Immunosuppressive plasma cells impede T-cell-dependent immunogenic chemotherapy

Shabnam Shalapour1,2, Joan Font-Burgada1,2, Giuseppe Di Caro1,2, Zhenyu Zhong1,2, Elsa Sanchez-Lopez1,2, Debanjan Dhar1,2, Gerald Willimsky3, Massimo Ammirante1,2, Amy Strasner1,2, Donna E. Hansel2, Christina Jamieson4, Christopher J. Kane4, Tobias Klatte5, Peter Birner6, Lukas Kenner6,7 & Michael Karin1,2

Cancer-associated genetic alterations induce expression of tumour antigens that can activate CD8+ cytotoxic T cells (CTLs), but the microenvironment of established tumours promotes immune tolerance through poorly understood mechanisms1,2. Recently developed therapeutics that overcome tolerogenic mechanisms activate tumour-directed CTLs and are effective in some human cancers1. Immune mechanisms also affect treatment outcome, and certain chemotherapeutic drugs stimulate cancer-specific immune responses by inducing immunogenic cell death and other effector mechanisms3,4. Our previous studies revealed that B cells recruited by the chemokine CXCL13 into prostate cancer tumours promote the progression of castrate-resistant prostate cancer by producing lymphotoxin, which activates an IkB kinase (IKK)-BMI1 module in prostate cancer stem cells5.6. Because castrate-resistant prostate cancer is refractory to most therapies, we examined B cell involvement in the acquisition of chemotherapy resistance. Here we focus on oxaliplatin, an immunogenic chemotherapeutic agent7,8 that is effective in aggressive prostate cancer9. We show that mouse B cells modulate the response to low-dose oxaliplatin, which promotes tumour-directed CTL activation by inducing immunogenic cell death. Three different mouse prostate cancer models were refractory to oxaliplatin unless genetically or pharmacologically depleted of B cells. The crucial immunosuppressive B cells are plasmacytoid (p) cells that express IgA, interleukin (IL)-10 and programmed death ligand 1 (PD-L1), the appearance of which depends on TGFβ receptor signalling. Elimination of these cells, which also infiltrate human-therapy-resistant prostate cancers1,2, allows CTL-dependent eradication of oxaliplatin-treated tumours.

Using the autochthonous transgenic adenocarcinoma of the mouse prostate (TRAMP) model of metastatic prostate cancer (PC), we examined how lymphocytes affect the response to low-dose oxaliplatin. Although early (≤0.2 g) tumours responded to oxaliplatin regardless of B cell status (Extended Data Fig. 1a, b), after reaching ≥0.7 g, wild-type tumours became largely resistant to ‘late’ chemotherapy (Fig. 1a). However, tumours arising in B-cell-deficient TRAMP;Jh−/− hybrid mice were oxaliplatin sensitive (Fig. 1a), although B cells had little effect on tumour progression and histology (Extended Data Fig. 1c, d). CD8+ cell-deficient TRAMP;Cda8a−/− mice bearing small tumours were less responsive to oxaliplatin, but large tumours were treatment-resistant (Fig. 1a and Extended Data Fig. 1b). Similar results were obtained by subcutaneous transplantation of mouse Myc-CaP (MC) prostate cancer cells3. Whereas small MC tumours (≤100 mm3) were chemotherapeutic responsive in wild-type mice (Extended Data Fig. 1e, f), large MC tumours (≥350–400 mm3) shrank after oxaliplatin treatment only in Jh−/− (also known as IgJ−/−) mice (Fig. 1b–d). No response was observed in Cda8a−/− mice. Oxaliplatin responsiveness was associated with enhanced caspase 3 activation, but the tumoral DNA damage response measured by histone H2AX phosphorylation was similarly activated by oxaliplatin, regardless of the host genotype (Fig. 1e and Extended Data Fig. 1g–i). Oxaliplatin treatment increased tumour-infiltrating CD45+ cells in wild-type and Jh−/− mice, but myofibroblast activation and CD31 infiltration was more pronounced in wild-type mice (Extended Data Fig. 1j–l). Low-dose oxaliplatin enhanced TRAMP mouse survival in a manner dependent on CTLs and inhibitable by B cells (Extended Data Fig. 1m, n). B cell immunodepletion also enhanced oxaliplatin-induced tumour regression and the effect was CTL-dependent (Fig. 1f).

Oxaliplatin stimulated CD8+ cell recruitment in TRAMP and TRAMP;Jh−/− mice, although more tumoral CD8+ cells were found in the latter (Fig. 2a and Extended Data Fig. 2a). B cell deficiency also enhanced oxaliplatin-induced CD8+ and CD4+ cell recruitment into MC tumours and induction of perforin, interferon-γ (IFNγ) and tumour necrosis factor (TNF) in CD8+ cells (Fig. 2b–e and Extended Data Fig. 2b–e). MC tumours in Jh−/− mice contained more CD8+ cells with activated STAT1, more proliferative CD8α+ CD444+GrzB+ Ki67+ cells and fewer exhausted CD8α+ CD444- PD-1+ Tim-3- and CD8+ BTLA- cells, the presence of which in wild-type tumours was increased by oxaliplatin (Fig. 2f–h and Extended Data Fig. 2f–i). B cell immunodepletion also enhanced tumoral CTL activation (Extended Data Fig. 2j–p). Oxaliplatin treatment greatly increased the number of tumoral B220+ CD19+ B cells (Fig. 3a and Extended Data Fig. 3a, b), after 3–4 treatment cycles, at least 40% of tumoral B cells were CD20low CD19+ B220lowCD138+ plasma cells, 40–80% of which expressed IgA (Fig. 3b, c and Extended Data Fig. 3c–l). IgA− B cells became detectable 48 h after the first treatment cycle, and their abundance increased to nearly 80% of B220low cells after additional cycles (Extended Data Fig. 3g, i). When cultured ex vivo, tumoral IgA− B cells released soluble IgA (Extended Data Fig. 4a). Oxaliplatin also increased serum IgA in both TRAMP and MC-tumour models, but had little effect on serum IgG (Extended Data Fig. 4b–e). Plasmocytic IgA+ cells were found adjacent to α-smooth muscle actin (α-SMA)-expressing myofibroblasts (Fig. 3d), which produce CXCL13 (ref. 10). Oxaliplatin-induced IgA− B cells from spleen and MC tumours expressed activation-induced cytidine deaminase (Extended Data Fig. 4f, g), suggesting recent class-switch recombination (CSR).

The IgA CSR is mainly induced by TGFβ together with CD40L, IL-21, IL-10 or IL-6 (ref. 11). Indeed, oxaliplatin increased the proportion of tumoral B cells containing phosphorylated SMAD2/3, and induced Tgfb1 messenger RNA in tumours (Fig. 3e and Extended Data Fig. 4h–j). Oxaliplatin also increased IL-21 expression and STAT3 phosphorylation in tumoral B cells (Extended Data Fig. 4k, l), as well as I Il10 mRNA in tumours, tumoral IL-10-producing B cells and IL-10 content per B cell (Fig. 3f, g and Extended Data Fig. 4m). Nearly 50% of

1Laboratory of Gene Regulation and Signal Transduction, Department of Pharmacology, School of Medicine, University of California San Diego (UCSD), 9500 Gilman Drive, San Diego, California 92093, USA. 2Department of Pathology, School of Medicine, University of California San Diego, 9500 Gilman Drive, San Diego, California 92093, USA. 3Institute of Immunology, Charité Campus Buch, 13125 Berlin, Germany. 4Department of Surgery, Urology Division, University of California San Diego, 3855 Health Sciences Drive, San Diego, California 92093, USA. 5Department of Urology, Medical University of Vienna, 1090 Vienna, Austria. 6Department of Pathology, Medical University of Vienna, 1090 Vienna, Austria. 7Clinical Institute of Pathology, Ludwig Boltzmann Institute for Cancer Research, Medical University of Vienna, Unit of Pathology of Laboratory Animals (UPLA), University of Veterinary Medicine Vienna, 1210 Vienna, Austria.
IgA+CD19+ plasmocytes contained IL-10 mRNA and protein (Fig. 3h, i and Extended Data Fig. 4n). Oxaliplatin induced the production of Fas ligand (Fas-L) and PD-L1 in about 50% of IgA+ plasmocytes, 40% of which expressed both PD-L1 and IL-10 (Fig. 3j, k and Extended Data Fig. 3f–j). Most PD-L1+ cells expressed IgA and contained phosphorylated SMAD2/3 (Extended Data Fig. 4j). However, lymphotoxin α/β (LTα/β)-producing B cells did not express IL-10 and their abundance was barely increased by oxaliplatin (Extended Data Fig. 4o, p). Tumoral CD19+ cells did not express CD5, a B regulatory (Breg) cell marker12 (Extended Data Fig. 4q). Oxaliplatin induced other immunoregulatory molecules, including NOS2, ARG1, IL-12p35 and IL-12p40, but no differences were observed between tumour-bearing wild-type and Jh−/− mice, although the latter expressed higher amounts of IL-12 (Extended Data Fig. 5a–d). B cell deficiency or depletion had no significant effect on tumoral normal killer cells, myeloid CD11b+Gr1+ cells, macrophages or Treg cells (Extended Data Fig. 5e–i). Thus, unlike mouse skin cancer, in which B cells modulate therapeutic responsiveness through macrophages13, B cells in mouse PC impede immunogenic chemotherapy by suppressing CTL activation.

Human PC samples (n = 110) were analysed for CD8+ and CD20+ cells (Extended Data Fig. 6a, b). Comparison of matched normal and tumour tissues from 87 patients with early-stage PC (E-PC) indicated higher CD8+ and CD20+ counts in tumours (Extended Data Fig. 6c, d). Patients with therapy-resistant PC (TR-PC) or metastatic PC (M-PC) exhibited reduced tumoral CD8+ cell density relative to patients with E-PC, the tumours of which contained fewer B cells than TR-PC and M-PC, in which B cells were most abundant (Extended Data Fig. 6e, f). E-PC specimens displayed higher CD8/CD20 ratios than specimens from TR-PC and M-PC (Extended Data Fig. 6g). Immunofluorescence and immunohistochemical analyses of human PC specimens revealed IgA+ cells in a scattered formation, frequently next to αSMA+ myofibroblasts, especially in the high-risk group (Fig. 3i and Extended Data Fig. 6h–j, n). CD20+ B cells were both scattered and clustered in lymphoid follicle-like areas (Extended Data Fig. 6b, k). Human PC also contained IL-10-producing IgA+ CD138+ cells and some IgA+ cells were adjacent to CD8+ T cells and expressed little CD20 (Extended Data Fig. 6i–k). 25% of IgA+ cells in fresh prostatectomy specimens expressed IL-10 and were enriched in the malignant tissue portion (Extended Data Fig. 6l, m). IgA+ CD138+ plasmocytes exhibited higher density in TR-PC and M-PC than E-PC and patients with higher IgA+CD138+ cell counts showed lower CD8/CD20 ratios (Extended Data Fig. 6n–p). Oncomine analysis of human IgA (IGHA1) mRNA revealed increased levels in malignant versus healthy prostates in 11 out of 15 data sets. Of these, five showed...
a significant increase (P < 0.05) and three showed a >twofold change. Results of one analysis14 are presented (Extended Data Fig. 6q) and fit earlier findings in mice15,13, suggesting that tumour infiltrating lymphocytes also control malignant progression and response to therapy in human PC.

Consistent with previous knowledge11 and SMAD2/3 activation in PD-L1+ cells, TGFBR2 ablation in B cells (Tgfr2+/-) enhanced oxaliplatin-induced tumour regression, mildly decreased tumour-infiltrating, but not splenic, B cells, and inhibited oxaliplatin-induced IgA+ plasmocyte generation without affecting IgGl+ or IgG2a+ cells (Fig. 4a–d and Extended Data Fig. 7a–e). IgA ablation also potentiated oxaliplatin responsiveness without reducing tumoral B cells (Fig. 4a, b). Both TGFBR2 and IgA ablations prevented induction of tumoral PD-L1+ or IgA+IL-10+ B cells by oxaliplatin, but barely affected IL-10 in B220+IgA- B cells (Extended Data Fig. 7f, g). TGFBR2 ablation or IgA deficiency also increased tumoral CT1 density, IFNγ production and surface CD107α expression by CD8+ T cells of oxaliplatin-treated mice (Fig. 4e, f). Suppressor B cells may attenuate T cell activation via PD-L1 (ref. 16). Treatment of mice bearing MC tumours with oxaliplatin plus anti-PD-L1, but not anti-PD-L1 alone, inhibited tumour growth, increased GrzB expression by effector T cells, downregulated PD-L1 expression on IgA+ cells, and reduced serum IgA, but not IgG (Extended Data Fig. 7h–m). Reconstitution of tumour-bearing Jhα–/– hosts with B cells lacking either PD-L1 or IL-10 failed to inhibit oxaliplatin-induced tumour regression (Fig. 4g and Extended Data Fig. 7n–p). PD-L1 ablation did not affect IL-10 expression, and IL-10 ablation had no effect on PD-L1 (Extended Data Fig. 10m, n), indicating that both molecules are needed for plasmocyte-mediated immunosuppression.

We used oxaliplatin because of its well-described immunogenic properties, which are not exhibited by the related compound cisplatin14. Both oxaliplatin and cisplatin induced apoptotic cell death but oxaliplatin was better in stimulating autophagy (Extended Data Fig. 8a, b). Importantly, only low-dose oxaliplatin induced regression of MC tumours in Jhα−/− mice, whereas low-dose cisplatin was ineffective, and only oxaliplatin increased the abundance of tumoral CD8+ and CD4+ cells (Extended Data Fig. 8c–e). Low-dose oxaliplatin did not increase intestinal permeability and had no effect on IgA production and other immune parameters in tumour-free wild-type or Tgfr2+/- mice (Extended Data Fig. 8f–k).

Immunogenic chemotherapy also potentiates the effectiveness of adoptively transferred T cells (ATCT). Immunogenic TRAMP-C2 cells15 were inoculated into B-cell-containing Tcrb−/− mice followed by oxaliplatin treatment and ATCT (Extended Data Fig. 9a). Bigger tumours in Tcrb−/− relative to wild-type mice confirmed TRAMP-C2 immunogenicity (Extended Data Fig. 9b). However, despite successful T cell take and increased CD8+ counts after oxaliplatin treatment, tumours were not rejected (Extended Data Fig. 9c–e). TRAMP-C2 tumours were also raised in Rag1−/−;OT-1 mice, which lack B cells and polyclonal T cells but contain CD8+ cells directed against chicken ovalbumin (Ova)18. Adoptively transferred CD8+ cells expanded and expressed GrzB in Rag1−/−;OT-1 hosts, especially after oxaliplatin treatment (Extended Data Fig. 9f–h). Consequently, tumour growth was inhibited by ATCT combined with oxaliplatin (Extended Data Fig. 9i, j). More notable results were obtained in TRAMP;Rag1−/− mice transplanted with CFSE-labelled splenocytes from either naive wild-type (B and T cell transfer) or Jhα−/− (T cell transfer) mice (Extended Data Fig. 9k). CD8+ cell proliferation in bone marrow, spleens and prostates of transplanted mice indicated successful T cell take (Extended Data Fig. 9l, m). Thirty days after lymphocyte transfer, prostate tumours were analysed. Oxaliplatin caused modest tumour shrinkage in mice receiving T and B cells, but in mice receiving only T cells it induced complete regression (Fig. 5a, b). Transplantation with T and B cells combined with oxaliplatin restored CD19+ cells in spleen and prostate and serum IgA and IgG, including IgA and IgG directed against SV40 T antigen, the TRAMP oncogene (Fig. 5c–e and Extended Data Fig. 9n), indicating a tumour-specific humoral response. Transferred B cells expressed TIM-1 (Extended Data Fig. 9o), a molecule involved in regulation of IL-10 expression and tolerance induction19. B and T cell co-transplantation led to the appearance of

---

**Figure 3** Oxaliplatin induces tumour infiltration with IgA+PD-L1+IL-10-producing plasmocytes. a, B220+CD19+ B lymphocytes in 20-week-old TRAMP mice prostates after four oxaliplatin cycles (n = 5–7 per group) normalized to prostate weights. b, c, B220, CD19, CD138 and IgA expression in tumoral B cells from a. Values are percentage of tumoral CD45+ (b) or CD19+ (c) cells. MC tumours (n = 4–5 per group) stained for zSMA (green) and IgA (red). Nuclei counterstained blue with DAPI (4',6-diamidino-2-phenylindole). Arrows denote IgA+ cells, the numbers of which per HMF are displayed on the bottom. e, Phosphorylated SMAD2/3 (p-SMAD2/3) in tumour-infiltrating B cells (n = 3–4 per group). f, Il10 mRNA in MC tumours (n = 5–6 per group). g, Tumour-infiltrating IL-10+CD19+ B cells in MC-WT mice, as percentages of CD45+ cells. h, Percentages of IL-10-producing cells in tumoral (MC-WT) CD19+IgA+ and CD19+IgA- cells. i, IL-10 expression by tumoral (MC-WT) IgA+ and IgA- B cells (n = 4–6 per group). j, PD-L1 and Fas-L expression in B cells from TRAMP tumours. k, Pdli mRNA in MC tumours (n = 5–6 per group). l, Low- (n = 5) and high- (n = 3) risk human PC specimens stained with IgA (red) and zSMA (green) antibodies. Arrows denote IgA+ cells. Results are mean ± s.e.m. of at least three independent experiments. *P < 0.05; **P < 0.01; ***P < 0.001 (Mann–Whitney and t-tests).
CD4+ and CD8+ cells in lymphoid organs, but T cell number was considerably lower in prostate tumours (Fig. 5f and Extended Data Fig. 9g–i). However, transplantation with B-cell-deficient splenocytes caused robust T cell infiltration into prostate tumours (Fig. 5f and Extended Data Fig. 9g–i). To confirm that IgA−B cells attenuate the response to immunogenic chemotherapy by inhibiting T cell activation, we raised MC tumours in Rag1−/− mice and transplanted them with T cells from wild-type mice immunized with MC cell lysate, with or without naive B cells from wild-type or Tgfbr2−/− spleens. In this case, oxaliplatin induced tumour regression and CTL activation only in mice receiving T cells, or T cells and TGF-B cells, which produced little IgA (Fig. 5g–i and Extended Data Fig. 10a–c). Hence, only B cells that have undergone TGFBR signalling and IgA CSR suppress CTL activation.

Figure 5 | Adoptively transferred B cells inhibit T-cell-dependent tumour eradication. a, b, TRAMP;Rag1−/− mice (16 weeks old) received weekly oxaliplatin. One day after the first treatment, CFSE-labelled splenocytes from wild-type or ifn−− mice were adoptively transferred into tumour-bearing mice (n = 4–5 per group). After three more oxaliplatin cycles, the prostates were photographed (a) and tumour weight was measured (b). c, Serum IgA in both adoptive cell transfer (ACT) groups and FVB wild-type mice. d, e, Serum anti-SV40-Tag IgA and IgG concentrations in indicated strains with or without ACT and/or oxaliplatin treatment. f, Frequency of CD8+ cells amongst CD45+ cells in TRAMP;Rag1−/− prostates after ACT and oxaliplatin treatment. g, MC tumour-bearing Rag1−/− mice were treated with oxaliplatin. One day later, mice (n = 4–5 per group) received activated T cells from wild-type mice immunized with MC cell extract without or with B cells from wild-type or Tgfbr2−/− mice. After two more treatments, mice were euthanized and tumour volumes determined. h, IFNγ in tumoral CD8+ cells of above mice. Cells were restimulated with PMA and ionomycin before determining the percentages of IFNγ-expressing cells in total CD8+ cells (n = 4–5 per group). i, Serum IgA in above mice. Results are mean ± s.e.m. *P < 0.05; **P < 0.01; ***P < 0.001 (Mann–Whitney and t-tests). ND, not detectable.
Our results show that successful eradication of large prostate tumours by immunogenic chemotherapy requires removal of immunosuppressive IgA+ plasmocytes that are present both in mouse and human PC. Spontaneous and transplantable PC models contain IgA+ plasmocytes that strongly suppress CTL activation after treatment with oxaliplatin, an immunogenic cell death inducer. Although oxaliplatin causes regression of small tumours, it does not activate CTLs or shrink large prostate tumours, despite inducing DNA damage, unless tumour-infiltrating immunosuppressive B cells are removed. These B cells are IgA-producing plasmocytes that express PD-L1, IL-10 and Fas-L. Genetic analysis confirms that much of the immunosuppressive activity derives from IgA+PD-L1+IL-10+ cells. Development of these cells, which differ from the lymphoxygen-producing CD20+ B cells that infiltrate androgen-deprived prostate tumours and stimulate castrate-resistant prostate cancer emergence through the IKKζ-BMI1 module, depends on TGFβR signalling. Nonetheless, CD20+IL-10+ B cells that are exposed to high TGFβ concentrations and antigen in the PC microenvironment after oxaliplatin treatment may eventually become IgA+ plasmocytes. A likely source of TGFβ is sStMA+ myofibroblasts that reside next to IgA+ cells in oxaliplatin-treated mouse tumours and human PC samples. Alternatively, lymphoxygen-producing B cells may stimulate the IgA CSR, as signalling via LTβ receptor on gut stromal cells is required for IgA production. Although the anti-inflammatory and regulatory activities of intestinal IgA-producing cells, as well as other plasmocytes, are well known, this is the first time, to our knowledge, IgA+ plasmocytes were found to suppress anti-tumour immunity.

IgA+ plasmocytes within prostate tumours induce CD8+ cell exhaustion and suppress anti-tumour CTL responses through PD-L1 and IL-10, either of which can induce anergy or exhaustion. Yet, B cells may regulate anti-tumour immunity by other mechanisms, including indirect control of T cell infiltration via macrophages and IL-10 production by Breg cells, although the latter only affect CD4+ T helper cells. Notably, IL-10-expressing IgA+ cells are most abundant in therapy-resistant and metastatic human PC and circulating IgA is a well-established adverse prognostic indicator in PC. We therefore suggest that elimination or inhibition of tumour infiltrating IgA+ plasmocytes may be the key to successful immunotherapy of PC, as long as an immunogenic chemotherapeutic, such as oxaliplatin, is also used. Immunogenic chemotherapeutic may also enhance response rates to PD-1 or PD-L1 blockade in other malignancies, including bladder cancer and cutaneous melanoma in which only 35% of the patients exhibit a response.

Online Content Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.

Received 4 August 2014; accepted 9 March 2015.

Published online 29 April 2015.

1. Chen, D. S. & Mellman, I. Oncology meets immunology: the cancer-immunity cycle. Immunity 39, 1–10 (2013).
2. Schietinger, A. & Greenberg, P. D. Tolerance and exhaustion: defining mechanisms of T cell dysfunction. Trends Immunol. 35, 51–60 (2014).
3. Zitvogel, L., Galluzzi, L., Smyth, M. J. & Kroemer, G. Mechanism of action of conventional and targeted anticancer therapies: reinstating immunosurveillance. Immunity 39, 74–88 (2013).
4. Kroemer, G., Galluzzi, L., Kepp, O. & Zitvogel, L. Immunogenic cell death in cancer therapy. Annu. Rev. Immunol. 31, 51–72 (2013).
5. Ammirante, M., Luo, J. L., Grivenny, S., Nedospasov, S. & Karin, M. B-cell-derived lymphomatrix promotes castration-resistant prostate cancer. Nature 464, 302–305 (2010).
6. Ammirante, M. et al. An IKKζ-ESF1-BMI1 cascade activated by infiltrating B cells controls prostate regeneration and tumor recurrence. Genes Dev. 27, 1435–1440 (2013).
7. Lee, J. L. et al. Gemcitabine-oxaliplatin plus prednisolone is active in patients with castration-resistant prostate cancer for whom docetaxel-based chemotherapy failed. Br. J. Cancer 110, 2472–2478 (2014).
8. Kaplan-Liebo, P. J. et al. Prognostic impact of an immunohistochemical analysis of prostate cancer in a preclinical transgenic mouse model. Prostate 55, 219–237 (2003).
9. Watson, P. A. et al. Context-dependent hormone-refractory progression revealed through characterization of a novel murine prostate cancer cell line. Cancer Res. 64, 11565–11571 (2004).
10. Ammirante, M., Shalapour, S., Yang, J., Jamieson, C. A. & Karin, M. Tissue injury and hypoxia promote malignant progression of prostate cancer by inducing CXCL13 expression in tumor myofibroblasts. Proc. Natl Acad. Sci. USA 111, 14776–14781 (2014).
11. Cerutti, A. The regulation of IgA class switching. Nature Rev. Immunol. 8, 421–438 (2008).
12. Yoshizaki, A. et al. Regulatory B cells control T-cell autoimmunity through IL-21-dependent cognate interactions. Nature 491, 264–268 (2012).
13. Affara, N. L. et al. B cells regulate macrophage phenotype and response to chemotherapy in squamous carcinomas. Cancer Cell 25, 809–821 (2014).
14. Yu, D. P. et al. Gene expression alterations in prostate cancer predicting tumor aggression and preceding development of malignancy. J. Clin. Oncol. 22, 2790–2799 (2004).
15. Luo, J. L. et al. Nuclear cytokine-activated IKKα controls prostate cancer metastasis by repressing Maspin. Nature 446, 690–694 (2007).
16. D"oi, T. et al. IgA plasma cells express the negative regulatory co-stimulatory molecule programmed cell death 1 ligand and have a potential tolerogenic role in the intestine. Biochem. Biophys. Res. Commun. 425, 918–923 (2012).
17. Foster, B. A., Gingrich, J. R., Kwon, E. D., Madias, C. & Greenberg, N. M. Characterization of prostatic epithelial cell lines derived from transgenic adenocarcinoma of the prostate (TRAMP) model. Cancer Res. 57, 3325–3330 (1997).
18. Hogquist, K. A. et al. T cell receptor antagonist peptides induce positive selection. Nature 376, 17–27 (1994).
19. Xiao, S. et al. Defect in regulatory B-cell function and development of systemic autoimmunity in T-cell Ig mucin 1 (Tim-1) mucin domain-mutant mice. Proc. Natl Acad. Sci. USA 109, 12015–12110 (2012).
20. Fang, H. S. et al. Signaling via LTβR on the lamina propria stromal cells of the gut is required for IgA production. Nature Immunol. 3, 576–582 (2002).
21. Feng, T., Elson, C. O. & Cong, Y. T cell- IgA axis in maintenance of host immune homeostasis with microbiota. Int. Immunopharmacol. 11, 589–592 (2011).
22. Kagan, H. et al. IL-35-producing B cells are critical regulators of immunity during autoimmune and infectious diseases. Nature 507, 366–370 (2014).
23. Parodi, D. M. The blockade of immune checkpoints in cancer immunotherapy. Nature Rev. Cancer 12, 252–264 (2012).
24. Ono, Z. et al. B cells inhibit induction of T cell-dependent tumor immunity. Nature Med. 4, 627–630 (1998).
25. Oltman, P. B. et al. Tumor-evoked regulatory B cells promote breast cancer metastasis by converting resting CD4+ T cells to T-regulatory cells. Cancer Res. 71, 3505–3515 (2011).
26. Freund, C., Schuett, F., Sohn, C., Beckhover, P. & Domshlie, C. B cell-regulated immune responses in tumor models and cancer patients. OncoImmunology 2, e25443 (2013).
27. Shah, N. Diagnostic significance of levels of immunoglobulin A in seminal fluid of patients with prostate disease. Urology 8, 270–272 (1976).
28. Schumacher, T. N., Kesmir, C. & van Buuren, M. M. Biomarkers in cancer immunotherapy. Cancer Cell 27, 12–14 (2015).

Supplementary Information is available in the online version of the paper.

Acknowledgements We thank L. Bastian, K. Wang, A. Umemura, M. K. Kim, M. Susani, E. Gurnhofer and F. Grizzi for discussions and research materials. Antibodies and MACS-beads were gifts from eBioscience, Biologens and Miltenyi Biotec. Anti-PD-L1 and IL-10 mouse were from L. Mellman (Genentech). Research was supported by the National Institutes of Health (NIH) (CA127923 and AI043477), DFG and German Cancer Consortium (TR36, DKTK to G.W.), the Genome Research-Austria project ‘Inflammbiota’ (FWF and P26011 to L.K.), the CureSearch Foundation (to D.D.) and postdoctoral research fellowships from the German Research Foundation (DFG, SFB721, 11565–3; SFB721-D1), the Austrian Science Fund (FWF and P26011 to L.K.) and the FIRC/ARC (to G.D.C.) M.K. is an ACS Research Professor and holds the Ben and Wanda Hildyard Chair for mitochondrial and Metabolic Diseases.

Author Contributions M.K. and S.S. conceived and designed the project. S.S. performed experiments. S.S. and M.K. analysed data. J.F.B., Z.Z., D.D., M.A. and S.S. were assisted with experiments and analysis. S.S., G.D.C., E.S.-L. and D.E.H. performed immunohistochemical analyses of human samples. O.W. performed Tag-specific ELISA. D.E.H., C.J., P.B., C.J.K., T.K. and L.K. collected and provided human specimens. M.K. and S.S. wrote the manuscript, with all authors contributing to writing and providing feedback.

Author Information Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests.

Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to M.K. (karinoffice@ucsd.edu).
METHODS

Animal models. C57BL/6 and FVB control mice were from Charles River Laboratories and CD45.1 mice were from the Jackson Laboratory, and all were bred at the University of California San Diego (UCSD) animal facility. C57BL/6-TgTRAMP8247Ng1 (TRAMP mice) were backcrossed to the FVB strain for more than ten generations. The median survival of TRAMP-FVB mice was 23 weeks compared to 52 weeks for TRAMP-C57BL/6 mice. TRAMP mice were crossed with B-cell-deficient (Jh-/-) mice, CTL-deficient (Cd8a-/-) mice or Rag1-/- mice, which lack both B and T cells, all in the FVB background. OT-I mice were obtained from Taicsonic. Tgβr2-/- (FVB-background) mice were obtained from H. Moses. Tcrb-/-, CD19-Cre, Il10-/- and CD45.1 mice were purchased from the Jackson Laboratory. IgM- and IgD-deficient (Igµ-/- and Igδ-/-) mice were obtained from Bayelor College of Medicine. Pdl1/2-/- mice were obtained from Genentech. CD19-Cre and Iga+/+ mice were backcrossed to the FVB strain for more than ten generations. All mice were maintained in filter-topped cages on autoclaved food and water at the UCSD animal facility and all experiments were performed in accordance with UCSD and NIH guidelines and regulations.

Mouse treatment studies were matched design control trials. Accordingly, mice were randomly chosen and paired based on sex (male), age and tumour size (Extended Data Fig. 1a, e). For transfected tumour models, tumour size was defined by the median tumour volume (for example, 400 mm3, for late treatments, Extended Data Fig. 1f). For TRAMP transgenic tumour models, treatment decisions were made based on age and mice were randomly chosen including a control littermate (Extended Data Fig. 1a). An identification code was assigned to each tumour-bearing mouse both in the transplanted and transgenic models, and the investigators were blinded to treatment allocation at the time of tumour volume measurement, autopsy and analysis. The number of mice used in each experiment and the number of experiments (replications) are shown in Supplementary Table 1.

Flow cytometry and lymphocyte isolation. For lymphocytes isolated from spleen and lymph nodes, standard protocols using filters have been used. Lymphocytes were isolated from human blood using Ficoll–Paque PLUS (GE Healthcare Life Science) according to manufacturer’s recommendations. For lymphocyte isolation from tumours (mouse and human), tumours were cut into small pieces and incubated in dissociation solution (RPMI medium supplemented with 5% FBS, collagenase type I (200 U ml-1), collagenase type IV (200 U ml-1) and DNase I (100 μg ml-1)) for 30 min at 37 °C. After incubation, cell suspensions were passed through a 50-μm cell strainer and washed twice. For large tumours (>0.7 g), haematopoietic cells were pre-enriched using density gradient centrifugation (Percoll or Ficoll). Small tumours (≤0.2 g) were pooled (two tumours per staining). Red blood cells were lysed (RBC Lysis buffer, multi-species; eBioscience). For blocking of Fc-mediated interactions, mouse cells were pre-incubated with FcR blocking reagent (Miltenyi Biotec). Isolated cells were stained with labelled antibodies of interest. Moreover, BD transcription factor buffer was used for Foxp3 and T-bet staining and BD Actinomycin DIII was used for Gzma staining (BD Biosciences). Dead cells were excluded based on staining with Live/Dead fixation dye (Invitrogen). For Foxp3 staining, a permeabilization solution (BD Perm/Wash buffer) was used. For intracellular cytokine staining, cells were permeabilized (Mycoprep solution; eBioscience, as indicated) in the presence of a protein transport inhibitor cocktail containing brefeldin A and monensin (eBioscience), as indicated. For CD107, a staining antibody was added to the culture during the stimulation. After 5 h, cells were fixed and permeabilized with BD Cytofix/Cytoperm reagent for cytokine staining. BD Transcription factor buffer was used for Foxp3 and T-bet staining and BD Phosflow was used for p-SMA/D2/3 and p-STAT staining (BD Biosciences) according to manufacturer’s recommendations. After fixation/permeabilization, cells were stained with labelled antibodies of interest. Moreover, Il10 and Actb mRNA expression were analysed on single cell level by flow cytometry in combination with CD45, IgA and IL-10 protein staining, using FlowRNA II Assay kit (Alfisystem biotechnology) according to manufacturer’s protocols137. Cells were analysed on a Beckman Coulter Cyan ADP flow cytometer. Data were analysed using FlowJo software (Treestar). Immune cell analysis of tumour-free mice of different genetic backgrounds (C57BL/6 and FVB) and different genetic ablations are shown in Extended Data Fig. 10d–p. The gating strategies and isotype controls for p-STAT1 and IL-10 staining are shown in Extended Data Fig. 10q–u.

Adoptive lymphocyte transfer. For ATCT, CD8+ T cells were isolated from single cell suspensions, prepared from spleens and lymph nodes as described above, using CD8α-specific microbeads and MACS-columns (both Miltenyi Biotec GmbH), and 5 × 106 CD8+ T cells were transferred intraperitoneally (Extended Data Fig. 9a–j). For adoptive B cell transfer (ABC), B cells (B220-CD19-) were isolated from single-cell suspensions prepared from spleens using CD19- and B220-specific microbeads and MACS-columns, and 5 × 106 B cells were transferred intraperitoneally (Fig. 4g and Extended Data Fig. 7n–p). For adoptive spleenocytes transfer (ACT), single cell suspensions prepared from spleens were transferred intraperitoneally, with one total spleen injected per mouse. Labelling with 5- and 6-carboxyfluorescein diacetate succinimidyl ester (CFSE; Molecular Probes) was done according to manufacturer’s protocol. Five million CD8+ T cells or 7 × 106 B cells were transferred (equal to one spleen per mouse; Fig. 5a–f and Extended Data Fig. 9k–r). For combined adoptive B and T cell transfer (Fig. 5g–i and Extended Data Fig. 10a–c), T cells were isolated from wild-type FVB mice immunized with a Myc-Cap cell lysate as previously described138. Specifically, Myc-Cap cells were incubated with oxaliplatin (40 μM) for 48 h. The extent of cell death was determined by flow cytometry, showing that more than 90% of cells were positive for annexin V and propidium iodide. The dead cells were injected subcutaneously into wild-type FVB mice. Seven days later, T cells were isolated from single cell suspensions of spleen and lymph nodes using a Pan T cell isolation kit (Miltenyi Biotec). B cells were isolated from spleens of naïve FVB wild-type or Tgβr2+/- mice using a Pan B cell isolation kit (Miltenyi Biotec). MC-tumour bearing Rag1-/- mice received 5 × 106 T cells with or without 5 × 106 B cells (98% pure) from wild-type or Tgβr2+/- mice. Purity was analysed on a Beckman Coulter Cyan ADP flow cytometer and was always >98%. Absolute numbers of particular immune cells in spleen were calculated by multiplying the CD45 cell number from one spleen by the percentages of the particular cell type amongst CD45+ cells. Absolute numbers of particular immune cells (for example, CD8+ cells) in tumours were calculated by multiplying the cell number in one tumour portion by the percentages of the corresponding cell type in vital tumour cells divided by the weight of the analysed tumour fragment.

Subcutaneous tumour models. Two million Myc-Cap+ or 3 × 106 TRAMP-C2 (ref. 17) cells (purchased from ATCC) were subcutaneously injected into the right flank. Tumours were measured every 2–3 days using a caliper. Tumour volumes were calculated as width2 × length/2.

Immunodepleted B cell depletion. B cells were depleted as previously described139. Mice were weekly injected (intraperitoneally) with a mixture of monoclonal antibodies, each at 150 μg per mouse: rat anti–mouse CD19 (clone 1D3), rat anti-mouse B220 (clone RA36B2), and mouse anti-mouse CD22 (clone CT34). After 48 h, the mice were injected with a secondary antibody (mouse anti-rat κ chain; GenTex) at 150 μg per mouse. In addition, mice were injected weekly, but not on the same day, with 250 μg per mouse anti-mouse CD20 (Genench). Rat anti-mouse IgG2a and IgG1 were used as isotype controls. Mice were treated for 3 weeks in total (Fig. 1f and Extended Data Fig. 2j–p).

Oncomine data analysis. In silico analysis of human IgA (IGHA1) mRNA expression was performed using 15 PC microarray gene data sets140–142 from the Oncomine database (Compendia Biosciences; http://www.oncomine.org)133 comparing a combined 126 carcinoma/adenocarcinoma specimens to 30 normal (either benign, disease-free normal and/or normal adjacent) tissue specimens. Evaluation criteria were set as P < 0.05, fold change >2.0, and gene rank in the top 10%.

Analysis of human specimens. Paraffin-embedded specimens from a total of 110 male patients were integrated into a tissue microarray system (TMA) constructed at the Clinical Institute of Pathology at the Medical University of Vienna (MUV). All of the human specimens used for TMA construction were approved by the MUV Research Ethics Committee (1753/2014). The cohort included 87 patients with E-PC, 9 patients with TR-PC, and 15 patients with M-PC. Patients’ demographic and histopathological features are shown in Supplementary Table 2.

TMA were designed to provide two cores of normal prostate tissue and four cores of PC tissue from each E-PC patient, and 3–6 cores of tumour tissue for each TR-PC and M-PC patient. Stained TMA slides were digitalized by virtual microscopy at 20× magnification with a fixed light intensity and resolution into a bright-field image using the Nanozoomer (Hamamatsu) scanner. Computer-aided analysis of individual TMA core images was used to quantify the percentage of CD8+ and CD20+ immune reactive area (IRA%) as a proportion of the total digitized haematoxylin-stained region, as previously described143. For each PC patient, the mean continuous values of CD8+ and CD20+ IRA% in TMA cores without technical artefact for normal and tumour prostate tissue were calculated and used for subsequent statistical analysis. The presence of CD138+ and IgA+ double immunoreactivity for plasma cells in the stromal compartment or directly contacting a cancer cell was semiquantitatively scored in TMA cores for each patient by an investigator who was blinded to the patients’ tumour features. A value of 0 was assigned to tissue cores without evidence of stromal CD138+IgA+ double immunoreactive cells and a value of 1 was recorded when CD138+IgA+ double immunoreactive cells were present in the stromal compartment. Furthermore, after approval from the UCSD Institutional Review Board, whole tissue slides were subjected to immunohistochemical (IHC) analysis of sMAMA+ and IgA. CD8+IgA and IL-10+IgA double staining from a cohort of formalin-fixed, paraffin-embedded radical prostatectomy specimens. As previously described133, this cohort included up to 50 patients, which were selected based on known clinical
outcome according to risk categories of low-, intermediate- and high-risk groups based on the D’Amico risk classification 34.

Anonymized free prostatectomy and blood samples from consented human subjects, and de-identified clinical information were provided under the UCSD Moores Cancer Center Biorepository and Tissue Technology Institutional Review Board approved protocol and provided to investigators (M.K., C.J.K., C.A.M.J. and D.E.H.) with Cancer Sample Banking Committee Approval. Fresh, de-identified samples of human prostate tissue and blood in 10-ml EDTA-coated tubes were collected from patients undergoing radical prostatectomy for clinically localized, intermediate or high risk PC, Gleason grade 3+4 or higher. A board-certified genitourinary pathologist (D.E.H.) collected samples of fresh prostate tumour and adjacent benign tissue, within 1 h of radical prostatectomy, that were 5-10 mm in diameter.

Immunostaining. Tissues were embedded in Tissue Tek OCT (Sakura Finetek) compound and snap-frozen. Tissue sections were fixed in cold acetonemethanol or 3% paraformaldehyde for 3-10 min and washed with PBS. Slides were blocked with PBS/1% normal donkey or goat serum for 1 h at room temperature. As negative controls, samples were incubated with isotype-matched control antibodies or secondary antibodies only. After staining with DAPI, sections were covered with Vectorshield Mounting Medium (Vector Laboratories). TMA tissue slides from formalin-processed and paraffin-embedded tumour sections were processed for immunohistochemistry. After de-paraffinization and rehydration, sections were immersed in a pre-heated antigen retrieval water bath at a pH 6.1 citrate buffer, or Dako Target Retrieval Solution for 20 min at 95–96 °C. ImmPRESS Polymer System Diaminobenzidine tetrahydrochloride (DAB) peroxidase substrate-based chromogens were used for single staining of CD8, CD20 and for IgA staining when combined with CD138 and for sSmA staining when combined with IHC for human samples. ImmPACT Vector NovaRED peroxidase substrate-based chromogens were used for CD138 staining when combined with IgA for IHC of human samples. All stainings were done according to the manufacturer’s protocols (Vector Laboratories). Nuclei were lightly counterstained with a freshly made haematoxylin solution then further washed in water and mounted. Sections were examined using an Axioplan 2 microscope with AxioVision Release 4.5 software (Zeiss) or TCS SPE Leica confocal microscope (Leica).

Antibodies. Antibodies specific for the following antigens were used: monoclonal rabbit antibody to cleaved caspase 3 (9696) or p-H2AX (S139; 203E) (Cell Signaling Technology); polyclonal rabbit antibody to: CD3 (Dako, IS03); sSmA (Dako); Tim-3 (B8.C21); Tim-1 (RMT1-4); p-SMAD2/3 (D27F4); LC3B (D11) and CD138 (syndecan-1) (anti-mouse Biolegend; anti-human rabbit antibody to cleaved caspase 3 (9661) or p–Il21 (R&D Systems). Anti-SV40 Tag immunoglobulin ELISA was performed as described 57.

Primers were used: Il21, forward 5’-TGGAGGTTGTTGACGAGGC-3’; reverse 5’-TGGAGGCGCTGATACGCA-3’, 32. Koh, D. R. et al. Cancer Res. 64, 920–929 (2004).

Oxaliplatin was diluted in 5% D.E.H.) with Cancer Sample Banking Committee approval. Fresh, de-identified samples of fresh prostate tumour and adjacent benign tissue, within 1 h of radical prostatectomy, that were 5-10 mm in diameter.

Immuno(Stechnology; polyclonal rabbit antibody to: CD3 (Dako, IS03); sSmA (Dako); Tim-3 (B8.C21); Tim-1 (RMT1-4); p-SMAD2/3 (D27F4); LC3B (D11) and CD138 (syndecan-1) (anti-mouse Biolegend; anti-human rabbit antibody to cleaved caspase 3 (9661) or p–Il21 (R&D Systems). Anti-SV40 Tag immunoglobulin ELISA was performed as described 57.

Primers were used: Il21, forward 5’-TGGAGGTTGTTGACGAGGC-3’; reverse 5’-TGGAGGCGCTGATACGCA-3’, 32. Koh, D. R. et al. Cancer Res. 64, 920–929 (2004).

Oxaliplatin was diluted in 5% D.E.H.) with Cancer Sample Banking Committee approval. Fresh, de-identified samples of fresh prostate tumour and adjacent benign tissue, within 1 h of radical prostatectomy, that were 5-10 mm in diameter.

Immuno(Stechnology; polyclonal rabbit antibody to: CD3 (Dako, IS03); sSmA (Dako); Tim-3 (B8.C21); Tim-1 (RMT1-4); p-SMAD2/3 (D27F4); LC3B (D11) and CD138 (syndecan-1) (anti-mouse Biolegend; anti-human rabbit antibody to cleaved caspase 3 (9661) or p–Il21 (R&D Systems). Anti-SV40 Tag immunoglobulin ELISA was performed as described 57.

Primers were used: Il21, forward 5’-TGGAGGTTGTTGACGAGGC-3’; reverse 5’-TGGAGGCGCTGATACGCA-3’, 32. Koh, D. R. et al. Cancer Res. 64, 920–929 (2004).

Oxaliplatin was diluted in 5% D.E.H.) with Cancer Sample Banking Committee approval. Fresh, de-identified samples of fresh prostate tumour and adjacent benign tissue, within 1 h of radical prostatectomy, that were 5-10 mm in diameter.

Immuno(Stechnology; polyclonal rabbit antibody to: CD3 (Dako, IS03); sSmA (Dako); Tim-3 (B8.C21); Tim-1 (RMT1-4); p-SMAD2/3 (D27F4); LC3B (D11) and CD138 (syndecan-1) (anti-mouse Biolegend; anti-human rabbit antibody to cleaved caspase 3 (9661) or p–Il21 (R&D Systems). Anti-SV40 Tag immunoglobulin ELISA was performed as described 57.

Primers were used: Il21, forward 5’-TGGAGGTTGTTGACGAGGC-3’; reverse 5’-TGGAGGCGCTGATACGCA-3’, 32. Koh, D. R. et al. Cancer Res. 64, 920–929 (2004).

Oxaliplatin was diluted in 5% D.E.H.) with Cancer Sample Banking Committee approval. Fresh, de-identified samples of fresh prostate tumour and adjacent benign tissue, within 1 h of radical prostatectomy, that were 5-10 mm in diameter.

Immuno(Stechnology; polyclonal rabbit antibody to: CD3 (Dako, IS03); sSmA (Dako); Tim-3 (B8.C21); Tim-1 (RMT1-4); p-SMAD2/3 (D27F4); LC3B (D11) and CD138 (syndecan-1) (anti-mouse Biolegend; anti-human rabbit antibody to cleaved caspase 3 (9661) or p–Il21 (R&D Systems). Anti-SV40 Tag immunoglobulin ELISA was performed as described 57.

Primers were used: Il21, forward 5’-TGGAGGTTGTTGACGAGGC-3’; reverse 5’-TGGAGGCGCTGATACGCA-3’, 32. Koh, D. R. et al. Cancer Res. 64, 920–929 (2004).

Oxaliplatin was diluted in 5% D.E.H.) with Cancer Sample Banking Committee approval. Fresh, de-identified samples of fresh prostate tumour and adjacent benign tissue, within 1 h of radical prostatectomy, that were 5-10 mm in diameter.

Immuno(Stechnology; polyclonal rabbit antibody to: CD3 (Dako, IS03); sSmA (Dako); Tim-3 (B8.C21); Tim-1 (RMT1-4); p-SMAD2/3 (D27F4); LC3B (D11) and CD138 (syndecan-1) (anti-mouse Biolegend; anti-human rabbit antibody to cleaved caspase 3 (9661) or p–Il21 (R&D Systems). Anti-SV40 Tag immunog...
46. Tomlins, S. A. et al. Integrative molecular concept modeling of prostate cancer progression. *Nature Genet.* 39, 41–51 (2007).
47. Welsh, J. B. et al. Analysis of gene expression identifies candidate markers and pharmacological targets in prostate cancer. *Cancer Res.* 61, 5974–5978 (2001).
48. Varambally, S. et al. Integrative genomic and proteomic analysis of prostate cancer reveals signatures of metastatic progression. *Cancer Cell* 8, 393–406 (2005).
49. Magee, J. A. et al. Expression profiling reveals hepsin overexpression in prostate cancer. *Cancer Res.* 61, 5692–5696 (2001).
50. Wallace, T. A. et al. Tumor immunobiological differences in prostate cancer between African-American and European-American men. *Cancer Res.* 68, 927–936 (2008).
51. Vanaja, D. K., Cheville, J. C., Iturria, S. J. & Young, C. Y. Transcriptional silencing of zinc finger protein 185 identified by expression profiling is associated with prostate cancer progression. *Cancer Res.* 63, 3877–3882 (2003).
52. Luo, J. H. et al. Gene expression analysis of prostate cancers. *Mol. Carcinog.* 33, 25–35 (2002).
53. Rhodes, D. R. et al. ONCOMINE: a cancer microarray database and integrated data-mining platform. *Neoplasia* 6, 1–6 (2004).
54. Di Caro, G. et al. Occurrence of tertiary lymphoid tissue is associated with T-cell infiltration and predicts better prognosis in early-stage colorectal cancers. *Clin. Cancer Res.* 20, 2147–2158 (2014).
55. Woo, J. R. et al. Tumor infiltrating B-cells are increased in prostate cancer tissue. *J. Transl. Med.* 12, 30 (2014).
56. D’Amico, A. V. et al. Biochemical outcome after radical prostatectomy, external beam radiation therapy, or interstitial radiation therapy for clinically localized prostate cancer. *J. Am. Med. Assoc.* 289, 969–974 (1998).
57. Czih, M. et al. The immune response to sporadic colorectal cancer in a novel mouse model. *Oncogene* 29, 6591–6602 (2010).
Extended Data Figure 1 | Treatment schemes and characterization of tumours and mouse survival before and after treatment. a, Early and late treatment schemes for TRAMP mice. TRAMP mice (n = 3–6 per group) were subjected to early oxaliplatin treatment as described in a and prostate weights were determined at 14 weeks, 1 week after completion of four treatment cycles. Dashed red line indicates prostate weight of tumour-free controls (n = 33 in total). Prostate weight in b is shown in a log2 scale. c, Representative images of haematoxylin-and-eosin-stained prostate sections from TRAMP mice are shown. Scale bars, 100 μm. PD, poorly differentiated adenocarcinoma; PIN, prostatic intraepithelial neoplasia; WD, well differentiated adenocarcinoma. d, Histopathological assessment of early and late treated TRAMP tumours in wild-type and Jh2/2 mice without or with oxaliplatin treatment. The percentages of the different histotypes shown in c are depicted (n = 3–7 per group). Fisher’s chi-square analysis was used to calculate statistical significance. e, Early and late treatment schemes for mice bearing subcutaneous (s.c.) MC tumours. MC cells were subcutaneously transplanted into wild-type and Jh2/2 mice (n = 3–7 per group) that were subjected to early oxaliplatin treatment when tumour volume was 100 mm³. Forty-eight hours after completion of three treatment cycles, mice were euthanized and tumour volumes (mm³) were measured (n = 19 in total). g, MC tumours from indicated mice were stained for CD45 (green) and cleaved caspase 3 (CC3; red) (n = 4–6 per group). h, MC tumours (n = 3–5 per group) grown in wild-type, Jh2/2 and Cd8a2/2 mice were stained for CD45 (green) and γH2AX (red), and the γH2AX foci in CD45− cells were enumerated (i). Scale bars, 100 μm. All results are mean ± s.e.m. j, Representative images of subcutaneous MC tumours (n = 5–6 per group) from wild-type and Jh2/2 mice, with or without oxaliplatin treatment stained for αSMA (red) and CD31 (green). k, l, Frequency of αSMA− (k) and CD31− (l) positive cells within tumours from j. Shown are median values ± s.e.m. *P < 0.05; **P < 0.01; ***P < 0.001 (Mann–Whitney and t-tests). m, TRAMP mice (wild-type, Cd8a−/− or Jh2/2; n = 6–14 per group) were treated weekly with low-dose oxaliplatin. Moribund mice were euthanized, and survival was compared by Kaplan–Meyer analysis and significance was determined (wild-type: P = 0.05; Cd8a−/−: P = 0.05; Jh2/2: **P = 0.002). n, Survival curves for the different TRAMP groups before and after oxaliplatin treatment. Significant differences in survival times are indicated on the right. No statistically significant differences in survival were found between wild-type and Jh2/− or Jh2/− and Cd8a−/− mice without treatment. Significant differences in survival times were observed between all three oxaliplatin-treated groups (wild-type, Cd8a−/− or Jh2/−; indicated on the right). *P < 0.05; **P < 0.01; ***P < 0.001 (Kaplan–Meyer analysis). The number of mice used in each experiment and the number of experiments (replications) are shown also in Supplementary Table 1.
Extended Data Figure 2 | B cells attenuate oxaliplatin-triggered CTL activation. a, Flow cytometry of CD8+ T lymphocytes in prostates of 20-week-old TRAMP mice after four cycles of oxaliplatin treatment (n = 4–6 per group) normalized to prostate weights. b, Late subcutaneous MC tumours from wild-type and Jh−/− mice were stained for CD8 and analysed by immunofluorescent microscopy. In the top left areas (white square), single CD8 staining (green) without DAPI counterstain is shown. Tumoral CD8+ cells were counted in 3–4 HMF (200×) per tumour (n = 4–5 tumours per group). Scale bars, 100 μm. c, d, Late subcutaneous MC tumours were analysed by flow cytometry for CD4+ lymphocytes in spleens (c) and tumours (d) after three oxaliplatin treatment cycles (n = 4–7 per group). The results show percentages of the corresponding CD8+ T cells in the CD8+ CD44+ population (n = 3–5 per group). e, Flow cytometric analysis of TNF and IFNγ expression by CD8+ cells in MC tumours from wild-type and Jh−/− mice treated as above (n = 6–8) and re-stimulated in vitro with tumour cell lysate. f, Flow cytometry of STAT1 phosphorylation in CD8+ cells from MC tumours of treated and untreated wild-type and Jh−/− mice (for isotype controls, see Extended Data Fig. 10u). The results are summarized in the right panel (n = 3–5 mice per group). g, Expression of GrzB and Ki67 in CD8+ T effector cells (CD8+ CD44+) from spleens of MC inoculated mice after oxaliplatin treatment. h, i, Flow cytometry of PD-1 and Tim-3 expression by CD8+ T effector cells (CD8+ CD44+) in spleen (h) and MC tumours (i) as indicated with or without oxaliplatin treatment. Shown are percentages of the corresponding CD8+ T cells in the CD8+ CD44+ population (n = 3–5 per group). j, The experimental scheme for B cell immunodepletion in tumour-bearing mice. MC tumours were raised in wild-type or Cd8a−/− mice, 16 days after subcutaneous tumour cell inoculation. B cells were depleted by twice weekly administration of antibodies directed against CD19, CD20, CD22 and B220. Four days after first antibody treatment, mice were treated with oxaliplatin (n = 4–7 mice per group, total: 44). After three weekly chemotherapy cycles, mice were euthanized. k, Flow cytometry analysis of tumour-infiltrating CD45+ CD8+ T cells stained for IFNγ (left) or IFNγ and TNF (right) after in vitro restimulation with PMA and ionomycin (n = 4–6 mice per group). l–n, Flow cytometry analysis of CD19+ (l, m) and IgA+ (n) cells in spleens and tumours isolated from the wild-type mice described above, confirming depletion of CD19+ B cells and oxaliplatin-induced IgA+ cells in spleen and tumours. o, Serum IgA concentrations in the mice described in j (n = 3–5 per group). p, Flow cytometry analyses of CD19+ B cells in tumours isolated from Cd8a−/− mice subjected to B cell depletion or not, confirmed the efficient depletion of tumoral CD19+ B cells. All results are mean ± s.e.m. *P < 0.05; **P < 0.01; ***P < 0.001 (Mann–Whitney and t-tests). ©2015 Macmillan Publishers Limited. All rights reserved
Extended Data Figure 3 | Immunogenic chemotherapy induces tumour infiltration by immunosuppressive CD19<sup>+</sup>CD20<sup>dim</sup>B220<sup>low</sup>IgA<sup>+</sup> B cells. a, b, MC tumours (n = 4–9 per group) raised in wild-type mice without or with oxaliplatin treatment were stained for B220 (a), and tumour-infiltrating B220<sup>+</sup> cells per HMF were enumerated (b). In a, single B220 staining (above) and combined staining B220/DAPI (below) are shown. Scale bars, 100 μm. c, d: The flow cytometry plots and gating strategy for analysis of splenic B cell populations using CD19, IgA, B220, CD138 and CD20 antibodies. Results from wild-type mice bearing MC tumours are shown in c and from oxaliplatin-treated mice in d (n = 8 mice per group). Oxaliplatin treatment modestly increased the amount of splenic IgA<sup>+</sup> cells. Splenic IgA<sup>+</sup> cells expressed CD138 as expected and showed lower levels of B220 and CD20, in either control or oxaliplatin-treated mice. e: The gating strategies for analysis of tumoral B cells using CD19, IgA, B220 and CD138 antibodies. Results from MC tumours in two representative oxaliplatin-treated wild-type mice are shown (n = 8 mice per group), demonstrating the presence of IgA<sup>+</sup> cells in oxaliplatin-treated tumours with a typical CD138<sup>+</sup>B220<sup>dim</sup> phenotype. f-i: Flow cytometry plots and gating strategies for analysis of tumoral B cell populations using CD19, B220, CD138, IgA and PD-L1 antibodies. Results from wild-type mice bearing MC tumours without (f) or with (g) oxaliplatin treatment (n = 6 mice per group) and Igα<sup>–/–</sup> mice bearing MC tumours without (h) or with (i) oxaliplatin treatment (n = 6 mice per group) are shown. Oxaliplatin treatment increased the amount of tumoral IgA<sup>+</sup>CD138<sup>+</sup>B220<sup>dim</sup>PD-L1<sup>+</sup> cells in wild-type mice. j: Flow cytometric analysis of PD-L1 and IL-10 expression in IgA<sup>+</sup>B220<sup>+</sup>Igα<sup>–</sup> and B220<sup>+</sup>Igα<sup>+</sup> B cells from oxaliplatin-treated TRAMP tumours (n = 4). k: Flow cytometric analysis of IgA and CD138 expression by TRAMP tumour-infiltrating B cells. Shown are percentages of IgA<sup>+</sup> cells among all tumour-infiltrating CD19<sup>+</sup>CD138<sup>+</sup> cells. l: Wild-type mice bearing MC tumours were treated with oxaliplatin as above. Two days after the first or last oxaliplatin cycle, mice were euthanized, tumours were isolated and analysed by flow cytometry as indicated (n = 6 per group). After dead-cell exclusion, tumour-infiltrating B cells were stained with CD19, CD20, B220, IgA and IgM antibodies. Shown are the results for control (left), one cycle (middle), and three cycles (right) of oxaliplatin treatment, demonstrating the presence of tumoral IgA<sup>+</sup> cells with a CD19<sup>+</sup>CD20<sup>dim</sup>B220<sup>dim</sup>IgA<sup>+</sup> cell phenotype within 48 h after oxaliplatin treatment.
Extended Data Figure 4 | Immunogenic chemotherapy induces tumoral and systemic IgA production through CSR. a, Ex vivo analysis of IgA released by tumour single cell suspension isolated from oxaliplatin-treated TRAMP tumours. Single cell suspension from non-treated tumours and culture medium without cells were used as controls. b, c, Serum IgA (b) and IgG (c) in treated and untreated TRAMP mice and age-matched naive FVB controls (n = 7–14 per group). d, Serum IgA amounts in control or oxaliplatin-treated mice bearing MC tumours (n = 5–7 per group) were determined and compared to age-matched naive FVB controls (n = 7). e, Serum IgG amounts in control or oxaliplatin-treated mice bearing MC tumours (n = 5–7 mice per group) were determined and compared to age-matched naive FVB controls. a–e, Results are mean ± s.e.m. Mann–Whitney and t-tests were used to calculate statistical significance. f, g, Immunofluorescence analysis of activation-induced cytidine deaminase (AID, green) and IgA (red) expression in spleen (f, used as a positive control) and MC tumours from oxaliplatin-treated wild-type mice (g). Scale bars: 10 µm (f, left and g) and 100 µm (f, right). Arrows point to IgA⁺ AID⁺ cells. Shown are representative results of spleens and tumours isolated from four mice per group. h, qRT–PCR analysis of Tgfb1 mRNA in MC tumours raised in wild-type or Jh−/− mice without or with oxaliplatin treatment (n = 3–7 mice per group). Results are mean ± s.e.m. i, Flow cytometry of SMAD2/3 phosphorylation in MC tumour-infiltrating B cells from wild-type mice before and after oxaliplatin treatment (n = 4 per group). Shown are the MFI and percentages (see Fig. 3e). j, Flow cytometry of SMAD2/3 phosphorylation and PD-L1 in MC tumour-infiltrating B cells from wild-type mice before and after oxaliplatin treatment (n = 4 per group). Shown are the percentages of PD-L1⁺ p-SMAD2/3⁺ cells within CD45⁺ CD19⁺ cells. k, qRT–PCR analysis of Il21 mRNA in MC tumours raised in wild-type or Jh−/− mice without or with oxaliplatin treatment (n = 4–5 mice per group). Chemotherapy-induced Il21 mRNA mainly in wild-type mice. l, m, Flow cytometry of tumour-infiltrating B cells stained for p-STAT3 and IL-10 (n = 5–6 per group). Shown are the mean ± s.e.m. qRT–PCR analysis of Il21 mRNA in MC tumours raised in wild-type or Jh−/− mice without or with oxaliplatin treatment. n, Flow cytometry analysis of Actb (β-actin) mRNA, IL-10 protein and Il10 mRNA in MC tumour-infiltrating IgA⁺ cells using PrimeFlow RNA technology (pooled data of 4 mice per group, after oxaliplatin treatment). Left, Actb mRNA gated on CD45⁻ cells; middle, Il10 mRNA and IL-10 protein expression after 1 h stimulation with PMA plus ionomycin and LPS gated on IgA⁺ cells, right panel: Il10 mRNA and IL-10 protein expression after 5 h stimulation with PMA plus ionomycin and LPS, gated on IgA⁺ cells. o, p, Flow cytometric analysis of tumour-infiltrating B cells in TRAMP mice (n = 4–5 per group) stained for p-STAT3 and IL-10 (n = 5–6 per group) stained for CD19, B220, IL-10 and LTαβ (o). The percentage of tumour-infiltrating LTαβ⁻ cells amongst all tumour-infiltrating B cells was determined (p). q, Flow cytometric analyses of CD5 expression by B cells from spleen and MC-tumour of wild-type mice after oxaliplatin treatment (n = 4–5 per group). Shown are the mean ± s.e.m. *P < 0.05; **P < 0.01; ***P < 0.001 (Mann–Whitney and t-tests).
Extended Data Figure 5 | Immunogenic chemotherapy or B cell deficiency has marginal effects on Treg, natural killer and myeloid cells. a, b, qRT–PCR analyses of Nos2 (a) and Arg1 (b) mRNA content of MC tumours (n = 4–7 mice per group). Chemotherapy induced Nos2 and Arg1 expression in wild-type and Jh−/− mice and no significant and consistent differences were found between both groups. c, d, qRT–PCR analyses of Il12p40 (c), Il12p35 (d) mRNA in MC tumours grown in wild-type and Jh−/− mice (n = 4–6 mice per group). e–i, Flow cytometry analyses of tumour-infiltrating or splenic lymphocytes and monocytes: tumoral Nk1.1+ cells (e), tumoral CD11b+CD11c−MHCII+ cells (f), tumoral CD11b+GR-1+ cells (g), CD4+FoxP3+ cells (splenic, h; tumoral, i). Cells in e–i are from tumour-bearing mice subjected to oxaliplatin treatment and/or B cell depletion as indicated (B cell depletion plus oxaliplatin; n = 4–6 mice per group). Results are mean ± s.e.m. Mann–Whitney and t-tests were used to calculate statistical significance.
Extended Data Figure 6 | Analyses of B and T cells in human prostate cancer specimens. a–g, Tissue microarrays of tumour and non-tumour tissue from 110 PC patients were stained for CD8 and CD20 (5–6 spots per patient = 3–4 tumour tissue and 2 non-tumour tissue). a, b, Representative examples of CD8 (a) and CD20 (b) IHC of PC tissue microarrays (left). Right, computer assisted image analysis with ad hoc developed image software. Tumour tissue is represented in yellow and CD8+ and CD20+ cells are represented in red. The percentages of immune reactive area (IRA) occupied by CD8+ or CD20+ cells are shown. Scale bars, 200 μm. c, d, Comparison of CD8 and CD20 IRAs in matched non-tumour and tumour tissues from each E-PC patient (n = 87). e–g, Patients were divided into three subgroups: E-PC (n = 86), TR-PC (n = 9) and M-PC (n = 15). e, CD8+/CD20+ cell infiltration into tumour tissues of the different groups. f, CD20+ cell infiltration into tumour tissues of the different groups. g, The CD8+/CD20+ ratio for the different groups. Each dot represents one patient. Line indicates the median value. Mann–Whitney test was used to calculate statistical significance between the two groups. Kruskal–Wallis test was used to calculate statistical significance between the three groups. h, IHC analysis of low-risk (n = 5) and high-risk (n = 5) human PC specimens using IgA (red) and αSMA (black). Nuclei were counterstained with haematoxylin. Scale bar, 100 μm. i, Immunofluorescence analysis of human PC showing IL-10-expressing (red) IgA+ (green) CD138+ (turquoise) plasma cells (n = 6). Representative images are shown. White arrow indicates IL-10-expressing IgA+ cells. Scale bars, 50 μm. j, Human normal prostate (n = 3–5) and human PC (n = 5) were stained for IgA and CD8. Typical images are shown. Red and green arrows indicate IgA+ and CD8+ cells, respectively. Scale bar, 100 μm. k, Human normal prostate (n = 3) and human PC (n = 5) were stained for IgA (red arrow) and CD20 (green arrow). Scale bar, 100 μm. l, Flow cytometric analysis of human prostate tumour-infiltrating CD19+ cells and IgA+ cells. The percentages of IL-10-expressing B cells in CD19+ IgA+ (two different samples) and CD19+ IgA+ B cells are shown. m, Summary of results obtained from human blood samples taken from healthy donors (n = 3) and patients with PC (n = 5) and prostate tissue specimens (benign, malignant; n = 4) analysed by flow cytometry for IL-10 expression in CD19+ IgA+ and CD19+ IgA+ B cells. n, o, Tissue microarrays from 110 patients with PC (described above) were stained for IgA and CD138. Patients were divided into three subgroups: E-PC (n = 86), TR-PC (n = 9) and M-PC (n = 15). n, Representative images of IgA (immunoperoxidase) and CD138 (alkaline phosphatase) double staining of tumour tissues from each group. CD138+ and IgA+ double-positive cells in the PC stroma are indicated by the white arrows (haematoxylin counterstain). Scale bar, 100 μm. o, Frequencies of IgA+ and CD138+ double-positive cells in the tumour stroma of the different PC patient groups. p, PC patient specimens were divided into two groups: IgA+/-low (n = 64) and IgA+/-hi (n = 46). Shown is the CD8+/CD20+ ratio for each group. Each dot represents one patient. Line indicates the median value. q, IgA mRNA expression (IGHA1) is significantly increased in human PC tissue relative to healthy or benign prostate tissue in 5 out of 15 studies evaluated via Oncomine. Results from one significant study14 are presented. *P < 0.05; **P < 0.01; ***P < 0.001 (chi-square test and Fisher’s exact test).
Extended Data Figure 7 | Effects of TGFβR2, IgA, PD-L1 and IL-10 ablations on tumour-infiltrating lymphocytes. MC tumours were raised in wild-type, Tgfbr2<sup>DB</sup> or Iga<sup>−/−</sup> mice (n = 5–11 per group). Mice were subjected to three cycles of late oxaliplatin treatment after which splenic and tumoral B cells were analysed. After dead cell exclusion, splenic (Spl; a, b) and tumoral (Tu; c, d) B cells were stained with CD19, B220, IgA, IgG2a and IgG1 antibodies and analysed by flow cytometry. e, Serum IgG concentrations in control or oxaliplatin-treated wild-type, Tgfbr2<sup>DB</sup> or Iga<sup>−/−</sup> mice bearing MC tumours (n = 5–9 per group). f, Flow cytometry of tumour-infiltrating CD19<sup>+</sup> B cells from wild-type, Tgfbr2<sup>DB</sup> or Iga<sup>−/−</sup> MC tumour-bearing mice (n = 4–7 per group) analysed for PD-L1 expression, revealing lower PD-L1 surface expression on Tgfbr2<sup>DB</sup> and Iga<sup>−/−</sup> B cells after oxaliplatin treatment. g, Flow cytometry of tumour-infiltrating B220<sup>+</sup> B cells (left) and IgA<sup>+</sup> B220<sup>+</sup> B cells (right) from wild-type, Tgfbr2<sup>DB</sup> or Iga<sup>−/−</sup> MC tumour-bearing mice (n = 4–7 per group) analysed for IL-10 expression, revealing no difference in IL-10 expression by B220<sup>+</sup>IgA<sup>−</sup> B cells in the corresponding groups, and lower IL-10 expression by Tgfbr2<sup>DB</sup> B cells after oxaliplatin treatment compared to wild-type mice. Results are mean ± s.e.m. Mann–Whitney and t-tests were used to calculate statistical significance. h, The experimental scheme. Wild-type mice bearing MC tumours were divided into four treatment groups (n = 7–8 per group): (1) isotype control (IgG2a), (2) oxaliplatin (weekly), (3) anti-PD-L1 (twice weekly), and (4) oxaliplatin plus anti-PD-L1 (weekly and twice weekly, respectively). After three treatment cycles, mice were euthanized and analysed. i, Tumour growth curves of tumour-bearing mice and gross appearance of untreated and treated mice. Significance was determined by Mann–Whitney and t-tests. j, Flow cytometric analysis for GrzB expression by tumour-infiltrating CD8<sup>+</sup> T effector cells (CD8<sup>+</sup>CD44<sup>+</sup>) from MC tumour-bearing mice treated as described above. Results are shown either as percentages of GrzB<sup>+</sup> cells amongst CD8<sup>+</sup> T cells (black), or percentages of GrzB<sup>+</sup>CD8<sup>+</sup>CD44<sup>+</sup> T cells amongst tumoral CD45<sup>+</sup> cells (red). k, Flow cytometry of PD-L1 expression on tumour-infiltrating IgA<sup>+</sup> CD19<sup>−</sup> B cells in the different treatment groups. l, m, Serum IgA (l) and IgG (m) concentrations in the different treatment groups described in h. n, The experimental scheme for the experiment whose results are shown in Fig. 4g. B cells were isolated from wild-type, Pdl1<sup>−/−</sup> and Il10<sup>−/−</sup> mice and 5 × 10<sup>6</sup> cells (purity 98%) were intraperitoneally transferred into MC tumour-bearing Jh<sup>−/−</sup> mice (16 days after MC cell inoculation). After 2 days (day 18), the mice were given three oxaliplatin treatment cycles and analysed. o, Flow cytometric analysis of splenocytes after staining with CD45 and CD19 antibodies, confirming presence of B cells in the ABCT groups. Shown are percentages and absolute B cell numbers in spleen. p, Tumour infiltrating CD8<sup>+</sup> cells from MC tumour-bearing Jh<sup>−/−</sup> mice transplanted with B cells and treated as above were re-stimulated for 4 h with PMA and ionomycin before flow cytometry (n = 4–6 mice per group). Results are mean ± s.e.m. *P < 0.05; **P < 0.01; ***P < 0.001 (Mann–Whitney and t-tests).
Extended Data Figure 8 | Low-dose cisplatin treatment is devoid of immunogenic activity and low-dose oxaliplatin does not affect gut barrier function. a, Flow cytometry of MC cells stained with annexin V and propidium iodide 24 h after treatment with either oxaliplatin or cisplatin (both at 20 μM). b, Flow cytometry analysis of MC cells treated as above and stained with antibody to the autophagy marker LC3A. c–e, MC tumours were raised in wild-type and Jh2/2 mice until 400 mm3 in size, after which the mice were treated with either cisplatin or oxaliplatin at 6 mg kg⁻¹ (n = 4–5 per group). After three weekly chemotherapy cycles, mice were euthanized. c, Tumour weights; left: wild-type mice; right: Jh2/2 mice. d, e, Flow cytometry of tumour-infiltrating CD8 (d) and CD4 (e) cells. Left: wild-type mice, right: Jh2/2 mice. f, Gut permeability was measured in wild-type mice before and after low- (LD) and high- (HD) dose oxaliplatin treatment using orally administered fluorescein isothiocyanate (FITC)-dextran. Shown are FITC-dextran concentrations in serum (μg ml⁻¹) (n = 5 mice per group). g, Serum IgA concentrations in naive wild-type (FVB) and Tgfbr2D mice before and after oxaliplatin treatment (n = 3–6 per group). h, IgA staining of colon sections of untreated or low-dose oxaliplatin-treated wild-type mice. Scale bars, 100 μm. i–k, Flow cytometry of CD8⁺ (i), CD4⁺ (j) and Nk1.1⁺ (k) cells in spleens of naive wild-type and Tgfbr2D mice without or with oxaliplatin treatment. All results are mean ± s.e.m. *P < 0.05; **P < 0.01; ***P < 0.001 (Mann–Whitney and t-tests).
Extended Data Figure 9 | Immunogenic chemotherapy supports adoptive T cell transfer only in the absence of B cells. a, The experimental scheme. Immunogenic TRAMP-C2 cells were subcutaneously inoculated into wild-type or Tcrb2/2 mice. After 30 days, the mice were divided into four groups (n = 4–5 per group): (1) control, (2) oxaliplatin (weekly), (3) ATCT, and (4) ATCT plus oxaliplatin (weekly). The first oxaliplatin cycle was given at day 31. Two days after the second cycle, CD8+ T cells from CD45.1 × CD45.2 wild-type mice (3 × 10^6 cells) were transferred into tumour-bearing mice and this was followed by two more oxaliplatin cycles after which mice were euthanized for analysis on day 59. b–d, Flow cytometric analysis of spleen (c) and tumour (d) cells after staining with CD45.1, CD45.2, CD8 and TCRab antibodies, confirming expansion of adoptively transferred T cells.

e, Tumour growth curves. f, The experimental scheme. Immunogenic TRAMP-C2 cells were subcutaneously inoculated into wild-type or Rag12/2;OT-1 mice (no B cells), that contain CD8+ T cells specific for chicken ovalbumin which is not expressed by TRAMP-C2 cells. After 30 days, tumour-bearing Rag1−/−;OT-1 mice were divided into four groups (n = 3–4 mice per group): (1) control, (2) oxaliplatin treatment, (3) ATCT, and (4) oxaliplatin treatment plus ATCT. The first oxaliplatin cycle was given at day 31. Two days after the second oxaliplatin cycle, CD8+ T cells (3 × 10^6) from CD45.1 × CD45.2 mice were adoptively transferred into tumour-bearing mice, which were euthanized on day 59 and analysed. g, Flow cytometric analysis of tumour-infiltrating cells stained with CD45.1, CD45.2, CD8 and TCRαβ antibodies, confirming infiltration of adoptively transferred T cells. h, Flow cytometric analysis of GrzB expression in adoptively transferred, tumour-infiltrating, CD8+ T effector cells (CD45.1+CD8+CD44+) from tumour-bearing mice treated as above. i, Tumour volumes (mm^3). j, Tumour growth curves. k, The experimental scheme for Fig. 5a–f. Sixteen-week-old TRAMP;Rag1−/− mice (no B and T cells) were treated with oxaliplatin (weekly). One day after the first treatment cycle, CFSE-labelled splenocytes from either wild-type (B and T cells, SP-WT) or Jh2/2 (T but no B cells, SP-Jh2/2) mice were transferred into the tumour-bearing mice (5 × 10^6 T cells per mouse; 4–5 mice per group). l, m, After 6 days, one mouse from each group was euthanized, and the proliferation of CD8+ (l) and CD4+ (m) cells in bone marrow (BM), spleen and prostates was analysed by CFSE staining and flow cytometry. n–r, After three more oxaliplatin cycles (4 weeks in total), the mice were euthanized and analysed. n, Frequency of adoptively transferred CD19+ cells amongst CD45+ cells in spleens and prostates 30 days after ACT. o, Flow cytometric analyses of CD19+ B lymphocytes for TIM-1 expression in spleens (left) and prostates (right) of above mice. p–r, Flow cytometric analyses of T cells. Percentages of CD8+ and CD4+ cells in lymph nodes (LN; p); spleens (q); prostates (r) of above TRAMP;Rag1−/− mice. Red: splenocytes from wild-type mice (T and B cell transfer), blue: splenocytes from Jh−/− mice (T cell transfer). Results are mean ± s.e.m. *P < 0.05; **P < 0.01; ***P < 0.001 (Mann–Whitney and t-tests).
Extended Data Figure 10 | Immunogenic chemotherapy supports adoptive T cell transfer only in the absence of B cells and analysis of lymphocytes and monocytes in tumour-free mice. a, The experimental scheme for Fig. 5g–i. MC tumour-bearing Rag1−/− mice (no B and T cells) were treated with oxaliplatin (weekly). One day after first oxaliplatin treatment, 5 × 10⁶ T cells (negative selection) from wild-type mice immunized with MC cell lysate were adoptively transferred into tumour-bearing mice (4–5 mice per group), alone or in combination with 5 × 10⁶ B cells from wild-type or Tgbr2+/− mice (purity 98%). After two more oxaliplatin cycles (3 weeks total), the mice were euthanized and analysed. b, Serum IgG analysis of above mice. c, Flow cytometric analysis of splenocytes after staining with CD45 and CD19 antibodies. d–p, Wild-type, Jh−/−, Iga−/− and Tgbr2+/− mice in the FVB background and wild-type, Pdl1−/−, Il10−/− and Iga−/− in the C57BL/6 background were analysed for the distribution of immune markers. d, Spleen weights of wild-type, Jh−/− and Tgbr2+/− mice in the FVB background. e, Flow cytometry of splenocytes for the following markers: CD3 (left), CD8 (middle) and CD4 (right), gated on the splenic CD45+ population. f, Absolute cell numbers of splenic CD3+ (left), CD8+ (middle) and CD4+ (right) cells are shown (percentage × cell count of whole spleen). g, h, Flow cytometry for TNF and IFNγ in CD8+ cells from tumour-free wild-type, Jh−/−, Tgbr2+/− and Iga−/− mice (n = 6–8) that were re-stimulated in vitro with PMA and ionomycin and the representative flow cytometry panels (h). i, j, Flow cytometry of splenocytes from wild-type and Tgbr2+/− for: CD19+ IgM+ cells (i) and IgA (j) gated on the splenic CD45+ population. k–n, Flow cytometry of splenocytes from wild-type, Pdl1−/−, Il10−/− and Iga−/− mice for: CD45+ CD19+ IgM+ cells (k), CD45+ IgA− cells (l), PD-L1 expression by CD19+ IgM+ cells (m), and IL-10 expression by CD19+ cells (n), as indicated. o, p, Serum IgA and IgG concentrations were analysed in wild-type, Pdl1−/− and Il10−/− mice (n = 4–5 mice per group). q–u, The different gating strategies and staining controls are shown. q, Gating strategies for tumour-infiltrating lymphocytes: lymphocyte gate, dead cell exclusion, doublets exclusion, and gating on the CD45+ population. r, Flow cytometric analysis of IL-10 and IgA expression, gated on the CD45+ population: (1) isotype control (no staining); (2) non-stimulated splenocytes: showing IgA staining, but not IL-10; (3) stimulated splenocytes from Il10−/− mice showing IgA staining, but not IL-10; and (4) stimulated splenocytes from wild-type mice showing IgA and IL-10 staining. s, Flow cytometric analysis of IL-10 and CD19 expression, gated on the CD19+ B220+ population. Left, stimulated cells from Il10−/− mice, showing B cell staining, but not IL-10; right: stimulated cells from wild-type mice showing B cell staining and IL-10 staining. t, Flow cytometric analysis of IL-10 and IgA expression, gated on the IgA+ population: left: stimulated cells from Il10−/− mice, showing IgA cell staining, but not IL-10. Right, stimulated cells from wild-type mice showing IgA and IL-10 staining. These results confirm IL-10 production by IgA− cells. u, Flow cytometric analysis of p-STAT1 staining with corresponding isotype control. All results are mean ± s.e.m. *P < 0.05; **P < 0.01; ***P < 0.001 (Mann–Whitney and t-tests).