SUPPORTING INFORMATION

Multiplex Detection of Functional G Protein-Coupled Receptors Harboring Site-Specifically Modified Unnatural Amino Acids

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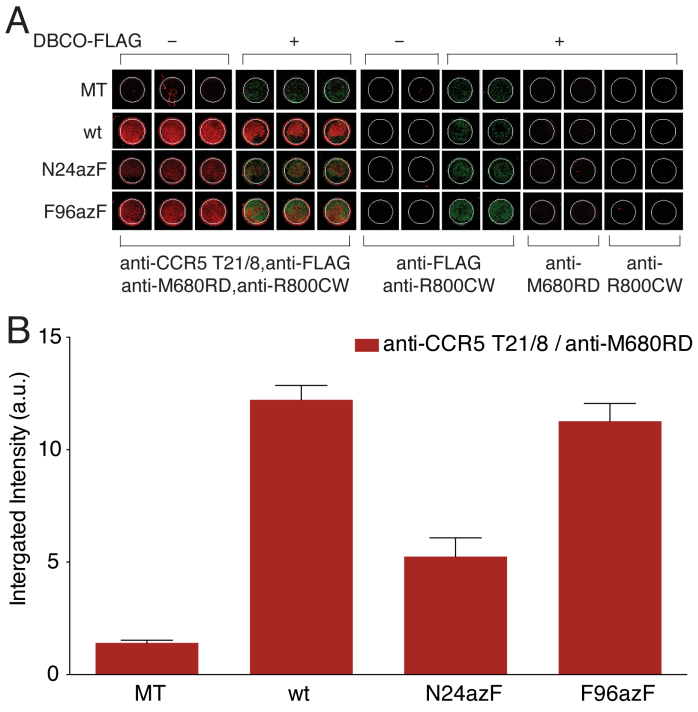
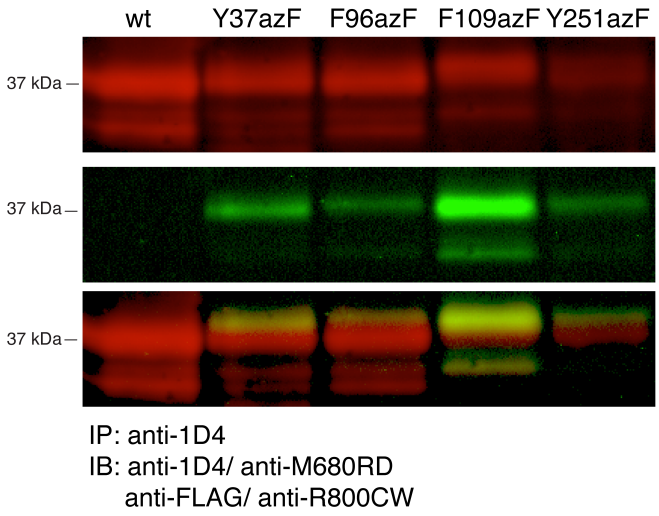
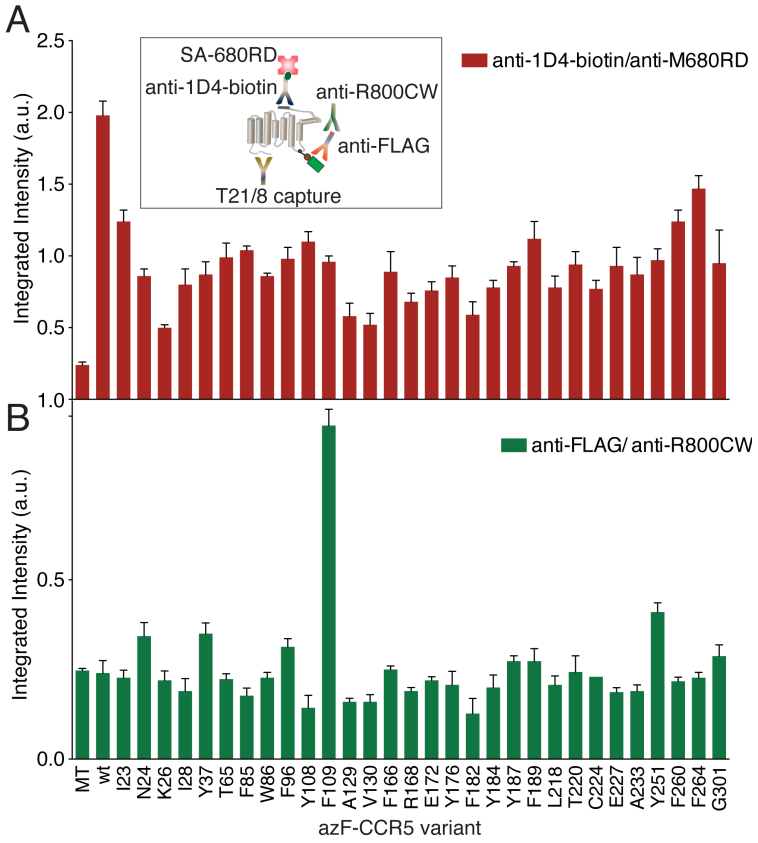
SUPPLEMENTARY FIGURES



Supplementary Figure S1. On cell-ISA for multiplex detection of cell-surface expressed labeled azF-CCR5. (A) The image of microtiter plate read on an Odyssey imager in two distinct spectral channels. The receptor expression is visualized in red by probing with an anti-CCR5 mAb T21/8 followed by IRDye 680RD conjugated to goat-anti-mouse IgG. The quantity of FLAG peptide in each well is probed using anti-FLAG pAb followed by IRDye 800CW goat-anti-rabbit IgG and visualized in green. The left panel shows the duplex detection of expressed receptor and label. The samples containing a yellow merge exhibit the presence of labeled receptor. (B) The integrated intensities of the wells probed for receptor expression (treated with DBCO-FLAG) is plotted. Error bars represent the standard error of the mean of triplicate measurements.



Supplementary Figure S2. Multiplex Western detection of immunoaffinity purified azF-CCR5 receptors. Key residues exhibiting above background labeling in the accessibility screen were immunoaffinity purified after DBCO-FLAG treatment. Samples were separated by SDS-PAGE followed by multiplex western detection to visualize receptor expression (upper panel, 700-nm channel, red) and FLAG-peptide incorporation into CCR5 (middle panel, 800-nm channel, green). In the merged image (lower panel), CCR5 appeared as two major adjacent bands. The lower band (red) corresponds to unlabeled receptor and upper band (yellow) to the FLAG-labeled receptor. The additional FLAG tag adds to the molecular weight of CCR5 by approximately 1.7 kDa.



Supplementary Figure S3. Accessibility screen for bioorthogonal site-specific modification of azF-CCR5 variants confirmed using the N-terminal capture multiplex sandwich ISA strategy (A, inset). azF-CCR5 is captured using the N-terminal specific anti-CCR5 mAb T21/8, followed by detection using (A) anti-1D4 mAb-biotin/IRDye 680RD streptavidin to measure receptor expression, and (B) anti-FLAG pAb/IRDye 800CW anti-rabbit IgG to measure DBCO-FLAG incorporation. The data represent means with the standard error from triplicate measurements.