**Figure S1**

**a,** Representative contour plots depicting the gating strategy used to identify individual immune cell populations within B16F10 or LLC tumours. **b,** Dot plot of selected genes that were used to identify and annotate the clusters in the B16F10 scRNA-seq dataset. The size of the dot encodes the percentage of cells within a cluster, while the colour encodes the average normalised expression level within a cluster, scaled per gene (blue is high). **c,** Growth curve of B16F10 tumour-bearing mice after IP treatment with 250 µg isotype or αPD-1 antibody. (n=3 mice per group) Representative data from two independent experiments. **d,** UMAP plot showing *Cd274* mRNA expression in the CD45+ fraction of B16F10 tumours. Significance of **c** evaluated using mixed-effects analysis and Tukey’s multiple comparisons test. Percentage of granzyme-B **(e)** and IFNg **(f)** producing CD8+ T cells within day 17 B16F10 tumours after isotype or αCD40 treatment. Representative data shown from three independent experiments (n=7). Statistical evaluation of **e,f,** performed by unpaired t-test.

**Figure S2**

**a,** Violin plot showing *Cd40* normalised mRNA expression within all CD45+ immune cells clusters identified of the sequenced B16F10 tumours. **b,** Histogram plots showing CD40 expression on different immune populations via flow cytometry from 100 mm3 B16F10 tumours **c,** Plot showing percentage of B cells within day 15 B16F10 tumours after isotype or aCD20 treatment on day 4. (n=5), Representative data from two independent experiments. **d,** Plot showing percentage of TAMs within day 20 B16F10 tumours after isotype or αCSF1R treatment on day 10 and 17 post tumour inoculation. (n=7) Representative data from two independent experiments. Significance of **c** and **d** was evaluated by unpaired t test

**Figure S3**

**a,** Percentage of cDC1 within B16F10 tumours in *Xcr1*wt/wt and *Xcr1*wt/dtr mice 24 hours after DT administration (n=6) Representative data from two independent experiments. **b,** Representative contour plots depicting the gating strategy used to identify individual cDC populations within B16F10 tumour draining lymph nodes in *Xcr1*wt/wt and *Xcr1*wt/dtrmice*.* **c,** Percentage of migratory (left) and resident (right) cDC1 in tumour-draining lymph nodes of *Xcr1*wt/wt and *Xcr1*wt/dtr mice 24 hours after DT administration. (n=3) Representative data from two independent experiments. Percentage of CD8+ T cells producing granzyme-B **(d)** and IFNg **(e)** within B16F10 tumours isolated from *Xcr1*wt/wt or *Xcr1*wt/dtr mice after isotype or aCD40 treatment. Representative data from two independent experiments (n=6). **f,** Percentage of CD8+ T cells within B16F10 tumours isolated from *Xcr1*wt/wt and *Xcr1*wt/dtr mice after treatment with DT in combination with isotype, αCD40, αCD8 or αCD40/αCD8 antibodies. (n=6) Representative data from two independent experiments. **g,** Percentage of TAMs within B16F10 tumours isolated from *Xcr1*wt/wt and *Xcr1*wt/dtr after treatment with DT in combination with isotype or αCD40/αCSF1R treatment. (n=5-9) Data from one experiment. **h,** Percentage of CD8+ T cells within B16F10 tumours isolated from *Xcr1*wt/wt and *Xcr1*wt/dtr after treatment with DT in combination with isotype or αCD40/αCSF1R treatment. (n=5-9) Data from one experiment. **i,** Ratio of CD44+ CD62L- effector to CD44- CD62L+ naïve tumour infiltrating CD8+ T cells within B16F10 tumours isolated from *Xcr1*wt/wt and *Xcr1*wt/dtr after treatment with DT in combination with isotype or αCD40/αCSF1R treatment. **j,** Volcano plot showing genes that are up- and down- regulated in the migDC population in comparison to all other clusters within CD45+ fraction B16F10 tumours. **k,** Gating strategy used for examining cDC1 and cDC2 with specific gating for MigDCs using CD200. **l-n** Abundance of MigDC, cDC1, and cDC2 within 100 mm3 B16F10 tumours24 hours after isotype or αCD40 administration, using gatings based on CD200 expression by MigDCs.(n=5) Representative data from two independent experiments. **o,** UMAP plot of the DC populations from a public scRNA-seq dataset of CD45+ sorted MC38 tumours 48 hours after isotype or αCD40 antibody administration. The UMAP plot is separated by treatment. **p,** UMAP plot of the DC populations from CD45+ sorted MC38 tumours 48 hours after isotype or αCD40 antibody administration, showing *Ccr7* gene expression and separated by treatment. **q,** Expression of MHC-I on B16F10 tumour-residing cDC2s 24 hours after isotype or aCD40 treatment. **r,** UMAP plot showing *Il12b* mRNA expression in the CD45+ fraction of B16F10 tumours. **s-u,** Abundance of cDC1 (**p**), cDC2 (**q**), and TAMs (**r**) in B16F10 tumours from *Itgax-*WT and *Itgax-*DTR bone marrow chimeras treated with DT and subsequent treatment with aCD40/aCSF1R or isotype control antibodies. (n=6), data from one experiment. Significance of **a,c,l-n,q** was evaluated by unpaired t test. Significance of **d-i, s-u** was evaluated by ordinary one-way ANOVA with Tukey’s multiple comparisons test.

**Figure S4**

**a,** Pie charts indicating the contribution of different immune populations to the CD45+ fraction of B16F10 tumours after delayed regrowth upon αCD40 or αCD40/αCSF1R. **b,** Percentage of TAMs within WT B16F10 tumours after treatment of αCD40 or αCD40/αCSF1R. **c,** Percentage of MMR+ TAMs within CD45+ cells of B16F10 tumours after αCD40 or αCD40/αCSF1R treatment. **d,** Percentage of Arginase1+ TAMs within CD45+ cells from B16F10 tumours after αCD40 or αCD40/αCSF1R treatment. **e,** Percentage of CD8+ T cells within B16F10 tumours after αCD40 or αCD40/αCSF1R treatment. **f,** Ratio of CD44+ effector to CD62L+ naïve tumour infiltrating CD8+ T cells after αCD40 or αCD40/αCSF1R treatment. **g,** Growth curve of B16F10 in WT mice treated with aCD40 or aCD40/aCSF1R in which aCD8+ T cells were depleted as from 4 days after aCD40 administration. (n=6) Data from one experiment. **b-f,** (n=3-5) Representative data from two independent experiments. Significance was evaluated by unpaired t test. Significance of **g** was calculated using 2way ANOVA with Tukey’s multiple comparisons test.

**Figure S5**

**a,** Dot plot of selected genes that were used to identify and annotate the clusters in the merged B16F10 and LLC dataset. UMAP plot**,** showing *Itgam* (**b)** and *Cd3e* (**c**) expression, separated by tumour type. **d,** Volcano plot showing the DE genes of TAM-1 cluster in comparison to all other clusters in the monocyte/TAM subset of the B16F10/LLC dataset. **e,** Volcano plot showing the DE genes of TAM-4 cluster in comparison to all other clusters in the monocyte/TAM subset of the B16F10/LLC dataset. **f,** Volcano plot showing the DE genes of mixed hypoxic cluster in comparison to all other clusters in the monocyte/TAM subset of the B16F10/LLC dataset. **g,** Percentage of cells from each sample that comprise each of the distinct lineages, detected by Slingshot trajectory inference analysis in the monocyte/TAM subset of the B16F10/LLC dataset.

**Figure S6**

**a,** CD4+ T cell infiltration into LLC tumours after treatment with isotype, αCD40, αCSF1R, or αCD40/αCSF1R antibodies. **b,** Percentage of CD4+ T cells that express the FoxP3 transcription factor across treatment groups. **c,** Neutrophil infiltration into LLC tumours after treatment with isotype, αCD40, αCSF1R, or αCD40/αCSF1R antibodies. **a-c,** (n=7) Representative data from three independent experiments. Percentage of cDC1s **(d)** and cDC2s **(e)** within LLC tumours after treatment with 30 µg of Flt3L for different durations during tumour growth. (n=4) Representative data from three independent experiments. **f,** Percentage of FoxP3+ cells within CD4+ T cells identified within LLC tumours after different treatment regimens using aCD25 targeting antibodies. (n=3) Data from one experiment. **g,** Percentage of neutrophils within day 15 LLC tumours after treatment with isotype or αLy6G/αMAR antibodies on d11, d12, d13 and d14 post tumour inoculation. (n=3) Representative data from two independent experiments. **h,** Percentage of neutrophils within day 17 LLC tumours after IP administration with αCSF1R on d10 and IP administration of αLy6G/αMAR on d11, d12, d13, d14, d15, d16 after tumour inoculation. (n=7) Data from one experiment. **i,** Percentage of neutrophils within d17 LLC tumours after IP treatment with vehicle control or CXCR2i beginning day 4 post tumour inoculation. (n=3) Data from one experiment. **j,** Percentage of neutrophils within d17 LLC tumours after treatment with isotype or αCSF1R together with CXCR2i. (n=4) Data from one experiment. **k,** Percentage of neutrophils expressing CXCR2 within LLC tumours after treatment with isotype, αCD40, αCSF1R, or αCD40/αCSF1R antibodies. (n=7) Data from one experiment. **l,** Percentage of neutrophil contribution to CD45+ tumour infiltrating cells in *Csf3r*+/+ or *Csf3r*-/- and treated with isotype or αCD40/αCSF1R therapy. (n=7), Representative data from two independent experiments. Significance of **a-f** and **k, l** was evaluated by ordinary one-way ANOVA with Tukeys’ multiple comparisons test. Significance of **g-j** was evaluated by unpaired t test.

**Figure S7**

Longitudinal *in vivo* lung micro-computed tomography (µCT)-derived biomarkers monitoring lung tumour burden including total lung volume **(a)** and non-aerated lung volume **(b)** of mice that had been injected intravenously with LLC-OVA. Data from one experiment (n=10). **c,** µCT images taken from two representative mice from each treatment condition twenty days after intravenous injection of LLC-OVA. Red arrows indicate tumour lesions. Data from one experiment (n=10). **d,** Growth curve of LLC tumours implanted in WT mice and treated with combinations of oxaliplatin and vehicle solutions and aCD40, aCSF1R and isotype control antibodies. Representative of one experiment (n=6). Changes to LLC tumour-infiltrating CD8+ T cells on day 15 after tumour implantation and treatment including abundance **(e)**,ratio of effector to naïve **(f)**, Ki67 expression **(g)**, and granzyme-B production **(h)**. Representative of one experiment (n=6). Percentage of FoxP3+ regulatory T cells **(i)** within LLC tumours on day 15 after tumour implantation and subsequence treatment along with the CCR8 expression on LLC tumour-infiltrating Tregs **(j)**.Representative of one experiment (n=6). **k,** Growth curve of LLC tumours implanted in *Xcr1*wt/wt and *Xcr1*wt/dtr mice and treated with combinations of oxaliplatin or vehicle solutions and aCD40, aCSF1R or isotype control antibodies. Representative of one experiment (n=6). Changes to LLC tumour-infiltrating CD8+ T cells on day 16 after tumour implantation and treatment including abundance **(l)**,ratio of effector to naïve **(m)**, and granzyme-B production **(n)**. Representative of one experiment (n=6). Percentage of FoxP3+ regulatory T cells **(o)** within LLC tumours on day 15 after tumour implantation and subsequence treatment along with the CCR8 expression on LLC tumour-infiltrating Tregs **(p)**. Representative of one experiment (n=6). Longitudinal *in vivo* lung micro-computed tomography (µCT)-derived biomarkers monitoring lung tumour burden including total lung volume **(q)** and non-aerated lung volume **(r)** of mice that had been injected intravenously with LLC. Red arrows indicate tumour lesions. Data from one experiment (n=10). **s,** µCT images taken from two representative mice from each treatment condition twenty days after intravenous injection of LLC. Data from one experiment (n=10).Statistical evaluation of **a,b,d,k,q,r,** performed by mixed-effects analysis with Tukey’s multiple comparisons test, **e,f,g,h,i,j,k,l,m,n,o,p** performed by ordinary one-way ANOVA with Tukey’s multiple comparisons test.