**VIVA SUGGESTED CORRECTIONS CHECKLIST**

1. **The candidate was provided with a copy of the thesis from the internal examiner where minor typographical errors were noted, for correction.**

I have amended all the typographic and grammatical mistakes that were marked in the hard copy of the thesis.

1. **Redefining dactylitis pathological process.**

I have re-written the paragraph under the sub-section “Histopathological alterations of skin and joints” and also included the reference of an updated dactylitis review.

Old: “As a result, alterations in bone remodeling lead to osteolysis, bone resorption and erosion at the affected joints (Mensah et al. 2008). Bone erosion is also the main histopathological process driving dactylatis, where bone lysis resolves in shortening of the digits (Gladman et al. 2005). Moreover, 35% of PsA patients also undergo inflammation of the connective tissue at the insertion of tendons or ligaments, a phenomenon known as enthesitis (McGonagle et al. 2011; Polachek et al. 2017). The inflammatory environment at the entheses favours bony spurs formation along the insertion sites, similar to RA, causing structural debilitation of the joints (Benjamin and McGonagle 2009; Finzel et al. 2014).”

New: “﻿As a result, alterations in bone remodelling lead to osteolysis, bone resorption and erosion at the affected joints (Mensah et al. 2008). Moreover, 35% of PsA patients also undergo inflammation of the connective tissue at the insertion of tendons or ligaments, a phenomenon known as enthesitis (McGonagle et al. 2011; Polachek et al. 2017). The inflammatory environment at the entheses favours bone spurs formation along the insertion sites, similar to RA, causing structural debilitation of the joints (Benjamin and McGonagle 2009; Finzel et al. 2014). Interestingly, enthesitis has been identified as the main mechanism driving dactylitis, which is importantly characterised by tenosynovitis of the flexor tendons that are constrained by accessory pulleys (sites of compressive and tensile biophysical stress). The accessory pulleys behave as functional entheses and high-resolution magnetic resonance imaging has shown inflammation of this structure in early dactylitis (McGonagle et al. 2019).”

1. **A sentence explaining why PsA and psoriasis may be increasing could be included on page 2.**

I have re-written page 2 paragraph:

Old: “On the other hand, cases of PsA in the general population varies between 0.04 and 1.2% occurring in 10 to 30% of psoriasis patients, evidencing the strong association between the two diseases (Gelfand et al. 2005; Reich et al. 2015; Perera et al. 2012). Overall, data suggest a steady increase in both psoriasis and PsA prevalence over the last 30 years (Springate et al. 2017; Organization 2016).”

New: “﻿For PsA, incidence in the general population varies between 0.04 and 1.2%, with peak age of onset between the 35 and 45 years of age. The estimation of psoriasis patients with concominant PsA ranges from 10 to 30%, with arthritis onset occurring approximately ten years after the onset of the skin disease (Greb2016). This clearly evidences the strong association between both diseases (Gelfand et al. 2005; Reich et al. 2015; Perera et al. 2012).”

I have moved the sentence and hypothesis for the increase of psoriasis incidence in the last 30 years to the subsection of Environmental factors and disease and added the following paragraph: “﻿Interestingly, epidemiological data suggests a steady increase in psoriasis and PsA prevalence over the last 30 years, particularly in older age groups (Springate et al. 2017; Organization 2016). This trend may be the result of the increase in frequency of various environmental risk factors over the same period of time (for example prevalence of obesity and beta blockers in patients with myocardial infarction), rather than a consequence of the improvement in diagnosis and access to medical care (Icen et al. 2009). Altogether this reinforces the role of environmental factors in the risk of developing psoriasis and PsA.”

1. **During the viva, Profe Anne Barton mentioned that a PsA twin study that I had not references.**

Following additional bibliographic search, the only twin study in PsA that I have found is the one conducted by Pedersen et al., 2008 (“On the heritability of psoriatic arthritis. Disease concordance among monozygotic and dizygotic twin”), which was already mentioned in the thesis. I have modified the paragraph to emphasise Pedersen study and I am happy to add another reference if Prof Anne Barton can provide it.

Old: “﻿The risk of developing psoriasis and PsA is not only influenced by environmental conditions but also by the genetic background of each individual. The concordance of psoriasis is greater in monozygotic (33-55%) compared to dizyogtic twins (13-21%), giving a heritability estimate of 80%, while no difference in concordance is reported for PsA, probably due to lack of statistical power and appropriate diagnosis (Farber et al. 1974; Duffy et al. 1993; Pedersen et al. 2008). In the general population, approximately 40% of patients with psoriasis or PsA have a family history in first degree relatives (Gladman et al. 1986). Interestingly, the recurrence rate in first-degree relatives has been shown to be greater in PsA (40%) compared to psoriasis (8%) in a study in the Icelandic population (Chandran et al. 2009). Altogether, this may suggest differences in the heritability between the two phenotypes and a stronger genetic contribution in PsA.”

New: “﻿The risk of developing psoriasis and PsA is not only influenced by environmental conditions but also by the genetic background of each individual. In fact, approximately 40% of patients with psoriasis or PsA have a family history in first degree relatives (Gladman et al. 1986). Twins studies in patients with psoriasis (only cutaneous lesions) have demonstrated that

concordance of disease is greater in monozygotic (33-55%) compared to dizygotic twins (13-21%), giving a heritability estimate between 50 to 80% in populations of European descendants (Farber et al. 1974; Duffy et al. 1993; Grjibovski et al. 2007; Pedersen et al. 2008; Lonnberg et al. 2013). Moreover, psoriasis prevalence is also greater amongst first degree relatives compared to the general population, ranging between 4-19% (Myers et al. 2005; Chandran et al. 2009).

Interestingly, the only twins study conducted to date in PsA did not find differences in concordance between monozygotic and dizygotic twins, which could be due to lack of power in the study (Pedersen et al. 2008). Nevertheless, heritability estimates in PsA are between 80 to 100% and the recurrence rate in first-degree relatives has been shown to be greater for PsA (40-47-fold) compared to psoriasis (8-fold) (Myers et al. 2005; Chandran et al. 2009; Karason et al. 2009). These observations may highlight differences in the heritability between the two phenotypes and a stronger genetic contribution in PsA compared to psoriasis.”

1. **In the introduction, it would be useful to include a short discussion on whether PsA may be a complication of psoriasis or a distinct disease entity and whether either or both are single diseases.**

In Chapter 1 section 1.3 I have added a subsection titled “﻿Psoriasis and PsA: the same or distinct disease entities”. I have placed this subsection here because it involves epidemiological, pathophysiological and also genetics data, which has been partially explained in previous subsections. Therefore, placing the subsection before finishing talking about all these aspects would be confusing/disruptive for the flow of the Introduction. Nevertheless, if you think it should be relocated somewhere else I am happy to do so.

**1.3.5 Psoriasis and PsA: the same or distinct disease entities**

It still remains unclear whether psoriasis and PsA are distinct entities or manifestations of a single disease since commonalities but also differences are found at the pathophysiological and genetic level, as previously reviewed. The fact the PsA prevalence amongst psoriasis patients is greater than the expected by chance and that skin lesions precede joint affection in 90% of the cases have supported the fact that PsA may be an extracutaneous manifestation of psoriasis. One of the links between skin and joint inflammation has been speculated to be biomechanical. The skin and the enthesis share associated tissue-specific factors since both structures integrate two different tissues, epidermis and dermis or tendon and ligaments, respectively, and they undergo high stress concentration at the tissue interface usually followed by microinflammation (McGonagle *et al.* 2007). Studying correlation between skin and joint symptoms has yielded opposing results. Some studies have shown lack of correlation between skin and joint lesions thus supporting independence of the two diseases, whereas others have demonstrated correlation within a subpopulation with concomitant onset of skin and joint inflammation suggesting a shared pathophysiology under particular clinical setting (Jones *et al.* 1994; Elkayam *et al*. 2000).

Regarding the genetic predisposition, the greater heritability of PsA compared to psoriasis have supported some independence between the two entities. Likewise, despite cutaneous psoriasis and PsA sharing a large number of GWAS risk loci, differences in the strength of association have also been identified for some of them, importantly the PsA association with the *HLA-B\*27* allele and the stronger association of *HLA-Cw\*0602* with cutaneous psoriasis (as previously detailed in 1.3.2). This heterogeneity could indicate that some of the associations are involved in disease susceptibility whereas other may have a role in modifying disease expression, which together with the influence of environmental factors, shape incidence and disease severity (Ciocon and Kimball 2007).

In terms of pathophysiology, the role of TNF-α and the IL-23/IL-17 axis has been demonstrated to be important for the establishment and perpetuation of skin and joint inflammation. Transcriptomic studies in skin and synovial membrane from the same patients have demonstrated a stronger IL-17 signature in the skin, consistent with the greater efficacy of anti-IL17 biologic therapies in the treatment of skin lesions compared to joints symptoms (Belasco *et al.* 2015; Furue *et al.* 2018). The role of T cells, particularly mCD8+ expansion in epidermis and synovial fluid, has been well documented in psoriasis and PsA, respectively (Wrone-Smith and Nickoloff 1996; Costello *et al.* 1999). Nevertheless, it remains unclear whether differences in T cells biological subsets and T cell migration exist between skin and joints. Two studies revealed biologically distinct T cell populations in the two compartments based on the higher levels of expression of the cutaneous lymphocyte-associated antigen (CLA) in skin T cells compared to the joints (Pitzalis *et al*. 1996; Jones et al. 1997). Such finding could be the result of a homogenous activated T cell population undergoing distinct differentiation to travel to specific tissue sites. Moreover, T cell clonality studies have revealed polyclonal and oligoclonal T cell populations in skin and joints, highlighting common antigens driving T cell activation across different patients (Tassiulas *et al*. 1999; Borgato *et al*. 2002). However, when comparing clonality between skin and joints of the same individual only in three of them shared clones were found across tissues, inconclusively supporting the existence of a homogenous T cell population driving psoriasis and PsA simultaneously.

Altogether, epidemiological, pathophysiological and genetic data have shown evidence for psoriasis and PsA to be consider as a common disease, but they have also revealed heterogeneity between the two conditions. Additional research in the genetics, cellular and molecular components contributing to both pathophysiological processes will be required to further understand the overlap in the aetiology of cutaneous and articular disease.

1. **The verbal explanation of the results of GWAS / immunochip studies comparing psoriasis and PsA was better than the written and I would suggest updating this in the thesis.**

Old: “The first psoriasis and PsA GWAS were published in 2007, with a total of 63 independent genetic associations identified at genome-wide significance (p-value<5x10x10-8) to date (Table 1.3), explaining 28% of the estimated psoriasis and PsA heritability (Tsoi et al. 2017). The majority of studies have been performed in Caucasian European or North American cohorts, but increasing numbers of GWAS in large Chinese cohorts are also being published (Zhang et al. 2009; Sun et al. 2010; Yin et al. 2015). Early GWAS with moderate power confirmed association with loci overlapping the PSOR1 and PSOR2 genomic regions (Cargill et al. 2007; Strange et al. 2010). HLA-C has been consistently identified as the most significant locus with the greatest effect size. Additional MHC-I and MHCII associations with disease risk have been identified for HLA-A, HLA-B and HLADQA1 through step-wise conditional analysis (Okada et al. 2014).

The informativeness of GWAS was significantly enhanced with the use of the Immunochip genotyping chip, which covers 186 immune relevant loci identified in previous GWAS across different inflammatory diseases at a greater genotyping density (Tsoi et al. 2012). The psoriasis Immunochip study uncovered 15 new associations, including CARD14 at the PSOR4, also included meta-analysis with the largest available psoriasis cohorts at the time (Tsoi et al. 2012). This meta-analysis has since been further expanded, yielding 16 additional associations and reinforcing the importance of NF\_B and cytotoxicity pathways in disease pathophysiology (Tsoi et al. 2015b; Tsoi et al. 2017). Meta-analysis of GWAS across Caucasian and Chinese populations revealed four new associations, as well as population-specific effect or allelic heterogeneity in 11 loci, including MHC-I genes, demonstrating the value of a trans-ethnic approach, to further understand the heterogeneous genetic susceptibility to psoriasis in different

populations (Yin et al. 2015).

Conducting independent GWAS for psoriasis and PsA has shown differences in HLA-C and HLA-B allele frequencies. Interestingly higher association with HLA-B has been found in PsA individuals compared to psoriasis patients without joint inflammation (Winchester et al. 2012; Okada et al. 2014). GWAS associations for previously identified psoriasis loci such as IFNLR1, IFIH1 and NFKBIA were also found as genome-wide significant when using PsA cases, and PsA-specific independent signals for IL23R and TNFAIP3 demonstrated stronger associations compared to psoriasis patients only with cutaneous affection (Ellinghaus et al. 2010; Stuart et al. 2015). Furthermore, PsA GWAS using Immunochip has also revealed a specific association in chromosome 5q31 not reported previously (Bowes et al. 2015).

Overall, GWAS have demonstrated shared and distinct genetic architectures for psoriasis and PsA. Nevertheless, it is important to take into account potential imprecision in the phenotyping of cases, one of the many challenges in the systematic comparison between the two disease, and the impact that this may have on the results of these studies.”

New: “The first psoriasis GWAS published in 2007 by Cargill et al. has been followed by other studies with larger sample sizes and meta-analysis across different cohorts (Table 1.3). The vast majority of these GWAS have combined psoriasis (only cutaneous lesions) and PsA patients in the cases group. Up to date, psoriasis GWAS studies have identified a total of 63

independent associations at genome-wide significance (p-value<5x10-8) in European population, which only account for 28% of the overall estimated psoriasis and PsA heritability (Tsoi et al. 2017). The majority of studies have been performed in Caucasian European or North American cohorts, but increasing numbers of GWAS in large Chinese cohorts are also being published, increasing the number of independent associated loci to 70

(Zhang et al. 2009; Sun et al. 2010; Yin et al. 2015). Early GWAS with moderate power confirmed association with loci overlapping the PSOR1 and PSOR2 genomic regions (Cargill et al. 2007; Strange et al. 2010).

The informativeness of GWAS was significantly enhanced with the use of the Immunochip genotyping chip, which covers 186 immune relevant loci identified in previous GWAS across different inflammatory diseases at a greater genotyping density (Tsoi et al. 2012). The psoriasis Immunochip study uncovered 15 new associations, including CARD14 at the PSOR4, and also performed a meta-analysis incorporating the largest available psoriasis cohorts at the time (Tsoi et al. 2012). This meta-analysis has since been further expanded, yielding sixteen additional associations and reinforcing the importance of NF-κB and cytotoxicity pathways in disease pathophysiology (Tsoi et al. 2015; Tsoi et al. 2017). Meta-analysis of GWAS across Caucasian and Chinese populations revealed four new associations as well as population-specific effect or allelic heterogeneity in eleven loci (including MHC-I genes), demonstrating the value of a trans-ethnic approach to further understand the heterogeneous genetic susceptibility to psoriasis in different populations (Yin et al. 2015).

**Non MHC genome-wide associations in psoriasis and PsA**

A limited number of PsA GWAS have been conducted,being the best powered the Immunochip study performed by Bowes and colleagues (Liu et al. 2008; Hffmeier et al. 2010; Ellinghaus et al. 2012; Bowes et al. 2015b). These studies identified a total of thirteen PsA associated loci at genome-wide significance (p-value<5x10-8). The Immunochip PsA GWAS also unveiled PsA-specific associations at the IL23R, 5q31 and *PTPN22* loci, which did not show significant associations (not even at nominal p-value <0.05) in psoriasis patients (Bowes et al. 2015b; Bowes et al. 2015a).

Following the Immunochip PsA GWAS, Stuart and colleagues published a ﻿larger study than the one conducted in 2010 comparing associations between PsA patients and those psoriasis patients with skin symptoms that had not developed PsA for at least ten years (Table 1.3). This study revealed nine regions associated with PsA (*IFNLR1, IL23R, REL, IFIH1, TNIP1, IFNLR1, IL12B, TRAF3IP2, NFKBIA* and *TYK2*) and ten with cutaneous psoriasis (*TNFRSF9, LCE3C/B, IL13, TNIP1, IL12B, TRAF3IP2, TNFAIP3, IL23A, NFKBIA* and *NOS2*) at genome-wide significance. Amongst the psoriasis (combined cutaneous psoriasis and PsA together) GWAS variants reported by Stuart and colleagues, those nearby *TNFRSF9* and *LCE3A* showed stronger association with cutaeous psoriasis than PsA whereas variants at the IL23R and TNFAIP3 loci presented stronger association with PsA compared to cutaneous psoriasis. Moreover,the PsA significantly associated variants at IL23R (previously reported by Bowes et al., 2015) and TNFAIP3 loci were independent from previously identified psoriasis variants at the same loci.

Interestingly, PsA-specific signals previously identified by less powered studies failed to show significant differences in association between cutaneous psoriasis and PsA in the Stuart and colleagues analysis (including the 5q31 loci reported by the Immunochip PsA GWAS), and only the *PTPN22* locus reached nominal stronger association in PsA (Stuart et al. 2015). The latest phenotype-specific association analysis published by Patrick and colleagues in 2018 has increased the number of genome-wide association with PsA and cutaneous psoriasis to thirteen and fifteen, respectively, with all of these loci previously identified as psoriasis associated (Patrick et al. 2018).

**Differences in the MHC associations between psoriasis and PsA**

Regarding MHC associations with psoriasis, *HLA-Cw\*06:02* has been consistently identified as the most significantly associated locus with the greatest effect size in psoriasis and also in independent analysis of cutaneous psoriasis and PsA compared to controls (Okada et al. 2014; Bowes et al. 2015b; Stuart et al. 2015). Step-wise conditional analysis has revealed additional HLA-I and HLA-II associations, including *HLA-C\*12:03*, HLA-B amino acid positions 67 and 9, HLA-A amino acid position 95, and HLA-DQA1 amino acid position 53, with similar results found in the independent analysis of cutaneous psoriasis and PsA compared to controls (Okada et al. 2014; Bowes et al. 2015b).

Dissecting the HLA-Cw\*06:02 association in psoriasis and PsA has remained challenging. *HLA-Cw\*06:02* has been demonstrated to increase risk of PsA when compared to controls. However, in the comparison between cutaneous psoriasis and PsA *HLA-Cw\*06:02* appeared as more strongly associated with cutaneous psoriasis and showed a protective effect for PsA within psoriasis (Whinchester2012; Eder et al. 2012; Stuart et al. 2015). Since *HLA-Cw\*06:02* is also associated with earlier age of onset in psoriasis, accounting for age of onset in the comparison of cutaneous psoriasis vs PsA *HLA-Cw\*06:02* did not show protective association in PsA (Bowes et al. 2017). Instead the most significant MHC association when comparing both phenotypes was HLA-B aminoacid 97 (asparagine, mostly found in HLA-B27), which differentiated PsA from cutaneous psoriasis (Bowes et al. 2017).

Overall, GWAS have demonstrated shared and distinct genetic architectures in MHC and non-MHC loci between cutaneous psoriasis and PsA, adding a layer of complexity in the understanding of the similarities and differences between the two pathologies.”

1. **In the section on missing heritability, how polygenic risk scores may capture some of this could be included.**

In Chapter 1 subsection 1.3.5: Limitations of GWAS, I have added a paragraph explaining the use of polygenic risk scores to capture some of the missing heritability and the use of polygenic risk scores to assess PsA risk in psoriasis patients.

New: “To overcome the need of increasingly larger cohorts, the calculation of genome-wide polygenic risk scores making use of existing GWAS data has started to be implemented in the study of complex diseases. New methodologies for the calculation of polygenic risk scores leverage the aggregation of GWAS variants under the genome-wide significant threshold to predict the genetic liability of disease on the basis of an individuals genotype and identify subgroups of the population at high risk (Khera et al. 2018). A validated genome-wide polygenic risk score across five common diseases has recently shown successful results in coronary artery disease, identifying 8% of the population at greater than three-fold increased risk (Khera et al. 2018). In psoriasis, a polygenic risk score based on 200 genetic markers has been developed to predict the risk of PsA amongst psoriasis patients, showing comparable accuracy (0.82) to that of polygenic risk scores used to discriminate IBD subtypes (Patrick et al. 2018).”

1. **Pros and cons of positive versus negative selection could be included.**

In Chapter 2 subsection 2.2.4 I have added a paragraph explaining why positive selection using magnetic-labelled antibodies was chosen over depletion, the advantages of the positive selection approach (step-wise isolation of several populations and higher purity) and also some of the limitations (mainly activation of T cells).

New: “MACS separation was chosen over fluorescence-associated cell sorting (FACS) due to time and logistic constraints during sample processing. Moreover, MACS positive selection was preferred over depletion as it allowed step-wise isolation of the four cell populations of interest with an expected purity of approximately 99%. Nevertheless, using positive selection with antibodies targeting CD4 and CD8 can cause T cell activation to some extent as both surface markers are co-receptors involved in the recognition by the T cell receptor (TCR) of antigens presented through MHC-I or MHC-II molecules, respectively. This pre-activation is of particular relevance when performing downstream cell culture and assays.”

1. **Some discussion about why peaks from CD4 cells always appear lower than CD14 could be included.**

I was a bit unsure about this suggested correction. The reason why in Figure 3.1 CD4+ T cell signal and peaks at the promoter of GAPDH and NOP2 genes (used as an example) are lower and less well-defined compare to the CD14+ ones is due to the lower CD4+ sample quality. Previously in the chapter, CD4+ T cells ATAC-seq libraries have been shown to have a lower sample quality in terms of signal-to-noise measures by enrichment of ATAC-seq reads across all the Ensembl TSSs compared to the CD14+ monocyte ones. The link between lower quality and lower signal/peaks in Figure 3.1 is explicitly mentioned in the following paragraph (page x): “Fold-enrichment signals over the TSS ranged from 5 to 7 for the CD4+ samples, and were much higher (17 to 20) in the CD14+ samples. The lower sample quality of the CD4+ compared to CD14+ samples indicated by the TSS enrichment values were further evidenced by visualising the ATAC-seq read pile up at the promoters of the glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) and the NOP2 Nucleolar Protein (*NOP2*) gene, showing more background reads and lower signal for the CD4+ samples (Figure 3.1C).”

1. **Fig 3.3 was incorrectly labelled and should be corrected.**

Old: “Figure 3.3: Peak calling filtering using IDR analysis in ATAC-seq samples. For each of the sequencing depths tested (from 5 to 30 million reads after filtering), an illustration of the percentage of peaks sharing IDR rank between the two pseudoreplicates is shown when using different p-value filtering thresholds in CTL2 (A) CD14+ monocytes and (B) CD4+, two representative samples differing in quality for this analysis.”

New: “Figure 3.3: Peak calling filtering using IDR analysis in ATAC-seq samples. For each of the sequencing depths tested (from 5 to 30 million reads after filtering), an illustration of the percentage of peaks sharing IDR rank between the two pseudoreplicates is shown when using different p-value filtering thresholds in CTL2 (A) CD4+ T cells and (B) CD14+ monocytes, two representative samples differing in quality for this analysis.”

1. **In the chapter comparing fresh vs frozen processing, some discussion as to whether this is feasible for all studies would be useful.**

I have added the following paragraph acknowledging the impossibility of using freshly processed samples for certain types of studies, in which case cryopreservation can be considered together with formaldehyde fixation.

New: “Fresh sample processing may not always be feasible, for example in studies aiming to maximise the use of existing biobank cryopreserved samples to achieve large sample sizes or in longitudinal studies looking at the same individual at different points in times (e.g pre- and post- disease or treatment). In such instances, studying chromatin accessibility in cells isolated from cryopreserved PBMCs represents a suitable alternative, where acknowledging that differences may be found when comparing to fresh processed samples can be useful when trying to validate these results. Importantly, the Buenrostro et al. 2013 ATAC-seq protocol with some modification has been successfully used in formaldehyde fixed samples and it may represent a better alternative to preserve freshly purified cell populations to study the epigenetic landscape of clinical samples retrospectively (Corces et al. 2017; Chen et al. 2016).”

1. **In the gene expression profile chapter discussion, it would be useful to include a brief discussion about separating cause from effect of the transcriptional changes.**

In Chapter 4 Section 4.3.X name I have added the following paragraph addressing separation of cause from effect in the transcriptional changes observed when comparing psoriasis circulating immune cells to healthy controls.

The characterisation of the transcriptomic differences between patients and controls in circulating immune cells conducted in this thesis provides some key insights into pathogenesis of disease. Nevertheless, separating those changes in disease cause and consequence remains challenging.

Integration of gene expression profiles with GWAS could help to identify which of those changes may be causal due to their link with genetic variants predisposing to disease. However, this approach is limited by the lack of complete functional characterisation of intergenic GWAS variants and the high number of susceptibility loci with small effect that have not yet been identified

1. **In the overall Discussion, a brief concluding paragraph discussing which are likely to be the driving cells based on this work and why monocytes were selected for scRNA-seq would be helpful. It would also be useful to summarise which technique(s) should be prioritised going forward and the immediate next steps.**
2. **Reference errors need corrections**

I have corrected all the reference mistakes marked in the hard copy of the thesis, which included wrong format of author names, missing journal name, volume, issue or page numbers.