1   **Supplementary Materials**

2   **Figure S1**

A

ERMS (RD)

B

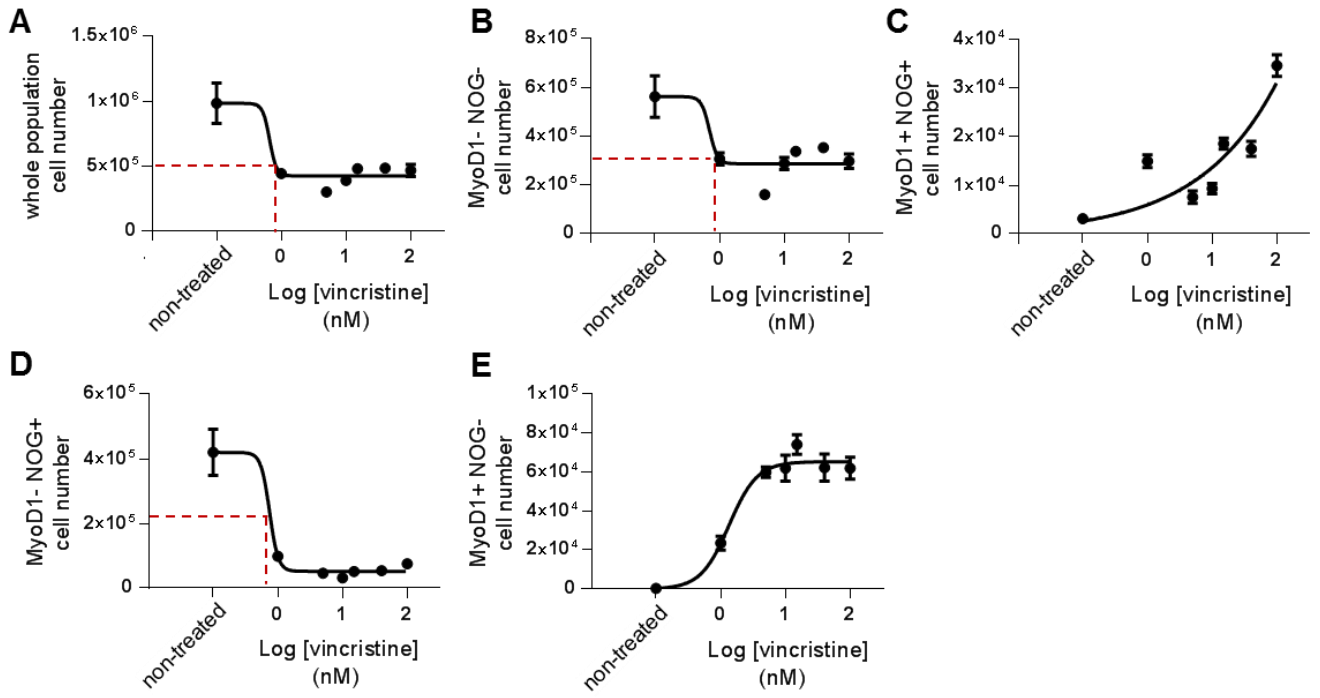**RT (A-204)**

3
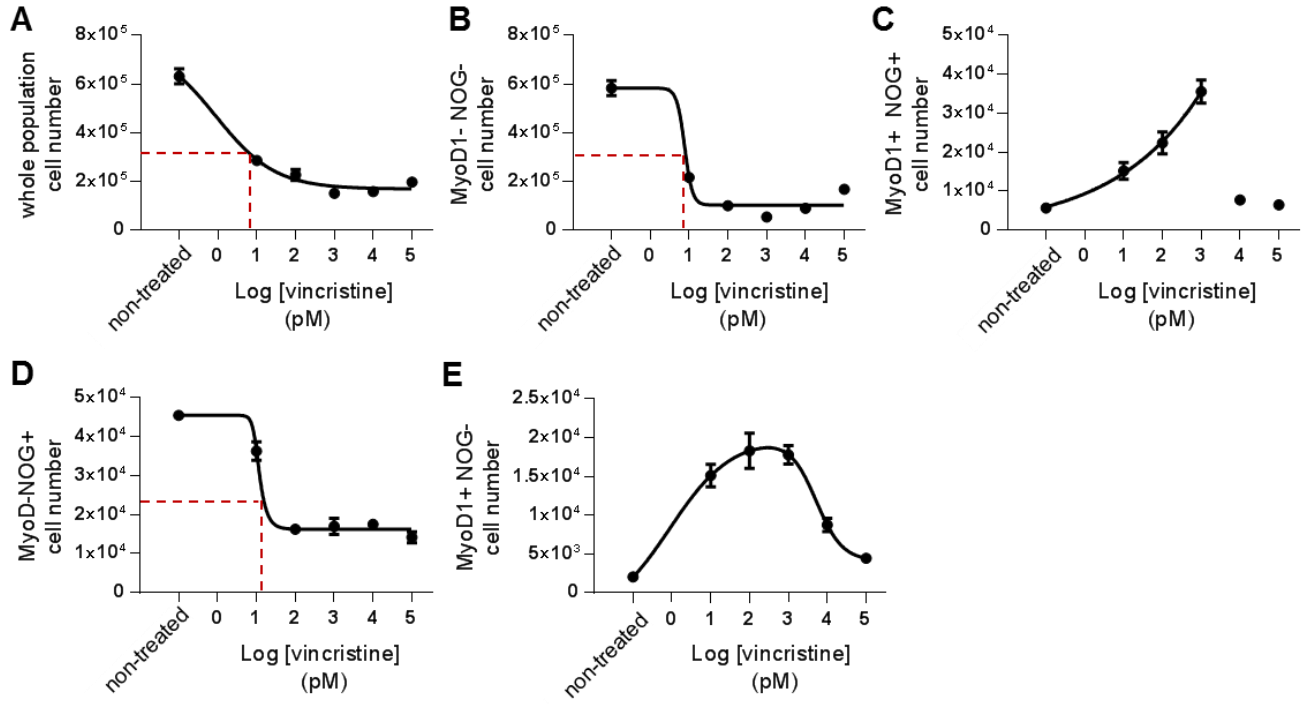
4
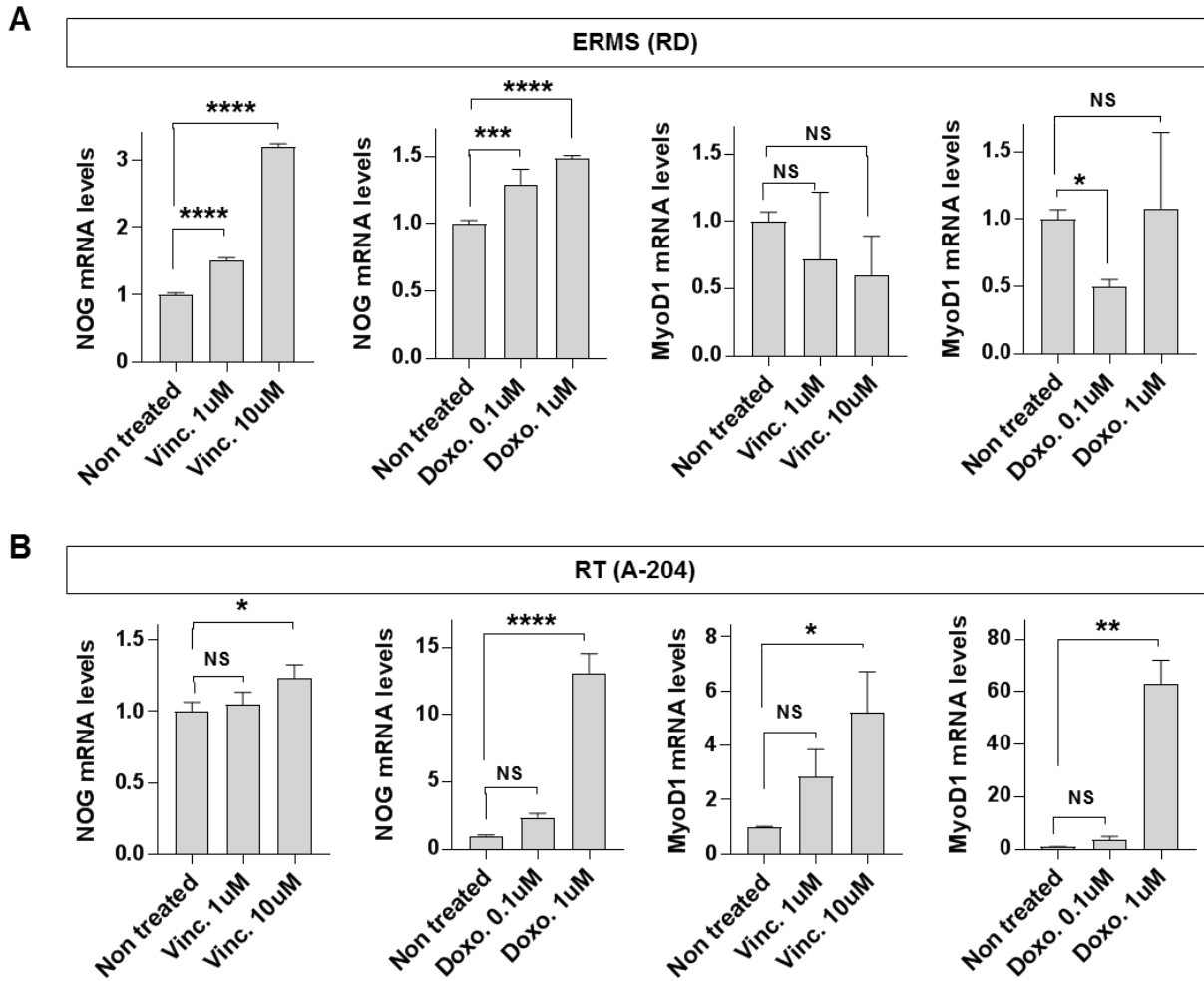
Figure S2

Figure S3

Supplementary figures legends

Figure S1. Relative MyoD1 and Noggin mRNA expression levels in treated ERMS and RT cell lines. Histograms show relative expression levels of Noggin (NOG) and MyoD1 normalized to non-treated control in **(A)** ERMS cell line RD and in **(B)** RT cell line A-204 (Mean ± standard deviation, N=3). *$p<0.05$ **$p<0.01$ ***$p<0.001$ ****$p<0.0001$,* ANOVA with Tukey's multiple comparisons test).

Figure S2. Dose reponse curves of ERMS RD cell line to vincristine treatment. Drug treatment dose responses for **(A)** whole cell population, $R^2=0.85$, $IC_{50}=0.7$ nM **(B)** double negative (MyoD1- NOG-), $R^2=0.8$, $IC_{50}=0.5$ nM **(C)** double positive (MyoD1+ NOG+), $R^2=0.76$ **(D)** MyoD1- NOG+ (single positive) subpopulation, $R^2=0.85$, $IC_{50}=0.4$ nM **(E)** MyoD1+ NOG- (single positive) subpopulation, $R^2=0.95$. Plots represent viable cell numbers versus drug concentrations transformed to logarithmic scale. Best fit curves (Mean ± Standard Deviation, N=3) are shown. Non-linear regression using least squares (ordinary) fit was applied for (A, B, D, E), and exponential growth using least squares (ordinary) fit for (C). Red dotted lines highlight the calculated IC_{50}.

Figure S3. Dose reponse curves of RT A-204 cell line to vincristine treatment. Drug treatment dose responses for **(A)** whole cell population, $R^2=0.98$, $IC_{50}=0.70$ pM **(B)** double negative (MyoD1- NOG-), $R^2=0.96$, $IC_{50}=0.8$ pM **(C)** double positive (MyoD1+ NOG+), $R^2=0.97$, **(D)** MyoD1-, NOG+ (single positive) subpopulation, $R^2=0.86$, $IC_{50}=17.7$pM **(E)** MyoD1+ NOG- (single positive) subpopulation, $R^2=0.97$. Plots represent viable cell numbers versus drug concentrations transformed to logarithmic scale. Best fit curves for 3 replicate experiments (Mean ± Standard Deviation) are shown. Non-linear regression dose-response (four parameters) using least squares (ordinary) fit was applied for (A, B, D), exponential growth using least squares (ordinary) fit for (C), and non-linear regression bell-shaped dose-response using least squares (ordinary) fit was applied for (E). Red dotted lines highlight the calculated IC_{50}.

4

34 **Supplementary methods**

35 **Real time qRT-PCR**

36 Total RNA was extracted using RNAeasy Plus Mini Kit (Qiagen) according to the manufacturer's

37 instructions. Optional on-column DNA digestion step was performed with RNase-Free DNase

38 according to the manufacturer's instructions (Qiagen). Reverse transcription was performed with

39 SuperScript IV First Strand Synthesis (Invitrogen). Real-time RT-PCR was performed with a

40 SYBR green master Mix kit (Applied Biosystems). Relative mRNA levels were normalized to

41 HPRT and calculated by $\Delta\Delta$CT method.

42 The primers are listed below:

43 MYOD1 (F: 5'-TGCTGGACAGGCAGTCTA-3', R: 5'-CTCCGACGGCATGATGG-3')

44 NOG (F:5'-GCAGCGTCTCGTTCAGAT-3', R: 5'-CCAGCACTATCTCCACATCC-3')

45 HPRT (F: 5'-GCGATGTCAATAGGACTCCAG-3', R:5'-TTGTTGTAGGATATGCCC-

46 TTG A-3'

47 **Drug dose-response curves**

48 Cell lines were seeded at 0.3 x 106 cells per well and treated with drug for 48 hours. RD cells

49 were treated with 1nM, 5nM, 10nM, 15nM, 40nM and 100nM of vincristine, A-204 cells - with

50 0.01nM, 0.1nM, 1nM, 10nM and 100nM of vincristine. Cells were collected at 48 hours after

51 twice washing wells with PBS to remove dead cells. Cells absolute numbers and viability were

52 obtained using Chemometec NC-200. Then cells were immediately processed for intracellular

53 flow cytometry staining as described in the corresponding Material and Methods section.

54 Percentages of each cell subpopulation (MyoD1- NOG-, MyoD1+ NOG+, MyoD1- NOG+ and

55 MyoD1+ NOG-) was obtained by flow cytometry analysis and absolute numbers of these cell

56 subpopulations were calculated.

5