Supplementary material

Long noncoding RNA TUG1 inhibits osteogenesis of bone marrow mesenchymal stem cells via Smad5 after irradiation

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Table S1

Noncoding RNAs were upregulated or downregulated by > 2-fold after irradiation (P < 0.05)

Gene_Symbol	24_Average	0_Average	FC	24-1	24-2	24-3	0-1	0-2	0-3
RP11-386G11.10	7.64	8.76	-2.17	7.48	7.84	7.59	8.81	8.64	8.82
linc_luo_1230	3.65	5.82	-4.52	3.85	3.50	3.56	5.95	5.71	5.79
L43846	5.66	6.81	-2.20	5.74	5.73	5.49	6.50	6.88	6.99
L32866	3.34	4.36	-2.02	3.23	3.37	3.40	4.19	4.20	4.66
VCAN-AS1	4.87	3.32	2.84	4.28	5.06	5.11	3.48	3.12	3.34
uc003pju.1	6.48	5.37	2.17	6.48	6.71	6.20	5.53	4.98	5.53
uc003kor.1	5.76	4.75	2.08	5.57	5.75	5.94	4.55	4.32	5.22
uc003jqp.2	8.98	7.80	2.29	8.78	9.04	9.10	7.97	7.42	7.95
uc003isk.1	5.37	4.37	2.09	5.21	5.31	5.57	4.50	3.67	4.73
uc001pye.1	7.27	5.47	3.73	7.33	7.27	7.21	6.11	4.93	5.07
uc001elt.1	3.95	2.73	2.38	3.94	3.92	3.99	3.10	2.46	2.54
TUG1	**8.57**	**7.55**	**2.04**	**8.50**	**8.58**	**8.61**	**7.81**	**7.25**	**7.55**
THAP9-AS1	5.98	4.96	2.02	5.72	5.99	6.18	5.09	4.78	4.97
RP11-787B4.2	5.21	3.66	2.91	5.41	5.35	4.81	3.78	3.77	3.39
RP11-611O2.5	4.21	2.65	3.01	4.00	4.27	4.35	3.02	2.42	2.41
PAPPA-AS1	6.84	5.41	2.75	6.70	7.03	6.76	5.77	5.03	5.32
NR_024415	7.02	6.00	2.06	6.95	7.12	6.99	6.14	5.59	6.19
NR_024020	5.63	4.52	2.18	5.32	5.64	5.88	4.51	4.09	4.87
NR_003254_2	5.49	4.02	2.74	5.24	5.45	5.73	3.94	4.11	4.01
NR_003252	6.10	4.92	2.32	6.02	6.34	5.89	5.29	4.40	4.92
MIR548O2	5.01	4.07	2.01	4.69	5.04	5.26	4.65	3.85	3.46
MIR548AA2	6.51	5.43	2.35	6.14	6.50	6.81	6.22	4.68	4.84
LOC285505	5.02	3.81	2.27	4.65	5.04	5.30	3.85	3.83	3.76
LOC100129550	5.25	4.16	2.20	5.07	5.24	5.43	4.37	3.55	4.41
LOC100129550	5.01	4.02	2.06	4.88	5.05	5.08	4.10	3.39	4.40
linc-ZNF543	6.52	5.49	2.10	6.72	6.41	6.41	5.97	5.22	5.13
linc-SLC39A10-10	3.24	2.08	2.28	3.28	3.26	3.17	2.34	1.68	2.12
linc-RAD23B-2	3.89	2.49	2.60	3.88	3.59	4.13	2.55	2.57	2.36
linc-POTED-3	6.34	4.70	3.15	6.10	6.31	6.58	4.47	4.47	5.08
linc-PAX8-3	5.73	4.66	2.12	5.78	5.71	5.70	4.89	4.36	4.68
linc-PAPPA-1	5.25	3.95	2.41	5.29	5.57	4.79	3.99	4.02	3.83
linc-NBPF15-1	7.58	5.93	3.19	7.51	7.62	7.61	6.21	5.64	5.87
linc-FAM75A6-8	7.67	6.64	2.05	7.55	7.67	7.80	6.74	6.38	6.79
linc-CGGBP1-3	5.62	4.57	2.06	5.27	5.53	5.97	4.80	4.18	4.66
linc-ANKRD20A1-8	6.66	4.92	3.32	6.48	6.50	6.94	4.79	4.76	5.17
LINC00657	7.91	6.87	2.07	7.78	7.97	7.97	7.12	6.65	6.79
linc_luo_504	6.46	5.27	2.39	6.34	6.47	6.56	5.81	4.89	4.91

linc_luo_185	6.11	4.35	3.54	5.78	6.09	6.40	4.92	3.92	3.97
linc_luo_1223	5.07	4.11	2.06	4.90	5.18	5.10	4.69	3.49	3.88
HNRNPU-AS1	4.23	2.97	2.52	4.35	4.23	4.11	3.49	2.38	2.82
DNM3OS	5.99	4.68	2.49	5.69	5.96	6.27	4.91	4.27	4.80
CR614809	6.78	5.75	2.07	6.61	6.87	6.85	5.94	5.39	5.86
CR613639	8.34	7.26	2.12	8.14	8.40	8.46	7.49	7.12	7.14
CR608583	6.34	5.22	2.18	6.22	6.26	6.52	5.37	4.97	5.30
CR607695	5.89	4.88	2.02	5.81	5.90	5.96	5.03	4.82	4.78
CR594753	4.17	2.90	2.42	3.81	4.31	4.34	3.21	2.70	2.72
CR590425	5.43	4.43	2.03	5.37	5.51	5.41	4.60	4.01	4.60
BC041400	5.53	4.48	2.07	5.29	5.58	5.69	4.65	4.26	4.51
BC035072	5.23	4.04	2.36	5.14	5.26	5.28	3.94	3.57	4.46
AY011601	9.56	7.99	2.97	9.59	9.46	9.61	8.10	8.00	7.86
AX746517	4.91	3.88	2.05	4.60	4.97	5.12	4.17	3.63	3.78
ANKRD10-IT1	4.27	3.05	2.39	4.18	4.32	4.30	3.40	3.00	2.62
AK129948	4.42	3.05	2.61	4.16	4.50	4.58	3.27	2.63	3.17
AK097984	6.50	4.83	3.94	6.36	6.62	6.52	5.79	4.22	3.56
AK095091	5.85	4.84	2.10	5.88	5.74	5.92	5.36	4.41	4.56
AK094510	4.78	3.68	2.15	4.75	4.81	4.78	3.70	3.59	3.74
AK001903	5.76	4.48	2.45	5.58	6.06	5.58	4.82	4.15	4.38

Table S2

The probles of TUG1

PROBE	PROBE (5'-> 3')	PROBE POSITION	PERCENT GC
1	cgagttctgcgtacgagaag	318	55.00%
2	cactaaggcggcataaggag	591	55.00%
3	attgtaccatatgcatcagc	1261	40.00%
4	cagaacacatccactcctaa	1306	45.00%
5	atggtcgtggaatatggtca	1420	45.00%
6	ctactgattttgaggttccg	1676	45.00%
7	agaacacaaggagggccaag	1727	55.00%
8	tctctgccctttaggaaaag	2029	45.00%
9	ttttgaggtacatccggatt	2120	40.00%
10	aagcatcttatcaccacagt	2148	40.00%
11	ttatctgctccaactattgc	2280	40.00%
12	tggaaatctggagtccatgg	2318	50.00%
13	tcacagcaaggtcaacagtc	2430	50.00%
14	gtctggacagaaactcaggt	2508	50.00%
15	ttcactttggatggagcatc	2545	45.00%

16	atagcacagggaaggtagtc	2611	50.00%
17	gcaaaaatccaggtaccagg	2647	50.00%
18	gtcagtagtaaggtggtagg	2678	50.00%
19	acaaactaggcctttgttgt	2855	40.00%
20	tgttttctcattcagtaggc	3099	40.00%
21	actgtgcctttgatttgatc	3135	40.00%
22	ggactccttggtagaatttc	3217	45.00%
23	tgtcttcttgcacttttcta	3674	35.00%
24	aaatgatcattcctggggtc	3705	45.00%
25	ggctgttaatctaggttcac	3742	45.00%
26	ccattctaggaatcactgga	3780	45.00%
27	cttaagggagtctgtcagtg	3832	50.00%
28	caagtaccagtagagcatct	3859	45.00%
29	aggtattcatggagggtcaa	3899	45.00%
30	atgaggcaccagcttcaaaa	3937	45.00%
31	caagttgctctaatatggcc	3960	45.00%
32	aagtagagtaccatggctga	3999	45.00%
33	ccaaggggcaatataactgc	4140	50.00%
34	tcacatgagaaggggtgttc	4332	50.00%
35	attgagactgatgtggttcc	4666	45.00%
36	ggcaaggaatgaagtcagct	4712	50.00%
37	gctggcctttaaattgactg	4882	45.00%
38	attcattcgagtagctcagg	4905	45.00%
39	aggctagtcaaccagatttt	5062	40.00%
40	actcgagcagatgatcatct	5263	45.00%
41	gcatggagacatggagacag	5360	55.00%
42	ccgatctctgagaatcatct	5445	45.00%
43	aggttctgtttaggagtcta	5560	40.00%
44	ctgccaaagaactttcagct	5611	45.00%
45	ctggaagaaccagcagatca	5759	50.00%
46	ggtgaatcggactcatttct	6174	45.00%
47	agtggtcatgagtctgagag	6341	50.00%
48	ccacagtttcaacacaagca	6366	45.00%

```
                   *        20         *        40
Smad5-1 : TTAGCAGTTCCCCAATCCTTGGGTTTTGAACCTGGGAACCT :  41
Smad5-2 : TTAGCAGTTCCCCAATCCTTGGGTTTTGAACCTGGGAACCT :  41
TUG1    : -TAGCAGTTCCCCAATCCTTGGGTTTTGAACCTGGGAACCT :  40
          tTAGCAGTTCCCCAATCCTTGGGTTTTGAACCTGGGAACCT

                  *        60         *        80
Smad5-1 : TGGATTGGAGTTGGGGATCCCCAAACTTCCTGAAATTGTGG :  82
Smad5-2 : TGGATTGGAGTTGGGGATCCCCAAACTTCCTGAAATTGTGG :  82
TUG1    : TGGATTGGAGTTGGGGATCCCCAAACTTCCTGAAATTGTGG :  81
          TGGATTGGAGTTGGGGATCCCCAAACTTCCTGAAATTGTGG

                 *       100         *
Smad5-1 : GAATGTGCGGTTTGGGGGAATGATGGGAATTTGT- : 116
Smad5-2 : GAATGTGCGGTTTGGGGGAATGATGGGAATTTGTG : 117
TUG1    : GAATGTGCGGTTTGGGGGAATGATGGGAATTTGTG : 116
          GAATGTGCGGTTTGGGGGAATGATGGGAATTTGTg
```

Figure S1. The RIP sequencing results

Smad5-1: The sequencing results of the indicated RNAs retrieved by Smad5-specific antibody in non-radiated BM-MSCs, Smad5-2: The sequencing results of the indicated RNAs retrieved by Smad5-specific antibody in radiated BM-MSCs

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                *        20         *        40
25   : ---ACGTCACCGGGC---CCAGTACAAGCAGCAGAACAC-AGCT :  37
50   : GGGGTATGAGTCGATTCACCAGTACACACAGAACTGTACCAGTT :  44
TUG1 : -------------------------------------------- :   -
             t a   g     ccagtaca  cag a    ac ag t

            *        60         *        80
25   : TGACGGGCCAAGGGACAAAAGGCCACTAAAAGGACGTACAGAAC :  81
50   : CAACCTAGCAAAAGAAGAAAAGTTTCCACTTGTACTTAAAATTT :  88
TUG1 : -------------------------------------------- :   -
         ac    caa  ga  aaa g   c a   g ac ta a

        *       100         *       120         *
25   : ACATCCACTCCTCAAG-----C-----GAATTATTTGATGTCCA : 115
50   : ACAGCTTGACTTCAAATTTGCCTCACAGAATTATTTGATGTA-A : 131
TUG1 : -------------------------------------------- :   -
       aca c    c tcaa      c     gaattatttgatgt  a

            140         *       160         *
25   : AAGGCTAGTTGTCTTACTTCAGATCAGCAGGACAGTTGGGCTCT : 159
50   : AAGGCTAGTTGTCTTACTTCAGATCAGCAGGACAGTTGGGCTCT : 175
TUG1 : -------------------------------------------- :   -
       aaggctagttgtcttacttcagatcagcaggacagttgggctct

        180         *       200         *       220
25   : CAAACTCATGACCACTGAGTTTGCTTGTGTTGAAACTGTGGTTT : 203
50   : CAGACTCATGACCACTGAGTTTGCTTGTGTTGAAACTGTGGTTT : 219
TUG1 : ---ACTCATGACCACTGAGTTTGCTTGTGTTGAAACTGTGGTTT :  41
       ca ACTCATGACCACTGAGTTTGCTTGTGTTGAAACTGTGGTTT

                *       240         *       260
25   : CATCCAACATATGCTATTGGACATGATTATTATTCCATTCAAAT : 247
50   : CATCCAACATATGCTATTGGACATGATTATTATTCCATTCAAAT : 263
TUG1 : CATCCAACATATGCTATTGGACATGATTATTATTCCATTCAAAT :  85
       CATCCAACATATGCTATTGGACATGATTATTATTCCATTCAAAT

            *       280         *       300
25   : GGATTACAGACTTCTTGAGGACAGGACAAACTTATCTCTCATGG : 291
50   : GGATTACAGACTTCTTGAGGACAGGACAAACTTATCTCTCATGG : 307
TUG1 : GGATTACAGACTTCTTGAGGACAGGACAAACTTATCTCTCATGG : 129
       GGATTACAGACTTCTTGAGGACAGGACAAACTTATCTCTCATGG

        *       320         *       340         *
25   : TGTTTTTTTAGAATACTTTTATAACCACCGAGG-ACCCC----- : 329
50   : TGTTTTTTTAGAATACTTTTATAACCAAGGAAGCAAACCAATGC : 351
TUG1 : TGTTTTTTTAGAATACTTTTATAACCA----------------- : 156
       TGTTTTTTTAGAATACTTTTATAACCA  ga g a  cc
```

Figure S2. The RIP sequencing results

25: The sequencing results of the indicated RNAs retrieved by Flag-specific antibody within BM-MSCs transfected with Δ25 deletion constructs, 50: The sequencing results of the indicated RNAs retrieved by Flag-specific antibody within BM-MSCs transfected with Δ50 deletion constructs

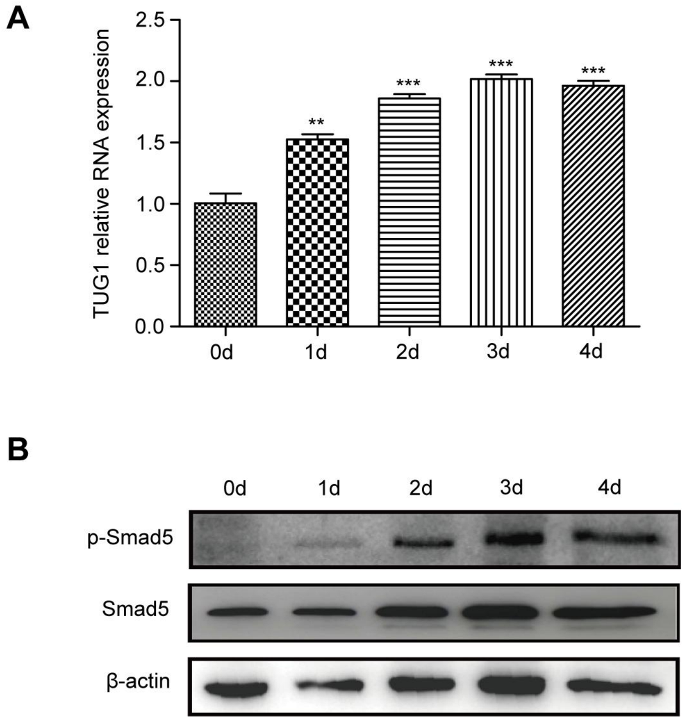

Figure S3. The expression levels of TUG1 and the phosphorylation level of Smad5 significantly increased in mice bone marrow cells after irradiation.

C57BL/6J mice were irradiated with 9 Gy Co-60 at a rate of 0.69 Gy/min. Mice bone marrows were harvested 1 day, 2 days, 3 days and 4 days after irradiation . (**A**) qRT-PCR was performed to detect the expression levels of TUG1 in mice bone marrow cells after irradiation. (**B**) Western blot was performed to detect the phosphorylation level of Smad5 in mice bone marrow cells after irradiation. All experiments were performed in triplicate, and the bars represent the mean ±SEM.**P* < 0.05, ***P* < 0.01, ****P* < 0.001

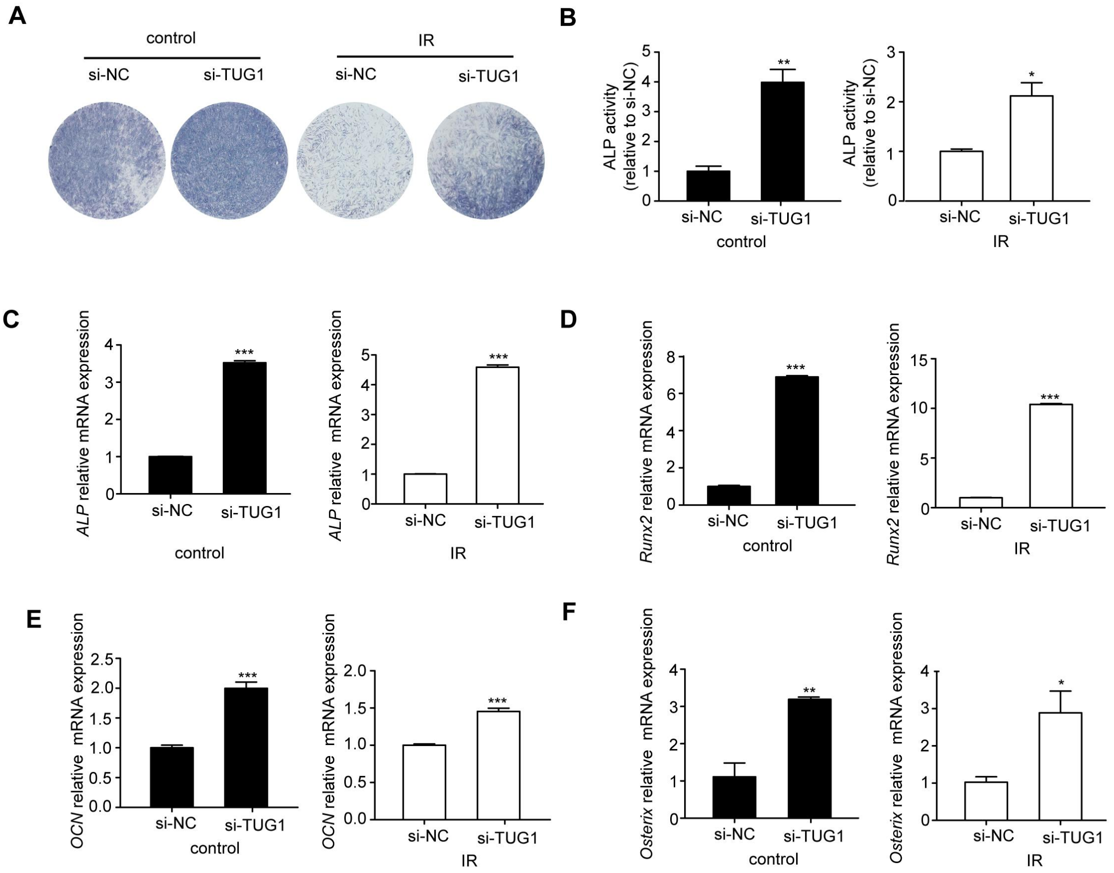

Figure S4. TUG1 inhibits the osteogenic differentiation of BM-MSCs at day 7 after osteogenic induction

(**A**) The alkaline phosphatase (ALP) staining was performed to detect the effect of TUG1 knockdown on osteogenic differentiation of non-irradiated BM-MSCs and irradiated BM-MSCs. (**B**) The ALP activity quantification. (**C-F**) qRT-PCR was performed to detect the effect of TUG1 knockdown on the mRNA levels of *ALP* (**C**), *Runx2* (**D**), *OCN* (**E**) and *osterix* (**F**) in BM-MSCs after 7 days of osteogenic induction.

All experiments were performed in triplicate, and the bars represent the mean ±SEM.*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$

Control: non-irradiated BM-MSCs; IR: irradiated BM-MSCs; si-NC: BM-MSCs transfected with empty vector, si-TUG1: BM-MSCs transfected with TUG1-siRNA vector.