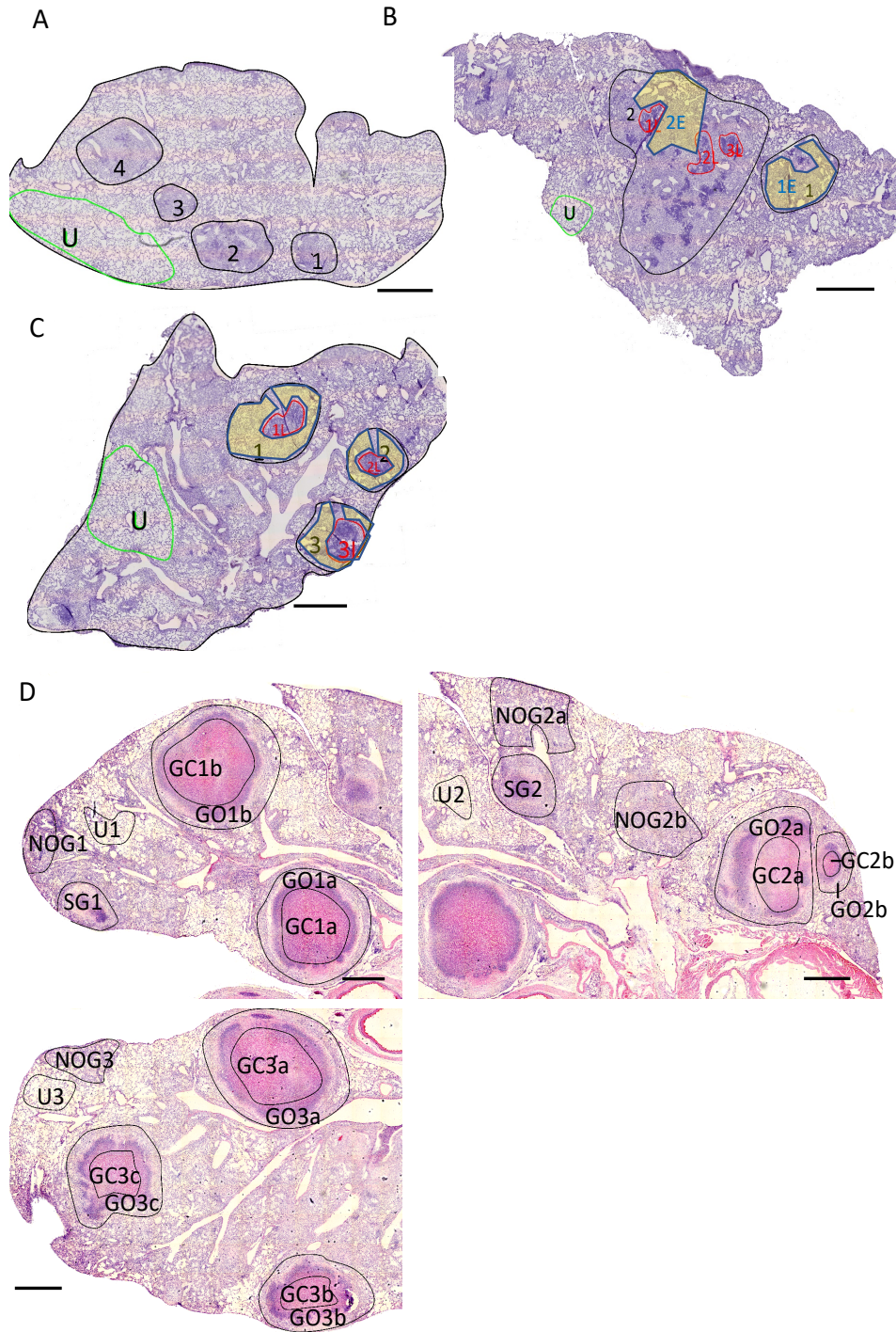


Supplementary Information

Spatial and temporal localization of immune transcripts defines hallmarks
and diversity in the tuberculosis granuloma

Carow et al.



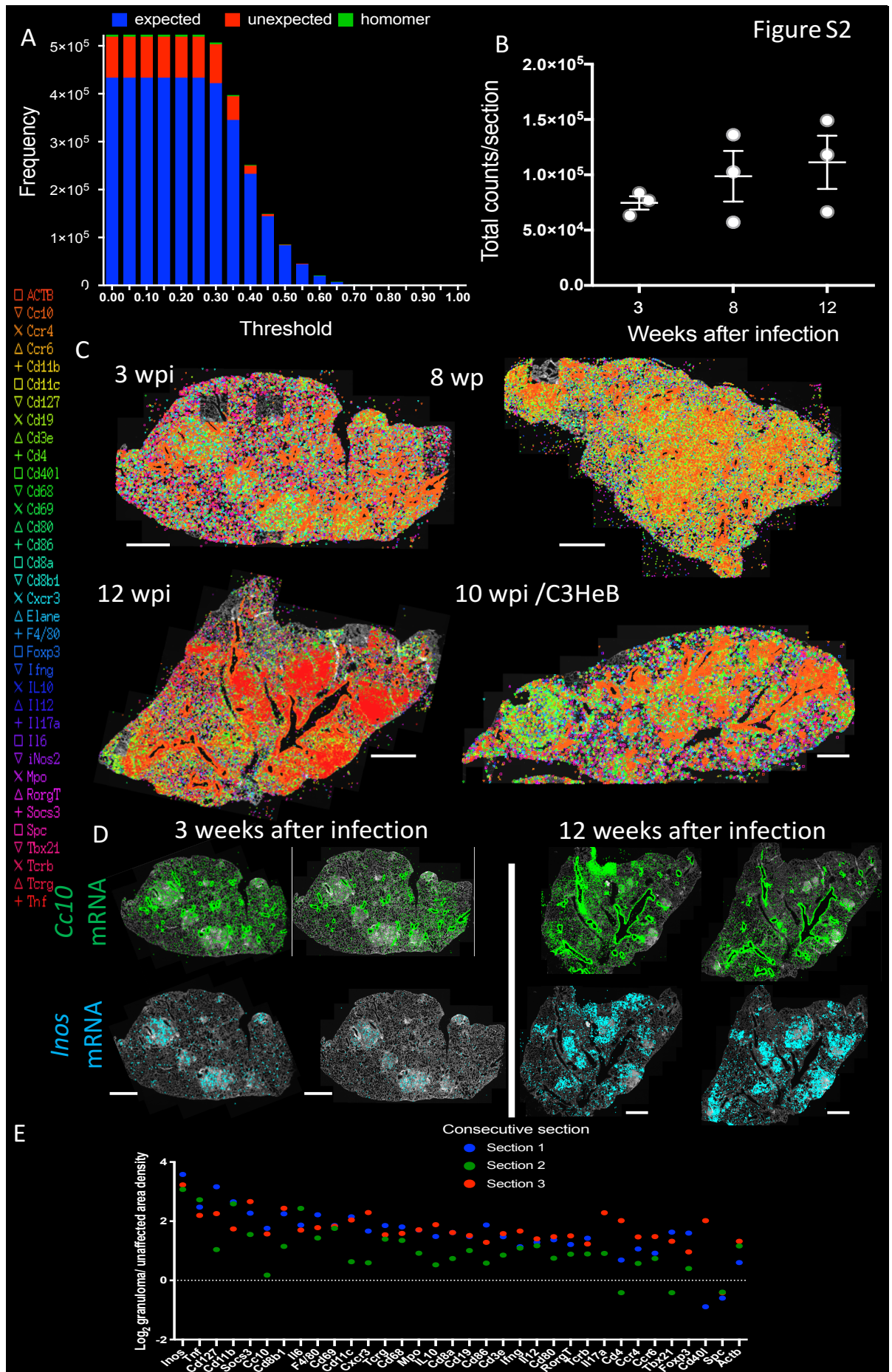
Supplementary Figure 1

Histological features of analyzed tissue sections

H&E stained sections from lung lobes from C57Bl/6 mice at 3 (A), 8 (B), 12 (C) wpi. In D, a section from C3HeB/FeJ mice 10 weeks after infection are shown. Areas that are marked were found and analyzed throughout the paper for all three consecutive sections. The areas occupied by lesions are marked and numbered in black. Unaffected

areas for all lungs are marked in green. In B and C, the lymphoid rich areas are marked in red and epitheloid areas shaded in yellow.

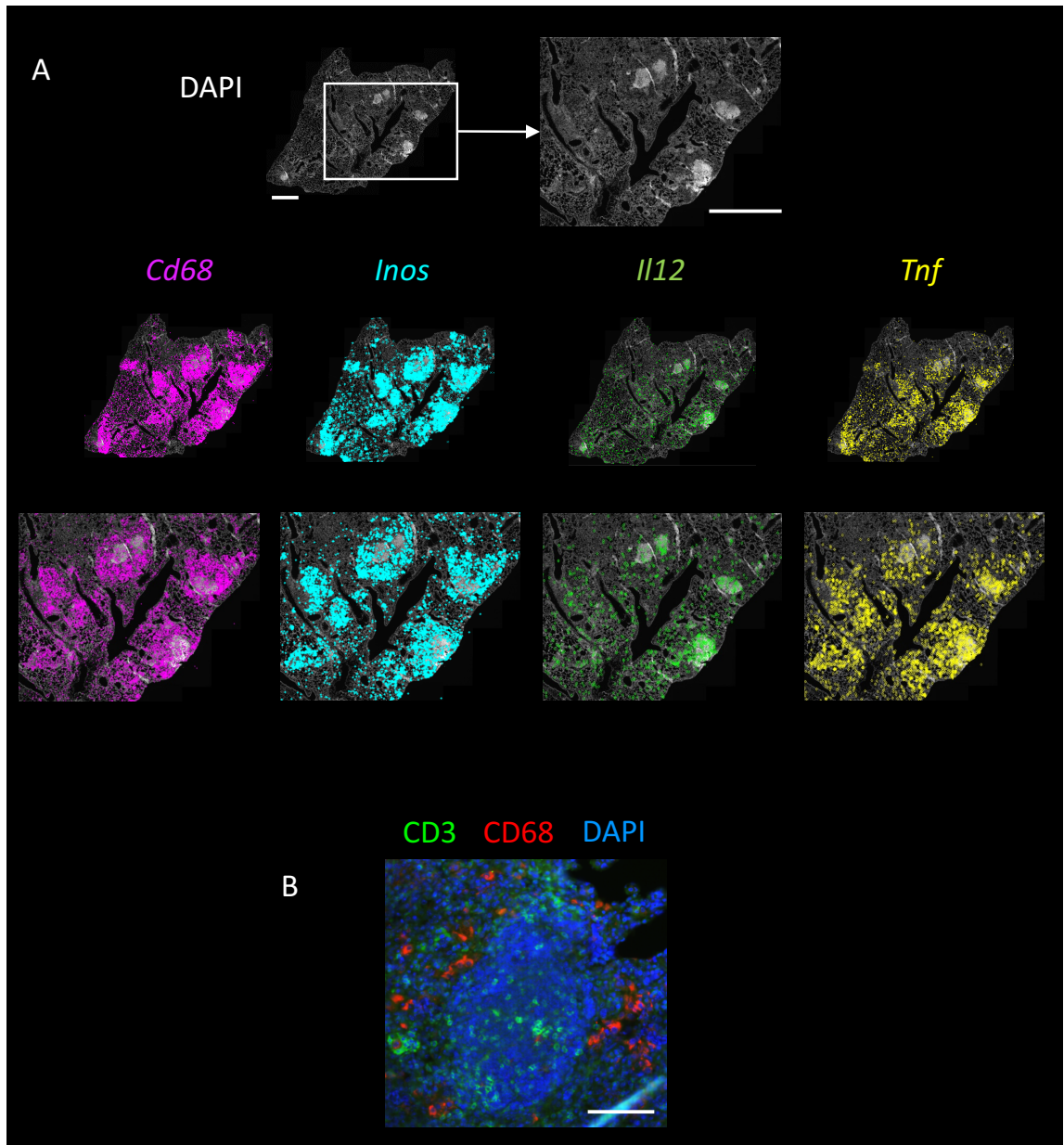
In D encapsulated granulomas and non-encapsulated lesions can be observed in three regions of a C3HeB/FeJ lung. U: unaffected area; GC: granuloma center; GO: granuloma outline; NOG: non-organized granuloma and SG: small necrotic granuloma. Scale bar: 1000 μm .



Supplementary Figure 2

Specificity and reproducibility of the in situ sequencing

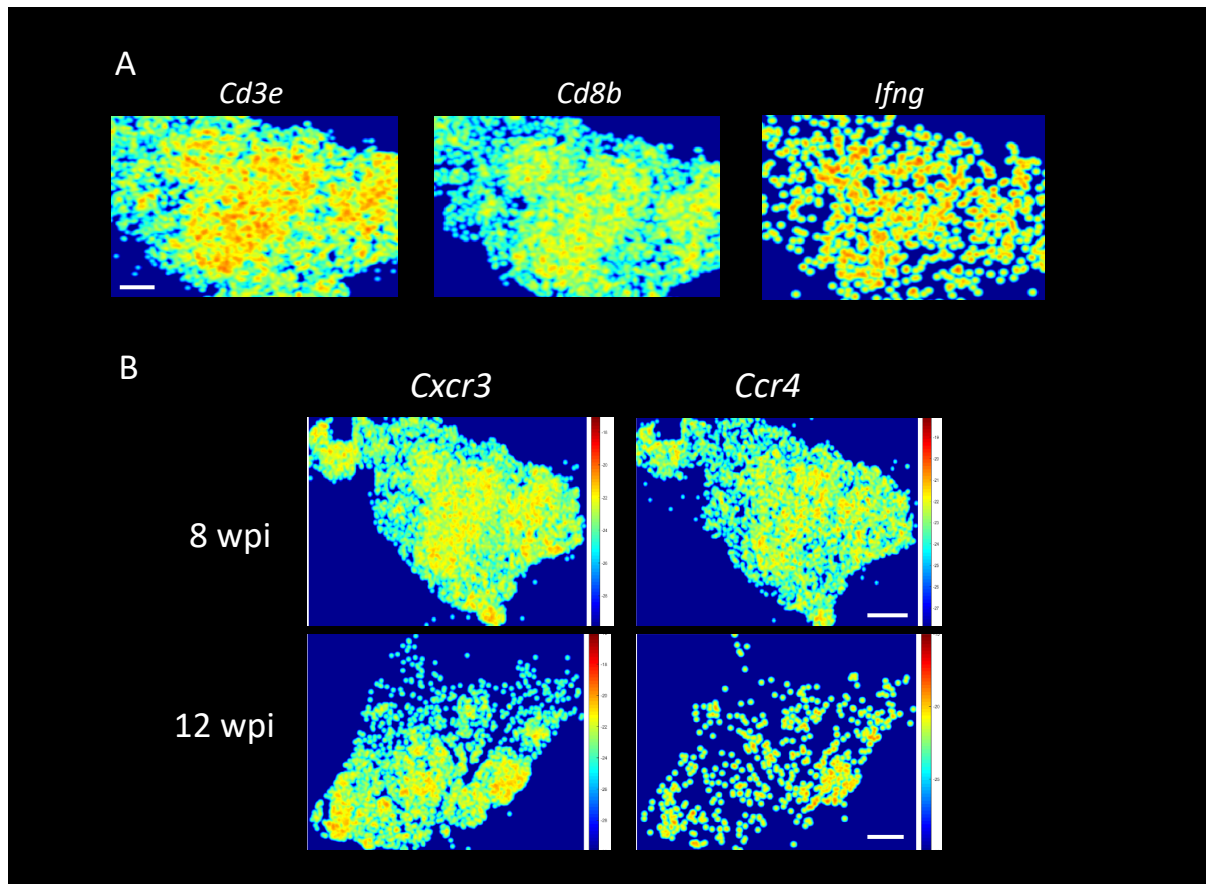
- A. The density of all barcoded sequences in relation to unexpected and homomers (unspecific) reads in each area at different signal intensity thresholds are depicted. The unexpected barcodes showed lower scores than true expected barcodes, and could thus be excluded from further analysis by setting a cut-off at a fixed threshold (0.45).
- B. The mean \pm SEM of the total number of amplified sequences per section in the different sections analysed are shown.
- C. Raw data showing the location of all decoded sequences in paraformaldehyde-fixed lung sections from *M. tuberculosis* infected mice obtained at the indicated time points after infection. Each dot represents one decoded sequence. The sequences are aligned against a DAPI staining. Note the differential localization of sequences. Scale bar: 1000 μ m.
- D. Raw data showing the location of *Cc10* and *Inos* decoded transcripts called from two consecutive lung sections from a *M. tuberculosis*-infected mouse obtained at the indicated time points after infection. Each dot represents one decoded sequence. The sequences are aligned against a DAPI staining. Note the differential localization of both sequences and the similarity of consecutive images. Scale bar: 1000 μ m
- E. The relative fold increase in the density of individual sequences in granuloma vs unaffected lung areas from consecutive lung sections at 3 wpi are shown. A dotted line indicates the mean relative density of *Actb* mRNA. We have excluded from this graph transcripts showing either no signals in the unaffected areas or those in which more than 2 different areas showed no signals. Source data are provided as a Source Data file.



Supplementary Figure 3

Spatial distribution of myeloid transcripts in lungs from M. tuberculosis-infected mice

- A. The raw signals for *Tnf*, *Inos*, *Il12* and *Cd68* (A) mRNAs in lungs from *M. tuberculosis* infected mice. Note in A that while *Inos* and *Cd68* sequences overlap, this is not the case for *Cd68* and *Il12* transcripts which locate in different areas of the granuloma, better shown in the zoomed area. Scale: 1000 μ m
- B. Double immunolabelling for CD3 and CD68 in DAPI stained C57BL/6 lung section 12 wpi with *M. tuberculosis*. A representative micrograph from 3 independent samples is shown. Scale: 100 μ m

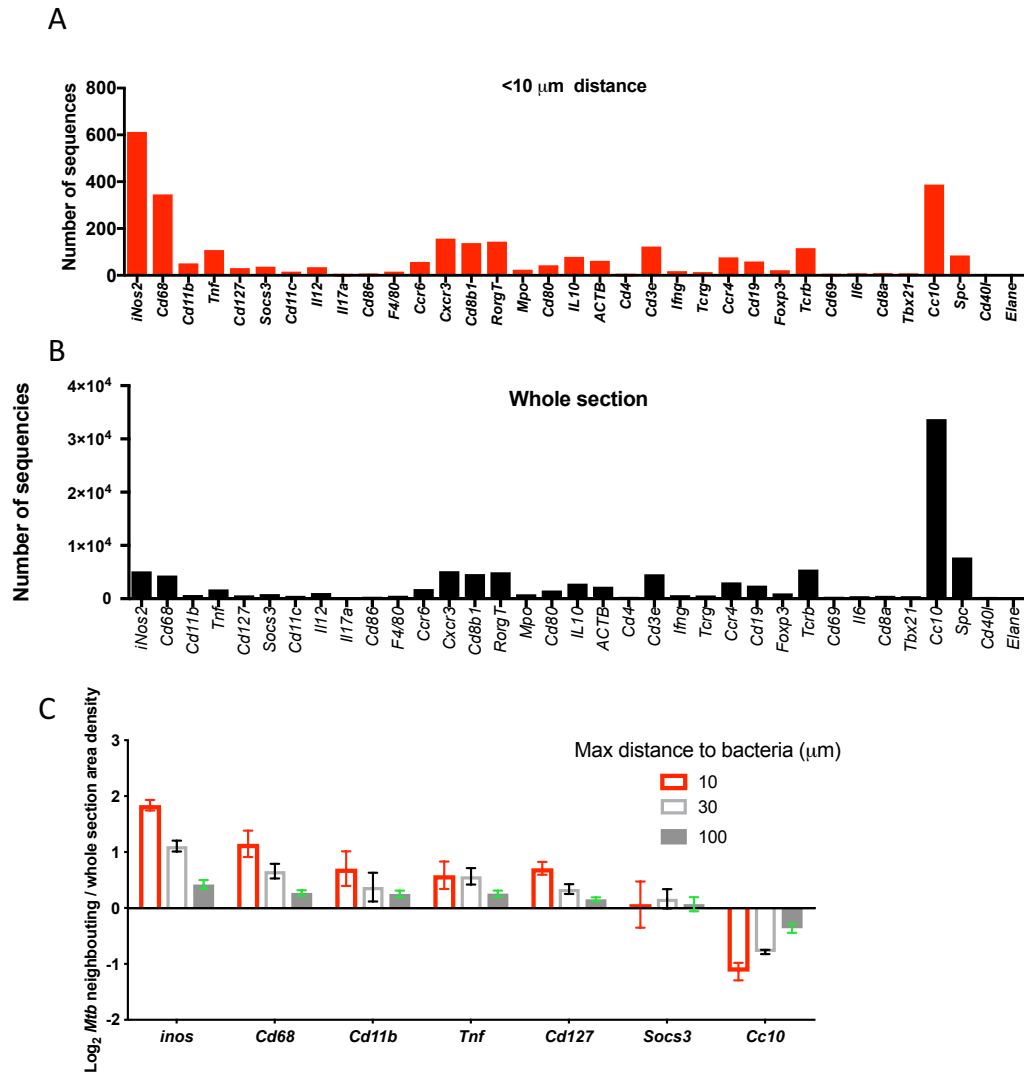


Supplementary Figure 4

Spatial distribution of lymphoid transcripts in lungs from M. tuberculosis-infected mice

A. Pseudocolor log₂ density plots of *Cd3e*, *Cd8b* and *Ifng* mRNA for one representative lung at 8 wpi. Scale: 1000 μm

B. Density plot of *Cxcr3* and *Ccr4* sequences in lungs at 8 and 12 wpi. Scale bar: 1000 μm.

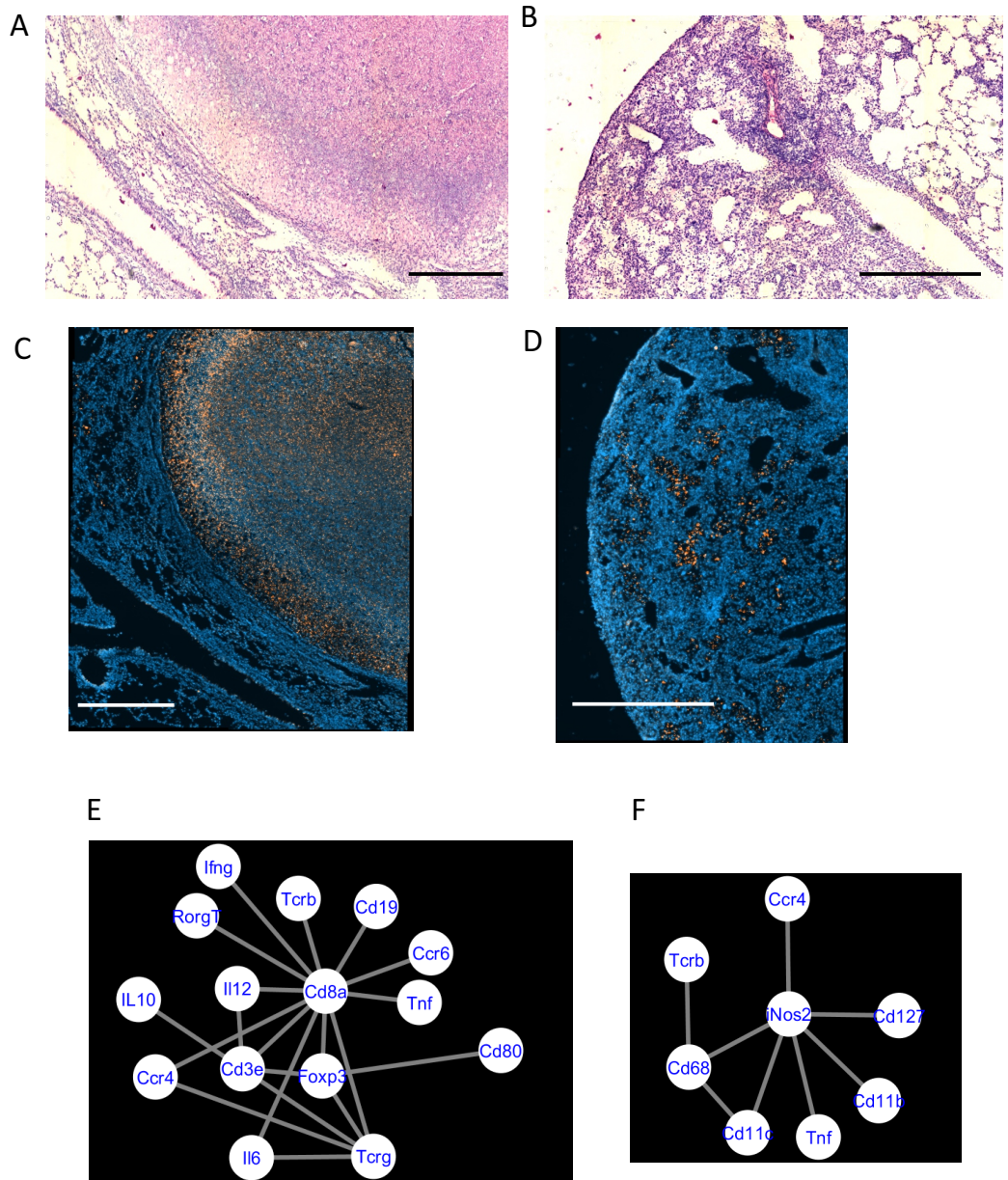


Supplementary Figure 5

Identification of sequences expressed at different distances from M. tuberculosis in the lung

- The transcript counts for each sequence located at <10 μm from *M. tuberculosis*.
- The transcript counts for each sequence in the whole area of the section in (A) are depicted.
- The frequency of selected transcripts located at <10, 30 or 100 μm from *M. tuberculosis* bacteria in relation to the total sequence count for each distance was determined. The fold increase of such frequencies was compared to the

frequencies of each sequence in the whole section. The mean fold increase at each distance vs the whole section of 7 selected transcripts \pm SEM of in 3 consecutive sections is depicted. Source data are provided as a Source Data file.



Supplementary Figure 6

In situ sequencing of the encapsulated granulomas

A-B. Micrograph showing the rim area from a necrotic encapsulated granuloma, showing the presence of a capsule, surrounding a layer of epitheloid and foamy cells and delineating compressed lung parenchyma and epitheloid and lymphoid cells (A). Area showing a perivascular lesion with numerous lymphocytes and epitheloid cells in the same H& E stained section from a C3HeB/FeJ lung (B). Scale bar: 500 μ m.

C-D. Auramine-Rhodamine staining of C3HeB/FeJ encapsulated (C) and non-encapsulated lesions (D). Note bacteria localizes in the rim of the encapsulated lesion. Scale bar: 500 μm .

E-F. The networks of co-expressed transcripts in an encapsulated granuloma (E) and non-encapsulated cellular granuloma (F) are shown.

Supplementary tables

Supplementary Table 1

Counts and statistical analysis of paired sequence combinations

Sequences	RIL count- section 1	p value RIL section 1	RIL count section 2	p value RIL section 2
<i>Ccr6-Cd19</i>	105	0	302	0
<i>Ccr6-Cd3e</i>	61	0	126	0
<i>Ccr6-Cd68</i>	45	8,63E-05	99	2,36E-07
<i>Ccr6-Cxcr3</i>	16	5,46E-05	30	1,11E-16
<i>Ccr6-IL10</i>	9	1,89E-09	43	0
<i>Ccr6-Il12</i>	14	8,88E-16	22	0
<i>Ccr6-RorgT</i>	20	3,65E-08	56	5,77E-15
<i>Cd19-Cd3e</i>	130	0	323	0
<i>Cd19-Cd68</i>	81	3,26E-05	229	0
<i>Cd19-Cd8b1</i>	25	2,35E-12	37	0
<i>Cd19-Cxcr3</i>	31	1,34E-07	70	0
<i>Cd19-Il12</i>	18	5,74E-11	67	0
<i>Cd19-RorgT</i>	43	0	106	0
<i>Cd19-Socs3</i>	12	9,06E-08	21	0
<i>Cd19-Tnf</i>	27	3,84E-05	140	0
<i>Cd3e-Cd68</i>	100	4,33E-05	221	1,22E-15
<i>Cd3e-Il12</i>	25	5,55E-16	32	0
<i>Cd68-iNos2</i>	146	2,33E-09	286	0
<i>Ifng-iNos2</i>	16	3,07E-07	22	0

Example of the counts and p values of statistically significant sequence pairs (χ^2 test) at 10 μm in 1 lymphoid region (RIL see Supplementary Figure 1) of 2 consecutive lung tissue sections at 12 wpi are here shown.

Supplementary Table 2

cDNA primers

name	sequence
mSoes3_prim_2	CTGGAAGTGTCCCGGCCGGTC
mSoes3_prim_3_AP2	CTCTCTTGGGGGTACTCCCG
mSoes3_prim_4_AP3	TGGGCTCCAAGATGGCTCAT
mMpo_prim_2	GTGTTGGTTAAACTGAGTG
mMpo_prim_3	AGTTGAGGCCAGTGAAGAAG
mMpo_prim_4	GTGCCAACTCCAGGTTCTTC
mIl10_prim_2	AGTGCTGAGCCAGGCATGAT
mIl10_prim_3	TCAAATTCATTCATGGCCTT
mIl10_prim_4	AAGACCCATGAGTTTCTTCA
mIfng_prim_2	AGGAGGAGAAGCCCAGAACT
mIfng_prim_3	TCCGGCAACAGCTGGTGGAC
mIfng_prim_4	CTCTTGAGACACTGCTTTCT
mFoxp3_prim_2	CATATACCAGGCACAGTGCC
mFoxp3_prim_3	GACCAGGCCGGGAGCACACT
mFoxp3_prim_4	GGCATTGCTTGAGGCTGCGT
mCxcr3_prim_2	CAGGGCGGGGAGTCAGAGAA
mCxcr3_prim_3	GGGGTCCCTGCGGTAGATCT
mCxcr3_prim_4	TGGTCAGAGCGGCCAGGCG
mCd80_prim_2	AAATGGAACAGAGTGTCTTT
mCd80_prim_3	ATATAAAGTCCGGTTCTTAT
mCd80_prim_4	GACGACGACTGTTATTACTG
mCd19_prim_2	CAGGAAGGGTGTGACTGGT
mCd19_prim_3	CCCGAGGGAGGCGTCACTTT
mCd19_prim_4	CCCTTGTGGGACACCATGGA
mCcr6_prim_2	AGAACTGTGAAGTTGTTTAC
mCcr6_prim_3	ACTGCCACACAGATGACCTT
mCcr6_prim_4	AGATGTAGCTTCCGAGTAA
mCcr4_prim_2	CTCCGGGTACCAGCAGGAGA
mCcr4_prim_3	GAAGAGTTGGGTGATGTACT
mCcr4_prim_4	ACTCCTGCCTCTGCCTCCAC
mCd11b_prim_2	GCCAGGTCCATCAAGCCATC

mCd11b_prim_3	CCAGCATCCTTGTTTTTTAA
mCd11b_prim_4	TGTTTCACATTTCTGCATCA
mCd3e_prim_2	CAGGATGCCCCAGAAAGTGT
mCd3e_prim_3	AGTCTGGGTGGGAACAGGT
mCd3e_prim_4	AGGAGGTATGGGGTGTGTAA
mF4/80_prim_2	AAGAGGAGCAGCCAAAAGCC
mF4/80_prim_3	GAGCCTGGTACATTGGTGCA
mF4/80_prim_4	ACAGCAGGAAGGTGGCTATG
mCd4_prim_2	CTCGAGACTTTGCAAACAGG
mCd4_prim_3	GTATCTTGAGGGTGAGTGGG
mCd4_prim_4	TCTTGCAAATTCAAAAGAGA
mCd8b1_prim_2	CATCTTGGCGCTTTTGGGGA
mCd8b1_prim_3	CTGGGTCTCTGGGTGGGGGA
mCd8b1_prim_4	TCCAGGGTCCCGGCTAGCTC
mCd8a_prim_2	TGGGGGAGGCGGTAGGGTC
mCd8a_prim_3	CTAGCTCTGGTGTTACAGTC
mCd8a_prim_4	CAGAAAGAGCCTGGGAATCT
mCd11c_prim_2	CCAGGTACAGCTCATGACTG
mCd11c_prim_3	CTGATTCTCAATATCCTTCA
mCd11c_prim_4	TAGTTGGGTCTTGGGGCTTT
mCd127_prim_2	TATAGCGAAAGCTCTACCCA
mCd127_prim_3	TGAATCTGGCAGTCCAGGAA
mCd127_prim_4	GACAGGTTTCATGGCAAGAGA
mI17a_prim_2	CAGATGAAGCTCTCCCTGGA
mI17a_prim_3	TGGGGGTTTCTTAGGGGTCA
mI17a_prim_4	AACATAAACTAAGTTTGGT
miNos2_prim_2	ATCTCTCCACTGCCCCAGTT
miNos2_prim_3	TCCAGGATGTTGTAGCGCTG
miNos2_prim_4	GTCTAAAGGCTCCGGGCTCT
mTerg_prim_2	GCAGGAAGTGCTCTGCAGGC
mTerg_prim_3	ATGACATCGGGAAAGAACTT
mTerg_prim_4	TTATGGCAGTGAGGATGAAA
mRorgT_prim_2	AGCTCCCGAGATGTCCGGTG
mRorgT_prim_3	ACTTCCTCTGGTAGCTGGTC
mRorgT_prim_4	TGTCAGTCTGTTTTTTATTT

mTerb_prim_2	GGGTGCCTGCCGCAAAGTAG
mTerb_prim_3	TCCAGGGTCCCCGGCTAGCTC
mTerb_prim_4	CTGGGTCTCTGGGTGGGGGA
mI16_prim_3	TCGTTCTTGGTGGGCTCCAG
mI16_prim_4	TTGTTCTTCATGTACTCCAG
mI16_prim_5	ACTTATACATTCCAAGAAAC
mI112_prim_3	GCAAGGGTGGCCAAAAAGAG
mI112_prim_4	TTGTCTAGAATGATCTGCTG
mI112_prim_5	GAGAGAAGCGATGGAGGGGA
mCd68_prim_3	TGCCTTCTCTTGGGAAGAGGA
mCd68_prim_4	AGGATTCGGATTTGAATTTG
mCd68_prim_5	AGTGGACTGGGGCAGATGCT
mTnf_prim_4	TTTCTGTTCTCCCTCCTGGC
mTnf_prim_5	AAGAGAACCTGGGAGTAGAC
mTnf_prim_6	CATCTTGTGTTTCTGAGTAG
mSCGB1A1_prim	TATCTCTGAAATCCAGTGAG
mSftpc_prim	CAGGTCTCTCCCGGAAGAAT
Cd69_prim1	GTGCTTTTGTFTTCTTCCTT
Cd69_prim2	ATCCTTGATGTGATTAGCAG
Cd69_prim3	ATGTCTGATTAGCTTCATTT
Cd40lg_prim1	TCAGATTGTAAGTTCTTAGG
Cd40lg_prim2	CAGTCACGTTGACAAACACA
Cd40lg_prim3	CTTGAGTGTAGACATAATAG
mCD86_ap1_prim	CCAGATCTTAAGAGTCTGCA
mCD86_ap2_prim	CAAATATAACCACTCCCATCC
mCD86_ap3_prim	CTGAAGTTGGCGATCACTGA
mElane_ap1_prim	CATTATGGCTTCGGATAATG
mElane_ap2_prim	TGGGCCACCTGCACGTTGGC
mElane_ap3_prim	GCCAGAGTCCGGCTGGATAG
mI112b_ap1_prim	GAGTGTGGCCATTGTGTCCT
mI112b_ap2_prim	GGTTGTCAAAAAGCTAATG
mI112b_ap3_prim	GCACGTGAACCGTCCGGAGT
mTbx21_ap1_prim	AGTGATGCAAAACAGAAGAA
mTbx21_ap2_prim	CCCTGTTCTCTGAGGATCC
mTbx21_ap3_prim	GTCGGGTCTGTGCGCCCGG

mCd4_5_prim	CTTGAGGTCTTTGGTGGACT
mCd4_6_prim	AGAAGGAGATCCAGGGCTGG
mCd4_7_prim	GAACCCTTAGGTTGGACATG

Supplementary Table 3

Padlock probes (all padlock probes are 5' phosphorylated)

name	sequence
mSocs3_GGAG	TCCAACGTGGCCACCCTCTTCCTCTATGATTACTGACTGCGTCT ATTTAGTGGAGCCGAGCTATCTTCTTTTACTGAGCCGACCTCTCTCC
mMpo_GAGG	CGATGACCCCTGCCTCCTCTTCCTCTATGATTACTGACTGCGTCT ATTTAGTGGAGCCGAGGCTATCTTCTTTTGCCCTTTGACAGCCTGCA
mIl10_CCGG	GCTAACCGACTCCTTAATGCTTCCTCTATGATTACTGACTGCGT CTATTTAGTGGAGCCCCGGCTATCTTCTTTGACCAGCTGGACAACATAC T
mIfng_CACA	GCTGTTTCTGGCTGTTACTGTTCCCTCTATGATTACTGACTGCGTC TATTTAGTGGAGCCCACACTATCTTCTTTCTTTGCAGCTCTTCCTCATG
mFoxp3_CAAC	CCTCCCACCACCTTCTGCTTTCCTCTATGATTACTGACTGCGTCT ATTTAGTGGAGCCCAACCTATCTTCTTTGACAACCCAGCCATGATCAG
mCxcr3_AGAA	GCCCTCTACAGCCTCCTCTTTTCCTCTATGATTACTGACTGCGTC TATTTAGTGGAGCCAGAACTATCTTCTTTTGACAGAACCTTCCTGCCA
mCd80_ACCA	CCGGGGCACATACAGCTGTTTCCTCTATGATTACTGACTGCGTC TATTTAGTGGAGCCACCACTATCTTCTTTCTGGGCTGGTCCCTTCAGA
mCd19_ACAC	GGACTCCTCACCTGTCTCTTTTCCTCTATGATTACTGACTGCGTC TATTTAGTGGAGCCACACCTATCTTCTTTTGCTGCCATGCCTCCC
mCcr6_AAGA	GTTACTCATGCCACCAACTTCCTCTATGATTACTGACTGCGT CTATTTAGTGGAGCCAAGACTATCTTCTTTACCCTACCGTTCTGGGCA
mCcr4_AACC	TTTGCTGTTTCGTCCTGTCCCTTCCTCTATGATTACTGACTGCGTC TATTTAGTGGAGCCAACCCTATCTTCTTTCTGAACCTGGCCATCTCGGA
mCd11b_AATT	AAACCCTAGCCCAAGATTCTCTATGATTACTGACTGCGTCTAT TTAGTGGAGCCAATTCTATCTTCTTTACCTTCAATGACTTCAAGAG
mCd3e_ACGT	CACCCTGCTACTCCTTTCTCTATGATTACTGACTGCGTCTATTT AGTGGAGCCACGTCTATCTTCTTTATCCAGCCCTCCGAG
mF4/80_ACTG	TGTCTGCTCAACCGTTCCTCTATGATTACTGACTGCGTCTATTTA GTGGAGCCACTGCTATCTTCTTTCTTTTCATCTTCCTCATTAC
mCd4_AGTC	TGCACCGTGACCCTGTCCTCTATGATTACTGACTGCGTCTATTTA GTGGAGCCAGTCTATCTTCTTTTCAGCGACTTCTGGAAC
mCd8b1_ATAT	GTTCAAACCAACCATACGTCCTCTATGATTACTGACTGCGTCTA TTTAGTGGAGCCATACTATCTTCTTTCCCTTCGTCCTGCTG
mCd8a_ATCG	AAGTGAACCTACTACTACCTCCTCTATGATTACTGACTGCGTC TATTTAGTGGAGCCATCGCTATCTTCTTTGTGCCAGTCCTTCAGA
mCd11c_ATGC	CTGCCACCAACCCTTTTCCTCTATGATTACTGACTGCGTCTATTTA GTGGAGCCATGCCTATCTTCTTTGCCGTGCCCTTGCTG
mCd127_ATTA	CTTCTCTATTCTTTCTCTTTCCTCTATGATTACTGACTGCGTCTA TTTAGTGGAGCCATTACTATCTTCTTTTCACATACAAGCGTGCTT
mIl17a_CATG	AGGCAGCCTAAACAGATCCTCTATGATTACTGACTGCGTCTATT

	TAGTGGAGCCCATGCTATCTTCTTTCCCTCGATTGTCCGCC
miNos2_GACT	AAGGCCACATCGGATTTCCCTCTATGATTACTGACTGCGTCTATT TAGTGGAGCCGACTCTATCTTCTTTTCATGACACTCTTACCAC
mTerg_GCCG	GACCTACCTTTGTCTCCTTCCCTCTATGATTACTGACTGCGTCTAT TTAGTGGAGCCGCCGCTATCTTCTTTAAATCTCCATAAGGCTGG
mRorgT_GCGC	TCCCTTTCTGCACTCTATTCCCTCTATGATTACTGACTGCGTCTAT TTAGTGGAGCCGCGCCTATCTTCTTTCCCTCACCAGCCTT
mTcrb_GTAC	GTCTTGTCTGCCACCATCCTCTATGATTACTGACTGCGTCTATTT AGTGGAGCCGTACCTATCTTCTTTACATCCTATCAACAAGGG
mActb_GCAT	TTACACCTTTCTTTGACAATCCGAGTAGTCTTTGTGCGTCTATT TAGTGGAGCCGCATCTATCTTCTTTGACTGTTACTGAGCTGCGTT
mI16_CGAT_1	CTCTACGAAGAACTGACAATTCCTCTATGATTACTGACTGCGTC TATTTAGTGGAGCCCGATCTATCTTCTTTGGTATCTGACTTATGTTGTT
mI16_CGAT_2	GCTCTCCTAACAGATAAGCTTCCCTCTATGATTACTGACTGCGTC TATTTAGTGGAGCCCGATCTATCTTCTTTTCCCTACCCCAATTTCCAAT
mI12_CAGT_1	TGACACCTTTGCTGATTTCTTCCCTCTATGATTACTGACTGCGTCT ATTTAGTGGAGCCAGTCTATCTTCTTTTCCAGTTTCCCTAAGTTCATCA
mI12_CAGT_2	TCTCCCTCAAGTTCCTTTGTTTCCCTCTATGATTACTGACTGCGTCT ATTTAGTGGAGCCAGTCTATCTTCTTTGCACTCCCCATTCTACT
1 mCd68_AGCT_	CTGTCTCTCATTCCCTTATCCTCTATGATTACTGACTGCGTCT ATTTAGTGGAGCCAGTCTATCTTCTTTGACGGTACCCATCCCCAC
2 mCd68_AGCT_	TCCTTACGATGACACCTTCCCTCTATGATTACTGACTGCGTCTAT TTAGTGGAGCCAGTCTATCTTCTTTGCCGTTACTCTCTGCCA
mTnf_CCAA_3	ATGGCCAGACCTCACACTTCCCTCTATGATTACTGACTGCGTC TATTTAGTGGAGCCCAACTATCTTCTTTGCCTCCCTCTCATCAGTCT
2_AP1 mSocs3_GGAG_	GACCGGCCGGGCAGTTCAGTCTACGATTTTACCAGTGGCTTTT GCGTCTATTTAGTGGAGCCGGAGCTATCTTCTTTGTGACTAAACATTAC AAGAA
3_AP2 mSocs3_GGAG_	CGGGAGTACCCCAAGAGAGCTGATTCCTTTGACTCACATTTT GCGTCTATTTAGTGGAGCCGGAGCTATCTTCTTTCCACCTGCCAGGCA CTCCC
4_AP3 mSocs3_GGAG_	ATGAGCCATCTTGGAGCCCATCTACGAGTTTGCAGTCACGTTTT GCGTCTATTTAGTGGAGCCGGAGCTATCTTCTTTGAAGGGAGGCAGATC AACAG
_AP1 mMpo_GAGG_2	CACTCAGTTTAACCAACTCTACGATTTTACCAGTGGCTTTTG CGTCTATTTAGTGGAGCCGAGGCTATCTTCTTTGTTCTTTCAAAGGATTT GGG
_AP2 mMpo_GAGG_3	CTTCTTCACTGGCCTCAACTCTGATTCCTTTGACTCACATTTT CGTCTATTTAGTGGAGCCGAGGCTATCTTCTTTGAGCCAGCTACCCGGTT CTC
_AP3 mMpo_GAGG_4	GAAGAACCTGGAGTTGGCACTCTACGAGTTTGCAGTCACGTTTT GCGTCTATTTAGTGGAGCCGAGGCTATCTTCTTTGGTGAGCTCGGCACG GTGCT
_AP1 mI10_CCGG_2	ATCATGCCTGGCTCAGCACTTCTACGATTTTACCAGTGGCTTTT GCGTCTATTTAGTGGAGCCCGGCTATCTTCTTTCTTGCAGAAAAGAGA GCTCC
_AP2 mI10_CCGG_3	AAGGCCATGAATGAATTTGACTGATTCCTTTGACTCACATTTT GCGTCTATTTAGTGGAGCCCGGCTATCTTCTTTTCCAAGACCAAGGTGT CTAC
_AP3 mI10_CCGG_4	TGAAGAACTCATGGGTCTTTCTACGAGTTTGCAGTCACGTTTT GCGTCTATTTAGTGGAGCCCGGCTATCTTCTTTTCCAGAGCTCCTAAGAG

	AGTTG
mIfng_CACA_2 _AP1	AGTTCTGGGCTTCTCCTCCTTCTACGATTTTACCAGTGGCTTTTG CGTCTATTTAGTGGAGCCACACTATCTTCTTTACCCTCTGACTTGAGAC AGA
mIfng_CACA_3 _AP2	GTCCACCAGCTGTTGCCGGACTGATTCCTTTGACTCACATTTTTG CGTCTATTTAGTGGAGCCACACTATCTTCTTTTCAATGAGCTCATCCGA GTG
mIfng_CACA_4 _AP3	AGAAAGCAGTGTCTCAAGAGTCTACGAGTTTGCAGTCACGTTTT GCGTCTATTTAGTGGAGCCACACTATCTTCTTTAGTAACAGGCTGTCCC TGAA
mFoxp3_CAAC _2_AP1	GGCACTGTGCCTGGTATATGTCTACGATTTTACCAGTGGCTTTT GCGTCTATTTAGTGGAGCCCAACCTATCTTCTTTCTCTGCAGGTTTAGTG CTGT
mFoxp3_CAAC _3_AP2	AGTGTGCTCCCGGCCCTGGTCCTGATTCCTTTGACTCACATTTTTG CGTCTATTTAGTGGAGCCCAACCTATCTTCTTTTAGCCACCAGTACTCAG GGC
mFoxp3_CAAC _4_AP3	ACGCAGCCTCAAGCAATGCCTCTACGAGTTTGCAGTCACGTTTT GCGTCTATTTAGTGGAGCCCAACCTATCTTCTTTACACAGGCATAACTG ATCAT
mCxcr3_AGAA _2_AP1	TTCTCTGACTCCCCGCCCTGTCTACGATTTTACCAGTGGCTTTTG CGTCTATTTAGTGGAGCCAGAACTATCTTCTTTATGGGGAAAACGAGAG CGAC
mCxcr3_AGAA _3_AP2	AGATCTACCGCAGGGACCCCTGATTCCTTTGACTCACATTTTT GCGTCTATTTAGTGGAGCCAGAACTATCTTCTTTGAGCATAGTGCACGC CACCC
mCxcr3_AGAA _4_AP3	CGCCTGGGCCGCTCTGACCATCTACGAGTTTGCAGTCACGTTTT GCGTCTATTTAGTGGAGCCAGAACTATCTTCTTTAAATGTGGATGTTGTT CACG
mCd80_ACCA_ 2_AP1	AAAGACACTCTGTTCCATTTTCTACGATTTTACCAGTGGCTTTTG CGTCTATTTAGTGGAGCCACCACTATCTTCTTTGGTTGTGAAACTCAACC TTC
mCd80_ACCA_ 3_AP2	ATAAGAACCGGACTTTATATCTGATTCCTTTGACTCACATTTTTG CGTCTATTTAGTGGAGCCACCACTATCTTCTTTACTAAAAGTGTGGCCCC AGT
mCd80_ACCA_ 4_AP3	CAGTAATAACAGTCGTCGCTCTACGAGTTTGCAGTCACGTTTT GCGTCTATTTAGTGGAGCCACCACTATCTTCTTTCTTTGGGGCAGGATTC GGCG
mCd19_ACAC_ 2_AP1	ACCAGTCAACACCCTCCTGTCTACGATTTTACCAGTGGCTTTT GCGTCTATTTAGTGGAGCCACACCTATCTTCTTTGCTGGCTTGGTATCGA GGTA
mCd19_ACAC_ 3_AP2	AAAGTGACGCCTCCCTCGGGCTGATTCCTTTGACTCACATTTTT GCGTCTATTTAGTGGAGCCACACCTATCTTCTTTACCCCGCCAGGAGATT CTTC
mCd19_ACAC_ 4_AP3	TCCATGGTGTCCCACAAGGGTCTACGAGTTTGCAGTCACGTTTT GCGTCTATTTAGTGGAGCCACACCTATCTTCTTTAATATGTCCAGCCGCT ACAT
mCcr6_AAGA_ 2_AP1	GTGAACAACCTTACAGTTCCTTCTACGATTTTACCAGTGGCTTTT GCGTCTATTTAGTGGAGCCAAGACTATCTTCTTTTGCTTCTTGCCACCG AGGT
mCcr6_AAGA_ 3_AP2	AAGGTCATCTGTGTGGCAGTCTGATTCCTTTGACTCACATTTTTG CGTCTATTTAGTGGAGCCAAGACTATCTTCTTTCCAGAACACTGACGCA CAGT

4_AP3	mCcr6_AAGA_	TTACTCGGAAAGCTACATCTTCTACGAGTTTGCAGTCACGTTTT GCGTCTATTTAGTGGAGCCAAGACTATCTTCTTTGGCTTCTCTGTGCC GGGT
_AP1	mCcr4_AACC_2	TCTCCTGCTGGTACCCGGAGTCTACGATTTTACCAGTGGCTTTT GCGTCTATTTAGTGGAGCCAACCCTATCTTCTTTCTCAACTGTTCTCATT GGCT
_AP2	mCcr4_AACC_3	AGTACATCACCCAACCTCTTCTGATTCCTTTGACTCACATTTTTG CGTCTATTTAGTGGAGCCAACCCTATCTTCTTTTCTCGGGGAGAAATTCC GCA
_AP3	mCcr4_AACC_4	GTGGAGGCAGAGGCAGGAGTTCTACGAGTTTGCAGTCACGTTT TGCCTCTATTTAGTGGAGCCAACCCTATCTTCTTTGTTATTGGGTATTGG GCATG
_2_AP1	mCd11b_AATT	GATGGCTTGATGGACCTGGCTCTACGATTTTACCAGTGGCTTTT GCGTCTATTTAGTGGAGCCAATTCTATCTTCTTTGGGGTAAGGATCTCAC AATG
_3_AP2	mCd11b_AATT	TTAAAAACAAGGATGCTGGCTGATTCCTTTGACTCACATTTTT GCGTCTATTTAGTGGAGCCAATTCTATCTTCTTTTGCATGTCAAGAACA AGTA
_4_AP3	mCd11b_AATT	TGATGCAGAAATGTGAAACATCTACGAGTTTGCAGTCACGTTTT GCGTCTATTTAGTGGAGCCAATTCTATCTTCTTTGCGCACCCAGGTCTTT GGAT
2_AP1	mCd3e_ACGT_	ACACTTTCTGGGGCATCCTGTCTACGATTTTACCAGTGGCTTTTG CGTCTATTTAGTGGAGCCACGTCTATCTTCTTTTCTGAGAGGATGCGGT GGA
3_AP2	mCd3e_ACGT_	ACCTGTTCCCAACCCAGACTCTGATTCCTTTGACTCACATTTTTG CGTCTATTTAGTGGAGCCACGTCTATCTTCTTTCAAACAAGGAGCGGC CACC
4_AP3	mCd3e_ACGT_	TTACACACCCATACCTCCTTCTACGAGTTTGCAGTCACGTTTTG CGTCTATTTAGTGGAGCCACGTCTATCTTCTTTCCCGGCCATCCTCCGA CTG
2_AP1	mF4/80_ACTG_	GGCTTTTGGCTGCTCCTCTTTCTACGATTTTACCAGTGGCTTTTG CGTCTATTTAGTGGAGCCACTGCTATCTTCTTTACTGCCACAGTACGATG TGG
3_AP2	mF4/80_ACTG_	TGCACCAATGTACCAGGCTCCTGATTCCTTTGACTCACATTTTTG CGTCTATTTAGTGGAGCCACTGCTATCTTCTTTAATGTGGACTGAATTCT GTC
4_AP3	mF4/80_ACTG_	CATAGCCACCTTCTGCTGTTCTACGAGTTTGCAGTCACGTTTTG CGTCTATTTAGTGGAGCCACTGCTATCTTCTTTCTGGTATGTCTTGCCTT GGC
_AP1	mCd4_AGTC_2	CCTGTTTGCAAAGTCTCGAGTCTACGATTTTACCAGTGGCTTTT GCGTCTATTTAGTGGAGCCAGTCCTATCTTCTTTACTCTCTTCTTCACTA GGTA
_AP2	mCd4_AGTC_3	CCCACTCACCTCAAGATACCTGATTCCTTTGACTCACATTTTTG CGTCTATTTAGTGGAGCCAGTCCTATCTTCTTTCTCCAGCTGAAGGAAAC GCT
_AP3	mCd4_AGTC_4	TCTCTTTTGAATTTGCAAGATCTACGAGTTTGCAGTCACGTTTTG CGTCTATTTAGTGGAGCCAGTCCTATCTTCTTTAGCTTCCCATGATGCCT GCT
_2_AP1	mCd8b1_ATAT	TCCCCAAAAGCGCCAAGATGTCTACGATTTTACCAGTGGCTTTT GCGTCTATTTAGTGGAGCCATATCTATCTTCTTTGAGCACTGAGGGGAA CAGTG
_3_AP2	mCd8b1_ATAT	TCCCCACCCAGAGACCCAGCTGATTCCTTTGACTCACATTTTT GCGTCTATTTAGTGGAGCCATATCTATCTTCTTTGAAGAAGAAGCAATG

	CCCGT
mCd8b1_ATAT_4_AP3	GAGCTAGCCGGGACCCTGGATCTACGAGTTTGCAGTCACGTTTT GCGTCTATTTAGTGGAGCCATATCTATCTTCTTTCTTTGGCCTTTCATGG AAAA
mCd8a_ATCG_2_AP1	GACCCTACACGCTCCCCATCTACGATTTTACCAGTGGCTTTT GCGTCTATTTAGTGGAGCCATCGCTATCTTCTTTGCTAAAGGAGCAGTTT CCCC
mCd8a_ATCG_3_AP2	GACTGTAACACCAGAGCTAGCTGATTCCTTTGACTCACATTTTT GCGTCTATTTAGTGGAGCCATCGCTATCTTCTTTTACAATGGGAGTAATG AGCA
mCd8a_ATCG_4_AP3	AGATTCCCAGGCTCTTTCTGTCTACGAGTTTGCAGTCACGTTTTG CGTCTATTTAGTGGAGCCATCGCTATCTTCTTTTAAAGATTTTATCTCA GTG
mCd11c_ATGC_2_AP1	CAGTCATGAGCTGTACCTGGTCTACGATTTTACCAGTGGCTTTT GCGTCTATTTAGTGGAGCCATGCCTATCTTCTTTCAGAGCCTGCTTCTGT TCTC
mCd11c_ATGC_3_AP2	TGAAGGATATTGAGAATCAGCTGATTCCTTTGACTCACATTTTT GCGTCTATTTAGTGGAGCCATGCCTATCTTCTTTCGTGGAGAACTTTGAT GCTT
mCd11c_ATGC_4_AP3	AAAGCCCCAAGACCCAACTATCTACGAGTTTGCAGTCACGTTTT GCGTCTATTTAGTGGAGCCATGCCTATCTTCTTTTGTCTGCCTTCATATT CATG
mCd127_ATTA_2_AP1	TGGGTAGAGCTTTCGCTATATCTACGATTTTACCAGTGGCTTTT GCGTCTATTTAGTGGAGCCATTACTATCTTCTTCTCTCAGAATGATG GCTC
mCd127_ATTA_3_AP2	TTCCTGGACTGCCAGATTCACTGATTCCTTTGACTCACATTTTTG CGTCTATTTAGTGGAGCCATTACTATCTTCTTTGAGTTTCAATCCCGAA AGT
mCd127_ATTA_4_AP3	TCTCTTGCCATGAACCTGTCTCTACGAGTTTGCAGTCACGTTTTG CGTCTATTTAGTGGAGCCATTACTATCTTCTTTACACATTCCAACCTCAA CCC
mI17a_CATG_2_AP1	TCCAGGGAGAGCTTCATCTGTCTACGATTTTACCAGTGGCTTTT GCGTCTATTTAGTGGAGCCCATGCTATCTTCTTTTCAGGACGCGCAAAC ATGAG
mI17a_CATG_3_AP2	TGACCCTAAGAAACCCCCACTGATTCCTTTGACTCACATTTTT GCGTCTATTTAGTGGAGCCCATGCTATCTTCTTTCCTAAACAGAGACCC GCGGC
mI17a_CATG_4_AP3	ACCAACTTAGTTTTATGTTTCTACGAGTTTGCAGTCACGTTTTG CGTCTATTTAGTGGAGCCCATGCTATCTTCTTCTCTCTGAATGGGGTG AAA
miNos2_GACT_2_AP1	AACTGGGGCAGTGGAGAGATTCTACGATTTTACCAGTGGCTTTT GCGTCTATTTAGTGGAGCCGACTCTATCTTCTTTCACAGTATGTGAGGAT CAAA
miNos2_GACT_3_AP2	CAGCGCTACAACATCCTGGACTGATTCCTTTGACTCACATTTTT GCGTCTATTTAGTGGAGCCGACTCTATCTTCTTTTTTCGAGACTTCTGTGA CACA
miNos2_GACT_4_AP3	AGAGCCCGGAGCCTTTAGACTCTACGAGTTTGCAGTCACGTTTT GCGTCTATTTAGTGGAGCCGACTCTATCTTCTTACAGCAATATAGGCTC ATCC
mTerg_GCCG_2_AP1	GCCTGCAGAGCACTTCCTGCTCTACGATTTTACCAGTGGCTTTT GCGTCTATTTAGTGGAGCCCGCTATCTTCTTTGCTGGTGACCTGAAAT TCCA

mTerg_GCCG_3 _AP2	AAGTTCTTTCCCGATGTCATCTGATTCCCTTTGACTCACATTTTTG CGTCTATTTAGTGGAGCCGCCGCTATCTTCTTTTCATACCTTTGTCTCCT GAA
mTerg_GCCG_4 _AP3	TTTCATCCTCACTGCCATAATCTACGAGTTTGCAGTCACGTTTTG CGTCTATTTAGTGGAGCCGCCGCTATCTTCTTTGGTACAGCAAGTCAGCT GGA
mRorgT_GCGC _2_AP1	CACCGGACATCTCGGGAGCTTCTACGATTTTACCAGTGGCTTTT GCGTCTATTTAGTGGAGCCGCCCTATCTTCTTTACAGGGCCCCACAGA GACAC
mRorgT_GCGC _3_AP2	GACCAGCTACCAGAGGAAGTCTGATTCCCTTTGACTCACATTTTT GCGTCTATTTAGTGGAGCCGCCCTATCTTCTTTCTCTTTTCACGGGAGG AGGT
mRorgT_GCGC _4_AP3	AAATAAAAAACAGACTGACATCTACGAGTTTGCAGTCACGTTTT GCGTCTATTTAGTGGAGCCGCCCTATCTTCTTTAGAGATAGGATGACC AAGTC
mTcrb_GTAC_2 _AP1	CTACTTTGCGGCAGGCACCCTCTACGATTTTACCAGTGGCTTTT GCGTCTATTTAGTGGAGCCGTACCTATCTTCTTTAGTCCGGTGAATTCGC CCCT
mTcrb_GTAC_3 _AP2	AAGTTCTTTCCCGATGTCATCTGATTCCCTTTGACTCACATTTTTG CGTCTATTTAGTGGAGCCGTACCTATCTTCTTTTCATACCTTTGTCTCCTTG AA
mTcrb_GTAC_4 _AP3	TTTCATCCTCACTGCCATAATCTACGAGTTTGCAGTCACGTTTTG CGTCTATTTAGTGGAGCCGTACCTATCTTCTTTGGTACAGCAAGTCAGCT GGA
mll6_CGAT_3_ AP1	CTGGAGCCCACCAAGAACGATCTACGATTTTACCAGTGGCTTTT GCGTCTATTTAGTGGAGCCCAGTCTATCTTCTTTTGTCTGTAGCTCATT TGCT
mll6_CGAT_4_ AP2	CTGGAGTACATGAAGAACAACACTGATTCCCTTTGACTCACATTTTT GCGTCTATTTAGTGGAGCCCAGTCTATCTTCTTTTTCTGGAGTACCATAG CTAC
mll6_CGAT_5_ AP3	GTTTCTTGAATGTATAAGTTCTACGAGTTTGCAGTCACGTTTT GCGTCTATTTAGTGGAGCCCAGTCTATCTTCTTTTTCTGTTACCTAGCCA GATG
mll12_CAGT_3 _AP1	CTCTTTTTGGCCACCCTTGCTCTACGATTTTACCAGTGGCTTTTG CGTCTATTTAGTGGAGCCCAGTCTATCTTCTTTTGTGTCATCAGCTAC CTC
mll12_CAGT_4 _AP2	CAGCAGATCATTCTAGACAACACTGATTCCCTTTGACTCACATTTTT GCGTCTATTTAGTGGAGCCCAGTCTATCTTCTTTCACTTCAGAATCACAA CCAT
mll12_CAGT_5 _AP3	TCCCCTCCATCGCTTCTCTCTACGAGTTTGCAGTCACGTTTTG CGTCTATTTAGTGGAGCCCAGTCTATCTTCTTTGCACAGCTACCTCAGCA TGG
mCd68_AGCT_ 3_AP1	TCCTCTTCCAAGAGAAGGCATCTACGATTTTACCAGTGGCTTTT GCGTCTATTTAGTGGAGCCAGCTCTATCTTCTTTGGGAAGTGAGGCTTTT CATT
mCd68_AGCT_ 4_AP2	CAAATTCAAATCCGAATCCTCTGATTCCCTTTGACTCACATTTTTG CGTCTATTTAGTGGAGCCAGCTCTATCTTCTTTCTTGTGTTACAGCTCCAA GCC
mCd68_AGCT_ 5_AP3	AGCATCTGCCCCAGTCCACTTCTACGAGTTTGCAGTCACGTTTT GCGTCTATTTAGTGGAGCCAGCTCTATCTTCTTTTCAACCTACCAGCCCC TCTG
mTnf_CCAA_4 AP1	GCCAGGAGGGAGAACAGAAATCTACGATTTTACCAGTGGCTTT TGCCTCTATTTAGTGGAGCCCCAACTATCTTCTTTGCGAGGACAGCAAG

	GGACTA
mTnf_CCAA_5_ AP2	GTCTACTCCCAGGTTCTCTTCTGATTCCCTTTGACTCACATTTTTG CGTCTATTTAGTGGAGCCCCAACTATCTTCTTTTCAGCCGATGGGTTGTAC CTT
mTnf_CCAA_6_ AP3	CTACTCAGAAACACAAGATGTCTACGAGTTTGCAGTCACGTTTT GCGTCTATTTAGTGGAGCCCCAACTATCTTCTTTGTGCTCAGAGCTTTCA ACAA
mCd40L_CGTA_1_ AP1	CCTAAGAACTTACAATCTGATCTACGATTTTACCAGTGGCTTTT GCGTCTATTTAGTGGAGCCCCTACTATCTTCTTTAATCCCAAGGGACCCT GCTC
mCd40L_CGTA_2_ AP2	TGTGTTTGTCAACGTGACTGCTGATTCCCTTTGACTCACATTTTTG CGTCTATTTAGTGGAGCCCCTACTATCTTCTTTGAATTACAAGCTGGTGC TTC
mCd40L_CGTA_3_ AP3	CTATTATGTCTACACTCAAGTCTACGAGTTTGCAGTCACGTTTT GCGTCTATTTAGTGGAGCCCCTACTATCTTCTTTACGGTTAAAAGAGAA GGACT
mCd69_CTAG_1_ AP1	AAGGAAGAAAACAAAAGCACTCTACGATTTTACCAGTGGCTTT TGCCTATTTAGTGGAGCCCTAGCTATCTTCTTTAAACCTCTGTAGCGT ATTTC
mCd69_CTAG_2_ AP2	CTGCTAATCACATCAAGGATCTGATTCCCTTTGACTCACATTTTTG CGTCTATTTAGTGGAGCCCTAGCTATCTTCTTTCTGAAACTGTCACCAAC TGA
mCd69_CTAG_3_ AP3	AAATGAAGCTAATCAGACATTCTACGAGTTTGCAGTCACGTTTT GCGTCTATTTAGTGGAGCCCTAGCTATCTTCTTTGAACATTGGATTGGGC TGAA
mSCGB1A1_GG CC_ AP4	CTCACTGGATTTTCAGAGATATGCTTGTGGTAGCAAATATTTTGC GTCTATTTAGTGGAGCCCGCCCTATCTTCTTTAAGCAAGATTTAAGATTC TGAAG
mSftpc_CGCG_ AP4	ATTCTTCCGGGAGAGACCTGTGCTTGTGGTAGCAAATATTTTGC GTCTATTTAGTGGAGCCCGCCTATCTTCTTTTGATACTGGTTCCGAGTC CG
mCD86_CTGA_ ap1	TGCAGACTCTTAAGATCTGGTCTACGATTTTACCAGTGGCTTTT GCGTCTATTTAGTGGAGCCCTGACTATCTTCTTTGTCCATCTTGATTCAC CCTG
mCD86_CTGA_ ap2	GGATGGGAGTGGTATATTTGCTGATTCCCTTTGACTCACATTTTT GCGTCTATTTAGTGGAGCCCTGACTATCTTCTTTGACAGCTACCTCTTCA GTCA
mCD86_CTGA_ ap3	TCAGTGATCGCCAACTTCAGTCTACGAGTTTGCAGTCACGTTTT GCGTCTATTTAGTGGAGCCCTGACTATCTTCTTTAACAGACATTAACAG AACTG
mElane_GATC_ ap1	CATTATCCGAAGCCATAATGTCTACGATTTTACCAGTGGCTTTT GCGTCTATTTAGTGGAGCCGATCCTATCTTCTTTTTTGCAGATTGGATCA ATTC
mElane_GATC_ ap2	GCCAACGTGCAGGTGGCCCACTGATTCCCTTTGACTCACATTTTT GCGTCTATTTAGTGGAGCCGATCCTATCTTCTTTATGGCTCCGCTACCAT TAAC
mElane_GATC_ ap3	CTATCCAGCCGGACTCTGGCTCTACGAGTTTGCAGTCACGTTTT GCGTCTATTTAGTGGAGCCGATCCTATCTTCTTTCCACCATGGCCCTTGG CAGA
mI12b_GCTA_a p1	AGGACACAATGGCCACACTCTCTACGATTTTACCAGTGGCTTTT GCGTCTATTTAGTGGAGCCGCTACTATCTTCTTTGAAGATACTACGGCTA CCTC

p2	mll12b_GCTA_a	CATTAGCTTTTGTGACAACCCTGATTCCTTTGACTCACATTTTTG CGTCTATTTAGTGGAGCCGCTACTATCTTCTTTCAATCAGGGCTGCGTAG GTA
p3	mll12b_GCTA_a	ACTCCGGACGGTTCACGTGCTCTACGAGTTTGAGTCACGTTTT GCGTCTATTTAGTGGAGCCGCTACTATCTTCTTTGAAGTGTGAAGCACC AAATT
_ap1	mTbx21_GTCA	TTCTTCTGTTTTGCATCACTTCTACGATTTTACCAGTGGCTTTTG CGTCTATTTAGTGGAGCCGCTACTATCTTCTTTAGAGTGGTGTCTGGATG TAT
_ap2	mTbx21_GTCA	GGATCCTCAGAGGAACAGGGCTGATTCCTTTGACTCACATTTTT GCGTCTATTTAGTGGAGCCGCTACTATCTTCTTTGCCATGGACCCGGG CCTG
_ap3	mTbx21_GTCA	CCGGGCGCACAGGACCCGACTCTACGAGTTTGCAGTCACGTTTT GCGTCTATTTAGTGGAGCCGCTACTATCTTCTTTATCGTTTCTTCTATCCC GAG
_AP1	mCd4_AGTC_5	AGTCCACCAAAGACCTCAAGTCTACGATTTTACCAGTGGCTTTT GCGTCTATTTAGTGGAGCCAGTCCTATCTTCTTTCAAAGAGGTGTCCGTA CAAA
_AP2	mCd4_AGTC_6	CCAGCCCTGGATCTCCTTCTCTGATTCCTTTGACTCACATTTTTG CGTCTATTTAGTGGAGCCAGTCCTATCTTCTTTGCAGAGAAGGATTCTTT CTT
_AP3	mCd4_AGTC_7	CATGTCCAACCTAAGGGTTCTCTACGAGTTTGCAGTCACGTTTT GCGTCTATTTAGTGGAGCCAGTCCTATCTTCTTTAGTGGTTCCAAAGTTC TCTC

Supplementary Table 4

Sequencing-by-ligation oligonucleotides

name	sequence	5' modification	3' modification
Anchor oligo B2_DO_27_U_5AF750	/5Alex750N/UGCGUCUAU UUAGUGGAGCC	Alexa Fluor 750	-
Cy5_0A_4N	ANNNCTATC	Phosphorylation	Cy5
Cy3_0G_4N	GNNNCTATC	Phosphorylation	Cy3
TR_0C_4N	CNNNCTATC	Phosphorylation	Texas Red
AF488_0T_4N	TNNNCTATC	Phosphorylation	Alexa Fluor 488
Cy5_1A_4N	NANNCTATC	Phosphorylation	Cy5
Cy3_1G_4N	NGNNCTATC	Phosphorylation	Cy3
TR_1C_4N	NCNNCTATC	Phosphorylation	Texas Red
AF488_1T_4N	NTNNCTATC	Phosphorylation	Alexa Fluor 488
Cy5_2A_4N	NNANCTATC	Phosphorylation	Cy5
Cy3_2G_4N	NNGNCTATC	Phosphorylation	Cy3
TR_2C_4N	NNCNCTATC	Phosphorylation	Texas Red
AF488_2T_4N	NNTNCTATC	Phosphorylation	Alexa Fluor 488
Cy5_3A_4N	NNNACTATC	Phosphorylation	Cy5
Cy3_3G_4N	NNNGCTATC	Phosphorylation	Cy3
TR_3C_4N	NNNCCTATC	Phosphorylation	Texas Red
AF488_3T_4N	NNNTCTATC	Phosphorylation	Alexa Fluor 488