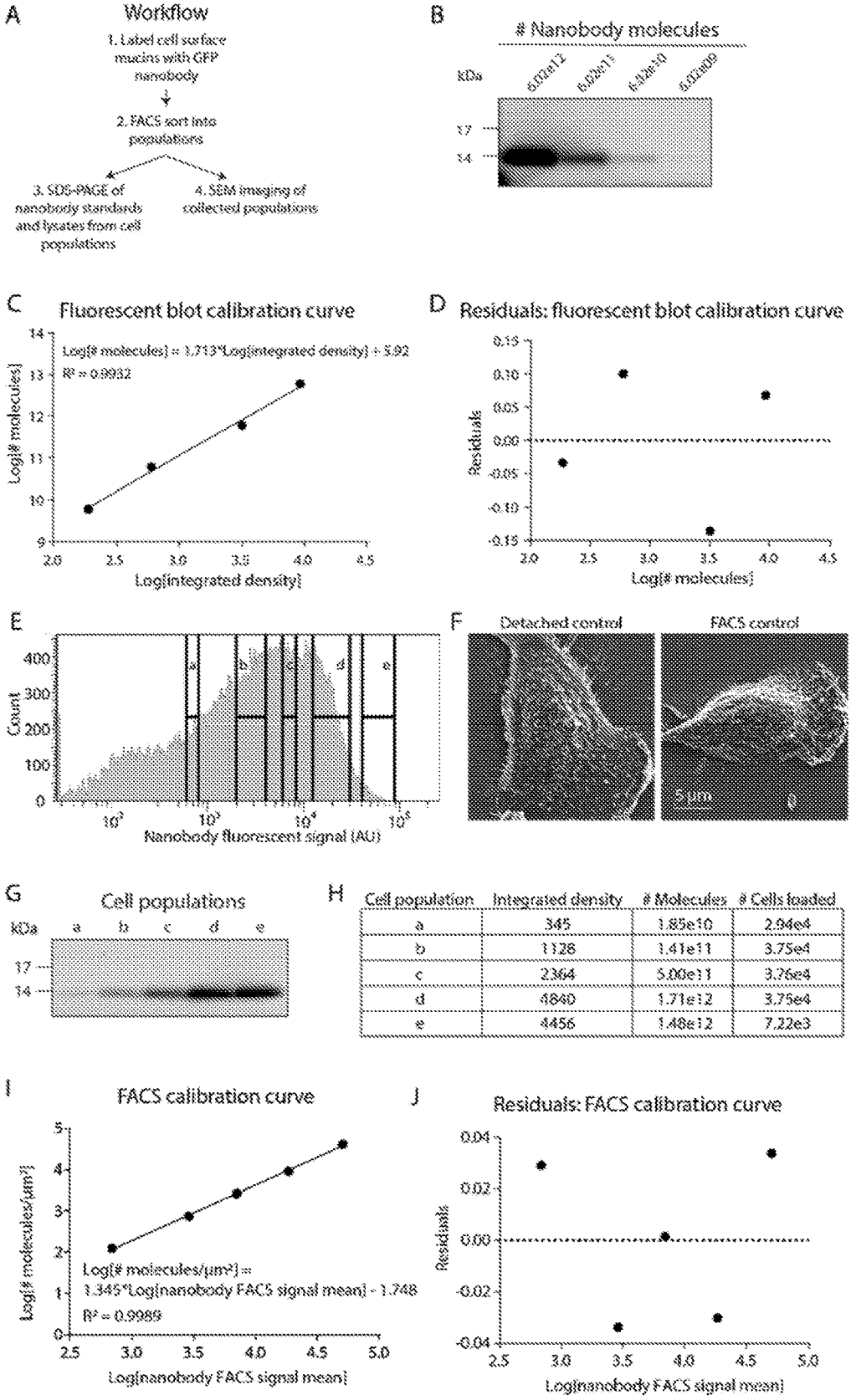
A
Workflow
1. Label cell surface mucins with GFP nanobody
2. FACS sort into populations
3. SDS-PAGE of nanobody standards and lysates from cell populations
4. SEM imaging of collected populations
B
Nanobody molecules
kDa
17
14
C
Fluorescent blot calibration curve
Log[# molecules] = 1.713*Log[integrated density] + 5.92
R² = 0.9932
Log[# molecules]
Log[integrated density]
D
Residuals: fluorescent blot calibration curve
Residuals
Log[# molecules]
E
Count
Nanobody fluorescent signal (AU)
F
Detached control
FACS control
G
Cell populations
kDa
a
b
c
d
e
H
Cell population | Integrated density | # Molecules | # Cells loaded
a | 345 | 1.85e10 | 2.94e4
b | 1128 | 1.41e11 | 3.75e4
c | 2364 | 5.00e11 | 3.76e4
d | 4840 | 1.71e12 | 3.75e4
e | 4456 | 1.48e12 | 7.22e3
I
FACS calibration curve
Log[# molecules/μm²] = 1.345*Log[nanobody FACS signal mean] - 1.748
R² = 0.9989
Log[# molecules/μm²]
Log[nanobody FACS signal mean]
J
Residuals: FACS calibration curve
Residuals
Log[nanobody FACS signal mean]

Figure 40