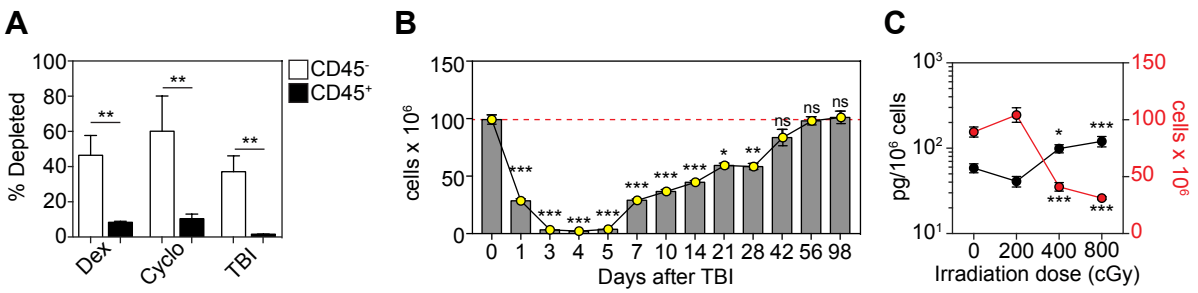
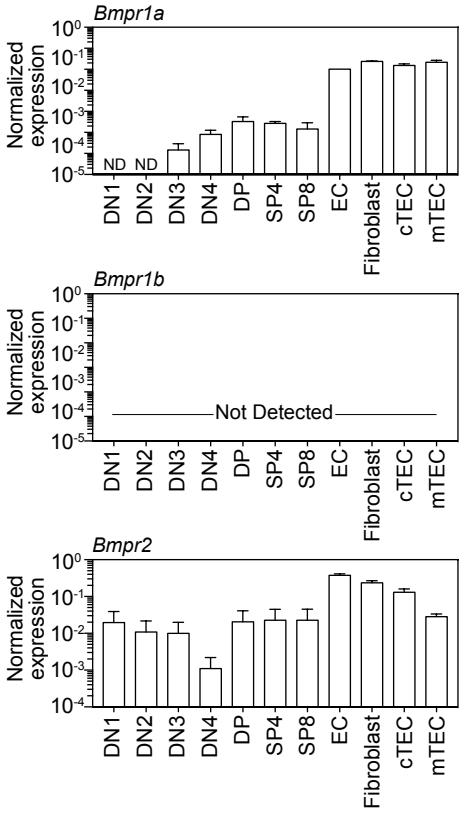


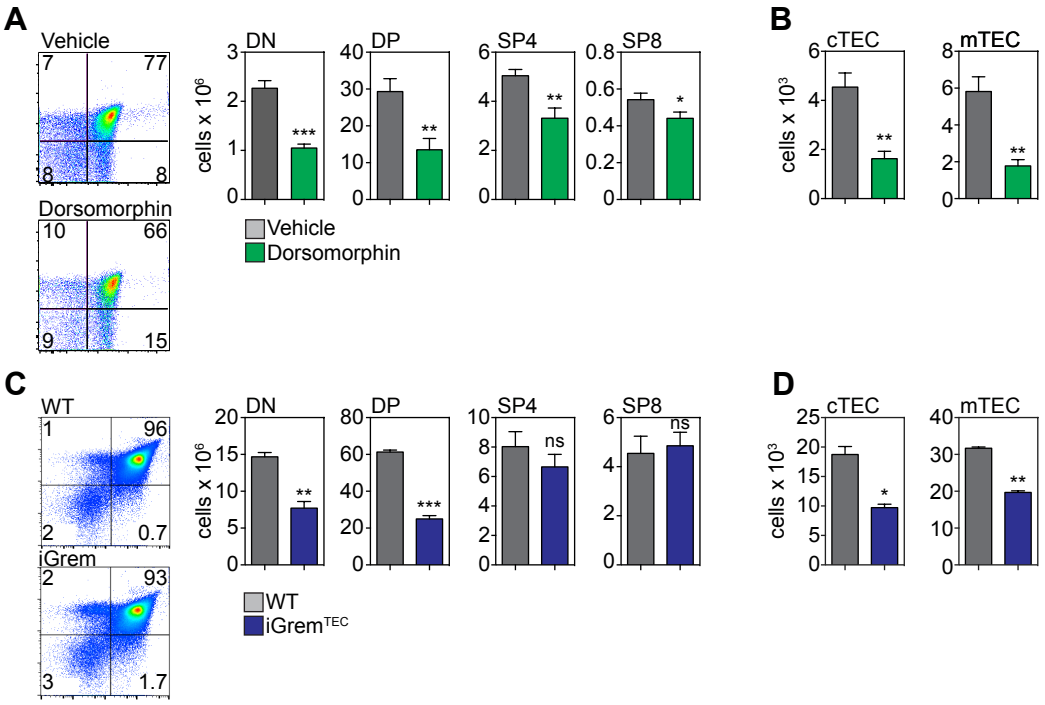
Supplementary Fig. 1: Thymic response to damage

(**A**) 6 week old female C57BL/6 mice were treated with PBS (n=10), Dexamethasone (Dex, 50mg/kg ip on day 0, n=10), cyclophosphamide (Cyclo, 100mg/kg/day ip on days -1 and 0, n=10) or SL-TBI (550 cGy on day 0, n=10). On day 4 absolute number of $CD45^+$ and $CD45^-$ cells were calculated and compared to untreated controls. (**B**) 6 week old female C57BL/6 mice were treated with SL-TBI (550 cGy on day 0) and thymic cellularity was measured at the indicated timepoints (n=5-16). (**C**) Graph of total thymus cellularity (right axis) and BMP4 measured by ELISA seven days after targeted thymic-irradiation (200, 400 or 800 cGy). Graphs represent combined mean ± SEM of at least 2 independent experiments. *, $p<0.05$; **, $p<0.01$, ***, $p<0.001$.



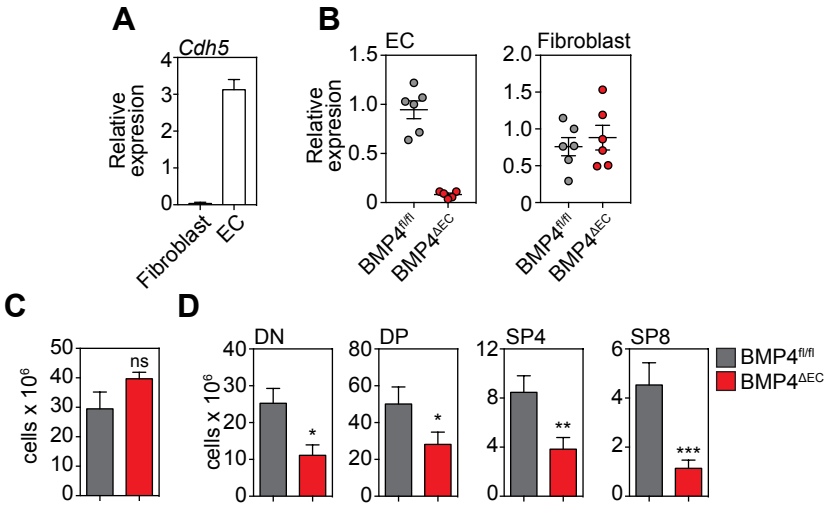
Supplementary Fig. 2: Distribution of BMP receptor subunits on thymic cell populations

(**A**) DN1 ($CD3^-CD4^-CD8^-CD44^+CD25^-$), DN2 ($CD3^-CD4^-CD8^-CD44^+CD25^+$), DN3 ($CD3^-CD4^-CD8^-CD44^-CD25^+$), DN4 ($CD3^-CD4^-CD8^-CD44^-CD25^-$), DP ($CD4^+CD8^+$), SP4 ($CD3^+CD8^-CD4^+$), SP8 ($CD3^+CD4^-CD8^+$), DCs ($CD11c^+MHCII^+$), fibroblasts ($CD45^-EpCAM^-MHCII^-CD31^-$VE-Cadherin$^-$ PDGFRα^+), ECs ($CD45^-EpCAM^-MHCII^-CD31^+$VE-Cadherin$^+$), cTECs ($CD45^-$ $EpCAM^+MHCII^+Ly51^{hi}$UEA-1^{lo}) and mTECs ($CD45^-EpCAM^+MHCII^+Ly51^{lo}$UEA-$1^{hi}$) were FACS purified from untreated 6 week-old female C57BL/6 mice and expression of the BMPR subunits *Bmpr1a, Bmpr1b,* and *Bmpr2* was measured by qPCR.



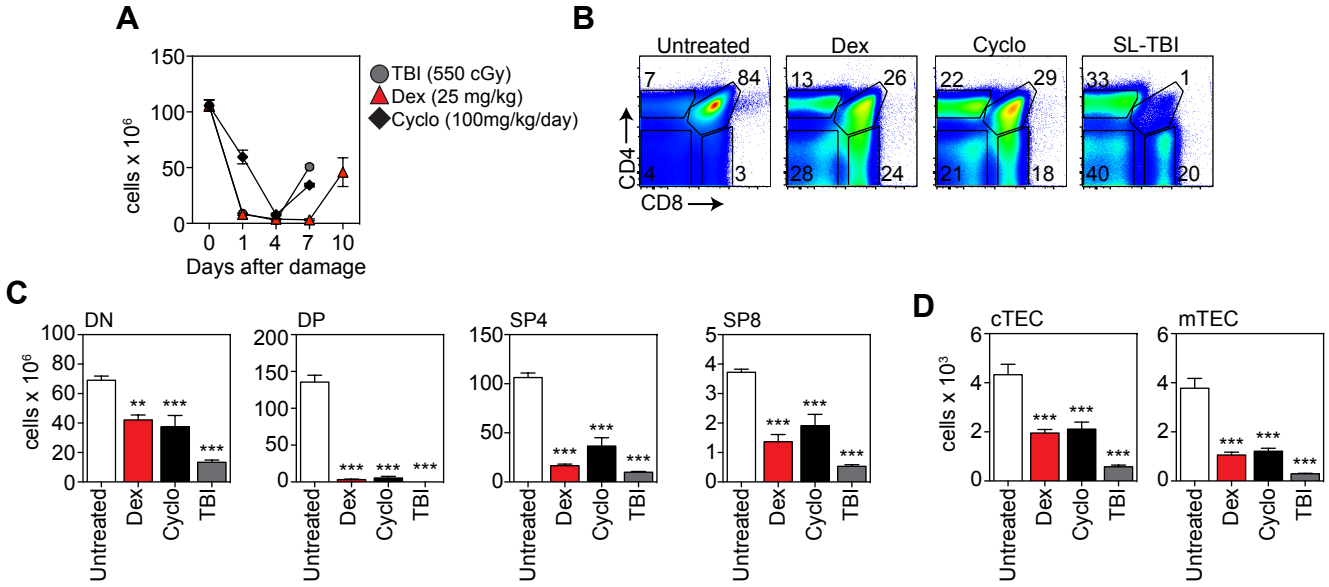
Supplementary Fig. 3: Inhibition of BMP signaling abrogates endogenous thymic regeneration

(**A-B**) 6 wo C57BL/6 mice were administered with the BMP type I receptor inhibitor Dorsomorphin dihydrochloride (12.5mg/kg) i.p. at day -1 before SL-TBI (550cGy single dose) and twice daily after SL-TBI. Thymus was harvested on day 7 and absolute number of (**A**) DN, DP, SP4, and SP8 as well as (**B**) cTEC and mTEC was assessed by flow cytometry. (**C-D**) iGremlin::K5-CreER ($Grem^{iTEC}$) mice were generated and tamoxifen was administered on days -1, 0 and +1 surrounding SL-TBI (550cGy). Thymus was harvested on day 9 and absolute number of (**c**) DN, DP, SP4, and SP8 as well as (**D**) cTEC and mTEC was assessed by flow cytometry. *, $p<0.05$; **, $p<0.01$, ***, $p<0.001$.



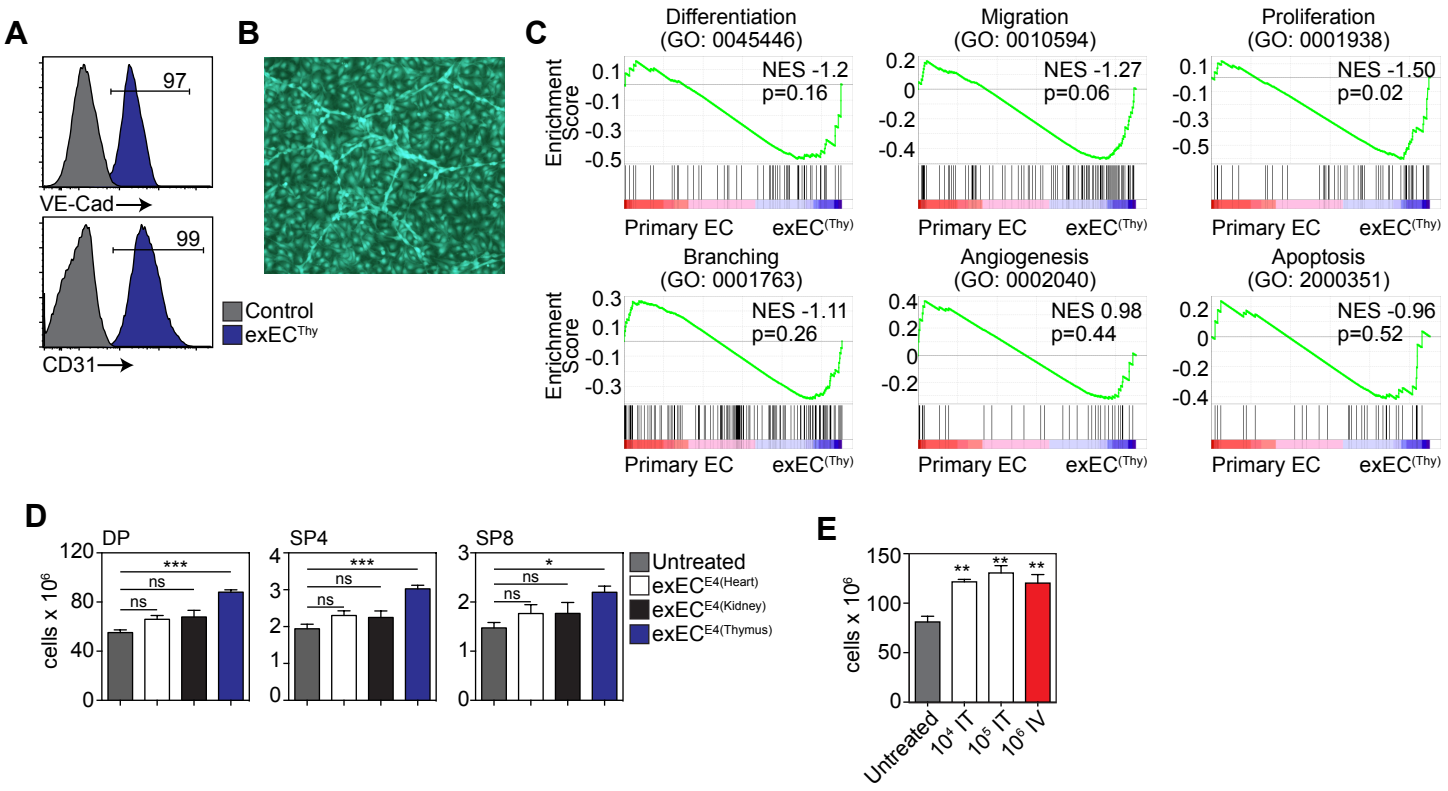
Supplementary Fig. 4: Deletion of BMP4 in ECs abrogates endogenous thymic regeneration

(**A**) Fibroblasts or ECs were FACS purified from from untreated 6 week-old female C57BL/6 mice and expression of *Cdh5* (VE-Cadherin) was measured by qPCR. (**B-D**) $BMP4^{fl/fl}$ mice were crossed with Cdh5-CreERT2 mice. (**B-C**) Tamoxifen was administered over 5 days to induce deletion of BMP4 in ECs. (**B**) Expression of *Bmp4* was assessed in FACS purified ECs and fibroblasts. (**C**) Total thymus cellularity. (**D**) Tamoxifen was administered on days -2, -1, 0, 1 and 2 surrounding SL-TBI (550cGy). Thymus was harvested on day 7 and absolute number of (DN, DP, SP4, and SP8 were assessed by flow cytometry. Graphs represent combined mean ± SEM. *, $p<0.05$; **, $p<0.01$, ***, $p<0.001$.



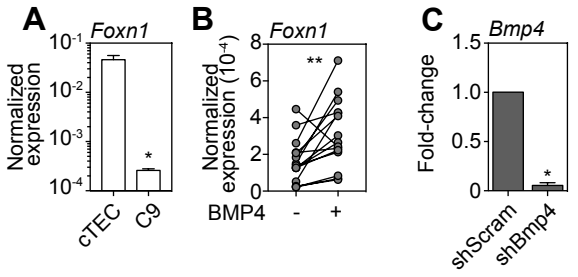
Supplementary Fig. 5: Damage response in the thymus to corticosteroids, chemotherapy and TBI

6 week old female C57BL/6 mice were treated with PBS (n=10), Dexamethasone (Dex, 50mg/kg ip on day 0, n=10), cyclophosphamide (Cyclo, 100mg/kg/day ip on days -1 and 0, n=10) or SL-TBI (550 cGy on day 0, n=10). On day 4, mice were perfused with 25µg anti-VE-cadherin antibody (BV13) conjugated to Alexa 647 and thymus harvested. (**A**) Total thymic cellularity at day 1, 4, 7, 10, and 14. (**B**) Concatenated flow cytometric plots detailing the proportion of thymocyte subsets. (**C-D**) Absolute number of (**C**) DN, DP, SP4, and SP8 as well as (**D**) cTEC and mTEC were assessed by flow cytometry. Graphs represent combined mean ± SEM. *, p<0.05; **, p<0.01, ***, p<0.001.



Supplementary Fig. 6: exECs can be propagated ex vivo and maintain an EC phenotype

ECs were FACS sorted from the thymus, heart or kidney based on expression of VE-cadherin and transduced with the viral gene E4ORF1. (**A**) Expression of VE-Cadherin and CD31 on exECs derived from thymus. (**B**) Phase contrast image of exECs derived from thymus. (**C**) Microarray analysis was performed on freshly isolated thymic ECs and on exEC$^{(Thymus)}$. GSEA analysis was performed looking signatures for EC Differentiation (GO: 0045446), EC Migration (GO: 0010594), EC Proliferation (GO: 0001938), EC Branching (0001763), Angiogenesis (GO: 0002040), and EC Apoptosis (2000351). (**D**) In order to model immune injury we exposed 6-8 weeks old C57/B6 mice to a single dose of sublethal TBI (550cGy) and 1 x 10^6 exEC were administered iv at day 3 after SL-TBI (n=10-15/group). Thymus was harvested on day 9 and absolute number of DP, SP4, and SP8 were assessed by flow cytometry. (**E**) On day 3 after SL-TBI, exEC$^{(Thymus)}$ were transplanted into mice either IV (3 x 10^6 cells), or via ultrasound guided intrathymic injection (3 x 10^4 or 3 x 10^5 cells). Total thymus cellularity was measured on day 9. Graphs represent combined mean ± SEM. *, p<0.05; **, p<0.01, ***, p<0.001.



Supplementary Fig. 7: Validating methods of inducing *Foxn1* and silencing *Bmp4*

(**A**) Expression of Foxn1 was measured by qPCR in freshly isolated FACS purified cTECs or the C9 (cTEC) cell line. (**B**) C9 cells were incubated for 24 hours with recombinant BMP4 (100ng/ml) and *Foxn1* expression was measured by qPCR. (**C**) Expression of *Bmp4*, measured by qPCR in thymus-derived exEC transduced to express either a *Bmp4* shRNA (shBMP4) or scrambled (shScram) control.