

Non-epigenetic induction of HEXIM1 by DNMT1 inhibitors and functional relevance

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Supplemental Figure 1. 5-AzadC dose dependent increase in the expression of HEXIM1, pCHK1, and pCHK2. C4-2 cells were treated with **(A)** 5-AzadC (5 μ M) at the indicated time points or **(B)** 5-AzadC at the indicated concentrations for 48 h and the expression of HEXIM1 normalized to GAPDH was assessed using western blots. **(C)** C4-2 cells were treated with 5-AzadC (5 μ M) at the indicated time points and the expression of DNMT1 normalized to GAPDH was assessed using western blots. **(D)** C4-2 cells were treated with Cladribine or Fludarabine at the indicated concentrations for 48 h and the expression of HEXIM1 normalized to GAPDH was assessed using western blots. **(E)** C4-2 cells were treated with 5-AzadC at the indicated concentrations for 48 h and the expression of pCHK1/CHK1 and pCHK2/CHK2 were assessed using western blots. Represented are blots cut into strips prior to blotting to minimize the amounts of antibodies required. In (A), (B), (C), and (E) figures are representative of at least 3 independent experiments. (D) is representative of 2 independent experiments. *P<0.05, **P<0.01 vs. Control.

Supplemental Figure 2. Treatment with ATM and ATR inhibitors attenuated 5-AzadC-induced pCHK1 and pCHK2 expression. **(A)** C4-2 cells were infected with control or ATR or ATM shRNA lentiviruses followed by puromycin selection. Cells were then treated with 5-AzadC (5 μ M) for 48 h. Cell lysates were prepared and expression of ATR and ATM normalized to GAPDH were analyzed by western blot. Figures are representative of at least 3 independent experiments. *P<0.05 versus Control. **(B)** C4-2 and LNCaP cells were pre-treated with an ATR inhibitor, VE-822 (1 μ M or 2 μ M), or an ATM inhibitor, caffeine (1 μ M or 2 μ M) for 2 h, followed by 5-AzadC treatment (5 μ M) for 48 h. Phospho-CHK1, total CHK1, phospho-CHK2, and total CHK2 levels were detected by western blot. Figures are representative of at least 3 independent experiments. *P<0.05 and **P<0.01 vs. Control. Represented are blots cut into strips prior to blotting to minimize the amounts of antibodies required.

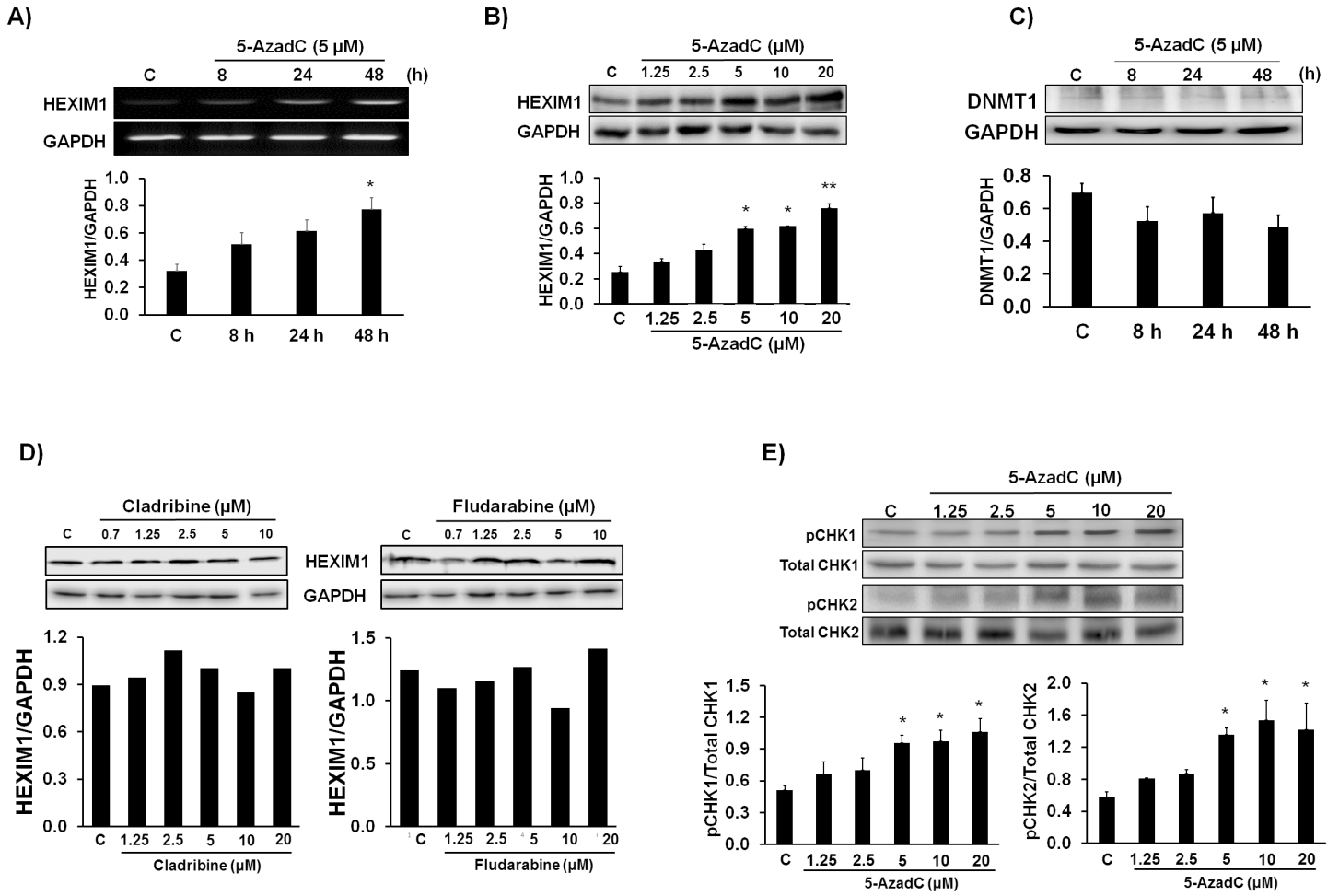
Supplemental Figure 3. CDK9 and NF- κ B protein levels in cells treated with 5- AzadC, VE-822, and/or caffeine. C4-2 and LNCaP cells were treated with VE-822 or caffeine for 2 h, followed by 5-AzadC for 2 h. Cells were **(A)** collected to assess CDK9 protein levels normalized by GAPDH using western blots, **(B)** processed for ChIP analyses of the occupancy of NF- κ B on the coding region of *HEXIM1*, and **(C)** collected to assess NF- κ B protein levels normalized by GAPDH using western blots. In (A) and (C), represented are blots cut into strips prior to blotting to minimize the amounts of antibodies required. In (B) input DNA was used as normalization control. IgG control was used as a negative control. Recruitment of CDK9 to the *HEXIM1* coding region was used as a positive control. Figures are representative of at least 3 independent experiments.

Supplemental Figure 4. CDK9 and NF- κ B protein levels in cells transduced with NF κ B shRNA lentiviruses. C4-2 and LNCaP cells were infected with control or NF- κ B shRNA lentiviruses and selected with puromycin. Some cells were treated with 5-AzadC (5 μ M, 2h) and then processed for western blotting to assess **(A)** NF- κ B and **(B)** CDK9 protein levels normalized by GAPDH. Represented are blots cut into strips prior to blotting to minimize the amounts of antibodies required. Figures are representative of at least 3 independent experiments. *P<0.05 versus Control.

Supplemental Figure 5. 5-AzadC did not induce necroptosis. C4-2 cells were treated with 5-AzadC (5 μ M) at the indicated time points and the expression of pMLKL normalized to total MLKL expression was assessed using western blots. Represented are blots cut into strips prior to blotting to minimize the amounts of antibodies required. Figures are representative of at least 3 independent experiments.

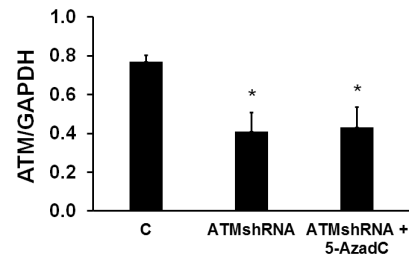
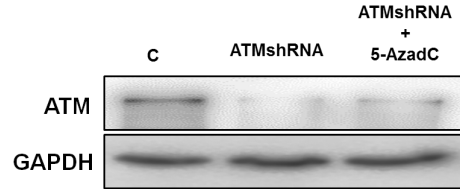
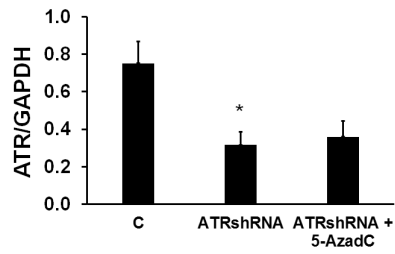
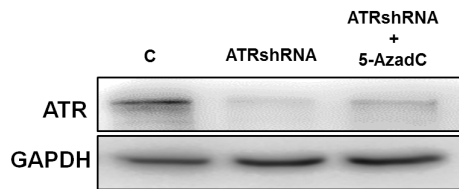
Supplemental Figure 6. 5-AzadC-induced HEXIM1 expression in breast cancer cells is mediated by ATM and NF- κ B. **(A)** MDA-MB-453 cells were infected with control or NF- κ B shRNA lentiviruses followed by puromycin selection. Cells were then treated with 5-AzadC (5 μ M) for 48 h. Cell lysates were prepared and expression of indicated proteins were analyzed by western blot. **(B)** MCF7 and T47D cells were treated with 5-AzadC at the indicated time points and the expression of HEXIM1 normalized to GAPDH was assessed using western blots. **(C)** MDA-MB-231 and MDA-MB-468 cells were treated with 5-AzadC (5 μ M) for 48 h in the absence or presence of pre-treatment with ATM inhibitor, caffeine (2 μ M). Expression of phospho-CHK2 normalized to total CHK2 was assessed by western blots. Represented are blots cut into strips prior to blotting to minimize the amounts of antibodies required. * $P < 0.01$ versus Control. Figures are representative of at least 3 independent experiments.

C4-2



A)

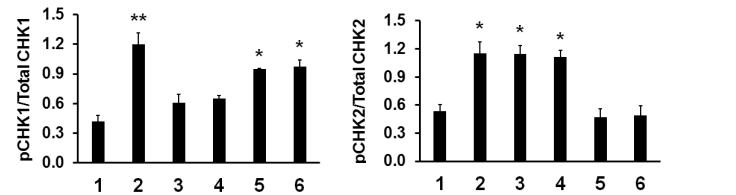
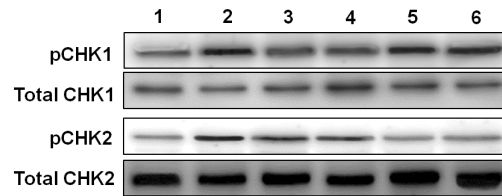
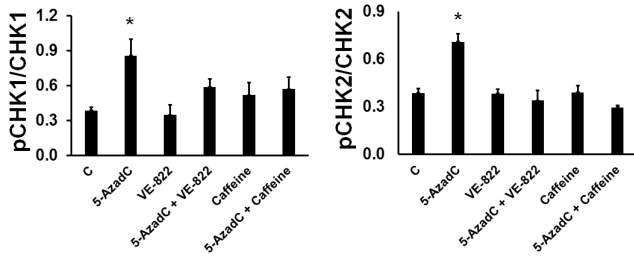
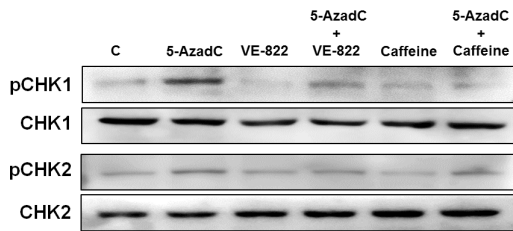
C4-2

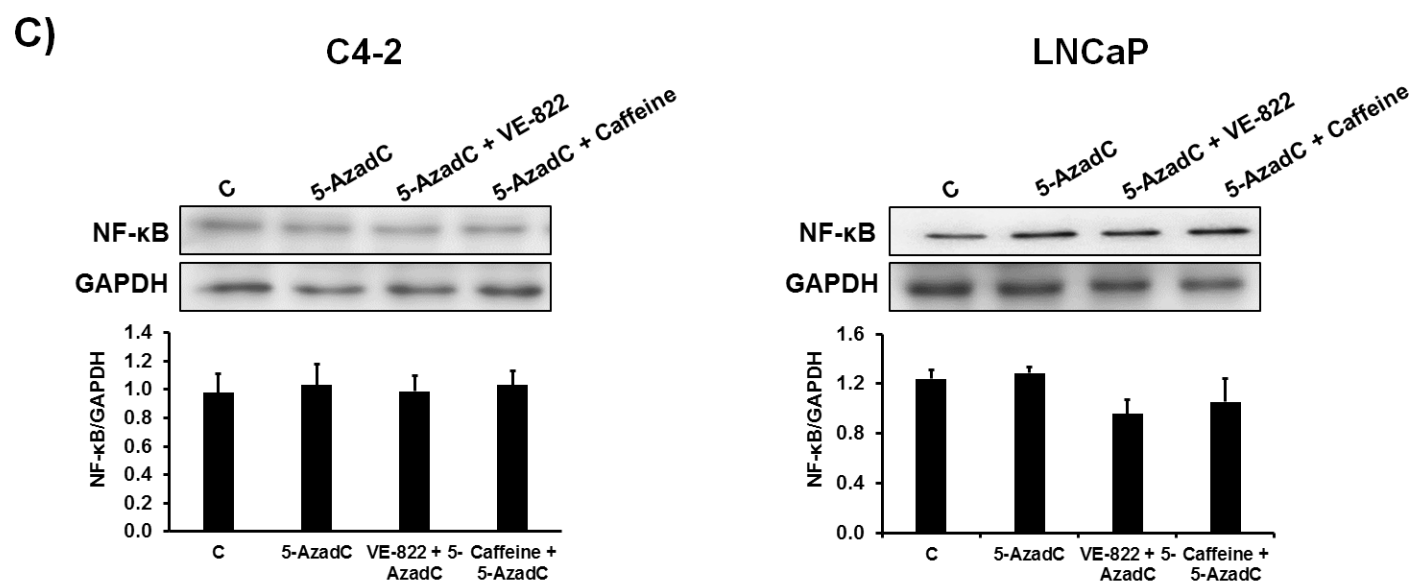
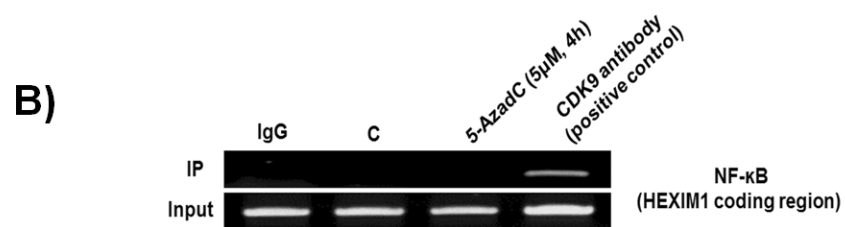
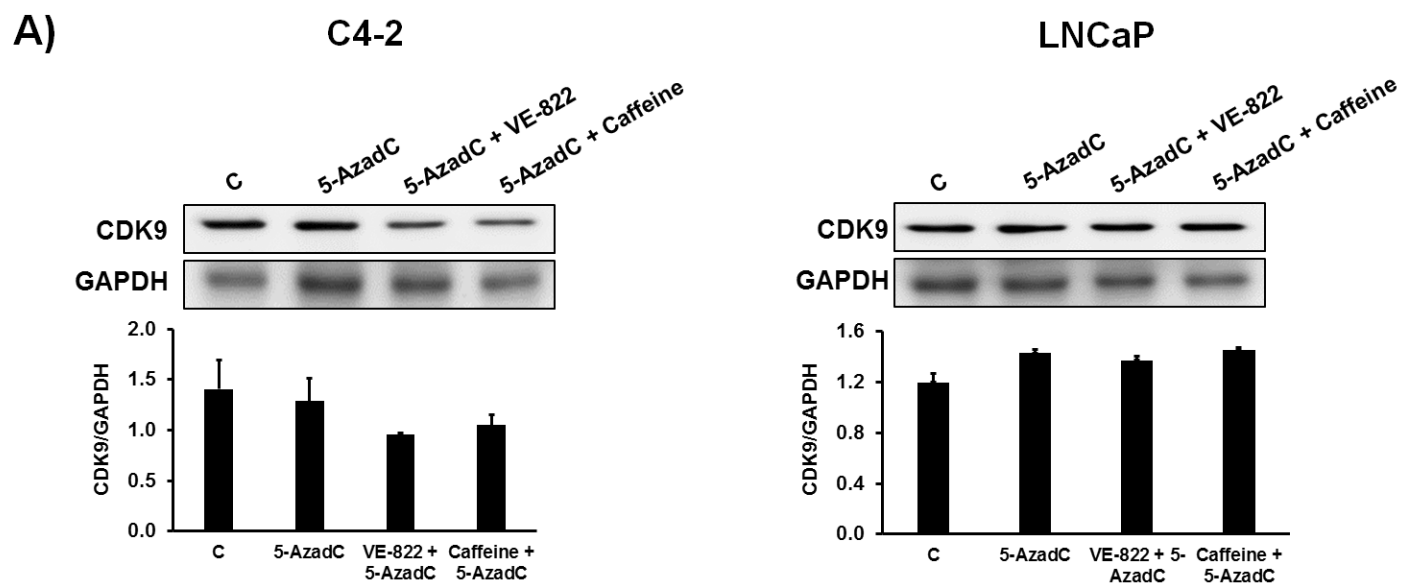


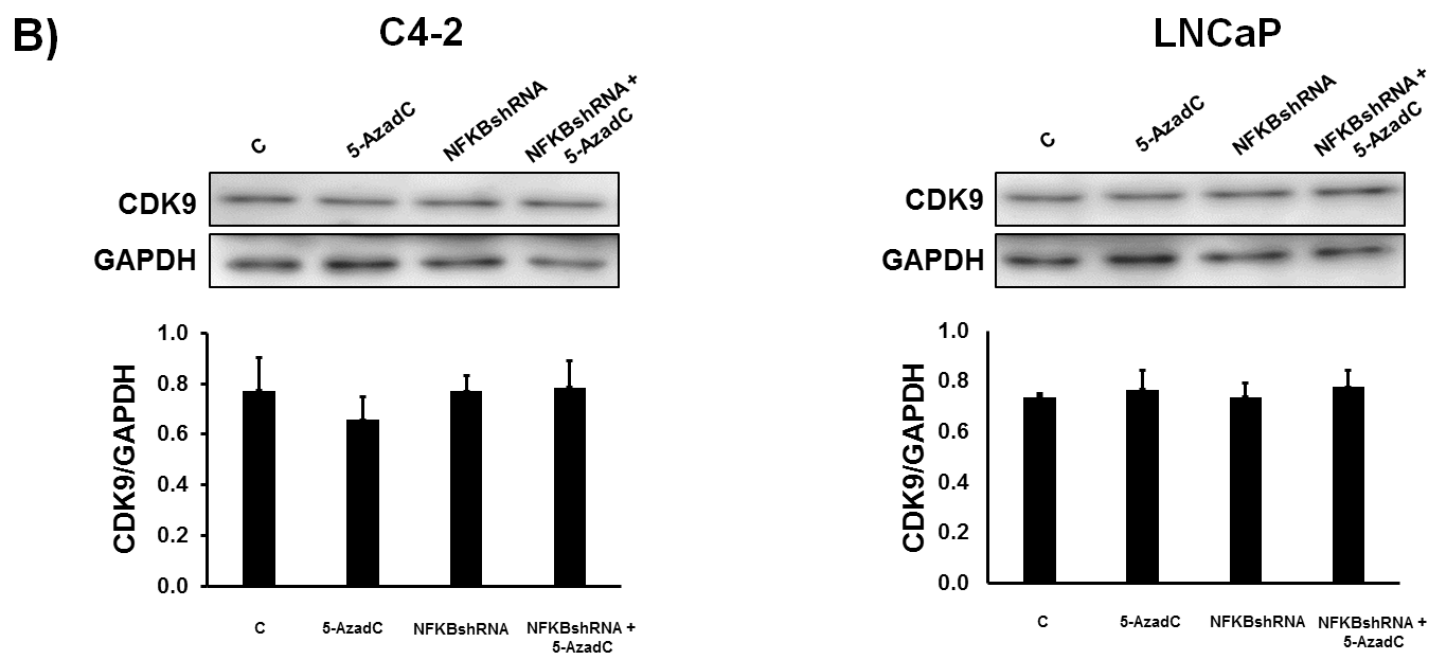
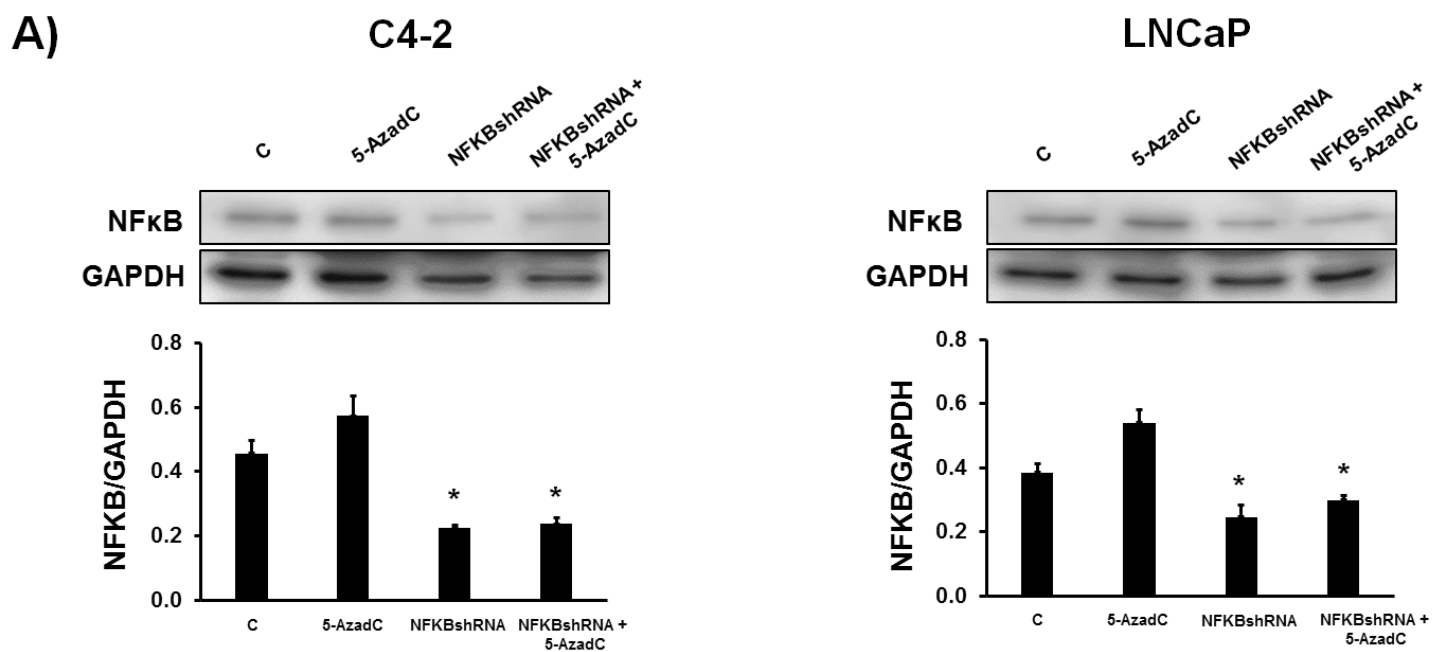
B)

C4-2

LNCaP

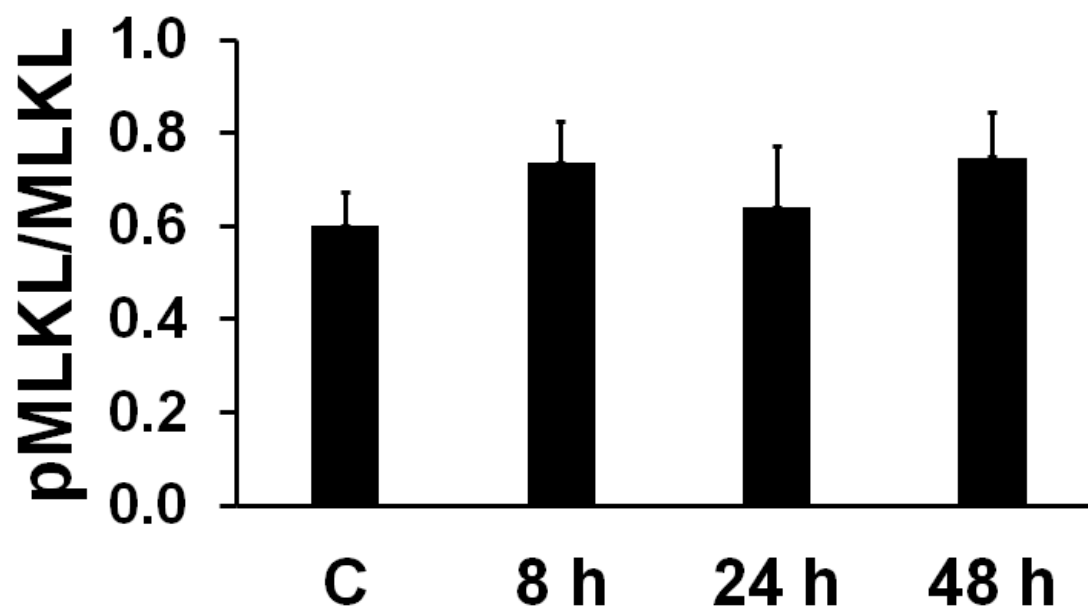
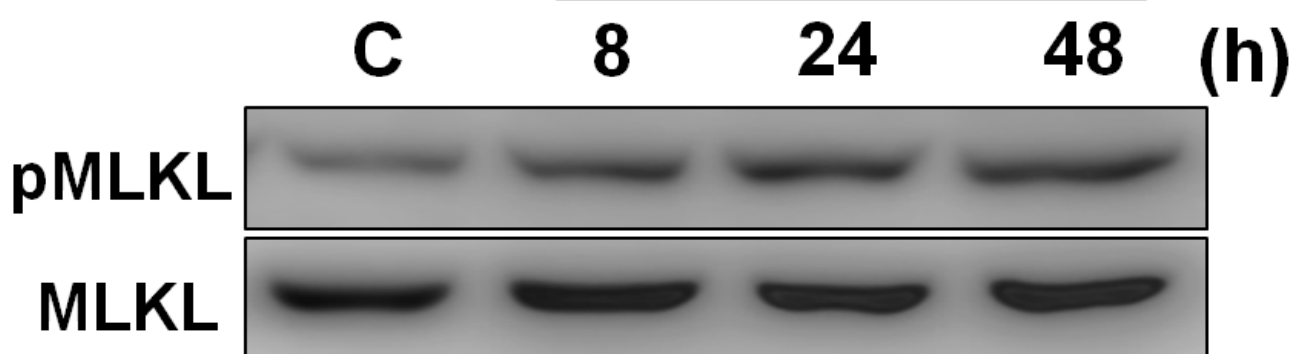




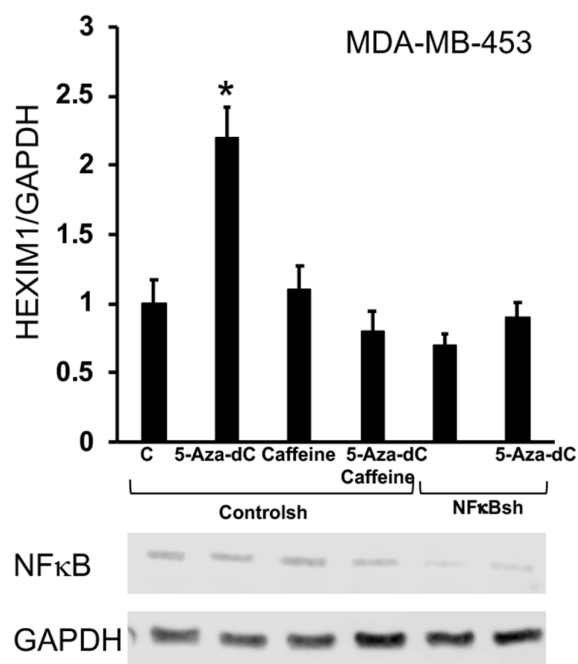


C4-2

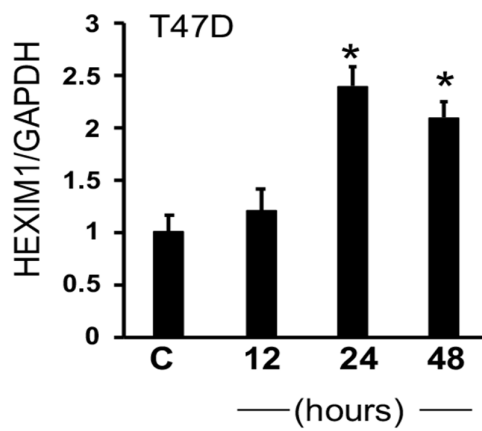
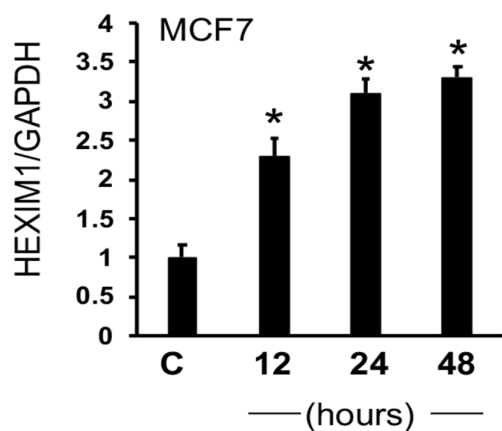
5-AzadC (5 μ M)



(A)



(B)



(C)

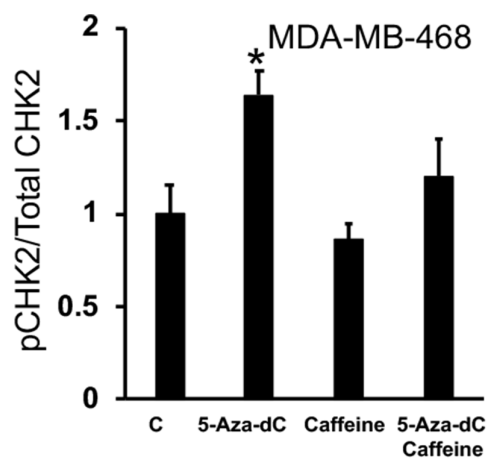
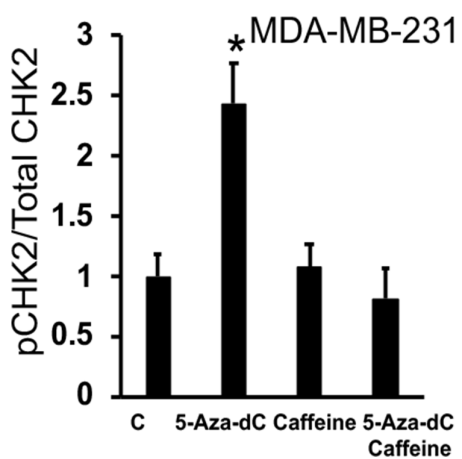
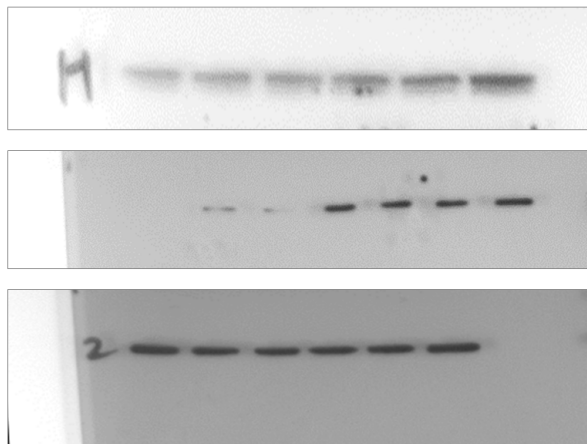


Figure 1

C4-2

LNCaP



HEXIM1

p21

GAPDH

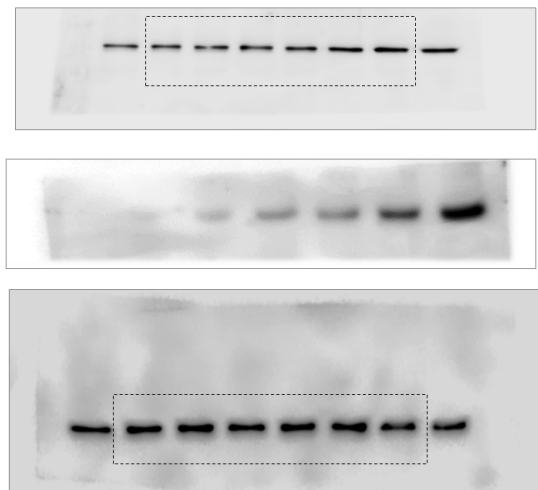


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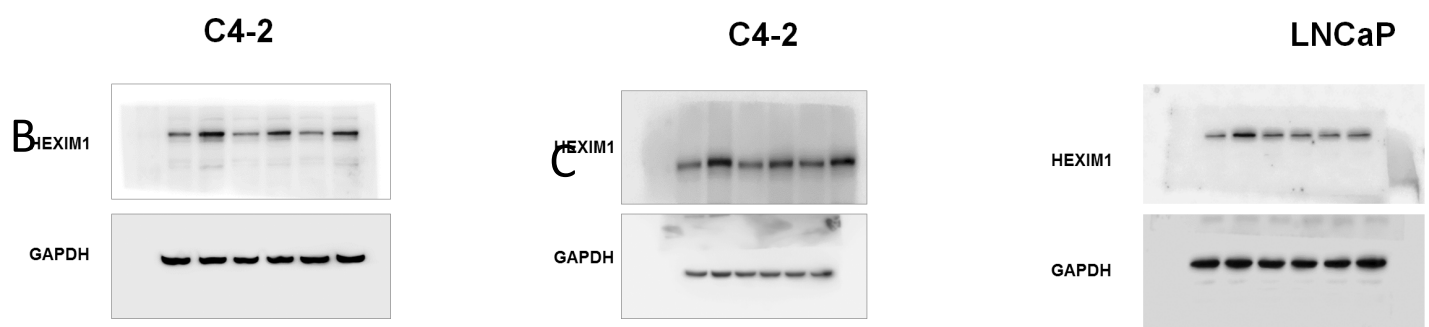
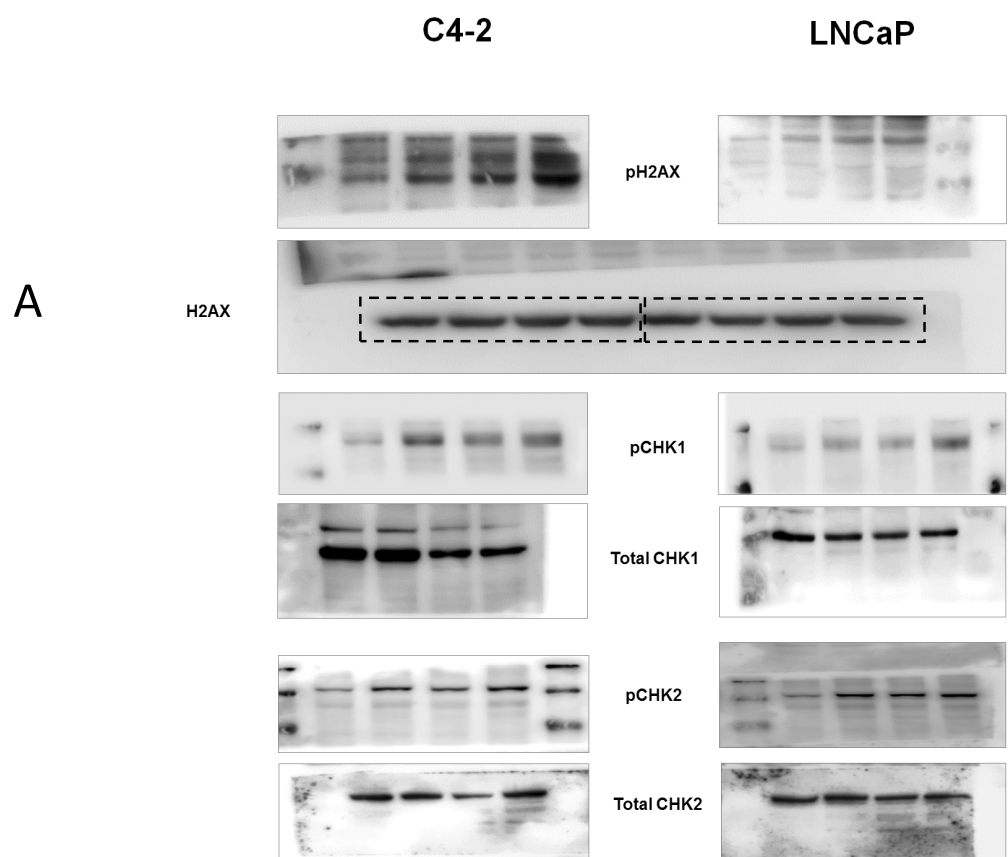


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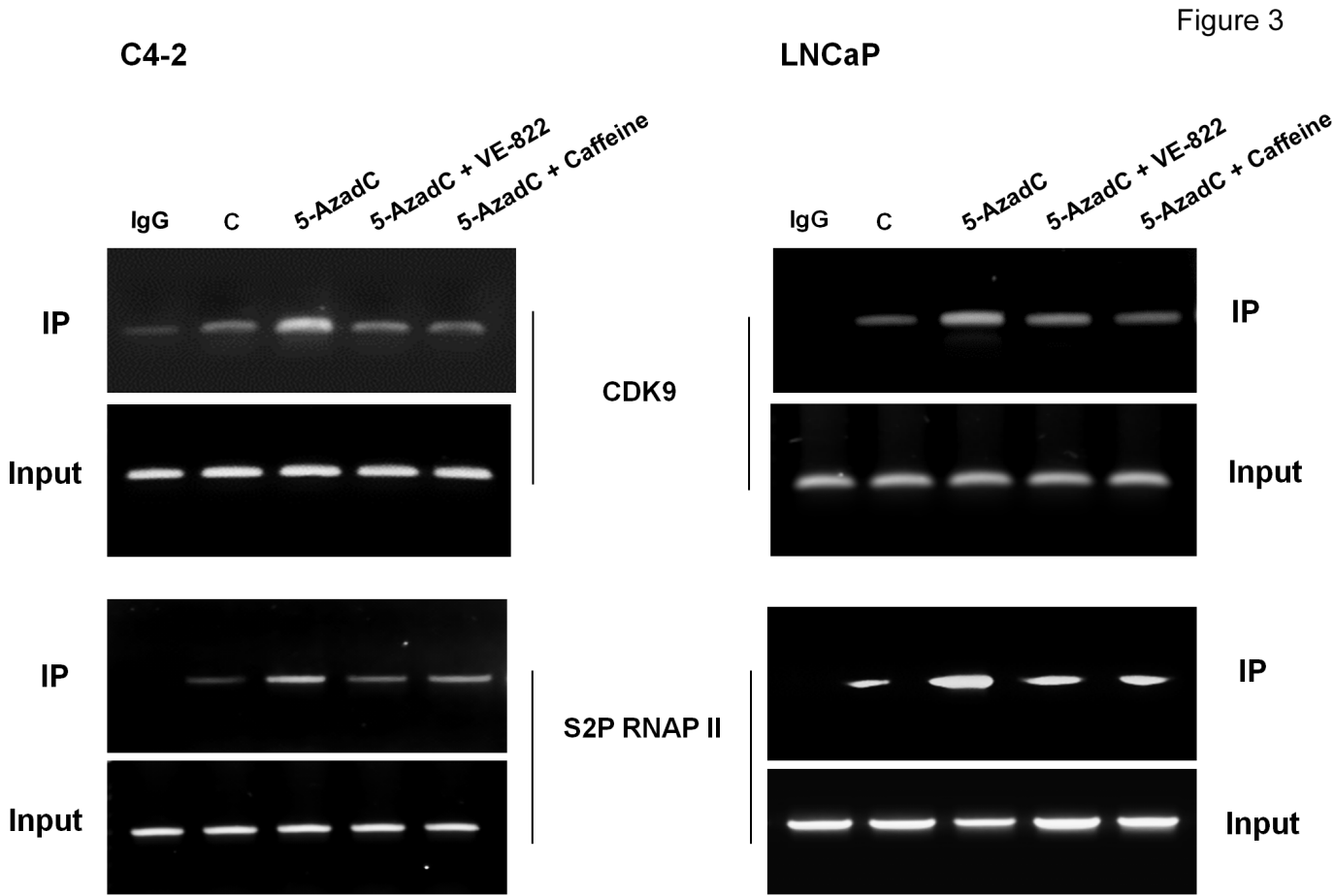


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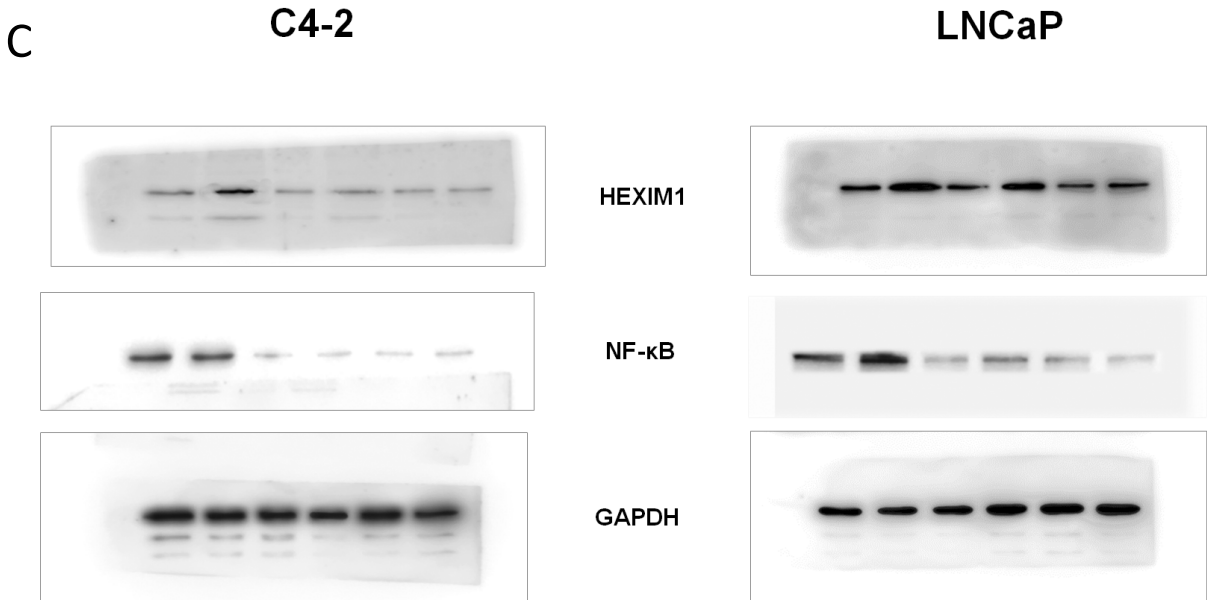
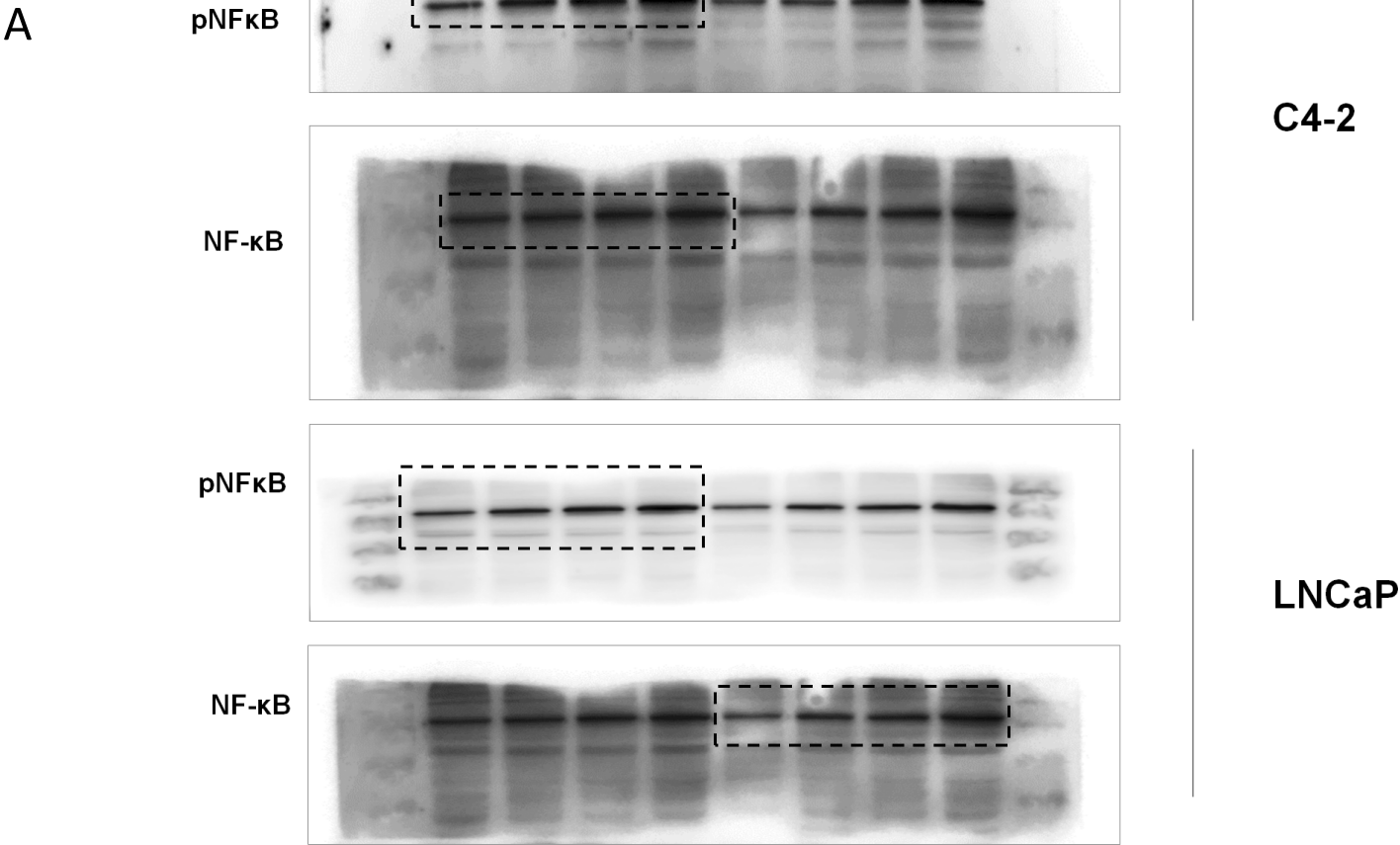
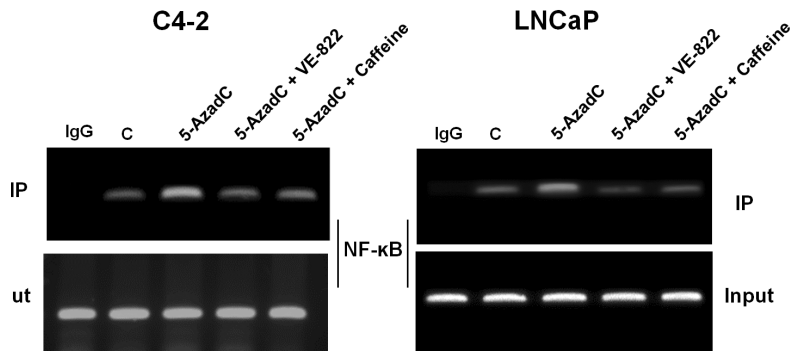


Figure 4

(B)



(D)

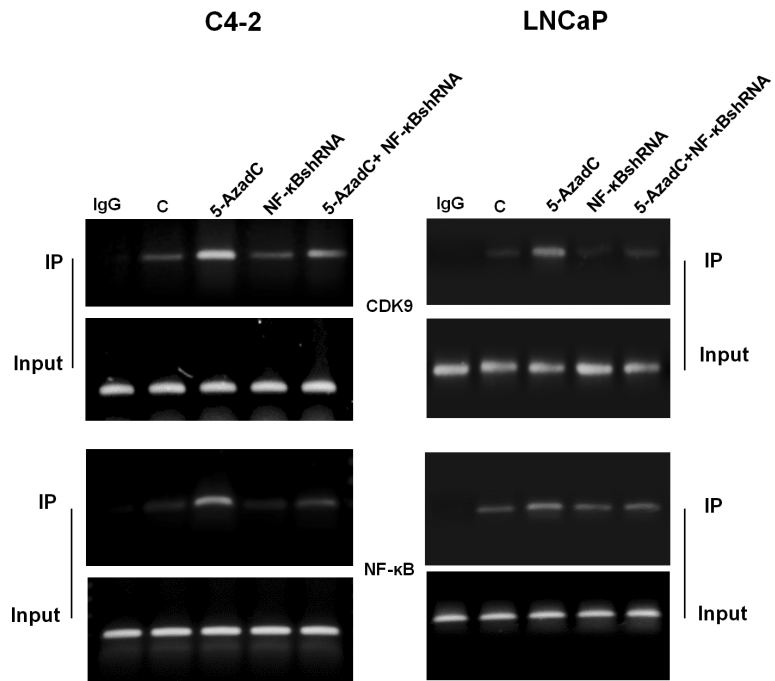
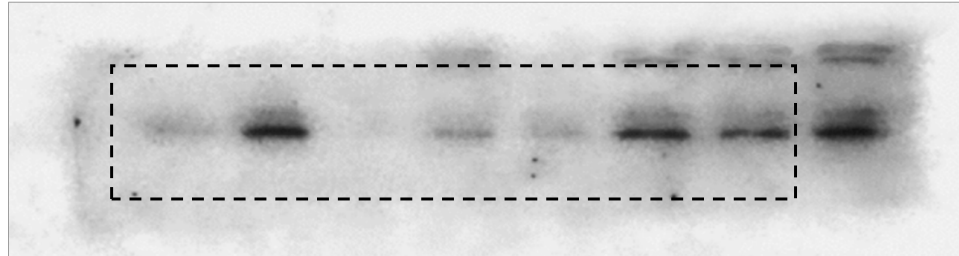


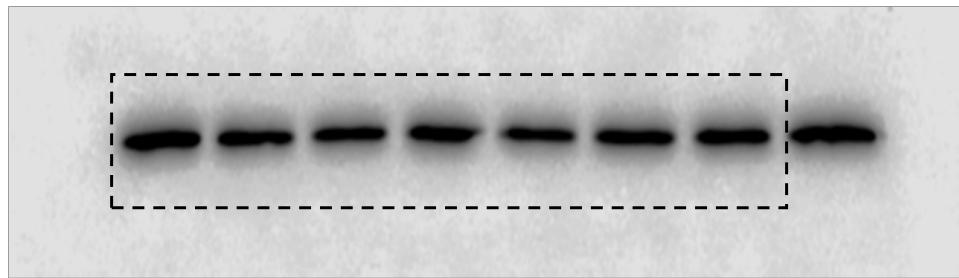
Figure 5A

C4-2

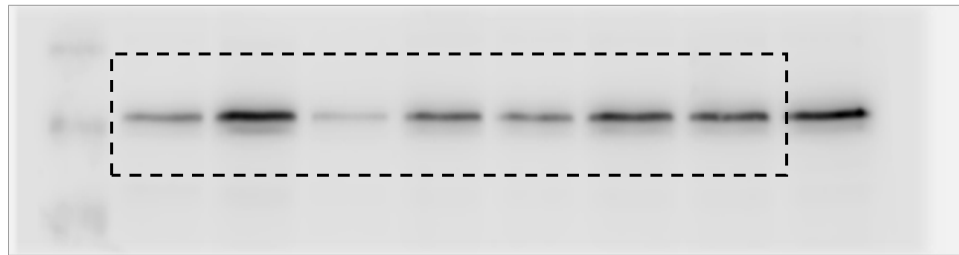
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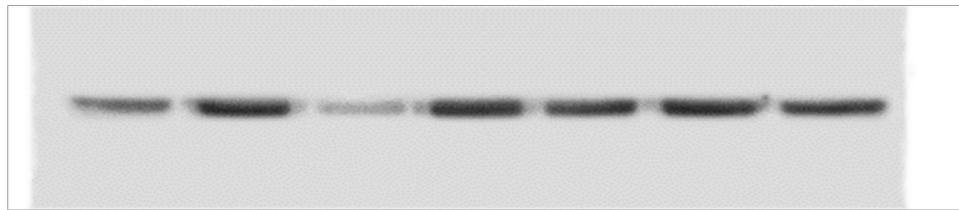
Caspase-3



HEXIM1



P53



GAPDH

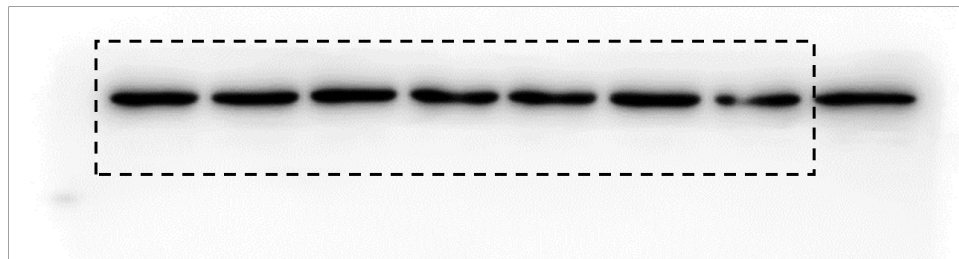
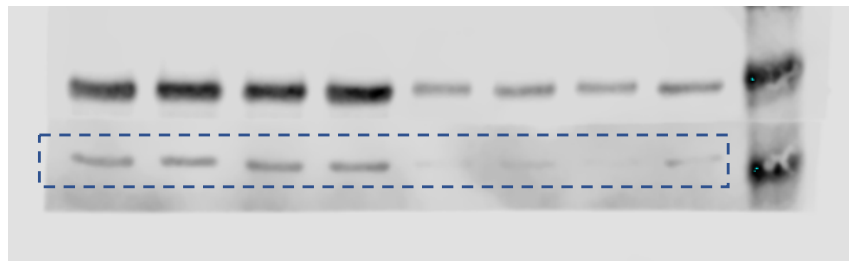


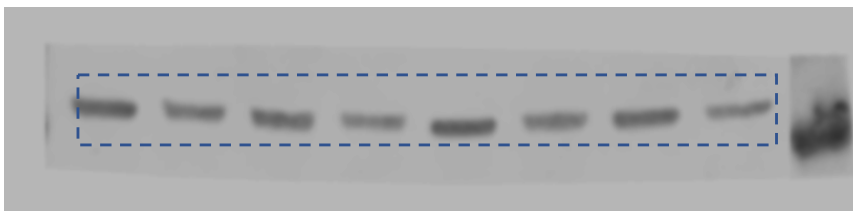
Figure 6A



NFκB

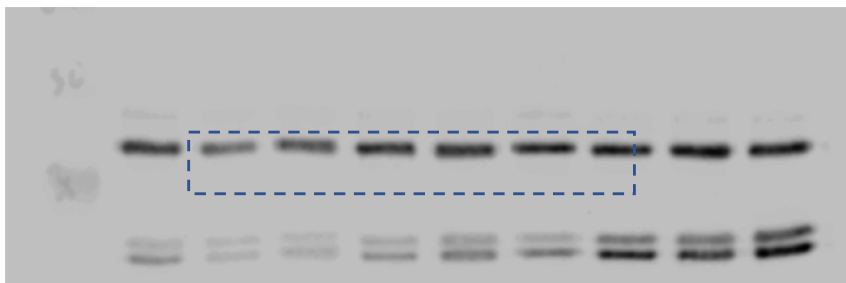
MDA-MB-231

GAPDH



NFκB

MDA-MB-468



GAPDH

Figure 6B

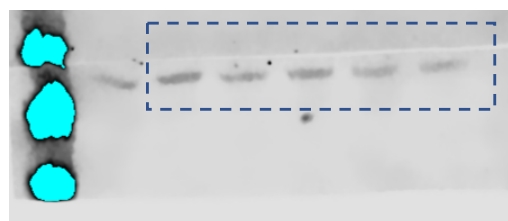
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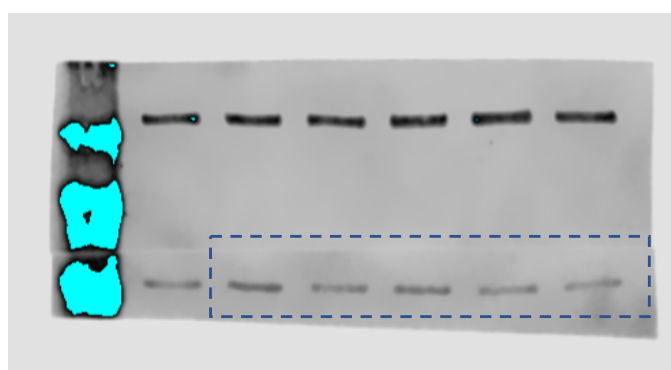
ATM



pCHK2



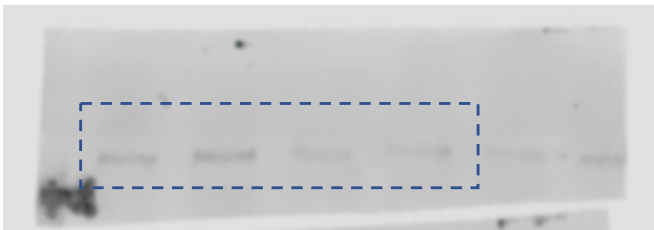
CHK2



GAPDH

Figure 6B

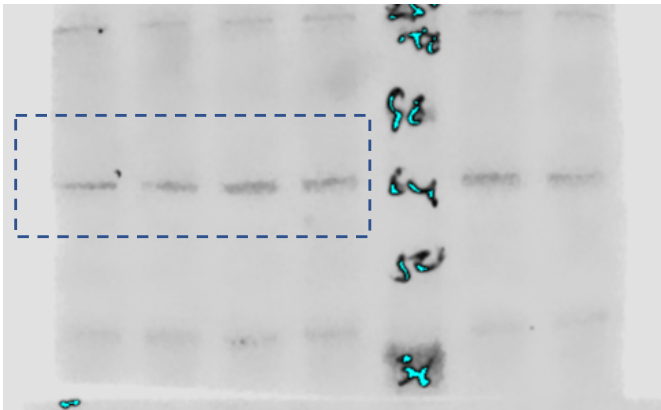
MDA-MB-468



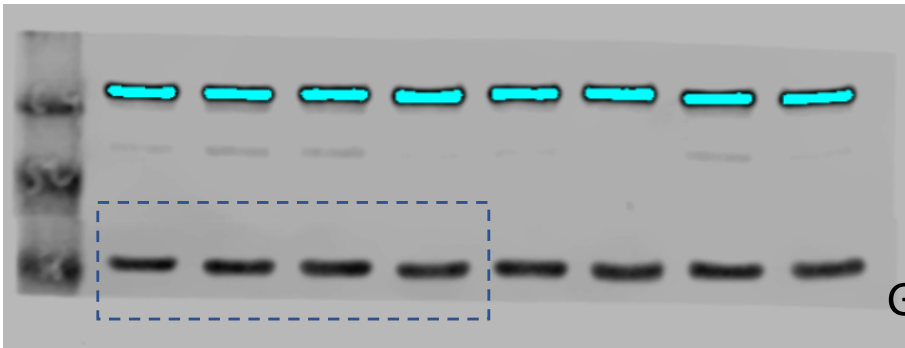
ATM



pCHK2



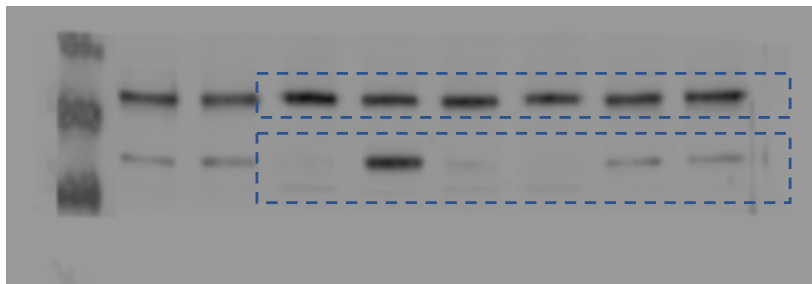
CHK2



GAPDH

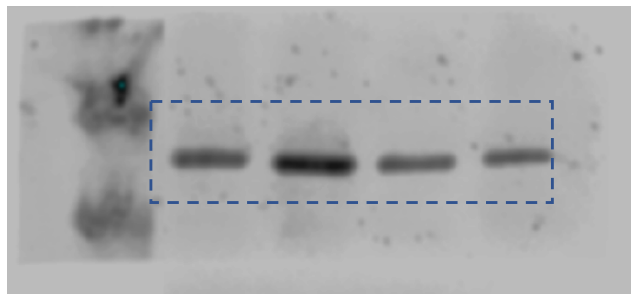
Figure 6C

MDA-MB-231

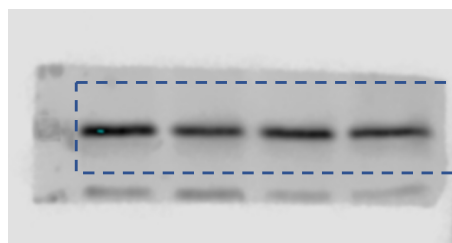


GAPDH
HEXIM1

MDA-MB-468

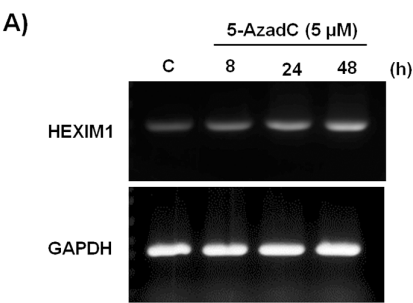


HEXIM1

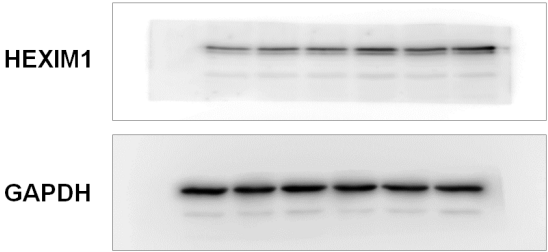


GAPDH

Supplementary Figure 1

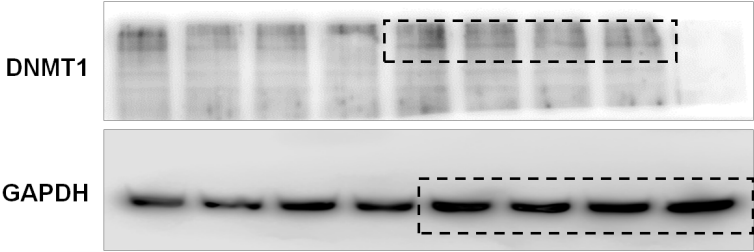


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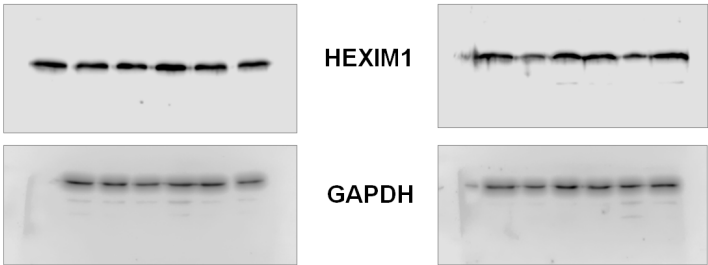


C4-2

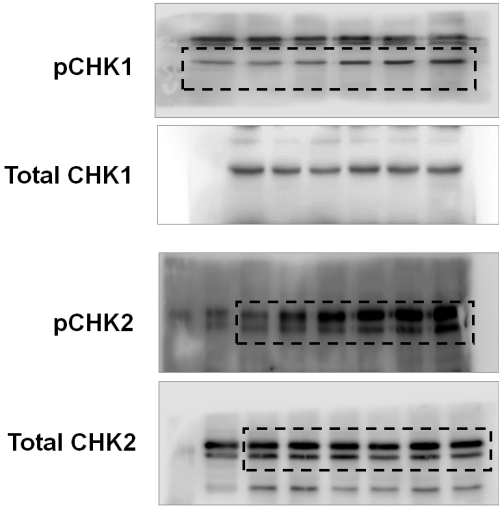
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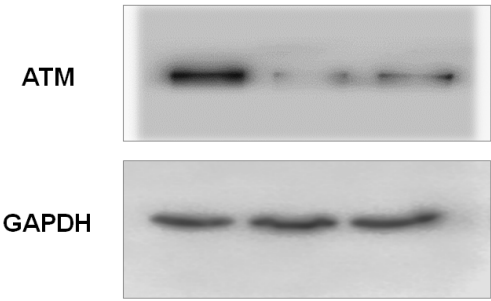
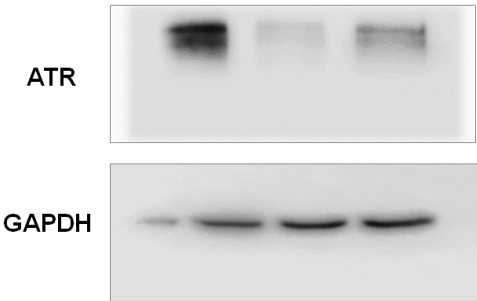
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Supplementary Figure 2

A)

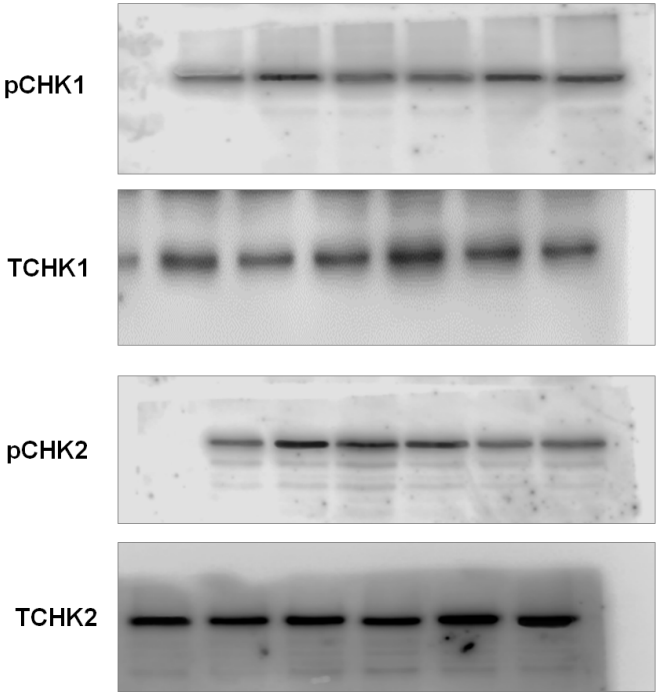
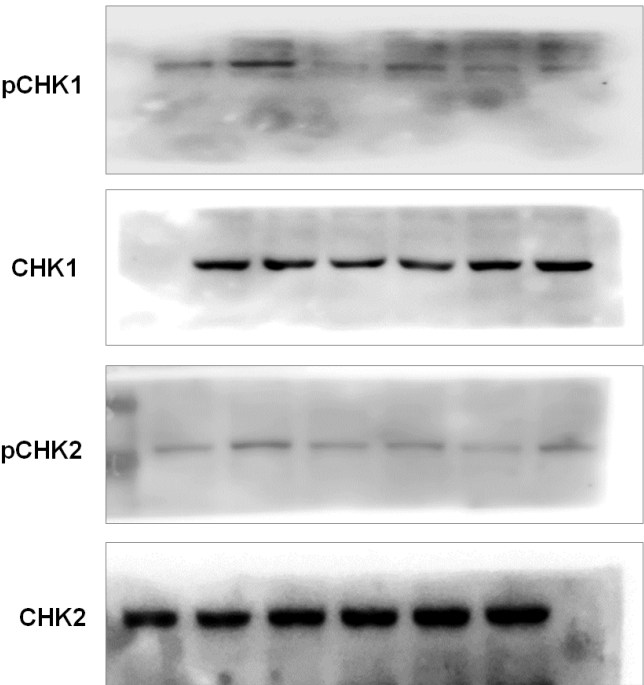
C4-2



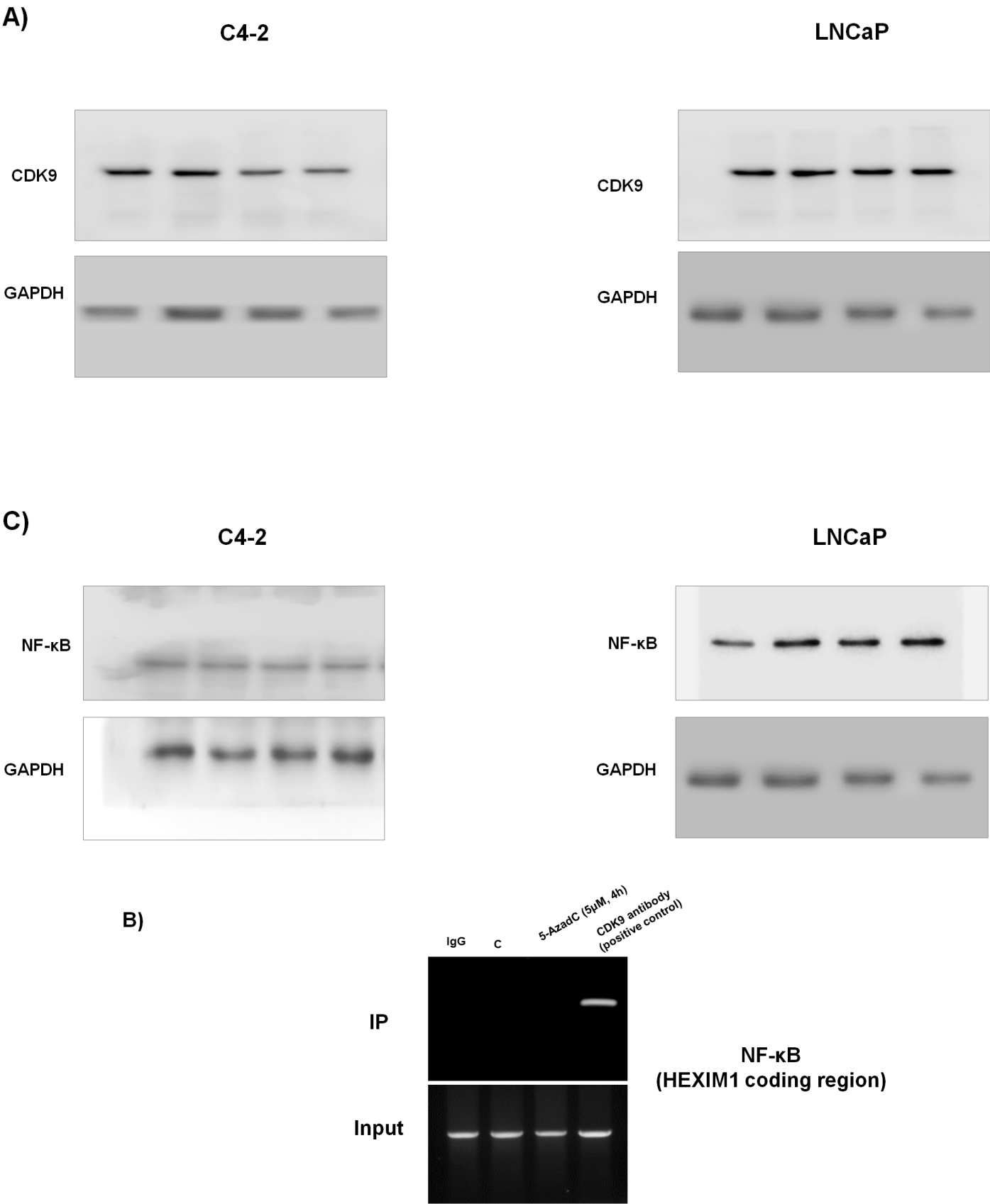
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C4-2

LNCaP

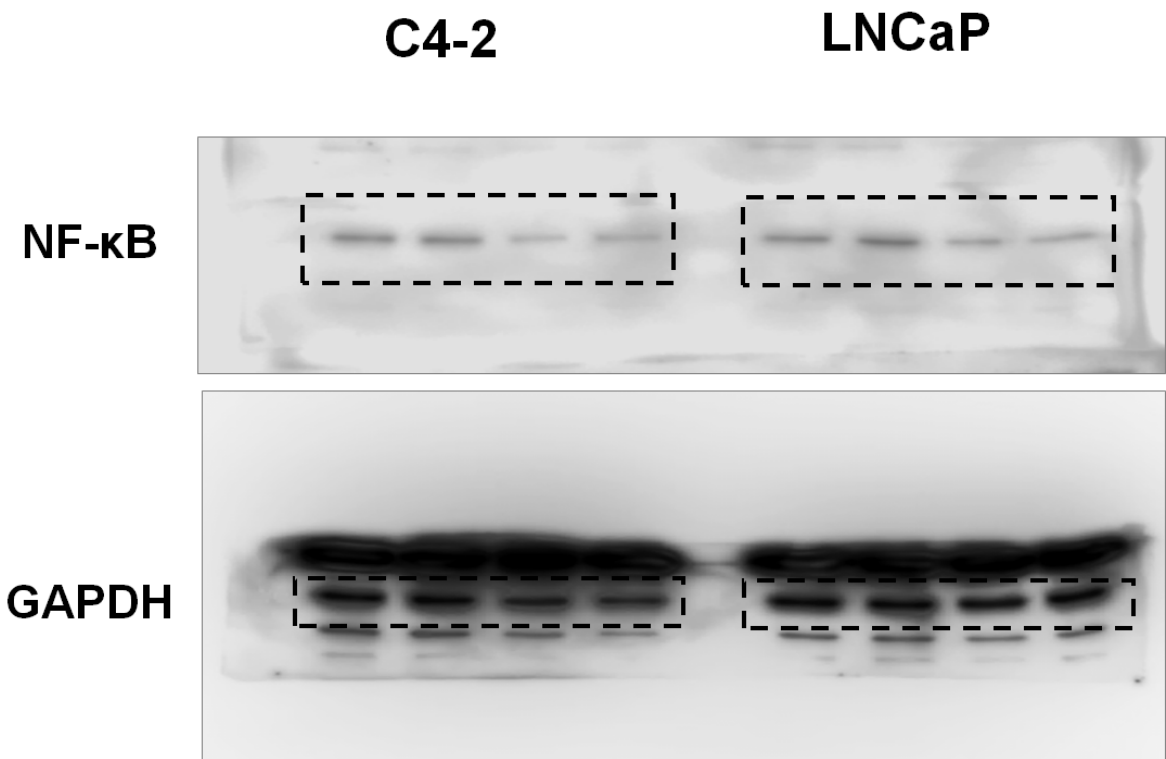


Supplementary Figure 3

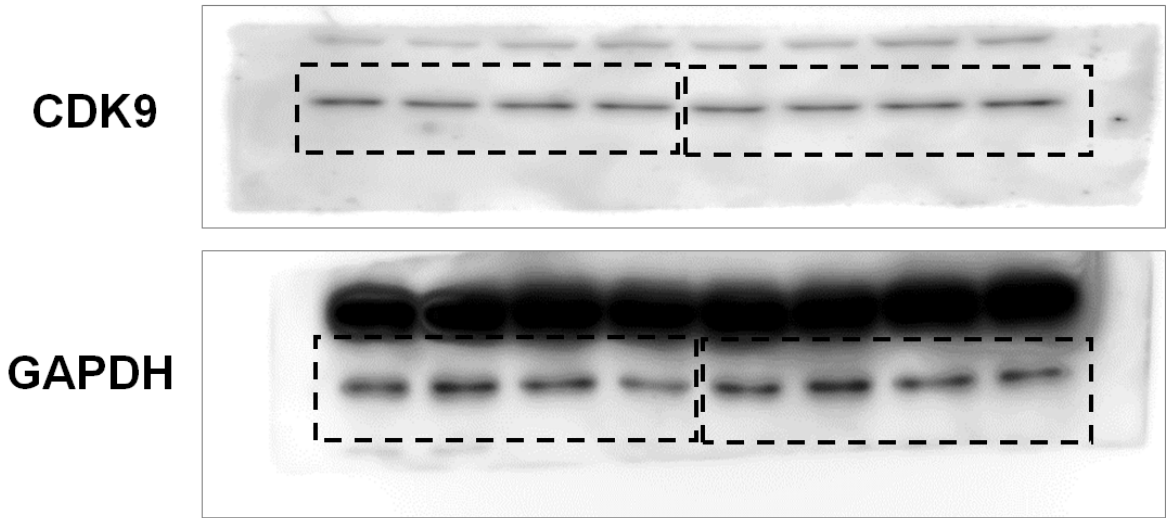


Supplementary Figure 4

A)



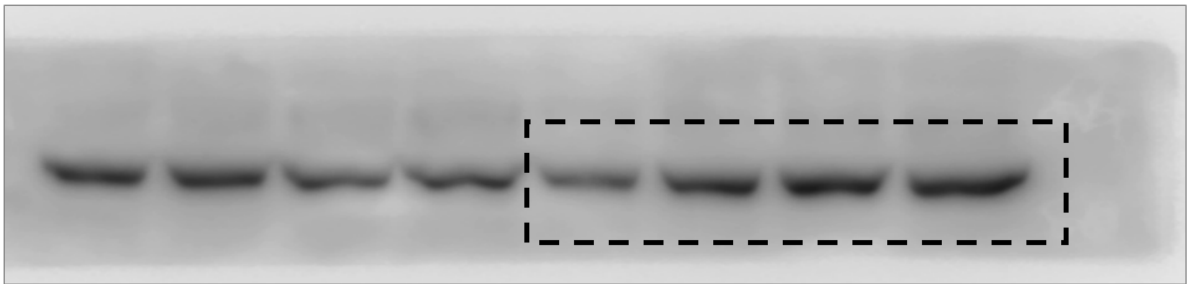
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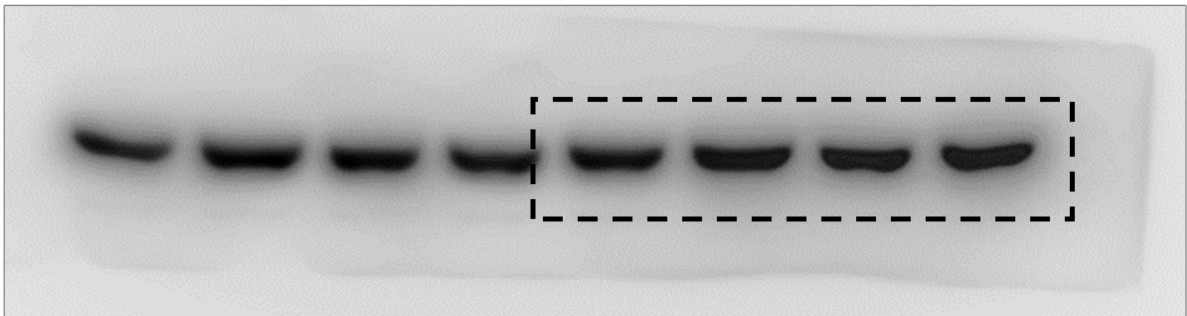
Supplementary Figure 5

C4-2

pMLKL



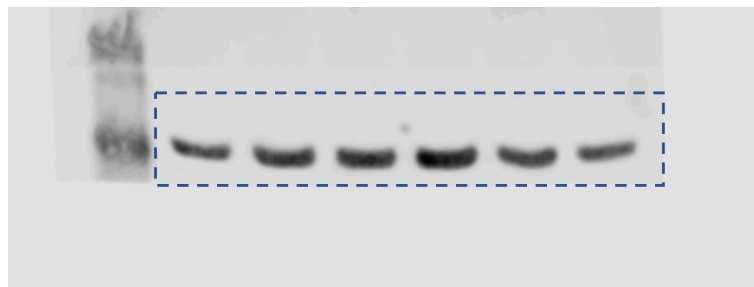
MLKL



Supplementary Figure 6



NFκB



GAPDH