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Supplemental Material to:

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The p27-Skp2 axis mediates glucocorticoid-induced cell cycle arrest in T-lymphoma cells

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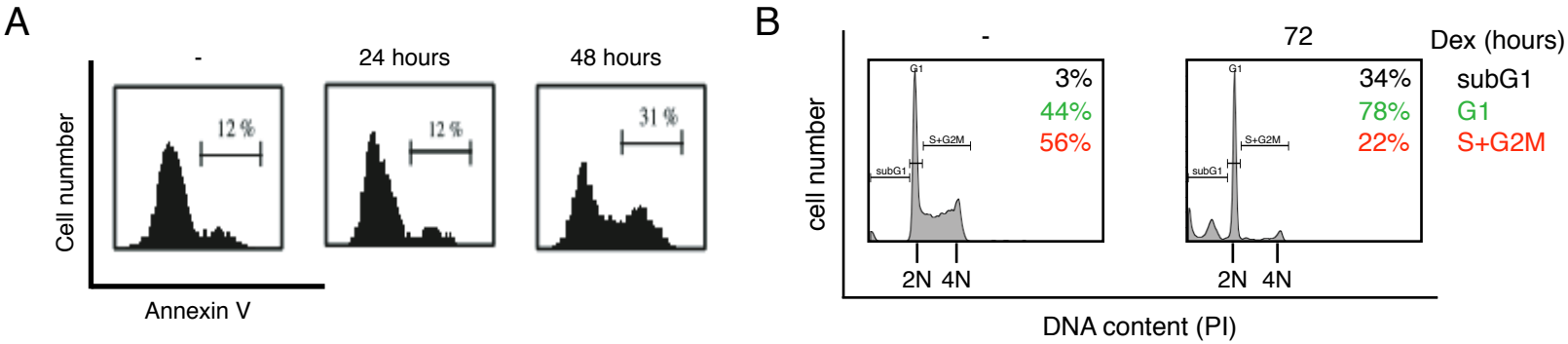
Suppl. figure 1



C

S49.1

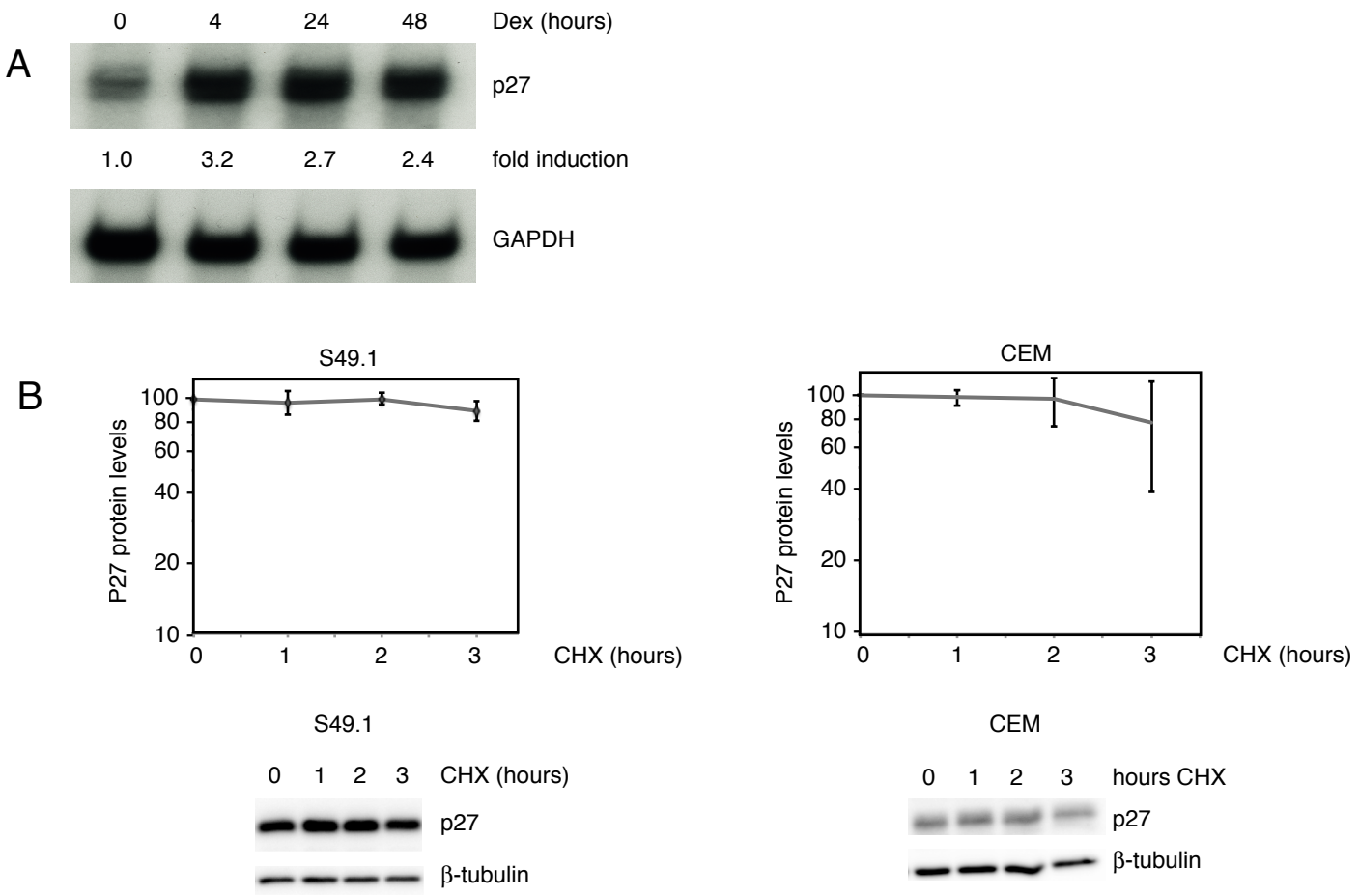
	control	24 h	48 h
subG1	3.7 ± 2.6	5.3 ± 2.6	12.1 ± 2.1
G1	34.4 ± 4.8	42.5 ± 2.9	54.9 ± 2.2
S+G2M	65.6 ± 4.8	57.5 ± 2.9	45.1 ± 2.9

CEM

	control	24 h	48 h
subG1	8.7 ± 4.8	11.9 ± 4.2	53 ± 7.6
G1	45.3 ± 8.4	66 ± 8.2	69.9 ± 9.5
S+G2M	54.7 ± 8.4	34 ± 8.2	30.1 ± 9.5

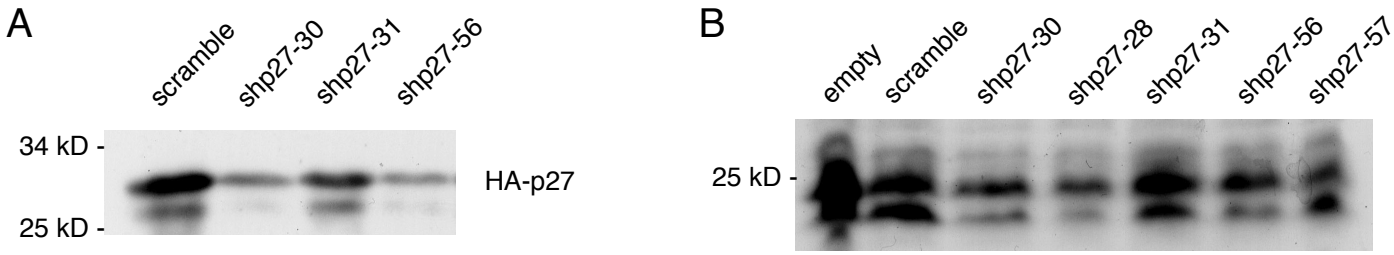
Suppl. figure 1. Induction of apoptosis by glucocorticoids in S49.1 cells. (A) Increased Annexin V staining during Dex-induced apoptosis. S49.1 cells were treated for 24 and 48 hours with Dex (100 nM). Approximately 10^6 S49.1 cells were processed for Annexin V staining and analyzed by flow cytometry. Annexin V positive cells are indicated by brackets in each panel. Percentage of positive cells are indicated above the brackets. (B) Increased amount of subG1-cells by Dex-treatment. Histogram of S49.1 cells treated for 72 hours with Dex (100 nM) and processed as described in figure 1A. (C) Apoptosis and cell cycle distribution in Dex-treated S49.1 and CEM cells. Tabular presentation of the data shown in the diagrams of figure 1A. Percent of cells in indicated phases of the cell cycle are expressed as mean +/- standard deviation from at least 3 independent experiments. Cell cycle distribution was calculated omitting apoptotic cells.

Suppl. figure 2



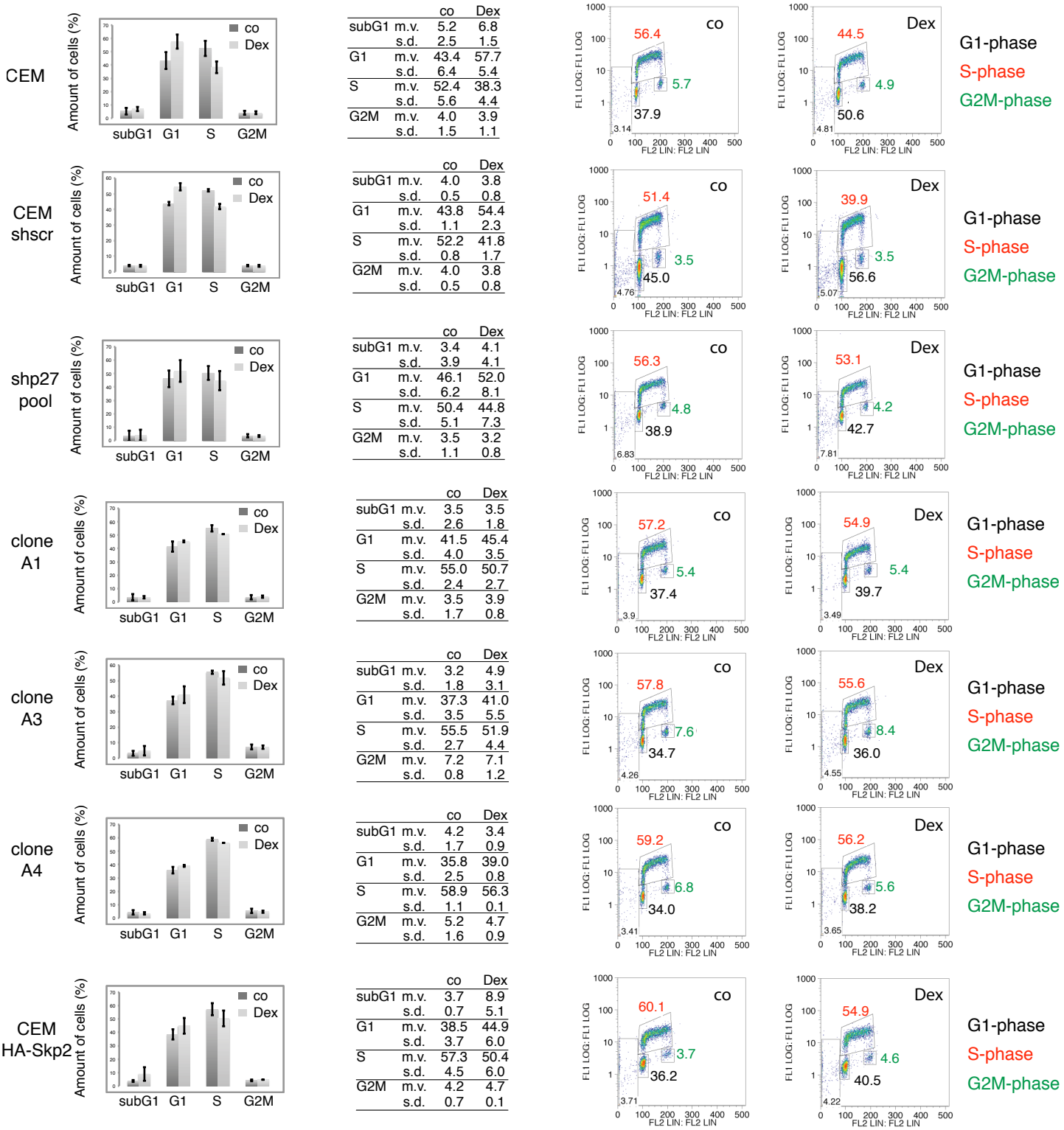
Suppl. figure 2. (A) Regulation of p27 mRNA by Dex in S49.1 and CEM cells. Northern blot analysis using poly A+ mRNA from non-treated or Dex-treated (100 nM, 4, 24 and 48 hours) cells. A p27-specific radioactive probe was used to determine p27-mRNA levels. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as loading control. Levels of p27-mRNA normalized to GAPDH are indicated below. Quantification was performed by PhosphoImaging. **(B)** Increased p27 protein stability by Dex in S49.1 and CEM cells. 24 hours Dex (100 nM) treated S49.1 and CEM cells were washed with a large volume of fresh culture media and resuspended in fresh media without Dex before treatment with cycloheximide (CHX, 20 μM). Thereafter the experiment was carried as described in figure 2C and 2D of the manuscript. Results in the diagram are derived from 3 independent experiments and error bars indicate SD.

Suppl. figure 3



Suppl. figure 3. (A) Characterization of a powerful p27 shRNA construct for p27 knock-down. The expression construct for HA-tagged p27 was transfected into 293T cells together with p27 mRNA targeting shRNA vectors (see Materials and Methods). Expression levels of p27 were determined by SDS-PAGE followed by immunoblotting using a HA-specific antibody. (B) Efficiency in downregulating endogenous p27 was analyzed by transfecting 293T cells with empty, control (scramble) or p27 shRNA constructs and processed as described above.

Suppl. figure 4



		co	Dex
subG1	m.v.	5.2	6.8
	s.d.	2.5	1.5
G1	m.v.	43.4	57.7
	s.d.	6.4	5.4
S	m.v.	52.4	38.3
	s.d.	5.6	4.4
G2M	m.v.	4.0	3.9
	s.d.	1.5	1.1

		co	Dex
subG1	m.v.	4.0	3.8
	s.d.	0.5	0.8
G1	m.v.	43.8	54.4
	s.d.	1.1	2.3
S	m.v.	52.2	41.8
	s.d.	0.8	1.7
G2M	m.v.	4.0	3.8
	s.d.	0.5	0.8

		co	Dex
subG1	m.v.	3.4	4.1
	s.d.	3.9	4.1
G1	m.v.	46.1	52.0
	s.d.	6.2	8.1
S	m.v.	50.4	44.8
	s.d.	5.1	7.3
G2M	m.v.	3.5	3.2
	s.d.	1.1	0.8

		co	Dex
subG1	m.v.	3.5	3.5
	s.d.	2.6	1.8
G1	m.v.	41.5	45.4
	s.d.	4.0	3.5
S	m.v.	55.0	50.7
	s.d.	2.4	2.7
G2M	m.v.	3.5	3.9
	s.d.	1.7	0.8

		co	Dex
subG1	m.v.	3.2	4.9
	s.d.	1.8	3.1
G1	m.v.	37.3	41.0
	s.d.	3.5	5.5
S	m.v.	55.5	51.9
	s.d.	2.7	4.4
G2M	m.v.	7.2	7.1
	s.d.	0.8	1.2

		co	Dex
subG1	m.v.	4.2	3.4
	s.d.	1.7	0.9
G1	m.v.	35.8	39.0
	s.d.	2.5	0.8
S	m.v.	58.9	56.3
	s.d.	1.1	0.1
G2M	m.v.	5.2	4.7
	s.d.	1.6	0.9

		co	Dex
subG1	m.v.	3.7	8.9
	s.d.	0.7	5.1
G1	m.v.	38.5	44.9
	s.d.	3.7	6.0
S	m.v.	57.3	50.4
	s.d.	4.5	6.0
G2M	m.v.	4.2	4.7
	s.d.	0.7	0.1

Suppl. figure 4. Reduced cell cycle arrest in p27 knock-down or Skp2 overexpressing cells. From left to right: diagBar graph showing relative amount of apoptotic (subG1) cells and cells in different phases of the cell cycle of control or Dex-treated cells. Error bars indicate s.d. of 3 independent experiments; table representing the numerical data of the bar graph. Graphs to the right: representive cell cycle distribution for the indicated cell lines upon Dex-treatment. Gated areas mark cells in different cell cycle phases and apoptotic (subG1) cells. Cell cycle profiles were determined upon BrdU incorporation and PI staining of DNA.