**Supplementary Tables**

**Table S1.** Gene-specific primers used in the qPCR experiments.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Gene | Sequence ID | Name of the primer | Sequence of the primer | Product size, bp |
| *IFNG* | NM\_000619.3 | IFNG forward | GGTCATTCAGATGTAGCGGA | 281 |
|  |  | IFNG reverse | TGCATCCTTTTTCGCCTTGC |  |
| *TNFA* | NM\_000594.4 | TNF forward | GTTGTAGCAAACCCTCAAGC | 148 |
|  |  | TNF reverse | TCACCCCGAAGTTCAGTAGA |  |
| *IL6* | NM\_000600.5 | IL6 forward | ATGTGTGAAAGCAGCAAAGAGG | 179 |
|  |  | IL6 reverse | TGCAAGTGCATCATCGTTGT |  |
| *IL1B* | NM\_000576.3 | IL1B forward | GATGCACCTGTACGATCACT | 229 |
|  |  | IL1B reverse | CACGGGAAAGACACAGGTAG |  |
| *IL12A* | NM\_000882.4 | IL12A forward | CTCCTGGACCACCTCAGTTTG | 89 |
|  |  | IL12A reverse | AGCTCCCTCTTGTTGTGGAA |  |
| *IL23A* | NM\_016584.3 | IL23A forward | CTTCTCTGCTCCCTGATAGC | 90 |
|  |  | IL23A reverse | TTCGAAGGATCTTGGAACGG |  |
| *IL10* | NM\_000572.3 | IL10 forward | GCAAAACCAAACCACAAGACAG | 194 |
|  |  | IL10 reverse | AGTCCTGCATTAAGGAGTCG |  |
| *TGFB* | NM\_000660.7 | TGFB forward | TGGTGGAAACCCACAACGAA | 113 |
|  |  | TGFB reverse | GTAGCGAAGTGCAGGTCAAT |  |
| *EGF* | NM\_001963.6 | EGF forward | GGATTGACACAGAAGGAACCA | 156 |
|  |  | EGF reverse | TCTATCCACTTCAGGGCTGT |  |
| *VEGFA* | NM\_001025366.3 | VEGFA forward | CAGCTACTGCCATCCAATCG | 165 |
|  |  | VEGFA reverse | AACACAAGTCCACAGCAGTC |  |
| *FGF2* | NM\_002006.6 | FGF2 forward | ATGTAGAAGATGTGACGCCG | 141 |
|  |  | FGF2 reverse | AGTTCGTTTCAGTGCCACAT |  |
| *ABCB1/MDR1* | NM\_001348945.2 | ABCB1 forward | GCAGCTGGAAGACAAATACACAA | 93 |
|  |  | ABCB1 reverse | CCCAACATCGTGCACATCA |  |
| *ABCG2/BCRP* | NM\_004827.3 | ABCG2 forward | GAAACCTGGTCTCAACGCCATCC | 193 |
|  |  | ABCG2 reverse | CGTCAGAGTGCCCATCACAACAT |  |
| *ABCC1/MRP1* | NM\_004996.4 | ABCC1 forward | CACCTGAAGGACTTCGTGTCAG | 460 |
|  |  | ABCC1 reverse | TGGTTTACCAGGGGTACTGACT |  |
| *ABCC5/MRP5* | NM\_005688.4 | ABCC5 forward | CAGGAGAACTCGACCGTTGG | 169 |
|  |  | ABCC5 reverse | AGTAGTCCGGATGGGCTTCA |  |
| *CYP3A4* | NM\_017460.6 | CYP3A4 forward | CACCCCCAGTTAGCACCATT | 292 |
|  |  | CYP3A4 reverse | CCCCACGCCAACAGTGATTA |  |
| *CYP3A5* | NM\_000777.5 | CYP3A5 forward | ACGTATGAAGGTCAACTCCCTG | 213 |
|  |  | CYP3A5 reverse | CTCCTTGAGTTTTCCGCTGGT |  |
| *CYP2C8* | NM\_000770.3 | CYP2C8 forward | CACCAAGCATCACTGGATGT | 293 |
|  |  | CYP2C8 reverse | GTGTAAGGCATGTGGCTCCT |  |
| *CYP2C9* | NM\_000771.4 | CYP2C9 forward | ATTTGTGTGGGAGAAGCCCT | 289 |
|  |  | CYP2C9 reverse | TAGTGAAAGATGGATAATGCCCC |  |
| *18SRNA* | NR\_003286.4 | 18SRNA forward | CAGCCACCCGAGATTGAGCA | 253 |
|  |  | 18SRNA reverse | GGACAGGACTAGGCGGAACA |  |

**Table S2.** The list of NSCLC tumor samples.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Sample ID | NSCLC subtype\* | Tumor volume (cm3) | Differentiation | Sex | Age | Smocking status |
| NSCLC\_001 | AC | 20 | High | 69 | F | No |
| NSCLC\_002 | SCC | 6 | Moderate | 66 | M | Yes |
| NSCLC\_003 | AC | 1.5 | n/a | 59 | F | n/a |
| NSCLC\_004 | AC | <1 | n/a | 63 | F | n/a |
| NSCLC\_005 | AC | 2 | n/a | 55 | F | n/a |
| NSCLC\_006 | LCC | <1 | Low | n/a | F | n/a |
| NSCLC\_007 | AC | <1 | Low | n/a | M | n/a |
| NSCLC\_008 | AC | 7 | n/a | 66 | M | Yes |
| NSCLC\_009 | AC | 11 | n/a | 65 | M | Yes |
| NSCLC\_010 | AC | 4.5 | n/a | 70 | M | n/a |
| NSCLC\_011 | AC | 10 | n/a | 67 | M | Yes |
| NSCLC\_012 | AC | 3 | n/a | 70 | M | Yes |
| NSCLC\_013 | AC | 3 | n/a | 65 | M | Yes |
| NSCLC\_014 | AC | 2.5 | n/a | 66 | M | Yes |
| NSCLC\_015 | AC | 2.5 | n/a | 65 | M | Yes |
| NSCLC\_016 | LCC | 3.5 | Low | 70 | M | n/a |
| NSCLC\_017 | AC | 5 | High | 56 | M | n/a |
| NSCLC\_018 | AC | 3 | Moderate | n/a | M | n/a |

\*AC – adenocarcinoma; LCC – large-cell carcinoma; SCC – squamous cell carcinoma

**Table S3.** Spearman’s correlation coefficients between growth factor/cytokine expression profiles in tumors and free-floating PDTOs.

|  |  |  |
| --- | --- | --- |
| Sample ID | Spearman’s correlation coefficient | p-value |
| NSCLC\_006 | 0.119 | 0.358 |
| NSCLC\_007 | -0.259 | 0.208 |
| NSCLC\_008 | -0.531 | 0.040 |
| NSCLC\_009 | **0.769** | **0.002** |
| NSCLC\_010 | **0.531** | **0.040** |
| NSCLC\_011 | **0.531** | **0.040** |
| NSCLC\_012 | **0.671** | **0.010** |
| NSCLC\_013 | 0.042 | 0.452 |
| NSCLC\_014 | **0.671** | **0.010** |
| NSCLC\_015 | **0.741** | **0.004** |
| NSCLC\_016 | 0.169 | 0.300 |
| NSCLC\_017 | **0.503** | **0.049** |

**Table S4.** Spearman’s correlation coefficients between expression of separate growth factors/cytokines in tumors and free-floating PDTOs.

|  |  |  |
| --- | --- | --- |
| Gene | Spearman’s correlation coefficient | p-value |
| *IFNG* | **0.900** | **<0.001** |
| *TNFA* | **0.714** | **0.005** |
| *IL6* | -0.062 | 0.425 |
| *IL1B* | **0.589** | **0.022** |
| *IL12A* | 0.103 | 0.375 |
| *IL23A* | 0.068 | 0.417 |
| *IL10* | 0.251 | 0.216 |
| *TGFB* | 0.019 | 0.477 |
| *EGF* | **0.949** | **<0.001** |
| *VEGFA* | **0.847** | **<0.001** |
| *FGF2* | **0.771** | **0.002** |
| *TSG6* | 0.287 | 0.183 |

**Supplementary Figures**



**Figure S1.** Production of free-floating NSCLC PDTOs. Pie charts showing (a) the subtypes of established 18 PDTOs and (b) the success rate of free-floating organoid establishment.



**Figure S2.** IC50 concentrations of cisplatin, paclitaxel, gemcitabine, etoposide, and pemetrexed, determined for free-floating PDTOs from ten NSCLC patients. The clinically relevant maximum concentrations (Cmax) are indicated with black dotted lines. The data are presented as mean ± standard error of the mean (SEM).