Stem Cell Reports, Volume *17*

Supplemental Information

Generation of SIV-resistant T cells and macrophages from nonhuman primate induced pluripotent stem cells with edited CCR5 locus

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Supplemental Materials for D'Souza et al.

Generation of SIV resistant T cells and Macrophages from Nonhuman Primate Induced Pluripotent Stem Cells with Edited CCR5 locus

Supplemental Experimental Procedures

Reprogramming NHP cells and demonstration of pluripotency

Fibroblasts were obtained from skin punch biopsies of MCM CY1 with M3/M3 MHC genotype and MCM CY3 with M6/M6 MHC genotype, while peripheral blood samples were obtained from CY1, CY2, and CY3 with corresponding M3/M3, M1/M3 and M6/M6 MHC genotypes. Fibroblasts were obtained from skin biopsy and reprogrammed using a combination of oriP/EBNA-1 episomal vectors expressing the 6 reprogramming factors OCT4, KLF4, SOX2, MYC, NANOG and LIN28 (OKSMNL) by electroporation as described previously (D'Souza et al., 2016; Yu et al., 2009) to generate fibroblast iPSCs (Fib-iPSCs). Peripheral blood T cells were reprogrammed using the CytoTune™-iPS 2.0 Sendai Reprogramming Kit (ThermoFisher Scientific) to generate T-iPSCs (Seki et al., 2010). Briefly, peripheral blood mononuclear cells were first separated by Ficoll centrifugation and then MACS enriched for $CD3^+$ cells. These cells were then activated using CD3 and CD28 antibodies in the presence of 100U/ml IL-2 (Peprotech). For reprogramming, $0.5x10^6$ activated cells were resuspended in 0.3ml of 10% RPMI1640 medium (ThermoFisher) containing 4μg/ml of hexadimethrine bromide (MilliporeSigma) and 20μl each of the 3 Sendai viruses expressing KOS (KLF4–OCT3/4–SOX2), MYC, and KLF4, and incubated overnight at 37°C in 5% CO_2. Cells were washed the following day and transferred onto mouse embryonic fibroblasts (MEFs). Colonies began to appear within 15 days and were subsequently transferred onto fresh MEFs for expansion and characterization. The iPSC lines (T-iPSCs and Fib-iPSCs) were maintained on MEFs in Primate ES cell medium (ReproCELL) supplemented with 4ng/ml bFGF (154 a.a.) (Peprotech) as previously described.(D'Souza *et al.*, 2016) Cells were passaged every 3-4 days using Collagenase Type IV (Life Technologies). Expression of pluripotency markers was analyzed by flow cytometry using SOX2 (Cell Signaling) and OCT3/4 (Santacruz Biotechnology) antibodies and the MACSQuant Analyzer 10 (Miltenyi) and FlowJo software (BD). Expression of pluripotency marker NANOG was analyzed by immunofluorescence using antibodies from Cell Signaling.

RT-PCR analysis of CCR5 expression

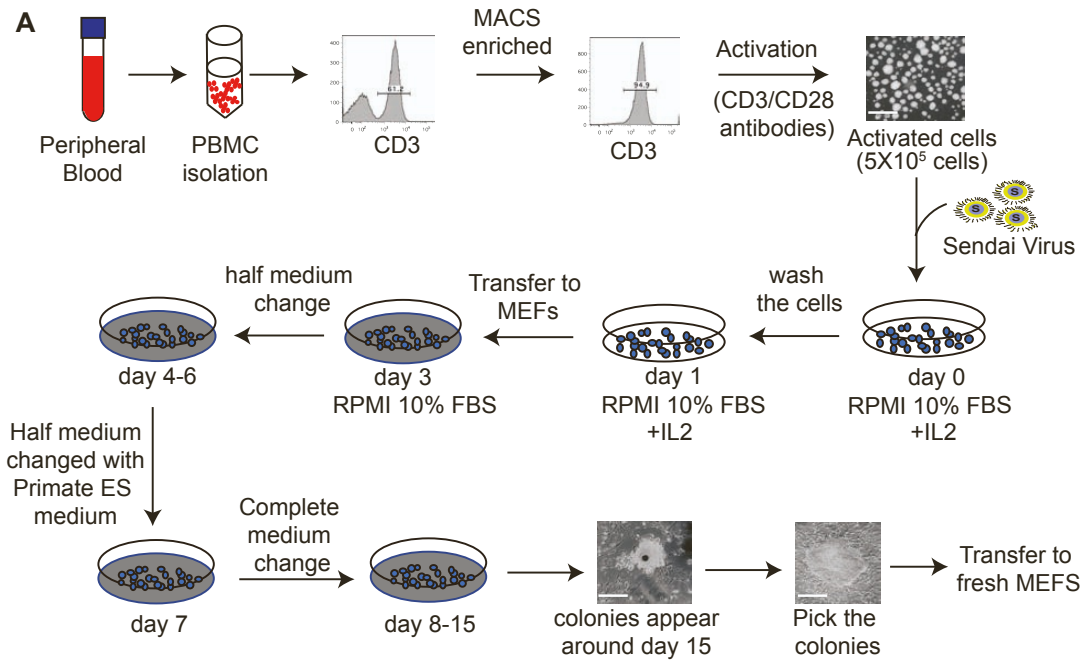
RNA was isolated using the RNeasy mini Kit (Qiagen) from macrophages and T cells generated from wild type and CCR5-mut iPSCs. cDNA was transcribed using the QuantiTect reverse transcription kit (Qiagen) from all the samples and amplified by PCR using Q5 master mix (New England Biolabs) and forward-TGTGTCAATGGAACTCTTGAC and reverse-TCGTTTCGACACCGAAGCAG CCR5 primers.

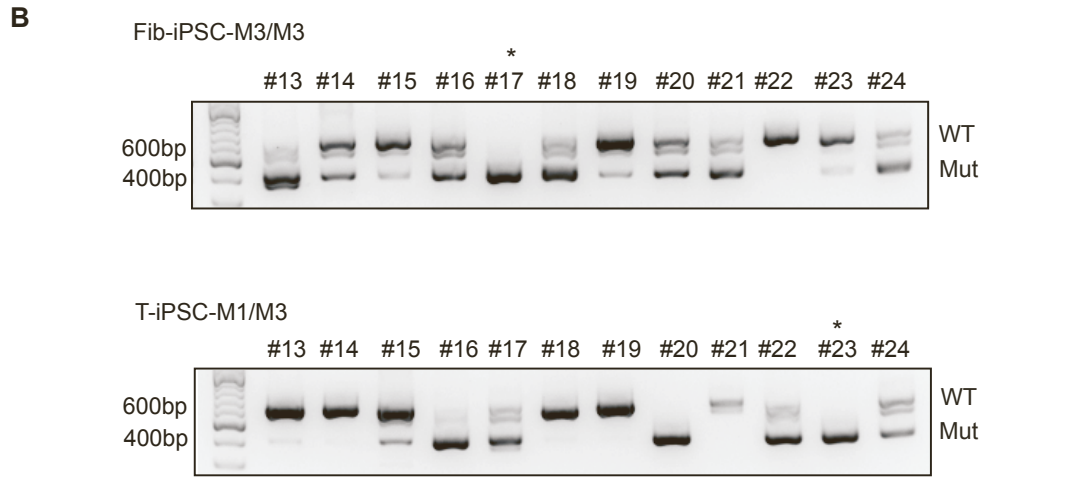
Supplemental References

D'Souza, S.S., Maufort, J., Kumar, A., Zhang, J., Smuga-Otto, K., Thomson, J.A., and Slukvin, II (2016). GSK3beta Inhibition Promotes Efficient Myeloid and Lymphoid Hematopoiesis from Non-human Primate-Induced Pluripotent Stem Cells. Stem Cell Reports *6*, 243-256. 10.1016/j.stemcr.2015.12.010.

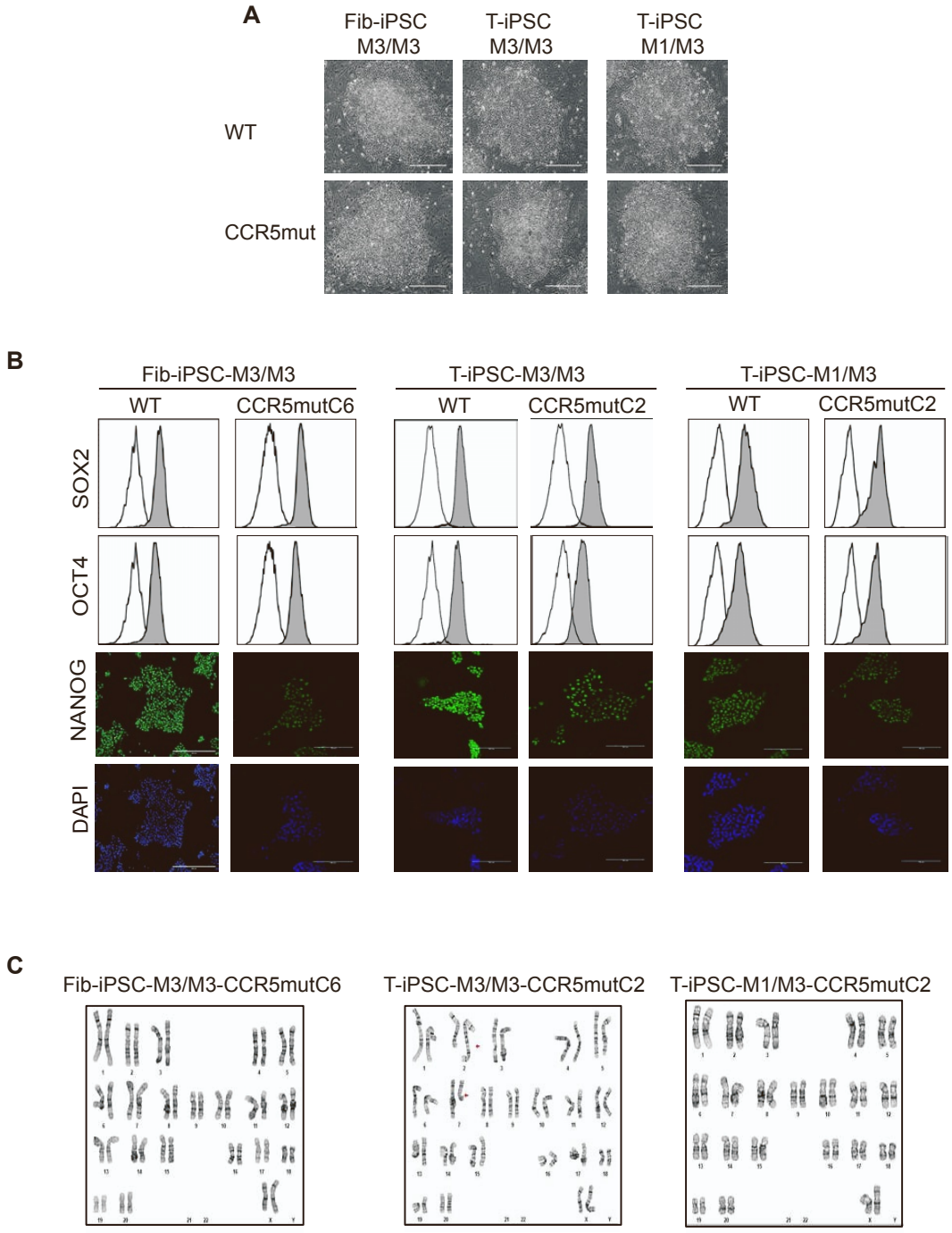
Seki, T., Yuasa, S., Oda, M., Egashira, T., Yae, K., Kusumoto, D., Nakata, H., Tohyama, S., Hashimoto, H., Kodaira, M., et al. (2010). Generation of induced pluripotent stem cells from human terminally differentiated circulating T cells. Cell Stem Cell *7*, 11-14. 10.1016/j.stem.2010.06.003.

Yu, J., Hu, K., Smuga-Otto, K., Tian, S., Stewart, R., Slukvin, II, and Thomson, J.A. (2009). Human induced pluripotent stem cells free of vector and transgene sequences. Science *324*, 797-801. 10.1126/science.1172482.

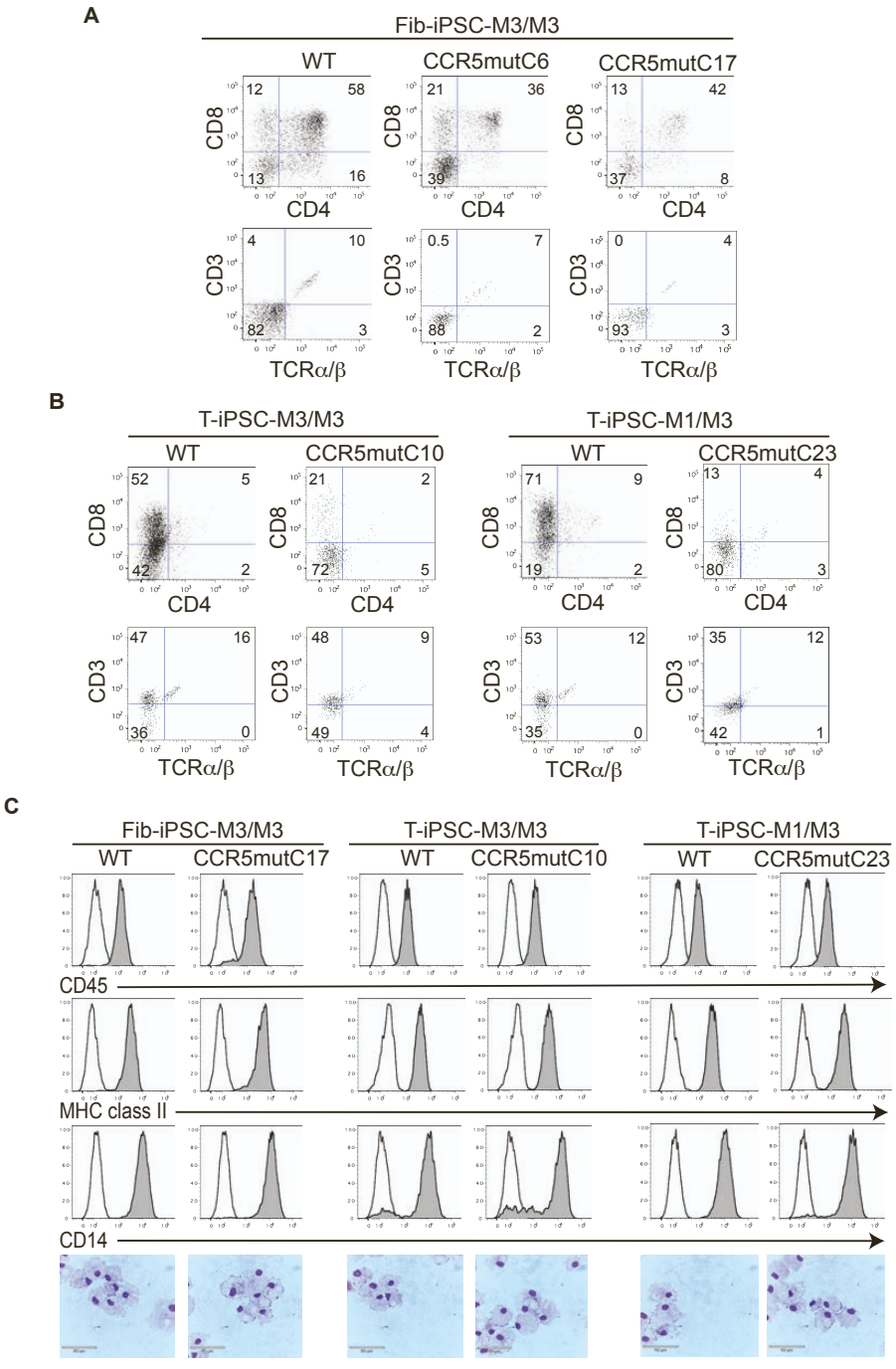




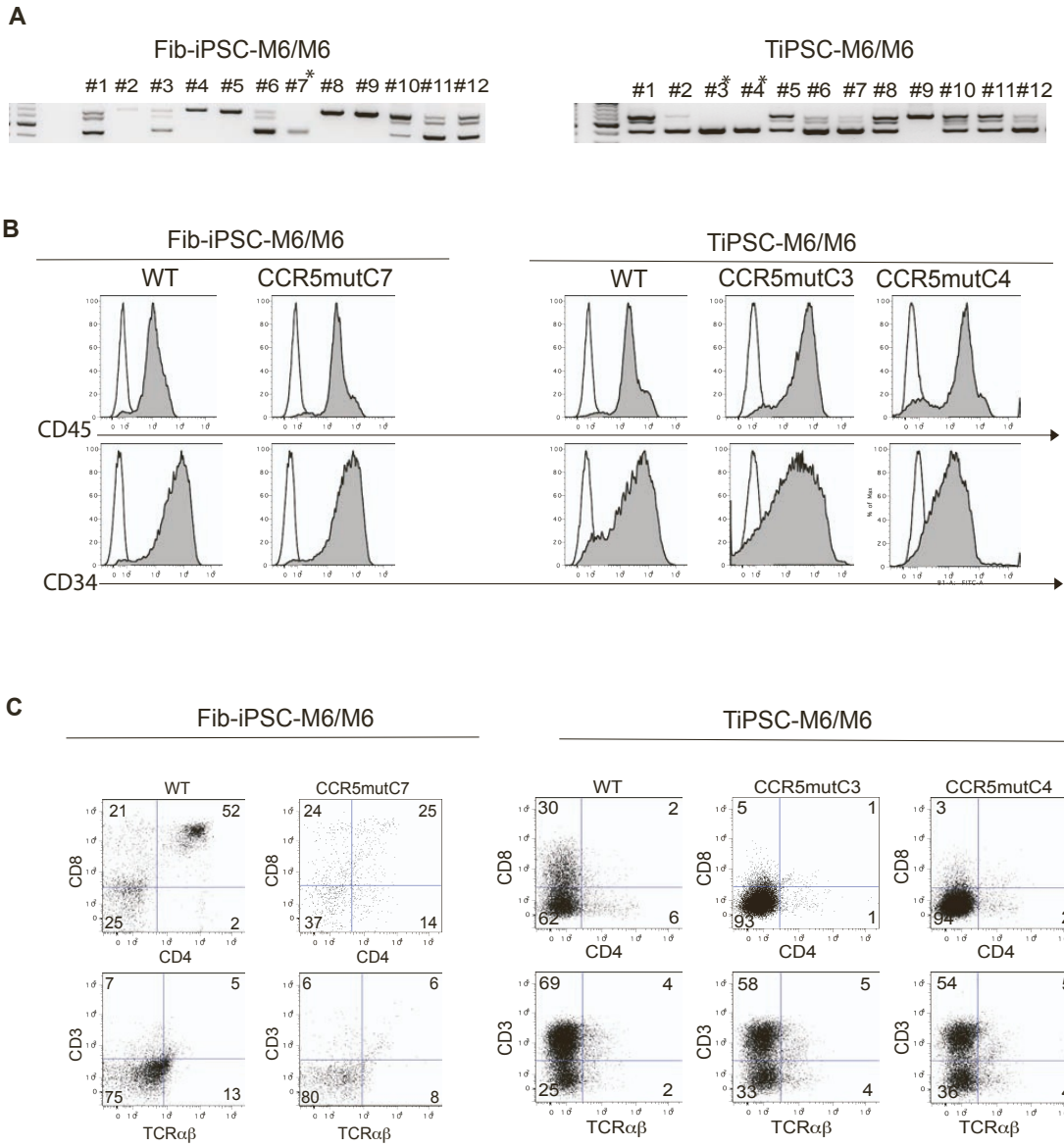
Supplementary Figure S1, related to Figure 1. Generation of CCR5mut iPSCs from MCM fibroblasts and T cells. (A) Schematic diagram of generation of T-iPSCs from peripheral blood T cells using Sendai virus kit. Scale bar in activated T cell images is 300 μm. Scale bar for colonies at day 15 is 150 μm and for pick up colonies is 740 μm. (B) Genomic PCR to detect deletion within CCR5 locus. * denotes clones selected in these studies.



Supplementary Figure S2, related to Figure 1. Generation and characterization of MCM-iPSCs. (A) Phase contrast images of WT and CCR5mut colonies on MEFs. Scale bar is 200 μm. (B) Expression of pluripotency markers by flow cytometry (OCT4 and SOX2) and by immunofluorescence (NANOG) in generated wild type and CCR5mut iPSC lines. Scale bars for WT Fib-iPSC-M3/M3 images are 400 μm. Scale bars for all other images are 100 μm. (C) Karyotype of CCR5mut iPSCs.



Supplemental Figure S3, related to Figure 2 and 3. Generation of T cells and macrophages from CCR5mut clones. (A) CCR5mutC6 and C17 clones from fib-iPSCs and (B) C10 and C23 CCR5mut clones from T-iPSC-M3/M3 and M1/M3 were differentiated into T cells. Expression of T cell markers was shown on $CD45^+$ gated cells after 3 weeks of culture of iPSC-derived MHP on OP9-DLL4 in the presence of SCF, IL7 and FLT3L. (C) Day 10 $CD34^+CD45^+$ MHPs from mutant and wild type NHP iPSCs were differentiated to macrophages. The phenotype and morphology of the cells was confirmed by flow cytometry and Wright stain of cytospins. Scale bar is 50 μm.



Supplemental Figure S4, related to Figure 1, 2 and 3. Generation of fib- and T-iPSCs from MCM with M6/M6 genotype and their hematopoietic differentiation. (A) Genomic DNA was isolated from single cell clones obtained after CCR5 editing and analyzed by PCR to detect deletion within the CCR5 locus. * Denotes the clones selected for further analysis. (B) Both wild type and CCR5mut, fib- and T-iPSCs efficiently generate $CD34^{+}CD45^{+}$ hematopoietic progenitors as analyzed by flow cytometry of floating cells collected on day10 of iPSC/OP9 cocultures. (D) $CD34^{+}CD45^{+}$ cells from WT and CCR5mut iPSCs were cultured on OP9-DLL4 in the presence of SCF, IL7 and FLT3L for 3 weeks and floating cells were analyzed by flow cytometry after gating on $CD45^{+}$ cells. Control staining with appropriate isotype matched antibodies were included to establish a threshold for positive staining. The graphs are representative of at least 3 independent experiments.

Supplementary Table S1. List of antibodies used in this study

Name	Clone	Vendor	Cat. Number
Anti NHP CD45	MB4-6D6	Miltenyi Biotech	130-119-764
Anti human CD34	563	BD Biosciences	550619
Anti human CD3ε	SP34	BD Biosciences	556612
Anti human CD4	L200	BD Biosciences	550630
Anti human CD5	UCHT2	Biolegend	300611
Anti human CD7	MT701	BD Biosciences	340656
Anti rat TCRα/β	R73	Biolegend	201110
Anti human CD8	SK1	Biolegend	980904
Anti human CD14	M5E2	BD Biosciences	555399
Anti human MHC class II	L243	BD Biosciences	340689
Anti monkey CD3-1	monoclonal	Mabtech	3610-1-50
Anti human CD28-A	monoclonal	Mabtech	3608-1-50
Oct 3/4	monoclonal	Santa Cruz	Sc-5279
Donkey anti mouse IgG (H+L) Alexa Fluor®488	polyclonal	Invitrogen	A-31570
Nanog	monoclonal	Cell Signaling Technology	4903
Donkey anti rabbit IgG (H+L) Alexa Fluor®488	polyclonal	Invitrogen	A-21206
Sox 2	monoclonal	Cell Signaling Technology	3579
Donkey anti rabbit IgG (H+L) Alexa Fluor®555	polyclonal	Invitrogen	A-31572