Immune-regulating strategy against rheumatoid arthritis by inducing tolerogenic dendritic cells with modified zinc peroxide nanoparticles

Han Qiao[1,2,#], Jingtian Mei[1,2,#], Kai Yuan[1,2], Kai Zhang[1,2], Feng Zhou[3], Tingting Tang[1,2,*], Jie Zhao[1,2,*]

1. Department of Orthopaedic Surgery, Shanghai Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China PR.

2. Shanghai Key Laboratory of Orthopaedic Implants, Shanghai Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China PR.

3. Department of Orthopaedic Surgery, The First Affiliated Hospital of Soochow University, Suzhou, Jiangsu Province, China PR.

# These authors contributed equally to this work.

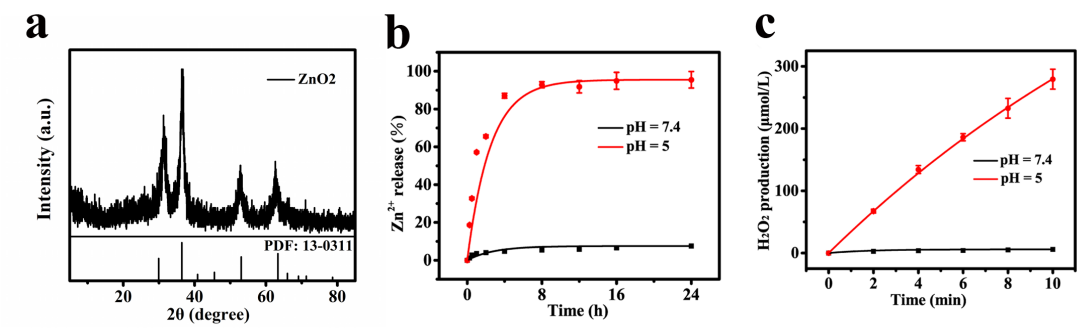
*Corresponding to:

Jie Zhao, Ph.D., M.D., Department of Orthopaedics, Shanghai Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai Key Laboratory of Orthopaedic Implants, 639 Zhizaoju Road, Shanghai 200011, China PR. E-mail, profzhaojie@126.com;

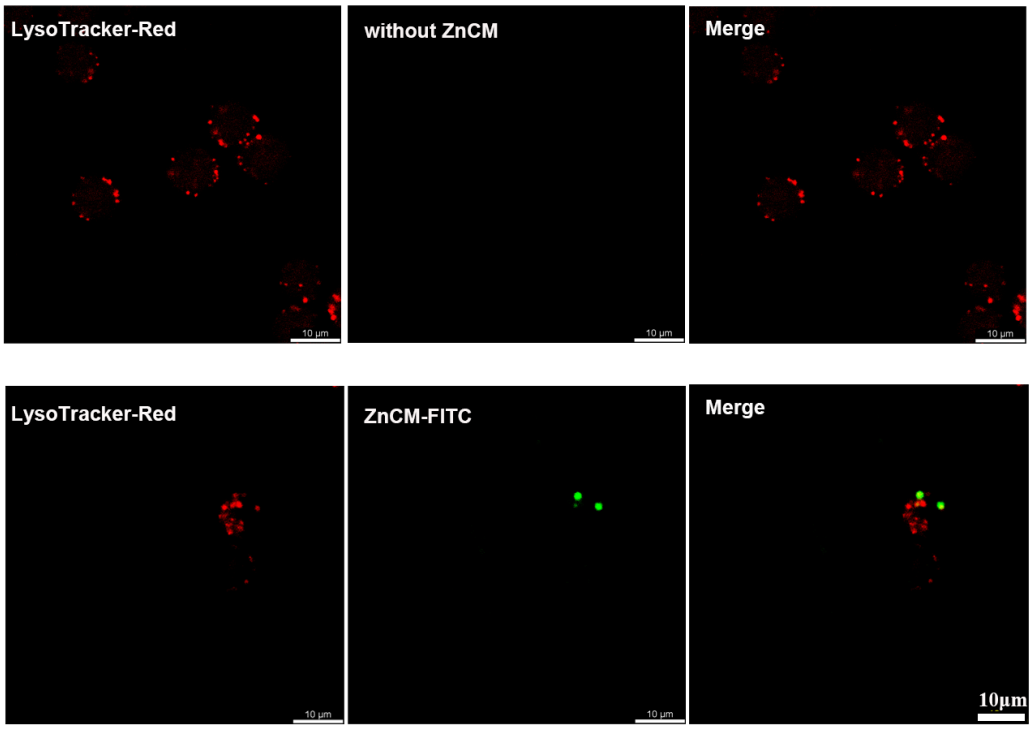
Also corresponding to:

Tingting Tang, Ph.D., M.D., Department of Orthopaedics, Shanghai Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai Key Laboratory of Orthopaedic Implants, 639 Zhizaoju Road, Shanghai 200011, China PR. E-mail, ttt@sjtu.edu.cn

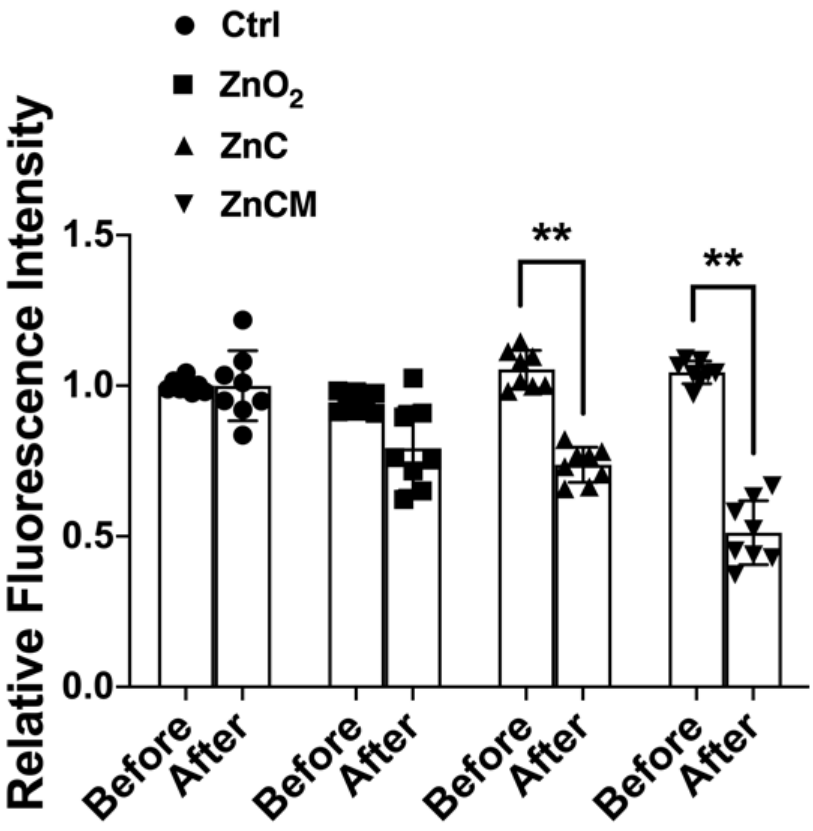
Supplementary Information



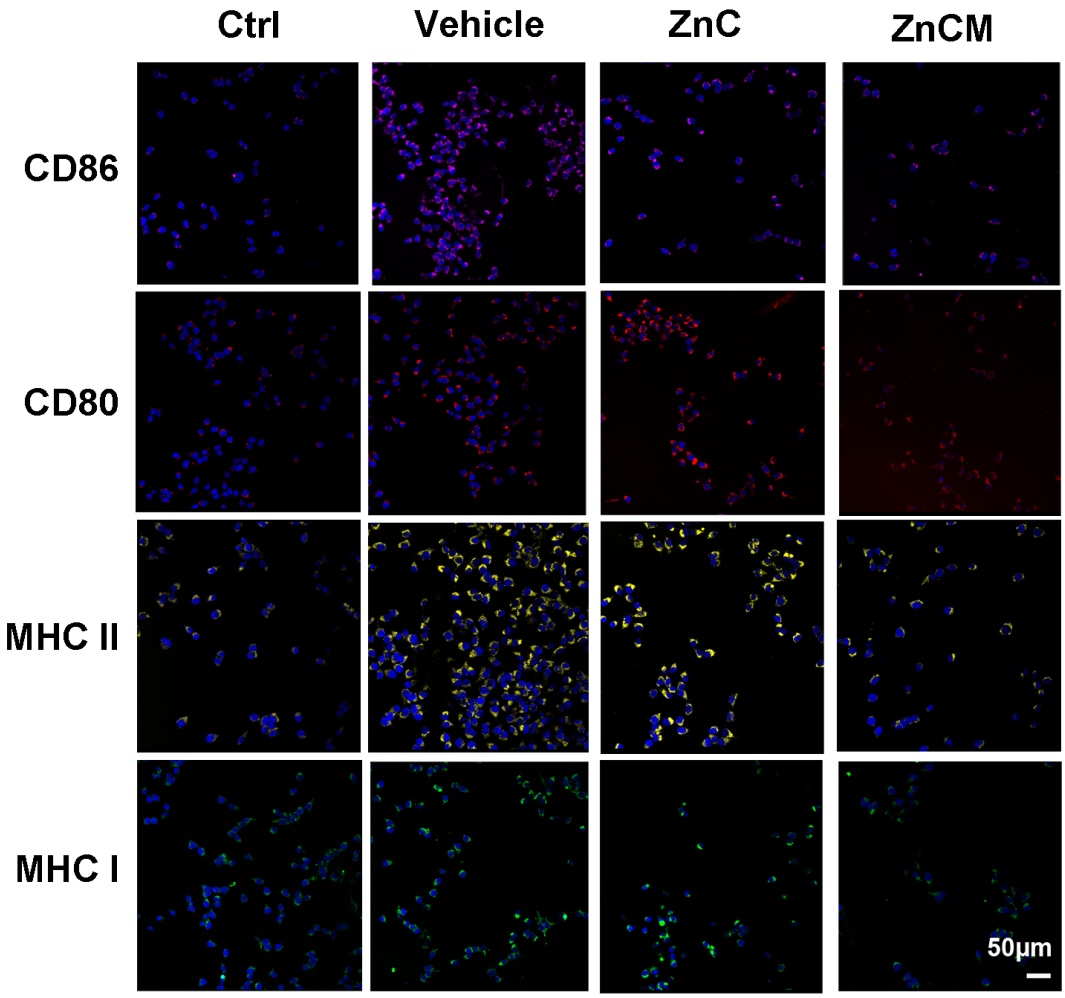
Supplementary Figure 1. (a) XRD pattern of ZnO_2 NPs. (b) Zn^{2+} released from ZnO_2 (100 μg/mL) NPs under different pH values. (c) H_2O_2 released from ZnO_2 (1 mg/mL) NPs under different pH values.



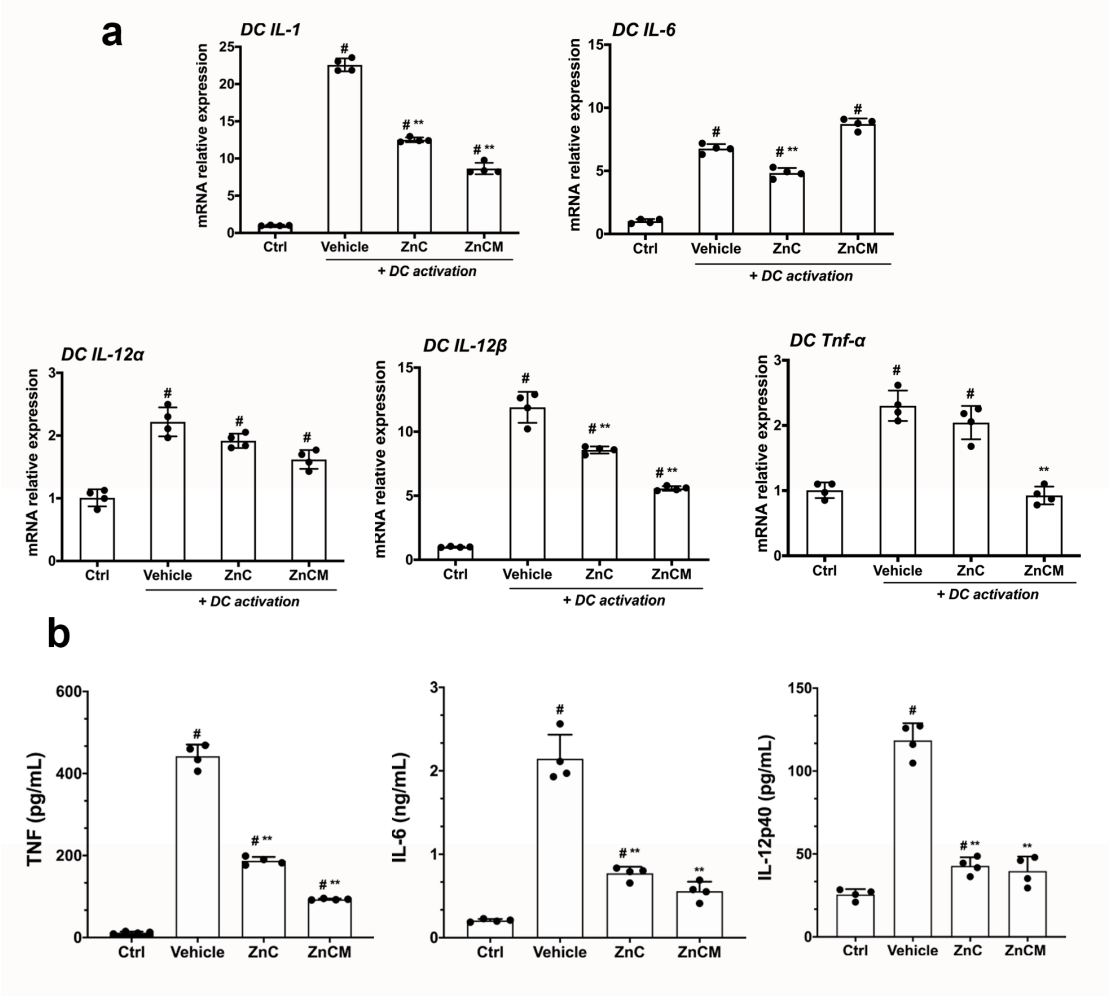
Supplementary Figure 2. Colocalization of ZnCM NPs within DCs lysosomes. CLSM observations after DCs were treated with non-toxic fluorescent ZnCM NPs.



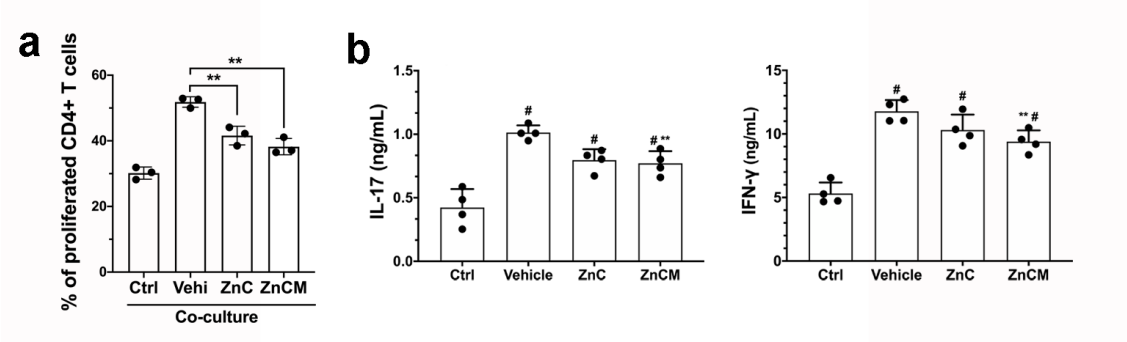
Supplementary Figure 3. Quantitative intracellular hypoxia state in DCs before and after various NPs treatments. ** indicated the significant difference of $p \leq 0.05$.



Supplementary Figure 4. Repression of igDCs (signal 1,2) after NPs treatment. CLSM observations of igDCs molecules after various NPs treatments.

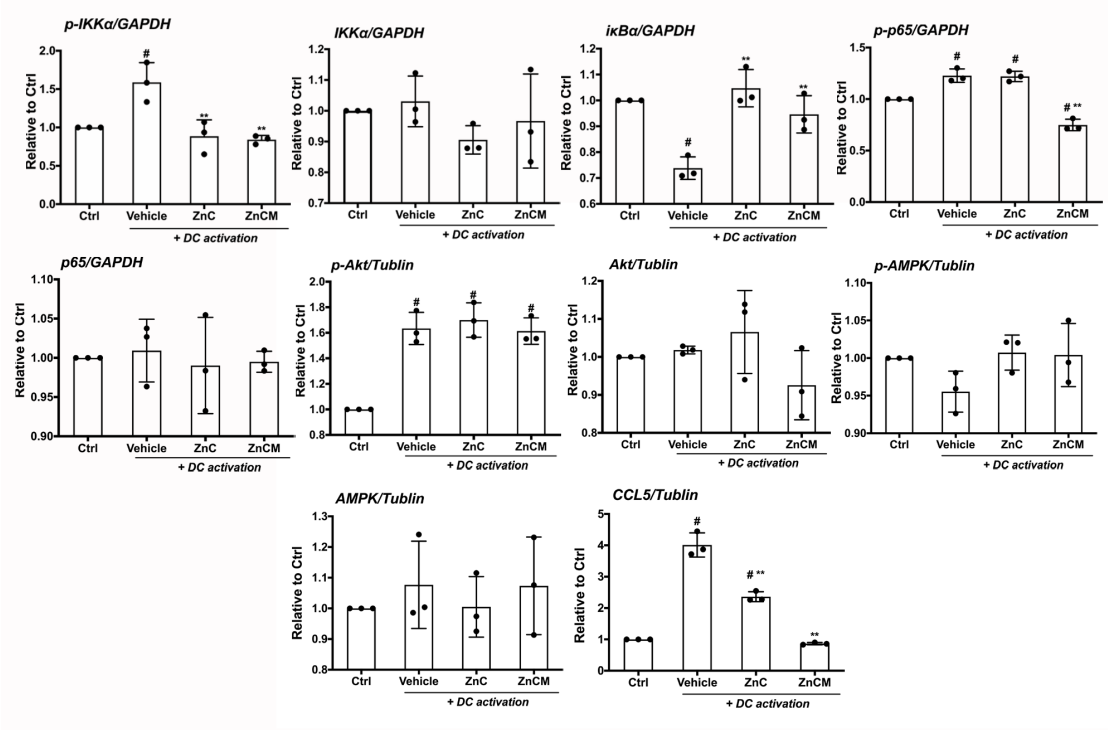


Supplementary Figure 5. Repression of igDCs (signal 3) after NPs treatment. (a) mRNA level of immune cytokines from igDCs treated with NPs towards T cell homeostasis regulation. (b) Cytometric bead array (CBA) immunoassay analyzing the levels of TNF, IL-6, and IL-12 from igDCs treated with NPs. ** indicated the significant difference of $p \leq 0.05$ compared with Vehicle, # indicated the significant difference of $p \leq 0.05$ compared with Ctrl.

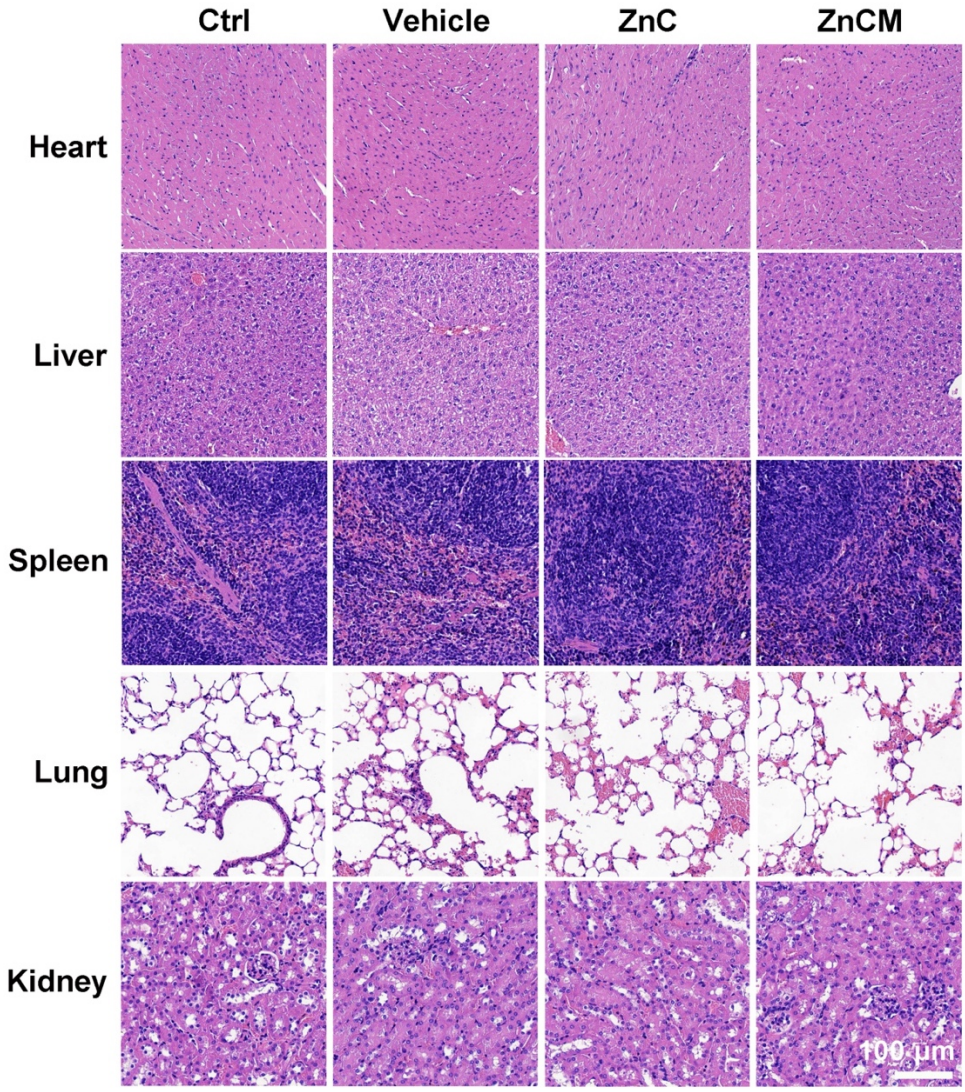


Supplementary Figure 6. Repression of T cells by tDCs after NPs treatment. (a) Proliferation of OT-II $CD4^+$ T cells cocultured with DCs treated with various NPs assessed by

flow cytometry. ** indicated the significant difference of p≤0.05. (b) CBA results showed the release of IL-17 and IFN-γ from $CD4^+$ T cells after co-culture with igDCs treated with NPs. ** indicated the significant difference of p≤0.05 compared with Vehicle, # indicated the significant difference of p≤0.05 compared with Ctrl.



Supplementary Figure 7. Quantification of expressed proteins in DCs after NPs treatments. ** indicated the significant difference of p≤0.05 compared with Vehicle, # indicated the significant difference of p≤0.05 compared with Ctrl.



Supplementary Figure 8. Biosafety of NPs. The HE histologic images of heart, liver, spleen, lung, and kidney in RA mice injected with varying NPs for 4 weeks.

Supplementary Table 1. Primers lists.

Primer	5' to 3'
UCHL3F	GCCCTGAAGAGAGAGCCAAA
UCHL3R	GGTGCCTCAGTCTGACCTTC
UCHL5F	ACTCAGTGCACGTTTGGCAT
UCHL5R	TGAAGAATGGAGTTTAGCCAGC
BAP1F	CCAAACGCCAGTGAGAACCT
BAP1R	ACATGTCACTCCCACTCCCA
UCHL1F	AGTCTGGGGAGAGAGAACCA
UCHL1R	GGGGCTGTAGAACGCAAGAA
USP15F	TCCCTCTACTCCTAATGTGAAAAAC
USP15R	AGGCCTGGCTGTTCATTGTT
USP46F	CTTCCCCCATCACACAGCTC
USP46R	GCAGGTCTCAACTCCACCTG
USP14F	CGGGAAATTACGTTTGGGTCC
USP14R	CTTGGGCTGAAAAGGCAAGG
USP21F	GAAGCCGGTGGCCGGT
USP21R	CCCCTCTTATCCCCAAACAGC
USP49F	AGAACATCAGTGCCCACAGG
USP49R	GCCACAAGCAAACGATCCAG
USP38F	GGCGTTGCACTCCAGATTCA
USP38R	TTAAAACCCCTGCCCTCTGG
USP4F	AGCCTGCAGTCAAATGGATCT
USP4R	GGCCAGGCTGTATATGAGGTG
USP25F	TCAATGAAGTGATGTTGTGAATGA
USP25R	CAAAAATAGATCGGTGGTAACATGA
USP44F	AGCATGTGCACGATGGAAGA
USP44R	TATTGGACAGGAGCTCGGGA
USP22F	TTGGTCTTTTTGCCTGCCCA
USP22R	GTCTCCAACTGGACCTCAGC
USP3F	TCAGCCTTTACAGCGGACAG
USP3R	CTACTAAAGACCTGAGTGACTGAA
USP26F	TCTAGATCCGTGGGGGTCTG
USP26R	CGGTCCTCACTCTGGTTCAC
USP5F	GGGAGACTGGCTACCCCTTA
USP5R	CTGGGGTCCAGAACCATGTC
USP13F	ACATTGAGGAGTTACCAGCCC
USP13R	ACTGGCACTTCGTTTTCCCA
USP12F	AGAAAGGATCGAGCGGATGT
USP12R	CAAAGTGAGCTACTGCCCTGA
USP18F	AGGGCGGCTGTGCCTA
USP18R	AATGACCCTCTCAAGCACGG

USP39F	GCTCCCTTCATCTCCTGTGG
USP39R	ACCCTTAACAAGCCTCAATGGG
USP27XF	GTGTTCTGGATGGGGAAGGG
USP27XR	TATCCTCGCCTCCTCCATGT
USP8F	ATATACACAGCGCGCAGAAG
USP8R	GCCCTTTCCTCATCACGATCT
USP33F	CCACACCTGCCAAATTGAGTT
USP33R	TGGACCTGGGGGATCCTTTA
PAN2F	CAGAGGTGGAACCGCTTCAT
PAN2R	TGAGCAGAAACTGCCTCCAG
USP20F	GCACTACTGTGACGCAGTCT
USP20R	AGTACAAGCTAGGGGCAGGA
USP45F	GAAGCTCCTGCGTGTGGAAG
USP45R	ATCATGAGGCAGCAGTGGTC
USP48F	ATCCCAGACCCGTAAACCCA
USP48R	CCTGGGCTTGCAGCCTATAA
USPL1F	ACAGCTCTCCACCATTGAGC
USPL1R	CCTTCCTGCTCCCTCAACAG
USP1F	GAAGGGCCATGACCGGATTT
USP1R	GACTGCCTCTTGAAAGCCCA
USP2F	CTCCATCCAGTGCCCCCAAC
USP2R	CCATAGCCCGGTTTGGCAT
USP10F	TTTGTGACTCCCCGCTCTTC
USP10R	CTGGTGCTCCTGTCCATCTG
USP36F	CGAAGGCTGACAGTCCAGG
USP36R	TCTCTGCAGCAGGTAAGGGA
SENP2F	CTGCTCAGGCCTGGAATGT
SENP2R	CAGCTGGAACGGGAATCCAA
CYLDF	CAGGTAGCAGGTTCGGCTG
CYLDR	ACTGGCAAAAAGGAGCCACT
YOD1F	TCAGACCTGTCCTCTGGGTT
YOD1R	TGCTCAAGTGGTAAAGGTAAGC
OTUD3F	GCTTAATGCCCCTTTGTGGC
OTUD3R	CCGGACGCTGTCATAGTGTT
OTUB2F	TCTGCCACCTAGGTCCTTCC
OTUB2R	GTGTCCTTGCCTCAGGTCTC
OTUD6AF	TCGAGAATATGCCTCCACGC
OTUD6AR	GCGACCTTTTCCTCCTCCTC
TNFAIP3F	CCTGCCAGCAGGTATATGGG
TNFAIP3R	CAAGGCCTGAAGAGGAAGGG
OTUB1F	TCCCCAGCTTTCCAACCATC
OTUB1R	GCCTATGGGAGCAGAACTCC

ZRANB1F	CCGCGGGGTTTATTTAGCTC
ZRANB1R	AGTCAGGACATGTTCTCCGC
ALG13F	CCTTTAATTCTAACCTTTGGGAGCC
ALG13R	CTCTGGGTAGAAAAGGATGGC
ATXN3F	CCCTCAAAGCAGACCTGGAG
ATXN3R	TACTGAGCTGAATGGCCCTG
JOSD1F	CACTGGCCTGCTTCTTCTCC
JOSD1R	CGAGGCCTCTGCCACTTG
JOSD2F	CCATTGCTAGCAGGAGCTGA
JOSD2R	CTTGCAGATTTCGTCGGCAG
STAMBP1F	TTCACGAAAGGTGGGAAGGG
STAMBP1R	AAGGAGTGTTGAGACCAGGC
PSMD14F	AAAGGAAGCCGAAGGAAGCC
PSMD14R	CTCGGAGAACAGGCGATGAA
BRCC3F	GGAGCAAAACCAGCAGCATT
BRCC3R	GCCAGGTATTTTCATCTGCCC
COPS5F	GCCTTGAGAGTCTATCACCACT
COPS5R	TGATGATCATGGTCTCGCCG
STAMBPL1F	CATCCTCACACCAAGGACCC
STAMBPL1R	TCACACACGTCCACAGATGG
EIF3FF	TCGAGGTTTTCACCACAGGG
EIF3FR	ATGCTTCTTTCTCCTGGCCG
UFSP1F	TGGGCCTGGCACAATGATAC
UFSP1R	CAGCTTGGCCTTGCTGTGTA
SENP5F	CCGGGAGCAAAGAACGTACA
SENP5R	GCTTCCAAACCCAGCATTCC
SENP8F	ACAAAGAATCCGCAATGCCC
SENP8R	ACTCAAGACCACAGGGTCCA
SENP7F	CGCGAATCTCACTTCTGGCT
SENP7R	ATCTCTGATGAAGCTCGCCG
SENP1F	GAGTAAAGAAGGTTCCGGTTCCC
SENP1R	CCGCCACTCACCGAACC
SENP3F	TGGCCTAGACGCGCTGA
SENP3R	CCAAAACCACCACCGGACT
ZC3H12AF	TGCTGTGTACAGAGGCGAGA
ZC3H12AR	CCACCATGTGGTTGCTGACA
UFD1LF	CAGTGCAGCATGAGGAGTCA
UFD1LR	GAACCAGAGAAGGCACGGAA
TAF1DF	ACTTGCTGGTGGAACGCTAA
TAF1DR	CTGCAGATGAGCAAAAGGGC
TRIM44F	ATGCCCATTCACACTCGGAA
TRIM44R	TCCTGGAAGGAACAATGAATACT

IL-1β	TTCAAGGGGACATTAGGCAG
IL-6	CAACGATGATGCACTTGCAGA
IL-12α	CTGTGCCTTGGTAGCATCTATG
IL-12β	GTGGAATGGCGTCTCTGTCT
TNF-α	CTCAGCGAGGACAGCAAGG