

Supplementary information

Targeting androgen receptor promotes prostate cancer metastasis through CCL2 induction via STAT3 activation

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Supplementary Figure legends

Figure S1: Cytokine array analysis of the CM isolated from C4-2 scr and siAR cells.

CM were collected after 24 h incubation (*left*). Increased CCL2 expression was observed in CM of C4-2 siAR cells (yellow square). The array map is shown at right. Ref=reference spot, Neg=negative control.

Figure S2: qPCR analysis of M2 markers expression in THP-1 cells.

CCL22, MRC1, IL-10 and Arg1 expression in THP-1 scr or THP-1 siAR cells with or without co-cultured with C4-2 cells are shown.

Figure S3: Western blot of CCL2, EMT markers, and AR in parental LNCaP and LAPC4

cells after treated with CM of THP-1 scr and siAR, or co-cultured with THP-1 scr and siAR cells for 24 h. Similar regulation with CCL2, EMT markers, and AR in C4-2 cells was noted in LNCaP (*left*) and LAPC4 (*right*) cells co-cultured with THP-1 siAR cells.

Figure S4: Western blot analysis of CCL2, EMT markers, and AR in monoculture

LNCaP and LAPC4 cells (scr and siAR). Similar regulation with CCL2, EMT markers, and AR in C4-2 scr and siAR cells was noted in LNCaP scr and siAR (*left*) and LAPC4 scr and siAR (*right*) cells.

Figure S5: IHC analysis of PIAS3 in TRAMP-C1 xenograft tumors (scr and siAR).

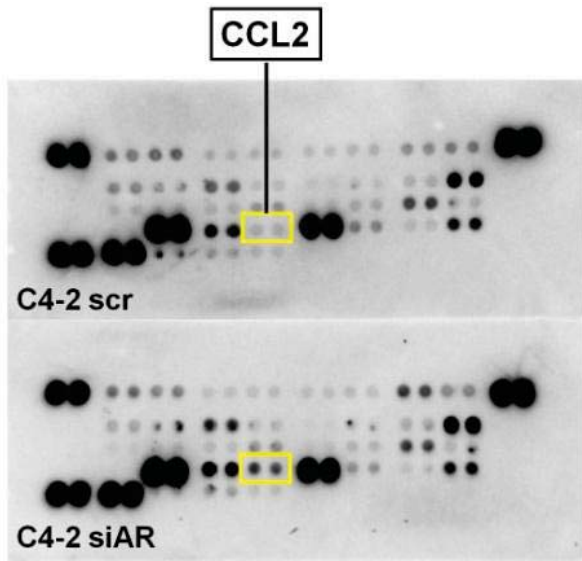
The strong staining of PIAS3 in sections of TRAMP-C1 scr tumors is noted (*left*). In contrast, only marginal PIAS3 immunoreactivity in TRAMP-C1 siAR tumors is observed (*right*), suggesting AR silencing in TRAMP-C1 cells reduces the expression of PIAS3 protein. The strong PIAS3 staining in the benign control prostate gland indicates that benign prostate in TRAMP-C1 siAR tumors has not lost PIAS3. Black arrows indicate PIAS3-positive tumor cells. **T**: tumor; **B**: benign gland of anterior prostate.

Figure S6: The clinical information of prostate biopsy samples at diagnosis and CRPC stage. **A.** The patient's information from biopsy samples is shown. PSA values at diagnosis and at CRPC stage were statistically analyzed with paired one-tailed t test. TNM=TNM classification by UICC 1997, GS=Gleason score, Dx=diagnosis, Re-Bx=re-biopsy, and N/A=not available. **B.** The number of CD68⁺ positive macrophages in prostate biopsy samples collected from patients during diagnosis or developing CRPC stage (*left*). The representative picture shows CD68⁺ cells (arrows) in Case E at CRPC stage (*right*).

Figure S7: IHC analysis of prostate biopsy samples at diagnosis and CRPC stage. IHC of CCL2, AR, PIAS3, and pSTAT3 in prostate biopsy samples at diagnosis and at CRPC stage are shown, neg=negative; mod=moderate; str=strong. The specimen of PIAS3 in Case C was not available.

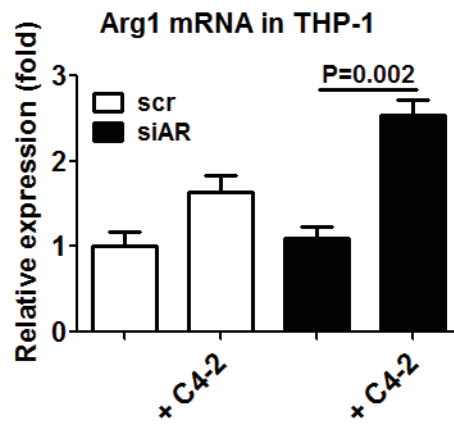
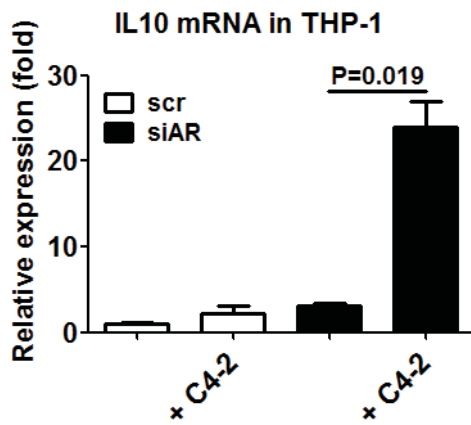
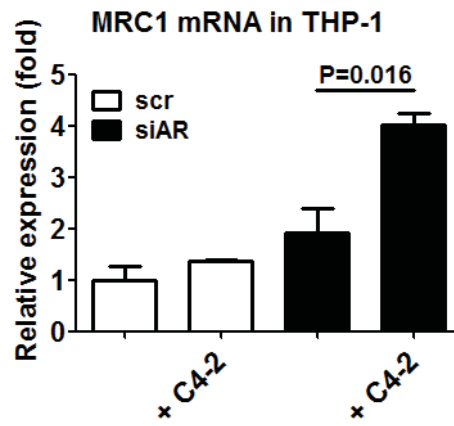
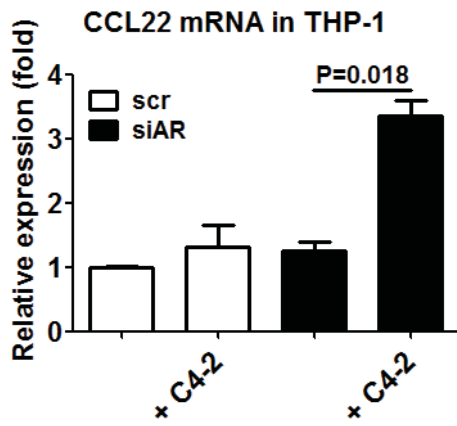
Figure S8: Gene profiling analyses using public database show increased CCL2 in human PCa tissues and androgen-deprived mouse prostates. **A.** GDS1439 database and **B-H.** GDS2562 database were obtained from the NCBI Gene Expression Omnibus (GEO) website. *Bars, Mean ± SEM.*

Supplementary Fig. S1

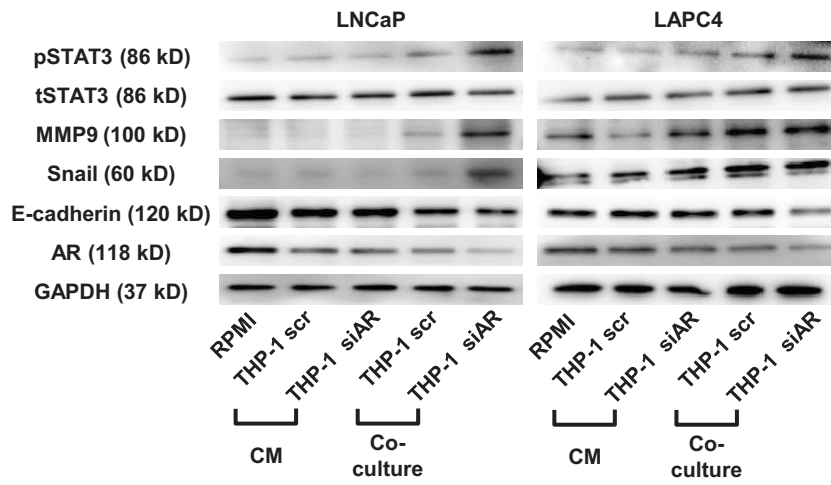


| Ref | C5a | CD 154 | G-CSF | GM-CSF | CXCL 1 | CCL1 | CD54 | IFN- γ | Ref |
|-----|----------------|--------------|---------------|---------|--------|--------|-------|---------------|-----|
| | IL-1 α | IL-1 β | IL-1ra | IL-2 | IL-4 | IL-5 | IL-6 | IL-8 | |
| | IL-10 | IL-12p70 | IL-13 | IL-16 | IL-17 | IL-17E | IL-23 | IL-27 | |
| | IL-32 α | CXCL 10 | CXCL 11 | CCL2 | MIF | CCL3 | CCL4 | PAI-1 | |
| Ref | CCL5 | SDF-1 | TNF- α | TREM -1 | | | | | Neg |

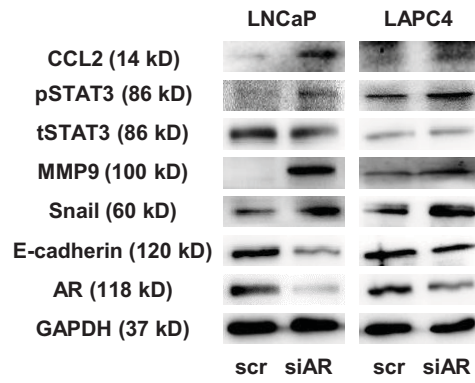
Supplementary Fig. S2



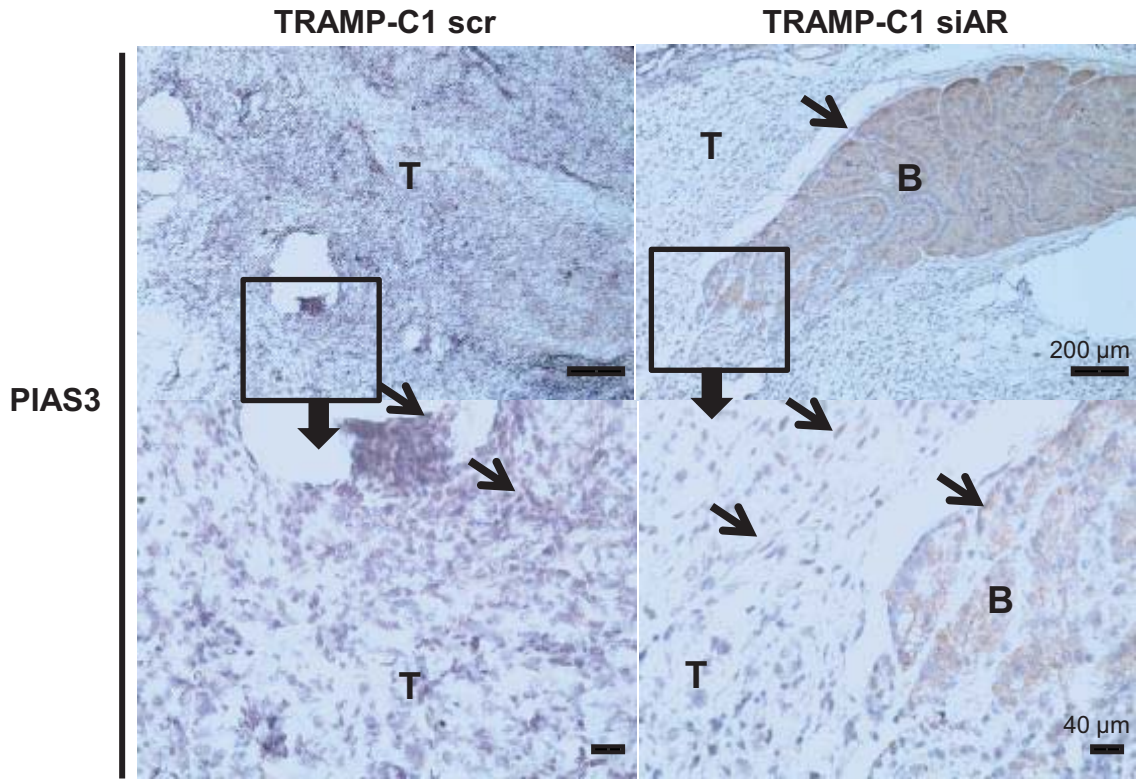
Supplementary Fig. S3



Supplementary Fig. S4



Supplementary Fig. S5



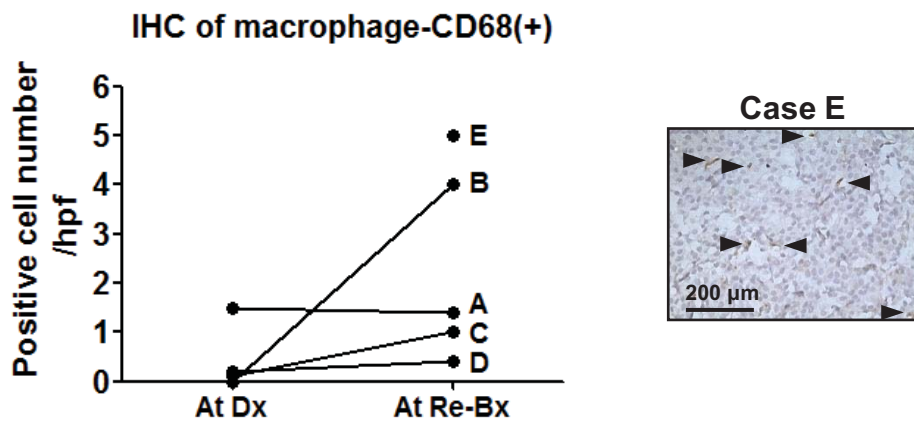
Supplementary Fig. S6

A

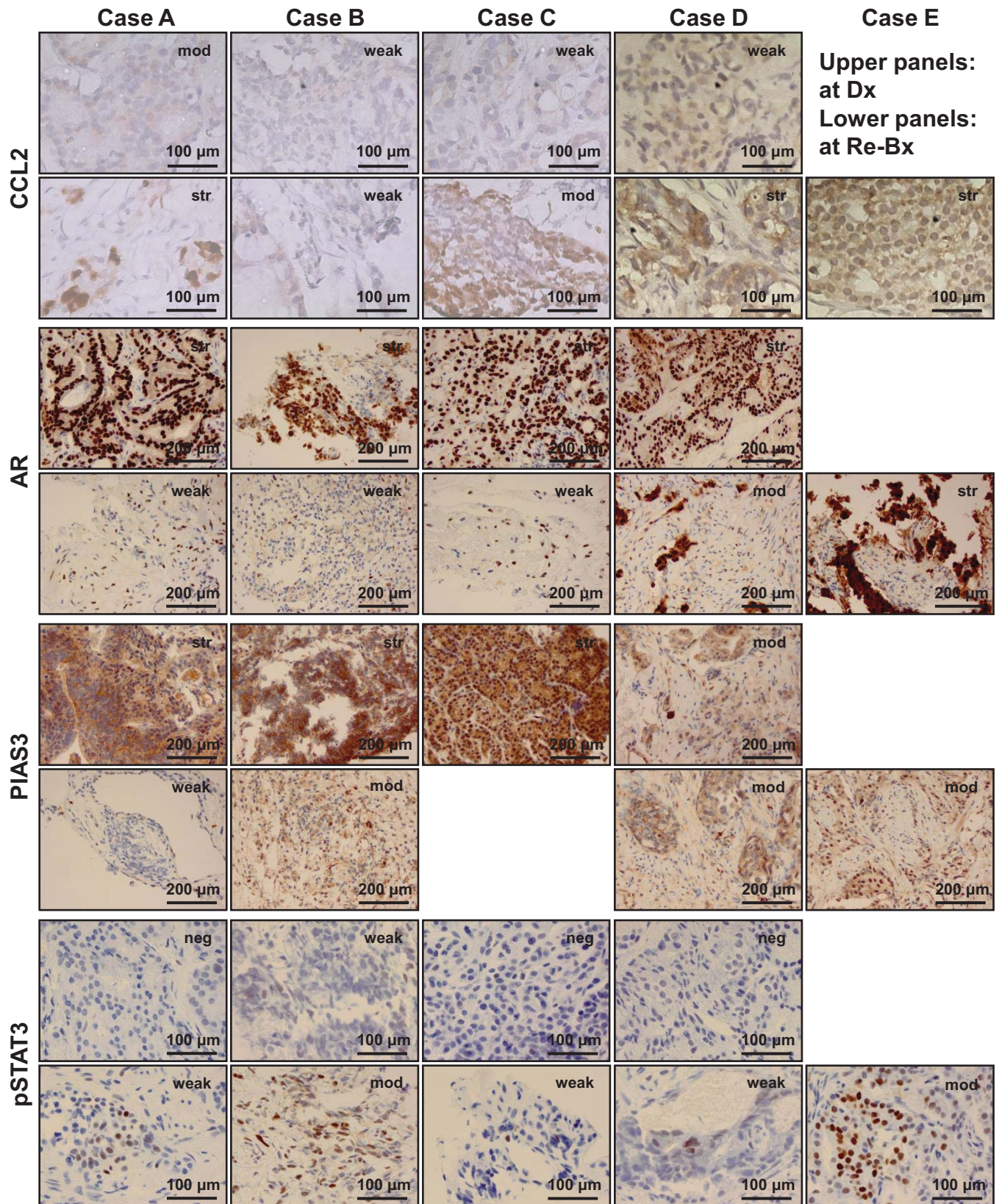
| | | Case A | Case B | Case C | Case D | Case E |
|-------------|-----------|---------|--------|---------|---------|--------|
| Age (year) | at Dx | 53 | 64 | 72 | 73 | N/A |
| | at Re-Bx | 57 | 68 | 76 | 77 | 80 |
| PSA (ng/ml) | at Dx | 46.4 | 196.6 | 71 | 246 | N/A |
| | at Re-Bx* | 0.017 | 11.326 | 0.194 | 198.079 | 185.25 |
| TNM | at Dx | T3aN1M0 | T4N1M0 | T3aN0M0 | T4N0M1b | N/A |
| GS | at Dx | 3+4 | 5+5 | 4+4 | 4+5 | N/A |

*P=0.038

B



Supplementary Fig. S7



Supplementary Fig. S8

