Cell Reports, Volume *36*

Supplemental information

Tumor-initiating stem cell shapes its microenvironment into an immunosuppressive barrier and pro-tumorigenic niche

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Figure S1

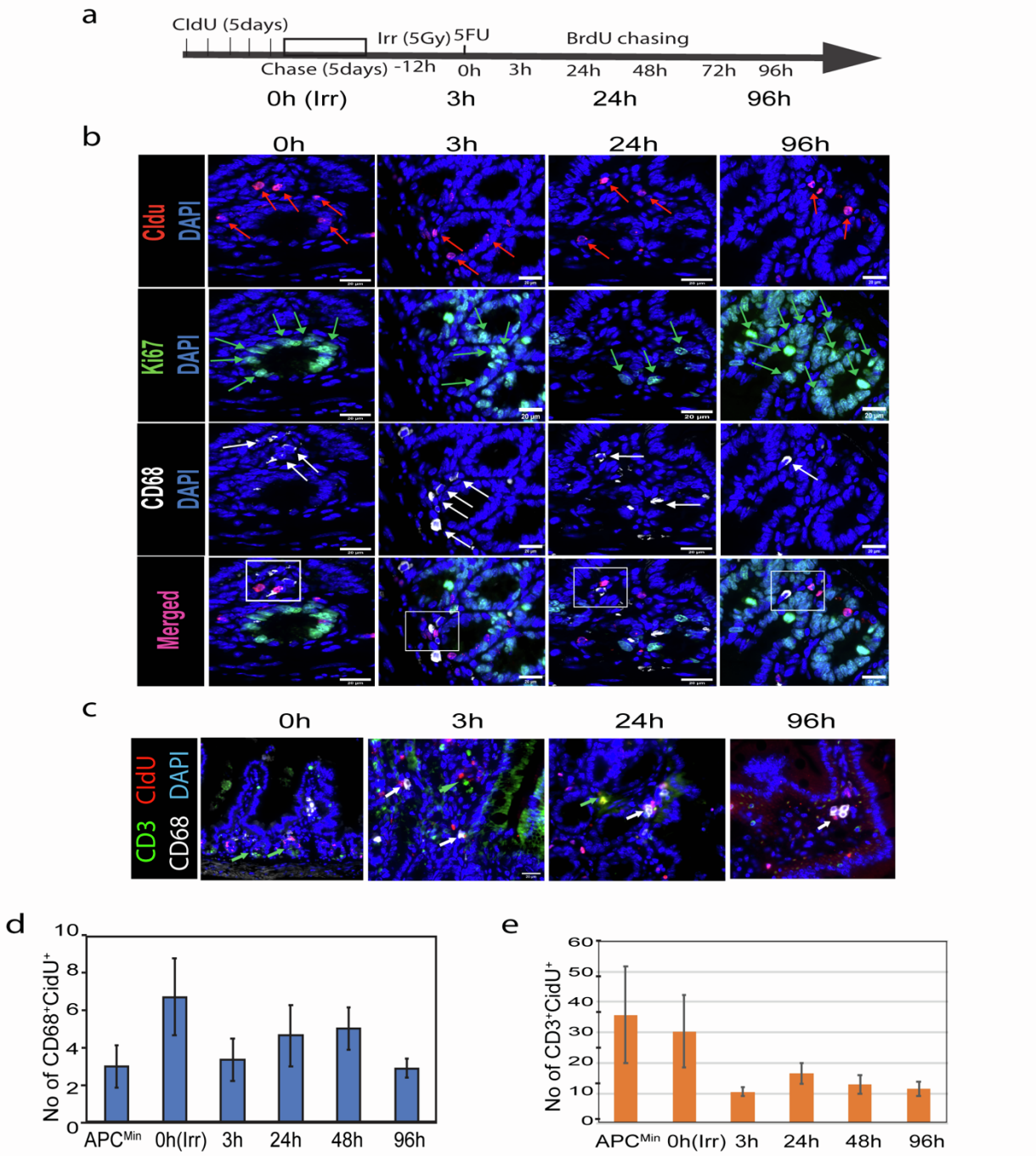


Figure S1. Differential responses of proliferating vs. slow-cycling tumor cells as well as MDCs vs T cells. Related to Figure 1G-J, 4A-C.

a. Illustration of the procedure for CIdU labeling, chemoradiotherapy (CRT), and subsequent detection of different states and types of cells. **b.** IF staining of CIdU (red) and Ki67 (green) to reveal slow- or active-cycling, activation of slow-cycling cells (Ki67+CIdU+), and CD68+ MDCs and CD3+ T cells. **c.** IF staining of CIdU, CD3, and CD68 positive cells. **d-e.** Quantification of pairing CIdU+ cells with CD68+ (D) or CD3+ (E) cells

Figure S2,

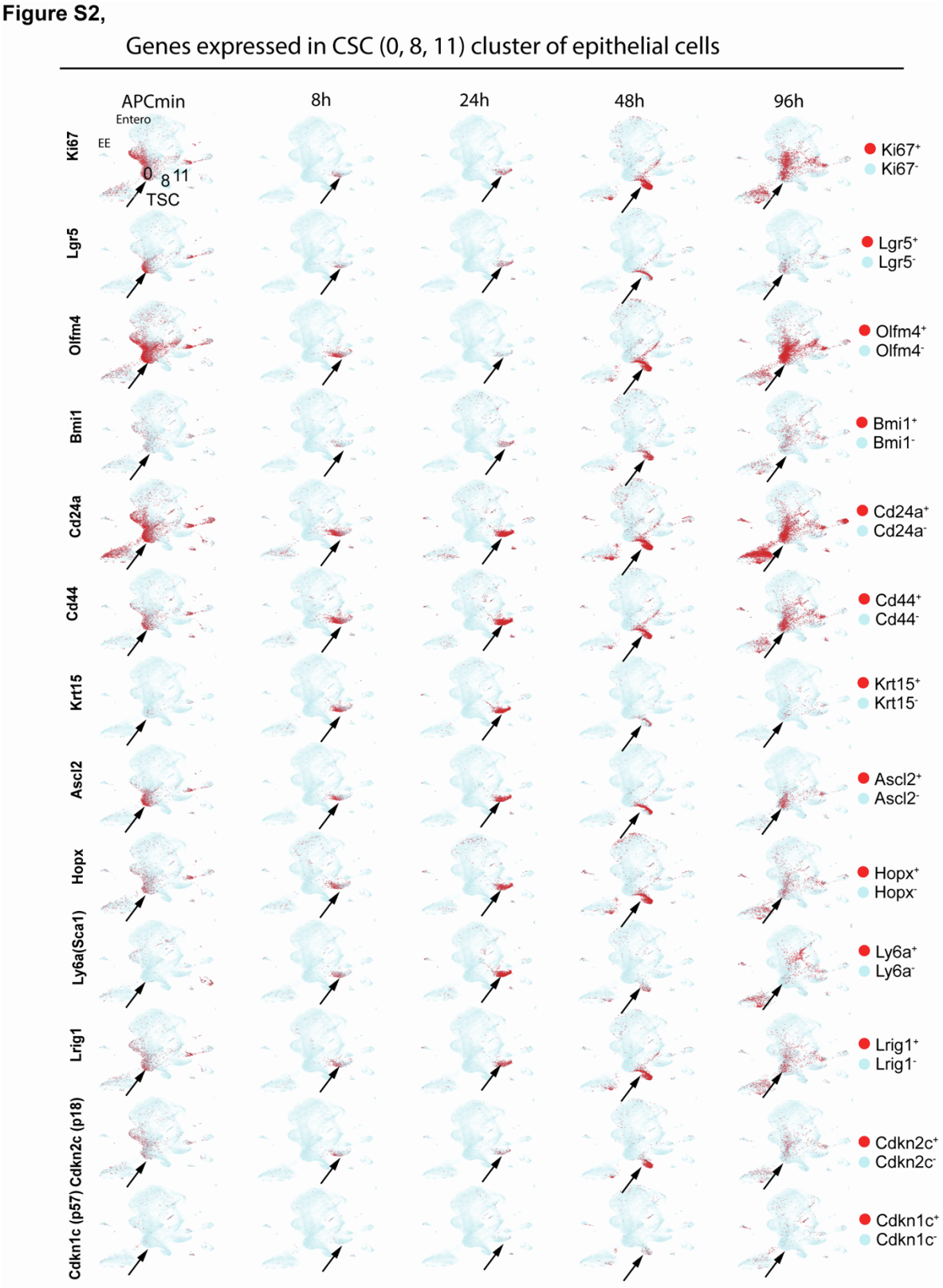


Figure S2. **Dynamic expression of different genes in CSC of adenoma (epithelial fraction) during the course of therapy in the UMAP analysis (Fig.1A.3A), arrows indicate CSC cluster at different times. Relarted to Figures 2, 3**

Figure S3.

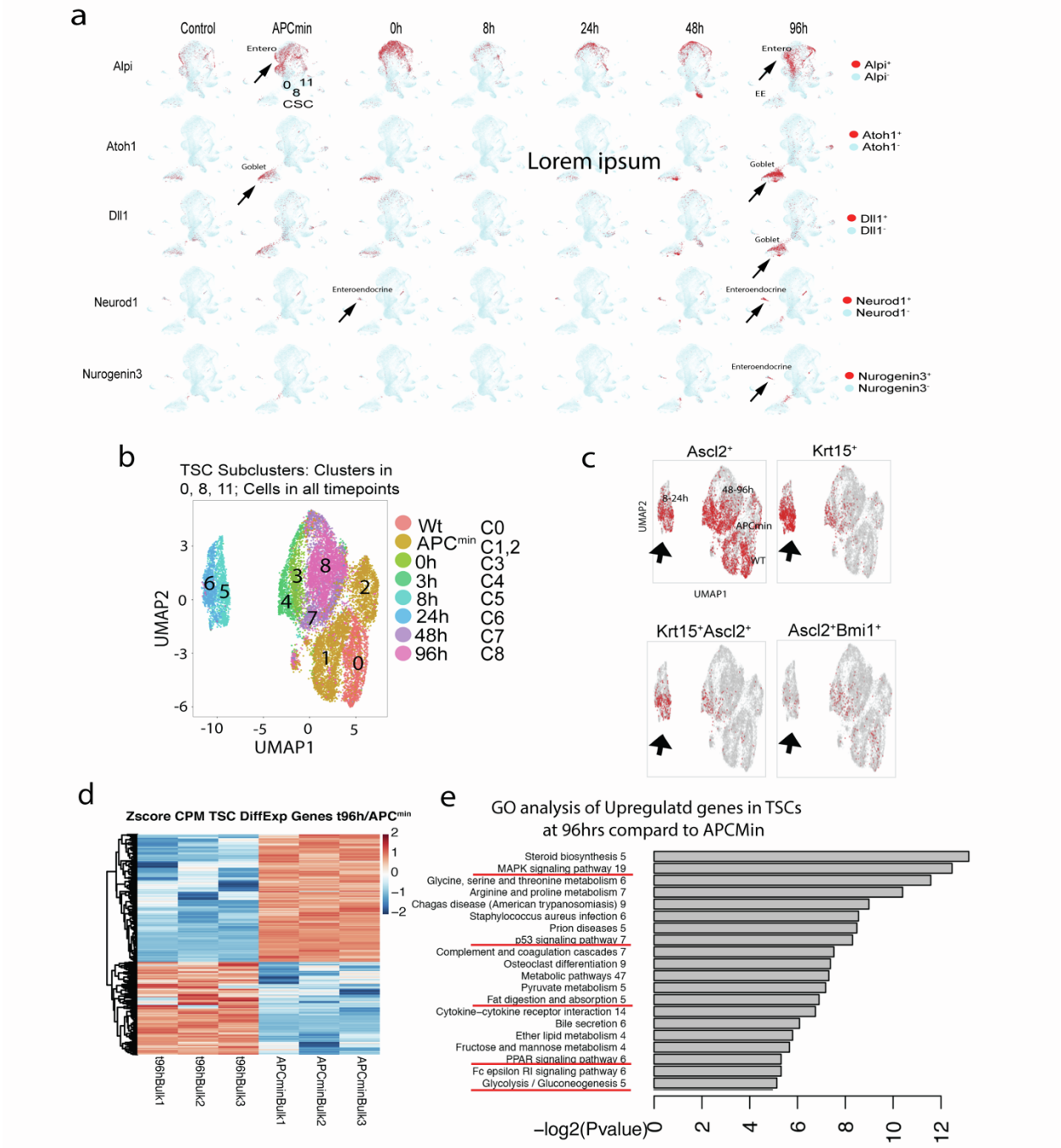
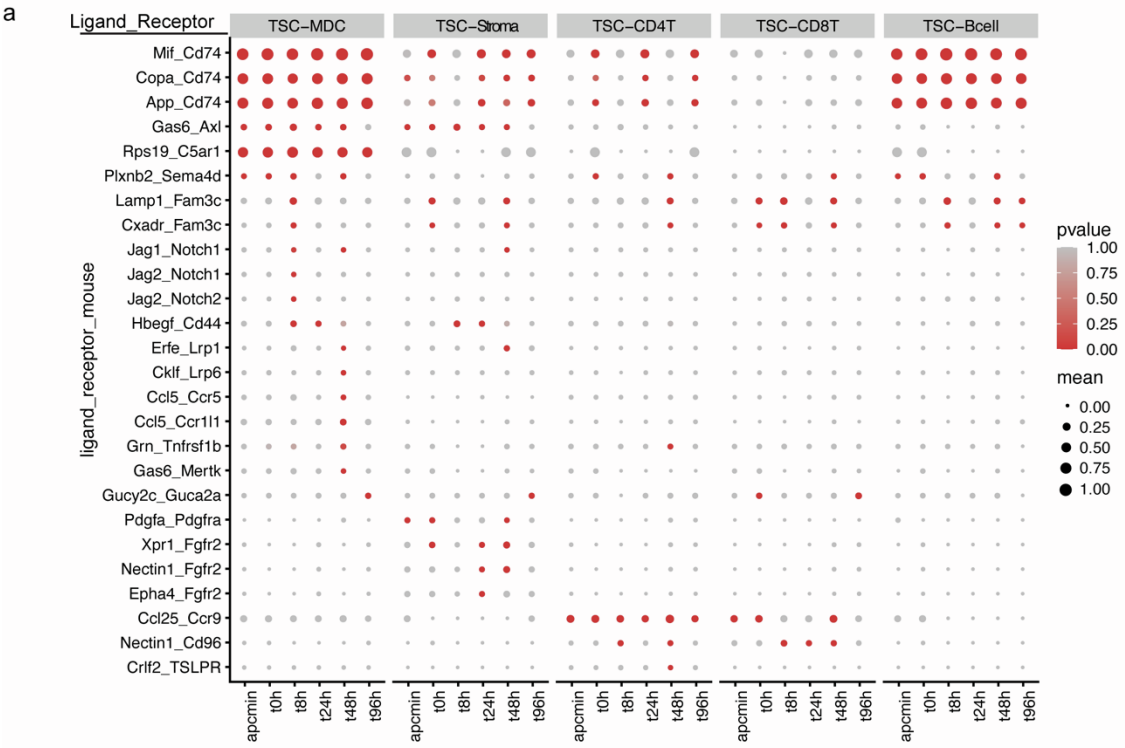


Figure S3. Representative gene expression and GO term analysis, Related to Figure 3.
a. Expression of representative genes of differentiated lineages including Alpi, Aloh1, Dll1, Neurod1, and Neurgenin3, none of these genes showed expression in the TRTSCs at 8-24 hrs post CRT, but Alpi and Dll1 express in TRTSCs at 48Hrs post CRT, indicating that survived TrTSCs (8-24hrs) do not express these genes, which however were detected in the reprogramed TrTSCs (48hrs). **b**. Further dissection of TRTSCs and revealed that TSCs (C1,2) is different from ISCs (C0), and Restored TSCs (C8) after CRT is different from initial TSC (C1,2). **c**. TrTSC at 24hrs coexpress Krt15 and Ascl2, as well as Ascl2 and Bmi1. **d-e**. Heatmap and GO-term analysis compare the difference between restored SCs (at 96hrs) and initial TSCs (APCmin/+). The former reveals enhanced MAPK and P53 pathway, and combined glycolysis and fatty acid metabolisms

Figure S4



b

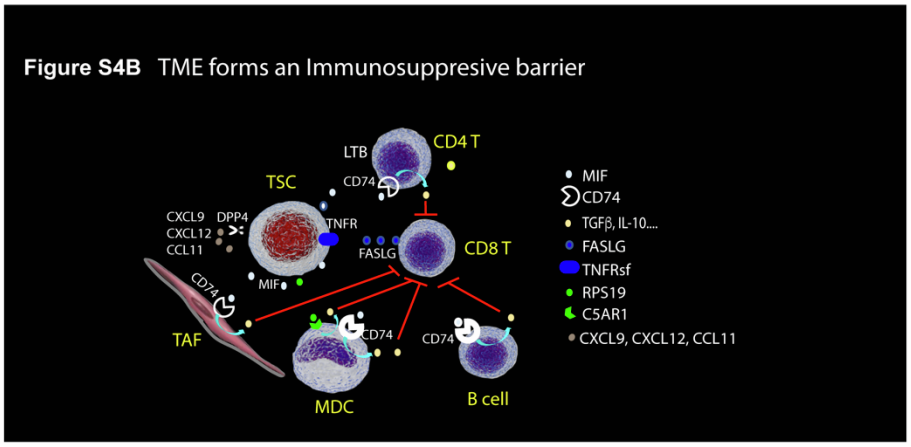


Figure S4. CellPhoneDB analysis revealed signaling modules from TSCs to a variety of TME cell types and in turn TME forms an Immune barrier. Related to Figure 4. a. CPDB analysis identifiied ligand-receptor inteeractions between TSC-TME. **b.** TSC sends out MIF which signaling via CD74 receptor to mediate a variety of functions including secreting immune suppressive signals such as TGFb1 and IL-10. Though CD74 is widely expressed, but it does not express in CD8 T cells and predominantly expresses in MDCs and B cells (Figueiredo et al., 2018). In response to CRT, while CD8T cell increased secretion of FAS ligand that has the potential to kill TSCs via TNF receptor (Gajate and Mollinedo, 2005). Inversely, stressed TSC sends out RPS19, which signaling via C5AR1 in MDCs to increase secreting immune suppressive signals including TGFb1 and IL-10 to inhibit CD8T cells (Kao et al., 2017; Markiewski et al., 2017).

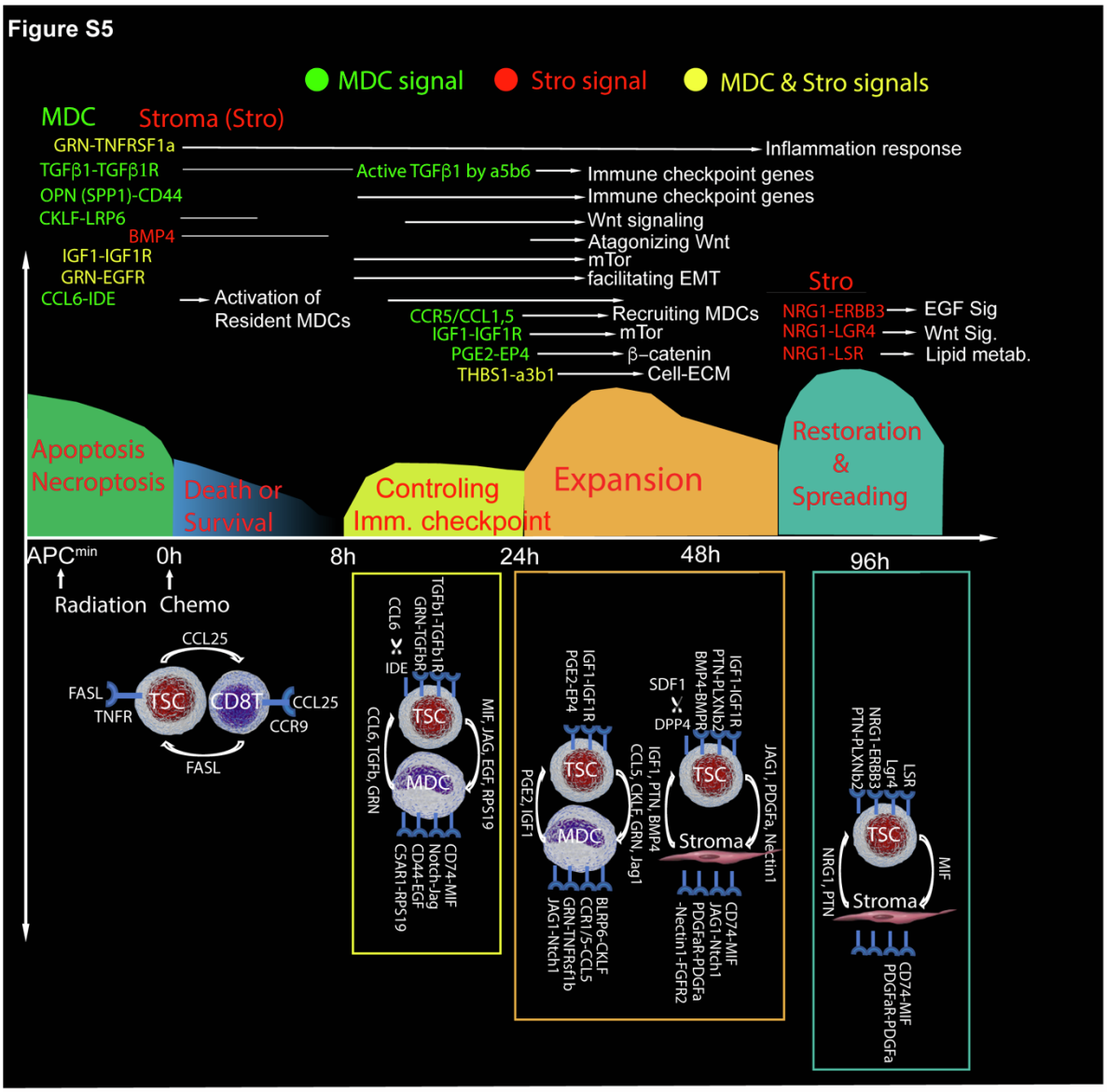


Figure S5. A summary of the events and the related signaling changes during CRT. Related to Figures 4, S4. MDC derived signaling shows as green, TAS derived signaling as red, the overlapped signaling of the two as yellow. TGFb1 and CKLF signaling from MDCs, T, and B cells to TSCs, regulatinh immune response. TAS cells upregulated BMP4 signaling to antagonize Wnt signaling (He et al., 2004). Granulin (GRN) signaling via TNFRSF1a that induces lysosomal activity and inflammation response (Kao et al., 2017). Upregulated IDE in TSCs activate CCL6 expressed in MDCs with a potential in mobilizing MDCs (Coelho et al., 2007). At 8hrs GRN-EGFR and COPA-EGFR signaling upregulated. Upregulation of Jag1 and Jag2 from TSCs play a role in converting MDCs to TAMs (Liu and Cao, 2015). OPN(SPP1)-CD44 signaling controlling of immune checkpoint (Klement et al., 2018). At 24hrs, TGFb1 with ITGa5b6 facilitates the conversion of the latent TGFb1 into the active form (Dong et al., 2017), coincided with upregulating TGFbR2 (Fig.3F). At 48hrs, CCL5-CCR1/5 chemotactic signaling recruites 2nd wave of MDCs (Walens et al., 2019). Upregulating PGE2 (reflected by Ptgs2 for Cox-2)-PTGER4 (EP4) signaling between MDCs and TSCs. Both IGF1 and PGE2 signaling might contribute to TSC expansion during 24-48hrs post CRT. At 96hrs, the majority of upregulated signals declined. The complex of IGF1 with ITGa6b4 is intriguing with a potential role in regulating cell migration (Bon et al., 2007). But an increasing in TAS-dependent signaling was a trend at 96hrs such as forming a protein complex of Nrg1 with ERBB3 (EGFR), LSR (Lipolysis stimulated lipoprotein receptor), Lgr4, and integrin-a6b4 between stromal cells and TSCs, thus promoting TSC further propagation and spreading.

Figure S6

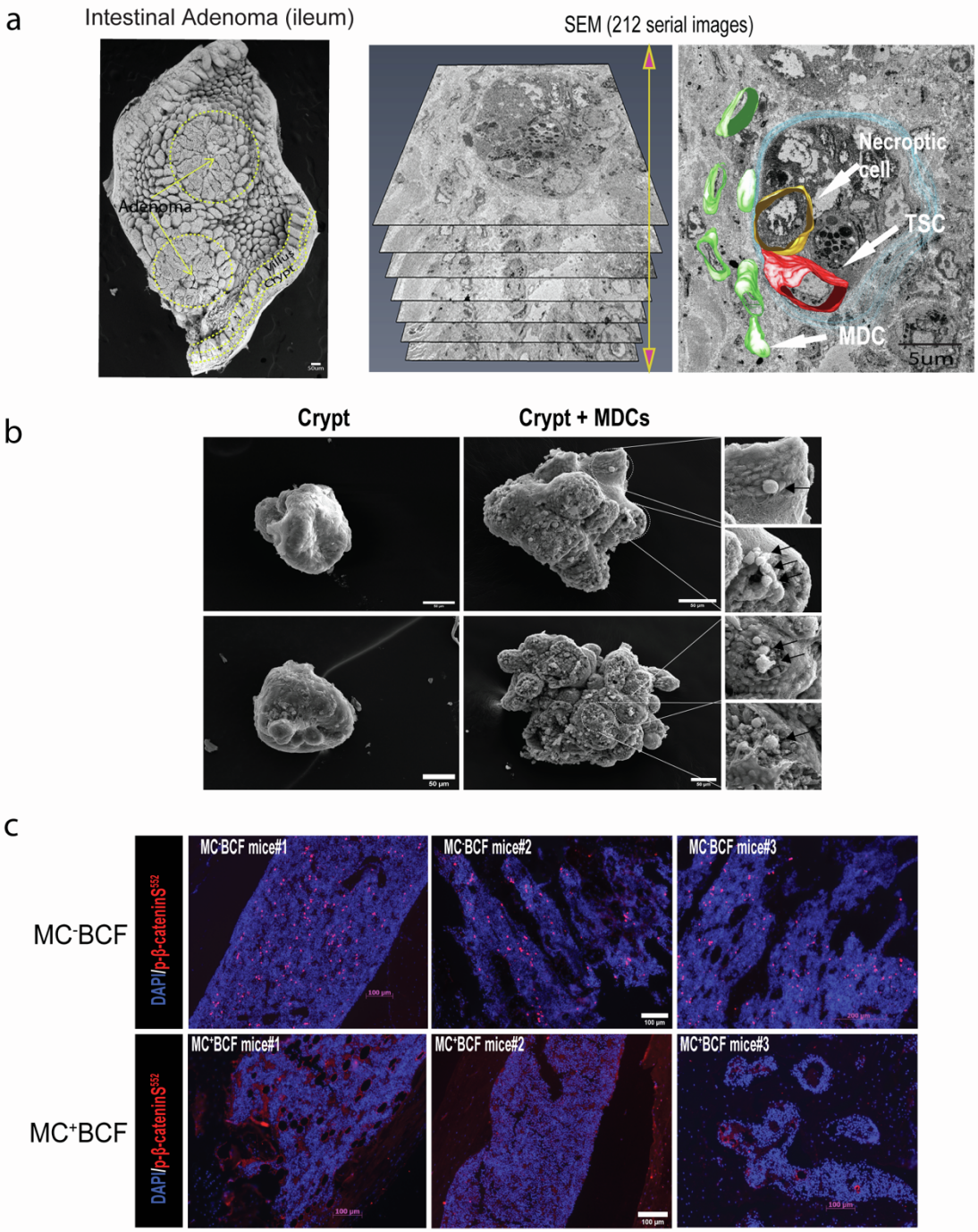


Figure S6. MDCs are recruited to the TSC niche, or support adenoma-organoid growth, as well as loss of anti-p-beta-cateninS^{552} staining in Ctnb KO bone marrow. Related to Figures 4A-C, 5A-C.

a. Distinguishing stem-like cells from surrounding necroptotic and differentiated cells as revealed using TEM (left panel), detection of recruiting MDCs to the tumor and TSC sites following CRT using SEM. The 3D model is composed of 212 images of SEM dataset (right panel:). **b**. Scanning EM Images of organoid attached by Macrophage-like cells (white). **c**. Failure to detect p-beta-cateninS^{552} in the section of bone marrow of Mx1-Cre:beta-catefl/fl mice compared to Wt BM. It is worthy to point out that the pattern of p-beta-cateninS552 positive cells reflects mitotic cell state, which is similar to but still different from that of p-Histon3+ cells. We recently also reported that beta-catenin interacts with chromatin-modulating proteins (Fang et al., 2020 Stem Cell Reports).

Figure S7

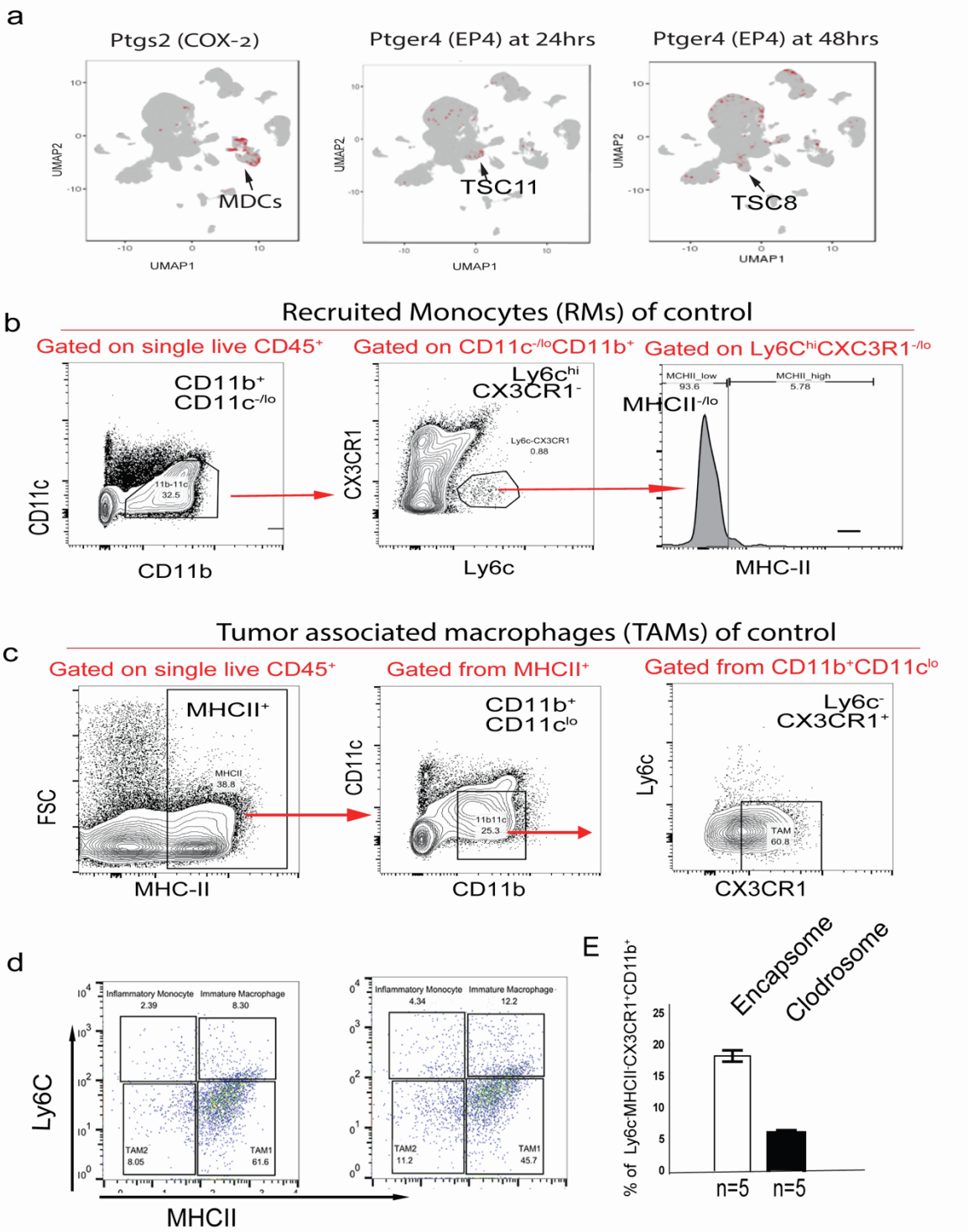


Figure S7. Expresison paterns of Ptgs2 and Ptger4 as well as flow cytometry analyses of RMS, TAMs. Related to Figure 5.

a. Expression of Pstg2 and Ptger4 between 24-48hrs revealed by Shiny map. **b-c.** Flow cytometry analysis of MDCs and TAMs. **d.** Further define TAMS in response to clodrosome and encapsome.

Figure S8

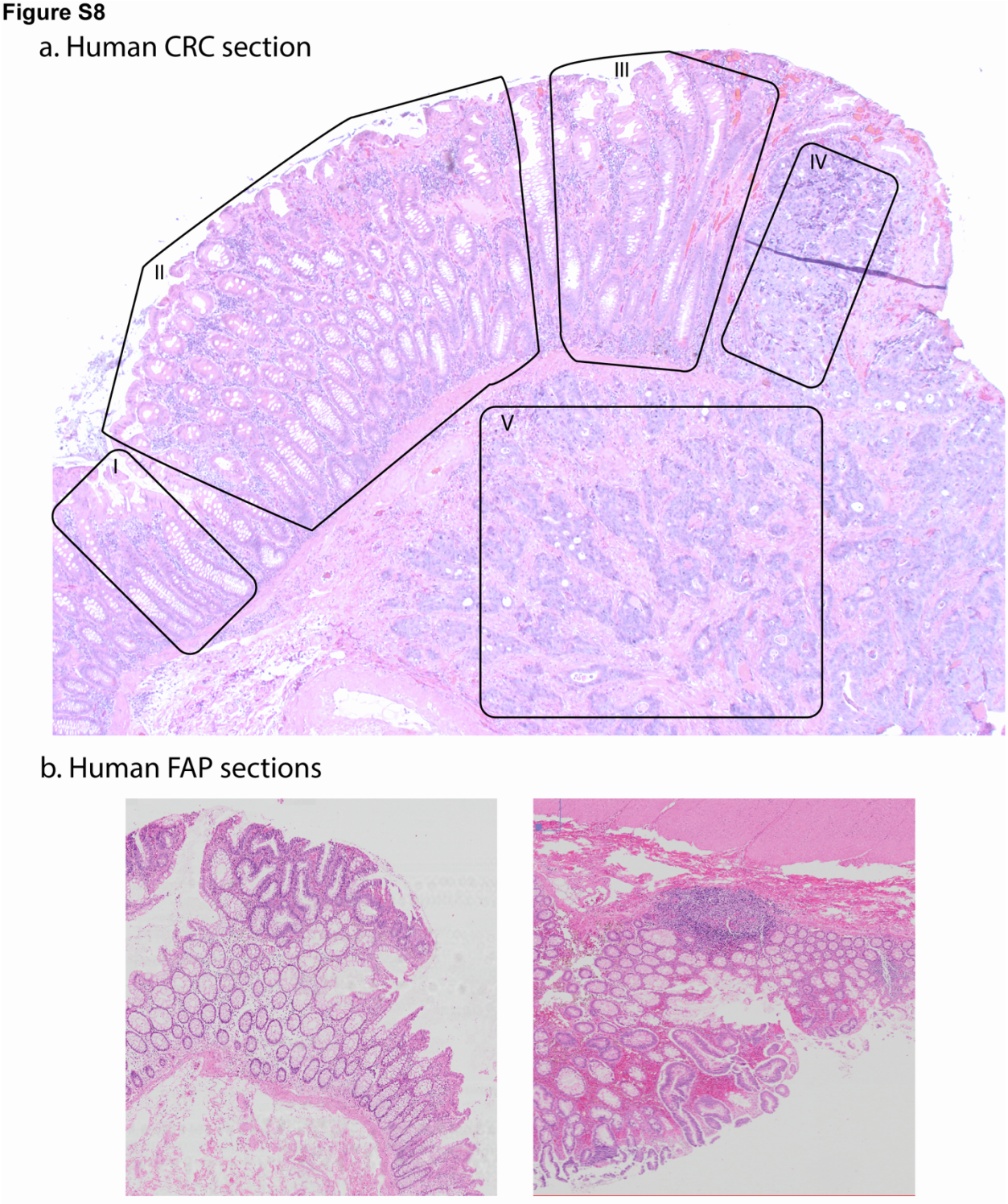


Figure S8. Representative HE sections from deidentified human CRC patient and human FAP patients. Related to Figure 7.

a. HE-staining of sections from human CRC patient with stages of "normal mucosa" (NM) (I), adenoma stages of hyper-Proliferation (II), Dysplasia (III), and carcinoma stages (IV.V).

b. Section of familiar adenoma polyposis (FAP).

Figure S9

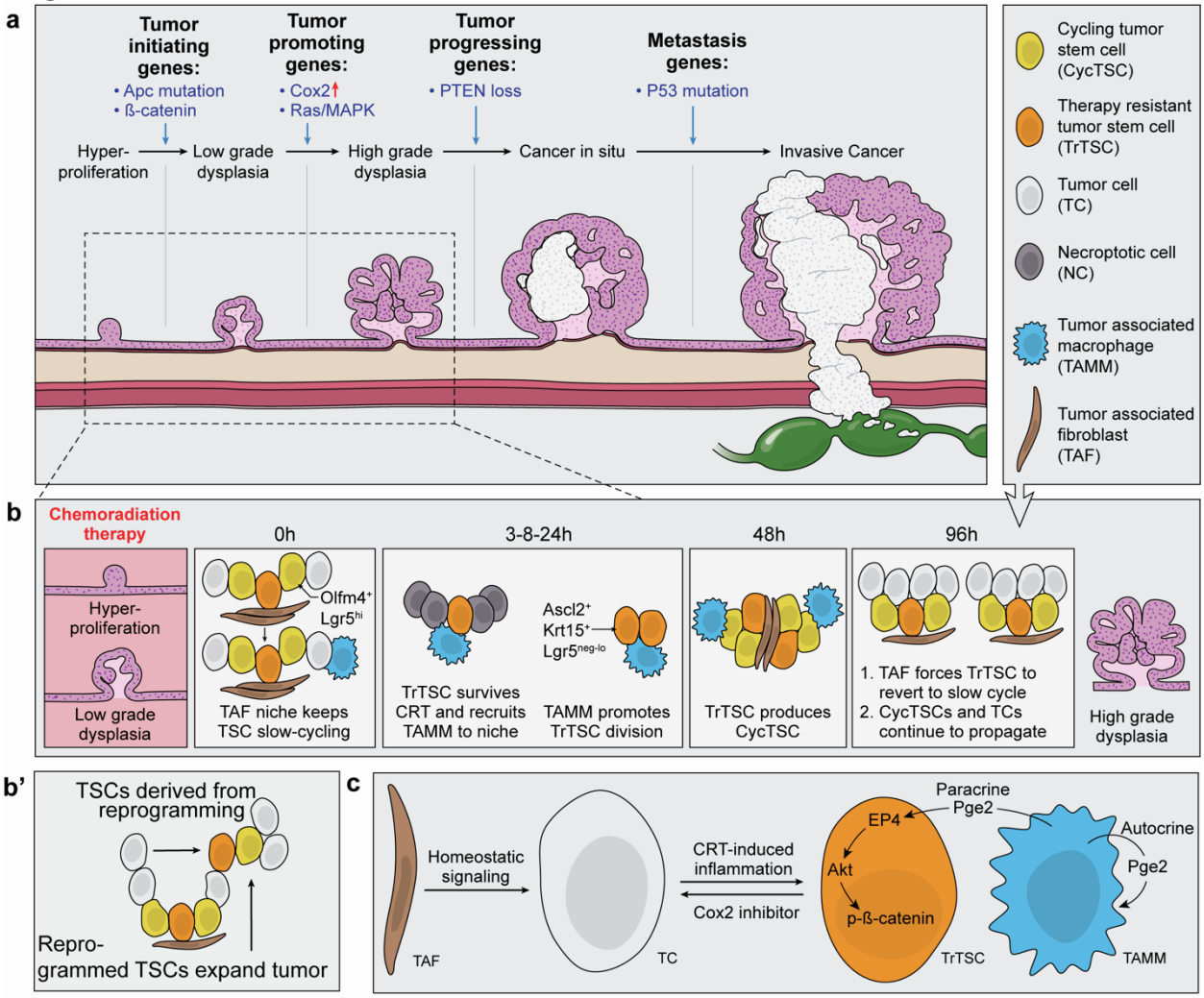


Figure S9. Summary of how microenvironment influences tumor initiating stem cell (TSC) survival, proliferation post chemoradiotherapy and restoring to normal maintenance. Related to Figures 1F-J, 3, 4A, 5.

a. The process of adenoma initiation to form an hyperproliferation (HyPro) foci, promotion to cloned abnormal crypts or tubular structure (low grad dysplasia, LGD), and progress to high grade dysplasia (HGD) with subcellular structure changes, to become carcinoma in situ (ACIS), and further into invasive carcinoma (InvAC). During this complex cascade process, it associates with different genetic mutations in oncogenes and tumor suppressors.

b. The chemoradiotherapy (CRT) induced transformation of adenoma from the early stage of Hypro and LGD to late stage of HGD. In response to the CRT, the majority of proliferating tumor cells and active-cycling TSCs (Lgr5hiOlfm4+) are eliminated, whereas slow-cycling TSCs (Ascl2+Krt15+,Lgr5lo) survive the CRT, and activate o produce daughter TSCs. Some daughter TSCs become active-cycling TSCs (Lgr5hiOlfm4+), some daughter TSCs revert to slow-cycling state. Active-cycling TSCs (Lgr5hiOlfm4+) drive the subsequent fast proliferation f tumor post CRT.

b'. Alternatively, TrTSCs can also be derived from differentiated cells called reprogrammed (repro-TSCs), which in turn can branching out to form new crypts and thus expanding tumor in mass.

c. Molecularly, TASs maintain TrTSCs slow-cycling state via proliferation-inhibitory signaling, TAMMs stimulate TrTSCs proliferation via, Pge2/EP signaling, IGF signaling, and potentially cytokines. There is also an autocrine ignaling of Pge2/EP in TAMMs.