# Additional file 1. Supplementary figures and supplementary tables

Figure S1. Schematic of drug-inducible sgRNA expression lentiviral vectors.

U6+2xTetO

GAGGGCCTATTTCCCATGATTCCTTCATATTTGCATATACGATACAAGGCTGTTAGAGAGATAATTAGAATTAATTTGACTGTAAACACAAAGATATTAGTACAAAATACGTGACGTAGAAAGTAATAATTTCTTGGGTAGTTTGCAGTTTTAAAATTATGTTTTAAAATGGACTATCATATGCTTACCGTAACTTGAAACTCCCTATCAGTGATAGAGATTATATATCTCCCTATCAGTGATAGACACC

U6+1xTetO

GAGGGCCTATTTCCCATGATTCCTTCATATTTGCATATACGATACAAGGCTGTTAGAGAGATAATTAGAATTAATTTGACTGTAAACACAAAGATATTAGTACAAAATACGTGACGTAGAAAGTAATAATTTCTTGGGTAGTTTGCAGTTTTAAAATTATGTTTTAAAATGGACTATCATATGCTTACCGTAACTTGAAACGTATTTCGATTTCTTGGCTTTATATATCTCCCTATCAGTGATAGACACC

U6+2xLacO

GAGGGCCTATTTCCCATGATTCCTTCATATTTGCATATACGATACAAGGCTGTTAGAGAGATAATTAGAATTAATTTGACTGTAAACACAAAGATATTAGTACAAAAAATTGTGAGCGGATAACAATTATTTCTTGGGTAGTTTGCAGTTTTAAAATTATGTTTTAAAATGGACTATCATATGCTTACCGTAACTTGAAAGTAATTGTGAGCGCTCACAATTATATATCTTGTGGAAAGGACGAAACACC

U6+1xLacO

GAGGGCCTATTTCCCATGATTCCTTCATATTTGCATATACGATACAAGGCTGTTAGAGAGATAATTAGAATTAATTTGACTGTAAACACAAAGATATTAGTACAAAATACGTGACGTAGAAAGTAATAATTTCTTGGGTAGTTTGCAGTTTTAAAATTATGTTTTAAAATGGACTATCATATGCTTACCGTAACTTGAAAGTAATTGTGAGCGCTCACAATTATATATCTTGTGGAAAGGACGAAACACC

Figure S2. Nucleotide sequence presentation of the DOX- and IPTG-inducible U6 promoter variants used in this study. The TATA box is underlined. TetO sequences are highlighted in yellow. LacO sequences are highlighted in red.

Figure S3. *EGFP* disruption activities using 1xTetO and 2xTetO constructs in response to DOX across different concentrations. Data represent mean ± SD (n = 3).

Figure S4. *EGFP* disruption activities using 1xLetO and 2xLetO constructs in response to IPTG across different concentrations. Data represent mean ± SD (n = 3).

Figure S5. Correlation of relative sgRNA expression and leakiness score (A) and activity score (B). Data represent mean ± SD (n = 3).

Figure S6. Treatment of chemical inducers including DOX (A), IPTG (B), Shield-1 (C) and TMP (D) does not affect the cell viability of MC-38 and HEK293T cells. Data represent mean ± SD (n = 3).

Figure S7. Treatment of chemical inducers does not affect EGFP fluorescence or Cas9 cleavage activity in MC-38 (A) or HEK293T (B) cells. Data represent mean ± SD (n = 3).

Figure S8. High efficiency of hematopoietic reconstitution as indicated by the percentage of CD45.2-positive cells from different tissues. Data represent mean ± SD (n = 5).

Figure S9. Composition of CD11b+, CD11c+ and CD19+ cells from the spleen (A), bone marrow (B) and blood (C) of the hematopoietic-system-reconstituted mice. Data represent mean ± SD (n = 5).

Figure S10. Treatment of DOX does not affect CD44 expression level in the hematopoietic-system-reconstituted mice. Data represent mean ± SD (n = 5).

Figure S11. Surface PD-L1 expression in MC-38 and MC-38-*Cd274-/-* cells with or without IFNγ (20 ng/mL) stimulation.

Figure S12. Abolishment of surface PD-L1 expression using constitutive, DOX-inducible and IPTG-inducible sgRNA expression vectors in MC-38 cells. Data represent mean ± SD (n = 3).

Figure S13. Scatter plots comparing the screening hits for positive PD-L1 regulators. (A) Correlation between induced and non-induced screening results using DOX-inducible sgRNA expression vector. Using median log2 fold change >1 as the cutoff, 3 out of 31 screening hits were identified in the non-induced conditions, indicating 10% leakniess. (B) Correlation between DOX-induced and constitutive screen results. (C) Correlation between induced and non-induced screening results using IPTG-inducible sgRNA expression vector. Using median log2 fold change >1 as the cutoff, 4 out of 31 screening hits were identified in the non-induced conditions, indicating 13 % leakniess. (D) Correlation between IPTG-induced and constitutive screen results.

Figure S14. Scatter plots comparing the screening hits for negative PD-L1 regulators. (A) Correlation between induced and non-induced screening results using DOX-inducible sgRNA expression vector. Using median log2 fold change >1 as the cutoff, no hits were identified, representing minimal leakiness. (B) Correlation between DOX-induced and constitutive screen results. (C) Correlation between induced and non-induced screening results using IPTG-inducible sgRNA expression vector. Using median log2 fold change >1 as the cutoff, no hits were identified, representing minimal leakiness. (D) Correlation between IPTG-induced and constitutive screen results.

Figure S15. FDRs of the top 200 screen hits in FACS-based CRISPR screening for PD-L1 regulators. 1 μg/mL DOX or 1 mM IPTG was used to induce the sgRNA expression.

Table S1. Leakiness scores and activity scores of the inducible systems in multiple cell lines.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Cell line** | **Organism** | **Tissue** | **U6+1xTetO** | **U6+2xTetO** | **U6+1xLacO** | **U6+2xLacO** |
| **Leakiness score** | **Activity score** | **Leakiness score** | **Activity score** | **Leakiness score** | **Activity score** | **Leakiness score** | **Activity score** |
| L-363 | Human | Blood | 0.51 | 1.00 | 0.09 | 0.99 | 0.10 | 0.51 | 0.17 | 0.77 |
| MC-38 | Mouse | Colon | 0.85 | 1.00 | 0.08 | 0.95 | 0.21 | 0.83 | 0.13 | 0.79 |
| A-498 | Human | Kidney | 0.73 | 1.00 | 0.03 | 0.95 | 0.06 | 0.53 | 0.00 | 0.41 |
| CT26 | Mouse | Colon | 0.50 | 0.93 | 0.12 | 0.89 | 0.15 | 0.97 | 0.13 | 0.93 |
| LL/2 | Mouse | Lung | 0.98 | 1.00 | 0.14 | 0.74 | 0.18 | 0.76 | 0.14 | 0.75 |
| HEK293T | Human | Kidney | 0.52 | 1.00 | 0.04 | 0.70 | 0.10 | 0.75 | 0.07 | 0.72 |
| LP-1 | Human | Blood | 0.73 | 1.00 | 0.00 | 0.68 | 0.12 | 0.72 | 0.11 | 0.65 |
| NCI-H1299 | Human | Lung | 0.78 | 1.00 | 0.07 | 0.60 | 0.18 | 0.55 | 0.13 | 0.52 |
| 4T1 | Mouse | Breast | 0.76 | 1.00 | 0.05 | 0.44 | 0.11 | 0.78 | 0.10 | 0.78 |
| 786-0 | Human | Kidney | 0.74 | 1.00 | 0.00 | 0.39 | 0.00 | 0.10 | 0.03 | 0.12 |
| OCI-LY10 | Human | Blood | N.T. | N.T. | 0.04 | 0.53 | N.T. | N.T. | 0.06 | 0.57 |
| THP1 | Human | Blood | N.T. | N.T. | 0.21 | 0.66 | N.T. | N.T. | 0.08 | 0.64 |

N.T.: not tested

Table S3. False discovery rates (FDRs) and median log2 fold changes (FC) of the known PD-L1 positive regulating genes in the constitutive and inducible CRISPR screens. The calculation is based on the comparison of the sgRNA abundances in PD-L1low versus pre-sort cells.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Gene** | **Constitutive** | **2xTetO -DOX** | **2xTetO +DOX** | **2xLacO -IPTG** | **2xLacO +IPTG** |
| **FDR** | **Log2FC** | **FDR** | **Log2FC** | **FDR** | **Log2FC** | **FDR** | **Log2FC** | **FDR** | **Log2FC** |
| ***Ifngr1*** | 0.00029 | 3.47 | 0.81 | 0.84 | 0.00071 | 3.12 | 0.75 | 1.72 | 0.00062 | 3.84 |
| ***Ifngr2*** | 0.00029 | 3.76 | 0.71 | 1.14 | 0.00071 | 3.33 | 0.99 | 0.70 | 0.00062 | 3.41 |
| ***Jak1*** | 0.00029 | 3.56 | 0.99 | 0.24 | 0.00071 | 2.79 | 0.99 | 0.43 | 0.00062 | 2.66 |
| ***Jak2*** | 0.00029 | 3.88 | 0.84 | 1.18 | 0.00071 | 3.47 | 0.99 | 1.32 | 0.00062 | 3.66 |
| ***Stat1*** | 0.00029 | 3.83 | 0.99 | 1.06 | 0.00071 | 3.46 | 0.99 | -0.27 | 0.00062 | 3.35 |
| ***Irf1*** | 0.00029 | 3.61 | 0.99 | 0.26 | 0.00071 | 3.06 | 0.99 | 1.43 | 0.40 | 3.08 |
| ***Cd274*** | 0.00029 | 3.65 | 0.96 | 0.57 | 0.00071 | 3.20 | 0.99 | 1.05 | 0.00062 | 3.47 |

Table S4. False discovery rates (FDRs) and median log2 fold changes (FC) of the known PD-L1 negative regulating genes in the constitutive and inducible CRISPR screens. The calculation is based on the comparison of the sgRNA abundances in PD-L1high versus pre-sort cells.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Gene** | **Constitutive** | **2xTetO -DOX** | **2xTetO +DOX** | **2xLacO -IPTG** | **2xLacO +IPTG** |
| **FDR** | **Log2FC** | **FDR** | **Log2FC** | **FDR** | **Log2FC** | **FDR** | **Log2FC** | **FDR** | **Log2FC** |
| ***Ptpn2*** | 0.00015 | 2.13 | 0.97 | 0.45 | 0.0012 | 1.81 | 0.99 | 0.52 | 0.0012 | 2.12 |
| ***Socs1*** | 0.00015 | 2.66 | 0.97 | 0.74 | 0.035 | 2.83 | 0.98 | 0.66 | 0.0012 | 2.65 |
| ***Irf2*** | 0.00015 | 1.84 | 0.53 | 0.11 | 0.028 | 1.50 | 1.00 | -0.85 | 0.0050 | 2.14 |