Figure S1: The binding affinity to Fc receptors and antibody-dependent cell-mediated cytotoxicity (ADCC) effect of BiTP. (a-e) enzyme linked immunosorbent assay to determine the binding affinity to Fc receptors. **(f)** Flow cytometry to measure ADCC activity. The molecular weights of the light chain and heavy chain. H358 cells were stained with 2.5 μM carboxyfluorescein diacetate succinimidyl ester (CFSE) buffer (containing 1% FBS) at 37 ℃ for 15 minutes. Then, H358 cells were washed by medium and seeded into 96 well plates. The next day, NK92MI-CD16A and serially diluted BiTP were added to plates. The plates were incubated at 37 ℃ for six hours. Afterward, after treatment with propidium iodide, the ratio of dead cells was measured by flow cytometry.

Figure S2: Mass spectrometry to measure the molecular weight of BiTP (a) The molecular weight of the intact antibody. **(b-c)** The molecular weights of heavy chain and light chain.

Figure S3: Surface Plasmon Resonance Biacore to determine the affinities and kinetics of BiTP. (a) The affinities and kinetics of anti-TGF-β moiety. **(b)** The affinities and kinetics of anti-PD-L1 moiety.

Figure S4: Flow cytometry and western blotting assays to measure the effect of BiTP on TGF-β-mediated Treg induction and epithelial-mesenchymal transition.
(a) Plates were coated with 10 μg/ml anti-human CD3 at 37℃ for two hours. The hPBMCs were maintained in RPMI-1640 with 10% FBS, 100 U/ml interleukin-2, 5 μg/ml anti-CD28, 20 ng/ml TGF-β1, and BiTP for six days. **(b)** MCF-7 and BT474 were cultured with RPMI-1640 with 10% FBS, 20 ng/ml TGF-β1, and BiTP for four days. Then, cells were harvested for western blotting.

Figure S5: Differentially expressed genes analysis for BiTP-treated 4T1-hPDL1 tumors. (a) Venn diagrams showing the overlap of differentially expressed genes (upregulated in BiTP-treated tumors). **(b)** Venn diagrams showing the overlap of differentially expressed genes (downregulated in BiTP-treated tumors). **(c)** Bubble diagrams showing enriched pathways of overlapping differentially expressed genes (upregulated in BiTP-treated tumors). **(d)** Bubble diagrams showing enriched pathways of non-overlapping differentially expressed genes (upregulated in BiTP-treated tumors).

(e) The principal component analysis based on expression profiles showing sample similarities.

Figure S6: Multiplex immunohistochemical (IHC) and IHC staining to explore the effect of BiTP on epithelial-mesenchymal transition in 4T1-hPD-L1 tumors. (a) Anti-Vimentin multiplex IHC staining. **(b)** Anti-E-cadherin IHC staining.

Figure S7: Immunohistochemical (IHC) staining to explore the effect of BiTP on tumor cell apoptosis and proliferation in 4T1-hPD-L1 tumors. (a) Anti-Cleaved Caspase 3 IHC staining. **(b)** Anti-Ki67 IHC staining. **(c)** Anti-PCNA IHC staining.

Figure S8: The expression of *Cd274* and *Tgfb1* in RNA-seq assay. (a) *Cd274* mRNA level. **(b)** *Tgfb1* mRNA level.
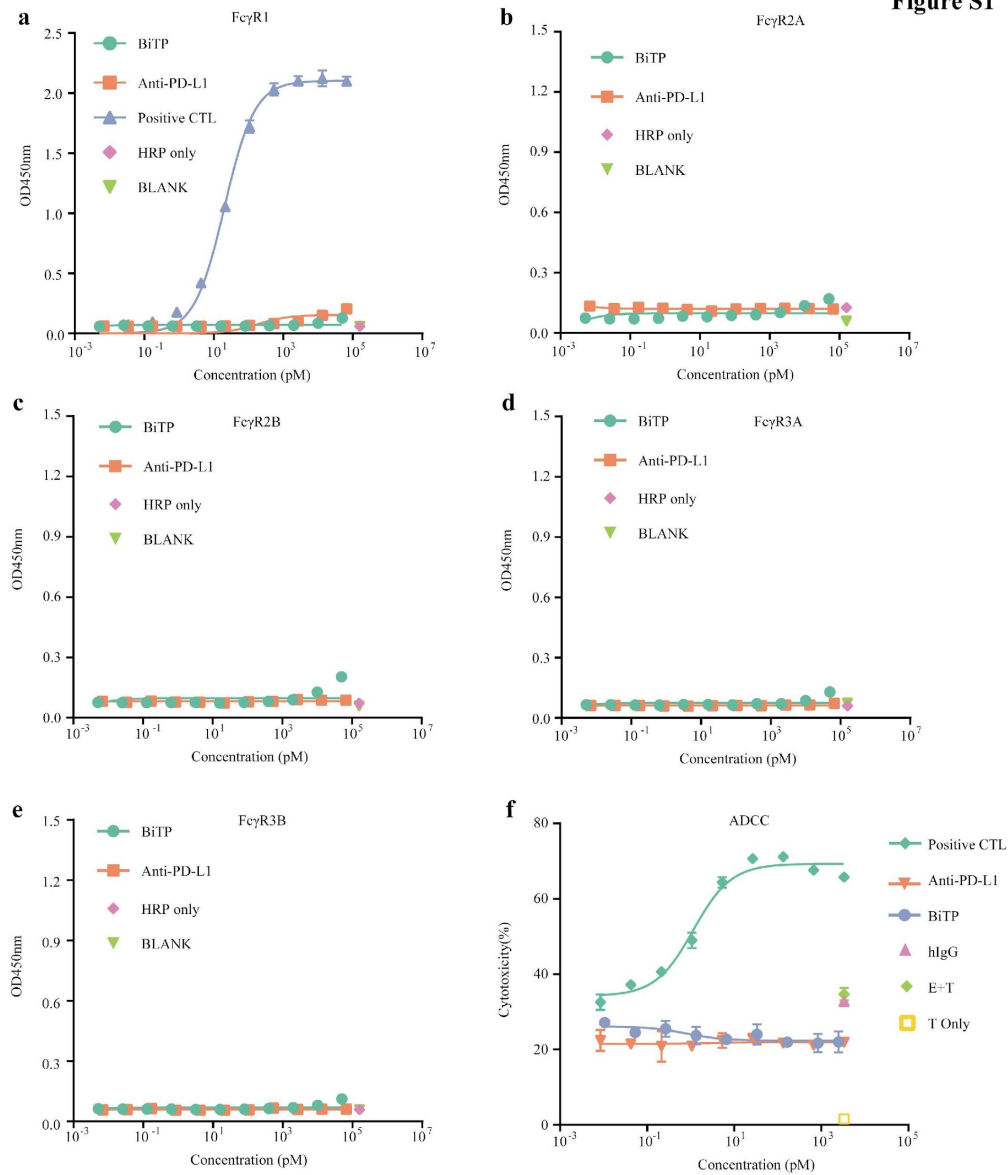
Figure S1

Figure S2

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Figure S3

a

TGF-β1

5 nM
2.5 nM
1.25 nM
0.625 nM
0.3125 nM

b

PD-L1

50 nM
25 nM
12.5 nM
6.25 nM
3.125 nM

5
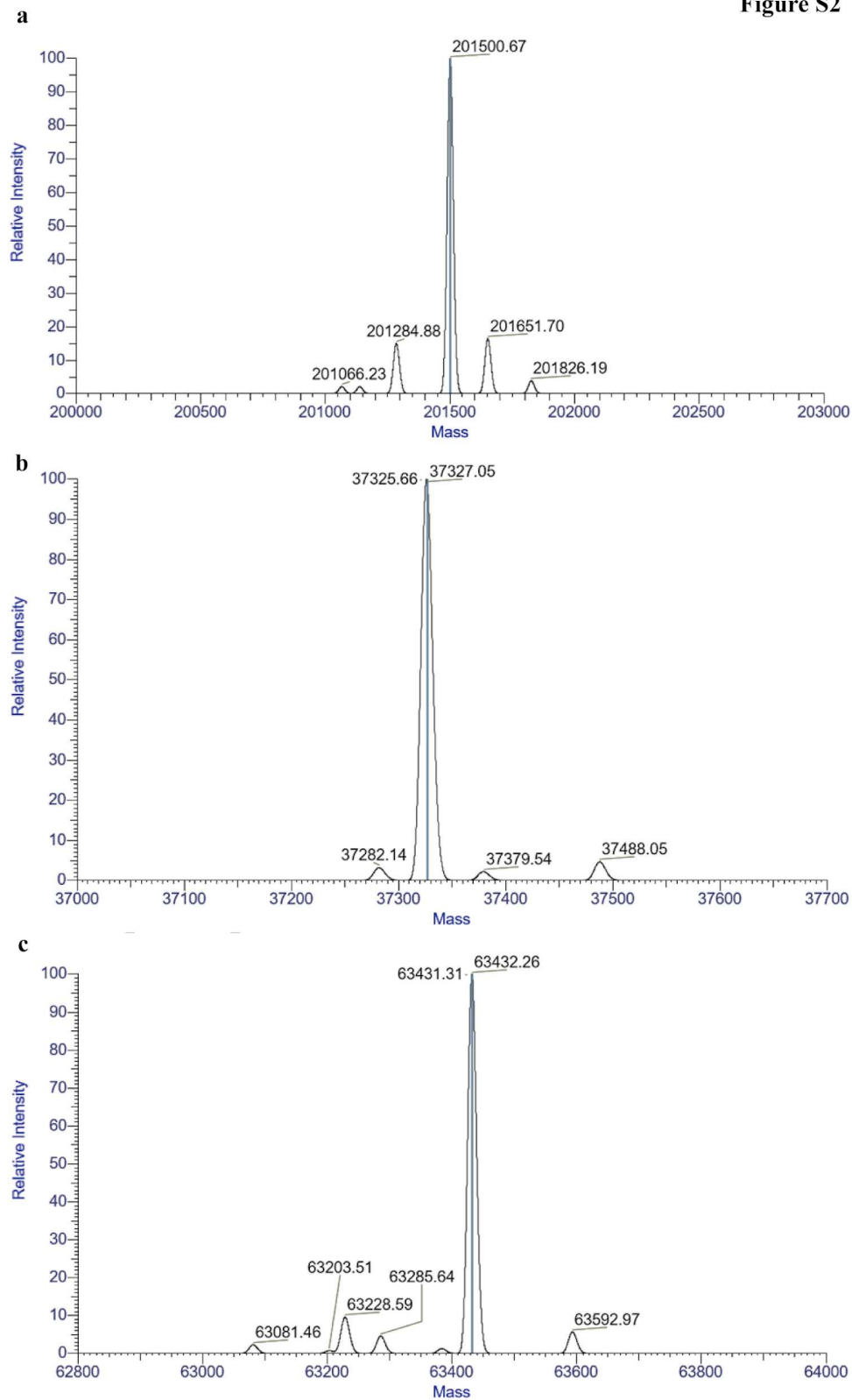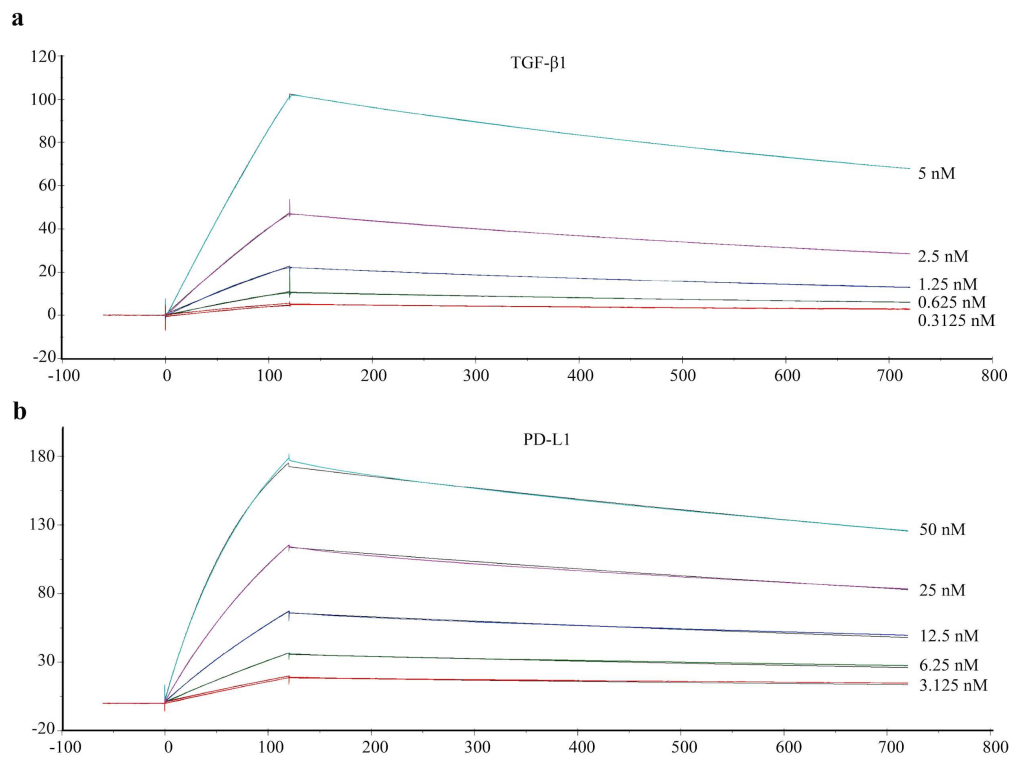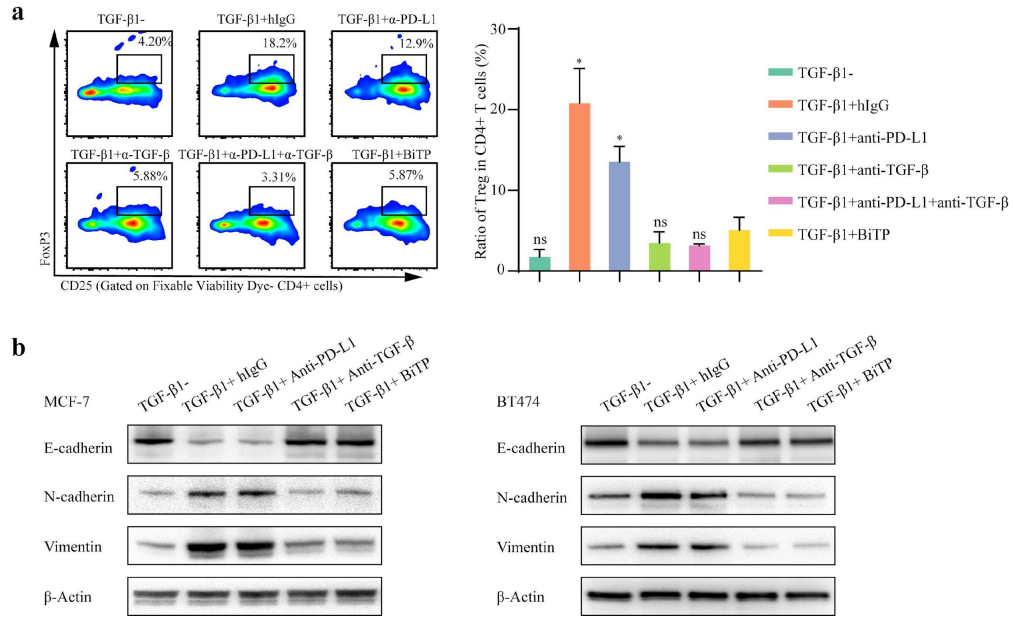
Figure S4

Figure S5

Figure S6

Figure S7

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Figure S8

9
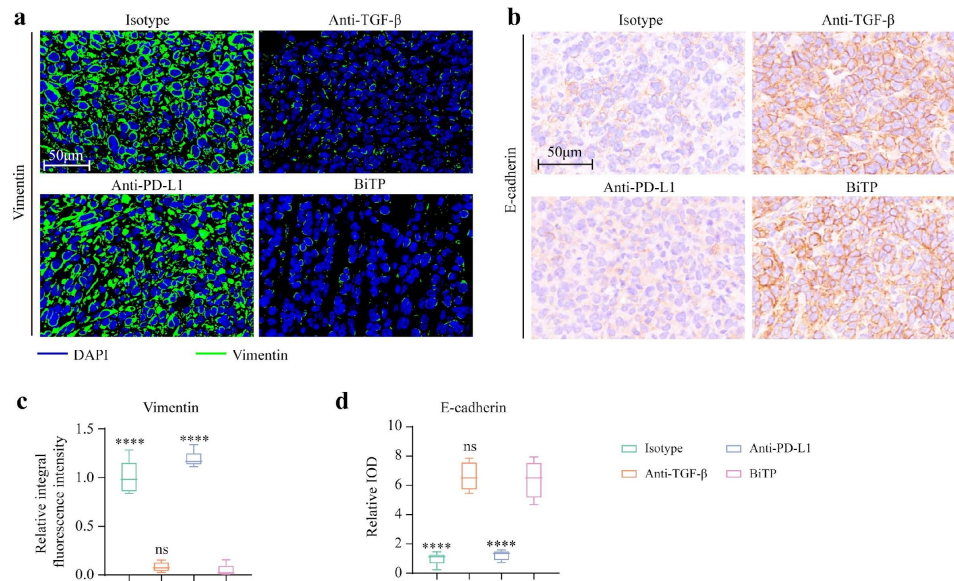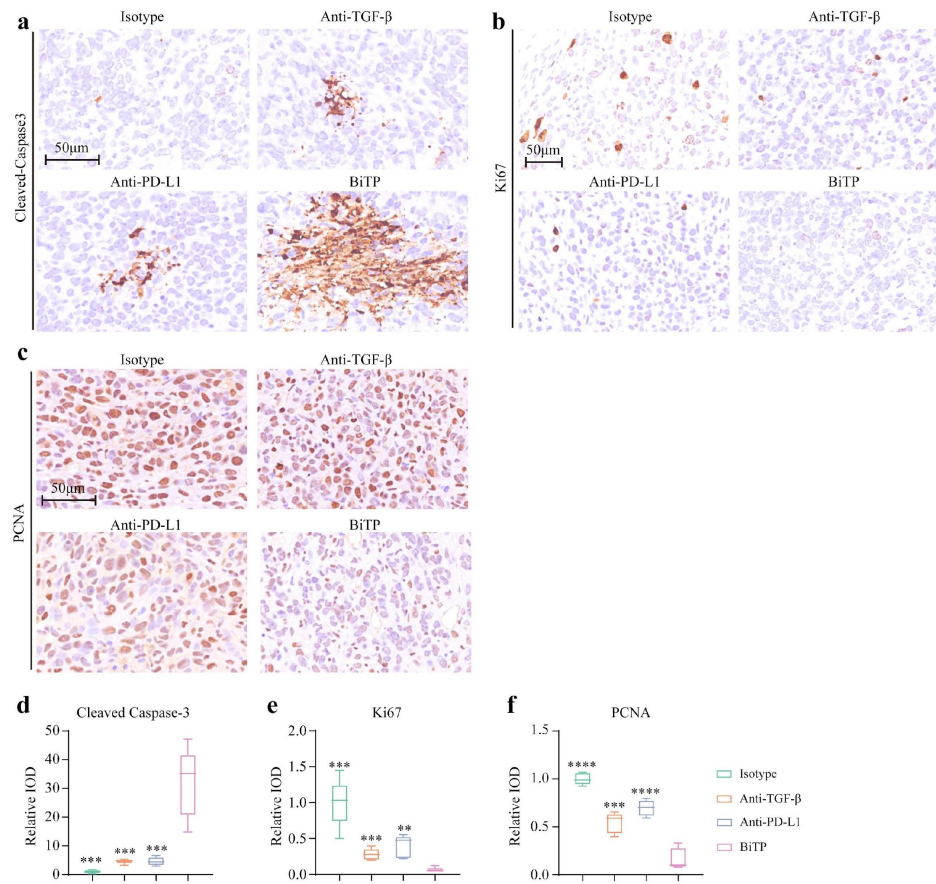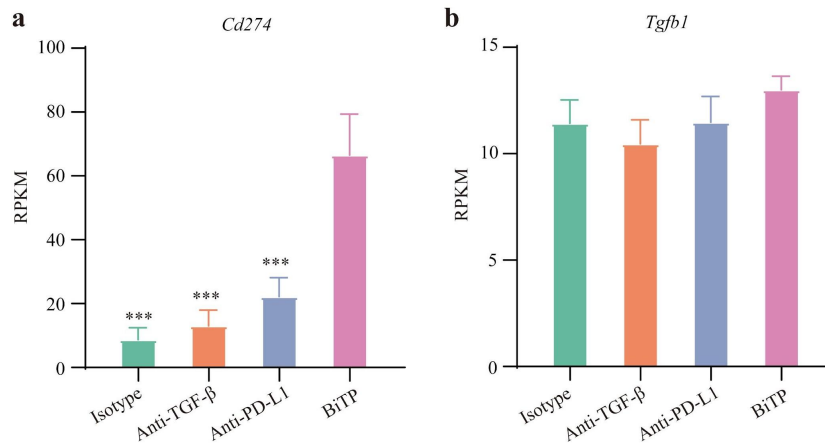
Table S1: The information about the RNA-seq library preparation and sequencing type.

Parameter	Value
Read length	PE-150
Strandness of the library	Strand-specific RNA-seq
Sequencing model	Paired-end
Enrichment strategy	polyA selection
Sequencing depth	6G
Gene annotation	Mus_musculus.GRCm38

Table S2: RNA-seq quality metrics of all samples in the RNA-seq assay.

Sample	Raw Reads	Raw Bases(G)	Raw Q20(%)	Raw Q30(%)	Raw GC(%)	Clean Reads	Clean Bases(G)
CTL1	42544940	6.38	96.82	92.26	53.57	36162334	5.24
CTL2	48089046	7.21	96.93	92.54	53.77	41460218	5.94
CTL3	44537806	6.68	97.08	92.89	53.48	38706156	5.58
CTL4	43858652	6.58	97.15	92.96	53.73	38203520	5.44
α-TGF1	42595106	6.39	97.32	93.28	53.51	37355198	5.41
α-TGF2	46827094	7.02	97.28	93.24	53.63	40970524	5.92
α-TGF3	40434076	6.07	97.1	92.84	53.79	34958072	5.02
α-TGF4	41247994	6.19	96.86	92.39	53.74	35233622	5.07
α-PDL1_1	44407064	6.66	97.09	92.91	53.86	38581268	5.58
α-PDL1_2	45478208	6.82	96.88	92.63	54.13	39372206	5.61
α-PDL1_3	52705070	7.91	96.95	92.53	53.65	45272198	6.53
α-PDL1_4	40712274	6.11	97.17	93.02	53.62	35433356	5.08
BiTP_1	44612128	6.69	96.99	92.75	53.75	38771740	5.59
BiTP_2	46358050	6.95	96.81	92.23	53.86	39337766	5.71
BiTP_3	40497030	6.07	96.82	92.39	54.01	34767182	4.96
BiTP_4	46178150	6.93	97.12	92.87	53.68	39922434	5.84

To be continued

Table S2 continued

Sample	Clean Q20(%)	Clean Q30(%)	Clean GC(%)	Effective Rate(%)	total_reads	total_mapped(%)
CTL1	98.85	95.56	53.69	85	26479738	25083120(94.73)
CTL2	98.9	95.74	53.81	86.22	29968542	28156180(93.95)
CTL3	98.97	95.94	53.58	86.91	28100438	26470435(94.20)
CTL4	98.99	96.01	53.77	87.11	27521604	25757652(93.59)
α-TGF1	99	96.04	53.61	87.7	31676780	29628810(93.53)
α-TGF2	99.02	96.09	53.75	87.49	33258248	31359501(94.29)
α-TGF3	98.96	95.9	53.85	86.46	29095570	27372344(94.08)

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α-TGF4	98.88	95.68	53.91	85.42	28449988	27130820(95.36)
α-PDL1_1	98.96	95.91	53.92	86.88	24369994	23068882(94.66)
α-PDL1_2	98.96	95.92	54.24	86.57	27644258	26120153(94.49)
α-PDL1_3	98.9	95.71	53.72	85.9	30672536	28951139(94.39)
α-PDL1_4	98.99	96.01	53.71	87.03	28416910	26697891(93.95)
BiTP_1	98.95	95.86	53.78	86.91	30713182	28994481(94.40)
BiTP_2	98.84	95.53	54.05	84.86	28146488	26747005(95.03)
BiTP_3	98.9	95.74	54.13	85.85	27438632	25737685(93.80)
BiTP_4	98.93	95.81	53.8	86.45	29343014	27654857(94.25)

To be continued

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Table S2 continued

Sample	non_unique(%)	unique(%)	un_mapped_reads(%)	read1(%)
CTL1	1045088(4.17)	24038032(95.83)	1396618(5.27)	12019003(50.00)
CTL2	1230045(4.37)	26926135(95.63)	1812362(6.05)	13463043(50.00)
CTL3	1133422(4.28)	25337013(95.72)	1630003(5.80)	12668480(50.00)
CTL4	1129309(4.38)	24628343(95.62)	1763952(6.41)	12314143(50.00)
α-TGF1	1230980(4.15)	28397830(95.85)	2047970(6.47)	14198894(50.00)
α-TGF2	1272643(4.06)	30086858(95.94)	1898747(5.71)	15043403(50.00)
α-TGF3	1076222(3.93)	26296122(96.07)	1723226(5.92)	13148041(50.00)
α-TGF4	987980(3.64)	26142840(96.36)	1319168(4.64)	13071396(50.00)
α-PDL1_1	948211(4.11)	22120671(95.89)	1301112(5.34)	11060315(50.00)
α-PDL1_2	1056525(4.04)	25063628(95.96)	1524105(5.51)	12531787(50.00)
α-PDL1_3	1178159(4.07)	27772980(95.93)	1721397(5.61)	13886467(50.00)
α-PDL1_4	1118910(4.19)	25578981(95.81)	1719019(6.05)	12789472(50.00)
BiTP_1	1129458(3.90)	27865023(96.10)	1718701(5.60)	13932489(50.00)
BiTP_2	970134(3.63)	25776871(96.37)	1399483(4.97)	12888426(50.00)
BiTP_3	1018225(3.96)	24719460(96.04)	1700947(6.20)	12359714(50.00)
BiTP_4	1159060(4.19)	26495797(95.81)	1688157(5.75)	13247879(50.00)

To be continued

Table S2 continued

Sample	reads_map_plus(%)	reads_map_minus(%)	non_splice_reads(%)	splice_reads(%)
CTL1	12019016(50.00)	12019016(50.00)	11397570(47.41)	12640462(52.59)
CTL2	13463060(50.00)	13463075(50.00)	12766655(47.41)	14159480(52.59)
CTL3	12668499(50.00)	12668514(50.00)	12030219(47.48)	13306794(52.52)
CTL4	12314168(50.00)	12314175(50.00)	11777913(47.82)	12850430(52.18)
α-TGF1	14198909(50.00)	14198921(50.00)	13778281(48.52)	14619549(51.48)
α-TGF2	15043422(50.00)	15043436(50.00)	14223887(47.28)	15862971(52.72)
α-TGF3	13148056(50.00)	13148066(50.00)	12544773(47.71)	13751349(52.29)
α-TGF4	13071412(50.00)	13071428(50.00)	12248516(46.85)	13894324(53.15)
α-PDL1_1	11060328(50.00)	11060343(50.00)	10437516(47.18)	11683155(52.82)
α-PDL1_2	12531805(50.00)	12531823(50.00)	11739231(46.84)	13324397(53.16)
α-PDL1_3	13886487(50.00)	13886493(50.00)	13070671(47.06)	14702309(52.94)
α-PDL1_4	12789491(50.00)	12789490(50.00)	12227885(47.80)	13351096(52.20)
BiTP_1	13932505(50.00)	13932518(50.00)	13328189(47.83)	14536834(52.17)
BiTP_2	12888435(50.00)	12888436(50.00)	11989806(46.51)	13787065(53.49)
BiTP_3	12359728(50.00)	12359732(50.00)	11860945(47.98)	12858515(52.02)

BiTP_4	13247894(50.00)	13247903(50.00)	12776084(48.22)	13719713(51.78)

To be continued

Table S2 continued

Sample	Ribosomal RNA(%)	CDS(%)	Intergenic(%)	Intron(%)
CTL1	1191942(2.8016)	32272736(79.06)	1108148(2.71)	2193525(5.37)
CTL2	1335490(2.7771)	35892328(78.72)	1283718(2.82)	2322260(5.09)
CTL3	1236392(2.7761)	33847854(78.83)	1205539(2.81)	2349160(5.47)
CTL4	1150178(2.6225)	32707437(78.69)	1154008(2.78)	2228266(5.36)
α-TGF1	1161648(2.7272)	33985867(79.54)	1130817(2.65)	2167668(5.07)
α-TGF2	1218798(2.6028)	37380457(79.23)	1238716(2.63)	2409929(5.11)
α-TGF3	1258866(3.1134)	33814806(78.82)	1183629(2.76)	2192367(5.11)
α-TGF4	1158354(2.8083)	34987404(78.35)	1239759(2.78)	2390527(5.35)
α-PDL1_1	1652868(3.7221)	36795906(79.29)	1237541(2.67)	2263656(4.88)
α-PDL1_2	1336120(2.9379)	37309967(78.33)	1281135(2.69)	2518135(5.29)
α-PDL1_3	1482514(2.8128)	40299172(79.05)	1358412(2.66)	2577555(5.06)
α-PDL1_4	1179774(2.8978)	33925250(78.68)	1180166(2.74)	2252495(5.22)
BiTP_1	1217224(2.7285)	37008941(78.78)	1196209(2.55)	2468287(5.25)
BiTP_2	1156220(2.4941)	34869998(79.62)	1017047(2.32)	2098543(4.79)
BiTP_3	1177266(2.907)	32712575(78.69)	1058493(2.55)	2101760(5.06)
BiTP_4	1397082(3.0254)	35293640(79.46)	1061350(2.39)	2187741(4.93)

To be continued

Table S2 continued

Sample	number of genes	PCR duplicates Reads	PCR replicates (%)
CTL1	26032	9682596	26.78%
CTL2	25595	11491676	27.72%
CTL3	26072	10605718	27.40%
CTL4	26078	10681916	27.96%
α-TGF1	26472	9710940	26.00%
α-TGF2	25765	10297988	25.14%
α-TGF3	26324	6789978	19.42%
α-TGF4	25480	5890608	16.72%
α-PDL1_1	25844	8480854	21.98%
α-PDL1_2	25339	7695426	19.55%
α-PDL1_3	24960	12013950	26.54%
α-PDL1_4	26162	7016446	19.80%
BiTP_1	25045	8058558	20.78%
BiTP_2	25667	11191278	28.45%
BiTP_3	24527	7328550	21.08%
BiTP_4	25352	11472446	28.74%

Note: Raw sequencing data was first filtered by fastp (version 0.23.1) (overall Q30% > 90%). Then, low-quality reads were discarded, and the reads contaminated with adaptor sequences were trimmed.