Supplemental Figure 1. **Gating strategy for evaluation of tumour cell PD-L1 expression.** A-C) Selection of cells by size and granularity (A), discrimination of live and dead cells by vital dye exclusion (B) and selection of CD45− fraction (C).
Supplemental Figure 2. Treatment of tumors with low-dose fractionated RT leads to increased PD-L1 expression on CD11b+GR1\textsuperscript{H}i monocyte-derived suppressor cells. A and B, median fluorescence intensity of PD-L1 expression on CD11b+GR1\textsuperscript{H}i (A) and CD11b+GR1\textsuperscript{L}o (B) cells isolated from tumors 1, 3 or 7 days after treatment with 10 Gy in 5 daily fractions of 2 Gy. Experimental groups contained at least 4 mice and are representative of at least 2 independent experiments. Data show mean ± SEM. *, \(P<0.05\) **, \(P<0.01\), Mann-Whitney test.
Supplemental Figure 3. **Blockade of both PD-1 and PD-L1 does not further enhance efficacy in combination with fractionated RT.** A) Survival curve following fractionated dose RT (as 10 Gy in 5 daily fractions of 2 Gy) alone or in combination with αPD-1, αPD-L1 or a combination of both mAb dosed 3qw for 3 weeks. n/s, $P>0.05$, log-rank; Mantel-Cox test. Experimental groups contained at least 5 mice and are representative of at least 2 independent experiments.
Supplemental Figure 4. **Combination therapy with fractionated RT and either αPD-1 or αPD-L1 mAb is well tolerated in mice.**, CT26 tumor bearing mice received 10 Gy RT delivered in 5 daily fractions of 2 Gy either alone or in combination with αPD-L1 mAb dosed at 10 mg/kg 3qw either for 3 weeks or 1 week. Experimental groups contained at least 7 mice and are representative of at least 2 independent experiments. n/s, *P*>0.05, Mann-Whitney test.
Supplemental Figure 5. Treatment of tumor cells with αPD-1 or αPD-L1 mAb does not sensitise to radiation-induced cell death in vitro. Clonogenic survival curves for CT26 cells (A), 4T1 cells (B) and 4434 cells (C) treated with RT (2.5-10 Gy) in the presence or absence of 2 μg/ml αPD1 or αPD-L1.
Supplemental Figure 6. **Dosing schedule is critical to outcome.** A) Tumour volumes following fractionated dose RT (as 10 Gy in 5 daily fractions of 2 Gy) alone or in combination with αPD-L1 mAb starting either on day 1 of RT cycle (schedule A), day 5 of RT cycle (schedule B) or 7 days post the last dose of RT (Schedule C). B and C) Tumour volumes of RT–treated mice demonstrating equivalent tumor volumes across different dosing schedules. n/s, P>0.05 (Mann-Whitney test). Experimental groups contained at least 5 mice and are representative of at least 2 independent experiments.
Supplemental Figure 7. **Acute versus chronic administration of αPD-L1 mAb does not impact efficacy of combination therapy.** A, CT26 tumor bearing mice received 10 Gy RT delivered in 5 daily fractions of 2 Gy either alone or in combination with αPD-L1 mAb dosed at 10 mg/kg 3qw either for 3 weeks or 1 week. Experimental groups contained at least 7 mice and are representative of at least 2 independent experiments. n/s, $P>0.05$, log-rank; Mantel-Cox test.