Supplemental Data

Intracellular Dynamics and Fate of a Humanized Anti-Interleukin-6 Receptor Monoclonal Antibody, Tocilizumab (TCZ)

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Supplemental Figure: 5

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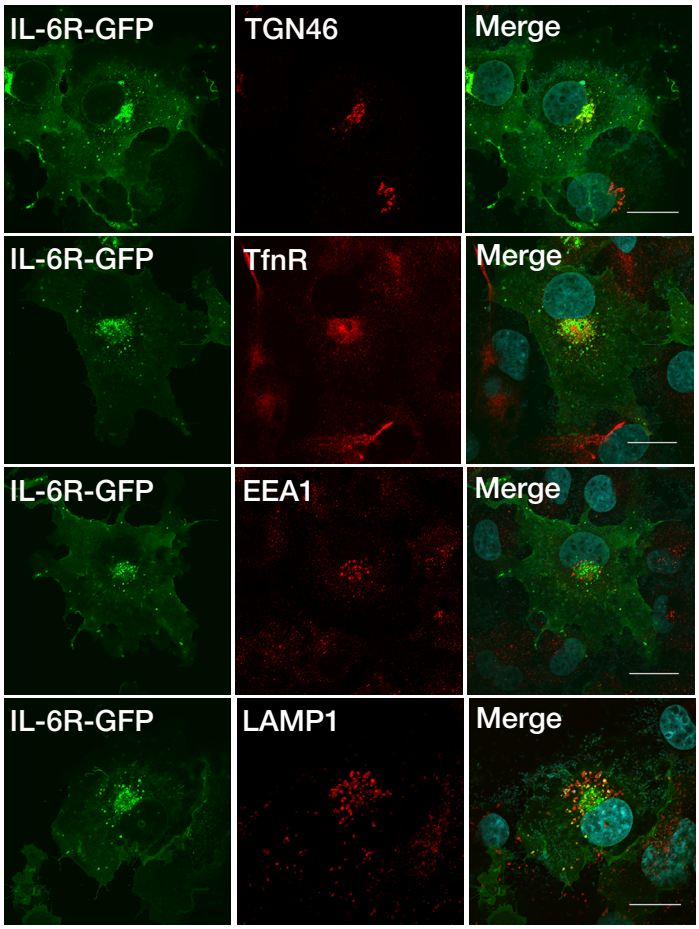
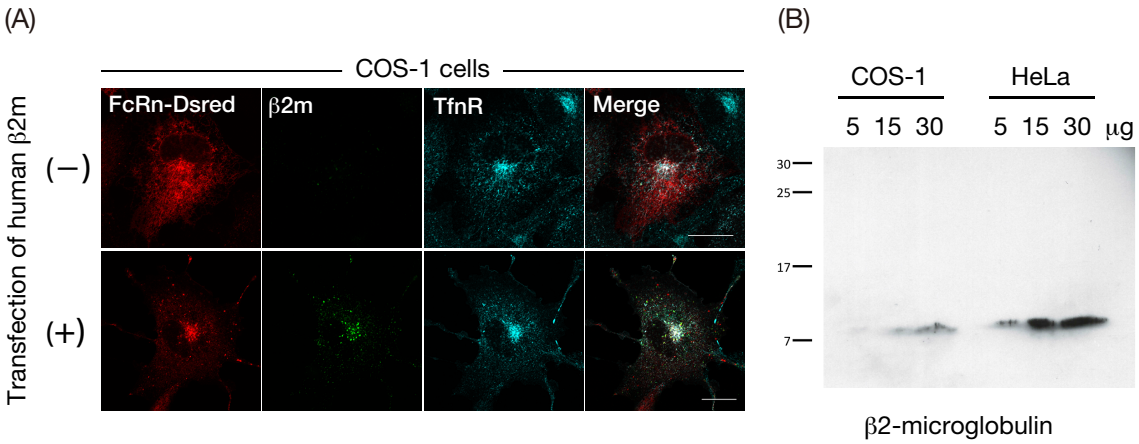
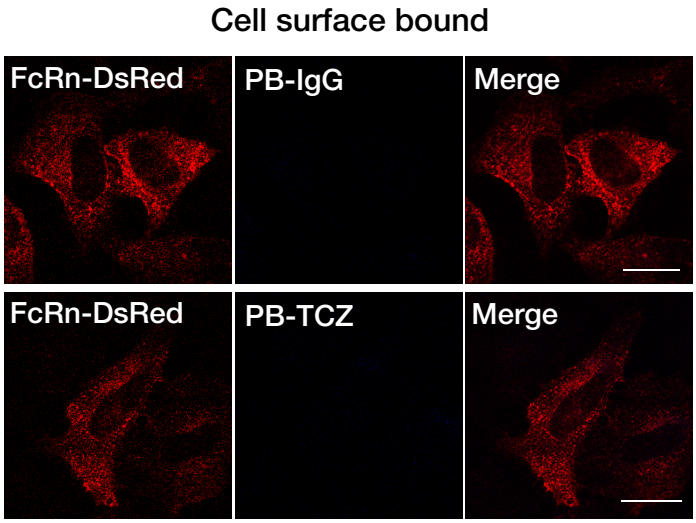


Supplemental Figure 1. Intracellular localization of IL-6R-GFP in COS-1 cells. COS-1 cells were transiently transfected with an expression plasmid encoding IL-6R-GFP, fixed and incubated with primary antibodies to TGN46, EEA1 or LAMP-1. The primary antibodies were revealed by incubation with Cy3-labeled secondary antibodies. Nuclei were labeled with DAPI. Cells were visualized by confocal microscopy. Right columns show the merged images for triple staining of IL-6R-GFP (green), each organelle marker (red) and DAPI (cyan). Bars, 20 μm.

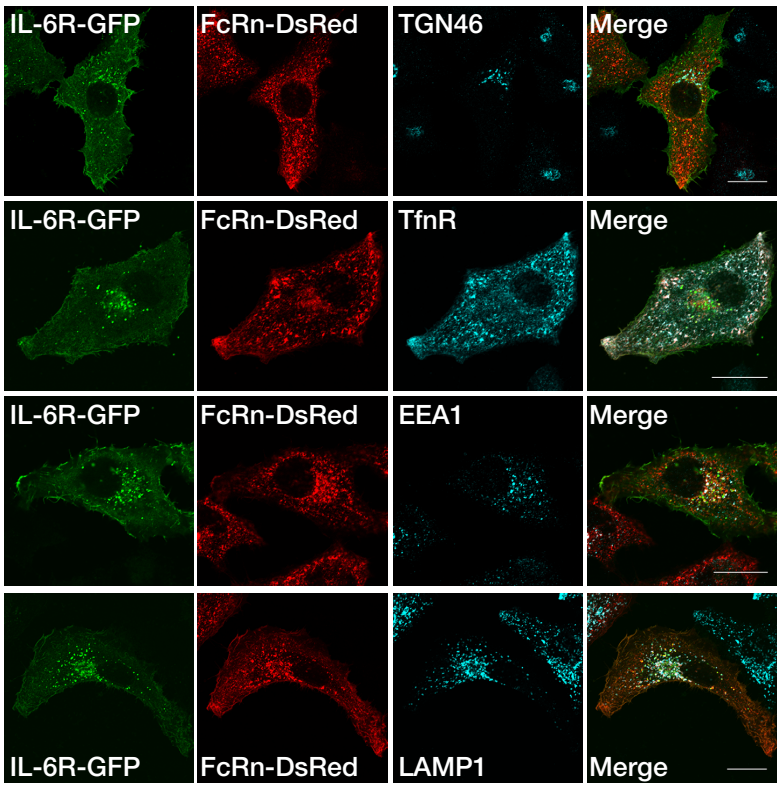


Supplemental Figure 2. Effect of expression of β2m on the localization of FcRn in COS-1 cells. A, COS-1 cells were transiently transfected with either an expression plasmid encoding FcRn-DsRed alone (top columns) or FcRn-DsRed and β2m (bottom columns), fixed and incubated with primary antibodies to β2m or TfnR. The primary antibodies were revealed by incubation with Cy3-labeled secondary antibodies. Cells were visualized by confocal microscopy. Right columns show the merged images for triple staining of FcRn-DsRed (red), β2m (green) and TfnR (cyan). Bars, 20 μm.

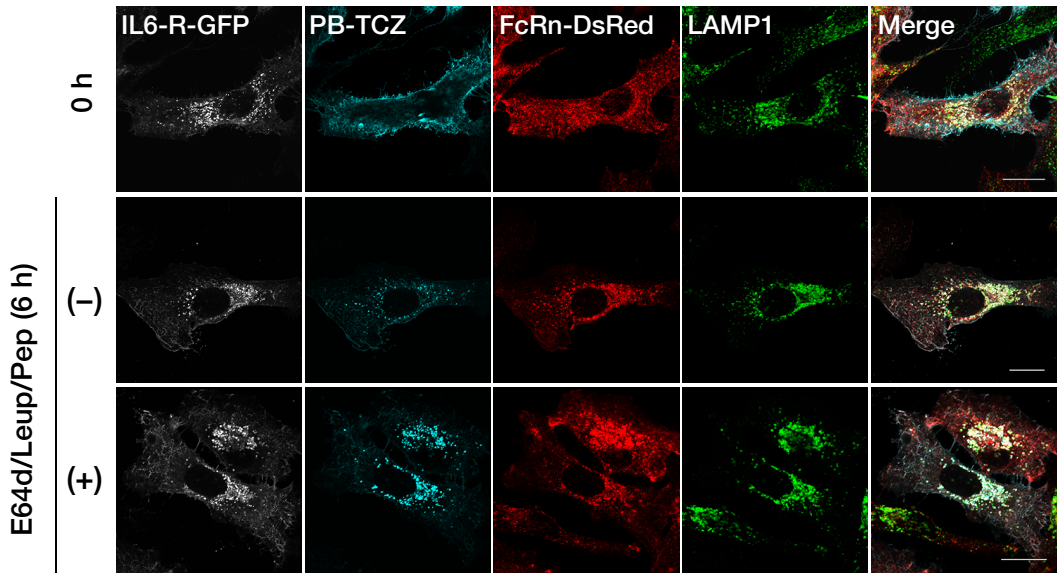
B, Western blotting of β2m from COS-1 and HeLa cells. The indicated amounts of COS-1 or HeLa cell lysates were subjected to SDS-PAGE, followed by Western blot analysis for β2m or β-actin.



Supplemental Figure 3. FcRn-DsRed is not involved in both binding with and internalization of PB-TCZ. HeLa cells transiently transfected with an expression plasmid encoding FcRn-DsRed were incubated with 2 μg/ml PB-IgG or PB-TCZ at 4 °C for 30 min. Cells were then fixed and fluorescence signals were visualized by confocal microscopy. Right columns show the merged images for double staining of FcRn-DsRed (red) and PB-IgG or PB-TCZ (cyan). Bars, 20 μm.



Supplemental Figure 4. Effect of IL-6R-GFP expression on the intracellular distribution of FcRn-DsRed. HeLa cells were transiently co-transfected with an expression plasmid encoding IL-6R-GFP and FcRn-DsRed. After 24 h, cells were fixed, permeabilized, and incubated with primary antibodies to TGN46, TfnR, EEA1, or LAMP-1. The primary antibodies were revealed by incubation with Cy5-labeled secondary antibodies. Cells were visualized by confocal microscopy. Right columns show the merged images for triple staining of IL-6R-GFP (green), FcRn-DsRed (red) and each organelle marker (cyan). Bars, 20 μm.



Supplemental Figure 5. Transport of FcRn-DsRed to late endosomes/lysosomes. HeLa cells co-transfected with an expression plasmid encoding IL-6R-GFP and FcRn-DsRed were incubated with PB-TCZ (2 μg/ml) at 4 °C for 30 min and allowed to internalize the antibody in the absence or presence of E64d/Leup/Pep for 6 h at 37 °C. Cells were then fixed, permeabilized, and incubated with primary antibodies to LAMP-1 and Cy5-labeled secondary antibodies. Cells were visualized by confocal microscopy. Right columns show the merged images for triple staining of PB-TCZ (cyan), FcRn-DsRed (red), and LAMP-1 (green). Left columns show the images for single staining of IL-6R-GFP in white. Bars, 20 μm.