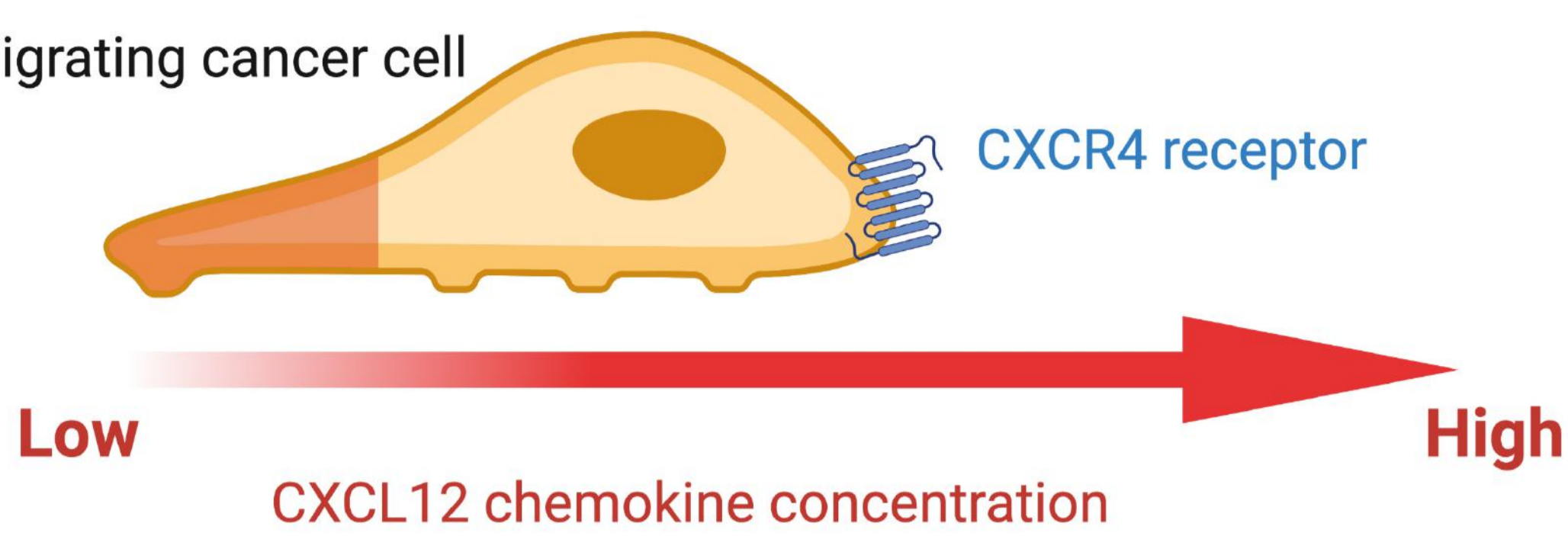
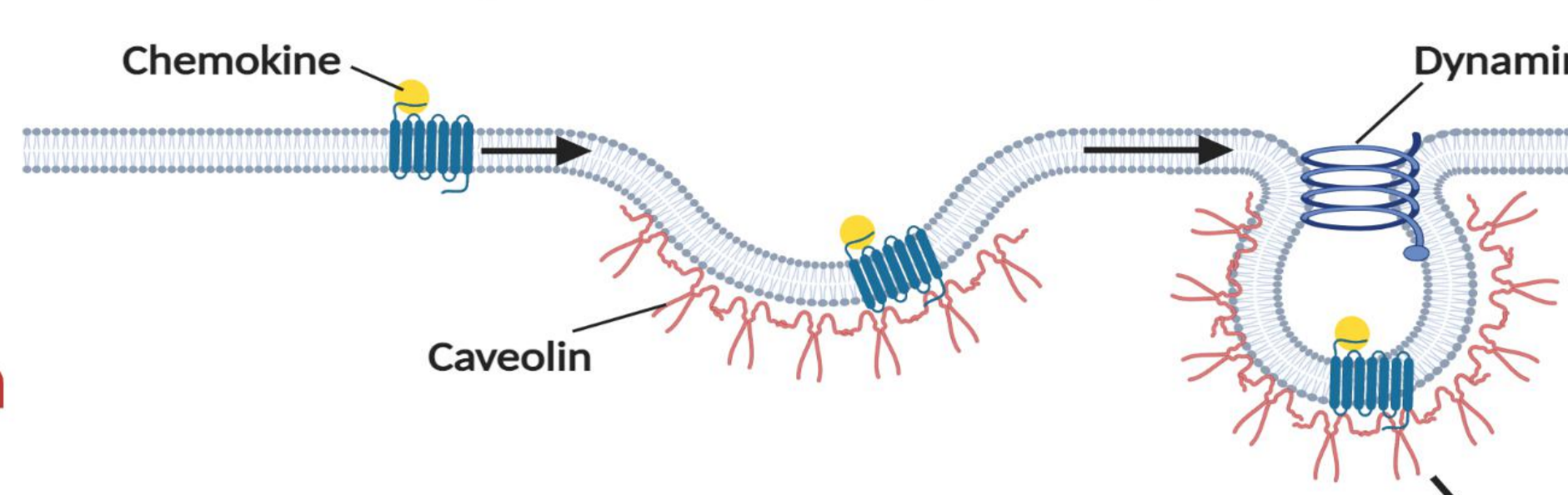


## Introduction

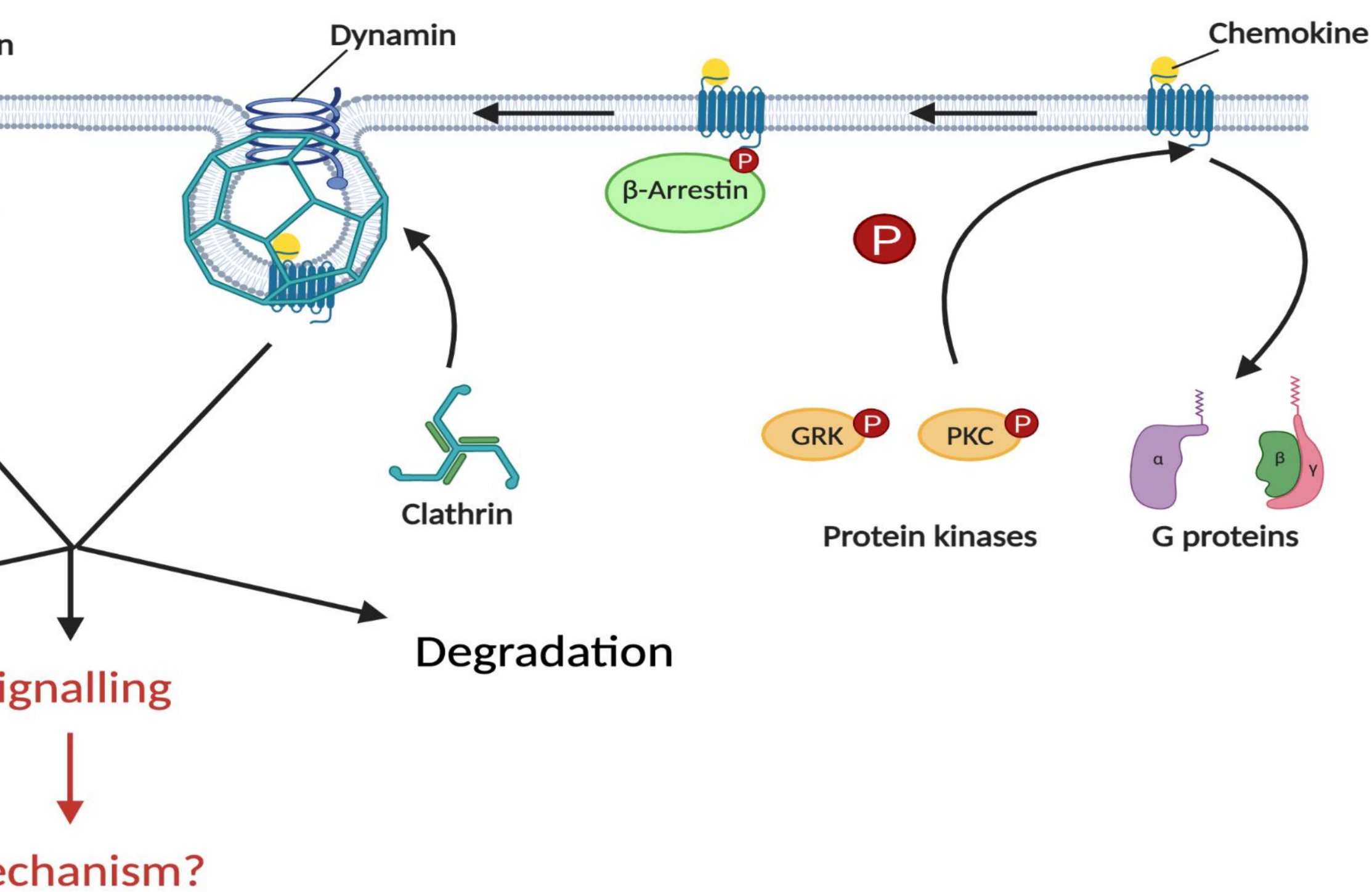
Migrating cancer cell



### I. Receptor trafficking through caveolae



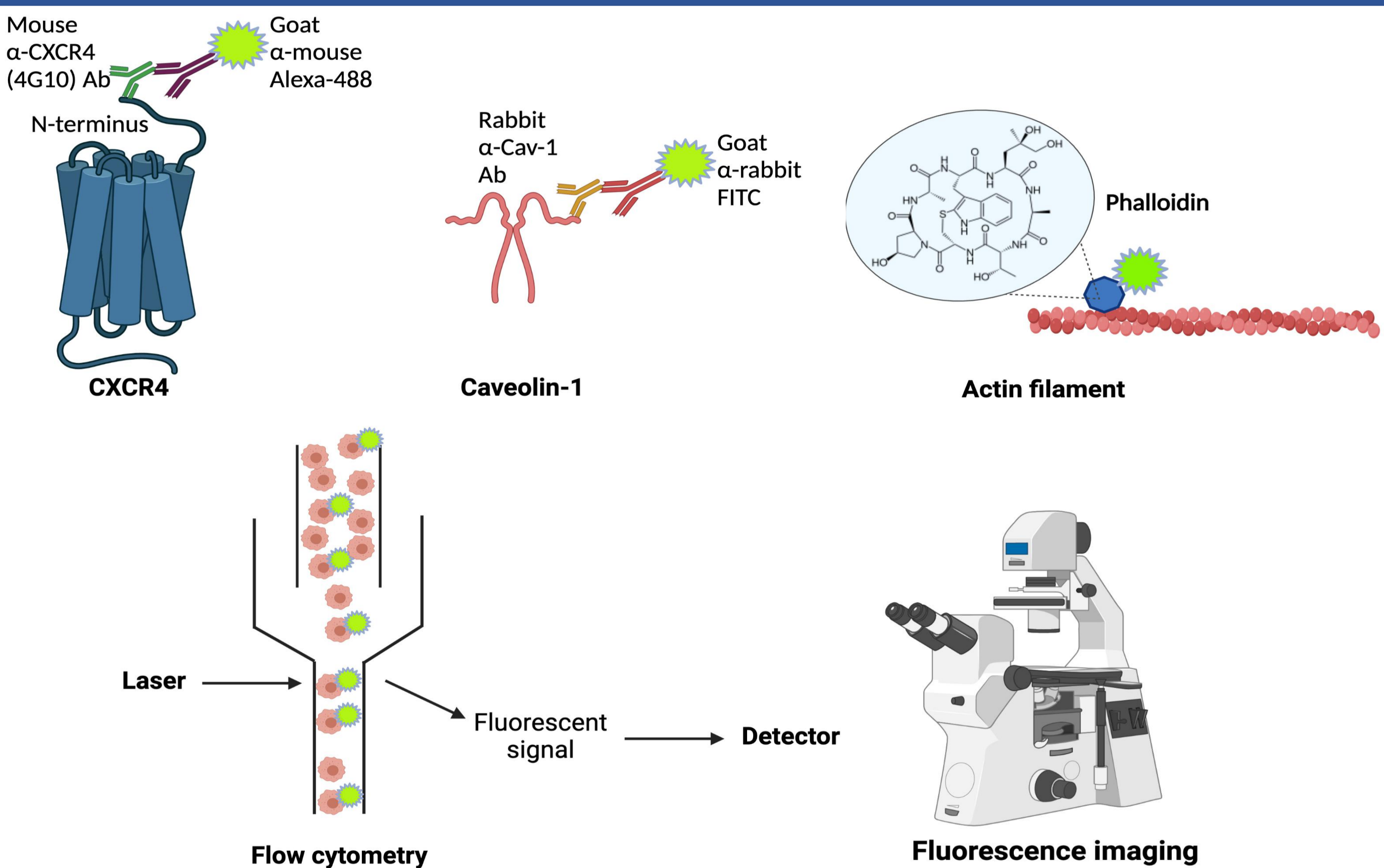
### II. Clathrin-dependent receptor trafficking



- CXCL12/CXCR4 signalling is crucial in regulating cancer metastasis.
- Downstream signalling upon receptor internalisation is a possibility to tune responsiveness to ligands for the development of precision medicine.

**Aim:** To investigate the downstream roles of protein kinase D (PKD) in CXCL12/CXCR4 signalling using breast cancer MCF-7 cells and leukaemic Jurkat cells.

## Results



### MCF-7 and Jurkat cells express CXCR4 and caveolin-1.

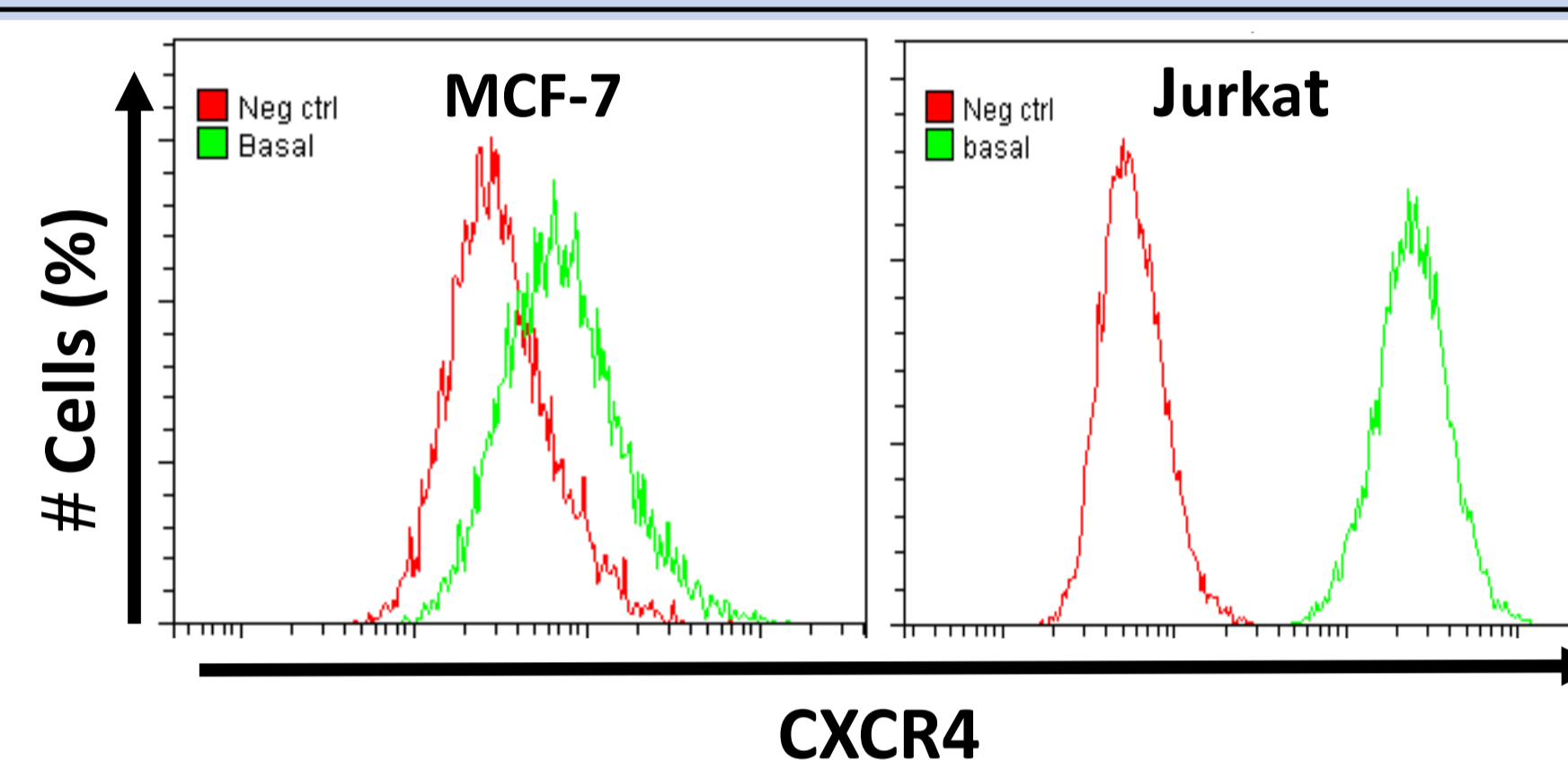


Figure 1: Expression of CXCR4 receptors in MCF-7 and Jurkat cells by flow cytometry

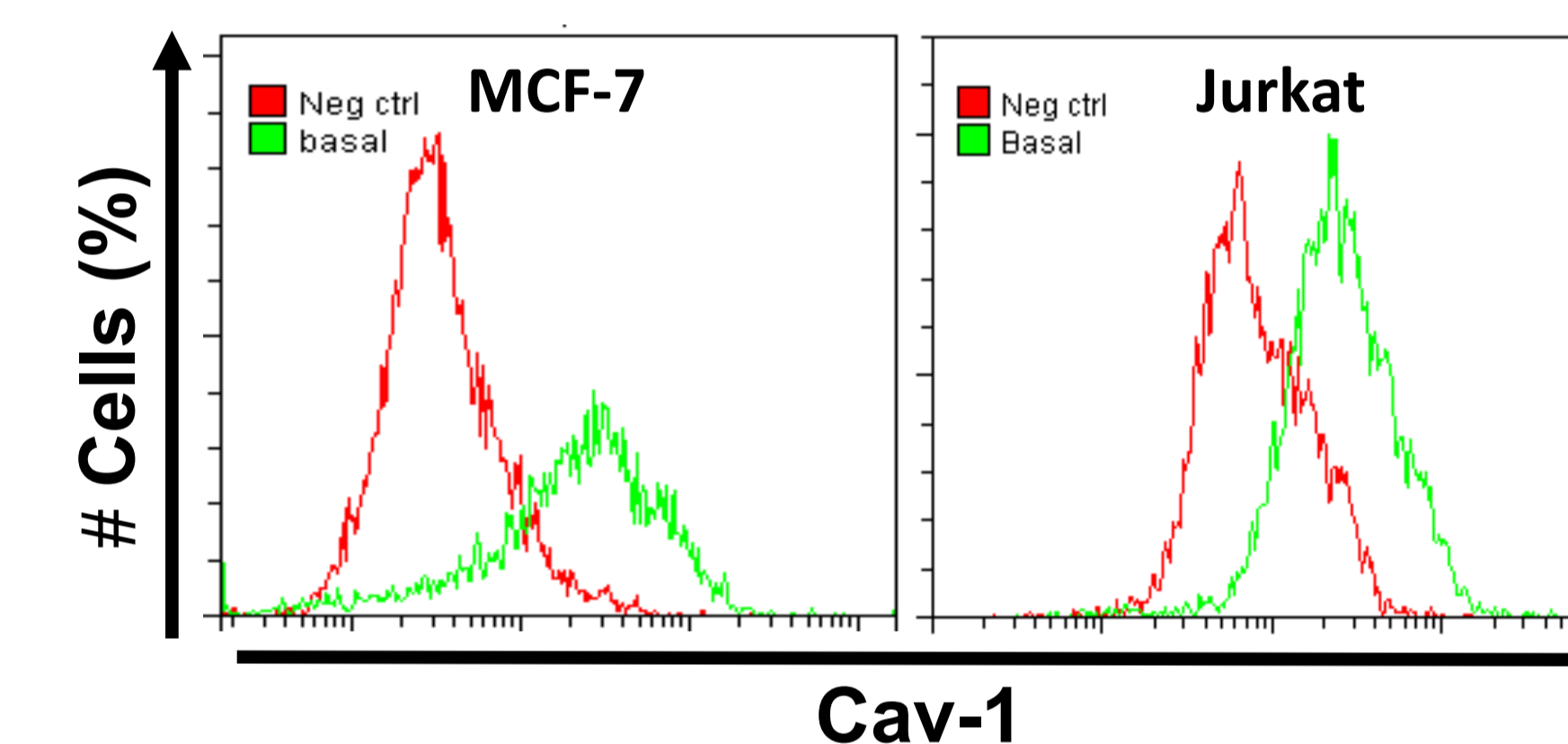


Figure 2: Expression of caveolin-1 (Cav-1) in MCF-7 and Jurkat cells by flow cytometry

**PKD inhibitors do not block CXCR4 internalization in both cell lines. Cholesterol-removal agents inhibit CXCR4 internalisation in Jurkat cells, but not in MCF-7 cells.**

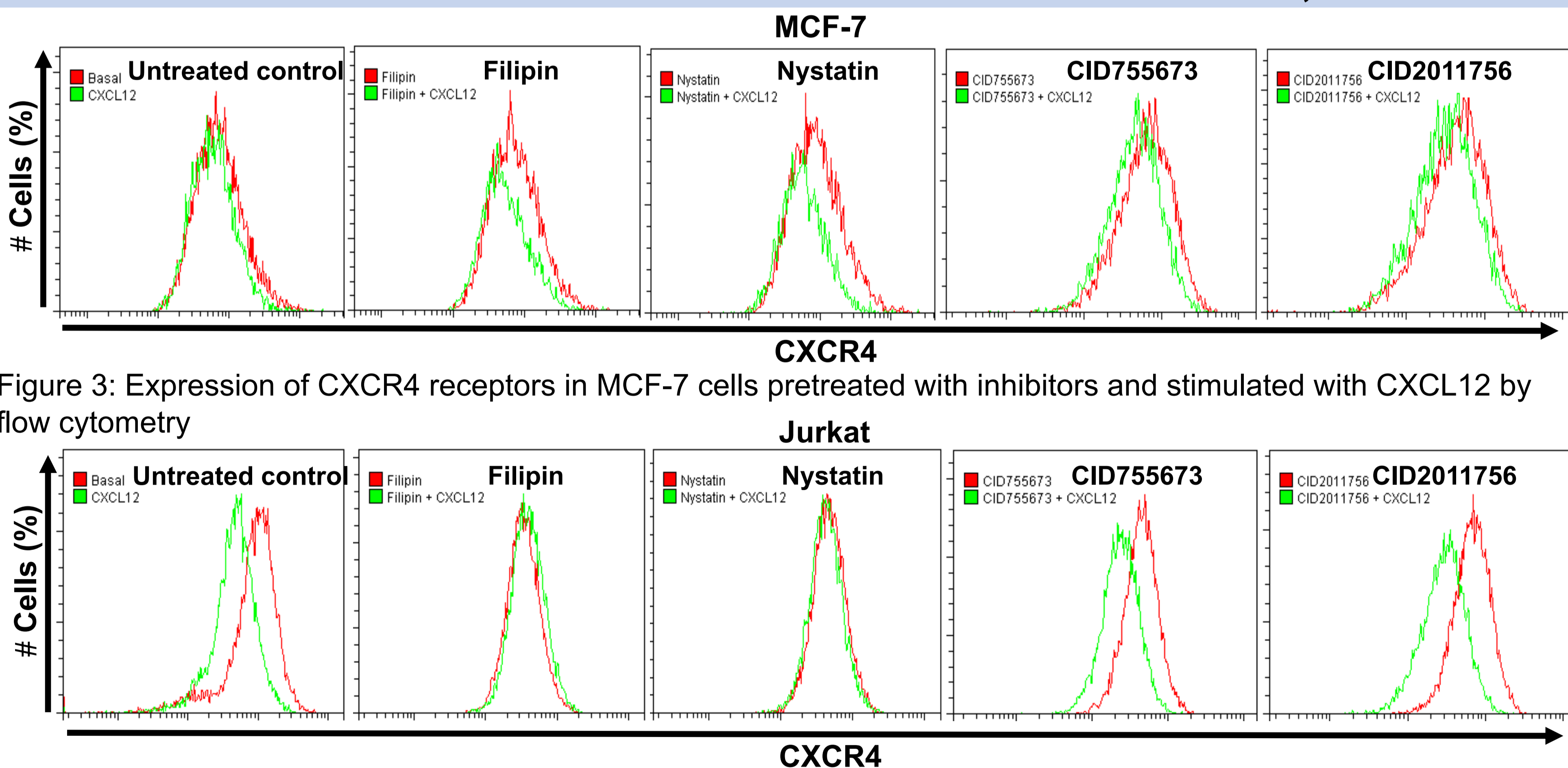


Figure 3: Expression of CXCR4 receptors in MCF-7 cells pretreated with inhibitors and stimulated with CXCL12 by flow cytometry

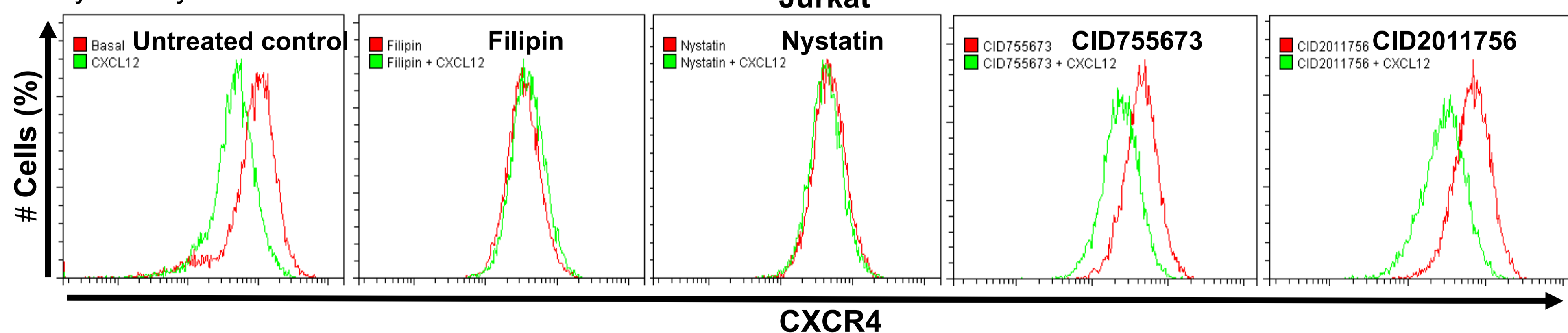


Figure 4: Expression of CXCR4 receptors in Jurkat cells treated with inhibitors and stimulated with CXCL12 by flow cytometry

Table 1: Quantitative CXCR4 surface expression on MCF-7 and Jurkat cells relative to unstimulated control

Treatment	Relative CXCR4 surface expression (%)	
	MCF-7 <sup>+</sup>	Jurkat <sup>++</sup>
Untreated control		
CXCL12	77.7	51.1
<b>Pretreated with inhibitors for 30 min</b>		
CID755673 (2.5 μM <sup>+</sup> ; 10 μM <sup>++</sup> )	73.5	56.9
CID2011756 (2.5 μM <sup>+</sup> ; 10 μM <sup>++</sup> )	75.4	57.9
Nystatin (50 μg/mL)	63.8	96.1 <sup>(****)</sup>
Filipin (5 μg/mL)	52.3	116.9 <sup>(****)</sup>

### PKD inhibitor reduces Cav-1 expression in Jurkat cells in CXCL12 stimulation

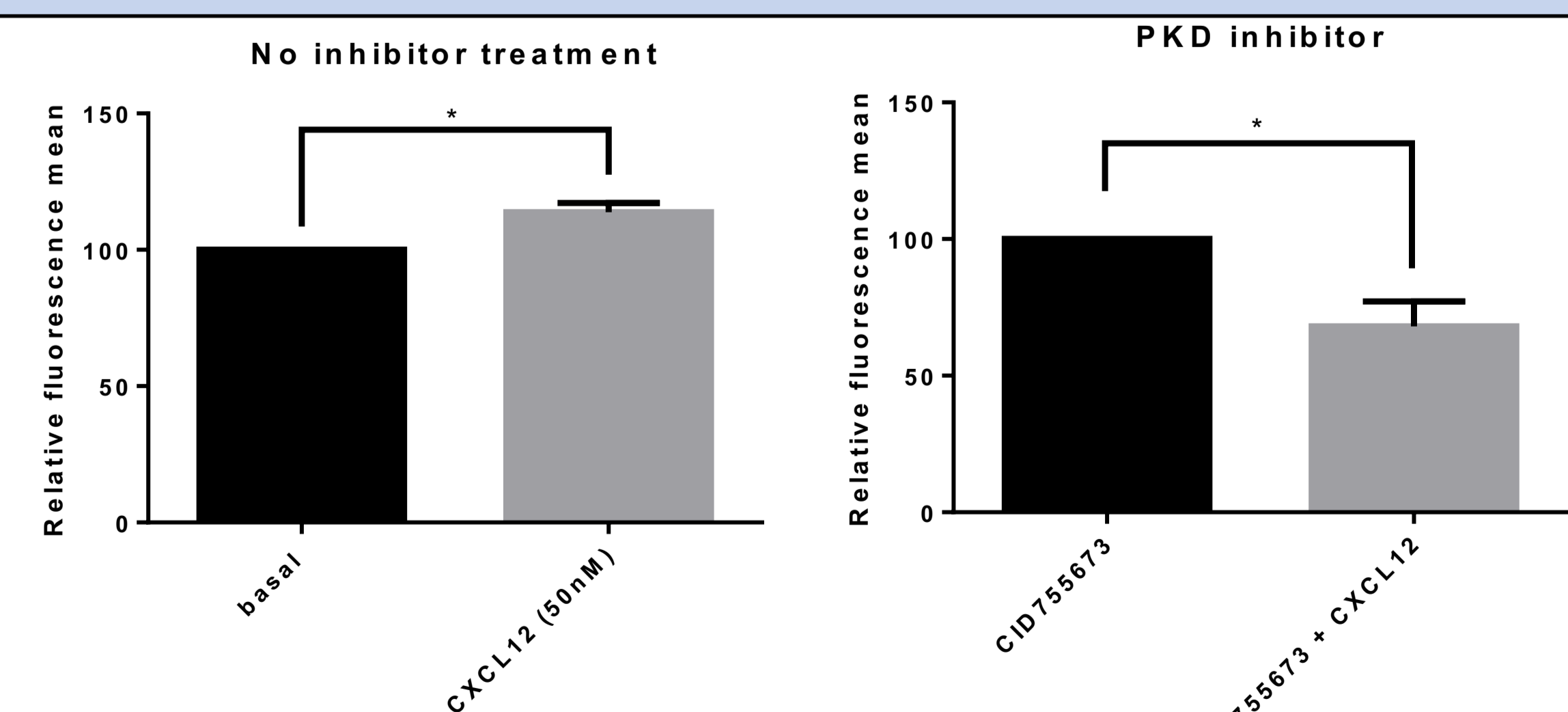


Figure 5: Cav-1 expression in Jurkat cells relative to unstimulated

### PKD inhibitors induce stress fibre formation in MCF-7 cells.

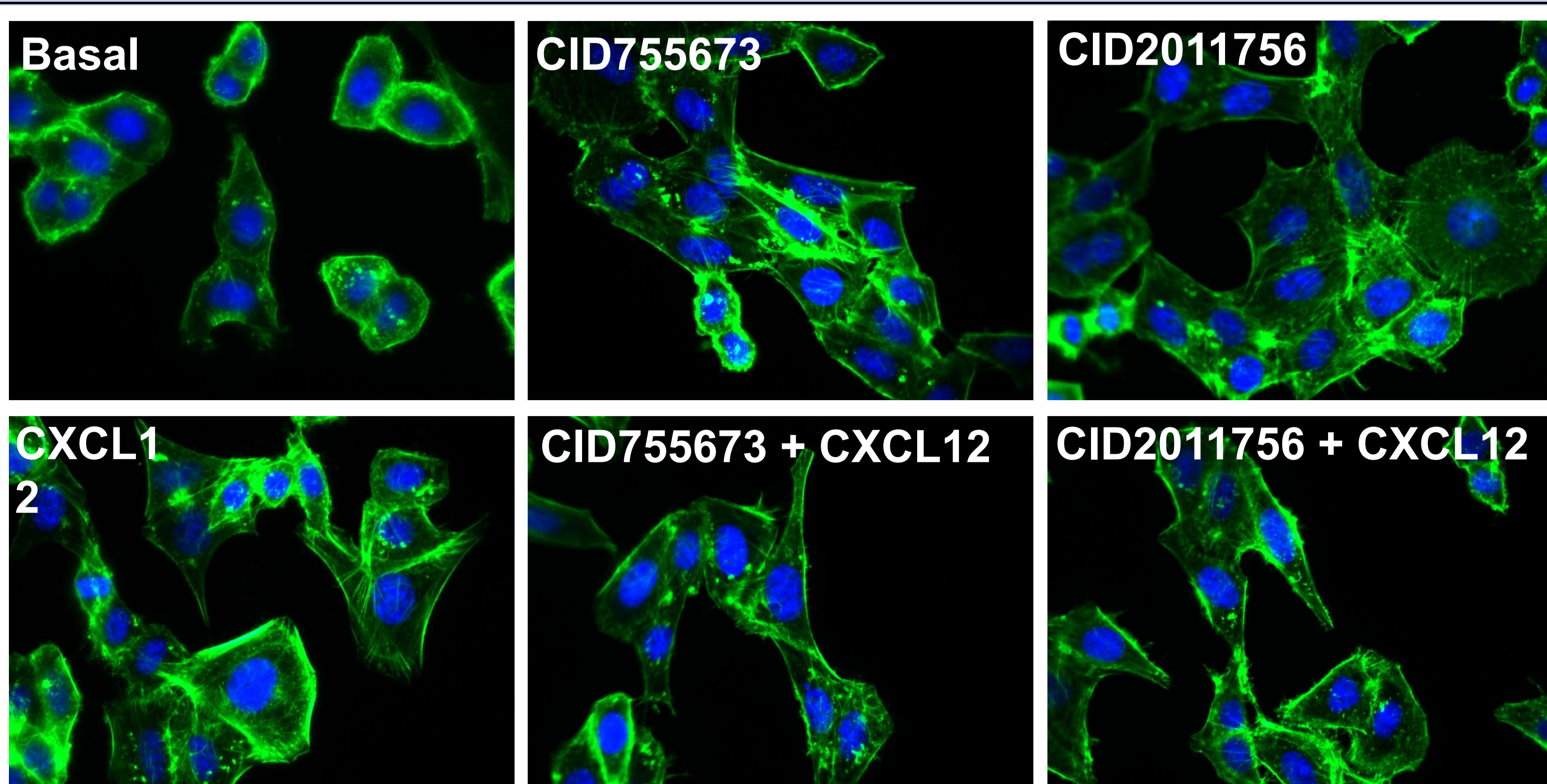


Figure 6: Actin cytoskeleton in MCF-7 cells visualised by Phalloidin stain (in green), cell nuclei (in blue)

## Conclusion

- PKD has **no direct effect** on CXCR4 internalisation.
- CXCR4 internalisation in Jurkat is **caveolin-dependent**.
- PKD might **regulate Cav-1 expression** during CXCR4 trafficking in Jurkat cells.
- PKD possess **inhibitory effects on cell motility** by regulating actin polymerisation in MCF-7 cells.