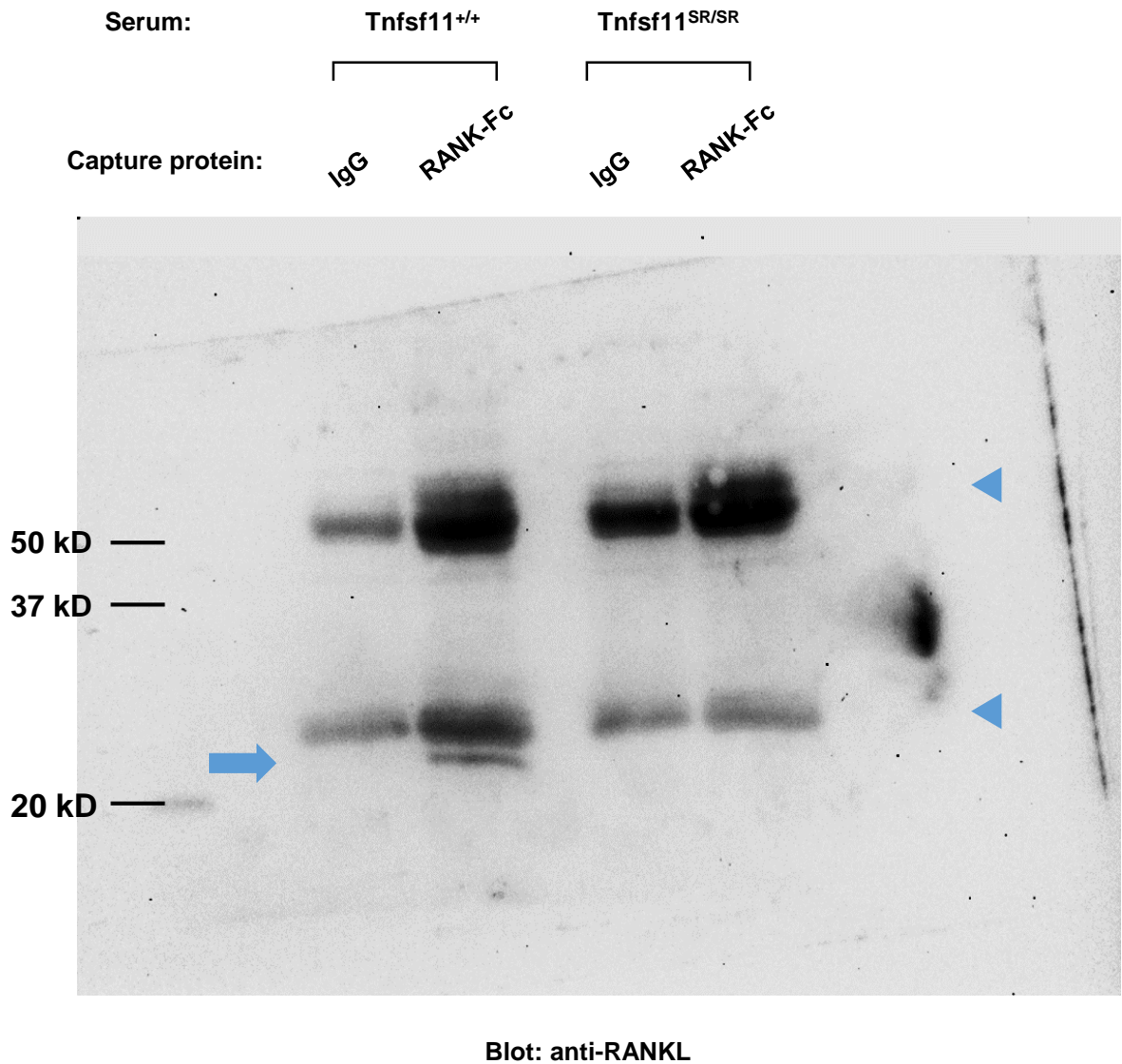
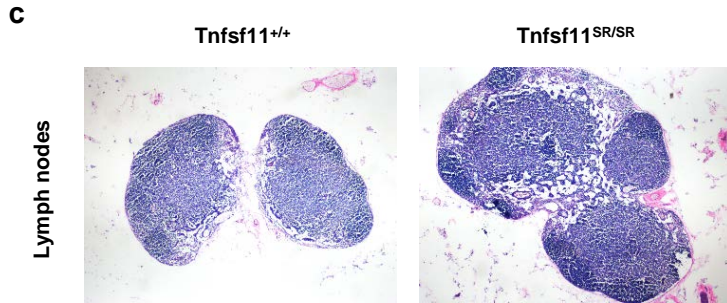
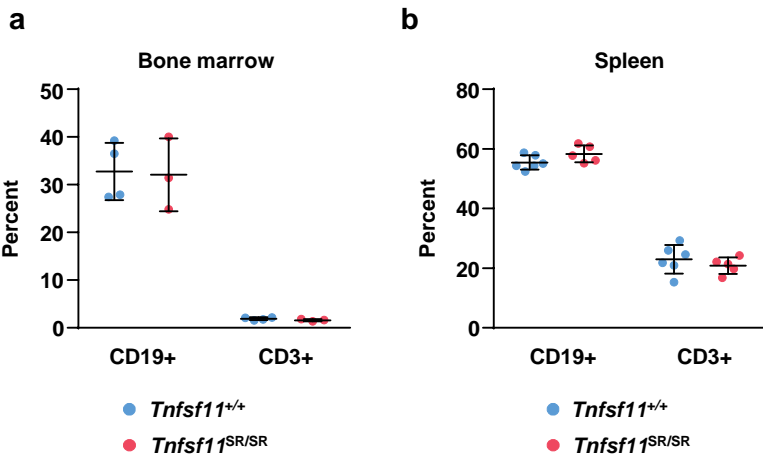


# **Soluble RANKL contributes to osteoclast formation in adult mice but not ovariectomy-induced bone loss**

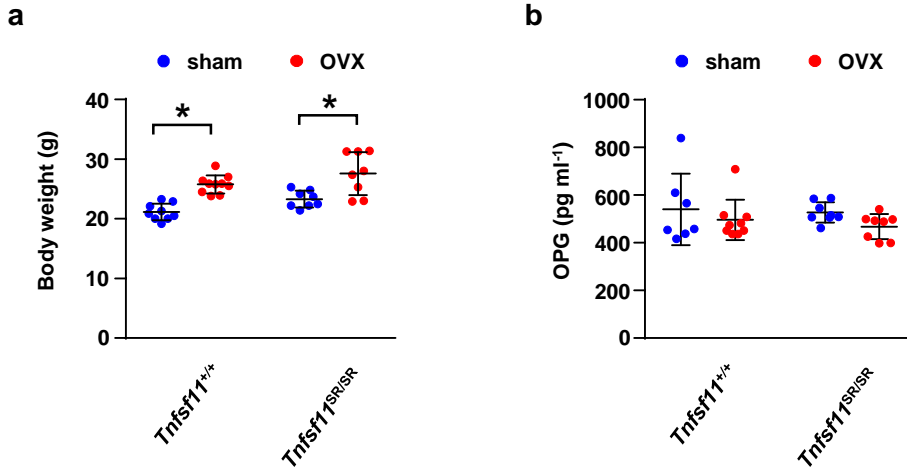
**Xiong et al**



**Supplementary Figure 1.  $Tnfsf11^{SR/SR}$  mice lack sRANKL as measured by affinity isolation.** Analysis of sRANKL from the serum of  $Tnfsf11^{+/+}$  and  $Tnfsf11^{SR/SR}$  mice by affinity isolation and immunoblot. The arrow indicates the location of sRANKL and the arrowheads indicate the location of IgG heavy and light chains.

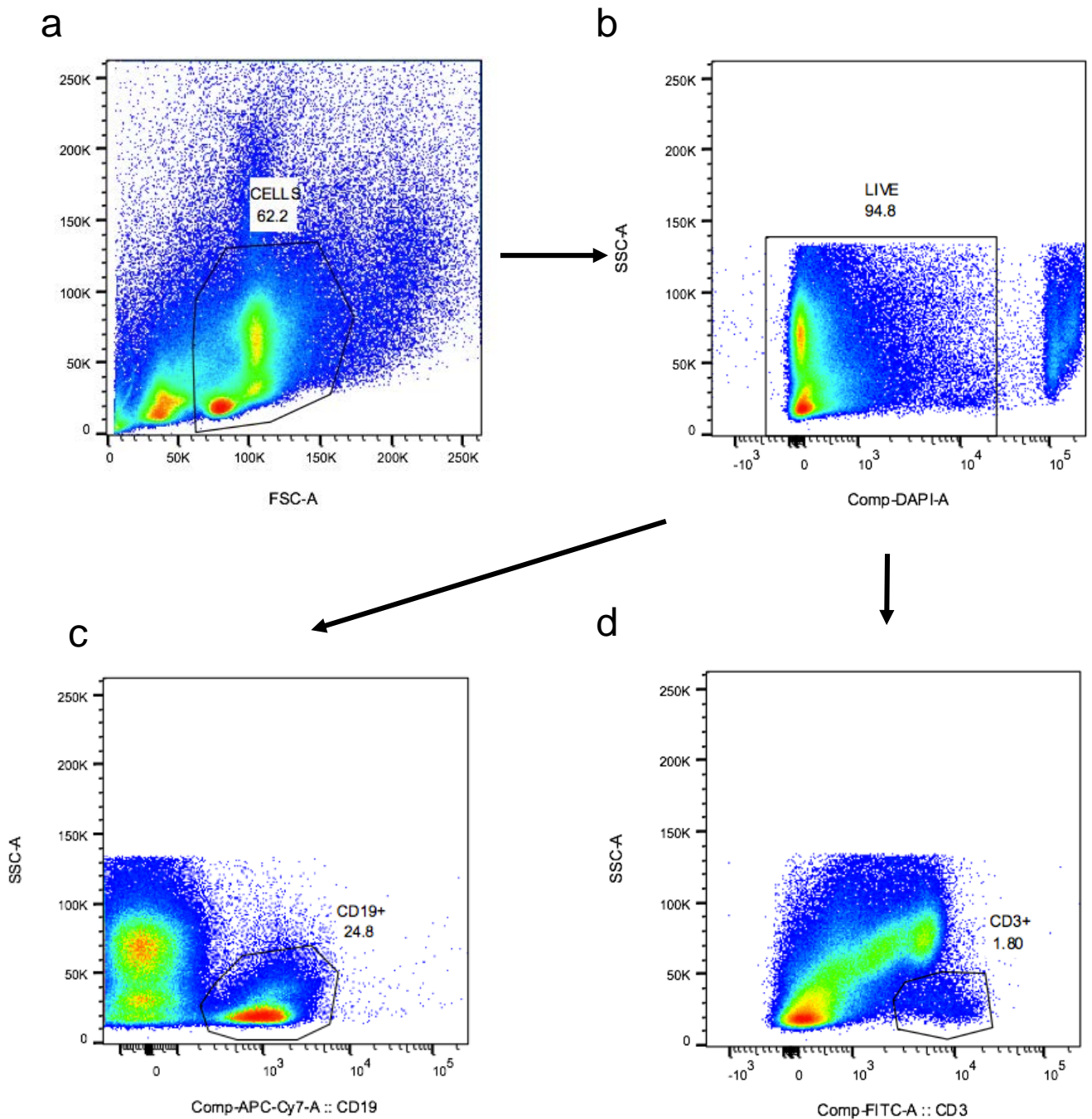


**Supplementary Figure 2. *Tnfsf11*<sup>SR/SR</sup> mice display normal lymphocyte and lymph node development.** (a) Quantification of B lymphocytes (CD19+) and T lymphocytes (CD3+) from bone marrow of *Tnfsf11*<sup>+/+</sup> ( $n = 4$ ) and *Tnfsf11*<sup>SR/SR</sup> ( $n = 3$ ) mice (values are mean  $\pm$  s.d.). (b) Quantification of B lymphocytes (CD19+) and T lymphocytes (CD3+) from the spleen of *Tnfsf11*<sup>+/+</sup> ( $n = 6$ ) and *Tnfsf11*<sup>SR/SR</sup> ( $n = 5$ ) mice (values are mean  $\pm$  s.d.). (c) Images of histological sections of inguinal lymph nodes from mice of the indicated genotypes.

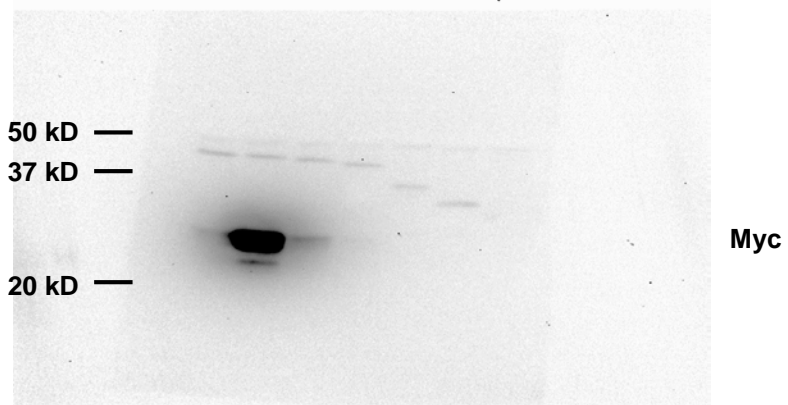
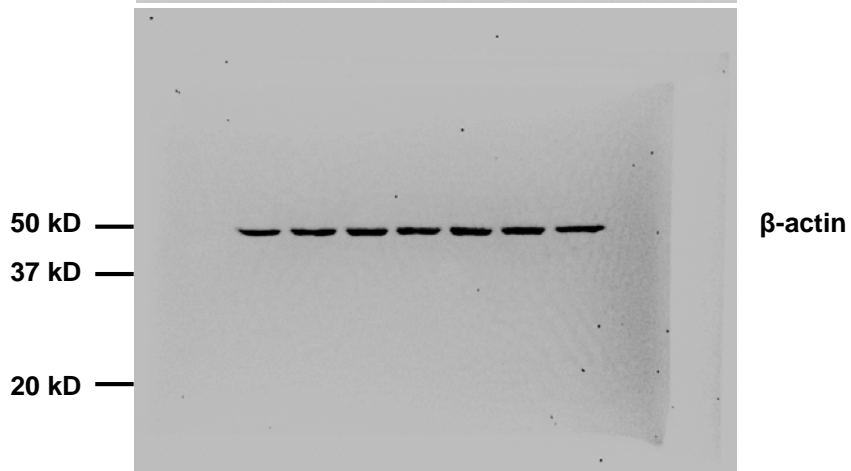
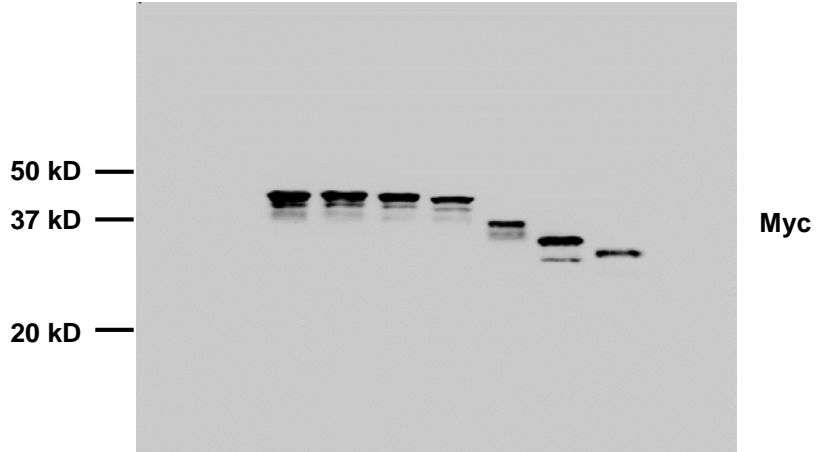
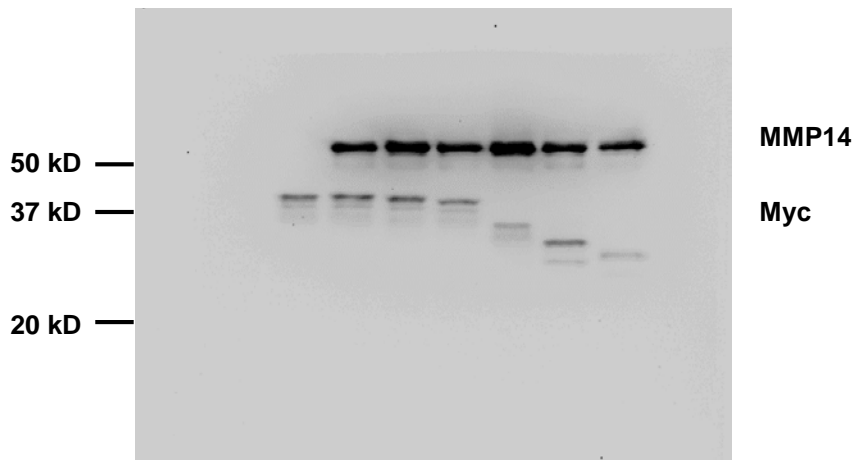


**Supplementary Figure 3. Body weight increases, but circulating OPG levels are unchanged, by ovariectomy in *Tnfsf11*<sup>+/+</sup> and *Tnfsf11*<sup>SR/SR</sup> mice.** Body weight (a), and OPG protein levels in the serum (b), of sham-operated (sham) and ovariectomized (ovx) *Tnfsf11*<sup>+/+</sup> ( $n = 7$  and  $9$  for sham and ovx, respectively) and *Tnfsf11*<sup>SR/SR</sup> ( $n = 8$  and  $8$  for sham and ovx, respectively). \* $P < 0.05$  using Student's t-test.

# Flow cytometry gating strategy



**Supplementary Figure 4. Representative gating strategy of a bone marrow sample.** Cells were gated on a forward scatter (FSC)/side scatter (SSC) plot (a), excluding cellular debris, and then live cells were selected by gating on the cells that excluded the viability dye (DAPI-negative) (b). B cells were recognized by gating on side scatter (SSC)/CD19-APC (c). T cells were recognized by gating on side scatter (SSC)/CD3-FITC (d). Percentages of sub-populations are shown for each graph.



Supplementary Figure 5. Uncropped western bots used in Fig. 1b.

50 kD —  
37 kD —  
20 kD —



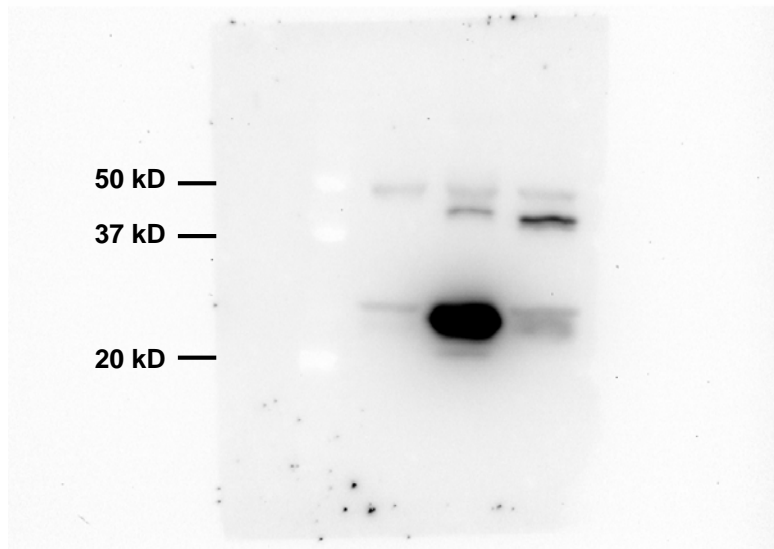
Myc

50 kD —  
37 kD —  
20 kD —



$\beta$ -actin

50 kD —  
37 kD —  
20 kD —



Myc

**Supplementary Figure 6. Uncropped western blots used in Fig. 1c.** Red box indicates the bands showed in Fig. 1c.

Supplementary Table 1

Primer Sequences			
No.	Name	Sequence	Product Size (bp)
1	ex3OT1-for ex3OT1-rev	cagggatccCCCCTTTCTCCGTAAGAAGC caggtcgacTGCATCATCATGGAACATCA	360
2	ex3OT2-for ex3OT2-rev	cagggatccTGTCCCCACCATCCTTTAAT caggtcgacTGGTAAGCCAGAAGGCTCAT	325
3	ex3OT3-for ex3OT3-rev	CATGCTTCAGGGATCCTCAT caggtcgacGGTTTGGTCCTCACTGGTCA	354
4	ex3OT4-for ex3OT4-rev	cagggatccGGACATTTGGGACTGGGTAA caggtcgacTTTTGAAGCCATTCTGACC	320
5	ex3OT5-for ex3OT5-rev	cagggatccCCACAACCCCTTACAGGATG caggtcgacAAGGAAAAGTAGCCCCAGGT	319
6	ex3OT6-for ex3OT6-rev	cagggatccGCCACTGGCACTTATCAAAA caggtcgacGCTCTGGAAAAATCTTGCTCAT	444
7	ex4OT1-for ex4OT1-rev	cagggatccCTGACACAGAAGCGGCACTA caggtcgacGGACTGCAGCTCTCAGCTCT	318
8	ex4OT2-for ex4OT2-rev	cagggatccTTTTTCTTGGCCATTTTACA caggtcgacGCAGATAATTAAGCCAGGCAAC	329
9	ex4OT3-for ex4OT3-rev	cagggatccTTTGCCATAGACTGTTTTCTTG caggtcgacCATCCCTCAGGAGCAAGTTC	323
10	ex4OT4-for ex4OT4-rev	cagggatccTTTTCTTTGACCCAGTATTACCA caggtcgacTCTGAGTTCCCTGCACAATG	305
11	ex4OT5-for ex4OT5-rev	cagggatccTTCCAAAAGGAGCCTTAAGAAG caggtcgacCAGGCCAAAATGTCAAAAAGA	402
12	ex4OT6-for ex4OT6-rev	cagggatccGGGACATTTCTGAGGACACAA caggtcgacAGCAGCACAGGGCATAGTG	349
13	ex4OT1mu-for ex4OT1mu-rev	CTGACACAGAAGCGGCACTA CCTTTCATCCCATTTATGAGC	160

Supplementary Table 2

Summary of Off-target Cleavage				
Sites	Sequence	Indel Frequency (Mutant/Total)	Locus	Overlapping Genes
Exon 3 sgRNA	TCAGGAAGTCAACACATTGTGG	0/5	chr14:78284445-78284470	No
Ex3OT1	GCAGTAAATTCAACACATTGTGG	0/5	chr8:96842918-96842943	No
Ex3OT2	TGAGGAGCTGCAACACATTCTGG	0/5	chrX:59562222-59562247	No
Ex3OT3	TAAGGAAGTACAACACATAGTGG	0/5	chr14:13074161-13074186	No
Ex3OT4	TTACCACCTGCAACACATTGAAG	0/5	chr7:26571291-26571316	Yes
Ex3OT5	TCAAGAACAGAAACACATTGGGG	0/5	chrX:136094828-136094853	No
Ex3OT6	TAAAGCATTGCAACACATTGCAG	0/5	chr13:82396661-82396686	No
Exon 4 sgRNA	TATGATGGAAGGCTCATGGTTGG	0/5	chr14:78284312-78284337	No
Ex4OT1	TAAGAAGGAAGGCTCATGGTTGG	2/5	chr12:71247138-71247163	No
Ex4OT2	TCTGATGAAAGGCTCATGGAAAG	0/5	chr5:98995982-98996007	No
Ex4OT3	TCTGGTGGAAAGCCTCATGGTAGG	0/5	chr14:35355852-35355877	No
Ex4OT4	TATGCTGGACAGCTCATGGTAGG	0/5	chr9:95577402-95577427	No
Ex4OT5	CATGTTGGAAGGCTCATGATCAG	0/5	chr14:102856819-102856844	No
Ex4OT6	TGTGAAGGAAAGCTCATGGTTAG	0/5	chr1:93292054-93292079	No



Supplementary Table 3

Sequences of cloned off-target PCR products		
	Exon 3	Exon 4
OT 1	GAGACTGCAGTAAATTCAACACATTGTGGTTTGGGA GAGACTGCAGTAAATTCAACACATTGTGGTTTGGGA GAGACTGCAGTAAATTCAACACATTGTGGTTTGGGA GAGACTGCAGTAAATTCAACACATTGTGGTTTGGGA GAGACTGCAGTAAATTCAACACATTGTGGTTTGGGA GAGACTGCAGTAAATTCAACACATTGTGGTTTGGGA GAGACTGCAGTAAATTCAACACATTGTGGTTTGGGA chr8:96842918-96842943 Overlapping Genes: None	ACCAAGTAAGAAGGAAGGCTCATGGTTGGATGATG ACCAAGTAAGAAGGAAGGCTCATGGTTGGATGATG ACCAAGTAAGAAGGAAGGCTCATA-----A--ATG ACCAAGTAAGAAGGAAGGCTCATGGTTGGATGATG ACCAAGTAAGAAGGAAGGCTCATGGTTGGATGATG ACCAAGTAAGAAGGAAGGCTCATGGTTGGATGATG ACCAAGTAAGAAGGAAGGCTCATA-----A--ATG chr12:71247130-71247172 Overlapping Genes: None
OT 2	TATATTTGAGGAGCTGCAACACATTCTGGTTGTTC TATATTTGAGGAGCTGCAACACATTCTGGTTGTTC TATATTTGAGGAGCTGCAACACATTCTGGTTGTTC TATATTTGAGGAGCTGCAACACATTCTGGTTGTTC TATATTTGAGGAGCTGCAACACATTCTGGTTGTTC TATATTTGAGGAGCTGCAACACATTCTGGTTGTTC TATATTTGAGGAGCTGCAACACATTCTGGTTGTTC chrX:59562222-59562247 Overlapping Genes: None	AGTGACTCTGATGAAAGGCTCATGGAAAGAAGAGG AGTGACTCTGATGAAAGGCTCATGGAAAGAAGAGG AGTGACTCTGATGAAAGGCTCATGGAAAGAAGAGG AGTGACTCTGATGAAAGGCTCATGGAAAGAAGAGG AGTGACTCTGATGAAAGGCTCATGGAAAGAAGAGG AGTGACTCTGATGAAAGGCTCATGGAAAGAAGAGG AGTGACTCTGATGAAAGGCTCATGGAAAGAAGAGG chr5:98995982-98996007 Overlapping Genes: None
OT 3	TTGTGATAAGGAACTACAACACATAGTGGATCACG TTGTGATAAGGAACTACAACACATAGTGGATCACG TTGTGATAAGGAACTACAACACATAGTGGATCAGCG TTGTGATAAGGAACTACAACACATAGTGGATCACG TTGTGATAAGGAACTACAACACATAGCGGATCACG TTGTGATAAGGAACTACAACACATAGTGGATCACG TTGTGATAAGGAACTACAACACATAGTGGATCACG chr14:13074161-13074186 Overlapping Genes: None	GTAGTTTCTGGTGAAGCCTCATGGTAGGTAGCAT GTAGTTTCTGGTGAAGCCTCATGGTAGGTAGCAT GTAGTTTCTGGTGAAGCCTCATGGTAGGTAGCAT GTAGTTTCTGGTGAAGCCTCATGGTAGGTAGCAT GTAGTTTCTGGTGAAGCCTCATGGTAGGTAGCAT GTAGTTTCTGGTGAAGCCTCATGGTAGGTAGCAT GTAGTTTCTGGTGAAGCCTCATGGTAGGTAGCAT chr14:35355852-35355877 Overlapping Genes: None
OT 4	TGGTTCCTTACCACCTGCAACACATTGAAGAGCTTA TGGTTCCTTACCACCTGCAACACATTGAAGAGCTTA TGGTTCCTTACCACCTGCAACACATTGAAGAGCTTA TGGTTCCTTACCACCTGCAACACATTGAAGAGCTTA TGGTTCCTTACCACCTGCAACACATTGAAGAGCTTA TGGTTCCTTACCACCTGCAACACATTGAAGAGCTTA TGGTTCCTTACCACCTGCAACACATTGAAGAGCTTA chr7:26571291-26571316 Overlapping Genes: Yes	TGCCTATATGCTGGACAGCTCATGGTAGGTGTTCT TGCCTATATGCTGGACAGCTCATGGTAGGTGTTCT TGCCTATATGCTGGACAGCTCATGGTAGGTGTTCT TGCCTATATGCTGGACAGCTCATGGTAGGTGTTCT TGCCTATATGCTGGACAGCTCATGGTAGGTGTTCT TGCCTATATGCTGGACAGCTCATGGTAGGTGTTCT TGCCTATATGCTGGACAGCTCATGGTAGGTGTTCT chr9:95577402-95577427 Overlapping Genes: None
OT 5	ATTTTCGTC AAGAACAGAAACACATTGGGGAACATA ATTTTCGTC AAGAACAGAAACACATTGGGGAACATA ATTTTCGTC AAGAACAGAAACACATTGGGGAACATA ATTTTCGTC AAGAACAGAAACACATTGGGGAACATA ATTTTCGTC AAGAACAGAAACACATTGGGGAACATA ATTTTCGTC AAGAACAGAAACACATTGGGGAACATA ATTTTCGTC AAGAACAGAAACACATTGGGGAACATA chrX:136094828-136094853 Overlapping Genes: None	ACTCGACATGTTGGAAGGCTCATGATCAGCTCCTC ACTCGACATGTTGGAAGGCTCATGATCAGCTCCTC ACTCGACATGTTGGAAGGCTCATGATCAGCTCCTC ACTCGACATGTTGGAAGGCTCATGATCAGCTCCTC ACTCGACATGTTGGAAGGCTCATGATCAGCTCCTC ACTCGACATGTTGGAAGGCTCATGATCAGCTCCTC ACTCGACATGTTGGAAGGCTCATGATCAGCTCCTC chr14:102856819-102856844 Overlapping Genes: None
OT 6	TGATAATAAAGCATTGCAACACATTGCAGCTCTAT TGATAATAAAGCATTGCAACACATTGCAGCTCTAT TGATAATAAAGCATTGCAACACATTGCAGCTCTAT TGATAATAAAGCATTGCAACACATTGCAGCTCTAT TGATAATAAAGCATTGCAACACATTGCAGCTCTAT TGATAATAAAGCATTGCAACACATTGCAGCTCTAT TGATAATAAAGCATTGCAACACATTGCAGCTCTAT chr13:82396661-82396686 Overlapping Genes: None	ACACAATGTGAAGGAAAGCTCATGGTTAGCGGTTT ACACAATGTGAAGGAAAGCTCATGGTTAGCGGTTT ACACAATGTGAAGGAAAGCTCATGGTTAGCGGTTT ACACAATGTGAAGGAAAGCTCATGGTTAGCGGTTT ACACAATGTGAAGGAAAGCTCATGGTTAGCGGTTT ACACAATGTGAAGGAAAGCTCATGGTTAGCGGTTT ACACAATGTGAAGGAAAGCTCATGGTTAGCGGTTT chr1:93292054-93292079 Overlapping Genes: None

\*Dashes (-) represent deleted bases