Supplemental File 2: Oligo Sequences Used

All oligos listed below were ordered from IDT. All oligos were resuspended in TE buffer at a concentration of 100 μM.

Universal Anchor: 5'-TGAATCTCTGGGTGCCAAGGGTAAGCATCCAGCTGTCACT -{Chol}-3’

Universal Co-Anchor: 5'-{Chol} AGTGACAGCTGGATGCTTAC -3'

The cholesterol modifications are only available with a 100nmole (or greater) synthesis order and HPLC purification.

Adapter Strands:

Overall Structure: 5’-CCTTGGCACCCAGAGATTCA (hybridizes with Universal Anchor) – poly T spacer (increasing oligo length improves efficiency of adhesion) - surface adhesion sequence -3’

Strand A: CCTTGGCACCCAGAGATTCA TTTTTTTTTTTTTTTTTTT ACTGACTGACTGACTGACTG

Strand A prime: CCTTGGCACCCAGAGATTCA TTTTTTTTTTTTTTTTTTT CAGTCAGTCAGTCAGTCAGT

Strand B: CCTTGGCACCCAGAGATTCA TTTTTTTTTTTTTTTTTTT AGAAGAAGAACGAAGAAGAA

Strand B prime: CCTTGGCACCCAGAGATTCA TTTTTTTTTTTTTTTTTTT TTCTTCTTCGTTCTTCTTCT

Strand C: CCTTGGCACCCAGAGATTCA TTTTTTTTTTTTTTTTTTT AGCCAGAGAGAGAGAGAGAG

Strand C prime: CCTTGGCACCCAGAGATTCA TTTTTTTTTTTTTTTTTTT CTCTCTCTCTCTCTCTGGCT

To create multilayered cellular structures, use complementary sets of oligos for the Adapter Strands (A and A prime, B and B prime, etc.) To pattern multiple cell populations without the cells adhering to each other, use orthogonal Adapter Strands (A and B, B prime and C prime, etc).

Designing Additional Adapter Strands:
Additional Adapter Strands can be created replacing the surface adhesion sequence following the poly T spacer with another 20-base oligo. Sequences that result in secondary structures of the oligo are undesirable.

It is recommended that the sequences for new Adapter Strands avoid CpG repeats, although TLR9 stimulation by CMO-labeling with CpG-containing Adapter Strands is low (Supplemental Figure 5).

When designing Adapter Strands to be orthogonal to each other, be aware that many mismatches are required for orthogonality. The presence of as few as six complementary bases

between oligos can be sufficient for hybridization and subsequent cell adhesion. To confirm that two Adapter Strands are sufficiently orthogonal, label two populations of cells with the CMOs as described in Step 5. Mix the two populations together and incubate with shaking for 5 minutes. If the cells form clusters, the Adapter Strands are hybridizing and are not sufficiently orthogonal to be used in the same experiment.

Adapter Strands Used to Test TLR9 Stimulation:
CMO-CPG: CCTTGGCACCCAGAGATTCA TTTTTTTTTTTTTTTTTTT TCCATGA**CG**TTCCTGA**CG**TT

CMO-GPC: CCTTGGCACCCAGAGATTCA TTTTTTTTTTTTTTTTTTT TCCATGA**GC**TTCCTGA**GC**TT