

**Supplemental Table 1:** Immunohistochemistry (IHC) antibodies and dilutions

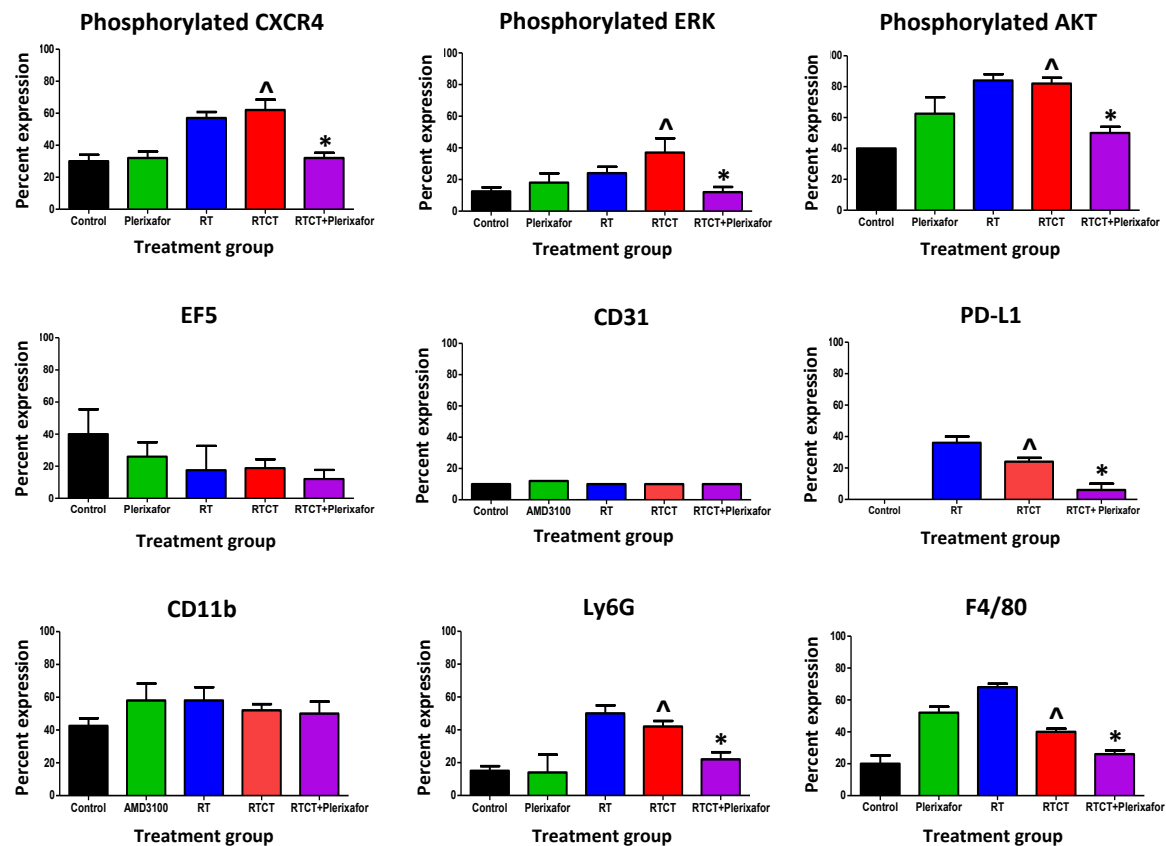
| <b>IHC Marker</b> | <b>Antibody</b>                                              | <b>Dilution</b> |
|-------------------|--------------------------------------------------------------|-----------------|
| CXCR4             | Santa Cruz<br>Goat anti-human                                | 1:400           |
| pCXCR4            | Abcam<br>Rabbit anti-human                                   | 1:300           |
| Ly6G              | Invitrogen<br>Rat anti-human                                 | 1:200           |
| PD-L1             | Roche<br>Rabbit anti-human                                   | 1:200           |
| pERK              | Cell Signalling<br>Rabbit anti-human                         | 1:50            |
| pAKT              | Cell Signalling<br>Rabbit anti-human                         | 1:50            |
| EF5               | Cameron Koch, University of Pennsylvania<br>Mouse anti-human | 1:50            |
| F4/80             | Bio-Rad<br>Rat anti-mouse                                    | 1:2000          |
| CD31              | Santa Cruz<br>Rabbit anti-human                              | 1:1000          |
| CD11b             | Abcam<br>Rabbit anti-human                                   | 1:100           |
| CD4               | Ebioscience<br>Mouse anti-human                              | 1:200           |
| CD8               | Abcam<br>Rabbit anti-mouse                                   | 1:1000          |

**Supplemental Table 2:** CXCL12 and housekeeping gene primer sequences for the RT-PCR gene expression studies

| <b>Primers</b> | <b>Forward Sequence (5`-3`)</b> | <b>Reverse Sequence (3`-5`)</b> |
|----------------|---------------------------------|---------------------------------|
| CXCL12         | TGCATCAGTGACGGTAAACCA           | CACAGTTTTGGAGTGTTGAGGAT         |
| L32            | GCCATTGTAGAAAGAGCAGCA           | TGCACACAAGCCATCTACTCA           |
| YWAZ           | CTGCCTACATATTGGTGTGTG           | TTTGTGTCACAGCCTCACAAG           |
| HSP90          | GAGGCAGACAAAAACGACAAA           | TGAGAAACCAGAGGAGAGCAG           |

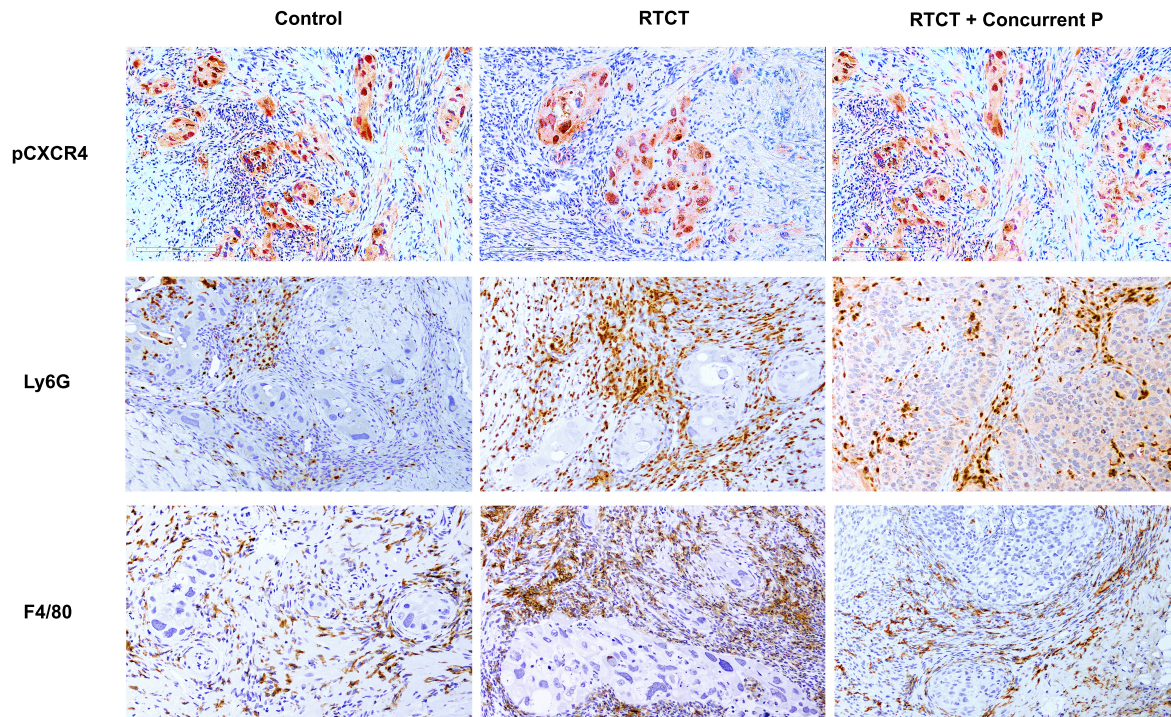
**Supplemental Table 3:** Radiation Injury Score (RIS) adapted from Langberg *et al.* 1992

| <b>Histologic Feature</b>                 | <b>Range</b> |
|-------------------------------------------|--------------|
| Epithelial atypia                         | 0-3          |
| Mucosal ulceration                        | 0-2          |
| Serosa thickening                         | 0-3          |
| Vascular sclerosis                        | 0-3          |
| Lymphatic congestion                      | 0-1          |
| Rectal wall fibrosis                      | 0-3          |
| Colitis cystica profunda                  | 0-3          |
| <b>Total Radiation Injury Score (RIS)</b> | <b>0-18</b>  |

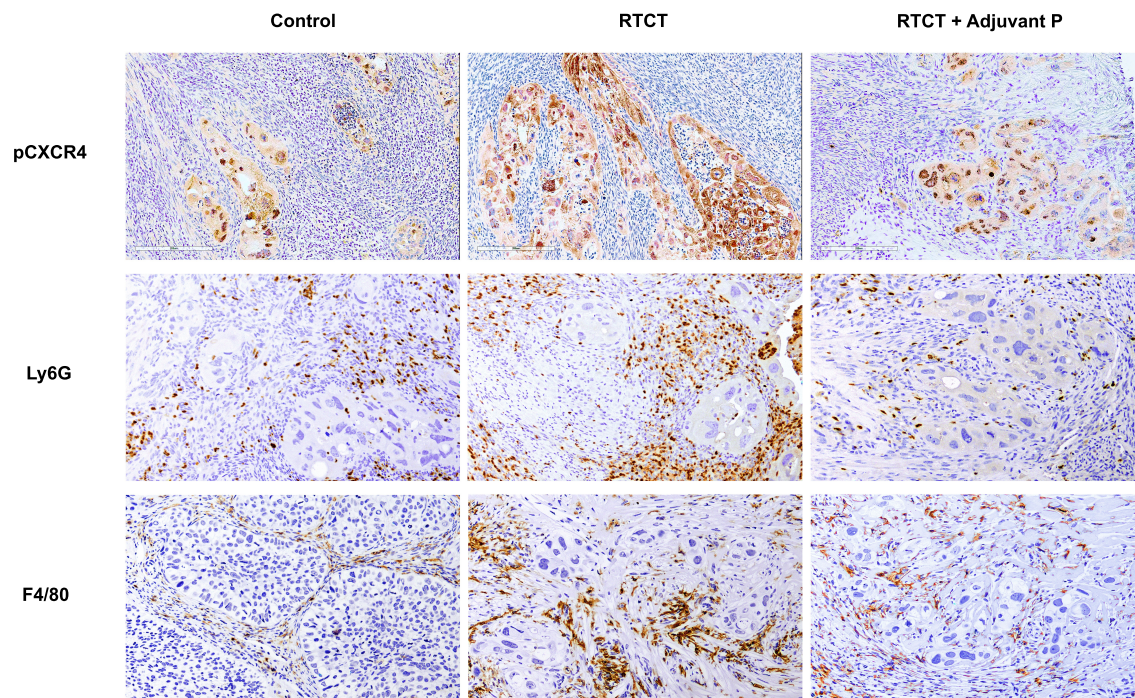


**Supplemental Figure 1:** Protein expression by IHC for pCXCR4, pAKT, pERK, vascular density (CD31), hypoxia (EF5), markers of myeloid cell (CD11b), MDSC (Ly6G) and macrophage (F4/80) intratumoral accumulation and PD-L1 at the end of RTCT (30 Gy in 2 Gy daily fractions for 3 weeks plus cisplatin 4 mg/kg weekly) and concurrent Plerixafor (5 mg/kg/day by continuous sc infusion). Protein expression is shown as the mean ( $\pm$  standard error) of the percentage of the tumor cell or stromal surface area that stained positively. pCXCR4, pAKT, pERK and PD-L1 were predominantly expressed in tumor cell regions, whereas CD11b, Ly6G and F4/80 were expressed in stroma. The results are for 7-12 tumors per treatment group. RT: Radiotherapy; RTCT: Radiotherapy and concurrent cisplatin; RTCT+P: RTCT + concurrent Plerixafor; <sup>^</sup>  $p < 0.05$  relative to controls; <sup>\*</sup>  $p < 0.05$  relative to RTCT



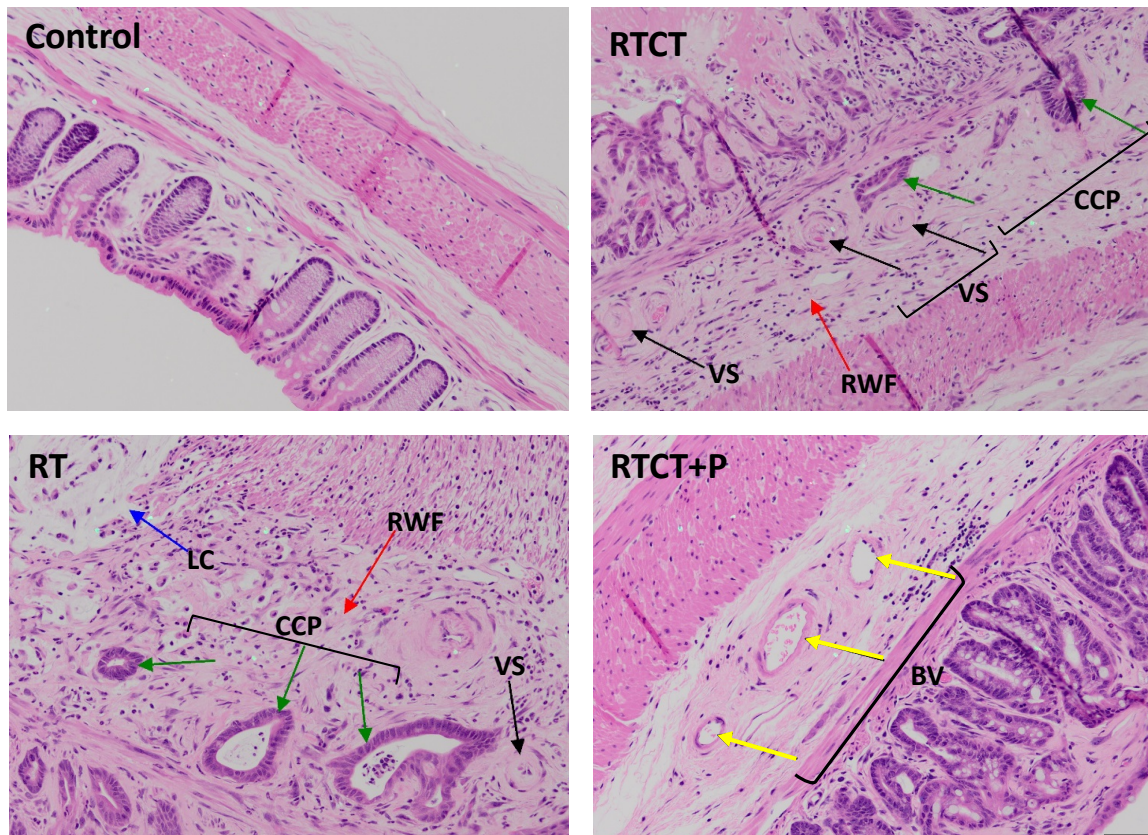


**Supplemental Figure 2(a) – RTCT and Concurrent Plerixafor:** Representative tumor sections showing immunohistochemical (IHC) staining for phosphorylated CXCR4 (pCXCR4), Ly6G+ MDSCs and F4/80+ macrophages in the control (no treatment), RTCT, and RTCT + plerixafor treatments arms. Mice in the control arm were euthanized when the tumors reached a size of 1-1.5 cm. Mice in the RTCT and RTCT + concurrent plerixafor arms were euthanized immediately after completing all treatment (3 weeks). Magnification 20X.



**Supplemental Figure 2(b) – RTCT and Adjuvant Plerixafor:** Representative tumor sections showing immunohistochemical (IHC) staining for phosphorylated CXCR4 (pCXCR4), Ly6G+ MDSCs and F4/80+ macrophages in the control (no treatment), RTCT, and RTCT + plerixafor treatments arms. Mice in the control arm were euthanized when the tumors reached a size of 1-1.5 cm. Mice in the RTCT arm were euthanized immediately after completing all treatment (3 weeks). Mice in the RTCT + adjuvant plerixafor arm were euthanized immediately after completing plerixafor (6 weeks). Magnification 20X.





**Supplemental Figure 3:** Representative sections of normal mouse colon/rectum showing histologic changes consistent with late radiation injury (Supplemental Table 3, Langberg *et al.* 1992) 90 days after RT, RTCT or RTCT + plerixafor. Green arrows: Colitis cystica profunda (CCP); Blue arrows: Lymphatic congestion (LC); Black arrows: Vascular sclerosis (VS); Red arrows: Rectal wall fibrosis (RWF); Yellow arrows: Histologically unremarkable (unaffected) blood vessels (BV). RT was administered as a single 20 Gy fraction. Cisplatin 4 mg/kg was administered as a single ip dose two hours before irradiation. Plerixafor 5 mg/kg/day was administered sc for 30 days beginning 3 days before irradiation. Magnification 20X.