# SUPPLEMENTARY INFORMATION

## ALPL-1 is a target for chimeric antigen receptor therapy in osteosarcoma

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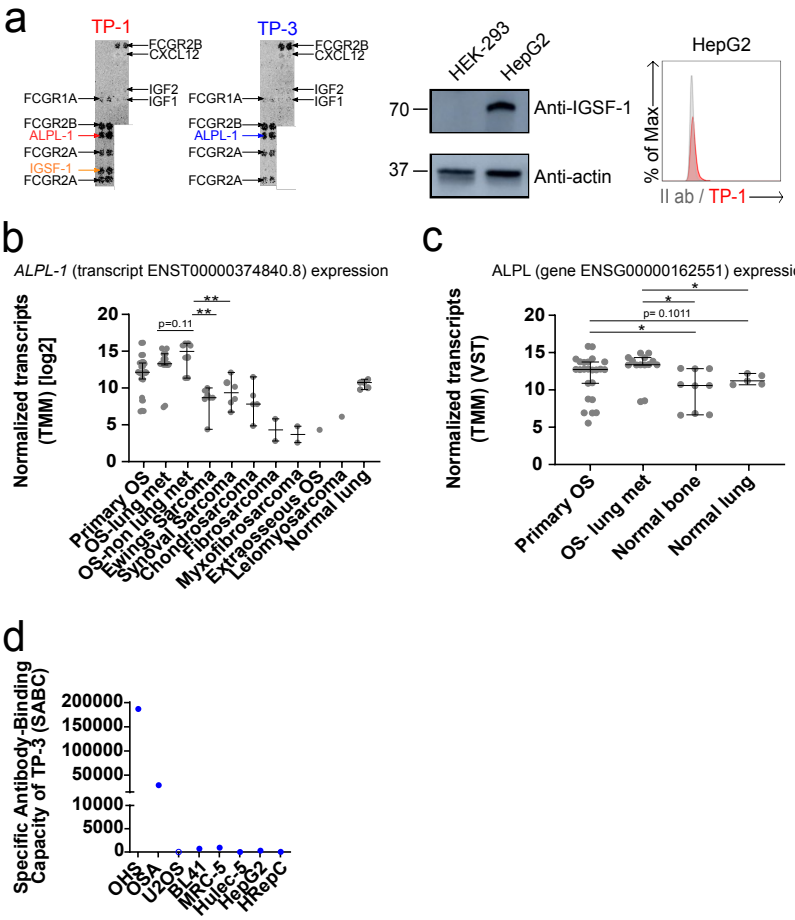
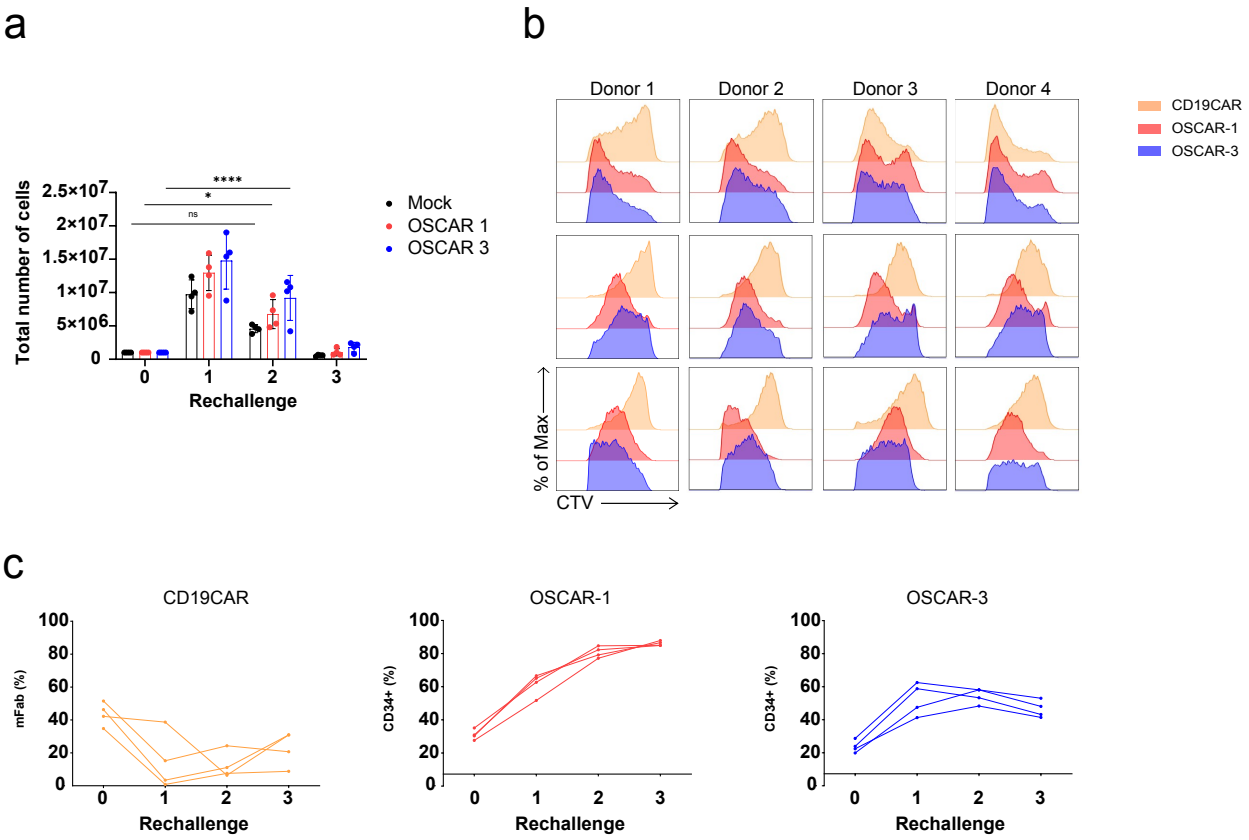
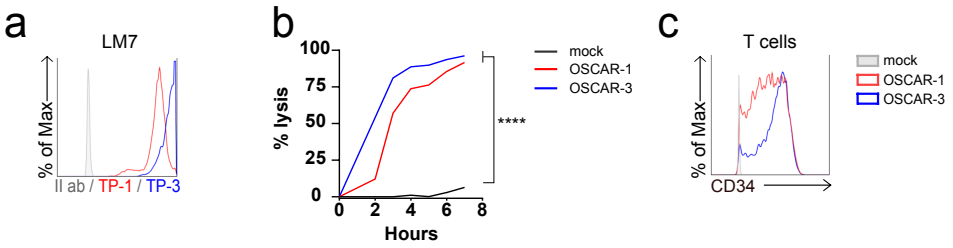


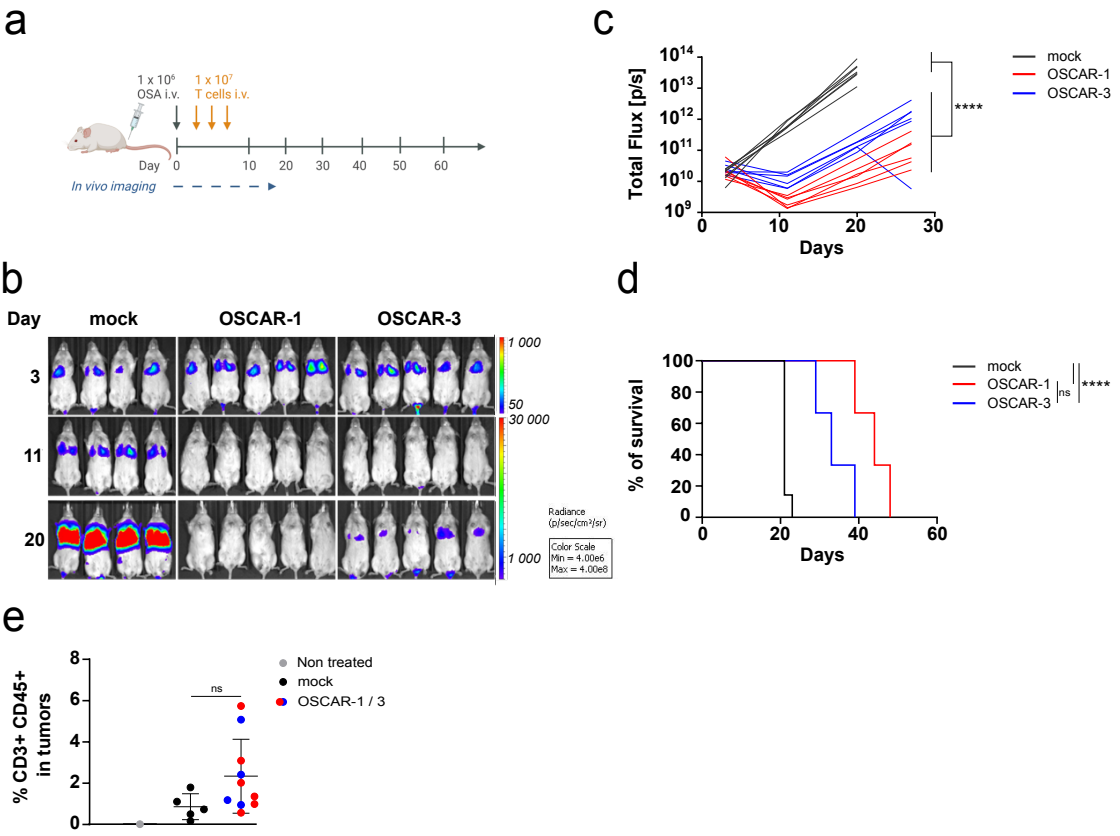

**Supplementary Figure 1. TP antibody specificity and affinity**. **a,** Target identification and cross-reactivity screening of TP-1 and TP-3 antibodies using Retrogenix screening technology (left, was repeated two times ); Western blotting showing presence of IGSF-1 proteins in Hep G2 (middle) (n=2, uncropped Western blotting in supplementary information ); flow cytometric histograms showing staining of HepG2 with TP-1 antibodies (right). b, Expression of ALPL-1 transcript ENST00000374840.8 in sarcomas (Primary OS n= 24, OS-lung met astastic (met) n=14, OS-non lung met n= 8, Ewings Sarcoma n=7, Synoval Sarcoma n= 6, Chondrosarcoma n=5, Fibrosarcoma n=2, Myxofibrosarcoma n= 2, Extraosseous OS n=1, Leiomyosarcoma n=1) and normal lung (n=5), (met, metastasis). The expression data is normalized by Trimmed mean of M values (TMM) method prior to log2 transformation . Statistical comparisons were performed with two-tailed Man-Whitney test ns, not significant p>0.05, *p<0.05, **p<0.01 ). **c**, Expression of *ALPL* gene ENSG00000162551 in primary (n=24) and metastatic (met) OS (n=14) and in normal tissues for bone (n=9) and lung (n=5). Normalized expression data presented using VST (variance stabilizing transformation). Statistical comparisons were performed with two-tailed Mann-Whitney test ns, not significant p>0.05, *p<0.05). **b,c,** Data are presented as median with 95% confidence intervalley. **d**, Specific antibody binding capacity of TP-3 against OS cell lines (OHS, OSA and U2OS), lymphoma (BL-41), healthy tissue cells representing lung (MRC-5, Hulec-5a, HPAEpiC), liver (HH), and kidney (HREpC).



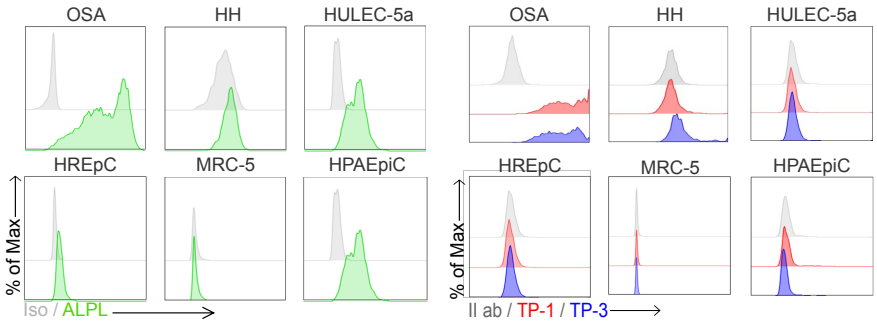

**Supplementary Figure 2. a**, Proliferation capability of OSCAR T cells upon rechallenge with OHS cell line. Data are shown as independent values of four donors (n=4). Two-way ANOVA with Tukey's multiple comparison test was used to calculate p values (ns, not significant p>0.05, *p<0.05, ****p<0.0001). **b**, Histograms of CTV staining of CAR T cells after 1st (top panel), 2nd (middle panel) and 3rd (bottom panel) antigen stimulation (HEK-293 $^{ALPL-1}$). Mean fluorescence intensity (MFI) of CTV in CD19CAR (orange), OSCAR-1 (red) and OSCAR-3 (blue) is shown in four donors. **c,** Monitoring of CAR expression after repeated antigen stimulation with HEK-293$^{ALPL-1}$ (three rechallenges). OSCAR-1 and 3 were detected by CD34 staining, and CD19CAR by mFab staining. Data are shown as independent values of four donors (n=4).




**Supplementary Figure 3.** ***In vitro*** **effector function of OSCAR -1 and OSCAR -3 T cellstowards LM7 OS cells. a**, Representative flow cytometric histograms showing staining of LM7 OS cells with TP-1 and TP-3 antibodies. **b**, Kinetics of *in vitro* cytotoxicity of OSCAR -1 and OSCAR -3 T cells against LM7 cells at an E:T ratio of 10:1 (in red and blue). Lysis of LM7 cells is monitored up to 7 hours. Data represents mean ± s.d. of two independent experiments (n=2). Statistical comparisons are performed with two-way ANOVA with Tukey's multiple comparison test (ns, not significant p>0.05, *** *p<0.0001). **c,** Expression level of OSCAR-1 and OSCAR-3 CAR in T cells used in the cytotoxicity assay.




**Supplementary Figure 4.** ***In vivo*** **effector function of OSCAR-1 and OSCAR-3 T cells in lung metastatic mouse models.** **a**, Experimental overview of OSA OS lung model. NSG mice were engrafted with GFP/Luc$^{+}$ OSA cells i.v. On day 10, mice were randomized and received 3 i.v. injections of mock, OSCAR-1, or OSCAR-3 T cells. **b**, Tumor burden measured by IVIS. Representative BLI images of the luminescent signal from each treatment group. **c**, Quantification of tumor progression for each individual mouse per each group measured by flux values acquired via BLI. Statistical comparisons were performed with two-way ANOVA with Tukey's multiple comparison test (ns, not significant ****p<0.0001). **d**, Kaplan-Meier survival curves using a Mantel-Cox (log-rank) test (ns, not significant p>0.05, ****p<0.0001). Experiment was performed once; five mice per group were included (n=5). **e**, T cell homing experiment showing percentage of CD3+/CD45+ cells found in tumor tissues from lungs. 3 mice per treatment group were injected i.v. with 1 x 10$^{6}$ GFP/Luc$^{+}$ OSA cells. Tumor-bearing mice were randomized and injected once with 1 x 10$^{7}$ mock, OSCAR-1, or OSCAR-3 T cells. Mice were euthanized 4 days after T cell injection and lungs were dissected. Data are presented as mean ± s.d. Statistical comparisons were performed with two-tailed unpaired Student t-test (ns, not significant p= 0.1849).




**Supplementary Figure. 5** Staining of target cells in figure 5b with anti ALPL, TP-1 and TP-3 antibodies. Isotype and secondary antibody controls (grey).

| Sample Id | Type | Sample Alias | Condition | Source |
|---|---|---|---|---|
| 10_S1 | Synovial sarcoma | HTT10 | Tumor | NSUC |
| 12_S2 | Extraoseos osteosarcoma | HTT12 | Tumor | NSUC |
| 16_S3 | Pleomorphic myxofibrosarcoma | HTT16 | Tumor | NSUC |
| 17_S4 | Primary OS | HTT17 | Tumor | NSUC |
| 21_S5 | Myxofibrosarcoma | HTT21 | Tumor | NSUC |
| 25_S6 | Pleaomorphic spindle cell sarcoma | HTT25 | Tumor | NSUC |
| 26_S7 | Chondrosarcoma | HTT26 | Tumor | NSUC |
| 29_S8 | Chondrosarcoma | HTT29 | Tumor | NSUC |
| 30_S1 | Chondrosarcoma | HTT30 | Tumor | NSUC |
| 31_S2 | High grade pleomorphic fibrosarcoma | HTT31 | Tumor | NSUC |
| 38_S3 | ST_sarcoma | HTT38 | Tumor | NSUC |
| 39_S4 | Primary OS | HTT39 | Tumor | NSUC |
| 41_S5 | Fibrosarcoma | HTT41 | Tumor | NSUC |
| 42_S6 | Fibrosarcoma | HTT42 | Tumor | NSUC |
| 47_S7 | Ewings sarcoma | HTT47 | Tumor | NSUC |
| 52_S8 | Myxofibrosarcoma | HTT52 | Tumor | NSUC |
| 58_S1 | Leiomyosarcoma | HTT58 | Tumor | NSUC |
| 62_S2 | Chondrosarcoma | HTT62 | Tumor | NSUC |
| 74_S3 | n.d | HTT74 | Tumor | NSUC |
| 75_S4 | n.d | HTT75 | Tumor | NSUC |
| 78_S5 | Primary OS | HTT78 | Tumor | NSUC |
| 79_S6 | Chondrosarcoma | HTT79 | Tumor | NSUC |
| 82_S7 | Primary OS | HTT82 | Tumor | NSUC |
| SRR10691631 | Normal kidney | GSM4217781 | Normal | ENA |
| SRR10691632 | Normal kidney | GSM4217782 | Normal | ENA |
| SRR10691633 | Normal kidney | GSM4217783 | Normal | ENA |
| SRR10691634 | Normal kidney | GSM4217784 | Normal | ENA |
| SRR10691635 | Normal kidney | GSM4217785 | Normal | ENA |
| SRR11804653 | Ewings sarcoma | GSM4557259 | Tumor | ENA |
| SRR11804654 | Ewings sarcoma | GSM4557260 | Tumor | ENA |
| SRR11804655 | Ewings sarcoma | GSM4557261 | Tumor | ENA |
| SRR11804656 | Ewings sarcoma | GSM4557262 | Tumor | ENA |
| SRR11804657 | Ewings sarcoma | GSM4557263 | Tumor | ENA |
| SRR11804658 | Ewings sarcoma | GSM4557264 | Tumor | ENA |
| SRR1265495 | Normal lung | GSM1375986 | Normal | ENA |
| SRR1265496 | Normal lung | GSM1375987 | Normal | ENA |
| SRR1265497 | Normal lung | GSM1375988 | Normal | ENA |
| SRR1265498 | Normal lung | GSM1375989 | Normal | ENA |
| SRR1265499 | Normal lung | GSM1375990 | Normal | ENA |
| SRR2748125 | Highly-met OS cell line | GSM1915018 | Tumor | ENA |
| SRR2748126 | Highly-met OS cell line | GSM1915019 | Tumor | ENA |
| SRR2748127 | Highly-met OS cell line | GSM1915020 | Tumor | ENA |
| SRR2748128 | Highly-met OS cell line | GSM1915021 | Tumor | ENA |
| SRR2748129 | Highly-met OS cell line | GSM1915022 | Tumor | ENA |
| SRR2748130 | Non-met OS cell line | GSM1915023 | Tumor | ENA |
| SRR2748131 | Non-met OS cell line | GSM1915024 | Tumor | ENA |
| SRR2748132 | Highly-met OS cell line | GSM1915025 | Tumor | ENA |
| SRR4343885 | OS-lung met | HO002M | Tumor | ENA |
| SRR4343886 | OS-lung met | HO002M | Tumor | ENA |
| SRR4343887 | OS-lung met | HO002M | Tumor | ENA |
| SRR4343888 | OS-lung met | HO002M | Tumor | ENA |
| SRR4343889 | OS-lung met | HO002M | Tumor | ENA |
| SRR4343890 | OS-lung met | HO002M | Tumor | ENA |
| SRR4343891 | Primary OS | HO003 | Tumor | ENA |
| SRR4343892 | Primary OS | HO003 | Tumor | ENA |
| SRR4343893 | Primary OS | HO003 | Tumor | ENA |
| SRR4343894 | Primary OS | HO003 | Tumor | ENA |
| SRR4343895 | Primary OS | HO003 | Tumor | ENA |

**Supplementary Table 1**

| Sample Id | Type | Sample Alias | Condition | Source |
|---|---|---|---|---|
| SRR4343896 | Primary OS | HO003 | Tumor | ENA |
| SRR4343897 | Primary OS | HO004 | Tumor | ENA |
| SRR4343898 | Primary OS | HO004 | Tumor | ENA |
| SRR4343899 | Primary OS | HO004 | Tumor | ENA |
| SRR4343900 | Primary OS | HO004 | Tumor | ENA |
| SRR4344033 | OS-lung met | HO043M | Tumor | ENA |
| SRR4344034 | OS-lung met | HO043M | Tumor | ENA |
| SRR4344035 | OS-lung met | HO044M | Tumor | ENA |
| SRR4344036 | OS-lung met | HO044M | Tumor | ENA |
| SRR4344039 | OS-met | HO046M-ch | Tumor | ENA |
| SRR4344040 | OS-met | HO046M-ch | Tumor | ENA |
| SRR4344041 | OS-met | HO047M-ch | Tumor | ENA |
| SRR4344042 | OS-met | HO047M-ch | Tumor | ENA |
| SRR4344043 | OS-lung met | HO052M | Tumor | ENA |
| SRR4344044 | OS-lung met | HO052M | Tumor | ENA |
| SRR4344045 | OS-met | HO055-ch | Tumor | ENA |
| SRR4344046 | OS-met | HO055-ch | Tumor | ENA |
| SRR4344047 | OS-met | HO056M-U | Tumor | ENA |
| SRR4344048 | OS-met | HO056M-U | Tumor | ENA |
| SRR4344049 | Primary OS | HO057 | Tumor | ENA |
| SRR4344050 | Primary OS | HO057 | Tumor | ENA |
| SRR4344051 | OS-lung met | HO058M | Tumor | ENA |
| SRR4344052 | OS-lung met | HO058M | Tumor | ENA |
| SRR4344053 | Primary OS | HO059 | Tumor | ENA |
| SRR4344054 | Primary OS | HO059 | Tumor | ENA |
| SRR4344055 | Primary OS | HO060 | Tumor | ENA |
| SRR4344056 | Primary OS | HO060 | Tumor | ENA |
| SRR4344057 | Primary OS | HO062 | Tumor | ENA |
| SRR4344058 | Primary OS | HO062 | Tumor | ENA |
| SRR4344059 | Primary OS | HO063 | Tumor | ENA |
| SRR4344060 | Primary OS | HO063 | Tumor | ENA |
| SRR4344073 | Normal bone | HO069B | Normal | ENA |
| SRR4344074 | Normal bone | HO069B | Normal | ENA |
| SRR4344075 | Normal bone | HO069B | Normal | ENA |
| SRR4344076 | Normal bone | HO070B | Normal | ENA |
| SRR4344077 | Normal bone | HO070B | Normal | ENA |
| SRR4344078 | Normal bone | HO070B | Normal | ENA |
| SRR4344079 | Normal bone | HO071B | Normal | ENA |
| SRR4344080 | Normal bone | HO071B | Normal | ENA |
| SRR4344081 | Normal bone | HO071B | Normal | ENA |
| SRR5576263 | Normal liver | hiPSC-hepatocyte | Normal | ENA |
| SRR5576264 | Normal liver | HepaRGTM | Normal | ENA |
| SRR5576266 | Normal liver | hESC-hepatocyte | Normal | ENA |
| SRR6373111 | Synovial sarcoma | GSM2887701 | Tumor | ENA |
| SRR6373112 | Synovial sarcoma | GSM2887702 | Tumor | ENA |
| SRR6373113 | Synovial sarcoma | GSM2887703 | Tumor | ENA |
| SRR6373114 | Synovial sarcoma | GSM2887704 | Tumor | ENA |
| SRR6373115 | Synovial sarcoma | GSM2887705 | Tumor | ENA |
| SRR6373116 | Synovial sarcoma | GSM2887706 | Tumor | ENA |
| Undetermined_S0 | n.d | n.d | Tumor | NSUC |

**Supplementary Table 1**

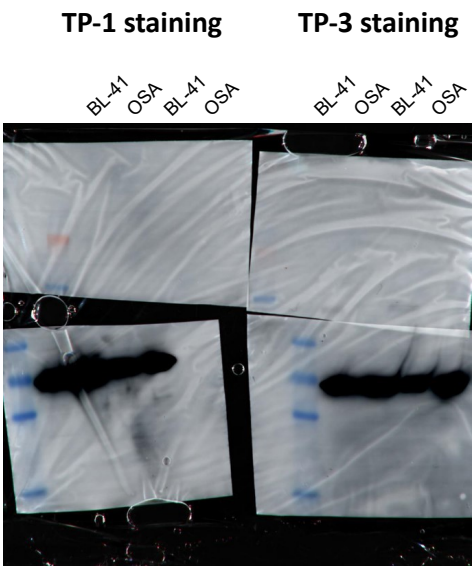
| Sample Id | Type | Sample Name | Condition | Source |
|---|---|---|---|---|
| 17_S4 | Primary OS | HTT17 | Tumor | NSUC |
| 39_S4 | Primary OS | HTT39 | Tumor | NSUC |
| 78_S5 | Primary OS | HTT78 | Tumor | NSUC |
| 82_S7 | Primary OS | HTT82 | Tumor | NSUC |
| SRR1265495 | Normal lung | GSM1375986 | Normal | ENA |
| SRR1265496 | Normal lung | GSM1375987 | Normal | ENA |
| SRR1265497 | Normal lung | GSM1375988 | Normal | ENA |
| SRR1265498 | Normal lung | GSM1375989 | Normal | ENA |
| SRR1265499 | Normal lung | GSM1375990 | Normal | ENA |
| SRR4343885 | OS-lung met | HO002M | Tumor | ENA |
| SRR4343886 | OS-lung met | HO002M | Tumor | ENA |
| SRR4343887 | OS-lung met | HO002M | Tumor | ENA |
| SRR4343888 | OS-lung met | HO002M | Tumor | ENA |
| SRR4343889 | OS-lung met | HO002M | Tumor | ENA |
| SRR4343890 | OS-lung met | HO002M | Tumor | ENA |
| SRR4343891 | Primary OS | HO003 | Tumor | ENA |
| SRR4343892 | Primary OS | HO003 | Tumor | ENA |
| SRR4343893 | Primary OS | HO003 | Tumor | ENA |
| SRR4343894 | Primary OS | HO003 | Tumor | ENA |
| SRR4343895 | Primary OS | HO003 | Tumor | ENA |
| SRR4343896 | Primary OS | HO003 | Tumor | ENA |
| SRR4343897 | Primary OS | HO004 | Tumor | ENA |
| SRR4343898 | Primary OS | HO004 | Tumor | ENA |
| SRR4343899 | Primary OS | HO004 | Tumor | ENA |
| SRR4343900 | Primary OS | HO004 | Tumor | ENA |
| SRR4344033 | OS-lung met | HO043M | Tumor | ENA |
| SRR4344034 | OS-lung met | HO043M | Tumor | ENA |
| SRR4344035 | OS-lung met | HO044M | Tumor | ENA |
| SRR4344036 | OS-lung met | HO044M | Tumor | ENA |
| SRR4344043 | OS-lung met | HO052M | Tumor | ENA |
| SRR4344044 | OS-lung met | HO052M | Tumor | ENA |
| SRR4344049 | Primary OS | HO057 | Tumor | ENA |
| SRR4344050 | Primary OS | HO057 | Tumor | ENA |
| SRR4344051 | OS-lung met | HO058M | Tumor | ENA |
| SRR4344052 | OS-lung met | HO058M | Tumor | ENA |
| SRR4344053 | Primary OS | HO059 | Tumor | ENA |
| SRR4344054 | Primary OS | HO059 | Tumor | ENA |
| SRR4344055 | Primary OS | HO060 | Tumor | ENA |
| SRR4344056 | Primary OS | HO060 | Tumor | ENA |
| SRR4344057 | Primary OS | HO062 | Tumor | ENA |
| SRR4344058 | Primary OS | HO062 | Tumor | ENA |
| SRR4344059 | Primary OS | HO063 | Tumor | ENA |
| SRR4344060 | Primary OS | HO063 | Tumor | ENA |
| SRR4344073 | Normal bone | HO069B | Normal | ENA |
| SRR4344074 | Normal bone | HO069B | Normal | ENA |
| SRR4344075 | Normal bone | HO069B | Normal | ENA |
| SRR4344076 | Normal bone | HO070B | Normal | ENA |
| SRR4344077 | Normal bone | HO070B | Normal | ENA |
| SRR4344078 | Normal bone | HO070B | Normal | ENA |
| SRR4344079 | Normal bone | HO071B | Normal | ENA |
| SRR4344080 | Normal bone | HO071B | Normal | ENA |
| SRR4344081 | Normal bone | HO071B | Normal | ENA |

*Note:*

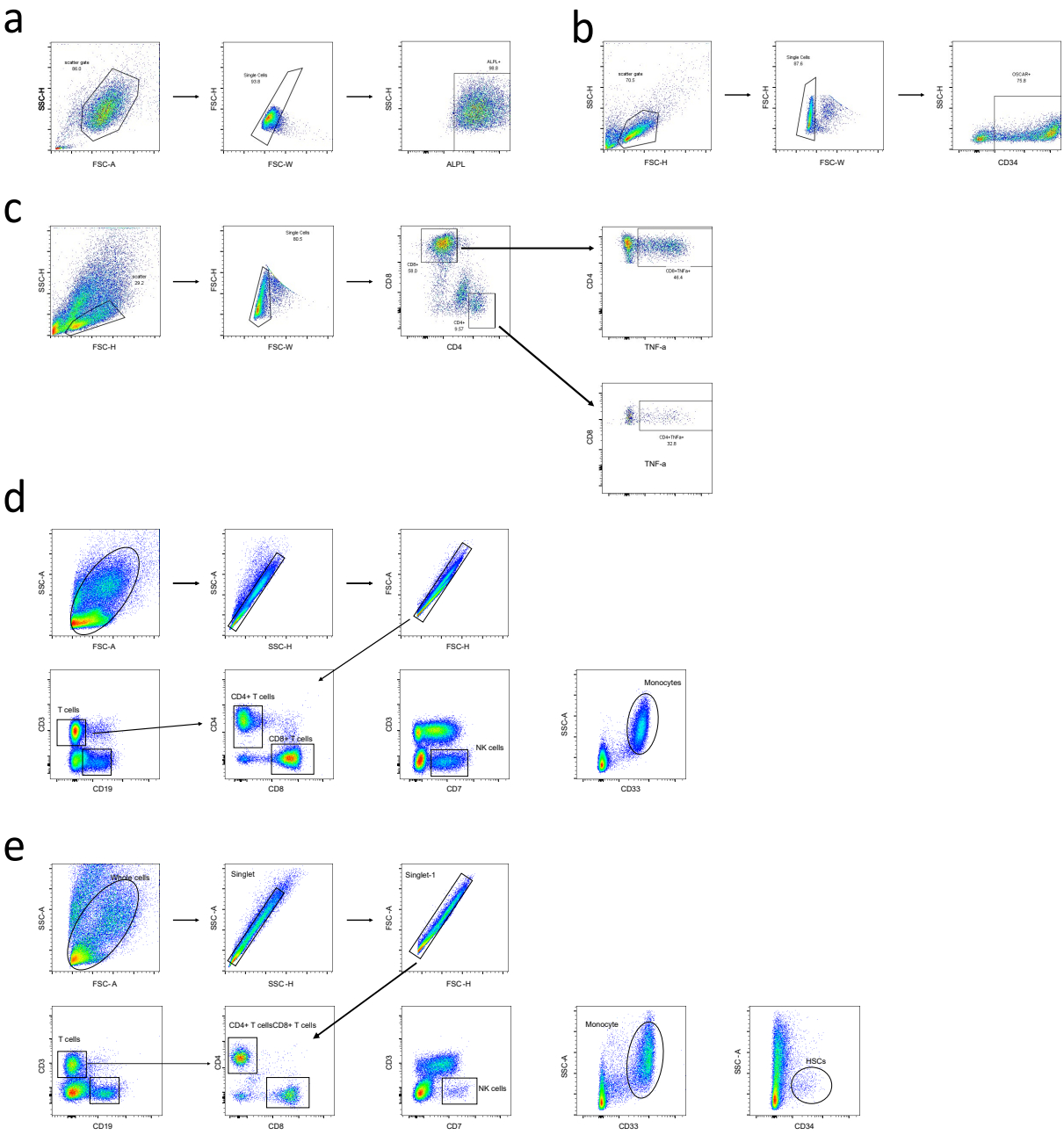
n.d = Not determined, NSUC = Nova Southeastern University Center, ENA = European Nucleotide Archive.

## Supplementary Table 1

# SUPPLEMENTARY METHODS




**Supplementary methods 1.** Western Blotting of BL-41 and OSA cells showing that TP-1 and TP-3 antibodies do not bind ALPL. The first column corresponds to the ladder, following by BL-41, OSA, BL-41, OSA (different lysate preparation or similar). Top gels correspond to TPs antibodies incubated and bottom gels correspond to B-actin staining.




**Supplementary methods 2. Gating strategy**. **a**, Gating strategies used for TPs and ALPL detection in OS cell lines (data shown in Fig. 1a,b,d,f and Fig. 5c). Cells were gated based on size and granularity ( FSC vs SSC). Singlets were gated by FSC-W vs FSC-H and TPs and ALPL were evaluated by the gating SSC vs ALPL/TPs. **b**, Gating strategies used to detect OSCAR+ T cells either by CD34 and/or aFab antibody staining (data shown in Fig. 2a). Cells were gated based on size and granularity (FSC vs SSC). Singlets were gated by FSC-W vs FSC-H and CD34 and/or aFab were evaluated by the gating SSC vs CD34 or SSC vs aFab. **c**, Gating strategies used to detect TNFa+ OSCAR CD8+ or CD4+ T cells (data shown in Fig. 5a, b). Cells were gated based on size and granularity (FSC vs SSC). Singlets were gated by FSC-W vs FSC-H. CD8+ or CD4+ T cells were evaluated by the gating CD4 vs CD8 and TNFa+ were obtained by the gating CD4 vs TNFa and CD8 vs TNFa. **d**, Gating strategies used TP3/ALPL detection in PBMCs (data shown in Fig. 5c). **e**, Gating strategies used TP3/ALPL detection in bone marrow, BM (data shown in Fig. 5c). For **d and e**, cells were gated based on size and granularity ( FSC vs SSC). Singlets were gated by SSC-H vs SSC-A, and singlet-1 by FSC-H vs FSC-A. T cells and B cells were evaluated by the gating CD3 vs CD19. CD8+ or CD4+ T cells were evaluated by the gating CD4 vs CD8, NK cells were gating on CD7+ CD3-, Monocytes on CD33+ and Human Stem cells (HSCs) CD34+.