G. Duclos et al. "perfect nematic order in confined monolayers..."

## Supplementary figures captions

**Supplementary Figure 1: NIH-3T3 cells are head-tail symmetric.** These cells move back and forth along their main axis with no preferential polarity. When time-averaged, they can be considered as effectively apolar. See also Supp. Video 1.

**Supplementary Figure 2: Orientation is conserved at divisions**. Cell division takes place along the major cell axis. After division, the two daughter cells are themselves aligned along the axis of the mother-cell.

Supplementary Figure 3: The morphology of the cells remains unchanged as they divide and cell density increases. Only a fraction (~15 %) of the cells are labeled to be able to resolve them at high density. Density was normalized by the final density. Cell surface and cell aspect ratio vary by less than 25 % during 100 h.

**Supplementary Figure 4: Topological defects characteristic of nematic phases**. Phase contrast image, color-coded orientation field and orientation field of the same FOV. Note the domains sharing a common orientation (same color) and the presence of typical nematic disclination defects of charge -1/2 (red circles) and +1/2 (blue circles).

**Supplementary Figure 5: Nematic ordering does not depend on the surface composition.** Despite differences in the dynamics, cells cultured on glass or fibronectin show very similar long time characteristics in terms of order parameter or correlation length. This can be verified in the distributions of these two quantities, but also on the direct relationship between them.

## **Supplementary videos**

**Supplementary Video 1: Movement of a single 3T3 cell on glass**. Cells move along their principal axis. At any time the cell show a distinct polarity that reflects in its direction of motion. However, no preferential direction was identified at the cell can be considered as effectively apolar.

**Supplementary Video 2**: Densification of a non-restricted monolayer of 3T3 cells. Cells were initially plated below confluence at which point, they adopt a random orientation. As the cells divide, they reorient themselves with their neighbors, forming large domains of cells sharing a common orientation. White spots are division events. Substrate: Fibronectin-coated glass.

**Supplementary Video 3**: Densification of a monolayer of 3T3 cells confined in a stripe of width 500  $\mu$ m. As the cells divide, they orient parallel to the edges. This orientation then propagates toward the center. Eventually, the orientation is perfect across the whole stripe.

**Supplementary Video 4**: Same as Supp. Video 3 with the added information of orientation as a color code.