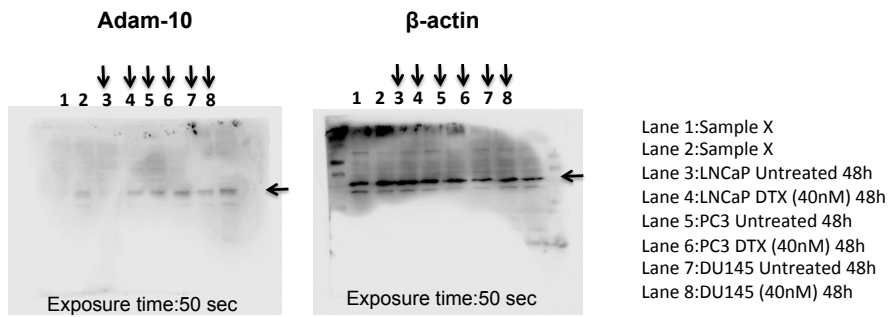


Supplementary Figures

Prostate cancer cells hyper-activate CXCR6 signaling by cleaving CXCL16 to overcome effect of docetaxel

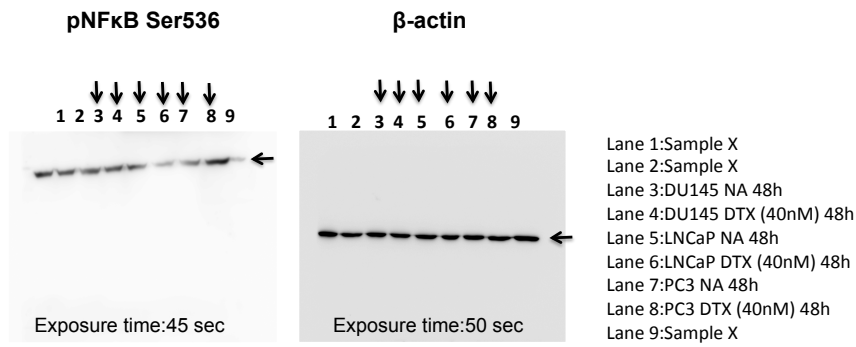
Neeraj Kapur, Hina Mir, Guru Sonpavde, Sanjay Jain, Sejong Bae, James W. Lillard Jr, and Shailesh Singh

Docetaxel induced ADAM-10 expression in prostate cancer cells.



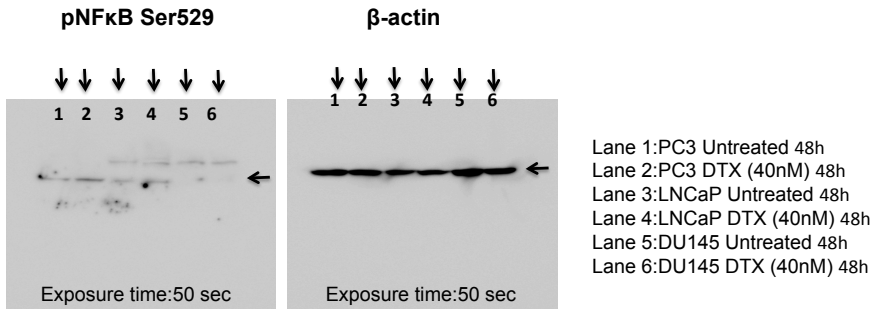
Supplementary figure 1. Western blot showing changes in expression of Adam-10 in PCa cells treated with DTX (40nM). Lane 1 and 2 are not used for this manuscript. Lane 3 and 4 represents untreated and DTX (40nM) treated LNCaP cells; Lane 5 and 6 represents untreated and DTX (40nM) treated PC3 cells; Lane 7 and 8 represents untreated and DTX (40nM) treated DU145 cells. Images for lane 3-8 were cropped and used for Figure 3B.

Docetaxel induced NF- κ B activation in prostate cancer cells.



Supplementary figure 2. Western blot showing changes in phosphorylation status of NFκB Ser-536 in prostate cancer cells treated with DTX (40uM). Lane 1, 2 and 9 are not used for this manuscript. Lane 3 and 4 represents untreated and DTX (40nM) treated DU145 cells; Lane 5 and 6 represents untreated and DTX (40nM) treated LNCaP cells; Lane 7 and 8 represents untreated and DTX (40nM) treated PC3 cells. Images for lane 3-8 were cropped and used for Figure 3C.

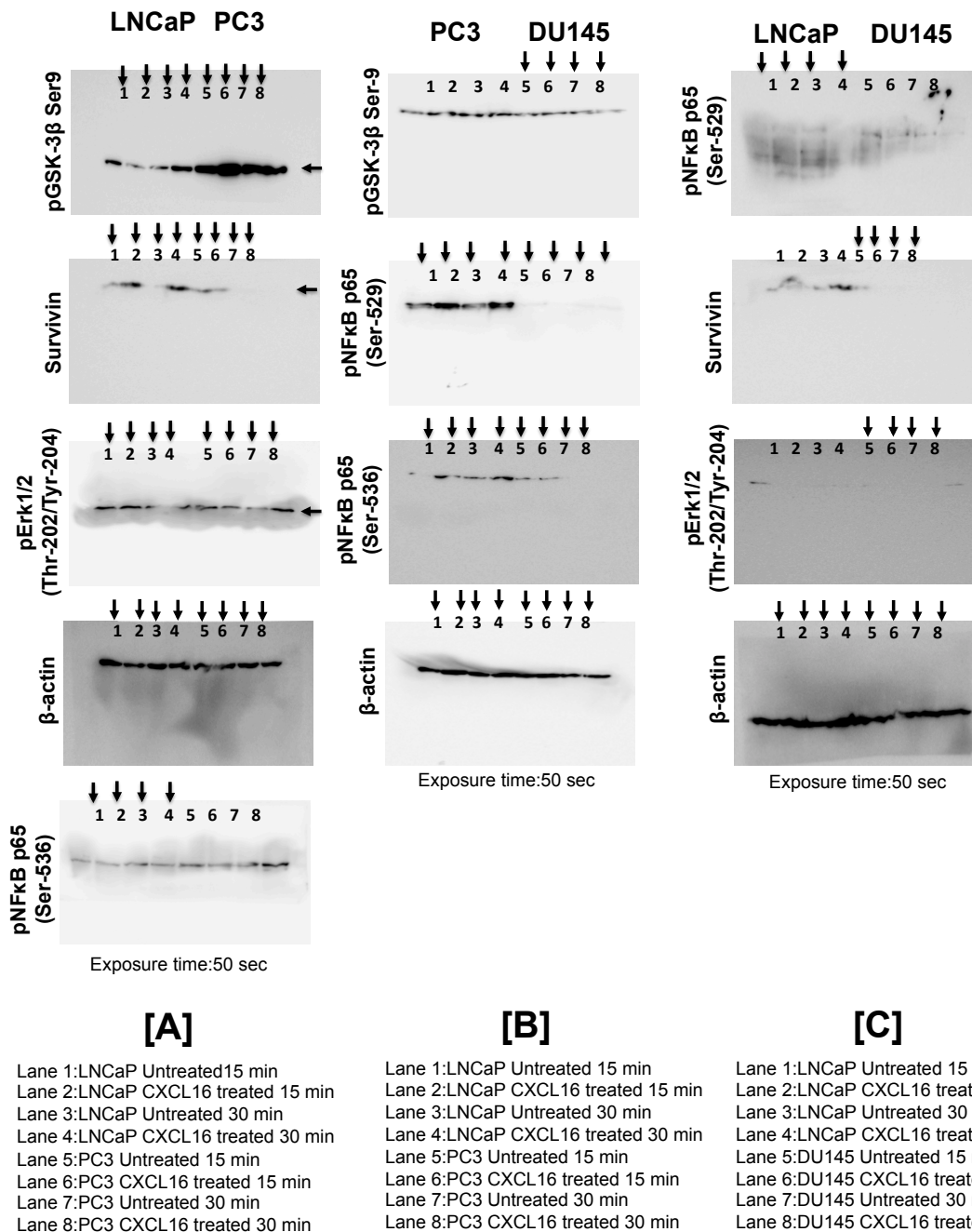
Docetaxel induced NF- κ B activation in prostate cancer cells.



Supplementary figure 3. Western blot showing changes in phosphorylation status of NFκB Ser-529 in prostate cancer cells treated with DTX (40uM). Lane 1 and 2 represents untreated and DTX (40nM) treated PC3 cells; Lane 3 and 4 represents untreated and DTX (40nM) treated LNCaP cells; Lane 5 and 6 represents untreated and DTX (40nM) treated DU145 cells. Images for lane 1-6 were cropped and used for Figure 3D.

Effect of CXCL16 treatment on phosphorylation and expression of signaling molecules in prostate cancer cells

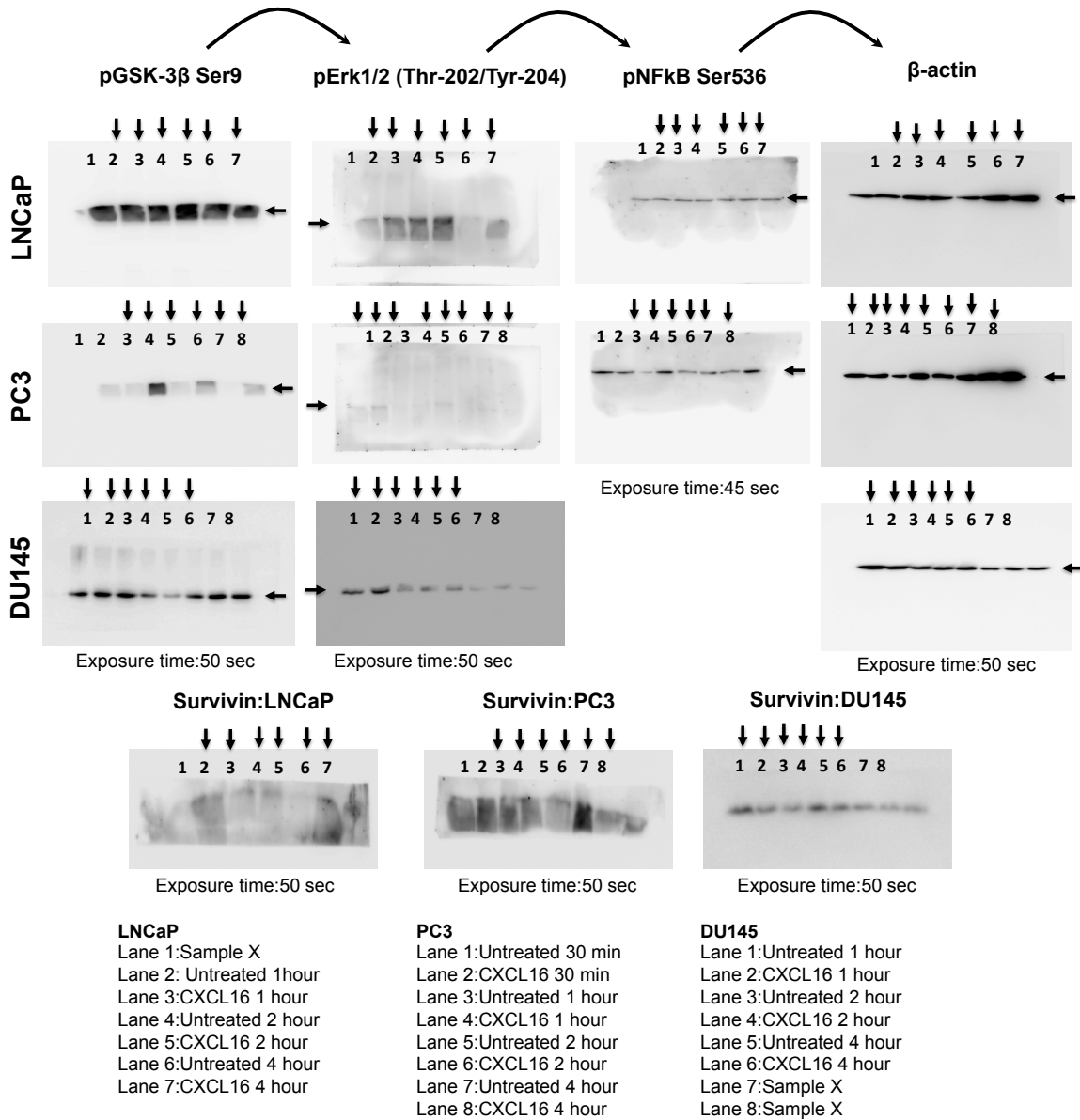
The blots were stripped using Restore PLUS western blot stripping (Thermo Fisher) buffer and re-probed for different protein.



Supplementary figure 4. Western blot showing changes in phosphorylation status of GSK-3 β Ser-536, pERK1/2 (Thr-202/Tyr-204), pNF κ B p65 Ser529/Ser536 and expression of survivin in prostate cancer cells following 15 min and 30 min of CXCL16 treatment. **Panel-A:** Lane 1 and 2 represents untreated and CXCL16 treated LNCaP cells after 15 min time interval; Lane 3 and 4 represents untreated and CXCL16 treated LNCaP cells after 30 min time interval; Lane 5 and 6 represents untreated and CXCL16 treated PC3 cells after 15 min time interval; Lane 7 and 8 represents untreated and CXCL16 treated PC3 cells after 30 min time interval. **Panel-B:** Lane 1 and 2 represents untreated and CXCL16 treated PC3 cells after 15 min time interval; Lane 3 and 4 represents untreated and CXCL16 treated PC3 cells after 30 min time interval; Lane 5 and 6 represents untreated and CXCL16 treated DU145 cells after 15 min time interval; Lane 7 and 8 represents untreated and CXCL16 treated DU145 cells after 30 min time interval. **Panel-C:** Lane 1 and 2 represents untreated and CXCL16 treated LNCaP cells after 15 min time interval; Lane 3 and 4 represents untreated and CXCL16 treated LNCaP cells after 30 min time interval; Lane 5 and 6 represents untreated and CXCL16 treated DU145 cells after 15 min time interval; Lane 7 and 8 represents untreated and CXCL16 treated DU145 cells after 30 min time interval. Images for respective lanes for pGSK-3 β , survivin, pERK1/2 and pNF κ B p65 Ser529/Ser536 levels in PCa cells were cropped (shown by arrow) and used in Fig 4B-E. Images from corresponding β -actin bands were cropped and used in the respective blot images.

Effect of CXCL16 treatment on phosphorylation and expression of signaling molecules in prostate cancer cells

The blots were stripped using Restore PLUS western blot stripping (Thermo Fisher) buffer.

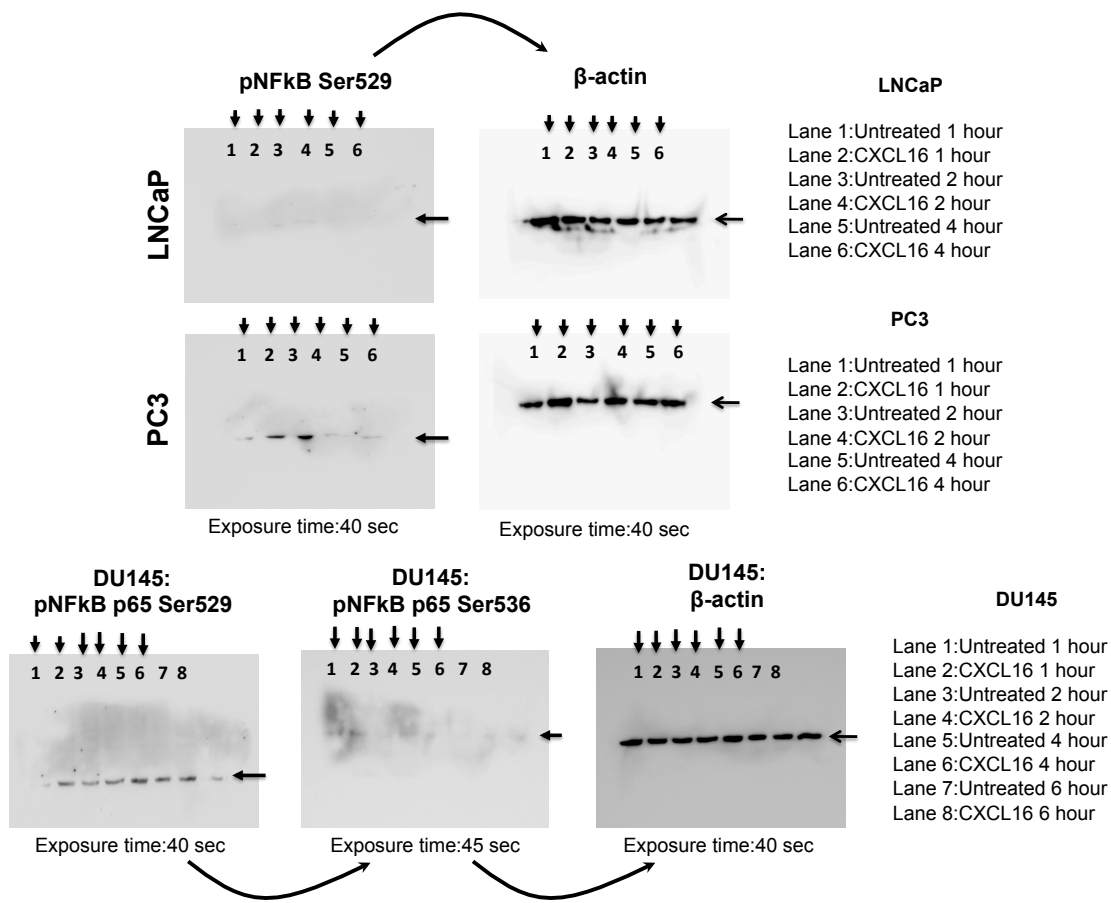


The original blots were cut at 25kDa marker band and lower blots (with proteins <25kDa) were probed for survivin. Upper blots (with proteins >25kDa) were probed for pGSK-3 β Ser-9, pERK1/2, pNF κ B Ser536 and lower blots were probed for survivin.

Supplementary figure 5. Western blot showing changes in phosphorylation status of GSK-3 β Ser-536, pERK1/2 (Thr-202/Tyr-204), pNF κ B p65 Ser536 and expression of survivin in prostate cancer cells following CXCL16 treatment. For **LNCaP** cells: Lane 2 and 3 represents untreated and CXCL16 treated cells, respectively, after 1h time interval; Lane 4 and 5 represents untreated and CXCL16 treated cells, respectively, after 2h time interval; Lane 6 and 7 represents untreated and CXCL16 treated cells, respectively, after 4h time interval. Images for Lane 4 and 5 were cropped for pGSK-3 β and used in Fig 4B, Lane 2 and 3 for pERK1/2 used in Fig 4D, Lane 6 and 7 for pNF κ B Ser536 and used in Fig 4E, Lane 2 and 3 for Survivin and used in Figure 4F. Images from corresponding β -actin bands were cropped and used in the respective figures. For **PC3** cells: Lane 1 and 2 represents untreated and CXCL16 treated cells, respectively after 30 min time interval; Lane 3 and 4 represents untreated and CXCL16 treated cells, respectively, after 1h time interval; Lane 5 and 6 represents untreated and CXCL16 treated cells, respectively, after 2h time interval; Lane 7 and 8 represents untreated and CXCL16 treated cells, respectively, after 4h time interval. Images for Lane 5 and 6 were cropped for pGSK-3 β and used in Fig 4B, Lane 1 and 2 for pERK1/2 used in Fig 4D, Lane 7 and 8 for pNF κ B Ser536 and used in Fig 4E, Lane 5 and 6 for Survivin and used in Figure 4F. Images from corresponding β -actin bands were cropped and used in the respective figures. For **DU145** cells: Lane 1 and 2 represents untreated and CXCL16 treated cells, respectively after 1h time interval; Lane 3 and 4 represents untreated and CXCL16 treated cells, respectively, after 2h time interval; Lane 5 and 6 represents untreated and CXCL16 treated cells, respectively, after 4h time interval; Lane 7 and 8 represents untreated and CXCL16 treated cells, respectively, after 6h time interval. Images for Lane 5 and 6 were cropped for pGSK-3 β and used in Fig 4B, Lane 1 and 2 for pERK1/2 used in Fig 4D, Lane 3 and 4 for Survivin and used in Figure 4F. Images from corresponding β -actin bands were cropped and used in the respective figures.

Effect of CXCL16 treatment on phosphorylation and expression of signaling molecules in prostate cancer cells

The blots were stripped using Restore PLUS western blot stripping (Thermo Fisher) buffer.



The blots were stripped using Restore PLUS western blot stripping (Thermo Fisher) buffer.

Supplementary figure 6 . Western blots showing changes in phosphorylation status of pNFkB p65 Ser529 in prostate cancer cells treated with CXCL16. Lane 1 and 2 represents untreated and CXCL16 (100ng/ml) treated PCa cells after 1h time interval; Lane 3 and 4 represents untreated and CXCL16 (100ng/ml) treated PCa cells after 2h time interval; Lane 5 and 6 represents untreated and CXCL16 (100ng/ml) treated PCa cells after 4h time interval, for the three PCa cell lines. For DU145 cells, Lane 7 and 8 represents untreated and CXCL16 (100ng/ml) treated cells after 6h time interval. Images for lane 1 and 2 were cropped and used for Figure 4C. Blot for DU145 cells was stripped using RestorePLUS western blot stripping buffer and reprobed for pNFkB p65 Ser536. Lane 3 and 4 were cropped and used for Figure 4E. Images from corresponding β-actin bands were cropped and used in the respective blot figure for each cell line.