

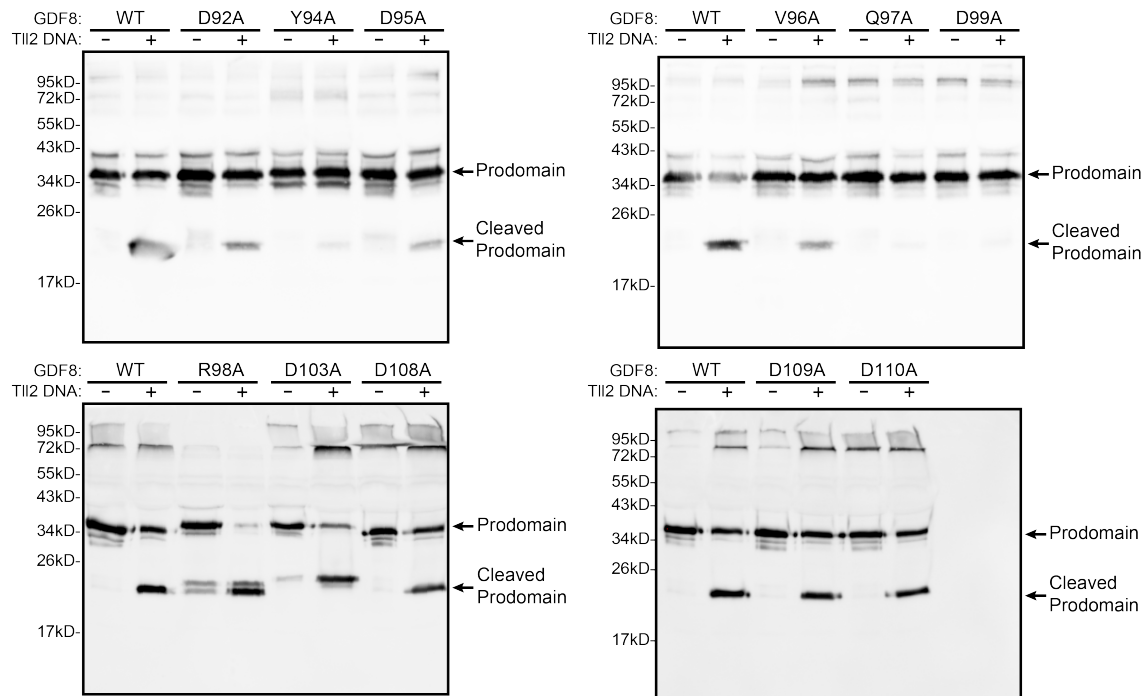
Supplement Table 1: CLUSTAL O(1.2.4)¹ multiple sequence alignment GDF8 residues adjacent to the tolloid cut site (D99). GDF8 sequences were identified using uniprot² and aligned using Clustal sequence alignment web software. Across 21 species the identity was 87%. Sequences are in FASTA format with the uniprot accession number followed by the species abbreviation in bold. D99 required for tolloid processing is red, bolded and underlined. Asterisks at the bottom of the sequences identify identical amino acid residues, colons and periods represent highly similar residues.

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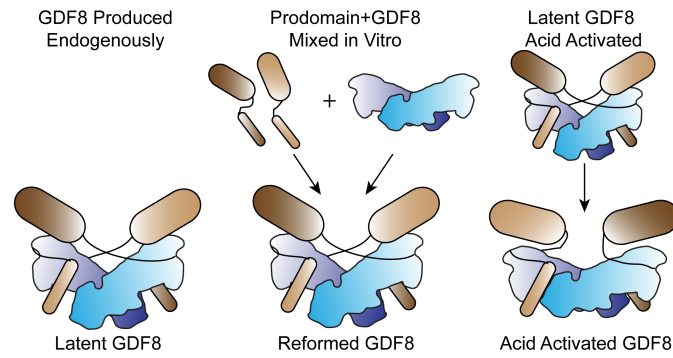
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>O42220-Chicken       89-ELIDQYDVQRDSSDGSLEDDDYH-112
>O08689-Mouse         90-ELIDQYDVQRDSSDGSLEDDDYH-113
>O35312-Rat           90-ELIDQYDVQRDSSDGSLEDDDYH-113
>O42221-Turkey        89-ELIDQYDVQRDSSDGSLEDDDYH-112
>Q6UKZ8-Dog           89-ELIDQYDVQRDSSDGSLEDDDYH-112
>A1C2U6-Macaque       89-ELIDQYDVQRDSSDGSLEDDDYH-112
>O18831-Pig           89-ELIDQYDVQRDSSDGSLEDDDYH-112
>O18830-Sheep         89-ELIDQYDVQRDSSDGSLEDDDYH-112
>Q6T5B8-Goat          89-ELIDQYDVQRDSSDGSLEDDDYH-112
>Q9GM97-Horse         89-ELIDQYDVQRDSSDGSLEDDDYH-112
>A1C2V0-Chimpanzee    89-ELIDQYDVQRDSSDGSLEDDDYH-112
>A1C2V5-Gorilla       89-ELIDQYDVQRDSSDGSLEDDDYH-112
>A1C2U7-Bonobo        89-ELIDQYDVQRDSSDGSLEDDDYH-112
>Q6J1J2-Artic Fox     89-ELIDQYDVQRDSSDGSLEDDDYH-112
>Q6DTL9-Red Fox       89-ELIDQYDVQRDSSDGSLEDDDYH-112
>A1C2U3-Orangutan     89-ELIDQYDVQRDSSDGSLEDDDYH-112
>Q8HY52-Brown Hare    89-ELIDQYDVQRDSSDGSLEDDDYH-112
>Q5USV5-Pronghorn     89-ELIDQYDVQRDSSDGSLEDDDYH-112
>Q6X5V1-Water Buffalo 89-ELIDQFDVQRDAGSDGSLEDDDYH-112
          *****:***** .*****

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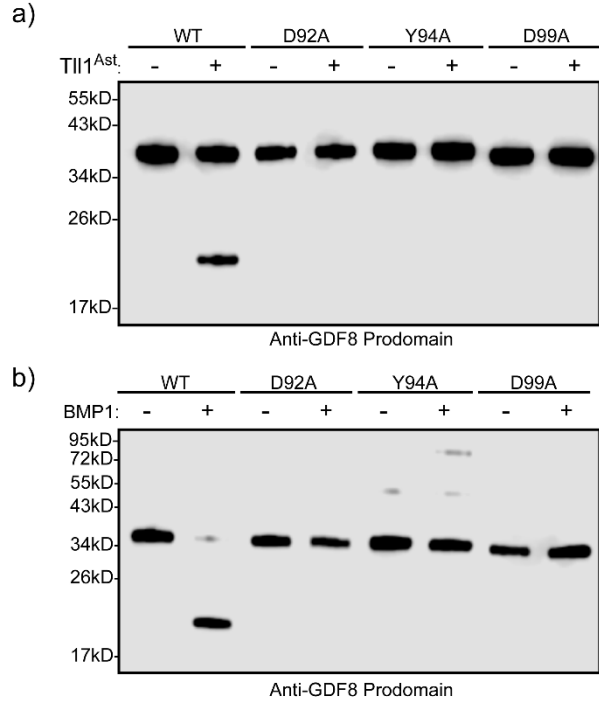
1. Larkin, M. A. et al. Clustal W and Clustal X version 2.0. *Bioinformatics* **23**, 2947-2948 (2007).
2. Bateman, A. et al. UniProt: the universal protein knowledgebase. *Nucleic Acids Res.* **45**, D158-D169 (2017).



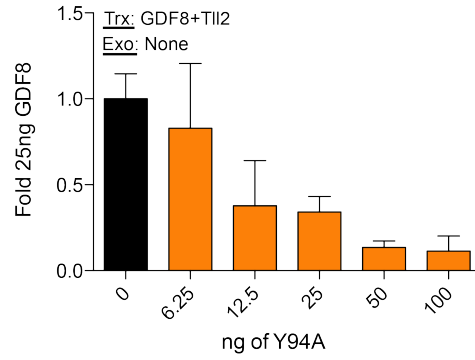
Supplement Figure 1: Expression and cleavage test of transfected GDF8. GDF8 DNA transfected is denoted at the top of each lane and if TII2 DNA was added (+) or not (-). The band corresponding to full length prodomain and the C-terminal cleavage product are labeled.



Supplement Figure 2: GDF8 States used in list study. *Left*) GDF8 endogenously produced forming a fully latent GDF8 procomplex termed “Latent GDF8”. *Middle*) “Reformed GDF8” made by mixing the GDF8 prodomain with the GDF8 mature ligand and subsequently purified using SEC. *Right*) “Acid activated GDF8” produced by exposed latent GDF8 to low pH followed by neutralization. The resulting complex is unable to inhibit GDF8 signaling.



Supplement Figure 3: Western blot analysis of latent GDF8 procomplex processing. Both westerns blots are anti-GDF8 prodomain run under non-reducing conditions **a)** Latent GD8 procomplexes incubated with the Tll1 astacin domain overnight at 37°C in a 2.4:1 molar ratio (astacin:prodomain). **b)** Latent GD8 procomplexes incubated with BMP1 overnight at 37°C in a 2.4:1 molar ratio (astacin:prodomain).



Supplement Figure 4: Titration of dominant negative GDF8 mutant, Y94A. 25 ng of WT GDF8, furin, Tll2, and a titration of Y94A GDF8 DNA (quantity denoted under the X-axis) was transfected into hEK293T (CAGA)₁₂ cells. Data plotted as mean±SD and were conducted three times with experimental triplicate. The DNA transfected (Trx) and exogenous (Exo) protein added is denoted in the top left each graph.