Determination of the physiological and pathological roles of E2F3 in adult tissues

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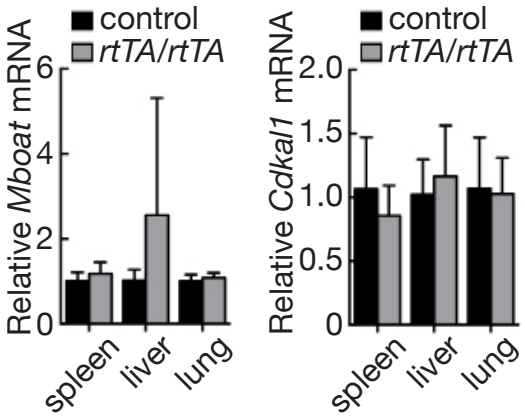
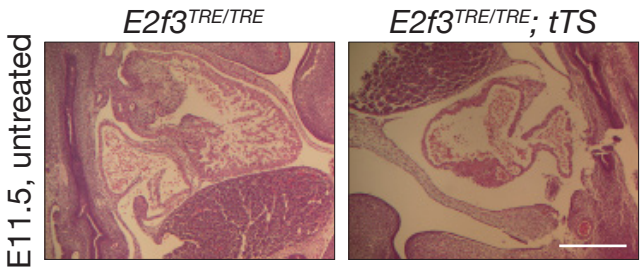
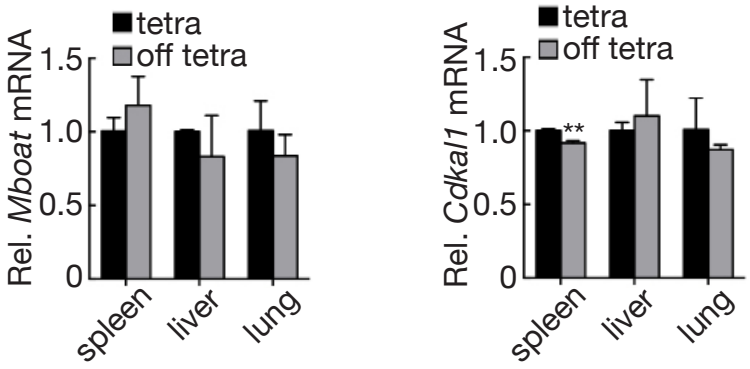
Supplementary Figures



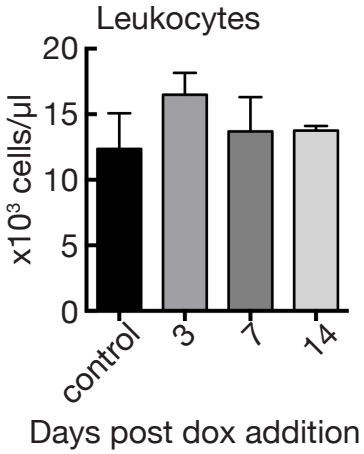
Supplementary Fig. 1| Expression of genes proximal to *E2f3* are unaffected by rtTA induced expression of *E2f3*.
Quantitative RT-PCR analysis of *Mboat* and *Cdkal1* in adult control (*n*=5 (spleen,lung), 4 (liver)) and $E2f3^{TRE/TRE};Rosa26^{rtTA/rtTA}$ (*n*=3) mouse tissues. Expression is normalized to *Actin* and relative to the mean of the control samples within each tissue. Error bars, s.e.m.



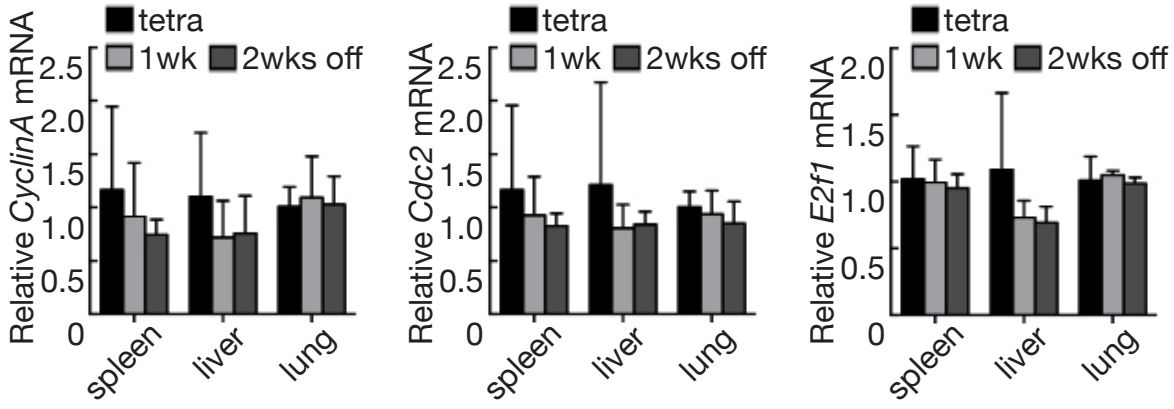
Supplementary Fig. 2| Repression of *E2f3* in $E2f3^{TRE/TRE}$;*β-actin-tTS* embryos results in reduced heart trabeculation.
Haematoxylin and eosin stained embryo hearts from $E2f3^{TRE/TRE}$;*β-actin-tTS* and $E2f3^{TRE/TRE}$ control 11.5 d.p.f. embryos developed in the absence of tetracycline.



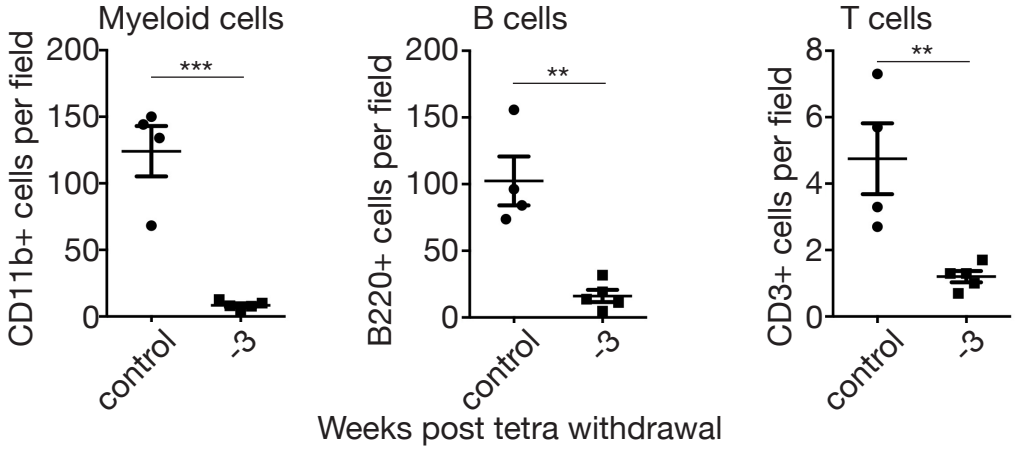
Supplementary Fig. 3| Expression of genes proximal to *E2f3* are unaffected by tTS-dependent repression of *E2f3*.
Quantitative RT-PCR analysis of *Mboat* and *Cdkal1* expression in *E2f3*$^{TRE/TRE}$*;β-actin-tTS* mouse tissues in the presence (n=2), or post removal of tetracycline for ≥1 week (n=3 (spleen, lung), 4 (liver)). Expression is normalized to *Actin* and relative to the mean of the control samples within each tissue. Error bars, s.e.m. Two-tailed t-test: tetra vs off tetra; *P*=0.009** (*Cdkal1*, spleen).



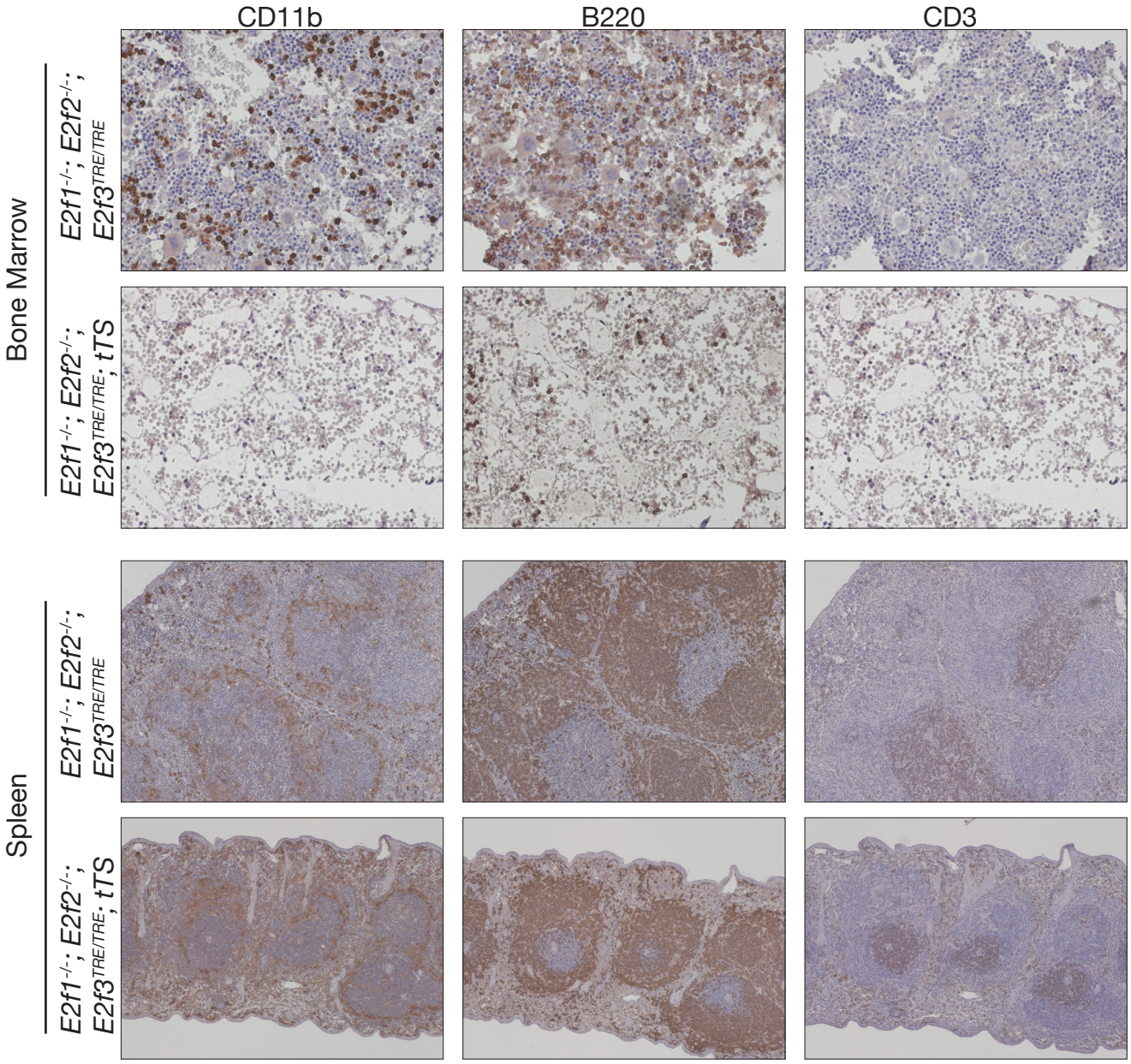
Supplementary Fig. 4| Peripheral leukocyte numbers trend towards a transient increase following rtTA induced expression of *E2f3*.
Leukocyte counts on peripheral blood isolated from control (*n*=2 three days post dox, *n*=2 seven days post dox) and *E2f3*$^{TRE/TRE}$*;Rosa26*$^{rtTA/rtTA}$ (*n*=3, three and seven days post dox; *n*=2, 14 days post dox) mice at the indicated number of days post addition of Doxcycline to the drinking water. Error bars, s.e.m. Two-tailed t-test: control vs 3 days; *P*=0.071.



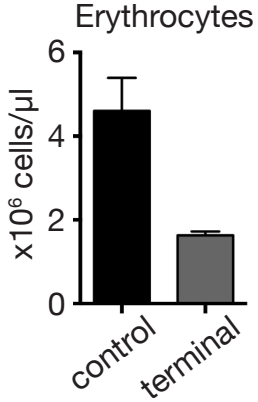
Supplementary Fig. 5| E2F target genes are unaffected by repression of *E2f3* in adult mouse tissues.
Quantitative RT-PCR analysis of *E2f1, Cdc2* and *CyclinA* expression in adult *E2f3*$^{TRE/TRE}$*;β-actin-tTS* mouse tissues maintained on tetracycline (tetra, *n*=3) and after removal from tetracycline for the times indicated (1wk, *n*=4; 2wks off, *n*=3). Expression is normalized to *HPRT* and relative to the mean of the control samples within each tissue. Error bars, s.e.m.



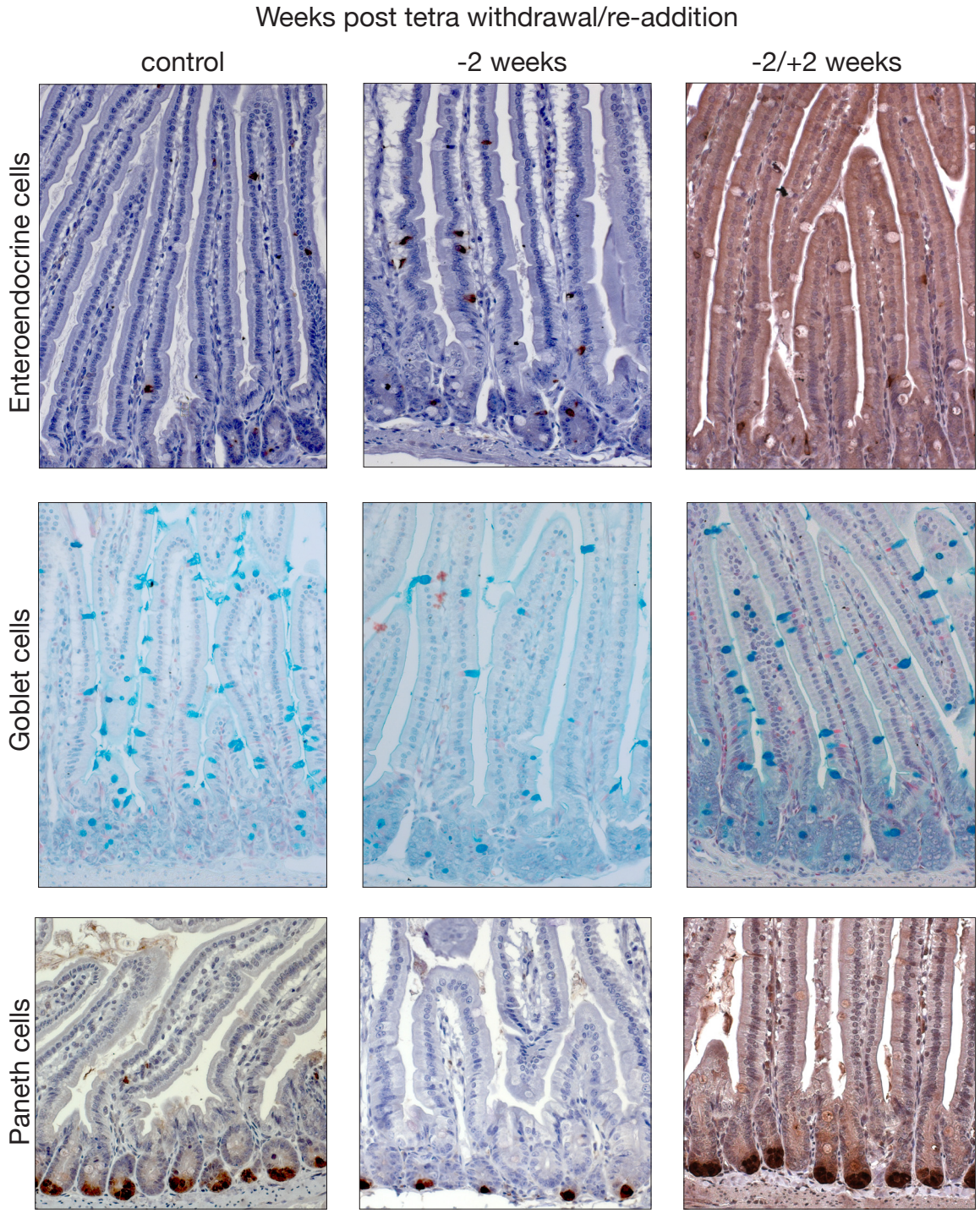
Supplementary Fig. 6| Repression of activator *E2fs* results in attrition of leukocytes in the bone marrow. Histogram of average numbers of CD11b (myeloid), B220 (B-cells) and CD3 (T-cells) positive cells from three fields of view in the bone marrow of *E2f1*$^{-/-}$*;E2f2*$^{-/-}$*;E2f3*$^{TRE/TRE}$ control (n=4) and *E2f1*$^{-/-}$*;E2f2*$^{-/-}$*;E2f3*$^{TRE/TRE}$*;β-actin-tTS* mice following withdrawal of 100 mg/L tetracycline for three weeks (-3, n=5). Two-tailed t-test: *P*=0.0002*** (myeloid), *P*=0.0014** (B cells), *P*=0.0076** (T cells).



Supplementary Fig. 7| Repression of activator *E2fs* results in attrition of leukocytes in the bone marrow and spleen. CD11b, B220 and CD3 stained images of the bone marrow and spleen for the indentification of myeloid, B and T cells respectively in *E2f1*$^{-/-}$;*E2f2*$^{-/-}$;*E2f3*$^{TRE/TRE}$ control and *E2f1*$^{-/-}$; *E2f2*$^{-/-}$;*E2f3*$^{TRE/TRE}$;*β-actin-tTS* mice 3 weeks after withdrawal of tetracycline. Representative images from n≥4 mice.

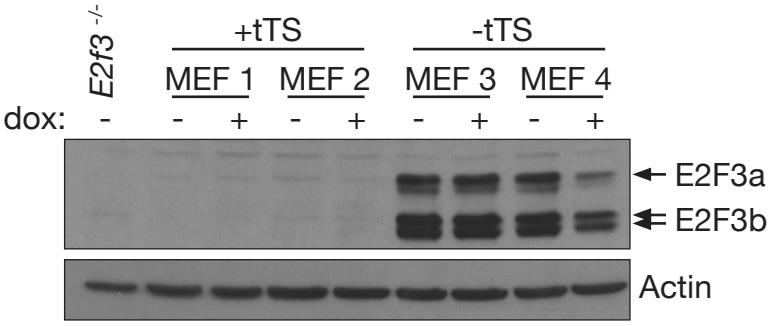


Supplementary Fig. 8| Repression of activator *E2fs* results in erythrocytopenia. Histogram of total numbers of erythrocytes in *E2f1*$^{-/-}$;*E2f2*$^{-/-}$;*E2f3*$^{TRE/TRE}$ control (n=8) and *E2f1*$^{-/-}$;*E2f2*$^{-/-}$;*E2f3*$^{TRE/TRE}$;*β-actin-tTS* mice following withdrawal of 100 mg/L tetracycline until mice presented as moribund (terminal, n=2).



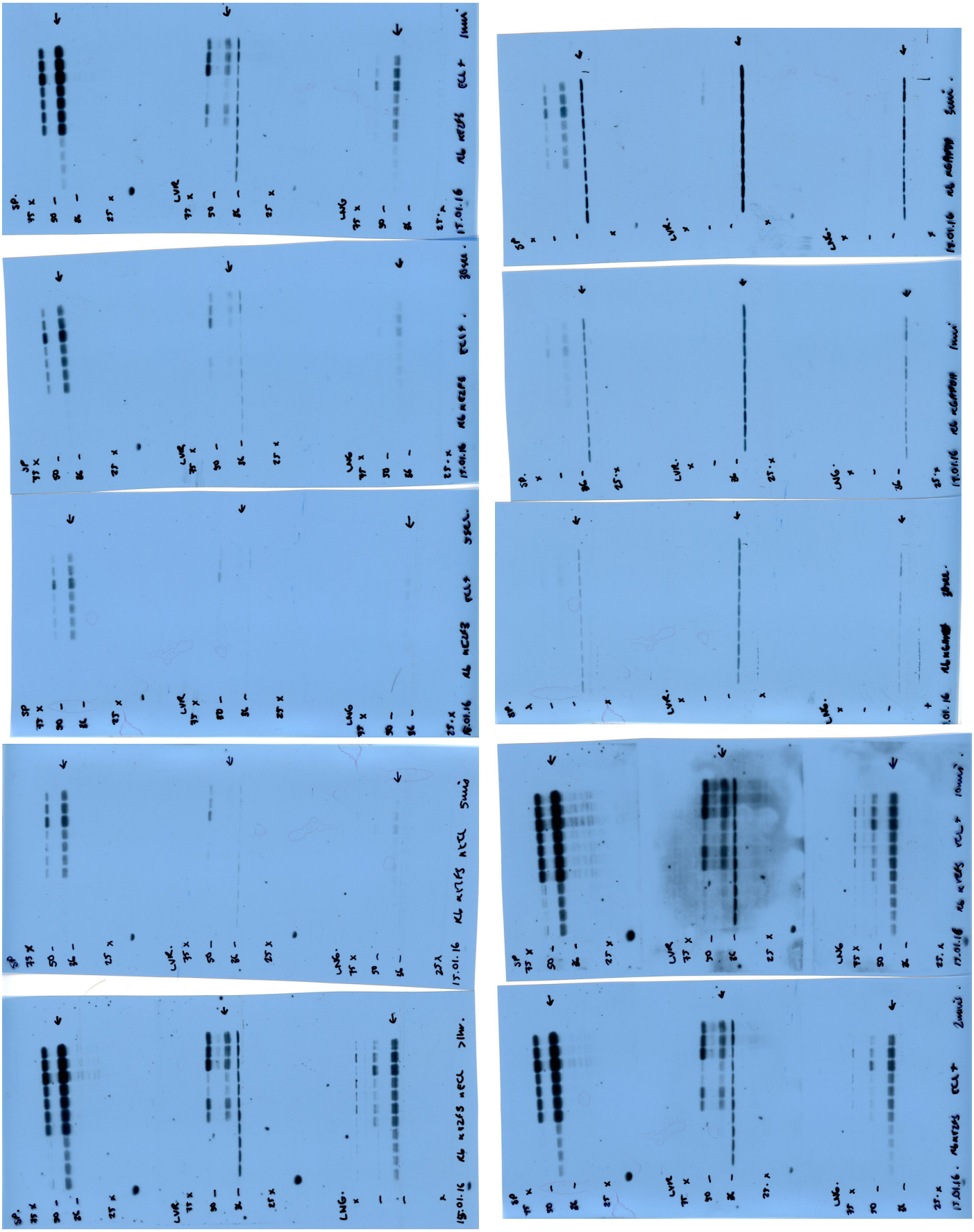
Supplementary Fig. 9| Repression of *E2f3* in the absence of *E2f1* and *E2f2* results in altered proportions of differentiated cell types in the intestinal epithelium that can be rescued with the restoration of *E2f3* expression.

Chromogranin A, Alcian blue and lysozyme stained images of the intestinal epitelium for the identification of Enteroendocrine, Goblet and Paneth cells respectively in *E2f1*$^{-/-}$;*E2f2*$^{-/-}$;*E2f3*$^{TRE/TRE}$ control and *E2f1*$^{-/-}$; *E2f2*$^{-/-}$;*E2f3*$^{TRE/TRE}$;*β-actin-tTS* mice 2 weeks after withdrawal of tetracycline and 2 weeks post re-addition. Representative images from n≥2 mice.

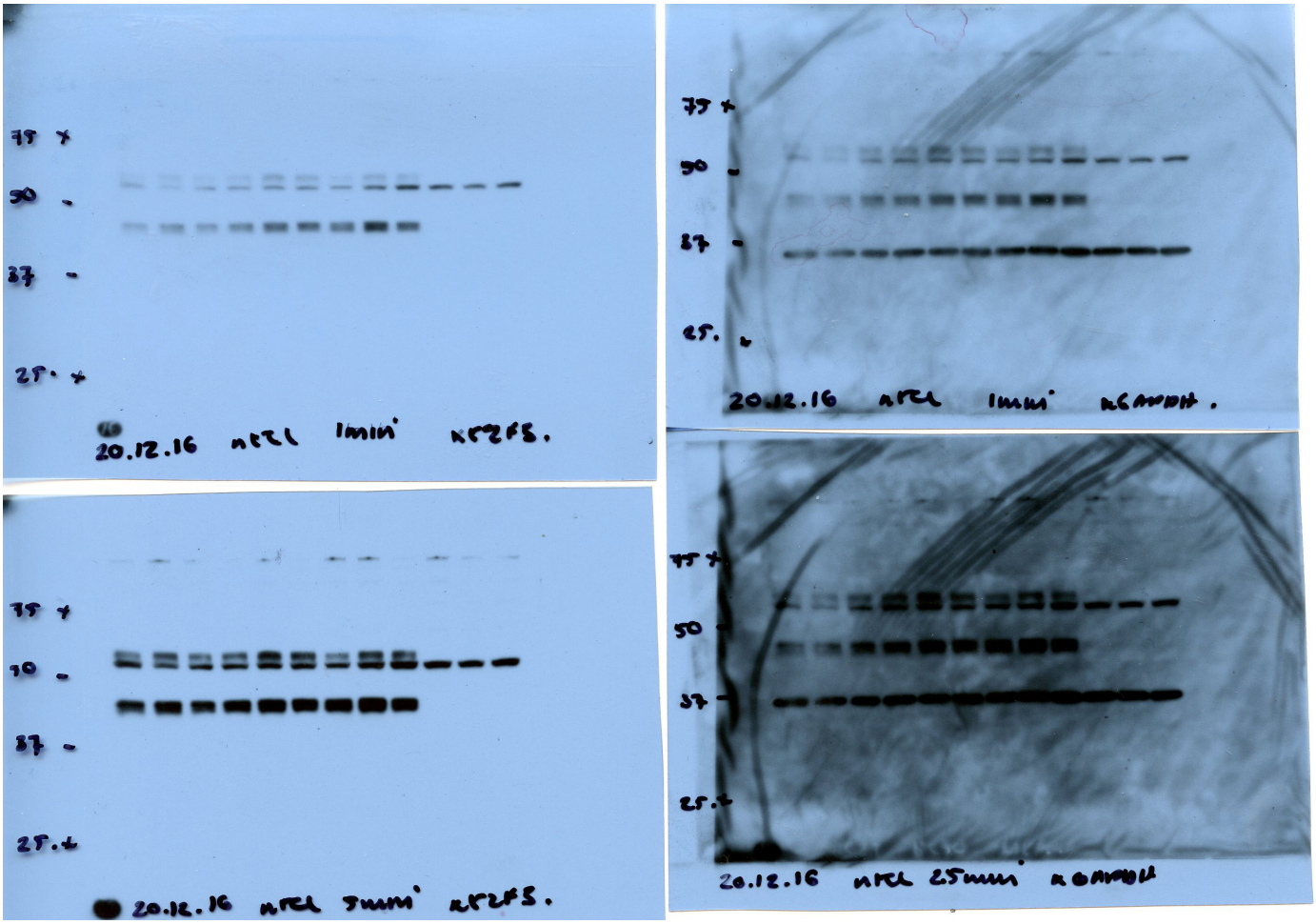


Supplementary Fig. 10| Repression of *E2f3* is irreversible when tTS is active during embryonic development.

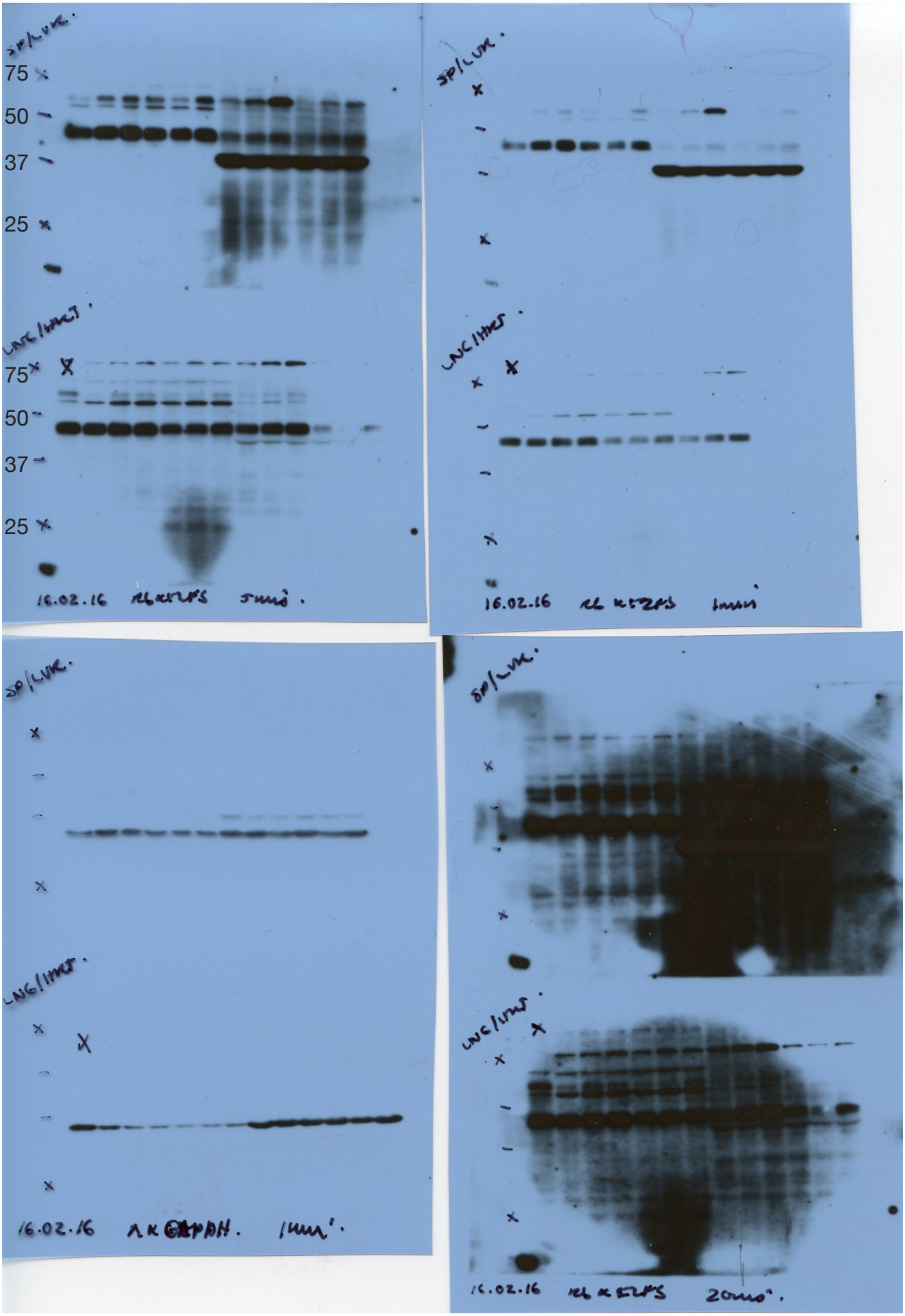
Western blot analysis of E2F3 protein levels in *E2f3*$^{TRE/TRE}$ (-tTS) and *E2f3*$^{TRE/TRE}$*; βactin-tTS* (+tTS) cultured mouse embryonic fibroblasts (MEFs), generated in the absence of doxycycline. MEFs were then grown in culture with (+) or without doxycycline (-), for at least two passages, as indicated. Replicate samples represent cultures derived from independently isolated embryos. Expression of Actin is included as a loading control.



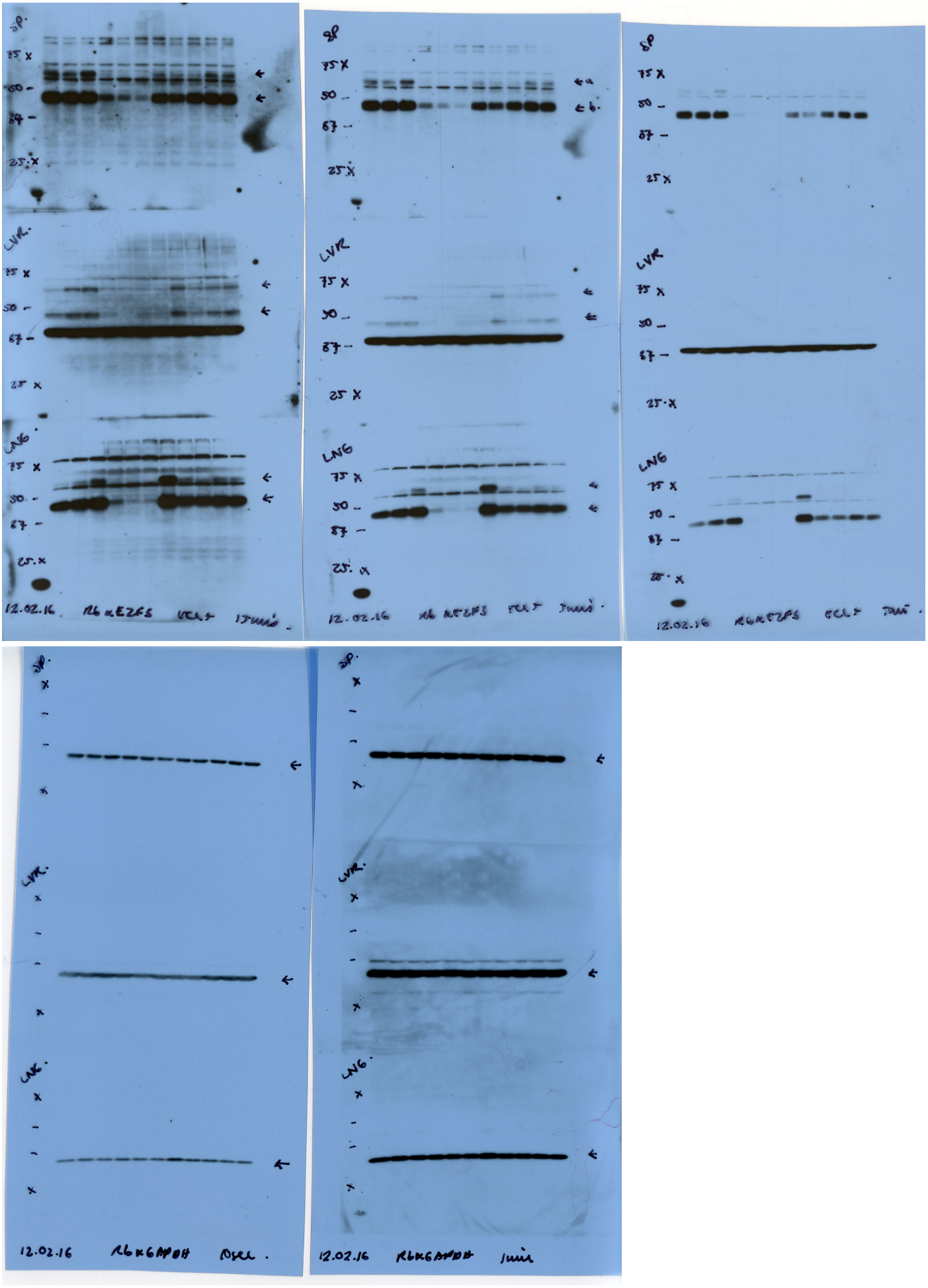
Supplementary Fig. 11| Uncropped images of blots represented in Figure 2c.
Multiple exposures also included.



Supplementary Fig. 12| Uncropped images of blots represented in Figure 3c.
Multiple exposures also included.



Supplementary Fig. 13| Uncropped images of blots represented in Figure 3d.
Multiple exposures also included. “X” on the first sample on LNG blot denotes a mis-loaded sample.



Supplementary Fig. 14| Uncropped images of blots represented in Figure 3f.
Multiple exposures also included.