Antibody Epitopes on G Protein-Coupled Receptors Mapped with Genetically Encoded Photoactivatable Cross-linkers

Sarmistha Ray-Saha, Thomas Huber, and Thomas P. Sakmar*

Laboratory of Chemical Biology and Signal Transduction,

The Rockefeller University, 1230 York Avenue, New York, NY 10065

www.sakmarlab.org

Supporting Information

I. Supplementary Figures

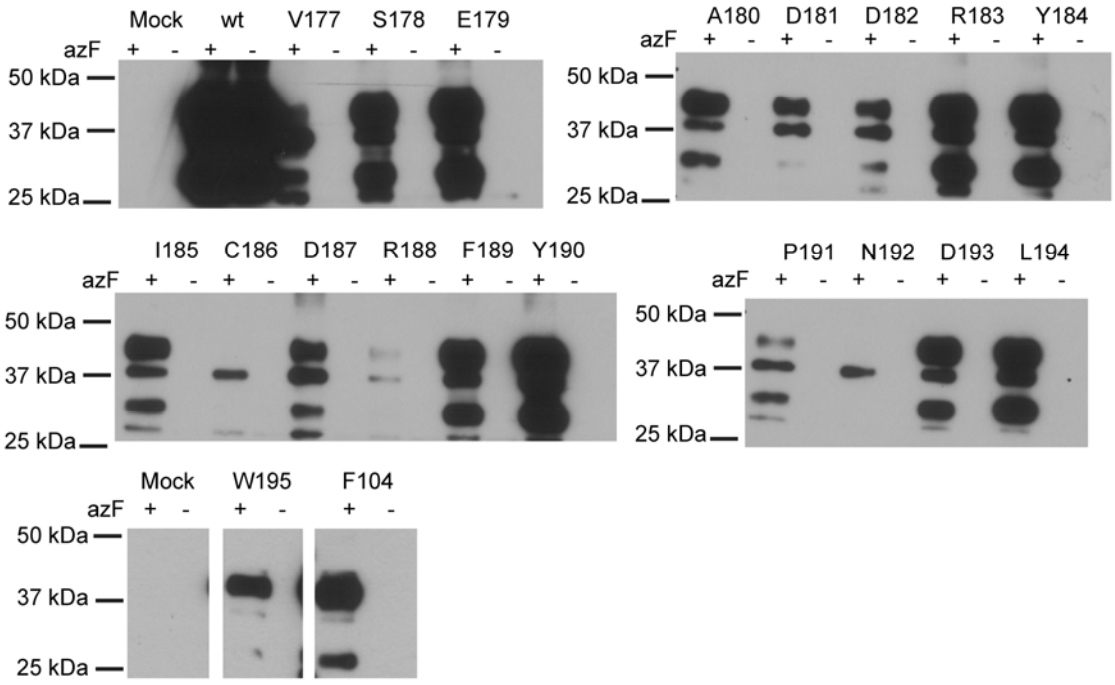

Figure S1. Expression analysis of azF-CXCR4 mutants. HEK293T cells co-transfected with wt or indicated amber mutants of CXCR4, along with suppressor tRNA and amino-acyl tRNA synthetase, were grown in the presence or absence of azF in the growth media. Full-length receptors were detected with the 1D4 antibody that recognizes a C-terminal epitope. Amber receptors were detected only in the presence of added azF, indicating the incorporation of azF at the indicated position.

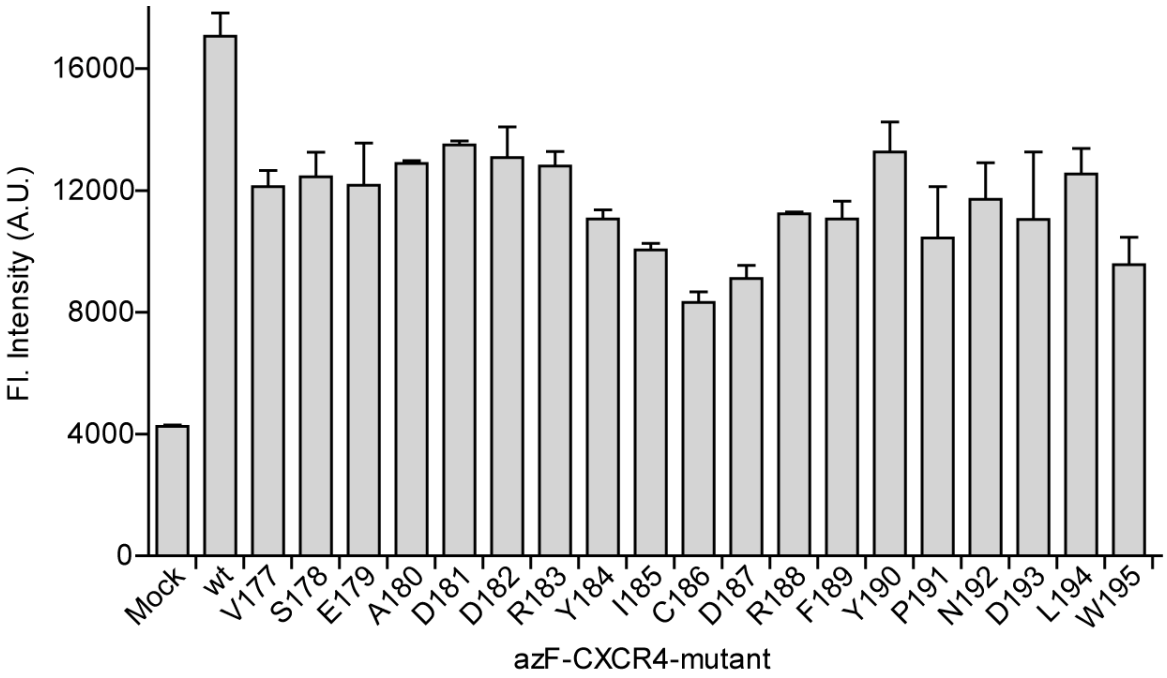

Figure S2. Expression analysis of azF-CXCR4 mutants by ELISA. HEK293T cells co-transfected with wt or indicated amber mutants of CXCR4, along with suppressor tRNA and amino-acyl tRNA synthetase, were grown in the presence of azF in the growth media. Full-length receptors were detected by ELISA after fixing and permeabilizing the cells by probing with the 1D4 antibody that recognizes a C-terminal epitope. Error bars represent the standard error from two independent trials, each performed in duplicate or triplicate.

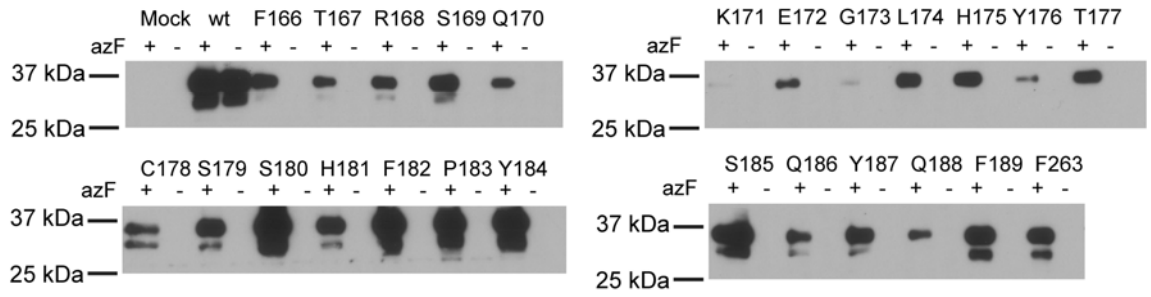

Figure S3. Expression analysis of azF-CCR5 mutants. HEK293T cells co-transfected with wt or indicated amber mutants of CCR5, along with suppressor tRNA and amino-acyl tRNA synthetase, were grown in the presence or absence of azF in the growth media. Full-length receptors were detected with the 1D4 antibody that recognizes a C-terminal epitope. Amber receptor mutants were detected only in the presence of added azF, indicating the incorporation of azF at the indicated position.

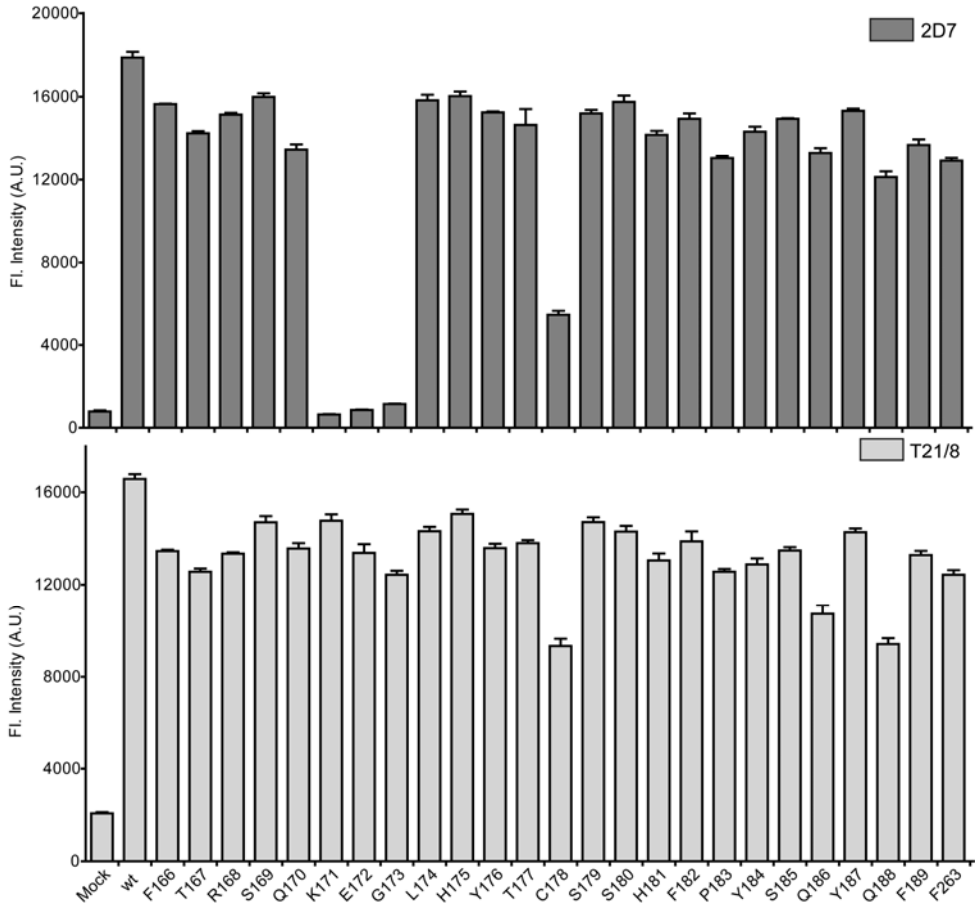

Figure S4. Test of antibody binding to azF-CCR5 variants at the cell surface. HEK293T cells expressing azF-CCR5 variants were incubated with 2D7 mAb and resultant fluorescence was detected in the whole-cell ELISA assay. Only positions 171, 172, 173 and 178 incorporating azF bound poorly to 2D7, in agreement with previous reports.[1-3] Cell surface expression was monitored with T21/8, which recognizes an N-terminal epitope. Error bars represent the standard error of the mean for 3 measurements

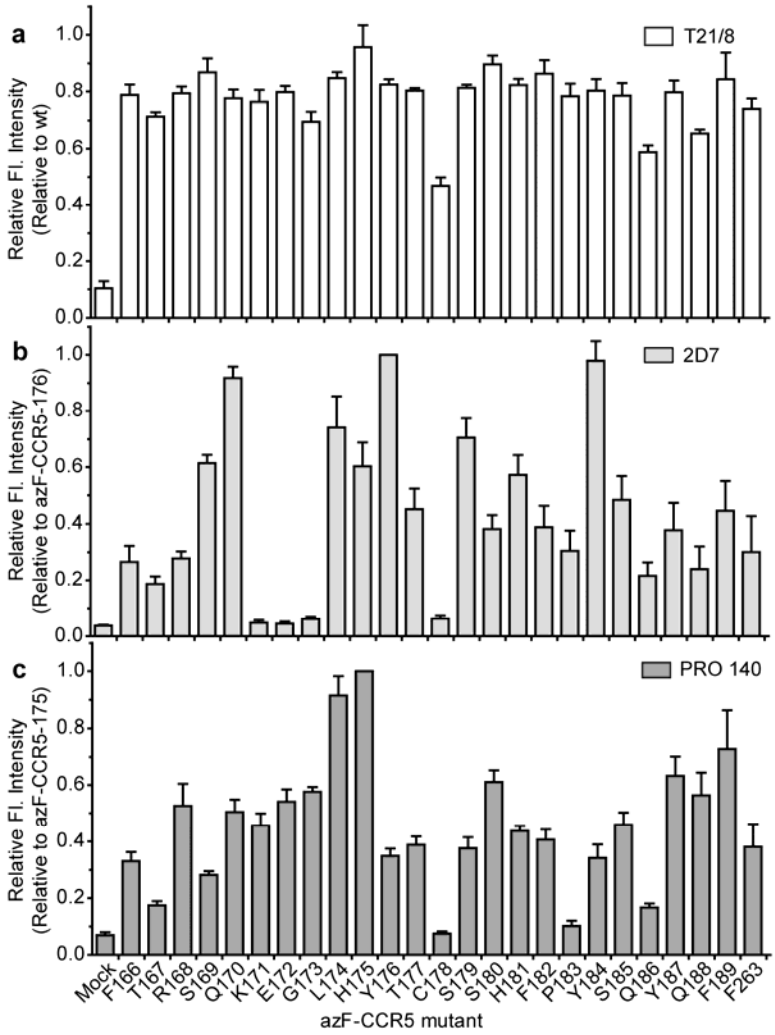

Figure S5. Analysis of binding and photo-cross-linking of azF-CCR5 mutants by ELISA. HEK293T cells expressing azF-CCR5 variants were incubated with mAbs. Expression levels in (a) were detected with T21/8, normalized to wt signal. Cells in the other wells were incubated with 2D7 or PRO 140, and exposed to UV light. After washing off excess un-cross-linked mAb, the fluorescence intensities were detected in a whole-cell ELISA assay. (b) After 2D7 incubation, signals normalized to azF-CCR5-176. (c) After PRO 140 incubation, signals normalized to azF-CCR5-175. Error bars represent the standard error of the mean from three or four independent trials, each performed in duplicate or triplicate.

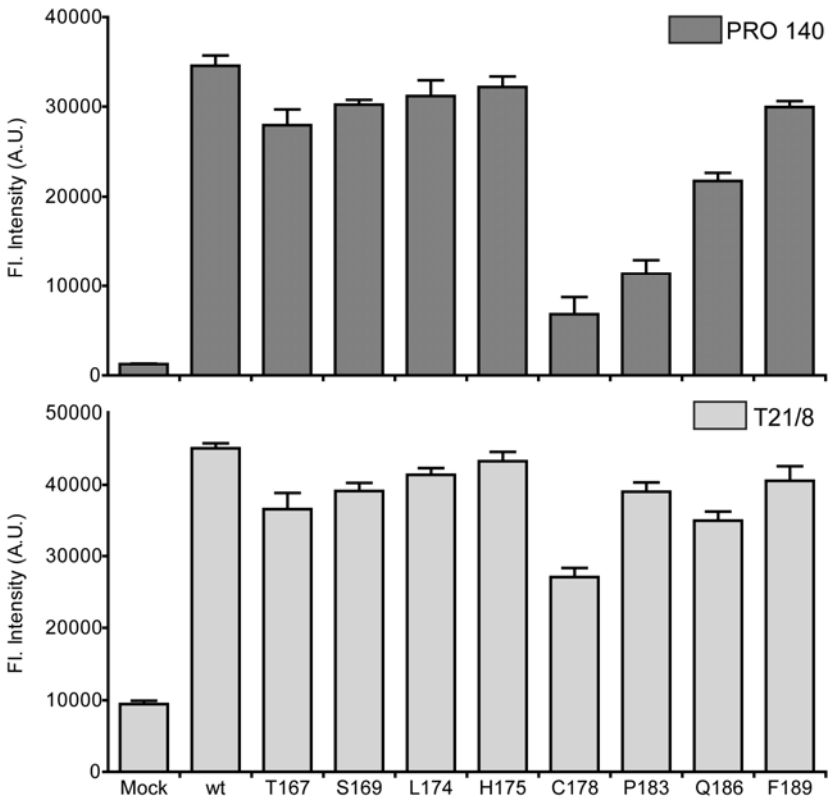

Figure S6. Analysis of azF-CCR5 interaction with PRO 140. HEK293T cells expressing CCR5 variants that incorporated azF at specific positions were incubated with PRO 140 mAb, and resultant fluorescence was detected in the whole-cell ELISA assay. Positions 178, 183, and 186 incorporating azF bound relatively weakly to PRO 140 compared to the other variants. Cell surface expression was monitored with T21/8 which recognizes an N-terminal epitope.

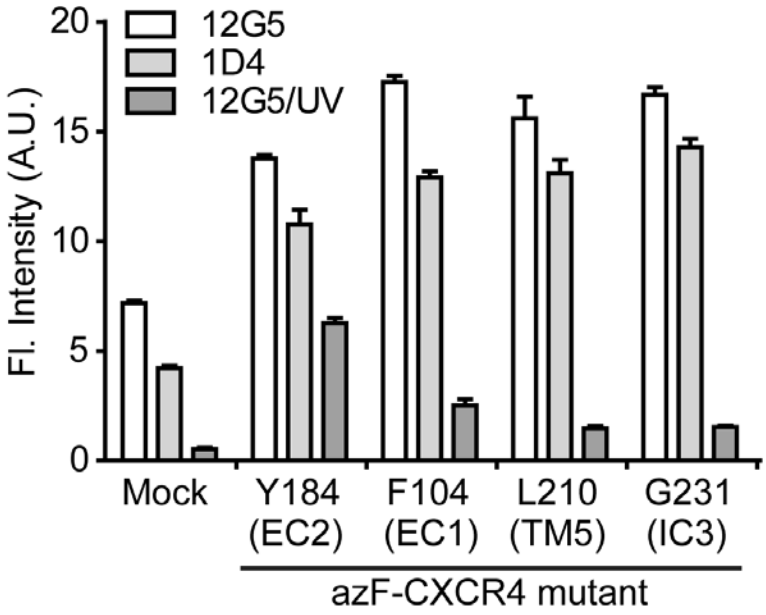

Figure S7. Analysis of photo-cross-linking efficiency of non-relevant sites in CXCR4. HEK293T cells expressing azF CXCR4 variants were incubated with 12G5, and then exposed to UV. After washing off excess non-cross-linked 12G5, fluorescence was detected in the whole-cell ELISA assay. Under these conditions, azF-104 (in EC1), L210 (in TM5) and G231 (in IC3) did not show appreciable signal.

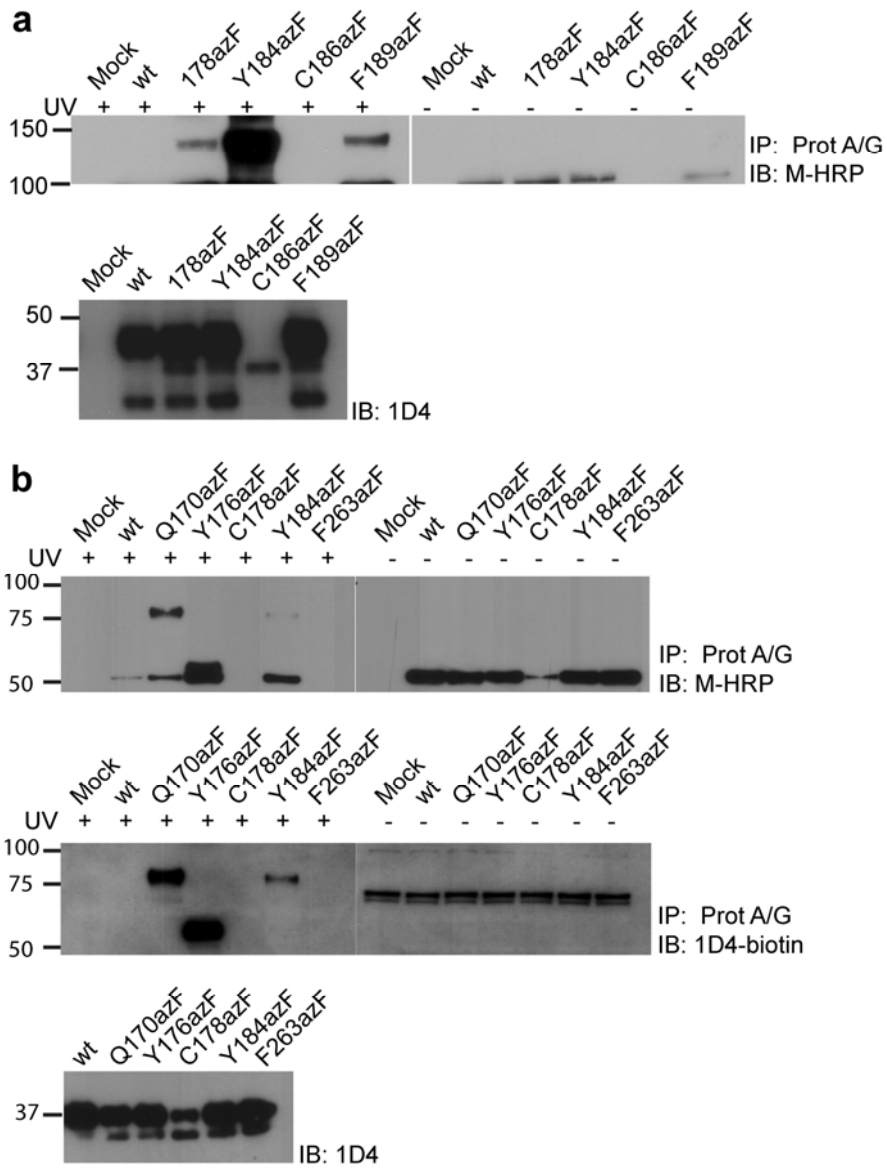

Figure S8. Analysis of photo-cross-linking efficiency by Western blot. HEK293T cells expressing (a) azF CXCR4 variants, or (b) azF CCR5 variants were incubated with 12G5 or 2D7 antibody, and then exposed to UV. After washing off excess un-cross-linked mAb, cells were solubilized in lysis buffer containing 1.5% DDM, and incubated with immobilized Protein A/G. Non-UV treated samples were not subjected to stringent

acidic washes. The eluate was analyzed by immunoblotting with HRP-conjugated anti-mouse secondary antibody alone in (a) or with 1D4-biotin/HRP-conjugated avidin (eBioscience, Cat#18-4100) in (b). UV-dependent higher molecular weight bands were detected in the mutants that were identified by ELISA to form cross-links.

II. References

(1) Timmerman, P.; Puijk, W. C.; Meloen, R. H. *Biopolymers* **2009**, *92*, 351.

(2) Blanpain, C.; Lee, B.; Vakili, J.; Doranz, B. J.; Govaerts, C.; Migeotte, I.; Sharron, M.; Dupriez, V.; Vassart, G.; Doms, R. W.; Parmentier, M. *J. Biol. Chem.* **1999**, *274*, 18902.

(3) Lee, B.; Sharron, M.; Blanpain, C.; Doranz, B. J.; Vakili, J.; Setoh, P.; Berg, E.; Liu, G.; Guy, H. R.; Durell, S. R.; Parmentier, M.; Chang, C. N.; Price, K.; Tsang, M.; Doms, R. W. *J. Biol. Chem.* **1999**, *274*, 9617.