Supplementary Table 1: Antibodies used for immunohistochemical and immunofluorescent imaging.

Target	Company	Clone
alpha-SMA	Dako	1A4
CD20	Dako	L26
CD31	Leica	1A10
CD68	Dako	PG-M1
DC-LAMP	Dendritics	1010E1.01
IL-1F5	Novus	polyclonal
IL-36g	Novus	polyclonal
PNAd	BD	MECA-79
Rabbit IgG isotype	Jackson Immunoresearch	polyclonal

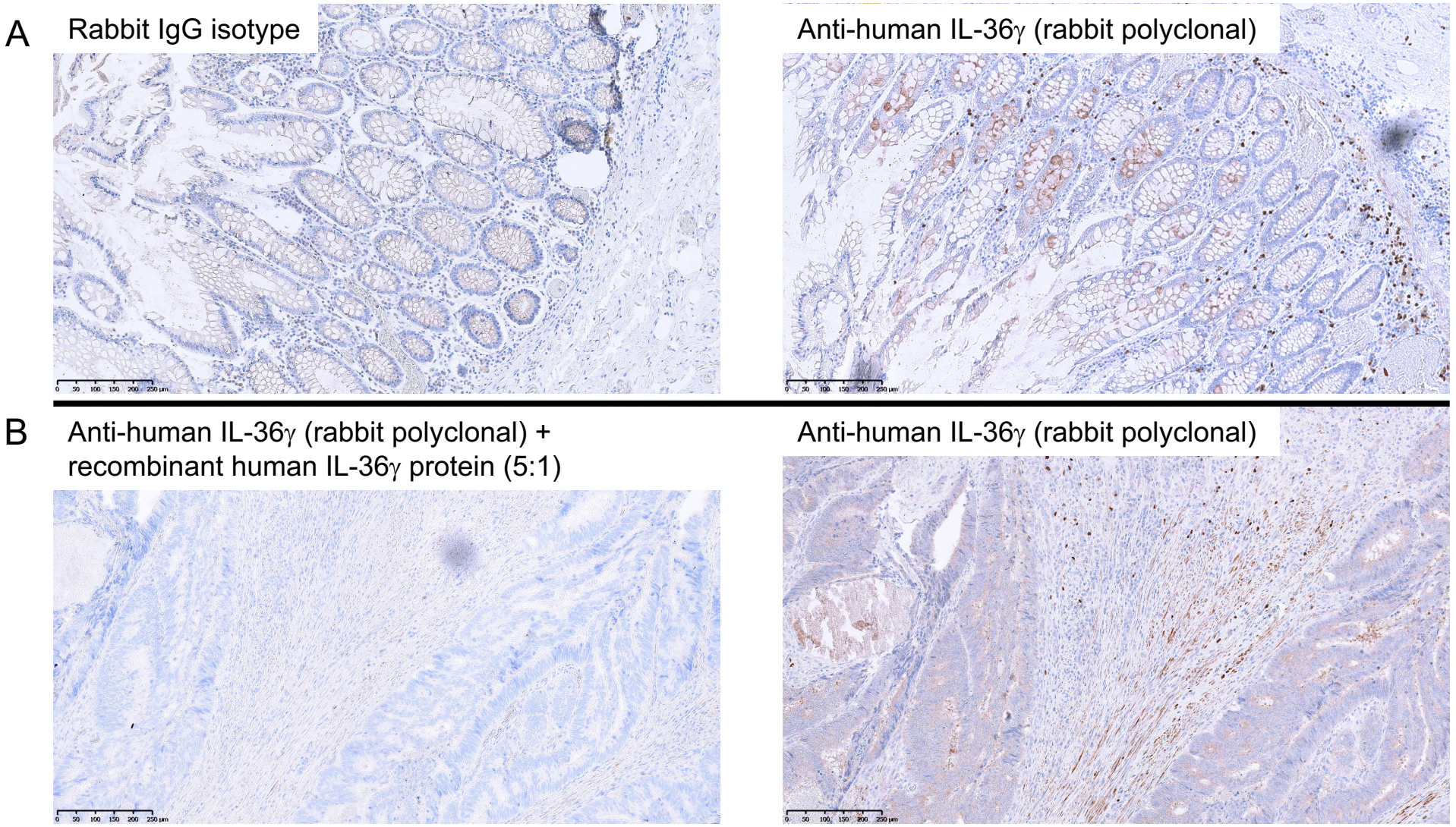
Supplementary Table 2: Antibodies used for flow cytometric analyses.

Target	Company	Clone	Conjugate
CCR7	Biolegend	G043H7	PE-Cy7
CD3	BD	UCHT1	AF700
CD4	Biolegend	OKT4	BV 605
CD45RA	Beckman Coulter	2H4	ECD
CD8	Biolegend	RPA-T8	BV 650

Supplementary Table 3: mRNA expression analysis of immune genes.

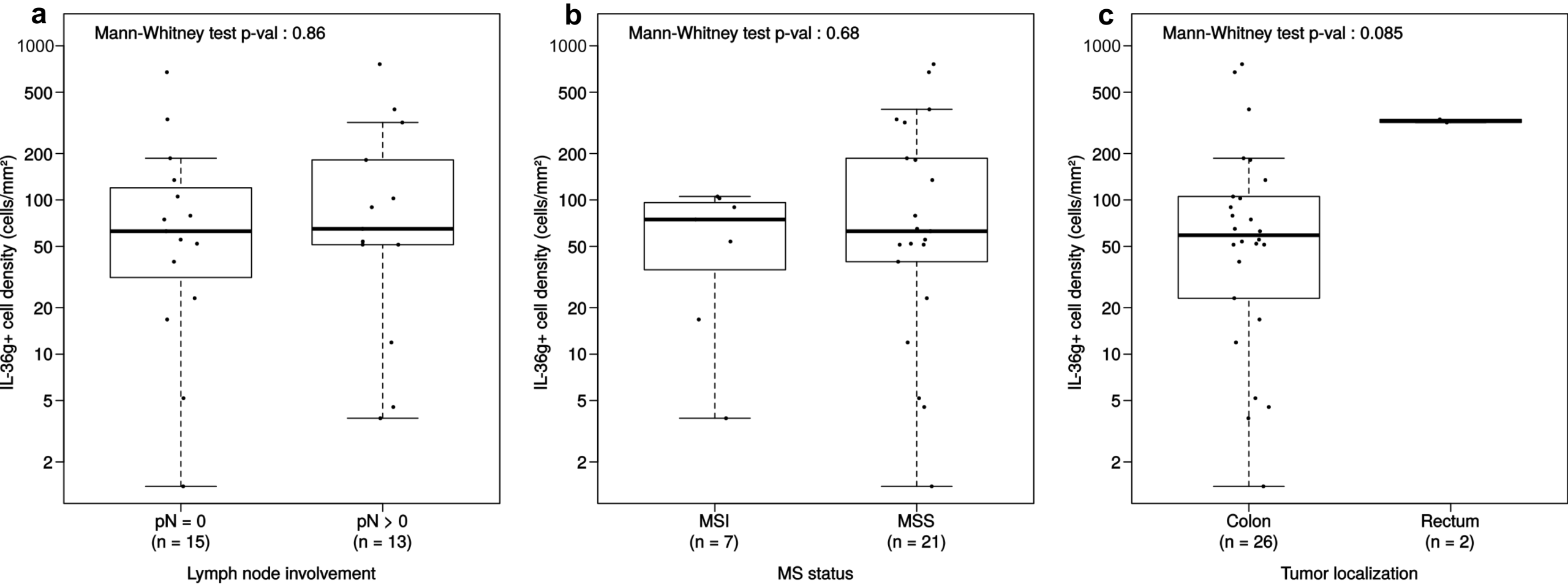
Gene target	
CCL19	*IL12A*
CCL2	*IL17A*
CCL20	*IL23*
CCL21	*IL6*
CD19	*LAG3*
CD274	*PDCD1*
CD68	*PDCD1LG2*
CSF1R	*TGFB1*
CTLA4	*TGFB2*
CXCL10	*TGFB3*
CXCL13	*TIE1*
EOMES	*TNF*
FLT3	*VEGFA*
ICOS	*VEGFB*
ICOSLG	*VEGFC*
IFNG	

Supplementary Figure 1



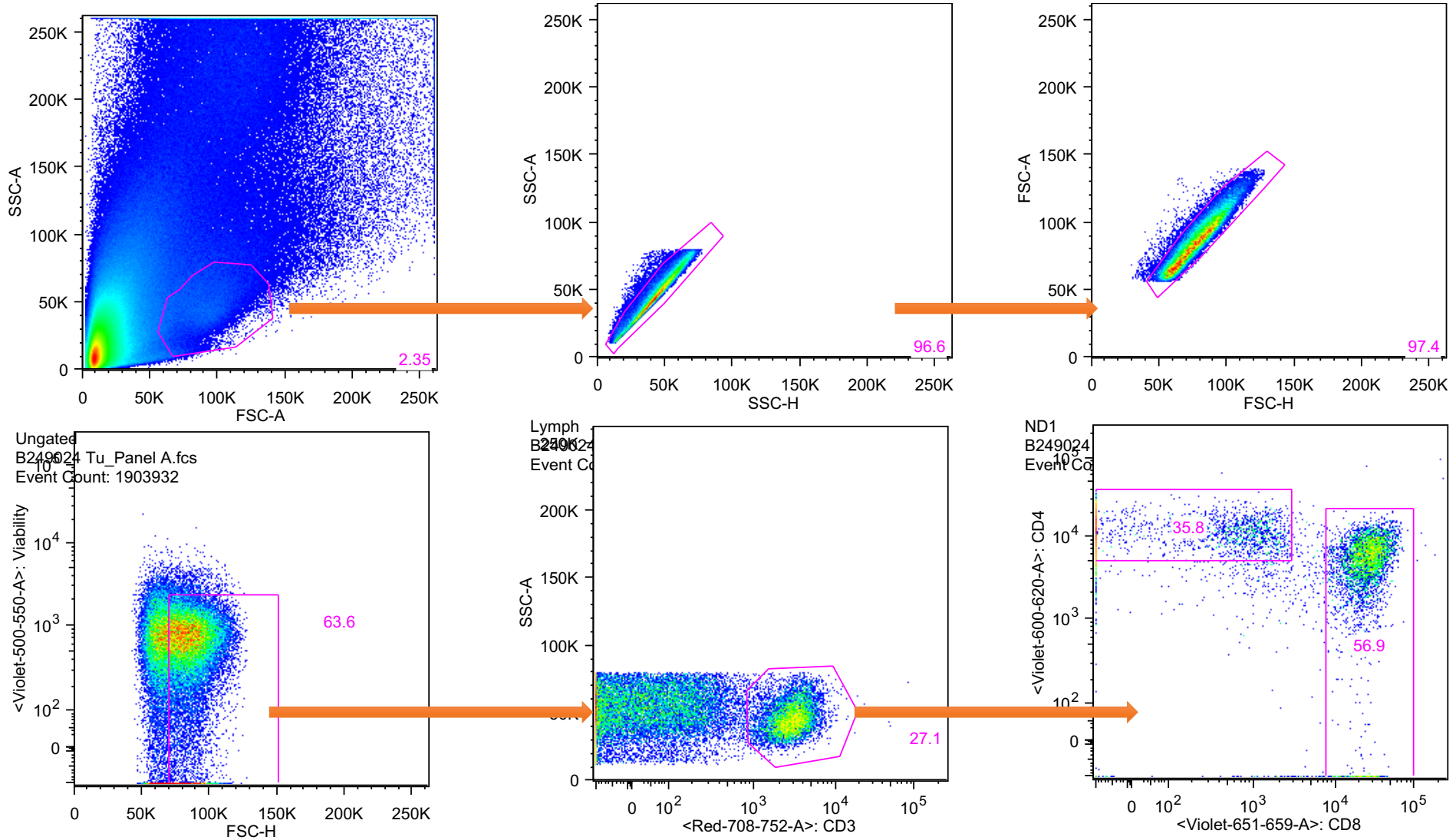
Supplementary Figure 1: Negative control staining. Colon sections were stained as described in Materials and Methods with a rabbit polyclonal anti-human IL-36g antibody (**A** and **B**, right)**.** To confirm antibody specificity, staining on serial sections was performed using either a rabbit IgG isotype control antibody (**A**) or by a mixture of the rabbit polyclonal anti-human IL-36g antibody with 5-times molar excess recombinant human IL-36g protein (R&D Systems). Scale bars = 250 microns.

Supplementary Figure 2



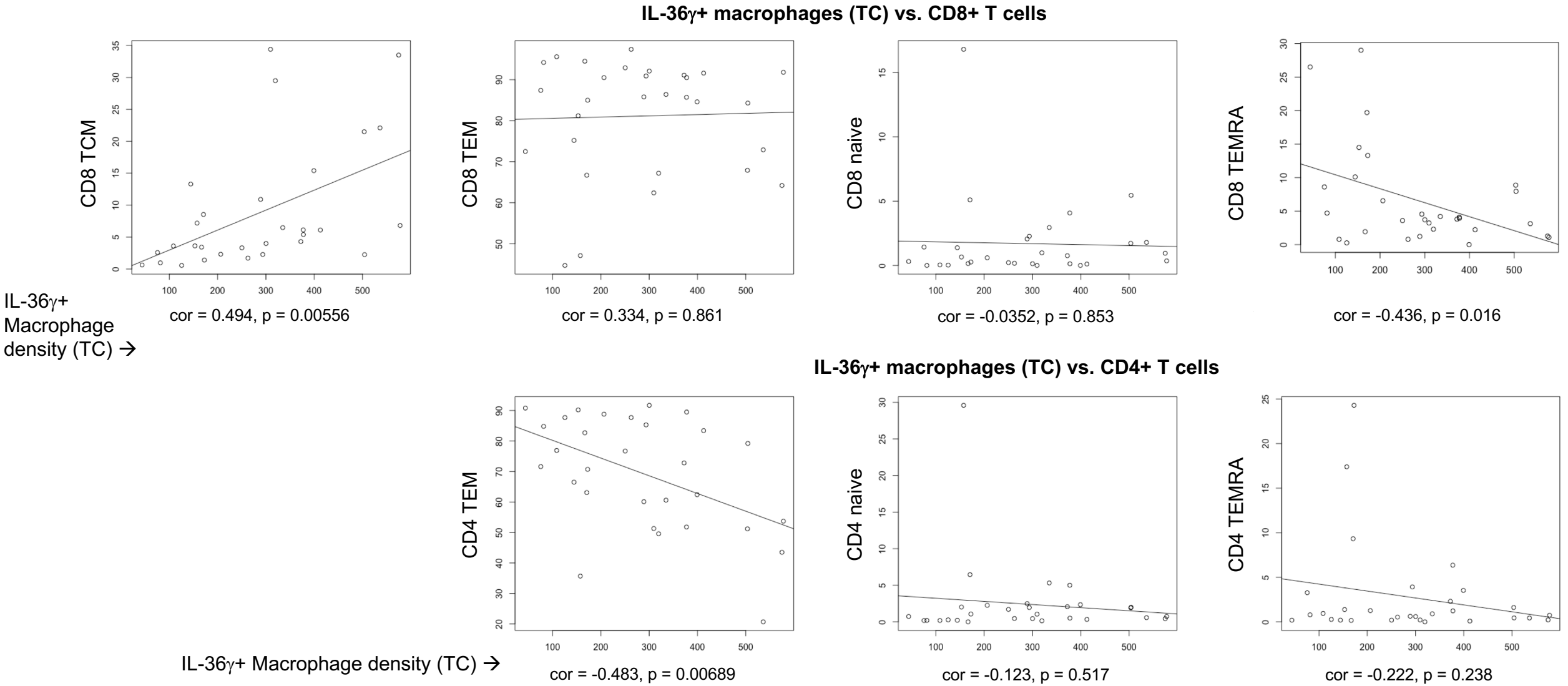
Supplementary Figure 2: IL-36γ expression by clinical parameters. Tumor sections were stained by immunohistochemistry with a rabbit polyclonal anti-human IL-36γ antibody and total IL-36γ^{+} surface area in the tumor was quantitated as described in Materials and Methods. Tumors were compared based by the presence or absence of lymph node metastases **(a)** or microsatellite status (**b**) and no significant difference was observed between these groups. Tumors localized to the colon, and tumors localized to the rectum, were compared to determine whether the location of the tumor affects the level of intratumoral IL-36γ, and results indicate that expression of IL-36γ in rectal tumors falls within the range of IL-36γ expression by colon tumors (**c**).

Supplementary Figure 3



Supplementary Figure 3: Representative gating strategy to identify T cells. After digestion and staining as described in Materials and Methods, cells were gated by size and by expression of CD3. T cells were then gated on expression of CD4 and CD8. To identify memory and naïve subsets, central memory T cells were considered CD45RA- CCR7+; effector memory RA (EMRA) T cells were CD45RA+ CCR7-; effector memory T cells were CD45RA- CCR7-; and naïve T cells were CD45RA+ CCR7+.

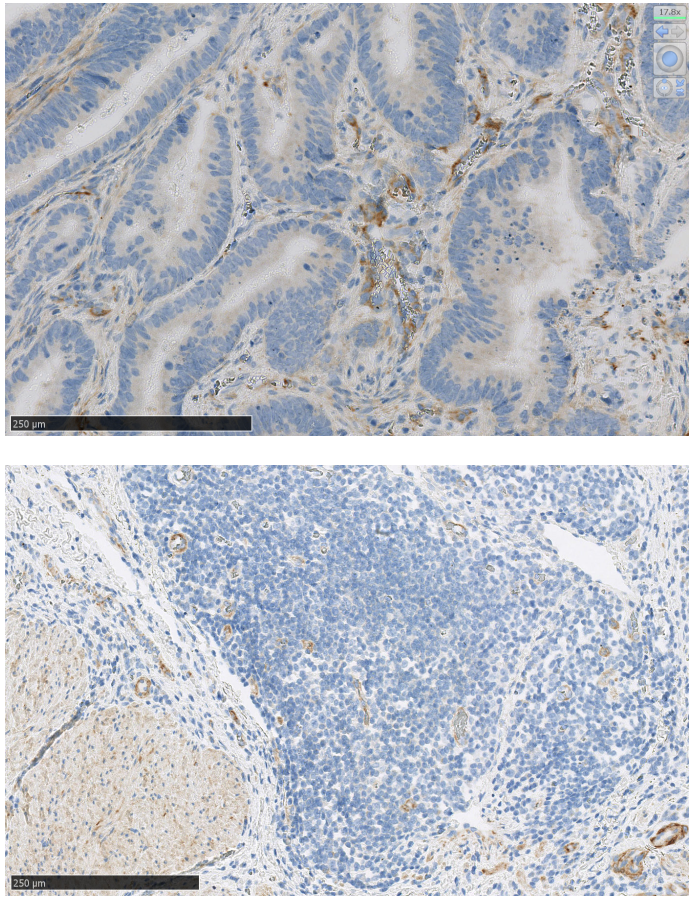
Supplementary Figure 4



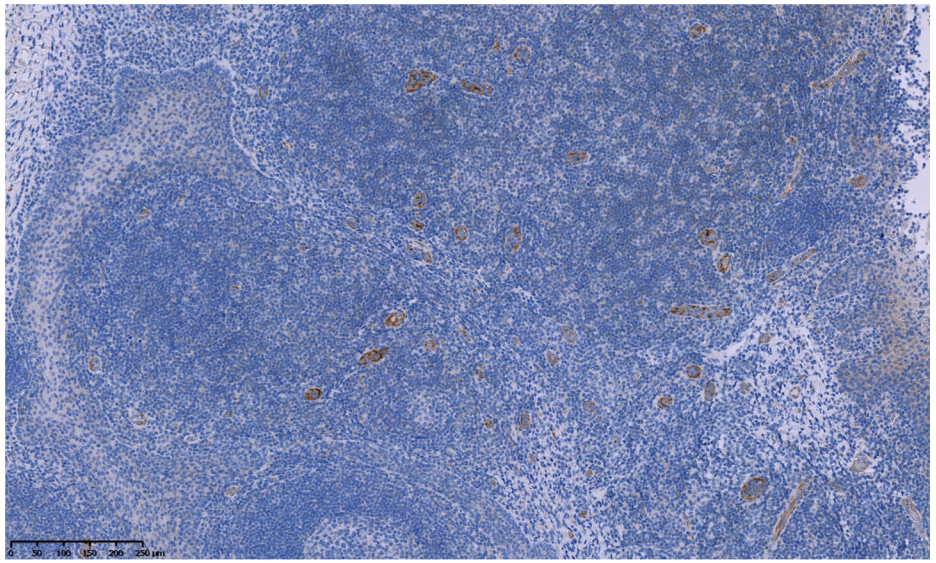
Supplementary Figure 4: IL-36g+ macrophages are not strongly correlated with most naïve and memory TIL subsets. FFPE tumor sections were visualized by immunofluorescence for CD68 and IL-36g or fresh tumor lysate was analyzed by flow cytometry for naïve and memory TIL populations. The correlation between IL-36g+ macrophages and all CD4+ and CD8+ naïve and memory cell populations (including naïve, central memory, effector memory, and EMRA, except for CD4+ TCM as shown in **Fig. 4**) was either not statistically significant or did not meet our threshold for correlation value of 0.5.

Supplementary Figure 5

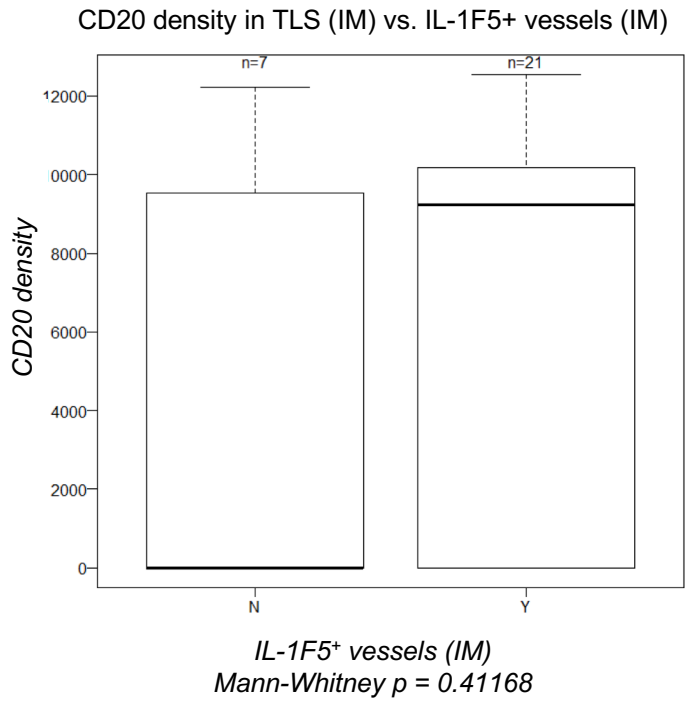
a



b

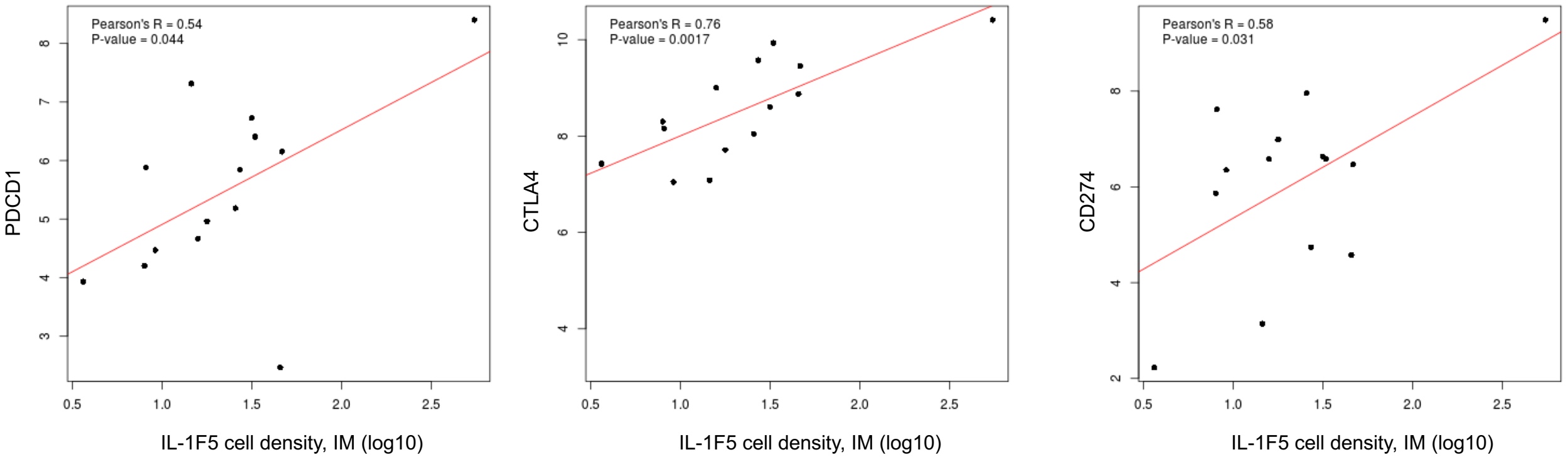


c



Supplementary Figure 5: Vasculature of tertiary and secondary lymphoid organs express IL-1F5. FFPE tumor sections were visualized by IHC for expression of IL-1F5, the natural antagonist to the IL-36 receptor. Like IL-36γ, IL-1F5 was observed to be expressed on the tumor vasculature (**a**). Bars = 250 microns. In **b**, FFPE sections of tonsil (a secondary lymphoid organ used as a control in this set of experiments) were visualized by IHC for expression of IL-1F5, which was observed to be expressed on the vasculature, especially surrounding germinal centers. Bar = 250 microns. When the intensity of IL-1F5 expression was correlated with the density of B cells in TLS within colon carcinoma lesions, no significant relationship was observed (**c**).

Supplementary Figure 6



Supplementary Figure 6: IL-1F5 expression positively-correlates with expression of immunosuppressive genes in the TME. IL-1F5 expression was visualized by IHC as described in **Fig. 7**, and transcript expression of *PDCD1* (PD1), *CTLA4*, *CD274* (PD-L1), *LAG3*, *ICOS*, and *ICOSL* was quantified by nCounter (Nanostring). Only expression of *PDCD1* (PD1), *CTLA4*, and *CD274* (PD-L1) was directly correlated with IL-1F5 expression in the IM of tumors.