Examining cellular and genetic changes in peritumoral microenvironment with anti-PD-1 treatment

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7/15/21

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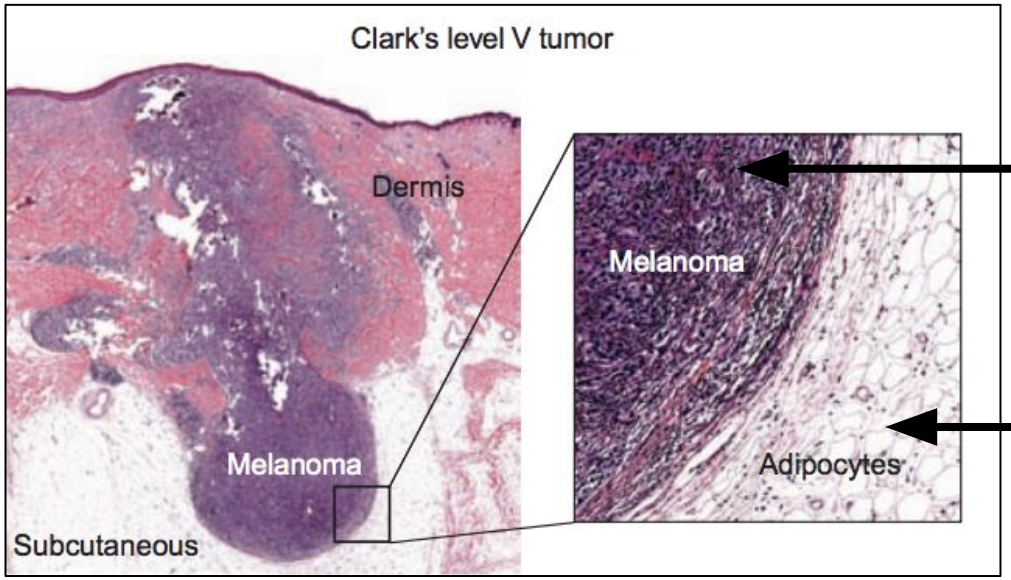
Adipose tissue undergoes crosstalk with tumor cells



Zhang et al. 2018. *Canc Disc*

- Tumor cells can use lipids from adipocytes as an energy source[1]
- Tumor cells promote adipocyte dedifferentiation into fibroblasts[2,3]
- Adipokines promote tumor growth via oncogenic signaling[4]
- Stroma and immune cells within adipose tissue may influence tumor biology[5,6]
  - Tumor associated suppressive CD11b+ myeloid cells

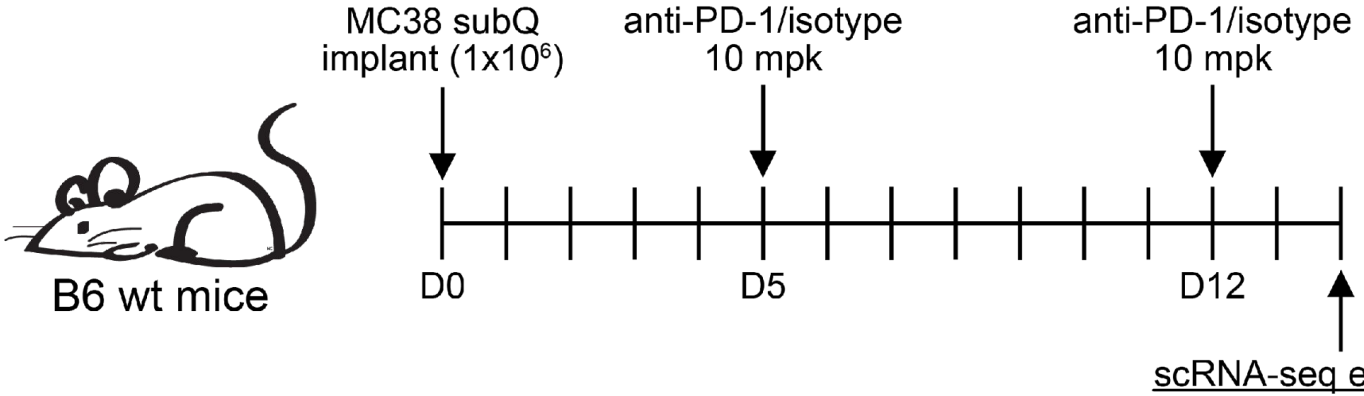
- White adipose tissue reserves memory T cells prior to infection at which point T cells switch to lipid metabolism
- Link between cancer, adipose tissue, and immune checkpoint blockade?

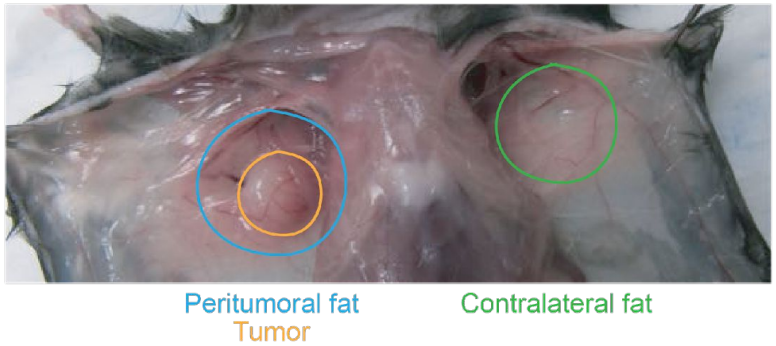
1. Zhang et al. 2018. *Cancer Disc*
2. Zoico et al. 2016. *Oncotarget*
3. Pearce et al. 2018. *Cancer Disc*
4. Khandekar et al. 2011. *Nat Rev Cancer*
5. Saha et al. 2017. *Cancer Res*
6. Wagner et al. 2012. *Angiogenesis*

How does response to immune checkpoint blockade influence cancer-adipose tissue crosstalk?

- Do cell populations in peritumoral fat change in activity during ICB response?
  - Does the genetic expression profile of these populations promote or repress response?

Experimental Setup





- Sorted by aPD-1 and isotype-treated mice
- 3 tissue type locations: peritumoral fat (FatT), contralateral fat (FatC), and Tumor
- Sorted by CD45+ immune cells or CD45-CD31+ stromal cells

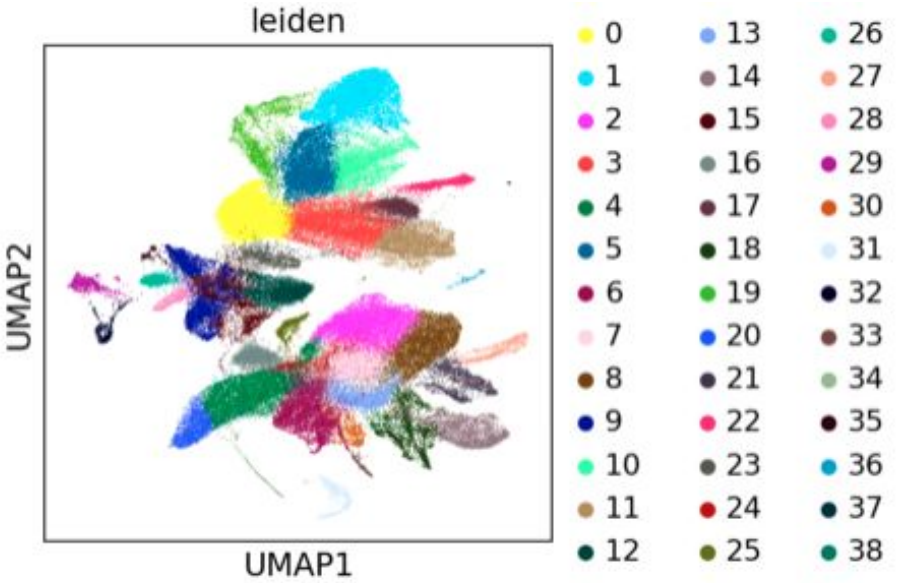
Data Preprocessing

1. scRNA-seq performed with CellRanger yielded 22.9M reads, 28K genes
2. Removal of 3 samples due to tumor contamination of fatT samples, slow response to aPD-1, and low barcode read count
3. Select for reads expressing <3500 genes and low levels of ribosomal, mitochondrial genes
4. Identify highly variable genes, select reads which express these genes
5. Regress out unwanted variation due to number of counts and percent mito. genes

PCA, Clustering, and Refinement

6. Calculate PCA, neighborhood graph for UMAP visualizations

- Leiden clustering algorithm for UMAPs to identify cell clusters
  - Improved community recognition and speed compared to Louvain algorithm[1]

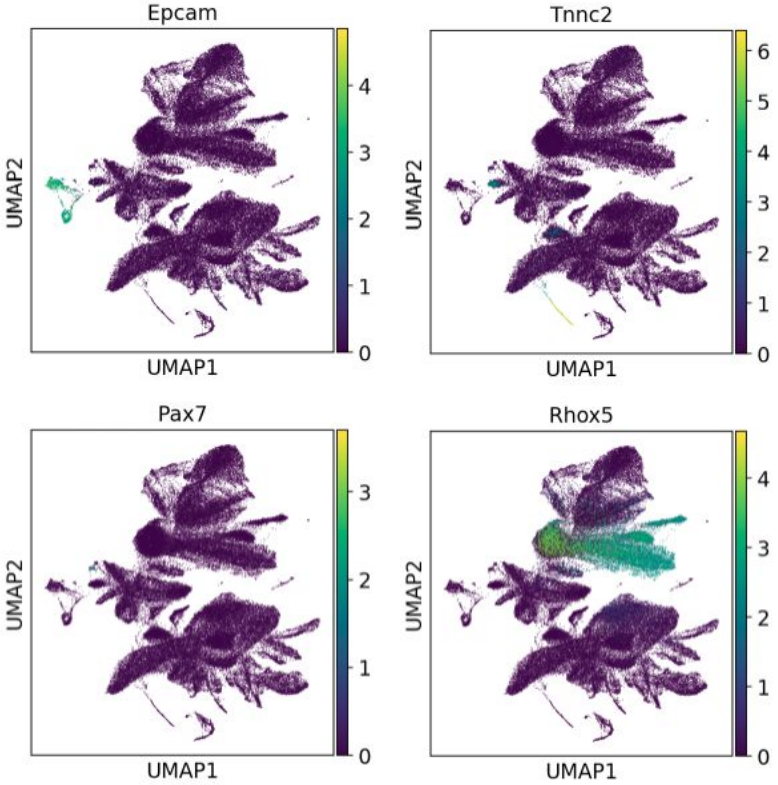


1. Traag et al. (2019), *Sci. Reports, 9*

PCA, Clustering, and Refinement

7. Remove clusters expressing markers for unwanted cell types
   - Epcam: epithelial cells
   - Pax7: satellite cells
   - Tnnc2: muscle cells
   - Hbb-bs: red blood cells

8. Remove cells that express no lineage markers for immune, stromal, or tumor cells



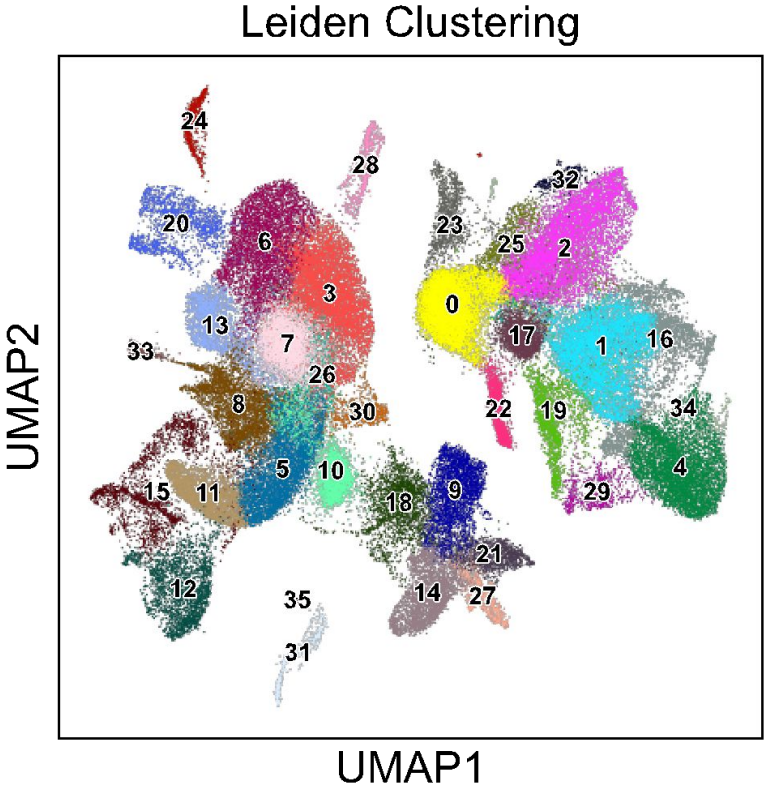
PCA, Clustering, and Refinement

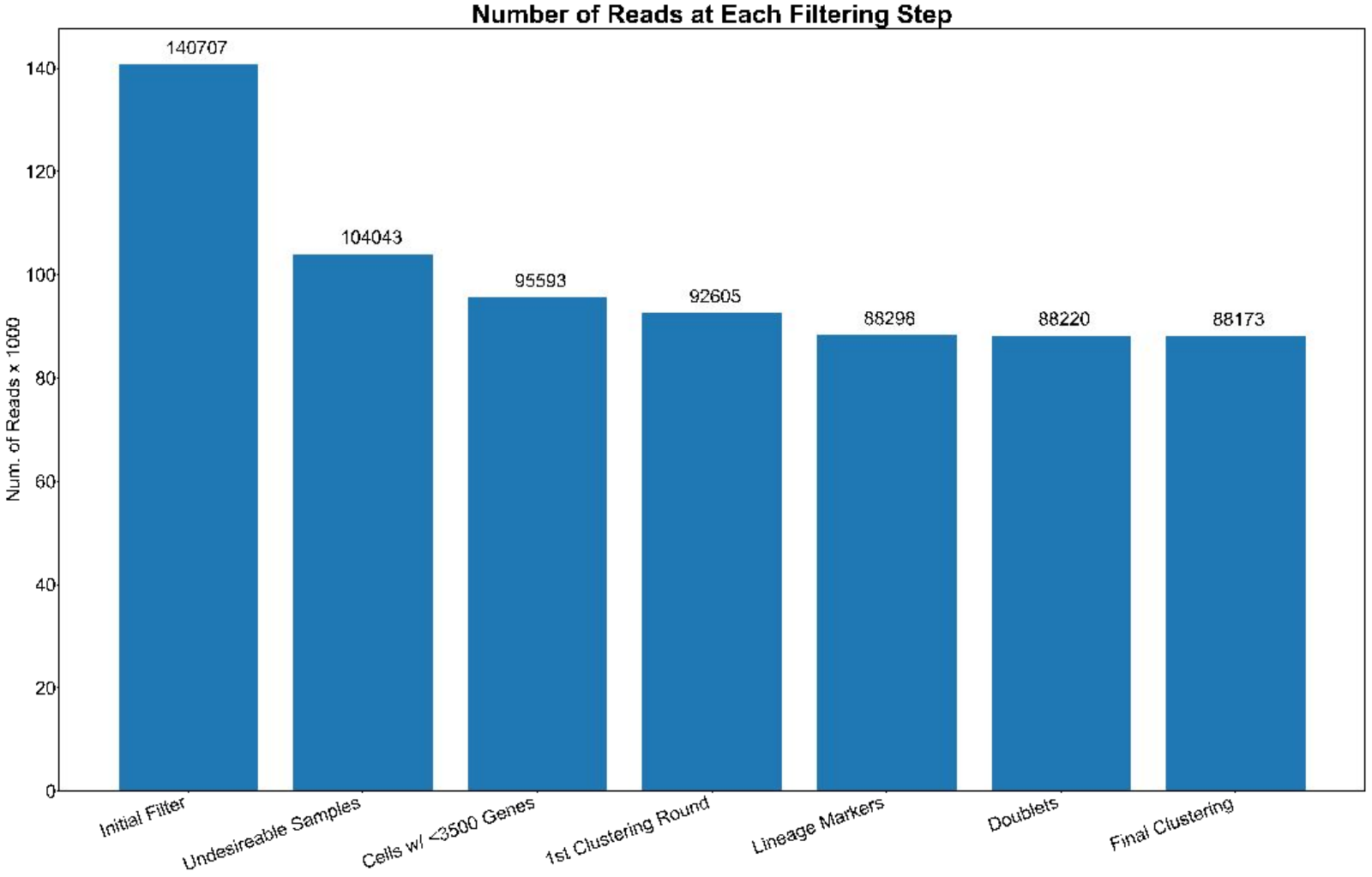
9. Filter out predicted doublet measurements from each sample

10. Rerun PCA, neighborhood graph, and Leiden clustering of UMAP

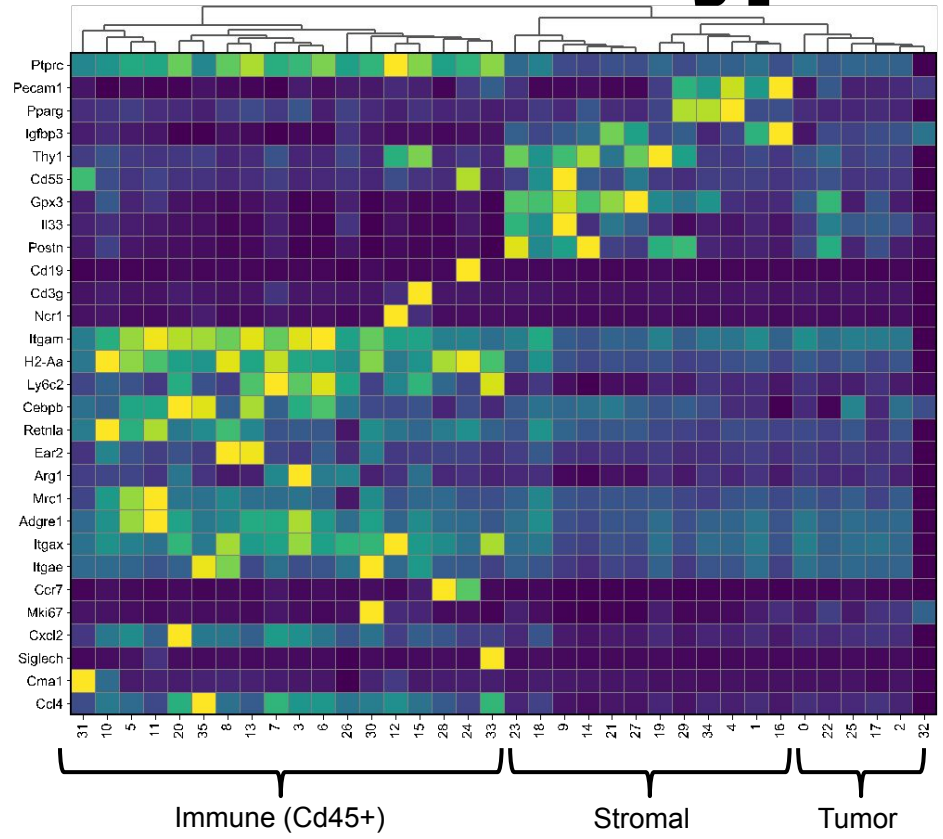
11. Check clusters for eosinophil marker SiglecF – clusters with < 100 cells that don't express gene are filtered

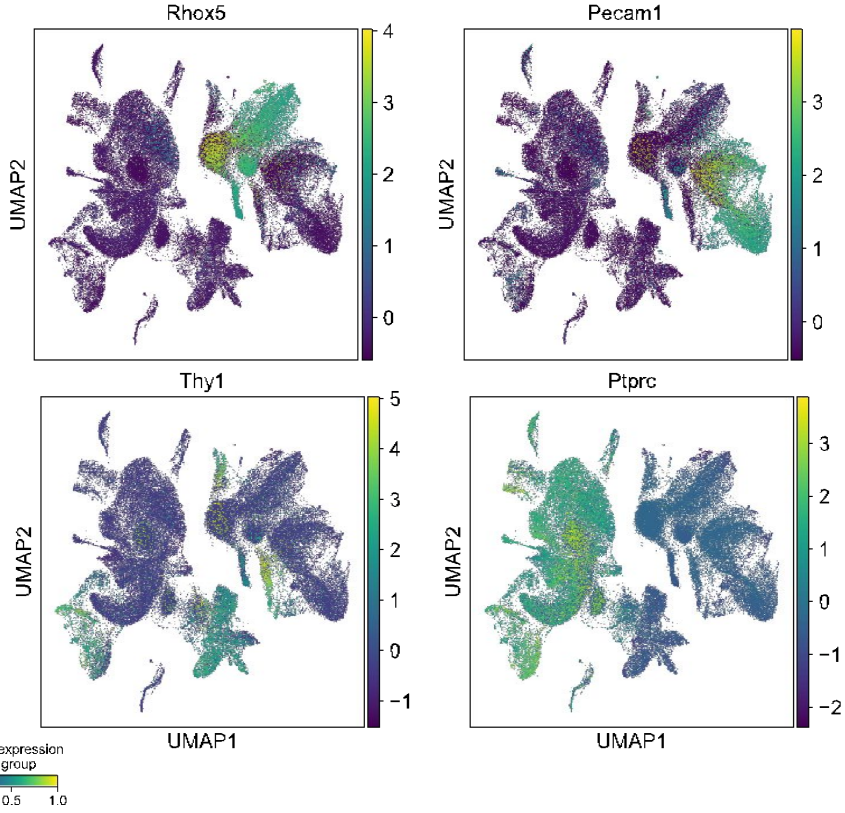
- Final UMAP: 36 clusters of 88173 cells




Number of Reads at Each Filtering Step
Num. of Reads x 1000
0
20
40
60
80
100
120
140
140707
104043
95593
92605
88298
88220
88173
Initial Filter
Undesireable Samples
Cells w/ <3500 Genes
1st Clustering Round
Lineage Markers
Doublets
Final Clustering

Cluster Cell Types





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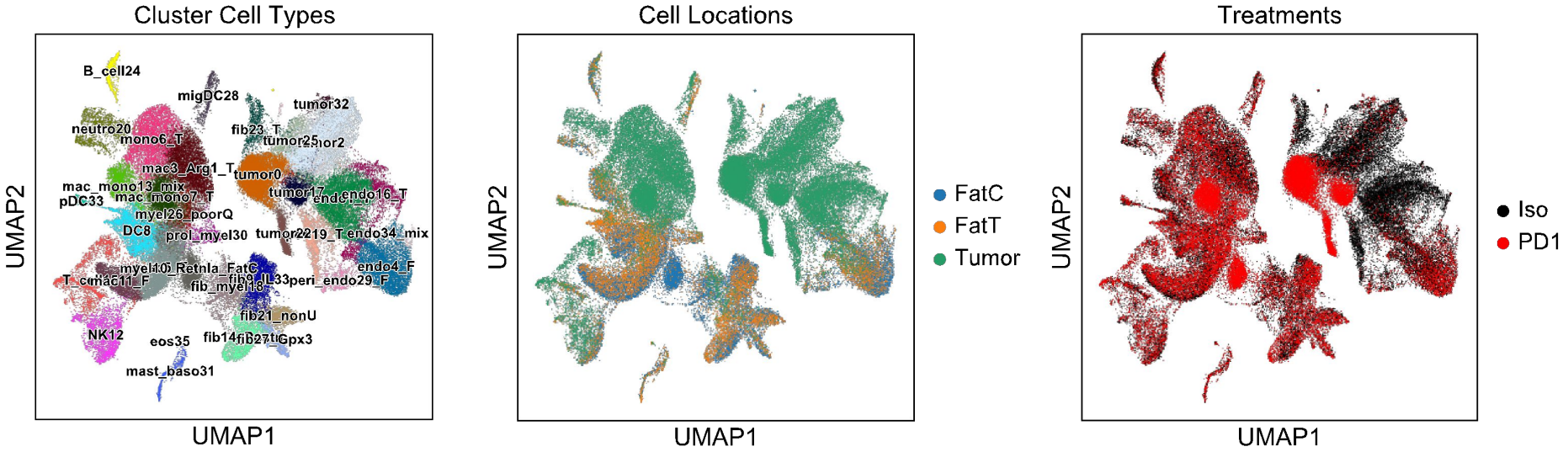
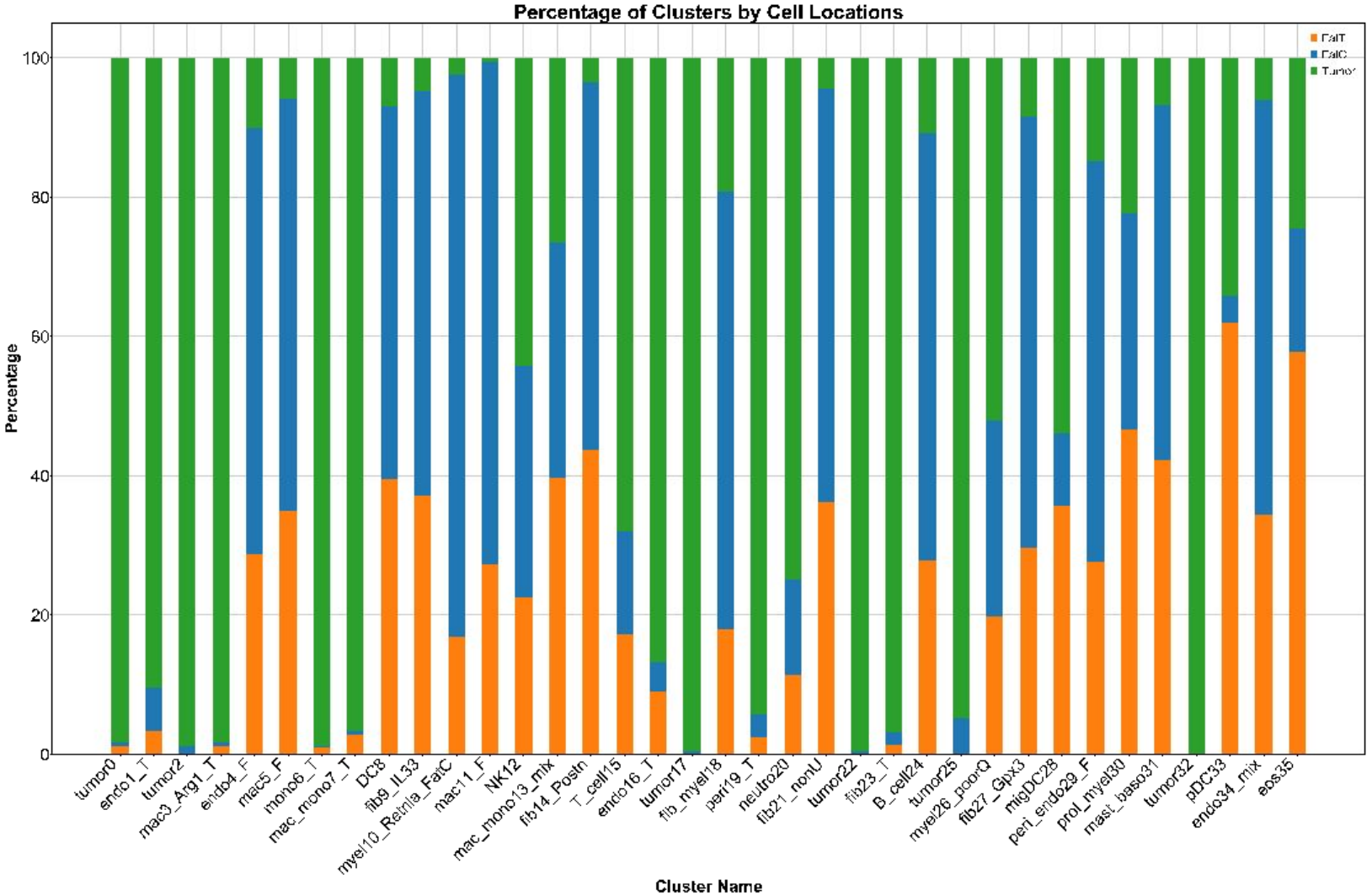
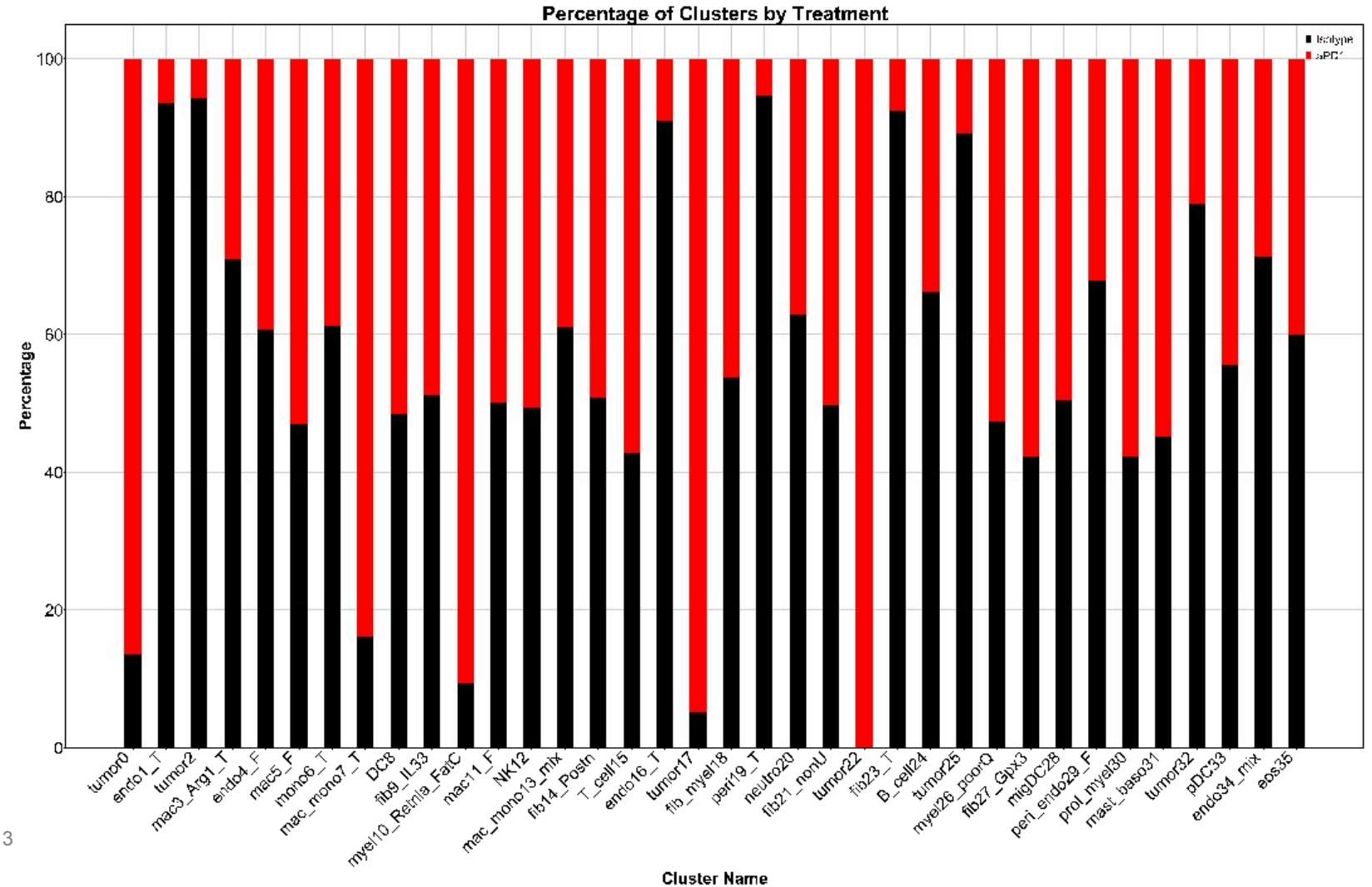
Cell Locations and Treatments



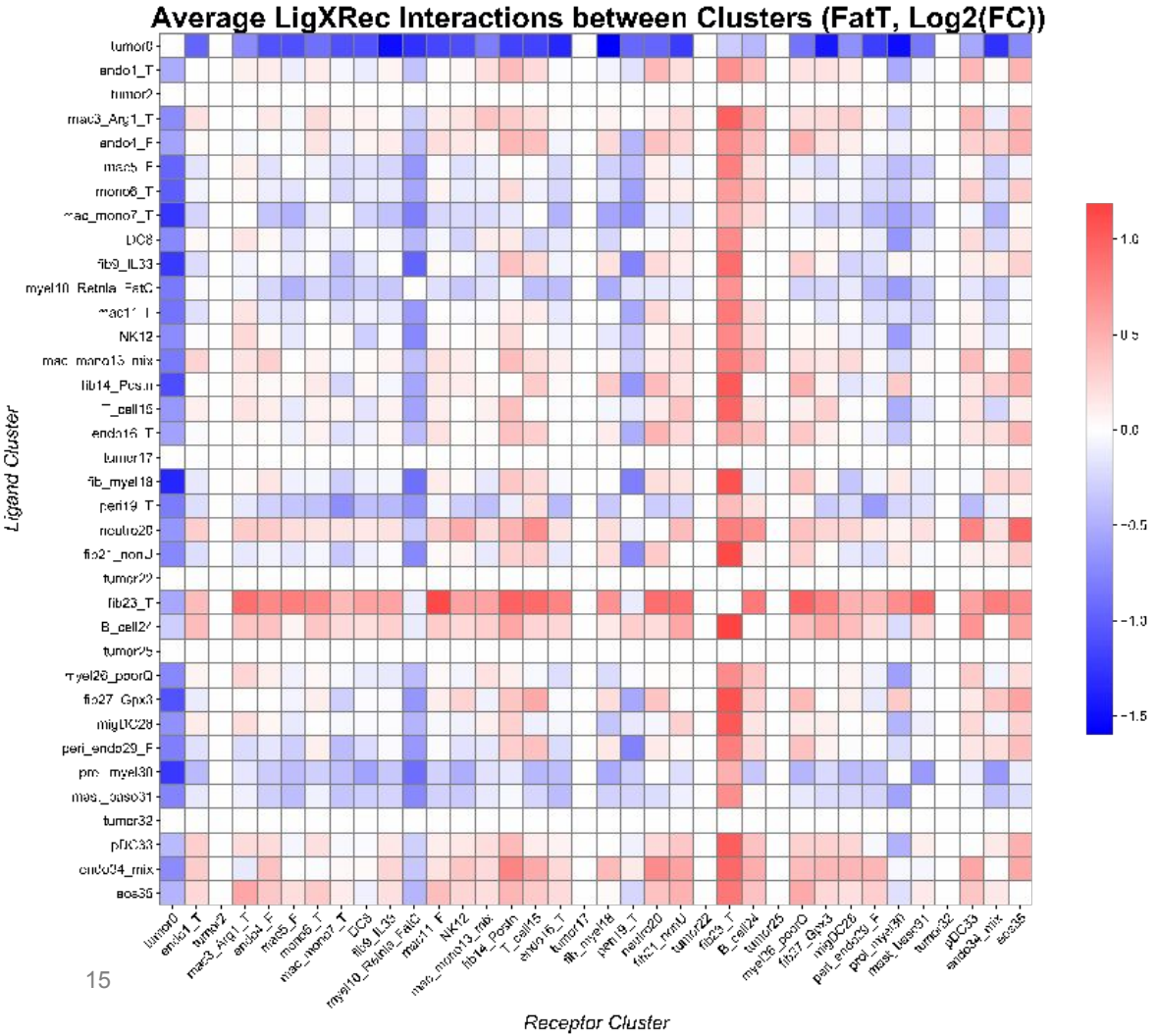

Percentage of Clusters by Cell Locations
FatT
FatC
Tumor
Percentage
100
80
60
40
20
0
tumor0
endo1_T
tumor2
mac3_Arg1_T
endo4_F
mac5_F
mono6_T
mac_mono7_T
DC8
fib9_IL33
myel10_Retnla_FatC
mac11_F
NK12
mac_mono13_mix
fib14_Postn
T_cell15
endo16_T
tumor17
fib_myel18
peri19_T
neutro20
fib21_nonU
tumor22
fib23_T
B_cell24
tumor25
myel26_poorQ
fib27_Gpx3
migDC28
peri_endo29_F
prol_myel30
masl_baso31
tumor32
pDC33
endo34_mix
eos35
Cluster Name


Percentage of Clusters by Treatment
Isotype
aPD1
Percentage
100
80
60
40
20
0
tumor0
endo1_T
tumor2
mac3_Arg1_T
endo4_F
mac5_F
mono6_T
mac_mono7_T
DC8
fib9_IL33
myel10_Retnla_FatC
mac11_F
NK12
mac_mono13_mix
fib14_Postn
T_cell15
endo16_T
tumor17
fib_myel18
peri19_T
neutro20
fib21_nonU
tumor22
fib23_T
B_cell24
tumor25
myel26_poorQ
fib27_Gpx3
migDC28
peri_endo29_F
prol_myel30
mast_baso31
tumor32
pDC33
endo34_mix
eos35
Cluster Name

LigXRec Interaction Analysis

- Quantify potential ligand-receptor interactions between cell clusters
- 2300 curated ligand-receptor gene pairs[1]
- LigXRec = (mean expression of gene in ligand cluster) x (mean expression of gene in receptor cluster)
- Performed for all combinations of clusters and for all ligand-receptor gene pairs
- Calculate difference and fold change (FC) of LigXRec values between aPD-1 and isotype treatments

1. Hou et al. (2020), doi: 10.1038/s41467-020-18873-z

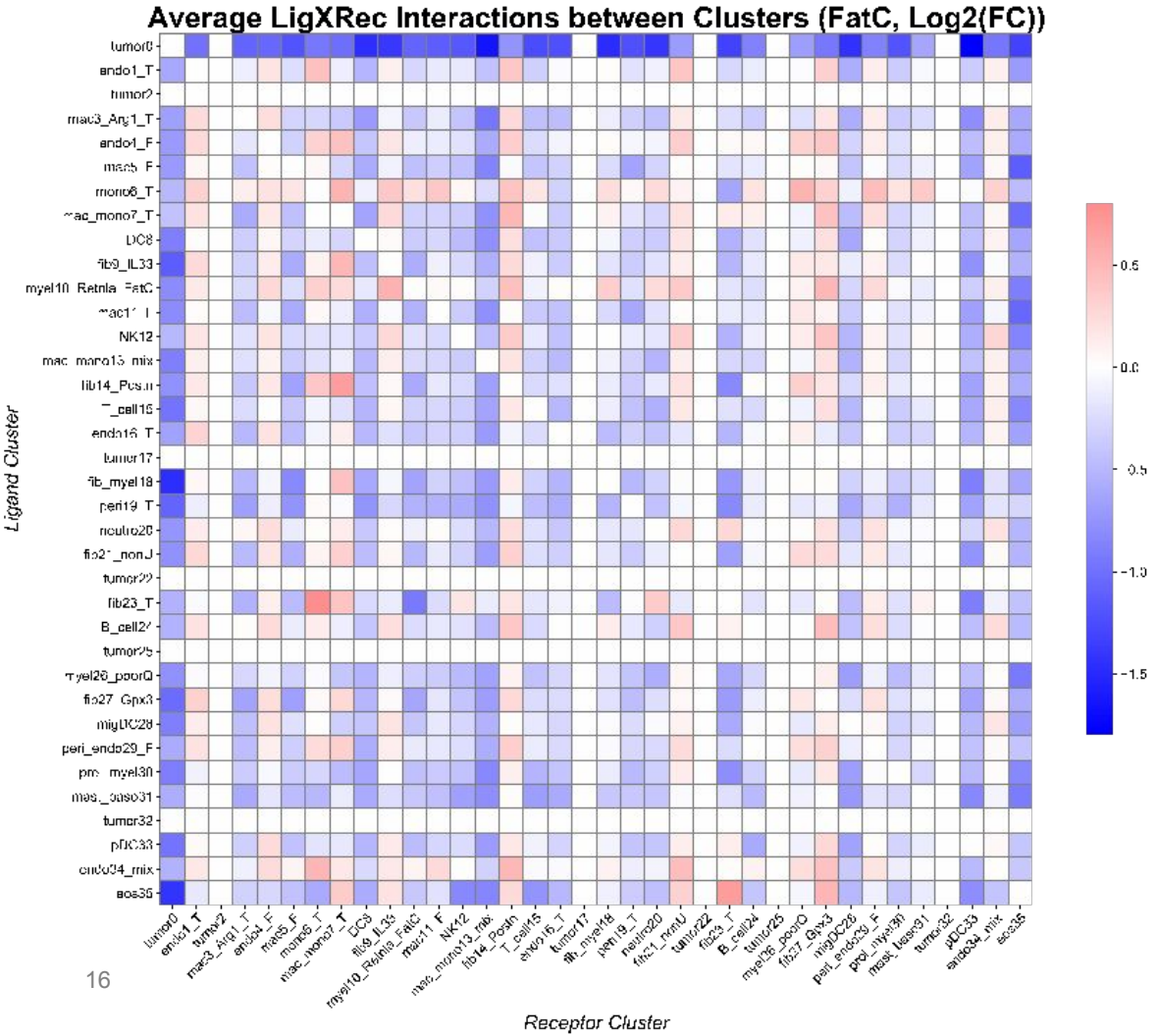


Fold Change in Average Cluster Interactions – FatT

Increased Interaction: aPD-1 vs. Iso

Top/Bottom 5 LigXRec Log2(aPD1/Iso)

Ligand	Receptor	Log2(FC)
B_cell24	fib23_T	1.18300181
fib23_T	mac11_F	1.12835925
fib21_nonU	fib23_T	1.12223948
fib27_Gpx3	fib23_T	1.07904404
fib_myel18	fib23_T	1.07853636
fib_myel18	tumor0	-1.3554015
tumor0	fib27_Gpx3	-1.4533958
tumor0	fib9_IL33	-1.4914748
tumor0	prol_myel30	-1.5212681
tumor0	fib_myel18	-1.5999584

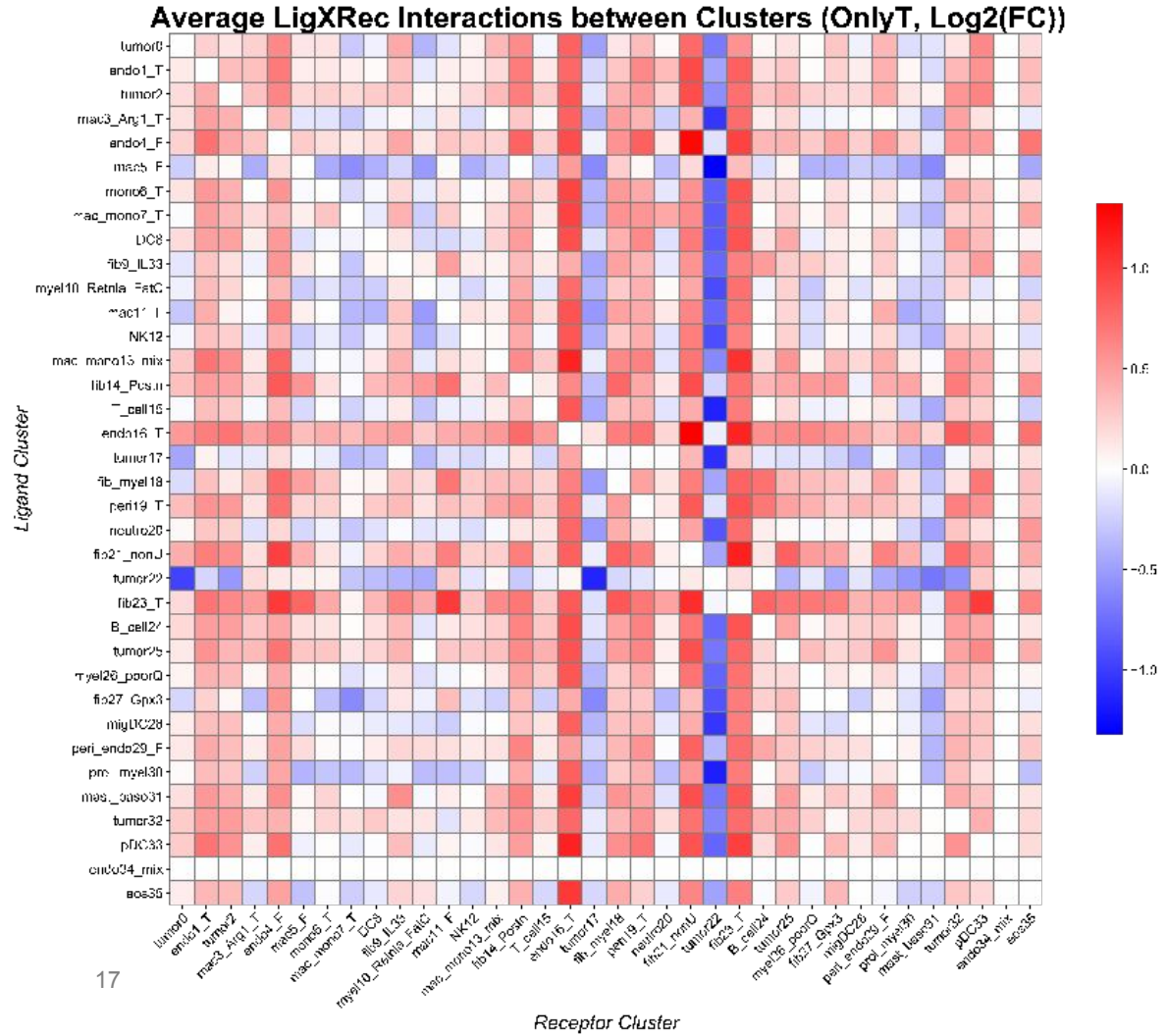


Fold Change in Average Cluster Interactions – FatC

Increased Interaction: aPD-1 vs. Iso

Top/Bottom 5 LigXRec Log2(aPD1/Iso)

Ligand	Receptor	Log2(FC)
fib23_T	mono6_T	0.79894195
eos35	fib23_T	0.6874007
fib14_Postn	mac_mono7_T	0.6866788
myel10_Retnla_FatC	fib9_IL33	0.52510349
mono6_T	myel26_poorQ	0.51578441
tumor0	DC8	-1.4632225
fib_myel18	tumor0	-1.4707097
tumor0	fib_myel18	-1.4808074
tumor0	mac_mono13_mix	-1.6521396
tumor0	pDC33	-1.7976665



Fold Change in Average Cluster Interactions – Tumor

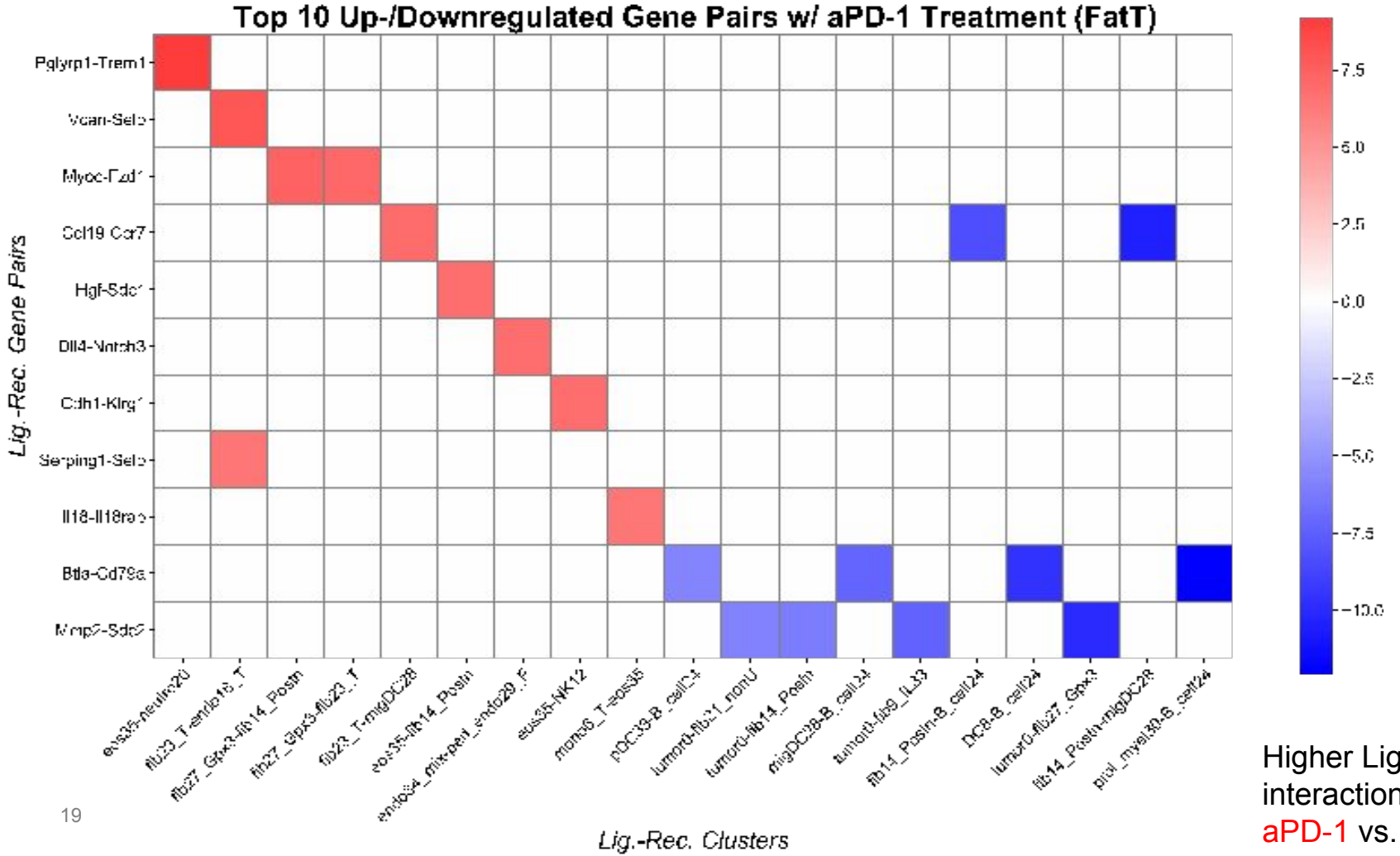
Increased Interaction: aPD-1 vs. Iso

Top/Bottom 5 LigXRec Log2(aPD1/Iso)

Ligand	Receptor	Log2(FC)
endo16_T	fib21_nonU	1.32082538
endo4_F	fib21_nonU	1.27436036
fib21_nonU	fib23_T	1.15069987
pDC33	endo16_T	1.13887923
endo16_T	fib23_T	1.12912548
tumor17	tumor22	-1.0794083
tumor22	tumor17	-1.1340537
T_cell15	tumor22	-1.1364758
prol_myel30	tumor22	-1.1654296
mac5_F	tumor22	-1.3217422

Changes between Cell and Gene Pairs Due to aPD-1 Treatment

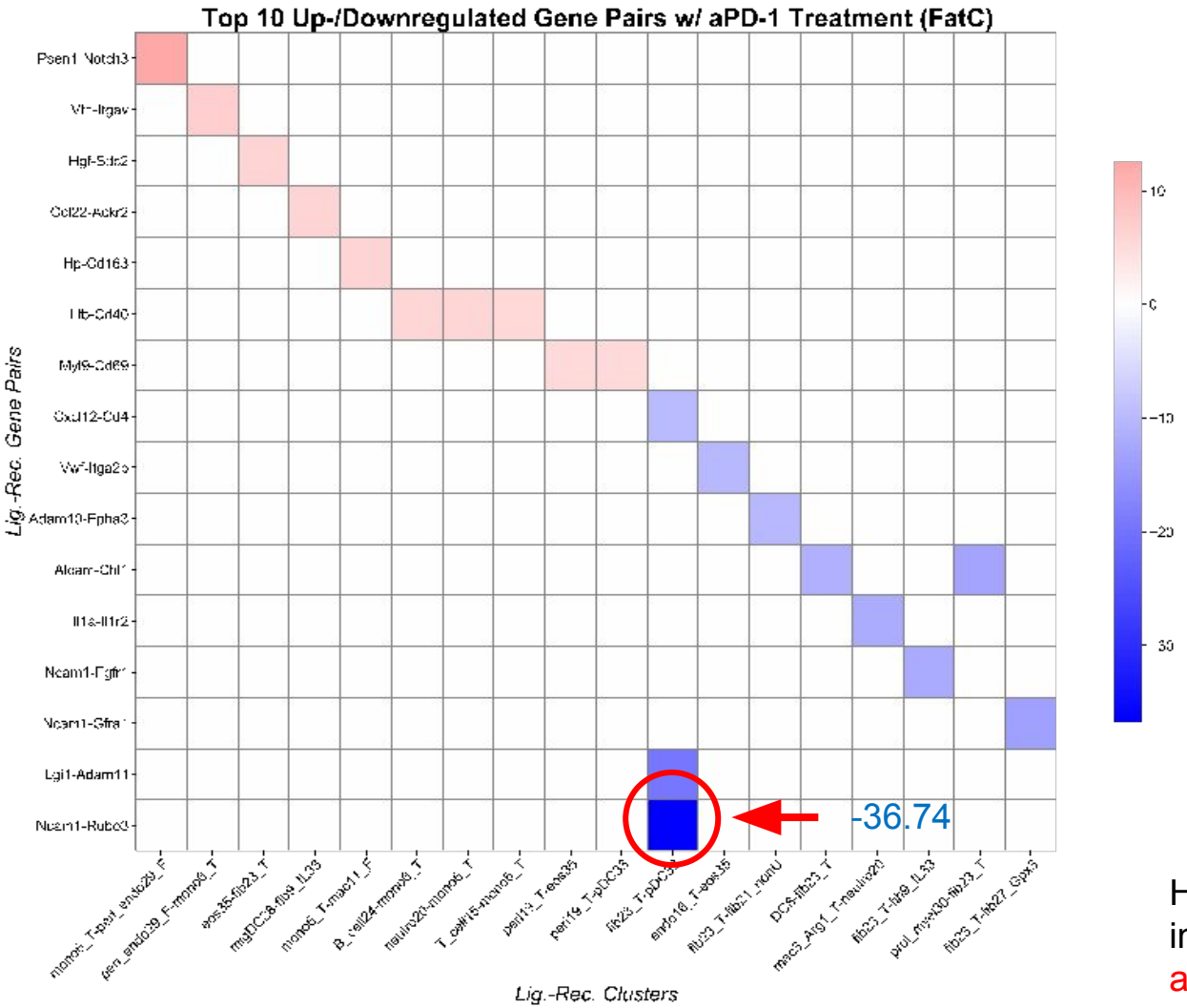
- What ligand-receptor genetic interactions between cell pairs are affected by aPD-1 treatment?
- Compare LigXRec values between isotype and aPD-1 treatments of matching gene-gene pairs and their corresponding cell-cell pairs
- Positive difference: increased genetic interaction between clusters
- Negative difference: decreased genetic interaction between clusters



Higher LigXRec interaction:
aPD-1 vs. Isotype

Top/Bottom LigXRec Differences – FatT (Greater LigXRec Value: aPD-1 vs. Isotype)

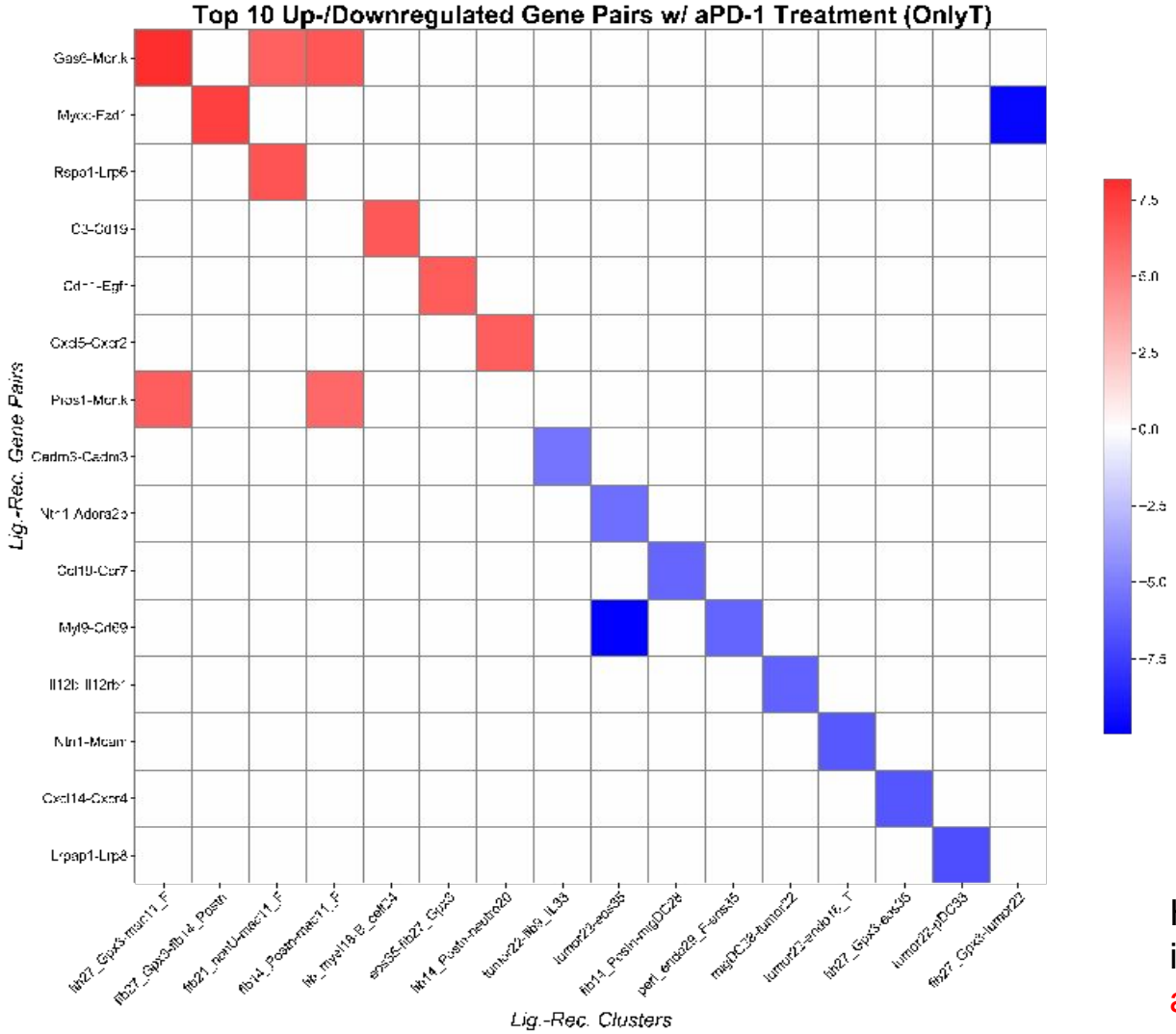
Category	Lig-Rec Genes	Functions	Lig-Rec Clusters
Immune Cell Activity	Pglyrp1-Trem1	Monocyte-mediated inflammation; high Trem1 activity poor cancer prognosis due to immunosuppression[1-4]	• Eos35-neutro20
	Btla-Cd79a	Negative regulation of B-cell, T-cell antigen recognition activity[5-9]	• DC8-B_cell24 • Prol_myel30-B_cell24
ECM/Stromal Interactions	Mmp2-Sdc2	Vasculature remodeling; tumor invasion, cell binding[10-11]	• Tumor0 – fib27_Gpx3
	Myoc-Fzd1	Regulation of ECM, cell-cell adhesion with actin cytoskeleton using Wnt pathway[12-13]	• fib27_Gpx3-fib14_Postn • fib27_Gpx3-fib23_T
	Vcan-Selp	Signals involved in ECM interactions between leukocytes and active endothelial cells[14-15]	• Fib23_T-endo16_T
Cell Signaling	Ccl19-Ccr7	Chemokinetic for naïve CD4, CD8 T-cells, B-cells[16-17]; potential increased T-cell, DC tumor infiltration with Ccl19+aPD-1 treatment[18-19]	• Fib23_T-migDC28 • Fib14_Postn-B_cell24 • Fib14_Postn-migDC28
Cell Cycle/Survival	Btla-Cd79a	Control of T-cell exhaustion, targeting Btla has shown increased T-cell activity[5-9]	• DC8-B_cell24 • Prol_myel30-B_cell24



Higher LigXRec interaction:
aPD-1 vs. Isotype

Top/Bottom LigXRec Differences – FatC (Greater LigXRec Value: aPD-1 vs. Isotype)

Category	Lig-Rec Genes	Genetic Function	Lig-Rec Clusters
Immune Cell Activity	Ncam1-Robo3	Cellular proliferation of CD8 T-cells, DCs; poor prognostic marker in AML[1-6]	• Fib23_T – pDC33
ECM/Stromal Interactions	Vtn-Itgav	Promotes cell adhesion to ECM components[7-8]	• Peri_endo29_F-mono6_T
	Alcam-Chl1	Cell adhesion molecules involved in neuron development[9-10]	• Prol_myel30-fib23_T
Cell Signaling	Psen1-Notch3	Regulation of Notch, Wnt pathways affecting cell fate; increased Notch3 associated with immunosuppression[11-15]	• Mono6_T-peri_endo29_F
	Ccl22-Ackr2	Regulated chemokinetic reaction for T-cell, B-cell, DC inflammation[16-17]	• migDC28-fib9_IL33
Cell Cycle/Survival	Hgf-Sdc2	Growth, proliferation of tissues; altered in some tumors[18-20]; high expression of genes can lead to increased tumor PD-L1, immunosuppression[21-22]	• Eos35-fib23_T
	Ncam1-Gfra1	Control of Ret proto-oncogene upon binding with neurotropic ligands[23-24]	• Fib23_T – fib27
	Ncam1-Fgfr1	Proliferation, differentiation, migration of fibroblasts[25-26]	• Fib23_T-fib9_IL33



Higher LigXRec interaction:
aPD-1 vs. Isotype

Top/Bottom LigXRec Differences – Tumor (Greater LigXRec Value: aPD-1 vs. Isotype)

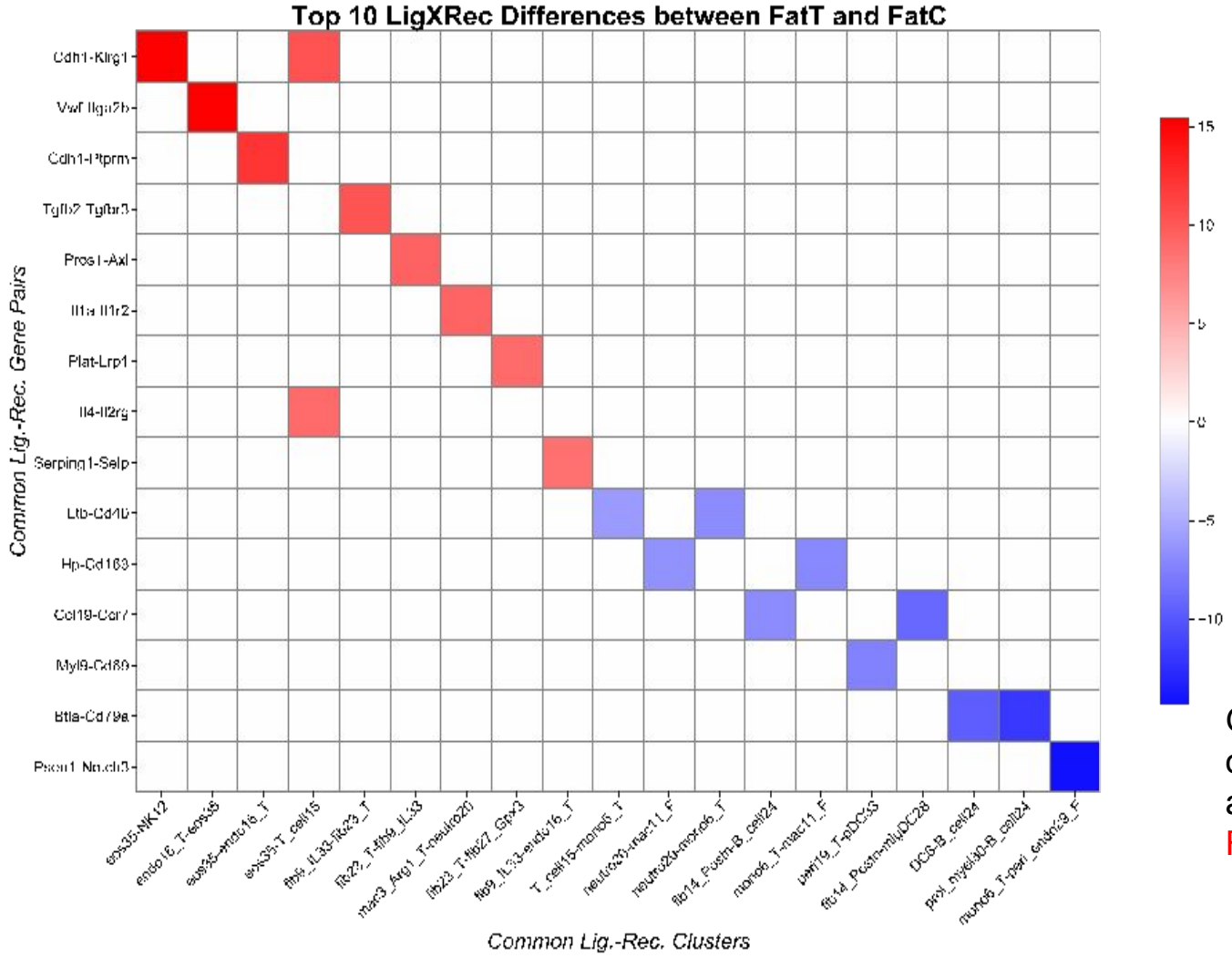
Category	Lig-Rec Genes	Function	Lig-Rec Clusters
Immune Cell Activity	C3-Cd19	Central complement protein; B-cell unique marker, triggers antibody response[1-2]	• Fib_myel18-B_cell24
	Myl9-Cd69	Myosin regulatory subunit, high expression shows low response to aPD-1 treatment[3-5] ; lymphocyte proliferation, blocking leads to tumor shrinkage due to longer lasting T-cells[6-9]	• Tumor22 – eos35
ECM/Stromal Interactions	Myoc-Fzd1	Regulation of ECM, cell-cell adhesion with actin cytoskeleton using Wnt pathway[10-11]	• fib27_Gpx3-fib14_Postn • fib27_Gpx3-tumor22
Cell Signaling	Ccl19-Ccr7	Chemokinetic for naïve CD4, CD8 T-cells, B-cells, potential for increased T-cell, DC tumor infiltration with Ccl19 + aPD-1 treatment[12-15]	• Fib23_T-migDC28 • Fib14_Postn-B_cell24 • Fib14_Postn-migDC28
Cell Cycle/Survival	Gas6-Mertk	Cell growth, survival, adhesion; Gas6 highly expressed in many cancers, anti-Mertk aids in immunotherapy[16-22]	• Fib27 – mac11_F • Fib14_Postn – mac11_F • Fib21-mac11_F
	Ntn1-Mcam	Survivor factor preventing apoptotic receptor interactions; seen in some cancers[23-24]	• Tumor22-endo16_T

Comparing Ligand-Receptor Interaction Changes between Tissues

- What changes in cluster and gene interactions due to aPD-1 are most distinctive for each tissue location?
  - i.e. larger degree in changes of LigXRec differences between tissue locations
  - (LigXRec diff. tissue location 1) - (LigXRec diff. tissue location 2)

Number of Unique and Shared Gene Pairs between Tissue Types

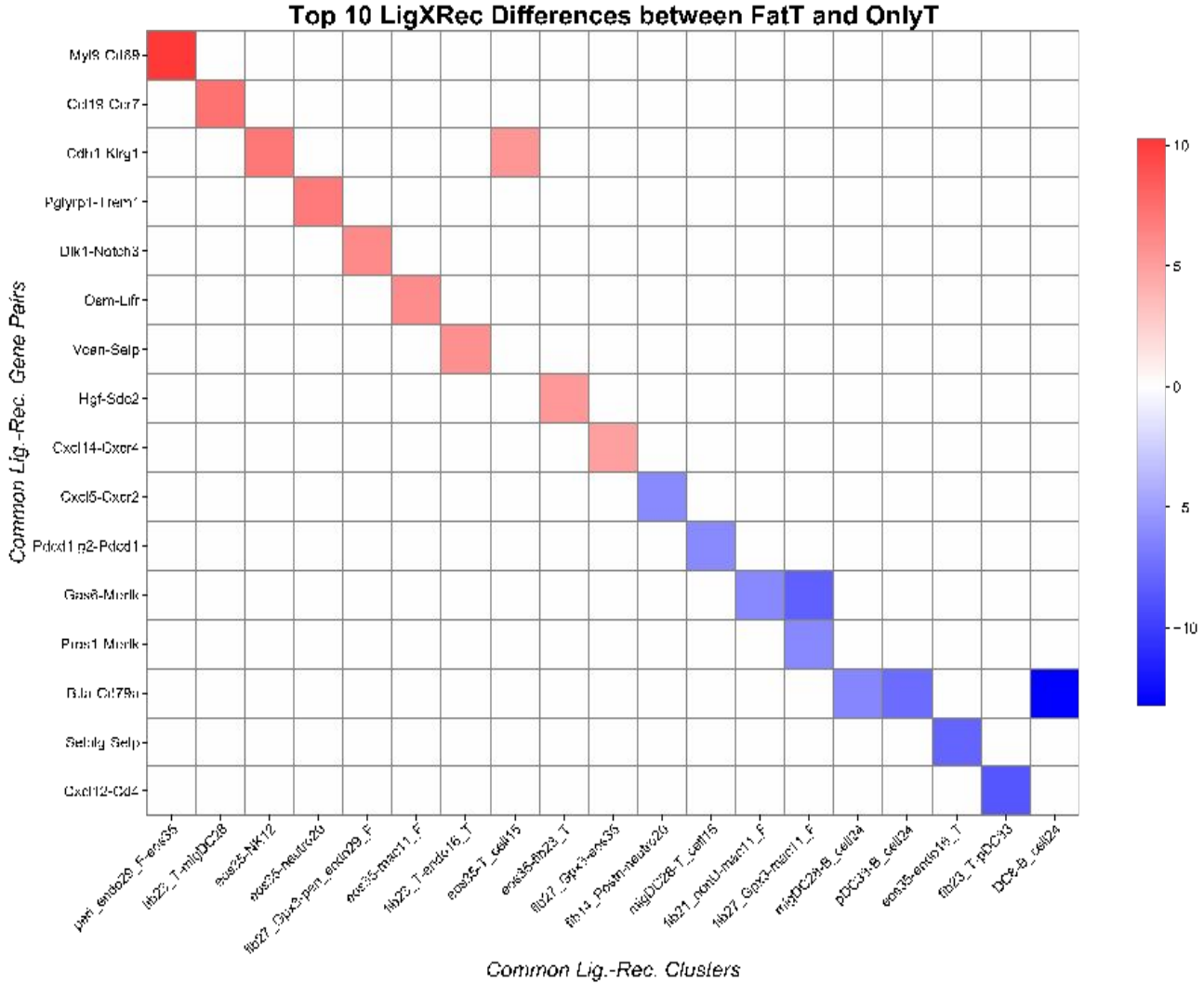
Comparison	Shared	FatT	FatC	Tumor
FatT vs. FatC	1428	70	61	-
FatC vs. Tumor	1398	-	91	68
FatT vs. Tumor	1400	98	-	66
All Tissues	1360	30	23	28



Greater degree of LigXRec diff. after aPD-1: FatT vs. FatC

Greater Degree of LigXRec Differences Due to aPD-1 – FatT vs. FatC

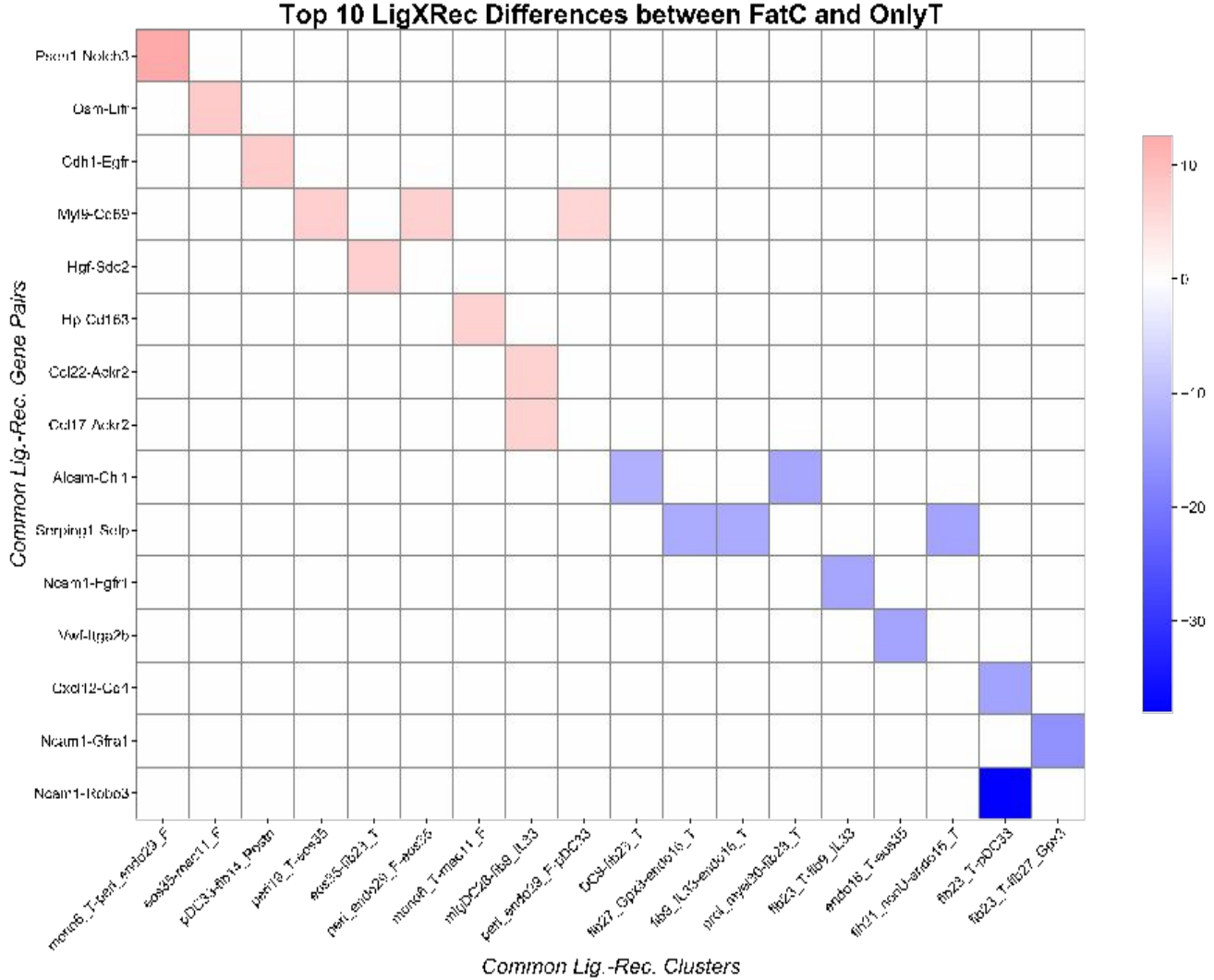
Category	Lig-Rec Genes	Function	Lig-Rec Clusters
Immune Cell Activity	Btla-Cd79a	Regulation of B-cell, T-cell antigen recognition activity; targeting Btla has shown increased T-cell activity[1-5]	• DC8-B_cell24 • Prol_myel30-B_cell24
	Myl9-Cd69	Myosin regulatory subunit, high expression shows low response to aPD-1 treatment[6-8] ; lymphocyte proliferation, blocking yields tumor shrinkage by longer lasting T-cells[9-12]	• Tumor22 – eos35
	Cdh1-Klrg1	Self-recognition and inhibition through E-cadherin recognition[13-14]; Klrg1+ effector CD8 T-cells less likely to infiltrate tumor, downregulate expression to become memory T-cells, increase antitumor immunity[15-16]	• Eos35-NK12 • Eos35-T_cell15
ECM/Stromal Interactions	Cdh1-Ptprm	Cell-cell adhesion, mobility, growth[17-18]; high Ptprm associated with EMT, metastasis, poor cancer prognosis[19]	• Eos35-endo16_T
	Tgfb2-Tgfbr3	Associated with EMT by cancer-associated fibroblasts; high Tgfb2 associated with poor prognosis in several cancers[20-22]	• fib9_IL33-fib23_T
Cell Signaling	Ccl19-Ccr7	Chemokinetic for naïve CD4, CD8 T-cells, B-cells, potential for increased T-cell, DC tumor infiltration with Ccl19+aPD-1 treatment[23-26]	• Fib14_Postn-B_cell24 • Fib14_Postn-migDC28
	Psen1-Notch3	Regulation of Notch, Wnt pathways affecting cell fate; increased Notch3 associated with immunosuppression[27-31]	• Mono6_T-peri_endo29_F
Cell Cycle/Survival	Tgfb2-Tgfbr3	Downregulation of Tgfb2 inhibits proliferation, promotes apoptosis of hepatocellular carcinoma[22]	• fib9_IL33-fib23_T



Greater degree of LigXRec diff. after aPD-1: FatT vs. Tumor

Greater Degree of LigXRec Differences Due to aPD-1 – FatT vs. Tumor

Category	Lig-Rec Genes	Function	Lig-Rec Clusters
Immune Cell Activity	Btla-Cd79a	Regulation of B-cell, T-cell antigen recognition activity; targeting Btla has shown increased T-cell activity[1-5]	• DC8-B_cell24 • pDC33-B_cell24 • migDC28-B_cell24
	Myl9-Cd69	Myosin regulatory subunit, high expression shows low response to aPD-1 treatment[6-8] ; lymphocyte proliferation, blocking yields tumor shrinkage by longer lasting T-cells[9-12]	• peri_endo29_F-eos35
	Cdh1-Klrg1	Self-recognition and inhibition through E-cadherin recognition[13-14]; Klrg1+ effector CD8 T-cells less likely to infiltrate tumor, downregulate expression to become memory T-cells, increase antitumor immunity[15-16]	• Eos35-NK12
	Pglyrp1-Trem1	Monocyte-mediated inflammation; high Trem1 activity poor cancer prognosis due to immunosuppression[17-20]	• Eos35-neutro20
Cell Signaling	Ccl19-Ccr7	Chemokinetic for naïve CD4, CD8 T-cells, B-cells, potential for increased T-cell, DC tumor infiltration with Ccl19+aPD-1 treatment[21-24]	• Fib23_T-migDC28
	Cxcl12-Cd4	Chemotaxis of T-cells, monocytes[25] ; Cxcl12 increases activation markers on CD3-stimulated CD4 T-cells in CLL[26]; prostate tumors express high Cxcl12 levels, correlation with intratumoral expression of MDSC/Treg markers[27]	• Fib23_T-pDC33
Cell Cycle/Survival	Gas6-Mertk	Cell growth, survival, adhesion; Gas6 highly expressed on cancers, anti-Mertk aids in immunotherapy[28-34]	• Fib27_Gpx3–mac11_F



Greater degree of LigXRec diff. after aPD-1: FatC vs. Tumor

Greater Degree of LigXRec Differences Due to aPD-1 – FatC vs. Tumor

Category	Lig-Rec Genes	Function	Lig-Rec Clusters (FatC vs. Tumor)
Immune Cell Activity	Ncam1-Robo3	Cellular proliferation of CD8 T-cells, DCs; poor prognostic marker in AML[1-6]	• Fib23_T – pDC33
	Myl9-Cd69	Myosin regulatory subunit, high expression shows low response to aPD-1 treatment[7-9] ; lymphocyte proliferation, blocking yields tumor shrinkage by longer lasting T-cells[10-13]	• peri_endo29_F-eos35
ECM/Stromal Interactions	Cdh1-Egfr	E-cadherin production and cell signaling based on extracellular cues[14-15]; Egfr upstream of Cdh1 in Wnt signaling axis, associated with poor tumor prognosis[16-17]	• pDC33-fib14_Postn
Cell Signaling	Psen1-Notch3	Regulation of Notch, Wnt pathways affecting cell fate; increased Notch3 associated with immunosuppression[18-22]	• Mono6_T-peri_endo29_F
	Cxcl12-Cd4	Chemotaxis of T-cells, monocytes[23] ; Cxcl12 increases activation markers on CD3-stimulated CD4 T-cells in CLL[24]; prostate tumors express high Cxcl12 levels, correlation with intratumoral expression of MDSC/Treg markers[25]	• Fib23_T-pDC33
Cell Cycle/Survival	Hgf-Sdc2	Growth, proliferation of tissues; altered in some tumors[26-28]; high expression of genes can lead to increased tumor PD-L1, immunosuppression[29-30]	• Eos35-fib23_T
	Ncam1-Gfra1	Control of Ret proto-oncogene upon binding with neurotropic ligands[31-32]	• Fib23_T – fib27
	Osm-Lifr	Regulates tumor growth and IL-6 cytokine production[33-34]; targeting Lifr interactions reduces tumor progression and drug resistance[35-36]	• Eos35-mac11_F

Concluding Remarks

- Certain gene pairs involved in immunosuppression show up repeatedly in top interaction changes between cell locations changes due to aPD-1
  - Btla-Cd79a, Myl9-Cd69, Psen1-Notch3, Hgf-Sdc2, Ccl19-Ccr7, Gas6-Mertk
- aPD-1 therapy affects a variety of cell types within and around the tumoral space
  - Fibroblasts, endothelial cells, myeloid cells, monocytes, B-cells, eosinophils, DCs
  - T-cells are not the only cells affected by ICB therapy in/around tumor
- Extent, targets of therapy efficacy depends on location of cells
  - How dependent are results on fat metabolism?
- How does cell location and treatment affect cell fate?
  - RNA velocity (Dynamo) for future tests on data