



Ministry of Education and Training
Vietnam International Education Cooperation Department

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Role of LRP-1 in Colon Cancer Cell Proliferation in 3D Culture Systems

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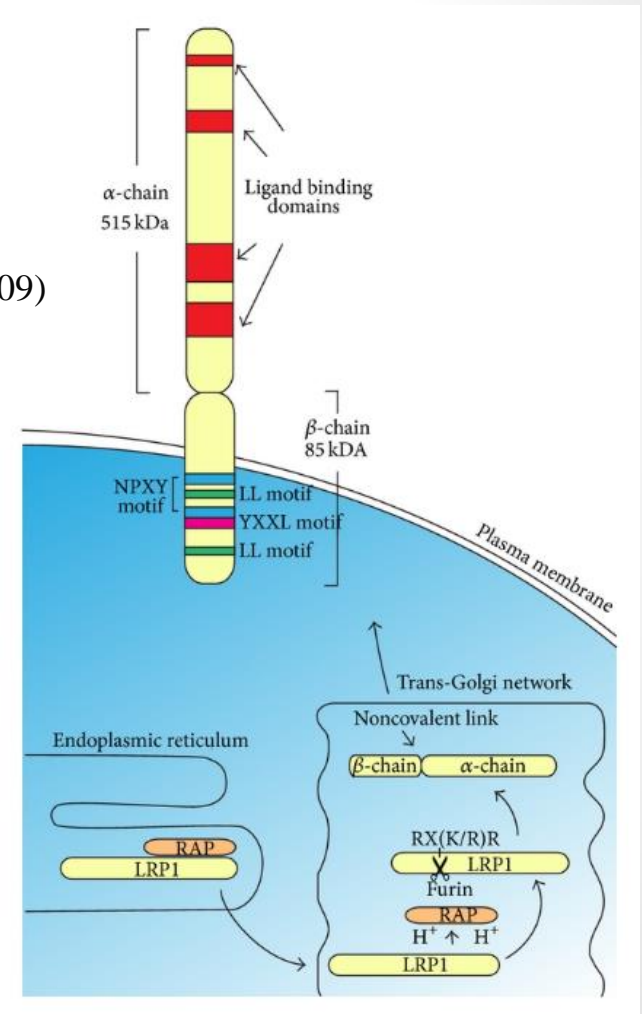
❖ Multifunctional endocytosis receptor

- Cell mobility (Xing *et al.*, 2016)
- Metabolism (Liu *et al.*, 2015; Risheng *et al.*, 2018)
- MAPK, AKT, ERK, JNK,..., (Roura *et al.*, 2014; Fuentealba *et al.*, 2009)

❖ Roles in various diseases

- Cancers (Leslie *et al.*, 2018, Boulagnon-Rombi *et al.*, 2018)
- Alzheimer,..., (Kanekiyo *et al.*, 2014; Shinohara *et al.*, 2017)

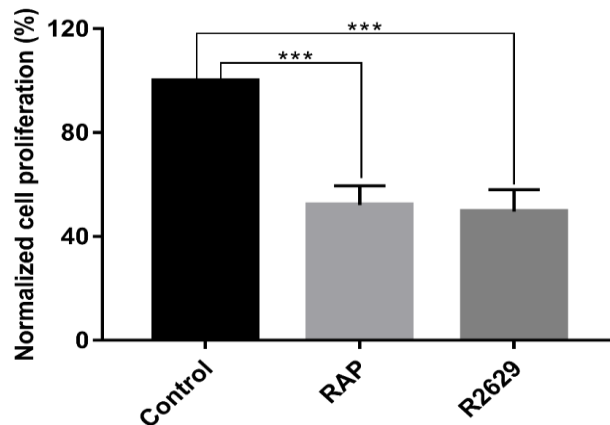
➔ *Effect on colon cancer cell proliferation ?*



Auderset *et al.*, 2016

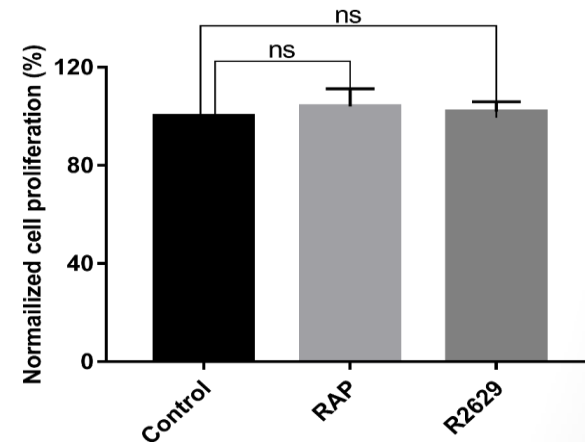
- **LS174T** cells in 3D type I collagen matrix or in 2D type I collagen coating.
- Treated by **RAP** (antagonist of LRP-1) or **R2629** (antibody against LRP-1)
- 5 days of treatment

Cell proliferation in 3D



***: $P = 0.0002$, $n=3$, one-way ANOVA using Dunnett's multiple comparisons test

Cell proliferation in 2D

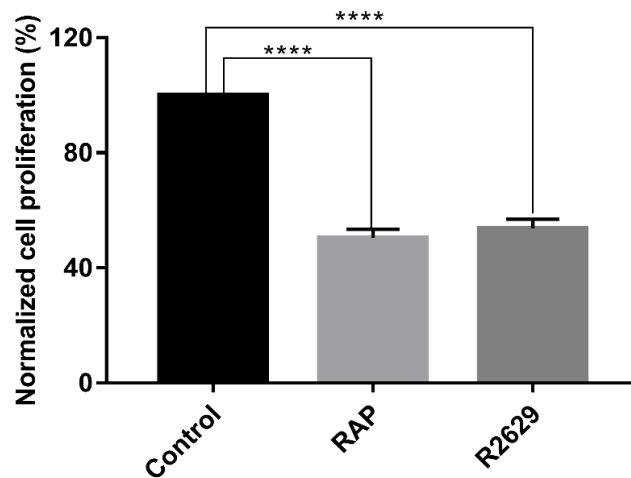


ns: not significant, $n=3$, one-way ANOVA using Dunnett's multiple comparisons test

LRP-1 could be involved in LS174T cell proliferation but only in 3D

- **HT-29** cells in 3D type I collagen matrix
- Treated by **RAP** (antagonist of LRP-1) or **R2629** (antibody against LRP-1)
- 5 days of treatment

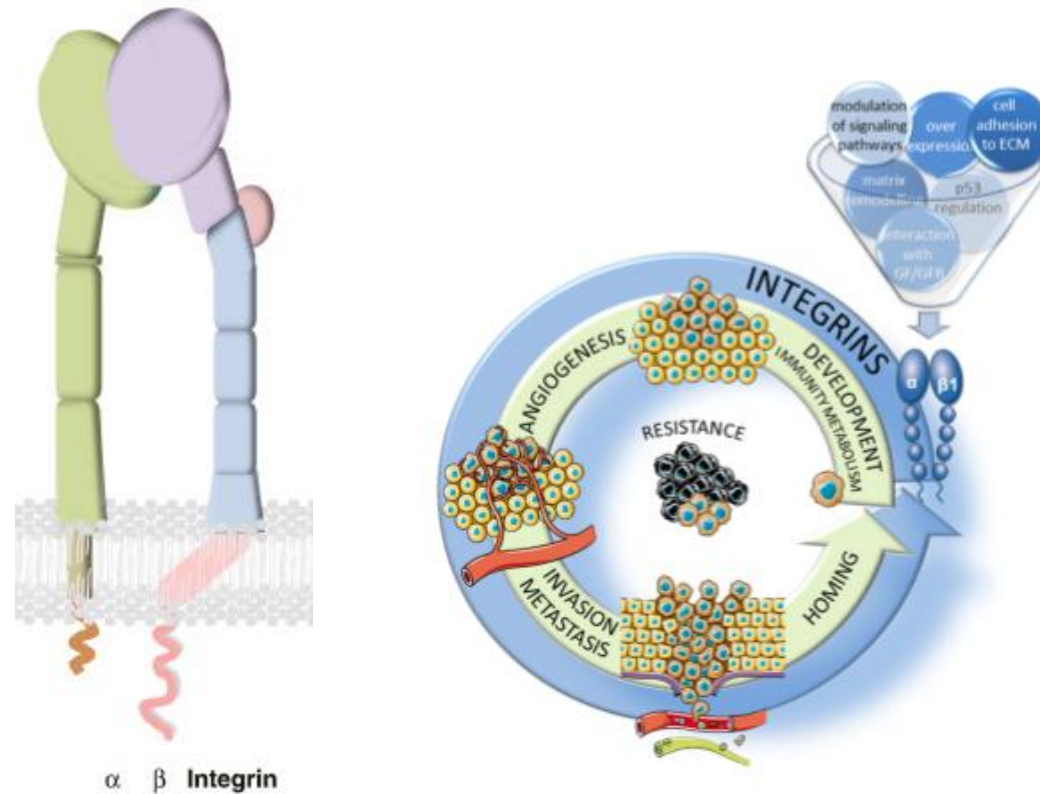
Cell proliferation in 3D



****: $P = 0.0001$, $n=3$, one-way ANOVA using Dunnett's multiple comparisons test

➔ LRP-1 has a positive effect on colon cancer cell proliferation in 3D only

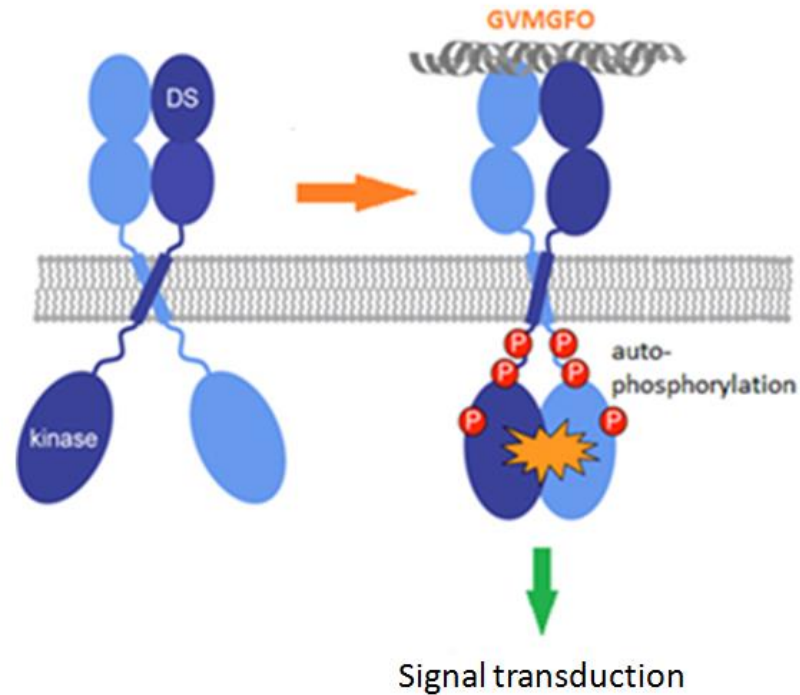
- The most studied receptors, 24 members
- 4 heterodimers recognize type I collagen: integrin $\alpha 1\beta 1$; $\alpha 2\beta 1$; $\alpha 10\beta 1$; $\alpha 11\beta 1$
- GFOGER motif



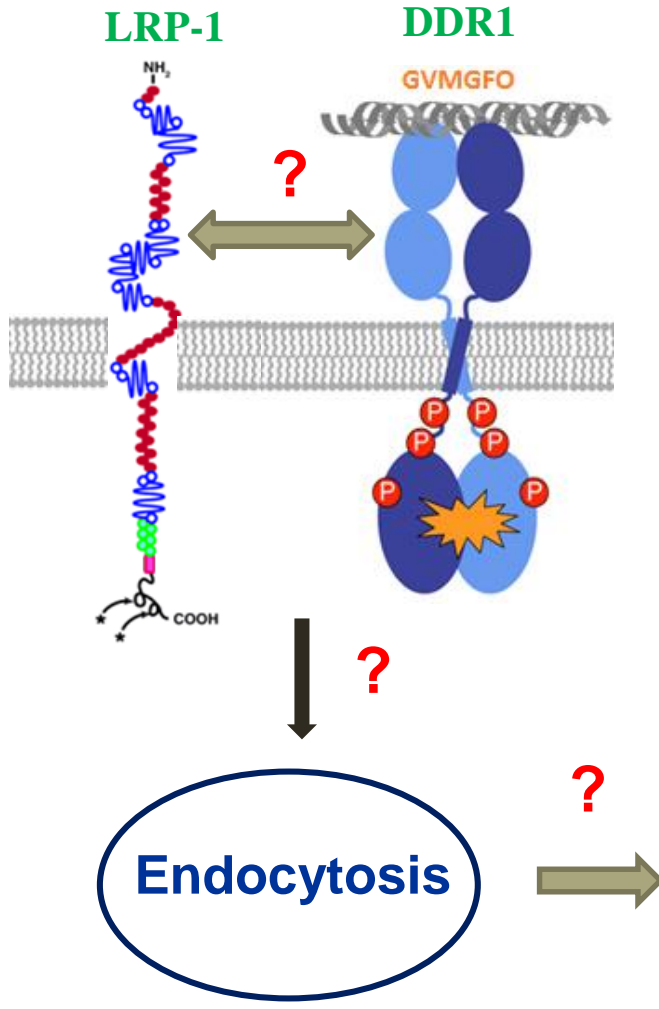
Humphries et al., 2006

Blandin et al., 2015

- ✓ Receptors with kinase activity domain
- ✓ Activated by fibrillar type I collagen
- ✓ GVMGFO motif: type I, II, III collagens
- ✓ Late and prolonged activation (2h – 18h)

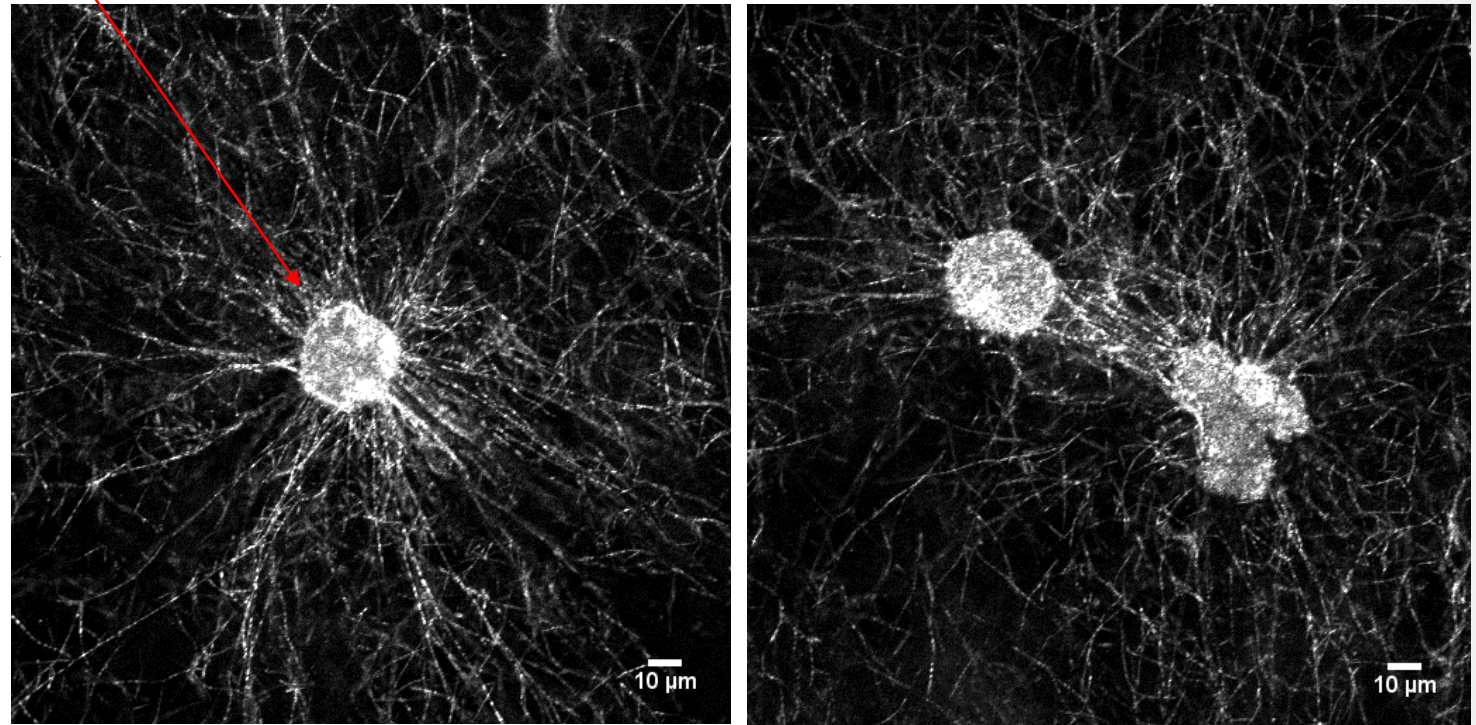


→ **DDR1 down-regulates cell proliferation in 3D collagen matrix**



- ✓ Regulation of DDR1 expression at the cell surface.
- ✓ Regulation of cell proliferation.

Colon cancer cells \pm *LRP-1* inhibitors/antagonists



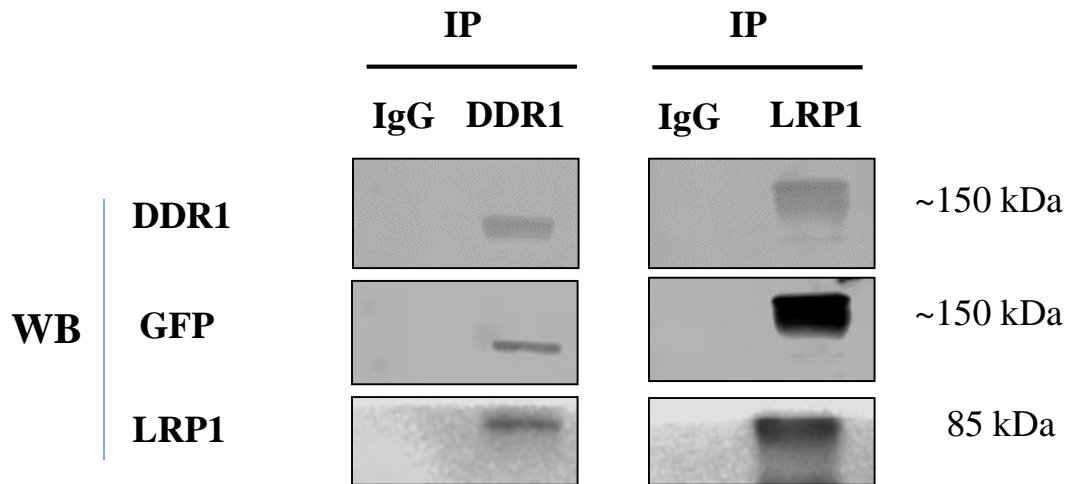
Cell proliferation

Cell cycle

Interaction between
LRP-1 and DDR1

DDR1 involvement

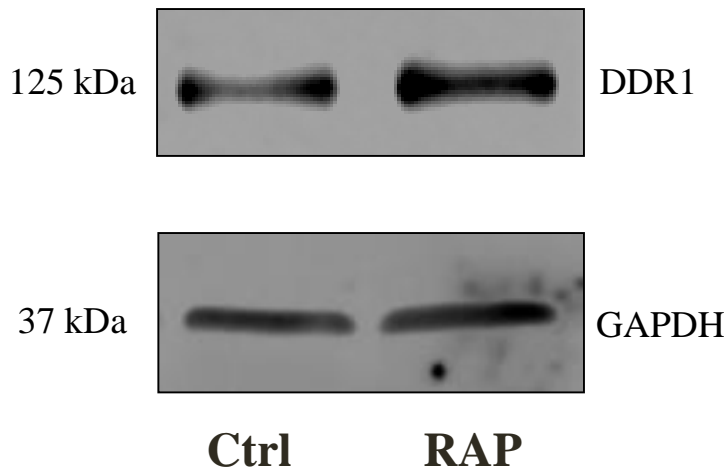
Co-immunoprecipitation between LRP-1 and DDR1 from **HT-29^{DDR1-GFP}** extract



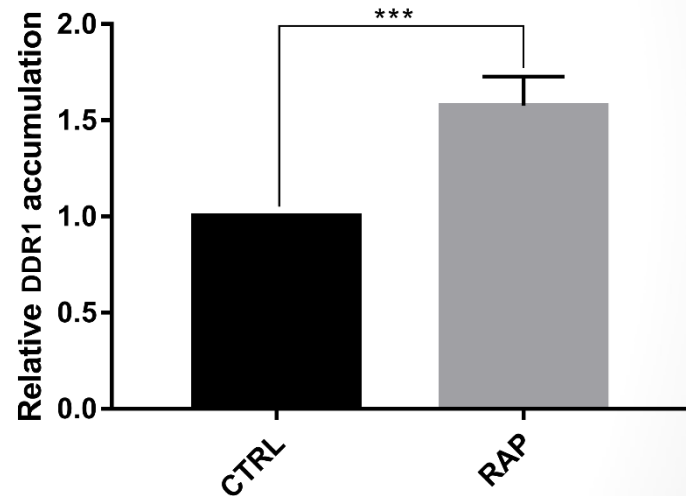
→ LRP-1 and DDR1 bind to each other

- LS174T cells
- Treated with RAP for 60 min
- Incubated with Sulfo-NHS-Biotin for 30 min, 4°C

DDR1 expression



Ctrl: Control for 60 min RAP treatment
RAP: 60 min-RAP treatment

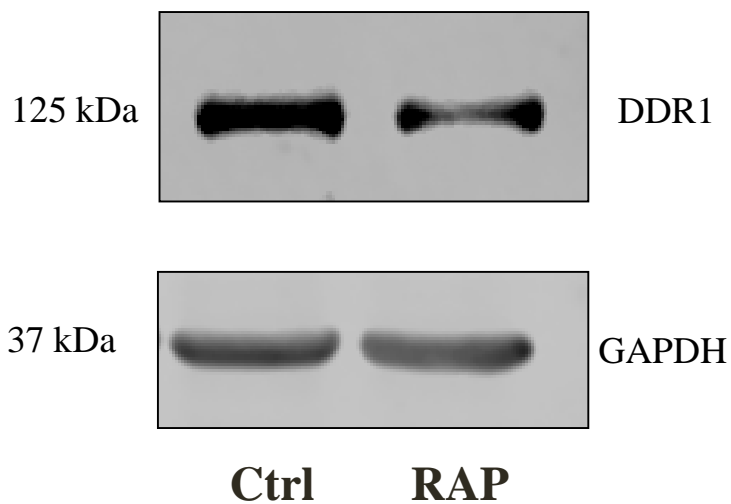


Mean \pm SD; ***: $P = 0.0006$,
 two samples t-test

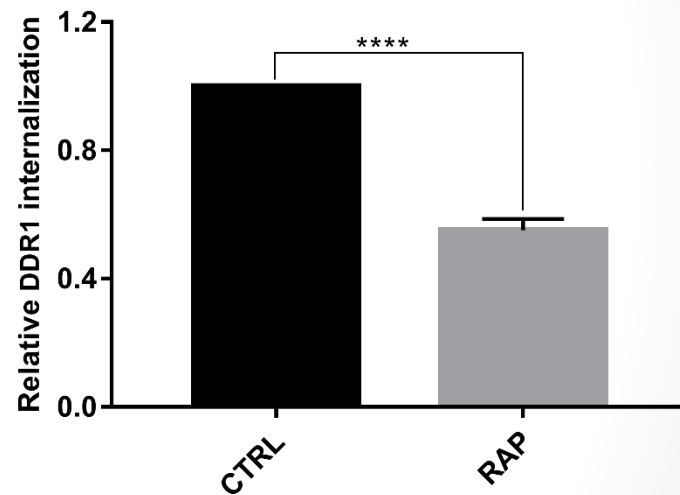
DDR1 is more expressed at cell membrane upon LRP-1 inhibition

- LS174T cells
- Treated with RAP for 60 min, incubated with Sulfo-NHS-biotin for 30 min, 4°C
- Endocytosis for 60 min

Endocytosis test



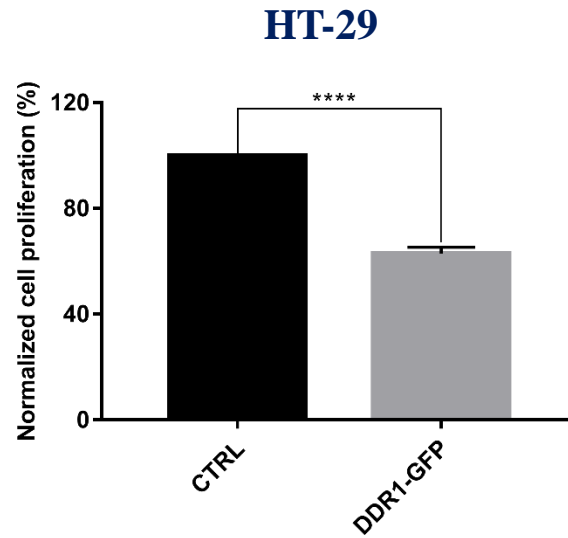
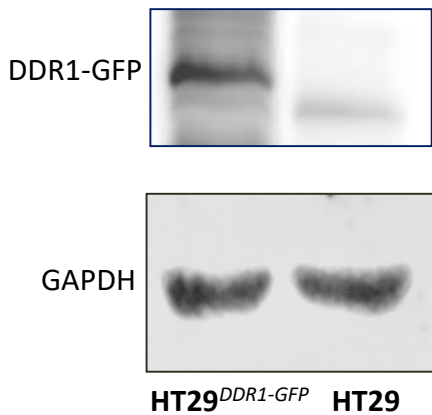
Ctrl: Control for 60 min RAP treatment
RAP: 60 min-RAP treatment



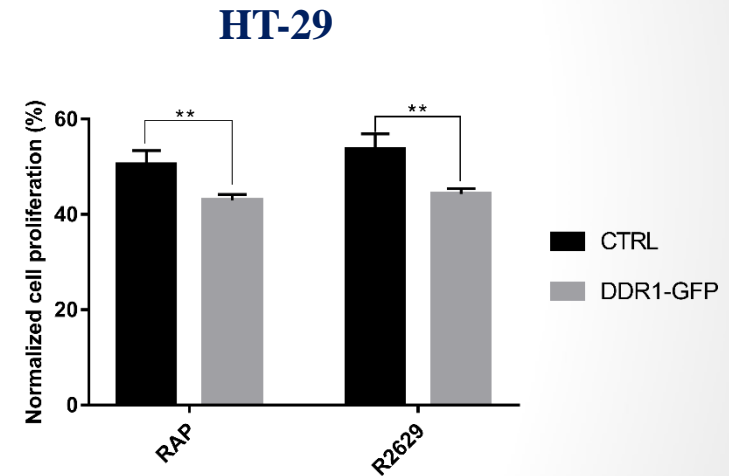
****: $P < 0.0001$, $n=3$, two sample t-test

→ DDR1 is internalized into cytoplasm by LRP-1

- Cells in 3D type I collagen matrix
- Treated by **RAP** (antagonist of LRP-1) or **R2629** (antibody against LRP-1)
- 5 days of treatment



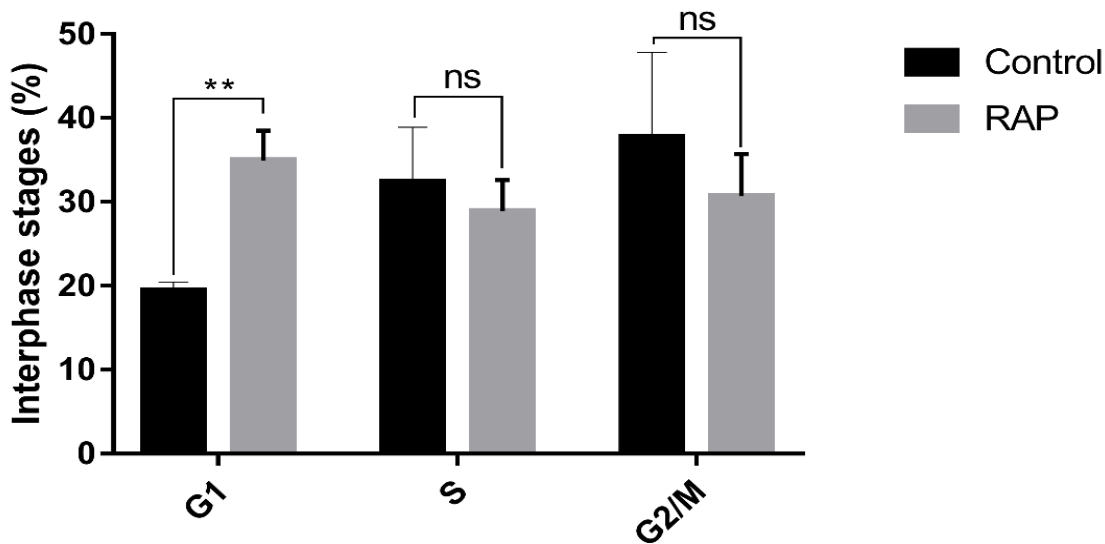
****: $P < 0.0001$, $n=3$, unpaired t-test



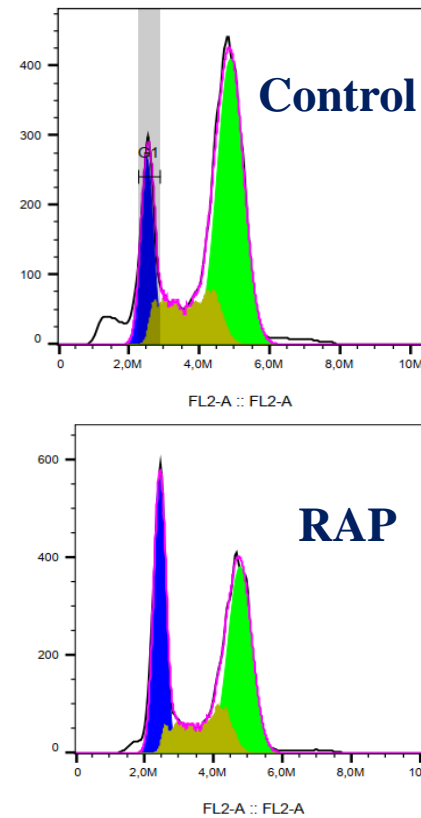
** : $P < 0.01$, $n = 3$, two way ANOVA test.

→ Overexpress DDR1-GFP causes an increase in efficiency of RAP and R2629 treatment

- HT-29 cells were synchronized by double thymidine blocking
- Synchronized cells were cultured in 3D for 24 hours with/without RAP treatment.

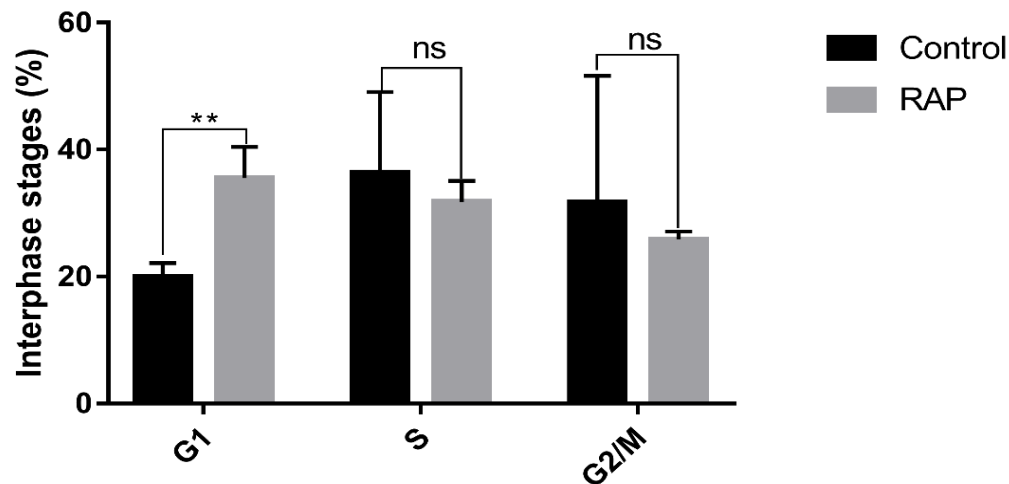


Mean \pm SD; **: $P < 0.005$, two sample t-test.

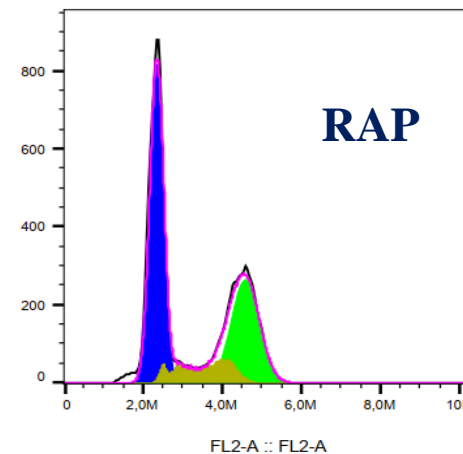
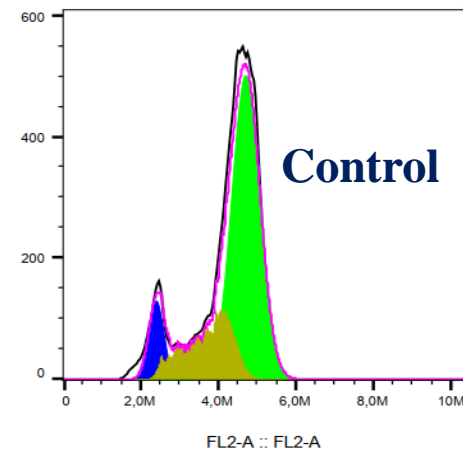


RAP treatment results in cell cycle arrest at late G1 phase

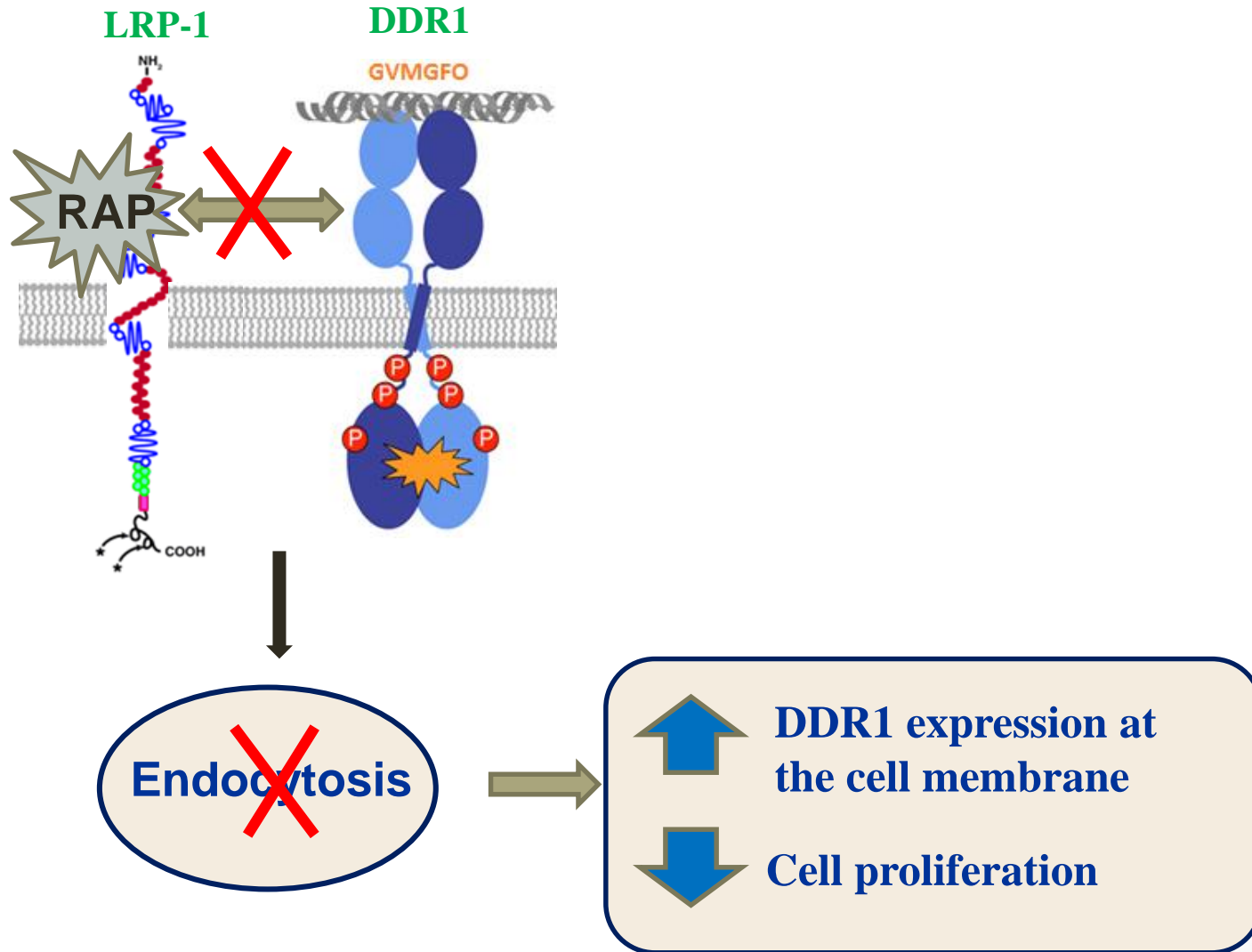
- HT-29^{DDRI-GFP} cells were synchronized by double thymidine blocking
- Synchronized cells were cultured in 3D for 24 hours with/without RAP treatment.



Mean \pm SD; **: $P < 0.005$, two sample t-test.



RAP treatment results in cell cycle arrest at late G1 phase



Acknowledgements

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