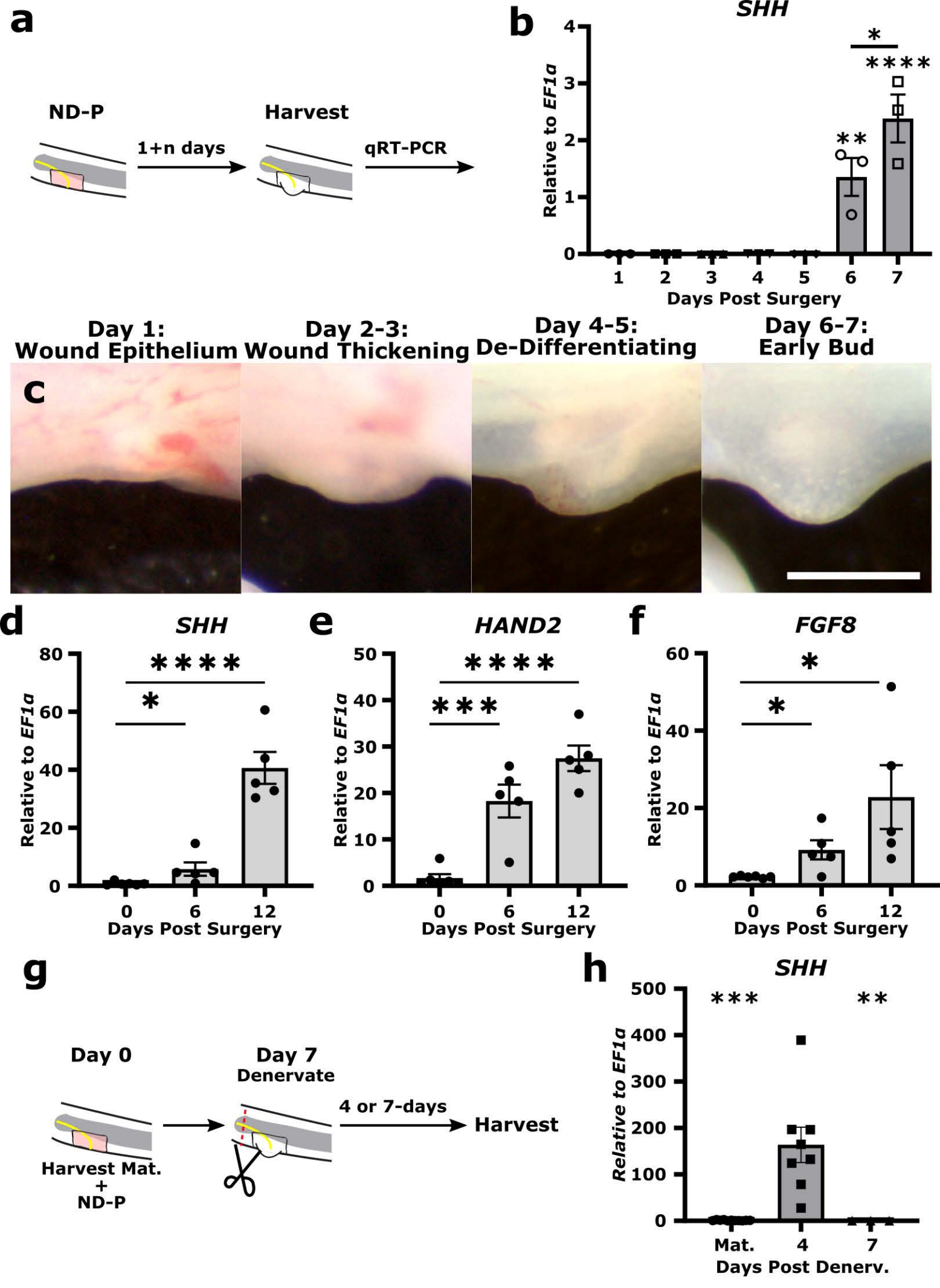
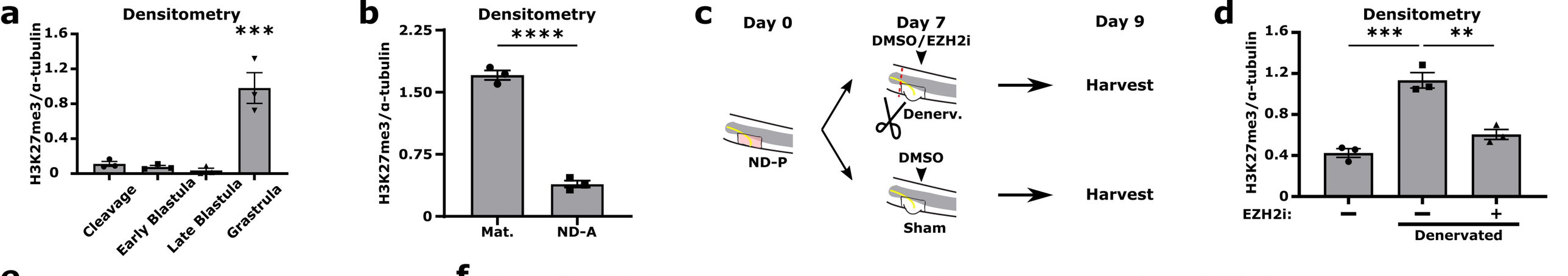
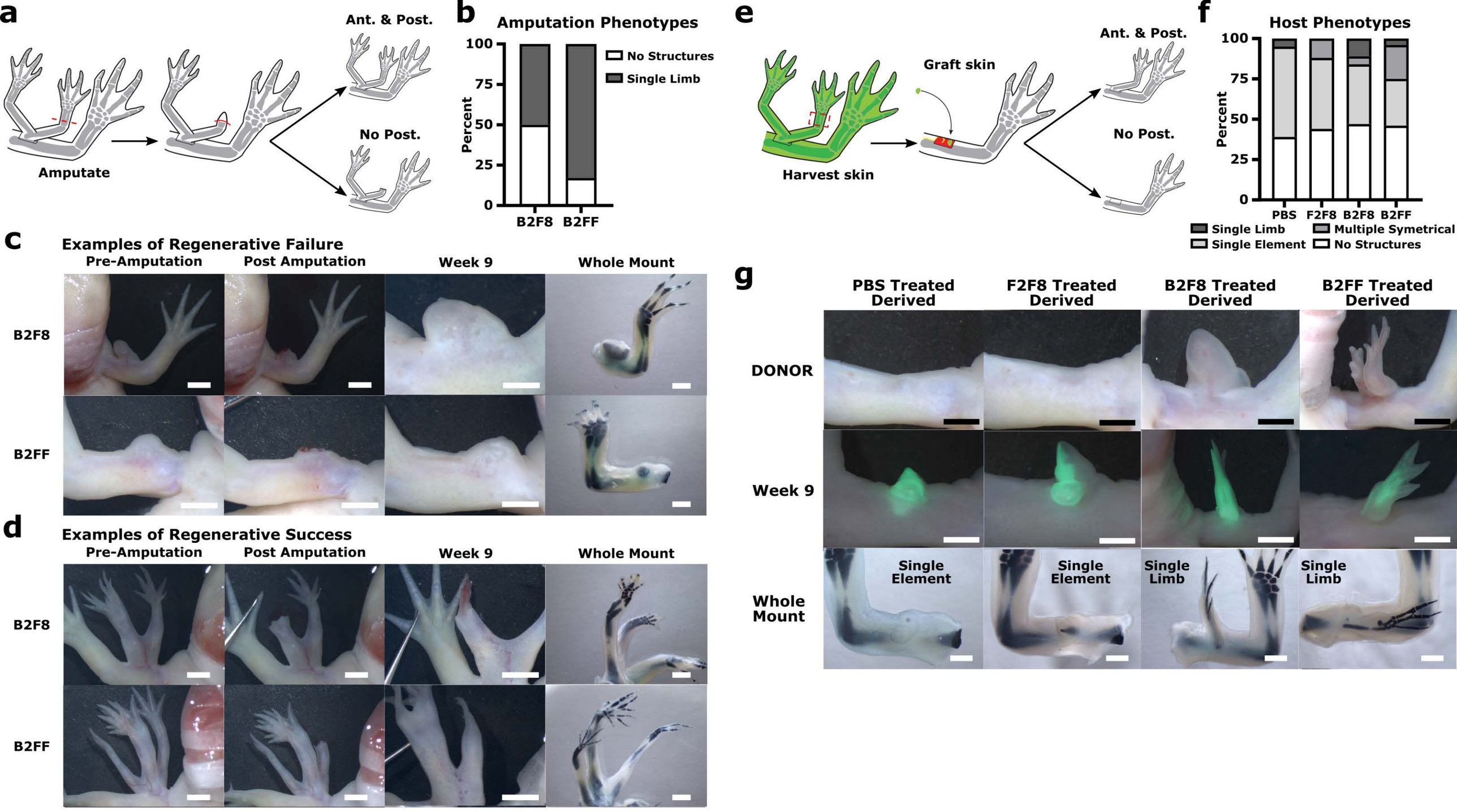


Supplemental Figure 1: Timing of limb patterning gene expression in ND-Ps. **a-b** ND-P samples were harvested at different timepoints after surgery and assessed by qRT-PCR for *SHH* expression. N=3 biological replicates per time point were collected and statistics performed was a One-Way ANOVA with a Tukey multiple comparisons test (*P≤0.05, **P≤0.01, ****P≤0.0001, error = SEM) **c** Representative brightfield images of example ND-P blastemas over time on live animals. **d-f** Histograms of qRT-PCR expression data collected of *SHH*, *HAND2*, and *FGF8* in ND-Ps overtime. N=5-6 biological replicates per sample were collected and statistics performed were the Unpaired t-test relative to day 0 was utilized (*P≤0.05, ***P≤0.0005, ****P≤0.0001, error = SEM). **g-h** Cartoon explaining sample collection and (h) histograms of qRT-PCR expression data collected of *SHH* in mature limb tissue and ND-P blastemas 4 or 7 days after denervation. N=3 biological replicates per time point were collected and statistics performed was a One-Way ANOVA with a Tukey multiple comparisons test (**P=0.0042, ***P=0.001, error = SEM).



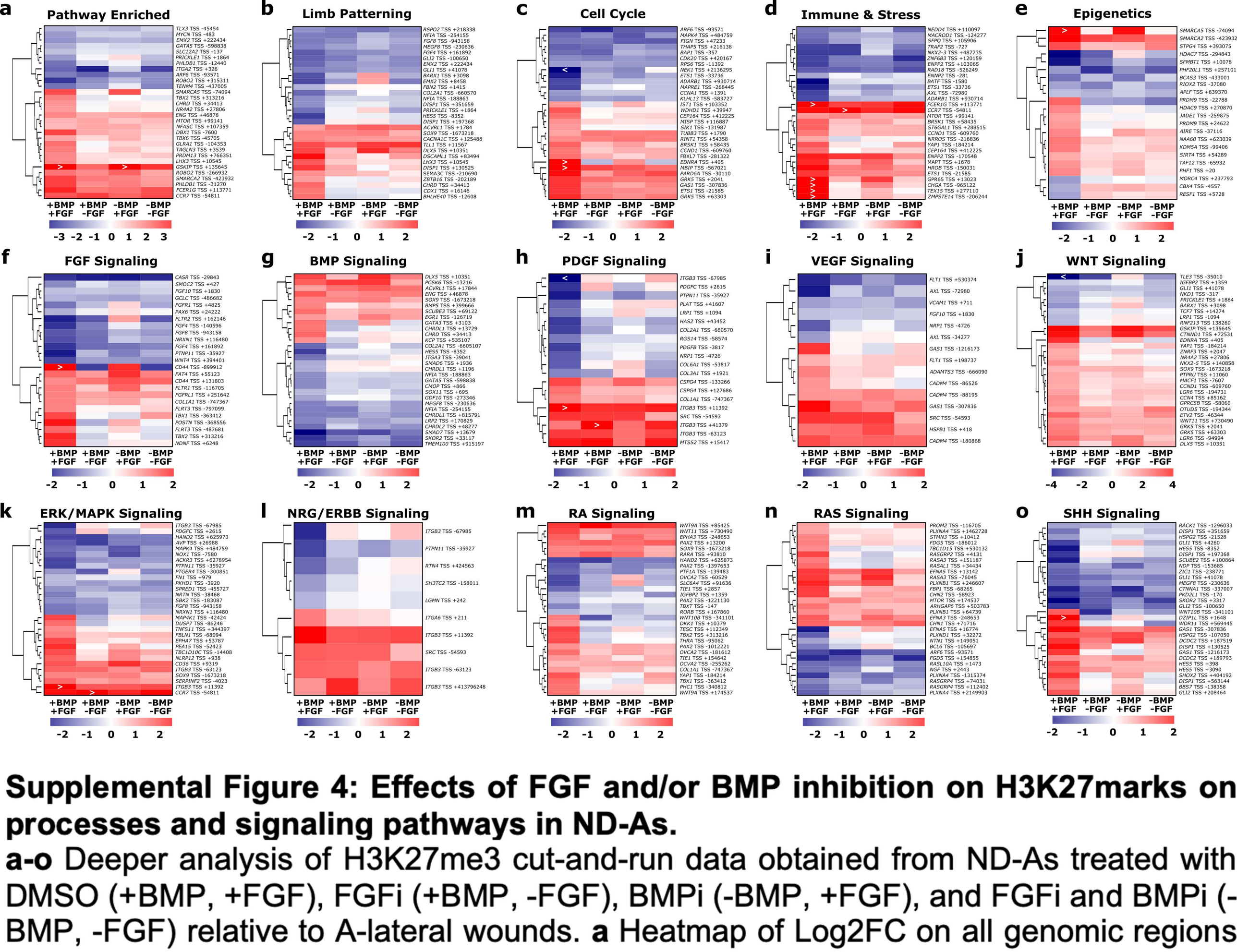
Supplemental Figure 2: anti-H3K27me3 antibody validation.

a-i All experiments were performed with the Epigentek Anti-H3K27me3 antibody. **a** Histogram of H3K27me3 abundance detected by densitometry of western blot images of samples of axolotl embryo stages. H3K27me3 bands were normalized to α-tubulin bands with N=3 biological replicates per sample, analyzed using a One-Way ANOVA with a Dunnett's multiple comparisons test (***P=0.0004, error = SEM). **b** Densitometry quantification of H3K27me3 abundance in western blot analysis of mature tissue and ND-As samples. H3K27me3 bands were normalized to α-tubulin bands with N=3 biological replicates per sample and analyzed using an Unpaired t-test (****P≤0.0001, error = SEM). **c-d** Cartoon of ND-P sample collection **c** and densitometry comparison of H3K27me3 abundance in denervated blastemas treated with DMSO or EZH2 inhibitor **d** and normalized to α-tubulin bands with N=3 biological replicates per sample and analyzed using a One-Way ANOVA with a Dunnett's multiple comparisons test (**P=0.0011, ***P=0.0002, error = SEM). **e** Example immunofluorescent images of immunostained tissue sections of mature and blastema cell nuclei (blue = DAPI, red = H3K27me3) at 63X magnification (scale = 5μm). **f** Pictural description of image processing algorithm used to quantify nuclear fluorescent signal for H3K27me3 in sections of limb tissues imaged with a 63x objective and using an Apotome to generate z-stacks. The user selects directories for nuclei/DAPI (Blue channel) and H3K27me3 (Red channel) folders containing each Z-stack in data set. The first image in the first blue channel z-stack is opened and is thresholded to count the total number of nuclei positive pixels. The corresponding image in the red channel is then opened and the number of overlapping red and blue channel pixels are counted. This count is appended to an output array and this loops until the first Z-stack is complete. This then iterates through every Z-stack in the data set and then the array is exported to a .csv format file. **g** Sample collection of amputation blastema and A-lateral wound time course and quantification of H3K27me3 nuclear abundance in immunofluorescent stained sections of blastema and A-lateral wounds over time. Time-course data points were compared to uninjured limb tissue (purple * indicates day 4 blastema vs. wound comparison). 3-6 biological replicates per sample were analyzed using an Unpaired t-test (*P≤0.05, error = SEM). **h** Sample collection of innervated and denervated amputation blastemas and quantification of H3K27me3 nuclear abundance in immunofluorescent stained sections. 3-5 biological replicates per sample were analyzed using an Unpaired t-test (*P≤0.05, error = SEM).



Supplemental Figure 3: Evaluation of stabilized posterior identity in growth factor/CALM-A induced accessory limbs.

a Cartoon describing regenerative assay performed on accessory limbs inducted in the growth factor/CALM-A. Note, accessory limbs were only induced in wounds treated with B2F8 or B2FF soaked beads. **b** Histogram representing the % of amputated accessory structures that regenerated or not (example images in c and d). N=4-6 biological replicates. **c** Examples of regenerative failure after amputation. **d** Examples of regenerative success after amputation. **e** Grafting of tissue from growth factor/CALM-A sites after 12 weeks into a host A-ALM to assess for stabilized posterior information. **f** Histogram representing the % of grafts that led to accessory limb induction in the new host site N=18-26 biological replicates. *Note: grafts of the growth factor wound sites were performed regardless of whether an accessory limb formed, and all were included in statistics.* Complexity of ectopic limb phenotypes were scored as described in (Vieira et al., 2019) **g** Representative images of ALM outcomes in the grafted wound sites. All data is available in Supplemental Data Table 1.3.



Supplemental Figure 4: Effects of FGF and/or BMP inhibition on H3K27marks on processes and signaling pathways in ND-As.

a-o Deeper analysis of H3K27me3 cut-and-run data obtained from ND-As treated with DMSO (+BMP, +FGF), FGFi (+BMP, -FGF), BMPi (-BMP, +FGF), and FGFi and BMPi (-BMP, -FGF) relative to A-lateral wounds. **a** Heatmap of Log2FC on all genomic regions that showed a significant change of H3K27me3 abundance in DMSO treated ND-As compared to A-lateral wounds. **b-o** Heatmaps of Log2FC values in top 30 significant genomic regions in the ND-As compared to A-lateral wound contrast for pathway enriched genes related to biological processes (limb patterning, cell cycle, immune and stress, epigenetics) and various signaling pathways. Heatmap keys indicate the Log2FC change of each sample group compared to DMSO treated A-lateral wounds that were significant in the ND-A (+BMP, +FGF) treated groups compared to A-lateral wound. Genes associated with each pathway were found by querying pathway associated GO-TERMS in ENSEMBL. White arrowheads indicates that enrichment score is outside of the range in a given heatmap. Full gene lists and data are provided in Supplemental Data Table 4.