Chapter 7: Concluding remarks and future work

7.1. Summary of experimental results

The objective of my thesis was to determine whether iTEC-based thymic organoids represent a suitable tool for recapitulating thymic function and T cell reconstituting athymic mice, as a model of the thymus-related PID, DiGeorge syndrome. The main findings of this thesis are outlined in Figure 7.1.

In Chapter 3, I characterised and optimised the MEFs to iTEC reprogramming system as related to the generation of iTEC-based RTOC capable of mediating thymopoiesis *in vivo*. Firstly, I investigated the effect of a longer reprogramming time and reaggregation period on the ability to transplant and successfully recover grafted iTEC-RTOC. From iTEC-RTOC transplantation experiments I showed that increasing the reprogramming period from four to eighteen days and increasing the reaggregation period from sixteen to sixty hours, allowed recovery of intact grafts two weeks post-transplantation. Further, I found that this increase in reprogramming time yielded a higher number of iTEC which allowed upscaling of experiments. To ensure this new optimised protocol did not compromise iTEC functionality, the ability of the grafts to support thymopoiesis *in vivo* was explored. I demonstrated that iTEC-RTOC were able to support thymopoiesis from the DN ($CD4^-CD8^-$) to DP ($CD4^-CD8^-$) and subsequently to the SP ($CD4^+CD8^-$ and $CD4^-CD8^+$) stages of T cell development, although variability between experiments remained high. Histological analysis of the recovered grafts also revealed that the improved iTEC protocol was able to generate grafts mimicking native thymic architecture with distinct cortical and medullary regions. The hypotheses that increasing the iTEC reprogramming period to eighteen days and the RTOC reaggregation period to sixty hours would yield macroscopic grafts capable of thymopoiesis was supported.

I also tested the hypothesis that enriching for $DLL4^+$ cells from the iTEC population would result in enhanced iTEC phenotype and functionality. Gene expression analysis revealed that this enrichment did not result in improved expression of TEC markers compared to unfractionated GFP^+ ($FOXN1^+$) iTEC. No significant differences were observed between iTEC-RTOC and $DLL4^+$ iTEC-RTOC in terms of capacity to support thymopoiesis or to generate grafts with clear cortex and medullary compartments, so enriched $DLL4^+$ iTEC were not utilised in further experiments.

Despite the improved functionality resulting from the protocol optimisation described above, the data presented in Chapter 3 indicated considerable batch to batch (and within-batch) variation, as assessed by both flow cytometry and histological read-outs, highlighting the need for further optimisation of the protocol.

In Chapter 4 I tested the hypothesis that fetal thymic mesenchyme (FTM) was an essential component of iTEC-RTOC. I established that FTM has an impact on thymopoiesis in grafted iTEC-RTOC, in terms of numbers and percentages of developing thymocytes, but that it is not required to mediate T cell development *per se*. As obtaining FTM is the most limiting factor in these experiments, efforts were made to eliminate its requirement from the system, with the expectation of also reducing variability among iTEC-RTOC. I established that the requirement for FTM could be at least partially compensated by increasing the number of iTEC-RTOC transplanted (iTEC-MultiRTOC) and number of iTEC within them, with regards to mediating thymocyte transition from DN to the DP stages and numbers. Additionally, I showed that FTM is not required for patterning of grafts into organised cortical regions, and medullary regions containing $AIRE^{+}$ mTEC.

I then focused on identifying factors that are differentially expressed between FTM and WT MEFs, in order to test whether provision of one or more of these factors could compensate for the absence of FTM in iTEC-RTOC. In a preliminary experiment, I tested twenty-five candidate factors identified as secreted proteins expressed by FTM but not MEFs. The factors were divided randomly into three pools for testing purposes. I found that, when supplemented in the media of *in vitro* cultures, Group 2 candidates augmented iTEC-RTOC function with regards to: (i) DN to SP progression, (ii) immature to mature T cell progression, and (iii) TCR activation. Strikingly, Group 2 iTEC-RTOC outperformed iTEC+FTM-RTOC in terms of all the aforementioned functions. These promising preliminary results suggest that the effect elicited by FTM on iTEC-RTOC can be replaced with the addition of soluble factors to the media and constitute the first step in understanding the role of FTM in iTEC-RTOC function.

In Chapter 5, I tested the hypothesis that optimised iTEC-MultiRTOC system could recruit host lymphoid progenitor cells (LPC) in the presence or absence of donor thymocytes. Through a series of transplantation experiments I confirmed the hypothesis that iTEC-RTOC that contained WT MEFs but not FTM could recruit host LPCs and support their T cell lineage commitment and development. Although

donor thymocytes were not required for the latter, provision of DN thymocytes within the iTEC-RTOC grafts resulted in improved histology of the recovered grafts, such that they resembled native thymi, and DN were therefore included in the iTEC-RTOC in subsequent experiments.

The combined results of Chapter 4 and 5 permitted the identification of the minimum cellular requirements for iTEC-RTOC transplantation: iTEC and WT MEFs. Complete exclusion of WT MEFs from the system was not tested but if performed could offer an even more clinically relevant approach that does not rely on any donor tissue material.

In Chapter 6, iTEC-MultiRTOC (iTEC + WT MEFs + DN thymocytes) were transplanted into *Foxn1*$^{G/G}$ nude T cell deficient hosts, and their ability to repopulate the peripheral immune system was tested. This work demonstrated that iTEC-MultiRTOC repopulate the periphery of the animal with T cells. Most importantly analysis of RNA-Seq data indicated the presence of a diverse TCR repertoire in some of the iTEC-RTOC recipients. Additionally, the two animals that became ill during the experiment contained a small number of dominant T cell clones, that could indicate development of autoimmunity, consistent with the known absence of medullary areas in some grafts. Histological analysis of recovered grafts at the experimental endpoint (twenty-two weeks post-grafting), however, showed that iTEC-RTOC grafts contained few cTEC and no mTEC. The latter was also the case for the two sick animals culled at seventeen and twenty weeks, respectively. These data demonstrate the limited longevity of iTEC generated using the current protocol following transplantation.

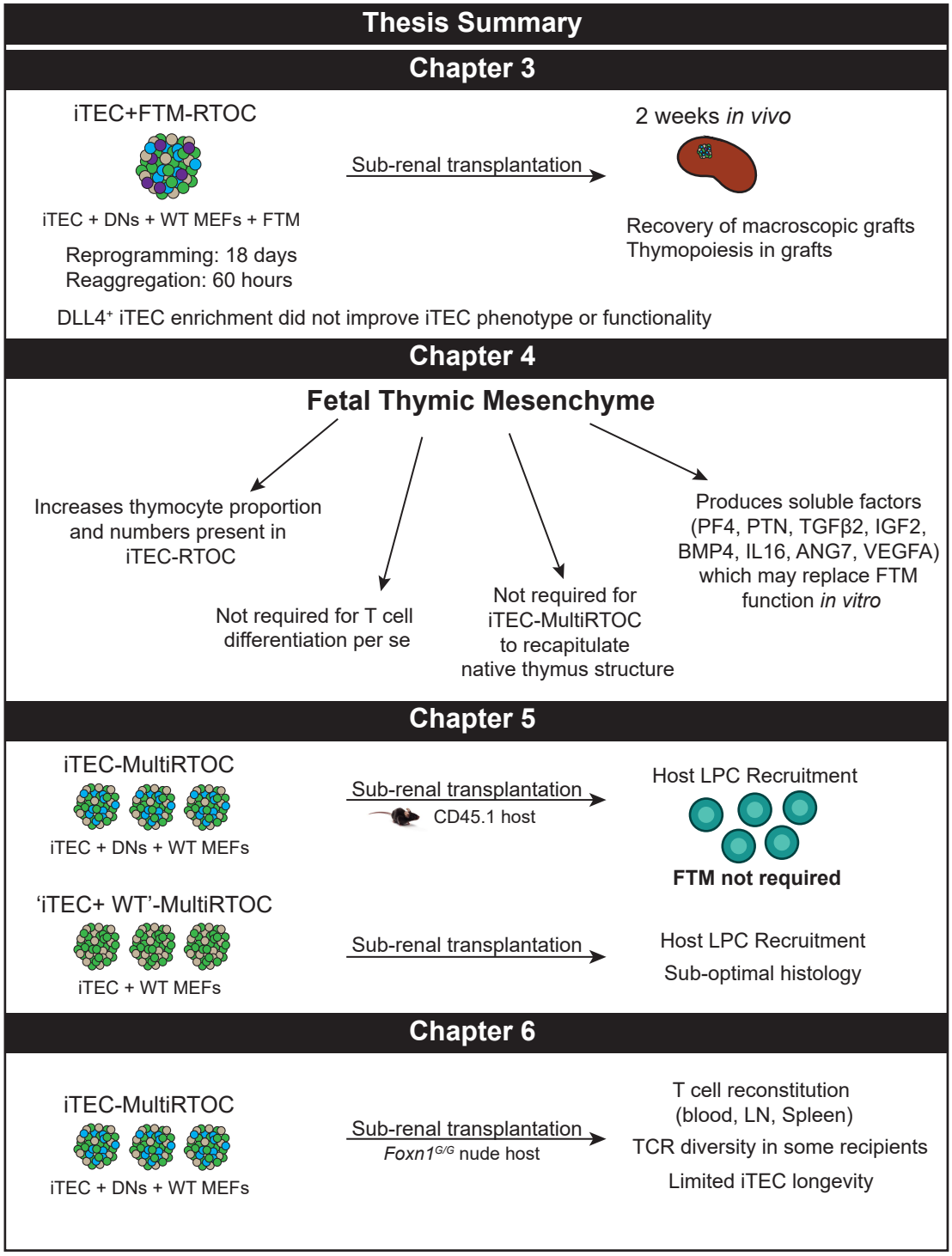


Figure 7.1. Graphical thesis abstract. Outline of thesis findings from each Chapter. FTM: Fetal Thymic Mesenchyme. LPC: Lymphoid Progenitor Cell. LN: Lymph Node.

7.2. Concluding remarks and future work

The overall hypothesis of this thesis was that iTEC-based thymic organoids represent a suitable tool for instating an adaptive immune system in athymic mice, essentially providing a means of overcoming the tissue-supply and ethical problems associated with transplantation of neonatal thymus tissue to treat thymus-related PIDs.

7.2.A. Strengths and weaknesses of the iTEC system

This thesis highlights several strengths and weaknesses of the iTEC system in relation to similar protocols for generating a transplantable source of TEC cells.

As outlined in this thesis the new optimised iTEC protocol is capable of mediating all major stages of thymopoiesis, mimicking thymus structure *in vivo* and repopulating the periphery of mice with a diverse pool of T cells. Upon transplantation, the iTEC system is capable of supporting SP4 and SP8 T cell, Treg, and γδ T cell development and can form a correctly patterned thymic structure. Strikingly iTEC grafts display distinct cortical and medullary regions expressing cTEC and mTEC functional markers (β5t and AIRE, respectively). The fact that iTEC express the correct machinery required for positive and negative selection of thymocytes offers a major advantage over other thymus-like structures generated thus far from other sources of pluripotent stem cells (PSC) (discussed in Chapter 1). In brief, in contrast to the iTEC-RTOC system, reports by Parent *et al*. (2013) and Sun *et al*. (2013) do not display corticomedullary organisation and Su *et al*. (2015) did not examine TCR diversity established by transplants. Additionally, Sun *et al*. PSC-derived TEPC fail to repopulate the host with SP8 T cells. The work detailed by Lai *et al*. (2009) described a low efficiency of directed differentiation (~25%), limited SP4 and SP8 T cell numbers within grafts and did not characterise the TCR repertoire produced. Lastly Otsuka *et al*. (2020) reported low numbers of T cells generated *in vivo* and did not investigate survival and histology of iPSC-derived TEC grafts. The iTEC system therefore represents the most promising means of generating an abundance of TEC *in vitro* for transplantation into *nude* hosts in comparison to similar methods described. Despite this, T cells generated from iTEC grafts remain to be functionally tested.

The success with which iTEC mediate thymopoiesis, mimic thymus-like architecture and establish TCR repertoire diversity is influenced by the large inherent variability found within the system (between technical replicates and independent

experiments). The factor(s) behind this variability has not been determined at this point, but vascularisation rate of grafts, presence of rare iTEC sub-populations, MEF batch variation from which iTEC are established, culture prior to transplantation and iTEC cell death or loss during transplantation may all play a role. These small variations at each step of this technically demanding protocol may accumulate and lead to significant differences between outcome of experiments.

A closer look into the transcriptomic and proteomic intricacies present in iTEC sub-populations, and comparison between technical replicates and batches of established iTEC, would be an interesting first step in elucidating some of this variation. As the current reprogramming approach involves a simple manipulation of enforced expression of a single transcription factor (FOXN1), it would be interesting to introduce further transcription factors involved in thymus organogenesis. This could result in less variation and improved TEC phenotype and function. Through single cell RNA-Seq of early and late-stage reprogramming iTEC, we could gain a better understanding of the TEC subpopulations present or lacking in the system and how these could be better bioengineered. These studies would also reveal if specific elements of the gene expression programmes regulating different TEC subpopulations were missing in iTEC, also assisting in elucidating the cause of the variation observed.

An additional factor requiring investigation is the elimination of serum during iTEC reprogramming. As serum is undefined, highly variable and impacts reproducibility and reliability of experiments, its elimination from the cell culture media would be a major step in reducing the variability. Further, transplantation of RTOC consisting solely of iTEC (without addition of WT MEFs and donor thymocytes) could be explored as a means to further reduce variability. Lastly, epigenetic changes could be explored during the reprogramming of MEFs, as a tool for understanding the molecular mechanisms of MEF-iTEC reprogramming and increasing the reproducibility of iTEC-based experiments.

With regards to the relevance of the work described in this thesis for human biology, the findings outlined can be taken forward and applied to a system that utilises standardised human induced pluripotent stem cells (hiPSC) for the generation of human iTEC. Therefore, this thesis points to the potential of iTEC as a TEC replacement to achieve thymopoiesis *in vivo*, in athymic individuals or in individuals who have lost thymus function due to cytoablative therapy or to involution. Before

the latter can be achieved a reduction in the variability of the system needs to be established, alongside elucidating ways of generating self-renewing iTEC or long-term iTEC progenitors. By elucidating the minimal cellular requirements for bioengineering iTEC organoids in a murine system, we are one step closer to generating a simplified clinically relevant system for future human application. In addition, this thesis further identifies the potential use of soluble factors produced by FTM that could be applied to boost thymopoiesis.

7.2.B. Future work

Although this thesis addresses many previously unanswered questions regarding the iTEC system (minimal cellular requirements of iTEC-RTOC, soluble factors provided by FTM, iTEC ability to recruit host lymphoid progenitors, iTEC longevity and TCR repertoire diversity established in nude hosts), further work is needed to fully elucidate the potential of the system for treating thymus-related immunodeficiencies.

A consistent observation between Chapters has been the overall trend that iTEC do not support the DP to SP progression as robustly as native TEC, and the success of this is highly variable between technical and biological replicates. The latter suggests more in-depth work needs to be carried out to fully understand the capacity with which iTEC are able to support positive selection of thymocytes and thymocyte proliferation at appropriate stages. A first attempt at this has been carried out by our lab whereby the transcriptional landscape of T cells within iTEC grafts was investigated and compared to T cells in an RFTOC graft using 10X single cell RNA-Seq. As this experiment was only carried out once it was not included in the main body of this thesis, but data indicate that some iTEC-RTOC may have issues in instigating TCR activation required for positive selection. It would be of interest to carry out further 10X scRNA-Seq experiments comparing ‘successful’ to ‘unsuccessful’ iTEC-RTOC and finding the component that varies between them and the consequences it has on thymopoiesis present. Single cell RNA-Seq on recovered iTEC would give valuable insight into the TEC-subpopulations present in grafts in relation to native thymic tissue transplants. The latter would allow better mechanistic understanding of the cellular reprogramming strategy described and ways in which it can be further fine-tuned to generate all necessary TEC subsets.

Regarding the work carried out on Fetal Thymic Mesenchyme (FTM), despite the promising preliminary results indicating that soluble factors supplemented *in vitro*

can replace FTM effect on iTEC-RTOC, this experiment must be repeated to confirm findings. Additionally, each substituted factor and its effect on the system need to be investigated individually and in different combinations with other factors exploring alternative groupings. This for example could be based on the signalling pathways utilised by each factor. Furthermore, as thymopoiesis was the only readout of these experiments it would be of interest to explore the implication and potential use of these factors in reprogramming of iTEC and early and late T cell development, separately, to generate a further optimised protocol.

Concerning iTEC ability to recruit host LPC, it would be of interest if future work employed analysis of the kinetics involved in this recruitment. Although donor thymocytes are no longer present in the iTEC-RTOC grafts by the two-week timepoint, additional experiments could elucidate whether these cells undergo apoptosis or whether they repopulate the periphery of the animal. The number of host LPC recruitment waves taking place, exact timepoint at which these occur and efficiency of this system versus native TEC would be critical in understanding the clinical application of the iTEC system for host T cell reconstitution. Although 'iTEC and WT MEF only' grafts were also able to recruit host LPCs, the preliminary histological examination of these grafts, however, suggests that donor thymocytes in the system improved thymus-like histology and cellularity. It cannot be excluded at this stage that variation observed is not due to inherent variation found within this reprogramming system. Future work will include further histological evaluation of grafts, a more detailed FACS panel for analysis of thymopoiesis stages and the use of TCR transgenic donor thymocytes with an early T cell developmental block. This will determine if thymic crosstalk with donor thymocytes prior to host LP recruitment plays a role in this process.

The reason behind the lack of TCR repertoire diversity described in some iTEC recipients and whether this is related to iTEC variability, library preparation or sequencing quality cannot be determined at this point. In order to fully understand this the latter experiment must be repeated, and possibly including a condition in the presence of FTM in RTOC. Finally, as the TCR repertoire of *nude* mice is not defined it would be beneficial to include ungrafted age and sex matched controls to determine whether clonotypes present arise from spontaneous maturation of DN thymocytes included in Cre MEF grafts, or if they are in fact extrathymically derived. In addition, the possible presence of autoimmunity in iTEC recipients remains to be

confirmed and the possibility that this may arise due to a lack of medullary formation within specific grafts must be considered as a factor. Detailed analysis of animal serum for presence of autoantibodies and detailed histological examination of recovered organs for immune infiltrates is also required.

Chapter 6 also highlighted the limited longevity of iTEC, raising many questions about the future applicability of such a system for treatment of T cell immunodeficiencies. Future work will include examination of whether a single round of transplantation is sufficient to reverse the T cell immunodeficiency associated with athymic recipients. Alternatively, several rounds of transplantation will be explored alongside the longevity of T cells generated within iTEC-RTOC and used as a tool to boost the adaptive immunity of recipients no longer undergoing active thymopoiesis in previous grafts. Exploration into engineering a TEPC/TEC stem cell niche into iTEC organoids could also be carried out alongside manipulation of *Foxn1* levels in reprogrammed iTEC to establish graft longevity.

Finally, it is critically important that T cell's functionality, originating from iTEC-RTOC, is tested to determine the applicability of the iTEC system. Further work will be carried out whereby T cell functionality will be determined through host infection with specific pathogens or through skin transplantation experiments.

7.2.C. Conclusion

With regards to the overall hypothesis of this thesis, evidence is presented that iTEC can be utilised to provide an unlimited source of cells that can form thymus-structures upon transplantation that accurately recapitulate the native thymus and reconstitute athymic recipients with a diverse TCR repertoire. This thesis extends previous findings from our laboratory to establish that iTEC-RTOC lacking FTM can recruit host LPC, support host-derived T cell development, and form a complex, organised and functional thymic organoid. The findings in this thesis are therefore crucial in establishing iTEC as a murine bioengineering tool for T cell development and reconstitution and form the basis for the development of thymus transplantation systems for future clinical use in immunocompromised patients.

8. Appendix

8.1. Full list of ligand-receptor candidates (FTM-TEC vs WT MEF-TEC)

Ligand - Receptor Pair	adjusted_p_values	Log2_Fold_Change_MEF_minus_FMT
CFH_SELL	4.60E-18	-0.8515
GDF10_ACVR1B	4.60E-18	-0.7446
FGF10_FGFR3	4.60E-18	-0.6608
FGF10_FGFR1	4.60E-18	-0.6608
FGF10_FGFR2	4.60E-18	-0.6608
CD34_SELL	4.60E-18	-0.6507
CXCL13_CCR10	4.60E-18	-0.6224
CXCL13_HTR2A	4.60E-18	-0.6224
CXCL13_ACKR4	4.60E-18	-0.6224
CCL11_ACKR4	4.60E-18	-0.5377
CCL11_CCR5	4.60E-18	-0.5377
RARRES2_CCRL2	4.60E-18	-0.5101
RARRES2_GPR1	4.60E-18	-0.5101
RARRES2_CMKLR1	4.60E-18	-0.5101
NID2_COL13A1	4.60E-18	-0.4956
SEMA6D_KDR	4.60E-18	-0.4622
SEMA6D_PLXNA1	4.60E-18	-0.4622
SEMA6D_TYROBP	4.60E-18	-0.4622
SEMA3D_NRP1	4.18E-17	-0.4621
HGF_CD44	4.60E-18	-0.4157
HGF_SDC1	4.60E-18	-0.4157
HGF_MET	4.60E-18	-0.4157
HGF_ST14	4.77E-18	-0.4157
PF4_FGFR2	4.60E-18	-0.4136
PF4_SDC2	4.60E-18	-0.4136
PF4_LDLR	4.60E-18	-0.4136
PF4_PROCR	4.60E-18	-0.4136
PF4_THBD	4.60E-18	-0.4136
ANGPTL4_TIE1	4.60E-18	-0.3902
MFAP5_NOTCH1	9.56E-18	-0.3894
DLL4_NOTCH2	4.60E-18	-0.3815
DLL4_NOTCH3	4.60E-18	-0.3815
DLL4_NOTCH1	4.60E-18	-0.3815
DLL4_NOTCH4	4.60E-18	-0.3815
IGF1_INSR	6.27E-16	-0.3815
IGF1_IGF1R	2.51E-17	-0.3815
PLAT_ITGB2	1.48E-14	-0.3676
PLAT_LRP1	1.45E-17	-0.3676

LAMA2_ITGA3	4.85E-18	-0.3629
LAMA2_ITGA1	6.10E-18	-0.3629
LAMA2_ITGB4	4.60E-18	-0.3629
LAMA2_ITGA6	4.60E-18	-0.3629
LAMA2_ITGA7	1.39E-17	-0.3629
SERPING1_LRP1	4.46E-13	-0.3302
SERPING1_SELE	3.22E-15	-0.3302
DCN_ERBB4	2.89E-16	-0.3285
DCN_EGFR	3.60E-17	-0.3285
DCN_MET	1.19E-16	-0.3285
IL15_IL15RA	4.85E-18	-0.3148
IL15_IL2RA	1.04E-17	-0.3148
IL15_IL2RG	3.42E-16	-0.3148
SERPINE2_LRP1	2.21E-15	-0.3098
NXPH1_NRXN2	4.60E-18	-0.3046
NXPH1_NRXN3	4.60E-18	-0.3046
NXPH1_NRXN1	8.52E-16	-0.3046
GNAS_ADCY1	7.22E-15	-0.3003
GNAS_ADCY9	1.22E-14	-0.3003
GNAS_ADORA1	4.60E-18	-0.3003
GNAS_ADRB3	8.42E-14	-0.3003
GNAS_PTGIR	4.46E-13	-0.3003
GNAS_TSHR	3.07E-15	-0.3003
GNAS_GCGR	1.28E-15	-0.3003
GNAS_VIPR1	2.89E-15	-0.3003
GNAS_ADCY7	8.60E-13	-0.3003
GNAS_ADCY8	4.60E-18	-0.3003
GNAS_HTR6	4.60E-18	-0.3003
BMP7_BMPR1A	4.60E-18	-0.2982
BMP7_ACVR2A	7.82E-18	-0.2982
BMP7_BMPR1B	8.70E-17	-0.2982
BMP7_ACVR2B	4.60E-18	-0.2982
BMP7_ENG	2.24E-17	-0.2982
BMP7_ACVR1	5.88E-18	-0.2982
GPC3_CD81	4.60E-18	-0.2979
GPC3_IGF1R	4.60E-18	-0.2979
PGF_FLT1	4.60E-18	-0.2978
PGF_NRP1	3.22E-14	-0.2978
PGF_NRP2	1.32E-12	-0.2978
IGFBP4_FZD8	5.34E-13	-0.2901
IGFBP4_LRP6	2.01E-13	-0.2901
FGF1_CD44	2.64E-16	-0.2747
FGF1_EGFR	4.04E-16	-0.2747

FGF1_FGFR1	3.96E-15	-0.2747
FGF1_FGFR3	3.19E-15	-0.2747
FGF1_FGFRL1	3.79E-14	-0.2747
FGF1_NRP1	6.85E-13	-0.2747
FGF1_FGFR2	2.23E-17	-0.2747
DLK1_NOTCH2	7.55E-15	-0.2673
DLK1_NOTCH3	9.66E-16	-0.2673
DLK1_NOTCH4	1.91E-15	-0.2673
DLK1_NOTCH1	2.20E-14	-0.2673
CXCL1_CXCR2	5.75E-18	-0.2594
FBLN1_ITGB1	4.60E-18	-0.2574
VTN_CD47	2.01E-17	-0.2567
VTN_ITGA3	5.04E-16	-0.2567
VTN_ITGA8	1.80E-07	-0.2567
VTN_ITGAV	2.06E-16	-0.2567
VTN_ITGB3	4.68E-16	-0.2567
VTN_ITGB5	5.84E-14	-0.2567
VTN_KDR	4.60E-18	-0.2567
VTN_PVR	3.02E-15	-0.2567
VTN_ITGA2B	1.97E-16	-0.2567
VTN_ITGA5	1.04E-16	-0.2567
VTN_ITGB1	1.41E-17	-0.2567
VTN_ITGB8	4.44E-17	-0.2567
VTN_PLAUR	2.14E-11	-0.2567
VTN_TNFRSF11B	3.50E-15	-0.2567
PROS1_AXL	6.10E-18	-0.2545
PROS1_TYRO3	4.60E-18	-0.2545
COL14A1_CD44	5.49E-08	-0.2499
FGF13_EGFR	4.83E-17	-0.2489
FGF13_FGFR2	1.08E-17	-0.2489
FGF13_FGFR3	4.31E-16	-0.2489
FGF13_SCN5A	5.22E-18	-0.2489
FGF13_SCN8A	1.08E-17	-0.2489
FGF13_FGFR1	8.37E-16	-0.2489
IL16_CCR5	2.52E-13	-0.2376
IL16_CD4	3.37E-13	-0.2376
IL16_GRIN2C	2.15E-13	-0.2376
IL16_KCNA3	1.01E-12	-0.2376
IL16_KCND1	1.99E-12	-0.2376
IL16_KCNJ15	4.41E-13	-0.2376
IL16_KCNJ4	3.22E-13	-0.2376
IL16_GRIN2D	1.89E-13	-0.2376
IL16_KCND2	2.10E-13	-0.2376

EDN3_EDNRA	1.79E-13	-0.2346
EDN3_EDNRB	9.20E-12	-0.2346
HAS2_HMMR	2.21E-15	-0.2331
HAS2_CD44	4.11E-16	-0.2331
SFRP1_FZD2	3.49E-11	-0.2324
SFRP1_FZD6	5.94E-11	-0.2324
SEMA4A_PLXND1	1.10E-14	-0.2303
NTNG1_LRRC4C	6.81E-09	-0.2199
MFAP2_NOTCH1	1.80E-07	-0.2144
NLGN3_NRXN3	4.60E-18	-0.2140
NLGN3_NRXN1	2.30E-11	-0.2140
NLGN3_NRXN2	4.60E-18	-0.2140
NLGN1_NRXN2	4.60E-18	-0.2137
NLGN1_NRXN3	4.60E-18	-0.2137
NLGN1_NRXN1	2.61E-11	-0.2137
ADAM12_ITGB1	6.54E-18	-0.2075
ADAM12_SDC4	2.56E-15	-0.2075
ADAM12_ITGA9	2.12E-16	-0.2075
FGF18_FGFR1	3.66E-12	-0.2057
FGF18_FGFR2	3.28E-13	-0.2057
FGF18_FGFR3	2.34E-12	-0.2057
SPON2_ITGB2	1.92E-06	-0.1974
ANGPT1_ITGA5	9.40E-12	-0.1946
ANGPT1_TEK	2.32E-09	-0.1946
ANGPT1_TIE1	2.63E-12	-0.1946
OLFM2_ROBO2	1.68E-13	-0.1943
PTN_PLXNB2	8.66E-12	-0.1937
PTN_PTPRB	5.70E-11	-0.1937
PTN_ALK	4.21E-14	-0.1937
PTN_CDH10	6.67E-12	-0.1937
PTN_PTPRS	3.66E-12	-0.1937
PTN_PTPRZ1	3.80E-11	-0.1937
PTN_SDC1	1.07E-11	-0.1937
PTN_SDC3	1.72E-12	-0.1937
SEMA6A_PLXNA4	5.40E-16	-0.1890
SEMA6A_PLXNA2	5.67E-16	-0.1890
NID1_COL13A1	1.16E-12	-0.1881
NID1_ITGA3	5.59E-14	-0.1881
NID1_ITGAV	1.11E-15	-0.1881
NID1_ITGB1	4.77E-18	-0.1881
NID1_ITGB3	2.78E-14	-0.1881
NID1_PTPRF	5.86E-17	-0.1881
EFNA2_EPHA1	2.34E-12	-0.1849

EFNA2_EPHA2	9.13E-18	-0.1849
EFNA2_EPHA5	7.30E-16	-0.1849
EFNA2_EPHA7	1.00E-10	-0.1849
EFNA2_EPHA3	4.17E-10	-0.1849
EFNA2_EPHA4	6.10E-18	-0.1849
EFNA2_EPHA8	4.60E-18	-0.1849
CX3CL1_CX3CR1	1.18E-11	-0.1815
ICAM2_ITGB2	5.23E-07	-0.1772
NTN3_CDON	2.50E-10	-0.1713
NTN3_NEO1	3.56E-10	-0.1713
LAMA4_ITGA3	4.33E-10	-0.1669
LAMA4_ITGAV	1.12E-10	-0.1669
LAMA4_ITGB1	1.07E-13	-0.1669
LAMA4_ITGA6	1.11E-11	-0.1669
DKK2_LRP6	1.28E-13	-0.1654
VEGFA_ITGAV	6.26E-15	-0.1649
VEGFA_EGFR	5.86E-17	-0.1649
VEGFA_FLT1	4.60E-18	-0.1649
VEGFA_GPC1	3.15E-13	-0.1649
VEGFA_NRP2	2.04E-06	-0.1649
VEGFA_SIRPA	9.96E-18	-0.1649
VEGFA_TYRO3	6.54E-18	-0.1649
VEGFA_EPHB2	5.28E-09	-0.1649
VEGFA_ITGA9	5.32E-17	-0.1649
VEGFA_ITGB1	4.60E-18	-0.1649
VEGFA_KDR	4.60E-18	-0.1649
VEGFA_NRP1	1.30E-08	-0.1649
VEGFA_RET	1.08E-10	-0.1649
IGF2_IGF1R	2.12E-16	-0.1609
IGF2_IGF2R	1.88E-12	-0.1609
IGF2_INSR	7.05E-14	-0.1609
HSPA1A_GRIN2D	2.54E-14	-0.1567
HSPA1A_TLR4	1.02E-13	-0.1567
TGFB2_ACVR1	1.47E-13	-0.1530
TGFB2_ENG	9.27E-13	-0.1530
TGFB2_TGFBR1	3.97E-17	-0.1530
TGFB2_TGFBR2	3.22E-15	-0.1530
TGFB2_TGFBR3	2.21E-16	-0.1530
EFEMP1_EGFR	7.87E-11	-0.1502
EFNB1_EPHA4	2.87E-13	-0.1485
EFNB1_EPHB2	3.11E-07	-0.1485
EFNB1_ERBB2	1.33E-07	-0.1485
EFNB1_EPHB1	1.24E-12	-0.1485

EFNB1_EPHB6	4.71E-12	-0.1485
COL3A1_DDR1	5.13E-12	-0.1468
COL3A1_ITGB1	2.96E-12	-0.1468
COL3A1_DDR2	2.51E-05	-0.1468
COL3A1_ITGA2	7.87E-07	-0.1468
COL3A1_MAG	2.63E-12	-0.1468
NRG2_ERBB4	4.80E-11	-0.1431
NRG2_ERBB2	4.74E-07	-0.1431
NRG2_ERBB3	2.74E-07	-0.1431
EFNA1_EPHA1	1.04E-06	-0.1374
EFNA1_EPHA2	7.86E-09	-0.1374
EFNA1_EPHA3	1.06E-05	-0.1374
EFNA1_EPHA5	4.77E-08	-0.1374
EFNA1_EPHA7	7.10E-06	-0.1374
EFNA1_EPHB6	2.07E-08	-0.1374
EFNA1_EPHA8	3.15E-09	-0.1374
EFNA1_EPHB1	6.46E-09	-0.1374
EFNA1_EPHA4	7.04E-09	-0.1374
SEMA3C_NRP1	5.47E-06	-0.1351
SEMA3C_NRP2	5.59E-05	-0.1351
SEMA3C_PLXND1	1.49E-09	-0.1351
COLQ_MUSK	2.80E-10	-0.1343
SEMA5A_MET	7.76E-08	-0.1336
SEMA5A_PLXNB3	1.67E-07	-0.1336
BMP4_BMPR2	4.71E-12	-0.1334
BMP4_ACVR1	1.02E-12	-0.1334
BMP4_ACVR2A	2.63E-13	-0.1334
BMP4_BMPR1A	7.22E-15	-0.1334
BMP4_BMPR1B	4.50E-10	-0.1334
FAT4_DCHS1	1.12E-05	-0.1328
MDK_PTPRZ1	3.64E-08	-0.1325
MDK_ALK	1.74E-09	-0.1325
MDK_GPC2	2.66E-09	-0.1325
MDK_ITGA4	1.44E-08	-0.1325
MDK_ITGA6	4.07E-09	-0.1325
MDK_ITGB1	2.92E-09	-0.1325
MDK_LRP1	1.56E-05	-0.1325
MDK_LRP2	0.001514226	-0.1325
MDK_SDC1	5.90E-09	-0.1325
MDK_SDC3	3.78E-09	-0.1325
MDK_SDC4	6.11E-09	-0.1325
MDK_PTPRB	1.80E-07	-0.1325
EFNB3_EPHA4	6.45E-11	-0.1273

EFNB3_EPHB3	3.35E-11	-0.1273
EFNB3_EPHB4	2.06E-10	-0.1273
EFNB3_EPHB6	1.73E-09	-0.1273
EFNB3_RHBDL2	6.98E-11	-0.1273
EFNB3_EPHB2	3.83E-06	-0.1273
SEMA4B_DCBLD2	2.08E-09	-0.1255
TSLP_IL7R	3.74E-15	-0.1235
TNFSF12_TNFRSF12A	9.46E-07	-0.1215
TNFSF12_TNFRSF25	2.32E-12	-0.1215
PTDSS1_SCARB1	9.42E-09	-0.1211
PTDSS1_JMJD6	1.85E-16	-0.1211
BMP5_ACVR2B	1.11E-12	-0.1195
BMP5_ACVR1	4.17E-10	-0.1195
BMP5_BMPR1A	4.53E-12	-0.1195
BMP5_ACVR2A	2.24E-10	-0.1195
BMP5_BMPR1B	4.31E-08	-0.1195
COL5A3_SDC3	1.49E-09	-0.1189
COL6A1_ITGA2	2.32E-07	-0.1160
COL6A1_ITGA1	2.66E-07	-0.1160
COL6A1_ITGA6	1.16E-11	-0.1160
COL6A1_ITGB1	5.67E-16	-0.1160
COL6A3_ITGA1	3.32E-06	-0.1092
COL6A3_ITGA2	3.51E-06	-0.1092
COL6A3_ITGB1	3.51E-14	-0.1092
COL6A2_ITGA1	1.81E-06	-0.1074
COL6A2_ITGB1	6.13E-14	-0.1074
COL6A2_ITGA2	1.50E-06	-0.1074
FGF11_FGFR1	1.32E-06	-0.1058
FGF11_FGFR2	1.06E-06	-0.1058
FGF11_FGFR3	9.11E-07	-0.1058
NPPC_NPR2	8.91E-06	-0.1037
NPPC_NPR3	0.000199755	-0.1037
LAMC1_ITGA3	2.85E-06	-0.1010
LAMC1_ITGA6	1.44E-08	-0.1010
LAMC1_ITGA7	1.98E-07	-0.1010
LAMC1_ITGAV	4.61E-07	-0.1010
LAMC1_ITGB4	3.03E-09	-0.1010
LAMC1_ITGA1	1.70E-05	-0.1010
LAMC1_ITGA2	1.51E-05	-0.1010
LAMC1_ITGB1	1.44E-11	-0.1010
NMB_GRPR	1.22E-16	-0.1000
NMB_NMBR	9.98E-09	-0.1000
CXCL3_CXCR2	4.53E-12	-0.0992

LAMC3_ITGA2	0.012462821	-0.0982
LAMC3_ITGA6	0.002169997	-0.0982
LAMC3_ITGB4	0.00236403	-0.0982
LAMC3_ITGA3	0.0027837	-0.0982
LAMC3_ITGB1	0.003768792	-0.0982
FGL1_EGFR	3.53E-08	-0.0954
PSEN1_CD44	1.14E-09	-0.0922
PSEN1_NOTCH1	7.48E-05	-0.0922
PSEN1_NOTCH2	0.000101626	-0.0922
PSEN1_NOTCH3	4.61E-07	-0.0922
PSEN1_NCSTN	3.22E-11	-0.0922
PSEN1_NOTCH4	2.44E-06	-0.0922
PODXL2_SELL	1.37E-08	-0.0912
MFGE8_ITGAV	1.02E-07	-0.0906
MFGE8_ITGB3	2.04E-06	-0.0906
MFGE8_PDGFRB	4.16E-07	-0.0906
DUSP18_ITGB1	2.32E-10	-0.0890
DUSP18_CD151	6.92E-10	-0.0890
DUSP18_ITGA1	5.74E-05	-0.0890
DUSP18_ITGA3	1.65E-05	-0.0890
DUSP18_ITGA6	1.38E-07	-0.0890
DUSP18_ITGB4	3.30E-08	-0.0890
DUSP18_RPSA	3.43E-10	-0.0890
DUSP18_ITGA2	5.15E-05	-0.0890
DUSP18_ITGA7	2.29E-06	-0.0890
LAMB3_CD151	1.89E-10	-0.0846
LAMB3_ITGA3	1.61E-05	-0.0846
LAMB3_ITGB4	1.58E-07	-0.0846
LAMB3_COL17A1	1.87E-06	-0.0846
LAMB3_ITGA6	1.21E-06	-0.0846
P4HB_GPR162	9.24E-17	-0.0841
TFPI_F3	7.53E-06	-0.0825
TFPI_LRP1	0.00085164	-0.0825
TFPI_SDC4	1.98E-06	-0.0825
TFPI_VLDLR	0.000664288	-0.0825
LRPAP1_LRP8	1.79E-05	-0.0816
LRPAP1_SORL1	3.55E-07	-0.0816
LRPAP1_VLDLR	0.003018945	-0.0816
LRPAP1_LDLR	1.52E-05	-0.0816
LRPAP1_LRP1	0.00183656	-0.0816
LRPAP1_SORT1	1.13E-07	-0.0816
SEMA3A_NRP1	0.004005713	-0.0745
SEMA3A_NRP2	0.004689036	-0.0745

SEMA3A_PLXNA4	3.63E-06	-0.0745
SEMA3A_PLXNA2	6.73E-07	-0.0745
EDA_EDA2R	0.041448075	-0.0728
EDA_EDAR	0.00697574	-0.0728
LIN7C_ABCA1	0.011673592	-0.0722
LIN7C_KCNJ4	2.35E-10	-0.0722
NRG1_ERBB2	0.000403609	-0.0717
NRG1_ERBB4	1.39E-05	-0.0717
NRG1_GPC1	0.000533955	-0.0717
NRG1_ERBB3	0.002128775	-0.0717
SEMA3F_NRP1	0.014686669	-0.0714
SEMA3F_NRP2	0.046642435	-0.0714
SEMA3F_PLXNA1	8.71E-05	-0.0714
SEMA3F_PLXNA3	0.001330924	-0.0714
CCL25_CCR10	0.028427432	-0.0702
CCL25_CCR9	0.005387864	-0.0702
DKK1_KREMEN1	3.16E-05	-0.0696
DKK1_LRP5	5.81E-05	-0.0696
DKK1_LRP6	0.000657573	-0.0696
DKK1_KREMEN2	0.000265158	-0.0696
ALOX5AP_ALOX5	5.27E-07	-0.0685
ITGB3BP_ITGB3	0.000302766	-0.0685
ITGB3BP_ITGB5	0.00273177	-0.0685
ADAM17_ITGB1	2.70E-09	-0.0682
ADAM17_NOTCH1	0.000533955	-0.0682
ADAM17_ERBB4	8.58E-07	-0.0682
ADAM17_ITGA5	1.18E-05	-0.0682
TCTN1_TMEM67	0.00273177	-0.0648
VCAM1_ITGA4	0.002835142	-0.0648
VCAM1_ITGA9	6.05E-05	-0.0648
VCAM1_ITGB1	1.01E-06	-0.0648
VCAM1_ITGB7	0.003342211	-0.0648
CXCL5_CXCR2	0.004253523	-0.0636
JAG1_NOTCH1	0.026398516	-0.0617
JAG1_NOTCH2	0.035542217	-0.0617
JAG1_NOTCH3	0.005990998	-0.0617
JAG1_NOTCH4	0.011673592	-0.0617
LAMB1_ITGB1	8.45E-09	-0.0614
LAMB1_ITGA1	0.002683578	-0.0614
LAMB1_ITGAV	0.000274766	-0.0614
LAMB1_ITGB4	3.73E-06	-0.0614
LAMB1_ITGA2	0.00036642	-0.0614
LAMB1_ITGA6	1.75E-05	-0.0614

LAMB1_ITGA7	1.12E-05	-0.0614
LAMB1_ITGA3	0.000621043	-0.0614
AGRN_ATP1A3	0.000545235	-0.0604
AGRN_LRP4	0.001762168	-0.0604
CTF1_LIFR	0.025556501	-0.0604
FBN1_ITGAV	0.003478951	-0.0578
FBN1_ITGB3	0.008218171	-0.0578
FBN1_ITGA5	0.001273388	-0.0578
FBN1_ITGB1	3.96E-05	-0.0578
PTHLH_PTH1R	0.007331051	-0.0573
PAPLN_SIRPA	0.007869866	-0.0545
BMP2_ACVR1	0.028125511	-0.0514
BMP2_ACVR2A	0.028532465	-0.0514
BMP2_ACVR2B	0.017982122	-0.0514
BMP2_BMPR1A	0.020315644	-0.0514
BMP2_BMPR2	0.034492955	-0.0514
RELN_ITGA3	0.045452514	-0.0492
RELN_ITGB1	0.02067702	-0.0492
RELN_LRP8	0.032410679	-0.0492
GRP_GRPR	1.30E-07	-0.0474
GRP_NMBR	0.003048787	-0.0474
NRG3_ERBB4	0.006043522	-0.0470
EFNA5_EPHA5	0.008374475	-0.0463
EFNA5_EPHA1	0.010656232	-0.0463
EFNA5_EPHA2	0.002417285	-0.0463
EFNA5_EPHA4	0.000728549	-0.0463
EFNA5_EPHB6	0.005265408	-0.0463
EFNA5_EPHB1	0.000603956	-0.0463
SEMA4G_PLXNB2	0.001689045	-0.0456
TGM2_SDC4	0.013696722	-0.0451
TGM2_ITGA4	0.037743564	-0.0451
TGM2_ITGB1	0.000432962	-0.0451
TGM2_ITGB3	0.040203932	-0.0451
TGM2_ITGA9	0.003846797	-0.0451
TGM2_TBXA2R	0.000545235	-0.0451
POMC_MC1R	0.000847553	-0.0445
POMC_MC2R	1.37E-08	-0.0445
NUCB2_ERAP1	0.007942135	-0.0442
EGF_ERBB4	0.026471289	-0.0437
LAMB2_RPSA	0.008077429	-0.0417
CCL19_CCR10	0.000191127	-0.0414
CCL19_ACKR4	0.000432962	-0.0414
FGF3_FGFR1	0.038887109	-0.0410

FGF3_FGFR2	0.028455484	-0.0410
FGF3_FGFR3	0.031743236	-0.0410
CLCF1_CNTFR	0.006857398	-0.0389
CLCF1_LIFR	0.000634767	-0.0389
CLCF1_CRLF1	0.000703961	-0.0389
CLCF1_IL6ST	0.00289888	-0.0389
HMGB1_SDC1	0.001798206	-0.0387
AGRP_SDC3	0.01845975	-0.0375
MIA_CDH19	3.03E-07	-0.0363
SCT_SCTR	0.00137138	-0.0354
SCT_VIPR1	0.008453793	-0.0354
IL34_CSF1R	0.029077556	-0.0309
PSPN_SDC3	0.037705474	-0.0250
SLIT2_DCC	0.034670327	-0.0248
PTMA_VIPR1	0.023118577	-0.0244
TCN2_CNR1	0.047914761	-0.0226
CXCL2_CXCR2	0.04565382	-0.0172
CXCL2_XCR1	3.16E-05	-0.0172
NLGN2_NRXN2	0.034348387	-0.0071
NLGN2_NRXN3	0.02928371	-0.0071
PYY_NPY2R	0.021912718	-0.0051
APOB_OLR1	0.021912718	0.0042
F2_GP1BB	0.030655388	0.0108
CNTF_LIFR	0.036913126	0.0134
LTF_GPR162	0.005396298	0.0146
CSF3_CSF1R	0.017539404	0.0157
PMCH_MCHR1	0.022466561	0.0164
FGF22_FGFR2	0.042760264	0.0172
DSCAM_DCC	0.017830135	0.0174
EFNA4_EPHA8	0.017889341	0.0233
ANGPTL2_TIE1	0.034670327	0.0303
COL1A1_ITGB1	0.003409954	0.0304
COL1A1_TMPRSS6	0.000545235	0.0304
COL1A1_CD44	0.030375978	0.0304
COL1A1_CD93	0.0313195	0.0304
COL1A1_DDR1	0.007942135	0.0304
COL1A1_FLT4	0.008053069	0.0304
COL1A1_ITGA5	0.040203932	0.0304
HSP90B1_TLR4	0.002683578	0.0340
HSP90B1_ERBB2	0.000797109	0.0340
HSP90B1_TLR2	0.000932576	0.0340
HSP90B1_TLR1	1.76E-07	0.0340
HSP90B1_TLR7	9.59E-10	0.0340

GPHA2_TSHR	0.035808035	0.0350
GNB3_GABBR2	0.004631178	0.0355
NAMPT_INSR	0.007659203	0.0362
OMG_TNFRSF1B	0.015164142	0.0365
OMG_LINGO1	0.023958784	0.0365
OMG_RTN4R	0.000121732	0.0365
OMG_RTN4RL1	0.000629356	0.0365
CALR_ITGA3	0.005173587	0.0373
CALR_ITGA2B	0.00752418	0.0373
CALR_LRP1	0.0459611	0.0373
CALR_ITGAV	0.013696722	0.0373
CALR_TSHR	3.74E-05	0.0373
CALR_SCARF1	1.12E-13	0.0373
COL5A2_DDR1	0.001091175	0.0379
COL5A2_ITGB1	0.000510842	0.0379
COL4A4_CD47	0.001240311	0.0384
COL4A4_ITGA2	0.021133481	0.0384
COL4A4_ITGAV	0.022945217	0.0384
COL4A4_CD93	0.001604166	0.0384
COL4A4_ITGB1	5.48E-05	0.0384
NGF_SORT1	0.005265408	0.0423
NGF_NTRK1	0.000545235	0.0423
NGF_KIDINS220	0.005265408	0.0423
NGF_MAGED1	0.027706843	0.0423
AMELX_LAMP1	0.000780548	0.0434
TIMP2_ITGA3	0.004783328	0.0435
TIMP2_ITGB1	4.77E-08	0.0435
BTC_EGFR	0.00146763	0.0468
BTC_ERBB2	0.006554271	0.0468
BTC_ERBB3	0.030243071	0.0468
BTC_ERBB4	0.001917885	0.0468
FGF9_FGFR1	0.02099199	0.0478
FGF9_FGFR2	0.000846151	0.0478
FGF9_FGFR3	0.02099199	0.0478
VEGFC_ITGB1	0.000210121	0.0504
VEGFC_FLT1	0.00013939	0.0504
VEGFC_FLT4	0.001038503	0.0504
VEGFC_ITGA9	0.001387107	0.0504
VEGFC_VIPR2	0.025144348	0.0504
VEGFC_KDR	0.000202621	0.0504
CALM1_ABCA1	0.005887801	0.0572
CALM1_ADCY8	4.60E-18	0.0572
CALM1_ADCYAP1R1	7.63E-10	0.0572

CALM1_FAS	0.02099199	0.0572
CALM1_KCNN4	9.96E-07	0.0572
CALM1_KCNQ5	1.68E-06	0.0572
CALM1_MIP	1.98E-09	0.0572
CALM1_PDE1A	3.44E-10	0.0572
CALM1_PDE1B	2.82E-10	0.0572
CALM1_PDE1C	0.000160628	0.0572
CALM1_GRM3	4.66E-11	0.0572
CALM1_GRM7	3.89E-16	0.0572
CALM1_VIPR1	4.56E-08	0.0572
CALM1_CACNA1C	0.0032757	0.0572
CALM1_GLP2R	1.46E-16	0.0572
CALM1_HMMR	1.12E-05	0.0572
CALM1_INSR	5.98E-06	0.0572
CALM1_MYLK	0.00056623	0.0572
CALM1_PTPRA	4.18E-05	0.0572
CALM1_TRPC3	2.60E-17	0.0572
CALM1_EGFR	1.50E-06	0.0572
COL5A1_ITGB1	1.21E-06	0.0576
COL5A1_SDC3	0.000121676	0.0576
COL5A1_ITGA1	0.007659203	0.0576
IL18_IL18RAP	5.81E-10	0.0621
IL18_IL1RL2	0.022636045	0.0621
IL18_IL1RAPL1	2.62E-05	0.0621
PODXL_SELL	3.15E-12	0.0655
PPBP_CXCR2	0.000137038	0.0658
GNAI2_ADCY7	0.000268061	0.0696
GNAI2_CCR5	6.03E-13	0.0696
GNAI2_CNR1	6.43E-08	0.0696
GNAI2_ADCY1	1.33E-07	0.0696
GNAI2_ADCY8	2.12E-14	0.0696
GNAI2_ADCY9	3.22E-07	0.0696
GNAI2_ADORA1	3.51E-14	0.0696
GNAI2_CAV1	0.00273177	0.0696
GNAI2_CXCR2	2.21E-11	0.0696
GNAI2_F2R	9.48E-06	0.0696
GNAI2_LPAR3	0.000465182	0.0696
GNAI2_PTPRU	6.33E-06	0.0696
GNAI2_S1PR3	7.10E-05	0.0696
GNAI2_TSHR	3.51E-08	0.0696
GNAI2_EDNRA	0.000607034	0.0696
GNAI2_EDNRB	0.002683578	0.0696
GNAI2_ADRA2A	1.15E-05	0.0696

GNAI2_CHRM1	3.64E-08	0.0696
GNAI2_IGF1R	2.01E-09	0.0696
GNAI2_S1PR1	1.17E-07	0.0696
GNAI2_ADRA2B	0.000205251	0.0696
GNAI2_EGFR	1.06E-07	0.0696
C4A_C3AR1	0.000298328	0.0702
C4A_C5AR2	5.12E-07	0.0702
RSPO1_ZNRF3	0.005990998	0.0716
RSPO1_FZD8	0.005074488	0.0716
RSPO1_LGR5	0.00417061	0.0716
RSPO1_LGR6	0.000108146	0.0716
RSPO1_LRP6	0.003771157	0.0716
RSPO1_LGR4	0.007248319	0.0716
FGF5_FGFR1	0.000165617	0.0718
FGF5_FGFR2	6.34E-06	0.0718
VEGFB_NRP1	0.008523102	0.0724
VEGFB_RET	0.00124449	0.0724
VEGFB_FLT1	4.65E-08	0.0724
VEGFB_TYRO3	1.79E-05	0.0724
ADAM2_CD9	2.49E-07	0.0752
ADAM2_ITGA6	8.67E-07	0.0752
ADAM2_ITGA9	1.92E-07	0.0752
ADAM2_ITGB1	1.12E-10	0.0752
ADAM2_ITGB7	0.000144998	0.0752
UBA52_ERBB2	1.10E-06	0.0754
UBA52_NOTCH1	0.000256047	0.0754
UBA52_TGFBR1	2.30E-11	0.0754
UBA52_ACVR1	5.74E-07	0.0754
UBA52_EGFR	4.47E-08	0.0754
UBA52_TGFBR2	2.08E-09	0.0754
UBA52_BMPR1B	5.33E-06	0.0754
ANGPT4_TEK	0.008971992	0.0782
ANGPT4_TIE1	7.85E-05	0.0782
FARP2_PLXNA4	1.15E-05	0.0784
FARP2_PLXNA1	1.05E-08	0.0784
FARP2_PLXNA2	3.12E-06	0.0784
FARP2_PLXNA3	7.85E-05	0.0784
UCN2_IL10RB	8.27E-06	0.0794
TNFSF11_TNFRSF11B	0.00018527	0.0799
TNFSF11_TNFRSF11A	9.13E-05	0.0799
TGFB3_TGFBR1	1.63E-07	0.0801
TGFB3_TGFBR3	1.68E-07	0.0801
TGFB3_ACVRL1	6.83E-06	0.0801

TGFB3_ENG	4.52E-05	0.0801
TGFB3_TGFBR2	8.91E-07	0.0801
CGN_F11R	0.000261527	0.0814
CGN_TGFBR1	1.63E-07	0.0814
CGN_TGFBR2	8.13E-07	0.0814
IL11_IL6ST	2.28E-10	0.0850
COL4A1_CD93	1.97E-07	0.0853
COL4A1_ITGA1	8.06E-05	0.0853
COL4A1_ITGA2	6.90E-05	0.0853
COL4A1_ITGAV	3.73E-06	0.0853
COL4A1_ITGB8	3.89E-08	0.0853
COL4A1_CD47	9.08E-09	0.0853
COL4A1_ITGB1	5.69E-10	0.0853
SEMA4D_MET	2.18E-07	0.0863
SEMA4D_PLXNB1	1.60E-08	0.0863
SEMA4D_CD72	0.001339768	0.0863
SEMA4D_ERBB2	5.33E-06	0.0863
SEMA4D_PLXNB2	2.04E-07	0.0863
APP_NCSTN	4.56E-09	0.0864
APP_CD74	2.18E-05	0.0864
APP_LRP1	0.000499639	0.0864
APP_NGFR	0.014433404	0.0864
APP_GPC1	9.75E-06	0.0864
APP_SLC45A3	2.12E-14	0.0864
APP_TNFRSF21	0.000366666	0.0864
APP_CAV1	0.000911596	0.0864
SEMA7A_ITGA1	3.87E-05	0.0893
SEMA7A_PLXNC1	4.55E-05	0.0893
CSF1_CSF1R	1.99E-07	0.0898
SORBS1_INSR	0.000797109	0.0936
SORBS1_ITGA1	0.001762168	0.0936
NTN1_ADORA2B	0.001246057	0.0940
NTN1_DCC	1.67E-07	0.0940
NTN1_NEO1	7.99E-06	0.0940
NTN1_UNC5B	5.47E-06	0.0940
NTN1_UNC5A	0.000101626	0.0940
NTN1_UNC5C	1.48E-05	0.0940
CXCL12_CXCR4	0.000341882	0.0946
CXCL12_SDC4	0.000177494	0.0946
CXCL12_ITGB1	4.89E-05	0.0946
CXCL12_CD4	0.000198103	0.0946
CXCL12_ACKR3	0.011247952	0.0946
CLEC11A_KIT	0.044669587	0.0948

GSTP1_TRAF2	1.48E-05	0.0952
NRTN_GFRA1	0.027264602	0.0955
NRTN_RET	0.023525256	0.0955
WNT4_FZD2	5.56E-07	0.0956
WNT4_FZD6	1.04E-06	0.0956
CCL8_ACKR4	5.55E-09	0.0961
CCL8_CCR5	3.14E-10	0.0961
ADM2_CALCRL	7.90E-11	0.1013
ADM2_RAMP1	4.10E-06	0.1013
OSTN_NPR3	0.002066671	0.1025
PTGS2_CAV1	0.043995705	0.1076
PTGS2_ALOX5	0.007942135	0.1076
TAC1_TACR1	1.18E-11	0.1082
FGF12_FGFR2	7.65E-05	0.1085
FGF12_FGFR1	0.000334381	0.1085
FGF12_FGFR3	0.000256047	0.1085
LCN2_LRP2	0.014116255	0.1086
COL18A1_GPC1	0.001358747	0.1086
COL18A1_ITGA5	0.000510842	0.1086
COL18A1_ITGB1	7.66E-05	0.1086
COL18A1_KDR	4.82E-05	0.1086
COL18A1_GPC4	0.002001203	0.1086
LAMC2_CD151	7.36E-07	0.1101
LAMC2_COL17A1	6.47E-06	0.1101
LAMC2_ITGA3	2.56E-05	0.1101
LAMC2_ITGA6	2.74E-06	0.1101
LAMC2_ITGB1	9.40E-07	0.1101
LAMC2_ITGB4	2.03E-06	0.1101
LAMC2_ITGA2	0.000510888	0.1101
FN1_IL17RC	7.86E-09	0.1128
FN1_ITGA5	2.16E-09	0.1128
FN1_ITGA2B	3.42E-08	0.1128
FN1_ITGAV	1.86E-08	0.1128
FN1_ITGB1	7.24E-13	0.1128
FN1_ITGB3	5.38E-07	0.1128
FN1_ITGB8	9.98E-11	0.1128
FN1_TNFRSF11B	2.95E-05	0.1128
FN1_CD44	1.91E-10	0.1128
FN1_COL13A1	5.33E-06	0.1128
FN1_FLT4	4.04E-11	0.1128
FN1_ITGA2	6.68E-06	0.1128
FN1_ITGA4	5.89E-07	0.1128
FN1_ITGA6	6.92E-10	0.1128

FN1_ITGA8	0.000797109	0.1128
FN1_ITGB7	9.76E-07	0.1128
FN1_SDC2	8.18E-11	0.1128
FN1_TMPRSS6	2.63E-12	0.1128
FN1_TSHR	1.12E-10	0.1128
FN1_ITGA9	1.00E-10	0.1128
BGN_LY96	2.39E-11	0.1150
BGN_TLR2	1.09E-10	0.1150
BGN_TLR1	3.89E-16	0.1150
BGN_TLR4	7.19E-10	0.1150
TNFSF15_TNFRSF25	1.50E-06	0.1153
FGF2_CD44	7.50E-08	0.1169
FGF2_FGFR1	1.29E-06	0.1169
FGF2_FGFR2	7.04E-09	0.1169
FGF2_FGFR3	6.73E-07	0.1169
FGF2_FGFRL1	3.48E-05	0.1169
FGF2_GPC4	5.81E-06	0.1169
FGF2_NRP1	0.000220402	0.1169
FGF2_SDC1	8.41E-07	0.1169
FGF2_SDC3	4.19E-08	0.1169
FGF2_SDC4	2.33E-07	0.1169
NPNT_ITGB1	3.02E-07	0.1198
NPNT_ITGA8	0.003699537	0.1198
TGFA_ERBB2	8.82E-07	0.1229
TGFA_EGFR	3.97E-11	0.1229
TGFA_ERBB4	3.94E-10	0.1229
C4B_CD46	0.000213585	0.1231
GDNF_EDNRB	6.34E-06	0.1249
GDNF_GFRA1	1.40E-05	0.1249
GDNF_SLC44A5	2.57E-14	0.1249
GDNF_GFRA2	4.57E-08	0.1249
GDNF_RET	8.89E-07	0.1249
C3_C3AR1	6.43E-09	0.1254
C3_CD19	3.14E-10	0.1254
C3_CD46	1.39E-05	0.1254
C3_CD81	1.12E-09	0.1254
C3_IFITM1	0.002723837	0.1254
C3_ITGB2	0.000323554	0.1254
C3_LRP1	1.35E-05	0.1254
IL1RN_IL1RL2	1.35E-05	0.1274
IL1RN_IL1R1	5.51E-11	0.1274
IL1RN_IL1R2	1.89E-09	0.1274
L1CAM_EGFR	3.96E-08	0.1298

L1CAM_EPHB2	1.31E-05	0.1298
L1CAM_ERBB2	2.20E-06	0.1298
L1CAM_FGFR2	1.25E-09	0.1298
L1CAM_CNTN1	4.78E-06	0.1298
L1CAM_ERBB3	2.56E-05	0.1298
L1CAM_ITGA5	9.05E-08	0.1298
L1CAM_ITGAV	8.30E-07	0.1298
COL4A2_CD93	6.67E-11	0.1317
LTBP1_ITGB5	3.12E-05	0.1410
THBS2_ITGA6	1.07E-13	0.1459
THBS2_ITGB1	9.77E-17	0.1459
THBS2_ITGA4	7.51E-11	0.1459
THBS2_NOTCH3	3.66E-12	0.1459
THBS2_NOTCH4	7.87E-11	0.1459
ADAM9_ITGAV	1.80E-09	0.1527
ADAM9_ITGB1	5.05E-11	0.1527
ADAM9_ITGB5	3.79E-07	0.1527
ADAM9_ITGA6	3.43E-10	0.1527
SEMA3B_NRP1	2.12E-05	0.1564
SEMA3B_NRP2	7.66E-05	0.1564
EDN1_EDNRB	1.15E-07	0.1590
EDN1_EDNRA	3.32E-09	0.1590
PLAU_IGF2R	8.51E-11	0.1590
PLAU_ITGA3	2.82E-10	0.1590
PLAU_ITGB2	5.02E-06	0.1590
PLAU_ITGB5	6.08E-08	0.1590
PLAU_MRC2	7.60E-12	0.1590
PLAU_PLAUR	2.10E-06	0.1590
PLAU_ST14	5.28E-09	0.1590
PLAU_VLDLR	6.34E-09	0.1590
PLAU_ITGA5	5.85E-12	0.1590
PLAU_ITGAV	2.60E-11	0.1590
PLAU_LRP1	1.85E-07	0.1590
HRAS_CAV1	3.83E-06	0.1605
HRAS_GRIN2D	8.29E-18	0.1605
HRAS_INSR	1.57E-15	0.1605
HRAS_TLR2	9.66E-16	0.1605
HRAS_SDC2	6.54E-18	0.1605
LIF_IL6ST	6.37E-13	0.1716
LIF_LIFR	5.83E-13	0.1716
CALM3_ADCY8	2.63E-12	0.1719
CALM3_GRM7	7.54E-13	0.1719
CALM3_KCNQ1	9.55E-13	0.1719

CALM3_MYLK	2.24E-09	0.1719
CALM3_PDE1A	1.80E-12	0.1719
CALM3_PDE1B	1.44E-12	0.1719
CALM3_INSR	3.92E-13	0.1719
CALM3_KCNQ5	4.62E-12	0.1719
CALM3_PDE1C	1.33E-05	0.1719
CALM3_EGFR	8.90E-14	0.1719
ECM1_CACHD1	8.68E-18	0.1735
NTF3_NGFR	2.07E-05	0.1746
NTF3_NTRK2	1.12E-08	0.1746
NTF3_NTRK1	3.74E-15	0.1746
NTF3_NTRK3	6.73E-07	0.1746
ADAM15_ITGA5	4.96E-16	0.1746
ADAM15_ITGB1	6.54E-18	0.1746
ADAM15_ITGB3	1.28E-13	0.1746
ADAM15_ITGA9	9.30E-17	0.1746
ADAM15_ITGAV	3.51E-15	0.1746
THBS1_ITGA3	3.37E-12	0.1788
THBS1_TNFRSF11B	4.17E-08	0.1788
THBS1_ITGA2B	3.75E-13	0.1788
THBS1_ITGA4	2.94E-12	0.1788
THBS1_ITGA6	4.64E-14	0.1788
THBS1_ITGB1	3.51E-15	0.1788
THBS1_LRP5	1.88E-14	0.1788
THBS1_ITGB3	4.34E-12	0.1788
THBS1_LRP1	7.04E-09	0.1788
THBS1_SDC1	1.61E-13	0.1788
THBS1_SDC4	9.77E-14	0.1788
THBS1_SCARB1	4.12E-11	0.1788
EREG_ERBB2	3.51E-08	0.1819
EREG_ERBB4	4.79E-13	0.1819
EREG_EGFR	1.17E-12	0.1819
HSP90AA1_EGFR	1.07E-16	0.1832
HSP90AA1_CFTR	7.48E-10	0.1832
HSP90AA1_FGFR3	1.28E-15	0.1832
PDGFC_FLT1	3.21E-15	0.1904
PDGFC_KDR	3.21E-15	0.1904
PDGFC_PDGFRA	5.01E-06	0.1904
PDGFC_PDGFRB	9.03E-10	0.1904
PDGFC_FLT4	3.28E-15	0.1904
VIM_CD44	1.08E-17	0.1926
LGALS3BP_ITGB1	4.60E-17	0.1995
LGALS3BP_VANGL1	3.37E-14	0.1995

PCSK9_VLDLR	1.09E-08	0.2003
SEMA4C_PLXNB2	1.39E-09	0.2016
HSPG2_CHRM3	6.33E-18	0.2030
HSPG2_COL13A1	8.95E-13	0.2030
HSPG2_ITGA2	1.90E-10	0.2030
HSPG2_ITGB1	9.56E-18	0.2030
HSPG2_SDC1	1.82E-15	0.2030
PDAP1_PDGFRB	5.44E-10	0.2071
PKM_CD44	1.32E-17	0.2104
HBEGF_CD82	5.94E-11	0.2104
HBEGF_ERBB2	2.40E-10	0.2104
HBEGF_PRLR	4.17E-12	0.2104
HBEGF_CD44	8.37E-16	0.2104
HBEGF_CD9	5.19E-16	0.2104
HBEGF_EGFR	7.67E-16	0.2104
HBEGF_ERBB4	3.02E-15	0.2104
BMP6_ACVR2B	3.20E-13	0.2152
BMP6_ACVR2A	3.29E-12	0.2152
BMP6_BMPR1A	4.36E-13	0.2152
BMP6_BMPR1B	4.19E-11	0.2152
BMP6_BMPR2	3.72E-12	0.2152
BMP6_ACVR1	2.90E-12	0.2152
FBLN2_ITGB3	3.74E-16	0.2254
LAMA5_BCAM	2.58E-13	0.2282
LAMA5_ITGA2	7.35E-11	0.2282
LAMA5_ITGA3	1.37E-14	0.2282
LAMA5_ITGA6	6.94E-17	0.2282
LAMA5_ITGB1	4.60E-18	0.2282
LAMA5_ITGB4	2.54E-17	0.2282
LAMA5_SDC1	9.82E-16	0.2282
PDGFA_PDGFRA	2.17E-07	0.2346
PDGFA_PDGFRB	1.32E-12	0.2346
APOD_LEPR	3.20E-05	0.2440
COL11A1_ITGB1	1.61E-13	0.2465
COL11A1_DDR1	2.75E-13	0.2465
COL11A1_ITGA2	1.66E-09	0.2465
CCL5_ACKR4	1.28E-13	0.2482
CCL5_CCR5	1.60E-14	0.2482
CCL5_GPR75	5.70E-13	0.2482
CCL5_SDC1	3.66E-12	0.2482
CCL5_SDC4	6.93E-13	0.2482
BDNF_DDR1	2.21E-16	0.2531
BDNF_NTRK2	4.81E-14	0.2531

BDNF_DRD4	1.22E-16	0.2531
BDNF_NGFR	3.14E-09	0.2531
BDNF_SORT1	2.21E-16	0.2531
B2M_HFE	3.51E-14	0.2641
B2M_KLRD1	4.60E-18	0.2641
B2M_TFRC	4.77E-18	0.2641
B2M_CD247	4.99E-18	0.2641
B2M_CD3G	4.99E-18	0.2641
WNT16_FZD6	5.88E-18	0.2670
TGFB1_ACVRL1	5.14E-18	0.2860
TGFB1_CAV1	1.38E-10	0.2860
TGFB1_ENG	6.54E-18	0.2860
TGFB1_ITGAV	6.54E-18	0.2860
TGFB1_ITGB8	4.60E-18	0.2860
TGFB1_TGFBR1	4.60E-18	0.2860
TGFB1_TGFBR2	4.60E-18	0.2860
TGFB1_CXCR4	8.29E-18	0.2860
TGFB1_CD109	8.60E-13	0.2860
TGFB1_SDC2	4.60E-18	0.2860
TGFB1_TGFBR3	4.60E-18	0.2860
COL7A1_ITGA2	1.56E-12	0.3077
COL7A1_ITGB1	8.37E-16	0.3077
PSAP_CELSR1	6.85E-17	0.3116
PSAP_LRP1	6.57E-15	0.3116
PSAP_GPR37	1.06E-14	0.3116
WNT5A_MCAM	4.60E-18	0.3195
WNT5A_FZD2	4.60E-18	0.3195
WNT5A_FZD6	4.60E-18	0.3195
WNT5A_FZD7	4.60E-18	0.3195
WNT5A_FZD8	6.38E-18	0.3195
WNT5A_ROR1	5.96E-18	0.3195
WNT5A_FZD4	3.57E-16	0.3195
WNT5A_FZD5	1.45E-17	0.3195
WNT5A_LRP5	4.60E-18	0.3195
WNT5A_ROR2	4.60E-18	0.3195
WNT5A_RYK	4.60E-18	0.3195
WNT5A_FZD1	8.29E-18	0.3195
GREM1_KDR	5.96E-18	0.3214
INHBB_ACVR2A	2.24E-16	0.3214
INHBB_ACVR2B	3.31E-17	0.3214
INHBB_ACVR1	5.26E-16	0.3214
INHBB_ACVR1B	4.82E-16	0.3214
EDIL3_ITGB5	4.00E-14	0.3276

EDIL3_ITGAV	2.10E-15	0.3276
ADM_RAMP2	2.52E-14	0.3426
ADM_CALCRL	3.60E-17	0.3426
ADM_GPR182	2.14E-17	0.3426
FST_BMPR1B	9.66E-16	0.3570
FST_BMPR2	2.43E-16	0.3570
CCL2_ACKR4	4.74E-16	0.3591
CCL2_CCR10	3.40E-16	0.3591
CCL2_CCR5	1.92E-16	0.3591
APOE_LDLR	5.86E-17	0.3646
APOE_LRP1	5.70E-15	0.3646
APOE_LRP5	1.04E-17	0.3646
APOE_LRP8	4.39E-17	0.3646
APOE_VLDLR	1.11E-15	0.3646
APOE_CHRNA4	2.51E-17	0.3646
APOE_LRP2	8.95E-13	0.3646
APOE_SCARB1	3.13E-16	0.3646
APOE_SORL1	9.96E-18	0.3646
TIMP1_CD63	4.60E-18	0.3675
ADAM23_ITGB3	4.60E-18	0.3690
ADAM23_ITGA5	4.60E-18	0.3690
RTN4_LINGO1	4.60E-18	0.3973
RTN4_GJB2	2.21E-16	0.3973
RTN4_CNTNAP1	4.60E-18	0.3973
RTN4_NGFR	1.28E-14	0.3973
RTN4_RTN4R	4.60E-18	0.3973
RTN4_RTN4RL1	4.60E-18	0.3973
RTN4_TNFRSF19	5.47E-18	0.3973
NCAM1_CACNA1C	4.60E-18	0.4134
NCAM1_FGFR1	4.60E-18	0.4134
NCAM1_FGFR2	4.60E-18	0.4134
NCAM1_GFRA1	1.08E-17	0.4134
NCAM1_ROBO1	4.60E-18	0.4134
NCAM1_ROBO3	4.60E-18	0.4134
NCAM1_PTPRA	4.60E-18	0.4134
NTN4_DCC	4.60E-18	0.4307
NTN4_UNC5A	1.87E-17	0.4307
TNC_CNTN1	1.52E-17	0.4463
TNC_EGFR	4.85E-18	0.4463
TNC_ITGA8	1.06E-15	0.4463
TNC_ITGB1	4.60E-18	0.4463
TNC_ITGA2	1.56E-16	0.4463
TNC_ITGA9	4.85E-18	0.4463

TNC_ITGAV	6.10E-18	0.4463
TNC_NT5E	9.96E-18	0.4463
TNC_SDC1	5.47E-18	0.4463
TNC_SDC4	5.26E-18	0.4463
TNC_ITGA5	5.47E-18	0.4463
TNC_ITGA7	1.32E-17	0.4463
TNC_PTPRB	8.68E-18	0.4463
TNC_PTPRZ1	6.88E-18	0.4463
INHBA_ACVR2A	4.60E-18	0.4598
INHBA_ACVR1	4.60E-18	0.4598
INHBA_ACVR1B	4.60E-18	0.4598
INHBA_TGFBR3	4.60E-18	0.4598
INHBA_BAMBI	4.60E-18	0.4598
SEMA3E_PLXND1	1.05E-14	0.4655
SERPINE1_LRP2	3.30E-15	0.5338
SERPINE1_ITGAV	1.06E-16	0.5338
SERPINE1_LRP1	8.31E-16	0.5338
SERPINE1_PLAUR	1.75E-15	0.5338
PRSS23_TMEM222	4.60E-18	0.5571
ANXA1_EGFR	4.60E-18	0.5616
ANXA1_DYSF	4.60E-18	0.5616
CCL7_ACKR4	4.85E-18	0.6130
CCL7_CCR10	4.60E-18	0.6130
CCL7_CCR5	4.60E-18	0.6130
GAS6_MERTK	4.85E-18	0.6301
GAS6_TYRO3	4.60E-18	0.6301
GAS6_AXL	4.60E-18	0.6301
SPP1_ITGA9	4.60E-18	0.9131
SPP1_S1PR1	4.60E-18	0.9131
SPP1_CD44	4.60E-18	0.9131
SPP1_ITGA5	4.60E-18	0.9131
SPP1_ITGAV	4.60E-18	0.9131
SPP1_ITGB1	4.60E-18	0.9131
SPP1_ITGA4	4.60E-18	0.9131

Table 8.1. Full list of Ligand-Receptor pairs significantly upregulated in FTM-TEC and MEF-TEC. Ligand-receptor pairs for FTM-TEC and the corresponding MEF-TEC pairs were compared and a Wilcoxon test was used to determine pairs that were significantly different between the two with a False Discovery Rate (FDR) < 0.1. Negative value of log2_FoldChange indicates ligand-receptor pair scored higher in FTM than MEFs. 445 ligand-receptor pairs were upregulated in FTM and 528 in MEFs.

8.2. Bioinformatic analysis for FTM-TEC and MEF-TEC Ligand-Receptor analysis: code

```
#### Change Lb path to local ####
.libPaths("C:/Program Files/R/R-4.0.2/library")

#### List of packages to install #############
BiocManager::install('Rsubread')
BiocManager::install('DESeq2')
BiocManager::install('vsn')
BiocManager::install('dplyr')
BiocManager::install('pheatmap')
BiocManager::install('RColorBrewer')
BiocManager::install('PoiClaClu')
BiocManager::install('biomaRt')
BiocManager::install('sva')
BiocManager::install('ReportingTools')
BiocManager::install('genefilter')
BiocManager::install('PCAtools')
install.packages('homologene')
install.packages('data.table')


#### List of Libraries to load ############
library("Rsubread")
library("DESeq2")
library("vsn")
library("dplyr")
library("ggplot2")
library("pheatmap")
library("RColorBrewer")
library("PoiClaClu")
library("biomaRt")
library("sva")
library("ReportingTools")
library("genefilter")
library("PCAtools")
library("data.table")
library("homologene")

#### Load in and Count FTM_MEF_RNA_seq ####
# Load in Samples
setwd("D:/Virtual_Shared_Folder/2019_12_17_Thymic_Mesenchyme_vs_WT_MEFs_FASTQ")
FTM_MEF_Sample_Table <-  read.table("Sample_TableBAM.txt", header = TRUE, sep ="\t")

# Change the Biological Replicates to a factor and level it so MEFs are bottum
FTM_MEF_Sample_Table$Biological_Replicate <-
as.factor(FTM_MEF_Sample_Table$Biological_Replicate)
FTM_MEF_Sample_Table$Technical_Replicate <-
as.factor(FTM_MEF_Sample_Table$Technical_Replicate)
FTM_MEF_Sample_Table$Sample <- factor(FTM_MEF_Sample_Table$Sample, c("MEFs", "FTM"))

# Change the names of the samples to make more sense
FTM_MEF_Sample_Table$Name <-
c("FTM1","FTM2","FTM3","MEF1","MEF2","MEF3","FTM4","FTM5","FTM6","MEF4","MEF5","MEF6","F
TM7","FTM8","FTM9","MEF7","MEF8","MEF9")

# Create a list of files and sample names
FTM_MEF_File_Names <- as.vector(FTM_MEF_Sample_Table$File_Name)
FTM_MEF_Sample_Names <- as.vector(FTM_MEF_Sample_Table$Name)

# Create a shortcut to find the BAM directory
```

```
BAM_dir <-
"D:/Virtual_Shared_Folder/2019_12_17_Thymic_Mesenchyme_vs_WT_MEFs_FASTQ/Aligned_Reads
/"

# Now create a vector with file path + file names
FTM_MEF_File_Directory <- paste0(BAM_dir,FTM_MEF_File_Names)
file.exists(FTM_MEF_File_Directory)

### Count the Features
# Use FeaturesCount of the Rsubread package to count the features
# Load in the reference Genome
gtffile <- paste0(BAM_dir,"Mus_musculus.GRCm38.99.gtf")
file.exists(gtffile)
#FTM_MEF_fc <- featureCounts(files=FTM_MEF_File_Directory,
#                 annot.ext=gtffile,
#                 isGTFAnnotationFile=TRUE,
#                 isPairedEnd=FALSE)

# Need to change the colnames of of fc to the sample IDs
# colnames(FTM_MEF_fc$counts) <- FTM_MEF_Sample_Names

# Save the fc at this point
#
setwd("D:/Virtual_Shared_Folder/2020_08_27_Ligand_Receptor_Analysis_TEC_FTM_MEF/RDS_File
s")
# saveRDS(FTM_MEF_fc, file = "Feature_Counts_MEF_FTM.rds")

# read in RDS file

setwd("D:/Virtual_Shared_Folder/2020_08_27_Ligand_Receptor_Analysis_TEC_FTM_MEF/RDS_File
s")
 FTM_MEF_fc <- readRDS("Feature_Counts_MEF_FTM.rds")

#### Load in and Count Franks TEC RNA-Seq ####
# Load in Samples
setwd("D:/Virtual_Shared_Folder/2020_06_16_Franks_Data/Aligned_Reads")
TEC_Sample_Table <-  read.csv("SampleTable.csv", header = TRUE)
colnames(TEC_Sample_Table) <- c("Sample", "Batch","Biological_Replicate", "File_Name")
TEC_Sample_Table$Name <- c("TEC_E12.5_1", "TEC_E12.5_2", "TEC_E12.5_3",
"TEC_E14.5_Pos_1", "TEC_E14.5_Pos_2", "TEC_E14.5_Pos_3", "TEC_E14.5_Neg_1",
"TEC_E14.5_Neg_2", "TEC_E14.5_Neg_3")
TEC_Sample_Table$Technical_Replicate <- rep("a", times = 9)
TEC_Sample_Table <-
TEC_Sample_Table[,c("Name","Sample","Biological_Replicate","Technical_Replicate", "File_Name")]

# Change the Biological Replicates to a factor
TEC_Sample_Table$Biological_Replicate <- as.factor(TEC_Sample_Table$Biological_Replicate)
TEC_Sample_Table$Sample <- as.factor(TEC_Sample_Table$Sample)

# Create a list of files and sample names
TEC_File_Names <- as.vector(TEC_Sample_Table$File_Name)
TEC_Sample_Names <- (TEC_Sample_Table$Names)

# Create a shortcut to find the BAM directory
BAM_dir <- "D:/Virtual_Shared_Folder/2020_06_16_Franks_Data/Aligned_Reads/"

# Now create a vector with file path + file names
Filenames <- paste0(BAM_dir,TEC_File_Names)
file.exists(Filenames)

### Count the Features
#Use FeaturesCount of the Rsubread package to count the features
#Load in the reference Genome
gtffile <- paste0(BAM_dir,"Mus_musculus.GRCm38.99.gtf")
```

```
file.exists(gtffile)

# Count the features
#TEC_fc <- featureCounts(files=Filenames,
#                        annot.ext=gtffile,
#                        isGTFAnnotationFile=TRUE,
#                        isPairedEnd=TRUE)

# Need to change the colnames of of fc to the sample IDs
#colnames(TEC_fc$counts) <- TEC_Sample_Table$Name

# Save the fc at this point
#setwd("D:/Virtual_Shared_Folder/2020_08_27_Ligand_Receptor_Analysis_TEC_FTM_MEF/RDS_Fil
es")
#saveRDS(TEC_fc , file = "Feature_Counts_Frank_Data.rds")

# read in RDS file
setwd("D:/Virtual_Shared_Folder/2020_08_27_Ligand_Receptor_Analysis_TEC_FTM_MEF/RDS_File
s")
TEC_fc <- readRDS("Feature_Counts_Frank_Data.rds")

# Remove all now obsolete objects
rm(list=setdiff(ls(), c("FTM_MEF_fc", "TEC_fc", "FTM_MEF_Sample_Table", "TEC_Sample_Table")))

#### Create a Merged FCs ####
ldt <- cbind(rownames(FTM_MEF_fc[["counts"]]), FTM_MEF_fc[["counts"]])
ldt <- data.table::data.table(ldt)
rdt <- cbind(rownames(TEC_fc[["counts"]]), TEC_fc[["counts"]])
rdt <- data.table::data.table(rdt)
Merged_Counts <- merge(ldt, rdt, by = "V1")
row.names <- Merged_Counts$V1
Merged_Counts <- Merged_Counts[,c(2:28)]
Merged_Counts <- as.matrix(Merged_Counts)
rownames(Merged_Counts) <- row.names
mode(Merged_Counts) = "numeric"

Merged_Sample_Table <- rbind(TEC_Sample_Table, FTM_MEF_Sample_Table)
Merged_Sample_Table$Sample <-as.factor(Merged_Sample_Table$Sample)
Merged_Sample_Table$Biological_Replicate <-as.factor(Merged_Sample_Table$Biological_Replicate)
Merged_Sample_Table$Technical_Replicate <-as.factor(Merged_Sample_Table$Technical_Replicate)

Merged_Counts <- Merged_Counts[,Merged_Sample_Table$Name]



rm(list=setdiff(ls(), c("Merged_Counts", "Merged_Sample_Table")))

# Then create the information required to create the input for DeSeq2 , called dds
dds <- DESeqDataSetFromMatrix(countData = Merged_Counts,
                              colData = Merged_Sample_Table,
                              design = ~ Sample)

dds <- DESeq(dds)

#### Annotation ####
ensembl <- useEnsembl(biomart = "genes",
                      dataset = "mmusculus_gene_ensembl")


ensembl<- useDataset(dataset = "mmusculus_gene_ensembl", mart = ensembl)

annotLookup <- getBM(
  mart = ensembl,
  attributes=c("ensembl_gene_id", "external_gene_name"),
```

```
  filter="ensembl_gene_id",
  values = rownames(dds),
  uniqueRows=TRUE)

# Merge together the looked up gene names with the Counts from the DDS
ensembl_gene_id <- row.names(dds)
ldt <- cbind(ensembl_gene_id, counts(dds))
ldt <- data.table::data.table(ldt)
rdt <- annotLookup
rdt <- data.table::data.table(rdt)
annotated_dds_Counts <- merge(ldt, rdt, by = "ensembl_gene_id")
annotated_dds_Counts <- as.matrix(annotated_dds_Counts)
# Clean up the annotated_dds_Counts by makeing the row names the external gene names
row.names(annotated_dds_Counts) <- make.names(annotated_dds_Counts[,"external_gene_name"],
unique = TRUE)
# Clean up the annotated_dds_Counts by removing the unnessecary columns
annotated_dds_Counts <- annotated_dds_Counts[,
c("TEC_E12.5_1","TEC_E12.5_2","TEC_E12.5_3","TEC_E14.5_Pos_1","TEC_E14.5_Pos_2","TEC_E
14.5_Pos_3","TEC_E14.5_Neg_1","TEC_E14.5_Neg_2","TEC_E14.5_Neg_3","FTM1","FTM2","FTM3"
,"MEF1","MEF2","MEF3","FTM4","FTM5","FTM6","MEF4","MEF5","MEF6","FTM7","FTM8","FTM9","ME
F7","MEF8","MEF9")]
mode(annotated_dds_Counts) = "numeric"

# Remove all now obsolete objects
rm(list=setdiff(ls(), c("annotated_dds_Counts", "Merged_Sample_Table", "dds", "annotLookup")))

#### Normalise Data using VSD ####
# Create Normalised Counts
vsd <- vst(dds, blind = FALSE)
# Annotate the Normalised Counts
ensembl_gene_id <- row.names(vsd@assays@data@listData[[1]])
ldt <- cbind(ensembl_gene_id, vsd@assays@data@listData[[1]])
ldt <- data.table::data.table(ldt)
rdt <- annotLookup
rdt <- data.table::data.table(rdt)
Counts <- merge(ldt, rdt, by = "ensembl_gene_id")
Counts <- as.matrix(Counts)
# Clean up the annotated_dds_Counts by making the row names the external gene names
row.names(Counts) <- make.names(Counts[,"external_gene_name"], unique = TRUE)
# Clean up the annotated_dds_Counts by removing the unnessecary columns
Counts <- Counts[,
c("TEC_E12.5_1","TEC_E12.5_2","TEC_E12.5_3","TEC_E14.5_Pos_1","TEC_E14.5_Pos_2","TEC_E
14.5_Pos_3","TEC_E14.5_Neg_1","TEC_E14.5_Neg_2","TEC_E14.5_Neg_3","FTM1","FTM2","FTM3"
,"MEF1","MEF2","MEF3","FTM4","FTM5","FTM6","MEF4","MEF5","MEF6","FTM7","FTM8","FTM9","ME
F7","MEF8","MEF9")]
mode(Counts) = "numeric"
# Now replace the unanotated VSD Count Matrix with this new anotated one
vsd@assays@data@listData[[1]] <- Counts

rm(list=setdiff(ls(), c("annotated_dds_Counts", "Merged_Sample_Table", "dds", "annotLookup", "vsd")))

### Create Heatmaps to show sample Clustering ###
#Take calculated sample distances using vsd
sampleDists <- dist(t(vsd@assays@data@listData[[1]]))
#Set Colours
col.pal <- rev(brewer.pal(9,"Spectral"))
# Put into a heat map
#setwd("D:/Virtual_Shared_Folder/2020_08_27_Ligand_Receptor_Analysis_TEC_FTM_MEF/Graphs")
#png("HeatMap_of_Sample_Distributions.png", units="mm", width= 145, height=130, res=600)
sampleDistMatrix <- as.matrix(sampleDists)
rownames(sampleDistMatrix) <- paste(vsd$Sample, vsd$Biological_Replicate,
vsd$Technical_Replicate, sep = " - ")
colnames(sampleDistMatrix) <- NULL
pheatmap(sampleDistMatrix,
```

```
        clustering_distance_rows = sampleDists,
        clustering_distance_cols = sampleDists,
        col = col.pal,
        main = "HeatMap Showing Sample Distances")
#dev.off()

#Create Heatmap to show sample Clustering without E12.5 TEC
#Take calculated sample distances using vsd
sampleDists <-
dist(t(vsd@assays@data@listData[[1]][,c("TEC_E14.5_Pos_1","TEC_E14.5_Pos_2","TEC_E14.5_Pos
_3","TEC_E14.5_Neg_1","TEC_E14.5_Neg_2","TEC_E14.5_Neg_3","FTM1","FTM2","FTM3","MEF1","
MEF2","MEF3","FTM4","FTM5","FTM6","MEF4","MEF5","MEF6","FTM7","FTM8","FTM9","MEF7","MEF
8","MEF9")]))
#Set Colours
col.pal <- rev(brewer.pal(9,"Spectral"))
#Put into a heat map
#setwd("D:/Virtual_Shared_Folder/2020_08_27_Ligand_Receptor_Analysis_TEC_FTM_MEF/Graphs")
#png("HeatMap_of_Sample_Distributions_without_E12.5_TEC.png", units="mm", width= 145,
height=130, res=600)
sampleDistMatrix <- as.matrix(sampleDists)
rownames(sampleDistMatrix) <- paste(vsd$Sample, vsd$Biological_Replicate,
vsd$Technical_Replicate, sep = " - ")
colnames(sampleDistMatrix) <- NULL
pheatmap(sampleDistMatrix,
        clustering_distance_rows = sampleDists,
        clustering_distance_cols = sampleDists,
        col = col.pal,
        main = "HeatMap Showing Sample Distances")

#dev.off()
rm(list=setdiff(ls(), c("annotated_dds_Counts", "Merged_Sample_Table", "dds", "annotLookup", "vsd")))

#### Principal Component Analysis All TEC ####
# Create the Matrix from which the PCA will be calculated
vsd_Matrix <- as.matrix(vsd@assays@data@listData[[1]])

rownames(Merged_Sample_Table) <- Merged_Sample_Table$Name
metadata <- cbind(Merged_Sample_Table[,c(1:4)], vsd_Matrix["Dll4",], vsd_Matrix["Dll1",],
vsd_Matrix["Pdgfra",], vsd_Matrix["Cxcl12",], vsd_Matrix["Ccl7",],
vsd_Matrix["Foxn1",],vsd_Matrix["Col1a1",], vsd_Matrix["Flt1",], vsd_Matrix["Twist2",],
vsd_Matrix["Wnt1",])
colnames(metadata) <-c("Name", "Sample", "Biological Replicate","Technical_Replicate", "Dll4
Expression", "Dll1 Expression", "Pdgfra Expression","Cxcl12 Expression","CCl7 Expression", "Foxn1
Expression", "Col1a1 Expression", "Flt1 Expression", "Twist2 Expression", "Wnt1 Expression")

p <- pca(vsd_Matrix, metadata = metadata , removeVar = 0.667) # use top 100,000 variable genes

# Screeplot to show much much varience each PC contributes to the data.
setwd("D:/Virtual_Shared_Folder/2020_08_27_Ligand_Receptor_Analysis_TEC_FTM_MEF/Graphs/All
TEC")
screeplot(p,
        gridlines.major = TRUE,
        gridlines.minor = FALSE,
        axisLabSize = 10,
        titleLabSize = 12,
        subtitleLabSize = 10,
        title = "PCA of FTM, MEF and TEC",
        subtitle = 'SceePlot of Principal Components in MEF, FTM and TEC DeSeq2',) +
  ggsave(file=paste0("Scree Plot FTM,  MEFs and TEC.png"), units="mm", width= 145, height=90,
dpi=600)

# Create Bi-plot showing the Sample and Biological replicate spread
setwd("D:/Virtual_Shared_Folder/2020_08_27_Ligand_Receptor_Analysis_TEC_FTM_MEF/Graphs/All
TEC")
```

```
biplot(pcaobj = p,
       lab = NULL,
       colby = 'Biological Replicate',
       shape = 'Sample',
       gridlines.major = TRUE,
       gridlines.minor = FALSE,
       drawConnectors = FALSE,
       pointSize = 2,
       legendLabSize = 8,
       axisLabSize = 10,
       titleLabSize = 12,
       subtitleLabSize = 10,
       labSize = 2,
       legendPosition = "right",
       legendIconSize = 2,
       title = "PCA of FTM, MEF and TEC",
       subtitle = 'Plot Highlighting Effect of Sample and Biological Replicate',) +
  ggsave(file=paste0("PCA_of_Sample_Distributions.png"), units="mm", width= 145, height=180,
dpi=600)


# Quick For Loop to print all graphs to R-Studio
setwd("D:/Virtual_Shared_Folder/2020_08_27_Ligand_Receptor_Analysis_TEC_FTM_MEF/Graphs/All
TEC")
Genes_of_Interest <- colnames(metadata)[5:length(colnames(metadata))]
for(i in 1:length(Genes_of_Interest)){
  print(
  biplot(pcaobj = p,
  lab = NULL,
  colby = Genes_of_Interest[i],
  shape = 'Sample',
  gridlines.major = TRUE,
  gridlines.minor = FALSE,
  drawConnectors = FALSE,
  pointSize = 2,
  legendLabSize = 10,
  axisLabSize = 10,
  titleLabSize = 12,
  subtitleLabSize = 10,
  labSize = 2,
  legendPosition = "none",
  legendIconSize = 2,
  title = "PCA of FTM, MEF and TEC",
  subtitle = paste0("Plot Highlighting ", Genes_of_Interest[i])) +
  scale_colour_gradient(low = 'gold', high = 'red2') +
  ggsave(file=paste0("Bi-plot showing Expression of", Genes_of_Interest[i],".png")), units="mm", width=
145, height=180, dpi=600)
  }


rm(list=setdiff(ls(), c("annotated_dds_Counts", "Merged_Sample_Table", "dds", "annotLookup", "vsd")))

#### Principal Component Analysis No E12.5 TEC ####
# Create the Matrix from which the PCA will be calculated and remove the E12.5 TEC
vsd_Matrix_No_E12.5 <- as.matrix(vsd@assays@data@listData[[1]])[,c("TEC_E14.5_Pos_1",
"TEC_E14.5_Pos_2", "TEC_E14.5_Pos_3",
                                                    "TEC_E14.5_Neg_1", "TEC_E14.5_Neg_2", "TEC_E14.5_Neg_3",
"FTM1", "FTM2", "FTM3", "MEF1", "MEF2", "MEF3",
                                                    "FTM4", "FTM5", "FTM6", "MEF4","MEF5", "MEF6", "FTM7",
"FTM8", "FTM9", "MEF7", "MEF8", "MEF9")]


# Remove the E12.5 TEc from the Merged_Sample_Table
rownames(Merged_Sample_Table) <- Merged_Sample_Table$Name
```

```
Merged_Sample_Table_No_E12.5 <- Merged_Sample_Table[c("TEC_E14.5_Pos_1",
"TEC_E14.5_Pos_2", "TEC_E14.5_Pos_3",
                                "TEC_E14.5_Neg_1", "TEC_E14.5_Neg_2", "TEC_E14.5_Neg_3",
"FTM1", "FTM2", "FTM3", "MEF1", "MEF2", "MEF3",
                                "FTM4", "FTM5", "FTM6", "MEF4","MEF5", "MEF6", "FTM7", "FTM8",
"FTM9", "MEF7", "MEF8", "MEF9"),]


metadata <- cbind(Merged_Sample_Table_No_E12.5[,c(1:4)], vsd_Matrix_No_E12.5["Dll4",],
vsd_Matrix_No_E12.5["Dll1",], vsd_Matrix_No_E12.5["Pdgfra",], vsd_Matrix_No_E12.5["Cxcl12",],
vsd_Matrix_No_E12.5["Ccl7",], vsd_Matrix_No_E12.5["Foxn1",],vsd_Matrix_No_E12.5["Col1a1",],
vsd_Matrix_No_E12.5["Flt1",], vsd_Matrix_No_E12.5["Twist2",], vsd_Matrix_No_E12.5["Wnt1",])
colnames(metadata) <-c("Name", "Sample", "Biological Replicate","Technical_Replicate", "Dll4
Expression", "Dll1 Expression", "Pdgfra Expression","Cxcl12 Expression","CCl7 Expression", "Foxn1
Expression", "Col1a1 Expression", "Flt1 Expression", "Twist2 Expression", "Wnt1 Expression")

p <- pca(vsd_Matrix_No_E12.5, metadata = metadata , removeVar = 0.667) # use top 100,000 variable
genes

# Screeplot to show much much varience each PC contributes to the data.
setwd("D:/Virtual_Shared_Folder/2020_08_27_Ligand_Receptor_Analysis_TEC_FTM_MEF/Graphs/N
o E12.5 TEC")
screeplot(p,
        gridlines.major = TRUE,
        gridlines.minor = FALSE,
        axisLabSize = 10,
        titleLabSize = 12,
        subtitleLabSize = 10,
        title = "PCA of FTM, MEF and E14.5 TEC",
        subtitle = 'SceePlot of Principal Components in MEF, FTM and E14.5 TEC',) +
  ggsave(file=paste0("No E12.5 TEC Scree Plot FTM and MEFs.png"), units="mm", width= 145,
height=90, dpi=600)

# Create Bi-plot showing the Sample and Biological replicate spread
setwd("D:/Virtual_Shared_Folder/2020_08_27_Ligand_Receptor_Analysis_TEC_FTM_MEF/Graphs/N
o E12.5 TEC")
biplot(pcaobj = p,
      lab = NULL,
      colby = 'Biological Replicate',
      shape = 'Sample',
      gridlines.major = TRUE,
      gridlines.minor = FALSE,
      drawConnectors = FALSE,
      pointSize = 2,
      legendLabSize = 8,
      axisLabSize = 10,
      titleLabSize = 12,
      subtitleLabSize = 10,
      labSize = 2,
      legendPosition = "right",
      legendIconSize = 2,
      title = "PCA of FTM, MEF and E14.5 TEC",
      subtitle = 'Plot Highlighting Effect of Sample and Biological Replicate',) +
  ggsave(file=paste0("No E12.5 TEC PCA_of_Sample_Distributions.png"), units="mm", width= 145,
height=180, dpi=600)


# Quick For Loop to print all graphs to R-Studio
setwd("D:/Virtual_Shared_Folder/2020_08_27_Ligand_Receptor_Analysis_TEC_FTM_MEF/Graphs/N
o E12.5 TEC")
Genes_of_Interest <- colnames(metadata)[5:length(colnames(metadata))]
for(i in 1:length(Genes_of_Interest)){
  print(
    biplot(pcaobj = p,
```

```
        lab = NULL,
        colby = Genes_of_Interest[i],
        shape = 'Sample',
        gridlines.major = TRUE,
        gridlines.minor = FALSE,
        drawConnectors = FALSE,
        pointSize = 2,
        legendLabSize = 10,
        axisLabSize = 10,
        titleLabSize = 12,
        subtitleLabSize = 10,
        labSize = 2,
        legendPosition = "none",
        legendIconSize = 2,
        title = "PCA of FTM, MEF and E14.5 TEC",
        subtitle = paste0("Plot Highlighting ", Genes_of_Interest[i])) +
    scale_colour_gradient(low = 'gold', high = 'red2') +
    ggsave(file=paste0("No E12.5 TEC Bi-plot showing Expression of", Genes_of_Interest[i],".png")),
units="mm", width= 145, height=180, dpi=600)
}


rm(list=setdiff(ls(), c("annotated_dds_Counts", "Merged_Sample_Table", "dds", "annotLookup", "vsd")))

#### Isolating the Ligand / Receptor Pairs ####
# read in table
setwd("D:/Virtual_Shared_Folder/2020_08_27_Ligand_Receptor_Analysis_TEC_FTM_MEF")
Ligand_Receptor_Pairs <- read.csv("Ligand-Receptor_Pairs.csv")

#### Converting Ligand Receptor Pairs into murine homologues ####
Mouse_Ligand_Names <- human2mouse(Ligand_Receptor_Pairs$Ligand.ApprovedSymbol)[,c(1,2)]

# Create the Murine_Ligand_Receptor_Pairs which will only have the ligands changed
Murine_Ligand_Receptor_Pairs <- matrix(nrow = 0,  ncol = ncol(Ligand_Receptor_Pairs))
for (t in 1:length(Mouse_Ligand_Names$humanGene)) {
# Search through the Mouse_Ligand_Names["humanGene"] and pull out any mouseGenes associated
with that human gene
Keep_list <- apply(Mouse_Ligand_Names["humanGene"], 1, function(r) any(r %in%
Mouse_Ligand_Names$humanGene[t]))
# Save the mouseGene names as mouseGene
mouseGene <- Mouse_Ligand_Names[Keep_list, "mouseGene"]
# Create a Keep_List2 to subset the Ligand_Receptor_Pairs
Keep_list2 <- Ligand_Receptor_Pairs$Ligand.ApprovedSymbol ==
Mouse_Ligand_Names$humanGene[t]
# So for some genes I cannot do a direct replacement as 1 Mouse_Ligand_Names$humanGene[t] =
>1 mouseGene
# If 1 Mouse_Ligand_Names$humanGene[t] = 1 mouseGene do the direct replacement - Easy! :)
if(length(mouseGene) == 1){
  New_rows_table <-Ligand_Receptor_Pairs[Keep_list2, ]
  New_rows_table$ï..Pair.Name <- gsub(Mouse_Ligand_Names$humanGene[t], mouseGene,
New_rows_table$ï..Pair.Name)
  New_rows_table$Ligand.ApprovedSymbol <- gsub(Mouse_Ligand_Names$humanGene[t],
mouseGene, New_rows_table$Ligand.ApprovedSymbol)
}
# But if 1 Mouse_Ligand_Names$humanGene[t] > than 1 mouseGene then :(
if(length(mouseGene) > 1){
  # multiply the rows in which Mouse_Ligand_Names$humanGene[t] appears in the
Ligand_Receptor_Pairs by the number of times mouseGene appears
  # Need to repeat the row by the number of mouseGenes and then do the replacement during this for
loop
  New_rows_table <- matrix(nrow = 0,  ncol = ncol(Ligand_Receptor_Pairs))
  for (i in 1:length(mouseGene)) {
    New_rows <- Ligand_Receptor_Pairs[Keep_list2, ]
```

```
    New_rows$ï..Pair.Name <- gsub(Mouse_Ligand_Names$humanGene[t], mouseGene[i],
New_rows$ï..Pair.Name)
    New_rows$Ligand.ApprovedSymbol <- gsub(Mouse_Ligand_Names$humanGene[t], mouseGene[i],
New_rows$Ligand.ApprovedSymbol)
    # I need to make a New_rows_table that I will add to the Ligand_Receptor_Pairs
    New_rows_table <- rbind(New_rows_table, New_rows)
  }
}
# Now I can use the new_rows_table to create a new, Murine_Ligand_Receptor_Pairs
Murine_Ligand_Receptor_Pairs <- rbind(Murine_Ligand_Receptor_Pairs, New_rows_table)
}

rm(list=setdiff(ls(), c("annotated_dds_Counts", "Merged_Sample_Table", "dds", "annotLookup", "vsd",
"Ligand_Receptor_Pairs", "Murine_Ligand_Receptor_Pairs")))
### Now convert the names of the receptors
Mouse_Receptor_Names <-
human2mouse(Ligand_Receptor_Pairs$Receptor.ApprovedSymbol)[,c(1,2)]

# create the loop that will change the receptors
Murine_Ligand_Receptor_Pairs2 <- matrix(nrow = 0,  ncol = ncol(Ligand_Receptor_Pairs))
for (t in 1:length(Mouse_Receptor_Names$humanGene)) {
  # Search through the Mouse_Receptor_Names["humanGene"] and pull out any mouseGenes
associated with that human gene
  Keep_list <- apply(Mouse_Receptor_Names["humanGene"], 1, function(r) any(r %in%
Mouse_Receptor_Names$humanGene[t]))
  # Save the mouseGene names as mouseGene
  mouseGene <- Mouse_Receptor_Names[Keep_list, "mouseGene"]
  # Create a Keep_List2 to subset the Ligand_Receptor_Pairs
  Keep_list2 <- Murine_Ligand_Receptor_Pairs$Receptor.ApprovedSymbol ==
Mouse_Receptor_Names$humanGene[t]
  # So for some genes I cannot do a direct replacement as 1 Mouse_Receptor_Names$humanGene[t]
= >1 mouseGene
  # If 1 Mouse_Receptor_Names$humanGene[t] = 1 mouseGene do the direct replacement - Easy! :)
  if(length(mouseGene) == 1){
    New_rows_table <- Murine_Ligand_Receptor_Pairs[Keep_list2, ]
    New_rows_table$ï..Pair.Name <- gsub(Mouse_Receptor_Names$humanGene[t], mouseGene,
New_rows_table$ï..Pair.Name)
    New_rows_table$Receptor.ApprovedSymbol <- gsub(Mouse_Receptor_Names$humanGene[t],
mouseGene, New_rows_table$Receptor.ApprovedSymbol)
  }
  # But if 1 Mouse_Ligand_Names$humanGene[t] > than 1 mouseGene then :(
  if(1:length(mouseGene) > 1){
    # multiply the rows in which Mouse_Ligand_Names$humanGene[t] appears in the
Ligand_Receptor_Pairs by the number of times mouseGene appears
    # Need to repeat the row by the number of mouseGenes and then do the replacement during this for
loop
    New_rows_table <- matrix(nrow = 0,  ncol = ncol(Ligand_Receptor_Pairs))
    for (i in 1:length(mouseGene)) {
      New_rows <- Murine_Ligand_Receptor_Pairs[Keep_list2, ]
      New_rows$ï..Pair.Name <- gsub(Mouse_Receptor_Names$humanGene[t], mouseGene[i],
New_rows$ï..Pair.Name)
      New_rows$Receptor.ApprovedSymbol <- gsub(Mouse_Receptor_Names$humanGene[t],
mouseGene[i], New_rows$Receptor.ApprovedSymbol)
      # I need to make a New_rows_table that I will add to the Ligand_Receptor_Pairs
      New_rows_table <- rbind(New_rows_table, New_rows)
    }
  }
  # Now I can use the new_rows_table to create a new, Murine_Ligand_Receptor_Pairs
  Murine_Ligand_Receptor_Pairs2 <- rbind(Murine_Ligand_Receptor_Pairs2, New_rows_table)
}

Ligand_Receptor_Pairs <- Murine_Ligand_Receptor_Pairs2
```

```
rm(list=setdiff(ls(), c("annotated_dds_Counts", "Merged_Sample_Table", "dds", "annotLookup", "vsd",
"Ligand_Receptor_Pairs")))

#### Create Interaction Scores ####
# Create a Matrix with Ligand Counts in
vsd_Matrix <- vsd@assays@data@listData[[1]]
Ligands <- Ligand_Receptor_Pairs$Ligand.ApprovedSymbol
Vsd_Genes <- rownames(vsd_Matrix)
ldt <- data.table::data.table(Ligands)
rdt <- data.table::data.table(Vsd_Genes)
colnames(rdt) <- "Ligands"
Ligands <- merge(ldt, rdt, by = "Ligands")
Ligands <- distinct(Ligands)
Ligand_Counts <- vsd_Matrix[unlist(Ligands),]

# Create a Matrix with Receptor Counts in
Receptors <- Ligand_Receptor_Pairs$Receptor.ApprovedSymbol
Vsd_Genes <- rownames(vsd_Matrix)
ldt <- data.table::data.table(Receptors)
rdt <- data.table::data.table(Vsd_Genes)
colnames(rdt) <- "Receptors"
Receptors <- merge(ldt, rdt, by = "Receptors")
Receptors <- distinct(Receptors)
Receptor_Counts <- vsd_Matrix[unlist(Receptors),]

# Isolate the Relevant Cell Populations for Each Comparison
# Create empty matrix to populate with interaction pairs
Calculated_Interaction_Scores <- matrix(nrow = 1, ncol =
ncol(Ligand_Counts)*ncol(Receptor_Counts)+1)
# Create a for loop to create colnames
Interaction_Pairs <- c()

for(i in 1:ncol(Ligand_Counts)){
  for(q in 1:ncol(Receptor_Counts)){
     tmp <- paste(colnames(Ligand_Counts)[i],"_",colnames(Receptor_Counts)[q])
     Interaction_Pairs <- c(Interaction_Pairs, tmp)
    }
}

# Make row one in the matrix the colnames
Calculated_Interaction_Scores[1,] <- c("Interaction Pair", Interaction_Pairs)

# For loop to multiple together every ligand - receptor pair.
tmpa <- c()
tmpb <- c()
tmpc <- c()

for(i in 1:length(Ligand_Counts)){
  for(q in 1:length(Receptor_Counts)){
    if(paste0(rownames(Ligand_Counts)[i],"_",rownames(Receptor_Counts)[q]) %in%
Ligand_Receptor_Pairs$i..Pair.Name){
      for(l in 1:ncol(Ligand_Counts)){
        for(r in 1:ncol(Receptor_Counts)){
          tmpa <- Ligand_Counts[i,l] * Receptor_Counts[q,r]
          tmpb <- c(tmpb, tmpa)
         }
      }
      tmpc <- c(paste0(rownames(Ligand_Counts)[i],"_",rownames(Receptor_Counts)[q]), tmpb)
      Calculated_Interaction_Scores <- rbind(Calculated_Interaction_Scores, tmpc)
      tmpa <- c()
      tmpb <- c()
      tmpc <- c()
  }
 }
```

```
}

# Tidy up the Calculated_Interaction_Scores
rownames(Calculated_Interaction_Scores) <- Calculated_Interaction_Scores[,1]
Calculated_Interaction_Scores <-
Calculated_Interaction_Scores[2:nrow(Calculated_Interaction_Scores),]
Calculated_Interaction_Scores <-
Calculated_Interaction_Scores[,2:ncol(Calculated_Interaction_Scores)]
Calculated_Interaction_Scores <- as.data.frame(Calculated_Interaction_Scores)
colnames(Calculated_Interaction_Scores) <- Interaction_Pairs

# Export the Calculated interaction scores as a CSV
setwd("D:/Virtual_Shared_Folder/2020_08_27_Ligand_Receptor_Analysis_TEC_FTM_MEF")
#write.csv(Calculated_Interaction_Scores, "Calculated_Interaction_Scores.csv", row.names = TRUE)
Calculated_Interaction_Scores <- read.csv("Calculated_Interaction_Scores.csv")
rownames(Calculated_Interaction_Scores) <- Calculated_Interaction_Scores[,1]
Calculated_Interaction_Scores <-
Calculated_Interaction_Scores[,2:ncol(Calculated_Interaction_Scores)]

#### Calculating which pairs are significantly different between MEF+E14.5_TEC and
FTM+E14.5_TEC ####
### Subset the FTM x E14.5 TECs and MEF x E14.5 TEC
# Create For loop to make colnames
tempa <- c("MEF","FTM")
tempb <- c("Neg","Pos")
Pairs <- c()
for(a in 1:2){
  for(b in 1:9){
    for(c in 1:2){
     for(d in 1:3){
       Pairs <- c(Pairs, paste0(tempa[a],b,"._.TEC_E14.5_",tempb[c],"_",d))
    }
  }
  }
}
# Subset the Calculated_Interaction_Scores table for just the MEF/FTM x E14.5 TEC data
Pairs_Interaction_Scores <- Calculated_Interaction_Scores[,Pairs]

# Remove all now obsolete objects
rm(list=setdiff(ls(), c("annotated_dds_Counts", "Merged_Sample_Table", "vsd",
"Pairs_Interaction_Scores","Receptor_Counts", "Ligand_Counts", "Ligand_Receptor_Pairs" )))

### Test for Normality
Pairs_Interaction_Scores <- data.matrix(Pairs_Interaction_Scores)
Normality <- c()
for(i in 1:nrow(Pairs_Interaction_Scores)){
  if(var(Pairs_Interaction_Scores[i,]) > 0 ){
      tmpa <- c(shapiro.test(Pairs_Interaction_Scores[i,]))
      Normality[i] <- tmpa$p.value
  }
}
all(Normality < 0.05)


### Run Wilcox Test
# Need to change the colnames into MEF (1) and FTM (2)
Data_List <- vector("list")
Groups <- c(rep("1", 54), rep("2", 54))
for(i in 1:nrow(Pairs_Interaction_Scores)){
  Value <- Pairs_Interaction_Scores[i,]
  Value <- t(rbind(Value, Groups))
  Data_List[[i]] <- Value
  names(Data_List)[i] <- rownames(Pairs_Interaction_Scores)[i]
  mode(Data_List[[i]]) = "numeric"
```

```
  colnames(Data_List[[i]]) <- c("Value","Groups")
}

# Run the Wilcox test
SigDif_List <- vector("list")
p_values <- c()
for(i in 1:length(Data_List)){
  SigDif_List[[i]] <- wilcox.test(Value ~ Groups, data=Data_List[[i]])
  names(SigDif_List)[i] <- rownames(Pairs_Interaction_Scores)[i]
  p_values <- c(p_values, SigDif_List[[i]]$p.value)
}

# Calculate adjusted p-values
adjusted_p_values <- p.adjust(p_values, method = "BH", n = length(p_values))

# Bind the p-values and adjusted p values to the Pairs_Interaction_Scores
Pairs_Interaction_Scores <- cbind(Pairs_Interaction_Scores, p_values)
Pairs_Interaction_Scores <- cbind(Pairs_Interaction_Scores, adjusted_p_values)

# Select only those pairs with a significant difference at a p-value of less than 0.5
Selected_Pairs <- subset(Pairs_Interaction_Scores, Pairs_Interaction_Scores[,"adjusted_p_values"] <
0.05)

# Remove all now obsolete objects
rm(list=setdiff(ls(), c("annotated_dds_Counts", "Merged_Sample_Table", "vsd",
"Pairs_Interaction_Scores","Receptor_Counts", "Ligand_Counts", "Ligand_Receptor_Pairs",
"SigDif_List", "Selected_Pairs")))

#### Calculate the Correlation between Ligand and Receptors ####
# Subset the VSD Matrix into E14.5 TEC, MEF and FTM
TEC_vsd <- vsd@assays@data@listData[[1]][,c("TEC_E14.5_Pos_1", "TEC_E14.5_Pos_2",
"TEC_E14.5_Pos_3", "TEC_E14.5_Neg_1", "TEC_E14.5_Neg_2", "TEC_E14.5_Neg_3")]
MEF_vsd <- vsd@assays@data@listData[[1]][,c("MEF1", "MEF2", "MEF3", "MEF4", "MEF5", "MEF6",
"MEF7", "MEF8", "MEF9")]
FTM_vsd <- vsd@assays@data@listData[[1]][,c("FTM1", "FTM2", "FTM3", "FTM4", "FTM5", "FTM6",
"FTM7", "FTM8", "FTM9")]

# Need to make the datasets have the same number of observations for the correlation to be
calculated
Bio_Reps_MEF <- matrix(nrow = 0, ncol = 4)
for(i in 1:nrow(MEF_vsd)){
  Bio_Reps_MEF <- rbind(Bio_Reps_MEF, c(rownames(MEF_vsd)[i],median(MEF_vsd[i,c(1:3)]),
median(MEF_vsd[i,c(4:6)]), median(MEF_vsd[i,c(7:9)])))
}
rownames(Bio_Reps_MEF) <- Bio_Reps_MEF[,1]
Bio_Reps_MEF <- Bio_Reps_MEF[,2:ncol(Bio_Reps_MEF)]
Bio_Reps_MEF <- cbind(Bio_Reps_MEF,Bio_Reps_MEF)
mode(Bio_Reps_MEF) <- "numeric"

Bio_Reps_FTM <- matrix(nrow = 0, ncol = 4)
for(i in 1:nrow(FTM_vsd)){
  Bio_Reps_FTM <- rbind(Bio_Reps_FTM, c(rownames(FTM_vsd)[i],median(FTM_vsd[i,c(1:3)]),
median(FTM_vsd[i,c(4:6)]), median(FTM_vsd[i,c(7:9)])))
}
rownames(Bio_Reps_FTM) <- Bio_Reps_FTM[,1]
Bio_Reps_FTM <- Bio_Reps_FTM[,2:ncol(Bio_Reps_FTM)]
Bio_Reps_FTM <- cbind(Bio_Reps_FTM,Bio_Reps_FTM)
mode(Bio_Reps_FTM) <- "numeric"

# Order into alphabetic order
Bio_Reps_MEF <- Bio_Reps_MEF[order(rownames(Bio_Reps_MEF)),]
Bio_Reps_FTM <- Bio_Reps_FTM[order(rownames(Bio_Reps_FTM)),]
TEC_vsd <- TEC_vsd[order(rownames(TEC_vsd)),]
```

```
# Create For loop to calculate the Pearson's Correlation between all ligand and receptor pairs
MEF_Calculated_Correlation_Scores <- matrix(nrow = 0, ncol = 2)
for(i in 1:nrow(TEC_vsd)){
  for(q in 1:nrow(Bio_Reps_MEF)){
    if(paste0(rownames(TEC_vsd)[i],"_",rownames(Bio_Reps_MEF)[q]) %in%
Ligand_Receptor_Pairs$i..Pair.Name){
      tmpa <- cor(x = TEC_vsd[i,],
                  y = Bio_Reps_MEF[q,],
                  method = "spearman")
      tmpb <- c(paste0(rownames(TEC_vsd)[i],"_",rownames(Bio_Reps_MEF)[q]), tmpa)
      MEF_Calculated_Correlation_Scores <- rbind(MEF_Calculated_Correlation_Scores, tmpb)
    }
  }
}

FTM_Calculated_Correlation_Scores <- matrix(nrow = 0, ncol = 2)
for(i in 1:nrow(TEC_vsd)){
  for(q in 1:nrow(Bio_Reps_FTM)){
    if(paste0(rownames(TEC_vsd)[i],"_",rownames(Bio_Reps_FTM)[q]) %in%
Ligand_Receptor_Pairs$i..Pair.Name){
      tmpa <- cor(x = TEC_vsd[i,],
                  y = Bio_Reps_FTM[q,],
                  method = "spearman")
      tmpb <- c(paste0(rownames(TEC_vsd)[i],"_",rownames(Bio_Reps_FTM)[q]), tmpa)
      FTM_Calculated_Correlation_Scores <- rbind(FTM_Calculated_Correlation_Scores, tmpb)
    }
  }
}


# Save Correlations as .csv
setwd("D:/Virtual_Shared_Folder/2020_08_27_Ligand_Receptor_Analysis_TEC_FTM_MEF")
#write.csv(MEF_Calculated_Correlation_Scores, "MEF_Calculated_Correlation_Scores.csv",
row.names = TRUE)
MEF_Calculated_Correlation_Scores <- read.csv("MEF_Calculated_Correlation_Scores.csv")
#write.csv(FTM_Calculated_Correlation_Scores, "FTM_Calculated_Correlation_Scores.csv",
row.names = TRUE)
FTM_Calculated_Correlation_Scores <- read.csv("FTM_Calculated_Correlation_Scores.csv")

# Tidy up the new matrixes
MEF_Calculated_Correlation_Scores <- MEF_Calculated_Correlation_Scores[,c(2,3)]
FTM_Calculated_Correlation_Scores <- FTM_Calculated_Correlation_Scores[,c(2,3)]

# Subset pairs with correlation greater than 0.4
MEF_Correlated_Pairs <- rbind(subset(MEF_Calculated_Correlation_Scores,
MEF_Calculated_Correlation_Scores[,"V2"] > 0.01), subset(MEF_Calculated_Correlation_Scores,
MEF_Calculated_Correlation_Scores[,"V2"] < -0.01))
FTM_Correlated_Pairs <- rbind(subset(FTM_Calculated_Correlation_Scores,
FTM_Calculated_Correlation_Scores[,"V2"] > 0.01), subset(FTM_Calculated_Correlation_Scores,
FTM_Calculated_Correlation_Scores[,"V2"] < -0.01))

# Subset the select pairs with the correlated pairs
Selected_Pairs <- unique(rbind(subset(Selected_Pairs, rownames(Selected_Pairs) %in%
MEF_Correlated_Pairs$V1),subset(Selected_Pairs, rownames(Selected_Pairs) %in%
FTM_Correlated_Pairs$V1)))


# Calculate mean log2 fold change in each pair between FTM and MEF
Log2_Fold_Change_MEF_minus_FMT <- c()
for(i in 1:nrow(Selected_Pairs)){
  Log2_Fold_Change_MEF_minus_FMT <- c(Log2_Fold_Change_MEF_minus_FMT,
mean(log2(Selected_Pairs[i,c(1:54)])) - mean(log2(Selected_Pairs[i,c(55:108)])))
}
Selected_Pairs <- cbind(Selected_Pairs, Log2_Fold_Change_MEF_minus_FMT)
```

```
P_values_only_Selected_Pairs <- Selected_Pairs[,c(109:111)]

# Export the Calculated interaction scores as a CSV
setwd("D:/Virtual_Shared_Folder/2020_08_27_Ligand_Receptor_Analysis_TEC_FTM_MEF")
write.csv(Selected_Pairs, "Selected_Pairs.csv", row.names = TRUE)
write.csv(P_values_only_Selected_Pairs, "P_values_only_Selected_Pairs.csv", row.names = TRUE)

rm(list=setdiff(ls(), c("annotated_dds_Counts", "Merged_Sample_Table", "dds", "annotLookup", "vsd",
"Pairs_Interaction_Scores", "Selected_Pairs")))

#### Create ggplot of an example Interaction score ####

data <- rbind(colnames(Pairs_Interaction_Scores)[1:108],
Pairs_Interaction_Scores["Fgf10_Fgfr1",c(1:108)])
data[1,] <- substr(data[1,], 1,3)
row.names(data) <- c("Group", "Data")
colnames(data) <- c(1:108)
data <- as.data.frame(t(data))
data$Group <- as.factor(data$Group)
data$Data <- as.numeric(data$Data)

ggplot(data, aes(x = Group, y = Data)) +
theme(plot.title = element_text(size = 15,
                          face="bold",
                          family="TT Arial",
                          color="Black",
                          hjust = 0,
                          lineheight = 1.2),

      plot.subtitle = element_text(size = 12,
                           family="TT Arial",
                           face="italic",
                           hjust = 0),

      plot.caption = element_text(size = 12,
                          family="TT Arial"),  # caption

      axis.title.x = element_text(vjust = 0,
                          size = 10,
                          family="TT Arial"),  # X axis title


      axis.title.y = element_text(vjust = 0,
                          size = 10,
                          family="TT Arial"),  # Y axis title

      axis.text.x = element_text(size = 8,
                          angle = 60,
                          vjust = 0.75,
                          family="TT Arial"),  # X axis text use less than 0 to bring downwards

      axis.text.y = element_text(size = 8,
                          angle = 90,
                          hjust = 0.5,
                          family="TT Arial"),

      axis.line.x = element_line(color="black",
                          size = 0.5),

      axis.line.y = element_line(color="black",
                          size = 0.5),

      legend.title = element_text(size = 0,
                           family="TT Arial"),
```

```
    legend.text = element_text(size = 8,
                               family="TT Arial"),

    legend.position = "bottom",

    panel.border = element_blank(),

    panel.background = element_blank(),

    panel.grid.major = element_blank(),

    panel.grid.minor = element_blank()
    ) +

geom_boxplot(fill = "#CCCCCC") +

geom_dotplot(binaxis='y',
             stackdir='center',
             dotsize = 0.5,
            fill = "#000000") +

  labs(title = "Comparison of Interaction Score",
    subtitle = "Fgf10_Fgfr1",
    y = "Interaction Score",
    x = "Group")
```

8.3. Bioinformatic analysis for TCR repertoire diversity: code

In [19]:

```
##Filter non-functional

import os
import re
for file in os.listdir("./"):
    if file.endswith(".TRB.txt"):
        filtered_output = open(file + "F", "w")
        with open(file) as new_file:
            header=next(new_file)
            non_productive_count = 0
            for line in new_file:
                line=line.rstrip('\n')
                data=line.split("\t")
                non_productive = re.findall(r"[_|*]", data[32])
                if len(non_productive) > 0:
                    non_productive_count += 1
                else:
                    filtered_output.write(line +"\n")
            print(str(non_productive_count) + " non-productive sequences removed from sample " + file)
```

```
2 non-productive sequences removed from sample iTEC-350Spleen.output.clonotypes.TRB.txt
0 non-productive sequences removed from sample iTEC-455LN.output.clonotypes.TRB.txt
65 non-productive sequences removed from sample iTEC-495Spleen.output.clonotypes.TRB.txt
2 non-productive sequences removed from sample iTEC-321Spleen.output.clonotypes.TRB.txt
33 non-productive sequences removed from sample Cre-416LN.output.clonotypes.TRB.txt
2 non-productive sequences removed from sample RFTOC-429Spleen.output.clonotypes.TRB.txt
63 non-productive sequences removed from sample Cre-486Spleen.output.clonotypes.TRB.txt
76 non-productive sequences removed from sample Cre-530LN.output.clonotypes.TRB.txt
9 non-productive sequences removed from sample iTEC-321LN.output.clonotypes.TRB.txt
0 non-productive sequences removed from sample iTEC-455Spleen.output.clonotypes.TRB.txt
52 non-productive sequences removed from sample iTEC-494LN.output.clonotypes.TRB.txt
147 non-productive sequences removed from sample RFTOC-499LN.output.clonotypes.TRB.txt
208 non-productive sequences removed from sample RFTOC-493LN.output.clonotypes.TRB.txt
0 non-productive sequences removed from sample RFTOC-493Spleen.output.clonotypes.TRB.txt
3 non-productive sequences removed from sample Cre-530Spleen.output.clonotypes.TRB.txt
64 non-productive sequences removed from sample iTEC-494Spleen.output.clonotypes.TRB.txt
71 non-productive sequences removed from sample RFTOC-411LN.output.clonotypes.TRB.txt
146 non-productive sequences removed from sample RFTOC-499Spleen.output.clonotypes.TRB.txt
15 non-productive sequences removed from sample iTEC-315LN.output.clonotypes.TRB.txt
347 non-productive sequences removed from sample RFTOC-429LN.output.clonotypes.TRB.txt
58 non-productive sequences removed from sample iTEC-501Spleen.output.clonotypes.TRB.txt
87 non-productive sequences removed from sample Cre-356Spleen.output.clonotypes.TRB.txt
12 non-productive sequences removed from sample iTEC-315Spleen.output.clonotypes.TRB.txt
46 non-productive sequences removed from sample RFTOC-417Spleen.output.clonotypes.TRB.txt
5 non-productive sequences removed from sample Cre-486LN.output.clonotypes.TRB.txt
89 non-productive sequences removed from sample iTEC-495LN.output.clonotypes.TRB.txt
365 non-productive sequences removed from sample RFTOC-411Spleen.output.clonotypes.TRB.txt
2 non-productive sequences removed from sample iTEC-350LN.output.clonotypes.TRB.txt
1 non-productive sequences removed from sample Cre-500Spleen.output.clonotypes.TRB.txt
10 non-productive sequences removed from sample Cre-500LN.output.clonotypes.TRB.txt
1 non-productive sequences removed from sample iTEC-501LN.output.clonotypes.TRB.txt
312 non-productive sequences removed from sample RFTOC-417LN.output.clonotypes.TRB.txt
2 non-productive sequences removed from sample Cre-416Spleen.output.clonotypes.TRB.txt
```

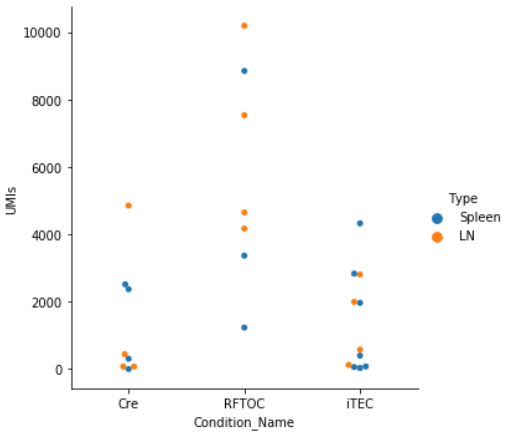
In [33]:

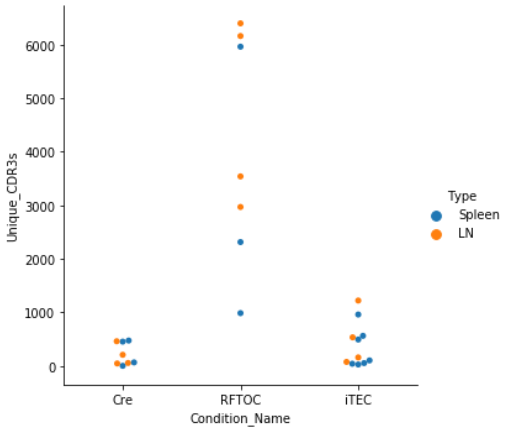
```
##Samples run on morning 24/22/20: 493_spleen, 501LN, 350LN, 416 SPleen, 411 LN, 455 LN.
##and in wrong tube: 429 Spleen.


##Note no file for Cre-356LN.output.clonotypes.TRB.txt as no fastq - no reads.

f_w=open('read_numbers.csv', 'w')
f_w.write('File,UMIs,Unique_CDR3s\n')

for file in sorted(os.listdir("./")):
    if file.endswith(".TRB.txtF"):
        UMIs=0
        Unique_CDR3s=0
        with open(file) as f:
            next(f) ##skip header
            for line in f:
                data=line.strip('\n').split('\t')
                UMIs+=int(float(data[1]))
                Unique_CDR3s+=1
        f_w.write(file+','+str(UMIs)+','+str(Unique_CDR3s)+'\n')
        print(file, UMIs, Unique_CDR3s)
```

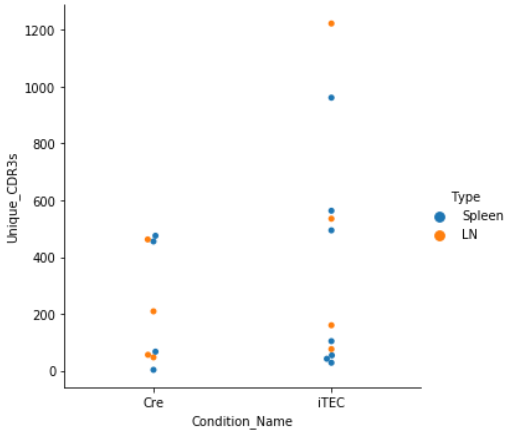
Cre-356Spleen.output.clonotypes.TRB.txtF 2516 475
Cre-416LN.output.clonotypes.TRB.txtF 444 209
Cre-416Spleen.output.clonotypes.TRB.txtF 25 22
Cre-486LN.output.clonotypes.TRB.txtF 77 47
Cre-486Spleen.output.clonotypes.TRB.txtF 2370 455
Cre-500LN.output.clonotypes.TRB.txtF 75 56
Cre-500Spleen.output.clonotypes.TRB.txtF 3 3
Cre-530LN.output.clonotypes.TRB.txtF 4851 462
Cre-530Spleen.output.clonotypes.TRB.txtF 309 67
RFTOC-411LN.output.clonotypes.TRB.txtF 1469 1336
RFTOC-411Spleen.output.clonotypes.TRB.txtF 8845 5962
RFTOC-417LN.output.clonotypes.TRB.txtF 7533 6161
RFTOC-417Spleen.output.clonotypes.TRB.txtF 1233 987
RFTOC-429LN.output.clonotypes.TRB.txtF 10187 6398
RFTOC-429Spleen.output.clonotypes.TRB.txtF 44 42
RFTOC-493LN.output.clonotypes.TRB.txtF 4648 3540
RFTOC-499LN.output.clonotypes.TRB.txtF 4168 2969
RFTOC-499Spleen.output.clonotypes.TRB.txtF 3366 2314
iTEC-315LN.output.clonotypes.TRB.txtF 579 160
iTEC-315Spleen.output.clonotypes.TRB.txtF 399 104
iTEC-321LN.output.clonotypes.TRB.txtF 126 76
iTEC-321Spleen.output.clonotypes.TRB.txtF 62 42
iTEC-350LN.output.clonotypes.TRB.txtF 18 13
iTEC-350Spleen.output.clonotypes.TRB.txtF 78 54
iTEC-455LN.output.clonotypes.TRB.txtF 0 0
iTEC-455Spleen.output.clonotypes.TRB.txtF 33 28
iTEC-494LN.output.clonotypes.TRB.txtF 1996 535
iTEC-494Spleen.output.clonotypes.TRB.txtF 2834 563
iTEC-495LN.output.clonotypes.TRB.txtF 2806 1222
iTEC-495Spleen.output.clonotypes.TRB.txtF 1966 961
iTEC-501LN.output.clonotypes.TRB.txtF 2 2
iTEC-501Spleen.output.clonotypes.TRB.txtF 4325 494

In [5]:

```
import seaborn as sns
import matplotlib.pyplot as plt
import os

##Samples run on morning 24/22/20: 493_spleen, 501LN, 350LN,  411 LN, 455 LN, ##and in wrong tube: 429 Spleen.
to_remove=['iTEC-501LN.output.clonotypes.TRB.txtF', 'iTEC-350LN.output.clonotypes.TRB.txtF', 'Cre-416Spleen.output.clonotypes.TRB.txtF', 'RFTO

reads=pd.read_csv('read_numbers.csv')

file_dict={}

for file in sorted(os.listdir("./")):
    if file.endswith(".TRB.txtF"):
        ID=file.split('.')[0]
        file_dict[ID]=file

metadata=pd.read_csv('JoSweetman_metadata.csv')
metadata['File']=metadata['ID.1'].map(file_dict)

merged_metadata=pd.merge(reads, metadata, on='File', how='outer')

merged_metadata=merged_metadata.loc[~merged_metadata['File'].isin(to_remove)]

g1=sns.catplot(x='Condition_Name', y='UMIs', kind='swarm', data=merged_metadata, hue='Type')
plt.show()

g2=sns.catplot(x='Condition_Name', y='Unique_CDR3s', kind='swarm', data=merged_metadata, hue='Type')
plt.show()

g3=sns.catplot(x='Condition_Name', y='Unique_CDR3s', order=['Cre', 'iTEC'], kind='swarm', data=merged_metadata, hue='Type')
plt.show()
```







In [76]:

```
def sort_human(l):
  convert = lambda text: float(text) if text.isdigit() else text
  alphanum = lambda key: [ convert(c) for c in re.split('([0-9]+)', key) ]
  l.sort( key=alphanum )
  return l

import os
from collections import OrderedDict

my_dict=OrderedDict()

for file in sort_human(os.listdir('./')):
    if file.endswith('clonotypes.TRB.txtF'):
        with open(file) as my_file:
            #next(my_file) #header
            for line in my_file:
                columns = line.rstrip('\n').split("\t")
                V = columns[5].split('*')
                V_allele=V[0]
                if not V_allele in my_dict:
                    my_dict[V_allele] = 0

for key in my_dict:
    print(key, my_dict[key])

print(len(my_dict))
```

TRBV13-1 0
TRBV17 0
TRBV12-2 0
TRBV14 0
TRBV16 0
TRBV20 0
TRBV12-1 0
TRBV13-2 0
TRBV13-3 0
TRBV1 0
TRBV15 0
TRBV26 0
TRBV5 0
TRBV3 0
TRBV19 0
TRBV2 0
TRBV4 0
TRBV29 0
TRBV31 0
TRBV23 0
TRBV24 0
TRBV21 0
TRBV30 0
TRBV22 0
24

In [81]:

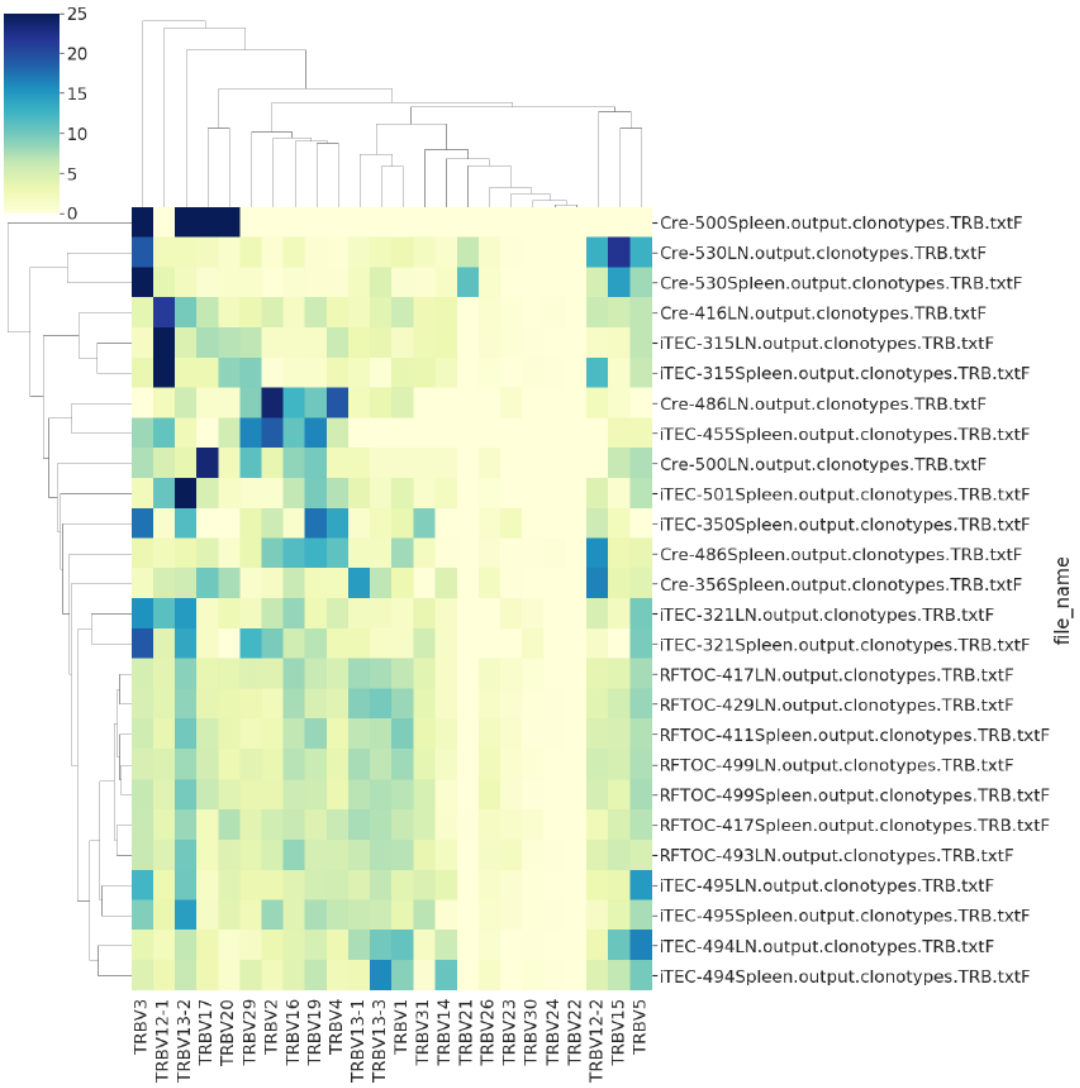
```
f_w = open("V_clonality.csv","w")
f_w.write('file_name,')
my_string = ''
for key in my_dict:
    my_string = my_string + key + ','
my_string = my_string.rstrip(',')
f_w.write(my_string + '\n')

for file in sort_human(os.listdir('./')):
    if file.endswith('.clonotypes.TRB.txtF'):
        temp_dict=OrderedDict(my_dict) #
        with open(file) as my_file:
            for line in my_file:
                columns = line.rstrip('\n').split("\t")
                V = columns[5].split('*')
                V_allele= V[0]
                temp_dict[V_allele]+=float(columns[1])
        my_string = ''
        for key in temp_dict.keys():
            my_string = my_string + str(temp_dict[key]) + ','
        my_string=my_string.rstrip(',')
        file_name=file.split('.')
        file_ID=file_name[0]
        f_w.write(file+','+my_string + '\n')

f_w.close()
```

In [99]:

```
# ##Vgene usage with proportion
# import matplotlib.pyplot as plt
# import seaborn as sns

# df=pd.read_csv('V_clonality.csv')

# df=df.loc[~df['file_name'].isin(to_remove)]


# df=df.set_index('file_name')
# df_p= (df.div(df.sum(axis=1), axis=0))*100

# fig, ax=plt.subplots(figsize=(20,20))
# g1=sns.heatmap(df_p, cmap='YlGnBu', vmax=25)
# plt.show()
```

In [8]:

```
##Vgene usage with zscores
import matplotlib.pyplot as plt
import seaborn as sns
import pandas as pd

sns.set(font_scale=2)

df=pd.read_csv('V_clonality.csv')

df=df.loc[~df['file_name'].isin(to_remove)]


df=df.set_index('file_name')
df_p= (df.div(df.sum(axis=1), axis=0))*100

# fig, ax=plt.subplots(figsize=(20,20))
# g1=sns.heatmap(df_p, cmap='coolwarm', vmax=25)
# plt.show()

g2=sns.clustermap(df_p, cmap='YlGnBu', vmax=25, figsize=(20,20))
plt.show()
```


25
20
15
10
5
0
Cre-500Spleen.output.clonotypes.TRB.txtF
Cre-530LN.output.clonotypes.TRB.txtF
Cre-530Spleen.output.clonotypes.TRB.txtF
Cre-416LN.output.clonotypes.TRB.txtF
iTEC-315LN.output.clonotypes.TRB.txtF
iTEC-315Spleen.output.clonotypes.TRB.txtF
Cre-486LN.output.clonotypes.TRB.txtF
iTEC-455Spleen.output.clonotypes.TRB.txtF
Cre-500LN.output.clonotypes.TRB.txtF
iTEC-501Spleen.output.clonotypes.TRB.txtF
iTEC-350Spleen.output.clonotypes.TRB.txtF
Cre-486Spleen.output.clonotypes.TRB.txtF
Cre-356Spleen.output.clonotypes.TRB.txtF
iTEC-321LN.output.clonotypes.TRB.txtF
iTEC-321Spleen.output.clonotypes.TRB.txtF
RFTOC-417LN.output.clonotypes.TRB.txtF
RFTOC-429LN.output.clonotypes.TRB.txtF
RFTOC-411Spleen.output.clonotypes.TRB.txtF
RFTOC-499LN.output.clonotypes.TRB.txtF
RFTOC-499Spleen.output.clonotypes.TRB.txtF
RFTOC-417Spleen.output.clonotypes.TRB.txtF
RFTOC-493LN.output.clonotypes.TRB.txtF
iTEC-495LN.output.clonotypes.TRB.txtF
iTEC-495Spleen.output.clonotypes.TRB.txtF
iTEC-494LN.output.clonotypes.TRB.txtF
iTEC-494Spleen.output.clonotypes.TRB.txtF
file_name
TRBV3
TRBV12-1
TRBV13-2
TRBV17
TRBV20
TRBV29
TRBV2
TRBV16
TRBV19
TRBV4
TRBV13-1
TRBV13-3
TRBV1
TRBV31
TRBV14
TRBV21
TRBV26
TRBV23
TRBV30
TRBV24
TRBV22
TRBV12-2
TRBV15
TRBV5

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