SUPPLEMENTAL MATERIAL O. V. SHLEPOVA, ET AL. "COMBINATION WITH A LOW DOSE OF DOXORUBICIN FURTHER BOOSTS THE ANTITUMOR EFFECT OF SLURP-1 *IN VIVO* AND ASSOCIATES WITH EGFR DOWN-REGULATION"

Table S1. Mortality rates for different treatment strategies

	Control (saline)	SLURP-1 0.5 mg/kg	SLURP-1 5 mg/kg	Doxorubicin 2.5 mg/kg	Doxorubicin 0.25 mg/kg + SLURP-1 0.5 mg/kg
Number of mice at the beginning of the experiment	10	9	8	10	10
Number of mice deaths during the experiment	2[1]	3[2,3,4]	0	0	2[5,6]

[1] – died on the 15th day, initially there were many metastases in the abdomen.
[2] – died on the 19th day, large tumor and metastasis.
[3] – died on the 11th day, cause of death is not clear.
[4] – died on the 18th day, large tumor.
[5,6] – died on the 10th day, initially tumor was growing inwards in the abdomen.

Tables S2. The parameters describing the dose-response curves of inhibition of A431 cell migration

	SLURP-1	Doxorubicin
EC_{50}, μM	9.4 ± 7.8	2.3 ± 1.7
A_1, %	-0.21 ± 0.84	-0.15 ± 0.3

Data are presented as the mean ± SEM, $n = 3–22$.

Table S3. The parameters describing the dose-response curves of inhibition of EGFR activation in A431 cells by SLURP-1

	-EGF	+EGF (25 nM)
EC_{50}, nM	40 ± 11	60 ± 17
A_1, %	50 ± 9*	74 ± 5*

Data are presented as the mean ± SEM, $n = 10–14$. *$p < 0.05$ indicate a significant difference between parameters according to the unpaired two-tailed t-test.

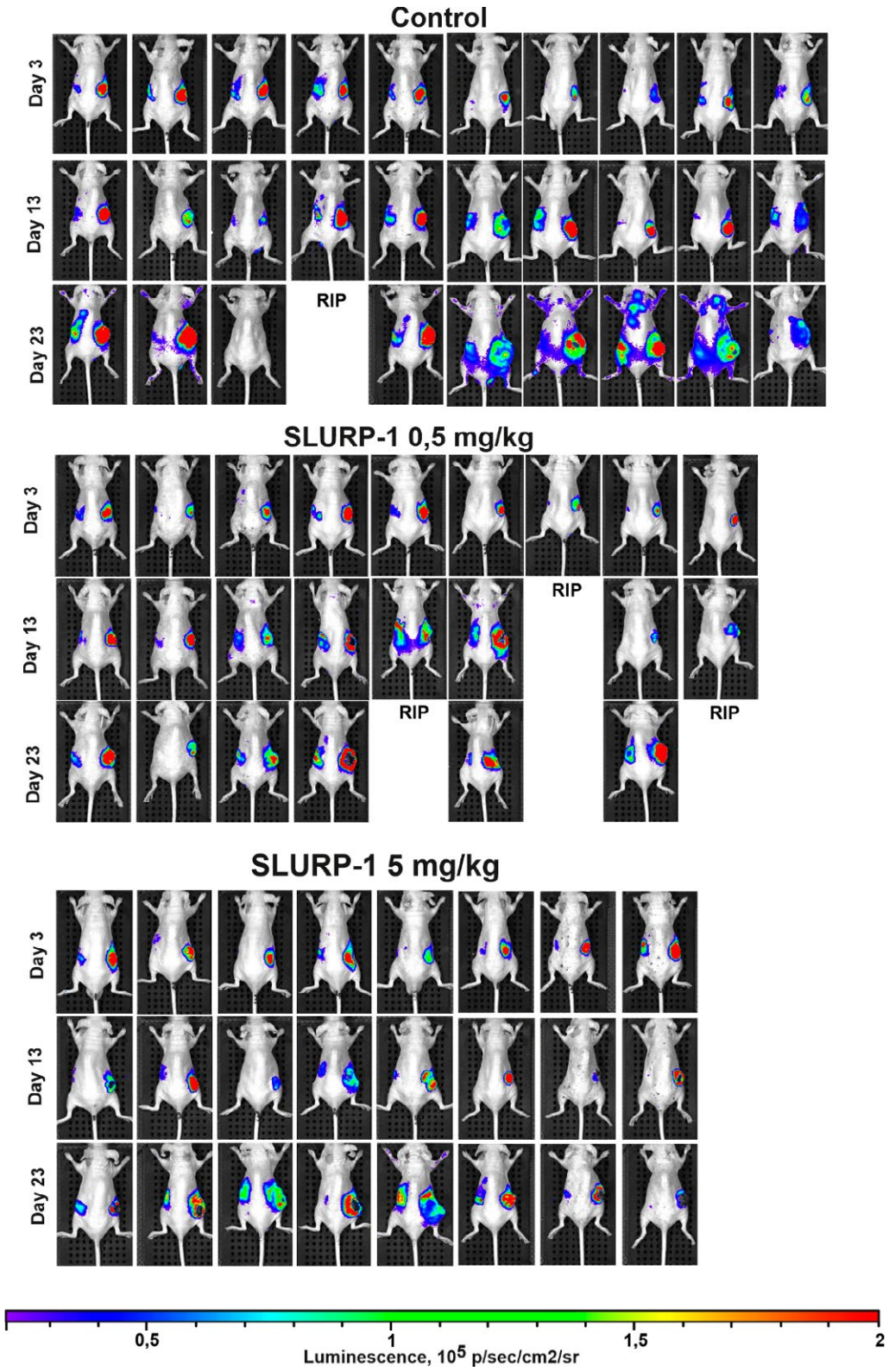
Control
Day 3
Day 13
Day 23
RIP
SLURP-1 0,5 mg/kg
Day 3
Day 13
Day 23
RIP
RIP
RIP
SLURP-1 5 mg/kg
Day 3
Day 13
Day 23
0,5
1
1,5
2
Luminescence, 10^5 p/sec/cm2/sr

Fig. S1 (start).

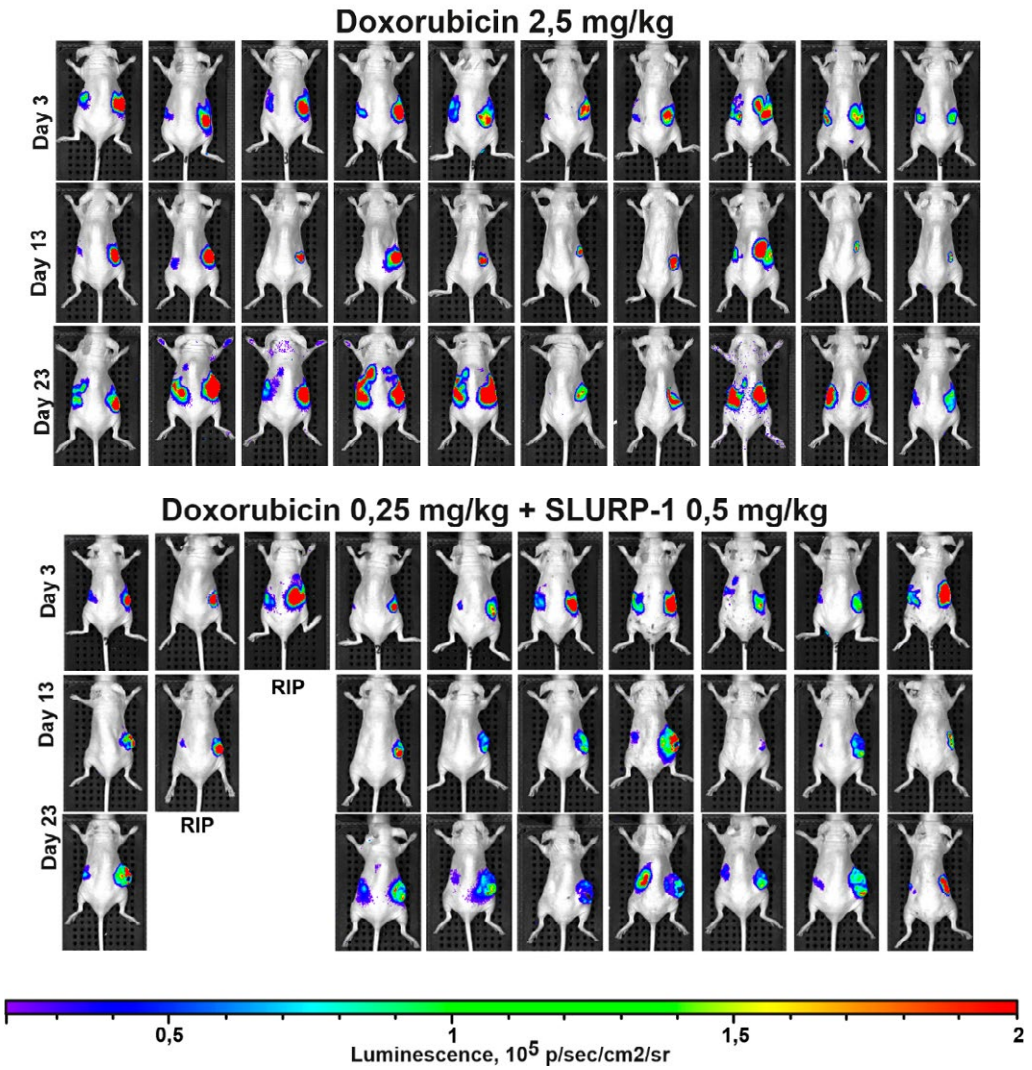

Fig. S1. Images of tumor bioluminescence (A431/NanoLuc cells) in mice before treatment (the 3rd day after tumor engraftment, the 1st day of the therapy), after treatment (the 13th day after tumor engraftment, the next day after the end of the 10-day therapy), and before sacrification (the 23rd day after tumor engraftment).
Five groups of animals are used:
1) Control, i.v. injection of saline ($n = 10$),
2) i.v. injection of 0.5 mg/kg of SLURP-1 ($n = 9$),
3) i.v. injection of 5 mg/kg of SLURP-1 ($n = 8$),
4) i.v. injection of 2.5 mg/kg of doxorubicin ($n = 10$),
5) i.v. injection of 0.5 mg/kg of SLURP-1 and 0.25 mg/kg of doxorubicin ($n = 10$).
Mice that looked weak and sick were not taken for bioimaging due to for fear of affecting their health. However they died only a few days later. The day and cause of death are shown in *Table S1*.

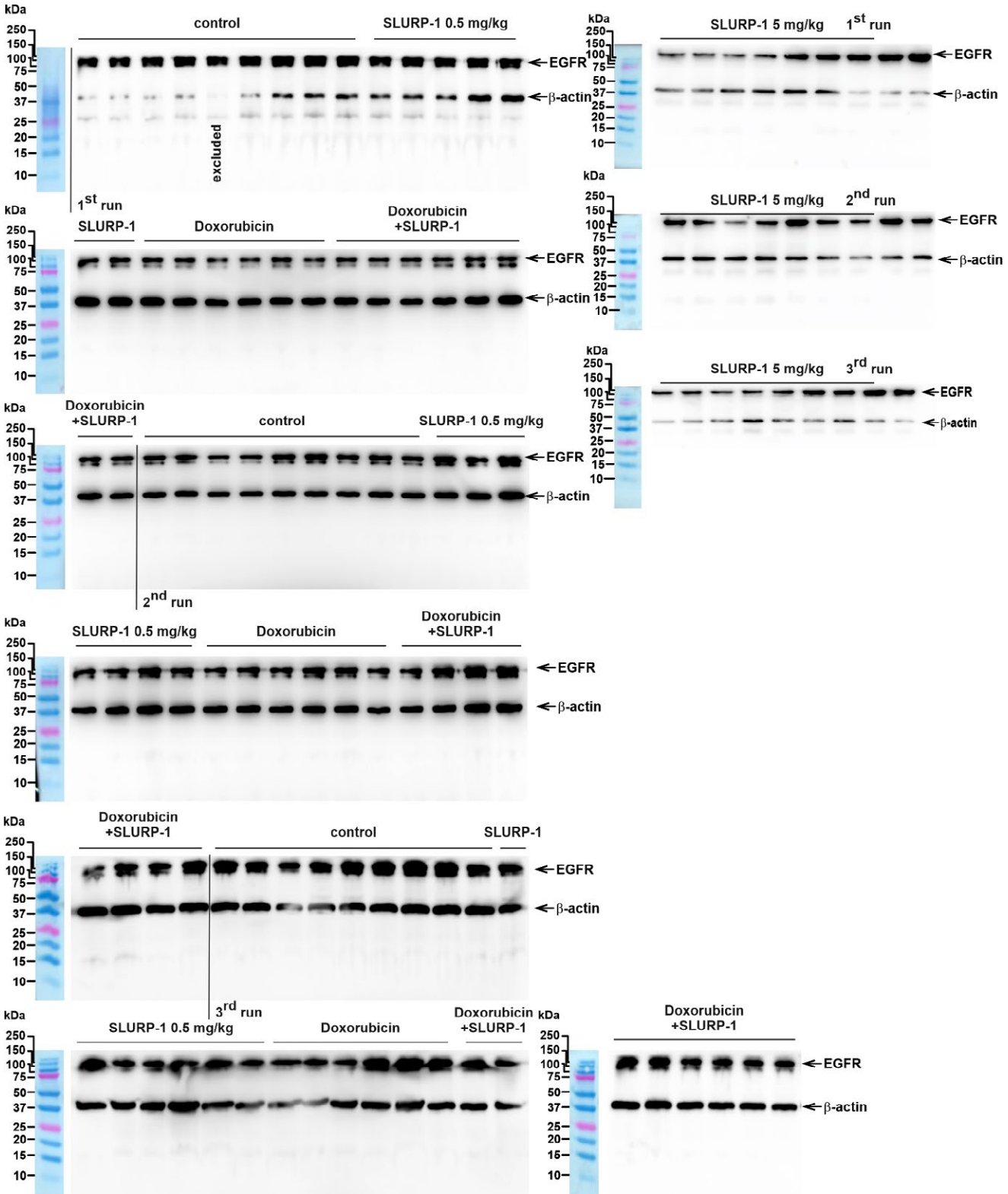

Fig. S2. Western blot membranes used for analysis of the EGFR and β-actin expression in tumors after treatment with saline (control), SLURP-1 (0.5 mg/kg), doxorubicin (2.5 mg/kg), and SLURP-1 (0.5 mg/kg) with doxorubicin (0.25 mg/kg) ($n = 6–9$ for a group, three different runs)

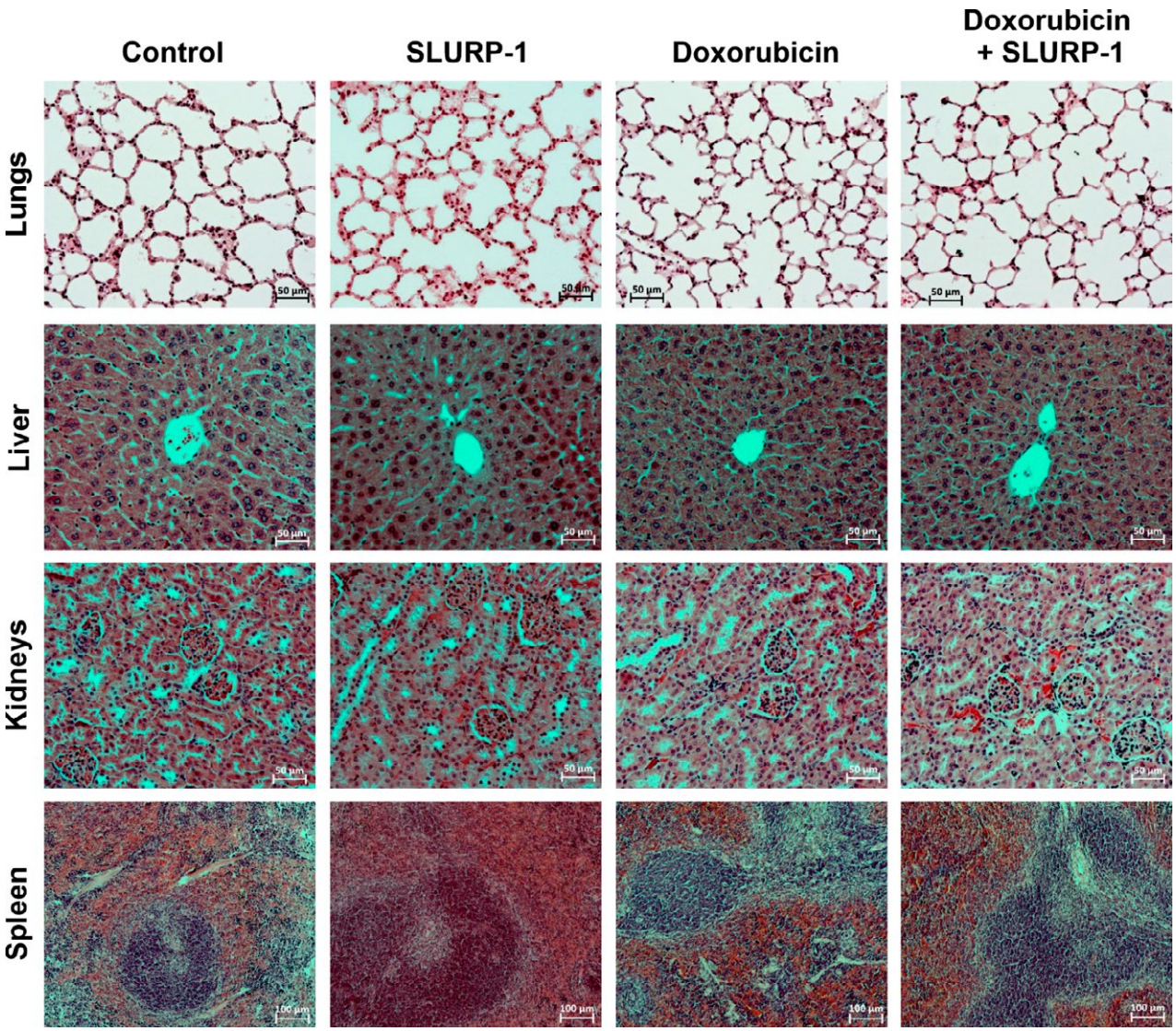

Fig. S3. Fragments of the lung, liver, kidney, and spleen of male mice from the saline (control), SLURP-1 (5 mg/kg), doxorubicin (2.5 mg/kg), or SLURP-1 (0.5 mg/kg) + doxorubicin (0.25 mg/kg) groups. No deviations from the norm were identified. Hematoxylin and eosin staining