chemical crosslinker. The cells were cultured at 37°C for 120 minutes, fixed and incubated with an anti-LAMP1 monoclonal antibody. Fluorescence labeling was carried out for IL-2Rα (red) and LAMP1 (green). Scale bars: 10 μm.

Fig. S2. Expression levels of IL-2Rβ and IL-4Rα in BAF-B03 transfectants. (A,B) The expression levels of IL-2Rβ and IL-4Rα on the cell surface of BAF transfectants were examined by flow cytometry. BAFβ-clone 15, BAFβ-clone 21, BAFβ-mH2-clone 10, BAFβ-mH2-clone 38, BAF-IL-4Rα-clone 18, BAF-IL-4Rα-clone 38, BAF-IL-4Rα-mH-clone 2 and BAF-IL-4Rα-mH-clone 48 were incubated with an anti-IL-2Rβ antibody (TU11) or anti-IL-4Rα antibody (MAB230), followed by incubation with a FITC-conjugated secondary antibody.

Fig. S3. Internalization and degradation of IL-2Rβ and IL-4Rα in MEF transfectants. (A,B) Internalization of IL-2Rβ and IL-4Rα in the transfectants. MEF transfectants were incubated with ^{125}I-anti-IL-2Rβ antibody (TU11) or ^{125}I- anti-IL-4Rα antibody (MAB230) at 0°C. The cells were incubated at 37°C and harvested at the indicated times. The radioactivities of the cell surface-bound acid-removable fractions (a) and intracellular acid-unremovable fractions (b) were counted. (C,D) Degradation of IL-2Rβ and IL-4Rα in the transfectants. MEF transfectants were incubated with ^{125}I-anti-IL-2Rβ antibody or ^{125}I- anti-IL-4Rα antibody at 0°C, followed by treatment with the chemical crosslinker DTSSP. The cells were incubated at 37°C and harvested at the indicated times. The radioactivities of the culture supernatants (a), cell precipitate fractions (b) and TCA-soluble fractions of the culture supernatants (c) were counted. The values represent the means ± SE of triplicate determinations.

Fig. S4. Cytokine-induced tyrosine phosphorylation of IL-2Rβ, IL-4Rα and STAT proteins in BAF-B03 transfectants. (A) BAFβ-clone 21 and BAFβ-mH2-clone 38 cells were incubated with

1nM human recombinant IL-2 for the indicated times. Total lysate: aliquots (10 μg) of the lysates were immunoblotted with an anti-phosphotyrosine STAT5 monoclonal antibody (Y694) (top panel) or anti-STAT5 antibody (C-17) (2nd panel). Aliquots (1 mg) of the cell lysates were immunoprecipitated with an anti-IL-2Rβ monoclonal antibody (TU11) and immunoblotted with an anti-phosphotyrosine monoclonal antibody (PY100) (3rd panel) or anti-IL-2Rβ antibody (C20) (bottom panel). (B) BAF-IL-4Rα-clone 38 and BAF-IL-4Rα-mH-clone 2 cells were incubated with 1nM human recombinant IL-4 for the indicated times. Total lysate: aliquots (10 μg) of the lysates were immunoblotted with an anti-phosphotyrosine STAT6 antibody (Y641) (top panel) or anti-STAT6 antibody (2nd panel). Aliquots (1 mg) of the cell lysates were immunoprecipitated with an anti- IL-4Rα antibody (C20) and immunoblotted with an anti-phosphotyrosine monoclonal antibody (PY100) (3rd panel) or anti-IL-4Rα antibody (C20) (bottom panel). IP: immunoprecipitation; IB: immunoblotting.

Fig. S5. Endosomal sorting of IL-2Rβ or IL-4Rα along with IL-2Rγc in MEF transfectants. (A,B) MEFβ, MEFβ-mH2, MEF-IL-4Rα and MEF-IL-4Rα-mH cells transiently expressing IL-2Rγc were grown on coverslips and incubated with 100nM human recombinant IL-2 or 5nM human recombinant IL-4 for 6 hours. Then the cells were fixed and double-labeled with an anti-IL-2Rβ antibody (TU11) or anti-IL-4Rα antibody (MAB230) and an anti-IL-2Rγc (TUGh4). The cells were incubated with fluorescently labeled secondary antibodies. (C) MEFβ and MEFβ-mH2 cells transiently expressing IL-2Rγc were grown on coverslips and the cell surface receptors were incubated with an anti-IL-2Rβ antibody (TU11) and anti-IL-2Rγc (TUGh4) along with100nM IL-2 at 0°C. After washing, The cells were cultured for 40 minutes at 37°C, fixed and incubated with an anti-mouse specific or anti-rat specific fluorescently labeled secondary antibody. Fluorescence labeling was carried out for IL-2Rβ (red) and IL-2Rγc (green). Fluorescence images were observed using a confocal laser microscope. Scale bars: 10 μm.

Fig. S6. IL-2Rβ and IL-4Rα sorting to transferrin receptor (TfR)-positive compartments. MEFβ, MEFβ-mH2, MEF-IL-4Rα and MEF-IL-4Rα-mH cells were grown on coverslips and the cell surface receptors were bound by an anti-IL-2Rβ antibody (TU11) or anti-IL-4Rα antibody (MAB230) at 0°C, followed by treatment with a chemical crosslinker. The cells were cultured for 40 minutes at 37°C, fixed and incubated with an anti-transferrin receptor monoclonal antibody (R17217.1.4). Fluorescence labeling was carried out for IL-2Rβ (red), IL-4Rα (red) and transferrin receptor (green). Scale bars: 10 μm.

Figure S1

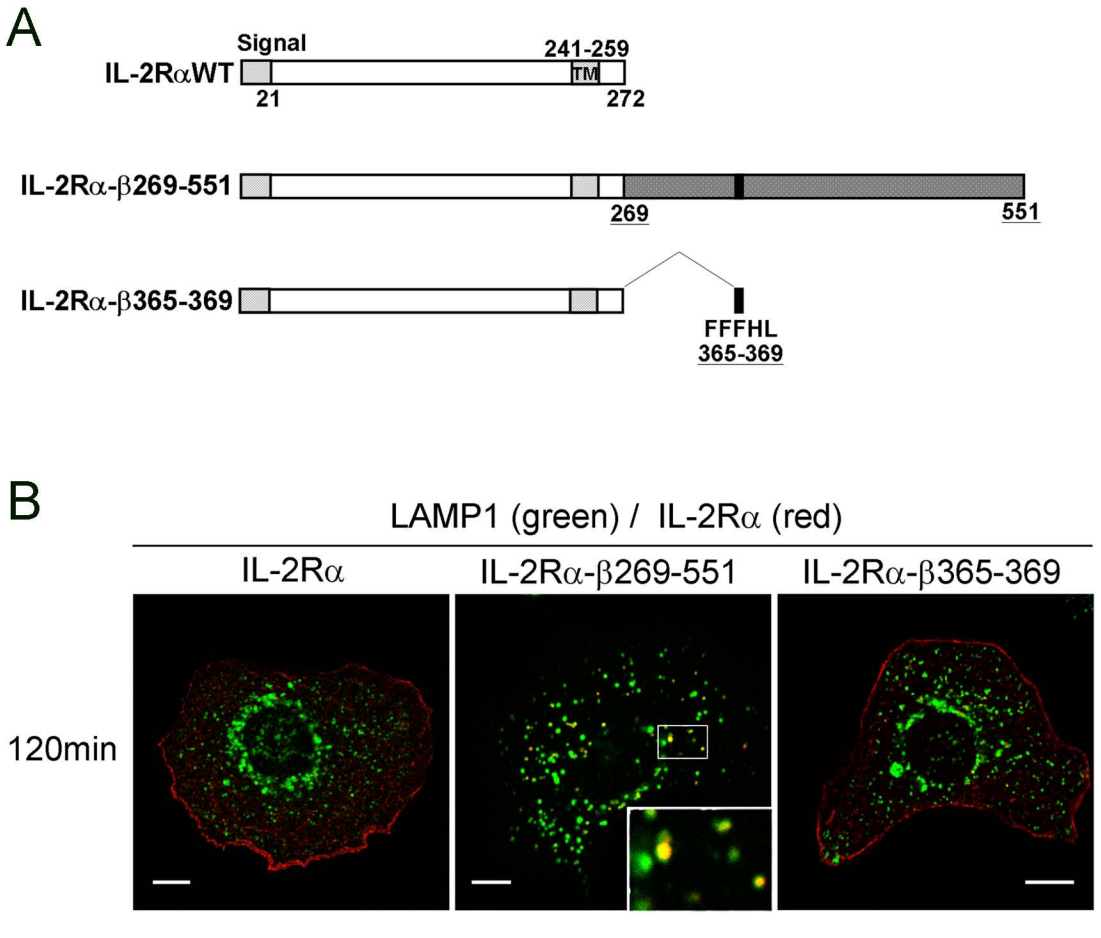

A
Signal
241-259
IL-2RαWT
TM
21
272
IL-2Rα-β269-551
269
551
IL-2Rα-β365-369
FFFHL
365-369
B
LAMP1 (green) / IL-2Rα (red)
IL-2Rα
IL-2Rα-β269-551
IL-2Rα-β365-369
120min

Figure S2

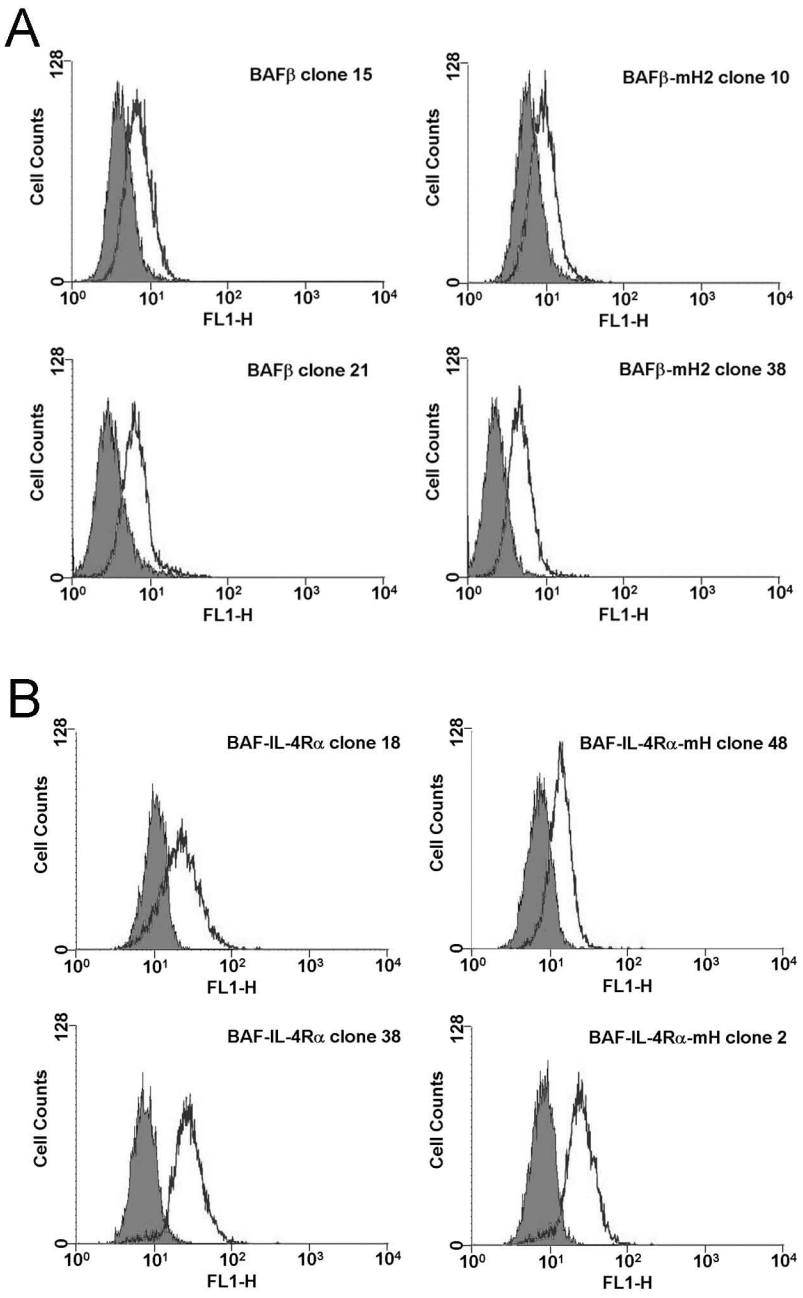

A
BAFβ clone 15
BAFβ-mH2 clone 10
BAFβ clone 21
BAFβ-mH2 clone 38
B
BAF-IL-4Rα clone 18
BAF-IL-4Rα-mH clone 48
BAF-IL-4Rα clone 38
BAF-IL-4Rα-mH clone 2
Cell Counts
FL1-H
0
128
10⁰
10¹
10²
10³
10⁴

Figure S3

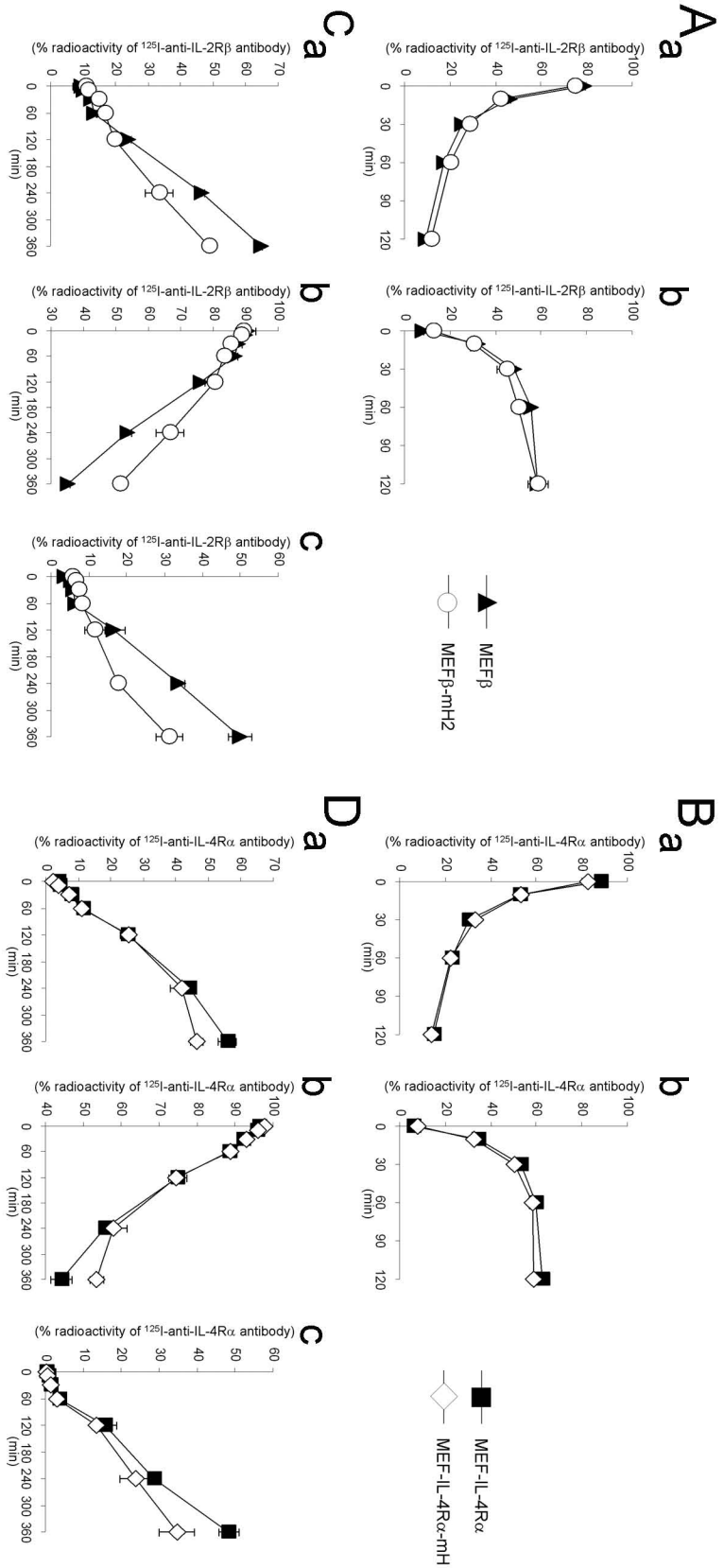

A
a
b
c
B
a
b
c
C
a
b
c
D
a
b
c
(% radioactivity of 125I-anti-IL-2Rβ antibody)
(% radioactivity of 125I-anti-IL-4Rα antibody)
(min)
MEFβ
MEFβ-mH2
MEF-IL-4Rα
MEF-IL-4Rα-mH

Figure S4

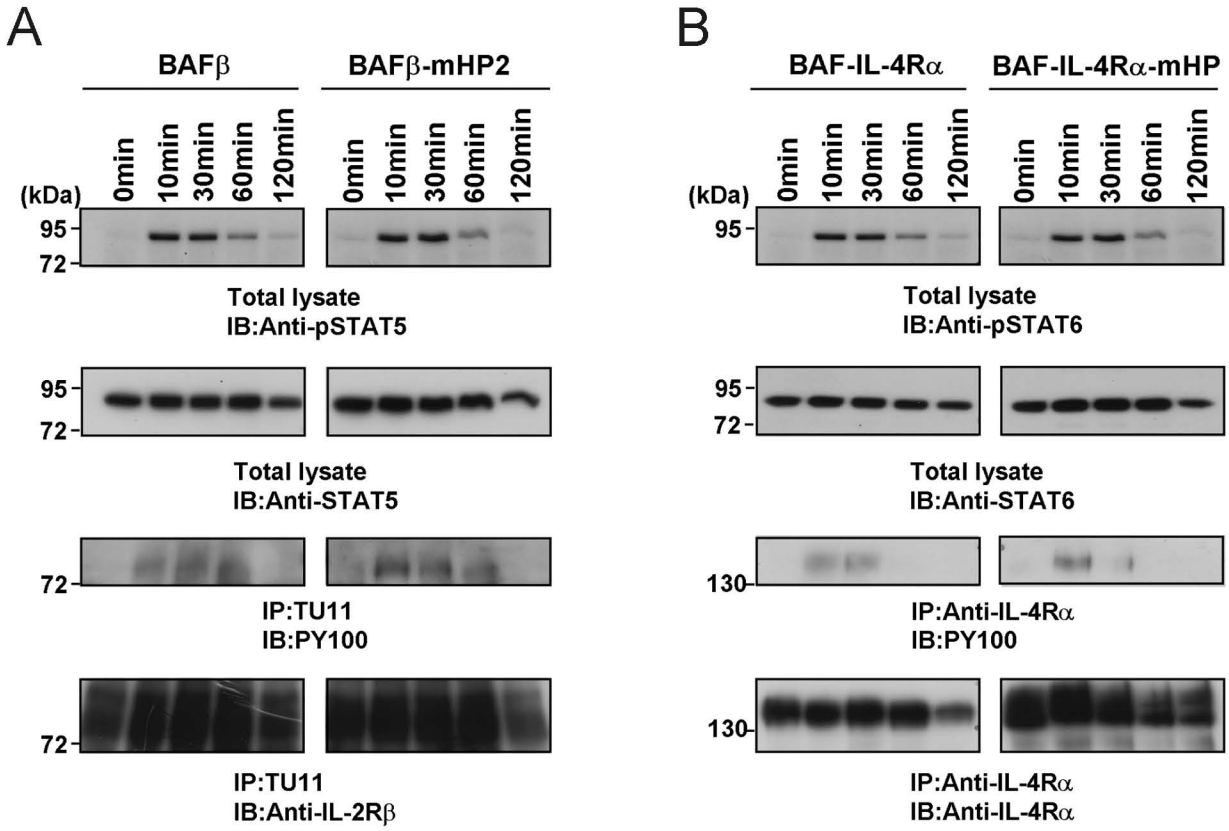

A
BAFβ
BAFβ-mHP2
0min
10min
30min
60min
120min
(kDa)
95
72
Total lysate
IB:Anti-pSTAT5
Total lysate
IB:Anti-STAT5
IP:TU11
IB:PY100
IP:TU11
IB:Anti-IL-2Rβ
B
BAF-IL-4Rα
BAF-IL-4Rα-mHP
130
Total lysate
IB:Anti-pSTAT6
Total lysate
IB:Anti-STAT6
IP:Anti-IL-4Rα
IB:PY100
IP:Anti-IL-4Rα
IB:Anti-IL-4Rα

Figure S5

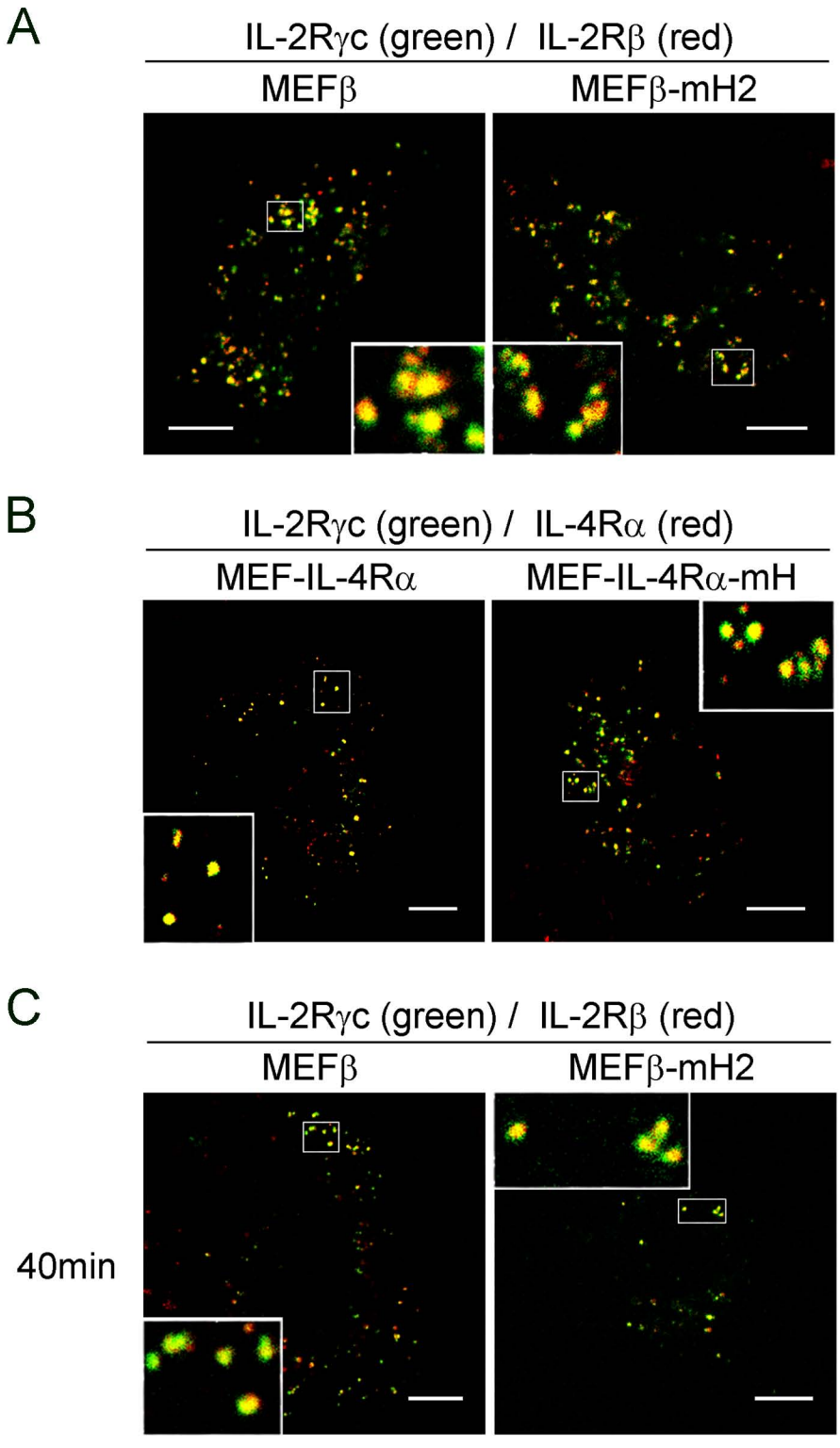
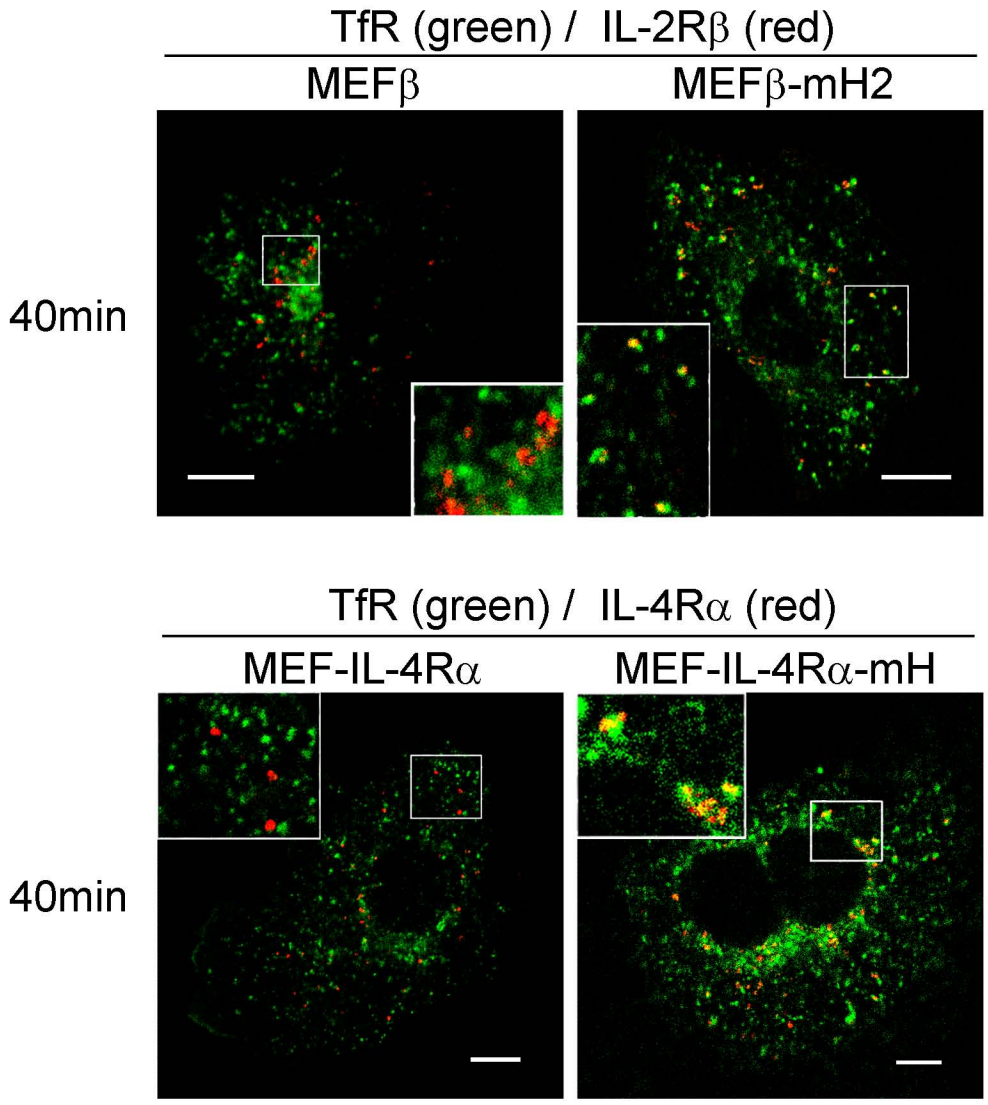

A
IL-2Rγc (green) / IL-2Rβ (red)
MEFβ
MEFβ-mH2
B
IL-2Rγc (green) / IL-4Rα (red)
MEF-IL-4Rα
MEF-IL-4Rα-mH
C
IL-2Rγc (green) / IL-2Rβ (red)
MEFβ
MEFβ-mH2
40min

Figure S6


TfR (green) / IL-2Rβ (red)
MEFβ
MEFβ-mH2
40min
TfR (green) / IL-4Rα (red)
MEF-IL-4Rα
MEF-IL-4Rα-mH
40min