Supplementary Figure 1

Schematic representation of Ad5-RV004 and Ad5-RV004.21 virus (1A)

Detection of p21shRNA sequence and Replication competent adenovirus (RCA) in the Ad5-RV004.21 preps: PCR amplification, using a set of primers specific to the H1 promoter and p21/Waf-1 region of shRNA were used to confirm the presence of p21/Waf-1 shRNA sequences in Ad5-RV004.21 preps along with controls. Hexon amplification was performed using same templates to confirm the virus DNA (1B). PCR amplification using set of primers against E1A promoter and E1A gene of adenovirus used to detect any RCA in Ad5-RV004.21 preps. Equal Pfu/ml (10^8 pfu/ml) of CN702 (wild type virus) and Ad5-RV004.21 were boiled at 94 C in PCR for 5 minutes. Two microliter s from each sample were used to amplify wild type E1A gene together with water to serve as a negative control (1C)

Supplemental Figure 2

Output to Input assay with CN702 Virus in p21/Waf-1 knockdown and intact C4-2 Cells.

Viral output of CN702 (MOI 1) from p21/Waf-1 knockdown and control C4-2 cell lines were tittered and reported as output to input ratios at 72 h PI. Data plots represent mean ± S.E of triplicate samples **(2A).**

Induction of ARE based promoter and enhancer by shRNA against GFP or p21/Waf-1 in C4-2 cells

Firefly luciferase assay was performed to study the induction of AREs based prostate specific enhance and rat probasin promoter (PSE/PBN) in C4-2 cells. Cells were tranfected with exogenous p21/Waf-1shRNA or an irrelevant control shRNA against GFP in C4-2 cells together with pCMV-RL (Renilla expressing plasmid). Firefly luciferase activity was normalized to Renilla luciferase expression and plotted as Fold luciferase expression. Data set represent mean ± S.E of the quadruplicate experiment **(2B)**

Supplemental Figure 3

Comparing the shRNA construct against Luciferase in two different regions of Adenovirus for functional knockdown.

Small hairpin RNA against Luciferase in the E1A region and fiber region of adenovirus driven by U6 promoter was compared for functional knockdown assay of the luciferase expression in C4-2 cells. C4-2 cells were co-infected with Ad5-TrackCMV-Luc (1 MOI) together with an Adenovirus that carries shRNA construct against luciferase after the Fiber region (AdTrack-Fex-shRNA-Luc) or in E1 region (Ad-Track-shRNALuc) of the virus at

10 MOI for 48 h. All of the infection assays were performed in sextuplicate. The luciferase expression was plotted as percent RLU after normalizing to the adenoviral GFP expression. There was no significant difference in the knockdown between the two virus using student t-test ($p<0.05$).

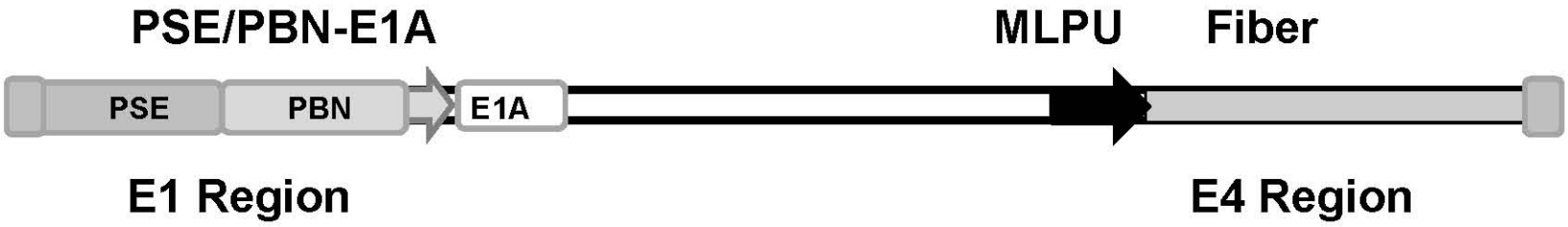
Supplemental Figure 4

Replication potency of non-armed Ad5-RV004 or p21/Waf-1 shRNA armed Ad5-RV004.21 virus in C4-2 or C4-2 p21/Waf-1 Knockdown cells.

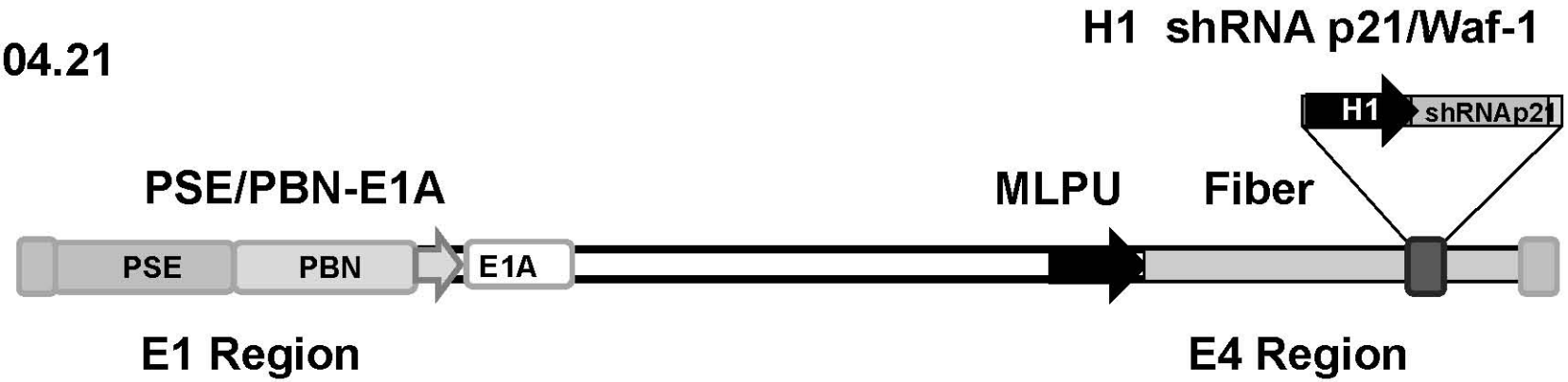
Viral output of Ad5-RV004 or Ad5-RV004.21 (MOI 1) from p21/Waf-1 knockdown and control C4-2 cell lines were tittered and reported as output to input ratios at 72 h PI. Data plots represent mean ± S.E of triplicate samples **(4).**

Supplementary Figure 1A

Ad5-RV004

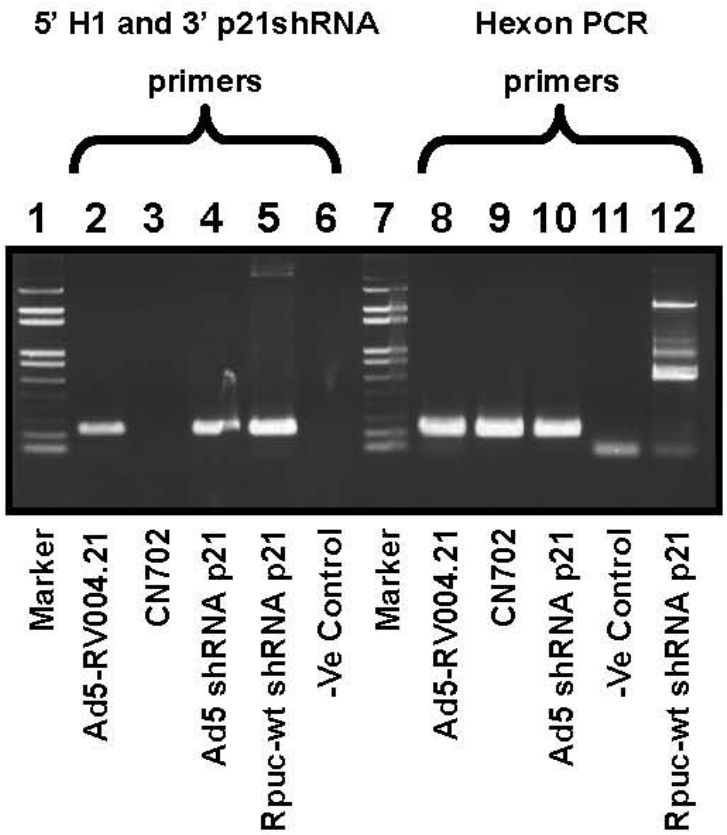


Ad5-RV004.21



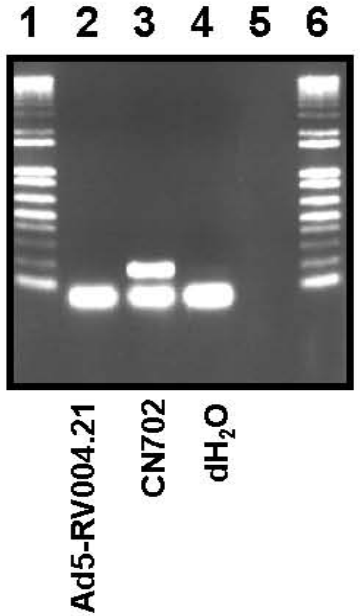
Supplementary Figure 1B

Detection of p21shRNA sequence in the Ad5-RV004.21 Prep

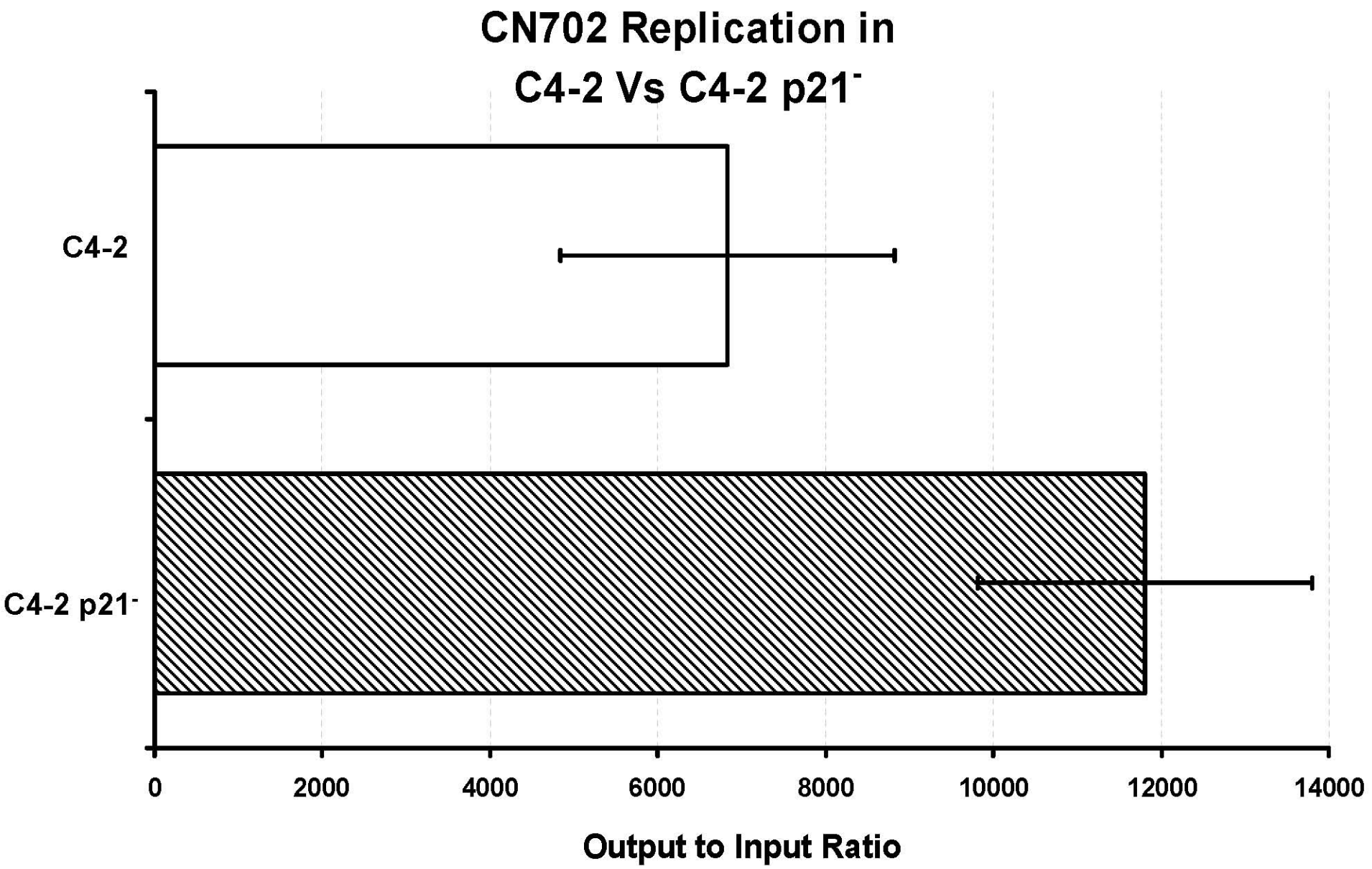


Supplementary Figure 1C

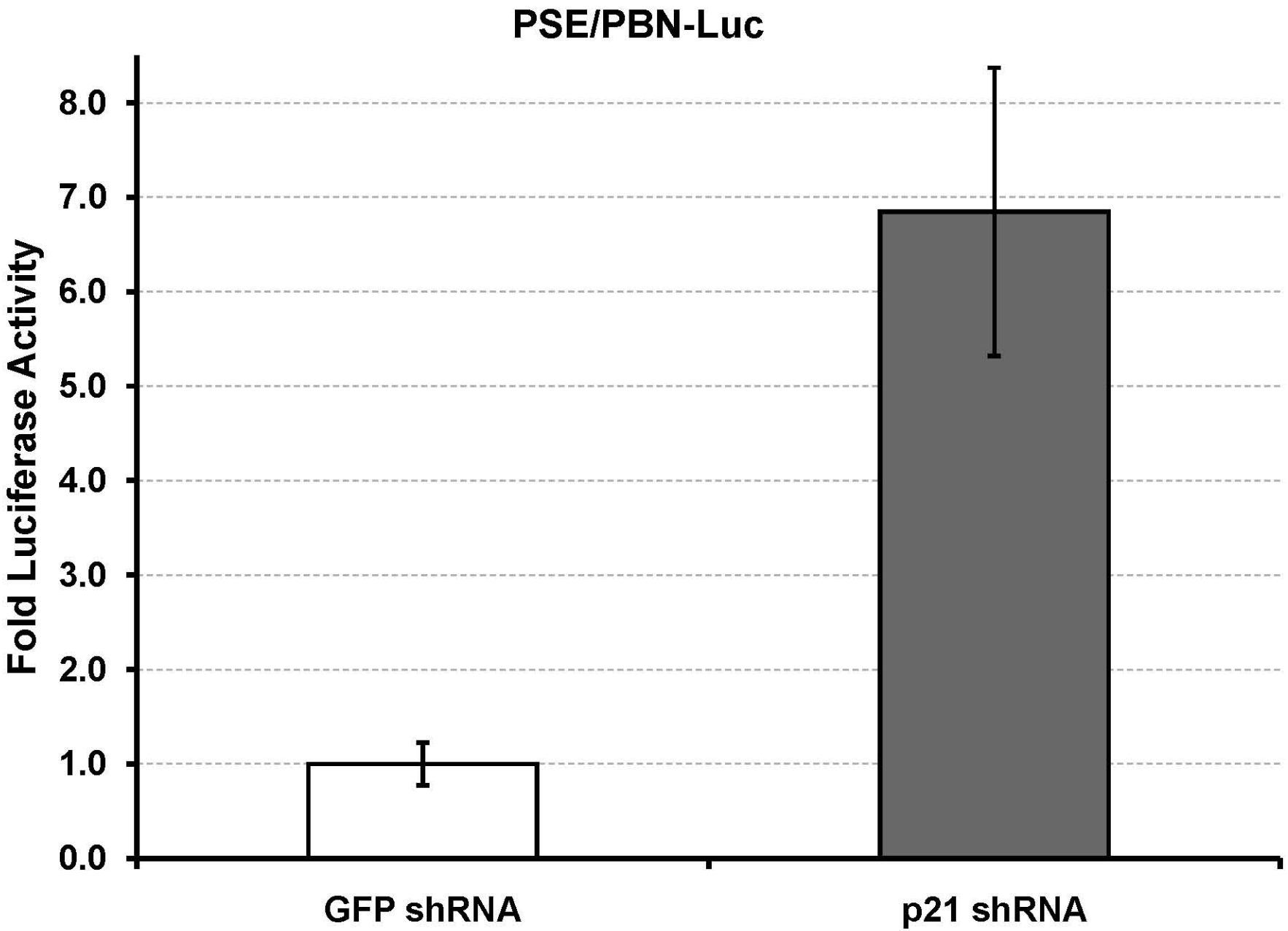
To Rule out RCA in the Ad5-RV004.21 Preps



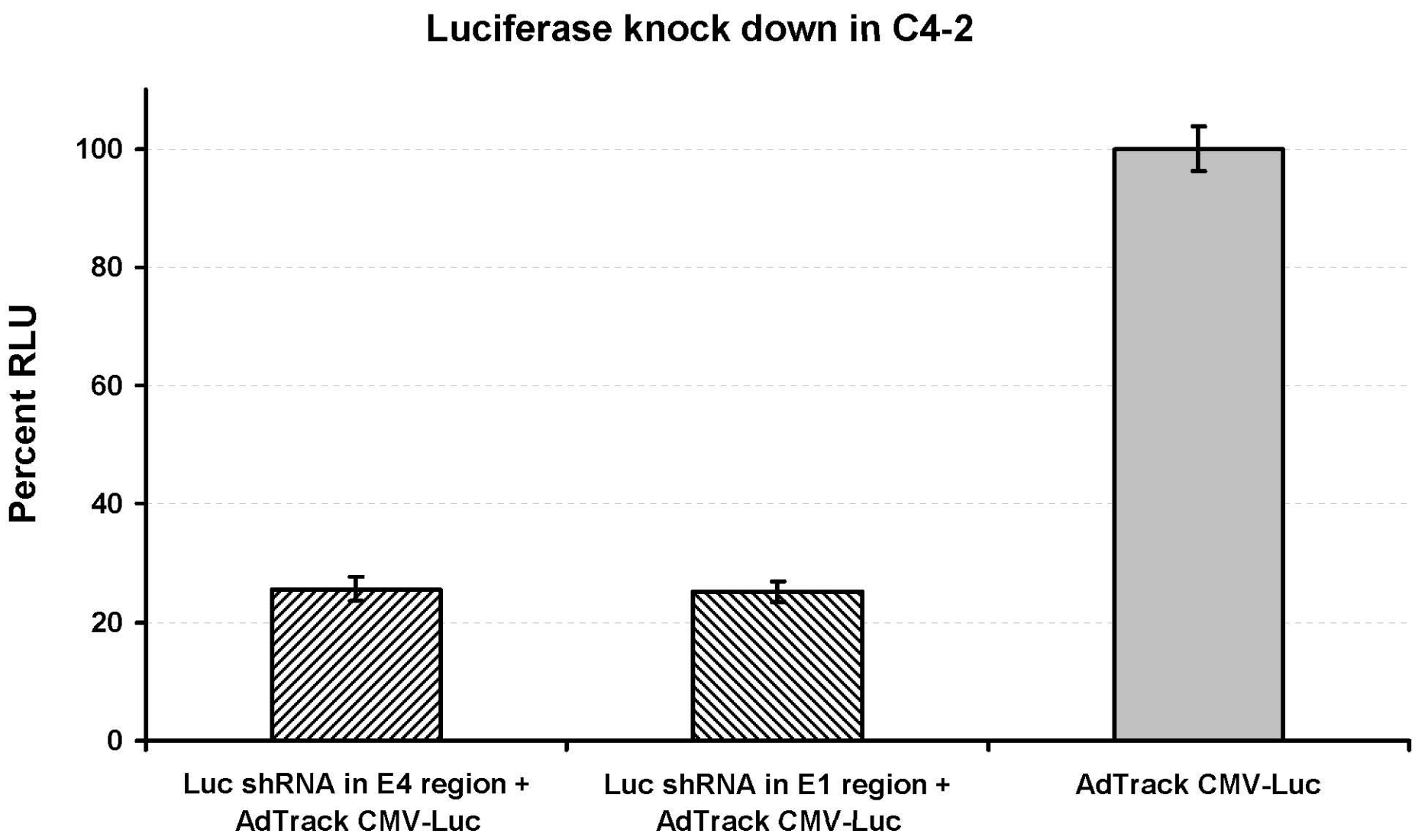
Supplementary Figure 2A



Supplementary Figure 2B



Supplementary Figure 3



Supplementary Figure 4