SUPPLEMENTARY INFORMATION

Dissecting the novel molecular interactions of solute carrier family 4 member 4 (SLC4A4) for prostate cancer (PCa) progression

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Supplementary Figure Legends

Figure S1. Western blot analysis of SLC4A4 in normal prostate epithelial cells (RWPE-1) and different prostate cancer (PCa) cells: PC-3, DU-145, 22RV1, and α tubulin as an internal loading control (lower panel). RWPE-1 normal prostate epithelial cells were maintained in Keratinocyte Serum Free Medium supplemented with bovine pituitary extract (BPE) and human recombinant epidermal growth factor (EGF) and 1% antibiotic-antimycotic (Gibco USA, Catalog # 15240062) in a 5% CO2 humidified atmosphere at 37°C. PC3 and DU145 cells were maintained in F12Kand EMEM respectively supplemented with 10% heat-inactivated fetal bovine serum and 1% antibiotic-antimycotic. All cells were maintained in a 5% CO2 humidified atmosphere at 37°C.

Fig. S1 (Original immunoblots)

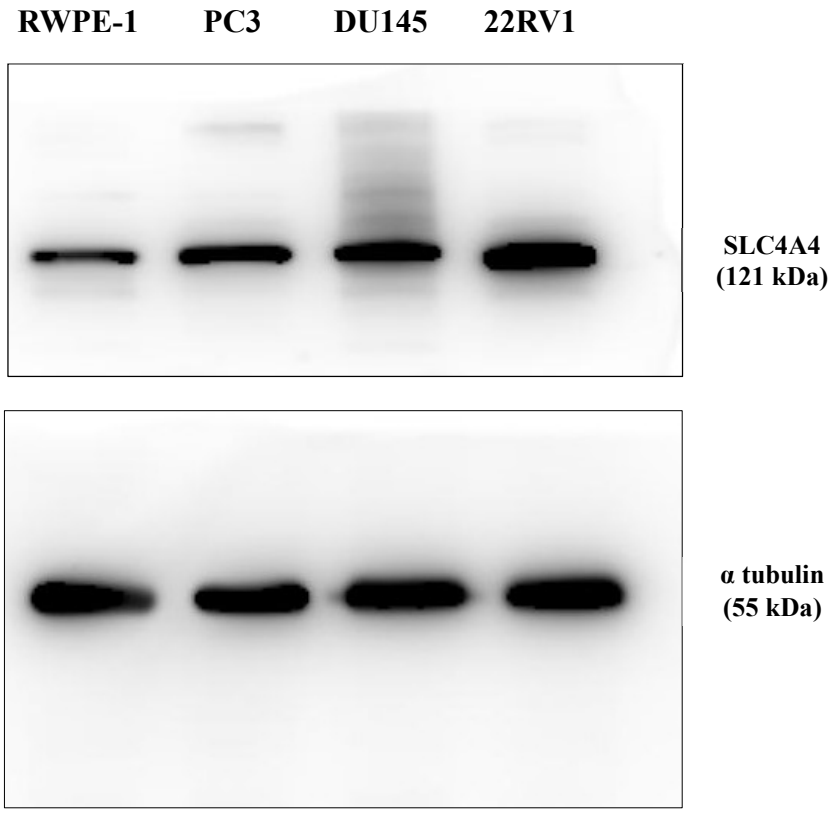
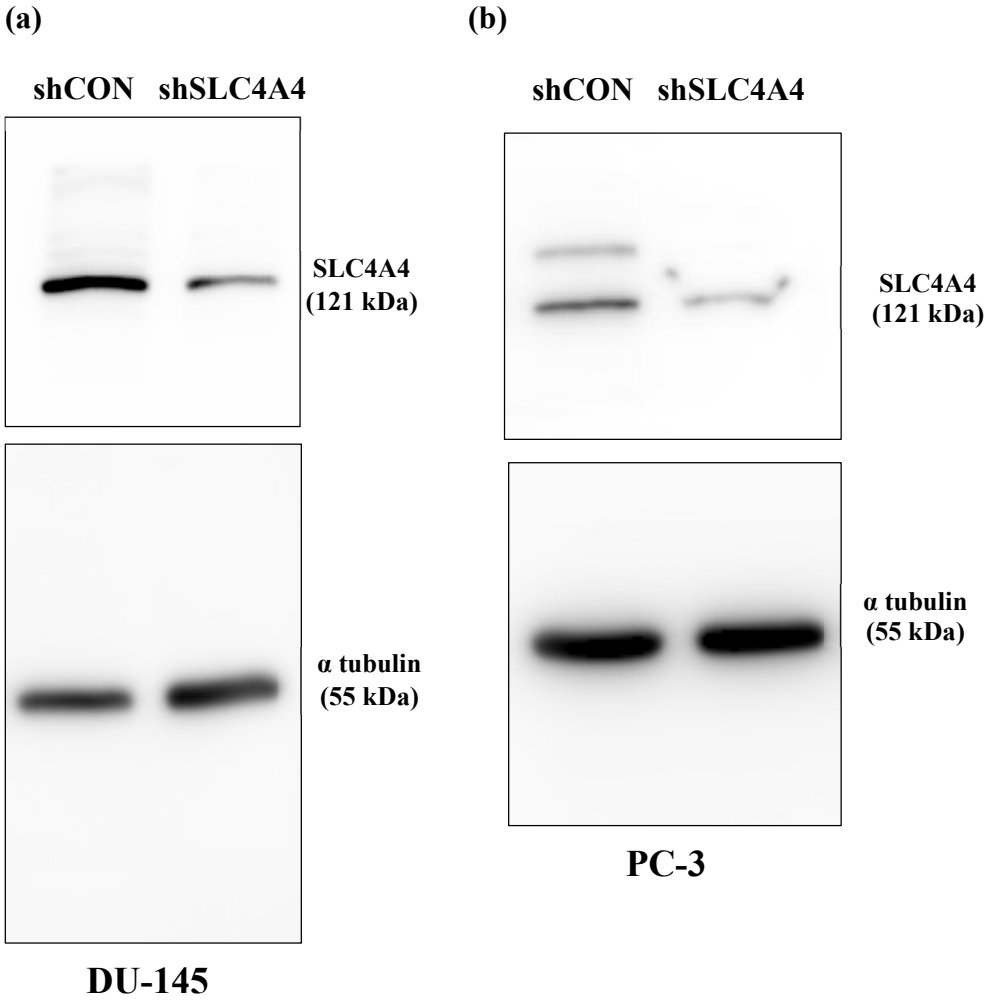


Figure S2. Western blot analysis for confirmation of SLC4A4 knockdown in Prostate Cancer (PCa) cells, and α tubulin as an internal loading control (lower panels)

Fig. S2 (Original immunoblots)



(c)

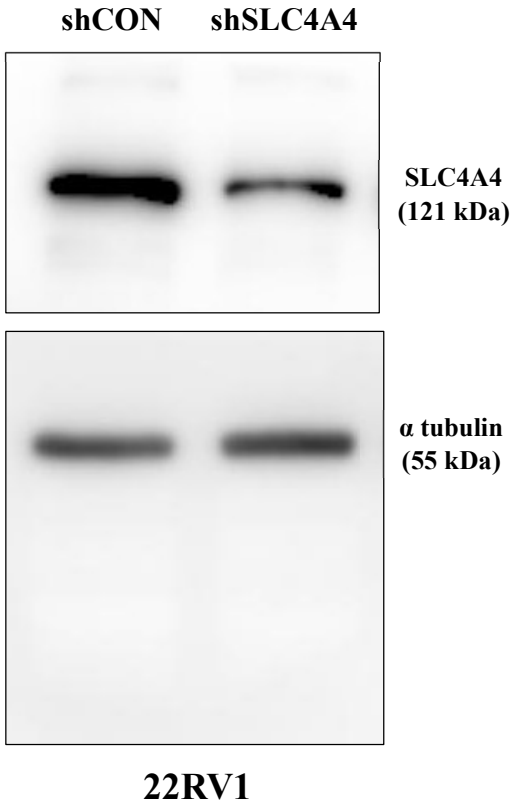


Figure S3. SLC4A4 knockdown effects Wnt/β-catenin signaling molecules in Prostate cancer (PCa) cells. (a) Immunoblots for Wnt/β-catenin signaling molecules after shSLC4A4 in PC-3 and DU-145 shows a decrease expression of β-catenin, meanwhile the level of GSK-3β was significantly increased which led to phosphorylation (S33/37) of β-catenin, and α tubulin as an internal loading control (lower panels) (b) Co-IP experiment showed that GSK-3β protein level started to increase to degrade endogenous β-catenin in shSLC4A4 PC3 cells compared to shCON

Fig. S3 (Original immunoblots)

(a)

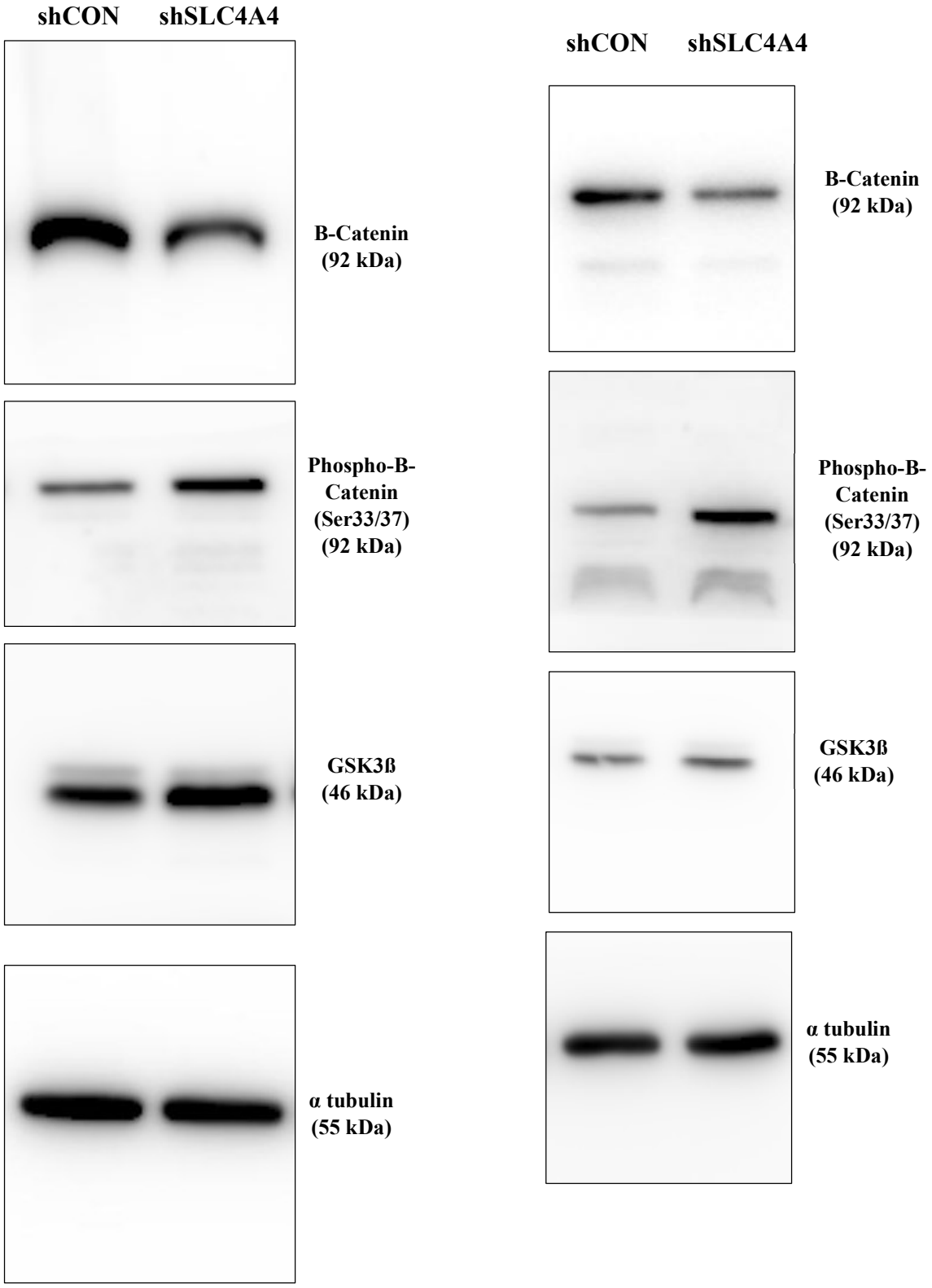


Fig. S3 (Original immunoblots)

(b)

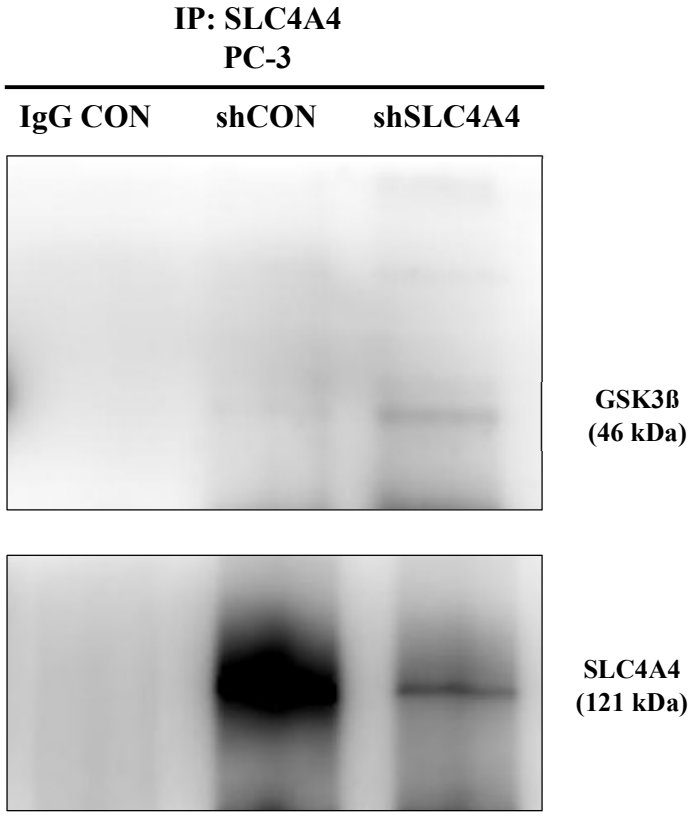
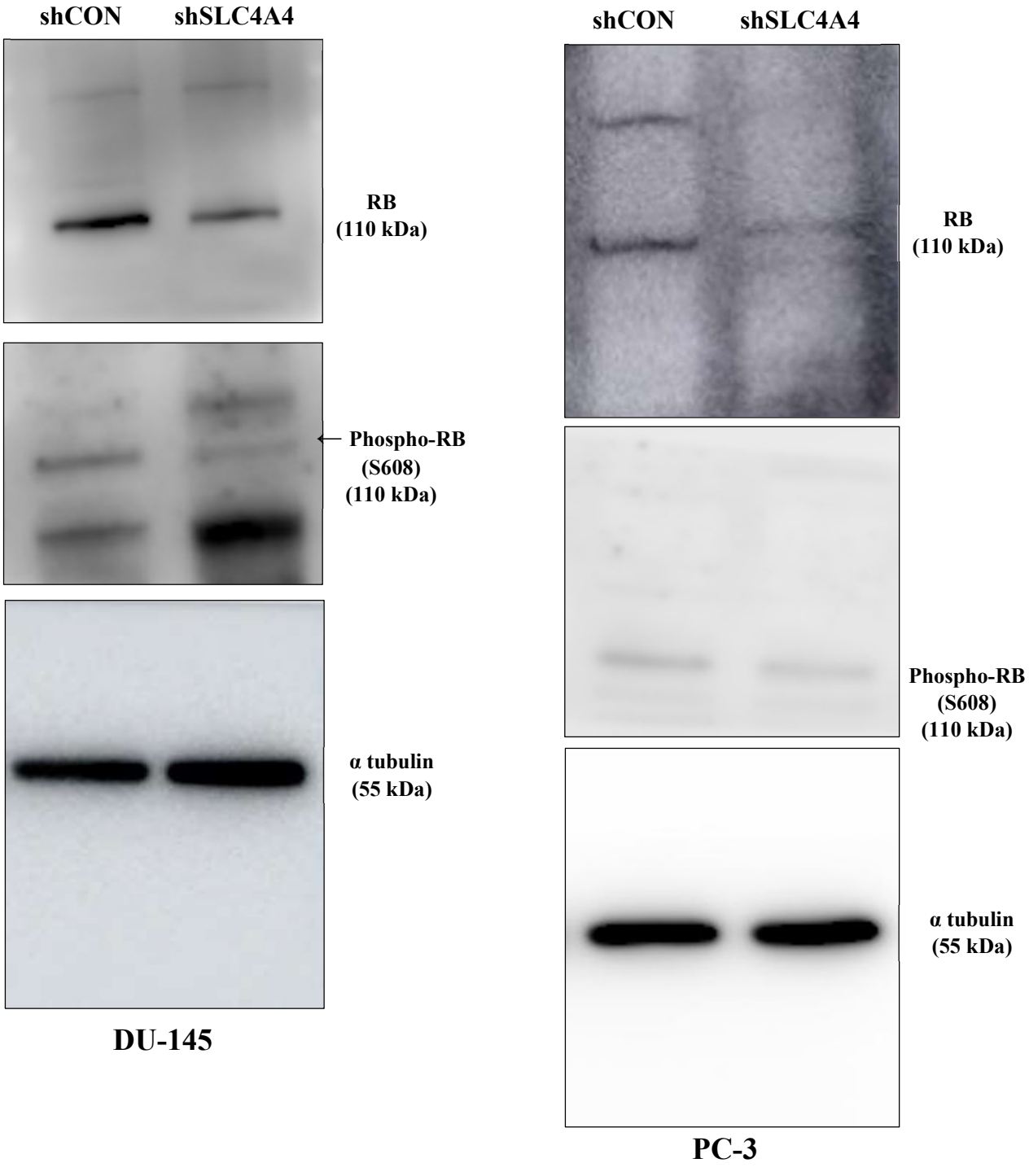


Figure S4. (a) Immunoblots for Wnt/β-catenin signaling molecules after shSLC4A4 in PC-3 and DU-145 shows a decrease expression of total RB expression and hypophosphorylation at serine 608 (S608) of RB, and α tubulin as internal loading control (lower panel) (b) Co-immunoprecipitation results show that SLC4A4 KD stable cells diminished total RB interaction with SLC4A4 protein compared to shCON

Fig. S4 (Original immunoblots)

(a)



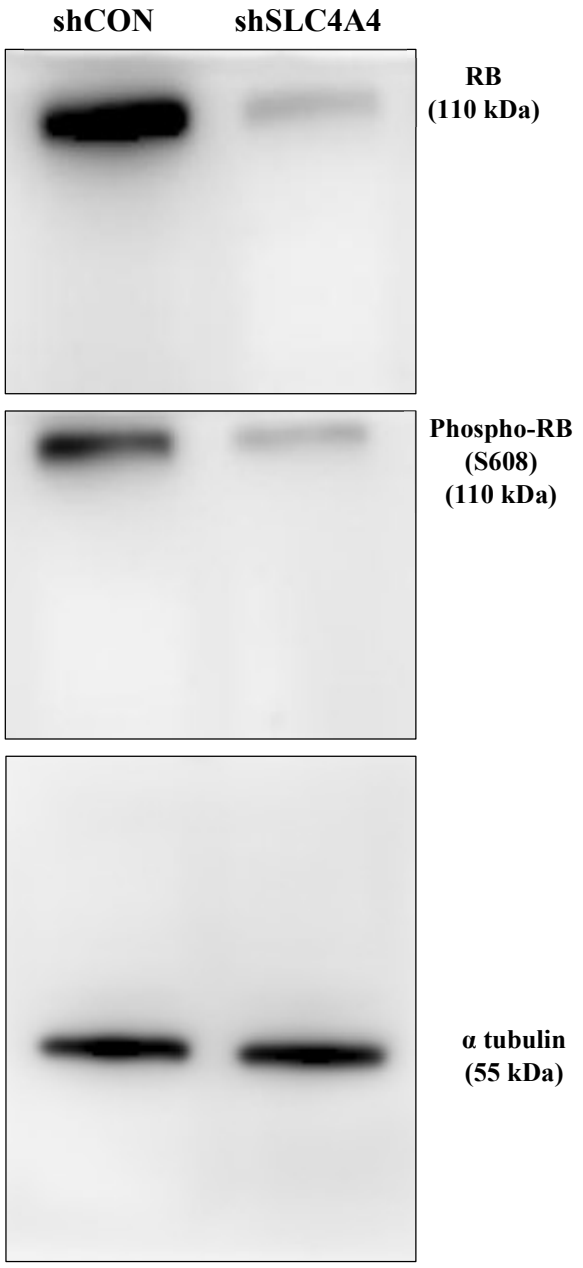

shCON
shSLC4A4
RB
(110 kDa)
Phospho-RB
(S608)
(110 kDa)
α tubulin
(55 kDa)
22RV-1

Fig. S4 (Original immunoblots)

(b)