Supplementary Figure 1. Representative electron micrograph of the ventral plasma membrane of a HEK-293T cell. CCP are marked by arrowheads, scale bar 500nm.

Supplementary Figure 2. Representative electron micrograph of the ventral plasma membrane of a HeLa cell. CCP are marked by arrowheads, FCL by L, scale bar 500nm.

Supplementary Figure 3. The topography of clathrin-coated structures. The electron intensity associated with CCS provides a surrogate measure of topography. A. A cartoon depicting the passage of an electron beam through a spherical CCP; where the beam passes through multiple surfaces fewer electrons are transmitted resulting in a distinct intensity profile. B. CCP displaying a characteristic ring of low electron intensity, the line plot reveals a distinct W-shaped profile. C. Mean radial electron intensity profiles from CCP (area <30,000nm² and circularity >0.7) and FCL (area >100,000nm²) in HEK-293T and HeLa cells, n= 716, 425 and 235 structures, respectively. Error bars indicate standard error of the mean. D. A representative image of FCL with a peripheral budding structure (*), scale bar 200nm. E. The mean radial electron intensity profile from budding structures within 50nm of the periphery of FCL, n= 54 buds. Error bars indicate standard error of the mean.

Supplementary Figure 4. CHO cells exhibit flat clathrin lattices. A. A representative electron micrograph of the ventral surface of a CHO-CCR5 cell, displaying individual CCP (arrowheads) and FCL (L) with peripheral budding structures (*), scale bar 500nm. B. Fixed CHO-CCR5 cells were permeabilised and stained with a mouse anti-clathrin heavy chain mAb, followed by secondary anti-mouse Alexa Fluor 647. CCS were imaged on the ventral cell surface by dSTORM using TIRF illumination. The dSTORM super-resolution image displays representative CCS in CHO-CCR5 cells, the image was reconstructed using a 25nm pixel size, scale bar 4µm. Insets display a standard diffraction-limited TIRF image of the same area (upper) and an enlarged area of the dSTORM image (lower), scale bar 1µm. Heat map intensity scale indicates density of localizations per µm². C. Morphometric analysis of clathrin heterogeneity was performed by automated image segmentation (see materials and methods). Scatter plots display the circularity and two-dimensional surface area of individual CCS. Shading indicates CCS defined as small: <30,000nm² (white), medium: 30,000-100,000nm² (light grey) or large: >100,000nm² (dark grey). D. CHO-CCR5 cells expressing LCb-RFP were imaged by TIRF microscopy for ten minutes at 0.33 frames per second. The lifetime of clathrin events were quantified using an automated tracking algorithm (see materials and methods). The histogram displays the distribution of CCS lifetimes using 9s wide bins, based on 10,094 events from six acquisitions across two independent experiments.

Supplementary Figure 5. CCR5 internalization in response to CCL5 stimulation. CHO-CCR5 cells, pre-labeled with mouse anti-CCR5 mAb directly conjugated to Atto 488, were imaged for 40 minutes at 2 frames per minute by TIRF microscopy. CCL5 was added after ten minutes at a final concentration of 125nM. Receptor internalization was quantified by measuring fluorescence
intensity over time (dots). The presented data is normalized to the signal at T=0, n=4 cells over two independent experiments, error bars indicate standard error of the mean. The data was fitted with a single-phase exponential decay curve (red line) $R^2=0.88$ (GraphPad Prism). The rate of internalization was independently confirmed in an endpoint assay using flow cytometry to measure cell surface levels of CCR5 (bar plots).

**Supplementary Figure 6. CCR5 agonist triggers persistent association of β-Arrestin with flat clathrin lattices.** CHO-CCR5 cells expressing Lcb-RFP and GFP-β-arrestin-2 were imaged by spinning disk microscopy for twenty minutes at six frames per minute. CCL5 was added after five minutes at a final concentration of 125nM. **A.** Stills taken from the beginning and after five minutes stimulation from a representative time course, images display the cell surface distribution of clathrin (magenta) and β-arrestin-2 (green), scale bar 10µm. **B.** β-arrestin-2 recruitment was quantified by measuring the GFP signal associated with CCS. The data were taken from representative experiments using either full length, or N-terminal domain truncated GFP-β-Arrestin-2 constructs and is presented as normalized fluorescence expressed relative to the signal at T=0. **C.** CHO-CCR5 cells expressing YFP-β-arrestin-1 were stimulated with 125nM CCL5 for two minutes. Cells were labeled on ice with mouse anti-CCR5 mAb + protein A 10nm gold, after which dorsal membrane sheets were prepared by rip off for EM. To visualize the distribution of YFP-β-arrestin-1, samples were stained with mouse anti-GFP mAb + protein A 15nm gold. CCR5 (arrows) and YFP-β-arrestin-1 (arrowheads) are highlighted, close juxtaposition of 10nm and 15nm gold particles (*) suggests individual CCR5/β-arrestin complexes. **D.** CHO-CCR5 cells expressing GFP-β-Arrestin-2 were incubated for one hour in the presence or absence of 125nM CCL5. Fixed cells were permeabilised and stained with mouse anti-GFP mAb followed by secondary anti-mouse Alexa Fluor 647. We imaged the ventral cell surface by dSTORM using TIRF illumination. The images display representative fields of the cell surface distribution of β-Arrestin-2, images were reconstructed with a 25nm pixel size, scale bar 1µm. Heat map intensity scale indicates density of localizations per µm².

**Fig. 3 Video 1. Flat clathrin lattices are long-lived.** HeLa cells expressing Lcb-RFP (magenta) and Dyn-2-EGFP (green) were imaged by TIRF microscopy (NSTORM; Nikon Inc.) for ten minutes at 0.33 frames per second. The right hand panel displays the merged signal. The white point has been set to allow low intensity transient events to be seen. Example CCP-type events are highlighted with arrows in the right hand panel.

**Fig. 4 Video 1. Flat clathrin lattices display molecular turnover.** HeLa cells expressing Lcb-RFP (magenta) and Dyn-2-EGFP (green) were imaged by spinning disk microscopy (UltraVIEW VoX; PerkinElmer Inc.) for six minutes at 0.33 frames per second. The CCS at the center of the field were selectively bleached after 1 minute.

**Fig. 4 Video 2. Clathrin turnover is ATP-dependent.** HeLa cells expressing Lcb-RFP (magenta) and Dyn-2-EGFP (green) were depleted of ATP for one hour and then imaged by spinning disk microscopy (UltraVIEW VoX; PerkinElmer...
Inc.) for six minutes at 0.33 frames per second. The CCS at the center of the field were selectively bleached after one minute.

**Fig. 5 Video 1. Agonist treatment triggers persistent association of CCR5 with flat clathrin lattices.** CHO-CCR5 cells expressing LCb-RFP (magenta) and pre-labeled with mouse anti-CCR5 mAb directly conjugated to Atto 488 (green) were imaged by TIRF microscopy (N-STORM; Nikon Inc.) for 60 minutes at two frames per minute. 125nM CCL5 was added at the beginning of the time course. The panels to the right are an enlargement of the boxed area and display both channels and the merged signal.

**Fig. 5 Video 2. CCR5 association with flat clathrin lattices and clathrin-coated pits in the presence of ligand.** CHO-CCR5 cells expressing LCb-RFP (magenta) and pre-labeled with mouse anti-CCR5 mAb directly conjugated to Atto 488 (green) were treated with 125nM CCL5 for one hour and then imaged by TIRF microscopy (N-STORM; Nikon Inc.) for ten minutes at 0.33 frames per second. The panels to the right are an enlargement of the boxed areas, and highlight two transient CCP-type events. Both channels and the merged signal are displayed.

**Supplementary Figure 6. Video 1. CCR5 agonist triggers persistent association of β-Arrestin-2 with flat clathrin lattices.** CHO-CCR5 cells expressing LCb-RFP (magenta) and GFP-β-Arrestin-2 (green) were imaged by spinning disk microscopy (UltraVIEW VoX; PerkinElmer Inc.) for twenty minutes at six frames per minute. CCL5 was added at a final concentration of 125nM after five minutes, as indicated.

**Supplementary Figure 6. Video 2. An N-terminal domain truncated GFP-β-Arrestin-2 construct does not respond to CCR5 agonist.** CHO-CCR5 cells expressing LCb-RFP (magenta) and an N-terminal domain truncated GFP-β-Arrestin-2 construct (green) were imaged by spinning disk microscopy (UltraVIEW VoX; PerkinElmer Inc.) for twenty minutes at six frames per minute. CCL5 was added at a final concentration of 125nM after five minutes, as indicated.
