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

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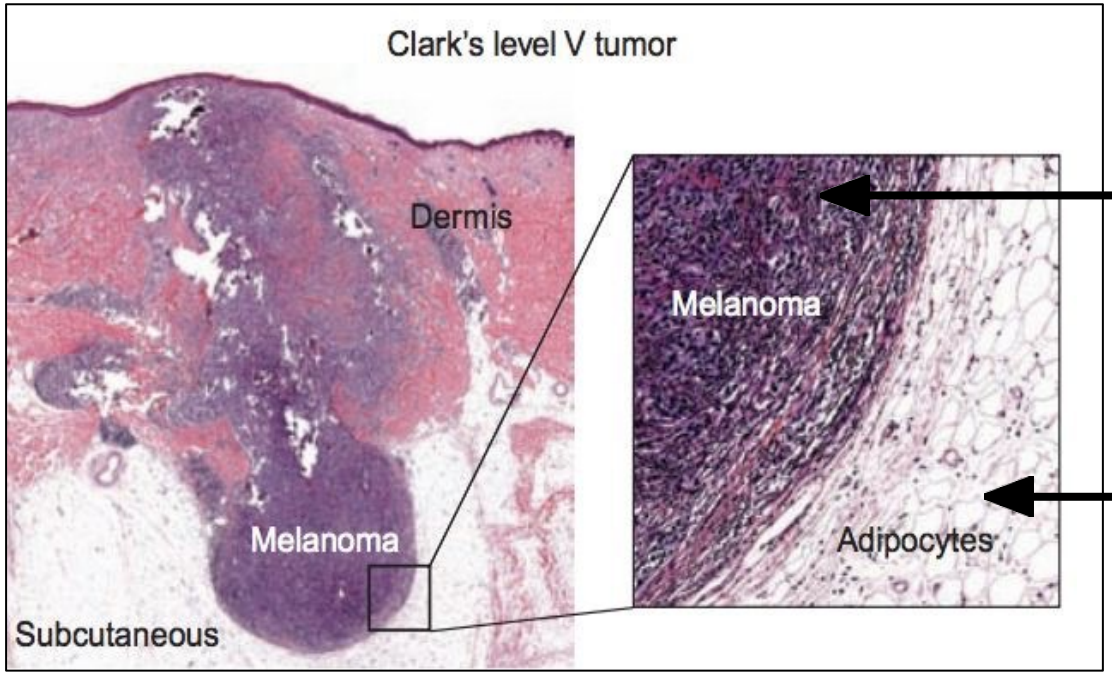
Adipose tissue undergoes crosstalk with tumor cells



Zhang et al. 2018. *Canc Disc*

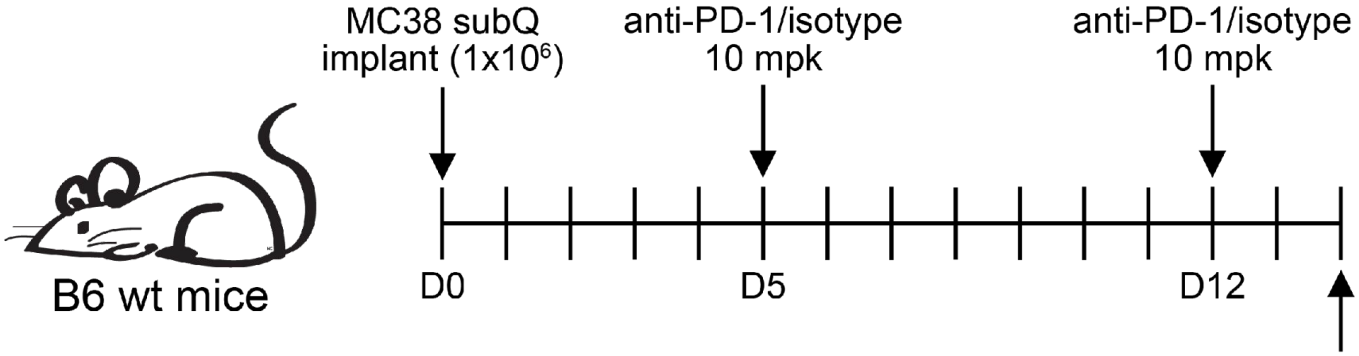
- Evidence of tumor cell – adipocyte interactions
  - Tumor cells can use lipids as energy source[1]
  - Tumor cells promote adipocyte dedifferentiation into fibroblasts[2,3]
  - Adipokines promote tumor growth via oncogenic signaling[4]
- Stromal and immune cells within adipose tissue may influence tumor biology[5,6]
  - Reserves of memory T cells in white adipose tissue
  - Tumor-associated suppressive CD11b+ myeloid cells

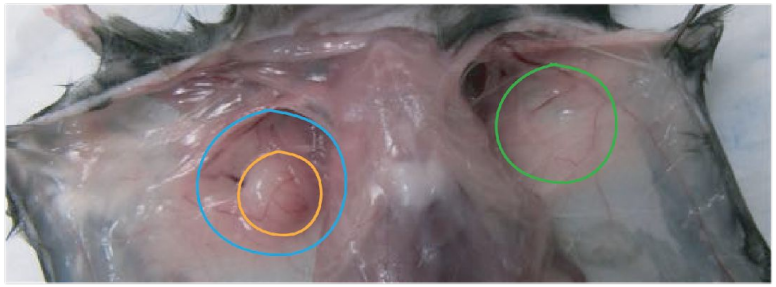
1. Zhang et al. 2018. *Cancer Disc*
2. Zoico et al. 2016. *Oncotarget*
3. Pearce et al. 2018. *Cancer Disc*
4. Khandekar et al. 2011. *Nat Rev Cancer*
5. Saha et al. 2017. *Cancer Res*
6. Wagner et al. 2012. *Angiogenesis*

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

- Do peritumoral cell populations have altered interactions during ICB?
- Are the genetic expression profiles of these populations promoted or repressed with ICB?

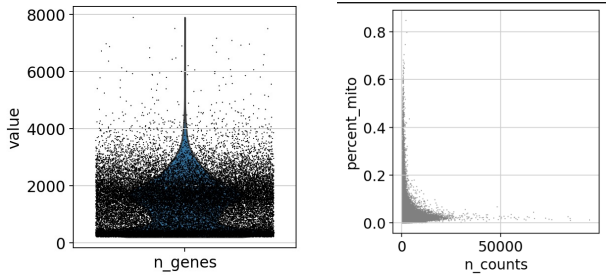
Experimental Setup

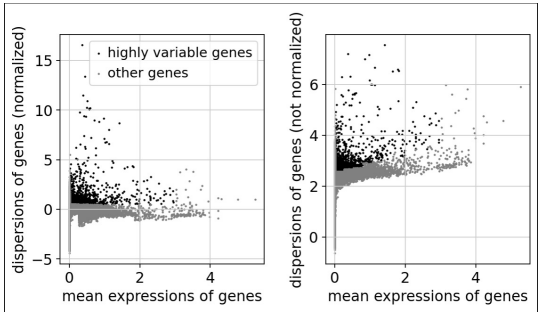




- Sorted by aPD-1 and isotype-treated mice
- 3 tissue type locations: peritumoral fat (FatT), contralateral fat (FatC), and Tumor
- Sorted by CD45+ immune cells or CD45-CD31+ stromal/tumor cells

Data Preprocessing, Filtering





scRNA-seq Tools

- 10X Genomics CellRanger for reading
- Scanpy library for analysis

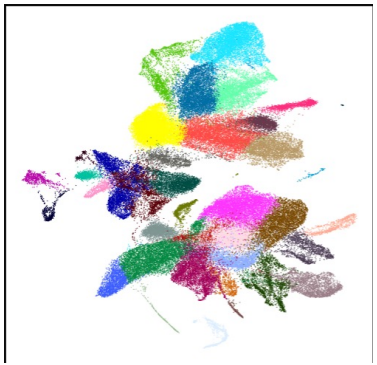
Initial Filtering

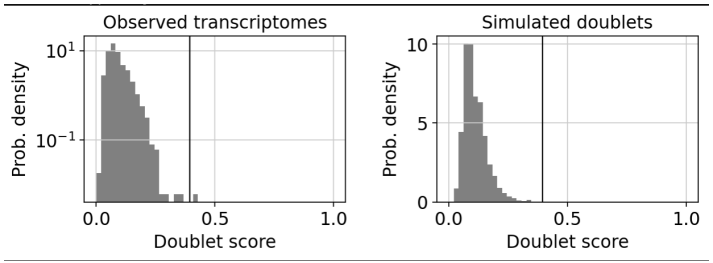
- Cells expressing 200-3500 genes
- Genes present in >3 cells
- Cells with <15% mito. gene content
- Normalize counts per cell for 10K counts

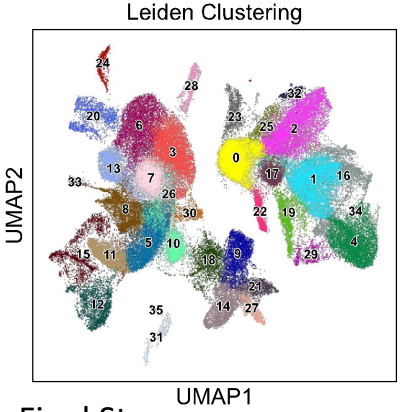
Variations

- Select for variable genes
- Mean expr. = 0.0125-3
- Min. disp. = 0.5
- Remove ribo. genes
- Regress out variations due to counts, mito. gene %

PCA, Clustering, Refinement







Initial Clustering

- PCA based on highly variable genetic expression
- Neighborhood graph (n=10, PCs=30)
- UMAP for dimension reduction
- Leiden clustering algorithm
- Initial: 39 clusters

Filtering

- Remove unwanted cell types
  - Epithelial (Epcam+)
  - Satellite (Pax7+)
  - Muscle (Tnnc2+)
  - RBCs (Hbb-bs+)
- Remove predicted doublet reads based on scoring by Scrublet library

Final Steps

- Rerun initial clustering steps
- Remove small clusters w/ <100 cells
- Cluster again
- Final: 36 clusters, 88K cells

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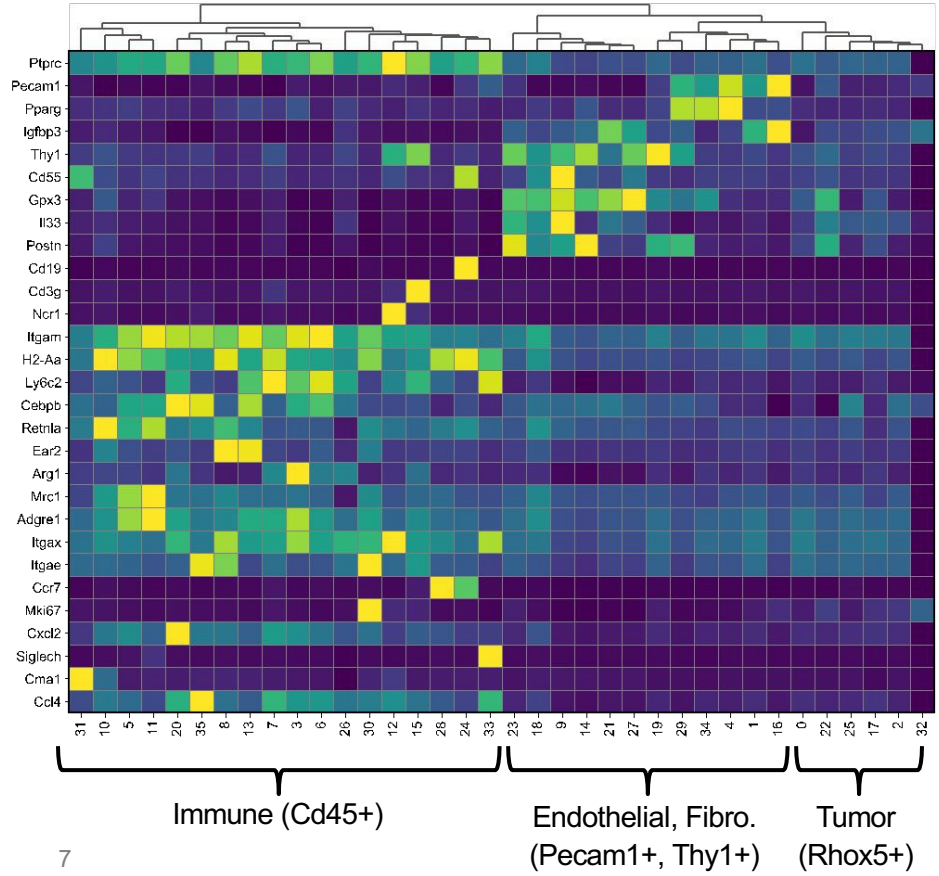
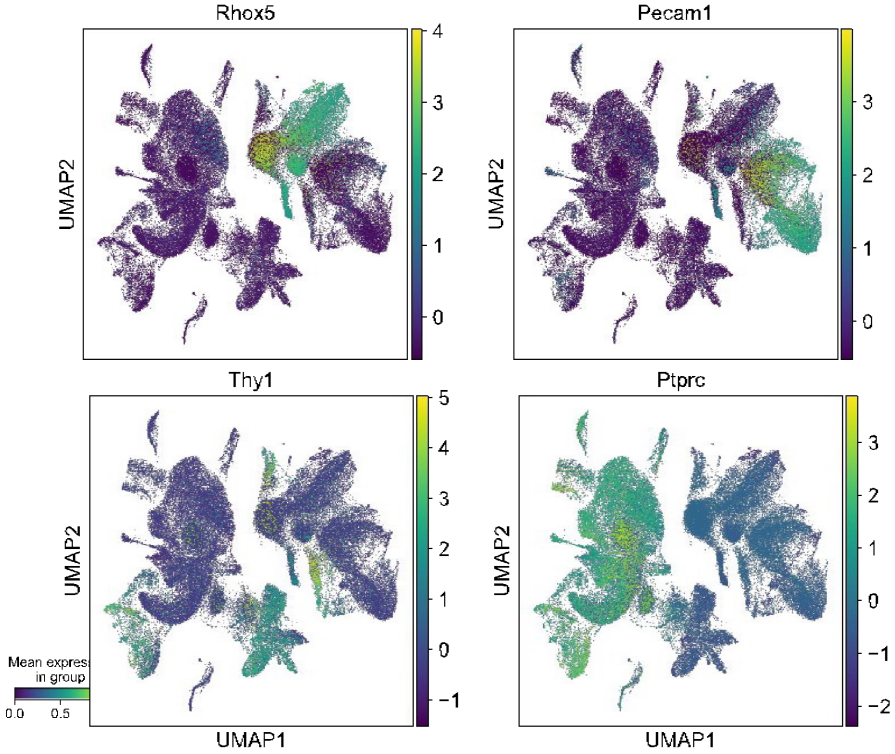
Cluster Gene Profiles





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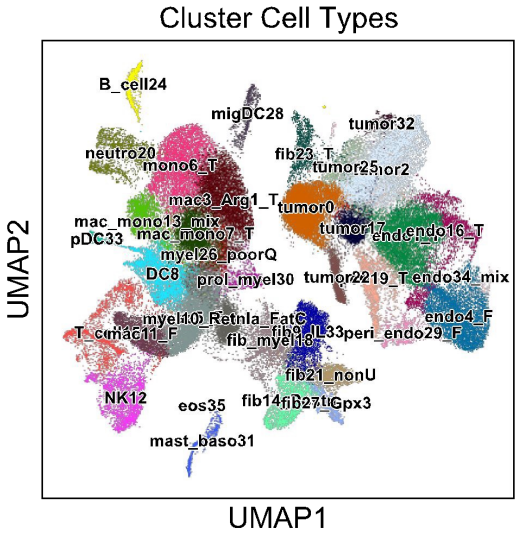
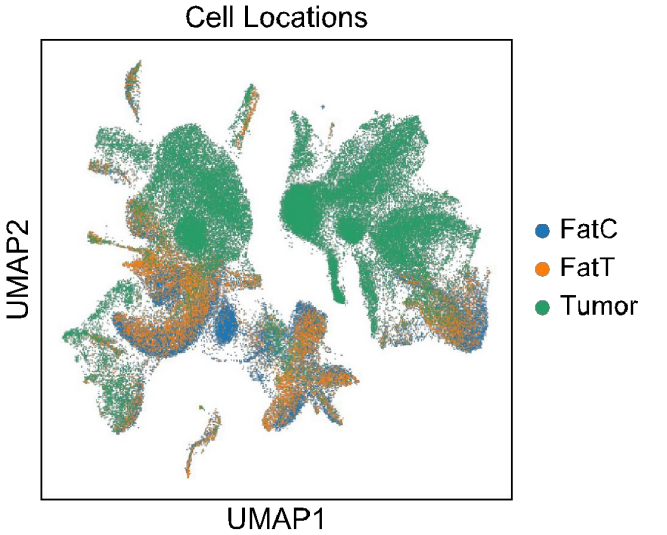
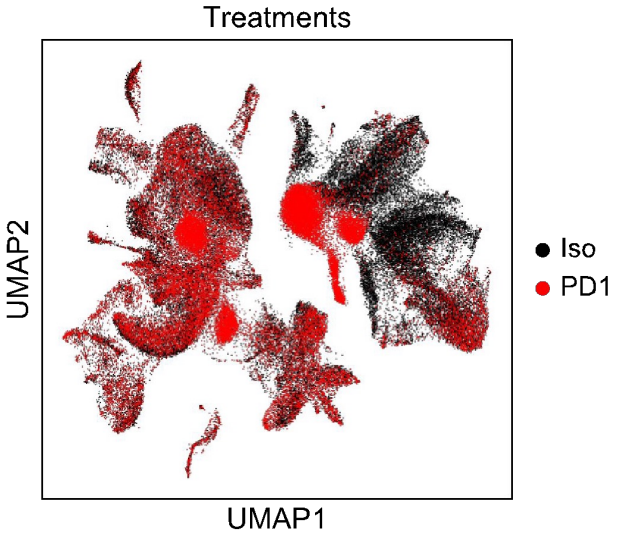
Cell Types, Locations, and Treatments







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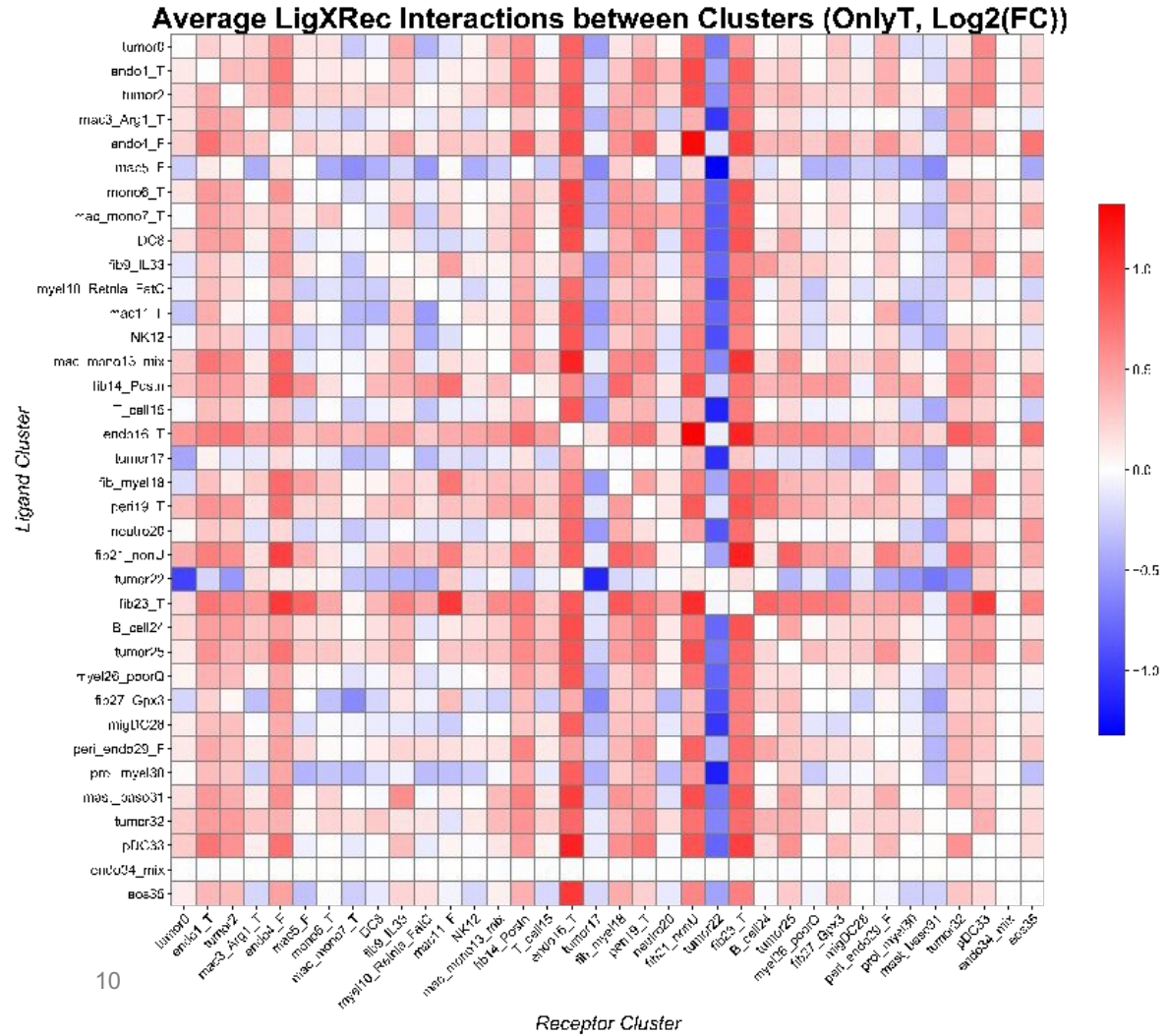
Ligand-Receptor Interaction Analysis

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

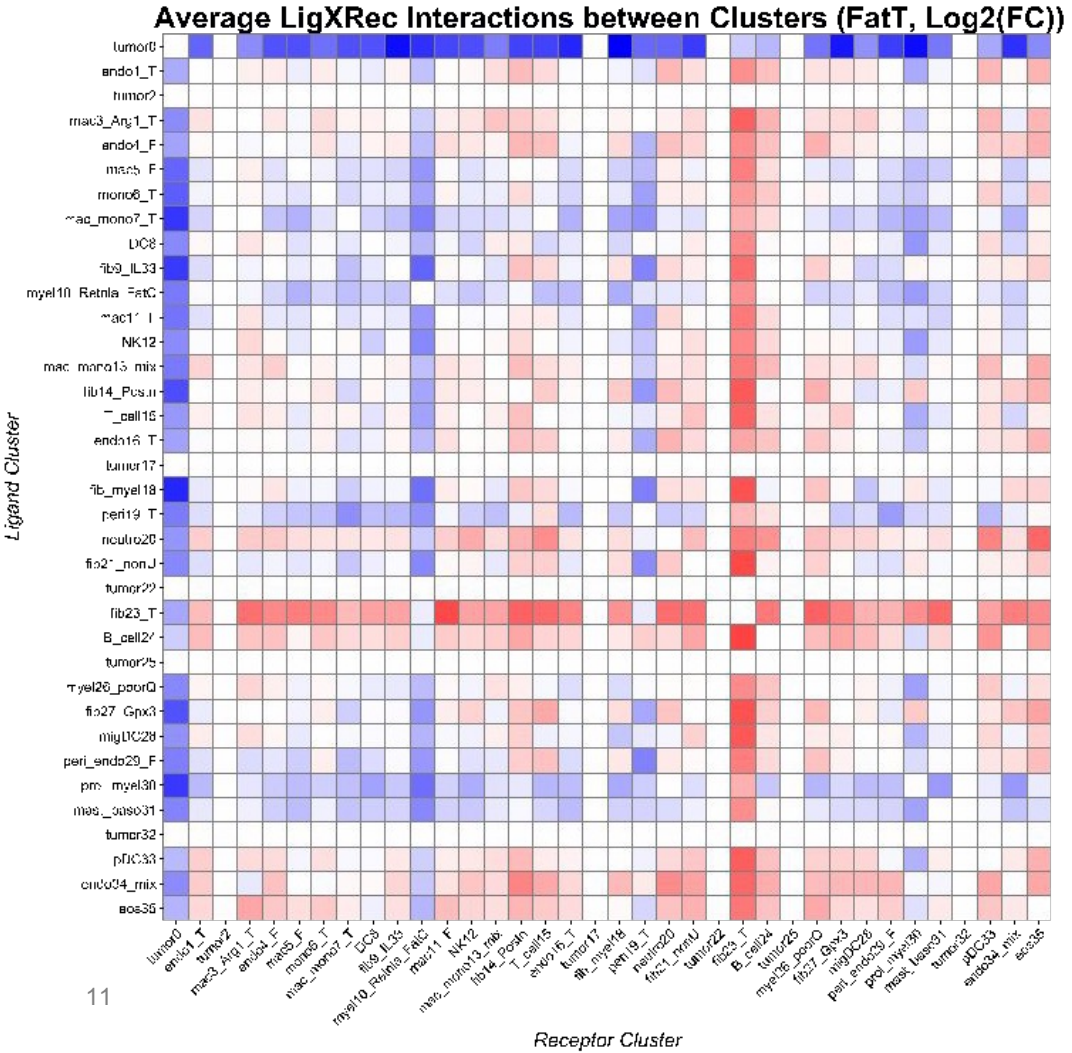
1. Hou et al. (2020), doi: 10.1038/s41467-020-18873-z



Fold Change in Average Cluster Interactions – Tumor

Change in Interaction w/ aPD-1:
Increase vs. Decrease

Ligand	Receptor	Log2(aPD1/Iso)
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



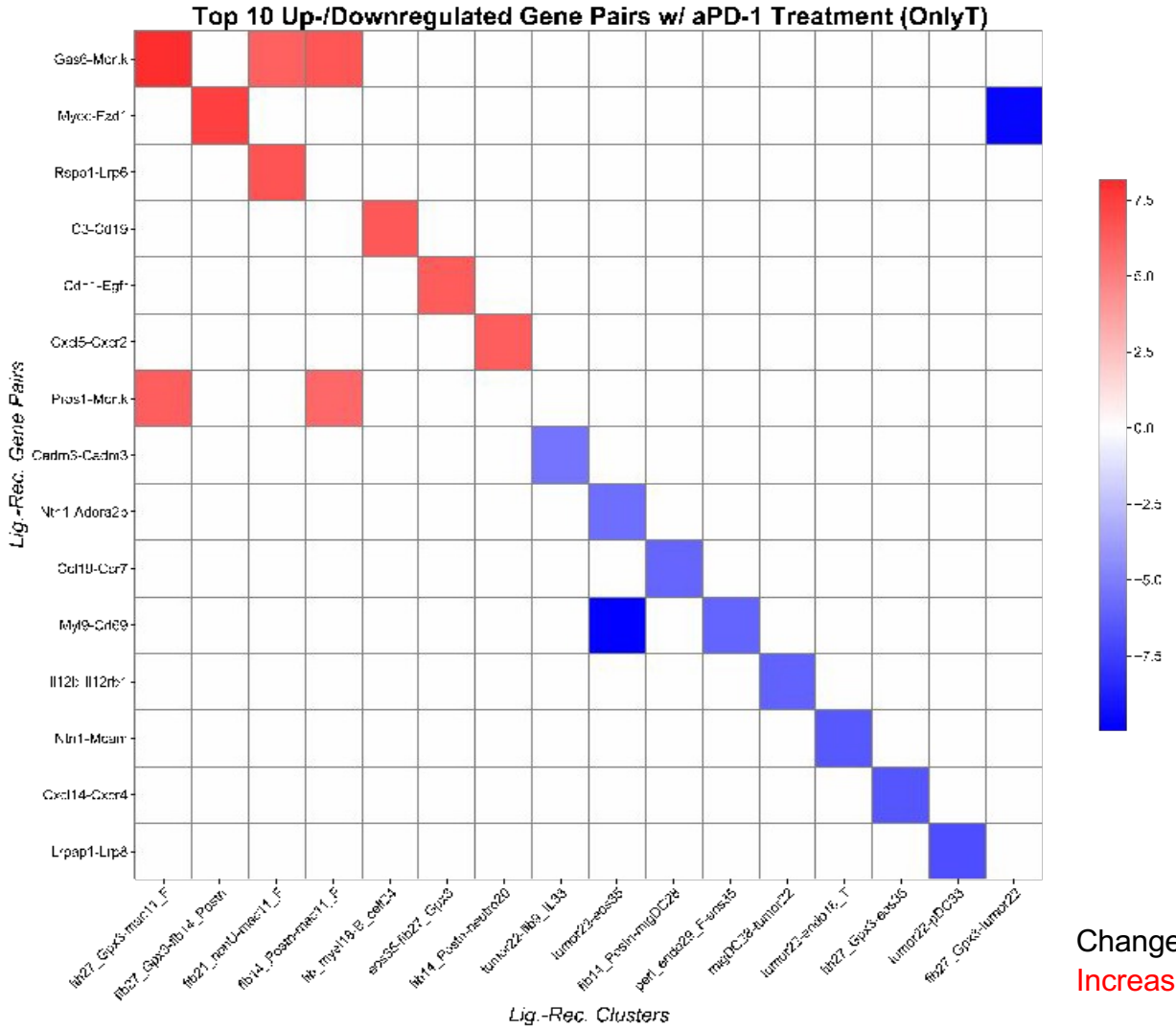
Fold Change in Average Cluster Interactions – FatT

Change in Interaction w/ aPD-1:
Increase vs. Decrease

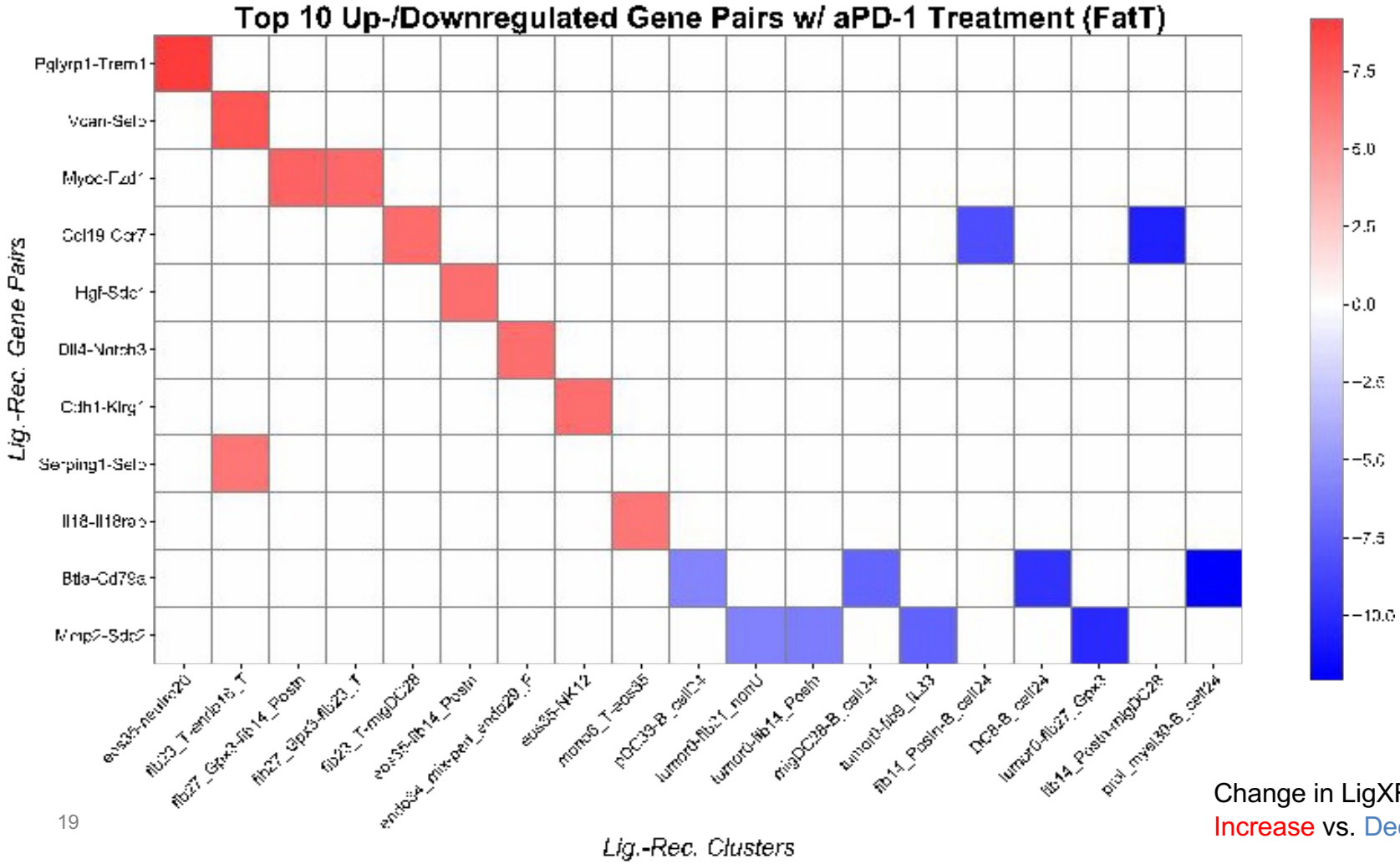
Ligand	Receptor	Log2(aPD1/Iso)
B_cell24	fib23_T	1.18300181
fib23_T	mac11_F	1.12835925
fib21_nonU	fib23_T	1.12223948
fib27_Gpx3	fib23_T	1.07904404
fib_myel18	fib23_T	1.07853636
fib_myel18	tumor0	-1.3554015
tumor0	fib27_Gpx3	-1.4533958
tumor0	fib9_IL33	-1.4914748
tumor0	prol_myel30	-1.5212681
tumor0	fib_myel18	-1.5999584

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

- What ligand-receptor genetic interactions between cluster pairs are affected most 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 w/ aPD-1
    - Negative difference: decreased genetic interaction between clusters w/ aPD-1



Change in LigXRec w/ aPD-1:
Increase vs. Decrease



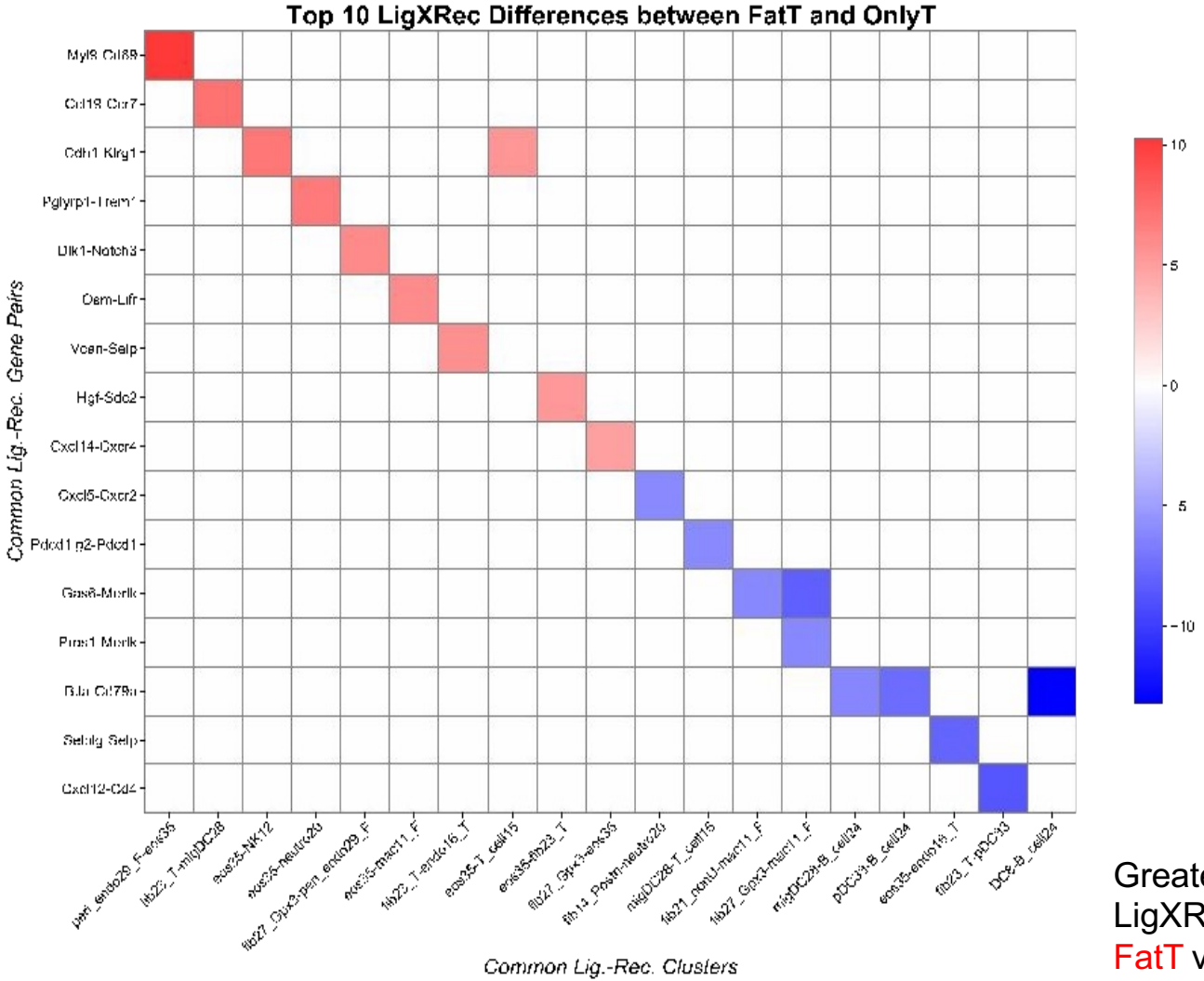
Change in LigXRec w/ aPD-1:
Increase vs. Decrease

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 abs. value of LigXRec diff. w/ aPD-1: FatT vs. Tumor

Notable Top LigXRec Differences Found Across Tissues

Category	Lig-Rec Genes	Function	LigXRec Interactions w/ aPD-1 (Increase vs. Decrease)
Immune Cell Activity	Myl9-Cd69	High expression shows low response to aPD-1 treatment; blocking yields tumor shrinkage via longer lasting T-cells	• peri_endo29_F – eos35 (Tumor) • Tumor22 – eos35 (Tumor)
	Btla-Cd79a	Negative regulation of B-cell, T-cell antigen recognition activity	• DC8 – B_cell24 (FatT) • Prol_myel30 – B_cell24 (FatT)
	Ncam1-Robo3	Cellular proliferation of CD8 T-cells, DCs; poor prognostic marker in AML	• Fib23_T – pDC33 (FatC)
Cell Signaling	Psen1-Notch3	Regulation of Notch, Wnt pathways affecting cell fate; increased Notch3 associated w/ immunosuppression	• Mono6_T – peri_endo29_F (FatC)
Cell Cycle/Survival	Hgf-Sdc2	High expression can lead to upregulated tumor PD-L1, immunosuppression	• Eos35 – fib23_T (FatC)
	Ccl19-Ccr7	Chemokinetic for naïve CD4, CD8 T-cells, B-cells; potential increased T-cell, DC tumor infiltration with Ccl19+aPD-1 treatment	• Fib23_T – migDC28 (FatT, Tumor) • Fib14_Postn – B_cell24 (FatT, Tumor) • Fib14_Postn – migDC28 (FatT, Tumor)
	Btla-Cd79a	Control of T-cell exhaustion, targeting Btla has shown increased T-cell activity	• DC8 – B_cell24 (FatT) • Prol_myel30 – B_cell24 (FatT)
	Gas6-Mertk	Cell growth, survival, adhesion; Gas6 highly expressed on cancers, anti-Mertk shown to aid in immunotherapy	• Fib27 – mac11_F (Tumor) • Fib14_Postn – mac11_F (Tumor) • Fib21 – mac11_F (Tumor)

Concluding Remarks

- Certain gene pairs involved in immunosuppression show up repeatedly in top interaction changes between cell locations changes with aPD-1 treatment
  - 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

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

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

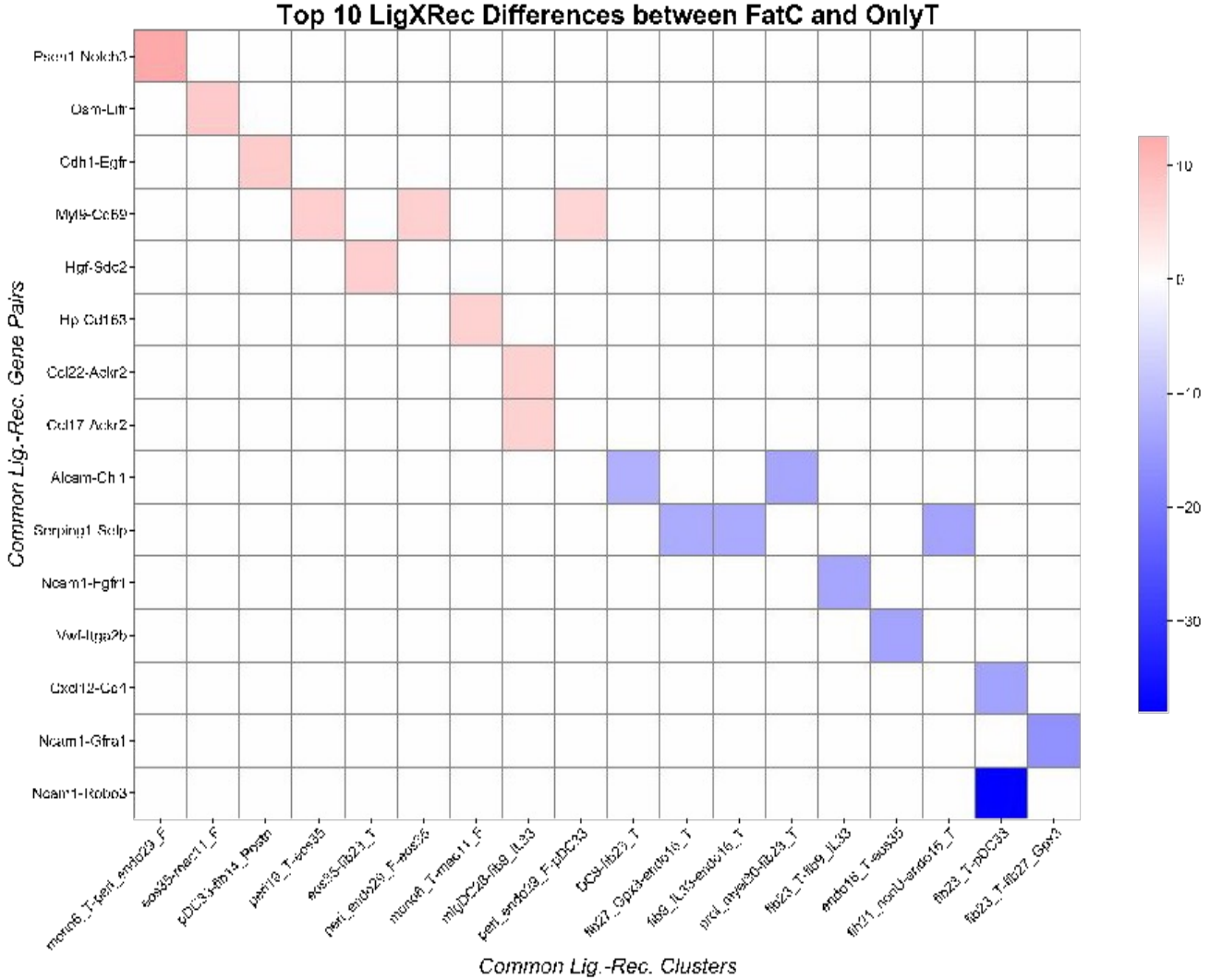
Category	Lig-Rec Genes	Genetic Function	Lig-Rec Clusters
Immune Cell Activity	Ncam1-Robo3	Cellular proliferation of CD8 T-cells, DCs; poor prognostic marker in AML[1-6]	• Fib23_T – pDC33
ECM/Stromal Interactions	Vtn-Itgav	Promotes cell adhesion to ECM components[7-8]	• Peri_endo29_F-mono6_T
	Alcam-Chl1	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

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

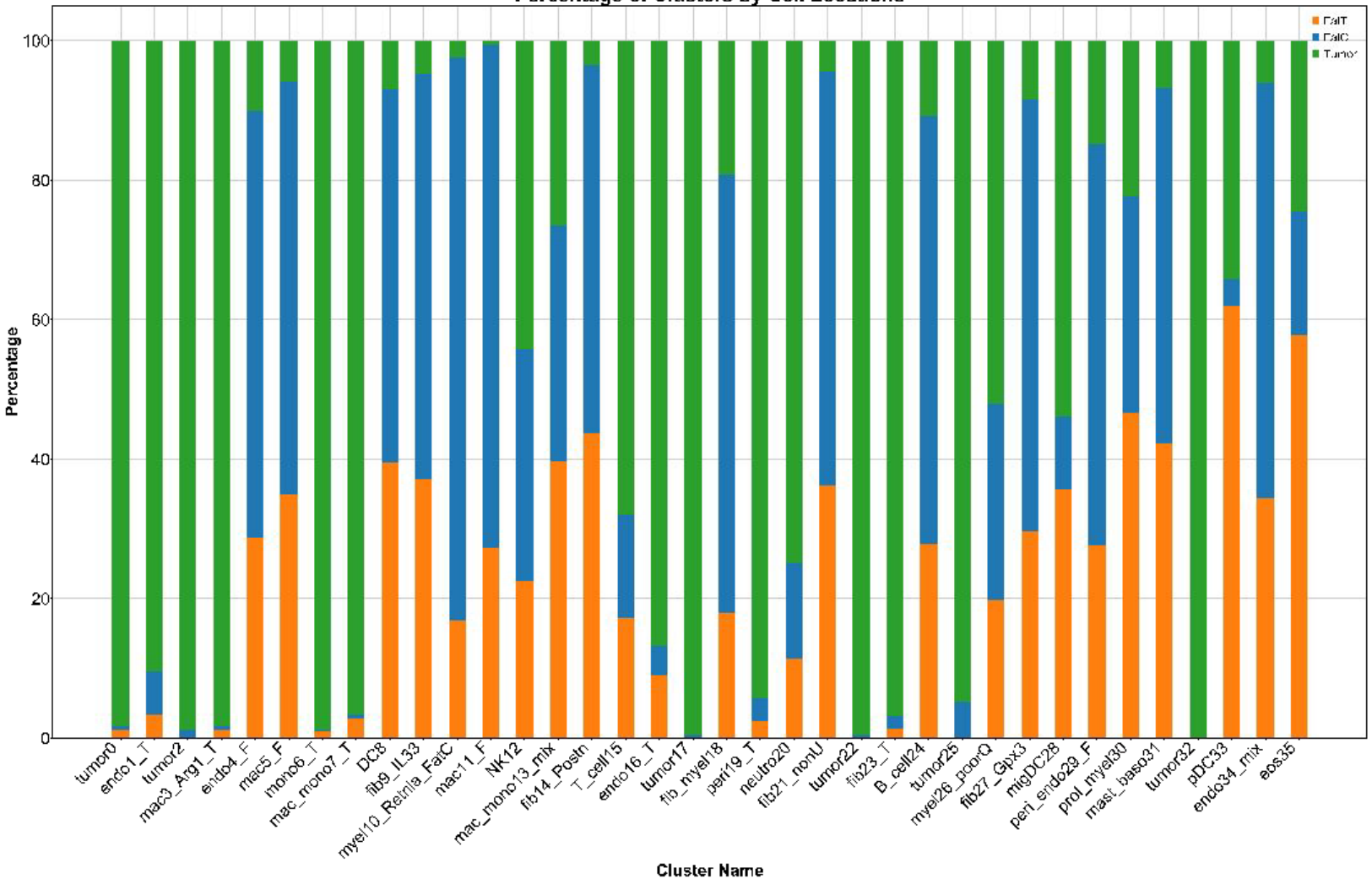
Category	Lig-Rec Genes	Functions	Lig-Rec Clusters
Immune Cell Activity	Pglyrp1-Trem1	Monocyte-mediated inflammation; high Trem1 activity poor cancer prognosis due to immunosuppression[1-4]	• Eos35-neutro20
	Btla-Cd79a	Negative regulation of B-cell, T-cell antigen recognition activity[5-9]	• DC8-B_cell24 • 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

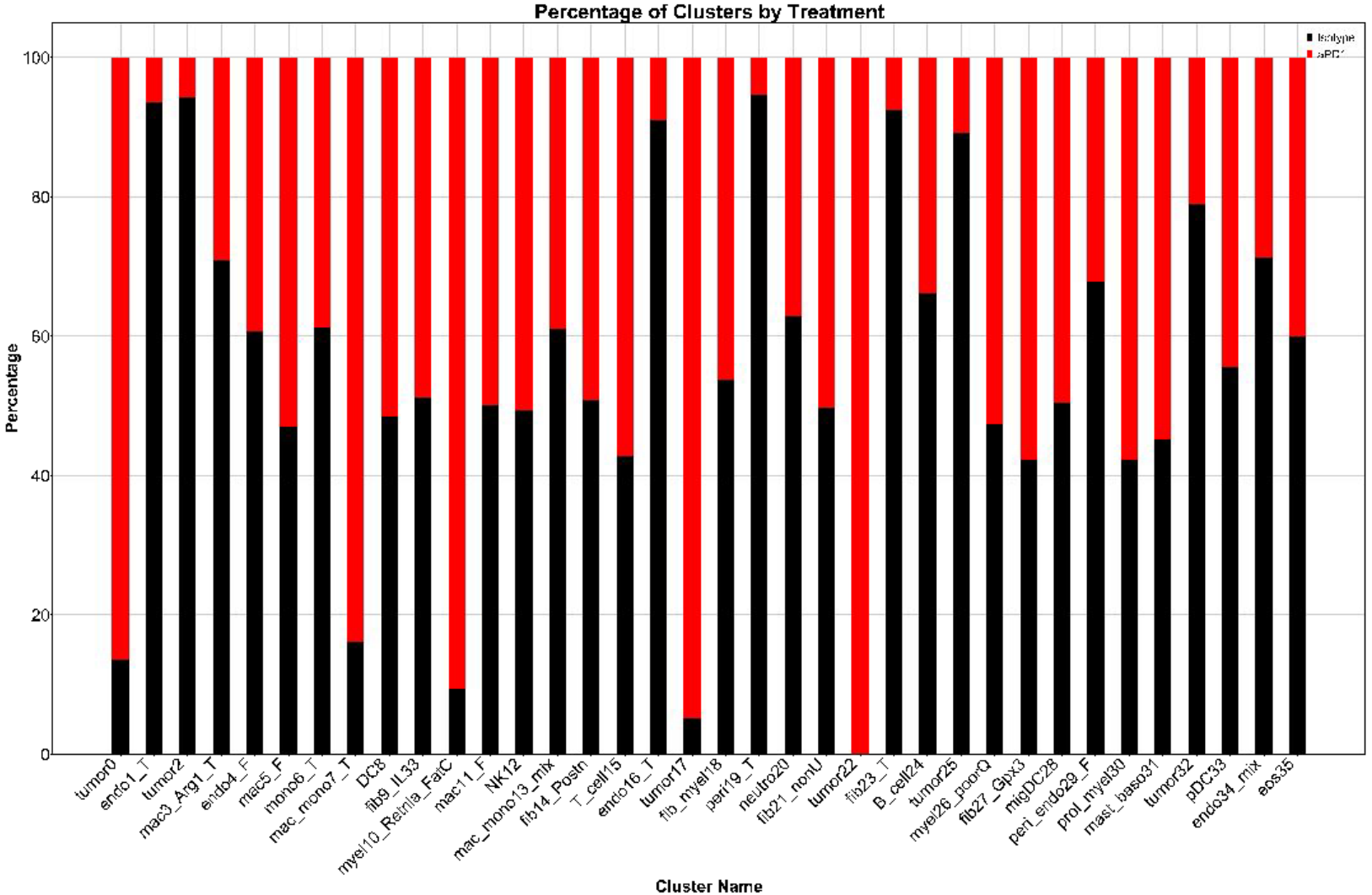
Greater Degree of LigXRec Differences Due to aPD-1 – FatC vs. Tumor

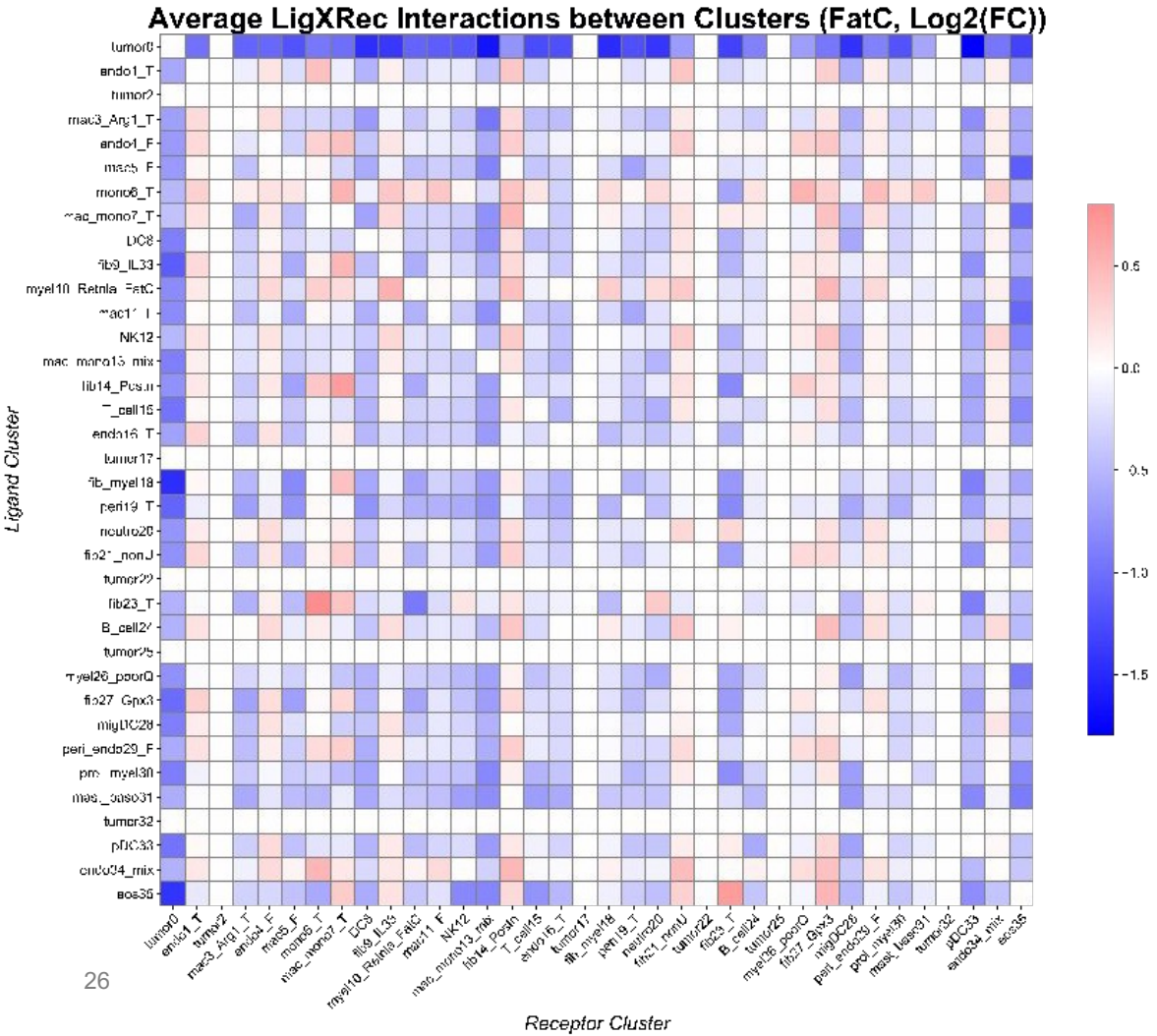
Category	Lig-Rec Genes	Function	Lig-Rec Clusters (FatC vs. Tumor)
Immune Cell Activity	Ncam1-Robo3	Cellular proliferation of CD8 T-cells, DCs; poor prognostic marker in AML[1-6]	• Fib23_T – pDC33
	Myl9-Cd69	Myosin regulatory subunit, high expression shows low response to aPD-1 treatment[7-9] ; lymphocyte 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



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


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
mast_baso31
tumor32
pDC33
endo34_mix
eos35
Cluster Name

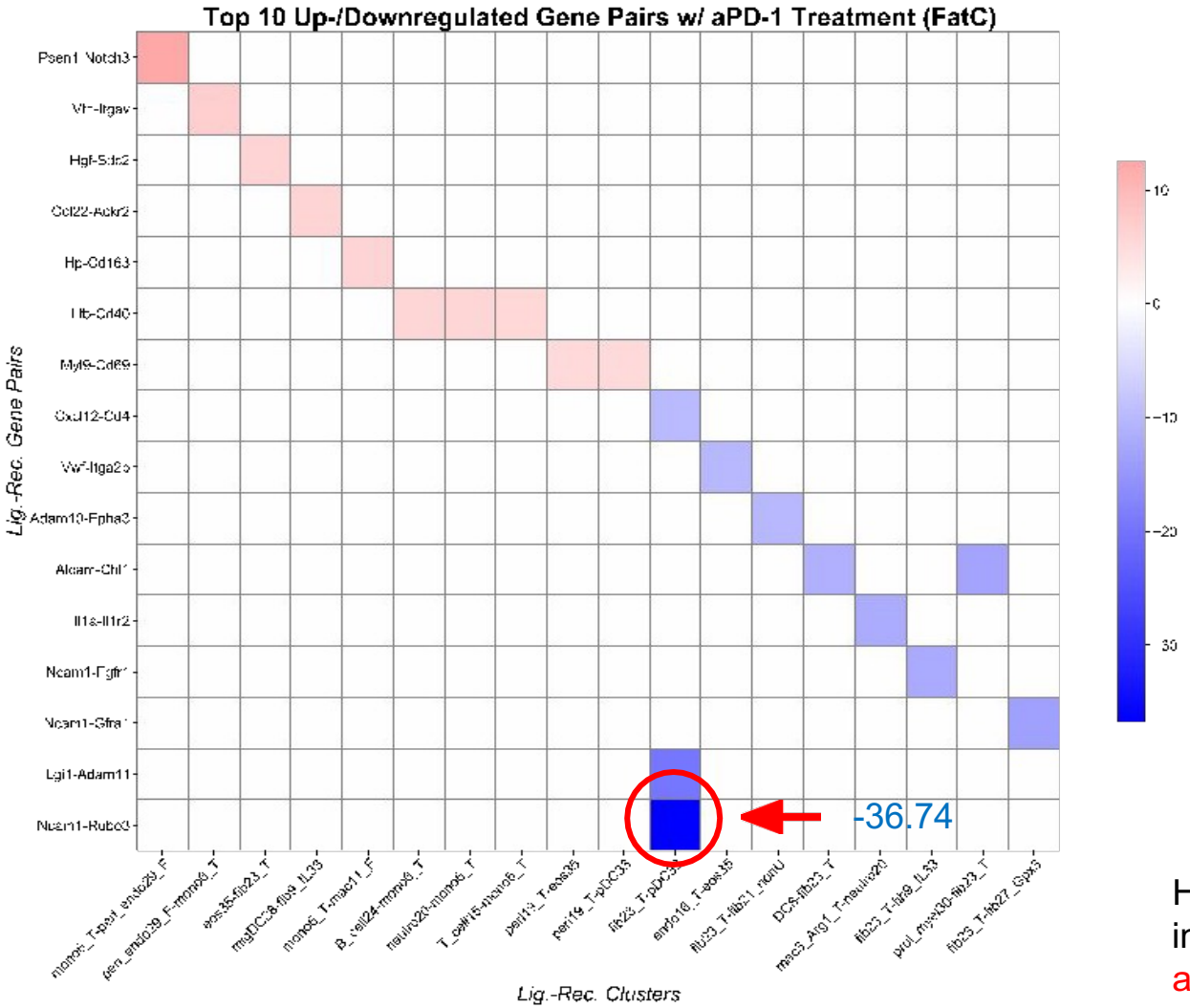

Percentage of Clusters by Treatment
Isotype
aPD1
Percentage
100
80
60
40
20
0
tumor0
endo1_T
tumor2
mac3_Arg1_T
endo4_F
mac5_F
mono6_T
mac_mono7_T
DC8
fib9_IL33
myel10_Retnla_FatC
mac11_F
NK12
mac_mono13_mix
fib14_Postn
T_cell15
endo16_T
tumor17
fib_myel18
peri19_T
neutro20
fib21_nonU
tumor22
fib23_T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



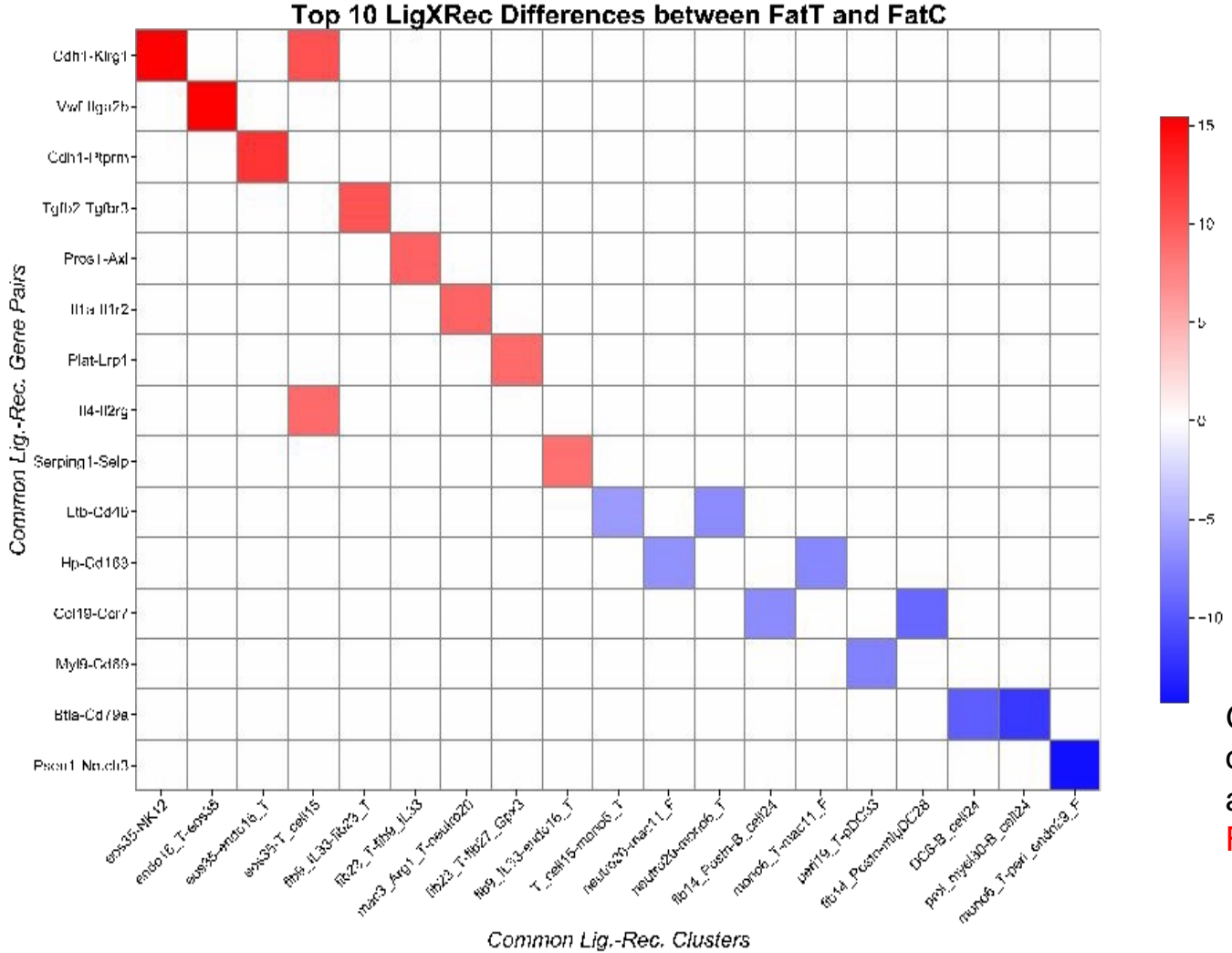
Fold Change in Average Cluster Interactions – FatC

Increased Interaction: aPD-1 vs. Iso

Ligand	Receptor	Log2(aPD1/Iso)
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



Higher LigXRec interaction:
aPD-1 vs. Isotype



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 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 immunother apy[28-34]	• Fib27_Gpx3–mac11_F