

Lapatinib and Obatoclax kill tumor cells through blockade of ERBB1 / 3 / 4 and through inhibition of BCL-XL and MCL-1.

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Figure S1. Lapatinib and obatoclax interact to kill GBM cells. GBM6 and GBM12 cells were plated as single cells in sextuplicate. Twelve h after plating cells were treated with vehicle control, obatoclax, or lapatinib at the indicated doses. Cells were treated with the drugs for 48h followed by culture in drug free media. Twelve days after drug treatment cells were fixed and stained and the number of colonies of > 50 cells counted. Data was placed into the CalcuSyn for windows program to determine the combination index (CI) values. The CI values are plotted in the Figure (n = 3, +/- SEM).

Figure S2. Expression and knock down of PTEN in tumor cells. GBM14 and GBM6 cells were transfected to either over-express GFP-PTEN or to knock down PTEN, respectively. Lysates were taken 24h after transfection followed by SDS PAGE and blotting for PTEN.

Figure S3. Activation of B-RAF weakly protects cells from the drug combination. DAOY cells were transfected with empty vector (CMV) or with plasmids to express activated B-RAF V600E and dominant negative MEK1, as indicated. Cells were treated with vehicle (DMSO) or with lapatinib (1 μ M) and obatoclax (GX, 50 nM). Cells were isolated 24h after exposure and viability determined by trypan blue exclusion (n = 3, +/- SEM).

Figure S4. Assessment of lapatinib + obatoclox toxicity in DAOY tumors. Tumors were established in the brains of athymic mice for 14 days. Animals were then treated with vehicle diluent or with lapatinib + obatoclox as described in Methods. Tumor volumes were measured every 2-3 days as indicated in the Figure. * $p < 0.05$ greater survival compared to vehicle control.

Figure S5. Assessment of lapatinib + obatoclox normal tissue toxicity in mice: total body mass. Animals were treated with vehicle diluent or with lapatinib + obatoclox as described in Methods. The body mass after 1 week and 2 weeks of drug exposure are plotted (+/- SEM).

Figure S6. Assessment of lapatinib + obatoclox normal tissue toxicity in mice: brain. Animals were treated with vehicle diluent or with lapatinib + obatoclox as described in Methods. Organs were removed and sections and H&E stained. A representative image of 3 separate organs / animals.

Figure S7. Assessment of lapatinib + obatoclox normal tissue toxicity in mice: heart. Animals were treated with vehicle diluent or with lapatinib + obatoclox as described in Methods. Organs were removed and sections and H&E stained. A representative image of 3 separate organs / animals.

Figure S8. Assessment of lapatinib + obatoclox normal tissue toxicity in mice: kidney. Animals were treated with vehicle diluent or with lapatinib + obatoclox as described in Methods. Organs were removed and sections and H&E stained. A representative image of 3 separate organs / animals.

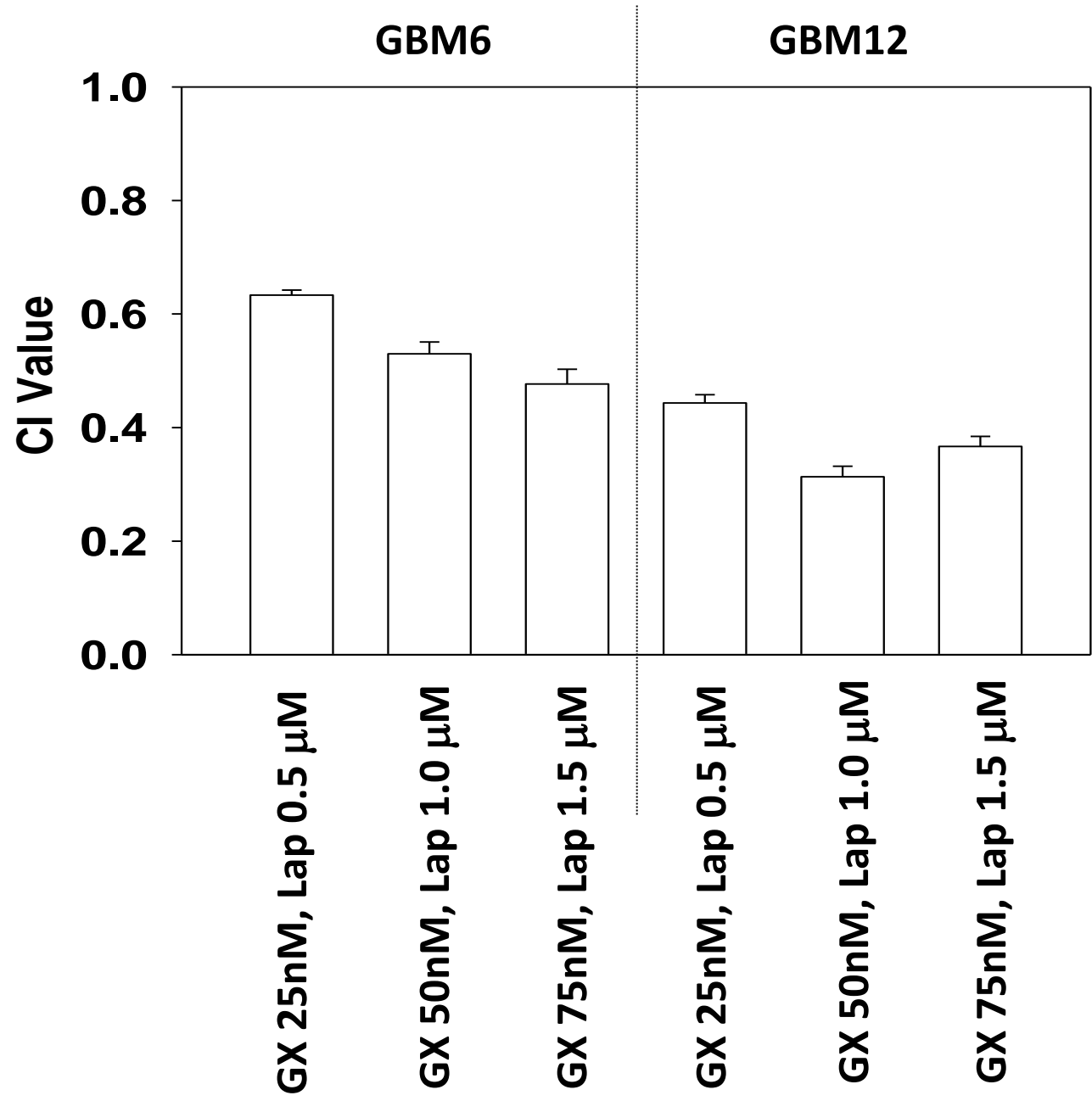
Figure S9. Assessment of lapatinib + obatoclox normal tissue toxicity in mice: liver. Animals were treated with vehicle diluent or with lapatinib + obatoclox as described in Methods. Organs were removed and sections and H&E stained. A representative image of 3 separate organs / animals.

Figure S10. Assessment of lapatinib + obatoclox normal tissue toxicity in mice: lung. Animals were treated with vehicle diluent or with lapatinib + obatoclox as described in Methods. Organs were removed and sections and H&E stained. A representative image of 3 separate organs / animals.

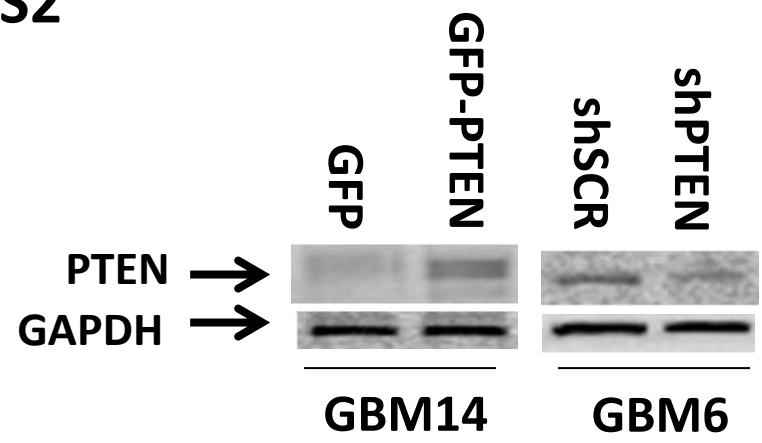
Figure S11. Assessment of lapatinib + obatoclox normal tissue toxicity in mice: spleen. Animals were treated with vehicle diluent or with lapatinib + obatoclox as described in Methods. Organs were removed and sections and H&E stained. A representative image of 3 separate organs / animals.

Figure S12. Possible signaling mechanisms by which lapatinib and obatoclox interact. Increase levels of PUMA and NOXA promote release of Beclin 1 from protective BCL-2 proteins such as MCL-1 and BCL-XL. Released Beclin 1 stimulates a toxic form of autophagy.

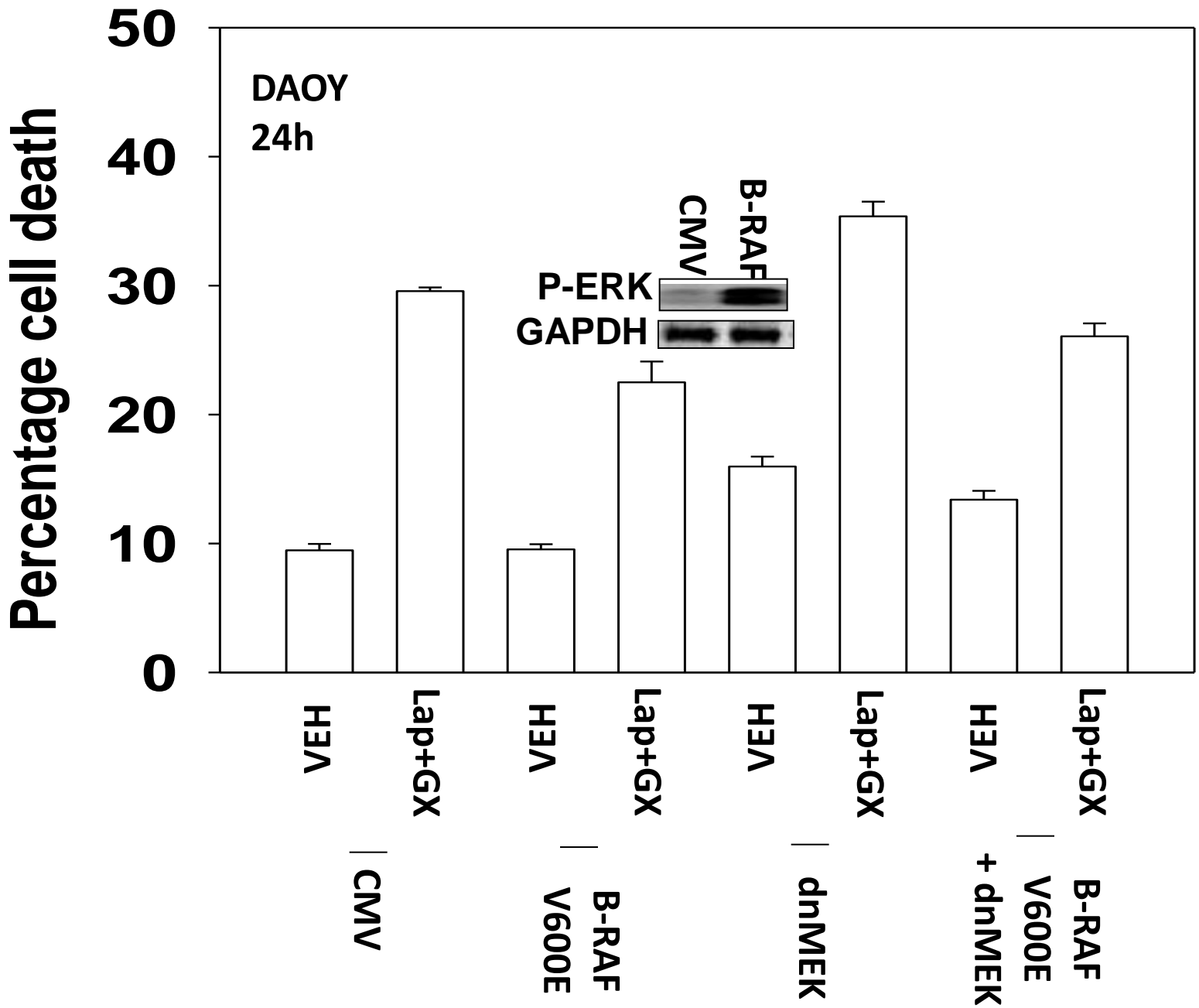
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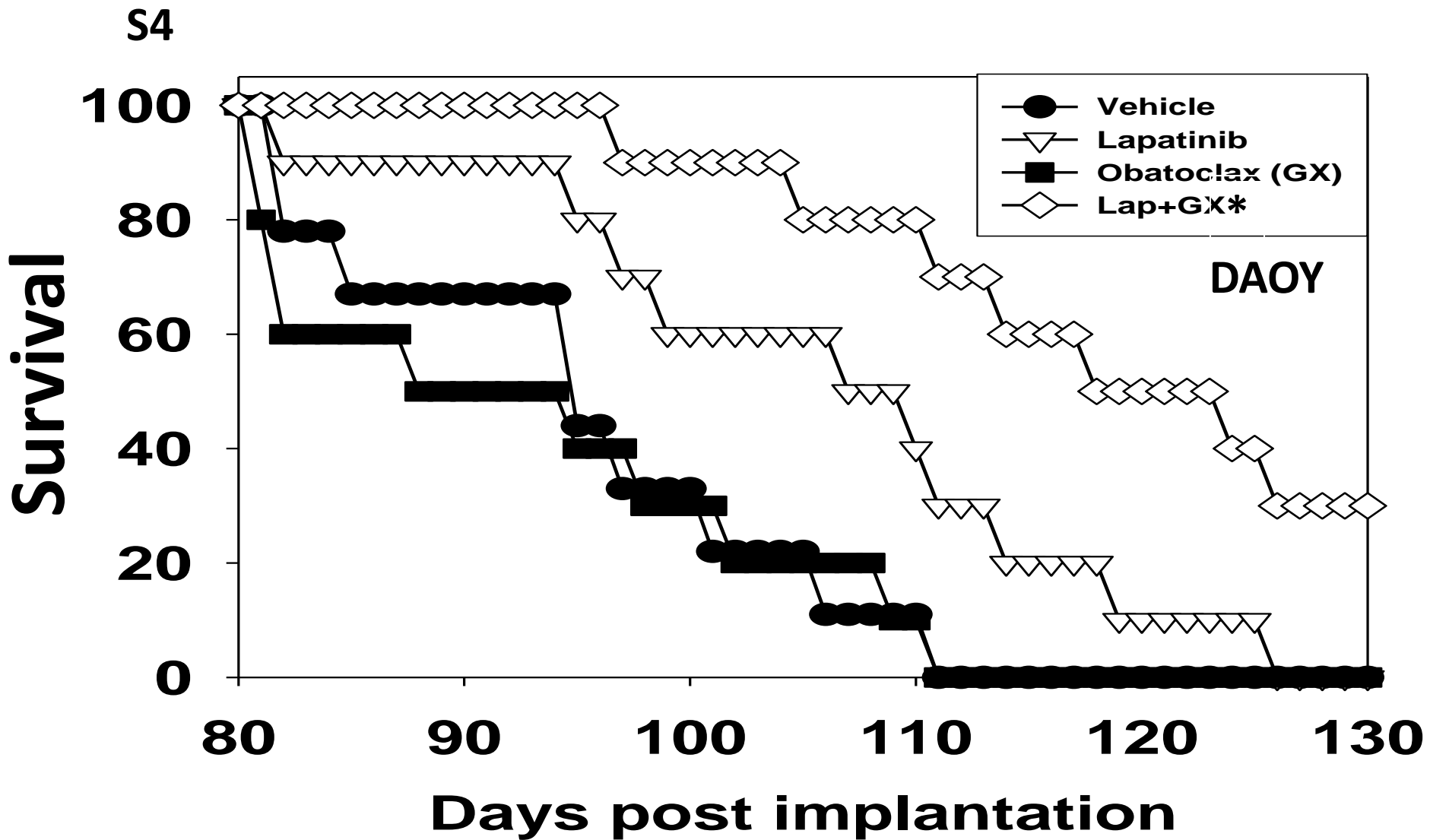


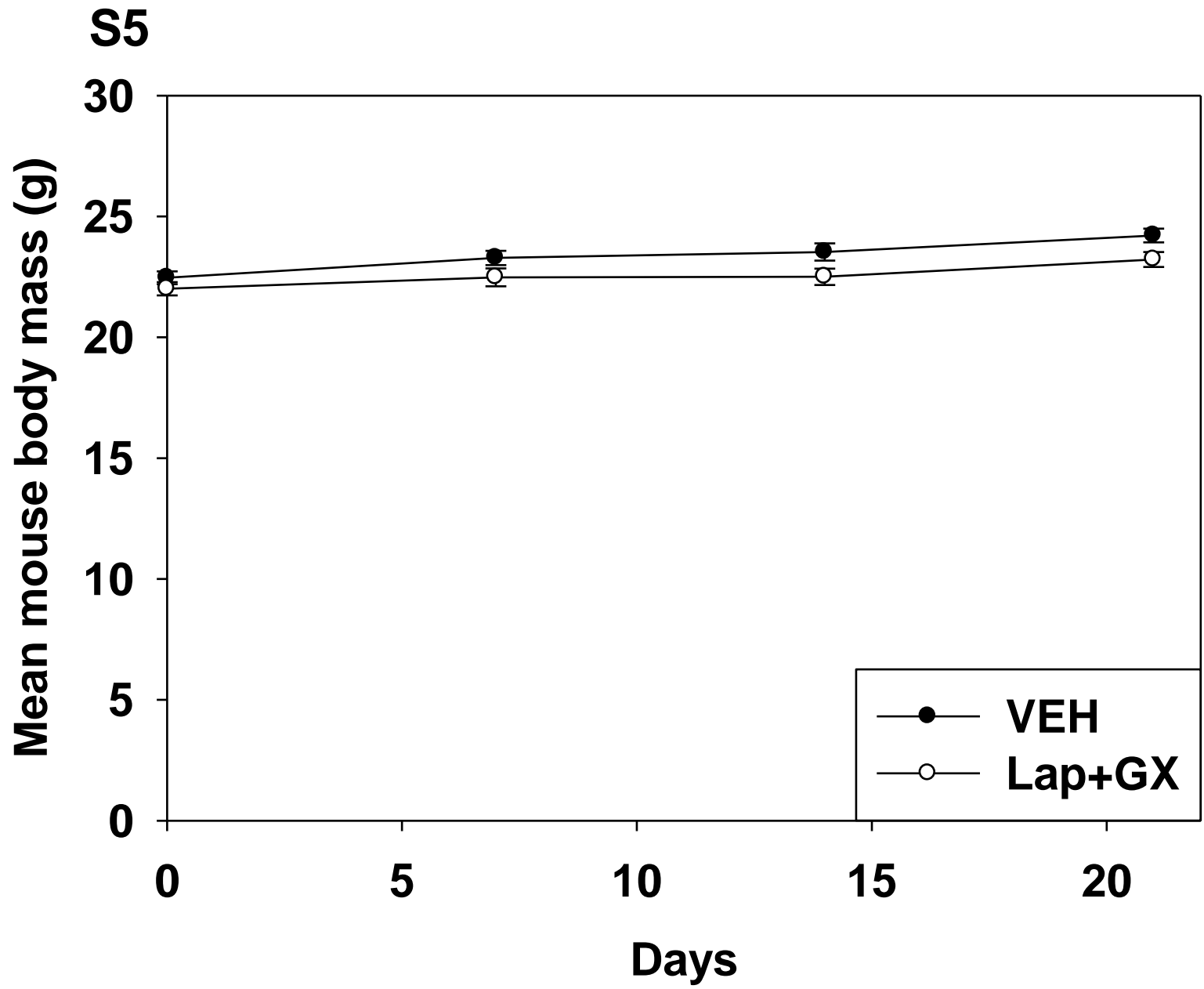
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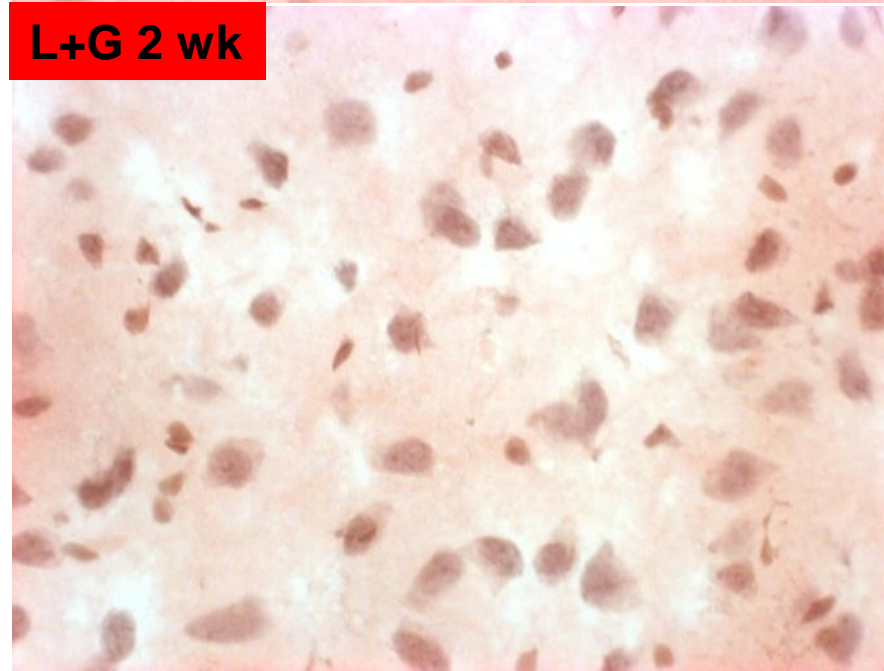
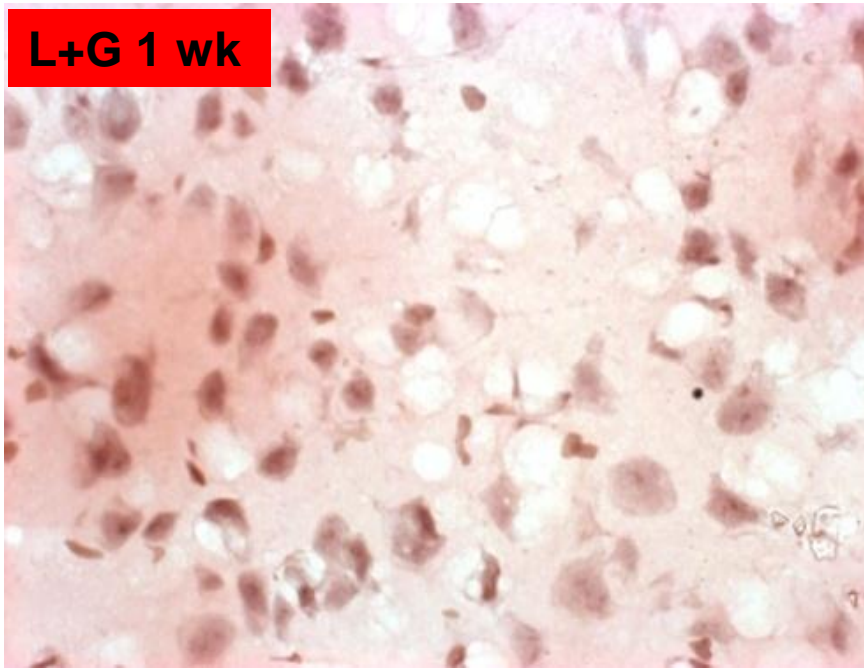
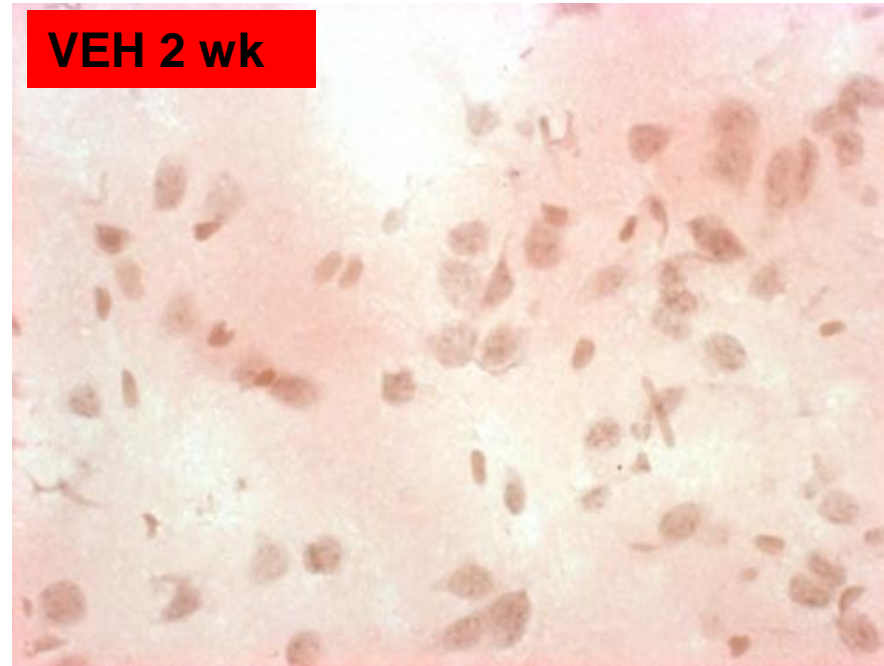
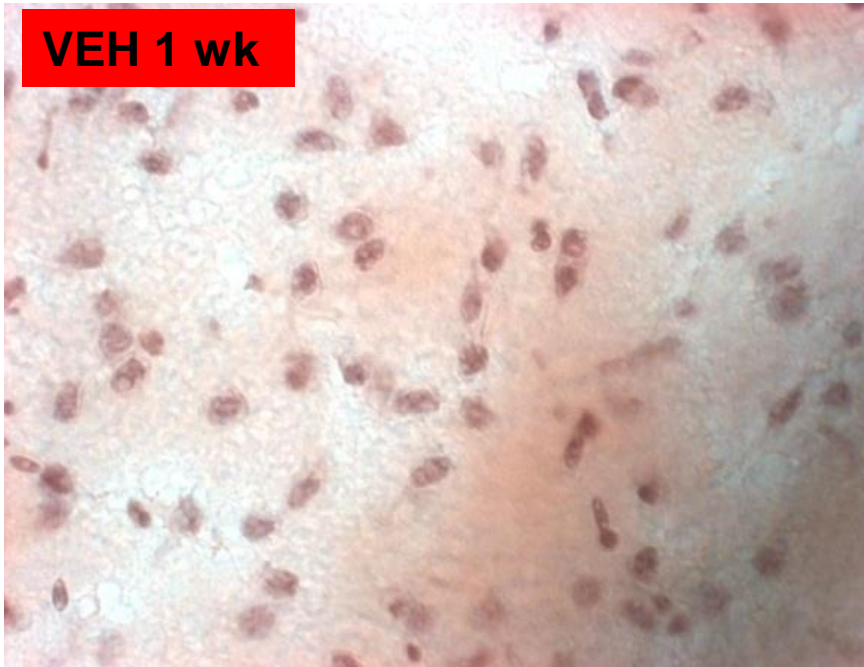






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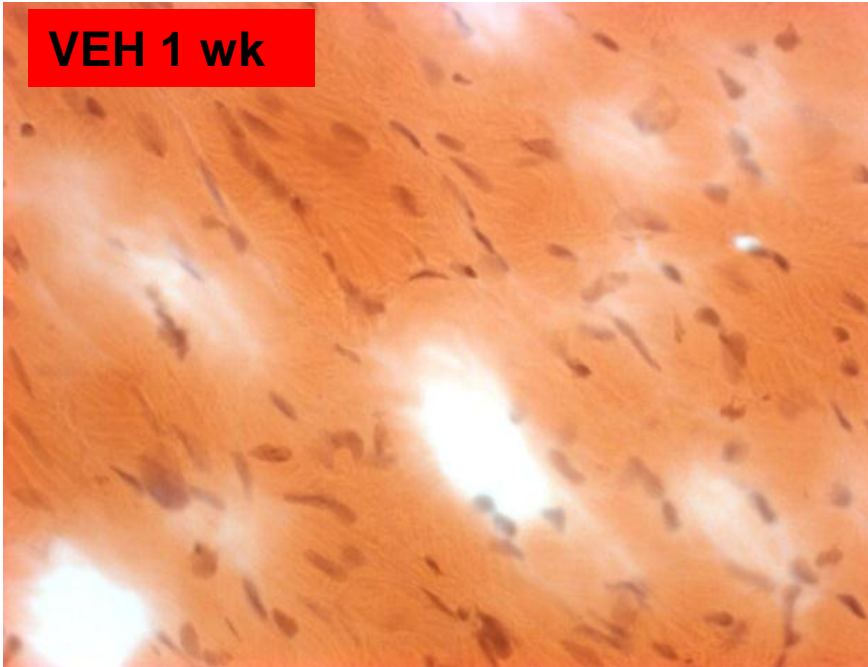
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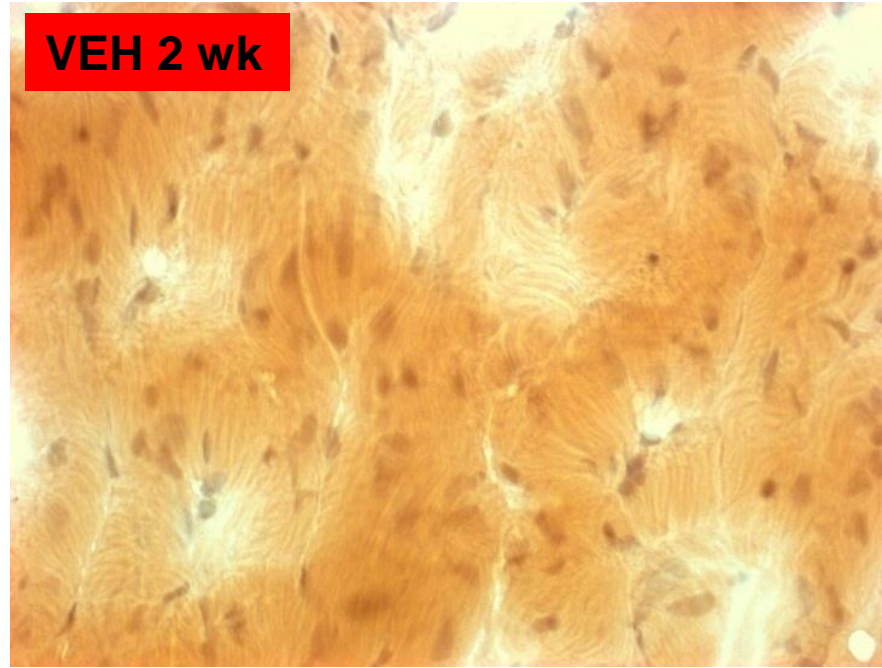
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heart

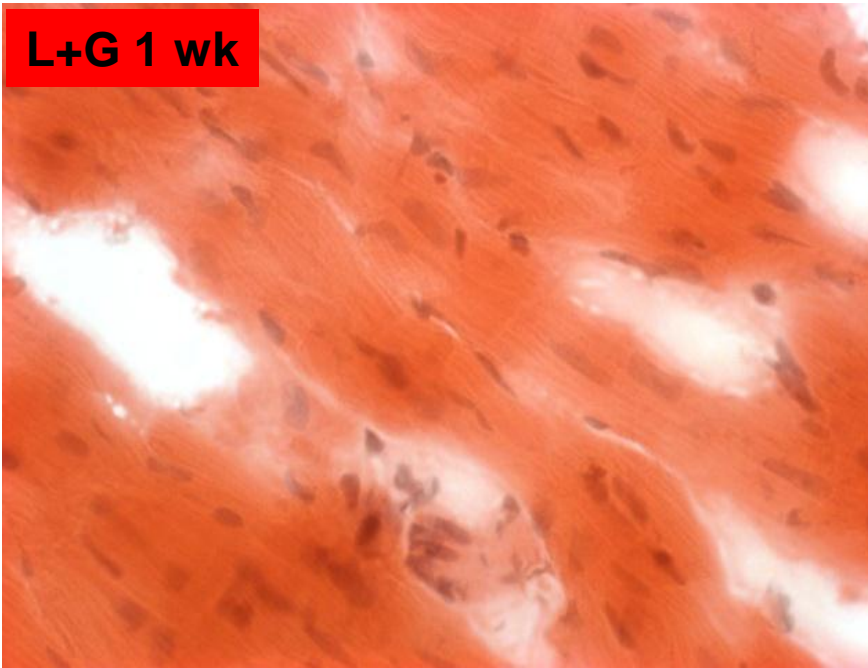
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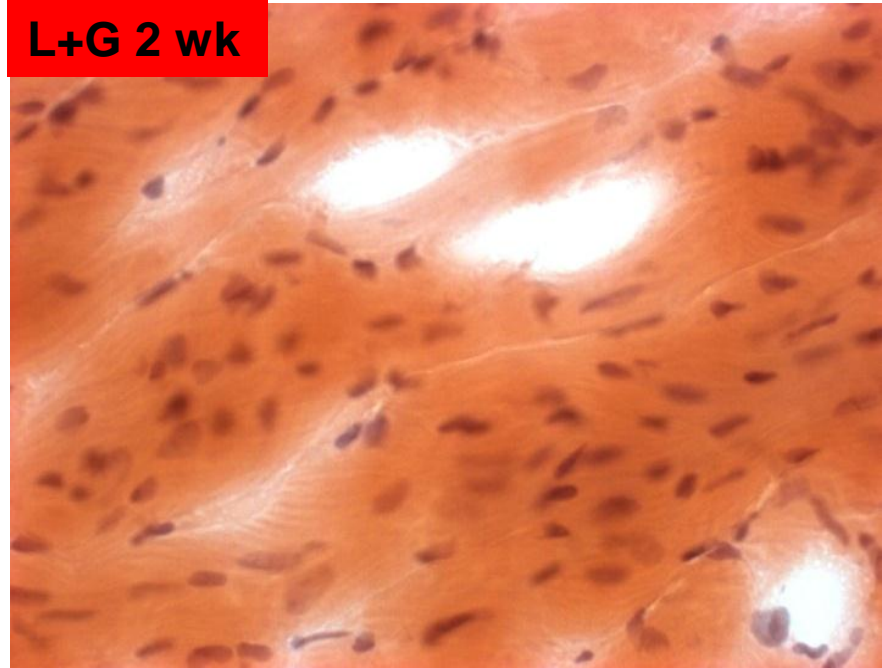
VEH 2 wk



L+G 1 wk

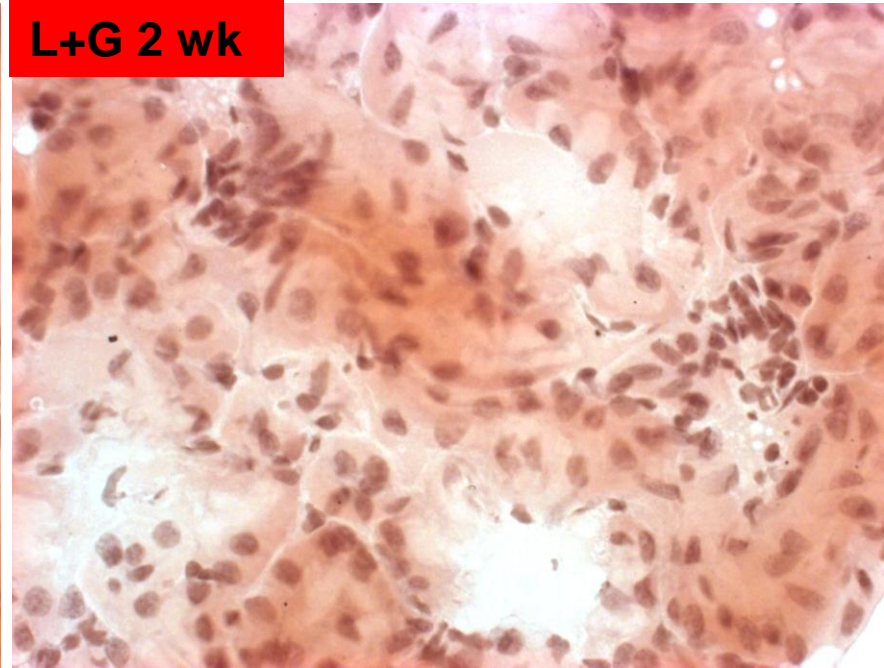
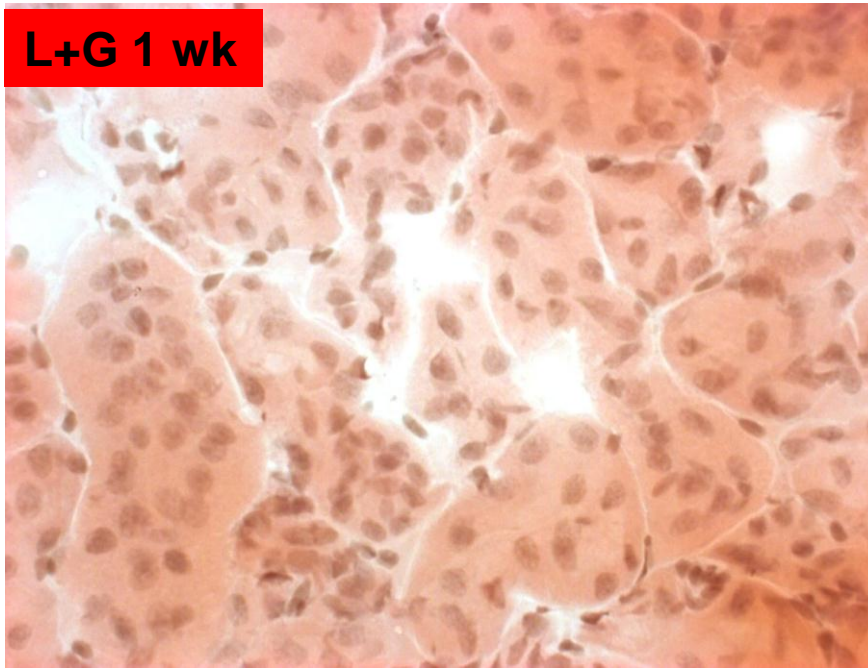
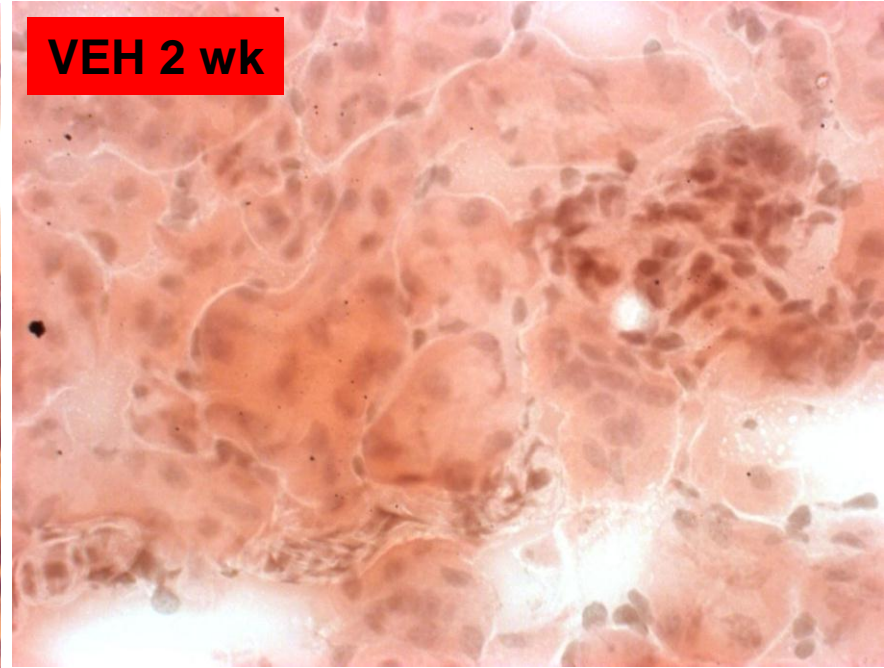
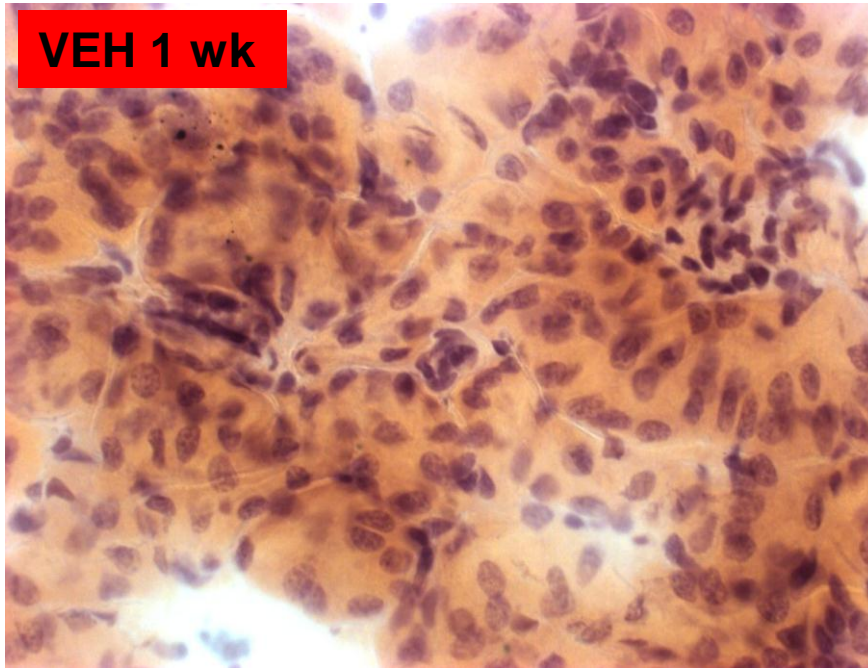


L+G 2 wk



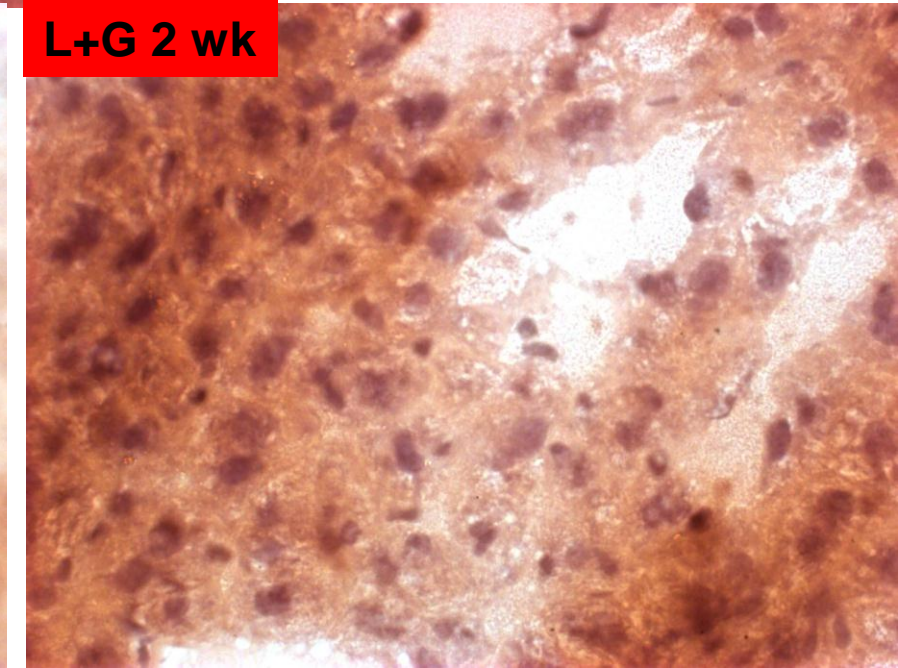
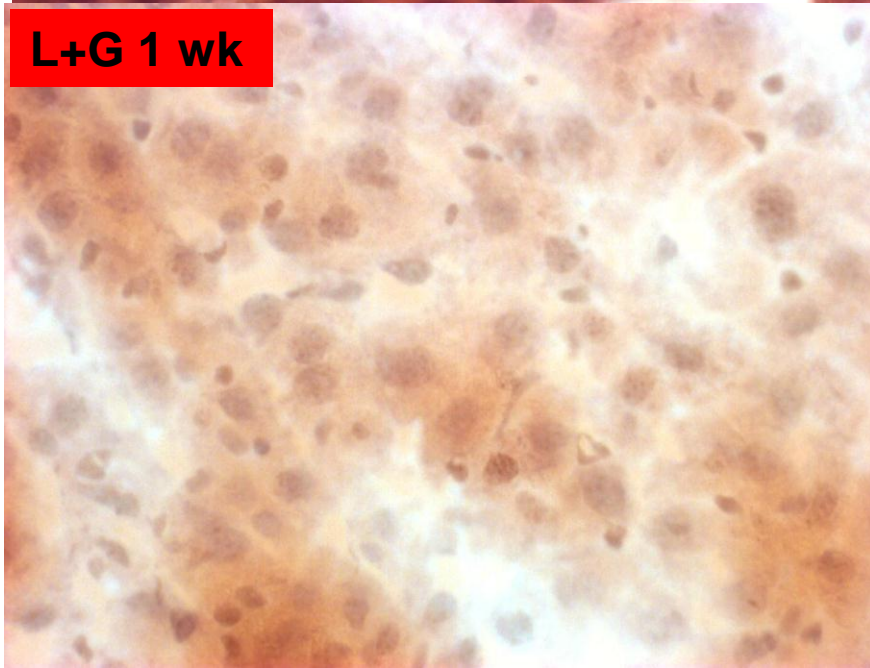
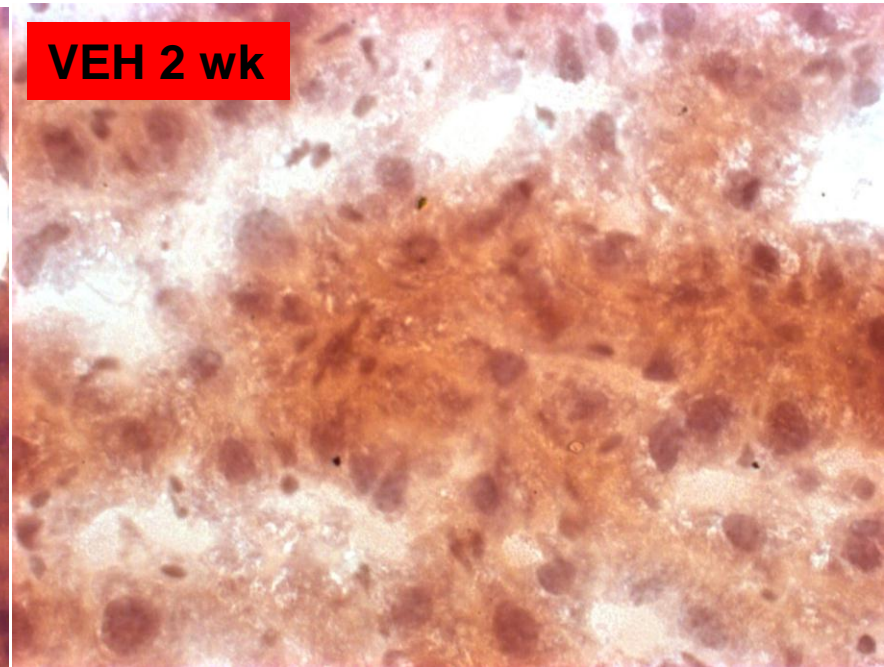
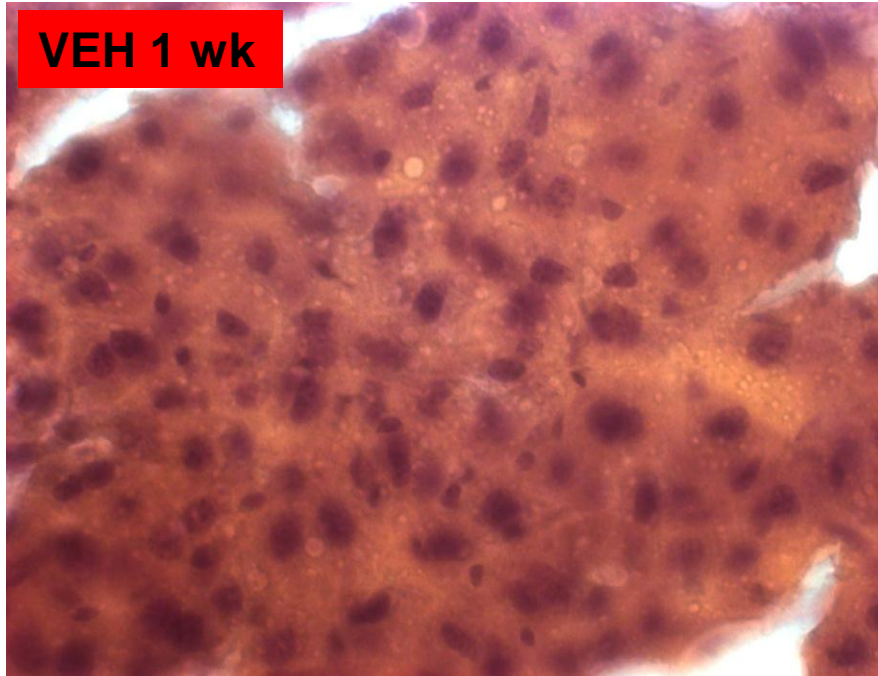
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kidney



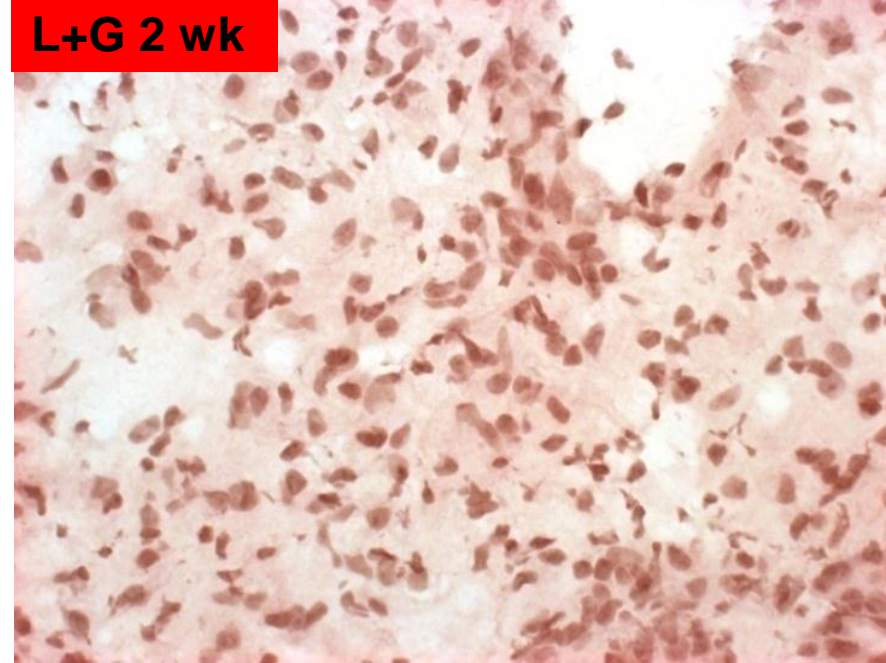
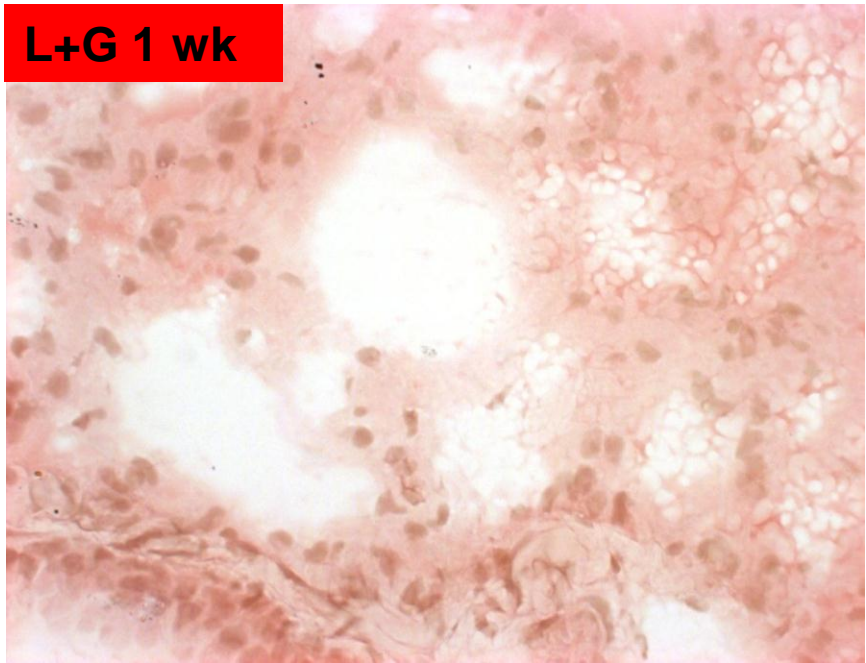
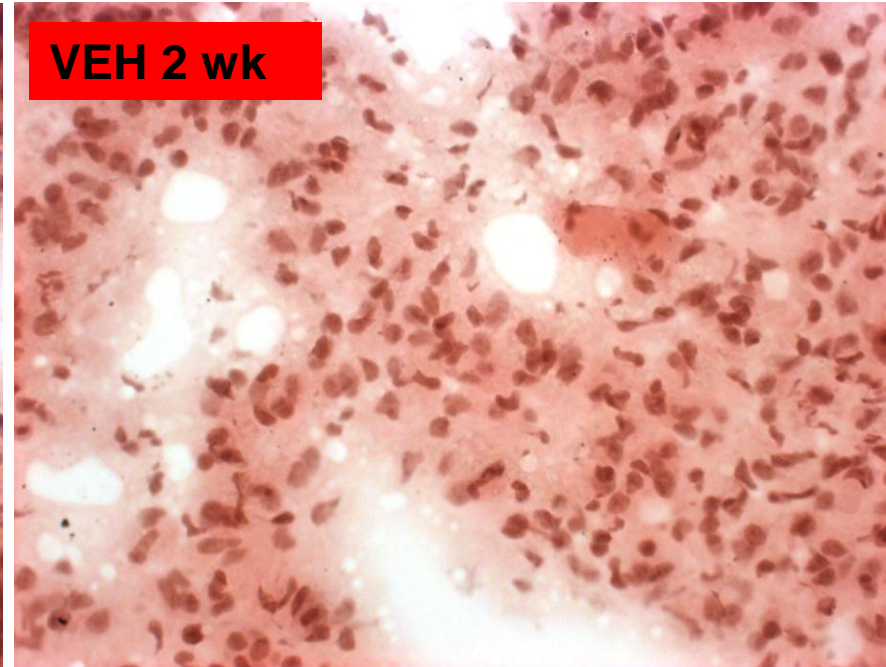
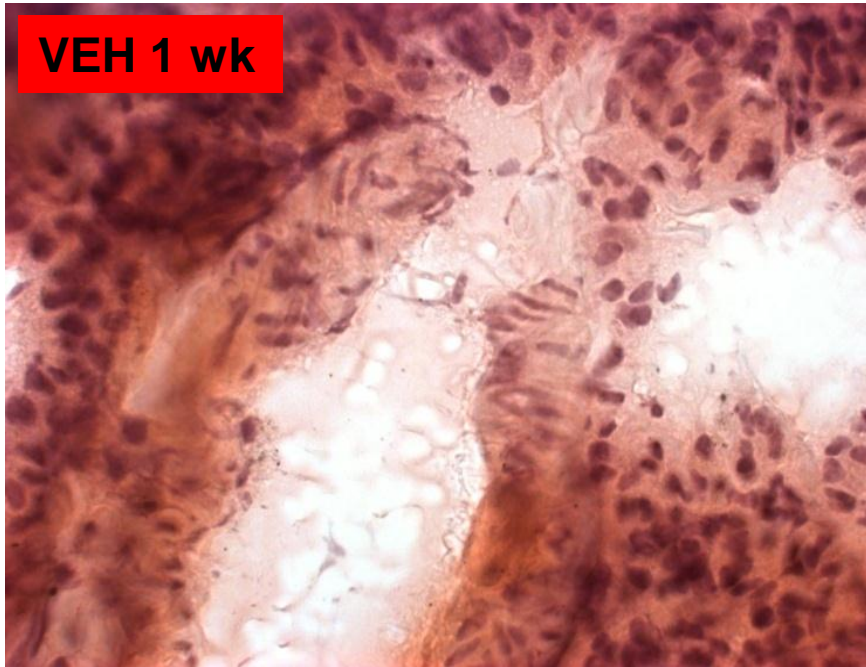
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liver



S10

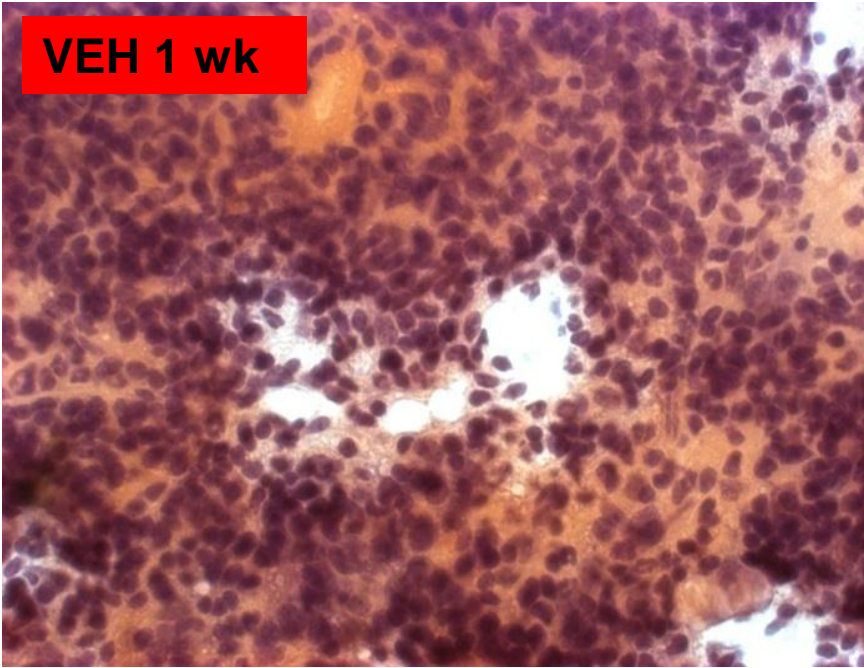
lung



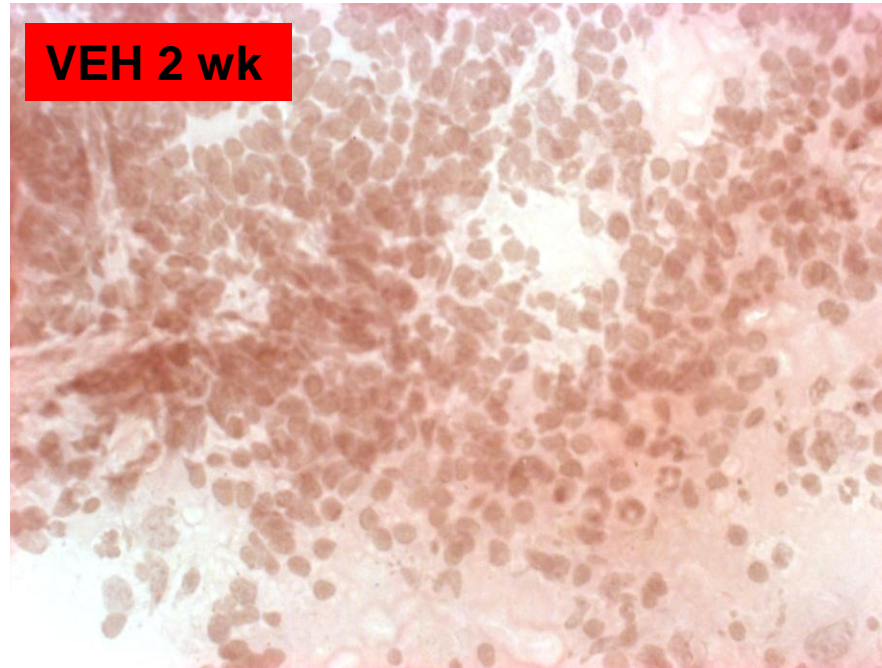
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spleen

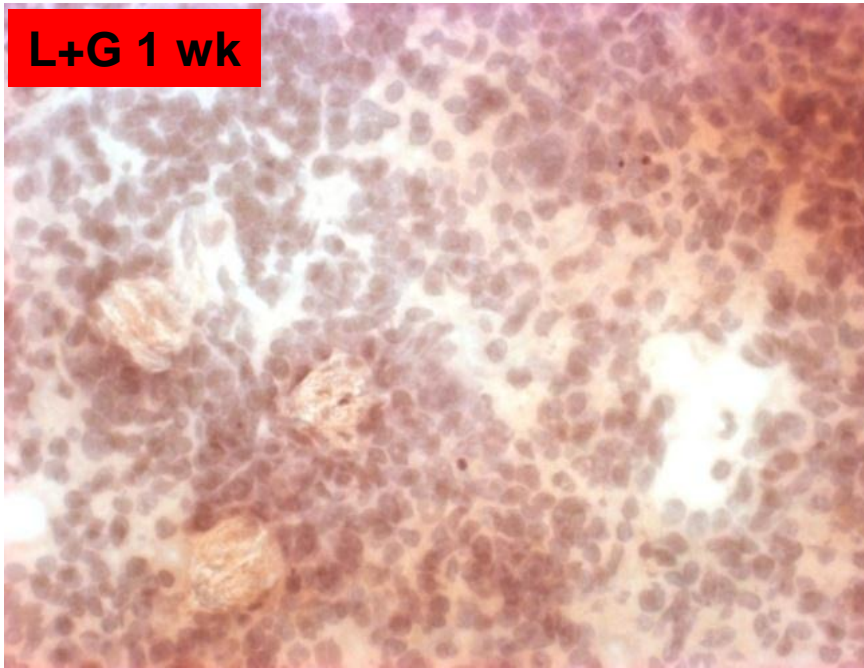
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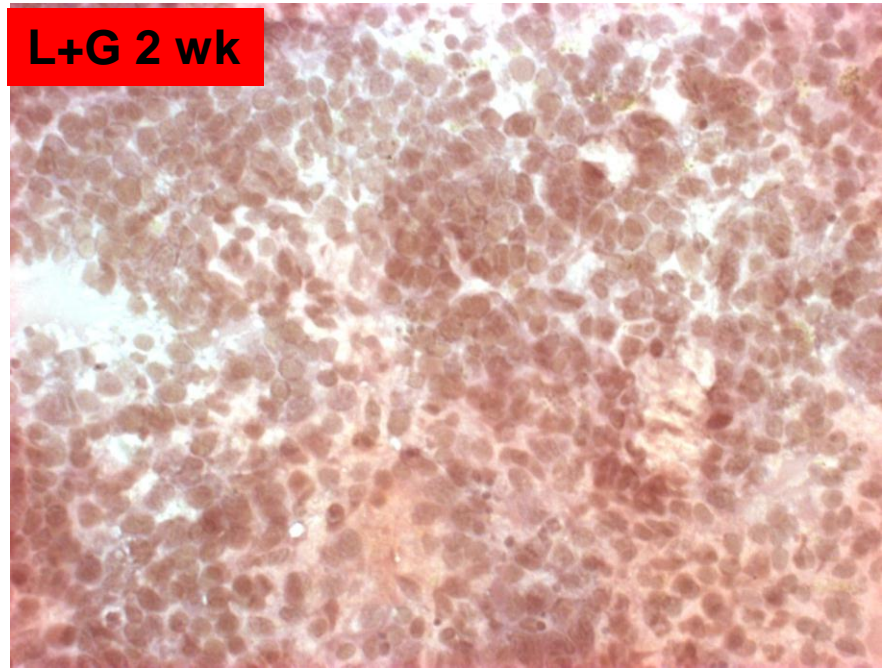
VEH 2 wk



L+G 1 wk



L+G 2 wk



S12

