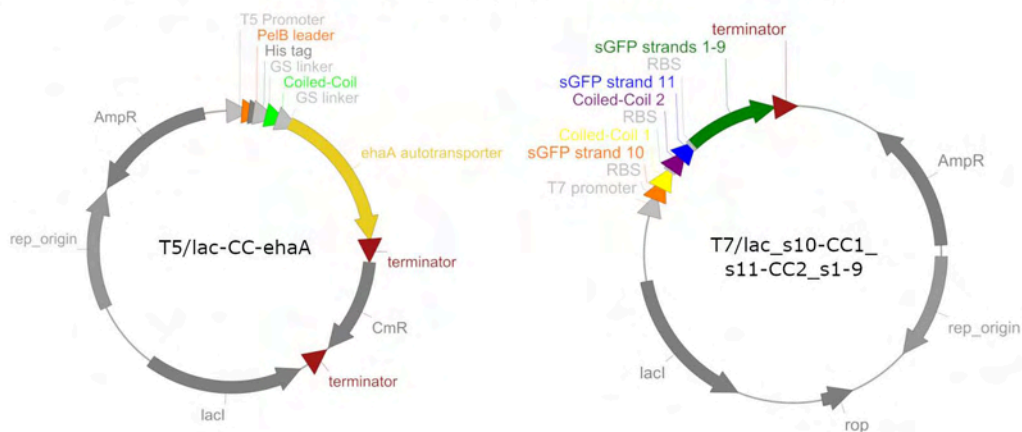
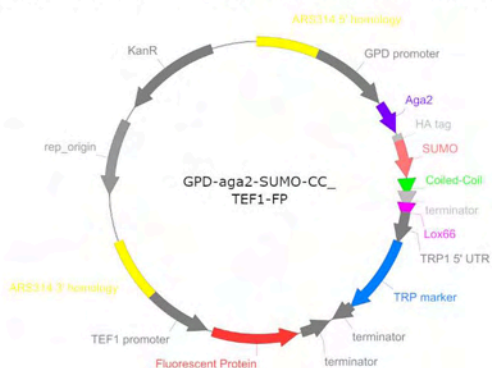


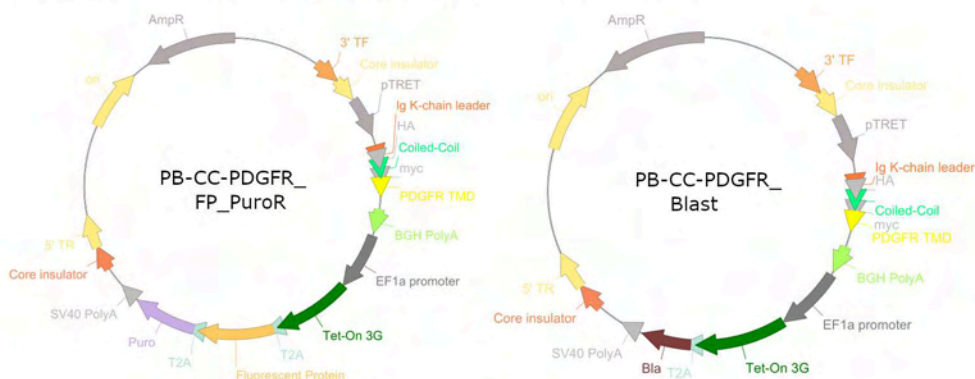
## a. Plasmids for *E. coli* helixCAM expression and screening



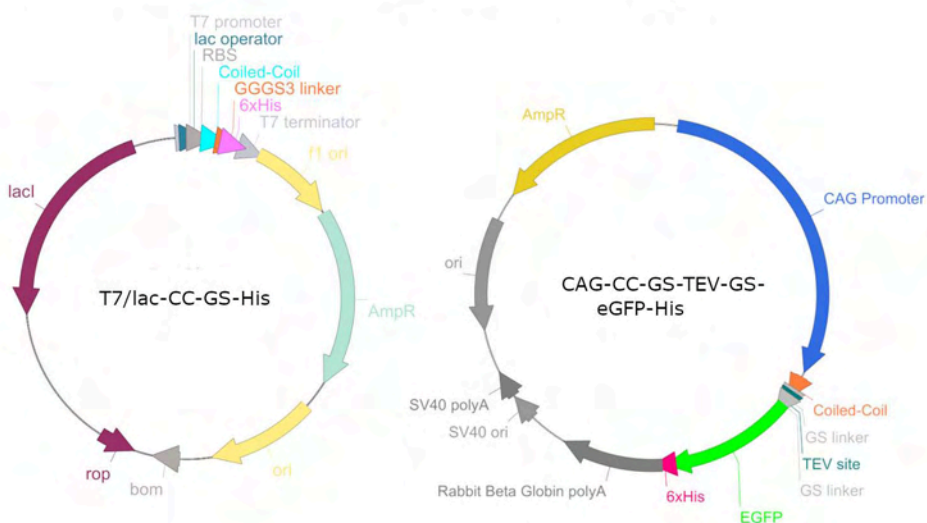
## b. Plasmid for Yeast SynAg Coiled-Coil Screen



## c. Plasmids for human helixCAM line construction



## d. Plasmids for Expression of His-tagged Coiled-Coils



**a.**

Uninduced

Induced

FoV 1

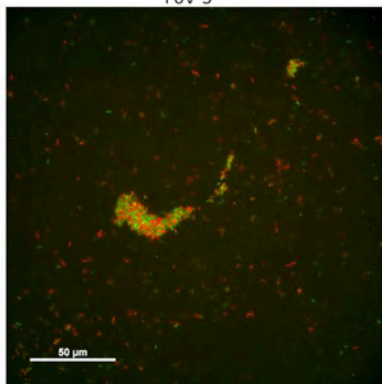
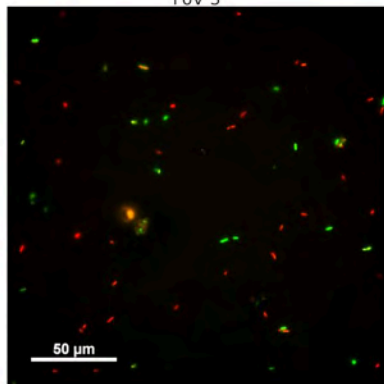
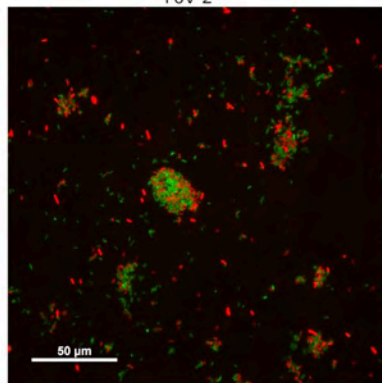
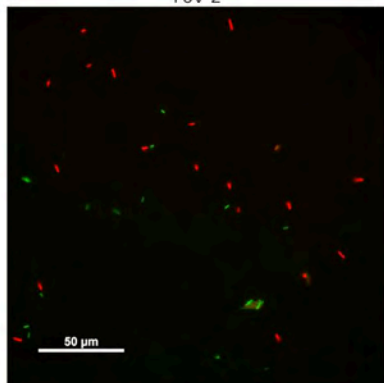
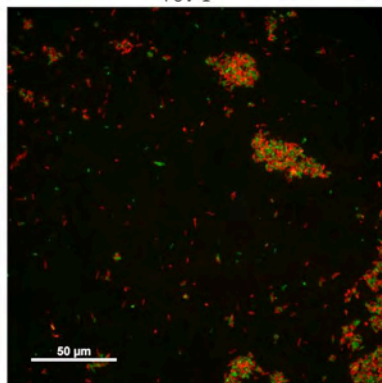
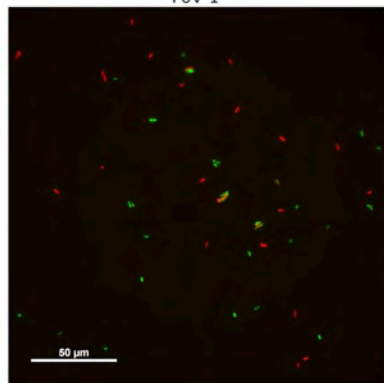
FoV 1

FoV 2

FoV 2

FoV 3

FoV 3



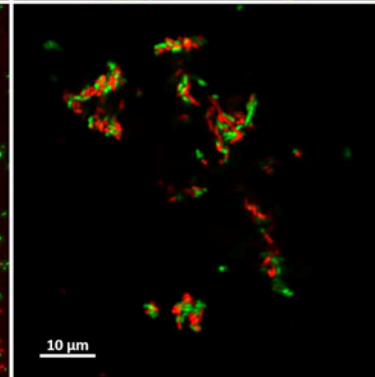
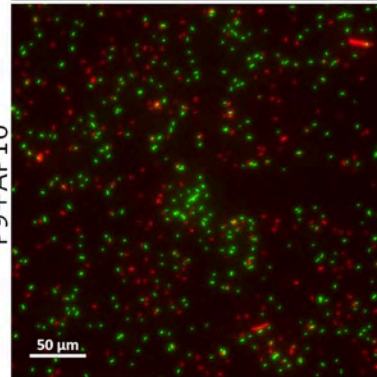
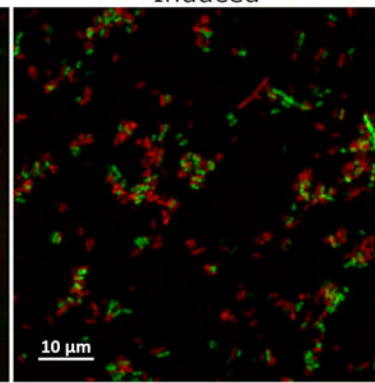
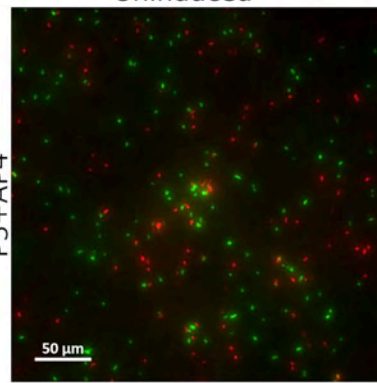
**b.**

Uninduced

Induced

P3+AP4

P9+AP10



# Pairwise helixCAM K562 cells

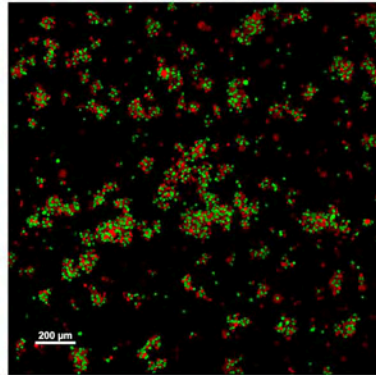
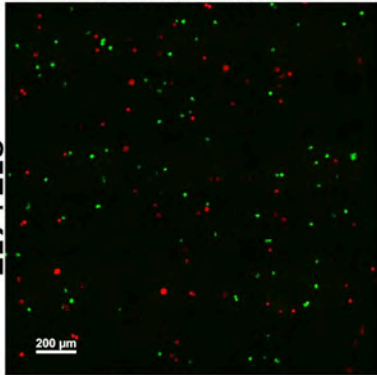
Uninduced

Induced

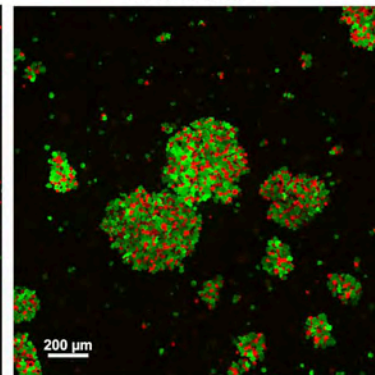
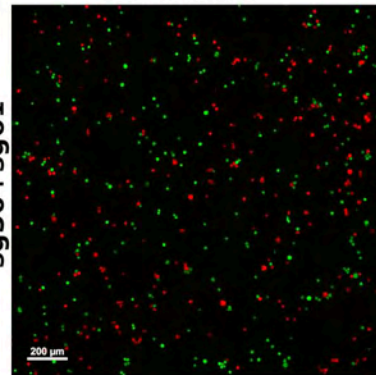
Uninduced

Induced

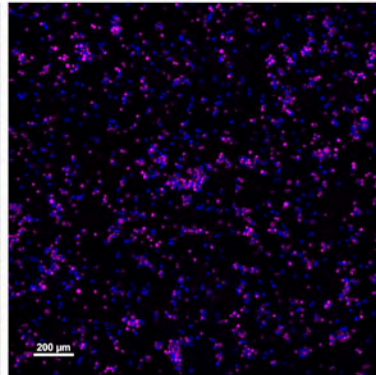
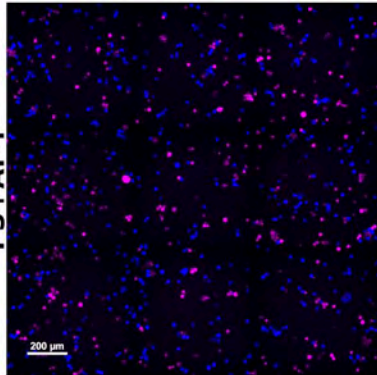
Z17+Z18



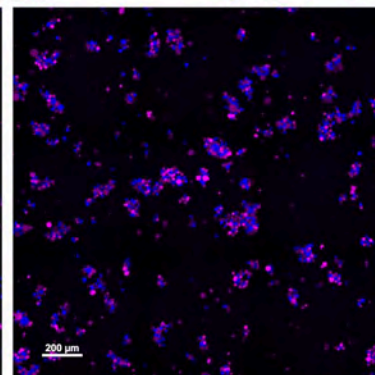
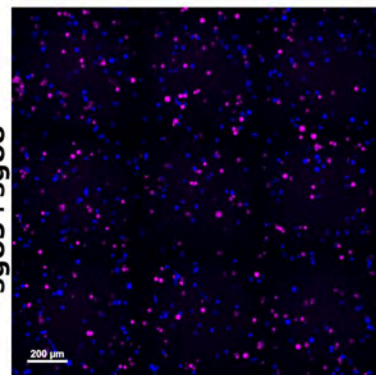
sg30+sg61



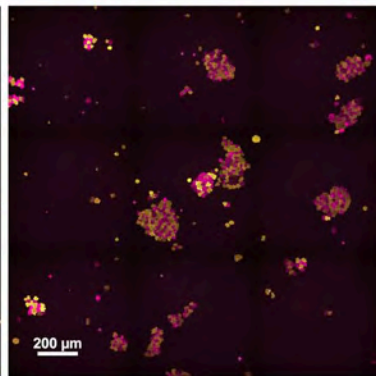
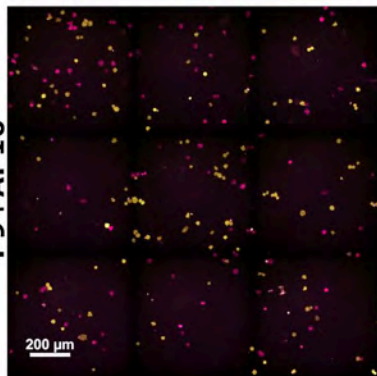
P3+AP4



sg83+sg88

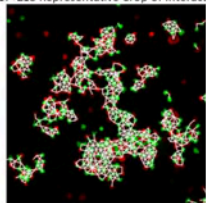


P9+AP10

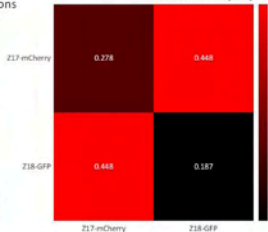


### Figure 1E

Z17+Z18 Representative Crop of Interactions

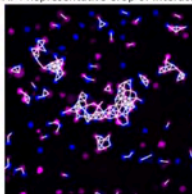


Z17<sup>mCherry</sup>+Z18<sup>GFP</sup> Observed Interaction Frequency

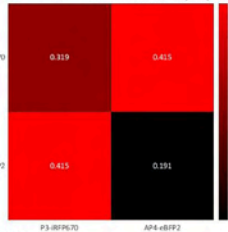


### Figure 2A

P3+AP4 Representative Crop of Interactions

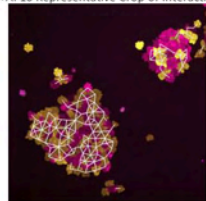


P3<sup>IRFP70</sup>+AP4<sup>eBFP2</sup> Observed Interaction Frequency

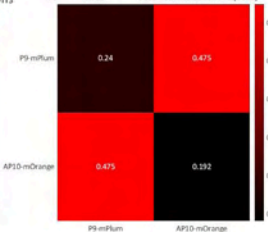


### Figure 2B

P9+AP10 Representative Crop of Interactions

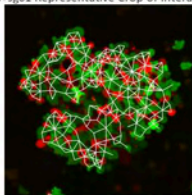


P9<sup>mEum</sup>+AP10<sup>mOrange</sup> Observed Interaction Frequency

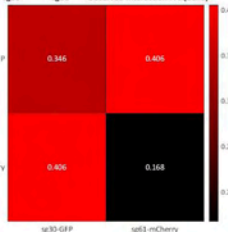


### Figure 3F

sg30+sg61 Representative Crop of Interactions

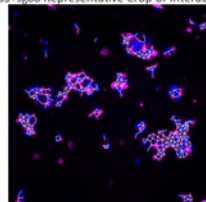


sg30<sup>GFP</sup>+sg61<sup>mCherry</sup> Observed Interaction Frequency

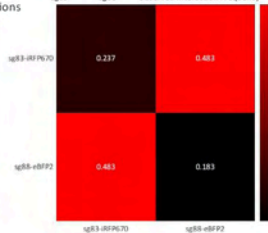


### Figure 3G

sg83+sg88 Representative Crop of Interactions

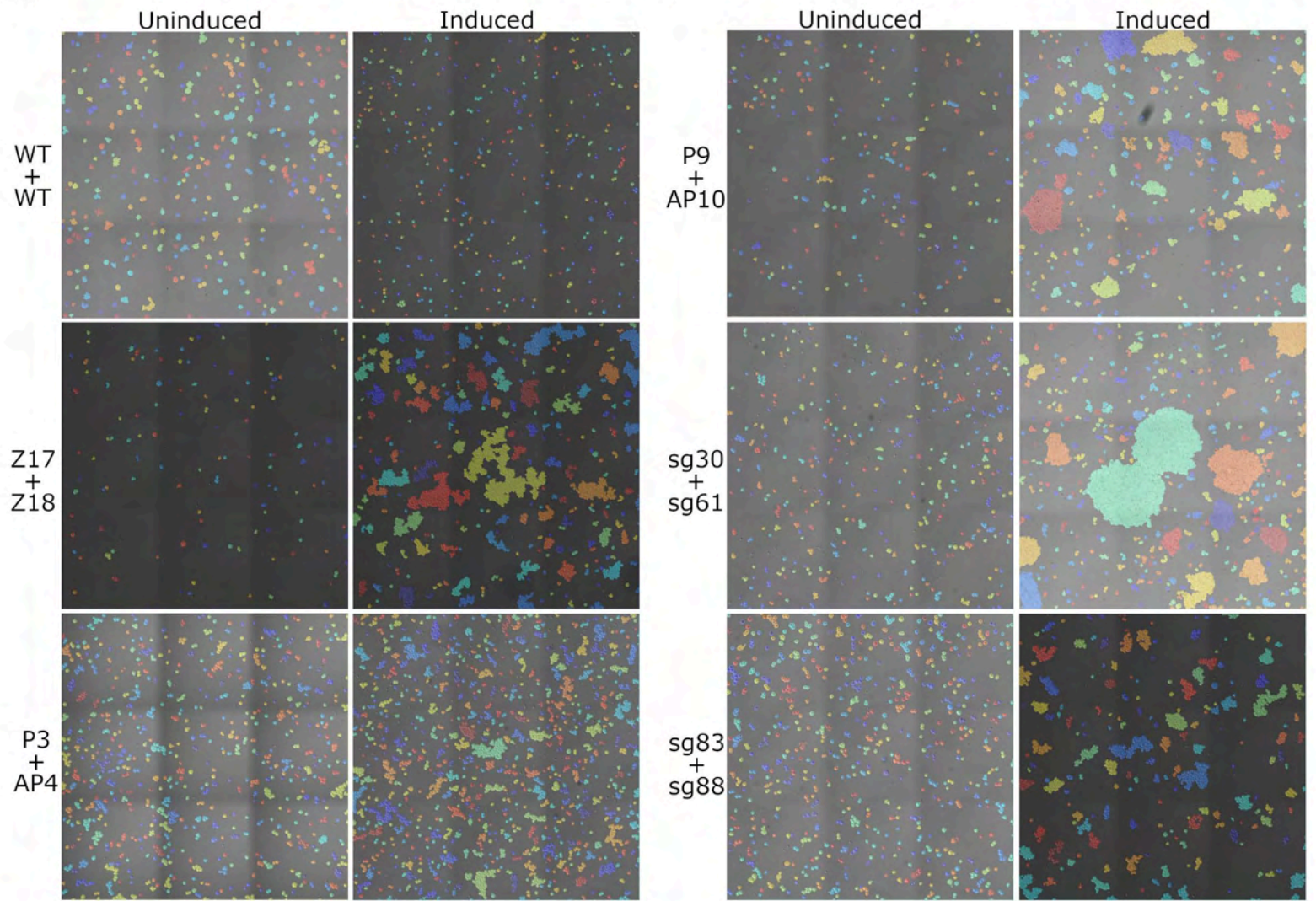


sg83<sup>IRFP70</sup>+sg88<sup>eBFP2</sup> Observed Interaction Frequency

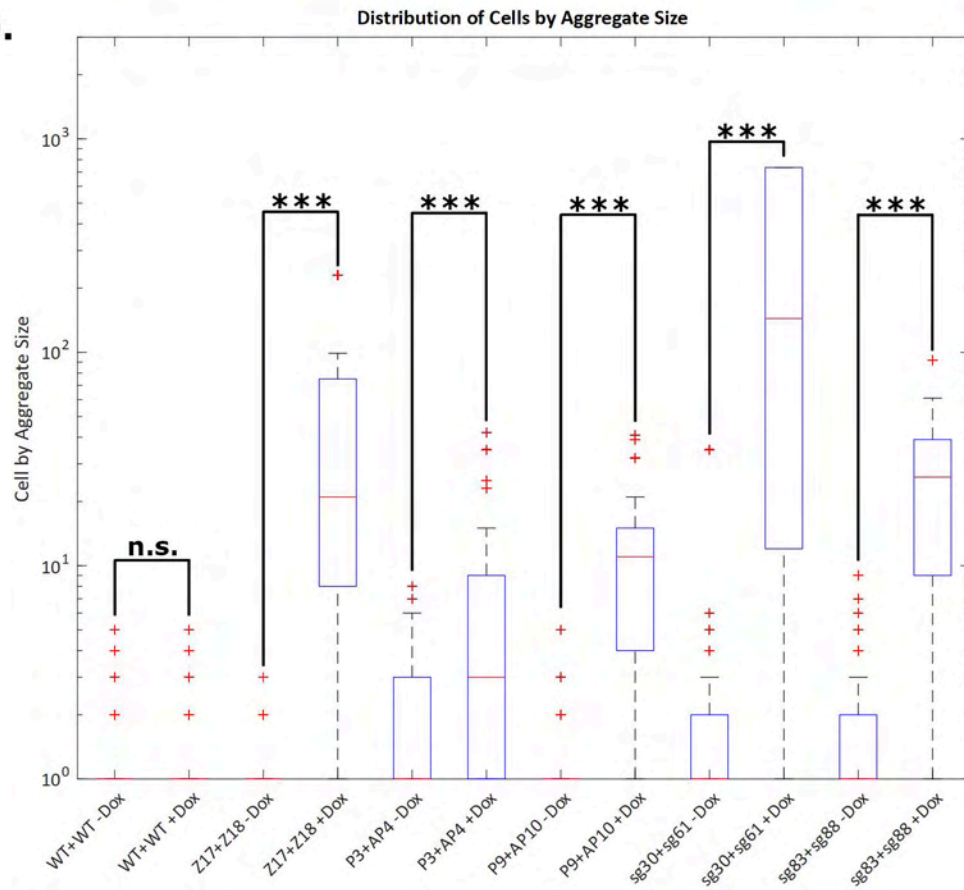


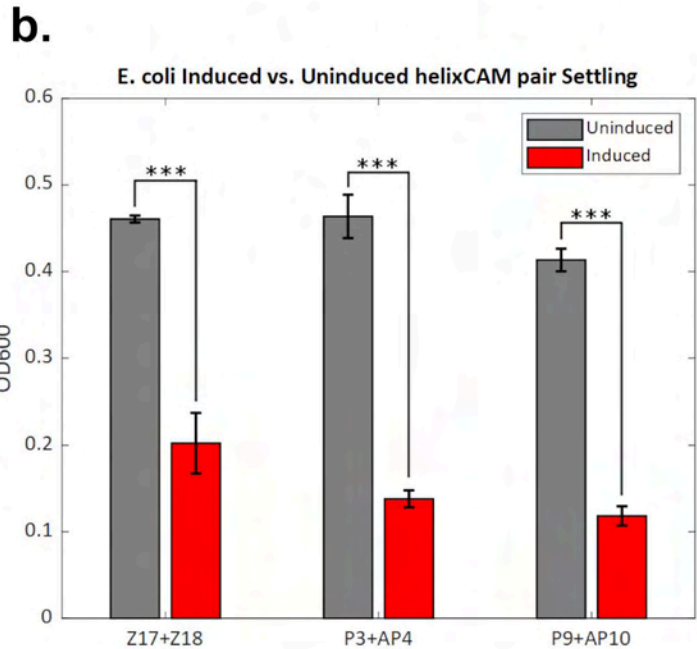
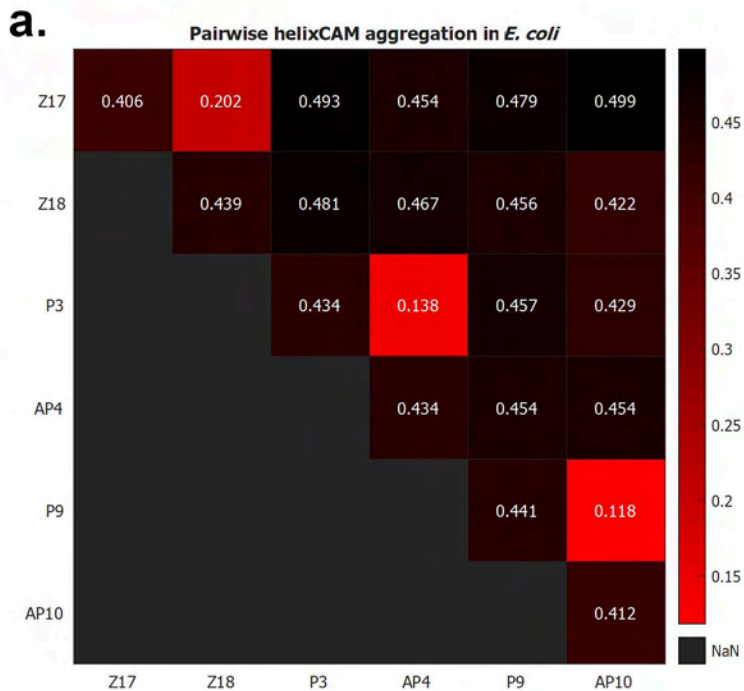
# Brightfield Images of K562 Co-culture with CellProfiler Overlay

**a.**



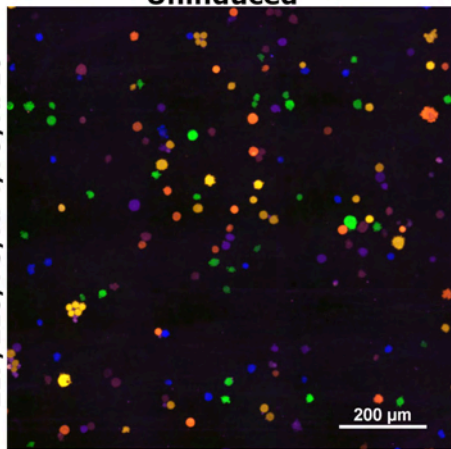
**b.**



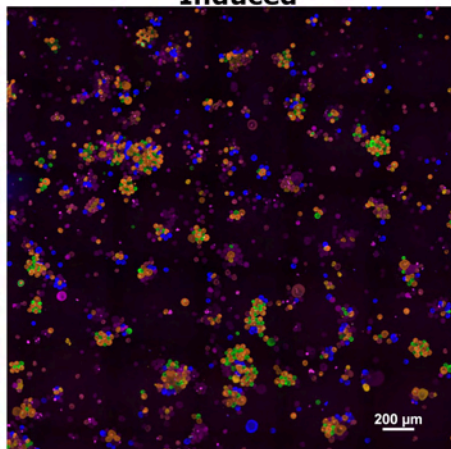


**Uninduced**

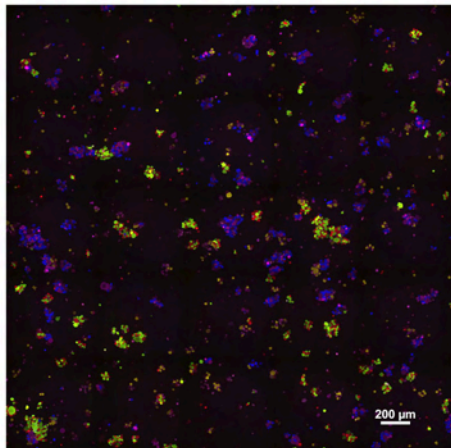
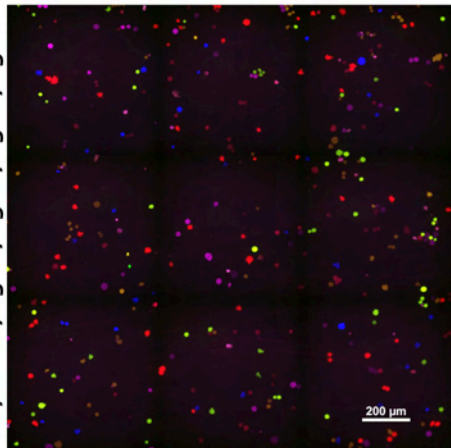
**Z17/Z18/P3/AP4/P9/AP10**

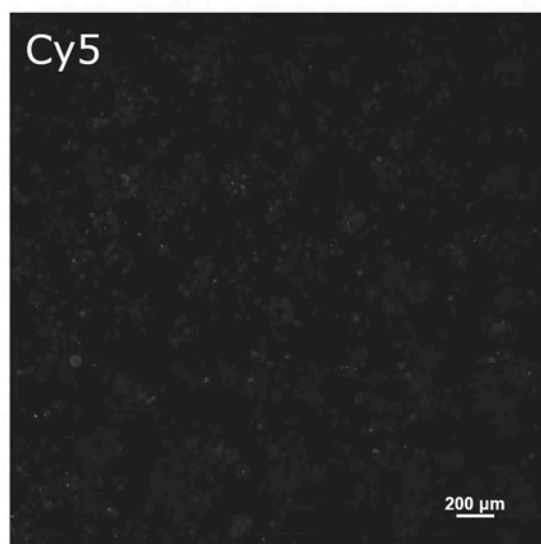
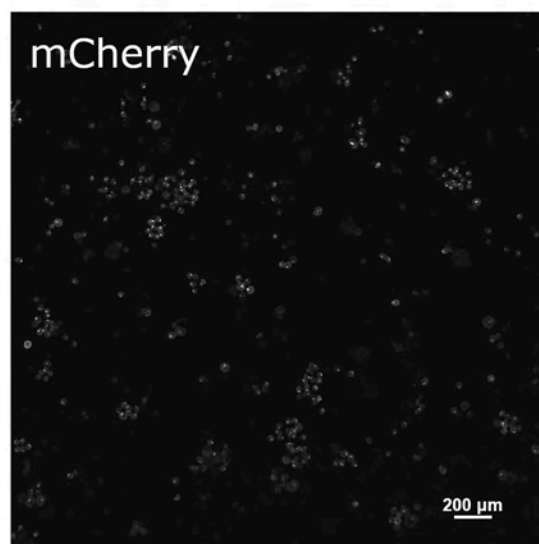
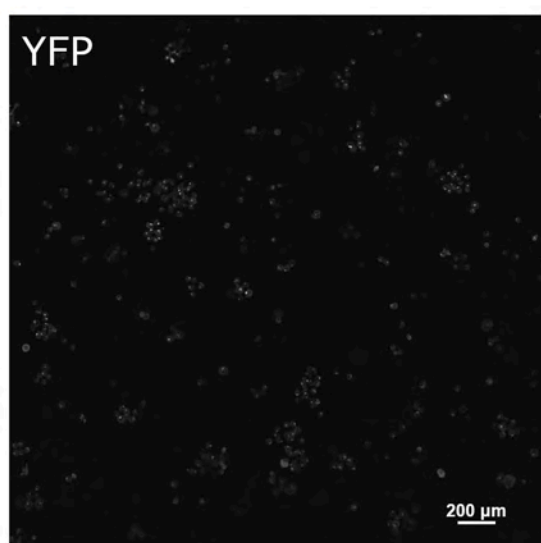
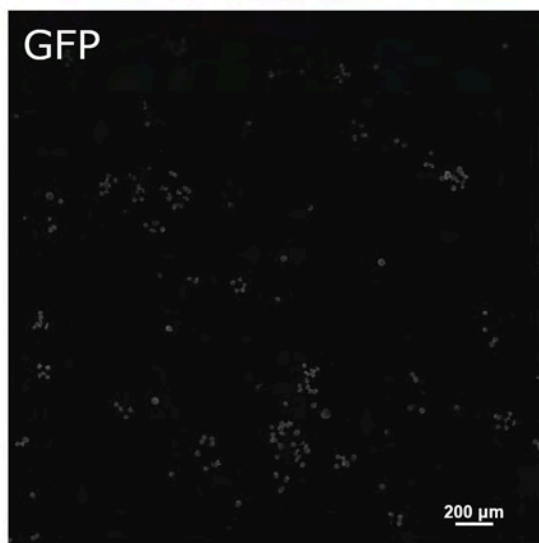
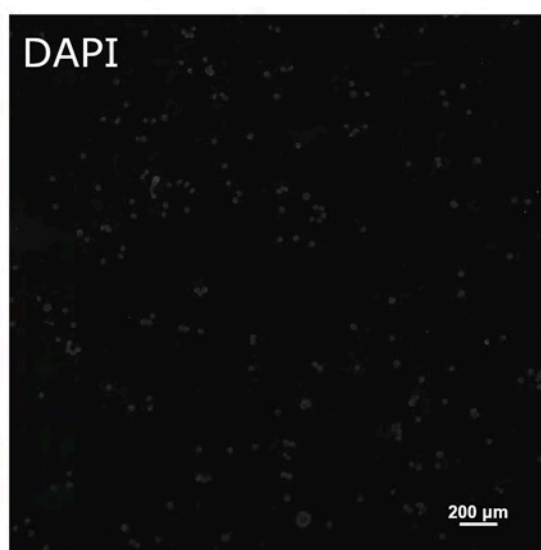
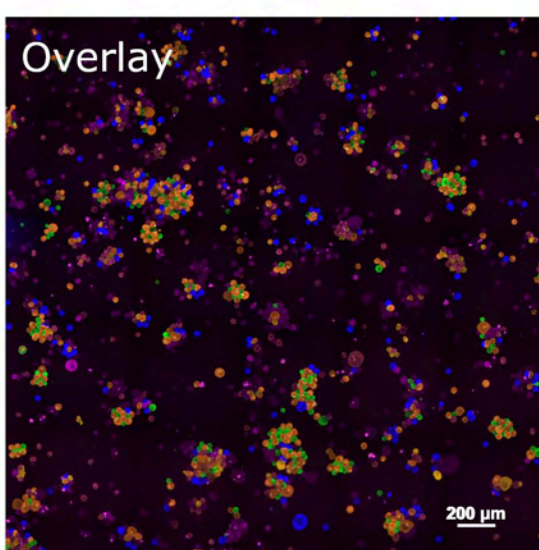


**Induced**

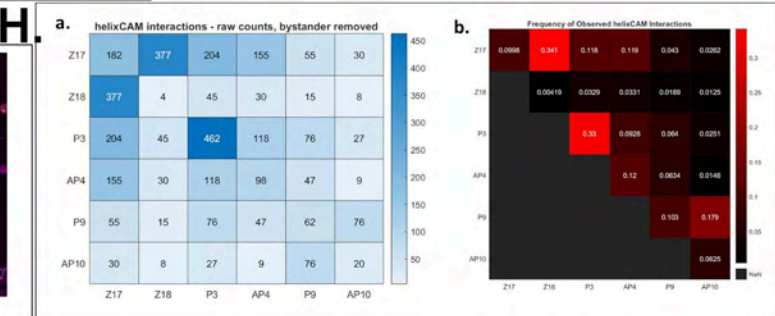
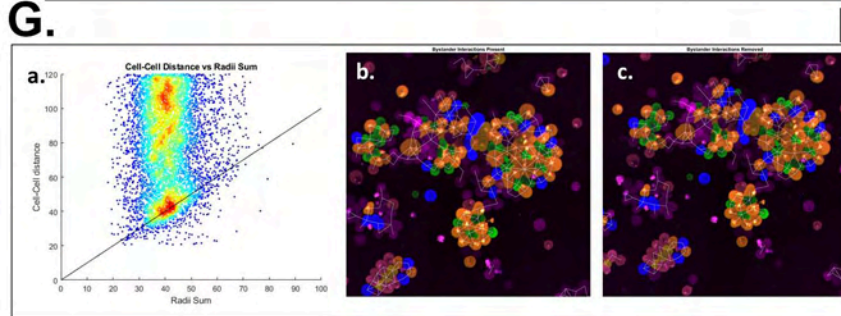
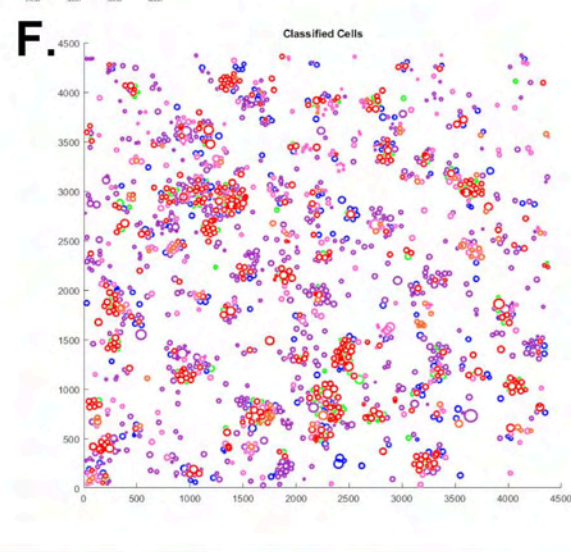
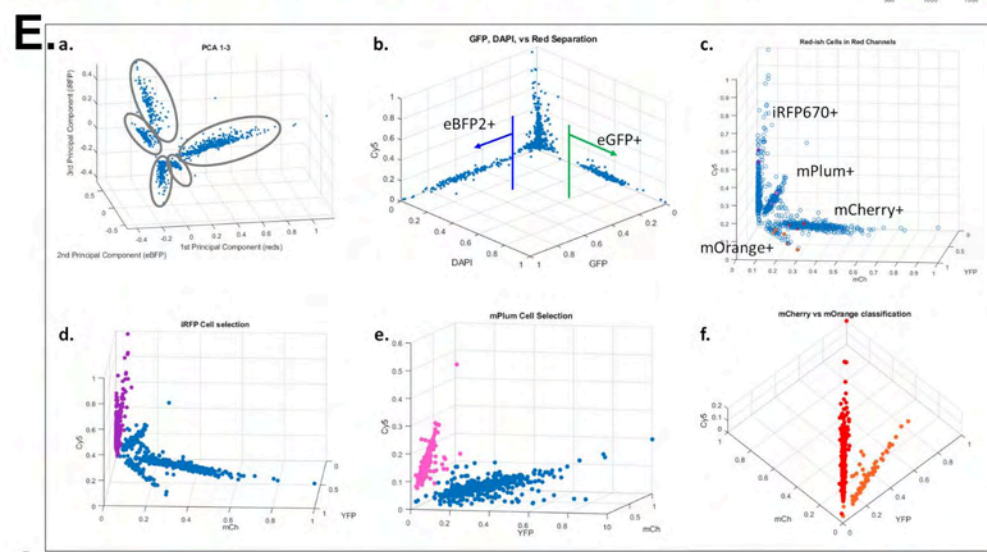
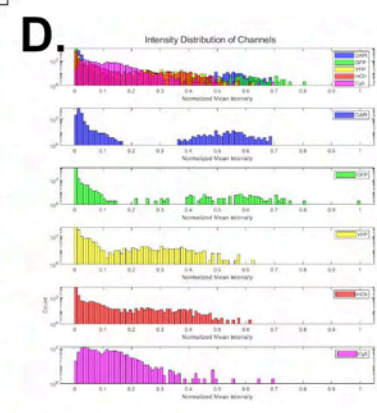
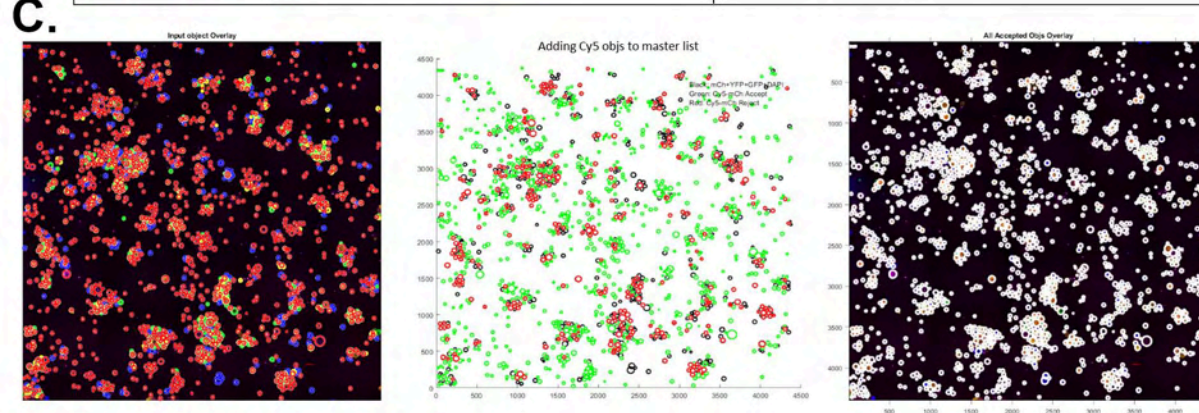
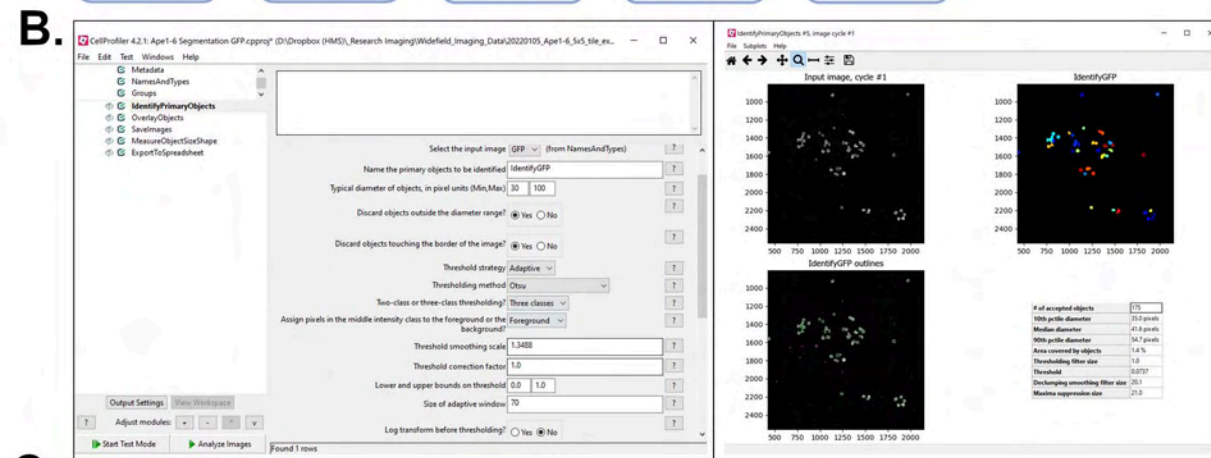
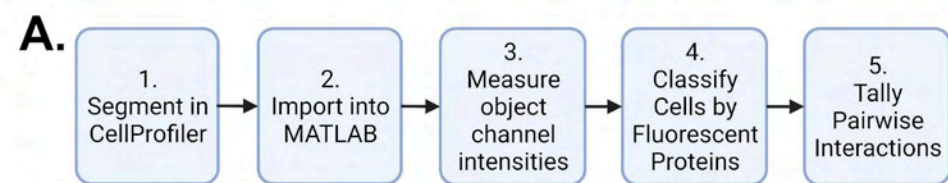


**P9/AP10/sg30/sg61/sg83/sg88**

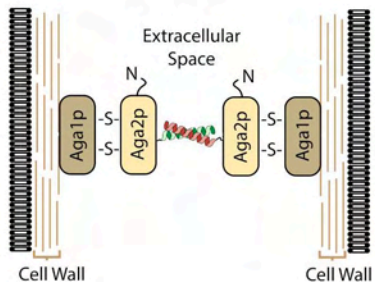




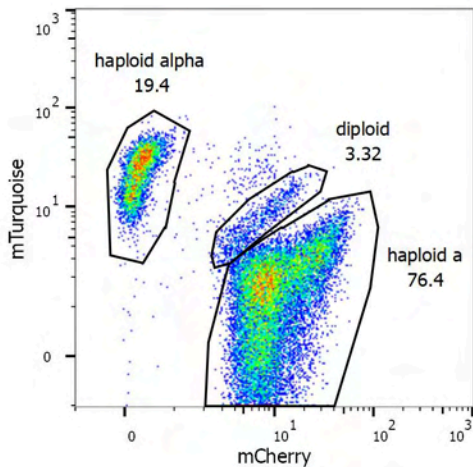
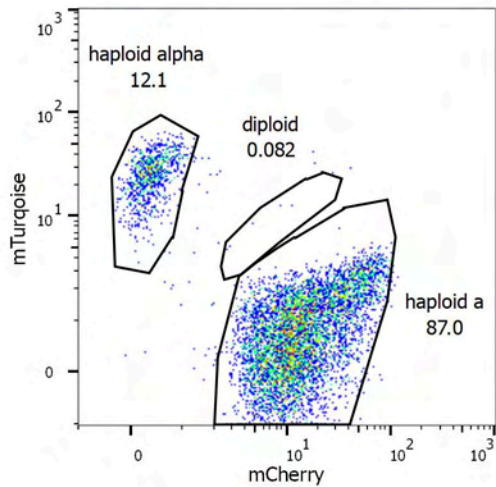
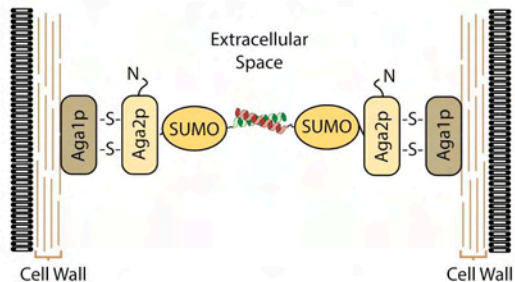




## Standard SynAg

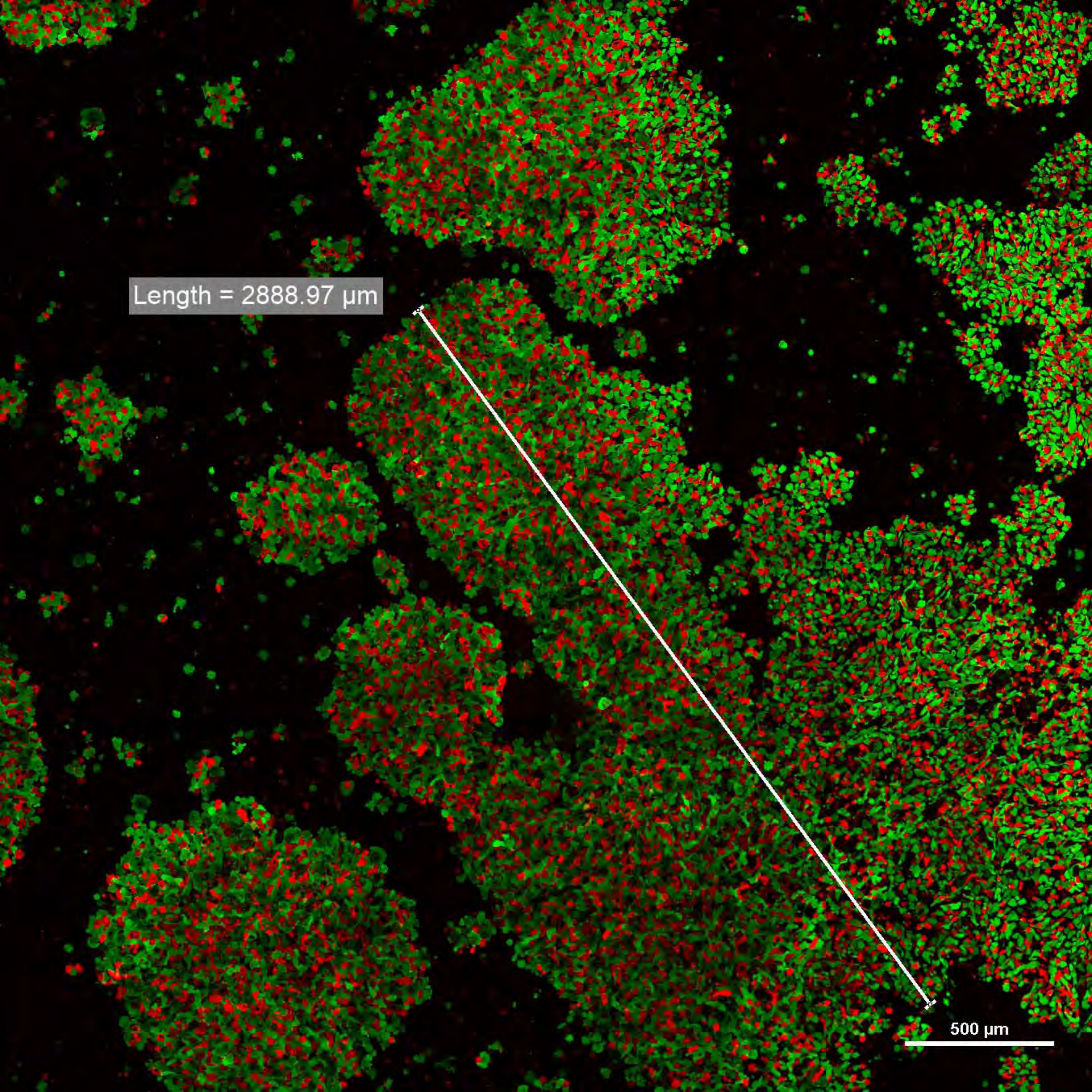


## SynAg + SUMO



Length = 2888.97  $\mu\text{m}$

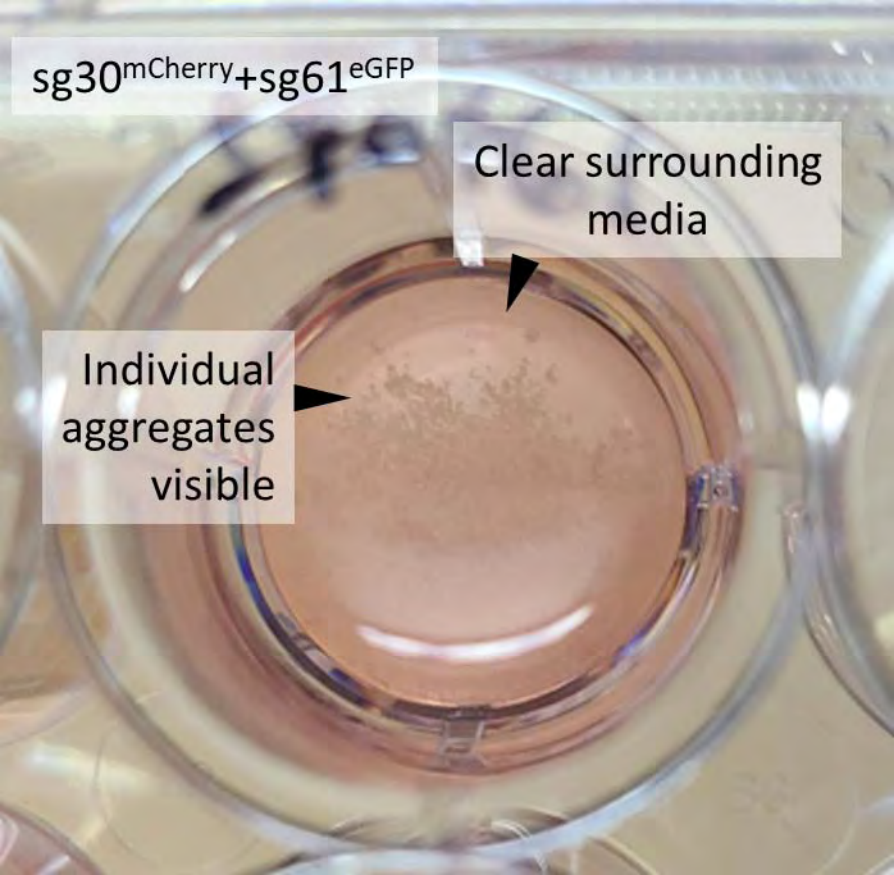
500  $\mu\text{m}$



sg30<sup>mCherry</sup>+sg61<sup>eGFP</sup>

Clear surrounding  
media

Individual  
aggregates  
visible

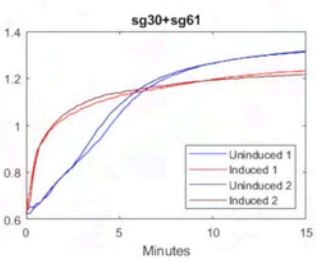
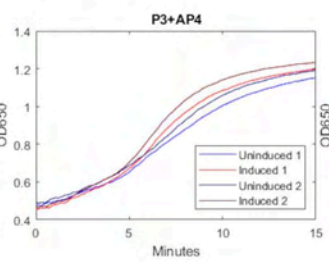
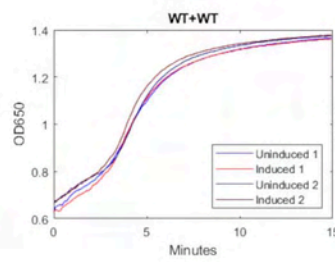


WT/No Affinity

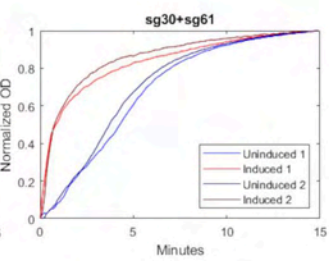
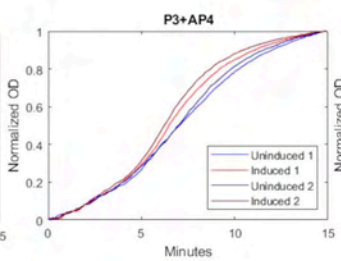
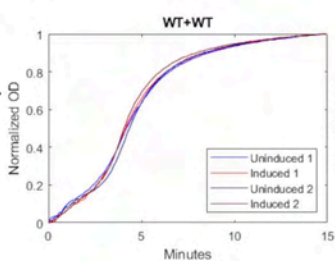
Weak Affinity

Strong Affinity

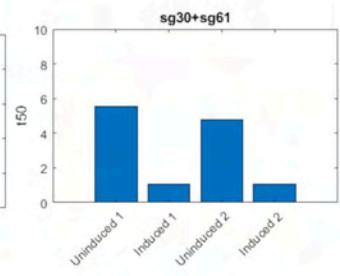
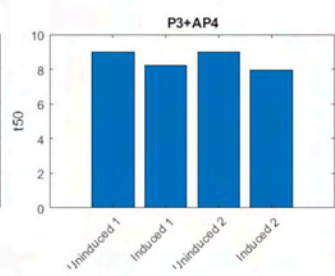
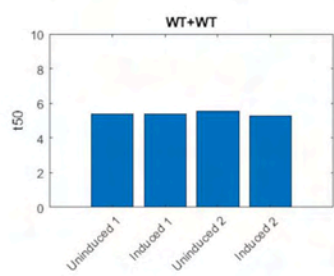
Raw  
OD650



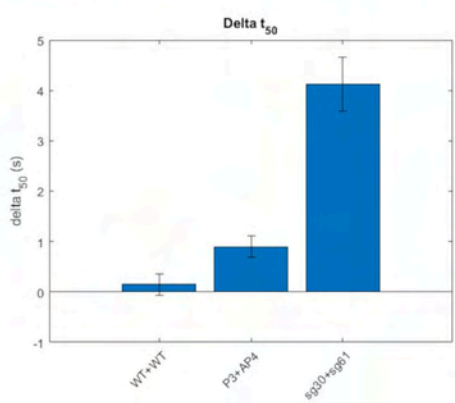
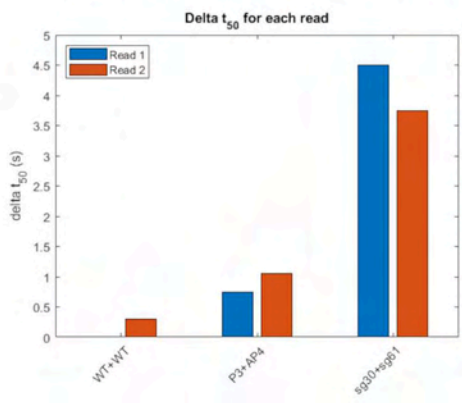
Normal-  
ized



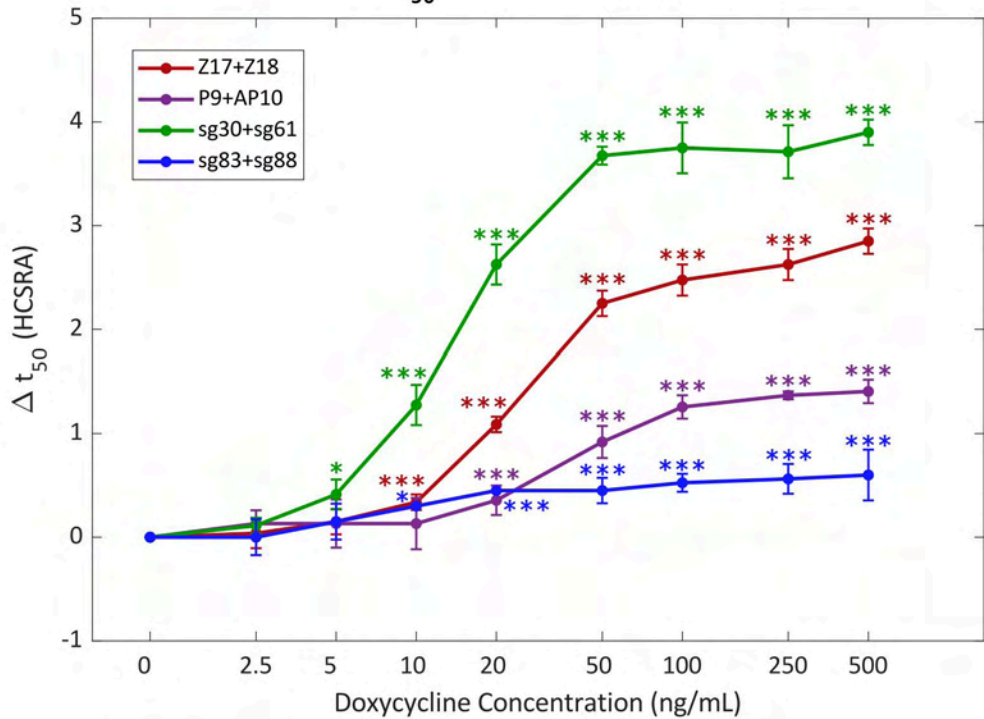
t50

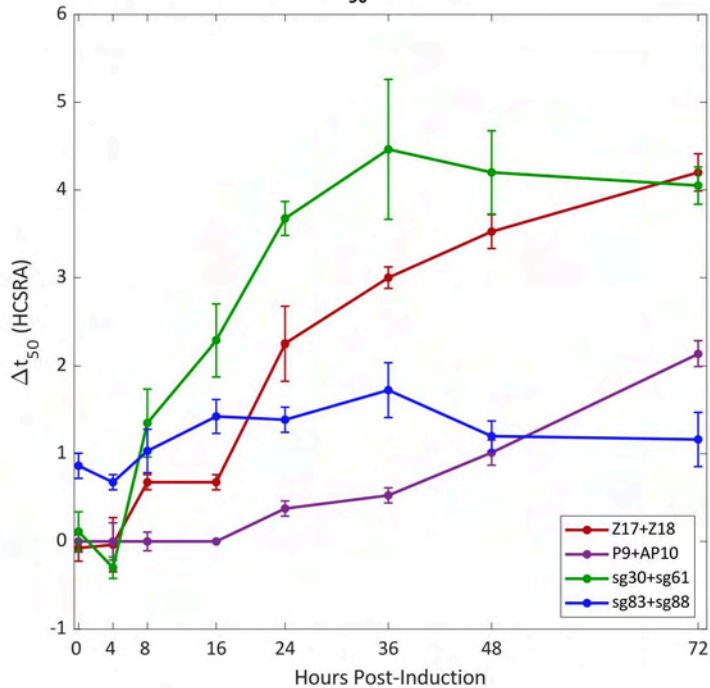
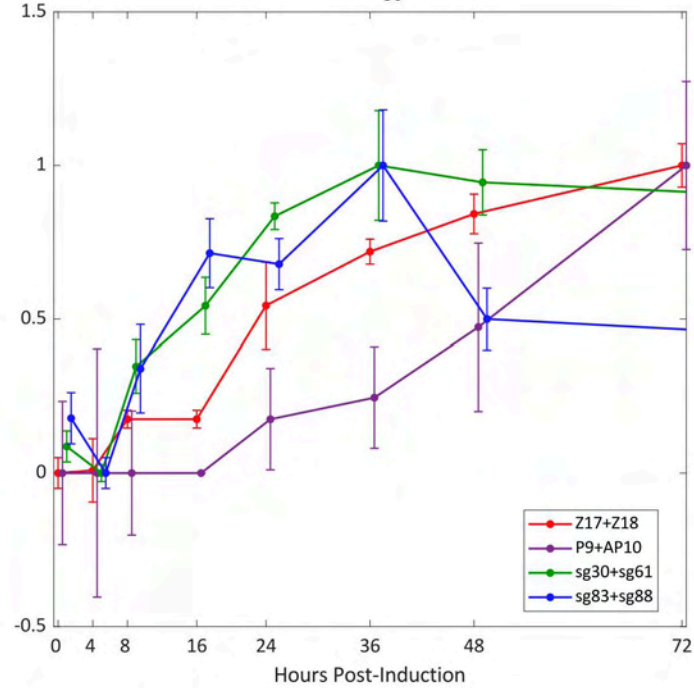


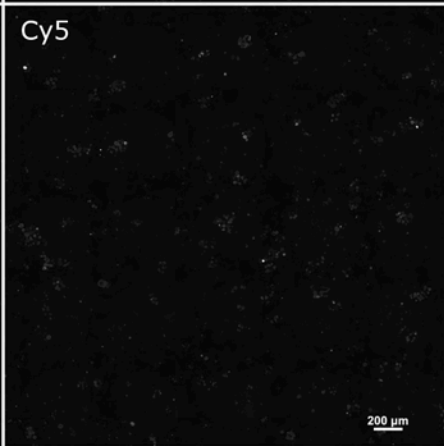
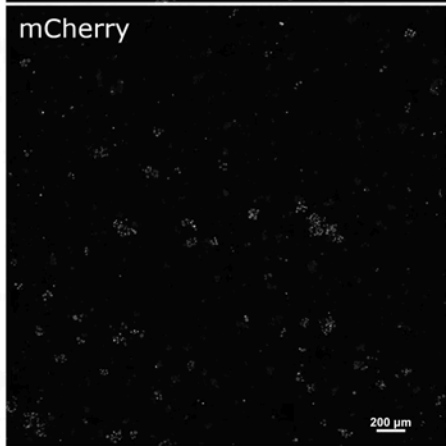
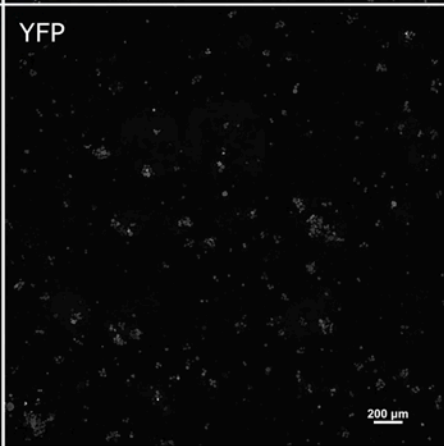
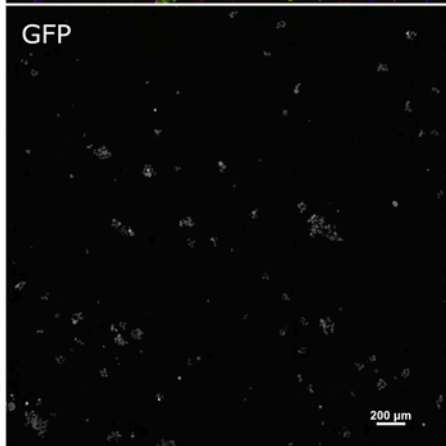
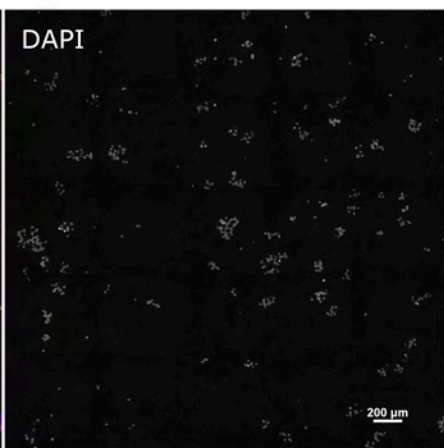
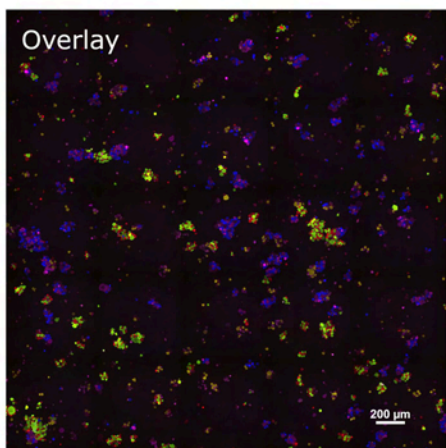
Δt50



# $\Delta t_{50}$ vs. Induction Strength

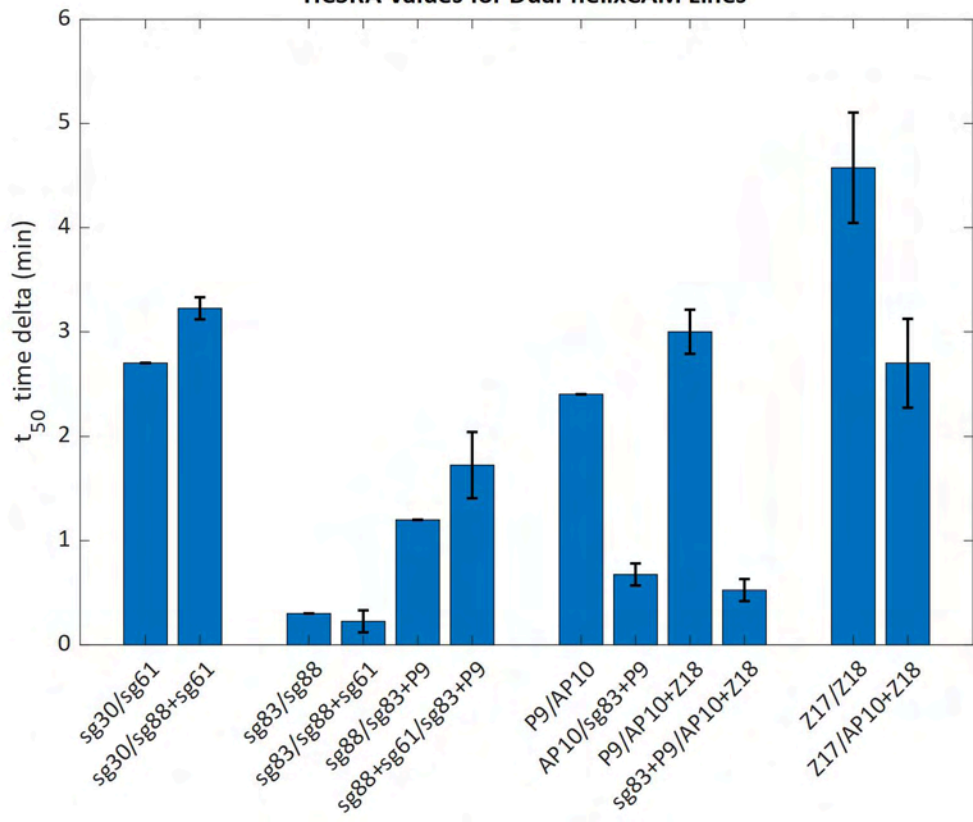


$\Delta t_{50}$  over timeNormalized  $\Delta t_{50}$  over time





### HCSRA Values for Dual-helixCAM Lines

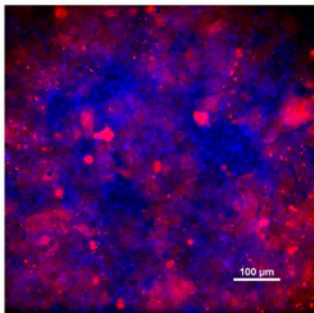
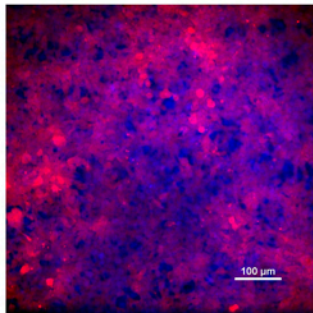
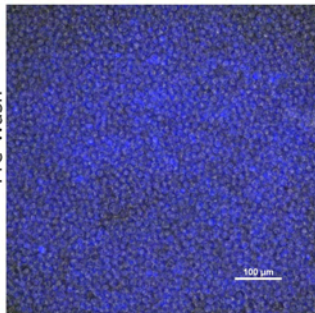


Wild-type HEK293AAV +  
sg61/sg88-eBFP2 (K562)  
48 hours induction  
12 hours incubation

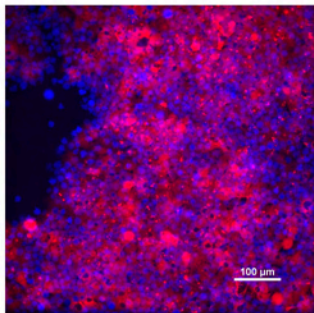
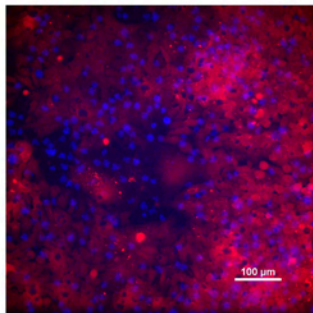
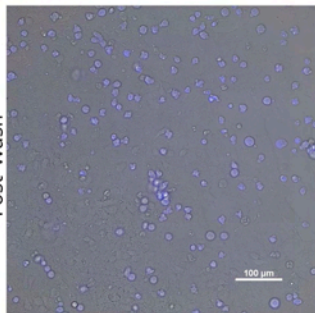
sg30-mCherry (HEK293AAV)+  
sg61/sg88-eBFP2 (K562)  
no induction  
12 hours incubation

sg30-mCherry (HEK293AAV)+  
sg61/sg88-eBFP2 (K562)  
48 hours induction  
12 hours incubation

Pre-Wash



Post-Wash

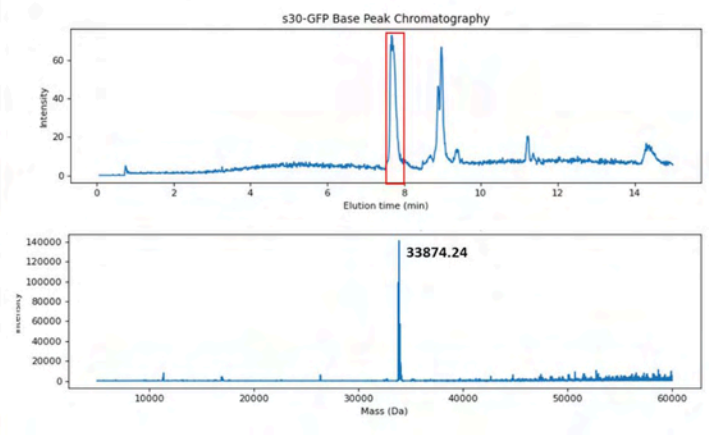
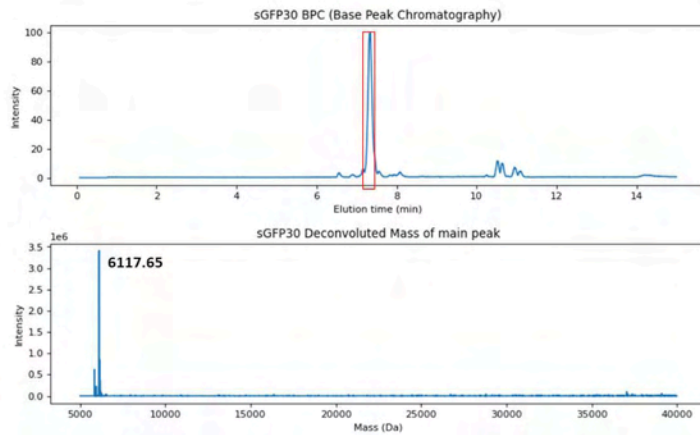


# LC-MS Analysis of His-tagged CCs

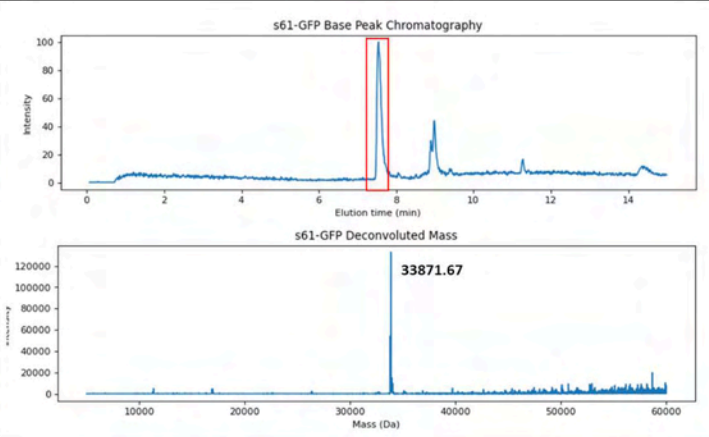
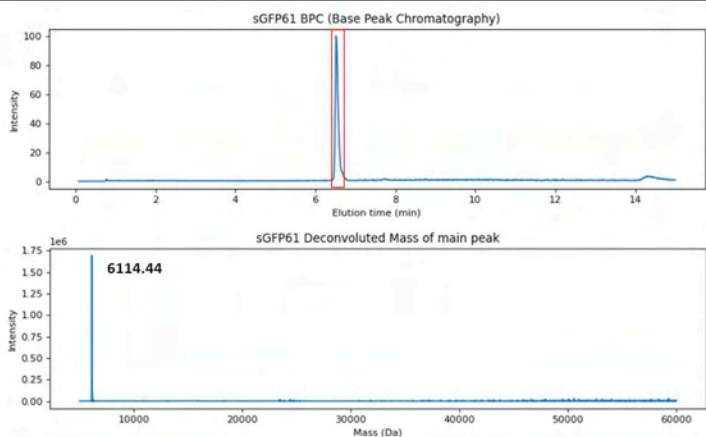
## CC-His

## CC-GFP-His

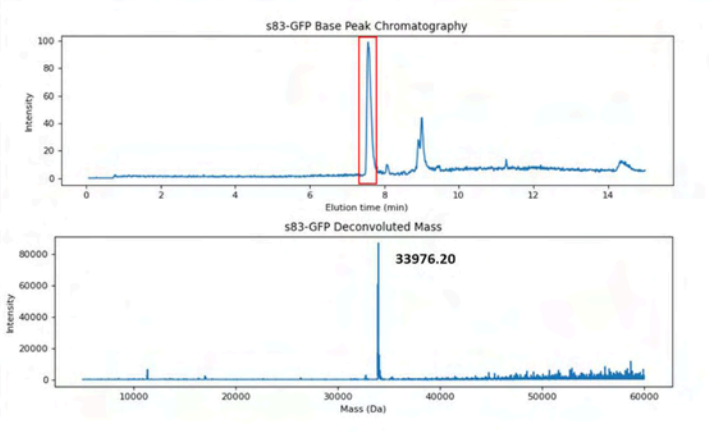
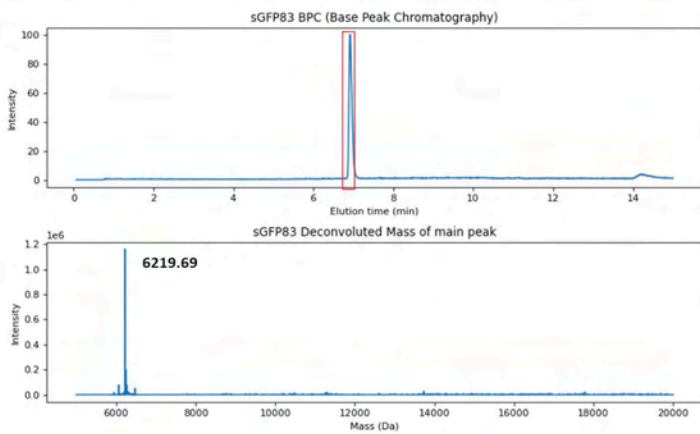
sg30



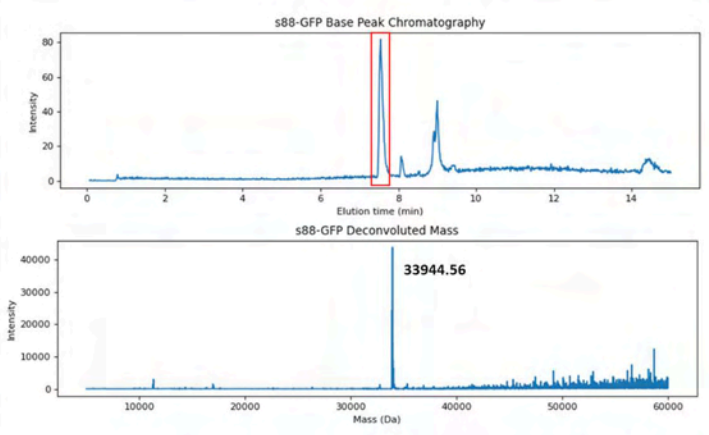
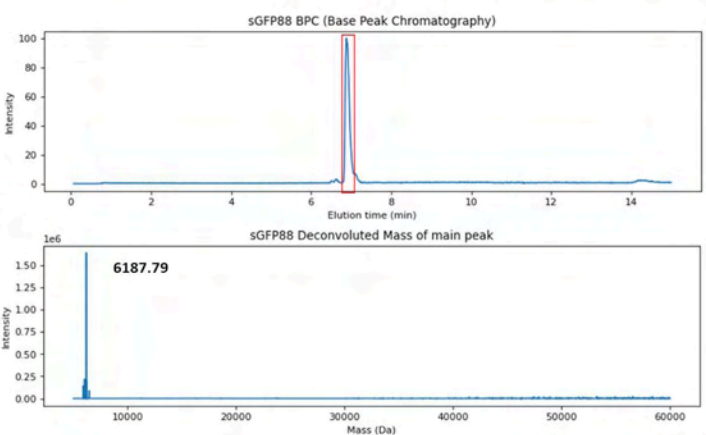
sg61



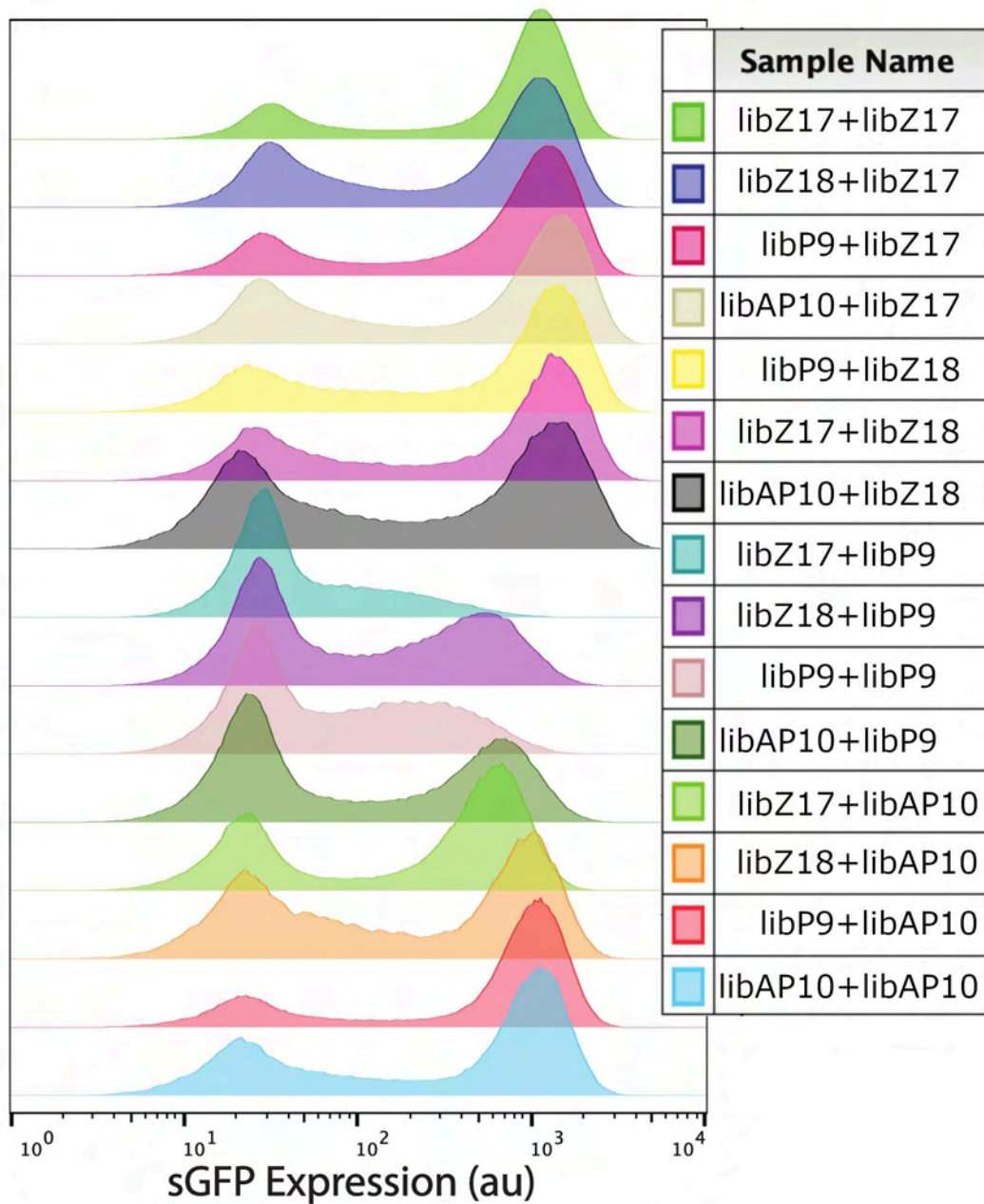
sg83

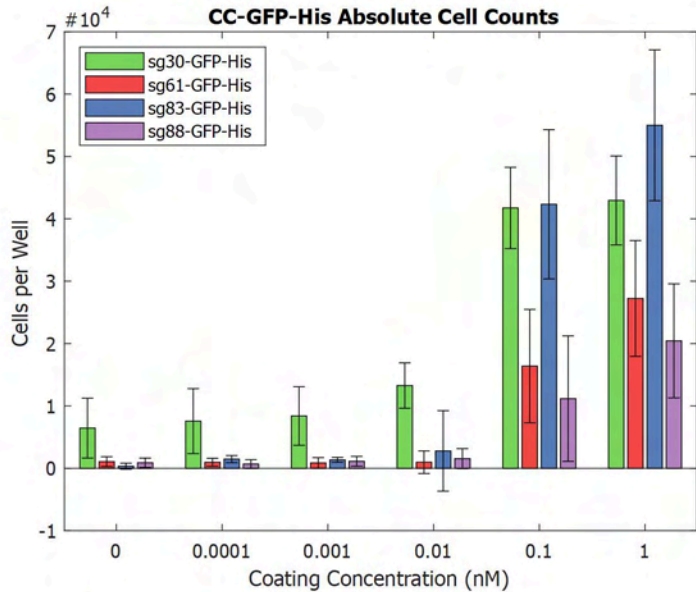
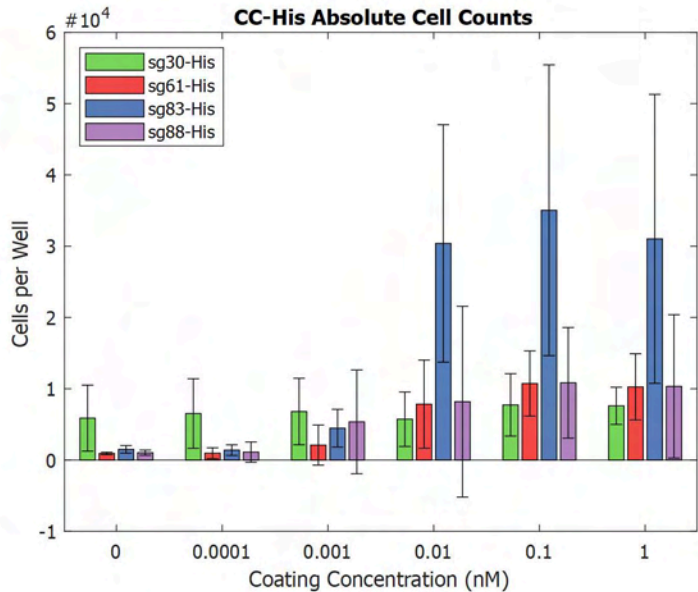


sg88



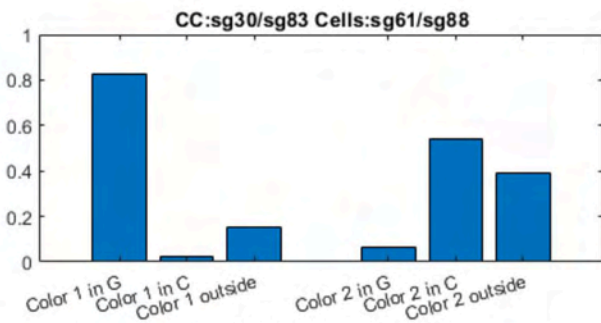
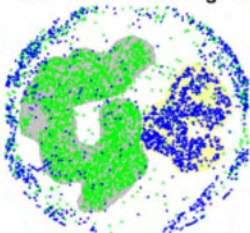
Scaled Cell Count



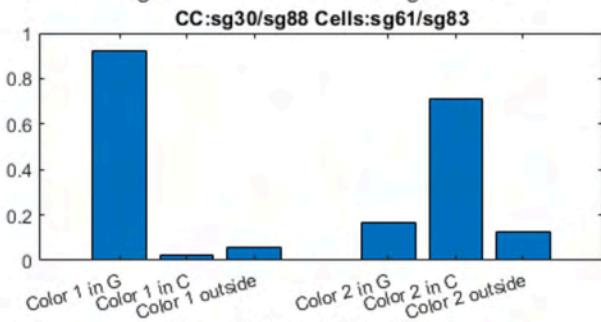


**a.**

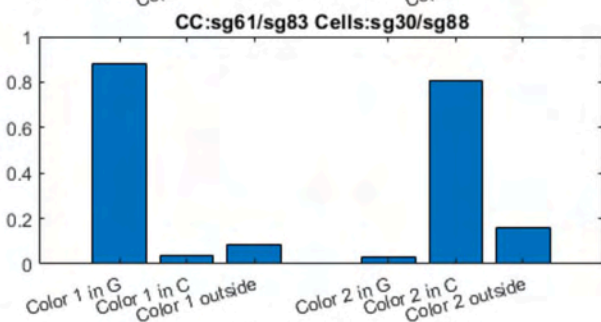
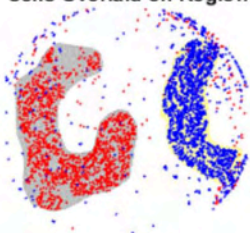
Cells Overlaid on Region



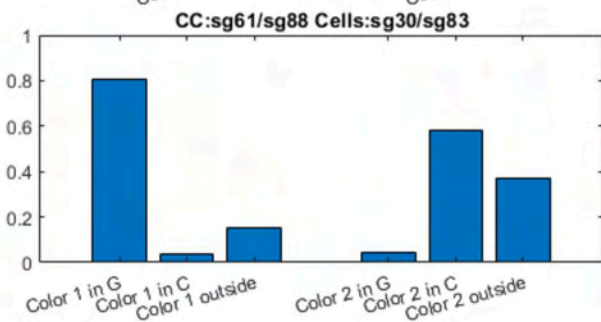
Cells Overlaid on Region



Cells Overlaid on Region

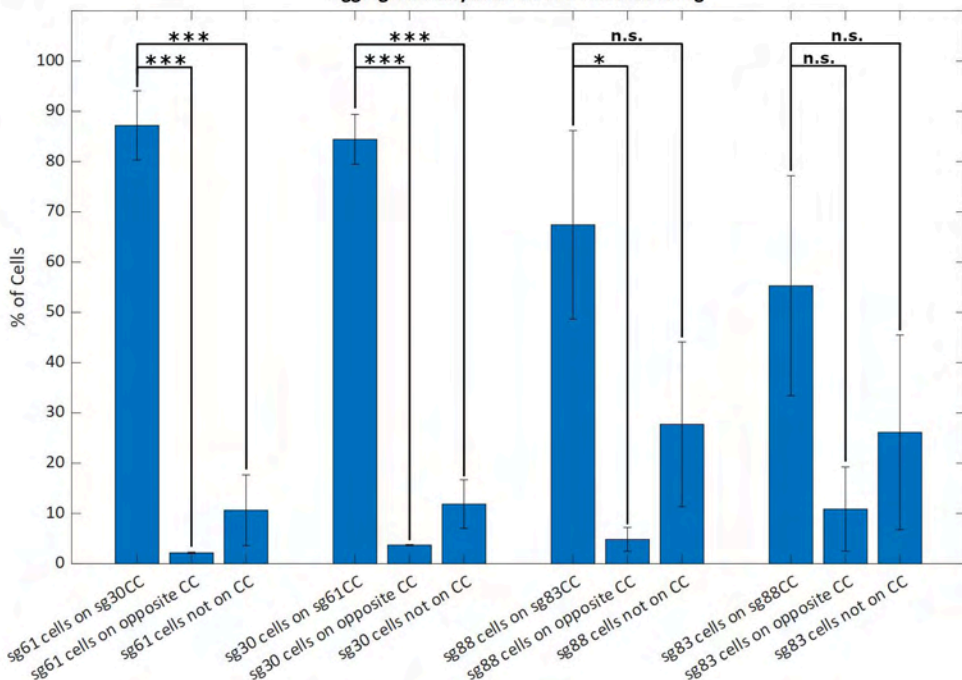


Cells Overlaid on Region



**b.**

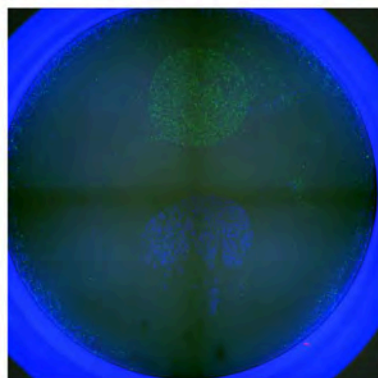
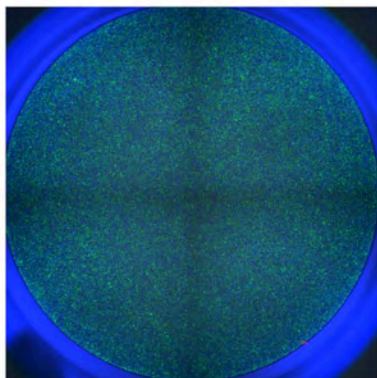
Aggregate Analysis of CC-GFP-His Patterning



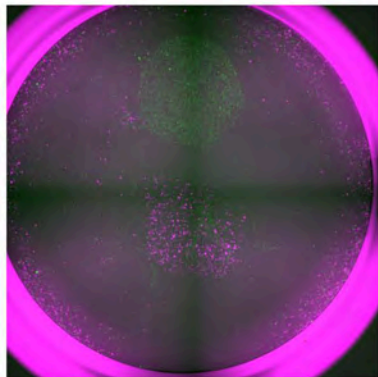
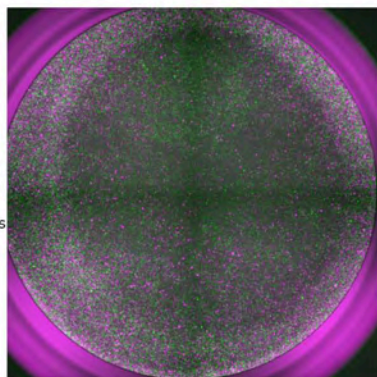
**Pre-Wash**

**Post-Wash**

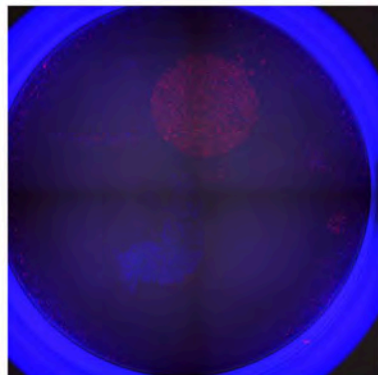
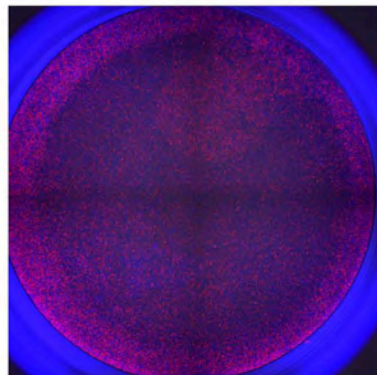
sg30 CC (top)  
sg83 CC (bottom)  
+  
sg61-GFP cells  
sg88-eBFP2 cells



sg30 CC (top)  
sg88 CC (bottom)  
+  
sg61-GFP cells  
sg83-IRFP670 cells



sg61 CC (top)  
sg83 CC (bottom)  
+  
sg30-GFP cells  
sg88-eBFP2 cells



sg61 CC (top)  
sg88 CC (bottom)  
+  
sg30-GFP cells  
sg83-IRFP670 cells

