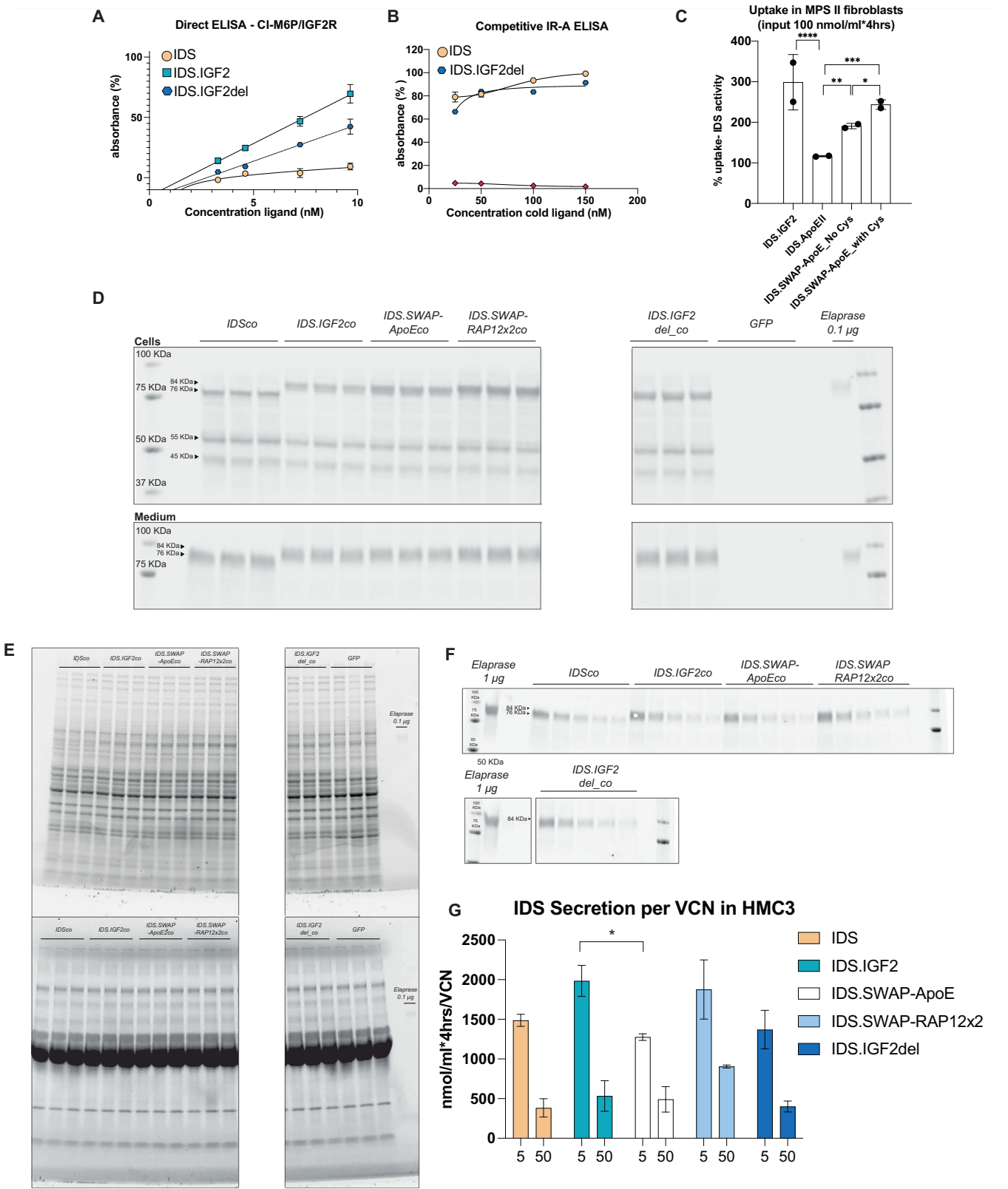


Expanded View Figures

Figure EV1. IDS.SWAP processing and secretion.

(A, B) Binding of IDS.IGF2del to CI-M6P/IGF2R and IR-A was assessed using two methods: a direct CI-M6P/IGF2R ELISA (A) and a competitive IR-A ELISA using biotinylated insulin (B). (C) Cellular uptake test of SWAP variants containing an ApoE insert into MPS II fibroblasts (24-h uptake). IDS.IGF2 and IDS.ApoEII refer to versions of the IDS protein tagged at the C-terminus with either IGF2 or ApoEII (tandem repeat of ApoE) tags via a flexible linker, as previously described (Gleitz et al, 2018; Catalano et al, 2023). IDS.SWAP-ApoE_No Cys refers to a SWAP version without a cysteine residue at each end of the insertion. IDS.SWAP-ApoE_with Cys refers to a SWAP version with a cysteine residue at each end of the insertion. The adjusted *P* values were as follows: IDS.IGF2 vs. IDS.ApoEII $P = <0.0001$, IDS.ApoEII vs. IDS.SWAP-ApoE_No Cys $P = 0.0085$, IDS.ApoEII vs. IDS.SWAP-ApoE_with Cys $P = 0.0004$, IDS.SWAP-ApoE_No Cys vs. IDS.SWAP-ApoE_with Cys $P = 0.0395$. (D) Immunoblot analysis of IDS proteins using an anti-IDS antibody. The upper panel shows SDS-PAGE analysis of the cell lysate of HMC3 cells 10 days after transduction at MOI 13.5 with lentiviral vectors encoding the indicated fusion proteins. The lower panel shows SDS-PAGE analysis of the medium supernatant of HMC3 transduced as above. Both upper and lower panels were separated to eliminate an irrelevant condition. Medium was refreshed 24 h before sample collection. HMC3 cells transduced with a GFP vector and 0.1 μ g of Elaprase served as negative and positive controls, respectively, during SDS-PAGE analysis. (E) Protein load for the western blot in Fig. EV1D and relative quantification (shown in Fig. 1C). Bottom panel was separated to eliminate an irrelevant condition. The stain free signal was quantified from the same gel used for immunoblot analysis to determine the total protein load. (F) Immunoblot analysis of IDS protein in five 2-fold dilutions of medium supernatant from HMC3 transduced with the indicated lentiviral vectors. 1 μ g of Elaprase served as control. This was used to measure specific IDS activity in Fig. 1E. Lower panel was separated to eliminate an irrelevant condition. (G) Secreted IDS protein activity per VCN after transduction of HMC3 cells at MOI 5 and MOI 50. The adjusted *p* value for MOI 5 IDS.IGF2 vs. IDS.SWAP-ApoE was 0.0283. Data information: data are presented as means $\pm$ SD. In (C), a total of 8 conditions were analyzed by one-way ANOVA; significant pairwise differences among the 4 relevant conditions are reported. In (G) data were analyzed by two-way ANOVA followed by Bonferroni's multiple testing correction. In (A–C) $n = 2$. In (G) $n = 3$. * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$; **** $P \leq 0.0001$. Significant comparisons are indicated by brackets.



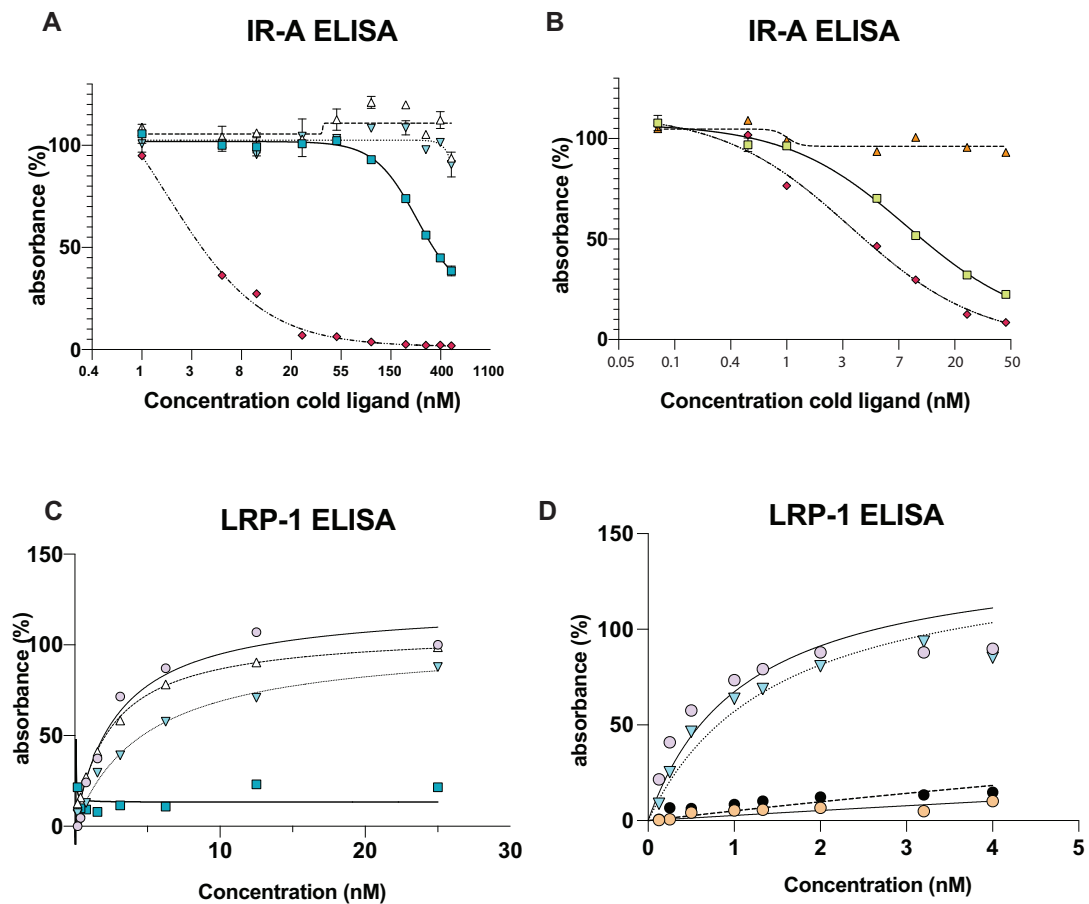
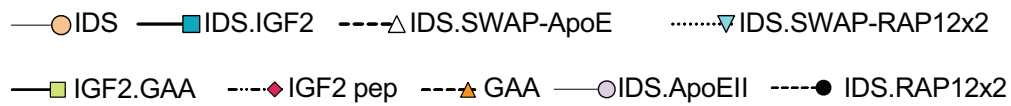
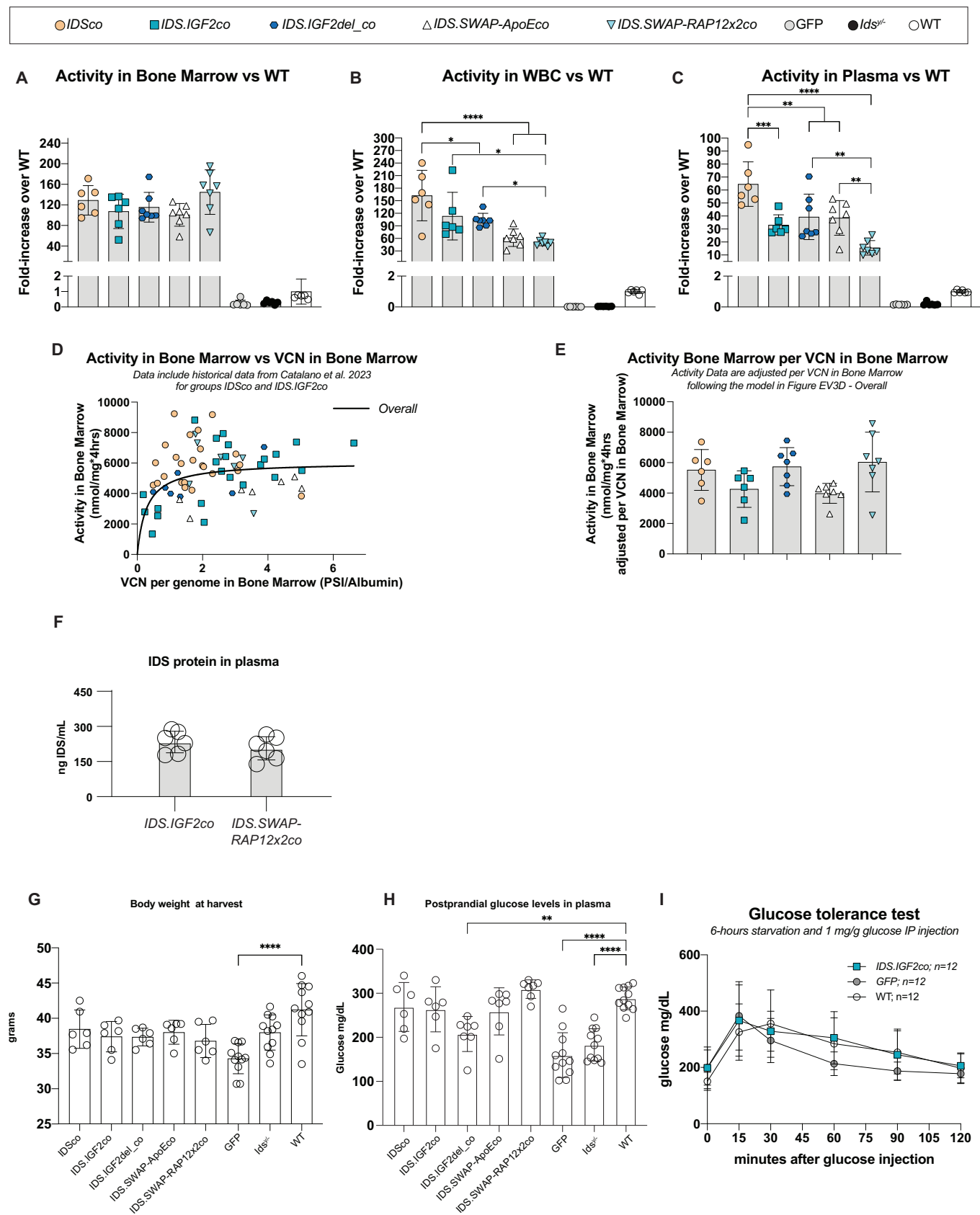


Figure EV2. CI-M6P/IGF2IGF2R, IR-A, LRP-1 ELISAs.

(A, B) competitive IR-A ELISA using biotinylated insulin. Concentrations of competing ligands are indicated. Ligands were derived from conditioned media produced in HEK 293T cells. (C, D) direct LRP-1 ELISA. Concentrations of ligands are indicated. Data information: data are means $\pm$ SD. Regression analysis is shown in Table EV1. $n = 2$ (A–C) or $n = 1$ (D).



◀ **Figure EV3. Supraphysiological levels of IDS activity in hematological samples, body weight, and glucose homeostasis.**

(A–C) IDS enzyme activity in hematological tissues. Fold-increase versus WT in bone marrow (A), WBC (B), and plasma (C). In (B, C) the adjusted *P* values were as follows: (B) *IDSco* vs. *IDS.SWAP-ApoEco* *P* = <0.0001, *IDSco* vs. *IDS.SWAP-RAP12x2co* *P* = <0.0001, *IDSco* vs. *IDS.IGF2del_co* *P* = 0.0317, *IDS.IGF2co* vs. *IDS.SWAP-RAP12x2* *P* = 0.0146, *IDS.IGF2del_co* vs. *IDS.SWAP-RAP12x2* *P* = 0.0492. (C) *IDSco* vs. *IDS.IGF2co* *P* = 0.0002, *IDSco* vs. *IDS.IGF2del_co* *P* = 0.0030, *IDSco* vs. *IDS.SWAP-ApoEco* *P* = 0.0020, *IDSco* vs. *IDS.SWAP-RAP12x2co* *P* = <0.0001, *IDS.IGF2del_co* vs. *IDS.SWAP-RAP12x2co* *P* = 0.0038, *IDS.SWAP-ApoEco* vs. *IDS.SWAP-RAP12x2co* *P* = 0.0058. (D, E) Relationship between IDS activity in bone marrow and VCN in bone marrow. (F) Sandwich ELISA to quantify IDS protein levels in plasma. (G) Body weight at harvest after gene therapy. The adjusted *P* value for *GFP* vs. WT was <0.0001. (H) Postprandial glucose levels at harvest. The adjusted *P* values were as follows: *IDS.IGF2del_co* vs. WT *P* = 0.0015, *GFP* vs. WT *P* = <0.0001, *Ids^{W/-}* vs. WT *P* = <0.0001. (I) Glucose tolerance test. Data information: data are means ± SD. Regression analysis of (D) is shown in Table EV1. In (A–C, E, G, H) data were analyzed by one-way ANOVA followed by Bonferroni's multiple testing correction. In (H), comparisons were made against WT. In (F) data were analyzed by *t* test. In (A–C, E) *n* = 6 for *IDSco*, *IDS.IGF2co*, *GFP*, *Ids^{W/-}*, and WT, *n* = 7 for *IDS.IGF2del_co*, *IDS.SWAP-ApoEco*, *IDS.SWAP-RAP12x2co*. In (F) *n* = 6. In (G, H) for *IDSco*, *IDS.IGF2co*, *IDS.IGF2del_co*, *IDS.SWAP-ApoEco*, *IDS.SWAP-RAP12x2co* *n* = 6; for *GFP*, *Ids^{W/-}*, and WT *n* = 11. In (I) *n* = 12. **P* ≤ 0.05; ***P* ≤ 0.01; ****P* ≤ 0.001; *****P* ≤ 0.001. Significant comparisons are indicated by brackets.

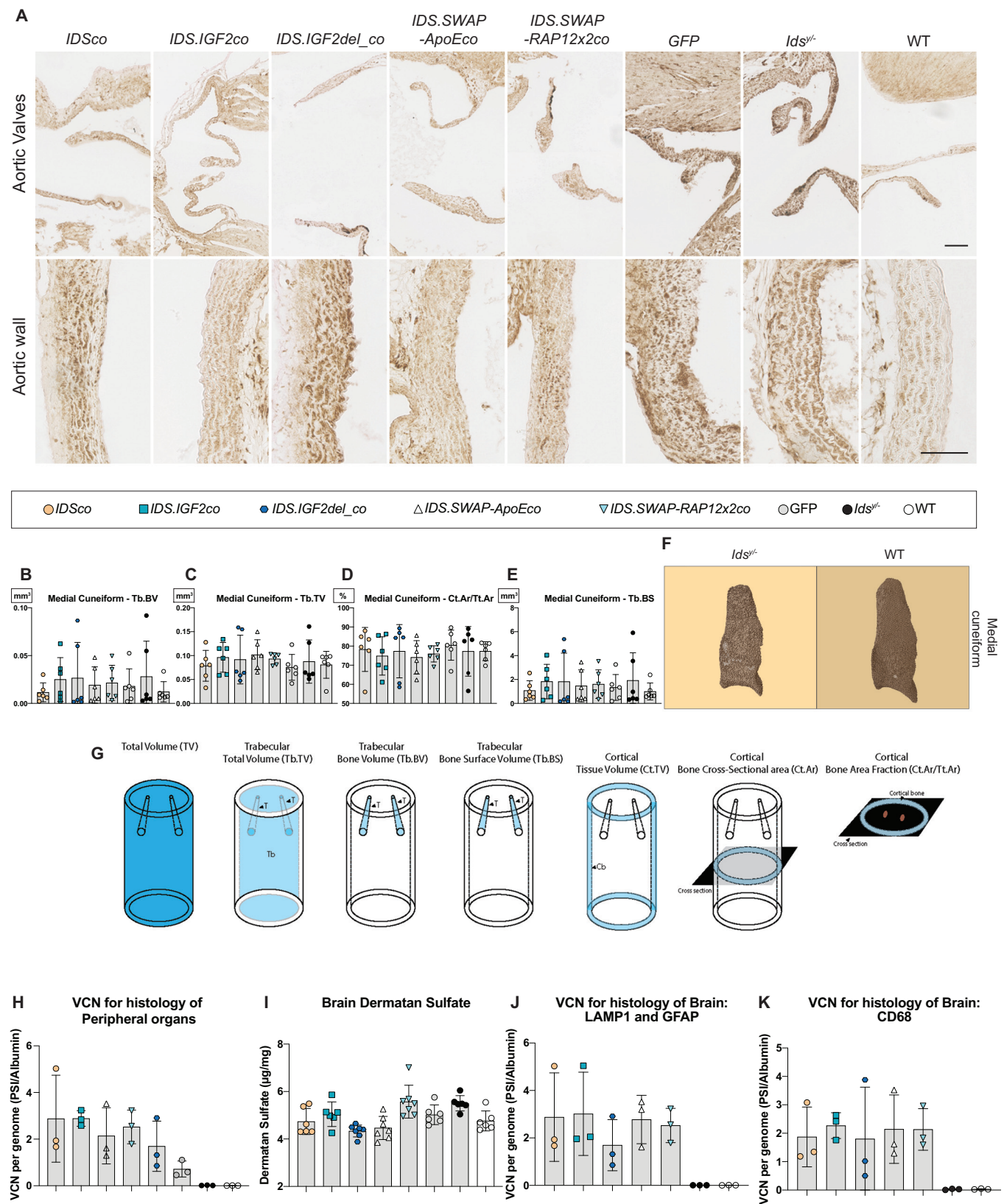




Figure EV4. LAMP1 staining in heart, microarchitecture of the medial cuneiform, brain dermatan sulfate levels, and bone marrow VCN for histology.

(A) LAMP1 staining in heart valves and aortic wall. $n = 3$. Scale bar = 100 μm . (B–E) Microarchitecture analysis of the medial cuneiform. (F) 3D rendering of reconstructed μCT scans of the medial cuneiform. (G) Explanatory drawings of μCT parameters. The volume/area measured by the μCT parameters is highlighted in blue. (H) VCN per genome in bone marrow of mice used for histological analysis of peripheral tissues. (I) Dermatan sulfate levels in total brain homogenates measured by mass-spectrometry. (J, K) VCN per genome in bone marrow of mice used for immunostaining of LAMP1 and GFAP (J), or CD68 (K) in brain. Data information: data are presented as means $\pm$ SD and were analyzed by one-way ANOVA with Bonferroni's correction. In (B–E) $n = 6$; in (H, J, K) $n = 3$; in (I) $n = 6$ (*IDSco*, *IDS.IGF2co*, *GFP*, *Id^{sc}/-*, WT) or $n = 7$ (*IDS.IGF2del_{co}*, *IDS.SWAP-ApoEco*, *IDS.SWAP-RAP12x2co*). Tb: trabecular bone; T: trabeculae; Cb: cortical bone. Data are presented as means $\pm$ SD and were analyzed by one-way ANOVA with Bonferroni's correction.

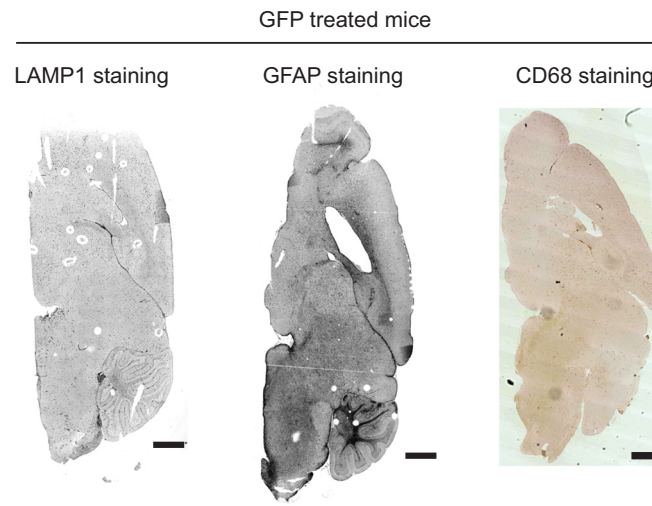


Figure EV5. Full scans of sagittal brain sections of GFP-treated mice stained for LAMP1, GFAP or CD68.

Example of sagittal brain sections from GFP-treated mice stained for LAMP1 (left panel), GFAP (central panel) or CD68 (right panel). $n = 3$. Scale bar = 1 mm.