# Supplemental Materials Molecular Biology of the Cell

Pageon et al.

## SUPPLEMENTARY INFORMATION

## Clus-DoC: A combined cluster detection and colocalization analysis for single-molecule localization microscopy data

Pageon S.V., Nicovich, P.R., Mollazade M., Tabarin T.& Gaus K.

#### С В Α 100-20,000 1.0 n.s. n.s. n.s Molecules in clusters (%) 80-0.8 Cluster area (nm<sup>2</sup>) 15,000 Circularity 60-0.6 10,000 0.4 40-5,000 20-0.2 0-0 0.0 TCR TCR TCR TCR TCR TCR (pTCR) (CD45) (pTCR) (CD45) (pTCR) (CD45) D Ε n.s. 60 4. n.s Rel. density in clusters Molecules per cluster 3. 40 2. 20 1 0 0 TCR TCR TCR TCR (pTCR) (CD45) (pTCR) (CD45)

## **Supplemental Figure 1**

Supplementary Figure 1: Clustering parameters for TCR in activated Jurkat T cells when imaged simultaneously with pTCR or with CD45. (A) Percentage of TCR molecules in clusters. (B) Average area of TCR clusters. (C) Circularity of clusters. (D) Average number of molecules per cluster. (E) Relative density of molecules within clusters. Relative density is calculated as the ratio of molecular density of TCR inside clusters to total molecular density.

## **Supplemental Figure 2**



**Supplementary Figure 2: Colocalization between TCR and CD45 for non-clustered molecules.** (A) Frequency distributions of DoC scores of non-clustered molecules, for TCR (top) and CD45 (bottom). (B) Distribution of total TCR between non-clustered molecules, molecules in non-colocalized clusters and molecules in colocalized clusters. (C) Distribution of colocalizedTCR molecules between non-clustered molecules, molecules in non-colocalized clusters and molecules in colocalized clusters. (D) Average DoC scores for TCR with CD45 fornon-clustered molecules, molecules in non-colocalized clusters and molecules in colocalized clusters.

### **Supplemental Figure 3**



**Supplementary Figure 3: Colocalization between paxillin and p-paxillin for non-clustered molecules.** (A) Frequency distributions of DoC scores of non-clustered molecules, for paxillin (top) and p-paxillin (bottom), with mean DoC scores for paxillin and p-paxillin indicated. (B) Percentage of non-clustered paxillin molecules colocalized with p-paxillin and percentage of non-clustered p-paxillin molecules colocalized with p-paxillin and percentage of non-clustered p-paxillin molecules between non-clustered molecules, molecules in non-colocalized clusters and molecules in colocalized clusters.