Figure S1, Related to Figure 1

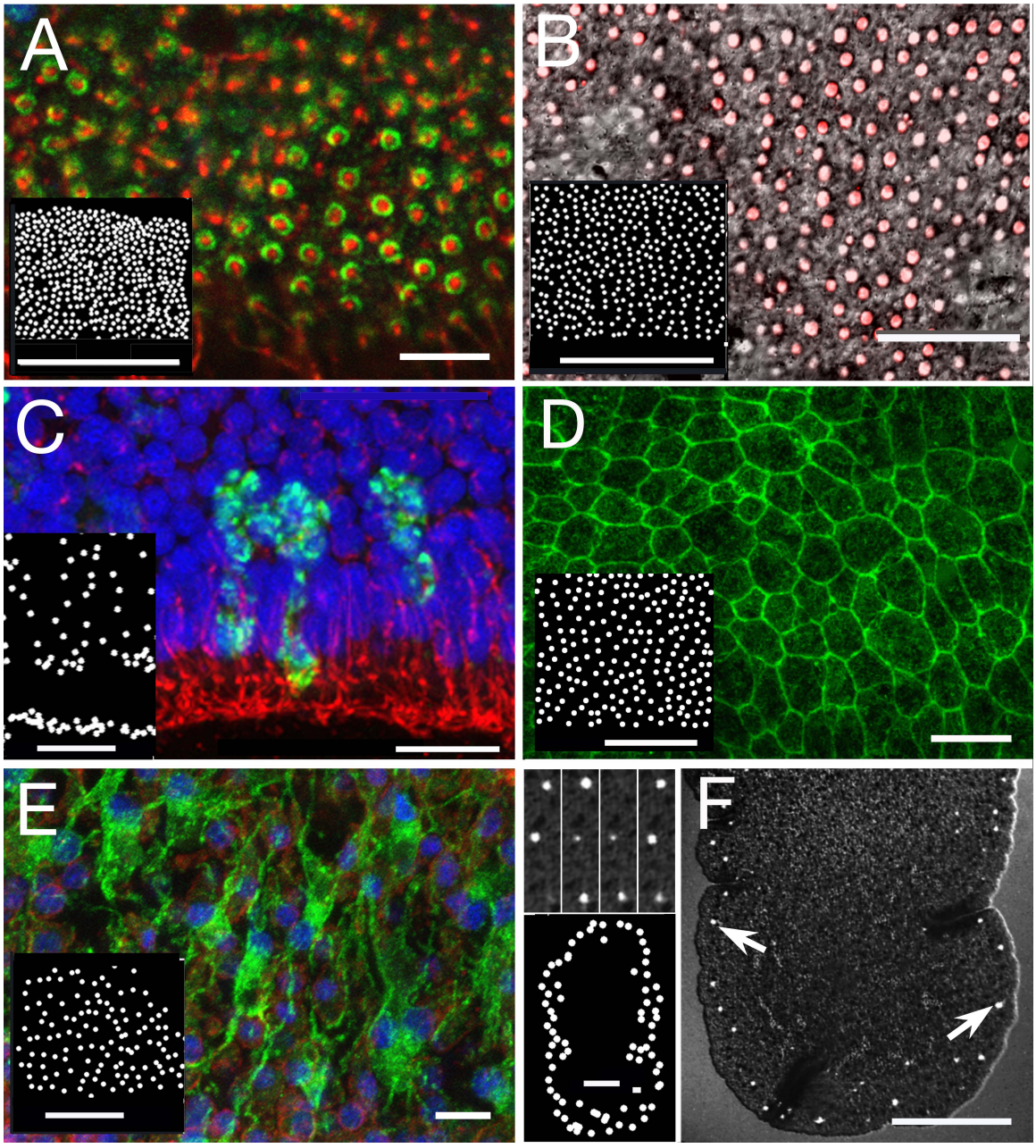

Figure S1. Counting and mapping the six major cell types in *Trichoplax* (A-F). Optical section near the surface of ventral epithelium labeled with antibodies that mark ciliary bases (green; CilB) and cilia (red; anti-tubulin). Each ciliated ventral epithelial cell presents a single cilium in a ciliary pit on the ventral surface. Map of

the pits in a ~50 μm square area of the ventral surface of a *Trichoplax* shows the dense packing of ciliated ventral epithelial cells. (B) Spherical inclusions of lipophils in a central region of the ventral surface visualized by confocal DIC (gray) and lysotracker staining (red overlay) in living *Trichoplax*. Map of spherical inclusions in a ~50 μm square region shows that they are several fold less common than ciliated epithelial cells. (C) Secretory granules in gland cells arrayed near the margin of a *Trichoplax* are stained by immunofluorescence for synaptobrevin (green). Tubulin red, nuclei blue. Map pinpoints stained gland cell bodies in a ~100 μm square region encompassing part of the margin. The densely packed linear array of cells at the bottom of the map is near the margin while the other cells are in more central regions of the ventral epithelium. (D) The flat tops of dorsal epithelial cells are outlined by staining with Alexa 488-phalloidin. Map shows distribution of dorsal epithelial cells in a ~100 μm square region of the dorsal surface. (E) Fiber cells in the interior of *Trichoplax* are outlined by immunofluorescence staining with an antibody against *Trichoplax* P2X-like receptor (green). Nuclei blue. Map shows all of the stained fiber cells in ~100 μm square region. (F) Crystal cells, arrayed around the margin, are detected and mapped by viewing their birefringent inclusions (arrows) in living *Trichoplax* with confocal polarization microscopy. Upper inset. Birefringent inclusions vary in brightness over time due to nutation of the crystal (time between panels, 6 s). Map shows positions of crystals during a 75 s time-lapse sequence (3 s/frame) while the *Trichoplax* remained stationary. Scale bars for micrographs represent 5 μm (A, C, E), 20 μm (B, D), 50 μm (A-E inset maps), 100 μm (F).

Figure S2, Related to Figure 2

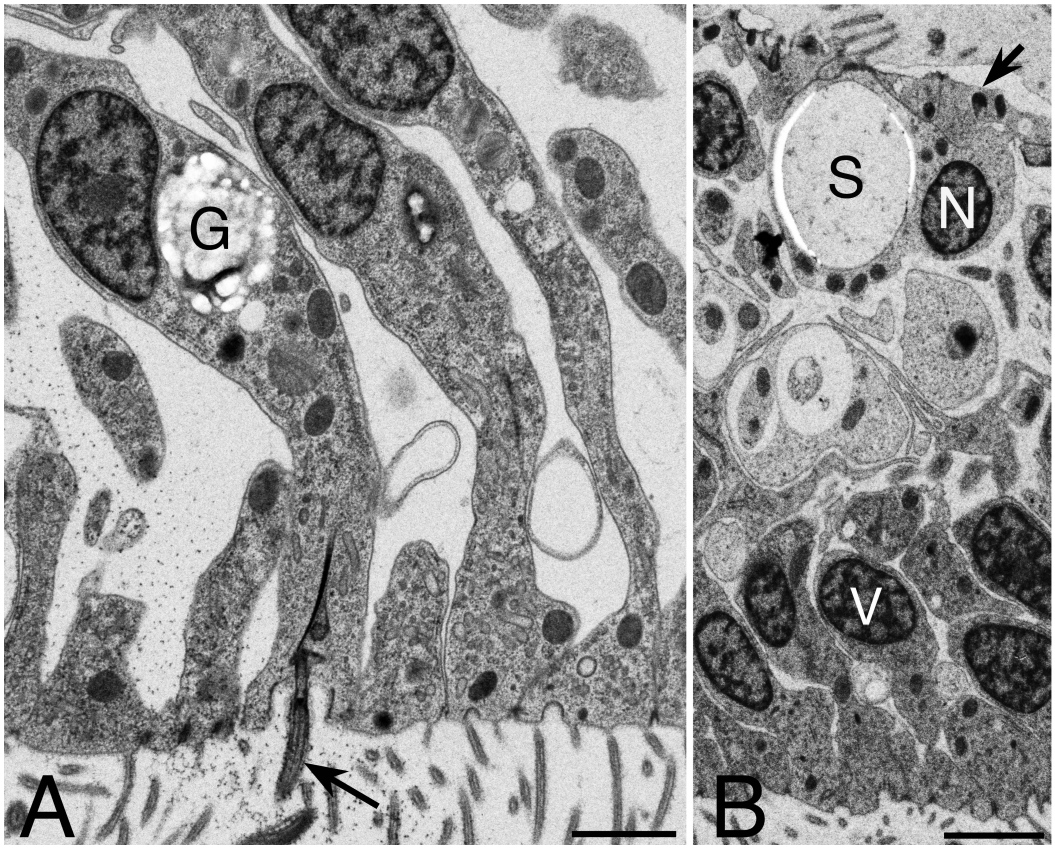

Figure S2. TEM micrographs of field-collected placozoan prepared by freeze-substitution. (A) *Ventral epithelial cells*, one giving rise to a cilium (arrow). The same cell has a large granule (G) near its nucleus, typical of ventral epithelial cells. (B) Cross section showing full thickness of the animal; dorsal epithelial cells with typical dense granules (arrow) above, ventral epithelial cells below (V). A large pale spherical inclusion (S) impinging on the dorsal epithelium is in a cell (nucleus, N) in the dorsal epithelium. Scale bars represent 0.5 μm (A) 1 μm (B).

Figure S3, Related to Figure 3

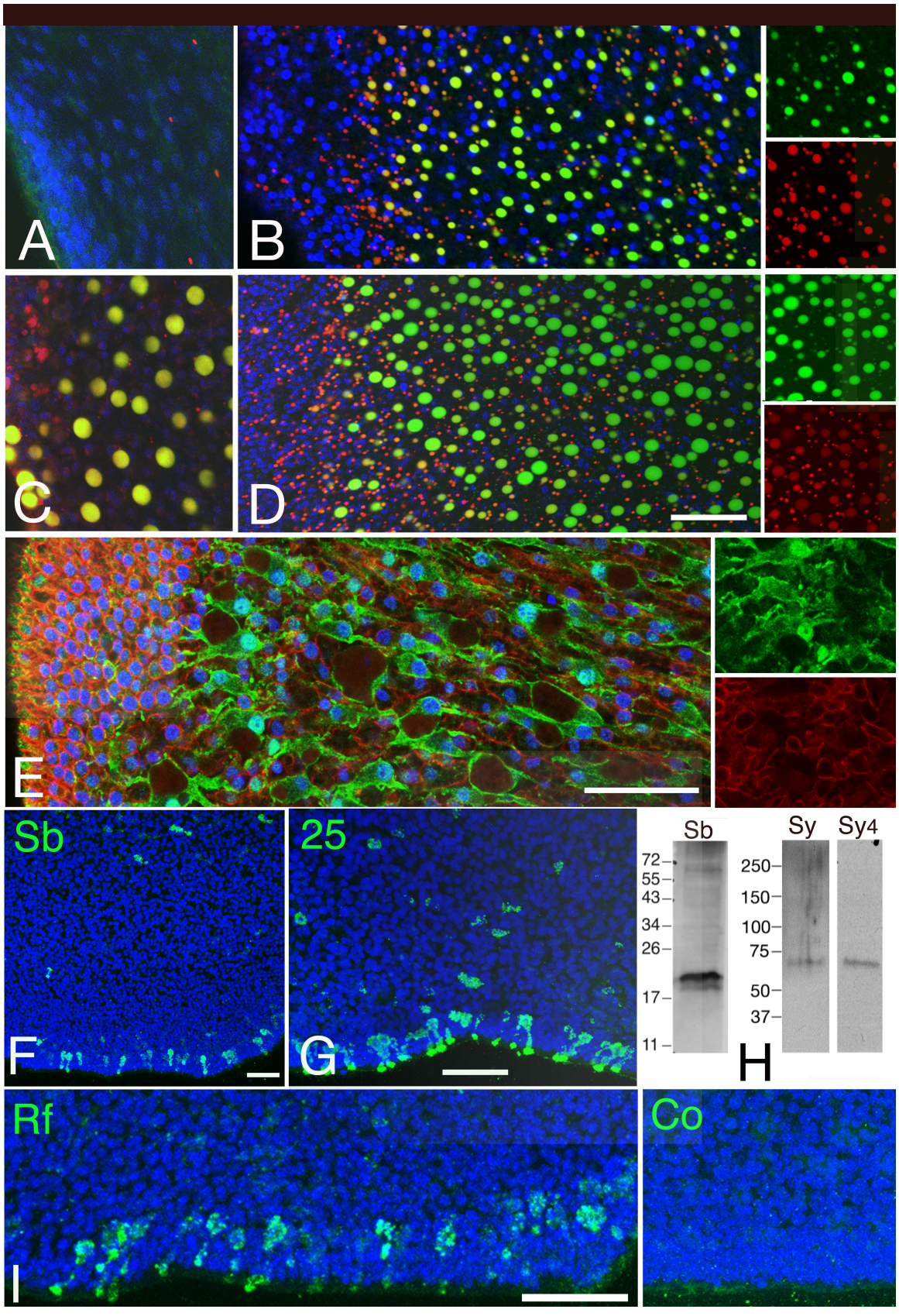
A
B
C
D
E
Sb
25
F
G
Sb
Sy
Sy4
72
55
43
34
26
17
11
250
150
100
75
50
37
H
Rf
Co
I

Figure S3. Horizontal distributions of lipid inclusions (A-D), fiber cells (E) and gland cells (F-H). (A-D) Comparison of the distributions of inclusions stained with C1-Bodipy-C12 fatty acid analog (green) and Lysotracker Red (red) in *Trichoplax* (A, B) and a field-collected placozoan (C, D). Single optical sections near the dorsal (A, C) and ventral (B, D) surfaces are shown. Large inclusions (~ 5 μm) that stain intensely with both dyes are present at the dorsal surface in the field-collected animal (C), but not in *Trichoplax* (A). In both organisms, slightly smaller inclusions (2-4 μm) that stain more intensely with C1-Bodipy-C12 than with Lysotracker Red are present throughout the ventral surface except near the rim (left). Insets show single color channels in a region near the center. Nuclei are blue, small intensely red granules may be lysosomes. **(**E) Double labeling for TaCDH (green; a marker for fiber cells) and MAGUK (red; a marker for epithelial and lipophil cells) shows that densely-packed epithelial cells at the rim (left) are interspersed with the processes of fiber cells and that fiber cells mingle with lipophils in the interior. Insets: single color channels from a region containing cell bodies of fiber cells (green) and lipophils (large red profiles). Nuclei are blue. (F-G) Immunostaining (green) for synaptobrevin (F; Sb) and SNAP-25 (G; 25) show prevalence of secretory cells near the rim (below) and scattered cells in more central regions. H. Western blot with anti-synaptobrevin (Sb) labels ~18 kDa protein and two synapsin antibodies (Sy and Sy4) label a ~72 kDa protein. (I) Secretory cells arrayed near the rim are labeled with anti-FMRF (Rf) not after the antibody is preabsorbed with FMRF peptide (Co). Scale bars represent 20 μm.